Synthesis of Phosphonate Analogues of the Antibiotic Moenomycin A$_{12}$

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بسم الله الرحمن الرحيم

to my parents

and my family

Abu Ajaj, Khalid Moh'd

Synthesis of Phosphonate Analogues of the
Antibiotic Moenomycin A$_{12}$

Synthese von Phosphonat-Analoga des
Antibiotikums Moenomycin A$_{12}$

Universität Leipzig, Dissertation

Diese Arbeit enthält 130 Seiten, 73 Abbildungen, 1 Tabelle, 156 Literaturangaben

Referat:

Im Rahmen der vorliegenden Arbeit wurden *C*-Glycosid-Di- und Trisaccharid-Bausteine des Antibiotikums Moenomycin A$_{12}$ ausgehend von β-D-Galactose-pentaacetat hergestellt. Das Ausgangmaterial wurde in D-Galactoheptonamid übergeführt. Die Einheit F des Disaccharidbausteins hat alle Substituenten, die die Einheit F des Moenomycins A$_{12}$ hat. Der ausgearbeitete Syntheseweg sollte zur Synthese anderer Analoga geeignet sein.

Die vorliegende Arbeit wurde in der Zeit von August 1998 bis März 2002 am Institut für Organische Chemie der Fakultät für Chemie und Mineralogie an der Universität Leipzig unter der Leitung von Herrn Prof. Dr. Peter Welzel angefertigt.

Contents

1 INTRODUCTION

In view of the problem of antibiotic resistance[1] antiinfectives with novel modes of action are desperately needed. The transglycosylation reaction,[2] the second last step in the biosynthesis of peptidoglycan (the main structural polymer of the bacterial cell wall) occurs at the outside of the cytoplasmic membrane and is catalysed by membrane proteins designated as *bifunctional (class A) high molecular mass penicillin-binding proteins (PBPs).*[3] The reaction came recently into focus as a promising new target for a number of reasons: (i) the methods for isolating the enzyme(s) involved have improved considerably,[4,5] (ii) one substrate of the transglycosylation step, the so-called lipid II, can now be made in sufficient amounts,[6] and (iii) new and efficient *in-vitro* test systems have been developed which conveniently allow to monitor the inhibition of the incorporation of lipid II into uncross-linked peptidoglycan,[6,7] and binding of inhibitors to the enzyme, respectively.[8]

The assembly of the peptidoglycan polysaccharide strands from lipid II is blocked by certain glycopeptides,[9] ramoplanin,[10] the lantibiotics,[11] and the moenomycin-type antibiotics.[12] Of these, the moenomycins (see structures of moenomycin A and moenomycin A_{12}) are the only compounds known to inhibit the enzyme itself[2] (i. e. the transglycosylase domain of the bifunctional high-molecular PBPs) whereas ramoplanin and the lantibiotics interfere with lipid II. The moenomycins are, thus, unique tools for investigating both the transglycosylation step and the corresponding enzyme(s). In addition, they are highly promising lead compounds for new antiinfectives.

1.1 Existence and structure of the moenomycin antibiotics

Moenomycins are phosphoglycolipid antibiotics isolated from various strains of *Streptomyces* (*S. bambergiensis, S. ghanaensis, S. ederensis and S. geysiriensis*) and are commercially available (Flavomycin®). Moenomycin A (**1**) is the main constituent of this product.[13,14] Moenomycin A_{12}[15] (**2**), C_1[16] (**3**), C_3 (**4**), C_4[17] (**5**), pholipomycin (**6**)[17,18] and AC326-α (**7**)[19] were also isolated. The family of moenomycin-type antibiotics also includes the prasinomycins,[18] diumycins (macarbomycins), 11837 R.P., 8036 R.P. (quebemycin), 19402 R.P., ensanchomycin, prenomycin and teichomycin[20] with mainly unknown structures.
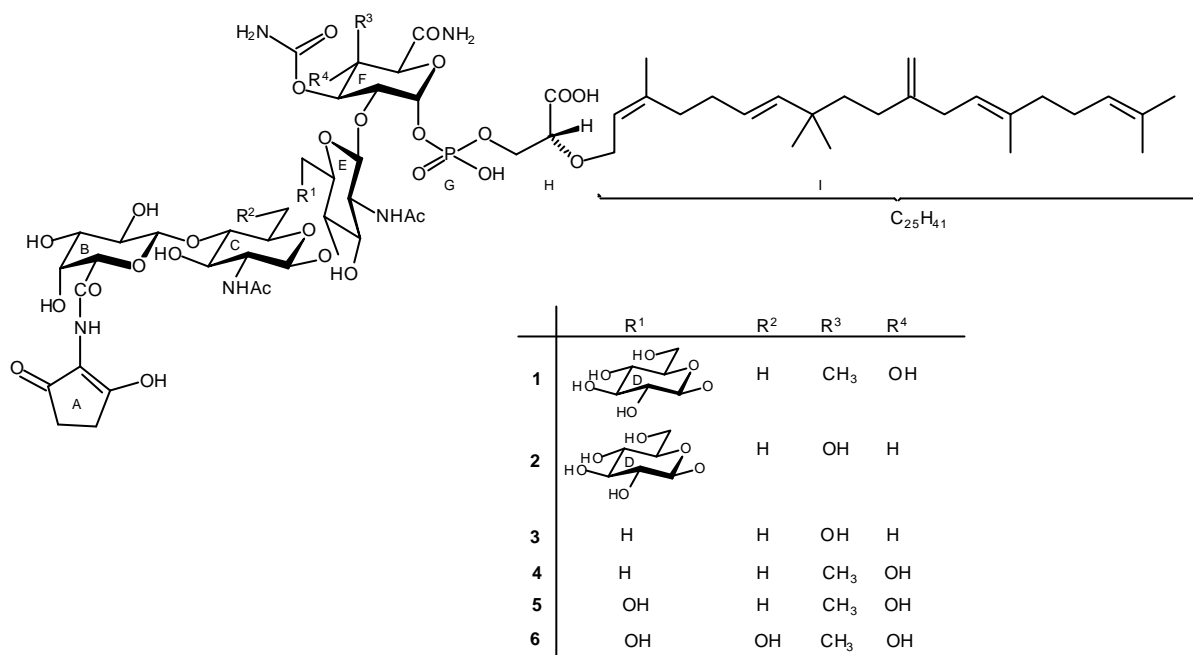
Figure 1.1: The moenomycins

All moenomycin-type members seem to contain an oligosaccharide chain linked via a phosphoric acid diester to a lipid unit, which may be either moenocinol (a C_{25}-lipid alcohol, as in the moenomycins and prasinomycins) or diumycinol (an isomer of moenocinol with a six-membered ring as in AC326-α and the diumycins (macarbomycins)).

The chemical structures of the isolated moenomycins have been established, based on chemical degradation combined with spectroscopic studies (fast atom bombardment MS and ^{13}C NMR).[15, 16,17, 21]
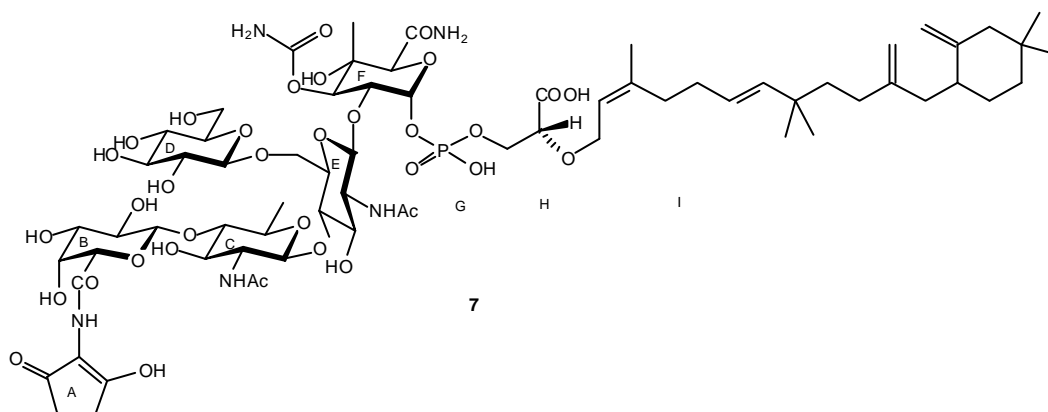
Figure 1.2: Moenomycin AC326-α

1.2 Structural properties of the moenomycin family

The moenomycin group is characterized by a relatively high molecular weight from 1600 to 2100 daltons and a phosphorus content of about 2%.[20]

Moenomycins and the related antibiotics are acids which form colourless, amorphous salts. They are readily soluble in water and lower alcohols, but insoluble in most organic solvents. In aqueous solution they are very stable at a pH range of 7-9 and against most enzymes.

Most of these antibiotics carry the chromophore moiety (UV: 257-258 nm) which is lacking in others that may contain glycine instead.[16,22]

All components show surface activity and form aggregates in aqueous solutions up to 70000 daltons dependent on the experimental conditions.[20,23]

They can be divided into two classes, depending on whether unit E carries a glucose moiety as in moenomycins A and A_{12} or not as in moenomycins C_1, C_3, C_4 and pholipomycin. In another classification compounds like moenomycin A, C_3, C_4 contain a glucuronic acid-derived unit F whereas the moenomycins A_{12} and C_1 have a D-galacturonic acid-derived ring F.

HCl-catalysed hydrolysis degrades moenomycin A completely into its components,[20] units B and F decompose under these conditions.[24,25,26]

The β-glycosidic linkage of the N-acetylated amino sugars can be cleaved specifically with trifluoroacetic acid. Degradation products containing units A-B-C, D-E, D-E-F-G-H, and F-G have been obtained using this method.[26,27]

The allyl ether bond between units H and I is acid-labile. The moenocinol part I can be cleaved off from the rest of the molecule and the remaining delipido moenomycin A was reported to be devoid of any antibiotic activity.[12,28]

The reaction of unit A of moenomycin A with an aromatic diazonium salt gave an azo compound which underwent a *Japp-Klingemann* cleavage of the five-membered ring to furnish an amidrazone which finally recycled to provide a triazole.[29]

Ozonolytic degradation of moenomycin A formed a compound lacking unit A and most of the lipid part. Reductive amination of the aldehyde furnished antibiotically inactive tertiary amines.[12]

$K_3[Fe(CN)_6]$ oxidation provides in high yield an antibiotically active compound lacking unit A.[12]

The introduction of polar groups at the end of the lipid side-chain destroys the antibiotic activity.[30]

1.3 Antimicrobial properties

Moenomycin A is active mainly against *Gram-positive* bacteria, and due to the presence of the outer membrane, it acts to a lower degree against *Gram-negative* bacteria.[13,25,31] Moenomycin is not applied in human medicine because it is not absorbed by oral application. After intravenous or intraperitoneal application the half life time in the body is about 9-10 days.[20] Moenomycin also diminishes the infection of cells with plasmids, this fact is especially important for the use of Flavomycin® as a growth promoter in animal nutrition.[20] It was reported that moenomycin as a feed additive has an excellent effectiveness, even in small doses.[32]

1.4 Mode of action

Based on structure-activity relationships,[33] a mechanism for their mode of action has been proposed.[4,34,35,36] Moenomycin and the related antibiotics are known to inhibit the biosynthesis of peptidoglycan, the main structural component of the bacterial cell wall. The peptidoglycan layer consists of a matrix of polysaccharide chains, composed of alternating N-acetylmuramic acid (MurNAc) and N-acetylglucosamine (GlcNAc) sugar residues, cross-linked through oligopeptide sidechains attached to the MurNAc residues (Figure 1.3). The sequence of the tetrapeptide in *E. coli* is L-Ala-γ-D-Glu-X-D-Ala, where X is usually meso-diaminopimelate (*m*-DAP) as usual for *Gram-negative* bacteria. In *Gram-positive* L-Lys bacteria is found instead.[37]

During the investigation of the mode of action of these moenomycin antibiotics, their antibiotic activity was compared to that of vancomycin and some other antibiotics which inhibit cell well synthesis. Vancomycin, as the phosphoglycolipid antibiotics, strongly inhibits the peptidoglycan synthesis and causes an accumulation of the lipid intermediate and of UDP-Mur NAc-pentapeptide.[20,38]
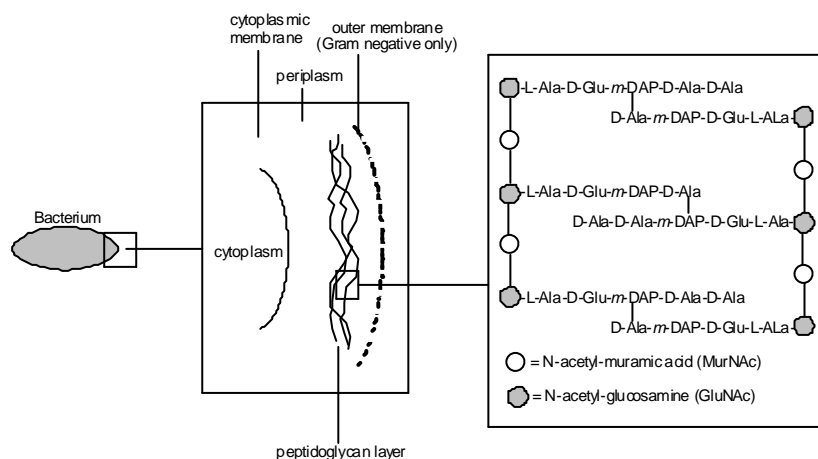
Figure 1.3: Simplified representation of the structure of the peptidoglycan

layer of bacterial cell walls

It is assumed that the moenomycins are anchored to the cytoplasmic membrane via the lipid part[23] and bind then highly selectively to the binding site of the growing polysaccharide strand at the enzyme via the C-E-F trisaccharide and inhibit the formation of the linear glycan strand of peptidoglycan.[39,40,41]

The two successive final reactions in the bacterial cell wall synthesis are the transglycosylation (D $\Rightarrow$ E) that extends the glycan chain and the transpeptidation (E $\Rightarrow$ F) that cross-links the glycan chain through two peptide units (Scheme 1.1).

A number of bifunctional enzymes (*penicillin-binding proteins*, PBP's) have been identified that catalyse both transglycosylation and transpeptidation.[39] With cell free systems from *E. coli*, it was demonstrated that the antibiotic moenomycin A selectively inhibits the transglycosylation step by its inhibitory effect on *penicillin-binding protein 1b* (PBP 1b). Both with the cell-free systems and purified PBP 1b moenomycin A was inhibitory at concentration between 10^{-8} and 10^{-7} mol/L.[39]

The mechanism of the glycopeptides that inhibit the transglycosylation reaction is not clear.[42] It has been proposed that both the glycan strand and the disaccharide intermediate (lipid II) are anchored by their lipid chains into the cytoplasmic membrane. The reaction proceed through the displacement of the phosphate of the growing strand by the 4-hydroxyl group of the GlcNAc unit of lipid II as indicated in Scheme 1.2.[34]
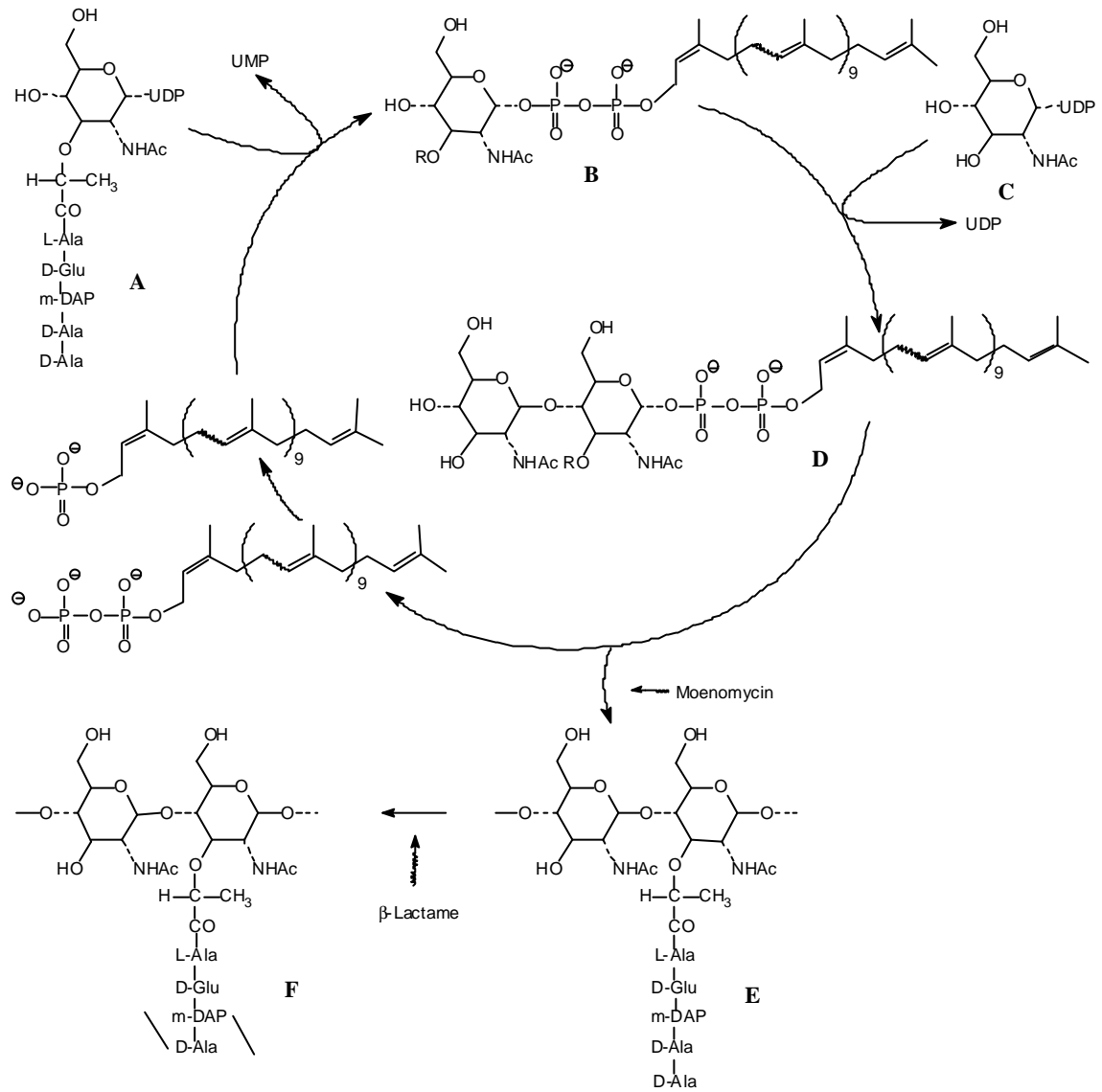
Scheme 1.1: The biosynthesis of the peptidoglycan of *E. coli*
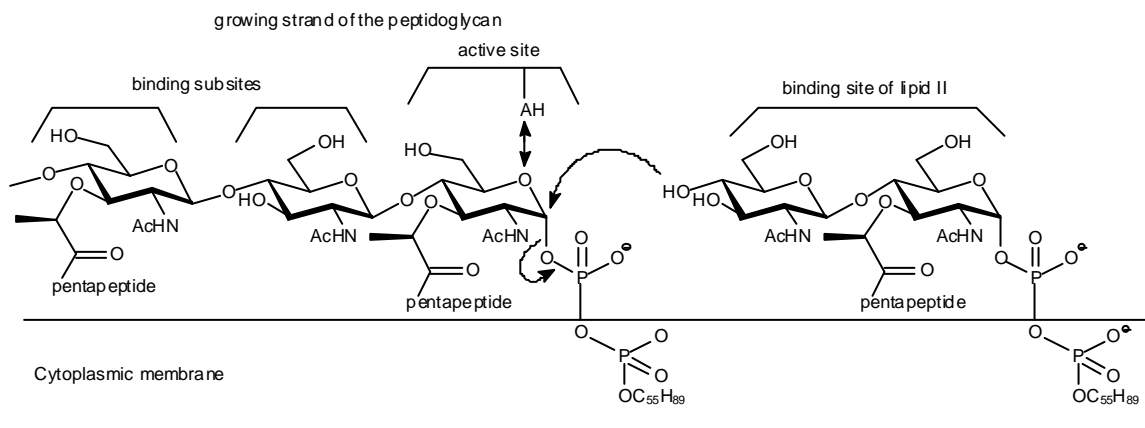
Scheme 1.2: Schematic representation of the transglycosylation reaction.

1.5 Structure-activity relationships of the moenomycins

Systematic stepwise degradation studies of moenomycins A, A_{12}, C_1, C_3, and C_4, in conjunction with assaying the degradation products for antibiotic activity both in the *in-vivo* and in the *E. coli* cell-free system have proved that:

- Units E, F, G, H, and I are essential,[21,28] in addition, unit C must be present in moenomycins A_{12}[15] and C_1.[16]

- The moenocinol part I may be saturated,[28] as well as cyclized in its terminal part.[19]

- The carbamoyl group in unit F must be present.[28, 12]

- The carboxyl group in unit H must be free,[21,39] and that in unit F in the carboxamide form.[28]

- The phosphate group in unit F must be α-oriented.[43]

Many disaccharide and trisaccharide analogues of moenomycins A and A_{12} have been prepared for the estimation of structure-activity relationship, and to define the minimal structural basis of the antibiotic activity and the exact mechanism of the transglycosylation reaction.

In the disaccharide series, unit F should have the D-*gluco* configuration.[44] Compounds **8**[28] and **9**[33] have full activity (when compared to moenomycin A) as transglycosylase inhibitors in *in-vitro* experiments. They are, however, antibiotically inactive. This result means that the methyl group at C-4 of unit F in moenomycin A is not involved in the activity. Thus, compound **9** is the smallest structural analogues of the moenomycins that elicits full transglycosylase inhibiting activity known to date. It was found that the N-acetyl group of unit E is needed as a prerequisite for the transglycosylase inhibiting properties.[45]
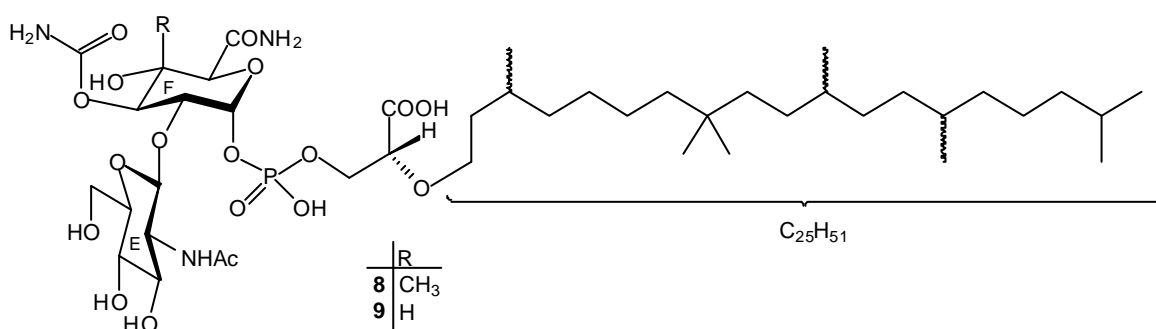
Figure 1.4: Antibiotic active disaccharide analogues of moenomycin A

In the trisaccharide series, compounds **10**[28], **11**[15], **12**[16], **13**[46] represent the minimum structural requirements for full *in vitro* activity as well as antibiotic activity. Unit F may have the D-*gluco* or D-*galacto* configuration. It was found that the N-acetyl group of unit C is essential.[34,47]

	R^1	R^2	R^3	R^4
10	CH_3	OH	OH	H
11	OH	H	OH	H
12	OH	H	H	H
13	OH	H	OH	OH
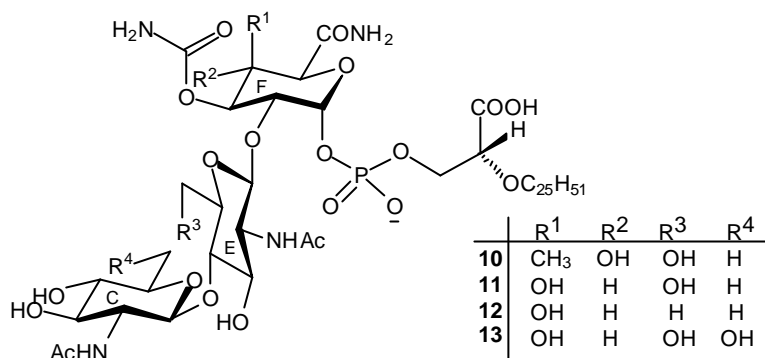
Figure 1.5: Antibiotic active trisaccharide analogues of moenomycins A and A_{12}

1.6 Enzymatic degradation moenomycin A

The moenomycins do not induce resistance readily. However, a weak point in this respect may be the phosphate bond at unit F. Its cleavage by a yet poorly characterized enzyme is the only enzymatic degradation reaction of the moenomycins that is known to-date.[48]

Enzymatic degradation of moenomycin A at the phosphoglycosidic linkage affords units MA, MB and MC. MB suffers further enzymatic cleavage to give MA, whereas MC probably contains units A through F of moenomycin A.[48] The structure of MA is closely related to moenocinol (the alcohol corresponding to unit I in moenomycin A).
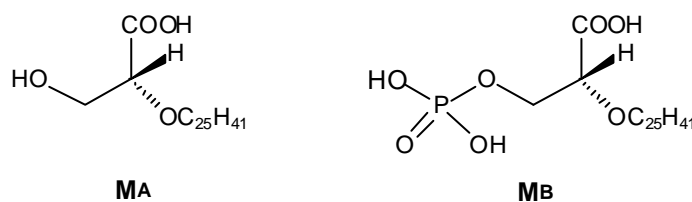
Figure 1.6: Enzymatic degradation products of moenomycin A

No inhibition of the transglycosylase reaction by Ma was observed, which reflects the importance of the phosphoglycosidic linkage to elicit antibiotic activity. Its cleavage furnishes two inactive parts. Furthermore, some moenomycin-type analogues which were expected to be antibiotically active, were devoid of antibiotic activity, although they contain most of the essential functional groups necessary for moenomycin-type activity. It has been speculated, that these compounds are used as substrates by the transglycosylase. [35]

1.7 *C*-glycosides

In *C*-glycosides the oxygen atom of the exo-glycosidic bond is replaced by a carbon atom. The methods for their synthesis have been extensively studied.[49,50,51] The structural modification involved in the transition from *O*-glycosides to *C*-glycosides results in a different nomenclature system. The *C*-glycoside derives its name from the largest continuous chain of carbons. Compound **14**, for example, derives its name from heptitol. As the heptitol is cyclized between C_2 and C_6 it is designated as 2,6-anhydro.

14

1,3,4,5-Tetra-*O*-acetyl-2,6-anhydro-7-deoxy-D-*glycero*-L-*galacto*-heptitol
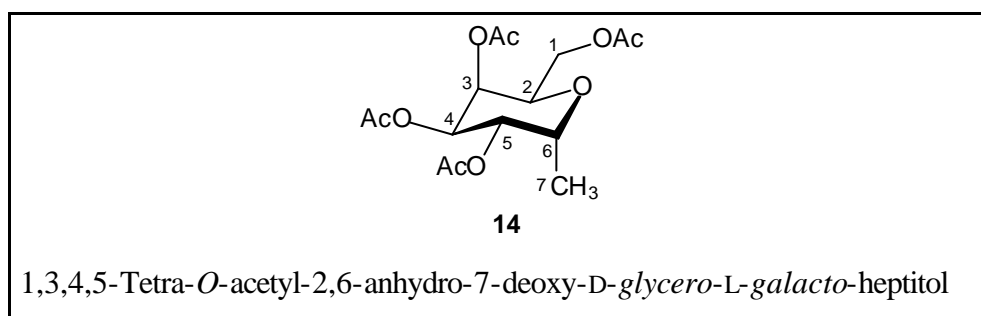
Figure 1.7: Representative *C*-glycoside and its nomenclature

C-glycosides have gained increasing interest in view of their occurrence as fragments in a number of natural products. Moreover, because of their resistance to hydrolysis, *C*-glycosides are expected to be stable mimics for natural *O*-glycosides with biological activity.[51] As a result, there is now interest in using such *C*-glycosides for enzymatic and metabolic studies.

The *C*-glycoside analogues may answer the question whether the replacement of the oxygen linkage between the anomeric carbon and the phosphorous moiety by a CH_2 group abolishes the transglycosylase inhibiting activity in moenomycin analogues or not.

2 PURPOSE OF THE PRESENT WORK

We started a programme aimed at synthesizing trisaccharide analogues of moenomycin A_{12} in which the phosphate oxygen at C-1 of unit F is replaced by a CH_2 group.[52]
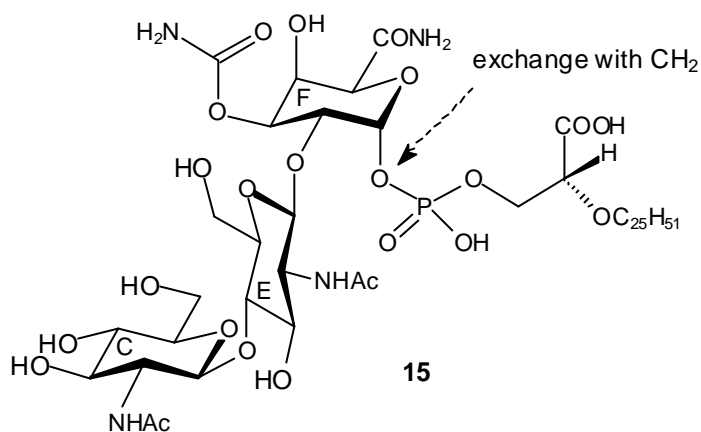
Figure 2. 1: Target molecule **15**

It seemed important to retain all other functional groups in ring F as present in moenomycin A_{12} since they are known to be of major importance as far as antibiotic activity is concerned.[33,53]

3 PREVIOUS WORK

Some mono- and disaccharide phosphonate models of moenomycin A have already been prepared using two different approaches. *Qiao and Vederas*[54] (Scheme 3.1) cyclized compound **16**, obtained via a *Wittig* approach from a protected glucose derivative by oxymercuration and subsequent iodination, whereas *Brooks et al.*[55] (Scheme 3.2) started from an allyl C-glycoside which on Pd-mediated rearrangement furnished the corresponding propenyl isomer alongside with other rearrangement products or from an allenyl C-glycoside. Both compounds on ozonolysis gave the corresponding alcohol in modest yield. Phosphonates were obtained by *Arbuzov* reaction.[56] *Qiao* and *Vederas* managed to prepare a disaccharide analogue but obviously the structure was too simple to elicit antibiotic activity.[54,55]
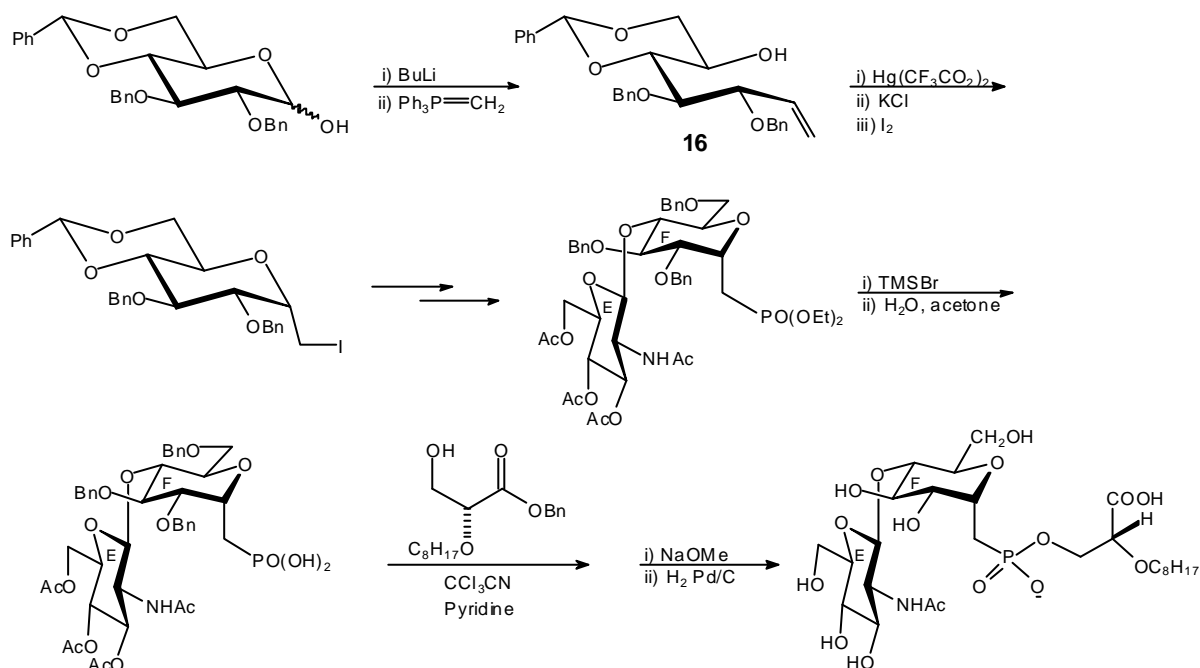
Scheme 3.1: Preparation of a phosphonate disaccharide by *Qiao et al*
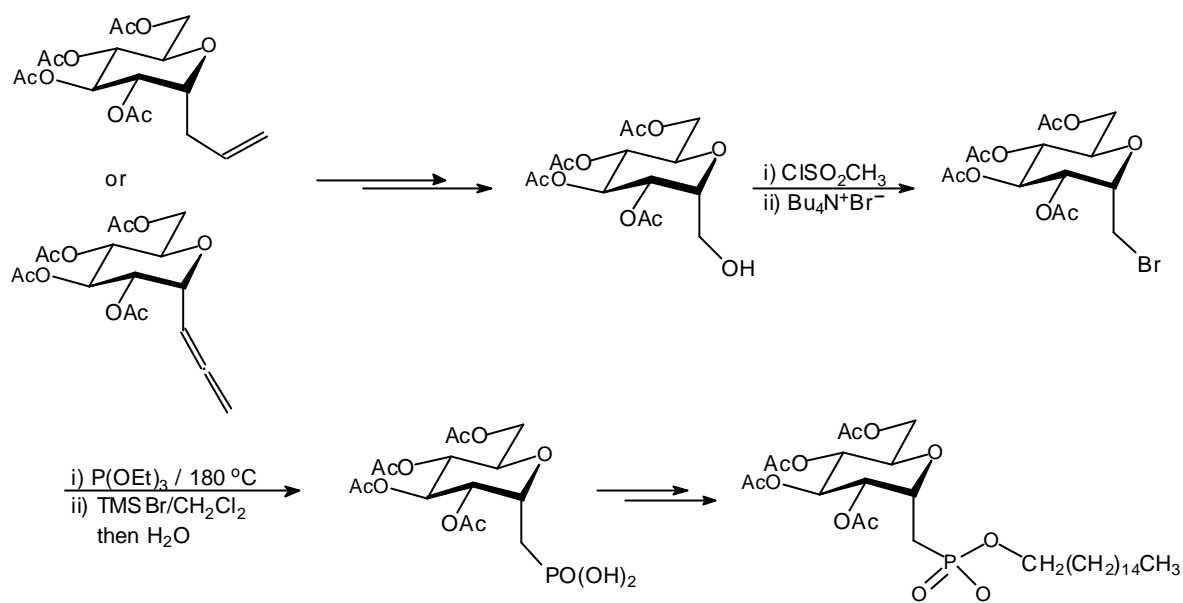
Scheme 3.2: Preparation of a phosphonate monosaccharide by *Brooks et al*

Some time ago *Welzel et al.*[57] (Scheme 3.3) reported on a new approach for the synthesis of such C-glycosides, which started from D-tartaric acid and a suitable C-3 allyl stannane and used also the mercury-induced cyclization. Disaccharide building block **17** was prepared via this route.
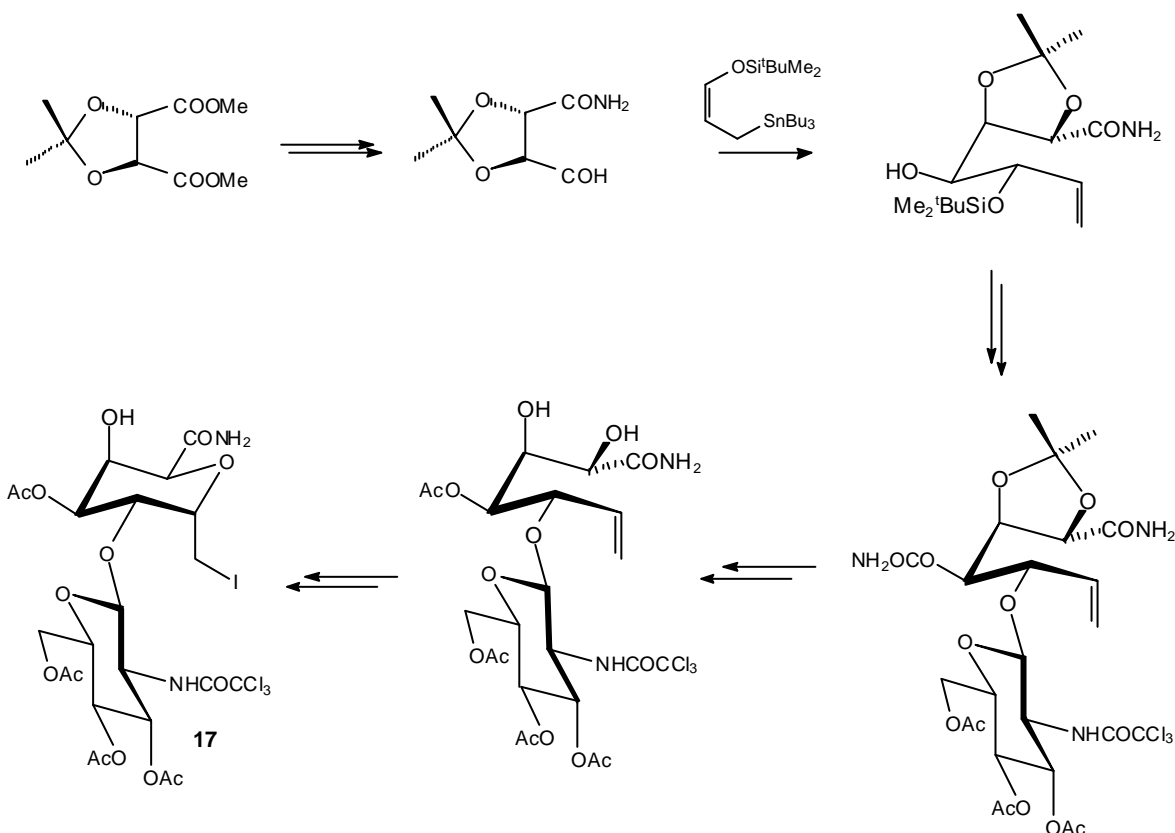
Scheme 3.3: Preparation of disaccharide building block **17**

Although this synthesis was conceptually new, there were some draw-backs due to difficulties in the protecting group chemistry. We decided, therefore, to explore a somewhat more traditional synthesis. Since according to all studies on structure-activity relationships, no antibiotic activity can be expected for disaccharide analogues, it was decided to include the synthesis of trisaccharide structures.

The present work describes the synthesis of the two building blocks **18** and **19** for phosphonate analogues of moenomycin A_{12}.
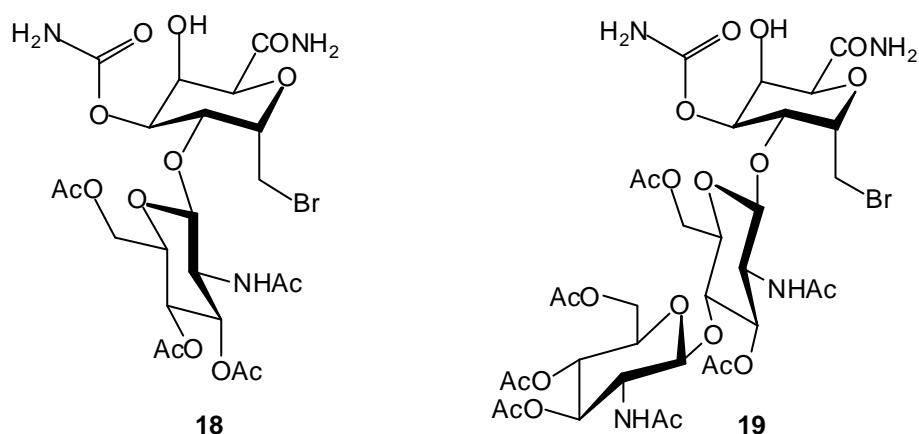
Figure 3.1: Building blocks **18** and **19**

4 SYNTHETIC DESIGN

4.1 Retrosynthetic analysis

A retrosynthetic analysis (Scheme 4.1) of the target compounds **20** and **15** leads to three precursors:

1. An E-F disaccharide **21** and a C-E-F trisaccharide **22**, respectively.

2. A nucleophilic phosphonic acid equivalent **23**, which could be obtained from triethylphosphite **24**.

3. A 2-*O*-alkylglyceric acid moiety **25**, which could be obtained from **26** either by degradation of moenomycin (Flavomycin complex[®])[17] (R = $C_{25}H_{51}$) or by synthesis.[58]

The combination of **21** as well as **22** and **24** should be possible getting benefit of the *Arbuzov* reaction.[56] The resulting posphonate esters should be converted into the corresponding phosphonic acids, and these in turn should couple with **26** after activation of the phosphonic acids. Subsequent removal of the protecting groups should furnish the target compound.

The precursors **21** and **22** could be synthesized by functional group conversion from **27** and **28**, respectively. It was planned to disconnect these di- and trisaccharide analogues at the glycosidic bonds, and to prepare the C-glycosidic homologue of unit F starting from a galactose-derived C-halomethyl of type **29**, which could be obtained from β-D-galactose-pentaacetate **30**. For the synthesis of the disaccharides, the glycosyl donor **31** could be obtained from D-glucosamine **32**, while for the trisaccharides, it could be obtained from chitobiose **33**.
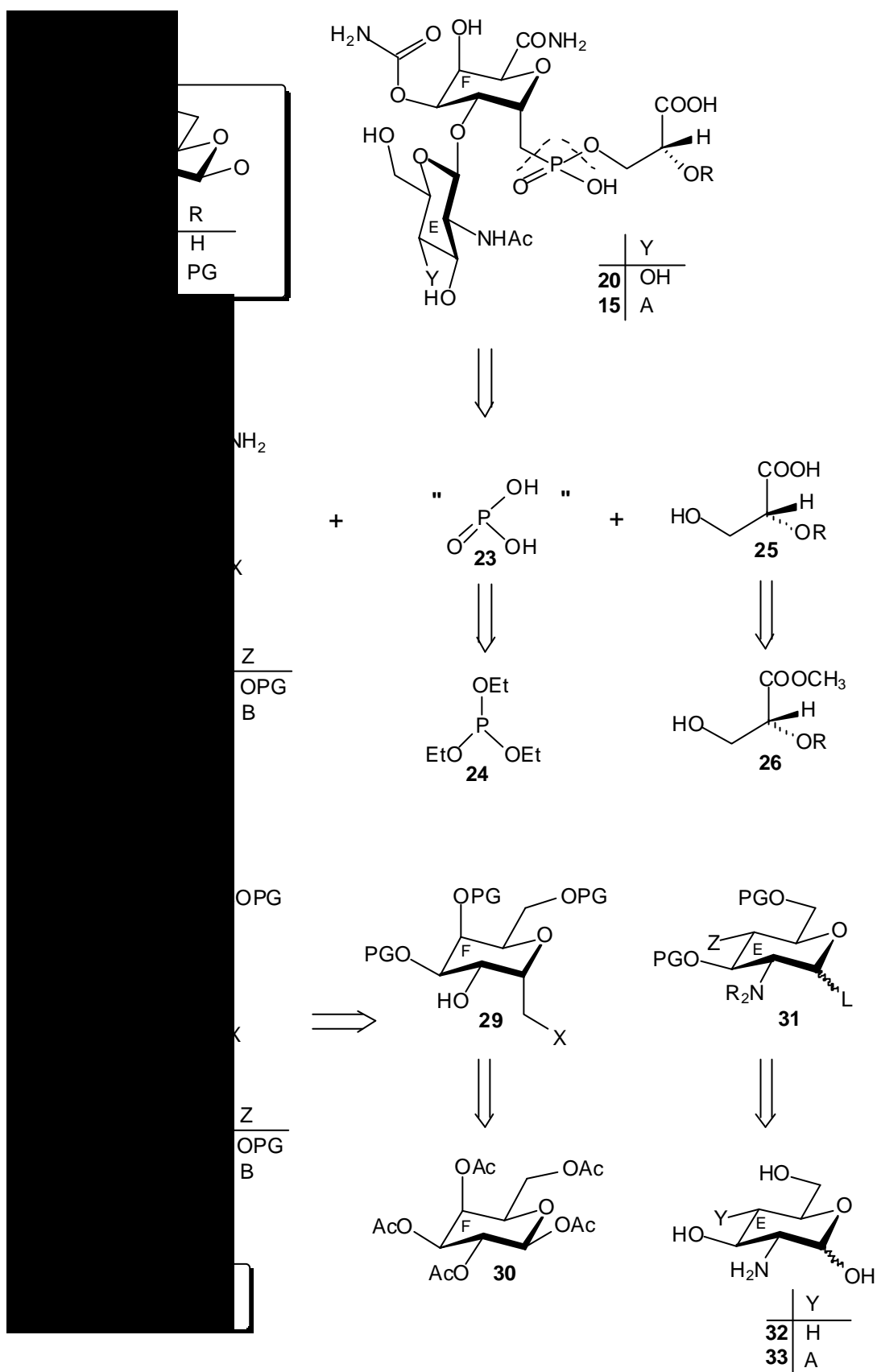
Scheme 4.1: Retrosynthetic analysis of target molecules **15** and **20**

4.2 Plan of synthesis

Synthesis of the target analogue of moenomycin A_{12} can be achieved after three major preparations:

1. Preparation of the glycosyl acceptor, which will represent unit F of the target molecule.

2. Glycosylation to achieve the desired β-oligosaccharide.

3. Linkage of the β-di- or trisaccharide via a phosphonic acid diester with a glyceric acid unit to a lipid chain.

Minimizing the number of functional group interchange reactions, as well as minimal use of protecting groups are highlights for the overall synthetic efficiency.

The glycosyl acceptor must be sufficiently protected prior to the glycosylation step; only one hydroxyl group should be glycosylated. A variety of protecting groups exists to perform the oligosaccharide synthesis. Our synthetic strategy dictated the use of **39** as the glycosyl acceptor.

The synthesis of the glycosyl acceptor **39** (Scheme 4.2) should begin with the commercially available and cheap β-D-galactose-pentaacetate, **30** in the following sequence. It will be transformed into the C-glycoside **34**[59] which should undergo a double bond rearrangement to yield its propenyl analogue **36**. Ozonolysis of this alkene followed by reduction of the produced aldehyde should afford the alcohol **35**, which should be converted into its halide derivative **37**, which is considered to be an important precursor to apply the *Arbuzov* reaction, through which the sugar unit should couple with the lipid unit moieties via a phosphonate bridge. Hydrolysis of the acetate groups in compound **37** without harming the primary bromide should afford compound **38**. â-Selective glycosylation at position-5 requires protection of the other free hydroxyl groups. The primary hydroxyl group in compound **38** could be selectively protected as a TBDPS ether. This protecting group was selected because of its stability and ease of selective introduction. It should be latter selectively deprotected, and the liberated hydroxyl group will be then converted into the corresponding amide functional group. Of the several options to protect *O*-3 and *O*-4 in **40**, we decided to select the isopropylidene acetal, a well-known protecting group stable during the glycosylation reaction. Thus, further protection of the hydroxyl groups at positions 3 and 4 in **40** should yield the glycosyl acceptor **39**.

Many methods exist for the 1,2-*trans* glycosylation.[60] In this work, disaccharide formation will be carried out according to the *Jacquinet and Blatter* method,[61] which uses **41** as the glycosyl donor. The advantage of this method is the high reactivity of the 2-trichloromethyloxazoline **41**. On the other hand, it constructs the disaccharide with an N-trichloroacetyl group, which must be transformed to an N- acetyl group, before
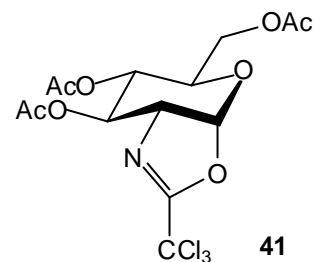
Figure 4.1:

Glycosyl donor **41**

applying the *Arbuzov* reaction. Thus, the reaction of the glycosyl acceptor **39** with the glycosyl donor **41** should give the desired disaccharide.

In another parallel project, trisaccharide formation will be carried out using the oxazoline **42**[62] as the glycosyl donor. It could be prepared from chitobiose octaacetate, and it generates the desired N-acetyl groups directly after the glycosylation step. Thus, the
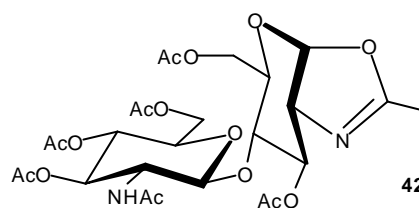
Figure 4.2: Glycosyl donor **42**

reaction between the glycosyl acceptor **39** and the glycosyl donor **42** should yield the desired trisaccharide.

The general synthetic plan for the target molecules is shown in Scheme 4.3.

Cleavage of the silyl ether in compound **43** could be selectively achieved under acidic conditions without disturbing the acetonide to give **44**. The primary hydroxyl group should then be oxidised to the corresponding acid **45**, and this in turn would finally be converted to the amide **46**. We expected that the 3,4-*O*-isopropylidene group of **46** can be selectively cleaved under acidic conditions furnishing **47**. The carbamoyl group was planned to be introduced at position 4 in unit F by different methods, depending on the protecting groups, thus giving **48** and leaving the axial hydroxyl group at position 3 free. Transformation of the N-trichloroacetyl group in **48** into an N-acetyl group should give **49**.

Construction of the phosphonic diester was to be achieved by applying the *Arbuzov* reaction. Thus, on its reaction with triethylphosphite, **49** should be converted into the *C*-phosphonate **50**, which should be in turn converted into the corresponding phosphonic acid **51** upon treatment with bromotrimethylsilane followed by reaction with water.

Coupling of **51** to the lipid bearing glycerol acid part using trichloroacetonitrile would complete the target skeleton formation **52**. Hydrolysis of the acetyl protecting groups under basic conditions should furnish the target compounds **20** and **15**.
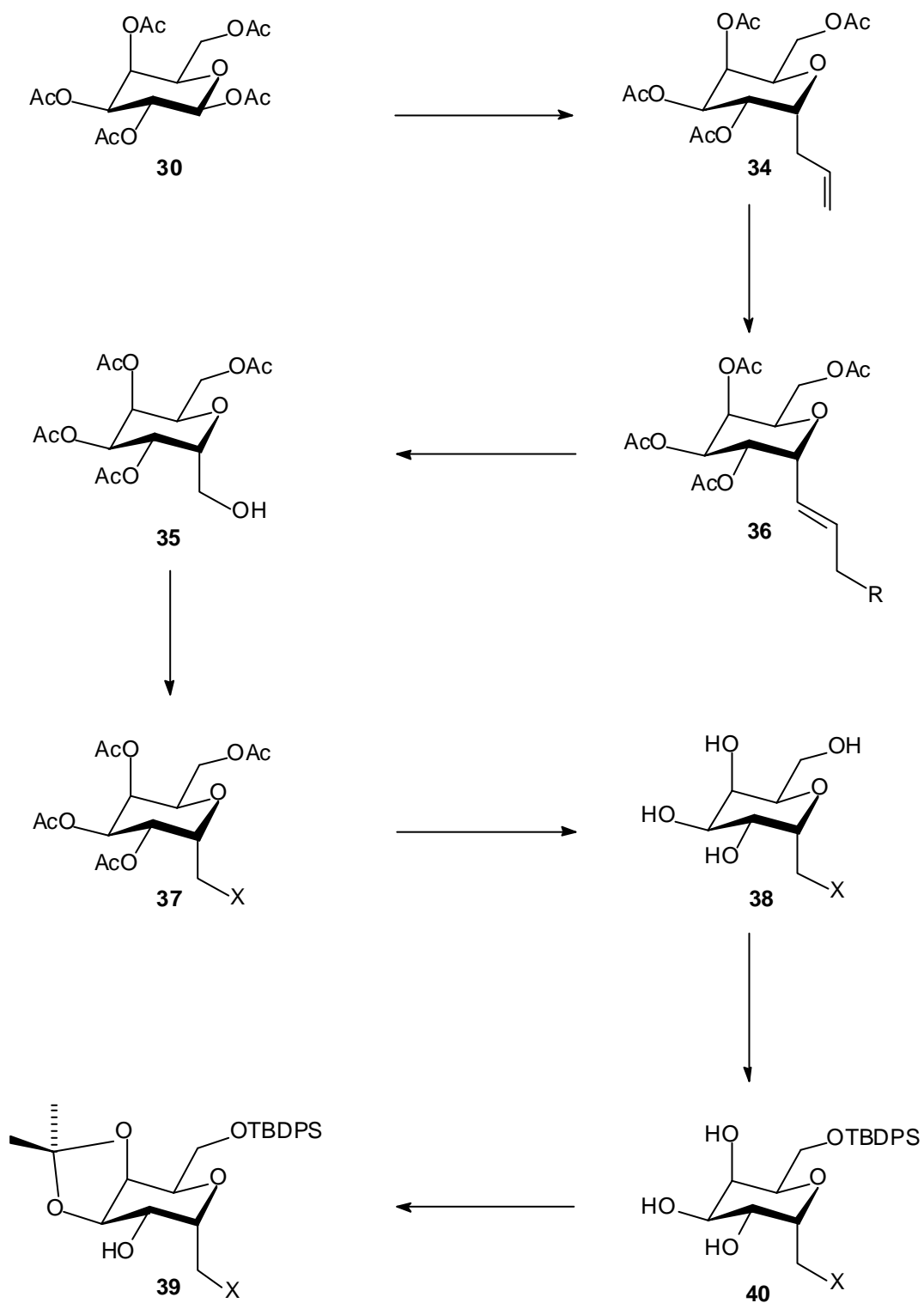
Scheme 4.2: Planned synthesis of the glycosyl acceptor **39**

	R
43	CH$_2$OSi^{t}BuPh$_2$
44	CH$_2$OH
45	COOH
46	CONH$_2$

	R^1	R^2
47	H	CCl$_3$
48	H$_2$NOC	CCl$_3$
49	H$_2$NOC	CH$_3$

	R
50	OEt
51	OH

	Y
20	OH
15	
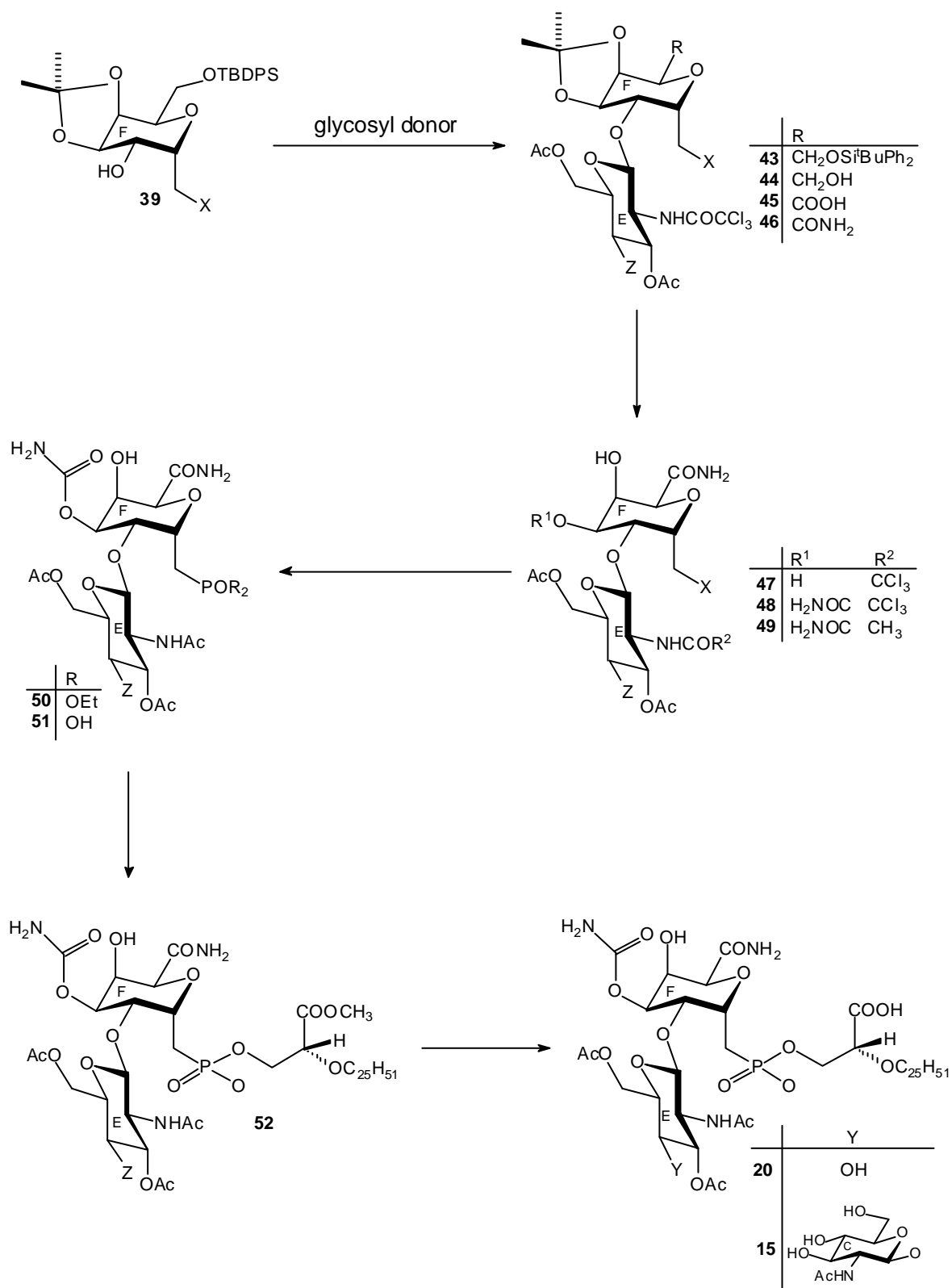
Scheme 4.3: Planned synthesis of the target molecules **15** and **20**

5 RESULTS AND DISCUSSION

5.1 Synthesis of unit F-derived glycosyl acceptor

The general structural prerequisites for the glycosyl acceptors were given by the retro synthesis, i.e.

- A halide group at C-7 as the phosphonate precursor

- A free hydroxyl group at position C-5 for the attachment of the other carbohydrate units

- Selectively cleavable protecting groups at C-3 and C-4

- A selectively removable protecting group for the 1-OH-group to allow oxidation at any appropriate stage in the synthesis.
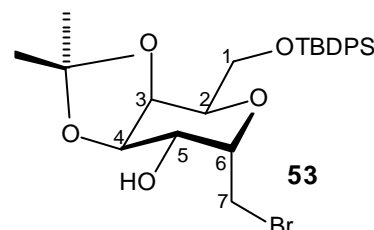
Figure 5. 1: Glycosyl acceptor

Compound **53** shown in Figure 5.1 was chosen as the glycosyl acceptor for this work.

5.1.1 Synthesis of allyl C-galactopyranoside 34

The synthesis began with the introduction of the C-glycoside appendage at position 1 of β-D-galactose-pentaacetate **30**. The peracetylated allyl C-galactopyranoside **34** has been prepared according to *Giannis et al.*[59] from **30** and allyltrimethylsilane in the presence of borontrifluoride ethyl etherate in 67 %. Compound **34** was contaminated with small amounts of the β-isomer, which has a slightly higher R_f value.
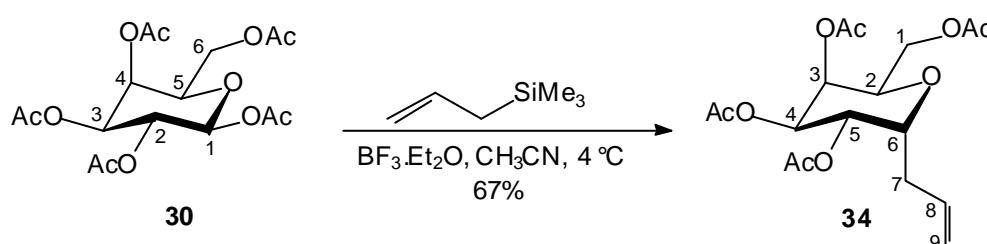
Scheme 5. 1: C-glycosylation with allyltrimethylsilane/ BF$_3$.Et$_2$O

5.1.2 Double bond rearrangement in compound 34

5.1.2.1 Palladium catalytic reaction of compound 34

It is well-known that double bonds can be shifted to thermodynamically more stable positions in Palladium-mediated reactions. This approach that has been employed by *Brooks et al.*[55] was described to be not completely satisfactory since product mixtures resulted.
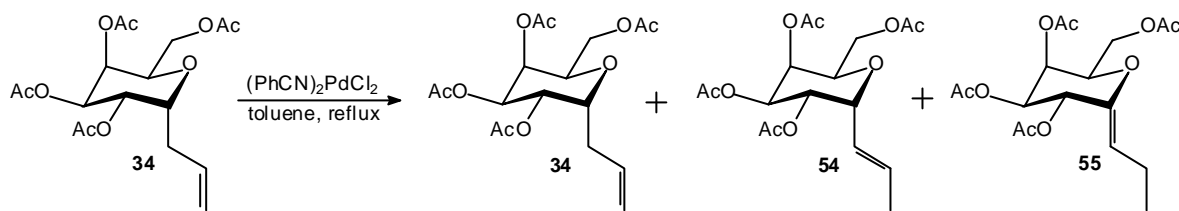
Scheme 5. 2: Palladium-catalysed rearrangement reaction of compound **34**

In the present work, the same observation was made. When compound **34** was refluxed in toluene with varying amounts of bis(benzonitrile)palladium(II) chloride, $(PhCN)_2PdCl_2$, TLC using several solvent mixtures showed a major spot with exactly the same R_f value as **34** and a minor spot similar to that of **34** but slightly higher. The major spot according to ^{13}C NMR corresponds to a mixture of **34** and **54**. The CH_3 signal of **54** appeared at 18 ppm, while the CH_2 vinylic signals of the starting material appeared at 31 ppm. In the 115-135 ppm range, the olefinic signals of the starting material **34** appeared at 118 and 133 ppm, whereas the signals at 119 and 123 are assigned to the olefinic carbons of product **54**. According to the ^{13}C NMR there was about a 1:1 ratio of the two isomers. Changing the amount of the catalyst and the reaction time did not affect the result.

The by-product was a white solid, and according to the spectra it has structure **55**.

The main problem was obviously the difficulty to separate and purify compound **54**. When a mixture of the two compounds **54** and **34** was ozonolyzed, again compounds with very close R_f values have been obtained (data not reported).

As a conclusion, we preferred to look for a more practical route for the double bond rearrangement in compound **34**.

5.1.2.2 Ene reaction

An ene reaction seemed to be a good choice for the rearrangement of the double bond. The first attempt was made using singlet oxygen as a reagent.

5.1.2.2.1 Attempted photooxygenation reaction of compound 34

The photolysis of **34** has been tested using two different sensitizers; methylene blue, and Rose bengal. Methanol and dichloromethane solutions of compound **34** and each of the sensitizers were illuminated with an external Philips 1000W halogen lamp while a stream of dry oxygen was passed through the solution. After 9 h at RT, no reaction was observed as indicated by TLC, using different eluents.

★ a) methylene blue, MeOH, 9 h, or b) methylene blue, CH₂Cl₂, 9 h, or
   b) Rose bengal, MeOH, 9 h   or d) Rose bengal, CH₂Cl₂, 9 h
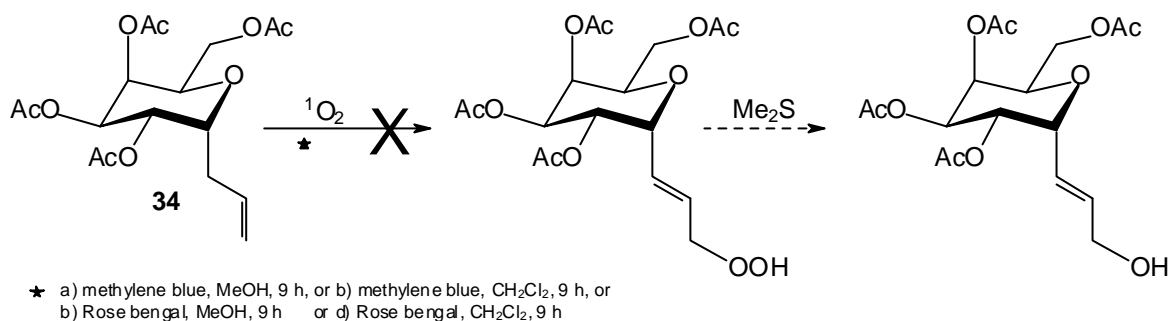
Scheme 5. 3: Attempted photooxygenation of compound **34**

Guided by this result we looked for a more reactive reagent.

5.1.2.2.2 Ene reaction of triazolinedione with compound 34

The ene reaction of 4-substituted-1,2,4-triazoline-3,5-diones (4-R-TAD) with alkenes was first studied by *Pirkle* and *Sticler*.[63] TADs are highly reactive neutral electrophiles, commonly used to detect unsaturation or to introduce nitrogen functionalities.[64] TADs undergo *Diels-Alder* reactions with dienes, and afford [2+2] adducts, or ene products with alkenes.[65]

The ene products were found to be very stable, and to be quite acidic. The NH group of the ene product is very reactive, and the product still possesses a carbon-carbon double bond, with interesting reactivity. This double bond can undergo reaction with bromine, or with ozone, but it has much lower reactivity toward a new molecule of TAD than the corresponding alkene.[66]

In our case, compound **34** on reaction with 4-phenyl-3H-1,2,4-triazoline-3,5-dione (PTAD)[66] in dichloromethane at RT provided **56** in 83 % yield. 13 % of unreacted alkene were recovered.
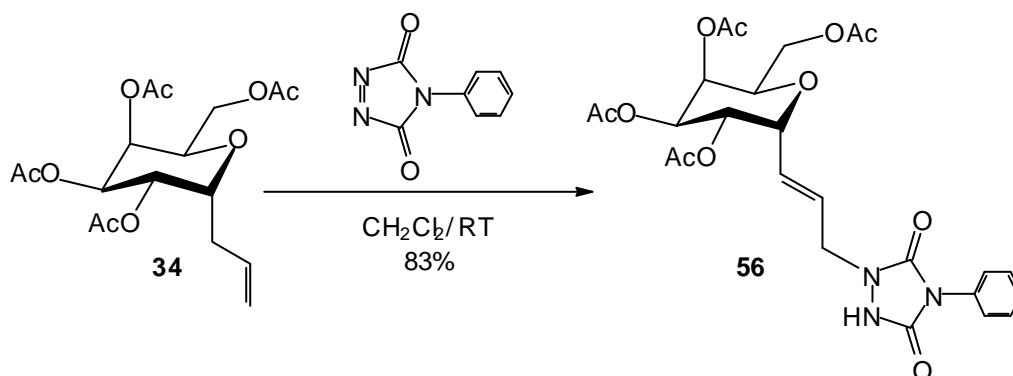
Scheme 5. 4: Reaction of compound **34** with PTAD

The typical red colour of PTAD was quickly discharged when PTAD was mixed with the alkene. The results of double bond rearrangement reactions of compound **34** are summarised in Scheme 5.5.
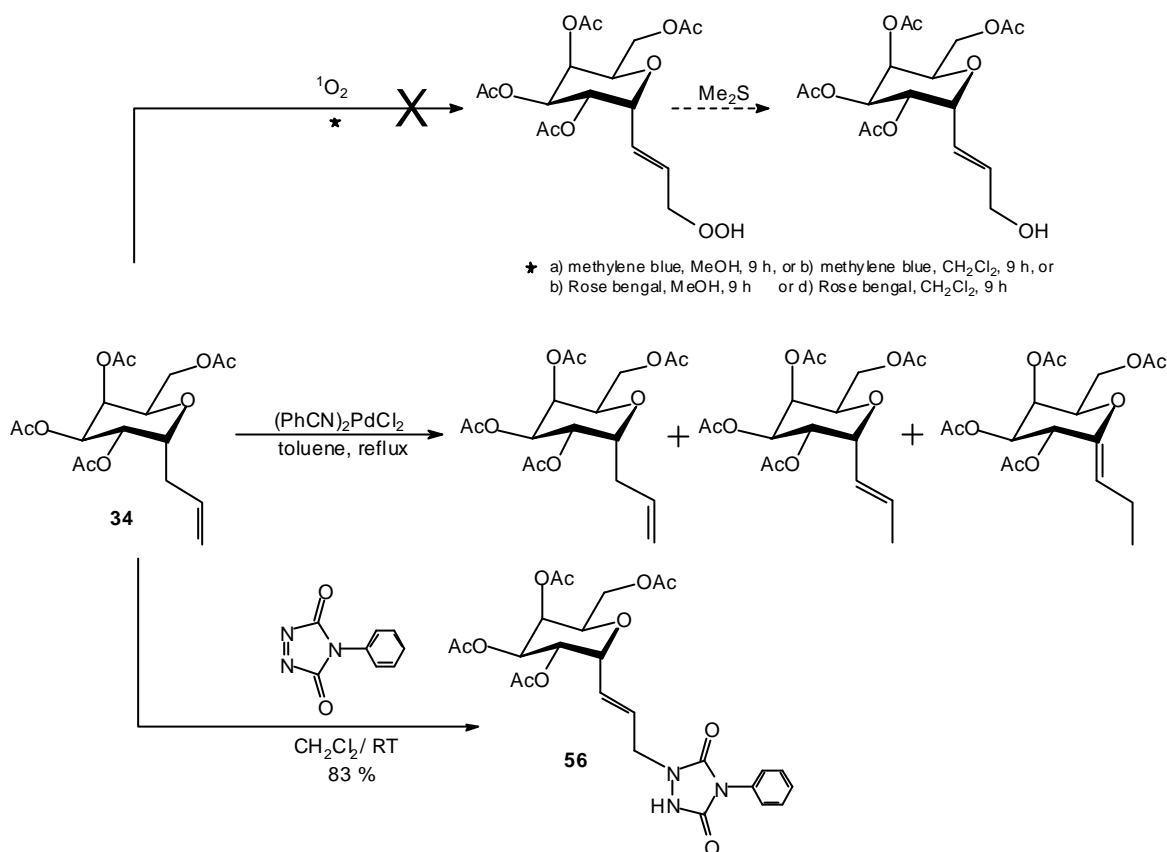
Scheme 5. 5: Double bond rearrangement of compound **34**

5.1.3 Preparation of alcohol 35

Compound **35** has been prepared from alkene **56** in two successive steps, ozonolysis followed by reduction. Ozonolysis of compound **56** in a dry CH_2Cl_2-MeOH (10:1) at -70 °C, and subsequent quenching with dimetyl sulfide should lead to the aldehyde **57**. No effort has been made to obtain pure samples of the aldehyde. *Brooks*[55] has reported that modest yields (48 %) of a gluco analogue of the desired alcohol **35** were obtained by using sodium acetoxyborohydride to reduce the crude ozonolysis products. In agreement with this, reduction of the crude ozonolysis products of **56** with sodium acetoxyborohydride (prepared in *situ* from $NaBH_4$ and AcOH in THF) furnished the primary alcohol **35** in a non satisfying yield of 42 %.

(i) O_3, CH_2Cl_2-MeOH, -70 °C, Me_2S
(ii) $NaBH_4$, AcOH, THF
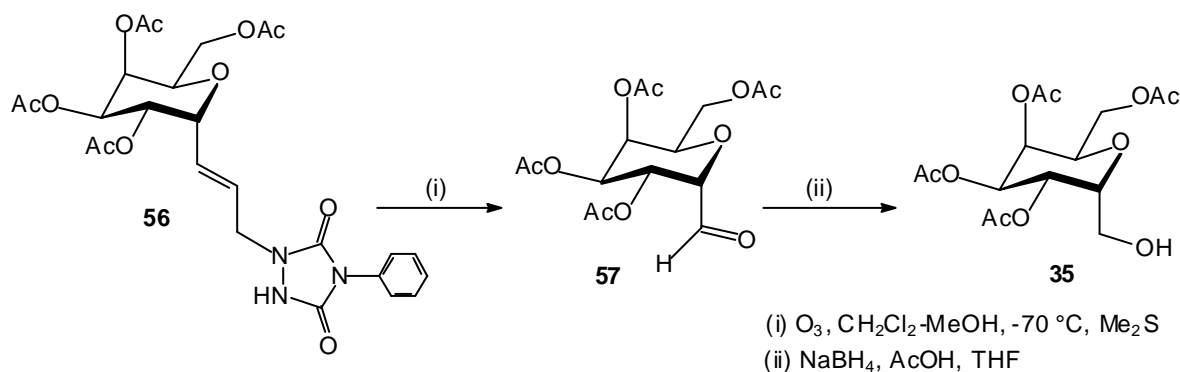
Scheme 5. 6: Preparation of alcohol **35**

An identified problem was the ready elimination of acetic acid to give the α,β-unsaturated aldehyde, **58**. The formation of this glycal was completely suppressed under dry conditions. When the reaction was performed using freshly distilled THF and acetic acid, the yield increased up to 85 % with no trace of the aldehyde **58** on the TLC.
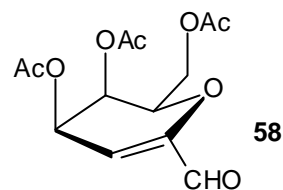
Figure 5. 2: Glycal **58**

5.1.4 Preparation of the halide derivatives

The halide derivative that we wanted to prepare is an important precursor for applying the *Arbuzov* reaction.[56] *Brooks et al.*[55] reported that OH → halide exchange in the gluco analogues of **35** by direct displacement of hydroxyl groups with thionyl bromide, or phosphorous tribromide in carbon tetrabromide was unsuccessful. They have used a two-step procedure via a sulfonate. On the contrary, compound **35** could be converted directly into compound **59** on reaction with iodine, in the presence of chlorodiphenylphosphine (Ph$_2$PCl) and imidazole[67], but the yield was low (31 %).
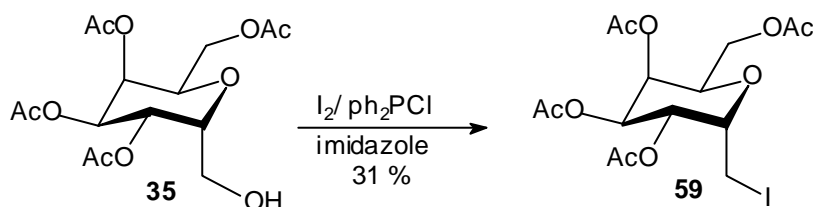
Scheme 5. 7: Synthesis of the iodide **59**

Eventually, the primary hydroxyl group in compound **35** was mesylated, using methanesulfonyl chloride in pyridine, in the presence of catalytic amount of *p*-dimethylaminopyridine (DMAP) at 4 °C to give methanesulphonate **60** in 60 % yield.
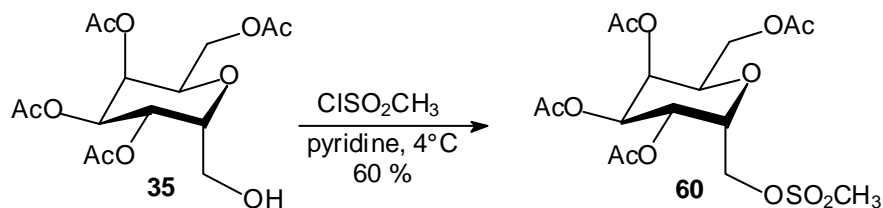
Scheme 5. 8: Synthesis of the methanesulphonate **60**

The mesylate **60** was converted in turn into the halide derivatives **59** (65 %) and **61** (80 %) when heated in toluene at 80 °C with tetrabutylammonium iodide and tetrabutylammonium bromide,[55] respectively.
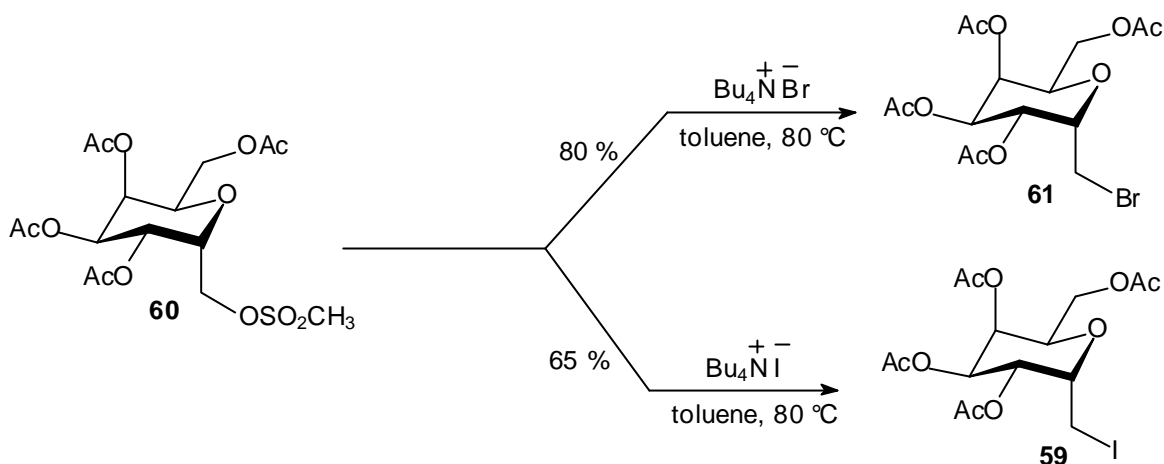
Scheme 5. 9: Conversion of the sulphonate **60** into the halides **59** and **61**

5.1.5 Hydrolysis of the acetate group

Hydrolysis of the acetate groups without harming the primary bromide was accomplished making use of the *Zemplén* method.[68] Thus, sodium methoxide was added to a solution of compound **61** in dry methanol (ca 10 mL MeOH for 1 g sugar) at 0 °C, and the mixture was then stirred at RT. The reaction mixture was then neutralized with Dowex 50-W X2 (H$^+$), affording the fully deprotected compound **62** in quantitative yield.
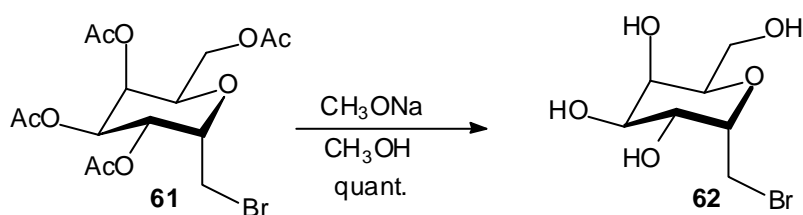
Scheme 5. 10: Ester hydrolysis of compound **61**

5.1.6 Preparation of the silyl ether 63

Silyl ethers have gained considerable importance as protecting groups in natural product chemistry. A large variety of triorganosilyl ethers are employed in organic chemistry. As the alkyl group becomes larger, silyl groups become more resistant to hydrolysis and also easier to place on primary hydroxyls regioselectively. They are normally formed by reaction of an alcohol with

the respective silyl chloride under basic catalysis, involving usually pyridine, triethylamine, DMAP or imidazole in DMF.

The need for regioselective protection of the primary hydroxyl group has led us to investigate the use of *t*-butyldiphenylsilyl chloride (TBDPSCl). The value of this reagent for preferentially blocking primary alcohols in sugar derivatives has been recognized. When **62** was treated with TBDPSCl, using weakly basic catalysts such as pyridine and triethylamine, in the presence of a catalytic amount of *p*-dimethylaminopyridine (DMAP) at 0 °C, the yield of **63** was only 33 %. On the other hand, treatment of compound **62** with TBDPSCl in N,N´-dimethylformamide (DMF) in the presence of imidazole at 0 °C afforded the 6-(*t*-butyldiphenylsilyl) ether **63** in 85 % yield.
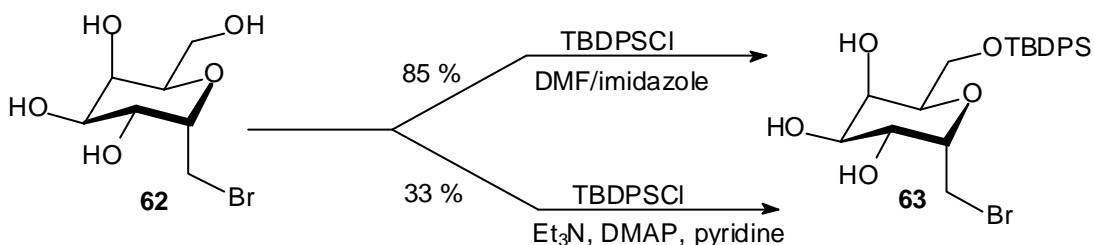
Scheme 5. 11: Selective 6-*O*-silylation in compound **62**

The *t*-butyldiphenylsilyl (TBDPS) ether is quite stable to acids, and it should survive the acidic conditions necessary for the formation and cleavage of isopropylidene acetals.[69] It is also stable to bases. Cleavage of silyl ethers can be achieved by fluoride ions, using a molar solution of TBAF in THF.

5.1.7 Preparation of the isopropylidene derivative 53

The free hydroxyl groups at *O*-3 and *O*-4 in compound **63** have been protected by acetonide formation.[70] The isopropylidene group can be introduced with dry acetone and an acid[71] (mineral acids[72] or Lewis acids such as ZnI_2 or dry $CuSO_2$,[73] $AlCl_3$[74] or $FeCl_3$[75]) or in DMF solution with 2,2-dimethoxypropane[76] or 2-ethoxypropene[77] and a catalytic amount of an acid, often *p*-toluenesulfonic acid (PTS). For energetic reasons, acetone normally reacts only with vicinal hydroxy groups which are positioned in a *cis*-arrangement at the sugar ring to give five-membered 1,3-dioxolanes. The axial methyl substituent at the acetal centre destabilizes the corresponding

1,3-dioxane. In this work, PTS-catalysed isopropylidenation with 2,2-dimethoxypropane in dry acetone was used and afforded the 3,4-*O*-isopropylidine acetal derivative **53** in 88 % yield.
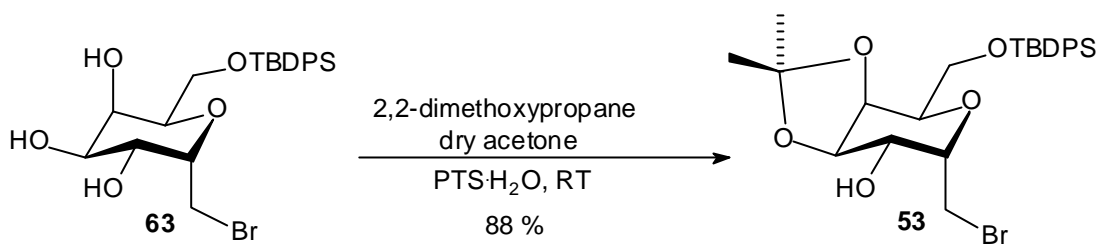
Scheme 5. 12: Synthesis of the isopropylidene derivative **53**

Work-up of the reactions was carried out using acid-free conditions to avoid acetal hydrolysis.

5.2 Synthesis of the disaccharide building block 18

5.2.1 Glycosylation

5.2.1.1 Choice of the glycosyl donor

A multitude of methods and versions is available for the glycosylation of carbohydrates, both with and without neighbouring group participation.[60] In our case, the glycosyl donor should contain a nitrogen functionality which in the final product has to be converted into an N-acetyl group. In principle, an N-acetylated donor **64** appears to be suitable. However, N-acetylated amino sugars are particularly unsuited as glycosyl donors, because under the glycosidation conditions they tend to form stable oxazoline derivatives.[78,79]
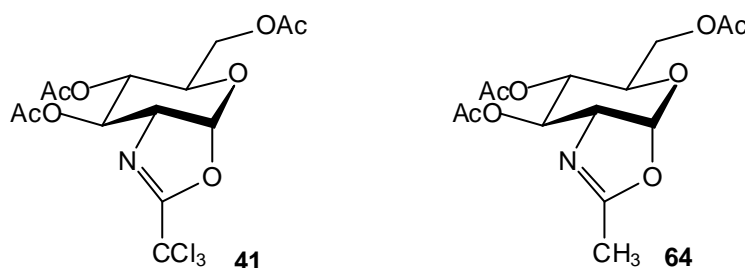
Figure 5. 3: Glycosyl donors **41** and **64**

The *Jacquinet* and *Blatter* method[61] solves this problem and uses the trichloromethyl oxazoline **41** as the glycosyl donor, and a Lewis acid as promoter. The use of trichloroacetamidates is one of the most commonly used glycosylation strategies. The general significance of *O*-glycosyl trichloroacetamidates lies in their ability to act as strong glycosyl donors under relatively mild acid catalysts such as borontrifluoride etherate complex ($BF_3.Et_2O$) or trimethylsilyl trifluoromethanesulfonate (TMSOTf). Despite the inductive effect of the halogens, trichoroacetamido derivatives such as **41** give rise to the formation of intermediate oxazolinium ions. The electron withdrawing effect of the trichloromethyl group in these intermediates greatly increases the electrophilic character of the anomeric carbon, and explains the much greater reactivity of **41**, compared to its N-acetyl congener **64**. However, this method constructs the disaccharide with a N-trichloroacetyl group, which must be transformed to a N-acetyl group as will be discussed latter.

5.2.1.2 Glycosylation mechanism

In the glycosylation reactions, neighbouring group participation of protecting groups at C-2 is usually the dominating effect in anomeric stereocontrol during glycosylation, giving rise to 1,2-*trans* glycosides. When non-participating protecting groups are selected, S_N2-type reactions can be carried out assisted by the use of nonpolar solvents, low reaction temperatures, and weak Lewis acid catalysts thus leading to 1,2-*trans* as well as 1,2-*cis* glycosides. The general glycosylation mechanism[60f] is shown in Scheme 5.13.
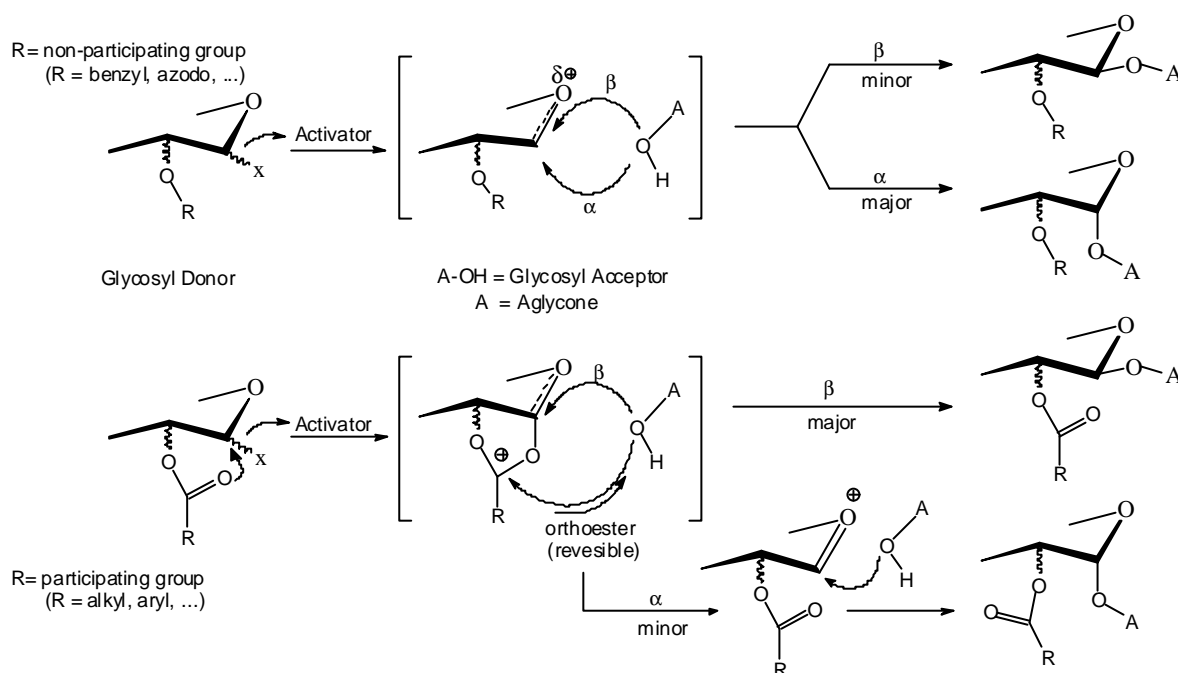
Scheme 5. 13: General glycosylation mechanism

5.2.1.3 Synthesis of the glycosyl donor

Blatter et al.[61] (Scheme 5.14) reported the use of the trichloromethyl oxazoline **41** as the glycoside donor (prepared in *situ* from **67**) using a catalytic amount of trimethylsilyl triflate (TMSOTf), as a promoter, which gave the best glycosidation results. D-glucosamine hydrochloride **32** was selectively N-substituted by treatment with trichloroacetyl chloride in aqueous media, thus giving **65** which undergoes an acetylation reaction to give **66** as an anomeric mixture of á and â isomers. Anomeric deprotection of the mixture with hydrazinium acetate in DMF, followed by treatment with trichloroacetonitrile and DBU afforded the trichloroacetamidate **67**.
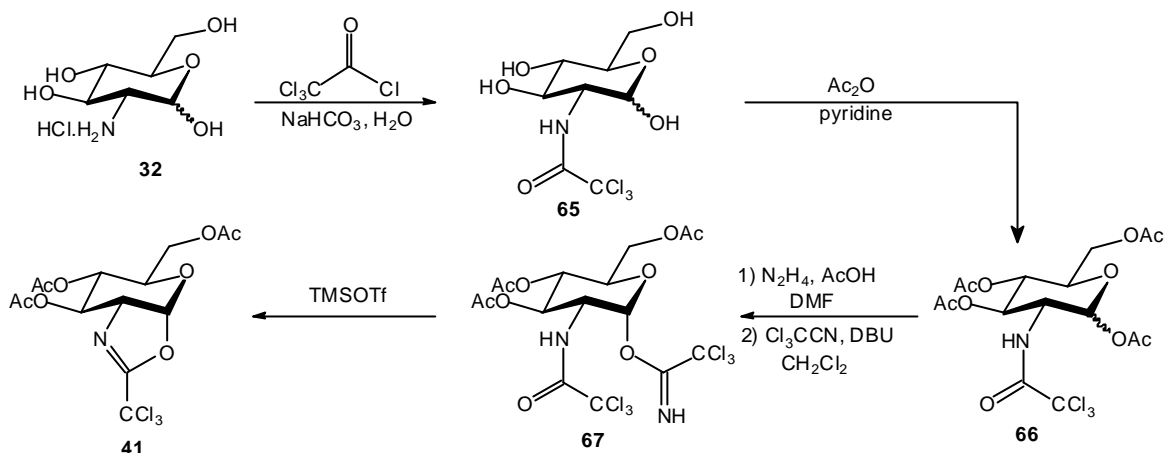
Scheme 5. 14: Synthesis of the glycosyl donor **41**

5.2.1.4 Disaccharide formation

In the glycosylation step, the glycosyl acceptor and donor are normally mixed in a dry inert solvent such as dichloromethane, acetonitrile or 1,2-dichloroethane, then the reaction is started by the addition of a catalytic amount of a Lewis acid. The glycoside is usually obtained in high yields with only a slight excess of the donor, and with a high degree of 1,2-*trans* stereoselectivity. In the present case, glycosyl acceptor **53** and a moderate excess of 1.2 eq of the glycosyl donor **67** were treated with 0.1 eq of TMSOTf in dry 1,2-dichloroethane in the presence of activated 3 Å molecular sieves at 0 °C for ca 10 min, followed by neutralization with triethylamine (TEA), and afforded the desired â-linked disaccharide **68** in 79 % yield.
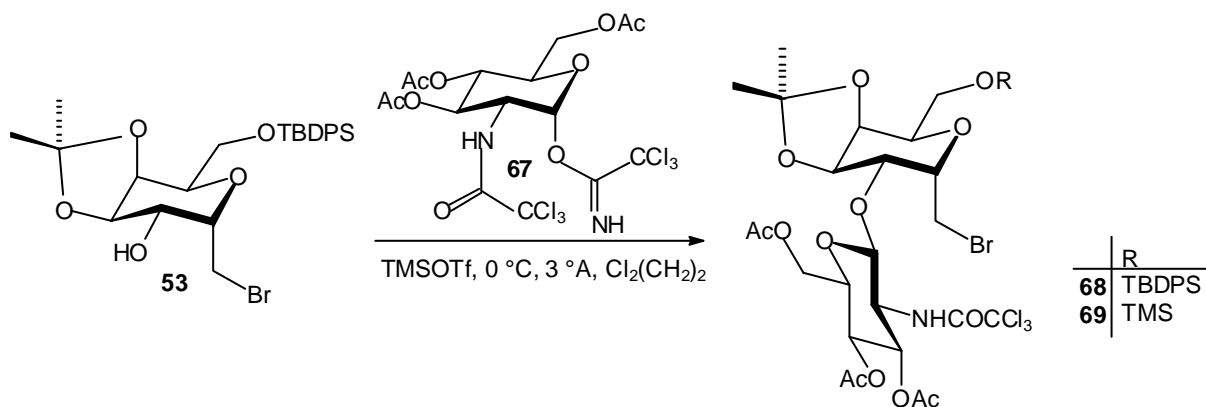
Scheme 5. 15: Glycosylation reaction of **53**

In one reaction (AJ 2-12) compound **69** was isolated as the main product in 44 % yield, contaminated with many by-products. Its formation could possibly be explained as shown in the following scheme. Compound **68** should be formed, and undergoes a reversible reaction with

TMSOTf leading to the intermediate **70**, which upon reaction with a nucleophile (possibly water) gives compound **68** (pathway a) or compound **69** (pathway b).
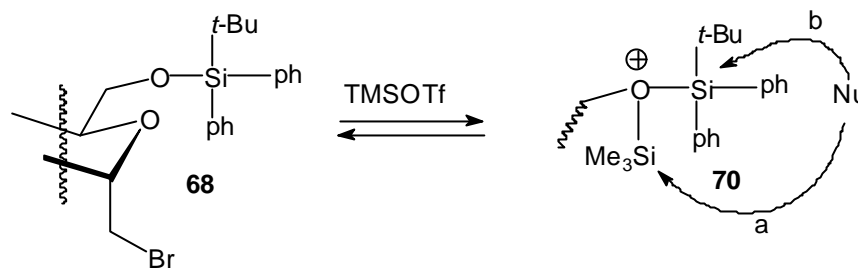
Scheme 5.16: Explanation for the formation of **69**

The reaction conditions have been carefully optimised. 0 °C and a reaction time not longer than 10 min are required to furnish the main product in a high yield. It was noticed by TLC that on longer reaction times decomposition occurs to give many products, and the desired disaccharide is obtained in low yields.

5.2.2 Cleavage of the silyl ethers in compounds 68 and 69

Selective cleavage of only one protecting group in a sugar can be accomplished if not all protecting moieties in a carbohydrate derivative have the same reactivity. Cleavage of silyl ethers can be achieved by fluoride ions using TBAF,[69] for example, which removes the silyl ethers without cleavage of other acid-sensitive groups in the same sugar. Removal of the TBDPS group from **68** was readily accomplished by treatment of **68** with a molar solution of TBAF in THF at room temperature, thus affording **71** in 89 % yield.
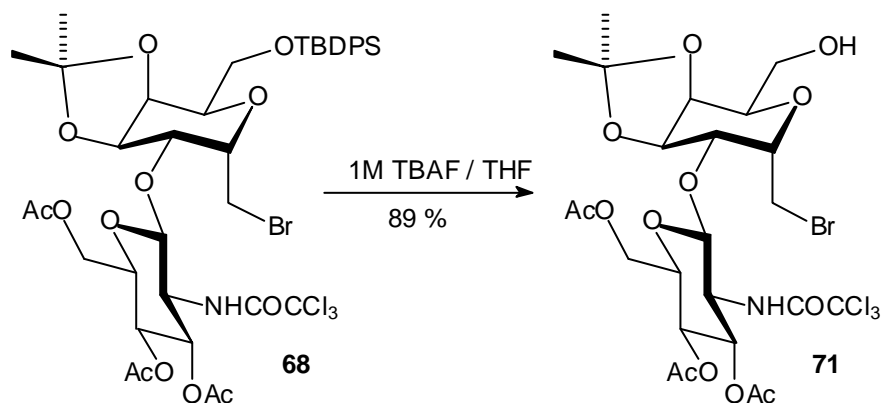
Scheme 5.17: Cleavage of the silyl ether in compound **68**

It should be noted that the bromomethyl group survives the reaction conditions.

On the other side, compound **71** was obtained in 95 % yield from **69**, upon its reaction with

Dowex 50-W X-8 (H^{+}) resin at room temperature in methanol.[80]
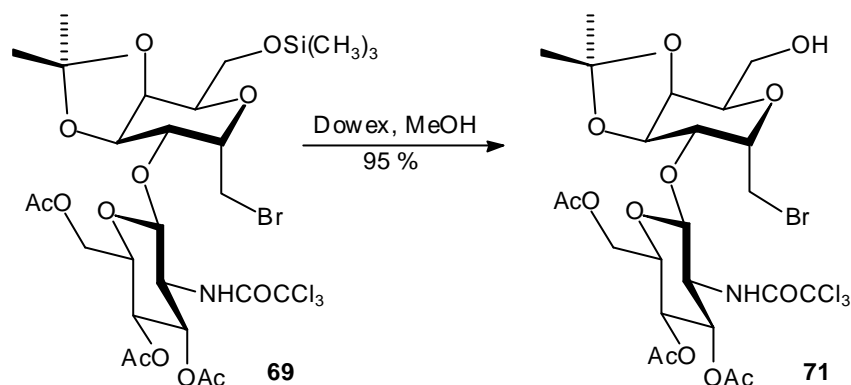
Scheme 5.18: Cleavage of the silyl ether in compound **69**

5.2.3 Synthesis of the uronamide 72

The oxidation of the primary hydroxyl group of carbohydrates to form the corresponding uronic

acid is often performed as a two-step reaction. For example, *Swern*-oxidation[81] (DMSO, oxalyl

chloride) to give an aldehyde, and sodium chlorite oxidation[82] of the latter to the uronic acid has

been successfully applied for the synthesis of a moenomycin analogue.[83] A further method to be

mentioned here, is the oxidation with *o*-iodoxybenzoic acid,[84] which has been successfully applied

by *Weigelt*[85] for the synthesis of an uronic aldehyde. A useful reagent is also 2,2,6,6-tetramethyl-

1-piperidinyloxy-radical (TEMPO). *Flitsch*[86] reported that the primary hydroxyl groups in

monosaccharides can selectively be oxidised with TEMPO[87] in the presence of a phase transfer

catalyst, leading even directly to the carboxylic acid. Tetrabutylammonium chloride is used as the

phase transfer catalyst. The consumed oxidizing reagent can be regenerated by hypobromite,

prepared in *situ* from hypochlorite and bromide.[88] Other workers could also achieve selective

oxidation of mono- and polysaccharides to the appropriate acids using this method.[89] However,

Kosmol[90] found that TEMPO-oxidation under phase transfer catalyst gave only the aldehyde,

which in turn had to be further oxidised with sodium chlorite to the corresponding acid. A version

of the TEMPO-oxidation using organic oxoammonium salts generated by acid-promoted

disproportionation of 4-acetylamino-TEMPO is reported by *Bobbitt*.[91]

The standard method to get the amide is the imidazolide procedure of *Staab*,[92] in which the acid

is activated with N,N´-carbonyldiimidazole (CDI) in dichloromethane to give the imidazolide,

which upon its reaction with ammonia[93] will be transferred to the amide. Other activating reagents

are dicyclohexylcarbodiimide and 1-hydroxybenzotriazol,[94] or the EEDQ in chloroform. In the

latter protocol the amide functional group was generated by the addition of ammonium hydrogencarbonate.[95]

In the present case the procedure of *Kosmol et al.*[35] was applied.

First, the primary alcohol **71** was oxidised to the corresponding aldehyde with sodium hypobromite (prepared in *situ* from sodium hypochlorite, and potassium bromide) in the presence of TEMPO and tetrabutylammonium chloride. The crude product was then oxidised with sodium chlorite to furnish the corresponding acid, which was immediately converted to the amide using *Staab's* method. The acid was dissolved in dry dichloromethane and activated with CDI. Gaseous ammonia was then bubbled through the solution at 0 °C. The required amide **72** was obtained in an overall yield of 98 % (based on alcohol **71**).
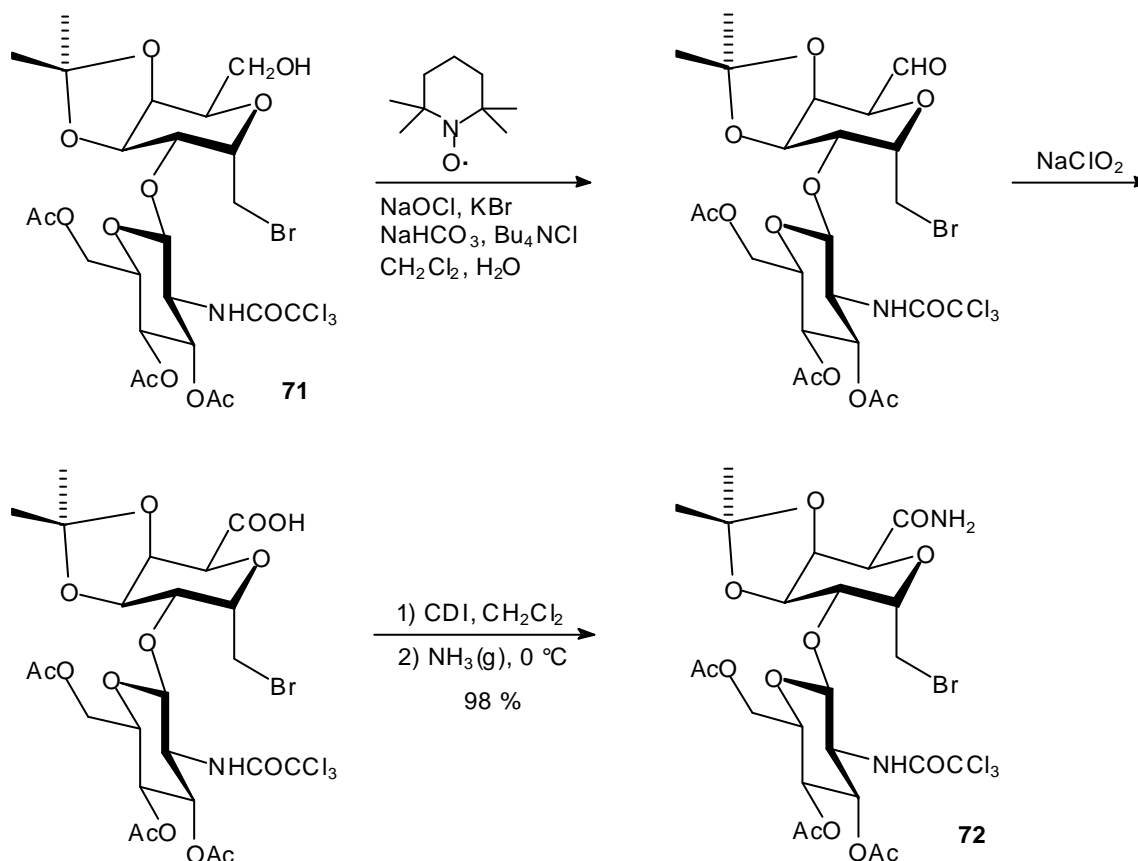
Scheme 5. 19: TEMPO, sodium chlorite oxidation and amide formation

5.2.4 Removal of the isopropylidene group from 72

The isopropylidene group, which is stable under basic-, oxidation- and reduction conditions,[96] can be hydrolysed under acidic conditions, using, for example, aqueous acetic acid, aqueous

trifluoroacetic acid (TFA), diluted HCl in THF, sulfuric acid in methanol, silica gel, or acidic ion exchange resin.[97] A newer method uses DDQ for the acetal cleavage in carbohydrates.[98]

The 3,4-*O*-isopropylidene group of compound **72** has shown resistance again Dowex 50 W X-8 (H^{+}), as shown in Scheme 5.18 for the cleavage of the trimethylsilyl ether in compound **69**. Additionally, no reaction was noticed when **72** was treated with 80 % AcOH at room temperature for 17 h. In contrast, the acetal was easily cleaved with TFA at room temperature, furnishing diol **73** in 89 % yield.
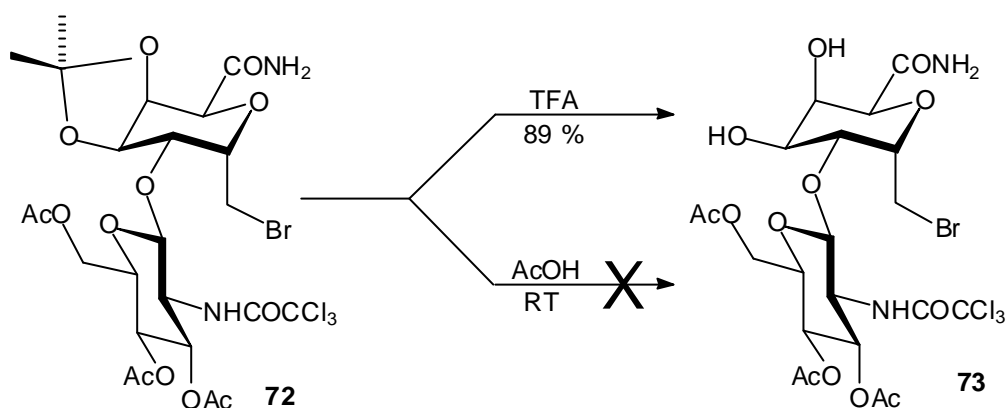
Scheme 5. 20: Removal of the isopropylidene group from **72**

5.2.5 Carbamoylation

There are different methods to introduce the carbamoyl functional group at position-4[F] which is necessary for the biological activity of moenomycin-type compounds.

The most versatile method is the addition of alcohols to isocyanates. The unsubstituted carbamoyl group can be obtained through the addition of chlorosulfonylisocyanate, followed by hydrolysis.[99] Through a reaction of mono[100]- or tri-chloroacetylisocyanate (TAI)[101] with the alcohol, the desired carbamoyl compound is obtained after removal of the mono- or tri-chloroacetyl groups. This is possible either hydrolytically in the presence of an ion exchange resin, with Al_2O_3,[101d] K_2CO_3,[102] or reductively with zinc dust in methanol.[103] TAI is very reactive and reacts with OH groups in the order:

primary > secondary > tertiary > phenol.
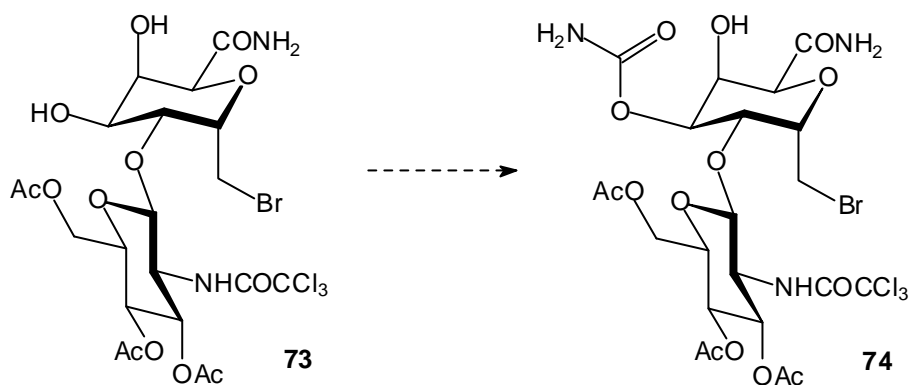
Scheme 5. 21: From diol **73** to urethane **74**

Another convenient method for the construction of the carbamoyl group is the *McLamore et al.* method.[104] The carbamoyl group is obtained via the phenyl carbonate, itself obtained from the alcohol and phenyl chloroformate. On reaction with ammonia,[105] the phenyl carbonate is cleaved to furnish the urethane. The introduction of the carbamoyl group with *p*-nitrophenylchloroformate and subsequent aminolysis with methanolic ammonia is likewise possible.[106,107,108]

An alternative method to form the urethane is the conversion of a 1,2-diol into the corresponding cyclic carbonate. The carbonate ring can then be opened with ammonia from both sides in a non-selective reaction, thus leading to two isomeric *O*-carbamoyl compounds.[106,109]

5.2.5.1 Trial based on TAI

Initial attempts to prepare the 4-*O*-carbamoyl compound were based on the prior reports stating that the reaction of the 3,4-diol grouping in a D-galactose-derived sugar with trichloroacetylisocyanate (TAI) leads regioselectively to the desired 4-*O*-carbamoyl compound.[34,44,110] The reaction was performed in dry CH_2Cl_2 using 3 Å molecular sieves, and 1 eq of TAI at -5 °C. To confirm dry conditions, compound **73** was dissolved in dry CH_2Cl_2, and the solution was refluxed over 3 Å molecular sieves before adding TAI. In spite of the high TAI reactivity, its reaction with alcohol **73** was fruitless. No reaction was observed, even using an excess amount of TAI, and performing the reaction at room temperature, up to 20 h. The result was reproducible. The reason for the low nucleophilicity of **73** in this reaction is unknown.
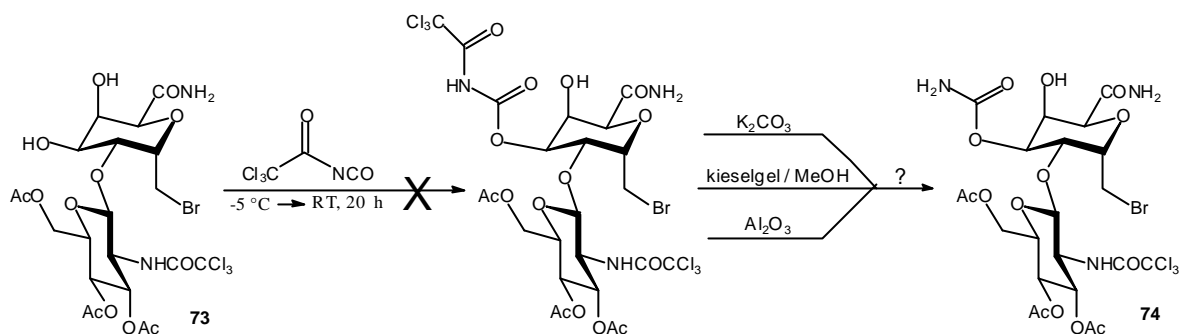
Scheme 5. 22: Trial to construct the carbamoyl group using TAI

5.2.5.2 Trial based on the phenyl carbonate 75

The alternative method of *McLamore et al.*[104] to form the urethane has been studied. Thus, compound **73** was dissolved in dry dichloromethane in the presence of DMAP, and TEA at 0 °C. Under these conditions, treatment of **73** with phenyl chloroformate furnished the undesired cyclic carbonate **76** in 72 % yield.
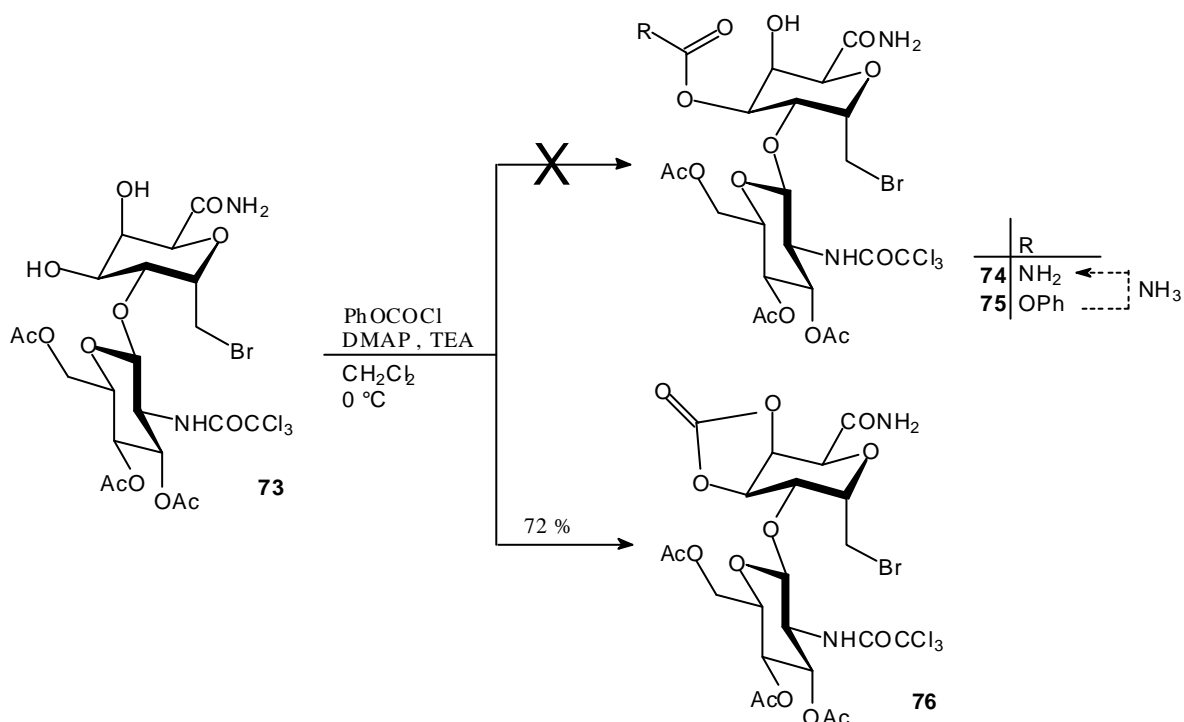
Scheme 5. 23: Trial to construct the carbamoyl group from the phenyl carbonate **75**

Formation of the five-membered ring carbonate **76** can be easily explained by the formation of **75**, followed by cyclization on reaction of the free OH group with the neighbouring phenylcarbonate.

5.2.5.3 Aminolysis of the cyclic carbonate 76

The cyclic carbonate **76** was also prepared from diol **73** on reaction with carbonyldiimidazole (CDI) in the presence of a catalytic amount of DMAP in dichloromethane. The yield was 84 %. Phosgene,[106] triphosgene[109] as well as trichloromethyl formyl chloride in pyridine[111] or in THF with collidine[112] have also been used to prepare such cyclic carbonates.

Selective opening of the cyclic carbonate was not a straightforward and the reaction was studied in some detail.
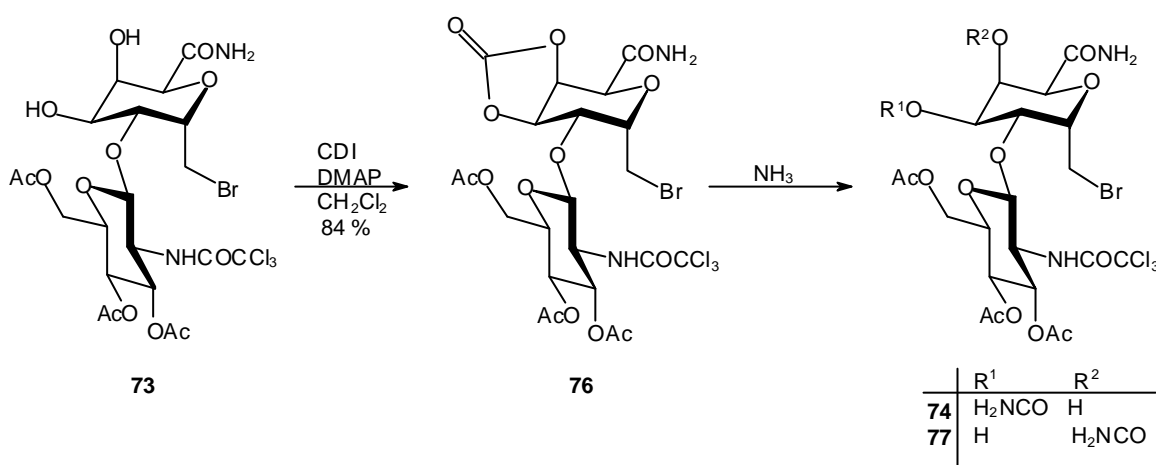
Scheme 5. 24: Aminolysis of the cyclic carbonate **76**

When gaseous ammonia was bubbled through an ethanolic solution of **76** at 0 °C for 1 h, the two isomers **74** (18 %), and **77** (20 %) were obtained. In another experiment, the reaction of **76** was performed with 0.5 M ethanolic ammonia at 0 °C for 5 h to afford **74** (3 %) and **77** (48 %). This result may reflect the greater lability of the equatorial urethane towards nucleophilic attack. When the reaction was run in dichloromethane solution (gaseous ammonia, 0 °C, 6 h), **74** was produced in a satisfying yield of 62 % alongside with 21 % of the unwanted isomer **77**.

These results are summarized in Table 5.1.

Table 5. 1: Results of ring opening of **76** with ammonia

Reaction code	Conditions	Yield (%)	
		74	**77**
AJ 2-35	1) EtOH, 0 °C	18	20
	2) NH$_3$ (g), 1 h		
AJ 2-46	0.5 M ethanolic	3	48
	ammonia, 0 °C, 5h		
AJ 2-45	1) CH$_2$Cl$_2$, 0 °C	<u>62</u>	21
	2) NH$_3$ (g), 6 h		

Separation of the two isomers was difficult and time consuming. Flash chromatography as well as reversed-phase chromatography were unsuccessful. They were separated by MPLC with difficulty. Both isomers were fully characterized by 2-dimensional NMR including, COSY, HMBC and HMQC. The 3- and the 4-*O*-carbamoyl derivatives were readily distinguishable. The 4-H signal of the 4-*O*-carbamoyl derivative **74** was observed at δ 5.68 as dd with coupling constants of J = 3.5 and 8.3 Hz, as expected for an axial proton. In contrast, the 3-H signal of the 3-*O*-carbamoyl derivative **77** was observed at δ 6.47 as a broad singlet, as expected for an equatorial proton.

5.2.6 Formation of the acetamide

The next step in the synthesis was the conversion of the N-trichloroacetyl group into an N-acetyl group. This can be achieved either by dehalogenation or by a sequence of amide cleavage and acetylation. Dehalogenation of chloro-substituted acetamides has been achieved with tributyltin hydride, Bu$_3$SnH,[61,113,114] in a radical reaction using AIBN[115] as initiator. (Me$_3$Si)$_3$SiH[116] can be used instead of Bu$_3$SnH. The reductive dehalogenation can also be accomplished by activated zinc-acetic acid in THF,[117] or activated Zn-Cu couple in acetic acid.[33,118]
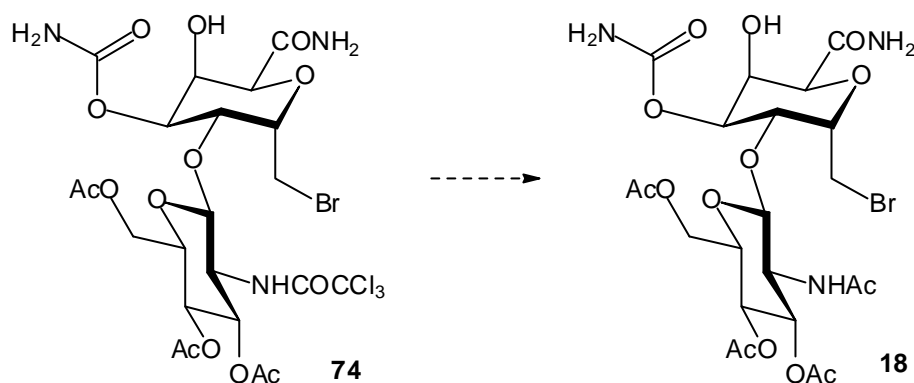
Scheme 5. 25: Dehalogenation of the N-trichloroacetyl group

On the other hand, N-trichloro- and N-trifluoro-acetyl groups have been removed to give the free amine on reaction with $NaBH_4$ in EtOH at 60 °C[119,120] via the hemiaminal, or hydrolytically on reaction with LiOH in MeOH-THF.[121]

To avoid the danger of harming the bromomethyl group, radical reactions seemed not appropriate and we preferred to use the second type of reactions.

Occasionally, hydrolysis of trifluoroacetamides was achieved by treatment with anhydrous methanolic ammonia.[122] This route seemed unsuitable for us, since as reported above, the trichloacetamidate group of **72** (Scheme 5.19), **74** and **77** (Scheme 5.24) was quite stable towards ammonia.

5.2.6.1 Removal of the trichloroacetyl group in compound 76

5.2.6.1.1 Hydrolysis with LiOH

Cyclic carbonates are stable towards acidic hydrolysis and were reported to be more stable to basic hydrolysis than acyclic esters.[123] Therefore, the hydrolysis of the N-trichloroacetamido group in compound **76** with LiOH has been studied (Scheme 5.26). Compound **76** was allowed to react with a solution of 0.5 M LiOH in MeOH-THF (1:1) at RT for ca 2 h. The crude product was then acetylated with Ac_2O in dry pyridine. Unfortunately, the reaction did not proceed as expected; instead of **78**, **80** was the sole product (73 %).
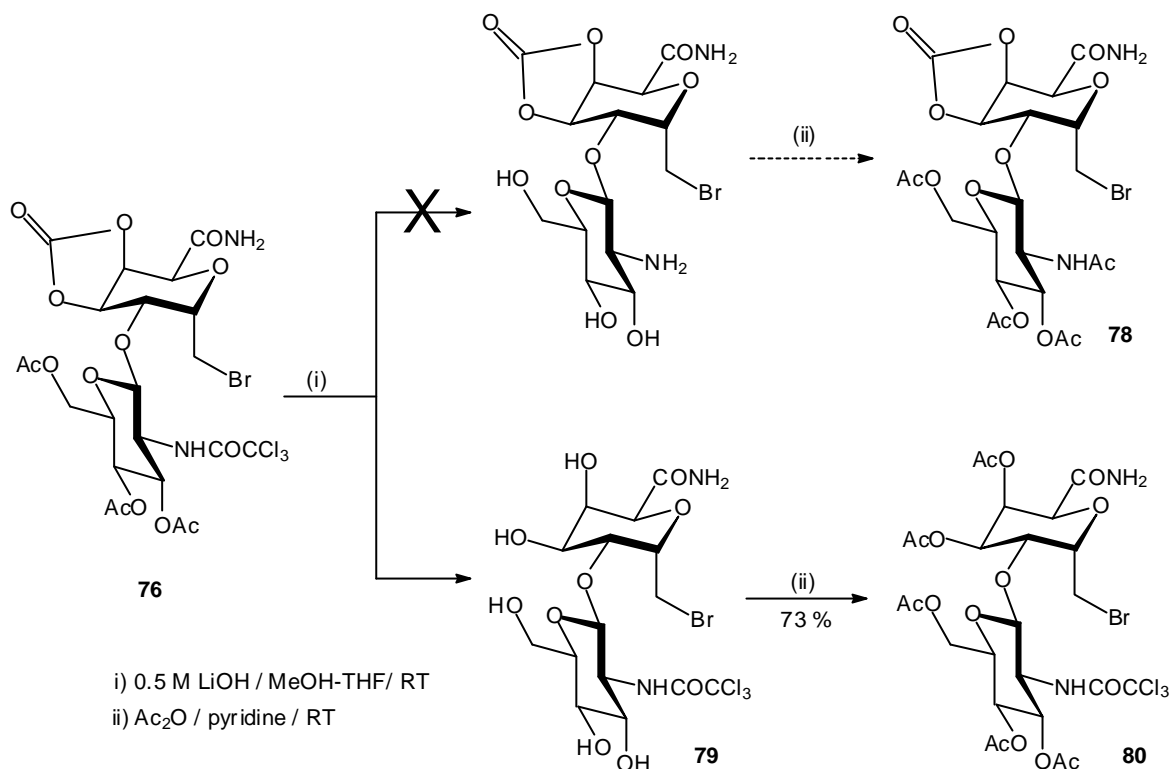
Scheme 5. 26: Hydrolysis of **76** with LiOH and subsequent acetylation

5.2.6.1.2 Reduction with NaBH$_4$

Reductive cleavage of N-trifluoro- and N-trichloro-acetyl groups with sodium borohydride into amines and 2,2,2-trihaloethanols should proceed according to *Weygand:*[119]

$$R\text{-}NH\text{-}COCX_3 \xrightarrow[\text{EtOH}]{\text{NaBH}_4} R\text{-}NH_2 + HO\text{-}CH_2CX_3 \ (X=F, Cl)$$
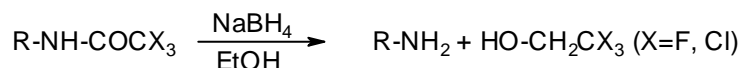
The reaction is stopped by adding acetic acid. The free amine can then be acetylated to furnish the acetamide.

Conversion of the N-trichloroacetyl group in compound **76** into the N-acetyl group was accomplished by the two-step procedure (Scheme 5.27). Reduction of **76** with 1 eq of NaBH$_4$ in absolute ethanol was performed at 60 °C for 8 h. The crude product was then acetylated with Ac$_2$O in dry pyridine. Under these conditions, the N-trichloroacetyl group was successfully converted into the N-acetyl group, but with an undesired interaction of the cyclic carbonate group of unit F.
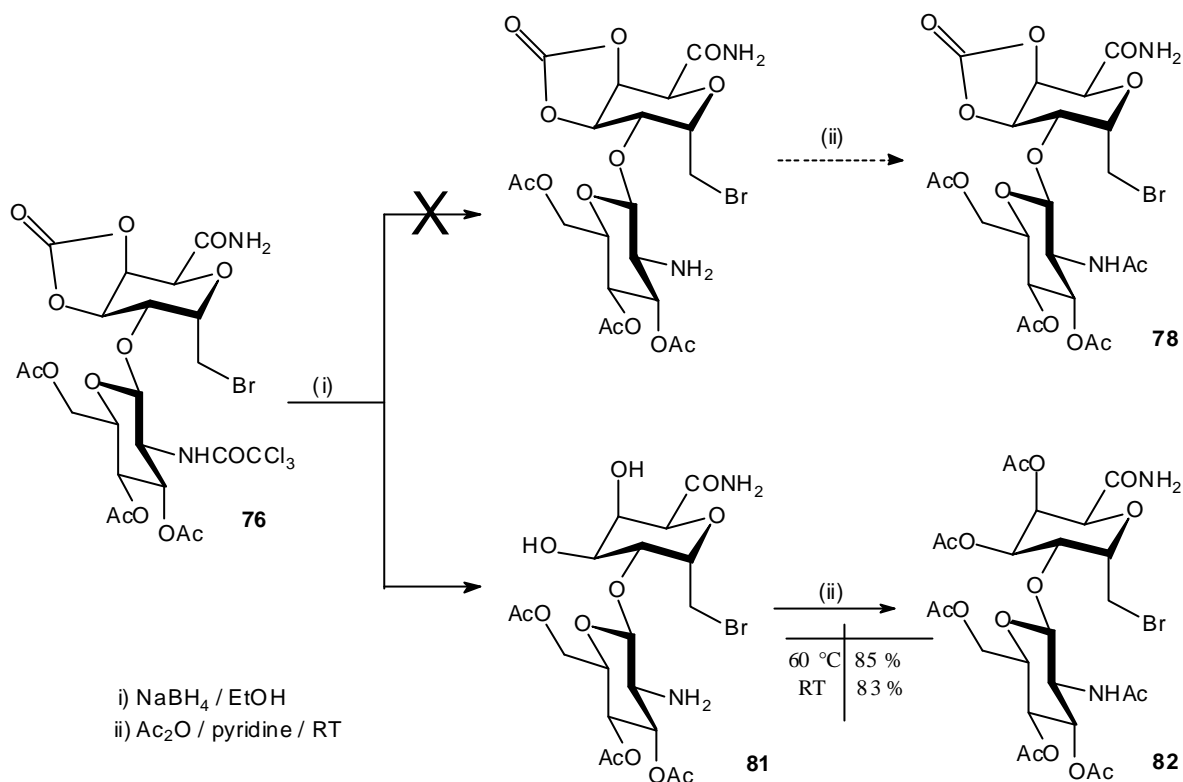
Scheme 5. 27: Reduction of **76** with NaBH$_4$ and subsequent acetylation

Obviously, the carbonate is so base-labile under the reaction conditions that it was cleaved under the reaction conditions. Acetylation of both the amino and hydroxy groups in **81** furnished then **82** in 85 % yield.

Changing the reaction temperature did not affect the result. No reaction was observed at 0 °C for 15 h using 1 eq of NaBH$_4$ in dry ethanol. When the reaction has been performed at RT for 10 h, again **82** was obtained in 83 % yield.

The product **82** is not a suitable precursor for structural analogues of monoemycins; since it seems difficult to hydrolyse selectively the acetate group at C-4^F to introduce the carbamoyl group which is necessary for the biological activity.

An alternative pathway had to be found which is the reduction of the N-trichloroacetyl group in compound **74**.

5.2.6.2 Reduction of the N-trichloroacetyl group in compound 74 with NaBH₄

Compound **74** has been allowed to react with 1 eq NaBH$_4$ in absolute ethanol at 60 °C for ca 9 h. The crude product was then acetylated using Ac$_2$O in dry pyridine.

Under these conditions, the N-trichloroacetyl group was converted into the N-acetyl group, but with the unwanted interaction of the carbamoyl group of unit F. Thus, instead of the desired product **83**, again compound **82** was obtained in 78 % yield. The result demonstrates the base-sensitivity of the urethane grouping.
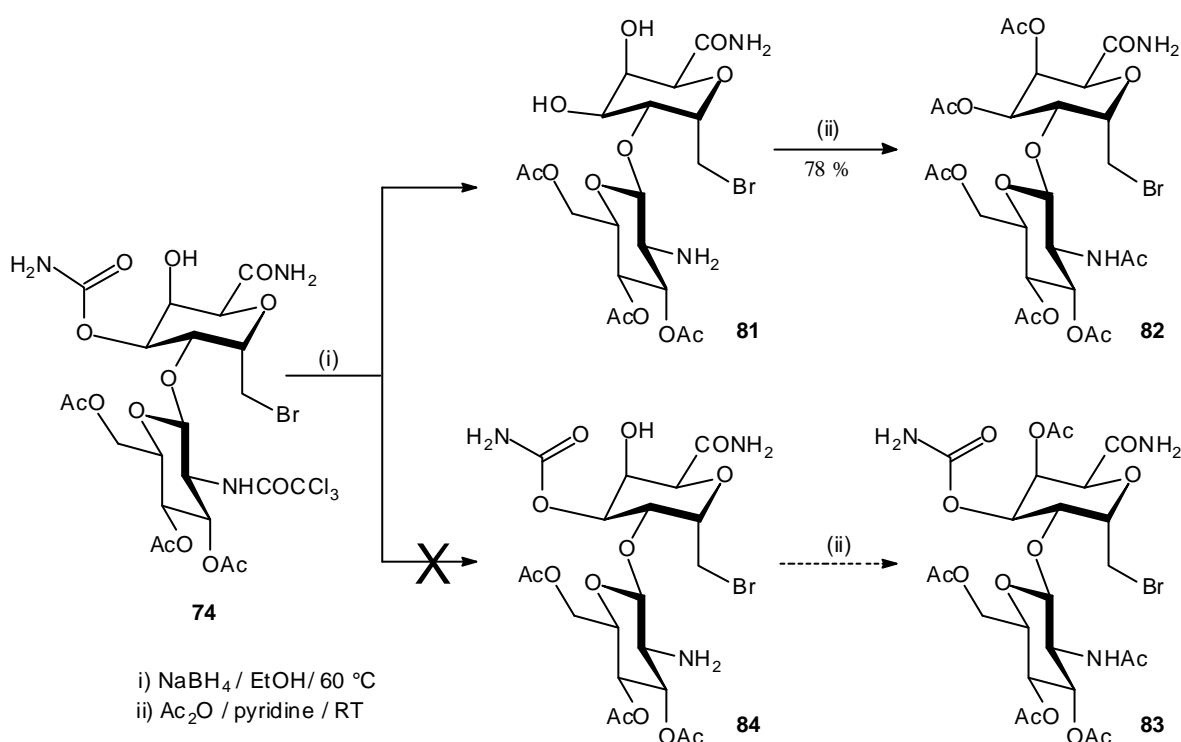
Scheme 5. 28: Reaction of compound **74** with NaBH$_4$ at 60 °C

Many factors as the molar ratio of NaBH$_4$ to **74**, dryness conditions, reaction temperature, reaction time and the reaction solvent have been taken into account. All the reactions were performed using a 1 molar ratio of NaBH$_4$ to **74**. The solvents as well as the acetic acid which was used to stop the reaction were freshly distilled. There was no observed reaction at 0 °C, even by leaving the reaction mixture for 48 h.

However, in one experiment (AJ 2-41), the N-trichloroacetyl group was successfully converted at RT (13 h) to the N-acetyl group, giving compound **83** in 42 % yield, in which the carbamoyl group is still present, and the *O*-3 hydroxyl group in unit F is acetylated. But again the fully acetylated product **82** was formed as well (46 % yield).
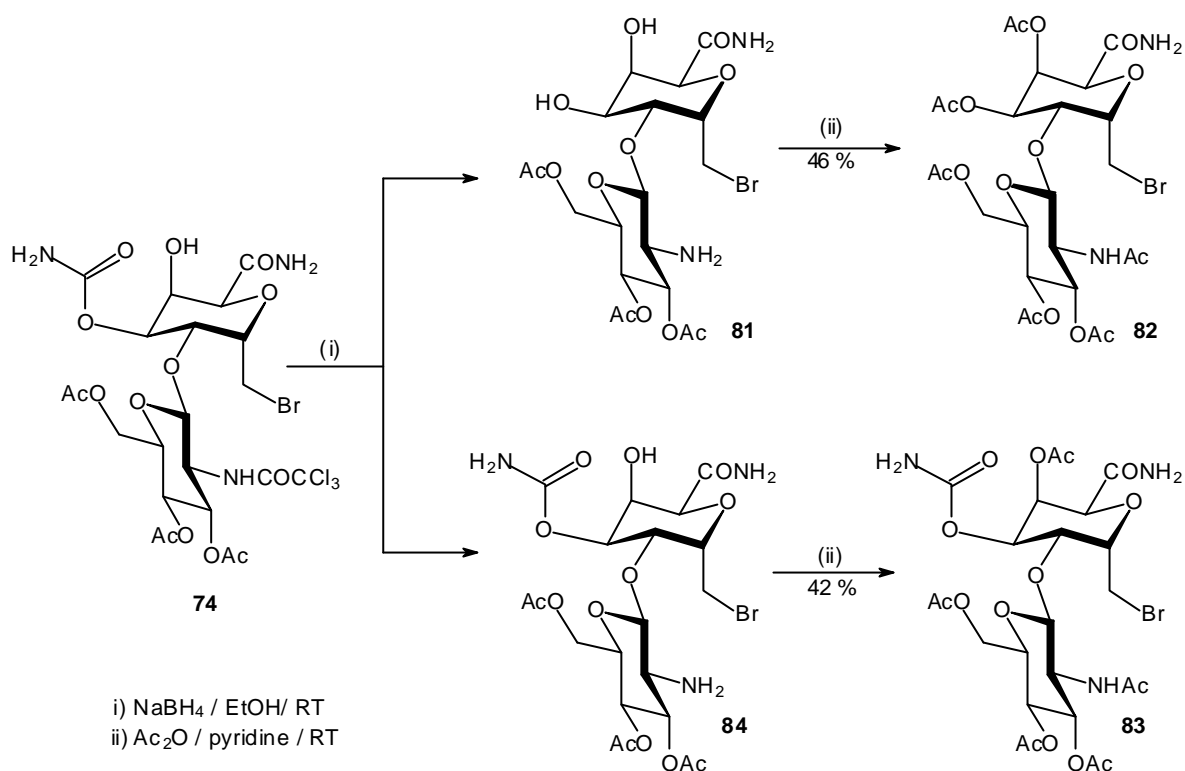
Scheme 5. 29: Formation of the N-acetyl group at RT

Unfortunately, the result could not be reproduced. Obviously, the carbamoyl group is so base-labile that the conditions of the NaBH$_4$ reduction are unsuitable for the selective removal of the N-trichloroacetyl group.

Upon repeating the reaction under conditions which were considered to be identical with the above conditions, even the carbamoyl group was removed preferentially furnishing **80** (65 % yield). The reaction was further studied in dry isopropanol at RT for 17 h, and in a mixture of dry THF-MeOH (4:1) at 5 °C for 19 h, and afforded only **80** in 73 % and 68 % yield, respectively.
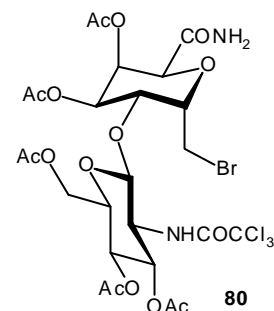
Figure 5. 4: Compound **80**

It should be mentioned here that in the course of the synthesis of moenomycin analogues, the introduction of the carbamoyl group in the case of 3,4-diol grouping in a D-galacto-derived unit F has been achieved getting benefit of the methods[34,44,110] which failed to give the urethane in this work (Schemes 5.22, 5.23). Worth mentioning is that the carbamoyl group survives the hydrolysis of the acetyl groups with LiOH in the last step of the synthesis.

It is possible that the transition from *O*-glycoside to *C*-glycoside results in problems in introducing the carbamoyl group and in its high base sensitivity.

Reduction of the N-trichloroacetyl group avoiding the undesired interaction of the other base sensitive groups under milder conditions led us to investigate the use of sodium cyanoborohydride. However, trials to reduce the N-trichloroacetyl group in **80** as a model substance by NaBH$_3$CN in the presence of HCl-ether were fruitless. **80** was conducted with 1 eq NaBH$_3$CN catalysed with HCl-dry ether. TLC showed a product formation, and the product of this step was acetylated. The reaction should give **82**, but the spectral data of the acetylated product were in accordance with those of **80**. Such a result may be explained by a possible cleavage of acetyl group(s) during the reduction step, followed by acetylation of the free hydroxyl group(s) thus recovering the starting material.

5.2.7 Phosphonate formation

Applying the *Arbuzov* reaction,[56] compound **82** was refluxed with triethylphosphite under an argon atmosphere, affording the expected product **85** (70 %).
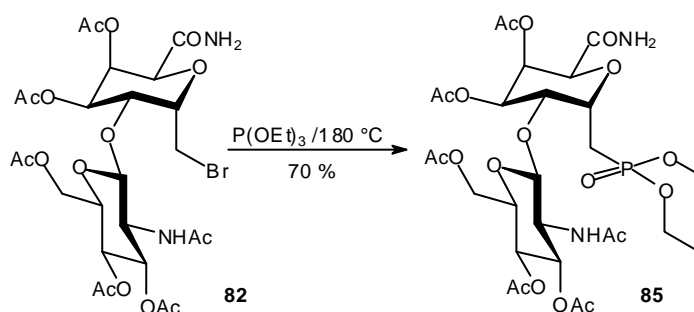
Scheme 5. 30: Phosphonate formation of compound **85**

The phosphite was stirred with 3 Å molecular sieves, and degassed under argon atmosphere for 1 h before use.

5.2.8 Conclusion and suggestions for further work

In conclusion, we were able to convert β-D-galactose-pentaacetate into galactoheptonamides. In this work, we developed the method to obtain the first *C*-glycosidic disaccharide analogues of moenomycin A$_{12}$ with all the substituents on unit F that fulfil the requirements of the moenomycin A$_{12}$ biological activity. This method can be used to prepare other analogues, as will be discussed in the coming section.

However, the synthetic method employed in this part warrants further investigation toward obtaining suitable conditions for the conversion of the N-trichloroacetyl group in compound **74**

into an N-acetyl group, which will enable us to apply the *Arbuzov* reaction, giving a compound that should lead to the target molecule.

5.3 Synthesis of the trisaccharide building block 19

5.3.1 Trials to prepare 87 based on oxazoline 42

Chitobiose octaacetate **86** can be prepared in moderate yield from chitin by microbial degradation followed by acetylation, or by chemical degradation.[62] The conversion of **86** into oxazoline **42** can be achieved with many reagents,[62] the best yield was obtained using trimethylsilyl trifluoromethanesulfonate-triethylamine.[124]
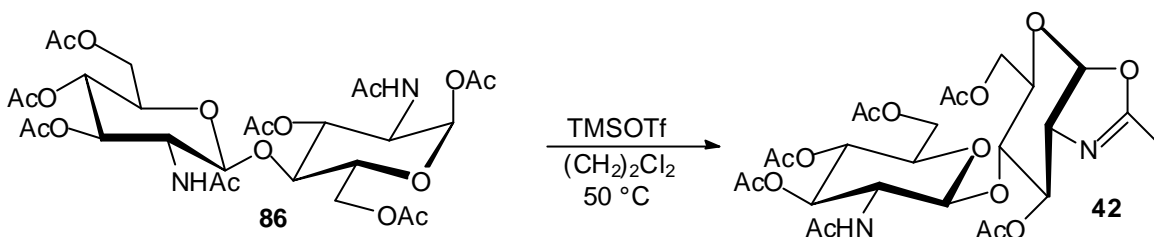
Scheme 5. 31: Synthesis of the oxazoline **42**

42 has been used as a key intermediate for the preparation of various 2-acetamido-2-deoxy-â-D-glycosides.[62] In trifluoromethanesulfonic acid (TfOH)[62] as well as camphorsulfonic acid (CSA)[46] promoted couplings, the yields were usually moderate.

The glycosylation reaction of **42** with the glycosyl acceptor **53** was intensely studied varying reaction times, reaction temperatures, and acceptor-donor ratios. When camphorsulfonic acid and trimethylsilyl trifluoromethanesulfonate (TMSOTf) were used as promoters, there was no reaction, and the glycosyl donor was recovered. In the case of trifluoromethanesulfonic acid, decomposition products were obtained as shown by TLC and NMR. Clearly the nucleophilicity of **53** is too low for the reaction with the notoriously unreactive oxazoline **42**.[57]
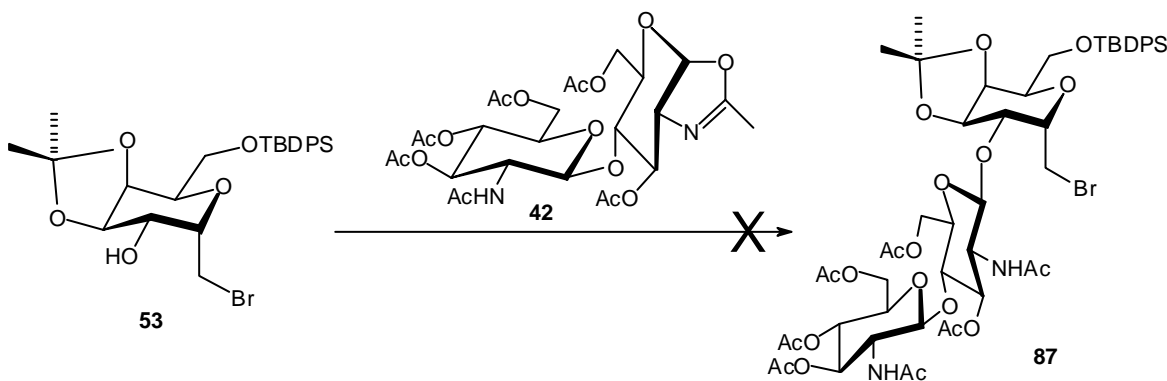
Scheme 5. 32: Glycosylation trial based on the oxazoline **42**

5.3.2 Synthesis based on the trichloroacetamidate donor 88

5.3.2.1 Synthesis of the donor 88

Compound **88** has recently been prepared in our group,[125] and has successfully been used as a glycosyl donor. It generates units C and E in the target product, while the acceptor represents unit F.
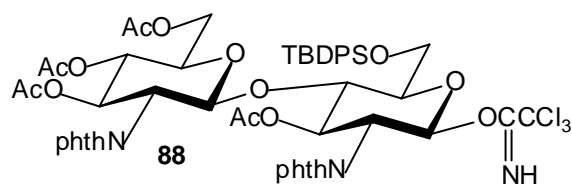
Figure 5. 5: Glycosyl donor **88**

88 was prepared in 14 steps from glucosamine hydrochloride **32** as shown in the following Scheme.
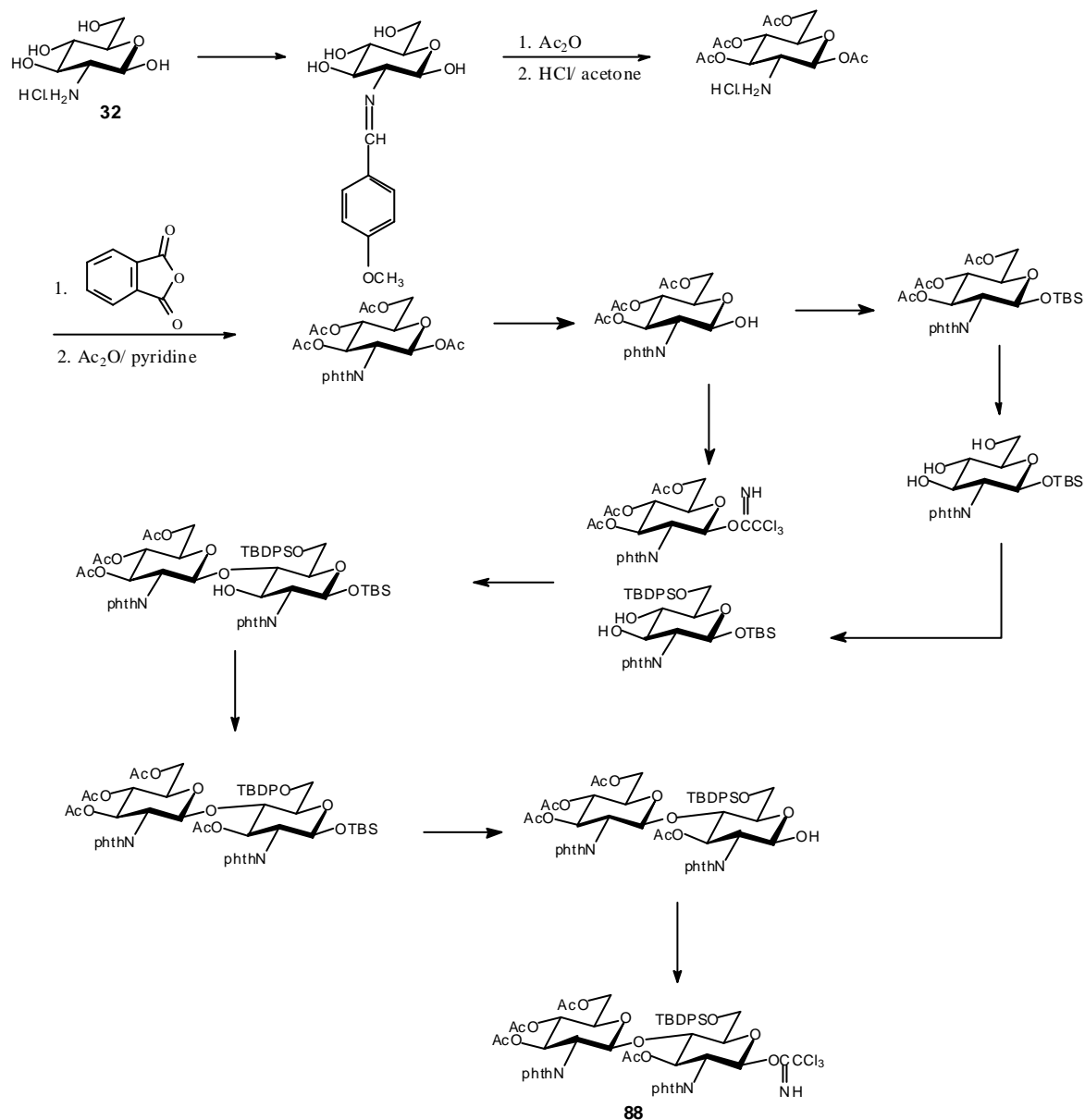
Scheme 5. 33: Synthesis of the donor **88**

88 is more reactive than the oxazoline derivative **42**. It survives the glycosylation conditions with a lower degree of decomposition and couples with the accessible glycosyl acceptor in moderate yields. The 2-phthalimido group directs the attack predominantly to the â face and prevents the formation of 1,2-oxazolines during the glycosylation.[60a,126] However, removal of the phthalimido group is often connected with problems.[126]

One of the primary hydroxyl groups in this donor is protected as the *t*-butyldiphenylsilyl ether, the same protecting group for the primary hydroxyl group in acceptor **53**, that we used for the synthesis of the disaccharide **68** (Scheme 5.15).

This would lead to a trisaccharide with two identical protecting groups at the primary hydroxyl groups which would have to be differentiated (as in Scheme 5.39) in the course of the synthesis of the uronamide **93**. In order to avoid deprotection complications we decided to use another glycosyl acceptor.
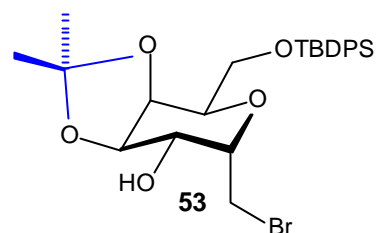
Figure 5. 6: Glycosyl acceptor **53**

5.3.2.2 Synthesis of the glycosyl acceptor 92

As discussed in section 1.5, the amide group at position 1 in unit F is essential for the biological activity. Therefore, acceptor **53** was converted into galacturanamide derivative **92**, which would be used as an alternative glycosyl acceptor. The conversion of **53** into **92** is shown in the following scheme.
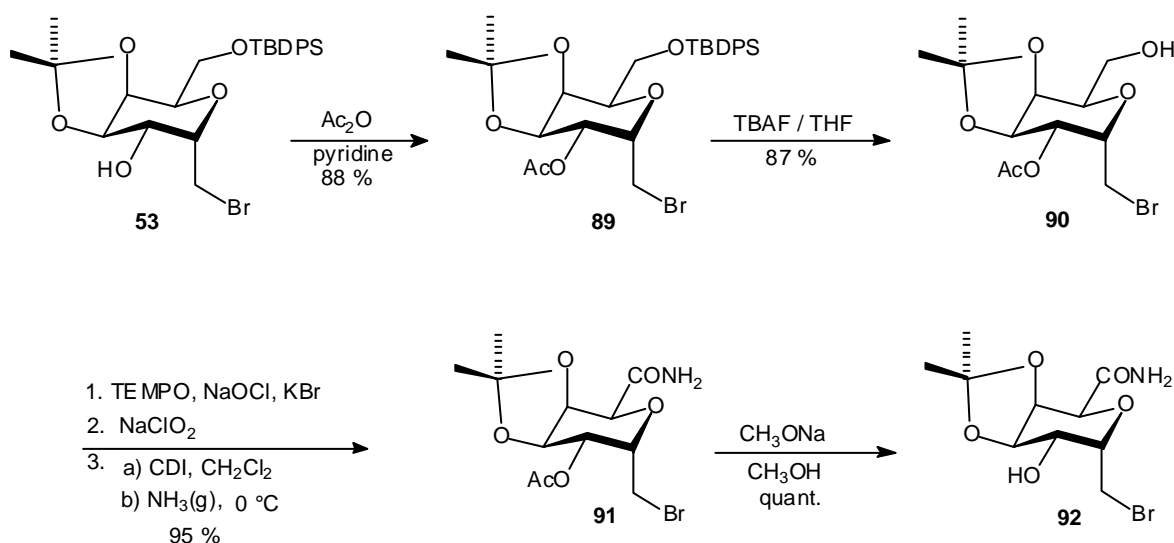
Scheme 5. 34: Synthesis of the acceptor **92**

The free hydroxyl group in compound **53** was protected as an acetate group on reaction with acetic anhydride in pyridine in the presence of DMAP. Compound **89** was obtained in 88 % yield. The silyl ether in **89** was cleaved with TBAF, as previously discussed, affording compound **90** in 87 % yield. The free hydroxyl group was then subjected to an oxidation using the TEMPO method affording the aldehyde which was in turn oxidised to the corresponding acid. The acid was converted to the amide **91**, making use of *Staab's* method, in an overall yield of 95 %. The required glycosyl acceptor **92** was obtained in quantitative yield by cleavage of the ester bond at position 5 under *Zemplén* conditions.

5.3.2.3 Glycosylation trial based on acceptor 92

It was hoped that the glycosylation reaction would take place according to Scheme 5.35 giving trisaccharide **93**, a precursor of the desired synthetic intermediate **19**.
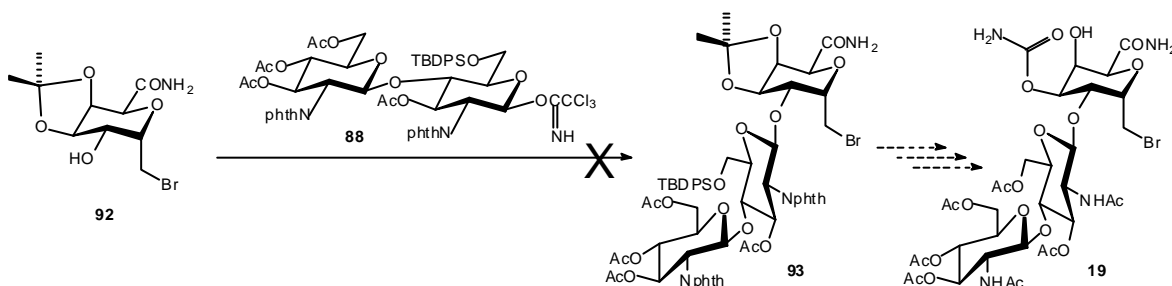
Scheme 5. 35: Attempted trisaccharide formation based on acceptor **92**

Unfortunately, the coupling reaction between acceptor **92** and donor **88** was unsuccessful. The reactants were submitted to glycosylation conditions that have been used for the disaccharide formation. TMSOTf and 3 Å molecular sieves in 1,2-dichloroethane and a mixture of 1,2-dichloroethane-ether were used. The reaction time, the reaction temperature and the molar ratios of acceptor-donor were varied, with no change in the result. TLC showed complete decomposition of the donor.

The reason is probably the low nucleophilicity of acceptor **92**, arising from the presence of the electron-withdrawing amide group. To confirm this assumption we allowed **92** to react with donor **67**. This glycosylation or even the inverse glycosylation reactions should give the previously prepared **72** (see Scheme 5.19).
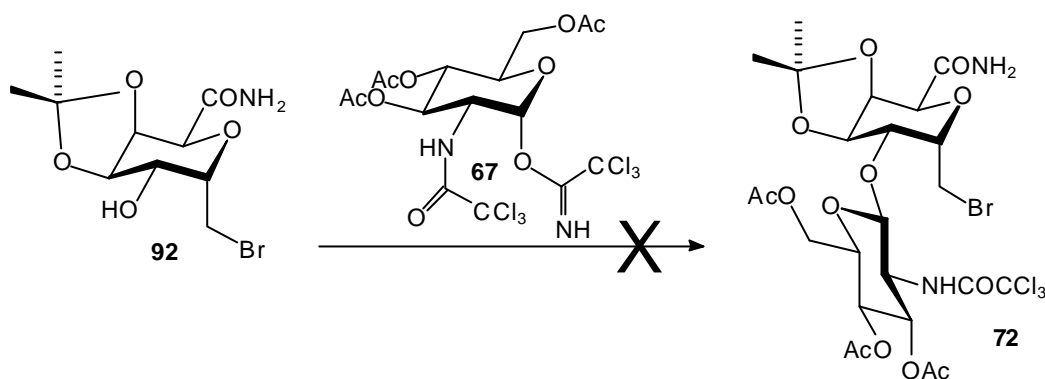
Scheme 5. 36: Attempted disaccharide formation based on acceptor **92**

Again, there was no reaction between **92** and **67**. Only decomposition of the donor **67** was observed confirming the low reactivity of **92**.

Thus, we had to look for another acceptor more reactive than the amide, in which the primary hydroxyl group is protected with a protecting group different from TBDPS. The TBDMS group was selected for this purpose.

5.3.2.4 Synthesis of the glycosyl acceptor 95

Compound **62** was converted in 87 % yield into its silyl ether derivative **94** upon reaction with *t*-butyldimethylsilyl chloride (TBDMSCl) at 0 °C in the presence of imidazole in dry DMF. Compound **94** has been subjected to acetonide formation, thus protecting the free hydroxyl groups at positions 3 and 4 furnishing the new acceptor **95** (90 %).
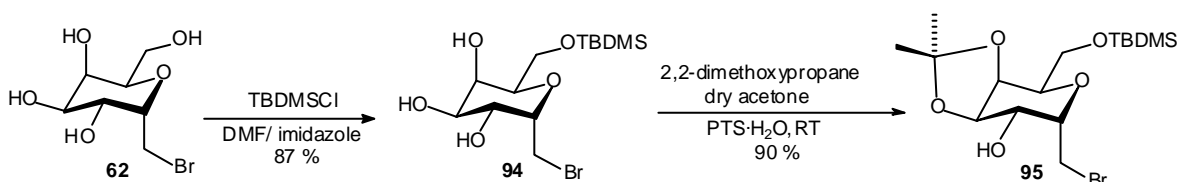
Scheme 5. 37: Synthesis of acceptor **95**

5.3.2.5 Glycosylation based on acceptor 95

The glycosidation reaction of donor **88** and the new acceptor **95** is shown in Scheme 5.38. The donor and a slight excess of the acceptor (1.2 eq) were dissolved in dry 1,2-dichloroethane containing 3 Å molecular sieves. Under the normal glycosylation conditions (TMSOTf-TEA, 0 °C), compound **96** was obtained in 10 % yield after chromatography. However, using low temperatures (-30 °C) the yield of the coupling reaction rose to 50 %, The reaction was accompanied by donor decomposition.
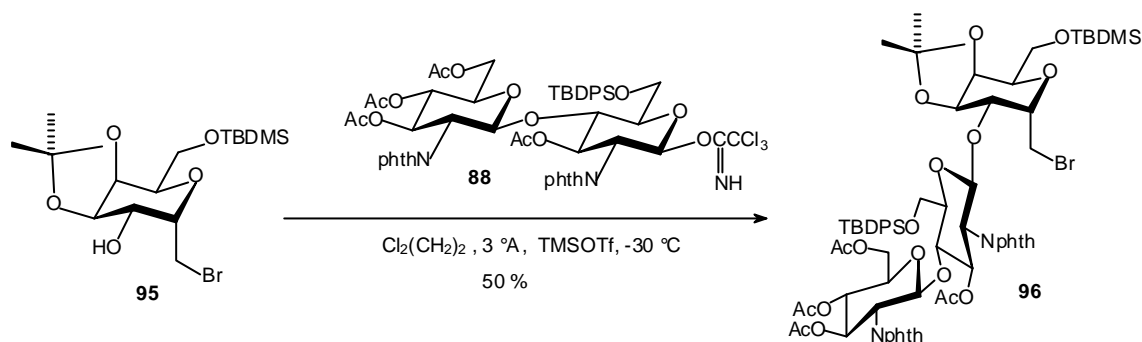
Scheme 5. 38: Synthesis of the trisaccharide **96**

The required reaction time to furnish the main product in a satisfying yield is not longer than 5 min. It was noticed by TLC that on longer reaction times decomposition occurs to give many products, and the desired trisaccharide is obtained in low yields. Such behaviour reflects the high reactivity of the glycosyl donor **88**.

5.3.3 Cleavage of the TBDMS group in 96

Selective deprotection of the TBDMS group in compound **96** was accomplished with TBAF at low temperatures. Thus, compound **96** was subjected to 1 eq of TBAF (1 M in THF) at -10 °C, and stirred at this temperature for ca 6 h. The solution was washed with water, extracted with chloroform, and evaporated. The residue which should be **97** was taken to the next reaction without further purification.
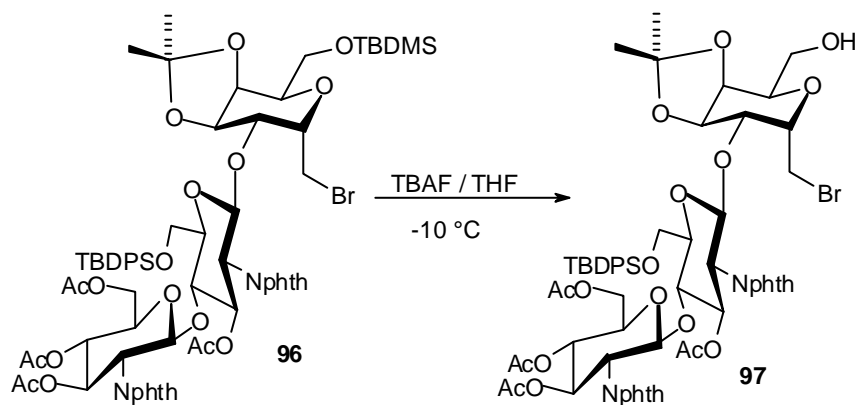
Scheme 5. 39: Selective cleavage of the TBDMS group in **96**

5.3.4 Synthesis of uronamide 93

The free hydroxyl group of **97** was subjected to an oxidation using the TEMPO method affording the aldehyde. After oxidation of the aldehyde with sodium chlorite, the corresponding acid was

converted according to *Staab's* method into the amide **93** in an overall yield of 95 % (based on **96**).
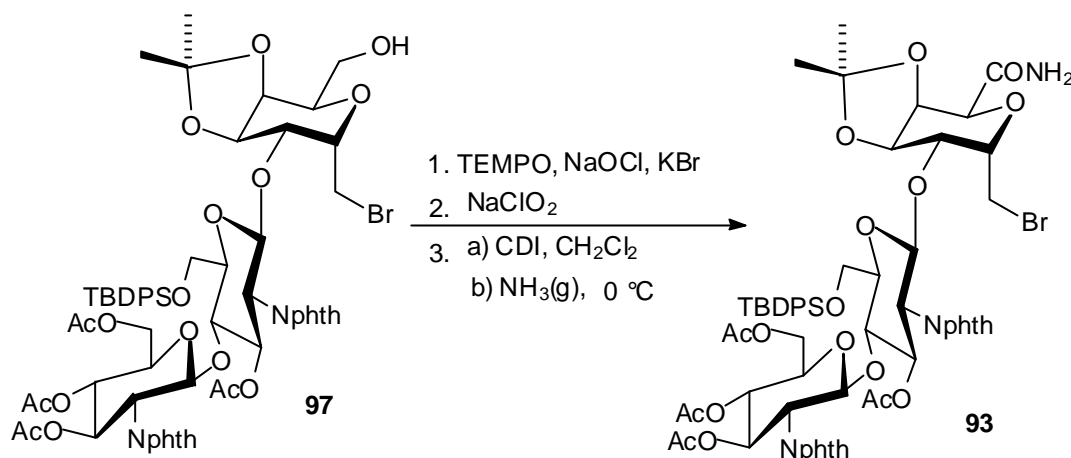
Scheme 5. 40: TEMPO, sodium chlorite oxidation and amide formation

5.3.5 Synthesis trial of 103

Cleavage of the TBDPS group, cleavage of the phthalimido group and acetylation of the hydroxyl groups as well as the primary amines are required to convert **93** into **103** (Scheme 5.41). The first step in this synthesis could be the cleavage of the TBDPS group in compound **93**. This could be accomplished with a molar solution of TBAF in THF at room temperature to give **98**. After work up, the residue was taken to the next reaction without further purification. The next step should be the cleavage of the phthalimido group in the crude product **98** which could be achieved by heating it with ethylenediamine in *n*-butanol. When the reaction was complete, the solvents were coevaporated with toluene and the residue was allowed to react with acetic anhydride in dry pyridine in the presence of a catalytic amount of DMAP. After chromatography, the overall yield of the separated product was 49 % (based on **93**). Unfortunately, the reaction did not proceed as wished. Instead of **103**, compound **102** with an elimination of HBr was obtained. HBr should be eliminated during the cleavage of the phthalimido group, since in an another experiment, leaving the TBDPS group at the moment, cleavage of the phthalimido group in **93** followed by acetylation led again to HBr elimination, thus giving **101** (58 %, based on **93**) instead of the expected compound **100**. This result may reflect the greater lability of the bromide group under the conditions that are required for the cleavage of the phthalimido group.
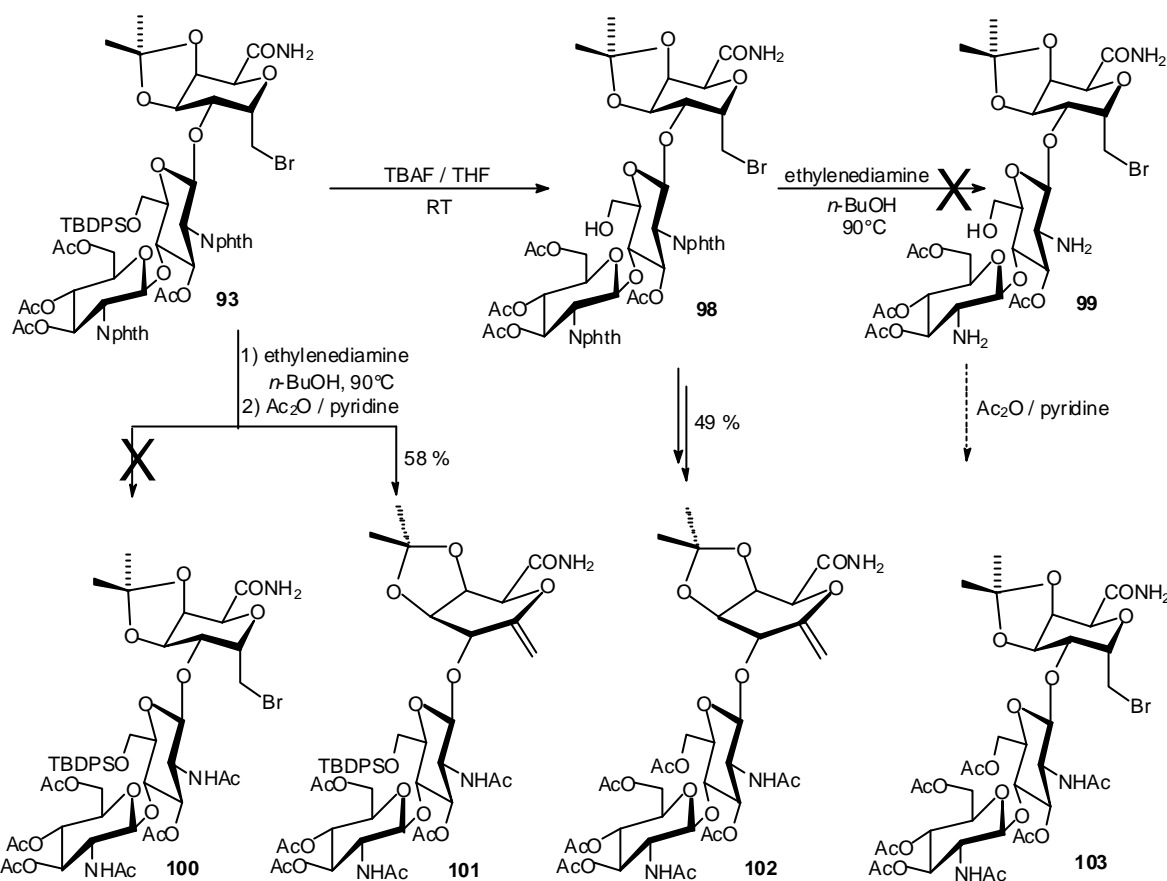
Scheme 5. 41: Trials to prepare **100** and **103**

5.3.6 Conclusion and suggestions for further work

In conclusion we were able to synthesize the C‑glycosidic trisaccharide analogues of moenomycin A_{12}, but we faced difficulty in removing the phthalimido group. This reaction warrants further investigation toward looking for the suitable reagent that does not harm the bromine, since this group seems to be an important precursor for applying the *Arbuzov* reaction, or even looking for another N‑protecting groups in the donor **88**.

We notice that in both di- and trisaccharides, the presence of the bromide limits us to find the suitable reagents for the removal of the N‑protecting groups. We suggest to protect the hydroxyl group in compound **35** rather than converting it into its corresponding halide derivative, and to follow the same sequence of reactions discussed previously. After conversion of the N‑protecting group into the desired N‑acetyl group, Br
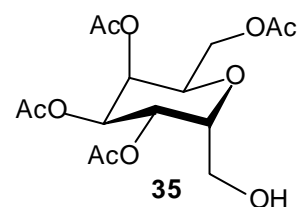
Figure 5.7: Compound **35**

could be introduced.

6 SUMMARY

The moenomycin-type compounds are known to inhibit selectively the enzyme *penicillin binding protein 1b* (PBP 1b) that catalyses the transglycosylation reaction in the biosynthesis of bacterial cell wall peptidoglycan. The moenomycins (see moenomycin A_{12}) have been shown to interfere with this biosynthetic step interacting with the enzyme(s).
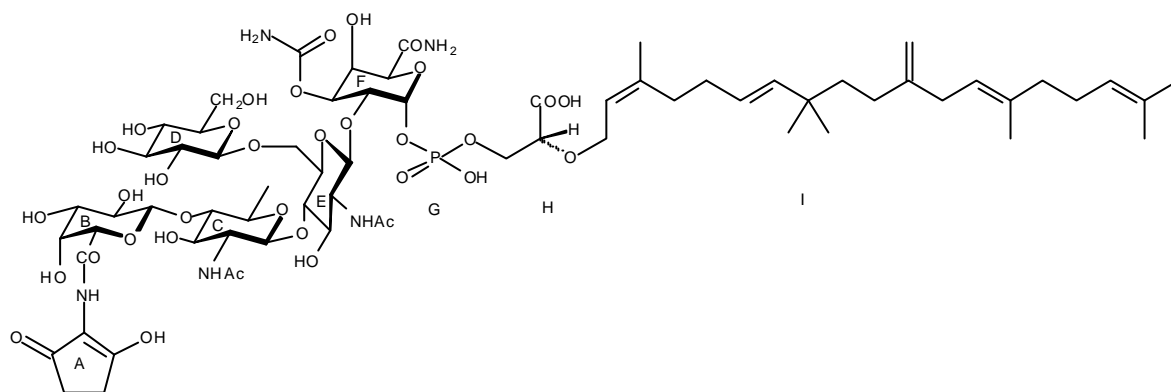
Figure 6.1: Moenomycin A_{12}

The moenomycins do not induce resistance readily. A weak point in this respect may, however, be the phosphate bond to unit F. Its cleavage by a yet poorly characterized enzyme is the only enzymatic degradation reaction of the moenomycins that is known to-date. With this in mind we started a programme aimed at synthesizing trisaccharide analogues of moenomycin A_{12} in which the phosphate oxygen at C-1 of unit F is replaced by a CH_2 group. It seemed important to retain all other functional groups in ring F as present in moenomycin since they are known to be of major importance as far as antibiotic activity is concerned.
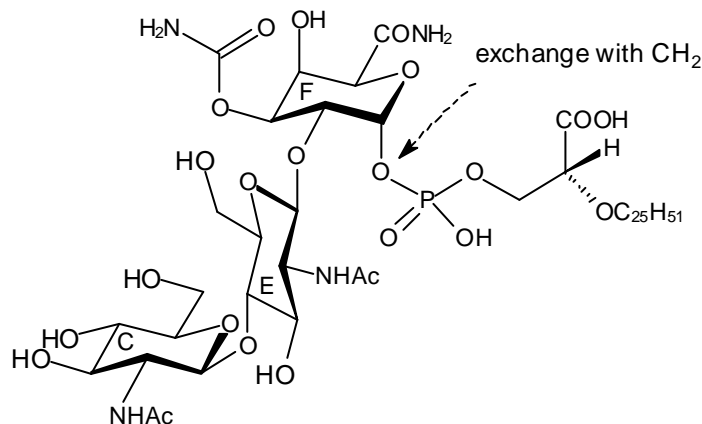
Figure 6.2: Target molecule

It appeared that the commercially available and cheap β-D-galactose-pentaacetate **30** would be an interesting starting material for this synthesis. In this work, the synthesis began with the introduction of the *C*-glycoside appendage at position 1 according to *Giannis et al.*, thus forming the allyl *C*-galactopyranoside **34**, a substance that represents the first *C*-glycosyl backbone for the synthesis of the glycosyl acceptors. The total synthesis of the glycosyl acceptors is shown in Scheme 6.1.

We wanted to convert the C-allyl glycoside **34** into its propenyl analogue. Attempts to achieve this with singlet oxygen and palladium-mediated reaction proved fruitless. On the other hand, ene reaction of **34** with 4-phenyltriazolin-3,5-dione in CH_2Cl_2 provided **56** in 83 % yield. Ozonolysis of this alkene (-70 °C, MeOH-CH_2Cl_2) and subsequent quenching with dimethyl sulfide, followed by reduction of the crude aldehyde with sodium acetoxyborohydride (prepared from $NaBH_4$ and AcOH in THF) furnished the primary alcohol **35** (85 %). This alcohol was converted into the mesylate **60** (60 %), and this in turn into the bromide **61** (80 %) by heating it at 80 °C with tetrabutylammonium bromide in toluene. The acetate groups were hydrolysed using *Zemplén* conditions to furnish **62** quantitatively. The primary hydroxyl group in **62** was protected as a $^tBuPh_2Si$ ether **63** (85 %) on reaction with TBDPSCl in DMF at 0 °C, and as a $^tBuMe_2Si$ ether **94** (87 %) on reaction with TBDMSCl in DMF at 0 °C in the presence of imidazole. PTS-catalysed isopropylidenation of the 3,4-diols **63** and **94** with 2,2-dimethoxypropane in dry acetone gave the 3,4-*O*-acetonide derivatives **53** (88 %) and **95** (90 %), respectively.

On the other hand, the glycosyl acceptor **53** was converted into the glycosyl acceptor **92**. The free hydroxyl group in compound **53** was protected as an acetate group on reaction with acetic anhydride in pyridine in the presence of DMAP giving **89** (88 %). The silyl ether in **89** was cleaved with a molar solution of TBAF in THF affording compound **90** in 87 % yield. The free hydroxyl group in **90** was then subjected to an oxidation using the TEMPO method affording the aldehyde which was in turn oxidised with sodium chlorite to the corresponding acid. The acid was converted to the amide **91**, making use of *Staab's* method, in which the acid was activated with CDI in dichloromethane to give the imidazolide, which upon reaction with ammonia furnished the amide **91** in an overall yield of 95 %. The required glycosyl acceptor **92** was obtained in quantitative yield by cleavage of the ester bond at position 5 under *Zemplén* conditions.
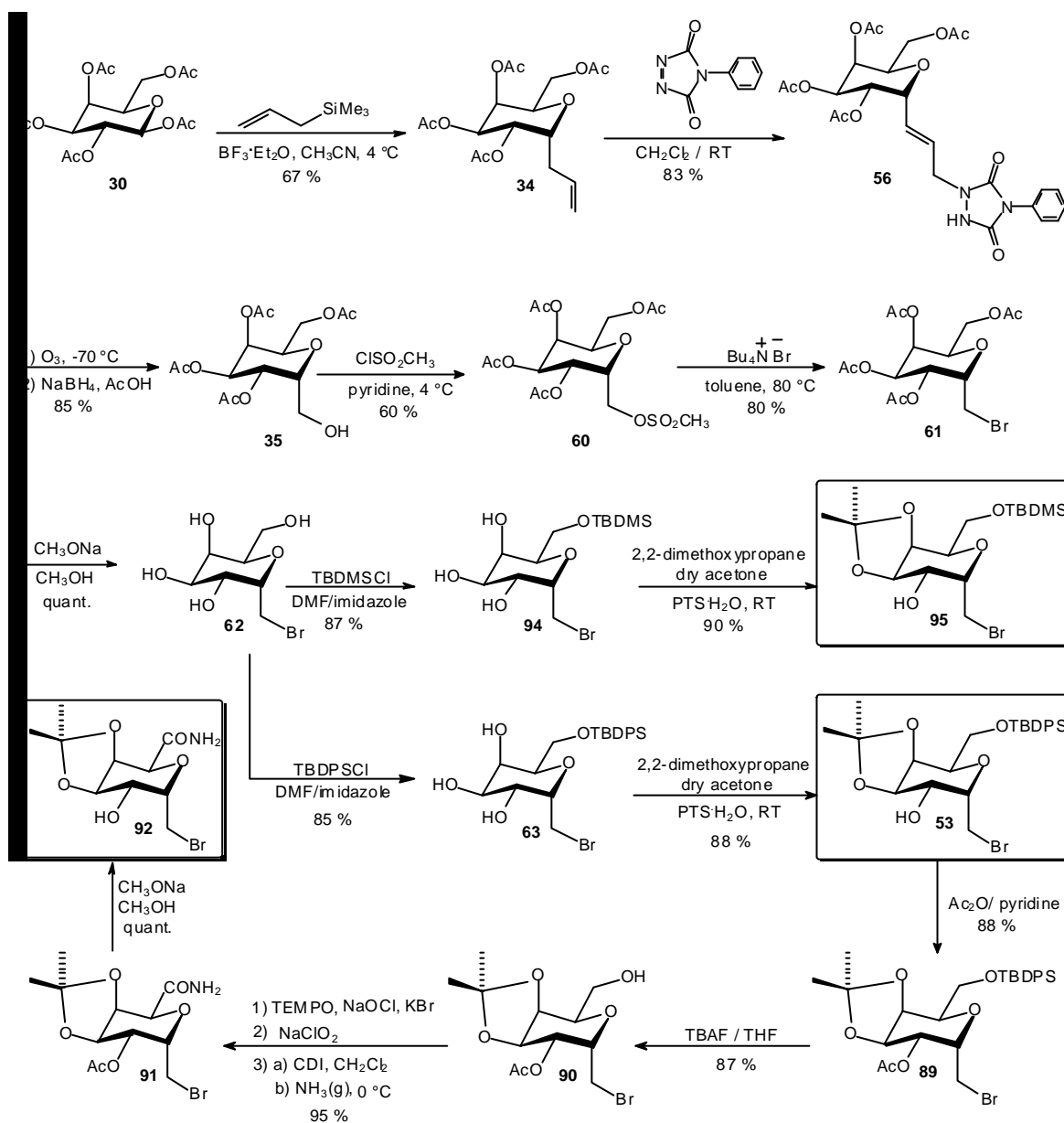
Scheme 6.1: Synthesis of the glycosyl acceptors

Disaccharide formation was achieved employing the *Jacquinet* and *Blatter* method, which involves the use of glycosyl donor **67** and TMSOTf. No reaction was observed between this donor and acceptor **92**, which may reflect the low nucleophilicity of the acceptor. On the contrary, glycosylation with acceptor **53** gave **68** (79 %). Deprotection of the silyl group in the disaccharide **68** was easily accomplished on treatment with a molar solution of TBAF in THF at RT affording **71** (89 %). Synthesis of the uronamide **72** was achieved after three major steps, in an overall yield of 98 %. Oxidation of the primary hydroxyl group in unit F to the corresponding aldehyde was accomplished with sodium hypochlorite and TEMPO. Oxidation of the crude aldehyde to the carboxylic acid with sodium chlorite followed by amide formation according to

Staab gave **72**. Removal of the isopropylidene group from **72** with trifluoroacetic acid (TFA) at RT furnished the diol **73** (89 %).
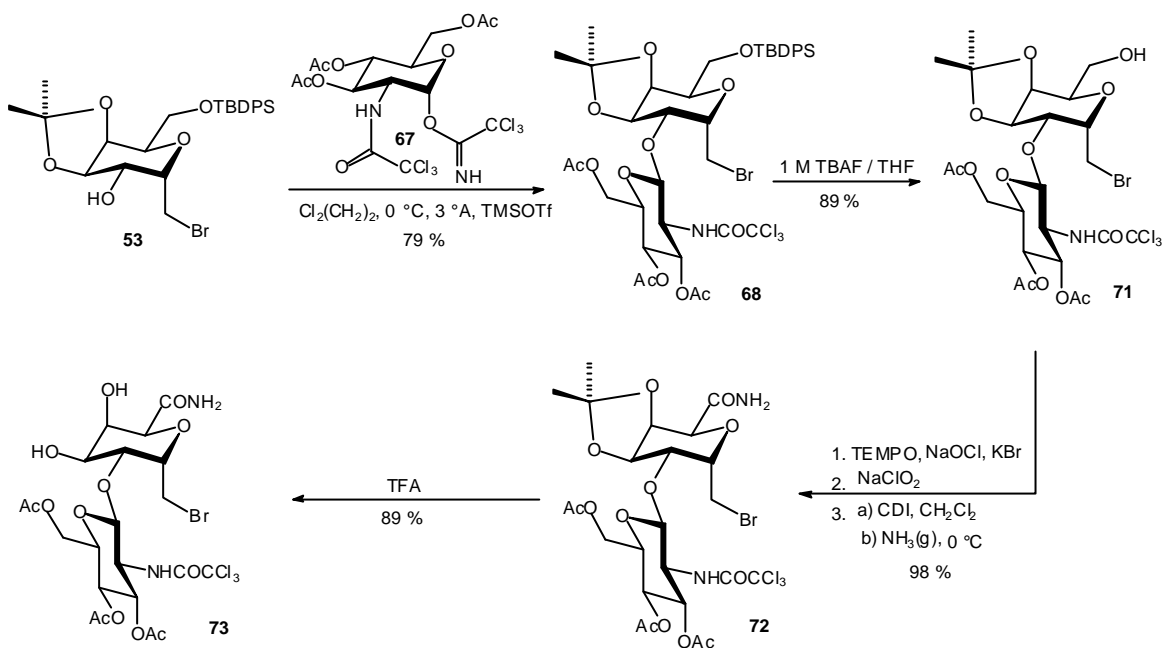
Scheme 6.2: Synthesis of disaccharide **73**

Introduction of the carbamoyl group at C-4^F position was achieved in two steps. Conversion of the diol **73** into the cyclic carbonate **76** with CDI in CH_2Cl_2 (84 %) and subsequent ring opening of this carbonate by bubbling a stream of gaseous ammonia into the CH_2Cl_2 solution at 0 °C gave **74** (62 %) as well as its isomer **77** (21 %).

Dehalogenation of the N-trichloroacetyl group was intensively studied, but interactions of other functional groups in the studied substances could not be avoided. The base-labile carbonate in **76** and the carbamoyl group in urethane **74** were cleaved under the reaction conditions. Hydrolysis of **76** with 0.5 M LiOH in MeOH-THF (1:1) followed by acetylation gave **80** (73 %), while its reduction with NaBH$_4$ in ethanol followed by acetylation gave **82** (60 °C, 85 %; RT, 83 %). On the other hand, reduction of **74** with NaBH$_4$ in ethanol at 60 °C followed by acetylation gave **82** (78 %), while performing the reduction step at 5 °C (THF-MeOH 4:1) or at RT (ethanol or isopropanol) gave **80** in an average yield of 65 %. In a non reproducible reaction (NaBH$_4$, EtOH, RT, then Ac$_2$O, pyridine, RT), the desired compound **83** (42 %) was obtained accompanied by **82** (46 %) The reaction between the N-trichloroacetyl group and NaBH$_3$CN was also fruitless. The phosphonate grouping was installed making use of *Arbuzov* reaction furnishing **85** (70 %).
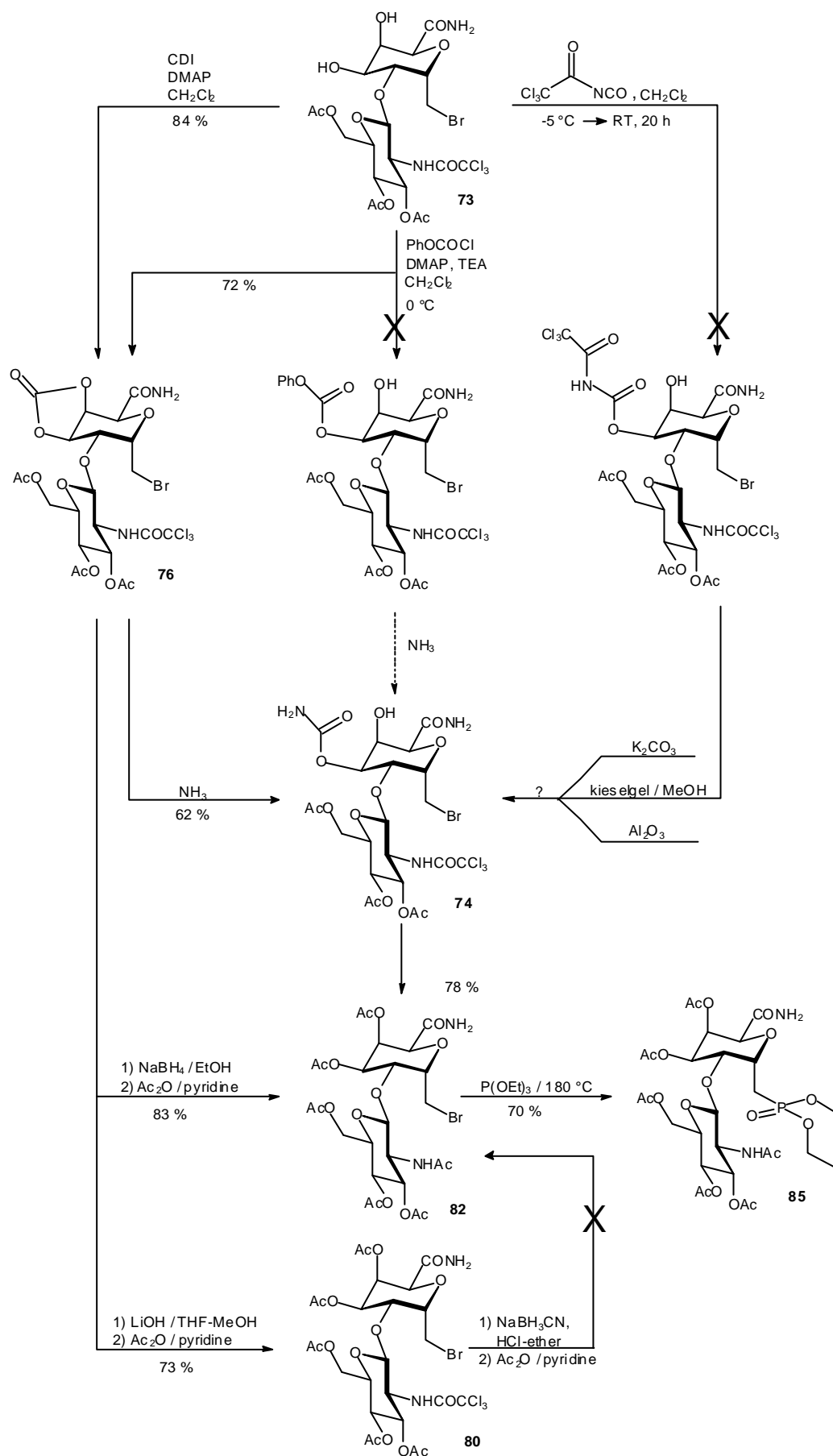
Scheme 6.3: from diol **73** to phosphonate **85**

Trisaccharides could not be obtained from the oxazoline donor **42** (prepared from chitobiose octaacetate **86**) through its reaction with acceptor **53**. There was also no coupling product between the recently synthesized donor **88** and the acceptor **92**. However, in this work, trisaccharide formation was achieved through the glycosylation reaction of donor **88** and acceptor **95** in 50 % yield (-30 °C, 1,2-dichloroethane, 3 Å, TMSOTf-TEA). Selective deprotection of the TBDMS group in compound **96** was accomplished at -10 °C with 1 eq of a molar solution of TBAF in THF. The free hydroxyl group of **97** was subjected to an oxidation using the TEMPO method affording the aldehyde. After oxidation of the aldehyde with sodium chlorite, the resulting carboxylic acid was converted according to *Staab's* method into the amide **93** in an overall yield of 95 % (based on **96**). There were difficulties in converting the N-phthalimido group in **93** to the N-acetyl group which is necessary for biological activity of moenomycin-type compounds, since the reactions were accompanied by elimination of HBr.

Scheme 6.4: Trisaccharide synthesis
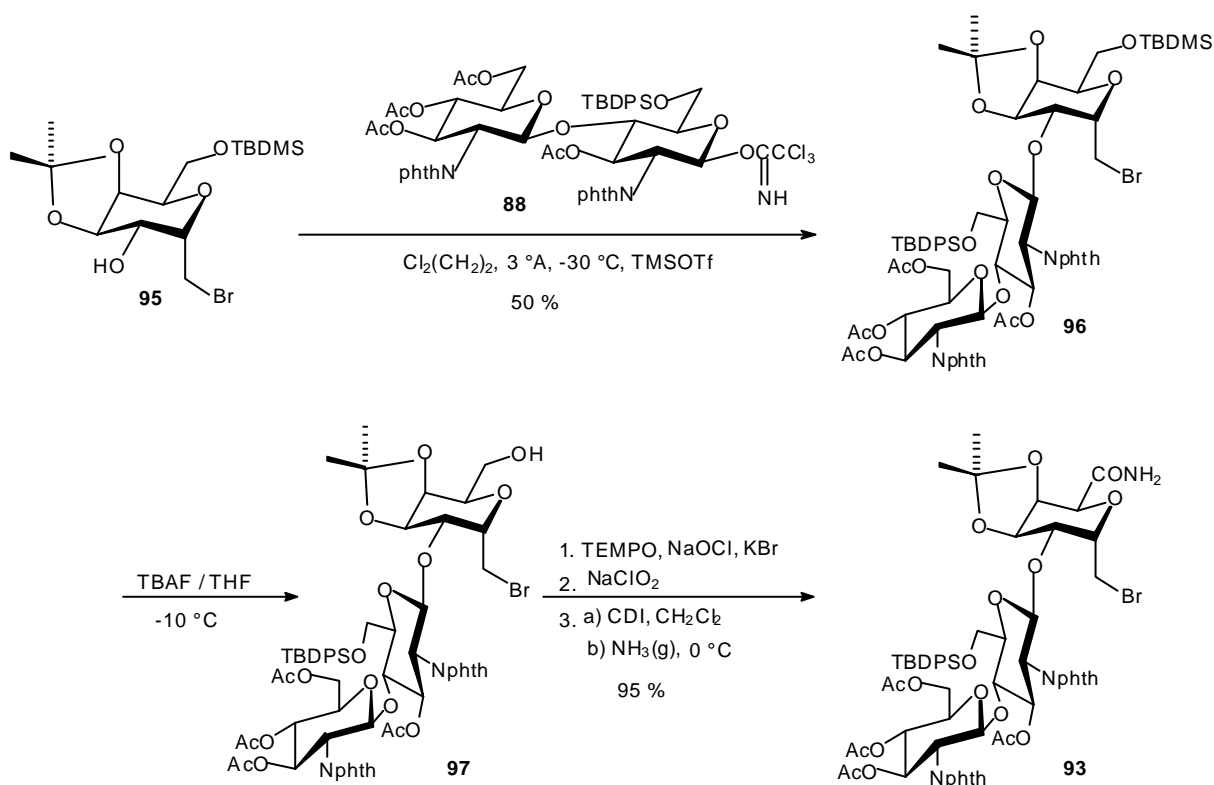
In conclusion, the synthetic methods employed in this work allow to prepare the di- and trisaccharides C-phosphonate analogues of moenomycin A_{12}.

7 EXPERIMENTAL PART

7.1 Devices, Methods and Materials

7.1.1 Devices used

NMR spectra were recorded on:

- Gemini 200 (Varian, ^{1}H: 199.975 MHz, ^{13}C: 50.289 MHz)

- Gemini 2000 (Varian, ^{1}H: 200.041 MHz, ^{13}C: 50.305 MHz)

- Gemini 300 (Varian, ^{1}H: 300.075 MHz, ^{13}C: 75.462 MHz, ^{31}P: 121.5 MHz)

- Avance DRX-400 (Bruker, ^{1}H: 399.952 MHz, ^{13}C: 100.577 MHz)

- DRX-600 (Bruker, ^{1}H: 600.133 MHz, ^{13}C: 150.918 MHz).

APT measurements were used to differentiate secondary and quaternary carbons from primary and tertiary carbons, which give rise to inverted signals (+ and -). The C- and CH_2- groups are marked as (+), while the CH- and CH_3 are marked as (-).

The ^{31}P-NMR shifts are based on external phosphoric acid. Chemical shifts are given in δ values (ppm) relative to tetramethylsilane = 0. The spectra were measured at 299 K, using 5 mm sample tubes. The deuterated solvents are given for each compound, separately.

Coupling constants (J) are reported in Hz, and have the following precision: (200 MHz-spectra ± 0.4, 300 MHz-spectra ± 0.3, 400 MHz-spectra ± 0.2, 600 MHz-spectra ± 0.2).

IR-spectra were determined on a Specord M80 grating spectrophotometer (Carl Zeiss Jena) and a FT-IR spectrometer (ATI Mattson Genesis). Solid compounds (ca 1 mg) were measured as KBr pellets, oily compounds (ca 1 mg) as films.

FAB MS spectra were recorded using VG AutoSpec FAB-mass-spectrometer (Fisons, cesium ion gun, 30 KeV, 1 μA primary-carrier flow, matrix: lactic acid or 3-nitrobenzyl alcohol).

ESI MS spectra were recorded using the FT-ICR-MS Apex II (Bruker Daltonics) in positive and negative modes.

Two molecular masses are always communicated; the first was calculated using the International Atomic Masses; the second refers to ^{12}C, ^{1}H, ^{16}O, ^{14}N, ^{31}P, ^{35}Cl, ^{80}Br, ^{32}S, ^{28}Si, ^{127}I (mono-isotopic masses).

Carbon and proton numbering in the subunits, as shown in the NMR data, follows the moenomycin nomenclature.[21]

Melting points were determined in capillary tubes, using a Büchi (B-540) melting point apparatus, and are corrected.

Specific optical rotation, [a]: the optical activity of a solution of the sample was observed in a Perkin-Elmer Model 141 polarimeter at a cell length of 0.5 dm, and measured as a rotation value, α. It is reported as specific rotation [α] obtained at a certain temperature, °C, and wavelength, λ. The observed α and the specific [α] are related in the manner shown in the formula

$$[a]_l^{\circ C} = \frac{100.a_l^{\circ C}}{l.c}$$

l: the length of the cell in decimeters,

c: the concentration of the compound in g per 100 mL,

where

λ: the wavelength used, usually being the wavelength of

sodium-D (double line), 589 nm.

Since the observed rotation value α depends on the concentration of the sample, the temperature and wavelength used, specific rotation values are reported together with these specifications, for example:

$$[a]_D^{23} = +46 \text{ (c 0.26, CH}_2\text{Cl}_2\text{)}$$

Ozonolysis was carried out using a Fischer Ozone Generator 500, connected to a dried- oxygen stream (ca 40 L/h), thus affording ozone (ca 2 g/h).

Photochemical reactions were performed in quartz tubes, an external Philips halogen lamp (1000W) was used.

Ultrasound (Bandelin, Sonorex Super RK 106) was used to degass the solvents when required, or to increase the solubility in the case of a suspension.

7.1.2 Methods used

O$_2$- and/or moisture-sensitive reactions were performed in an argon atmosphere, using glassware oven-preheated at 130 °C, sealed with septa (size NS 29 and NS 14.5), evacuated, and cooled down under argon pressure. Liquids and solutions were transferred by syringe. During the reaction, a constant argon pressure was ensured. Small-scale reactions were performed in Wheaton serum bottles (1, 2, 5, 10 mL) sealed with aluminium caps with open top Teflon-faced septum (Aldrich).

O$_2$- and/or moisture-sensitive reagents were taken either under a positive pressure of argon, or under an argon bell.

Analytical TLC was used to monitor reactions for completeness:

- Normal phase TLC: Merck precoated silica gel, 60F$_{254}$ plates, 0.2 mm.

- Reversed phase TLC: Merck RP-18 precoated silica gel, F$_{254S}$ plates, 0.2 mm.

Visualization of TLC plates was accomplished by one or several suitable detection methods; spots were identified:

- by UV light absorption at λ 254 and 366 nm, as far as the substances absorb UV,

- and by dipping into a solution of *p*-anisaldehyde (1mL) and conc. H2SO4 (4 mL) in ethanol (95 mL),[127] and subsequent heating at 140 °C,

- or in a molybdatophosphoric acid/ Ce(IV)-sulphate dipping reagent {$Ce(SO_4)_2 \times 4H_2O$ (10 g) and $H_3[PO_4(Mo_3O_9)_4] \times H_2O$ (25 g) in water (940 mL) and conc. H_2SO_4 (60 mL)},[128] and subsequent heating at 140 °C to colour the TLC-plates.

Column Chromatography was used for the purification of the compounds:

- **Flash-Chromatography (FC)**[129,130] was performed using columns of diameters of 1 cm (ca 6 g silica gel), 2 cm (ca 25 g silica gel), 3 cm (ca 55 g silica gel), 4 cm (ca 100 g silica gel), 5 cm (ca 150 g silica gel). When not mentioned, 32-63 μm silica gel (ICN Biomedicals) was used. To produce pressure, optima pump (Model 10007) was used in this process. The samples were dissolved in a small amount of the eluent, or dissolved in a suitable solvent, and deposited on twice their weights of Kieselguhr (Merck).

- **Medium-Pressure Liquid Chromatography (MPLC)** was carried out using a column of size B, filled with 35-70 μm Silica gel (75 g, about 3000 plates, Amicon). The samples were deposited on twice their weights Kieselguhr (Merck), and were applied to a pre-column (3-5 g kieselgel, 63-100 μm) and were eluted at a pressure of 2-3 bar using a dosage pump (Kronlab, Chromatographie und Labortechnik, Sinsheim).

Distilled solvents, at least technical degree of purity, were used for chromatography. The high-boiling fraction of petroleum ether (80-120 °C) was used.

Organic solvent evaporations were performed *in vacuo* at water bath temperature of 40 °C using a rotatory evaporator. Evaporation of dimethyl sulfide, and triethylphosphite was carried out in an effective fume hood. Water was removed by **lyophilization** using the Leybold-Heraeus GT2, and the Christ Alpha 1-2 apparatus.

Low temperatures down to -78 °C were achieved either by isopropanol-dry ice bath, or by using a cryostat Model RL6 CS (Lauda).

7.1.3 Chemicals and solvents used

All commercial materials were used without further purification unless otherwise stated.

Compounds 34[59], 42[62], 67[61], 88[125] were prepared according to the literature.

Drying of the solvents have been carried using standard procedures:

For the reactions performed under anhydrous conditions, the solvents have been dried according to the following standard procedures:

- acetonitrile, 1,2-dichloroethane, dichloromethane, N,N´-dimethylformamide, diethyl ether, pyridine, toluene, and triethylamine were refluxed over calcium hydride (3 h), and distilled immediately prior to use.

- THF was refluxed with sodium; and distilled immediately before use; benzophenone was used as an indicator.

- acetone was refluxed over P_2O_5.

- ethanol (absolute) was left overnight over activated 3 Å molecular sieves.

- methanol was refluxed with Mg chips, distilled and kept over 3 Å molecular sieves.

- isopropanol was refluxed over sodium and phthalic acid diethyl ester, distilled, and further dried over 4 Å molecular sieves.

- acetic acid was refluxed over phosphorous pentoxide, and distilled.

Degassing of the solvents, when required, was performed in an ultrasound bath (Bandelin, Sonorex Super RK 106) under an argon atmosphere.

Argon, oxygen, or ammonia gas streams were allowed to pass over phosphorous pentoxide before use. When not mentioned, ammonia was bubbled directly into the reaction solution.

Non volatile carbohydrates, fused in O_2- and/or moisture-sensitive reactions and before the analysis, were dried at 0.1 mbar in a dynamic oil pump vacuum over phosphorus pentoxide at RT for 12 h.

Molecular sieves were activated for 24 h at 320 °C in a Muffel oven at 0.1 mbar in a dynamic oil pump vacuum, and kept in a dessiccator over phosphorous pentoxide.

Dowex 50 WX 2 was regenerated with 5 % HCl, and was then washed neutral with bidistilled water.

CSA was dried at 0.1 mbar in a dynamic oil pump vacuum at 100 °C for 12 h over phosphorus pentoxide.

Anhydrous MgSO₄ was used as the drying agent for the organic layers in the extraction steps.

7.2 Preparation of the glycosyl acceptor 53

7.2.1 Preparation of 34[59]

AJ 1-05

β-D-Galactose-pentaacetate (3.12 g, 8.0 mmol) was dissolved in dry acetonitrile (40 mL) under an argon atmosphere. Allyltrimethylsilane (3.82 mL, 3.0 eq; 24.0 mmol) and $BF_3.Et_2O$ (7.65 mL, 5.0 eq; 40.0 mmol) were added at 4 °C. The reaction mixture was stirred at this temperature for 48 h. The orange mixture was poured into a saturated aqueous solution of sodium hydrogencarbonate. The product was extracted with CH_2Cl_2 (3 × 40 mL). The combined organic phases were dried, and the solvent was evaporated leaving a crude oil, which was repeatedly chromatographed (FC) eluting with petroleum ether-ethyl acetate-toluene 2 : 2 : 1 to give **34** as an oil (2.00 g, 67 % yield), which slowly crystallized to a pale yellow solid. Spectral data (¹H NMR, ¹³C NMR, MS) agree with those in the literature.[59]

1,3,4,5-Tetra-*O*-acetyl-2,6-anhydro-7,8,9-trideoxy-D-*glycero*-L-*galacto*-non-8-enitol (34)
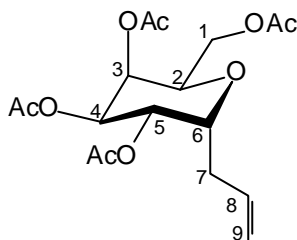
$C_{17}H_{24}O_9$ [372.37]
Ecact Mass [372.14]

7.2.2 Double bond rearrangement reaction of 34

7.2.2.1 Photooxygenation reaction of 34

7.2.2.1.1 using methylene blue

AJ 1-07

Compound **34** (0.100 g, 0.26 mmol) was added to a solution of methylene blue (0.007 g) in methanol (10 mL). The mixture was placed into a flask with an inlet for a dry oxygen gas stream, and illuminated with a halogen lamp (1000 W). Irradiation was continued for 9 h. The reaction was controlled by TLC (different eluents); no reaction was observed.

AJ 1-12

The same procedure was repeated in CH_2Cl_2. TLC showed also that no reaction had occurred.

7.2.2.1.2 using Rose bengal

AJ 1-08

Compound **34** (0.100 g, 0.26 mmol) was added to a solution of Rose bengal (0.007 g) in methanol (10 mL). The mixture was placed into a flask with an inlet for a dry oxygen gas stream, and illuminated with a halogen lamp (1000 W). Irradiation was continued for 9 h. The reaction was controlled by TLC (different eluents); no reaction was observed.

AJ 1-11

The same procedure was repeated in CH_2Cl_2, irradiation continued for 9 h. After 7 h, the reaction mixture was supported with excess amount of Rose bengal. TLC showed that there was no reaction.

7.2.2.2 Palladium catalytic reaction of 34

AJ 1-13

Compound **34** (0.960 g, 2.58 mmol) was added to a solution of bis(benzonitrile)-palladium(II) chloride (0.100 g, 0.1 eq; 0.26 mmol) in dry toluene (40 mL) under an argon atmosphere. The reaction mixture was refluxed for 63 h, and the reaction progress was controlled by TLC (ethyl acetate-petroleum ether 1 : 2) which showed the presence of a major spot with the same R_f value as the reactant **34**. The reaction mixture was filtered, evaporated, and chromatographed twice with ethyl acetate-petroleum ether 1 : 2. Evaporation of the solvents gave 0.4 g of a yellow oil, which appeared as a single spot, but really it was a mixture of both the reactant, **34**, and the product, **54**, in an equal ratio as indicated by ^{13}C NMR.

AJ 1-19, AJ 1-21, AJ 1-25

The same procedure was repeated with 15 % mol percent of the catalyst. Thus, compound **34** (1.00 g, 2.69 mmol) was added to a solution of bis(benzonitrile)palladium(II) chloride (0.15 gm, 0.15 eq; 0.40 mmol) in dry toluene (200 mL) under an argon atmosphere. The reaction mixture was refluxed for 5 days. Then it was filtered, evaporated, and applied to FC eluting with ethyl acetate-petroleum ether 1 : 2, giving an impure yellow oil (0.98 g). ^{13}C NMR showed that it was a mixture of an equal ratio of **34** and **54**. The by-product **55** was obtained (0.005 g).

1,3,4,5-Tetra-*O*-acetyl-2,6-anhydro-7,8,9-trideoxy-D-*glycero*-L-*galacto*-non-6-enitol (55)
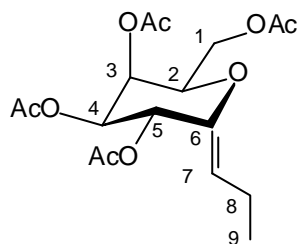
C$_{17}$H$_{24}$O$_9$ [372.37]
Exact Mass [372.14]

- colour and physical state: white solid

- R$_f$: 0.26 (ethyl acetate-petroleum ether 1:2)

- ESI-MS: *m/z* 373.1 [M+H]$^+$, 395.1 [M+Na]$^+$

- IR (KBr): $\tilde{n}$ = 1754 cm^{-1}

- ^{1}H NMR (HH-COSY, 200 MHz, CDCl$_3$):

δ = 0.95 (t, 3H, CH$_3$-9, J 7.6), 2.00, 2.06, 2.12, 2.15 (4s, 12H, 4 × CH$_3$COO), 2.22-2.25 (m, 2H, CH$_2$-8), 3.95 (ddd, 1H, 2-H, J 1.7, 5.7, 7.2), 4.14 (dd, 1H, 1-H, J 5.7, 11.4), 4.24 (dd, 1H, 1-H´, J 7.2, 11.4), 4.89 (dt, 1H, 7-H, J 1.8, 7.4), 5.02 (dd, 1H, 4-H, J 3.4, 9.9), 5.50 (dd, 1H, 3-H, J 1.7, 3.4), 5.62 (m, 1H, 5-H)

- ^{13}C NMR (50.30 MHz, CDCl$_3$):

δ = 14.48 (C-9), 17.99 (C-8), 21.04, 21.21 (4 × $\underline{C}$H$_3$COO), 62.13 (C-1), 67.68, 68.12, 71.97, 75.88, 114.71 (C-7), 145.56 (C-6), 170.08, 170.56, 170.71, 171.02 (4 × CH$_3\underline{C}$OO)

7.2.2.3 Ene reaction of compound 34 with PTAD

AJ 1-36

To a solution of compound **34** (0.518 g, 1.39 mmol) in dry dichloromethane (20 mL), a solution of PTAD (0.244 g, 1.0 eq; 1.39 mmol) in dichloromethane (20 mL) was added slowly via a dropping funnel with stirring. The reaction mixture was stirred at RT overnight, until the red colour had disappeared. The reaction progress was controlled by TLC (ethyl acetate-petroleum ether 1 : 1). Evaporation of the solvent left a slightly yellow semisolid, which was purified by FC eluting with a gradient ethyl acetate-petroleum ether 2 : 1 → ethyl acetate . Evaporation left **56** (0.631 g, 83 %). Unreacted **34** (0.067 g, 13 %) was recovered.

(7*E*)-1,3,4,5-Tetra-*O*-acetyl-2,6-anhydro-7,8,9-trideoxy-9-(3,5-dioxo-4-phenyl-1,2,4-triazolidin-1-yl)-D-*glycero*-L-*galacto*-non-7-enitol (56)
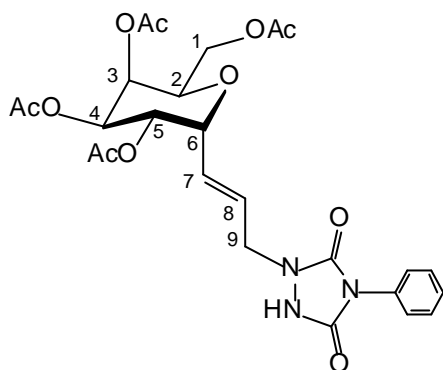
$C_{25}H_{29}N_3O_{11}$ [547.52]
Exact Mass   [547.18]

- colour and physical state: pale yellow solid

- M.p.: 75-76 °C (ethyl acetate-petroleum ether)

- $[\alpha]_D^{23}$ = +46.15 (c 0.26, CH_2Cl_2)

- R_f: 0.36 (ethyl acetate)

- FAB-MS: *m/z* 548.1 $[M+H]^+$, 570.1 $[M+Na]^+$

- ESI-MS: *m/z* 548.2 $[M+H]^+$, 570.0 $[M+Na]^+$

- IR (KBr): $\tilde{n}$ = 1229, 1709, 1749, 3440 cm^{-1}

- ^{1}H NMR (HH-COSY, 300 MHz, $CDCl_3$):

δ = 2.06, 2.07, 2.09, 2.18 (4s, 12H, 4 × CH_3COO), 3.99 (m, 1H, 1-H), 4.13 (m, 1H, 1-H´), 4.23 (m, 1H, 2-H), 4.28-4.32 (m, 2H, CH_2-9), 4.84 (m, 1H, 6-H), 5.13 (dd, 1H, 4-H, J 3.3, 9.9), 5.32 (m, 1H), 5.36 (m, 1H), 5.96-6.02 (m, 2H, 7-H, 8-H), 7.50-7.54 (m, 5H, aromatic)

- ^{13}C NMR (APT, HETCOR, 75.45 MHz, $CDCl_3$):

δ = 20.96, 21.00, 21.05, 21.09 (-, 4 × $\underline{C}H_3COO$), 49.04 (+, C-9), 62.14 (+, C-1), 68.07 (-), 68.19 (-), 68.62 (-, C-4), 68.88 (-, C-2), 71.99 (-, C-6), 125.85, 128.52, 128.68, 128.76, 129.49 (-, 5 × C^{Ar}, C-7 and C-8), 131.35 (+, NC^{Ar}), 153.00, 154.20 (+, 2 × NCON), 170.26, 170.34, 170.39 (+, 4 × $CH_3\underline{C}OO$)

7.2.3 Preparation of alcohol 35

AJ 1-26

A mixture of compounds **34** and **54** (0.24 g, 0.645 mmol) was dissolved in dry dichloromethane (30 mL) and dry methanol (3 mL), the solution was cooled to -70 °C. Ozone was bubbled through the solution for ca 1 h, when the solution became saturated with the gas. Progress of the reaction was controlled by TLC (ethyl acetate-petroleum ether 1 : 1). Excess ozone was removed by purging with oxygen and then with argon. Dimethyl sulfide (2 mL) was added, and

the solution was left overnight at RT. The solvents were evaporated, and the crude product was redissolved in THF (10 mL), and added to a mixture of sodium borohydride (0.027 g, 1.1 eq; 0.710 mol) and acetic acid (44 ìL, 1.1 eq; 0.710 mmol) in THF (2 mL). The mixture was left overnight, diluted with brine, and extracted with ethyl acetate (3×20 mL). The extract was dried, evaporated leaving 0.19 g as a crude mixture. TLC (ethyl acetate-petroleum ether 2 : 1) showed many products, the major two with similar R_f values. Upon repeated FC, no pure samples were obtained.

AJ 1-42

Compound **56** (0.254 g, 0.464 mmol) was dissolved in dry dichloromethane (20 mL) and dry methanol (2 mL), the solution was cooled to -70 °C. Ozone was bubbled through the solution for ca 1 h, when the solution became saturated with the gas. Progress of the reaction was controlled by TLC (ethyl acetate-petroleum ether, 1 : 1). Excess ozone was removed by purging with oxygen and with argon. Dimethyl sulfide (1 mL) was added, and the solution was left overnight at RT. The solvents were evaporated, and the crude product was redissolved in THF (10 mL), and added to a mixture of sodium borohydride (0.019 g, 1.1 eq; 0.510 mol) and acetic acid (40 ìL, 1.1 eq; 0.510 mmol) in THF (2 mL). The mixture was left overnight, diluted with brine, and extracted with ethyl acetate (3×20 mL). The extract was dried, evaporated and left 0.192 gm as a crude, which was chromatographed, eluting with ethyl acetate-petroleum ether 2 : 1. Evaporation left **58** (0.070 g, 50 %) and **35** (0.071 g, 42 %).

AJ 1-44

Compound **56** (0.116 g, 0.212 mmol) was dissolved in dry dichloromethane (20 mL) and dry methanol (2 mL), the solution was cooled to -70 °C. Ozone was bubbled through the solution for ca 10 min, when the solution became saturated with the gas. The reaction was judged complete by the disappearance of the starting material (TLC, ethyl acetate-petroleum ether 1 : 1). Excess ozone was removed by purging with oxygen and with argon. Dimethyl sulfide (1 ml) was added, and the solution was left overnight at RT. The solvents were evaporated, and the crude product was redissolved in freshly distilled THF (10 mL), and added to a mixture of sodium borohydride (0.009 g, 1.1 eq; 0.233 mmol) and freshly distilled acetic acid (18 ìL, 1.1 eq; 0.233 mmol) in dry THF (10 mL). The mixture was left overnight, diluted with brine, and extracted with ethyl

acetate (3×15 mL). The extract was dried, evaporated and chromatographed, eluting with ethyl acetate-petroleum ether 1 : 1 to give **35** (0.065 g, 85 %).

4,5,7-Tri-*O*-acetyl-2,6-anhydro-3-deoxy-D-*lyxo*-hept-2-enose (58)
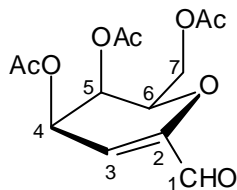
$C_{13}H_{16}O_8$ [300.27]
Exact Mass [300.08]

- colour and physical state: pale yellow oil
- R_f: 0.60 (ethyl acetate)
- ^{1}H NMR (200 MHz, CDCl$_3$):

δ = 2.07, 2.09, 2.12 (3s, 9H, $3 \times$ CH$_3$COO), 4.28-4.34 (m, 2H), 4.45 (m, 1H), 5.51 (m, 1H), 5.70 (m, 1H), 5.77 (m, 1H), 9.23 (s, 1H, 1-H)

- ^{13}C NMR (50.30 MHz, CDCl$_3$):

δ = 21.03, 21.09, 21.19 ($3 \times$ <u>C</u>H$_3$COO), 61.76, 62.98, 65.16, 74.31, 116.23 (C-3), 152.54 (C-2), 170.39, 170.52, 170.89 ($3 \times$ CH$_3$<u>C</u>OO), 185.69 (C-1)

1,3,4,5-Tetra-*O*-acetyl-2,6-anhydro-D-*glycero*-L-*galacto*-heptitol (35)
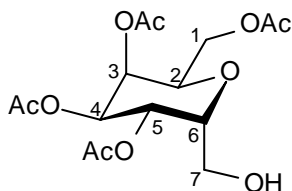
$C_{15}H_{22}O_{10}$ [362.33]
Exact Mass [362.12]

- colour and physical state: pale yellow oil
- $[\boldsymbol{a}]_D^{23}$ = +44.44 (c 0.18, CH$_2$Cl$_2$)
- R_f: 0.45 (ethyl acetate)
- FAB-MS: *m/z* 303.1 [M+H-AcOH]$^+$, 345.1 [M+H-H$_2$O]$^+$, 363.1 [M+H]$^+$, 385.1 [M+Na]$^+$
- ESI-MS: *m/z* 385.1 [M+Na]$^+$
- IR (Film): $\tilde{\boldsymbol{n}}$ = 1080, 1142, 1296, 1724, 2929, 3491 cm^{-1}
- ^{1}H NMR (HH-COSY, 200 MHz, CDCl$_3$):

δ = 2.05, 2.06, 2.10, 2.11 (4s, 12H, $4 \times$ CH$_3$COO), 3.65 (dd, 1H, 7-H, J 4.6, 12.0), 3.83 (dd, 1H, 7-H´, J 7.5, 12.0), 4.07 (dd, 1H, 1-H, J 4.0, 11.0), 4.27 (m, 1H, 6-H), 4.31 (m, 1H, 2-H),

4.41 (dd, 1H, 1-H´, J 7.8, 11.0), 5.27 (m, 1H, 5-H), 5.29 (m, 1H, 4-H), 5.44 (t, 1H, 3-H, J 3.0)

- ^{13}C NMR (APT, HETCOR, 50.29 MHz, CDCl$_3$):

δ = 21.15, 21.22 (-, 4 × C̲H$_3$COO), 60.37 (+, C-7), 61.48 (+, C-1), 67.50 (-, C-3), 68.37 (-, C-4), 68.58 (-, C-5), 70.82 (-, C-2), 71.77 (-, C-6), 170.20, 170.40, 170.63, 171.30 (+, 4 × CH$_3$C̲OO)

7.2.4 Preparation of methanesulfonate 60

AJ 1-56

Compound **35** (0.046 g, 0.127 mmol) was dissolved in dry pyridine (4 mL) containing DMAP (0.002 g, cat) and the mixture was cooled to 4 °C. Methanesulfonyl chloride (12 ìL, 1.2 eq; 0.152 mmol) was added, and the orange-red mixture was stirred at 4°C for 5 h (TLC, ethyl acetate). The mixture was diluted with dichloromethane and 2 M sulfuric acid. The organic layer was washed with aqueous sodium hydrogencarbonate and with brine. Drying, evaporation, and FC eluting with ethyl acetate-petroleum ether 1 : 1 gave **60** (0.034 g, 60 %).

1,3,4,5-Tetra-*O*-acetyl-2,6-anhydro-7-*O*-methysulfonyl-D-*glycero*-L-*galacto*-heptitol (60)
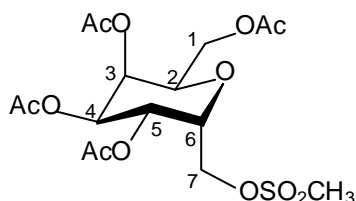
C$_{16}$H$_{24}$O$_{12}$S [440.42]
Exact Mass [440.10]

- colour and physical state: pale yellow oil

- $[\boldsymbol{a}]_D^{23}$ = +39.28 (c 0.56, CH$_2$Cl$_2$)

- R$_f$: 0.56 (ethyl acetate)

- FAB-MS: *m/z* 345.1 [M+H-MeSO$_3$H]$^+$, 381.0 [M+H-AcOH]$^+$, 441.1 [M+H]$^+$, 463.0 [M+Na]$^+$

- ESI-MS: *m/z* 463.1 [M+Na]$^+$

- IR (Film): $\tilde{\boldsymbol{n}}$ = 1055, 1175, 1227, 1367, 1749, 3446 cm^{-1}

- ^{1}H NMR (HH-COSY, 200 MHz, CDCl$_3$):

δ = 2.05, 2.06, 2.11, 2.12 (4s, 12H, 4 × CH$_3$COO), 3.09 (s, 3H, SO$_2$CH$_3$), 4.11 (dd, 1H, 1-H, J 2.6, 8.8), 4.20 (m, 1H, 2-H), 4.30 (m, 1H, 1-H´), 4.32 (m, 1H, 7-H), 4.48 (m, 1H, 6-H),

4.51 (m, 1H, 7-H´), 5.21 (dd, 1H, 4-H, J 3.1, 8.5), 5.31 (dd, 1H, 5-H, J 4.0, 8.5), 5.43 (m, 1H, 3-H)

- ^{13}C NMR (APT, HETCOR, 50.29 MHz, CDCl$_3$):

δ = 20.73, 20.79, 20.84 (-, 4 × $\underline{C}$H$_3$COO), 38.05 (-, SO$_2$CH$_3$), 60.95 (+, C-1), 65.59 (+, C-7), 67.02 (-, C-3), 67.11 (-, C-5), 67.81 (-, C-4), 69.81 (-, C-6), 70.36 (-, C-2), 169.64, 169.69, 169.89, 170.64 (-, 4 × CH$_3$$\underline{C}$OO)

7.2.5 Preparation of the halide derivatives 59 and 61

7.2.5.1 Preparation of compound 61

AJ 1-63

The methanesulfonate **60** (0.054 g, 0.122 mmol) was dissolved in dry toluene (5 mL) containing tetrabutylammonium bromide (0.098 g, 2.5 eq; 0.305 mmol), and the mixture was heated at 80 °C for 20 h (TLC, ethyl acetate-petroleum ether 80 : 20). The mixture was partitioned between water (10 mL) and ethyl acetate (20 mL), the organic layer was washed with aqueous sodium hydrogencarbonate, and with brine. Drying and evaporation left a crude mixture, which was chromatographed eluting with ethyl acetate-petroleum ether 80 : 20, to give **61** (0.042 g, 80 %).

1,3,4,5-Tetra-*O*-acetyl-2,6-anhydro-7-bromo-7-deoxy-D-*glycero*-L-*galacto*-heptitol (61)

C$_{15}$H$_{21}$BrO$_9$ [425.23]
Exact Mass [424.04]

- colour and physical state: pale yellow oil

- $[\boldsymbol{a}]_D^{23}$ = +64.86 (c 0.3, CH$_2$Cl$_2$)

- R$_f$: 0.60 (ethyl acetate-petroleum ether 80:20)

- FAB-MS: *m/z* 345.1 [M+H-HBr]$^+$, 365.0 [M+H-AcOH]$^+$, 425.0 [M+H]$^+$, 447.0 [M+Na]$^+$

- ESI-MS: *m/z* 447.1 [M+Na]$^+$

- IR (Film): $\tilde{\boldsymbol{n}}$ = 1049, 1227, 1371, 1749, 3435 cm^{-1}

- ^{1}H NMR (HH-COSY, 200 MHz, CDCl$_3$):

δ = 2.05, 2.08, 2.11, 2.13 (4s, 12H, 4 × CH$_3$COO), 3.44 (dd, 1H, 7-H, J 5.1, 11.4), 3.55 (dd, 1H, 7-H´, J 9.2, 11.4), 4.11 (m, 1H, 2-H), 4.16 (m, 1H, 1-H), 4.30 (m, 1H, 1-H´), 4.45 (m,

1H, 6-H), 5.19 (dd, 1H, 4-H, J 3.3, 8.7), 5.35 (dd, 1H, 5-H, J 4.9, 8.7), 5.43 (t, 1H, 3-H, J 2.9)

- ^{13}C NMR (APT, HETCOR, 50.29 MHz, CDCl$_3$):

δ = 20.77, 20.82 (-, 4 × $\underline{C}$H$_3$COO), 27.49 (+, C-7), 61.06 (+, C-1), 67.22 (-, C-3), 67.71 (-, C-5), 68.13 (-, C-4), 69.39 (-, C-2), 71.74 (-, C-6), 169.62, 169.79, 170.02, 170.74 (+, 4 × CH$_3$$\underline{C}$OO)
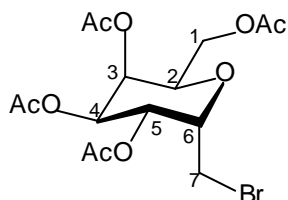
7.2.5.2 Preparation of compound 59

AJ 1-61

Iodine (0.028 g, 1.3 eq; 0.112 mmol) was added at RT to a mixture of **35** (0.031 g, 0.086 mmol), chlorodiphenylphosphine (0.02 mL, 1.3 eq; 0.112 mmol) and imidazole (0.013 g, 2.2 eq; 0.189 mmol) in toluene (5 mL). The reaction mixture was stirred at 50 °C for 4 h (TLC, ethyl acetate). The reaction mixture was poured at RT into an equal volume of saturated aqueous sodium hydrogencarbonate in a separating funnel, and shacked for few minutes, while adding iodine portionwise, until the organic layer remained iodine-coloured. The organic layer was separated, washed with aqueous sodium thiosulphate, to remove the excess iodine, and washed with water. Drying, and evaporation left a crude product, which was chromatographed eluting with ethyl acetate-petroleum ether 80 : 20 to give **59** (0.013 g, 31 %).

AJ 1-62

The methanesulfonate **60** (0.077 g, 0.175 mmol) was dissolved in toluene (10 mL) containing tetrabutylammonium iodide (0.161 g, 2.5 eq; 4.37 mmol), and the mixture was heated at 80 °C for 20 h (TLC, ethyl acetate-petroleum ether 80 : 20). The mixture was partitioned between water (10 mL) and ethyl acetate (30 mL), the organic layer was washed with aqueous sodium hydrogencarbonate, and with brine. Drying and evaporation left a crude mixture, which was chromatographed eluting with ethyl acetate-petroleum ether 80 : 20, to give **59** (0.054 g, 65 %).

1,3,4,5-Tetra-*O*-acetyl-2,6-anhydro-7-deoxy-7-iodo-D-*glycero*-L-*galacto*-heptitol (59)
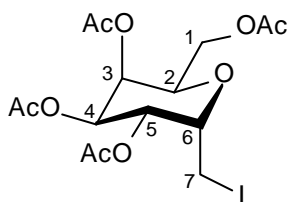
C$_{15}$H$_{21}$IO$_9$ [472.23]
Exact Mass [472.02]

- colour and physical state: pale yellow oil

- $[\boldsymbol{a}]_D^{23}$ = +70 (c 0.20, CH$_2$Cl$_2$)

- R$_f$: 0.60 (ethyl acetate-petroleum ether 80:20)

- FAB-MS: *m/z* 345.1 [M+H-HI]$^+$, 413.0 [M+H-AcOH]$^+$, 473.0 [M+H]$^+$, 494.9 [M+Na]$^+$

- ESI-MS: *m/z* 495.0 [M+Na]$^+$

- IR (Film): $\tilde{\boldsymbol{n}}$ = 1057, 1227, 1745 cm^{-1}

- ^{1}H NMR (HH-COSY, 300 MHz, CDCl$_3$):

δ = 2.03, 2.09, 2.10, 2.13 (4s, 12H, 4 × CH$_3$COO), 3.28-3.38 (m, 2H, CH$_2$-7), 4.03 (ddd, 1H, 2-H, J 2.6, 4.9, 7.7), 4.13 (dd, 1H, 1-H, J 4.9, 11.6), 4.27 (dd, 1H, 1-H´, J 7.7, 11.6), 4.36 (m, 1H, 6-H), 5.18 (dd, 1H, 4-H, J 3.3, 9.3), 5.33 (dd, 1H, 5-H, J 5.2, 9.3), 5.42 (t, 1H, 3-H, J 3.1)

- ^{13}C NMR (APT, HETCOR, 50.29 MHz, CDCl$_3$):

δ = 0.20 (+, C-7), 21.00, 21.04, 21.16, 21.25 (-, 4 × <u>C</u>H$_3$COO), 61.63 (+, C-1), 67.77 (-, C-3), 67.88 (-, C-4), 68.42 (-, C-5), 69.02 (-, C-2), 72.84 (-, C-6), 170.12, 170.36, 170.55, 171.16 (+, 4 × CH$_3$<u>C</u>OO)

7.2.6 Hydrolysis of the acetate group in 61

AJ 1-68

To a solution of compound **61** (0.050 g, 0.118 mmol) in dry methanol (10 mL) at 0 °C, a solution of sodium methoxide (0.013 g, 2.0 eq; 0.236 mmol) in dry methanol (5 mL) was added. The reaction mixture was stirred at RT for about 3 h until the reactant was accomplished, as shown by TLC (CH$_3$OH-CHCl$_3$ 15 : 85). The mixture was diluted with methanol, neutralized with Dowex 50-W X2 (H$^+$). The mixture was stirred for a few minutes, and then the resin was filtered off, washed with methanol (3 × 5 mL). The combined filtrate was concentrated. The residue was applied to FC eluting with CH$_3$OH-CHCl$_3$ 15 : 85, and provided after solvent evaporation **62** (0.030 g, quant).

2,6-Anhydro-7-bromo-7-deoxy-D-*glycero*-L-*galacto*-heptitol (62)
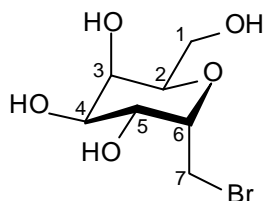
$C_7H_{13}BrO_5$ [257.08]
Exact Mass [255.99]

- colour and physical state: white solid

- M.p.: 115-116 °C (EtOH-petroleum ether)

- $[\boldsymbol{a}]_D^{23} = +73.68$ (c. 0.38, EtOH)

- R_f: 0.16 (CHCl$_3$ -MeOH 85:15)

- FAB-MS: *m/z* 257.0 [M+H]$^+$

- ESI-MS: *m/z* 278.8 [M+Na]$^+$

- IR (KBr): $\tilde{\boldsymbol{n}}$ = 1055, 1080, 3365, 3419 cm^{-1}

- ^{1}H NMR (HH-COSY, 200 MHz, pyridine-d$_5$):

δ = 4.20-4.30 (m, 2H, CH$_2$-7), 4.32-4.38 (m, 2H), 4.52-4.55 (m, 2H, CH$_2$-1), 4.75-4.85 (m, 3H)

- ^{13}C NMR (APT, HETCOR, 50.29 MHz, pyridine-d$_5$):

δ = 31.09 (+, C-7), 61.40 (+, C-1), 69.44 (-), 69.77 (-), 71.79 (-), 74.51 (-), 76.03 (-)

7.2.7 Preparation of the silyl ether 63

AJ 2-01

To a solution of **62** (0.032 g, 0.125 mmol) in dry pyridine (3 mL) and dry triethylamine (3 mL) at 0 °C TBDPSCl (26 μL, 1.2 eq; 0.150 mmol) and DMAP (0.002 g, cat) were added. The solution was stirred at 0 °C, and after about 4 h the reaction came to completion as indicated by TLC (CH$_3$OH-CHCl$_3$ 10 : 90). The reaction mixture was quenched at 0 °C with MeOH (2mL), and then coevaporated with toluene. The residue was purified by FC (CH$_3$OH-CHCl$_3$ 5 : 95) to yield **63** (0.020 g, 33 % yield).

AJ 2-10

To a cold (0 °C, bath) stirred solution of **62** (0.200 g, 0.781 mmol) and imidazole (0.105 g, 2.0 eq; 1.56 mmol) in dry DMF (4 mL) TBDPSCl (0.16 mL, 1.2 eq; 0.937 mmol) was added. The

mixture was stirred at 0 °C for about 2 h until the reaction was complete (TLC, CH_3OH-$CHCl_3$ 10 : 90). The mixture was poured into ice-water (10 mL) and extracted with chloroform (5 × 20 mL). The organic layer was extracted with saturated aqueous sodium hydrogencarbonate, water, dried, and evaporated. The residue was applied to FC, and eluted with a gradient $CHCl_3$ → CH_3OH-$CHCl_3$ 2 : 98. On evaporation, the fractions corresponding to the product afforded **63** (0.328 g, 85 %).

2,6-Anhydro-7-bromo-1-*O*-(*tert*-butyldiphenylsilyl)-7-deoxy-D-*glycero*-L-*galacto*-heptitol (63)
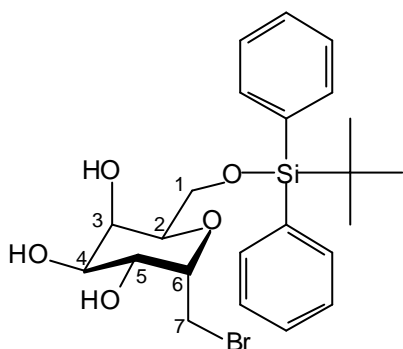
C$_{23}$H$_{31}$BrO$_5$Si [495.49]
Exact Mass   [494.11]

- colour and physical state: white solid

- M.p.: 77-78 °C ($CHCl_3$-petroleum ether)

- $[\boldsymbol{a}]_D^{23}$ = +50 (c 0.24, CH_2Cl_2)

- R$_f$: 0.42 ($CHCl_3$-MeOH 90:10)

- FAB-MS: *m/z* 413.2 [M+H-HBr]$^+$, 437.1 [M+Na-HBr]$^+$, 495.0 [M+H]$^+$, 517.0 [M+Na]$^+$

- IR (KBr): $\tilde{\boldsymbol{n}}$ = 703, 1079, 1110, 2930, 3405-3456 cm^{-1}

- ^{1}H NMR (HH-COSY, 200 MHz, CDCl$_3$):

δ = 1.07 (s, 9 H, (CH$_3$)$_3$CSi), 3.38 (m, 1H, 7-H), 3.50-3.55 (m, 2H), 3.65 (dd, 1H, 7-H´, J 3.4, 11.6), 3.80-3.91 (m, 2H, CH$_2$-1), 4.08-4.10 (m, 2H), 4.25 (m, 1H), 7.26-7.43 (m, 6H, aromatic), 7.66-7.73 (m, 4H, aromatic)

- ^{13}C NMR (APT, HETCOR, 50.29 MHz, CDCl$_3$):

δ = 19.54 (+, (CH$_3$)$_3$<u>C</u>Si), 27.25 (-, (<u>C</u>H$_3$)$_3$CSi), 28.24 (+, C-7), 64.04 (+, C-1), 69.45 (-), 70.29 (-), 71.15 (-), 71.59 (-), 76.24 (-), 128.39, 128.44, 130.51 (-, C^{Ar}), 133.18, 133.40 (+, SiC^{Ar}), 136.10, 136.22 (-, C^{Ar})

7.2.8 Preparation of the acceptor 53

AJ 2-08

To a solution of compound **63** (0.045 g, 0.091 mmol) in dry acetone (5 mL) 2,2-dimethoxypropane (2 mL) and *p*-toluenesulfonic acid monohydrate (0.009 g, cat) were added. The reaction mixture was stirred at RT for 2 h until the starting material was consumed (TLC, ethyl acetate-petroleum ether 1 : 1). The reaction mixture was neutralized by adding triethylamine, and concentrated. The residue was applied to FC, eluting with ethyl acetate-petroleum ether 1 : 1 to give **53** (0.043 g, 88 %).

2,6-Anhydro-7-bromo-1-*O*-(*tert*-butyldiphenylsilyl)-3,4-*O*-isopropylidene-7-deoxy-D-glycero-L-galacto-heptitol (53)
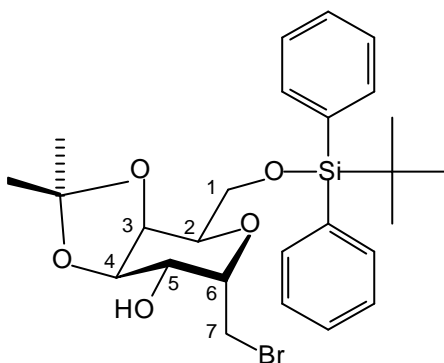
$C_{26}H_{35}BrO_5Si$ [535.55]
Exact Mass [534.14]

- colour and physical state: pale yellow oil

- $[\boldsymbol{a}]_D^{23}$ = +12.5 (c 0.32, CH_2Cl_2)

- R_f: 0.50 (ethyl acetate-petroleum ether 1:1)

- FAB-MS: *m/z* 535.1 [M+H]$^+$, 557.1 [M+Na]$^+$

- IR (Film): $\tilde{\boldsymbol{n}}$ = 1225, 1745 cm^{-1}

- ^{1}H NMR (HH-COSY, 200 MHz, CDCl$_3$):

δ = 1.06 (s, 9H, (CH$_3$)$_3$CSi), 1.36, 1.46 (2s, 6H, (CH$_3$)$_2$COO), 3.37 (dd, 1H, 7-H, J 5.4, 9.9), 3.51 (dd, 1H, 7-H´, J 8.8, 9.9), 3.78-3.84 (m, 2H, CH$_2$-1), 4.12 (m, 1H, 2-H), 4.17 (m, 1H, 6-H), 4.18 (m, 1H, 5-H), 4.36 (dd, 1H, 4-H, J 2.6, 7.6), 4.47 (dd, 1H, 3-H, J 1.6, 7.6), 7.33-7.43 (m, 6H, aromatic), 7.66-7.73 (m, 4H, aromatic)

- ^{13}C NMR (APT, HETCOR, 50.30 MHz, CDCl$_3$):

δ = 19.76 (+, (CH$_3$)$_3$CSi), 24.91, 27.11 (-, (CH$_3$)$_2$COO), 27.31 (-,(CH$_3$)$_3$CSi), 30.46 (+, C-7), 63.63 (+, C-1), 67.53 (-, C-5), 71.24 (-), 71.33 (-), 72.11 (-), 74.59 (-), 110.02 (+,

$(CH_3)_2\underline{C}OO)$, 128.05, 128.12, 130.10 (-, C^{Ar}), 134.04, 134.07 (+, SiC^{Ar}), 136.11, 136.15 (-, C^{Ar})

7.3 Disaccharide synthesis

7.3.1 Formation of disaccharide 68

AJ 2-12

A mixture of **53** (0.019 g, 0.035 mmol), **67** (0.025 g, 1.2 eq; 0.042 mmol) and activated 3 Å molecular sieves in anhydrous 1,2-dichloroethane (8 mL) was stirred for 1 h at RT under a dry argon atmosphere, then cooled to 0 °C. Trimethylsily triflate (1 µL, 0.1 eq; 0.004 mmol) was added, and the mixture was stirred at 0 °C for ca 10 min (TLC, ethyl acetate-petroleum ether 1 : 1). Triethylamine (0.1 mL) was added, and the mixture was diluted with CH_2Cl_2 (10 mL) and filtered. The molecular sieves were washed with CH_2Cl_2 (3 × 5 mL), and the combined filtrate was concentrated. The residue was chromatographed eluting with petroleum ether-ethyl acetate 1 : 1 to give **69** (0.028 g, 44 %).

3,4,6-Tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroacetamido)-ᵬ-D-glucopyranosyl-(1®5)-2,6-anhydro-7-bromo-7-deoxy-3,4-*O*-isopropylidene -1-*O*-(trimethylsilyl)-D-*glycero*-L-*galacto*-heptitol (69)
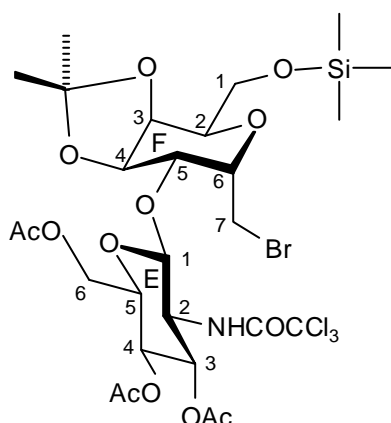
$C_{27}H_{41}BrCl_3NO_{13}Si$ [801.97]
Exact Mass [799.05]

- colour and physical state: white solid
- R_f: 0.35 (ethyl acetate-petroleum ether 1:1)
- FAB-MS: *m/z* 800 [M+H]⁺, 823 [M+Na]⁺
- ¹H NMR (HH-COSY, 600 MHz, CDCl₃):

δ = 0.06 (s, 9H, Si(CH$_3$)$_3$), 1.21, 1.38 (2s, 6H, (CH$_3$)$_2$COO), 1.92, 1.93, 2.00 (3s, 9H, 3 × CH$_3$COO), 3.25 (dd, 1H, 7^F-H, J 6.0, 9.9), 3.34 (dd, 1H, 7^F-H′, J 7.8, 9.9), 3.64 (m, 1H, 5^E-H), 3.73 (m, 1H, 2^F-H), 3.78 (dd, 1H, 4^F-H, J 3.4, 12.0), 3.82 (bs, 1H, 3^F-H), 3.97 (m, 1H, 2^E-H), 4.01 (m, 1H, 6^F-H), 4.05 (m, 1H, 5^F-H), 4.06-4.08 (2H, CH$_2$-1^F), 4.09 (m, 1H, 6^E-H), 4.21 (dd, 1H, 6^E-H′, J 4.5, 12.3), 4.90 (d, 1H, 1^E-H, J 8.4), 5.04 (dd, 1H, 4^{F}H, J 9.4, 9.9), 5.13 (dd, 1H, 3^E-H, J 9.4, 10.5), 6.77 (d, 1H, NHCOCCl$_3$, J 8.9)

- ^{13}C NMR (APT, HETCOR, 150.91 MHz, CDCl$_3$):

δ = 0.51 (-, Si(CH$_3$)$_3$), 20.93, 20.94, 21.16 (-, 3 × $\underline{C}$H$_3$COO), 24.62, 26.91 (-, ($\underline{C}$H$_3$)$_2$COO), 33.27 (+, C-7^F), 56.17 (-, C-2^E), 62.35 (+, C-6^E), 68.41 (-, C-3^F), 68.69 (-, C-4^E), 70.04 (+, C-1^F), 70.88 (-, C-4^F, C-2^F), 71.34 (-, C-6^F), 72.45 (-, C-3^E), 72.58 (-, C-5^E), 74.61 (-, C-5^F), 93.00 (+, NHCO$\underline{C}$Cl$_3$), 101.66 (-, C-1^E), 110.40 (+, (CH$_3$)$_2$$\underline{C}$OO), 162.55 (+, NH$\underline{C}$OCCl$_3$), 169.89, 171.34 (+, 3 × CH$_3$$\underline{C}$OO)

AJ 2-19

A mixture of **53** (0.034 g, 0.064 mmol), **67** (0.045 g, 1.2 eq; 0.077 mmol) and activated 3 Å molecular sieves in anhydrous 1,2-dichloroethane (5 mL) was stirred for 1 h at RT under an argon atmosphere, then cooled to 0 °C. Trimethylsilyl triflate (1.5 µL, 0.1 eq, 0.006 mmol) was added, and the mixture was stirred at 0 °C for ca 5 min (TLC, ethyl acetate-petroleum ether 1 : 1). Triethylamine (0.1 mL) was added to stop the reaction, and the mixture was diluted with CH$_2$Cl$_2$ (10 mL) and filtered. The molecular sieves were washed with CH$_2$Cl$_2$ (3 × 5 mL) and the combined filtrate was concentrated. The residue was subjected to FC eluting with petroleum ether-ethyl acetate 2 : 1 to furnish **68** (0.048 g, 79 %).

3,4,6-Tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroacetamido)-β-D-glucopyranosyl-(1→5)-2,6-anhydro-7-bromo-7-deoxy-3,4-*O*-isopropylidene-1-*O*-(*tert*-butyldiphenylsilyl)-D-*glycero*-L-*galacto*-heptitol (68)
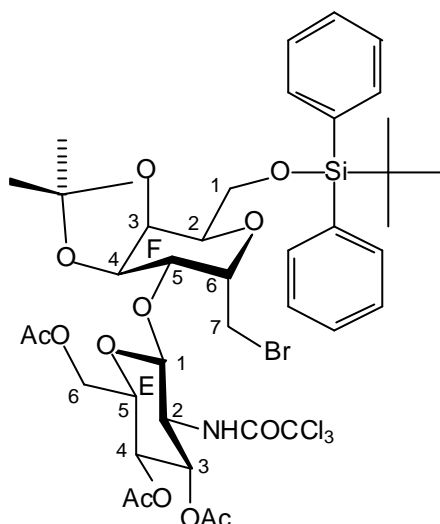
$C_{40}H_{51}BrCl_3NO_{13}Si$ [968.19]
Exact Mass   [965.14]

- colour and physical state: white solid

- M.p.: 227-228 °C (CHCl$_3$-petroleum ether), decomposition

- $[\boldsymbol{a}]_D^{23}$ = -16 (c 0.25, CH$_2$Cl$_2$)

- R$_f$: 0.36 (ethyl acetate-petroleum ether 1:1)

- FAB-MS: *m/z* 966.1 [M+H]$^+$, 988.1 [M+Na]$^+$

- ESI-MS: *m/z* calculated [M+NH$_4$]$^+$ 983.17169, found 983.17325

- IR (KBr): $\tilde{\boldsymbol{n}}$ = 1047, 1108, 1237, 1526, 1750, 3437 cm^{-1}

- ^{1}H NMR (HH-COSY, 300 MHz, CDCl$_3$):

δ = 1.03 (s, 9H, SiC(CH$_3$)$_3$), 1.31, 1.46 (2s, 6H, (C$\underline{H}$$_3$)$_2$COO), 2.05 (s, 6H, 2 × CH$_3$COO), 2.09 (s, 3H, CH$_3$COO), 3.30 (dd, 1H, 7^F-H, J 5.9, 10.0), 3.55 (dd, 1H, 7^F-H´, J 8.1, 10.0), 3.75 (m, 1H, 5^E-H), 3.77-3.80 (m, 2H, CH$_2$-1^F), 3.94 (m, 1H, 2^E-H), 4.02 (m, 1H, 2^F-H), 4.10 (m, 1H, 5^F-H), 4.15 (m, 1H, 6^F-H), 4.21 (m, 1H, 4^F-H), 4.24-4.27 (m, 2H, CH$_2$-6^E), 4.47 (dd, 1H, 3^F-H, J 1.4, 7.1), 4.89 (d, 1H, 1^E-H, J 8.5), 5.14 (dd, 1H, 4^{E}H, J 9.6, 9.9), 5.39 (dd, 1H, 3^E-H, J 9.6, 10.7), 7.02 (d, 1H, NHCOCCl$_3$, J 8.8), 7.35-.7.41 (m, 6H, aromatic), 7.65-7.70 (m, 4H, aromatic)

- ^{13}C NMR (APT, HETCOR, 75.45 MHz, CDCl$_3$):

δ = 19.51 (+, Si$\underline{C}$(CH$_3$)$_3$), 20.85, 20.91, 21.09 (-, 3 × $\underline{C}$H$_3$COO), 24.62, 27.15 (-, ($\underline{C}$H$_3$)$_2$COO), 27.06 (-, SiC($\underline{C}$H$_3$)$_3$), 30.33 (+, C-7^F), 56.42 (-, C-2^E), 62.29 (+, C-6^E), 63.05 (+, C-1^F), 68.55 (-, C-4^E), 70.19 (-), 71.09 (-, C-6^F), 71.69 (-, C-3^E), 71.96 (-, C-3^F), 72.37 (-, C-4^F), 72.49 (-, C-5^E), 75.29 (-), 93.00 (+, NHCO$\underline{C}$Cl$_3$), 99.92 (-, C-1^E), 109.86 (+, (CH$_3$)$_2$$\underline{C}$OO), 127.87, 127.93, 129.87, 129.91 (-, C-^{Ar}), 133.60, 133.67 (+, SiC^{Ar}), 135.86, 135.97 (-, C-^{Ar}), 162.19 (+, NH$\underline{C}$OCCl$_3$), 169.60, 171.05, 171.34 (+, 3 × CH$_3$$\underline{C}$OO)

7.3.2 Cleavage of the silyl ethers in 68 and 69

7.3.2.1 Reaction of compound 69 with Dowex resin

AJ 2-14

To a solution of **69** (0.021 g, 0.027 mmol) in dry methanol (7 mL), Dowex 50-W X-8 (H$^+$) resin (0.060 g) was added at RT. The mixture was stirred for 1 h and the reaction progress was monitored by TLC (ethyl acetate-petroleum ether 1 : 1). The reaction mixture was filtered, the resin was washed with CH$_2$Cl$_2$ (3 × 5 mL), and the combined filtrate was evaporated. The residue was purified by FC eluting with petroleum ether-ethyl acetate 1 : 1 to furnish after solvent evaporation **71** (0.018 g, 95 %).

7.3.2.2 Reaction of compound 68 with TBAF

AJ 2-21

To a solution of **68** (0.300 g, 0.311 mmol) in THF (30 mL) a TBAF solution (1.0 M in THF, 0.37 mL, 1.2 eq; 0.373 mmol) was added. The mixture was stirred at RT for 21 h (TLC, ethyl acetate-petroleum ether 1 : 1). Water (15 mL) was added and the mixture was extracted with chloroform (5 × 10 mL). The combined organic layers were dried, and the solvent was evaporated. The residue was chromatographed (FC) eluting with petroleum ether-ethyl acetate 1 : 1 to give **71** (0.201 g, 89 %).

3,4,6-Tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroacetamido)-b-D-glucopyranosyl-(1®5)-2,6-anhydro-7-bromo-7-deoxy-3,4-*O*-isopropylidene-D-*glycero*-L-*galacto*-heptitol (71)

C$_{24}$H$_{33}$BrCl$_3$NO$_{13}$ [729.79]
Exact Mass   [727.02]

- colour and physical state: white solid

- M.p.: 230-231 °C (CHCl$_3$-petroleum ether), decomposition.

- $[\mathbf{a}]_D^{23}$ = -30 (c 0.20, CH$_2$Cl$_2$)

- R$_f$: 0.13 (ethyl acetate-petroleum ether 1:1)

- FAB-MS: *m/z* 728.0 [M+H]$^+$, 750.0 [M+Na]$^+$

- ESI-MS: *m/z* calculated [M+Na]$^+$ 750.00931, found 750.00868

- IR (KBr): $\tilde{\mathbf{n}}$ = 1044, 1109, 1162, 1236, 1374, 1529, 1750, 2923, 3393, 3492 cm^{-1}

- ^{1}H NMR (HH-COSY, 200 MHz, pyridine-d$_5$):

δ = 1.32, 1.54 (2s, 6H, (CH$_3$)$_2$COO), 2.01, 2.02. 2.10 (3s, 9H, 3 × CH$_3$COO), 3.79 (dd, 1H, 7^F-H, J 7.0, 10.3), 3.92 (dd, 1H, 7^F-H′, J 6.6, 10.3), 4.05 (ddd, 1H, 5^E-H, J 2.2, 4.8, 9.9), 4.21 (m, 1H, 1^F-H), 4.24 (m, 1H, 1^F-H′), 4.35 (m, 1H, 2^E-H), 4.41 (m, 1H, 6^E-H), 4.51 (m, 1H, 3^F-H), 4.53 (m, 1H, 6^E-H′), 4.58 (m, 1H, 2^F-H), 4.58 (m, 1H, 6^F-H), 4.74 (dd, 1H, 4^F-H, J 3.1, 7.2), 4.88 (dd, 1H, 5^F-H, J 1.3, 7.2), 5.51 (m, 1H, 4^{E}H), 5.67 (d, 1H, 1^E-H, J 8.4), 6.14 (dd, 1H, 3^E-H, J 9.2, 10.6), 10.67 (d, 1H, NHCOCCl$_3$, J 8.1)

- ^{13}C NMR (APT, HETCOR, 50.29 MHz, pyridine-d$_5$):

δ = 20.49, 20.65 (-, 3 × CH$_3$COO), 24.55, 27.09 (-, (CH$_3$)$_2$COO), 31.86 (+, C-7^F), 57.18 (-, C-2^E), 62.08 (+, C-1^F), 62.43 (+, C-6^E), 69.61 (-, C-4^E), 71.73 (-, C-3^E, C-2^F), 71.96 (-, C-6^F), 72.39 (-, C-5^E), 72.82 (-, C-4^F), 72.92 (-, C-5^F), 75.51 (-, C-3^F), 93.73 (+, NHCOCCl$_3$), 99.37 (-, C-1^E), 109.78 (+, (CH$_3$)$_2$COO), 163.28 (+, NHCOCCl$_3$), 169.81, 170.36, 170.43 (+, 3 × CH$_3$COO)

7.3.3 Preparation of the uronamide 72

AJ 2-27

a. Tempo oxidation

To a mixture of **71** (0.068 g, 0.094 mmol), TEMPO (0.016 g, 1.06 eq; 0.099 mmol), tetrabutylammonium chloride (0.026 g, 1.0 eq; 0.094 mmol), potassium bromide (0.011 g, 1.0 eq; 0.095 mmol) and dichloromethane (2 mL) at 0 °C, a mixture of satd. aq. sodium chloride (1 mL), satd. aq. sodium hydrogencarbonate (1.1 mL), and satd. aq. sodium hypochlorite (1 mL, 12-14 % active chlorine) was added slowly. The reaction mixture was vigorously stirred at RT for 3 h (TLC, petroleum ether-CHCl$_3$-EtOH 5 : 2 : 2). The pH was adjusted to 2-3 with conc.

HCl. The organic layer was separated and the aqueous layer was extracted with dichloromethane (5×10 mL). The combined organic extracts were dried, and the solvent was removed.

b. Sodium chlorite oxidation

The crude product of the TEMPO oxidation (max 0.094 mmol), sodium chlorite (0.085 g, 10 eq; 0.94 mmol), and sodium dihydrogenphosphate monohydrate (0.097 g, 7.5 eq; 0.70 mmol) were placed in a reaction flask, and with stirring successively 2-methyl-2-butene (0.4 mL), *t*-butanol (1.8 mL) and water (0.7 mL) were added. The reaction mixture was stirred at RT for 4 h (TLC, petroleum ether-$CHCl_3$-EtOH 5 : 2 : 2), then it was diluted with water (2 mL) and dichloromethane (5 mL). The organic layer was separated, and the aqueous layer was extracted with dichloromethane (4×10 mL). The aqueous layer was adjusted to pH 2 with conc. HCl, and extracted with dichloromethane (3×10 mL). The combined organic extracts were dried, and the solvent was removed. The residue was dried at 0.1 mbar for ca 2 h.

c. Amide formation according to Staab

The crude acid (max 0.094 mmol), and CDI (0.039 g, 2.5 eq; 0.24 mmol) were dissolved in dry dichloromethane (10 mL), and the mixture was stirred at RT for 5 h. Through this solution at 0 °C, gaseous ammonia was bubbled for 40 min (TLC, petroleum ether-$CHCl_3$-EtOH 5 : 2 : 2), then the mixture was stirred at RT for 1 h. The solvent was evaporated, and the residue was chromatographed eluting with petroleum ether-$CHCl_3$-EtOH 8 : 2 : 2 to furnish amide **72** (0.068g, 98 % based on **71**).

3,4,6-Tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroacetamido)-β-D-glucopyranosyl-(1→5)-2,6-anhydro-7-bromo-7-deoxy-3,4-*O*-isopropylidene-1-*O*-D-*glycero*-L-*galacto*-heptonamide (72)

$C_{24}H_{32}BrCl_3N_2O_{13}$ [742.78]
Exact Mass   [740.02]

- colour and physical state: white solid
- M.p.: 152-153 °C ($CHCl_3$-petroleum ether), decomposition

- $[\boldsymbol{a}]_D^{23}$ = -80 (c 0.15, CH_2Cl_2)

- R_f: 0.49 (petroleum ether-$CHCl_3$-EtOH 5:2:2)

- FAB-MS: *m/z* 741.0 $[M+H]^+$, 763.0 $[M+Na]^+$

- ESI-MS: *m/z* $[M+H]^+$ (calculated 741.022261, found 741.02190), $[M+Na]^+$ (calculated 763.00456, found 763.00636)

- IR (KBr): $\tilde{\boldsymbol{n}}$ = 1046, 1108, 1148, 1233, 1374, 1531, 1685, 1750, 3378, 3475 cm^{-1}

- ^{1}H NMR (HH-COSY, 600 MHz, $CDCl_3$):

δ = 1.33, 1.48 (2s, 6H, $(C\underline{H}_3)_2COO$), 2.03, 2.05, 2.11 (3s, 9H, $3 \times C\underline{H}_3COO$), 3.45, (dd, 1H, 7^F-H, J 6.5, 10.2), 3.57 (dd, 1H, 7^F-H´, J 7.1, 10.2), 3.77 (ddd, 1H, 5^E-H, J 2.5, 4.7, 9.9), 3.95 (m, 1H, 2^E-H), 4.07 (m, 1H, 5^F-H), 4.21 (dd, 1H, 6^E-H, J 2.5, 12.2), 4.25 (dd, 1H, 6^E-H´, J 4.7, 12.2), 4.29 (m, 1H, 6^F-H), 4.37 (d, 1H, 2^F-H, J 2.1), 4.41 (dd, 1H, 4^F-H, J 2.4, 7.6), 4.70 (dd, 1H, 3^F-H, J 1.6, 7.3), 4.95 (d, 1H, 1^E-H, J 8.4), 5.12 (dd, 1H, 4^EH, J 9.4, 9.9), 5.39 (dd, 1H, 3^E-H, J 9.4, 10.5), 6.07, 6.72 (2d, 2H, H_2NCO, J 3.7), 7.66 (d, 1H, $NHCOCCl_3$, J 8.4)

- ^{13}C NMR (APT, HETCOR, HMBC, HMQC, 150.92 MHz, $CDCl_3$):

δ = 20.99, 21.05, 21.21 (-, $3 \times \underline{C}H_3COO$), 24.65, 26.97 (-, $(\underline{C}H_3)_2COO$), 31.30 (+, C-7^F), 56.60 (-, C-2^E), 62.36 (+, C-6^E), 68.75 (-, C-4^E), 71.41 (-, C-6^F), 71.49 (-, C-2^F), 71.55 (-, C-4^F), 71.84 (-, C-3^E), 72.75 (-, C-5^E), 73.04 (-, C-3^F), 74.08 (-, C-5^F), 92.65 (+, $NHCO\underline{C}Cl_3$), 99.48 (-, C-1^E), 110.85 (+, $(CH_3)_2\underline{C}OO$), 162.97 (+, $NH\underline{C}OCCl_3$), 169.92, 171.26, 171.49 (+, $3 \times CH_3\underline{C}OO$), 172.28 (+, H_2NCO)

7.3.4 Removal of the isopropylidene group from 72

AJ 2-29

Compound **72** (0.015 g, 0.02 mmol) was dissolved in 80 % AcOH-H_2O (10 mL), and stirred at RT for 17 h, after which no reaction was observed (TLC, petroleum ether-$CHCl_3$-EtOH 5 : 2 : 2). The solution was coevaporated 3 times with toluene (2 mL). The residue was submitted to FC, eluting with petroleum ether-$CHCl_3$-EtOH 8 : 2 : 2 to recover the starting material (0.013 g)

AJ 2-30

Compound **72** (0.040 g, 0.054 mmol) was dissolved in CH_2Cl_2 (10 mL) and trifluoroacetic acid (21 ìL, 5 eq; 0.270 mmol) was added. The reaction mixture was stirred at RT for 2 h (TLC, petroleum ether-$CHCl_3$-EtOH 5 : 2 : 2). The solution was coevaporated 3 times with toluene (3 mL). The residue was submitted to FC eluting with petroleum ether-$CHCl_3$-EtOH 8 : 2 : 2 to give **73** (0.034 g, 89 %).

3,4,6-Tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroacetamido)-β-D-glucopyranosyl-(1→5)-2,6-anhydro-7-bromo-7-deoxy-D-*glycero*-L-*galacto*-heptonamide (73)

$C_{21}H_{28}BrCl_3N_2O_{13}$ [702.72]
Exact Mass [699.98]

- colour and physical state: white solid

- M.p.: 241-242 °C (EtOH-petroleum ether), decomposition

- $[\alpha]_D^{23}$ = -50 (c 0.12, CH_2Cl_2)

- R_f: 0.23 (petroleum ether-$CHCl_3$-EtOH 5:2:2)

- FAB-MS: *m/z* 700.9 [M+H]$^+$, 722.9 [M+Na]$^+$

- ESI-MS: *m/z* calculated [M+Na]$^+$ 722.97326, found 722.97544

- IR (KBr): $\tilde{n}$ = 1635, 1678, 1746, 3443 cm^{-1}

- ^{1}H NMR (HH-COSY, 400 MHz, pyridine-d$_5$):

δ = 1.96, 1.99, 2.07 (3s, 9H, 3 × C$\underline{H}_3$COO), 3.82 (ddd, 1H, 5^E-H, J 2.5, 4.6, 9.8), 4.12 (dd, 1H, 7^F-H, J 3.7, ca 11.7), 4.25 (dd, 1H, 7^F-H´, J 3.0, ca 11.7), 4.42-4.50 (m, 2H, CH$_2$-6^E), 4.51 (m, 1H, 5^F-H), 4.65 (dd, 1H, 2^E-H, J 8.6, 10.6), 4.79 (d, 1H, 3^F-H, J 2.4), 4.83 (m, 1H, 2^F-H), 4.91 (m, 1H, 6^F-H), 5.01 (m, 1H, 4^F-H), 5.49 (m, 1H, 4^{E}H), 5.58 (d, 1H, 1^E-H, J 8.4), 5.95 (dd, 1H, 3^E-H, J 9.3, 10.6), 8.00, 8.78 (2bs, 2 H, H$_2$NCO), 10.63 (d, 1H, N$\underline{H}$COCCl$_3$, J 8.6)

- ^{13}C NMR (APT, HMBC, HMQC 100.58 MHz, pyridine-d$_5$):

δ = 20.28, 20.37, 20.48 (-, 3 × $\underline{C}$H$_3$COO), 30.83 (+, C-7^F), 56.64 (-, C-2^E), 62.15 (+, C-6^E), 69.40 (-, C-4^E), 69.86 (-, C-4^F), 70.07 (-, C-5^F), 72.00 (-, C-5^E), 72.55 (-, C-3^E), 73.39 (-,

C-3^F), 75.11 (-, C-6^F), 78.19 (-, C-2^F), 93.76 (+, NHCO$\underline{C}$Cl$_3$), 101.46 (-, C-1^E), 163.18 (+, NH$\underline{C}$OCCl$_3$), 169.63, 170.30, 170.38 (+, 3 × CH$_3$$\underline{C}$OO), 173.59 (+, H$_2$NCO)
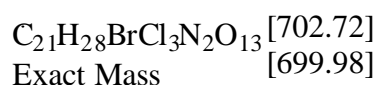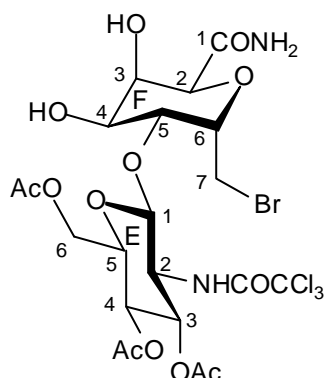
7.3.5 Preparation of cyclic carbonate 76

AJ 2-34

To a solution of **73** (0.313 g, 0.45 mmol) in dry CH$_2$Cl$_2$ (20 mL), CDI (0.146 g, 2.0 eq; 0.90 mmol) and DMAP (0.055 g, 1.0 eq; 0.45 mmol) were added. The reaction mixture was stirred at RT for 2 h (TLC, petroleum ether-CHCl$_3$-EtOH 5 : 2 : 2). Evaporation, FC eluting with petroleum ether-CHCl$_3$-EtOH 8 : 2 : 2 afforded **76** (0.272 g, 84 %).

3,4,6-Tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroacetamido)-β-D-glucopyranosyl-(1→5)-2,6-anhydro-7-bromo-3,4-*O*-carbonyl-7-deoxy-D-*glycero*-L-*galacto*-heptonamide (76)
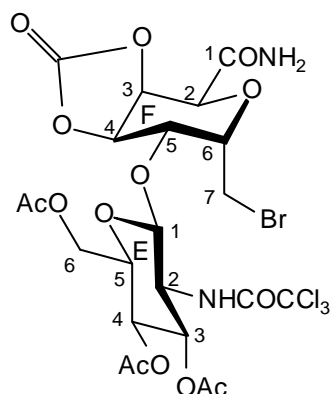
C$_{22}$H$_{26}$BrCl$_3$N$_2$O$_{14}$ [728.72]
Exact Mass   [725.96]

- colour and physical state: white solid

- M.p: 153-154°C (CHCl$_3$-petroleum ether)

- $[\alpha]_D^{23}$ = -13.33 (c 0.15, CH$_2$Cl$_2$)

- R$_f$: 0.41 (petroleum ether-CHCl$_3$-EtOH 5:2:2)

- FAB-MS: *m/z* 727.1 [M+H]$^+$,749.1 [M+Na]$^+$

- ESI-MS: *m/z* [M+Na]$^+$ (calculated 748.95252, found 748.95186), [M+K]$^+$ (calculated 764.92646, found 764.92556)

- IR (KBr): $\tilde{n}$ = 1047, 1069, 1236, 1696, 1749, 1816, 3420, 3439 cm^{-1}

- ^{1}H NMR (HH COSY, 400 MHz, pyridine-d$_5$):

δ = 2.02, 2.03, 2.12 (3s, 9H, 3 × C$\underline{H}_3$COO), 3.76 (dd, 1H, 7^F-H, J 7.9, 10.9), 3.92 (dd, 1H, 7^F-H′, J 5.9, 10.9), 4.12 (ddd, 1H, 5^E-H, J 2.6, 4.8, 9.9), 4.38 (dd, 1H, 2^E-H, J 8.1, 10.6), 4.47 (dd, 1H, 6^E-H, J 2.6 12.0), 4.51 (dd, 1H, 6^E-H′, J 4.8, 12.0), 4.63 (ddd, 1H, 6^F-H, J 3.7,

5.9, 7.9), 4.93 (t, 1H, 5^F-H, J 3.7), 5.01 (d, 1H, 2^F-H, J 1.7), 5.53 (m, 1H, 4^{E}H), 5.59 (m, 1H, 4^F-H), 5.82 (d, 1H, 1^E-H, J 8.4), 5.95 (dd, 1H, 3^F-H, J 1.7, 8.4), 6.17 (dd, 1H, 3^E-H, J 9.2, 10.6), 7.92, 8.90 (2s, 2H, H$_2$NCO), 10.78 (d, 1H, NHCOCCl$_3$, J 8.6)

- ^{13}C NMR (APT, HMBC, HMQC, 100.57 MHz, pyridine-d$_5$):

δ = 21.60, 21.77 (-, 3 × <u>C</u>H$_3$COO), 31.79 (+, C-7^F), 58.11 (-, C-2^E), 63.42 (+, C-6^E), 70.64 (-, C-4^E), 72.02 (-, C-2^F), 72.72 (-, C-3^E), 73.28 (-, C-5^F), 73.43 (-, C-4^F), 73.76 (-, C-5^E), 74.66 (-, C-6^F), 76.52 (-, C-3^F), 94.70 (+, NHCO<u>C</u>Cl$_3$), 100.08 (-, C-1^E), 155.16 (+, OCOO), 164.80 (+, NH<u>C</u>OCCl$_3$), 170.71 (+, H$_2$NCO), 171.00, 171.57, 171.60 (+, 3 × CH$_3$<u>C</u>OO)

7.3.6 Synthesis of urethane 74

7.3.6.1 Trial based on TAI

AJ 2-32

A solution of **73** (0.046 g, 0.066 mmol) in dry dichloromethane (8 mL) was refluxed over activated 3 Å molecular sieves under an argon atmosphere for 2 h, then it was cooled to -5 °C. Trichloroacetylisocyanate, TAI, (5   L, 1.0 eq, 0.071 mmol) was added, and the solution was stirred at -5 °C for 6 h, during which TLC (petroleum ether-CHCl$_3$-EtOH 5 : 2 : 2) showed no reaction. The temperature was raised gradually to RT, with addition of excess of TAI (10 eq), but also no reaction was observed. Excess reagent was destroyed with MeOH (2 mL). Solvents were evaporated, and the residue was taken to a FC, and eluted with petroleum ether-CHCl$_3$-EtOH 8 : 2 : 2. Solvent evaporation provided reactant recovery (0.039 g).

7.3.6.2 Trial based on phenyl carbonate

AJ 2-36

A solution of **73** (0.020 g, 0.028 mmol) and DMAP (0.004 g, 1.0 eq; 0.028 mmol) in dry CH$_2$Cl$_2$ (5 mL) was cooled to 0 °C, then dry triethylamine (4 µL, 1.0 eq; 0.028 mmol) was added. Phenyl chloroformate (3.8 µL, 1.1 eq, 0.031 mmol) was added dropwise at 0 °C, and the reaction mixture was stirred at RT for 1 h (TLC, petroleum ether-CHCl$_3$-EtOH 5 : 2 : 2). The

solvent was evaporated, and the residue was separated by FC, eluting with petroleum ether-CHCl$_3$-EtOH 8 : 2 : 2 to give compound **76** (0.015 g, 72 %).

7.3.6.3 Ring opening of 76 with ammonia

AJ 2-35

Dry gaseous ammonia was bubbled through a solution of **76** (0.313 g, 0.43 mmol) in dry ethanol (20 mL) at 0 °C for 1 h (TLC, petroleum ether-CHCl$_3$-EtOH 5 : 2 : 2). The solvent was evaporated, and the residue was separated by repeated MPLC eluting with petroleum ether-ethyl acetate-EtOH 5 : 2 : 1 to give the two isomers **74** (0.058 g, 18 %) and **77** (0.064 g, 20 %).

AJ 2-45

Dry ammonia gas was bubbled through a solution of **76** (0.150 g, 0.207 mmol) in CH$_2$Cl$_2$ (10 mL) at 0 °C for 6 h (TLC, petroleum ether-CHCl$_3$-EtOH 5 : 2 : 2). The solvent was evaporated, and the residue was separated using repeated MPLC eluting with petroleum ether-ethyl acetate-EtOH 5 : 2 : 1 to give **74** (0.095 g, 62 %) and **77** (0.032 g, 21 %).

AJ 2-46

76 (0.250 g, 0.344 mmol) was dissolved at 0 °C in 0.5 M ethanolic ammonia (10 mL), and stirred at this temperature for 5 h (TLC, petroleum ether-CHCl$_3$-EtOH 5 : 2 : 2). The solvent was evaporated, and the residue was separated by repeated MPLC eluting with petroleum ether-ethyl acetate-EtOH 5 : 2 : 1 to leave **74** (0.008 g, 3 %) and **77** (0.122 g, 48 %).

3,4,6-Tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroacetamido)-β-D-glucopyranosyl-(1→5)-2,6-anhydro-7-bromo-4-carbamoyl-7-deoxy-D-*glycero*-L-*galacto*-heptonamide (74)
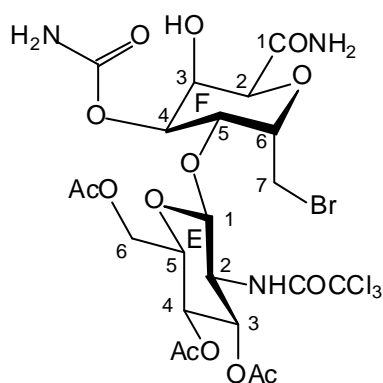
$C_{22}H_{29}BrCl_3N_3O_{14}$ [745.74]
Exact Mass   [742.99]

- colour and physical state: white solid

- M.p.: 261-262 °C (EtOH-petroleum ether), decomposition

- R_f: 0.14 (petroleum ether-CHCl$_3$-EtOH 5:2:2)

- FAB-MS: *m/z* 744.2 [M+H]$^+$, 766.2 [M+Na]$^+$

- ESI-MS: *m/z* calculated [M+H]$^+$ 743.99712, found 743.99616

- IR (KBr): $\tilde{n}$ = 1690, 1706, 1743, 3310-3521 cm^{-1}

- ^{1}H NMR (HH-COSY, 400 MHz, pyridine-d$_5$):

δ = 2.00, 2.03, 2.10, (3s, 9H, 3 × CH$_3$COO), 3.84 (m, 1H, 5^E-H), 4.10 (dd, 1H, 7^F-H, J 3.3, 11.4), 4.30 (dd, 1H, 7^F-H´, J < 1, 11.4), 4.31 (m, 1H, 6^E-H,), 4.42 (m, 1H, 6^E-H´), 4.43 (m, 1H, 2^E-H), 4.84 (d, 1H, 2^F-H, J 1.8), 4.96 (m, 1H, 6^F-H), 5.03 (m, 1H, 5^F-H), 5.40 (dd, 1H, 3^F-H, J 1.8, 3.5), 5.50 (dd, 1H, 4^{E}H, J 9.5, 9.9), 5.57 (d, 1H, 1^E-H, J 8.4), 5.68 (dd, 1H, 4^F-H, J 3.5, 8.3), 6.08 (dd, 1H, 3^E-H, J 9.5, 10.6), 7.50 (bs, 2H, H$_2$NCOO), 7.80, 8.49 (2s, 2H, H$_2$NCO), 10.57 (d, 1H, NHCOCCl$_3$, J 8.1)

- ^{13}C NMR (APT, HMBC, HMQC, 100.58 MHz, pyridine-d$_5$):

δ = 20.12, 20.21, 20.31 (-, 3 × $\underline{C}$H$_3$COO), 30.32 (+, C-7^F), 56.76 (-, C-2^E), 61.83 (+, C-6^E), 67.63 (-, C-3^F), 69.27 (-, C-4^E), 71.58 (-, C-3^E), 71.73 (-, C-5^E), 72.71 (-, C-4^F), 73.17 (-, C-2^F), 75.11 (-, C-5^F), 75.66 (-, C-6^F), 93.46 (+, NHCO$\underline{C}$Cl$_3$), 100.57 (-, C-1^E), 156.83 (+, H$_2$NCOO), 162.89 (+, NH$\underline{C}$OCCl$_3$), 169.43, 170.06, 170.14 (+, 3 × CH$_3$$\underline{C}$OO), 171.98 (+, H$_2$NCO)

3,4,6-Tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroacetamido)-β-D-glucopyranosyl-(1→5)-2,6-anhydro-7-bromo-3-carbamoyl-7-deoxy-D-*glycero*-L-*galacto*-heptonamide (77)

$C_{22}H_{29}BrCl_3N_3O_{14}$ [745.74]
Exact Mass   [742.99]

- colour and physical state: white solid

- M.p.: 257-258 °C (EtOH-petroleum ether), decomposition

- R_f: 0.12 (petroleum ether-CHCl$_3$-EtOH 5:2:2)

- FAB-MS: *m/z* 744.2 [M+H]$^+$, 766.2 [M+Na]$^+$

- ESI-MS: *m/z* calculated [M+H]$^+$ 743.99712, found 743.99605

- IR (KBr): $\tilde{n}$ = 1721, 3306-3445 cm^{-1}

- ^{1}H NMR (HH-COSY, 400 MHz, pyridine-d$_5$):

δ = 2.01, 2.06, 2.10, (3s, 9H, 3 × CH$_3$COOO), 3.84 (m, 1H, 5^E-H), 4.17 (dd, 1H, 7^F-H, J 3.2, 11.5), 4.29 (dd, 1H, 7^F-H´, J < 1, 11.5), 4.34 (m, 1H, 6^E-H), 4.47 (dd, 1H, 6^E-H, J 4.7, 12.2), 4.64 (m, 1H, 4^F-H), 4.65 (m, 1H, 5^F-H), 4.71 (ddd, 1H, 2^E-H, J 8.5, 8.8, 10.4), 4.95 (m, 1H, 6^F-H), 5.47 (d, 1H, 1^E-H, J 8.5), 5.01 (d, 1H, 2^F-H, J 1.7), 5.52 (dd, 1H, 4^{E}H, J 9.6, 9.9), 5.93 (dd, 1H, 3^E-H, J 9.6, 10.4), 6.47 (bs, 1H, 3^F-H), 7.60 (bs, 2H, H$_2$NCOO), 7.88, 8.54 (2s, 2H, H$_2$NCO), 10.73 (d, 1H, NHCOCCl$_3$, J 8.8)

- ^{13}C NMR (APT, HMBC, HMQC, 100.58 MHz, pyridine-d$_5$):

δ = 20.28, 20.40, 20.53 (-, 3 × CH$_3$COO), 29.83 (+, C-7^F), 56.48 (-, C-2^E), 62.24 (+, C-6^E), 68.71 (-, C-4^F), 69.40 (-, C-4^E), 71.93 (-, C-2^F), 72.14 (-, C-5^E), 72.54 (-, C-3^F), 72.71 (-, C-3^E), 76.52 (-, C-6^F), 78.58 (-, C-5^F), 93.79 (+, NHCOCCl$_3$), 102.20 (-, C-1^E), 157.78 (+, H$_2$NCOO), 163.22 (+, NHCOCCl$_3$), 169.60, 170.32, 170.45 (+, 3 × CH$_3$COO), 170.84 (+, H$_2$NCO)

7.3.7 Dehalogenation of the trichloroacetamido group

7.3.7.1 Trials based on 76

7.3.7.1.1 Hydrolysis with LiOH

AJ 2-43

Compound **76** (0.015 g, 0.021 mmol) was treated with 0.5 M LiOH in THF- MeOH 1 : 1 (10 mL) for 2 h (TLC, petroleum ether-$CHCl_3$-EtOH 5 : 2 : 2). The solution was diluted with EtOH (10 mL), cooled to 0 °C and neutralized with dry acetic acid. The solution was coevaporated with MeOH (3 × 2 mL). The crude product was dissolved in dry pyridine (5 mL) and stirred overnight with Ac_2O (5 mL). The solution was coevaporated with toluene, and the residue was purified by FC eluting with petroleum ether-$CHCl_3$-EtOH 8 : 2 : 2 to give **80** (0.012 g, 73 %).

3,4,6-Tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroacetamido)-β-D-glucopyranosyl-(1→5)-3,4-di-*O*-acetyl-2,6-anhydro-7-bromo-7-deoxy-D-*glycero*-L-*galacto*-heptonamide (80)

$C_{25}H_{32}BrCl_3N_2O_{15}$ [786.80]
Exact Mass   [784.00]

- colour and physical state: white solid

- $[\alpha]_D^{23}$ = +61.54 (c 0.13, CH_2Cl_2)

- R_f: 0.44 (petroleum ether-$CHCl_3$-EtOH 5:2:2)

- ESI-MS: *m/z* calculated $[M+Na]^+$ 806.99439, found 806.99427

- IR (KBr): $\tilde{n}$ = 1043, 1234, 1749, 3558 cm^{-1}

- ^{1}H NMR (HH-COSY, 400 MHz, pyridine-d_5):

δ = 2.01, 2.02, 2.05, 2.13, 2.21 (5s, 15H, 5 × CH_3COO), 4.05 (m, 1H, 5^E-H), 4.15 (dd, 1H, 7^F-H, J 3.3, 11.7), 4.27 (m, 1H, 2^E-H), 4.43-4.47 (m, 2H, CH_2-6^E), 4.54 (dd, 1H, 7^F-H′, J < 1, 11.7), 4.90 (m, 1H, 5^F-H), 5.07 (m, 1H, 6^F-H), 5.09 (d, 1H, 2^F-H, J 1.5), 5.52 (dd, 1H, 4^E-H, J 9.2, 9.9), 5.77 (d, 1H, 1^E-H, J 8.1), 5.87 (dd, 1H, 4^F-H, J 3.6, 10.6), 6.20 (dd, 1H, 3^E-H, J 9.2, 10.6), 6.57 (dd, 1H, 3^F-H, J 1.5, 3.6), 8.02, 8.84 (2s, 2H, H_2NCO), 10.67 (d, 1H, NHCOCCl₃, J 7.7)

- ^{13}C NMR (APT, HMBC, HMQC, 100.58 MHz, pyridine-d_5):

δ = 20.04, 20.08, 20.12, 20.26, 20.94 (-, 5 × $\underline{C}H_3COO$), 29.45 (+, C-7^F), 57.24 (-, C-2^E), 62.20 (+, C-6^E), 69.52 (-, C-4^E), 69.77 (-, C-3^F), 70.22 (-, C-4^F), 70.94 (-, C-2^F), 71.38 (-,

C-3^E), 72.24 (-, C-5^E), 75.04 (-, C-5^F), 76.79 (-, C-6^F), 93.00 (+, NHCO$\underline{C}$Cl$_3$), 100.85 (-, C-1^E), 162.67, 169.44, 169.84, 169.90, 169.98, 170.08 (+, 5 $\times$ CH$_3$$\underline{C}OO, NH\underline{C}$OCCl$_3$, H$_2N\underline{C}$O)
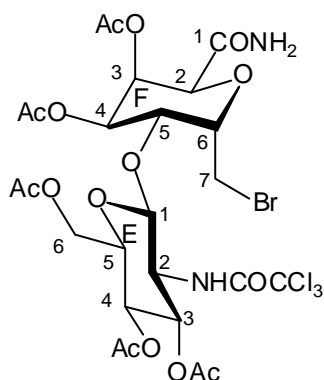
7.3.7.1.2 Reduction with NaBH$_4$

AJ 2-40

A solution of **76** (0.040 g, 0.055 mmol) and NaBH$_4$ (0.002 g, 1.0 eq; 0.055 mmol) in dry ethanol (10 mL) was stirred for 8 h at 60 °C. The reaction progress was controlled by TLC (petroleum ether-CHCl$_3$-EtOH 5 : 2 : 2). The solution was diluted with dry EtOH (10 mL), cooled to 0 °C and neutralized with dry acetic acid. The solution was coevaporated with MeOH (3 $\times$ 2 mL). The crude product was dissolved in dry pyridine (5 mL), and stirred overnight with Ac$_2$O (3 mL). The solution was coevaporated with toluene, and the residue was purified by FC eluting with petroleum ether-CHCl$_3$-EtOH 8 : 2 : 2 to give **82** (0.032 g, 85 %).

AJ 2-42

A solution of **76** (0.025g, 0.034 mmol) and NaBH$_4$ (0.001 g, 1.0 eq; 0.034 mmol) in dry ethanol (10 mL) was stirred at 0 °C for 15 h. The reaction progress was controlled by TLC (petroleum ether-CHCl$_3$-EtOH 5 : 2 : 2), which showed no reaction.

The temperature was then raised to RT, and the reaction mixture was stirred for further 10 h. The solution was diluted with dry EtOH (10 mL), cooled to 0 °C and neutralized with dry acetic acid. The solution was coevaporated twice with MeOH (5 mL portions), and taken up in dry pyridine (5 mL), and stirred overnight with Ac$_2$O (3 mL). The solution was coevaporated with toluene, and the residue was purified by FC eluting with petroleum ether-CHCl$_3$-EtOH 8 : 2 : 2 to furnish **82** (0.023 g, 83 %).

2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→5)-3,4-di-*O*-acetyl-2,6-anhydro-7-bromo-7-deoxy-D-*glycero*-L-*galacto*-heptonamide (82)
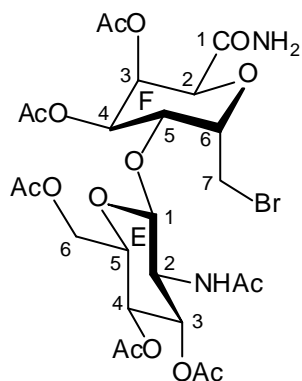
$C_{25}H_{35}BrN_2O_{15}$ [683.46]

Exact Mass   [682.12]

- colour and physical state: white solid

- M.p.: 179-180 °C (CHCl$_3$-petroleum ether), decomposition

- $[a]_D^{23}$ = +142.8 (c 0.07, CH$_2$Cl$_2$)

- R$_f$: 0.28 (petroleum ether-CHCl$_3$-EtOH 5:2:2)

- FAB-MS: *m/z* 683.1 [M+H]$^+$, 705.11 [M+Na]$^+$

- ESI-MS: *m/z* [M+H]$^+$ (calculated 683.12936, found 683.12523), [M+Na]$^+$ (calculated 705.11185, found 705.11001)

- IR (KBr): $\tilde{n}$ = 1236, 1745, 3423 cm^{-1}

- ^{1}H NMR (HH-COSY, 400 MHz, pyridine-d$_5$):

δ = 2.00, 2.01, 2.04, 2.05, 2.09, 2.16 (6s, 18H, 5 × CH$_3$COO, NHCOC$\underline{H}_3$), 4.03 (m, 1H, 5^E-H), 4.08 (m, 1H, 2^E-H), 4.15 (m, 1H, 7^F-H), 4.43-4.44 (m, 2H, CH$_2$-6^E), 4.55 (m, 1H, 7^F-H´), 4.80 (dd, 1H, 5^F-H, J 3.3, 10.5), 5.02 (m, 1H, 6^F-H), 5.08 (d, 1H, 2^F-H, J 1.5), 5.45 (dd, 1H, 4^E-H, J 9.5, 10.3), 5.64 (d, 1H, 1^E-H, J 8.4), 5.85 (dd, 1H, 4^F-H, J 3.4, 10.5), 6.08 (dd, 1H, 3^E-H, J 9.5, 10.6), 6.48 (dd, 1H, 3^F-H, J 1.5, 3.4), 7.90, 8.80 (2bs, 2H, H$_2$NCO), 9.27 (d, 1H, N$\underline{H}$COCH$_3$, J 8.1)

- ^{13}C NMR (APT, HMBC, HMQC 100.58 MHz, pyridine-d$_5$):

δ = 20.45, 20.49, 20.49, 20.67, 20.83 (-, 5 × $\underline{C}$H$_3$COO), 23.13 (-, NHCO$\underline{C}$H$_3$), 29.52 (+, C-7^F), 56.15 (-, C-2^E), 62.50 (+, C-6^E), 69.68 (-, C-4^E), 69.87 (-, C-3^F, C-4^F), 71.04 (-, C-2^F), 72.09 (-, C-5^E), 72.55 (-, C-3^E), 75.72 (-, C-5^F), 76.83 (-, C-6^F), 101.89 (-, C-1^E), 169.84, 169.87, 170.14, 170.47 (+, 5 × CH$_3$$\underline{C}OO, NH\underline{C}OCH_3$, H$_2N\underline{C}$O)

7.3.7.2 Reduction of 74 with NaBH$_4$

AJ 2-39

A solution of **74** (0.041 g, 0.055 mmol) and $NaBH_4$ (0.002 g, 1.0 eq; 0.055 mmol) in dry ethanol (10 mL) was stirred at 60 °C for 9 h (TLC, petroleum ether-$CHCl_3$-EtOH 5 : 2 : 2). The solution was diluted with dry EtOH (6 mL), cooled to 0 °C and neutralized with dry acetic acid. The solution was coevaporated with MeOH (3 × 2 mL). The crude product was dissolved in dry pyridine (10 mL), and stirred overnight with Ac_2O (3 mL). The solution was coevaporated with toluene, and the residue was purified by FC eluting with petroleum ether-$CHCl_3$-EtOH 8 : 2 : 2 giving **82** (0.029 g, 78 %).

AJ 2-41

A solution of **74** (0.040 g, 0.054 mmol) and $NaBH_4$ (0.002 g, 1.0 eq; 0.054 mmol) in dry ethanol (10 mL) was stirred at 0 °C for 48 h, after which TLC (petroleum ether-$CHCl_3$-EtOH 5 : 2 : 2) showed no reaction.

The temperature was then raised to RT, and the solution was stirred for further 17 h. The solution was diluted with dry EtOH (6 mL), cooled to 0 °C and neutralized with dry acetic acid. The solution was coevaporated with MeOH (2 × 2 mL). The crude product was dissolved in dry pyridine (10 mL), and stirred overnight with Ac_2O (3 mL). The solution was coevaporated with toluene, and the residue was purified by FC eluting with petroleum ether-$CHCl_3$-EtOH 8 : 2 : 2 furnishing **82** (0.017 g, 46 %) and **83** (0.015 g, 42 %).

2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→5)-3-*O*-acetyl-2,6-anhydro-7-bromo-4-*O*-carbamoyl-7-deoxy-D-*glycero*-L-*galacto*-heptonamide (83)

$C_{24}H_{34}BrN_3O_{15}$ [684.45]
Exact Mass   [683.12]

- colour and physical state: white solid

- R_f: 0.12 (petroleum ether-$CHCl_3$-EtOH 5:2:2)

- ESI-MS: *m/z* calculated $[M+Na]^+$ 706.106551, found 706.10766

- ^{1}H NMR (HH-COSY, 400 MHz, pyridine-d_5):

δ = 1.99, 2.00, 2.06, 2.07, 2.16 (5s, 15H, 4 × CH$_3$COO, NHCOC$\underline{H}_3$), 4.00 (m, 1H, 5^E-H), 4.08 (m, 1H, 2^E-H), 4.14-4.18 (m, 2H, CH$_2$-7^F), 4.40-4.45 (m, 2H, CH$_2$-6^E), 4.76 (dd, 1H, 5^F-H, J 6.4, 10.8), 4.97 (m, 1H, 6^F-H), 5.07 (d, 1H, 2^F-H, J 1.5), 5.43 (dd, 1H, 4^E-H, J 9.1, 10.2), 5.70 (d, 1H, 1^E-H, J 8.2), 5.93 (dd, 1H, 4^F-H, J 3.2, 11.0), 6.20 (dd, 1H, 3^E-H, J 9.1, 10.8), 6.65 (dd, 1H, 3^F-H, J 1.5, 3.2), 7.70 (bs, 2H, H$_2$NCOO), 7.90, 8.60 (2s, 2H, H$_2$NCO), 9.31 (d, 1H, N$\underline{H}$COCH$_3$, J 7.8)

- ^{13}C NMR (APT, HETCOR, 100.63 MHz, pyridine-d$_5$):

δ = 20.60, 20.71, 20.95 (-, 4 × $\underline{C}$H$_3$COO), 23.29 (+, NHCO$\underline{C}$H$_3$), 29.92 (+, C-7^F), 56.34 (-, C-2^E), 62.53 (+, C-6^E), 69.73, 69.87 (-, C-4^F, C-4^E), 70.32 (-, C-3^F), 71.32 (-, C-5^F), 71.96 (-, C-2^F), 72.46 (-, C-5^E), 76.66 (-, C-3^E), 77.01 (-, C-6^F), 102.09 (-, C-1^E), 157.08 (+, H$_2$N$\underline{C}$OO), 169.51, 169.89, 170.11, 170.40, 170.50, 171.01 (-, 4 × CH$_3$$\underline{C}OO, NH\underline{C}OCH_3$, H$_2N\underline{C}$O)
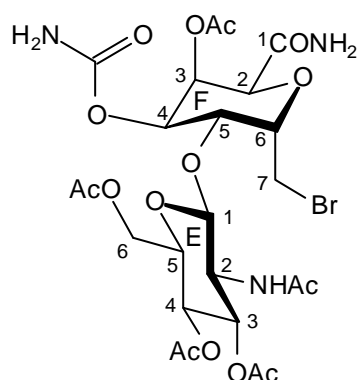
This reaction could not be reproduced. Repeating the previous reaction using the same scale and under conditions considered to be identical gave only **80** in an average yield of 65 %.

AJ 2-52

A solution of **74** (0.025 g, 0.034 mmol) and NaBH$_4$ (0.001 g, 1.0 eq; 0.034 mmol) in dry isopropanol (8 mL) was stirred at RT for 17 h (TLC, petroleum ether-CHCl$_3$-EtOH 5 : 2 : 2). The solution was diluted with dry isopropanol (6 mL), cooled to 0 °C and neutralized with dry acetic acid. The solution was coevaporated with toluene (2 × 2 mL). The crude product was dissolved in dry pyridine (10 mL), and stirred overnight with Ac$_2$O (4 mL). The solution was coevaporated with toluene, and the residue was purified by FC eluting with petroleum ether-CHCl$_3$-EtOH 8 : 2 : 2 to provide **80** (0.019 g, 73 %).

AJ 2-53

A solution of **74** (0.021 g, 0.028 mmol) and NaBH$_4$ (0.001 g, 1.0 eq; 0.028 mmol) in a mixture of dry THF-MeOH 4 : 1 (5 mL) was stirred at 5 °C for 19 h (TLC, petroleum ether-CHCl$_3$-EtOH 5 : 2 : 2). The solution was diluted with dry THF (5 mL), and neutralized with dry acetic acid. The solution was coevaporated with toluene (2 × 2 mL). The crude product was dissolved in dry pyridine (10 mL), and stirred overnight with Ac$_2$O (3 mL). The solution was coevaporated

with toluene, and the residue was purified by FC eluting with petroleum ether-CHCl$_3$-EtOH 8 : 2 : 2 to furnish **80** (0.015 g, 68 %).

7.3.7.3 Reduction of 80 with NaBH$_3$CN

AJ 2-54

Compound **80** (0.010 g, 0.013 mmol) was dissolved in dry THF (5 mL), then 4 Å molecular sieves and NaBH$_3$CN (0.001 g, 1.0 eq; 0.013 mmol) were added. After stirring at RT for 20 min, the mixture was added to a saturated HCl-dry ether solution (0.5 mL) until the bubbles disappeared. The mixture was stirred for another 2 h (TLC, petroleum ether-CHCl$_3$-EtOH 5 : 2 : 2). The mixture was diluted with ether (10 mL) and filtered. The molecular sieves were washed with ether (3 × 5 mL) and the combined filtrate was concentrated. The residue was dissolved in dry pyridine (5 mL), and stirred overnight with Ac$_2$O (2 mL). The solution was coevaporated with toluene, and the residue was purified by FC eluting with petroleum ether-CHCl$_3$-EtOH 8 : 2 : 2 to recover **80** (0.015 g, 68 %). The spectral data (ESI-MS, ^{1}H NMR, ^{13}C NMR) of the separated product agree with those of the starting material **80**.

7.3.8 Synthesis of phosphonate 85

AJ 2-47

Compound **82** (0.010 g, 0.015 mmol) was dissolved in degassed triethyl phosphite (5 mL) and heated overnight in an oil bath to 180 °C, while purging the solution with an argon stream. The solution was cooled, and triethyl phosphite was coevaporated with toluene under reduced pressure. FC eluting with petroleum ether-CHCl$_3$-EtOH 8 : 2 : 2 gave **85** (0.007 g, 70 %).

2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→5)-3,4-di-*O*-acetyl-2,6-anhydro-7-deoxy-7-(diethoxyphosphoryl)-D-*glycero*-L-*galacto*-heptonamide (85)
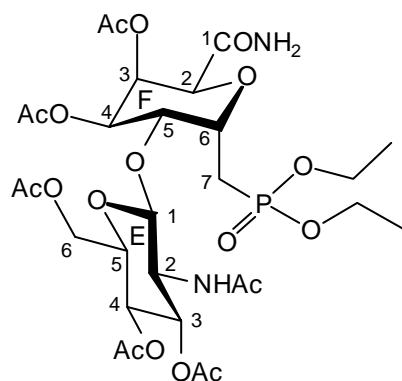
C$_{29}$H$_{45}$N$_2$O$_{18}$P   [740.65]
Exact Mass   [740.24]

- colour and physical state: white solid

- R_f: 0.19 (petroleum ether-CHCl$_3$-EtOH 5:2:2)

- FAB-MS: m/z 741.2 [M+H]$^+$, 763.2 [M+Na]$^+$

- ESI-MS: m/z [M+H]$^+$ (calculated 741.24778, found 741.24687), [M+Na]$^+$ (calculated 763.22972, found 763.22925)

- IR (KBr): $\tilde{\boldsymbol{n}}$ = 1236, 1747, 3437 cm^{-1}

- ^{1}H NMR (HH-COSY, 600 MHz, pyridine-d$_5$):

δ = 1.22 (t, 3H, POCH$_2$CH$_3$, J 7.0), 1.26 (t, 3H, POCH$_2$CH$_3$, J 7.0), 2.00, 2.03, 2.04, 2.08, 2.11, 2.19 (6s, 18H, 5 × CH$_3$COO; NHCOCH$_3$), 2.69 (m, 1H, 7^F-H), 3.06 (m, 1H, 7^F-H´), 4.03 (m, 1H, 5^E-H), 4.06 (m, 1H, 2^E-H), 4.17-4.22 (m, 4H, PO(OCH$_2$CH$_3$)$_2$), 4.39 (dd, 1H, 6^E-H, J 2.4, 12.2), 4.53 (dd, 1H, 6^E-H´, J 4.4, 12.2), 4.75 (m, 1H, 5^F-H), 5.16 (m, 1H, 2^F-H, partially hidden by the water signal), 5.34 (m, 1H, 6^F-H), 5.45 (t, 1H, 4^{E}H, J 9.7), 5.68 (d, 1H, 1^E-H, J 8.4), 5.89 (dd, 1H, 4^F-H, J 3.3, 9.9), 6.11 (t, 1H, 3^E-H, J 9.8), 6.48 (m, 1H, 3^F-H), 8.22, 8.62 (2s, 2H, H$_2$NCO), 9.36 (d, 1H, NHCOCH$_3$, J 8.1)

- ^{13}C NMR (50.29 MHz, pyridine-d$_5$):

δ = 16.44 (d, CH$_3$CH$_2$OP, ^{3}J$_{C,P}$ 6.1), 16.51 (d, CH$_3$CH$_2$OP, ^{3}J$_{C,P}$ 6.1), 20.45, 20.52, 20.61, 20.61, 20.94 (5 × CH$_3$COO), 23.16 (NHCOCH$_3$), 26.98 (C-7^F), 56.20 (C-2^E), 61.59 (d, CH$_3$CH$_2$OP, ^{2}J$_{C,P}$ 6.5), 61.91 (d, CH$_3$CH$_2$OP, ^{2}J$_{C,P}$ 6.5), 62.57 (C-6^E), 69.54, 70.58, 71.80, 72.03, 72.54 (probably more than one signal), 75.67, 75.96, 101.85 (C-1^E), 169.84, 170.21, 170.27, 170.44, 170.47, 170.60 (5 × CH$_3$COO, NHCOCH$_3$, H$_2$NCO)

- ^{31}P NMR (121.5 MHz, pyridine-d$_5$):

δ = 27.94

7.4 Trisaccharide synthesis

7.4.1 Trials to prepare trisaccharide 87 based on oxazoline 42

7.4.1.1 Synthesis of the oxazoline donor 42[62]

AJ 3-00

A solution of chitobiose octaacetate **86** (0.082 g, 0.12 mmol) in dry 1,2-dichloroethane (10 mL) containing activated 3 Å molecular sieves was treated with trimethylsilyl trifluoromethanesulfonate (25 ìL, 1.08 eq; 0.13 mmol) under an argon atmosphere, and the mixture was stirred for 5 h at 50 °C. Triethylamine (1 mL) was added, and the mixture was applied to FC eluting with $CHCl_3$-MeOH 20 : 1 to give **42** (0.066 g, 90 %). The spectral data agree with those in the literature.[62]

7.4.1.2 Trials based on the oxazoline 42

AJ 3-01

To a solution of **53** (0.260 g, 4.0 eq; 0.48 mmol) in dry CH_2Cl_2 (10 mL) containing activated 4 Å molecular sieves, anhydrous camphorsulfonic acid (0.011 g, 0.4 eq; 0.005 mmol) dissolved in dry CH_2Cl_2 (2 mL) was added, and 2.5 mL of a solution of **42** (0.074 g, 0.12 mmol) in dry CH_2Cl_2 (5 mL). The mixture was stirred at 60 °C under an argon atmosphere for 3 h, after which the remaining portion of the solution of **42** was added. No reaction was observed (TLC, $CHCl_3$-MeOH 20 : 1). Triethylamine (1 mL) was added, and stirring was continued for 10 min at RT. Solvent evaporation followed by FC led to the recovery of compound **42** (0.065 g).

AJ 3-02

To a solution of **42** (0.021 g, 0.034 mmol) and **53** (0.200 g, 11 eq; 0.37 mmol) in dry CH_2Cl_2 (7 mL) containing activated 4 Å molecular sieves, trifluoromethanesulfonic acid (3 ìL, 0.4 eq; 0.016 mmol) was added. The mixture was stirred at RT under an argon atmosphere for 2 h (TLC, $CHCl_3$-MeOH 20 : 1). Triethylamine (1 mL) was added and stirring was continued for 10 min at RT. Solvent evaporation followed by FC furnished many products in small fractions. ^{1}H NMR and ^{13}C NMR spectra proved that they were decomposition products. No effort was made to identify them.

AJ 3-03

A mixture of **42** (0.042 g, 0.068 mmol), **53** (0.400 g, 11 eq, 0.75 mmol) and activated 3 Å molecular sieves in anhydrous 1,2-dichloroethane (5 mL) was stirred for 1 h at RT under a dry argon atmosphere, then cooled to 0 °C. Trimethylsilyl triflate (12 μL, 1 eq; 0.068 mmol) was added, and the mixture was stirred 1 h at 0 °C and further 3 h at RT. No reaction was observed as indicated by TLC (CHCl$_3$-MeOH 20 : 1). Triethylamine (0.5 mL) was added, and the mixture was diluted with CH$_2$Cl$_2$ (10 mL), filtered, concentrated and applied to FC. **42** (0.035 g) was recovered.

7.4 2 Preparation of the acceptor 92

7.4.2.1 Acetylation of compound 53

AJ 3-11

Compound **53** (1.00 g, 1.87 mmol) was dissolved in dry pyridine (10 mL) and acetic anhydride (5 mL) and was stirred at RT for 3 h, during which the reaction progress was monitored by TLC (petroleum ether-ethyl acetate 2 : 1). Coevaporation with toluene, followed by FC eluting with petroleum ether-ethyl acetate 2 : 1 gave the acetylated compound **89** (0.950 g, 88 %).

5-*O*-Acetyl-2,6-anhydro-7-bromo-1-*O*-(*tert*-butyldiphenylsilyl)-7-deoxy-3,4-*O*-isopropylidene-D-*glycero*-L-*galacto*-heptitol (89)
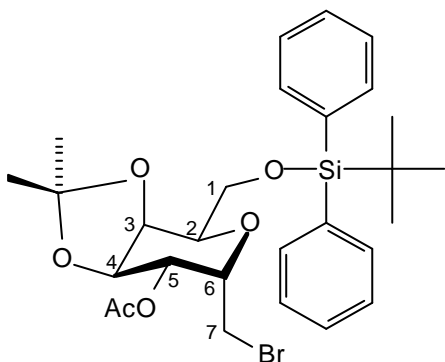
C$_{28}$H$_{37}$BrO$_6$Si [577.59]
Exact Mass   [576.15]

- colour and physical state: pale yellow oil

- $[\alpha]_D^{23}$ = -16.67 (c 0.48, CH$_2$Cl$_2$)

- R$_f$: 0.51 (ethyl acetate-petroleum ether 2:1)

- ESI-MS: *m/z* calculated [M+Na]$^+$ 599.14350, found 599.14379

- IR (Film): $\tilde{n}$ = 1068, 1105, 1153, 1221,1751, 2854, 3498 cm^{-1}

- ^{1}H NMR (HH-COSY, 600 MHz, CDCl$_3$):

δ = 1.08 (s, 9H, (CH$_3$)$_3$CSi), 1.35, 1.49 (2s, 6H, (CH$_3$)$_2$COO), 2.14 (s, 3H, CH$_3$COO), 3.27-3.31 (m, 2H, CH$_2$-7), 3.83 (dd, 1H, 1-H, J 6.2, 9.9), 3.86 (dd, 1H, 1-H´, J 7.7, 9.9), 3.98 (m, 1H, 2-H), 4.29 (ddd, 1H, 6-H, J 2.6, 6.2, 8.7), 4.34 (dd, 1H, 4-H, J 2.6, 7.5), 4.45 (dd, 1H, 3-H, J 1.3, 7.5), 5.25 (t, 1H, 5-H, J 2.6), 7.38-7.44 (m, 6H, aromatic), 7.68-7.76 (m, 4H, aromatic)

- ^{13}C NMR (APT, HMBC, HMQC, 100.58 MHz, CDCl$_3$):

δ = 19.42 (+, (CH$_3$)$_3$<u>C</u>Si), 20.98 (-, <u>C</u>H$_3$COO), 24.48, 26.68 (-, (<u>C</u>H$_3$)$_2$COO), 26.99 (-, (<u>C</u>H$_3$)$_3$CSi), 29.39 (+, C-7), 63.26 (+, C-1), 68.63 (-, C-5), 70.08 (-, C-6), 71.19 (-, C-2), 71.56 (-, C-3), 71.76 (-, C-4), 110.02 (+, (CH$_3$)$_2$<u>C</u>OO), 127.70, 127.75 , 129.71, 129.78 (-, C^{Ar}), 133.62, 133.74 (+, SiC^{Ar}), 135.72, 135.77 (-, C^{Ar}), 169.51 (+, CH$_3$<u>C</u>OO)

7.4.2.2 Cleavage of the silyl group in 89

AJ 3-12

To a solution of compound **89** (0.550 g, 0.955 mmol) in THF (20 mL) at RT a TBAF solution (1.0 M in THF, 1.15 mL, 1.2 eq; 1.146 mmol) was added. The reaction was stirred at RT for 3 h (TLC, petroleum ether-ethyl acetate 1 : 1). Water was added and the mixture was extracted with chloroform (5 × 20 mL). The combined organic layers were dried, and the solvent was removed by evaporation. The residue was submitted to FC eluting with petroleum ether-ethyl acetate 1 : 1, and gave **90** (0.281 g, 87 %).

5-*O*-Acetyl-2,6-anhydro-7-bromo-7-deoxy-3,4-*O*-isopropylidene -D-*glycero*-L-g*alacto*-heptitol (90)
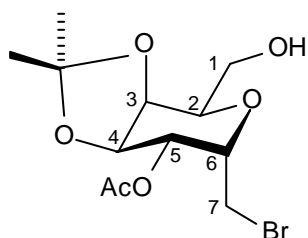
C$_{12}$H$_{19}$BrO$_6$ [339.18]
Exact Mass [338.04]

- colour and physical state: pale yellow oil

- $[a]_D^{23}$ = +8 (c 0.25, CH$_2$Cl$_2$)

- R$_f$: 0.28 (ethyl acetate-petroleum ether 1:1)

- ESI-MS: *m/z* calculated [M+Na]$^+$ 361.02572, found 361.02609

- IR (Film): $\tilde{n}$ = 1061, 1221, 1745 cm^{-1}

- ^{1}H NMR (HH-COSY, 400 MHz, CDCl$_3$):

δ = 1.26, 1.43 (2s, 6H, (CH$_3$)$_2$COO), 2.06 (s, 3H, CH$_3$COO), 2.50 (bs, 1H, OH), 3.25-3.35 (m, 2H, CH$_2$-7), 3.67 (dd, 1H, 1-H, J 4.7, 11.6), 3.78 (dd, 1H, 1-H´, J 6.7, 11.6), 3.93 (ddd, 1H, 2-H, J 1.6, 4.7, 6.7), 4.23 (dd, 1H, 3-H, J 1.6, 7.5), 4.27 (m, 1H, 4-H), 4.30 (m, 1H, 6-H), 5.21 (m, 1H, 5-H)

- ^{13}C NMR (APT, HMBC, HMQC, 100.57 MHz, CDCl$_3$):

δ = 20.91(-, $\underline{C}$H$_3$COO), 24.36, 26.46 (-, ($\underline{C}$H$_3$)$_2$COO), 29.31 (+, C-7), 63.10 (+, C-1), 67.98 (-, C-5), 70.13 (-, C-4), 70.99 (-, C-2), 71.70 (-, C-6), 72.35 (-, C-3), 110.47 (+, (CH$_3$)$_2$$\underline{C}$OO), 169.54 (+, CH$_3$$\underline{C}$OO)

7.4.2.3 Preparation of the uronamide 91

AJ 3-13

a. Tempo oxidation

To a mixture of **90** (0.330 g, 0.940 mmol), TEMPO (0.161 g, 1.06 eq; 0.996 mmol), tetrabutylammonium chloride (0.261 g, 1.0 eq; 0.940 mmol), potassium bromide (0.113 g, 1.0 eq; 0.95 mmol) and dichloromethane (20 mL) at 0 °C, a mixture of satd. aq. sodium chloride (10 mL), satd. aq. sodium hydrogencarbonate (11 mL), and satd. aq. sodium hypochlorite (1 mL, 12-14 % active chlorine) was added slowly. The reaction mixture was vigorously stirred at RT for 3 h (TLC, petroleum ether-CHCl$_3$-EtOH 5 : 2 : 2). The pH was adjusted to 2-3 with conc. HCl. The organic layer was separated and the aqueous layer was extracted five times with dichloromethane (10 mL portions). The combined organic extracts were dried, and the solvent was removed.

b. Sodium chlorite oxidation

The crude product of the TEMPO oxidation (max 0.940 mmol), sodium chlorite (0.846 g, 10.0 eq; 9.4 mmol), and sodium dihydrogenphosphate monohydrate (0.97 g, 7.5 eq; 7.0 mmol) were placed in a reaction flask, and with stirring successively 2-methyl-2-butene (4.0 mL), *t*-butanol (18 mL) and water (7.0 mL) were added. The reaction mixture was stirred at RT for 4 h (TLC, petroleum ether-CHCl$_3$-EtOH 5 : 2 : 2), and then it was diluted with water (20 mL) and dichloromethane (50 mL). The organic layer was separated and the aqueous layer was extracted with dichloromethane (2 × 20 mL). The aqueous layer was adjusted to pH 2 with conc. HCl and

extracted with dichloromethane (3 × 10 mL). The combined organic extracts were dried, and the solvent was removed. The residue was dried at 0.1 mbar for ca 2 h.

c. Amide formation according to Staab

The crude acid (max 0.94 mmol), and CDI (0.386 g, 2.5 eq; 2.4 mmol) were dissolved in dry dichloromethane (30 mL), and the mixture was stirred at RT for 5 h. Through this solution at 0 °C, gaseous ammonia was bubbled for 40 min (TLC, petroleum ether-CHC1$_3$-EtOH 5 : 2 : 2), then the mixture was stirred at RT for 1 h. The solvent was evaporated, and the residue was separated by FC eluting with petroleum ether-CHC1$_3$-EtOH 8 : 2 : 2 to give amide **91** (0.286 g, 95 % based on **90**).

5-*O*-Acetyl-2,6-anhydro-7-bromo-7-deoxy-3,4-*O*-isopropylidene -D-*glycero*-L-*galacto*-heptonamide (91)
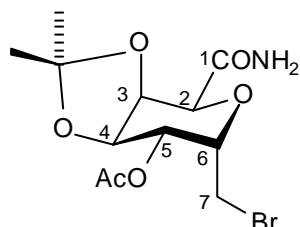
$C_{12}H_{18}BrNO_6$ [352.18]
Exact Mass   [351.04]

- colour and physical state: pale yellow oil

- $[\boldsymbol{a}]_D^{23}$ = -18.18 (c 0.22, CH$_2$Cl$_2$)

- R$_f$: 0.50 (petroleum ether-CHCl$_3$-EtOH 5:2:2)

- ESI-MS: *m/z* [M+H]$^+$(calculated 352.03903, found 352.03928), [M+Na]$^+$ (calculated 374.02097, found 374.02106), [M+K]$^+$ (calculated 389.99491, found 389.99513)

- IR (Film): $\tilde{\boldsymbol{n}}$ = 1752, 1697, 1367, 1215, 1151, 1060 cm^{-1}

- ^{1}H NMR (HH-COSY, 400 MHz, CDCl$_3$):

δ = 1.26, 1.43 (2s, 6H, (CH$_3$)$_2$COO), 2.06 (s, 3H, CH$_3$COO), 3.26-3.36 (m, 2H, CH$_2$-7), 4.31 (dd, 1H, 4-H, J 2.7, 7.5), 4.35 (d, 1H, 2-H, J 1.6), 4.37 (dd, 1H, 6-H, J 2.2, 7.0), 4.65 (dd, 1H, 3-H, J 1.6, 7.5), 5.15 (t, 1H, 5-H, J 2.2), 6.15, 6.65 (2bs, 2H, H$_2$NCO)

- ^{13}C NMR (APT, HMBC, HMQC, 100.57 MHz, CDCl$_3$):

δ = 20.82 (-, <u>C</u>H$_3$COO), 24.19, 26.50 (-, (<u>C</u>H$_3$)$_2$COO), 30.06 (+, C-7), 68.38 (-, C-5), 70.47 (-, C-6), 71.56 (-, C-4), 71.67 (-, C-2), 72.50 (-, C-3), 110.60 (+, (CH$_3$)$_2$<u>C</u>OO), 169.33 (+, CH$_3$<u>C</u>OO), 171.50 (+, H$_2$N<u>C</u>O)

7.4.2.4 Preparation of the acceptor 92

AJ 3-14

A solution of compound **91** (0.300 g, 0.855 mmol) in dry methanol (10 mL) was treated at RT with sodium methoxide (0.092 g, 2.0 eq; 1.71 mmol). The reaction mixture was stirred for 1 h (TLC, petroleum ether-CHCl$_3$-EtOH, 5 : 2 : 2). The mixture was diluted with methanol and neutralized with Dowex 50-W X2 (H$^+$). The mixture was stirred for a few minutes, and then the resin was filtered off, washed with methanol (5 × 10 mL), and the filtrate concentrated. The residue was chromatographed (FC) eluting with petroleum ether-CHCl$_3$-EtOH 8 : 2 : 2 to furnish compound **92** (0.264 g, quant).

2,6-Anhydro-7-bromo-7-deoxy-3,4-*O*-isopropylidene -D-*glycero*-L-*galacto*-heptonamide (92)
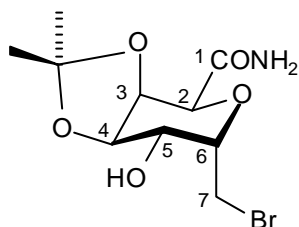
C$_{10}$H$_{16}$BrNO$_5$ [310.14]
Exact Mass   [309.02]

- colour and physical state: pale yellow oil

- $[a]_D^{23}$ = -31.58 (c 0.19, CH$_2$Cl$_2$)

- R$_f$: 0.47 (petroleum ether-CHCl$_3$-EtOH 5:2:2)

- ESI-MS: *m/z* [M+H]$^+$ (calculated 310.02846, found 310.02861), [2M+H]$^+$ (calculated 619.05019, found 619.05060)

- IR (Film): $\tilde{n}$ = 1687, 3432 cm^{-1}

- ^{1}H NMR (HH-COSY, 400 MHz, CDCl$_3$):

δ = 1.27, 1.41 (2s, 6H, (CH$_3$)$_2$COO), 3.41 (dd, 1H, 7-H, J 6.6, 10.2), 3.48 (dd, 1H, 7-H′, J 7.8, 10.2), 4.07 (bs, 1H, 5-H), 4.20 (m, 1H, 6-H), 4.35 (dd, 1H, 4-H, J 2.5, 7.8), 4.50 (bs, 1H, 2-H), 4.66 (d, 1H, 3-H, J 7.8), 5.74, 6.64 (2bs, 2H, H$_2$NCO)

- ^{13}C NMR (APT, HMBC, HMQC, 100.57 MHz, CDCl$_3$):

δ = 24.28, 26.52 (-, (CH$_3$)$_2$COO), 30.98 (+, C-7), 66.39 (-, C-5), 71.33 (-, C-2), 71.65 (-, C-6), 72.69 (-, C-3), 73.83 (-, C-4), 110.11 (+, (CH$_3$)$_2$COO), 172.69 (+, H$_2$NCO)

7.4.3 Trials to prepare trisaccharide 93 based on acceptor 92

AJ 3-15-A

A mixture of **92** (0.019 g, 0.061 mmol), **88** (0.084 g, 1.2 eq; 0.073 mmol) and activated 3 Å molecular sieves in anhydrous 1,2-dichloroethane (5 mL) was stirred for 1 h at RT under an argon atmosphere, then cooled to 0 °C. Trimethylsilyl triflate (1.1 µL, 0.1 eq, 0.006 mmol) was added, and the mixture was stirred at 0 °C for ca 20 min (TLC, petroleum ether-ethyl acetate 1 : 1) after which only composition of the glycosyl donor was observed.

AJ 3-15-B

The reaction was repeated using a mixture of dry ether and 1,2-dichloroethane (1 : 1) as the reaction solvent, with no change in the result.

Trials to prepare compound 72

AJ 3-16-A

A mixture of **92** (0.019 g, 0.061 mmol), **67** (0.043 g, 1.2 eq; 0.073 mmol), and activated 3 Å molecular sieves in anhydrous 1,2-dichloroethane (5 mL) was stirred for 1 h at RT under an argon atmosphere, then cooled to 0 °C. Trimethylsilyl triflate (1.1 µL, 0.1 eq; 0.006 mmol) was added, and the mixture was stirred at 0 °C for ca 20 min (TLC, petroleum ether-ethyl acetate 1 : 1), after which decomposition of the donor was observed.

AJ 3-16-B

The glycosyl acceptor **92** (0.065 g, 0.210 mmol) was dissolved in anhydrous 1,2-dichloroethane (5 mL) and stirred with activated 3 Å molecular sieves for 1 h at RT under an argon atmosphere, and the solution was cooled to 0 °C. Trimethylsily triflate (3.8 µL, 0.1 eq; 0.021 mmol) was added. The donor **67** (0.150 g, 1.2 eq; 0.252 mmol) was then added, and the mixture was stirred at 0 °C for ca 10 min (TLC, petroleum ether-ethyl acetate 1 : 1). TLC indicated that there was only donor decomposition.

7.4.4 Synthesis of the acceptor 95

7.4.4.1 Preparation of the silyl ether 94

AJ 3-18

To a solution of compound **62** (0.500 g, 1.95 mmol) in dry pyridine (20 mL) containing DMAP (0.010 g, cat) at 0 °C TBDMSCl (0.320 g, 1.1 eq; 2.15 mmol) was added, and the reaction was stirred at 0 °C (TLC, MeOH-CHCl₃ 15 : 85). After 5 h, the reaction mixture was coevaporated with toluene. FC of the residue eluting with MeOH-CHCl₃ 15 : 85 afforded compound **94** (0.630 g, 87 %).

2,6-Anhydro-7-bromo-1-*O*-(*tert*-butyldimethylsilyl)-7-deoxy-D-*glycero*-L-*galacto*-heptitol (94)
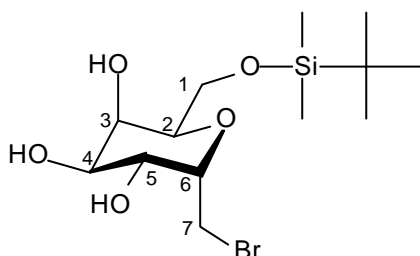
$C_{12}H_{18}BrNO_6$ [371.34]
Exact Mass [370.08]

- colour and physical state: white oil

- $[a]_D^{23}$ = +56.25 (c 0.32, CH₂Cl₂)

- R$_f$: 0.35 (CHCl₃-MeOH 95:15)

- ESI-MS: *m/z* calculated [M+H]⁺ 371.08839, found 371.08867

- IR (Film): $\tilde{n}$ = 839, 1082, 2929, 3392 cm⁻¹

- ¹H NMR (HH-COSY, 300 MHz, pyridine-d₅):

δ = 0.15, 0.17 (2s, 6H, Si(CH₃)₂), 0.95 (s, 9H, SiC(CH₃)₃), 4.23-4.35 (m, 4H containing CH₂-7), 4.38-4.43 (m, 2H), 4.63 (m, 1H, 3-H), 4.71-4.81 (m, 2H)

- ¹³C NMR (APT, HETCOR, 75.45 MHz, pyridine-d₅):

δ = -5.41, -5.22 (-, Si(CH₃)₂), 18.74 (+, SiC(CH₃)₃), 26.34 (-, SiC(CH₃)₃), 31.52 (+, C-7), 62.68 (+, C-1), 69.41 (-), 70.23 (-), 72.32 (-), 74.75 (-), 76.60 (-, C-3)

7.4.4.2 Preparation of the acceptor 95

AJ 3-19

To a solution of compound **94** (0.40 g, 1.08 mol) in dry acetone (10 mL) and 2,2-dimethoxyacetone (4 mL) *p*-toluenesulfonic acid monohydrate (0.015 g, cat) was added and the mixture was stirred at RT. After 1 h (TLC, petroleum ether-ethyl acetate, 1 : 1) the reaction mixture was neutralized with triethylamine, the solvents were evaporated, and the residue was submitted to FC eluting with petroleum ether-ethyl acetate 2 : 1 to afford compound **95** (0.40 g, 90 %).

2,6-Anhydro-7-bromo-1-*O*-(t*ert*-butyldimethylsilyl)-7-deoxy-3,4-*O*-isopropylidene-D-*glycero*-L-*galacto*-heptitol (95)
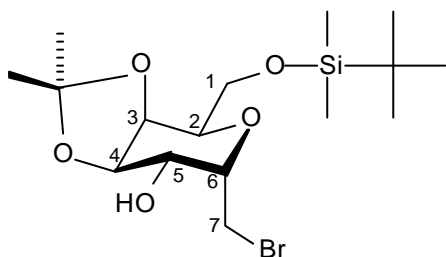
$C_{16}H_{31}BrO_5Si$ [411.41]
Exact Mass   [410.11]

- colour and physical state: pale yellow oil

- $[a]_D^{23}$ = -12.90 (c 0.31, CH_2Cl_2)

- R_f: 0.62 (ethyl acetate-petroleum ether 1:1)

- ESI-MS: *m/z* calculated $[M+H]^+$ 411.11969, found 411.12010

- IR (Film): $\tilde{n}$ = 839, 1255, 2929, 3494 cm^{-1}

- ^{1}H NMR (HH-COSY, 300MHz, CDCl$_3$,):

δ = 0.06 (s, 6H, Si(CH$_3$)$_2$), 0.88 (s, 9H, SiC(CH$_3$)$_3$), 1.32, 1.47 (2s, 6H, (CH$_3$)$_2$COO), 2.22 (d, 1H, OH, J 4.7), 3.39 (dd, 1H, 7-H, J 5.5, 9.9), 3.52 (dd, 1H, 7-H´, J < 1, 9.9), 3.69 (dd, 1H, 1-H, J 6.3, 9.5), 3.75 (dd, 1H, 1-H´, J 7.3, 9.5), 4.03 (m, 1H, 2-H), 4.14 (m, 1H, 5-H), 4.16 (m, 1H, 6-H), 4.34 (dd, 1H, 4-H, J 2.3, 7.7), 4.36 (dd, 1H, 3-H, J 1.1, 7.7)

- ^{13}C NMR (APT, HETCOR, 75.45 MHz, CDCl$_3$):

δ = -5.02, -5.17 (-, Si(CH$_3$)$_2$), 18.60 (+, SiC(CH$_3$)$_3$), 24.63, 26.91 (-, (CH$_3$)$_2$COO), 26.14 (-, SiC(CH$_3$)$_3$), 30.27 (+, C-7), 63.72 (+, C-1), 67.27 (-, C-5), 71.07 (-), 71.11 (-), 71.76 (-, C-3), 74.33 (-, C-4), 109.77 (+, (CH$_3$)$_2$COO)

7.4.5 Preparation of trisaccharide 96 based on acceptor 95

AJ 3-20

A mixture of **95** (0.043 g, 1.2 eq; 0.105 mmol), **88** (0.101 g, 1.0 eq; 0.088 mmol) and activated 3 Å molecular sieves in anhydrous 1,2-dichloroethane (5 mL) was stirred for 1 h at RT under an argon atmosphere, then cooled to 0 °C. Trimethylsilyl triflate (1.6 µL, 0.1 eq; 0.009 mmol) was added, and the mixture was stirred at 0 °C for ca 5 min (TLC, petroleum ether-ethyl acetate 1 : 1). The reaction mixture was neutralized with triethylamine, the solvents were evaporated, and the residue was submitted to FC eluting with petroleum ether-ethyl acetate 2 : 1 to give **96** (0.012 g, 10 %).

AJ 3-21

A mixture of **95** (0.190 g, 1.2 eq; 0.463 mmol), **88** (0.443 g, 1.2 eq; 0.385 mmol) and activated 3 Å molecular sieves in anhydrous 1,2-dichloroethane (5 mL) was stirred for 1 h at RT under an argon atmosphere, then cooled to -30 °C. Trimethylsilyl triflate (8 µL, 0.1 eq; 0.039 mmol) was added, and the mixture was stirred at -30 °C for ca 5 min (TLC, petroleum ether-ethyl acetate 1 : 1). The reaction mixture was neutralized with triethylamine, the solvents were evaporated, and the residue was submitted to FC eluting with petroleum ether-ethyl acetate 2 : 1 to provide compound **96** (0.269 g, 50 %).

3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→5)-2,6-anhydro-7-bromo-1-*O*-(*tert*-butyldimethylsilyl)-7-deoxy-3,4-*O*-isopropylidene-D-*glycero*-L-*galacto*-heptitol (96)
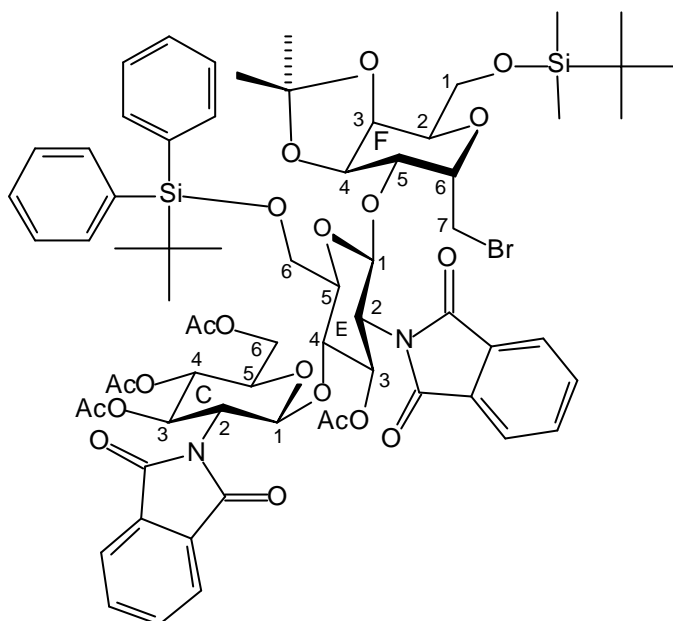
$C_{68}H_{83}BrN_2O_{21}Si_2$ [1400.48]
Exact Mass   [1398.42]

- colour and physical state: white solid

- $[\boldsymbol{a}]_D^{23}$ = -22.22 (c 0.18, CH_2Cl_2)

- R_f: 0.36 (ethyl acetate-petroleum ether 1:1)

- ESI-MS: *m/z* calculated [M+H]$^+$ 1399.42830, found 1399.43009

- IR (KBr): $\tilde{\boldsymbol{n}}$ = 1045, 1072, 1106, 1151, 1226, 1384, 1720, 1751 cm^{-1}

- ^{1}H NMR (HH-COSY, 600 MHz, CDCl$_3$):

δ = -0.07 (s, 6H, Si(C$\underline{H}$$_3$)$_2$C(CH$_3$)$_3$), 0.77 (s, 9H, Si(CH$_3$)$_2$C(C$\underline{H}$$_3$)$_3$), 1.02, 1.28 (2s, 6H, (CH$_3$)$_2$COO), 1.12 (s, 9H, SiPh$_2$C(C$\underline{H}$$_3$)$_3$), 1.83, 1.96, 2.01, 2.06 (4s, 12H, 4 × CH$_3$COO), 3.10 (dd, 1H, 7^F-H, J 8.4, 10.8), 3.19 (dd, 1H, 7^F-H′, J 5.4, 10.8), 3.44 (m, 1H, 5^E-H), 3.47 (m, 1H, 2^F-H), 3.54-3.55 (m, 2H, CH$_2$-1^F), 3.60 (ddd, 1H, 5^C-H, J 2.1, 4.5, 9.9), 3.77 (dd, 1H, 6^E-H, J 3.3, 11.7), 3.80 (t, 1H, 5^F-H, J 3.6), 3.82 (dd, 1H, 3^F-H, J 1.5, 7.2), 3.86 (dd, 1H, 4^F-H, J 3.6, 7.2), 3.87 (m, 1H, 6^E-H′), 3.97 (ddd, 1H, 6^F-H, J 3.3, 5.4, 8.4), 4.03 (dd, 1H, 6^C-H, J 1.8, 12.6), 4.19 (2dd, 2H, 2^C-H and 2^E-H, J 8.4, 10.8), 4.37 (dd, 1H, 6^C-H′, J 4.5, 12.3), 4.38 (t, 1H, 4^E-H, J 9.6), 5.10 (dd, 1H, 4^C-H, J 9.0, 10.2), 5.35 (d, 1H, 1^E-H, J 8.4), 5.56 (d, 1H, 1^C-H, J 9), 5.69 (dd, 1H, 3^C-H, J 8.7, 11.1), 5.72 (dd, 1H, 3^E-H, J 9.3, 10.5), 7.43-7.44 (m, 3H, aromatic), 7.47-7.48 (m, 3H, aromatic), 7.70-7.74 (m, 6H, aromatic), 7.78-7.80 (m, 4H, aromatic), 7.83-7.84 (m, 2H, aromatic)

- ^{13}C NMR (APT, HETCOR, HMBC, HSQC, 75.45 MHz, CDCl$_3$):

δ = -5.51, -5.26 (-, Si($\underline{C}$H$_3$)$_2$C(CH$_3$)$_3$), 18.32 (+, Si(CH$_3$)$_2$$\underline{C}$(CH$_3$)$_3$), 19.74 (+, SiPh$_2$$\underline{C}$(CH$_3$)$_3$) 20.55, 20.71, 20.75, 20.78 (-, 4 × $\underline{C}$H$_3$COO), 24.47, 26.89 (-, ($\underline{C}$H$_3$)$_2$COO), 25.93 (-, Si(CH$_3$)$_2$C($\underline{C}$H$_3$)$_3$), 27.07 (-, SiPh$_2$C($\underline{C}$H$_3$)$_3$), 30.81(+, C-7^F), 55.02 (+, C-2^C), 55.28 (-, C-2^E), 61.72 (+, C-6^C), 62.08 (+, C-1^F), 62.51 (+, C-6^E), 68.68 (-, C-4^C), 70.06 (-, C-2^F), 70.44 (-, C-3^E), 70.80 (-, C-3^C), 71.73, 71.80 (-, C-3^F, C-4^F, C-5^C, C-6^F), 72.71 (-, C-4^E), 75.44 (-, C-5^F), 75.73 (-, C-5^E), 96.02 (-, C-1^C), 97.53 (-, C-1^E), 109.47 (+, (CH$_3$)$_2$$\underline{C}$OO), 123.54, 123.78, 127.83, 127.92, 129.96, 129.99 (-, C^{Ar}), 131.42 (+, 4 × NCO$\underline{C}^{Ar}$), 133.19, 133.58 (+, 2 × Si$\underline{C}^{Ar}$), 134.46, 136.00, 136.11 (-, C^{Ar}), 167.53 (+, 4 × N$\underline{C}$OC^{Ar}), 169.58, 170.23, 170.72 (+, 4 × CH$_3$$\underline{C}$OO)

7.4.6 Cleavage of the TBDMS ether in 96

AJ 3-22

Compound **96** (0.100 g, 0.072 mmol) was dissolved in THF (10 mL), and the solution was cooled to -10 °C. The reaction mixture was treated with a TBAF solution (1.0 M in THF, 86 ìL, 1.2 eq, 0.086 mmol). After 6 h (TLC, petroleum ether-ethyl acetate 1 : 1) water was added and the mixture was extracted with chloroform (3 × 20 mL). The combined organic layers were dried, evaporated and the residue (**97**) taken to the next step without further purification.

3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3-*O*-acetyl-6-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→5)-2,6-anhydro-7-bromo-7-deoxy-3,4-*O*-isopropylidene-D-*glycero*-L-*galacto*-heptitol (97)
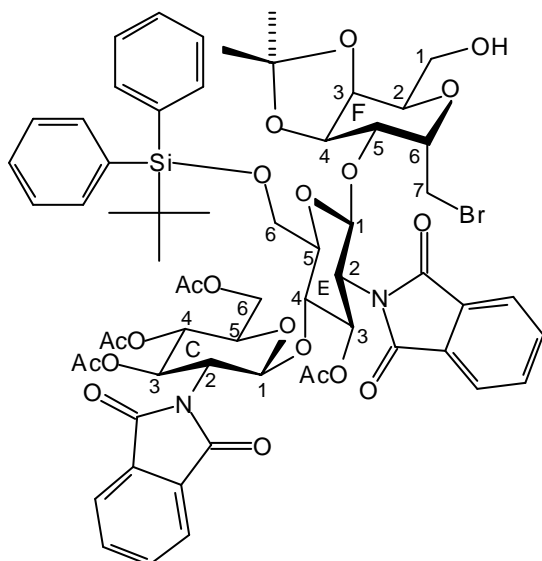
C$_{68}$H$_{83}$BrN$_2$O$_{21}$Si$_2$ [1286.22]
Exact Mass   [1284.33]

7.4.7 Synthesis of the uronamide 93

AJ 3-23

a. Tempo oxidation

To a mixture of crude **97** (max 0.072 mmol), TEMPO (0.012 g, 1.06 eq; 0.076 mmol), tetrabutylammonium chloride (0.020 g, 1.0 eq; 0.072 mmol), potassium bromide (0.009 g, 1.0 eq; 0.076 mmol) and dichloromethane (10 mL) at 0 °C, a mixture of satd. aq. sodium chloride (0.76 mL), satd. aq. sodium hydrogencarbonate (0.84 mL), and satd. aq. sodium hypochlorite (0.76 mL, 12-14 % active chlorine) was added slowly. The reaction mixture was vigorously stirred at RT for 1 h (TLC, petroleum ether-$CHCl_3$-EtOH 5 : 5 : 2). The pH was adjusted to 2-3 with conc. HCl. The organic layer was separated and the aqueous layer was extracted with dichloromethane (5 × 10 mL). The combined organic extracts were dried, and the solvent was removed.

b. Sodium chlorite oxidation

The crude product of the TEMPO oxidation (max 0.072 mmol), sodium chlorite (0.066 g, 10 eq; 0.72 mmol), and sodium dihydrogenphosphate monohydrate (0.075 g, 7.5 eq; 0.54 mmol) were placed in a reaction flask, and with stirring successively 2-methyl-2-butene (0.31 mL), *t*-butanol (1.4 mL) and water (0.54 mL) were added. The reaction mixture was stirred at RT for 1 h (TLC, petroleum ether-$CHCl_3$-EtOH 5 : 2 : 2), and then it was diluted with water (10 mL) and dichloromethane (20 mL). The organic layer was separated, and the aqueous layer was extracted with dichloromethane (2 × 10 mL). The aqueous layer was adjusted to pH 2 with conc. HCl, and extracted with dichloromethane (3 × 10 mL). The combined organic extracts were dried, and the solvent was removed. The residue was dried at 0.1 mbar for ca 2 h.

c. Amide formation according to Staab

The crude acid (max 0.072 mmol) and CDI (0.029 g, 2.5 eq; 0.18 mmol) were dissolved in dry dichloromethane (10 mL), and the mixture was stirred at RT for 1 h. Through this solution at 0 °C gaseous ammonia was bubbled for 40 min (TLC, petroleum ether-$CHCl_3$-EtOH 5 : 2 : 2), then the mixture was stirred at RT for 1 h. The solvent was evaporated, and the residue was chromatographed (FC), eluting with petroleum ether-$CHCl_3$-EtOH 8 : 2 : 2 to furnish the amide **93** (0.089 g, 95 % based on **96**).

3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-b-D-glucopyranosyl-(1®4)-3-*O*-acetyl-6-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-2-phthalimido-b-D-glucopyranosyl-(1®5)-2,6-anhydro-7-bromo-7-deoxy-3,4-*O*-isopropylidene-D-*glycero*-L-*galacto*-heptonamide (93)
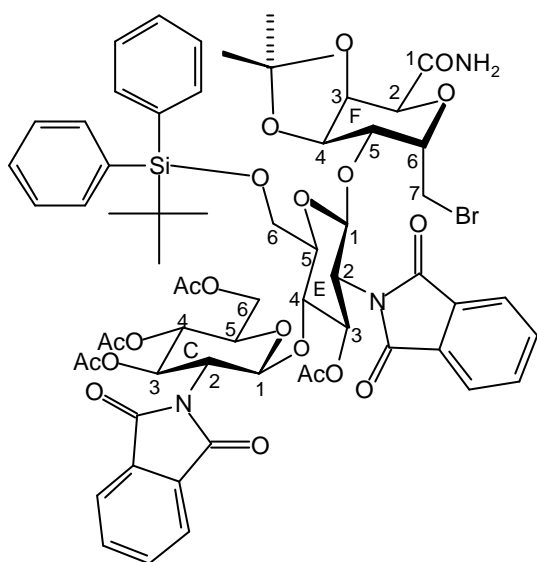
$C_{62}H_{68}BrN_3O_{21}Si$ [1299.22]
Exact Mass   [1297.33]

- colour and physical state: white solid

- $[\mathbf{a}]_D^{23}$ = +42.86 (c 0.14, CH_2Cl_2)

- R_f: 0.55 (petroleum ether-$CHCl_3$-EtOH 5:2:2)

- ESI-MS: *m/z* [M+H]$^+$ (calculated 1298.33707, found 1298.33860), [M+NH$_4$]$^+$ (calculated 1315.36362, found 1315.36310)

- IR (KBr): $\tilde{\boldsymbol{n}}$ = 1049, 1228, 1383, 1718, 1749, 3469 cm^{-1}

- ^{1}H NMR (HH-COSY, 300 MHz, CDCl$_3$):

δ = 1.10 (s, 12H, SiPh$_2$C(C$\underline{H}_3$)$_3$, (C$\underline{H}_3$)(CH$_3$)COO), 1.33 (s, 3H, (CH$_3$)(C$\underline{H}_3$)COO), 1.82, 1.95, 2.01, 2.06 (4s, 12H, 4 × CH$_3$COO), 3.07 (dd, 1H, 7^F-H, J 5.2, 10.5), 3.16 (dd, 1H, 7^F-H´, J 7.8, 10.5), 3.48 (m, 1H, 5^E-H), 3.62 (m, 1H, 5^C-H), 3.74 (m, 1H, 6^E-H), 3.77 (t, 1H, 5^F-H, J 2.4), 3.81 (d, 1H, 2^F-H, J 1.8), 3.88 (m, 1H, 6^E-H´), 3.98 (dd, 1H, 4^F-H, J 2.7, 7.5), 4.03 (ddd, 1H, 6^F-H, J 2.2, 4.4, 7.8), 4.04 (m, 1H, 6^C-H), 4.12 (dd, 1H, 3^F-H, J 1.8, 7.4), 4.17 (dd, 1H, 2^E-H, J 8.5, 10.7), 4.19 (dd, H, 2^C-H, J 8.2, 10.7), 4.32 (t, 1H, 4^E-H, J 9.8), 4.37 (dd, 1H, 6^C-H´, J 4.6, 13.0), 5.10 (dd, 1H, 4^C-H, J 9.2, 10.0), 5.37 (d, 1H, 1^E-H, J 8.5), 5.45, (d, 1H, $\underline{H}$HNCO, J 4.0), 5.54 (d, 1H, 1^C-H, J 8.3), 5.68 (dd, 1H, 3^C-H, J 9.1, 10.7), 5.71 (dd, 1H, 3^E-H, J 9.1, 10.7), 6.55 (d, 1H, H$\underline{H}$NCO, J 4.0) , 7.42-7.48 (m, 6H, aromatic), 7.69-7.88 (m, 12H, aromatic)

- ^{13}C NMR (APT, HETCOR, 50.33 MHz, CDCl$_3$):

δ = 19.68 (+, SiPh$_2$C(CH$_3$)$_3$), 20.51, 20.67, 20.71, 20.75 (-, 4 × CH$_3$COO), 23.99, 26.46 (-, (CH$_3$)$_2$COO), 26.99 (-, SiPh$_2$C(CH$_3$)$_3$), 32.00 (+, C-7^F), 54.96 (+, C-2^C), 55.10 (-, C-2^E), 61.64 (+, C-6^C), 62.54 (+, C-6^E), 68.59 (-, C-4^C), 70.29 (-, C-3^E), 70.79 (-, C-2^F, C-3^C, C-4^F), 71.17 (-, C-6^F), 71.88 (-, C-5^C), 72.30 (-, C-3^F), 72.91 (-, C-4^E), 73.72 (-, C-5^F), 75.93 (-, C-5^E), 96.00 (-, C-1^C), 96.71 (-, C-1^E), 110.19 (+, (CH$_3$)$_2$COO), 123.76, 127.82, 127.92, 130.01 (-, C^{Ar}), 131.34 (+, 4 × NCOC^{Ar}), 133.15, 133.44 (+, 2 × SiC^{Ar}), 133.83, 134.47, 135.89, 136.00 (-, C^{Ar}), 167.51 (+, 4 × NCOC^{Ar}), 169.55, 170.21, 170.69 (+, 4 × CH$_3$COO), 171.05 (+, H$_2$NCO)

7.4.8 Synthesis trial of 103

a. Cleavage of the TBDPS ether in 93 (AJ 3-24)

To a solution of compound **93** (0.076 g, 0.059 mmol) in THF (10 mL) a TBAF solution (1.0 M in THF, 71 ìL, 1.2 eq; 0.071 mmol) was added. The reaction mixture was stirred at RT for 3 h (TLC, petroleum ether-CHCl$_3$-EtOH 5 : 2 : 2). Water was added and the mixture was extracted with chloroform (3 × 10 mL). The combined organic layers were dried, evaporated and the residue (**98**) was taken to the next step without further purification.

b. Cleavage of the phthalimido group (AJ 3-25)

The crude alcohol **98** (max 0.059 mmol) was dissolved in *n*-butanol (15 mL), followed by the addition of ethylenediamine (2 mL). The reaction mixture was heated at 90 °C. After 16 h (TLC, CHCl$_3$-MeOH 85 : 15) total conversion of the starting material was observed. The solution was coevaporated with toluene, and the residue was taken to the next step without further purification.

c. Acetylation (AJ 3-26)

The crude compound (max 0.059 mmol) was dissolved in dry pyridine (12 mL) and acetic anhydride (2 mL) and stirred at RT for 5 h (TLC, CHCl$_3$-MeOH 85 : 15). Coevaporation with toluene, followed by FC eluting with CHCl$_3$-MeOH 90 : 10 left compound **102** (0.027 g, 49 % based on **93**).

2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamimido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→5)-2,6-anhydro-7-deoxy-3,4-*O*-isopropylidene-D-*glycero*-L-*galacto*-hept-6-enonamide (102)
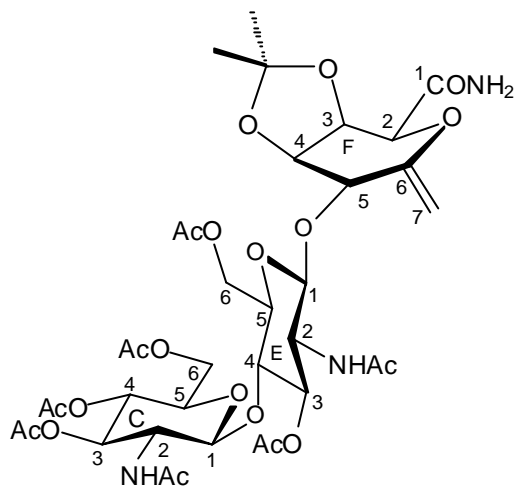
C$_{36}$H$_{51}$N$_3$O$_{20}$ [845.81]
Exact Mass   [845.31]

colour and physical state: white solid

- $[\alpha]_D^{23}$ = +35.29 (c 0.17, CH$_2$Cl$_2$)

- R$_f$: 0.36 (CHCl$_3$-MeOH 9:1)

- ESI-MS: *m/z* calculated [M+H]$^+$ 846.31447, found 846.31458

- ^{1}H NMR (HH-COSY, 400 MHz, CDCl$_3$):

δ = 1.31, 1.40 (2s, 6H, (CH$_3$)$_2$COO), 1.92, 1.95, 1.99, 2.00, 2.04, 2.07, 2.11 (7s, 21H, 5 × CH$_3$COO, 2 × NHCOCH$_3$), 3.62-3.70 (m, 3H, 4^E-H, 5^C-H, 5^E-H), 3.83 (q, 1H, 2^C-H, J 9.3), 3.94 (q, 1H, 2^E-H, J 9.0), 4.01 (dd, 1H, 6^C-H, J < 1, 10.7), 4.16 (d, 1H, 5^F-H, J 2.0), 4.23 (dd, 1H, 6^E-H, J 5.9, 11.7), 4.29 (s, 1H, 7^F-H), 4.31-4.38 (m, 2H, 6^C-H′, 6^E-H′), 4.49 (dd, 1H, 4^F-H, J 2.0, 7.7), 4.57 (s, 1H, 7^F-H′), 4.60 (d, 1H, 1^C-H, J 8.3), 4.71 (d, 1H, 1^E-H, J 8.3), 4.76 (d, 1H, 3^F-H, J 7.7), 4.80 (s, 1H, 2^F-H), 5.03 (t, 1H, 4^C-H, J 9.7), 5.10 (t, 1H, 3^E-H, J 9.1), 5.20 (t, 1H, 3^C-H, J 10.0), 6.10 (d, 1H, HHNCO, J 2.4), 6.37 (d, 1H, NHCOCH$_3$, J 8.8), 6.46 (d, 1H, NHCOCH$_3$, J 8.8), 6.63 (d, 1H, HHNCO, J 2.4)

- ^{13}C NMR (HMBC, 100.62 MHz, CDCl$_3$):

δ = 20.73, 20.77, 20.84, 21.07, 23.31 (5 × CH$_3$COO, 2 × NHCOCH$_3$), 24.73, 26.36 ((CH$_3$)$_2$COO), 53.91 (C-2^E), 54.93 (C-2^C), 62.00 (C-6^C), 62.50 (C-6^E), 68.33 (C-4^C), 71.99 (C-5^C), 72.28 (C-4^F), 72.54 (C-3^C, C-3^E), 72.92 (C-2^F), 73.19 (C-3^F, C-5^E), 75.74 (C-5^F), 76.09 (C-4^E), 94.45 (C-7^F), 99.12 (C-1^E), 101.20 (C-1^C), 110.60 ((CH$_3$)$_2$COO), 152.46 (C-6^F), 169.46, 170.51, 170.69, 170.86, 170.95, 170.99, 171.13 (5 × CH$_3$COO, 2 × NHCOCH$_3$, H$_2$NCO).

7.4.9 Synthesis trial of 100

a. Cleavage of the phthalimido group (AJ 3-27)

Compound **93** (0.015 g, 0.012 mmol) was dissolved in *n*-butanol (10 mL), followed by the addition of ethylenediamine (1 mL). The reaction mixture was heated at 90 °C for 15 h (TLC, $CHCl_3$-MeOH 85 : 15). The solution was coevaporated with toluene, and the residue was taken to the next step without further purification.

b. Acetylation (AJ 3-28)

The crude compound (max 0.012 mmol) was dissolved in dry pyridine (8 mL) and acetic anhydride (2 mL) and stirred at RT for 4 h (TLC, $CHCl_3$-MeOH 85 : 15). Coevaporation with toluene, followed by FC eluting with $CHCl_3$-MeOH 90 : 10 left compound **101** (0.007 g, 58 % based on **93**).

2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-ß-D-glucopyranosyl-(1→4)-2-acetamimido-3-*O*-acetyl-6-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-ß-D-glucopyranosyl-(1→5)-2,6-anhydro-7-deoxy-3,4-*O*-isopropylidene-D-*glycero*-L-*galacto*-hept-6-enonamide (101)
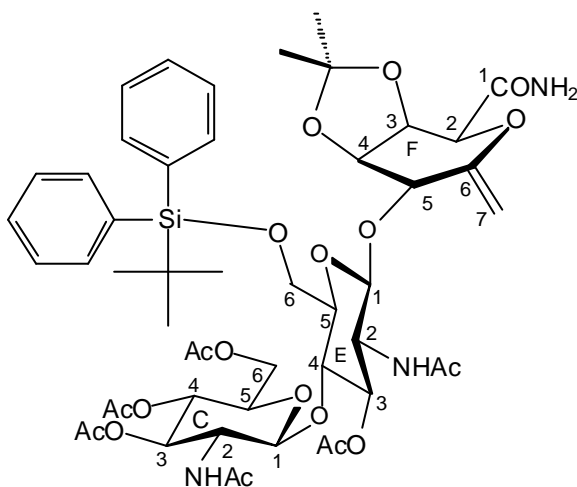
$C_{50}H_{67}N_3O_{19}Si$ [1042.18]
Exact Mass   [1041.41]

- colour and physical state: white solid
- R_f: 0.55 (petroleum ether-$CHCl_3$-EtOH 5:2:2)
- ESI-MS: *m/z* calculated $[M+Na]^+$ 1064.40357, found 1046.40429
- ^{1}H NMR (HH-COSY, 600 MHz, $CDCl_3$):

δ = 1.13 (s, 9H, SiPh₂C(CH₃)₃), 1.33, 1.48 (2s, 6H, (CH₃)₂COO), 1.98, 2.00, 2.01, 2.04, 2.05 (5s, 18H, 4 × CH₃COO, 2 × NHCOCH₃), 3.40 (m, 1H, 4^F-H), 3.49 (m, 1H, 5^C-H), 3.58-3.60 (m, 2H, 2^C-H, 5^E-H), 3.74 (m, 1H, 3^F-H), 3.82 (m, 1H, 5^F-H), 3.95-3.99 (m, 3H, CH₂-6^E, 2^F-H), 4.20 (s, 1H, 7^F-H), 4.21-4.30 (m, 3H, CH₂-6^C, 2^E-H), 4.61 (s, 1H, 7^F-H´), 4.77 (d, 1H, 1^E-H, J 7.6), 4.85 (d, 1H, 1^C-H, J 8.4), 4.97 (t, 1H, 4^C-H, J 9.6), 5.03 (m, 1H,

4^E-H), 5.23 (t, 1H, 3^C-H, J 10.0), 5.35 (d, 1H, 3^C-H, J 9.0), 5.65 (bs, 1H, $\underline{H}$HNCO), 5.96-6.02 (m, 2H, 2 × N$\underline{H}$COCH$_3$), 6.60 (bs, 1H, H$\underline{H}$NCO), 7.71-7.77 (m, 10H, aromatic)

8 ABBREVIATIONS

Ac   acetyl

AJ   code of the reactions

AIBN   α,α´-azoisobutyronitrile

APT   attached proton test (NMR)

aq.   aqueous

Ar   aromatic

bs   broad singlet (NMR)

Bu   butyl

cat   catalytic

CDI   *N*,*N*´-carbonyldiimidazole

conc.   concentrated

COSY   correlated spectroscopy (NMR)

CSA   DL-10-Camphersulfonic acid

d   doublet (NMR)

DBU   1,8-diazabicyclo[5.4.0]undec-7-ene

dd   doublet of doublet (NMR)

ddd   doublet of doublet of doublet (NMR)

DDQ   2,3-dichloro-5,6-dicyano-benzoquinone

DMAP   *p*-dimethylaminopyridine

DMF   N,N´-dimethylformamide

dt   doublet of triplet (NMR)

E. coli   Escherichia Coli

EEDQ   ethyl-2-ethoxy-1,2-dihydrochinoln-1-carboxylate

eq   equivalent

ESI   Electron Spray Ionisation (MS)

Et   ethyl

FAB   fast atom bombardment (MS)

FC   flash chromatography

h   hour(s)

HETCOR ^{1}H, ^{13}C-Heterocorrelated spectroscopy (NMR)

HMBC   Heteronuclear Multiple Bond Correlation (NMR)

HMQC   Heteronuclear Multiple Quantum Coherence (NMR)

HSQC   Heteronuclear Single Quantum Coherence (NMR)

IR   infrared spectroscopy, infrared spectrum

J   coupling constant (NMR) given in hertz

m   multiplet (NMR)

m   meta

M   molarity

max   maximum

Me   methyl

MHz   Mega Hertz

min   minute

MS   mass spectroscopy, mass spectrum

M.p.   melting point

MPLC   medium-pressure liquid chromatography

n   normal

NMR   nuclear magnetic resonance

o   ortho

p   para

PBP   penicillin-binding protein

Ph   phenyl

PG   protecting group

ppm   part per million (NMR)

PTAD   4-phenyl-3H-1,2,4-triazoline-3,5-dione

PTS   *p*-toluenesulfonic acid

q   quartet (NMR)

quant   quantitative

R   Rest

R_f ratio of front

RP   Reversed Phase (Column Chromatography)

RT   room temperature

s   singlet (NMR)

satd.   saturated

t   triplet (NMR)

t   tertiary

TAD   1,2,4-triazoline-3,5-diones

TAI   trichloroacetylisocyanate

TBAF   tetrabutylammonium fluoride

TBDMSCl   *t*-butyldimethylsilyl chloride

TBDPSCl   *t*-butyldiphenylsilyl chloride

TEA   triethylamine

TEMPO   2,2,6,6-tetramethyl-1-piperidinyloxy-radical

TFA   trifluoroacetic acid

THF   tetrahydrofuran

TLC   thin layer chromatography

TMS   trimethylsilyl

TMSOTf   trimethylsilyl triflate

UV   ultra violet

X   halide

Additionally, the IUPAC abbreviations for the amino acids were used.

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