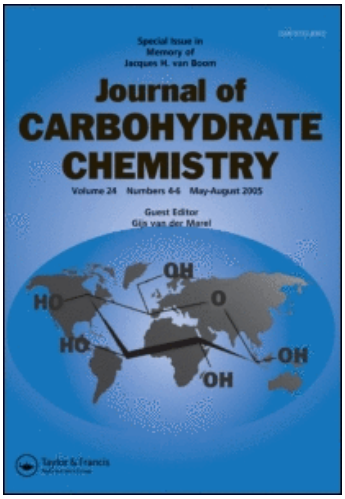


Journal of Carbohydrate Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

Synthesis of 2-Acetamido-2-Deoxy-D-Mannose Analogues as Potential Inhibitors of Sialic Acid Biosynthesis

H. J. G. Broxterman; G. A. van der Marel; J. H. van Boom

To cite this Article Broxterman, H. J. G. , van der Marel, G. A. and van Boom, J. H.(1991) 'Synthesis of 2-Acetamido-2-Deoxy-D-Mannose Analogues as Potential Inhibitors of Sialic Acid Biosynthesis', Journal of Carbohydrate Chemistry, 10: 2, 215 — 237

To link to this Article: DOI: 10.1080/07328309108543902

URL: http://dx.doi.org/10.1080/07328309108543902

J. CARBOHYDRATE CHEMISTRY, 10(2), 215-237 (1991)

SYNTHESIS OF 2-ACETAMIDO-2-DEOXY-D-MANNOSE ANALOGUES AS POTENTIAL INHIBITORS OF SIALIC ACID BIOSYNTHESIS

H. J. G. Broxterman, G. A. van der Marel and J. H. van Boom*

Gorlaeus Laboratories, P.O. Box 9502,
2300 RA Leiden, The Netherlands

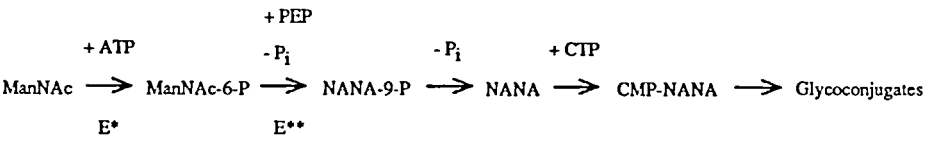
Received July 16, 1990 - Final Form November 16, 1990

ABSTRACT

Anomeric deoxygenation of D-glucopyranoside derivatives afforded suitable starting compounds for the synthesis of protected 2-azido-1,5-anhydro-2-deoxy-D-mannitols. The latter intermediates were converted after removal of the protecting groups, into the following analogues of 2-acetamido-2-deoxy-D-mannose: *i.e.* 2-acetamido-1,5-anhydro-2,6-dideoxy-D-mannitol, 2-acetamido-1,5-anhydro-2-deoxy-D-mannitol, 2-acetamido-1,5-anhydro-2-deoxy-D-mannitol 6-(disodium phosphate) and 2-acetamido-1,5-anhydro-2-deoxy-D-mannitol 6-(hexadecyl sodium phosphate).

INTRODUCTION

The most widely distributed sialic acid, 5-*N*-acetylneuraminic acid (NANA), is an important constituent of glycoconjugates [*i.e.*, glycoproteins (lipids)] and plays a pivotal role in various biological recognition processes.[1-4] Incorporation of NANA in glycoconjugates proceeds *in vivo* by specific sialyltransferases.[5] These enzymes catalyze the transfer of NANA from its donor sialyl cytidine monophosphate (CMP-NANA) to a specific location in a mature acceptor molecule. The biosynthetic pathway[6] resulting in the β-ketosidically linked CMP-NANA is illustrated in Scheme 1. In the first enzymatic step, 2-acetamido-2-deoxy-D-mannopyranose (*N*-acetyl-D-mannosamine) is phosphorylated at C-6 by a specific kinase (E*) in the presence of

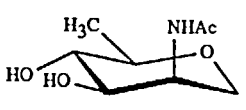


Scheme 1

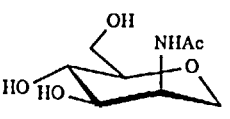
ATP to give *N*-acetyl-D-mannosamine-6-phosphate (ManNAc-6-P). Subsequently, *N*-acetylneuraminate-9-phosphate synthase (E^{**}) assisted aldol condensation of ManNAc-6-P with phosphoenolpyruvate (PEP) affords NANA-9-P, which is then converted by dephosphorylation and coupling with CTP into CMP-NANA.[3] In order to gain a better insight into the possible inhibition[7] of the two enzymes E^{*} and E^{**} involved in the early stage of the biosynthesis of CMP-NANA, we report herein on the synthesis of the D-mannitol derivatives **1-4**.

RESULTS AND DISCUSSION

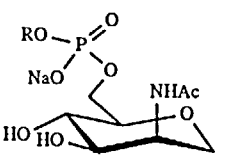
The incentive to prepare the particular compounds **1-3** and the phosphodiester derivative **4**, which is a lipophilic analogue of **3**, was based on the assumption that the removal of the anomeric hydroxyl group from ManNAc, or derivatives thereof, will afford compounds (*e.g.* compounds **1-4**) that may inhibit the E^{**}-assisted aldol condensation of ManNAc with PEP. Furthermore, compound **2**, but not **1**, may be converted by the kinase E^{*} resulting in the formation of **3**. Finally, it is not excluded that the passive diffusion of the potential inhibitor **3** through membranes of living cells would be increased by the more lipophilic analogue **4**.



1

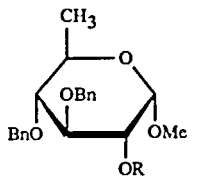


2

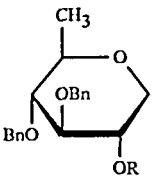


3 R = Na

4 R = $C_{16}H_{33}$

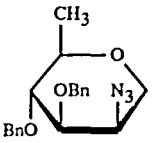


5 R = H

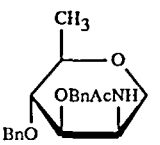


6 R = H

7 R = CF_3SO_2



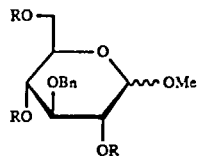
8



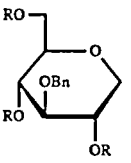
9

The first step in the approach towards the dideoxy derivative **1** consisted of deoxygenation of methyl 3,4-di-*O*-benzyl-6-deoxy-α-D-glucopyranoside[8,9,10] (**5**) following the procedure of *Bennek* and *Gray*.[11] Thus, silylation of **5** with *N,O*-bis(trimethylsilyl)trifluoroacetamide followed by *in situ* deoxygenation of intermediate **5** (R=$SiMe_3$) with triethylsilane/trimethylsilyl triflate, and subsequent acidic hydrolysis of the trimethylsilyl group from **6** (R=$SiMe_3$), afforded the 1,5-anhydro-D-glucitol **6** (R=H). Triflation of **6** (R=H) with trifluoromethanesulfonic anhydride resulted in a nearly quantitative formation of the triflate **7**. *Walden* inversion at C-2 of crude **7** occurred smoothly with lithium azide to afford the 2-azido-D-mannitol **8**. Unfortunately, the simultaneous removal of the benzyl groups and reduction of the azido function in **8** by hydrogenolysis over palladium on carbon was not successful. Therefore, the azido group was first converted into the acetamido function by reduction with sodium borohydride-nickel(II) chloride[12] followed by acetylation with acetic anhydride, to furnish **9**. Hydrogenolytic cleavage of the benzyl groups from **9** with palladium on carbon gave 2-acetamido-1,5-anhydro-2,6-dideoxy-D-mannitol (**1**) in an overall 72% yield based on **5**.

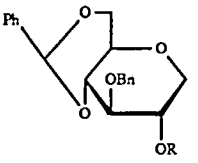
The successful synthesis of compound **1** urged us to use methyl 3-*O*-benzyl-α/β-D-glucopyranoside (**10**) as a starting compound for the preparation of the derivatives **2** and **3**. Thus, anomeric deoxygenation[11] of **10** gave **11** which, after acetylation, was fully characterized as the triacetate **12**. Acid-catalyzed acetalation of **11** with α,α-dimethoxytoluene yielded **13**. Triflation of **13**, and inversion of the configuration at C-2 in **14** with lithium azide gave **15**, the D-manno configuration of which was *inter alia* confirmed by the small value (3.8 Hz) of the $^3J_{2,3}$ coupling constant. Hydrogenolysis of **15** under acid conditions with palladium(II) hydroxide on carbon, and subsequent acetylation with acetic anhydride, furnished **16**. Finally,



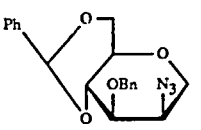
10 R = H



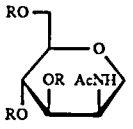
11 R = H
12 R = Ac



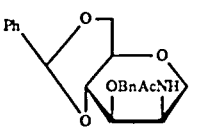
13 R = H
14 R = CF_3SO_2



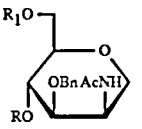
15



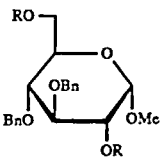
16 R = Ac



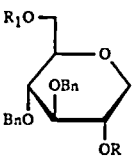
17



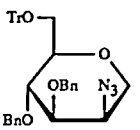
18 R_1 = R = H
19 R_1 = R = Ac
20 R_1 = DMTr , R = H
21 R_1 = DMTr , R = Bn
22 R_1 = H , R = Bn



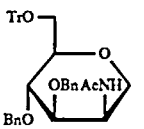
23 R = H



24 R_1 = R = H
25 R_1 = Tr, R = H
26 R_1 = Tr, R = CF_3SO_2



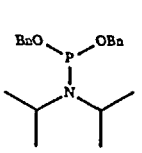
27



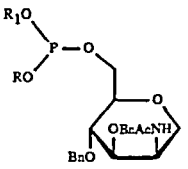
28

Zemplén deacetylation of **16** resulted in the formation of **2** which was isolated in 35% yield based on **10**.

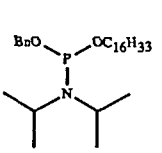
In order to obtain the phosphorylated target molecule **3**, two different routes to its properly protected precursor 2-acetamido-1,5-anhydro-3,4-di-*O*-benzyl-2-deoxy-D-mannitol (**22**) were explored. In the first approach, the azido function in **15** was reduced with hydrogen sulfide,[13] and the crude product was acetylated with acetic anhydride to give **17**. Removal of the 4,6-*O*-benzylidene group in **17** was effected with *p*-toluenesulfonic acid in methanol. The resulting diol **18** was isolated in a nearly quantitative yield, and its identity was confirmed by ^{1}H- and ^{13}C-NMR spectroscopy of the corresponding fully acetylated derivative **19**. Regio-selective dimethoxytritylation of the hydroxyl group at C-6 in **18** with 4,4'-dimethoxytrityl chloride followed by benzylation of the C-4 hydroxyl, and acid hydrolysis of the 4,4'-dimethoxytrityl group, gave **22** in 34% yield, based on **10**.



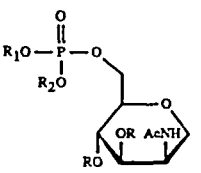
29



30 R = R_1 = Bn
31 R = Bn, R_1 = $C_{16}H_{33}$



32



33 R = R_1 = R_2 = Bn
34 R = R_1 = Bn, R_2 = $C_{16}H_{33}$

In the second approach to **22**, methyl 3,4-di-*O*-benzyl-α-D-glucopyranoside[8,9,10] **23** was first deoxygenated, as mentioned before, at C-1 to give **24**. Tritylation of the latter gave **25**, which was converted into the 2-azido derivative **27** by triflation and subsequent *Walden* inversion of **26** with lithium azide. In this respect it is of interest to note that triflation of **25** in the presence of pyridine was accompanied by partial loss of the trityl group. Nonetheless, **27** could be obtained in an acceptable yield. Reduction of the azido group at C-2 in **27** with hydrogen sulfide,[13] followed by acetylation of the amine function with acetic anhydride, yielded **28**. Finally, acid hydrolysis of the trityl group in **28** with benzenesulfonic acid in methanol/dichloromethane[14] afforded key compound **22** in overall 31% yield based on **23**. In terms of the overall yields and the number of steps involved in both procedures leading to **22** it seems evident that the second approach is more convenient. However, taking into consideration the easy accessibility of starting compound **10**, the first strategy is in our opinion superior over the second one.

The required monophosphate **3** was now prepared as follows. Phosphitylation of **22** with *bis*(benzyloxy)-*N,N*-diisopropylaminophosphine[15,16] (**29**) in the presence of 1-*H*-tetrazole gave the phosphite **30** which, upon *in situ* oxidation with *t*-butyl hydroperoxide,[17] led to the phosphotriester **33** in 77% yield. Removal of the benzyl groups from the phosphotriester and O-3,4 with palladium on carbon gave, after further processing, the homogeneous sodium salt of the D-mannitol-6-phosphate derivative **3** in an excellent yield.

The synthesis of the lipophilic phosphodiester derivative **4** could be accomplished by phosphitylation of **22** with the monofunctional reagent **32**, which was easily prepared by treating a slight excess of benzyloxy-*bis*(*N,N*-diisopropylamino)phosphine[18,19] with hexadecanol in the presence of 1-*H*-tetrazole.

Thus, 1-*H*-tetrazole-mediated phosphitylation of **22** with **32** gave, after *in situ* oxidation with *t*-butyl hydroperoxide of the intermediate phosphite **31**, the fully protected phosphotriester **34** (mixture of diastereoisomers) in 93% yield. Hydrogenolysis of **34** over palladium on carbon resulted, after further processing, in the isolation of the homogeneous sodium salt of **4** in an excellent yield.

In conclusion, the results presented in this paper indicate that the potential inhibitors **1-4** can be conveniently prepared starting from easily accessible starting compounds. The inhibitory effect of **1-4** on the biosynthetic formation of CMP-NANA is at present under investigation and will be published elsewhere.

EXPERIMENTAL

General Procedures. Acetonitrile and triethylamine were dried by refluxing with CaH_2 for 16 h, then distilled and stored over molecular sieves (0.4 nm). Pyridine was dried by refluxing with CaH_2 for 16 h and then distilled, redistilled from *p*-toluenesulfonyl chloride (60 g/L), redistilled from KOH (40 g/L) and stored over molecular sieves (0.4 nm). Dioxane was dried by refluxing with CaH_2 for 16 h, then distilled and redistilled from $LiAlH_4$ directly before use. Toluene and dichloromethane were dried by refluxing with P_2O_5 (5 g/L) for 2 h and then distilled. Toluene was stored over sodium wire. Dichloromethane was stored over basic aluminum oxide. *N,N*-Dimethylformamide was stirred with CaH_2 for 16 h and then distilled under reduced pressure and stored over molecular sieves (0.4 nm). Methanol and ethanol were dried by refluxing with magnesium, distilled and stored over molecular sieves (0.3 nm). All solvents were stored under a nitrogen atmosphere.

Melting points were uncorrected. TLC analysis was performed on silica gel (Schleicher & Schull, F 1500 LS 254). Thus compounds were visualized by UV light or by spraying with the appropriate reagents. Compounds containing alkene functions were visualized by spraying the TLC plates with $KMnO_4$ (1%) in aqueous Na_2CO_3 (2%); saccharides were visualized by treatment with concd. H_2SO_4/methanol (2:8, v/v) followed by charring at 140 °C for a few minutes. Compounds containing NH functions were visualized by spraying the TLC plates with a tolidine solution after treatment of the TLC plates with chlorine. Column chromatography was

performed on Merck Kieselgel (230-400 mesh, ASTM) and elutions were effected by applying linear gradients at a flowrate of 0.2 mL/s. Evaporations were carried out below 40 °C under reduced or low pressure (15 mm or 0.5 mm Hg, respectively).

^{1}H NMR spectra were measured at 300 MHz using a Bruker WM-300 spectrometer equipped with an ASPECT-2000 computer operating in the Fourier transform mode. ^{13}C NMR and ^{31}P NMR spectra were measured at 50.1 and 80.7 MHz, respectively, using a Jeol JNM-FX 200 spectrometer on line with a JEC 980 B computer. ^{1}H and ^{13}C chemical shifts are given in ppm (δ) relative to tetramethylsilane (TMS) as internal standard, and ^{31}P chemical shifts are given in ppm (δ) relative to 85% H_3PO_4 as external standard. The concentration of the NMR-samples was 20±1 mg/mL unless stated otherwise.

1,5-Anhydro-3,4-di-*O*-benzyl-6-deoxy-D-glucitol (6). A solution of methyl 3,4-di-*O*-benzyl-6-deoxy-α-D-glucopyranoside (**5**, 1.5 mmol) in acetonitrile (10 mL) was concentrated (2 x) and the residue was redissolved in the same solvent (1.5 mL), after which *N,O*-bis(trimethylsilyl)trifluoroacetamide (1.5 mmol, 0.4 mL) was added. The mixture was heated for 2 h at 75-80 °C under a nitrogen atmosphere in a sealed flask. After cooling to room temperature, triethylsilane (5 mmol, 0.8 mL) and trimethylsilyl triflate (5 mmol, 1 mL) were added to the mixture which was then allowed to stand for 16 h at ambient temperature. TLC analysis (ethanol/toluene, 3:97, v/v) of the solution showed complete conversion of the starting material into **6**. The solution was poured into water (25 mL, 0 °C) and stirred vigorously for 15 min. The mixture was diluted with dichloromethane (40 mL), washed with saturated aqueous sodium bicarbonate (15 mL) and water (15 mL). The organic layer was dried with magnesium sulfate, filtered and concentrated *in vacuo*. The residue was applied to a column of Kieselgel (20 g) suspended in toluene which, after elution with ethanol/toluene (0:1 to 3:97, v/v), gave compound **6** (1.35 mmol, 91%) as a colourless oil: R_f 0.30 (ethanol/toluene, 3:97, v/v); ^{1}H NMR ($CDCl_3$) δ 7.37-7.31 (m, 10 H, $H_{arom.}$ 2 × benzyl), 4.96 (d, 1 H, CH_2 benzyl, $J_{A,B}$ = 11.5 Hz), 4.87 (d, 1 H, CH_2 benzyl, $J_{A,B}$ = 10.9 Hz), 4.77 (d, 1 H, CH_2 benzyl), 4.67 (d, 1 H, CH_2 benzyl), 3.93 (dd, 1 H, H-1, $J_{1,1'}$ = 11.1 Hz, $J_{1,2}$ = 5.4 Hz), 3.68 (ddd, 1 H, H-2, $J_{2,3}$ = 9.1 Hz), 3.43 (t, 1 H, H-3, $J_{3,4}$ = 8.8 Hz), 3.35 (dq, 1 H, H-5, $J_{5,6}$ = 6.2 Hz), 3.19 (t, 1 H, H-1', $J_{1',2}$ = 10.7 Hz), 3.14 (t, 1 H, H-4, $J_{4,5}$ = 9.0 Hz), and 1.30 (d,

3 H, 3 × H-6); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 138.6 and 137.9 (2 × $C_{arom.}$ benzyl), 128.2-127.4 ($CH_{arom.}$ benzyl), 86.4, 83.3, 75.8 and 70.4 (C-2, C-3, C-4 and C-5), 74.8 and 74.7 (2 × CH_2Ph), 69.3 (C-1) and 17.9 (C-6).

Anal. Calcd for $C_{20}H_{24}O_4$ (328.41): C, 73.15; H, 7.37. Found: C, 74.0; H, 7.35.

1,5-Anhydro-2-azido-3,4-di-*O*-benzyl-2,6-dideoxy-D-mannitol (8). A solution of compound **6** (1.3 mmol) in dry dichloromethane (2.6 mL) was added dropwise to a cooled (-20 °C) suspension of trifluoromethanesulfonic anhydride (2.6 mmol, 0.44 mL), pyridine (3 mmol, 0.24 mL) and 1.3 g activated molecular sieves (0.4 nm) in dry dichloromethane (8 mL). The mixture was stirred for 0.5 h at -20 °C under a nitrogen atmosphere, after which TLC analysis (ether/light petroleum ether, 2:1, v/v) showed complete conversion of the starting material into **7**. The reaction mixture was filtered, washed with an ice cold aqueous solution of saturated sodium bicarbonate (100 mL) and ice cold water (100 mL). The organic layer was dried with magnesium sulfate, filtered, concentrated and the residue was twice concentrated with dry toluene (2 × 15 mL) (water bath temperature 20 °C). The resulting syrup was dissolved in dry *N,N*-dimethylformamide (4 mL) and lithium azide (8 mmol) was added, the mixture was stirred for 1 h at ambient temperature under a nitrogen atmosphere. TLC analysis (ether/light petroleum ether, 2:1, v/v) showed complete conversion of the 2-*O*-trifluoromethane sulfonate **7** into **8**. The reaction mixture was concentrated *in vacuo,* diluted with dichloromethane (15 mL), washed twice with water (5 mL), dried with magnesium sulfate, concentrated and applied to a column of Kieselgel (15 g). Elution was effected with diethyl ether/hexane (0:1 to 1:1, v/v) compound **8** (1.1 mmol, 83%) as a colourless oil: R_f 0.71 (ethanol/toluene, 3:97, v/v) and 0.45 (diethyl ether/hexane, 2:1, v/v); IR (KBr) υ 2100 cm^{-1} (s, N=N=N stretch); ^1H NMR ($CDCl_3$) δ 7.41-7.29 (m, 10 H, $H_{arom.}$ 2 × benzyl), 4.93 (d, 1 H, CH_2 benzyl, $J_{A,B}$ = 10.8 Hz), 4.74 (AB, 1 H, CH_2 benzyl, $J_{A,B}$ = 11.6 Hz), 4.65 (d, 1 H, CH_2 benzyl), 3.90 (dd, 1 H, H-1, $J_{1,1'}$ = 12.1 Hz, $J_{1,2}$ = 2.3 Hz), 3.87 (m, 1 H, H-2), 3.69 (dd, 1 H, H-3, $J_{3,2}$ = 3.7 Hz, $J_{3,4}$ = 9.0 Hz), 3.47 (t, 1 H, H-4, $J_{4,5}$ = 9.0 Hz), 3.42 (d, 1 H, H-1'), 3.25 (dq, 1 H, H-5, $J_{5,6}$ = 6.1 Hz) and 1.31 (d, 3 H, 3 × H-6); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 137.9 and 137.4 (2 × $C_{arom.}$ benzyl), 128.1-127.3 ($CH_{arom.}$ benzyl), 82.1, 79.9 and 76.0 (C-3, C-4 and C-5), 75.1 and 71.5 (2 × CH_2Ph), 67.3 (C-1), 59.0 (C-2) and 17.8 (C-6).

2-Acetamido-1,5-anhydro-3,4-di-*O*-benzyl-2,6-dideoxy-D-mannitol (9). Compound **8** (0.27 mmol) was dissolved in ethanol (10 mL) together with nickel(II) chloride (40 mg) and H_3BO_3 (20 mg). To the solution a suspension of $NaBH_4$ (10 mg) in ethanol (2 mL) was added dropwise over a period of 30 min. The mixture was concentrated, dissolved in dry pyridine (2 mL) and acetic anhydride (1 mL) was added. The mixture was stirred at ambient temperature. After 2 h, TLC analysis (ethanol/toluene, 3:97, v/v) of the suspension showed complete conversion of the starting material into **9**. The mixture was concentrated with toluene, diluted with dichloromethane (25 mL) and washed twice with water. The organic layer was dried (magnesium sulfate), concentrated and the residue was applied to a column of Kieselgel (10 g). Elution was effected with ethanol/toluene (0:1 to 3:97, v/v), and concentration of the appropriate fractions gave compound **9** (0.26 mmol, 96%) as a colourless oil: R_f 0.19 (ethanol/toluene, 3:97, v/v); ^1H NMR ($CDCl_3$) δ 7.38-7.27 (m, 10 H, $H_{arom.}$ 2 × benzyl), 5.90 (d, 1 H, *N*-H, $J_{NH,2}$ = 8.5 Hz), 4.94 (d, 1 H, CH_2 benzyl, $J_{A,B}$ = 10.8 Hz), 4.79 (d, 1 H, CH_2 benzyl, $J_{A,B}$ = 11.1 Hz), 4.61 (d, 1 H, CH_2 benzyl), 4.59 (m, 1 H, H-2), 4.50 (d, 1 H, CH_2 benzyl), 3.95 (dd, 1 H, H-1, $J_{1,1'}$ = 12.3 Hz, $J_{1,2}$ = 2.1 Hz), 3.70 (dd, 1 H, H-3, $J_{3,2}$ = 4.8 Hz, $J_{3,4}$ = 9.1 Hz), 3.48 (dd, 1 H, H-1', $J_{1',2}$ = 1.8 Hz), 3.29 (dq, 1 H, H-5, $J_{5,6}$ = 6.1 Hz), 3.15 (t, 1 H, H-4, $J_{4,5}$ = 9.2 Hz), 2.07 (s, 3 H, CH_3 acetamido), 1.32 (d, 3 H, 3 × H-6); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 170.0 ($COCH_3$), 138.2 and 137.7 (2 × $C_{arom.}$ benzyl), 128.3-127.5 ($CH_{arom.}$ benzyl), 80.5, 80.4 and 76.3 (C-3, C-4 and C-5), 75.3 and 70.8 (2 × CH_2Ph), 69.0 (C-1), 47.1 (C-2), 23.5 ($COCH_3$) and 18.3 (C-6).

2-Acetamido-1,5-anhydro-2,6-dideoxy-D-mannitol (1). A solution of compound **9** (0.19 mmol) in *tert*-butyl alcohol/water (3:1, v/v, 5 mL) was shaken with 10% palladium on carbon (30 mg) under a hydrogen atmosphere (P_{H_2} = 0.4 MPa) for 48 h at room temperature. TLC analysis (methanol/dichloromethane, 4:96, v/v) showed complete conversion of the starting material into **1**. The palladium catalyst was removed by filtration. The filtrate was concentrated *in vacuo* and concentrated several times with water. Lyophilization of the residue gave homogeneous 2-acetamido-1,5-anhydro-2,6-dideoxy-D-mannitol (**1**) (0.19 mmol, 99%) as a white foam: R_f 0.76 (1-propanol/water, 6:4, v/v); ^1H NMR (CD_3OD) δ 4.21 (dt, 1 H, H-2, $J_{2,3}$ = 4.9 Hz), 3.78 (dd, 1 H, H-1, $J_{1,1'}$ = 12.2 Hz, $J_{1,2}$ = 2.0 Hz), 3.64 (dd, 1 H, H-3, $J_{3,4}$ = 9.4 Hz), 3.54 (dd, 1 H, H-1' $J_{1',2}$ = 1.8 Hz), 3.30 (t, 1 H, H-4, $J_{4,5}$ =

9.3 Hz), 3.18 (dq, 1 H, H-5, $J_{5,6}$ = 6.1 Hz), 2.02 (s, 3 H, CH_3 acetamido), 1.29 (d, 3 H, 3 × H-6); $^{13}C\{^1H\}$ NMR (CD_3OD) δ 174.0 ($COCH_3$), 78.4, 74.4 and 74.1 (C-3, C-4 and C-5), 69.9 (C-1), 52.6 (C-2), 22.6 ($COCH_3$), 18.3 (C-6).

Anal. Calcd for $C_8H_{15}NO_4$ (189.21): C, 50.78; H, 7.99; N, 7.40. Found: C, 50.1; H, 8.03; N, 7.2.

1,5-Anhydro-3-*O*-benzyl-D-glucitol (11). A solution of methyl 3-*O*-benzyl-α/β-D-glucopyranoside (**10**, 20.3 mmol) in acetonitrile (20 mL) was concentrated twice and the residue was dissolved in the same solvent (20 mL), after which *N,O*-bis(trimethylsilyl)trifluoroacetamide (45.7 mmol, 12 mL) was added. The mixture was heated for 4 h at 75-80 °C under a nitrogen atmosphere in a sealed flask. After cooling to room temperature, triethylsilane (101.5 mmol, 16.2 mL) and trimethylsilyl triflate (101.5 mmol, 19.6 mL) were added to the mixture which was allowed to stand for 16 h at ambient temperature. Progress of the reaction was monitored by TLC analysis (methanol/dichloromethane, 8:92, v/v). The solution was poured into water (100 mL, 0 °C) and stirred vigorously for 15 minutes. The mixture was treated with excess of Amberlist IRA 400 [OH^-] and filtered. The filtrate was then neutralized with Dowex 50 XW [H^+], filtered and concentrated *in vacuo* to yield crude **11** (5.9 g) as light yellow oil, which was without further purification used for the synthesis of compounds **12** and **13**.

2,4,6-Tri-*O*-acetyl-1,5-anhydro-3-*O*-benzyl-D-glucitol (12). Crude **11** (300 mg) was dissolved in a mixture of dry pyridine (4 mL) and acetic anhydride (2 mL). The mixture was stirred at ambient temperature. After 2 h, TLC analysis (acetone/toluene, 3:97, v/v) of the solution showed complete conversion of the starting material into **12**. The mixture was concentrated *in vacuo* and concentrated several times with toluene (40 mL). The residue was applied to a column of Kieselgel (15 g) which, after elution with acetone/dichloromethane (0:1 to 2:98, v/v) and concentration of the appropriate fractions, gave **12** (0.91 mmol, 91%) as a colourless oil. Compound **12** was crystallized from dichloromethane and hexane (mp 99 °C); R_f 0.40 (toluene/acetone, 97:3, v/v); ^1H NMR ($CDCl_3$) δ 7.34-7.27 (m, 5 H, $H_{arom.}$ benzyl), 5.04 (t, 1 H, H-4, J4,5 = 9.6 Hz), 4.99 (ddd, 1 H, H-2, $J_{2,3}$ = 9.4 Hz), 4.87 (AB, 2 H, CH_2 benzyl, $J_{A,B}$ = 11.8 Hz), 4.16 (dd, 1 H, H-6, $J_{6,6'}$ = 12.3 Hz), 4.14 (dd, 1 H, H-1, $J_{1,2}$ = 5.3 Hz), 4.11 (dd, 1 H, H-6'), 3.68 (t, 1 H, H-3, $J_{3,4}$ = 9.2 Hz), 3.50 (ddd, 1 H, H-5, $J_{5,6}$ = 4.9 Hz, $J_{5,6'}$ = 2.7 Hz), 3.20 (t, 1 H, H-1', $J_{1',2}$ =

10.8 Hz), 2.09, 2.00 and 1.99 [3 × (s, 3 H, $COCH_3$)]; $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 170.4, 169.4 and 169.2 (3 × CO acetyl), 137.8 ($C_{arom.}$ benzyl), 128.2, 127.5 and 127.4 ($CH_{arom.}$ benzyl), 80.7, 76.5, 71.0 and 69.5 (C-2, C-3, C-4 and C-5), 74.2 (CH_2 benzyl), 66.6 (C-1), 62.3 (C-6) and 20.5 (3 × $COCH_3$).

Anal. Calcd for $C_{19}H_{24}O_8$ (380.39): C, 59.99; H, 6.36. Found: C, 59.69; H, 6.31.

1,5-Anhydro-3-*O*-benzyl-4,6-*O*-benzylidene-D-glucitol (13). A solution of crude **11** (11 g) in dry *N,N*-dimethylformamide (25 mL) was concentrated and the residue was redissolved in the same solvent (100 mL). α,α-Dimethoxytoluene (24.1 mmol, 3.6 mL) and a catalytic amount of *p*-toluenesulfonic acid (pH ≈ 4) were added to the solution and the mixture was stirred at 50 °C. After 20 h, TLC analysis (methanol/dichloromethane, 4:96, v/v) showed complete conversion of the starting material to a product with a higher mobility. The reaction mixture was cooled, neutralised with aqueous sodium bicarbonate and concentrated *in vacuo*. The residue was dissolved in dichloromethane (200 mL), washed two times with water (100 mL), dried (magnesium sulfate, 25 g) and concentrated *in vacuo*. The crude product was crystallised from dichloromethane and hexane to give **13** (5.6 mmol, 29%) as white needles. The mother liquor was concentrated *in vacuo* and applied to a column of Kieselgel (300 g) which, after elution with dichloromethane/acetone (1:0, to 95:5, v/v) and concentration of the appropriate fractions gave another portion of homogeneous **13** (7.3 mmol, 39%): mp 132 °C; R_f 0.57 (acetone/dichloromethane, 5:95, v/v); ^1H NMR ($CDCl_3$) δ 7.53-7.26 (m, 10 H, $H_{arom.}$ 2 × Ph), 5.58 (s, 1 H, CHPh), 5.04 (d, 1 H, CH_2 benzyl, $J_{A,B}$ = 11.5 Hz), 4.72 (d, 1 H, CH_2 benzyl), 4.34 (dd, 1 H, H-6, $J_{6,6'}$ = 10.4 Hz), 4.06 (dd, 1 H, H-$1_{(eq)}$, $J_{1,1'}$ = 11.1 Hz, $J_{1,2}$ = 5.6 Hz), 3.78 (m 12 lines, 1 H, H-2), 3.72 (t, 1 H, H-6'), 3.66 (t, 1 H, H-4, $J_{4,5}$ = 8.5 Hz), 3.58 (t, 1 H, H-3, $J_{3,4}$ = 8.7 Hz), 3.42 (ddd, 1 H, H-5, $J_{5,6}$ = 4.9 Hz, $J_{5,6'}$ = 9.7 Hz), 3.34 (t, 1 H, H-$1'_{(ax)}$, $J_{1',2}$ = 10.8 Hz) and 2.36 (d, 1 H, OH, $J_{OH,2}$ = 2.6 Hz); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 138.2 and 137.2 (2 × $C_{arom.}$ Ph), 128.9-125.9 ($CH_{arom.}$ Ph), 101.1 (CHPh), 82.6, 82.0, 71.4 and 69.4 (C-2, C-3, C-4 and C-5), 74.5 (CH_2 benzyl), 69.8 and 68.7 (C-1 and C-6).

Anal. Calcd for $C_{20}H_{22}O_5$ (342.39): C, 70.16; H, 6.48. Found: C, 70.01; H, 6.60.

1,5-Anhydro-2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-D-mannitol (15). A solution of compound **13** (10 mmol) in dry dichloromethane (20 mL) was added dropwise to a cooled (-20 °C) suspension of trifluoromethanesulfonic anhydride (20 mmol, 3.36 mL), pyridine (23 mmol, 1.85 mL) and 10 g activated molecular sieves (0.4 nm) in dry dichloromethane (60 mL). The mixture was stirred for 0.5 h at -20 °C under a nitrogen atmosphere, after which TLC analysis (ether/light petroleum ether, 2:1, v/v) showed complete conversion of the starting material into **14**. The reaction mixture was filtered, washed with an ice cold aqueous solution of saturated sodium bicarbonate (100 mL) and ice cold water (100 mL). The organic layer was dried with magnesium sulfate, filtered, concentrated and the residue was concentrated with dry toluene (2 × 15 mL) (water bath temperature 20 °C). The resulting syrup was dissolved in dry *N,N*-dimethylformamide (30 mL) and lithium azide (60 mmol) was added, the mixture was stirred for 1 h at ambient temperature under a nitrogen atmosphere. TLC analysis (ether/light petroleum ether, 1:1, v/v) showed complete conversion of the 2-*O*-trifluoromethane sulfonate **14**. The reaction mixture was concentrated *in vacuo*, diluted with dichloromethane (100 mL), washed twice with water (50 mL), dried with magnesium sulfate, concentrated and applied to a column of Kieselgel (60 g). Elution was effected with ether/light petroleum ether (1:2 to 2:1, v/v) to give **15** as a colourless oil (7.15 mmol, 72%): R_f 0.41 (diethyl ether/petroleum ether, 1:1, v/v); IR (NaCl) υ 2100 cm^{-1}, N=N=N stretch; ^1H NMR ($CDCl_3$) δ 7.48-7.22 (m, 10 H, $H_{arom.}$ 2 × Ph), 5.61 (s, 1 H, CHPh), 4.87 (d, 1 H, CH_2Ph, J_{AB} = 12.3 Hz), 4.74 (d, 1 H, CH_2Ph), 4.25 (dd, 1 H, H-6, $J_{6,6'}$ = 10.5 Hz), 4.08 (t, 1 H, H-4, $J_{4,5}$ = 9.4 Hz), 3.91 (dd, 1 H, H-1, $J_{1,1'}$ =12.5 Hz, $J_{1,2}$ = 1.8 Hz), 3.87 (dt, 1 H, H-2, $J_{2,3}$ = 3.8 Hz), 3.80 (dd, 1 H, H-3, $J_{3,4}$ = 9.5 Hz), 3.77 (t, 1 H, H-6'), 3.50 (dd, 1 H, H-1', $J_{1',2}$ = 1.6 Hz) and 3.28 (dt, 1 H, H-5, $J_{5,6}$ = 4.9 Hz, $J_{5,6'}$ = 9.7 Hz); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 137.8 and 137.3 (2 × $C_{arom.}$ Ph), 128.7-127.2 (10 × $HC_{arom.}$ Ph), 101.1 (CHPh), 78.9, 77.8 and 71.9 (C-3, C-4 and C-5), 72.5 (CH_2Ph), 68.8 and 68.1 (C-1 and C-6) and 60.4 (C-2).

Anal. Calcd for $C_{20}H_{21}N_3O_4$ (367.40): C, 65.38; H, 5.76; N, 11.44. Found: C, 65.34; H, 5.96; N, 11.24.

2-Acetamido-3,4,6-tri-*O*-acetyl-1,5-anhydro-2-deoxy-D-mannitol (16). A solution of compound **15** (1.04 mmol) in *tert*-butyl alcohol/water/acetic acid (3:1:1, v/v/v, 10 mL) was shaken with palladium(II) hydroxide on carbon 20% (moisture

ca. 50%, 0.7 g) under a hydrogen atmosphere (P_{H_2} = 0.4 MPa) for 48 h at room temperature. TLC analysis (1-propanol/water/triethylamine, 600:400:5, v/v/v) showed complete conversion of the starting material into **15**. The palladium catalyst was removed by filtration and the filtrate was concentrated to near dryness. The residue was concentrated several times with dry pyridine (15 mL), dissolved in the same solvent (6 mL) and acetic anhydride (3 mL) was added. The solution was stirred for 2 h at ambient temperature. The reaction mixture was diluted with toluene (25 mL), concentrated several times with the same solvent and applied to a column of Kieselgel (2.5 g). Elution was effected with dichloromethane/methanol (1:0 to 95:5, v/v) to give **16** as a colourless foam (0.89 mmol, 88%): R_f 0.23 (methanol/dichloromethane, 4:96, v/v); ^{1}H NMR ($CDCl_3$) δ 6.06 (d, 1 H, N-H, $J_{NH,2}$ = 8.8 Hz), 5.10 (t, 1 H, H-4, $J_{4,5}$ = 9.8 Hz), 5.02 (dd, 1 H, H-3, $J_{3,4}$ = 10.0 Hz), 4.63 (ddd, 1 H, H-2, $J_{2,3}$ = 4.2 Hz), 4.24 (dd, 1 H, H-6, $J_{6,6'}$ = 12.4 Hz), 4.09 (dd, 1 H, H-6'), 3,94 (dd, 1 H, H-1, $J_{1,1'}$ = 12.4 Hz, $J_{1,2}$ = 2.0 Hz), 3.70 (dd, 1 H, H-1', $J_{1',2}$ = 1.9 Hz), 3.58 (ddd, 1 H, H-5, $J_{5,6}$ = 6.0 Hz, $J_{5,6'}$ = 2.5 Hz) and 2.11-2.02 (4 × s, 12 H, 4 × CH_3 acetyl); ^{13}C{^{1}H} NMR ($CDCl_3$) δ 170.1, 169.9 and 169.3 (4 × *C*OCH_3), 76.4, 71.6 and 66.0 (C-3, C-4 and C-5), 68.6 (C-1), 62.6 (C-6), 47.1 (C-2), 22.4 (CH_3 acetamido) and 20.2 (3 × CH_3 *O*-acetyl).

2-Acetamido-1,5-anhydro-2-deoxy-D-mannitol (2). Compound **16** (0.71 mmol) was dissolved in dry methanol (7 mL) and sodium methoxide (1 mmol) was added. The solution was stirred for 2 h at ambient temperature, and the reaction was monitored by TLC analysis (1-propanol/water, 6:4, v/v). The reaction mixture was treated with Dowex 50 XW [H^+] filtered and concentrated *in vacuo*. Lyophilization of the residue gave homogeneous 2-acetamido-1,5-anhydro-2-deoxy-D-mannitol (**2**) (0.58 mmol, 82%) as a white foam: R_f 0.71 (1-propanol/water, 6:4, v/v); ^{1}H NMR (D_2O, pD = 9.0, T = 303 K) δ 4.28 (dt, 1 H, H-2, $J_{2,3}$ = 4.7 Hz), 3.86 (dd, 1 H, H-6, $J_{6,6'}$ = 12.3 Hz), 3.83 (dd, 1 H, H-1, $J_{1,1'}$ = 12.4 Hz, $J_{1,2}$ = 1.9 Hz), 3.80 (dd, 1 H, H-3, $J_{3,4}$ = 9.7 Hz), 3.74 (dd, 1 H, H-6'), 3.67 (dd, 1 H, H-1', $J_{1',2}$ = 1.8 Hz), 3.54 (t, 1 H, H-4, $J_{4,5}$ = 9.7 Hz), 3.30 (ddd, 1 H, H-5, $J_{5,6}$ = 2.4 Hz, $J_{5,6'}$ = 5.3 Hz) and 2.02 (s, 3 H, NHCOC*H*$_3$); ^{13}C{^{1}H} NMR (D_2O, pD = 9.0) δ 175.4 (*C*OCH_3), 81.4, 73.3 and 70.0 (C-5, C-4 and C-3), 69.5 (C-1), 61.5 (C-6), 51.4 (C-2) and 22.8 (CO*C*H_3).

Anal. Calcd for $C_8H_{15}NO_5$ (205.21): C, 46.82; H, 7.37; N, 6.83. Found: C, 47.28; H, 7.48; N, 6.63.

2-Acetamido-1,5-anhydro-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-D-mannitol (17). Compound **15** (6 mmol) was treated with hydrogen sulfide in a mixture of pyridine, triethylamine and water (120 mL, 4:1:1, v/v/v). The mixture was stirred for 3 h at ambient temperature. TLC analysis (ether/hexanes, 2:1, v/v) showed complete conversion of the starting material. The mixture was concentrated *in vacuo* and the residue was diluted with dichloromethane (100 mL), washed with water (2 × 25 mL) and dried with magnesium sulfate. The organic layer was concentrated *in vacuo,* concentrated with dry pyridine (2 × 10 mL), and dissolved in the same solvent (30 mL). The solution was treated with acetic anhydride (15 mL) and stirred at ambient temperature. After 0.5 h, TLC analysis (ether/hexane, 2:1, v/v) of the solution showed complete conversion of the starting material. The mixture was concentrated *in vacuo* and concentrated several times with toluene (40 mL). The residue was applied to a column of Kieselgel (50 g) which, after elution with ethanol/toluene (0:1 to 5:95, v/v) and concentration of the appropriate fractions, gave **17** (5.8 mmol, 96%) as a colourless oil. Compound **17** was crystallized from diethyl ether and hexane: mp 188 °C; R_f 0.45 (toluene/acetone, 9:1, v/v); ^1H NMR ($CDCl_3$) δ 7.52-7.24 (m, 10 H, $H_{arom.}$ phenyl), 6.09 (s, 1 H, N-H), 5.59 (s, 1 H, C*H*Ph), 4.75 (d, 1 H, CH_2 benzyl, $J_{A,B}$ = 12.1 Hz), 4.61 (d, 1 H, CH_2 benzyl), 4.55 (ddt, 1 H, H-2, $J_{2,3}$ = 4.2 Hz, $J_{2,NH}$ = 7.8 Hz), 4.28 (dd, 1 H, H-6, $J_{6,6'}$ = 10.4 Hz), 4.09 (dd, 1 H, H-1, $J_{1,1'}$ = 12.4 Hz, $J_{1,2}$ = 1.7 Hz), 3.80-3.75 (m, 2 H, H-3 and H-4), 3.72 (t, 1 H, H-6'), 3.57 (dd, 1 H, H-1', $J_{1',2}$ = 2.0 Hz), 3.33 (dt, 1 H, H-5, $J_{5,4} \approx J_{5,6'}$ = 9.5 Hz, $J_{5,6}$ = 4.8 Hz) and 2.05 (s, 3 H, CH_3 acetamido); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 170.1 (C =O acetamido), 137.6 and 137.1 (2 × $C_{arom.}$ Ph), 128.7-125.7 ($CH_{arom.}$ Ph), 101.1 (PhCHOO), 78.5, 75.4 and 71.9 (C-3, C-4 and C-5), 70.9, 69.9 and 68.2 (C-1, C-6 and CH_2Ph), 47.9 (C-2) and 23.0 (CH_3 acetyl).

Anal. Calcd for $C_{22}H_{25}NO_5$ (383.44): C, 68.91; H, 6.57; N, 3.65. Found: C, 68.63; H, 6.63; N, 3.49.

2-Acetamido-1,5-anhydro-3-*O*-benzyl-2-deoxy-D-mannitol (18). Compound **17** (5 mmol) was dissolved in a mixture of methanol and water (50 mL, 99:1, v/v) and *p*-toluenesulfonic acid (300 mg) was added. The mixture was stirred for 3 h at ambient temperature until TLC analysis (dichloromethane/methanol, 9:1, v/v) indicated complete conversion of compound **17** into a single product having a lower mobility. The solution was treated with Amberlite IRA 400 [OH^-] (10 mL), filtered,

concentrated *in vacuo*, and the product precipitated from dichloromethane (3 mL) and hexane (50 mL). After drying *in vacuo* compound **18** (1.45 g) was used without further purification for the synthesis of compounds **19** and **20**.

2-Acetamido-4,6-di-O-acetyl-1,5-anhydro-3-*O*-benzyl-2-deoxy-D-mannitol (19). Crude **18** (113 mg≈ 0.3 mmol) was dissolved in dry pyridine (2 mL) and acetic anhydride (1 mL). The mixture was stirred at ambient temperature. After 1 h, TLC analysis (methanol/dichloromethane, 1:9, v/v) of the solution showed complete conversion of the starting material into **19**. The mixture was concentrated *in vacuo* and concentrated several times with toluene (20 mL). The residue was applied to a column of Kieselgel (5 g) which, after elution with methanol/dichloromethane (0:1 to 6:94, v/v) and concentration of the appropriate fractions, gave **19** (0.27 mmol, 90%) as a colourless oil: R_f 0.61 (methanol/dichloromethane, 1:9, v/v); ^{1}H NMR ($CDCl_3$) δ 7.37-7.26 (m, 5 H, $H_{arom.}$ benzyl), 6.03 (d, 1 H, *N*-H), 5.01 (t, 1 H, H-4, $J_{4,5}$ = 9.3 Hz), 4.70 (d, 1 H, CH_2 benzyl, $J_{A,B}$ = 12.2 Hz), 4.58 (ddt, 1 H, H-2, $J_{2,NH}$ = 8.2 Hz, $J_{2,3}$ = 4.8 Hz), 4.46 (d, 1 H, CH_2 benzyl), 4.23 (dd, 1 H, H-6, $J_{6,6'}$ = 12.3 Hz), 4.08 (dd, 1 H, H-6'), 4.06 (dd, 1 H, H-1, $J_{1,1'}$ = 12.3 Hz, $J_{1,2}$ = 2.7 Hz), 3.63 (dd, 1 H, H-3, $J_{3,4}$ = 9.2 Hz), 3.52 (dd, 1 H, H-1'), 3.50 (ddd, 1 H, H-5, $J_{5,6}$ = 6.5 Hz, $J_{5,6'}$ = 2.8 Hz), 2.09, 2.06 and 2.03 (3 × s, 9 H, 3 × $COCH_3$).

2-Acetamido-1,5-anhydro-3-*O*-benzyl-2-deoxy-6-*O*-(4,4'-dimethoxytrityl)-D-mannitol (20). A solution of compound **18** (4.5 mmol) in pyridine (10 mL) was concentrated, the residue dissolved in the same solvent (21 mL) and treated with 4,4'-dimethoxytrityl chloride (4.95 mmol). The mixture was stirred at 20 °C and monitored by TLC analysis (ethanol/toluene, 5:95, v/v). After 2 h, methanol (1 mL) was added to the mixture. After 0.5 h, the mixture was concentrated *in vacuo*, diluted with dichloromethane (50 mL), washed with saturated aqueous sodium bicarbonate (15 mL) and water (15 mL). The organic layer was dried with magnesium sulfate, filtered and concentrated *in vacuo*. The residue was applied to a column of Kieselgel (40 g) which, after elution with ethanol/toluene (0:1 to 2:98, v/v) and concentration of the appropriate fractions, gave **20** (4.4 mmol, 98%) as a light yellow foam: R_f 0.36 (ethanol/toluene, 5:95, v/v); ^{1}H NMR ($CDCl_3$) δ 7.46-6.79 (m, 18 H, $H_{arom.}$ benzyl and DMTr), 5.94 (d, 1 H, NH, $J_{NH,2}$ = 7.7 Hz), 4.83 (d, 1 H, CH_2 benzyl, $J_{A,B}$ = 11.0 Hz), 4.66 (m, 1 H, H-2), 4.45 (d, 1 H, CH_2 benzyl), 4.03 (dd, 1 H, H-1, $J_{1,1'}$ = 12.2 Hz, $J_{1,2}$ = 2.8 Hz), 3.76 (s, 6 H, OCH_3

DMTr), 3.72 (br t, 1 H, H-4, $J_{4,5}$ = 9.2 Hz), 3.54-3.46 (m, 2 H, H-6 and H-3), 3.40-3.30 (m, 3 H, H-1', H-5 and H-6'), 2.71 (br s, 1 H, OH), 2.03 (s, 3 H, $COCH_3$); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 169.7 ($COCH_3$), 158.1 and 144.6 (3 × $C_{arom.}$ DMTr), 137.4 ($C_{arom.}$ benzyl), 135.6 ($C_{arom.}$ DMTr), 129.7-124.9 and 112.8 ($CH_{arom.}$ benzyl and DMTr), 85.8 ($OCAr_3$ DMTr), 79.8, 79.3 (C-3 and C-5), 70.4 (CH_2Ph), 68.7 (C-1), 67.6 (C-4), 63.4 (C-6), 54.7 (OCH_3 DMTr), 46.0 (C-2), 22.9 ($COCH_3$).

2-Acetamido-1,5-anhydro-3,4-di-*O*-benzyl-2-deoxy-6-*O*-(4,4'-dimethoxytrityl)-D-mannitol (21). Compound **20** (4.2 mmol) was dissolved in *N,N*-dimethylformamide (15 mL). To the solution, barium oxide (16 mmol), $Ba(OH)_2.H_2O$ (1 mmol) and benzyl bromide (25 mmol) were added and the suspension was stirred at 50 °C. The mixture was monitored by TLC analysis (ethanol/toluene, 5:95, v/v). After 48 h, the mixture was diluted with dichloromethane (20 mL) and washed with water (3 × 40 mL). The organic layer was dried with sodium sulfate, concentrated *in vacuo* and applied to a column of Kieselgel (80 g). Elution was effected with dichloromethane/acetone (1:0 to 95:5, v/v) and concentration of the appropriate fractions gave **21** (3.3 mmol, 79%) as a colourless syrup: R_f 0.46 (ethanol/toluene, 5:95, v/v), 0.37 (dichloromethane/acetone, 95:5, v/v); ^1H NMR ($CDCl_3$) δ 7.49-6.76 (m, 23 H, $H_{arom.}$ 2 × benzyl and DMTr), 6.09 (d, 1 H, NH), 4.85 (d, 1 H, CH_2 benzyl, $J_{A,B}$ = 11.0 Hz), 4.76 (d, 1 H, CH_2 benzyl, $J_{A,B}$ = 10.3 Hz), 4.66 (dddd, 1 H, H-2, $J_{2,NH}$ = 9.0 Hz, $J_{2,3}$ = 4.2 Hz), 4.50 (d, 1 H, CH_2 benzyl), 4.20 (d, 1 H, CH_2 benzyl), 4.03 (dd, 1 H, H-1, $J_{1,1'}$ = 12.1 Hz, $J_{1,2}$ = 2.2 Hz), 3.75 (t, 1 H, H-4, $J_{4,5}$ = 9.2 Hz), 3.74 (s, 6 H, OCH_3 DMTr), 3.68 (dd, 1 H, H-3, $J_{3,4}$ = 9.3 Hz), 3.58 (dd, 1 H, H-6, $J_{6,6'}$ = 10.0 Hz), 3.52 (dd, 1 H, H-1', $J_{1',2}$ = 1.3 Hz), 3.36 (ddd, 1 H, H-5, $J_{5,6}$ = 2.1 Hz, $J_{5,6'}$ = 3.5 Hz), 3.23 (dd, 1 H, H-6'), 2.12 (s, 3 H, $COCH_3$); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 169.9 ($COCH_3$), 158.4 and 144.9 (3 × $C_{arom.}$ DMTr), 137.9 and 137.7 (2 × $C_{arom.}$ benzyl), 135.9 and 135.5 (2 × $C_{arom.}$ benzyl), 130.1-126.6 and 113.0 ($CH_{arom.}$ benzyl and DMTr), 85.7 ($OCAr_3$ DMTr), 81.1, 79.5 and 74.5 (C-3, C-4 and C-5), 75.0 and 71.0 (2 × CH_2Ph), 68.9 (C-1), 62.1 (C-6), 55.0 (OCH_3 DMTr), 46.8 (C-2), 23.6 ($COCH_3$).

Further elution gave, after concentration of the appropriate fractions, unreacted **20** (0.8 mmol, 18%) as a colourless foam.

2-Acetamido-1,5-anhydro-3,4-di-*O*-benzyl-2-deoxy-D-mannitol (22). Method A: To a solution of compound **21** (3.1 mmol) in methanol/dichloromethane (15 mL,

3:7, v/v) was added a solution of benzenesulfonic acid (4%, w/v) in methanol/dichloromethane (15 mL, 3:7, v/v). After 6 min., the mixture was diluted with dichloromethane (30 mL), washed with aqueous sodium bicarbonate (30 mL, 10% w/v) and water (30 ml). The organic phase was dried with magnesium sulfate, filtered, concentrated and applied to a column of Kieselgel (25 g). Elution was effected with dichloromethane/methanol (1:0 to 9:1, v/v) and concentration of the appropriate fractions gave **22** (2.9 mmol, 93%) as a colourless foam: R_f 0.18 (dichloromethane/methanol, 96:4, v/v), 0.53 (methanol/dichloromethane, 9:1, v/v); ^1H NMR ($CDCl_3$) δ 7.38-7.25 (m, 10 H, $H_{arom.}$ 2 × benzyl), 6.13 (d, 1 H, NH, $J_{NH,2}$ = 8.1 Hz), 4.95 (d, 1 H, CH_2 benzyl, $J_{A,B}$ = 11.0 Hz), 4.80 (d, 1 H, CH_2 benzyl, $J_{A,B}$ = 11.2 Hz), 4.62 (d, 1 H, CH_2 benzyl), 4.60 (m, 1 H, H-2), 4.51 (d, 1 H, CH_2 benzyl), 4.01 (dd, 1 H, H-1, $J_{1,1'}$ = 12.2 Hz, $J_{1,2}$ = 2.0 Hz), 3.85 (dd, 1 H, H-6, $J_{6,6'}$ = 11.8 Hz), 3.75 (dd, 1 H, H-3, $J_{3,2}$ = 4.6 Hz, $J_{3,4}$ = 9.3 Hz), 3.72 (dd, 1 H, H-6'), 3.56 (t, 1 H, H-4, $J_{4,5}$ = 9.4 Hz), 3.50 (dd, 1 H, H-1', $J_{1',2}$ = 1.7 Hz), 3.25 (ddd, 1 H, H-5, $J_{5,6}$ = 2.7 Hz, $J_{5,6'}$ = 3.5 Hz), 2.09 (s, 1 H, *O*H), 2.05 (s, 3 H, CH_3 acetyl); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 170.4 (*C*OCH_3), 137.9 and 137.5 (2 × $C_{arom.}$ benzyl), 128.2-127.2 ($CH_{arom.}$ benzyl), 80.4, 79.8 and 73.7 (C-3, C-4 and C-5), 74.8 and 70.5 (2 × CH_2Ph), 68.9 (C-1), 61.2 (C-6), 46.5 (C-2), 22.7 (*C*H_3CO).

Method B: Compound **28** (0.45 mmol) was treated as described under A until TLC analysis (dichloromethane/methanol, 9:1, v/v) indicated complete conversion (15 min.) of compound **28** into **22**. Work-up and purification as described under A gave **22** (0.43 mmol, 95%). Analytical and spectral data of compound **22** thus obtained, were in excellent agreement with the data reported under A.

1,5-Anhydro-3,4-di-*O*-benzyl-D-glucitol (24). A solution of methyl 3,4-di-*O*-benzyl-α-D-glucopyranoside (**23**, 4 mmol) in acetonitrile (10 mL) was concentrated (2 x) and the residue redissolved in the same solvent (4 mL), after which *N,O*-bis-(trimethylsilyl)trifluoroacetamide (6 mmol, 1.6 mL) was added. The mixture was heated for 2 h at 75-80 °C under a nitrogen atmosphere in a sealed flask. After cooling to room temperature, triethylsilane (20 mmol, 3.2 mL) and trimethylsilyl triflate (20 mmol, 3.8 mL) were added to the mixture, which was then allowed to stand for 16 h at ambient temperature. TLC analysis (methanol/dichloromethane, 4:96, v/v) of the solution showed complete disappearance of the starting material. The solution was poured into water (100 mL, 0 °C) and stirred vigorously for 15

min. The mixture was diluted with dichloromethane (40 mL), washed with saturated aqueous sodium bicarbonate (15 mL) and water (15 mL). The organic layer was dried with magnesium sulfate, filtered and concentrated *in vacuo*. The residue was applied to a column of Kieselgel (30 g) which, after elution with acetone/dichloromethane (0:1 to 5:95, v/v) and concentration of the appropriate fractions, gave **24** (2.96 mmol, 74%) as a colourless oil: R_f 0.45 (methanol/dichloromethane, 4:96, v/v); ^1H NMR ($CDCl_3$) δ 7.36-7.32 (m, 10 H, $H_{arom.}$ 2 × benzyl), 4.95 (d, 1 H, CH_2Ph, J_{AB} = 11.5 Hz), 4.85 (d, 1 H, CH_2Ph, J_{AB} = 11.0 Hz), 4.77 (d, 1 H, CH_2Ph), 4.67 (d, 1 H, CH_2Ph), 3.95 (dd, 1 H, H-1, $J_{1,1'}$ = 11.1 Hz, $J_{1,2}$ = 5.4 Hz), 3.83 (dd, 1 H, H-6, $J_{6,6'}$ = 11.9 Hz), 3.68 (dd, 1 H, H-6'), 3.67 (m, 1 H, H-2, $J_{2,3}$ = 8.7 Hz), 3.51 (t, 1 H, H-3, $J_{3,4}$ = 8.7 Hz), 3.46 (t, 1 H, H-4, $J_{4,5}$ = 8.7 Hz), 3.30 (ddd, 1 H, H-5, $J_{5,6}$ = 2.6 Hz, $J_{5,6'}$ = 4.6 Hz) and 3.21 (t, 1 H, H-1', $J_{1',2}$ = 10.7 Hz); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 138.3 and 137.7 (2 × $C_{arom.}$ Ph), 128.3-127.7 (10 × $HC_{arom.}$ Ph), 86.5, 79.8 and 77.4 (C-3, C-4 and C-5), 75.0 and 74.7 (2 × CH_2Ph), 70.0 (C-2), 69.2 (C-1) and 61.5 (C-6).

1,5-Anhydro-3,4-di-*O*-benzyl-6-*O*-trityl-D-glucitol (25). A solution of compound **24** (2.8 mmol) in pyridine (10 mL) was concentrated, the residue redissolved in the same solvent (12 mL) and treated with chlorotriphenylmethane (3.3 mmol). The mixture was stirred at 80 °C and monitored by TLC analysis (ethanol/toluene, 3:97, v/v). After 16 h, the mixture was diluted with dichloromethane (50 mL), washed with saturated aqueous sodium bicarbonate (15 mL) and water (15 mL). The organic layer was dried with magnesium sulfate, filtered and concentrated *in vacuo*. The residue was applied to a column of Kieselgel (50 g) which, after elution with ethanol/toluene (0:1 to 2:98, v/v) and concentration of the appropriate fractions, gave **25** (2.24 mmol, 81%) as a colourless foam: R_f 0.58 (ethanol/toluene, 3:9, v/v); ^1H NMR ($CDCl_3$) δ 7.50-6.87 (m, 25 H, $H_{arom.}$ trityl and 2 × benzyl), 4.94 (d, 1 H, CH_2 benzyl, $J_{A,B}$ = 11.4 Hz), 4.73 (d, 1 H, CH_2 benzyl), 4.66 (d, 1 H, CH_2 benzyl, $J_{A,B}$ = 10.3 Hz), 4.35 (d, 1 H, CH_2 benzyl), 4.09 (dd, 1 H, H-1, $J_{1,1'}$ = 11.0 Hz, $J_{1,2}$ = 5.3 Hz), 3.79 (br dt, 1 H, H-2, $J_{2,3}$ = 9.4 Hz), 3.75 (t, 1 H, H-3, $J_{3,4}$ = 9.2 Hz), 3.58 (dd, 1 H, H-6, $J_{6,6'}$ = 10.3 Hz), 3.42 (t, 1 H, H-4, $J_{4,5}$ = 8.8 Hz), 3.39 (ddd, 1 H, H-5, $J_{5,6}$ = 1.6 Hz, $J_{5,6'}$ = 4.7 Hz), 3.23 (t, 1 H, H-1', $J_{1',2}$ = 10.9 Hz), 3.20 (dd, 1 H, H-6') and 2.19 (br s, 1 H, OH); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 143.7 ($C_{arom.}$ trityl), 138.4 and 137.6 (2 × $C_{arom.}$ benzyl), 128.8-125.1 ($CH_{arom.}$ Ph), 86.7, 79.4,

77.8 and 70.2 (C-2, C-3, C-4 and C-5), 75.1 and 74.6 (2 × CH_2Ph), 69.5 (C-1) and 62.4 (C-6).

1,5-Anhydro-2-azido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-trityl-D-mannitol (27). Compound **25** (2.0 mmol) was converted as described for the synthesis of compound **15** and yielded, after work-up and purification by short column chromatography (Kieselgel, 20 g; diethyl ether/petroleum ether 40/60 °C, 0:1 to 1:1, v/v), compound **27** (1.3 mmol, 66%) as a colourless oil: R_f 0.59 (diethyl ether/hexane, 1:1, v/v); IR (KBr) υ 2095 cm^{-1} (s, azide); ^1H NMR ($CDCl_3$) δ 7.50-6.87 (m, 25 H, $H_{arom.}$ trityl and 2 × benzyl), 4.72 (d, 1 H, CH_2 benzyl, $J_{A,B}$ = 10.4 Hz), 4.71 (AB, 2 H, CH_2 benzyl), 4.31 (d, 1 H, CH_2 benzyl), 4.08 (dd, 1 H, H-1, $J_{1,1'}$ = 12.7 Hz, $J_{1,2}$ = 1.9 Hz), 3.97 (t, 1 H, H-4, $J_{4,5}$ = 9.3 Hz), 3.85 (dt, 1 H, H-2, $J_{2,3}$ = 3.8 Hz), 3.68 (dd, 1 H, H-3, $J_{3,4}$ = 9.2 Hz), 3.52 (dd, 1 H, H-6, $J_{6,6'}$ = 10.1 Hz), 3.48 (dd, 1 H, H-1', $J_{1',2}$ = 1.9 Hz), 3.34 (ddd, 1 H, H-5, $J_{5,6}$ = 1.8 Hz, $J_{5,6'}$ = 4.5 Hz) and 3.21 (dd, 1 H, H-6'); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 143.8 ($C_{arom.}$ trityl), 137.8 and 137.6 (2 × $C_{arom.}$benzyl), 128.9-126.8 ($CH_{arom.}$trityl and benzyl), 86.3 ($OCPh_3$), 82.5, 79.9 and 74.7 (C-3, C-4 and C-5), 75.2 and 72.1 (2 × CH_2Ph), 67.8 (C-1), 62.8 (C-6) and 59.2 (C-2).

2-Acetamido-1,5-anhydro-3,4-di-*O*-benzyl-2-deoxy-6-*O*-trityl-D-mannitol (28). Compound **27** (0.6 mmol) was converted with hydrogen sulfide as described for the synthesis of compound **17**, and yielded, after work-up and purification by short column chromatography (Kieselgel, 15 g; diethyl ether/hexane (0:1 to 1:1, v/v), compound **28** (0.5 mmol, 82%) as a colourless oil: R_f 0.50 (ethanol/toluene, 5:95, v/v); ^1H NMR ($CDCl_3$) δ 7.52-6.48 (m, 25 H, $H_{arom.}$ 2 × benzyl and trityl), 6.06 (d, 1 H, NH), 4.86 (d, 1 H, CH_2 benzyl, $J_{A,B}$ = 11.0 Hz), 4.75 (d, 1 H, CH_2 benzyl, $J_{A,B}$ = 10.4 Hz), 4.66 (10 lines, 1 H, H-2, $J_{2,3}$ = 4.1 Hz, $J_{2,NH}$ = 9.0 Hz), 4.50 (d, 1 H, CH_2 benzyl), 4.21 (d, 1 H, CH_2 benzyl), 4.02 (dd, 1 H, H-1, $J_{1,1'}$ = 12.1 Hz, $J_{1,2}$ = 2.2 Hz), 3.76 (t, 1 H, H-4, $J_{4,5}$ = 9.2 Hz), 3.68 (dd, 1 H, H-3, $J_{3,4}$ = 9.1 Hz), 3.60 (dd, 1 H, H-6, $J_{6,6'}$ = 10.0 Hz), 3.51 (dd, 1 H, H-1', $J_{1',2}$ = 1.6 Hz), 3.37 (ddd, 1 H, H-5, $J_{5,6}$ = 2.1 Hz, $J_{5,6'}$ = 3.7 Hz), 3.22 (dd, 1 H, H-6'), 2.12 (s, 3 H, CH_3 acetyl); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 169.8 ($COCH_3$), 143.6 ($C_{arom.}$ trityl), 137.8 and 137.6 (2 × $C_{arom.}$ benzyl), 128.7-126.8 ($CH_{arom.}$ benzyl), 86.2 (CPh_3), 81.0, 79.3 and 74.3 (C-3, C-4 and C-5), 74.9 and 70.9 (2 × CH_2Ph), 68.8 (C-1), 62.2 (C-6), 46.6 (C-2), 23.4 (CH_3CO).

Benzyloxy-hexadecyloxy-*N,N*-diisopropylamino-phosphine (32). To a mixture of dry hexadecanol (4.1 mmol) and dry 1-*H*-tetrazole (2 mmol) was added a 1 M solution of benzyloxy-*bis*(*N,N*-diisopropylamino)phosphine[18,19] (4.5 mmol) in dichloromethane. The mixture was stirred for 1 h at room temperature under a nitrogen atmosphere. The mixture was poured in an aqueous solution of triethylammonium hydrogen carbonate (1 M, 20 mL) and extracted with dichloromethane (40 mL). The organic layer was dried with magnesium sulfate, filtered and concentrated *in vacuo*. The residue was applied to a column of Kieselgel (60 g) suspended in hexane/triethylamine (39:1, v/v). Elution was effected with hexane/triethylamine (39:1, v/v). After concentration of the appropriate fractions, compound **32** (3 mmol, 73%) was isolated as a colourless oil: ^{1}H NMR ($CDCl_3$) δ 7.36-7.20 (m, 5 H, $H_{arom.}$ benzyl), 4.70 (t, 2 H, CH_2 benzyl), 3.63 (m, 4 H, CH_2 hexadecyl and N[C*H*$(CH_3)_2]_2$), 1.61 (m, 2 H, CH_2 hexadecyl), 1.26 (s, 26 H, 13 × CH_2 hexadecyl) 1.19 and 1.17 (2 × d, 12 H, N[CH(C*H*$_3)_2]_2$) and 0.89 (t, 3 H, CH_3 hexadecyl); ^{13}C{^{1}H} NMR ($CDCl_3$) δ 139.5 ($C_{arom.}$ benzyl), 128.0, 127.0 and 126.8 ($CH_{arom.}$ benzyl), 65.2 and 63.6 (2 × d, CH_2Ph and CH_2 hexadecyl, $^2J_{C,P}$ = 18 Hz and $^2J_{C,P}$ = 16 Hz), 42.8 (d, N[*C*H$(CH_3)_2]_2$, $^2J_{C,P}$ = 13 Hz), 31.9-22.7 (14 × CH_2 hexadecyl), 24.6 (d, N[CH(*C*H$_3)_2]_2$, $^3J_{C,P}$ = 7 Hz) and 14.0 (CH_3 hexadecyl); ^{31}P{^{1}H} NMR ($CDCl_3$) δ 147.2.

2-Acetamido-1,5-anhydro-3,4-di-*O*-benzyl-2-deoxy-D-mannitol 6-(dibenzyl phosphate) (33). A solution of compound **22** (0.5 mmol) and 1-*H*-tetrazole (0.9 mmol) in acetonitrile (10 mL) was concentrated (2 x) to dryness. The residue was dissolved in dry acetonitrile (5 mL) and to the cooled (0 °C) solution *bis*(benzyloxy)-*N,N*-diisopropylaminophosphine (**29**, 0.75 mmol) was added. After 1 min., a white solid precipitated from the solution, and TLC analysis (acetone/dichloromethane, 1:9, v/v) indicated complete disappearance of the starting material. After 5 min., *tert*-butyl hydroperoxide (2.5 mmol, 10 M solution in di-*tert*-butyl peroxide) was added and the mixture was stirred for 10 min. at 0 °C. The mixture was poured in an aqueous solution of triethylammonium hydrogen carbonate (1 M, 10 mL) and extracted with dichloromethane (50 mL). The organic layer was washed with an aqueous solution of sodium sulfite (15 mL, 5 w/v) and water (15 mL). The organic layer was dried (magnesium sulfate), filtered, concentrated with toluene (50 mL) and applied to a column of Kieselgel (20 g). Elution was effected with ethyl

acetate/toluene (0:1 to 2:1, v/v), and concentration of the appropriate fractions **33** (0.39 mmol, 77%) was isolated as a colourless oil: R_f 0.26 (acetone/dichloromethane, 1:9, v/v), 0.35 (ethyl acetate/toluene, 2:1, v/v); ^{1}H NMR ($CDCl_3$) δ 7.40-7.25 (m, 20 H, $H_{arom.}$ 4 × benzyl), 5.07-4.96 (m, 4 H, 2 × CH_2 benzyl), 4.95 (d, 1 H, CH_2 benzyl, $J_{A,B}$ = 10.2 Hz), 4.89 (d, 1 H, CH_2 benzyl, $J_{A,B}$ = 11.1 Hz), 4.69 (ddt, 1 H, H-2, $J_{2,3}$ = 4.2 Hz, $J_{2,NH}$ = 9.3 Hz), 4.57 (d, 1 H, CH_2 benzyl), 4.52 (d, 1 H, CH_2 benzyl), 4.29 (ABMX, 2 H, H-6 and H-6'), 3.88 (dd, 1 H, H-1, $J_{1,1'}$ = 12.1 Hz, $J_{1,2}$ = 2.3 Hz), 3.82 (t, 1 H, H-4, $J_{4,5}$ = 9.5 Hz), 3.69 (dd, 1 H, H-3, $J_{3,4}$ = 9.3 Hz), 3.50 (dd, 1 H, H-1', $J_{1',2}$ = 1.3 Hz), 3.33 (dq, 1 H, H-5, $J_{5,6} \approx J_{5,6'} \approx J_{5,P} \approx$ 1.5 Hz), 2.07 (s, 3 H, CH_3 acetyl); ^{13}C{^{1}H} NMR ($CDCl_3$) δ 170.3 ($COCH_3$), 138.0 and 137.7 (2 × $C_{arom.}$ benzyl), 135.6 (d, $C_{arom.}$ benzyl, $^3J_{P,C}$ = 7.3 Hz), 128.3-127.4 ($CH_{arom.}$ benzyl), 80.6, 77.9 and 73.2 (C-3, C-4 and C-5), 75.0 and 70.5 (2 × CH_2Ph), 69.0 (C-1), 67.8 (d, C-6, $^2J_{P,C}$ = 5.9 Hz), 45.8 (C-2), 22.9 (CH_3CO); ^{31}P{^{1}H} NMR ($CDCl_3$) δ -1.30.

2-Acetamido-1,5-anhydro-2-deoxy-D-mannitol 6-(disodium phosphate) (3). A solution of compound **33** (0.38 mmol) in *tert*-butyl alcohol/water (5:1, v/v, 6 mL) was shaken with 10% palladium on carbon (25 mg) under a hydrogen atmosphere (P_{H_2} = 0.4 MPa) for 48 h at room temperature. The palladium catalyst was removed by filtration and the filtrate was concentrated to a small volume (1 mL). The residue was applied to a column of SP-Sephadex C-25 [Na^+] (3 g) and elution was effected with water (150 mL). The fractions containing **3** were pooled and concentrated *in vacuo*. Lyophilization of the residue gave homogeneous **3** (0.38 mmol, 99%) as a white powder: ^{1}H NMR (D_2O, pD = 5.3) δ 4.30 (dt, 1 H, H-2, $J_{2,3}$ = 4.6 Hz), 4.08 (ABMX, 2 H, H-6 and H-6'), 3.85 (dd, 1 H, H-1, $J_{1,1'}$ = 12.3 Hz, $J_{1,2}$ = 1.8 Hz), 3.83 (dd, 1 H, H-3, $J_{3,4}$ = 9.9 Hz), 3.70 (dd, 1 H, H-1', $J_{1',2}$ = 1.9 Hz), 3.65 (t, 1 H, H-4, $J_{4,5}$ = 9.9 Hz), 3.41 (dt, 1 H, H-5, $J_{5,6} \approx J_{5,6'}$ = 3.3 Hz), 2.05 (s, 3 H, CH_3 acetyl); ^{13}C{^{1}H} NMR (D_2O, pD = 5.3) δ 175.5 ($COCH_3$), 80.3 (d, C-5, $^3J_{C,P}$ = 7.3 Hz), 73.0 and 67.6 (C-3 and C-4), 69.7 (C-1), 64.8 (d, C-6, $^2J_{C,P}$ = 4.4 Hz), 51.3 (C-2), 22.8 ($COCH_3$); ^{31}P{^{1}H} NMR (D_2O, pD = 5.3) δ 1.54.

Anal. Calcd for $Na_2C_8H_{14}NO_8P$ (329.15): C, 29.19, H, 4.29, N, 4.26, P, 9.41. Found: C, 29.10, H, 4.27, N, 4.24, P, 9.49.

2-Acetamido-1,5-anhydro-3,4-di-*O*-benzyl-2-deoxy-D-mannitol 6-(benzyl hexadecyl phosphate) (34 {R/S mixture}). Compound **22** (0.25 mmol) was treated with 1-*H*-tetrazole, benzyl hexadecyl *N,N*-diisopropyl phosphoramidite (**32**) and *tert*-butyl hydroperoxide as described for the synthesis of compound **33**. After work-up and column chromatography, compound **34** (diastereoisomeric mixture, 0.24 mmol, 93%) was isolated as a colourless oil: R_f 0.51 (methanol/dichloromethane, 2:8, v/v); ^{1}H NMR ($CDCl_3$) δ 7.46-7.24 (m, 15 H, $H_{arom.}$ 3 × benzyl), 5.11-4.87 (m, 4 H, 2 × CH_2 benzyl), 4.69 (m, 1 H, H-2), 4.64-4.49 (m, 2 H, CH_2 benzyl), 4.32-4.25 and 4.06-3.67 (m, 7 H, H-1, H-3, H-4, H-6, H-6' and CH_2 hexadecyl), 3.48 (d, 1 H, H-1', $J_{1,1'}$ = 11.1 Hz), 3.32 (m, 1 H, H-5), 2.09 and 2.05 (2 × s, 3 H, $COCH_3$), 1.60 (m, 2 H, CH_2 hexadecyl), 1.25 (m, 26 H, 13 × CH_2 hexadecyl), 0.87 (t, 3 H, CH_3 hexadecyl); ^{13}C{^{1}H} NMR ($CDCl_3$) δ 170.3 ($COCH_3$), 138.1 and 137.8 (2 × $C_{arom.}$ benzyl), 128.3-127.4 ($CH_{arom.}$ benzyl), 80.7, 78.0 and 73.3 (C-3, C-4 and C-5), 75.0 and 70.5 (2 × CH_2Ph), 69.5, 69.0, 68.4 and 67.7 (C-1, C-6, CH_2Ph and CH_2 hexadecyl), 45.9 (C-2), 31.6-22.4 (14 × CH_2 hexadecyl), 23.0 ($COCH_3$), 13.9 (CH_3 hexadecyl); ^{31}P{^{1}H} NMR ($CDCl_3$) δ -1.09 and -1.28.

2-Acetamido-1,5-anhydro-2-deoxy-D-mannitol 6-(hexadecyl sodium phosphate) (4). A solution of compound **34** (0.12 mmol) in *tert*-butyl alcohol/water (5:1, v/v, 6 mL) was shaken with 10% palladium on carbon (25 mg) under a hydrogen atmosphere (P_{H_2} = 0.4 MPa) for 24 h at room temperature. The palladium catalyst was removed by filtration and the filtrate was concentrated to a small volume (1 mL). The solution was applied to a column of SP-Sephadex C-25 [Na^+] (2 g) and elution was effected with water (100 mL). The fractions containing **4** were combined and concentrated *in vacuo*. Lyophilization of the residue from dioxane/water (1:1, v/v) gave homogeneous **4** (0.12 mmol, 100%) as a white fluffy material: ^{1}H NMR (D_2O) δ 4.32 (d, 1 H, H-2, J = 4.6 Hz), 4.13 (m, 1 H, H-6), 3.97 (m, 1 H, H-6'), 3.88-3.63 (m, 6 H, H-1, H-1', H-3, H-4 and CH_2 hexadecyl), 3.30 (d, 1 H, H-5, J = 9.5), 2.05 (s, 3 H, $COCH_3$), 1.62 (m, 2 H, CH_2 hexadecyl), 1.27 (m, 26 H, 13 × CH_2 hexadecyl), 0.87 (t, 3 H, CH_3 hexadecyl, J = 6.9 Hz); ^{13}C{^{1}H} NMR (D_2O) δ 174.7 ($COCH_3$), 81.2 (d, C-5, $^3J_{C,P}$ = 7.3 Hz), 73.6 and 68.1 (C-3 and C-4), 70.1 (C-1), 66.9 and 65.5 (2 × d, $^2J_{C,P}$ = 5.9 Hz $^2J_{C,P}$ = 4.4 Hz respectively, C-6 and CH_2 hexadecyl), 51.8 (C-2), 32.9 (CH_2 hexadecyl), 31.6 (d, CH_2 hexadecyl, $^3J_{C,P}$ = 8.8 Hz), 30.7, 30.5, 30.4, 26.8 and 23.6 (12 × CH_2 hexadecyl), 22.9 ($COCH_3$), 14.7 (CH_3 hexadecyl); ^{31}P{^{1}H} NMR (D_2O) δ 2.19.

REFERENCES

1. A. Gottschalk, *The Chemistry and Biology of Sialic Acids and Related Substances*; Cambridge University Press, 1960, p 115.

2. R. Schauer, *Angew. Chem. Intern. Ed.* **12**, 127 (1973).

3. N. Sharon, *Complex Carbohydrates: Their Chemistry, Biosynthesis, and Functions*; Addison-Wesley Publishing Company, Massachusetts, 1975.

4. J. Montreuil, *Adv. Carbohydr. Chem. Biochem.*, **37**, 157 (1980).

5. T. A. Beyer, J. E. Sadler, J. I. Rearick, J. C. Paulson and R. L. Hill, *Adv. Enzymol.*, **52**, 23, (1981).

6. R. Schauer, *Adv. Carbohydr. Chem. Biochem.*, **40**, 131 (1982).

7. R. J. Bernacki and W. Korytnyk, in *The Glycoconjugates*, 4; M. Horowitz Ed.; Academic Press, Inc. New York, San Francisco and London, 1982, p 245.

8. T. Ogawa, Y. Takahashi, and M. Matsui, *Carbohydr. Res.*, **102**, 207 (1982).

9. S. Koto, N. Morishima, T. Yoshida, M. Uchino, and S. Zen, *Bull. Chem. Soc. Jpn.*, **56**, 1171 (1983).

10. S. Koto, N. Morishima, Y. Mori, H. Tanaka, S. Hayashi, Y. Iwai, and S. Zen, *Bull. Chem. Soc. Jpn.*, **60**, 2301 (1987).

11. J. A. Bennek, and G. R. Gray, *J. Org. Chem.*, **52**, 892 (1987).

12. H. Paulsen, and J-P. Hölck, *Carbohydr. Res.*, **109**, 89 (1982).

13. T. Adachi, Y. Yamada, and I. Inoue, *Synthesis*, 45 (1977).

14. J. Stawinski, T. Hozumi, S. A. Narang, C. P. Bahl, and R. Wu, *Nucl. Acids Res.*, **4**, 353, (1977).

15. J. W. Perich, and R. B. Johns, *Tetrahedron Lett.*, **28**, 101, (1987).

16. C. E. Dreef, R. J. Tuinman, C. J. J. Elie, G. A. van der Marel, and J. H. van Boom, *Recl. Trav. Chim. Pays-Bas*, **107**, 395, (1988), and references cited therein.

17. J. Engels, and A. Jäger, *Angew. Chem. Suppl.*, 2010 (1982).

18. C. E. Dreef, C. J. J. Elie, P. Hoogerhout, G. A. van der Marel, and J. H. van Boom, *Tetrahedron Lett.*, **29**, 6513, (1988).

19. C. J. J. Elie, C. E. Dreef, R. Verduyn, G. A. van der Marel, and J. H. van Boom, *Tetrahedron*, **45**, 3477, (1989).