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Synthesis of Alpha-GalCer Analogues as iNKT-Cell Agonists and Reactions of 2-Methyleneoxetanes

Donald Raymond Caldwell
University of Connecticut - Storrs, donald.caldwell@uconn.edu

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and

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Donald R. Caldwell, Ph.D.

University of Connecticut, 2017

Glycolipids, particularly α-galactosylceramides, have gained considerable attention due to their ability to modulate immune responses. The discovery of a synthetic glycolipid antigen, KRN7000, has enhanced our understanding of iNKT cell function. Since KRN7000 stimulates iNKT cells to release both Th1 and Th2 cytokines in relative amounts at which the biological activity of either one is antagonized by the other, tremendous effort has been devoted toward synthesizing analogues of KRN7000 that bias cytokine release, as such targets may offer new approaches to treating viral infections, bacterial infections, cancer, and autoimmune conditions. The first part of this proposal describes the synthesis of a series of novel carbohydrate modified analogues of α-galactosylceramide. These analogues have modifications at C6" of the sugar moiety as well as on the sphingoid base. Development of these analogues was aimed at inducing Th1 biased cytokine release in iNKT cells.

For the past two decades, the Howell group has made contributions towards the novel synthesis and application of 4-membered heterocyclic compounds such as oxetanes and 2-methyleneoxetanes. The second part of this dissertation will discuss the development of Lewis acid mediated transformations utilizing 2-methyleneoxetanes to access synthetically valuable products. Herein, we present the first example of 2-methyleneoxetanes as nucleophiles in carbon-carbon bond forming reaction.

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Reactions of 2-Methyleneoxetanes

Donald R. Caldwell

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M.A., Indiana University of Pennsylvania, **2011**

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Reactions of 2-Methyleneoxetanes

Presented by

Donald R. Caldwell, B.S., M.A.

Major Advisor _____

Amy R. Howell, Ph. D.

Associate Advisor _____

Mark W. Peczuh, Ph. D.

Associate Advisor _____

Gaël Ung, Ph. D.

University of Connecticut

2017

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Table of Contents

List of Figures

List of Schemes

List of Tables

List of Abbreviations

$[\alpha]_D^{23}$	Specific rotation (at 23 °C)
AAC	Acyl halide-aldehyde cyclocondensation
Ac	Acetyl
Ac_2O	Acetic anhydride
AcCl	Acetyl chloride
AcOH	Acetic acid
$AgClO_4$	Silver perchlorate
APC	Antigen-presenting cell
Ar	Aromatic
Asn	Asparagine
Asp	Aspartic acid
β_2m	β_2-microglobulin
$BF_3{\cdot}Et_2O$	Boron trifluoride diethyl etherate
BINOL	1,1'-Bi-2-naphthol
Bn	Benzyl
Boc	*tert*-Butyloxycarbonyl
BODIPY	Boron dipyrromethene
br	broad
br s	Broad singlet
Bu	Butyl
Bz	Benzyl
Calcd	Calculated
CD	Cluster of differentiation
$CDCl_3$	Chloroform-d
$CHCl_3$	Chloroform
Cp	Cyclopentadienyl
Cp_2TiMe_2	Bis(η^5-cyclopentadienyl)dimethyltitanium
CSA	Camphor sulfonic acid
d	day
DAST	Diethylaminosulfur trifluoride
DBU	1,8-Diazabicycloundec-7-ene
DC	Dendritic cell
DCC	*N*,*N'*-Dicyclohexylcarbodiimide
DCM	Dichloromethane
DEAD	Diethyl azodicarboxylate
Δ	Heat
DIAD	Diisopropyl azodicarboxylate
DIBAL-H	Diisobutylaluminium hydride
DIC	*N*,*N'*-Diisopropylcarbodiimide
DIPA	*N*,*N*-Diisopropylamine
DIPEA	*N*,*N*-Diisopropylethylamine
DMAP	4-Dimethylaminopyridine

DMDO	Dimethyldioxirane
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
DPPA	Diphenylphosphorazidate
dr	Diastereomeric ratio
E^+	Electrophile
EDC	*N*-Ethyl-*N'*-(3-dimthylaminopropyl)carbodiimide
eq	Equivalent
ESI	Electrospray ionization
Et	Ethyl
Et_2O	Diethyl ether
EtOAc	Ethyl acetate
EtOH	Ethanol
FTIR	Fourier Transform Infrared
α-GalCer	α-Galactosylceramide
Gal	Galactosyl
Gly	Glycine
GSLs	Glycosphingolipids
h	Hour
H_2SO_4	Sulfuric acid
HCl	Hydrochloric acid
HMDS	Hexamethyldisilazane
HMDS	Hexamethyldisilazane
HRMS	High resolution mass spectroscopy
Hz	Hertz
hν	Light
IC_{50}	50% Inhibition concentration
IFN-γ	Interferon-gamma
IL	Interleukin
IR	Infrared
J	Coupling constant value
K_2CO_3	Potassium carbonate
KH	Potassium hydride
$KMnO_4$	Potassium permanganate
KO*t*-Bu	Potassium *tert*-butoxide
LA	Lewis Acid
LDA	Lithium diisopropylamide
LDBB	Lithium 4,4'-di-*tert*-butylbiphenylide
LG	Leaving group
$LiAl(Ot\text{-}Bu)_3H$	Lithium tri-*tert*-butoxyaluminum hydride
$LiAlH_4$	Lithium aluminium hydride
m/z	Mass to charge ratio
ManCer	Mannosylceramide
MeCN	Acetonitrile
MeOH	Methanol

$Mg(OTf)_2$	Magnesium trifluoromethanesulfonate (triflate)
$MgSO_4$	Magnesium sulfate
MHC	Major histocompatibility complex
min	Minutes
mol, mmol	Mole, millimole
MS	Molecular sieve
MsCl	Methanesulfonyl chloride
Na_2SO_4	Sodium sulfate
NaH	Sodium hydride
$NaHCO_3$	Sodium bicarbonate
NaN_3	Sodium azide
NaOH	Sodium Hydroxide
NaOMe	Sodium Methoxide
nap	Naphthalene
NBS	*N*-Bromosuccinimide
NEt_3	Triethylamine
NH_4Cl	Ammonium chloride
NKT	Natural killer T
NMM	*N*-Methylmorpholine
NMR	Nuclear magnetic resonance
Nu	Nucleophile
$Pd(OH)_2$	Palladium hydroxide
PEG	Polyethylene glycol
PG	Protecting group
Ph	Phenyl
$PhCH_3$	Toluene
Phe	Phenylalanine
PhH	Benzene
PMB	*p*-Methoxybenzyl
PNP	*p*-Nitrophenyl
PPh_3	Triphenylphosphine
PPL	Porcine pancreatic lipase
ppm	Parts per million (NMR)
Py	Pyridine
PyBOP	(Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate
quant.	Quantitative
rt	Room temperature
SAR	Structure activity relationship
s-BuMgCl	*sec*-Butylmagnesium chloride
Ser	Serine
S_N2	Bimolecular nucleophilic substitution
$SnCl_2$	Tin(II) chloride
$SnCl_4$	Tin(IV) chloride
TBACN	Tetrabutylammonium cyanide
TBAF	Tetrabutylammonium fluoride

TBAI	Tetrabutylammonium iodide
TBDPS	*tert*-Butyldiphenylsilyl
TBS	*tert*-Butyldimethylsilyl
TCR	T cell receptor
TFA	Trifluoroacetic acid
TfN_3	Trifluoromethanesulfonic azide
Th	T helper
THF	Tetrahydrofuran
Thr	Threonine
$TiCl_4$	Titanium tetrachloride
TLC	Thin Layer Chromatography
TMS	Trimethylsilyl
$TMSN_3$	Trimethylsiyl azide
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
TNF	Tumor necrosis factor
TOF	Time of flight
Tr	Triphenylmethyl (trityl)
TrCl	Triphenylmethyl chloride (trityl chloride)
Ts	4-Toluensulfonyl (tosyl)
TsOH	*p*-Toluensulfonic acid
UV	Ultraviolet
$ZnCl_2$	Zinc chloride

Chapter 1. Synthesis of α-GalCer analogues as NKT-cell agonists

1.1 General introduction to glycosylceramides

1.1.1 Glycosphingolipids

Glycosphingolipids (GSLs) are a diverse class of glycolipids found in the lipid membranes of plants, animal cells, and bacterial cells. They have varied molecular structures and play key roles in cell-cell communication, viral and bacterial infections, immune system activation and modulation, and cell proliferation.[1] GSLs are comprised of a ceramide backbone covalently linked to one or several saccharide residues. GSLs are generally classified into four categories:[2]

1. Cerebrosides	Containing one sugar residue
2. Neutral glycosphingolipids	Containing one or more uncharged sugar residues
3. Gangliosides	Containing one or more neuraminic acid residues
4. Sulfatides	Containing one sulfated residue on the saccharide

Ceramides contain a sphingoid base with an amide linkage to a fatty acid chain (Figure 1A), which can vary in saturation, length, branching, and hydroxylation. Natural sphingoid bases consist of sphingosine (2*S*,3*R*-D-*erythro*-2-amino-1,3-octadec-4*E*-enediol), sphinganine (2*S*,3*R*-D-*erythro*-2-amino-1,3-octadecanediol) and phytosphingosine (2*S*,3*S*,4*R*-D-*ribo*-2-amino-1,3,4-octadecane triol) (Figure 1B). The sphingoid base of GSLs has a 1”-1’ glycosidic linkage to the sugar head group, which is most commonly glucose or galactose (Figure 1C, D). This glycosidic linkage of the anomeric carbon (C1”) to the sugar can be in either α- or β-linkage (Figure 1E). Mammalian glycolipids usually exist in the β orientation.[3]

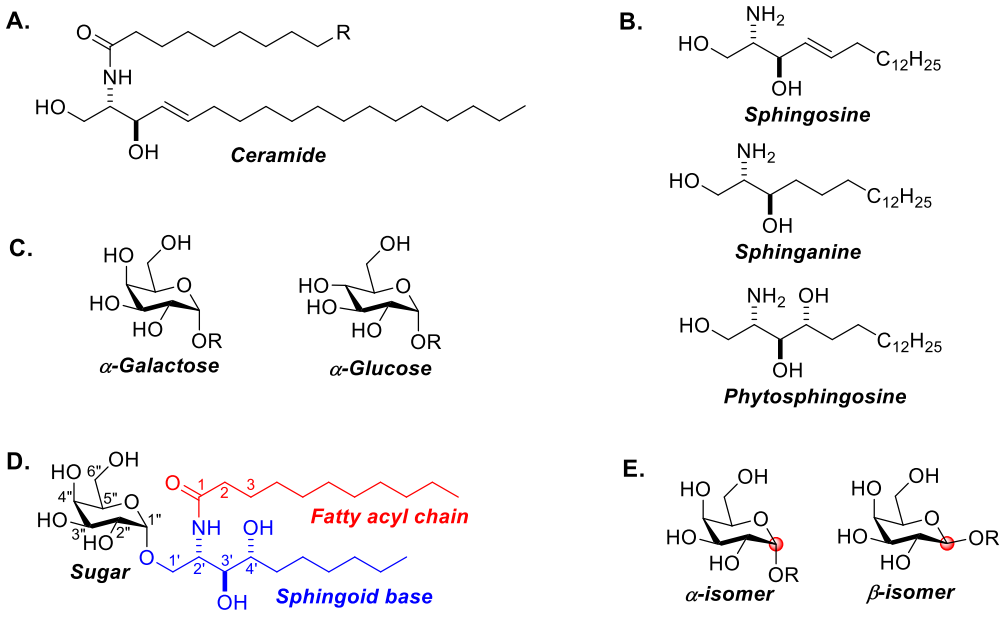


Figure 1. (A) General structure of ceramide; (B) Structures of natural sphingoid bases; (C) Structures of galactose and glucose; (D) General structure of galactosylceramide; (E) α and β-anomers of D-galactose.

1.1.2 History of α-galactosylceramides

In 1993, the pharmaceutical division of Kirin Brewery Inc. isolated a series of novel α-galactosylceramides called agelasphins (AGLs) from the marine sponge, *Agelas mauritianus*.[4,5] Of these agelasphins, agelasphin-9b (Figure 2) exhibited potent anti-tumor activity in *in vivo* models of several murine tumor lines.[6] Agelasphins consist of a saccharide that is α- or β-linked to a phytosphingosine-containing ceramide backbone, and diversity in the compositions and lengths of the lipid chains lead to different agelasphins (Figure 2). Initial screenings demonstrated that α-linked galactose-containing agelasphin glycolipids were significantly more potent against B16 mouse melanoma cells than β-linked agelasphins.

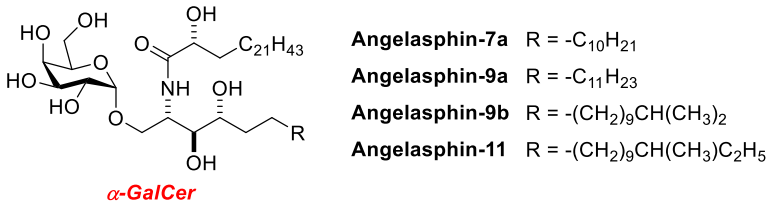


Figure 2. Chemical structure of some natural AGLs from the *Agelas mauritianus*.

While agelasphin-9b was the most potent agelasphin identified in the marine sponge, it could only be isolated in very small amounts. The promising anti-tumor activities of agelasphin-9b led Kirin Brewery Co.[7] to perform a structure-activity relationship (SAR) study with the intention of finding a potent, commercially-viable anti-tumor agent. SAR studies led to the synthesis of KRN7000 (Figure 3), more commonly referred to as α-galactosylceramide (α-GalCer), which was selected as a candidate for clinical application.[8] The strategic simplifications included removing the fatty acid C2 hydroxyl group and the terminal branching of the sphingoid base. Importantly, by extending the sphingoid base chain to 18-carbons and the fatty acid chain to 26-carbons, the stimulatory activity of agelasphin-9b was maintained for α-GalCer. In 1997, it was found that KRN7000 formed a complex with CD1d and stimulated NKT cells to produce equally high levels of both Th1 and Th2 cytokines; these cytokines antagonize each other's biological functions and limit the therapeutic effects of KRN7000.[33]

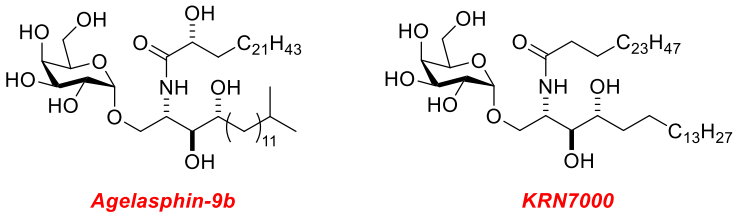


Figure 3. Structures of agelasphin-9b and KRN7000.

1.1.3 Activation of NKT cells by the complex CD1d/Glycolipid/TCR

α-GalCer activates NKT cells (natural killer T cells) in a CD1d-restricted manner.[9] First, α-GalCer binds to CD1d, a major histocompatibility complex (MHC) class I-like molecule on the

surface of antigen-presenting cells (APCs), to form a glycolipid/CD1d binary complex (Figure 4). This α-GalCer/CD1d complex is then presented to the T cell receptor (TCR) of the NKT cell, and upon recognition, rapid secretion of T helper 1 and 2 (Th1 and Th2) cytokines occurs. Th1 cytokines include IFN-γ, IL-2, and TNF-α, and the production of these cytokines is associated with anti-tumor, anti-viral, antibacterial, and adjuvant effects of α-GalCer. Th2 cytokines include IL-4 and IL-10, and the production of these cytokines is associated with certain autoimmune diseases such as type 1 diabetes. In the process of NKT activation, α-GalCer induces the secretion of Th1 and Th2 cytokines indiscriminately.

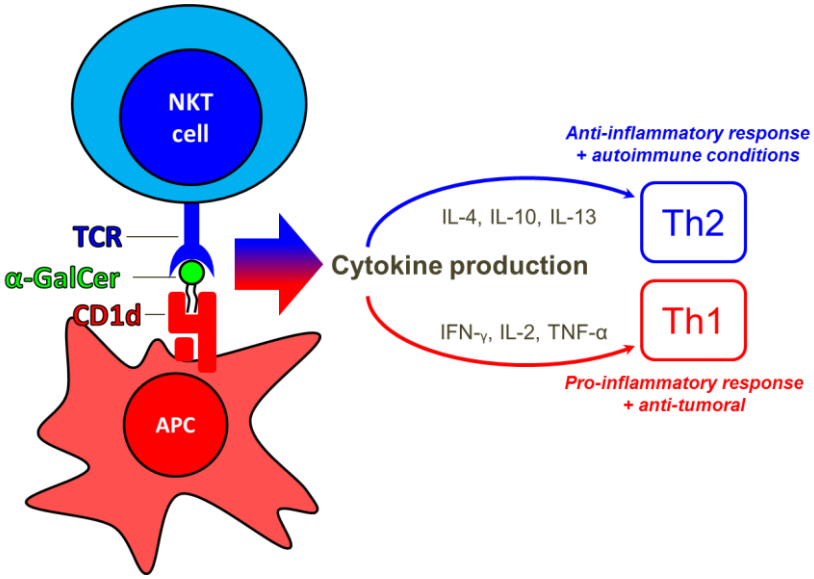


Figure 4. Activation of NKT cells by CD1d/glycolipid/TCR complexation.

1.1.3.1 Binary complex

X-ray crystallography analysis of the mouse[10,11] and human[12] CD1d/KRN7000 complexes revealed both the architecture of CD1d and the binding mode for the glycolipid. The CD1d protein consists of a C-terminus α3 domain composed of β-sheets in two layers and a more complex *N*-terminus domain, which consists of two α-helices, α1 and α2 (Figure 5). The α1 and α2 helices of CD1d lie on top of seven β strands and form two large hydrophobic pockets, named A' and C' (F' for mouse CD1d). The glycolipid specifically binds to CD1d with the 26 carbon acyl chain of

α-GalCer occupying the longer A' pocket and the shorter, 18 carbon sphingoid base chain occupying the C' pocket. Thus, hydrophobic interactions are the principle factors generating the binding energy between the lipid chains and the CD1d binding groove. Meanwhile, the hydrophilic galactose head group protrudes from the CD1d groove for recognition by the TCR of NKT cells.

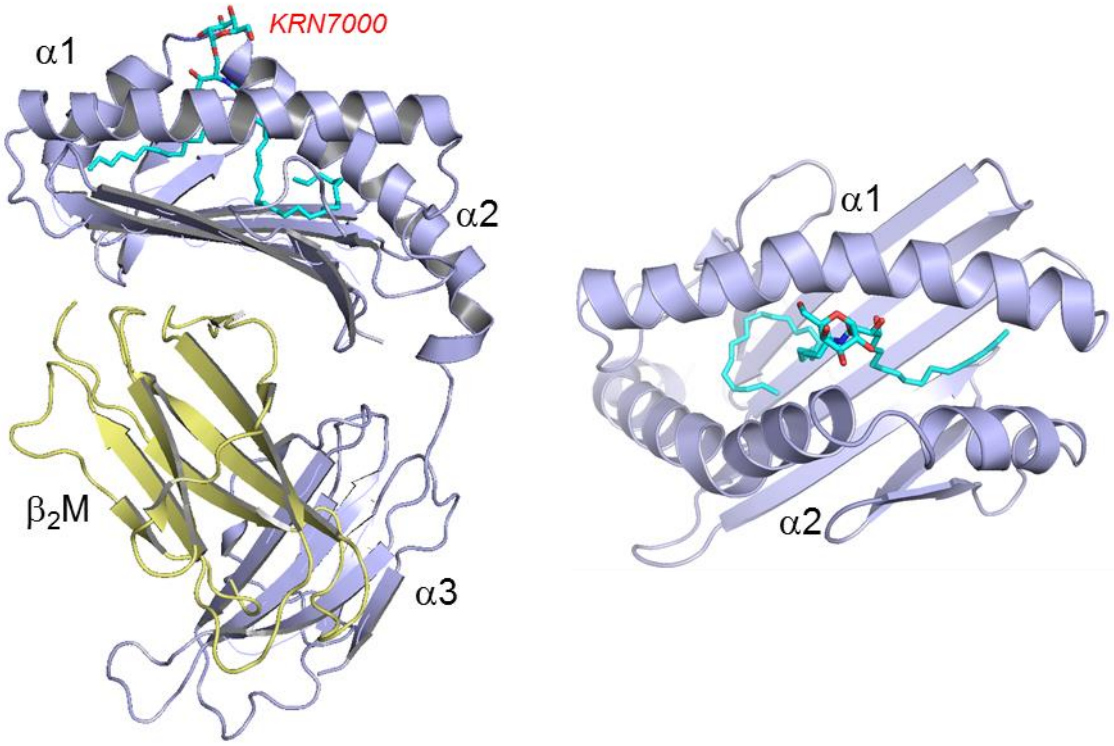


Figure 5. Crystal structure of hCD1d-KRN7000 binary complex. Figure generated from 1ZT4[12] with PyMol.

Analysis of the crystal structure of the hCD1d/KRN7000 complex has identified key hydrogen bonds between KRN7000 and CD1d (Figure 6). Notably, three amino acids of the CD1d engage in hydrogen bonds with KRN7000. The 2"- and 3"-OH of the galactose hydrogen bond to the two oxygen atoms on Asp151 (mouse Asp153), located on the CD1d α2 helix. The glycosidic oxygen atom 1"-O on KRN7000 can form a hydrogen bond with the hydroxide group of Thr154 (mouse Thr156); alternately, Thr154 can act as a hydrogen acceptor with the 2"-NH amide group. The 3'-OH of the sphingoid base hydrogen bonds to the oxygen atom on Asp80, located on the CD1d α1 helix. However, in mouse CD1d, Asp80 binds to the 3'- and 4'-OH groups of the

sphingoid base.[44] These interactions are believed to orient the sugar in a specific position for presentation with the TCR of NKT cells.

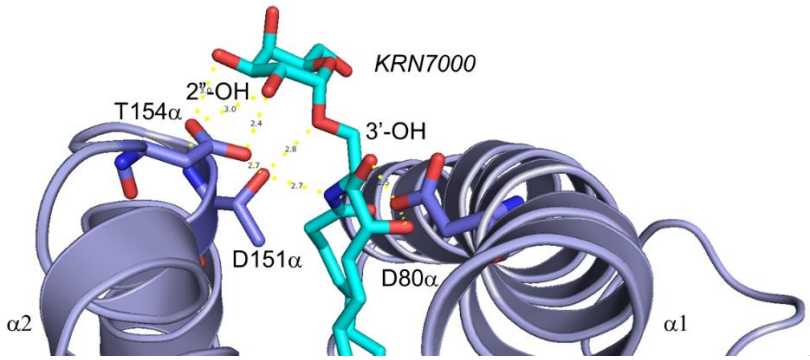


Figure 6. Hydrogen bonds formed between KRN7000 (blue) and amino acid residues of hCD1d (purple). Figure generated from 1ZT4[12] with PyMol.

1.1.3.2 Ternary complex

The X-ray crystallographic structure of the ternary human CD1d/KRN7000/TCR complex, published by Rossjohn and co-workers[13] in 2007, has been instrumental for understanding the interactions that occur within the TCR/CD1d/KRN7000 complex (Figure 7). The crystal structures of both human and mouse[14] ternary CD1d/KRN7000/TCR complexes showed conformational conservation, indicating a lock-and-key style binding mechanism. Even more surprisingly, the NKT TCR was bound to the CD1d-antigen binding cleft in a parallel manner, which differed from the diagonal footprints observed for MHC class-I-restricted TCRs.[15] This now positions the TRC α-chain above the exposed end of the CD1d binding groove, and the TCR β-chain is positioned above the enclosed end of the F' pocket. Consequently, binding of the TCR and KRN7000 happens solely with the TCR α-chain (CDR1α and CDR3α loops).

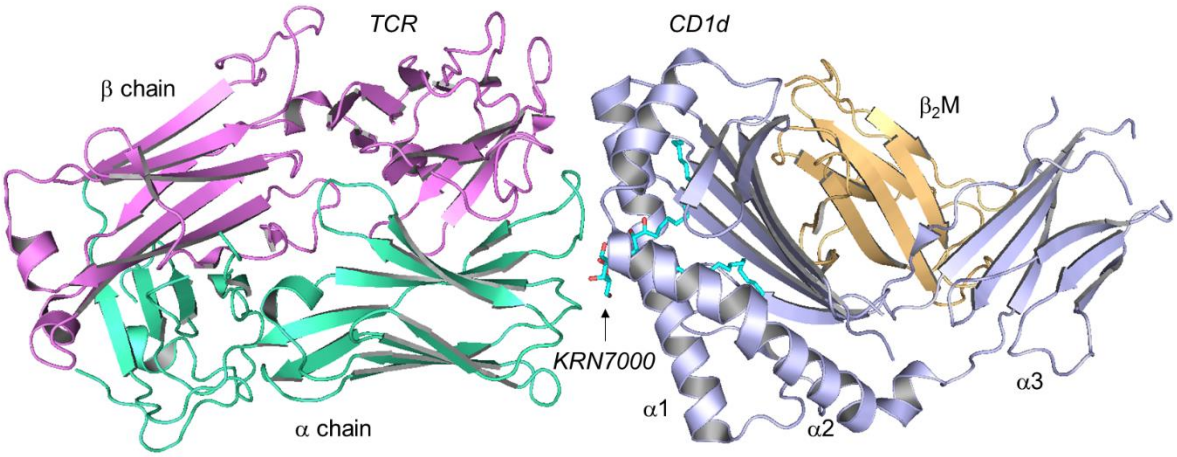


Figure 7. Crystal structure of hCD1d-KRN7000-TCR ternary complex. Figure generated from 2PO6[13] with PyMol.

Analysis of the crystal structure of hCD1d/KRN7000/TCR complex identified key hydrogen bonds formed when the 2”-, 3”-, and 4”-OH groups on the galactose interact with amino acid residues Gly96α, Ser30α, and Phe29α, respectively (mouse 2”- and 3”-OH hydrogen bond with Asn30) (Figure 8). Additionally, the 3’-OH of the sphingoid base forms a hydrogen bond with Arg95. Formation of these hydrogen bonds appears to be important for the recognition of the TCR complex and activation of NKT cells. These results also explain why glycosyl ceramides with other sugar head groups, such as α-mannosylceramide (α-ManCer), do not activate NKT cells.[16] The orientations of the 2”- and 4”-OH groups in mannose (2”-axial and 4”-equatorial) differs compared to those of galactose, resulting in the loss of two hydrogen bonds between CD1d and the TCR of the NKT cell. Additionally, the α-linkage of KRN7000 was shown to be more important for TCR recognition than the β-linkage of β-galactosylceramide.[17]

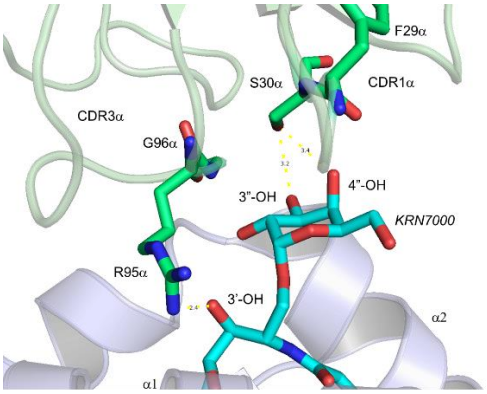


Figure 8. Hydrogen bonds formed between KRN7000 (blue), amino acid residues of hCD1d, and the TCR receptor of NKT cells. Figure generated from 2PO6[13] with PyMol.

1.1.4 Analogues of KRN7000

The limited positive therapeutic outcome of KRN7000 in clinical trials was believed to be due to unbiased production of both Th1 and Th2 cytokines. It is suggested that CD1d-ligands must be capable of biasing either a Th1 or Th2 response for therapeutic applications.[18] KRN7000 inspired researchers to design analogues of KRN7000 that bias towards either Th1 or Th2 cytokine response. Modifications of KRN7000 are mainly based on four types: (1) nature of the ceramide moiety; (2) variation on the lipid chains; (3) modification of the sugar head moiety; and (4) nature and configuration of the anomeric bond (Figure 9).[19,20,21] The different biological activities of various KRN7000 analogues provides a better understanding of the interactions of the CD1d/glycolipid/TCR complex and immune response. Below, a brief overview of notable and/or relevant analogues is given.

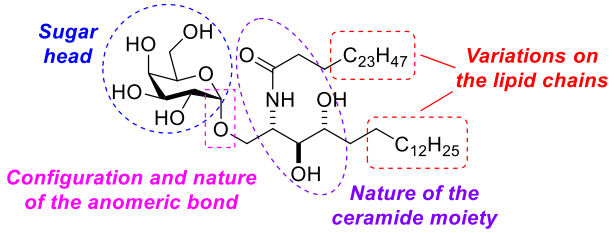


Figure 9. Four commonly modified moieties in KRN7000.

1.1.4.1 Modification of the sphingoid base

To understand the structural requirements of the ceramides necessary for anti-tumor activity, Morita and co-workers synthesized different 3'- and 4'-OH analogues, as well as different acyl chain length analogues, of α-GalCer (Figure 10).[22,23,24,25] These initial SAR studies showed an α-GalCer analogue lacking the 3'- and 4'-OH group (AGL-535) was inactive in stimulating NKT cells. However, the four diastereomers of the sphinganine analogues (AGL-555, AGL-556, AGL-558, and AGL-559) showed only slightly decreased anti-tumor activity, with the natural (2*S*,3*R*)-isomer, AGL-558, having similar effects to KRN7000.[23] It was also noted that a longer acyl chain enhanced the bioactivity.

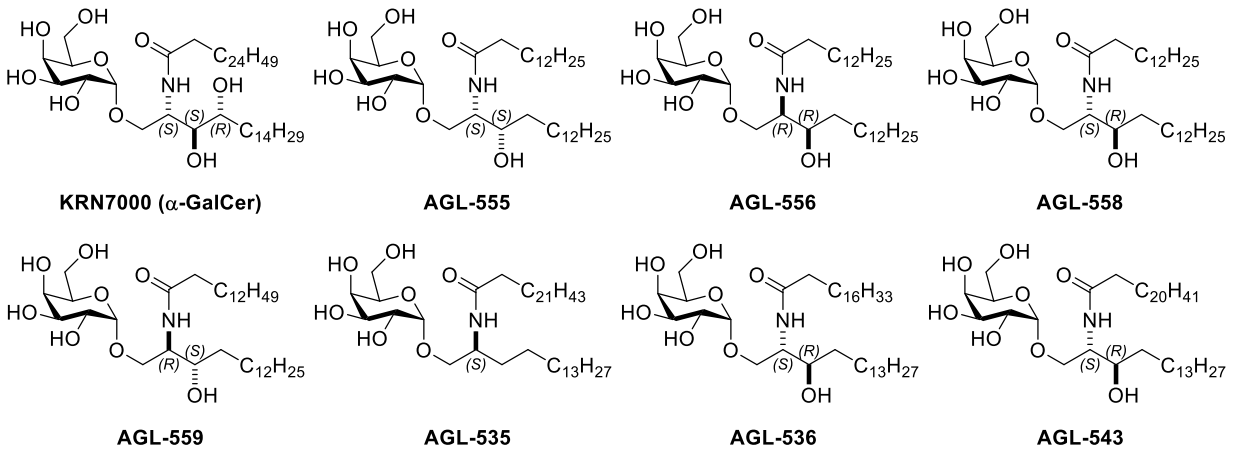


Figure 10. Structure of KRN7000 and ceramide modified analogues.

The importance of the 3'-OH group was later explained by the crystal structure of hCD1d/KRN7000/TCR. The crystal structure revealed that the 3'-OH of KRN7000 formed a hydrogen bond with Asp80 of the CD1d and with Arg95 of the TCR, whereas the 4'-OH of KRN7000 showed no significant interaction with CD1d or the TCR. To further explore the importance of the 3'- and 4'-OH groups, modifications at these positions have been prepared (Figure 11).[26,27] Replacement of the 4'-OH with *gem*-difluorides (**1**) provided a compound that showed a minimal loss in cytokine production yet had an increased bias toward Th1 cytokine polarization.[26] This was attributed to the *gem*-difluorides increasing the hydrogen bond donating

ability of the 3'-OH with Asp80 of the CD1d. Furthermore, Van Calenbergh and co-workers investigated replacing the 3'- and 4'-OH (H-bond donor) with an amine group (H-bond acceptor).[27] It was found that replacement of the 3'-OH with an amine group (**2**) (Figure 11) resulted in a significant drop in cytokine response compared to KRN7000, despite compound **2** having a good affinity for the TCR. Replacement of the 4'-OH with an amino (**3**) induced a moderate cytokine production, however lower than that induced by KRN7000. The 3'-OH analogues (**3**) was shown to be more effective at activating NKT cells than the 4'-OH analogues (**2**).

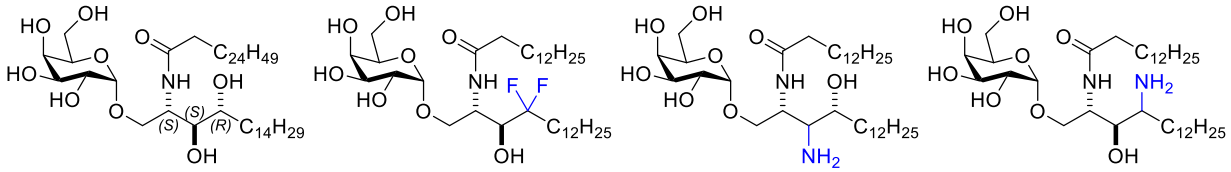


Figure 11. Structures of KRN7000 and sphingoid base analogues.

In 2008, Chung and co-workers[28] were able to synthesize and evaluate all eight stereoisomers on the phytosphingosine chain of KRN7000 (Figure 12). The biological analysis of these eight stereoisomers revealed the *S* configuration of the amide and 3'-OH group was important for NKT cell activation. However, the stereochemistry at the 4'-OH position was not significant for the stimulation of NKT cells. Van Calenbergh and co-workers also reported the synthesis of three similar diastereoisomers with truncated acyl chains.[29] Interestingly, *in vitro* data demonstrate that truncated diastereoisomer (2*S*,3*S*,4*S*)-**4** of α-GalCer induced similar levels of IFN-γ and IL-4 compared to KRN7000. These results suggest changing the stereochemistry at the 4'-OH of the phytosphingosine chain could result in similar NKT cell activation.

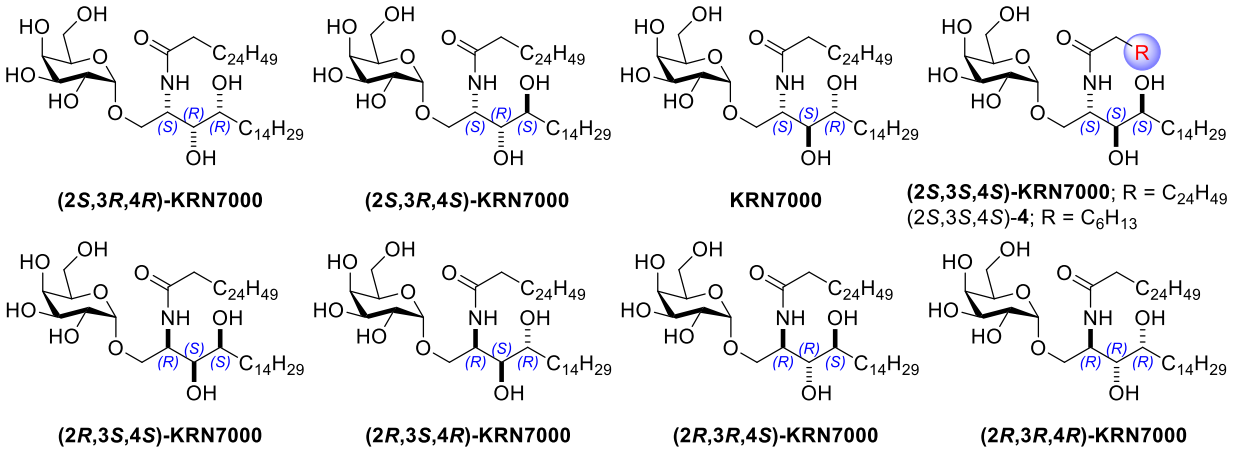


Figure 12. Structure of the eight KRN7000 stereoisomers and truncated analogue.

In 2011, Kim and co-workers reported the first synthesis of the 3'-deoxy analogue of α-GalCer.[30] Unexpectedly, they found that mouse NKT cell activation by 3'-deoxy-α-GalCer was comparable to activation by both 4'-deoxy-α-GalCer and KRN7000. Additional docking studies with hCD1d/glycolipid/TCR suggested that the 3'-deoxy analogue was able to shift itself lower in the CD1d pocket and establish a new hydrogen bond between the 4'-OH and CD1d. This indicates that the 4'-OH can also play an important role, and the two hydroxyl groups could play cooperative or individual roles in orienting the glycolipid.

In 2012, a conflicting reports were published on the importance of the 4'-OH.[31] Stocker and co-workers reported the phytosphingosine, sphinganine, and sphingosine analogues of α-GalCer stimulate human and mouse NKT cells differently (Figure 13). These three glycolipids showed similar activation of mouse NKT cells; however, human NKT cells showed a significant decrease in activity for the sphinganine and sphingosine analogues in several assay systems. These results suggested that the 4'-OH is important for the activation of human NKT cells and should be considered when comparing the CD1d-glycolipid binding between different species.

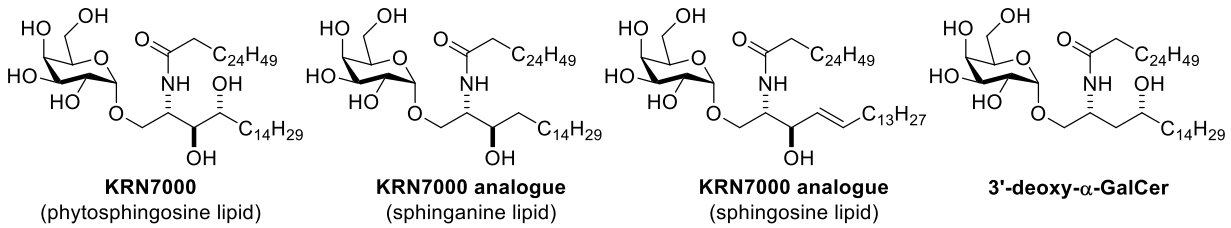


Figure 13. Structure of KRN7000 and ceramide modified analogues.

The importance of the 4'-OH was further explored by Pipelier and co-workers,[32] who synthesized 3',4'-dideoxy-α-GalCer analogues combined with the introduction of a mono or difluoro substituent at C3' position (Figure 14). Biological evaluations were performed *in vitro* on human cells and *in vivo* on mice. Replacement of the 3'-OH with one isoelectronic fluorine was not able to restore the effects lost with the 3'-OH group, but the presence of two fluorine groups at the C3' position significantly restored activation of human NKT cells. Moreover, in support of previous reports, human NKT cell response shows a divergence from mice NKT cell response and might not be reliable models between species.

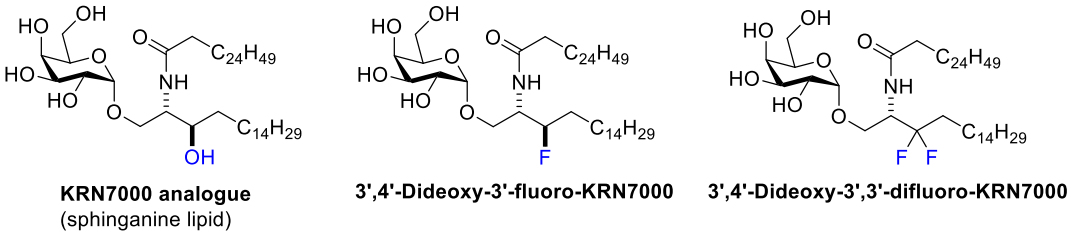


Figure 14. Structure of sphinganine and 3',4'-dideoxy-fluoro-α-GalCer analogues.

1.1.4.2 Modification of the sugar head group

After the discovery of the potent anti-tumor activities of KRN7000, several studies were performed to investigate structure-activity relationships (SAR) based on modifications of the sugar head group. In 1997, Taniguchi and co-workers[33] synthesized a number of disaccharide analogues of α-GalCer with the second sugar at the 2"-, 3"-, 4"-, and 6"-positions of the galactose fragment (Figure 15). Interestingly, the activity for each of the disaccharides was similar to that of KRN7000. Following this finding, Kronenberg and co-workers[34] showed that disaccharide

Gal(α1→6)GalCer was able to stimulate NKT cells without lysosomal processing to the monosaccharide. In contrast, the corresponding disaccharides of Gal(α1→2)GalCer, Gal(β1→3)GalCer, and Gal(α1→4)GalCer[35] required enzymatic processing to the monosaccharide for NKT cell stimulation.

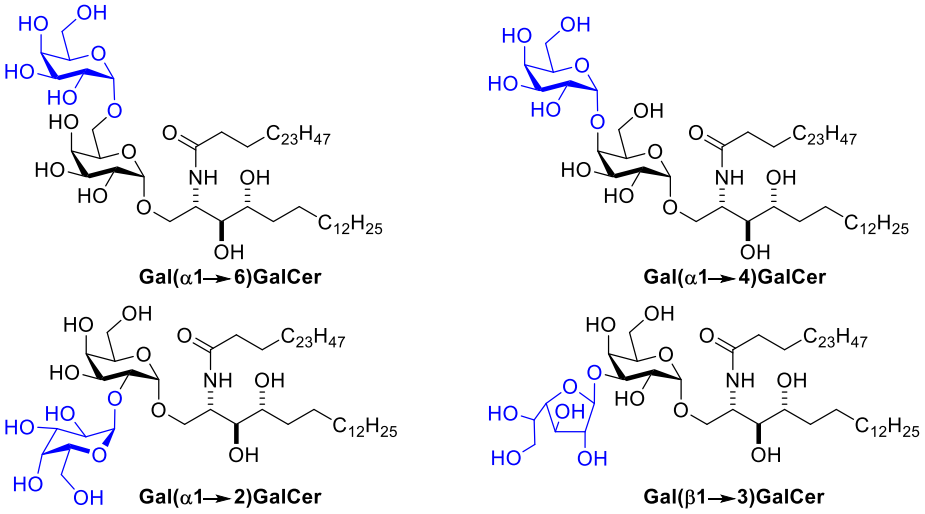


Figure 15. Disaccharide analogues of KRN7000.

The importance of hydroxyl groups in positions 2”, 3”, and 4” of the galactose fragment was examined by deletion or modification of the functional group (Figure 16). Removal of the 2”-OH[36] or substitution with a methoxy[37], fluoro[38], or acetamide[39] group resulted in a dramatic decrease in cytokine response. Modification to the 3”-OH and 4”-OH of the galactose has also been explored by evaluation of 3”- or 4”-deoxy[40,41] or fluoro[41] analogues of α-GalCer. Unlike the 2”-OH analogues that dramatically decreased the immune response, small modifications at the 3”- and 4”-OH subtly decreased the cytokine response. The 3”-*O*-sulfate[39,42] and 4”-alkylaryl[43] analogues gave a response comparable to KRN7000.

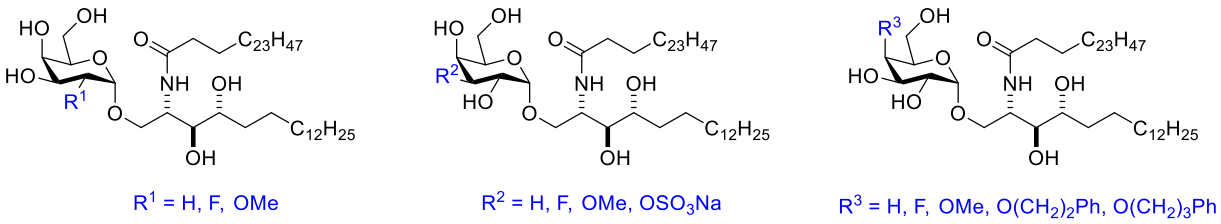


Figure 16. Structures of sugar head group modifications of α-GalCer.

Successful modifications of the sugar head group of α-GalCer are rather limited and mostly restricted to the 6”-position. The crystal structure of the CD1d/α-GalCer/TCR complex showed hydrogen-bonding between the 2”-, 3”-, and 4”-hydroxyls of the galactose and the amino acids of the TCR and/or CD1d.[44,45] However, the primary 6”-OH of the galactose is positioned away from the TCR/CD1d interface and does not participate in hydrogen bonding, suggesting that modification at the 6”-OH may be well tolerated. These findings have led to the design of various C6” analogues of α-GalCer with diverse properties and applications (Figure 17), including amide probes[49,54], arylamides[57,58,59], disaccharides[34], triazole analogues[60,61], PEGylated analogues[56], thio/peptide-conjugates[46], and small functional groups[47,48,55].

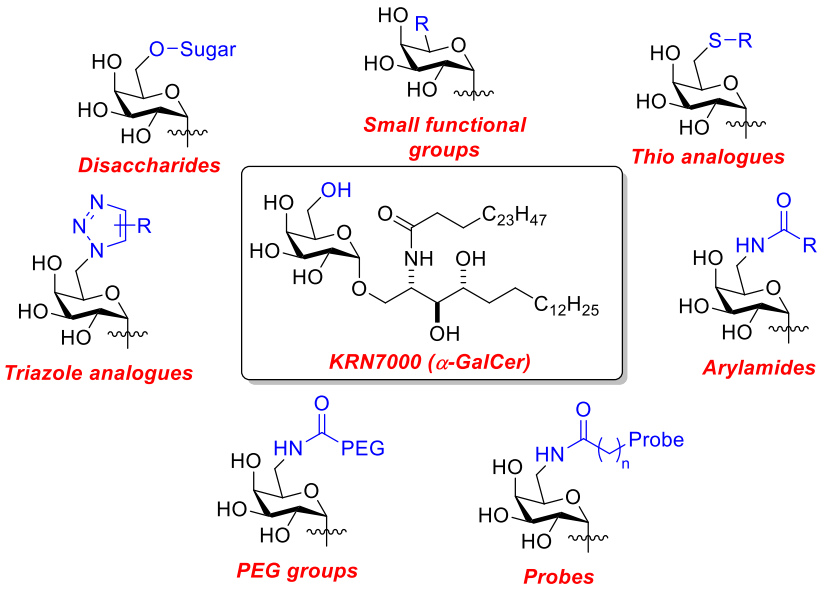


Figure 17. Modifications at the C6”-position of α-GalCer.

In 2002, Savage and co-workers converted the 6”-OH of α-GalCer to an amine group, which allowed for efficient coupling of fluorophores such as prodan and dansyl or small molecules like biotin (Figure 18).[49] These analogues were designed as probes to observe the trafficking of glycolipids and to quantify their association with CD1d and NKT cell receptors. Probes for studying glycolipid trafficking have been prepared previously (fluorophore,[50] biotin,[51] and BODIPY[52]). These probes were incorporated on the end of the fatty acyl chain that binds within the deep hydrophobic pocket of the CD1d receptor. However, the incorporation of probes on the acyl chain is not ideal as the probe may interfere with the association formed between the acyl chain and the CD1d receptor. Analogues with incorporation of probes on the C6” galactose were able to stimulate NKT cells similarly to KRN7000. Wang and co-workers reported the visibility of a biotin-labelled α-GalCer analogue by flow cytometry.[53] Soon after, Stocker and co-workers demonstrated the ability of dansyl α-GalCer to activate NKT cells and to be used as a fluorescent probe with flow cytometry.[54] These results, among others, led to further exploration in designing C6”-α-GalCer analogues that can bias cytokine release.

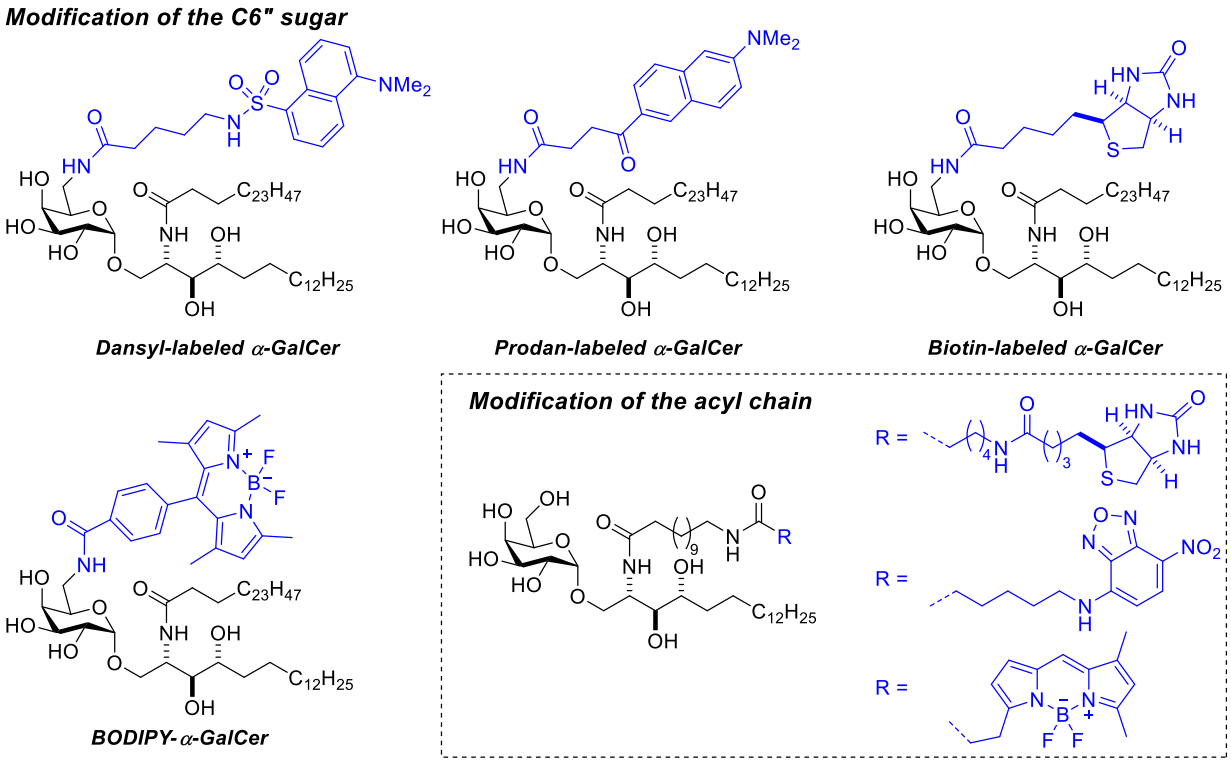


Figure 18. Fluorophore-, BODIPY-, and biotin-appended α-GalCer analogues.

One practical limitation of α-GalCer is its poor solubility in aqueous and organic solvents. Savage and co-workers found that the incorporation of an acetamide group at C6" (PBS-57) with the incorporation of a *cis*-double bond on the acyl chain provided a compound with increased solubility in DMSO (Figure 19).[55] Also, *in vivo* and *in vitro* studies of PBS-57 exhibited increased NKT cell activation compared to KRN7000. Gunzán and co-workers demonstrated that the solubility of KRN7000 could be improved by the addition of a polyethylene glycol (PEG) group.[56] The PEGylated 6"-analogue of KRN7000 (MPEG-α-GalCer) had increased solubility and stimulated NKT cells more efficiently than KRN7000. Interestingly, MPEG-α-GalCer skewed the cytokine release profile toward a Th2 response, whereas many C6" analogues to date have skewed a Th1 cytokine response.

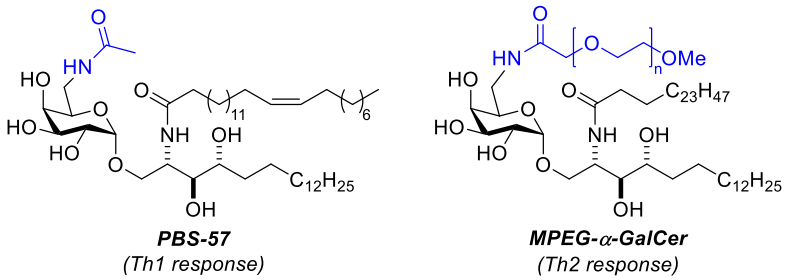


Figure 19. Structures of PBS-57 and MPEG-α-GalCer.

Another application in modifying the C6" position of KRN7000 was demonstrated when Van Calenbergh and co-workers synthesized a number of C6"-analogues of amide- or urea-linked aromatic motifs to obtain additional interactions with the CD1d receptor.[57] Remarkably, many of these analogues provided a skewed Th1 response. In particular, the promising analogue Nu-α-GalCer (Figure 20) gave diminished production of IL-4 and increased production of IFN-γ *in vivo* compared to KRN7000. The significant Th1-skewed cytokine response of Nu-α-GalCer was later explained when Elewaut and co-workers[58] obtained the X-ray crystal structure of mouse Nu-α-GalCer complexed with CD1d and the TCR. The study revealed that all of the hydrogen bonding interactions of α-GalCer with CD1d and the TCR are conserved with Nu-α-GalCer. In addition to these hydrogen bonds, another hydrogen bond was formed between the carbonyl oxygen of the urea linker and Thr159 of CD1d. Moreover, the structure revealed that the naphthyl group sat in a small hydrophobic pocket on top of the A' pocket. This induced-fit binding pocket acted as a third anchor to the CD1d, along with the A' and F' pockets. The addition of the hydrogen bond together with the induced-fit binding pocket imparted a higher affinity of Nu-α-GalCer to CD1d, which resulted in a longer interaction with the NKT cell and is thought to account for the Th1 cytokine bias. Elewaut and co-workers also synthesized three new α-GalCer analogues (PyrC-α-GalCer, NC-α-GalCer, and 4ClPhC-α-GalCer) that were more potent in preventing lung metastasis in B16 mice compared to Nu-α-GalCer and KRN7000. [59]

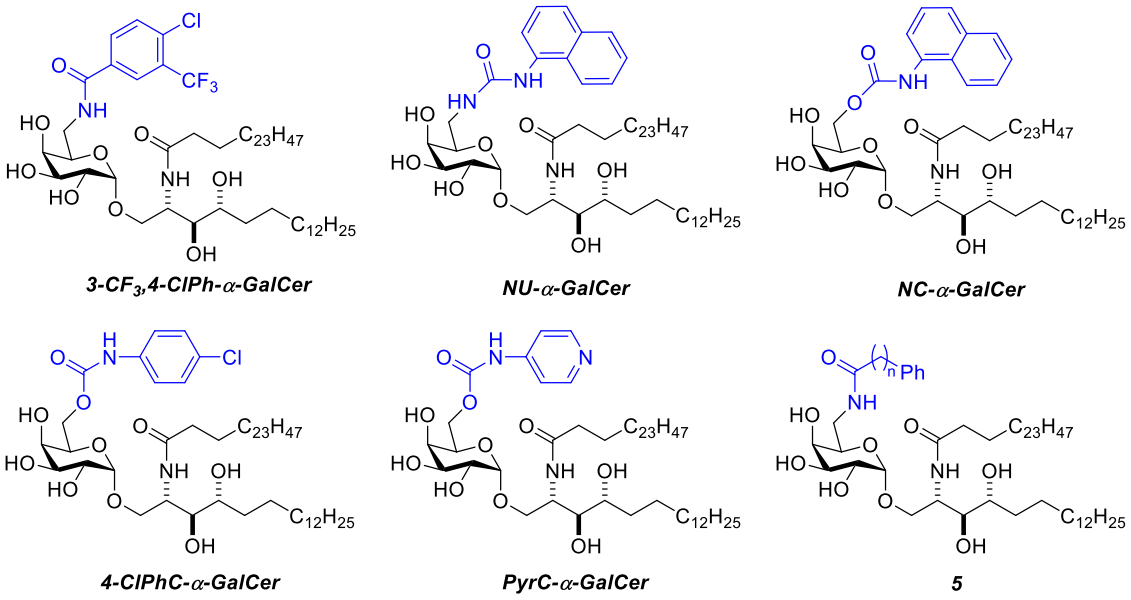


Figure 20. Structures of 6”-α-GalCer analogues.

The C6” position of galactose has also been explored by Besra and coworkers, who designed a series of 1,2,3-triazole moieties (Figure 21).[60] 1,4-Disubstituted and 1,5-disubstituted triazole group are considered as bioisosteres of the amide bond. Using click chemistry, a library of 1,2,3-triazole-α-GalCer analogues was synthesized from a diverse range of aromatic-substituted alkynes. Surprisingly, these 6”-triazole-substituted α-GalCer analogues stimulated both IFN-γ and IL-4 production, which elicited either a Th1 bias similar to KRN7000 or a small Th2 cytokine biasing result. These results demonstrated that subtle structural changes to the 6”-position can impart a significant difference in cytokine-biasing response. Besra and coworkers further explored utilizing click chemistry to synthesize homodimeric α-GalCer analogues (Figure 21) in hopes of mimicking results that multimeric versions of monomeric ligands have higher affinity for target receptors.[61] However, homodimeric analogues of α-GalCer proved to be less active than α-GalCer.

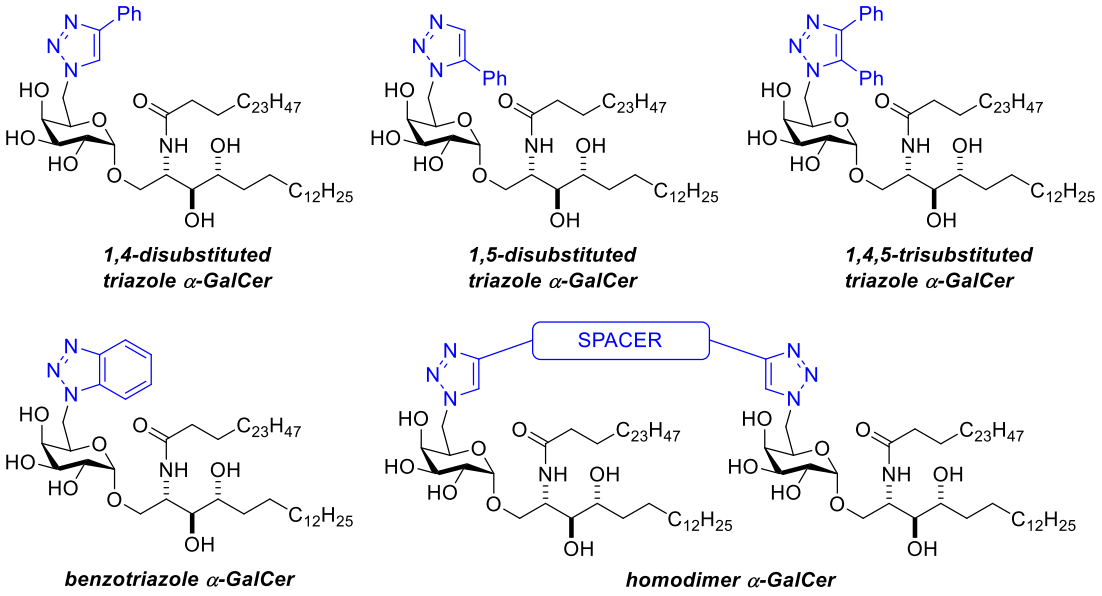
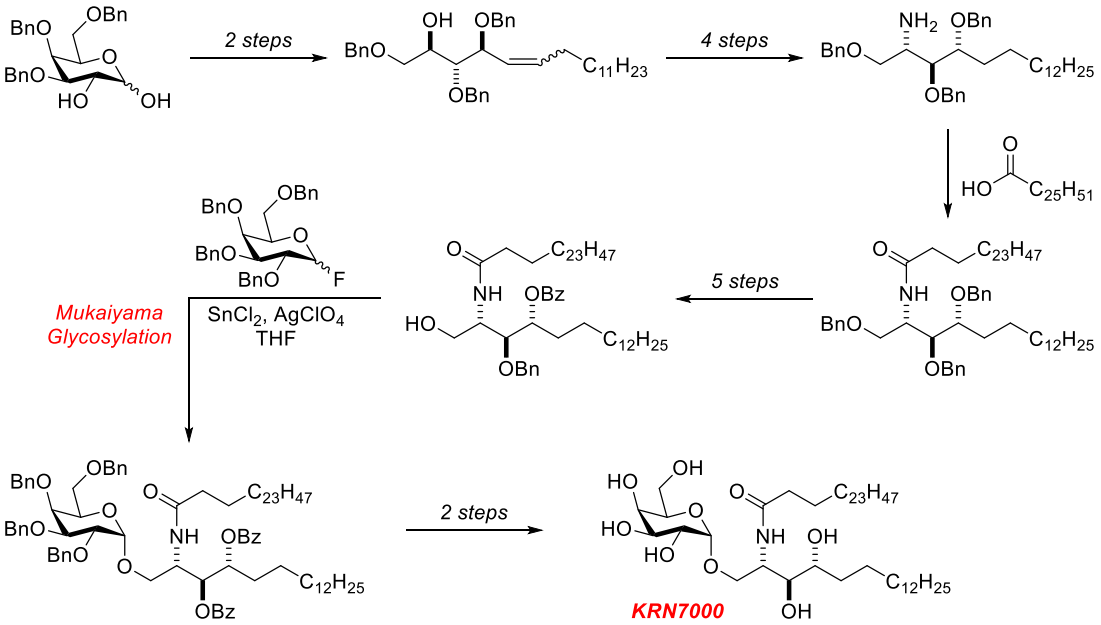


Figure 21. Structures of 6”-trazole α-GalCer analogues.

1.1.5 Synthesis of α-GalCer

Kirin Brewery co. initially isolated several agelasphins from the extracts of the Okinawan marine sponge, *Agelas mauritianus*.[4] The anti-tumor activity of agelaphin-9b prompted Morita and co-workers to synthesize agelaphin-9b.[5] However, due to the minute amounts of agelasphin-9b present in the marine sponge and difficulty in the scale-up, new analogues were probed. This led Morita and co-workers to the first synthesis of KRN7000 and other analogues (Scheme 1).[6] Notably, glycosylation was done under Mukaiyma reaction conditions with predominantly α-selectivity. [62] Since then, many synthetic strategies have been developed to optimize the synthesis of KRN7000 (α-GalCer), including a practical synthesis of it by Kirin Brewery Co.[63]



Scheme 1. Synthesis of KRN7000 (α-GalCer).

α-GalCer and its analogues are generally composed of three fragments, the sugar head group (galactose), the sphingoid base, and the fatty acyl chain. Typically, one of two strategies is implemented when coupling these fragments (Figure 22): (1) incorporation of the acyl chain with the sphingoid base for total functionalization of the ceramide moiety before coupling with the protected sugar; or (2) coupling of the protected sugar with the sphingoid base moiety, followed by *N*-acylation.[19] In the two strategies, the major difficulty in synthesizing KRN7000 and its analogues is the formation of the glycosidic bond with high α-selectivity. The success of the glycosylation depends on many things, including the reactivity of the donor and acceptor (both the sugar and ceramide), the substituents on the donor and acceptor (protecting groups and amide precursor), and the promotor or catalyst.[64] Noteworthy, before the recent commercially availability of D-*ribo*-phytosphingosine, much research focused on constructing the asymmetric centers of the sphingoid base.

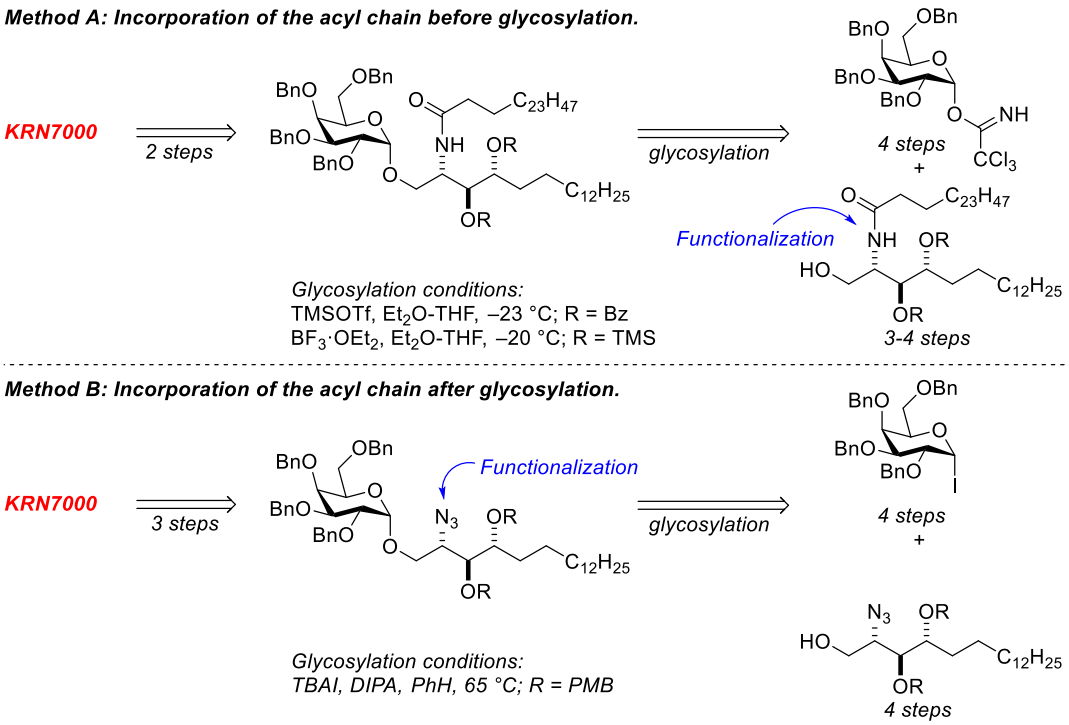


Figure 22. Major pathways for the synthesis of KRN7000.

From a mechanistic viewpoint, the major challenge in the stereochemical control of glycosidic bond formation stems from the formation of an oxocarbenium ion intermediate. Glycosylation reactions typically involve four distinct steps (Figure 23): (1) activation of a glycosyl donor with an activator or promoter (Lewis acid or Bronsted acid); (2) departure of the leaving group results in the formation of a glycosyl cation, which is stabilized as an oxocarbenium ion intermediate; (3) nucleophilic attack by the glycosyl acceptor on the top or the bottom face of the flattened ring; and (4) proton transfer to provide the neutral 1,2-*trans* (β) and/or 1,2-*cis* (α) glycoside(s).[64]

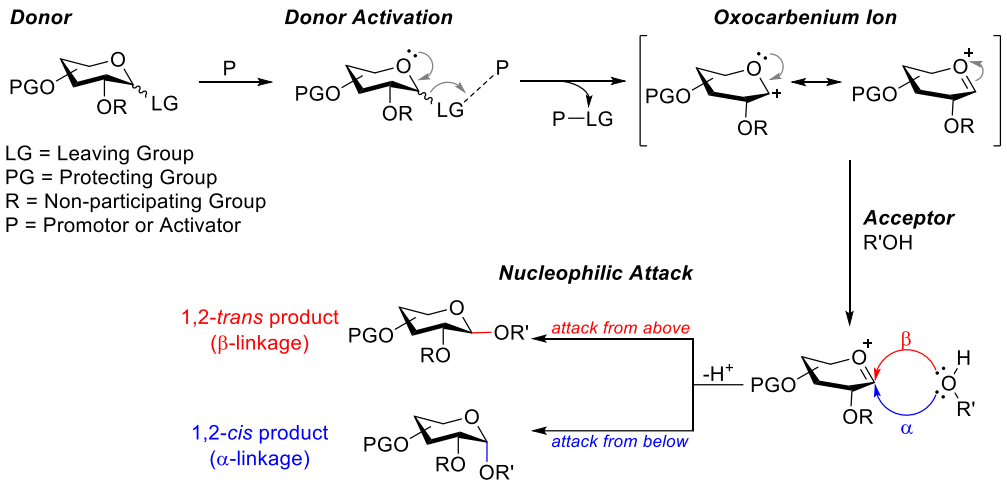


Figure 23. General mechanisms of glycosylation.

The formation of a 1,2-*trans* linkage glycoside can be reliably achieved using the participatory effect of the neighboring 2-acyl substituent (Figure 24). For instance, 2-*O*-acyl protected donors often lead to the formation of 1,2-*trans* glycosides (β-linkage) via the formation of a bicyclic acyloxonium intermedate. Since the bottom face of the ring is blocked, nucleophilic attack from the top face by the glycosyl acceptor would occur. Typically, the use of neighboring group assistance leads to very high or complete stereoselectivity of 1,2-*trans* linkage products. Alternatively, the formation of 1,2-*cis* linkages is typically much more challenging. The use of non-participating groups at C-2 leads to the formation of the oxocarbenium ion, resulting in the formation of 1,2-*cis* and/or 1,2-*trans* glycosides. Nonetheless, biasing of the α-anomeric selectivity has been achieved with appropriate choice of donor, acceptor, promotor/activator, solvent, temperature, and protecting groups.

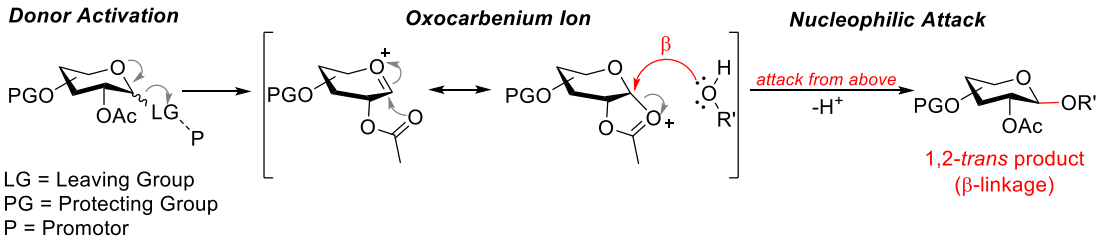
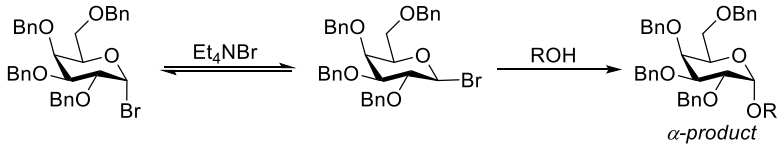


Figure 24. Stereoselective synthesis of 1,2-*trans* linkage via neighboring group assistance.

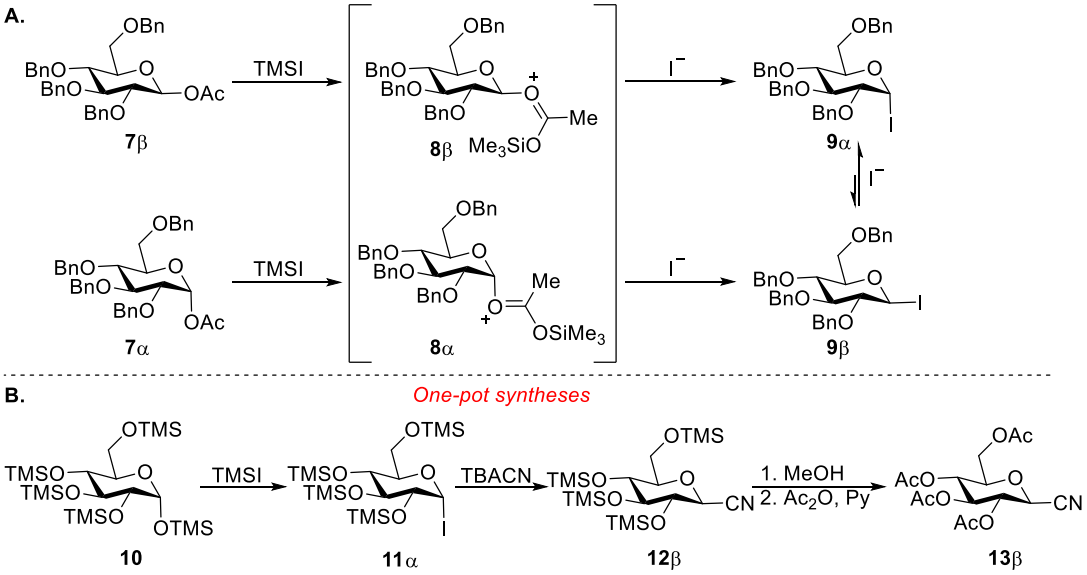
In recent years, glycosyl bromides and especially glycosyl iodides have been applied to *in situ* anomerization for the synthesis of α-glycosides. Lemieux and coworkers discovered that tetra-*O*-benzylated α-glycosyl bromide is in equilibrium with the more reactive β-glycosyl bromide, the equilibrium is catalyzed in the presence of excess bromide ions from tetraalkylammonium bromide (tetraethylammonium bromide), and the reaction proceeds in an S_N2-like manner to afford the α-glycosyl product (Scheme 2).[65] Mechanistic and kinetic studies have been conducted to support an S_N2-type mechanism.[66]



Scheme 2. Lemieux's *in situ* anomerization procedure.

Although the first report of utilizing glycosyl iodides in a glycosylation reaction dates back to 1929, these glycosyl donors have been underutilized in comparison to glycosyl bromides.[67] Glycosyl iodides were avoided due to their high level of reactivity, instability, and difficulties in purification. Recently, Gervay-Hague and co-workers discovered that glycosyl iodides can be utilized in glycosylation reactions with excellent yields and high α-selectivity.[66a,68,69,70] In the late 1990s, Gervay-Hague and co-workers reported the synthesis and mechanistic studies of both α- and β-glycosyl iodides using NMR spectrometry (Scheme 3A).[66] Treatment of α-acetate **7**α with TMSI at –100 °C resulted in the formation of the β-glycosyl iodide (**9**β). However, equilibration to the β-anomer occurs rapidly as the temperature is raised. Formation of α-glycosyl iodide (**9**α) from both α- and β-acetates (**7**α and **7**β) likely occurs via *O*-silylation of the acetyl group to afford the trimethylsilyl acetoxonium ion intermediates (**8**α and **8**β), followed by S_N2-like displacement to afford the glycosyl iodide. This technique is highly useful for the formation of glycosyl iodides because the byproduct, trimethylsilyl acetate, is volatile and easily removed. This methodology was also utilized to generate glycosyl iodides (**11**α) directly from per-*O*-silylated sugar with TMSI

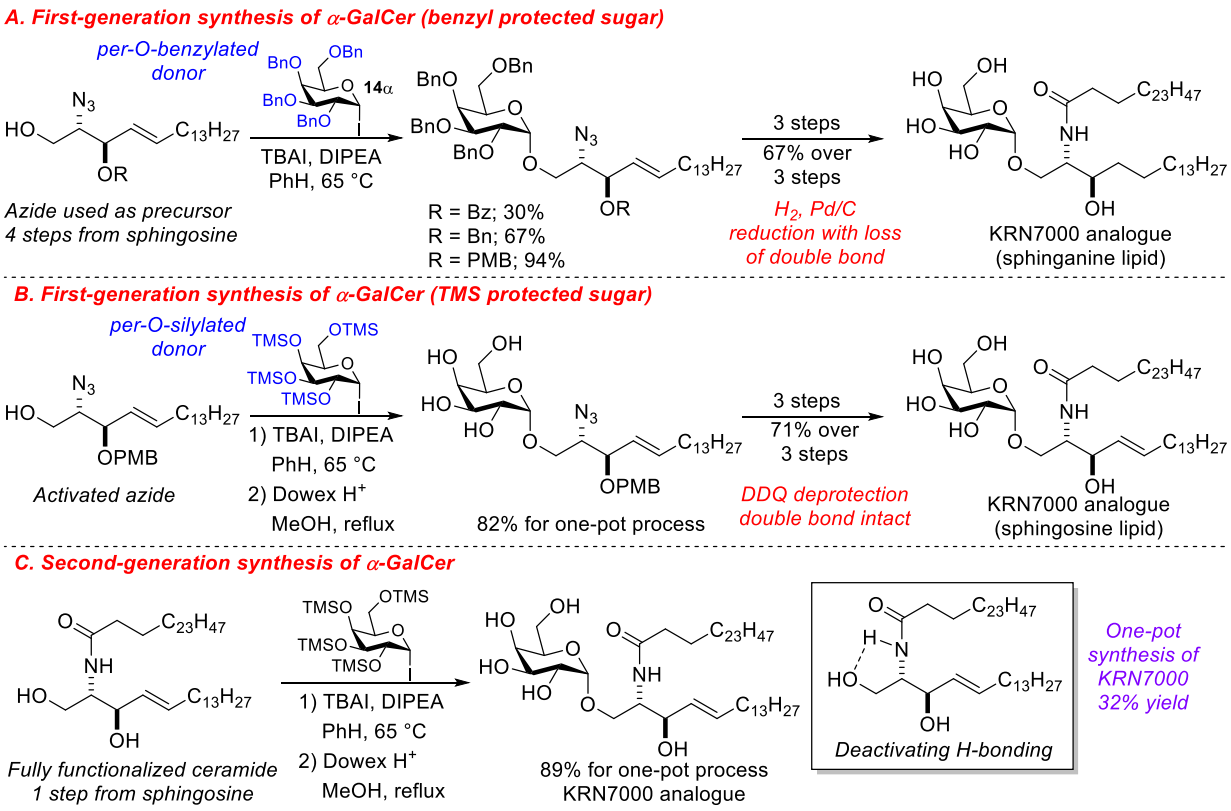
(Scheme 3B).[71] Subsequent treatment with TBACN (CN^-) provided intermediate β-cyanoglucoside (**12**β). The reaction with TMS ether is also advantageous since the byproducts are volatile, and multiple reactions can be carried out in one pot without isolation of intermediates. Thus, per-*O*-silylated β-cyanoglucoside (**12**β) was converted to per-*O*-acetylated β-cyanoglucoside (**13**β) in a one-pot, four-step process.



Scheme 3. (A) Proposed mechanism of glycosyl iodide formation; (B) Synthesis of per-*O*-acetylated β-cyanoglucose in a one-pot, four-step process.

This methodology was effectively used by Gervay-Hague and co-workers for the synthesis of α-GalCer analogues (Scheme 4). Many glycosylation methods involve installation of the amide linkage of the fatty acyl chain after glycosylation due to the poor nucleophilicity of the primary hydroxyl group as a result of intramolecular hydrogen bonding. Thus, azides have proven to be functionally useful amine surrogates without the deactivation of intramolecular hydrogen bonding. Gervay-Hague and co-workers utilized azido sphingosine analogues with per-*O*-benzylgalactosyl iodide (**14**α) in efficient syntheses of α-galactosyl ceramides (Scheme 4A).[68] The stereoselectivity was achieved from the *in situ* anomerization of the α-glycosyl iodide to the more reactive β-anomer by the addition of TBAI, which subsequently undergoes an S_N2-type reaction to

exclusively produce α-glycoside. Correlation between the yields and protecting groups on the secondary alcohol(s) of the sphingoid base was observed. Protecting the secondary hydroxyl groups with electron withdrawing groups, such as benzoates (Bz), gave a 30% yield. Electron donating groups, such as benzyl (Bn) and *p*-methoxybenzyl (PMB), as protecting groups provided higher yields (67% and 94%, respectively). Similarly, changing the protecting groups on the galactose from benzyl to trimethylsilane groups increased the reactivity of the donor. These per-*O*-TMS glycosides are particularly attractive due to their ease of synthesis, scalability, and deprotection under methanolysis conditions. Gervay-Hague and co-workers demonstrated a one-pot process for the synthesis of α-glycosides with removal of the TMS groups (Scheme 4B). [68] Shortly after, Gervay-Hague and co-workers were able to achieve glycosylation between glycosyl iodides with fully functionalized ceramide acceptors (Scheme 4C). [69,70] In a one-pot, three-step process, α-linked glycosyl ceramides were efficiently synthesized in good yields.



Scheme 4. Progress towards a one-pot synthesis of α-linked glycolipids; A) First-generation synthesis with azido sphingosine and per-*O*-benzylated galactosyl iodide; B) Second-generation synthesis with azido sphingosine and per-*O*-silylated galactosyl iodide; C) Second-generation synthesis with fully functionalized ceramide and per-*O*-silylated galactosyl iodide.

1.2 Aims and objectives

It is widely accepted that a stronger glycolipid-CD1d interaction provides a proportionally higher release of Th1-cytokines, which is important in treating infectious diseases and cancer. In an effort to simplify SAR studies, the Howell research group designed and synthesized a sphinganine analogue of KRN7000, AH03-1 (Figure 25).[72] This analogue was able to activate mice NKT cells; in addition, it induced a Th1 biased response. However, it did not show a noticeable effect on the activity of human NKT cells.

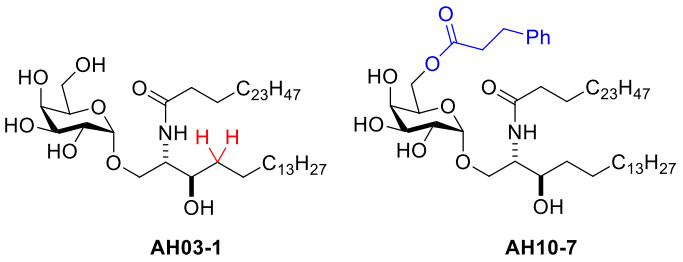


Figure 25. Sphingoid base and C6" modified analogues of α-GalCer.

Many analogues of α-GalCer in which the 6"-hydroxyl(methyl) group is modified have been developed. As mentioned previously PBS-57[55], NU-α-GalCer[58], NC-α-GalCer[59], and PyrC-GalCer[59] showed potent Th1-type immunostimulatory activities. Previous work in the Howell group led to the design and synthesis of a set of carbohydrate-modified analogues of AH03-1 and KRN7000 that incorporated aromatic rings at varying distances from C6" and C4" of the galactose moiety. An initial biological study of these compounds showed that AH10-7 (Figure 25) elicited a strong Th1 bias. Current efforts have centered on further exploration of compounds related to AH10-7. Thus, we decided to synthesize four new target compounds (Figure 26) of different length linkers between a C6" amide bond and the aromatic moiety. These compounds will also provide further information about the role of 4'-OH group on the ceramide for NKT cell activation in mice and human cells. The route towards the synthesis of 6"-*N*-derivatized α-galactosyl ceramides is described.

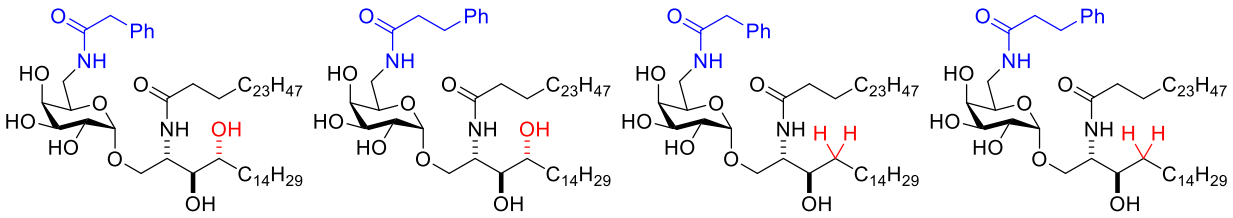


Figure 26. Target molecules **15**-**18**.

1.3 Results and discussion

1.3.1 Synthetic routes to target compounds

In devising a synthetic route to access targets **15**-**18** compounds, many aspects were evaluated. Firstly, the most important aspect in the synthetic strategy is to obtain α-stereoselectivity in the glycosylation process. Stereoselectivity in the anomeric bond formations can effectively be controlled by the choice of glycosyl donor and suitable reaction conditions. Ideally, a short route that involves minimal protecting group manipulations is desired, as protecting group manipulations can factor into poor overall yields. It is also ideal to incorporate the C6"-amide functionality at a later stage to avoid completely repeating the synthetic sequence for each target compound. With this in mind, the retrosynthesis of our target compounds is shown in Figure 27. It was envisioned that the compounds **15**-**18** could be accessed by reduction and acylation of an azide at the C6" of galactose. Key azides **19** could be obtained by global deprotection and acylation of glycosylated sphingoid bases **20**. The fully protected azide **20** could be accessed via a Mitsunobu reaction from the selective monodeprotection of primary hydroxyl **21**. α-Glycosides **21** could be obtained via a Gervay-Hague glycosylation of glycosyl donor **22** and acceptors **23**.

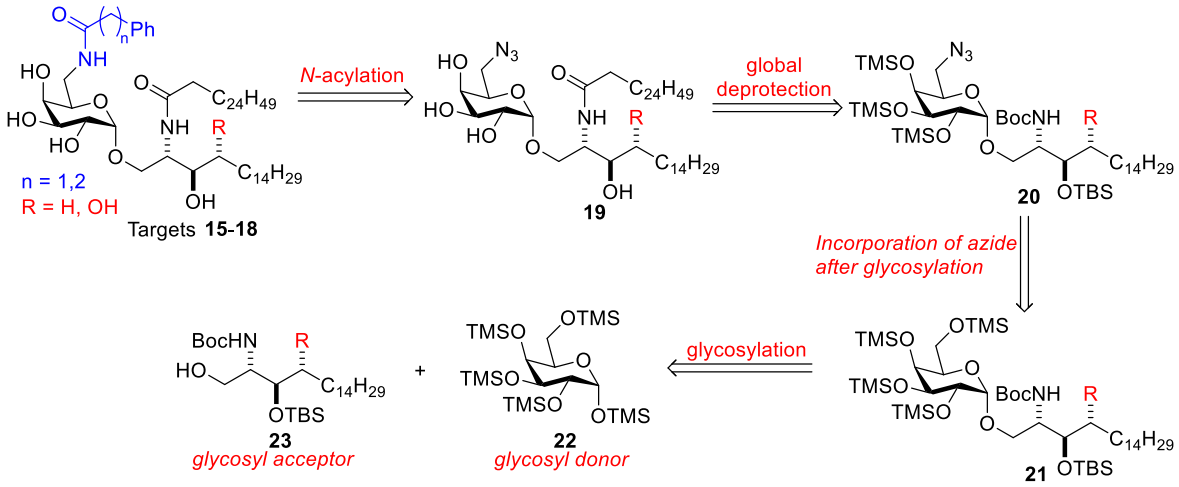


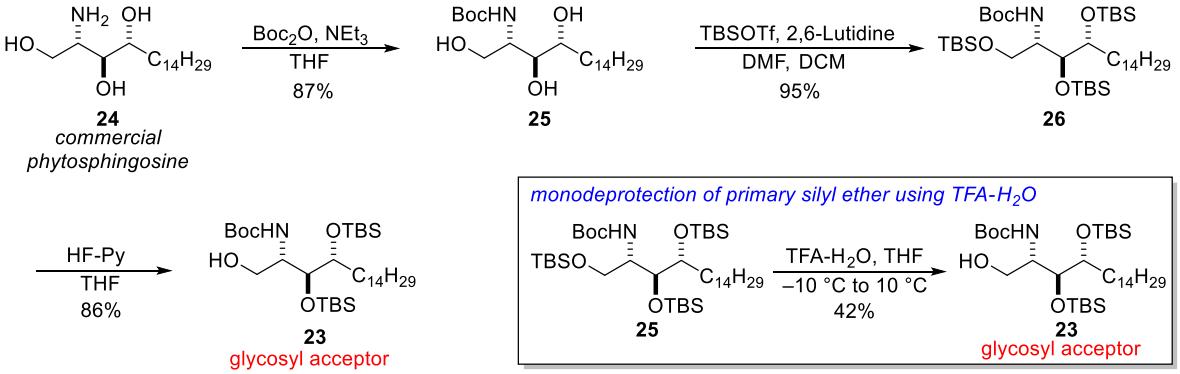
Figure 27. Retrosynthetic strategy for target compounds.

The three protecting groups (TMS, TBS, and Boc) were chosen for many reasons; (1) their ease of attachment and removal; (2) TMS incorporation on the galactose sugar donor increases reactivity in glycosylation reactions; (3) selective monodeprotection of the primary silyl ether; (4) removal of the protecting groups in a one step process; (5) excellent stereoselectivity in glycosylation.

1.3.2 Synthesis of 6"-*N*-derivatized α-galactosyl ceramide

1.3.2.1 Synthesis of glycosyl acceptor

The synthesis of sphingoid base fragment **23** was achieved in three steps using a procedure similar to that developed by Besra and co-workers (Scheme 5).[73] First, commercially available phytosphingosine (**24**) was Boc-protected using di-*tert*-butyldicarbonate to provide **25**, in excellent yields. Next, both the primary and secondary hydroxyl groups were silylated with TBSOTf in the presence of 2,6-lutidine to give silyl ether **26**. Selective monodeprotection of the primary silyl ether using HF-pyridine provided glycosyl acceptor **23** in 86% yield. Noteworthy, monodeprotection of primary silyl ether using TFA-H_2O mixture resulted in complete deprotection of all silyl ethers or much lower yields obtained for the glycosyl acceptor.

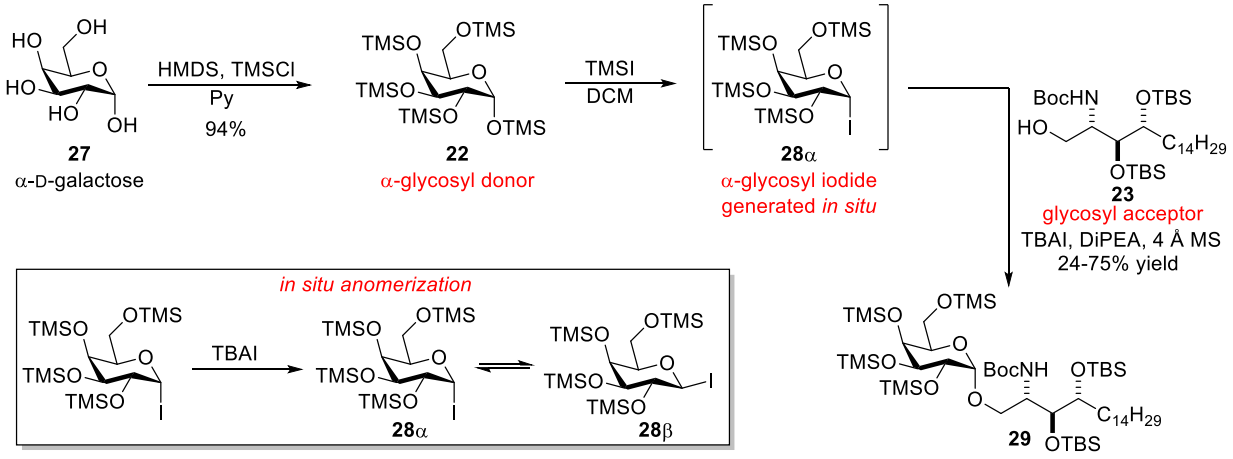


Scheme 5. Synthesis of glycosyl acceptor.

1.3.2.2 Synthesis of glycosyl donor and glycosylation reaction

The glycosyl donor **22** (per-*O*-silylated galactose) was obtained in one step from commercially available D-galactose (**27**) in quantitative yield (Scheme 6). The key step in the

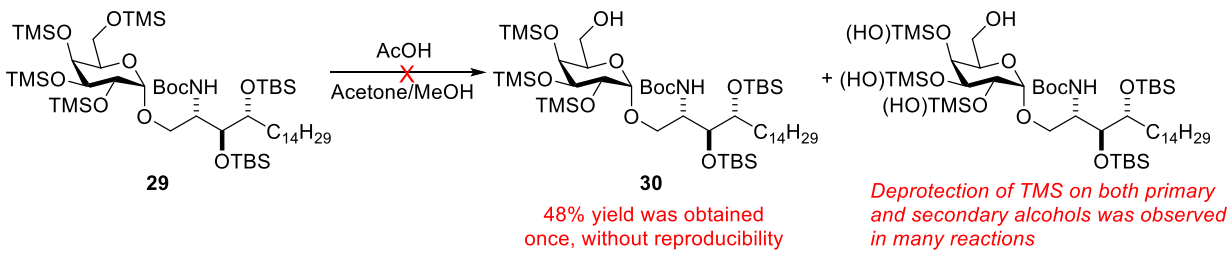
synthesis of the target glycolipids employed Gervay-Hague's glycosylation methodology, in which the glycosyl iodide donor **28**α was generated *in situ* by treating per-*O*-silylated galactose **22** with iodotrimethysilane (TMSI).[68] The stereoselectivity was achieved from the *in situ* anomerization of the α-glycosyl iodide to the more reactive β-anomer (**28**β) by the addition of TBAI, which subsequently undergoes S_N2-type reaction with glycosyl acceptor **23** to provide high α-selectivity of glycoside **29** in up to 75% yield.



Scheme 6. Synthesis of glycosyl donor **22** and α-glycoside **29**.

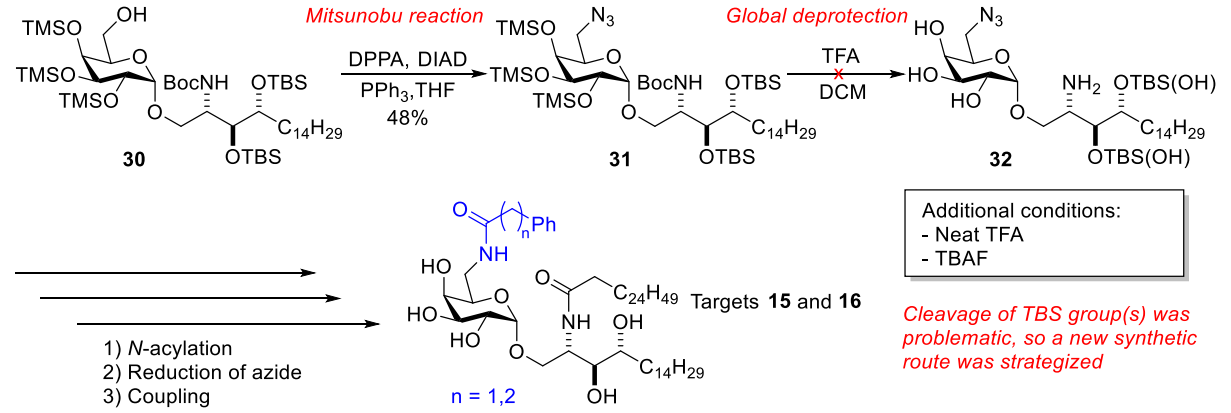
1.3.2.3 Synthesis of 6"-*N*-derivatized α-galactosyl ceramide targets 15-18

With α-glycoside **29** in hand, attempts were made to perform the selective monodeprotection of the primary silyl ether group. To this end, following the literature procedure of Besra and co-workers, treatment of α-glycoside **29** with acetic acid in acetone/methanol was not a success (Scheme 7).[73] Many reactions gave deprotection of TMS groups from both primary and secondary alcohols. Reaction conditions such as concentration, equivalents of acetic acid, temperature, and time were probed without formation of the product **30**. However, one reaction gave the desired compound in 48% yield without reproducibility.



Scheme 7. Selective monodeprotection of the primary silyl ether group of α-glycoside **30**.

With the small amount of product acquired, alcohol **30** was converted to azide **31** (Scheme 8) by a Mitsunobu reaction using diphenylphosphoryl azide (DPPA), triphenylphosphine (PPh_3), and diisopropyl azodicarboxylate (DIAD).[74] Azide **31** was then treated with 10% trifluoroacetic acid (TFA) in dichloromethane (DCM) overnight to cleave the Boc, TMS, and TBS-groups in one step. The Boc and TMS groups were successfully removed, although surprisingly, the TBS group(s) on the secondary alcohol was not removed. Additional attempts to cleave the TBS group(s) with neat TFA or TBAF were unsuccessful. Due to the challenges encountered in selective monodeprotection and the global deprotection steps, a new synthetic route was strategized.



Scheme 8. Attempted synthesis azido α-glycoside **32**.

1.3.3 Synthesis of 6"-*N*-derivatized α-galactosyl ceramide (route 2)

Our new retrosynthetic analysis for the preparation of the four target α-GalCer analogues is presented in Figure 28. Using a modified procedure from Van Calenbergh and co-workers, we

planned to prepare targets **15-18** from the benzyl ether protected 6”-azido-α-GalCer analogue **33**.[57] We chose to protect the secondary hydroxyl groups of the α-GalCer as benzyl ethers to streamline the final deprotection. Furthermore, to avoid solubility problems, the final debenzylation step was performed after modification of the 6”-amino group. The amide functionality could be obtained by reduction of azide **33**, follow by EDC coupling of the amine with the appropriate acid. The fully protected glycolipid **33** could be obtained using a Mukaiyama glycosylation of sugar donor **34** with ceramides **35** or **36**. By incorporating the acyl chain to the sphingoid base and having the amine functionality on the sugar masked as an azide, minimal transformations were required after glycosylation. In addition, sugar donor **34** and ceramides **35** and **36** could be obtained from readily available starting materials.

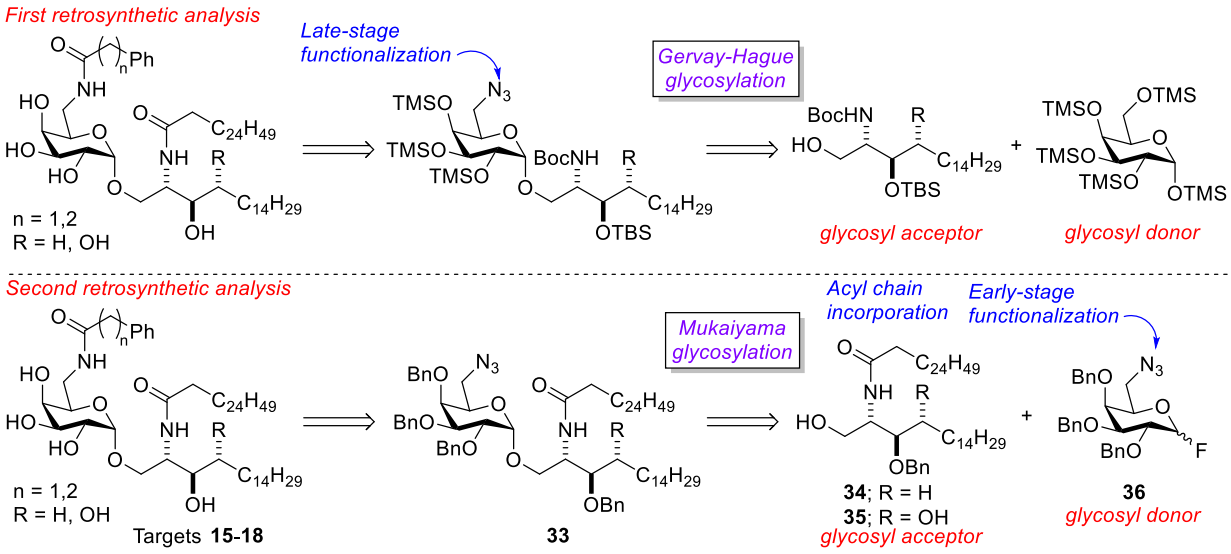
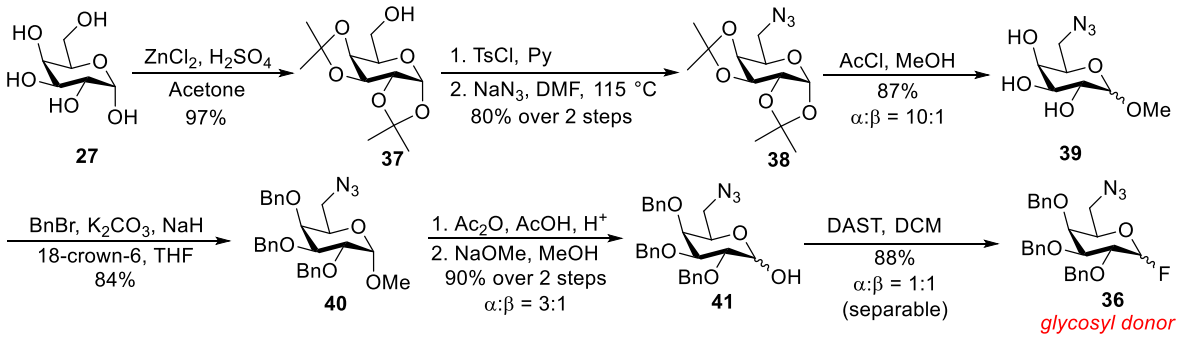


Figure 28. New retrosynthetic strategy for target compounds.

1.3.3.1 Synthesis of sugar donor

With our synthetic strategy in place, the first goal was the preparation of the azido galactose donor.[7] The synthesis of the galactose donor was accomplished in accordance with published methods (Scheme 9).[75] Compound **37** was formed in an excellent yield by the selective installation of isopropylidenes at the 1,2 and 3,4 positions of commercially available D-galactose

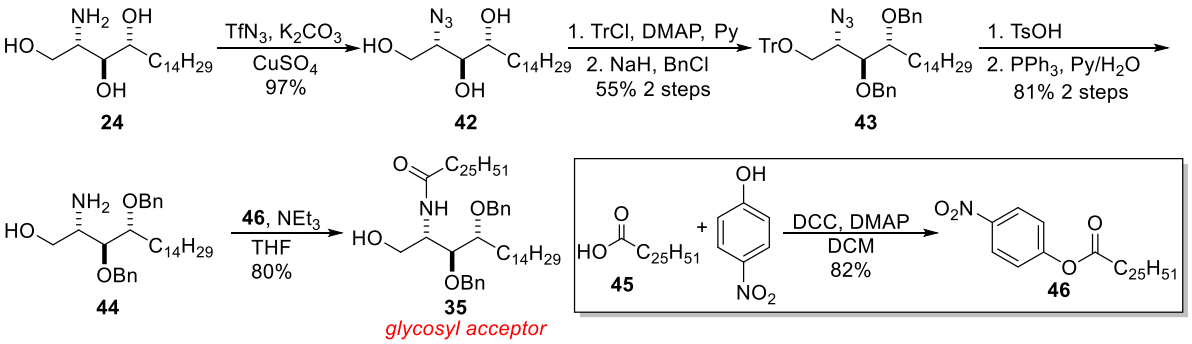
(**27**). Selective installation of the tosylate at the primary position, followed by azide substitution, gave compound **38**. Next, simultaneous cleavage of the isopropylidenes and Fisher glycosylation using a 5% solution of AcCl in MeOH furnished compound **39** in 87% yield over three steps. Subsequent treatment with NaH, followed by the addition of benzyl bromide, gave compound **40**. Acetolysis of **40**, followed by Zémplen deacetylation, gave hemiacetal **41**, which was then transformed into fluorides **36** using diethylaminosulfurtrifluoride (DAST).



Scheme 9. Synthesis of glycosyl donor **36**.

1.3.3.2 Synthesis of glycosyl acceptor for targets 15 and 16

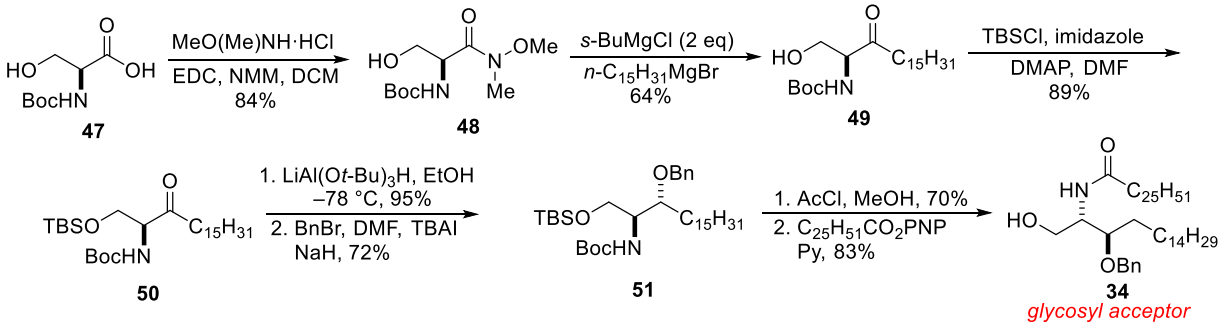
The key phytosphingosine ceramide acceptor **35** (Scheme 10) for glycosylation was prepared by use of a method similar to Schmidt's,[76] with modifications. The amino group of commercially available phytosphingosine **24** was converted into an azido group (**42**) in a metal-catalyzed diazotransfer reaction with freshly prepared TfN_3.[77] Selective tritylation of the primary alcohol, followed by benzylation of the two secondary alcohols with sodium hydride and benzyl chloride, gave **43**. Deprotection of the primary alcohol was achieved using TsOH, and reduction of the azide under Staudinger conditions gave amino alcohol **44**. Lastly, acylation of the free amine with activated hexacosanic acid (**45**) furnished glycosyl acceptor **46**. The activated hexacosanic acid (**45**) was accessed in one step via DCC coupling of commercially available cerotic acid (**46**) with *para*-nitrophenol.



Scheme 10. Synthesis of glycosyl acceptor **35**.

1.3.3.3 Synthesis of sphingoid base acceptor for targets 17 and 18

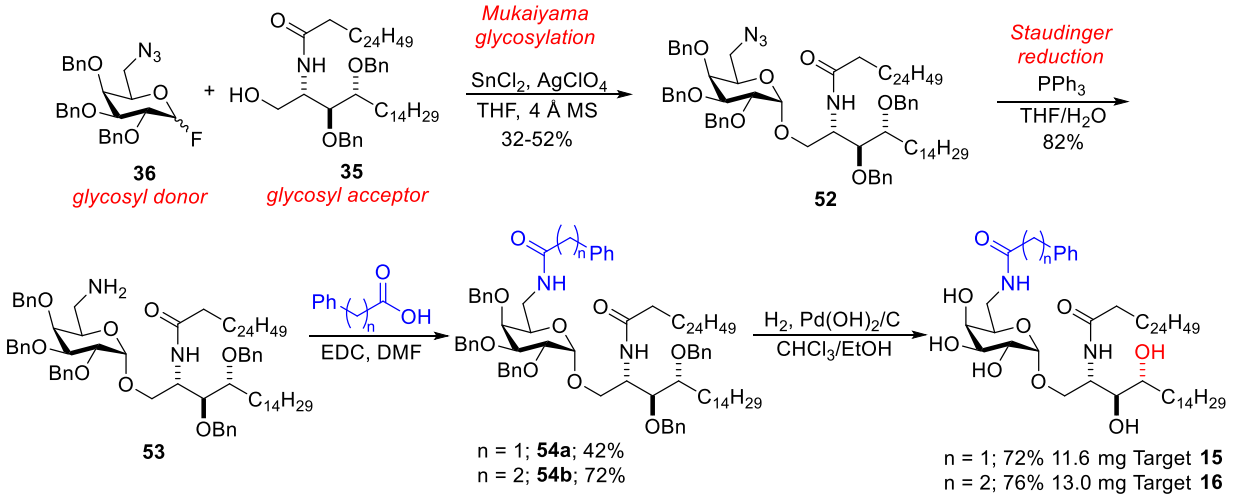
Sphinganine ceramide **34** was synthesized using a well-developed protocol from the Howell group starting from commercially available Boc-L-serine **47** (Scheme 11).[72] The serine-derived Weinreb amide **48** was reacted with isopropylmagnesium bromide (sacrificial base), followed by the nucleophilic addition of pentadecyl magnesium bromide, to give moderate yield of ketone **49**. Silyl protection of the primary hydroxyl group gave compound **50** in 89% yield. Diastereoselective reduction of ketone **50** using lithium tri-*tert*-butoxyaluminum hydride, followed by benzyl protection of the secondary alcohol, furnished **51** in good yield. Deprotection of the silyl and Boc groups was accomplished in one step by *in situ* generation of HCl using acetyl chloride and methanol; the resulting free amine underwent acylation with an activated ester of cerotic acid to give ceramide **34**.



Scheme 11. Synthesis of sphingoid base glycosyl acceptor **34**.

1.3.3.4 Synthesis of targets 15 and 16

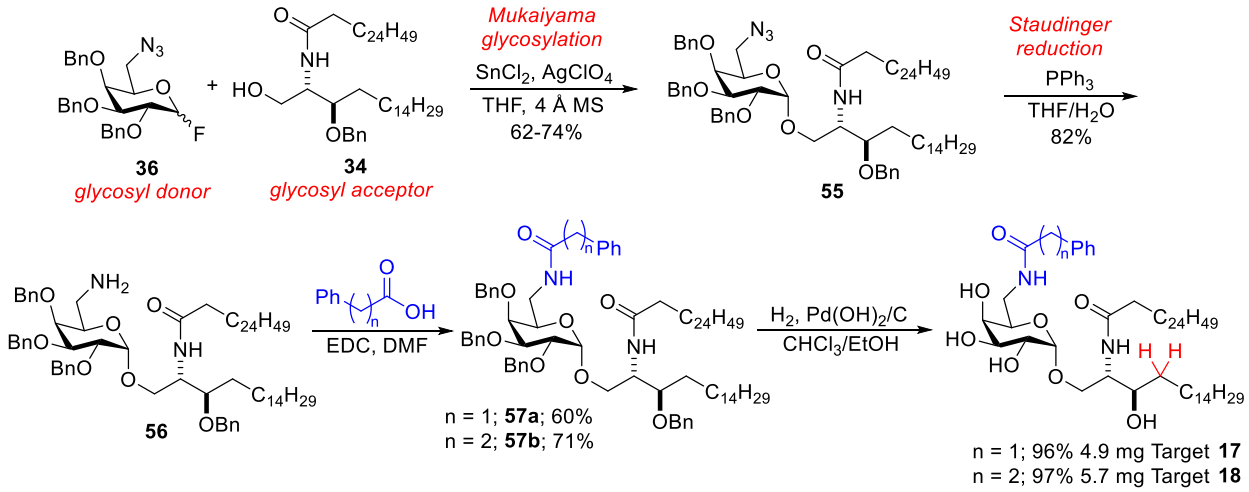
Glycosylation of donor **36**β and acceptor **35** was achieved via Mukaiyama glycosylation,[78] using $SnCl_2/AgClO_4$ as the activator in anhydrous THF with 4 Å MS, to afford the desired α-glycoside **52** with an α/β ratio of 4/1 in 52% yield (Scheme 12). Glycosylation using the α-anomer of the glycosyl donor (**36**α) gave the desired α-glycoside (**52**) with similar selectivity, but a lower yield at 32%. Staudinger reduction of azide **52** provided the free 6"-amino-group (**53**) in good yield. Next, coupling of amine **53** with the appropriate acid and EDC as the coupling reagent provided amides **54a** and **54b**. Removal of the benzyl groups in compounds **54a** and **54b** by hydrogenolysis gave the desired amide targets **15** and **16**.



Scheme 12. Synthesis of α-GalCer analogues targets **15** and **16**.

1.3.3.5 Synthesis of targets 17 and 18

Glycosylation was performed on donor **36** and ceramide acceptor **34** using the same conditions as those in Sec 1.3.3.4 (Scheme 13). In this case, the glycosylation yields were improved to 62-72% of the desired α-glycoside (**55**). Reduction of the azide (**55**), followed by coupling with the appropriate acid and removal of the benzyl groups, gave targets **17** and **18**.



Scheme 13. Synthesis of α-GalCer analogues targets **17** and **18**.

1.3.4 Biological testing

The evaluation of the target analogues **15**-**18** is currently under way by our collaborators Dr. Weiming Yuan from Keck School of Medicine of USC, CA and Dr. Steven A. Porcelli Albert Einstein College of Medicine, NY. Preliminary *in vitro* assay in mice for their ability to stimulate NKT cells as measured by IL-2 production demonstrated that all four compounds (**15**-**18**) were significantly active. Interestingly, compounds **17** and **18** which lack the 4'-OH seem to be more potent. Additionally, *in vitro* assay in human cell lines showed the ability of compounds **17** and **18** to stimulate IFN-γ production. The immunostimulatory studies of these analogues (**15**-**18**) as well as related compounds is currently under investigation by our collaborators.

1.4 Conclusion

The synthesis of the four target compounds **15**-**18** proved to be less straightforward than initially expected due to several difficulties encountered during the first synthetic route. Nonetheless, an alternative route was devised and target compounds **15**-**18** were obtained from commercially available starting materials. The key glycosylation step of the second route was accomplished utilizing a Mukaiyama glycosylation method with high α-selectivity. The incorporation of the C6" amide functionally in a later stage avoided the need to repeat the synthetic

sequence for each target compound. The preliminary biological evaluation of these four glycolipid analogues in collaboration with Dr. Steven A. Porcelli and Dr. Weiming Yuan found that these compounds were able to stimulate cytokine production of IL-2 and IFN-γ. Further *in vitro* and *in vivo* studies will provide a better understanding for the nature of the immune response elicited by these compounds.

1.5 Experimental

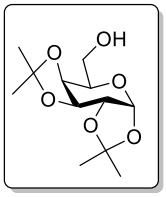
1.5.1 General experimental

All moisture sensitive reactions were run in a flame-dried flask under an atmosphere of N_2. Methylene chloride (DCM), dimethylforamide (DMF), toluene, and pyridine were dried over CaH_2 or over 4 Å molecular sieves (MS). Tetrahydrofuran (THF) was dried using a J. C. Meyer Solvent Dispensing System (SDS). Deuterated chloroform was dried over 4 Å MS. Zinc(II) chloride was freshly fused prior to use. Commercially available reagents were purchased from Acros, Aldrich, Alfa Aesar, or TCI America and were purified as necessary.

Flash column chromatography was performed using silica gel, 40 microns flash silica. Silica gel was deactivated with trimethylamine when necessary. Thin layer chromatography was carried out with silica gel (Silica Gel 60 F_{254}) glass plates, and compounds were visualized by UV (254 nm), 0.5% $KMnO_4$ in 0.1 M aqueous NaOH solution, 5% phosphomolybdic acid in EtOH, or a 2.5% solution of *p*-anisaldehyde in 95% EtOH. ^{1}H NMR spectra were recorded on a Bruker AVANCE 300, 400, or 500 MHz spectrometer. ^{13}C NMR spectra were recorded on a Bruker AVANCE 75, 100, or 125 MHz spectrometer. ^{1}H NMR chemical shifts are reported as δ values in ppm calibrated to residual $CHCl_3$ (7.26 ppm) or pyridine-d_5 (8.71, 7.56 and 7.18 ppm). ^{1}H NMR coupling constants (*J*) are reported in Hertz (Hz). ^{13}C NMR chemical shifts are calibrated to the $CDCl_3$ peak at 77.23 ppm or pyridine-d_5 at 149.9, 135.5 and 123.5 ppm. Infrared spectra were obtained on a FTIR spectrometer. High-resolution mass spectra (HRMS) were obtained using

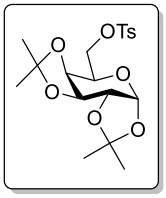
DART AccuTOF or JEOL JMS-AX505HA mass spectometers. Optical rotations were recorded on JASCO P-2000 polarimeter in a 5 mL cell.

1,2:3,4-Di-*O*-isopropylidene-α-D-galactopyranose (37)



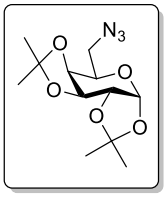
D-Galactose (5.00 g, 27.7 mmol) was dissolved in dry acetone (125 mL), followed by the addition of anhydrous zinc chloride (3.78 g, 27.7 mmol) and concentrated H_2SO_4 (0.50 mL). The mixture was stirred at rt for 20 h. Then potassium carbonate (1.0 g) was added, and the resultant mixture was stirred for 10 min. The reaction mixture was filtered through a pad of celite and the filter cake washed with acetone (2 × 200 mL). The filtrate and washings were concentrated to afford 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**37**) as a colorless syrup (7.01 g, 97%):[79] $[\alpha]_D^{23}$ = –56.9 (*c* = 1.0, $CHCl_3$); IR (neat) 3441, 2930, 1451, 1375, 1209 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 5.57 (d, *J* = 5.1 Hz, 1H), 4.62 (dd, *J* = 8.0, 2.4 Hz, 1H), 4.34 (dd, *J* = 5.1, 2.4 Hz, 1H), 4.28 (d, *J* = 8.0 Hz, 1H), 3.92–3.71 (m, 2H), 2.45 (dd, *J* = 10.2, 3.0 Hz, 1H), 2.31 (br s, 1H), 1.54 (s, 3H), 1.46 (s, 3H), 1.34 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 109.3, 108.6, 92.1, 71.5, 70.6, 70.4, 68.1, 62.2, 26.0, 25.9, 24.9, 24.1; HRMS (ESI) *m/z* calcd for $C_{12}H_{20}NaO_6$ $[M + Na]^+$ 283.1157, found 283.0957.

1,2:3,4-Di-*O*-isopropylidene-6-*O*-*p*-toluenesulfonyl-6-α-D-galactopyranose (38a)



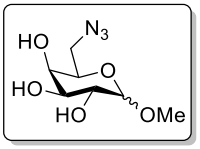
1,2:3,4-Di-*O*-isopropylidene-α-D-galactopyranose (**37**) (3.10 g, 11.9 mmol) was dissolved in dry pyridine (25 mL), and the solution was cooled to 0 °C. Then *p*-toluenesulfonyl chloride (2.72 g, 14.2 mmol) was added in 3 portions over 20 min. The solution was then allowed to warm to rt. After 20 h, the solution was poured into an ice-water mixture (20 g). The solution was extracted with $CHCl_3$ (3 × 25 mL), washed with H_2O (2 × 20 mL) and dried ($MgSO_4$). Evaporation of the solvent gave a black syrup which crystallized from diethyl ether-petroleum ether (1:1) to afford 1,2:3,4-di-*O*-isopropylidene-6-*O*-*p*-toluenesulfonyl-α-D-galactopyranose (**38a**) as a white solid (4.34 g, 88%):[80] mp = 90–92 °C; $[\alpha]_D^{23}$ = –67.0 (*c* = 1.0, $CHCl_3$); IR (neat) 2993, 2958, 2936, 1359, 1212, 1119, 1067, 956 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, *J* = 8.3 Hz, 2H), 7.33 (d, *J* = 8.1 Hz, 2H), 5.45 (d, *J* = 5.0 Hz, 1H), 4.58 (dd, *J* = 7.9, 2.5 Hz, 1H), 4.29 (dd, *J* = 5.0, 2.5 Hz, 1H), 4.22–4.18 (m, 2H), 4.11–4.02 (m, 2H), 2.44 (s, 3H), 1.50 (s, 3H), 1.34 (s, 3H), 1.31 (s, 3H), 1.28 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.9, 133.1, 130.0, 128.3, 109.8, 109.2, 96.3, 70.8, 70.6, 70.6, 68.4, 66.1, 26.2, 26.0, 25.1, 24.6, 21.8; HRMS (ESI) *m/z* calcd for $C_{19}H_{27}O_8S$ $[M + H]^+$ 415.1427, found 415.1424.

6-Azido-6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (38)



Sodium azide (0.91 g, 13.9 mmol) was added to a solution of 1,2:3,4-di-*O*-isopropylidene-6-*O*-*p*-toluenesulfonyl-α-D-galactopyranose (**38a**) (4.43 g, 10.7 mmol) in dry DMF (78 mL). The mixture was heated at 130 °C for 48 h. The mixture was cooled to rt and filtered. The filtrate was concentrated to 30 mL, and H_2O (35 mL) was added. The solution was extracted with $CHCl_3$ (4 × 40 mL), and the combined extracts were dried (Na_2SO_4) and concentrated to give a syrup. Purification by flash chromatography on silica gel (petroleum ether/EtOAc, 80:20) afforded 6-azido-6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**38**) as a pale yellow oil (2.77 g, 91%):[80] $[\alpha]_D^{23}$ = –95.1 (*c* = 1.0, $CHCl_3$); IR (neat) 2988, 2936, 2099, 1210, 1065, 1004 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.52 (d, *J* = 5.1 Hz, 1H), 4.60 (dd, *J* = 7.8, 2.4 Hz, 1H), 4.29 (dd, *J* = 4.8, 2.4 Hz, 1H), 4.15 (dd, *J* = 7.8, 2.0 Hz, 1H), 3.88 (ddd, *J* = 7.8, 5.4, 1.9 Hz, 1H), 3.47 (dd, *J* = 12.8, 7.8 Hz, 1H), 3.32 (dd, *J* = 12.7, 5.4 Hz, 1H), 1.51 (s, 3H), 1.42 (s, 3H), 1.30 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 109.7, 108.9, 96.5, 71.3, 70.9, 70.5, 67.1, 50.8, 26.1, 26.1, 25.0, 24.5; HRMS (ESI) *m/z* calcd for $C_{12}H_{20}N_3O_5$ $[M + H]^+$ 286.1403, found 286.1411.

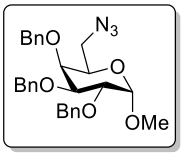
6-Azido-6-deoxy-1-*O*-methyl-α-D-galactopyranoside (39)



6-Azido-6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**38**) (4.30 g, 15.1 mmol) was dissolved in MeOH (45 mL) and cooled to 0 °C. Acetyl chloride (10.2 mL, 143 mmol) was added to the solution dropwise, which was allowed to warm to rt. After 12 h, the solution was

concentrated, and the residue was purified by flash chromatography on silica gel (DCM/MeOH, 90:10), yielding 6-azido-6-deoxy-1-*O*-methyl-α-D-galactopyranoside (**39**) (about 9% β-isomer) as a white solid (2.87 g, 87%):[49] mp = 172.4–173.1 °C; IR (neat) 3371, 3237, 2933, 2092, 1641, 1460, 1349, 1297 cm^{-1}; ^1H NMR (400 MHz, pyridine-d_5) δ 6.56 (br s, 3H), 5.18 (d, *J* = 3.7 Hz, 1H), 4.60 (dd, *J* = 10.1, 3.7 Hz, 1H), 4.42 (dd, *J* = 10.1, 3.3 Hz, 1H), 4.30 (m, 1H), 4.21 (dd, *J* = 3.3, 1.1 Hz, 1H), 4.02 (dd, *J* = 12.8, 8.8 Hz, 1H), 3.58 (dd, *J* = 12.8, 4.0 Hz, 1H), 3.48 (s, 3H); ^{13}C NMR (100 MHz, pyridine-d_5) δ 102.3, 71.8, 71.4, 71.4, 70.6, 55.8, 53.0; HRMS (ESI) *m/z* calcd for $C_7H_{14}N_3O_5$ $[M + H]^+$ 220.0934, found 220.0954.

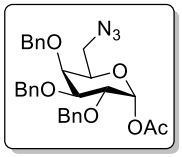
6-Azido-2,3,4-tri-*O*-benzyl-6-deoxy-1-*O*-methyl-α-D-galactopyranoside (40)



6-Azido-6-deoxy-1-*O*-methyl-α/β-D-galactopyranoside (**39**) (1.07 g, 4.88 mmol, α/β ratio 10:1) was dissolved in dry THF (65 mL), followed by the addition of benzyl bromide (3.48 mL, 29.3 mmol), K_2CO_3 (5.39 g, 39.1 mmol) and 18-crown-6 (90 mg, 0.07 mmol). The suspension was stirred for 15 min, and NaH (60% in mineral oil, 1.46 g, 36.6 mmol) was added. After 12 h, brine (50 mL) was added and the solution was extracted with Et_2O (3 × 50 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc, 80:20) afforded 6-azido-2,3,4-tri-*O*-benzyl-6-deoxy-1-*O*-methyl-α-D-galactopyranoside (**40**) as a colorless oil (2.01 g, 84%):[49] $[\alpha]_D^{23}$ = +4.8 (*c* = 1.0, $CHCl_3$); IR (neat) 3063, 3030, 2905, 2097, 1092, 1042, 1027, 734, 696 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.46–7.29 (m, 15H), 5.04 (d, *J* = 11.4 Hz, 1H), 4.93 (d, *J* = 11.8 Hz, 1H), 4.85 (d, *J* = 12.0 Hz, 1H), 4.79 (d, *J* = 11.9 Hz, 1H), 4.77 (d, *J* = 3.6 Hz, 1H), 4.72 (d, *J* = 12.0 Hz, 1H), 4.15–3.78 (m, 4H), 3.60–3.53 (m, 1H), 3.43 (s, 3H), 2.97 (dd, *J* = 12.6, 4.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.5, 138.2, 138.0, 128.2, 128.1, 127.8, 127.6, 127.5, 127.4, 127.3, 127.3, 98.5, 78.6, 76.1, 75.2, 74.4,

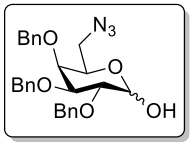
73.3, 73.2, 69.6, 55.1, 51.2; HRMS (ESI) *m/z* calcd for $C_{28}H_{31}N_3NaO_5$ $[M + Na]^+$ 512.2161, found 512.2179.

1-*O*-Acetyl-6-azido-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-galactopyranoside (41a)



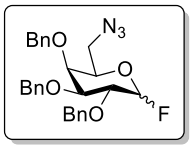
6-Azido-2,3,4-tri-*O*-benzyl-6-deoxy-1-*O*-methyl-α-D-galactopyranoside (**40**) (505 mg, 1.03 mmol) was dissolved in acetic acid (0.42 mL) and cooled to 0 °C. The suspension was stirred for 10 min, and acetic anhydride (0.56 mL, 5.98 mmol) was added, followed by the addition of concentrated H_2SO_4 (9 µL). The mixture was stirred at 0 °C for 8 h, followed by the addition of ice cold H_2O (10 mL). The mixture was extracted with DCM (4 × 15 mL), dried (Na_2SO_4), and concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc, 95:5) afforded 1-*O*-acetyl-6-azido-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-galactopyranoside (**41a**) as a colorless oil (427 mg, 80%):[81] $[\alpha]_D^{23}$ = +44.4 (*c* = 1.0, $CHCl_3$); IR (neat) 3063, 3031, 2874, 2102, 1749, 1227, 1126, 1104, 737, 698 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.35–7.28 (m, 15H), 6.40 (d, *J* = 3.9 Hz, 1H), 5.03 (d, *J* = 11.4 Hz, 1H), 4.90 (d, *J* = 11.7 Hz, 1H), 4.78 (d, *J* = 12.0 Hz, 1H), 4.72 (s, 2H), 4.61 (d, *J* = 11.4 Hz, 1H), 4.21–4.16 (m, 1H), 3.94–3.90 (m, 3H), 3.49 (dd, *J* = 12.3, 7.2 Hz, 1H), 3.17 (dd, *J* = 12.3, 6.3 Hz, 1H), 2.14 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.3, 138.3, 137.9, 137.8, 128.2, 128.1, 128.0, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 90.3, 78.3, 74.8, 74.6, 74.5, 73.0, 72.9, 71.6, 50.6, 20.6; HRMS (ESI) *m/z* calcd for $C_{29}H_{31}N_3NaO_6$ $[M + Na]^+$ 540.2111, found 540.2101.

6-Azido-2,3,4-tri-*O*-benzyl-6-deoxy-α/β-D-galactopyranoside (41)



6-Azido-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-galactopyranoside (**41a**) (377 mg, 0.728 mmol) was dissolved in dry MeOH (3 mL). Then sodium methoxide solution in MeOH (25 wt. %, 0.10 mL) was added dropwise, and the solution was stirred at rt for 5 min. The mixture was neutralized with 1.0 M HCl and extracted with $CHCl_3$ (4 × 5 mL). The combined extracts were washed with water (2 × 5 mL), dried (Na_2SO_4), and concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc, 85:15 to 70:30) afforded 6-azido-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-galactopyranoside (**41**) (mixture of anomers, α/β ratio 3:1) as a colorless oil (329 mg, 96%):[81] α-isomer: $[\alpha]_D^{23}$ = +8.8 (*c* = 1.0, $CHCl_3$); IR (neat) 3421 , 3030, 2924, 2102, 1454, 1091, 1063, 1028, 737, 698 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.42–7.29 (m, 15H), 5.29 (d, *J* = 3.5 Hz, 1H), 5.00 (d, *J* = 11.4 Hz, 1H), 4.85 (d, *J* = 11.8 Hz, 1H), 4.84 (d, *J* = 11.7 Hz, 1H), 4.81 (d, *J* = 11.2 Hz, 1H), 4.72 (d, *J* = 11.7 Hz, 1H), 4.61 (d, *J* = 11.4 Hz, 1H), 4.07–4.03 (m, 2H), 3.94 (dd, *J* = 7.7, 2.7 Hz, 1H), 3.85 (br s, 1H), 3.50 (dd, *J* = 12.4, 7.5 Hz, 1H), 3.11 (dd, *J* = 12.5, 4.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.6, 138.3, 138.2, 128.7, 128.6, 128.4, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 92.0, 78.7, 76.6, 74.8, 74.7, 73.8, 73.4, 69.9, 51.3; HRMS (ESI) *m/z* calcd for $C_{27}H_{29}N_3NaO_5$ $[M + Na]^+$ 498.2005, found 498.2028.

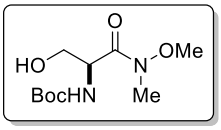
6-Azido-2,3,4-tri-*O*-benzyl-6-deoxy-α/β-D-galactosyl fluoride (36)



6-Azido-2,3,4-tri-*O*-benzyl-6-deoxy-α/β-D-galactopyranoside (**41**) (500 mg, 1.05 mmol, α/β ratio 3:1) was dissolved in dry DCM (8 mL), and the solution was cooled to –30 °C. Then

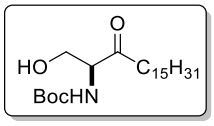
diethylaminosulfur trifluoride (508 mg, 3.15 mmol) was added dropwise, and stirring was continued for for 20 min. MeOH (2 mL) was added, and the solution was poured into cold saturated aqueous $NaHCO_3$ (6 mL). The mixture was extracted with $CHCl_3$ (3 × 10 mL). The combined organic extracts were washed with brine (15 mL), dried (Na_2SO_4) and concentrated. Purification and separation of diastereomers by flash chromatography on silica gel (petroleum ether/EtOAc, 90:10) afforded 6-azido-2,3,4-tri-*O*-benzyl-6-deoxy-α-galactosyl fluoride (**36**α) as a colorless oil (220 mg, 44%):[81] α-isomer as a colorless oil: IR (neat) 3088, 3064, 3031, 2914, 2872, 2102, 1454, 1126, 1109, 1049, 738, 698 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.43–7.31 (m, 15H), 5.61 (dd, *J* = 53.6, 2.6 Hz, 1H), 5.04 (d, *J* = 11.3 Hz, 1H), 4.91–4.74 (m, 4H), 4.62 (d, *J* = 11.3 Hz, 1H), 4.12–3.96 (m, 3H), 3.91 (br s, 1H), 3.53 (dd, *J* = 12.4, 7.2 Hz, 1H), 3.17 (dd, *J* = 12.5, 6.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.4, 138.1, 128.7, 128.7, 128.6, 128.5, 128.2, 128.2, 128.1, 128.0, 127.8, 106.2 (d, $J_{C\text{-}F}$ = 225.5 Hz), 78.4, 75.8 (d, $J_{C\text{-}F}$ = 23.5 Hz), 75.0, 74.5, 74.0, 73.7, 72.1 (d, $J_{C\text{-}F}$ = 2.7 Hz), 51.0; HRMS (ESI) *m/z* calcd for $C_{27}H_{28}FN_3NaO_4$ $[M + Na]^+$ 500.1962, found 500.1958: β-isomer 6-azido-2,3,4-tri-*O*-benzyl-6-deoxy-β-galactosyl fluoride (**36**β) was obtained as a colorless oil (220 mg, 44%): IR (neat) 3029, 2881, 2103, 1454, 1101, 1055, 70, 696 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.37–7.31 (m, 15H), 5.23 (dd, *J* = 52.6, 6.3 Hz, 1H), 4.96 (d, *J* = 11.6 Hz, 1H), 4.84–4.72 (m, 4H), 4.61 (d, *J* = 11.5, 1H), 3.94 (ddd, *J* = 12.2, 9.0, 6.3 Hz, 1H), 3.83–3.80 (m, 1H), 3.72–3.58 (m, 3H), 3.51 (s, 2H), 3.27 (dd, *J* = 11.6, 5.04 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.2, 138.1, 138.0, 128.7, 128.6, 128.4, 128.3, 128.2, 128.1, 128.1, 127.8, 109.9 (d, $J_{C\text{-}F}$ = 216.4 Hz), 80.2 (d, $J_{C\text{-}F}$ = 9.9 Hz), 78.5 (d, $J_{C\text{-}F}$ = 22.0 Hz), 75.0, 74.4, 73.9 (d, $J_{C\text{-}F}$ = 4.2 Hz), 73.6, 73.1, 51.2; HRMS (ESI) *m/z* calcd for $C_{27}H_{28}FN_3NaO_4$ $[M + Na]^+$ 500.1962, found 500.1981.

(*S*)-[2-Hydroxy-1-(methoxymethylcarbamoyl)ethyl]-carbamic acid *tert*-butyl ester (48)



(*S*)-2-(*tert*-Butoxycarbonyl)-3-hydroxypropanoic acid (4.00 g, 19.5 mmol) was dissolved in dry DCM (77 mL), and the solution was cooled to –15 °C. Next, *N*,*O*-dimethylhydroxyl amine hydrochloride (1.96 g, 20.0 mmol) was added, followed by *N*-methylmorpholine (2.21 mL, 20.1 mmol). After 5 min EDC (3.85 g, 20.1 mmol) was added in 5 equal portions over 30 min (1 portion per 6 min). The reaction mixture was allowed to stir an additional 1 h at –15 °C. Then, ice cold aqueous HCl (1.0 M, 25 mL) was added, the layers were separated, and the aqueous layer was extracted with DCM (3 × 25 mL). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ (2 × 25 mL), H_2O (2 × 25 mL) and brine (1 × 25 mL). The organic phase was dried ($MgSO_4$) and concentrated to give (*S*)-[2-hydroxy-1-(methoxymethylcarbamoyl)ethyl]-carbamic acid *tert*-butyl ester (**48**) as a white solid (4.06 g, 84%):[82] ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.62 (br s, 1H), 4.78 (br s, 1H), 3.81–3.77 (m, 5H), 3.22 (s, 3H), 2.77 (br s, 1H) 1.44 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.2, 156.1, 80.3, 63.9, 61.8, 52.6, 52.6, 32.3, 28.5.

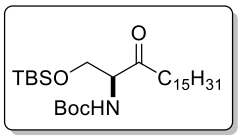
(2*S*)-2-(*N*-*tert*-Butoxycarbonyl)amino-1-hydroxyoctadecan-3-one (49)



(*S*)-[2-Hydroxy-1-(methoxymethylcarbamoyl)ethyl]-carbamic acid *tert*-butyl ester (**48**) (2.00 g, 8.06 mmol) was dissolved in dry THF (16 mL) under N_2. The resulting solution was cooled to –15 °C via an ice/salt water bath. Next, *s*-BuMgCl (2.0 M in Et_2O, 8.05 mL, 16.1 mmol) was added dropwise. After 5 min, pentadecylmagnesium bromide (**48a**) (0.42 M in THF, 24.9 mL, 10.5 mmol) was added dropwise at –15 °C. The resulting solution was allowed to warm to rt and stir overnight. The reaction was cooled to –15 °C and ice cold aqueous HCl (1.0 M, 20 mL) was added, followed

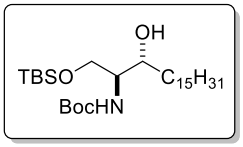
by EtOAc (20 mL). The two layers were separated, and the aqueous layer was extracted with DCM (3 × 35 mL). The combined organic extracts were washed with H_2O (40 mL), dried ($MgSO_4$), and concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc, 80:20) afforded (2*S*)-2-(*N*-*tert*-butoxycarbonyl)amino-1-hydroxyoctadecan-3-one (**49**) as a white solid (2.06 g, 64%):[72] ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.63 (br s, 1H), 4.34 (s, 1H), 3.97–3.90 (m, 2H), 2.63 (br s, 1H), 1.57 (m, 2H), 1.45 (s, 9H), 1.27–1.22 (m, 24H), 0.88 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 208.1, 164.0, 80.3, 70.1, 68.9, 63.3, 61.6, 52.3, 49.8, 39.9, 31.9, 29.7, 29.6, 29.4, 29.3, 29.2, 28.3, 23.5, 22.7, 14.1.

(2*S*)-2-(*N*-*tert*-Butoxycarbonyl)amino-1-*tert*-butyldimethylsilanyloxyoctadecan-3-one (50)



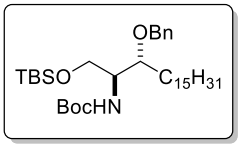
(2*S*)-2-(*N*-*tert*-Butoxycarbonyl)amino-1-hydroxyoctadecan-3-one (**49**) (1.22 g, 3.05 mmol) was dissolved in dry DMF (8 mL) under N_2. Next, imidazole (0.63 g, 3.05 mmol) was added, followed by a catalytic amount of DMAP. After 20 min TBSCl (0.95 mL, 3.66 mmol) was added, and the reaction was stirred for 14 h. The reaction mixture was diluted with saturated aqueous NH_4Cl (15 mL), and the aqueous layer was extracted with DCM (3 × 20 mL). The combined organic extracts were washed with H_2O (20 mL) and brine (20 mL), dried ($MgSO_4$), and concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc, 99:1) afforded (2*S*)-2-(*N*-*tert*-butoxycarbonyl)amino-1-*tert*-butyldimethylsilanyloxyoctadecan-3-one (**50**) as a colorless oil (1.73 g, 89%):[72] ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.49 (d, *J* = 7.0 Hz, 1H), 4.26 (t, *J* = 3.3 Hz, 1H), 4.05 (d, *J* = 10.3 Hz, 1H), 3.82 (dd, *J* = 10.2, 3.6 Hz, 1H), 2.62–2.43 (m, 2H), 1.58– (m, 2H), 1.45 (s, 9H), 1.26 (m, 24H), 0.88 (t, *J* = 5.7 Hz, 3H), 0.86 (s, 9H), 0.03 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 208.0, 155.5, 79.8, 63.6, 61.3, 40.3, 32.1, 29.8, 29.8, 29.8, 29.6, 29.6, 29.5, 29.4, 28.5, 25.9, 23.5, 22.9, 18.3, 14.3, –5.4.

(2*S*,3*R*)-2-(*N*-*tert*-Butoxycarbonyl)amino-1-*tert*-butyldimethylsilanyloxyoctadecan-3-ol (51a)



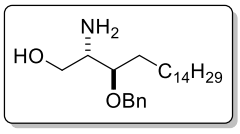
Dry EtOH (28 mL) was cooled to –78 °C and stirred under N_2 for 20 min. Then, LiAl(O*t*-Bu)$_3$H (5.13 g, 20.2 mmol) was added, and the mixtue was stirred for 20 min. Next, a solution of (2*S*)-2-(*N*-*tert*-butoxycarbonyl)amino-1-*tert*-butyldimethylsilanyloxyoctadecan-3-one (**50**) (2.18 g, 3.4 mmol) in dry EtOH (28 mL) was added dropwise over 15 min to the stirred solution. The temperature was maintained –78 °C for 6 h. The reaction mixture was diluted with DCM (20 mL), and 10% aqueous citric acid solution (35 mL) was added. The mixture was stirred for 1.5 h at rt, and the aqueous layer was extracted with DCM (3 × 25 mL). The combined organic extracts were washed with H_2O (25 mL) and brine (25 mL), dried ($MgSO_4$), and concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc, 97:3) afforded (2*S*,3*R*)-2-(*N*-*tert*-butoxycarbonyl)amino-1-*tert*-butyldimethylsilanyloxyoctadecan-3-ol (**51a**) as a colorless oil (2.07 g, 95%):[72] ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.32 (d, *J* = 6.6 Hz, 1H), 3.97 (dd, *J* = 10.6, 2.8 Hz, 1H), 3.83 (d, *J* = 9.3 Hz, 1H), 3.65 (m, 1H), 3.51 (br s, 1H), 3.01 (br s, 1H), 1.53 (br s, 2H), 1.46 (s, 9H), 1.26 (m, 24H), 0.92 (s, 9H), 0.89 (t, *J* = 7.7 Hz, 3H), 0.09 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.9, 79.5, 74.5, 63.7, 54.1, 35.1, 32.1, 29.9, 29.8, 29.8, 29.6, 26.2, 26.0, 22.9, 18.3, 14.3, –5.4, –5.4.

(2*S*,3*R*)-2-(*N*-*tert*-Butoxycarbonyl)amino-1-*tert*-butyldimethylsilanyloxy-3-benzyloxyoctadecane (51)



(2*S*,3*R*)-2-(*N*-*tert*-Butoxycarbonyl)amino-1-*tert*-butyldimethylsilanyloxyoctadecan-3-ol (**51a**) (889 mg, 1.39 mmol) was dissolved in dry DMF (10 mL), and the solution was cooled to 0 °C and stirred under N_2. Then, tetrabutylammonium iodide (759 mg, 2.06 mmol) was added, followed by NaH (60% in mineral oil, 69 mg, 1.73 mmol). After 10 min benzylbromide (0.245 mL, 2.06 mmol) was added dropwise. After the addition the ice bath was removed, and the reaction mixture was stirred at rt for 4 h. The reaction was quenched with saturated aqueous NH_4Cl (15 mL), and the mixture was extracted with Et_2O (4 × 20 mL). The combined organic extracts were washed with H_2O (25 mL) and brine (25 mL), dried ($MgSO_4$), and concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc, 99:1) afforded (2*S*,3*R*)-2-(*N*-*tert*-butoxycarbonyl)amino-1-*tert*-butyldimethylsilanyloxy-3-benzyloxyoctadecane (**51**) as a clear oil (756 mg, 72%):[72] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (m, 5H), 4.65 (br s, 1H), 4.49 (s, 2H), 3.79 (m, 2H), 3.58 (m, 1H), 3.47 (br s, 1H), 1.49 (m, 2H), 1.39 (s, 9H), 1.21 (br s, 26H), 0.85 (s, 9H), 0.82 (t, *J* = 4.5 Hz, 3H), 0.00 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.8, 138.9, 128.8, 128.3, 127.8, 79.2, 78.6, 72.6, 62.2, 53.8, 32.1, 30.7, 30.1, 29.9, 29.9, 29.8, 29.8, 29.6, 28.6, 25.5, 25.1, 22.9, 18.4, 18.3, 14.3, –4.3, –5.2.

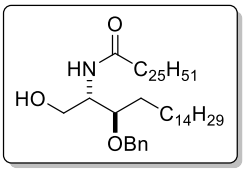
(2*S*,3*R*)-2-Amino-3-benzyloxyoctadecan-1-ol (34a)



Acetyl chloride (3.2 mL, 34.6 mmol) was added dropwise to MeOH (39.1 mL) at 0 °C. The solution was slowly warmed to rt under N_2. (2*S*,3*R*)-2-(*N*-*tert*-Butoxycarbonyl)amino-1-*tert*-

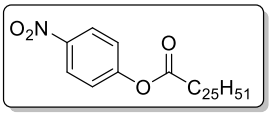
butyldimethylsilanyloxy-3-benzyloxyoctadecane (**51**) (1.01 g, 13.2 mmol) dissolved in dry Et_2O (39 mL) was slowly added, and the solution was stirred for 2 d at rt. The reaction mixture was concentrated then dissolved in DCM (10 mL). Saturated aqueous $NaHCO_3$ (15 mL), followed by aqueous NaOH (1.0 M, 10 mL) was added. The mixture was stirred for 1 h, then diluted with DCM (75 mL). The aqueous layer was extracted with DCM (3 × 50 mL), and the combined organic extracts were dried ($MgSO_4$) and concentrated. Purification by flash chromatography on silica gel (DCM/MeOH, 90:10) afforded (2*S*,3*R*)-2-amino-3-benzyloxyoctadecan-1-ol (**34a**) as a colorless oil (363 mg, 70%):[72] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (m, 5H), 4.50 (m, 2H), 3.69 (m, 3H), 3.01 (br s, 1H), 2.47 (br s, 3H), 1.35 (m, 2H), 1.20 (m, 26H), 0.82 (t, *J* = 5.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.6, 128.7, 128.0, 128.0, 82.5, 72.5, 63.7, 54.5, 32.1, 30.7, 30.1, 29.9, 29.9, 29.8, 29.8, 29.6, 25.6, 22.9, 14.3.

(2*S*,3*R*)-3-Benzyloxy-2-(*N*-hexacosanoylamino)octadecan-1-ol (34)



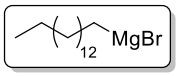
p-Nitrophenyl hexacosanoate (**46**) (132 mg, 0.254 mmol) was added to a stirred solution under N_2 of (2*S*,3*R*)-2-amino-3-benzyloxyoctadecan-1-ol (**34a**) (83 mg, 0.212 mmol) in dry pyridine (4.15 mL). The reaction was stirred for 1 d at rt. The solution was concentrated, and the residue was purified by flash chromatography on silica gel (petroleum ether/EtOAc, 85:15) to afford (2*S*,3*R*)-3-benzyloxy-2-(*N*-hexacosanoylamino)octadecan-1-ol (**34**) as a white solid (84 mg, 83%):[72] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 (m, 5H), 6.09 (d, *J* = 7.2 Hz, 1H), 4.62 (d, *J* = 11.6 Hz, 1H), 4.36 (d, *J* = 11.6 Hz, 1H), 3.94 (m, 2H), 3.63 (br s, 1H), 3.55 (d, *J* = 9.1 Hz, 1H), 2.04 (m, 2H), 1.68 (m, 1H), 1.53 (m, 3H), 1.22 (br s, 70H), 0.85 (t, *J* = 5.6 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.6, 138.2, 128.9, 128.3, 128.1, 82.2, 73.0, 62.4, 52.3, 36.9, 32.1, 31.6, 30.0, 29.9, 29.8, 29.7, 29.6, 29.5, 25.9, 25.9, 22.9, 14.3.

***p*-Nitrophenyl hexacosanoic acid (46)**



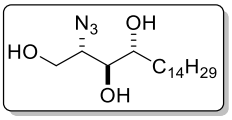
Hexacosanoic acid (2.00 g, 5.04 mmol) was dissolved in dry DCM (285 mL) under N_2. Then, *p*-nitrophenol (637 mg, 4.58 mmol) was added, followed by DMAP (112 mg, 0.92 mmol) and DCC (1.00 g, 4.82 mmol). The reaction mixture was stirred at rt for 18 h. The solution was concentrated, and the residue was purified by flash chromatography on silica gel (petroleum ether/ EtOAc, 98:2 to 95:5) to afford *p*-nitrophenyl hexacosanoic acid (**46**) as a white solid (2.14 g, 82%):[83] ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (d, *J* = 9.0 Hz, 2H), 7.28 (d, *J* = 9.1 Hz, 2H), 2.60 (t, *J* = 7.5 Hz, 2H), 1.80-1.73 (m, 2H), 1.26 (s, 44H), 0.89 (t, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.5, 155.8, 145.5, 125.4, 122.6, 34.6, 32.1, 29.9, 29.8, 29.7, 29.6, 29.4, 29.3, 25.0, 22.9, 14.3.

***n*-Pentadecylmagnesium bromide (48a)**



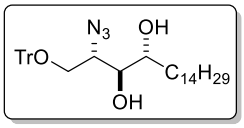
Magnesium turnings (381 mg, 15.7 mmol) were placed in an oven-dried, three neck flask equipped with a condenser under N_2, and anhydrous THF (2 mL) was added, followed by a crystal of iodine. The flask was heated in a preheated oil bath at 75 °C, until the pink solution turned colourless. Approximately 25% of a solution of 1-bromopentadecane (3.05 g, 10.5 mmol) in dry THF (8.5 mL) was added. After 10 min or until the brown color changed to gray, the rest of the 1-bromopentadecane solution was added over 10 min, and the mixture was heated at reflux for 1.5 h. The mixture was cooled to rt and the Grignard reagent was titrated with salicylaldehyde phenylhydrazone.[84] The concentration of the generated *n*-pentadecylmagnesium bromide (**48a**)[72] solution was determined to be 0.44 M in THF.

(2*S*,3*S*,4*R*)-2-Azido-1,3,4-octadecanetriol (42)



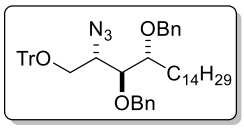
Sodium azide (5.00 g, 96.0 mmol) was dissolved in H_2O (20 mL), followed by the addition of DCM (27 mL). The biphasic mixture was cooled to 0 °C and triflic anhydride (7.96 mL, 48.0 mmol) was added dropwise over 20 min, with vigorous stirring of the mixture. The flask was stoppered and stirred at 0 °C for 2.5 h. The mixture was slowly quenched with saturated aqueous $NaHCO_3$ (15 mL), while stirring was continued until gas evolution had ceased. The organic layer was separated, and the aqueous layer was extracted with DCM (2 × 25 mL). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ (15 mL). The resulting triflyl azide solution in DCM was used in the azidation step without further purification as follows; (2*S*,3*S*,4*R*)-2-amino-1,3,4-octadecanetriol (5.00 g, 15.8 mmol), $CuSO_4 \cdot 5H_2O$ (16 mg, 0.065 mmol), and potassium carbonate (2.27 g, 16.5 mmol) were dissolved in the same volume of H_2O as the volume of triflyl azide solution (42 mL). The DCM solution of TfN_3 was then added with vigorous stirring, followed by the addition of MeOH (285 mL) over 5 min. After 20 h, the reaction mixture was diluted with H_2O (130 mL) and extracted with EtOAc (4 × 100 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography on silica gel (EtOAc), to afford (2*S*,3*S*,4*R*)-2-azido-1,3,4-octadecanetriol (**42**) as a white solid (5.24 g, 97%):[85] mp = 92.0–93.1 °C; $[\alpha]_D^{23}$ = +15.0 (*c* = 1.0, $CHCl_3$); IR (neat) 3343, 2919, 2847, 2120, 1463 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 4.02 (dd, *J* = 11.4, 5.1 Hz, 1H), 3.88 (dd, *J* = 11.4, 4.4 Hz, 1H), 3.83–3.74 (m, 2H), 3.70–3.66 (m, 1H), 1.61–1.51 (m, 3H), 1.41–1.26 (m, 23H), 0.88 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 74.8, 72.5, 63.0, 62.0, 31.9, 29.6, 29.5, 29.3, 25.7, 22.6, 14.1; HRMS (ESI) *m/z* calcd for $C_{18}H_{37}N_3NaO_3$ $[M + Na]^+$ 366.2727, found 366.2722.

(2*S*,3*S*,4*R*)-2-Azido-1-*O*-triphenylmethyl-1,3,4-octadecanetriol (43a)



(2*S*,3*S*,4*R*)-2-Azido-1,3,4-octadecanetriol (**42**) (2.30 g, 6.5 mmol) was dissolved in dry pyridine (30 mL), followed by the addition of TrCl (7.30 g, 26.2 mmol) and DMAP (200 mg). The reaction mixture was stirred at 50 °C for 14 h. The solution was concentrated, and the residue was dissolved in EtOAc (50 mL) and washed with H_2O (25 mL). The organic layer was dried (Na_2SO_4) and concentrated, and the residue was purified by flash chromatography on silica gel (petroleum ether/EtOAc, 90:10 to 85:15) to afford (2*S*,3*S*,4*R*)-2-azido-1-*O*-triphenylmethyl-1,3,4-octadecanetriol (**43a**) as a colorless oil (2.30 g, 60%):[86] $[\alpha]_D^{23}$ = +9.7 (*c* = 1.0, $CHCl_3$); IR (neat) 3416, 2907, 2849, 1449, 1215, 1070 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.48–7.42 (m, 6H), 7.36–7.23 (m, 9H), 3.66–3.61 (m, 2H), 3.56–3.51 (m, 2H), 3.41 (dd, *J* = 10.5, 5.4 Hz, 1H), 2.35 (d, *J* = 5.4 Hz, 1H), 1.81 (d, *J* = 5.4 Hz, 1H), 1.56–1.24 (m, 26H), 0.88 (t, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 143.3, 128.5, 127.9, 127.2, 87.4, 74.1, 72.0, 63.5, 62.1, 31.4, 29.6, 29.5, 29.3, 25.6, 22.6, 14.0; HRMS (ESI) *m/z* calcd for $C_{37}H_{52}N_3O_3$ $[M + H]^+$ 586.4015, found 586.4061.

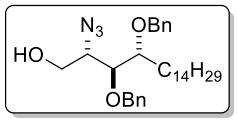
(2*S*,3*S*,4*R*)- 2-Azido-3,4-di-*O*-benzyl-1-*O*-triphenylmethyl-1,3,4-octadecanetriol (43)



(2*S*,3*S*,4*R*)-2-Azido-1-*O*-triphenylmethyl-3,4-octadecanetriol (**43a**) (2.74 g, 4.68 mmol) was dissolved in dry DMF (40 mL), followed by the addition of NaH (60% in mineral oil, 1.68 g, 14.0 mmol). The mixture was stirred at rt for 1 h. Then, the mixture was cooled to 0 °C, and benzyl-chloride (1.67 mL, 14.0 mmol) was added dropwise. The resulting mixture was allowed to warm to rt and stirred for 20 h. The excess NaH was quenched with ice-cooled water (100 mL), and the reaction mixture was extracted with EtOAc (3 × 100 mL). The combined organic extracts were

washed with brine (3 × 50 mL), dried ($MgSO_4$) and concentrated. The residue was purified by flash chromatography on silica gel (petroleum ether/EtOAc, 99:1) to afford (2*S*,3*S*,4*R*)-2-azido-3,4-di-*O*-benzyl-1-*O*-triphenylmethyl-1,3,4-octadecanetriol (**43**) as a colorless oil (3.17 g, 92%):[86] $[\alpha]_D^{23}$ = +9.9 (*c* = 1.0, $CHCl_3$); IR (neat) 2914, 2849, 1450, 1086, 1028, 745 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.49–7.07 (m, 25H), 4.60 (d, *J* = 11.1 Hz, 1H), 4.48 (d, *J* = 11.1 Hz, 1H), 4.45 (s, 2H), 3.82–3.77 (m, 1H), 3.60–3.50 (m, 3H), 3.40 (dd, *J* = 10.1, 8.1 Hz, 1H), 1.68–1.20 (m, 26H), 0.91 (t, *J* = 6.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 144.0, 138.6, 138.2, 128.9, 128.5, 128.4, 128.1, 128.0, 127.9, 127.7, 127.6, 127.2, 79.6, 79.4, 73.7, 72.3, 64.5, 63.5, 31.8, 30.0, 29.9, 29.6, 29.3, 25.5, 22.9, 14.3; HRMS (ESI) *m/z* calcd for $C_{51}H_{63}N_3NaO_3$ $[M + Na]^+$ 788.4767, found 788.4780.

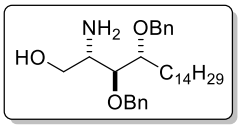
(2*S*,3*S*,4*R*)-2-Azido-3,4-di-*O*-benzyl-1,3,4-octadecanetriol (44a)



(2*S*,3*S*,4*R*)-2-Azido-3,4-di-*O*-benzyl-1-*O*-triphenylmethyl-1,3,4-octadecanetriol (**43**) (1.15 g, 1.56 mmol) and 4-toluenesulfonic acid (131 mg, 0.76 mmol) were dissolved in dry DCM/MeOH (12 mL, 2:1) and stirred at rt. After 12 h the reaction mixture was washed with saturated aqueous $NaHCO_3$ (15 mL) and extracted with EtOAc (2 × 15 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated. The residue was purified by flash chromatography on silica gel (petroleum ether/EtOAc, 90:10) to afford (2*S*,3*S*,4*R*)-1,3,4-di-*O*-benzyl-1-*O*-triphenylmethyl-2-azido-3,4-octadecanetriol (**44a**) as a colorless oil which slowly solidified (740 mg, 96%):[76] $[\alpha]_D^{23}$ = –5.7 (*c* = 1.0, $CHCl_3$); IR (neat) 3430, 2923, 2853, 2095, 1496, 1095, 1062, 734, 697 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.39–7.29 (m, 10H), 4.73–4.56 (m, 4H), 3.93–3.88 (m, 1H), 3.83–3.77 (m, 1H), 3.73–3.63 (m, 3H), 2.54 (t, *J* = 6.4 Hz, 1H), 1.74–1.27 (m, 26 H), 0.90 (t, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.2, 137.9, 128.7, 128.6, 128.3, 128.2, 128.0, 80.7, 79.3, 73.9,

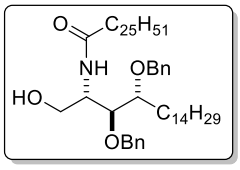
72.8, 63.3, 62.5, 32.1, 30.4, 29.9, 29.9, 29.8, 29.7, 29.6, 25.7, 22.9, 14.3; HRMS (ESI) *m/z* calcd for $C_{32}H_{50}N_3O_3$ $[M + H]^+$ 524.3852, found 524.3863.

(2*S*,3*S*,4*R*)-2-Amino-3,4-di-*O*-benzyl-1,3,4-octadecanetriol (44)



(2*S*,3*S*,4*R*)-2-Azido-3,4-di-*O*-benzyl-1,3,4-octadecanetriol (**44a**) (627 mg, 1.27 mmol) and triphenylphosphine (682 mg, 2.60 mmol) were dissolved in stirring pyridine/water (36 mL, 9:1). After 12 h the solvent was removed under reduced pressure and co-distillation with toluene (2 × 30 mL). The residue was purified by flash chromatography on silica gel (DCM/MeOH, 95:5 to 80:20) to afford (2*S*,3*S*,4*R*)-2-amino-3,4-di-*O*-benzyl-1,3,4-octadecanetriol (**44**) as a colorless oil (531 mg, 84%):[76] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34–7.26 (m, 10H), 4.76–4.55 (m, 4H), 3.76–3.71 (m, 2H), 3.59–3.55 (m, 2H), 3.04–3.00 (m, 1H), 2.32 (br s, 3H), 1.75–1.27 (m, 26H), 0.88 (t, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.5, 138.4, 133.1, 132.2, 132.1, 132.1, 132.0, 83.2, 73.6, 72.5, 64.7, 53.7, 32.0, 30.7, 29.9, 29.8, 29.7, 29.5, 26.0, 22.8, 14.2.

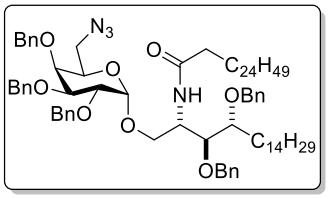
(2*S*,3*S*,4*R*)-3,4-Di-*O*-benzyl-2-hexacosanoylamino-1,3,4-octadecanetriol (35)



(2*S*,3*S*,4*R*)-2-Azido-3,4-di-*O*-benzyl-1,3,4-octadecanetriol (**44**) (402 mg, 0.807 mmol) was dissolved in dry THF (25 mL), and NEt_3 (0.41 mL, 2.90 mmol) and *p*-nitrophenyl hexacosanoic acid (**46**) (502 mg, 0.969 mmol) were added. The reaction mixture was stirred at 50 °C for 14 h. The solvent was removed under reduced pressure, and the residue was purified by flash chromatography on silica gel (petroleum ether/EtOAc, 99:1 to 95:5) to afford (2*S*,3*S*,4*R*)-3,4-di-*O*-benzyl-2-hexacosanoylamino-1,3,4-octadecanetriol (**35**) as a white solid (604 mg, 85%):[76] mp

= 75.1–76.2 °C; $[\alpha]_D^{23}$ = +25.9 (*c* = 1.0, $CHCl_3$); IR (neat) 2917, 2850, 1637, 1618, 1548, 1468, 1102, 1053 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.39–7.28 (m, 10H), 4.72 (d, *J* = 11.7 Hz, 1H), 4.67 (d, *J* = 11.4 Hz, 1H), 4.61 (d, *J* = 11.4 Hz, 1H), 4.46 (d, *J* = 11.7 Hz, 1H), 4.17–4.12 (m, 1H), 4.02–3.98 (m, 1H), 3.72–3.67 (m, 2H), 3.61 (ddd, *J* = 12.7, 8.5, 4.4 Hz, 1H), 3.05 (dd, *J* = 8.4, 4.4 Hz, 1H), 2.06–1.93 (m, 2H), 1.71–1.26 (m, 72H), 0.90–0.87 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.0, 138.4, 138.0, 129.0, 128.7, 128.4, 128.3, 128.2, 128.0, 82.5, 79.3, 73.3, 73.1, 63.2, 50.8, 37.0, 32.1, 31.1, 30.0, 29.8, 29.7, 29.6, 29.5, 26.3, 25.9, 22.9, 14.3; HRMS (ESI) *m/z* calcd for $C_{58}H_{102}NO_4$ $[M + H]^+$ 876.7809, found 876.7797.

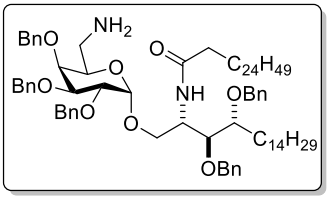
(2*S*,3*S*,4*R*)-3,4-Di-*O*-benzyl-1-*O*-(6-azido-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-galactopyranosyl)-2-hexacosylaminooctadecane-1,3,4-triol (52)



6-Azido-2,3,4-tri-*O*-benzyl-6-deoxy-β-galactosyl fluoride (**36**β) (352 mg, 0.736 mmol) and (2*S*,3*S*,4*R*)-3,4-di-*O*-benzyl-2-hexacosanoylamino-1,3,4-octadecanetriol (**35**) (461 mg, 0.526 mmol) were azeotroped with toluene (3 × 15 mL), dissolved in dry THF and stirred with activated powdered 4 Å MS for 30 min. The reaction mixture was cooled to 0 °C; $AgClO_4$ (349 mg, 1.68 mmol) and $SnCl_2$ (319 mg, 1.68 mmol) were introduced into the solution with protection from light. The mixture was allowed to warm to rt over 3 h under N_2, then filtered through a pad celite. The filter cake was washed with Et_2O (200 mL). The combined filtrate was concentrated and the resulting residue was purified by flash chromatography on silica gel (petroleum ether/EtOAc, 7:1) to afford (2*S*,3*S*,4*R*)-1-*O*-(6-azido-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-galactopyranosyl)-3,4-di-*O*-benzyl-2-hexacosylaminooctadecane-1,3,4-triol (**52**) as a white solid (365 mg, 52%): mp = 59.1–60.3 °C; $[\alpha]_D^{23}$ = +35.1 (*c* = 1.0, $CHCl_3$); IR (neat) 2922, 2853, 2100, 1454, 1093, 1056, 1027, 732,

696 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.44–7.28 (m, 25H), 5.91 (d, *J* = 8.7 Hz, 1H), 5.03 (d, *J* = 11.4 Hz, 1H), 4.90–4.78 (m, 5H), 4.69 (d, *J* = 11.8 Hz, 1H), 4.65 (m, 2H), 4.56 (d, *J* = 11.6 Hz, 1H), 4.54 (d, *J* = 11.7 Hz, 1H), 4.37–4.31 (m, 1H), 4.08 (dd, *J* = 10.0, 3.6 Hz, 1H), 3.95–3.85 (m, 3H), 3.83–3.80 (m, 3H), 3.60–3.56 (m, 1H), 3.55–3.50 (m, 1H), 3.04 (dd, *J* = 12.4, 5.0 Hz, 1H), 1.98–1.93 (m, 2H), 1.78–1.63 (m, 2H), 1.59–1.51 (m, 2H), 1.50–1.42 (m, 1H), 1.41–1.24 (m, 68H), 0.93 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.9, 138.9, 138.8, 138.7, 138.6, 138.3, 128.6, 128.6, 128.5, 128.1, 128.0, 127.9, 127.9, 127.8, 127.7, 127.7, 99.3, 80.1, 79.5, 79.0, 76.7, 75.1, 74.8, 73.7, 73.5, 72.0, 70.2, 69.0, 51.6, 50.3, 36.9, 32.1, 30.3, 30.0, 29.9, 29.8, 29.7, 29.6, 26.1, 25.9, 22.9, 14.3; HRMS (ESI) *m/z* calcd for $C_{85}H_{129}N_4O_8$ $[M + H]^+$ 1333.9810, found 1333.9789.

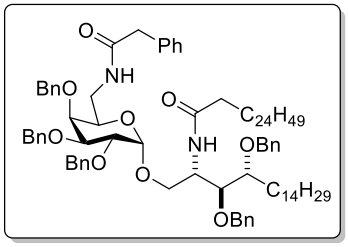
(2*S*,3*S*,4*R*)-1-*O*-(6-Amino-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-galactopyranosyl)-3,4-di-*O*-benzyl-2-hexacosylaminooctadecane-1,3,4-triol (53)



(2*S*,3*S*,4R)1-*O*-(6-Azido-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-galactopyranosyl)-3,4-di-*O*-benzyl-2-hexacosylaminooctadecane-1,3,4-triol (**52**) (74 mg, 0.055 mmol) in THF (3 mL) was added H_2O (0.7 mL) and triphenylphosphine (22 mg, 0.083 mmol). The reaction mixture was stirred at rt for 18 h. The solution was concentrated under reduce pressure to afford (2*S*,3*S*,4*R*)-1-*O*-(6-amino-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-galactopyranosyl)-3,4-di-*O*-benzyl-2-hexacosylaminooctadecane-1,3,4-triol (**53**) as a colorless oil (59 mg, 82%): IR (neat) 2922, 2852, 1665, 1545, 1454, 1117, 744, 694 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.37–7.24 (m, 25H), 5.98 (d, *J* = 9.1 Hz, 1H), 4.94 (d, *J* = 11.3 Hz, 1H), 4.82–4.70 (m, 5H), 4.62 (d, *J* = 11.8 Hz, 1H), 4.58 (d, *J* = 11.4 Hz, 1H), 5.54 (d, *J* = 11.4 Hz, 1H), 5.49 (d, *J* = 11.5 Hz, 1H), 4.46 (d, *J* = 11.8 Hz, 1H), 4.26–4.19 (m, 1H), 3.99 (dd, *J* = 10.1, 3.6 Hz, 1H), 3.93 (dd, *J* = 11.4, 7.6 Hz, 1H), 3.84–3.78 (m, 3H), 3.69–3.67 (m,

2H), 3.55–3.51 (m, 1H), 2.90 (dd, *J* = 12.9, 8.0 Hz, 1H), 2.64 (dd, *J* = 13.1, 3.4 Hz, 1H), 1.93–1.82 (m, 2H), 1.68–1.58 (m 2H), 1.48–1.39 (m, 2H), 1.32–1.15 (m, 68H), 0.90–0.86 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.3, 138.7, 138.6, 138.4, 133.2, 132.3, 132.2, 132.2, 132.1, 132.1, 128.1, 128.7, 128.6, 128.6, 128.6, 128.1, 128.1, 128.0, 128.0, 127.9, 127.8, 127.8, 127.8, 127.6, 99.7, 80.0, 79.9, 79.5, 76.8, 75.1, 74.7, 73.7, 73.4, 72.0, 70.7, 69.2, 50.8, 50.8, 42.1, 36.9, 32.1, 30.3, 30.0, 29.9, 29.9, 29.7, 29.6, 29.6, 26.0, 25.9, 22.9, 14.3; HRMS (ESI) *m/z* calcd for $C_{85}H_{131}N_2O_8$ $[M + H]^+$ 1307.9905, found 1307.9968.

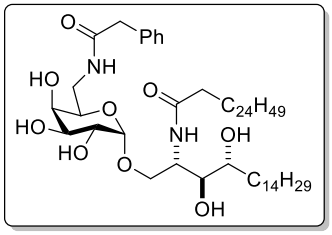
(2*S*,3*S*,4*R*)-3,4-Di-*O*-benzyl-1-*O*-(2,3,4-tri-*O*-benzyl-6-deoxy-6-phenylacetamido-α-D-galactopyranosyl)-2-hexacosylaminooctadecane-1,3,4-triol (54a)



A mixture of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (19 mg, 0.098 mmol) and phenylacetic acid (13 mg, 0.098 mmol) in dry DMF (3 mL) was stirred at rt for 1 h. Then, (2*S*,3*S*,4*R*)-1-*O*-(6-amino-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-galactopyranosyl)-3,4-di-*O*-benzyl-2-hexacosylaminooctadecane-1,3,4-triol (**53**) (130 mg, 0.098 mmol) was added, and the reaction mixture was allowed to stirred for an additional 3 h. DMF was removed, and the residue was purified by flash chromatography on silica gel (hexane/EtOAc, 90:10) to afford (2*S*,3*S*,4*R*)-3,4-di-*O*-benzyl-1-*O*-(2,3,4-tri-*O*-benzyl-6-deoxy-6-phenylacetamido-α-D-galactopyranosyl)-2-hexacosylaminooctadecane-1,3,4-triol (**54a**) as a white solid (59 mg, 42%): mp = 112.6–115.0 °C; $[\alpha]_D^{23}$ = +29.4 (*c* = 1.0, $CHCl_3$); IR (neat) 3290 (br), 2919, 2852, 1647, 1453, 1094, 731, 696 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38–7.14 (m, 30H), 5.92 (d, *J* = 8.6 Hz, 1H), 5.66 (dd, *J* = 7.0, 3.9 Hz, 1H), 4.91 (d, *J* = 11.4 Hz, 1H), 4.81–4.71 (m, 5H), 4.62 (d, *J* = 11.8 Hz, 1H), 4.59 (d, *J* = 11.6 Hz, 1H), 4.57 (d, *J* = 11.3 Hz, 1H), 4.49–4.46 (m, 2H), 4.23–4.17 (m, 1H), 3.99 (dd, *J* = 10.0, 3.6 Hz, 1H),

3.85–3.73 (m, 4H), 3.68 (dd, *J* = 6.3, 6.3 Hz, 1H), 3.58 (dd, *J* = 10.9, 3.8 Hz, 1H), 3.54–3.44 (m, 2H), 3.37 (s, 2H), 3.10 (ddd, *J* = 11.6, 7.2, 4.0 Hz, 1H), 2.05–1.88 (m, 2H), 1.69–1.40 (m, 7H), 1.32–1.21 (m, 65H), 0.88 (t, *J* = 6.6 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.1, 171.3, 138.9, 138.7, 138.6, 138.5, 135.2, 129.5, 129.0, 128.8, 128.7, 128.6, 128.6, 128.2, 128.1, 128.0, 127.9, 127.9, 127.8, 127.7, 127.4, 99.4, 80.1, 79.4, 79.4, 77.4, 76.6, 75.1, 74.8, 73.8, 73.7, 73.3, 72.0, 69.3, 68.7, 50.5, 43.8, 40.5, 36.9, 32.2, 30.2, 30.1, 30.0, 29.7, 29.7, 29.6, 26.3, 26.0, 22.9, 14.3; HRMS (ESI) *m/z* calcd for $C_{93}H_{137}N_2O_9$ $[M + H]^+$ 1426.0324, found 1426.0309.

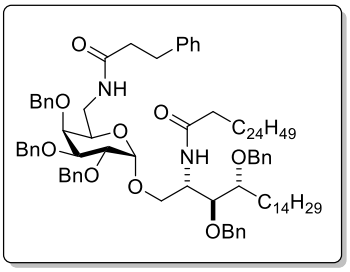
(2*S*,3*S*,4*R*)-1-*O*-(6-Deoxy-6-phenylacetamido-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (15)



$Pd(OH)_2$/C (20 wt% Pd dry basis on carbon, 30 mg) was added to a solution of (2*S*,3*S*,4*R*)-3,4-di-*O*-benzyl-1-*O*-(2,3,4-tri-*O*-benzyl-6-deoxy-6-phenylacetamido-α-D-galactopyranosyl)-2-hexacosylaminooctadecane-1,3,4-triol (**54a**) (23 mg, 0.016 mmol) dissolved in in EtOH/$CHCl_3$ (8 mL, 3:1), and the mixture was hydrogenated under atmospheric pressure for 14 h. The mixture was purged for 10 min with N_2 and filtered through a pad of celite. The filter cake was washed with EtOH/$CHCl_3$ (25 mL, 3:1). The combined filtrate was concentrated, and the resulting solid was triturated with EtOAc (3 × 3 mL) to afford (2*S*,3*S*,4*R*)-1-*O*-(6-deoxy-6-phenylacetamido-α-D-galactopyranosyl)-2-hexacosylaminooctadecane-1,3,4-triol (**15**) as a white solid (12 mg, 72%): mp = 179.4–180.4 °C; $[\alpha]_D^{23}$ = +37.4 (*c* = 1.0, $CHCl_3$/MeOH 1:1); IR (neat) 3273 (br), 2916, 2849, 1640, 1550, 1467, 1136, 1077, 1031 cm^{-1}; ^{1}H NMR (400 MHz, pyridine-d_5) δ 8.88 (dd, *J* = 5.4, 5.4 Hz, 1H), 8.58 (d, *J* = 8.5 Hz, 1H), 7.38–7.25 (m, 5H), 6.33 (br s, 5H), 5.51 (d, *J* = 3.6 Hz, 1H), 5.28–5.21 (m, 1H), 4.59–4.55 (m, 2H), 4.47–4.44 (m, 1H), 4.35–4.29 (m, 5H), 4.21–4.14 (m, 1H),

3.96–3.88 (m, 3H), 2.56–2.43 (m, 2H), 2.36–2.26 (m, 1H), 1.99–1.80 (m, 4H), 1.76–1.64 (m, 1H), 1.35–1.20 (m, 66H), 0.88 (t, *J* = 6.2 Hz, 6H); ^{13}C NMR (100 MHz, pyridine-d_5) δ 173.8, 172.1, 137.6, 130.3, 129.3, 127.4, 101.8, 77.2, 73.0, 71.6, 71.1, 70.5, 69.0, 51.9, 44.2, 41.9, 37.3, 34.9, 32.6, 30.9, 30.7, 30.5, 30.3, 30.3, 30.1, 27.0, 26.9, 23.4, 14.8; HRMS (ESI) *m/z* calcd for $C_{58}H_{107}N_2O_9$ $[M + H]^+$ 975.7977, found 975.8001.

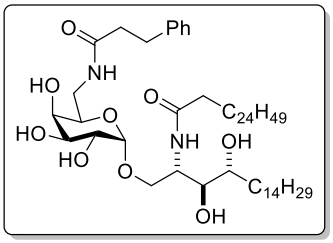
(2*S*,3*S*,4*R*)-3,4-Di-*O*-benzyl-1-*O*-[2,3,4-tri-*O*-benzyl-6-deoxy-6-(3-phenylpropylamido)-α-D-galactopyranosyl]-2-hexacosylaminooctadecane-1,3,4-triol (54b)



A mixture of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (10.6 mg, .056 mmol) and phenylpropanoic acid (8.35 mg, 0.056 mmol) in dry DMF (1.7 mL) was stirred at rt for 1 h. Then, (2*S*,3*S*,4*R*)-1-*O*-(6-amino-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-galactopyranosyl)-3,4-di-*O*-benzyl-2-hexacosylaminooctadecane-1,3,4-triol (**53**) (56 mg, 0.043 mmol) was added, and the reaction mixture was stirred for an additional 3 h. DMF was removed, and the residue was purified by flash chromatography on silica gel (hexane/EtOAc, 90:10) to afford (2*S*,3*S*,4*R*)-3,4-di-*O*-benzyl-1-*O*-[2,3,4-tri-*O*-benzyl-6-deoxy-6-(3-phenylpropylamido)-α-D-galactopyranosyl]-2-hexacosylamino-octadecane-1,3,4-triol (**54b**) as a white solid (44 mg, 72%): mp = 113.6–115.0 °C; $[\alpha]_D^{23}$ = +30.4 (*c* = 1.0, $CHCl_3$); IR (neat) 3293 (br), 2921, 2851, 1644, 1534, 1454, 1261, 1093, 1049, 1027, 731, 696 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39–7.10 (m, 25H), 6.12 (d, *J* = 8.4 Hz, 1H), 5.35 (dd, *J* = 6.4, 6.4 Hz, 1H), 4.92 (d, *J* = 11.4 Hz, 1H), 4.85 (d, *J* = 11.8 Hz, 1H), 4.81–4.72 (m, 4H), 4.64–4.60 (m, 3H), 4.51–4.47 (m, 2H), 4.24–4.16 (m, 1H), 4.02–3.97 (m, 2H), 3.83 (dd, *J* = 10.0, 2.7 Hz, 1H), 3.78–3.64 (m, 4H), 3.53 (ddd, *J* = 7.2, 3.5, 3.5 Hz, 1H), 3.34 (ddd, *J* = 13.7, 6.9, 6.9 Hz,

1H), 3.14 (ddd, *J* = 13.7, 5.2, 5.2 Hz, 1H), 2.81 (t, *J* = 7.8 Hz, 2H), 2.30–2.12 (m, 2H), 2.02–1.89 (m, 2H), 1.66–1.40 (m, 8H), 1.34–1.12 (m, 67H), 0.90–0.86 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.3, 172.4, 141.1, 138.8, 138.7, 129.2, 128.7, 128.6, 128.6, 128.6, 128.5,128.2, 128.1, 128.1, 128.0, 127.9, 127.6, 126.4, 99.9, 79.9, 79.5, 77.4, 74.7, 74.5, 73.8, 73.8, 73.6, 72.1, 68.9, 51.0, 40.1, 38.2, 36.9, 32.2, 31.7, 30.3, 30.0, 29.7, 29.7, 29.6, 26.1, 26.0, 22.9, 14.3; HRMS (ESI) *m/z* calcd for $C_{94}H_{139}N_2O_9$ $[M + H]^+$ 1440.0481, found 1440.0443.

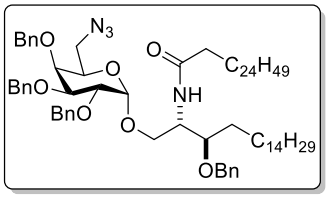
(2*S*,3*S*,4*R*)-1-*O*-[6-Deoxy-6-(3-phenylpropylamido)- α-D-galactopyranosyl]-2-hexacosyl-aminooctadecane-1,3,4-triol (16)



(2*S*,3*S*,4*R*)-3,4-Di-*O*-benzyl-1-*O*-[2,3,4-tri-*O*-benzyl-6-deoxy-6-(3-phenylpropylamido)-α-D-galactopyranosyl]-2-hexacosylaminooctadecane-1,3,4-triol (**54b**) (25 mg, 0.017 mmol) was dissolved in EtOH/$CHCl_3$ (8 mL, 3:1), $Pd(OH)_2$/C (20 wt% Pd dry basis on carbon, 30.4 mg) was added. The mixture was hydrogenated under atmospheric pressure for 14 h. The solution was filtered through a pad of celite, and the filter cake was washed with EtOH/$CHCl_3$ (25 mL, 3:1). The filtrate was concentrated, and the resulting residue was purified by trituration with EtOAc (3 × 3 mL) to afford (2*S*,3*S*,4*R*)-1-*O*-[6-deoxy-6-(3-phenylpropylamido)- α-D-galactopyranosyl]-2-hexacosylaminooctadecane-1,3,4-triol (**16**) as a white solid (13 mg, 76%): mp = 184.8–185.7 °C; $[\alpha]_D^{23}$ = +36.9 (*c* = 1.0, $CHCl_3$/MeOH 1:1); IR (neat) 3295 (br), 2917, 2849, 1641, 1549, 1306, 1018 cm^{-1}; ^{1}H NMR (400 MHz, pyridine-d_5) δ 8.87 (dd, *J* = 5.4, 5.4 Hz, 1H), 8.58 (d, *J* = 8.4 Hz, 1H), 7.35–7.28 (m, 5H), 6.31 (br s, 5H), 5.49 (d, *J* = 3.4 Hz, 1H), 5.28–5.20 (m, 1H), 4.63–4.56 (m, 2H), 4.47 (dd, *J* = 6.4, 6.4 Hz, 1H), 4.34–4.27 (m, 5H), 4.20–4.15 (m, 1H), 3.92–3.86 (m, 1H), 3.22–3.18 (m, 2H), 2.88–2.75 (m, 2H), 2.51–2.46 (m, 2H), 2.35–2.23 (m, 1H), 2.01–1.77 (m, 4H),

1.76–1.62 (m, 2H), 1.32–1.26 (m, 66H), 0.89–0.86 (m, 6H); ^{13}C NMR (100 MHz, pyridine-d_5) δ 173.8, 173.5, 142.6, 129.4, 129.3, 126.9, 101.8, 77.2, 73.0, 71.7, 71.4, 71.0, 70.6, 68.9, 52.0, 41.6, 38.9, 37.3, 34.9, 32.8, 32.6, 30.9, 30.7, 30.5, 30.4, 30.3, 30.3, 30.1, 27.0, 26.9, 23.4, 14.8; HRMS (ESI) *m/z* calcd for $C_{59}H_{109}N_2O_9$ $[M + H]^+$ 989.8133, found 989.8123.

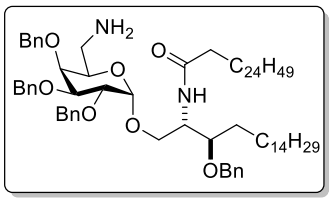
(2*S*,3*R*)-1-*O*-(6-Azido-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-galactopyranosyl)-3-benzyloxy-2-hexacosylaminooctadecane-1,3-diol (55)



6-Azido-2,3,4-tri-*O*-benzyl-6-deoxy-β-galactosyl fluoride (**36**β) (152 mg, 0.318 mmol) and (2*S*,3*R*)-3-benzyloxy-2-(*N*-hexacosanoylamino)octadecan-1-ol (**34**) (245 mg, 0.318 mmol) were co-evaporated with toluene (3 × 15 mL), dissolved in dry THF (6 mL) and stirred with activated powdered 4 Å MS for 30 min. The reaction mixture was cooled to 0 °C, and $AgClO_4$ (198 mg, 0.954 mmol) and $SnCl_2$ (181 mg, 0.954 mmol) were introduced into the solution with protection from light. The mixture was allowed to warm to rt over 3 h under N_2, then filtered through a pad celite, and the filter cake was washed with Et_2O. The combined filtrate was concentrated, and the resulting residue was purified by flash chromatography on silica gel (petroleum ether/EtOAc, 7:1) to afford (2*S*,3*R*)-1-*O*-(6-azido-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-galactopyranosyl)-3-benzyloxy-2-hexacosylaminooctadecane-1,3-diol (**55**) as a white solid (288 mg, 74%): mp = 57.9–58.8 °C; $[\alpha]_D^{23}$ = +33.4 (*c* = 1.0, $CHCl_3$); IR (neat) 3308, 2918, 2852, 2101, 1640, 1542, 1461, 1097, 1046 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41–7.26 (m, 20H), 5.83 (d, *J* = 8.8 Hz, 1H), 4.99 (d, *J* = 11.5 Hz, 1H), 4.87–4.84 (m, 2H), 4.80 (d, *J* = 11.8 Hz, 1H), 4.76 (d, *J* = 11.8 Hz, 1H), 4.66 (d, *J* = 11.8 Hz, 1H), 4.60 (d, *J* = 11.4 Hz, 1H), 4.59 (d, *J* = 11.6 Hz, 1H), 4.44 (d, *J* = 11.6 Hz, 1H), 4.28 (m, 1H), 4.04 (dd, *J* = 10.0, 3.5 Hz, 1H), 3.89 (dd, *J* = 10.1, 2.6 Hz, 1H), 3.85–3.75 (m, 4H), 3.60–3.56

(m, 1H), 3.54–3.49 (m, 1H), 2.97 (dd, *J* = 12.5, 4.6 Hz, 1H), 2.02 (m, 2H), 1.62–1.51 (m, 4H), 1.43–1.16 (m, 70H), 0.90 (t, *J* = 6.5 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.0, 138.8, 138.7, 138.6, 138.3, 128.6, 128.6, 128.1, 128.0, 128.0, 127.9, 127.8, 127.7, 99.0, 79.2, 78.9, 76.7, 75.2, 74.8, 73.6, 72.3, 70.4, 68.0, 51.7, 51.4, 37.0, 32.1, 31.0, 30.1, 29.9, 29.8, 29.6, 29.6, 25,9, 25.6, 22.9, 14.3; HRMS (ESI) *m/z* calcd for $C_{78}H_{123}N_4O_7$ $[M + H]^+$ 1227.9392, found 1227.9417.

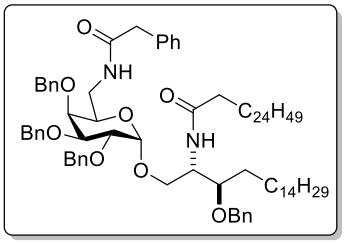
(2*S*,3*R*)-1-*O*-(6-Amino-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-galactopyranosyl)-3-benzyloxy-2-hexacosylaminooctadecane-1,3-diol (56)



(2*S*,3*R*)-1-*O*-(6-Azido-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-galactopyranosyl)-3-benzyloxy-2-hexacosylaminooctadecane-1,3-diol (**55**) (78 mg, 0.064 mmol) was dissolved in THF (3 mL) and H_2O (0.7 mL), and triphenylphosphine (25 mg, 0.095 mmol) was added. The reaction mixture was stirred at rt for 18 h. The solution was concentrated, and the residue was purified by flash chromatography on silica gel (DCM/MeOH, 80:20) to afford (2*S*,3*R*)-1-*O*-(6-amino-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-galactopyranosyl)-3-benzyloxy-2-hexacosylaminooctadecane-1,3-diol (**56**) as a colorless oil (64 mg, 82%): mp = 71.8–74.0 °C; $[\alpha]_D^{23}$ = +26.8 (*c* = 1.0, $CHCl_3$); IR (neat) 3294 (br), 2921, 2851, 1640, 1454, 1094, 1045, 1027, 731, 696 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39–7.22 (m, 20H), 6.02 (d, *J* = 8.6 Hz, 1H), 4.95 (d, *J* = 11.4 Hz, 1H), 4.89 (d, *J* = 3.2 Hz, 1H), 4.83 (d, *J* = 11.7 Hz, 1H), 4.77 (d, *J* = 11.9 Hz, 1H), 4.73 (d, *J* = 11.8 Hz, 1H), 4.64 (d, *J* = 11.9 Hz, 1H), 4.60 (d, *J* = 11.4 Hz, 1H), 4.55 (d, *J* = 11.6 Hz, 1H), 4.43 (d, *J* = 11.5 Hz, 1H), 4.25–4.19 (m, 1H), 4.25–4.19 (m, 1H), 4.01 (dd, *J* = 9.3, 3.0 Hz, 1H), 3.87–3.74 (m, 4H), 3.65 (s, 1H), 3.55 (dd, *J* = 11.8, 6.0 Hz, 1H), 2.99–2.94 (m, 1H), 2.74–2.69 (m, 1H), 2.10–1.95 (m, 2H), 1.60–1.46 (m, 8H), 1.45–1.10 (m, 66H), 0.90–0.86 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.4, 138.5,

138.5, 138.4, 138.0, 128.5, 128.5, 128.4, 128.4, 128.0, 127.9, 127.8, 127.8, 127.7, 127.5, 99.3, 79.6, 78.9, 76.3, 75.0, 74.5, 73.4, 73.4, 72.4, 70.6, 68.5, 51.8, 41.6, 36.9, 31.9, 31.1, 29.9, 29.7, 29.5, 29.4, 25.7, 25.5, 22.7, 14.3; HRMS (ESI) *m/z* calcd for $C_{78}H_{125}N_4O_7$ $[M + H]^+$ 1201.9487, found 1201.9442.

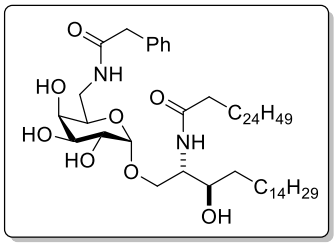
(2*S*,3*R*)-3-Benzyloxy-1-*O*-[2,3,4-tri-*O*-benzyl-6-deoxy-6-phenylacetamido-α-D-galacto-pyranosyl]-2-hexacosylaminooctadecane-1,3-diol (57a)



A mixture of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (7.8 mg, 0.041 mmol) and phenylacetic acid (5.5 mg, 0.041 mmol) in dry DMF (2 mL) was stirred at rt for 1 h. Then, (2*S*,3*R*)-1-*O*-(6-amino-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-galactopyranosyl)-3-benzyloxy-2-hexacosylamino-octadecane-1,3-diol (**56**) (33 mg, 0.027 mmol) was added, and the reaction mixture stirred for an additional 3 h. DMF was removed, and the residue was purified by flash chromatography on silica gel (hexane/EtOAc, 90:10) to afford (2*S*,3*R*)-3-benzyloxy-1-*O*-[2,3,4-tri-*O*-benzyl-6-deoxy-6-phenylacetamido-α-D-galactopyranosyl]-2-hexacosylaminooctadecane-1,3-diol (**57a**) as a white solid (21 mg, 60%): mp = 112.6–115.0 °C; $[\alpha]_D^{23}$ = +35.1 (*c* = 1.0, $CHCl_3$); IR (neat) 3317 (br), 2920, 2851, 1648, 1638, 1545, 1467, 1454, 1050 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.37–7.18 (m, 25H), 5.78–5.72 (m, 2H), 4.91 (d, *J* = 11.3 Hz, 1H), 4.81–4.75 (m, 3H), 4.71 (d, *J* = 11.8 Hz, 1H), 4.62 (d, *J* = 11.7 Hz, 1H), 4.56 (d, *J* = 11.4 Hz, 1H), 4.54 (d, *J* = 11.5 Hz, 1H), 4.41 (d, *J* = 11.6 Hz, 1H), 4.23–4.19 (m, 1H), 3.98 (dd, *J* = 10.0, 3.6 Hz, 1H), 3.81 (dd, *J* = 9.9, 2.5 Hz, 1H), 3.74–3.73 (m, 1H), 3.69–3.66 (m, 2H), 3.56–3.46 (m, 3H), 3.43 (s, 2H), 3.16–3.10 (m, 1H), 2.10–1.94 (m, 2H), 1.59–1.49 (m, 6H), 1.32–1.25 (m, 74H), 0.88 (t, *J* = 6.6 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.1, 171.2, 138.9, 138.8, 138.7, 138.6, 138.5, 129.5, 129.1, 128.7, 128.7, 128.6,

128.1, 127.9, 127.9, 127.7, 127.4, 99.3, 79.3, 79.0, 76.6, 75.3, 74.8, 73.7, 73.4, 72.2, 69.3, 68.0, 51.6, 43.9, 40.5, 37.0, 32.2, 31.0, 30.2, 30.0, 29.7, 29.6, 26.0, 25.5, 22.9, 14.3; HRMS (ESI) *m/z* calcd for $C_{86}H_{131}N_2O_8$ $[M + H]^+$ 1319.9905, found 1319.9865.

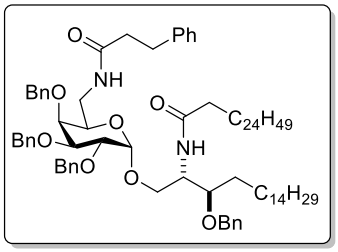
(2*S*,3*R*)-1-*O*-[6-Deoxy-6-phenylacetamido-α-D-galactopyranosyl]-2-hexacosylamino-octadecane-1,3-diol (17)



(2*S*,3*R*)-3-Benzyloxy-1-*O*-[2,3,4-tri-*O*-benzyl-6-deoxy-6-phenylacetamido-α-D-galactopyranosyl]-2-hexacosylaminooctadecane-1,3-diol (**57a**) (7.0 mg, 0.0050 mmol) was dissolved in dry EtOH/$CHCl_3$ (8 mL, 3:1) was added $Pd(OH)_2$/C (20 wt% Pd dry basis on carbon, 30 mg), and the mixture was hydrogenated under atmospheric pressure for 14 h. The solution was filtered through a pad of celite, and the filter cake was washed with EtOH/$CHCl_3$ (3:1). The combined filtrate was concentrated, and the resulting residue was purified by trituration with EtOAc (3 × 3 mL) to afford (2*S*,3*R*)-1-*O*-[6-deoxy-6-phenylacetamido-α-D-galactopyranosyl]-2-hexacosylaminooctadecane-1,3-diol (**17**) as a white solid (4.9 mg, 96%): mp = 168.3–170.0 °C; $[\alpha]_D^{23}$ = +36.4 (*c* = 1.0, $CHCl_3$/MeOH 1:1); IR (neat) 3418 (br), 3289 (br), 2918, 2850, 1641, 1551, 1466, 1076, 1033 cm^{-1}; ^1H NMR (400 MHz, pyridine-d_5) δ 8.82 (dd, *J* = 5.4, 5.4 Hz, 1H), 8.61 (d, *J* = 8.6 Hz, 1H), 7.63–7.59 (m, 1H), 7.38–7.34 (m, 2H), 7.28–7.24 (m, 1H), 5.68 (br s, 4H), 5.40 (d, *J* = 3.5 Hz, 1H), 4.77–4.68 (m, 1H), 4.58 (dd, *J* = 9.6, 3.6 Hz, 1H), 4.49–4.46 (m, 1H), 4.42–4.38 (m, 1H), 4.35–4.25 (m, 3H), 4.21–4.15 (m, 1H), 4.00–3.86 (m, 3H), 2.59–2.43 (m, 2H), 1.91–1.85 (m, 5H), 1.61–1.28 (m, 69H), 0.89–0.86 (m, 6H); ^{13}C NMR (100 MHz, pyridine-d_5) δ 173.6. 171.6, 137.1, 129.9, 128.9, 127.0, 102.0, 72.0, 71.3, 70.7, 70.3, 69.6, 54.9, 43.8, 41.6, 36.8, 35.3, 32.2, 30.2, 30.1,

30.1, 29.9, 29.8, 29.6, 26.6, 26.5, 23.0, 14.3; HRMS (ESI) *m/z* calcd for $C_{58}H_{86}N_2NaO_8$ $[M + Na]^+$ 981.7847, found 981.7848.

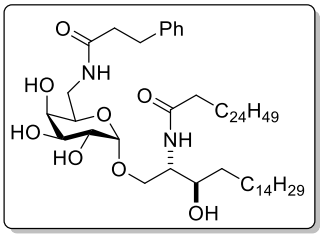
(2*S*,3*R*)-3-Benzyloxy-1-*O*-[2,3,4-tri-*O*-benzyl-6-deoxy-6-(3-phenylpropylamido)-α-D-galactopyranosyl]-2-hexacosylaminooctadecane-1,3-diol (57b)



A mixture of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (9.4 mg, 0.049 mmol) and phenylpropanoic acid (7.4 mg, .049 mmol) in dry DMF (1 mL) was stirred at rt for 1 h. Then (2*S*,3*R*)-1-*O*-(6-amino-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-galactopyranosyl)-3-benzyloxy-2-hexacosylaminooctadecane-1,3-diol (**56**) (40 mg, 0.033 mmol) was added, and the reaction mixture was allowed to stir for an additional 3 h. DMF was removed, and the residue was purified by flash chromatography on silica gel (hexane/EtOAc, 90:10) to afford (2*S*,3*R*)-3-benzyloxy-1-*O*-[2,3,4-tri-*O*-benzyl-6-deoxy-6-(3-phenylpropylamido)-α-D-galactopyranosyl]-2-hexacosylaminooctadecane-1,3-diol (**57b**) as a white solid (31 mg, 71%): mp = 113.8–114.7 °C; $[\alpha]_D^{23}$ = +38.0 (*c* = 1.0, $CHCl_3$); IR (neat) 3318 (br), 2921, 2853, 1642, 1545, 1459, 1148, 1100, 1052 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.40–7.14 (m, 25H), 5.91 (d, *J* = 8.6 Hz, 1H), 5.51 (t, *J* = 5.6 Hz, 1H), 4.93 (d, *J* = 11.4 Hz, 1H), 4.85 (d, *J* = 11.7 Hz, 1H), 4.82 (d, *J* = 3.7 Hz, 1H), 4.78 (d, *J* = 11.7 Hz, 1H), 4.73 (d, *J* = 11.7 Hz, 1H), 4.64 (d, *J* = 11.8 Hz, 1H), 4.62 (d, *J* = 11.4 Hz, 1H), 4.57 (d, *J* = 11.6 Hz, 1H), 4.43 (d, *J* = 11.5 Hz, 1H), 4.22–4.16 (m, 1H), 4.01 (dd, *J* = 10.0, 3.6 Hz, 1H), 3.89–3.80 (m, 2H), 3.70–3.63 (m, 3H), 3.55 (dd, *J* = 11.5, 5.8 Hz, 1H), 3.36 (ddd, *J* = 13.6, 6.6, 6.6 Hz, 1H), 3.20 (ddd, *J* = 13.5, 5.5, 5.5 Hz, 1H), 2.87 (t, *J* = 7.7 Hz, 2H), 2.37–2.19 (m, 2H), 1.64–1.24 (m, 4H), 1.64–1.24 (m, 73 H), 0.90–0.87 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.3, 172.3, 141.1, 138.7,

138.6, 138.6, 129.1, 128.8, 128.8, 128.7, 128.7, 128.6, 128.5, 128.1, 127.9, 127.9, 127.8, 127.6, 126.4, 99.8, 79.4, 79.3, 74.6, 73.7, 73.6, 72.4, 69.0, 68.8, 52.0, 40.1, 38.3, 37.0, 32.1, 31.8, 31.2, 30.1, 29.9, 29.8, 29.7, 29.6, 26.0, 25.5, 22.9, 14.3; HRMS (ESI) *m/z* calcd for $C_{87}H_{133}N_2O_8$ [M + H]$^+$ 1334.0062, found 1333.9927.

(2*S*,3*R*)-1-*O*-[6-Deoxy-6-(3-phenylpropylamido)-α-D-galactopyranosyl]-2-hexacosylamino-octadecane-1,3-diol (18)



(2*S*,3*R*)-3-Benzyloxy-1-*O*-[2,3,4-tri-*O*-benzyl-6-deoxy-6-(3-phenylpropylamido)-α-D-galactopyranosyl]-2-hexacosylaminooctadecane-1,3-diol (**57b**) (7.4 mg, 0.0050 mmol) was dissolved in dry EtOH/$CHCl_3$ (8 mL, 3:1). Then, $Pd(OH)_2$/C (20 wt% Pd dry basis on carbon, 30 mg) was added, and the mixture was hydrogenated under atmospheric pressure for 14 h. The solution was filtered through a pad of celite, and the filter cake was washed with EtOH/$CHCl_3$ (25 mL, 3:1). The filtrate was concentrated, and the resulting residue was purified by trituration with EtOAc (3 × 3 mL) to afford (2*S*,3*R*)-1-*O*-[6-deoxy-6-(3-phenylpropylamido)-α-D-galactopyranosyl]-2-hexacosylamino-octadecane-1,3-diol (**18**) as a white solid (5.7 mg, 97%): mp = 174.2–175.5 °C; $[\alpha]_D^{23}$ = +35.2 (*c* = 1.0, $CHCl_3$/MeOH 1:1); IR (neat) 3417 (br), 3292 (br), 2919, 2850, 1640, 1557, 1468, 1138, 1082, 1042 cm^{-1}; ^{1}H NMR (400 MHz, pyridine-d_5) δ 8.85 (t, *J* = 5.3 Hz, 1H), 8.62 (d, *J* = 8.4 Hz, 1H), 7.38–7.36 (m, 2H), 7.33–7.29 (m, 2H), 5.45 (br s, 4H), 4.72 (m, 1H), 4.60 (dd, *J* = 9.8, 3.6 Hz, 1H), 4.51–4.44 (m, 2H), 4.38 (dd, *J* = 10.0, 3.1 Hz, 1H), 4.30–4.27 (m, 3H), 4.19 (ddd, *J* = 13.4, 6.6, 6.6 Hz, 1H), 3.95 (ddd, *J* = 13.2, 6.8, 6.8 Hz, 1H), 3.23 (t, *J* = 7.4 Hz, 2H), 2.92–2.77 (m, 2H), 2.60–2.47 (m, 2H), 1.90–1.85 (m, 5H), 1.58–1.53 (m, 1H), 1.48–1.18 (m, 69H), 0.89–0.86 (m, 6H); ^{13}C NMR (100 MHz, pyridine-d_5) δ 173.6, 173.0, 142.2, 128.9, 128.8, 126.4, 102.0,

72.0, 71.3, 71.0, 70.6, 70.3, 69.5, 55.0, 41.2, 38.4, 36.8, 35.3, 32.3, 32.2, 30.2, 30.1, 30.1, 29.9, 29.8, 29.6, 26.7, 26.5, 23.0, 14.3; HRMS (ESI) *m/z* calcd for $C_{59}H_{109}N_2O_8$ $[M + H]^+$ 973.8184, found 973.8209.

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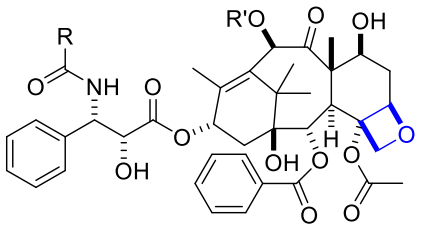
Chapter 2 New reactions of 2-methyleneoxetanes

2.1 Introduction

As a class of structurally unique organic compounds, oxetanes have been the focus of much attention in organic synthesis. Such interest in this structural motif stems from its applications in material and polymer science; cationic ring-opening polymerization;[1] and as crosslinkers.[2] Strategies employing oxetanes as synthetic intermediates include: (a) ring opening with nucleophiles; (b) ring expansions; and (c) rearrangements.[3,4] The oxetane moiety is found in natural products, biologically active compounds, and agrochemicals.[4] Oxetanes have also emerged as important structures in medicinal chemistry due to their physicochemical properties.[5]

2.1.1 Oxetanes in natural products

One of the most well-known oxetane-containing natural products is paclitaxel, also known by its brand name, Taxol (Figure 29). Taxol is an anti-cancer chemotherapy drug that was first isolated in 1971 from the bark of the pacific yew tree, *Taxus brevifolia*.[6] Between 1992 and 1994, Taxol was approved by the FDA for the treatment of both ovarian and breast cancer.[7] Taxol, along with a structurally related compound called docetaxel which is sold under the brand name Taxotere, are presently used in chemotherapy (Figure 29).[8]



paclitaxel (Taxol) R = Ph; R' = Ac
docetaxel (Taxotere) R = O*t*Bu; R' = H
chemotherapy agents

Figure 29. Structures of paclitaxel (Taxol) and docetaxel (Taxotere).

Only a few natural products contain oxetane rings, but when present, this scaffold is often important for biological activity (Figure 30). Oxetanocin A is a nucleoside analogue that was

isolated from the fermentation broth of bacteria, *Bacillus megaterium*,[9,10] and showed potent inhibition against HIV,[11] hepatitis B virus,[12] herpes simplex virus,[9] and human cytomegalovirus.[13] Oxetin was isolated from the soil bacterium, *Streptomyces sp.* OM-2317, and exhibited herbicidal and antibacterial activity.[14] Thromboxane A_2 is produced by activated platelets to promote vasoconstriction and platelet aggregation.[15] Ramariolide B was isolated from the mushroom, *Ramaria cystidiophora*, and contains an unusual spiro oxetane moiety; it showed *in vitro* antimicrobial activity against *Mycobacterium tuberculosis* and *Mycobacterium smegmatis*.[16] Maoyecrystal I and Mitrephorone A are both polycyclic diterpenoids shown to be cytotoxic.[17,18] Laureatin is a major metabolite of the red marine algae genus, *Laurencia nipponica*, and has shown potent insecticidal activity against mosquitos.[19] Dictyoxetane is a marine diterpene isolated from a sample of the brown algae, *Dictyota dichotoma;* its biological activity is currently unknown.[20] Merrilactone A was isolated from *Illicium merrillianum* and has shown neurotropic activity in cultures of fetal rat cortical neurons.[21] Bradyoxetin is produced by the soil bacterium, *Bradyrhizobium japonicum,* and is involved in gene regulation in the soybean plant.[22] Finally, Pennigritrem is a tremorgenic mycotoxin isolated from the fungus, *Penicillium nigricans*.[23]

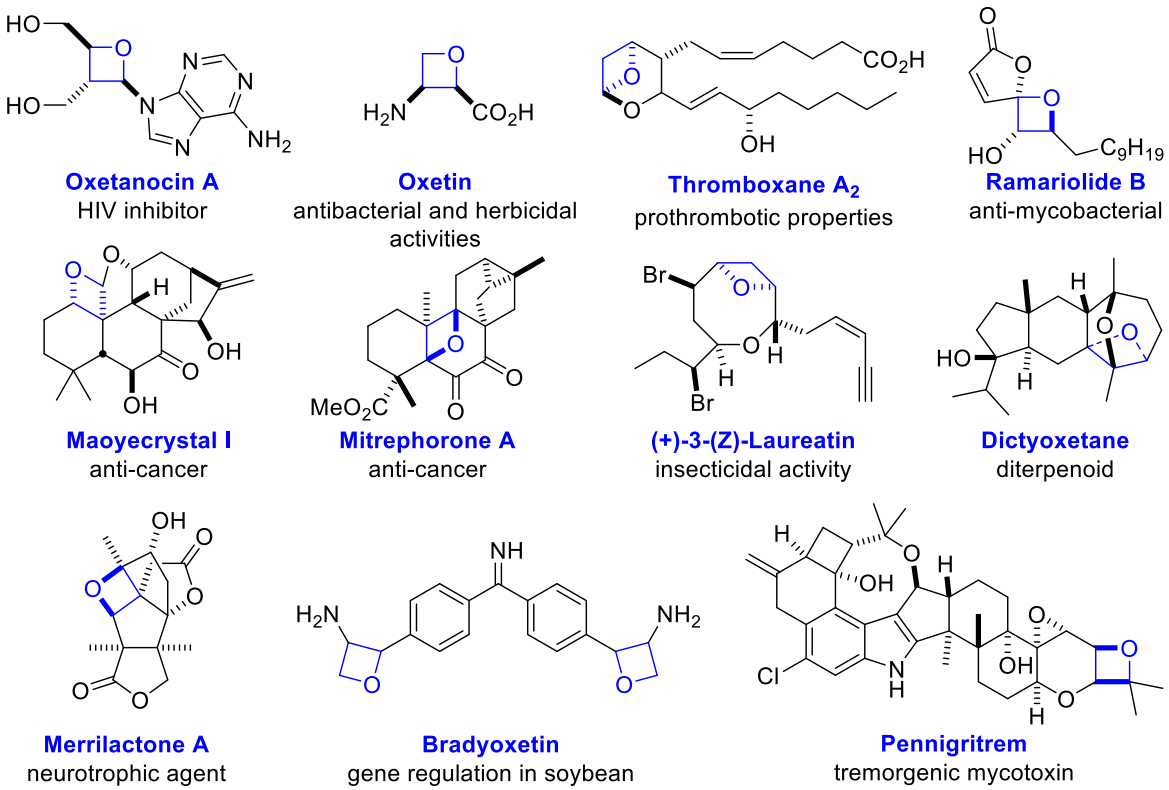


Figure 30. Oxetane-containing natural products.

2.1.2 Oxetanes as replacement groups

In recent years, incorporation of the oxetane moiety has surged, mainly due to its ability to influence solubility, lipophilicity, metabolic stability, basicity, and other physicochemical properties of drugs and drug candidates. In 2006, Carreira and co-workers demonstrated the remarkable abilities of the 3,3-disubstituted oxetane motif as a replacement group (isostere) for *gem*-dimethyl groups in medicinal chemistry (Figure 31A).[24] In drug discovery, a *gem*-dimethyl group is commonly used to block metabolically unstable methylene groups. However, the replacement of methylene hydrogens with methyl groups can result in increased lipophilicity, which may have adverse effects on the physicochemical and pharmacokinetic properties of compounds. Results showed substituting a *gem*-dimethyl group with an oxetane group increased the polar surface area without changing the overall conformation or molecular size; this decreased lipophilicity and increased metabolic stability.

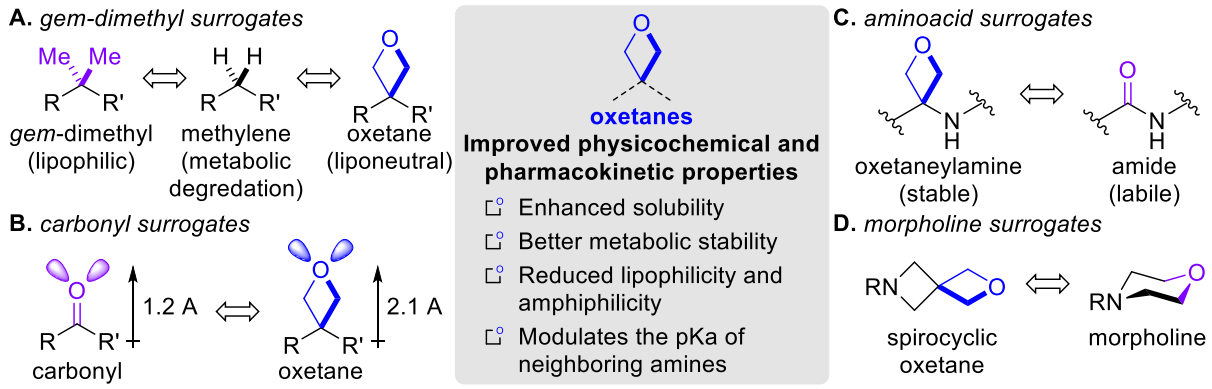


Figure 31. Oxetanes as surrogates for (A) *gem*-dimethyl, (B) carbonyl groups, (C) aminoacids, and (D) morpholines.

Carreira and co-workers later investigated using oxetane as carbonyl surrogates (Figure 31B).[25] The lone pair of electrons on the oxygen of oxetane and on the carbonyl group share similar spatial orientation, dipoles, and H-bonding properties. Replacement of the carbonyl group with an oxetane could be beneficial due to the susceptibility of carbonyl compounds (aldehydes, ketones, and esters) to undergo enzymatic modification and α-deprotonation/epimerization of stereogenic centers. This methodology was later utilized in the synthesis of oxetanyl peptides, where the amide bond is replaced by a non-hydrolyzable aminooxetane fragment (Figure 31C).[26]

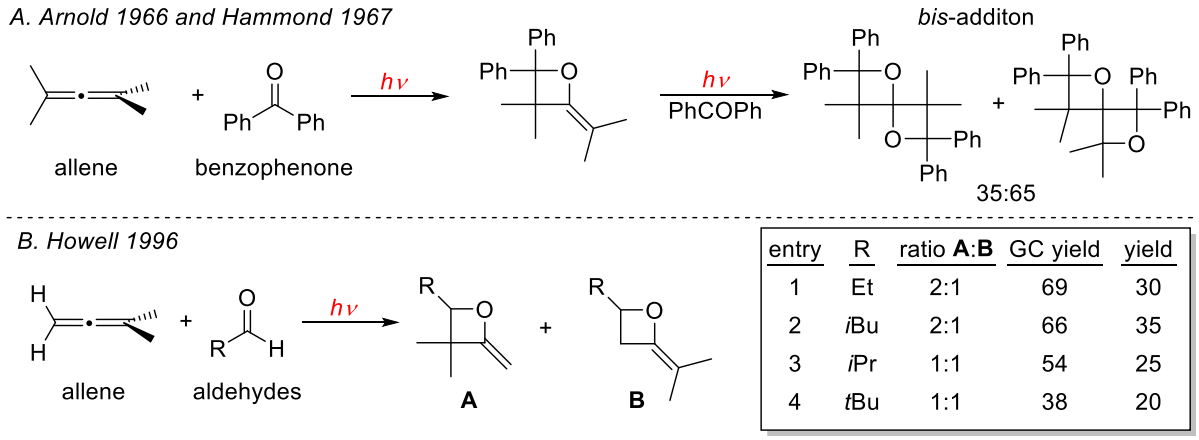
Carreira and co-workers also demonstrated that a spirocyclic oxetane could serve as a surrogate for morpholine, a common motif in pharmaceutical drugs (Figure 31D).[25,27] Morpholine is often incorporated into drug scaffolds to increase solubility, however it can be susceptible to oxidative metabolism. Many drug candidates fail to reach clinical trials due to poor solubility, poor lipophilicity, or metabolic instability; thus, incorporation of the oxetane motif into small molecules can enhance physicochemical expand the pool of potential drug candidates.

2.1.3 Introduction to 2-methyleneoxetanes

2-Methyleneoxetanes, oxetanes that bear an exocyclic double bond at the C2 position, are a largely unexplored class of strained heterocycles. The unique combination of functionalities and reactivities contained in oxetanes and an exocyclic double bond offers intriguing possibilities for further manipulation.

2.1.3 Synthesis of 2-methyleneoxetanes

The synthesis of 2-alkylideneoxetanes was first reported in the 1960s utilizing a Paternò-Büchi reaction. Arnold and Glick showed that benzophenone could be added to tetramethylallene under a high-pressure mercury arc lamp to give photochemical cycloaddition products (Scheme 14A),[28] although yields were low, and the major products tended to be the *bis*-addition of the carbonyl compounds to give dioxaspiroheptanes. Concurrently, Hammond and co-workers extended the substrate scope of the Paternò-Büchi reaction with various allene and carbonyl compounds.[29] The yields for the formation 2-alkylideneoxetanes were higher than that of benzophenone, likely due to the stability of the oxetanes formed.

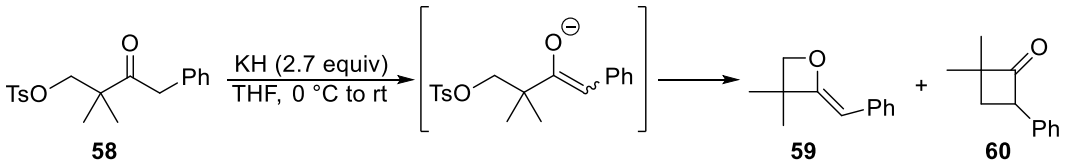


entry	R	ratio **A**:**B**	GC yield	yield
1	Et	2:1	69	30
2	*i*Bu	2:1	66	35
3	*i*Pr	1:1	54	25
4	*t*Bu	1:1	38	20

Scheme 14. (A) Synthesis of 2-alkylideneoxetanes by the Paternò-Büchi reaction. (B) Synthesis of 2-methyleneoxetanes by the Paternò-Büchi reaction.

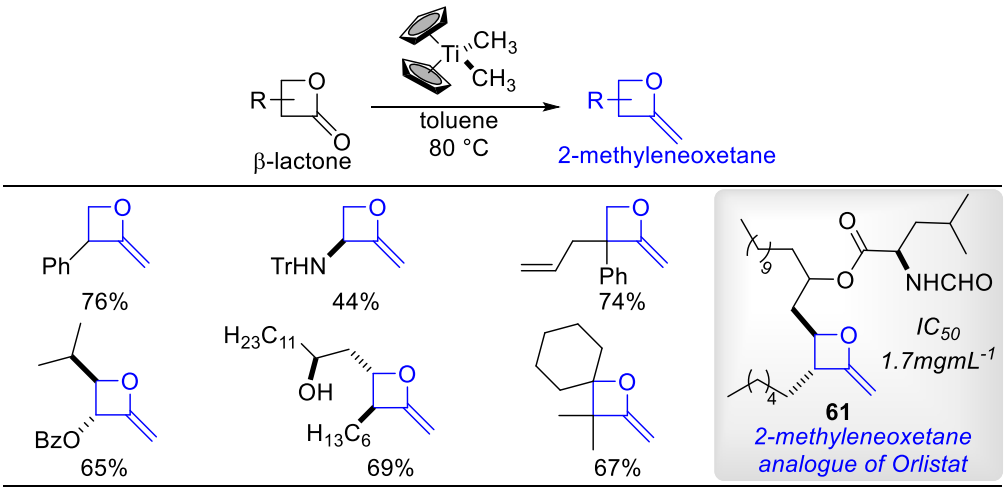
In 1996, Howell and co-workers utilized the Paternò-Büchi reaction with aliphatic aldehydes and a range of allenes (Scheme 14B).[30] Reaction of 3-methyl-1,2-butadiene (dimethylallene) with various aldehydes gave 2-methyleneoxetanes and 2-alkylideneoxetanes as the major products. However, these reactions had significant disadvantages in poor selectivity and generally low yields. In addition, require a large excess of allene was required, and slow reacting aldehydes exhibited more side-products.

In the 1970s, Hudrlik and Mohtady demonstrated that 2-alkylideneoxetanes could be synthesized through intramolecular *O*-alkylation of ketone enolates (Scheme 15).[31] Treatment of ketone **58** with potassium hydride afforded the *O*-alkylation product, 2-benzylideneoxetane (**59**), along with the *C*-alkylation product, cyclobutanone (**60**), in estimated yields of 36% and 25%, respectively.



Scheme 15. Synthesis of 2-alkylideneoxetanes through intramolecular *O*-alkylation.

In 1996, Howell and co-workers reported the first general protocol for the synthesis of 2-methyleneoxetanes (Scheme 16).[32] It was found that 2-methyleneoxetanes could be obtained through the methylenation of β-lactones using a Petasis reagent, dimethyltitanocene. This approach proved to be a useful way to generate various substituted 2-methyleneoxetanes in good isolated yields. This protocol was also used for the synthesis of a 2-methyleneoxetane analogue of orlistat, an anti-obesity drug (Scheme 16).[33] In comparing the IC_{50} values of orlistat and analogue **61** in an assay against porcine pancreatic lipase (PPL), lower activity was found for the methyleneoxetane analogue **61**. The lower activity of **61** is significant considering the carbonyl group of orlistat is believed to be integral for its biological activity.



Scheme 16. Synthesis of 2-methyleneoxetanes via methylenation of β-lactones.

2.1.4 The reactivity of 2-methyleneoxetanes

Over the last two decades, the Howell group has extensively investigated the reactivity of 2-methyleneoxetanes (Figure 32). The reactivity of 2-methyleneoxetanes can be separated into two types of transformations; 1) ring opening of the oxetane; and 2) oxetane-intact functionalization of the exocyclic double bond. For instance, treatment of 2-methyleneoxetanes **62** with lithium diisopropylamide (LDA) and an electrophile resulted in the efficient formation of homopropargylic alcohols **63**.[34] Reductive ring opening of 2-methyleneoxetanes **62** with lithium di-*tert*-butyldiphenyl (LDBB), followed by addition of an electrophile, gave functionalized ketones **64**.[35] Nucleophilic ring opening of **62** with strong nucleophiles, such as carbanion or heteroatom nucleophiles, afforded β-substituted ketones **65**.[36,37] Additionally, 2-methyleneoxetanes **62** have been reported to undergo rearrangement to α,β-unsaturated methylketones **66** at high temperatures.[38]

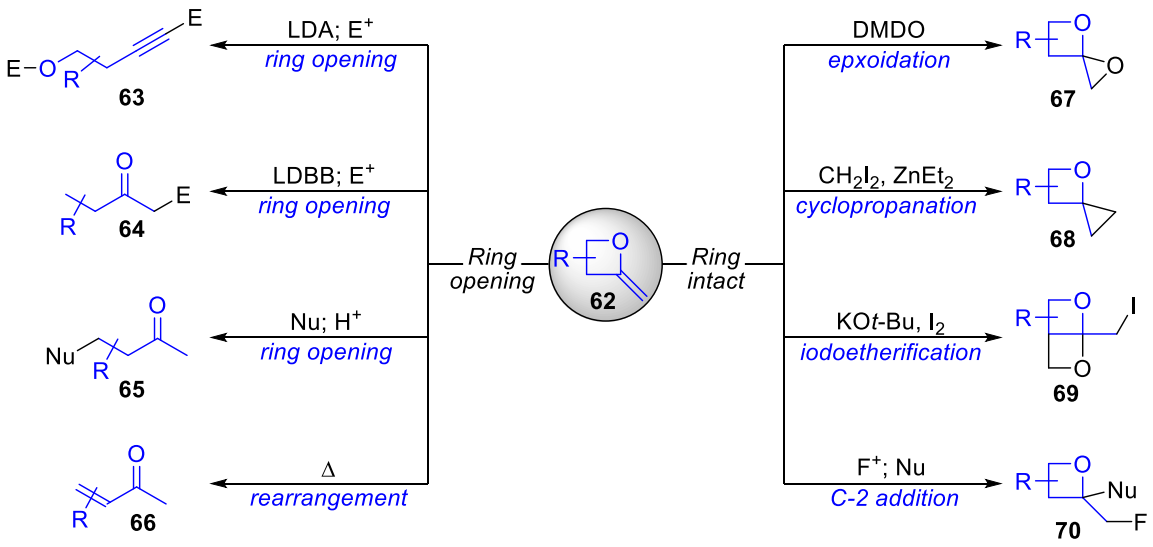
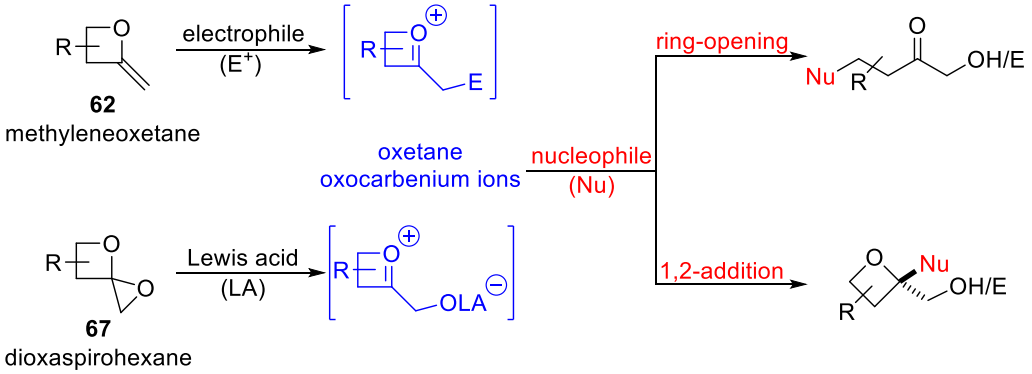


Figure 32. Reactivity of 2-methyleneoxetanes.

In addition to ring opening reactions, 2-methyleneoxetanes can be converted to a variety of oxetane-intact species. For instance, treatment 2-methyleneoxetanes **62** with anhydrous dimethyldioxirane (DMDO) afforded a very reactive species, 1,5-dioxaspiro[3.2]hexanes **67**, in excellent yields.[39,40] Cyclopropanation of the exocyclic double bond on 2-methyleneoxetanes **62** via modified Simmons-Smith conditions provided 4-oxaspiro[2.3]hexanes **68**.[41] Intramolecular iodetherification of 2-methyleneoxetanes **62** with potassium *tert*-butoxide (KO*t*Bu) and I_2 provided the first example of a [2.2.0]-fused ketal **69**.[42] 2-Methyleneoxetanes **62** were also used to access oxetanocin-type analogues **70** by electrophilic addition of F^+ followed by nucleophilic attack of the nucleobase.[43]

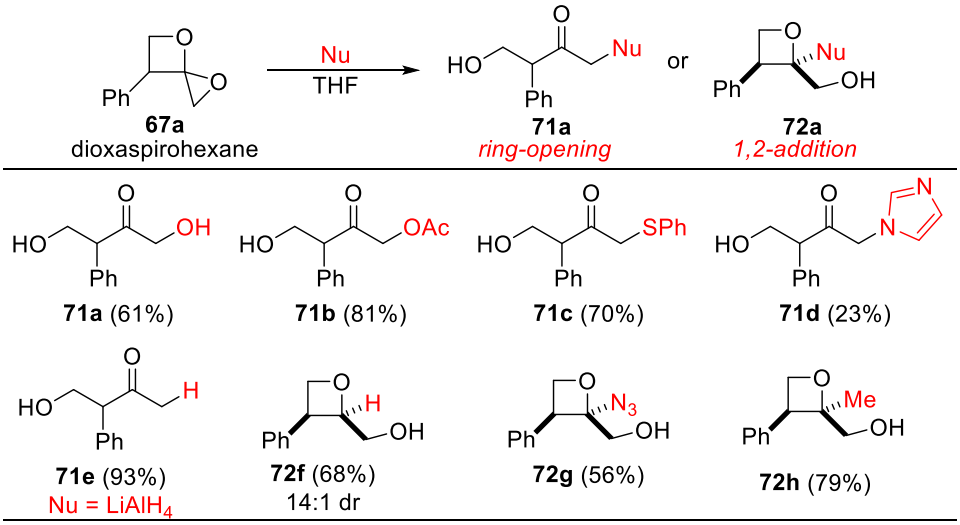
2.1.4.1 Generation and transformations of oxetane oxocarbenium ions

In exploring the reactivity of 2-methyleneoxetanes **62** and 1,5-dioxaspiro[3.2]hexanes **67**, similar reactivity was observed in the generation of oxetane oxocarbenium ions using suitable electrophiles or Lewis acids (Scheme 17). Subsequent addition of various nucleophiles gave two distinct reaction pathways, ring opening or 1,2-addition. In this section, our work on the generation of oxetane oxocarbenium ions from 2-methyleneoxetanes and 1,5-dioxaspiro[3.2]hexanes, and their reactivity with various nucleophiles, will be discussed.



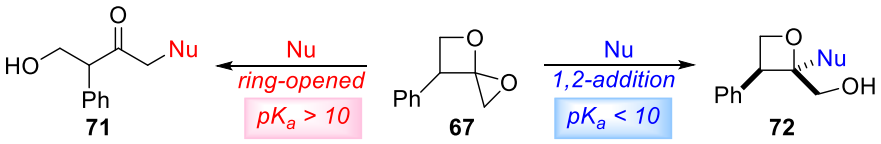
Scheme 17. Generation of oxetane oxocarbenium ion from 2-methyleneoxetane or 1,5-dioxaspiro[3.2]hexane, and reaction with nucleophiles, to obtain ring open or oxetane intact products.

The reactivity of 1,5-dioxaspiro[3.2]hexanes **67a** was first investigated with a variety of heteroatom nucleophiles, hydride donors, and organoaluminum reagents (Scheme 18).[40] It was found that some nucleophiles, such as water, tetrabutylammonium acetate, sodium thiolate, and imidazole, gave the corresponding hydroxyl ketones **71a**-**71d**. Lithium aluminum hydride also gave ring opening followed by reduction to give 1,3-diol **71e**. Interestingly, treatment of 1,5-dioxaspiro[3.2]hexanes **67a** with diisobutylaluminum hydride (DIBAL-H) gave nucleophilic attack at the internal position of the ring to give oxetane-intact product **72f**. Ring opening of just the epoxide also occurred when $TMSN_3$ and $AlMe_3$ were used as nucleophiles, giving oxetane-intact products **72g** and **72h**, respectively. In these reactions, coordination of the epoxide oxygen to the Lewis acid was proposed with generation of an oxetane oxocarbenium ion (see Scheme 17).



Scheme 18. Reactions of 1,5-dioxaspiro[3.2]hexanes with various nucleophiles.

In 2003, Howell and co-workers reported the reaction of 1,5-dioxaspiro[3.2]hexanes **67** with heteroaromatic nucleophiles. The pK_a of the nucleophile was correlated to the reaction outcome (Table 1).[44] It was shown that more acidic nucleophiles (pK_a < 10) led to oxetane-intact products **72** via 1,2-addition, while more basic nucleophiles (pK_a > 10) gave ring-opened products **71**.



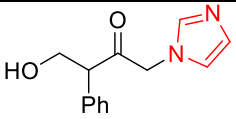
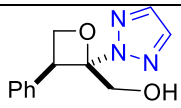
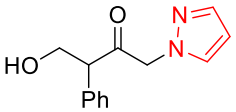
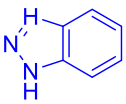
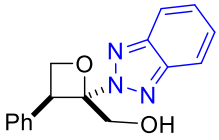
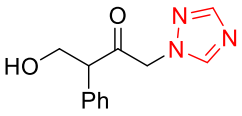
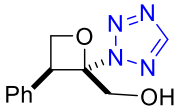
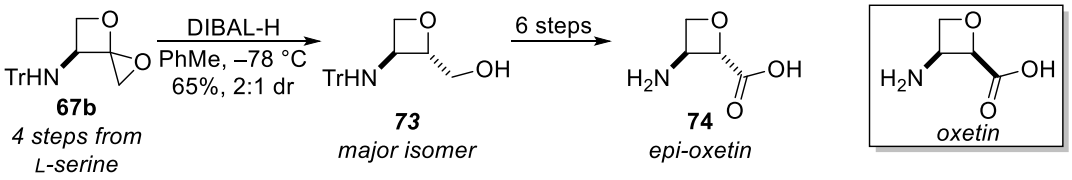
nucleophile	*pK*$_a$	product	% yield	*nucleophile*	*pK*$_a$	product	% yield
	14.5		50%		9.3		59%
	14.2		90%		8.2		45%
	10.3		45%		4.9		42%

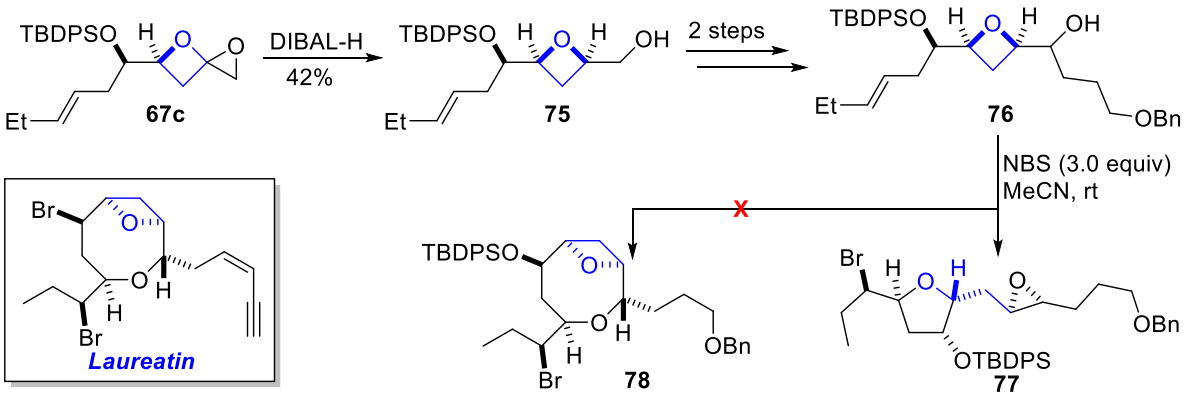
Table 1. Reactions of dioxaspirohexanes with nucleophiles and the effects of pK_a.

Formation of the oxetane oxocarbenium ion from dioxaspirohexanes has been utilized in the syntheses of C-2 functionalized oxetanes (Scheme 19).[45] *epi*-Oxetin **74** was synthesized from an L-serine derived 1,5-dioxaspiro[3.2]hexane **67b**, which underwent DIBAL-H epoxide ring opening to provide 2-hydroxymethyloxetane **73** as a key intermediate.



Scheme 19. 1,5-Dioxaspiro[3.2]hexane **67b** utilized in the synthesis of *epi*-oxetin **74**.

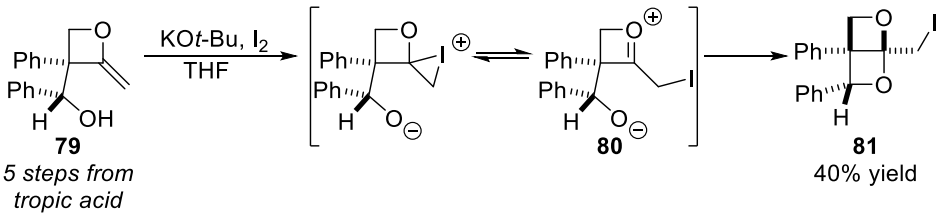
In 2012, epoxide ring opening of 1,5-dioxaspiro[3.2]hexane **67c** with DIBAL-H was used to generate hydroxymethyloxetane **75** as a possible key intermediate in the synthesis of laureatin (Scheme 20).[46] However, attempted *N*-bromosuccinimide (NBS)-mediated cyclization of oxetane alcohol **76**, provided an unexpected rearrangement, yielding epoxytetrahydrofuran **77**, rather than the expected laureatin core **78**.



Scheme 20. Unexpected rearrangement of oxetane alcohol **76** afforded epoxytetrahydrofuran **77**.

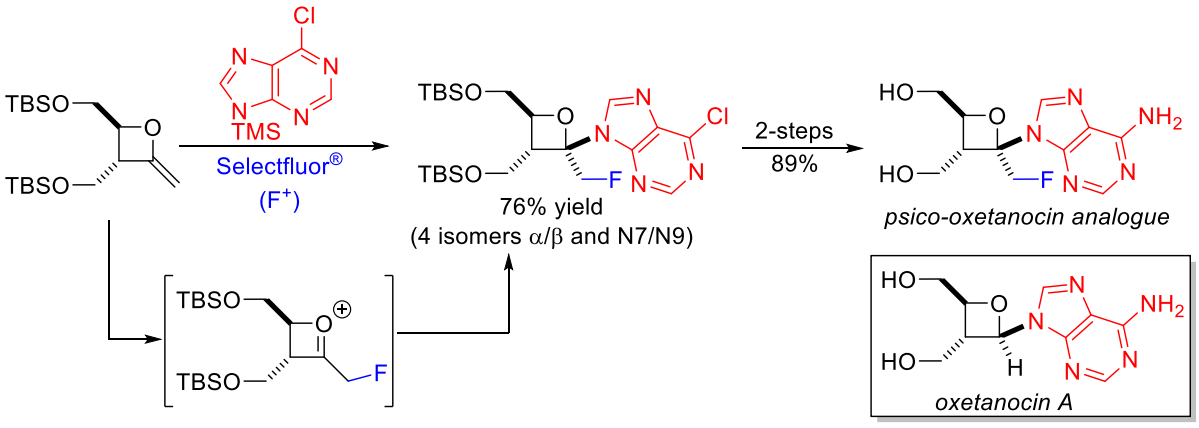
Similarly, 2-methyleneoxetanes, the precursors of 1,5-dioxaspiro[3.2]hexanes, have been utilized in the generation of oxetane oxocarbenium ions with suitable electrophiles. In particular, we reported an intramolecular iodoetherification of 2-methyleneoxetane **79** to provide an

intermediate oxetane oxocarbenium ion **80** (Scheme 21).[42] Subsequent trapping of **80** with the pendant alcohol provided the first synthesis of a [2.2.0]-fused ketal, **81**, in 40% yield.



Scheme 21. Synthesis of [2.2.0]-fused ketal **81** via oxocarbenium ion with *O*-nucleophile.

A similar strategy was used in the first *psico*-oxetanocin analogue of the potent antiviral natural product, oxetanocin A.[43] This was achieved through F^+-mediated oxetane oxocarbenium ion formation followed by 1,2-additon of the nucleobase (Scheme 22).



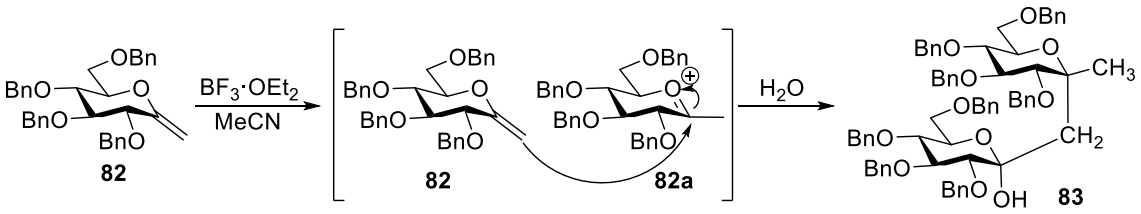
Scheme 22. Utility of oxetane oxocarbenium ion in the synthesis of *psico*-oxetanocin analogue.

2.1.5 Exocyclic enol ethers as nucleophiles in carbon-carbon bond formation

Exocyclic enol ethers are useful in many reaction for introducing diverse functionality to the exocyclic double bond. Exocyclic enol ethers have been used as substrates in pericyclic reactions, radical reactions, rearrangements, hydrogenation, oxidative additions, hydroboration, and as both electrophiles and nucleophiles. However, exocyclic enol ethers as nucleophiles in carbon-carbon bond forming reactions has received limited attention. Carbon-carbon bond formation is widely regarded by synthetic organic chemistry for applications in natural product

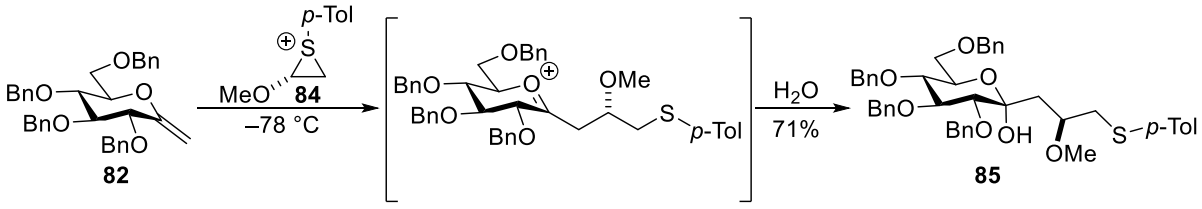
synthesis and medicinal chemistry. This section will demonstrate the utility of exocyclic enol ethers as nucleophiles in carbon-carbon bond formation reactions.

In 1992, Nicotra and co-workers synthesized *C*-disaccharides by a Lewis acid catalyzed dimerization of *exo*-glycals (Scheme 23).[47] This reaction was proposed to proceed via the nucleophilic attack of the exocyclic enol ether **82** to the oxonium ion **82a** formed *in-situ* from another molecule of the **82**. Subsequent addition of water provided *bis*-tetrahydropyran **83** in 66% yield with excellent stereoselectivity.



Scheme 23. Synthesis of *C*-disaccharides through dimerization of *exo*-glycals.

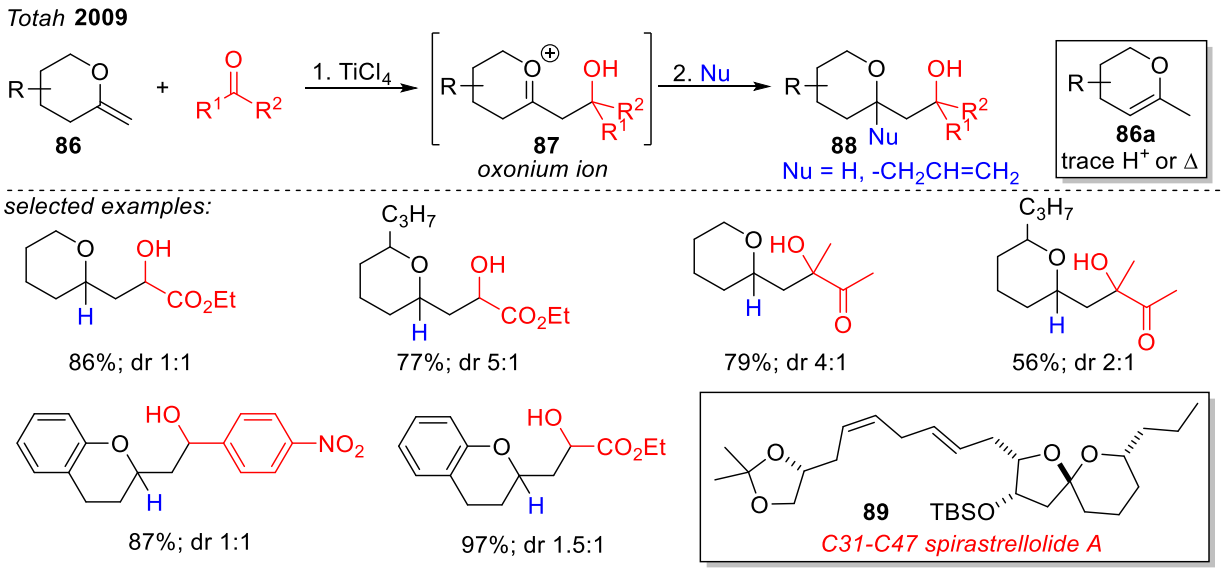
Smoliakova and co-workers demonstrated the nucleophilic properties of *exo*-glucal for a one-pot synthesis of *β-C*-glucopyranosides (Scheme 24).[48] This was achieved through the nucleophilic addition of *exo*-glycal **82** to episulfonium ion **84**, followed by trapping of the oxonium ion intermediate with water to provide *β-C*-glucopyranoside **85**. The observed stereoselectivity of the addition of water from the opposite face of the C-6 substituent to afford the β-anomer is based upon steric and electronic factors.[49]



Scheme 24. Synthesis of *β-C*-glucopyranosides from *exo*-glycal.

Totah and co-workers demonstrated the feasibility of using exocyclic enol ethers in nucleophilic addition processes for the synthesis of tetrahydropyranyl ketides (Scheme 25).[50] In

a three component coupling reaction, 2-methylenetetrahydropyrans **86** underwent nucleophilic addition to an activated aldehyde or ketone with trapping of the oxonium ion intermediate **87** by a secondary nucleophile providing β-hydroxy-tetrahydropyrans **88**. This protocol used equimolar amounts of the aldehyde and Lewis acid to facilitate rapid addition of the exocyclic enol ether prior to competing double bond isomerization **86a**. This transformation was highlighted in the synthesis of a C31-C47 fragment of spirastrelloide A (**89**), a potent and selective inhibitor of protein phosphatase 2A.[51]

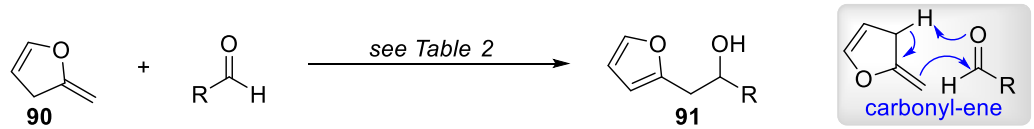


Scheme 25. Three component coupling reaction of 2-methylenetetrahydropyrans and application in the synthesis of the C31-C47 fragment of spirastrelloide A.

2.1.6 Carbonyl-ene reactions of exocyclic enol ethers

Carbonyl-ene reactions of acyclic enol ethers in the presence of Lewis acid catalysts have been extensively studied.[52] However, exocyclic enol ethers are rarely used in carbonyl-ene reactions. To date, only one general methodology for exocyclic enol ether carbonyl-ene reactions has been published. Furthermore, syntheses which utilize this chemistry include only two examples: product formation driven by aromatization[53,54] and a Diels-Alder reaction gave some byproduct formation.[56] Thus, exocyclic enol ethers have been under-utilized in carbonyl-ene reactions.

In 2005, Miles and co-workers demonstrated that β-hydroxyfurans **91** could be obtained via the carbonyl-ene reaction of 2-methylene-2,5-dihydrofuran **90** with various enophiles (Table 2).[53] Reaction of **90** with the highly activated enophile, ethyl glyoxylate, provided β-hydroxyfuran **91a** in 89% yield, without the presence of a catalyst (Entry 1). Non-activated enophiles in the presence of Lewis acid catalysts, zinc(II) chloride and Yb(fod)$_3$, gave β-hydroxyfurans **91b** and **91c**, respectively (Entries 2 and 4) in good to excellent yields. Interestingly, the carbonyl-ene reaction of **90** with decanal using titanium(IV)/(*S*)-BINOL catalyst provided (*S*)-**91b** with good enantioselectivity (Entry 3). An important driving factor in this carbonyl-ene methodology is the development of the furan aromaticity.



90 **91**

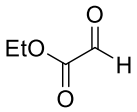
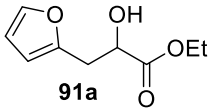
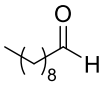
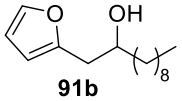
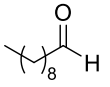
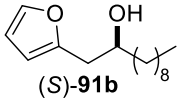
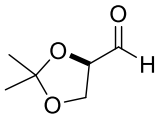
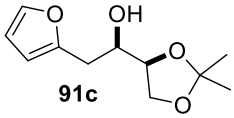
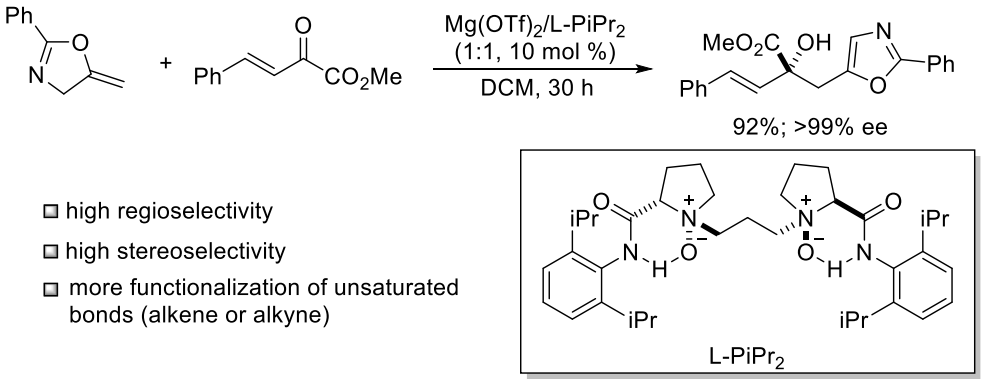
entry	substrate	conditions (temp; solvent; catalyst)	product	yield (%) [% ee or de]
1		0°C; DCM; none	 **91a**	89
2		22°C; DCM; $ZnCl_2$	 **91b**	78
3		22°C; Et_2O; Ti(IV)/(*S*)-BINOL	 (*S*)-**91b**	65 [94]
4		0°C; DCM; $Yb(fod)_3$	 **91c**	93 [65]

Table 2. Carbonyl-ene reaction of **90** with various enophiles to provide β-hydroxy furans.

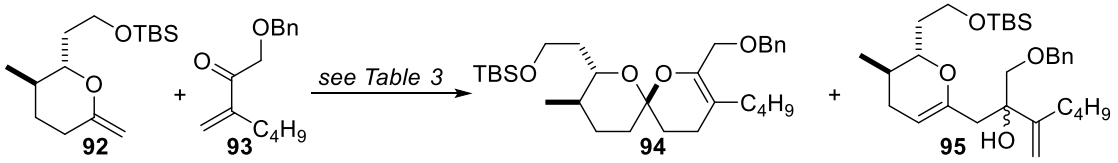
Similarly, Feng and coworkers demonstrated a catalytic asymmetric carbonyl-ene reaction of β,γ-unsaturated α-ketoesters with 5-methyleneoxazolines in the presence of a chiral *N*,*N'*-dioxide-Mg^{II} complex (Scheme 26).[54] A series of chiral α-hydroxyesters with a quaternary center were obtained with excellent enantioselectivites. The utility of this methodology is highlighted by

the unsaturated bonds (alkene or alkyne) in the desired products, which allows further derivatization, and the resulting oxazole units are found in various natural products and biologically active compounds.[55]



Scheme 26. Catalytic asymmetric carbonyl-ene reaction of β,γ-unsaturated α-ketoesters with 5-methyleneoxazolines.

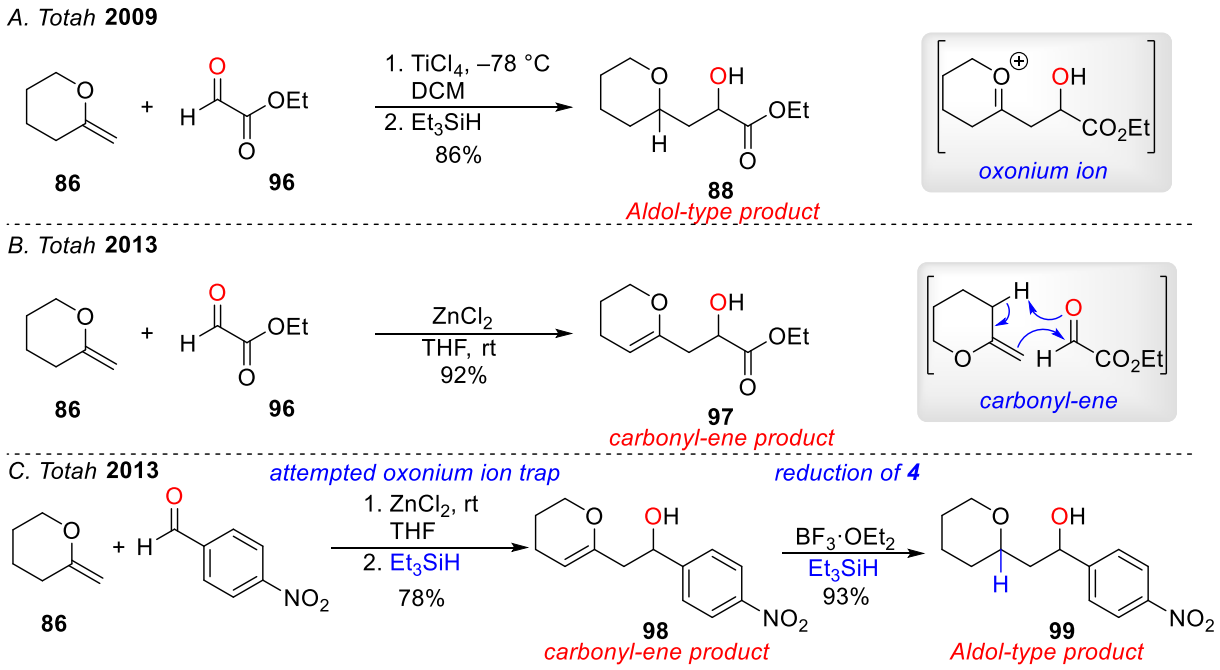
Rizzacasa and co-workers observed the formation of carbonyl-ene adduct **95** as a byproduct in the Lewis acid catalyzed hetero-Diels-Alder reaction of a 2-methylenetetrahydropyran **92** (Table 3).[56] Reaction of exocyclic enol ether **92** with enone **93** at high temperatures gave the desired Diel-Alder product (**3**), in low yields (Entries 1 and 2). Addition of the Lewis acid catalyst, $Eu(fod)_3$, improved the yield of the spiroketal **94;** however, the formation of a carbonyl-ene byproduct (**95**) also resulted (Entries 3 and 4).



entry	additive (mol%)	solvent	temp (°C)	time (h)	yield (%)	ratio (**3**:**4**)
1	K_2CO_3 (50)	toluene	110	24	11	1:0
2	K_2CO_3 (50)	neat	110	24	26	1:0
3	$Eu(fod)_3$ (15)	MeCN	0	48	35	1:1.3
4	$Eu(fod)_3$ (15)	neat	0	72	59	2:1

Table 3. Reaction of 2-methylenepyran **92** with enone **93**.

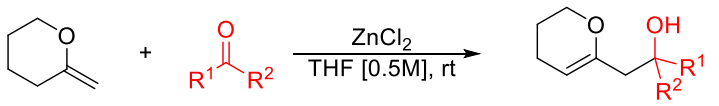
In 2013, Totah and co-workers reported an interesting result upon further evaluation of the reaction conditions associated with the three component coupling reaction (Scheme 27).[57] Reaction of exocyclic enol ether **86** with ethyl glyoxylate **96** in the presence of 1 equiv zinc chloride at room temperature gave the carbonyl-ene product **97**, instead of the anticipated aldol-type product (**88**). Similar results were obtained when the reaction was conducted at concentrations of 0.5 M using only 5 mol % of $ZnCl_2$. Support for the carbonyl-ene type mechanism comes from the formation of the carbonyl-ene product **98** in the presence of 3 equiv triethylsilane (Et_3SiH) (Scheme 27C). However, treatment of product **98** with $BF_3{\cdot}OEt_2$ and triethylsilane provided the tetrahydropyran product **99** in 93% yield.



Scheme 27. Lewis acid and temperature dependent transformations of 2-methylenetetrahydropyrans.

The scope of this transformation was further evaluated by varying the enophile component (Table 4). Good to excellent yields were obtained when the reaction was carried out in THF at room temperature with catalytic $ZnCl_2$. As shown, the reaction was successful with activated aldehydes and ketones (Entries 1 and 2), aromatic aldehydes bearing either an electron withdrawing or an electron donating group (Entries 3 and 4), and α,β-unsaturated (Entry 6),

aliphatic (Entry 7), or sterically hindered (Entry 8) substrates. Higher yields were obtained for less activated aldehydes (Entry 5) by increasing the amount of $ZnCl_2$ to 20 mol %.



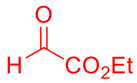
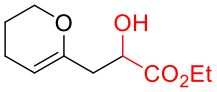
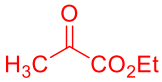
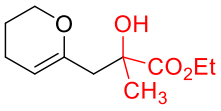
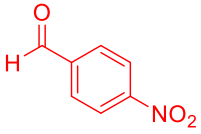
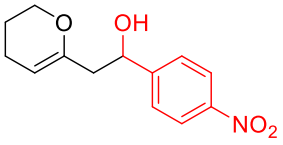
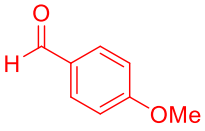
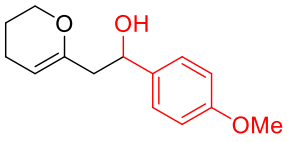
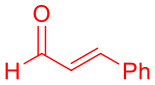
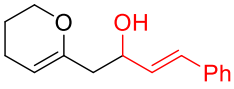
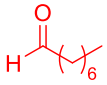
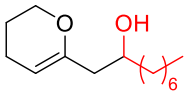
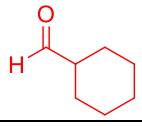
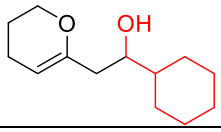
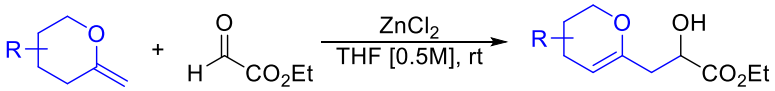
entry	enophile	time	$ZnCl_2$	product	yield
1		2 h	5 mol %		92%
2		15 h	5 mol %		90%
3		2 h	5 mol %		90%
4		24 h	5 mol %		68%
5		24 h	20 mol %		95%
6		24 h	20 mol %		75%
7		12 h	5 mol %		93%
8		24 h	5 mol %		85%

Table 4. Reaction of 2-methylenetetrahydropyran with various enophiles.

This methodology was further explored by reacting various exocyclic enol ethers with ethyl glyoxylate (Table 5). Under the optimized conditions, 2-methylenetetrahydropyrans with substitution at C-3 (Entries 1 and 2) or C-6 (Entries 3 and 4) and the electron-deficient dihydrocumarin enol ether (Entry 5) underwent reaction with ethylglyoxylate to provide the carbonyl-ene adducts in excellent yields. Interestingly, reaction of a seven-membered enol ether (Entry 6) with ethyl glyoxylate afforded the hydrolyzed carbonyl-ene adduct in moderate yield.



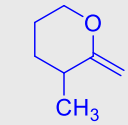
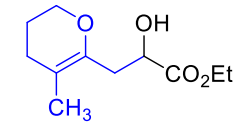
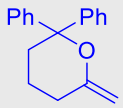
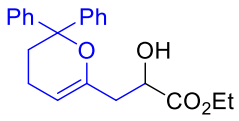
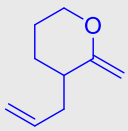
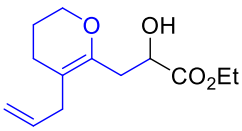
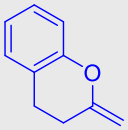
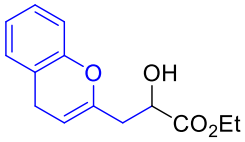
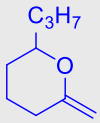
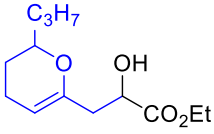
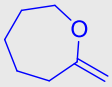
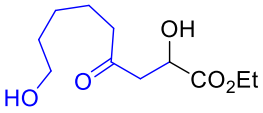
entry	enophile	product	yield	entry	enophile	product	yield
1			97%	4			90%
2			93%	5			91%
3			92%	6			67%

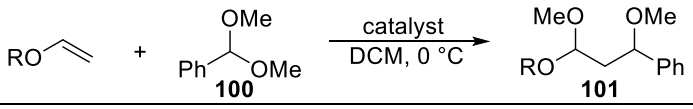
Table 5. Carbonyl-ene reaction with various exocyclic enol ethers.

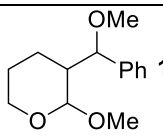
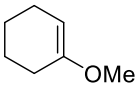
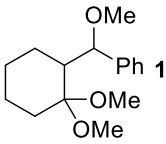
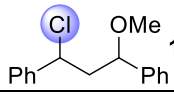
2.1.7 Endocyclic enol ethers as nucleophiles in carbon-carbon bond formation

Exocyclic enol ethers have only been utilized sporadically as nucleophiles in carbon-carbon bond forming reactions, likely due to the susceptibility of these systems to undergo ring opening, isomerization, and hydrolysis. Nonetheless, chemical transformations have been successfully applied to the double bond of exocyclic enol ether systems (*vide infra*). On the other hand, endocyclic enol ethers have been more extensively utilized as nucleophiles in carbon-carbon bond forming reactions and provide insight on the reactivity of these structurally similar motifs. This section will review the use of endocyclic enol ethers as nucleophiles in coupling reactions.

In 1988, Mukiyama and co-workers reported the utility of endocyclic enol ethers as nucleophiles for a carbon-carbon bond-forming transformation.[58] This methodology employed the nucleophilic addition of olefin substrates such as styrene, endocyclic ethers, and vinyl ethers, to acetals under mild conditions (Table 6). The reaction of endocyclic enol ethers with acetal **100** in the presence of tin(II) chloride and trimethylsilyl chloride (Entry 1) or tin(II) trifilate and trityl

chloride (Entry 2) afforded the corresponding methoxy adduct **101a** and **101b**. When styrene was employed as an olefin with an equimolar amount of tin(II) chloride and trimethylsilyl chloride, the result was the exclusive formation of the corresponding chloride adduct **102** in 80% yield (Entry 3).

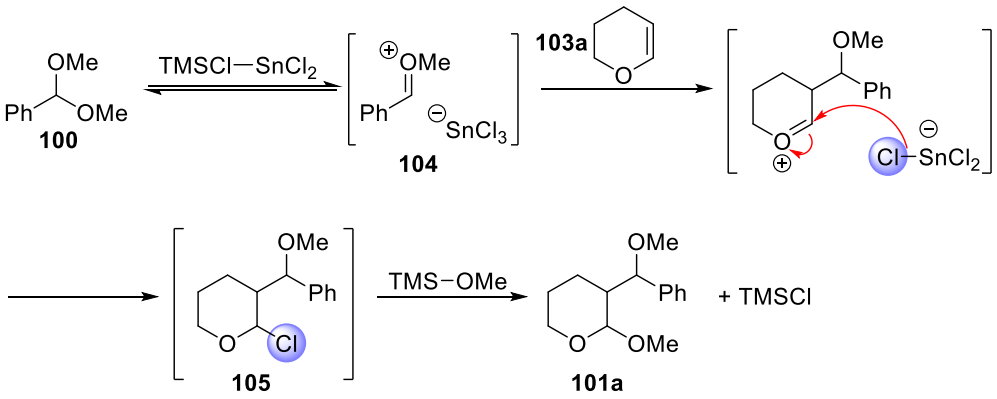


entry	olefin	catalyst	product		yield(%)	dr
1		$SnCl_2$ + TMSCl[a]		**101a**	84	70:14:13:3
2		$Sn(OTf)_2$ + TrCl[b]		**101b**	74	60:40
3		$SnCl_2$ + TMSCl[c]		**102**	80	55:45

[a] 1 equiv. of $SnCl_2$ and 10 mol % TMSCl used
[b] 1 equiv. of $Sn(OTf)_2$ and 10 mol % TrCl used
[c] 1 equiv. of $SnCl_2$ and 1 equiv. of TMSCl used

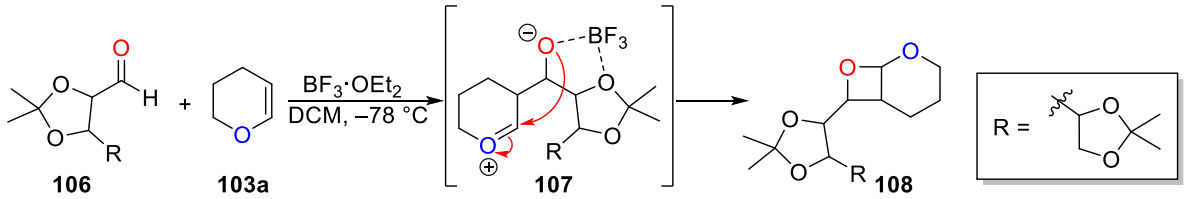
Table 6. Mukaiyama electrophilic addition of acetals to activated olefins under mild conditions.

These result suggest the formation of a chloride species as a key intermediate in the reaction pathway (Scheme 28). The proposed reaction mechanism starts with the activation of acetal **100**, using a tin catalyst, resulting in formation of oxocarbenium ion **104**. Nucleophilic addition of dihydropyran **103a** to intermediate **104** and subsequent chlorine addition provides the intermediate chloride **105**. Lastly, replacement of the intermediate chloride **105** with a methoxide furnishes tetrahydropyran **101a**.



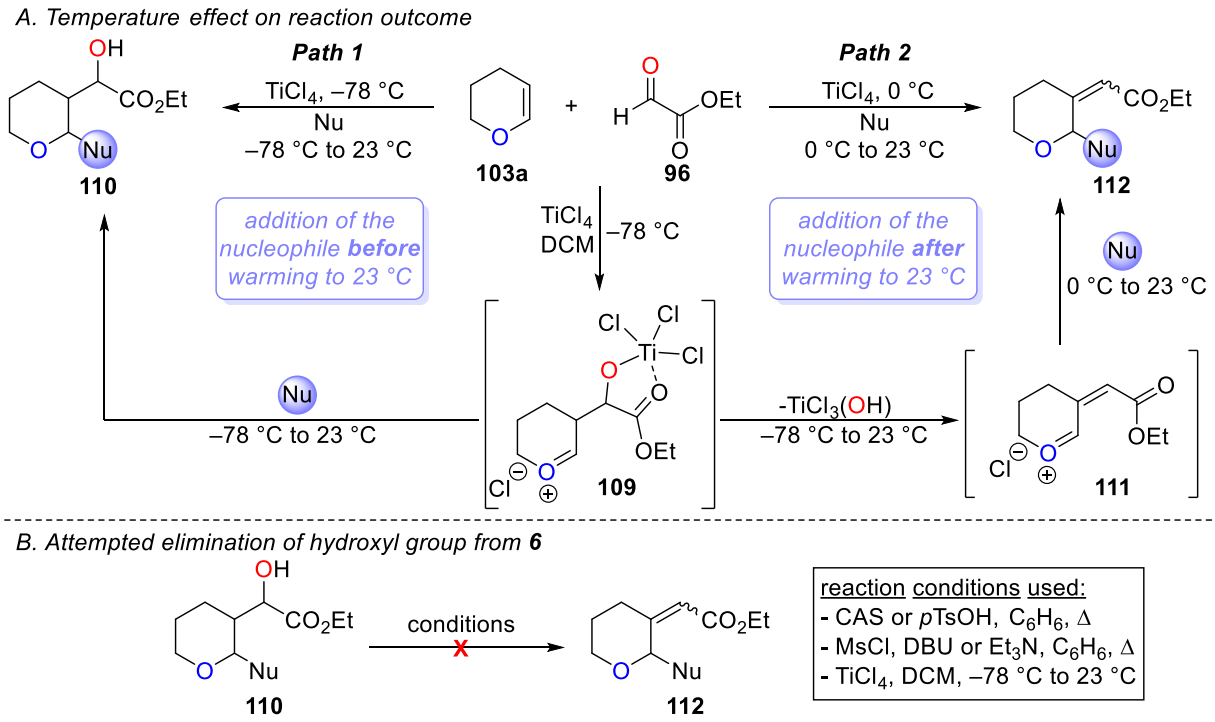
Scheme 28. Proposed mechanism for the addition reaction of acetal **100** with olefin **103a**.

Similarly, Sugimura and co-workers developed a methodology for the synthesis of chiral oxetanes by an intramolecular Prins reaction with enol ethers and aldehydes (Scheme 29).[59] It was proposed that reaction of dihydropyran **103a** with the boron trifluoride etherate activated aldehyde **106** formed the zwitterion **107** at –78 °C. Intramolecular cyclization by oxocarbenium ion provided fused oxetane **108**.



Scheme 29. Formation of oxetane **108** from dihydrofuran **103a**.

In 1999, Ghosh and co-workers reported a titanium(IV) chloride mediated three component coupling reaction for the synthesis of 2,3-disubstituted tetrahydropyrans.[60] Presumably, reaction of dihydropyran **103a** and ethyl glyoxylate **96** in the presence of titanium(IV) chloride at –78 °C led to the formation of oxocarbenium ion intermediate **109**, which was trapped with a secondary nucleophile to give 2,3-disubstituted tetrahydropyrans **110** (Scheme 30A, Path 1).



Scheme 30. (A) Temperature dependent formation of tetrahydropyran **110** and tetrahydropyrilidene acetate **112**. (B) Probed reaction conditions for the attempted elimination of the hydroxyl group from **110**.

Ghosh and co-workers found that, when the coupling reaction was carried out at 0 °C, the eliminated product, substituted tetrahydropyrilidene acetate **112**, was isolated as the major product (Scheme 30A, Path 2).[61] Interestingly, the hydroxyl group from tetrahydropyran **6** was found not to be prone to elimination (Scheme 30B). Subjection of tetrahydropyran **110** to *p*-toluenesulfonic acid or camphorsulphonic acid in refluxing benzene, as well as treatment of **110** with excess $TiCl_4$ at –78 °C to 23 °C, provided only recovered starting material and no eliminated product **112**. Furthermore, treatment of the mesylate analogue of **110** with trimethylamine or DBU in refluxing benzene for several hours did not yield eliminated product **112**. The stability of the hydroxyl group in **110** suggests that the elimination occurs from titanium complex **109** upon warming to 23 °C, forming a new oxocarbenium ion intermediate **111** which then yields eliminated product **112** upon addition of a secondary nucleophile.

This three-component coupling methodology developed by Ghosh and co-workers, has been applied to a number of systems, including pyruvates,[62] functionalized α-amino acids,[63] benzo-fused oxabicyclooctanes,[64] flavone analogues,[65] substituted pyrrolidines and prolines,[66] cyclopentenoids,[67] modified nucleosides,[68] and the natural product eburnamonine.[69]

2.2 Research design and mechanistic hypothesis

Strained heterocycles possessing unique structural features display interesting reactivity and have been shown to be valuable in organic syntheses.[70] Over the past two decades, we have pioneered research on one member of this important class of heterocycles, 2-methyleneoxetanes (**62**), for the preparation of bioactive natural products and synthetic analogues.[71] Our group has demonstrated that 2-methyleneoxetanes can be converted to oxetane oxocarbenium ions when treated with a suitable electrophiles (Figure 33). This oxocarbenium ion can react with a nucleophile in one of two distinct reaction pathways, ring opening or 1,2 addition.

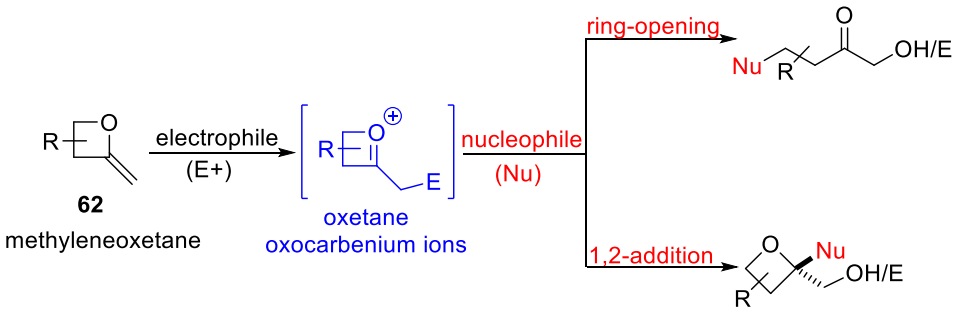


Figure 33. Generation and reactivity of oxetane oxocarbenium ions.

2.2.1 Mechanistic hypothesis

Our interest in utilizing 2-methyleneoxetanes as synthetic scaffolds led us to examine the electron rich exocyclic double bond for the development of nucleophilic transformations. We anticipated that nucleophilic addition of **62** to an aldehyde or ketone would generate an oxetane oxocarbenium ion and a nucleophilic species (Figure 34). Our previous work[58b,58c] led us to anticipate two reaction outcomes: intramolecular ring opening at C-4 or 1,2-addition. Reaction at

C-4 would provide tetrahydropyran-4-ones (THPOs),[72] and 1,2-addition would give dioxaspiroheptanes[73]; either reaction would lead to synthetically useful products.

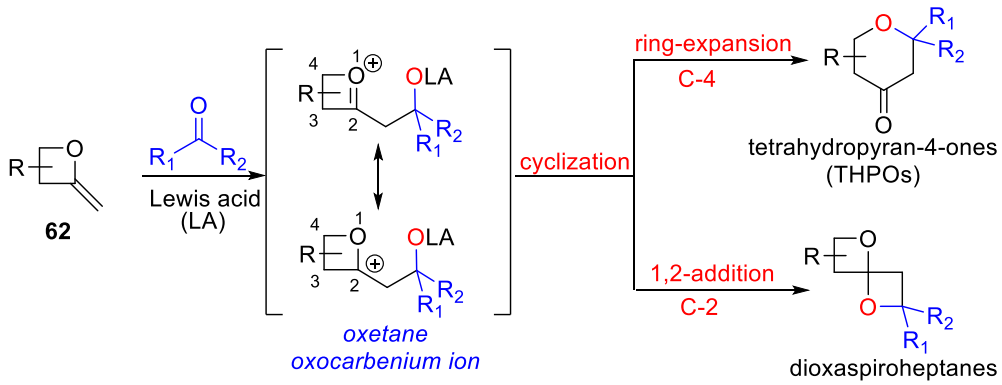
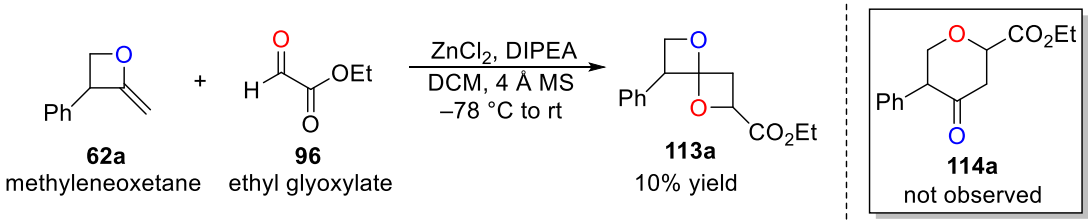


Figure 34. Generation and proposed reaction pathways of oxetane oxocarbenium ions from 2-methyleneoxetanes.

2.3 Results

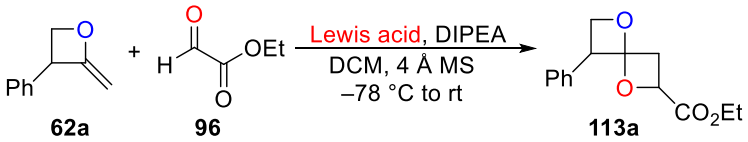
2.3.1 Initial studies

In order to evaluate the feasibility of using 2-methyleneoxetanes in nucleophilic addition processes, we examined the reaction of 3-phenyl-2-methyleneoxetane (**62a**) with ethyl glyoxylate (**96**). The initial reaction was carried out by adding zinc(II) chloride (1.0 equiv) and DIPEA (1.0 equiv) to DCM at –78 °C (Scheme 31). A premixed solution of methyleneoxetane **62a** (1.0 equiv) with ethyl glyoxylate (**96**) (1.5 equiv) in dry DCM was then added dropwise. The reaction was slowly warmed to room temperature over 12 h by allowing the dry ice to sublime. To our delight, formation of the dioxaspiroheptane **113a** was observed in 10% yield without the formation of tetrahydropyranone **114a**. Encouraged by these results, we decided to optimize the reaction and explore the scope of this transformation. Preliminary studies included screening of Lewis acids, variation of the reaction stoichiometry, temperature, and concentration, and evaluation of reagent addition order/time.



Scheme 31. Initial reaction of methyleneoxetane **62a** with ethyl glyoxylate **96** to give dioxaspiroheptane **113a**.

Initial efforts in optimizing the formation of dioxaspiroheptane **113a** focused on screening various Lewis acids (Table 7). Reactions conducted with Lewis acids $EtAlCl_2$, Et_2AlCl, $BF_3{\cdot}OEt_2$, $MgCl_2$, or $Mg(OTf)_2$ (Entries 4-7) gave decomposition of the starting material, no reaction, or undesired byproducts. $ZnCl_2$ and $Zn(OTf)_2$ gave formation of the desired product **113a**, albeit in very low yields (Entries 1 and 2). The best results were obtained using stronger Lewis acids such as $TiCl_4$ or $SnCl_4$ (Entries 8 and 9). These results suggested the need for a strong Lewis acid to efficiently promote the addition of the 2-methyleneoxetane.



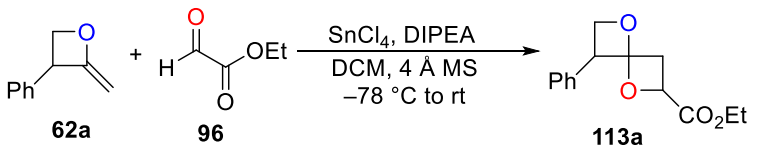
entry[a,b]	Lewis acid	yield (%)[c]
1	$ZnCl_2$	10
2	$Zn(OTf)_2$	<5
3	$EtAlCl_2$	—
4	Et_2AlCl	—
5	$BF_3{\cdot}OEt_2$	—
6	$MgCl_2$	—
7	$Mg(OTf)_2$	—
8	$TiCl_4$	71
9	$SnCl_4$	74

[a]Reactions were run in anhydrous DCM (0.25 M) at –78 °C to rt with 0.50 mmol **113a**, 1.2 equiv ethyl glyoxylate **96**, 1.0 equiv DIPEA, and 1.0 equiv Lewis acid. [b]Reaction time was recorded until complete conversion of the starting material or until reaction time reaches 24 h. [c]Isolated yield after flash column chromatography.

Table 7. Effect of Lewis acids on the reaction of 3-phenyl-2-methyleneoxetane (**62a**) with ethyl glyoxylate (**96**).

Further optimization in the formation of dioxaspiroheptane **113a** from 2-methyleneoxetane **62a** included changing the reaction concentration and the reaction stoichiometry, whereby relative amounts of Lewis acid, electrophile, and base were varied to increase the yield (Table 8). Evaluation of the quantity of tin(II) chloride revealed that using one equivalent with respect to the methyleneoxetane gave the best yield (Entry 4). Decreasing the amount of tin(II) chloride showed a direct correlation with a decrease in yield (Entries 1-3). Additionally, increasing the amount of tin(II) chloride to two molar equivalents gave a decreased yield (Entry 5).

Examination of the reaction stoichiometry, where ethyl glyoxylate (**96**) and 2-methyleneoxetane (**113a**) were varied, showed an insignificant effect on the reaction yield (Entries 4, 6, and 7). Nonetheless, excess ethyl glyoxylate was used due to ethyl glyoxylate existing in a partly polymerized form, which is problematic for accurately measuring exact amounts. Surprisingly, when the reaction was conducted without DIPEA, no product formation was observed (Entry 8). The effect of increasing the amount of DIPEA to 1.5 equivalents was insignificant on the reaction yield (Entry 9). Furthermore, the best yields for the formation of dioxaspirohexane **113a** were obtained at concentrations of 0.25 M with respect to **62a** (Entries 4, 10, 11, and 12).



entry[a,b]	equiv LA	E^+	DIPEA	conc. (M)[e]	yield (%)[c]
1	0.1	1.5	1.0	0.25	14
2	0.2	1.5	1.0	0.25	24
3	0.5	1.5	1.0	0.25	46
4	1.0	1.5	1.0	0.25	74
5	2.0	1.5	1.0	0.25	56
6	1.0	2.0	1.0	0.25	69
7	1.0	0.9	1.0	0.25	67[d]
8	1.0	1.5	—	0.25	—
9	1.0	1.5	1.5	0.25	69
10	1.0	1.5	1.0	0.05	34
11	1.0	1.5	1.0	0.10	39
12	1.0	1.5	1.0	0.50	67

[a]Reactions were run in anhydrous DCM with 4 Å molecular sieves at –78 °C to rt. [b]Reaction time was monitored until complete conversion of the starting material or until reaction time reached 24 h. [c]Isolated yield after flash column chromatography. [d]Yield was calculated using ethyl glyoxylate as limiting reagent.[e]Concentrations with respect to 2-methyleneoxetane.

Table 8. Optimization of conditions for formation of dioxaspiroheptane **113a**.

2.3.2 Preparation of β-lactones and 2-methyleneoxetane substrates

A variety of 2-methyleneoxetanes was prepared to explore the scope of dioxaspiroheptane ring formation. The starting β-lactones were prepared from previously reported lactonization procedures or using modified procedures. The formation of β-lactones can be classified into three types of transformations: (1) lactonization by oxygen-acyl bond formation; (2) lactonization by oxygen-alkyl bond formation; and (3) [2+2] cycloaddition of ketenes (or ketene equivalents) and carbonyl compounds (Figure 35). Methylenations of various β-lactones were conducted using our previously reported protocol by reacting the β-lactones with the Petasis reagent.[34,37]

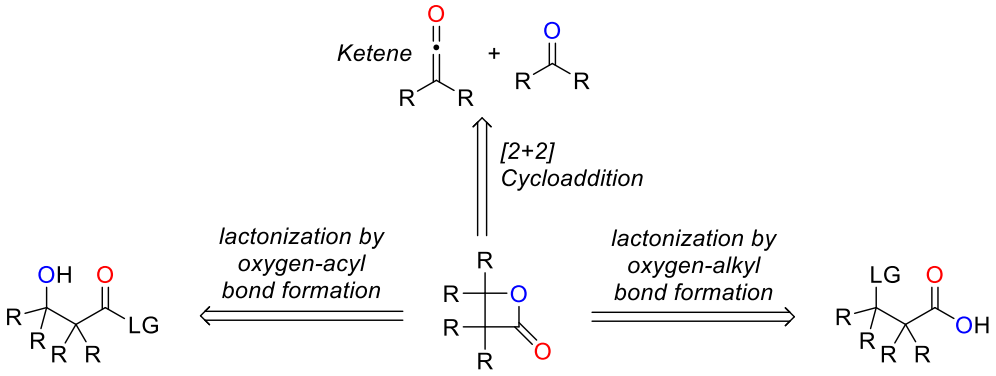
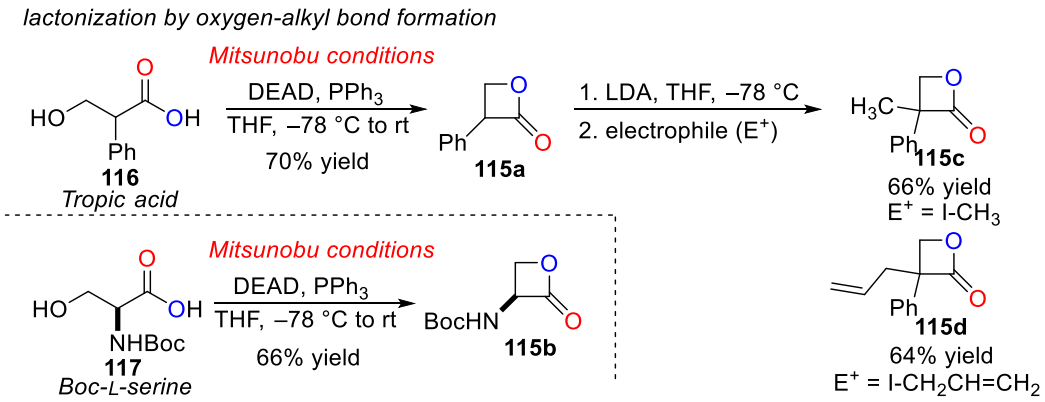


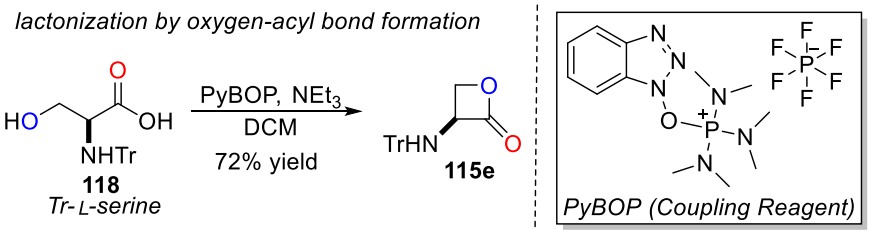
Figure 35. General methods for the formation of β-lactones.

The syntheses of C-3 monosubstituted β-lactones **115a** and **115b** was achieved by the lactonization of tropic acid **116** and *N-tert*-butyloxycarbonyl substituted L-serine **117**, respectively, using modified Mitsunobu conditions (Scheme 32).[74,75] β-Lactone **115a** was then utilized for the synthesis of 3,3-disubstituted β-lactones. Formation of the β-lactone enolate of **115a** with LDA at – 78 °C, followed by treatment with an electrophile, provided **115c** and **115d** in good yields.[32]



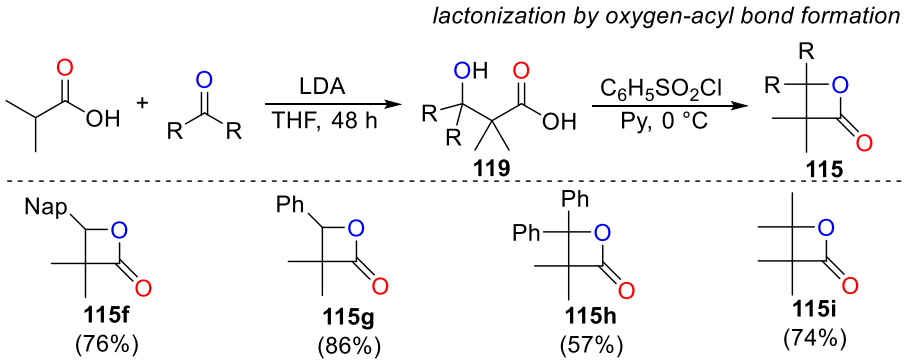
Scheme 32. Synthesis of β-lactones using modified Mitsunobu conditions and formation of 3,3-disubstituted β-lactones.

The synthesis of β-lactone **62e** was accomplished by employing the coupling reagent, (benzotriazol-1-yloxy)tris(dimethylamino) phosphonium hexafluorophosphate (PyBOP), with N-trityl-protected L-serine **118** (Scheme 33).[76,77] Other coupling agents, such as *N*,*N*-diisopropylcarbodiimide (DIC)[78] and *N*,*N*-dicyclohexylcarbodiimide (DCC),[79] have also been reported for the synthesis of **115e**, but in much lower yields.



Scheme 33. Synthesis of β-lactone **115e** using the PyBOP coupling reagent.

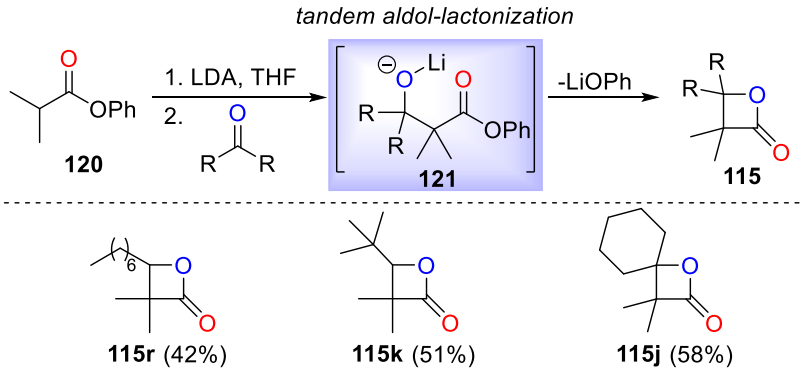
The syntheses of gem-dimethyl substitiuted β-lactones **115f**-**115i** were accomplished by utilizing a methodology developed by Adam and co-workers (Scheme 34).[80] This method commenced with formation of β-hydroxy acid **119** via the condensation of α-lithio carboxylate salts with various ketones or aldehydes. Subsequent lactonization of the β-hydroxy acids (**119**) with benzenesulfonyl chloride in pyridine at low temperatures (0-5 °C) afforded tri- and tetra-substituted β-lactones **115f**-**115i**.



Scheme 34. Synthesis of gem-dimethyl substituted β-lactones.

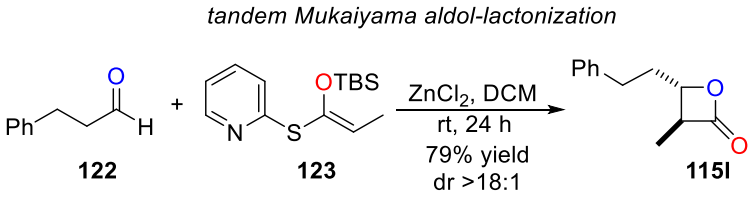
Additional tri- and tetra-substituted β-lactones **115j** and **115k** were synthesized using an aldol-lactonization methodology developed by Schick and co-workers (Scheme 35).[81] Deprotonation of phenyl isobutyrate (**120**) with lithium diisopropylamide (LDA) and aldolization of heptanal, pivaldehyde or cyclohexanone afforded β-lactones **115r**, **115k** and **115j**, respectively. The activation of the carbonyl group with a phenoxy group assisted in the in situ intramolecular

cyclization of *O*-lithiated phenyl β-hydroxyalkanoates **121** intermediates to a β-lactone by elimination of lithium phenoxide.



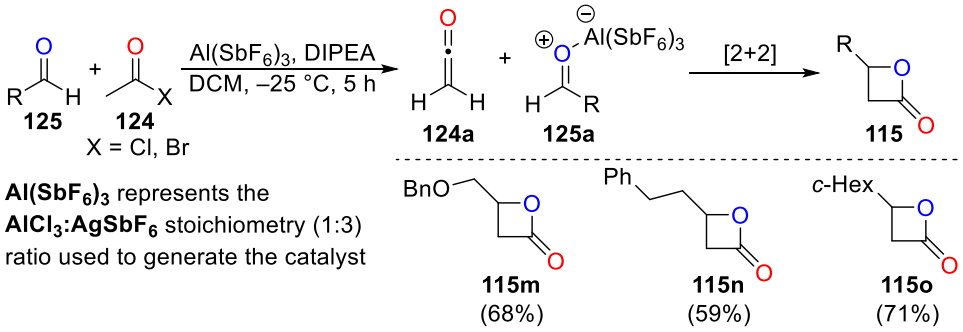
Scheme 35. Synthesis of β-lactones via a tandem aldol-lactonization reaction.

trans-Disubstituted β-lactone **115l** was made using a $ZnCl_2$-mediated tandem Mukaiyama aldol-lactonization reaction developed by Romo and co-workers (Scheme 36).[82] Reaction of hydrocinnamaldehyde **122** and TBS thiopyridyl ketene acetal **123** (*E*/*Z*, 19:1) with $ZnCl_2$ delivered β-lactone **115l** in 79% yield and with excellent diastereoselectivity (>18:1) favoring the *trans*-β-lactone.



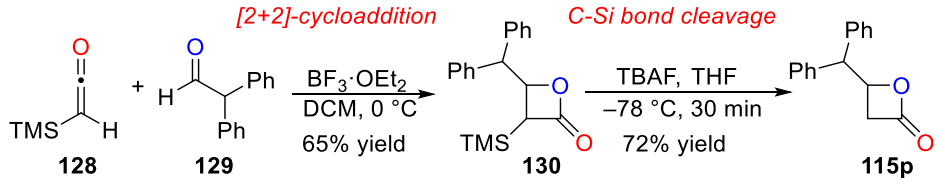
Scheme 36. Synthesis of β-lactone **115l** via tandem Mukaiyama aldol-lactonization.

The syntheses of C-4 monosubstituted β-lactones **115m**, **115n**, and **115o** were accomplished with a catalyzed acyl halide-aldehyde cyclocondensation (AAC) reaction developed by Nelson and co-workers (Scheme 37).[83] This reaction proceeds by *in situ* ketene **124a** formation via *N*,*N*-diisopropylethylamine (DIPEA) mediated dehydrohalogenation of acyl halide **124**. Next, [2+2] cycloaddition of the ketene with aldehyde **125**, activated by the Lewis acid catalyst $Al(SbF_6)_3$, delivered the desired β-lactones **115m**, **115n**, and **115o**.



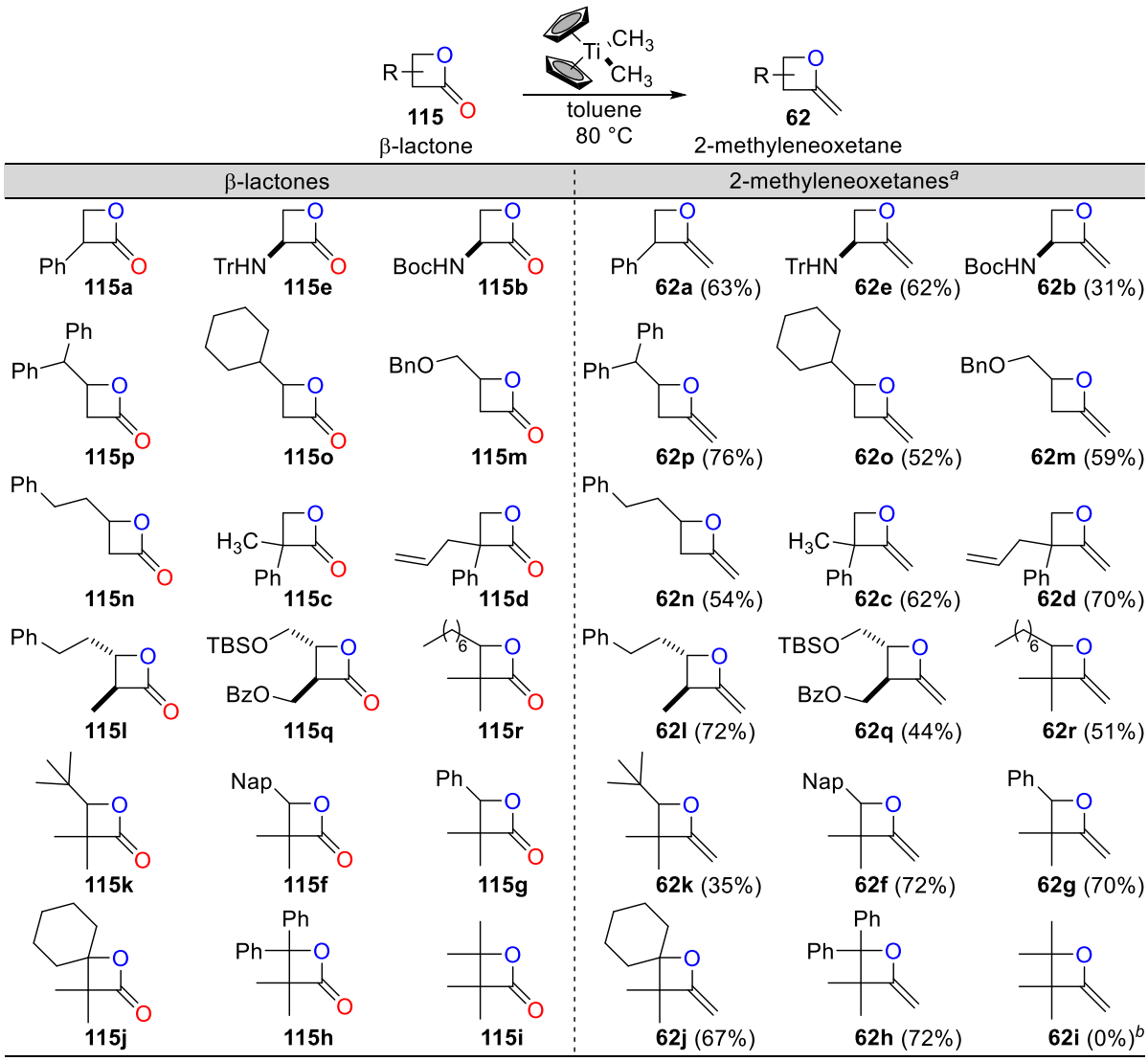
Scheme 37. Synthesis of β-lactone using Lewis acid-catalzed acyl halide aldehyde cyclocondensation.

Similarly, α-silyl-β-lactone **127** was prepared from a [2+2]-cycloaddition of silyketene **128** and diphenylacetaldehyde **129** in the presence of catalytic $BF_3{\cdot}OEt_2$ (Scheme 38).[84] Treatment of α-silyl-β-lactone **130** with TBAF at –78 °C provided C-Si bond cleavage to afford C-4 monosubstituted β-lactone **115p** in good yield (Scheme 38).[85]



Scheme 38. Synthesis of β-lactone **115p** by [2+2]-cycloaddition and subsequent C-Si bond cleavage with TBAF.

Methylenation of the β-lactones using the Petasis reagent provided a library of 2-methyleneoxetanes in fair to good yields (Table 9). One exception was the methylenation of tetra-substituted β-lactone **115i** which gave only the ring-opened methylene product. It should also be noted that 2-methyleneoxetanes are prone to ring-opening with silica gel chromatography. To minimize their propensity for ring-opening during column chromatography, the silica gel was deactivated with triethylamine.



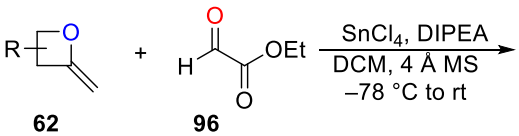
[a]Reactions were monitored by TLC with 1.5-3.0 equiv Petasis reagent between 2-12 h.
[b]Ring-opening product of 2-methyleneoxetane was only observed.

Table 9. Preparation of 2-methyleneoxetanes from methylenation of β-lactones.

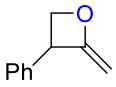
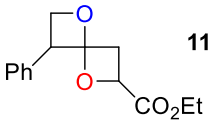
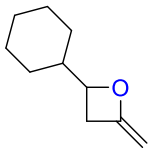
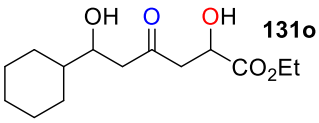
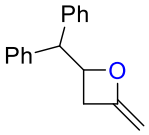
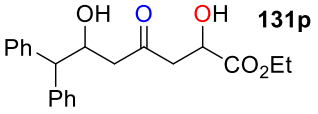
2.3.3 Scope of the reaction

The preliminary experiments demonstrated the feasibility of 2-methyleneoxetanes **62a** to act as neutral nucleophiles in carbon-carbon bond-forming reactions with ethyl glyoxylate **96** (Table 10, Entry 1). The optimized conditions using one equivalent of tin(IV) chloride were applied to a variety of mono-, di-, tri-, and tetrasubstituted 2-methyleneoxetanes.

We first explored mono-substituted 2-methyleneoxetanes **62o** and **62p**, with substituents at the C-4 position of the oxetane ring. When **62o** or **62p** were treated with tin(IV) chloride, no dioxaspirohexane resulted; instead, ring opened diol products **131o** and **131p** were isolated (Table 10, Entries 2 and 3) as a 1:1 mixture of diastereomers after column chromatography. The same reaction outcome was also observed when the Lewis acid was changed to titanium(IV) chloride. Hydrolyzed ring opened products were observed possibly, due to instability of the spiroketal moiety. It must be noted that diastereomeric ratios are typically determined from crude ^{1}H NMR spectra; however, impurities present in the reaction mixtures made these measurements difficult for many reactions. Even though a majority of the column fractions were analyzed, in most reactions, it is possible that other diastereomers and products formed. Additionally, the diastereomeric ratio could be affected by the purification process.



62 **96**

entry[a,b]	2-methyleneoxetane	product	yields[c]
1	 **62a**	 **113a**	74%
2	 **62o**	 **131o**	78%[e] (70%)[d,e]
3	 **62p**	 **131p**	83% (65%)[d,e]

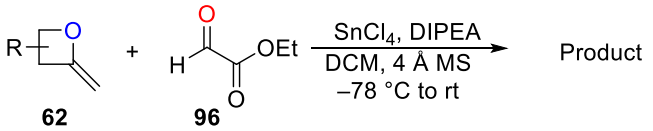
[a]Reactions were run in anhydrous DCM with 4 Å molecular sieves at –78 °C to rt. [b]Reaction time was recorded until complete conversion of the starting material with aqueous work-up. [c]Isolated yield after flash column chromatography. [d]Reaction was conducted with 1 equiv $ZnCl_2$.[e]dr 1:1.

Table 10. Reaction of mono-substituted 2-methyleneoxetanes with ethyl glyoxylate.

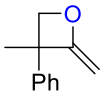
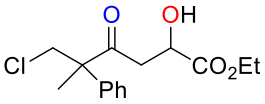
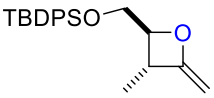
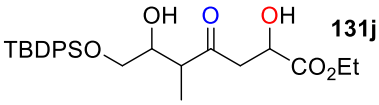
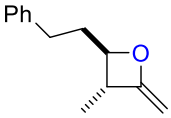
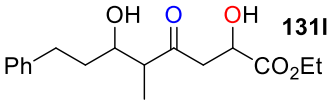
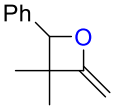
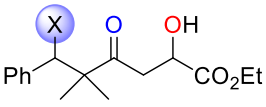
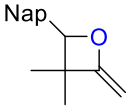
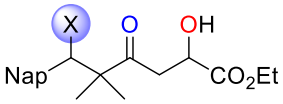
The reaction of di- and tri-substituted 2-methyleneoxetanes (**62**) with ethyl glyoxylate (**96**) also provided ring-opening products under optimized conditions (Table 11). When disubstituted

2-methyleneoxetane **62c** was used, the unexpected ring-opened chloride product **132c** was isolated as one diastereoisomer in 66% yield (Entry 1). Reaction outcomes with **62c** were the same with and without aqueous workups. Reactions conducted with *trans*-substituted 2-methyleneoxetanes **62j** and **62l** provided the ring-opened diol products as a mixture of diastereoisomers in 45% and 49% yields, respectively (Entries 2 and 3).

Reactions with trisubstituted 2-methyleneoxetanes **62g** and **62f** provided ring-opened products in good yields (Entries 4–6). Reaction with **62g** provided diol **131g** as mixture of diastereomers (3:2 ratio) that were inseparable by column chromatography (Entry 4). Diastereomeric ratios were determined by ^{1}H NMR intergrations of purified product, and the relative stereochemistry of the products was not determined. Additionally, reaction of **62g** in the presence of $TiCl_4$ gave the ring-opened chloride product **132g** as a mixture of diastereomers (Entry 5). The reaction of 2-methyleneoxetane **62f** in the presence of $SnCl_4$ gave a mixture of alcohol and chloride substituted ring-opened products **133f** and **133f'** (Entry 6) that were separated by column chromatography. Surprisingly, the crude reaction mixture only showed the formation of the chloride compound. Thus, substitution most likely occurred during the purification process.



62 **96**

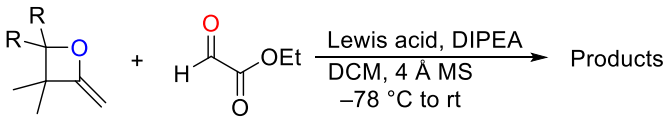
entry[a,b]	2-methyleneoxetane		product		yields[c]
1		**62c**		**132c**	66%
2		**62j**		**131j**	45%[d]
3		**62l**		**131l**	49%[d]
4		**62g**		**131g** X = OH	83%[e]
5				**132g** X = Cl	52%[f,g]
6		**62f**		**133f** X = OH **133f'** X = Cl	74%[h]

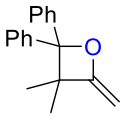
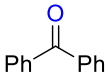
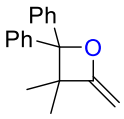
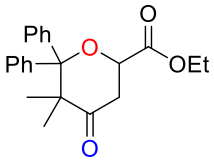
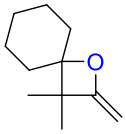
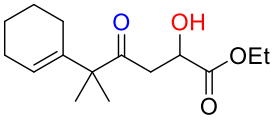
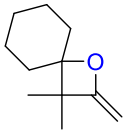
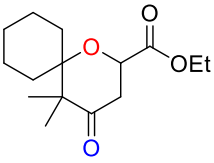
[a]Reactions were run in anhydrous DCM with 4 Å molecular sieves at –78 °C to rt. [b]Reaction time was recorded until complete conversion of the starting material with aqueous work-up. [c]Isolated yield after flash column chromatography. [d]Mixture of diastereoisomers.[e]dr 3:2. [f]Reaction used $TiCl_4$ as the Lewis acid. [g]dr 2:1. [h]Ratio of OH to Cl 4:1.

Table 11. Reaction of di- and trisubstituted 2-methyleneoxetanes with ethyl glyoxylate.

The reaction of ethyl glyoxylate with tetrasubstituted 2-methyleneoxetanes was also examined (Table 12). Reaction with 2-methyleneoxetane **62h** in the presence of $SnCl_4$ or $TiCl_4$ suggested a cycloreversion to form benzophenone (**134**) in quantitative yield had occurred (Entry 1). However, reaction of **62h** in the presence of $ZnCl_2$ resulted in the formation of tetrahydropyranone **114h** (Entry 2).

Similarly, reaction of 2-methyleneoxetane **62j** in the presence of $SnCl_4$ or $ZnCl_2$ provided different reaction outcomes (Entries 3 and 4). Reaction of **62j** using the stronger Lewis acid, $SnCl_4$, led to the ring-opened alkene product **135** in 58% yield (Entry 3). On the other hand, reaction of **62j** in the presence of a weaker Lewis acid, $ZnCl_2$, led to the formation of tetrahydropyranone **114j** (Entry 4).



entry[a,b]	2-methyleneoxetane		Lewis acid	product		yield[c]
1		**62h**	$SnCl_4$		**134**	99%[d]
2		**62h**	$ZnCl_2$		**114h**	29%
3		**62j**	$SnCl_4$		**135**	58%
4		**62j**	$ZnCl_2$		**114j**	53%

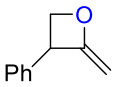
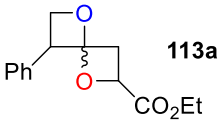
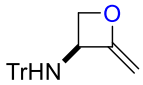
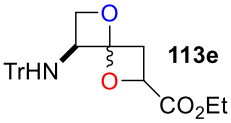
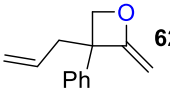
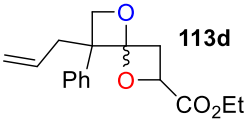
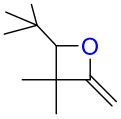
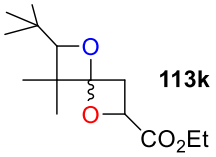
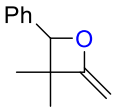
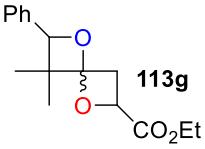
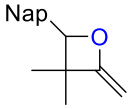
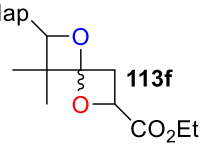
[a]Reactions were run in anhydrous DCM with 4 Å molecular sieves at –78 °C to rt. [b]Reaction time was recorded until complete conversion of the starting material with aqueous work-up. [c]Isolated yield after flash column chromatography. [d]Reaction using $TiCl_4$ gave similar yields.

Table 12. Reaction of tetrasubstituted 2-methyleneoxetanes with ethyl glyoxylate.

Up until this point, reactions conducted under optimized or slightly modified conditions did not lead to formation of the desired dioxaspiroheptanes in a significant amount. However, the formation of dioxaspiroheptanes was achieved using certain 2-methyleneoxetanes under the standard reaction conditions or with slight modifications (Table 13). Reaction of *N*-trityl-protected 2-methyleneoxetane **62e** with ethyl glyoxylate **96** and the titanium BINOL catalyst provided dioxaspiroheptane **113e** as a diastereomic mixture (Entry 2). As previously discussed, purification of the crude mixture was required in order to determine the diastereomer ratio, which could be altered during purification. The relative stereochemistry for the mixture of **113e** was not determined.

Reaction of C-3 disubstituted 2-methyleneoxetane **62d** in the presence of $ZnCl_2$ gave dioxaspiroheptane **113d** as a mixture of diastereomers (Entry 3). Additionally, trisubstituted 2-

methyleneoxetane **62k** also provided dioxaspiroheptane **113k** in low yields (Entry 4). Surprisingly, reactions with 2-methyleneoxetanes **62g** and **62f** in the presence of $SnCl_4$ gave diaoxspiroheptanes **113g** and **113f** in moderate yields (Entries 5 and 6). Previous reactions of **62g** and **62f** using the same electrophile and Lewis acid led to the formation of ring-opened products (see Table 11). It is possible the formation of the dioxaspiroheptanes could be related to the reaction temperature. Formation of dioxaspirohetanes **113g** and **113f** was observed when the reactions were slowly warmed from –78 °C to room temperature by allowing the dry ice to sublime.

entry	2-methyleneoxetane	Lewis acid	product	yield[a,b]	dr
1	 **62a**	$SnCl_4$	 **113a**	74%	7:1
2	 **62e**	$Ti(OiPr)_2Cl_2$ BINOL	 **113e**	56%[c]	3:2
3	 **62d**	$ZnCl_2$	 **113d**	65%	3:2
4	 **62k**	$ZnCl_2$	 **113k**	21%	5:4
5	 **62g**	$SnCl_4$	 **113g**	54%	5:4
6	 **62f**	$SnCl_4$	 **113f**	58%	4:1.5

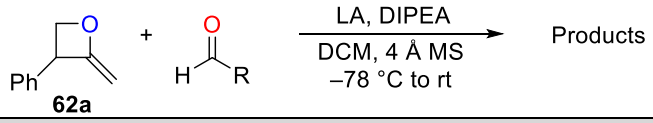
[a]Isolated yields are combined mixture of diastereomers.[b]Reaction was slowly warmed to room temperture.
[c]Reaction conditions used 10% $Ti(OiPr)_2Cl_2$, 10% BINOL.

Table 13. Formation of dioxaspiroheptanes from 2-methyleneoxetanes.

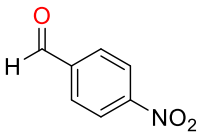
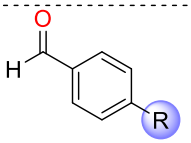
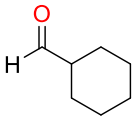
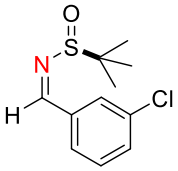
2.3.4 Reaction with different electrophiles

After examining a variety of 2-methyleneoxetanes, we sought to evaluate the scope of electrophiles that could be used in this aldol-type addition. At the outset of this study, 3-phenyl-2-methyleneoxetane **62a** was used as a substrate model and reacted with various electrophiles (Table 14). Reactions of 2-methyleneoxetane **62a** with *p*-nitrobenzaldehyde, a relatively activated aldehyde, using $SnCl_4$, resulted in messy reactions and low conversions to coupled products (Entry 1). Changing the Lewis acid to $ZnCl_2$ or $TiCl_4$ also resulted in very messy reactions with

significant formation of coupled product(s) (Entries 2 and 3). Along these lines, reaction of 2-methyleneoxetanes with moderately or non-activated electrophiles did not provide coupled product(s) in appreciable yields (Entries 4-8). These results suggest a need for activated electrophiles and/or 2-methyleneoxetanes that have a greater level of stability.



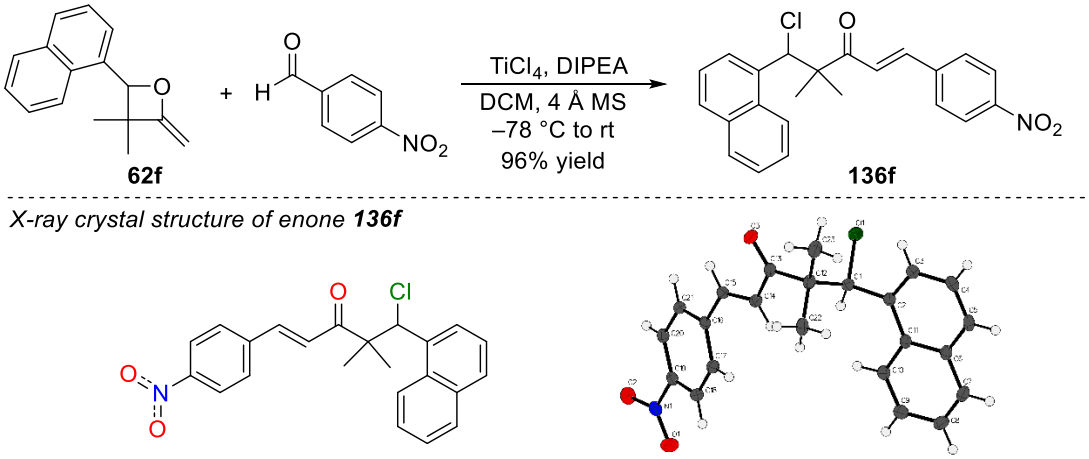
62a

entry	electrophile		Lewis acid	results
1			$SnCl_4$	very messy but about 30-40% conversion
2			$TiCl_4$	possible addition products present in small amounts
3			$ZnCl_2$	no significant addition product formation
4		R = Br	$SnCl_4$	very messy but about 30% conversion
5		R = CF_3	$SnCl_4$	very messy but small addition product formed (<20%)
6		R = H	$SnCl_4$	very small addition product seen (<15%)
7			$SnCl_4$	very small addition product seen (<15%)
8			$SnCl_4$	no reaction; only decomposition of S.M.

[a]Reactions were run using 1.5 equiv. of the electrophile in anhydrous DCM with 4 Å molecular sieves at –78 °C to rt. [b]Reaction time was recorded until complete conversion of the starting material or until 24 h.

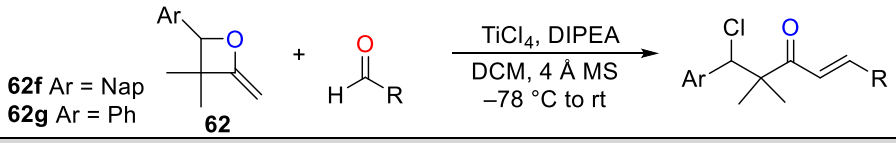
Table 14. Reaction of 2-methyleneoxetane **62a** with various electrophiles.

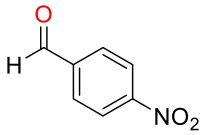
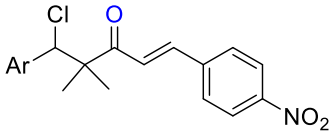
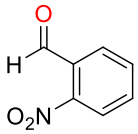
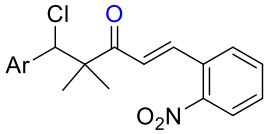
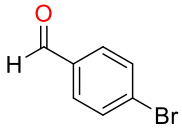
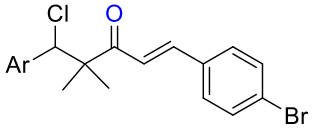
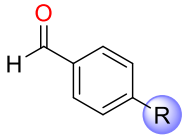
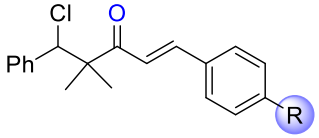
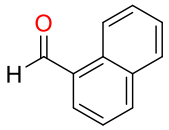
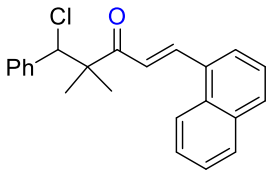
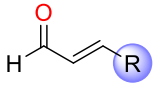
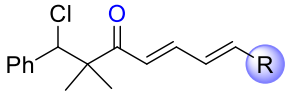
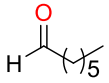
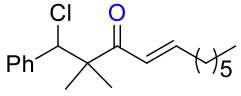
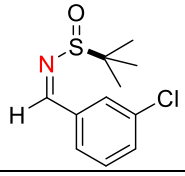
Further exploration using 2-methyleneoxetane **62f** with *p*-nitrobenzaldehyde and $TiCl_4$ gave enone **136f** in 96% yield (Scheme 39). A single X-ray crystal structure was obtained for enone **136f**. The incorporation of chloride can occur via intramolecular attack by a chloride from the titanium or by loss of the Cl^- followed by attack.



Scheme 39. Reaction of 2-methyleneoxetane **62f** with *p*-nitrobenzaldehyde.

This transformation was further explored with enophiles of different reactivities in the presence of $TiCl_4$ (Table 15). Reaction of 2-methyleneoxetanes **62f** or **62g** with moderately activated aldehydes, such as *p*-nitrobenzaldehyde, *o*-nitrobenzaldehyde, and *p*-bromobenzaldehyde, gave similar ring-opened enone products (Entries 1-6) in good to excellent yields. 2-Methyleneoxetane **62g** also reacted with aromatic aldehydes bearing slight activating groups (Entry 8), non-activated (Entry 7), and somewhat deactivating groups (Entries 9 and 10), albeit in lower yields. The reaction with conjugated aldehydes and **62g** also provided aldol-type, ring-opened products (Entries 11 and 12). Low conversion or no product formation was observed with heptanal and *tert*-butylsulfinamide (Entries 13 and 14).



entry	Ar	electrophile	product		yield
1	Nap			**136f**	96%
2	Ph			**136g**	92%
3	Nap			**137f**	68%
4	Ph			**137g**	82%
5	Nap			**138f**	80%
6	Ph			**138g**	94%
7	Ph	 R = H		**139g**	32%
8	Ph	R = CF_3		**140g**	42%[a]
9	Ph	R = OMe		**141g**	31%[a]
10	Ph			**142g**	40%[a]
11	Ph	 R = CH_3		**143g**	72%[a]
12	Ph	R = Ph		**144g**	87%
13	Ph			**145g**	13%[a]
14	Ph		NR		—

[a]Aldehyde present after flash column chromatography. Yield calculated based on ^{1}H proton NMR ratio of product to aldehyde.

Table 15. Reaction of tri-substituted 2-methyleneoxetanes with various electrophiles.

2.3.5 5-Membered rings

In light of the results obtained with four-membered exocyclic enol ethers, we wondered what effect ring size would have on the reaction outcome. Previous research has shown that under similar reaction conditions 2-methylenetetrahydropyrans gave either the carbonyl-ene

adduct or the aldol-type adduct (see Scheme 27). However, reaction of five-membered exocyclic enol ether **146** with ethyl glyoxyate **96** in the presence of a Lewis acid has not been reported (Figure 36).

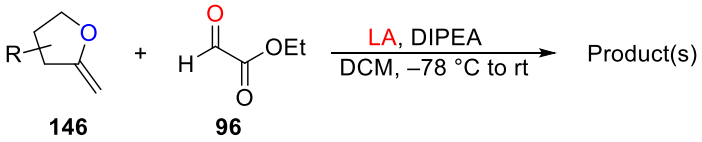
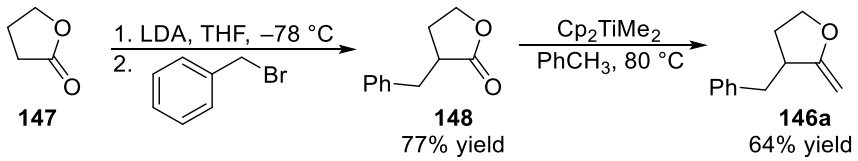


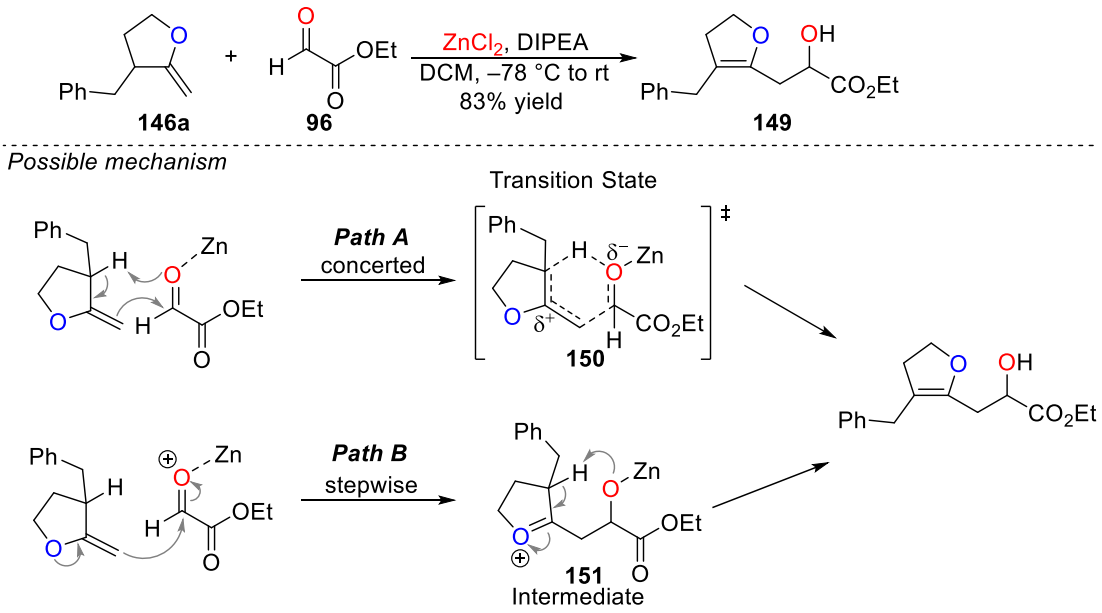
Figure 36. Probing of five-membered exocyclic enol ethers **146** with ethyl glyoxylate **96** and various Lewis acids.

We decided to synthesize benzyl substituted 2-methylenetetrahydrofuran **146a** as a model substrate due to the low boiling point of the unsubstituted 2-methylenetetrahydrofuran (Scheme 40). Benzyl substituted γ-lactone **148** was prepared by alkylation of γ-butryolactone (**147**) using LDA and benzyl bromide.[86] Methylenation of γ-lactone **148** using the Petasis reagent provided 2-methylenetetrahydrofuran **146a** in 64% yield.



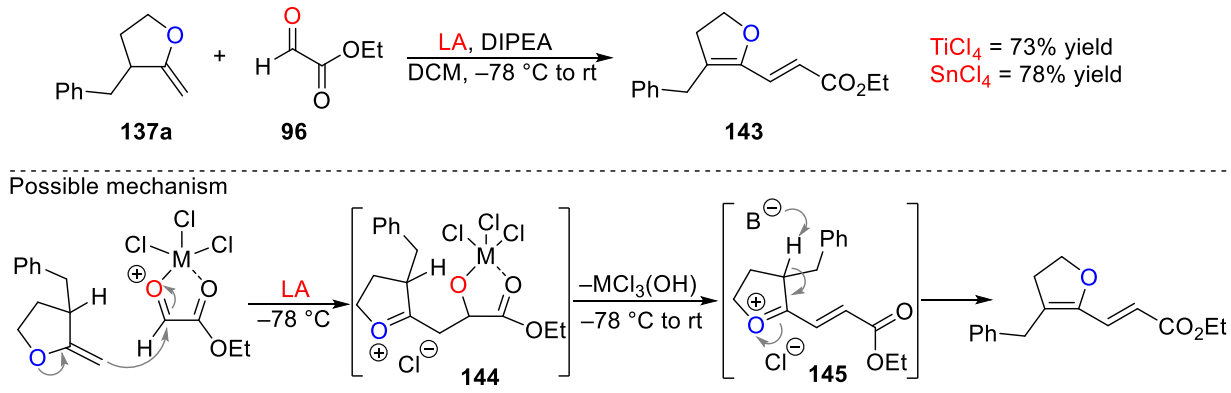
Scheme 40. Synthesis of 2-methylenetetrahydrofuran **146a**.

We first examined the reaction of 2-methylenetetrahydrofuran **146a** and ethyl glyoxylate (1.2 equiv) with stoichiometric $ZnCl_2$ at –78 °C (Scheme 41). After the addition of $ZnCl_2$, the reaction was allowed to slowly warm to room temperature over 12 hours. Not unexpectedly, formation of the β-hydroxydihydrofuran **149** was observed in 72% yield. Based on literature related to cyclic enol ether reactions with Lewis acid catalysts, we hypothesized two potential pathways for the formation of **149** (Scheme 41). This Lewis acid catalyzed reaction can proceed via a concerted mechanism with a polar transition state, **150**, or a stepwise mechanism, forming an oxocarbenium ion intermediate, **151**. Similar transformations are often classified as falling somewhere in between a concerted and stepwise mechanism.



Scheme 41. Reaction of 2-methylenetetrahydrofuran with ethyl glyoxylate in the presence of $ZnCl_2$ and the possible mechanism.

Reaction of 2-methylenetetrahydrofuran **146a** with ethyl glyoxylate (**96**) and stoichiometric $SnCl_4$ or $TiCl_4$ provides a different reaction outcome, the formation of diene **152** (Scheme 42). The formation of the elimination product **152**, rather than hydroxide **149**, is likely due to the use of stronger Lewis acids. Based on literature related to endocyclic enol ethers reactions (*vide infra*), we hypothesize that the reaction goes via a stepwise mechanism (Scheme 42). The first step in the reaction mechanism is the activation of ethyl glyoxylate (**96**) by the Lewis acid, followed by nucleophilic addition of 2-methylenetetrahydrofuran **146a** to give oxonium intermediate **153**. Elimination of the oxonium ion-titanium complex would give oxonium ion intermediate **154**. Deprotonation of **154** would provide diene **152**.



Scheme 42. Reaction of 2-methylenetetrahydrofuran with ethyl glyoxylate in the presence of $SnCl_4$ or $TiCl_4$ and the possible mechanism.

2.4 Conclusion

2-Methyleneoxetanes were utilized for the first time as nucleophiles in carbon-carbon bond-forming reactions. Notably, a number of 2-methyleneoxetanes were shown to react with ethyl glyoxylate to provide dioxaspiroheptanes in the presence of a Lewis acid. Reaction of tetrasubstituted 2-methyleneoxetanes provided a different reaction outcome in that tetrahydropyranones were formed. Furthermore, certain 2-methyleneoxetanes reacted with ethyl glyoxylate to provide ring-opened products. These reaction conditions were also applied to 2-methylenetetrahydrofuran for the formation of β-hydroxydihydrofuran or its eliminated equivalent. In exploring aldehydes other than ethyl glyoxylate, it was found that only activated aldehydes, such as *p*-nitrobenzaldehyde reacted productively under typical conditions.

2.5 Experimental

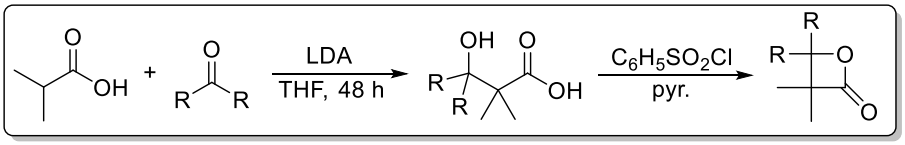
2.5.1 General Information

All moisture sensitive reactions were run in a flame-dried flask under and atmosphere of nitrogen. Methylene chloride (DCM), dimethylforamide (DMF), toluene, and pyridine were dried over CaH_2 of over 4 Å molecular sieves. Tetrahydrofuran (THF) was dried using J. C. Meyer Solvent Dispensing System (SDS) under nitrogen. Deuterated chloroform was dried over 4 Å

molecular sieves. Zinc(II) chloride was freshly fused prior to use. Commercially available reagents were purchased from Acros, Aldrich, Alfa Aesar, or TCI America and were purified as necessary.

Flash column chromatography was performed using silica gel, 40 microns flash silica. Silica gel was deactivated with triethylamine when necessary. Thin layer chromatography was carried out with silica gel (Silica Gel 60 F_{254}) glass plates, and the compounds were visualized by UV (254 nm), 0.5% $KMnO_4$ in 0.1 M aqueous NaOH solution, 5% phosphomolybdic acid in EtOH, or a 2.5% solution of *p*-anisaldehyde in 95% EtOH. ^{1}H NMR spectra were recorded on a Bruker AVANCE 300, 400, or 500 MHz spectrometer. ^{13}C NMR spectra were recorded on a Bruker AVANCE 75, 100, or 125 MHz spectrometer. ^{1}H NMR chemical shifts are reported as δ values in ppm calibrated to residual $CHCl_3$ (7.26 ppm). ^{1}H NMR coupling constants (*J*) are reported in Hertz (Hz). ^{13}C NMR chemical shifts are calibrated to the $CDCl_3$ peak at 77.23 ppm. Infrared spectra were obtained on a FTIR spectrometer. High-resolution mass spectra (HRMS) were obtained using DART AccuTOF or JEOL JMS-AX505HA mass spectometers.

General procedure for the preparation of 3,3-dimethyl-β-lactones

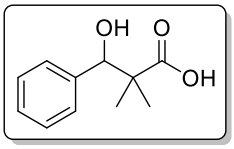


n-Butyllithium (2.5 M in hexanes, 2.0 equiv) was added dropwise to a stirred solution of diisopropylamine (2.0 equiv) in dry THF (0.15 M) at 0 °C under N_2. The solution was maintained at 0 °C for 15 min, and then isobutyric acid (1.0 equiv, 1.0 M solution in dry THF) was added dropwise at 0 °C. The ice bath was removed and reaction was stirred for 1 h at rt. Then, the aldehyde (1.0 equiv, 2.5 M solution in dry THF) was added dropwise at rt, and the solution was stirred for 48 h at rt. The reaction mixture was poured onto ice (equal to the total volume of the solution) and acidified with HCl (6 N) to pH 2. The mixture was extracted with Et_2O (3 × equal volumes), and the combined organic extracts were washed with brine (2 × 1/3 total volume), dried

($MgSO_4$), and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel.

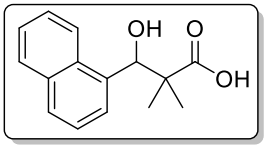
Benzenesulfonyl chloride (3.0 equiv) was added drop-wise to a solution of β-hydroxyacid (1.0 equiv) in dry pyridine (0.15 M) at 0 °C under N_2. The resulting solution was stirred at 0 °C for 48 h. Then, it was poured onto crushed ice (10 mL per 1.0 mmol) and stirred for 15 min. Et_2O (5 mL per 1.0 mmol) was added, and the layers were separated. The aqueous layer was extracted with Et_2O (3 × 7 mL per 1.0 mmol). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ (2 × 5 mL per 1.0 mmol), 10% aqueous $CuSO_4$ (4 × 10 mL per 1.0 mmol) and H_2O (1 × 5 mL per 1.0 mmol), dried ($MgSO_4$) and concentrated. The residue was purified by flash chromatography on silica gel.

3-Hydroxy-2,2-dimethyl-3-phenylpropanoic acid (119g)



3-Hydroxy-2,2-dimethyl-3-phenylpropanoic acid (**119g**) was prepared from benzaldehyde (5.50 g, 53.5 mmol) using the general procedure. Purification by flash chromatography on silica gel (DCM/MeOH, 95:5) afforded 3-hydroxy-2,2-dimethyl-3-phenylpropanoic acid (**119g**) as a white solid (7.89 g, 70%):[87] ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35–7.31 (m, 5H), 4.95 (s, 1H), 1.17 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 182.9, 139.8, 128.2, 128.2, 127.9, 78.8, 47.8, 23.6, 18.9.

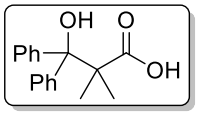
3-Hydroxy-2,2-dimethyl-3-(naphthalene-1-yl)propanoic acid (119f)



3-Hydroxy-2,2-dimethyl-3-(naphthalene-1-yl)propanoic acid (**119f**) was prepared from 1-naphthaldehyde (8.36 g, 53.5 mmol) using the general procedure. Purification by flash

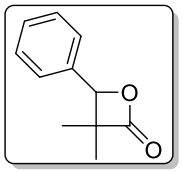
chromatography on silica gel (DCM/MeOH, 95:5) afforded 3-hydroxy-2,2-dimethyl-3-(naphthalene-1-yl)propanoic acid (**119f**) as a white solid (9.94 g, 76%): IR (neat) 3571, 3467, 1707, 1050, 806, 785 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31–8.29 (m, 1H), 7.89–7.87 (m, 1H), 7.83–7.81 (m, 1H), 7.76–7.74 (m, 1H), 7.53–7.44 (m, 3H), 6.03 (s, 1H), 4.92 (br s, 1H), 1.23 (s, 3H), 1.03 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.8, 137.2, 133.6, 132.0, 128.4, 127.7, 125.9, 125.3, 124.9, 124.5, 123.6, 72.2, 48.3, 23.0, 18.1; HRMS (ESI) *m/z* calcd for $C_{15}H_{15}O_3$ $[M - H]^+$ 243.1021, found 243.1019.

3-Hydroxy-2,2-dimethyl-3,3-diphenylpropanoic acid (119h)



3-Hydroxy-2,2-dimethyl-3,3-diphenylpropanoic acid (**119h**) was prepared from benzophenone (10.0 g, 54.8 mmol) using the general procedure. Purification by flash chromatography on silica gel (DCM/MeOH, 95:5) afforded 3-Hydroxy-2,2-dimethyl-3,3-diphenylpropanoic acid (**119h**) as a white solid (7.6 g, 51%):[88] ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.49–7.14 (m, 10H), 1.34 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 185.7, 145.1, 128.9, 127.8, 127.5, 82.7, 49.1, 24.1;

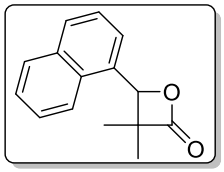
3,3-Dimethyl-4-phenyloxetan-2-one (115g)



3,3-Dimethyl-4-phenyloxetan-2-one (**115g**) was prepared from 3-hydroxy-2,2-dimethyl-3-phenylpropanoic acid (**119g**) (5.70 g, 29.3 mmol) using the general procedure. Purification by flash chromatography on silica gel (petroleum ether/EtOAc, 90:10) afforded 3,3-dimethyl-4-phenyloxetan-2-one (**115g**) as a white solid (4.45 g, 86%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41–

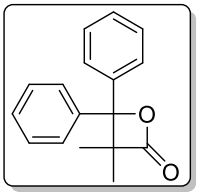
7.24 (m, 5H), 5.31 (s, 1H), 1.55 (s, 3H), 0.87 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.0, 135.4, 128.7, 128.5, 125.3, 82.8, 56.7, 22.5, 18.0.

3,3-Dimethyl-4-(naphthalene-1-yl)oxetan-2-one (115f)



3,3-Dimethyl-4-(naphthalene-1-yl)oxetan-2-one (**115f**) was prepared from 3-hydroxy-2,2-dimethyl-3-(naphthalene-1-yl)propanoic acid (**119f**) (3.00 g, 12.5 mmol) using the general procedure. Purification by flash chromatography on silica gel (petroleum ether/EtOAc, 90:10), afforded 3,3-dimethyl-4-(naphthalene-1-yl)oxetan-2-one (**115f**) as a white solid (1.73 g, 76%): IR (neat) 1812, 1085, 921, 866, 789, 773 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94–7.91 (m, 1H), 7.85–7.83 (m, 1H), 7.69–7.67 (m, 1H), 7.62–7.49 (m, 4H), 5.99 (s, 1H), 1.81 (s, 3H), 0.83 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.5, 133.2, 131.6, 129.7, 129.0, 128.4, 126.6, 126.0, 125.3, 122.5, 121.9, 81.3, 56.8, 22.0, 16.9; HRMS (ESI) *m/z* calcd for $C_{15}H_{15}O_2$ $[M + H]^+$ 227.1072, found 227.1086.

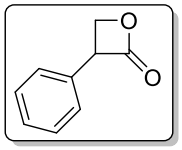
3,3-Dimethyl-4,4-diphenyloxetan-2-one (115h)



3,3-Dimethyl-4,4-diphenyloxetan-2-one (**115h**) was prepared from 3-hydroxy-2,2-dimethyl-3,3-diphenylpropanoic acid (**119h**) (7.55 g, 27.9 mmol) using the general procedure. Purification by flash chromatography on silica gel (petroleum ether/EtOAc, 90:10), afforded 3,3-dimethyl-4,4-diphenyloxetan-2-one (**115h**) as a white solid (4.02 g, 57%):[89] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51–

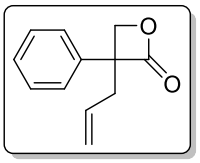
7.27 (m, 10H), 1.25 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.0, 139.9, 128.6, 127.9, 125.8, 88.7, 60.0, 21.7.

3-Phenyloxetan-2-one (115s)



Triphenylphosphine (3.18 g, 12.0 mmol) was dissolved in dry THF (40 mL) under N_2, and the solution was cooled to –78 °C. Then diethyl azodicarboxylate (2.10 g, 12.0 mmol) was added to the solution dropwise and the solution stirred for 20 min. A solution of tropic acid (2.00 g, 12.0 mmol) in dry THF (40 mL) was added dropwise, and the reaction mixture was stirred at –78 °C. After 30 min the mixture was slowly warmed to 0 °C in an ice bath over 2 h. The mixture was preloaded on silica gel and purified by flash chromatography on silica gel (petroleum ether/EtOAc, 90:10), yielding 3-phenyloxetan-2-one (**115a**) as a colorless syrup (1.78 g, 70%):[74] IR (neat) 1814, 1104, 880, 698 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42–7.29 (m, 5H) 4.94 (dd, *J* = 6.6, 4.9 Hz, 1H), 4.67 (dd, *J* = 6.8, 5.2 Hz, 1H), 4.36 (dd, *J* = 5.0, 5.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.7, 132.8, 129.4, 128.5, 127.3, 66.6, 57.1; HRMS (ESI) *m/z* calcd for $C_9H_9O_2$ $[M + H]^+$ 149.0603, found 149.0603.

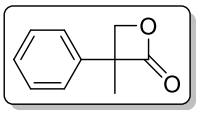
3-Allyl-3-phenyloxetan-2-one (115d)



n-Butyllithium (2.5 M in hexanes, 3.05 mL, 7.63 mmol) was added dropwise to a stirred solution of diisopropylamine (1.07 mL, 7.63 mmol) in dry THF (44 mL) at 0 °C under N_2. The solution was maintained at 0 °C for 15 min, then cooled to –78 °C. A solution of 3-phenyloxetan-2-one (**115a**)

(1.13 g, 7.63 mmol) in dry THF (22 mL) was added dropwise, and the solution was stirred for 30 min more. Allyl iodide (3.52 g, 21.0 mmol) in dry THF (16.5 mL) was added dropwise over 15 min, and the solution was maintained at –78 °C for 2 h. The reaction was quenched with saturated aqueous NH_4Cl (40 mL). The organic layer was separated, and the aqueous layer was extracted with Et_2O (3 × 50 mL). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated. The residue was purified by flash chromatography on silica gel (petroleum ether/EtOAc, 95:5) to afford 3-allyl-3-phenyloxetan-2-one (**115d**) as a yellow oil (0.91 g, 64%):[32] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37–7.25 (m, 5H), 5.78–5.67 (m, 1H), 5.18–5.13 (m, 2H), 4.48 (d, *J* = 5.3 Hz, 1H), 4.46 (d, *J* = 5.2 Hz, 1H), 2.76 (dd, *J* = 14.1, 7.0 Hz, 1H), 2.68 (dd, *J* = 14.1, 7.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.8, 137.2, 131.3, 128.8, 127.8, 126.1, 120.3, 69.6, 65.0, 41.6.

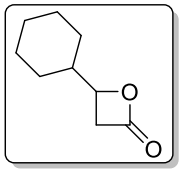
3-Methyl-3-phenyloxetan-2-one (115c)



n-Butyllithium (2.5 M in hexanes, 5.40 mL, 13.5 mmol) was added dropwise to a stirred solution of diisopropylamine (1.50 g, 14.8 mmol) in dry THF (78 mL) at 0 °C under N_2. The solution was maintained at 0 °C for 15 min, then cooled to –78 °C. A solution of 3-phenyloxetan-2-one (**115a**) (2.00 g, 13.5 mmol) in dry THF (39 mL) was added dropwise, and the solution was stirred for 30 min more. Methyl iodide (5.80 g, 40.8 mmol) in dry THF (29 mL) was added dropwise over 20 min, and the solution was maintained at –78 °C for 2 h. The reaction was quenched with saturated aqueous NH_4Cl (70 mL). The organic layer was separated, and the aqueous layer was extracted with Et_2O (3 × 90 mL). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated. The residue was purified by flash chromatography on silica gel (petroleum ether/EtOAc, 95:5) to afford 3-methyl-3-phenyloxetan-2-one (**115c**) as a yellow oil (1.45 g, 66%):[32]

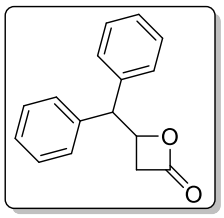
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41–7.29 (m, 5H), 4.52 (d, *J* = 4.7 Hz, 1H), 4.39 (d, *J* = 4.7 Hz, 1H), 1.77 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.1, 138.6, 129.0, 127.8, 125.5, 73.1, 61.2, 23.6.

4-Cyclohexyloxetan-2-one (115o)



A solution of $Ag(SbF_6)_3$ (3.03 g, 8.87 mmol) in dry DCM (20 mL) was added to a solution of $AlCl_3$ (0.39 g, 2.96 mmol) and *i*-Pr_2NEt (1.53 mL, 8.87 mmol) in DCM (20 mL) at –25 °C. Then *i*-Pr_2NEt (2.57 mL, 14.8 mmol), acetyl chloride (1.57 mL, 22.2 mmol), and a solution of cyclohexylcarboxaldehyde (1.67 g, 14.8 mmol) in DCM (5 mL) were added. The reaction mixture was stirred at –25 °C for 4 h. The reaction mixture was filtered through a pad of Celite, and the pad was washed with DCM (15 mL). The filtrate was then concentrated to give a yellow oil. The residue was purified by flash chromatography on silica gel (petroleum ether/EtOAc, 90:10) to afford 4-cyclohexyloxetan-2-one (**115o**) as a colorless oil (1.60 g, 71%):[82] ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.21–4.16 (m, 1H), 3.41 (dd, *J* = 16.3, 5.8 Hz, 1H), 3.10 (dd, *J* = 16.3, 4.4 Hz, 1), 1.97–1.55 (m, 6H), 1.33–0.93 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.8, 75.0, 42.1, 41.2, 28.3, 27.3, 26.1, 25.6, 25.3.

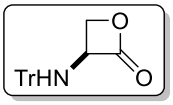
4-(2-Diphenylmethyl)oxetan-2-one (115p)



4-(2-Diphenylmethyl)-3-trimethylsilyloxetane-2-one[84] (2.36 g, 7.60 mmol) was dissolved in dry THF (50 mL) under nitrogen and cooled to –78 °C. A solution of TBAF (1.0 M in THF, 8.0 mL, 8.0 mmol) was added to the flask dropwise and the solution stirred at –78 °C. After stirring for 30 min,

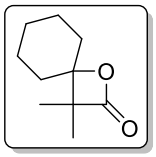
the reaction was quenched with pH 7 buffer (30 mL), and the solution was extracted with Et_2O (3 × 50 mL). The combined organic extracts were then dried ($MgSO_4$), filtered and concentrated, yielding a yellow oil. The crude oil was purified by flash chromatography on silica gel (petroleum ether/EtOAc, 90:10), to afford 4-(2-diphenylmethyl)oxetan-2-one (**115p**) as a colorless oil (1.35 g, 72%): IR (neat) 1825, 1118, 740, 697 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.36–7.22 (m, 10H), 5.15 (ddd, *J* = 8.3, 5.6, 4.4 Hz, 1H), 4.26 (d, *J* = 8.3 Hz, 1H), 3.51 (dd, *J* = 16.5, 5.7 Hz, 1H), 3.15 (dd, *J* = 16.5, 4.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.7, 139.6, 139.5, 129.2, 128.9, 128.7, 128.6, 127.7, 127.5, 72.3, 55.2, 42.7; HRMS (ESI) *m/z* calcd for $C_{16}H_{15}O_2$ $[M + H]^+$ 239.1072, found 239.1090.

(*S*)-3-(Tritylamino)oxetan-2-one (115e)



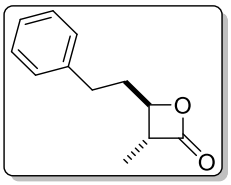
N-Tritylserine (2.00 g, 5.76 mmol) was suspended in dry DCM (30 mL) under N_2 at rt. Then triethylamine (2.29 mL, 16 mmol) was added, and the mixture became homogeneous. Benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (3.57 g, 8.07 mmol) was added in three portions over 15 min, and the solution stirred for 1 h. The reaction was then diluted with H_2O (30 mL) and stirred for an additional 20 min. The organic layer was separated, and the aqueous layer was extracted with DCM (3 × 30 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated, yielding a yellow solid. The crude solid was purified by flash chromatography on silica gel (petroleum ether/EtOAc, 85:15) to afford (*S*)-3-(tritylamino)-oxetan-2-one (**115e**) as a white solid (1.35 g, 72%):[45] ^1H NMR (400 MHz, $CDCl_3$) δ 7.42–7.29 (m, 5H), 4.94 (dd, *J* = 6.6, 4.9 Hz, 1H), 4.67 (dd, *J* = 6.8, 5.2 Hz, 1H), 4.36 (dd, *J* = 5.0, 5.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.3, 145.4, 128.7, 128.6, 128.2, 128.0, 127.5, 127.2, 71.0, 70.8, 64.7.

3,3-Dimethyloxetan-2-one-4-spirocyclohexane (115j)



n-Butyllithium (2.5 M in hexanes, 5.85 mL, 14.7 mmol) was added dropwise to a stirred solution of diisopropylamine (2.30 mL, 16.5 mmol) in dry THF (25 mL) at 0 °C under N_2. The solution was maintained at 0 °C for 15 min, then cooled to –78 °C. A solution of phenyl isobutyrate (2.00 g, 12.5 mmol) in dry THF (5 mL) was added dropwise, and the solution was left to stir to 30 min. Cyclohexanone (1.25 mL, 12.3 mmol) in dry THF (5 mL) was added dropwise, and the solution was maintained at –78 °C for 30 min. The reaction was slowly warmed to 0 °C over 2.5 h and stirred for an additional 1 h at 0 °C. The reaction was quenched with saturated aqueous NH_4Cl (10 mL), and the reaction mixture was diluted with Et_2O (10 mL). The organic layer was separated, and the aqueous layer was extracted with Et_2O (3 × 15 mL). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated to give a yellow oil. The residue was purified by flash chromatography on silica gel (petroleum ether/EtOAc, 95:5) to afford 3,3-dimethyloxetan-2-one-4-spirocyclohexane (**115j**) as a white solid (1.22 g, 58%):[90] ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.00–1.92 (m, 2H), 1.68–1.61 (m, 7H), 1.30 (br s, 7H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.3, 85.3, 54.6, 32.5, 25.0, 22.9, 18.3.

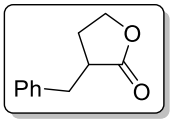
***trans*-4-(2-Phenylethyl)-3-methyloxetan-2-one (115l)**



Anhydrous $ZnCl_2$ (5.04 g, 37.0 mmol) was freshly fused (under vacuum at 0.5 mmHg) and after cooling to rt was broken into small pieces with a spatula. Then dry DCM (190 mL) was added, followed by the addition of hydrocinnamaldehyde (3.46 g, 25.8 mmol) dissolved in dry DCM (12

mL). After stirring for 20 min, TBS-thiopyridylketene acetal (**123**)[82] (8.00 g, 28.4 mmol) in dry DCM (12 mL) was added, and the reaction mixture was stirred for 24 h at rt. Freshly made phosphate buffer (pH = 7, 30 mL) was added, and the resulting mixture was stirred vigorously for 20 min. The mixture was filtered through a pad of Celite, and the Celite was washed with DCM (3 × 15 mL). The organic layer was separated, and the aqueous layer was extracted with DCM (3 × 30 mL). The combined organic extracts were dried ($MgSO_4$), and $CuBr_2$ (7.52 g, 33.6 mmol) was added and stirred for 1.5 h at rt. The mixture was filtered through a pad of Celite, and the Celite was washed with DCM (3 × 15 mL). The filtrate was washed with saturated aqueous K_2CO_3 (40 mL) and brine (40 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated to give a sticky oil. Purification by flash chromatography on silica gel (petroleum ether/EtOAc, 95:5) afforded *trans*-4-(2-phenylethyl)-3-methyloxetan-2-one (**115l**) as a colorless oil (3.76 g, 79%):[91] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36–7.22 (m, 5H), 4.20 (ddd, *J* = 7.4, 6.0, 4.1 Hz, 1H), 3.23 (dq, *J* = 7.6, 4.0 Hz, 1H), 2.89–2.70 (m, 2H), 2.26–2.07 (m, 2H), 1.34 (d, *J* = 7.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.0, 140.3, 128.8, 128.5, 126.6, 78.8, 51.0, 36.0, 31.5, 12.6.

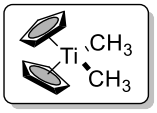
3-Benzyltetrahydrofuran-2-one (148)



n-Butyllithium (2.5 M in hexanes, 10.4 mL, 26.0 mmol) was added to diisopropylamine (2.67 g, 26.0 mmol) in dry THF (30 mL) under N_2 at –78 °C. The resulting solution was stirred 20 min. γ-Butyrolactone (2.59 g, 26.0 mmol) was added neat over 20 min; then benzyl bromide (4.90 g, 28.6 mmol) in THF (15 mL) was added dropwise over 20 min. The resulting reaction mixture was stirred for 1.5 h at –78 °C. The reaction was quenched with saturated aqueous NH_4Cl (30 mL) and extracted with diethyl ether (50 mL × 3). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated. The residue was purified by flash chromatography on silica gel

(petroleum ether/EtOAc, 85:15) to afford 3-benzyltetrahydrofuran-2-one (**148**) as a colorless oil (3.52 g, 77%):[86] IR (neat) 1762, 1144, 1019, 699 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.32–7.19 (m, 5H), 4.20 (ddd, *J* = 8.8, 8.8, 3.0 Hz, 1H), 4.12 (ddd, *J* = 9.2, 9.2, 6.7 Hz, 1H), 3.23 (dd, *J* = 13.5, 3.9 Hz, 1H), 2.84 (ddd, *J* = 13.4, 9.2, 4.2 Hz, 1H), 2.74 (dd, *J* = 13.5, 9.3 Hz, 1H), 2.26–2.19 (m, 1H), 2.02-1.92 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.8, 138.5, 128.9, 128.6, 126.8, 66.6, 41.1, 36.2, 28.1; HRMS (ESI) *m/z* calcd for $C_{11}H_{13}O_2$ $[M + H]^+$ 177.0916, found 177.0916.

Preparation of dimethyltitanocene



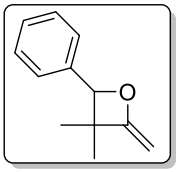
Methyllithium (1.60 M in Et_2O, 116 mL, 185 mmol) was added dropwise under N_2 to a stirred slurry of bis(cyclopentadienyl)titanocene dichloride (20.0 g, 80.3 mmol) in dry toluene (160 mL) at 0 °C. The mixture was stirred at 0 °C for 1 h and allowed to warm to rt over 1 h. Then the mixture was cooled to 0 °C and quenched carefully with ice-cold aqueous NH_4Cl (6%, 70 mL). The organic layer was separated and washed with H_2O (2 × 50 mL) and brine (2 × 50 mL), dried $MgSO_4$, and filtered to provide an orange solution. The solution was concentrated to one-third the volume and stored as a 0.50 M solution in toluene in the freezer.[32] ^1H NMR (400 MHz, $CDCl_3$) δ 6.27–6.24 (m, 10H), 0.19–0.17 (m, 6H).

General procedure for the preparation of 2-methyleneoxetanes

A solution of dimethyltitanocene (0.50 M in toluene, 1.5–2.5 equiv) and β-lactone (1.0 equiv) was stirred in the dark at 75 °C in a pressure tube. The reaction was monitored over a period of 4–12 h by TLC until the disappearance of the starting material. The reaction mixture was cooled to rt and poured into petroleum ether (10 volumes) and stirred overnight. The orange precipitate was filtered through a pad of Celite, and the filter cake was washed with petroleum ether until the filtrate was colorless. The filtrate was concentrated to one-tenth the volume, and the residue was

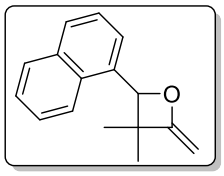
purified by flash column chromatography on silica gel (deactivated by 5% NEt_3 in petroleum ether).

3,3-Dimethyl-4-phenyl-2-methyleneoxetane (62g)



3,3-Dimethyl-4-phenyl-2-methyleneoxetane (**62g**) was prepared from 3,3-dimethyl-4-phenyloxetan-2-one (**115g**) (234 mg, 1.33 mmol) using the general procedure (2.0 eq of dimethyltitanocene). Purification by flash chromatography on silica gel (petroleum ether/NEt_3 99:1) afforded 3,3-dimethyl-4-phenyl-2-methyleneoxetane (**62g**) as a yellow oil (162 mg, 70%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45–7.34 (m, 5H), 5.56 (s, 1H), 4.23 (d, *J* = 3.7 Hz, 1H), 3.84 (d, *J* = 3.7 Hz, 1H), 1.55 (s, 3H), 0.87 (3, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.2, 138.8, 128.2, 127.8, 125.2, 90.3, 76.1, 46.9, 26.5, 21.7.

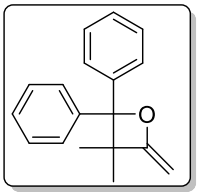
3,3-Dimethyl-4-(naphthalene-1-yl)-2-methyleneoxetane (62f)



3,3-Dimethyl-4-(naphthalen-1-yl)-2-methyleneoxetane (**62f**) was prepared from 3,3-dimethyl-4-(naphthalen-1-yl)oxetan-2-one (**115f**) (310 mg, 1.37 mmol) using the general procedure (2.0 eq of dimethyltitanocene). Purification by flash chromatography on silica gel (petroleum ether/ NEt_3 99:1) afforded 3,3-dimethyl-4-(naphthalen-1-yl)-2-methyleneoxetane (**62f**) as a yellow oil (221 mg, 72%): IR (neat) 3061, 3026, 2978, 2896, 1683, 959, 769, 697 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97–7.86 (m, 3H), 7.73–7.70 (m, 1H), 7.64–7.55 (m, 3H), 6.34 (s, 1H), 4.38 (d, *J* = 3.8 Hz, 1H), 3.95 (d, *J* = 3.7 Hz, 1H), 1.82 (s, 3H), 0.87 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.2, 134.3,

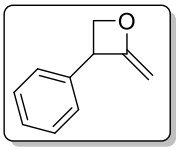
133.4, 130.2, 129.1, 128.0, 126.3, 125.8, 125.7, 123.1, 122.5, 88.6, 76.5, 47.4, 26.4, 21.2; HRMS (ESI) *m/z* calcd for $C_{16}H_{15}O$ $[M + H]^+$ 223.1123, found 223.1144.

3,3-Dimethyl-4,4-diphenyl-2-methyleneoxetane (62h)



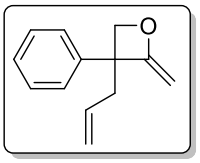
3,3-Dimethyl-4,4-diphenyl-2-methyleneoxetane (**62h**) was prepared from 3,3-dimethyl-4,4-diphenyloxetan-2-one (**115h**) (0.90 g, 3.6 mmol) using the general procedure (1.5 eq of dimethyltitanocene). Purification by flash chromatography on silica gel (petroleum ether/NEt_3 99:1) afforded 3,3-dimethyl-4,4-diphenyl-2-methyleneoxetane (**62h**) as a white solid (0.65 g, 72%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55–7.25 (m, 10H), 4.30 (d, *J* = 3.7 Hz, 1H), 3.83 (d, *J* = 3.7 Hz, 1H), 1.20 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.8, 142.4, 128.4, 127.2, 125.7, 93.8, 76.2, 51.2, 25.6.

3-Phenyl-2-methyleneoxetane (62a)



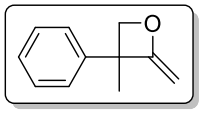
3-Phenyl-2-methyleneoxetane (**62a**) was prepared from 3-phenyloxetan-2-one (**115a**) (0.50 g, 3.4 mmol) using the general procedure (1.5 eq of dimethyltitanocene). Purification by flash chromatography on silica gel (petroleum ether/NEt_3 99:1) afforded 3-phenyl-2-methyleneoxetane (**62a**) as a colorless oil (0.31 g, 63%):[32] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48–7.35 (m, 5H), 5.02 (dd, *J* = 7.6, 5.1 Hz, 1H), 4.75–4.72 (m, 1H), 4.64 (dd, *J* = 5.1, 5.1 Hz, 1H), 4.37–4.36 (m, 1H), 3.91–3.90 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.5, 138.7, 128.9, 127.5, 127.5, 80.4, 76.0, 47.3.

3-Allyl-3-phenyl-2-methyleneoxetane (62d)



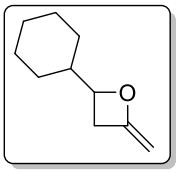
3-Allyl-3-phenyl-2-methyleneoxetane (**62d**) was prepared from 3-allyl-3-phenyloxetan-2-one (**115d**) (365 mg, 1.94 mmol) using the general procedure (1.8 eq of dimethyltitanocene). Purification by flash chromatography on silica gel (petroleum ether/NEt_3/EtOAc, 97.5:0.5:2) afforded 3-allyl-3-phenyl-2-methyleneoxetane (**62d**) as a yellow oil (254 mg, 70%):[32] IR (neat) 3061, 3026, 2978, 2896, 1683, 959, 769, 697 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.43–7.29 (m, 5H), 5.87–5.76 (m, 1H), 5.20–5.16 (m, 2H), 4.80 (d, *J* = 5.0 Hz, 1H), 4.76 (d, *J* = 5.0 Hz, 1H), 4.38 (d, *J* = 4.0 Hz, 1H), 4.08 (d, *J* = 4.0 Hz, 1H), 2.84–2.75 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5, 142.0, 133.3, 128.6, 127.0, 126.3, 118.9, 80.5, 79.2, 54.1, 44.1; HRMS (ESI) *m/z* calcd for $C_{13}H_{15}O$ $[M + H]^+$ 187.1123, found 187.1123.

3-Methyl-3-phenyl-2-methyleneoxetane (62c)



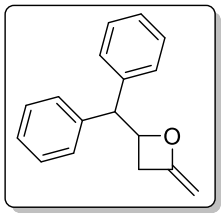
3-Methyl-3-phenyl-2-methyleneoxetane (**62c**) was prepared from 3-methyl-3-phenyloxetan-2-one (**115c**) (540 mg, 3.33 mmol) using the general procedure (1.8 eq of dimethyltitanocene). Purification by flash chromatography on silica gel (petroleum ether/NEt_3/EtOAc, 97.5:0.5:2) afforded 3-methyl-3-phenyl-2-methyleneoxetane (**62c**) as a yellow oil (332 mg, 62%):[32] ^1H NMR (400 MHz, $CDCl_3$) δ 7.53–7.33 (m, 5H), 4.77 (d, *J* = 4.9 Hz, 1H), 4.73 (d, *J* = 4.9 Hz, 1H), 4.36 (d, *J* = 4.0 Hz, 1H), 4.03 (d, *J* = 4.0 Hz, 1H), 1.82 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.9, 143.3, 128.6, 126.9, 125.7, 112.1, 82.3, 78.8, 50.5, 26.3.

4-Cyclohexyl-2-methyleneoxetane (62o)



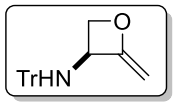
4-Cyclohexyl-2-methyleneoxetane (**62o**) was prepared from 4-cyclohexyloxetan-2-one (**115o**) (0.50 g, 3.3 mmol) using the general procedure (1.8 eq of dimethyltitanocene). Purification by flash chromatography on silica gel (petroleum ether/NEt_3 99:1) afforded 4-cyclohexyl-2-methyleneoxetane (**62o**) as a colorless oil (0.26 mg, 52%):[71c] ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.40 (ddd, *J* = 7.9, 6.7, 5.3 Hz, 1H), 4.09–4.07 (m, 1H), 3.72–3.70 (m, 1H), 3.17–3.10 (m, 1H), 2.88–2.82 (m, 1H), 1.94–1.88 (m, 1H), 1.78–1.58 (m, 5H), 1.31–1.10 (m, 3H), 0.99–0.83 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.0, 83.0, 79.6, 43.3, 32.4, 27.9, 26.6, 26.5, 25.7, 25.5.

4-(2-Diphenylmethyl)-2-methyleneoxetane (62p)



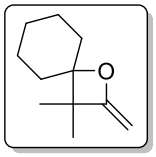
4-(2-Diphenylmethyl)-2-methyleneoxetane (**62p**) was prepared from 4-(2-diphenylmethyl)oxetan-2-one (**115p**) (0.33 g, 1.4 mmol) using the general procedure (1.8 eq of dimethyltitanocene). Purification by flash chromatography on silica gel (petroleum ether/NEt_3 99:1) afforded 4-(2-diphenylmethyl)-2-methyleneoxetane (**62p**) as a yellow oil (0.25 g, 76%): IR (neat) 3061, 1689, 951, 697 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39–7.24 (m, 10H), 5.45 (ddd, *J* = 9.2, 6.5, 5.2 Hz, 1H), 4.36 (d, *J* = 9.2 Hz, 1H), 4.24 (ddd, *J* = 3.4, 2.4, 2.4 Hz, 1H), 3.83 (ddd, *J* = 3.5, 1.8, 1.8 Hz, 1H), 3.25 (dddd, *J* = 15.1, 4.0, 1.9, 1.9 Hz, 1H), 2.97 (dddd, *J* = 7.2 4.5, 2.1, 2.1 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.7, 140.7, 140.0, 128.9, 128.7, 128.6, 128.5, 127.2, 127.0, 80.5, 80.1, 56.5, 33.7; HRMS (ESI) *m/z* calcd for $C_{17}H_{17}O$ $[M + H]^+$ 237.1279, found 237.1290.

(*S*)-3-Tritylamino-2-methyleneoxetane (62e)



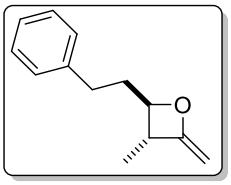
(*S*)-3-Tritylamino-2-methyleneoxetane (**62e**) was prepared from (*S*)-3-tritylaminooxetan-2-one (**115e**) (280 mg, 0.855 mmol) using the general procedure (1.8 eq of dimethyltitanocene). Purification by flash chromatography on silica gel (petroleum ether/NEt_3 99:1) afforded (*S*)-3-tritylamino-2-methyleneoxetane (**62e**) as a white solid (174 mg, 62%):[32] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 (d, *J* = 7.5 Hz, 6H), 7.42–7.30 (m, 9H), 4.61–4.55 (m, 1H), 4.29 (dd, *J* = 3.4, 2.5 Hz, 1H), 4.14 (dd, *J* = 3.4, 1.8 Hz, 1H), 4.02 (dd, *J* = 6.0, 6.0 Hz, 1H), 3.64 (dd, *J* = 5.5, 5.5 Hz, 1H), 2.69 (d, *J* = 11.7 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0, 146.1, 129.0, 128.6, 127.0, 80.3, 79.6, 70.4, 55.4.

3,3-Dimethyl-2-methyleneoxetane-4-spirocyclohexane (62j)



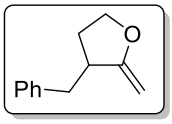
3,3-Dimethyl-2-methyleneoxetane-4-spirocyclohexane (**62j**) was prepared from 3,3-dimethyloxetan-2-one-4-spirocyclohexane (**115j**) (500 mg, 2.96 mmol) using the general procedure (1.5 eq of dimethyltitanocene). Purification by flash chromatography on silica gel (petroleum ether/NEt_3 99:1) afforded 3,3-dimethyl-2-methyleneoxetane-4-spirocyclohexane (**62j**) as a yellow oil (335 mg, 67%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.93 (d, *J* = 3.4 Hz, 1H), 3.61 (d, *J* = 3.4 Hz, 1H), 1.97–1.92 (m, 2H), 1.63–1.52 (m, 8H), 1.22 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.2, 90.1, 75.2, 46.4, 33.0, 25.3, 22.7, 22.1.

trans-3-Methyl-4-(2-phenylethyl)-2-methyleneoxetane (62l)



trans-3-Methyl-4-(phenylethyl)-2-methyleneoxetane (**62l**) was prepared from *trans*-4-(phenylethyl)-3-methyloxetan-2-one (**115l**) (0.50 g, 2.7 mmol) using the general procedure (2.0 eq of dimethyltitanocene). Purification by flash chromatography on silica gel (petroleum ether/NEt_3/EtOAc, 98.5:0.5:1) afforded *trans*-3-methyl-4-(phenylethyl)-2-methyleneoxetane (**62l**) as a yellow oil (0.36 mg, 72%):[37] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34–7.22 (m, 5H), 4.42 (ddd, *J* = 7.5, 5.3, 5.3 Hz, 1H), 4.13 (dd, *J* = 3.4, 2.3 Hz, 1H), 3.78 (dd, *J* = 3.5, 1.8 Hz, 1H), 3.11–3.03 (m, 1H), 2.79 (ddd, *J* = 14.2, 9.5, 5.6 Hz, 1H), 2.67 (ddd, *J* = 14.2, 9.1, 7.0 Hz, 1H), 2.23–2.14 (m, 1H), 2.09–2.00 (m, 1H), 1.27 (d, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.6, 141.2, 128.6, 128.6, 126.2, 86.6, 78.0, 42.1, 37.3, 30.9, 16.8.

3-Benzyl-2-methylenetetrahydrofuran (146a)



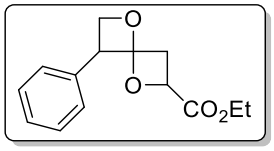
3-Benzyl-2-methylenetetrahydrofuran (**146a**) was prepared from 3-benzyltetrahydrofuran-2-one (**148**) (305 mg, 1.73 mmol) using the general procedure (1.5 eq of dimethyltitanocene). Purification by flash chromatography on silica gel (petroleum ether/NEt_3, 99:1) afforded 3-benzyl-2-methylenetetrahydrofuran (**146a**) as a white solid (193 mg, 64%): IR (neat) 1708, 1085, 1019, 732, 698 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34–7.22 (m, 5H), 4.30 (dd, *J* = 1.6, 1.6 Hz, 1H), 4.09 (ddd, *J* = 7.9, 7.9, 4.2 Hz, 1H), 3.96 (ddd, *J* = 8.4, 8.4, 6.5 Hz, 1H), 3.86 (dd, *J* = 1.6, 1.6 Hz, 1H), 3.06 (dd, *J* = 13.3, 5.2 Hz, 1H), 3.02–2.94 (m, 1H), 2.62 (dd, *J* = 13.4, 9.6 Hz, 1H), 2.03–1.95 (m, 1H), 1.78–1.69 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.3, 140.1, 129.0, 128.6, 126.5,

112.2, 79.0, 68.9, 42.7, 39.7, 31.2; HRMS (ESI) *m/z* calcd for $C_{12}H_{15}O$ $[M + H]^+$ 175.1123, found 175.1112.

General procedure for the preparation of aldol type reaction

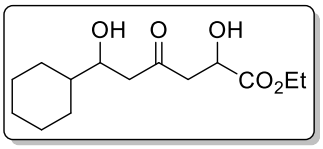
Anhydrous DCM (0.25 M) was added to a reaction tube containing 4 Å MS (50 mg / 1 mL DCM) under N_2. The mixture was cooled to –78 °C and the enol ether (1.0 mmol, 1 eq.) and the carbonyl derivative (1.4 mmol, 1.4 eq.) was added. Then *N*,*N*-diisopropylethylamine (1.0 mmol, 1 eq.) and the Lewis acid (1.0 mmol, 1 eq.) were added dropwise respectively. The solution was slowly warmed to rt over 2 h and stirred at rt for 12-24 h. The reaction mixture was concentrated *in vacuo* and the residue was purified directly by flash column chromatography on silica gel (petroleum ether/EtOAc).

Ethyl 7-phenyl-1,5-dioxaspiro[3.3]heptane-2-carboxylate (113a)



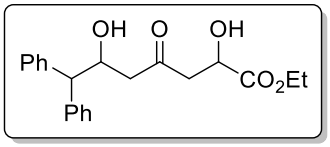
Ethyl 7-phenyl-1,5-dioxaspiro[3.3]heptane-2-carboxylate (**113a**) was prepared from 3-phenyl-2-methyleneoxetane (**62a**) (146 mg, 1.0 mmol) using the general procedure. Purification by flash chromatography on silica gel (petroleum ether/EtOAc, 90:10) afforded ethyl 7-phenyl-1,5-dioxaspiro[3.3]heptane-2-carboxylate (**113a**) as a colorless oil (184 mg, 74%): IR (neat) 1730, 1205, 1029, 940, 700 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.42–7.26 (m, 5H), 4.80 (dd, *J* = 8.0, 5.9 Hz, 1H), 4.57 (dd, *J* = 7.9, 6.0 Hz, 1H), 4.41 (dd, *J* = 5.8, 5.8 Hz, 1H), 4.24 (q, *J* = 7.1 Hz, 2H), 4.15 (dd, *J* = 7.7, 6.4 Hz, 1H), 2.79 (dd, *J* = 13.1, 8.1 Hz, 1H), 2.58 (dd, *J* = 13.1, 5.7 Hz, 1H), 1.28 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1, 136.5, 129.3, 128.0, 127.8, 117.0, 69.6, 68.9, 61.6, 52.5, 36.4, 14.3; HRMS (ESI) *m/z* calcd for $C_{14}H_{17}O_4$ $[M + H]^+$ 249.1127, found 249.1123.

Ethyl 6-cyclohexyl-2,6-dihydroxy-4-oxohexanoate (131o)



Ethyl 6-cyclohexyl-2,6-dihydroxy-4-oxohexanoate (**131o**) was prepared from 4-cyclohexyl-2-methyleneoxetane (**62o**) (129 mg, 0.847 mmol) using the general procedure. Purification by flash chromatography on silica gel (petroleum ether/EtOAc, 70:30) afforded ethyl 6-cyclohexyl-2,6-dihydroxy-4-oxohexanoate (**131o**) as a colorless oil (230 mg, 78%, dr 1:1): IR (neat) 3459 (br), 2921, 1712, 1203, 1095, 1027 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.50–4.44 (m, 1H), 4.28–4.17 (m, 2H), 3.85–3.78 (m, 1H), 3.00–2.82 (m, 3H), 2.59–2.57 (m, 2H), 1.82–1.60 (m, 6H), 1.37–0.92 (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 209.7, 209.6, 173.8, 71.9, 71.8, 67.1, 67.1, 62.1, 47.5, 47.4, 47.0, 43.3, 29.0, 28.4, 26.6, 26.3, 26.2, 14.3; HRMS (ESI) *m/z* calcd for $C_{14}H_{25}O_5$ $[M + H]^+$ 273.1702, found 273.1702.

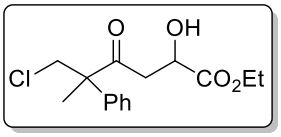
Ethyl 2,6-dihydroxy-4-oxo-7,7-diphenylheptanoate (131p)



Ethyl 2,6-dihydroxy-4-oxo-7,7-diphenylheptanoate (**131p**) was prepared from 4-(2-diphenyl-methyl)-2-methyleneoxetane (**62p**) (156 mg, 0.660 mmol) using the general procedure. Purification by flash chromatography on silica gel (petroleum ether/EtOAc, 70:30) afforded ethyl 2,6-dihydroxy-4-oxo-7,7-diphenylheptanoate (**131p**) as a colorless oil (210 mg, 83%, dr 1:1): IR (neat) 3442 (br), 1720, 1095, 1031, 746, 704 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40–7.17 (m, 10H), 4.88 (dd, *J* = 8.7, 8.7 Hz, 1H), 4.48–4.41 (m, 1H), 4.23 (q, *J* = 7.2 Hz, 1H), 3.92 (d, *J* = 9.0 Hz, 1H), 3.17 (dd, *J* = 4.9, 3.4 Hz, 1H), 2.94–2.78 (m, 2H), 2.66–2.51 (m, 3H), 1.28–1.24 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 208.6, 173.8, 141.9, 141.3, 129.1, 129.0, 128.9, 128.5, 127.1, 70.2,

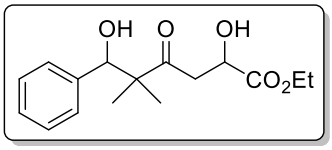
70.2, 67.1, 67.0, 62.2, 58.0, 48.7, 48.6, 47.2, 47.1, 14.3; HRMS (ESI) *m/z* calcd for $C_{21}H_{25}O_5$ [M + H]$^+$ 357.1702, found 357.1689.

Ethyl 6-chloro-2-hydroxy-5-methyl-4-oxo-5-phenylhexanoate (132c)



Ethyl 2,6-dihydroxy-5-methyl-4-chloro-5-phenylhexanoate (**132c**) was prepared from 3-methyl-3-phenyl-2-methyleneoxetane (**62c**) (110 mg, 0.686 mmol) using the general procedure. Purification by flash chromatography on silica gel (petroleum ether/EtOAc, 90:10) afforded ethyl 2,6-dihydroxy-5-methyl-4-oxo-5-phenylhexanoate (**132c**) as a colorless oil (135 mg, 66%): IR (neat) 3507 (br), 1734, 1707, 1207, 1097, 1025, 699 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40–7.23 (m, 5H), 4.41–4.38 (m, 1H), 4.28–4.12 (m, 2H), 4.06 (d, *J* = 11.3 Hz, 1H), 3.82 (dd, *J* = 11.3, 2.2 Hz, 1H), 3.08 (dd, *J* = 15.2, 5.2 Hz, 1H), 2.93–2.80 (m, 1H), 2.73–2.65 (m, 1H), 1.71 (s, 3H), 1.26–1.23 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 208.0, 207.8, 173.9, 138.8, 129.2, 128.2, 126.8, 67.0, 66.8, 62.1, 56.9, 51.3, 42.1, 42.0, 19.7, 14.3; HRMS (ESI) *m/z* calcd for $C_{15}H_{20}ClO_4$ [M + H]$^+$ 299.1050, found 299.1045.

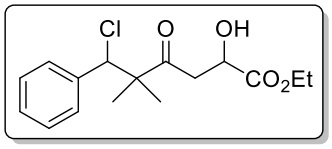
Ethyl 2,6-dihydroxy-5,5-dimethyl-4-oxo-6-phenylhexanoate (131g)



Ethyl 2,6-dihydroxy-5,5-dimethyl-4-oxo-6-phenylhexanoate (**131g**) was prepared from 3,3-dimethyl-4-phenyl-2-methyleneoxetane (**62g**) (174 mg, 1.00 mmol) using the general procedure. Purification by flash chromatography on silica gel (petroleum ether/EtOAc, 90:10 to 70:30) afforded ethyl 2,6-dihydroxy-5,5-dimethyl-4-oxo-6-phenylhexanoate (**131g**) as a colorless oil (244 mg, 83%, dr 3:2). The diastereomers were inseparable during column chromatography: IR (neat)

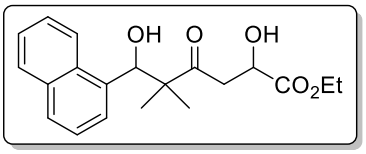
3480 (br), 1733, 1710, 1200, 1096, 1054, 1028, 702 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.35–7.32 (m, 5H), 5.32 (s, 0.6H), 5.31 (s, 0.4 H), 4.51–4.46 (m, 1H), 4.28–4.23 (m, 2H), 3.18–3.02 (m, 2H), 1.30–1.25 (m, 6H), 1.01 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 211.1, 211.0, 173.9, 137.1, 129.3, 128.6, 128.1, 68.0, 67.9, 67.1, 67.0, 62.0, 53.7, 53.6, 42.6, 42.5, 23.8, 23.7, 18.7, 18.6, 14.3; HRMS (ESI) *m/z* calcd for $C_{16}H_{22}O_5$ $[M]^+$ 294.1467, found 294.1730.

Ethyl 6-chloro-5,5-dimethyl-2-hydroxy-4-oxo-6-phenylhexanoate (132g)



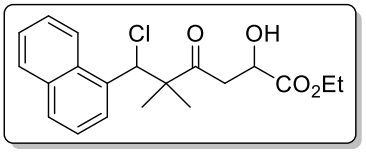
Ethyl 6-chloro-5,5-dimethyl-2-hydroxy-4-oxo-6-phenylhexanoate (**132g**) was prepared from 3,3-dimethyl-4-phenyl-2-methyleneoxetane (**62g**) (174 mg, 1.00 mmol) using the general procedure with $TiCl_4$ as the Lewis acid. Purification by flash chromatography on silica gel (petroleum ether/EtOAc, 90:10 to 80:20) afforded ethyl 6-chloro-5,5-dimethyl-2-hydroxy-4-oxo-6-phenylhexanoate (**132g**) as a colorless oil (163 mg, 52%, dr 2:1). The diastereomers were inseparable during column chromatography: ^1H NMR (400 MHz, $CDCl_3$) δ 7.38–7.29 (m, 7.5H), 4.92 (s, 1H), 4.86 (dd, *J* = 8.0, 3.6 Hz, 1H), 4.41 (s, 0.5H), 4.38 (dd, *J* = 12.4, 3.2 Hz, 0.5H), 4.29–4.17 (m, 3H), 3.08 (dd, *J* = 15.4, 8.0 Hz, 1H), 3.04 (dd, *J* = 14.6, 12.4 Hz, 0.5H), 2.84 (dd, *J* = 15.3, 3.6 Hz, 1H), 2.65 (dd, *J* = 14.6, 3.2 Hz, 0.5H), 1.31 (t, *J* = 9.2 Hz, 1.5H), 1.26 (t, *J* = 7.2 Hz, 3H), 1.08 (s, 1.5H), 1.06 (s, 3H), 1.03 (s, 3H), 0.97 (s, 1.5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 210.0, 209.7, 170.8, 169.4, 136.6, 136.3, 128.4, 128.3, 128.1, 128.1, 128.0, 86.4, 83.2, 76.9, 76.0, 72.9, 61.8, 61.8, 50.8, 50.3, 40.8, 38.7, 29.9, 20.9, 19.9, 19.9, 19.5, 14.4; HRMS (ESI) *m/z* calcd for $C_{16}H_{25}ClNO_4$ $[M + NH_4]^+$ 330.1472, found 330.1471.

Ethyl 2,6-dihydroxy-5,5-dimethyl-6-(naphthalene-1-yl)-4-oxohexanoate (133f)



Ethyl 2,6-dihydroxy-5,5-dimethyl-6-(naphthalene-1-yl)-4-oxohexanoate (**133f**) was prepared from 3,3-dimethyl-4-(naphthalen-1-yl)-2-methyleneoxetane (**62f**) (224 mg, 1.00 mmol) using the general procedure. Purification by flash chromatography on silica gel (petroleum ether/EtOAc, 90:10) afforded ethyl 2,6-dihydroxy-5,5-dimethyl-6-(naphthalene-1-yl)-4-oxohexanoate (**133f**) as a colorless oil (185 mg, 59%): IR (neat) 2975, 1746, 1718, 1135, 1023, 792 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, *J* = 7.6 Hz, 1H), 7.89–7.82 (m, 3H), 7.57–7.44 (m, 3H), 5.38 (s, 1H), 4.51 (dd, *J* = 12.4, 3.3 Hz, 1H), 4.29–4.24 (m, 2H), 3.14 (dd, *J* = 14.6, 12.4 Hz, 1H), 2.73 (dd, *J* = 14.6, 3.3 Hz, 1H), 1.31 (t, *J* = 7.2 Hz, 3H), 1.22 (s, 3H), 0.89 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 210.1, 169.5, 133.8, 132.4, 131.5, 129.2, 129.0, 127.5, 126.2, 125.5, 125.2, 123.8, 76.3, 61.9, 52.1, 40.8, 20.5, 20.3, 14.3; HRMS (ESI) *m/z* calcd for $C_{20}H_{24}O_5$ $[M]^+$ 344.1624, found 344.1837.

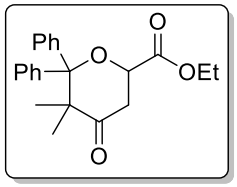
Ethyl 6-chloro-5,5-dimethyl-2-hydroxy-6-(naphthalene-1-yl)-4-oxohexanoate (133f')



Ethyl 6-chloro-5,5-dimethyl-2-hydroxy-6-(naphthalene-1-yl)-4-oxohexanoate (**133f'**) was obtained as the minor product from the above reaction. Compound **133f'** was obtained as a colorless oil (54 mg, 15%): IR (neat) 2924, 1713, 1127, 906, 727 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (d, *J* = 8.3 Hz, 1H), 7.88–7.84 (m, 2H), 7.57–7.46 (m, 4H), 5.92 (s, 1H), 4.72 (dd, *J* = 7.0, 5.6 Hz, 1H), 4.24–4.13 (m, 2H), 3.56 (dd, *J* = 15.6, 7.2 Hz, 1H), 1.22–1.20 (m, 6H), 1.15 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 210.5, 170.8, 134.1, 132.1, 131.9, 129.3, 129.1, 126.9, 126.6,

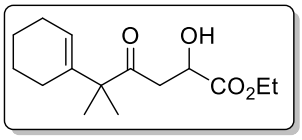
125.8, 124.8, 123.9, 78.3, 72.2, 61.8, 50.8, 39.1, 23.3, 20.5, 14.3; HRMS (ESI) *m/z* calcd for $C_{20}H_{23}ClO_4$ $[M]^+$ 362.1285, found 362.2022.

Ethyl 5,5-dimethyl-4-oxo-6,6-diphenyloxane-2-carboxylate (114h)



Ethyl 5,5-dimethyl-4-oxo-6,6-diphenyloxane-2-carboxylate (**114h**) was prepared from 3,3-dimethyl-4,4-diphenyl-2-methyleneoxetane (**62h**) (137 mg, 0.548 mmol) using the general procedure with $ZnCl_2$ as the Lewis acid. Purification by flash chromatography on silica gel (petroleum ether/EtOAc, 90:10) afforded ethyl 5,5-dimethyl-4-oxo-6,6-diphenyloxane-2-carboxylate (**114h**) as a colorless oil (56 mg, 29%): IR (neat) 2920, 1738, 1709, 1657, 1446, 1275, 698 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.36–7.23 (m, 10H), 4.31–4.12 (m, 2H), 3.87 (dd, *J* = 11.8, 4.4 Hz, 1H), 2.99 (dd, *J* = 16.0, 11.8 Hz, 1H), 2.58 (dd, *J* = 16.0, 4.3 Hz, 1H), 1.40 (s, 3H), 1.28 (t, *J* = 7.1 Hz, 3H), 1.13 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 211.2, 170.5, 142.3, 141.3, 132.6, 130.8, 130.3, 129.3, 128.5, 128.4, 127.4, 127.0, 87.8, 68.9, 61.5, 52.8, 39.3, 24.3, 19.7, 14.3; HRMS (ESI) *m/z* calcd for $C_{22}H_{25}O_4$ $[M + H]^+$ 353.1752, found 353.1747.

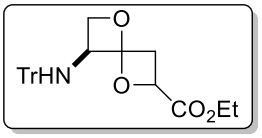
Ethyl 5-(cyclohex-1-en-1-yl)-2-hydroxy-5-methyl-4-oxohexanoate (135)



Ethyl 5-(cyclohex-1-en-1-yl)-2-hydroxy-5-methyl-4-oxohexanoate (**135**) was prepared from 3,3-dimethyl-4-phenyl-2-methyleneoxetane (**62j**) (174 mg, 1.00 mmol) using the general procedure. Purification by flash chromatography on silica gel (petroleum ether/EtOAc, 95:5) afforded ethyl 5-(cyclohex-1-en-1-yl)-2-hydroxy-5-methyl-4-oxohexanoate (**135**) as colorless oil (281 mg, 58%):

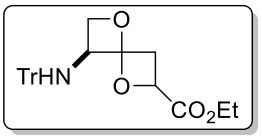
IR (neat) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.72–5.65 (m, 1H), 4.40 (ddd, *J* = 7.3, 7.3, 7.3 Hz, 1H), 4.23 (q, *J* = 9.5 Hz, 2H), 3.16 (d, *J* = 7.6 Hz, 1H), 2.93 (dd, *J* = 23.8, 5.5 Hz, 1H), 2.86 (dd, *J* = 23.8, 7.4 Hz, 1H), 2.13–2.05 (m, 2H), 1.84–1.74 (m, 2H), 1.62–1.50 (m, 4H), 1.28 (t, *J* = 9.5 Hz, 3H), 1.18 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 212.3, 174.3, 139.7, 123.1, 67.4, 61.9, 53.6, 40.6, 26.0, 25.8, 23.3, 23.1, 23.0, 22.4, 14.3; HRMS (ESI) *m/z* calcd for $C_{15}H_{25}O_4$ $[M + H]^+$ 269.1753, found 269.1760.

Ethyl 7-tritylamino-1,5-dioxaspiro[3.3]heptane-2-carboxylate (113e)



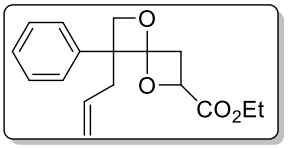
Ethyl 7-tritylamino-1,5-dioxaspiro[3.3]heptane-2-carboxylate (**113e**) was prepared from (*S*)-3-tritylamino-2-methyleneoxetane (**62e**) (220 mg, 0.67 mmol) using the general procedure. Purification by flash chromatography on silica gel (petroleum ether/EtOAc, 90:10) afforded ethyl 7-tritylamino-1,5-dioxaspiro[3.3]heptane-2-carboxylate (**113e**) as a colorless oil (114 mg, 40%): IR (neat) 1732, 1447, 1260, 1031, 700 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50–7.46 (m, 6H), 7.29–7.17 (m, 9H), 4.93 (dd, *J* = 8.6, 6.0 Hz, 1H), 4.39–4.25 (m, 2H), 3.88–3.82 (m, 2H), 3.78–3.73 (m, 1H), 3.20–3.10 (m, 1H), 2.91 (dd, *J* = 13.1, 8.6 Hz, 1H), 2.42 (dd, *J* = 13.2, 6.0 Hz, 1H), 1.33 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1, 146.0, 128.6, 128.4, 126.9, 119.1, 73.4, 70.8, 70.4, 61.7, 58.9, 34.0, 14.6; HRMS (ESI) *m/z* calcd for $C_{27}H_{28}NO_4$ $[M + H]^+$ 430.2018, found 430.1991.

Ethyl 7-tritylamino-1,5-dioxaspiro[3.3]heptane-2-carboxylate (113e')



Ethyl 7-tritylamino-1,5-dioxaspiro[3.3]heptane-2-carboxylate (**113e'**) was obtained as the minor isomer from the above reaction. Compound **113e'** was obtained as a colorless oil (76 mg, 26%): IR (neat) 1730, 1205, 1029, 940, 700 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54–7.52 (m, 6H), 7.34–7.21 (m, 9H), 5.05 (dd, *J* = 8.3, 6.1 Hz, 1H), 3.91 (ddd, *J* = 12.7, 6.4, 6.4 Hz, 1H), 3.62 (dd, *J* = 12.5, 6.1 Hz, 1H), 3.45 (dd, *J* = 6.4, 6.4 Hz, 1H), 2.93–2.88 (m, 2H), 2.18 (d, *J* = 12.3 Hz, 1H) , 1.34 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1, 146.0, 128.6, 128.4, 126.9, 119.1, 73.4, 70.8, 70.4, 61.7, 58.9, 34.0, 14.6; HRMS (ESI) *m/z* calcd for $C_{27}H_{28}NO_4$ $[M + H]^+$ 430.2018, found 430.2020.

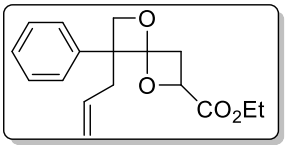
Ethyl 7-phenyl-7-(prop-2-en-1-yl)-1,5-dioxaspiro[3.3]heptane-2-carboxylate (113d)



Ethyl 7-phenyl-7-(prop-2-en-1-yl)-1,5-dioxaspiro[3.3]heptane-2-carboxylate (**113d**) was prepared from 3-allyl-3-phenyl-2-methyleneoxetane (**62d**) (310 mg, 1.66 mmol) using the general procedure with $ZnCl_2$ as the Lewis acid. Purification by flash chromatography on silica gel (petroleum ether/EtOAc, 90:10) afforded ethyl 7-phenyl-7-(prop-2-en-1-yl)-1,5-dioxaspiro[3.3]heptane-2-carboxylate (**113d**) as a colorless oil (186 mg, 39%): IR (neat) 2920, 1742, 1203, 1032, 949 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41–7.28 (m, 5H), 5.54–5.43 (m, 1H), 5.06 (dd, *J* = 17.1, 1.4 Hz, 1H), 4.98 (d, *J* = 10.1 Hz, 1H), 4.90 (dd, *J* = 8.5, 5.8 Hz, 1H), 4.70 (d, *J* = 5.6 Hz, 1H), 4.38 (d, *J* = 5.7 Hz, 1H), 4.30–4.21 (m, 2H), 3.07–2.96 (m, 2H), 2.73 (dd, *J* = 13.2, 8.6 Hz, 1H), 2.53 (dd, *J* = 13.2, 5.8 Hz, 1H), 1.31 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz,

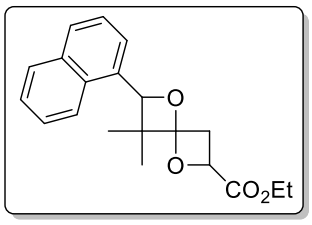
$CDCl_3$) δ 172.0, 139.3, 133.5, 128.9, 127.2, 127.2, 118.5, 118.0, 71.7, 69.8, 61.6, 55.9, 39.0, 36.8, 14.4; HRMS (ESI) *m/z* calcd for $C_{17}H_{21}O_4$ $[M + H]^+$ 289.1440, found 289.1416.

Ethyl 7-phenyl-7-(prop-2-en-1-yl)-1,5-dioxaspiro[3.3]heptane-2-carboxylate (113d')



Ethyl 7-phenyl-7-(prop-2-en-1-yl)-1,5-dioxaspiro[3.3]heptane-2-carboxylate (**113d'**) was obtained as the minor isomer from the above reaction. Compound **113d'** was obtained as a colorless oil (125 mg, 26%): IR (neat) 1731, 1206, 1029, 944, 914, 700 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.44–7.23 (m, 5H), 5.51–5.41 (m, 1H), 5.06 (dd, *J* = 17.1, 1.3 Hz, 1H), 5.01–4.98 (m, 1H), 4.68 (dd, *J* = 7.9, 6.2 Hz, 1H), 4.64 (dd, *J* = 5.6, 1.4 Hz, 1H), 4.38 (d, *J* = 5.6 Hz, 1H), 4.26 (q, *J* = 7.2 Hz, 2H), 3.10 (dd, *J* = 14.5, 8.3 Hz, 1H), 2.87–2.80 (m, 2H), 2.53 (dd, *J* = 13.2, 6.2 Hz, 1H), 1.29 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1, 139.7, 133.3, 129.1, 127.4, 126.7, 118.8, 116.4, 71.6, 68.9, 61.8, 55.6, 38.8, 37.0, 14.4; HRMS (ESI) *m/z* calcd for $C_{17}H_{21}O_4$ $[M + H]^+$ 289.1440, found 289.1418.

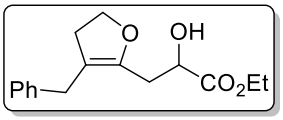
Ethyl 7,7-dimethyl-6-(naphthalene-1-yl)-1,5-dioxaspiro[3.3]heptane-2-carboxylate (113f)



Ethyl 7,7-dimethyl-6-(naphthalene-1-yl)-1,5-dioxaspiro[3.3]heptane-2-carboxylate (**113f**) was prepared from 3,3-dimethyl-4-(naphthalen-1-yl)-2-methyleneoxetane (**62f**) (224 mg, 1.00 mmol) using the general procedure. Purification by flash chromatography on silica gel (petroleum ether/EtOAc, 90:10) afforded ethyl 7,7-dimethyl-6-(naphthalene-1-yl)-1,5-dioxaspiro[3.3]heptane-2-carboxylate (**3c**) as a colorless oil (189 mg, 58%, dr 4:1). The diastereomers were inseparable

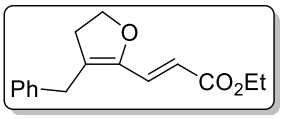
during column chromatography: IR (neat) 2966, 1736, 1015, 948, 733 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90–7.86 (m, 1.2H), 7.79–7.77 (m, 1H), 7.72–7.70 (m, 0.8H), 7.62–7.60 (m, 1H), 7.56–7.46 (m, 3H), 6.07 (s, 0.8H), 5.86 (s, 0.2H), 5.01–4.97 (m, 1H), 4.32–4.17 (m, 2H), 3.16–3.06 (m, 0.4H), 3.08 (dd, *J* = 12.8, 8.5 Hz, 0.8H), 2.87 (dd, *J* = 13.0, 5.4 Hz, 0.8H), 1.74 (s, 2.4H), 1.64 (s, 0.6H), 1.31 (t, *J* = 7.2 Hz, 2.4H), 1.29 (t, *J* = 7.2 Hz, 0.6H), 0.75 (s, 0.6H), 0.66 (s, 2.4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.2, 172.1, 137.6, 135.6, 135.2, 133.5, 133.4, 133.3, 130.3, 129.1, 129.0, 127.9, 127.8, 126.3, 126.1, 125.9, 125.7, 123.7, 122.8, 122.6, 122.5, 117.3, 117.0, 85.2, 84.5, 69.6, 61.5, 47.9, 47.5, 35.8, 35.6, 24.0, 22.1, 18.8, 16.7, 14.3; HRMS (ESI) *m/z* calcd for $C_{20}H_{23}O_4$ $[M + H]^+$ 327.1596, found 327.1603.

Ethyl 3-(3-benzyl-4,5-dihydrofuran-2-yl)-2-hydroxypropanoate (149)



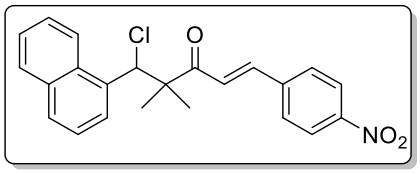
Ethyl 3-(3-benzyl-4,5-dihydrofuran-2-yl)-2-hydroxypropanoate (**149**) was prepared from 3-benzyl-2-methylenetetrahydrofuran (**146a**) (115 mg, 0.660 mmol) using the general procedure with $ZnCl_2$ as the Lewis acid. Purification by flash chromatography on silica gel (petroleum ether/EtOAc, 90:10) afforded ethyl 3-(3-benzyl-4,5-dihydrofuran-2-yl)-2-hydroxypropanoate (**149**) as a colorless oil (182 mg, 83%): IR (neat) 3454, 2899, 1731, 1196, 1149, 1091 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31–7.17 (m, 5H), 4.40 (dd, *J* = 11.2, 6.4 Hz, 1H), 4.29–4.11 (m, 4H), 3.37 (s, 2H), 3.13 (d, *J* = 7.1 Hz, 1H), 2.78–2.66 (m, 2H), 2.49–2.44 (m, 2H), 1.28 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.2, 147.3, 140.0, 128.6, 128.6, 126.3, 109.9, 69.2, 68.4, 61.8, 33.1, 32.8, 31.2, 14.4; HRMS (ESI) *m/z* calcd for $C_{16}H_{21}O_4$ $[M + H]^+$ 277.1440, found 277.1422.

Ethyl (*2E*)-3-(3-benzyl-4,5-dihydrofuran-2-yl)prop-2-enoate (152)



Ethyl (*2E*)-3-(3-benzyl-4,5-dihydrofuran-2-yl)prop-2-enoate (**152**) was prepared from 3-benzyl-2-methylenetetrahydrofuran (**146a**) (115 mg, 0.660 mmol) using the general procedure with $SnCl_4$ (or $TiCl_4$) as the Lewis acid. Purification by flash chromatography on silica gel (petroleum ether/EtOAc, 90:10) afforded ethyl (*2E*)-3-(3-benzyl-4,5-dihydrofuran-2-yl)prop-2-enoate (**152**) as a colorless oil (133 mg, 78%): IR (neat) 1717, 1299, 1275, 1178, 1027, 983, 700 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.40 (d, *J* = 15.4 Hz, 1H), 7.31–7.15 (m, 5H), 6.16 (d, *J* = 15.4 Hz, 1H), 4.31 (t, *J* = 9.4 Hz, 2H), 4.23 (q, *J* = 7.1 Hz, 2H), 3.58 (s, 2H), 2.62 (t, *J* = 9.4 Hz, 2H), 1.31 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.6, 148.4, 138.8, 129.7, 128.8, 128.7, 126.7, 122.1, 118.9, 68.3, 60.6, 34.3, 33.2, 14.5; HRMS (ESI) *m/z* calcd for $C_{16}H_{19}O_3$ $[M + H]^+$ 259.1334, found 259.1329.

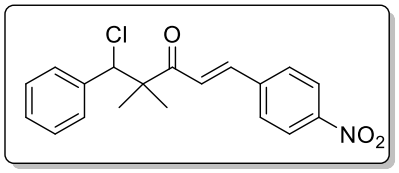
(1*E*)-5-Chloro-4,4-dimethyl-5-(naphthalene-1-yl)-1-(4-nitrophenyl)pent-1-en-3-one (136f)



(1*E*)-5-Chloro-4,4-dimethyl-5-(naphthalene-1-yl)-1-(4-nitrophenyl)pent-1-en-3-one (**136f**) was prepared from 3,3-dimethyl-4-(naphthalen-1-yl)-2-methyleneoxetane (**62f**) (224 mg, 1.00 mmol) using the general procedure with $TiCl_4$ as the Lewis acid. Purification by flash chromatography on silica gel (petroleum ether/EtOAc, 96:4) afforded (1*E*)-5-chloro-4,4-dimethyl-5-(naphthalene-1-yl)-1-(4-nitrophenyl)pent-1-en-3-one (**136f**) as colorless needles (378 mg, 96%): IR (neat) 1601, 1515, 1337, 1053, 786 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.25–8.16 (m, 3H), 7.87–7.83 (m, 3H), 7.65–7.47 (m, 6H), 7.18 (d, *J* = 15.6 Hz, 1H), 6.45 (br s, 1H), 1.57 (br s, 3H), 1.13 (br s, 3H); ^{13}C

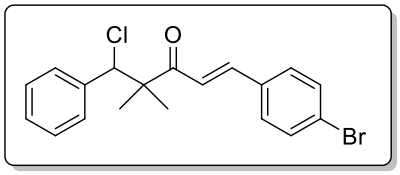
NMR (100 MHz, $CDCl_3$) δ 201.5, 148.8, 141.1, 141.1, 133.9, 133.6, 131.7, 129.4, 129.3, 129.1, 128.8, 126.8, 125.9, 125.2, 124.9, 124.3, 123.1, 62.2, 54.0, 24.2, 20.0; HRMS (ESI) *m/z* calcd for $C_{23}H_{20}ClNO_3$ $[M]^+$ 393.1132, found 393.1132.

(1*E*)-5-Chloro-4,4-dimethyl-1-(4-nitrophenyl)-5-phenylpent-1-en-3-one (136g)



(1*E*)-5-Chloro-4,4-dimethyl-1-(4-nitrophenyl)-5-phenylpent-1-en-3-one (**136g**) was prepared from 3,3-dimethyl-4-phenyl-2-methyleneoxetane (**62g**) (174 mg, 1.00 mmol) using the general procedure with $TiCl_4$ as the Lewis acid. Purification by flash chromatography on silica gel (petroleum ether/EtOAc, 95:5) afforded (1*E*)-5-chloro-4,4-dimethyl-1-(4-nitrophenyl)-5-phenylpent-1-en-3-one (**136g**) as colorless oil (316 mg, 92%): IR (neat) 1684, 1602, 1516, 1338, 1052 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.25–8.23 (m, 2H), 7.71–7.68 (m, 3H), 7.41–7.30 (m, 5H), 7.22 (d, *J* = 15.6 Hz, 1H), 5.38 (br s, 1H), 1.40 (br s, 3H), 1.16 (br s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 200.9, 148.7, 141.2, 141.1, 137.3, 129.2, 128.7, 128.1, 124.6, 124.3, 68.2, 53.1, 23.4, 19.6; HRMS (ESI) *m/z* calcd for $C_{19}H_{22}ClN_2O_3$ $[M + NH_4]^+$ 361.1319, found 361.1335.

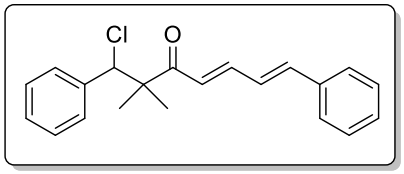
(1*E*)-1-(4-Bromophenyl)-5-chloro-4,4-dimethyl-5-phenylpent-1-en-3-one (138g)



(1*E*)-1-(4-Bromophenyl)-5-chloro-4,4-dimethyl-5-phenylpent-1-en-3-one (**138g**) was prepared from 3,3-dimethyl-4-phenyl-2-methyleneoxetane (**62g**) (174 mg, 1.00 mmol) using the general procedure with $TiCl_4$ as the Lewis acid. Purification by flash chromatography on silica gel (petroleum ether/EtOAc, 96:4) afforded (1*E*)-1-(4-bromophenyl)-5-chloro-4,4-dimethyl-5-

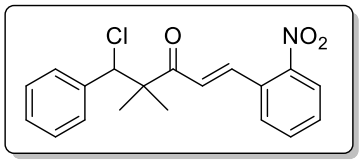
phenylpent-1-en-3-one (**138g**) as colorless oil (347 mg, 94%): IR (neat) 1681, 1601, 1482, 1054, 996, 818, 754, 701 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, *J* = 15.5 Hz, 1H), 7.54–7.51 (m, 2H), 7.44–7.38 (m, 4H), 7.35–7.30 (m, 3H), 7.10 (d, *J* = 15.5 Hz, 1H), 5.39 (s, 1H), 1.40 (s, 3H), 1.14 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.2, 143.0, 137.6, 133.9, 132.4, 130.0, 129.2, 128.6, 128.1, 125.0, 121.4, 68.4, 53.0, 23.4, 19.9; HRMS (ESI) *m/z* calcd for $C_{19}H_{19}BrClO$ $[M + H]^+$ 377.0308, found 377.0330.

(4*E*,6*E*)-1-Chloro-2,2-dimethyl-1,7-diphenylhepta-4,6-dien-3-one (143g)



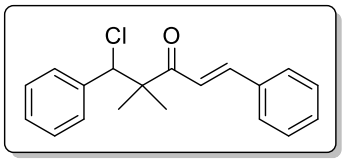
(4*E*,6*E*)-1-Chloro-2,2-dimethyl-1,7-diphenylhepta-4,6-dien-3-one (**143g**) was prepared from 3,3-dimethyl-4-phenyl-2-methyleneoxetane (**62g**) (174 mg, 1.00 mmol) using the general procedure with $TiCl_4$ as the Lewis acid. Purification by flash chromatography on silica gel (petroleum ether/EtOAc, 95:5) afforded (4*E*,6*E*)-1-chloro-2,2-dimethyl-1,7-diphenylhepta-4,6-dien-3-one (**143g**) as colorless oil (282 mg, 87%): IR (neat) 1680, 1581, 1052, 1000, 749, 700 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54–7.48 (m, 3H), 7.40–7.30 (m, 8H), 7.02–6.93 (m, 2H), 6.71 (d, *J* = 14.8 Hz, 1H), 5.39 (s, 1H), 1.37 (s, 3H), 1.10 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.5, 144.5, 142.3, 137.8, 136.3, 129.4, 129.2, 129.1, 128.5, 128.0, 127.5, 127.0, 124.2, 68.5, 52.7, 23.4, 19.9; HRMS (ESI) *m/z* calcd for $C_{21}H_{22}ClO$ $[M + H]^+$ 325.1359, found 325.1375.

(1*E*)-5-Chloro-4,4-dimethyl-1-(2-nitrophenyl)-5-phenylpent-1-en-3-one (137g)



(1*E*)-5-Chloro-4,4-dimethyl-1-(2-nitrophenyl)-5-phenylpent-1-en-3-one (**137g**) was prepared from 3,3-dimethyl-4-phenyl-2-methyleneoxetane (**62g**) (174 mg, 1.00 mmol) using the general procedure with $TiCl_4$ as the Lewis acid. Purification by flash chromatography on silica gel (petroleum ether/EtOAc, 90:10) afforded (1*E*)-5-chloro-4,4-dimethyl-1-(2-nitrophenyl)-5-phenylpent-1-en-3-one (**137g**) as colorless oil (281 mg, 82%): IR (neat) 1685, 1603, 1521, 1342, 1055, 753, 701 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.07–8.02 (m, 3H), 7.67–7.53 (m, 3H), 7.40–7.30 (m, 5H), 6.98 (d, *J* = 15.3 Hz, 1H), 5.36 (s, 1H), 1.40 (s, 3H), 1.16 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 200.6, 139.4, 137.5, 133.6, 131.4, 130.5, 129.5, 129.2, 128.6, 128.2, 125.8, 125.1, 68.4, 53.1, 23.1, 20.0; HRMS (ESI) *m/z* calcd for $C_{19}H_{22}ClN_2O_3$ $[M + NH_4]^+$ 361.1319, found 361.1333.

(1*E*)-5-Chloro-4,4-dimethyl-1,5-diphenylpent-1-en-3-one (139g)



(1*E*)-5-Chloro-4,4-dimethyl-1,5-diphenylpent-1-en-3-one (**139g**) was prepared from 3,3-dimethyl-4-phenyl-2-methyleneoxetane (**62g**) (174 mg, 1.00 mmol) using the general procedure with $TiCl_4$ as the Lewis acid. Purification by flash chromatography on silica gel (petroleum ether/EtOAc, 95:5) afforded (1*E*)-5-chloro-4,4-dimethyl-1,5-diphenylpent-1-en-3-one (**139g**) as colorless oil (281 mg, 82%): IR (neat) 3061, 3028, 1680, 1603, 1055, 762, 701 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.73 (d, *J* = 15.5 Hz, 1H), 7.61–7.57 (m, 2H), 7.42–7.39 (m, 5H), 7.35–7.30 (m, 3H), 7.16 (d, *J* = 15.5 Hz, 1H), 5.44 (s, 1H), 1.41 (s, 3H), 1.15 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.2, 144.4,

137.7, 137.9, 130.7, 129.2, 129.1, 128.6, 128.5, 128.0, 120.8, 68.4, 52.9, 23.3, 19.9; HRMS (ESI) *m/z* calcd for $C_{19}H_{20}ClO$ $[M + H]^+$ 299.1203, found 299.1212.

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