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Syntheses of Lewis^X and Dimeric Lewis^X: Construction of Branched Oligosaccharides by a Combination of Preactivation and Reactivity Based Chemoselective One-Pot Glycosylations

Adeline Miermont,[†,‡] Youlin Zeng,[†,‡] Yuqing Jing,[†] Xin-shan Ye,[§] and Xuefei Huang*[,†]

Department of Chemistry, The University of Toledo, 2801 West Bancroft Street, MS 602, Toledo, Ohio 43606,[§]The State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, Xue Yuan Rd. 38, Beijing 100083, China

xuefei.huang@utoledo.edu

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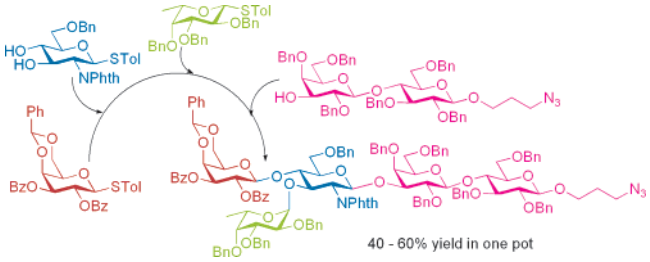
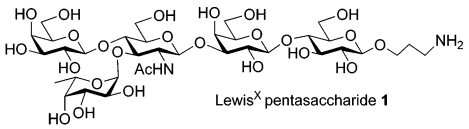
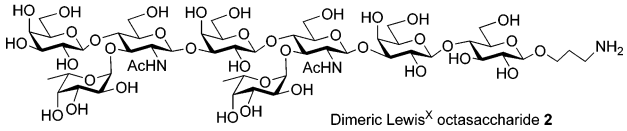


Two asymmetrically branched oligosaccharides, Lewis^X and dimeric Lewis^X, were assembled in one pot with high yields and exclusive regio- and stereoselectivities. *p*-Tolyl thioglycosides were utilized as the sole type of building blocks, thus simplifying the overall synthetic design. The reactivity-independent nature of the preactivation based method allows modular assembly of the dimeric Lewis^X octasaccharide without the need for tedious protective group manipulation to achieve exact anomeric reactivities.

The Lewis family of oligosaccharides, as represented by Lewis^X pentasaccharide **1** and dimeric Lewis^X octasaccharide **2**, is involved in a wide array of biological events, such as modulation of the immune system toward a Th2 response[1] and bacterial and viral infection.[2,3] In addition, they are known to be overexpressed on tumor cell surface,[4,5] thus providing a promising target for carbohydrate based anticancer vaccine studies.[6] With their biological significance and structural and stereochemical complexities, Lewis antigens have served as targets for the development of new synthetic methodologies,[7,8] including automated solid-phase synthesis,[9] automated parallel synthesis in solution,[7] soluble polymer supported synthesis,[10] and reactivity based chemoselective glycosylation.[11,12]

Recently, we have developed a preactivation based chemoselective one-pot glycosylation method, where a thioglycosyl donor is activated in the absence of an acceptor.[13] Upon completion of the activation, addition of a thioglycosyl acceptor will lead to the formation of a disaccharide containing a thioaryl aglycon, ready for the next round of preactivation and glycosylation. Multiple glycosylations can be performed in a single reaction flask without intermediate oligosaccharide purifications, thus significantly expediting the glycoassembly process. We have demonstrated that this is a powerful methodology, which has been successfully applied in syntheses of linear oligosaccharides, including hyaluronic acid oligosaccharides,[14] heparin trisaccharides,[15] chitotetraose,[16] and GloboH.[17] Herein, we report the application of the preactivation based methodology to one-pot construction of *branched* oligosaccharides.





It is a challenging task to assemble Lewis antigens **1** and **2** in one pot. It is well-known that the 4-hydroxyl group of glucosamine derivatives has very low nucleophilicity.[18] In addition, fucosylation on 3-OH needs to be carried out with high α selectivity for efficient one-pot synthesis. The in situ anomerization procedure for introducing α fucosyl linkage[19,20]

† The University of Toledo.
‡ These authors have contributed equally to this work.
§ Peking University.

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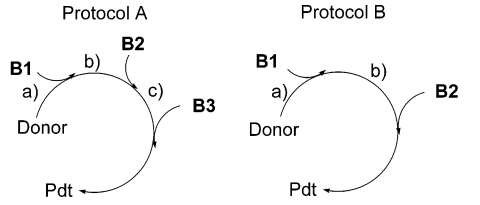
TABLE 1. One-Pot Synthesis of Oligosaccharides 10−12 and 14



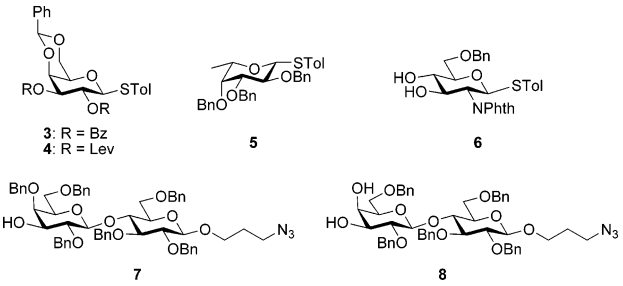
Reagents and conditions: a) AgOTf (4 eq), *p*-TolSCl, - 78 °C, 10 min; then **B1**, TTBP, 20 min; b) **B2**, *p*-TolSCl, TTBP, - 78 °C - 0 °C in 45 min; c) **B3**, *p*-TolSCl, AgOTf, TTBP - 78 °C - 0 °C in 45 min.

	protocol	donor	B1	B2	B3	Pdt	yield (%)
1[a]	A	**3**	**6**	**5**	**7**	**11**	40−60
2[b]	B	**3**	**6**	**5**		**10**	68
3[c]	B	**4**	**6**	**5**		**12**	71
4[d]	B	**10**	**13**	**7**		**14**	44−61

[a] Donor:B1:B2:B3 = 1.1:1.0:1.1:0.8. [b] Donor:B1:B2 = 1.1:1.0:1.1. [c] Donor:B1:B2 = 1.4:1.0:1.2. [d] Donor:B1:B2 = 1.1:1.0:0.7.

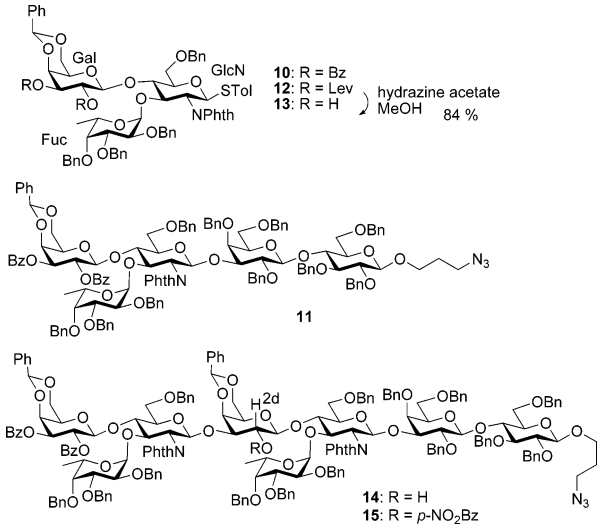
is not applicable since the reaction condition cannot be extended to β glycosylations. Furthermore, the rate of glycosylation with the in situ anomerization procedure is low requiring room temperature overnight, which is undesirable for multiple sequential glycosylations in one pot. To overcome these difficulties, we designed building blocks **3**−**8** with glucosamine diol **6** serving as a key compound. Keeping the C3 hydroxyl group of **6** unprotected reduces the steric hindrance to the C4 hydroxyl group thus increasing its nucleophilicity. In addition, this allows fucosylation on C3-OH immediately following β-glycosylation of C4-OH without the need to remove the C3 protective group. The *N*-Phth moiety in **6** is crucial to ensure exclusive regioselectivity for β-galactosylation of 4-OH, as smaller Troc,[21] azido, or acetamido groups[22] on C2 led to regioisomers. Diol **8** was examined initially as the lactoside acceptor with its axial 4-OH assumed to be much less reactive than the equatorial 3-OH.[10,12,23,24] However, glycosylation of **8** produced two regioisomeric oligosaccharides in similar quantities.[25] The lack of regioselectivity led to the use of lactoside acceptor **7** for our synthesis.[26]

Preactivation of galactoside **3** in dichloromethane (DCM) at −78 °C by *p*-TolSOTf,[13] formed in situ through reaction of *p*-TolSCl and AgOTf, was followed by addition of acceptor **6** and a sterically hindered base, tri-*tert*-butylpyrimidine (TTBP)[27] (Table 1, entry 1). TLC analysis indicated that acceptor **6** disappeared within just a few minutes. Fucosyl donor **5** was then added to the reaction mixture as a solution in diethyl ether, a solvent known to favor the formation of thermodynamically more stable axial product.[28−30] Because the armed fucosyl donor **5** has high anomeric reactivity,[31] addition of another equivalent of *p*-TolSCl chemoselectively activated **5** leading to trisaccharide **10**. Lactoside **7** was then added followed by AgOTf and *p*-TolSCl producing the fully protected Lewis^X pentasaccharide **11** with 40−60% yield in just 4 h for this four-component one-pot synthesis.



In a separate experiment, the one-pot reaction was stopped prior to addition of the lactoside acceptor **7**, from which trisaccharide **10** was isolated as the sole trisaccharide in an excellent 68% yield (Table 1, entry 2). Trisaccharide **10** was characterized by extensive NMR experiments. Correlations of the anomeric carbon of the Gal unit (99.8 ppm) with H_4 of GlcN (4.25 ppm) and the anomeric carbon of Fuc (98.0 ppm) with the H_3 of GlcN (4.68 ppm) were observed in its gHMBC spectrum, confirming that galactosylation occurred exclusively on the C4-OH of diol **6**. The anomeric configurations of newly formed glycosyl linkages were established by coupling constants of anomeric protons (Gal 4.99 ppm, $^3J_{H1,H2} = 7.8$ Hz indicating β linkage; Fuc 4.64 ppm, $^3J_{H1,H2} = 4.2$ Hz suggesting α linkage).

Recently, an elegant synthesis of Lewis^X pentasaccharide[9] was reported with use of the automated solid-phase synthesis method pioneered by Seeberger and co-workers.[32] The glycoassembly process on the carbohydrate synthesizer took 18 h with 10−15 equiv of each glycosyl building block with a 12.7% overall yield. As a comparison, through one-pot synthesis, Lewis^X was assembled rapidly with higher yield without resorting to the usage of a large excess of building blocks, which can be very tedious to prepare. Furthermore, the progress of the one-pot synthesis can be easily followed by TLC allowing convenient reaction monitoring and the intermediate oligosaccharide can be readily characterized as demonstrated by trisaccharide **10**. This highlights that our one-pot method complements well the existing automated solid-phase synthesis method.



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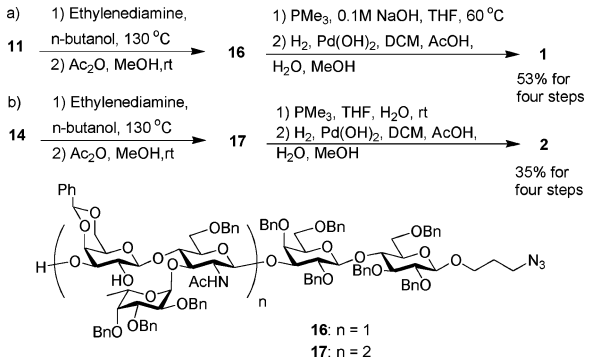
SCHEME 1



For a highly convergent synthesis of dimeric Lewis^X, we designed three modules, trisaccharide donor **10**, bifunctional trisaccharide acceptor **13**, and lactoside **7**. To simplify the overall synthetic design without relying on selective activation of different types of glycosyl donors,[7] the same aglycon leaving group *p*-STol was used for **10** and **13**. If the traditional armed−disarmed chemoselective glycosylation approach[31,33] were to be applied, the acceptor must be less reactive than the glycosyl donor. This would require manipulations of the protective groups to significantly decrease the relative anomeric reactivity of the trisaccharide acceptor **13**, which can be difficult due to the large number of protective groups present. Because donor activation and addition of acceptor occur at two distinct steps with the preactivation based method, anomeric reactivities of glycosyl donor and acceptor are independent of each other.[13] This would allow the direct glycosylation of trisaccharide **13** by trisaccharide **10**, even though **10** is less reactive.

Diol **13** was prepared by hydrazine acetate treatment of trisaccharide **12**, which was obtained by one-pot sequential reactions of galactoside **4** with glucosamine **5** and fucose **6** in a similar fashion as the formation of **10** (Table 1, entry 3). With all the necessary building blocks in hand, the synthesis of dimeric Lewis^X octasaccharide was carried out in one pot (Table 1, entry 4). The trisaccharide donor **10** was preactivated by *p*-TolSCl/AgOTf, followed by addition of acceptor **13**. After complete consumption of **13** was confirmed by TLC analysis, the lactose acceptor **7** was added followed by another equivalent of *p*-TolSCl/AgOTf. The desired fully protected dimeric Lewis^X octasaccharide **14** was successfully acquired in 44−61% yield. Glycosylation on 3-OH of trisaccharide **13** was confirmed by ^{1}H NMR analysis of the *p*-nitrobenzoate derivative of **14** (octasaccharide **15**) with its H_{2d} proton appearing at 5.25 ppm as a triplet (^3J = 9.0 Hz).

Deprotection of Lewis^X pentasaccharide **11** was performed by removal of benzoyl and Phth with ethylenediamine, followed by selective acetylation of the free amine leading to pentasaccharide **16** (Scheme 1a). Attempts to simultaneously reduce the azido moiety and benzyl groups in **16** through catalytic hydrogenation with Pd/C or Pd(OH)$_2$ under atmospheric pressure or high-pressure (100−250 psi) hydrogen gas failed to yield any desired product. ^{1}H NMR of the reaction mixture showed a complex mix of compounds. Instead, Staudinger reduction of the azide moiety in **16** by trimethylphosphine under basic condition gave the amine,[17] which underwent smooth hydrogenolysis with Pd(OH)$_2$ to produce the desired fully deprotected Lewis^X **1** as an acetate salt.

For dimeric Lewis^X deprotection, removal of benzoyl and Phth from **14** and selective acetylation were performed under the same conditions as those for Lewis^X **11**. Subsequent Staudinger reduction of **17** under basic condition, however, led to partial removal of one acetamido moiety. As an alternative, compound **17** was treated with trimethylphosphine in aqueous THF without any base to give a free amine, which was hydrogenated to produce dimeric Lewis^X octasaccharide **2** in 35% overall yield for the four steps (Scheme 1b).

In conclusion, we have demonstrated that branched oligosaccharides can be constructed by using the combination of preactivation and reactivity based one-pot synthesis with exclusive regio- and stereoselectivities. High synthetic efficiency was achieved without requiring a large excess of building blocks. A single type of glycosyl donors, i.e., *p*-tolyl thioglycosides, was used for all glycosylations, thus significantly simplifying overall synthetic design. With its anomeric reactivity independent nature, the preactivation based chemoselective glycosylation method presents a powerful strategy for modular synthesis of complex oligosaccharides.

Experimental Section

3-Azidopropyl (2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→3)]-(6-*O*-benzyl-2-deoxy-2-*N*-phthalimido-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (11): Galactose **3** (100 mg, 0.172 mmol) was dissolved in DCM (3 mL) and stirred at −78 °C with freshly activated molecular sieves MS 4 Å (100 mg) for 30 min. Silver triflate (173 mg, 0.67 mmol) dissolved in acetonitrile (0.3 mL) was added to the reaction mixture. Five minutes later, orange-colored *p*-TolSCl (27 μL, 0.172 mmol) was added directly into the reaction mixture. This needs to be performed quite quickly to prevent the *p*-TolSCl from freezing inside the syringe tip or on the flask wall. The yellow color of the solution quickly dissipated within 1 min, indicating the complete consumption of *p*-TolSCl, and the complete activation of galactose donor **3** was confirmed by TLC analysis. The glucosamine acceptor **4** (78 mg, 0.155 mmol) along with TTBP (43 mg, 0.172 mmol) dissolved in DCM (2 mL) was then added dropwise to the reaction mixture. This was stirred for 20 min at which point the glucosamine acceptor **4** was completely consumed. The fucose donor **5** (93 mg, 0.172 mmol) and TTBP (43 mg, 0.172 mmol) dissolved in Et_2O (2 mL) were added to the mixture. After 10 min, *p*-TolSCl (27 μL, 0.172 mmol) was added into the reaction mixture, which was stirred for an additional 45 min. When complete consumption of the fucose donor **5** was confirmed by TLC analysis, the lactose acceptor **6** (116 mg, 0.120 mmol) and TTBP (43 mg, 0.172 mmol) were dissolved in DCM (2 mL) and added to the reaction mixture. This was stirred for 10 min at −78 °C and then silver triflate (44 mg, 0.172 mmol) was added. After 5 min, *p*-TolSCl (27 μL, 0.172 mmol) was added into the reaction mixture, which was stirred for 45 min. The mixture was then filtered through Celite and the Celite was washed with DCM until no organic compounds were present in the filtrate. The filtrate was extracted with a saturated solution of $NaHCO_3$. The organic layer was then dried over Na_2SO_4 and concentrated to dryness. The residue was purified by silica gel column chromatography (hexanes/DCM/EtOAc, 5:3:2). The desired product was obtained in 40−60% yield as a white solid. $[\alpha]^D_{25}$ −44 (*c* 1.5, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.04−6.76 (m, 69H), 5.85 (t,

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1H, ^3J = 9.0 Hz), 5.56 (s, 1H), 5.19 (d, 1H, ^3J = 7.8 Hz), 5.17 (dd, 1H, ^3J = 4.8, 13.8 Hz), 5.12 (d, 1H, ^3J = 12 Hz), 5.02 (d, 1H, ^3J = 8.4 Hz), 4.88 (m, 2H), 4.81 (t, 2H, ^3J = 10.2 Hz), 4.75 (d, 1H, ^3J = 10.8 Hz), 4.61 (d, 1H, ^3J = 4.8 Hz), 4.54−4.49 (m, 4H), 4.44−4.29 (m, 6H), 4.27 (d, 1H, ^3J = 11.4 Hz), 4.19−4.06 (m, 7H), 4.03−3.99 (m, 2H), 3.96 (s, 1H), 3.89 (dd, 1H, ^3J = 2.4, 10.2 Hz), 3.84−3.80 (m, 2H), 3.79 (s, 1H), 3.76 (d, 1H, ^3J = 4.8 Hz), 3.62 (d, 1H, ^3J = 10.2 Hz), 3.55 (dd, 1H, ^3J = 4.8, 10.2 Hz), 3.49−3.43 (m, 2H), 3.41−3.37 (m, 5H), 3.32−3.29 (m, 4H), 3.28 (q, 1H, ^3J = 7.8, 9.0 Hz), 3.20 (d, 1H, ^3J = 10.8 Hz), 3.16 (s, 1H), 2.86 (d, 1H, ^3J = 8.4 Hz), 1.80−1.77 (m, 2H), 1.26 (d, 3H, ^3J = 6.6 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.3, 164.9, 139.7, 139.7, 139.6, 139.2, 138.8, 138.6, 138.6, 138.5, 138.4, 138.2, 137.8, 130.1, 129.9, 129.5, 129.3, 128.9, 128.8, 128.7, 128.6, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.3, 128.2, 128.1, 128.1, 128.1, 128.0, 127.9, 127.9, 127.9, 127.9, 127.8, 127.8, 127.7, 127.6, 127.4, 127.3, 127.2, 127.1, 127.0, 126.9, 126.4, 125.9, 103.6, 102.8 ($^1J_{C-H}$ = 163.4 Hz), 100.0 ($^1J_{C-H}$ = 161.3 Hz), 99.9 ($^1J_{C-H}$ = 158.2 Hz), 99.8 ($^1J_{C-H}$ = 164.6 Hz), 97.6 ($^1J_{C-H}$ = 174.1 Hz), 83.1, 82.2, 81.8, 79.2, 78.7, 77.5, 77.2, 77.0, 76.8, 76.2, 75.3, 75.2, 75.0, 74.9, 74.8, 74.1, 73.6, 73.5, 73.2, 73.0, 72.9, 71.6, 66.7, 66.6, 66.5, 48.5, 29.4, 16.5; HRMS $[M + Na]^+$ *m*/*z* calcd for $C_{132}H_{132}N_4NaO_{28}$ 2244.8926, found 2244.8960.

3-Aminopropyl β-D-galactopyranosyl-(1→4)-[(α-L-fucopyranosyl)-(1→3)]-(2-*N*-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside (1): The fully protected pentasaccharide **11** (200 mg, 0.09 mmol) was mixed with ethylenediamine (0.5 mL) in *n*-butanol (5 mL). The reaction was stirred at 130 °C for 20 h. The mixture was then concentrated and the residue was dissolved in DCM and extracted with a saturated solution of NH_4Cl. The organic layer was dried over Na_2SO_4 and concentrated to dryness. The crude residue was purified by silica gel column chromatography. The desired product was obtained in its pure form as an off-white solid. The newly formed compound was then dissolved in MeOH (5 mL) along with triethylamine (0.1 mL) and acetic anhydride (0.1 mL, 15 equiv). The reaction mixture was stirred at room temperature for 4 h. It was then concentrated and the residue was dissolved in DCM and extracted with a saturated solution of NH_4Cl. The organic layer was dried over Na_2SO_4 and concentrated to dryness. The crude residue was purified by silica gel column chromatography (hexanes/EtOAc, 1:1→1:3) to afford the pentasaccharide **16** as a white solid. A mixture of **16** (100 mg, 0.052 mmol), a 1 M solution of PMe_3 in THF (0.360 mL, 7 equiv), and 0.1 M NaOH (0.5 mL) in THF was stirred at 60 °C overnight. The mixture was then concentrated and the resulting residue was dissolved in DCM and extracted twice with H_2O. The organic layer was dried over Na_2SO_4 and concentrated to dryness. The crude product was purified by silica gel column chromatography (DCM → DCM/MeOH 8:1). The desired product bearing a terminal free amine was obtained as an off-white solid. Finally a mixture of this product (80 mg, 0.042 mmol) and $Pd(OH)_2$ (80 mg) in DCM/MeOH/H_2O/AcOH (1 mL:1 mL:2 mL:2 mL) was stirred at room temperature under atmospheric pressure H_2 for 24 h. The mixture was filtered through Celite, concentrated, and extracted with DCM (3 times) and EtOAc (3 times). The water layer was then lyophilized to afford a white solid, which was purified by Sephadex G-10 size exclusion column. The pure Lewis^X pentasaccharide **1** was obtained in acetate form as a solid in 53% yield over four steps. $[\alpha]^{25}_D$ −217 (*c* 0.5, H_2O); ^{1}H NMR (600 MHz, D_2O) δ 4.94 (d, 1H, ^3J = 3.6 Hz), 4.52 (d, 1H, ^3J = 8.4 Hz), 4.33 (d, 1H, ^3J = 8.4 Hz), 4.28 (d, 1H, ^3J = 8.4 Hz), 4.25 (d, 1H, ^3J = 8.4 Hz), 3.97 (d, 1H, ^3J = 2.4 Hz), 3.80−3.75 (m, 4H), 3.73−3.69 (m, 4H), 3.63−3.37 (m, 20H), 3.31 (t, 1H, ^3J = 7.2 Hz), 3.16−3.13 (m, 2H), 2.97 (t, 2H, ^3J = 7.2 Hz), 1.83 (s, 3H), 0.99 (d, 3H, ^3J = 6.0 Hz); ^{13}C NMR (150 MHz, D_2O) δ 181.7, 174.8, 103.0, 102.7, 102.2, 101.9, 98.7, 82.2, 78.4, 75.2, 75.1, 75.0, 74.9, 74.4, 73.1, 72.8, 72.6, 72.0, 71.1, 70.0, 69.3, 68.5, 68.4, 68.0, 67.8, 66.8, 61.6, 61.1, 60.1, 59.7, 56.1, 37.7, 26.8, 23.4, 22.4, 15.4; HRMS $[M + Na]^+$ *m*/*z* calcd for $C_{35}H_{62}N_2NaO_{25}$ 933.3539, found 933.3531.

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Supporting Information Available: Experimental procedures and selected ^{1}H, ^{13}C, and 2D NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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