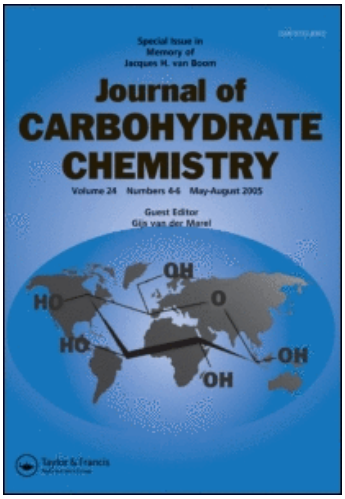


## Journal of Carbohydrate Chemistry

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

# Syntheses of Heterobifunctional Candidate Ligands of P-Selectin Containing Both Sulfated Lewis X Trisaccharide and Various Sulfated Peptides

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Online publication date: 12 November 2003

**To cite this Article** Dagron, Franck and Lubineau, André(2003) 'Syntheses of Heterobifunctional Candidate Ligands of P-Selectin Containing Both Sulfated Lewis X Trisaccharide and Various Sulfated Peptides ', Journal of Carbohydrate Chemistry, 22: 7, 481 — 500
**To link to this Article: DOI:** 10.1081/CAR-120026453
**URL:** http://dx.doi.org/10.1081/CAR-120026453

JOURNAL OF CARBOHYDRATE CHEMISTRY
Vol. 22, Nos. 7 & 8, pp. 481–500, 2003

# Syntheses of Heterobifunctional Candidate Ligands of P-Selectin Containing Both Sulfated Lewis X Trisaccharide and Various Sulfated Peptides[†]

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## ABSTRACT

Various heterobifunctional glycopeptides containing both 3′-sulfated Lewis x and peptides containing at least one sulfated tyrosine were constructed onto a pentaerythritol backbone following a common strategy that relies on the condensation, through reductive amination, of aldehydes **9** or **17** with various peptides having a free terminal amino group. The corresponding homodimer of 3′-sulfated Lewis x linked to pentaerythritol was prepared for comparison of the inhibitory activities.

*Key Words:* Selectins; Selectin ligands; Sulfated Lewis x; Sulfated tyrosine; Heterobifunctional ligand.

[†]This paper is dedicated to Professor Gérard Descotes on the occasion of his 70th birthday.

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DOI: 10.1081/CAR-120026453
0732-8303 (Print); 1532-2327 (Online)


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## INTRODUCTION

The selectins (E, P and L selectins) are mammalian C-type lectins which mediate cellular adhesion between blood cells or cancer cells and vascular endothelium. They participate in a variety of normal and pathological phenomena.[1,2] In particular, P-selectin which is stored in platelet α-granules and in Weibel–Palade bodies of endothelial cells is redistributed, within minutes, in the plasma membranes upon activation of the vascular endothelium. Once there, it mediates the initial tethering and rolling of leucocytes at inflammation sites.[3] The major counter-receptor of P-selectin, PSGL-1 (P-Selectin Glycoprotein Ligand-1), is a dimeric mucin present on leucocytes. It interacts with P-selectin with high affinity through its *N*-terminal portion which contains a cluster of three sulfated tyrosines (at positions 5, 7 and 10) and a sialyl Lewis x (sLex) attached to threonine 16. Indeed, a synthetic glycosulfopeptide corresponding to the *N*-terminal part of PSGL-1 was found to bind as efficiently as the dimeric mucin structure.[4] However, whereas sLex was found essential for tight binding, sulfations of tyrosine could be modulated. Indeed, only one of the three tyrosines needs to be sulfated, even if the corresponding polysulfated peptides gave better interactions.[5] Recently, this was confirmed by analysis of the crystal structure of a construct containing the lectin and EGF domains in complex with the *N*-terminal domain of human PSGL-1.[6] Several glycopeptides[7,8] have already been synthesized starting from a glyco-aminoacid derived from sLex, and extending most of the time the peptide chain through solid phase peptide synthesis.[9–12] In order to get more insights into the interaction and to generate more diversity, we decided to develop a strategy compatible with parallel syntheses, in which the oligosaccharide moiety could be linked, through reductive amination, to any peptide having a free terminal amino group. Moreover, the fact that a sulfate, 3-linked to the terminal galactose, can substitute sialic acid advantageously in several selectin ligands as demonstrated for P-selectin,[13,14] prompted us to use 3′-sulfated Lewis x as the sugar part. We used the pentaerythritol backbone[15] as a linker between the oligosaccharide and the peptide chains to get a chance to add, if needed, a third bioactive part. Finally, to distinguish between the relative contribution of the two components parts, we synthesized the homobifunctional dimer of 3′-sulfated Lewis x on the same pentaerythritol backbone.

## RESULTS AND DISCUSSION

We first needed a pentaerythritol derivative in which the hydroxyl groups were differentiated. Starting from the known *p*-methoxybenzylidene derivative **1**,[16] benzylation under standard conditions followed by reductive opening of the *p*-methoxybenzylidene ($NaBH_3CN$, TFA, $CH_2Cl_2$) gave **3** in 88% yield for the two steps. Then, condensation of the known trichloroacetimidate **5**[17] in the presence of TMSOTf gave **6** in 86% yield. The *N*-phthalimido protecting group was then replaced with *N*-acetamido by treating **6** with hydrazine acetate and subsequent peracetylation to give **7** in 89% overall yield. Removal of the *p*-methoxybenzyl group with cerium ammonium nitrate gave **8** (84%). Next, Swern oxidation gave the aldehyde **9** (96%) ready for coupling with the dipeptide H–Gly–Tyr(OH)–OBn (**30**). Compond **30** was prepared from commercial Boc–Gly–OH (**27**) and Tyr(OH)–OBn (**28**) according to a





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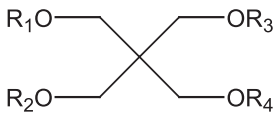
routine procedure in peptide chemistry in the presence of EDC and HOBt.[18] Thus, coupling reaction between **27** and **28**, gave the dipeptide **29** in 73% yield in which the Boc protecting group was removed with trifluoroacetic acid to give **30** in 78% yield. The reductive amination was performed as usual with $NaBH_3CN$ in a THF–phosphate buffer (pH 5) mixture to give **10**. Subsequent sulfation of crude **10** with $SO_3$–$NMe_3$ in DMF gave the *N*,*O*-disulfate **11** in 64% yield for the two steps. The benzyl ester was then selectively cleaved by catalytic hydrogenation ($H_2$, Pd/C) in the presence of ammonium acetate.[19] The resulting **12** was directly deacetylated with NaOMe in MeOH to give **13** in 80% overall yield from **11**. The 3′-sulfate was then introduced through the stannylene methodology[20] to give **14** in 49% yield (95% from consumed **13**). The reaction was not pushed to completion to avoid concomitant sulfation at C-6. Finally, complete debenzylation under standard conditions afforded compound **15** in 95% yield bearing an additionnal *N*-sulfate. Although the later is a priori undesirable, it deserves nevertheless to be tested as an inhibitor of P-selectin PSGL-1 interaction and the selective *N*-desulfation was not attempted. We preferred to focus on an alternative route in which the sulfate groups on both galactose and tyrosine were introduced before the reductive amination. We have therefore prepared the aldehyde **17** already sulfated at O–3′, as a key intermediate to be condensed with sulfated peptides **33** and **41**. The aldehyde **17** was prepared from the aldehyde **9** after deacetylation (NaOMe in MeOH) into **16** (94%) and sulfation using the stannylene methodology (43%, 81% from consumed **16**). Sulfated dipeptide **33** was first prepared from **30** according to standard procedures. The Fmoc protecting group was introduced with *N*-(9-fluorenylmethoxycarbonyloxy)-succinimide to give **31** in 80% yield. Then, sulfation

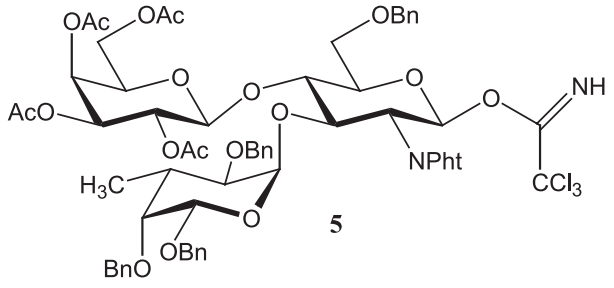



$R^1 = R^2 = H$, $R^3$, $R^4$ = CHPh-*p*-OMe **1**

$R^1 = R^2 = Bn$, $R^3$, $R^4$ = CHPh-*p*-OMe **2**

$R^1 = R^2 = Bn$, $R^3 = H$, $R^4$ = *p*-MBn **3**

$R^1 = R^2 = Bn$, $R^3 = R^4 = H$ **4**




**5**

***Scheme 1.***





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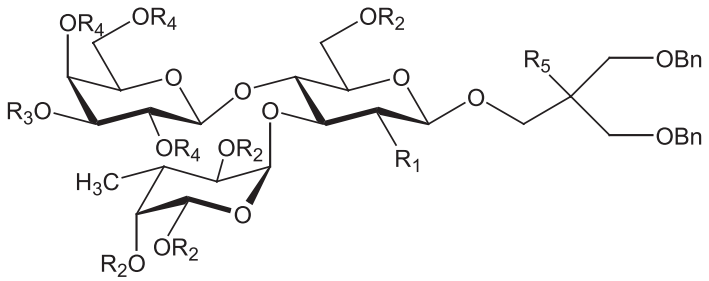
of tyrosine with $SO_3$–DMF complex in DMF afforded **32** (91%) which was hydrogenolysed quantitatively to give the sulfated peptide **33**. Second, we prepared the oligopeptide **41** containing a cluster of three contiguous sulfated tyrosines linked to 6-aminohexanoic acid as a spacer. Boc–[Tyr(OH)]$_3$–OBn (**36**) was prepared step by step in 64% overall yield, from commercial Boc–Tyr(OH)–OH (**34**) and H–Tyr(OH)–OBn (**28**) as described for peptide **30**. The Boc protecting group in **36** was removed with trifluoroacetic acid to give **37** in 82% yield. Then, conventional coupling with Fmoc–NH($CH_2$)$_5$–COOH **38**[21] gave **39** (82%). Sulfation of the three tyrosines with $SO_3$–DMF complex gave the intermediate derivative **40** from which the Fmoc protecting group was removed with morpholine in DMF to afford **41** in 77%




$R^1$ = NPhth, $R^2$ = Bn, $R^3$ = $R^4$ = Ac, $R^5$ = $CH_2O$-*p*-MBn **6**

$R^1$ = NHAc, $R^2$ = Bn, $R^3$ = $R^4$ = Ac, $R^5$ = $CH_2O$-*p*-MBn **7**

$R^1$ = NHAc, $R^2$ = Bn, $R^3$ = $R^4$ = Ac, $R^5$ = $CH_2OH$ **8**

$R^1$ = NHAc, $R^2$ = Bn, $R^3$ = $R^4$ = Ac, $R^5$ = CHO **9**

$R^1$ = NHAc, $R^2$ = Bn, $R^3$ = $R^4$ = Ac, $R^5$ = $CH_2NHCH_2CO$[Tyr(OH)]-OBn **10**

$R^1$ = NHAc, $R^2$ = Bn, $R^3$ = $R^4$ = Ac, $R^5$ = $CH_2N(SO_3Na)CH_2CO$[Tyr($OSO_3Na$)]-OBn **11**

$R^1$ = NHAc, $R^2$ = Bn, $R^3$ = $R^4$ = Ac, $R^5$ = $CH_2N(SO_3Na)CH_2CO$[Tyr($OSO_3Na$)]-OH **12**

$R^1$ = NHAc, $R^2$ = Bn, $R^3$ = $R^4$ = H, $R^5$ = $CH_2N(SO_3Na)CH_2CO$[Tyr($OSO_3Na$)]-OH **13**

$R^1$ = NHAc, $R^2$ = Bn, $R^3$ = $SO_3Na$, $R^4$ = H, $R^5$ = $CH_2N(SO_3Na)CH_2CO$[Tyr($OSO_3Na$)]-OH **14**

$R^1$ = NHAc, $R^2$ = H, $R^3$ = $SO_3Na$, $R^4$ = H, $R^5$ = $CH_2N(SO_3Na)CH_2CO$[Tyr($OSO_3Na$)]-OH **15**

$R^1$ = NHAc, $R^2$ = Bn, $R^3$ = $R^4$ = H, $R^5$ = CHO **16**

$R^1$ = NHAc, $R^2$ = Bn, $R^3$ = $SO_3Na$, $R^4$ = H, $R^5$ = CHO **17**

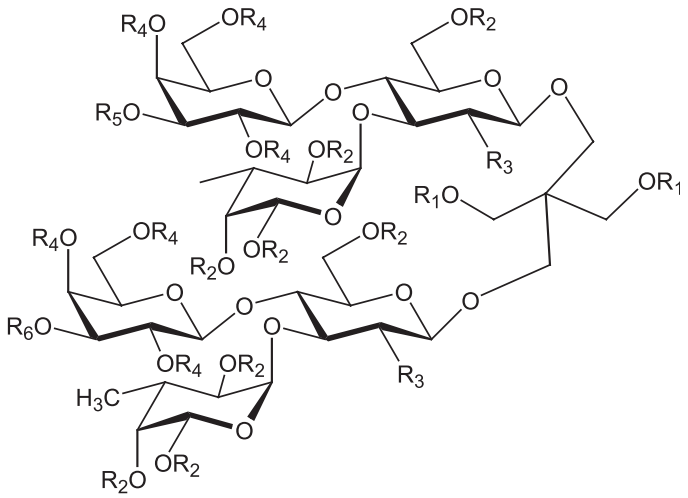
$R^1$ = NHAc, $R^2$ = Bn, $R^3$ = $SO_3Na$, $R^4$ = H, $R^5$ = $CH_2NHCH_2CO$[Tyr($OSO_3Na$)]-OH **18**

$R^1$ = NHAc, $R^2$ = H, $R^3$ = $SO_3Na$, $R^4$ = H, $R^5$ = $CH_2NHCH_2CO$[Tyr($OSO_3Na$)]-OH **19**

$R^1$ = NHAc, $R^2$ = Bn, $R^3$ = $SO_3Na$, $R^4$ = H, $R^5$ = $CH_2NH(CH_2)_5CO$[Tyr($OSO_3Na$)]$_3$-OBn **20**

$R^1$ = NHAc, $R^2$ = H, $R^3$ = $SO_3Na$, $R^4$ = H, $R^5$ = $CH_2NH(CH_2)_5CO$[Tyr($OSO_3Na$)]$_3$-OH **21**

***Scheme 2.***



$R^1 = R^2 = Bn$, $R^3 = NPhth$, $R^4 = R^5 = R^6 = Ac$ **22**

$R^1 = R^2 = Bn$, $R^3 = NHAc$, $R^4 = R^5 = R^6 = H$ **23**

$R^1 = R^2 = Bn$, $R^3 = NHAc$, $R^4 = H$, $R^5 = R^6 = SO_3Na$ **24**

$R^1 = R^2 = Bn$, $R^3 = NHAc$, $R^4 = R^5 = H$, $R^6 = SO_3Na$ **25**

$R^1 = R^2 = R^4 = H$, $R^3 = NHAc$, $R^5 = R^6 = SO_3Na$ **26**

***Scheme 3.***

yield. The coupling of both sulfated peptides **33** and **41** with aldehyde **17** through reductive amination, gave **18** (79%) and **20** (77%). Finally, hydrogenolysis led to the heterobifunctional derivatives **19** and **21** both in 95% yield (Schemes 1–4).

The homobifunctional derivative **26** was prepared through a straightforward sequence. Thus, trichloroacetimidate **5** was condensed with the known diol **4**[22] in the presence of TMSOTf to give **22** in 80% yield. Subsequent treatment with hydrazine acetate, followed by selective *N*-acetylation gave **23** (66%) which was further sulfated at O–3′, using the above described methodology, to give the disulfate **24** (40%) along with the monosulfate **25** (50%). Additional **24** could be obtained by treating **25** with 1 equivalent of dibutyltin oxide and 1 equivalent of $SO_3$–DMF complex to give finally **24** in a total yield of 70%. Lastly, hydrogenolysis of the benzyl group in **24** afforded **26** in 94% yield.

Compounds **15**, **19**, **21** and **26** are currently being tested as P-selectin ligands.

## EXPERIMENTAL

**General procedures.** All moisture-sensitive reactions were performed under argon using oven-dried glassware. If necessary, solvents were dried and distilled prior to

| | | | |
|---|---|---|---|
| Boc-Gly-OH | **27** | Boc-Tyr(OH)-OH | **34** |
| Tyr(OH)-OBn | **28** | Boc-$[Tyr(OH)]_2$ -OBn | **35** |
| Boc-Gly-Tyr(OH)-OBn | **29** | Boc-$[Tyr(OH)]_3$ -OBn | **36** |
| H-Gly-Tyr(OH)-OBn | **30** | H-$[Tyr(OH)]_3$ -OBn | **37** |
| Fmoc-Gly-Tyr(OH)-OBn | **31** | Fmoc-$NH(CH_2)_5$ -COOH | **38** |
| Fmoc-Gly-Tyr($OSO_3Na$)-OBn | **32** | Fmoc-$NH(CH_2)_5$ -CO-$[Tyr(OH)]_3$ -OBn | **39** |
| H-Gly-Tyr($OSO_3Na$)-OH | **33** | Fmoc-$NH(CH_2)_5$ -CO-$[Tyr(OSO_3Na)]_3$ -OBn | **40** |
| | | $H_2N(CH_2)CO$-$[Tyr(OSO_3Na)]_3$ -OBn | **41** |

***Scheme 4.***



use. NMR spectra were recorded with Bruker AM250, AC200, AC250 or DRX 400 spectrometers. In $CDCl_3$, $^1H$ and $^{13}C$ chemical shifts (δ) are reported relative to tetramethylsilane (δ 0.00 ppm) and internal chloroform (δ 77.0 ppm), respectively. In $D_2O$, acetone (δ 2.22 and 30.5 ppm, respectively) was used as internal reference. Signal multiplicity is indicated as follow: s for singlet, bs for broad singlet, d for doublet, t for triplet, q for quadruplet, m for multiplet. In the listing of the NMR data, I, II, III refers respectively to *N*-acetylglucosamine, fucose, and galactose. IR spectra were recorded on a FT-IR Bruker IFS-66 spectrometer. Optical rotations were determined using a Jasco DIP-370 digital polarimeter. Mass spectra were recorded on a Finnigan MAT 95 S spectrometer using electrospray ionization. Flash chromatography was performed using 6–35 μ silica gel (60) purchased from S.D.S. Company. TLC was run using Merck 60 $F_{254}$ plates, and visualized first with UV light and second by heating after alcoholic sulfuric acid treatment. Melting points were measured with a Reichert apparatus and are uncorrected. Elemental analyses were performed at the Service Central de Microanalyses, CNRS, Gif sur Yvette, France.

**2,2-Bis-benzyloxymethyl-1,3-*p*-methoxybenzylidenepropan-1,3-diol (2).** NaH (60% in oil, 2.05 g, 51 mmol) was added to a cooled (0°C) solution of compound **1** (5.0 g, 19.68 mmol) in THF (36 mL). After 5 min, BnBr (5.6 mL, 43 mmol) was added dropwise. The mixture was then stirred at rt until completion of the reaction. Methanol was then carefully added, and the mixture was concentrated. Flash chromatography (Petroleum ether/EtOAc, 9/1) of the residue gave **2** (8.26 g, 98%) as colorless oil. $^1H$ NMR (250 MHz, $CDCl_3$): δ 7.20–7.40 (m, 12 H), 6.85 (d, 2 H, J = 8.8 Hz), 5.35 (s, 1 H), 4.56 (s, 2 H), 4.40 (s, 2 H), 4.14 (d, 2 H, J = 12.0 Hz), 3.87 (d, 2 H), 3.84 (s, 2 H), 3.75 (s, 3 H), 3.31 (s, 2 H). $^{13}C$ NMR (62.9 MHz, $CDCl_3$): δ 159.9, 138.2, 130.8, 128.3, 128.2, 127.5, 127.4, 127.33, 113.5, 101.6, 73.3, 73.2, 70.2, 70.0, 68.7, 55.2, 38.8. IR($cm^{-1}$) ν 3030, 2972, 2876, 2844, 2787, 1497, 1477, 1455, 1398, 1361, 1199, 1178, 1112, 1098, 1074, 1019, 981, 968, 956, 914, 759, 739, 697.



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Anal. Calcd for $C_{27}H_{30}O_5$ (434.54): C, 74.63; H, 6.96; O, 18.41. Found: C, 74.81; H, 7.04; O, 18.24.

**2,2-Bis-benzyloxymethyl-3-*p*-methoxybenzyloxymethylpropan-1-ol (3).** Trifluoroacetic acid (1.5 mL, 19.7 mmol) was added to a cooled (0°C) solution of compound **2** (1 g, 2.3 mmol) and $NaBH_3CN$ (2.2 g, 3.5 mmol) in THF (14 mL). The mixture was stirred for 20 min, then diluted with $CH_2Cl_2$, neutralized with $NEt_3$ and finally washed with satd aq $NaHCO_3$ and water. The aq layers were extracted with $CH_2Cl_2$. The organic phases were pooled, filtered off silicone treated paper and concentrated. Flash chromatography of the residue (EtOAc/petroleum ether, 2/8) gave **3** (0.9 g, 90%) as a colorless oil. $^1$H NMR (250 MHz, $CDCl_3$): δ 7.23–7.38 (m, 10 H), 7.19 (d, 2 H, J = 8.8 Hz), 6.83 (d, 2 H), 4.47 (s, 4 H), 4.40 (s, 2 H), 3.77 (s, 3 H), 3.76 (d, 2 H, J = 6 Hz), 3.55 (s, 4 H), 3.53 (s, 2 H), 2.89 (t, 1 H, $CH_2OH$). $^{13}$C NMR (62.9 MHz, $CDCl_3$): δ 159.1, 138.3, 130.3, 129.0, 128.3, 127.5, 127.3, 113.7, 73.4, 73.1, 70.8, 70.5, 66.1, 55.2, 44.9. IR ($cm^{-1}$): ν 3497, 3063, 3030, 3004, 2863, 1717, 1612, 1586, 1513, 1496, 1453, 1422, 1362, 1302, 1248, 1207, 1173, 1091, 1030, 820, 737, 698.

Anal. Calcd for $C_{27}H_{32}O_5$ (436.55): C, 74.29; H, 7.39; O, 18.32. Found: C, 74.26; H, 7.42; O, 18.59.

**2,2-Bis-benzyloxymethyl-1-*O*-{2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-*O*-[2,3,6-tri-*O*-benzyl-α-L-fucopyranosyl-(1 → 3)]-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyloxy}-3-*p*-methoxybenzyloxypropane (6).** Molecular sieves (4 Å, 100 mg) were added to a stirred solution of the trichloroacetimidate **5** (219 mg, 0.17 mmol) and compound **3** (364 mg, 0.85 mmol) in $CH_2Cl_2$ (1.2 mL). After 5 min, the mixture was cooled to −30°C, and a solution of trimethylsilyl triflate in $CH_2Cl_2$ (0.1 M, 170 μL) was added. The stirred mixture was then allowed to warm up to 0°C over a period of 3 h. $NEt_3$ (50 μL) was then added and the mixture was concentrated. Flash chromatography of the residue (petroleum ether/EtOAc, 7/3) gave **6** (228 mg, 86%) as a colorless foam. $[\alpha]_D^{28}$ −24 (*c* 1.07, $CH_2Cl_2$). $^1$H NMR (250 MHz, $CDCl_3$): δ 7.60 (m, 4 H), 7.42–6.98 (m, 32 H), 6.77 (d, 2 H, J = 8.5 Hz), 5.23 (d, 1 H, $J_3^{III},_4^{III}$ = 3.5 Hz, H-4$^{III}$), 5.00 (dd, 1 H, $J_1^{III},_2^{III}$ = 8.0, $J_2^{III},_3^{III}$ = 10.0 Hz, H-2$^{III}$), 4.99 (d, 1 H, $J_1^{I},_2^{I}$ = 8.5 Hz, H-1$^{I}$), 4.83 (d, 1 H, $J_1^{II},_2^{II}$ = 3.5 Hz, H-1$^{II}$), 4.81 (d, 1 H, $J_{gem}$ = 12.0 Hz, $OCH_2Ph$), 4.78 (d, 1 H, $J_{gem}$ = 12.0 Hz, $OCH_2Ph$), 4.77 (m, 1 H, H-3$^{I}$), 4.76 (dd, 1 H, $J_3^{III},_4^{III}$ = 3.5 Hz, H-3$^{III}$), 4.72 (d, 1 H, H-1$^{III}$), 4.68 (s, 2 H, $OCH_2Ph$), 4.65 (m, 1 H, H-5$^{II}$), 4.59 (d, 1 H, J = 12.0 Hz, $OCH_2Ph$), 4.44 (dd, 1 H, $J_2^{I},_3^{I}$ = 10.5 Hz, H-2$^{I}$), 4.42 (d, 2 H, J = 12.0 Hz, 2 $OCH_2Ph$), 4.27 (d, 2 H, J = 12.0 Hz, $OCH_2Ph$), 4.22–4.05 (m, 8 H, H-4$^{I}$, H-6$^{III}$, 2 $OCH_2Ph$, $OCH_2Ph$–OMe), 3.97 (d, 1 H, J = 9.0 Hz), 3.96 (dd, 1 H, $J_5^{III},_{6'}^{III}$ = 5.5 Hz, $J_6^{III},_{6'}^{III}$ = 10.5 Hz, H-6′$^{III}$), 3.95–3.72 (m, 7 H, H-2$^{II}$, H-3$^{II}$, H-6$^{I}$, H-6′$^{I}$, $CH_3$–O), 3.63 (bs, 1 H, H-4$^{II}$), 3.56 (m, 2 H, H-5$^{I}$, H-5$^{III}$), 3.42 (d, 1 H, J = 9.0 Hz), 3.36–3.21 (m, 6 H), 2.01 (s, 6 H, 2 OAc), 1.94, 1.81 (2 s, 6 H, 2 OAc), 1.20 (d, 3 H, $J_5^{II},_6^{II}$ = 6.5 Hz, H-6$^{II}$). $^{13}$C NMR (62.9 MHz, $CDCl_3$): δ 170.0, 169.8, 168.7, 138.8, 138.7, 138.1, 137.7, 130.7, 134.0, 128.7, 128.6, 128.2, 128.1, 127.9, 127.8, 127.4, 127.1, 127.0, 126.9, 123.4, 113.5, 99.4, 98.7, 97.2, 79.7, 76.9, 75.1, 74.5, 71.9, 70.9, 70.2, 68.8, 66.7, 66.7, 74.0, 73.4, 72.9, 72.7, 72.3, 69.1, 68.7, 68.5, 67.3, 60.1, 56.5, 55.2, 45.12, 20.7, 20.6, 20.5. IR ($cm^{-1}$): ν 3451, 3063, 3030, 2934, 2871, 1755, 1716, 1613, 1514, 1497, 1454, 1386, 1368, 1302, 1249, 1220, 1171, 1135, 1047, 955, 912, 821, 738, 723, 698.





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Anal. Calcd for $C_{89}H_{97}NO_{24}$ (1564.76): C, 68.32; H, 6.25; N, 0.90; O, 24.54. Found: C, 68.23; H, 6.38; N, 0.92; O, 24.51.

**2,2-Bis-benzyloxymethyl-1-{*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-*O*-[2,3,6-tri-*O*-benzyl-α-L-fucopyranosyl-(1 → 3)]-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyloxy}-3-*p*-methoxybenzyloxypropane (7).** A solution of compound **6** (256 mg, 0.16 mmol) in a mixture of hydrazine hydrate and ethanol (1/9, 3 mL) was refluxed for 20 h. The mixture was then cooled to rt then concentrated. Pyridine (3 mL) and acetic anhydride (2 mL) were then added to the residue. After 18 h at rt, the reaction mixture was concentrated. Flash chromatography of the residue (petroleum ether/EtOAc, 6/4) gave **7** (216 mg, 89%) as a colorless powder. $[\alpha]_D^{24}$ −50 (*c* 1.06, $CH_2Cl_2$). $^1$H NMR (200 MHz, $CDCl_3$): δ 7.42–7.20 (m, 30 H, Ph), 7.18 (d, 2 H, J = 8.5 Hz), 6.81 (d, 2 H), 5.63 (d, 1 H, $J_{NH,2}^{I}$ = 8.5 Hz, NH), 5.28 (d, 1 H, $J_{3}^{III},_{4}^{III}$ = 3.5 Hz, H-4$^{III}$), 5.10 (d, 1 H, $J_{1}^{II},_{2}^{II}$ = 3.5 Hz, H-1$^{II}$), 5.03 (dd, 1 H, $J_{1}^{III},_{2}^{III}$ = 8.0, $J_{2}^{III},_{3}^{III}$ = 10.5 Hz, H-2$^{III}$), 4.95 (d, 1 H, J = 12.0 Hz, OCHPh), 4.84 (dd, 1 H, H-3$^{III}$), 4.83 (d, 1 H, J = 12.0 Hz, OCHPh), 4.81–4.64 (m, 4 H, 4 OCHPh), 4.61 (d, 1 H, $J_{1}^{I},_{2}^{I}$ = 6.5 Hz, H-1$^{I}$), 4.58 (d, 1 H, J = 12.0 Hz, OCHPh), 4.54 (d, 1 H, H-1$^{III}$), 4.48–4.29 (m, 8 H, H-5$^{II}$, 3 $OCH_2$Ph, OCHPh), 4.18–3.90 (m, 4 H, H-2$^{II}$, H-6$^{III}$, H-6′$^{III}$, H-4$^{I}$), 3.88 (dd, 1 H, $J_{2}^{II},_{3}^{II}$ = 10.5 Hz, $J_{3}^{II},_{4}^{II}$ = 3.5 Hz, H-3$^{II}$), 3.80–3.66 (m, 6 H, H-2$^{I}$, H-6$^{I}$, H-3$^{I}$, $CH_3O$), 3.63 (bs, 1 H, H-4$^{II}$), 3.60–3.40 (m, 9 H, H-5$^{I}$, H-5$^{III}$), 2.01, 1.98, 1.96 and 1.92 (4 s, 12 H, 4 OAc), 1.68 (s, 3 H, NHAc), 1.15 (d, 3 H, $J_{5}^{II},_{6}^{II}$ = 6.5 Hz, H-6$^{II}$). $^{13}$C NMR (50 MHz, $CDCl_3$): δ 169.9, 169.7, 169.4 (C═O), 158.8, 138.8, 138.5, 137.9, 130.8, 128.8, 128.4, 128.4, 128.3, 127.8, 127.7, 127.6, 127.2, 127.0, 113.5, 100.7, 99.3, 97,3 (C-1$^{I}$, C-1$^{II}$, C-1$^{III}$), 79.6, 77.0, 76.3, 73.9, 70.5, 70.3, 68.8, 66.7, 66.4, 74.3, 74.2, 73.3, 72.8, 72.6, 69.2, 68.9, 68.7, 68.2, 60.3, 55.5, 55.1, 45.4, 23.1, 20.7, 20.5, 16.6. IR($cm^{-1}$): ν 3410, 3088, 3063, 3030, 2931, 2871, 1754, 1672, 1612, 1586, 1514, 1497, 1454, 1396, 1302, 1249, 1220, 1171, 1099, 1056, 954, 911, 819, 738, 698.

Anal. Calcd for $C_{83}H_{97}NO_{23}$ (1476.69): C, 67.51; H, 6.62; N, 0.95; O, 24.92. Found: C, 67.49; H, 6.73; N, 1.11; O, 24.63.

**2,2-Bis-benzyloxymethyl-3-{*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-*O*-[2,3,6-tri-*O*-benzyl-α-L-fucopyranosyl)-(1 → 3)]-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyloxy}-propan-1-ol (8).** Ammonium cerium nitrate (349 mg, 0.64 mmol) was added to a vigorously stirred solution of compound **7** (119 mg, 0.08 mmol) in a 9/1 mixture of acetonitrile and water (1 mL). After 5 min, $CH_2Cl_2$ was added, and the mixture washed with satd aq $NaHCO_3$, then with water. The aq layers were washed with $CH_2Cl_2$. Then, the organic phases were pooled, filtered using silicone treated paper and concentrated. Flash chromatography of the residue (EtOAc/petroleum ether, 65/35) gave **8** (92 mg, 84%) as a colorless powder. $[\alpha]_D^{26}$ −53 (*c* 1.13, $CH_2Cl_2$). $^1$H NMR (250 MHz, $CDCl_3$): δ 7.55–7.10 (m, 30 H, Ph), 5.86 (d, 1 H, $J_{NH,2}^{I}$ = 8.5 Hz, NH), 5.28 (d, 1 H, $J_{3}^{III},_{4}^{III}$ = 3.5 Hz, H-4$^{III}$), 5.27 (d, 1 H $J_{1}^{II},_{2}^{II}$ = 3.0 Hz, H-1$^{II}$), 2.00, 1.98, 1.96, 1.93 (4 s, 12 H, 4 OAc), 1.72 (s, 3 H, NHAc), 1.14 (d, 3 H, $J_{5}^{II},_{6}^{II}$ = 6.5 Hz, H-6$^{II}$). $^{13}$C NMR (62.9 MHz, $CDCl_3$): δ 170.1, 169.9, 169.2 (C═O), 138.7, 138.6, 138.5, 138.3, 137.7, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.5, 127.4, 127.3, 127.1, 100.6, 99.3, 97.8 (C-1$^{I}$, C-1$^{II}$, C-1$^{III}$), 79.7, 76.9, 76.4, 74.4, 74.3, 73.8, 70.5, 68.8, 73.5, 73.3, 72.5, 70.4, 69.6, 68.2, 66.6, 66.4, 64.6, 60.3, 55.5, 45.0, 23.1,





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20.7, 20.5, 16.6. IR ($cm^{-1}$): ν 3418, 3088, 3063, 3031, 2934, 2873, 1754, 1672, 1524, 1497, 1454, 1369, 1221, 1168, 1134, 1100, 1054, 954, 910, 814, 739, 698.

Anal. Calcd for $C_{75}H_{89}NO_{22}$ (1356.54): C, 66.41; H, 6.61; N, 1.03; O, 25.95. Found: C, 66.54; H, 6.44; N, 1.18; O, 25.76.

**2,2-Bis-benzyloxymethyl-3-{*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-*O*-[2,3,6-tri-*O*-benzyl-α-L-fucopyranosyl)-(1 → 3)]-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyloxy}-propanal (9).** DMSO (81.5 μL, 1.15 mmol) was added dropwise to a cooled (−78°C) solution of oxalyl chloride (20 μL) in $CH_2Cl_2$ (500 μL). After 15 min, a solution of compound **8** (97 mg, 77 μmol) in $CH_2Cl_2$ (0.5 mL) was added and the resulting mixture was stirred at −78°C for 30 min. Then, $NEt_3$ (160 μL, 1.15 mmol) was added and the reaction mixture was slowly warmed up to 0°C over 3 h. The reaction was then diluted with $CH_2Cl_2$, washed with a 5% aq solution of $KH_2PO_4$ then with water. The aq layers were extracted with $CH_2Cl_2$. Then, the organic phases were pooled, filtered using silicone treated paper, and concentrated. Flash chromatography of the residue (EtOAc/petroleum ether, 1/1) gave **9** (94 mg, 96%) as a colorless syrup. $[\alpha]_D^{24}$−53 (*c* 1.05, $CH_2Cl_2$). $^1$H NMR (250 MHz, $CDCl_3$): δ 9.65 (s, 1 H, CHO), 7.45–7.15 (m, 30 H, Ph), 5.72 (d, 1 H, $J_{NH,2}^{I}$ = 8.5 Hz, NH), 5.27 (d, 1 H, $J_{3}^{III},_{4}^{III}$ = 3.5 Hz, H-4$^{III}$), 5.04 (d, 1 H, $J_{1}^{II},_{2}^{II}$ = 3.5 Hz, H-1$^{II}$), 5.02 (dd, 1 H, $J_{1}^{III},_{2}^{III}$ = 8.0 Hz, $J_{2}^{III},_{3}^{III}$ = 11.0 Hz, H-2$^{III}$), 4.95 (d, 1 H, J = 12 Hz, CHPh), 4.85 (d, 1 H, J = 11.5 Hz, CHPh), 4.82 (d, 1 H, H-3$^{III}$), 4.76 (d, 1 H, CHPh), 4.70 (d, 1 H, J = 12 Hz, CHPh), 4.69 (d, 1 H, $J_{1}^{I},_{2}^{I}$ = 7.0 Hz, H-1$^{I}$), 4.68 (d, 1 H, CHPh), 4.60 (d, 1 H, CHPh), 4.52 (d, 1 H, H-1$^{III}$), 4.44 (s, 2 H, $OCH_2Ph$), 4.39 (s, 2 H, $OCH_2Ph$), 4.38 (d, 1 H, CHPh), 4.32 (m, 1 H, H-5$^{II}$), 4.12 (dd, 1 H, $J_{5}^{III},_{6}^{III}$ = 8.0 Hz, $J_{6}^{III},_{6'}^{III}$ = 11.0 Hz, H-6$^{III}$), 4.09 (d, 1H, J = 9.5 Hz), 4.09 (dd, 1 H, $J_{2}^{II},_{3}^{II}$ = 10.5 Hz, H-2$^{II}$), 4.05 (t, 1 H, $J_{2}^{I},_{3}^{I}$ = 7.0 Hz, $J_{3}^{I},_{4}^{I}$ = 7.0 Hz, H-3$^{I}$), 3.97 (dd, 1 H, $J_{5}^{III},_{6'}^{III}$ = 6.0 Hz, H-6′$^{III}$), 3.93 (t, 1 H, $J_{4}^{I},_{5}^{I}$ = 7.0 Hz, H-4$^{I}$), 3.87 (dd, 1 H, $J_{3}^{II},_{4}^{II}$ = 2.5 Hz, H-3$^{II}$), 3.80–3.53 (m, 10 H), 3.48 (m, 1 H, H-5$^{I}$), 2.00, 1.99, 1.96, 1.93 (4 s, 4 OAc), 1.72 (s, 1 H, NHAc), 1.13 (d, 1 H, $J_{5}^{II},_{6}^{II}$ = 6.5 Hz, H-6$^{II}$). $^{13}$C NMR (62.9 MHz, $CDCl_3$): δ 203.7 (CHO), 170.1, 170.0, 169.4 (C═O), 138.8, 138.7, 138.5, 138.0, 137.8, 128.5, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.6, 127.4, 127.4, 127.1 (arom), 100.5, 99.4, 97.9 (C-1$^{I}$, C-1$^{II}$, C-1$^{III}$), 79.8, 76.9, 76.4, 74.2, 74.1, 73.8, 70.5, 70.4, 68.8, 66.7, 66.5, 74.4, 73.1, 73.4, 72.6, 68.3, 67.8, 67.6, 67.5, 60.4, 56.1, 55.1, 23.1, 20.7, 20.6, 20.5, 16.7. IR ($cm^{-1}$): ν 3411, 3063, 3030, 2872, 1754, 1676, 1523, 1497, 1454, 1369, 1220, 1168, 1134, 1100, 1057, 955, 910, 815, 739, 698.

Anal. Calcd for $C_{75}H_{87}NO_{22}$ (1354.52): C, 66.51; H, 6.47; N, 1.03; O, 25.99. Found: C, 65.67; H, 6.53; N, 1.12; O, 25.75.

**Sodium salt of *N*-Sulfo-*N*-{2,2-bis-benzyloxymethyl-3-(*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-*O*-[(2,3,6)-tri-*O*-benzyl-α-L-fucopyranosyl)-(1 → 3)]-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyloxy)propyl}-Gly-Tyr($SO_3^-$)-*O*-benzyl (11).** Dipeptide **30** (25 mg, 0.057mmol) was added to a solution of aldehyde **9** (70 mg, 0.052 mmol) in THF (0.9 mL). Then, were added successively, phosphate buffer (1 M, pH 5, 0.1 mL) and sodium cyanoborohydride (4 mg, 0.062 mmol). After 3 h, the mixture was diluted with $CH_2Cl_2$ and washed with water. The organic phase was then concentrated and dried in vacuo. A solution of $SO_3$–$NMe_3$





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(143 mg, 1.03 mmol) in DMF (1 mL) was added, and the mixture was stirred at 50°C for 10 h. After cooling to rt, excess of sulfur trioxide was destroyed with MeOH (0.25 mL). After 1 h, the mixture was diluted with ethyl acetate (10 mL) and washed with satd aq $NaHCO_3$, then with water. Aqueous phases were extracted with ethyl acetate and the combined organic phases were dried ($Na_2SO_4$) then concentrated. Flash chromatography of the residue (EtOAc/MeOH/$NEt_3$, from 9/1/0.01 to 8/2/0.01) gave **11** (62 mg, 64%) as a white solid. $[\alpha]_D^{29}$ −28 (*c* 1.22, MeOH). $^1$H NMR [250 MHz, $CDCl_3$–$CD_3OD$ (8/2)]: δ 7.45–7.15 (m, 37 H), 7.02 (d, 2 H, J = 8.5 Hz), 2.03, 1.99, 1.96, 1.92, 1.87 (5 s, 15 H, 4 OAc and NHAc), 1.21 (d, 3 H, $J_{5^{II},6^{II}}$ = 6.5 Hz, H-6$^{II}$). $^{13}$C NMR [62.9 MHz, $CDCl_3$–$CD_3OD$ (8/2)]: δ 172.4, 172.0, 170.8, 170.6, 170.4, 169.8 (C═O), 151.8, 139.0, 138.8, 138.6, 138.0, 137.8, 135.3, 132.7, 130.5 128.9, 128.8, 128.6, 128.3, 128.1, 128.0, 127.9, 127.6, 127.1, 122.1, 102.3, 98.8 and 97.1 (C-1$^I$, C-1$^{II}$, C-1$^{III}$), 79.6, 77.8, 77.4, 75.3, 75.1, 74.7, 74.4, 74.0, 73.7, 73.6, 73.5, 73.1, 72.8, 72.6, 71.1, 70.6, 69.6, 69.4, 69.1, 68.3, 67.8, 67.7, 67.6, 67.0, 66.6, 60.5, 55.9, 54.6, 53.5, 51.1, 45.5, 37.1, 23.3, 20.8, 20.7, 20.6, 16.8. IR (cm$^{-1}$): ν 3450, 3063, 3032, 2928, 1755, 1669, 1541, 1507, 1455,1371, 1218, 1173, 1100, 1049, 955, 914, 864, 840, 819, 741, 698, 643. MS (electrospray, negative ion mode) *m*/*z* calcd for $C_{93}H_{105}N_3O_{31}S_2Na_2$: $[M-Na]^-$ 1846.6, $[M-2\ Na]^{2-}$ 911.8. Found: 1846.7, 911.8.

**Sodium salt of *N*-Sulfo-*N*-{2,2-bis-benzyloxymethyl-3-(*O*-(β-D-galactopyranosyl)-(1 → 4)-*O*-[2,3,6-tri-*O*-benzyl-α-L-fucopyranosyl)-(1 → 3)]-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyloxy)propyl}–Gly–Tyr($SO_3^-$)–OH (13).** Ammonium acetate (4 mg) and Pd/C (5%, 50 mg) were added to a solution of compound **11** (50 mg, 0.028 mmol) in MeOH (3 mL). The mixture was stirred under $H_2$ atmosphere (1 atm) for 30 min, then filtered through a pad of celite and concentrated. Sodium methylate in MeOH (0.5 M, 1 mL) was added to the residue and the solution was left at rt for 2 h. The mixture was then neutralized with acetic acid and concentrated. Reverse phase chromatography of the residue ($C_{18}$ silica gel, water/MeOH from 7/3 to 4/6) gave **13** (34 mg, 80%) as a colorless foam. $[\alpha]_D^{29}$ −41 (*c* 2.1, MeOH). $^1$H NMR (200 MHz, $CDCl_3$): δ 7.50–7.10 (m, 34 H), 5.34 (d, 1 H, $J_{1^{II},2^{II}}$ = 3.5 Hz, H-1$^{II}$), 1.98 (s, 3 H, NHAc), 1,18 (d, 3 H, $J_{5^{II},6^{II}}$ = 6.5 Hz, H-6$^{II}$). $^{13}$C NMR (62.9 MHz, $CDCl_3$): δ 178.1, 173.5, 173.2 (C═O), 152.4, 140.4, 140.2, 140.1, 140.0, 139.6, 139.3, 136.2, 131.2, 129.6, 129.4, 129.3, 128.8, 128.7, 128.5, 128.4, 122.4, 103.6, 102.9, 97.8 (C-1$^I$, C-1$^{II}$, C-1$^{III}$), 80.0, 79.7, 77.1, 76.4, 76.3, 75.2, 74.8, 74.7, 74.6, 74.2, 74.1, 73.6, 73.2, 72.6, 71.0, 70.9, 69.9, 69.1, 68.4, 67.8, 63.2, 57.1, 56.8, 55.2, 52.2, 46.3, 39.1, 23.9, 16.9. IR (cm$^{-1}$): ν 3423, 1637, 1558, 1541, 1506, 1456, 1418, 1209, 553, 520, 507. HRMS (electrospray, negative ion mode) *m*/*z* calcd for $C_{78}H_{90}N_3O_{27}S_2Na_3$: $[M-3\ Na + H]^{2-}$ 1565.52814. Found: 1565.52818.

**Sodium salt of *N*-Sulfo-*N*-{2,2-bis-benzyloxymethyl-3-(*O*-(3-sulfo-β-D-galactopyranosyl)-(1 → 4)-*O*-[2,3,6-tri-*O*-benzyl-α-L-fucopyranosyl)-(1 → 3)]-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyloxy)propyl}–Gly–Tyr($SO_3^-$)–OH (14).** A suspension of dibutyltin oxide (4 mg, 15 μmol) and compound **13** (19 mg, 12 μmol) in toluene (5 mL) was heated under reflux for 12 h with continual removal of water using a Dean–Stark apparatus and concentrated. A solution of $SO_3$–$NMe_3$ in DMF (0.1 M, 125 μL) was added to the residue, and the resulting mixture was stirred for 5 h at rt. The mixture was then diluted with EtOAc and washed with a satd aq

$NaHCO_3$ solution. The aq phase was extracted with EtOAc, and the combined organic layers were dried ($Na_2SO_4$) then concentrated. Reverse phase chromatography (silica gel $C_{18}$, water/MeOH, from 1/0 to 6/4) gave **14** along with the starting material **13** (9 mg). Fractions containing compound **14** were passed through a cation exchange resin column (Biorad AG50WX-8, sodium form) which was eluted with MeOH to give **14** as the pure sodium salt (10 mg, 49%, 95% from consumed **13**). $[\alpha]_D^{29}$ −25 (*c* 1.92, MeOH). $^1$H NMR (200 MHz, $CD_3OD$): δ 7.45–7.12 (m, 34 H), 5.34 (d, 1 H, $J_{1^{II},2^{II}}$ = 3.5 Hz, H-1$^{II}$), 1.98 (s, 3 H, NHAc), 1.15 (d, 3 H, $J_{5^{II},6^{II}}$ = 6.5 Hz, H-6$^{II}$). $^{13}$C NMR (50 MHz, $CD_3OD$): δ 177.6, 173.5, 173.2 (C═O), 152.4, 140.6, 140.2, 140.1, 139.7, 139.5, 136.2, 131.4, 129.6, 129.5, 129.3, 129.2, 129.0, 128.8, 128.6, 128.4, 128.3, 122.4, 103.2, 103.1, 97.8 (C-1$^I$, C-1$^{II}$ and C-1$^{III}$), 81.8, 79.9, 77.1, 76.9, 76.4, 75.3, 74.3, 74.2, 73.6, 71.1, 71.0, 70.2, 69.1, 68.4, 67.7, 63.4, 57.5, 57.1, 57.05, 52.6, 46.3, 39.2, 24.0, 16.8. IR ($cm^{-1}$): ν 3435, 2928, 2089, 1644, 1506, 1454, 1402, 1235, 1164, 1098, 1049, 867, 736, 697, 577, 560, 552, 519, 503, 499. HRMS (electrospray, negative ion mode) *m/z* calcd for $C_{78}H_{89}N_3O_{30}S_3Na_4$: $[M-4\ Na + H]^{3-}$ 1644.47713. Found: 1644.47631.

**Sodium salt of *N*-Sulfo-*N*-{2,2-bis-hydroxymethyl-3-(*O*-(3-sulfo-β-D-galactopyranosyl)-(1 → 4)-*O*-[α-L-fucopyranosyl)-(1 → 3)]-2-acetamido-2-deoxy-β-D-glucopyranosyloxy)propyl}–Gly–Tyr($SO_3^-$)–OH (15).** A solution of compound **14** (6 mg, 3.4 μmol) and $Pd(OH)_2$/C (20%, 10 mg) in a mixture of methanol and water (1/1, 0.5 mL) was stirred under $H_2$ atmosphere (1 atm) for 24 h. The reaction mixture was then filtered through a pad of celite and eluted with water from a cation exchange resin column (Biorad AG50WX-8, sodium form). Freeze-drying of the resulting aq solution gave **15** (4 mg, 95%) as a white powder. $[\alpha]_D^{29}$ −25 (*c* 1.02, $H_2O$). $^1$H NMR (250 MHz, $D_2O$): δ 7.33 (d, 2 H, J = 8.0 Hz), 7.25 (d, 2 H, J = 8.0 Hz), 5.12 (d, 1 H, $J_{1^{II},2^{II}}$ = 3.5 Hz, H-1$^{II}$), 4.58 (d, 1 H, $J_{1^{III},2^{III}}$ = 8.0 Hz, H-1$^{III}$), 4.48 (dd, 1 H, J = 5.5, 6.5 Hz), 4.32 (dd, 1 H, $J_{2^{III},3^{III}}$ = 9.0 Hz, $J_{3^{III},4^{III}}$ = 2.5 Hz, H-3$^{III}$), 4.32 (d, 1 H, $J_{1^I,2^I}$ = 8.0 Hz, H-1$^I$), 4.27 (d, 1 H, H-4$^{III}$), 2.04 (s, 3 H, NHAc), 1.17 (d, 3 H, $J_{5^{II},6^{II}}$ = 6.5 Hz, H-6$^{II}$). $^{13}$C NMR (62.9 MHz, $D_2O$): δ 174.5–172.3 (C═O), 150.3, 135.5, 130.9, 121.8, 101.7, 101.3, 98.8 (C-1$^I$, C-1$^{II}$, C-1$^{III}$), 80.4, 75.2, 74.9, 74.8, 73.5, 72.2, 69.5, 69.4, 68.0, 66.9, 68.3, 61.6, 60.9, 59.8, 56.2, 56.1, 50.9, 45.6, 37.4, 22.6, 15.5. HRMS (electrospray, negative ion mode) *m/z* calcd for $C_{36}H_{53}N_3O_{30}S_3Na_4$: $[M-4\ Na + H]^{3-}$ 1104.19543. Found: 1104.19545.

**2,2-Bis-benzyloxymethyl-3-{*O*-(β-D-galactopyranosyl)-(1 → 4)-*O*-[2,3,6-tri-*O*-benzyl-α-L-fucopyranosyl)-(1 → 3)]-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyloxy}propanal (16).** A solution of NaOMe in MeOH (0.2M, 0.65 mL) was added to the aldehyde **43** (88 mg, 0.065 mmol). The mixture was stirred for 1 h, then neutralized with AcOH (7 μL) and concentrated. Flash chromatography of the residue (EtOAc/ MeOH from 1/0 to 95/5) gave **16** (73 mg, 94 %) as a white solid. $[\alpha]_D^{28}$ −66 (*c* 2.75, MeOH). $^1$H NMR (200 MHz, $CDCl_3$): δ 9.65 (s, 1 H, CHO), 7.5–7.05 (m, 30 H, Ph), 5.23 (bs, 1 H, H-1$^{II}$), 4.96 (m, 1 H, H-5$^{II}$), 1.79 (s, 3 H, NHAc), 1.12 (d, 3 H, $J_{5^{II},6^{II}}$ = 6.5 Hz, H-6$^{II}$). $^{13}$C NMR [62.9 MHz, $CDCl_3$–$CD_3OD$ (8/2)]: δ 204.2 (CHO), 171.2 (CONH), 139.0, 138.8, 138.2, 138.1, 138.0, 128.7, 127.4, 101.8, 101.6, 97.0 (C-1$^I$, C-1$^{II}$, C-1$^{III}$), 79.2, 78.1, 76.2, 75.7, 74.7, 74.0, 73.6, 72.5, 71.8, 69.2, 66.9, 75.3, 73.6, 73.4, 73.3, 68.5, 68.0, 67.8, 67.7, 62.5, 56.3, 23.2, 16.4. IR ($cm^{-1}$): ν 3420 ; 3063 ; 3030 ; 2925 ; 2872 ; 1727 ; 1656 ; 1540 ; 1497 ; 1454 ; 1366 ; 1312 ; 1208 ; 1098 ; 1066 ;

912 ; 812 ; 737 ; 697. MS (electrospray, positive ion mode) *m/z* calcd for $C_{67}H_{79}NO_{18}$: $[M + Na]^+$ 1208.5. Found: 1208.4.

Anal. Calcd for $C_{67}H_{79}NO_{18}$, 0.5 $H_2O$: C, 67.32; H, 6.75; N, 1.17; O, 24.27. Found: C, 67.37; H, 6.53; N, 1.06; O, 25.15.

**Sodium salt of 2,2-bis-benzyloxymethyl-3-{*O*-(3-*O*-sulfo-β-D-galactopyranosyl)-(1 → 4)-*O*-[2,3,6-tri-*O*-benzyl-α-L-fucopyranosyl)-(1 → 3)]-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyloxy}propanal (17).** A suspension of dibutyltin oxide (31 mg, 126 μmol) and aldehyde **15** (75 mg, 63 μmol) in toluene (15 mL) was heated under reflux for 18 h with continual removal of water using a Dean–Stark apparatus and concentrated. A solution of $SO_3$–$NMe_3$ (10 mg) in DMF (0.6 mL) was added to the residue, and the resulting mixture was stirred for 5 h at rt. The mixture was diluted with ethyl acetate and washed with a satd aq $NaHCO_3$ solution. The aq phase was extracted with ethyl acetate, and the combined organic layers were dried ($Na_2SO_4$) and concentrated. Flash chromatography of the residue (EtOAc/MeOH, 9/1) gave **17** along with the starting material **15** (17 mg). Fractions containing **17** were passed through a cation exchange resin column (Biorad AG50WX-8, sodium form) which was further eluted with MeOH to give **17** as the pure sodium salt (51 mg, 63%, 81% from consumed **15**). $[\alpha]_D^{28}$ −49 (*c* 1.21, $CH_2Cl_2$). $^1$H NMR [250 MHz, $CDCl_3$–$CD_3OD$ (8/2)]: δ 9.64 (s, 1 H, CHO), 7.50–7.10 (m, 30 H, Ph), 5.18 (d, 1 H, $J_{1^{II},2^{II}}$ = 3.5 Hz, H-1$^{II}$), 1.83 (s, 3 H, NHAc), 1.15 (d, 3 H, $J_{5^{II},6^{II}}$ = 7.5 Hz, H-6$^{II}$). $^{13}$C NMR [62.9 MHz, $CDCl_3$–$CD_3OD$ (8/2)]: δ 204.2 (CHO), 171.2 (NHAc), 139.0, 138.8, 138.2, 138.1, 138.0, 128.7, 127.4, 101.8, 101.6, 97.0 (C-1$^{I}$, C-1$^{II}$, C-1$^{III}$), 79.2, 78.1, 76.2, 75.7, 74.7, 74.0, 73.6, 72.5, 71.8, 69.2, 66.9, 75.3, 73.6, 73.4, 73.3, 68.5, 68.0, 67.8, 67.7, 62.5, 56.3, 23.2, 16.4. IR ($cm^{-1}$): ν 3426, 3088, 3063, 3031, 2925, 2858, 1956, 1881, 1813, 1728, 1658, 1545, 1497, 1454, 1367, 1258, 1213, 1157, 1100, 1058, 1027, 875, 810, 737, 697. MS (electrospray, negative ion mode) *m/z* calcd for $C_{67}H_{78}NO_{21}SNa$: $[M–Na]^-$ 1264.5. Found: 1264.2.

Anal. Calcd for $C_{67}H_{78}NO_{21}SNa$, $H_2O$: C, 61.60; H, 6.17; N, 1.09; S, 2.45; Found: C, 61.56; H, 6.51; N, O.99; S, 2.27.

**Sodium salt of *N*-{2,2-bis-benzyloxymethyl-3-(*O*-(3-sulfo-β-D-galactopyranosyl)-(1 → 4)-*O*-[2,3,4,tri-*O*-benzyl-α-L-fucopyranosyl)-(1 → 3)]-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyloxy}propyl]–Gly–Tyr($SO_3^-$)–OH (18).** A solution of aldehyde **17** (13 mg, 10 μmol) and dipeptide **33** (17 mg, 49 μmoL) in a 1/1/1 mixture of THF, MeOH and pH5 phosphate buffer (0.5 mL) was heated to 50°C. After 5 min, $NaBH_3CN$ (2 mg) was added and the mixture was kept at 50°C for 15 h. The mixture was then cooled to rt, diluted with $CH_2Cl_2$ and washed with water. The aq phase was extracted with $CH_2Cl_2$ and the combined organic layers were dried ($Na_2SO_4$) and concentrated. Reverse phase chromatography ($C_{18}$ silica gel, water/MeOH, from 90/10 to 75/25) followed by cation exchange resin column (Biorad AG50WX-8, sodium form) eluted with MeOH gave **18** as pure sodium salt (13 mg, 79%). $[\alpha]_D^{26}$ −47 (*c* 1.00, MeOH). $^1$H NMR [250 MHz, $CDCl_3$–$CD_3OD$ (1/1)]: δ 7.45–7.10 (m, 34 H), 5.31 (d, 1 H, $J_{1^{II},2^{II}}$ = 3.5 Hz, H-1$^{II}$), 1.91 (s, 3 H, NHAc), 1.14 (d, 3 H, $J_{5^{II},6^{II}}$ = 6.5 Hz, H-6$^{II}$). $^{13}$C NMR [62.9 MHz, $CDCl_3$–$CD_3OD$ (1/1)]: δ 175.0, 173.1 (C═O), 152.2, 139.8, 139.5, 138.7, 138.5, 138.3, 134.5, 130.7, 129.1, 128.9, 128.5, 128.0,





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122.1, 102.5, 97.3 (C-$1^{I}$, C-$1^{II}$, C-$1^{III}$), 62.9, 56.6, 55.5, 55.4, 52.2, 43.4 et 38.0, 30.3, 23.6, 16.6. IR ($cm^{-1}$): ν 3440, 3088, 3066, 3032, 2925, 2855, 1733, 1683, 1676, 1654, 1636, 1558, 1541, 1507, 1498, 1455, 1384, 1261, 1213, 1159, 1094, 1049, 1028, 738, 698. HRMS (electrospray, negative ion mode) *m/z* calcd for $C_{78}H_{91}N_3O_{27}S_2Na_2$: $[M-Na]^{2-}$, 1565.52814. Found: 1565.52798.

**Sodium salt of *N*-{2,2-bis-hydroxymethyl-3-(*O*-(3-sulfo-β-D-galactopyranosyl)-(1 → 4)-*O*-[α-L-fucopyranosyl)-(1 → 3)]-2-acetamido-2-deoxy-β-D-glucopyranosyloxy)propyl}-Gly-Tyr($SO_3^-$)-OH (19).** A suspension of compound **18** (8 mg, 5 μmol) and $Pd(OH)_2/C$ (20%, 10 mg) in a 1:1 mixture of MeOH and water was stirred under $H_2$ atmosphere (1 atm) for 24 h. The reaction mixture was then filtered through a pad of celite and eluted with water from a cation exchange resin column (Biorad AG50WX-8, sodium form). Freeze-drying of the resulting aq solution gave **19** (5 mg, 95%) as a white powder. $[\alpha]_D^{29}$ −19 (*c* 1.00, $H_2O$). $^1H$ NMR (250 MHz, $D_2O$): δ 7.48–7.15 (m, 4 H), 5.12 (bs, 1 H, H-$1^{II}$), 4.57 (d, 1 H, $J_{1^{III},2^{III}}$ = 7.5 Hz, H-$1^{III}$), 4.48 (dd, 1 H, $J_{a,b}$ = 5.5 Hz, $J_{a,b'}$ = 6.5 Hz), 4.32 (dd, 1 H, $J_{2^{III},3^{III}}$ = 9.0 Hz, $J_{3^{III},4^{III}}$ = 2.5 Hz, H-$3^{III}$), 4.32 (d, 1 H, $J_{1^{I},2^{I}}$ = 8.0 Hz, H-$1^{I}$), 4.27 (d, 1 H, H-$4^{III}$), 2.04 (s, 3 H, NHAc), 1.18 (d, 3 H, $J_{5^{II},6^{II}}$ = 6.5 Hz, H-$6^{II}$). $^{13}C$ NMR (50 MHz, $D_2O$): δ 174.5, 150.6, 135.1, 131.3, 121.6, 101.7, 98.8 (C-$1^{I}$, C-$1^{II}$, C-$1^{III}$), 80.4, 75.4, 75.1, 74.9, 73.7, 72.2, 69.5, 68.0, 63.1, 61.6, 59.8, 56.1, 55.1, 46.3, 36.9, 22.6, 15.5. IR ($cm^{-1}$): ν 3437, 2922, 2860, 1620, 1383, 1253, 1053, 868, 820, 778. HRMS (electrospray, negative ion mode) *m/z* calcd for $C_{36}H_{55}N_3O_{27}S_2Na_2$: $(M-2\ Na^+)^{2-}$, 1057.21847; found: 1057.21802.

**Tetrabutylammonium salt of *N*-{2,2-bis-benzyloxymethyl-3-(*O*-(3-sulfo-β-D-galactopyranosyl)-(1 → 4)-*O*-[2,3,4,tri-*O*-benzyl-α-L-fucopyranosyl)-(1 → 3)]-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyloxy)propyl}-6-aminohexanoyl-[Tyr($SO_3^-$)]$_3$-*O*-benzyl (20).** A solution of aldehyde **17** (40 mg, 30 μmol) and peptide **41** (55 mg, 33 μmol) in a water–THF mixture (1/1, 0.2 mL) was heated to 40°C. After 10 min, $NaBH_3CN$ (4 mg) was added and the resulting mixture was kept at 40°C for 18 h. After cooling to rt, the mixture was diluted with EtOAc and washed with water. Water was extracted with EtOAc and the organic layers were combined, dried ($Na_2SO_4$) then concentrated. Column chromatography (LH20, $CH_2Cl_2$/MeOH, 1/1) followed by elution with MeOH from a cation exchange resin column (Biorad AG50WX-8, tetrabutylammonium form) gave **20** as pure tetrabutylammonium salt (68 mg, 77%). $[\alpha]_D^{29}$ −31 (*c* 1.52, $CH_2Cl_2$). $^1H$ NMR [250 MHz, $CDCl_3$–$CD_3OD$ (8/2)]: δ 7.45–7.00 (m, 47 H), 5.33 (d, 1 H, $J_{1^{II},2^{II}}$ = 3.0 Hz, H-$1^{II}$), 5.12 (s, 2 H, $OCH_2Ph$), 2.08–1.85 (m, 2 H), 1.90 (s, 3 H, NHAc), 1.13 (d, 3 H, $J_{5^{II},6^{II}}$ = 6.5 Hz, H-$6^{II}$). $^{13}C$ NMR [62.9 MHz, $CDCl_3$–$CD_3OD$ (8/2)]: δ 174.8, 172.2, 172.1, 172.0, 171.6, 171.3 (C═O), 152.2, 151.9, 151.8, 139.4, 139.1, 138.3, 138.1, 137.9, 137.8, 135.4, 133.5, 133.0, 132.5, 132.4, 121.5, 121.4 et 121.1, 102.0, 101.7, 96.7 (C$1^{I}$, C$1^{II}$, C$1^{III}$), 81.2, 79.2, 78.5, 77.8, 76.1, 75.9, 75.5, 75.1, 73.7, 73.2, 73.0, 72.6, 70.9, 70.3, 70.2, 69.8, 68.0, 67.9, 67.4, 66.8, 62.9, 58.8 ($NBu_4^+$), 56.5 (C-$2^{I}$), 54.3, 54.2, 54.1, 52.4, 50.9, 42.9, 37.0, 36.1, 35.9, 25.5, 25.3, 25.0, 23.9 ($NBu_4^+$), 23.4 (NHAc), 19.8 ($NBu_4^+$), 16.4 (C-$6^{II}$), 13.7 ($NBu_4^+$). IR ($cm^{-1}$): ν 3471, 3063, 3030, 2937, 2875, 1756, 1716, 1497, 1454, 1427, 1387, 1368, 1316, 1218, 1170, 1134, 1100, 1048, 955, 911, 874.0, 740, 722, 698, 643. HRMS (electrospray,





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negative ion mode) *m/z* calcd for $C_{107}H_{122}N_5O_{37}S_4$: $[M-3\ N^+Bu_4]^{3-}$ 2196.67015. Found: 2196.66564.

**Sodium salt of *N*-{2,2-bis-hydroxymethyl-3-(*O*-(3-sulfo-β-D-galactopyranosyl)-(1 → 4)-*O*-[α-L-fucopyranosyl)-(1 → 3)]-2-acetamido-2-deoxy-β-D-glucopyranosyloxy)propyl}-6-aminohexanoyl-[Tyr($SO_3^-$)]$_3$−OH (21).** A suspension of compound **20** (18 mg, 6.2 μmol) and $Pd(OH)_2$/C (20%, 20 mg) in a mixture of MeOH and water (1/1, 1 mL) was stirred under $H_2$ atmosphere (1 atm) for 24 h. The reaction mixture was then filtered through a pad of celite and eluted with water from a cation exchange resin column (Biorad AG50WX-8, sodium form). Freeze-drying of the resulting aq solution gave **21** (10 mg, 95%) as a white powder. $[\alpha]_D^{29}$−32 (*c* 0.5, $H_2O$). $^1$H NMR (400 MHz, $D_2O$): δ 7.35–7.10 (m, 12 H), 5.13 (d, 1 H, $J_{1^{II},2^{II}}$ = 3.5 Hz, H-1$^{II}$), 4.88–4.76 (m, 1 H, H-5$^{II}$), 4.70–4.53 (m, 3 H), 4.50 (d, 1 H, $J_{1^I,2^I}$ = 8.0 Hz, H-1$^I$), 4.32 (dd, 1 H, $J_{2^{III},3^{III}}$ = 9.5 Hz, H-3$^{III}$), 4.26 (d, 1 H, H-4$^{III}$), 2.16–2.02 (m, 2 H), 2.04 (s, 3 H, NHAc), 1.64–1.48 (m, 2 H), 1.42–1.24 (m, 2 H), 1.17 (d, 3 H, $J_{5^{II},6^{II}}$ = 6.5 Hz, H-6$^{II}$), 1.01–0.80 (m, 2 H). $^{13}$C NMR (62.9 MHz, $D_2O$): δ 176.3, 174.6, 172.8, 171.8 (C═O), 150.2, 135.7, 134.8, 134.4, 130.8, 130.7, 121.8, 121.6, 101.7 (C-1$^{III}$), 101.5 (C-1$^I$), 98.8 (C-1$^{II}$), 80.4 (C-3$^{III}$), 75.4, 74.9, 74.0, 73.6, 72.2, 69.4, 68.0, 67.0, 62.0, 61.6, 61.4, 59.9, 56.0 (C-2$^I$), 54.5, 54.4, 54.2, 50.3, 49.2, 43.2, 37.4, 36.7, 36.3, 35.4, 25.1, 25.0, 24.9, 22.6 (NHAc), 15.5. IR ($cm^{-1}$): ν 3423, 2927, 2852, 1734, 1653, 1558, 1540, 1507, 1384, 1254, 1165, 1051, 1018, 871, 834, 643, 624. HRMS (electrospray, negative ion mode) *m/z* calcd for $C_{58}H_{79}N_5O_{37}S_4Na_4$: $[M-4\ Na-SO_3+H]^{3-}$ 1486.38469. Found: 1486.38414.

**2,2-Bis-benzyloxymethyl-1,3-bis-{*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-*O*-[2,3,6-tri-*O*-benzyl-α-L-fucopyranosyl)-(1 → 3)]-6-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyloxy}propane (22).** Compound **4** (59 mg, 0.19 mol) was added to a solution of trichloroacetimidate **5** (630 mg, 0.49 mmol) in $CH_2Cl_2$ (2.5 mL). The stirred solution was cooled to 0°C, and a solution of TMSOTf in $CH_2Cl_2$ (0.1M, 38 μL) was added dropwise. The mixture was kept at 0°C for 1 h, quenched with $NEt_3$ (50 μL) and concentrated. Flash chromatography of the residue (toluene/EtOAc, 3.5/1) gave **22** (392 mg, 80%) as a white foam. $[\alpha]_D^{27}$−35 (*c* 1.38, $CH_2Cl_2$). $^1$H NMR (250 MHz, $CDCl_3$): δ 7.58 (m, 8 H, 2 NPhth), 7.42–6.94 (m, 50 H, Ph), 5.21 (d, 2 H, $J_{3^{III},4^{III}}$ = 3.5 Hz, 2 H-4$^{III}$), 4.99 (dd, 2 H, $J_{1^{III},2^{III}}$ = 8.5, $J_{2^{III},3^{III}}$ = 10.5 Hz, 2 H-2$^{III}$), 4.81 (d, 2 H, $J_{1^I,2^I}$ = 8.5 Hz, 2 H-1$^I$), 4.80 (d, 2 H, $J_{gem}$ = 12.0 Hz, 2 OCHPh), 4.77–4.69 (m, 6 H, 2 H-1$^{II}$, 2 H-3$^{III}$, 2 OCHPh), 4.68–4.59 (m, 10 H, 2 H-1$^{III}$, 2 H-3$^I$, 2 H-5$^{II}$, 2 $OCH_2Ph$), 4.58 (d, 2 H, J = 12.0 Hz, 2 OCHPh), 4.41 (d, 2 H, J = 12.0 Hz, 2 OCHPh), 4.37 (d, 2 H, J = 12.0 Hz, 2 OCHPh), 4.33 (dd, 2 H,$J_{2^I,3^I}$ = 10.5 Hz, 2 H-2$^I$), 4.23 (d, 2 H, J = 12.0 Hz, 2 OCHPh), 4.11 (t, 2 H, $J_{3^I,4^I}$ = 10.5 Hz, 2 H-4$^I$), 4.11 (dd, 2 H, $J_{6^{III},6'^{III}}$ = 10.5, 2 H-6$^{III}$), 3.93 (dd, 2 H, $J_{5^{III},6'^{III}}$ = 5.5 Hz, 2 H-6′$^{III}$), 3.91–3.68 (m, 14 H, 2 H-2$^{II}$, 2 H-3$^{III}$, 2 H-6$^I$, 2 H-6′$^I$, 2 OCH–$C_8$, 4 OCHPh), 3.61 (bs, 2 H, 2 H-4$^{II}$), 3.47 (ddd, 2 H, $J_{4^{III},5^{III}}$ = 1.0, $J_{5^{III},6^{III}}$ = 8.5 Hz, 2 H-5$^{III}$), 3.40 (d, 2 H, $J_{5^I,6^I}$ = 10.0 Hz, 2 H-5$^I$), 3.19 (d, 2 H, J = 9.0 Hz, 2 OCH–$C_8$), 2.00, 1.99, 1.93, 1.79 (4 s, 24 H, 8 OAc), 1.20 (d, 6 H, $J_{5^{II},6^{II}}$ = 6.5 Hz, 6 H-6$^{II}$). $^{13}$C NMR (50 MHz, $CDCl_3$): δ 170.0, 169.8, 168.8 (C═O), 138.9, 138.6, 138.1, 137.8, 131.5, 134.1, 128.6, 128.3, 128.1, 127.9, 127.8, 127.4, 127.1, 126.9, 126.7, 123.4, 99.4, 98.6. 97.2 (C-1$^I$, C-1$^{II}$, C-1$^{III}$), 79.7, 77.2, 76.9, 75.0, 74.4, 71.8,

70.9, 70.2, 68.8, 66.6, 66.2, 74.1, 73.4, 72.7, 72.3, 68.6, 67.9, 67.4, 60.1, 56.4, 44.9, 20.5, 16.7. IR ($cm^{-1}$): ν 3471, 3063, 3030, 2937, 2875, 1756, 1716, 1497, 1454, 1427, 1387, 1368, 1316, 1218, 1170, 1134, 1100, 1048, 955, 911, 874.0, 740, 722, 698, 643.

Anal. Calcd for $C_{143}H_{154}N_2O_{42}$ (2572.81): C, 66.76; H, 6.03; N, 1.09; O, 26.12. Found: C, 66.37; H, 6.37; N, 1.28; O, 26.38.

**2,2-Bis-benzyloxymethyl-1,3-bis-{*O*-(β-D-galactopyranosyl)-(1 → 4)-*O*-[2,3,6-tri-*O*-benzyl-α-L-fucopyranosyl)-(1 → 3)]-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyloxy}propane (23).** A solution of **22** (563 mg, 0.219 mmol) in a 1/2 mixture of ethylenediamine and ethanol (2 mL) was heated under reflux for 18 h, then concentrated. A 1/1 mixture of pyridine and acetic anhydride (2 mL) was then added to the residue, and the resulting mixture was stirred at 40°C for 12 h. Solvents were coevaporated with toluene and the residue diluted with $CH_2Cl_2$. The solution was washed with satd aq $NaHCO_3$ and water, then concentrated. Flash chromatography of the residue (EtOAc/petroleum ether, 52/48) gave the peracetylated intermediate (353 mg) which was added to a solution of sodium methanolate in MeOH (0.1 M, 1.5 mL). After 1 h at rt, acetic acid (8 μL) was added and the mixture concentrated. Flash chromatography of the residue (EtOAc/$CH_2Cl_2$ /MeOH, 50/42/8) gave **23** (296 mg, 66%) as a colorless powder. $[\alpha]_D^{25}$ −68 (*c* 1.12, $CH_2Cl_2$). $^1$H NMR [400 MHz, $CDCl_3$–$CD_3OD$ (8/2)]: δ 7.40–7.50 (m, 50 H), 5.19 (d, 2 H, $J_{1^{II},2^{II}}$ = 3.0 Hz, 2 H-$1^{II}$), 1.90–1.60 (m, 6 H, 2 NHAc), 1.09 (d, 6 H, $J_{5^{II},6^{II}}$ = 5.0 Hz, 6 H-$6^{II}$). $^{13}$C NMR [50 MHz, $CDCl_3$–$CD_3OD$ (8/2)]: δ 175.8, 170.8 (NHAc), 138.9, 138.8, 138.7, 138.5, 138.4, 138.2, 137.9, 137.8, 137.7, 128.4, 128.3, 128.2, 128.1, 128.0, 127.8, 127.5, 127.4, 127.3, 127.1, 101.9, 101.3, 100.8, 100.0, 97.2, 97.0 (C-$1^{I}$, C-$1'^{I}$, C-$1^{II}$, C-$1'^{II}$, C-$1^{III}$, C-$1'^{III}$), 59.5 (C-$2^{I}$), 45.3, 23.3, 21.3 (NHAc), 16.6, 16.5 (C-$6^{II}$, C-$6'^{II}$). IR ($cm^{-1}$): ν 3418, 3088, 3063, 3030, 3005, 2930, 2874, 1720.4, 1660, 1585, 1540, 1497, 1454, 1368, 1313, 1280, 1209, 1157, 1094, 1054, 1028, 913, 805, 737, 697. HRMS (electrospray, positive ion mode) *m/z* calcd for $C_{115}H_{138}N_2O_{32}$: $[M + 2Na]^{2+}$, 2104.90281. Found: 2104.90162.

Anal. Calcd for $C_{115}H_{138}N_2O_{32}$, 2 $H_2O$: C, 65.89; H, 6.83; N, 1.34; O, 25.95. Found: C, 65.89; H, 6.79; N, 1.21; O, 25.57.

**Sodium salt of 2,2-bis-benzyloxymethyl-1,3-bis-(*O*-{3-*O*-sulfo-β-D-galactopyranosyl)-(1 → 4)-*O*-[2,3,6-tri-*O*-benzyl-α-L-fucopyranosyl)-(1 → 3)]-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyloxy}propane (24).** A suspension of **23** (103 mg, 0.05 mmol) and dibutyltin oxide (27 mg, 0.11mmol) in toluene (10 mL) was heated under reflux for 12 h with continual removal of water using a Dean–Stark apparatus and concentrated. A solution of $SO_3$–$NMe_3$ (15 mg, 0.11 mmol) in THF (0.5 mL) was added to the residue, and the mixture was kept at rt for 14 h. The reaction mixture was diluted with EtOAc and washed with satd aq $NaHCO_3$ and water. The aq phases were extracted with EtOAc and the combined organic layers were dried ($Na_2SO_4$) and concentrated. Flash chromatography of the residue (EtOAc/MeOH, from 95/5 to 85/15) gave **24** along with the monosulfated compound **25**. Separately, fractions containing **24** and fractions containing **25** were passed through a cation exchange resin column (Biorad AG50WX-8, sodium form) eluted with MeOH to give the bis sulfate **24** (45 mg, 40%) and the mono sulfate **25** (54 mg, 50%) as pure sodium salts. Following the same procedure, but using 1 equiv of dibutyltin oxide and 1 equiv of $SO_3$–$NMe_3$,

additional **24** (33 mg) could be obtained from the monosulfate **25** in 60% yield giving a 70% total yield of **24** from **23**. $[\alpha]_D^{30}$ −52 (*c* 1.37, $CH_2Cl_2$). $^1H$ NMR [250 MHz, $CDCl_3$–$CD_3OD$ (8/2)]: δ 7.18–7.42 (m, 50 H), 5.22 (d, 2 H, $J_{1^{II},2^{II}}$ = 2.5 Hz, 2 H-$1^{II}$), 1.86 (s, 6 H, NHAc), 1.14 (d, 6 H, $J_{5^{II},6^{II}}$ = 6.5 Hz, H-$6^{III}$). $^{13}C$ NMR [50 MHz, $CDCl_3$–$CD_3OD$ (8/2)]: δ 171.9 (C═O), 138.9, 138.6, 138.5, 138.1, 137.9, 137.7, 128.7, 128.5, 128.0, 127.8, 127.7, 127.6, 127.4, 102.6, 101.7, 97.0 (C-$1^{I}$, C-$1^{II}$, C-$1^{III}$), 62.3 (C-$6^{III}$), 55.8 (C-$2^{I}$), 45.5, 23.3 (NHAc), 16.4 (C-$6^{II}$). IR ($cm^{-1}$): ν 3422, 3088.5, 3064, 3032, 2925, 2874, 2856, 1959, 1882, 1720.2, 1665, 1552, 1497, 1454, 1370, 1261, 1212, 1157, 1099, 1059, 1027, 805, 737, 697. MS (electrospray, negative ion mode) *m*/*z* calcd for $C_{115}H_{136}N_2O_{38}S_2Na_2$: $[M-2\ Na]^+$, 1108.4. Found: 1108.9.

Anal. Calcd for $C_{115}H_{136}N_2O_{38}S_2Na_2$, 4 $H_2O$: C, 59.12; H, 6.21; N, 1.20; S, 2.74; Found: C, 59.16; H, 6.81; N, 1.09; S, 2.51.

**Sodium salt of 2,2-bis-benzyloxymethyl-1,3-bis-{*O*-(3-*O*-sulfo-β-D-galactopyranosyl)-(1 → 4)-*O*-[2,3,6-tri-*O*-benzyl-α-L-fucopyranosyl)-(1 → 3)]-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyloxy}propane (26).** A suspension of compound **24** (39 mg, 17 μmol) and $Pd(OH)_2$/C (20%, 40 mg) in a mixture of MeOH and water (1/1, 1 mL) was stirred under $H_2$ atmosphere (1 atm) for 36 h. The reaction mixture was then filtered through a pad of celite and eluted with water from a cation exchange resin column (Biorad AG50WX-8, sodium form). Freeze-drying of the resulting aq solution gave **26** (22 mg, 94%) as a white powder. $[\alpha]_D^{27}$ −51 (*c* 0.375, $H_2O$). $^1H$ NMR (400 MHz, $D_2O$): δ 5.13 (d, 2 H, $J_{1^{II},2^{II}}$ = 3.5 Hz, 2 H-$1^{II}$), 4.84–4.80 (m, 2 H, 2 H-$5^{II}$), 4.57 (d, 2 H, $J_{1^{III},2^{III}}$ = 8.0 Hz, 2 H-$1^{III}$), 4.45 (d, 2 H, $J_{1^{I},2^{I}}$ = 8.0 Hz, 2 H-$1^{I}$), 4.32 (dd, 2 H, $J_{2^{III},3^{III}}$ = 10.0 Hz, $J_{3^{III},4^{III}}$ = 3.0 Hz, 2 H-$3^{III}$), 4.26 (d, 2 H, 2 H-$4^{III}$), 3.91 (dd, 2 H, 2 H-$2^{I}$), 3.71–3.60 (m, 4 H, 2 H-$3^{I}$, 2 H-$2^{III}$), 2.04 (s, 6 H, 2 NHAc), 1.17 (d, 6 H, $J_{5^{II},6^{II}}$ = 6.5 Hz, H-$6^{II}$). $^{13}C$ NMR (50MHz, $D_2O$): δ 174.4 (NHAc), 102.0 (C-$1^{I}$), 101.7 (C-$1^{III}$), 98.8 (C-$1^{II}$), 80.4 (C-$3^{III}$), 75.4, 74.8, 73.6, 72.2, 69.4, 68.0, 66.9, 61.6, 60.9, 59.9, 56.2 (C-$2^{I}$), 22.5 (NHAc), 15.5. IR ($cm^{-1}$): ν 3429, 2934, 1652.1, 1559, 1507, 1388, 1248, 1163, 1072, 1035, 871, 810, 771. HRMS (electrospray, negative ion mode) *m*/*z* calcd for $C_{45}H_{76}N_2O_{38}S_2Na_2$: $(M-2\ Na^+)^{2-}$, 1316.35175. Found: 1316.35216.

**Boc–Gly–Tyr(OH)–OBn (29).** Diisopropyl ethylamine (2 mL, 11.42 mmol) was added to a solution of commercial Boc–Gly–OH (**27**) (1g, 5.71 mmol) and H–Tyr(OH)–OBn (**28**) (2.3 g, 5.19 mol) in $CH_2Cl_2$ (18 mL). HOBt (841 mg, 6.23 mmol) and EDC (1.19 g, 6.23 mmol) were then added. After 18 h, the reaction mixture was diluted with $CH_2Cl_2$ and washed with satd aq $NaHCO_3$. Aqueous solution was extracted with $CH_2Cl_2$ and the combined organic layers were dried ($Na_2SO_4$) and concentrated. Flash chromatography of the residue (toluene/acetone, 77/23) gave **29** (1.6 g, 73%) as a colorless gum. $[\alpha]_D^{29}$ + 7 (*c* 1, $CH_2Cl_2$). $^1H$ NMR (200 MHz, $CDCl_3$): δ 7.42–7.25 (m, 5 H, Ph), 6.79 (d, 2 H, J = 8.0 Hz), 6.63 (d, 2 H, J = 8.0 Hz), 5.17 (d, 1 H, J = 12.0 Hz, OCHPh), 5.09 (d, 1 H, OCHPh), 4.86 (m, 1 H), 3.74 (m, 2 H), 3.12 (m, 4 H), 1.43 (s, 9 H, 3 $CH_3$). $^{13}C$ NMR (50 MHz, $CDCl_3$): δ 171.4, 169.5 (C═O), 155.5, 134.9, 130.3, 128.6, 126.4, 115.6, 80.4, 67.3, 53.2, 44.0, 36.9, 28.2. IR ($cm^{-1}$): ν 3370, 3067, 3044, 2979, 2931, 1742, 1672, 1615, 1595, 1516, 1455, 1392, 1367, 1251, 1213, 1166, 1116, 1050, 1029, 944, 914, 858, 827.

Anal. Calcd for $C_{23}H_{28}N_2O_6$, O.5 $H_2O$: C, 63.14; H, 6.68; N, 6.40; O, 23.77. Found: C, 63.20; H, 6.77; N, 6.49; O, 23.18.





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**H–Gly–Tyr(OH)–OBn (30).** Trifluoroacetic acid (1.5 mL) was added to a cooled (0°C) solution of compound **29** (948 mg, 2.21 mmol) in $CH_2Cl_2$ (3 mL) and the reaction mixture was allowed to warm up at rt. After 1 h, solvents were coevaporated three times with $CH_2Cl_2$. Flash chromatography of the residue ($CH_2Cl_2$/MeOH, from 88/12 to 85/15) gave **30** as trifluoroacetate (2.545 g, 78%). $[\alpha]_D^{28}$ + 3 (*c* 1, MeOH). $^1$H NMR [250 MHz, $CDCl_3$–$CD_3OD$ (8/2)]: δ 7.41–7.24 (m, 5 H, Ph), 6.92 (d, 2 H, J = 8.5 Hz), 6.70 (d, 2 H, J = 8.5 Hz), 5.23 (s, 2 H, O–$CH_2$–Ph), 4.75 (dd, 1 H, J = 6.0, 8.0 Hz), 3.61 (bs, 2 H), 3.07 (dd, 1 H, J = 14.5, 6.0 Hz), 2.91 (dd, 1 H, J = 14.5, 8 Hz). $^{13}$C NMR [62.9 MHz, $CDCl_3$–$CD_3OD$ (8/2)]: δ 171.6, 166.1 (C═O), 156.1, 135.2, 130.4, 128.8, 128.6, 126.8, 115.6, 67.6 ($OCH_2$Ph), 54.5, 40.4, 36.9. IR ($cm^{-1}$): ν 3318, 3093, 2974, 1736, 1724, 1664, 1612, 1596, 1549, 1514, 1458, 1441, 1378, 1350, 1287, 1190, 1135, 1021, 958, 914, 841, 799, 752, 724, 698.

Anal. Calcd for $C_{20}H_{21}N_2O_6F_3$, 1.5 $H_2O$: C, 51.17; H, 5.15; N, 5.97. Found: C, 50.78; H, 5.01; N, 5.35.

**H–Gly–Tyr($OSO_3Na$)–OH (33).** *N*-(9-Fluorenylmethoxycarbonyloxy)succinimide (534 mg, 1.58 mmol) and $NaHCO_3$ (133 mg, 1.6 mmol) were added to a cooled (0°C) and stirred solution of dipeptide **30** (350 mg, 0.79 mmol) in a mixture of acetone/water (4/1, 3 mL). After 15 h, the reaction mixture was diluted with $CH_2Cl_2$ and washed with water. The aq phases were extracted with $CH_2Cl_2$ and the combined organic layers were dried ($Na_2SO_4$) and concentrated. Flash chromatography of the residue (petroleum ether/EtOAc, 6/4 to 4/6) gave **31** (350 mg, 80%). A solution of of $SO_3$–DMF complex (486 mg, 3.178 mmol) in DMF (2.5 mL) was then added, and the mixture was stirred at 70°C for 12 h. After cooling, satd aq $NaHCO_3$ was added and the resulting mixture was extracted with EtOAc. The combined organic layers were dried ($Na_2SO_4$) and concentrated. Flash chromatography of the residue (EtOAc/MeOH, 1/0 to 9/1) followed by cation exchange chromatography (Biorad AG50WX-8, sodium form, MeOH) gave **32** (376 mg, 90 % from **31**) as a colorless foam. The residue was then dissolved in MeOH (25 mL) and $Pd(OH)_2$/C (20%, 350 mg) was added. The resulting mixture was stirred under an $H_2$ atmosphere (1 atm) for 16 h, then filtered through a pad of celite washed thoroughly with MeOH. Solvents were evaporated and the residue was dissolved in a mixture of ether and water. The aq phase was extracted several time with ether, then freeze-dried to give **33** (196 mg, 100% from **32**, 72% from **30**) as a white powder. $[\alpha]_D^{29}$ + 15 (*c* 1.40, $H_2O$). $^1$H NMR (250 MHz, $D_2O$): δ 7.3 (d, 2 H, J = 9.0 Hz), 7.24 (d, 2 H, J = 9.0 Hz), 4.53 (dd, 1 H, J = 4.5, 9.0 Hz), 3.78 (d, 1 H, J = 16.0 Hz), 3.65 (d, 1 H, J = 16 Hz), 3.26 (dd, 1 H, J = 14.0 Hz), 2.94 (dd, 1 H). $^{13}$C NMR (62.9 MHz, $D_2O$): δ 177.7 (NHCO), 166.4 (COOH), 150.2, 135.8, 130.6, 121.8, 56.5, 40.5, 37.2. IR ($cm^{-1}$): ν 3469, 3299, 3090, 2959, 2632, 1912, 1685, 1608, 1506, 1442, 1400, 1262, 1234, 1164, 1106, 1049, 1017, 916, 867, 780, 746, 700, 644, 623. HRMS (electrospray, negative ion mode) *m/z* calcd for $C_{11}H_{13}N_2O_7SNa$: $[M-Na]^-$, 317.044348. Found: 317.044260.

**Boc–Gly–[Tyr(OH)]$_3$–OBn (36).** Diisopropylethylamine (1.57 mL, 9 mmol) was added to a solution of Boc–Tyr(OH)–OH (**34**) (761 mg, 2.71 mmol) and Tyr(OH)–OBn (**28**) (1 g, 2.26 mmol) in $CH_2Cl_2$ (7.5 mL). After 5 min, HOBt (366 mg, 2.71 mmol) and EDC (519 mg, 2.71 mmol) were added. After stirring for 18 h, the mixture was diluted with $CH_2Cl_2$ then washed with satd aq $NaHCO_3$ and water. The aq phases were extracted with $CH_2Cl_2$, and the combined organic layers were dried ($Na_2SO_4$) then





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concentrated. Flash chromatography of the residue (toluene/acetone, 77/23) gave **35** (1.06 g, 88%) as a gum. The residue was dissolved in $CH_2Cl_2$ (5mL) then cooled to 0°C. Trifluoroacetic acid (1 mL) was then added and the mixture was stirred overnight at rt. Solvents were coevaporated three time with $CH_2Cl_2$, and the residue dried in vacuo. The resulting trifluoroacetate salt was dissolved in $CH_2Cl_2$ (10 mL) and Boc–Tyr(OH)–OH (0.67 g, 2.38 mmol), then diisopropylethylamine (1 mL, 5.74 mmol) were added, followed after 5 min by HOBt (322 mg, 2.38 mmol) and EDC (456 mg, 2.38 mmol). After 15 h, the mixture was diluted with $CH_2Cl_2$, then washed with satd aq $NaHCO_3$ and with water. The aq phases were extracted with $CH_2Cl_2$, then the combined organic layers were concentrated. Flash chromatography of the residue (Toluene/acetone, 68/32) gave **36** (1.00 g, 72%) as a colorless gum. $[\alpha]_D^{27}$ −13 (*c* 1.03, acetone). $^1$H NMR (250 MHz, $CDCl_3$): δ 8.30 (bs, 3 H, OH), 7.59 (d, 1 H, J = 7.5 Hz, NH), 7.45–7.25 (m, 5 H), 7.10–6.90 (m, 6 H), 6.78–6.62 (m, 6 H), 6.07 (d, 1 H, J = 7.5 Hz, NH), 5.11 (s, 2 H, OBn), 4.78–4.63 (m, 2 H), 4.35–4.22 (m, 1 H), 3.22–2.70 (m, 6 H), 1.28 (bs, 9 H, $CH_3$). $^{13}$C NMR (62.9 MHz, $CD_3COCD_3$): δ 172.3, 171.8 and 171.5 (3 C═O), 157.1, 156.8, 156.2, 136.8, 131.3, 131.1, 129.2, 129.0, 128.9, 128.5, 127.9, 116.0, 115.8, 79.4 ($OC(CH_3)_3$), 67.1, 57.1, 55.0, 37.9, 37.7, 37.5, 28.4 ($CH_3$). IR ($cm^{-1}$): ν 3319, 3028, 2977, 2928, 2363, 1654, 1614, 1516, 1446, 1368, 1232, 1171, 1104, 1049, 1018, 892, 827, 752, 698.

Anal. Calcd for $C_{39}H_{43}N_3O_9$, $H_2O$: C, 65.44; H, 6.34; N, 5.87. Found: C, 65.09; H, 6.65; N, 5.51.

**Fmoc–NH$(CH_2)_5$CO[Tyr(OH)]$_3$–OBn (39).** Trifluoroacetic acid (3 mL) was added to a cooled (0°C) solution of the tripeptide **36** (411 mg, 0.59 mmol) in $CH_2Cl_2$ (3 mL). After 3 h the reaction mixture was concentrated. Flash chromatography of the residue (EtOAc/MeOH, from 95/5 to 85/15) gave **37** (0.343 g, 82%) as a colorless gum, a part of which was directly used in the coupling reaction. Thus, $NEt_3$ (0.42 mmol, 74 μL) was added to a solution of **37** (151 mg, 0.21 mmol) and acid **38** (75 mg, 0.21 mmol) in $CH_2Cl_2$ (800 μL). After 5 min, EDC (45 mg, 0.23 mmol) and HOBt (31 mg, 0.23 mmol) were added. The mixture was stirred for 18 h, diluted with EtOAc and washed successively with satd aq $NaHCO_3$, 10% aq $KH_2PO_4$ and water. The organic phase was dried ($Na_2SO_4$) then concentrated. Flash chromatography of the residue (toluene/acetone, 6/4) gave **39** (161 mg, 82%) as a white powder. $[\alpha]_D^{27}$ −13 (*c* 1.5, MeOH). $^1$H NMR (250 MHz, $CD_3OD$): δ 7.78 (d, 2 H, J = 7.0 Hz), 7.62 (d, 2 H, J = 7.0 Hz), 7.42–7.20 (m, 9 H), 7.03–6.88 (m, 6 H), 6.69–6.61 (m, 6 H), 5.05 (s, 2 H, $OCH_2Ph$), 4.63 (t, 1 H, J = 6.5 Hz), 4.59–4.50 (m, 2 H), 4.34 (d, 2 H, J = 7.0 Hz), 4.20 (t, 1 H), 3.12–2.62 (m, 8 H), 2.10 (d, 2 H, J = 7.5 Hz), 1.60–1.00 (m, 6 H). $^{13}$C NMR (62.9 MHz, $CD_3OD$): δ 176.0, 173.5, 173.1, 172.5 (4 C═O), 158.4 (O–CONH), 157.4, 157.3, 157.2, 145.3, 142.6, 136.9, 131.3, 131.2, 131.1, 129.5, 129.7, 128.3, 128.1, 126.2, 68.0, 67.5, 56.0, 55.7, 48.5, 41.6, 38.3, 37.8, 36.7, 30.5, 27.1, 26.5. IR ($cm^{-1}$): ν 3284, 3067, 2924, 2856, 1735, 1687, 1638, 1614, 1539, 1515, 1450, 1384, 1231, 1172, 1104, 1002, 825, 758, 740, 712, 695. MS (electrospray, positive ion mode) *m/z* calcd for $C_{55}H_{56}N_4O_{10}$: $[M + Na]^+$ 955.4. Found: 955.9.

Anal. Calcd for $C_{55}H_{56}N_4O_{10}$, $H_2O$: C, 69.46; H, 6.15; N, 5.89. Found: C, 69.42; H, 6.24; N, 5.65.

**$H_2N$–$(CH_2)_5$CO–[Tyr($OSO_3NBu_4$)$_3$–OBn (41).** Compound **39** (200 mg, 0.21 mmol) was added to a solution of $SO_3$–DMF complex (503 mg, 3.2 mmol) in DMF





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(2 mL). The mixture was maintained at 50°C for 20 h then cooled to 0°C. A solution of $NBu_4HCO_3$ (prepared by passing $CO_2$ through an 0.75 M aq solution of $NBu_4OH$) was then added until neutrality. The reaction mixture was extracted with $CH_2Cl_2$ and the combined organic layers were filtered using silicone treated paper then concentrated. Column chromatography of the residue (LH20, $CH_2Cl_2$/MeOH, 1/1) gave the intermediate **40** (450 mg) slightly contaminated with tetrabutyl ammonium salts. Part of the residue (50 mg, 26 μmol) was then dissolved in a mixture of morpholine and DMF (1/4, 200 μL). After 20 h, the reaction mixture was directly subjected to a column chromatography (LH20, $CH_2Cl_2$/MeOH, 1/1). Elution with the same solvent mixture gave **41** (34 mg, 77%). $[\alpha]_D^{29}$ −13 (*c* 1.03, MeOH). $^1$H NMR [250 MHz, $CDCl_3$–$CD_3OD$ (1/1)]: δ 7.40–7.05 (m, 17 H), 5.15 (d, 2 H, $OCH_2Ph$), 4.75 (t, 1 H, J = 7.0 Hz), 4.62 (dd, 1 H, J = 9.0, 6.0 Hz), 4.54 (dd, 1 H, J = 3.5, 12.0 Hz), 3.29–2.97 (m, 29 H), 2.85 (dd, 1 H, J = 9.0, 14.5 Hz), 2.68 (t, 2 H, J = 7.0 Hz), 2.07 (t, 2 H, J = 7.0 Hz), 1.70–1.54 (m, 24 H), 1.52–1.25 (m, 30 H), 1.02 (t, 36 H, J = 7.0 Hz, $(CH_3–C_3H_6)_4N^+$). $^{13}$C NMR (62.9 MHz, $CDCl_3$): δ 175.4, 172.7, 172.2, 171.7 (4 C═O), 152.3, 152.1, 151.9, 135.9, 134.4, 133.5, 133.0, 130.5, 130.4, 129.1, 129.0, 121.8, 121.7, 121.6, 67.7, 59.0, 55.2, 54.8, 54.4, 39.9, 37.4, 37.2, 36.7, 35.7, 26.8, 25.4, 25.0, 24.2, 20.1, 13.8. IR ($cm^{-1}$): ν 3422, 3307, 3060, 3034, 2963, 2936, 2876, 1746, 1667, 1611, 1507, 1488, 1464, 1383, 1268, 1238, 1218, 1171, 1044, 1018, 863, 741, 697, 642, 618. HRMS (electrospray, negative ion mode) *m*/*z* calcd for $C_{56}H_{79}N_5O_{17}S_3$: $[M–2\ NBu_4]^{2-}$ 1189.46331. Found: 1189.46410.

## ACKNOWLEDGMENTS

University of Paris-Sud and CNRS are acknowledged for their financial support and the Ministère de l'Education Nationale de la Recherche et de la Technologie is acknowledged for a fellowship to FD.

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Received January 16, 2003
Accepted June 18, 2003