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CARBOHYDRATE RESEARCH

Note

Synthesis of oligosaccharides containing β-D-Gal-(1→3)-O-(6-O-sulfo-β-D-GlcNAc) as a terminal unit[1]

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Abstract

The chemical synthesis of β-D-Gal-(1→3)-6-O-SO_3Na-β-D-GlcNAc-(1→6)-α-D-Man-O-$C_6H_4NO_2$ (**1**) and β-D-Gal-(1→3)-6-O-SO_3Na-β-D-GlcNAc-(1→2)-α-D-Man-OMe (**2**) is reported using a key glycosyl donor, phenyl O-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)-4,6-di-*O*-chloroacetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**3**).

Carcinoembryonic antigen (CEA) has been shown to contain numerous *N*-linked oligosaccharides of both high mannose and complex type [2]. Yamashita [3] reported the β-D-Gal-(1→3)-6-O-SO_3-β-D-GlcNAc moiety to be present in the complex type *N*-linked oligosaccharide structure of CEA. This moiety is expressed in adult colonic endothelial cells. This finding prompted us to develop the chemical synthesis of β-D-Gal-(1→3)-6-O-SO_3Na-β-D-GlcNAc-(1→6)-α-D-Man-O-$C_6H_4NO_2$(*p*) (**1**) and β-D-Gal-(1→3)-6-O-SO_3Na-β-D-GlcNAc-(1→2)-α-D-Man-OMe (**2**), which will be used for examining the biosynthetic pathway of the β-D-Gal-(1→3)-6-O-SO_3-GlcNAc moiety in CEA. The assembly of complex carbohydrate structures bearing the 6-O-SO_3-GlcNAcβ1→2/6Manα determinant will be established by confirming the reactions shown in Reaction 1.

The *p*-nitrophenyl derivative **1** can also be employed in immunological studies after reducing the nitro group and subsequently linking to protein.

This communication reports the chemical synthesis of target molecules **1** and **2** through the employment of a key glycosyl donor; phenyl *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)-4,6-di-*O*-chloroacetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**3**). The building block **3** was prepared from known compound **6** [4] in two steps (Scheme 1). Cleavage of the benzylidene group in **6**

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[1] Synthetic studies in carbohydrates, Part 106. For Part 105 see ref. [1].

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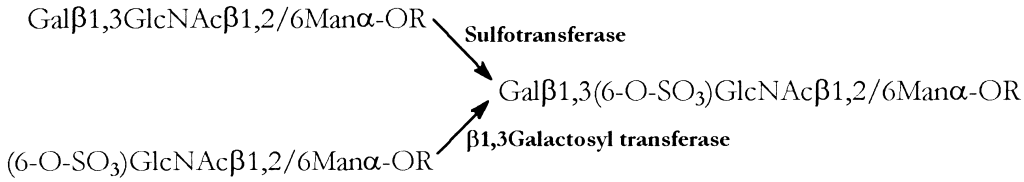
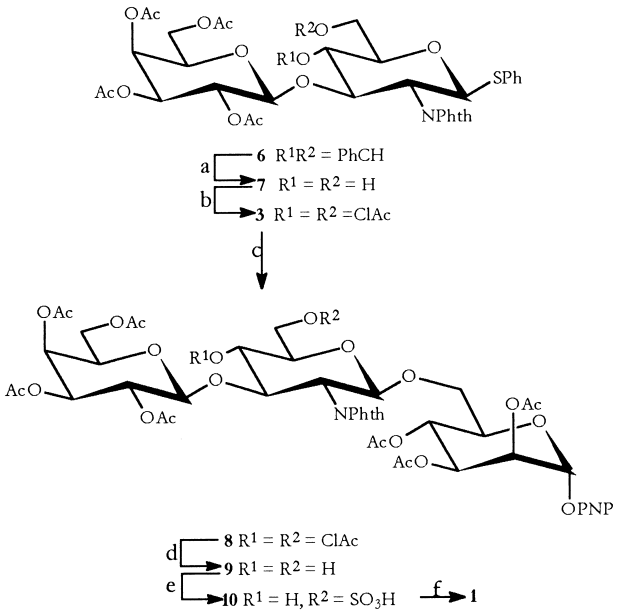


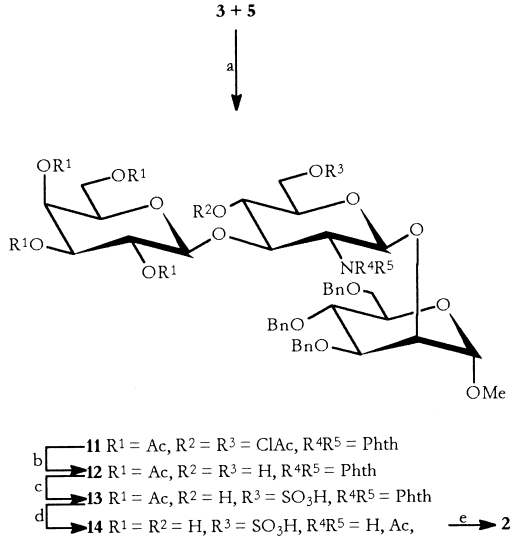
Reaction 1.

with 60% aq acetic acid followed by treatment with chloroacetic anhydride–$NaHCO_3$–DMF [5] afforded **3** in 63% yield, after silica gel chromatography. Glycosylation of 4-nitrophenyl 2,3,4-tri-*O*-acetyl-α-D-mannopyranoside (**4**) with **3** in CH_2Cl_2 promoted by *N*-iodosuccinimide-triflic acid [6] gave the β-linked trisaccharide **8** in 48% yield. Selective removal of chloroacetyl groups in **8** with thiourea and 2,6-lutidine in EtOH–CH_2Cl_2 (1:1, v/v) provided the diol **9** in 75% yield after chromatographic purification. Selective sulfation of **9** with SO_3–pyridine complex in DMF followed by removal of the phthalimido group and *N*-acetylation with MeOH–Ac_2O–triethyl amine produced the target molecule **1** in 33% yield. Similarly, reaction of methyl 3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (**5**) [7,8] with **3** in the presence of NIS-triflic acid provided the trisaccharide **11** (Scheme 2). Treatment of **11** in a manner analogous to that described in the synthesis of **1** from **8** afforded compound **2** in 39% yield after hydrogenolysis to remove *O*-benzyl groups.

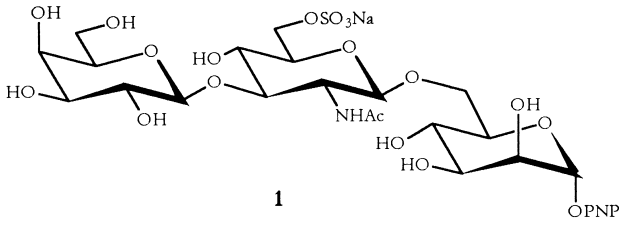
The products **1** and **2** were characterized unambiguously by NMR spectroscopy (see Tables 1 and 2), FAB mass spectroscopy and elemental analysis (see Experimental section). The ^1H and ^{13}C NMR assignments for **1** and **2** were confirmed through the application of the following two-dimensional NMR techniques: H,H-COSY (H-assignment), H,C-COSY (C-assignment) and long-range H,C-COSY (glycosidic linkage). The C-6 resonances of both Man (δ 68.1) and GlcNAc (δ 66.3) in compound **1** exhibited downfield shifts, confirming that they are substitution sites. Similarly, the C-2 (δ 75.7) of Man and C-6 of GlcNAc (δ 66.0) in compound **2** displayed downfield shifts, confirming these positions as sites of glycosylation and sulfation.



Scheme 1. Reagents and conditions: (a) 60% HOAc, 60 °C, 1 h; (b) $ClAc_2O$, $NaHCO_3$, DMF, 0 °C–room temperature, 3 h, 63%; (c) **4** (1.0 equiv), NIS (3.0 equiv)-triflic acid, MS-4 Å, CH_2Cl_2, −20 °C, 0.5 h, 37%; (d) thiourea, 2,6-lutidine, EtOH–CH_2Cl_2 (1:1), 80 °C, 12 h, 75%; (e) SO_3–pyridine, DMF, 0 °C, 4 h; (f) 85% hydrazine hydrate, EtOH, 80 °C, 3 h; MeOH–TEA–Ac_2O (4:2:1), 0 °C–room temperature, 2 h; Na^+ resin, 33% from **9**.



Scheme 2. Reagents and conditions: (a) NIS (3.0 equiv)-triflic acid, MS-4 Å, CH_2Cl_2, 0 °C, 2 h; (b) thiourea, 2,6-lutidine, EtOH–CH_2Cl_2 (1:1), 80 °C, 12 h, 14% for a and b; (c) SO_3–Pyridine complex, DMF, 0 °C, 3.5 h; (d) 85% hydrazine hydrate, EtOH, 80 °C, 2 h; MeOH–TEA–Ac_2O (4:2:1), 0 °C–room temperature, 2 h; (e) 10% Pd/C, MeOH–H_2O (9:1), H_2, room temperature, 12 h; Na^+ resin, 39% from **12**.



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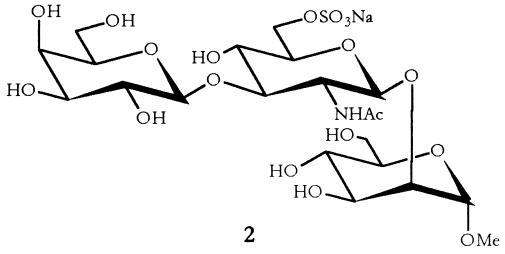

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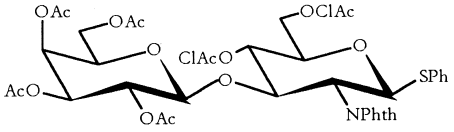

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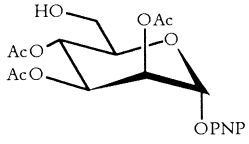

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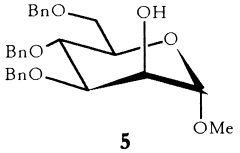

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1. Experimental

General methods.—Optical rotations were measured at 25 °C with a Perkin–Elmer 241 Polarimeter. TLC was conducted on glass plates, precoated with a 0.25 mm layer of Silica Gel 60F-254 (Analtech GHLF uniplates). The compounds were visualized by exposure to UV light and/or by spraying with 5% H_2SO_4 in EtOH and charring. Baker Analyzed (60–200 mesh) silica gel was used for column chromatography. The solvents used for chromatography are given below: Solvent A, hexanes–EtOAc = 1:1; Solvent B, CH_2Cl_2–acetone = 9:1; Solvent C, CH_2Cl_2–acetone = 4:1; Solvent D, CH_2Cl_2–MeOH = 4:1; Solvent E, CH_2Cl_2–MeOH–H_2O = 13:6:1; Solvent F, CH_2Cl_2–MeOH–H_2O = 5:4:1. NMR spectra were recorded at 30 °C. ^1H NMR and ^{13}C NMR spectra were obtained with a Bruker AM-400 (400 MHz) and Bruker AMX-600 (600 MHz) NMR spectrometers. Solutions in organic solvents were generally dried over anhydrous Na_2SO_4. Dichloromethane and DMF were kept dry over molecular sieves (4 Å). Elemental analyses were performed by Robertson Laboratories, Madison, NJ.

Phenyl O-*(2,3,4,6-tetra*-O-*acetyl*-β-D-*galacto-pyranosyl)-(1→3)-4,6-di*-O-*chloroacetyl-2-deoxy-2-phthalimido-1-thio*-β-D-*glucopyranoside* (**3**).—Compound **6** (410 mg, 0.5 mmol) in 60% aq acetic acid

Table 1
^1H and ^{13}C chemical shift assignments (ppm)[a] of **1** and **2**

	Gal		GlcNAc		Man	
Assignment	H	C	H	C	H	C
Compound **1**						
1	4.42	102.5	4.58	100.3	5.76	96.8
2	3.56	69.7	3.75	53.4	4.22	68.7
3	3.66	71.6	3.76	81.3	4.09	69.4
4	3.94	67.6	3.43	67.7	3.72	65.9
5	3.72	74.3	3.66	72.2	3.89	71.8
6	3.77,3.81	60.0	4.07,4.32	66.3	3.84,4.08	68.1
$NHCOCH_3$			2.03	21.3		
Compound **2**						
1	4.49	102.4	4.67	98.4	4.80	97.0
2	3.56	69.7	3.90	53.4	4.11	75.7
3	3.67	71.5	3.86	80.5	3.81	68.6
4	3.95	67.6	3.66	67.3	3.53	66.3
5	3.73	74.3	3.73	72.4	3.61	71.7
6	3.77,3.82	60.0	4.28,4.40	66.0	3.66,3.94	60.6
$NHCOCH_3$			2.08	21.4		
OMe					3.45	53.8

[a] Obtained from spectra of aqueous (D_2O) solutions of **1** or **2**. Expressed relative to TSP for ^1H, and relative to acetone (at 29.2 ppm) for ^{13}C.

Table 2
^1H–^1H coupling constants (Hz)[a] in **1** and **2**

	Gal	GlcNAc	Man
Compound **1**			
$^3J_{1,2}$	7.7	8.3	1.9
$^3J_{2,3}$	9.9	[b]	3.5
$^3J_{3,4}$	3.5	8.6	9.7
$^3J_{4,5}$	1.0	10.0	9.8
$^3J_{5,6}$	4.0	6.0	6.2
$^3J_{5,6'}$	8.2	2.2	1.8
$^2J_{6,6'}$	−11.8	−11.2	−11.7
Compound **2**			
$^3J_{1,2}$	7.8	8.3	1.8
$^3J_{2,3}$	10.0	10.4	3.5
$^4J_{2,4}$	−0.4	[c]	[c]
$^3J_{3,4}$	3.4	8.3	9.6
$^3J_{4,5}$	1.1	10.0	9.7
$^3J_{5,6}$	3.9	5.3	7.3
$^3J_{5,6'}$	8.1	2.2	2.1
$^3J_{6,6'}$	−11.8	−11.3	−11.8

[a] Obtained by first-order analysis of spectra acquired on aqueous (D_2O) solutions of **1** or **2**. Probable error is ±−0.1 Hz. Couplings through three bonds are assumed to be positive, and those through two or four bonds are assumed to be negative.
[b] Complex patterns exhibited by H-2 and H-3 of GlcNAc were not amenable to first-order analysis.
[c] Not observed.

(20 mL) was stirred at 60 °C for 1 h. The solvents were evaporated and coevaporated with toluene. The resulting residue (**7**) was treated with chloroacetyl anhydride (427 mg, 2.50 mmol) in DMF (10 mL) containing sodium bicarbonate (504 mg, 6 mmol) at 0 °C, then kept at room temperature for 3 h. The mixture was poured into ice water and the white solid product was collected by filtration. Chromatographic purification using Solvent A as eluent gave **3** (280 mg, 63%). R_f 0.26 (Solvent A), $[\alpha]_D = +11°$ (*c* 0.54, $CHCl_3$), ^1H NMR (CD_2Cl_2): δ 7.91–7.23 (m, 9H, arom.), 5.50 (d, 1H, $J = 10.6$ Hz, H-1), 4.16 (m, 4H, 2×CH_2Cl), 2.14–1.84 (4 s, 12H, 4×OAc). Anal. Calcd for $C_{38}H_{39}Cl_2NO_{17}S$: C, 51.59; H, 4.44; N, 1.58; Found: C, 51.30; H, 4.23; N, 1.55.

4-Nitrophenyl O-*(2,3,4,6-tetra*-O-*acetyl*-β-D-*galactopyranosyl)-(1→3)-4,6-di*-O-*chloroacetyl-2-deoxy-2-phthalimido*-β-D-*glucopyranosyl)-(1→6)-2,3,4-tri*-O-*acetyl*-α-D-*mannopyranoside* (**8**).—A solution of compound **3** (700 mg, 1.1 mmol) and **4** (320 mg, 1.0 mmol) in CH_2Cl_2 (20 mL) was stirred with 4 Å molecular sieves (5.0 g) at −20 °C for 30 min, after which NIS (680 mg, 3.0 mmol) was added to the mixture. A solution of trifluoromethanesulfonic acid (0.1 mL) in 20 mL of CH_2Cl_2 was then added dropwise. After stirring for 30 min at the same temperature, satd $NaHCO_3$ was added and the mixture was filtered through a bed of Celite. The organic layer was successively washed with H_2O, satd $NaHCO_3$ and 10% $Na_2S_2O_3$, dried, and concentrated. The residue was subjected to column chromatography to provide **8** (440 mg, 37%). R_f 0.35 (Solvent B), $[\alpha]_D = +32°$ (*c* 0.40, $CHCl_3$), ^1H NMR (CD_2Cl_2): δ 8.21–7.10 (m, 8H, arom.), 5.44 (d, 1H, $J = 1.82$ Hz, H-1), 4.16 (2 s, 4H, 2×CH_2Cl), 2.20–1.84 (cluster of s, 21H, 7×OAc). Anal. Calcd for $C_{50}H_{54}Cl_2N_2O_{28}$: C, 49.97; H, 4.53; N, 2.33; Found: C, 49.74; H, 4.34; N, 2.16.

4-Nitrophenyl O-*(2,3,4,6-tetra*-O-*acetyl*-β-D-*galactopyranosyl)-(1→3)*-O-*(2-deoxy-2-phthalimido*-β-D-*glucopyranosyl)-(1→6)-2,3,4-tri*-O-*acetyl*-α-D-*manno-pyranoside* (**9**).—A mixture of compound **8** (330 mg, 0.27 mmol), thiourea (206 mg, 2.7 mmol) and 2,6-lutidine (0.16 mL, 1.38 mmol) in EtOH–CH_2Cl_2 (40 mL, 1:1) was stirred at 80 °C overnight. The solvents were evaporated and the residue was taken up in CH_2Cl_2 (20 mL) and washed with H_2O, dried, and concentrated. The residue was subjected to column chromatography to provide **9** (216 mg, 75%). R_f 0.18 (Solvent D), $[\alpha]_D = +46°$ (*c* 1.40, $CHCl_3$), ^1H NMR (CD_2Cl_2): δ 8.20–7.11 (m, 8H, arom.), 5.48 (d, 1H, $J = 1.74$ Hz, H-1), 2.21–1.40 (cluster of s, 21H, 7×OAc). Anal. Calcd for $C_{46}H_{52}N_2O_{26}$: C, 52.67; H, 5.00; N, 2.67; Found: C, 52.90; H, 4.92; N, 2.59.

4-Nitrophenyl O-*(*β-D-*galactopyranosyl)-(1→3)*-O-*(2-acetamido-2-deoxy-6*-O-*sulfo*-β-D-*glucopyranosyl sodium salt)-(1→6)*-α-D-*mannopyranoside* (**1**).—To a stirred solution of compound **9** (220 mg, 0.20 mmol) in dry DMF (10 mL) at 0 °C was added SO_3–pyridine complex (38 mg, 0.24 mmol). After 2 h, more reagent (38 mg) was added to the mixture and stirring was continued for an additional 2 h at 0 °C. Methanol and pyridine were added to consume excess reagent. The solvents were evaporated and the residue was subjected to column chromatography to provide **10**. Compound **10** was then treated with 85% hydrazine hydrate (4 mL) in ethanol (20 mL) at 80 °C for 3 h. The solvent was evaporated and coevaporated with toluene. To a solution of this compound in methanol (20 mL) containing triethylamine (10 mL) was added acetic anhydride (5 mL) at 0 °C. After the mixture was stirred at room temperature for 2 h, the solvent was evaporated and the product was purified by column chromatography with Solvent F as the eluent. The fractions corresponding to the product were combined and concentrated, and the residue so

obtained was dissolved in a small amount of water and passed through Amberlite IR-120P (Na^+) cation-exchange resin. Lyophilization of the fractions corresponding to **1** gave a hygroscopic amorphous solid (50 mg, 33%). R_f 0.26 (CH_2Cl_2–MeOH–H_2O = 5:4:1), $[\alpha]_D = +45°$ (*c* 0.73, H_2O), for ^1H and ^{13}C NMR data, see Table 1; MS *m/z*, 745.1 $(M-Na)^-$; Anal. Calcd for $C_{26}H_{37}N_2NaO_{21}S$: C, 40.63; H, 4.85; N, 3.64; Found: C, 40.52; H, 5.09; N, 3.42.

Methyl O-*(2,3,4,6-tetra*-O-*acetyl*-β-D-*galacto-pyranosyl)-(1→3)*-O-*(2-deoxy-2-phthalimido*-β-D-*glucopyranosyl)-(1→2)-3,4,6-tri*-O-*benzyl*-α-D-*mannopyranoside* (**12**).—A solution of compound **3** (2.30 g, 2.60 mmol) and **5** (1.09 g, 2.35 mmol) in CH_2Cl_2 (100 mL) was stirred with 4 Å molecular sieves (12 g) at 0 °C for 30 min, after which time NIS (1.60 g) was added to the mixture. A solution of trifluoromethanesulfonic acid (0.2 mL) in 20 mL of CH_2Cl_2 was then added dropwise and the mixture was stirred at 0 °C for an additional 1 h. Satd $NaHCO_3$ was added and the mixture was filtered through a bed of Celite. The organic layer was successively washed with H_2O, satd $NaHCO_3$ and 10% $Na_2S_2O_3$, dried, and concentrated. The residue (**11**) was treated with thiourea (2.8 g) and 2,6-lutidine (2.0 mL) in EtOH–CH_2Cl_2 (50 mL, 1:1) at 80 °C overnight. The solvents were evaporated and the residue was subjected to column chromatography to provide **12** (350 mg, 14% from **5**). R_f 0.43 (Solvent C), $[\alpha]_D = +15°$ (*c* 0.57, $CHCl_3$), ^1H NMR (CD_2Cl_2): δ 7.67–7.16 (m, 19H, arom.), 3.13 (s, 3H, OCH_3), 2.12–1.55 (cluster of s, 12H, 4×OAc). Anal. Calcd for $C_{56}H_{63}NO_{21}$: C, 61.93; H, 5.85; N, 1.29; Found: C, 62.30; H, 6.02; N, 1.22.

Methyl O-*(*β-D-*galactopyranosyl)-(1→3)*-O-*(2-acetamido-2-deoxy-6*-O-*sulfo*-β-D-*glucopyranosyl sodium salt)-(1→2)*-α-D-*mannopyranoside* (**2**).—To a stirred solution of compound **12** (300 mg, 0.27 mmol) in dry DMF (10 mL) at 0 °C was added SO_3–pyridine complex (50 mg, 0.32 mmol). After 2 h, more reagent (50 mg) was added to the mixture and stirring continued for an additional 16 h at 0 °C. Methanol and pyridine were added to consume excess reagent. The solvents were evaporated and the residue was subjected to column chromatography to provide **13**. The compound **13** was then treated with 85% hydrazine hydrate (8 mL) in ethanol (40 mL) at 80 °C for 2 h. The solvents were evaporated and coevaporated with toluene. To a solution of this compound in methanol (30 mL) containing triethylamine (15 mL) was added acetic anhydride (7.5 mL) at 0 °C. After the mixture was stirred at room temperature for 2 h, the solvents were evaporated and the product was purified by column chromatography with Solvent E as the eluent. The fractions corresponding to the product (**14**) were combined and concentrated, and the residue (~100 mg) so obtained was hydrogenolyzed in MeOH–H_2O (20 mL, 9:1) in the presence of 10% Pd/C (300 mg) overnight. The suspension was filtered through a bed of Celite. The solids were thoroughly washed with MeOH–H_2O. The filtrate and washings were combined and evaporated under reduced pressure. The residue was purified by column chromatography using Solvent F as the eluent. The fractions corresponding to the product were combined and concentrated, and passed through Amberlite IR-120P (Na^+) cation-exchange resin. Lyophilization of the fractions corresponding to **2** gave a hygroscopic amorphous solid (70 mg, 39% from **12**). R_f 0.20 (Solvent F), $[\alpha]_D = +8°$ (*c* 0.55, H_2O), for ^1H and ^{13}C NMR data see Table 1; MS *m/z* 638.2 $(M-Na)^-$.

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