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Synthesis of a common trisaccharide fragment of glycoforms of the outer core region of the *Pseudomonas aeruginosa* lipopolysaccharide

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Abstract—The first synthesis of the common trisaccharide of glycoforms of the outer core region of the *Pseudomonas aeruginosa* lipopolysaccharide is reported. A fully protected trisaccharide precursor was prepared via a highly efficient α-(1→4)-glucosylation of a β-(1→3)-linked 6-*O*-benzyl-2-azido-2-deoxy-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α-D-galactopyranoside. In contrast, an alternative sequence of glycosylations, which involves β-glucosylation of an α-(1→4)-linked Glc-GalN_3 unit, did not lead to the target trisaccharide backbone. Further O-deacetylation, azido group reduction and debenzylation of the protected trisaccharide precursor gave the corresponding trisaccharide amine. The latter structure was used in the synthesis of a series of trisaccharides bearing an acetyl group, an L-alanine or an N-acetylated L-alanine residue on its amino group at C-2 of GalN.

Cystic fibrosis (CF) is a congenital disease wherein there is a high susceptibility to *Pseudomonas aeruginosa* infection in the lungs. It was previously shown[1] that the outer core region of the lipopolysaccharide of *P. aeruginosa* influences a critical step in the elimination of this bacterium from a normal host by binding to the CF transmembrane conductance regulator (CFTR), the protein that is missing or is dysfunctional in CF. In order to ascertain which of the two naturally occurring glycoforms[2] **I** and **II** (Fig. 1) is recognized by the CFTR receptor on respiratory epithelial cells, we performed a systematic synthesis of the oligosaccharides representing these two glycoforms.

In this letter, we describe the synthesis of a common precursor for both glycoforms, branched *N*-(L-alanyl)trisaccharide **34**, as well as its *N*-acetyl- **36** and *N*-(*N*-acetyl-L-alanyl)-analogues **35** (Scheme 3). Compounds **34** and **35** were prepared to elucidate the role of the L-alanine residue and its amino group in the host recognition of the presence of *P. aeruginosa* bacterial cells.

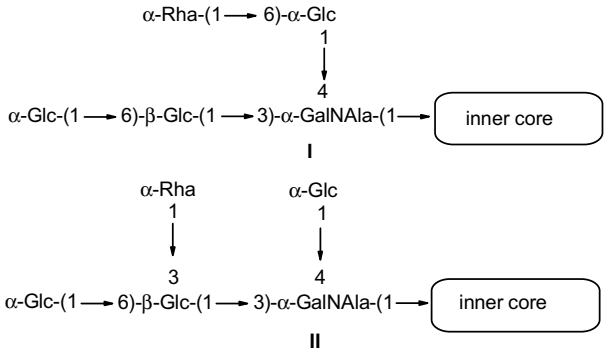

Figure 1. Glycoforms of the outer core region of the *Pseudomonas aeruginosa* lipopolysaccharide.

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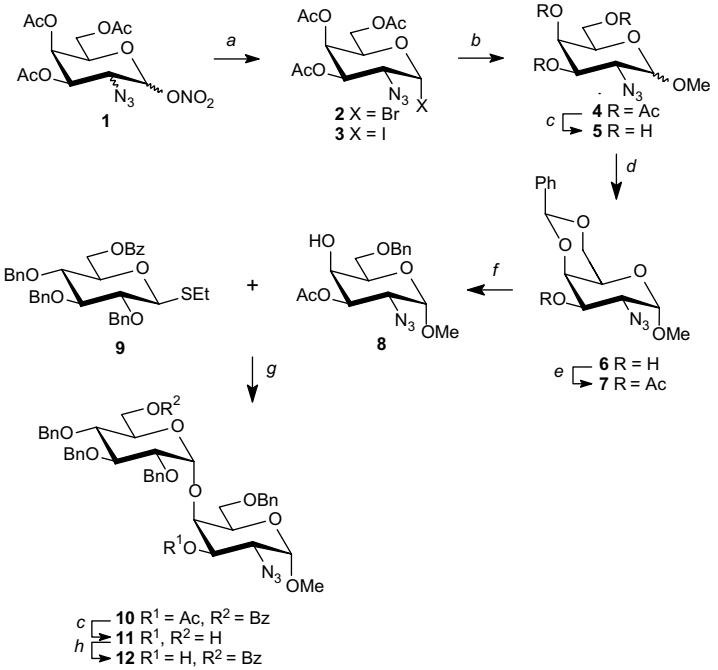
A mixture of azidonitrates **1**,[3] which contained both α- and β-anomers of 1-*O*-nitro-2-azido-2-deoxy-D-galactopyranose and 1-*O*-nitro-2-azido-2-deoxy-α-D-talopyranose in a ratio of 3:6:1 was used as the starting material. No efficient and stereoselective procedure for conversion of this mixture into α-methyl glycoside has been reported so far. We found that methanolysis of 2-azido-2-deoxy-D-galactopyranosyl halides **2** or **3** in the presence of tetraalkylammonium halides in CH_2Cl_2 gave **4** with high α-stereoselectivity. Thus, treatment of bromide **2**[4] with MeOH (5 equiv) in the presence of Bu_4NBr (1.5 equiv) in CH_2Cl_2 afforded an inseparable mixture of **4** and its β-anomer in a ratio of 5:1, whereas the reaction of iodide **3**[3] with MeOH (20 equiv) in the presence of Bu_4NI (2 equiv) gave an improvement in the ratio of α:β = 9:1.

Deacetylation of the latter mixture gave **5** (α:β = 9:1) in 65% overall yield (from **1**). Triol **5** was converted into benzylidene derivative **6**; an admixture of the corresponding β-anomer could be easily removed by column chromatography at this step. The benzylidene derivative **6** was acetylated and the acetal ring in **7** was opened[5] regioselectively to give **8**.

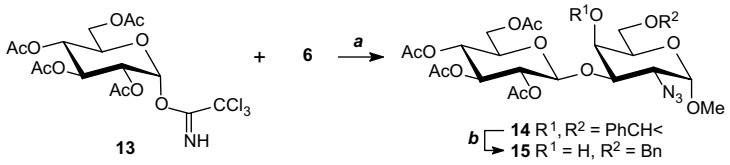
α-Glucosylation of the acceptor **8** with per-O-benzylated glucopyranosyl donors of ethyl thioglycoside (NIS/TfOH or MeOTf in CH_2Cl_2 or Et_2O), bromide (Bu_4NBr or AgOTf in CH_2Cl_2), fluoride ($SnCl_2/AgClO_4$ in Et_2O) or trichloracetimidate (TMSOTf in CH_2Cl_2) gave unsatisfactory results in terms of the yield and stereoselectivity. Glucosylation with ethyl thioglycoside **9** (Scheme 1) possessing a 6-*O*-benzoyl group, which was thought to be capable of remote anchimeric participation, was more efficient. Although a large excess (3- to 10-fold) of **9** was required to complete the conversion of the acceptor **8**, the reaction led to the disaccharide **10** stereospecifically. Liberation of the 3-OH group in the 2-azidogalactoside residue was achieved in two steps (Scheme 1): first the acyl protecting groups in **10** were removed with MeONa in MeOH, then the primary OH group of the resulting structure **11** was selectively benzoylated to give the disaccharide acceptor **12** (67% yield in two steps). Direct removal of the acetyl group from **10** by mild acidic methanolysis[6] proved to be slow (several days) and less efficient (50%).

Glycosylation of the acceptor **12** with peracetylated glucosyl donors **13**, **26** and **27** (Scheme 3, path A) was studied next. Reactions of **12** with imidate **13** ($BF_3{\cdot}Et_2O$ or TMSOTf with MS AW-300), bromide **26** (AgOTf, MS AW-300 or without MS), or thioglycoside **27** (NIS/TfOH, MS AW-300 or MS 4 Å) resulted in complete decomposition of the starting glycosyl donors; no formation of the target trisaccharide **28** was detected. In contrast, glycosylation of **12** with **13** or **26** in the presence of relatively basic MS 4 Å (Scheme 3) gave orthoester **30**. Attempts at acid-catalyzed transformation[7] of the orthoester **30** into glycoside **28** failed also. The low reactivity of the 3-OH group in **12** may be accounted for by the combined effects of the neighboring electron-withdrawing azido group[8] and the bulky glucosyl substituent.

Therefore, an alternative approach to the assembly of the target trisaccharide was explored where the (1→3)-bond was constructed first and the resulting disaccharide was 4-O-α-glucosylated. Unlike the disaccharide



Scheme 1. Reagents and conditions: (a) for **2**: LiBr, CH_3CN (65%); for **3**: NaI, CH_3CN; (b) from **2**: MeOH (5 equiv), Bu_4NBr (1.5 equiv), CH_2Cl_2 (α:β = 5:1; 59% over two steps); from **3**: MeOH (20 equiv), Bu_4NI (2 equiv), CH_2Cl_2 (α:β = 9:1; 68% over two steps); (c) MeONa, MeOH; (d) $PhCH(OMe)_2$ (2 equiv), TsOH (cat.), CH_3CN (76% for α-isomer); (e) Ac_2O, Py; (f) $Me_3N{\cdot}BH_3$, $AlCl_3$, H_2O, THF (78%); (g) **9** (>3 equiv), NIS (2.5 equiv), TfOH (0.5 equiv), CH_2Cl_2, −5 °C (78% based on **8**); (h) BzCl, Py, −20 °C (67% two steps).

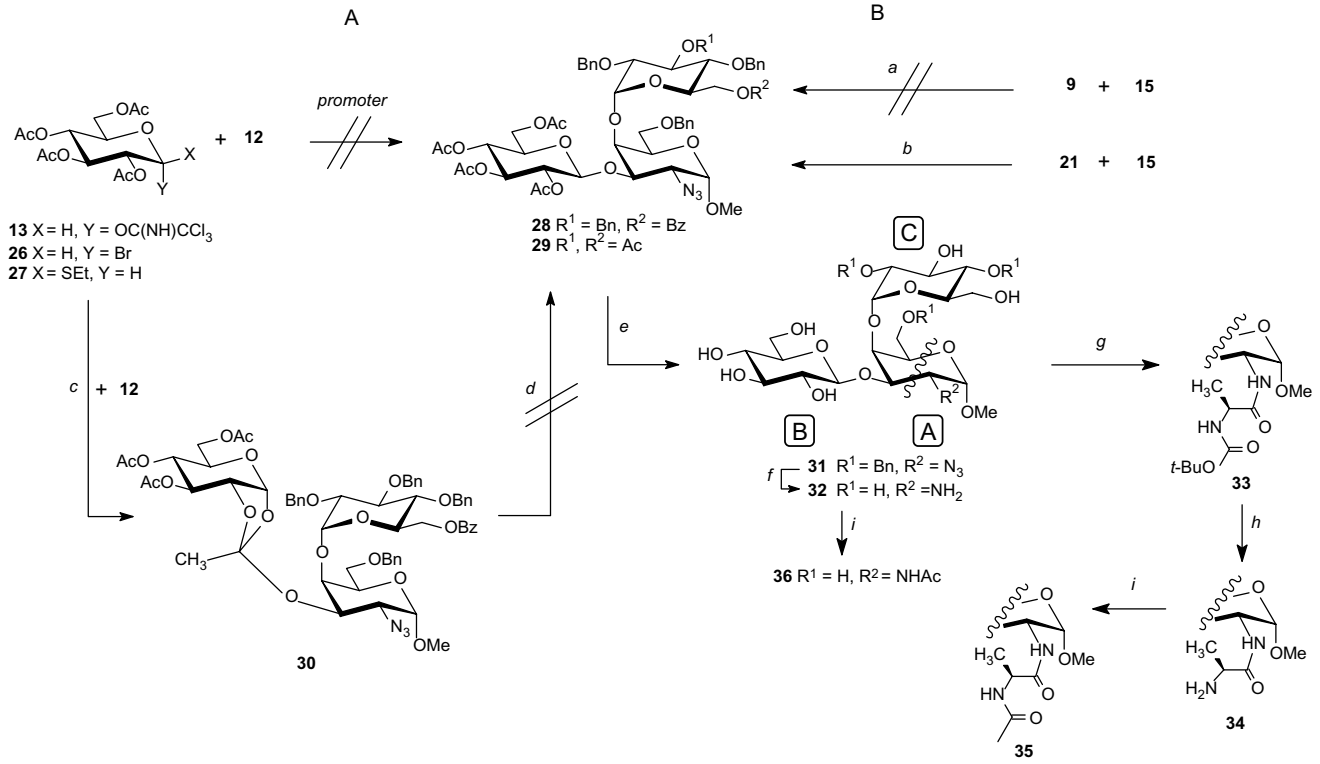


Scheme 2. Reagents and conditions: (a) TMSOTf, CH_2Cl_2, AW-300 (82%); (b) $Me_3N{\cdot}BH_3$, $AlCl_3$, H_2O, THF (86%).

acceptor **12**, 3-O-glucosylation of the benzylidene derivative **6** with the donor **13** (TMSOTf, MS AW-300) proceeded smoothly and gave disaccharide **14** in 80% yield (Scheme 2). Reductive opening of the benzylidene acetal ring in **14** produced the acceptor **15**. However, reaction of **15** with a large excess of the thioglycoside **9**, which previously showed high α-stereoselectivity in reaction with **8**, did not lead to any trisaccharide product (Scheme 3).

Consequently, we had to find an effective and α-stereoselective method for 4-O-glucosylation of **15**. The low reactivity of the 4-OH in **15** and fast decomposition of the donor under the conditions used seem to have been responsible for the low yields of the α-glycosylation with **9** in the presence of NIS–TfOH. Hence, a mild and slow glycosylation procedure was considered as a preferred route of synthesis. AgOTf-promoted glycosylation with trichloroacetimidates[9] seemed to satisfy these requirements. To verify this, a model glucosylation of the monosaccharide acceptor **8** with various imidate-type glucosyl donors in the presence of AgOTf and MS AW-300 was initially studied. The results are summarized in Table 1. Reaction of the trichloroacetimidate **16** (Fig. 2) bearing a participating acetyl group at O-6 resulted in a mixture of the anomeric disaccharides with good α-stereoselectivity but in moderate yield (entry 1). Glucosylation with the recently introduced perbenzylated *N*-phenyl-trifluoracetimidate **17**[10] was much more efficient in terms of yield but the α-stereoselectivity was poor (entry 2). Taking into account the result of the glycosylation with the 6-O-acetylated trichloracetimidate **16**, the anchimeric participation of remote acyl protecting groups was studied in more detail with the aim of improving the stereoselectivity.

The concept of remote group participation was validated with the improvement of the stereoselectivity of 1,2-*cis*-glycoside formation in L-*fuco*-,[11] D-*galacto*-,[12] and D-*manno*-series.[13] Glycosylation with *N*-phenyl-trifluoracetimidate **18**, which has a 6-*O*-benzoyl participating group (entry 3) gave a mixture of **10** and its β-isomer



Scheme 3. Reagents and conditions: (a) NIS, TfOH, MS AW-300, CH_2Cl_2; (b) **21** (1.2 equiv), AgOTf (0.6 equiv), MS AW-300, CH_2Cl_2–toluene (6.5:1) (62% for α); (c) **13** (1.2 equiv), AgOTf (1.2 equiv), MS AW-300, CH_2Cl_2:toluene (1:1); (d) TMSOTf, CH_2Cl_2; (e) MeONa, MeOH; (f) H_2, 20% $Pd(OH)_2$/C, HCl, MeOH (64%); (g) Boc-L-AlaOSu (1.5 equiv), Amberlyst A-26 (HCO_3^-), DMF, H_2O (70%); (h) CF_3COOH, H_2O (67%); (i) Ac_2O, Amberlyst A-26 (HCO_3^-), MeOH (95%).

Table 1. Effect of acyl protecting groups in glucosyl donors on the stereochemical outcome of the glycosylation of acceptor **8**

Entry	Donor	Products	α:β ratio[a]	Total yield (%)
1	**16**	**23a**, **23b**	4:1	48
2	**17**	**22a**, **22b**	2:1	95
3	**18**	**10** + β-Isomer	6:1	84
4	**19**	**23a**, **23b**	5:1	90
5	**20**	**24a**, **24b**	4:1	90
6	**21**	**25a**, **25b**	8:1	96

[a] The α:β ratio was determined according to signal intensities in the ^{1}H NMR spectra.

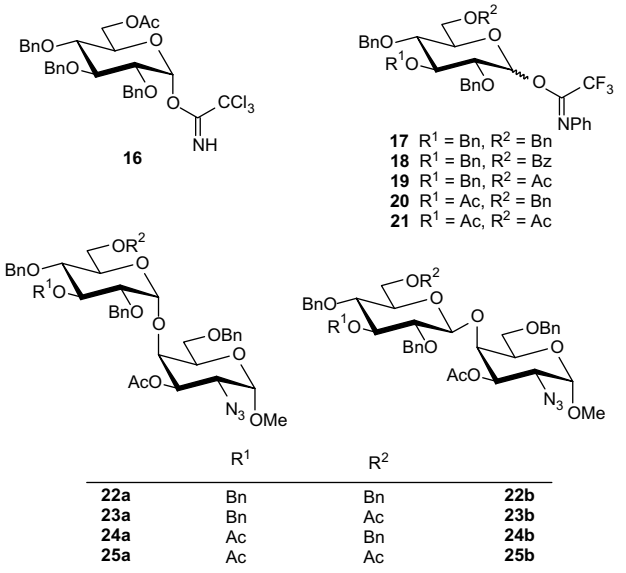


Figure 2.

with an α:β ratio of 6:1. Replacement of the 6-*O*-benzoyl group with 6-*O*-acetyl (entry 4, donor **19**) did not change the proportion of anomers (α:β = 5:1). Participation of a 3-*O*-acetyl group seemed to be slightly less efficient than that occurred with the 6-*O*-acetyl group as the reaction of **20** with **8** led to a mixture of **24a** and **24b** with an α:β ratio of 4:1. Finally, the combined effect of both 3-*O*- and 6-*O*-acetyl groups in donor **21** achieved an α:β ratio of 8:1 (entry 6).[14] The most efficient donor **21** was then successfully used for the preparation of the target, protected trisaccharide **29** (Scheme 3). Reaction of **15** with **21** in the presence of AgOTf and MS AW-300 afforded **29**[15] in 62% yield. Trisaccharide **29** was deacetylated and the benzyl groups in the resulting structure **31** were subjected to catalytic hydrogenolysis with concomitant reduction of the azido group to furnish amine **32** in 64% yield. The latter was either N-alanylated or N-acetylated to provide *N*-acyl derivatives **33** and **36**, respectively. Removal of the *N*-Boc protecting group from **33** with aqueous CF_3COOH afforded the target trisaccharide **34** in 60% yield over two steps. N-Acetylation of **34** with Ac_2O yielded another target structure, *N*-(*N*-acetyl-L-alanyl)-derivative **35**.[15]

In conclusion, this first synthesis of the common trisaccharide fragment of both glycoforms of the outer core region of the *P. aeruginosa* lipopolysaccharide and some structural analogs was efficiently carried out with the use of the concept of remote group participation during the key α-glucosylation step.

Acknowledgements

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14. *General procedure for AgOTf promoted glycosylations with N-phenyltrifluoroacetimidates*: To a solution of donor **21** (42.9 mg, 0.07 mmol) and acceptor **8** (21 mg, 0.06 mmol) in dry CH_2Cl_2 (0.85 mL), MS AW-300 (80 mg) was added. The reaction mixture was stirred at room temperature for 1 h after which a solution of AgOTf (9.5 mg, 0.04 mmol) in toluene (110 μL) was added. After 18 h the reaction was quenched with pyridine, then filtered through Celite, washed with $Na_2S_2O_3$ (1 M) and concentrated. Column chromatography ($CHCl_3$–Et_2O, 20:1) afforded **25a/b** (47 mg, 96%) as a foam, ^{1}H NMR (500 MHz, $CDCl_3$) for **25a**: 7.20–7.40 (15H, m, Ar), 5.59 (1H, t, H-3 B, $J_{3,2} = J_{3,4} = 9.7$ Hz), 5.22 (1H, dd, H-3 A, $J_{3,4} = 2.7$ Hz), 4.86 (1H, d, H-1 A, $J_{1,2} = 3.5$ Hz), 4.84 (1H, d, H-1 B, $J_{1,2} = 3.4$ Hz), 4.45–4.60 (4H, m, PhCH_2), 4.36 (1H, d, PhCH_2, $J = 12.0$ Hz), 4.28 (2H, m, H-6 B), 4.23 (1H, d, PhCH_2, $J = 12.0$ Hz), 4.17 (1H, d, H-4 A, $J_{4,3} = 2.7$ Hz), 4.14 (1H, m, H-5 B), 4.00 (1H, t, H-5 A, $J_{5,6A} = J_{5,6B} = 6.5$ Hz), 3.77 (1H, dd, H-6A A, $J_{6A,6B} = 10.2$ Hz, $J_{6A,5} = 6.55$ Hz), 3.56 (1H, m, H-6B A), 3.52 (1H, t, H-4 B, $J_{4,3} = J_{4,5} = 9.7$ Hz), 3.43 (1H, dd, H-2 B, $J_{2,3} = 10.2$ Hz,

$J_{2,1} = 3.4$ Hz), 3.40 (3H, s, OMe), 2.10, 2.04, 1.94 (9H, s, Ac); ^{13}C NMR (125 MHz, $CDCl_3$) for α: 169.6–170.5 (C═O), 137.2–138.1 (*ipso*-Ph), 127.2–129.5 (Ph), 99.1 (C-1 B), 98.7 (C-1 A), 78.2 (C-2 B), 76.7 (C-4 A), 76.3 (C-4 B), 74.6 ($PhCH_2$), 73.4 (C-3, B), 73.3 ($PhCH_2$), 72.8 ($PhCH_2$), 70.9 (C-3 A), 69.3 (C-5 B), 69.3 (C-5 A), 68.3 (C-6 A), 62.6 (C-6 B), 57.9 (C-2 A), 55.3 (OMe), 20.8–21.0 (Ac). Anal. Calcd (%) for $C_{40}H_{47}N_3O_{12}$: C, 61.77; H, 6.09. Found: C, 61.86; H, 6.24.

15. Data for compounds **29**, **34**, **35**, **36**. Compound **29**: $[\alpha]_D$ 81 (*c* 1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): 7.10–7.40 (15H, m, Ar), 5.67 (1H, t, H-3 C, $J_{3,2} = J_{3,4} = 9.7$ Hz), 5.27 (1H, t, H-3 B, $J_{3,2} = J_{3,4} = 9.6$ Hz), 5.25 (1H, d, H-1 C, $J_{1,2} = 3.4$ Hz), 5.16 (1H, t, H-4 B, $J_{4,3} = J_{4,5} = 10.0$ Hz), 5.12 (1H, m, H-2 B), 4.90 (1H, d, H-1 A, $J_{1,2} = 3.2$ Hz), 4.82 (1H, d, H-1 B, $J_{1,2} = 8.0$ Hz), 4.63 (1H, d, PhCH_2, $J = 12.2$ Hz), 4.61 (2H, s, PhCH_2), 4.55 (1H, d, PhCH_2, $J = 12.1$ Hz), 4.46 (1H, dd, H-6 C, $J_{6,5} = 2.8$ Hz, $J_{6,6'} = 12.2$ Hz), 4.43 (1H, d, PhCH_2, $J = 12.0$ Hz), 4.36 (1H, dd, H-6′ C, $J_{6',5} = 1.6$ Hz, $J_{6,6'} = 12.2$ Hz), 4.31 (1H, m, H-6 B), 4.30 (1H, d, PhCH_2, $J = 12.0$ Hz), 4.23 (1H, br s, H-4 A), 4.21 (1H, m, H-5 C), 4.20 (1H, dd, H-6′ B, $J_{6',5} = 1.7$ Hz, $J_{6,6'} = 11.8$ Hz), 4.02 (1H, dd, H-3 A, $J_{3,2} = 10.8$ Hz, $J_{3,4} = 2.0$ Hz), 3.97 (1H, m, H-5 A), 3.84 (1H, dd, H-6 A, $J_{6,5} = 5.6$ Hz, $J_{6,6'} = 10.2$ Hz), 3.75 (1H, m, H-5 B), 3.72 (1H, m, H-2 A), 3.68 (1H, m, H-6′ A), 3.54 (1H, t, H-4 C, $J_{4,3} = J_{4,5} = 9.6$ Hz), 3.54 (1H, dd, H-2 C, $J_{2,1} = 3.4$ Hz $J_{2,3} = 9.6$ Hz), 3.46 (3H, s, OMe), 2.13 (3H, s, Ac), 2.01–2.09 (9H, 3s, Ac), 1.99 (6H, s, Ac); ^{13}C NMR (125 MHz, $CDCl_3$): 169.0–170.3 (C═O), 137.6–138.3 (*ipso*-Ph), 127.4–129.5 (Ph), 102.3 (C-1 C), 98.8 (C-1 A), 98.0 (C-1 B), 78.5 (C-2 C), 78.3 (C-4 A), 77.7 (C-3 A), 76.2 (C-4 C), 74.1 ($PhCH_2$), 73.4 (C-3 C), 73.0 ($PhCH_2$), 72.8 (C-3 B), 72.7 ($PhCH_2$), 72.0 (C-5 B), 70.9 (C-2 B), 70.1 (C-5 A), 69.1 (C-6 A), 68.9 (C-5 C), 68.4 (C-4 B), 62.8 (C-6 C), 69.9 (C-6 B), 59.9 (C-2 A), 55.2 (OMe), 20.3–21.3 (Ac). Anal. Calcd (%) for $C_{52}H_{63}N_3O_{21}$: C, 58.59; H, 5.96; N, 3.94. Found: C, 58.70; H, 5.99; N, 3.85. Compound **34**: $[\alpha]_D$ 36 (*c* 0.5, H_2O); ^{1}H NMR (500 MHz, D_2O): 5.00 (1H, d, H-1 C, $J_{1,2} = 3.5$ Hz), 4.82 (1H, d, H-1 A, $J_{1,2} = 3.5$ Hz), 4.46 (1H, d, H-1 B, $J_{1,2} = 7.5$ Hz), 4.45 (1H, dd, H-2 A, $J_{2,1} = 3.5$ Hz, $J_{2,3} = 11.5$ Hz), 4.31 (1H, d, H-4 A, $J_{4,3} = 2.5$ Hz), 4.22 (1H, m, H-5 C), 4.13 (1H, dd, H-3 A, $J_{3,2} = 11.5$ Hz, $J_{3,4} = 2.5$ Hz), 4.04 (2H, m, H-5 A, Ala), 3.89 (1H, d, H-6 B, $J_{6,6'} = 12.5$ Hz), 3.88 (1H, m, H-6 A), 3.85 (2H, m, H-6′ A, H-6 C), 3.82 (1H, t, H-3 C, $J_{3,2} = J_{3,4} = 9.5$ Hz), 3.77 (1H, dd, H-6′ C, $J_{6',5} = 1.3$ Hz, $J_{6',6} = 12.7$ Hz), 3.71 (1H, dd, H-6′ B, $J_{6',5} = 4.0$ Hz, $J_{6',6} = 12.5$ Hz), 3.52 (1H, t, H-4, $J_{4,3} = J_{4,5} = 9.5$ Hz), 3.42 (1H, m, H-3 B), 3.38 (3H, s, OMe), 3.37 (2H, m, H-4 B, H-5 B), 3.17 (1H, dd, H-2 B, $J_{2,1} = 7.5$ Hz, $J_{2,3} = 9.5$ Hz), 1.54 (3H, d, Ala, $J = 7.1$ Hz); ^{13}C NMR (125 MHz, D_2O): 172.3 (C═O), 105.4 (C-1 B), 100.0 (C-1 C), 99.2 (C-1 A), 76.9 (C-5 B), 76.7 (C-4 A), 76.6 (C-3 B), 76.4 (C-3 A), 74.0 (C-2 A), 73.6 (C-3 C), 72.9 (C-2 C), 72.6 (C-5 A, C-5 C), 70.9 (C-4 B), 70.2 (C-4 C), 61.8 (C-6 B), 61.4 (C-6 A), 61.2 (C-6 C), 56.3 (OMe), 50.5 (C-2 A, CH Ala), 17.7 (Me, Ala); HRMS $C_{22}H_{40}N_2O_{16}$ $[M+H]^+$; calculated: 589.246, found: 589.233. Compound **35**: $[\alpha]_D$ 70 (*c* 1, H_2O); ^{1}H NMR and ^{13}C NMR spectra of **35** are identical to those of **34** except for the downfield shift of the signal corresponding to the α-proton of alanine (δ 4.06→4.44) and the presence of a singlet at δ 2.00 (3H) in the ^{1}H NMR spectrum and signals at δ 22.8 and 176.7 in the ^{13}C NMR spectrum corresponding to the acetyl group; HRMS $C_{24}H_{42}N_2O_{17}$ $[M+Na]^+$; calculated: 653.238, found: 653.230. Compound **36**: $[\alpha]_D$ 52 (*c* 0.5, H_2O); ^{1}H NMR and ^{13}C NMR spectra of **36** were identical to those of **34** except for the absence of the signals corresponding to alanine and the presence of a singlet at δ 2.00 (3H) in the ^{1}H NMR spectrum and signals at δ 23.2 and 176.1 in the ^{13}C NMR spectrum corresponding to the acetyl group; HRMS $C_{21}H_{37}NO_{16}$ $[M+Na]^+$; calculated: 560.219, found: 560.218.