Vitamin D Analogues
via
Dynamic Combinatorial Chemistry

by

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car grâce à elle, tout est possible.

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The road of life twists and turns and no two directions are ever the same.
Yet our lessons come from the journey, not the destination.

Don Williams, Jr., American Novelist and Poet, b.1968

What a journey it has been !!!! Even though I had a clear idea of my destination, I only had a vague notion of what my journey through graduate studies would be like. I had come to McGill mostly hoping to improve my English and left with a lifetime of memories and invaluable life lessons. During this journey, I had the chance to meet incredible people : amazing scientists, generous labmates, caring administrative staff and trusting friends... More than any scientific achievements, I feel that those encounters and the friendships they eventually evolved into are the main goals any graduate student should strive to achieve. Today, as I reflect upon my journey, I would like to take the time to tell those precious persons how much I appreciated their presence in my life. With all my heart, I would like to say thank you.

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ABSTRACT

Calcitriol (1α,25-$(OH)_2D_3$, 1,25D) is known for its calcium regulatory function, but also for being a powerful inhibitor of cell growth in a variety of normal and neoplastic cells. As such, it is a lead structure in the development of new cancer drugs. The goal of our project is to identify analogues of calcitriol which bind tightly to the vitamin D receptor (VDR) yet do not exhibit unwanted hypercalcemic effects. The development of novel analogues using conventional chemistry and dynamic chemistry was investigated. First, the numerous building blocks required for the exploration of the different libraries were created through the use of conventional techniques. We then developed different synthetic schemes based primarily on thiol alkylation and amide couplings, enabling rapid parallel synthesis of potential 1,25D analogues. Preliminary investigations allowed us to implement with our collaborators a set of biological assays to evaluate our compounds. Although some analogues appeared to bind to the VDR, it was found that none of the 8 analogues tested in the preliminary studies seemed to be vitamin D receptor (VDR) agonists.

In a second part, our research focused on the study of dynamic combinatorial chemistry as a tool for the synthesis of 1,25D analogues. Our first dynamically-generated libraries were created via thioester exchange. It was found that the rate of thioester exchange was dependent on the nature of the thiols involved and the acyl portion of the thioesters. Aliphatic acid thioesters exchanged more slowly than aromatic acid thioesters. The use of branched (α-substituted) thiols also slowed the rate compared to primary ones. However, the use of large concentration of aliphatic or aromatic thiols could accelerate the thioester exchange. When thioesters were placed in presence of protein targets in a dynamic system, their inherent electrophilic nature rendered them prone to chemical decomposition (hydrolysis and acylation of protein nucleophilic residues) at basic pHs. Nonetheless, we created thioester libraries of up to 40 members from as few as 7 building blocks under near neutral

conditions. We showed that these dynamic libraries ere influenced by the presence of proteins. We have not been able to confirm if thioester libraries were influenced exclusively by the VDR via its binding site. Some of our observations challenged the viability of the thioester exchange as a reversible process in the context of VD_3 analogue synthesis.

Finally, we created dithiol building blocks, amenable to disulfide exchange in dynamic libraries. Chemically stable, these disulfide entities easily generated libraries of at least 30 members from as few as 7 building blocks. Our preliminary disulfide libraries seemed uninfluenced by VDR's presence. These results still allowed us to understand better the characteristics and limitations of dynamic systems applied to the development of novel vitamin D analogues.

RÉSUMÉ

Calcitriol (1α,25-dihydroxyvitamine D_3, 1,25D) est connu pour sa fonction dans la régulation du métabolisme du calcium, mais aussi comme étant un puissant inhibiteur de la prolifération cellulaire dans un éventail de cellules normales et néoplastiques. Comme tel, il représente le point de départ pour le développement de nouvelles molécules anticancéreuses. Le but de notre projet est d'identifier des analogues du calcitriol capables de se lier au récepteur de la vitamine D (RVD) sans causer d'hypercalcémie.

Le développement de nouveaux analogues de la vitamine D_3 a été exploré en utilisant la chimie conventionelle et la chimie dynamique. Dans un premier temps, de nombreux synthons nécessaires à la création des différentes librairies ont été fabriqués par des techniques de synthèse traditionnelles. Ensuite, des routes de synthèse basées sur l'alkylation de thiols et la formation d'amides ont été élaborées, permettant une synthèse en parallèle efficace d'analogues de la vitamine D. En collaboration avec nos partenaires, des études préliminaires ont permis d'établir la validité d'une série de tests pour valuer l'activit biologique de nos analogues sur le récepteur de la vitamine D. Bien que plusieurs composés semblent se lier au récepteur, aucun des 8 analogues évalués de façon préliminaire n'ont révélé une activité agoniste du RVD.

Dans un deuxième temps, nous avons axé notre recherche sur l'étude de la chimie combinatoire dynamique (CCD) comme outil pour la création et l'évaluation d'analogues de la vitamine D. La CCD est basée sur le fait qu'une protéine peut influencer la composition de bibliothèques dynamiques en favorisant la création de ses propres ligands, et ce, proportionnellement à leur constante d'affinité. Les premières bibliothèques dynamiques furent créées à partir d'échanges entre thioesters. Il a été évalué qualitativement que la vitesse de ce processus est dépendante de la nature des thiols impliqués ainsi que de la na-

ture de la portion acyl des thioesters. L'échange des thioesters d'acides aliphatiques est plus lent que celui des thioesters d'acides aromatiques. Aussi, plus les thiols sont encombrés stériquement, plus le processus est lent. Toutefois, l'utilisation de grandes concentrations de thiols aromatiques et aliphatiques peut accélérer le processus d'échange. Lorsque des thioesters sont utilisés en présence de protéines à pH basique, leur caractère électrophilique les rend propices à la dégradation chimique par hydrolyse ou par acylation des fonctionnalités nucléophiles des protéines. Néanmoins, à pH neutre, des bibliothèques de thioesters possédant plus de 40 composantes furent facilement réalisées à partir de seulement 7 synthons. Il a pu être démontré que la composition de ces bibliothèques est influencée par la présence de protéines. Cependant, nous n'avons pas été en mesure de confirmer si le RVD a véritablement influencé la nature des bibliothèques de thioesters grâce à son site de liaison. Certaines de nos observations mettent en doute l'utilisation de la transthioestérification comme processus dynamique viable pour la création d'analogues de la vitamine D en présence de protéines.

Finalement, des synthons dithiols furent créés et leur combinaison a permis la formation de bibliothèques dynamiques de plus de 30 composés, grâce à l'échange de disulfures. Les études préliminaires ont montré que les bibliothèques de disulfures ne semblent pas être influencées par la présence du RDV. Ces résultats ont permis de connaître les caractéristiques et les limites de ces systèmes dynamiques appliqués au développement de nouveaux analogues de la vitamine D.

List of Abbreviations, Chemical Formulas and Symbols

1,25D	calcitriol or 1α,25-$(OH)_2$ D_3
25D	calcidiol or 25-OH D_3
1α,25-$(OH)_2$ D_3	calcitriol
BMBA	3,5-bis(mercaptomethyl)benzoic acid
*t*BuOMe	methyl tert-butyl ether
AcCN	acetonitrile
AcOH	acetic acid
aq.	aqueous
cat.	catalytic
CDI	1,1'-carbonyldiimidazole
CH_2Cl_2	dichloromethane or methylene chloride
CT	calcitonine
DBP	vitamin D binding protein
DCC	dynamic combinatorial chemistry
DCL	dynamic combinatorial library
DCM	CH_2Cl_2 or dichloromethane or methylene chloride
ddH_2O	double-distilled H_2O
DEAD	diethylazodicarboxylate
DIAD	diisopropylazodicarboxylate
DIBALH	Diisobutylaluminum hydride
DIPEA	diisopropylethylamine

DMAP	dimethylaminopyridine
DMF	dimethylformamide
DMPU	*N,N'*-dimethylpropylene urea
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
DPPA	diphenylphosphoryl azide
EDTA	ethylenediaminetetraacetic acid
ElGST	equine liver glutathione-S transferase
EtOAc	ethyl acetate
Et_2O	diethyl ether
Et_3N	triethylamine
GST	glutathione-S transferase
GST-*h*VDR	glutathione-S transferase - human vitamin D receptor conjuguate
HBTU	*O*-(Benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate
HEPES	4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid
HOBt	1-hydroxybenzotriazole
hrs	hours
*h*VDR	human vitamin D receptor
IPTG	isopropyl β-D-1-thiogalactopyranoside
LBD	ligand-binding domain
LBP	ligand-binding pocket
LDA	lithium diisopropylamide
LiDBB	lithium di-tert-butylbiphenyl
MCD	(1S,3S)-5-mercaptocyclohexane-1,3-diol
MCD_2	(1S,3S)-5-mercaptocyclohexane-1,3-diol disulfide
MeCN	acetonitrile
MeLi	methyl lithium
MeMPAA	methyl mercaptophenylacetate
MESNa	sodium 2-mercaptoethanesulfonate
MMB	4-mercapto-2-methylbutan-2-ol
MMB_2	4-mercapto-2-methylbutan-2-ol disulfide

MMHe	6-mercapto-2-methylhexan-2-ol
MMPe	5-mercapto-2-methylpentan-2-ol
$MMPe_2$	5-mercapto-2-methylpentan-2-ol disulfide
MMPr	1-mercapto-2-methylpropan-2-ol
$MMPr_2$	1-mercapto-2-methylpropan-2-ol disulfide
MMHp	6-mercapto-2-methylheptan-2-ol
min.	minutes
MOPS	3-(*N*-morpholino)propanesulfonic acid sodium salt
mp	melting point
MPAA	mercaptophenylacetic acid
NaHMDS	sodium hexamethyldisilazide
NCL	native chemical ligation
NMI	*N*-methylimidazole
NMM	*N*-methylmorpholine
NMR	nuclear magnetic resonance
NR	nuclear receptor
o/n	overnight
OD_{600}	optical density of a sample measured at a wavelength of 600 nm
PIPES	1,4-piperazinediethanesulfonic acid
PTG	parathyroid gland
PTH	parathyroid hormone
RPM	rotation per minute
rt	room temperature
Rt	retention time
RXR	retinoid-x receptor
sat.	saturated
TBDMS	tert-butyldimethylsilyl
TBS	tert-butyldimethylsilyl
TCDI	thiocarbonyldiimidazole
TCEP	tris[2-carboxyethyl]phosphine
TEA	triethanolamine

TfOH	trifluorosulfonic acid
THF	tetrahydrofuran
TLC	thin-layer chromatography
TRIS or Tris	tris(hydroxymethyl)aminomethane
TsCl	*p*-toluenesulfonyl chloride
UV	ultra-violet
VD_2	vitamin D_2
VD_3	vitamin D_3
VDR	vitamin D receptor
VDRE	vitamin D response elements

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CHAPTER 1

General Introduction

1.1 Vitamin D

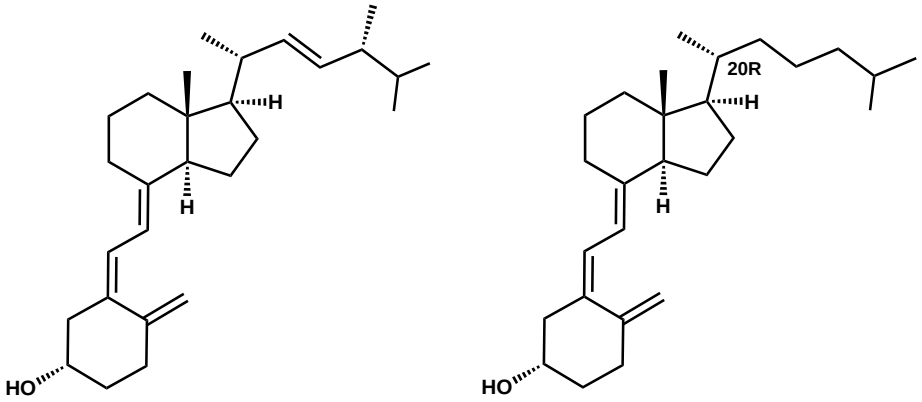
1.1.1 History

> Jack was suffering and he was not alone ; many of his friends were also sick. It was quite common for kids to be sick in the polluted cities of 18th century Europe. A doctor came and examined the young boy. Diagnosis : rickets.

Characterized by the presence of soft and deformed bones, muscle spasms and seizures, this condition of unknown origins and to which no treatment existed at the time is now well understood. Rickets arises from the body's inability to use calcium and phosphorus normally. By the beginning of the 19th century, it was known that cod liver oil and sun-bathing (even using artificially produced ultraviolet light) had antirachitic effects which cured rickets. Something from the diet, something from the sun, same curative properties. Approximately 100 years later, the common source of the cure was identified as a nutrient

that occurs naturally in only a few foods but that can also be manufactured in the skin when exposed to the ultraviolet rays. This new nutrient was named during the heart of the discovery of the multiple *vital amines*, which the body crucially needed to function properly, and received the now popular name, vitamin D.[1]

1.1.2 Sources and metabolism



vitamin D_2 (ergocalciferol) **vitamin D_3 (cholecalciferol)**

Scheme 1.1. Structures of vitamin D_2 (VD_2) and vitamin D_3 (VD_3)

By the end of the 1930's, it was known that there were actually several vitamin Ds. The principal ones were ergocalciferol or vitamin D_2 (VD_2) and cholecalciferol or vitamin D_3 (VD_3) (Scheme 1.1). There is no vitamin D_1 because after being named thus, it was found to be a 1:1 mixture of lumisterol and vitamin D_2 rather than a pure substance. The vitamin Ds structures were elucidated and found to be secosteroids, steroids which had undergone breakage of the B-ring. Vitamin D_2 is a steroid that originates in plants and can be produced by ultraviolet irradiation of ergosterol. It is vitamin D_2 that is now incorporated as a supplement in certain foods such as milk. Vitamin D_3 is the result of a photochemical reaction of 7-dehydrocholesterol that occurs in the skin (Figure 1.1). Humans can

[1] For the exact nomenclature of the molecules in the vitamin D family, read Dixon's IUPAC report.[1]

synthesize 7-dehydrocholesterol (also called provitamin D_3), which is also a precursor of cholesterol, from acetyl-coenzyme A. Thus, by definition, the substances called vitamin D are not really vitamins because they can be produced by the body. However, for nutritional and public health purposes, vitamin D_3 continues to be classified as a vitamin.

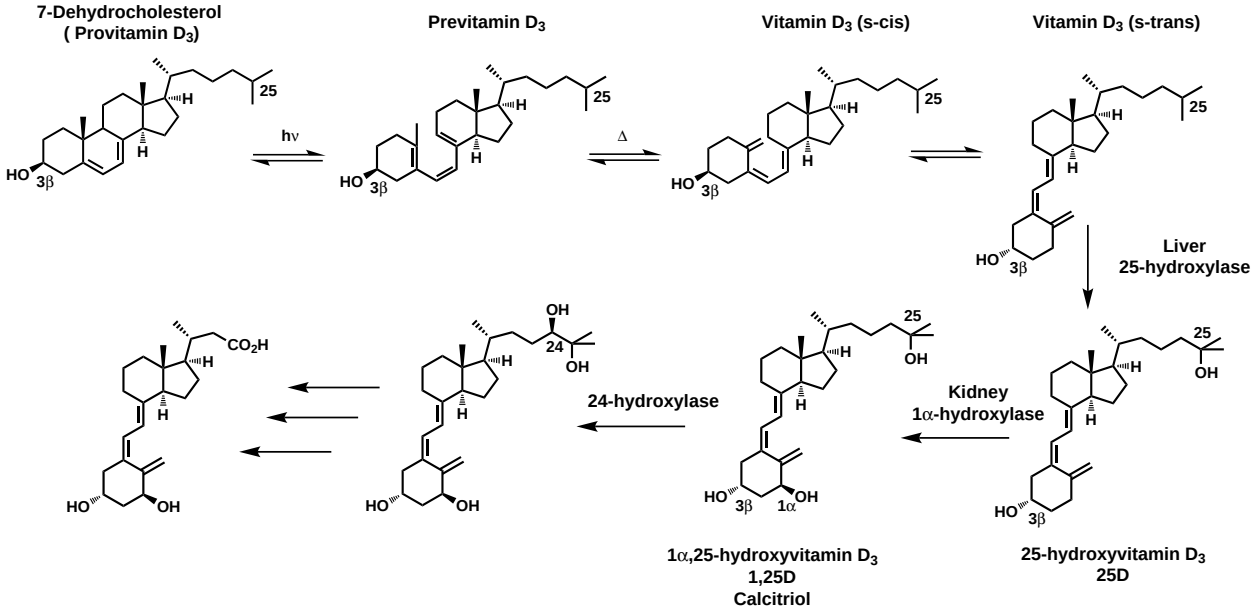


Figure 1.1. Metabolism of vitamin D_3, adapted from [2].

Today, we know more about the mechanism that converts 7-dehydrocholesterol into vitamin D_3 (see Figure 1.1). The skin is the only tissue in the human body where this photochemical reaction pathway can occur. The photosynthesis starts with UV light conversion of 7-dehydrocholesterol into previtamin D_3 via conrotatory electrocyclic ring opening of ring B. Subsequent thermal isomerization of previtamin D_3 into vitamin D_3 occurs via a 1,7-sigmatropic hydrogen shift. It is interesting to note that these two key reactions to produce vitamin D_3 are pure chemical reactions that occur without any enzyme catalysis. Before any of the vitamin Ds can act upon the body (neither of them is biologically active), they need to undergo structural modifications via metabolism. Through the circulatory sys-

tem and with the help of vitamin D binding protein (DBP)[2], both cholecalciferol (VD_3) and ergocalciferol (VD_2) can reach the liver where they are oxidized into 25-hydroxyvitamin D (25D) by the enzyme CYP27A1[4], a cytochrome P450 enzyme that also functions in bile acid metabolism. After reentering circulation, 25D undergoes another hydroxylation and becomes 1α,25-$(OH)_2$ D (1,25D or calcitriol) through a process catalyzed by 1α-hydroxylase such as CYP27B1.[5–8] The bulk of the 1,25-$(OH)_2$ D_3 is produced in the kidneys but immune cells and several other tissues (keratinocytes, brain (glial cells), bone, pneumocytes) express 1α-hydroxylase needed to achieve the transformation as well. It is the 1,25D form of the vitamin Ds that mediates the physiological activity attributed to these molecules.

1.1.3 Pleiotropic actions of vitamin D

General mode of action : binding to the vitamin D receptor and triggering of transactivation

Small lipophilic molecules such as steroids, vitamin D, thyroid hormones, and retinoic acid play an important role in the growth, differentiation, metabolism, reproduction, and morphogenesis of higher organisms and humans. Most cellular actions of these molecules are mediated through binding to their corrsponding nuclear receptors (NRs) that act as ligand-dependent transcription factors. Accordingly, the active vitamin D expresses its functions by binding to the vitamin D receptor (VDR). 1,25D is a molecule presenting structural features that ensure a good fit into the ligand-binding pocket (LBP) of VDR.[9–11] This receptor binds its hormone with high affinity (K_D = 0.1 nM). [12]

[2]DBP binds and transports vitamin D and its metabolites through serum.[3]

Vitamin D transported into the nucleus of a target cell binds to the VDR. This binding is mainly mediated by the 25-hydroxyl (OH) group, which hydrogen bonds to two histidine residues (His305 and His397), and the 1α-hydroxyl and 3-hydroxyl groups both on the A-ring portion, which bind respectively to arginine 274 (R274) and to serine 278 (S278) and tyrosine 143 (Y143).[13]

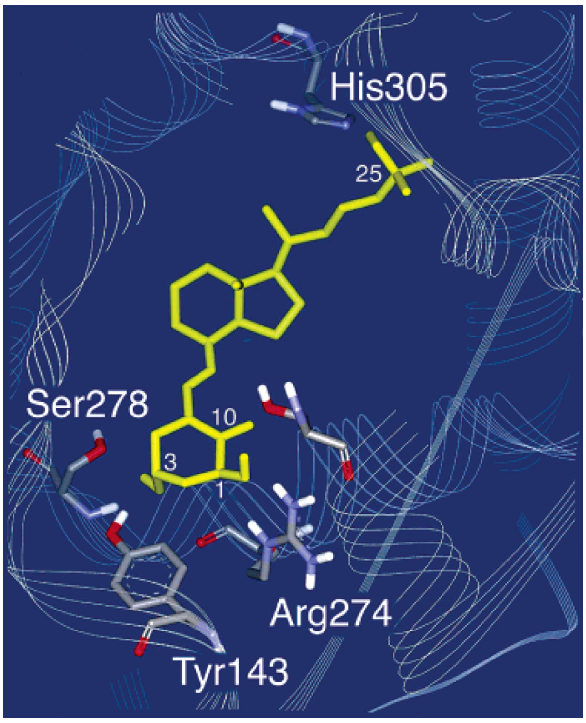


Figure 1.2. Important interactions between 1,25D and *h*VDR according to reported cocrystal structure.[13] (Figure adapted from [14].)

The binding of 1,25D to the VDR triggers a chain of events that culminates in the transactivation of target genes (Figure 1.3). Upon binding of its ligand, the VDR changes its conformation to an active form that forms a heterodimer with retinoic acid X receptor (RXR). This dimer has a zinc finger domain that binds to specific DNA sequences, termed vitamin D responsive elements (VDRE), in the promoter region of target genes. Recruitment of coactivator proteins occurs on the active form of the VDR and this triggers a complex series of events leading to transactivation.[15]

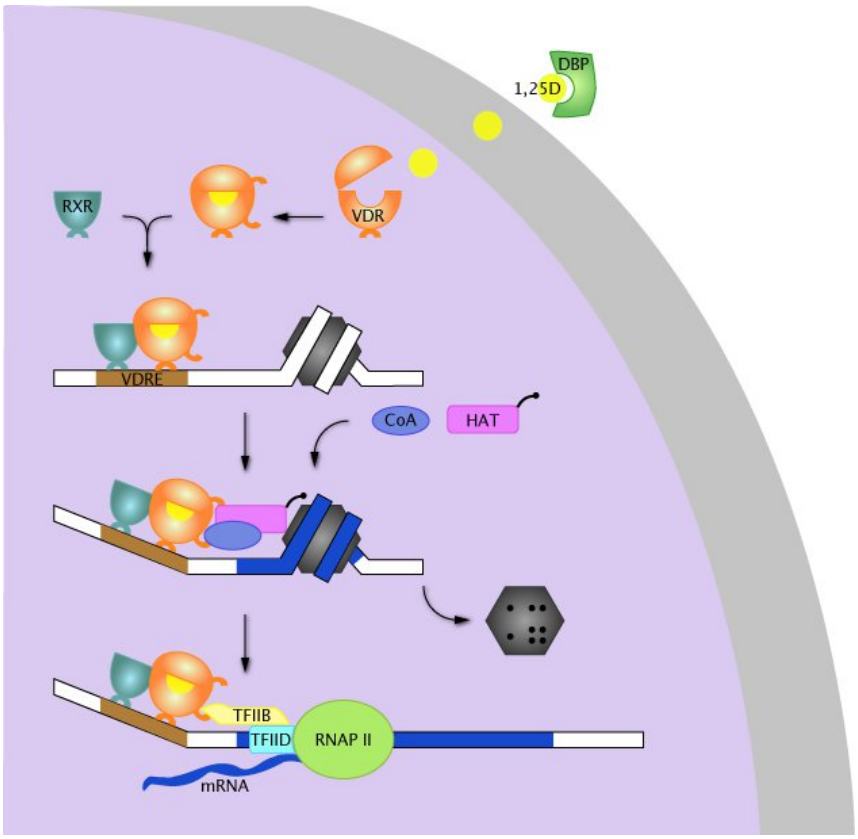


Figure 1.3. Gene switching mechanism by 1,25D-binding to VDR. Figure 4. The proposed mechanism for 1,25D mediated upregulation of VDR target genes. 1,25D is transported in the blood to target tissues by the vitamin D binding protein (DBP). On binding to VDR in the cell nucleus, the VDR-1,25D complex partners with another nuclear hormone, the retinoid X receptor (RXR). The receptor heterodimer associates with the regulatory regions of target genes, termed vitamin D response elements (VDREs), and recruits coactivator (CoA) protein complexes. Coactivator complexes may facilitate gene expression by modifying histone acetylation through the activity of histone acetyl transferases (HATs) within the complex, which serve to reveal the DNA to the transcriptional machinery of the cell, such as RNA polymerases (RNAP II).(Melanie Burger, ©2010)

Broad-action gene switch

Calcium and phosphorus homeostasis Vitamin D is widely known for its primary role in regulating calcium and phosphorus homeostasis. 1,25D regulates Ca and P blood levels via target genes that encode proteins such as calbindin, osteocalcin, and osteopontin. The high potency of 1,25D in elevating serum calcium and phosphate levels requires a mechanism to attenuate its activity. This is accomplished within virtually all target cells by the $1,25(OH)_2D_3$-inducible $25(OH)D_3$-24-hydroxylase (encoded by the gene CYP24), which catalyzes a series of oxidation reactions at carbons 24 and 23, leading to side chain cleavage and inactivation.[16, 17]

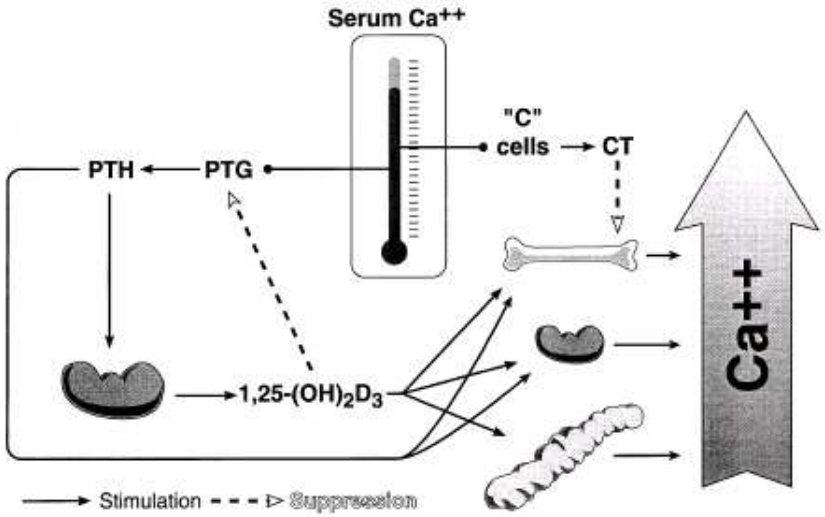


Figure 1.4. Calcium regulation mechanism. PTH, parathyroid hormone; $1,25(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3 ; PTG, parathyroid gland; C cells, parafollicular cells of the thyroid that secrete calcitonin (CT).(taken from [2]).

Figure 1.4 shows the mechanism by which calcium homeostasis occurs. In the case of hypocalcemia (deficiency of calcium in the blood), the parathyroid glands (PTG) get activated, secrete a parathyroid hormone (PTH) which activates the 25-OH-D_3-1αOHase and results in the conversion of 25-OH D_3 into hormonally active $1,25(OH)_2D_3$. PTH also

deactivates 25-OH-D-24-OHase to minimize 1,25$(OH)_2D_3$ destruction. The net result is the elevation of plasma levels of 1,25$(OH)_2D_3$, which in turn causes a redistribution or a capture of more Ca in the blood from the bones, liver, and intestines. In case of hypercalcemia (excess of calcium in the blood resulting in the medium-long term by calcification of soft tissues (kidneys, heart, aorta, intestines) causing organ failure and death), the C cells (parafolicular cells) of the thyroid gland secrete the hormone calcitonine (CT). CT acts on osteocytes and osteoclasts (bones) reducing the calcium mobilizing activity from the skeleton. This reduces the Ca plasma level.

Diverse other roles The virtually ubiquitous presence of vitamin D receptors (VDRs) suggests a more universal function for 1,25D. The combined presence of 25$(OH)D_3$-1α-hydroxylase and specific receptors in several tissues (e.g., bone cells, keratinocytes, immune cells) introduced the idea of a paracrine[3] role for 1,25D. So it wasn't a complete surprise when researchers found that, in addition to its calcemic effects, vitamin D is a potent regulator of proliferation and differentiation of cells and can also stimulate an immune response.[2, 18] This finding has led to the analysis of the anticancer potential of VD_3 analoguess. The direct use of 1,25$(OH)_2$ VD_3 in cancer treatment is hindered by a problem of activity separation. At doses required to achieve chemopreventive efficacy, vitamin D exerts toxic effects such as hypercalcemia, weight loss, and tissue calcification, as was noted in preclinical studies.[19] The strong calcemic activity of vitamin D_3 limits the therapeutic potential of vitamin D compounds in non-classical uses. Design of new analogues has focused on modifications to separate antitumor properties from other biological influences, mainly calcium regulation.

To date, there are more than 3000 1α,25-$(OH)_2D_3$ analogues (or sometimes called

[3] *Paracrine signalling is a form of cell signaling in which the target cell is near (para = near) the signal-releasing cell. For example, growth factors and clotting factors are paracrine signaling agents. Wikipedia, http://en.wikipedia.org/wiki/Paracrine_signalling*

deltanoids) that have been synthesized and their biological activity evaluated.[20–23] Most of those analogues show agonistic potential, but they differ greatly in their effciency [20]. Some of these agonists have even been shown to be superagonists, i.e. they act in living cells at much lower concentrations than 1α,25$(OH)_2D_3$. Among the many structural analogues of calcitriol prepared and biologically tested, some exhibit an interesting separation of calcemic and differentiation activities. Such selectivity is of potential therapeutic value.

1.1.4 Characteristics of vitamin D receptor modulators (VDRM)

Vitamin D can be structurally divided into of four portions : the side-chain (top) portion, the core C,D-ring structure (middle), the seco-B ring (triene) portion and the A-ring (bottom) portion (see Figure 1.5)

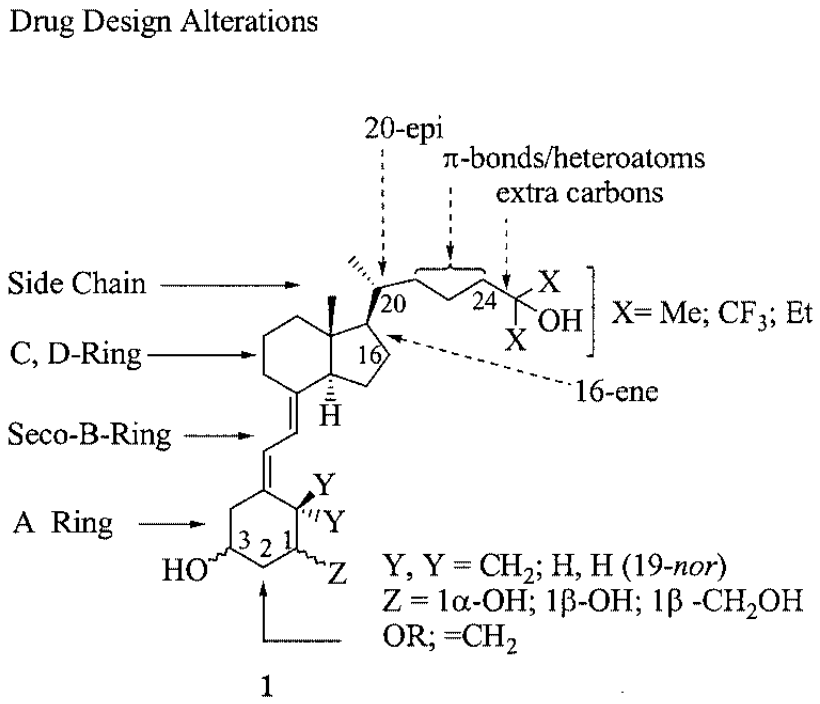


Figure 1.5. The four sections of vitamin D and its main alterations leading to relevant biologically active analogues (taken from [24]).

Many vitamin D_3 analogues have been created by modifying these 4 different regions

of this general chemical structure. With the accumulated data from those analogues, we now have a better idea of the influence of each of those modifications on the biological activity exerted by 1,25D.

It is important however to realize that many variations of seemingly important features of the secosteroid structure are acceptable. Moreover, as one examines the reports on the biological activities of deltanoids, it becomes clear that a significant body of literature points toward a poor correlation between binding affinities of the analogues to the VDR and their efficacy in antiproliferative, differentiative or even calcemic evaluation assays.[25] There is a gray zone around the direct comparison of binding affinities and the range and degree of the biological competence of deltanoids.

It is therefore important to keep in mind that modifications and their effects reported below are not absolutes. Also, while some modifications can be combined to create super efficient analogues, there is rarely a general way of predicting how or when a modification will increase affinity or efficacy.

Side chain modifications

In the study of the structure-function relationship of vitamin D analogues, the focus has been overwhelmingly put on side chain modifications and for good reason. A large variety of modifications in this part of the molecule are known to have beneficial effects on biological potency and the activity spectrum of vitamin D (Figure 1.5).[26] Among those modifications that elevate potency are epimerization at C(20), substitution of C(22) with oxygen, elongation of the side chain, methylation at the terminal carbons C(26) and C(27) and at C(20), perfluorination at C(24) and C(26) and C(27), introduction of a double bond at C(16)-C(17) and C(22)-C(23) and a triple bond at C(23)-C(24), and removal of the

angular C(18) methyl group between the C and D rings. Molecule EB-1089 is an example of a vitamin D_3 analogue obtained from side chain modification (Figure 1.6).

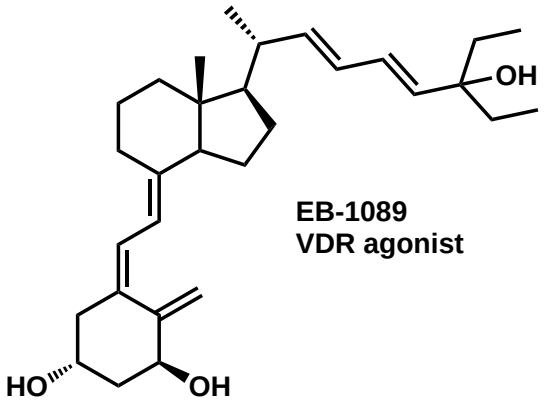


Figure 1.6. EB1089, a vitamin D_3 side chain analogue possessing high biological potency.

A-ring modifications

The main characteristics of the A-ring concern the two hydroxyl groups positioned at C(1) and C(3) on the ring (see Figure 1.5). Their presence, especially at C(1), is vitally important for the biological activity of VD_3, as exemplified by the fact that it is the enzyme-catalyzed hydroxylation at position C(1) that generates the physiologically active form of vitamin D. The stereochemistry of those OH groups is also crucial, as all other stereoisomers exhibit significantly reduced biological activity.[27]

Also of note is the exocyclic alkene C(19). Its presence is not necessarily essential for biological activity and many analogues called 19-*nor* deltanoids are created without it. Some molecules sporting various alkoxy groups [28, 29] or a terminal alkene [30] at position C(2) have also shown interesting activity.

There also exists a category of analogues bearing an extremely simplified aromatic or heteroaromatic A-ring portion (see Posner, Figure 1.7).[31, 32] Although they mostly bind

poorly to the calf thymus VDR even at micromolar concentrations, they all show substantial antiproliferative activities in murine keratinocytes at 1 μM.

Some other analogues are completed devoid of hydroxyl groups on the A-ring (see Figure 1.7). One of them, HY- 11, is a 19-*nor* vitamin D_3 analog whose A-ring bears a nitrogen where C(2) would be located.[18] It inhibits the proliferation of human acute myelogenous leukemia (AML) and HL-60 cell lines, through induction of cell cycle arrest and triggering of apoptosis, without producing hypercalcemia (at least in mice).

Not all modifications are accepted. 19-nor-analogues of 1,25D prepared by replacing C(5)-C(6) with an amide function did not bind to the VDR, even at a high concentrations (see Suhara, Figure 1.7).

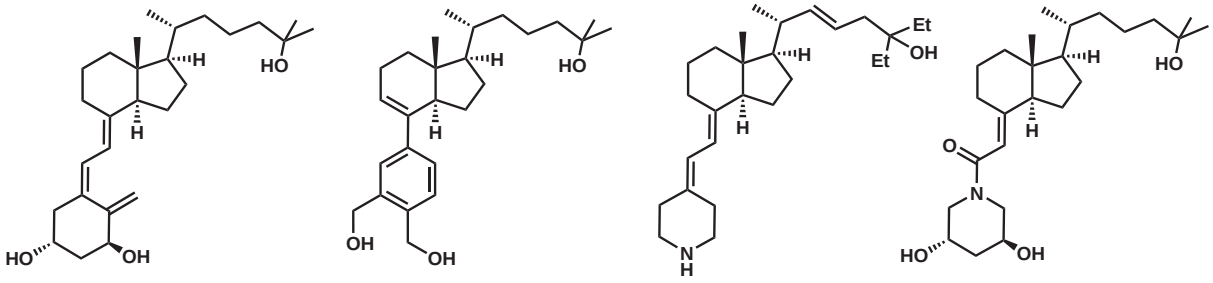


Figure 1.7. A-ring modified deltanoids

C,D-rings modifications

While structural modifications of the A-ring and side chain have been intensively studied, less is known about the influence of the C,D-rings. The limited research in this area revealed that this type of alteration of the vitamin D skeleton is viable. As shown in Figure 1.8, the removal of the C and D rings lead to very linear structures (Plonska). Some of those compounds retained some transcriptional activity and binding affinity to the vitamin

D receptor albeit decreased by two orders of magnitude in comparison with the analogous vitamins possessing intact C,D-rings.[33] Most of them were at least devoid of calcemic activity. Some other deltanoids retained one cyclic structure and presented equivalent affinities for the VDR and significantly better ratios of cell proliferation activities versus calcemic effect compared to calcitriol (Wu and Vrielynck).[34, 35]

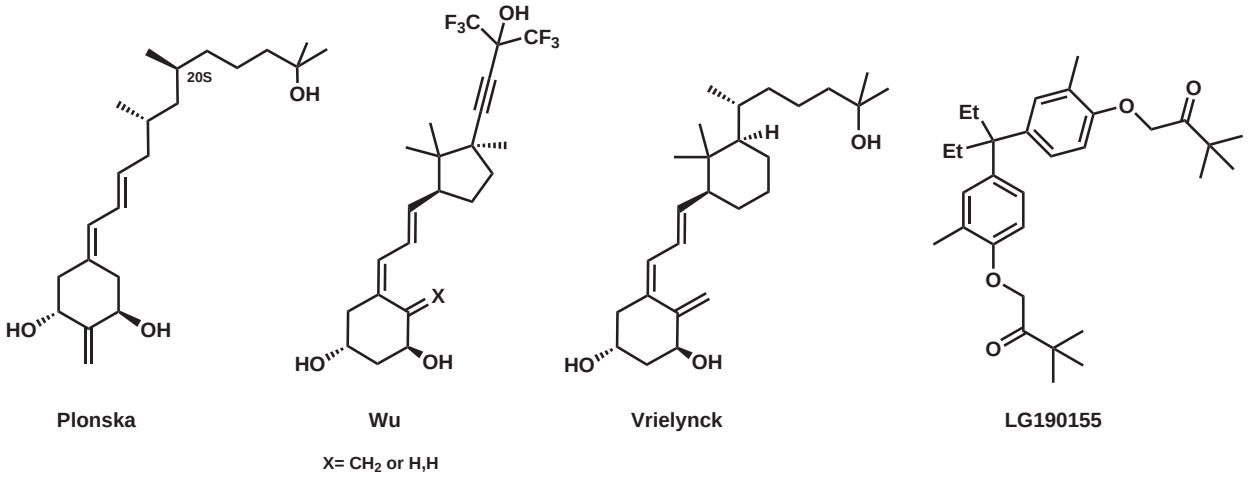


Figure 1.8. C,D-rings modified deltanoids and NSVDRMs

Since it is known that C,D-rings of some analogues are surrounded in their crystalline complex with the LBD of the VDR by strongly hydrophobic amino acids[36], there is probably still room to create new calcitriol analogues having quasi-intact side chain and A-ring moieties but much simpler (and still hydrophobic) center portions in replacement of the C,D-rings.

Non-secosteroidal molecules as vitamin D receptor modulators (VDRM)

There have been a few reports of VDR agonists where the entire secosteroid is replaced by two aromatic rings linked by either one or two atoms (see LG190155, Figure 1.8).[37] These mimics were detected by the screening of small-molecules libraries in cotransfection-cotransactivation (CTF) assays. Several of these structures have decreased

effects on serum calcium and resistance to oxidation by CYP24 while maintaining moderate VDR agonist and antiproliferative activity *in vitro*. However, there is still room for improvement as none showed higher potency in hyperproliferative assays than 1,25D and all had reduced affinity for VDR. Moreover, some of those nonsteroidal analogues of the VDR are significant binders of a mutated form of vitamin D receptor whose presence leads to human vitamin D-resistant rickets (*h*VDRR) and results in high serum 1,25$(OH)_2D_3$ concentrations and severe bone underdevelopment.[14] For patients suffering from *h*VDRR, the optimization and the therapeutic use of those non-secosteroidal VDRM might reestablish 1,25$(OH)_2D_3$ responsiveness and, therefore, the very important ligand-dependent transactivation functions of VDR.

1.1.5 Vitamin D-derived drugs

A large number of 1α,25$(OH)_2D_3$ mimics have been created over the years. A few of these analogues with adequate pharmacokinetic profiles are in the market as treatment for certain conditions (Figure 1.9). For example, although calcipotriol (Daivonex) is not a superagonist, it is the clinically most successful analog being applied topically against keratinocyte dysfunction in psoriasis.[38] Most of the 1,25 $(OH)_2D_3$ analogues that are currently in (or were in and failed) clinical trials are designed for oral administration against different types of cancers[39], osteoporosis[40], and immune disorders.[41]

1.1.6 Synthesis of vitamin D analogues

The preparation of calcitriol mimics *de novo* is a slow, expensive, and inefficient process. Due to the complex structure of itamin D_3, most structural changes focus on new side chains as well as A-ring modifications. These modifications are easier to accomplish

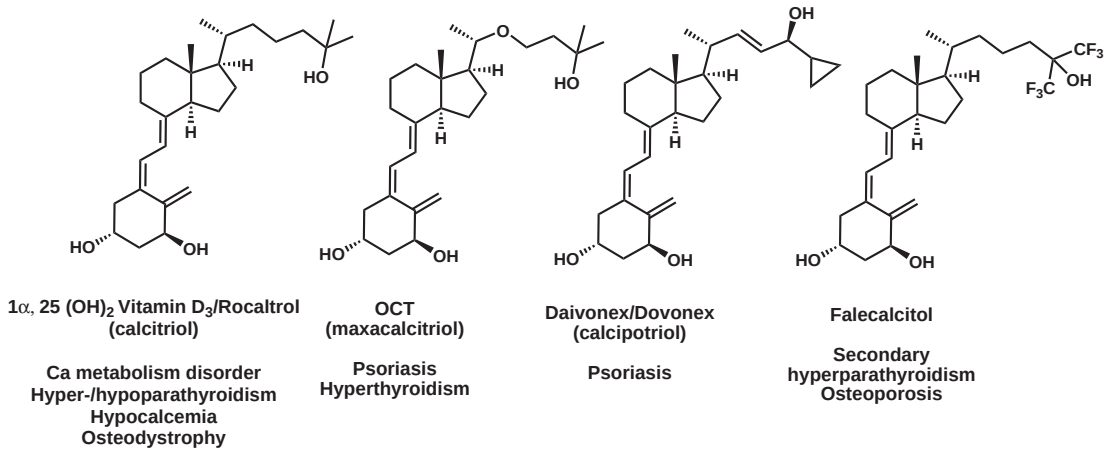


Figure 1.9. Deltanoids in use as drugs.

because chemical oxidation of inexpensive vitamin D_2 can provide the C,D-rings portion equipped with handles for the modifications of the top and bottom portion of the secosteroid structure (Figure 1.10).

In the interest of finding new deltanoids, it would be desirable to be able to synthesize a large number of analogues very quickly. Researchers have tried to apply combinatorial chemistry and its techniques to this situation. In 2001, Hijikuro and coworkers have published[42] their conditions for the synthesis of a 72-member library of vitamin D_3 analogues (from 6 top chain mimics, 4 A-ring portions and 3 C,D-rings units) via a split and pool methodology utilizing radiofrequency encoded combinatorial (REC) chemistry.[4]

While other researchers have used solid phase techniques as a tool to create deltanoids [46–48], we are aware of no other publications reporting combinatorial chemistry being used in the area of deltanoid synthesis. It is interesting to note that, among all the efforts using these techniques in this context, none of them aimed at varying the core structure of VD_3 analogues. As we were interested in creating deltanoids bearing new C,D-rings struc-

[4]For more details about radiofrequency encoded combinatorial chemistry (REC), please read the work of Nicolaou[43, 44] and Xiao[45].

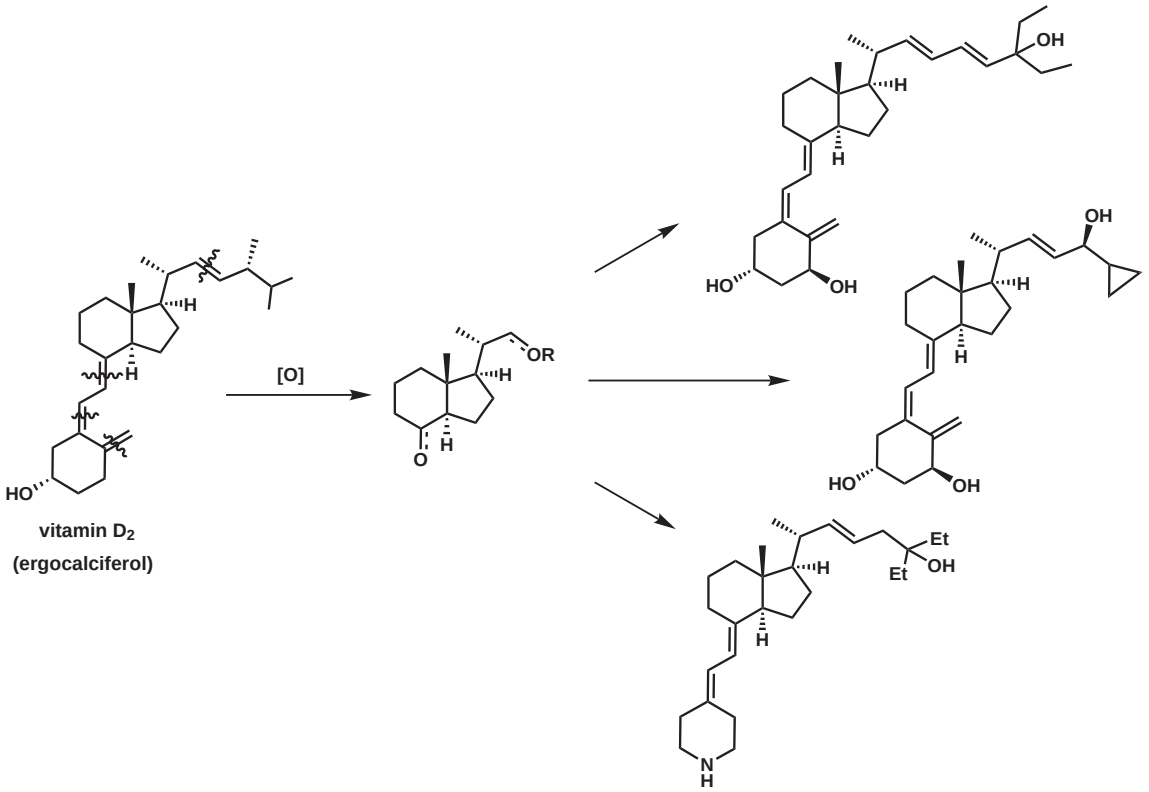


Figure 1.10. Vitamin D_2 as a source of the structurally complex C,D-rings system for the synthesis of deltanoids

tures, it became clear that we would need libraries containing a wide variety of C,D-rings surrogates, in order to obtain molecules with even greater therapeutic potential. In general, however, the combinatorial preparation of analogues containing significant modifications to the central core has not been explored extensively due to the long synthetic sequences required to prepare such molecules. We then realized that we would have to design new synthetic routes and schemes in order to achieve our goals.

1.1.7 Summary

In summary, the widespread presence of the vitamin D receptor (VDR) throughout the human body makes the study of vitamin D_3 analogues highly interesting for future drug development. There is considerable interest in academia and the pharmaceutical industry to create vitamin D-related drugs that exhibit specific actions applicable to the treatment of immune disorders, malignant tumors, and bone formation. Moreover, there are many indications that molecules of lower molecular complexity than VD_3 are able to reproduce some, if not all, of the biological activity of the sunshine vitamin.

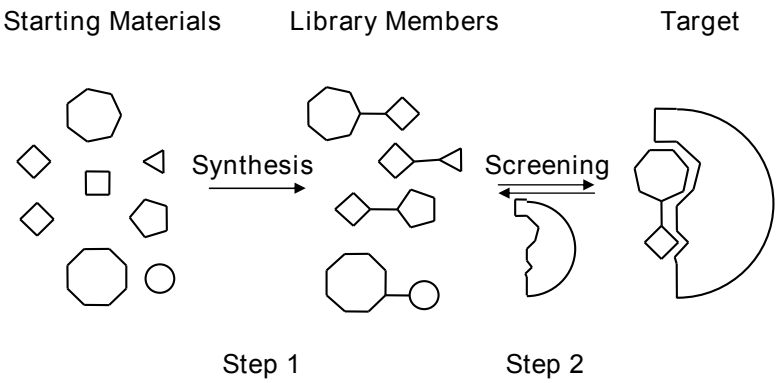
Because of it's length and low structural yield, linear, classical synthesis of analogues is not well suited for our goal : **finding structurally new and simpler analogues of calcitriol**. Unfortunately, the lack of a simple synthetic scheme has prevented widespread use of the combinatorial library approach to the creation of 1α,25$(OH)_2D_3$ analogues, particularly those which incorporate modified C,D-rings systems. Some of the combinatorial chemistry techniques could potentially alleviate some of these problems but one is still faced with the somewhat difficult situation of separating and testing the library members.

With these observations in mind, we decided to explore a new way of finding vitamin D_3 analogues.

1.2 Dynamic Combinatorial Chemistry

In recent years, a new approach in combinatorial chemistry called dynamic combinatorial chemistry (DCC) has emerged to address the inconveniences of the traditional process of discovering bioactive molecules. Whereas traditional and combinatorial chemistry utilize a two-step process to discover bioactive molecules (irreversible synthesis **followed** by screening, see Figure 1.11 top part), DCC is designed as a one-step process (reversible synthesis **and** screening at the same time, Figure 1.11 bottom part).

Combinatorial Chemistry



Dynamic Combinatorial Chemistry

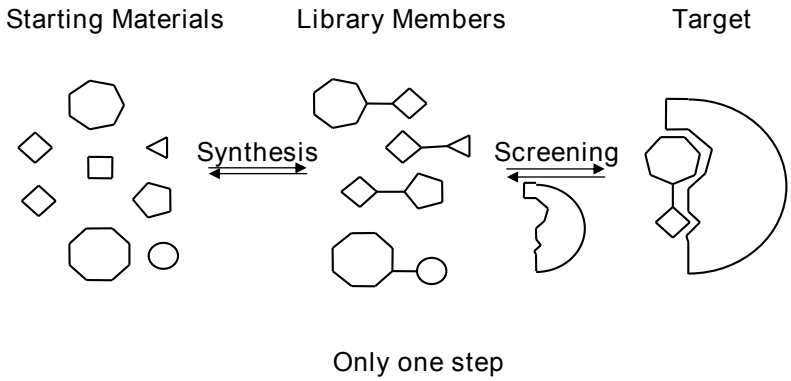


Figure 1.11. Comparaison of the process of combinatorial chemistry and dynamic combinatorial chemistry

In DCC, a dynamic combinatorial library (DCL) of molecules is generated from a set of building blocks that can reversibly form bonds with each other. Because the population distribution of those libraries is governed by the thermodynamic stability of the members, when subjected to the influence of a target, a selection/adaptation occurs which alters the equilibrium state of the DCL (in accordance with Le Châtelier's principle[49]). Binding of library members to a target can be viewed as either stabilizing them or removing them from the synthetic equilibrium. The library adapts itself to the selection pressure and the constituents bound to the receptor are enriched (amplified) in relation to the unbound compounds (Figure 1.12. Careful analysis of the library composition differences between DCLs generated in presence and in absence of a target (a biological receptor or a small molecule template) —through the use of high-pressure liquid chromatography (HPLC) or nuclear magnetic resonance (NMR), for instance— can reveal which members have been amplified by the target being present (in other words, which ones are the best binders).

In drug discovery applications of DCC, the target to which the library members should bind is called a receptor. Receptors can be cell membrane receptors, enzymes, interfaces for protein–protein interaction, sites on RNA or DNA, etc. In supramolecular receptor-building applications, targets are small molecules molding a supramolecular structures.[50] In both types of experiments, the amplification of a library member refers to its concentration in the presence of the target divided by its concentration in the absence of the target [51], while the selectivity of a dynamic system is defined by the ratio of the amplification of two library members A and B (where A is the stronger binder) divided by the ratio of their relative binding constants.[50]

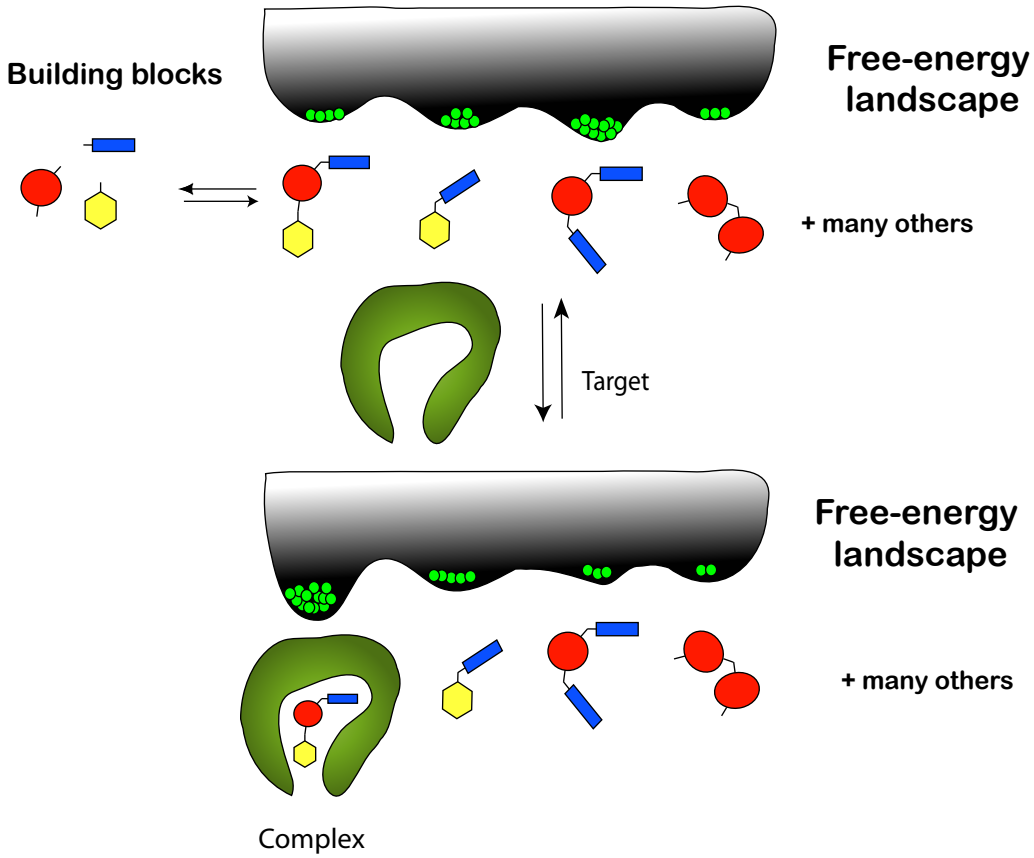


Figure 1.12. Generation of a dynamic combinatorial library under thermodynamic equilibrium and influence of a target on the library composition

Selection approaches

Three main classes of experiments, casting, molding, and external physical stimuli experiments, can be defined in dynamic combinatorial chemistry, depending on the source of the pressure applied to the system. When an experiment employs a relatively large target such as a protein, DNA, or supramolecular entity to influence the thermodynamic equilibrium of a dynamic library of comparatively small molecules, it is termed **casting** (see Figure 1.13). Experiments that use a biological target to find its own binders/substrates fall in that category. Dynamic **molding** experiments, on the other hand, use small target

molecules to template the formation of large receptors, cages, or supramolecular entities (see Figure 1.13). The major and more numerous applications of DCLs published to this day are in the molding of macro- and supramolecular receptors category, not in the casting of biologically active small molecules.

The third category is composed of external physical stimuli that can also be used to influence the composition of a DCL or to select certain library members over others (not shown). Examples of this process includes phase transitions [52], crystallization [53], temperature and pH changes [54], electrical field [55] or influence of light. [56].

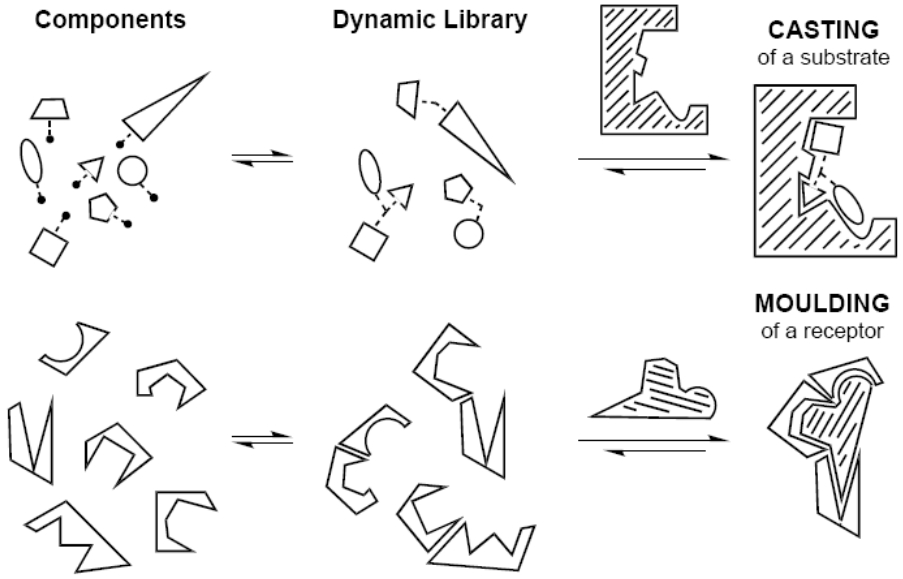
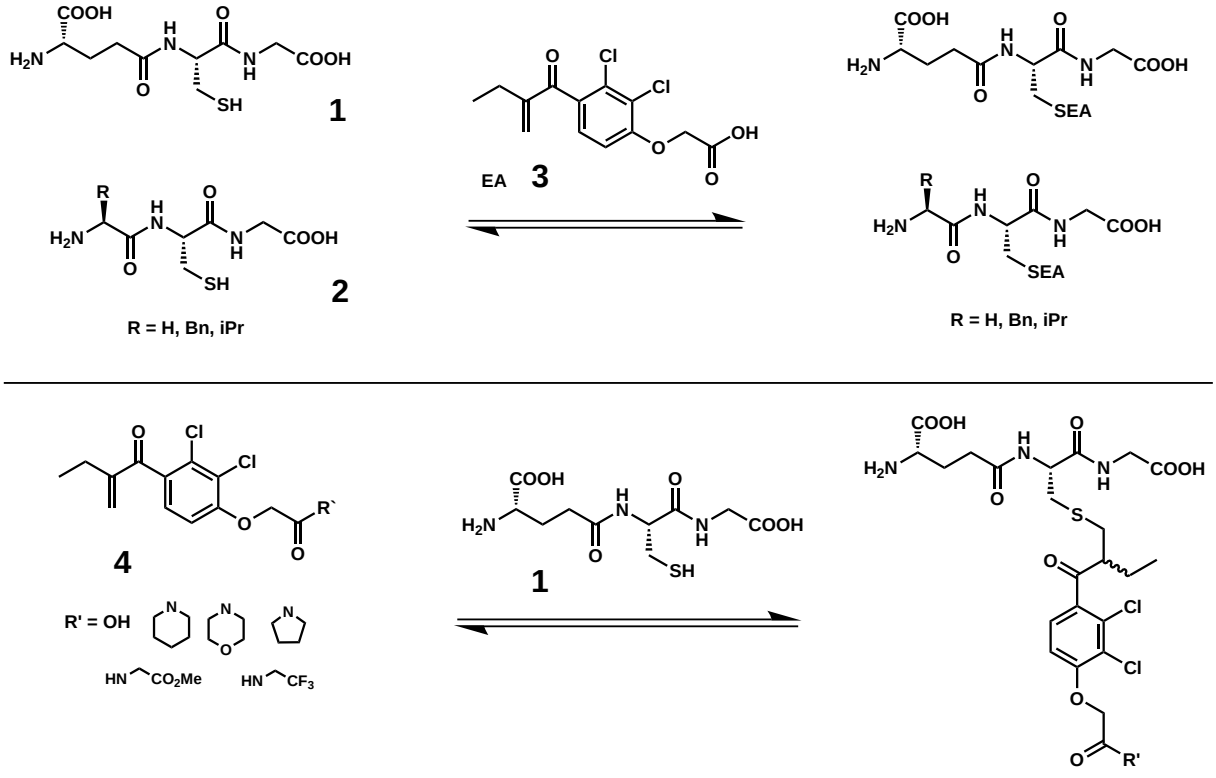


Figure 1.13. Example of casting and molding experiments (figure taken from [57]).

Examples of dynamic casting of small molecule ligands

In their 2005-2006 studies, Greaney and Campopiano presented dynamic combinatorial libraries (DCLs) directed by the active site of the enzyme glutathione S-transferase (GST), a target for the design of inhibitors in cancer therapy and the treatment of parasitic diseases. They explored and implemented the conjugate addition of thiols to enones

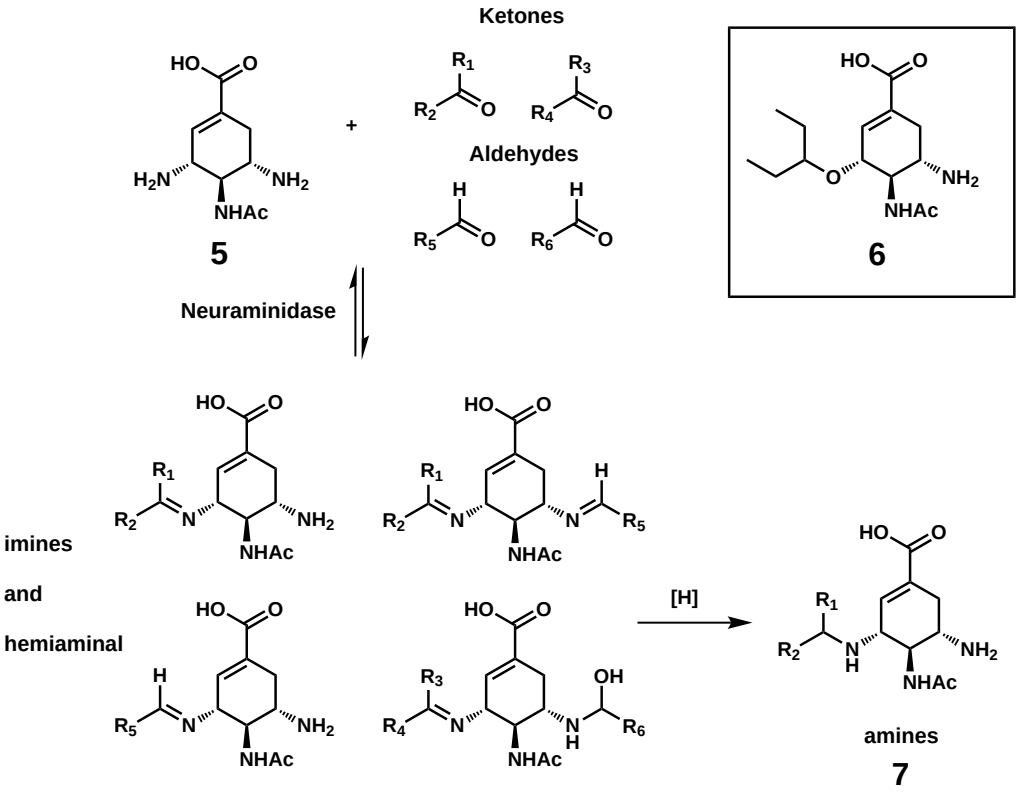
as a fast, freely reversible reaction at basic pH.[58] Using tripeptide glutathione (γ-Glu-Cys-Gly, GSH, **1**) and one of the best characterized inhibitors of GSTs, an enone called ethacrynic acid (EA, **3**), they created two DCLs influenced by the presence of GST (Scheme 1.2).[59] The first one used EA and GSH analogues of type **2** to explore the GSH binding region and the second probed the hydrophobic acceptor binding region of GST using GSH and ethacrynic acid derivatives of type **4**. In both cases the strongest binding DCL components were identified due to molecular amplification by GST. Their studies lead to the identification of two new inhibitors for the GST enzyme.



Scheme 1.2. Dynamic combinatorial library composed of ethacrynic acid and glutathione analogues (top). Dynamic combinatorial library composed of ethacrynic acid analogues and glutathione (bottom).

In 2002 and 2003, Eliseev and coworkers reported the discovery of nanomolar inhibitors of neuramidase (NA) through dynamic casting libraries (Scheme 1.3).[60, 61]. Their libraries were composed of amine **5**, which was known to be structurally similar to

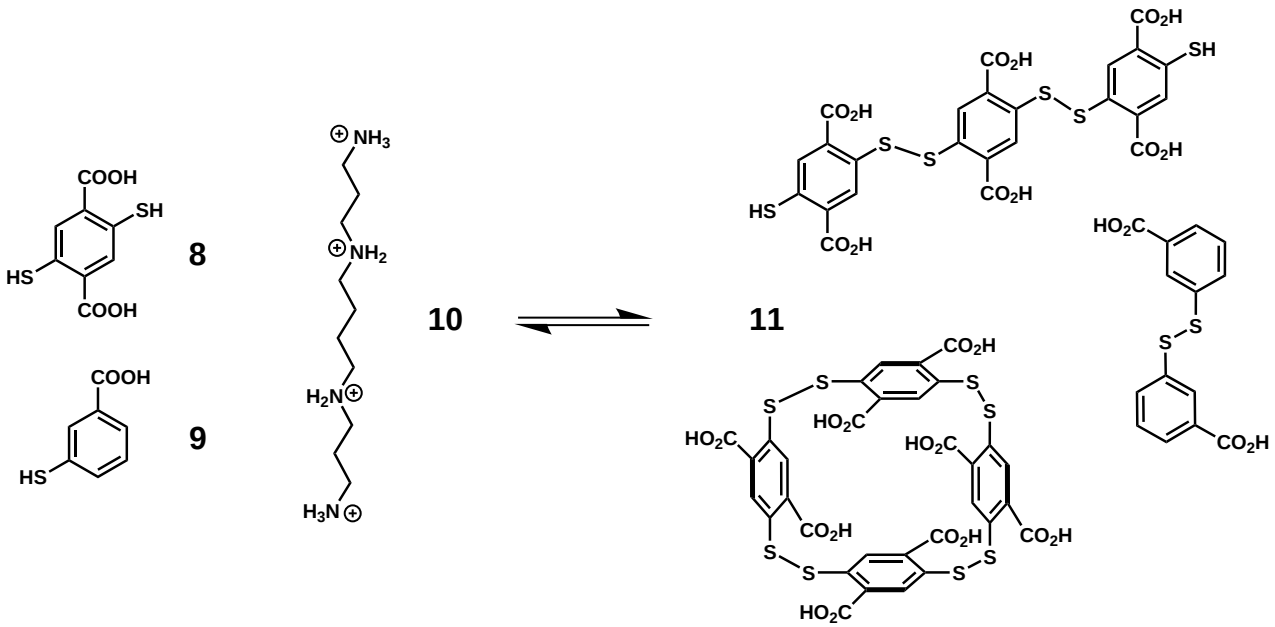
the active component of commercial influenza drug tamiflu **6**, and aldehydes or ketones. They used a key enzyme involved in the influenza virus propagation, neuraminidase, as the DCC target to influence the library composition. The dynamic process was imine exchange and because imines are easily hydrolyzed they cannot be recovered from the libraries at the end of the experiment. To circumvent this problem, they were reduced to amines prior to analysis of the library composition by LC-MS. When the library was equilibrated in the presence of NA, some of the amine products **7** were amplified. To show that the amplification was related to active site binding and not allosteric interactions, the authors repeated the experiment with a non binding protein called bovine serum albumine (BSA) instead of NA and with NA but in the presence of a known inhibitor. In both cases no amplification was observed. This library was a good example of a virtual library, where the concentration of the library members was just below the detection limit of the assay and only the presence of the receptor revealed the good binders by amplification. Finally, the authors synthesized pure samples of some of the amplified members and then quantified their K_i. The DCL experiment allowed the discovery of amines with inhibition constants (85 nM - 92 nM) much lower than the K_i of starting amine **5** (31300 nM). This DCL example showed both the advantages and the shortcomings of the DCC method. In a few experiments, a new structure can be revealed by the protein itself. However, the necessity of reducing the imines to amines prior to library analysis can create false results, since the target-selected imine components and the amine products have different binding properties. This example also showed that starting with a molecule related to known inhibitors and improving its affinity for a protein is possible via dynamic combinatorial chemistry. It is also a good example of a library based on a double-point exchange, which has amine **5** as the center. This type of library is much more uncommon than single-point exchange experiment.



Scheme 1.3. Inhibitors of neuraminidase (NA) found through dynamic libraries mediated by imine exchange.

Examples of dynamic molding of receptors

Otto et al. used dynamic combinatorial chemistry to develop a synthetic receptor for spermine, a polyamine that plays an important role in numerous cellular processes including apoptosis and cancer.[62] The DCL was based on disulfide exchange and included monothiol building block **9** and dithiol **8** (Scheme 1.4). The dynamic exchange generated many linear (**193**) and cyclic structures (**194**) and when the DCL was exposed to spermine **10**, cyclic tetramer **11** was greatly amplified. This compound had such a high affinity for spermine and that it was strong enough to sequester spermine from one of its natural hosts, DNA.



Scheme 1.4. Oxidation of thiol building blocks **8** and **9** produced a DCL of linear and macrocyclic disulfides that contained receptors for protonated spermine **10**.

Dynamic combinatorial chemistry has been successfully exploited in the discovery of new molecules in very diverse areas : development of synthetic receptors for small molecule guests [63–67], study of self-replication[68–70], formation of dynamic polymers[71, 72], materials science[73, 74], complex molecule synthesis[75–77], study of new entities as analytical tools[78], and, of course, the discovery of ligands for biomolecules. [60, 61, 79–83]

Library composition analysis The analysis of a dynamic experiment usually involves the comparison of an equilibrated library in the absence of the receptor with one in the presence of the receptor to identify amplified library members. Since DCLs are typically very complex mixtures of species in interconversion with each other, the quantification of the amplification quickly becomes difficult as the size of the library increases. For this reason, only a few examples of large libraries (for now, anything >than 50 members can be considered large) are known. An effective screening method would require an ana-

lytic tool combining both high sensitivity and large range of action (sensitive and precise over many orders of magnitude). Thus far, the most commonly used technique is liquid chromatography-mass spectrometry (LC-MS), because it allows for simultaneous separation and identification of the library components. In particular, MS-MS fragmentations pattern analysis can aid identification. The main example of LC-MS as a promising technique for DCL characterization is the work of Otto et al. where a library of >than 9000 virtual members was created from only 8 different thiol building blocks.[66] Another feat was accomplished by Poulsen with the use of mass spectrometry alone, specifically Fourier transform ion cyclotron resonance mass spectrometry (FTICR MS) together with sustained off-resonance irradiation collision activated dissociation (SORI-CAD), to detect noncovalent protein-DCL ligand complexes under native conditions. This allowed the identification of all ligands of interest from a large DCL in the presence of the enzyme in a single, rapid experiment.[84] Although MS does not allow for straightforward quantitation, its high resolution and its ability to provide the masses of the library members make it an attractive tool to analyze DCLs. Schofield and coworkers made use of a very similar technique (termed *dynamic combinatorial mass spectrometry*, DCMS) to find new disulfide-based metallo-β-lactamase inhibitors under non-denaturing conditions.[85]

Nuclear magnetic resonance (NMR) is also an often-used technique. In relatively small libraries, certain types of NMR experiments such as time–resolved heteronuclear single quantum correlation (HSQC) NMR [86] or diffusion–ordered spectroscopy (DOSY) NMR [87] have found application.

Finally, new approaches have been recently developed that involve the use of alternative analytical detection methods and spectroscopic methods : fluorescence, UV–vis and polarimetry. Although not as widely applicable, these techniques offer fast and inexpensive spectroscopic methods that have also the advantage of a very high sensitivity (see [78] and

references therein).

Target concentration The premise behind using DCLs to identify ligands for a target depends on the amplification of those ligands by the target. Since amplification is proportional to and limited by target concentration, there is a question as to how much target is needed/optimal to maximize amplification. An experiment needs a certain amount of target to provoke amplification but too much target could render the selection between binders difficult. At least when searching for binders to proteins or enzymes with only one binding or active site, an equimolar quantity of target versus the total amount of potential library members might be optimal. Competition for target between binders of similar binding affinities might cause amplification to be too small to be detectable if excess library members is used. The use of excess target is not necessarily a better option as available binding sites might cause a very small amplification of each of the many good binders thus preventing the observer from seeing those small amplifications. While some theoretical studies on this particular issue have been published in the field of supramolecular dynamic assembly (the molding of a receptor, see reference [88] for an example), the application of DCLs for small-molecule ligand identification has not been explored in sufficient details to propose an optimal amount of target to use.

Reversible Connections

Dynamic combinatorial chemistry (DCC) relies on reversible chemical reactions to generate libraries of compounds. While a large number of reversible covalent connections have been developed for molding style DCLs, not all of those dynamic processes actually are applicable to drug discovery. DCL generation requires reaction conditions that do not disrupt the target's function or structure. Thus, the reversible reaction must comply with

a few important criteria. The process must be freely reversible on a reasonable time scale and occur in aqueous media, at biologically relevant temperature and physiological pH. The exchange should be controllable, with a mechanism allowing for easy switching between an ”on” and ”off” state. Last but not least, it should be compatible with the target's functional groups.

Few classes of reactions have so far proven to be effective at generating dynamic combinatorial libraries under such constraints (see Figure 1.14) but more are developed/explored every year. Examples of important competent processes are disulfide exchange [entry *a*], imine-type exchanges (imine [entry *b*], oxime [entry *d*], and acylhydrazones [entry *c*] exchanges), alkene metathesis [entry *i*], and conjuguate addition of thiols to enones [entry *g*].

Sometimes, a reversible connection is not immediately applicable to dynamic chemistry in presence of biological targets because the conditions under which library equilibration could occur are not compatible with those targets. In those cases, further development is necessary to adapt the conditions to ensure physiological compatibility. The acylhydrazone exchange (see Figure 1.14, (c)) is an example of this. At first, it required an acidic pH (<4) to be a useful mean of library generation and was used in many abiological examples by Sanders and Lehn ([89–94]). However, others groups found ways to accelerate the rate of acylhydrazone exchange under physiologically compatible conditions, either by the use of an enzyme (carbonic anhydrase, Poulsen et al. [84]) or a nucleophilic catalyst (aniline, Greaney et al. [95]). The use of a nucleophilic catalyst to increase exchange reaction rates has also been demonstrated in the case of oxime [96] and acylhydrazone [97] exchanges, both using the aromatic amine aniline.

Sometimes, the outcome of a dynamic combinatorial library is linkage dependent. [98–

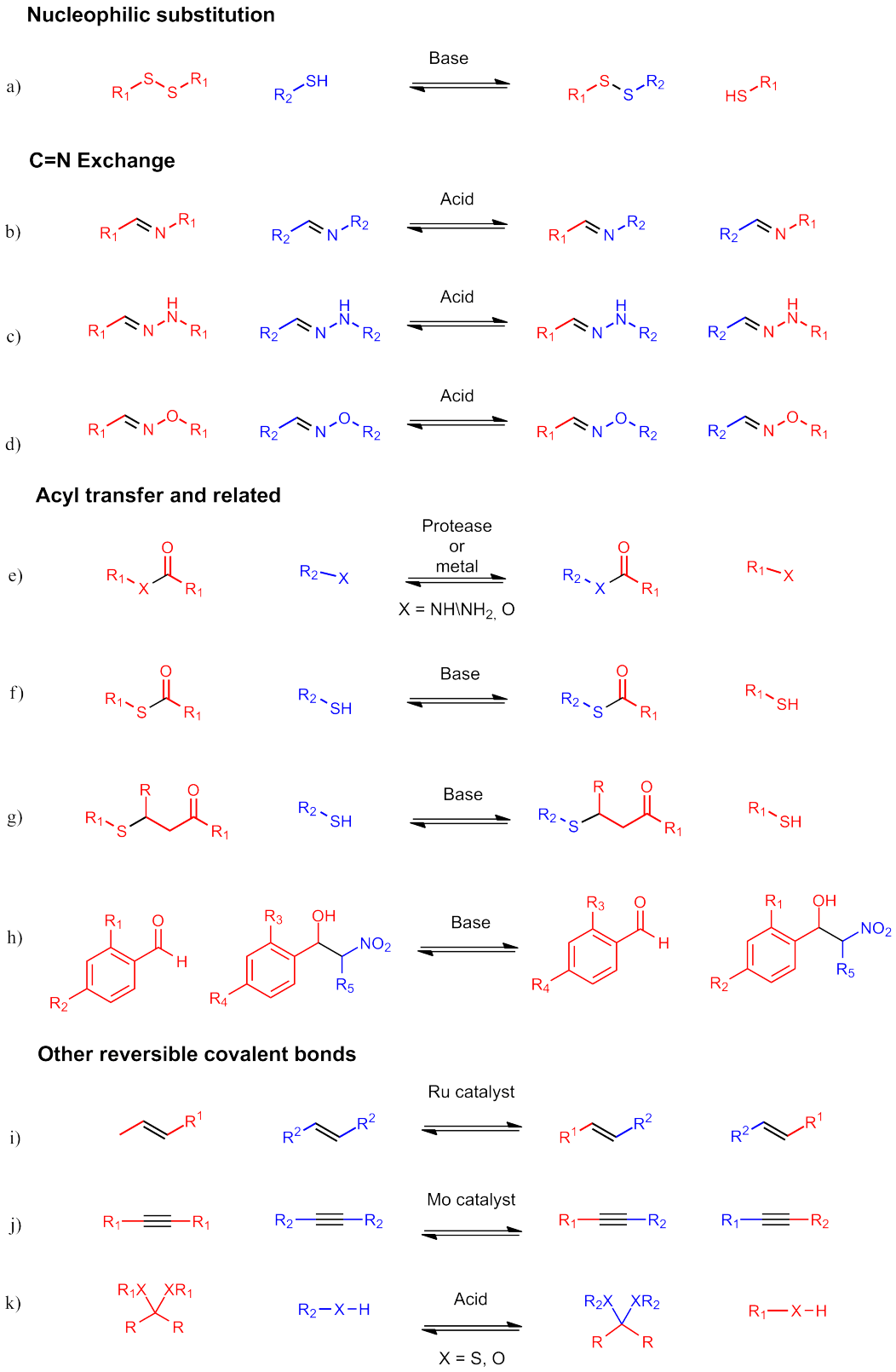


Figure 1.14. Non-extensive collection of reversible covalent connections used in DCLs. (a) disulfide exchange, (b) imine exchange, (c) hydrazone exchange, (d) oxime exchange, (e) transesterification or transamidation, (f) transthioesterification, (g) conjuguate/retro-conjuguate addition, (h) nitroaldol exchange, (i) alkene metathesis, (j) alkyne metathesis, (k) acetal or thioacetal exchange.

101] Thus, when probing active/binding sites of a particular target with a DCL, it is advisable to consider a greater diversity, both of building block constitution and of reversible connection used.

Combination of reversible reactions

Dynamic combinatorial libraries presenting a high level of complexity can easily be created when a combination of reversible reactions are used. A library based on more than one type of reversible connections is termed a multi-level library. The advantage of such multi-level systems is the extension of the diversity within the library members since the building blocks are held together by a variety of linkages. Depending on their characteristics such as chemical functionalities, rate of exchange, conditions for equilibration or compatibility with other processes, the dynamic reactions used are either orthogonal to each other or communicating with each other.

Double-level orthogonal libraries A library is termed *double-level orthogonal* when it is possible to selectively activate one of the two exchange processes while the other occurs slowly enough to be negligible on the same timescale. Since they operate in different pH regions, the disulfide metathesis (basic pH) and the hydrazone exchange (acidic pH) form one pair of orthogonal processes. As such, they have been studied by Otto [102], used in 2001 by Lehn and Eliseev with terpyridine-based ligand coordination to a transition metal template [103] and more recently by Escalante and coworkers.[104, 105]

Double-level communicating libraries An equally interesting case of multi-level system is the one where two or more dynamic processes actually occur at the same time. Because they use common building blocks, the two processes are influencing each other

and are thus termed *communicating*. The main example of such a nonorthogonal system was reported by Otto where they used building blocks carrying a thiol and a thioester functionality.[106] At first, thiol reacts with thioester in a thioester exchange but then, exposition to atmospheric oxygen causes disulfide formation via oxidation and, subsequently, disulfide exchange. Thus, the two exchange processes can be activated consecutively but then allowed to proceed simultaneously.

DCC limitations

After all that has been said, let's examine some limitations of DCL-based approaches to drug discovery and a few solutions that accompany them.

1. **Library size** : The larger the number of tight binders in a DCL, the more difficult it is to detect the reorganization of the library when exposed to the target. Computational simulations estimate that libraries of 10-10^6 members may be used in supramolecular dynamic assembly experiments. [107]

2. **Small amplification** : Even within small libraries, it might be difficult to detect amplification of compounds already present in large proportion in absence of the target. In order to maximize amplification, a library competitor (also called *dummy building block*) used in excess quantity can "occupy" the useful building blocks and thus lower the concentration of all the library members prior to selection and amplification by the target. [78]

3. **Unwanted pressure** : There is always the possibility that the pressure influencing the equilibrium is NOT the binding to the target but something else like solubility issues of some library members/building blocks, affinity of those components to the experimental apparatus (filters, vials, membranes, etc.) or other undesired events.

1.2.1 Examples of DCL in the search for ligands for biomolecules

When we started this project, we became interested in using DCC because it had shown potential as a technique for the discovery of binders of therapeutically relevant proteins. However, even after a decade of research in this area, the field is still at the stage of proof-of-principle experiments. A selection of those DCC experiments in drug discovery is presented in Table 1.1 and Table 1.2 .

Maybe one of the most important example is the discovery by Miller et al. of lead compounds targeting myotonic dystrophy type 1 (DM1).[82] Miller and his group made use of resin-bound dynamic combinatorial chemistry (RB-DCC) based on disulfide exchange and found four high-affinity ligands by competitive binding and isolation by retention on a solid support. With this approach, they managed to identify several peptide/small-molecule hybrid disulfides that bind to CUG-repeat mRNAs and also inhibit its interaction with a RNA-binding protein called MBNL1 with low micromolar activity.

1.2.2 Summary

In summary, a new combinatorial method has emerged in the past few years, dynamic combinatorial chemistry (DCL). DCL uses biological targets as templates to form drug-like molecules from smaller building blocks. In an equilibrating mixture of potential drug-like structures, binding to the target removes the best molecules from the solution. In response, the equilibrium shifts to make more of those tight-binding library members. The result is that the synthesis and screening steps of the drug discovery process are combined in a single reaction flask and the concentration of the tightest binding library members is amplified through the binding process. Examples of the usefulness of the method abound

Table 1.1: Dynamic combinatorial libraries in drug discovery
Adapted from [127]

Target	Reversible chemistry	Library size	Hit(s)	Reference
Non-enzyme proteins				
GalNAc - specific lectins	metal coordination	4	tris - GalNAc	[108, 109]
Con A	thiol - disulfide	21	bis - mannoside	[99]
Con A	acyl hydrazone	>474	tris - mannoside	[100]
peanut lectin	thiol - disulfide	>10	divalent galactoside	[110]
wheat germ agglutinin	thiol - disulfide	13	N- acetylglucosamine	[111]
human galectins	thiol - disulfide	21	glycosyldisulfides	[112]
gal - selective plant lectins	thiol - disulfide	21	glycosyldisulfides	[112]
wheat germ agglutinin	aldol reaction	4	sialic acid	[113, 114]
calmodulin	thiol – disulfide	15	inhibitor	[115]
gramidicin A	thiol - disulfide	3	disulfide phospholipids	[116]
Nucleotides				
DNA	transimination/metal coordination	ND	salicylaldimine - Zn(II) complex	[117]
RNA	metal coordination	>27	salicylamide - Cu(II) complex	[118]
DNA/RNA	transimination	ND	oligonucleotide derivatives	[119]
DNA	thiol - disulfide	6	peptide derivatives	[120]
DNA	thiol - disulfide	54	peptide derivatives	[121]
RNA	thiol - disulfide exchange	11325	peptide derivatives	[81]
RNA	transimination	>1015	oligonucleotide derivatives	[122]
DNA	thiol - disulfide exchange	7	polyamides	[123]
DNA	thiol - disulfide exchange	5	oxazole - peptide macrocycles	[124]
Cells				
MRSA	thiol - disulfide exchange	3828	psammaplin A analogues	[125]
Ac_2 -L- Lys -D- Ala -D- Ala	metathesis/thiol - disulfide exchange	36	vancomycin dimers	[126]
Ac_2 -L- Lys -D- Ala -D- Ala	metathesis/thiol - disulfide exchange	36	vancomycin dimers	[98]

Table 1.2: Dynamic combinatorial libraries in drug discovery
Adapted from [127]

Target	Reversible chemistry	Library size	Hit(s)	Reference
enzyme				
trypsin	alcohol boronate exchange	ND	tripeptidyl - boronate	[128]
carbonic anhydrase	transimination	12	sulfamoylbenzaldimine	[129]
carbonic anhydrase	metathesis	ND	bis - sulfonamide derivatives	[130]
AChE	acyl hydrazone exchange	66	bis - pyridinium structure	[90]
AChE	transthiolesterification	10	substrates	[131]
AChE	transthiolesterification	25	substrates	[132]
HPr kinase/phosphatase	acyl hydrazone exchange	440	2 - aminobenzimidazole	[91]
cyclin - dependent kinase 2	hydrazone exchange	ND	oxindole structures	[133]
Aurora kinase	thiol disulfi de exchange	ND	3 - amidobenzamide structure	[134]
glutathione S- transferase	conjugate addition	5	glutathione derivatives	[58]
glutathione S- transferase	conjugate addition	ND	inhibitors	[59]
glutathione S- transferase	acyl hydrazone exchange	10	inhibitors	[95]
neuraminidase	transimination	>40000	Tamiflu analogues	[60]
neuraminidase	transimination	ND	Tamiflu analogues	[61]
hen egg white lysozyme	transimination	6	N- acetylglucosamine derivative	[135]
hen egg white lysozyme	transimination	12	N- acetylglucosamine derivative	[80]
P. cepacia lipase	nitroaldol reaction	16	- nitroacetates	[136]
galactosyl transferase	transimination	ND	UDP - galactose mimics	[137]
galactosyl transferase	transimination	ND	UDP - galactose mimics	[51]
subtilisin	thiol disulfide exchange	3	thiocolchicine/ podophyllotoxin conjugates	[138]
$\beta - lactamase$	thiol disulfide exchange	ND	N- benzoyl -D- cysteine	[85]

and it appears to be a promising technique to find to bioactive molecules.

1.3 The Aim of this Thesis

This thesis describes research directed towards the development of both simple molecular libraries and dynamic combinatorial libraries (DCLs) of vitamin D-like molecules. The goal is ultimately to expose the DCLs to the vitamin D receptor to identify high-affinity ligands which might eventually be used in treatment of cancer. Reaching that penultimate goal, however, requires the synthesis of key components and development of appropriate techniques to prepare static and dynamic libraries for use in screening.

The design of the molecular and dynamic libraries demands synthetically complex building blocks. More specifically, our project requires the synthesis of synthons possessing characteristics of the A-ring and side chain portions of vitamin D, as well as original central core components that can be clipped together both irreversibly (static) and reversibly (dynamic). Therefore, new synthetic routes will have to be conceived, tested and implemented.

Once the synthesis of the building blocks is accomplished, the production of various static molecular libraries will involve the development of optimal experimental conditions to ensure high product yield and efficient isolation and purification procedures.

The dynamic libraries to be developed will use two exchange points so that, ultimately, vitamin D analogues with novel core frameworks might be identified. Two-point exchange libraries are uncommon in casting-based DCLs. Since our dynamic libraries will be target-influenced equilibrium mixtures of molecules, many variables will need examination. We have to explore and control the experimental conditions under which the dual-point disulfide exchange and the transthioesterification will take place. To influence the composition of our dynamic libraries, we require the presence of stoechiometric amounts of the hu-

man vitamin D receptor-ligand binding domain, *h*VDR(LBD), which is not commercially available in a suitable form. Therefore, we have to conduct a large-scale production of this protein by expression from plasmids. Special care should then be taken to ensure the chemical stability of the library members and of the vitamin D receptor under the conditions developed.

Ultimately, in line with our long-term goal of identifying a tight-binding molecule, we will view this Thesis as a success if we are able to present a preliminary test of the concept of VDR-influenced dynamic libraries. This test should show our ability to control the creation of libraries of molecules from the thermodynamic assembly of well-designed building blocks. We should also be able to characterize the composition of those libraries and attempt to manipulate them using the vitamin D receptor. In summary, Chapter 2 narrates our work to synthesize library building blocks and the preliminary studies for conventional synthesis of vitamin D-like molecules. Chapter 3 tells our research concerning thiol/thioester-based dynamic combinatorial libraries, as well as the synthesis of additional building blocks. Chapter 4 elaborates on our work involving disulfide exchange-based DCLs.

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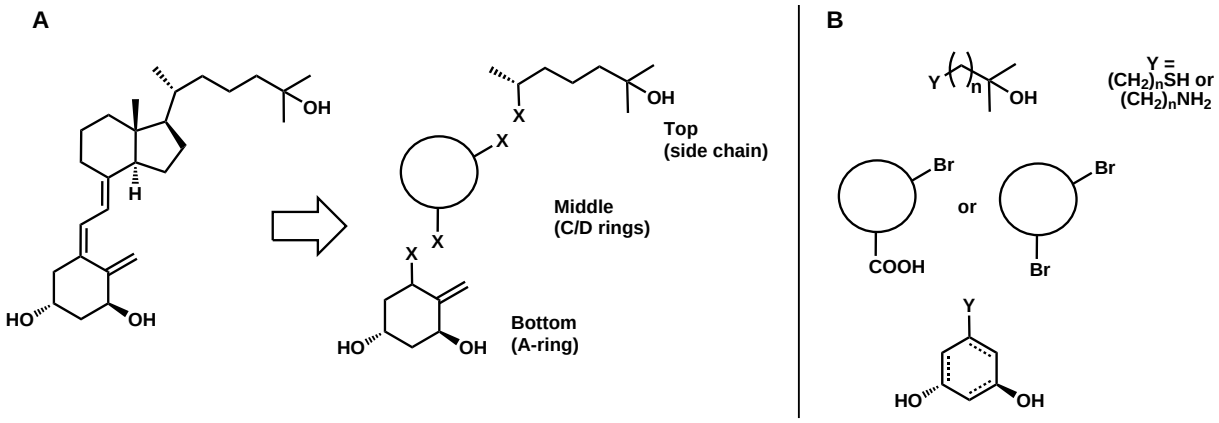
CHAPTER 2

Static Library of Vitamin D_3 Analogues.

2.1 Library design

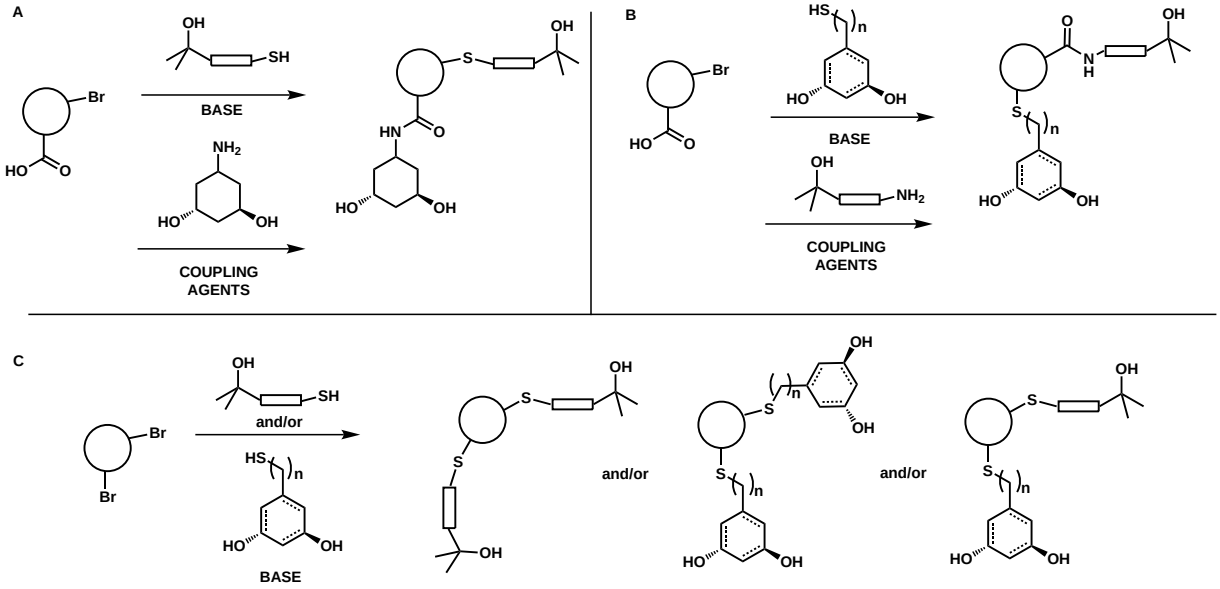
As mentioned in the introduction chapter, the binding of 1α,25-$(OH)_2D_3$ to the vitamin D receptor (VDR) is mainly mediated by hydrogen-bonding of critically located OH groups. In the search for new, structurally-simple analogues of vitamin D_3, we decided to focus our efforts on simplifying the complex bicyclic C,D-rings structure while keeping a consistent top chain and A-ring portion to preserve the three known H-bonding anchor points required for binding. Replacing the C,D-rings with simpler and smaller linkers has been demonstrated to yield reasonably potent 1α,25-$(OH)_2D_3$ analogues (see Introduction). Accordingly, we envisaged using simple, commercially-available or easily prepared synthons in lieu of linkers between a top chain tertiary OH group and an A-ring diol, both known to be excellent for binding to VDR (Scheme 2.1, part A).

To accomplish the assembly of those analogues, we decided that core molecules would be designed to be the electrophilic portions of the library. They would bear a combination of alkyl halide and carboxylic acid functionalities or simply be bis-alkyl halides (Scheme 2.1, part B). Thus, top chain and A-ring synthons could either be thiols, reacting via SN_2 displacement on the alkyl bromide portions to form thioethers, or amines, being coupled to the carboxylic acid to generate amides. Accordingly, these bifunctional cores (Scheme 2.2) would react with either top chain-mimic thiol and amine A-ring analogue (part A), a thiol A-ring analogue and a top chain-mimic amine (part B) or top chain thiols and A-ring thiols



Scheme 2.1. Breaking down of calcitriol into fragments suitable for static library construction.

(part C).



Scheme 2.2. Alternative designs of thioether-amide vitamin D_3-like molecules

Before we could test this modular approach to highly-diversified, but simplified VD_3 analogues, we had to synthesize the required building blocks, an A-ring thiol, multiple top chain thiols and top chain amines and the requisite bifunctional electrophilic cores. Their synthesis is described in the next section.

2.2 Synthesis of components

2.2.1 A-ring amine : D-(-)-quinic acid approach

(1R,3R,4R,5R)-1,3,4,5-tetrahydroxycyclohexane-l-carboxylic acid (or D-(-)-quinic acid) is a plant metabolite originating from D-glucose (Figure 2.1). It is a highly versatile chiron. Its accessibility at a relatively low cost and its multiple stereocenters have spurred its use as a starting point for many natural product syntheses.[1] In the VD_3 context, quinic acid is a valuable molecule mainly because its hydroxyl groups at position 1 and 3 possess matching stereochemistry with the two OHs on the 1,25-$(OH)_2VD_3$ A-ring (Figure 2.2). As early as 1985, Desmaele and Tanier have used quinic acid to make a nor-19 A-ring VD_3 analogue.[2] DeLuca used it for several types of A-ring analogues.[3]

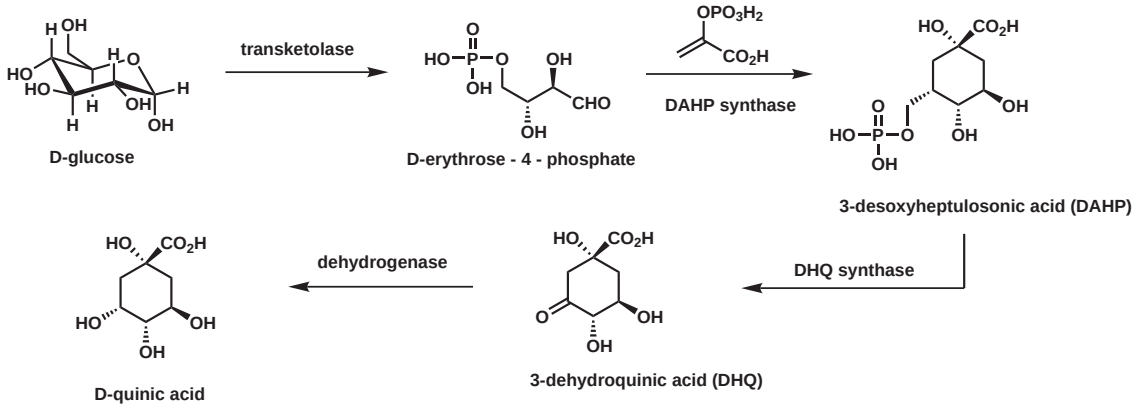


Figure 2.1. Biosynthetic production of D-quinic acid (adapted from [1]).

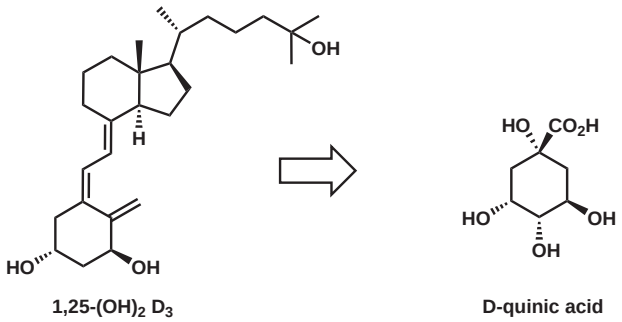
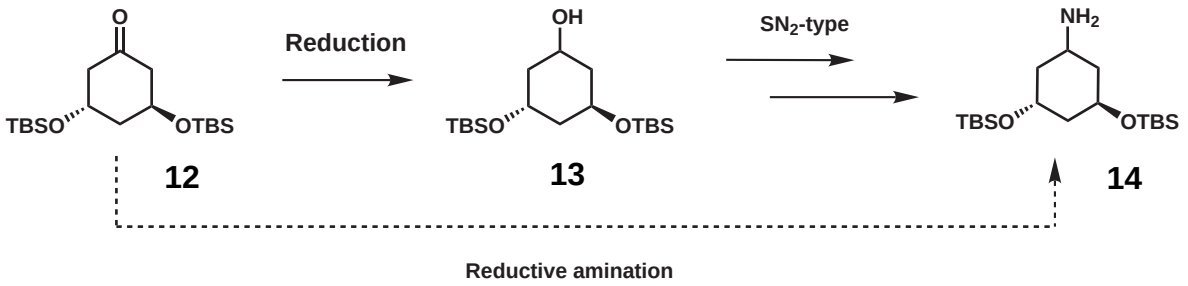


Figure 2.2. 1,25D_3 A-ring from D-quinic acid

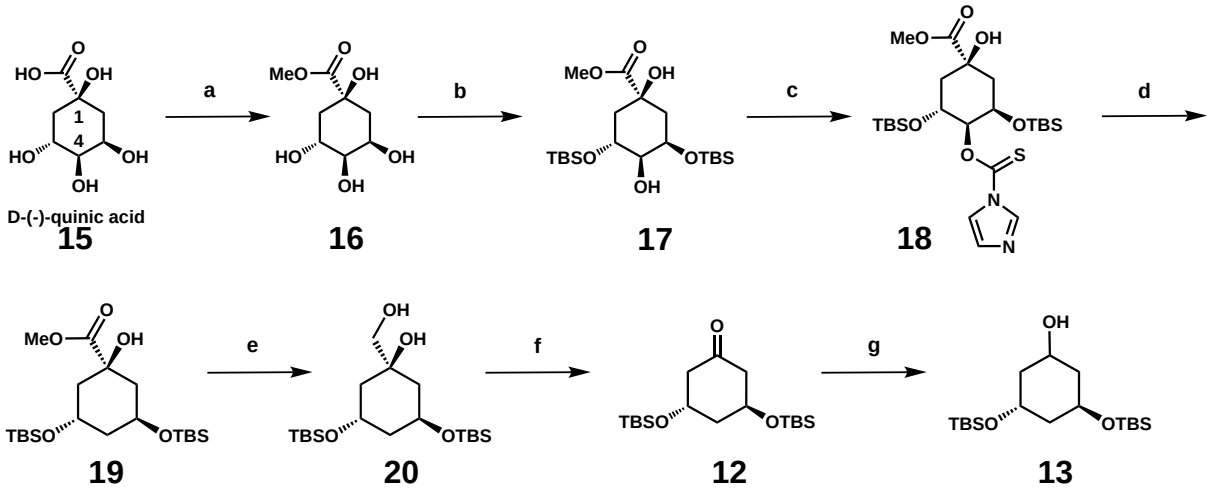
We envisioned the creation of A-ring amine **14** from either A-ring ketone **12** via reductive amination or from A-ring alcohol **13** via nucleophilic displacement of a suitable

leaving group (Scheme 2.3).



Scheme 2.3. A-ring amine **14** from A-ring ketone **12** or alcohol **13**.

Many synthetic schemes transforming D-(-)-quinic acid into A-ring analogues are available. It was decided to try the chemistry developed by DeLuca and colleagues for the synthesis of 19-nor-vitamin D compounds to obtain A-ring ketone **12** (Scheme 2.4).



Scheme 2.4. Route to A-ring ketone from D-quinic acid. *Reagents*. (a) AcCl, MeOH, $0\ ^{\circ}\mathrm{C}$ to rt, 16hrs, quant. ; (b) TBSCl, DMAP, TBABr, Et_3N, DMF, $0\ ^{\circ}\mathrm{C}$ to rt, 16hrs, 73 %, 2 steps; (c) TCDI, DMAP, CH_2Cl_2, rt, 72hrs, 92 % ; (d) $NaH_2PO_2xH_2O$, AIBN, 2-methoxyethanol, $90\ ^{\circ}\mathrm{C}$, 3hrs, 84 % ; (e) $NaBH_4$, EtOH, $0\ ^{\circ}\mathrm{C}$ to rt, 16hrs, 94 % ; (f) $NaIO_4$, THF/H_2O, $0\ ^{\circ}\mathrm{C}$ to rt, 16hrs, quant. ; (g) $NaBH_4$, EtOH, $0\ ^{\circ}\mathrm{C}$ to rt, 2hrs, 88 %.

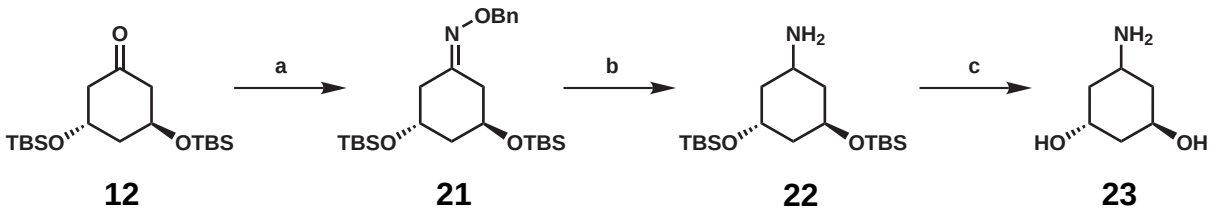
A-ring ketone Following the procedure described, esterification of quinic acid via acid-catalysis and protection of tetraol **16** as a bis- TBDMS ether afforded protected derivative **17**. As 1α,25-$(OH)_2D_3$ bears no functional groups at position 2, the corresponding OH group on quinic acid, 4-OH, had to be removed. This transformation could be accomplished via deoxygenation of thiocarbamate **18**. Derivatization of alcohol **17** at position

4 using thiocarbonyldiimidazole (TCDI) was fairly unreliable under the conditions mentioned in DeLuca's report.[3] A literature search indicated that we were not the first ones to experience these problems and that a solution had been found.[4] The reaction had to be carried at very high concentration, using only TCDI from a freshly opened bottle. This procedure generated thiocarbamoyl derivative **18** in both high yield and purity.

Radical deoxygenation using Bu_3SnH[3] worked well but as we planned to perform this transformation frequently and on a multigram scale, we looked for a less toxic and less expensive alternative. A recent review of the possible alternatives to Sn species in radical reactions[5], lead us to a report by Graham and coworkers at Abbott Laboratories (Illinois). In this article, tin hydride species were replaced with phosphorus hydrides as the hydrogen source in radical deoxygenations involving thiocarbonyls in the large scale synthesis of erythromycin derivatives.[6] In this report, the inorganic salt sodium hypophosphite (NaH_2PO_2) and high boiling point alcoholic solvents, such as methoxyethanol ($MeOCH_2CH_2OH$) were used in radical deoxygenation in place of Bu_3SnH and toluene. Applied to thiocarbonyl derivative **18**, conditions employing 5.0 equiv NaH_2PO_2, 0.3 equiv AIBN and 1.0 equiv thiocarbonyl in $MeOCH_2CH_2OH$ at reflux afforded an 84 % yield of deoxygenated product after purification. This procedure was effectively less expensive and significantly greener than the tin hydride alternative and was thus adopted. Hydroxyester **19** was reduced to diol **20** via $NaBH_4$ reduction in ethanol. Oxidative cleavage of this diol using $NaIO_4$ in a mixture of THF and water gave ketone **12** in quantitative yield. Compared to the reported yield of 25 % by DeLuca[3], our synthetic route with an equal number of steps provided an overall yield of 53 %. A-ring ketone **12** proved to be an extremely useful precusor to numerous compounds we required. If necessary, alcohol **13** is easily accessible from the corresponding ketone via reduction with sodium borohydride in 88 % yield.

A-ring amine In order to form an A-ring amine building block, we envisioned that a reductive amination of ketone **12** would be very efficient. After finding that A-ring ketone **12** was not a good substrate for acid-catalyzed imine formation (decomposing when mixed with benzyl amine and different acid catalysts), we transformed it into oxime **21** in high yield by mixing the ketone, *O*-benzylhydroxylamine hydrochloride, and sodium acetate in toluene at elevated temperature (see Scheme 2.5). Oxime **21** proved to be somewhat

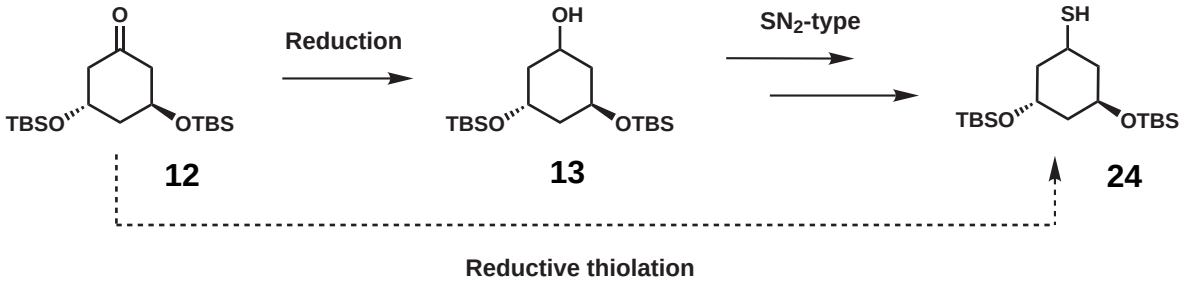
difficult to reduce cleanly to an amine, either in a single step or in a stepwise fashion. Finally, we found that Raney nickel was the most efficient catalyst for the transformation. Active forms of Raney nickel are easily prepared from nickel-aluminum alloys of different ratios.[7] For example, when a 50 % Ni-50 % Al alloy is reacted with NaOH (weight ratio alloy/NaOH of 1:1.28) at $50\ ^{\circ}\mathrm{C}$ for 50 minutes, it yields an excellent hydrogenation catalyst for phenols, ketones and other functional groups and is named Raney nickel W-7. In our hands, this catalyst was able to reduce oxime **21** to amine **22** in one step and with high yields and purity. However, it is known that the activity of Raney Ni thus prepared can highly vary from batch to batch and this was observed in our hands. Batches of Raney-Ni were unequally competent to achieve high conversion in reasonable time and yields. Final deprotection of amine **22** to yield amine **23** (Scheme 2.5) was accomplished by repeating twice the following treatment : a small drop of concentrated HCl added to a solution of amine in MeOH followed by solvent removal in vacuo. However, due to synthetic considerations, we almost exclusively used the protected version of the amine, compound **22** in VD_3 analogues synthesis.



Scheme 2.5. A-ring amine synthesis via oxime reduction. *Reagents*. (a) *O*-benzylhydroxylamine hydrochloride, NaOAc, toluene, reflux, 3hrs, 85 % ; (b) W-7 Raney Nickel, H_2, EtOH, rt, 16hrs, 86 % ; (c) conc. HCl, MeOH, rt, 15 min., 81 %.

2.2.2 Secondary A-ring thiol

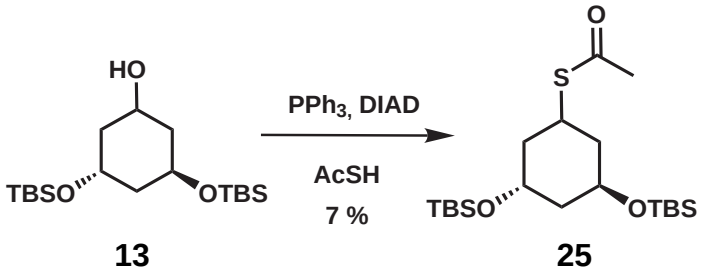
We envisioned the synthesis of secondary A-ring thiol **24** (Scheme 2.6) from two possible angles. First, we would attempt its synthesis via an SN_2 displacement of a suitably modified alcohol **13**, a molecule easily accessible via carbonyl reduction from ketone **12**. If we met resistance, we would then try via a reductive thiolation directly from ketone **12**.



Scheme 2.6. Sulfur introduction into an A-ring analogue

Secondary thiols, approach 1 : Sulfur incorporation via nucleophilic displacement

The Mitsunobu reaction is a very powerful tool for hydroxyl group transformation.[8] It was known that thioacetic acid (AcSH, a sulfur atom-containing equivalent of acetic acid) serves as a competent nucleophile under those conditions and affords thioacetate esters in high yields.[9] It was thus envisioned that we could use the Mitsunobu reaction to introduce a sulfur atom into the A-ring portion (Scheme 2.7) and form thioacetate **25**. Multiple attempts at this transformation were made by varying crucial reaction conditions such as reagent stoichiometry, reaction temperature and time, solvent, and even order of addition (exact reaction conditions not shown). However hard we tried, our best yield of thioacetate was 7 %, the remainder being starting material or very complex mixtures.

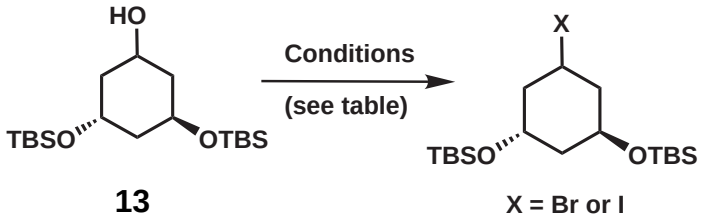


Scheme 2.7. Attempted Mitsunobu reactions on alcohol **13** to yield thioacetate **25**

Realizing that Mitsunobu conditions would not provide thioacetate in one high-yielding step, we tried to perform the incorporation of sulfur in two steps : the conversion of alcohol **13** into an electrophile (mesylate, triflate, halide) and then nucleophilic substitution (S_N2) with a sulfur-based nucleophile.

Our first approach at transforming alcohol **13** into an electrophile involved the creation

of an alkyl halide (Scheme 2.8). There are several ways to accomplish this transformation and we were quite optimistic. In summary, our attempts in this direction (Table 2.1) also failed miserably. We first tried using PPh_3 and *N*-bromosuccinimide (NBS) or iodine (I_2) under various reaction conditions (entries 1 to 5). Although these sets of reagents are known to be competent in the formation of alkyl bromides and iodides from alcohols, they did not work for our substrate. We even tried less traditional conditions, such as Me_3SiCl/LiBr, but to no avail.



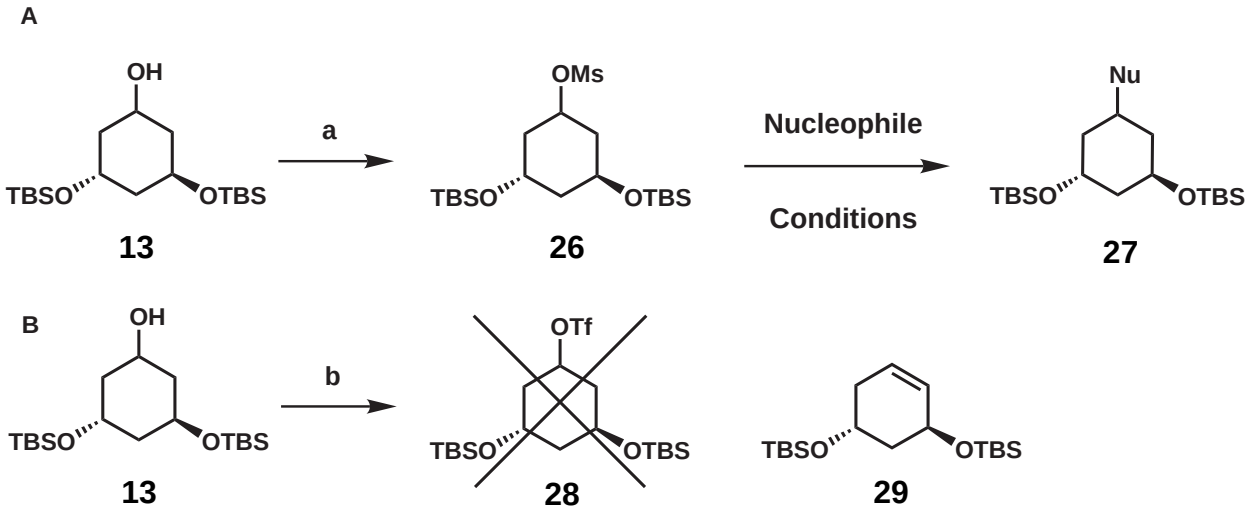
Scheme 2.8. Attempted formation of halides

Table 2.1: Attempted formation of halides

Entries	Conditions	Expected product	References
1	PPh_3, NBS, DMF, 0 and 50 °C	Alkyl Bromide	[10]
2	PPh_3, NBS, DCM, r.t	Alkyl Bromide	-
3	PPh_3, NBS, THF, r.t	Alkyl Bromide	[11]
4	PPh_3, I_2, 2,4,6-collidine, toluene, rt	Alkyl Iodide	-
5	PPh_3, I_2, imidazole, toluene, 70 °C	Alkyl Iodide	[12]
6	Me_3SiCl, LiBr, CH_3CN, 60 °C	Alkyl Bromide	[13]

Our second approach entailed the creation of a sulfonate ester, either a methanesulfonate (Ms) or a trifluoromethanesulfonate (Tf) ester. Formation of mesylate **26** proceeded optimally using MsCl and Et_3N (Scheme 2.9, part A). Quite surprisingly however, nucleophilic substitution of the mesylate **26** proved to be another daunting task (see Scheme 2.9 and Table 2.2). The secondary mesylate resisted to a plethora of S_N2 displacement conditions that included elevated temperature, excess reagents, and even particularly competent nucleophiles such as iodide (Table 2.2, entry 1), hydrosulfide anion (entry 2), sulfide anion (entry 3), thioacetate anion (entry 4), and cyanide (entry 5). We then tried morphing alcohol **13** into an even better electrophile : a trifluoromethanesulfonate ester (triflate). It involved the reaction of the alcohol with Tf_2O and base (Scheme 2.9, part B). While we wanted to create and isolate triflate ester **28**, we found that the exclusive product coming out of this reaction corresponded to an alkene, identified as alkene **29**, created cleanly in a 74

% yield. Unfortunately, attempts at *in situ* derivatization of a possible triflate intermediate only yielded alkene product.

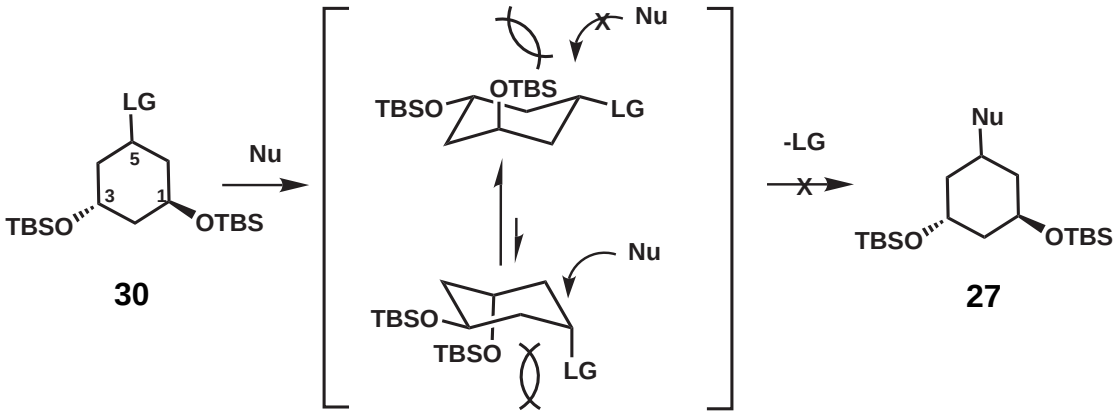


Scheme 2.9. Formation and attempted nucleophilic substitution of mesylate **26**. *Reagents*. (a) MsCl, Et_3N, CH_2Cl_2, 0 °C, 30 min., 95 %. ; (b) Tf_2O, DMAP, pyridine, CH_2Cl_2, 0 °C to rt, 15 min., 74 %.

Table 2.2: Attempted nucleophilic displacements on mesylate **26**

Entries	Conditions	Expected product
1	NaI, acetone, 50 °C	Alkyl Iodide
2	NaSH, MeOH/THF, 60 °C	Thiol
3	Na_2S, MeOH, 60 °C	Thiol
4	CsSAc or KSAc, DMF, 60 °C	Thioacetate
5	KCN	Nitrile

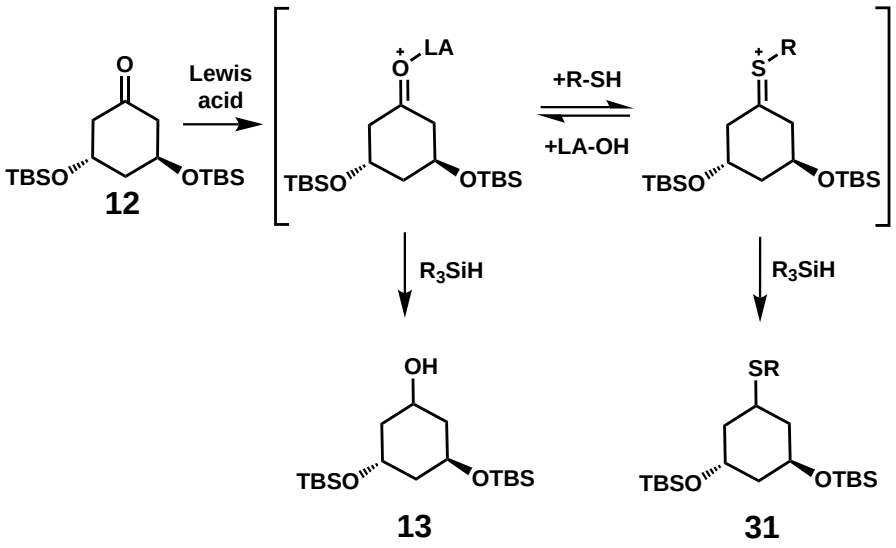
In the light of all these results and looking at the three-dimensional chair structure of a generic A-ring molecule ready for substitution **30** (Scheme 2.10), we think that the presence of at least one t-butyldimethylsilyloxy group (TBSO) in an axial orientation makes it very difficult for a nucleophilic attack at position 5. If we were to introduce sulfur into this chemical structure, it would be necessary to resort to a different strategy.



Scheme 2.10. Presence of intense steric interactions as possible explanation for low reactivity of A-ring electrophiles

Secondary thiols, approach 2 : Sulfur incorporation via reductive thiolation

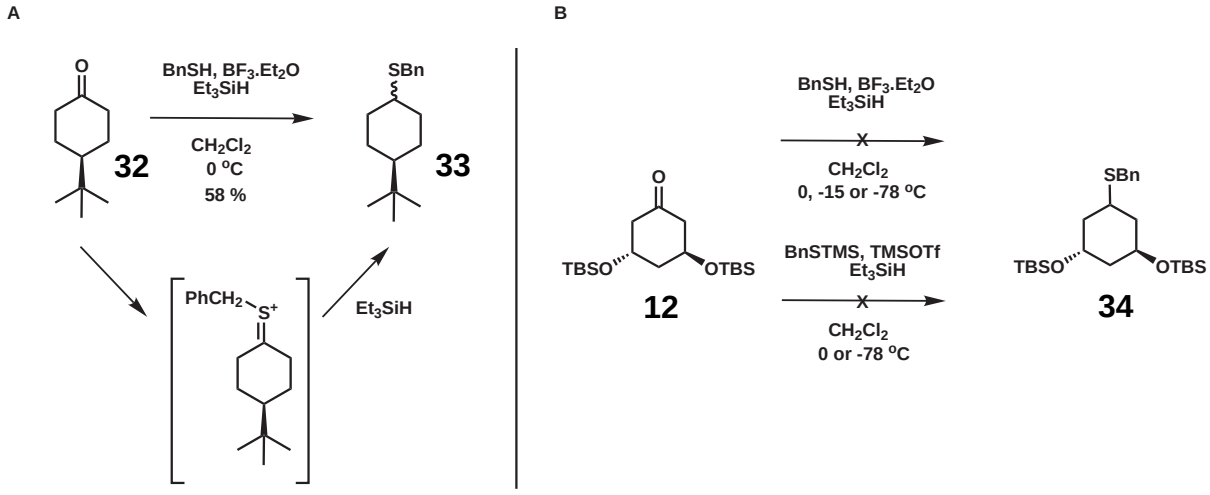
Having met fierce resistance from alcohol **13** to undergo nucleophilic substitution, we decided to investigate a reaction originally reported by Olah[14] which utilizes Lewis acid catalysis to trap thionium ions derived from simple ketones and thiols on their route to hemithioacetals with trialkylsilanes as reducing agents. The overall transformation is a reductive thiolation reaction occurring directly from a ketone to yield a thioether. This method had already been used in the stereocontrolled synthesis of novel enantiomerically pure sulfides and selenides from (+)-camphor and (+)-camphor-10-sulfonyl chloride.[15] In our case, ketone **12** would yield a thioether like **31**, as can be seen in Scheme 2.11. Note that since the formation of the thionium ion has to go through an oxonium ion, premature trapping with a silane can yield alcohols to a certain extent.



Scheme 2.11. General scheme for a reductive thiolation of ketone **12** to give a thioether of type **31**.

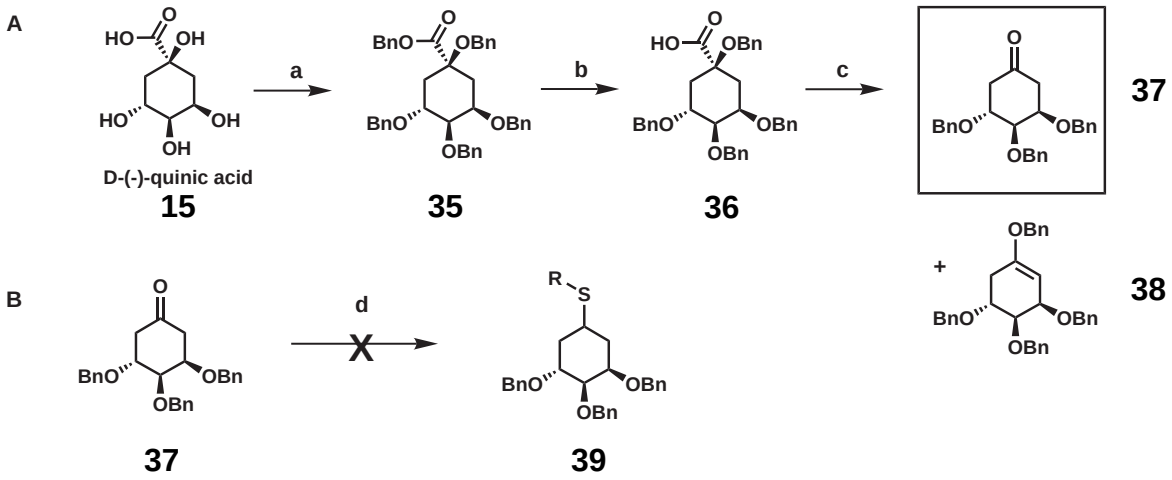
Employing t-butylcyclohenxanone as a model ketone, we found that we could accomplish the transformation using $BF_3 \cdot Et_2O$ as the Lewis acid, benzylmercaptan as the thiol and Et_3SiH acting as the reducing silane (Scheme 2.12, part A). The somewhat average yield of 58 % comes from the expected production of t-butylcyclohexanol as a side product from premature reduction of the corresponding oxonium. We immediately transfered those conditions to our ketone **12** (Scheme 2.12, part B). Unfortunately, we were thwarted by the decomposition of the starting ketone under those conditions, even when lowering

the reaction temperature down to -78 °C. Contemplating the idea that the decomposition was triggered/mediated by the available protons (H^+) in the reaction, we decided to try the following conditions : BnSTMS instead of BnSH as the nucleophile and TMSOTf instead of $BF_3 \cdot Et_2O$ as the Lewis acid. We transformed benzylmercaptan into thiosilylether BnSTMS using imidazole-catalysis of the reaction of BnSH with hexamethyldisilazane ($HN(TMS)_2$) according to Glass' method[16], while TMSOTf was prepared as described by Demuth.[17] Even under those conditions, we met no success : ketone **12** and its OTBS ethers seemed too unstable. Almost invariably, we would witness the apparition of a myriad of side products by TLC, some of them being identifiable as TBSO elimination products. Most of the time however, the reaction mixtures were simply impossible to analyze. We then decided to verify if protecting groups of another nature (such as benzyl ethers) could protect the hydroxyl groups and survive the reaction conditions. For this, we prepared from D-quinic acid a model substrate on which the only protecting groups were benzylethers (Scheme 2.13, part A). The synthesis took three steps and yielded ketone **37** via a $Pb(OAc)_4$-mediated radical decarboxylation. When we subjected this trisbenzyloxycyclohexanone **37** to the 2 sets of reductive thiolation conditions mentioned earlier (Scheme 2.13, part B), we found that it too did not survive the reaction. Clearly, the problem resided in the structure of the 3,5-bis(t-butyltyldimethylsilyloxy)cyclohexanone **12**.



Scheme 2.12. Attempts at reductive thiolation of ketone **12**.

Faced with failure, we turned our effort towards a well-precedented reaction to incorporate a sulfur atom into a molecule : conjuguate addition of a thiol onto a α, β-unsaturated

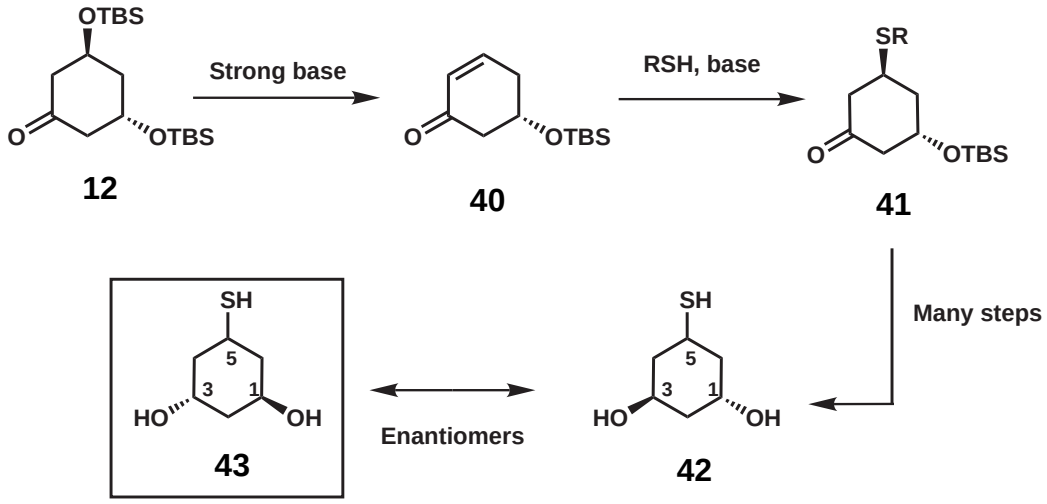


Scheme 2.13. (A) Synthesis of ketone **37** and (B) attempted reductive thiolation to give thioether **39**. *Reagents* : (a) BnBr, NaH, DMF, rt, 16hrs, 67 %; (b) aq. NaOH, THF, $65\ ^{\circ}\mathrm{C}$, 2hrs, 87 %; (c) $Pb(OAc)_4$, DMF, rt, o/n, 40 % ; (d) BF_3-Et_2O, Et_3SiH, BnSH, CH_2Cl_2, $0\ ^{\circ}\mathrm{C}$, 15 min or BnSTMS, TMSOTf, Et_3SiH, $0\ ^{\circ}\mathrm{C}$, 15 min : decomposition.

ketone.

Secondary thiols, approach 3 : Sulfur incorporation via conjugate addition

Preliminary work We reasoned that the known facile conjuguate addition of thiols to α, β-unsaturated ketones was probably an other way of creating a sulfur-containing A-ring analogue (Scheme 2.14).

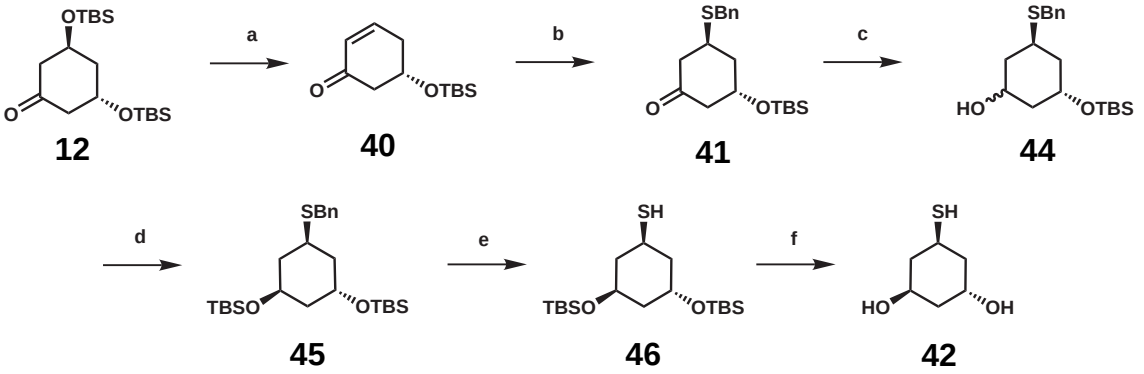


Scheme 2.14. Proposed conjuguate addition of a thiol to α, β-unsaturated ketones to create a sulfur-containing A-ring analogue, albeit an enantiomer of our desired molecule.

We were aware that β-alkoxy-ketones such as ketone **12** can be converted to enones readily via elimination of the β-alkoxy moiety. Moreover, it was well known in vitamin D chemistry that A-ring ketones similar to ketone **12** easily suffer elimination (and aromatization) when subjected to basic conditions.[18, 19] We thought we could easily and quickly test our new route by producing enone **40**. To test our hypothesis and see whether it would generate (S)-enone **40**, we submitted ketone **12** to strong bases.[1] Optimal reaction conditions were 1.0 equiv of ketone added to 0.5 equiv of generated lithium diisopropylamide (LDA) or NaHMDS, at $-78\ ^{\circ}\mathrm{C}$ for 15 minutes yielding about 15-35 % of enone and the remainder was recovered starting material. Longer reaction times, the use of a full equivalent of or higher temperatures to increase conversion led to formation of phenol in very good yield, probably via double elimination. Repeating the reaction several times afforded us enough material to test the sequence leading to the A-ring secondary thiol, as presented

[1]Note here that this enone **40** is the enantiomer of the one that would lead to the A-ring thiol **42** of proper stereochemistry at position 1 and 3 (Scheme 2.14).

in Scheme 2.15. Using freshly obtained enone **40**, benzyl mercaptan and a base (EtONa, MeONa or Et_3N), we accomplished sulfur incorporation via a facile thiol conjugate addition : thioether-ketone **41** was obtained cleanly in 88 % yield. By careful analysis of the ^1H NMR spectum of the thioether and comparison to known molecules, we were certain of the anti relationship between the TBSO and the BnS group, this observation being consistent with axial attack on the enone. Next the ketone was reduced using $NaBH_4$ giving a 1:1 mixture of syn:anti diol **44** from which the antidiol could be isolated by chromatographic separation. A few protecting group manipulation involving protection of the alcohol as a t-butyltyldimethylsilyl derivative **45**, reductive deprotection of the benzyl group using Li in ammonia or LiDBB in THF and acid-mediated TBS ethers deprotection generated thiol **42**.



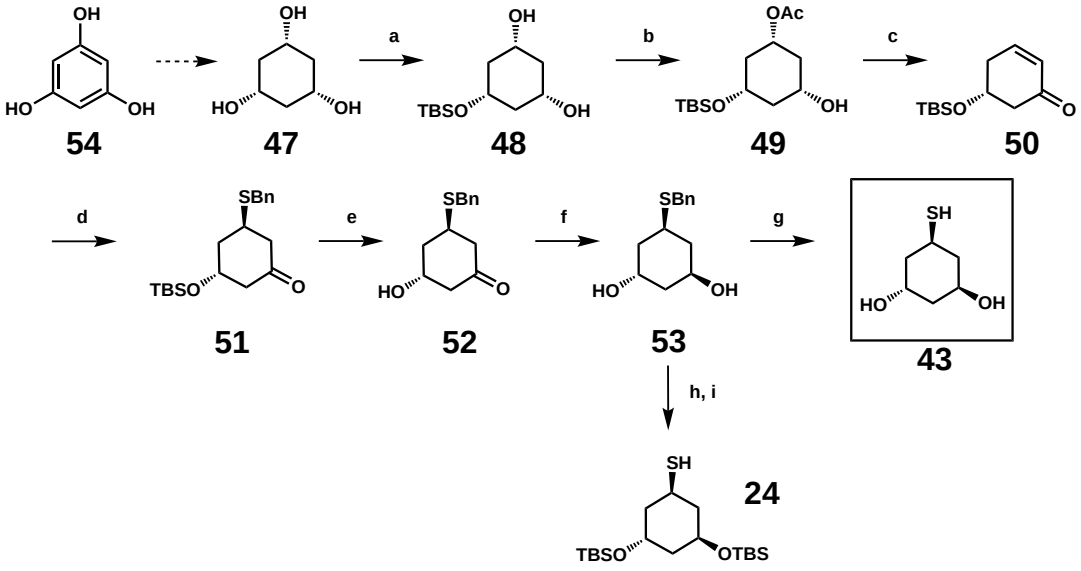
Scheme 2.15. Construction of ent-A-ring thiol **42** from ketone **12**. *Reagents*. (a) LDA, THF, different solvents and temperatures, 15 min., 15-35 % (b) BnSH, cat. NaOEt, CH_2Cl_2, rt, 16hrs, 83 %; (c) $NaBH_4$, MeOH, THF, $0\ ^\circ C$ to rt, 2hrs, 89 %; (d) TBSCl, imidazole, Et_3N, DMF, $0\ ^\circ C$ to rt, 8hrs, 85 %; (e) Li, NH_3, $-78\ ^\circ C$, 1.5hrs, 76 % ; (f) conc. HCl, MeOH, rt, 30 min., 79 %.

Since this route worked, all we needed was a way to access enone **50** possessing the proper stereochemistry. Scanning the literature we came upon a method based on enzymmatic desymmetrization of a meso-diol compound.[19] The next section details our efforts using this method to create a chiral enone.

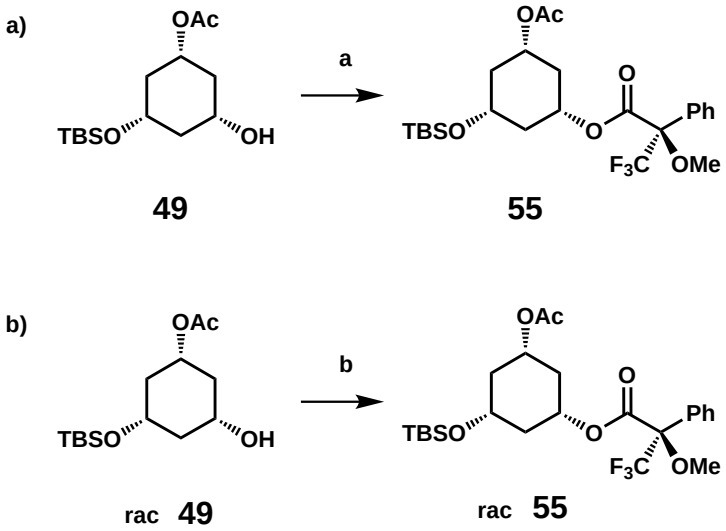
A-ring thiol via enzymatic desymmetrization Our new route to making (1S,3S)-5-mercaptocyclohexane-1,3-diol (MCD) **43** (Scheme 2.16) is based primarily on an enzyme-catalysed desymmetrization of the meso-diol **48** via its conversion to monoacetate **49**. The

starting material is the achiral cis,cis-1,3,5-cyclohexanetriol **47**, a.k.a cis−phloroglucitol. Although commercially available[2], cis−phloroglucitol **47** was reported to be accessible through heterogeneous catalytic hydrogenation of cis−phloroglucinol **195**.[20] For the reduction, we used hydrogen and freshly prepared W-7 Raney nickel from commercially available 50/50 nickel-aluminum alloy. Amenable to large scale preparation (>50 g), this reduction gives in average a 43 % yield of cis,cis-1,3,5-cyclohexanetriol after recrystallization from water of the crude 2:1 cis/trans mixture. The next step was the monosilylation of **47**. Although reported with a yield of 93 %[19], our numerous attempts and variations of the reported optimized conditions (a combination of Et_3N and NaH as bases) never afforded more than a 45 % yield, with the remainder being a mixture of products resulting from double or even triple protection. Fortunately, chromatographic separation was straightforward and we were then ready to test lipase-mediated desymmetrization of the meso-diol **48**. This transformation was extensively investigated by Wirz et al. in a study involving a broad panel of commercially available enzymes. Among the ones tested, lipase QL seemed to be suiting our needs best.[21] In a reaction involving lipase, meso-diol **48**, and vinyl acetate in a weight ratio of 0.1:1:10 in EtOAc ([diol] = 0.04 M), it was found that within 48 hours, the diol was fully and cleanly converted to monoalcohol-monoacetate **49**. To verify that we had created the correct enantiomer via enzymatic acetylation, we converted the product into a Mosher ester using S-MTPA-Cl (Scheme 2.17). By comparing its NMR spectra (^1H and ^{13}C) with those of a Mosher ester made from racemic monoacetate rac-**49** (obtained using straightforward monoacetylation using acetic anhydride), we could calculate an ee of above 99 %. Furthermore, measurement of alcohol **49**'s α_D matched in sign that of alcohol reported by Wirz[19] (+15, $CHCl_3$, 1% w/v for the former vs +5, $CHCl_3$, 1% w/v for the latter). If we were concerned that the magnitude was different from the reported one, we were reassured about the identity of the enantiomer fabricated by the fact that all lipases tested in the initial study by Hilpert and Wirz [21] generated this particular enantiomer preferentially.

[2] As of June 2010, **47** and **195** were sold by Sigma-Aldrich : cis,cis-1,3,5-Cyclohexanetriol dihydrate (cis-phloroglucitol), 359017 (98 %, 57 $/g) and 1,3,5-Trihydroxybenzene dihydrate (cis-phloroglucinol), P38005 (97 %, 0.83$/g).



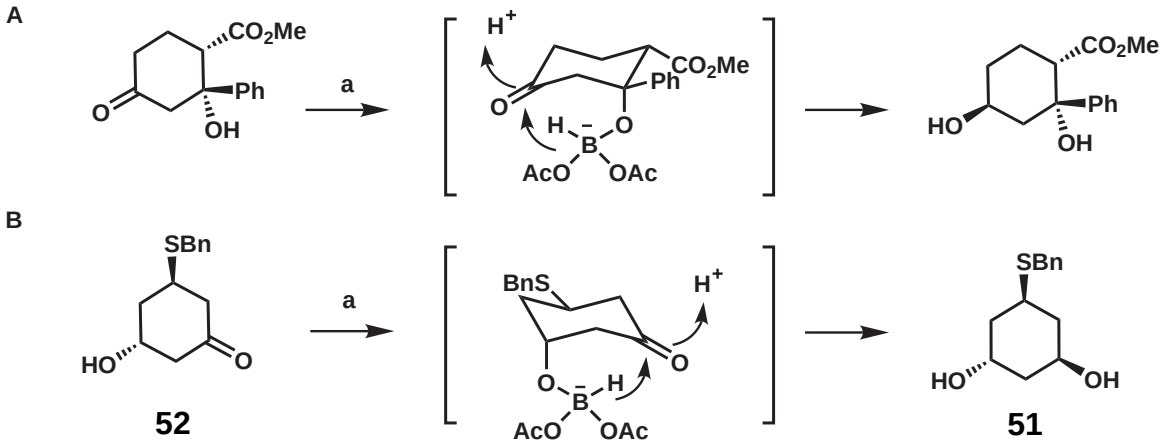
Scheme 2.16. Route to A-ring thiol **43** via chiral (R)-5-(tert-butyldimethylsilyloxy)cyclohex-2-enone **50**. *Reagents*: (a) TBSCl, imidazole, Et_3N, DMF, $0\ ^{\circ}C$ to rt, 16hrs, 43 %; (b) Lipase QL, vinyl acetate, EtOAc, rt, 48hrs, 97 %; (c) i: $COCl_2$, DMSO, $-78\ ^{\circ}C$, 1hr. ii: Et_3N, $-78\ ^{\circ}C$ to rt, 16hrs, 98 %; (d) BnSH, NaOEt (cat.), CH_2Cl_2, rt, 16hrs, 88 %; (e) 48 % aqueous HF, CH_3CN, $-15\ ^{\circ}C$, 4hrs ; (f) $NaBH(OAc)_3$, EtOAc, rt, 5hrs, 76 % over 2 steps (g) Li, NH_3, $-78\ ^{\circ}C$, 30 min., 67 % ; (h) TBSCl, cat. DMAP, Et_3N, DMF, $0\ ^{\circ}C$ to rt, 16hrs, 69 %; (i) Li, NH_3, $-78\ ^{\circ}C$, 30 min., 79 %.



Scheme 2.17. Establishment of the absolute configuration at C-1. *Reagents*: (a) (S)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride, pyridine/CH_2Cl_2 (1:6), cat. DMAP, rt, o/n, 71 % (b) (S)-(+)-MTPA-Cl, pyridine/CH_2Cl_2 (1:6), DMAP cat., rt, o/n, 81 %.

Next, we envisioned a two-step process to create (R)-5-(tert-butyldimethylsilyloxy)-cyclohex-2-enone **50** : first, an oxidation of alcohol **49** to a ketone and second, a basic alumina-mediated elimination of the β-acetoxy group. When we proceeded with the oxidation via a Swern protocol[22], we were please to see that prolongated reaction time after the standard addition of Et_3N promoted elimination of the acetoxy moiety and clean enone **50** could be isolated in 98 % yield. We immediately tried our previously elaborated conditions (BnSH and cat. EtONa, section 2.2.2) for benzylthiol addition to enone **50** and found that thioether **51** was formed in 80 % yield. The trans selectivity observed previously with compound **45** was also observed.

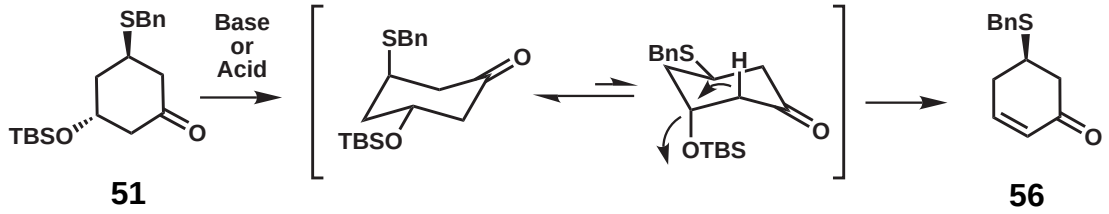
Recalling the approximately 1:1 ratio of syn:anti diol obtained in the $NaBH_4$ reduction of ketone **41** (Scheme 2.15), we decided to try to increase the overall yield of anti-diol **43**. To do this, we chose to attempt a directed reduction of the ketone using a free hydroxyl group at position 3 and a suitable reducing agent. According to the work of Turnbull et al.[23], such directed reduction seemed possible. In their worked, they took advantage of a the presence of a OH group in a β-hydroxyketone and used $NaBH(OAc)_3$ to effect a directed ketone reduction via a transition state shown in Scheme 2.18, part A. As is visible in part B of that scheme, we envisioned a similar transition state to take place for a putative directed reduction of a ketone on a suitably deprotected β-hydroxyketone **52**.



Scheme 2.18. Rationale behind the predicted stereochemical outcome of directed reduction of hydroxyketone **51** : (a) $NaBH(OAc)_3$

With precedent backing us, we needed to assess the problem of the deprotection of the t-butyltyldimethylsilyl ether to free the required hydroxyl group. Multiple attempts

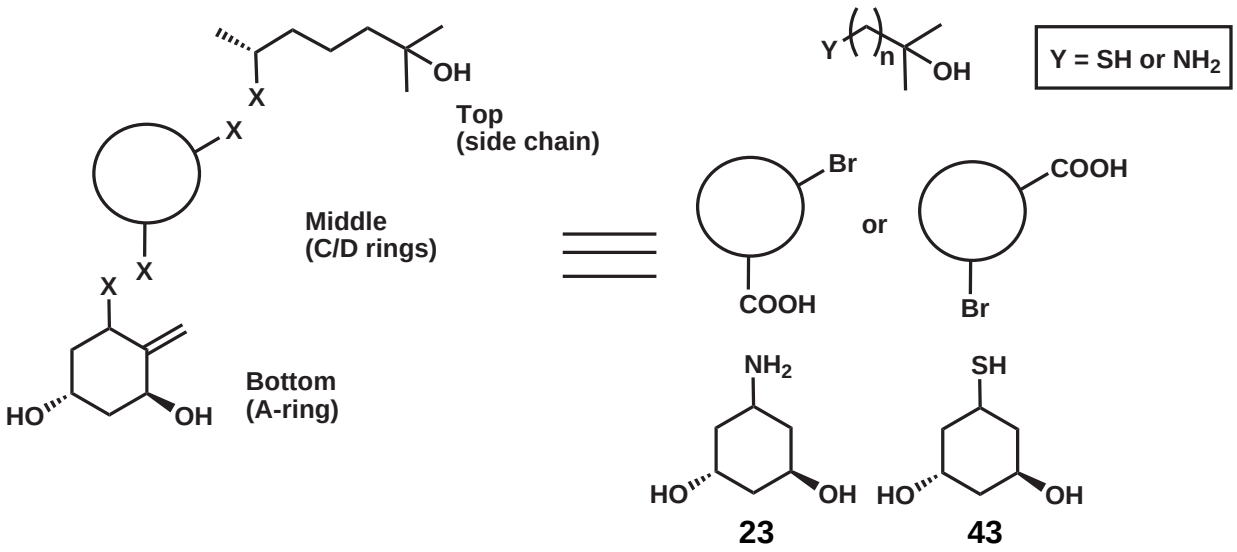
were made using a variety of standard conditions to achieve the deprotection (TBAF, HCl/MeOH, HF · Et_3N, PPTS, AcOH, TMSOTf, etc.) but often the conditions yielded side product enone **56**, as shown in Scheme 2.19. This product comes from an elimination reaction that occurs readily on 5-tert-butyldimethylsilanyloxy-3-alkyl-cyclohexanone derivatives, as reported by Sato et al. [24] (they report elimination of the OTBS moiety and formation of enones with 92 to 98 % yield from similar products to ours, by using DBU in CH_2Cl_2 at rt in 5h). To our satisfaction, optimal conditions were found to be the use of 48 % aqueous HF in acetonitrile as solvent, at approximately $-30\ ^\circ C$ (Scheme 2.16 on page 63). The ketone **52** was typically used a crude product in the directed reduction step, even though it was possible to purify and isolate via silica column chromatography (Scheme 2.16). We were pleased to find that, on our first try, a clean reduction product was obtained using $NaBH(OAc)_3$. After purification, we could observe by NMR (via coupling constants measurements and comparison with previously isolated products **44** and **45**) that the reduction proceeded to give exclusively anti diol, in a directed fashion as expected.



Scheme 2.19. Side reaction of **51** yields product **56**

Thioether diol **53** was thus one deprotection step away from the much needed A-ring thiol building block MCD **43** (Scheme 2.16). We conducted the benzyl group deprotection using Li reduction in condensed ammonia at $-78\ ^\circ C$ and isolated the polar (1S,3S)-5-mercapto-1,3-cyclohexanediol in 67 % yield. It was also possible to create an hydroxyl groups-protected A-ring thiol, most likely very useful to ensure future selective reactions of the thiol over the alcohols. Diol **53** was TBS protected and the benzyl thioether cleaved using standard procedures to afford bis-TBS A-ring thiol **24**.

So far, we have created two main pieces that are going to be used to mimic the bottom A-ring portion of 1α,25-$(OH)_2$ VD_3 in our analogues (Scheme 2.20) : A-ring amine **23** and A-ring thiol **43**. Next is presented the work on the top chain portions, followed by the creation of the middle core building blocks.



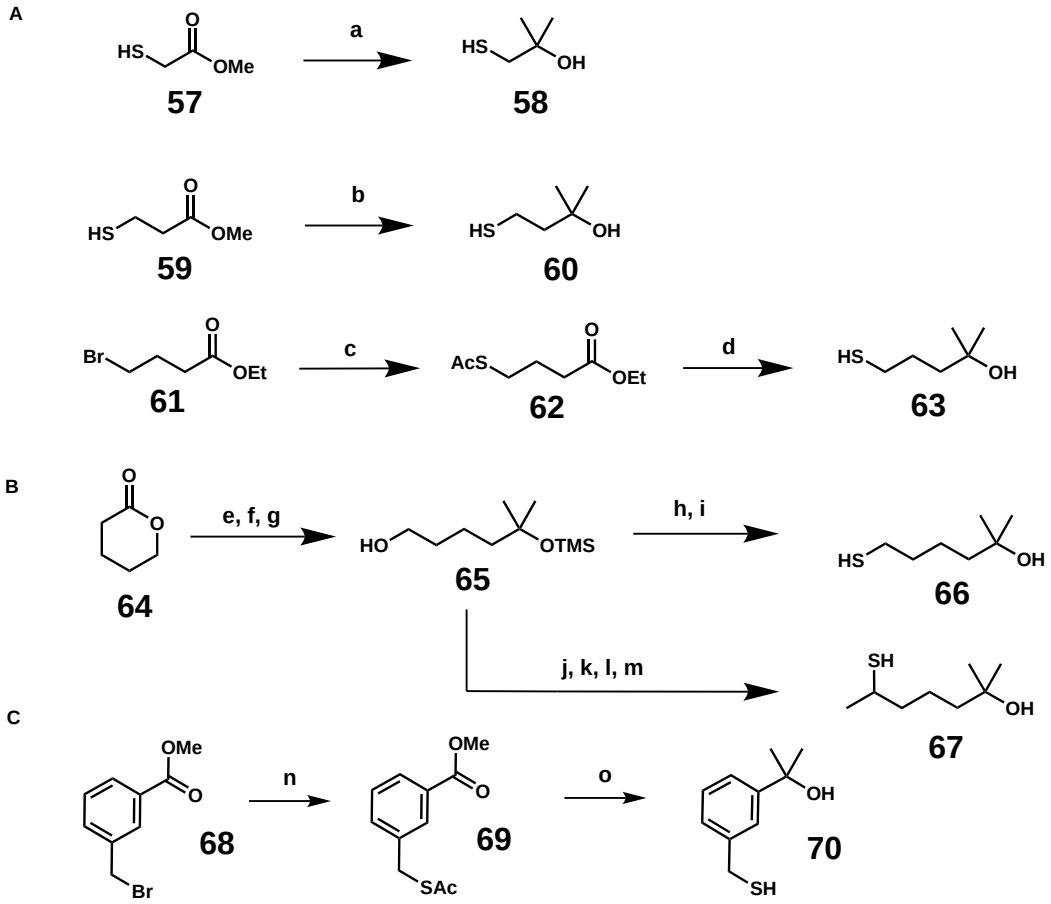
Scheme 2.20. Fragments suitable for static library construction.

2.2.3 Top chain thiols

Top chain thiol pieces[3] of different lengths terminating in dimethylalkyl tertiary hydroxyl groups reminiscent of the 25-OH group in 1α,25-$(OH)_2$ VD_3 were selected as potentially useful candidates for library synthesis. Thus, following the work of Takahashi and coworkers in the preparation of a parallel synthesis of a vitamin D_3 library in the solid-phase[25], we envisioned the double addition of methyl lithium to esters as the critical transformation to access the needed thiols (Scheme 2.21). As can be seen in part A, thiols bearing tertiary OH groups can easily be obtained from the addition of MeLi to mercapto methyl esters. Varying the chain length between the thiol group and the ester functionality in the starting material affords thiols of different sizes in one or two steps from commercial reagents. In two cases, thiol **66** and thiol **67** (part B), the synthesis required 6 or 7 straightforward steps. We also became intrigued by a report by Norman in 1991 describing VD_3 analogues with an aromatic side chain.[26] Since these analogues had some antiproliferative activity and low calcemic effects, we decided to prepare side chain thiol **70**. We synthesized it from methyl 3-bromomethylbenzoate **68**, a compound made from radical bromination of the corresponding methyl 3-methylbenzoate (see section 2.2.5 for more

[3]Top chain thiol pieces have been prepared mainly by a postdoctoral fellow, Dr Tan Quach. Although I repeated his work on many occasions to replenish my building block collection, Dr Quach is responsible for the elaboration of the synthetic work described for this category of thiols.

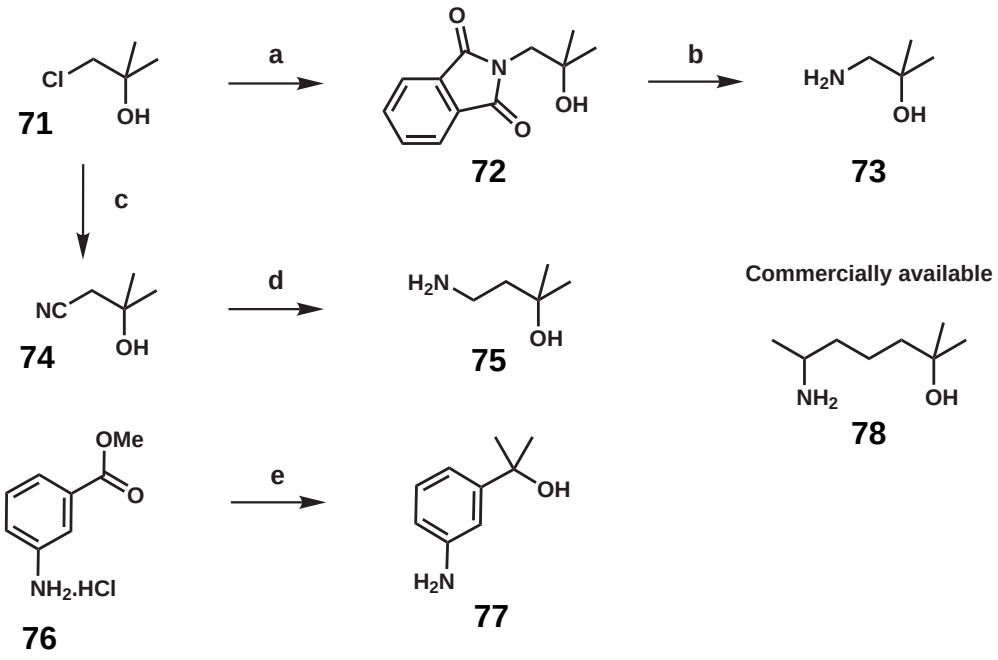
details about this chemical transformation). Incorporation of sulfur via S_N2 reaction of thioacetate anion with the benzylic bromide afforded **69**, while the tertiary alcohol group was produced again via MeLi addition to an ester group.



Scheme 2.21. Synthesis of the top chain thiols. *Reagents*: (a) MeLi, THF/Et_2O, $-78\ ^\circ C$ to rt, o/n, 88 % ; (b) MeLi, THF/Et_2O, $-78\ ^\circ C$ to rt, o/n, 90 % ; (c) AcSK, EtOH, rt, 18hrs, 95 % ; (d) MeLi, THF/Et_2O, $-78\ ^\circ C$ to rt, o/n, 49 % ; (e) MeLi, dried $CeCl_3 \cdot 7\,H_2O$, Et_2O, $-78\ ^\circ C$ to rt, 16hrs, 58 % ; (f) TMSCl, imidazole, CH_2Cl_2, rt, o/n, ; (g) K_2CO_3, MeOH, rt, 30 min. 96 % over 2 steps ; (h) PPh_3, DIAD, AcSH, THF, $0\ ^\circ C$ to rt, 3hrs, 69 % (i) NaOEt, EtOH, rt, 15 min., 83 % ; (j) Dess-Martin, CH_2Cl_2, rt, 2hrs, 63 % ; (k) MeMgBr, THF, $-78\ ^\circ C$ to rt, 16hrs, 84 % ; (l) PPh_3, DIAD, AcSH, THF, $0\ ^\circ C$ to rt, 6hrs, 62 % ; (m) NaOEt, EtOH, rt, 15 min., 86 % ; (n) AcSK, EtOH, rt, 29hrs, 82 % ;(o) MeLi, THF/Et_2O, $-78\ ^\circ C$ to rt, o/n, 86 %.

2.2.4 Top chain amines

To complement the A-ring thiols as nucleophilic components, we designed an orthogonal nucleophile in the side chain portion of VD_3 analogues : amines. We expected that side chain amines with a dimethylalkyl alcohol functional group could be selectively incorporated into halo acid central cores. Although some amines were commercially available, we easily prepared them via simple chemistry presented in Scheme 2.22. For two amines, we started from 1-chloro-2-methylpropan-2-ol **71** as it was both a source of the dimethylalkyl tertiary alcohol group needed and a competent electrophile. Its reaction with potassium phthalimide afforded N-protected alcohol **72**. Removal of the phthalimide group was accomplished with the use of excess hydrazine (NH_2-NH_2) to give amine **73**. The reaction of 1-chloro-2-methylpropan-2-ol with cyanide anion afforded nitrile **74**, which was reduced to amine **75** using $LiAlH_4$. In addition, we prepared aromatic top chain-containing VD_3 analogues by reaction of methyl 3-aminobenzoate hydrochloride **76** with excess MeLi reagent to afford aniline-alcohol **77**. We complemented our set of top chain amine with a commercial amine already possessing the required tertiary alcohol called heptaminol **78**. We will use this amine quite frequently in our preliminary studies.

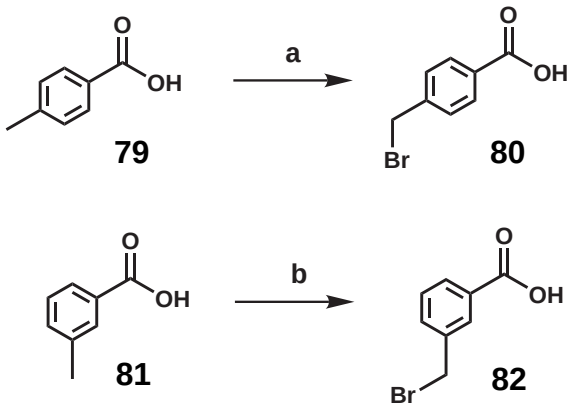


Scheme 2.22. Synthesis of the top chain amines. *Reagents* : (a) Potassium phthalimide, NaI, DMF, reflux, 24hrs, 76 % ; (b) Hydrazine hydrate, EtOH, reflux, 17hrs, 68 % ; (c) NaCN, EtOH/H_2O, rt, 14hrs, 82 % ; (d) $LiAlH_4$, Et_2O, $0\ ^{\circ}C$ to rt to reflux, 7hrs, 89 % ; (e) MeLi, Et_2O, $-78\ ^{\circ}C$ to rt, o/n, 27 %.

2.2.5 Bromoalkyl-carboxylic acid cores

Bromomethylbenzoic acids

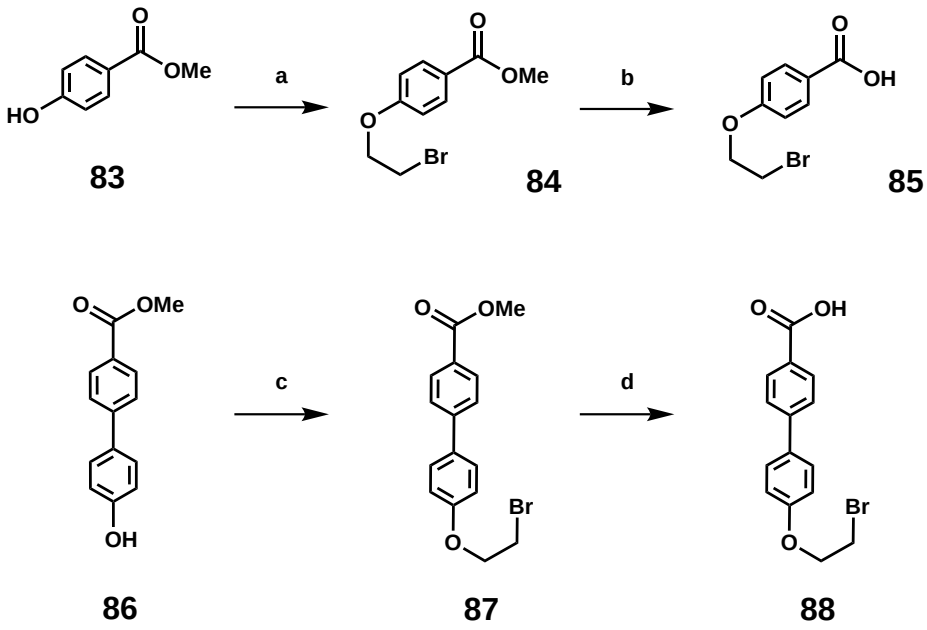
Core building blocks that interested us included bromomethylbenzoic acids. They can be created by functionalization of toluic acids via radical bromination of benzylic C-H bonds. Ishii and coworkers have found a very selective procedure for the formation of benzyl bromides.[27] Their studies found that $NaBrO_3$ combined with a reducing agent, $NaHSO_3$, generates hypobromous acid (HOBr) and serves as an effective brominating agent of alkylbenzenes under a biphasic system using EtOAc and water as solvents. Using their method (Scheme 2.23), we were able to produce bromomethylbenzoic acids **80** and **82** from m-toluic acid and p-toluic acid respectively. As was reported, we observed no functionalization of aromatic C-Hs. Careful analysis of NMR spectra allowed us to determined that the ratio of monobromination versus dibromination of the benzylic position was fairly high, with approximately only 10 % of dibromination observed.



Scheme 2.23. Synthesis of bromomethylbenzoic acids via radical bromination of corresponding toluic acids. *Reagents* : (a) $KBrO_3$, $NaHSO_3$, EtOAc/H_2O, rt, 4hrs, 65 %. (b) $KBrO_3$, $NaHSO_3$, EtOAc/H_2O, rt, 4hrs, 47 %.

Bromoethoxybenzoic acids

Bromoethoxybenzoic acids were our next targets. We chose to make bromoethoxybenzoic acids **85** and **88** (Scheme 2.24). They could easily be prepared in two steps by alkylation of phenolic esters **83** and **86** with excess 1,2-dibromoethane[28], followed by ester hydrolysis using LiOH.

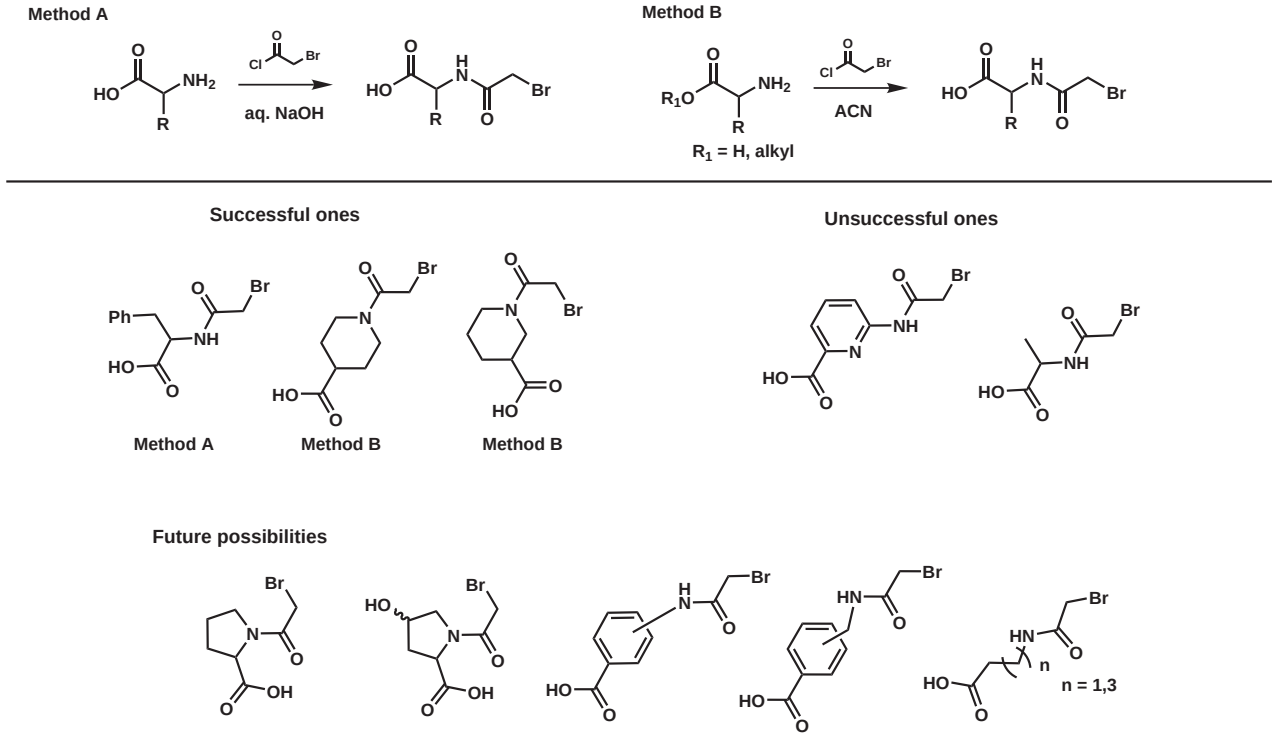


Scheme 2.24. Synthesis of bromoethoxybenzoic acids via alkylation of corresponding phenolic acid derivatives with 1,2-dibromoethane. *Reagents* : (a) $BrCH_2CH_2Br$, K_2CO_3, acetone, reflux, 3 days ; (b) $LiOH \cdot H_2O$, THF/MeOH/H_2O, rt, 6hrs, 67 % over 2 steps ; (c) $BrCH_2CH_2Br$, K_2CO_3, acetone, reflux, 3 days ; (d) $LiOH \cdot H_2O$, THF/MeOH/H_2O, rt, 7hrs, 35 % over 2 steps ;

Bromoacetamide acids

Due to their great structural diversity and commercial availability, we wanted to find a way to incorporate amino acid derivatives as core building blocks. To do so, we needed to transform amino acids into bis-electrophilic molecules, i.e. convert the amino group from a nucleophile to an electrophile. Possibly the fastest and most obvious way of doing this was to form α-bromoacetylamides from aminoacids (Scheme 2.25). Many methods are known to effect this transformation. We used mainly two different protocols : the N-acylation of amino acids under aqueous conditions (Schotten-Baumann conditions, method A)[29] and the N-acylation of aminoacids or aminoesters with bromoacetyl chloride under anhydrous conditions (method B)[30]. As is shown in Scheme 2.25, three examples of bromoacetamide acids were successfully created, one derived from phenylalanine using method A and two derived from 3- and 4-piperidine carboxylic acids using method B. Interestingly, attempted acylation of both alanine and 2-amino-5-carboxypyridine failed

under both sets of conditions.[4] Depending on the results of the preliminary studies on the behavior of those bromacetamide acids, we could extend the diversity of the building blocks by creating various other bromoacids (Scheme 2.25, bottom part).

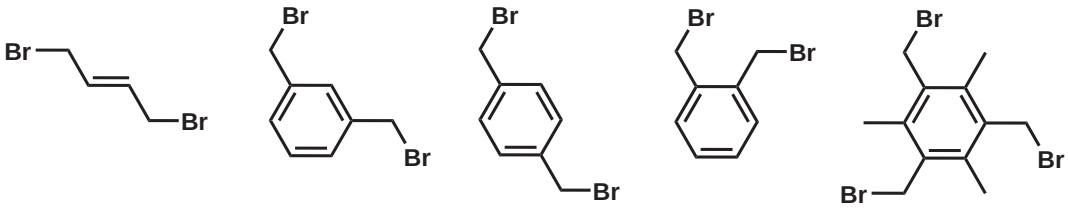


Scheme 2.25. Synthesis of bromoacetylamides. Reagents : **Method A** (modified Schotten-Baumann conditions) : amino acid (1 equiv), 4 N NaOH (3 equiv), bromoacetyl chloride (1.1 equiv), toluene, 0 °C, 50 minutes. **Method B** : amino acid (1 equiv), bromoacetyl chloride, CH_3CN, reflux, 2hrs.

2.2.6 Bifunctional electrophilic cores for thioether formation

The first series of dihalides was composed of commercially available electrophiles as they did not need chemical modification and could be used directly to find the best reaction conditions for this library. They are presented in Scheme 2.26. We chose simple linear alkyl and aromatic dihalides **89**, **90**, **91**, **92** and **93** for their reactivity (allylic and benzylic halides are more reactive towards substitution than alkyls halides) and for their availability.

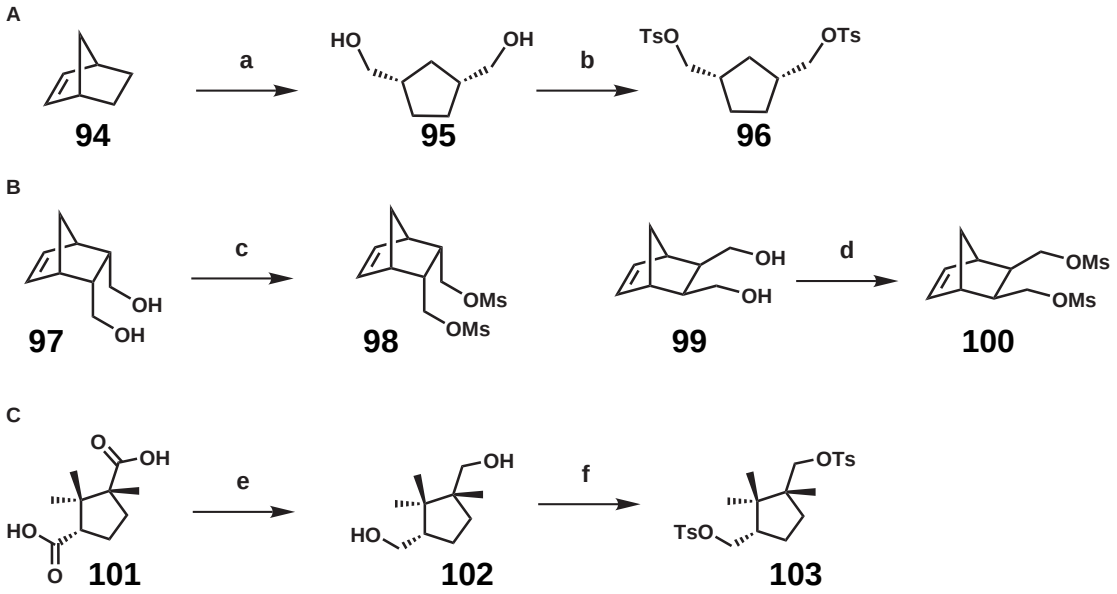
[4]We are convinced that the pyridine nitrogen, being in close proximity to the α-bromoacetamide, reacts with it and creates a charged species. As for the alanine derivative, it has been reported to be recovered untouched under those acylating conditions.[29]



89 **90** **91** **92** **93**

Scheme 2.26. Commercially available electrophilic cores for preliminary studies of thioether library generation

Some effort was invested to create more elaborate bifuctional electrophilic cores. We focused on four simple cyclic molecules (**96**, **98**, **100**, **103**) easily accessible from commercially available reagents (Scheme 2.27). While their reactivity would be significantly lower than that of allylic and benzylic electrophiles presented in Scheme 2.26, their structural features were similar enough to known VD_3 analogues that it seemed reasonable to include them. Bifunctional molecule **96** was created from norbornene via ozonolytic cleavage, followed by reductive work-up with $NaBH_4$. The resulting bis-(hydroxymethyl)cyclopentane **95** was then tosylated under standard conditions to create bifunctional core **96**. Bis-mesylates **98** and **100** resulted from the simple mesylation reaction of their corresponding commercially available alcohols. Finally, we chose to prepare highly congested cyclopentane derivative **103** because of its resemblance to known VD_3 analogues reported by Vandewalle [31] which possess significant binding affinities for VDR, low calcemic activities and antiproliferative activity comparable to calcitriol. The synthesis of this bifunctional electrophile was straightforward and started from inexpensive (+)-camphoric acid. Reduction of the two carboxylic acid groups to alcohols using $LiAlH_4$ and subsequent tosylation of the diol following an improved procedure for the reaction of congested alcohols [32] formed **103** in two steps. Although there were many other structures that were possible, this small assortment was deemed sufficient for a preliminary study.



Scheme 2.27. Synthesis of elaborate bifunctional cores for bis-thioether molecules. *Reagents* : (a) (i) O_3, MeOH, $-78\ ^\circ C$, 1hr. (ii) $NaBH_4$, $-78\ ^\circ C$ to rt, 16hrs, 51 % ; (b) TsCl, pyridine, $0\ ^\circ C$ to rt, 16 hrs, 66 % ; (c) MsCl, Et_3N, CH_2Cl_2, $0\ ^\circ C$ to rt, 90 % ; (d) MsCl, Et_3N, CH_2Cl_2, $0\ ^\circ C$ to rt, 83 % ; (e) $LiAlH_4$, THF/Et_2O, reflux, 4 hrs, 83 % ; (f) TsCl, NMI, Et_3N, chlorobenzene/toluene, $0\ ^\circ C$ to rt, 3 days, 52 %.

2.3 Synthesis of analogues : preliminary studies

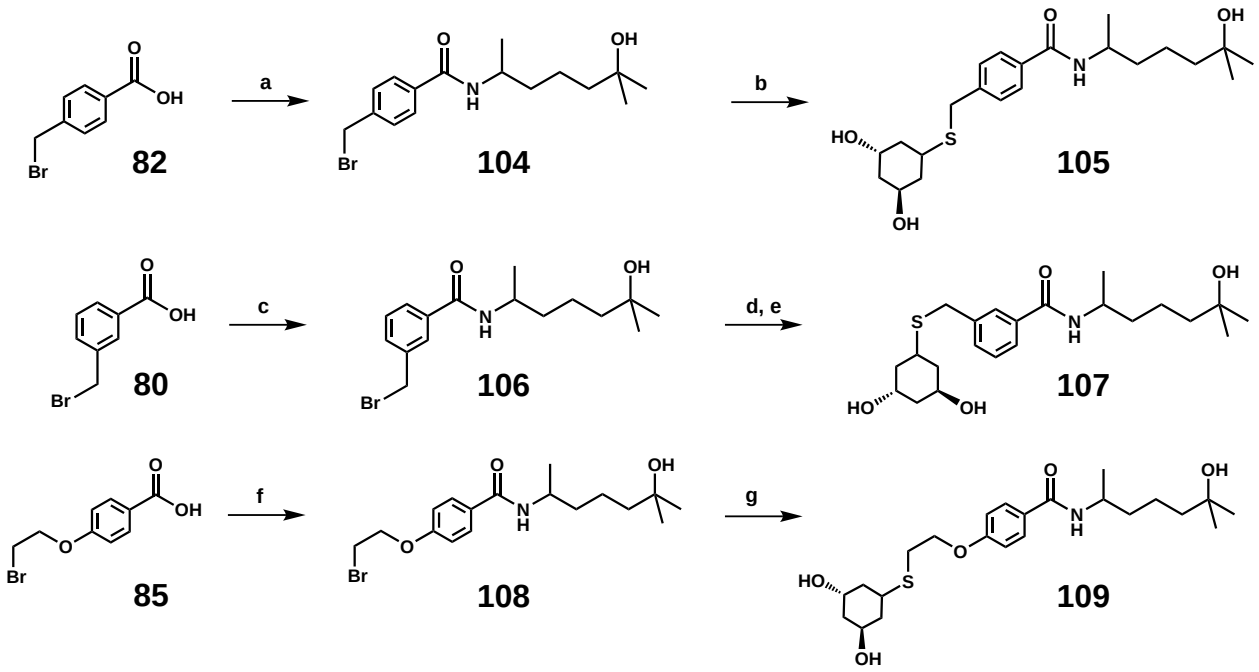
2.3.1 Thioether-amides

Our objective was the creation of large libraries of thioether-amides based on a thioether formation and an amide coupling as the two main reactions. We decided to perform test reactions to know which sequence between thioether formation followed by amide coupling or amide coupling followed by thioether formation would be the best sequence in terms of yield, number of steps, practicality, etc. Also, a shortage of A-ring amine **23**, caused by inconsistent yield of the reduction of oxime **21**, forced us to designed our experiments only using thiols as the source of the A-ring diol.

Amide bond followed by thioether formation

We chose to test the sequence amide coupling followed by thioether formation using bromomethylbenzoic acids **80** and **82** and bromoethoxybenzoic acid **85** (Scheme 2.28) because we wanted to find a way of creating amide bonds in the presence of reactive benzylic bromide moieties and compare the result with amide coupling of somewhat less reactive bromoalkylbenzoic acids. We found that such benzamides were best formed by the reaction of an amine (for instance, heptaminol **78**) and acyl chlorides freshly prepared from carboxylic acids using $(COCl)_2$)/cat. DMF. Under those conditions, amides **104**, **106** and **108** were easily created in greater than 90 % isolated yield. Alkylation of A-ring thiol **43** with the resulting bromomethylamides proceeded moderately well using Et_3N as base in MeOH, with typical yields around 25 %. Such low reaction yield could not be increase significantly by the use of stronger bases such as DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) or K_2CO_3. It seems that the already observed low nucleophilicity of this thiol is the main cause for a general lack of reactivity. Alkylation of A-ring thiol monoTBS ether **119**[5] with the meta-bromomethyl benzamide **106** proceeded in similar yield to give thioether amide **107**. By this two-step route, thioether-amides **109**, **105** and **107** were obtained and their biological activity is presented in section 2.4.

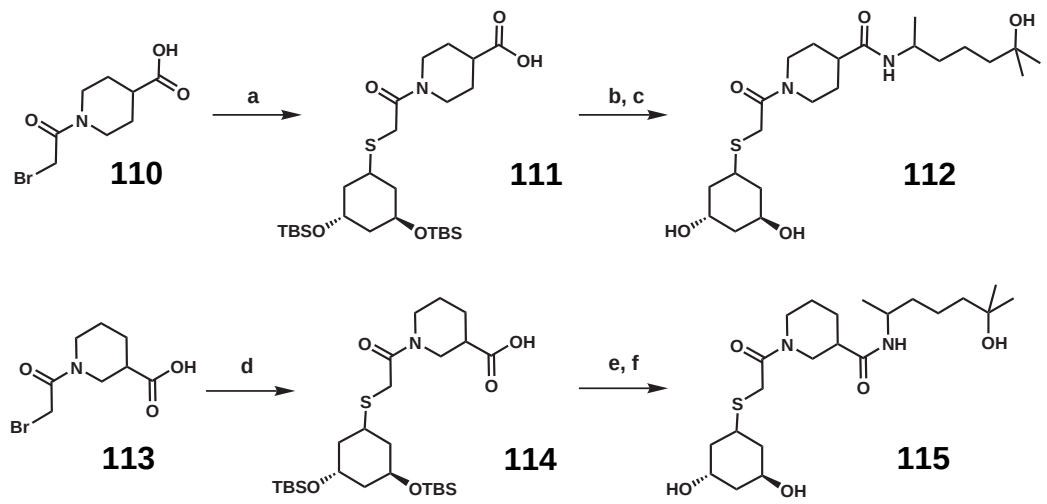
[5]This monoTBS-protected A-ring thiol **119** comes from decomposition of fully protected A-ring thiol **24** which sees one of its TBS ether hydrolyze over time when stored at room temperature. To test its reactivity and reuse this precious thiol, we used it in this test reaction with great success.



Scheme 2.28. Synthesis of the thioether-amide VD_3 analogues, testing amide-thioether sequence. *Reagents* : (a) (i) $(COCl)_2$, cat. DMF, CH_2Cl_2, $0\ ^\circ C$ to rt, 2hrs ; (ii) heptaminol.HCl, DIPEA, rt, 16hrs ; (b) A-ring thiol **43**, K_2CO_3, MeOH, rt, 2 days, 35 % over two steps ; (c) (i) $(COCl)_2$, cat. DMF, CH_2Cl_2, $0\ ^\circ C$ to rt, 2hrs ; (ii) heptaminol.HCl, DIPEA, rt, 16hrs ; (d) monoprotected A-ring thiol **119**, Et_3N, CH_3CN, rt, 2 days, 23 % over 2 steps ; (e) conc. HCl, MeOH, rt, 78 % (f) (i) $(COCl)_2$, cat. DMF, CH_2Cl_2, $0\ ^\circ C$ to rt, 2hrs ; (ii) heptaminol.HCl, DIPEA, rt, 16hrs ; (g) A-ring thiol **43**, K_2CO_3, MeOH, rt, 2 days, 27 % over two steps.

Thioether formation followed by amide bond

We tested the sequence starting with thioether formation followed by amide coupling using bromoacetamide acids **110** and **113** (Scheme 2.29). Thiol alkylation proceeded as described previously with Et_3N as base in MeOH, with 24 % and 83 % respective yields of thioethers **111** and **114**. The high yield of thioether **114** stands out as it is the only reaction of A-ring thiol alkylation with such a high success. We could not find a rationalization for this observation. Amide couplings of these carboxylic acids with heptaminol **78** using HBTU/HOBt in DMF followed by acid cleavage of the TBS ether gave thioether-amides **112** and **115** in 6.9 % and 32 % yield respectively). The biological activity of these substrates is presented in section 2.4.

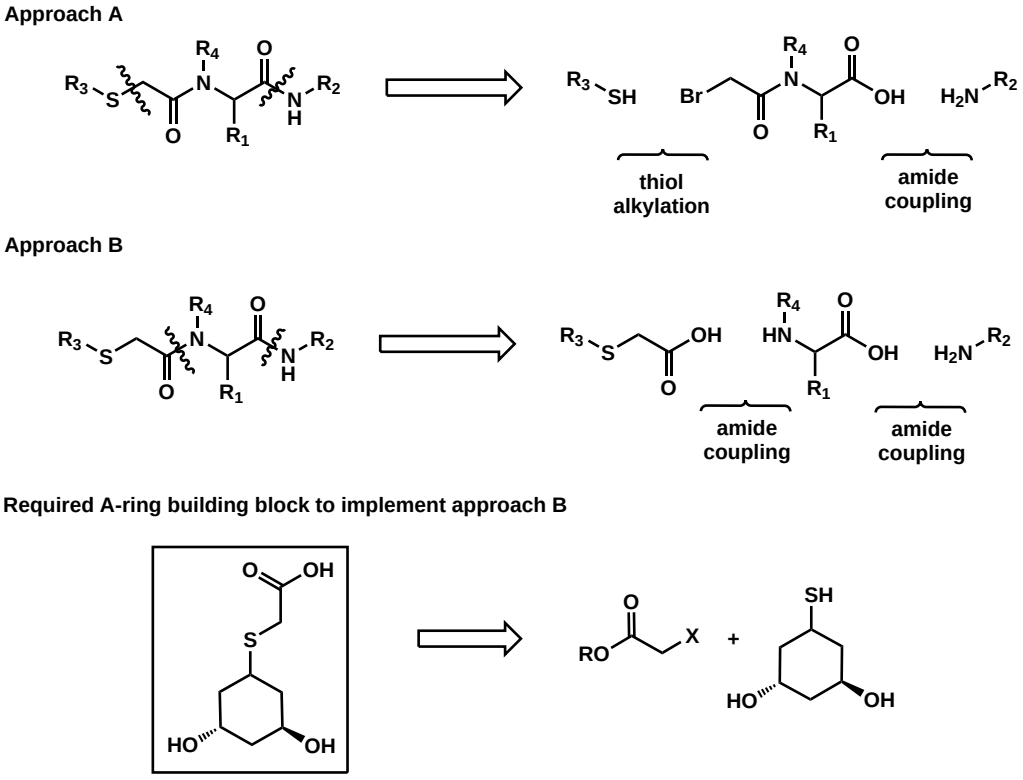


Scheme 2.29. Synthesis of the thioether-amide VD_3 analogues, testing thioether-amide sequence. *Reagents* : (a) Bis-protected A-ring thiol **24**, DIPEA, MeOH/$CHCl_3$, rt, 2 days, 22 % ; (b) heptaminol hydrochloride, DIPEA, HOBt, HBTU, DMF, $0\ ^{\circ}C$ to rt, 2 hrs, 7 % ; (c) conc. HCl, MeOH, rt, 1 hr, 45 %. (d) Bis-protected A-ring thiol **24**, DIPEA, MeOH/$CHCl_3$, rt, 2 days, 83 % ; (e) heptaminol hydrochloride, DIPEA, HOBt, HBTU, DMF, $0\ ^{\circ}C$ to rt, 2 hrs ; (f) conc. HCl, MeOH, rt, 1 hr, 39 % over two steps.

Double amide bond formation using A-ring thiol as a carboxylic acid derivative

In the previous section, we have mentioned how A-ring secondary thiols (e.g. thiols **43** or **119** or **24**) gave low alkylation yield. This was viewed as unacceptable for an eventual parallel synthesis. We reasoned that if we modified slightly the building blocks, it would be possible to assemble the thioether-amides using amino acid cores via a different route. Shown in Scheme 2.30 are two possible retrosynthetic analyses of the thioether-amides VD_3 analogues. Approach A shows the disconnection described above with thiol alkylation and amide formation. Approach B replaces the thiol alkylation by an amide coupling reaction. The resulting required building blocks are then an A-ring carboxylic acid and an amino acid (shown at the bottom, Scheme 2.30). It should be possible to produce an A-ring carboxylic acid from straightforward alkylation of an A-ring thiol with an α-haloester or α-halocarboxylic acid. The higher reactivity of these electrophiles would presumably result in more acceptable yields.

To test this approach, we decided to synthesize thioamide **112**, already created earlier (see section 2.3.1), in order to compare its synthesis using different methods. We also



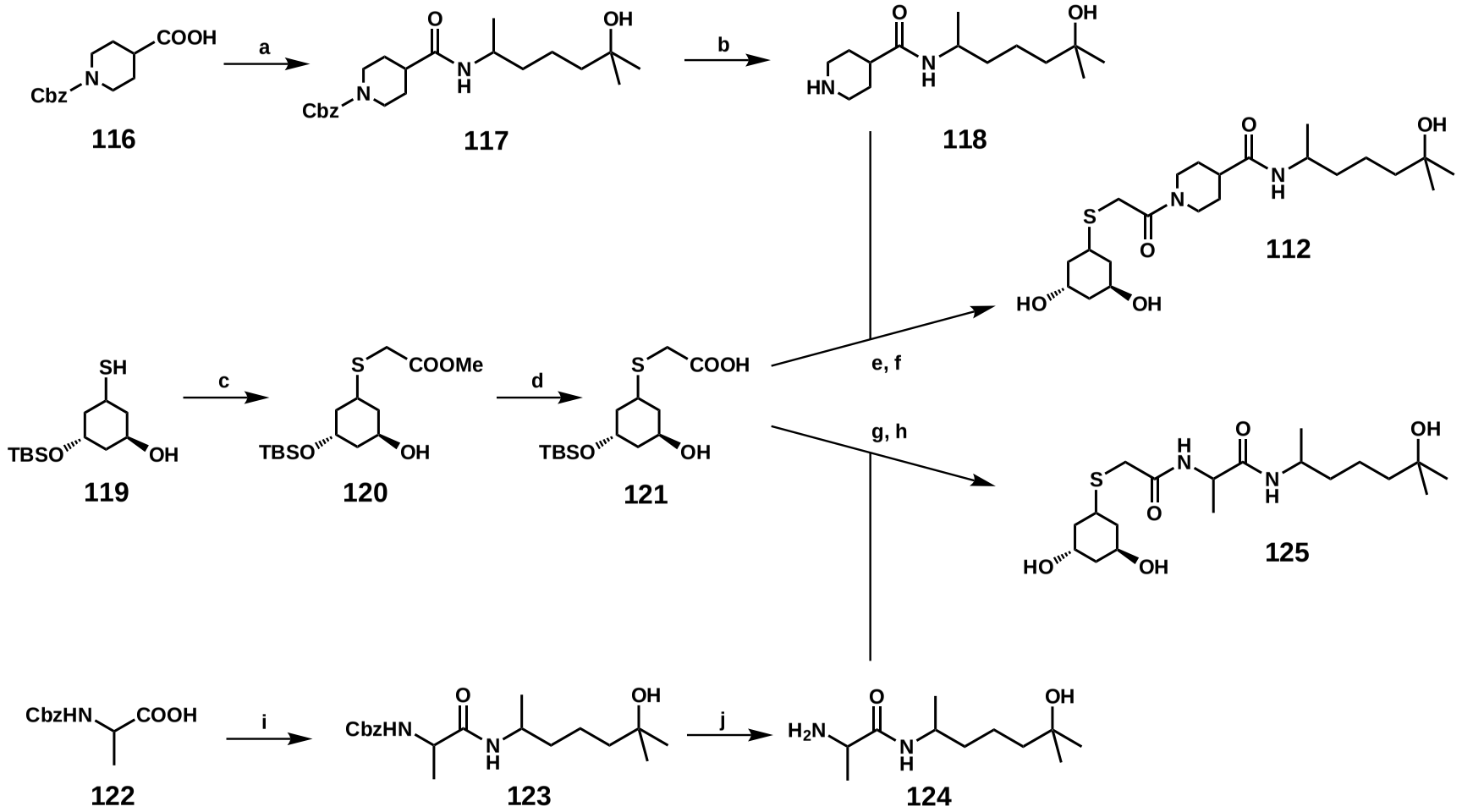
Scheme 2.30. New retrosynthetic analysis of thioether-amides.

set our eyes on an alanine-based core fragment, as we had been unable to produce an α-bromoacetamide from alanine.

We started our investigation by transforming A-ring thiol **119** into a carboxylic acid (Scheme 2.31) by alkylating the thiol with methyl 2-bromoacetate in 77 % yield. The use of a large excess of the very active alkylating agent and long reaction times helped in affording a good yield. Subsequent ester hydrolysis was efficient (87 %) and thus the two-step sequence was high-yielding.

To complete our test, we had to see how easy and efficient was the completion of the thioetheramide VD_3 analogues. For this, we used N-protected amino acids N-Cbz-4-piperidine carboxylic acid and N-Cbz-Alanine. To both, we coupled top chain amine heptaminol **78** using standard coupling conditions. Following suitable deprotection reactions, we were ready to couple A-ring acid **121** to these amines. Both couplings worked with limited success, affording thioether amides **112** and **125**, in respective overall yields of 2.0 % and 12 %. These yields are similar to the 6.9 % yield of thioether-amide **112**

obtained earlier (Scheme 2.29) using a different reaction scheme. Both methods are thus low-yielding but remain possible avenues for the creation of VD_3-like analogues.



Scheme 2.31. Synthesis of the thioether-amide VD_3 analogues, testing A-ring derivatization-amide-amide sequence. *Reagents* : (a) heptaminol hydrochloride, DIPEA, HOBt, HBTU, DMF, $0\ ^{\circ}C$ to rt, 2 hrs ; (b) 10 % Pd/C, H_2, MeOH, rt, 24 hrs, 45 % over 2 steps ; (c) Methyl α-bromoacetate, Et_3N, CH_3CN, rt, 2 days, 77 % ; (d) aq. NaOH, MeOH/THF, 2hrs, rt, 87 % ; (e) DIPEA, HOBt, HBTU, DMF, $0\ ^{\circ}C$ to rt, 2 hrs, 15 % ; (f) conc. HCl, MeOH, rt, 30 min., 45 % ; (g) DIPEA, HOBt, HBTU, DMF, $0\ ^{\circ}C$ to rt, 2 hrs, 75 % ; (h) conc. HCl, MeOH, rt, 30 min., 66 % ; (i) heptaminol hydrochloride, DIPEA, HOBt, HBTU, DMF, $0\ ^{\circ}C$ to rt, 2 hrs ; (j) 10 % Pd/C, H_2, MeOH, rt, 24 hrs, 35 % over two steps.

2.3.2 Bis-thioethers

We had in mind that new VD_3 analogues needed to be accessed through easy, simple chemistry. The reaction of bis-electrophilic molecules such as bis-(bromoalkyl)benzenes or bis-(bromoalkyl)cycloalkanes with thiols to form unsymmetrical VD_3-like bis-thioethers meets these criteria. Of course, as discussed earlier, thiol alkylation is sometimes less efficient than required, particularly for our A-ring secondary thiol. Nevertheless, we envisioned that a static library of bis-thioethers could be rapidly put together and would quite possibly scan a large proportion of the possible chemical diversity available to novel, non-secosteroidal VD_3 analogues.

To establish whether we could accomplish rapid synthesis and efficient purification of the complex mixtures arising from the reaction multiple thiols with bis-electrophiles, we tested our idea using 4 thiols (3 top chain thiols + 1 A-ring thiol equivalent) and 5 bis- or tris-(bromoalkyl)benzenes generating a 20-member library constructed in parallel (Scheme 2.32). We purposely chose to conduct those experiments using only one thiol per reaction, thus avoiding the formation of mixtures of VD_3-like bis-thioethers. This decision was motivated by that fact that during our preliminary studies, we specifically wanted to test the feasibility of the overall process of synthesis and purification in as little time as possible. The creation of complex mixtures of symmetrical and unsymmetrical bis-thiothers, while potentially more fruitful in terms of VD_3-like molecules, comes with more elaborate and time-consuming purification procedures.

We conducted those 20 experiments in a parallel fashion, mixing stock solutions of thiols, alkylbromides to K_2CO_3 suspended in MeOH in screw cap vials and under argon (Figure 2.3). In this figure, one can see the reaction vessels (vials) prior to being sealed in a Ziploc bag filled with Ar (picture A). Monitoring the reactions was done using analytical thin-layer chromatography (picture B) and isolation of crude product was done by liquid-liquid extractions, vial by vial, followed by batch-drying in dessicators under argon and then vacuum (picture C). Purification of crude mixtures was finally done using preparative thin-layer chromatography (picture D) but complemented by semi-preparative HPLC when necessary.

When looking at Scheme 2.32, one can immediately see that the reaction yield values

are rather widely spread, ranging from 11 to 75 %, with an average of 47 %. The main cause is the thiol-to-disulfide oxidation that occurred in some cases in spite of all precautions. This unwanted oxidation reduced the amount of thiol present to react with the electrophiles.

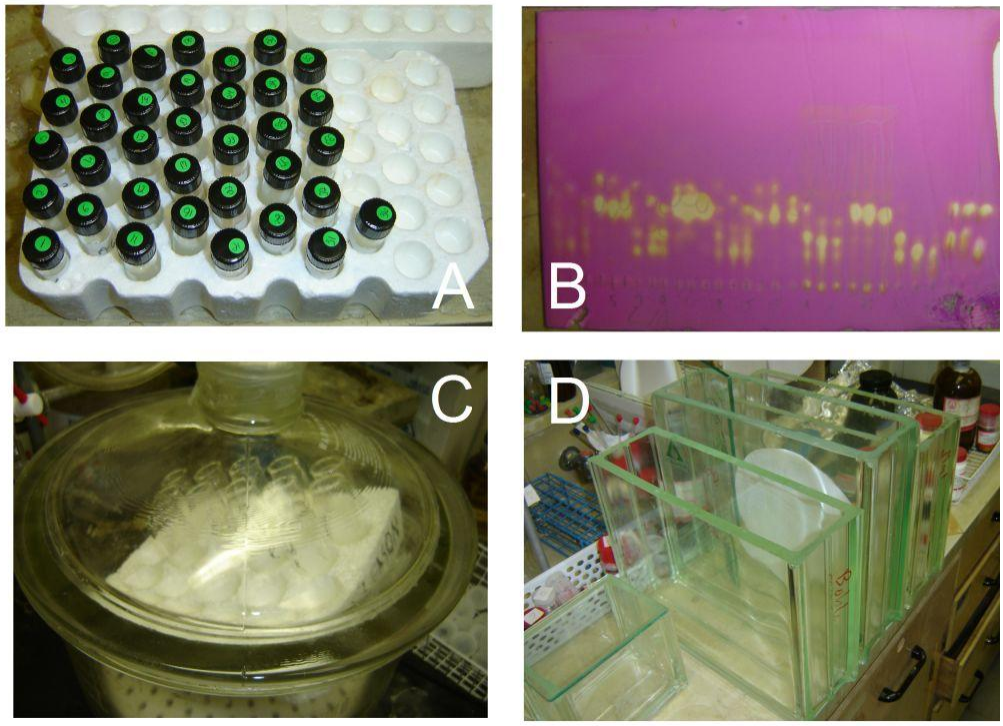
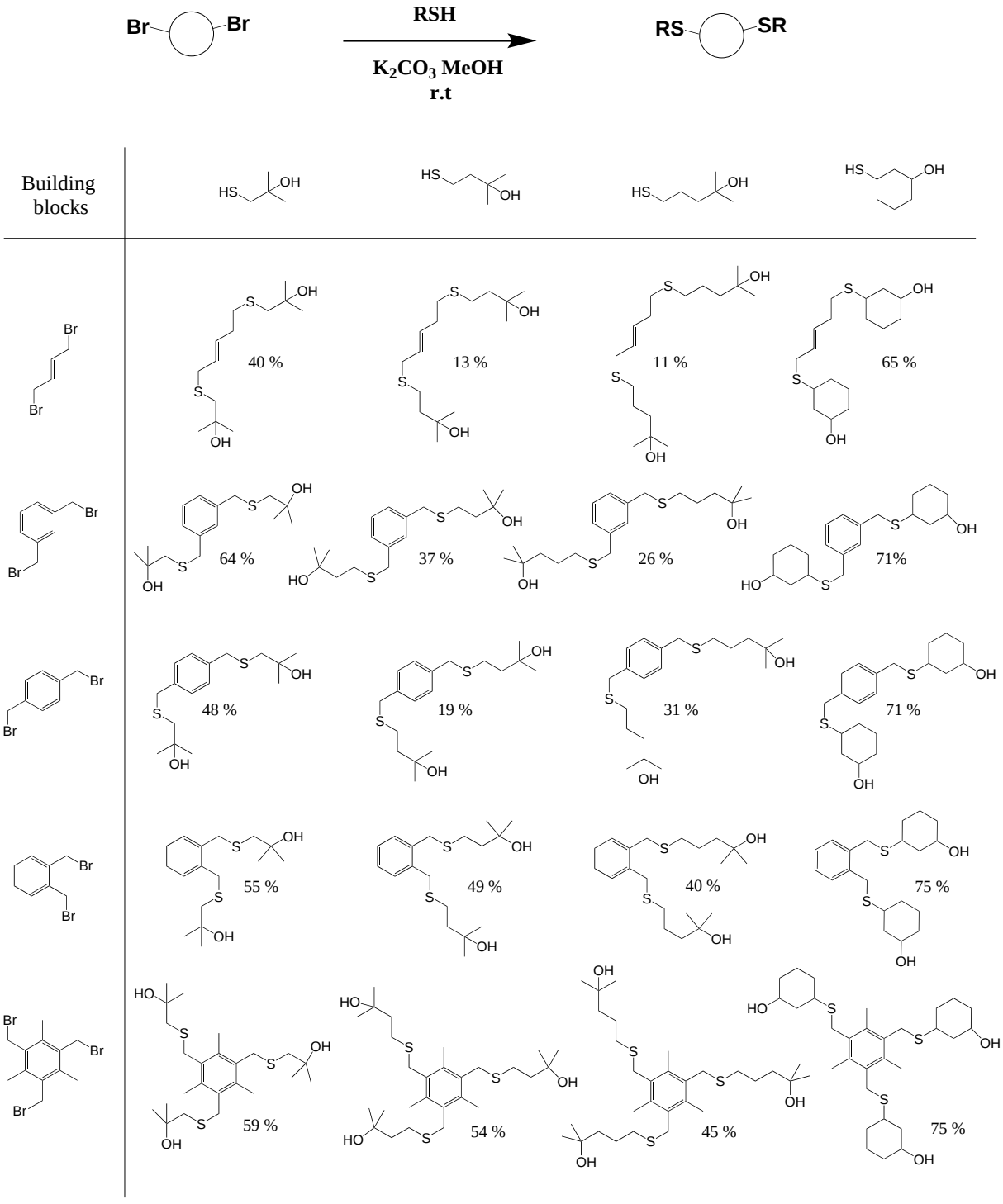


Figure 2.3. Process of parallel synthesis of the bis-thioether library

Of course, as already mentioned, this rather odd-looking library was not designed to be highly VD_3-like, with only symmetrical bis-thioethers being generated. However, with this simple set-up, we have tested our ability to perform parallel synthesis and rapid purification of this category of substrates and this exercise laid the ground for future focused static libraries of bis-thioethers.



Scheme 2.32. Synthesis of the symmetrical bis-thioether analogues. Conditions : dibromide (1 equiv), thiol (4 equiv), K_2CO_3 (10 equiv), MeOH, rt, 3 days.

2.4 Biological tests and results

CYP24 induction assays

CYP24 is the enzyme that catabolizes the hormonally active form of vitamin D_3 (1,25D). In effect, when 1,25D levels are high, CYP24 activation brings vitamin D levels back to homeostatic concentrations (through a feedback loop). One very simple way we can test the vitamin D analogues for agonism is by assaying for levels of CYP24 gene expression *in vitro*. If CYP24 levels are comparable in both analogues and 1,25D treatment, then we know we have a hit. The assay is a black-and-white detection method for 1,25D agonism. In this type of assay, if a molecule is a VDR agonist, one should expect to see a white band corresponding to expressed CYP24 (in the black region of the CYP24 27x test, see Figure 2.4). If a molecule does not bind to VDR, no band is visible. This is unfortunately what we observed for the 6 analogues tested[6] (5 made by the author, **112** labeled 1264, **109** labeled 1200, **105** labeled 1199, **107** labeled 1257, **125** labeled 1261 and 1 (labeled 78) made in collaboration with a coworker, Jean-François Lacroix). It would have been quite surprising to find a hit among a set of 6 molecules randomly chosen for the synthetic preliminary studies.

2.5 Summary

In summary, these preliminary studies on the synthesis of analogues of calcitriol through the use of conventional chemistry proved particularly fruitful. Rapidly, we could elaborate the design of possible libraries of non-secosteroidal VD_3 analogues. We have synthesized the required building blocks, while maximizing their potential as useful synthons in other projects. Along the way, we tested the chemistry involved and the potential procedures by making some analogues which were analyzed for VDR agonism. Finally, these results are left in the hands of another coworker for the implementation of the libraries.

Realizing that medicinal chemistry is a long process when trying to explore without any actual idea of structure-activity relationship (SAR), we decided to switch to dynamic

[6]These assays were performed by Ari J. Bitton, a collaborator from John H. White's laboratory, at McGill University.

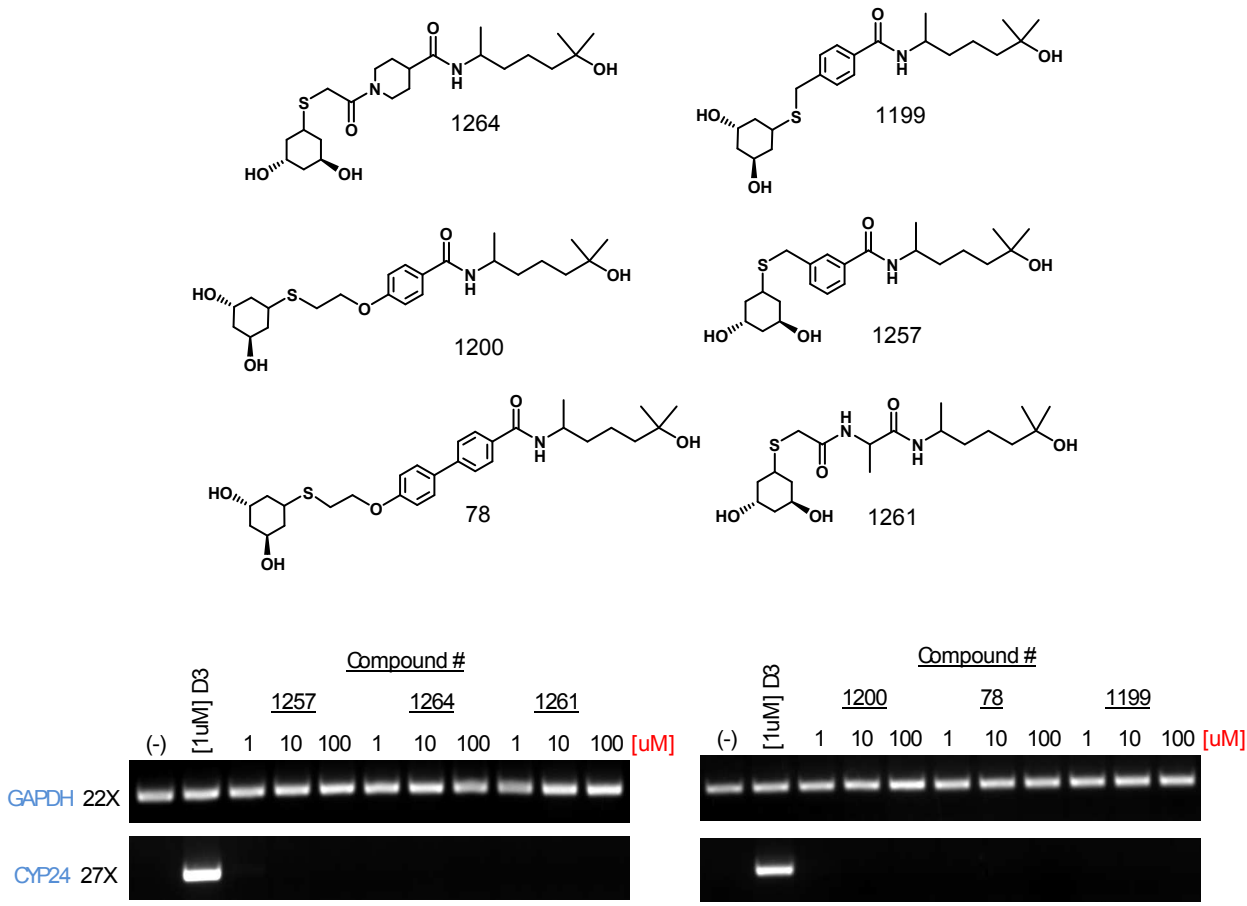


Figure 2.4. CYP24 induction assays on thioether-amide analogues

combinatorial chemistry as a possibly faster and more efficient method of creating VD_3 analogues.

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CHAPTER 3

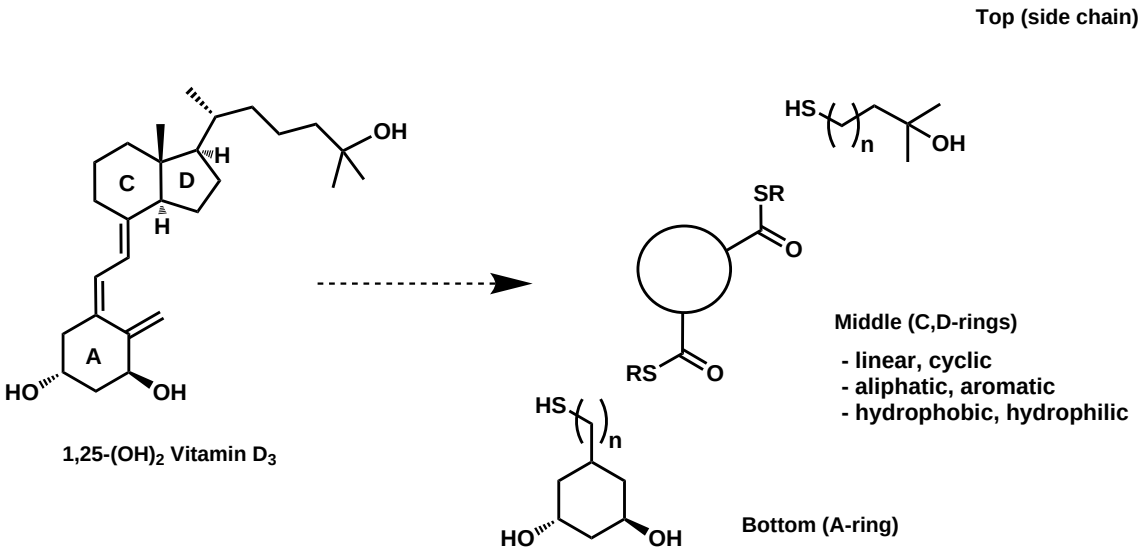
Dynamic Libraries of Vitamin D_3 Analogues Based on Transthioesterification

3.1 Introduction

Dynamic combinatorial chemistry (DCC) has developed into a unique tool in the search for bioactive molecules. In order to find molecules possessing 1α,25 $(OH)_2$ vitamin D_3 anticancer properties but lacking its calcemic activity, we planned the creation of a library where the chemical and structural diversity would be generated via dynamic exchange of suitably chosen building blocks. Submission of the library to the presence of the vitamin D receptor (VDR) would influence the synthetic equilibrium and thus possibly reveal the best binders via an increase of their concentrations when compared to a control dynamic library without the receptor.

Keeping with our goal of simplifying the core portion of 1α,25 $(OH)_2$ vitamin D_3, we decided to separate VD_3 in three parts (Scheme 3.1) : top chain mimics that include a tertiary OH group capable of H-bonding, an A-ring equivalent bearing two hydroxyl groups and a variable middle portion mimicking the C,D-rings core. Considering all the known reversible connections that have been employed in dynamic combinatorial libraries, we opted for thiol-thioester exchange for reasons discussed below. Thus, our setup would have the first two parts (top chain and bottom ring) as thiols, while the variable middle piece

would be a bis-thioester, thus representing the center of a two-point-of-exchange library. These key components could potentially assemble themselves into a myriad of diverse molecules, some of them having vitamin D_3-like structures.



Scheme 3.1. Breaking down of calcitriol into fragments suitable for dynamic combinatorial libraries based on thiol-thioester exchange

The reasons behind our choice of the thioester functionality were simple. First, the building blocks were easily accessible. We considered DCC as a way to shorten the time between the idea occurring event and actual experimental testing. Being able to buy commercially available building blocks (such as diacids for center pieces or thiols for the top and botton portions) or to simply pick it off the shelf because of its commonness seemed a great advantage. In addition, and unlike some other reversible linkages, the thioester exchange was expected to work under mild conditions compatible with the vitamin D receptor.

Second, thioesters offered structural diversity. There was the possibility that the overall 3-dimensional structures of potential dithioesters arising from a dynamic combinatorial library (DCL) based on thioester exchange might be different than those from bis-disulfide or bis-imine molecules. It was both difficult to predict and possibly irrelevant to match closely the structure of the natural ligand for the receptor. What was certain was that the various would-be center cores of our library members, dicarboxylic acids, were commercially available in extremely diverse substitution patterns, lengths, compositions, etc.

Finally, there was the novelty argument. At the onset of our research, only a few articles described the use of thioester exchange in a DCL context. As of 2008, thioester linkage had been used far less often than other reversible covalent connections. For example, it had appeared in less than 4 % (only 7 articles [1–6]) out of the 204 published on dynamic combinatorial chemistry) compared to 25 % for the disulfide exchange and approximately 20 % each for the transimination and the hydrazone exchange. Since then, at least three more articles have appeared[7–9], but the total number remains low.

3.1.1 Thioester exchange

The transthioesterification reaction is the sulfur analogue reaction of the transesterification. The former occurs more readily under slightly basic aqueous conditions than the latter. It is reversible at room temperature, in water, at physiological pHs. The typical rate of exchange is faster when the pH is above 7, but slows down when it is below 5, thus making the process stoppable. Mechanistically, it involves the base-catalyzed reaction between a thiol nucleophile and a thioester derivative (see Scheme 3.2). As already mentioned, the transthioesterification reaction has been successfully implemented as a convenient and efficient route to dynamic libraries in a few occasions. Furthermore, being a planar functional group, the thioester provides a linkage which structurally mimics, and is subsequently replaceable by, the more robust amide or ester functionalities.

Scheme 3.2. Thiol-thioester exchange

Thioester exchange in DCL was first introduced by Ramström and coworkers in 2004 when they demonstrated that acetylcholinesterase catalysis could be used as a mean to self-screen thioester substrates from a dynamic combinatorial library.[1] For the first time, thioester exchange of very simple components (based on aliphatic acyl chains and two aliphatic primary thiols) was shown to be able to generate dynamic libraries compatible with mild conditions in aqueous media (Scheme 3.3). They were not interested in characterizing the library composition at equilibrium but were probing the specificity of acetylcholinesterase. In a complementary study published in 2005 [2], they made key observations that are important to application of thioesters in other systems. First, branched acyl

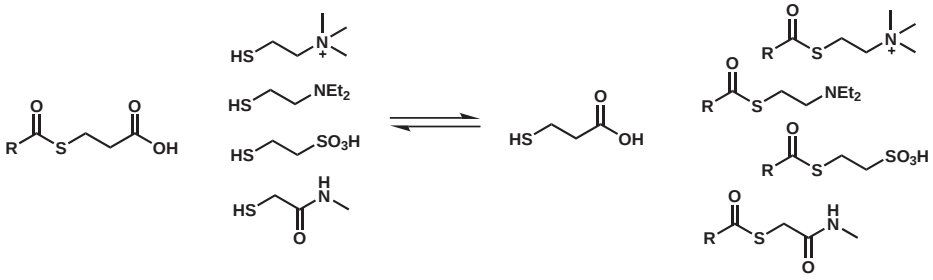
groups reduce the exchange rate of the libraries, the carbonyl group being more sterically hindered than linear acyl groups. Second, secondary thiols are competent partners, albeit showing slower kinetics or unfavorable equilibria. Third, the rate of transthioesterification can be increased for less reactive thiols by increasing the basicity of the solution. However, this also increases competing and unproductive hydrolysis of the thioesters. For example, undesired thioester hydrolysis in the absence of any protein is more pronounced at pH 8.0 (350 % more, after 3 days) or at pH 9.0 (500 % more, after 3 days) than at pH 7.0.

In 2005, Sanders, Otto and coworkers demonstrated the simultaneous use of disulfide and thioester linkages for library generation (Scheme 3.3).[3] Although they elegantly showed that the two reversible connections are communicating with each other (i.e. the overall equilibrium is governed by the thermodynamics of the disulfide exchange AND the thioester exchange), they did not use a biological target to influence their library composition.

Transthioesterification was used again in 2004 [4], 2006 [5, 6] and 2007 [10] by Gellman et al. to compare conformational stabilities of proteins via backbone thioester exchange (BTE) (Scheme 3.3). BTE is implemented by replacing a single backbone amide linkage in a polypeptide with a thioester (i.e., a single R-amino-acid residue is replaced by an R-thio-acid residue). Conformational stability can then be probed by monitoring equilibration between a full-length molecule and peptide fragments that cannot form a complete tertiary core via a thioester-thiol exchange process. The equilibrium constant (K_{BTE}) provides insight on the free energy of tertiary structure formation. Although these examples are not very similar to our design, they still represent the generation of a library (albeit very small) using transthioesterification.

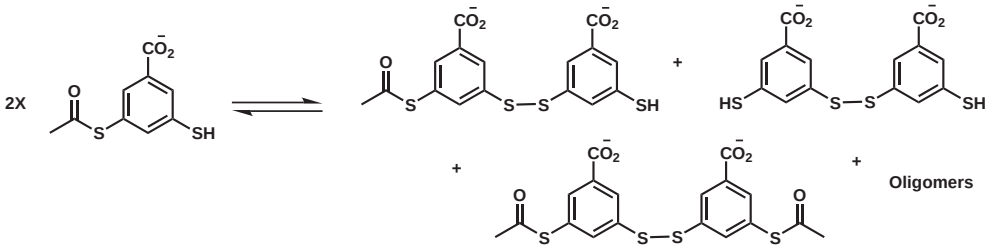
Ghadiri and coworkers made a very clever use of thioester exchange for the preparation of self-assembling sequence-adaptive peptide nucleic acids, as explained in a Science article published [7] and highlighted [11] in 2009 (Scheme 3.3). The concept is simple : an oligo-dipeptide backbone made of any amino acid alternated with cysteine is incubated with nucleic acids functionalized as thioesters. Very quickly, thioester peptide nucleic acids (tPNAs) are formed and they undergo dynamic sequence modification in response to the pressure caused by changing templates (DNA or RNA of certain sequences) in solution. The oligomers specifically self-paired with complementary tPNA strands and cross-paired with RNA and DNA in Watson-Crick fashion.

Ramström

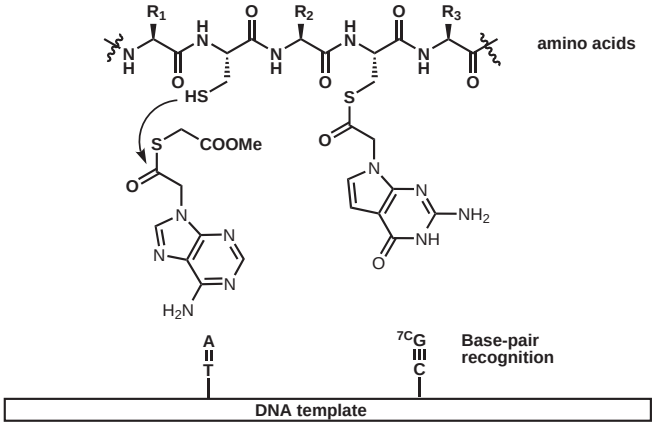


Gellman

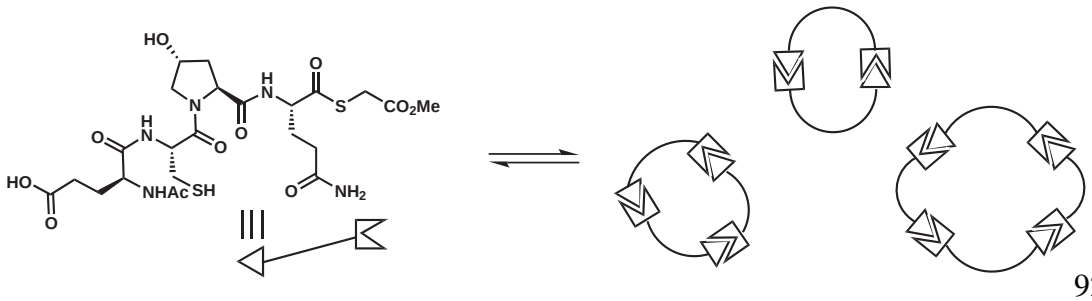
Sanders and Otto



Ghadiri



Waters/Gagné



Scheme 3.3. Examples of thioester exchange in literature

Until recently, all reported cases of thioester exchange in DCC used thioesters that were unsubstituted at the alpha-position, which would significantly limit the diversity of structures possible in a library. Waters and Gagné decided to assess the situation and used thiol-thioester exchange to generate libraries of cyclic thiodepsipeptides under thermodynamic control (Scheme 3.3).[8] During their studies with small tetrapeptide thioesters, they observed that bulkier side chains were slowing the rate of exchange at the carbonyl next to them. For example, a thioester group on glycine (unsubstituted at the α-position) underwent complete exchange within 3-4 hours, during which time a corresponding thioester group on valine (substituted at the α-position by an isopropyl group (iPr)) had exchanged to the extent of only about 5 % (and only 20 % complete after 20 hours). Although other factors might influence the results just presented, they certainly point at the slower exchange of α-substituted thioesters. They also found that the macrocyclic thiodepsipeptides were hydrolyzed over extended periods, but not before equilibrium was reached (about 18h). This seems to correspond well with the observations made by Ramström that hydrolysis was present and took some time to represent a real problem. Finally, no acylation of lysine side chain-NH_2 occurred at pH 6.75.

The thioester exchange examples presented above showed the wide variety of research that can be explored successfully using this linkage. In the development of DCL of VD_3 analogues, there are many issues that need to be addressed, such as thioester stability (hydrolysis, interaction with the amino acid residues of the target) and rate of exchange. The first step in examining the suitability of thioesters was to prepare a variety of VD_3 mimics.

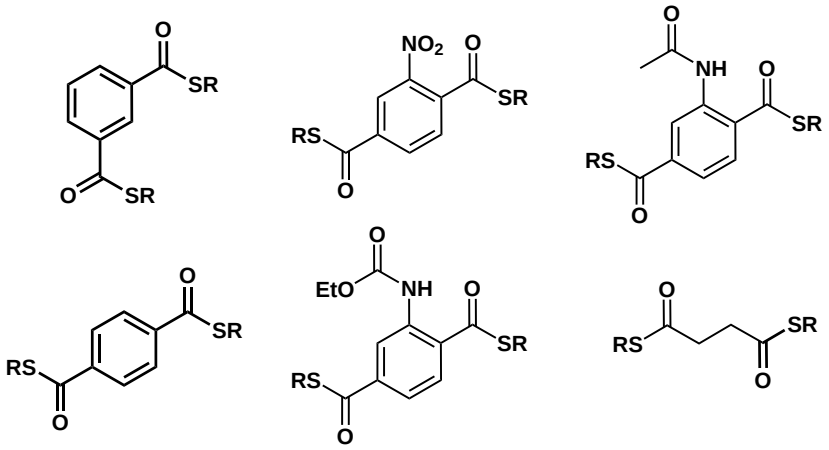
3.2 Synthesis of building blocks and library members

Ideally, building blocks should be simple, inexpensive to synthesize and straightforward to characterize. Also, the design of building block scaffolds should include the incorporation of solubilizing groups and chromophores. Analyzing the composition of a library is frequently the most challenging aspect of DCC, and therefore all building blocks should ideally have unique masses and/or spectroscopic signatures. With these criteria in mind, we finally based our choice of cores on mainly three things. First, there should be structural and functional diversity in a large scale library. Second, thioester cores should be able to *somewhat* match 1,25D structure in terms of spatial arrangement and length between important H-bonding functional groups. Third, thioester cores should be easily created and

manipulated.

In accordance with these criteria, we initially chose to examine an aromatic core based on isophthalic acid.Following the initial studies, we could consider other aromatic cores based on terephthalic acid bearing substituents, such as nitro, N-ethoxycarboxyl or acetamido groups (Scheme 3.13). A third option to increase diversity would be to create dithioesters from aliphatic cores although these would be more problematic for analysis due to the lack of a chromophore.

The building blocks that we decided to create are described below and a summary is presented in Scheme 3.4.



Scheme 3.4. Examples of potential dithioester center cores

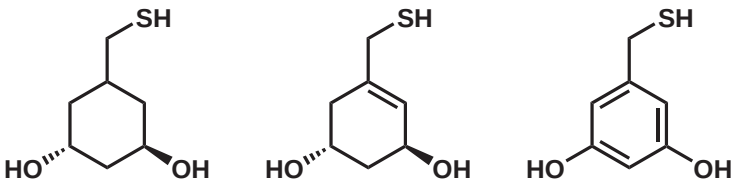
3.2.1 Synthesis of thiols

Our previous work on static VD_3 analogues (chapter 2) had provided us with secondary A-ring thiol (1S,3S)-5-mercaptocyclohexane-1,3-diol (MCD) **43** and all of the side chain thiols we needed. However, we desired additional A-ring thiols to maximize a best fit with C,D-ring cores of varying sizes and lengths.

Primary thiols

One A-ring structure that was desirable was one possessing a primary thiol, as these were expected to be better nucleophiles in thioester exchange than secondary thiols. We had

three different molecules in mind : **126**, **127**, and **128** coming from very diverse synthetic routes.



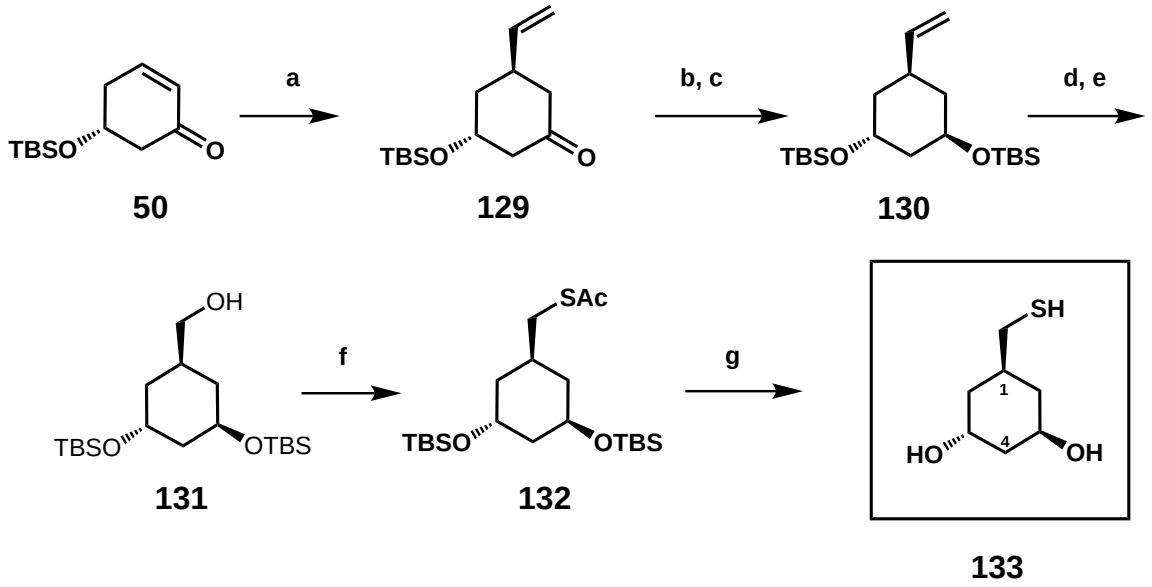
126 **127** **128**

Scheme 3.5. Primary thiols as building blocks for dynamic libraries

Primary thiol via conjugate addition and directed reduction For thiol **126**, our idea was to perform a conjuguate addition of a vinyl-cuprate species onto (R)-5-tert-butyldimethyl silanyloxy-2-cyclohexenone **50** (Scheme 3.6). Further chemical transformations would allow us to obtain a primary thiol possessing the much-needed 1,3-diols mimicking the A-ring of 1α,25-$(OH)_2$ vitamin D_3. We started from enone **50** and allowed it to react with vinyltributyltin and higher order cyanocuprate $Bu_2Cu(CN)Li_2$ in an *in situ* formation of a higher order vinyl cuprate species as reported by Campbell and Lipshutz.[12] In less than 4 hrs, we could isolated vinylketone **129** in 71 % yield. Careful analysis of the crude mixture indicated that some butyl group addition on our starting enone lowered the yield. Although it did not matter in our situation (the final product primary thiol **133** has an axis of rotation going through C-1 and C-4 and is thus C_2 symmetric, Scheme 3.6), NMR analysis showed that the product was coming at more than 95 % from trans addition. This is consistent with prior observations by Sato et al.[13]

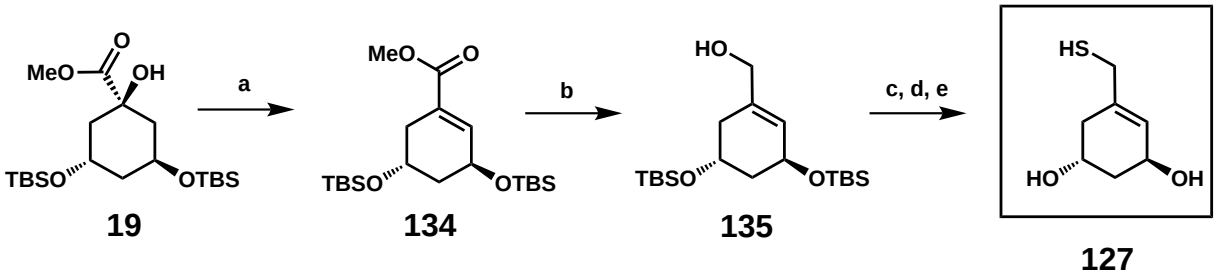
Transformation of ketone **129** into bis-protected diol **130** was accomplished via chemistry mentioned previously in the conversion ketone **51** into thiol **24**. First, the ketone was reduced with $NaBH_4$ and the resulting 1:1 mixture of alcohols was protected as silyl ethers using TBSCl, before being separated via flash chromatography. The identification of the anti diol compound was accomplished by NMR techniques and comparison of the spectra with previously synthesized molecules. Next, the oxidation of the vinyl moiety into a primary alcohol was accomplished in two steps. First, ozonolytic cleavage of the double bond to an aldehyde which was then reduced with $NaBH_4$ to afford a primary alcohol. Second, a Mitsunobu reaction with thioacetate as the nucleophile gave thioester **132**. Finally, primary thiol **133** was isolated after deprotection of the thioester group and the two TBS groups

using hydrazine for the former and HCl in methanol for the latter.



Scheme 3.6. Formation of primary thiol **133** via vinyl cuprate conjugate addition onto enone **50** : (a) $Bu_2CuCNLi_2$, $Bu_3SnCHCH_2$, $-65\ ^\circ C$, 5 min., 45 %; (b) $NaBH_4$, MeOH, $0\ ^\circ C$, 2 hrs, 94 % (mixture of 2 alcohols) ; (c) TBSCl, imidazole, Et_3N, DMF, $0\ ^\circ C$ to rt, 10 hrs, 67 % (3 steps); (d) (i) O_3, CH_2Cl_2, -78$^\circ C$, 45 min. : (ii) PPh_3, rt, 16 hrs.; (e) $NaBH_4$, MeOH, THF, $0\ ^\circ C$ to rt, 2 hrs.; (f) PPh_3, DIAD, AcSH, THF, $0\ ^\circ C$ to rt, time, 55 % (3 steps); (g) (i) N_2H_4, MeOH, rt, 2 hrs (ii) conc. HCl, MeOH, rt, 1 hr, 90% (2 steps).

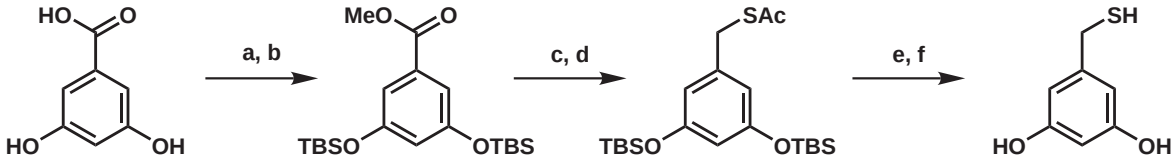
Primary thiol from quinic acid Our prior experience working with D-(-)-quinic acid as a source of the cyclohexane anti diol suggested an easy access to another primary thiol building block. It made use of the hydroxyester **19** prepared on our way to A-ring amine (Chapter 2). Employing a dehydration method already published for the synthesis of quinic and shikimic acid derivatives[14], we knew that hydroxyester **19** was susceptible to undergo elimination of the OH moiety and yield enoate ester **134** (Scheme 3.7). When we conducted the reaction using $POCl_3$ in pyridine, we were pleased to see that elimination reaction was clean and gave enoate **134** in high yield. Ester reduction was accomplished using $LiAlH_4$ and installation of sulfur atom was done via the Mitsunobu reaction as before. Relevant deprotection procedures followed and primary thiol **127** could be isolated in 9 steps overall from D-quinic acid.



19 **134** **135** **127**

Scheme 3.7. Formation of primary thiol **192**. *Reagents* : (a) $POCl_3$, pyridine, $70\ ^\circ C$, 16 hrs, 95 % ; (b) DIBALH, THF, $0\ ^\circ C$ to rt, 16 hrs, 73 % ; (c) PPh_3, DIAD, AcSH, THF, $0\ ^\circ C$ to rt, o/n, 63 %; (d) 2 M HCl, MeOH, $-25\ ^\circ C$, 24 hrs, 72 %; (e) N_2H_4, MeOH, rt, 2hrs, 85 %.

Primary benzylic thiol Synthesis of an A-ring primary thiol surrogate having an aromatic ring was straightforward. Suitably protected methyl ester **137** made from commercially available 3,5-dihydroxybenzoic acid underwent lithium aluminum hydride reduction to the primary alcohol. Nucleophilic substitution under Mitsunobu conditions afforded thioacetate **138** in good yield. Cleavage of the phenolic TBS ethers required a combination of KF and HBr[15]. Finally, treatment of the thioester with hydrazine efficiently revealed thiol **128**.

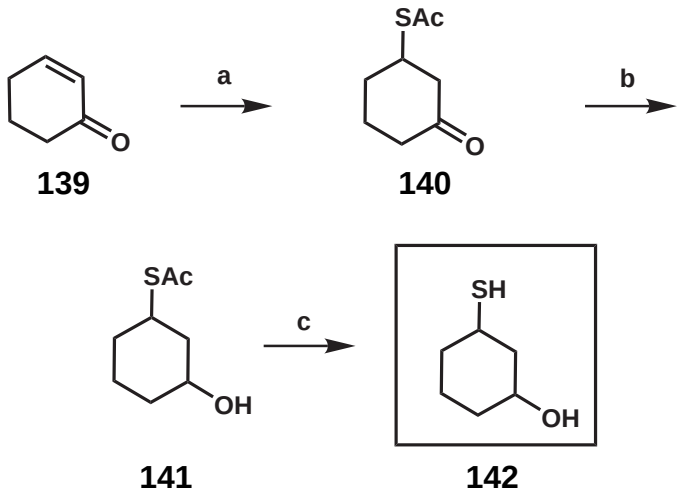


136 **137** **138** **128**

Scheme 3.8. Formation of primary benzylic thiol **128**. *Reagents* : (a) conc. HCl, MeOH, reflux, 16h ; (b) TBSCl, imidazole, DMF, $0\ ^\circ C$ to rt, 16 hrs, 52 % over 2 steps ; (c) $LiAlH_4$, THF, $0\ ^\circ C$ to rt, 7 hrs ; (d) PPh_3, DIAD, AcSH, THF, $0\ ^\circ C$ to rt, o/n, 61 % over 2 steps; (e) $KF \cdot 2\,H_2O$, 48 % aq. HBr, rt, o/n ; (f) N_2H_4, MeOH, rt, 2h, 56 % over 2 steps.

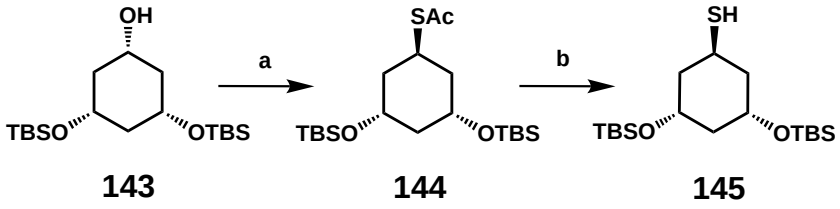
To introduce additional diversity into the available pool of exchangeable building blocks, we decided to create secondary thiols lacking the anti-1,3-diol moiety that provides binding points to the vitamin D receptor. We chose to create a thiol possessing only one hydroxyl group and one where 2 hydroxyls were present but in a syn relation to each other.

The simplest of the two extra thiols is 3-mercaptocyclohexanol **142**. It was made from commercially available reagents in three steps and 23 % overall yield via a conjugate addition of potassium thioacetate onto cyclohexenone **139**, a carbonyl reduction using $NaBH_4$ and a thiol deprotection using propylamine.



Scheme 3.9. Synthesis of building block secondary thiol **142** : (a) AcSH, cat NaOMe, CH_2Cl_2/DMF (8:1), $0\ ^{\circ}C$ to rt, 3 days ; (b) $NaBH_4$, THF/DMF, $0\ ^{\circ}C$ to rt, 3 hrs, 28 % over 2 steps ; (c) propylamine, THF, rt, o/n, 63 %.

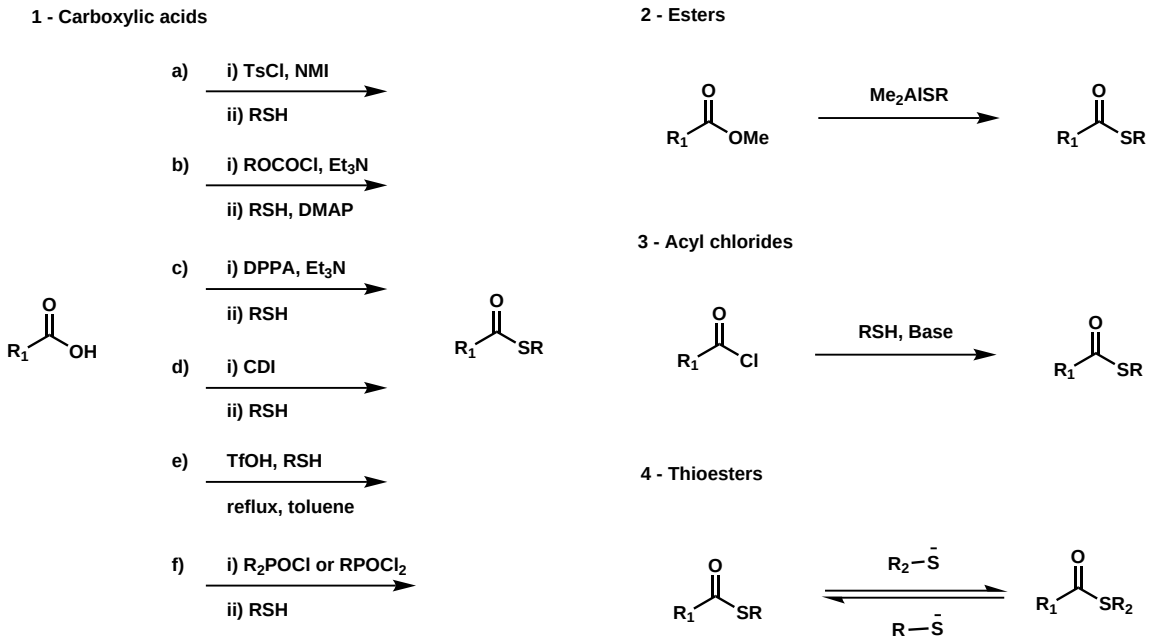
The synthesis of syn diol 5-mercapto-cyclohexane-1,3-diol was accomplished in three simple steps from previously isolated alcohol **143**, a by-product in the synthesis of mono-protected alcohol **48**. In stark contrast to alcohol **13**, Mitsunobu reaction of alcohol **143** with thioacetic acid as the nucleophile proceeded smoothly (Scheme 3.10). The resulting thioester could be partially deprotected with NaOH to remove the acetyl protection group. However, we kept it in this form, as we envisioned to also use it in thioester formation reactions requiring alcohol protection in place.



Scheme 3.10. Synthesis of building block secondary thiol **145** *Reagents* : (a) PPh_3, DIAD, AcSH, THF, $0\ ^{\circ}C$ to rt, 16 hrs ; (b) NaOH, MeOH, rt, 2h, 68 % over 2 steps.

3.2.2 Synthesis of dithioesters

As a prelude to DCL experiments, it was necessary to prepare bis-thioesters as standards and starting materials for dynamic exchange. Methods to make thioesters abound and mostly involve the reaction of a carboxylic acid (or a derivative of) with a thiol. The main categories of thioester forming reactions are : (1) reactions starting from a carboxylic acid, (2) reactions starting from an ester, (3) reactions using acyl chlorides, and (4) reactions involving thioester exchange. The major difference between them is the method of activation of the carboxylic acid (or derivative) to increase it's reactivity towards a nucleophilic attack from a thiol. Scheme 3.11 presents a summary of the different methods available to make thioesters. We have used a variety of these procedures at one point or another during the course of our studies and we will refer to this scheme when necessary.

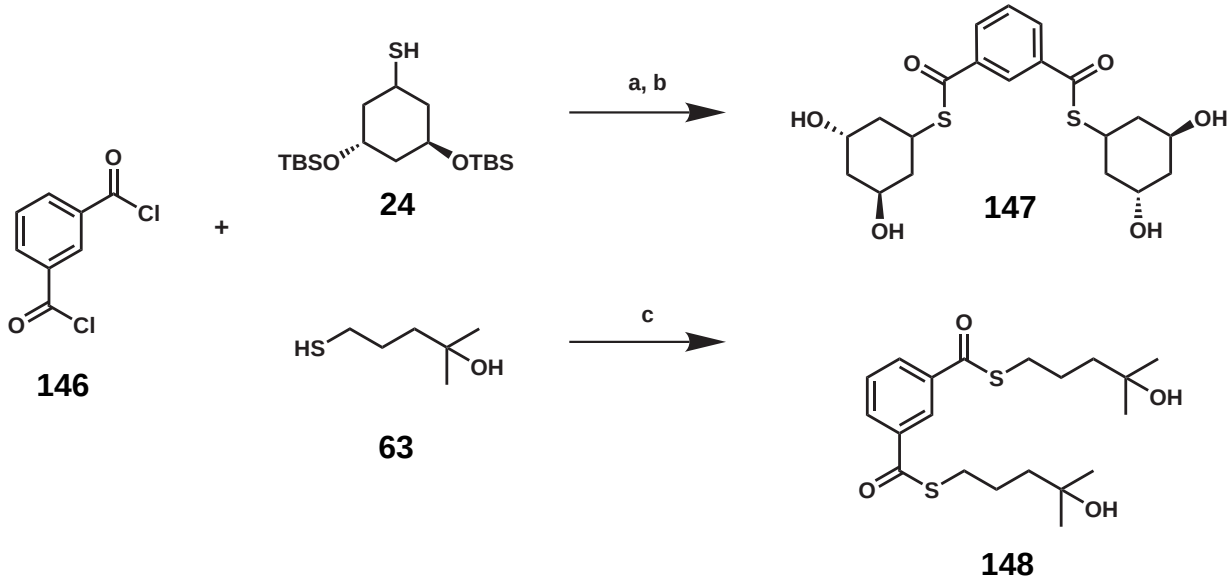


Scheme 3.11. Some synthetic methods used to construct thioesters presented in this thesis.

Synthesis of dithioesters bearing an aromatic core

For our intial studies, we decided to employ aromatic center cores as they offered many advantages. They were good chromophores, many were commercially available, and their rigidity would hold the A-ring and side chain in definite positions in space.

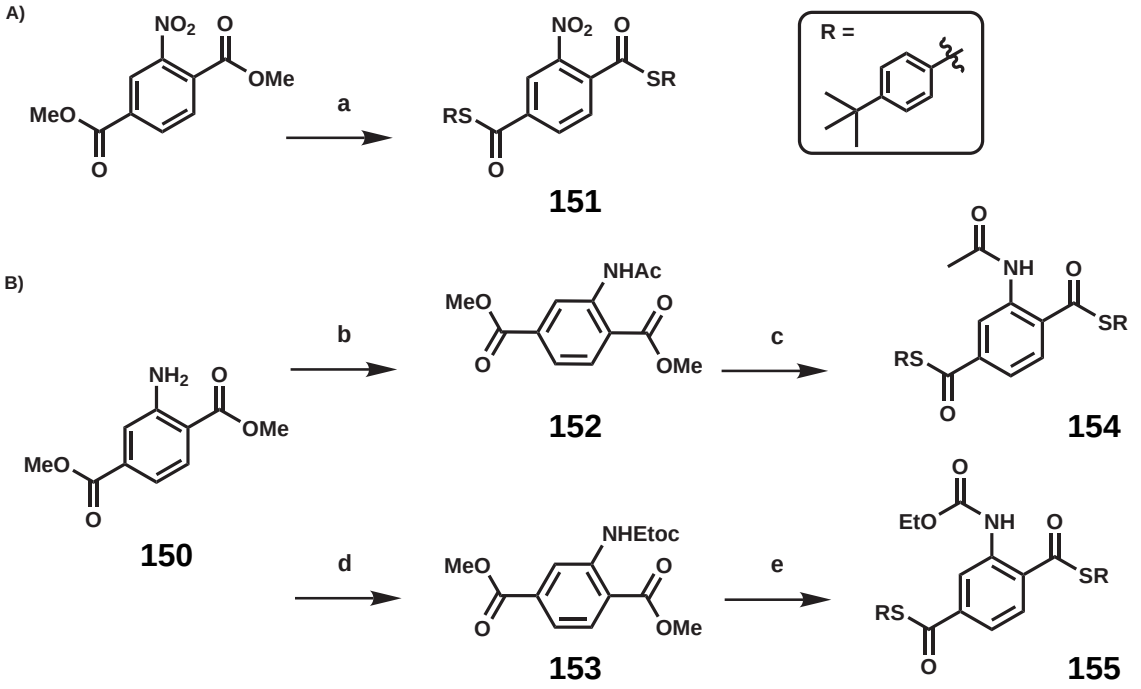
Aromatic dithioesters **147** and **148** (Scheme 3.12) were obtained by mixing isophthaloyl chloride with either secondary thiol **24** or primary thiol **63** under basic conditions. If required, silicon-based TBS protecting groups could be rapidly and cleanly removed under acidic conditions via treatment with excess dilute HCl in MeOH. Thioesters invariably remained untouched under those conditions.



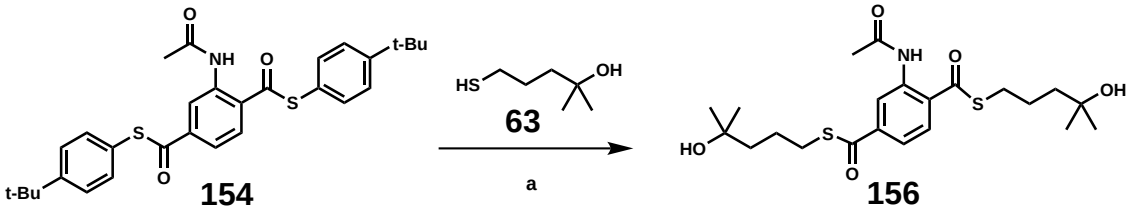
Scheme 3.12. Synthesis of bis-thioesters based on isophthalic acid. *Reagents* : (a) Et_3N, toluene, rt, o/n ; (b) conc. HCl, MeOH, rt, 30 min., 60 % over 2 steps ; (c) Et_3N, toluene, rt, o/n, 37 %.

We also prepared thioesters from 2-substituted terephthalate esters (Scheme 3.13). As described above, thioesters can be made directly from methyl esters with the use of $AlMe_2SR$, prepared in situ from a thiol RSH and $AlMe_3$.[16] Using 4-t-butylthiophenol as the thiol, we created bis-thioesters **151**, **154** and **155** via this synthetic method.

With the double objective of creating a new dithioester bearing significant thiols and testing the thioester exchange under anhydrous conditions, we attempted the synthesis of bis-alkylthiol-dithioester **156** from previously made bis-arylthiol-dithioester **154** (Scheme 3.14). We proceeded by mixing top chain mimic thiol MMPe **63** and Et_3N in degassed THF and adding dithioester **154** at ambient temperature and under argon. After 8 days of stirring, treatment and purification of the reaction yielded bis-alkyl dithioester **156** in 34 % yield.



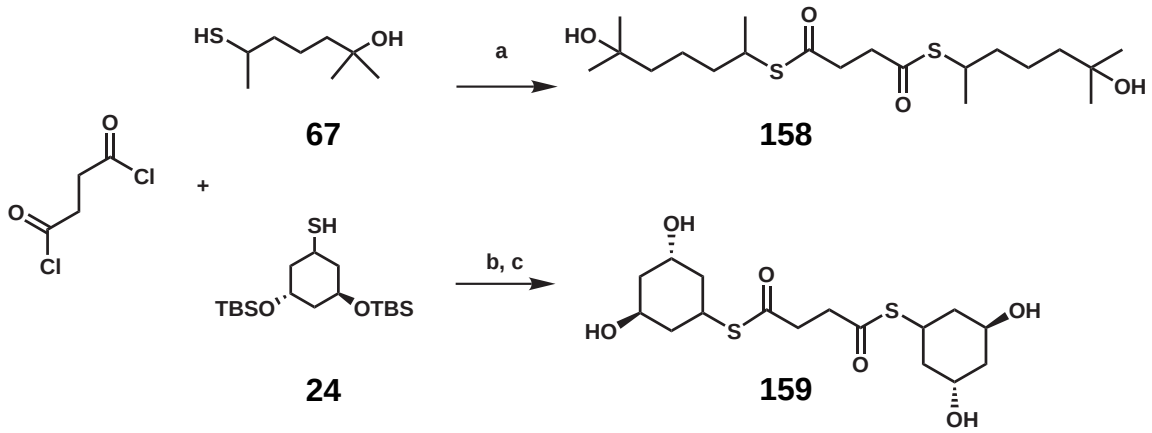
Scheme 3.13. Synthesis of 4-t-butylthiophenolbis-thioesters based on 2-substituted terephthalic acids. *Reagents* : (a) t-butylthiophenol, $AlMe_3$, CH_2Cl_2, $0\ ^{\circ}C$ to rt, overnight, 71 % ;(b) AcCl, DMAP, pyridine, CH_2Cl_2, $0\ ^{\circ}C$, 3 hrs, 52 % (c) t-butylthiophenol, $AlMe_3$, CH_2Cl_2, $0\ ^{\circ}C$ to rt, overnight, 62 % ;(d) ethyl chloroformate, pyridine, DMAP, CH_2Cl_2, $0\ ^{\circ}C$ to rt, 3 days, 97 % ;(e) t-butylthiophenol, $AlMe_3$, CH_2Cl_2, $0\ ^{\circ}C$ to rt, overnight, 68 %.



Scheme 3.14. Synthesis of bis-alkyldithioesters via thioester exchange from bis-aryldithioesters. *Reagents* : (a) thiol MMPe **63**, Et_3N, THF, rt, 8 days, 34 %.

Synthesis of dithioesters possessing an aliphatic core

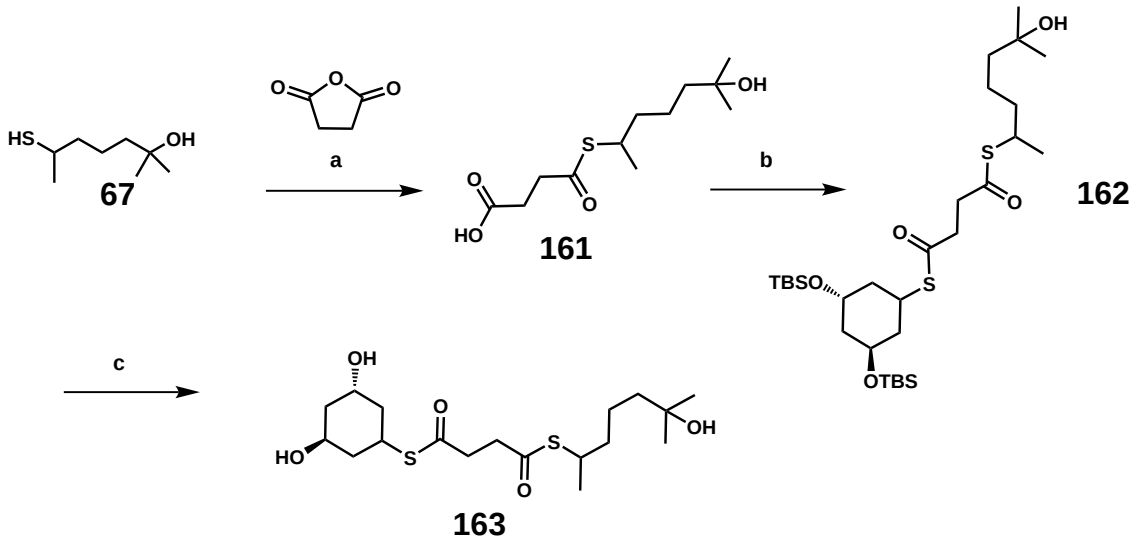
Creation of symmetrical aliphatic core-based dithioesters was easily accomplished from the mixing of a thiol (either top chain thiol **67** or A-ring thiol **24**) with a bis-acyl chloride such as succinoyl chloride. The two reactions shown in Scheme 3.15 proceeded in low to moderate yield.



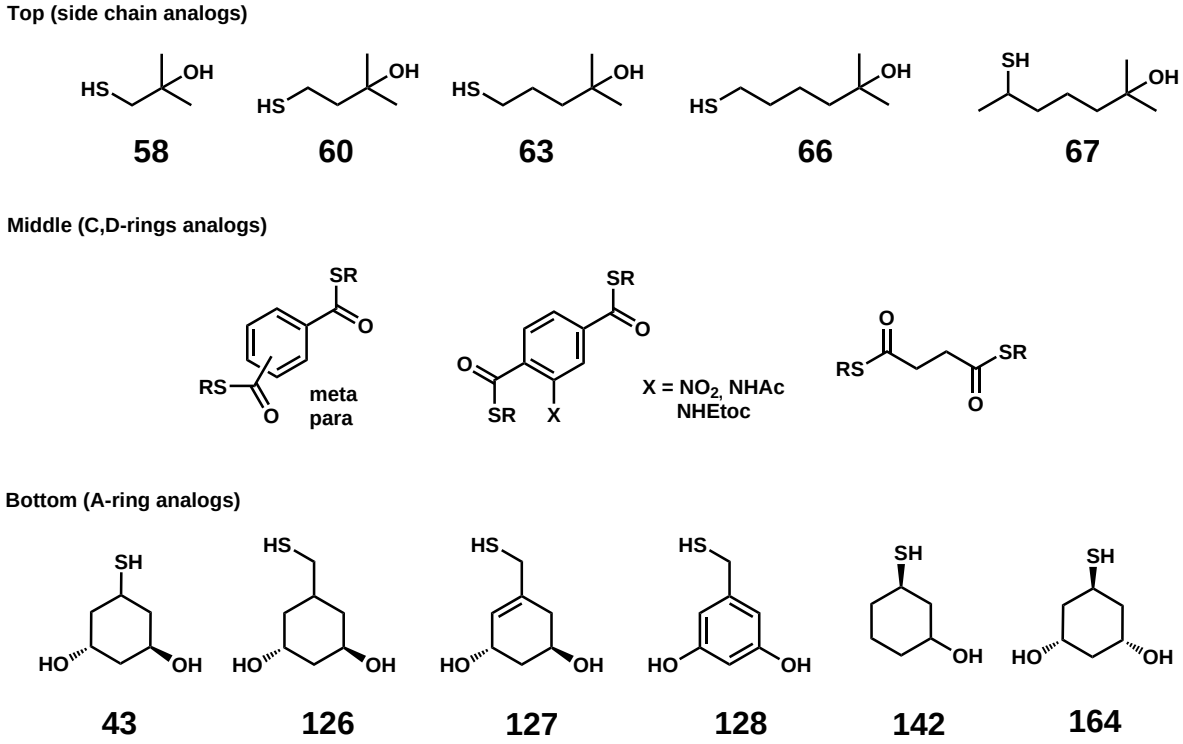
Scheme 3.15. Synthesis of bis-thioesters based on aliphatic core succinic acid. *Reagents* : (a) Et_3N, CH_3CN, rt, 22 hrs, 32 % ; (b) Et_3N, DMF, rt, 24 hrs, 66 % ; (c) conc. HCl, MeOH, rt, 45 min., 77 %.

It is also possible to create mixed aliphatic dithioesters via known synthetic methods (Scheme 3.16). We made dithioester **163** in 3 steps. First, the reaction of top chain analogue thiol MMHp **67** with succinic anhydride gave thioester-acid **161**. This acid was reacted with the complementary A-ring thiol **24** to yield mixed (or unsymmetrical) bis-thioester **163** using the TsCl/NMI method.[17]

Summary Our goal was to conduct a dynamic combinatorial experiment to find new, simple molecules via selection by the vitamin D receptor. After intense work, the required building blocks had been made and were now ready to be used in actual dynamic libraries (Scheme 3.17). To start, we needed to explore the experimental conditions under which the exchange could be conducted and we chose to investigate a simple, three component system (one A-ring, one C,D-rings core and one side chain analogs). Our results are described in the next section.



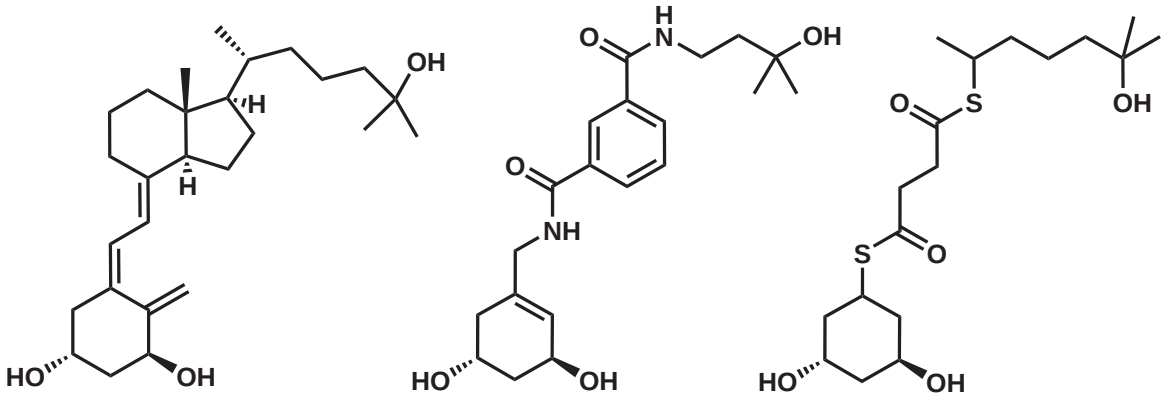
Scheme 3.16. Synthesis of unsymmetrical bis-thioester based on aliphatic core succinic acid. *Reagents* : (a) Et_3N, DMF, $0\ ^{\circ}C$ to rt, 22 hrs, 96 % ; (b) TsCl, NMI, A-ring thiol **24**, CH_3CN, $0\ ^{\circ}C$ to rt, 46 % ; (c) conc. HCl, MeOH, rt, 1 hrs, 74 %.



Scheme 3.17. All available building blocks and library members for a dynamic combinatorial library based on thioester exchange.

3.3 Biological evaluation of some library members

As a preliminary examination, we prepared a few selected compounds to be generated in the libraries, two examples of which are shown in Scheme 3.18. The evaluation of their binding affinity to the receptor was important to assess the potential of VDR to act as a target in the dynamic libraries. Binding constants were assessed by the Kremer laboratory (Richard Kremer, Department of Medicine, Royal Victoria Hospital, McGill University) via a standard analysis using ^{3}H labeled 1α,25(OH)$_2$ D$_3$. Scatchard plot analysis indicated that the aromatic amide **TQ** had modest binding affinity (K$_d$ of 10^{-7} M) while aliphatic bis-thioester **163** had a much stronger binding affinity (K$_d$ of 5x10^{-10} M). This affinity is about half that of calcitriol. Subsequently, a cell growth inhibition assay was conducted using immortalized (HPK1A) and ras-transfected (HPK1A) keratinocytes. Effectiveness was assayed using cell count by coulter counter, Formazan assay and ^{3}H-thymidine incorporation. While the aromatic diamide showed, at best, minimal effects, the aliphatic dithioester showed growth inhibition similar to that observed with calcitriol. The inhibition was not as sustained as long as with 1,25-(OH)$_2$ D$_3$, possibly due to slow hydrolysis of the thioesters.



calcitriol **TQ** **163**

Scheme 3.18. Thioester and amide analogues tested.

Given the *in vitro* results, an *in vivo* assay of calcemic activity was conducted. Balb/c mice were administered with a constant infusion of either calcitriol or the aliphatic dithioester **163**. Infusion of 1,25-(OH)$_2$ D$_3$ resulted in a rapid and sustained elevation of serum calcium as compared to vehicle alone. In contrast, no significant change in serum calcium concentrations were observed at any doses tested of the dithioester analogue.

The effectiveness of the aliphatic thioester analogue was quite surprising, as we had expected these thioester structures to be labile. While the *in vivo* results must be followed up, the cell growth inhibition assay indicates that these compounds may represent lead compounds for future development and be viable substrates for dynamic libraries.

We decided to synthesize a metabolically more stable version of dithioester **163** by replacing the thioesters with amides and test it in a CYP24 induction assay (Figure 3.1). This assay was performed on both compounds, thioester **163** and amide **191**, again by Ari J. Bitton, from John H. White's laboratory at McGill University. If the molecules were VDR antagonists, one would not see much induction of gene expression. But if they were even only partial agonists, there would be a measurable effect. It can be seen on Figure 3.1 that neither the dithioester analogue **163** (labeled 1274) nor the diamide version **191** (labeled 1276) showed agonistic effect on VDR. Also, aromatic dithioesters **147** (labeled 643) and **148** (labeled 624) were tested under the same conditions and presented the very same results : none of them are VDR agonists (Figure 3.2). Even though the somewhat ambiguous diagnostic given by the two different assays (direct binding constants measurements and CYP24 induction assays) was puzzling, it was believed that agonist activity was not directly related to binding affinity. There remained the possibility that our molecules bound VDR but did not allow it to perform its task as a gene regulator. From that point on, our goal was to build on the positive results obtained by the binding assay and establish conditions for the dithioester exchange so that we would be able to let the vitamin D receptor point to us its preferred binder.

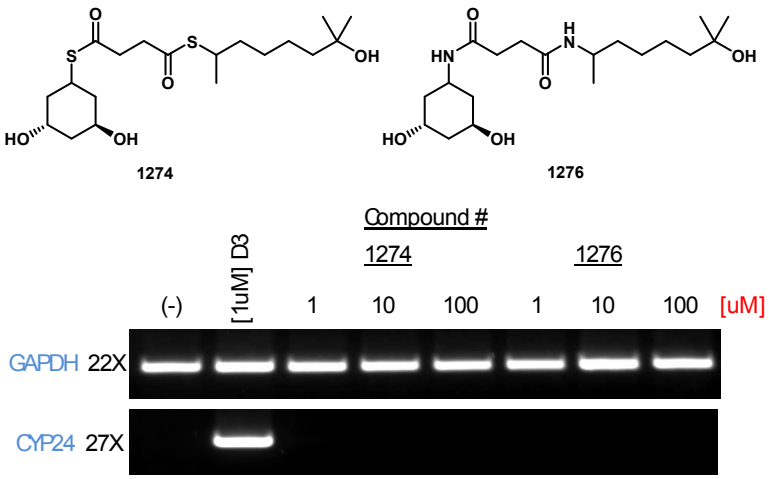


Figure 3.1. CYP 24 induction assays on aliphatic thioesters **163** and diamide **191**.

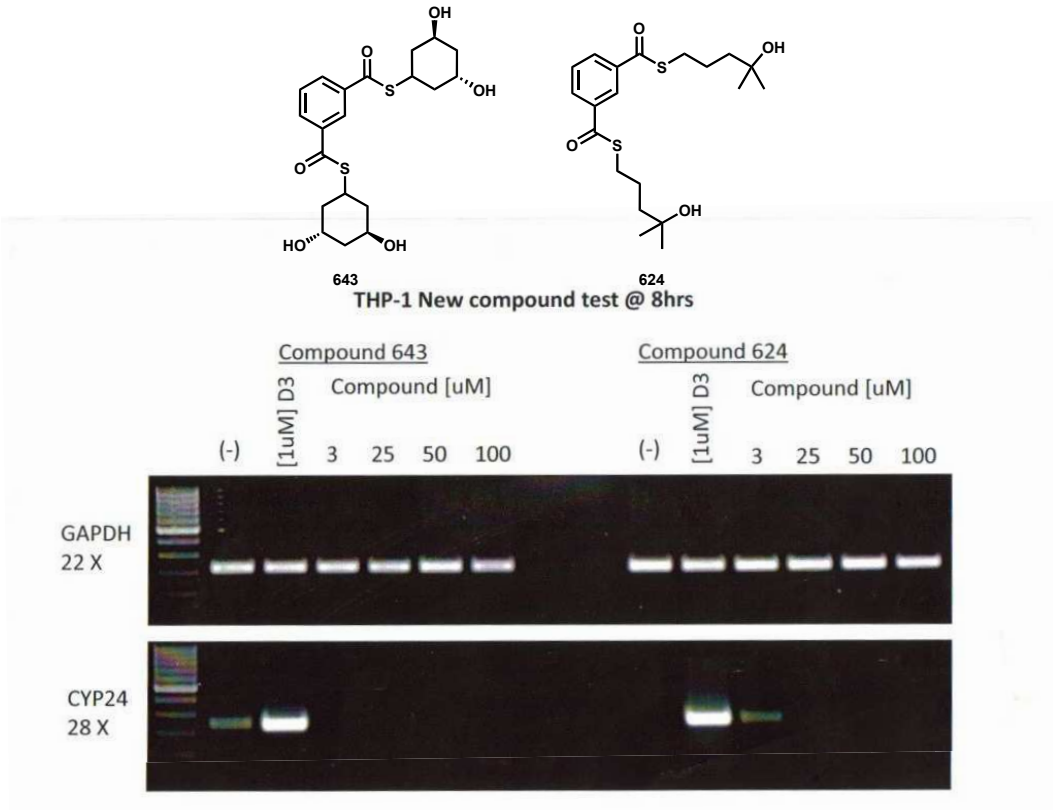
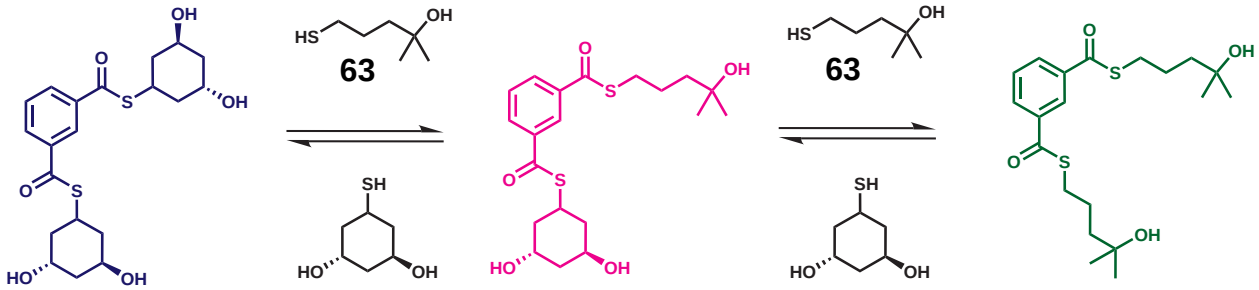


Figure 3.2. CYP 24 induction assays on aromatic thioesters **147** (643) and **148** (624).

3.4 Validation of the method : sample dynamic libraries in the absence of receptor

The next step we took toward the development of dynamic libraries of vitamin D analogues was to develop conditions under which the thioester exchange could occur in the presence of theVDR. For successful dynamic combinatorial experiments to occur, many practical aspects needed to be verified. First, we had to look for the existence of a reversible chemical synthesis of the library members from dynamic assembly of the building blocks. Then, we needed to assess the stability of the library members under the experimental conditions for the duration of the experiment. Also, we had to confirm the ability of the library to reach equilibrium. Finally, the selective binding to the target should be the only influence on the equilibrium composition of the library, not solubility or allosteric interactions or something else.

We set out to explore these aspects using a very simplified system composed of A-ring aromatic dithioester **147**, side chain thiol 5-mercapto-2-methylpentan-2-ol (MMPe) **63** and A-ring thiol (1S,3S)-5-mercaptocyclohexane-1,3-diol (MCD) **43** as shown in Scheme 3.19. This set of components should give rise to two other dithioesters, **165** and **148**, making this experiment a three-component library.



Scheme 3.19. Set up of the preliminary thioester exchange experiments using aromatic thioesters : side chain dithioester **148** and A-ring dithioester **147**.

3.4.1 Rate of exchange and hydrolytic stability versus pH

The first dynamic experiment was prepared as a mixture of A-ring dithioester **147** with side chain thiol MMPe **63**, dissolved respectively at 0.04 mM and 0.08 mM concentration in 10 mM triethanolamine (TEA) buffer at pH 7.8, with 1 mM EDTA, 300 mM KCl and 0.2 mM TCEP · HCl. The TCEP · HCl (tris[2-carboxyethyl]phosphine hydrochloride)[18], a known water-soluble trialkylphosphine reducing agent, was added to prevent disulfide formation from free thiolates, while the buffer, EDTA and KCl are standard conditions for maintaining stability of VDR. Under those basic conditions, we expected thiol-thioester interchange to proceed by the reaction of a thiolate with a thioester. Starting the experiment with only one dithioester (**147**), we could monitor using UV-HPLC the appearance of the mixed dithioester **165** and then, as thiols reacted with it further, appearance of symmetrical side chain dithioester **148**. The results are shown in Figure 3.3.

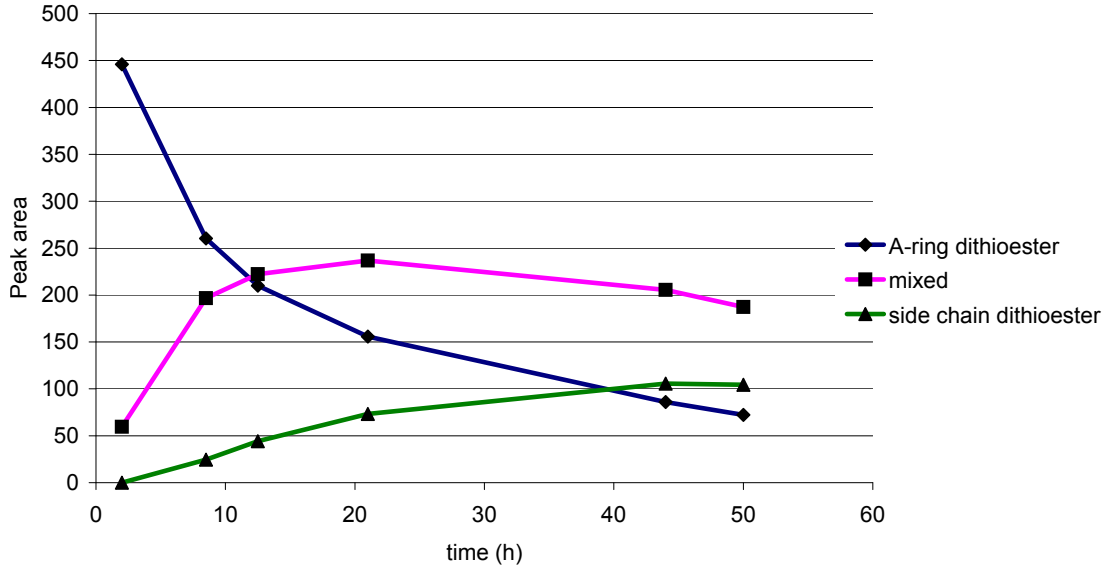


Figure 3.3. Thioester exchange at pH 7.8 . Initial conditions : A-ring dithioester **147** (0.04 mM, 1 equiv), side chain thiol MMPe **63** (0.08 mM, 2 equiv). Buffer : 10 mM TEA, pH 7.8, 1 mM EDTA, 300 mM KCl, 0.2 mM TCEP · HCl.

Assessing the results of the experiment, it appeared that equilibrium was reached in about 40 hrs. However, there was a slight decrease in the total amount of library members present, pointing toward instability of the dithioesters to the reaction conditions. As can be seen on Figure 3.4, absorbance analysis shows that the sum of the dithioester components compared to the total content of the library had decreased by almost 30 % in less than 50

hrs (From a total of around 500 absorbance units at time 2 hrs down to 363 units at 50 hrs). In dynamic equilibrium experiments, chemical instability and degradation have to be avoided as much as possible to maximize the impact of the presence of receptor. Otherwise, distinguishing receptor-mediated amplification from background chemical influence becomes very difficult.

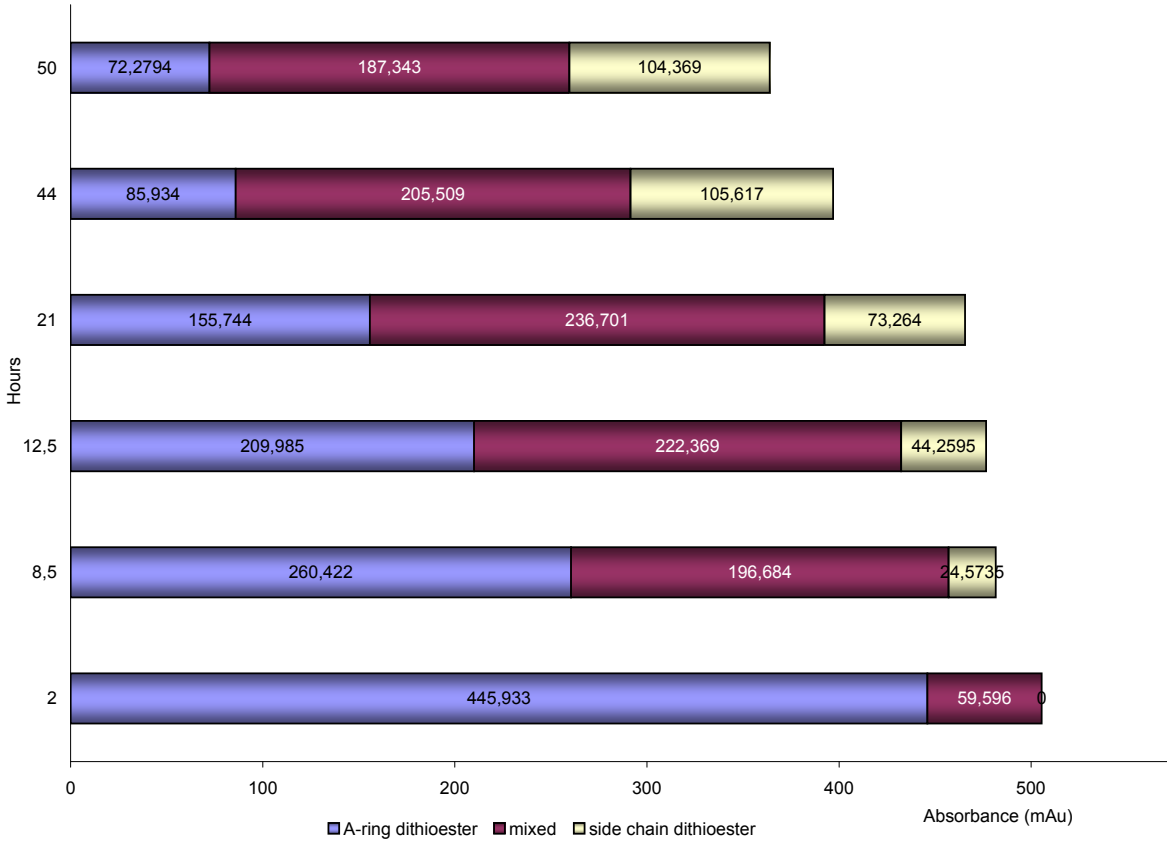


Figure 3.4. Decrease in the total amount of library members present in solution during the course of the experiment of thioester exchange at pH 7.8 .

To investigate the problem we conducted experiments at lower pHs (7.0 and 6.2) and examined the extent of exchange and hydrolysis under those conditions. In order to keep the pH constant, the buffer was switched from TEA to 1,4-piperazinediethanesulfonic acid (PIPES). We also increased the concentration of TCEP · HCl from 0.2 mM to 1.6 mM in the reaction to ensure that a sufficient amount remained over the extended period of time we intended to monitor the reaction.

While the library conducted at pH 7.8 showed a good exchange rate, the ones conducted at lower pHs were much slower. Figure 3.5 presents the result at pH 7.0. Even

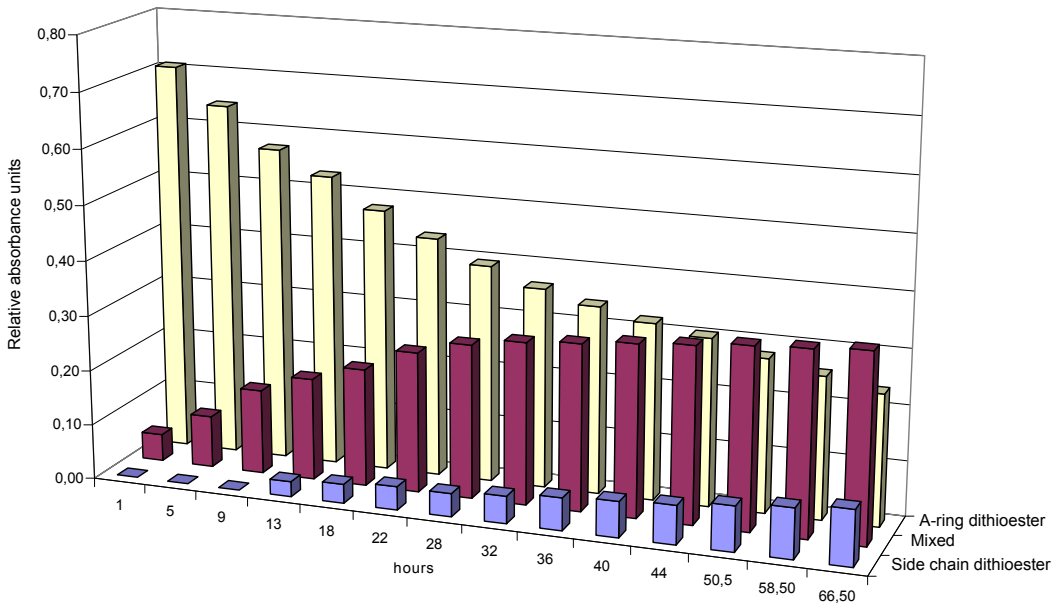


Figure 3.5. Thioester exchange at pH 7.0 ; Initial conditions : A-ring dithioester **147** (0.04 mM, 1 equiv), side chain thiol MMPe **63** (0.08 mM, 2 equiv). Buffer : 10 mM PIPES, pH 7.0, 1 mM EDTA, 330 mM KCl, 1.6 mM TCEP · HCl.

after 66 hours, the mixture still had not reached equilibrium. The good news was that according to our estimates, hydrolysis was significantly reduced at pH 7.0, with only 10 % disappearance over 66hrs (Figure 3.6). Conducting the experiment at pH 6.2 also showed no hydrolysis but the exchange is completely shut down (data not shown).

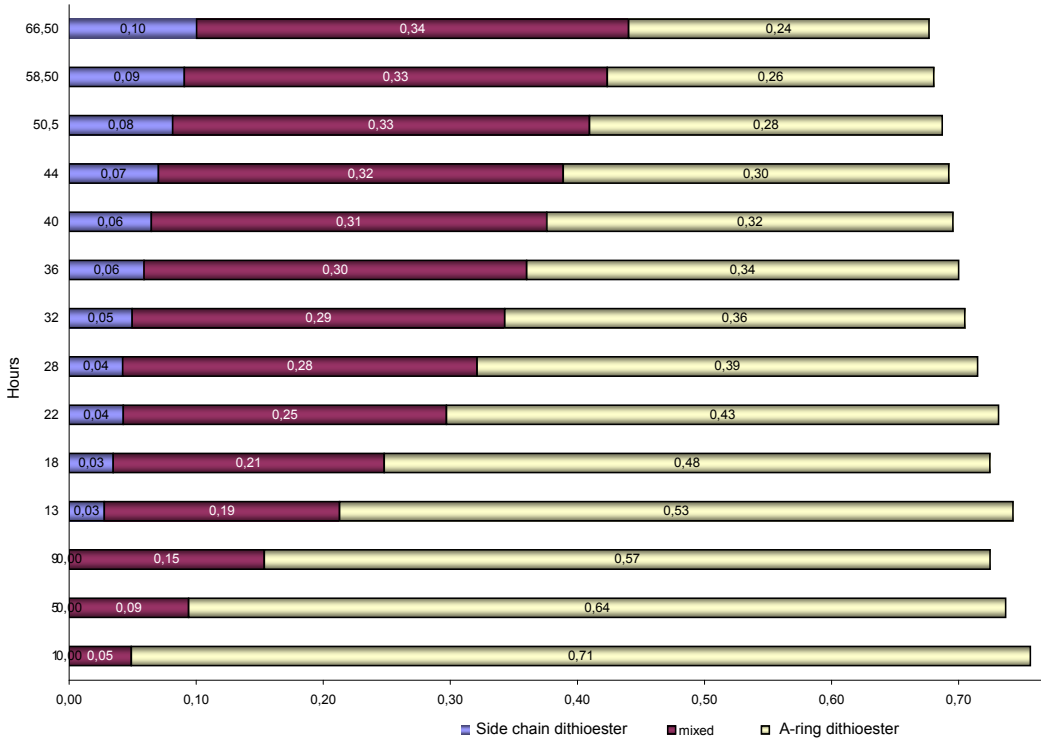


Figure 3.6. Total amount of library members present in solution during the course of the experiment of thioester exchange at pH 7.0.

3.4.2 Additional factors influencing the rate of exchange

Stoechiometry

Aromatic dithioester cores As discussed above, thioester exchange was slow when conducted using 2 equivalents of side chain thiol MMPe **63** against A-ring dithioester **147** at pH 7.0 . For a DCL based on transthioesterification to become practical, the rate of exchange needed to be increased. Assuming that the rate of forward and backward reactions that combine to yield an equilibrium state were to some extent governed by the concentrations of the species involved in those reactions (basic bimolecular kinetics), we decided that we could use a large excess of thiols to accelerate the reactions.

We proceeded by conducting experiments where the total amount of thiols included in the library was varied relative to a static amount of A-ring dithioester **147** and the rate at which the library reached equilibrium was monitored. It was found that the more thiols were present, the faster equilibrium was reached. For example, while the equilibrium was not reached even after 66 hrs when using 2 equiv of only side chain thiol MMPe **63** (Figure 3.5), it was attained in less than 40 hrs using 100 equiv of total thiols (50 equiv of MMPe **63** and 50 equiv of A-ring thiol MCD **43**, see Figure 3.7). Moreover, an experiment using side chain dithioester **148** instead of A-ring dithioester **147** and 200 equiv total of thiols (100 equiv each of MMPe **63** and MCD **43**), but under otherwise equivalent conditions, reached equilibrium in less than 12 hrs (Figure 3.8).

It is noteworthy that when comparing the library composition of the two latter experiments, we can conclude that the library composition at equilibrium is *independent* of the initial composition in terms of dithioesters. As was expected, the fact that both experiments started either with side chain dithioester **148** or A-ring dithioester **147** did not influence the final composition. However, the library composition at equilibrium should be (and, in fact, is) *dependent* of the ratio of thiols present in the reaction. To verify and illustrate that fact, we performed two experiments, both of them using side chain dithioester **148** as the starting point and 50 equiv total of thiols but in different ratios. The first reaction (exp A) had a 4 to 1 ratio of thiol MCD **43** to thiol MMPe **63** and the second (exp B) a ratio of 10 to 1. As shown in Figure 3.9, neither of them had reached equilibrium after about 360 hrs but clearly, they are leading to different library compositions.

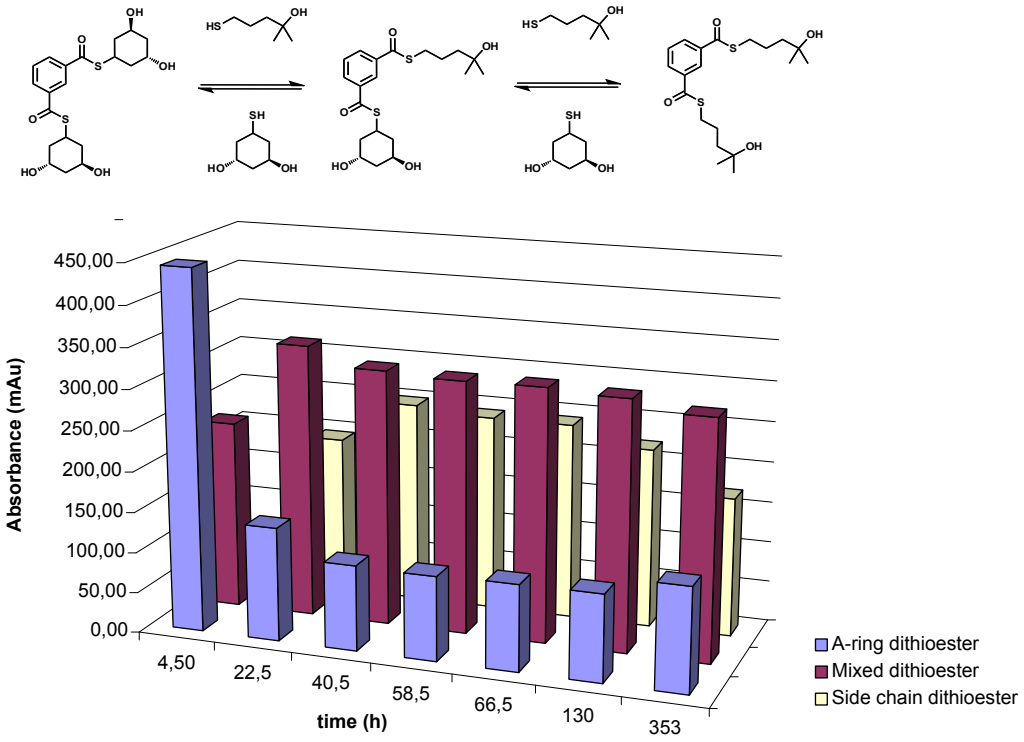


Figure 3.7. Thioester exchange at pH 7.0 using 50 equiv of each thiol. Initial conditions : A-ring dithioester **147** (0.04 mM, 1 equiv), side chain thiol MMPe **63** (1.00 mM, 50 equiv), A-ring thiol MCD **43** (1.00 mM, 50 equiv). Buffer : 11 mM PIPES, pH 7.0, 1 mM EDTA, 330 mM KCl, 1.6 mM TCEP · HCl.

Aliphatic dithioester cores We also investigated the behaviour of aliphatic A-ring dithioester **159** when mixed with A-ring thiol MCD **43** and side chain thiol 6-mercapto-2-methylheptan-2-ol (MMHp) **67** (Scheme 3.20). Using 100 equiv of each thiol in an experiment at pH 7.0 (Figure 3.10), it was observed that while the thiol-thioester exchange took place, it had not reached equilibrium within 100 hrs and was thus significantly slower than aromatic dithioesters-based exchanges where equilibrium is reached in about 40 hrs under the same conditions. Moreover, as the experiment went on, there seemed to be a slow but noticeable disappearance of the dithioesters. We could not confirm without a doubt the source of this phenomenon. However, it is most likely a combination of chemical hydrolysis and physical causes such as the insolubility of non-polar dithioesters, the attractive interaction with vial container, etc.

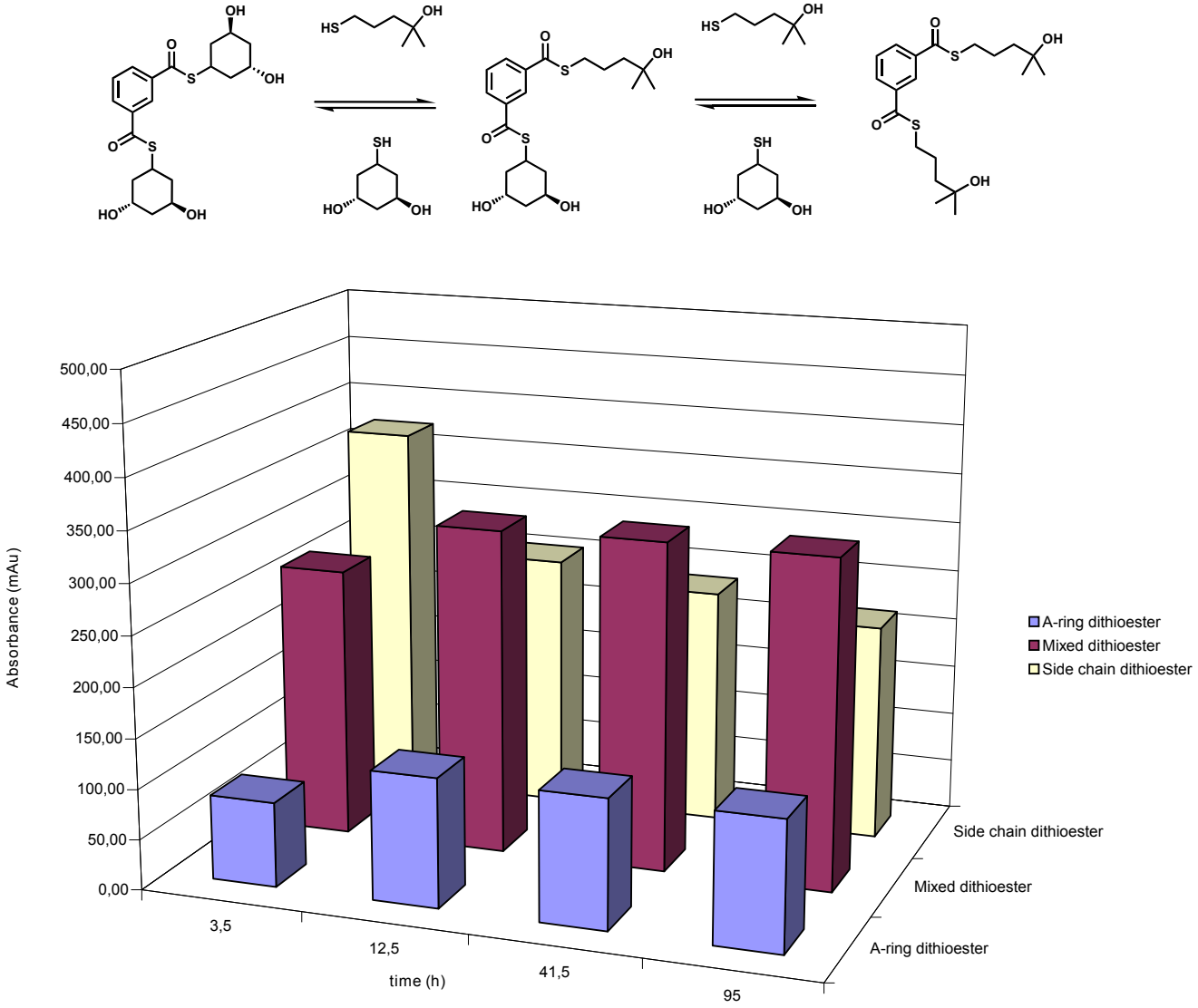


Figure 3.8. Thioester exchange at pH 7.0 using 100 equiv of each thiol (total 200 equiv thiols). Initial conditions : Side chain dithioester **148** (0.04 mM, 1 equiv), side chain thiol MMPe **63** (2.00 mM, 100 equiv), A-ring thiol MCD **43** (2.00 mM, 10 equiv). Buffer : 11 mM PIPES, pH 7.0, 1 mM EDTA, 330 mM KCl, 1.6 mM TCEP · HCl.

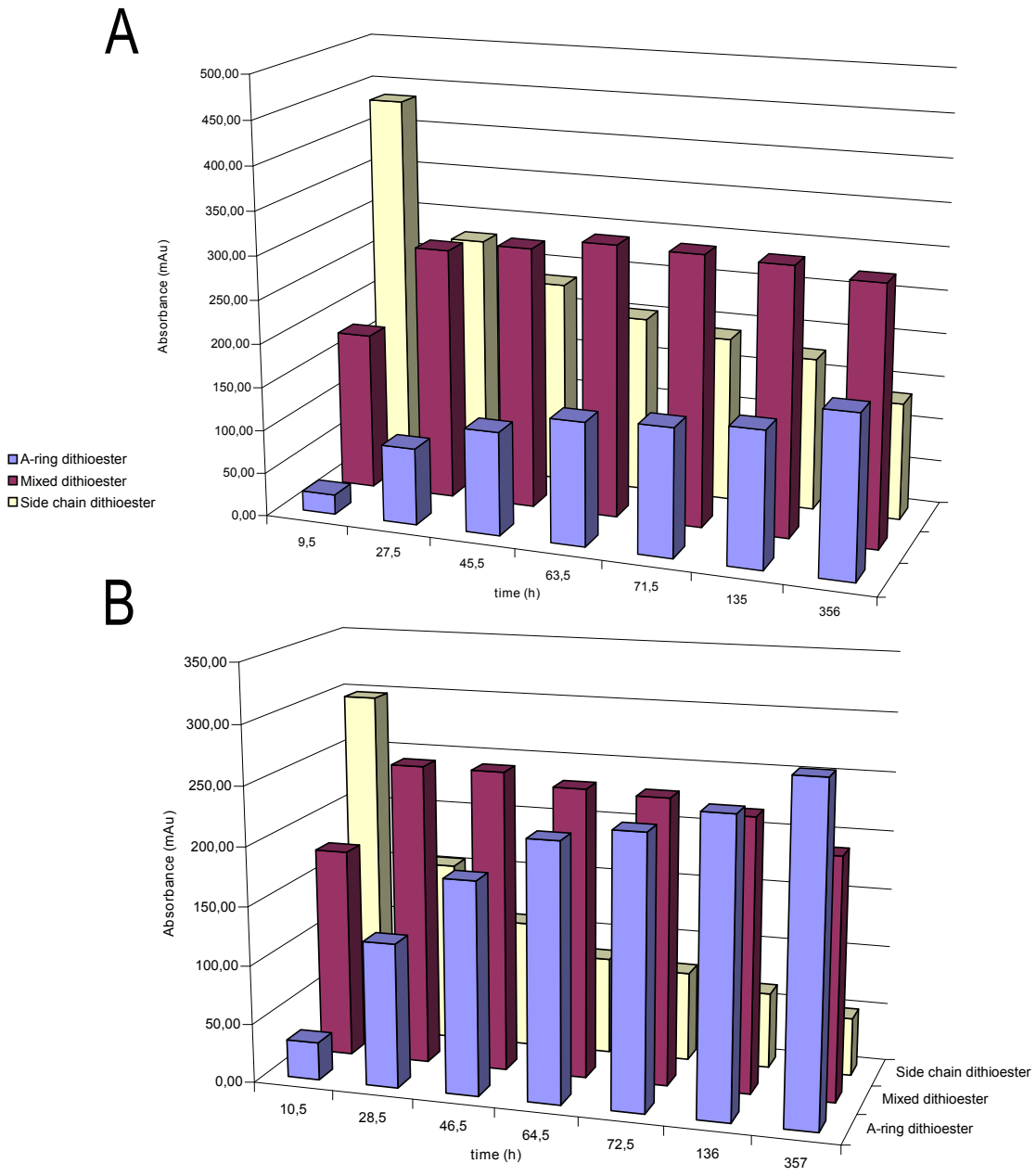


Figure 3.9. Different ratios of thiols lead to different library compositions. Total of 50 equiv of thiols divided between the A-ring thiol MCD **43** and the side chain thiol MMPe **63**. Conditions : (A) A-ring thiol MCD **43** (0.40 mM, 10 equiv), side chain thiol MMPe **63** (1.6 mM, 40 equiv), **148** (0.04 mM, 1 equiv). (B) A-ring thiol MCD **43** (0.182 mM, 4.6 equiv), side chain thiol MMPe **63** (1.82 mM, 45.4 equiv), **148** (0.04 mM, 1 equiv). Buffer : 11 mM PIPES, pH 7.0, 1 mM EDTA, 330 mM KCl, 1.6 mM TCEP · HCl.

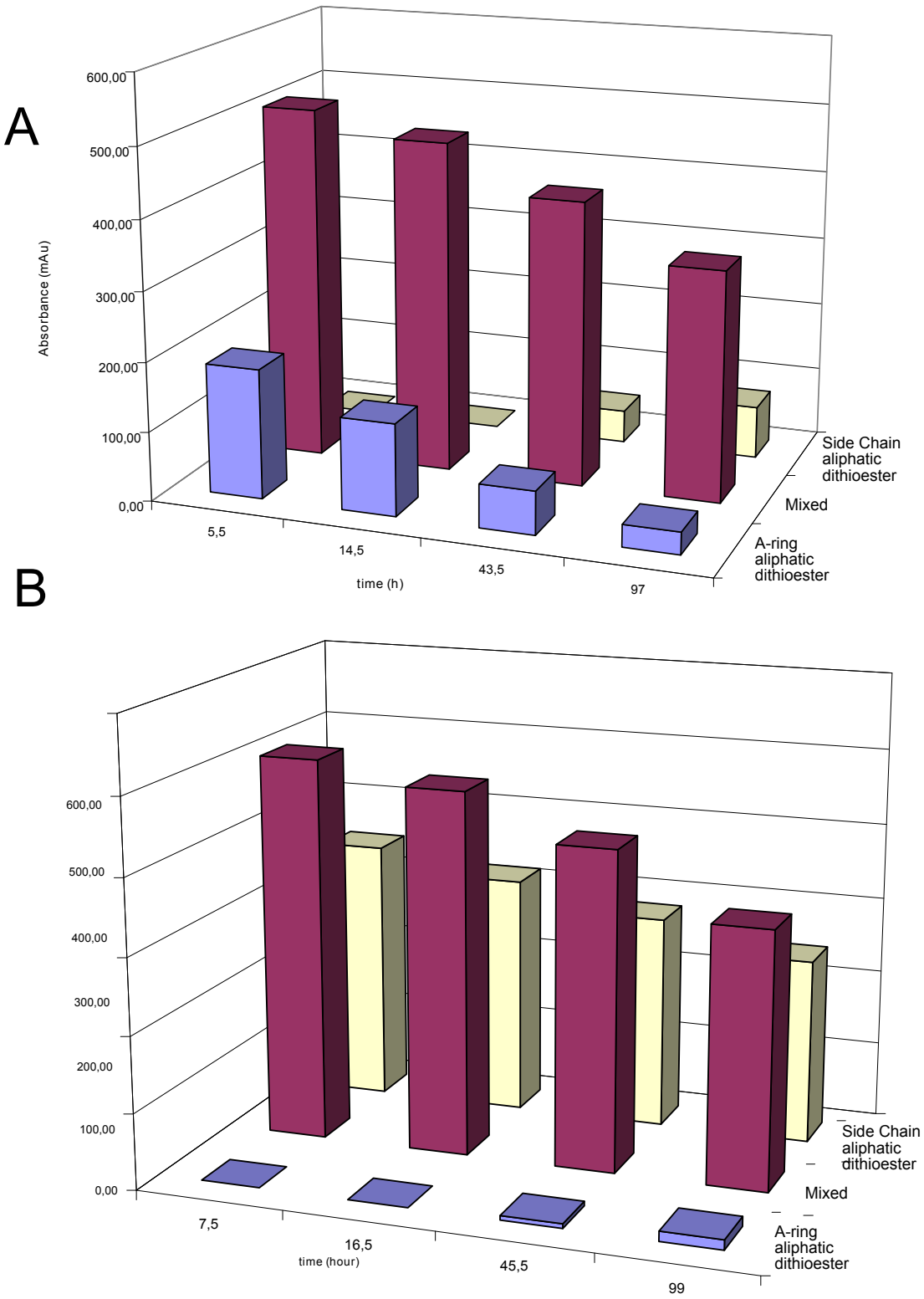
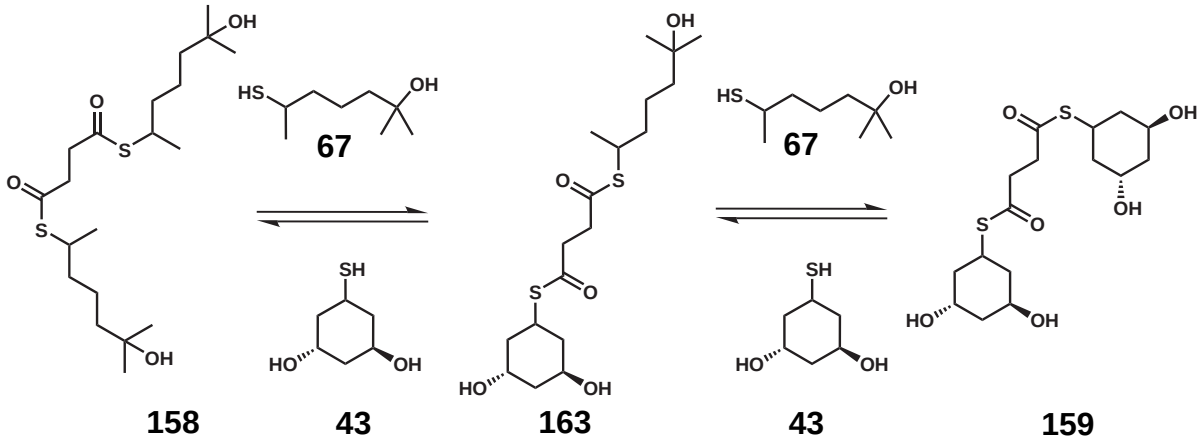


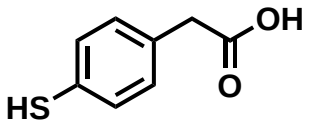
Figure 3.10. Thioester exchange using 200 equiv of total thiols. (A) Starting from A-ring aliphatic dithioester **159** (B) Starting from side chain aliphatic dithioester **158**. Conditions for both reactions : dithioester (0.04 mM, 1 equiv), side chain thiol MMHp **67** (4.00 mM, 100 equiv), A-ring thiol MCD **43** (4.00 mM, 100 equiv). Buffer : 11 mM PIPES, pH 7.0, 1 mM EDTA, 330 mM KCl, 1.6 mM TCEP · HCl.



Scheme 3.20. Set up of the preliminary exchange experiments using aliphatic thioesters : side chain dithioester **158** and A-ring dithioester **159**.

Using an aromatic thiol to increase the rate of exchange

In order to conduct dynamic combinatorial experiments with our aliphatic dithioesters and thiols, we had to find additional ways to increase the rate of reaction to render the process viable. Seeing this situation, we searched the literature for ways to achieve our goals. A possible avenue came from the work of Kent et al.[19] They have found that 4-carboxymethylthiophenol **167** (or designated by its trivial name, mercaptophenylacetic acid (MPAA)) Scheme 3.21, an aromatic thiol, was an excellent native chemical ligation rate enhancer. Native chemical ligation (NCL) uses thioester exchange between an N-terminal cysteine and a thioester to form an amide. Compared to benzyl mercaptan, thiophenol or 2-mercaptoethane sulfonate sodium (MESNa), the aromatic thiol MPAA is perhaps the perfect balance between leaving-group reactivity, water solubility and rapid thiol-thioester exchange capability.[19]



167

Scheme 3.21. Structure of the aromatic thiol 4-carboxymethylthiophenol (mercaptophenylacetic acid (MPAA)) **167**

Since MPAA is supposed to accelerate the rate of thioester exchange, we attempted to

verify if this effect could be combined with the rate enhancing effect of excess library thiols. The use of a new thiol, catalyst or not, aromatic or aliphatic, in a thioester-based library will influence its composition by creating 3 new dithioester entities (A-ring-S–CO-Core-CO–S-MPAA, side chain-S–CO-Core-CO–S-MPAA and MPAA-S–CO-Core-CO–S-MPAA). Because they simply are different dynamic environment, it is impossible to directly compare the compositions of libraries with and without MPAA. However, rates to equilibrium can effectively be compared.

We set up a series of dynamic experiments using aromatic dithioesters **147**, **148** and aliphatic dithioester **159** at 0.04 mM and 200 equiv total of A-ring and side chain thiols (8 mM total) with the addition of 20 mM of MPAA. Figure 3.11 and Figure 3.12 show the resulting libraries from the aromatic dithioesters and Figure 3.13 presents the ones involving aliphatic dithioesters.

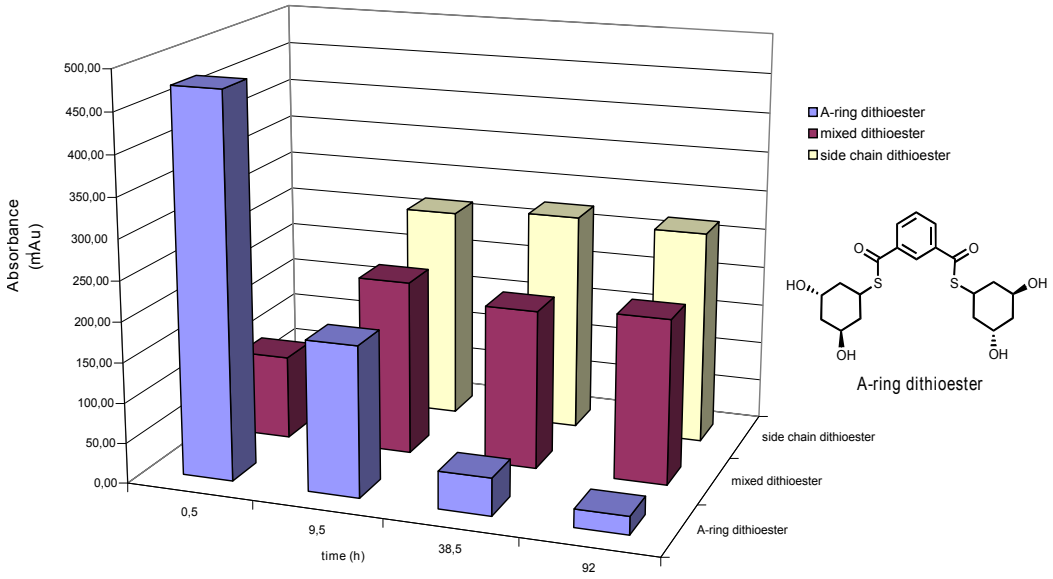


Figure 3.11. Influence of aromatic thiol MPAA on rate of thioester exchange, starting from A-ring aromatic dithioester **147**. Initial conditions : Aromatic A-ring dithioester **147** (0.04 mM, 1 equiv), side chain thiol MMPe **63** (4.00 mM, 100 equiv), A-ring thiol MCD **43** (4.00 mM, 100 equiv), aromatic thiol MPAA (20 mM, 500 equiv). Buffer : 11 mM PIPES, pH 7.0, 1 mM EDTA, 330 mM KCl, 16 mM TCEP · HCl.

When looking at the two libraries based on aromatic dithioesters (Figure 3.11 and Figure 3.12), we find that they converge to sufficiently similar equilibrium compositions and in about the same time (probably less than 40 hrs) but the equilibrium is not reached

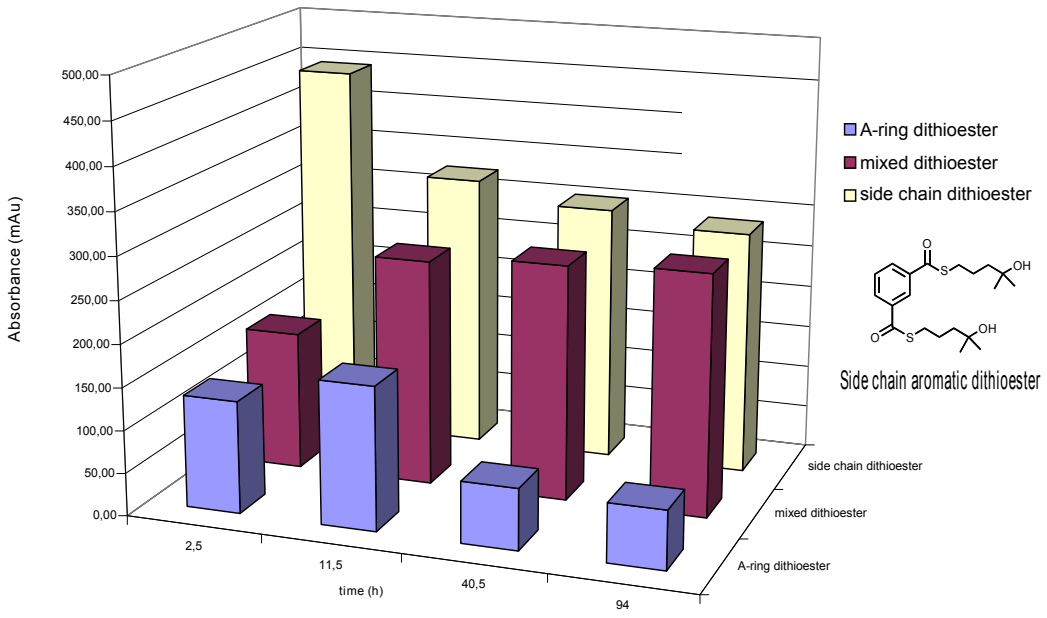


Figure 3.12. Influence of aromatic thiol MPAA on rate of thioester exchange starting from side chain aromatic dithioester **148**. Initial conditions : Side chain dithioester **148** (0.04 mM, 1 equiv), side chain thiol MMPe **63** (4.00 mM, 100 equiv), A-ring thiol MCD **43** (4.00 mM, 100 equiv), aromatic thiol MPAA (20 mM, 500 equiv). Buffer : 11 mM PIPES, pH 7.0, 1 mM EDTA, 330 mM KCl, 16 mM TCEP · HCl.

necessarily faster than without MPAA (approx. 40 hrs, see Figure 3.7). Although these experiments could not pinpoint the exact moment when the equilibrium was reached due to insufficient data points (especially between 10 and 40 hrs), the important conclusion was that MPAA was not necessarily more effective than excess thiols in our dynamic libraries of aromatic dithioesters. In addition, there seems to be greater disappearance of the 3 aromatic dithioesters over time compared to control libraries.

The analysis of the two libraries based on aliphatic dithioesters (Figure 3.13 and Figure 3.14) is particularly interesting. Even though they seem to be evolving in the same direction (converging to similar equilibrium compositions), neither of them attained a state of equilibrium even after a little under 100 hrs. We knew these libraries of aliphatic dithioesters were slower to exchange, but it seemed that, even in the presence of excess building block thiols AND large excess of aromatic catalyst MPAA, equilibrium still cannot be reached. Also, as with the aromatic thioesters, there is an unexplained disappearance of total library members over time.

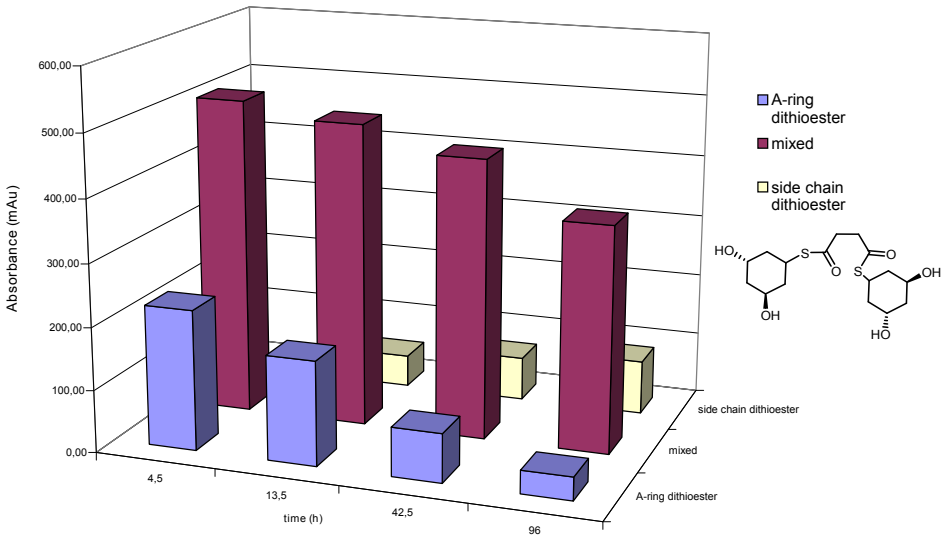


Figure 3.13. Influence of MPAA on rate of thioester exchange. Starting from A-ring aliphatic dithioester **159**. Initial conditions : dithioester **159** (0.04 mM, 1 equiv), side chain thiol MMHp **67** (4.00 mM, 100 equiv), A-ring thiol MCD **43** (4.00 mM, 100 equiv), MPAA (20 mM, 500 equiv). Buffer : 11 mM PIPES, pH 7.0, 1 mM EDTA, 330 mM KCl, 16 mM TCEP · HCl.

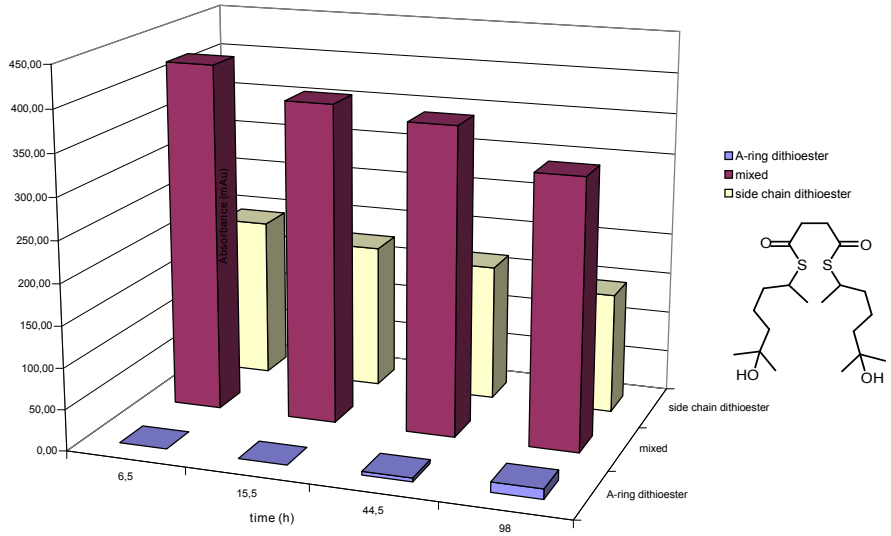


Figure 3.14. Influence of MPAA on rate of thioester exchange. Starting from A-ring aliphatic dithioester **158**. Initial conditions : dithioester **158** (0.04 mM, 1 equiv), side chain thiol MMHp **67** (4.00 mM, 100 equiv), A-ring thiol MCD **43** (4.00 mM, 100 equiv), MPAA (20 mM, 500 equiv). Buffer : 11 mM PIPES, pH 7.0, 1 mM EDTA, 330 mM KCl, 16 mM TCEP · HCl.

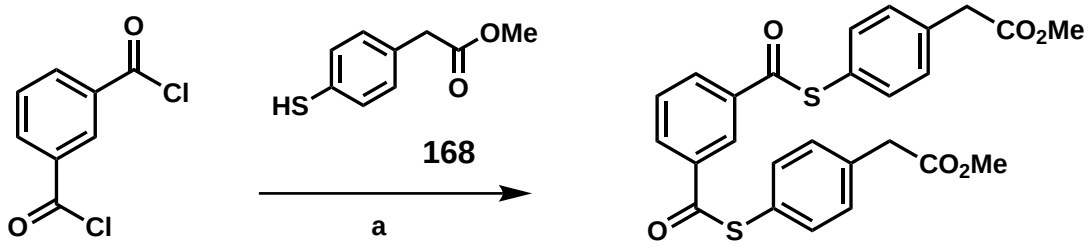
3.4.3 Feedstock experiments

Contrary to many other reversible processes that can be triggered by simple addition of the precursors to a specific functional group (for example, transimination can arise from mixing aldehydes and amines, disulfide exchange can be started by air oxidation of thiols and conjugate addition spontaneously occurs when thiolates are presented with enones), the thioester exchange cannot be implemented by mixing thiols and carboxylic acids. In order to achieve thioester exchange, one has to create a thioester and then mix it with appropriate thiols. In our case of double-level communicating dynamic library (see chapter 1), every core molecule bears two functional groups to be transformed into dithioesters, increasing synthetic complexity and chances of wasting precious thiols. With the goal of preserving thiols and reducing the number of different chemical transformations needed, the next best method to create dithioesters is possibly having dithioester core units as feedstock molecules. These would be easily synthesized, undergo exchange very quickly and not be made from precious homemade thiols.

As a future expansion to our thioester exchange-based system where central diacid cores are investigated, we envisioned that dithioesters based on the mercaptophenylacetic acid thiol (MPAA) could fulfill all of our criteria for an ideal system. Their synthesis was adequately simple and the creation of dynamic libraries of expected composition was easily achieved when starting from these feedstock MPAA-based dithioesters upon addition of other homemade precious thiols.

Synthesis of feedstock molecules

The synthesis of the prototype feedstock dithioester **169** is shown in Scheme 3.22 and consists of a standard reaction between acyl chloride **146** and a thiol **168**, this time using methyl mercatophenylacetate (MeMPAA). The use of the methyl ester instead of the carboxylic acid stems from the fact that the final dithioester product was very easy to create and handle and was used for initial examination. If results with the system were promising, extension to the acid should be straightforward.



146 **169**

Scheme 3.22. Synthesis of bis-(MeMPAA) isophthalate **169**, a prototype dithioester for testing the concept of feedstock dithioester. *Reagents* : (a) pyridine, CH_2Cl_2, cat. DMAP, rt, 3 days. 83 %.

Experiments with feedstock molecules

To verify the potential of this method, we dissolved bis-MeMPAA-aromatic dithioester **169** in PIPES buffer at pH 7.0 and added 50 equiv each of A-ring thiol MCD **43** and side chain thiol MMPe **63** to it. Monitoring the library immediately upon addition, we observed that the main species present was the bis-MeMPAA dithioester **169**, but the three dithioesters of interest, side chain aromatic dithioester **148**, mixed aromatic dithioester **165** and A-ring aromatic dithioester **147** were starting to be formed (Figure 3.15). After 9 hours, the feedstock dithioester had been completely consumed, probably to form half-MeMPAA/half-alkyl dithioesters and ultimately library members that were full alkyldithioesters. Following the library composition over time, the total amount of alkyldithioester increased steadily (purple). Although we do not have quantifiable results on this experiment (the % of feedstock molecule that is transfered into library members vs other thioesters, actual time to reached equilibrium and equilibrium composition), we feel that the concept of feedstock dithioester core molecules is worthy of consideration if one intends to achieve a double-point of exchange DCL.

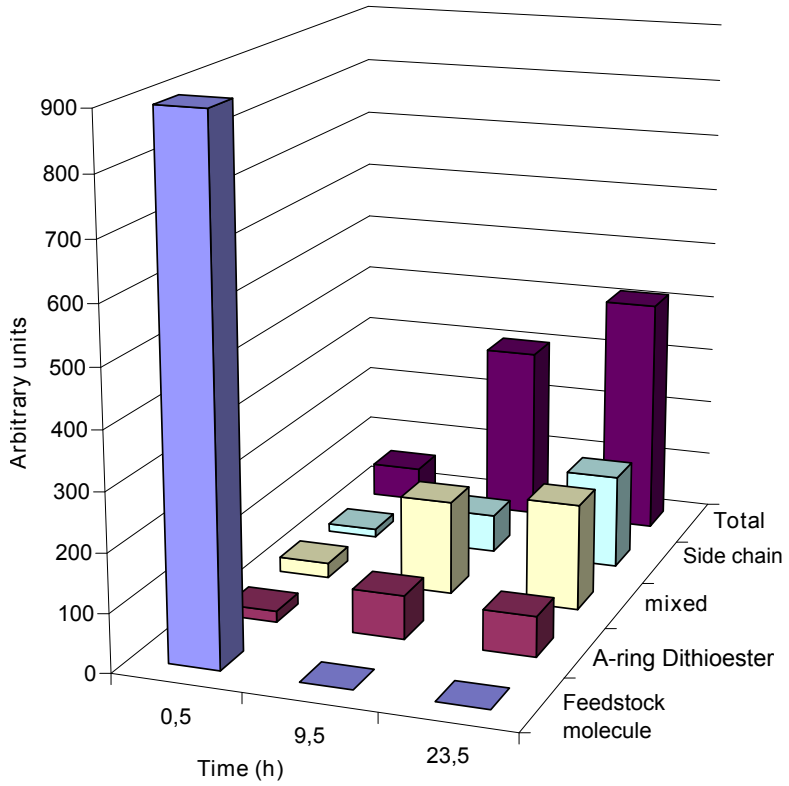
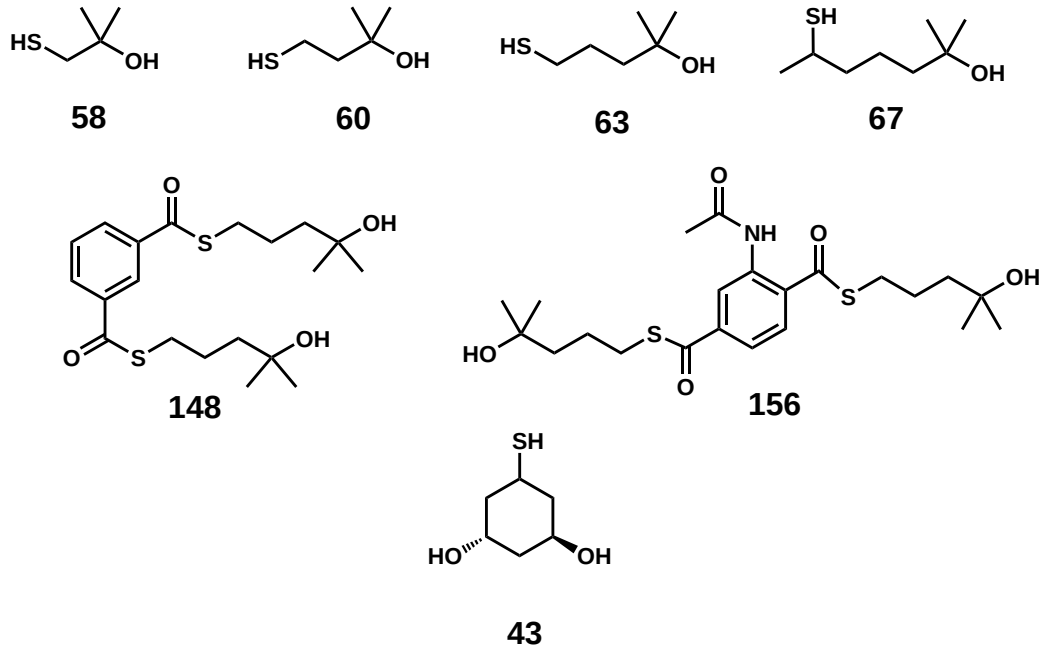


Figure 3.15. Formation of a dithioester dynamic library from feedstock molecule bis-(MeMPAA)isophthalate **169** Initial conditions : Feedstock dithioester **169** (0.0394 mM, 1 equiv), side chain thiol MMPe **63** (1.97 mM, 50 equiv), A-ring thiol MCD **43** (1.97 mM, 50 equiv). Buffer : 25 mM PIPES, pH 7.0, 1 mM EDTA, 300 mM KCl, 16 mM TCEP · HCl, 0.15 mM penicillin G.

3.4.4 Extended library

Previously, we experimented with a library containing only 2 thiols (a side chain and an A-ring mimics) and one core molecule. In order to gain experience and increase the complexity of our library, we conducted an exchange with 4 side chain molecules, two dithioester cores, and one A-ring building block. Shown in Scheme 3.23 are the 7 building blocks involved in this extended library. Under standard conditions, we mixed thiols MCD **43**, MMHp **67** and MMPr **58** and MMB **60** and MMPe **63** with side chain dithioester **148** and side chain 2-acetamido substituted dithioester **156** and monitored the exchange using HPLC.



Scheme 3.23. Building blocks used in the elaboration of extended dithioester library.

Figure 3.16 presents the evolution of the library over time. An experiment containing this many building blocks is expected to create a large number of distinctive entities. In this case, a total to 40 members can be created from these seven building blocks. As early as 17.5 hrs, the main components are already present and only minor changes seem to occur during the course of the experiment. The equilibrium is probably reached early on and the observable changes between the different HPLC traces (at 21.5 and 23.0 min., for instance) is possibly due chemical degradation of the library components. To verify if

the library was still amenable to equilibrium shift, we added a large amount of side chain dithioester **148**. This addition brought very little change in the visible library composition, as can be seen by comparing the *190 hrs* HPLC trace and the *190 hrs+thioester+21 hrs* HPLC trace. Either very few thiols were still present at 190 hrs to react with the newly added thioester or the 21 hours between its addition and the sampling were sufficient for the library to reach a new equilibrium position which corresponded almost exactly to the previous one. Nevertheless, this extended library proved successful in the fact that we could generate a high level of diversity in a dynamic library from only few thioesters and thiols. Precise characterization of the dynamic library using LC-MS was attempted several times but technical difficulties only related to the apparatus prevented us from gathering more information on those libraries. More efforts will be placed in this direction in future attempts.

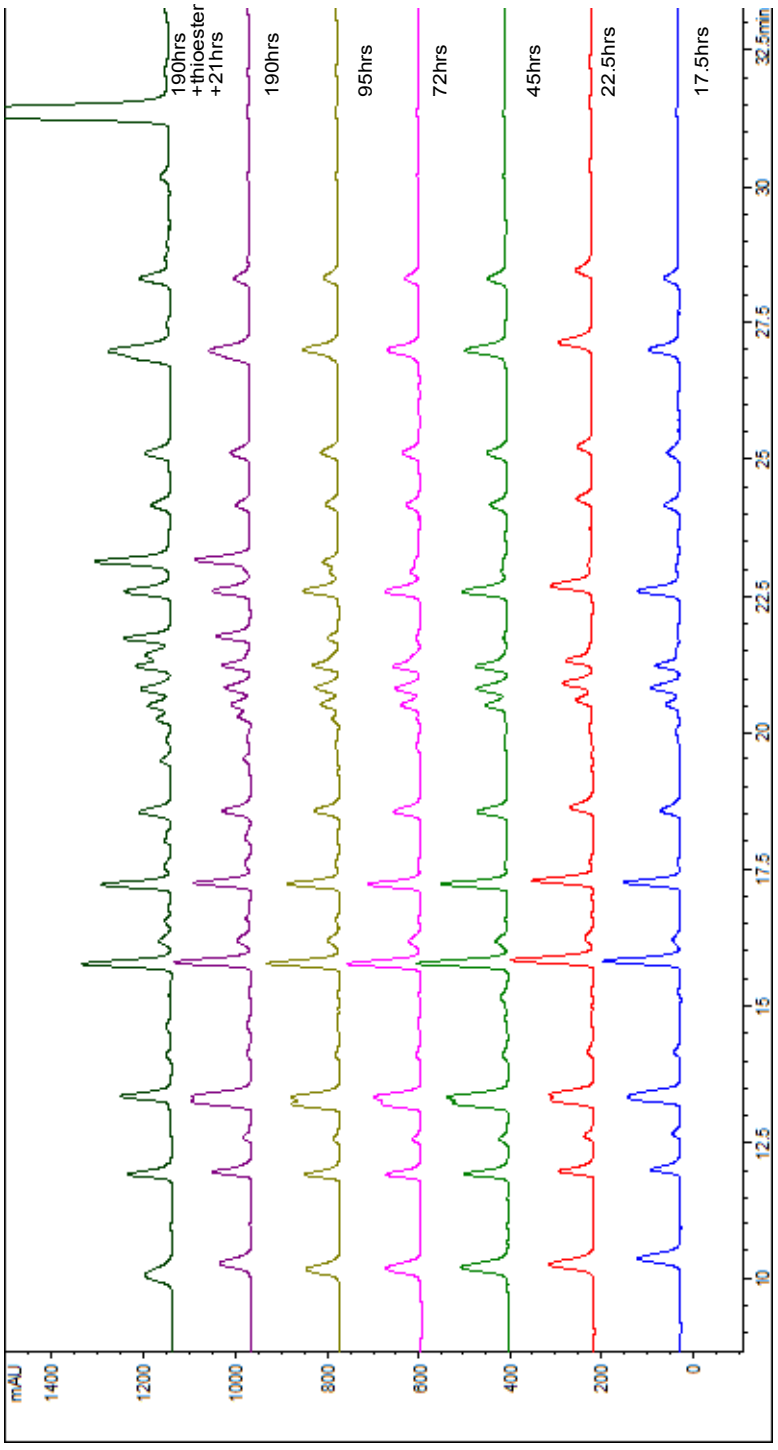


Figure 3.16. Overlay of HPLC traces of extended dithioester library. Initial conditions : side chain dithioester **148** (0.16 mM, 1 equiv) and substituted side chain dithioester **156** (0.16 mM, 1 equiv), side chain thiols MMPr **58** (12.97 mM, 400 equiv), MMB **60** (12.97 mM, 400 equiv), MMPe **63** (12.97 mM, 400 equiv), MMHp **67** (12.97 mM, 400 equiv) and A-ring thiol MCD **43** (12.97 mM, 400 equiv). Buffer : 50 mM MOPS, pH 7.3, 1 mM EDTA, 300 mM KCl, 0.15 mM penicillin G.

3.4.5 Effect of temperature

One concern was the thermal stability of the VDR. If stability was an issue, maybe the dynamic experiment could be performed at sub-ambient temperature to maybe prolong VDR lifetime. Using the same conditions and components of the extended library just mentioned, we tested the thioester exchange at different temperatures in order to judge whether it was feasible or not. Shown in Figure 3.17 are the results of the three libraries after 72 hrs of exchange at 5, 15, and 25 °C. Those libraries are sufficiently similar in composition that it seems viable to use a temperature lower than 25 °C if necessary.

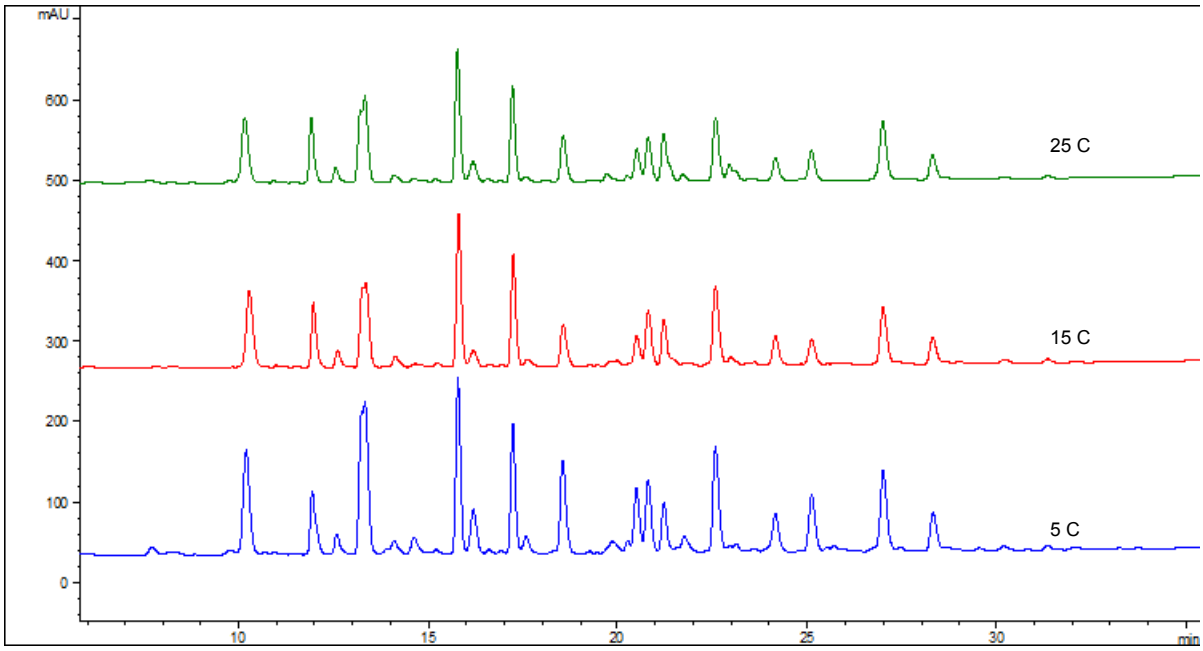


Figure 3.17. HPLC traces of thioester exchanges at 5, 15, and 25 °C. Data points taken at 72 hrs.

3.5 Dynamic libraries in the presence of VDR to identify new analogues

With optimal conditions for thioester exchange in hand, we were ready to test the concept of a vitamin D receptor targeted dynamic combinatorial library.

3.5.1 Preparation of GST- *h*VDR(LBD)

In dynamic combinatorial chemistry, the amplification of a library member is proportional to the target's concentration and thus, to maximize amplification and simplify detection, the target must be used in stoichiometric amounts. Thus, since DCL synthesis requires large amount of highly purified protein, we needed access to large quantities of the vitamin D receptor (5-10 mg). To prepare usable quantities, we prepared a glutathione-S-transferase (GST) conjugate of the human vitamin D receptor ligand binding domain, *h*VDR(LBD). The GST fusion system is a very common method for the expression, purification and detection of proteins that can be produced in *Eschericia coli*.[20] In collaboration with Prof. Karine Auclair and her student Amélie Ménard, we settled on a method published by Kumar which reported the isolation of adequate quantities of the protein.[21] The first step was to clone the DNA coding for the glutathione-S-tranferase (GST) tagged vitamin D receptor (received as a generous gift from Dr. Rajiv Kumar, Mayo Clinic). Next, BL21 (*E.coli*) cells were used to express this plasmid using standard procedures. The GST-*h*VDR(LBD) was purified from bacterial lysates by affinity chromatography using immobilized tripeptide glutathione (GSH), the natural ligand for GST.

3.5.2 Exchanges in presence of GST- *h*VDR(LBD)

With the receptor in hand, we were ready to perform the dynamic library experiment. However, when a dynamic library made from core aromatic dithioester **147**, top chain thiol MMPe **63** and A-ring thiol MCD **43** was combined with GST-VDR, we did not witness an equilibrium shift as desired. Instead, **we observed a rapid and non-selective destruction of the library components**. In less than 2 hrs, 90 % of our dithioesters had disappeared (data not shown). To probe the origin of the destruction, we executed multiple control

experiments.

Bovine serum albumine (BSA)

As a first step, we addressed the question of how much of the observed loss of thioester was caused by nucleophilic functionalities on the protein backbone. We suspected from the beginning that our thioesters could be degraded to some extent by any proteins (either via acylation of nucleophilic amines or via reaction with cysteines). However, we did not anticipate the magnitude of the loss observe in our first experiment. As a control, we used bovine serum albumin (BSA), a commercially available protein. BSA is a single 66 kDa polypeptide chain consisting of about 583 amino acid residues. At pH 5-7, it contains 17 intrachain disulfide bridges and 1 thiol group.[22] We subjected A-ring aromatic dithioester **147** to long exposures to BSA and monitored its concentration over time.

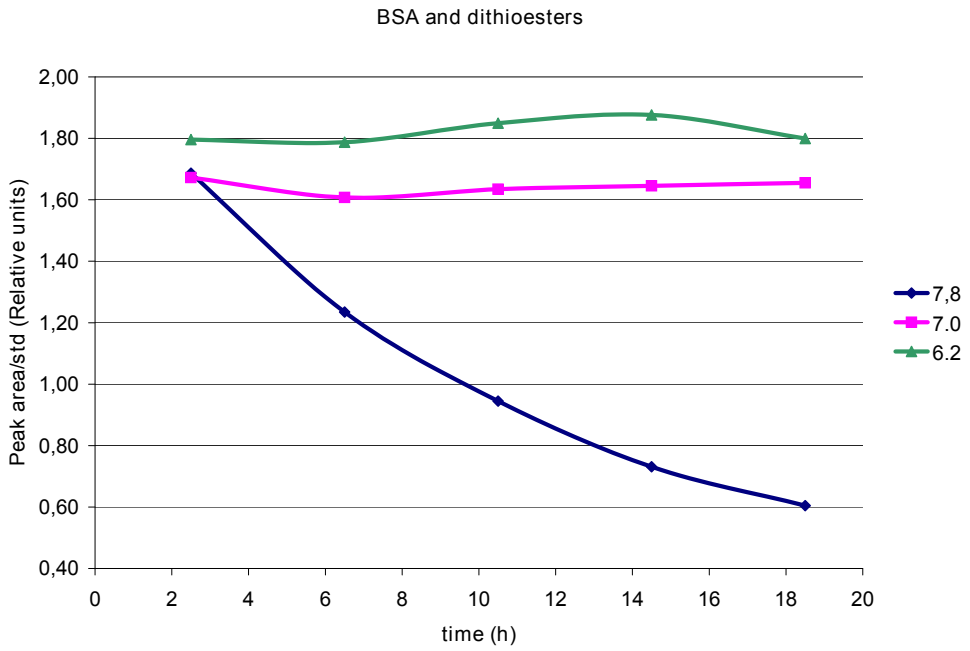


Figure 3.18. Control experiment using bovin serum albumine to evaluate the possible destruction of library components by proteins. Conditions : BSA (0.60 mg, 0.0364 mM, 1 equiv), A-ring dithioester **147** (0.0364 mM, 1 equiv). Buffer : 10 mM TEA, 1 mM EDTA, 300 mM KCl, 16 mM TCEP · HCl.

As presented in Figure 3.18, the presence of BSA seems to cause some disappearance of the dithioester, especially at pH = 7.8, as was expected (see section 3.4.1). However, it did not cause significant destruction of dithioester in conditions used in the GST-

*h*VDR(LBD) dynamic library (near neutral pH or lower). Thus, the mere presence of a protein should not cause the complete destruction of the library thioesters under our standard conditions.

Equine liver Glutathione-S-Transferase (ElGST)

In a second step, we set up a dynamic library using A-ring dithioester **147** with 2 equiv of side chain thiol MMPe **63** and let it exchange for 9 hours. Then, a stoichiometric amount of commercially available equine liver GST (ElGST) and its bound ligand (GSH) were added to the mix and the fate of the dithioesters was monitored. The outcome is shown in Figure 3.19. It shows a rapid destruction of the library members following addition of ElGST. The rate of destruction was such that less than 11 hours after the addition of ElGST, no more than 28 % of the dithioesters remained.

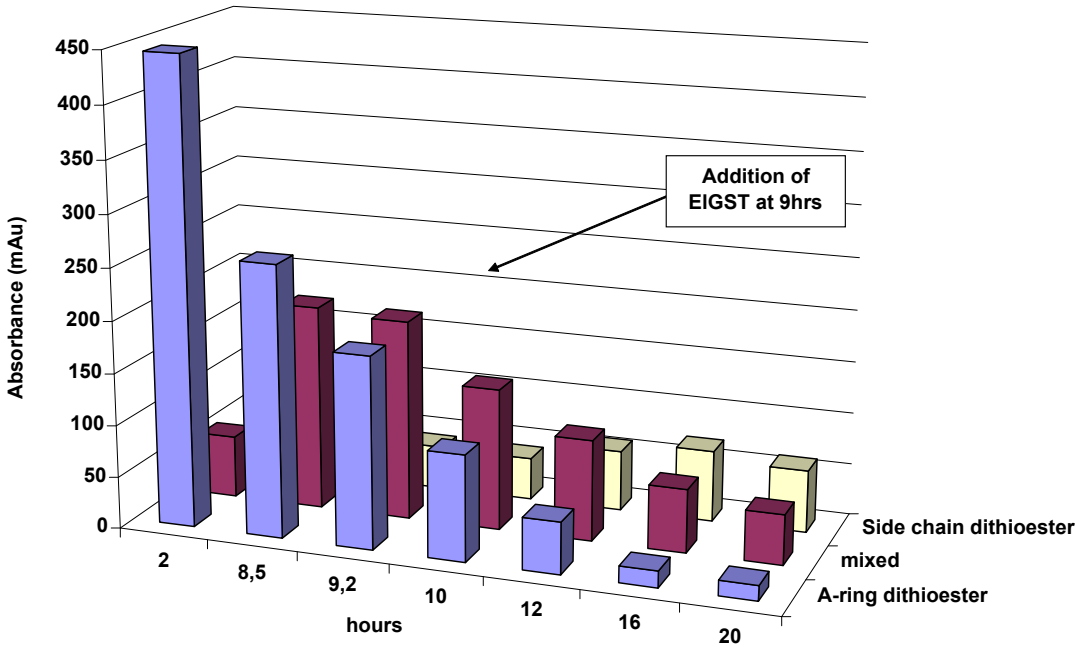


Figure 3.19. Evaluation of a thioester library composition in presence of equine liver glutathione-S-transferase (ElGST). Initial conditions : A-ring dithioester **147** (0,04 mM, 1 equiv), side chain thiol MMPe **63** (0,08 mM, 2 equiv). After 9 hrs, addition of ElGST (0.27 mg of 70% enzyme powder, 1 equiv). Buffer : 10 mM TEA pH 7.0, 1 mM EDTA, 300 mM KCl, 0.2 mM TCEP · HCl.

These two experiments and other controls using ElGST with various amounts of added glutathione (GSH) (not shown) established that the degradation observed in the GST-VDR

experiment was most likely due to the presence of GST, both as part of the conjugate (GST-VDR) and in free form in the VDR preparation (approximately 30 % of the protein content, as judged by gel analysis). After some investigations into the GST gene fusion system, we found that the GST portion of fusion proteins retains its enzymatic activity in the conjugate, activity that consists in living organisms in the catalysis of the conjugation of glutathione (a tripeptide containing cysteine, a thiol) to electrophilic molecules (such as prostaglandins, aromatic xenobiotics or thioesters !) to render them more water soluble and thus facilitating excretion. Without a doubt, the GST tag had to be removed.

3.5.3 Exchanges in presence of *h*VDR(LBD)

We thus implemented a known method for isolating pure VDR by immobilizing the GST-conjugate on a glutathione affinity column and cleaving the fusion protein by treatment with thrombine, releasing pure VDR into the eluent. Concentration and buffer exchange to remove undesirable constituents gave us *h*VDR(LBD) in small aliquots, ready to be used in dynamic experiments. The production afforded approximately 0.64 mg of *h*VDR(LBD) per liter of culture.

With this pure *h*VDR(LBD) (1 equiv), we set up a dynamic experiment using A-ring aromatic dithioester **147** (1 equiv), side chain thiol MMPe **63** (100 equiv) and A-ring thiol MCD **43** (100 equiv). This mixture was then split and VDR was added to one portion. As mentioned before, we used the receptor in stoichiometric amount. The evolution of the library was monitored over time by HPLC with UV detection at 240 nm.

Direct comparison of the two HPLC traces is presented in Figure 3.20. The three dithioesters are labeled *AA*, *AS* and *SS*, in recognition of the nature of the thiols attached to the core unit : *A* stands for A-ring thiol MCD **43** and *S* for side chain thiol MMPe **63**. It is difficult to quantitatively compare the two traces using visual analysis. A representation of the relevant peak areas is shown in Figure 3.21. The results are organized to compare the peak areas of the three dithioesters during the without- (in blue, label *n*) and with-VDR (in purple, label *w*) experiments over time. The graph presents the beginning of the experiment at the back and the 91 hours-point at the front.

Looking at Figure 3.21, we can make several observations. First, at every point during

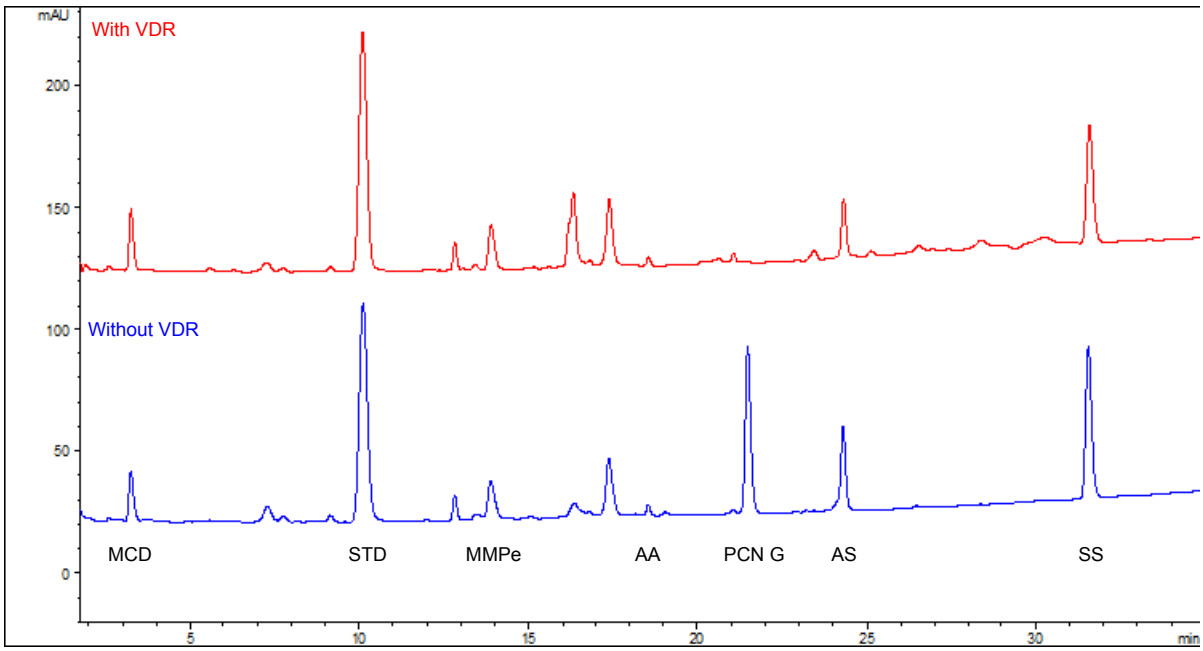


Figure 3.20. Direct comparison of the dynamic experiments in the absence and in the presence of the vitamin D receptor after 34hrs. Initial conditions : A-ring dithioester **147** (0.0309 mM, 1 equiv), side chain thiol MMPe **63** (3.09 mM, 100 equiv), A-ring thiol MCD **43** (3.09 mM, 100 equiv), *h*VDR(LBD) (80 μg, 0.0309 mM, 1 equiv). Buffer : 50 mM HEPES, 10 % glycerol, pH 7.3, 1 mM EDTA, 300 mM KCl, 16 mM TCEP · HCl, 0.56 mM penicillin G.

the experiment, the library with the VDR seems to have less thioesters (AAw, ASw and SSw) present in the mixture compared to the one without the receptor (purple bars are always smaller than blue ones). This complicates analysis as we must examine the relative concentration ratios of the library components and not the absolute amplification of the library member in the with- and without-VDR libraries. Second, the two libraries seemed to have attained equilibrium at around the 34 hrs mark since the composition of each of them at 34 hrs was fairly similar to the one at 64 hrs (also visible when comparing the HPLC traces directly, data not shown). Third, looking at the 34 hrs time point, our proposed point of equilibrium, the side chain dithioester SS is the major one in the mixture for both libraries, followed by AS and AA. (In other words, [SS] >[AS] >>[AA]). Finally, the dithioester SS has been amplified from 66,1 % of total dithioester concentration in the absence of VDR (experiment *a*) to 70,7 % at equilibrium in the presence of VDR (experiment *b*), as shown in Figure 3.22. This represents a very small amplification, quite possibly within the range of uncertainty. It is clear that the presence of the VDR receptor has little

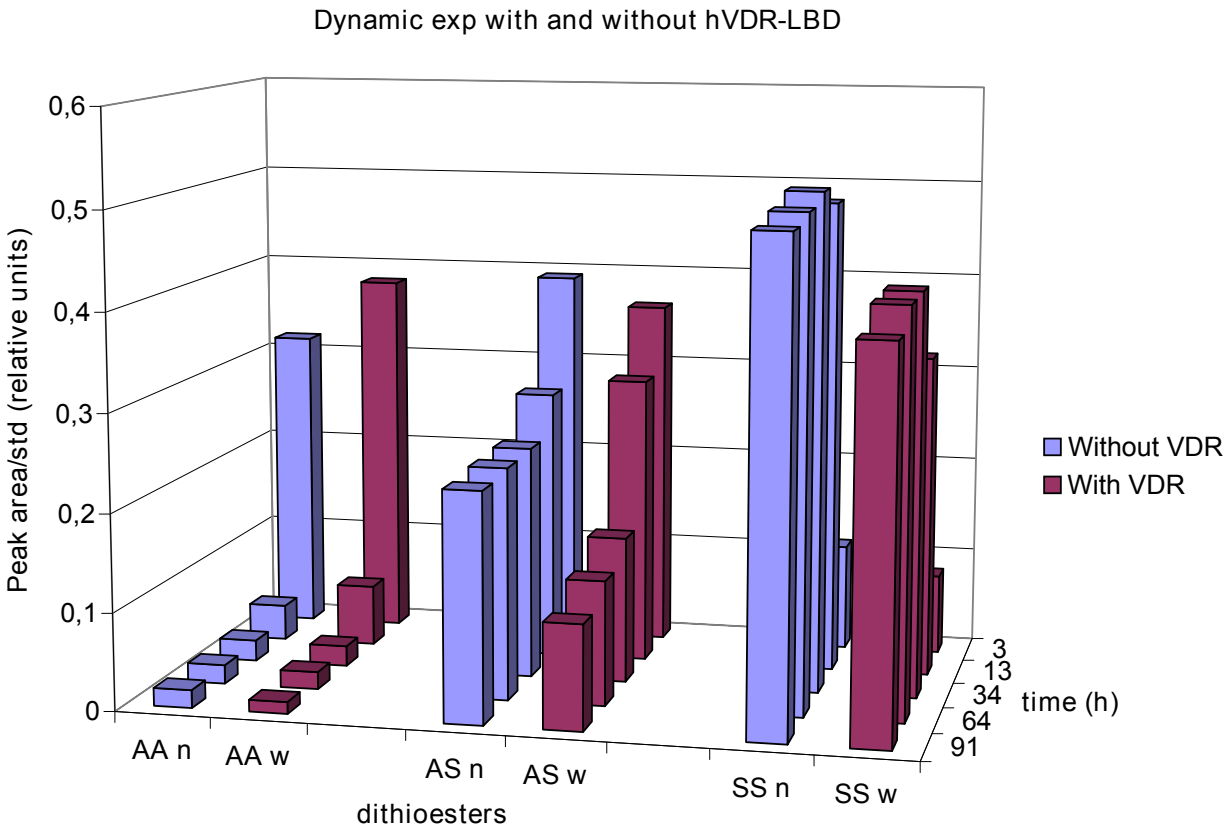


Figure 3.21. Composition of dynamic combinatorial libraries with and without the vitamin D receptor, in terms of dithioesters concentration over time.

to no influence on the library composition. If anything, it shows that dithioester SS is very lightly favored by the VDR. Also, we noticed that the HPLC trace of the library with VDR presents some other singularities (Figure 3.20): the appearance of a tall new peak (Rt : 16.5 minutes) and the disappearance of the peak corresponding to penicillin G (Rt : 22.5 minutes), an antibiotic added to the buffer to prevent bacterial growth. There is the possibility that these two phenomenons were linked. Penicillin G could be consumed over time in the library with VDR and degraded to give some metabolites that eluted at Rt = 16.5 min. On the other hand, there is the possibility that there existed no link between these phenomenons. For one part, penicillin G could be consumed over time and could have disappeared from the HPLC trace and the new peak at 16.5 minutes could be a product of dithioester hydrolysis. Although not proven, this scenario was supported by the observed disappearance of the peaks corresponding to dithioesters.

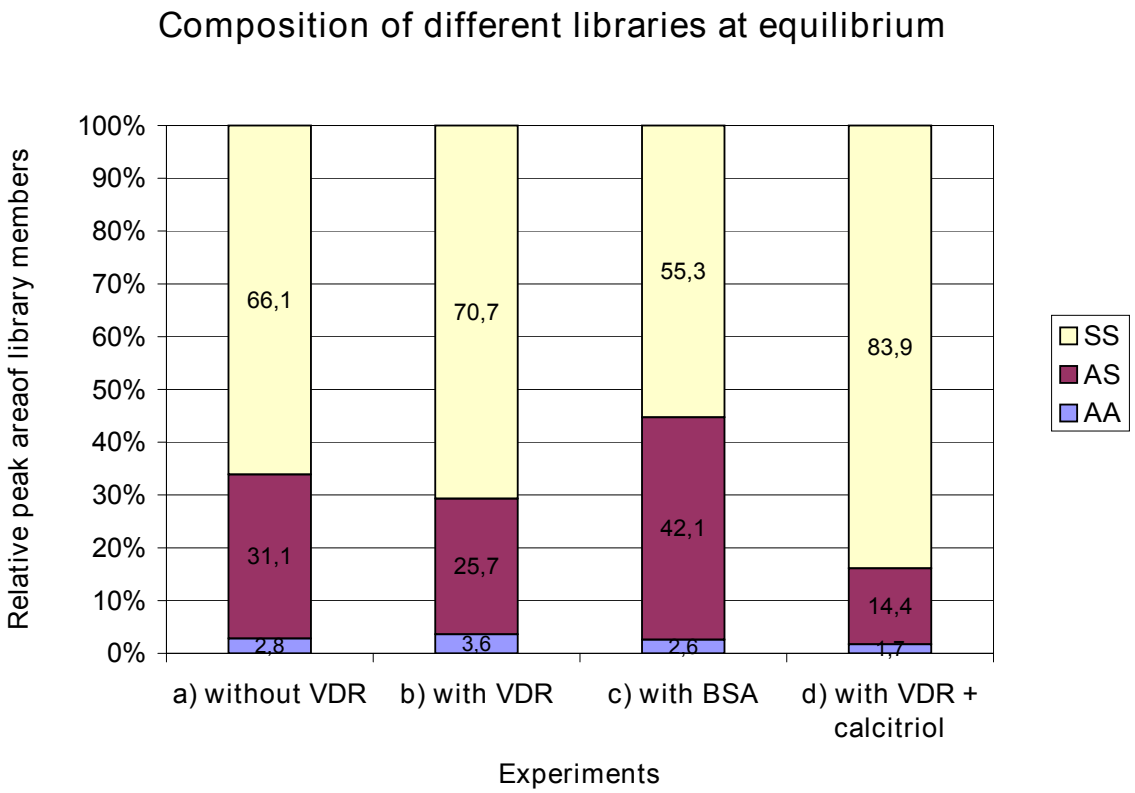


Figure 3.22. Composition of dynamic combinatorial libraries in the presence or absence of different proteins, in terms of relative dithioester content at equilibrium.

Control Libraries

Bovine serum albumin To examine whether the amplification was related to active binding to VDR or to some nonspecific interactions with the protein, we repeated the dynamic experiment replacing the nuclear protein with bovine serum albumin (BSA) (experiment *c*, Figure 3.22). BSA is often used as a control in DCL experiments.[2, 23–27] To our surprise, it appeared as if BSA had a greater influence on the library composition than VDR itself. This does not imply that VDR had no effect. However, it brought up the question as to whether its effect, small as it was, was really from binding dithioesters via its ligand binding domain (LBD) or just from protein surface interaction. We had to keep in mind that a generic protein like BSA had the ability to interact with a DCL and cause amplification by nonspecific interactions of the members with the protein surface.[1]

[1] In this regard, BSA had been used as a convenient mean to eliminate false positives from DCC hits.[27]

Vitamin D receptor and calcitriol To further probe a dynamic library, some researchers have used ligands or inhibitors of their particular receptor or enzyme and reported on the lack ability of the protein in question to influence the outcome of a DCL under those conditions. We performed again a dynamic experiment, this time adding excess natural ligand calcitriol (1α,25-$(OH)_2$ vitamin D_3) to the mix of dithioesters and VDR.[2] We expected calcitriol to be a much better ligand for VDR than any of our dithioesters. If VDR influenced the library through its ligand binding domain, adding calcitriol should impede this influence. Thus, a library with VDR and calcitriol should look like a library without the VDR. However, this was not the case (experiment *d*, Figure 3.22). In this control experiment, dithioester SS was even more favored when VDR and calcitriol were present then when only VDR is used (83.9 % of the total dithioesters concentration compared to 70.7 %) and this again at the expense of the dithioester AS (went from 25.7 % to 14.4 %).

In every experiment where either VDR or BSA were involved, we noticed a significant decrease in visible dithioester concentrations (Figure 3.23). Both experiments employing VDR (*b* and *d*, Figure 3.23) show a 25 % reduction of their dithioester content compared to experiment without any protein, experiment *a*. In the case of the BSA-containing experiment, the disappearance is actually twice as much, with barely 50 % of the starting dithioesters.

While we could not conclusively explain all the results obtained from the various dynamic library experiments with aromatic dithioesters in the presence of the *h*VDR(LDB), we could propose possible explanations. The first possible conclusion from those experiments is that we observed the inability of VDR to influence the library to a significant extent. If the binding affinities of A-ring dithioesters AA **147** and side chain dithioester SS **148** correlate well with their poor ability to induce expression of CYP24 (as found by CYP24 induction assays (section 3.3)), then they might not be ligands for the vitamin D receptor at all. This might explain why the *h*VDR(LBD) could not influence significantly a thioester library made from those molecules. A larger library made from additional dithioester cores and other thiols, in combination with a better analysis method, would help the definitive assessment of the viability of the VDR in the context of a thioester-based DCL.

[2]The amount of calcitriol added to the library was determined from the maximum binding capacity (Bmax), as measured by Kumar et al.[21]

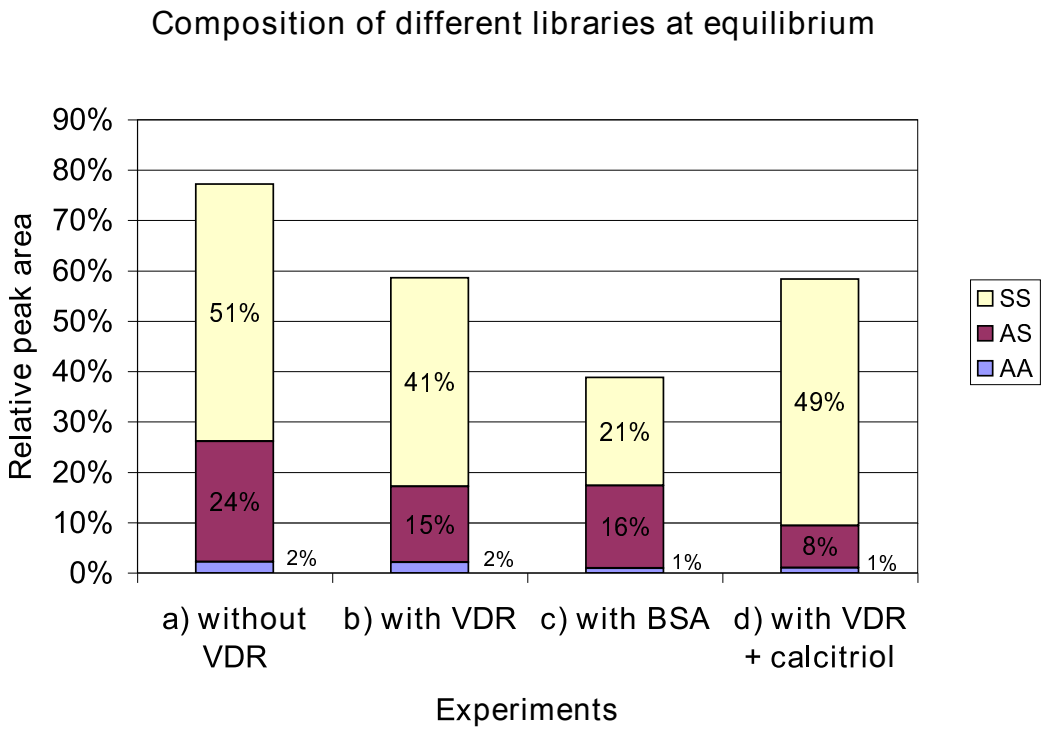


Figure 3.23. Composition of dynamic combinatorial librariesin the presence or absence of different proteins, in terms of absolute dithioester content at equilibrium.

Another explanation to the observed results can come from possible degradation of the receptor. Since we have not been able to test whether the vitamin D receptor was stable or not under the conditions displayed in our dynamic combinatorial libraries (buffer, pH, temperature, etc.), we could not be sure that the VDR was still intact after some time. Employing VDR in binding assays before and after incubation in standard library conditions could possibly tell us to expect a loss or not in the VDR activity over time. Similar tests have been performed in the context of DCL experiments.[23]

3.5.4 Summary

We started from the simple idea of using the vitamin D receptor to select and amplify its own preferred ligand from a pool of reversibly connected building blocks. We created the thiol and thioester building blocks required to implement a double-point exchange dynamic library. After the optimal experimental conditions were found, we performed the dynamic combinatorial experiment in the presence of the VDR. Although the preliminary results were at best ambiguous, we were undeniably getting close to a fully working dy-

namic system using thioesters. However, realizing that the instability of the thioesters might have been complicating the analysis of the dynamic libraries, we decided to pursue our initial goal of creating new 1,25D analogues by testing DCLs made of chemically stable disulfides.

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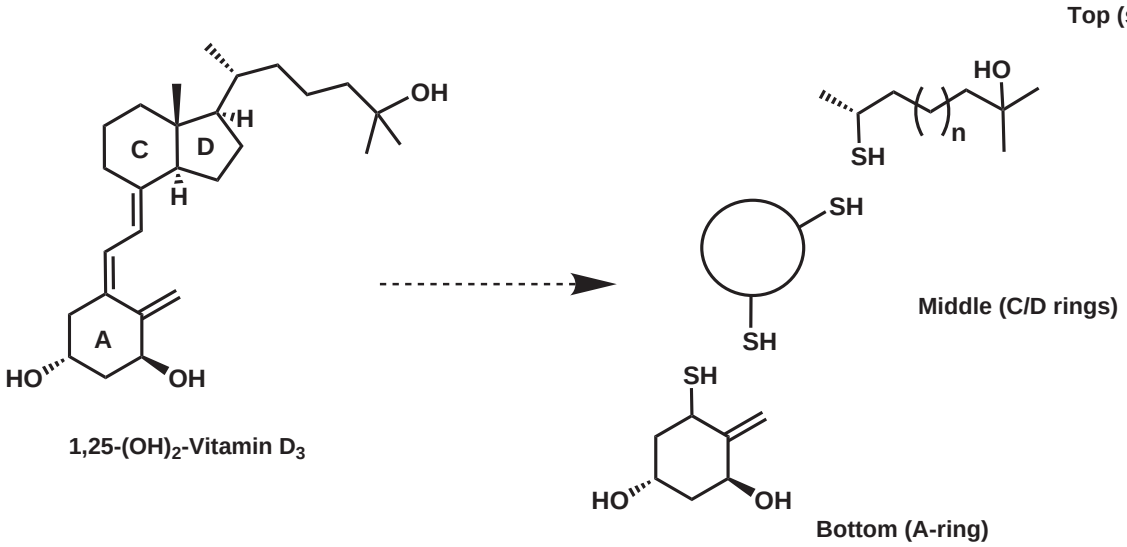
CHAPTER 4

Dynamic Libraries of Vitamin D_3 Analogues Based on Disulfide Exchange

4.1 Introduction

After examination of the possible functional groups possessing the requirements for a dynamic exchange to occur, we chose to examine a system that revolves around the disulfide exchange. Keeping with our goal of simplifying the core portion of 1α,25$(OH)_2$ vitamin D_3, we decided to separate 1α,25-$(OH)_2$ vitamin D_3 in three parts : top chain mimics that include a tertiary OH group capable of H-bonding, an A-ring equivalent bearing two hydroxyl groups and a variable middle portion mimicking the C,D-rings core. As with the previous thioester setup, this one would present the first two parts (top chain and bottom ring) as thiols, while the variable middle piece would now be a dithiol instead of a dithioester. The dithiol core would represent the center of the two-point-of-exchange library (figure Scheme 4.1). These key components could potentially assemble themselves into diverse molecules and hopefully some of them having vitamin D_3-like structures.



Scheme 4.1. Breaking down of calcitriol into fragments suitable for dynamic combinatorial library construction based on disulfide exchange

Scheme 4.2. Thiol-disulfide exchange

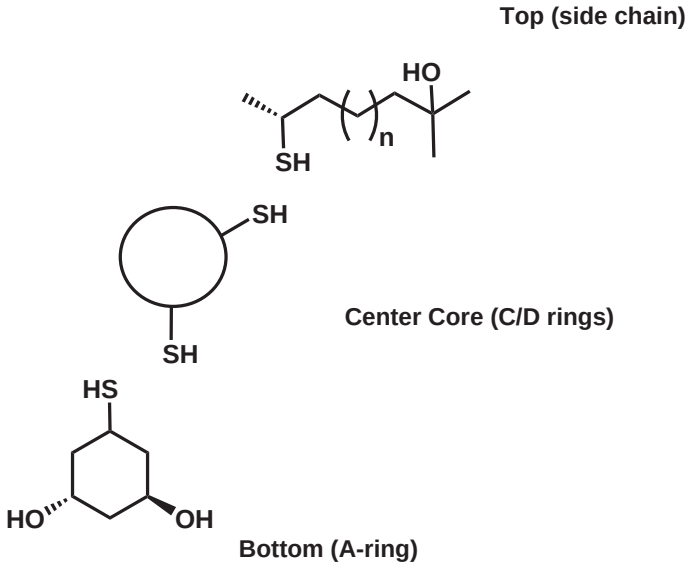
4.1.1 Conditions for disulfide exchange or metathesis reaction

Thiols readily oxidize to disulfide in aqueous solution upon exposure to air or oxidizing agents.[1, 2] When oxidation is taking place, the reaction goes through a moment where thiols and disulfide both exist and can react with each other ; the reversible exchange called thiol-disulfide exchange (or disulfide metathesis) can occur (Scheme 4.2). Disulfide exchange proceeds through an uncomplicated S_N2-type nucleophilic attack of a thiolate anion on a disulfide bond, thus liberating another thiolate as the leaving group.[3] Since thiolates are required for both the oxidation and attack on a disulfide, the reversible exchange requires basic conditions. Accordingly, disulfide exchange is a switchable process. At alkaline pH ($>$7), exchange is rapid. It is increasingly slower at more acidic pHs and totally turned off below pH = 5. Complete oxidation of all available thiols to disulfides also stops the exchange. One last but not negligible characteristic of disulfides is their stability toward many different functional groups. In practice, disulfides DCLs are often prepared in water from thiol building blocks, which are allowed to oxidize to form the desired disulfides.

In our present system with the vitamin D receptor as the template, the disulfide interchange could be expected to occur without any interference with the protein, even though it contains 6 cysteines (but no disulfide bridge). This concern is one of minor importance since disulfide formation is reversible and most cysteines are probably unaccessible to solution phase disulfides at low concentrations.

4.2 Building block synthesis

Our dynamic library design based on disulfide exchange aimed at finding new cores between the A-ring portion and the top side chain in vitamin D_3 analogues. It thus required these center pieces to present two functional groups allowing for a double exchange process (see Scheme 4.3). Our previous work (chapter 2 and 3) already provided a sufficient number of A-ring thiols and side chain thiols. The only additional components necessary were dithiol cores.

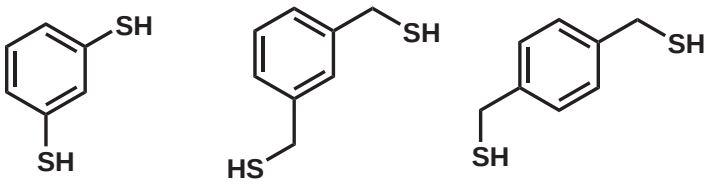


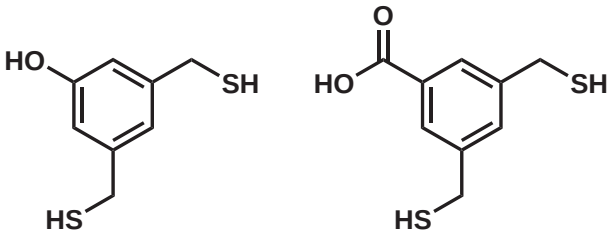
Scheme 4.3. Dynamic library design : an A-ring thiol analogue, a bifunctional center core dithiol and a top chain analogue thiol.

4.2.1 Center cores dithiols

Building blocks based on an aromatic ring such as the ones shown in Scheme 4.4 were selected on both the basis of their ease-of-synthesis and reasonable ultra-violet absorp-

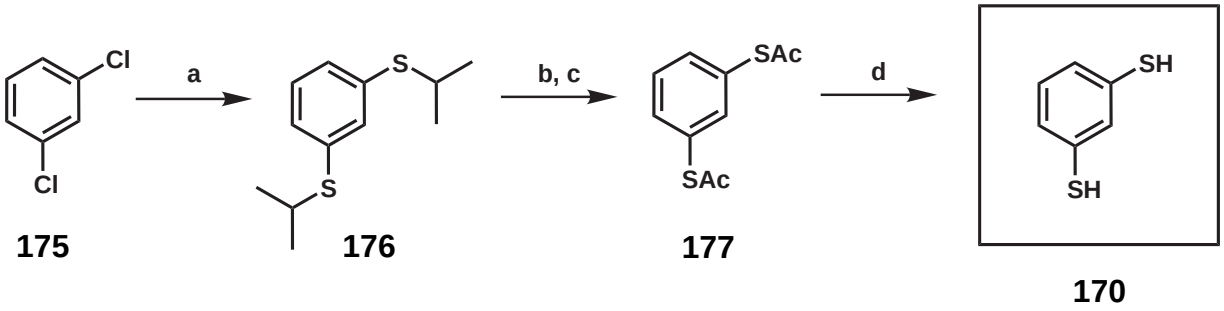
tion, which would greatly simplify the library analysis via HPLC. If necessary, we would also employ molecules bearing polar functional group such as carboxylic acids or phenols. Their individual synthesis are described below and outlined in Scheme 4.6, Scheme 4.7 and Scheme 4.8.



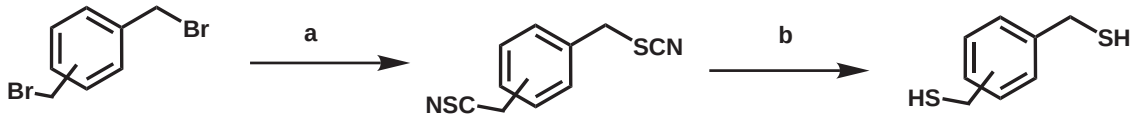


Scheme 4.4. Examples of potential dithiol center cores

Benzenedithiol **170** is commercially available but can also be created in three steps from commercially available 1,3-dichlorobenzene **175** (Scheme 4.5).[4, 5] Nucleophilic aromatic substitution of **175** with sodium 2-propylthiolate at 90 °Cin DMPU as solvent afforded dithioether **176**. Reductive cleavage of the thioether S-C_{alkyl} bond using Li, followed by trapping of the resulting thiolate anions with acetic anhydride afforded bisthioester **177**. Simple deprotection gave access to the desired benzenedithiol **170**. Dithiols **171** and **172** were created from commercially available meta and para α, α'-dibromoxylenes, according to Scheme 4.6. When mixed in acetone with a silica gel-immobilized potassium thiocyanate reagent[6], these benzylic bromides form bisalkylthiocyanates rapidly and efficiently. This KSCN $\cdot$ SiO_2 reagent is easily prepared by drying the product resulting from mixing an aqueous solution of KSCN and silica gel for 30 minutes at room temperature. Moreover, it could be stored on the bench for extended periods without losing its quality. The thiocyanates were subjected to lithium aluminum hydride reduction to cleanly afford benzylic thiols **171** and **172**. No trace of the disulfides (or cyclic/linear polydisulfides) could be detected.



Scheme 4.5. Synthesis of 1,3-benzenedithiol. *Reagents* (a) *i*PrSNa, DMPU, 80 °C, 48hrs, 95 % ; (b) (i) Li, NH_3, -78°C, 20 minutes ; (ii) Ac_2O, DMAP, pyridine, THF, rt, 16hrs ; (c) N_2H_4, MeOH, rt, 1h.

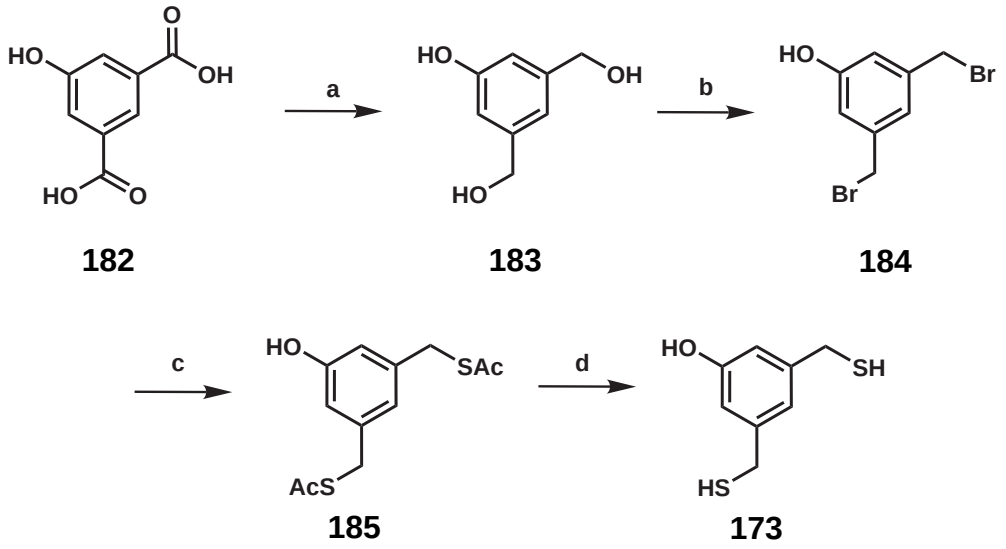


178 or **179** **180** or **181** **171** or **172**

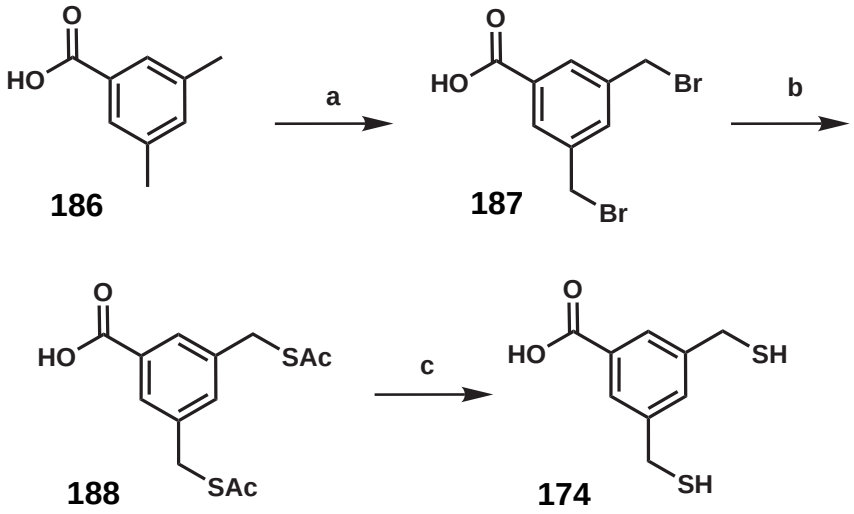
Scheme 4.6. Synthesis of *meta*- and *para*-phenylenedimethanethiols *Reagents* (a) KSCN · SiO_2, acetone, rt, 30 min., 88-90 %.; (b) $LiAlH_4$, THF, 0 °C to rt, 16hrs, 92-95 %.

Dithiol **173** containing a phenolic hydroxyl group was synthesized through similar techniques (Scheme 4.7). A borane reduction of 5-hydroxyisophthalic acid **182** to diol **183** and a functional group conversion by phosphorus tribromide afforded bis(bromomethyl)benzene **184** in 45% yield over two steps.[7] The previously described sequence using KSCN · SiO_2 and $LiAlH_4$ provided dithiol **173** via the thiocyanate **185** in high yield and selectivity (no disulfides were found).

Finally, carboxylic acid-equipped dithiol **174** (Scheme 4.8) was synthesized from 3,5-dimethylbenzoic acid **186** using NBS-mediated radical bromination[8], bromide displacement on **187** with potassium thioacetate and thioester deprotection of **188** with hydrazine.



Scheme 4.7. Synthesis of 5-OH-*meta*-phenylenedimethanethiol **173**. *Reagents* : (a) $BH_3 \cdot DMS$, THF, rt, 5 days, 10.5 % ; (b) PBr_3, $MeCN/Et_2O$, $0\ ^{\circ}C$ to rt, 48hrs ; (c) $KSCN \cdot SiO_2$, acetone, rt, 30 min. ; (d) $LiAlH_4$, THF, $0\ ^{\circ}C$ to rt, 16hrs, 48 % over 3 steps.



Scheme 4.8. Synthesis of 5-carboxy-*meta*-phenylenedimethanethiol **174** : (a) NBS, Bz_2O_2, CCl_4, reflux, 16hrs ; (b) AcSK, MeOH/EtOH (1:1), $0\ ^{\circ}C$ to rt, 48hrs, 35 % over 2 steps ; (c) N_2H_4, MeOH, $0\ ^{\circ}C$ to rt, 4hrs, 85 %.

4.3 Validation of the method : sample dynamic libraries in the absence of receptor

Having secured the necessary building blocks, we were in position to proceed with library generation experiments. First, we needed to establish conditions under which a small set of components would equilibrate and reach equilibrium. Scanning the literature for ways of creating libraries via the disulfide exchange, we found that there are two main categories of conditions that differ in the initial library composition and the process by which the equilibration is initiated. These were the oxidation of mixtures of thiols under air or with chemical oxidant and simple mixing of thiols and disulfides under strict anaerobic conditions. We set out to explore which one most suited our needs.

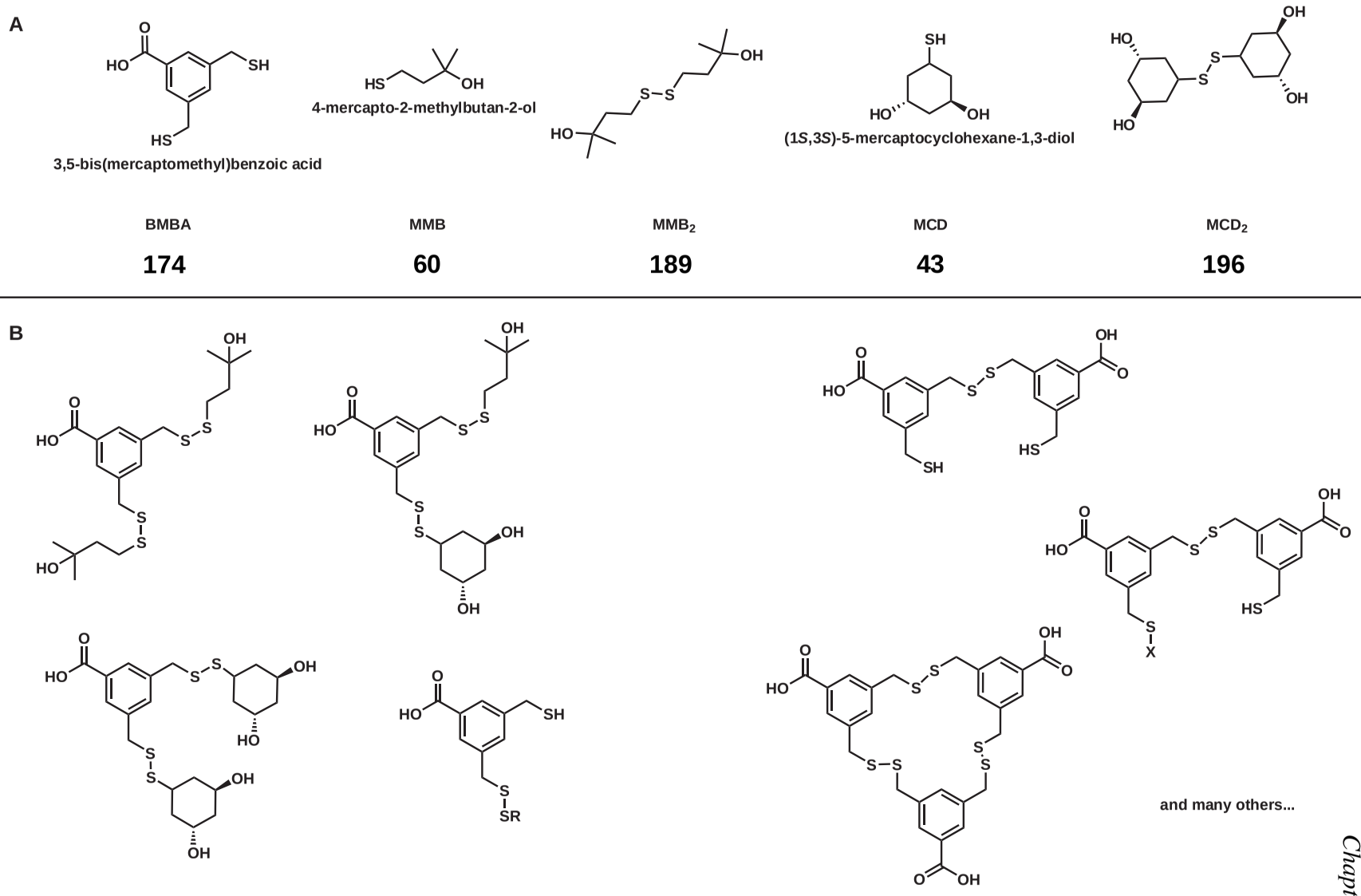
4.3.1 Comparison of disulfide library creation methods

To examine the optimum conditions for library generation, we used 3,5-bis(mercaptomethyl) benzoic acid (BMBA) **174**, side chain thiol 4-mercapto-2-methylbutan-2-ol (MMB) and its disulfide (MMB_2) and A-ring thiol (1S,3S)-5-mercaptocyclohexane-1,3-diol **43** (MCD) and its disulfide MCD_2 (see part **A** of Scheme 4.9). While the size of a disulfide library based on those building blocks is relatively small, it can still generate a tremendous amount of diversity. Part **B** of Scheme 4.9 shows a small number of the potential library members that are expected to be formed.

Disulfide exchange via oxidation of mixtures of thiols

First, we investigated the use of air oxidation starting from mixtures of thiols using a simple setup of Tris buffer in capped 2-mL eppendorf vials equipped with a needle piercing the cap. This setup allowed for air-mediated (O_2-mediated) oxidation of thiols to disulfides, effectively starting the equilibration process. Eventually, the exchange would come to a halt when all thiols were converted to disulfides.

We tested four different starting compositions to generate libraries, as can be seen in Table 4.1. All experiments contained the core dithiol BMBA **174** at 0.4 mM concentration with either two thiols (thiols MCD and MMB, experiment 1), a thiol and a disulfide (thiol



Scheme 4.9. Building blocks used to test conditions for disulfide library generation.

MCD and disulfide MMB_2, experiment 2 and thiol MMB and disulfide MCD_2, experiment 3) or two disulfides (MCD_2 and MMB_2, experiment 4).

Table 4.1: Composition at the start of disulfide exchange experiments 1 to 4 under aerobic conditions.

	Experiments			
	1	2	3	4
Substances	Concentration (mM)			
BMBA **174**	0.4	0.4	0.4	0.4
MCD **43**	20	20	-	-
MMB **60**	20	-	20	-
MCD_2 **196**	-	-	10	10
MMB_2 **189**	-	10	-	10

The formation of multiple new products was observed by HPLC in all four experiments. As expected from the numerous possible combinations of the starting building blocks (see Scheme 4.9), HPLC traces were quite complex (see Figure 4.1). Tracking of the library composition over time for library composition to remain constant showed that an average of 105 hours was necessary for complete oxidation of the components in the four libraries (not shown). Either the equilibrium had been reached or all thiols had been converted to disulfides by oxidation thus stopping the exchange process at an arbitrary point. All four experiments were set up with identical amounts of each component and, in theory, all four libraries should converge to the same library composition at equilibrium. Clearly, however, the HPLC traces were all quite different indicating that the exchange stopped by air oxidation before equilibrium had been reached (Figure 4.2).

Disulfide exchange via mixing of thiols and disulfides under anaerobic conditions

As an alternative, we examined the simple mixing of thiols and disulfides under strictly anaerobic conditions to prevent premature thiol oxidation to disulfide. Contrary to the method explored above where an equilibrated library would only contain disulfides, running a library experiment under anaerobic conditions should equilibrate to a mixture containing the same proportion of thiols and disulfides it started with.

As before, several parallel experiments were initiated with the same total amount of each component but starting from different amounts of thiols and disulfides. Thiols BMBA,

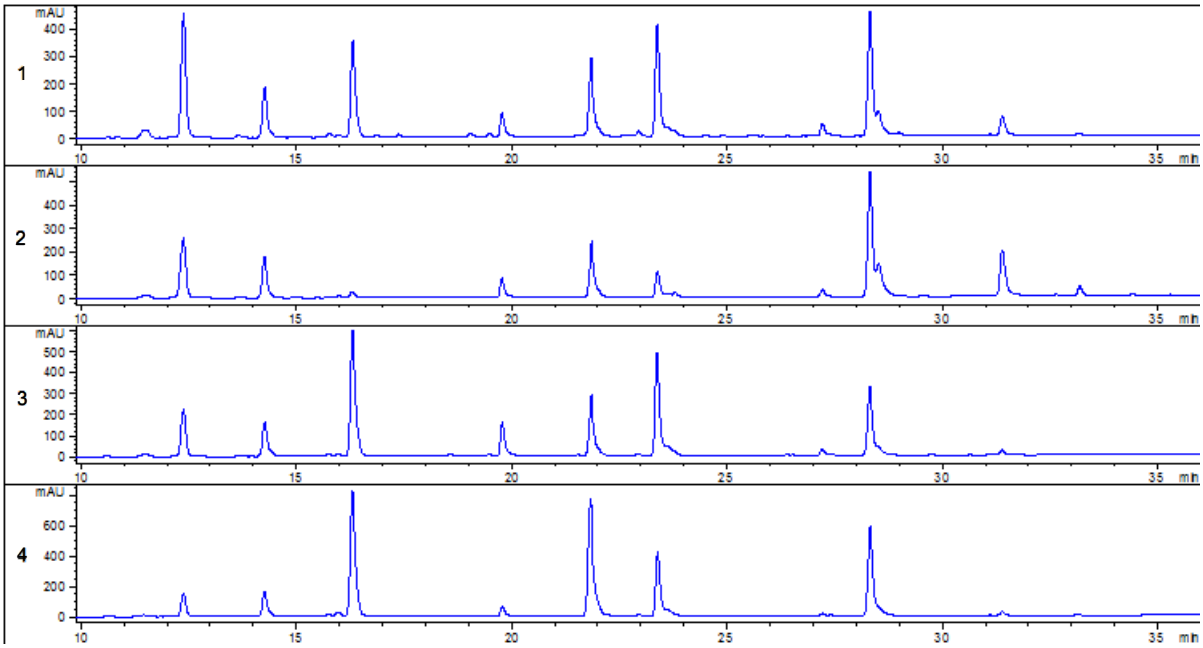


Figure 4.1. HPLC traces of all 4 disulfide libraries generated under aerobic conditions, when no more change was visible. As can be seen, they show different library compositions, probably caused by complete oxidation of thiols before thermodynamic equilibrium was reached. Initial conditions : Components (see Table 4.1 for actual library compositions), Buffer : 50 mM Tris, pH 7.8, 1 mM EDTA, 300 mM KCl, 0.15 mM penicillin G.

MCD and MMB and disulfides MCD_2 and MMB_2 were combined according to the proportions described in table Table 4.2, in degassed Tris buffer and shaken under argon in a glove bag for a total of 261 hours.

Table 4.2: Composition at the start of disulfide exchange experiments 1 to 3 under anaerobic conditions.

	Experiments		
	1	2	3
Substance	Concentration (mM)		
BMBA **174**	0.4	0.4	0.4
MCD **43**	-	20	-
MMB **60**	-	-	20
MCD_2 **196**	10	-	10
MMB_2 **189**	10	10	-

Analysis by HPLC-UV allowed us to monitor the evolution of the library composition over time (Figure 4.3). The four sampling times shown demonstrate the typical behavior

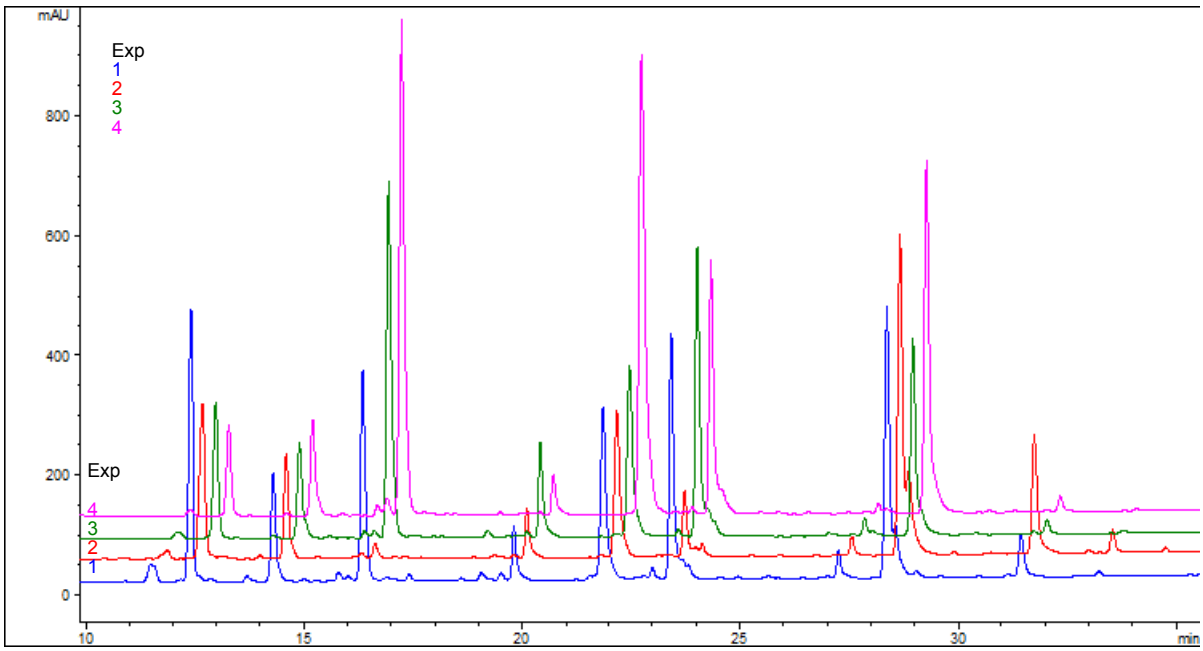


Figure 4.2. HPLC traces of all 4 libraries generated under aerobic conditions, when no more change was visible. As can be seen, they show different library compositions, probably caused by complete oxidation of thiols before thermodynamic equilibrium was reached. Initial conditions : Components (see Table 4.1 for actual library compositions), Buffer : 50 mM Tris, pH 7.8, 1 mM EDTA, 300 mM KCl, 0.15 mM penicillin G.

of a dynamic library. The change in composition is particularly visible between the 18hrs- and 94hrs-data points. Upon integration of the peaks, a graph was made of each of the experiments. One of them representing experiment 1 is shown in Figure 4.4. All three experiments seemed to have reached an equilibrium within 94 hrs (not shown). However, the sampling frequency used does not allow for the determination of the exact moment when a state of equilibrium was reached. For each library, the composition at 94 hrs was very similar to the ones at 162 and 261 hrs (also visible in Figure 4.3).

The three experiments had different initial compositions (shown in Figure 4.5, part **A**) but because they contained the same building blocks (albeit in different proportions and forms (thiols or disulfides)), we expected to see them converge to the same equilibrium composition. The analysis shown in part **B** demonstrates that, despite some minor differences, all three experiments equilibrated to very similar library composition after as soon as 94 hrs.

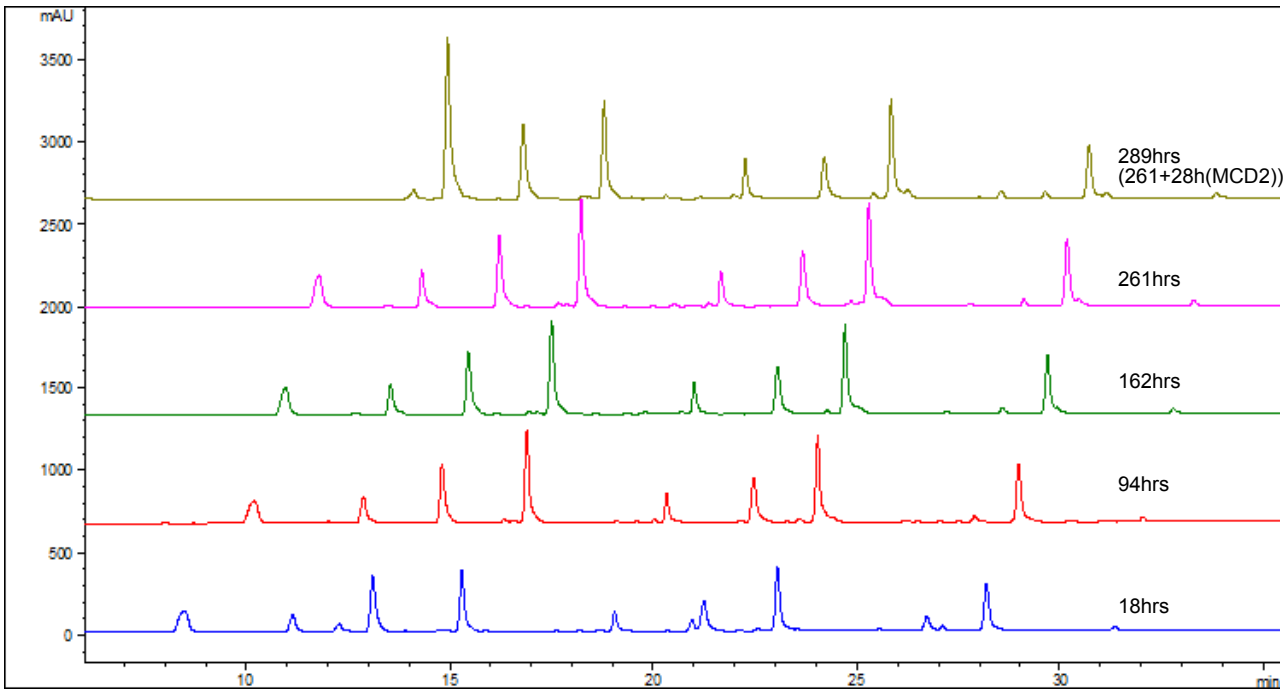


Figure 4.3. HPLC trace of disulfide exchange under anaerobic conditions (experiment 1) over time. Initial conditions : Components (see Table 4.2 for actual library compositions), Buffer : 50 mM Tris, pH 7.8, 1 mM EDTA, 300 mM KCl, 0.15 mM penicillin G.

Furthermore, to prove that the experiments were at the point of equilibrium and thus still contained free thiols able to react with disulfides, we added a large amount of disulfide to each experiment : MCD_2 **196** to experiment 1 and 3 at 261h and MMB_2 **189** to experiment 2 at 261h. As can be seen in Figure 4.6, addition of a new amount of MCD_2 to experiment 1 produced an increase in its concentration, which in turn provoked the expected equilibrium shift. Similar effects were observed for experiments 2 and 3.

In summary, disulfide exchange from a mixture of thiols and disulfides, under argon to prevent air-oxidation, efficiently produces dynamic libraries. It was then decided that we should use this set of condition.

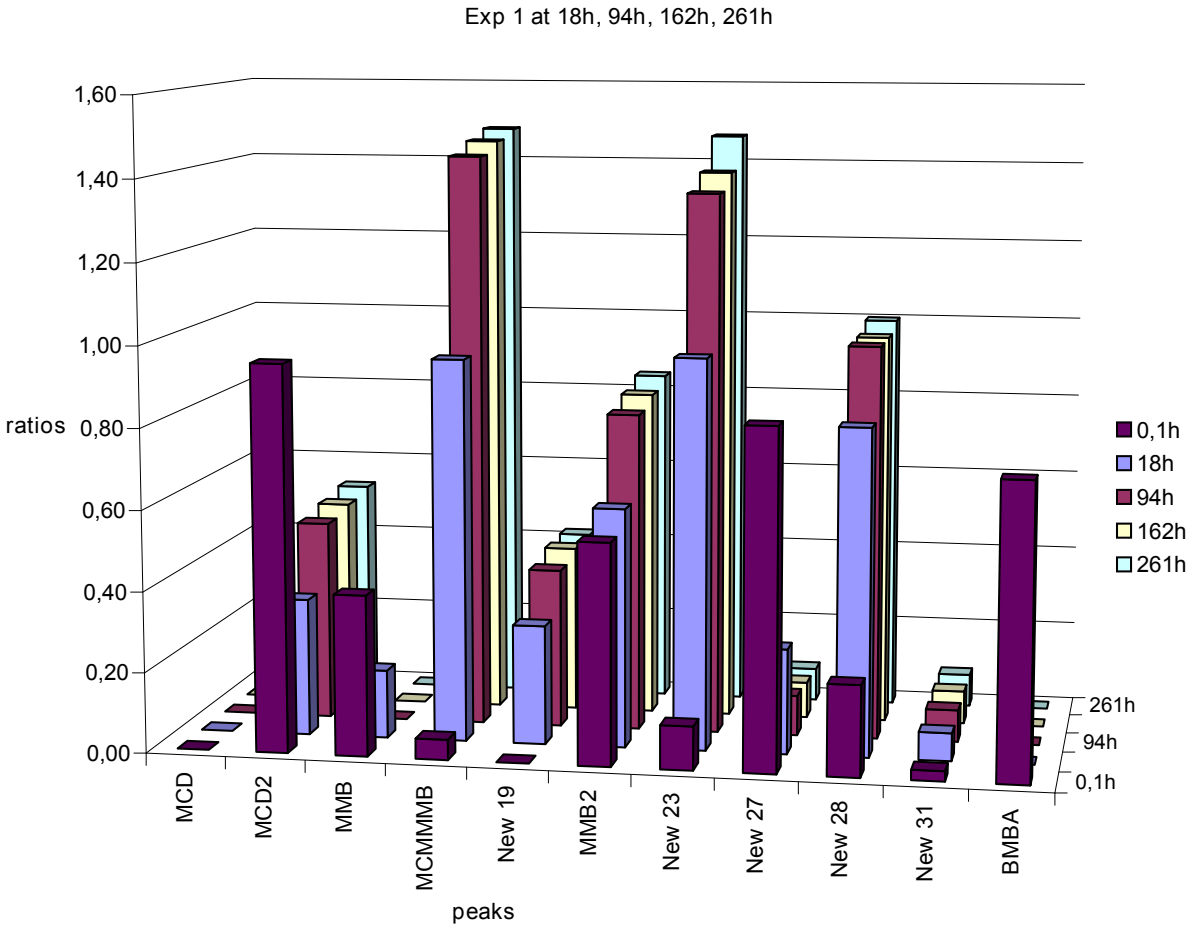


Figure 4.4. Alternative representation of disulfide exchange under anaerobic conditions (experiment 1, Figure 4.3) over time.

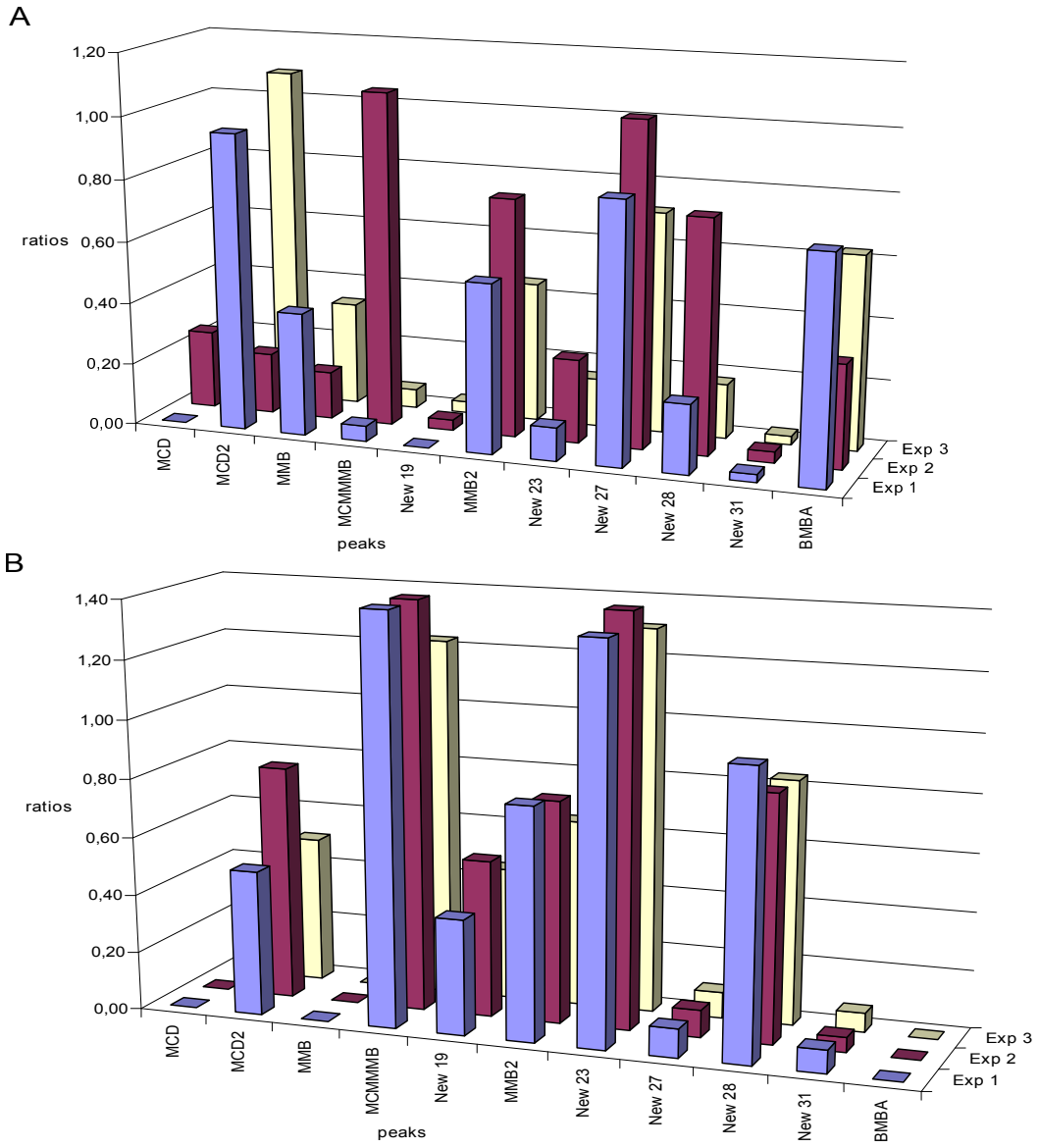


Figure 4.5. Representation of three disulfide exchange experiments under anaerobic conditions over time. **Part A** Initial library composition (0.1 hr). **Part B** Library composition at equilibrium (94 hrs)

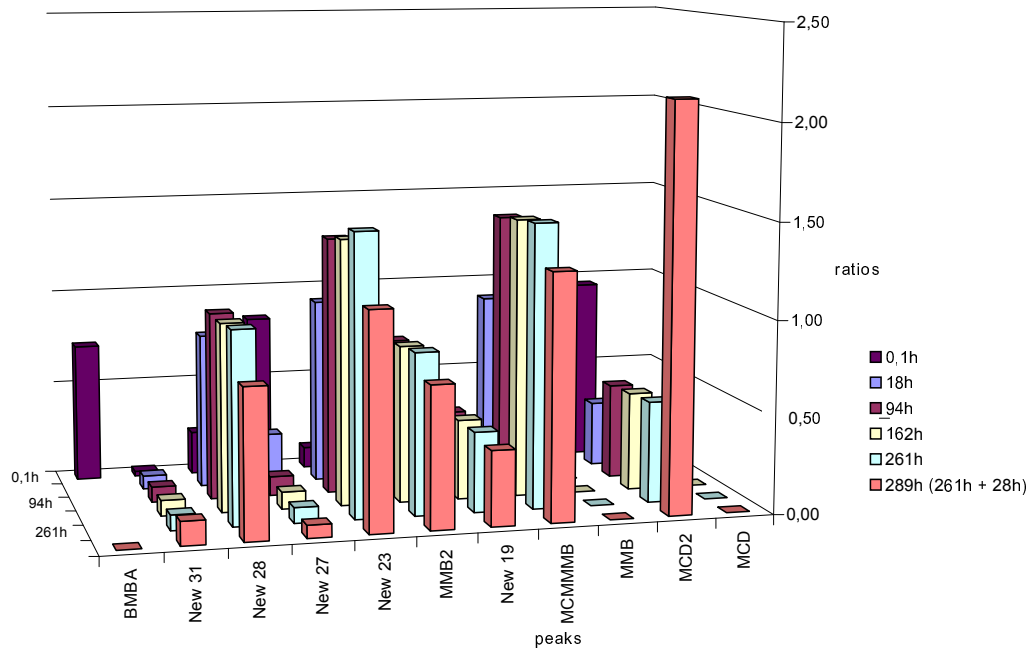
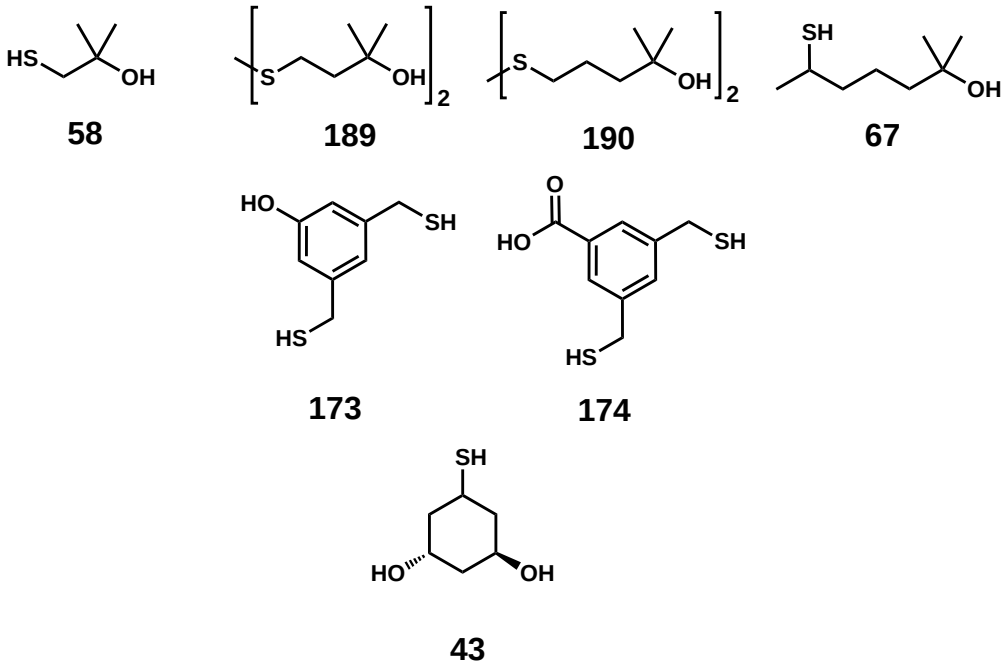


Figure 4.6. Representation of equilibrium shift caused by addition of disulfide MCD_2 to exchange experiment 1. (Note the change of perspective vs Figure 4.4)

4.3.2 Extended library and effect of temperature on rate of exchange

Above, we examined libraries consisting of one core dithiol molecule (BMBA) and two other building blocks, a side chain analogue (MMB) and an A-ring mimic (MCD), the last two in a thiol or a disulfide form. In an effort to increase the level of diversity generated in the disulfide libraries, we conducted an exchange with 4 side chains molecules, two dithiol cores, and one A-ring building block. Shown in Scheme 4.10 are the 7 building blocks involved in this extended library.

Under similar conditions as described above (section 4.3.1), dithiols 3,5-bismercaptomethyl phenol (BMP) **173** and BMBA **174**, A-ring thiol MCD **43**, MMHp **67** and MMPr **58** and disulfides MMB_2 **189** and $MMPe_2$ **190** were combined and the exchange process monitored using HPLC.



Scheme 4.10. Building blocks used in the elaboration of extended disulfide library.

Figure 4.7 presents the evolution of the library over time. An experiment containing this many building blocks is expected to create a large number of distinctive entities, exemplified by the fact that, although we initially only included 7 thiols/disulfides, more than 30 different peaks are visible by HPLC by the 190th hour. As early as 17.5 h, the main

components are already present and only minor changes seem to occur during the remaining course of the experiment. We also verified that the library still contained thiols by adding a large amount of disulfide $MMPe_2$ **190**. This addition brought some change in the visible library composition, as can be seen by comparing the *190hrs* HPLC trace and the *190hrs+22hrs RSSR* HPLC trace. Most likely, thiols were still present at 190hrs to react with the newly added disulfide.

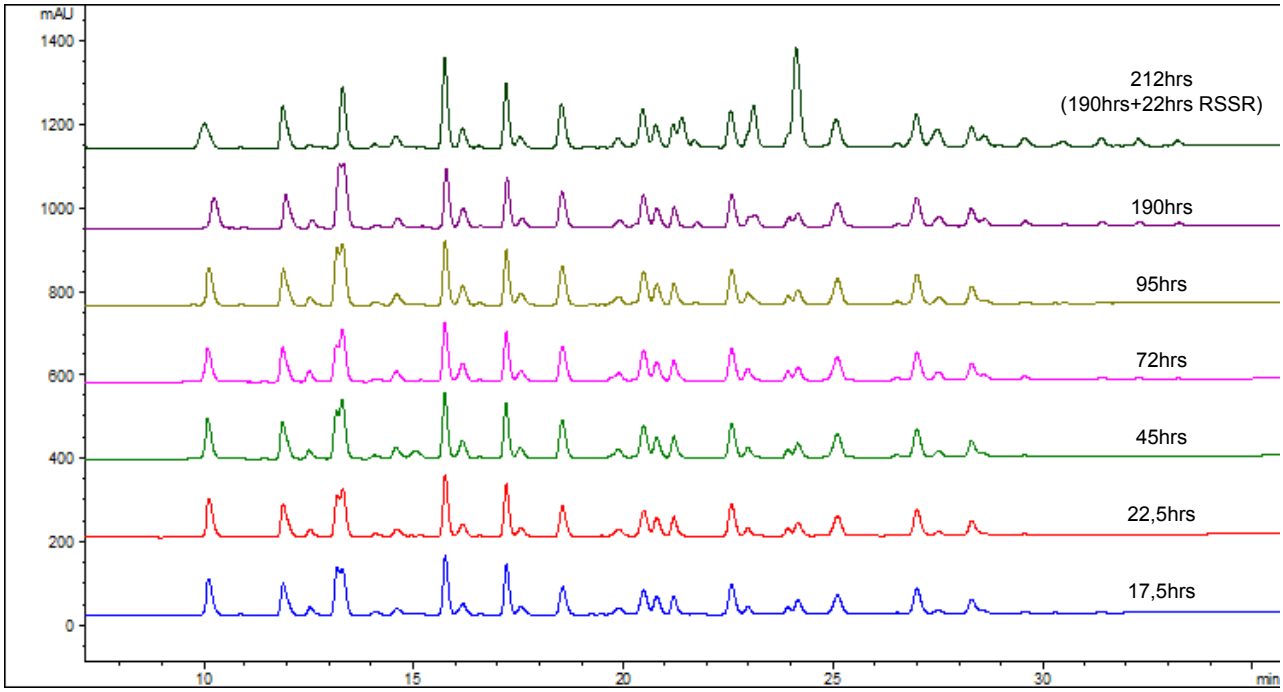


Figure 4.7. Overlay of HPLC traces of the extended disulfide dynamic experiment over time. Initial conditions : dithiols BMBA **174** (0.16 mM, 1 equiv) and BMP **173** (0.16 mM, 1 equiv), side chain thiols MMHp **67** (12.97 mM, 400 equiv) and MMPr **58** (12.97 mM, 400 equiv) side chain disulfides $MMPe_2$ **190** (6.49 mM, 200 equiv) and MMB_2 **189** (6.49 mM, 200 equiv), A-ring thiol MCD **43** (12.97 mM, 400 equiv). Buffer : 50 mM MOPS, pH 7.8, 1 mM EDTA, 300 mM KCl, 0.15 mM penicillin G.

Effect of temperature on rate We were still concerned about the thermal stability of the VDR over the course of a long experiment at 25 °C. Exactly as we had done for the thioester exchange, we tested whether we could perform a disulfide exchange at sub-ambient temperature and prolong VDR lifetime. Using the same conditions and components of the extended library, we tested the disulfide exchange at different temperatures. Figure 4.8 shows the results of the three libraries after 72 hrs of exchange at 5, 15, and 25 °C. Again, those libraries are sufficiently similar in composition that it seems viable to use a temperature

lower than 25 °C if necessary.

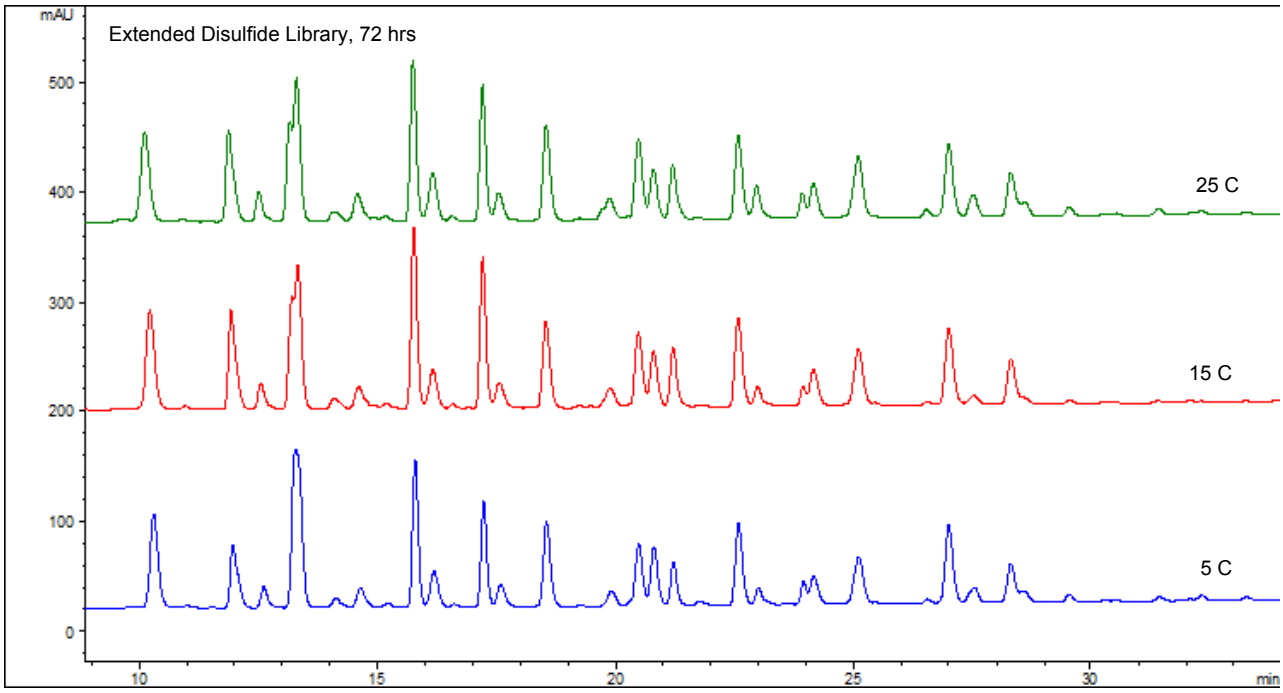


Figure 4.8. HPLC traces of the extended disulfide experiment performed at 5, 15, and 25 °C. Data points taken from at 72hrs.

4.4 Dynamic library experiments in the presence of VDR

4.4.1 Dynamic experiment with *h*VDR(LBD)

We were now in position to perform a dynamic disulfide experiment in the presence of the *h*VDR(LBD). Using dithiol BMBA **174**, A-ring thiol MCD **43** and disulfide MMB_2 **189**, we started two small parallel disulfide libraries, one with the receptor and one without. The results of the experiment with the receptor are shown in Figure 4.9 while the ones from the experiment in absence of VDR are presented in Figure 4.10.

As can be seen from both figures, equilibrium was reached early and the library compositions did not change significantly after around 24 hours. When we compared the two experiments (Figure 4.11), we could see small differences in their chemical compositions. First, the library with VDR did not show the peak corresponding to the antibiotic ampicillin

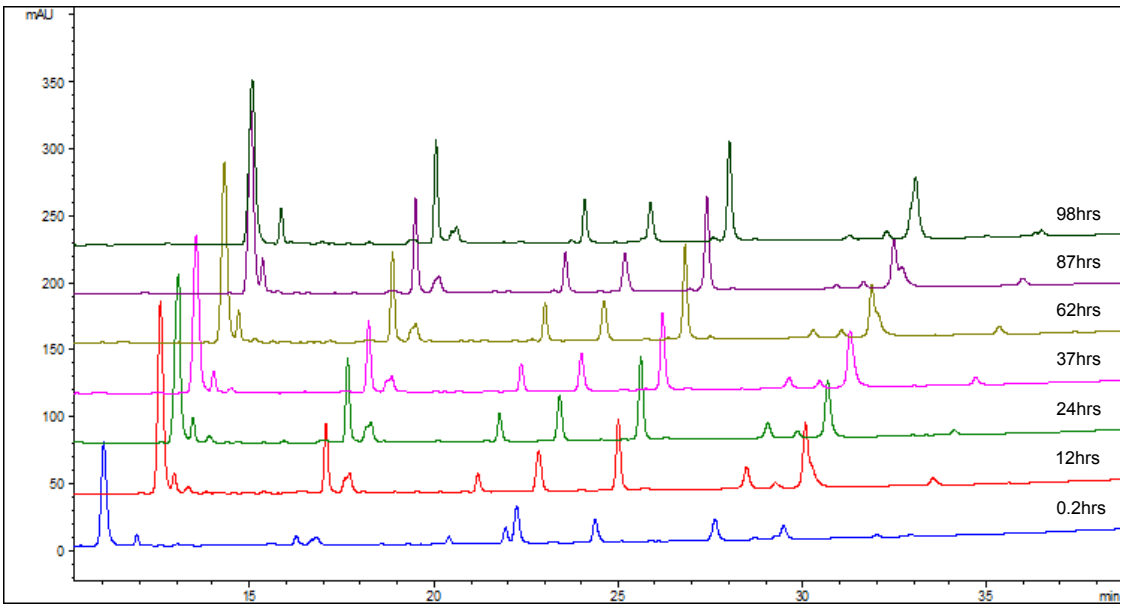


Figure 4.9. Overlay of the HPLC traces of the disulfide experiment in presence of *h*VDR(LBD) performed at 25 °C. Initial conditions : dithiols BMBA **174** (0.112 mM, 10 equiv), side chain disulfide MMB_2 **189** (0.280 mM, 25 equiv), A-ring thiol MCD **43** (0.560 mM, 50 equiv), *h*VDR(LBD) (0.0112 mM, 1 equiv). Buffer : 50 mM PIPES, 10 % glycerol, pH 7.3, 1 mM EDTA, 300 mM KCl, 0.15 mM ampicillin.

used in the buffer (Figure 4.11, top part, Rt : 14.2 minutes). Moreover, when we overlaid the two HPLC traces (Figure 4.11, bottom part) and used the peak of the internal standard as a reference (Rt = 12.0 minutes), four other peaks were different when VDR was present (at 13.5, 15.0, 18.5 and 21.7 minutes). Because the identity of the various new visible peaks was unknown, it was impossible to draw definitive conclusions from those experiments. However, we could propose two explanations. The first was that VDR did not seem to significantly shift the equilibrium of the system and the new peaks are either ampicillin degradation products or some other impurities. The second, slightly more positive, was that the small new peaks were possibly structures being selected by the receptor. They were invisible in the absence of the receptor because their concentration was below the limit of detection but the presence of the receptor amplified them. Such a scenario is possible and has been seen many times in DCLs (see Eliseev's example, introduction [9, 10]). The true explanation probably lies between the two explanations, with some new peaks belonging to ampicillin degradation products and others from amplified library members.

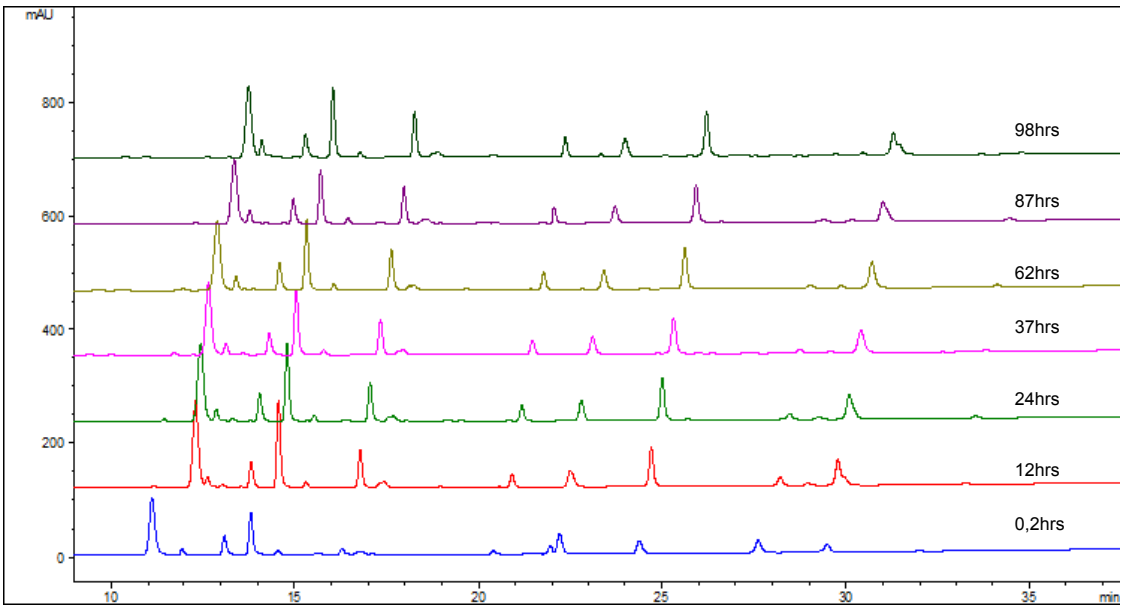


Figure 4.10. Overlay of the HPLC traces of the disulfide experiment in absence of *h*VDR(LBD) performed at 25 °C. Initial conditions : dithiols BMBA **174** (0.112 mM, 10 equiv), side chain disulfide MMB_2 **189** (0.280 mM, 25 equiv), A-ring thiol MCD **43** (0.560 mM, 50 equiv), *h*VDR(LBD) (0.0112 mM, 1 equiv). Buffer : 50 mM PIPES, 10 % glycerol, pH 7.3, 1 mM EDTA, 300 mM KCl, 0.15 mM ampicillin.

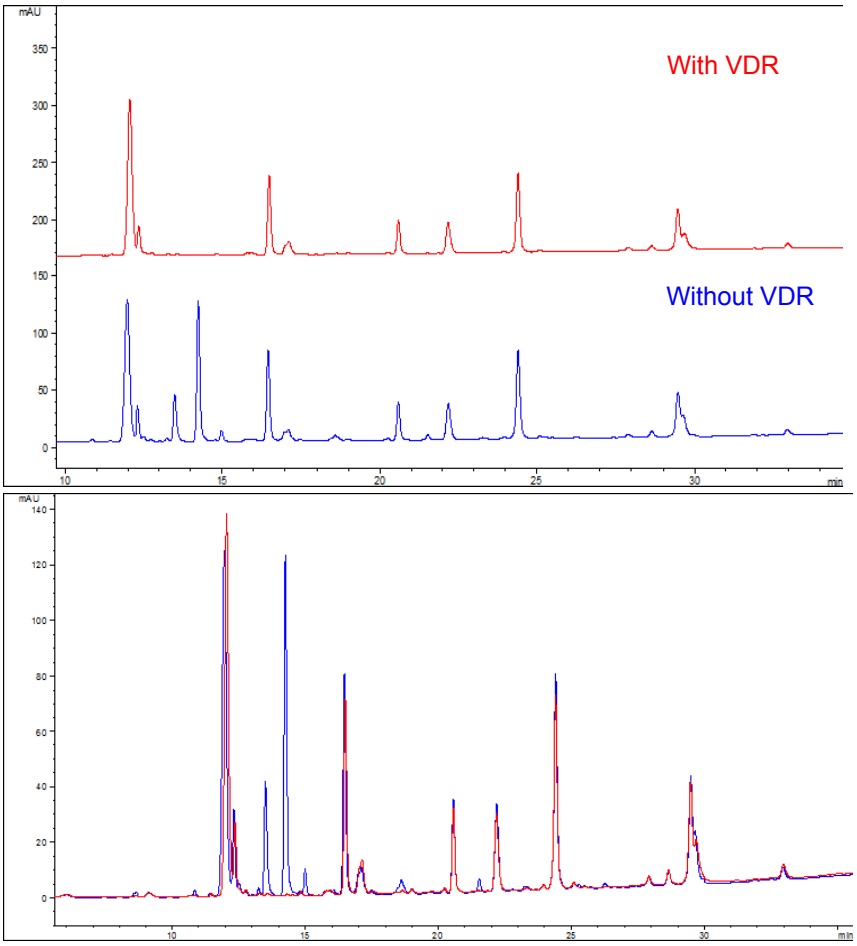


Figure 4.11. Overlay of the HPLC traces of the disulfide experiment in presence and absence of *h*VDR(LBD) performed at 25 °C. Data points taken from at 87hrs.

4.5 Summary

Having met ambiguous results from the dithioester-based dynamic libraries, we set out to explore the use of the disulfide exchange in the discovery of new 1,25D analogues. Dithiol building blocks were rapidly created and since the disulfide interchange is widely known and well characterized in this context, we were able to create medium-size dynamic libraries efficiently. After examining the influence of the temperature on the library composition, we were ready to examine the influence of the VDR on a disulfide library. We found that the vitamin D receptor very lightly shifted the library composition but until further investigations and experimental improvements are made, it is impossible for us to conclude on the nature and the extent of the influence of the receptor.

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CHAPTER 5

Conclusions, Contributions to Original Knowledge, Future Work and Outlook

Conclusion

The research reported in this Thesis developed various methodologies that possess the potential to greatly simplify the synthesis of 1,25 $(OH)_2$ vitamin D (1,25D) analogues. These methodologies have explored different ways to access new bioactive molecules, such as the synthesis of static libraries via conventional chemistry and the creation of dynamic libraries from thioester exchange and disulfide interchange.

First, multiple small scale static libraries were synthesized. The chemistry of the static libraries established the thiol alkylation and the amide coupling as viable sequential reactions in a parallel synthesis scheme. The results showed that the order in which the sequential reaction were accomplished did not have a significant impact on the overall yield of the sequence, while the steric nature of the nucleophilic thiol greatly influenced the yield of thioether formation.

Second, we developed two-point exchange dynamic libraries based on the disulfide exchange and the transthioesterification reaction. The study of the dynamically generated libraries revealed the various advantages and disadvantages of the thioester exchange. For example, we showed that thioesters exchange more rapidly but are less stable at basic pH

than neutral pH. The rate of exchange can be accelerated with either the use of an excess of aromatic thiols or a large excess of less acidic aliphatic thiols. However, the study of dynamic combinatorial libraries was impeded by our inability to sufficiently accelerate the exchange of aliphatic acid thioesters and had to be conducted using only aromatic acid thioesters. We found that thioester libraries could be conveniently generated from core molecules possessing thiols closely related to rate accelerating aromatic thiols. The various thioester libraries were subject to chemical degradation of the thioesters, which highly complicated the analysis of their composition. Nevertheless, a small dynamic thioester library was conducted with and without the vitamin D receptor and the results seem to indicate that the receptor did not influence the library via its binding site. Thioesters were then replaced by disulfides in dynamic libraries. Preliminary experiments under various sets of conditions explored and confirmed the dynamic nature of the disulfide interchange. Again, the vitamin D receptor seemed unable to influence the chemical composition of a small disulfide library.

Contribution to Knowledge

This work contributed to the design and implementation of various synthetic schemes for the synthesis of thiol, dithioester, dithiol, disulfide and amine building blocks. Their synthesis and characterization will now be available to other researchers who wish to use them in the context of synthesis of vitamin D analogues as well as in other research areas.

Our work mainly contributed to knowledge by the development of dynamic libraries of vitamin D-like molecules that exchange at two points under conditions compatible with the viability of the vitamin D receptor. Our work explored and documented the factors influencing the dynamic thioester exchange. It presented methods to control the rate of exchange and the stability of the thioester components involved. It also introduced the use of feedstock molecules to start thioester libraries. The work then examined disulfide exchange as an alternative to transthioesterification. In both types of libraries, we verified that different experiments converged towards the same library composition, confirming a thermodynamic control over them. Experiments leading to libraries of moderate size were created and characterized (e.g., a 40-member thioester library and more than 30-member disulfide library). We also confirmed a method to synthesize large amounts of the human vitamin D receptor ligand binding domain. Our preliminary dynamic experiments with the

receptor showed a probable limitation of the method. The search of high affinity molecules via dynamic combinatorial libraries would be greatly simplified if we were starting from known receptor-binding components, even weak ones. Once possible binders are identified, their optimization through our methodology could easily be accomplished.

Overall, even without being able to identify any new ligand of the VDR with high confidence, our research contributed to the overall development of dynamic combinatorial libraries by gathering data and knowledge on the underexploited thioester interchange and by confirming the disulfide exchange as a privileged reversible process in DCLs. Our research lays the foundation for eventual working libraries where tight-binding molecules will be identified using the vitamin D receptor.

Future Work

Static libraries of calcitriol analogs

We can make some recommendations concerning the project described in chapter 2. First, a larger scale library of thioether-amides should include a greater diversity of synthons, particularly of thiols and bromoacids. Also, the synthesis of unsymmetrical bis-thioethers should figure among the priorities. It would be highly advisable to use the more complex, but most likely less reactive, bis-electrophilic cycloalkyls we created.

Dynamic libraries

Regarding our dynamic libraries, we had realized early that HPLC-UV was possibly not the most adequate method to analyze our experiments. LC-MS is a technique that would not only allow thorough characterization of library composition, but also allow the analysis of larger ones. We researched in that direction but time and technical challenges prevented us to present a working system in this Thesis. Therefore, the first step of a follow-up project would be to implement LC-MS as the analysis method. Another future task would be to test whether the vitamin D receptor is stable under the conditions displayed in our dynamic combinatorial libraries (buffer, pH, temperature, etc.). Until we perform such test, we cannot be sure whether or not the VDR stays active over the entire duration

of the experiment. Employing VDR in binding assays before and after submission to a similar set of conditions would probably allow to quantify a possible loss in activity over time. Tests like this have been performed in the context of DCL experiments.[1] If the very unbiased approach taken initially proved still unsuccessful, it would advisable to use a known VDR ligand and simplify/optimize it using DCLs in presence of the receptor. The main advantage of this approach is that one should expect some influence over dynamic libraries from the target if a known, high-affinity synthon is included in a library.

There are some aspects specific to disulfide-based dynamic libraries that would be worth exploring. First, it seems that disulfide exchange can be accelerated by the action of phosphines, both in organic solvents[2] and in water(coming soon in 2010). It would interesting to test whether the presence of phosphine or aromatic thiols could allow the equilibrium to be reached faster in our setup. Finally, to address the possible issue of small amplification linked to small binding affinities to the target, there exists the idea of using a sacrificial or dummy thiol to occupy core molecules. That way, any amplification of real positives in the presence of the target would be highly visible. Such an amelioration might be crucial to the detection of effective 1,25D analogues in our dynamic libraries.

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CHAPTER 6

Experimental section

6.1 General synthetic methods

CH_3CN was distilled from 4ÅMS. Et_3N and CH_2Cl_2 were distilled from CaH_2 under argon. Toluene, THF and Et_2O were distilled from sodium metal/benzophenone ketyl under argon. All other commercial solvents and reagents were used as received from the Aldrich Chemical Company, Fischer Scientific Ltd., EMd Chemicals Inc., Strem or BdH. All glassware was flame dried and allowed to cool under a stream of dry argon.

Silica gel (60 Å, 230-400 mesh) used in flash column chromatography was obtained from Silicycle and was used as received. Analytical thin-layer chromatography (TLC) was performed on pre-coated silica gel plates (Ultra Pure Silica Gel Plates purchased from Silicycle), visualized with a Spectroline UV254 lamp, and stained with an acidic ceric ammonium molybdate solution, or a basic $KMnO_4$ solution. Solvent systems associated with Rf values and flash column chromatography are reported as percent by volume values.

^1H and ^{13}C NMR, recorded at 300 MHz and 75 MHz respectively, were performed on a Varian Mercury 300 spectrometer. ^1H and ^{13}C NMR, recorded at 400 MHz and 100 MHz respectively, were performed on a Varian Mercury 400 spectrometer. Proton chemical shifts were internally referenced to the residual proton resonance in $CDCl_3$ (7.26 ppm), CD_3OD (3.31 ppm), CD_3CN (1.94 ppm), or d_6-DMSO (2.50 ppm). Carbon chemical shifts were internally referenced to the deuterated solvent signals in $CDCl_3$ (77.2 ppm), CD_3OD (49.0 ppm), CD_3CN (118.3 ppm and 1.3 ppm) or d_6-DMSO (39.5 ppm).

6.1.1 List of Abbreviations and Symbols

1,25D	calcitriol or 1α,25-$(OH)_2$ D_3
25D	calcidiol or 25-OH D_3
1α,25-$(OH)_2$ D_3	calcitriol
BMBA	3,5-bis(mercaptomethyl)benzoic acid
*t*BuOMe	methyl tert-butyl ether
AcCN	acetonitrile
AcOH	acetic acid
aq.	aqueous
cat.	catalytic
CDI	1,1'-carbonyldiimidazole
CH_2Cl_2	dichloromethane or methylene chloride
CT	calcitonine
DBP	vitamin D binding protein
DCC	dynamic combinatorial chemistry
DCL	dynamic combinatorial library
DCM	CH_2Cl_2 or dichloromethane or methylene chloride
ddH_2O	double-distilled H_2O
DEAD	diethylazodicarboxylate
DIAD	diisopropylazodicarboxylate
DIBALH	Diisobutylaluminum hydride
DIPEA	diisopropylethylamine
DMAP	dimethylaminopyridine
DMF	dimethylformamide
DMPU	*N,N'*-dimethylpropylene urea
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
DPPA	diphenylphosphoryl azide
EDTA	ethylenediaminetetraacetic acid
ElGST	equine liver glutathione-S transferase
EtOAc	ethyl acetate
Et_2O	diethyl ether
Et_3N	triethylamine
GST	glutathione-S transferase
GST-*h*VDR	glutathione-S transferase - human vitamin D receptor conjuguate
HBTU	*O*-(Benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate
HEPES	4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid
HOBt	1-hydroxybenzotriazole

hrs	hours
*h*VDR	human vitamin D receptor
IPTG	isopropyl β-D-1-thiogalactopyranoside
LBD	ligand-binding domain
LBP	ligand-binding pocket
LDA	lithium diisopropylamide
LiDBB	lithium di-tert-butylbiphenyl
MCD	(1S,3S)-5-mercaptocyclohexane-1,3-diol
MCD_2	(1S,3S)-5-mercaptocyclohexane-1,3-diol disulfide
MeCN	acetonitrile
MeLi	methyl lithium
MeMPAA	methyl mercaptophenylacetate
MESNa	sodium 2-mercaptoethanesulfonate
MMB	4-mercapto-2-methylbutan-2-ol
MMB_2	4-mercapto-2-methylbutan-2-ol disulfide
MMHe	6-mercapto-2-methylhexan-2-ol
MMPe	5-mercapto-2-methylpentan-2-ol
$MMPe_2$	5-mercapto-2-methylpentan-2-ol disulfide
MMPr	1-mercapto-2-methylpropan-2-ol
$MMPr_2$	1-mercapto-2-methylpropan-2-ol disulfide
MMHp	6-mercapto-2-methylheptan-2-ol
min.	minutes
MOPS	3-(*N*-morpholino)propanesulfonic acid sodium salt
mp	melting point
MPAA	mercaptophenylacetic acid
NaHMDS	sodium hexamethyldisilazide
NCL	native chemical ligation
NMI	*N*-methylimidazole
NMM	*N*-methylmorpholine
NMR	nuclear magnetic resonance
NR	nuclear receptor
o/n	overnight
OD_{600}	optical density of a sample measured at a wavelength of 600 nm
PIPES	1,4-piperazinediethanesulfonic acid
PTG	parathyroid gland
PTH	parathyroid hormone
RPM	rotation per minute
rt	room temperature

Rt	retention time
RXR	retinoid-x receptor
sat.	saturated
TBDMS	tert-butyldimethylsilyl
TBS	tert-butyldimethylsilyl
TCDI	thiocarbonyldiimidazole
TCEP	tris[2-carboxyethyl]phosphine
TEA	triethanolamine
TfOH	trifluorosulfonic acid
THF	tetrahydrofuran
TLC	thin-layer chromatography
TRIS or Tris	tris(hydroxymethyl)aminomethane
TsCl	*p*-toluenesulfonyl chloride
UV	ultra-violet
VD_2	vitamin D_2
VD_3	vitamin D_3
VDR	vitamin D receptor
VDRE	vitamin D response elements

6.1.2 General amide bond formation

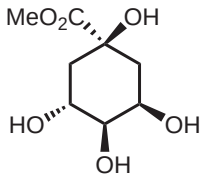
Reagents were mixed neat together in that order : 1.1 equiv amine, 1.0 equiv acid, 3.0 equiv DIPEA (4.0 equiv if amine is an acid salt) and 2 equiv HOBt. The mix was diluted with DMF to obtain a 1M solution (relative to acid) and 1.1 equiv HBTU added to the mixture of all the other reagents at rt. Once it is all dissolved, the reaction is usually done but one additional hour is usually sufficient to achieve completion. The mixture is then quenched with brine (10 volumes relative to DMF) and, if a precipitate appeared, it is filtered and washed with water or, in absence of a precipitate, the biphasic mix is extracted with EtOAc (3 x 10 volumes relative to DMF). The combined organic layers are then washed with 0.1 M aq. HCl and 0.1 M sat. aq. $NaHCO_3$. The organic phase is then dried ($MgSO_4$) and concentrated *in vacuo*.

6.1.3 General acid-mediated TBS deprotection

To a solution of compound in MeOH (15 mL MeOH/1 mmol compound) is added conc. HCl (200 μL per 0.50 mmol of TBS ether) at rt. The reaction flask is then concentrated *in vacuo* on a rotavap to dryness. The process is repeated once then repeated twice more, but excluding the concentrated acid. Crude residues are then dried on high vacuum.

6.1.4 W-7 Raney nickel preparation

Solid NaOH (12.80 g) was suspended in distilled water (50 mL) and the resulting mixture cooled in ice bath and stirred until all NaOH was dissolved. 50 %-50 % nickel aluminum alloy (10 g) was added to the stirred basic solution in portions of 2-3 g, with heating or cooling applied to keep the internal temperature at 50 °C. When addition is over, the falsk is keep at 50 °Cfor however long it takes to reach an overall time of 80 minutes at that temperature since the beginning of the addition. The resulting black powder suspension is poured in a separatory funnel and let to settle. The top clear liquid layer was pipetted out with vacuum. The remaining powder was shaken with water and semi-dried by pipetting twice more. The powder was shaken with 95 % EtOH and semi-dried 3 times, followed by the same procedure with absolute EtOH (3 times). The powder is finally suspended and stored in absolute EtOH.



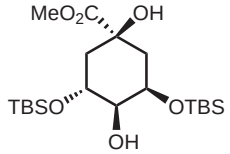
Chemical Formula: $C_8H_{14}O_6$
Exact Mass: 206,08
Molecular Weight: 206,19

6.1.5 Quinic acid route

16 :
(1S,3R,4S,5R)-methyl 1,3,4,5-tetrahydroxycyclohexanecarboxylate

Acetyl chloride (2.044 g, 1.850 mL, 26.02 mmol) was added to a stirring solution of MeOH (37 mL) in flame dried round bottom flask charged with argon at 0 °C. (-)-Quinic acid (10.00 g, 52.04 mmol) was added to the mixture, and the suspension stirred for 16 hr while warming to room temperature. The solid reactant dissolved as the reaction proceeded to afford a pale yellow solution. The reaction was concentrated in vacuo, and the residue dissolved in $CHCl_3$ then concentrated again, repeating this process three times to remove the excess MeOH via azeotropic distillation. The product **16** was isolated as a viscous yellow oil in quantitative yield (10.80 g, 52.38 mmol). Rf = 0.10 (30% EtOAc in hexanes); ^{1}H NMR (300 MHz, CD_3CN) δ 4.04-3.97 (1H, m), 3.96-3.84 (1H, m), 3.65 (3H, s), 3.34-3.26 (1H, m), 2.09-1.88 (3H, m), 1.74-1.63 (1H, m), 4 exchangeable protons unobserved; ^{13}C NMR (75 MHz, CD_3CN) δ 175.0, 76.7 (2C), 71.3, 67.6, 53.0, 42.1, 38.0.

17 :
(1S,3R,4S,5R)-methyl 3,5-bis(tert-butyldimethylsilyloxy)-1,4-dihydroxy cyclohexane carboxylate

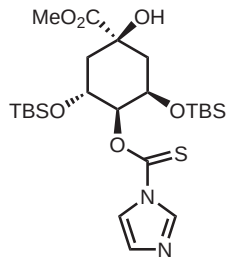


Chemical Formula: $C_{20}H_{42}O_6Si_2$
Exact Mass: 434,25
Molecular Weight: 434,71

In a flame dried, round bottom flask flushed with argon, dMAP (0.6358 g, 5.204 mmol), TBABr (1.730 g, 5.204 mmol), and TBSCl (17.26 g, 114.5 mmol) were added to a solution of methyl quinicate **16** (10.73 g, 52.04 mmol) in DMF (200 mL). The flask

was sealed with a rubber septum and cooled to 0 °C, at which point Et_3N (11.85 g, 16.30 mL, 117.1 mmol) was added to the reaction via syringe. A fine white precipitate formed upon addition of the amine. The reaction was stirred under argon for 16 hr while warming to room temperature. The reaction mixture was then filtered to remove the precipitate, and the filtrate diluted with EtOAc (200 mL) and washed with sat. NH_4Cl (3 x 100 mL), distilled H_2O (100 mL) and brine (100 mL). The organic layer was then separated, dried ($MgSO_4$), and concentrated in vacuo to give the crude product as a yellow, viscous oil. Product **17** was isolated as a fluffy, white solid via FCC (30% EtOAc in hexanes) in 73% yield (16.42 g, 37.76 mmol). Rf = 0.60 (30% EtOAc in hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.52 (1H, br s), 4.36 (1H, dt, J = 4.5, 2.5 Hz), 4.11 (1H, ddd, J = 13.0, 8.5, 4.5 Hz), 3.76 (3H, s), 3.42 (1H, dt, J = 8.5, 2.5 Hz), 2.32 (1H, d, J = 2.5 Hz), 2.18 (1H, ddd, J = 13.0, 4.5, 2.5 Hz), 2.09 (1H, dd, J = 14.0, 2.5 Hz), 2.01 (1H, ddd, J = 14.0, 4.5, 2.5 Hz), 1.82 (1H, dd, J = 13.0, 10.5 Hz), 0.90 (18H, d, J = 6.0 Hz), 0.15 (6H, d, J = 7.0 Hz), 0.11 (6H, d, J = 5.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.8, 76.3, 76.1, 71.6, 68.7, 52.8, 42.8, 38.0, 26.1 (6C), 18.4 (2C), -4.0, -4.3, -4.4, -4.7.

18 :
(1S,3R,4S,5R)-methyl 4-(1H-imidazole-1-carbonothioyloxy)- 3,5-bis(tert-butyldimethylsilyloxy)-1-hydroxycyclohexanecarboxylate

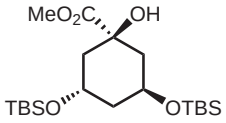


Chemical Formula: $C_{24}H_{44}N_2O_6SSi_2$
Exact Mass: 544,25
Molecular Weight: 544,85

Alcohol **17** (5.534 g, 12.73 mmol) was dissolved in CH_2Cl_2 (14 mL) in a flamed dried round bottom flask. To this stirring solution was added dMAP (0.1555 g, 1.273 mmol) and TCdI (3.402 g, 19.09 mmol) which dissolved into solution after several hours of stirring. The reaction vessel was sealed with a rubber septum and flushed with argon, and the reaction was stirred at room temperature for 3 days. The reaction mixture was then concentrated to give the crude product as a dark orange, viscous oil which was loaded directly on the silica gel. **18** was isolated as a pale yellow, viscous oil via FCC (gradient 30% to 50% EtOAc in hexanes) in 92% yield (6.401 g, 11.75 mmol). Rf = 0.20 (30% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.28 (1H, s), 7.54 (1H, s), 6.95 (1H, s), 5.43 (1H, dd, J = 8.5, 3.0 Hz), 4.61-4.54 (2H, m), 4.50-4.41 (1H, m), 3.70 (3H, s), 2.27-2.13 (2H, m), 2.04-1.92 (2H, m), 0.82 (9H, s), 0.70 (9H, s), 0.01 (3H, s), 0.00 (3H, s), -0.05 (3H, s), -0.17

(3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 183.6, 173.2, 137.0, 130.7, 117.7, 86.0, 75.1, 68.3, 65.4, 52.7, 43.0, 38.0, 25.8, 25.7 (2C), 25.5 (2C), 25.4, 17.8, 17.7, -4.2, -4.7, -4.9, -5.6.

19 :
(3S,5S)-methyl 3,5-bis(tert-butyldimethylsilyloxy)-1-hydroxy cyclohexanecarboxylate

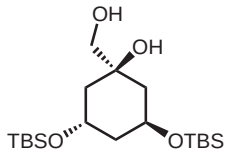


Chemical Formula: $C_{20}H_{42}O_5Si_2$
Exact Mass: 418,26
Molecular Weight: 418,72

Thiocarbonyl **18** (9.490 g, 17.42 mmol) and $NaH_2PO_2xH_2O$ (7.660 g, 87.09 mmol) were dissolved in 2-methoxy-ethanol (230 mL) under argon in a flamed dried round bottom flask equipped with a reflux condenser, and heated to reflux with a heating mantle. In a separate flask, AIBN (0.5714 g, 3.484 mmol) was dissolved in 2-methoxy-ethanol (20 mL), Et_3N (approx. 2 mL) was added to this solution until a pH of 8 was obtained. Half the AIBN solution was added to the refluxing reaction mixture. The reaction was refluxed for 3hrs with addition of the second half of the AIBN solution after 1 h. The reaction was then cooled to room temperature and diluted with EtOAc (200 mL) and washed with sat. NH_4Cl (3 x 100 mL), distilled H_2O (100 mL) and brine (100 mL). The organic layer was then separated, dried ($MgSO_4$), and concentrated in vacuo to give the crude product as a clear, viscous oil. **19** was isolated as a white solid via FCC (30% EtOAc in hexanes) in 84% yield (6.110 g, 14.59 mmol). Rf = 0.60 (30% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.76 (1H, s), 4.43-4.37 (1H, m), 4.32 (1H, tt, J = 11.0, 4.5 Hz), 3.76 (3H, s), 2.25-2.16 (1H, m), 2.09-1.99 (1H, m), 1.97-1.92 (2H, m), 1.71 (1H, dd, J = 13.0, 11.0 Hz), 1.51-1.42 (1H, m), 0.90 (9H, s), 0.88 (9H, s), 0.12 (3H, s), 0.10 (3H, s), 0.07 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.3, 70.0, 63.7, 52.8, 44.9, 42.4, 39.7, 38.6, 26.2 (3C), 26.0 (3C), 18.5, 18.1, -4.2, -4.3, -4.7, -4.8.

20 :
(3S,5S)-3,5-bis(tert-butyldimethylsilyloxy)-1-(hydroxymethyl)cyclohexanol

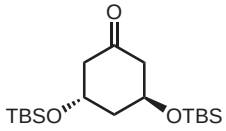
Ester **19** (6.110 g, 14.59 mmol) was dissolved in EtOH (150 mL) in a round bottom flask and cooled to 0 °C. $NaBH_4$ (1.656 g, 43.78 mmol) was added to this stirring solution. After 30 min of stirring at 0 °C, the reaction was warmed to room temperature and stirred over night. The reaction was then quenched with sat. NH_4Cl (50 mL) and diluted with EtOAc (100 mL). The layers were separated and the aqueous layer extracted with EtOAc (2 x 50 mL). The combined organic layers where further extracted with sat. NH_4Cl (2 x 50 mL), distilled H_2O (50 mL) and brine (50 mL), then separated, dried ($MgSO_4$), and



Chemical Formula: $C_{19}H_{42}O_4Si_2$
Exact Mass: 390,26
Molecular Weight: 390,71

concentrated in vacuo to give the crude product as a translucent, grey solid in 94 % yield (5.368 g, 13.74 mmol). The diol **20** was carried forward without further purification. Rf = 0.40 (30% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.58 (1H, s), 4.42-4.26 (2H, m), 3.44-3.28 (2H, m), 2.21 (1H, dd, J = 8.5, 4.5 Hz), 2.10-1.85 (3H, m), 1.50-1.36 (2H, m), 1.27 (1H, dd, J = 12.5, 11.0 Hz), 0.92 (9H, s), 0.90 (9H, s), 0.13 (3H, s), 0.12 (3H, s), 0.09 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 74.7, 71.0, 70.0, 64.2, 44.1, 43.0, 38.1, 26.2 (3C), 25.1 (3C), 18.4, 18.0, -4.3, -4.4, -4.7, -5.0.

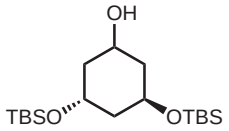
12 :
(3S,5S)-3,5-bis[tert-butyl(dimethyl)silyloxy]cyclohexanone



Chemical Formula: $C_{18}H_{38}O_3Si_2$
Exact Mass: 358,24
Molecular Weight: 358,66

To a stirring solution of diol **20** (5.368 g, 13.74 mmol) in THF (100 mL) cooled to 0 °Cwas added an aqueous solution of $NaIO_4$ (4.408 g, 20.61 mmol) in water (50 mL). A fine, white precipitate forms as the reaction proceeds. The reaction mixture was then warmed to room temperature and stirred over night. The reaction was then diluted with distilled H_2O until all of the precipitate dissolved. The layers were separated and the aqueous layer extracted with EtOAc (2 x 50 mL). The organic layers were combined and extracted with sat. NH_4Cl (2 x 50 mL), distilled H_2O (50 mL) and brine (50 mL), then dried ($MgSO_4$), and concentrated in vacuo to give the crude product as a white, crystalline solid in quantitative yield (4.938 g, 13.77 mmol). If necessary, the ketone **12** was purified via flash chromatographie (column : 3,5 cm x 10 cm, eluent : 10 % EtOAc/hexanes) Rf = 0.40 (10% EtOAc in hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.34 (2H, m), 2.55 (2H, dd, J = 14.0, 4.0 Hz), 2.35 (2H, ddd, J = 14.0, 7.0, 1.0 Hz), 1.94 (2H, t, J = 5.5 Hz), 0.87 (18H, s), 0.07 (6H, s), 0.06 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 207.7, 67.0 (2C), 50.4 (2C), 42.3, 25.9 (6C), 18.2 (2C), -4.6 (2C), -4.7 (2C).

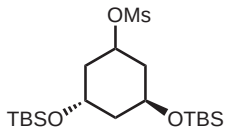
13 :
(3R,5R)-3,5-bis(tert-butyldimethylsilyloxy)cyclohexanol



Chemical Formula: $C_{18}H_{40}O_3Si_2$
Exact Mass: 360,25
Molecular Weight: 360,68

In a 250 mL round bottom flask, solid $NaBH_4$ (205 mg, 5,43 mmol, 3,0 equiv) was added to a stirring solution of crude ketone **12** (648 mg, 1,81 mmol, 1,0 equiv) in 95 % EtOH (70 mL) cooled in an ice bath. The reaction was let warmed to room temperature over 20 minutes then stirred until TLC showed completion. The reaction was quenched with a sat. aqueous NH_4Cl solution (50 mL) and diluted with CH_2Cl_2 (200 mL). The layers were separated and the aqueous layer extracted with CH_2Cl_2 (2x50 mL). The combined organic layers were washed with aq. sat. NH_4Cl (50 mL), brine (50 mL), dried ($MgSO_4$) and concentrated in vacuo to give a crude oil which solidified upon storage. Purification via FCC (column : 3,5 cm x 10 cm, eluent : 0, 1, 3 % acetone/toluene, 150 mL each) provided **13** as a clear oil in 88 % (574 mg, 1,59 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.36 4.27 (m, 1H), 4.25 (s, 1H), 4.13 4.03 (m, 1H), 1.93 (m, 1H), 1.82 (m, 1H), 1.73 (m, 2H), 1.57 (m, 2H), 0.91 (s, 9H), 0.90 (s, 9H), 0.10 (s, 6H), 0.08 (s, 6H). ESI-HRMS found 361.25901 $\pm$ 0.00 (MH^+ calc. 361.25887).

26 :
(3S,5S)-3,5-bis(tert-butyldimethylsilyloxy)cyclohexyl methanesulfonate

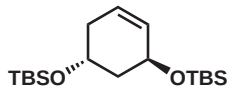


Chemical Formula: $C_{19}H_{42}O_5SSi_2$
Exact Mass: 438,23
Molecular Weight: 438,77

Neat methanesulfonyl chloride (16.5 μL, 0.212 mmol, 1.5 equiv) was added to a stirring solution of alcohol **13** (51 mg, 0.141 mmol) and Et_3N (29.5 μL, 0.212 mmol, 1.5 equiv) in CH_2Cl_2 (10 mL) cooled to 0 °C. The reaction was stirred at this temperature for 30 minutes. TLC showed completion. Reaction mixture diluted with 0.05 M HCl (2.5 mL) and CH_2Cl_2 (5 mL). Organic layer was washed with H_2O (2 x 3 mL) and brine (1 x 3 mL), dried over $MgSO_4$ and concentrated to afford crude product as a yellow oil. The residue was purified via flash chromatography (column : 1.5cm x 10 cm, eluent : 5, 10,

15 % EtOAc/hexanes, 50 mL each) to provide 59 mg of mesylate **26** as a clear oil. (95 %, 0.158 mmol). Rf : 0.8 (20 % EtOAc/Hexanes). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.96 (tt, J = 11.2, 4.5 Hz, 1H), 4.23 (s, 1H), 4.07 (ddd, J = 15.0, 10.7, 4.3 Hz, 1H), 2.99 (s, 3H), 2.42 2.28 (m, 1H), 2.12 (dd, J = 7.4, 5.1 Hz, 1H), 1.88 (dd, J = 9.1, 3.9 Hz, 1H), 1.63 1.47 (m, 2H), 1.34 (ddd, J = 13.0, 10.8, 2.3 Hz, 1H), 0.88 (d, J = 1.0 Hz, 18H), 0.05 (dd, J = 4.0, 1.7 Hz, 12H) ; ^{13}C NMR (75 MHz, $CDCl_3$) δ 66.49, 65.32, 42.30, 42.05, 39.53, 38.61, 25.79, 25.63, 18.07, 17.89, -4.76, -5.09.

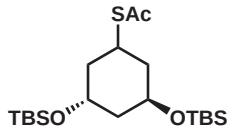
29 :
(1R,3S)-cyclohex-4-ene-1,3-diylbis(oxy)bis(tert-butyldimethylsilane)



Chemical Formula: $C_{18}H_{38}O_2Si_2$
Exact Mass: 342,24
Molecular Weight: 342,66

Tf_2O (11 μL, 60 μmol, 2.0 equiv) was added neat to a solution of alcohol **13** (10.8 mg, 30 μmol, 1.0 equiv) and dMAP (37 mg, 60 μmol, 2.0 equiv) in CH_2Cl_2 (3.5 mL) at 0 °C. The reaction was stirred 15 minutes at 0 °Cwhen TLC showed completion (Rf = 0.5, 15 % acetone/toluene). The solvent was removed *in vacuo* and the residue was diluted with EtOAc (10 mL). The layers were separated and the organic layer was washed 1N HCl (0.5 mL), H_2O (5 mL), brine (5 mL) then dried ($MgSO_4$) and concentrated *in vacuo* to give 7.2 mg of crude product as an oil. NMR confirmed clean alkene product in 74 % yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.65 (s, 1H), 4.36 (s, 1H), 4.12 (d, J = 4.0 Hz, 1H), 2.30 (dd, J = 15.9, 4.1 Hz, 1H), 2.02 1.61 (m, 3H), 0.90 (s, 18H), 0.09 (s, 12H).

25 :
S-(3S,5S)-3,5-bis(tert-butyldimethylsilyloxy)cyclohexyl ethanethioate

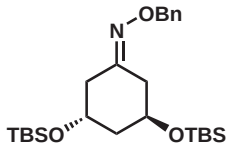


Chemical Formula: $C_{20}H_{42}O_3SSi_2$
Exact Mass: 418,24
Molecular Weight: 418,78

In a flame dried 10 mL round bottom flask in an ice bath, dEAd (33 μL, 0.195 mmol, 2.0 equiv) was added neat to a solution of PPh_3 (51 mg, 0.195 mmol, 2.0 equiv) in THF (1.1 mL). A milky solution formed after 5 minutes and the yellow color of dEAd remained. The reaction was stirred at 0 °Cfor 30 minutes overall before being charged with a solution of alcohol **13** (35 mg, 0.097 mmol, 1.0 equiv) and AcSH (14 μL, 0.195 mmol, 2.0 equiv) in

THF (0.5 + 0.5 mL). Precipitate appearance changed from milky yellow to greenish yellow to silvery pale yellow. The reaction was left at 0 °Cfor 15 minutes then warmed to room temperature and put (under water condenser) in an oil bath at 65 °C. TLC showed complete disappearance of alcohol after 2 h. The mixture was concentrated to dryness in vacuo. The pinky white solid was purified via flash chromatography (column : 1.5 cm x 15 cm, eluent : 0, 2, 4, 7, 9, 12, 15, 20 % acetone/toluene, 20 mL each). The least polar fraction was purified further using preparative TLC (100 % hexanes) and provided thioacetate **25** as an oil in 7.5 % (3.0 mg, 7.3 μmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.17 (s, 1H), 4.10 (ddd, J = 14.1, 9.8, 4.1 Hz, 1H), 3.86 (tt, J = 11.9, 3.9 Hz, 1H), 2.30 (d, J = 1.1 Hz, 3H), 2.11 (d, J = 12.1 Hz, 1H), 1.92 1.80 (m, 2H), 1.55 (s, 1H), 1.41 (ddd, J = 24.2, 14.8, 7.1 Hz, 4H), 0.98 0.81 (m, 18H), 0.07 (ddd, J = 3.2, 2.4, 0.7 Hz, 10H). ; ^{13}C NMR (101 MHz, $CDCl_3$) δ 195.06, 67.34, 66.65, 42.61, 41.46, 39.58, 36.15, 30.75, 29.82, 26.00, 25.86, 18.30, 18.15, -4.52, -4.56, -4.77, -4.84.

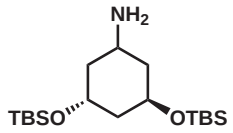
21 :
(3R,5R)-3,5-bis(tert-butyldimethylsilyloxy)cyclohexanone O-benzyl oxime



Chemical Formula: $C_{25}H_{45}NO_3Si_2$
Exact Mass: 463,29
Molecular Weight: 463,8

O-Benzylhydroxylamine hydrochloride (93 mg, 0.585 mmol, 1.05 equiv) and sodium acetate (51 mg, 0.614 mmol, 1.10 equiv) were added to a solution of ketone **12** (200 mg, 0.558 mmol, 1.00 equiv) in toluene (20 mL). The mixture was heated under reflux condenser in oil bath (temperature : 90 °C) for 3 hrs after which time TLC showed completion (3 % acetone/toluene). The mixture was diluted with EtOAc (10 mL) and H_2O (10 mL). The layers were separated and the aqueous layer was extracted with EtOAc (2 x 5 mL). The combined organic layers were washed with 0.5 M aqueous HCl (5 mL), H_2O (5 mL), brine (5 mL) then dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product as an oil. The residue was purified via flash chromatography (column : 2,5 cm x 15 cm, eluent : 3, 6, 9, 12 % acetone/toluene, 30 mL each) to provide 219 mg of oxime **21** as a clear oil (85 %, 0.474 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 7.26 (m, 5H), 5.06 (s, 2H), 4.23 4.11 (m, 2H), 2.67 (dd, J = 14.1, 6.2 Hz, 1H), 2.48 (ddd, J = 20.8, 14.0, 3.8 Hz, 2H), 2.15 (dd, J = 13.7, 7.7 Hz, 1H), 1.80 (m, 1H), 1.72 (m, 1H), 0.87 and 0.86 (s, 9H each), 0.04 (m, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.360, 138.267, 128.230, 127.788, 127.460, 66.818, 66.597, 42.987, 40.449, 33.659, 25.771, 25.740, 18.035, -4.790, -4.859, -4.950, -4.981. ESI-HRMS found 464.30135 $\pm$ 0.00 (MH^+ calc. 464.30107).

22 :
(3R,5R)-3,5-bis(tert-butyldimethylsilyloxy)cyclohexanamine

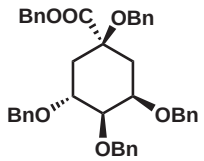


Chemical Formula: $C_{18}H_{41}NO_2Si_2$
Exact Mass: 359,27
Molecular Weight: 359,69

About 2 mL (around 33 % powder) of the W7-Raney Nickel preparation (6.1 on page 167) was added as a slurry to a solution of oxime **21** (71 mg, 0.153 mmol) in absolute ethanol (5 mL). The flask was flushed three times with H_2 and then was stirred at 50 °Cunder H_2 atmosphere for 20 hrs or until TLC showed completion. The suspension was filtered over paper, then over Celite. The filtrate was concentrated *in vacuo* to afford an oily residue which was purified via flash chromatography (column : 1.5 cm x 15 cm, eluent : 0, 5, 10, 15, 20 % acetone/toluene, 20 mL each) and provided 47 mg of amine **22** as an oil (86 %, 0.132 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.16 (s, 1H), 4.10 3.96 (m, 1H), 3.13 (m, 1H), 2.14 (br s, 2H), 2.03 (d, J = 11.6 Hz, 1H), 1.81 (m, 2H), 1.33 (t, J = 11.5 Hz, 1H), 1.26 1.09 (m, 2H), 0.87 and 0.85 (s, 9H each), 0.04 and 0.02 (4 x s, 3H each) ; ^{13}C NMR (101 MHz, $CDCl_3$) δ 66.933, 66.521, 44.801, 42.728, 42.522, 25.900, 25.725, 18.188, 17.936, -4.745, -4.729, -4.966, -5.042. ESI-HRMS found 360.27481 $\pm$ 0.000 (MH^+ calc. 360.27486).

Test reductive thiolation on quinic perbenzylated ketone

35 :
(1S,3R,4S,5R)-benzyl 1,3,4,5-tetrakis(benzyloxy)cyclohexanecarboxylate

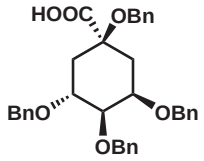


Chemical Formula: $C_{42}H_{42}O_6$
Exact Mass: 642,3
Molecular Weight: 642,78

A solution of d-quinic acid (200 mg, 1.04 mmol, 1.0 equiv) in DMF (10 mL) was added to a suspension of NaH (250 mg, 10.4 mmol, 10.0 equiv, 95 %) in DMF (5 mL) at 0 °C. The mixture was stirred 20 minutes before being charged with a solution of benzyl bromide (1.23 mL, 10.4 mmol, 10.4 equiv) in DMF (5 mL), let warmed to rt and stirred overnight. The cloudy mixture was quenched with MeOH (1 mL) and diluted with H_2O

(80 mL) and EtOAc (80 mL). The biphasic mixture was separated and the aqueous layer was reextracted with EtOAc (3 x 25 mL). Combined organic layers were washed with 0.5 M aq. $NaHCO_3$ (20 mL) and brine (20 mL) and then dried ($MgSO_4$) and concentrated **in vacuo** to afford clear, oily mixture. Purification via flash chromatography (column : 1,5 cm x 13 cm, eluent : 0, 5, 20 % EtOAc/hexanes) provided 440 mg of perbenzylated product **35** a clear oil (67 %, 0.697 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 7.18 (m, 25H), 5.01 (d, J = 12.2 Hz, 1H), 4.81 (dd, J = 14.7, 12.2 Hz, 2H), 4.63 (dd, J = 12.0, 8.7 Hz, 2H), 4.54 (t, J = 11.0 Hz, 2H), 4.36 (dd, J = 48.3, 12.0 Hz, 1H), 4.21 4.05 (m, 2H), 3.83 3.74 (m, 2H), 2.74 (s, 1H), 2.56 (s, 1H), 2.16 2.06 (m, 2H).

36 :
(1S,3R,4S,5R)-1,3,4,5-tetrakis(benzyloxy)cyclohexanecarboxylic acid

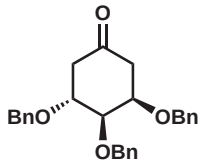


Chemical Formula: $C_{35}H_{36}O_6$
Exact Mass: 552,25
Molecular Weight: 552,66

Aqueous NaOH (1.6 mL, 2 M, 3.2 mmol, 5.14 equiv) was added to a solution of benzyl ester **35** in THF (10 mL) at rt. The reaction was heated in oil bath at $65\ ^\circ C$ for 2hrs, after which time it was quenched with MeOH (2 mL) and stirred for 5hrs. After 15 min, a precipitate started to appear and the reaction was diluted with Et_2O (50 mL). The layers were separated and the aqueous was reextracted with Et_2O (2 x 15 mL). Combined organic layers were washed with brine (25 mL), dried ($MgSO_4$) and concentrated *in vacuo* to yield 300 mg of oily product **36** (87 %, 0.541 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 7.03 (m, 20H), 4.77 (d, J = 12.1 Hz, 1H), 4.68 4.52 (m, 5H), 4.41 (d, J = 12.2 Hz, 1H), 4.29 (d, J = 10.5 Hz, 1H), 4.13 4.04 (m, 1H), 3.86 (d, J = 3.1 Hz, 1H), 3.74 (dd, J = 4.8, 2.7 Hz, 1H), 2.59 (d, J = 11.1 Hz, 1H), 2.43 (d, J = 13.7 Hz, 1H), 2.21 2.11 (m, 2H) ; ^{13}C NMR (101 MHz, $CDCl_3$) δ 179.26, 140.02, 138.69, 138.63, 138.47, 128.05, 127.92, 127.84, 127.70, 127.48, 127.24, 127.05, 126.91, 126.57, 80.87, 74.53, 74.35, 72.40, 71.62, 65.96, 64.32, 32.27.

37 :
(3R,5R)-3,4,5-tris(benzyloxy)cyclohexanone

$Pb(OAc)_4$ (448 mg, 1.01 mmol, 2.0 equiv) was added to a solution of acid **36** in DMF (8 mL) at rt. The reaction was stirred overnight at rt. TLC showed completion (40 % EtOAc/Hexanes). The reaction wa treated with H_2O (50 mL) and EtOAc (50 mL). The layers were separated and the aqueous was reextracted with EtOAc (2 x 15 mL). Combined

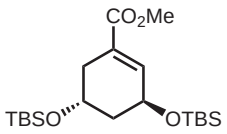


Chemical Formula: $C_{27}H_{28}O_4$
Exact Mass: 416,2
Molecular Weight: 416,51

organic layers were washed with sat. aq. $NaHCO_3$ (25 mL), brine (25 mL), dried ($MgSO_4$) and concentrated *in vacuo* to yield 220 mg of crude product which was purified using flash chromatography (eluent : 5, 10, 12, 15, 20 % EtOAc/hexanes) and afforded 85 mg of pure ketone **37** (40 %, 0.202 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 7.10 (m, 15H), 4.87 (d, J = 12.0 Hz, 1H), 4.70 (d, J = 12.0 Hz, 1H), 4.56 (dt, J = 12.0, 7.9 Hz, 3H), 4.42 (d, J = 11.8 Hz, 1H), 4.05 (ddd, J = 22.0, 6.7, 3.1 Hz, 3H), 2.83 (ddd, J = 19.1, 14.5, 7.2 Hz, 2H), 2.64 (d, J = 14.9 Hz, 1H), 2.49 (d, J = 14.6 Hz, 1H) ; ^{13}C NMR (75 MHz, $CDCl_3$) δ 206.51, 149.75, 138.44, 138.18, 137.76, 128.32, 128.08, 127.68, 127.61, 127.51, 76.07, 75.45, 73.33, 71.41, 71.36, 43.70, 42.29, 30.86, 29.78.

134 :
(3S,5R)-methyl 3,5-bis(tert-butyldimethylsilyloxy)cyclohex-1-enecarboxylate

[1]

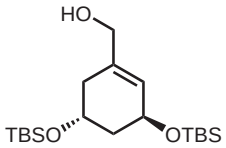


Chemical Formula: $C_{20}H_{40}O_4Si_2$
Exact Mass: 400,25
Molecular Weight: 400,7

To a solution of alcohol **19** (238 mg, 0.568 mmol) in dry pyridine (10 mL) was added neat $POCl_3$ (159 μL, 1.71 mmol). The reaction was stirred at 70-75 °Cunder water condenser and argon for 16hrs after which time the reaction had turned brownish. The mixture was quenched by addition of saturated aqueous NH_4Cl (3 mL) and diluted with EtOAc (20 mL). Layers were separated and aqueous reextracted with EtOAc (3 x 10 mL). The combined organic layers were washed with H_2O (10 mL), brine (10 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product as a brown oil. The residue was diluted in toluene (20 mL) and concentrated in vacuo (3x) to get rid of pyridine affording ester **134** as a tan oil (216 mg, 95 %) of sufficient purity. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.787 (d, J = 1.5 Hz, 1H), 4.530 (m, 1H), 4.177 (m, 1H), 3.729 (s, 3H), 2.533 (td, J = 2.3, 18 Hz, 1H), 2.152 (dd, J = 5.5, 18 Hz, 1H), 1.808 (m, 1H), 1.705 (m, 1H), 0.894 and 0.869 (s, 9H each), 0.095, 0.080, 0.056, 0.047 (s, 3H each). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.25, 139.87,

128.43, 65.39, 64.96, 51.77, 39.75, 33.76, 25.88, 25.85, 23.43, 18.21, -4.56, -4.63, -4.66, -4.74.

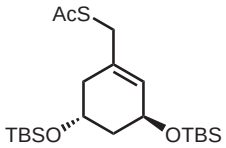
135 :
((3S,5R)-3,5-bis(tert-butyldimethylsilyloxy)cyclohex-1-enyl)methanol



Chemical Formula: $C_{19}H_{40}O_3Si_2$
Exact Mass: 372,25
Molecular Weight: 372,69

In a flame dried 10 mL round bottom flask, a solution of ester **134** (13.7 mg, 0.034 mmol, 1.0 equiv) in THF (1+1 mL) was added to a 1.5 M solution of dIBAL in toluene (119 μL, 0.178 mmol, 5.2 equiv) in THF (1.5 mL) in an ice bath. The reaction was stirred 90 minutes at 0 °Cand 16hrs at room temperature. The mixture was stirred vigorously and quenched with Et_2O (2 mL), H_2O (0.3 mL), 2 M aq. NaOH (0.5 mL) and then H_2O (0.5 mL) to afford two clear layers. The mixture was diluted with EtOAc (10 mL). The layers were separated and the aqueous layer was extracted with EtOAc (2 x 5 mL). The combined organic layers were washed with saturated aqueous NH_4Cl (5 mL), brine (5 mL) then dried ($MgSO_4$) and concentrated in vacuo to give the crude product as an oil. The residue was purified via flash chromatography (column : 1,5 cm x 13 cm, eluent : 10, 20, 30 % EtOAc/hexanes, 50 mL each) provided 9.3 mg of alcohol **135** a clear oil (73 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.66 (s, 1H), 4.43 (s, 1H), 4.20 (m, 1H), 4.02 (d, J = 4.5 Hz, 2H), 2.27 (dd, J = 4.7, 16.4 Hz, 1H), 1.95 (dd, J = 7.0, 16.4 Hz, 1H), 1.76 (m, 1H), 1.34 (t, J = 6.3 Hz, 1H), 0.91 (s, 9H), 0.90 (s, 9H), 0.10 (s, 6H), 0.09 (s, 3H), 0.08 (s, 3H).

197 :
S-((3S,5R)-3,5-bis(tert-butyldimethylsilyloxy)cyclohex-1-enyl)methyl ethanethioate

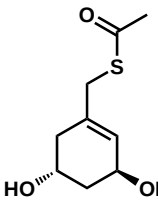


Chemical Formula: $C_{21}H_{42}O_3SSi_2$
Exact Mass: 430,24
Molecular Weight: 430,79

In a flame dried 10 mL round bottom flask in an ice bath, DIAD (16 μL, 0.08 mmol, 1.0 equiv) was added neat to solid PPh_3 (31 mg, 0.12 mmol, 1.5 equiv) immediately followed

by THF (2 mL). A milky solution formed after 5 minutes and the yellow color of DIAD disappeared after 25 minutes. The reaction was stirred at 0 °Cfor 30 minutes overall before being charged with a solution of allylic alcohol **135** (30 mg, 0.08 mmol, 1.0 equiv) in THF (2+1 mL). Precipitate appearance changed from milky white to silvery white. The reaction was stirred at 0 °Cfor 30 minutes overall before being charged with neat AcSH (12 μL, 0.16 mmol, 2.0 equiv). Cloudiness immediately disappeared. The reaction was warmed to room temperature and stirred overnight. The mixture was quenched with H_2O (10 mL) and diluted with EtOAc (15 mL). The layers were separated and the aqueous layer was extracted with EtOAc (2 x 5 mL). The combined organic layers were washed with 5 % aqueous $NaHCO_3$ (5 mL), H_2O (5 mL), brine (5 mL) then dried ($MgSO_4$) and concentrated in vacuo to give the crude product as a solid. Purification via flash column chromatography (column : 1.5 cm x 15 cm, eluent : 0, 2, 5, 10, 15, 20 % EtOAc/hexanes, 100 mL (0 and 2 %) then 50 mL from 5 %) provided 21.8 mg of thioacetate **197** as a clear oil (63 %, 95 % based on recovered starting alcohol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.63 (s, 1H), 4.36 (m, 1H), 4.18 4.11 (m, 1H), 3.51 (q, J = 13.7 Hz, 2H), 2.33 (s, 3H), 2.18 (dd, J = 17.2, 4.5 Hz, 1H), 1.97 (dt, J = 17.2, 6.3 Hz, 1H), 1.76 1.63 (m, 2H), 0.88 and 0.86 (s, 18H), 0.06 (s, 12H). ^{13}C NMR (101 MHz, $CDCl_3$ and CD_3OD) δ 201.23, 144.56, 125.73, 68.93, 64.81, 39.54, 38.45, 36.97, 29.89, 25.81, 25.62, 18.12, -4.09, -4.71.

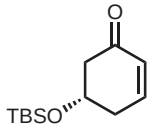
198 :
S-(((3S,5R)-3,5-dihydroxycyclohex-1-en-1-yl)methyl) ethanethioate



Chemical Formula: $C_9H_{14}O_3S$
Exact Mass: 202,0664
Molecular Weight: 202,2707

Hydrochloric acid (225 μL, 0.45 mmol, 3.00 equiv, 2 mol/L) was added to a solution of bis-silyl ether **197** (65 mg, 0.15 mmol, 1.00 equiv) in MeOH (5 mL) at around -30 °C in an ice-acetone bath. The mixture was stirred for 5 minutes then put in freezer (-30 °C) without stirring for 4 hrs, after which time TLC showed completion (10 % EtOAc/hexanes). The mixture was then diluted with EtOAc (10 mL) and H_2O (5 mL). The layers were separated and the aqueous layer was extracted with EtOAc (2 x 5 mL). The combined organic layers were washed with H_2O (5 mL), brine (5 mL) then dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product as an oil. The residue was purified via flash chromatography (column : 2,5 cm x 15 cm, eluent : 2.5, 2.5, 5, 10, 15 % Methanol/CH_2Cl_2) to provide 22 mg of thioacetate **198** as a clear oil (72 %, 0.108 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.79 (s, 1H), 4.41 (s, 1H), 4.17 (s, 1H), 3.54 (s, 2H), 2.40 (s, 1H), 2.35 (s, 3H), 2.00 (m, 1H), 1.90 (m, 1H), 1.88 (m, 1H), 1.80 (m, 1H).

40 :
(S)-5-(tert-butyldimethylsilyloxy)cyclohex-2-enone

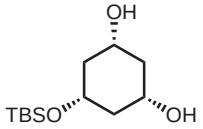


Chemical Formula: $C_{12}H_{22}O_2Si$
Exact Mass: 226,14
Molecular Weight: 226,39

To diisopropylamine (11 uL, 77 umol) in dry THF (1 mL) at -78 °Cwas added n-BuLi (29 uL, 2,5M hexanes). Stirred 30 minutes at that temperature before a solution of ketone (25 mg, 70 umol) in toluene (2+1 mL) was added. Quenched at -78 °Cusing sat. aq. NH_4Cl (2mL). diluted with EtOAc (5mL) TLC after 4 minutes is identical to a TLC after 2h30 at -78 °C. Layers were separated and the aqueous layer extracted with EtOAc (2x50 mL). Combined organic layers were washed with aq. sat. NH_4Cl (3 mL), H_2O (2 mL), brine (5 mL), dried over $MgSO_4$ and concentrated in vacuo to give 21 mg as a crude yellow oil. ^{1}H NMR ($CDCl_3$, 300 MHz) showed a 2.3:1 mixture of starting ketone and unsaturated ketone. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.87 (ddd, J = 10.0, 5.1, 3.3 Hz, 1H), 6.05 (d, J = 10.1 Hz, 1H), 4.22 (ddd, J = 12.1, 8.7, 4.4 Hz, 1H), 2.76 2.26 (m, 5H), 1.00 0.76 (m, 10H), 0.08 (d, J = 12.3 Hz, 6H).

6.1.6 Cyclohexane triol desymmetrization route

48 :
(1S,3R,5s)-5-(tert-butyldimethylsilyloxy)cyclohexane-1,3-diol

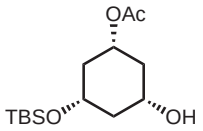


Chemical Formula: $C_{12}H_{26}O_3Si$
Exact Mass: 246,17
Molecular Weight: 246,42

Tert-butyldimethylsilyl chloride (942 mg, 6.25 mmol, 1.1 equiv), NEt_3 (871 μL, 6.25 mmol, 1.1 equiv), the cis-triol **47** (750 mg, 5,68 mmol, 1.0 equiv) followed by 1 drop of H_2O were added to a washed (2 x 15 mL n-hexanes) suspension of NaH (382 mg, 60% in oil, 9.54 mmol, 1.7 equiv) in THF (38 mL) at 22 °C. Temperature was kept at 60 °Cfor 16h. The suspension was cooled to 10 °Cand filtered over paper to recuperate unreacted starting triol. The filtrate was evaporated and the crude residue was purified by flash chromatography on silica gel (column : 3,5 cm x 10 cm, eluent : 0, 1, 2, 4, 4 %

methanol/dichloromethane, 100 mL each) to provide **48** as a white solid in 45 % (625 mg, 2.53 mmol). mp 121-122 °C. ^{1}H NMR (400 MHz, CD_3OD): 3.78 (m, 3H), 2.16 (d, J = 10.9 Hz, 3H), 1.16 (dd, J = 23.1, 11.5 Hz, 3H) , 0.89 (s, 9H), 0.08 (s, 6H). ; ^{13}C NMR (101 MHz, $CDCl_3$) δ 67.02, 66.11, 42.88, 42.71, 25.75, 18.01, -4.84.

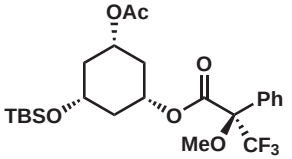
49 :
(1R,3S,5S)-3-(tert-butyldimethylsilyloxy)-5-hydroxycyclohexyl acetate



Chemical Formula: $C_{14}H_{28}O_4Si$
Exact Mass: 288,18
Molecular Weight: 288,46

Lipase QL (994 mg) was added to a stirred solution of the diol **48** (850 mg, 3.44 mmol, 1.0 equiv) in vinyl acetate (8.5 mL) and ethyl acetate (85 mL) at 22 °C. The reaction was stirred at 22 °Cfor 48hrs (or until TLC showed completion). The reaction mixture was filtered on a 3-cm deep bed of diatomaceous earth, the filtrate concentrated *in vacuo* and the residue dried at 0.01 mbar overnight to give a clear oil which was purified by flash chromatography on silica gel (column : 3,5 cm x 18 cm, eluent : 10, 15, 20, 25, 30 % EtOAc/n-hexanes) to provide **49** as a clear oil in 97 % (960 mg, 3.33 mmol). Rf=0.8 (10 % MeOH/dichloromethane). $[\alpha]_d$ = 14.988 ($CHCl_3$, 1% w/v). ^{1}H NMR (300 MHz, $CDCl_3$): 4.76 (m, 1H), 3.73 (m, 2H), 2.22–2.03 and 1.54–1.37 (m each, 4H and 3H), 2.04 (s, 3H), 0.88 (s, 9H), 0.07 and 0.06 (s each, 3H each) ; ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.729, 67.829, 66.197, 65.587, 43.522, 39.891, 39.648, 25.723, 21.280, 18.009, -4.799. ESI-HRMS found 289.18254 $\pm$ 0.00 (MH^+ calc. 289.18296).

199 :
(R)-((1S,3R,5R)-3-acetoxy-5-(tert-butyldimethylsilyloxy)cyclohexyl) 3,3,3-trifluoro-2-methoxy-2-phenylpropanoate

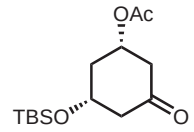


Chemical Formula: $C_{24}H_{35}F_3O_6Si$
Exact Mass: 504,22
Molecular Weight: 504,61

Neat (S)-(+)-α-Methoxy-α-trifluoromethylphenylacetyl chloride (23 μL, 122.3 μmol, 1.4 equiv) was added to a stirred solution of **49** (25.2 mg, 87.4 μmol, 1.0 equiv) and dMAP

(1.0 mg, 8.2 μmol, 0.1 equiv) in CH_2Cl_2 (2 mL) and pyridine (0.300 mL). The reaction was stirred at rt for 24h. Another portion of neat (S)-(+)-α-Methoxy-α-trifluoromethylphenylacetyl chloride (23 μL, 122.3 μmol, 1.4 equiv) was added to the reaction. The reaction was stirred at rt for another 24h. TLC (15 % acetone/toluene) showed completion. The reaction mixture was quenched with water (2 mL), EtOAc (2 mL), the layers were separated, and the aqueous layer extracted with EtOAc (2 x 3 mL). The combined organic layers were washed with brine, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The pale yellow oily residue (125 mg) was not purified by flash chromatography and was directly analyzed by ^{13}C NMR in $CDCl_3$ and d*6*-benzene. ^{13}C NMR racemic (75 MHz, d6-benzene) : 169.014, 168.980, 165.538, 70.000, 69.966, 66.955, 66.868, 65.719, 65.677, 55.218, 40.931, 40.634, 40.321, 36.341, 36.089, 25.878, 20.631, 20.616, 18.086, -4.603. ; ^{13}C NMR non racemic (75 MHz, d6-benzene) 169.231, 165.450, 69.993, 66.990, 65.662, 55.241, 40.908, 40.294, 36.314, 30.239, 25.889, 20.654, 18.101, 14.625, -4.596.

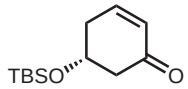
200 :
(1S,3R)-3-(tert-butyldimethylsilyloxy)-5-oxocyclohexyl acetate



Chemical Formula: $C_{14}H_{26}O_4Si$
Exact Mass: 286,16
Molecular Weight: 286,44

To a solution of oxalyl chloride (0.96 mL, 11.01 mmol, 1.5 equiv) in CH_2Cl_2 (5 ml) in an acetone-dry ice bath (-78 °C) was added precooled (acetone-dry ice bath) d_6-DMSO (1.56 mL, 22.02 mmol, 3.0 equiv) in CH_2Cl_2 (5 mL). The resulting mixture was stirred at that temperature for 30 minutes before precooled (acetone-dry ice) alcohol **49** (2.12 g, 7.34 mmol, 1.0 equiv) in CH_2Cl_2 (10 + 5 ml) was added dropwise. After an additional 30 minutes, Et_3N (5.12 mL, 36.7 mmol, 5.0 equiv) was added neat. The reaction was allowed to warm to room temperature over 1 hour before being quenched with water (10 mL) and diluted with ethyl acetate (50 mL). The layers were separated and the aqueous layer was extracted with EtOAc (2 x 10 mL). The combined organic layers were washed with H_2O (10 mL), brine (15 mL) then dried ($MgSO_4$) and concentrated *in vacuo* to give a mixture of title compound **200** and enone **50**, separable via flash chromatography (column : 1.5 x 13 cm, 10 % EtOAc/hexanes). However, the mixture was typically not purified and used directly in the next reaction (OAc elimination with Al_2O_3 or Et_3N). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.009–4.959 (m, 1H), 4.031–3.967 (m, 1H), 2.730–2.578 (m, 2H), 2.468–2.335 (m, 3H), 2.063 (s, 3H), 1.937–1.829 (m, 1H), 0.885 (s, 9H), 0.078 and 0.073 (s, 3H each). ^{13}C NMR (75 MHz, $CDCl_3$) δ 205.513, 170.105, 67.577, 66.307, 50.738, 46.384, 39.763, 25.996, 21.459, 21.352, 18.284, -4.451, -4.474.

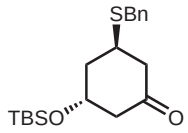
50 :
(R)-5-(tert-butyldimethylsilyloxy)cyclohex-2-enone



Chemical Formula: $C_{12}H_{22}O_2Si$
Exact Mass: 226,14
Molecular Weight: 226,39

To a solution of oxalyl chloride (1.7 mL, 19.14 mmol, 1.5 equiv) in CH_2Cl_2 (10 ml) in an acetone-dry ice bath (-78 °C) was added precooled (acetone-dry ice bath) d_6-DMSO (2.70 mL, 38.24 mmol, 3.0 equiv) in CH_2Cl_2 (10 mL). The resulting mixture was stirred at that temperature for 30 minutes before precooled (acetone-dry ice) alcohol **49** (3.68 g, 12.76 mmol, 1.0 equiv) in CH_2Cl_2 (15 + 5 ml) was added dropwise. After an additional 30 minutes, Et_3N (8.90 mL, 63.80 mmol, 5.0 equiv) was added neat. The reaction was allowed to warm to room temperature over 1 hour and left at rt overnight. The reaction was quenched with water (20 mL) and diluted with ethyl acetate (100 mL). The layers were separated and the aqueous layer was extracted with EtOAc (2 x 20 mL). The combined organic layers were washed with H_2O (20 mL), brine (25 mL) then dried ($MgSO_4$) and concentrated *in vacuo* to give title compound enone **50**. Purification via flash chromatography (column : 1.5 x 13 cm, 10 % EtOAc/hexanes) afforded 2.85 g of enone as a clear oil (98 %, 12.63 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.87 (ddd, J = 10.0, 5.1, 3.3 Hz, 1H), 6.05 (d, J = 10.1 Hz, 1H), 4.22 (ddd, J = 12.1, 8.7, 4.4 Hz, 1H), 2.76 2.26 (m, 5H), 1.00 0.76 (m, 9H), 0.08 (d, J = 12.3 Hz, 6H) ; ^{13}C NMR (101 MHz, $CDCl_3$) δ 198.511, 146.960, 130.193, 67.858, 48.362, 35.909, 26.093, 18.387, -4.294, -4.362.

51 :
(3R,5S)-3-(benzylthio)-5-(tert-butyldimethylsilyloxy)cyclohexanone

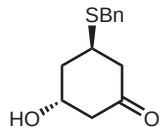


Chemical Formula: $C_{19}H_{30}O_2SSi$
Exact Mass: 350,17
Molecular Weight: 350,59

Benzyl thiol (324 μL, 2.76 mmol, 1.20 equiv) and NaOMe (7.8 mg, 0.12 mmol, 0.05 equiv) were added to a solution of enone **50** (520 mg, 2,30 mmol, 1.00 equiv) in degassed CH_2Cl_2 (10 mL) and degassed THF (1 mL) at rt. The mixture was stirred for 4h or until SM is no longer visible by TLC (5 % acetone in toluene). The reaction is quenched with H_2O (10 mL) and the layers were separated. The organic layer was washed with H_2O (5

mL), dried ($MgSO_4$) and then concentrated *in vacuo* to yield a smelly, non-homogeneous yellow oil. The residue was purified by flash chromatography on silica gel (column : 4 cm x 12 cm, eluent : 1, 2 % acetone/toluene, 250 mL each) to provide **51** as a clear oil in 88 % (709 mg, 2.02 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30 (m, 5H), 4.38 (s, 1H), 3.77 (s, 2H), 3.30 (ddd, J = 15.1, 10.9, 4.2 Hz, 1H), 2.80 2.54 (m, 1H), 2.54 2.17 (m, 3H), 2.08 (dd, J = 13.5, 8.9 Hz, 1H), 1.97 1.65 (m, 1H), 0.92 0.70 (s, 9H), 0.03 (2x s, 6H) ; ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.380, 137.643, 128.429, 128.330, 126.851, 58.113, 49.136, 47.818, 39.282, 36.896, 35.022, 25.640, 17.889, -4.907, -4.945.

52 :
(3R,5S)-3-(benzylthio)-5-hydroxycyclohexanone

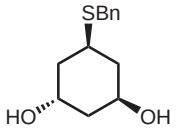


Chemical Formula: $C_{13}H_{16}O_2S$
Exact Mass: 236,09
Molecular Weight: 236,33

To a solution of ketone **51** (125 mg, 0.357 mmol) in acetonitrile (6 mL) cooled at -35 °Cin dry ice acetonitrile bath was added aq HF (1 mL, aqueous 48 % w/w, d=1.15, 60 equiv HF). Temperature raised to -15 °Cin brine-ice bath and reaction stirred until no starting material was visible by TLC (Rf = 0.85, 15 % acetone/toluene, 4 to 6hrs at -15 °Cor 16hrs in a freezer at -30 °C). Reaction diluted with CH_2Cl_2 (30 mL) and H_2O (10 mL). Layers were separated and aqueous phase reextracted with CH_2Cl_2 (3 x 5 mL), combined organic layers washed with H_2O (3 x 5 mL). Organic layer dried ($MgSO_4$) and concentrated *in vacuo* (cold water bath) to yield an oil. Product mixture is usually carried to the next step but can be purified via flash column chromatography (eluent : 15 % acetone/toluene). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (m, 5H), 4.42 (s, 1H), 3.77 (s, 2H), 3.33 (m, 1H), 2.75 2.66 (m, 1H), 2.61 2.54 (m, 1H), 2.43 2.37 (m, 2H), 2.22 2.17 (br m, 2H), 1.97 1.85 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 207.01, 137.57, 128.59, 128.45, 127.03, 67.50, 48.92, 47.65, 38.41, 37.33, 35.24.

53 :
(1S,3S)-5-(benzylthio)cyclohexane-1,3-diol

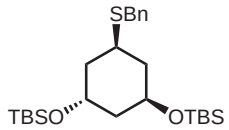
$NaBH(OAc)_3$ (1.06 mmol, 3.0 equiv) was added to a solution of alcohol **52** (0.357 mmol, 1.0 equiv) in EtOAc (10 ml) at room temperature. The suspension was stirred at rt until no more starting material was visible by TLC (5-7hrs). Reaction was quenched with 1 M NaKtartrate (5 mL) and diluted with EtOAc (10 mL). Layers were separated and the aqueous phase reextracted with EtOAc (5 mL). The combined organic phases were



Chemical Formula: $C_{13}H_{18}O_2S$
Exact Mass: 238,1
Molecular Weight: 238,35

washed with saturated aqueous NH_4Cl (10 mL), H_2O (10 mL) and brine (10 mL), dried ($MgSO_4$) and concentrated *in vacuo* to afford the crude product as an oil. Purification via flash chromatography (column : 1,5 cm x 10 cm, eluent : 30, 40, 50 % acetone/toluene, 40 mL each) provided 65 mg of a white solid (76 % over 2 steps, 0.272 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 7.11 (m, 5H), 4.27 (s, 1H), 4.08 3.92 (m, 1H), 3.79 (s, 2H), 3.11 2.91 (m, 1H), 2.24 (d, J = 12.2 Hz, 1H), 1.98 (dd, J = 29.7, 13.4 Hz, 2H), 1.82 1.04 (m, 4H) ; ^{13}C NMR (101 MHz, $CDCl_3$) δ 128.75, 128.52, 127.00, 66.66, 66.05, 41.35, 39.21, 36.13, 34.95.

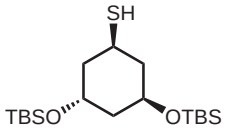
201 :
((1S,3S)-5-(benzylthio)cyclohexane-1,3-diyl)bis(oxy)bis(tert-butyldimethylsilane)



Chemical Formula: $C_{25}H_{46}O_2SSi_2$
Exact Mass: 466,28
Molecular Weight: 466,87

Tert-butyldimethylsilyl chloride (4.63 g, 30.71 mmol, 3.0 equiv) was added to a solution of NEt_3 (5.7 mL, 40.95 mmol, 4.0 equiv) and diol **53** (2.44 g, 10.24 mmol, 1.0 equiv) in DMF (25 mL) at rt. The mixture was left at rt for 16hrs and then quenched using H_2O (50 mL) and tBuOMe (120 mL). The layers were separated and the aqueous layer reextracted using tBuOMe (2 x 20 mL). The combined organic layers were washed with 0.05 M aq. HCl (2 x 30 mL), H_2O (2 x 30 mL) and brine (30 mL), dried ($MgSO_4$) and concentrated *in vacuo* to afford a crude brown oil. The residue was purified via flash chromatography (column : 10 cm x 25 cm, eluent : 1, 5, 12 % acetone/toluene, 250 mL each) to provide 3.30 g of a clear oil (69 %, 7.07 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 7.10 (m, 5H), 4.14 (s, 1H), 4.01 3.84 (m, 1H), 3.75 (s, 2H), 3.02 2.85 (m, 1H), 2.20 2.05 (m, 1H), 1.92 1.75 (m, 2H), 1.38 1.21 (m, 3H), 0.87 (s, 9H), 0.83 (s, 9H), 0.04 (d, J = 2.5 Hz, 6H), 0.01 (s, 3H), -0.02 (s, 3H) ; ^{13}C NMR (101 MHz, $CDCl_3$) δ 128.725, 128.504, 126.919, 67.291, 66.269, 42.103, 39.831, 35.861, 34.779, 25.709, -5.042.

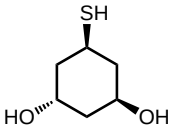
24 :
(3S,5S)-3,5-bis(tert-butyldimethylsilyloxy)cyclohexanethiol



Chemical Formula: $C_{18}H_{40}O_2SSi_2$
Exact Mass: 376,23
Molecular Weight: 376,75

A solution of benzylthioether **201** (213 mg, 0.456 mmol, 1.0 equiv) in dry THF (5 + 5 mL) was added to a solution of Li (22 mg, 3.2 mmol, 7.0 equiv, first rinced in n-hexanes, THF, and methanol) in condensed ammonia (15 mL) at -78 °C. Characteristic blue color remained and the reaction was stirred 30 minutes at -78 °Cbefore being quenched slowly until total disappearance of blue color with saturated aqueous NH_4Cl (5 mL). Mixture allowed to warm to rt under a strong stream of Ar for 4hrs before being diluted with Et_2O (20 mL) and H_2O (10 mL). The layers were separated, and the aqueous layer extracted with Et_2O (2 x 15 mL) and combined organics were washed with saturated aqueous NH_4Cl (5 mL), H_2O (5 mL) and brine (10 mL) before being dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product as a brown oil. The residue was purified by flash chromatography on silica gel (column : 2 cm x 12 cm, eluent : 0, 50, 100 % n-hexanes/heptane, 100 mL each) to provide **24** as a yellow-clear oil in 79 % (136 mg, 0.360 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.14 (s, 1H), 3.99 (ddd, J = 15.0, 10.7, 4.2 Hz, 1H), 3.22 3.03 (m, 1H), 2.23 (d, J = 12.4 Hz, 1H), 1.91 (dd, J = 26.8, 13.0 Hz, 2H), 1.53 (d, J = 7.7 Hz, 1H), 1.44 1.24 (m, 3H), 0.88 (d, J = 0.8 Hz, 18H), 0.04 (dd, J = 4.1, 2.2 Hz, 12H). ^{13}C NMR (300 MHz, $CDCl_3$) δ 67.771, 66.635, 47.383, 44.510, 42.201, 31.577, 18.315, 18.056, -4.526, -4.815, -4.869.

43 :
(1S,3S)-5-mercaptocyclohexane-1,3-diol

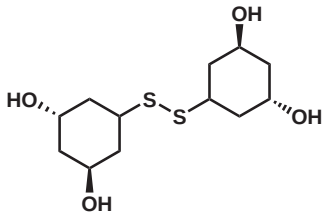


Chemical Formula: $C_6H_{12}O_2S$
Exact Mass: 148,06
Molecular Weight: 148,22

Concentrated HCl (50 μL, mmol, 36.5-38.0 %) was added to a solution of thiol **24** (50 mg, 0.133 mmol, 1 equiv) in MeOH (10 ml) at rt. The mixture was concentrated *in vacuo* on a rotatory evaporator. The procedure was repeated twice more. The dry residue was

concentrated from pure dry MeOH (15 mL) twice before being dried under pump vacuum. The residue was purified by flash chromatography on silica gel (column : 1 cm x 11 cm, eluent : 0, 50, 100 % methanol/CH_2Cl_2, 25 mL each) to provide 15 mg of **43** as a solid (76 %, 0.101 mmol). ^{1}H NMR (400 MHz, CD_3OD) δ 4.134 (m, 1H), 3.895 (m, 1H), 3.069 (m, 1H), 2.268 (m, 1H), 2.031 (m, 2H), 1.413 (m, 1H), 1.276 (m, 2H). ^{13}C NMR (101 MHz, CD_3OD) δ 67.961, 66.331, 48.002, 44.526, 41.325, 31.906.

196 :
(1S,1'S,3S,3'S)-5,5'-disulfanediyldicyclohexane-1,3-diol

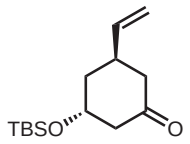


Chemical Formula: $C_{12}H_{22}O_4S_2$
Exact Mass: 294,1
Molecular Weight: 294,43

Solid iodine (I_2) (0.155 g, 0.61 mmol, 1.05 equiv) was added to a solution of thiol **43** (86 mg, 0.582 mmol, 1.00 equiv) and Et_3N (0.12 mL, 0.873 mmol, 1.5 equiv) in CH_2Cl_2 (10 mL) at rt. The dark brown reaction mixture was stirred 2 hr until TLC showed completion. The reaction was diluted with EtOAc (15 mL) and water (10 mL) and saturated aqueous $Na_2S_2O_8$ (1.5 mL). The layers were separated and the aqueous layer was extracted with EtOAc (15 mL). The combined organic layers were washed with saturated aqueous $Na_2S_2O_8$ (2 x 10 mL), H_2O (10 mL), brine (15 mL) then dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product as an oil. The residue was purified via flash chromatography (column : 1,5 cm x 13 cm, eluent : 0, 10, 20, 30 % MeOH/CH_2Cl_2, 20 mL each) to provide 68 mg of disulfide **196** as a white solid (79 %, 0.460 mmol). ^{1}H NMR (400 MHz, CD_3OD) δ 4.25 4.14 (m, 1H), 4.00 3.80 (m, 1H), 3.05 (ddd, J = 12.4, 8.0, 3.5 Hz, 1H), 2.36 2.23 (m, 1H), 2.11 1.96 (m, 2H), 1.33 (dddd, J = 38.9, 35.4, 18.3, 7.1 Hz, 4H) ; ^{13}C NMR (100 MHz, CD_3OD) 67.786, 66.407, 43.978, 42.926, 41.691, 39.542. ESI-HRMS found 317.08512 $\pm$ 0.00 (MNa^+ calc. 317.08517).

129 :
(3R,5R)-3-(tert-butyldimethylsilyloxy)-5-vinylcyclohexanone

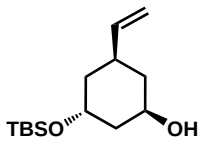
A 2.63 M solution of *n*BuLi in hexanes (2.60 mL, 6.84 mmol, 3.08 equiv) was added to a suspension of flame-dried CuCN in THF (5 mL) at 0 °C. To this mixture brought to rt was added via cannula a solution of vinyltributyltin ($CH_2CHSnBu_3$) (1.0 mL, 3.42 mmol, 1.6 equiv) in THF (5 + 5 mL). The reaction mixture was stirred at rt for 1.5 hr then cooled



Chemical Formula: $C_{14}H_{26}O_2Si$
Exact Mass: 254,17
Molecular Weight: 254,44

to -65 °Cin isopropanol-dry ice bath. To this solution was added a solution of enone **50** (500 mg, 2.21 mmol, 1.0 equiv) in THF (5 + 3 + 2 mL). TLC showed completion in 5 minutes (15 % EtOAc/hexanes). The reaction mixture was quenched by pouring reaction into a 9:1 saturated ammonium chloride/ammonium hydroxide solution (30 mL total) at rt. The mixture was diluted with Et_2O (10 mL). The layers were separated and the aqueous layer was extracted with Et_2O (10 mL). The combined organic layers were washed with H_2O (5 mL), brine (5 mL) then dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product as a pale yellow oil. The residue was purified via flash chromatography (column : 2,5 cm x 25 cm, eluent : 15 % EtOAc/hexanes) to provide 400 mg of vinylketone **129** as a clear oil (71 %, 1.57 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.80 (ddd, J = 17.0, 10.4, 6.4 Hz, 1H), 5.10 4.92 (m, 2H), 4.46 4.36 (m, 1H), 3.08 2.85 (m, 1H), 2.43 (dddd, J = 17.9, 14.2, 8.4, 2.0 Hz, 3H), 2.15 (dd, J = 13.9, 12.0 Hz, 1H), 1.93 (d, J = 13.5 Hz, 1H), 1.75 1.57 (m, 1H), 0.91 (s, 9H), 0.06 and 0.04 (s, 3H each) ; ^{13}C NMR (101 MHz, $CDCl_3$) δ 209.09, 141.25, 113.58, 68.57, 49.24, 46.52, 38.45, 36.01, 25.61, 17.90, -5.00, -5.03. ESI-HRMS found 255.17724 $\pm$ 0.000 (MH^+ calc. 255.17748).

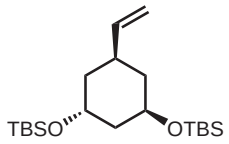
202 ; (1R,3R,5S)-3-(tert-butyldimethylsilyloxy)-5-vinylcyclohexanol



Chemical Formula: $C_{14}H_{28}O_2Si$
Exact Mass: 256,1859
Molecular Weight: 256,4564

Solid $NaBH_4$ (230 mg, 6.0 mmol, 5.0 equiv) was added to a stirring solution of ketone **129** (305 mg, 1.2 mmol, 1.0 equiv) in MeOH (10 mL) at 0 °C. The mixture was warmed to room temperature over 20 minutes then stirred for 2 hrs or until TLC showed completion (15 % EtOAc/hexanes). The reaction mixture was quenched with sat. aqueous NH_4Cl (5 mL) and diluted with EtOAc (30 mL). The layers were separated and the aqueous layer extracted with EtOAc (3 x 15 mL). The combined organic layers were washed with aq. sat. NH_4Cl (5 mL), brine (5 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give 290 mg of crude oil which solidified upon storage. The crude residue was directly taken to the next step without purification.

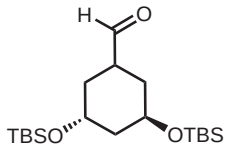
130 ; ((1R,3R)-5-vinylcyclohexane-1,3-diyl) bis(oxy)bis (tert-butyldimethylsilane)



Chemical Formula: $C_{20}H_{42}O_2Si_2$
Exact Mass: 370,27
Molecular Weight: 370,72

Et_3N (1.30 mL, 9.35 mmol, 8.3 equiv) and TBSCl (740 mg, 4.91 mmol, 4.3 equiv) were added to a solution of crude alcohol **202** (290 mg, 1.13 mmol, 1.0 equiv) in DMF (20 mL) at 0 °C. The reaction was warmed to room temperature and stirred for 6 hrs or until TLC showed completion (15 % EtOAc/hexanes). The reaction mixture was diluted with t-BuOMe (250 mL) and water (100 mL) and the layers were separated. The organic phase was washed with water (4 x 50 mL), brine (100 mL), dried ($MgSO_4$), and concentrated in vacuo to give 527 mg of crude product as an oil. rated *in vacuo* to give the crude product as a pale yellow oil. The residue was purified via flash chromatography (column : 2,5 cm x 27 cm, eluent : 2 % EtOAc/hexanes, no gradient) to provide 226 mg of bis-protected antidiol **130** as a clear oil (51 % over two steps, 0.609 mmol) and 145 mg of bis-protected syndiol (33 % over two steps, 0.391 mmol). 84 % yield of bis-protected diols over two steps. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.87 5.69 (m, 1H), 5.07 4.84 (m, 2H), 4.22 4.11 (m, 1H), 4.11 3.94 (m, 1H), 2.51 (s, 1H), 1.97 1.80 (m, 2H), 1.68 1.55 (m, 1H), 1.33 1.05 (m, 3H), 0.95 0.80 (m, 18H), 0.08 -0.07 (m, 12H).

203 :
(3R,5R)-3,5-bis(tert-butyldimethylsilyloxy)cyclohexanecarbaldehyde

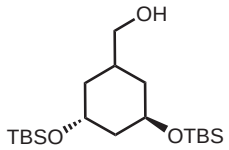


Chemical Formula: $C_{19}H_{40}O_3Si_2$
Exact Mass: 372,25
Molecular Weight: 372,69

Ozone gas was bubbled through a solution of vinyl diTBS **130** (18.4 mg, 49.63 μmol, 1.0 equiv) in MeOH (1 ml) and CH_2Cl_2 (5 ml) at -78 °C until a pale blue color persisted (20 min.) and then left for another 10 min. Argon was then bubbled through the reaction mixture until the solution turned clear. PPh_3 (39 mg, 149 μmol, 3.0 equiv) was added to the reaction mixture at -78 °C and the reaction stirred for 1 h, then warmed to room temperature and stirred for 16 hrs. The reaction mixture was concentrated *in vacuo* and immediately purified by preparative chromatography (eluent : 6 % EtOAc/hexanes) to afford

15 mg of aldehyde **203** (81 %, 40.20 μmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.65 (s, 1H), 4.19 (s, 1H), 4.15 4.06 (m, 1H), 2.68 (m, 1H), 2.03 (m, 1H), 1.70 (m, 3H), 1.60 1.41 (m, 2H), 0.89 and 0.87 (s, 9H each), 0.06 (m, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 203.63, 66.92, 65.82, 44.64, 42.70, 34.56, 32.70, 25.79, -4.81.

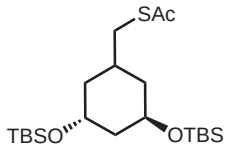
131 :
((3R,5R)-3,5-bis(tert-butyldimethylsilyloxy)cyclohexyl)methanol



Chemical Formula: $C_{19}H_{42}O_3Si_2$
Exact Mass: 374,27
Molecular Weight: 374,71

Solid $LiAlH_4$ (23 mg, 0.605 mmol, 5.0 equiv) was added to a stirring solution of a crude aldehyde **203** (max 0.121 mmol, 1.0 equiv) in THF (100 mL) cooled in an ice bath. The reaction was let warmed to room temperature over 20 minutes then stirred overnight. The reaction was quenched with water (0.5 mL), 1M NaOH (0.5 mL) and water again (1 mL) (Fieser work up). The mixture stirred vigorously in the presence of ($MgSO_4$) for 30 minutes then filtered and concentrated *in vacuo* to give a crude oil. The residue was purified using preparative chromatography (eluent : 15 % EtOAc/hexanes) which provided 13.2 mg of alcohol **131** as a clear oil (29 % over two steps, 0.035 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.20 (s, 1H), 4.13 3.99 (m, 1H), 3.50 (s, 2H), 2.04 (s, 1H), 1.97 1.82 (m, 2H), 1.68 1.57 (m, 1H), 1.41 1.27 (m, 2H), 1.16 0.96 (m, 2H), 0.88 (d, J = 0.9 Hz, 18H), 0.12 -0.03 (m, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 68.15, 67.40, 66.99, 43.18, 38.71, 35.87, 33.70, 25.91, 25.74, -4.69.

132 ; S-((3R,5R)-3,5-bis(tert-butyldimethylsilyloxy)cyclohexyl)methyl ethanethioate



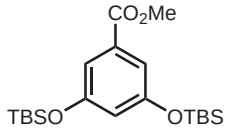
Chemical Formula: $C_{21}H_{44}O_3SSi_2$
Exact Mass: 432,25
Molecular Weight: 432,81

DIAD (8 μL, 0.042 mmol, 1.21 equiv) was added neat to solid PPh_3 (11 mg, 0.042 mmol, 1.20 equiv) immediately followed by THF (1 mL) at 0 °C. A milky solution formed after 5 minutes and the yellow color of DIAD disappeared after 10 minutes at 0 °C. The

reaction was charged with a solution of alcohol **131** (13.2 mg, 0.035 mmol, 1.0 equiv) in THF (1+0.5 mL). Reaction appearance changed from milky white to clear. The reaction was stirred at 0 °Cfor 30 minutes overall before being charged with neat AcSH (7.5 μL, 0.105 mmol, 3.0 equiv). The reaction was warmed to room temperature and stirred overnight. The mixture was then concentrated *in vacuo* and purified via preparative chromatography (eluent : 15 % EtOAc/hexanes) to provide 5.6 mg of thioacetate **132** as a clear oil (37 % (77 % based on recovered starting alcohol), 0.013 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.14 (s, 1H), 4.07 3.89 (m, 1H), 2.84 (qd, J = 13.3, 6.6 Hz, 2H), 2.33 (s, 3H), 2.01 (s, 1H), 1.88 (t, J = 14.4 Hz, 2H), 1.66 (d, J = 13.2 Hz, 1H), 1.36 1.23 (m, 2H), 1.10 0.97 (m, 2H), 0.876 and 0.862 (s, 9H each), 0.047 and 0.043 and 0.025 and 0.019 (s, 3H each). ^{13}C NMR (101 MHz, $CDCl_3$) δ 202.45, 68.83, 41.40, 40.11, 35.76, 29.89, 25.81, 25.42, 18.08, -4.52.

6.1.7 Miscellaneous mimics

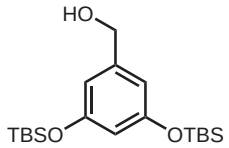
137 :
methyl 3,5-bis(tert-butyldimethylsilyloxy)benzoate



Chemical Formula: $C_{20}H_{36}O_4Si_2$
Exact Mass: 396,22
Molecular Weight: 396,67

Imidazole (6.1 g, 89.28 mmol) and TBSCl (17.26 g, 114.5 mmol) were added to a solution of methyl quinicate **16** (10.73 g, 52.04 mmol) in DMF (200 mL). The flask was sealed with a rubber septum and cooled to 0 °C, at which point Et_3N (11.85 g, 16.30 mL, 117.1 mmol) was added to the reaction via syringe. A fine white precipitate formed upon addition of the amine. The reaction was stirred under argon for 16 hr while warming to room temperature. The reaction mixture was then filtered to remove the precipitate, and the filtrate diluted with EtOAc (200 mL) and washed with sat. NH_4Cl (3 x 100 mL), distilled H_2O (100 mL) and brine (100 mL). The organic layer was then separated, dried ($MgSO_4$), and concentrated in vacuo to give the crude product as a yellow, viscous oil. Product **17** was isolated as a fluffy, white solid via FCC (30% EtOAc in hexanes) in 73% yield (16.42 g, 37.76 mmol). Rf = 0.60 (30% EtOAc in hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.08 (d, J = 2.2 Hz, 2H), 6.48 (t, J = 2.2 Hz, 1H), 3.85 (s, 3H), 0.94 (s, 18H), 0.21 (s, 12H) EI-MS m/z 396 ($M^{\bullet+}$).

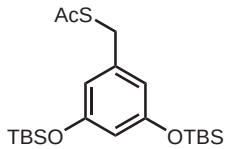
204 :
(3,5-bis(tert-butyldimethylsilyloxy)phenyl)methanol



Chemical Formula: $C_{19}H_{36}O_3Si_2$
Exact Mass: 368,22
Molecular Weight: 368,66

Solid $LiAlH_4$ (1.44 g, 37.83 mmol, 1.5 equiv) was added to a stirring solution of a ester **137** (10 g, 25.22 mmol, 1.0 equiv) in THF (200 mL) cooled in an ice bath. The reaction was let warmed to room temperature over 20 minutes then stirred overnight. The reaction was quenched with water according to the Fieser work-up (water (3.70 mL), 1M NaOH (3.7 mL) and water again (30 mL)). The mixture stirred vigorously in the presence of ($MgSO_4$) for 60 minutes then filtered and concentrated *in vacuo* to give a crude oil. The residue was purified using flash chromatography (column : 16 cm x 3.5 cm, eluent : 15, 20, 25, 35 % EtOAc/hexanes) and provided 5.57 g of alcohol **204** as a clear oil (60 % over two steps, 15.11 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.47 (d, J = 2.2 Hz, 2H), 6.25 (t, J = 2.2 Hz, 1H), 4.57 (d, J = 6.0 Hz, 2H), 0.98 (s, 18H), 0.19 (s, 12H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.66, 143.02, 111.73, 111.16, 65.12, 25.65, 18.17, -4.41.

138 :
S-3,5-bis(tert-butyldimethylsilyloxy)benzyl ethanethioate

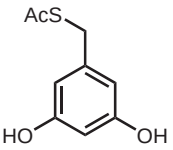


Chemical Formula: $C_{21}H_{38}O_3SSi_2$
Exact Mass: 426,21
Molecular Weight: 426,76

DIAD (192 μL, 0.977 mmol, 1.2 equiv) was added neat to solid PPh_3 (254 mg, 0.969 mmol, 1.19 equiv) immediately followed by THF (10 mL) at 0 °C. A milky solution formed after 5 minutes and the yellow color of DIAD disappeared after 25 minutes at 0 °C. The reaction was charged with a solution of alcohol **204** (356 mg, 0.814 mmol, 1.0 equiv) in THF (5+1 mL). Precipitate appearance changed from milky white to silvery white. The reaction was stirred at 0 °Cfor 30 minutes overall before being charged with neat AcSH (175 μL, 2.44 mmol, 3.0 equiv). Cloudiness immediately disappeared. The reaction was warmed to room temperature and stirred overnight. The mixture was then concentrated in vacuo and purified via flash chromatography (column : 3.0 cm x 20 cm, eluent : 0, 2, 5, 10,

15, 20 % EtOAc/hexanes, 100 mL each) to provide 318 mg of thioacetate **138** as a clear oil (94 %, 0.768 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.38 (d, J = 2.1 Hz, 2H), 6.21 (d, J = 2.1 Hz, 1H), 3.99 (s, 2H), 2.34 (s, 3H), 0.96 (s, 18H), 0.18 (s, 12H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 195.03, 156.53, 139.18, 113.82, 110.96, 33.32, 30.26, 25.64, 18.17, -4.44.

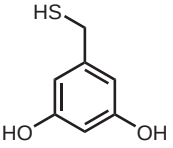
205 :
S-3,5-dihydroxybenzyl ethanethioate



Chemical Formula: $C_9H_{10}O_3S$
Exact Mass: 198,04
Molecular Weight: 198,24

Aqueous HBr (200 μL, 1.78 mmol, 48 %, 0.92 equiv) was added to a solution of thioester **138** (827 mg, 1.94 mmol, 1.0 equiv) and potassium fluoride dihydrate ($KF \cdot 2\,H_2O$) (731 mg, 7.77 mmol, 4.0 equiv) in DMF (10 mL) at rt. The reaction was stirred overnight at room temperature. The mixture went from yellow to brown. TLC showed completion (10 % AcCN/$CHCl_3$, DMF removed under vaccum for 30 minutes prior to elution). The mixture was quenched with 2 N aq. HCl (2 mL) and diluted with *t*BuOMe (100 mL). The layers were separated and the aqueous layer was extracted with *t*BuOMe (2 x 15 mL). The combined organic layers were washed with H_2O (2 x 15 mL), brine (15 mL) then dried ($MgSO_4$) and concentrated *in vacuo*. The product **205** was obtained crude as a brown oil containing DMF that was immediately taken to the next step (see product **128**). ^{1}H NMR (400 MHz, CD_3OD) δ 6.21 (s, 2H), 6.13 (s, 1H), 3.49 (s, 2H), 2.31 (s, 3H). ^{13}C NMR (75 MHz, CD_3OD) δ 194.12, 159.53, 137.96, 111.34, 105.51, 35.41, 30.26.

128 :
5-(mercaptomethyl)benzene-1,3-diol

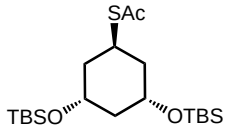


Chemical Formula: $C_7H_8O_2S$
Exact Mass: 156,02
Molecular Weight: 156,2

Aqueous NaOH (32 mL, 97 mmol, 50 equiv, 3 M) was added to a yellow solution of thioacetate **205** (max 1.94 mmol, 1.0 eq) in dry MeOH (40 mL) and under argon. The

yellow color discharged immediately. The reaction was stirred 30 min then the reaction was acidified to pH 3.0 with 2 N HCl. The biphasic mixture was concentrated *in vacuo* to remove all organics and the aqueous phase was diluted with EtOAc (100 mL) and washed with H_2O (50 mL), brine (50 mL), then dried (MgSO4) and concentrated *in vacuo* to give the crude product as a yellow oil. the residue was purified by flash chromatography (column : 2.5 cm x 21 cm, eluent : 15, 20, 30, 40, 50 % $CH_3CN/CHCl_3$, 100 mL each) to provide 190 mg of thiol **128** as a pale yellow oil (63 %, 1.22 mmol). ^{1}H NMR (300 MHz, CD_3OD) δ 6.26 (s, 2H), 6.12 (s, 1H), 3.54 (br s, 2H), 2.02 (br s, 1H). ^{13}C NMR (75 MHz, CD_3OD) δ 157.31, 138.98, 106.45, 102.42, 29.67.

144 :
S-((1r,3R,5S)-3,5-bis((tert-butyldimethylsilyl)oxy)cyclohexyl) ethanethioate

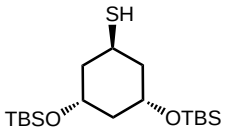


Chemical Formula: $C_{20}H_{42}O_3SSi_2$
Exact Mass: 418,2393
Molecular Weight: 418,7817

DIAD (32.8 μL, 0.1664 mmol, 2.00 equiv) was added neat to solid PPh_3 (43 mg, 0.1621 mmol, 1.95 equiv) then THF (1 mL) was added at 0 °C. A milky solution formed after 5 minutes and the yellow color of DIAD remained. The reaction was stirred at 0 °Cfor 20 minutes overall before being charged with a solution of (1r,3R,5S)-3,5-bis((tert-butyldimethylsilyl)oxy)cyclohexanol (30 mg, 0.0832 mmol, 1.0 equiv) and AcSH (12.0 μL, 0.1664 mmol, 2.0 equiv) in THF (3+1 mL) was added dropwise at 0 °C. Precipitate disappeared and solution became homogeneous. The reaction was left at 0 °Cfor 15 minutes then warmed to room temperature overnight. The mixture was quenched with sat. aq. $NaHCO_3$ (5 mL) and diluted with EtOAc (15 mL). The layers were separated and the aqueous layer was extracted with EtOAc (2 x 10 mL). The combined organic layers were washed with brine (10 mL) then dried ($MgSO_4$) and concentrated *in vacuo*. The crude oily residue was used directly in the next step without purification.

145 :
(1r,3R,5S)-3,5-bis((tert-butyldimethylsilyl)oxy)cyclohexanethiol

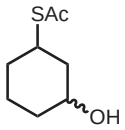
A solution of aq. NaOH (132 μL, 0.33 mmol, 4.0 equiv, 2.5 M) was added to a solution of crude thioacetate **144** (max 0.0832 mmol, 1.0 equiv) in 1:1 MeOH/THF (2 mL) at 25 °Cunder argon. The reaction was stirred for 2 hr at 25 °C. TLC showed completion (5 % EtOAc/hexanes). The mixture was quenched with 0.1N HCl (1 mL) and diluted with EtOAc (5 mL). The layers were separated and the aqueous layer was extracted with EtOAc



Chemical Formula: $C_{18}H_{40}O_2SSi_2$
Exact Mass: 376,2288
Molecular Weight: 376,7450

(2 x 5 mL). The combined organic layers were washed with H_2O (5 mL), brine (5 mL) then dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product. The residue was purified via preparative TLC with 2 % EtOAc/hexanes to give 21 mg of thiol **145** (68 %, 0.056 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.15 (m, 1H), 3.98 (m, 1H), 3.18 (m, 1H), 2.22 (m, 1H), 1.90 (m, 2H), 1.54 (d, J = 9.2 Hz, 1H), 1.35 (m, 3H), 0.95 (s, 18H), 0.04 (s, 12H).

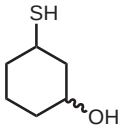
141 :
S-3-hydroxycyclohexyl ethanethioate



Chemical Formula: $C_8H_{14}O_2S$
Exact Mass: 174,07
Molecular Weight: 174,26

AcSH (7.79 mL, 105 mmol, 1.05 equiv) was added to a solution of cyclohexenone (9.73 mL, 100 mmol, 1.00 equiv) and cat. NaOMe (100 mg, 1.85 mmol, 0.0185 equiv) in 8:1 DCM/DMF (600 mL) at 0 °C. The reaction was stirred at room temperature for 3 days before being concentrated *in vacuo* to remove the DCM. The DMF layer was diluted with THF (300 mL). $NaBH_4$ (1.20 g, 34.3 mmol 0.34 equiv) was added to the mixture at 0 °C and kept at that temperature for 4 hrs before being quenched with H_2O (150 mL) and diluted with EtOAc (150 mL). The layers were separated and the aqueous layer was extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine (50 mL) then dried ($MgSO_4$) and concentrated *in vacuo* to give a crude oil. The residue was purified via flash chromatography (column : 10 x 19 cm, eluent : 5, 10, 15 % $CH_3CN/CHCl_3$, 1000 mL each) to give 4.808 g of compound **141** (28 %, 27.6 mmol) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) 3.67 (m, 1H), 3.38 (m, 1H), 2.29 (s, 3H), 2.20 (m, 1H), 1.85 (m, 3H), 1.25 (m, 4H) ; ^{13}C NMR (101 MHz, $CDCl_3$) 211.21, 69.80, 41.70, 39.78, 34.46, 31.93, 30.66, 23.59.

142 :
3-mercaptocyclohexanol

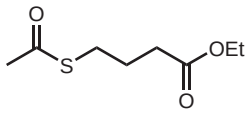


Chemical Formula: $C_6H_{12}OS$
Exact Mass: 132,06
Molecular Weight: 132,22

Propylamine (12 mL, 145 mmol, 10.0 equiv) was added to a solution of thioacetate **141** (2.53 g, 14.49 mmol, 1.0 equiv) in THF (30 mL) at rt under argon. The reaction was stirred overnight and then concentrated to a yellow oil. The oil was diluted with EtOAc (50 mL) and was washed with water (3 x 50 mL), brine (50 mL) then dried ($MgSO_4$) and concentrated *in vacuo* to give 1.21 g of an oil of sufficient purity (63 %, 9.13 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.57 (m, 1H), 2.72 (m, 1H), 2.29 (m, 1H), 1.94 (m, 2H), 1.81 (m, 1H), 1.59 (d, J = 6.6 Hz, 1H), 1.24 (s, 4H) ; ^{13}C NMR (101 MHz, CD_3OD) δ 67.96, 48.00, 44.52, 41.32, 31.90, 25.47.

6.1.8 Top chain thiols and amines

62 :
ethyl 4-(acetylthio)butanoate

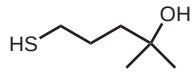


Chemical Formula: $C_8H_{14}O_3S$
Exact Mass: 190,07
Molecular Weight: 190,26

Ethyl 4-bromobutanoate (2.38 mL, 16.450 mmol, 1.0 equiv) was added to a suspension of potassium thioacetate (AcSK) (2.067 g, 18.095 mmol, 1.1 equiv) in EtOH (100 mL) at rt. The reaction was stirred at rt overnight. The mixture was quenched with H_2O (150 mL) and diluted with EtOAc (150 mL). The layers were separated and the aqueous layer was extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with sat. aq. $NaHCO_3$ (100 mL), brine (50 mL) then dried ($MgSO_4$) and concentrated *in vacuo* to give a crude oil. The residue was purified via flash chromatography (eluent : 20, 30, 40 % EtOAc/hexanes, 200 mL each) to give 2.98 g of compound **62** (95 %, 15.66 mmol) as a pale red oil. ^{1}H NMR (400 MHz, $CDCl_3$) 4.093 (quartet, J = 7.3 Hz, 2H), 2.882 (t, J =

7.0 Hz, 2H), 2.342 (t, J = 6.3 Hz, 2H), 2.296 (s, 3H), 1.870 (quintet, J = 7.0 Hz, 2H), 1.226 (t, J = 7.0 Hz). ; ^{13}C NMR (101 MHz, $CDCl_3$) 195.331, 172.631, 60.536, 33.127, 30.761, 28.468, 25.068, 14.406.

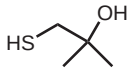
63 :
5-mercapto-2-methylpentan-2-ol



Chemical Formula: $C_6H_{14}OS$
Exact Mass: 134,08
Molecular Weight: 134,24

A solution of ester **62** (2.98 g, 15.66 mmol, 1.0 equiv) in THF (10+10 mL) was added via cannula over a period of 30 min. to a solution of MeLi (46.98 mL, 70.47 mmol, 4.5 equiv, 1.5 M in Et_2O) in THF (125 mL) at $-78\ ^\circ C$. The reaction was stirred at that temperature for 1h then warmed to room temperature and stirred for 1h. The mixture was quenched carefully with 1 M aq. HCl (50 mL) and H_2O (50 mL). The layers were separated and the aqueous layer was extracted with EtOAc (50 mL). The combined organic layers were washed with brine (50 mL) then dried ($MgSO_4$) and concentrated *in vacuo* to give 1.029 g of thiol **63** (49 %, 7.67 mmol). The residue could be purified via flash chromatography (eluent : 20 % EtOAc/hexanes). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.501 (m, 2H), 1.664 (m, 3H), 1.507 (m, 2H), 1.339 (t, J = 7.8 Hz, 1H) 1.178 (s, 6H) ; ^{13}C NMR (101 MHz, $CDCl_3$) 70.737, 42.950, 42.599, 34.863, 29.483, 29.460, 29.109, 25.314, 24.148, 15.673

58 :
1-mercapto-2-methylpropan-2-ol

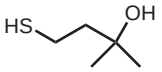


Chemical Formula: $C_4H_{10}OS$
Exact Mass: 106,05
Molecular Weight: 106,19

Methyl thioglycolate (3.561 g, 33.55 mmol, 1.0 equiv) was added to a solution of MeLi (83.9 mL, 134.2 mmol, 4.0 equiv, 1.6 M in Et_2O) in THF (80 mL) at $-78\ ^\circ C$. The reaction was warmed to room temperature and the reaction followed by TLC. The mixture was quenched carefully with a mixture of sat. aq. NH_4Cl (50 mL) and H_2O (50 mL). The layers were separated and the aqueous layer was extracted with EtOAc (50 mL). The combined organic layers were washed with brine (50 mL) then dried ($MgSO_4$) and concentrated *in vacuo* to give 3.1214 g of thiol **58** (88 %, 29.39 mmol) as a yellow oil smelling

like burned onions. The residue could be purified via flash chromatography (eluent : 10 % EtOAc/hexanes). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.610 (d, J = Hz, 2H), 2.328 (br s, 1H), 1.373 (t, J = Hz, 1H), 1.261 (s, 6H) ; ^{13}C NMR (75 MHz, $CDCl_3$) δ 69.748, 38.500, 28.929, 27.949

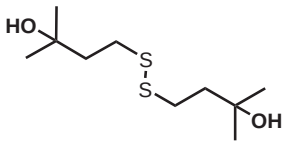
60 :
4-mercapto-2-methylbutan-2-ol



Chemical Formula: $C_5H_{12}OS$
Exact Mass: 120,06
Molecular Weight: 120,21

Methyl 3-mercaptopropanoate (4.436 g, 36.91 mmol, 1.0 equiv) was added to a solution of MeLi (92.3 mL, 147.7 mmol, 4.0 equiv, 1.6 M in Et_2O) in THF (95 mL) at -78 °C. The reaction was warmed to room temperature and the reaction followed by TLC. The mixture was quenched carefully with a mixture of sat. aq. NH_4Cl (50 mL) and H_2O (50 mL). The layers were separated and the aqueous layer was extracted with EtOAc (50 mL). The combined organic layers were washed with brine (50 mL) then dried ($MgSO_4$) and concentrated *in vacuo* to give 4.01 g of thiol **60** (90 %, 33.36 mmol) as a yellow oil. The residue could be purified via flash chromatography (eluent : 10 % EtOAc/hexanes). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.559 (dd, J = 7.83, 16.0, 2H), 2.216 (br s, 1H), 1.739 (m, 2H), 1.384 (t, J = 7.4 Hz, 1H), 1.165 (s, 6H) ; ^{13}C NMR (101 MHz, $CDCl_3$) δ 70.829, 48.193, 29.368, 19.872

189 :
4,4'-disulfanediylbis(2-methylbutan-2-ol)

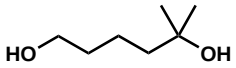


Chemical Formula: $C_{10}H_{22}O_2S_2$
Exact Mass: 238,11
Molecular Weight: 238,41

Solid iodine (I_2) (1.55 g, 6.1 mmol, 1.05 equiv) was added to a solution of thiol **60** (780 mg, 5.82 mmol, 1.00 equiv) and Et_3N (1.2 mL, 8.73 mmol, 1.5 equiv) in CH_2Cl_2 (20 mL) at rt. The dark brown reaction mixture was stirred 4 hr until TLC showed completion. The reaction was diluted with EtOAc (50 mL) and water (40 mL) and saturated aqueous $Na_2S_2O_8$ (15 mL). The layers were separated and the aqueous layer was extracted with EtOAc (15 mL). The combined organic layers were washed with saturated aqueous $Na_2S_2O_8$ (2 x 10

mL), H_2O (10 mL), brine (15 mL) then dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product as an oil. The residue was purified via flash chromatography (column : 1,5 cm x 13 cm, eluent : 0, 10, 20 % EtOAc/hexanes, 20 mL each) to provide 1.08 g of disulfide **189** as a clear oil (74 %, 4.51 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.85 2.69 (m, 4H), 1.90 1.77 (m, 4H), 1.75 (s, 2H), 1.29 1.16 (s, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 70.751, 43.116, 33.819, 29.429. ESI-HRMS found 261.09487 $\pm$ 0.002 (MNa^+ calc. 261.09534).

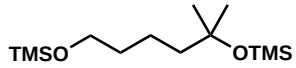
206 :
5-methylhexane-1,5-diol



Chemical Formula: $C_7H_{16}O_2$
Exact Mass: 132,12
Molecular Weight: 132,20
m/z: 132.12 (100.0%), 133.12 (7.8%)
Elemental Analysis: C, 63.60; H, 12.20; O, 24.20

delta-valerolactone (20.0 mL, 165.53 mmol, 1.0 equiv, tech. grade : 75 %) was added to a suspension of dried $CeCl_3 \cdot 7\,H_2O$ (6.166 g, 16.55 mmol, 0.1 equiv) and MeLi (331.0 mL, 496.6 mmol, 3.0 equiv, 1.5 M in Et_2O) in Et_2O (50 mL) at $-78\ ^\circ C$. The reaction was warmed to room temperature and the reaction followed by TLC. The mixture was quenched carefully with a mixture of sat. aq. NH_4Cl (150 mL) and H_2O (150 mL). The layers were separated and the aqueous layer was extracted with EtOAc (3 x 150 mL). The combined organic layers were washed with brine (150 mL) then dried ($MgSO_4$) and concentrated *in vacuo* to give 12.80 g of crude alcohol **206** (crude yield : 90 % , 96.83 mmol).

207 :
2,2,4,4,10,10-hexamethyl-3,9-dioxa-2,10-disilaundecane

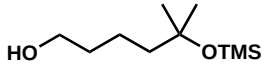


Chemical Formula: $C_{13}H_{32}O_2Si_2$
Exact Mass: 276,19
Molecular Weight: 276,56

TMSCl (36.9 mL, 290.5 mmol, 3.0 equiv) and imidazole (19.70 g, 290.5 mmol, 3.0 equiv) were added to a solution of diol **206** (12.80 g, 96.83 mmol, 1.0 equiv) in CH_2Cl_2 (400 mL) at rt. The reaction was stirred under argon for 3hrs. The reaction mixture was quenched by H_2O (200 mL) and the layers separated. The aqueous phase was reextracted using CH_2Cl_2 (100 mL) then the combined organic layers were washed with distilled H_2O (100 mL) and brine (100 mL), dried ($MgSO_4$), and concentrated *in vacuo* to give 12.65

g of crude product as a yellow oil. Product **207** was used in the next reaction without purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.627 (m, 2H), 1.556 (m, 2H), 1.424 (m, 4H), 1.200 (s, 6H), 0.096 (s, 18H) ; ^{13}C NMR (75 MHz, $CDCl_3$) δ 74.132, 63.028, 44.723, 33.379, 30.009, 20.779, 2.882.

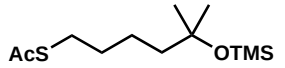
65 :
5-methyl-5-((trimethylsilyl)oxy)hexan-1-ol



Chemical Formula: $C_{10}H_{24}O_2Si$
Exact Mass: 204,15
Molecular Weight: 204,38

K_2CO_3 (0.1086 g, 0.7857 mmol, 0.1 equiv) was added to a solution of BisTMSdiol **207** (2.173 g, 7.857 mmol, 1.0 equiv) in MeOH (50 mL) at rt. The reaction was stirred under argon for 30 min. The reaction mixture was then filtered over paper, quenched by H_2O (20 mL), diluted with EtOAc (100 mL) and the layers separated. The aqueous phase was reextracted using EtOAc (30 mL) then the combined organic layers were washed with distilled H_2O (40 mL) and brine (40 mL), dried ($MgSO_4$), and concentrated *in vacuo* to give a crude product as a yellow oil. Product **65** was purified via flash chromatography (eluant : 20 % EtOAc/hexanes) to yield 1.538 g (96 %, 7.525 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.621 (t, J = 6.45 Hz, 2H), 1.567 (m, 2H), 1.423 (m, 4H), 1.198 (s, 6H), 0.0421 (s, 9H) ; ^{13}C NMR (75 MHz, $CDCl_3$) 74.128, 63.009, 44.739, 33.394, 30.006, 20.786, 2.871.

208 :
S-(5-methyl-5-((trimethylsilyl)oxy)hexyl) ethanethioate

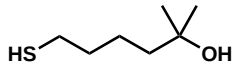


Chemical Formula: $C_{12}H_{26}O_2SSi$
Exact Mass: 262,14
Molecular Weight: 262,48

DIAD (0.276 mL, 1.401 mmol, 1.0 equiv) was added neat to solid PPh_3 (0.5513 g, 2.102 mmol, 1.5 equiv) then THF (3 mL) was added at 0 °C. A milky solution formed after 5 minutes and the yellow color of DIAD remained. The reaction was stirred at 0 °Cfor 30 minutes overall before being charged with a solution of alcohol **65** (0.2864 g, 1.401 mmol, 1.0 equiv) in THF (3+1 mL) at 0 °C. The reaction was stirred 30 minutes and a solution of AcSH (200 μL, 2.802 mmol, 2.0 equiv) in THF (3+1 mL) was added. Precipitate appearance changed from milky yellow to greenish yellow to silvery pale yellow. The reaction was left at 0 °Cfor 15 minutes then warmed to room temperature overnight. The mixture was quenched with sat. aq. $NaHCO_3$ (5 mL). The layers were separated and the

aqueous layer was extracted with EtOAc (2 x 10 mL). The combined organic layers were washed with brine (10 mL) then dried ($MgSO_4$) and concentrated *in vacuo*. Purification using flash chromatography (eluent : 30 % toluene/hexanes) gave 0.254 g of thioacetate **208** (69 %, 0.9688 mmol).

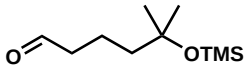
66 :
6-mercapto-2-methylhexan-2-ol



Chemical Formula: $C_7H_{16}OS$
Exact Mass: 148,09
Molecular Weight: 148,27

Thioester **208** (0.5000 g, 1.907 mmol, 1.0 equiv) was added to a solution of NaOEt (freshly created by the addition of NaH (91.5 mg, 3.814 mmol, 2.0 equiv) to EtOH) in EtOH (20 mL) at rt. Reaction was stirred at rt until TLC showed completion. The reaction was quenched by 1 M aq. citric acid (15 mL) and diluted with Et_2O. Layers were separated and aqueous reextracted using Et_2O (2 x 20 mL). The combined organic phases were washed with brine (20 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Crude residue was purified through a 4-cm plug of silica (eluent : 10 % EtOAc/hexanes) to yield 0.212 mg of thiol **66** (75 %, 1.43 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.873 (t, J = 7.0 Hz, 2H), 2.318 (s, 3H), 1.556 (m, 2H), 1.409 (sharp m, 4H), 1.195 (s, 6H), 0.100 (s, 9H) ; ^{13}C NMR (75 MHz, $CDCl_3$) δ 195.896, 73.926, 44.410, 30.872, 30.231, 30.059, 29.395, 23.885, 2.898.

209 :
5-methyl-5-((trimethylsilyl)oxy)hexanal

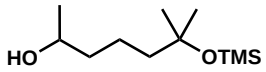


Chemical Formula: $C_{10}H_{22}O_2Si$
Exact Mass: 202,14
Molecular Weight: 202,37

dess-Martin reagent (8.64 g, 20.31 mmol, 2.0 equiv) was added to a solution of alcohol **65** (2.081 g, 10.182 mmol, 1.0 equiv) in CH_2Cl_2 (50 mL) at rt. Reaction was followed by TLC (eluent : 10 % EtOAc/hexanes) until completion. The reaction was quenched by addition of Et_2O (40 mL), sat. aq. $Na_2S_2O_3$ (40 mL) and sat. aq. $NaHCO_3$ (120 mL) and stirred for 30 min. The clear organic layer and the cloudy aqueous layer were separated and the aqueous phase was reextracted using Et_2O (3 x 40 mL). The combined organic layers were washed with distilled H_2O (40 mL) and brine (40 mL), dried ($MgSO_4$), and concentrated *in vacuo* to give a crude product was purified via flash chromatography (eluant : 10 % EtOAc/hexanes) to yield 1.30 g (63 %, 6.405 mmol). ^{1}H NMR (300 MHz,

$CDCl_3$) δ 9.741 (t, J = 1.8 Hz, 1H), 2.422 (dt, J = 1.8 Hz, 7.3 Hz, 2H), 1.695 (m, 2H), 1.425 (m, 2H), 1.218 (s, 6H), 0.097 (s, 9H) ; ^{13}C NMR (101 MHz, $CDCl_3$) δ 202.787, 73.773, 44.475, 44.365, 29.979, 17.306, 2.852.

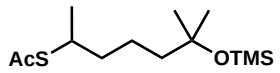
210 :
6-methyl-6-((trimethylsilyl)oxy)heptan-2-ol



Chemical Formula: $C_{11}H_{26}O_2Si$
Exact Mass: 218,17
Molecular Weight: 218,41

MeMgBr (0.384 mL, 1.152 mmol, 1.25 equiv, 3.0 M in Et_2O) was added to a solution of aldehyde **209** (0.187 g, 0.9216 mmol, 1.0 equiv) in THF (15 mL) at -78 °C. The reaction was warmed to room temperature and stirred overnight. The mixture was quenched carefully with a mixture of sat. aq. NH_4Cl (25 mL) and H_2O (25 mL). The layers were separated and the aqueous layer was extracted with EtOAc (50 mL). The combined organic layers were washed with brine (50 mL) then dried ($MgSO_4$) and concentrated *in vacuo* to give 0.1685 g of alcohol **210** (84 %, 0.7715 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.790 (m, 1H), 1.764 (s, 1H), 1.423 (m, 6H), 1.200 (sharp m, 9H), 0.096 (s, 9H) ; ^{13}C NMR (75 MHz, $CDCl_3$) 74.121, 68.168, 44.872, 39.923, 30.101, 29.960, 23.690, 20.790, 2.898.

211 :
S-(6-methyl-6-((trimethylsilyl)oxy)heptan-2-yl) ethanethioate

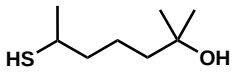


Chemical Formula: $C_{13}H_{28}O_2SSi$
Exact Mass: 276,16
Molecular Weight: 276,51

DIAD (1.17 mL, 5.96 mmol, 1.0 equiv) was added neat to solid PPh_3 (1.7196 g, 6.556 mmol, 1.1 equiv) then THF (25 mL) was added. A milky solution formed after 5 minutes and the yellow color of DIAD remained. The reaction was stirred at 0 °Cfor 30 minutes overall before being charged with a solution of alcohol **210** (0.9624 g, 5.960 mmol, 1.0 equiv) in THF (15 mL) at 0 °C. The reaction was stirred 30 minutes and a solution of AcSH (469 μL, 6.556 mmol, 1.1 equiv) in THF (15 + 0.5 mL) was added. Precipitate appearance changed from milky yellow to greenish yellow to silvery pale yellow. The reaction was left at 0 °Cfor 15 minutes then warmed to room temperature overnight. The mixture was quenched with sat. aq. $NaHCO_3$ (25 mL). The layers were separated and the aqueous layer was extracted with EtOAc (50 mL). The combined organic layers were washed with brine (50 mL) then dried ($MgSO_4$) and concentrated *in vacuo*. Purification

using flash chromatography (eluent : 5 % EtOAc/hexanes) gave 1.12 g of thioacetate **211** (62 %, 4.07 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.549 (sextet, J = 7.0 Hz, 1H), 2.296 (s, 3H), 1.530 (m, 2H), 1.404 (sharp m, 4H), 1.296 (d J = 6.7 Hz, 3H), 1.192 (s, 6H), 0.098 (s, 9H) ; ^{13}C NMR (75 MHz, $CDCl_3$) 195.945, 73.979, 44.559, 39.782, 37.038, 31.013, 30.147, 30.051, 22.057, 21.603, 2.902.

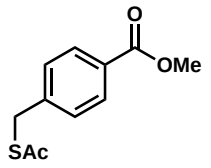
67 :
6-mercapto-2-methylheptan-2-ol



Chemical Formula: $C_8H_{18}OS$
Exact Mass: 162,11
Molecular Weight: 162,29

Thioester **211** (0.9413 g, 3.4042 mmol, 1.0 equiv) was added to a solution of NaOEt (freshly created by the addition of NaH (0.2723 g, 6.8084 mmol, 2.0 equiv) to EtOH) in EtOH (20 mL) at rt. Reaction was stirred at rt until TLC showed completion. The reaction was quenched by 1 M aq. citric acid (15 mL) and diluted with Et_2O. Layers were separated and aqueous reextracted using Et_2O (2 x 20 mL). The combined organic phases were washed with brine (20 mL), dried ($MgSO_4$) and concentrated *in vacuo*. Crude residue was purified through a 5-cm plug of silica (eluent : 10 % EtOAc/hexanes) to yield 0.475 of thiol **67** (86 %, 2.923 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.95 (m, 1H), 1.50 (m, 8H), 1.34 (d, J = 6.6 Hz, 3H), 1.22 (s, 6H) ; ^{13}C NMR (75 MHz, $CDCl_3$) δ 70.940, 43.449, 41.329, 35.555, 29.696, 29.304, 29.263, 25.685, 22.250, 1.018.

212 :
methyl 4-(acetylthiomethyl)benzoate

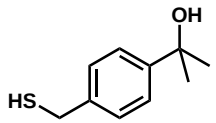


Chemical Formula: $C_{11}H_{12}O_3S$
Exact Mass: 224,05
Molecular Weight: 224,28

Potassium thioacetate (AcSK) (1.19 g, 10.43 mmol, 1.2 equiv) was added to a solution of methyl p-bromomethylbenzoate (2.00 g, 8.69 mmol, 1.0 equiv) in a EtOH (50 mL) at rt. The reaction was stirred 29hrs at rt when TLC showed completion (20 % EtOAc/hexanes). The mixture was concentrated *in vacuo* to give the crude product as a brown solid. The residue was purified via flash chromatography (eluent : 15 % EtOAc/hexanes) to give 1.60

g of thioester **212** as a white solid (82 %, 7.09 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, J = 8.0 Hz, 1H), 7.35 (d, J = 8.1 Hz, 1H), 4.14 (s, 2H), 3.90 (s, 3H), 2.36 (s, 3H) ; ^{13}C NMR (101 MHz, $CDCl_3$) δ 194.56, 166.67, 142.98, 129.88, 129.07, 128.78, 52.05, 33.05, 30.26.

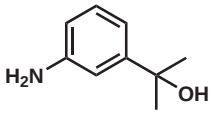
70 :
2-(4-(mercaptomethyl)phenyl)propan-2-ol



Chemical Formula: $C_{10}H_{14}OS$
Exact Mass: 182,08
Molecular Weight: 182,28

Thioester **212** (100 mg, 0.444 mmol, 1.0 equiv) was added to a solution of MeLi (1.39 mL, 2.22 mmol, 5.0 equiv, 1.6 M in Et_2O) in THF (5 mL) at -78 °C. The reaction was warmed to room temperature and the reaction followed by TLC. The mixture was quenched carefully with a mixture of sat. aq. NH_4Cl (5 mL) and H_2O (5 mL). The layers were separated and the aqueous layer was extracted with EtOAc (5 mL). The combined organic layers were washed with brine (5 mL) then dried ($MgSO_4$) and concentrated *in vacuo* to give a crude residue that could be purified via flash chromatography (eluent : 10 % EtOAc/hexanes) to give 70 mg of thiol **70** (86 %, 0.384 mmol) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (m, 2H), 7.30 (m, 2H), 3.74 (d, J = 7.5 Hz, 2H), 1.76 (t, J = 7.6 Hz, 1H), 1.63 1.52 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$ and CD_3OD) δ 147.9, 136.5, 128.5, 126.0, 72.9, 31.2, 28.0.

77 :
2-(3-aminophenyl)propan-2-ol



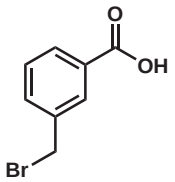
Chemical Formula: $C_9H_{13}NO$
Exact Mass: 151,1
Molecular Weight: 151,21

MeLi (6.83 mL, 10.94 mmol, 4.1 equiv, 1.6 M in Et_2O) was added to a suspension of methyl 3-aminobenzoate hydrochloride **76** (0.500 g, 2.67 mmol, 1.0 equiv) in Et_2O (10 mL) at -78 °C. The reaction was warmed to room temperature and stirred overnight. The mixture was quenched carefully with a mixture of sat. aq. NH_4Cl (25 mL) and H_2O (25 mL). The layers were separated and the aqueous layer was extracted with EtOAc (15 mL).

The combined organic layers were washed with brine (15 mL) then dried ($MgSO_4$) and concentrated *in vacuo*. The crude residue was purified via flash chromatography to give 109 mg of amine **77** (27 %, 0.727 mmol, 49 % based on recovered starting material). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.09 (t, J = 7.9 Hz, 1H), 6.82 (s, 2H), 6.52 (d, J = 7.1 Hz, 1H), 3.42 (br s, 3H), 1.50 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$ and CD_3OD) δ 150.1, 147.5, 128.2, 119.4, 117.2, 112.4, 71.4, 30.5.

6.1.9 Bromoalkyl-carboxylic acid cores

80 :
3-(bromomethyl)benzoic acid

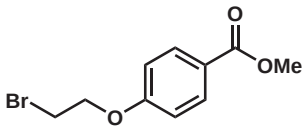


Chemical Formula: $C_8H_7BrO_2$
Exact Mass: 213,96
Molecular Weight: 215,04

To a solution of $KBrO_3$ (6.67 g, 40 mmol, 3.0 equiv) in water (25 mL) was added commercial *m*-toluic acid (2.0 g, 13.3 mmol, 1.0 equiv) in ethyl acetate (18 mL), followed by a solution of $NaHSO_3$ (6.56 g, 40.0 mmol, 3.0 equiv) in water (27 mL) over a period of about 15 min, and the mixture was stirred at room temperature for 4 h. The mixture was poured into 50 mL of ether. After separation of the phases, the aqueous layer was extracted twice with ether, and the combined organic layer was washed with saturated aqueous $Na_2S_2O_3$ solution (20 mL), brine (15 mL) and dried ($MgSO_4$). The solvents were then removed *in vacuo* to provide 1.81 g of bromide **80** (47 %, 6.29 mmol) that was used crude in the next reactions. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.45 (br s, 1H), 8.15 (s, 1H), 8.08 (t, J = 9.3 Hz, 1H), 7.66 (d, J = 7.6 Hz, 1H), 7.55 7.44 (m, 1H), 4.54 (s, 2H). ; ^{13}C NMR ($CDCl_3$) 171.4, 137.9, 134.3, 130.7, 130.1, 130.1, 128.9. MS (ESI) calcd for $C_8H_7BrO_2$ - H (M-H): 212.9551; found: 212.9562

84 :
methyl 4-(2-bromoethoxy)benzoate

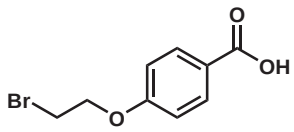
To a solution of methyl 4-hydroxybenzoate (3 g, 19.74 mmol, 1.0 equiv) in acetone (100 mL) were added potassium carbonate (K_2CO_3) (8.18 g, 59.22 mmol, 3 equiv) and 1,2-dib- romoethane (6.8 mL, 78.95 mmol, 4e quiv) and the mixture was refluxed for 48 h. The progress of the reaction was monitored by TLC using 20 % EtOAc/hexanes. After



Chemical Formula: $C_{10}H_{11}BrO_3$
Exact Mass: 257,99
Molecular Weight: 259,1

completion of the reaction, potassium carbonate was removed by filtration and the solvent was evaporated under vacuum to get the crude product. This was directly used in the next without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 7.93 (m, 2H), 7.00 6.80 (m, 2H), 4.34 (t, J = 6.2 Hz, 2H), 3.89 (s, 3H), 3.66 (t, J = 6.2 Hz, 2H).

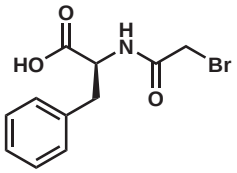
85 :
4-(2-bromoethoxy)benzoic acid



Chemical Formula: $C_9H_9BrO_3$
Exact Mass: 243,97
Molecular Weight: 245,07

To a solution of ester **84** (0.500 g, 1.93 mmol, 1.0 equiv) in THF/MeOH/H_2O (3:1:1, 25 mL) was added solid LiOH · H_2O (0.203 g, 4.83 mmol, 2.5 equiv) at rt. The mixture was stirred until TLC showed completion (20 % AcCN/$CHCl_3$). After most of the THF and methanol had been evaporated, the aqueous phase was acidified with 12 N HCl to pH 7 and extracted with EtOAc to give the compound **85** as a white solid (320 mg, 67 % over 2 steps, 1.30 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, J = 8.8 Hz, 2H), 6.96 (d, J = 8.8 Hz, 2H), 4.37 (t, J = 6.2 Hz, 2H), 3.67 (t, J = 6.2 Hz, 2H) ; ^{13}C NMR (101 MHz, d_6-DMSO and CD_3OD) δ 171.2, 161.0, 132.4, 122.1, 117.9, 65.2, 29.3.

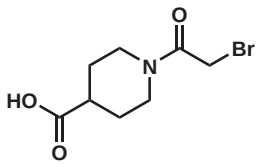
213 :
(S)-2-(2-bromoacetamido)-3-phenylpropanoic acid



Chemical Formula: $C_{11}H_{12}BrNO_3$
Exact Mass: 285
Molecular Weight: 286,12

A solution of bromoacetyl chloride (98 μL, 1.18 mmol, 1.00 equiv) in toluene (3 mL) was added dropwise to a biphasic mixture of L-Phenylalanine (195 mg, 1.18 mmol, 1.00 equiv) and NaOH (250 μL, 5 M aq., 1.25 mmol, 1.25 equiv) in toluene (2 mL) at 0 °C. 5M aq. NaOH is added in parallel to keep the pH above 10. The reaction was kept at 0 °C for 1hr (pH 10). The mixture was then quenched with 1 N q. HCl (enough to bring pH to 6). The phases were separated and aqueous phase was acidified to pH 2 with 1 N aq. HCl and extracted with EtOAc (3 x 5 mL). The combined organic layers were washed once with brine (10 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give 328 mg of a white solid of sufficient purity (97 %, 1.14 mmol). ^{1}H NMR (300 MHz, d_6-DMSO) δ 8.61 (d, J = 7.9 Hz, 1H), 7.24 (dt, J = 15.4, 7.6 Hz, 5H), 4.40 (d, J = 5.0 Hz, 1H), 3.84 (s, 2H), 2.96 (ddd, J = 22.9, 14.1, 7.1 Hz, 2H) ; ^{13}C NMR (75 MHz, d_6-DMSO and CD_3OD) δ 177.3, 168.3, 136.1, 129.3, 128.3, 127.2, 56.7, 38.1, 31.7.

214 :
1-(2-bromoacetyl)piperidine-4-carboxylic acid

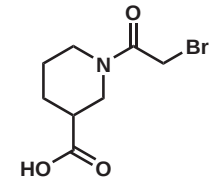


Chemical Formula: $C_8H_{12}BrNO_3$
Exact Mass: 249
Molecular Weight: 250,09

Bromoacetyl chloride (0.416 mL, 5.00 mmol, 1.05 equiv) was added to a solution of 4-piperidinecarboxylic acid (0.615 g, 4.76 mmol, 1.00 equiv) and sodium acetate (1.19 g, 4.76 mmol, 1.00 equiv) in AcCN (20 mL) at rt. The reaction was refluxed 2 h. The mixture was then concentrated *in vacuo* to give a oily solid. Trituration of the solid with cold AcCN afforded 1.07 g of product **214** as a solid (91 %, 4.32 mmol). It was carried forward without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.15 (br s, 1H), 4.33 (d, J = 12.9 Hz, 1H), 3.89 (s, 2H), 3.95 3.75 (m, 1H), 3.24 (t, J = 11.3 Hz, 1H), 2.97 (t, J = 11.3 Hz, 1H), 2.72 2.55 (m, 1H), 2.03 (m, 2H), 1.92 1.59 (m, 2H). ^{13}C NMR (101 MHz, d_6-DMSO and CD_3OD) δ 182.0, 167.3, 47.6, 46.9, 44.5, 43.1, 29.0.

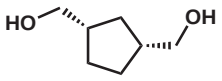
215 :
1-(2-bromoacetyl)piperidine-3-carboxylic acid

Bromoacetyl chloride (1.11 mL, 13.52 mmol, 1.05 equiv) was added to a solution of (+/-)-ethyl nipecotate (2.024 g, 12.88 mmol, 1.00 equiv) and sodium acetate (1.216 g, 14.17 mmol, 1.10 equiv) in AcCN (20 mL) at rt. The reaction was refluxed 2 h. The mixture was then concentrated *in vacuo* to give a mixture of a solid and an oily residue. Trituration of the solid with cold AcCN afforded the product **215** as a solid.



Chemical Formula: $C_8H_{12}BrNO_3$
Exact Mass: 249
Molecular Weight: 250,09

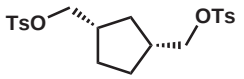
95 :
(1S,3R)-cyclopentane-1,3-diyldimethanol



Chemical Formula: $C_7H_{14}O_2$
Exact Mass: 130,1
Molecular Weight: 130,18

Ozone gas was bubbled through a solution of norbornene (10 g, 106 mmol, 1.0 equiv) in MeOH (125 ml) $-78\ ^{\circ}\mathrm{C}$ until a pale blue color persisted (30 min.) and then left for another 10 min. Argon was then bubbled through the reaction mixture until the solution turned clear. $NaBH_4$ (8 g, 211 mmol, 2.0 equiv) was added to the reaction mixture in portions at $-78\ ^{\circ}\mathrm{C}$ over 45 minutes. When the addition was over, the reaction was stirred for 1 hr, then warmed to room temperature and stirred for 16 hrs. The reaction mixture quenched with H_2O (30 mL) and stirred 2 hrs at rt before being evaporated to near dryness and the resulting residue extracted with EtOAc (5 X 50 mL). The combined organic layers were washed with water (20 mL), brine (25 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give 7.04 g of an oily crude product which was directly taken to the next step without purification (51 %, 54.1 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.55 (d, J = 6.7 Hz, 4H), 2.24 2.07 (m, 2H), 2.01 1.91 (m, 1H), 1.84 1.66 (m, 2H), 1.57 1.43 (m, 2H, OHs), 1.42 1.30 (m, 2H), 1.01 0.87 (m, 1H). ; ^{13}C NMR (100 MHz, $CDCl_3$) δ 67.0, 42.3, 32.9, 28.3.

96 :
(1S,3R)-cyclopentane-1,3-diylbis(methylene) bis(4-methylbenzenesulfonate)

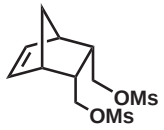


Chemical Formula: $C_{21}H_{26}O_6S_2$
Exact Mass: 438,12
Molecular Weight: 438,56

p-Toluenesulfonyl chloride (2.68 g, 14.06 mmol, 3.0 equiv) was added to a cooled (ice bath) solution of pyridine (24 mL) and diol **95** (610 mg, 4.69 mmol, 1.0 equiv). The reaction was stirred overnight under argon at room temperature. The pyridine was removed

by evaporation and the white solid residue was diluted with 2 M HCl (5 mL) and CH_2Cl_2 (50 mL). The organic layer was removed and the aqueous layer was extracted with CH_2Cl_2 (4 30 mL). The combined organic layers were washed with aq. sat. $NaHCO_3$ (25 mL), water (25 mL) and brine (25 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product as a white solid. The product was further purified via trituration with cold diethyl ether to yield 1.36 g of bis-tosylate **96** (66 %, 3.10 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 7.68 (m, 4H), 7.35 (d, J = 8.0 Hz, 4H), 3.87 (d, J = 5.9 Hz, 4H), 2.46 (s, 6H), 2.32 2.09 (m, 2H), 1.96 1.79 (m, 1H), 1.79 1.63 (m, 2H), 1.39 1.17 (m, 2H), 0.91 0.72 (m, 1H) ; ^{13}C NMR (75 MHz, $CDCl_3$) δ 141.9 131.9, 130.9, 128.9, 72.4, 37.9, 32.4, 27.8, 21.5.

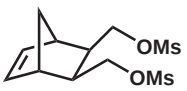
98 :
bicyclo[2.2.1]hept-5-ene-2,3-diylbis(methylene) dimethanesulfonate (endo)



Chemical Formula: $C_{11}H_{18}O_6S_2$
Exact Mass: 310,05
Molecular Weight: 310,39

Neat methanesulfonyl chloride (994 μL, 7.13 mmol, 4.0 equiv) was added to a stirring solution of cis-endo-2,3-bis(hydroxymethyl) bicyclo[2.2.1]hept-5-ene (275 mg, 1.78 mmol, 1.0 equiv) and Et_3N (554 μL, 7.13 mmol, 4.0 equiv) in CH_2Cl_2 (50 mL) cooled to 0 °C. The reaction was stirred at this temperature for 30 minutes. TLC showed completion. Reaction mixture diluted with 0.05 M HCl (2.5 mL) and CH_2Cl_2 (25 mL). Organic layer was washed with H_2O (2 x 3 mL) and brine (1 x 3 mL), dried over $MgSO_4$ and concentrated to afford crude product as a yellow solid. The product was further purified via trituration with cold diethyl ether to provide 500 mg of bis-mesylate **98** as yellow solid. (90 %, 1.61 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.24 (s, 2H), 4.10 3.98 (m, 2H), 3.98 3.85 (m, 2H), 3.02 (s, 6H), 2.75 2.63 (m, 2H), 1.65 1.51 (m, 1H), 1.41 (d, J = 8.7 Hz, 1H) ; ^{13}C NMR 135.4, 69.6, 49.4, 45.3, 41.2, 36.9.

100 :
bicyclo[2.2.1]hept-5-ene-2,3-diylbis(methylene) dimethanesulfonate (exo)



Chemical Formula: $C_{11}H_{18}O_6S_2$
Exact Mass: 310,05
Molecular Weight: 310,39

Bis-mesylate **100** was prepared according to the same procedure as the previous compound, bis-mesylate **98**, from cis-exo-2,3-bis(hydroxymethyl) bicyclo[2.2.1]hept-5-ene (275 mg, 1.78 mmol, 1.0 equiv). Yield : 442 mg (83 %, 1.48 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.22 (s, 2H), 4.45 4.30 (m, 2H), 4.27 4.12 (m, 2H), 3.05 (s, 6H), 2.85 (dd, J = 8.5, 6.7 Hz, 2H), 2.08 1.92 (m, 1H), 1.47 (q, J = 9.5 Hz, 1H).

102 :
((1R,3S)-1,2,2-trimethylcyclopentane-1,3-diyl)dimethanol

[2]

Chemical Formula: $C_{10}H_{20}O_2$
Exact Mass: 172,15
Molecular Weight: 172,26

A solution of (+)-camphoric acid (2 g, 10 mmol, 1.0 equiv) in anhydrous THF (10 + 2 mL) was added dropwise to a suspension of $LiAlH_4$ (1.2 g, 32.4 mmol, 3.24 equiv) in anhydrous diethyl ether (25 mL) at 0 °C. The reaction was refluxed under a condenser for 4 hrs condenser. Then, the reaction was cooled to room temperature and quenched by the dropwise addition of water (1.2 mL), followed by 15 % aq. NaOH (1.2 mL) and finally by water (3.5 mL). The precipitate was filtered and washed with copious amounts of THF. The filtrate was dried quickly (anhydrous Na_2SO_4), and concentrated *in vacuo* to afford 1.42 g of diol **102** as a solid (83 %, 8.3 mmol). The crude product was used directly in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.79 3.68 (m, 1H), 3.62 3.55 (m, 1H), 3.55 3.42 (m, 2H), 2.15 2.02 (m, 1H), 2.02 1.89 (m, 1H), 1.67 1.52 (m, 1H), 1.41 1.32 (m, 2H), 1.25 1.13 (m, 2H), 1.01 (s, 6H), 0.78 (s, 3H).

103 :
((1R,3S)-1,2,2-trimethylcyclopentane-1,3-diyl)bis(methylene) bis(4-methylbenzenesulfonate)

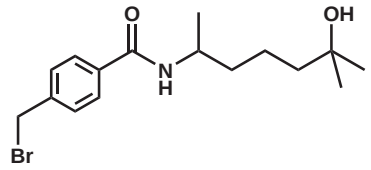
Chemical Formula: $C_{24}H_{32}O_6S_2$
Exact Mass: 480,16
Molecular Weight: 480,64

A solution of p-toluenesulfonyl chloride (1.32 g, 6.97 mmol, 3.0 equiv) in toluene (2+1 mL) was added dropwise to a cooled (ice bath) solution of diol **102** (400 mg, 2.32 mmol,

1.0 equiv), Et_3N (0.97 mL, 6.97 mmol, 3.0 equiv) and N-methylimidazole (NMI) (0.56 mL, 6.96 mmol, 3.0 equiv) in chlorobenzene (16 mL) and toluene (8 mL). The reaction was stirred 3 days under argon at room temperature. The mixture was then concentrated *in vacuo*, redissolved and reconcentrated from toluene (50 mL) then $CHCl_3$ (50 mL) to give the crude product as a solid. The product was further purified via trituration with cold diethyl ether to yield 580 mg of bis-tosylate **96** (52 %, 1.21 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (dd, J = 8.3, 1.7 Hz, 4H), 7.34 (d, J = 7.9 Hz, 4H), 4.01 (dd, J = 9.5, 6.1 Hz, 1H), 3.86 (dd, J = 16.3, 8.8 Hz, 2H), 3.74 (d, J = 9.4 Hz, 1H), 2.45 (s, 6H), 2.23 2.10 (m, 1H), 1.90 1.77 (m, 1H), 1.52 1.41 (m, 1H), 1.25 (m, 2H), 0.91 (d, J = 1.2 Hz, 6H), 0.61 (s, 3H).

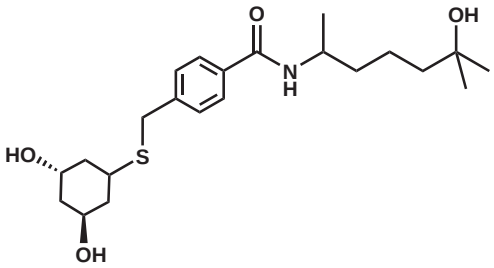
6.1.10 Thioetheramides

104 :
4-(bromomethyl)-N-(6-hydroxy-6-methylheptan-2-yl)benzamide



Chemical Formula: $C_{16}H_{24}BrNO_2$
Exact Mass: 341,1
Molecular Weight: 342,27

Oxalyl chloride ($(COCl)_2$) (131 μL, 1.5 mmol, 1.5 equiv) was added to a solution of commercially available acid **82** (215 mg, 1.0 mmol, 1.0 equiv) in CH_2Cl_2 (10 mL) with a drop of DMF at 0 °C. The reaction was stirred 1h at room temperature. The mixture was charged with amine hydrochloride **78** (200 mg, 1.1 mmol, 1.1 equiv) and DIPEA (523 μL, 3.0 mmol, 3.0 equiv) at room temperature. The reaction was stirred overnight at room temperature. The mixture was quenched with 0.1N HCl (2 mL), H_2O (10 mL) and diluted with EtOAc (20 mL). The layers were separated and the aqueous layer was extracted with EtOAc (2 x 10 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (5 mL), H_2O (5 mL), brine (5 mL) then dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product as an oil. The crude product was taken directly to the next step without purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.79 7.67 (m, 2H), 7.44 (d, J = 8.2 Hz, 2H), 5.91 (d, J = 7.6 Hz, 1H), 4.60 (s, 2H), 4.31 4.14 (m, 2H), 1.65 1.31 (m, 6H), 1.25 (d, J = 6.6 Hz, 3H), 1.20 (d, J = 2.6 Hz, 6H).

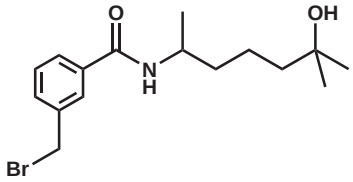


Chemical Formula: $C_{22}H_{35}NO_4S$
Exact Mass: 409,23
Molecular Weight: 409,58

105 :
4-(((3S,5S)-3,5-dihydroxycyclohexylthio)methyl)-N-(6-hydroxy-6-methylheptan-2-yl)benzamide

A solution of thiol **216** (10 mg, 68 μmol, 1.0 equiv) in MeOH was added to a suspension of K_2CO_3 (56 mg, 405 μmol, 6 equiv) and bromide **104** (28 mg, 81 μmol, 1.2 equiv) in degassed MeOH (3 mL) at 25 °C. The reaction was stirred 2 days at 25 °C. The reaction was carefully filtered through a 1-cm plug of cotton wool and concentrated *in vacuo* to give the crude products. The residues were purified via preparative TLC with 10 % MeOH/toluene to give the title thioether-amide **105** (35 % over 2 steps). ^{1}H NMR (400 MHz, CD_3OD) δ 7.74 (d, J = 8.1 Hz, 2H), 7.43 (d, J = 8.1 Hz, 2H), 4.22 4.05 (m, 2H), 3.83 (s, 2H), 2.93 (t, J = 12.4 Hz, 1H), 2.21 (d, J = 11.6 Hz, 1H), 1.99 (t, J = 14.3 Hz, 2H), 1.69 1.26 (m, 9H), 1.23 (d, J = 6.4 Hz, 3H), 1.16 (s, 6H). ^{13}C NMR (101 MHz, d_6-DMSO) δ 167.5, 142.5, 132.4, 129.4, 127.9, 72.8, 63.1, 48.6, 44.7, 44.2, 42.1, 38.2, 36.5, 29.3, 29.0, 21.0, 15.4.

106 :
3-(bromomethyl)-N-(6-hydroxy-6-methylheptan-2-yl)benzamide

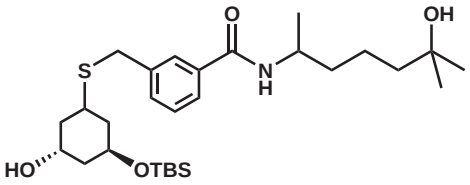


Chemical Formula: $C_{16}H_{24}BrNO_2$
Exact Mass: 341,1
Molecular Weight: 342,27

Oxalyl chloride ($(COCl)_2$) (131 μL, 1.5 mmol, 1.5 equiv) was added to a solution of homemade acid **80** (215 mg, 1.0 mmol, 1.0 equiv) in CH_2Cl_2 (10 mL) with a drop of DMF at 0 °C. The reaction was stirred 1h at room temperature. The mixture was charged with amine hydrochloride **78** (200 mg, 1.1 mmol, 1.1 equiv) and DIPEA (523 μL, 3.0 mmol, 3.0 equiv) at room temperature. The reaction was stirred overnight at room temperature. The

mixture was quenched with 0.1N HCl (2 mL), H_2O (10 mL) and diluted with EtOAc (20 mL). The layers were separated and the aqueous layer was extracted with EtOAc (2 x 10 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (5 mL), H_2O (5 mL), brine (5 mL) then dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product as an oil. The product was used in this next step without purification despite being contaminated with heptaminol amide of meta-toluic acid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.77 (s, 1H), 7.69 (d, J = 7.7 Hz, 1H), 7.52 (d, J = 7.6 Hz, 1H), 7.42 (t, J = 7.7 Hz, 1H), 5.92 (d, J = 8.6 Hz, 1H), 4.62 (s, 2H), 4.30 4.15 (m, 1H), 1.63 1.40 (m, 6H), 1.28 1.22 (d, J = 6.6 Hz, 3H), 1.21 and 1.20 (s, 3H).

217 :
3-(((3S,5S)-3-(tert-butyldimethylsilyloxy)-5-hydroxycyclohexylthio)methyl)-N-(6-hydroxy-6-methylheptan-2-yl)benzamide

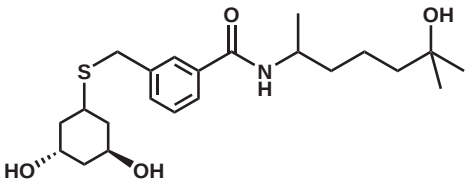


Chemical Formula: $C_{28}H_{49}NO_4SSi$
Exact Mass: 523,32
Molecular Weight: 523,84

A solution of thiol **119** (25 mg, 95 μmol, 1.0 equiv) in degassed AcCN was added to a solution of Et_3N (20 μL, 143 μmol, 1.5 equiv) and bromide **106** (37 mg, 110 μmol, 1.3 equiv) in degassed AcCN (3 mL) at 25 °C. The reaction was stirred 2 days at 25 °C. The reaction was carefully filtered through a 1-cm plug of cotton wool and concentrated *in vacuo* to give the crude products. The residues were purified via preparative TLC with 2 % MeOH/toluene to give 11.5 mg of thioether **217** (23 % over 2 steps, 22 μmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 7.68 (m, 1H), 7.60 (dd, J = 12.1, 7.7 Hz, 1H), 7.44 (dd, J = 11.6, 5.8 Hz, 1H), 7.34 (t, J = 7.7 Hz, 1H), 5.96 (t, J = 9.2 Hz, 1H), 4.23 (s, 1H), 4.15 (s, 1H), 4.00 3.86 (m, 1H), 3.77 (t, J = 9.2 Hz, 2H), 3.02 2.82 (m, 1H), 2.24 (d, J = 11.8 Hz, 1H), 1.95 (d, J = 11.0 Hz, 1H), 1.80 (m, 3H), 1.50 (m, 6H), 1.30 1.22 (m, 4H), 1.21 and 1.20 (s, 3H each), 0.80 (s, 9H), 0.03 and -0.06 (s, 3H each).

107 :
3-(((3S,5S)-3,5-dihydroxycyclohexylthio)methyl)-N-(6-hydroxy-6-methylheptan-2-yl)benzamide

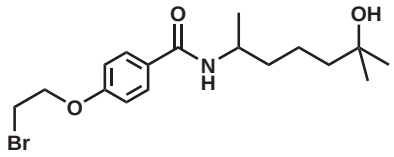
Prepared from compound **217** (3.8 mg, 7.3 μmol, 1 equiv) according to General acid-mediated TBS deprotection method. The crude product was purified via preparative TLC (eluent : 10 % MeOH/CH_2Cl_2) to provide 2.3 mg of **107** (78 %, 5.7 μmol). ^{1}H NMR (400 MHz, CD_3OD) δ 7.77 (s, 1H), 7.66 (d, J = 6.6 Hz, 1H), 7.51 (d, J = 7.5 Hz, 1H), 7.39 (t,



Chemical Formula: $C_{22}H_{35}NO_4S$
Exact Mass: 409,23
Molecular Weight: 409,58

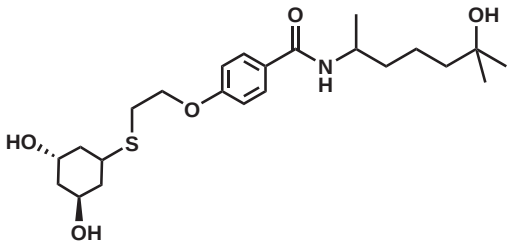
J = 7.7 Hz, 1H), 4.15 (s, 2H), 3.88 3.75 (s, 2H), 2.94 (t, J = 12.3 Hz, 1H), 2.27 2.18 (m, 1H), 2.10 1.95 (m, 2H), 1.69 1.26 (m, 9H), 1.54 (d, J = 5.1 Hz, 3H), 1.17 and 1.15 (s, 3H) ; ^{13}C NMR (101 MHz, d_6-DMSO) δ 168.7, 139.2, 133.8, 131.1, 127.4, 126.3, 72.3, 62.3, 61.0, 48.3, 46.7, 42.7, 42.0, 38.0, 36.4, 29.8, 29.4, 21.0, 14.9.

218 :
4-(2-bromoethoxy)-N-(6-hydroxy-6-methylheptan-2-yl)benzamide



Chemical Formula: $C_{17}H_{26}BrNO_3$
Exact Mass: 371,11
Molecular Weight: 372,3

Oxalyl chloride ($(COCl)_2$) (206 μL, 2.36 mmol, 1.5 equiv) was added to a solution of acid **85** (215 mg, 1.0 mmol, 1.0 equiv) in CH_2Cl_2 (10 mL) with a drop of DMF at 0 °C. The reaction was stirred 1h at room temperature. The mixture was charged with amine hydrochloride **78** (200 mg, 1.1 mmol, 1.1 equiv) and DIPEA (523 μL, 3.0 mmol, 3.0 equiv) at room temperature. The reaction was stirred overnight at room temperature. The mixture was quenched with 0.1N HCl (2 mL), H_2O (10 mL) and diluted with EtOAc (20 mL). The layers were separated and the aqueous layer was extracted with EtOAc (2 x 10 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (5 mL), H_2O (5 mL), brine (5 mL) then dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product as an oil. The crude product was taken directly to the next step without purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71 (d, J = 7.0 Hz, 2H), 6.93 (d, J = 8.8 Hz 2H), 5.87 (d, J = 7.7 Hz, 1H), 4.32 (t, J = 6.2 Hz, 2H), 3.64 (t, J = 6.2 Hz, 2H), 1.64 1.35 (m, 6H), 1.23 (d, J = 6.6 Hz, 3H), 1.19 (d, J = 3.3 Hz, 9H).

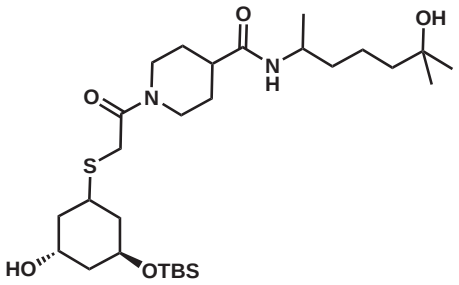


Chemical Formula: $C_{23}H_{37}NO_5S$
Exact Mass: 439,24
Molecular Weight: 439,61

109 :
4-(2-((3S,5S)-3,5-dihydroxycyclohexylthio)ethoxy)-N-(6-hydroxy-6-methylheptan-2-yl)benzamide

A solution of thiol **216** (12 mg, 81 μmol, 1.0 equiv) in degassed MeOH was added to a suspension of K_2CO_3 (67 mg, 486 μmol, 6.0 equiv) and bromide **108** (45 mg, 122 μmol, 1.5 equiv) in degassed MeOH (3 mL) at 25 °C. The reaction was stirred 2 days at 25 °C. The reaction was carefully filtered through a 1-cm plug of cotton wool and concentrated *in vacuo* to give the crude products. The residues were purified via preparative TLC with 10 % MeOH/toluene to give thioether-amide **109** (27 % over two steps). ^{1}H NMR (300 MHz, CD_3OD) δ 7.78 (d, J = 8.9 Hz, 2H), 6.98 (d, J = 8.8 Hz, 2H), 4.20 (t, J = 6.5 Hz, 2H), 4.15 4.07 (m, 1H), 3.93 (m, 1H), 2.96 (t, J = 6.7 Hz, 2H), 2.28 (m, 1H), 2.05 (d, J = 14.2 Hz, 2H), 1.66 1.25 (m, 9H), 1.22 (d, J = 6.7 Hz, 3H), 1.15 (s, 6H) ; ^{13}C NMR (101 MHz, d_6-DMSO) δ 167.2, 163.1, 129.3, 128.1, 116.1, 71.2, 69.8, 66.1, 48.8, 43.7, 41.3, 38.7, 35.4, 32.1, 29.2, 21.4, 19.0.

219 :
1-(2-((3S,5S)-3-(tert-butyldimethylsilyloxy)-5-hydroxycyclohexylthio)acetyl)-N-(6-hydroxy-6-methylheptan-2-yl)piperidine-4-carboxamide

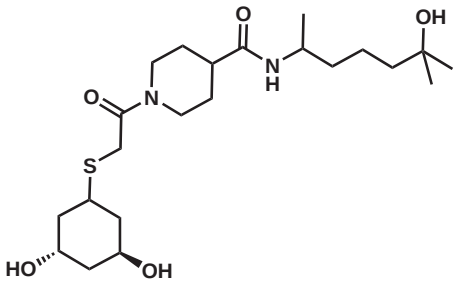


Chemical Formula: $C_{28}H_{54}N_2O_5SSi$
Exact Mass: 558,35
Molecular Weight: 558,89

Prepared from acid **121** (11 mg, 34.3 μmol) and amine **118** (10.6 mg, 41.1 μmol) according to General amide bond formation method. The crude product was purified via

preparative TLC (eluent : 7 % MeOH/CH_2Cl_2) to provide 2.9 mg of amide **219** (15 %, 5 μmol). ^{1}H NMR (300 MHz, CD_3OD) δ 4.49 (d, J = 11.8 Hz, 1H), 4.25 (s, 1H), 4.07 3.80 (m, 3H), 3.48 3.39 (m, 2H), 3.13 (m, 2H), 2.69 (t, J = 12.1 Hz, 1H), 2.48 2.25 (m, 2H), 1.91 (m, 2H), 1.79 (m, 2H), 1.58 (dd, J = 23.8, 13.3 Hz, 1H), 1.50 1.20 (m, 8H), 1.15 (s, 6H), 1.10 (d, J = 6.5 Hz, 3H), 0.90 (s, 9H), 0.07 (s, 6H).

112 :
1-(2-((3S,5S)-3,5-dihydroxycyclohexylthio)acetyl)-N-(6-hydroxy-6-methylheptan-2-yl)piperidine-4-carboxamide

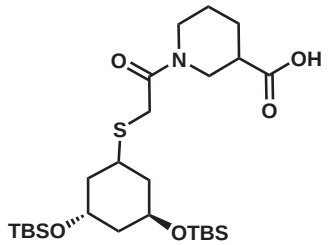


Chemical Formula: $C_{22}H_{40}N_2O_5S$
Exact Mass: 444,27
Molecular Weight: 444,63

Prepared from **219** (2.9 mg, 5 μmol) according to General acid-mediated TBS deprotection method. The crude product was purified via preparative TLC (eluent : 10 % MeOH/CH_2Cl_2) to provide 1.2 mg of thioether-amide **112** (45 %, 2.2 μmol). ^{1}H NMR (400 MHz, CD_3OD) δ 4.50 (d, J = 12.2 Hz, 1H), 4.18 (s, 1H), 4.01 (d, J = 13.5 Hz, 1H), 3.88 (m, 2H), 3.49 3.43 (m, 2H), 3.13 (m, 2H), 2.69 (t, J = 13.1 Hz, 1H), 2.44 (m, 1H), 2.30 (m, 1H), 2.04 (d, J = 13.4 Hz, 2H), 1.79 (s, 3H), 1.66 1.54 (m, 1H), 1.51 1.19 (m, 9H), 1.16 (s, 6H), 1.11 (d, J = 6.6 Hz, 3H) ; ^{13}C NMR (101 MHz, d_6-DMSO) δ 171.2, 168.2, 71.5, 66.4, 49.2, 47.5, 44.2, 42.7, 40.7, 40.1, 39.9, 36.7, 30.1, 29.1, 28.2, 27.2, 21.0, 19.2.

114 :
1-(2-((3S,5S)-3,5-bis(tert-butyldimethylsilyloxy)cyclohexylthio)acetyl)piperidine-3-carboxylic acid

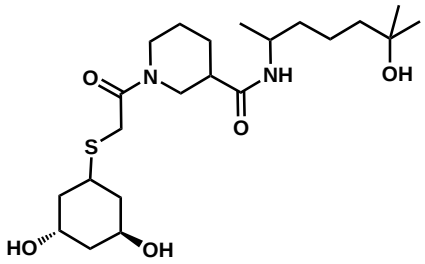
A solution of thiol **24** (20 mg, 53.2 μmol, 1.0 equiv) in degassed MeOH (5 mL) was added to a solution of DIPEA (27 μL, 160 μmol, 3.0 equiv) and bromide **215** (21 mg, 85.1 μmol, 1.6 equiv) in degassed $CHCl_3$ (3 mL) at 25 °C. The reaction was stirred 2 days at 25 °C. The reaction was carefully filtered through a 1-cm plug of cotton wool and concentrated *in vacuo* to give the crude products. The residues were purified via preparative TLC with 2 % MeOH/toluene to give 24 mg of thioether-acid **114** (83 %, 44 μmol). ^{1}H NMR (300



Chemical Formula: $C_{26}H_{51}NO_5SSi_2$
Exact Mass: 545,3
Molecular Weight: 545,92

MHz, $CDCl_3$) δ 4.34 (d, J = 13.2 Hz, 1H), 4.17 (s, 1H), 3.99 (m,1H), 3.83 (d, J = 13.4 Hz, 1H), 3.36 (s, 2H), 3.15 (dd, J = 24.6, 11.9 Hz, 2H), 2.96 2.78 (m, 1H), 2.57 (m, 1H), 2.27 2.11 (m, 1H), 2.04 1.55 (m, 6H), 1.33 (m, 4H), 0.87 (s, 18H), 0.04 (s, 12H). ^{13}C NMR (75 MHz, $CDCl_3$ and CD_3OD) δ 178.0, 162.1, 69.8, 49.2, 48.1, 44.6, 41.3, 39.2, 38.1, 32.4, 30.5, 26.7, 26.1, 18.5, -4.7.

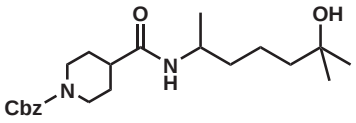
115 :
1-(2-((3S,5S)-3,5-dihydroxycyclohexylthio)acetyl)-N-(6-hydroxy-6-methylheptan-2-yl)piperidine-3-carboxamide



Chemical Formula: $C_{22}H_{40}N_2O_5S$
Exact Mass: 444,2658
Molecular Weight: 444,6284

Prepared from acid **114** (24 mg, 44 μmol) and heptaminol hydrochloride **78** (16 mg, 88 μmol) according to General amide bond formation method. The crude product was taken directly to next step following General acid-mediated TBS deprotection method. The residue was finally purified via preparative TLC (eluent : 10 % MeOH/CH_2Cl_2) to provide 7.7 mg of amide **115** (39 %, 17.2 μmol). ^{1}H NMR (300 MHz, CD_3OD) δ 4.56 (d, J = 9.8 Hz, 1H), 4.21 (s, 1H), 4.12 3.83 (m, 3H), 3.37 (s, 2H), 3.12 (m, 2H), 2.66 (m, 1H), 2.43 2.18 (m, 2H), 1.91 (m, 4H), 1.77 1.27 (m, 14H), 1.20 and 1.18 (s, 3H each), 1.16 (d, 7.0 Hz, 3H). ^{13}C NMR (101 MHz, d_6-DMSO and CD_3OD) δ 174.0, 165.1, 72.7, 68.2, 51.6, 49.7, 46.3, 43.6, 43.2, 41.0, 38.21, 37.1, 36.5, 33.1, 29.7, 38.4, 24.1, 22.1, 19.9.

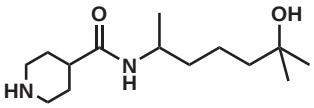
117 :
benzyl 4-(6-hydroxy-6-methylheptan-2-ylcarbamoyl)piperidine-1-carboxylate



Chemical Formula: $C_{22}H_{34}N_2O_4$
Exact Mass: 390,25
Molecular Weight: 390,52

Prepared from homemade N-Cbz-4-carboxy-piperidine (500 mg, 1.90 mmol) and heptaminol hydrochloride **78** (378 mg, 2.09 mmol) according to General amide bond formation method. The crude product **117** was immediately used without further purification.

118 :
N-(6-hydroxy-6-methylheptan-2-yl)piperidine-4-carboxamide

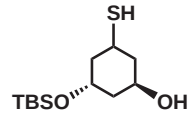


Chemical Formula: $C_{14}H_{28}N_2O_2$
Exact Mass: 256,22
Molecular Weight: 256,38

Carbobenzyloxy-protected amine **117** (110 mg, 0.282 mmol, 1.0 equiv) was added to a suspension of 10 % Pd on C (10 mg, 10 % weight-dry basis on activated carbon, wet. degussa-type E101. Water around 50 %) in MeOH under H_2 atmosphere. The reaction was conducted for 24h or until TLC showed completion (10 % MeOH/CH_2Cl_2). The mixture was filtered twice on Celite and concentrated *in vacuo*. The residue was purified using flash chromatography (column : 1,5 cm x 15 cm, eluent : 10 % MeOH/CH_2Cl_2, 500 mL total) to yield 22 mg of amine **118** as a clear oil (45 % over two steps, 0.086 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.35 (br s, 3H), 4.05 3.94 (m, 1H), 3.19 3.10 (m, 2H), 2.70 2.59 (m, 2H), 2.24 2.17 (m, 1H), 1.88 1.25 (m, 10H), 1.20 and 1.18 (s, 3H each) 1.12 (d, J = 6.8 Hz, 3H) ; ^{13}C NMR (75 MHz, $CDCl_3$ and CD_3OD) δ 171.2, 72.8, 49.5, 48.2, 43.6, 43.5, 37.4, 29.2, 27.6, 22.0, 19.3.

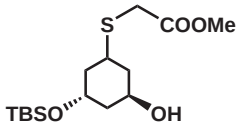
119 :
(1S,3S)-3-(tert-butyldimethylsilyloxy)-5-mercaptocyclohexanol

Not created, but isolated as a side product of decomposition and reused. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.23 4.13 (m, 1H), 4.07 3.94 (m, 1H), 3.26 3.07 (m, 1H), 2.44 2.27 (m, 1H), 2.07 1.91 (m, 2H), 1.46 1.21 (m, 4H), 0.868 (s, 9H), 0.04 and 0.03 (s, 3H each).



Chemical Formula: $C_{12}H_{26}O_2SSi$
Exact Mass: 262,14
Molecular Weight: 262,48

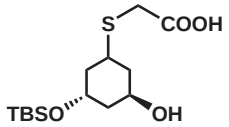
120 :
methyl 2-((3S,5S)-3-(tert-butyldimethylsilyloxy)-5-hydroxycyclohexylthio)acetate



Chemical Formula: $C_{15}H_{30}O_4SSi$
Exact Mass: 334,16
Molecular Weight: 334,55

Methyl α-bromoacetate (22 μL, 0.229 mmol, 1.2 equiv) was added to a solution of thiol **119** (50 mg, 0.190 mmol, 1.0 equiv) and Et_3N (40 μL, 0.285 mmol, 1.5 equiv) in degassed-via-Ar-bubbling AcCN (1 mL) at 25 °C. The reaction was stirred at 450 rpm on Thermomixer for 2 days at 25 °C. The mixture was concentrated *in vacuo* purified via preparative TLC using 30 % EtOAc/hexanes to provide 50 mg of ester **120** as a clear oil (77 %, 0.147 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.21 4.17 (m, 1H), 4.10 3.91 (m, 1H), 3.71 (s, 3H), 3.26 (s, 2H), 3.22 3.10 (m, 1H), 2.35 2.23 (m, 1H), 2.04 1.84 (m, 2H), 1.76 (s, 1H), 1.43 1.17 (m, 4H), 0.856 (s, 9H), 0.03 and 0.02 (s, 3H each). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.06, 67.23, 66.06, 52.43, 41.95, 39.45, 37.15, 31.96, 25.68, 17.91, -5.02, -5.05.

121 :
2-((3S,5S)-3-(tert-butyldimethylsilyloxy)-5-hydroxycyclohexylthio)acetic acid

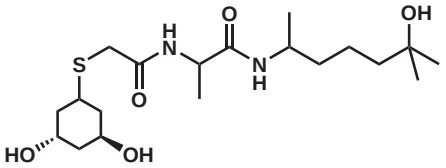


Chemical Formula: $C_{14}H_{28}O_4SSi$
Exact Mass: 320,15
Molecular Weight: 320,52

A solution of aq. NaOH (1.22 mL, 3.05 mmol, 10.0 equiv, 2.5 M) was added to a solution of ester **119** (102 mg, 0.305 mmol, 1.0 equiv) 1:2 MeOH/THF (6 mL) at 25 °C. The reaction was stirred for 2 hr at 25 °C. TLC showed completion (30 % EtOAc/hexanes). The mixture was quenched with 0.1N HCl (2 mL) and diluted with EtOAc (15 mL). The layers were separated and the aqueous layer was extracted with EtOAc (2 x 10 mL). The combined organic layers were washed with H_2O (10 mL), brine (10 mL) then dried ($MgSO_4$)

and concentrated *in vacuo* to give the crude product. The residue was purified via preparative TLC with 50 % EtOAc/hexanes (1 % AcOH) to give 85 mg of acid **121** (87 %, 0.265 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.22 (s, 1H), 4.03 (m, 1H), 3.33 (s, 2H), 3.21 (m, 1H), 2.35 (m, 1H), 1.99 (m, 2), 1.35 (m, 3H), 0.83 (s, 9H), 0.06 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.48, 67.23, 66.22, 41.85, 41.62, 39.41, 37.42, 32.13, 25.73, 17.94, -4.99, -5.01.

125 :
2-(2-((3S,5S)-3,5-dihydroxycyclohexylthio)acetamido)-N-(6-hydroxy-6-methylheptan-2-yl)propanamide



Chemical Formula: $C_{19}H_{36}N_2O_5S$
Exact Mass: 404,23
Molecular Weight: 404,56

Prepared from amine **124** (8.9 mg, 41 μmol) and acid **121** (11 mg, 34 μmol) according to General amide bond formation method . The crude product **220** was immediately used without further purification in General acid-mediated TBS deprotection method. The crude product was purified via preparative TLC (eluent : 10 % MeOH/CH_2Cl_2) to provide 6.9 mg **125** as an oil (50 % over two steps, 17 μmol). ^{1}H NMR (400 MHz, CD_3OD) δ 4.39 4.26 (m, 1H), 4.17 (s, 1H), 4.01 3.79 (m, 2H), 3.69 3.61 (m, 1H), 3.28 (s, 2H), 3.15 3.02 (m, 1H), 2.27 (d, J = 12.1 Hz, 1H), 2.03 (d, J = 12.9 Hz, 2H), 1.50 1.23 (m, 15H), 1.19 1.07 (m, 6H) ; ^{13}C NMR (101 MHz, d_6-DMSO and CD_3OD) δ 175.1, 173.2, 71.5, 66.8, 55.4, 48.2, 46.7, 43.5, 40.9, 39.1, 36.5, 34.9, 29.2, 21.1, 18.4, 17.2.

6.1.11 Dithioether library

General comments :

All solvents were degassed with Ar-bubbling method. All vials were filled with Ar before the beginning of the procedures. Reactions were conducted in Ar-filled Ziploc bags stirred at 200 rpm in 5-dram screw cap vials.

Stock solutions (ss):

Thiols : Stock solutions were created to have 1 equiv of thiols per 125 μL of MeOH. dibromides stock solutions were created to contain 10 mg of dibromide per 500 μL of MeOH (for dibromides A, B, d) and 1000 μL of 1:1 MeOH/EtOAc (for dibromides C, E).

Procedure:

A solution of thiol in MeOH (500 μL (4 equiv) for dibromides or 750 μL (6 equiv) for tribromides) was added to a suspension of K_2CO_3 (10 equiv) and dibromide (10 mg, 1.0 equiv) in MeOH or 1:1 MeOH/EtOAc (500 μL or 1000 μL, respectively) at 25 °C. The reactions were stirred 3 days at 25 °C. The reaction were carefully quenched with enough 0.5 N aq. HCl to dissolve all solids. The mixtures were diluted with EtOAc (5 mL). The layers were separated and the aqueous layer was extracted with EtOAc (2 x 1 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (1 mL), H_2O (1 mL), brine (1 mL) then dried ($MgSO_4$) and concentrated *in vacuo* to give the crude products. The residues were purified first via preparative TLC with either 50 % EtOAc/hexanes (1 % AcOH) or 30 % acetone/toluene. When necessary, further purification via semi-preparative HPLC was performed.

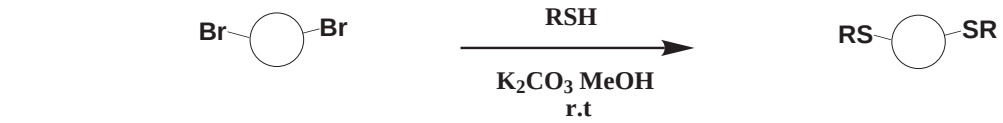
Table 6.1: Yields of the thioether library.

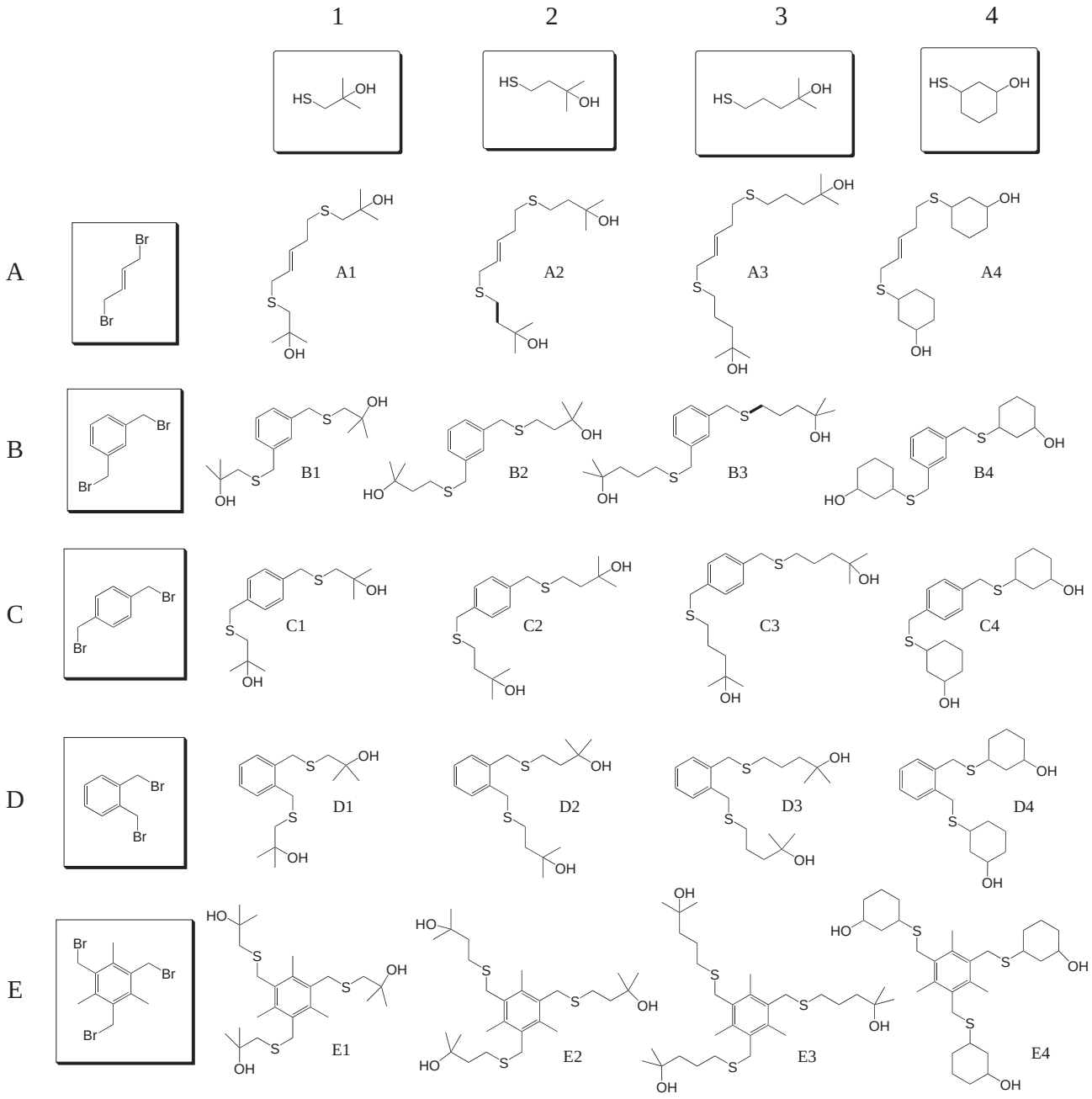
	Thiols							
dibromides	1		2		3		4	
	mg	%	mg	%	mg	%	mg	%
A	4.9	40	1.8	13	1.6	11	9.6	65
B	7.2	65	19.8	37	3.6	26	9.8	71
C	5.7	48	10.1	19	4.3	31	9.9	71
δ	6.6	55	6.3	49	5.1	40	10.4	75
E	7.1	59	7.0	54	6.3	45	10.4	75

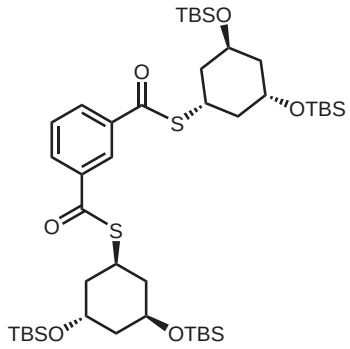
6.1.12 Dithioesters

221 :
S,S-bis((3S,5S)-3,5-bis(tert-butyldimethylsilyloxy)cyclohexyl) benzene-1,3-bis(carbothioate)

Et_3N (38 μL, 0.271 mmol, 2.20 equiv) was added to a solution of isophthaloyl dichloride (25 mg, 0.123 mmol, 1.00 equiv) and thiol **24** (95 mg, 0.252 mmol, 2.05 equiv) in toluene (5 mL) at room temperature. The reaction was stirred room temperature overnight. The mixture was quenched with H_2O (1 mL) and diluted with EtOAc (5 mL). The layers were separated and the aqueous layer was extracted with EtOAc (2 x 5 mL). The combined organic layers were washed with H_2O (5 mL), brine (5 mL) then dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product as an oil which was directly taken to the next step. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (t, J = 1.7 Hz, 1H), 8.10 (dd, J = 7.8, 1.8 Hz, 2H), 7.51 (t, J = 7.8 Hz, 1H), 4.28 4.02 (m, 6H), 2.22 (d, J = 12.5 Hz, 2H), 1.92 (dd, J = 33.8, 13.0 Hz, 4H), 1.51 (ddd, J = 31.0, 22.5, 13.2 Hz, 6H), 0.92 (d, J = 9.1 Hz, 36H), 0.08 (s, 24H).

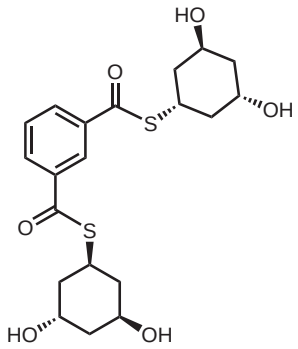

Br
Br
RSH
K_2CO_3 MeOH
r.t
RS
SR


1
2
3
4
A
B
C
D
E
A1
A2
A3
A4
B1
B2
B3
B4
C1
C2
C3
C4
D1
D2
D3
D4
E1
E2
E3
E4



Chemical Formula: $C_{44}H_{82}O_6S_2Si_4$
Exact Mass: 882,46
Molecular Weight: 883,59

147 :
S,S-bis((3S,5S)-3,5-dihydroxycyclohexyl) benzene-1,3-bis(carbothioate)

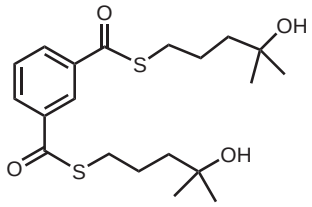


Chemical Formula: $C_{20}H_{26}O_6S_2$
Exact Mass: 426,12
Molecular Weight: 426,55

Prepared from dithioester **221** (crude, max 0.123 mmol) according to general acid-mediated TBS deprotection method. The crude product was purified via flash chromatography (column : 2,5 cm x 8 cm, eluent : 10, 15, 20 % MeOH/CH_2Cl_2, 100 mL each) to provide 32 mg of dithioester **147** as a white solid (60 % over 2 steps, 0.074 mmol). ^{1}H NMR (400MHz, CD_3OD) δ 8.419 (s, 1H), 8.151 (d, J = 1.6 Hz, 2H), 7.619 (t, J = 7.8 Hz, 1H), 4.235 (m, 2H), 4.08 (m, 4H), 2.315 (m, 2H), 2.051 (m, 4H), 1.642 (dt, J = 2.7, 14.3 Hz, 2H), 1.458 (q, J = 11.7 Hz, 4H). ESI-HRMS found 425.10845 $\pm$ 0.00 (M$-$H calc. 425.10871).

148 :
S,S-bis(4-hydroxy-4-methylpentyl) benzene-1,3-bis(carbothioate)

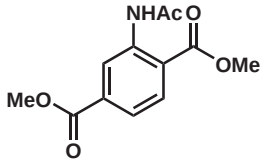
Et_3N (755 μL, 5.42 mmol, 2.20 equiv) was added to a solution of isophthaloyl dichlo-



Chemical Formula: $C_{20}H_{30}O_4S_2$
Exact Mass: 398,16
Molecular Weight: 398,58

ride (500 mg, 2.46 mmol, 1.00 equiv) and thiol **63** (680 mg, 5.05 mmol, 2.05 equiv) in toluene (50 mL) at room temperature. The reaction was stirred room temperature overnight. The mixture was quenched with H_2O (20 mL). The layers were separated and the aqueous layer was extracted with EtOAc (2 x 5 mL). The combined organic layers were washed with H_2O (5 mL), brine (5 mL) then dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product as a reddish oil. The residue was purified via flash chromatography (column : 2,5 cm x 19 cm, eluent : 25, 35, 42, 47, 55 % EtOAc/hexanes, 50 mL each) to provide 361 mg of dithioester **148** as a clear oil (37 %, 0.91 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55 (d, J = 1.7 Hz, 1H), 8.15 (dd, J = 7.8, 1.8 Hz, 2H), 7.55 (t, J = 7.8 Hz, 1H), 3.13 (t, J = 7.2 Hz, 4H), 1.86 1.73 (m, 4H), 1.65 1.58 (m, 4H), 1.25 (s, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 191.15, 137.43, 131.41, 128.96, 125.86, 70.68, 42.67, 29.58, 29.26, 24.52. ESI-HRMS found 421.14730 $\pm$ 0.00 (MNa^+ calc. 421.14777).

152 :
dimethyl 2-acetamidoterephthalate

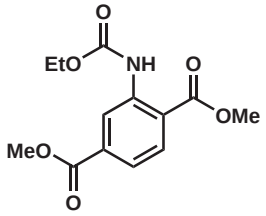


Chemical Formula: $C_{12}H_{13}NO_5$
Exact Mass: 251,08
Molecular Weight: 251,24

Neat acetyl chloride (3.1 mL, 43.05 mmol, 3.0 equiv) was added to a solution of methyl 2-amino terephthalate **150** (3.00 g, 14.34 mmol, 1.0 equiv), pyridine (4.7 mL, 57.36 mmol, 4.0 equiv) and cat. dMAP (a pinch) in CH_2Cl_2 (100 mL) at 0 °C and the reaction is stirred for 3 days. The mixture is concentrated in vacuo before being diluted with EtOAc (100 mL). washed with 2N aq. HCl (2 x 20 mL), water (20 mL) and brine (20 mL), dried ($MgSO_4$) and concentrated **in vacuo** to give dark red-orange solids. The solids were triturated (crushed, swirled and rinced) with a mixture of Et_2O/EtOAc/EtOH (approx. 10:2:1) and once filtered, washed again with the same solvent mixture. The resulting pinkish solid was dried **in vacuo** to afford 1.885 g of acetamide **152** (52 %, 7.46 mmol). ^{1}H NMR (400

MHz, $CDCl_3$) δ 10.99 (s, 1H), 9.30 (d, J = 1.6 Hz, 1H), 8.08 (d, J = 8.3 Hz, 1H), 7.72 (dd, J = 8.3, 1.7 Hz, 1H), 3.95 (s, 3H), 3.93 (s, 3H), 2.25 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.98, 168.05, 166.06, 141.42, 135.29, 130.74, 123.06, 121.25, 117.93, 52.61, 52.46, 25.38.

153 :
dimethyl 2-(ethoxycarbonylamino)terephthalate

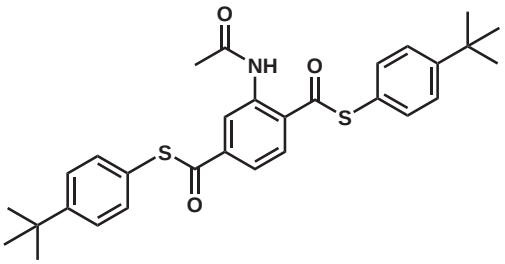


Chemical Formula: $C_{13}H_{15}NO_6$
Exact Mass: 281,09
Molecular Weight: 281,26

Neat ethyl chloroformate (2.9 mL, 30.11 mmol, 2.1 equiv) was added to a solution of methyl 2-amino terephthalate **150** (3.00 g, 14.34 mmol, 1.0 equiv), pyridine (4.7 mL, 57.36 mmol, 4.0 equiv) and cat. dMAP (a pinch) in CH_2Cl_2 (100 mL) at 0 °C and the reaction is stirred for 3 days. The mixture is concentrated in vacuo before being diluted with EtOAc (100 mL). washed with 2N aq. HCl (2 x 20 mL), water (20 mL) and brine (20 mL), dried ($MgSO_4$) and concentrated **in vacuo** to give solids. The solids were triturated (crushed, swirled and rinced) with Et_2O, filtered and rinced with Et_2O. The resulting solid was dried **in vacuo** to afford 3.88 g of carbamate **153** (97 %, 13.81 mmol). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.41 (s, 1H), 9.07 (s, 1H), 8.04 (d, J = 8.3 Hz, 1H), 7.65 (d, J = 8.3 Hz, 1H), 4.24 (quartet, J = 6.8 Hz, 2H), 3.92 (s, 6H), 1.32 (t, J = 6.8 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.83, 166.10, 162.65, 153.49, 141.811, 135.23, 130.863, 122.07, 119.76, 117.56, 61.36, 52.53, 52.35, 14.42.

154 :
S,S-bis(4-tert-butylphenyl) 2-acetamidobenzene-1,4-bis(carbothioate)

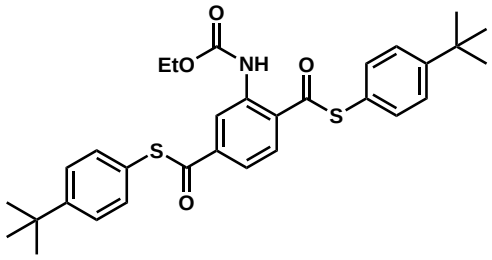
4-tert-Butylthiophenol (2.07 mL, 12.0 mmol, 6.0 equiv) was added to a solution of $AlMe_3$ (6 mL, 2 M, 12.0 mmol, 6.0 equiv) in CH_2Cl_2 (25 mL) at 0 °C. The reaction was stirred at that temperature for 15 min. before being charged dropwise with a solution of diester **152** (500 mg, 2.0 mmol, 1.0 equiv) in CH_2Cl_2/toluene/THF (2:4:5, 30 mL) at 0 °C. The reaction was let warmed to rt and stirred overnight before being diluted with Et_2O (150 mL) and quenched by dropwise addition of 2N aq. HCl (20 mL). The mixture was further treated with H_2O (30 mL) and the aqueous residue was extracted with Et_2O (3 x 50 mL) and the combined organic layers were washed with 2N aq. HCl (20 mL), H_2O (40



Chemical Formula: $C_{30}H_{33}NO_3S_2$
Exact Mass: 519,19
Molecular Weight: 519,72

mL), brine (40 mL), dried ($MgSO_4$) and concentrated **in vacuo** to a yellow semi-solid. The residue was purified using flash chromatography (column : 23 x 2.5 cm, eluent : 0, 5, 8, 10, 17, 25, 37 % EtOAc/hexanes, 200 mL each) and 644 mg of pure solid dithioester **154** was obtained (62 %, 1.23 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.46 (s, 1H), 8.29 (d, J = 8.5 Hz, 1H), 7.71 (d, J = 8.4 Hz, 1H), 7.55 - 7.43 (m, 8H), 2.19 (s, 3H), 1.37 (s, 9H), 1.35 (s, 9H).

222 :
S,S-bis(4-(tert-butyl)phenyl) 2-((ethoxycarbonyl)amino)benzene-1,4-bis(carbothioate)

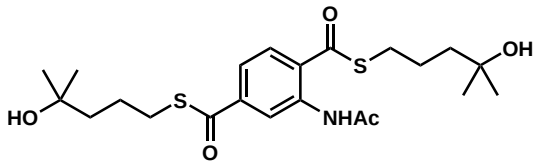


Chemical Formula: $C_{31}H_{35}NO_4S_2$
Exact Mass: 549,2007
Molecular Weight: 549,7439

4-tert-Butylthiophenol (2.07 mL, 12.0 mmol, 6.0 equiv) was added to a solution of $AlMe_3$ (6 mL, 2 M, 12.0 mmol, 6.0 equiv) in CH_2Cl_2 (25 mL) at 0 °C. The reaction was stirred at that temperature for 15 min. before being charged dropwise with a solution of diester **153** (563 mg, 2.0 mmol, 1.0 equiv) in CH_2Cl_2/toluene/THF (2:4:5, 30 mL) at 0 °C. The reaction was let warmed to rt and stirred overnight before being diluted with Et_2O (150 mL) and quenched by dropwise addition of 2N aq. HCl (20 mL). The mixture was further treated with H_2O (30 mL) and the aqueous residue was extracted with Et_2O (3 x 50 mL) and the combined organic layers were washed with 2N aq. HCl (20 mL), H_2O (40 mL), brine (40 mL), dried ($MgSO_4$) and concentrated **in vacuo** to a brown semi-solid. The residue was purified using flash chromatography (column : 22 x 2.5 cm, eluent : 0, 3, 6, 10.5, 17, 25 % EtOAc/hexanes, 200 mL each) and 745 mg of pure solid dithioester **154**

was obtained (68 %, 1.35 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.18 (s, 1H), 9.23 (s, 1H), 8.28 (d, J = 9.1 Hz, 1H), 7.62 (d, J = 9.2 Hz, 1H), 7.55 - 7.41 (m, 8H), 4.21 (quartet, J = 6.5 Hz, 2H), 1.38 (s, 18H), 1.37 (m, 3H).

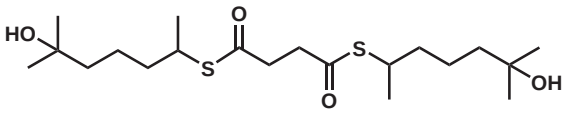
156 :
S,S-bis(4-hydroxy-4-methylpentyl) 2-acetamidobenzene-1,4-bis(carbothioate)



Chemical Formula: $C_{22}H_{33}NO_5S_2$
Exact Mass: 455,1800
Molecular Weight: 455,6311

Et_3N (696 μL, 4.81 mmol, 5.0 equiv) was added to a solution of dithioester **154** (500 mg, 0.962 mmol, 1.0 equiv) and thiol MMPe **63** (517 mg, 3.85 mmol, 4.0 equiv) in THF (25 mL) at rt. The reaction was stirred under argon at rt for 8 days. Then the reaction mixture was quenched by adding neat HCOOH (formic acid, 300 μL) to bring the pH to 3 and concentrated **in vacuo**. The residue was purified using flash chromatography (column : 15 x 6 cm, eluent : 20, 30, 40, 60 and 80 % EtOAc/hexanes) and 151 mg of pure oily dithioester was obtained (34 %, 0.327 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.73 (s, 1H), 9.23 (d, J = 1.5 Hz, 1H), 8.01 (d, J = 8.4 Hz, 1H), 7.54 (dd, J = 8.4, 1.7 Hz, 1H), 3.03 (dd, J = 15.6, 7.4 Hz, 4H), 2.19 (s, 3H), 1.99 (s, 2H), 1.81 1.63 (m, 4H), 1.62 1.43 (m, 4H), 1.19 and 1.17 (s, 6H each). ^{13}C NMR (101 MHz, $CDCl_3$) δ 195.342, 191.325, 169.201, 141.193, 138,503, 129.708, 125.128, 120.410, 119.580, 70.431, 70.392, 42.621, 42.598, 30.031, 29.665, 29.192, 29.131, 25.275, 24.284, 24.071.

158 :
S,S-bis(6-hydroxy-6-methylheptan-2-yl) butanebis(thioate)

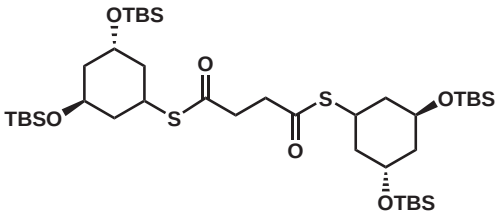


Chemical Formula: $C_{20}H_{38}O_4S_2$
Exact Mass: 406,22
Molecular Weight: 406,64

Et_3N (180 μL, 1.29 mmol, 3.00 equiv) was added to a solution of top chain thiol MMHp **67** (150 mg, 0.926 mmol, 2.15 equiv) and succinoyl chloride (48 μL, 0.43 mmol, 1.00 equiv) in AcCN (15 mL) stirred vigorously at rt. The reaction was stirred at rt overnight (total of 22hrs). The reaction mixture was quenched by 1M aq. HCl (1 mL)

and diluted with EtOAc (30 mL). The layers were separated and the aqueous layer was extracted with EtOAc (5 x 15 mL). The combined organic layers were washed with 1M aq. HCl (10 mL), H_2O (15 mL), brine (5 mL) then dried ($MgSO_4$) and concentrated *in vacuo*. The crude brown oil was purified via flash chromatography (column : 2,5 cm x 15.5 cm, eluent : 24, 35, 50, 55, 70, 85, 100 % EtOAc/hexanes then 10 % acetone/toluene, all 100 mL) and provided 49 mg of dithioester **158** as a clear oil (28 %, 0.120 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.59 (quartet, J = 6.9 Hz, 2H), 2.84 (s, 4H), 1.71 1.39 (m, 12H), 1.29 (d, J = 8.1 Hz, 6H), 1.20 (s, 12H) ; ^{13}C NMR (101 MHz, $CDCl_3$) δ 197.646, 70.947, 70.855, 46.588, 43.638, 43.390, 43.379, 39.408, 19.389, 38.716, 36.995, 36.969, 36.647, 29.963, 29.345, 29.330, 29.297, 29.275, 29.234, 29.175, 21.873, 21.728, 21.340, 20.688, 20.662. ESI-HRMS found 407.22844 $\pm$ 0.00 (MH^+ calc. 407.22843).

223 :
S,S-bis((3S,5S)-3,5-bis(tert-butyldimethylsilyloxy)cyclohexyl) butanebis(thioate)

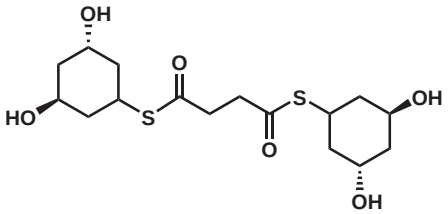


Chemical Formula: $C_{40}H_{82}O_6S_2Si_4$
Exact Mass: 834,46
Molecular Weight: 835,55

Et_3N (55 μL, 0.396 mmol, 2.20 equiv) was added to a solution of A-ring thiol **24** (142 mg, 0.378 mmol, 2.10 equiv) and succinoyl chloride (20 μL, 0.180 mmol, 1.00 equiv) in DMF (5 mL) stirred vigorously at 0 °C. The reaction was stirred at rt overnight. The reaction mixture was diluted with *t*BuOMe (50 mL) and 0.01 M aq. HCl (10 mL). The layers were separated and the aqueous layer was extracted with *t*BuOMe (2 x 15 mL). The combined organic layers were washed with 0.01 M aq. HCl (2 x 10 mL), H_2O (3 x 5 mL), brine (5 mL) then dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified via flash chromatography (column : 1,5 cm x 16 cm, eluent : 0, 1, 2, 3, 4, 6, 8, 10 % EtOAc/hexanes, 50 mL each) provided 100 mg of dithioester **223** as a clear oil (66 %, 0.12 mmol). Taken to the next step without characterization.

159 :
S,S-bis((3S,5S)-3,5-dihydroxycyclohexyl) butanebis(thioate)

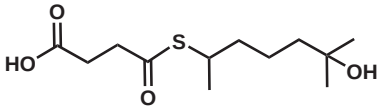
Prepared from **223** (100 mg, 0.120 mmol) according to General acid-mediated TBS deprotection. The crude product was purified via flash chromatography (column : 1,5 cm x 16 cm, eluent : 10, 12, 12, 17, 25, 75 % MeOH/CH_2Cl_2, 100 mL each) to provide 35



Chemical Formula: $C_{16}H_{26}O_6S_2$
Exact Mass: 378,12
Molecular Weight: 378,5

mg of dithioester **159** as a solid (77 %, 0.093 mmol). ^{1}H NMR (300 MHz, CD_3OD) δ 4.26 4.11 (m, 2H), 4.10 3.90 (m, 2H), 3.90 3.75 (m, 2H), 2.86 (s, 4H), 2.26 2.10 (m, 2H), 1.95 (m, 4H), 1.62 1.22 (m, 6H) ; ^{13}C NMR (126 MHz, CD_3OD) δ 196,998, 66.058, 64.737, 41.340, 40.067, 37.935, 37.798, 35.422. ESI-HRMS found 401.10627 $\pm$ 0.00 (MNa^+ calc. 401.10630).

161 :
4-(6-hydroxy-6-methylheptan-2-ylthio)-4-oxobutanoic acid

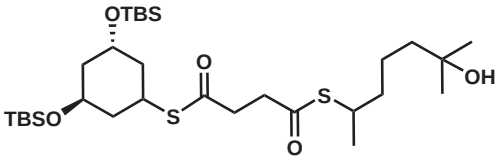


Chemical Formula: $C_{12}H_{22}O_4S$
Exact Mass: 262,12
Molecular Weight: 262,37

Et_3N (95 μL, 0.679 mmol, 1.0 equiv) was added to a solution of side chain thiol **67** (110 mg, 0.679 mmol, 1.00 equiv) and succinic anhydride (75 mg, 0.747 mmol, 1.1 equiv) in DMF (5 mL) stirred vigorously at 0 °C. The reaction was stirred at rt overnight. The reaction mixture was diluted with *t*BuOMe (50 mL) and 0.01 M aq. HCl (10 mL). The layers were separated and the aqueous layer was extracted with *t*BuOMe (2 x 15 mL). The combined organic layers were washed with 0.01 M aq. HCl (2 x 10 mL), H_2O (3 x 5 mL), brine (5 mL) then dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product **161** as a clear oil in 96 % (170 mg, 0.648 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.66 3.59 (m, 1H), 2.89 2.60 (m, 4H), 1.62 1.36 (m, 6H), 1.29 (d, J = 8.0 Hz, 3H), 1.21 (s, 6H).

162 :
S^1-(3S,5S)-3,5-bis(tert-butyldimethylsilyloxy)cyclohexyl S^4-6-hydroxy-6-methylheptan-2-yl butanebis(thioate)

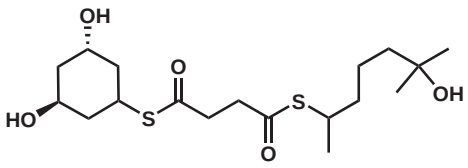
A solution of freshly recrystallized TsCl (109 mg, 0.572 mmol, 1.5 equiv) in AcCN (1.0 mL) was added slowly to a stirred solution of acid **161** (100 mg, 0.381 mmol, 1.0 equiv) and N-methylimidazole (91.0 μL, 1.14 mmol, 3.0 equiv) in AcCN (3.0 mL) at 0 °C.



Chemical Formula: $C_{30}H_{60}O_5S_2Si_2$
Exact Mass: 620,34
Molecular Weight: 621,1

The mixture was stirred at that temperature for 72 min. To the stirred mixture, a solution of A-ring thiol **24** (144 mg, 0.381 mmol, 1.0 equiv) in AcCN (3.0 mL) was added at 0 °Cand the mixture was stirred at the same temperature for 2 hr then overnight at room temperature. The reaction was quenched with 0.01 M aq. HCl (10 mL) ans diluted with EtOAc (50 mL). The layers were separated and the aqueous layer was extracted with EtOAc (2 x 5 mL). The combined organic layers were washed with H_2O (5 mL), brine (5 mL) then dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product as a brown oil. The residue was purified via flash chromatography (column : 1,5 cm x 16 cm, eluent : 15, 15, 20, 25, 30 % EtOAc/hexanes, 50 mL each) provided 110 mg of dithioester **162** as a clear oil (46 %, 0.177 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.16 (s, 1H), 4.13 4.03 (m, 1H), 3.87 (m, 1H), 3.59 (dd, J = 13.7, 6.8 Hz, 1H), 2.92 2.80 (m, 4H), 2.09 (d, J = 11.5 Hz, 1H), 1.84 (d, J = 12.8 Hz, 2H), 1.55 (m, 3H), 1.49 1.33 (m, 6H), 1.30 (d, J = 6.9 Hz, 3H), 1.20 (s, 6H), 0.90 and 0.88 (s, 9H each), 0.05 (m, 12H).

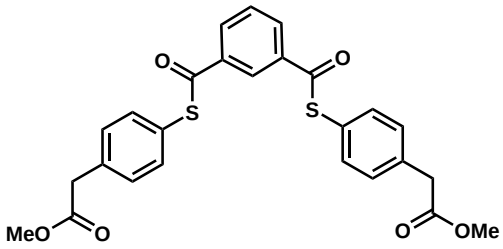
163 :
S^1-(3S,5S)-3,5-dihydroxycyclohexyl S^4-6-hydroxy-6-methylheptan-2-yl butanebis(thioate)



Chemical Formula: $C_{18}H_{32}O_5S_2$
Exact Mass: 392,17
Molecular Weight: 392,57

Prepared from compound **162** (25 mg, 40.3 μmol, 1 equiv) according to General acid-mediated TBS deprotection method. The crude product was purified via preparative TLC (eluent : 10 % MeOH/CH_2Cl_2) to provide 10.3 mg of **163** (65 %, 26.2 μmol). ^{1}H NMR (400 MHz, CD_3OD) δ 4.20 4.14 (m, 1H), 4.00 (m, 1H), 3.83 (m, 1H), 3.56 (dd, J = 13.8, 6.9 Hz, 1H), 2.86 (s, 4H), 2.19 (d, J = 12.0 Hz, 1H), 2.01 (m, 1H), 1.92 (d, J = 13.4 Hz, 1H), 1.62 1.32 (m, 9H), 1.29 (d, J = 6.9 Hz, 3H), 1.16 (s, 6H) ; ^{13}C NMR (101 MHz, CD_3OD) δ 198.92, 198.36, 71.31, 67.45, 66.10, 44.31, 42.76, 41.45, 40.50, 39.42, 39.38, 39.21, 38.21, 36.79, 29.23, 29.10, 22.91, 21.72.

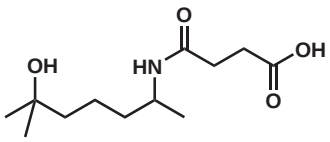
169 :
bis(MPAA-OMe) isophathloyl dithioester



Chemical Formula: $C_{26}H_{22}O_6S_2$
Exact Mass: 494,0858
Molecular Weight: 494,5793

Thiol methyl 2-(4-mercaptophenyl)acetate **168** (1000 mg, 5.49 mmol, 2.20 equiv) was added to a solution of isophthaloyldichloride (507 mg, 2.50 mmol, 1.00 equiv) and dMAP (20 mg, 0.164 mmol, 0.066 equiv) in CH_2Cl_2 (25 mL) and pyridine (0.30 mL) at 25 °C. The reaction was stirred 72 hrs at room temperature. The mixture was concentrated *in vacuo* to a an oily residue. The residue was diluted with EtOAc (50 mL) and 0.5 M aq. HCl (10 mL). The layers were separated and the aqueous layer was extracted with EtOAc (2 x 5 mL). The combined organic layers were washed with 0.5 M aq. HCl (10 mL), H_2O (5 mL), brine (5 mL) then dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product as a yellow oil. The residue was purified via flash chromatography (column : 3,5 cm x 20 cm, eluent : 30, 30, 40, 40 % EtOAc/hexanes, 100 mL each) provided 1015 mg of dithioester **169** as a clear oil (82 %, 2.05 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65 (s, 1H), 8.24 (d, J = 7.7 Hz, 2H), 7.64 (t, J = 7.8 Hz, 1H), 7.54 7.33 (m, 8H), 3.72 (s, 6H), 3.70 (s, 4H).

224 :
4-(6-hydroxy-6-methylheptan-2-ylamino)-4-oxobutanoic acid

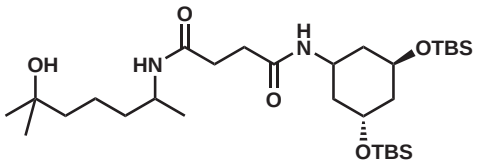


Chemical Formula: $C_{12}H_{23}NO_4$
Exact Mass: 245,16
Molecular Weight: 245,32

Et_3N (5.77 mL, 41.4 mmol, 2.50 equiv) was added to a suspension of heptaminol hydrochloride (3.00 g, 16.5 mmol, 1.00 equiv) and succinic anhydride (2.07 g, 20.7 mmol, 1.25 equiv) in DMF (90 mL) stirred vigorously at 0 °C. The reaction was stirred at 0 °Cfor 1h then 4 days at rt. The reaction mixture was concentrated *in vacuo* down to 10 mL. The residue was diluted with EtOAc (50 mL) and 0.5 M aq. HCl (10 mL). The layers were separated and the aqueous layer was extracted with EtOAc (2 x 5 mL). The combined

organic layers were washed with 0.5 M aq. HCl (10 mL), H_2O (5 mL), brine (5 mL) then dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product as a yellow oil containing 50 % DMF. The residue used as is in the next reactions. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.81 (s, 1H), 4.02 (m, 1H), 2.75 (m, 1H), 2.66 2.54 (m, 1H), 2.51 2.39 (m, 2H), 1.56 1.32 (m, 6H), 1.20 (s, 6H), 1.13 (d, J = 6.6 Hz, 3H).

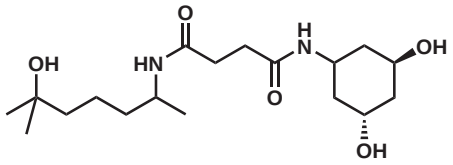
225 :
(3R,5R)-3,5-bis(tert-butyldimethylsilyloxy)cyclohexyl 4-(6-hydroxy-6-methylheptan-2-ylamino)-4-oxobutanoate



Chemical Formula: $C_{30}H_{62}N_2O_5Si_2$
Exact Mass: 586,42
Molecular Weight: 586,99

Ethyl chloroformate (2.5 μL, 26 μmol, 1.1 equiv) was added neat to a solution of acid **224** (5.8 mg, 23.6 μmol, 1.0 equiv) and NMM (3.9 μL, 35 μmol, 1.5 equiv) in THF (2 mL) at 0 °C. The reaction was stirred 30 minutes at 0 °C. A solution of amine **22** (17 mg, 47 μmol, 2.0 equiv) was added to the reaction and the mixture was stirred overnight at rt. The mixture was concentrated *in vacuo* to give the crude product as an oil which was directly taken to the next step. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.65 (s, 1H), 6.01 (dd, J = 23.4, 8.1 Hz, 1H), 4.28 (s, 1H), 4.20 (s, 1H), 4.12 (s, 1H), 3.97 (s, 1H), 2.51 2.38 (m, 4H), 1.84 1.29 (m, 12H), 1.25 (s, 1H), 1.22 1.15 (m, 4H), 1.11 (d, J = 6.5 Hz, 3H), 0.91 and 0.87 (s, 9H each), 0.08 0.04 (4x s, 3H each).

191 :
N^1-((3R,5R)-3,5-dihydroxycyclohexyl)-N^4-(6-hydroxy-6-methylheptan-2-yl)succinamide



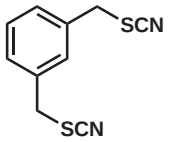
Chemical Formula: $C_{18}H_{34}N_2O_5$
Exact Mass: 358,25
Molecular Weight: 358,47

Prepared from crude compound **225** (max 23.6 μmol, 1.0 equiv) according to General acid-mediated TBS deprotection method. The crude product was purified via preparative TLC (eluent : 10 % MeOH/CH_2Cl_2) to provide 6.3 mg of **191** (35 % over two steps, 17.7

μmol). ^{1}H NMR (400 MHz, CD_3OD) δ 4.15 (m, 2H), 4.00 (m, 1H), 3.85 (m, 1H), 2.43 (s, 3H), 2.16 2.04 (m, 1H), 1.99 (m, 1H), 1.86 (m, 1H), 1.50 1.33 (m, 9H), 1.16 (s, 6H), 1.10 (d, J = 6.5 Hz, 3H) ; ^{13}C NMR (101 MHz, d_6-DMSO and CD_3OD) δ 173.2, 172.1, 71.0, 62.7, 49.7, 47.2, 45.1, 43.4, 39.3, 37.8, 32.4, 29.8, 21.1, 18.6.

6.1.13 Center core dithiols

180 :
1,3-bis(thiocyanatomethyl)benzene



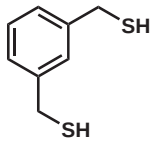
Chemical Formula: $C_{10}H_8N_2S_2$
Exact Mass: 220,01
Molecular Weight: 220,31

KSCN adsorbed on silica was prepared as mentioned in Ref [3]. A 100 mL round bottom flask was charged with potassium thiocyanate (1.46 g, 15 mmol) dissolved in distilled water (5 mL) and silica gel (1.50 g, 100-200 mesh) is added in one portion. The water is removed under reduced pressure using a rotary evaporator, keeping the bath temperature below 60 °C. The resulting powder was dried under pump vacuum overnight.

KSCN/silica (2.2 g, 11.37 mmol, [5.15 mmol KSCN/g solid support], 6.0 equiv) was added to a solution of bis(bromomethyl)benzene (500 mg, 1.89 mmol, 1.0 equiv) in acetone (30 mL). Color changed immediately from clear yellow to pale pink. The reaction mixture was stirred 1h then it was filtered through paper, concentrated *in vacuo* and redissolved in EtOAc (20 mL), refiltered and concentrated again to give crude product **180** as an oil in 88 % (288 mg, 1.31 mmol), which was taken directly to next step without purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.436–7.363 (m, 4H), 4.153 (s, 4H). ESI-HRMS found 243.00171 $\pm$ 0.002 (MNa^+ calc. 243.00211).

171 :
1,3-phenylenedimethanethiol

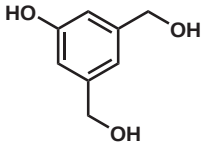
Solid $LiAlH_4$ (48 mg, 1.27 mmol, 2.0 equiv) was added to a solution of bis(thiocyanate) **180** (140 mg, 0.635 mmol, 1.0 equiv) in THF (20 mL) in an ice bath. The reaction was brought to rt over 30 minutes and 16 hr at room temperature. The mixture was stirred vigorously and quenched with 1N HCl (10 mL) and diluted with EtOAc (25 mL). The layers were separated and the aqueous layer was extracted with EtOAc (3 x 15 mL). The combined organic layers were washed with brine (15 mL) then dried ($MgSO_4$) and concentrated



Chemical Formula: $C_8H_{10}S_2$
Exact Mass: 170,02
Molecular Weight: 170,29

in vacuo to give dithiol **171** as a smelly oil 95 % (103 mg, 0.603 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 (m, 3H), 3.74 (d, J = 7.6 Hz, 4H), 1.77 (t, J = 7.6 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 141.51, 128.97, 127.64, 126.70, 28.78.

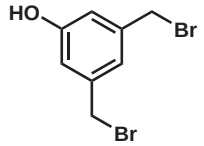
183 :
(5-hydroxy-1,3-phenylene)dimethanol



Chemical Formula: $C_8H_{10}O_3$
Exact Mass: 154,06
Molecular Weight: 154,16

$BH_3 \cdot$ dMS (21 mL,210 mmol, 3 equiv, 10.0 M) was added dropwise to a solution of 5-hydroxyisophthalic acid (12.74 g, 70 mmol, 1 equiv) in THF (50 mL) in ice bath. The reaction was diluted with THF (40 mL) before being allowed to warm to room temperature and was stirred 5 days affording a white precipitate which was filtered off and dissolved in 3N NaOH (50 mL) then stirred 15 minutes. The solution was neutralized using glacial acetic acid to pH = 6,7 then saturated with brine (100 mL) and extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine (50 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give 1.13 g of white solid. (10.5 %, 7.35 mmol). ^{1}H NMR (400 MHz, CD_3OD) δ 6.80 (s, 1H), 6.70 (s, 2H), 4.52 (s, 4H).

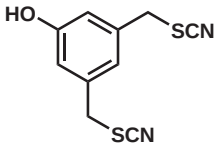
184 :
3,5-bis(bromomethyl)phenol



Chemical Formula: $C_8H_8Br_2O$
Exact Mass: 277,89
Molecular Weight: 279,96

Neat PBr_3 (0.842 mL, 8.96 mmol, 2.5 equiv) was added dropwise to a solution of diol **183** (552 mg, 3.85 mmol, 1.0 equiv) in 1:1 Et_2O/MeCN (30 mL) at 0 °C. The reaction was warmed to rt over 1 hr and left at stirring for 48 hr at room temperature. The mixture was cooled in ice and stirred vigorously and quenched with H_2O (20 mL). The mixture was diluted with EtOAc (30 mL). The layers were separated and the aqueous layer was extracted with EtOAc (2 x 15 mL). The combined organic layers were washed with H_2O (15 mL), brine (15 mL) then dried ($MgSO_4$) and concentrated *in vacuo* to give 713 mg of crude product as a beige oil. This crude mixture was taken directly to next step. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.97 (s, 1H), 6.78 (s, 2H), 4.72 (br s, 1H), 4.40 (s, 4H) ; ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.8, 139.2, 122.4, 116.2, 32.5.

185 :
3,5-bis(thiocyanatomethyl)phenol



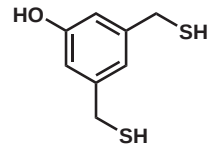
Chemical Formula: $C_{10}H_8N_2OS_2$
Exact Mass: 236,01
Molecular Weight: 236,31

KSCN adsorbed on silica was prepared as mentioned in Ref [3]. A 100 mL round bottom flask was charged with potassium thiocyanate (1.46 g, 15 mmol) dissolved in distilled water (5 mL) and silica gel (1.50 g, 100-200 mesh) is added in one portion. The water is removed under reduced pressure using a rotary evaporator, keeping the bath temperature below 60 °C. The resulting powder was dried under pump vaccum overnight.

KSCN/silica (2.97 g, 15.29 mmol, [5.15 mmol KSCN/g solid support], 6.0 equiv) was added to a solution of crude bis(bromomethyl) **184** (713 mg, max 2.55 mmol, 1.0 equiv) in acetone (30 mL). Color changed immediately from clear yellow to pale pink. The reaction mixture was stirred 1h then filtered through paper and concentrated *in vacuo* to a pink oil (Rf = 0.1, 25 % EtOAc/Hexanes) which was taken directly to next step without purification. ^{1}H NMR (300 MHz, CD_3OD) δ 6.89 (s, 1H), 6.82 (s, 2H), 4.05 (s, 4H).

226 :
3,5-bis(mercaptomethyl)phenol

$LiAlH_4$ (284 mg, 7.5 mmol, 3.0 equiv) was added as a solid to a solution of bis(thiocyanate) **185** (max 2.55 mmol, 1.0 equiv) in THF (20 mL) in an ice bath. The reaction was brought to rt over 30 minutes and 16 hr at room temperature. The mixture was stirred vigorously and quenched with 1N HCl (10 mL) and diluted with EtOAc (25 mL). The layers were

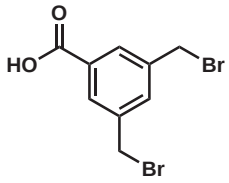


Chemical Formula: $C_8H_{10}OS_2$
Exact Mass: 186,02
Molecular Weight: 186,29

separated and the aqueous layer was extracted with EtOAc (3 x 15 mL). The combined organic layers were washed with brine (15 mL) then dried ($MgSO_4$) and concentrated in vacuo to give a smelly solid. The residue was purified via flash chromatography (dry loading, column : 2.5 cm x 20 cm, eluent : 25, 30 40 50 % EtOAc/hexanes, 200 mL each) to provide 221 mg of dithiol **226** as a white solid (48 % over 3 steps, 1.19 mmol). ^{1}H NMR (300 MHz, CD_3OD) δ 6.76 (s, 1H), 6.63 (s, 2H), 3.62 (s, 4H). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.85 (s, 1H), 6.71 (s, 2H), 3.65 (d, J = 7.1 Hz, 4H), 1.78 (t, J = 7.2 Hz, 2H).

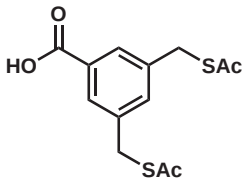
187 :
3,5-bis(bromomethyl)benzoic acid

[4]



Chemical Formula: $C_9H_8Br_2O_2$
Exact Mass: 305,89
Molecular Weight: 307,97

N-Bromosuccinimide (5.37 g, 32.50 mmol) and a catalytic amount of benzoyl peroxide (360 mg, 1.48 mmol, 0.1 equiv) were added to a degassed solution of 3,5-dimethylbenzoic acid (2.22 g, 14.78 mmol) in CCl_4 (100 mL). The suspension was refluxed under nitrogen for 16hrs after which time succinimide was observed floating on the surface of the solvent when the solution was cooled down to room temperature. The succinimide was filtered off under vacuum and the filtrate was concentrated *in vacuo* to 4.9 g of yellowish solids. The solids were a mixture of dibromide and monobromide-alkylsuccinimide. directly taken to next step.

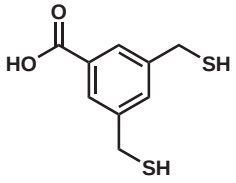


Chemical Formula: $C_{13}H_{14}O_4S_2$
Exact Mass: 298,03
Molecular Weight: 298,38

188 :
3,5-bis(acetylthiomethyl)benzoic acid

Potassium thioacetate (AcSK) (3.37 g, 29.6 mmol, 2.0 equiv) was added to a solution of bis(bromomethyl)benzoic acid **187**(crude mixture from previous reaction, max 4.55 g, 14.78 mmol, 1.0 equiv) in a 1:1 MeOH/EtOH (250 mL) at 0 °C. The reaction was brought to room temperature over 30 minutes. The mixture was stirred vigorously for 48h and then concentrated *in vacuo* to give the crude product as a brown solid. The residue was purified via flash chromatography (column : 5,0 cm x 40 cm, eluent : 10, 20, 30 % EtOAc/hexanes with 1 % acetic acid, 250 mL each) to provide 1.54 g of bisthioacetate **188** as a pale beige solid (35 % over 2 steps, 5.17 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (s, 2H), 7.47 (s, 1H), 4.14 (s, 4H), 2.37 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 194.76, 171.43, 138.80, 134.64, 129.99, 129.43, 32.82, 30.34.

174 :
3,5-bis(mercaptomethyl)benzoic acid



Chemical Formula: $C_9H_{10}O_2S_2$
Exact Mass: 214,01
Molecular Weight: 214,3

Neat hydrazine hydrate ($N_2H_4 \cdot H_2O$) (292 μL, 6.03 mmol, 15 equiv) was added to a degassed solution of bisthiacetate **188** (120 mg, 0.40 mmol, 1.0 equiv) in MeOH (15 mL) at 0 °C. The reaction was brought to room temperature over 30 minutes. The mixture was stirred vigorously for 4 hr and then quenched with concentrated HCl (30 μL, 36.5-38 %). The mixture was diluted with EtOAc (10 mL) and H_2O (10 mL). The layers were separated and the aqueous layer was extracted with EtOAc (2 x 5 mL). The combined organic layers were washed with 0.05 M aq. HCl (5 mL), H_2O (5 mL), brine (5 mL) then dried ($MgSO_4$) and concentrated *in vacuo* to give the crude product as a sufficiently pure

solid. It represented 75 mg of dithiol **174** as a white solide (87 %, 0.35 mmol). ^{1}H NMR (400 MHz, CD_3OD) δ 7.87 (d, J = 1.6 Hz, 1H), 7.56 (s, 1H), 3.77 (s, 2H). ^{13}C NMR (75 MHz, CD_3OD) δ 169.40, 143.99, 133.52, 132.52, 128.92, 28.72. ESI-HRMS found 213.003 $\pm$ 0.002 (M$-$H calc. 213.00408).

6.2 Protein expression (*h*VDR(LDB))

All manipulations are adapted from [5].

Expression

DAY 1 - Transformation into BL21 (DE3)

Thaw a 50 μL aliquot of One Shot BL21 (DE3) Chemically Competent Cells (Invitrogen) on ice. Add 0.5 μL pGEX-VDR plasmid and incubate on ice for 30 min. Heat shock at 42 °C for 30 s. Incubate on ice 3 min. Add 250 μL SOC medium and incubate at 37 °C, 225 RPM for 1 h. Plate 50 μL and 100 μL on LB/Amp (100 μg/mL) agar plates. Incubate at 37 °Cfor 15-17 hrs (overnight). Note: It is necessary to use the plate to start overnight cultures the same day as they are taken out of the incubator.

Expression

DAY 2 - Starter cultures and preparation of bulk culture media Prepare 5 L 2x YT media. For 1 L: 16 g Tryptone, 10 g Yeast Extract, 5 g NaCl in 900 mL ddH_2O Adjust to pH 7.0, Make volume up to 1 L with ddH_2O.

Place 750 mL into each of six (6) 2 L baffled culture flasks and autoclave. Place 50 mL into a 250 mL Erlenmeyer flask and autoclave. Place a single colony into the above 250 mL Erlenmeyer flask containing 50 mL 2x YT to which has been added 100 μg/mL ampicillin. Incubate 22-23 hrs at 37 °C, 250 RPM.

DAY 3 - Bulk culture

Add ampicillin to the culture flasks to a final concentration of 100 μg/mL. Add 7.5 mL of starter culture to each flask. Incubate at 37°C, 250 RPM until $OD_{(}600)$ is about 1.0. Add IPTG to a final concentration of 0.1 mM. Incubate at 20 °C, 250 RPM for 13-14 hrs (preferably 12 hrs). Harvest cells by centrifuging at 7750 g, 10 min, 4 °C. Store pellet at -80 °C (obtained about 65 g).

Purification

Note: Perform all steps on ice or in the cold box.

- Resuspend 30 g cell pellet with 45 mL ice-cold lysis buffer (total volume : 70 mL).
- Sonicate on ice 15 x 10 s (3 sets of 5 cycles, 30 s rests between cycles, 3 min rests between sets), 80 % cycle duty.
- Clarify homogenates by centrifuging twice serially at 30,000 g at 4 °C, for 10 min and then 15 min then transfer in new containers then 53,000 g (21,000 rpm) for 15 min.
- Equilibrate GSTrap FF column (5 mL) using 25 mL binding buffer (2 mL/min).
- Load supernatant onto 5 mL GSTrap FF column (0.2-0.5 mL/min) Actual 0.5 mL/min.
- Wash with 50 mL binding buffer (1.3 mL/min), 50 mL TD-ATP buffer (1.8 mL/min) and 50 mL Td buffer (1-2 mL/min).
- In the meantime, prepare the Thrombin mix: dissolve Thrombin powder in 5 mL Thrombin cleavage buffer (TD buffer).
- Load Thrombin mix onto column (1-2 mL/min).
- Seal the column and incubate at 4 °Covernight.
- Equilibrate HiTrap Benzamidine FF column with 5 mL doubly distilled H_2O and 5 mL binding buffer HB (1 mL/min).
- Connect to outlet of GSTrap FF column.
- Elute with 15 mL binding buffer (1 mL/min) while collecting 0.5-1 mL fractions.
- Analyze fractions by SDS-PAGE (12.5 % polyacrylamide) and pool those containing pure *h*VDR(LBD) (around 37 kDa).
- Concentrate (using 0.5 mL centrifugal filters, 10 kDa MWCO) and store in single experiment aliquots (9 x 170 μL + 1 x 130μL), concentration mesured via micro-Bradford assay to be around 400 μg/mL.

Buffers **Lysis buffer** (according to Kumar[5]): 1x PBS adjust pH to 7.3 (0.14 M NaCl (8.18g/L), 2.7 mM KCl (200mg/L), 10 mM Na_2HPO_4 (1.419g/L)) 10 mM dTT MW 154.25 (1.543 mg/mL) 5 mM EdTA MW 292.24 (1.461 mg/mL) 1 mM PMSF MW 174.2 (0.174 mg/mL, 0.1 M in EtOH (0.5 mL))
Binding buffer: 1x PBS + 10mM dTT (77 mg/50 mL)

TD buffer 50 mM Tris-HCl (1.576g /200mL) 10 mM dTT (308mg/200mL) pH 8.0
ATP buffer: Td buffer + 4 mM ATP (220.45 mg/100 mL) 10 mM $MgCl_2$ (203.31 mg/ 100 mL) pH 7.5

Thrombin cleavage buffer: TD buffer

Binding buffer HB: 50 mM Tris-HCl 0.5 M NaCl (730 mg/ 25 mL) pH 7.5

GSH elution buffer: 50 mM Tris HCl 10 mM GSH pH 8.0

VDR storage buffer 25 mM PIPES (MW 302.4, 3.78g in 500mL), 300 mM KCl (MW 74.56, 11.18g in 500mL), 1 mM EDTA (MW 292.24, 146mg in 500mL), 10 % glycerol, pH 7.3 (Note the absence of DTT or any thiol reducing agent).

6.3 Typical dynamic combinatorial library experiment

Setup of the DCLs:

- Human vitamin D receptor (*h*VDR) was created and stored as aliquots as described earlier.
- Equine liver Glutathione-S-transferase (ElGST) was purchased from Aldrich (G6511 batch 125K7405, 70 % enzyme (remainder is Tris buffer, GSH, phosphates, EDTA)). Used assuming a MW of 50 kDa (Aldrich mentions 45-50kDa).

Solutions of *h*VDR(LBD) were thawed and used immediately. Stock solutions of thioesters/dithiols (around 1 mM each), thiols (around 100 mM each) and disulfides (100 mM each), HPLC standard (around 0.15 mM), TCEP · HCl (around 450 mM) were prepared in degassed dimethylsulfoxide (DMSO) or degassed water (for TCEP · HCl). Proper amounts of each reagent stock solutions, sufficient for two experiments, were introduced into an Eppendorf tube and mixed. Separation of this solution into two Eppendorf tubes is followed by dilution with either a buffer solution (for the control experiment without the receptor) or *h*VDR(LBD) in buffer solution and dilution with 10 % volume of glycerol. The

resulting mixtures were equilibrated at ambient temperature. Sampling method was either (a) aliquots (10 μL) were removed and diluted with a 1:1 mixture of AcCN and TFA (2 μL), rapidly frozen (-78 °C) and stored at -30 °C in freezer until HPLC analysis of aliquots was performed (b) equilibration was directly performed on HPLC sampling tray and injections directly taken from reaction vials (2-3 μL). In this case, reaction was quenched by the TFA present in the HPLC eluent.

6.3.1 Measurement of evolution of dynamic experiments

The evolution of dynamic exchanges was measured by taking aliquots of reacting mixture over time (see typical DCL experiment described above for sampling method). The evolution was monitored by HPLC-UV. Separation was performed using water (0.1 % trifluoroacetic acid)–acetonitrile (0.075 % trifluoroacetic acid) eluent, from 0 % to 100 % AcCN over 45 to 60 minutes and a flow rate of 0.25 mL/min. Multiple wavelengths were monitored for analysis (214 nm, 240 nm, and 254 nm) but analysis always performed with data from 240 nm, as this wavelength was the best compromise between the absorptions of the thioesters and thiols-disulfides species. Amount of species in each HPLC trace was determined by manual integration of the area under the peak. Each amount was then corrected with the internal HPLC standard absorption and, for the three thioesters **148**, **227**, **228**, correction also included their molar absorption coefficients as determined by us.

References

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