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Synthesis of new peptidic glycoclusters derived from β-alanine

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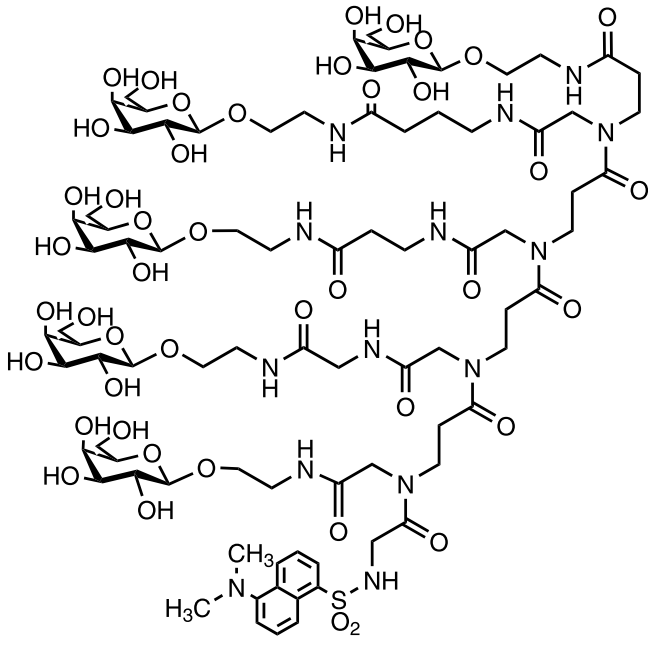
Abstract—The synthesis of an asymmetric glycocluster **1** has been achieved by coupling of a sugar unit with the β-alanine polypeptide, the principal chain, and combining a carbohydrate chain with the side chain causing it to branch from the N terminal. The synthesis of this side chain multivalent ligands is based on the scaffolding of some ω-amino acid (glycine, β-alanine, and GABA) derivatives. This method facilitated the synthesis of the cluster, of which the length of each unit differs.

The carbohydrate portions of glycoconjugates in the cell surface participates at a macromolecular level in many biological recognition processes, such as those involving immune defense response, viral replication, parasite infection, cell–cell adhesion and inflammation.[1] The research of many glycobiologists is, therefore, often concentrated on detailed investigation of carbohydrate–protein interactions which might eventually lead to the development of carbohydrate-based drugs and prove to be an important tool for investigation of their function. With the carbohydrate chain localized intracellularly to form a microdomain, reciprocal interactions demonstrate increased binding affinity with certain proteins when compared with the individual monomeric unit.[2] By using the cluster carbohydrate chain (glycocluster) in a synthetic carbohydrate, it is reported that the bioactivation is strengthened. Glycoclusers have proved to be advantageous in many instances, as the multipresentation of a specific sugar epitope in one molecule can result in remarkably increased adhesion. It is expected that multivalent carbohydrates would bind to the cell surface adhesion molecule more tightly than monovalent ones. The past method for the synthesis of glycoclusters[3] has an advantage over ours in view of the repeated simultaneous equivalent reactions needed in ours for the polyvalence functional group to amplify the carbohydrate chain exponentially. However, in the previous method only the symmetrical structure could be taken.

This paper will focus on a newly designed glycocluster derived from β-alanine. Glycoclusters of this type are now more than 10 years old, without considering the pioneering work of Y. C. Lee et al.[4] who made analogous structures based on aspartic acid and that of R. Roy et al.[5] on lysine and glycines. The new glycocluster which we synthesized involves making the β-ala-



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Scheme 1.

Keywords: glycocluster; β-peptide; D-galactose; unit synthesis; carbohydrate–protein interaction.

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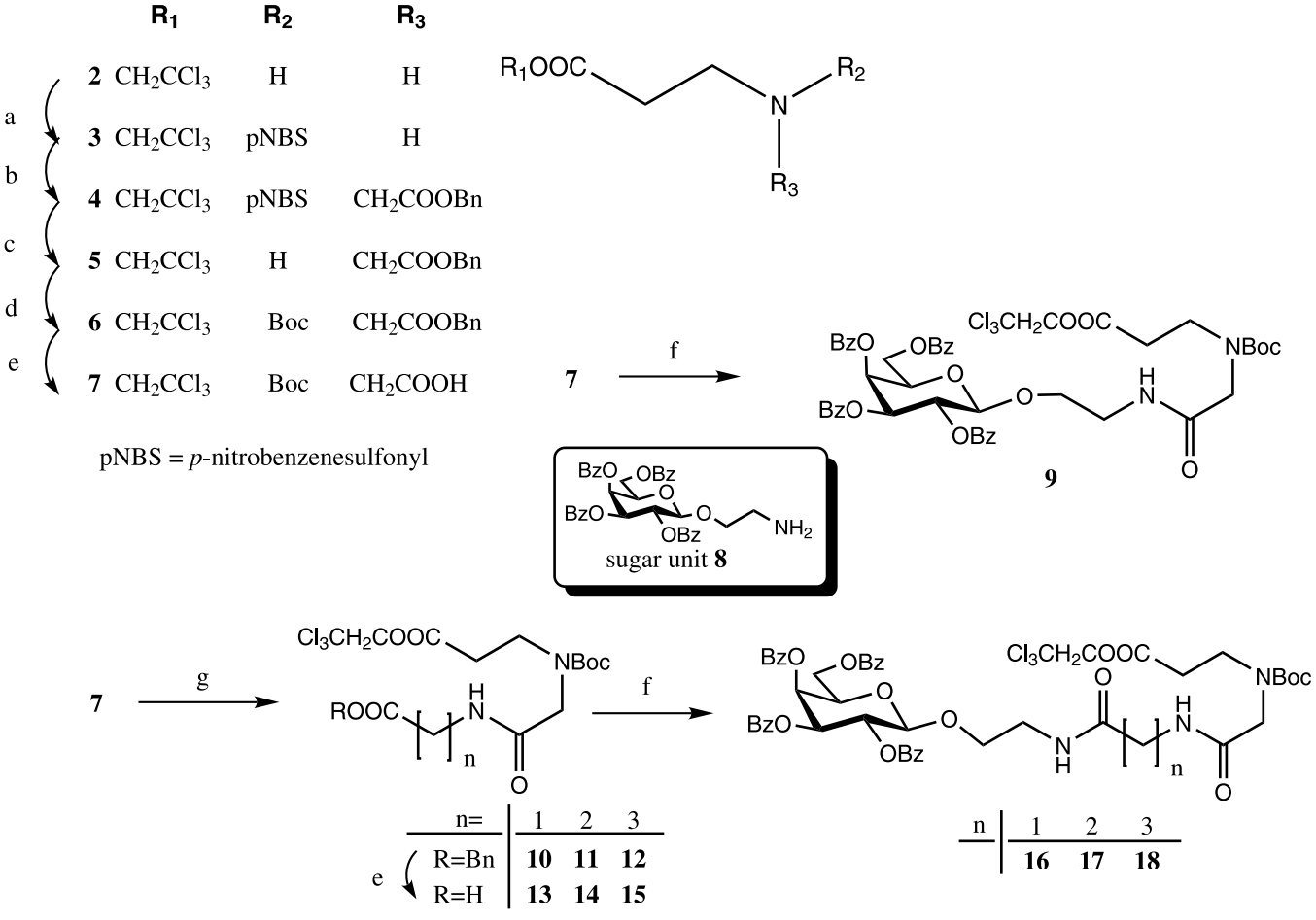
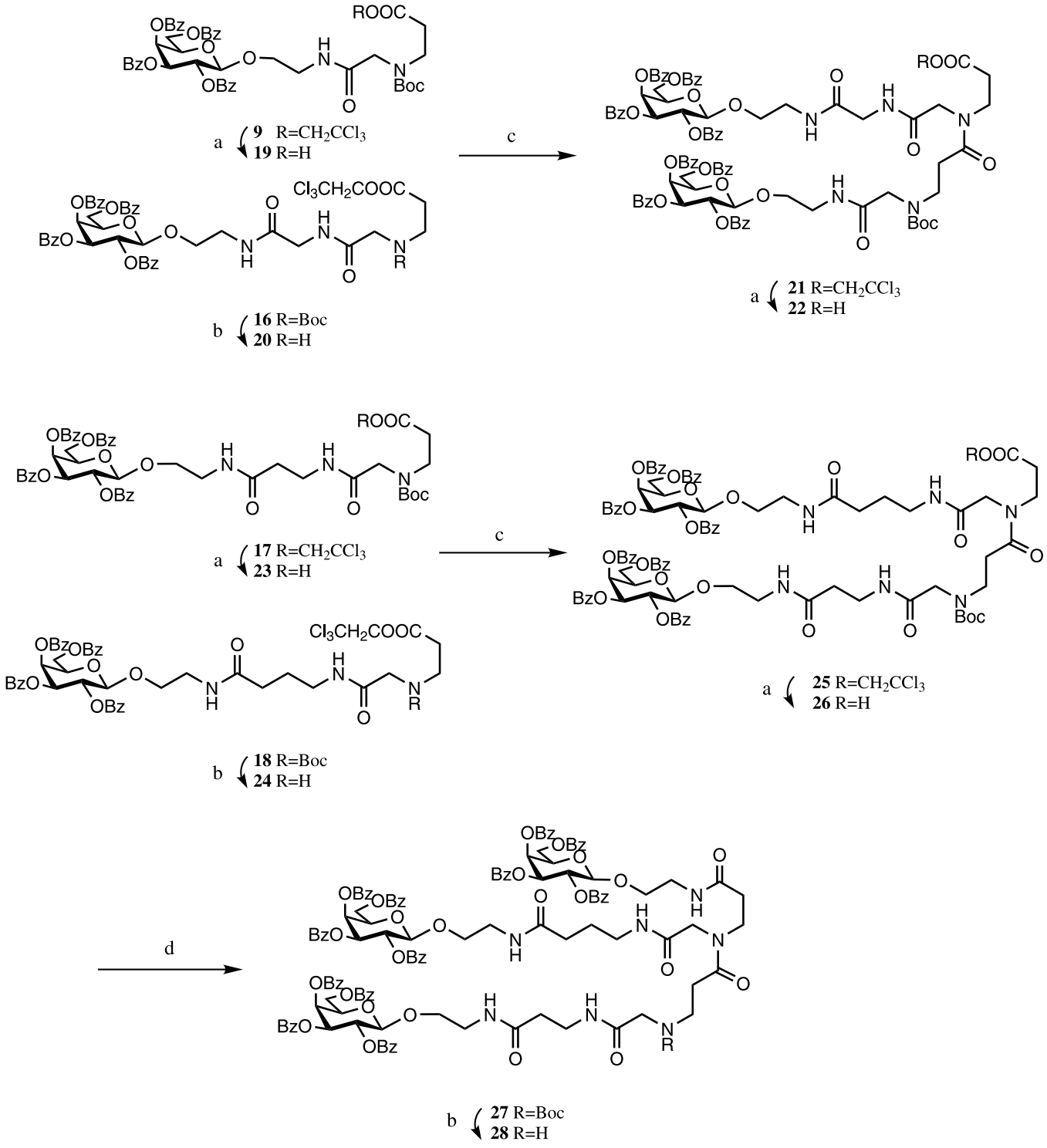
nine polypeptide the principal chain, and combining a carbohydrate chain with the side chain causing it to branch from the N terminal. Unlike the cluster using the usual amino acid, the problem of racemization in the amide condensation was avoided by using the N terminal β-alanine. Furthermore, by using some ω-amino acid (glycine, β-alanine, and GABA) it facilitated the synthesis of the cluster, of which the length of each unit differs. We describe the synthesis of new asymmetric peptide glycoclusters of which the length partially differs.

In this paper we report the synthesis of the glycocluster **1** of dancyl-labelled β-alanine trees, depicted in Scheme 1. The dancyl probe will allow flow cytometric analysis of the interaction and the uptake of the constructions by human dendritic cells.

First, the synthesis of the four desired compounds **9**, **16**, **17**, and **18**, which were selected as the glycocluster units, is shown in Scheme 2. Preparation of the *N*-carboxymethyl β-alanine derivative **7** was prepared from β-alanine by the following six-step procedure. β-Alanine was protected with 2,2,2-trichloroethanol and further, the amino group was protected with *p*-nitrobenzenesulfonyl (*p*NBS) chloride to give **3**. *N*-Carboxymethylation of **3** was carried out by the Mitsunobu protocol[6] with benzyl glycolate to give compound **4**. Removal of the *p*NBS group by PhSH and K_2CO_3 in DMF at 25°C[7] and subsequent *t*-butoxycarbonylation by $(Boc)_2O$ gave compound **6**. This compound **6** has two carboxylic acid derivatives, and it is a chemical compound with the features which can expand the sugar units by being, respectively, independent. Condensation of **7** with sugar unit **8**, which is a simple galactose derivative, in the presence of typical peptide coupling conditions [DEPC (diethyl cyanophosphonate) and Et_3N] was carried out for 16 h at 25°C to give the coupled compound **9**.[8] On the other hand, coupling of **7** with each ω-amino acid (glycine, β-alanine, and GABA) benzyl ester under DEPC for 16 h at 25°C gave dipeptide derivative **10**, **11** and **12**. Subsequent removal of the benzyl ester of compound **10**, **11** and **12** by catalytic hydrogenolysis over 10% Pd–C afforded compounds **13**, **14** and **15**. Furthermore, coupling of **8** with **13**, **14** and **15** in the presence of DEPC as described for **9** gave the different length of each side chain of derivatives **16**, **17** and **18**.[9]

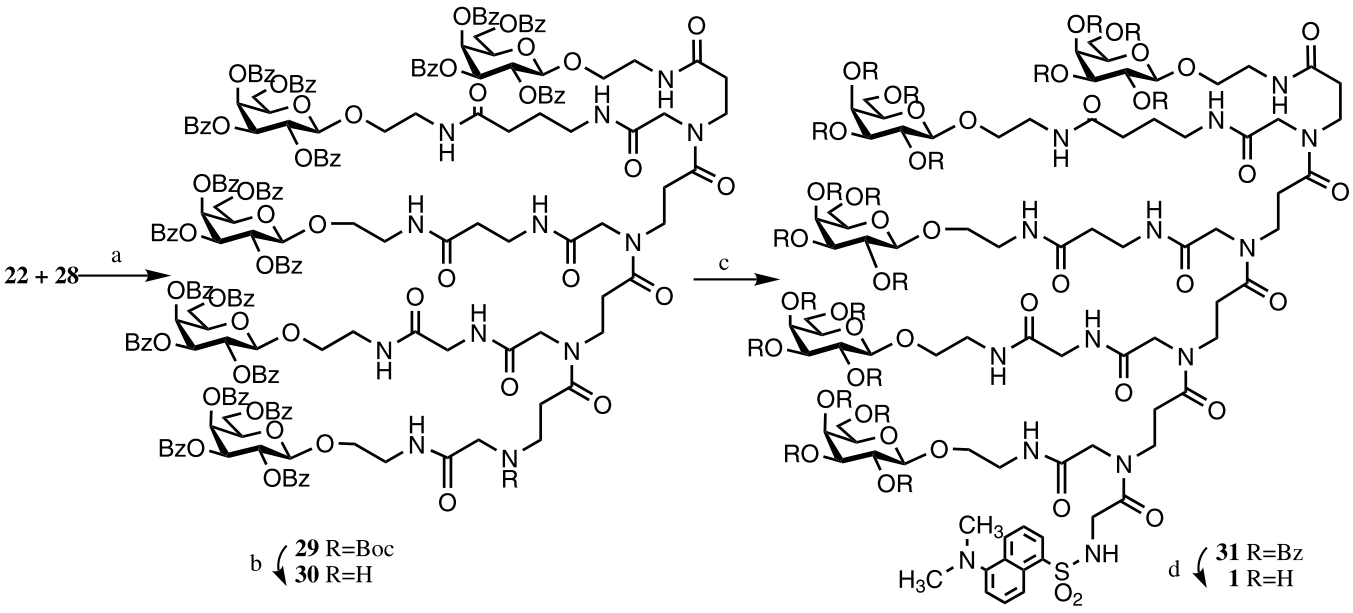


Scheme 2. *Reagents and conditions*: (a) *p*-Nitrobenzenesulfonyl chloride, Et_3N, CH_2Cl_2, 86%; (b) benzyl glycolate, DEAD, PPh_3, CH_2Cl_2, 90%; (c) PhSH, K_2CO_3, DMF, 81%; (d) $(Boc)_2O$, Et_3N, CH_2Cl_2, 94%; (e) Pd-C, H_2, THF, **7**: 84%, **13**: 80%, **14**: 99%, **15**: 82%; (f) sugar unit **8**, DEPC, Et_3N, DMF, **9**: 78%, **16**: 80%, **17**: 76%, **18**: 80%; (g) ω-amino acid benzylester, DEPC, Et_3N, DMF, **10**: 84%, **11**: 82%, **12**: 92%.



Scheme 3. *Reagents and conditions*: (a) Zn–AcOH, **19**: 75%, **22**: 75%, **23**: 89%, **26**: 76%; (b) 50% TFA, **20**: 79%, **24**: 76%, **28**: 82%; (c) DEPC, Et_3N, DMF, **21**: 98%, **25**: 94%; (d) sugar unit **8**, DEPC, Et_3N, DMF, 88%.

Next, compounds **22** and **28** were selected as the N-terminal block and C-terminal block, respectively, and are shown in Scheme 3. Glycocluster unit **19** derived from **9** by reductive removal of the trichloroechyl group using Zn–AcOH was treated with another glycocluster unit **20** derived from **16** by removal of the Boc group using 50% TFA under coupling conditions as described for **9** to afford cluster block **21**. Then, removal of the trichloroechyl group from **21** by Zn–AcOH gave the N-terminal block compound **22**. On the other hand, compound **25** was obtained by coupling of **23** from **17** with **24** from **18** in the presence of DEPC as described for **21**, and subsequent removal of the trichloroechyl group from **25** gave compound **26**. Furthermore, coupling of **26** with sugar unit **8** gave **27** and removal of the Boc group using 50% TFA gave the target C-terminal block compound **28**.



Scheme 4. *Reagents and conditions*: (a) DEPC, Et_3N, DMF, 80%; (b) 50% TFA, 83%; (c) dansyl glycine, DEPC, Et_3N, DMF, 77%; (d) NaOMe, MeOH, 1,4-dioxane, 94%.

Finally, coupling of **22** with **28** in the presence of DEPC as described for **21** gave the pentamer derivative **29**. The Boc group of **29** was removed under acidic conditions with 50% TFA giving a free secondary amino group, which was subsequently treated with dancyl glycine under DEPC giving **31**. Then, complete de-*O*-benzoylation of **31** afforded the target compound **1** with free hydroxyl groups on all the asymmetric glycoclusters.[10] (Scheme 4) At this time, each step gave, respectively, a high yield and there were no by-products generated in the peptide condensation.

In conclusion, efficient and widely applicable synthetic strategies in glycoconjugate chemistry have given access to a new glycocluster. The glycocluster was synthesized this time in order to optionally change the distance from the core to the carbohydrate chain and the distance between carbohydrate chains, thus becoming the cluster with the optimum structure which fits the structure of the carbohydrate chain recognition domain, and higher biological activities are expected.

Typical procedure: To a solution of compound **13** (81.2 mg, 0.19 mmol) and compound **8** (143.0 mg, 0.22 mmol) in DMF (2 mL) were added DEPC (34 μL, 0.22 mmol) and triethylamine (52 μL, 0.37 mmol). The reaction mixture was stirred for 16 h at 25°C. After completion of the reaction, the mixture was extracted with ethyl acetate, washed with water, dried (Mg_2SO_4), and concentrated. The product was purified by silica-gel column chromatography (benzene: acetone = 5:1) as elute to give compound **16** (156.8 mg, 79.6%).

Acknowledgements

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8. Physical data for **9**: $[\alpha]_D^{23}=+59.0°$ ($c=1.9$, $CHCl_3$) ^1H NMR (500 MHz, $CDCl_3$): δ 8.10–7.23 (20H, m, Ar-H), 6.25 (1H, s, NH), 6.00 (1H, d, H-4), 5.77 (1H, dd, $J=10.4$ Hz, H-2), 5.63 (1H, dd, $J=3.1$ Hz, H-3), 4.87 (1H, d, $J=7.9$ Hz, H-1), 4.72 (2H, s, CH_2CCl_3), 4.67 (1H, dd, H-6a), 4.45 (1H, dd, H-6b), 4.36 (1H, m, H-5), 3.98 and 3.76 (2H, m, OCH_2 of sugar unit), 3.65 (2H, s, NCH_2CO), 3.55–3.49 (4H, m, NCH_2 of sugar unit, NCH_2 of β-alanine), 2.72 (2H, t, $J=6.7$ Hz, $COCH_2$ of β-alanine), 1.43 (9H, s, *t*-Bu). ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.3, 166.0, 165.5, 165.3, 133.7, 133.5, 133.4, 133.3, 130.0, 129.7, 129.3, 129.2, 129.0, 128.7, 128.6, 128.5, 128.3, 101.7, 94.8, 80.9, 74.1, 71.6, 71.5, 69.8, 68.9, 68.1, 62.1, 51.7, 44.9, 39.2, 33.0, 28.3. MALDI-TOFMS: calcd for $C_{48}H_{49}Cl_3N_2O_{15}$: 998, found 1021$[M+Na]^+$.
9. Physical data for **16** $[\alpha]_D^{23}=+57.5°$ ($c=1.9$, $CHCl_3$) ^1H NMR (500 MHz, $CDCl_3$): δ 8.10–7.23 (20H, m, Ar-H), 6.42 (1H, s, NH), 6.01 (1H, s, NH), 6.00 (1H, d, H-4), 5.75 (1H, dd, $J_{2,3}=10.4$ Hz, H-2), 5.64 (1H, dd, $J_{3,4}=3.1$ Hz, H-3), 4.84 (1H, d, $J_{1,2}=7.9$ Hz, H-1), 4.71 (2H, s, CH_2CCl_3), 4.69 (1H, dd, H-6a), 4.44 (1H, dd, H-6b), 4.36 (1H, t, H-5), 3.99, 3.75 (2H, m, CH_2O of sugar unit), 3.89 (2H, s, NCH_2CO), 3.68–3.40 (6H, m, $NHCH_2CO$, NCH_2C, $CONHCH_2$), 2.79 (2H, t, $COCH_2C$), 1.46 (9H, s, *t*-Bu). ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.5, 166.1, 165.5, 165.5, 133.7, 133.4, 130.0, 129.7, 129.7, 129.3, 129.0, 128.7, 128.5, 128.3, 101.6, 94.7, 81.3, 74.1, 71.6, 71.4, 70.0, 68.6, 68.1, 52.3, 45.0, 42.5, 39.2, 33.4, 28.3. HR-FABMS: calcd for $C_{50}H_{52}Cl_3N_3O_{16}$: 1055.2413, found 1056.2516$[M+H]^+$.
Physical data **17** $[\alpha]_D^{23}=+53.0°$ ($c=1.8$, $CHCl_3$) ^1H NMR (500 MHz, $CDCl_3$): δ 8.10–7.23 (20H, m, Ar-H), 6.79 (1H, s, NH), 6.00 (1H, d, H-4), 5.96 (1H, s, NH), 5.76 (1H, dd, $J_{2,3}=10.4$ Hz, H-2), 5.65 (1H, dd, $J_{3,4}=3.6$ Hz, H-2), 4.84 (1H, d, $J_{1,2}=7.9$ Hz, H-1), 4.74 (2H, s, CH_2CCl_3), 4.68 (1H, dd, H-6a), 4.44 (1H, dd, H-6b), 4.36 (1H, t, H-5), 4.01, 3.70 (2H, m, OCH_2 of sugar unit), 3.85 (2H, s, $COCH_2N$), 3.60 (2H, t, NCH_2 of β-alanine), 3.57-3.34 (4H, m, NCH_2 of sugar unit, NCH_2 of β-alanine), 2.78 (2H, t, CH_2CO), 2.11 and 1.99 (2H, m, CH_2CO), 1.44 (9H, s, *t*-Bu). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 171.4, 169.1, 166.0, 165.5, 165.4, 133.4, 130.0, 129.7, 129.7, 129.3, 128.9, 128.7, 128.5, 128.3, 101.6, 94.8, 81.0, 74.0, 71.6, 71.3, 69.9, 68.9, 68.0, 62.0, 52.0, 44.8, 39.0, 35.2, 34.7, 33.2, 28.2. HR-FABMS: calcd for $C_{51}H_{54}Cl_3N_3O_{16}$: 1069.2570, found 1070.2609$[M+H]^+$.
Physical data **18** $[\alpha]_D^{23}=+53.5°$ ($c=1.8$, $CHCl_3$) ^1H NMR (500 MHz, $CDCl_3$): δ 8.10–7.23 (20H, m, Ar-H), 6.69 (1H, s, NH), 6.10 (1H, s, NH), 6.01 (1H, d, H-4), 5.77 (1H, dd, H-2), 5.65 (1H, dd, H-3), 4.86 (1H, d, H-1), 4.73 (2H, s, CH_2CCl_3), 4.69 (1H, dd, H-6a), 4.44 (1H, m, H-6b), 4.37 (1H, t, H-5), 4.01 and 3.72 (2H, OCH_2 of sugar unit), 3.85 (2H, s, $COCH_2N$), 3.62 (2H, t, NCH_2 of β-alanine), 3.56-3.34 (2H, m, NCH_2 of sugar unit), 3.18 (2H, m, NCH_2 of GABA), 2.77 (2H, t, CH_2CO of β-alanine), 2.01–1.85 (2H, m, CH_2CO of GABA), 1.67 (2H, m, CCH_2C), 1.44 (9H, s, *t*-Bu). ^{13}C NMR (125 MHz, $CDCl_3$): δ 172.6, 169.5, 166.0, 165.5, 165.4, 133.64, 133.61, 133.3, 120.0, 129.7, 129.3, 129.04, 128.95, 128.64, 128.61, 128.5, 128.3, 101.6, 94.7, 81.0, 74.1, 71.5, 71.4, 69.9, 69.0, 68.1, 62.0, 52.1, 44.9, 39.1, 39.0, 33.3, 28.3, 24.8. HR-FABMS: calcd for $C_{52}H_{56}Cl_3N_3O_{16}$: 1083.2726, found 1084.2780$[M+H]^+$.
10. MALDI-TOFMS of final compound **1**: calcd for $C_{83}H_{134}N_{14}O_{44}$: 2063, found: 2086 $[M+Na]^+$.