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Synthesis of novel oligosaccharides

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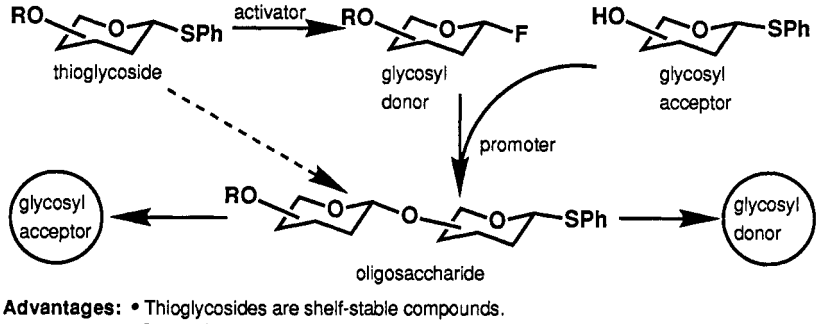
Abstract - The synthesis of the Le^x family of glycosphingolipids (monomeric, dimeric, and trimeric Le^x) is outlined. The strategy involves enantioselective construction of a sphingosine equivalent which is then coupled to oligosaccharide fragments to build the skeletons of the targeted molecules utilizing the two-stage activation procedure. The careful definition of protecting groups allowed easy differentiation of functional groups and stereospecific construction of the glycoside bonds in these complex targets culminating in highly efficient entries into these structures. Model studies in the area of the antibiotic calicheamicin γ_1^I oligosaccharide are also described. A key [3,3]-sigmatropic rearrangement was utilized as a central operation to set the stage for the successful construction of a model for the ABC ring system of this oligosaccharide. Thus solutions for the construction of the most crucial bonds of this novel oligosaccharide have been demonstrated.

INTRODUCTION

Due to the process of photosynthesis, carbohydrates comprise most of the biomass present on earth. Saccharides were long underappreciated in the sciences because they were assumed to possess only structural support and energy-storing functions. Over the last three decades, however, carbohydrate-containing compounds have been found to have many interesting and useful biological activities. For example, carbohydrate units are found in many antibiotics and anticancer agents such as the macrolides, the anthracyclins and the enediyne classes (refs. 1-3). Furthermore, oligosaccharides have been found to control the growth, development and defense mechanisms of plants (ref. 4). More recently, glycosphingolipids, which are key constituents of membranes of most types of cells, were established as fundamental mediators of cell-cell recognition and communication, cell-growth regulation, cell immune response, and cell oncogenic transformations (refs. 5-7).

With an increased appreciation for the role of carbohydrates in the biological and pharmaceutical sciences, came a resurgence of interest in carbohydrate chemistry particularly from synthetic laboratories (refs. 8-13). Our efforts concentrated on strategies for the construction of complex oligosaccharides with particular emphasis on stereocontrol and overall efficiency (refs. 14-19). Two recent syntheses, the total synthesis of the Le^x family of tumor-associated glycosphingolipids (ref. 20) and the construction of an ABC ring model of the oligosaccharide fragment of calicheamicin γ_1^I (ref. 21) are illustrative of these efforts. The Le^x synthesis features glycoside bond construction utilizing the two-stage activation process (ref. 18) which we advanced a number of years ago by combining chemistries of thioglycosides (ref. 22) and glycosyl fluorides (ref. 23) as outlined in **Scheme 1**. This



Advantages:
- Thioglycosides are shelf-stable compounds.
- Glycosyl fluorides can be generated from thioglycosides under mild, neutral conditions in high yield and are stable to chromatography conditions.
- Glycosyl fluorides serve as efficient glycosyl donors under mild conditions.
- Thioglycosides serve as excellent acceptors under the coupling conditions.
- The process is iterative and is adaptable to single unit or block-type synthesis.

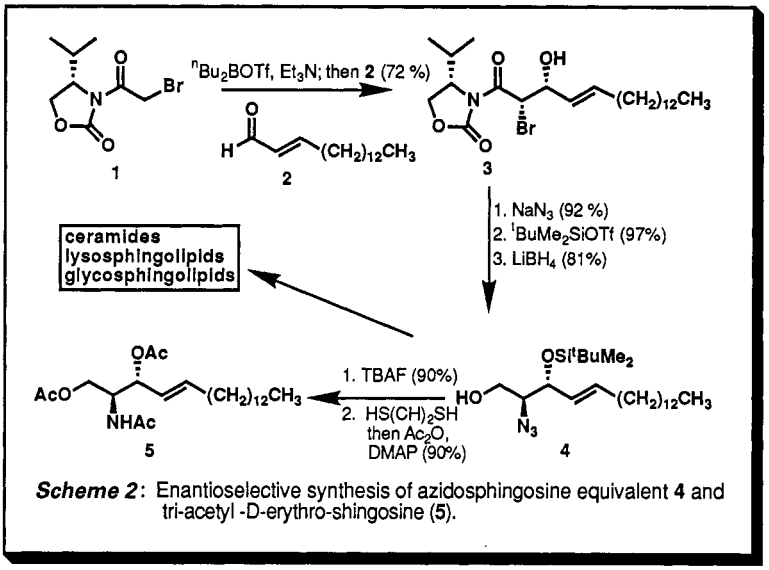
Scheme 1: The two-stage activation procedure for oligosaccharide Synthesis. (Ref. 14)

procedure, involving activation of thioglycosides to glycosyl fluorides under neutral conditions followed by coupling of the resulting glycosyl fluorides to glycosyl acceptors upon further activation, allows the continuous growth of an oligosaccharide chain without damage to preexisting glycoside bonds (ref. 18).

The oligosaccharide fragment of calicheamicin γ_1^I (ref. 3) is one of the most novel and synthetically challenging oligosaccharides found in nature thus far and offers a unique opportunity for the development of new and novel synthetic technologies and strategies. Indeed such a novel strategy was developed as will be discussed below with model studies in this area.

ENANTIOSELECTIVE SYNTHESIS OF SPHINGOSINE AND SPHINGOSINE EQUIVALENTS

Our strategy for the synthesis of glycosphingolipids, including the Le^x family, was based on (a) an asymmetric construction of a suitable sphingosine equivalent and (b) stereospecific coupling of this equivalent to carbohydrate fragments according to the two-stage activation procedure previously advanced from these laboratories (ref. 18). The developed synthesis (refs. 14, 24) of sphingosine was based on advances made by Evans et al (ref. 25) and Pridgen et al (ref. 26) and is outlined in **Scheme 2**. Thus, after conversion to its boron enolate, oxazolidinone **1** was reacted with aldehyde **2** affording derivative **3** as the major product. Substitution of the bromide in **3** with NaN_3 proceeded with complete inversion of configuration leading, after silylation and reduction, to the desired sphingosine equivalent **4** in high overall yield. Standard chemistry allowed the conversion of **4** to sphingosine and sphingosine triacetate **5** whereas coupling reactions to carbohydrate fragments and further manipulations led to various ceramides, lysosphingolipids and glycosphingolipids (**Scheme 2**). The application of this strategy to the Le^x family of antigens is summarized below.



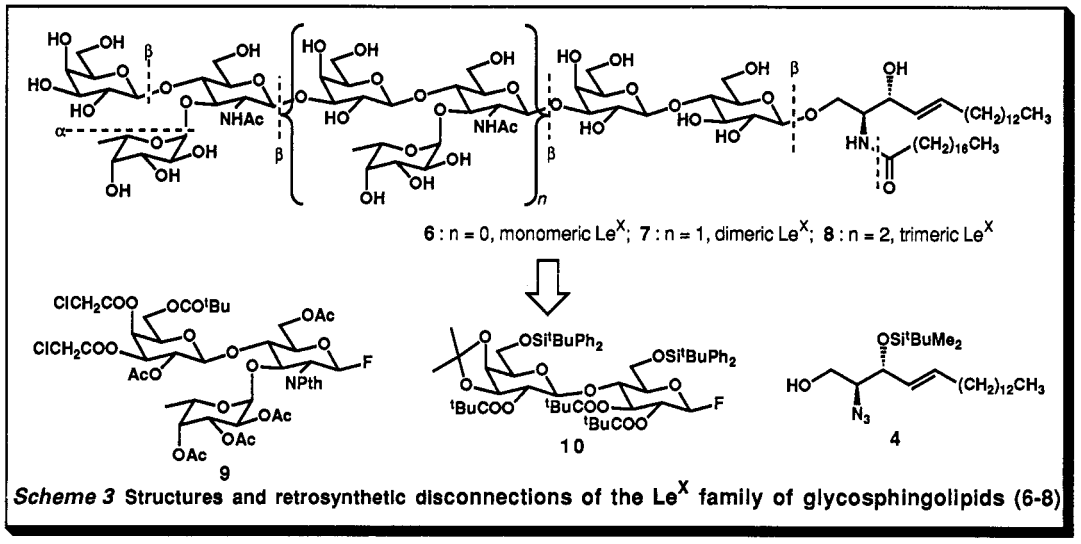
Scheme 2: Enantioselective synthesis of azidosphingosine equivalent **4** and tri-acetyl -D-erythro-shingosine (**5**).

SYNTHESIS OF THE Le^x FAMILY OF GLYCOSPHINGOLIPIDS

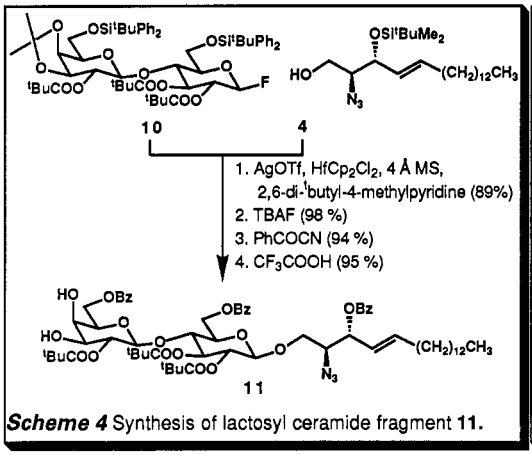
Several glycosphingolipids and lysosphingolipids have been synthesized in these laboratories (refs. 7, 14, 24). Herein we outline the synthesis of the most complex of these targets, namely the Le^x family of antigens (**6-8**) shown in **Scheme 3**. The design used to synthesize these molecules was based on the strategic bond disconnections shown in **Scheme 3** (dotted lines). Careful definition of protecting groups allowed for both high selectivity in the sequence and exclusive formation of the desired stereochemistry of all glycoside bonds. The retrosynthetic analysis led to the use of intermediates **4**, **9** and **10** (**Scheme 3**) as common building blocks for the construction of all three members of the Le^x family of compounds. The synthesis of the trimeric Le^x (**8**) is summarized in **Schemes 4-6**. The syntheses of the monomeric (**6**) and dimeric (**7**) Le^x proceeded along similar lines and are described elsewhere (ref. 20).

Scheme 4 summarizes the synthesis of the lactosyl fragment **11** from lactosyl fluoride **10** and sphingosine equivalent **4**. Thus β-directed (by the C-2 ester) coupling of **10** and **4** followed by standard functional group chemistry led to **11** ready for coupling to the repeating Le^x segment. The appropriately functionalized Le^x repeating segment **9** was constructed from glucosamine derivative **12**, galactosyl fluoride intermediate **13** and fucosyl derivative **16** as summarized in **Scheme 5**. Coupling of **12** and **13** under standard conditions (ref. 23) followed by removal of the allyl group gave the β-glycoside **15** which was further coupled with **16** to afford the trisaccharide derivative **9** (with the α-configuration at the newly established glycoside bond) after conversion to the glycosyl fluoride with NBS-DAST (refs. 18, 24) and protecting group exchange (**Scheme 5**).

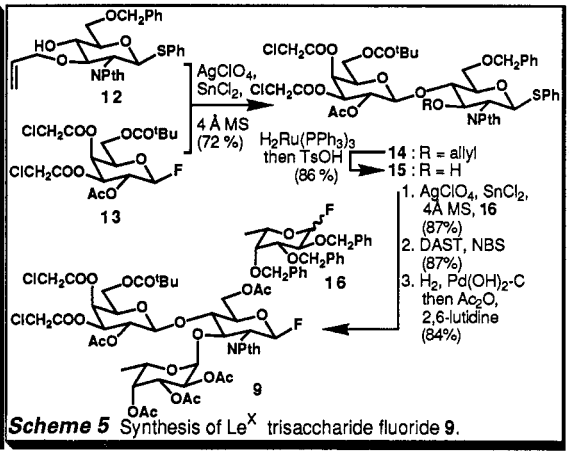
With advanced intermediates **9** and **11** at hand, the completion of the synthesis proceeded as outlined in **Scheme 6**. Thus regiospecific coupling of **9** with **11** under the influence of $AgOTf\text{-}HfCp_2Cl_2$ (ref. 27) took place at the more reactive C-3 position and stereospecifically in the β-sense to afford, after thiourea-induced removal of the monochloroacetate groups, the pentasaccharide **17** in high yield. Repetition of the coupling and deprotection



Scheme 3 Structures and retrosynthetic disconnections of the Le^X family of glycosphingolipids (6-8)



Scheme 4 Synthesis of lactosyl ceramide fragment **11**.



Scheme 5 Synthesis of Le^X trisaccharide fluoride **9**.

procedures led to octasaccharide **18** and thence to undecasacharide **19**. Acetylation of **19** followed by reduction of the azido group and amide formation with octadecanoic acid gave amide **20** in high overall yield.

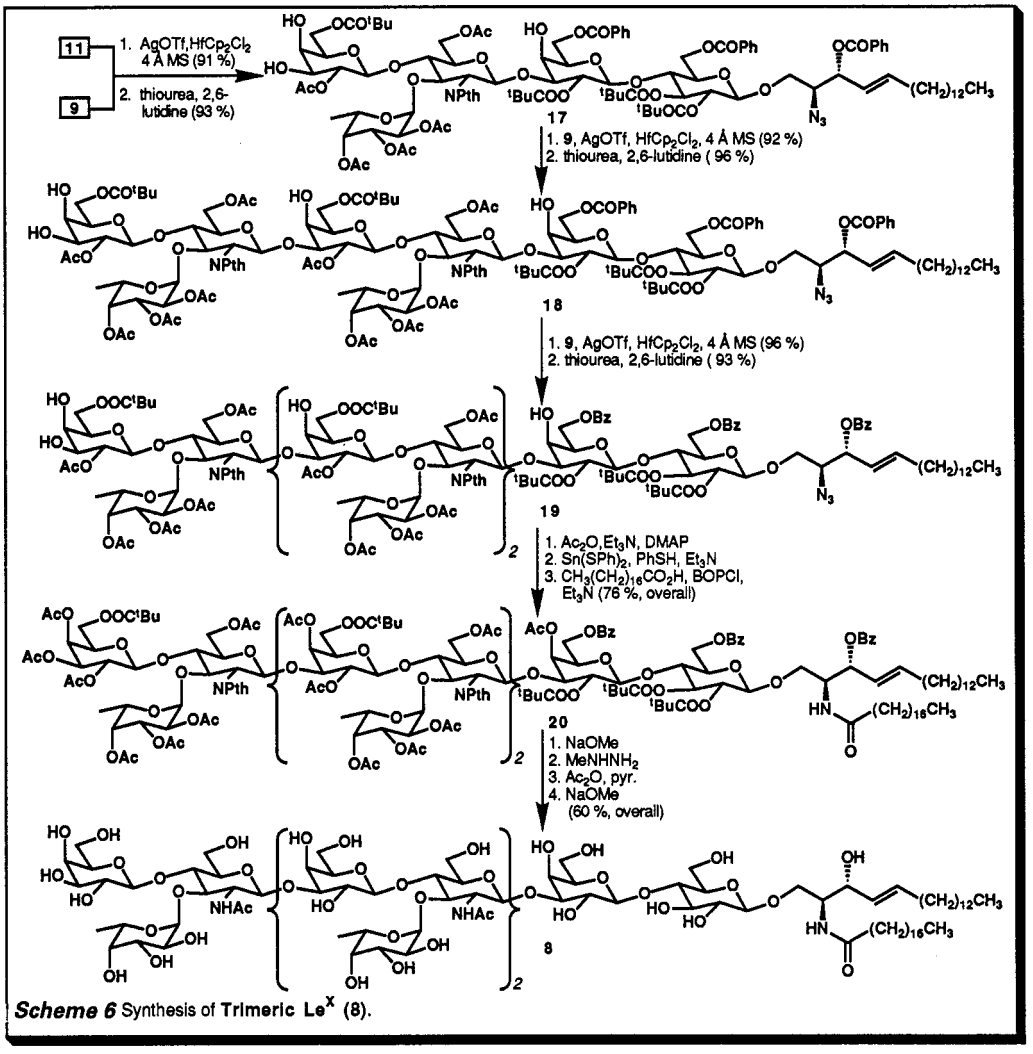
Finally generation of trimeric Le^X (**8**) from **20** proceeded via (i) NaOMe-induced ester cleavage; (ii) removal of the phthalimido groups; (iii) exhaustive acetylation for purification purposes; and (iv) deacetylation.

MODEL STUDIES IN THE AREA OF CALICHEAMICIN γ_1^I OLIGOSACCHARIDE

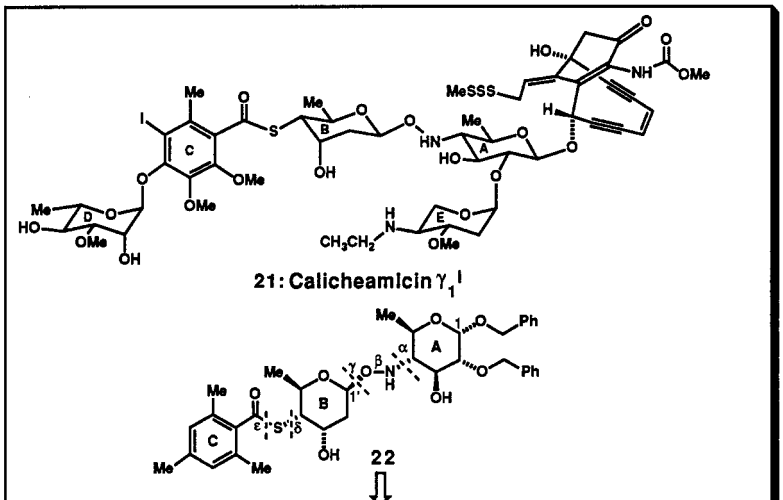
The rather unusual structures of the calicheamicins (ref. 3) coupled with their interesting biological activity have stimulated a flurry of research investigations. Focusing on the novel oligosaccharide fragment of calicheamicin γ_1^I (**21**, **Scheme 7**), the most prominent member of this class of antibiotics, we initiated model studies in order to explore strategies for its total synthesis. Model system **22** (**Scheme 7**) was chosen as the initial target to explore this chemistry which led to solutions for the stereoselective construction of the crucial bonds α-ε (structure **22**) present in the calicheamicin γ_1^I oligosaccharide. The synthesis evolved as follows (ref. 21).

Inspection of the oligosaccharide fragment of calicheamicin γ_1^I, revealed the following challenging synthetic features (shown in structure **22**, **Scheme 7**): (a) the unusual alkoxylamine bond (β) linking carbohydrate units A and B via bonds α and γ; (b) the β-stereochemistry of the glycoside bond γ, which, in combination with the 2-deoxy nature of unit B, offers a serious challenge to synthetic construction; (c) the sulfur bridge, linking carbohydrate moiety B with a highly substituted aromatic system via bonds δ and ε; and (d) the α-stereochemistry of the N- and S-carrying stereogenic centers of units A and B, respectively.

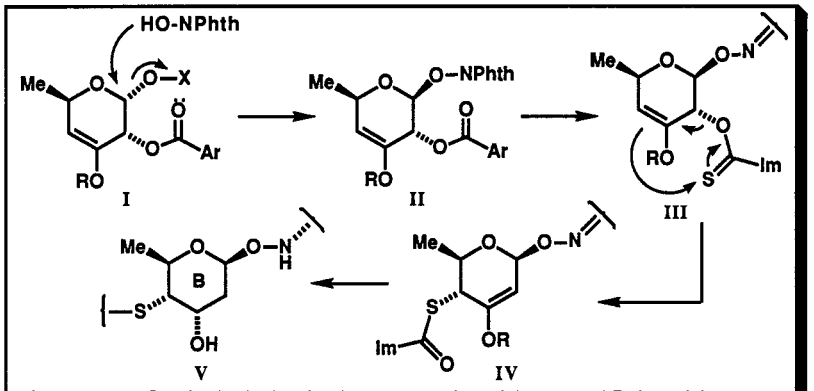
The synthetic plan was based on the strategic bond disconnections indicated in structure **22**, which defined thiocarbonyldiimidazole ($Im_2C{=}S$) as the sulfur source, N-hydroxyphthalimide (HO-NPhth) as the origin of the O-NH group, and equivalents to rings A (**A**), B (**B**), and C as potential starting points (**Scheme 7**). The strategy devised from this analysis is shown in **Scheme 8**. In addition to addressing the above mentioned problems, this strategy avoids a potentially treacherous deoxygenation step for establishing the methylene group of ring B. Thus derivative **I** (**Scheme 8**) was designed with an ester moiety at C-2 to direct the stereochemical outcome of



Scheme 6 Synthesis of **Trimeric Le^X (8)**.



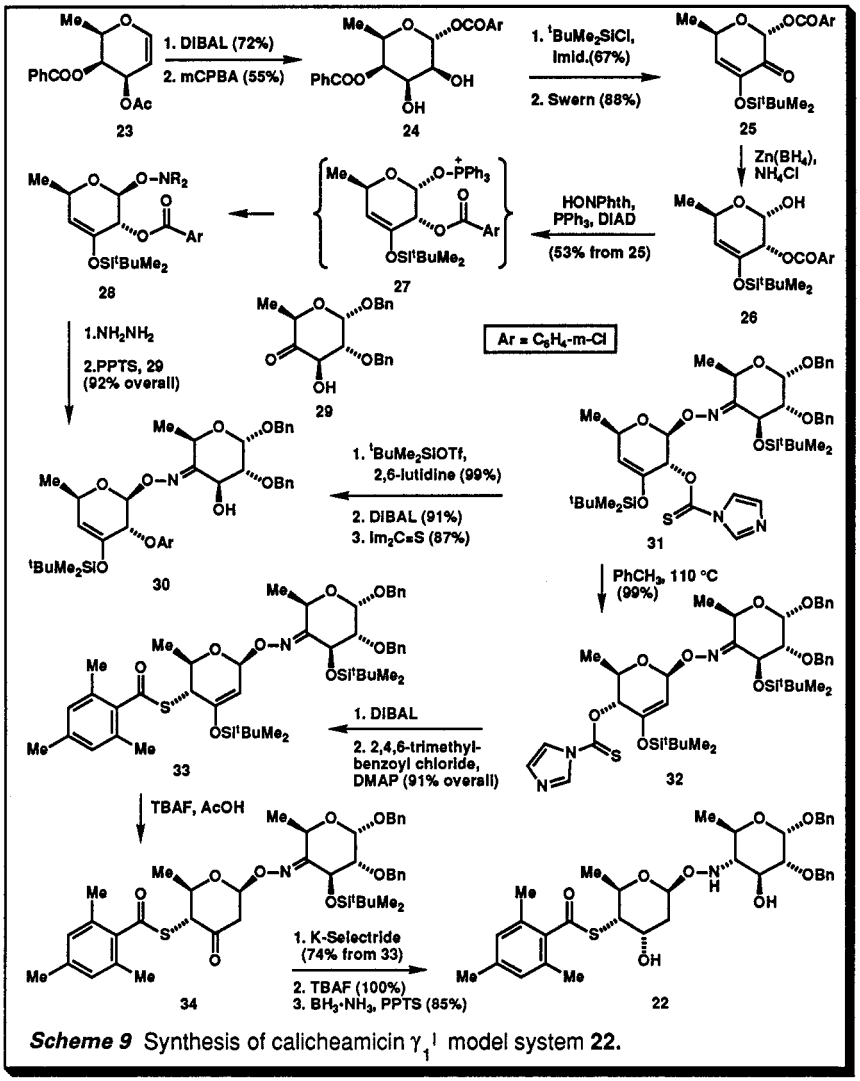
Scheme 7 Retrosynthetic analysis of calicheamicin γ_1^I oligiosaccharide model system **22**.



Scheme 8 Synthetic design for the construction of the central B ring of the calicheamicin γ_1^I oligosaccharide fragment.

the glycosidation reaction (**I**⇒**II**, β-configuration) as well as to stereospecifically deliver the sulfur atom at position 4 via a [3,3]-sigmatropic rearrangement (**II**⇒**III**⇒**IV**). Compound **IV** was then expected to serve as a precursor to the desired system **V**.

Scheme 9 summarizes the successful sequence to model **22** starting with the readily available diester **23**. Thus selective DIBAL-induced ester cleavage in **23** followed by stereoselective epoxidation and concommitant opening of the resulting epoxide afforded diol **24**. Chemoselective monosilylation of **24** followed by Swern



Scheme 9 Synthesis of calicheamicin γ_1^I model system **22**.

oxidation and elimination gave the enone **25**. This enone (**25**) underwent smooth 1,2-reduction from the β-face with $Zn(BH_4)_2$-NH_4Cl and the resulting intermediate suffered, in *situ*, ester migration to afford the α-lactol **26** as the major product. Reaction of this lactol with HONPhth-PPh_3-$^iPrOOCN{=}NCOO^iPr$ resulted in the formation of the β-glycoside **28**, presumably via the intermidiacy of **27**. The amino group was then liberated in **28** by the action of NH_2NH_2 and the resulting hydroxylamine derivative was condensed with ketone **29** to afford compound **30** (single, unassigned geometry) in high overall yield. This intermediate was then converted to the desired thionoimidazolide derivative **31** by (i) silylation; (ii) DIBAL-induced ester cleavage; (iii) exposure to thiocarbonyldiimidazole. The expected [3,3]-sigmatropic rearrangement of **31** proceeded smoothly in refluxing toluene to afford, stereospecifically and in excellent yield, the targeted intermediate thioimidazolide **32**. DIBAL reduction of **32** followed by coupling with 2,4,6-trimethylbenzoyl chloride under basic conditions led to the expected coupling product **33** which was selectively desilylated at the enol ether site furnishing ketone **34**. Finally, stereoselective reduction of the carbonyl group in **34** was achieved utilizing the bulky reagent K-selectride, whereas desilylation and exposure to $BH_3{\cdot}NH_3$-PPTS resulted in stereoselective reduction of the oxime functionality leading to the desired model system **22**.

CONCLUSION

The completed total synthesis of trimeric Le^x (**8**) and its relatives **6** and **7** demonstrated the usefulness of the two-stage activation procedure (ref. 18) for complex oligosaccharide synthesis and made available, in pure form, these important glycosphingolipids. The described model studies in the calicheamicin γ_1^I saccharide area provided stereocontrolled solutions to the most crucial bond constructions of the oligosaccharide fragment of this important antibiotic. The opened avenues to the targeted oligosaccharides promise to aid further chemical and biological investigations.

Acknowledgement

The contributions of our collaborators whose names are mentioned in the references are deeply appreciated. This work was financially supported by the National Institutes of Health, Merck Sharp and Dohme (USA) Hoffmann-La Roche (USA) Nippon Zeon (Japan) the University of Pennsylvania and the Research Institute of Scripps Clinic.

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