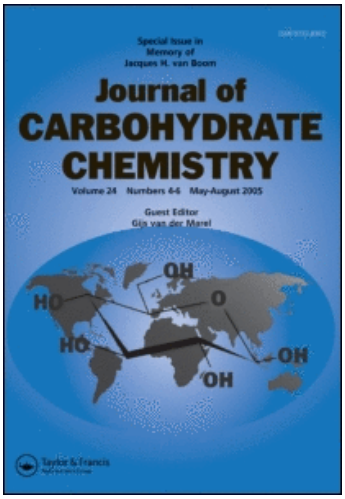

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Solid-Phase Synthesis of Sialyl Tn Antigen

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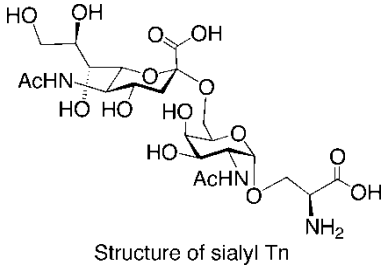
Solid-phase synthesis of sialyl Tn [α-Neu5Ac-(2 $\rightarrow$ 6)-α-GalNAc-(1 $\rightarrow$ *O*)-Ser] antigen with Kenner's acylsulfonamide linker is described. The acylsulfonamide bond was found to be stable under glycosylation reactions using dimethyl(methylthio)sulfonium triflate (DMTST) as a promoter and basic conditions used for the removal of protecting groups. The solid-phase reaction was monitored by the inverse gated decoupling ^{13}C NMR technique, which enabled quantitative analysis of the reaction progress. At the end of the synthesis, the sulfamyl group of the linker was activated by treatment with (trimethylsilyl)diazomethane to provide a *N*-methyl-*N*-acylsulfonamide. The acyl group was displaced with hydroxide to give the corresponding precursors of sialyl Tn antigen and its anomeric isomers, which were deprotected to afford the target molecules.



Structure of sialyl Tn

Keywords Oligosaccharide synthesis, Solid-phase synthesis, Thioglycosides, Monitoring, Kenner's linker

INTRODUCTION

A disaccharide, namely sialyl Tn, is known as a tumor-associated antigen present in glycoproteins expressed on the surface of cancer cells[1] and is also found in the envelope glycoprotein gp 120 of the human immunodeficiency virus (HIV).[2] A

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synthetic cancer vaccine[3–6] and a potential target for HIV immuno intervention[7] based on sialyl Tn antigen has thus been a target of investigations.

Although several chemical or enzymatic syntheses of sialyl Tn epitope have been reported,[8–12] there is no report based on solid-phase synthesis. One of the reasons for this is the difficulty associated with the sialylation reaction. As a part of our ongoing investigation of the solid-phase synthesis of oligosaccharides and a non-destructive monitoring method using inverse gated decoupling ^{13}C NMR,[13,14] we selected an αNeuAc(2 $\rightarrow$ 6)αGalNAc $\rightarrow$ Ser (sialyl Tn-Ser) epitope to demonstrate the applicability of our solid-phase synthetic method in the practical synthesis of an oligosaccharide. Also, we decided to synthesize all of its possible anomers. The primary aim of a current trend in the chemical synthesis of oligosaccharides that includes solid-phase synthesis is to synthesize a "desired" product, which has been isolated and structurally defined, and found to play important biological roles. However, it may be possible to find useful structures in nonnatural oligosaccharides. Merging with the concept of combinatorial chemistry, the synthesis of an oligosaccharide library should be recognized as a potential pool for finding lead compounds in the development of pharmaceuticals, especially those for treating infectious diseases.

Despite the importance of solid-phase synthesis of oligosaccharides,[15–26] there is still room for improvement. One of the important factors is a linker that is stable during glycosylation and deprotection reactions, which can be cleaved easily as needed. Also, the quantitative nondestructive monitoring of the reaction process remains to be solved. Regarding a suitable linker for our solid-phase oligosaccharide synthesis, we have selected Kenner's acylsulfonamide linker,[27–29] which is stable under basic and strongly nucleophilic reaction conditions. Examination of the utility of the linker in oligosaccharide synthesis revealed that the acylsulfonamide bond was stable in a glycosylation reaction using DMTST[30,31] as a promoter and basic conditions used for the removal of acetyl groups. Furthermore, the sulfamyl group of the resin could be activated successfully by treatment with (trimethylsilyl)diazomethane to provide an *N*-methyl-*N*-acylsulfonamide. The acyl group was displaced with hydroxide to give the corresponding free acid. For the monitoring of solid-phase oligosaccharide synthesis, we have been investigating a nondestructive monitoring technique using an inverse gated decoupling ^{13}C NMR.[32] The ^{13}C-enriched protecting groups were used for the glycosylating agents together with ^{13}C-enriched linker as an internal integral marker.[13,14] Hence, the reaction yields are given as relative to the internal standard, and the actual chemical yields for the corresponding reactions are obtained.

We report herein the solid-phase synthesis of sialyl Tn antigen as a Boc-protected form and all its stereoisomers using acylsulfonamide linker, the reaction course of which is quantitatively monitored by an inverse gated decoupling ^{13}C NMR technique.

RESULTS AND DISCUSSION

Synthesis of L-Serine Attachment to Acylsulfonamide Linker

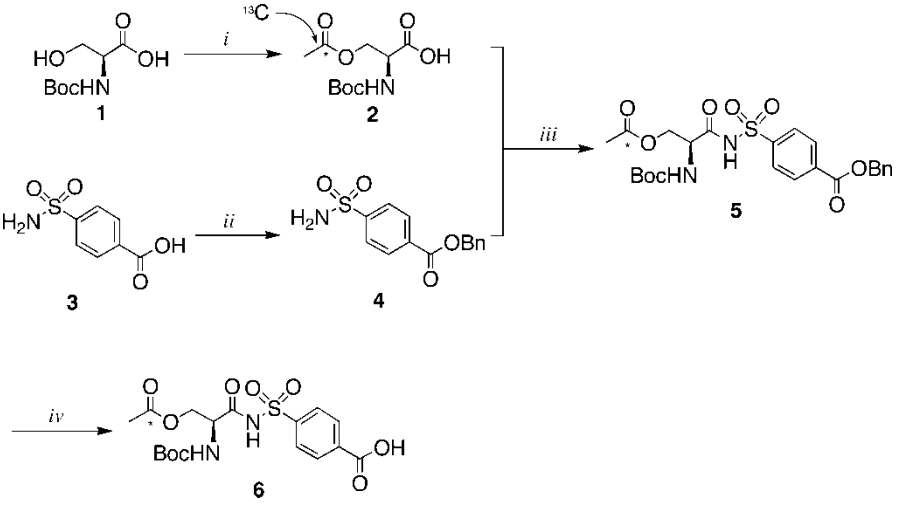
N-(*t*-butoxycarbonyl)-L-serine (**1**) was treated with acetic-*1*-^{13}C anhydride, prepared from acetic-*1*-^{13}C acid, in the presence of 4-dimethylaminopyridine (DMAP) in CH_2Cl_2 to give compound **2**, which was coupled with 4-sulfamoyl-benzoic acid benzyl ester (**4**) using benzotriazole-1-yl-oxy-tris(pyrrolidino) phosphonium hexafluorophosphate (PyBop) in CH_2Cl_2 to obtain a conjugate of L-serine-linker with a ^{13}C-enriched tag (**5**) in 72% yield. Compound **5** was debenzylated by hydrogenolysis to give compound **6** (Scheme 1).

Synthesis of GalN Glycosyl Donor

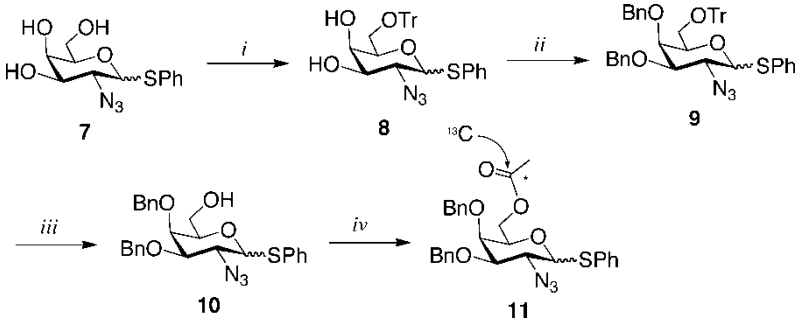
The primary OH group of the phenylthioglycoside of 2-azide-2-deoxy-galactose **7**[10] was tritylated and the remaining hydroxy groups were subsequently benzylated to give **9**. Compound **9** was detritylated with TFA to give compound **10**, which was converted to the GalN glycosyl donor (**11**) after acetylation with acetic-*1*-^{13}C acid for solid-phase synthesis (Scheme 2).

Solid-Phase Synthesis of Sialyl Tn Antigen

The ^{13}C acetyl-protected L-serine attached sulfonamide linker **6** was first incorporated into the TentaGel carrying a *1*-^{13}C-glycine (**12**) as an internal



Scheme 1: Synthesis of L-Ser attached to the acylsulfonamide linker. *i.* acetic-*1*-^{13}C anhydride, DMAP/CH_2Cl_2, rt, 12 h, 92%; *ii.* BnOH, DIPC, HOBt / DMF, rt, 12 h, 85%; *iii.* PyBop, $^{i}Pr_2EtN/CH_2Cl_2$, −20°C, 10 h, 72%; *iv.* 10% Pd/C, H_2/MeOH, rt, 12 h, 88%.



Scheme 2: Synthesis of GalN donor. *i.* TrCl, pyridine, rt, 24 h, 90%; *ii.* BnBr, NaH/DMF, rt, 2 h, 90%; *iii.* 10% TFA/CH_2Cl_2, rt, 1 h, 83%; *iv.* acetic-*1*-^{13}C acid, DCC, DMAP/CH_2Cl_2, rt, 4 h, 94%.

integral standard. The coupling yield of the reaction was estimated by gated decoupling ^{13}C NMR (97%) after filtration. Subsequent deacetylation of **13** afforded a glycosyl acceptor **14** quantitatively. The solid-bound L-Ser **14** was glycosylated with GalN donor **11** (2 equiv.) in the presence of DMTST (8 equiv.) in CH_2Cl_2-Et_2O at 0°C. The reaction was carried out twice to obtain a resin, **15**, in 82% yield, which was confirmed by gated decoupling ^{13}C NMR. The remaining hydroxyl groups of resin-bound L-Ser were capped using *t*-butyldimethylsilylchloride (TBDMS-Cl) to eliminate the sequence deletion. [33] After deprotection of the acetyl group (**16**, quant.), a second glycosylation reaction was performed using methyl (^{13}C) 5-acetoamide-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-methylthio-D-*glycero*-β-D-*galacto*-2-nonulopyranosylonate.[14] The sialylation reaction was carried out in the presence of DMTST at -15°C in CH_3CN. Solvent selection was based on some preliminary experiments to obtain all anomers where the desired distribution of the mixture was equal. Cleavage of the solid-bound compounds was achieved by a sequential treatment with TMS-diazomethane and NaOH to give the glycopeptides **19a–d** as a mixture of the combination of the possible anomers. The yield for the cleavage reaction was 62% based on gravimetric analysis of the released products and on the reaction yields estimated by the gated decoupling technique. The resin should be glycosylated in various degrees of order since each glycosylation reaction was not quantitative, and individual compounds are given as a mixture of anomers. In order to determine the anomeric configurations in each glycosidic bond, compounds **19a–d** released from the resin were purified by gel permeation chromatography and repeated silica gel column chromatography. The product was found to consist of four isomers based on the anomeric configurations of the Gal and Sia residues, and it was revealed that the desired anomeric combination [α α(Sia/Gal)] was a major component among the anomers: α/α (**19a**, 25%), α/β (**19b**, 19%), β/α (**19c**, 12%), and β/β(**19d**, 6%). Each compound was analyzed by HPLC, the chromatogram of which is shown

in Figure 1. Assignment of the anomeric configurations for the sialic acid residue was based on an empirical rule where the chemical shifts of the equatorial proton of H-3 appeared in the lower field for the α-glycosides.[34] The observed chemical shifts of the proton for compounds **19a–d** were δ 2.85, 2.89, 2.41, and 2.47, respectively (Scheme 3).

Deprotection of Sialyl Tn Antigen

The isolated compounds **19a–d** were first subjected to hydrogenolysis to deprotect benzyl groups and at the same time to convert the azide into an amine. Subsequent *N*-acetylation gave sialyl Tn antigen and its anomeric isomers (**20a–d**) as a Boc-protected form, which could be used in peptide synthesis (Scheme 4).

Conclusion

The synthesis of sialyl Tn-Ser antigen and its stereoisomers with a Boc-protecting group was achieved on the solid support, where we have used two ^{13}C enriched markers for the inverse gated decoupling ^{13}C NMR technique. The strategy should fit suitably into the small-scale solid-phase synthesis of oligosaccharides and perhaps other organic compounds, especially for optimization of the reaction conditions. The advantage of this method is that actual chemical yields are obtained since the yield is given directly from integrals in ^{13}C NMR without any manipulation, which sometimes results in inaccurate calculations due to resin breakdown. For the cleavage of the synthesized molecule from resin, Kenner's acylsulfonamide linker was used. The acylsulfonamide bond was found to be stable to the glycosylation reaction conditions using DMTST as a promoter and basic conditions used for the removal of protecting groups.

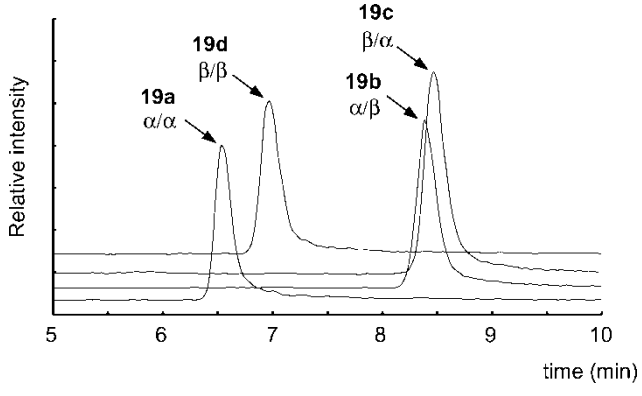
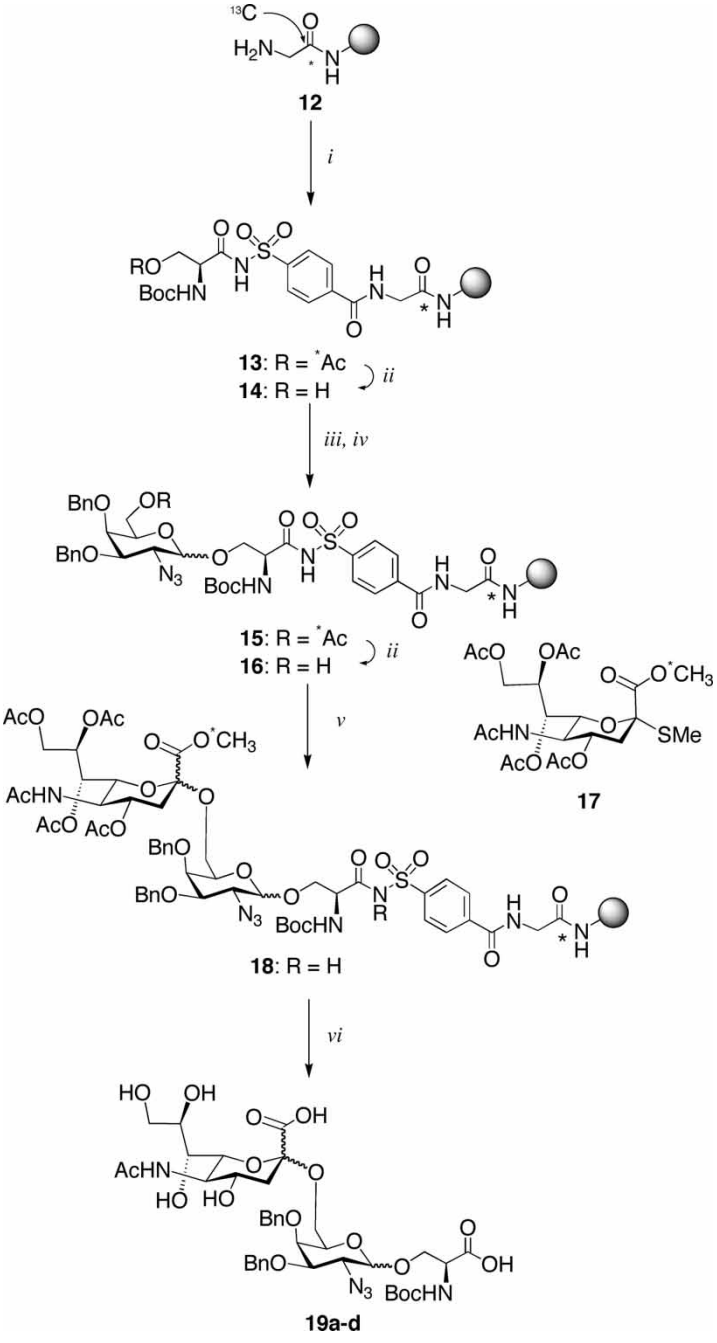
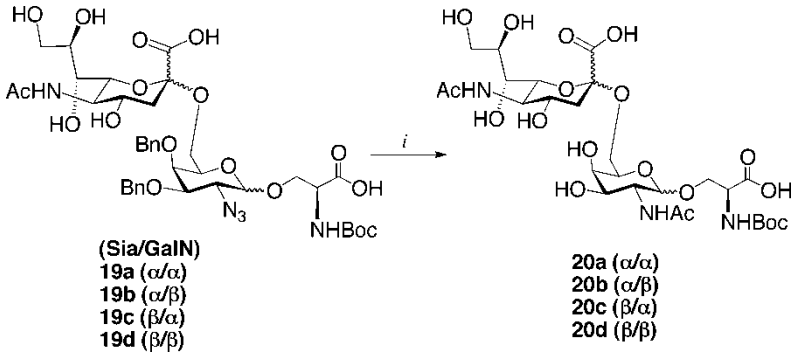


Figure 1: Chromatograms of purified compounds **19a–d**.



Scheme 3: Solid-phase synthesis of sialyl Tn antigen. *i.* **6**, DIPC-HOBt-*i*Pr_2EtN/DMF, rt, 12 h, 97%; *ii.* 0.05 M NaOMe/MeOH-DMF (1:1), rt, 12 h, quant.; *iii.* **11**, DMTST/CH_2Cl_2-Et_2O (1 : 1), 0°C, 12 h, twice, 82%; *iv*. TBDMSCl-imidazole / CH_2Cl_2, rt, 12 h; *v.* **17**, DMTST/CH_3CN, −15°C, 12 h, twice, 80%; *vi.* a) TMS-diazomethane, *i*Pr_2EtN/THF, rt, 24 h; b) 0.05 N NaOH/H_2O-THF (1 : 1), rt, 12 h, 62% (in two steps).



Scheme 4: Deprotection reactions. *i.* (a) $Pd(OH)_2$-H_2/MeOHH_2O-AcOH, rt, 12 h, (b) Ac_2O, pyridine, rt, 12 h, (c) 0.05M NaOMe / MeOH, r.t., 3 h.

EXPERIMENTAL

General Methods

TentaGel™ S-NH_2 resin was purchased from Fluka. Dried solvents were used for all reactions. Solvents were evaporated under reduced pressure at a bath temperature not exceeding 50°C. Analytical thin layer chromatography (TLC) was performed on Merck Art. 5715, Kieselgel 60 F_{254}/0.25-mm thickness plates. Visualization was accomplished with UV light, phosphomolybdic acid, or sulfuric acid solution followed by heating. Column chromatography was performed with silica gel FL-100D (Fuji Silysia Co.). Optical rotations were measured on a Horiba SEPA-200 polarimeter with sodium lamp ($\lambda = 589$ nm). ^{1}H NMR (270 MHz) and ^{13}C NMR (67.5 MHz) spectra were recorded with a JEOL EX-270 spectrometer in deuterated solvents using tetramethylsilane (δ 0.00 for ^{1}H NMR), $CDCl_3$ (δ 77.00 for ^{13}C NMR), or CD_3OD (δ 49.00 for ^{13}C NMR) as an internal standard. MALDI-TOF mass spectra were recorded on a Perceptive Voyager mass spectrometer with 2,5-dihydroxybenzoic acid as matrix. High-resolution mass spectra were recorded on a JEOL JMS-700 spectrometer under FAB conditions. Compounds **19a–d** were analyzed by LC-MS (Waters 2525 pump system and Micromass ZQ). The conditions used were as follows. Column: Imtakt Unizon UK-C18 (4.6 × 75 mm); flow rate: 1.0 mL/min; column temp.: 40°C: elution: 0.1% AcOH in CH_3CN (Merck Hyper Grade)/H_2O (LC/MS Grade) = 35/65; ionization: ESI; detection: m/z = 862 $[M\text{-}H]^-$ (negative ion mode).

Materials

Compound **17** was synthesized according to the procedure described in ref. 14.

General Methods for Monitoring of Solid-phase Compounds with Inverse-gated Decoupling ^{13}C NMR Measurement

The dried resin (ca. 60 mg) was slurried in $CDCl_3$, and the sample was prepared to contain a relaxation agent, chromium (III) 2,4-pentanedionate [$Cr(AcAc)_3$, 0.1 M], in an ordinary 5-mm ϕ NMR tube. ^{13}C NMR was measured on a JEOL EX-270 spectrometer at 67.5 MHz and operated with a 9-s relaxation delay and gated decoupling without NOE (160 transients, 0.6-s acquisition time). The spectra were referred to resonance for TMS. ^{13}C NMR spin-lattice relaxation times (T_1) were measured by using the inversion recovery method at 298 K (16 data points, 16 scans per point). T_1 values for methyl and carbonyl groups attached to the resin were shorter than 1 s in the presence of $Cr(AcAc)_3$.

Procedures

***N*-(*tert*-Butoxycarbonyl)-*O*-acetyl(*1*-^{13}C)-L-serine.**[2] To a solution of DCC (780 mg, 3.78 mmol) in dry CH_2Cl_2 (8.0 mL) was added acetic-*1*-^{13}C acid (400 μL, 6.87 mmol) at rt and the mixture was stirred for 30 min and then filtered to remove urea. To the filtrate, a solution of *N*-(*tert*-butoxycarbonyl)-L-serine (800 mg, 3.90 mmol) and DMAP (48 mg, 0.39 mmol) in CH_2Cl_2 (15.0 mL) and compound **1** were added at rt and stirred for 12 h. The mixture was concentrated in vacuo. The residue was purified by silica gel column chromatography using toluene/acetone/AcOH (10 : 5 : 0.2) as an eluent to provide **2** (784 mg, 92%): $[\alpha]_D$ +15.6° (*c* 1.0, $CHCl_3$); R*f* 0.19 (toluene/acetone/AcOH 10 : 5 : 0.2); ^1H NMR ($CDCl_3$) δ 5.53–5.41 (m, 1H, Ser α), 4.60–4.40 (m, 2H, Ser β), 2.08 (d, 3H $J = 6.9$ Hz, Ac), 1.46 (s, 9H, *t*Bu-CH_3); ^{13}C NMR ($CDCl_3$) δ 172.97, 170.76, 155.58, 80.79, 64.17, 52.80, 28.27, 20.66 (d, $J = 59.9$ Hz). MALDI-TOF MS: Calcd for $C_9\,^{13}CH_{17}NO_6$ (M): 248.1. Found m/z: 271.1 $(M + Na)^+$; HR-FAB MS: Calcd for $C_9\,^{13}CH_{17}NO_6$ (M): 248.1089. Found m/z: 247.1026 $(M\text{-}H)^-$.

4-Sulfamoylbenzoic acid benzyl ether.[4] 1-Hydroxybenzotriazole (HOBt, 3.7 g, 27.4 mmol) and *N, N*′-diisopropylcarbodiimide (DIPC, 3.45 g, 27.3 mmol) were added at rt to a solution of 4-sulfamoylbenzoic acid (**3**) (5.0 g, 24.9 mmol) and benzylalcohol (6.0 mL, 58.0 mmol) in DMF (12 mL). After stirring for 12 h, the reaction mixture was concentrated. The residue was purified by silica gel column chromatography using toluene/acetone (5 : 1) as an eluent to provide **4** (6.13 g, 85%) as a white mass: $[\alpha]_D$ −30.4° (*c* 0.5, MeOH); R*f* 0.35 (toluene/acetone 4 : 1); ^1H NMR (CD_3OD) δ 8.19–7.96 (m, 4H, Ar), 7.48–7.30 (m, 5H, Ar), 5.38 (s, 2H, Bn-CH_2); ^{13}C NMR (CD_3OD) δ 167.30, 150.01, 138.08, 135.33, 131.97, 130.46, 130.25, 130.17, 128.18, 69.10. Anal calcd for $C_{14}H_{13}NO_4S$: C, 57.72; H, 4.50; N, 4.81; S, 11.01. Found: C, 57.70; H, 4.48; N, 4.80; S, 10.89.

***N*-(*tert*-Butoxycarbonyl)-*O*-acetyl(*1*-^{13}*C*)-L-seryl-4-sulfamoylbenzoic acid benzyl ether.**[5] To a solution of compound **2** (655 mg, 2.65 mmol) and **4** (1540 mg, 5.3 mmol) in dry CH_2Cl_2 (15 mL) was added *N, N*-diisopropylethylamine (iPr$_2$EtN, 0.9 mL, 5.3 mmol) at rt. After stirring for 20 min, the mixture was cooled to -20°C. PyBop (2100 mg, 4.0 mmol) was added to the mixture and stirred for 10 h, then diluted with CH_2Cl_2, which was washed with brine (three times), dried with Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography using toluene/acetone (1 : 1) as an eluent to provide **5** (996 mg, 72 %): $[\alpha]_D$ -30.4° (*c* 0.5, $CHCl_3$); R*f* 0.17 (toluene/acetone 1 : 1); ^{1}H NMR ($CDCl_3$) δ 8.24–8.11 (m, 4H, Ar), 7.45–7.28 (m, 5H, Ar), 5.40 (s, 2H Bn-CH_2), 4.35–4.21 (m, 3H, Ser α and Ser β), 2.02 (d, 3H $J = 6.9$ Hz, Ac), 1.44 (s, 9H, tBu-CH_3); ^{13}C NMR ($CDCl_3$) δ 170.80, 167.44, 164.76, 156.12, 142.10, 135.35, 135.13, 130.21, 128.72, 128.57, 128.54, 128.36, 82.14, 67.49. 62.50, 54.13, 28.16, 20.59 (d, $J = 59.8$ Hz). Anal calcd for C_{23} $^{13}CH_{28}N_2O_9S$: C, 55.46; H, 5.41; N, 5.37; S, 6.15. Found: C, 55.12; H, 5.36; N, 5.28; S, 6.73.

***N*-(*tert*-Butoxycarbonyl)-*O*-acetyl(*1*-^{13}*C*)-L-seryl-4-sulfamoylbenzoic acid.**[6] A mixture of compound **5** (800 mg, 1.53 mmol) in MeOH (10 mL) was stirred at rt in the presence of 10% Pd/C (50 mg) under an H_2 atmosphere for 12 h. The reaction mixture was filtered through a pad of celite and the filtrate was concentrated. The residue was purified by Sephadex LH-20 column chromatography using MeOH as an eluent to provide **6** (580 mg 88%): $[\alpha]_D$ -48.7° (*c* 0.5, CHCl3); R*f* 0.47 (CH_2Cl_2/MeOH/AcOH 10 : 1 : 0.5); ^{1}H NMR (CD_3OD) δ 8.20–8.07 (m, 4 H, Ar), 4.25–4.14 (m, 3H, Ser α and Ser β), 1.91 (d, 3H $J = 6.9$ Hz, Ac), 1.39 (s, 9H, tBu-CH3); ^{13}C NMR (CD_3OD) δ 172.73, 171.31, 168.74, 158.26, 145.07, 137.62, 131.90, 130.19, 81.97, 64.77, 56.18, 29.35, 21.20 (d, $J = 59.8$ Hz). HR-FAB MS: Calcd for C_{16} $^{13}CH_{22}N_2O_9S$ (M): 431.1080. Found m/z: 454.0977 $(M + Na)^+$.

Phenyl 2-azido-2-deoxy-1-thio-6-*O*-trityl-α,β,-D-galactopyranoside.[8] A solution of compound **7** (230 mg, 0.77 mmol) and trityl chloride (440 mg, 1.58 mmol) in pyridine (4.0 mL) was stirred at rt for 24 h. The reaction mixture was concentrated. The residue was purified by silica gel column chromatography using toluene/acetone (10 : 1) as an eluent to provide **8** as a mixture of α and β anomers (374 mg, 90%): R*f* 0.48 (toluene/acetone 4 : 1); ^{1}H NMR (CD_3OD) δ 7.66–7.22 (m, Ar), 5.68 (d, $J_{1,2} = 5.3$ Hz, H-1α), 4.45 (ddd, $J_{4,5} = 1.5$, $J_{5,6} = 4.3$ Hz, H-5α), 4.41 (d, $J_{1,2} = 9.9$ Hz, H-1β), 4.36 (dd, $J_{2,3} = 10.6$ Hz, H-2α), 4.04 (dd, $J_{3,4} = 3.0$ Hz, H-4α), 3.93 (dd, $J_{3,4}$= 2.7, $J_{4,5} = 1.0$ Hz, H-4β), 3.80 (dd, H-3α), 3.55–3.40 (m, H-2β, 3β, 5β, 6α, 6β). MALDI-TOF MS: Calcd for $C_{31}H_{29}N_3O_4S$ (M): 539.2. Found m/z: 562.5 $(M + Na)^+$; Anal calcd for $C_{31}H_{29}N_3O_4S$: C, 69.00; H, 5.42; N, 7.79. Found: C, 68.83; H, 5.56; N, 7.65

Phenyl 2-azido-3,4-di-*O*-benzyl-2-deoxy-1-thio-6-*O*-trityl-α,β-D-galactopyranoside.[9] To a solution of compound **8** (414 mg, 0.77 mmol) in DMF (6.0 mL) was added NaH (60%, in mineral oil, 120 mg, 3.0 mmol) at 0°C, and the mixture was stirred for 30 min. Benzyl bromide (360 μL, 3.0 mmol) was added to the mixture and the temperature of the mixture was allowed to increase to rt. After stirring for 2 h the excess NaH was carefully destroyed by adding MeOH. After evaporation, a solution of the residue in CH_2Cl_2 was successively washed with H_2O (3 times), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography using hexane/ethyl acetate (10 : 1) as an eluent to provide a mixture of α and β anomers (495 mg, 90%): R*f* 0.47 (hexane/ethyl acetate 6 : 1); ^{1}H NMR ($CDCl_3$) δ 7.56–7.02 (m, Ar), 5.57 (*d*, $J_{1,2} = 5.3$ Hz, H-1α), 4.79–4.64 (m, Bn-CH_2), 4.45–4.33 (m, H-1β, 2α, 5α, Bn-CH_2), 3.94 (br d, $J_{3,4} = 2.0$ Hz, H-4α), 3.84 (br d, $J_{3,4} = 2.3$ Hz, H-4β), 3.79–3.73 (m, H-2β, 3α), 3.55 (dd, $J_{5,6} = 5.6$, $J_{6a,6b} = 9.2$ Hz, H-6aβ), 3.43 (dd, $J_{5,6} = 5.9$, $J_{6a,6b} = 9.6$ Hz, H-6aα), 3.37–3.30 (m, H-3β, 5β), 3.24–3.16(m, H-6bα, 6bβ). MALDI-TOF MS: Calcd for $C_{45}H_{41}N_3O_4S$ (M): 719.3. Found *m*/*z*: 742.5 $(M + Na)^+$; Anal calcd for $C_{45}H_{41}N_3O_4S$: C, 75.08; H, 5.74; N, 5.84. Found: C, 74.84; H, 5.65; N, 5.81.

Phenyl 2-azido-3,4-di-*O*-benzyl-2-deoxy-1-thio-α- and - β-D-galactopyranoside.[10] Compound **9** (490 mg, 0.68 mmol) was treated with 10% TFA in CH_2Cl_2 at rt for 1 h and then the reaction mixture was concentrated. The residue was purified by silica gel column chromatography using hexane/ethyl acetate (2 : 1) as an eluent to provide α and β anomers, respectively: Data for α anomer: $[\alpha]_D$ +152.6° (*c* 1.0, $CHCl_3$); R*f* 0.39 (hexane/ethyl acetate 2 : 1); ^{1}H NMR ($CDCl_3$) δ 7.50–7.24 (m, 15H, Ar), 5.65 (d, 1H, $J_{1,2} = 5.3$ Hz, H-1), 4.91 and 4.57 (2d, 2H, $J_{gem} = 11.6$ Hz, Bn-CH_2), 4.78 (dd, 2H, $J_{gem} = 13.8$, 11.5 Hz, Bn-CH_2), 4.44 (dd, 1H, $J_{2,3} = 10.6$ Hz, H-2), 4.25 (ddd, 1H, $J_{5,6} = 6.6$ Hz, H-5), 3.94 (br s, 1H H-4), 3.80 (dd, 1H, $J_{3,4} = 2.6$ Hz, H-3), 3.71 (dd, 1H, $J_{6a,6b} = 11.5$ Hz, H-6a), 3.53 (br m, 1H, H-6b); ^{13}C NMR ($CDCl_3$) δ 137.77, 137.23, 132.99, 132.45, 129.06, 128.59, 128,50, 128.28, 128.09, 128.05, 127.87, 127.75, 87.35, 79.23, 74.61, 73.28, 72.74, 71.88, 62.03, 60.38. MALDI-TOF MS: Calcd for $C_{26}H_{27}N_3O_4S$ (M): 477.2. Found *m*/*z*: 500.5 $(M + Na)^+$. Data for β anomer: $[\alpha]_D$ −35.6° (*c* 1.0, $CHCl_3$); R*f* 0.22 (hexane/ethyl acetate 2:1); ^{1}H NMR ($CDCl_3$) δ 7.59–7.21 (m, 15H, Ar), 4.88 and 4.54 (2d, 2H, $J_{gem} = 11.6$ Hz, Bn-CH_2), 4.72 (s, 2H, Bn-CH_2), 4.40 (d, 1H, $J_{1,2} = 10.2$ Hz, H-1), 3.88–3.79 (m, 3H, H-2, 4, 6a), 3.54 (br m, 1H, H-6b), 3.44–3.39 (m, 2H, H-3, 5); ^{13}C NMR ($CDCl_3$) δ 138.04, 137.29, 132.81, 131.77, 128.91, 128.57, 128.39, 128.10, 127.98, 127.91, 127.85, 86.45, 82.64, 78.96, 74.20, 72.72, 72.01, 62.14, 61.69. HR-FAB MS: Calcd for $C_{26}H_{27}N_3O_4S$ (M): 477.1722. Found *m*/*z*: 478.1797 $(M + H)^+$.

Phenyl 6-*O*-acetyl(*1*-^{13}*C*)-2-azido-3,4-di-*O*-benzyl-2-deoxy-1-thio-α- and -β-D-galactopyranoside.[11] Acetic-*1*-^{13}*C* acid (15 μL, 0.26 mmol) was added at rt to a solution of compound **10** (as a mixture of α and β anomers) (120 mg, 0.25 mmol), DCC (78 mg, 0.38 mmol), and DMAP (6 mg, 0.05 mmol) in dry CH_2Cl_2 (5 mL), and the mixture was stirred for 4 h. The reaction mixture was filtered and the filtrate was diluted with CH_2Cl_2, washed with brine (three times), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography using hexane/ethyl acetate (4 : 1) to provide **11** (123 mg, 94%): Data for α anomer: $[\alpha]_D$ +138.6° (*c* 1.0, $CHCl_3$); R*f* 0.61 (hexane/ethyl acetate 2 : 1); ^{1}H NMR ($CDCl_3$) δ 7.51–7.24 (m, 15H, Ar), 5.65 (d, 1H, $J_{1,2} = 5.3$ Hz, H-1), 4.92 and 4.56 (2d, 2 H, $J_{gem} = 11.2$ Hz, Bn-CH_2), 4.79 (dd, 2H, $J_{gem} = 11.5$, 12.9 Hz, Bn-CH_2), 4.47–4.41 (m, 2H, H-2, 5), 4.21–4.04 (m, 2H, H-6), 3.92 (br s, 1H H-4), 3.79 (dd, 1H, $J_{2,3} = 10.6$, $J_{3,4} = 2.6$ Hz, H-3), 1.92 (d, 3H, $J = 6.9$ Hz, Ac); ^{13}C NMR ($CDCl_3$) δ 170.42, 137.72, 137.22, 133,17, 132.09, 128.93, 128.59, 128.39, 128.14, 128.09, 127.91, 127.85, 127.57, 87.19, 79.16, 74.68, 73.17, 72.80, 69.63, 63.24, 60.25, 20.64 (d, $J = 59.8$ Hz). MALDI-TOF MS: Calcd for C_{27} $^{13}CH_{29}N_3O_5S$ (M): 520.2. Found *m*/*z*: 543.4 $(M + Na)^+$. Data for β anomer: $[\alpha]_D$ −18.5° (*c* 1.0, $CHCl_3$); R*f* 0.49 (hexane/ethyl acetate 2 : 1); ^{1}H NMR ($CDCl_3$) δ 7.59–7.18 (m, 15H, Ar), 4.89 and 4.53 (2d, 2 H, $J_{gem} = 11.6$ Hz, Bn-CH_2), 4.72 (s, 2H, Bn-CH_2), 4.37 (d, 1H, $J_{1,2} = 9.9$ Hz, H-1), 4.25 (dd, 1H, $J_{5,6a} = 3.3$, $J_{6a,6b}$= 11.2 Hz, H-6a), 4.10 (dd, 1H, $J_{5,6b} = 3.0$ Hz, H-6b), 3.84 (dd, 1H, $J_{2,3} = 9.6$ Hz, H-2), 3.79 (br d, 1H, $J_{3,4} = 2.6$ Hz, H-4), 3.55 (br dd, 1H, H-5), 3.41 (dd, 1H, H-3), 1.98 (d, 3H, $J = 6.9$ Hz, Ac); ^{13}C NMR ($CDCl_3$) δ 170.42, 137.86, 137.20, 132.87, 131.73, 128.90, 128.73, 128.48, 128.21, 128.01, 127.89, 127.80, 127.64, 86.31, 82.50, 76.12, 74.20, 72.72, 71.93, 63.22, 61.44, 20.67 (d, $J = 58.6$ Hz). MALDI-TOF MS: Calcd for $C_{27}{}^{13}CH_{29}N_3O_5S$ (M): 520.2. Found *m*/*z*: 543.5 $(M + Na)^+$ Anal calcd for $C_{27}{}^{13}CH_{29}N_3O_5S$: C, 64.79; H, 5.61; N, 8.07. Found: C, 64.72; H, 5.60; N, 7.89.

Solid-Phase Synthesis

Resin 13. To a suspension of resin **12** (500 g, 0.13 mmol) and compound **6** (112 mg, 0.26 mmol) in dry DMF (10 mL) were added *i*Pr_2EtN (67 μL, 0.39 mmol), HOBt (52 mg, 0.39 mmol) and DIPC (60 μL, 0.39 mmol), at rt. After shaking for 4 h, the resins were washed with DMF, H_2O, MeOH, and CH_2Cl_2, then dried in vacuo to give sulfonamide linker-attached resin **12** (97%). The yield was estimated by integral obtained by inverse gated decoupling as relative to internal ^{13}C marker at 168.9 ppm: ^{13}C NMR ($CDCl_3$) δ 170.0, 168.9.

Deacetylation of Solid-phase Compounds. ^{13}C-enriched acetyl protected resin-bound compound was treated with 5 mM NaOMe in MeOH-DMF mixed

solvent (1 : 1, v/v) at rt for 12 h, washed with DMF, H_2O, DMF-AcOH (10 : 1, v/v), MeOH, and CH_2Cl_2, and then dried in vacuo to give the resin-bound compounds with a free hydroxyl group. The deprotection was confirmed by the disappearance of carbonyl signals that originated from the acetyl group in ^{13}C NMR spectra.

Resin 14. ^{13}C NMR ($CDCl_3$) δ 168.9.

Resin 16. ^{13}C NMR ($CDCl_3$) δ 169.5.

Glycosylation Reaction on Solid-phase. DMTST (8 equiv.) was added to a mixture of resin-bound acceptor and glycosyl donor (2 equiv.) in dry CH_2Cl_2-Et_2O mixed solvent or CH_3CN (20 mL/g-resin). The mixture was shaken under a nitrogen atmosphere at the temperature designated in Scheme 3 for 24 h. The resin was washed with CH_2Cl_2, MeOH, H_2O, DMF, and CH_2Cl_2, and this procedure was repeated twice. The resin was then suspended in CH_2Cl_2, $tBuMe_2SiCl$, and imidazole added at rt, and the mixture shaken for 24 h. The resins were washed with CH_2Cl_2, MeOH, water, DMF, and CH_2Cl_2. The reaction yields were estimated based on the integrals of signals obtained by ^{13}C NMR experiments.

Resin 15. ^{13}C NMR ($CDCl_3$) δ 171.0, 168.3.

Resin 18. ^{13}C NMR ($CDCl_3$) δ 170.0, 52.3.

Cleavage of Compounds from Solid-phase. iPr$_2$EtN (65 μL, 0.37 mmol) and (trimethylsilyl)diazomethane (2.0 M solution in hexane, 2.0 mL) were added to a suspension of resin **18** (560 mg) in dry THF (10 mL). The mixture was shaken in the dark under a nitrogen atmosphere for 24 h. The resins were washed with THF and DMF. Subsequently, the resin was suspended in 0.05 M NaOH/H_2O-THF (1 : 1) and shaken at rt for 12 h. The resins were washed with DMF and MeOH. The filtrate was neutralized by Amberlite IR-120 (H^+), filtered, and concentrated. The residue was passed through a Sephadex LH-20 gel permeation column with MeOH as a solvent to isolate a disaccharide-serine fraction. The fraction was purified by repeated silica gel column chromatography using CH_2Cl_2/MeOH/H_2O/AcOH (20 : 10 : 1 : 1) as an eluent to provide **19a** (17.9 mg), **19b** (13.6 mg), **19c** (8.6 mg), and **19d** (4.3 mg): Data for **19a**: $[\alpha]_D$ +30.2° (c 0.35, MeOH); Rf 0.43 (CH_2Cl_2/MeOH/H_2O/AcOH 20 : 10 : 1 : 1); ^1H NMR (CD_3OD) δ 7.40–7.27 (m, 10H, Ar), 4.84 (br s, 1H, H-1a), 2.85 (br d, 1H, J = 12.9 Hz, H-3b-eq), 2.01 (s, 3H, Ac), 1.66 (t, 1H, H-3b-ax), 1.45 (s, 9 H, tBu-CH3). MALDI-TOF MS: Calcd for $C_{39}H_{53}N_5O_{17}$ (M): 863.3. Found m/z: 886.3 $(M + Na)^+$; HR-FAB MS: Calcd for $C_{39}H_{53}N_5O_{17}$ (M): 863.3436. Found m/z: 886.3331 $(M + Na)^+$. Data for **19b**:

$[\alpha]_D$ +33.4° (*c* 0.34, MeOH); R*f* 0.41 (CH_2Cl_2/MeOH/H_2O/AcOH 20 : 10 : 1 : 1); ^{1}H NMR (CD_3OD) δ 7.42–7.25 (m, 10H, Ar), 4.27 (d, 1H, J = 7.6 Hz, H-1a), 2.89 (dd, 1H, J = 11.2, 3.0 Hz, H-3b-eq), 2.01 (s, 3H, Ac), 1.61 (t, 1H, H-3b-ax), 1.42 (s, 9H, *t*Bu-CH_3). MALDI-TOF MS: Calcd for $C_{39}H_{53}N_5O_{17}$ (M): 863.3. Found *m*/*z*: 886.3 $(M + Na)^+$. Data for **19c**: $[\alpha]_D$ +63.2° (*c* 0.32, MeOH); Rf 0.36 (CH_2Cl_2/MeOH/H_2O/AcOH 20 : 10 : 1 : 1); ^{1}H NMR (CD_3OD) δ 7.39–7.25 (m, 10H, Ar), 4.89 (br s, 1H, H-1a), 2.41 (br d, 1H, J = 12.1 Hz, H-3b-eq), 2.03 (s, 3H, Ac), 1.63 (t, 1H, H-3b-ax), 1.44 (s, 9H, *t*Bu-CH3). MALDI-TOF MS: Calcd for $C_{39}H_{53}N_5O_{17}$ (M): 863.3. Found *m*/*z*: 886.3 $(M + Na)^+$. Data for **19d**: $[\alpha]_D$ +3.4° (*c* 0.44, MeOH); Rf 0.35 (CH_2Cl_2/MeOH/H_2O/AcOH 20 : 10 : 1 : 1); ^{1}H NMR (CD_3OD) δ 7.38–7.22 (m, 10H, Ar), 4.27 (d, 1H, J = 8.9 Hz, H-1a), 2.47 (br d, 1H, J = 12.0 Hz, H-3b-eq), 1.98 (s, 3H, Ac), 1.66 (t, 1H, H-3b-ax), 1.43 (s, 9H, *t*Bu-CH_3). MALDI-TOF MS: Calcd for $C_{39}H_{53}N_5O_{17}$ (M): 863.3. Found *m*/*z*: 886.5 $(M + Na)^+$.

Typical Procedure for Hydrogenolysis and *N*-Acetylation Reaction. AcOH (1 drop) and $Pd(OH)_2$ (15 mg) were added at rt to a solution of compound **19a** (17.9 mg, 0.021 mmol) in 4 : 1 MeOH-H_2O (5 mL). The suspension was stirred under an H_2 atmosphere for 12 h. The reaction mixture was filtered through a pad of celite and the filtrate was concentrated in vacuo. The residue was treated with pyridine (3 mL) and Ac_2O (1.5 mL) at rt for 12 h. The reaction mixture was concentrated in vacuo. The residue was then treated with 0.05 M NaOMe in MeOH (3 mL) at rt for 3 h, neutralized with Amberlite IR-120 (H^+), filtered, and concentrated. The residue was purified by silica gel column chromatography using CH_2Cl_2/MeOH/AcOH (40 : 1 : 0.5) as an eluent to provide **20a** (8.0 mg 55%): Data for **20a**: $[\alpha]_D$ +44.8° (*c* 0.30, H_2O); R*f* 0.45 (CH_2Cl_2/MeOH/H_2O/AcOH 20 : 10 : 1 : 1); ^{1}H NMR (D_2O) δ 4.88 (br s, 1H, H-1a), 2.77 (br d, 1H, J = 12.6 Hz, H-3b-eq), 2.04 (s, 3H, Ac), 2.01 (s, 3H, Ac), 1.66 (t, 1H, H-3b-ax), 1.44 (s, 9H, *t*Bu-CH_3). MALDI-TOF MS: Calcd for $C_{27}H_{45}N_3O_{18}$ (M): 699.3. Found *m*/*z*: 622.8 $(M + Na)^+$; HR-FAB MS: Calcd for $C_{27}H_{45}N_3O_{18}$ (M): 699.2698. Found *m*/*z*: 722.2593 $(M + Na)^+$. Data for **20b**: $[\alpha]_D$ +42.1° (*c* 0.21, H_2O); R*f* 0.44 (CH_2Cl_2/MeOH/H_2O/AcOH 20 : 10 : 1 : 1); ^{1}H NMR (D_2O) δ 4.25 (d, 1H, J = 8.0 Hz, H-1a), 2.75 (dd, 1H, J = 12.2, 3.0 Hz, H-3b-eq), 2.04 (s, 3H, Ac), 2.01 (s, 3H, Ac), 1.65 (t, 1H, H-3b-ax), 1.41 (s, 9H, *t*Bu-CH3). MALDI-TOF MS: Calcd for $C_{27}H_{45}N_3O_{18}$ (M): 699.3. Found *m*/*z*: 722.7 (M + Na)+. Data for **20c**: $[\alpha]_D$ +50.3° (*c* 0.28, H_2O); Rf 0.44 (CH_2Cl_2/MeOH/H_2O/AcOH 20 : 10 : 1 : 1); ^{1}H NMR (D_2O) δ 4.89 (br s, 1H, H-1a), 2.49 (br d, 1H, J = 12.1 Hz, H-3b-eq), 2.05 (s, 3H, Ac), 2.03 (s, 3H, Ac), 1.70 (t, 1H, H-3b-ax), 1.44 (s, 9 H, *t*BuCH3). MALDI-TOF MS: Calcd for $C_{27}H_{45}N_3O_{18}$ (M): 699.3. Found *m*/*z*: 722.7 $(M + Na)^+$. Data for **20d**: $[\alpha]_D$ +10.4° (*c* 0.10, H_2O); R*f* 0.40 (CH_2Cl_2/MeOH/H_2O/AcOH 20 : 10 : 1 : 1); ^{1}H NMR (D_2O) δ 4.27 (d, 1H, J = 8.5 Hz, H-1a), 2.51 (br d, 1H, J = 12.1 Hz, H-3b-eq), 2.04 (s, 3H, Ac), 2.00 (s, 3H, Ac), 1.66

(t, 1H, H-3b-ax), 1.42 (s, 9H, *t*Bu-CH_3). MALDI-TOF MS: Calcd for $C_{27}H_{45}N_3O_{18}$ (M): 699.3. Found m/z: 722.9 $(M + Na)^+$.

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