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A facile and efficient method for synthesis of macrocyclic lipoglycopeptide

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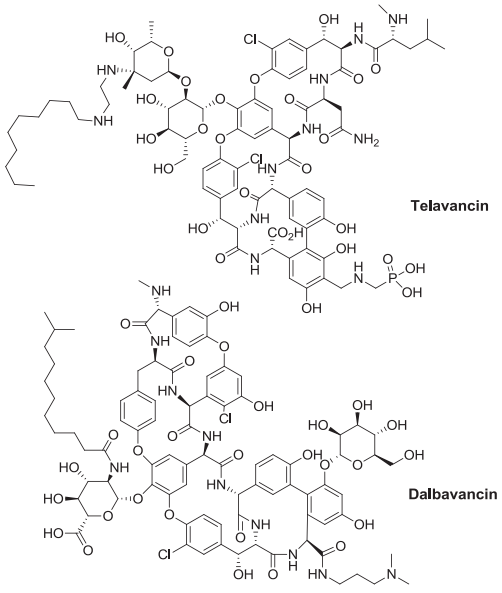
ABSTRACT

An efficient and practical method for macrocyclic lipoglycopeptide synthesis was developed and utilized to synthesize lipoglycosylated derivatives of Tyrocidine A. The method is based on solid-phase peptide synthesis using 2-chlorotrityl resin as the solid-phase support and lipoglycosyl amino acids as building blocks. This synthetic method should be generally applicable to various macrocyclic lipoglycopeptides.

1. Introduction

Lipoglycopeptide antibiotics are actinomycete-derived antibiotics with unique tricyclic or tetracyclic heptapeptide cores that are usually glycosylated and sometimes have additional lipophilic fatty acid side,[1] such as telavancin[2] and dalbavancin[3] (Fig. 1). The glycopeptides antibiotics are the most important drugs in current use for the treatment of Gram-positive bacterial infections.[4] Dalbavancin and telavancin contain a heptapeptide core, common to all glycopeptides, which enables them to inhibit transglycosylation and transpeptidation (cell wall synthesis), and their lipophilic side chains can prolong their half-life, help to anchor the agents to the cell membrane and increase their activity against Gram-positive cocci. In addition to inhibiting cell wall synthesis, telavancin and oritavancin are also able to disrupt bacterial membrane integrity and increase membrane permeability, oritavancin also inhibits RNA synthesis.[2,5] However, Lipoglycopeptides can only be acquired through extraction of natural products,[6] biotransformation,[7] and semisynthesis,[8] which limit the application of lipoglycopeptides in clinic.

Recently, great efforts have been made to obtain macrocyclic peptides and their mimics.[9] In 2009, our group has successfully realized the first total synthesis of macrocyclic glycopeptides and this method has been applied to prepare other macrocyclic glycopeptides.[10] However, chemical synthesis of macrocyclic lipoglycopeptide was not reported and attracted our attention.



Figiure 1. Chemical structure of telavancin and dalbavancin.

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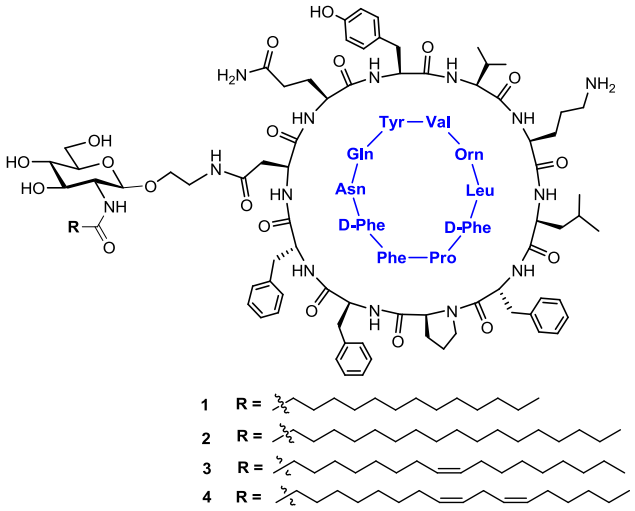
Tyrocidine A is a cyclic decapeptide isolated from *Bacillus* bacteria that exhibits strong bactericidal activities and it has been suggested to primarily target the bacterial membrane. In spite of its severe side effects, Tyrocidine A represents an attractive lead compound for the development of new antibacterial drugs.[11] Herein, a facile and efficient method of synthesizing macrocyclic lipoglycopeptide is described and four lipoglycosyl Tyrocidine A derivatives were designed and synthesized. Further biological evaluation was in progress (Fig. 2).



Figiure 2. Chemical structure of Tyrocidine A lipoglycosylated derivatives.

2. Results and discussion

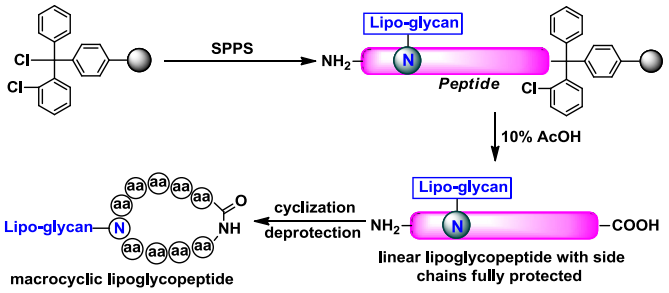
2.1. Synthetic design

Our plan was to first construct lipoglycosylated liner decapeptides by solid-phase peptide synthesis (SPPS) and then unite their C-terminus and N-terminus via solution phase with good yield to obtain macrocyclic lipoglycopeptides. It has been reported that glycosylated amino acids could be directly used as building blocks, just as other simple amino acids in the SPPS.[12] Therefore, in our research, the lipoglycosylated amino acids were directly coupled to the peptide backbone for solid-phase lipoglycosylated liner peptides assembly.

In addition, as the free amino and/or carboxylic groups within their amino acid side chains contained in the title compounds, it was necessary to fully protect the active group within the side chains during the cyclization reaction, which ensured the efficiency of the coupling reactions between the peptide C- and N-termini.[13] In this regard, the extremely acid-sensitive 2-chlorotrityl resin can be an applicable SPPS support, as peptides can be released from this resin using 10% acetic acid, which does not affect the protecting groups of amino acid side chains. Another advantage of using fully protected glycopeptides for cyclic glycopeptide synthesis is that these substrates would be easily soluble in organic solvents, which should be particularly helpful for the cyclization reaction. On the basis of the above considerations, we envisioned a synthetic strategy as outlined in Scheme 1.

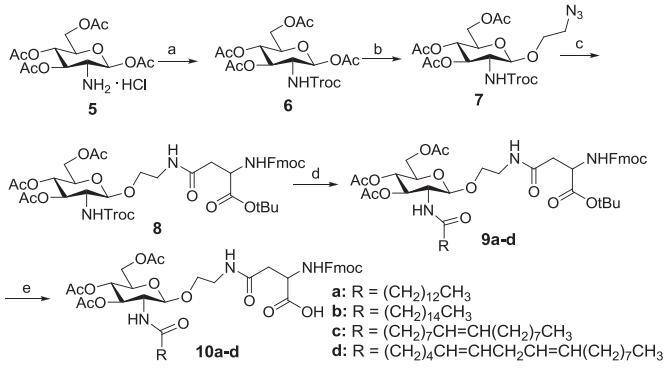
2.2. Synthetic design

Synthetic targets **10a**–**d** have monosaccharide with a long aliphatic chain attached to their asparagine side chain. They were key intermediates for the synthesis of target compounds, and our first undertaking was to prepare **10a**–**d** (Scheme 2). The synthesis of



Scheme 1. Synthesis of full protected lipo-glyco asparagine derivatives.

10a–**d**, as shown in Scheme 2, started from 2-amino-2-deoxy-D-glucose hydrochloride **5**. Firstly, **5** was transformed into compound **6** by well established procedures.[14] Then, the free amino group was protected with a 2,2,2-trichloroethoxycarbonyl (Troc) group, which could be easily removed by zinc.[15] The azide ethyl side chain was introduced into the glucosamine using $BF_3 \cdot Et_2O$ as a catalyst to afford the compound **7**. Next, the azido group was selectively reduced in the condition of Pd/C (10%)/CH_3OH, followed by coupling with Fmoc-Asp-OtBu to afford the compound glycosylated amino acid **8**. Further, when the Troc protected group was cleavaged in the condition of Zn/CH_3COOH, the free amino group can be coupled with fatty acids to afford the key intermediate **9a**–**d**. Finally, the ^{t}Bu group was removed with TFA and the key intermediate **9a**–**d** were converted into the full acetyl protected lipoglycosylated amino acids **10a**–**d**.



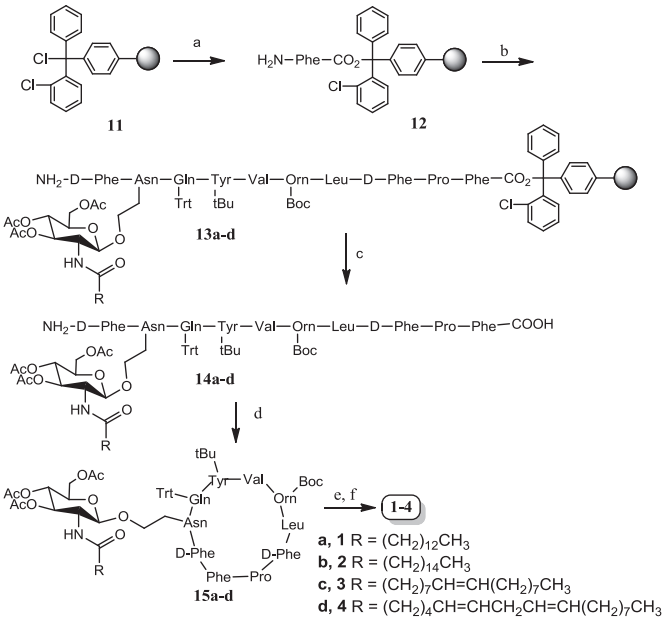
Scheme 2. The synthesis of full protected lipo-glyco amino acids. *Reagents and conditions*: (a) 1,1,1-trichloro-2-(chloromethoxy) ethane, $NaHCO_3$, DCM, 70%; (b) 2-azidoethanol, BF_3Et_2O, DCM, 68%; (c) Pd/C (10%), CH_3OH, 78%; then Fmoc-Asp-OtBu, DIC, HOBt, DCM, DMF, 48%; (d) Zn, CH_3COOH; then lipo-acid, DIC, HOBt, DCM, DMF; (e) TFA: DCM=1:3.

2.3. Synthesis of the title compounds

From a retrosynthetic point of view, any peptide bond of the cyclic peptide can be cleaved to offer a linear structure to work with, so theoretically SPPS can start from any amino acid in the ring. Practically, however, various synthetic plans can give different results.[16] In the case of lipoglycopeptides synthesis, it is readily imaginable that the lipoglycosylated amino acids should be installed at a later stage so as to reduce any potential interferences caused by the lipo-glycans and to facilitate manual installation of the lipoglycosylated amino acids, if necessary.

In our synthesis of the title compounds, we chose to construct the linear peptide/glycopeptides with 2-chlorotrityl resin as the solid-phase support and Phe as the first amino acid to install.[17] In

this case, the full acetyl protected lipoglycosylated amino acids **10a**–**d** would be the second-to-last amino acid to be introduced (Scheme 3). Thus, Phe was firstly anchored to the solid-phase support via reacting trityl resin **11** with Fmoc-Phe-OH. Next, the lipoglycosylated liner decapeptides were assembled via sequential introduction of Fmoc-Pro-OH, Fmoc-D-Phe-OH, Fmoc-Leu-OH, Fmoc-Orn(Boc)-OH, Fmoc-Val-OH, Fmoc-Tyr(^{t}Bu)-OH, Fmoc-Gln(Trt)-OH, Asn derivatives **10a**–**d**, and Fmoc-D-Phe-OH. Fully protected lipoglycosylated liner decapeptides assembly were completely constructed by SPPS using 20% piperidine for the deprotection of Fmoc and DCC and HOBt as the condensation reagents. The coupling processes were monitored by detecting Fmoc released in each deprotection step, which suggested that all coupling reactions were very effective. Finally, the loaded resins were treated with 10% HOAc in trifluoroethanol (TFE) and DCM (1:8) to give linear peptides **14a**–**d**. The released linear peptides were directly used in the next step without further purification.



Scheme 3. The synthesis of the title compounds. *Reagents and conditions*: (a) Fmoc-Phe-OH, DIPEA, DCM; (b) Fmoc-amino acids, DCC, HOBt, DMF, DCM; (c) HOAc, TFE, DCM (1: 2: 16); (d) PyBOP, HOBt, DIPEA, DCM; (e) TFA, Et_3SiH, DCM (2: 1: 8); (f) MeOH, MeONa.

The cyclization of **14a**–**d** was realized via benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP), HOBt, and DIPEA as the condensation reagents. The substrate linear peptides **14a**–**d** were added very slowly (0.5 mg/ml) to the DCM solution of condensation reagents to promote intramolecular reaction. When MS results indicated that the cyclization reactions were done and the linear peptides **14a**–**d** were transferred fully into the cyclic peptides **15a**–**d**, the side chain protecting groups of **15a**–**d** were removed with 20% TFA in DCM containing 10% triethylsilane (TES) and MeONa/MeOH (pH=8) to afford the title compound **1**–**4**. The final products were thoroughly purified by reverse phase HPLC to afford pure **1**–**4** in overall yields (15–22%, starting from the step to load Phe onto the resin).

3. Conclusion

In summary, a highly efficient and versatile synthetic method was developed for macrocyclic lipoglycopeptides. This method is based on solid-phase peptide synthesis using 2-chlorotrityl resin as the solid-phase support and lipoglycosylated amino acids as building blocks. The cyclization reactions of these glycopeptides gave the desired cyclic glycopeptides in practically quantitative yields. This method was employed to synthesize four lipoglycosylated derivatives of tyrocidine A, and it should be applicable to other macrocyclic lipoglycopeptides as well.

4. Experimental section

4.1. General experimental methods

NMR spectra were recorded in $CDCl_3$, CD_3OD or DMSO-d_6 unless otherwise indicated with a Bruker AC-600P spectrometer, using TMS as internal standard. ESI mass spectra were performed on an API-3000 LC-MS spectrometer. HR-Q-TOF-MS were measured on an Agilent 6538 UHD Accurate Mass Q-TOF LC/MS mass spectrometer. Column chromatography was carried out on silica gel (200–300 mesh). The solvents and reagents were used as received or dried prior to use as needed. All reactions were monitored using thin-layer chromatography (TLC) on silica gel plates. Detection was effected by examination under UV light.

4.2. General procedure for full protected lipo-glyco amino acids

4.2.1. 2-(2,2,2-Trichloroacetamido)-1,3,4,6-tetra-O-acetyl-2-deoxy-β-D-glucopyranose ***6***. After $NaHCO_3$ (2.2 g), compound **5** (5 g, 13.1 mmol) were dissolved in water (120 mL), and then 2,2,2-trichloroethyl chloroformate (3.03 g, 14.3 mmol) was added slowly at 0 °C. The reaction mixture was stirred rt overnight. The reaction was washed with water and extracted with DCM. The organic phase was dried over Na_2SO_4, and then condensed in vacuum and solidified with ethyl ether to give the product **6** (3.6 g, 66%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.80 (d, J=4.4 Hz, 1H), 5.43 (d, J=4.7 Hz, 1H), 5.32 (t, J=4.8 Hz, 1H), 5.18 (t, J=4.8 Hz, 1H), 4.79 (s, 2H), 4.35 (dd, J=6.2, 2.2 Hz, 1H), 4.19 (dd, J=6.2, 0.8 Hz, 1H), 4.00 (t, J=5.0 Hz, 1H), 3.93–3.90 (m, 1H), 2.17 (s, 3H), 2.15 (s, 3H), 2.11 (s, 6H). HR-QTOF-MS Calcd for $C_{17}H_{23}Cl_3NO_{11}^+$, $(M+H)^+$, 522.0331, found, 522.0388.

4.2.2. 2-Azidoethoxyl-2-(2,2,2-trichloroacetamido)-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranoside ***7***. After compound **6** (3 g, 5.76 mmol) was dissolved in DCM (40 mL), 2-Azidoethanol (1.43 g, 16.4 mmol) was added at 0 °C. After 30 min, the $BF_3{\cdot}Et_2O$ (2.2 mL) was added slowly and the reaction was stirred at 0 °C for 1 h and then at rt overnight. The reaction mixture was filtered and the filtrate was washed with saturated $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated in vacuum. The residue was purified on a silica gel column (petroleum ether/EtOAc, 7:2) to give pure product **7** as white solid (2.1 g, 68%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.36 (t, J=10.0 Hz, 1H), 5.21 (d, J=8.7 Hz, 1H), 5.08 (t, J=9.9 Hz, 1H), 4.84–4.75 (m, 2H), 4.61 (d, J=8.7 Hz, 1H), 4.27 (dd, J=12.4, 4.6 Hz, 1H), 4.14 (dd, J=12.3, 2.4 Hz, 1H), 4.08–4.03 (m, 1H), 3.74–3.47 (m, 4H), 3.34–3.27 (m, 1H), 2.10 (s, 3H), 2.04 (s, 6H). HR-QTOF-MS Calcd for $C_{17}H_{24}Cl_3N_4O_{10}^+$, $(M+H)^+$, 549.0553, found, 549.0558.

4.2.3. Glycosylated amino acid ***8***. After compound **7** (0.77 g, 1.48 mmol) was dissolved in CH_3OH (15 mL), 10% Pd/C (154 mg) was added into the reaction and H_2 was passed into the mixture overnight. The reaction mixture was filtered and the filtrate was concentrated and dried in vacuum to give crude primary amine. Then, Fmoc-Asp-OtBu (745 mg, 1.81 mmol) was dissolved in DCM (10 mL) and DMF (1 mL), HOBt (305 mg, 2.26 mmol) and DIC (350 μL, 2.26 mmol) were added and the reaction was stirred for 40 min. The obtained primary amine previously and DCM (30 mL) were added

into the reaction mixture and the reaction was stirred overnight. After the reaction was completed, the solution was concentrated and the residue was purified on a silica gel column (petroleum ether/EtOAc, 7:3) to give **8** as white solid, yield: 0.61 g, 48%. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.77 (d, J=7.5 Hz, 2H), 7.62 (d, J=7.3 Hz, 2H), 7.41 (t, J=7.4 Hz, 2H), 7.32 (t, J=7.0 Hz, 2H), 6.18–6.12 (m, 1H), 5.89–5.58 (m, 2H), 5.21 (t, J=9.7 Hz, 1H), 5.06 (t, J=7.3 Hz, 1H), 4.82–4.80 (m, 1H), 4.72 (d, J=12.1 Hz, 1H), 4.54–4.43 (m, 3H), 4.33–4.32 (m, 1H), 4.25 (t, J=6.9 Hz, 2H), 4.16–4.12 (m, 1H), 3.90–3.85 (m, 1H), 3.63–3.54 (m, 4H), 3.32–3.25 (m, 1H), 2.98–2.86 (m, 1H), 2.71–2.66 (m, 1H), 2.08 (s, 3H), 2.01 (s, 6H), 1.51 (s, 9H). HR-QTOF-MS Calcd for $C_{40}H_{49}Cl_3N_3O_{15}^+$, $(M+H)^+$, 916.2224, found, 916.2240.

*4.2.4. General synthetic procedure for glycosylated amino acid **9a–d**.* After compound **8** (0.6 mmol) was dissolved in CH_3COOH (10 mL), Zn (500 mg) was added and the reaction was stirred at rt overnight. After the reaction was completed monitored by TLC, CH_3COOH was removed and the residue was washed with water and extracted with DCM. The organic phase was dried over Na_2SO_4, and then condensed in vacuum and used directly in next step. The lipo acid (0.5 mmol) was dissolved in DCM (10 mL) and DMF (1 mL), HOBt (0.5 mmol) and DIC (0.5 mmol) were added and the reaction was stirred for 40 min. Then the amine obtained previously (0.5 mmol) were added into the reaction mixture and the reaction was stirred overnight. After the reaction was completed, the solution was concentrated and the residue was purified on a silica gel column (DCM/MeOH, 25:1) to give pure product.

Compound **9a**: Yield 262 mg, 55%. ^{1}H NMR (600 MHz, MeOD): δ 7.80 (d, J=7.5 Hz, 2H), 7.60 (d, J=7.4 Hz, 2H), 7.56 (s, 1H), 7.43 (t, J=7.3 Hz, 2H), 7.34 (t, J=7.4 Hz, 2H), 5.21 (t, J=9.6 Hz, 1H), 5.05 (t, J=9.5 Hz, 1H), 4.58 (d, J=8.5 Hz, 1H), 4.51–4.48 (m, 1H), 4.45–4.42 (m, 1H), 4.38–4.35 (m, 1H), 4.31–4.26 (m, 2H), 4.19–4.12 (m, 1H), 4.00 (t, J=9.8 Hz, 1H), 3.89–3.82 (m, 1H), 3.78–3.72 (m, 1H), 3.67–3.62 (m, 1H), 3.50–3.42 (m, 1H), 3.35–3.31 (m, 1H), 2.82–2.72 (m, 2H), 2.20–2.14 (m, 2H), 2.10 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 1.62–1.56 (m, 2H), 1.50 (s, 9H), 1.34–1.25 (m, 20H), 0.91 (t, J=6.9 Hz, 3H). HR-QTOF-MS Calcd for $C_{51}H_{74}N_3O_{14}^+$, $(M+H)^+$, 952.5165, found, 952.5184.

Compound **9b**: Yield 284 mg, 58%. ^{1}H NMR (600 MHz, MeOD): δ 7.78 (d, J=7.5 Hz, 2H), 7.63 (d, J=7.4 Hz, 2H), 7.52 (s, 1H), 7.40 (t, J=7.3 Hz, 2H), 7.34 (t, J=7.5 Hz, 2H), 5.20 (t, J=9.5 Hz, 1H), 5.03 (t, J=9.6 Hz, 1H), 4.54 (d, J=8.4 Hz, 1H), 4.53–4.47 (m, 1H), 4.45–4.42 (m, 1H), 4.38–4.35 (m, 1H), 4.31–4.26 (m, 2H), 4.19–4.12 (m, 1H), 4.02 (t, J=9.8 Hz, 1H), 3.89–3.84 (m, 1H), 3.79–3.72 (m, 1H), 3.68–3.62 (m, 1H), 3.50–3.42 (m, 1H), 3.35–3.31 (m, 1H), 2.92–2.84 (m, 2H), 2.22–2.14 (m, 2H), 2.12 (s, 3H), 2.08 (s, 3H), 2.03 (s, 3H), 1.65–1.54 (m, 2H), 1.52 (s, 9H), 1.36–1.22 (m, 24H), 0.89 (t, J=6.9 Hz, 3H). HR-QTOF-MS Calcd for $C_{53}H_{78}N_3O_{14}^+$, $(M+H)^+$, 980.5484, found, 980.5434.

Compound **9c**: Yield 287 mg, 57%. ^{1}H NMR (600 MHz, MeOD): δ 7.78 (d, J=7.5 Hz, 2H), 7.64 (d, J=7.4 Hz, 2H), 7.43 (s, 1H), 7.41 (t, J=7.3 Hz, 2H), 7.33 (t, J=7.4 Hz, 2H), 5.39–5.33 (m, 2H), 5.18 (t, J=9.5 Hz, 1H), 5.04 (t, J=9.7 Hz, 1H), 4.55 (d, J=8.4 Hz, 1H), 4.49–4.47 (m, 1H), 4.45–4.40 (m, 1H), 4.37–4.31 (m, 2H), 4.29–4.25 (m, 2H), 4.17–4.12 (m, 2H), 3.99 (t, J=9.8 Hz, 1H), 3.86–3.82 (m, 1H), 3.74–3.70 (m, 1H), 3.64–3.58 (m, 1H), 3.46–3.41 (m, 1H), 3.36–3.30 (m, 1H), 2.84–2.69 (m, 2H), 2.20–2.11 (m, 2H), 2.09 (s, 3H), 2.07–2.00 (m, 10H), 1.61–1.54 (m, 2H), 1.49 (s, 9H), 1.40–1.25 (m, 20H), 0.91 (t, J=6.9 Hz, 3H). HR-QTOF-MS Calcd for $C_{55}H_{80}N_3O_{14}^+$, $(M+H)^+$, 1006.5635, found, 1006.5684.

Compound **9d**: Yield 266 mg, 53%. ^{1}H NMR (600 MHz, MeOD): δ 7.80 (d, J=7.5 Hz, 2H), 7.64 (d, J=7.4 Hz, 2H), 7.47 (s, 1H), 7.45 (t, J=7.3 Hz, 2H), 7.33 (t, J=7.4 Hz, 2H), 5.49–5.37 (m, 4H), 5.18 (t, J=9.5 Hz, 1H), 5.09 (t, J=9.7 Hz, 1H), 4.59 (d, J=8.4 Hz, 1H), 4.49–4.45 (m, 1H), 4.45–4.39 (m, 1H), 4.35–4.31 (m, 2H), 4.29–4.25 (m, 2H), 4.19–4.12 (m, 2H), 4.02 (t, J=9.8 Hz, 1H), 3.86–3.80 (m, 1H), 3.74–3.70 (m, 1H), 3.64–3.60 (m, 1H), 3.46–3.41 (m, 1H), 3.36–3.28 (m, 1H), 2.84–2.69 (m, 2H), 2.64–2.59 (m, 2H), 2.20–2.11 (m, 2H), 2.09 (s, 3H), 2.01–1.95 (m, 10H), 1.61–1.54 (m, 2H), 1.49 (s, 9H), 1.40–1.25 (m, 14H), 0.95 (t, J=6.9 Hz, 3H). HR-QTOF-MS Calcd for $C_{55}H_{78}N_3O_{14}^+$, $(M+H)^+$, 1004.5478, found, 1004.5404.

*4.2.5. General synthetic procedure for glycosylated amino acid **10a–d**.* After the full protected glycosylated amino acid (0.1 mmol) was dissolved in TFA/DCM (1:3) (15 mL) and the reaction was stirred for 1 h, the solution was concentrated and the residue was purified on a silica gel column (DCM/MeOH, 15:1) to give pure product **10a–d** as white solid.

Compound **10a**: Yield 85%. ^{1}H NMR (600 MHz, DMSO): δ 7.95 (d, J=7.5 Hz, 2H), 7.93–7.86 (m, 2H), 7.75 (d, J=7.4 Hz, 2H), 7.55 (s, 1H), 7.47 (t, J=7.5 Hz, 2H), 7.38 (t, J=7.4 Hz, 2H), 5.14 (t, J=9.3 Hz, 1H), 4.88 (t, J=9.4 Hz, 1H), 4.69 (d, J=8.5 Hz, 1H), 4.43–4.35 (m, 1H), 4.34–4.29 (m, 2H), 4.29–4.20 (m, 2H), 4.09–4.02 (m, 1H), 3.91–3.85 (m, 1H), 3.85–3.77 (m, 1H), 3.74–3.67 (m, 1H), 3.58–3.52 (m, 1H), 3.32–3.26 (m, 1H), 3.24–3.17 (m, 1H), 2.68–2.62 (m, 1H), 2.54–2.48 (m, 1H), 2.09–2.04 (m, 5H), 2.02 (s, 3H), 1.94 (s, 3H), 1.53–1.43 (m, 2H), 1.30–1.24 (m, 20H), 0.89 (t, J=6.9 Hz, 3H). HR-QTOF-MS Calcd for $C_{47}H_{66}N_3O_{14}^+$, $(M+H)^+$, 896.4539, found, 896.4552.

Compound **10b**: Yield 83%. ^{1}H NMR (600 MHz, DMSO): δ 7.94 (d, J=7.5 Hz, 2H), 7.93–7.86 (m, 2H), 7.75 (d, J=7.4 Hz, 2H), 7.55 (s, 1H), 7.47 (t, J=7.5 Hz, 2H), 7.38 (t, J=7.4 Hz, 2H), 5.14 (t, J=9.2 Hz, 1H), 4.88 (t, J=9.5 Hz, 1H), 4.68 (d, J=8.7 Hz, 1H), 4.43–4.30 (m, 2H), 4.29–4.21 (m, 2H), 4.09–4.03 (m, 1H), 3.91–3.85 (m, 1H), 3.83–3.78 (m, 1H), 3.73–3.67 (m, 1H), 3.59–3.52 (m, 1H), 3.32–3.26 (m, 1H), 3.24–3.17 (m, 1H), 2.68–2.60 (m, 1H), 2.54–2.47 (m, 1H), 2.09–2.03 (m, 5H), 2.01 (s, 3H), 1.94 (s, 3H), 1.52–1.45 (m, 2H), 1.31–1.25 (m, 24H), 0.92–0.89 (m, 3H). HR-QTOF-MS Calcd for $C_{49}H_{70}N_3O_{14}^+$, $(M+H)^+$, 924.4852, found, 924.4885.

Compound **10c**: Yield 90%. ^{1}H NMR (600 MHz, MeOD): δ 7.95 (d, J=7.5 Hz, 2H), 7.93–7.86 (m, 2H), 7.75 (d, J=7.4 Hz, 2H), 7.58 (d, J=7.1 Hz, 1H), 7.47 (t, J=7.3 Hz, 2H), 7.38 (t, J=7.4 Hz, 2H), 5.42–5.31 (m, 2H), 5.13 (t, J=9.5 Hz, 1H), 4.88 (t, J=9.7 Hz, 1H), 4.69 (d, J=8.6 Hz, 1H), 4.44–4.37 (m, 1H), 4.34–4.20 (m, 4H), 4.09–4.02 (m, 1H), 3.90–3.84 (m, 1H), 3.83–3.77 (m, 1H), 3.73–3.66 (m, 1H), 3.59–3.52 (m, 1H), 3.32–3.27 (m, 1H), 3.23–3.18 (m, 1H), 2.69–2.62 (m, 1H), 2.10–1.98 (m, 12H), 1.52–1.44 (m, 2H), 1.36–1.23 (m, 20H), 0.90 (t, J=7.1 Hz, 3H). HR-QTOF-MS Calcd for $C_{51}H_{72}N_3O_{14}^+$, $(M+H)^+$, 950.5009, found, 950.4990.

Compound **10d**: Yield 88%. ^{1}H NMR (600 MHz, MeOD): δ 7.94 (d, J=7.5 Hz, 2H), 7.92–7.88 (m, 2H), 7.75 (d, J=7.4 Hz, 2H), 7.60 (d, J=7.1 Hz, 1H), 7.47 (t, J=7.3 Hz, 2H), 7.38 (t, J=7.4 Hz, 2H), 5.47–5.34 (m, 4H), 5.13 (t, J=9.5 Hz, 1H), 4.88 (t, J=9.7 Hz, 1H), 4.69 (d, J=8.6 Hz, 1H), 4.49–4.45 (m, 1H), 4.45–4.38 (m, 1H), 4.35–4.31 (m, 2H), 4.29–4.25 (m, 2H), 4.16–4.12 (m, 2H), 4.02 (t, J=9.4 Hz, 1H), 3.86–3.80 (m, 1H), 3.74–3.70 (m, 1H), 3.64–3.60 (m, 1H), 3.46–3.41 (m, 1H), 3.36–3.28 (m, 1H), 2.84–2.69 (m, 2H), 2.66–2.59 (m, 2H), 2.19–2.13 (m, 2H), 2.12–2.08 (m, 4H), 2.01–1.95 (m, 9H), 1.57–1.44 (m, 2H), 1.40–1.25 (m, 14H), 0.95 (t, J=6.9 Hz, 3H). HR-QTOF-MS Calcd for $C_{51}H_{70}N_3O_{14}^+$, $(M+H)^+$, 948.4852, found, 948.4870.

4.3. Synthesis of the title compounds 1–4 (common procedures)

After a mixture of trityl resin **11** (100 mg, 0.1 mmol) and Fmoc-Phe-OH (98 mg, 0.24 mmol) was shaken on a vortex mixer at rt for 0.5 h, the resin was filtered off and washed several times with MeOH and DCM. Then piperidine/DMF(v/v, 1:4) (10 mL) was added and the resin was shaken at rt for 3 h. The resin was filtered off and washed several times with DCM to give the product **12**. Then HOBt

(40 mg, 0.3 mmol), Fmoc-Pro-OH (67 mg, 0.2 mmol) and DCC (62 mg, 0.3 mmol) were dissolved in DCM (8 mL) and DMF (2 mL) and stirred for 0.5 h, the residue was mixed with the resin and the resin was shaken for 3 h. After that, the resin was filtered off and washed several times with DMF and DCM, and then piperidine/DMF(v/v, 1:4) (10 mL) was added and the resin was shaken at rt for 3 h. The resin was filtered off and washed several times with DCM, here the first Pro had been loaded on the resin. Then amino acids Fmoc-D-Phe-OH, Fmoc-Leu-OH, Fmoc-Orn(Boc)-OH, Fmoc-Val-OH, Fmoc-Tyr(^{t}Bu)-OH, Fmoc-Gln(Trt)-OH, full protected lipo-glyco amino acids **10a**–**d** and Fmoc-D-Phe-OH were sequentially loaded to construct the resins, which contains the linear lipo-glyco peptides **13a**–**d**.

To cleave the linear peptides from the resin, the lipo-glyco peptide loaded resin was treated with a mixture of HOAc and TFE in DCM (1:2:16, 15 mL) for 1 h at rt. The resin was filtered off and was washed with DCM. The washings were combined and condensed together with toluene three times in vacuum to give **14a**–**d**. These crude products were applied to cyclization without any purification.

For lipo-glyco peptides cyclization, when PyBOP (3 equiv), HOBt (3 equiv) and DIPEA (6 equiv) were dissolved in DCM (200 mL) in a round-bottom flask, compounds **14a**–**d** that dissolved in DCM (0.5 mg/ml) were added to the solution over a period of 4 h. After the addition was finished, the mixture was stirred at rt overnight. Then, DCM was removed and the resulting residue was briefly purified by Sephadex LH-20 to afford **15a**–**d**. Compounds **15a**–**d** were dissolved in TFA/DCM/Et_3SiH (2:8:1) (15 mL) and the reaction was stirred at rt for 1 h. Then the reaction mixture was concentrated to dryness. At last, the residues were dissolved in MeONa/MeOH (pH=8) (15 mL), the reaction was stirred at rt for another hour, and then the solution was concentrated in vacuum, finally the residues were purified by HPLC (Supelco Discovery C18 250 mm×25 mm column, Solution A was 0.1% TFA in water, and solution B was 0.1% TFA in MeCN. Gradient: A linear gradient of 25%–25% B in first 5 min, then a linear gradient of 25%–90% B in 5–30 min, then 90% in last 10 min, 10 mL/min) to give title compounds **1**–**4**.

Title compound **1**, retention time: 16 min, white solid (7.5 mg, 19%). ^{1}H NMR (600 MHz, MeOD): δ 7.88–6.45 (m, 19H), 5.84 (s, 1H), 5.44 (s, 1H), 4.95 (s, 1H), 4.74 (s, 1H), 4.68–4.57 (m, 3H), 4.49 (s, 1H), 4.39 (d, J=7.4 Hz, 1H), 4.21–4.16 (m, 1H), 4.13–4.09 (m, 1H), 3.84 (d, J=10.0 Hz, 2H), 3.81–3.63 (m, 4H), 3.62–3.57 (m, 1H), 3.56–3.51 (m, 1H), 3.45 (t, J=10.0 Hz, 1H), 3.36–3.32 (m, 2H), 3.30–3.17 (m, 6H), 3.09–3.02 (m, 2H), 2.89–2.80 (m, 1H), 2.49–2.41 (m, 1H), 2.27–2.17 (m, 5H), 2.10–1.83 (m, 7H), 1.78–1.70 (m, 2H), 1.64–1.49 (m, 5H), 1.45–1.22 (m, 32H), 1.20–1.05 (m, 9H), 0.98–0.5 (m, 6H). HR-QTOF-MS Calcd for $C_{88}H_{129}N_{14}O_{19}^+$, $(M+H)^+$, 1686.9626, found 1686.9624.

Title compound **2**, retention time: 20 min, white solid (6.5 mg, 22%). ^{1}H NMR (600 MHz, MeOD): δ 7.92–6.44 (m, 19H), 5.89 (d, J=11.1 Hz, 1H), 5.56–5.49 (m, 1H), 5.05–5.02 (m, 1H), 4.81–4.77 (m, 2H), 4.72–4.62 (m, 4H), 4.57–4.52 (m, 2H), 4.39 (d, J=9.8 Hz, 1H), 4.23–4.12 (m, 2H), 3.87–3.86 (m, 1H), 3.84–3.81 (m, 1H), 3.80–3.76 (m, 1H), 3.75–3.71 (m, 1H), 3.69–3.65 (m, 1H), 3.62–3.56 (m, 2H), 3.48–3.42 (m, 2H), 3.36–3.23 (m, 5H), 3.20 (s, 1H), 3.12–3.06 (m, 1H), 2.91–2.83 (m, 1H), 2.51–2.44 (m, 1H), 2.33–2.16 (m, 5H), 2.11–2.02 (m, 2H), 1.88–1.81 (m, 4H), 1.76–1.67 (m, 3H), 1.63–1.57 (m, 2H), 1.54–1.48 (m, 1H), 1.41–1.25 (m, 29H), 1.26–1.14 (m,12H), 0.96 (t, J=7.0 Hz, 3H). HR-QTOF-MS Calcd for $C_{90}H_{133}N_{14}O_{19}^+$, $(M+H)^+$, 1714.9939, found 1714.9902.

Title compound **3**, retention time: 23 min, white solid (7.0 mg, 15%). ^{1}H NMR (600 MHz, MeOD): δ 9.30–8.38 (m, 11H), 7.75–7.25 (m, 19H), 6.97–6.91 (m, 1H), 6.74–6.66 (m, 1H), 5.88–5.63 (m, 3H), 5.40–5.35 (m, 1H), 4.71–4.65 (m, 4H), 4.40 (d, J=7.8 Hz, 1H), 4.24–4.15 (m, 5H), 4.11–4.05 (m, 2H), 3.81–3.50 (m, 3H), 3.29–3.21 (m, 17H), 2.85–2.46 (m, 5H), 2.24 (t, J=7.6 Hz, 6H), 2.05 (s, 8H), 1.62–1.33 (m, 20H), 1.08–0.92 (m, 9H), 0.58–0.51 (m, 6H). HR-QTOF-MS Calcd for $C_{92}H_{135}N_{14}O_{19}^+$, $(M+H)^+$, 1741.0095, found, 1741.0041.

Title compound **4**, retention time: 22 min, white solid (9.5 mg, 19%). ^{1}H NMR (600 MHz, MeOD): δ 9.23–8.57 (m, 11H), 7.92–7.01 (m, 19H), 6.72–6.67 (m, 1H), 6.47–6.43 (m, 1H), 5.50–5.26 (m, 6H), 4.94–4.78 (m, 5H), 4.59–4.24 (m, 5H), 4.24–3.96 (m, 3H), 3.43–3.32 (m, 20H), 2.73–2.68 (m, 2H), 2.40–2.09 (m, 7H), 1.91–1.61 (m, 18H), 1.60–1.41 (m, 3H), 1.41–1.05 (m, 14H), 1.19–1.00 (m, 9H), 0.89–0.54 (m, 6H). HR-QTOF-MS Calcd for $C_{92}H_{133}N_{14}O_{19}^+$, $(M+H)^+$, 1738.9938, found, 1738.9932.

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Supplementary data

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