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Note

Multigram-scale synthesis of an orthogonally protected 2-acetamido-4-amino-2,4,6-trideoxy-D-galactose (AAT) building block

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ABSTRACT

Reported is the gram-scale synthesis of *tert*-butyldiphenylsilyl 4-(*N*-benzyloxycarbonyl)-amino-2-azido-2,4,6-trideoxy-β-D-galactopyranoside, which represents an orthogonally protected 2,4-diamino-D-fucose building block, a common constituent of various zwitterionic polysaccharides. The building block has been synthesized from D-glucosamine in 19% overall yield over 14 steps, requiring 5 chromatographic purifications. The key step in the synthesis is the introduction of the C-4 amino substituent, which has been accomplished by a one-pot three step procedure, involving regioselective C-3-*O*-trichloroacetimidate formation, C-4-O-triflation, and intramolecular substitution. The building block can be used as an acceptor and is readily transformed into a donor glycoside.

2-Acetamido-4-amino-2,4,6-trideoxy-D-galactose (AAT)[†] is a rare carbohydrate residue found in various polysaccharides, present in infectious bacteria, such as *Shigella sonnei*,[1] *Streptococcus pneumonia*,[2] *Bacteroides fragilis*,[3] *Streptococcus mitis*[4] and *Proteus vulgaris*.[5] AAT represents an important constituent of many zwitterionic polysaccharides (ZPs), which are capable of eliciting a T-cell dependent immune response.[6] Key to this activity is the presence of both negative and positive charges on the polysaccharide backbone. The negative charges in these polysaccharides originate from either uronic acid constituents or pyruvate moieties, where the positive charge is often found on the C-4 amino function of the AAT-residues. To gain insight into the role of these AAT-containing polysaccharides in bacterial pathogenicity and immunogenicity, the availability of (fragments of) pure polysaccharides is of importance and therefore the synthesis of these polysaccharides has attracted ample attention.[7–10] In these syntheses one of the obstacles is presented by the procurement of sufficient amounts of a suitable AAT-building block. Over the years several syntheses have been reported, most of which start to form a glucosamine precursor, as summarized in Scheme 1. Transformation of the glucosamine core (Scheme 1A) into an AAT-building block requires deoxygenation of C-6 and introduction of the second amino functionality with concomitant inversion at C-4. Lönngren and co-workers employed a di-mesylate to accomplish these two steps in the first synthesis of an orthogonally protected AAT building block in 1984.[11] Other syntheses typically employ the installment of a C-6 tosylate, which is subsequently displaced by iodine prior to hydride substitution (Sharon 1974,[12] Pozsgay 1997,[13,7] Schmidt 2010[14]). Introduction of the C-4 amino functionality has most often been accomplished through the S_N2-type displacement of a C-4 mesylate,[11,12] tosylate[13] or triflate[7,8,14] with azide[15,16] or phthalimide.[14]

Syntheses starting from different precursors have also been developed, as exemplified by the synthetic efforts of van Boom and co-workers, who started from D-mannose (Scheme 1B).[17] Recently, Bundle reported an elegant procedure starting from D-glucal (Scheme 1C).[18] Deoxygenation of C-6 was followed by the regioselective introduction of a C-3 benzyl carbamate. Intramolecular displacement of the subsequently installed C-4 mesylate led to a C-4-amino galactal, protected with a cyclic carbamate, which was subjected to azidonitration to install the required C-2 azide functionality. Seeberger and co-workers employed a conceptually different approach and used Cbz-protected L-threonine as a precursor to generate a Cbz-protected C-4-amino galactal intermediate in a de novo strategy (Scheme 1D).[19] We have previously reported on the use of an intramolecular displacement strategy to obtain a suitably protected AAT-building block, featuring a non-participating azide group at C-2.[7] This strategy is based on the regioselective installment of a C-3-O-imidate functionality, followed by the introduction of a C-4-triflate and subsequent oxazoline formation.[20] We here describe an optimized synthetic route, using this approach, for the multi-gram synthesis of AAT building block **9**.

Our synthesis started from glucosamine hydrochloride (**1**) as depicted in Scheme 2. Introduction of the required C-2 azide was accomplished by an azidotransfer reaction using imidazole-1-sulfonyl

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[†] Other abbreviations found in literature include: AATGal and D-FucNAc4N.

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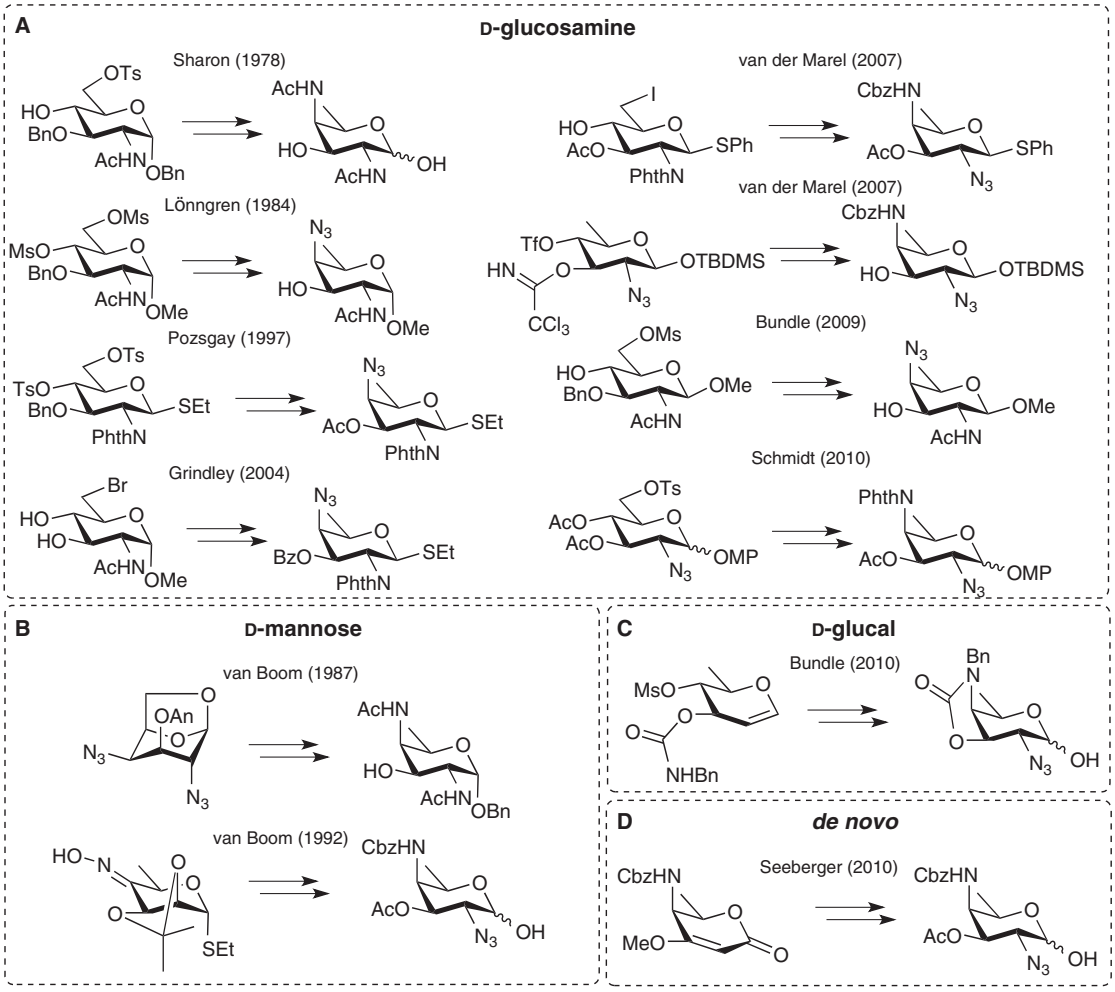
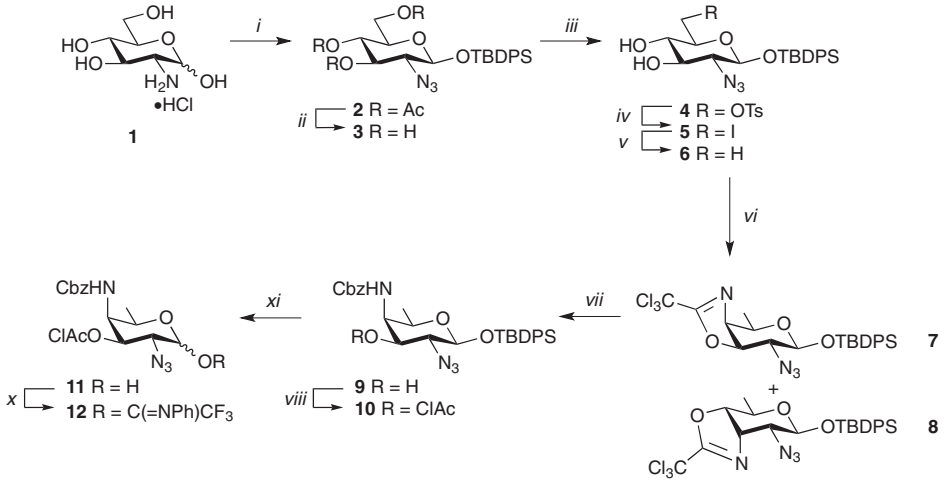


Scheme 1. Previous syntheses of AAT-building blocks.



Scheme 2. Reagents and conditions: (i) (1) Imidazole-1-sulfonyl azide·HCl, MeOH, $CuSO_4$ (cat.); (2) pyridine, Ac_2O; (3) piperidine, THF; (4) *t*-$BuPh_2SiCl$, imidazole, DMF (60%, 4 steps); (ii) NaOMe (cat.), MeOH, DCM (quant.); (iii) tosylchloride, pyridine (83%); (iv) NaI, butanone (92%); (v) $NaCNBH_3$, diethylene glycol diethyl ether, reflux (88%); (vi) Cl_3CCN, DBU, DCM, −13 °C then Tf_2O, pyridine then DiPEA (**I**: 24%, **J**: 63%); (vii) (1) AcOH, H_2O, EtOAc; (2) *N*-(benzyloxycarbonyloxy)succinimide, triethylamine, DCM (75%); (viii) $(ClAc)_2O$, pyridine, DCM, (quant.); (ix) triethylamine·3HF, THF, (98%); (x) $ClC(=NPh)CF_3$, Cs_2CO_3, H_2O, acetone, (83%, α/β 1:3).

azide·HCl, introduced by Goddard-Borger and Stick.[21] Global acetylation was then followed by liberation of the anomeric hydroxyl through the aegis of piperidine in THF. In our previous synthesis of an AAT building block (see Scheme 1A) we employed a *tert*-butyldimethylsilyl group to mask the anomeric hydroxyl.[7] However, we found that this silyl ether was not completely stable to the acidic

reaction conditions employed later on in the synthesis to cleave the intermediate oxazoline and therefore we switched to the use of the more acid stable *tert*-butyldiphenylsilyl ether.[22] Introduction of the anomeric TBDPS ether using TBDPS-Cl and imidazole in DCM led to the fully protected crystalline glucosazide **2**, which was obtained in 60% yield over four steps without chromatographic purification (300 mmol scale). Next, the three acetyl groups were removed and a tosylate was regioselectively installed at the C-6-OH. Substitution of the tosylate for an iodine function then set the stage for the crucial deoxygenation step, which required substantial optimization. It was found that the use of $NaBH_4$ as a reducing agent in DMSO led to partial reduction of the azide functionality and we therefore switched to the use of a milder reducing agent, $NaCNBH_3$, at higher temperature. It was found that diethylene glycol was the optimal solvent for the reaction and at reflux temperature iodide **5** was uneventfully reduced to give the key intermediate **6** in 88% yield. The required C-4 amino group was installed using an intramolecular displacement strategy.[20] Thus, in a one-pot three step procedure diol **6** was treated with trichloroacetonitrile and a catalytic amount of DBU to give the intermediate C-3-O-imidate. Next, triflic anhydride and pyridine (5 equiv) were added to the reaction mixture to form the C-4 trifluoromethanesulfonyl ester. Finally treatment of this species with an excess DiPEA furnished oxazoline **7**,[‡] which was isolated in 63% yield. We also isolated the *allo*-configured oxazoline **8**, formed from the regioisomeric imidate, by C-3-O-triflation and intramolecular substitution, in 23% yield. Hydrolysis of the oxazoline moiety in **7** with acetic acid and water gave an intermediate amino alcohol, which was directly transformed into benzylcarbamate **9**. As anticipated the anomeric TBDPS ether was unaffected during cleavage of the oxazoline moiety. *tert*-Butyldiphenylsilyl4-(*N*-benzyloxycarbonyl)-amino-2-azido-2,4,6-trideoxy-β-D-galactopyranoside **9** was obtained in 19% yield from D-glucosamine in 14 steps, requiring 5 chromatographic purifications. AAT building block **9** was further converted into 1-hydoxyl donor **11** by installation of a chloroacetyl ester at the C-3-OH and subsequent removal of the anomeric silyl group using HF.Et_3N (98% over two steps). Imidate donor **12** was obtained from this lactol by treatment with *N*-phenyltrifluoroacetamidoyl chloride in acetone in the presence of Cs_2CO_3 and a few drops of water. Building blocks **9**, **11,** and **12** represent a set of versatile building blocks for the construction of various AAT containing (zwitterionic) oligosaccharides.

In conclusion we have described an optimized synthetic route for the multi-gram synthesis of orthogonally protected AAT-building blocks starting from D-glucosamine. Key steps in the synthesis include the deoxygenation of a C-6-iodo glucosazide and the subsequent one-pot three step tethered nucleophilic inversion procedure to introduce the C-4 amino functionality. The usefulness of AAT synthons **9**, **11**, **12** in the construction of zwitterionic oligosaccharides has previously been described in the context of the assembly of all three repeating units of the ZP of *S. pneumonia* Sp1.[9]

1. Experimental section

1.1. General procedures

All chemicals were used as received. Trifluoromethanesulfonic anhydride (Tf_2O) was distilled from P_2O_5 and stored in a Schlenk flask. TLC analysis was conducted on silica gel-coated aluminum TLC sheets (Merck, silica gel 60, F_{245}). Compounds were visualized by UV absorption (245 nm), by spraying with 20% H_2SO_4 in ethanol or with a solution of $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ 25 g/L, $(NH_4)_4Ce(SO_4)_4{\cdot}2H_2O$10 g/L, 10% H_2SO_4 in H_2O followed by charring at ~140 °C. Flash chromatography was performed on silica gel (screening devices, 40–63 μm 60 Å, www.screeningdevices.com) using technical grade, distilled solvents. NMR spectra were recorded on a Bruker AV400. For solutions in $CDCl_3$ chemical shifts (δ) are reported relative to tetramethylsilane (^1H) or $CDCl_3$ (^{13}C). Peak assignments were made based on HH-COSY and HSQC measurements. Optical rotation was measured using a Propol automatic polarimeter. The IR absorbance was recorded using a Shimadzu FTIR-83000 spectrometer. Mass analysis was performed using a PE/SCIEX API 165 with an Electrospray Interface (Perkin–Elmer).

1.2. *tert*-Butyldiphenylsilyl 3,4,6-tri-*O*-acyl-2-azido-2-deoxy-β-D-glucopyranoside(2)

To a mixture of 107.8 g D-glucosamine·HCl (500 mmol, 1 equiv) in 2 L MeOH was added 174 mL triethylamine (1.25 mol, 2.5 equiv), 1.25 g $CuSO_4{\cdot}H_2O$ (5 mmol, 0.01 equiv), and 125.8 g imidazole-1-sulfonyl azide·HCl[22] (600 mmol, 1.2 equiv). The reaction was stirred for 1.5 h and the solvents were evaporated. The crude material was coevaporated with pyridine and subsequently stirred overnight in 2 L pyridine/Ac_2O (4/1 v/v). The solvent was evaporated and the residue was partitioned between H_2O and EtOAc. The organic layer was washed with aq 1 M HCl solution, satd aq $NaHCO_3$ solution, and brine. The organic phase was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. To a mixture of the crude product in 1 L THF was added 117 mL piperidine (1.19 mol, 2.4 equiv) and the reaction was run for 2.5 h. The mixture was diluted with 1.5 L EtOAc and washed with aq 1 M HCl solution, satd aq $NaHCO_3$ solution, and brine. The organic phase was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude hemiacetal was coevaporated with toluene and dissolved in 700 mL DMF. To this solution 47.5 g imidazole (697 mmol, 1.4 equiv) and 135.6 mL *t*-$BuPh_2SiCl$ (523 mmol, 1.05 equiv) were added and the mixture was stirred for 2 h at 60 °C. Next, 1.5 L H_2O was added and the mixture was extracted with EtOAc. The combined organic layers were washed with aq 1 M HCl solution, satd aq $NaHCO_3$ solution, and brine. The organic phase was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. Crystallization from EtOH yielded 171.9 g of the title compound (**2**) (302.0 mmol, 60% over 4 steps). Spectral data were in accordance with those reported in the literature.[23]

1.3. *tert*-Butyldiphenylsilyl 2-azido-2-deoxy-β-D-glucopyranoside(3)

To a solution of 66.11 g *tert*-butyldiphenylsilyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-D-glucopyranoside **2** (116.1 mmol, 1 equiv) in 500 ml methanol/DCM (9/1 v/v) was added 1.27 g NaOMe (23.6 mmol, 0.2 equiv). The mixture was stirred until TLC indicated complete conversion of the starting material to a single lower running spot. The mixture was neutralized with Amberlite H^+ resin and filtered. The filtrate was evaporated to dryness yielding 51.4 g of the title compound (115.8 mmol, quant.). R_f 0.25 (EtOAc/PE, 3/2, v/v); IR (neat, cm^{-1}) 3370 (br), 2932, 2860, 2110, 1428, 698; ^1H NMR (400 MHz, $CDCl_3$) δ 7.73–7.66 (m, 4H, H_{arom}), 7.46–7.32 (m, 6H, H_{arom}), 4.51 (d, J = 7.7 Hz, 1H, H-1), 4.22 (s, 2H, OH), 3.49–3.36 (m, 3H, H-6, H-4), 3.30 (dd, J = 10.0, 7.7 Hz, 1H, H-2), 3.19 (br t, J = 9.4 Hz, 1H, H-3), 2.85–2.78 (m, 1H, H-5), 1.90 (s, 1H, OH), 1.11 (s, 9H, CH3 *t*-Bu); ^{13}C NMR (101 MHz, $CDCl_3$) δ 135.7 (CH_{arom}), 133.6, 132.4 (C_q), 130.1, 129.9, 127.7, 127.4 (CH_{arom}), 96.9 (C-1), 75.0 (C-5), 74.6 (C-3), 69.6 (C-4), 68.6 (C-2), 61.4 (C-6), 26.7 (CH_3*t*-Bu), 19.0 (C_q*t*-Bu); $[\alpha]_D^{22}$ +25 (*c* 1.0, $CHCl_3$); HRMS [M+Na]$^+$ calcd for $C_{22}H_{29}N_3O_5SiNa$ 466.17687, found 466.17659.

[‡] Ring closure can also be effected by stirring overnight without addition of DiPEA.

1.4. *tert*-Butyldiphenylsilyl 2-azido-2-deoxy-6-*O*-tosyl-β-D-glucopyranoside (4)

8.45 g Tosylchloride (44.3 mmol, 3.0 equiv) was added to an ice-cooled solution of 6.55 g *tert*-butyldiphenylsilyl 2-azido-2-deoxy-β-D-glucopyranoside (**3**) (14.8 mmol, 1.0 equiv) in 75 mL pyridine. The mixture was stirred for 2 h at 0 °C and quenched by the addition of MeOH. After evaporation of the solvents the crude mixture was partitioned between EtOAc and water and the organic layer was washed with aq 1 M HCl solution, satd aq $NaHCO_3$ solution, and brine. The organic phase was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. Flash column chromatography using EtOAc/ PE (3/7 → 2/3) gave 7.31 g (12.2 mmol, 83%) of the title compound (**4**) as a colorless oil. R_f 0.29 (EtOAc/PE, 2/3, v/v); $[\alpha]_D^{22}$ +11 (*c* 1.0, CH_2Cl_2); IR (neat, cm^{-1}) 3400 (br), 2932, 2858, 2110, 1174, 812; ^1H NMR (400 MHz, $CDCl_3$, HH-COSY, HSQC) δ 7.68–7.62 (m, 6H, H_{arom}), 7.44–7.21 (m, 8H, H_{arom}), 4.36 (d, *J* = 7.6 Hz, 1H, H-1), 4.05 (dd, *J* = 10.5, 4.5 Hz, 1H, H-6), 3.85 (d, *J* = 10.5 Hz, 1H, H6), 3.77 (s, 1H, OH), 3.67 (s, 1H, OH), 3.46 (t, *J* = 9.2 Hz, 1H, H-4), 3.30 (dd, 1H, *J* = 9.2, 7.6 Hz, H-2), 3.20 (t, *J* = 9.3 Hz, 1H, H-3), 2.99 (dd, *J* = 9.7, 4.2 Hz, 1H, H-5), 2.41 (s, 3H, CH_3Ts), 1.08 (s, 9H, CH_3 *t*-Bu); ^{13}C NMR (100 MHz, $CDCl_3$, HH-COSY, HSQC) δ 144.9 (C_qTs), 135.8, 135.7 (CH_{arom}), 132.8, 132.4, 132.2 (C_qPh), 129.9, 129.8, 129.7, 127.9, 127.5, 127.4 (CH_{arom}), 96.7 (C-1), 74.5 (C-3), 73.0 (C-5), 69.3 (C-4), 68.3 (C-2), 68.1 (C-6), 26.7 (CH_3 *t*-Bu), 21.6 (CH_3Ts), 19.0 (C_q *t*-Bu); HRMS $[M+Na]^+$ calcd for $C_{29}H_{35}N_3O_7SSiNa$ 620.18572, found 620.18562.

1.5. *tert*-Butyldiphenylsilyl 2-azido-2,6-dideoxy-6-iodo-β-D-glucopyranoside (5)

Tosylate **4** (7.22 g, 12.1 mmol, 1 equiv) was refluxed for 6 h in 60 mL butanone together with 3.98 g NaI (26.6 mmol, 2.2 equiv). After cooling to room temperature EtOAc was added and the mixture was washed with aq 1 M $Na_2S_2O_3$ solution and H_2O. The organic phase was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. Flash column chromatography using EtOAc/PE (1/4 → 3/7) afforded 6.12 g of the title compound **5** (11.1 mmol, 92%) as a yellow oil. R_f 0.46 (EtOAc/PE, 2/3, v/v); $[\alpha]_D^{22}$ +8 (*c* 1.0, CH_2Cl_2); IR (neat, cm^{-1}) 3400, 2858, 2110, 1078, 812; ^1H NMR (400 MHz, $CDCl_3$, HH-COSY, HSQC) δ 7.79–7.69 (m, 4H, H_{arom}), 7.45–7.34 (m, 6H, H_{arom}), 4.49 (d, *J* = 7.6 Hz, 1H, H-1), 3.51 (s, 1H, OH), 3.41 (s, 1H, OH), 3.40 (t, *J* = 8.8 Hz, 1H, H-4), 3.37(dd, *J* = 9.6, 7.6, Hz, 1H, H-2), 3.28 (dd, *J* = 9.6, 8.8 Hz, 1H, H-3), 3.24 (dd, *J* = 10.8, 4.8 Hz, 1H, H6), 3.15 (dd, *J* = 10.8, 2.8 Hz, 1H, H-6), 2.62–2.53 (m, 1H, H-5), 1.13 (s, 9H, CH_3 *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$, HH-COSY, HSQC) δ 136.1, 135.9 (CH_{arom}), 132.8, 132.5 (C_qPh), 130.0, 129.7, 127.6, 127.5, 127.4, 127.3 (CH_{arom}), 96.4 (C-1), 74.3 (C-3), 73.6 (C-4), 73.1 (C-5), 68.6 (C-2), 26.8 (CH_3 *t*-Bu), 19.1 (C_q *t*-Bu), 5.8 (C-6); HRMS $[M+Na]^+$ calcd for $C_{22}H_{28}IN_3O_4SiNa$ 576.07860, found 576.07845.

1.6. *tert*-Butyldiphenylsilyl 2-azido-2,6-dideoxy-β-D-glucopyranoside (6)

To a solution of 10.56 g (19.1 mmol, 1 equiv) *tert*-butyldiphenylsilyl 2-azido-2,6-dideoxy-6-iodo-β-D-glucopyranoside **5** in 110 mL diethylene glycol diethyl ether was added 11.9 g (190 mmol, 10 equiv) of $NaCNBH_3$ and the mixture was refluxed for 7 h. After cooling to room temperature, the mixture was diluted with 1 L of EtOAc, washed with water and brine. dried ($MgSO_4$), and concentrated in vacuo. Flash column chromatography using EtOAc/PE (1/4 v/v) afforded 7.2 g of the title compound **6** (16.8 mmol, 88%). R_f 0.38 (EtOAc/PE, 2/3, v/v); $[\alpha]_D^{22}$ +22 (*c* 1.0, CH_2Cl_2); IR (neat, cm^{-1}) 3364, 2932, 2862, 2361, 2114, 1111, 1072, 818; ^1H NMR (400 MHz, $CDCl_3$, HH-COSY, HSQC) δ 7.70 (t, *J* = 7.9 Hz, 4H, H_{arom}), 7.42–7.31 (m, 6H, H_{arom}), 4.38 (d, *J* = 7.9 Hz, 1H, H-1), 4.11 (s, 1H, OH), 3.83 (s, 1H, OH), 3.27 (t, *J* = 8.5 Hz, 1H, H-2), 3.14–3.02 (m, 2H, H-3, H-4), 2.88–2.81 (m, 1H, H-5), 1.12 (s, 9H, CH_3 *t*-Bu), 1.02 (d, *J* = 6.9 Hz, 3H, H-6). ^{13}C NMR (100 MHz, $CDCl_3$, HH-COSY, HSQC) δ 135.9, 135.8 (CH_{arom}), 133.2, 132.7 (C_qPh), 129.8, 129.7, 127.5, 127.3 (CH_{arom}), 96.5 (C-1), 75.4 (C-4), 74.7 (C-3), 71.4 (C-5), 68.9 (C-2), 26.8 (CH_3 *t*-Bu), 19.1 (C_q *t*-Bu), 17.1 (C-6); HRMS $[M+Na]^+$ calcd for $C_{22}H_{31}N_3O_4SiNa$ 450.18195, found 450.18171.

1.7. 2-Trichloromethyl-4,5-dihydro-(2-azido-2,4,6-trideoxy-1-*O*-*tert*-Butyldiphenylsilyl-β-D-galactopyranoso)[4,3-d]-1,3-oxazole (8) and 2-trichloromethyl-4,5-dihydro-(2-azido-2,4,6-trideoxy-1-*O*-*tert*-butyldiphenylsilyl-β-D-allopyranoso)[3,4-d]-1,3-oxazole (8)

Diol (**6**) (3.49 g, 8.18 mmol, 1 equiv) and 984 μL Cl_3CCN (9.82 mmol, 1.2 equiv) were dissolved in 80 mL DCM, stirred over activated 3 Å molecular sieves, and cooled to −13 °C. After addition of 122 μL DBU (818 μmol, 0.1 equiv) the reaction mixture was allowed to stir for 1 h. Then 3.30 mL pyridine (40.9 mmol, 5 equiv) and 1.64 mL triflic anhydride (9.82 mmol, 1.2 equiv) were added at −30 °C and the reaction mixture was allowed to warm to ambient temperature. Two hours later 13.52 mL DiPEA (81.8 mmol, 10 equiv) was injected and the mixture was stirred overnight. H_2O was added and the organic layer was separated from the aqueous phase, which was extracted with DCM. Drying over $MgSO_4$, filtration, and concentration under reduced pressure, filtration over Celite (eluent: EtOAc/PE 1/99) and again removal of the solvents gave a crude mixture. Purification was done by flash column chromatography (silica was pretreated with triethylamine/PE (1/19 → 0/1)) using Et_2O/PE (0/1 → 5/95) as eluent to furnish the title compounds (**8**) (1.07 g, 1.94 mmol, 24%) R_f 0.80 (EtOAc/PE, 1/9, v/v); $[\alpha]_D^{22}$ −27 (*c* 1.0, CH_2Cl_2); IR (neat, cm^{-1}) 2932, 2860, 2108, 1653, 1427, 978, 698; ^1H NMR (400 MHz, $CDCl_3$, HH-COSY, HSQC) δ 7.72–7.65 (m, 4H, H_{arom}), 7.45–7.33 (m, 6H, H_{arom}), 4.87 (d, *J* = 5.8 Hz, 1H, H-1), 4.75 (dd, *J* = 8.5, 5.7 Hz, 1H, H-3), 4.59 (t, *J* = 8.8 Hz, 1H, H-4), 3.93 (t, *J* = 5.7 Hz, 1H, H-2), 3.43–3.36 (m, 1H, H-5), 1.14 (d, *J* = 6.2 Hz, 3H, H-6), 1.11 (s, 9H, CH_3 *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$, HH-COSY, HSQC) δ 163.7 (C=N), 135.7, 135.6 (CH_{arom}), 132.8, 132.5(C_qPh), 130.0, 129.8, 127.8, 127.7, 127.4 (CH_{arom}), 94.6 (C-1), 84.0 (C-4), 68.8 (C-5), 66.5 (C-3), 61.4 (C-2), 26.7 (CH_3 *t*-Bu), 19.0 (C_q *t*-Bu), 18.9 (C-6); HRMS $[M+H]^+$ calcd for $C_{24}H_{28}Cl_3N_4O_3Si$ 553.09908, found 553.09909; and (**7**) (2.83 g, 5.13 mmol, 63%) R_f 0.58 (EtOAc/PE, 1/9, v/v); $[\alpha]_D^{22}$ +42 (*c* 1.0, CH_2Cl_2); IR (neat, cm^{-1}) 2860, 2116, 1655, 1427, 1063, 700; ^1H NMR (400 MHz, $CDCl_3$, HH-COSY, HSQC) δ7.83–7.61 (m, 4H, H_{arom}), 7.50–7.32 (m, 6H, H_{arom}), 4.64 (t, *J* = 8.1 Hz, 1H, H-4), 4.31 (d, *J* = 8.1 Hz, 1H, H-1), 3.90 (dd, *J* = 8.3, 3.2 Hz, 1H, H-4), 3.44–3.38 (m, 1H, H-5), 3.38 (t, *J* = 8.0 Hz, 1H, H-2), 1.32 (d, *J* = 6.3 Hz, 3H, H-6), 1.13 (s, 9H, CH_3 *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$, HH-COSY, HSQC) δ 162.3 (C=N), 135.9, 135.8 (CH_{arom}), 132.8, 132.5 (C_qPh), 130.0, 129.8, 127.6, 127.4 (CH_{arom}), 95.4 (C-1), 84.5 (C-3), 69.9 (C-5), 67.0 (C-4), 66.6 (C-2), 26.7 (CH_3 *t*-Bu), 19.1 (C_q *t*-Bu), 17.3 (C-6); HRMS $[M+H]^+$ calcd for $C_{24}H_{28}Cl_3N_4O_3Si$ 553.09908, found 553.09892.

1.8. *tert*-Butyldiphenylsilyl 4-(*N*-benzyloxycarbonyl)-amino-2-azido-2,4,6-trideoxy-β-D-galactopyranoside (9)

1.61 g Dihydro-oxazole (**7**) (2.92 mmol, 1 equiv) was stirred overnight in 18 mL AcOH/H_2O/EtOAc (4/1/1). The solvents were removed and the residue was coevaporated with toluene. The crude amine was dissolved in 15 mL of DCM and 526 μL triethylamine (3.79 mmol, 1.3 equiv) and 800 mg *N*-(benzyloxycarbonyloxy)succinimide (3.21 mmol, 1.1 equiv) were added. Stirring was allowed for 45 min followed by quenching with MeOH. Product **9** (1.22 g,

2.19 mmol, 75%) was obtained in pure form by flash column chromatography using EtOAc/PE (1/4 → 1/3). R_f 0.59 (EtOAc/PE, 7/13, v/v); $[\alpha]_D^{22}$ +19 (*c* 1.0, CH_2Cl_2); IR (neat, cm^{-1}) 3410 (br), 2939, 2862, 2114, 1705, 1512, 1111, 1065; ^1H NMR (400 MHz, $CDCl_3$, HH-COSY, HSQC) δ7.71–7.68 (m, 4H, H_{arom}), 7.44–7.31 (m, 11H, H_{arom}), 5.17–5.06 (m, 2H, CH_2Cbz), 4.97 (d, *J* = 9.4 Hz, 1H, NH), 4.37 (d, *J* = 7.8 Hz, 1H, H1), 3.83 (dd, *J* = 9.3, 3.4 Hz, 1H, H-4), 3.51 (dd, *J* = 10.0, 2.8 Hz, 1H, H-3), 3.31–3.20 (m, 2H, H-2, H-5), 3.17 (s, 1H, OH), 1.10 (s, 9H, CH_3 *t*-Bu), 0.96 (d, *J* = 6.4 Hz, 3H, H-6); ^{13}C NMR (100 MHz, $CDCl_3$, HH-COSY, HSQC) δ 157.8 (C=O Cbz), 135.9 (C_qPh), 135.8, 135.7 (CH_{arom}), 133.2, 132.7 (C_qPh), 129.8, 129.7, 128.5, 128.2, 128.1, 127.4, 127.3 (CH_{arom}), 97.0 (C-1), 72.2 (C-3), 69.3 (C-5), 67.3 (CH_2Cbz), 66.8 (C-2), 54.8 (C-4), 26.7 (CH_3 *t*-Bu), 19.0 (C_q *t*-Bu), 16.1 (C-6). HRMS $[M+H]^+$ calcd for $C_{30}H_{37}N_4O_5Si$ 561.25277, found 561.25250.

1.9. *tert*-Butyldiphenylsilyl 4-(*N*-benzyloxycarbonyl)-amino-2-azido-3-*O*-chloroacetyl-2,4,6-trideoxy-β-D-galactopyranoside (10)

To a mixture of alcohol **9** (860 mg, 1.54 mmol, 1 equiv), 5 mL DCM and 607 µl pyridine (7.68 mmol, 5 equiv) was added 525 mg chloroacetic anhydride (3.07 mmol, 2 equiv). After 1 h, 500 µl H_2O was added and the mixture was stirred for another 15 min. After evaporation the residue was taken up in EtOAc and washed with aq 1 M HCl, satd aq $NaHCO_3$ and brine. The organic phase was dried over $MgSO_4$, filtered, and evaporated to dryness yielding title compound **10** (984 mg, 1.54 mmol, quant.). R_f 0.79 (EtOAc/PE, 1/3, v/v); $[\alpha]_D^{22}$ −9 (*c* 1.0, CH_2Cl_2); IR (neat, cm^{-1}) 2114, 1713, 1504, 1165, 1057, 733. ^1H NMR (400 MHz, $CDCl_3$, HH-COSY, HSQC) δ 7.71–7.68 (m, 4H, H_{arom}), 7.46–7.25 (m, 11H, H_{arom}), 5.14 (d, *J* = 12.2 Hz, 1H, CH_2Cbz), 5.02 (d, *J* = 12.2 Hz, 1H, CH_2Cbz), 4.93 (d, *J* = 9.5 Hz, 1H, NH), 4.64 (dd, *J* = 10.7, 3.7 Hz, 1H, H-3), 4.44 (d, *J* = 7.7 Hz, 1H, H-1), 4.00 (dd, *J* = 9.5, 3.4 Hz, 1H, H-4), 3.95–3.81 (m, 2H, CH_2, ClAc), 3.48 (dd, *J* = 10.4, 8.0 Hz, 1H, H-2), 3.36 (q, *J* = 6.2 Hz, 1H, H-5), 1.11 (s, 9H, CH_3 *t*-Bu), 0.97 (d, *J* = 6.3 Hz, 3H, H-6). ^{13}C NMR (100 MHz, $CDCl_3$, HH-COSY, HSQC) δ 166.5, 156.45 (C=O), 136.2 (C_qPh), 135.7 (CH_{arom}), 132.9, 132.4 (C_qPh), 129.9, 128.5, 128.3, 128.1, 127.5, 127.4 (CH_{arom}), 97.0 (C-1), 74.6 (C-3), 68.9 (C-5), 67.1 (CH_2Cbz), 63.5 (C-2), 51.6 (C-4), 40.5 (CH_2ClAc), 26.7 (CH_3 *t*-Bu), 19.0, (C_q *t*-Bu), 16.0 (C-6); HRMS $[M+Na]^+$ calcd for $C_{32}H_{37}ClN_4O_6SiNa$ 659.20631, found 659.20672.

1.10. 4-(*N*-Benzyloxycarbonyl)-amino-2-azido-3-*O*-chloroacetyl-2,4,6-trideoxy-D-galactopyranose (11)

1.03 g galactosazide **10** (1.62 mmol, 1 equiv) in 10 mL THF was treated with 527 µl $N_3Et{\cdot}3HF$ (3.23 mmol, 2 equiv) and the mixture was stirred at 70 °C for 30 min. When the reaction mixture had cooled to ambient temperature EtOAc was added and the organic mixture was washed with satd aq $NaHCO_3$. The aqueous layer was extracted with DCM and the combined organic layers were dried over $MgSO_4$, filtered, and evaporated. Purification by flash column chromatography using EtOAc/PE (1/3 → 3/7) yielded galactopyranose **11** (632 mg, 1.58 mmol, 98%, α/β 1:2) with a minor unidentified side-product. R_f 0.42 (EtOAc/PE, 2/3, v/v); IR (neat, cm^{-1}) 3356 (br), 2361, 2114, 1701, 1526, 1061; ^1H NMR (400 MHz, $CDCl_3$, HH-COSY, HSQC) δ 7.42–7.29 (m, 5H, H_{arom}), 5.44 (d, *J* = 9.6 Hz, 0.7H, NH-α), 5.35–5.28 (m, 0.6H, H-1α, H-3α), 5.17–5.04 (m, 2H, CH_2Cbz-α, CH_2Cbz-β), 4.75 (dt, *J* = 13.2, 6.6 Hz, 0.7H, H-3β), 4.63 (d, *J* = 8.0 Hz, 0.7H, H-1β), 4.52–4.47 (m, 0.3H, H-5α), 4.33 (s, 0.7H, OH-β), 4.26–4.22 (m, 0.3H, H-4α), 4.18–4.12 (m, 0.7H, H-4β), 3.96–3.86 (m, 2H, CH_2ClAc), 3.81–3.74 (m, 0.7H, H-5β), 3.56 (dd, *J* = 11.1, 3.7 Hz, 0.3H, H-2α), 3.50 (dd, *J* = 10.8, 8.0 Hz, 0.7H, H-2β), 3.42 (s, 0.3H, OH-α), 1.24 (d, *J* = 6.4 Hz, 0.7H, H-6β), 1.18 (d, *J* = 6.5 Hz, 0.3H, H-6α). ^{13}C NMR (100 MHz, $CDCl_3$, HH-COSY, HSQC) δ 166.9, 157.1, 157.0 (C=O), 136.1, 136.0 (C_qPh), 128.6, 128.5, 128.3, 128.1, 127.9 (CH_{arom}), 96.2 (C-1β), 91.8 (C-1α), 74.6 (C-3β), 72.2 (C-3α), 69.2 (C-5β), 67.3, 67.2 (CH_2Cbz), 64.1 (C-5α), 61.8 (C-2β), 58.0 (C-2α), 52.5 (C-4α), 51.8 (C-4β), 40.6, 40.5 (CH_2ClAc), 16.4 (C-6β), 16.3 (C-6α); HRMS $[M+H]^+$ calcd for $C_{16}H_{20}ClN_4O_6$ 399.10659, found 399.10647.

1.11. 4-(*N*-Benzyloxycarbonyl)-amino-2-azido-3-*O*-chloroacetyl-2,4,6-trideoxy-α/β-D-galactopyranosyl (*N*-phenyl)trifluoroacetimidate (12)

To a solution of 511 mg hemiacetal **11** (1.28 mmol, 1 equiv) in 6.1 mL acetone and 0.3 mL H_2O were added 460 mg Cs_2CO_3 (1.41 mmol, 1.1 equiv) and 532 mg $ClC(C{=}NPh)CF_3$ (2.56 mmol, 2 equiv). When TLC analysis showed complete consumption of the starting material, the mixture was coevaporated with toluene. Purification by flash column chromatography using EtOAc/PE (1/9 → 3/7) yielded 606 mg of imidate **12** (1.06 mmol, 83%, anomers α/β 1:3). R_f 0.54 (EtOAc/PE, 1/3, v/v); IR (neat, cm^{-1}) 2116, 1717, 1524, 1211, 1163, 1072, 696; ^1H NMR (400 MHz, $CDCl_3$, HH-COSY, HSQC) (*T* = 333 K) δ 7.41–7.23 (m, 9.3H, H_{arom}), 7.09 (m, 1.4H, H_{arom}), 6.83 (m, 2.7H, H_{arom}), 6.35 (s, 0.3H, H-1α), 5.49 (d, *J* = 7.5 Hz, 1H, H-1β), 5.28 (dd, *J* = 11.1, 3.5 Hz, 0.3H, H-3α), 5.20–4.96 (m, 4H, CH_2Cbz, NH), 4.81 (dd, *J* = 10.7, 3.9 Hz, 1H, H-3β), 4.38–4.26 (m, 0.7H, H-4α, H-5α), 4.16 (dd, *J* = 9.7, 3.1 Hz, 1H, H-4β), 3.89 (s, 2.7H, CH_2ClAc), 3.81 (dd, *J* = 10.9, 3.9 Hz, 0.3H, H-2α), 3.75–3.59 (m, 2H, H-2β, H-5β), 1.20 (m, 4H, H-6). ^{13}C NMR (100 MHz, $CDCl_3$, HH-COSY, HSQC) (*T* = 333 K) δ 166.4, 156.7 (C=O), 143.1, 143.0, 136.3 (C_qPh), 128.8, 128.6, 128.3, 128.0, 124.7, 124.6, 119.3, 119.2 (CH_{arom}), 95.9 (C-1β), 93.7 (C-1α), 74.6 (C-3β), 72.2 (C-3α), 70.6 (C-5β), 67.5 (C-5α), 67.4 (CH_2Cbz), 60.2 (C-2β), 57.2 (C-2α), 52.4 (C-4α), 51.8 (C-4β), 40.3 (CH_2ClAc), 16.2 (C-6); HRMS $[M-(C(N{=}Ph)CF_3)+H+Na]^+$ calcd for $C_{16}H_{19}ClN_4O_6$ 421.08853, found 421.08845.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2012.02.015.

References

1. Kenne, L.; Lindberg, B.; Petersson, K.; Katzenellenbogen, E.; Romanowska, E. *Carbohydr. Res.* **1980**, *78*, 119–126.
2. (a) Lindberg, B.; Lindqvist, B.; Lönngren, J.; Powell, D. A. *Carbohydr. Res.* **1980**, *78*, 111–117; (b) Stroop, C. J. M.; Xu, Q.; Retzlaff, M.; Abeygunawardana, C.; Bush, C. A. *Carbohydr. Res.* **2002**, *337*, 335–344; (c) Karlsson, C.; Jansson, P.-E.; Sørensen, U. B. S. *Eur. J. Biochem.* **1999**, *265*, 1091–1097; (d) Behr, T.; Fischer, W.; Peter-Katalinic, J.; Egge, H. *Eur. J. Biochem.* **1992**, *207*, 1063–1075; (e) Fischer, W.; Behr, T.; Hartmann, R.; Peter-Katalinic, J.; Egge, H. *Eur. J. Biochem.* **1993**, *215*, 851–857.
3. Baumann, H.; Tzianabos, A. O.; Brisson, J.-R.; Kasper, D. L.; Jennings, H. J. *Biochemistry* **1992**, *31*, 4081–4089.
4. Bergström, N.; Jansson, P.-E.; Kilian, M.; Sørensen, U. B. S. *Eur. J. Biochem.* **2000**, *267*, 7147–7157.
5. Arbatsky, N. P.; Kondakova, A. N.; Senchenkova, S. N.; Siwinska, M.; Shashkov, A. S.; Zych, K.; Knirel, Y. A.; Sidorczyk, Z. *Carbohydr. Res.* **2007**, *342*, 2061–2066.
6. (a) Mazmanian, S. K.; Kasper, D. L. *Nat. Rev. Immunol.* **2006**, *6*, 849–858; (b) Tzianabos, A. O.; Onderdonk, A. B.; Rosner, B.; Cisneros, R. L.; Kasper, D. L. *Science* **1993**, *262*, 416–419; (c) Cobb, B. A.; Kasper, D. L. *Cell Microbiol.* **2005**, *7*, 1398–1403; (d) Cobb, B. A.; Wang, Q.; Tzianabos, A. O.; Kasper, D. L. *Cell* **2004**, *117*, 677–687; (e) Wang, Q.; McLoughlin, R. M.; Cobb, B. A.; Charrel-Dennis, M.; Zaleski, K. J.; Golenbock, D.; Tzianabos, A. O.; Kasper, D. L. *J. Exp. Med.* **2006**, *203*, 2853–2863.
7. van den Bos, L. J.; Boltje, T. J.; Provoost, T.; Mazurek, J.; Overkleeft, H. S.; van der Marel, G. A. *Tetrahedron Lett.* **2007**, *48*, 2697–2700.
8. Wu, X. Y.; Cui, L. N.; Lipinski, T.; Bundle, D. R. *Chem. Eur. J.* **2010**, *16*, 3476–3488.
9. Christina, A. E.; van den Bos, L. J.; Overkleeft, H. S.; van der Marel, G. A.; Codée, J. D. C. *J. Org. Chem.* **2011**, *76*, 1692–1706.
10. Pragani, R.; Seeberger, P. H. *J. Am. Chem. Soc.* **2011**, *133*, 102–107.
11. Lönn, H.; Lönngren, J. *Carbohydr. Res.* **1984**, *132*, 39–44.
12. Liav, A.; Hildesheim, J.; Zehavi, U.; Sharon, N. *Carbohydr. Res.* **1974**, *33*, 217–227.

13. Medgyes, A.; Farkas, E.; Lipták, A.; Pozsgay, V. *Tetrahedron* **1997**, *53*, 4159–4178.
14. (a) Pedersen, C. M.; Figueroa-Perez, I.; Lindner, B.; Ulmer, A. J.; Zahringer, U.; Schmidt, R. R. *Angew. Chem., Int. Ed. Engl.* **2010**, *49*, 2585–2590; (b) Pedersen, C. M.; Figueroa-Perez, I.; Boruwa, J.; Lindner, B.; Ulmer, A. J.; Zahringer, U.; Schmidt, R. R. *Chemistry* **2010**, *16*, 12627–12641.
15. Liang, H.; Grindley, T. B. *J. Carbohydr. Chem.* **2004**, *23*, 71–82.
16. Cai, Y.; Ling, C. C.; Bundle, D. R. *J. Org. Chem.* **2009**, *74*, 580–589.
17. (a) Hermans, J. P. G.; Elie, C. J. J.; van der Marel, G. A.; van Boom, J. H. *J. Carbohydr. Chem.* **1987**, *6*, 451–462; (b) Smid, P.; Jörning, W. P. A.; van Duuren, A. M. G.; Boons, G. J.; van der Marel, G. A.; van Boom, J. H. *J. Carbohydr. Chem.* **1992**, *11*, 849–865.
18. Iynkkaran, I.; Bundle, D. R. *Carbohydr. Res.* **2010**, *345*, 2323–2327.
19. Pragani, R.; Stallforth, P.; Seeberger, P. H. *Org. Lett.* **2010**, *12*, 1624–1627.
20. van den Bos, L. J.; Codée, J. D. C.; van Boom, J. H.; Overkleeft, H. S.; van der Marel, G. A. *Org. Biomol. Chem.* **2003**, *1*, 4160–4165.
21. Goddard-Borger, E. D.; Stick, R. V. *Org. Lett.* **2007**, *9*, 3797–3800; For caution for use: Goddard-Borger, E. D.; Stick, R. V. *Org. Lett.* **2011**, *13*, 2514.
22. (a) *Greene's Protective Groups in Organic Synthesis*; Wuts, P. G. M., Greene, T. W., Eds.; John Wiley & Sons: Hoboken, New Jersey, 2007; (b) Nelson, T. D.; Crouch, R. D. *Synthesis* **1996**, 1031–1069.
23. Takatani, M.; Nakama, T.; Kubo, K.; Manabe, S.; Nakahara, Y.; Ito, Y.; Nakahara, Y. *Glycoconjugate J.* **2000**, *17*, 361–375.