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Graphical Abstract

Conformational studies of glycosylated cyclic oligomers of furanoid sugar amino acids

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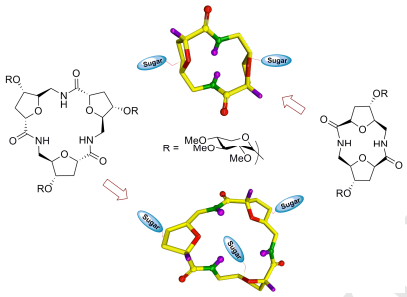

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ABSTRACT

Glycosylation of molecules improve their pharmacological and pharmacokinetic properties. In the current manuscript, we have explored the effect of glycosylation on the structure and function of conformationally well-defined small ring homooligomers derived from a structurally diverse library of sugar amino acids (SAA). Conformational analyses carried out by NMR suggested that these cyclic dimers and trimers have well-defined structures in solution. MD simulations performed based on the restraints obtained from NMR revealed that C2H and CO are positioned outside the plane of the ring and NHs are pointed inside the ring. It was encouraging to note that while the cyclic non-glycosylated homooligomers did not show any antimicrobial activity at all, their glycosylated counterparts showed relatively better activity. The modular design developed here is amenable to further improvement and can serve as a tool to investigate many molecular recognition processes.

1. Introduction

Glycosylated natural products are abundant displaying wide ranging antimicrobial, antifungal and/or anticancer activities.[1] Many bacteria also use glycosylated small molecules as chemical weapons to gain a selective advantage or as signaling molecules for intra- and interspecie[1]s communication.[2] Sugar moieties in these natural products and metabolites dramatically improve their pharmacological and pharmacokinetic properties, such as solubility, cellular permeability, distribution and metabolic stability.[3] They also impact the delivery of the natural product to the target, present high affinity and specificity for a given target, tissue, cell, as well as modulate both mechanism and *in vivo* properties.[4] Given that carbohydrates occupy a very large chemical space, differential glycosylation of natural products and/or synthetic small molecules offers a viable strategy to produce new chemical entities with improved pharmacological properties and biological activities.[5] This encouraged us to explore the effect of glycosylation on the structure and function of conformationally well-defined small ring homooligomers derived from a structurally diverse library of sugar amino acids (SAA). To begin with, the choice of the carbohydrate to be attached to these cyclic peptides stemmed from many naturally occurring C_2-symmetric cyclic diolides isolated from marine

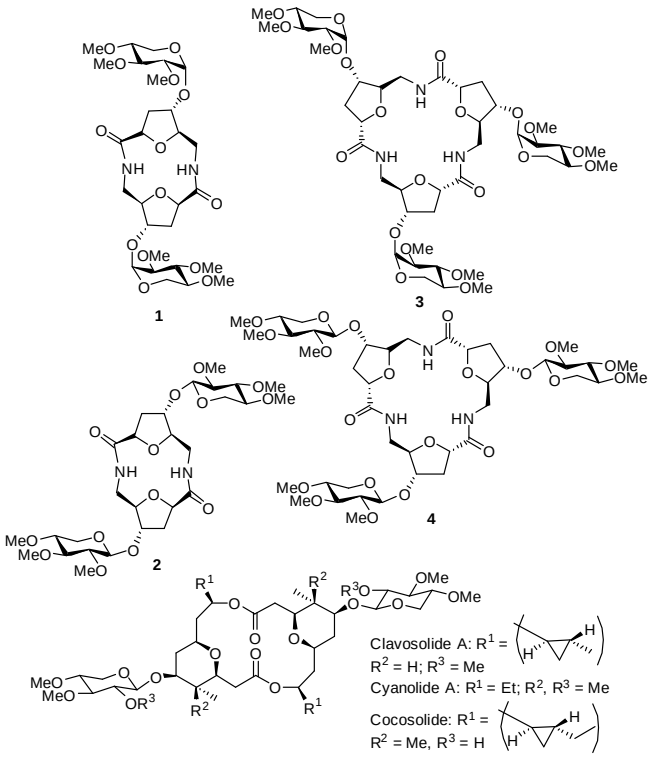

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Figure 1. Structures of glycosylated cyclic peptides of sugar amino acids, **1-4** and C_2-symmetric diolide natural products.

cyanobacteria, like clavosolides,[6] cyanolides,[7] cocosolide,[8] etc. (Figure 1), all of which carry methylated xylopyranosides. In this paper, we describe the synthesis, conformational studies and biological activities of cyclic homooligomers **1-4** of furanoid sugar amino acids,[9] glycosylated with tri-*O*-methyl-D-xylopyranosyl sugar.

2. Results and discussion

The synthesis of the cyclic glycopeptides **1-4** was carried out in a similar fashion as reported by us and others[10] using solution phase peptide synthesis in which the glycosylated sugar amino acid (SAA) monomers **5-8** (Figure 2) were converted to their cyclic homooligomer counterparts efficiently. The construction of the monomers **5-8** (**5** for **1**, **6** for **2**, **7** for **3** and **8** for **4**) was initiated using 2-deoxy-D-ribose (for glycosyl acceptor residues **12** and **13**) and D-xylose (for glycosyl donor residue **14**) as the starting raw materials (Scheme 1). The monomers **5-6** differ from monomers **7-8** in the configuration of the stereocenter at C2 of the furanoid rings of their constituent *δ*-SAA moieties (Scheme 1). But the monomers **5** (or **7**) and **6** (or **8**) departed from each other with respect to the type of *O*-glycosidic linkage present in them, *i.e.* monomer **5** (or **7**) possesses *α*-*O*-glycosidic linkage while monomer **6** (or **8**) carry *β*-*O*-glycosidic linkage. While monomers **5** and **6** were used to get solely the C_2-symmetric glycosylated homooligomers **1** and **2**, respectively, monomers **7** and **8** furnished C_3-symmetric glycosylated homooligomers **3** and **4**, respectively as the major products (Schemes 2 and 3).

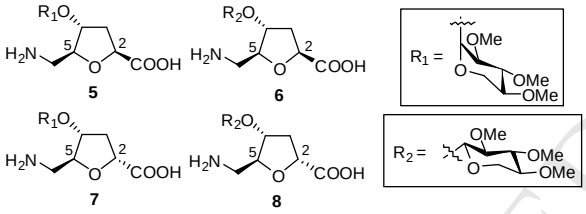
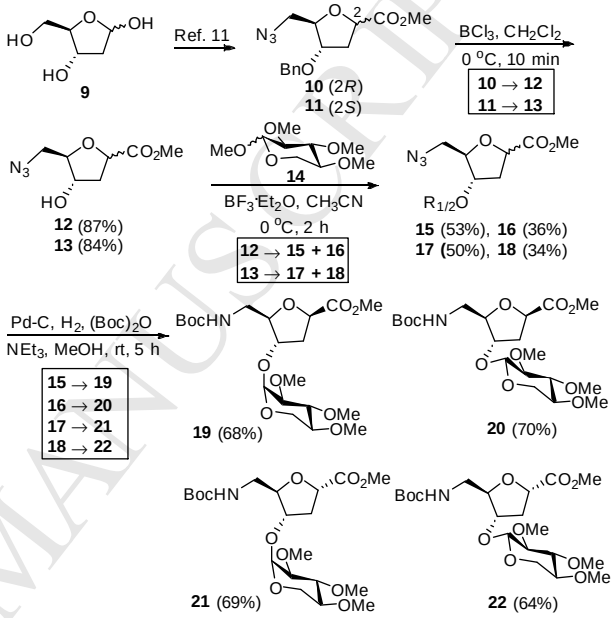


Figure 2. Glycosylated *δ*-SAA monomers **5-8**.

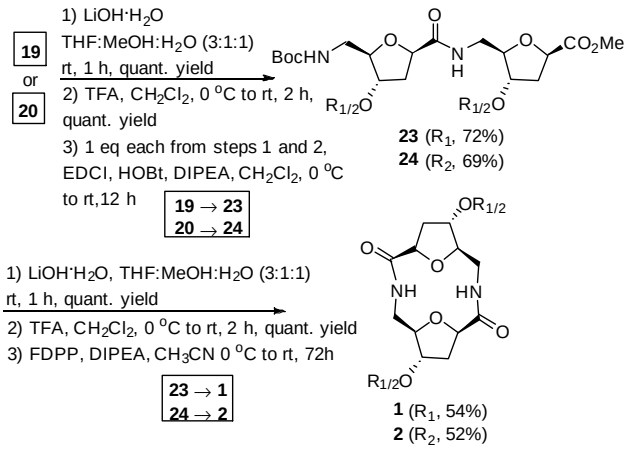
The synthesis of the protected SAA precursors (**19-22**), each representing the monomers **5-8** sequentially, was carried out by *O*-glycosylation of **12-13** using the glycosyl donor **14** (Scheme 1). The preparation of **12-13** commenced from commercially available 2-deoxy-D-ribose (**9**) which was easily and efficiently converted in to diastereomeric intermediates **10** and **11** as reported earlier from our lab (Scheme 1).[11] BCl_3-mediated benzyl ether deprotection[12] of intermediates **10** (C2-*R*-isomer) and **11** (C2-*S*-isomer) furnished **12** and **13**, respectively, in excellent yields. Thereafter, $BF_3 \cdot Et_2O$ mediated *O*-glycosyaltion reaction[13] was implemented between substrates **12-13** and the glycosyl donor substrate, permethylated-D-xylose[14] **14**, resulting in the formation of *α*-*O*-glycosylated product **15** along with *β*-*O*-glycosylated product **16** (from **12**, overall 89% yield) and *α*-*O*-glycosylated product **17** along with *β*-*O*-glycosylated product **18** (from **13**, overall 84% yield), respectively in a ratio of 1.5:1 (*α*:*β*) after chromatographic separation. The azido moiety in the intermediates **15-18** were subjected to *in situ* one-pot reduction and Boc-protection reactions using Pd-C/H_2 and $(Boc)_2O$-NEt_3, furnishing compounds **19-22** in good yields (Scheme 1).

After acquiring the monomeric building blocks **19-22**, we proceeded for the synthesis of glycosylated cyclic homooligomers **1-4**. Firstly, syntheses of C_2-symmetric cyclic peptides **1** and **2** were accomplished from the monomers **19** and **20**, respectively (Scheme 2). As we had reported in our earlier paper[10d] that similar kind of substrates with '2,5-*cis*' ring lead to the bicyclic lactam formation, but linear dimers from such substrates furnished the requisite products on cyclization. Thus, the monomer **19** was subjected, separately in equimolar amounts, to LiOH-mediated saponification in THF-MeOH-H_2O solvent system and TFA-mediated Boc-deprotection in CH_2Cl_2 to provide the acid and the TFA-salt, respectively, in quantitative yields. Next, the acid and TFA-salt were coupled using conventional solution phase peptide synthesis method using 1-ethyl-3-(3-(dimethylamino)propyl)-carbodiimide hydrochloride (EDCI) and 1-hydroxybenzotriazole (HOBt) as coupling reagents and diisopropylethylamine (DIPEA) as base in CH_2Cl_2 to provide the linear precursor **23** in 72% yield (Scheme 2).



Scheme 1. Synthesis of *O*-glycosylated and protected precursors **19-22**.

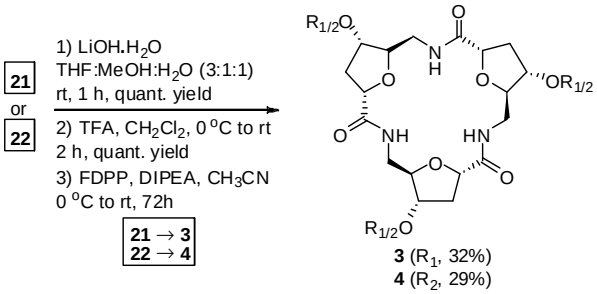
The monomer **20** led to the other linear precursor **24** in 69% yield using the same reagents under identical reaction conditions. Both the linear precursors **23** and **24**, separately, were subjected sequentially to LiOH-mediated ester hydrolysis followed by TFA-mediated Boc-deprotection to furnish TFA-salt which underwent intramolecular cyclization in the presence of pentafluorophenyl diphenyphosphinate (FDPP) as coupling reagent[15] and DIPEA as base in dry CH_3CN (5 x 10^{-3} M) to furnish the required products **1** in 54% yield and **2** in 52% yield,



respectively, as the only isolable products (Scheme 2).

Scheme 2. Synthesis of the C_2-symmetric glycosylated cyclodimers **1** and **2**.

The syntheses of C_3-symmetric glycosylated homooligomers **3** and **4** were commenced from the monomers **21** and **22**, respectively (Scheme 3). Both the monomeric precursors were, separately and sequentially, subjected to LiOH-mediated ester hydrolysis in THF-MeOH-H_2O solvent system to provide the acids which further underwent TFA-mediated Boc-deprotection in CH_2Cl_2 to give TFA-salts in quantitative yields. The TFA-salts were then subjected to homooligomerization using the same reagents under identical reaction conditions as described above (for the synthesis of compounds **1** and **2**) to furnish the glycosylated tricyclic peptides **3** and **4** as the major products in 32% and 29% yield, respectively (the corresponding C_2-symmetric glycosylated dimers were also formed in both cases, but in negligible yields).



Scheme 3. Synthesis of the C_3-symmetric glycosylated cyclotrimers **3** and **4**.

3. Conformational Analysis: NMR studies.

Conformational studies of cyclic glycopeptides **1-4** were carried out in $CDCl_3$ with the sample concentrations of 2–5 mM. Both ^1H, ^{13}C spectra of cyclodimers **1** and **2** showed only one set of sharp resonances corresponding to the constituent δ-SAA moiety indicating C_2-symmetry among these cyclic peptides. The vicinal coupling constant values ($^3J_{NH\text{-}C6H}$) for **1** and **2** showed either < 3 Hz or > 10 Hz, which indicated that the dihedral angle φ (CO–N–C6–C5) was about 120°. $^3J_{C6H\text{–}C5H}$ values of 4 Hz and 0 Hz clearly supported the dihedral angle θ (N–C6–C5–O) as –30° (Figure 3). Observations of nOe cross peaks between NH↔C6H$_{(pro\text{-}R)}$, C5H↔C6H$_{(pro\text{-}S)}$ and C5H↔C6H$_{(pro\text{-}R)}$ suggested a (–) synclinal (*sc*) rotation about C5–C6 bond in both the glycopeptides **1** and **2**. Additionally, nOes between NH↔C5H, C5H↔C2H, C5H↔C2H and C4H↔C6H$_{(pro\text{-}R)}$ confirmed the (–) *sc* rotation in both these glycopeptides (Figures 3 and 4). Both, nOe correlations [C5H–C3H$_{(pro\text{-}R)}$] and vicinal coupling constant values $^3J_{C2H\text{–}C3H(pro\text{-}S)}$ of 1.1 Hz and $^3J_{C2H\text{–}C3H(pro\text{-}R)}$ of 7.4 Hz suggested that the five-membered furanoid ring was with C_4-*endo* ring puckering in both **1** and **2**.

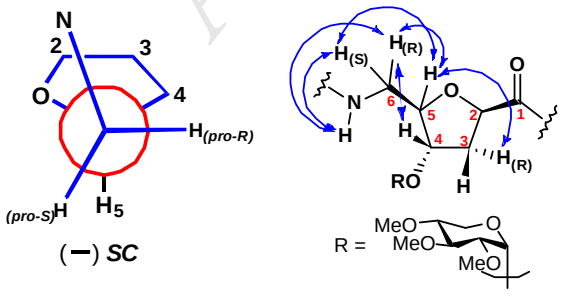
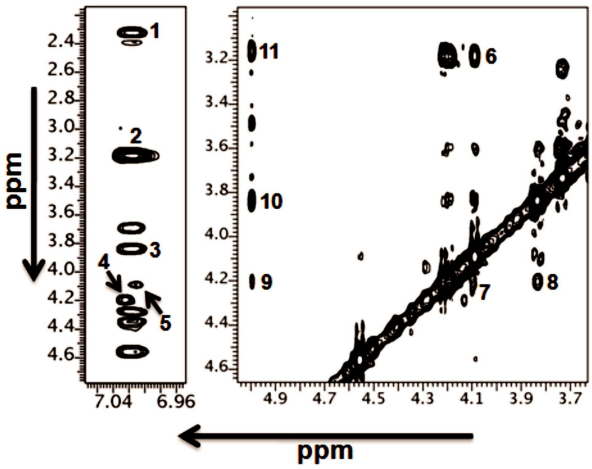


Figure 3. Newman projection and nOe representation for **1**.

Aggregation studies of glycopeptides **1** and **2**, carried out with



a concentration range of 0.625 mM to 20 mM in $CDCl_3$,

Figure 4. 2D-ROESY expansion and nOe representations for **1**; characteristic nOes between NH-C3H$_{(pro\text{-}R)}$, NH-C6H$_{(pro\text{-}S)}$, NH-C4H, NH-C6H$_{(pro\text{-}R)}$, NH-C5H, C5H-C6H$_{(pro\text{-}S)}$, C5H-C6H$_{(pro\text{-}R)}$, C6H$_{(pro\text{-}R)}$-C4H, C6H$_{(pro\text{-}R)}$-SugC1H, C4H-SugC1H, C6H$_{(pro\text{-}S)}$-SugC1H are marked as **1**-**11**, respectively.

revealed variations in the chemical shifts of the amide protons in both **1** and **2** (0.038 and 0.037 ppm, respectively). These observations suggested that the amide protons may be participating in intermolecular H-bonding and thus both the glycopeptides were self-aggregating at concentrations > 5 mM (Figure 5).

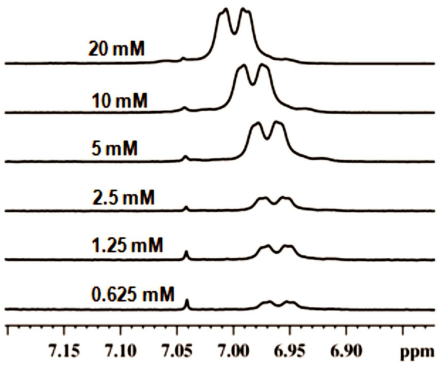


Figure 5. ^1H-NMR spectra showing NH peaks as a function of concentration for glycopeptide **1** in $CDCl_3$.

Conformational studies were also carried out on glycopeptides **3** and **4**. The ^1H, ^{13}C NMR spectra of compounds **3** and **4** in $CDCl_3$ also showed only one set of sharp resonances indicating C_3-symmetry in these cyclotrimeric glycopeptides. The vicinal coupling constant values ($^3J_{NH\text{-}C6H}$) were either < 3 Hz or > 6 Hz, which indicated that the dihedral angle φ (CO–N–C6–C5) is about 140°. $^3J_{C6H\text{-}C5H}$ value of either < 2 Hz or > 10 Hz indicated that the dihedral angle θ (N–C6–C5–O) was about +30° (Figure 6). Observations of nOe cross peaks between NH↔C6H, C5H↔C6H$_{(pro\text{-}S)}$ and C5H↔C6H$_{(pro\text{-}R)}$ suggested a (+) synclinal (*sc*) rotation about C5–C6 bond in both the glycopeptides. Further nOes between NH↔C5H, NH↔C4H, C5H↔C2H, C4H↔C2H and C4H↔C6H, C2H↔C6H$_{(pro\text{-}R)}$ and C2H↔C6H$_{(pro\text{-}S)}$ confirmed the (+) *sc* rotation in both glycopeptides (Figure 6 and 7). Additionally, nOe correlations

between C2H↔C6H$_{(pro\text{-}S)}$, C2H–C5H, C2H–C4H and the coupling constant values of the protons[16] further suggested that the five-membered furanoid ring was with C_2-*endo* ring puckering in both the cyclotrimeric glycopeptides **3** and **4**.

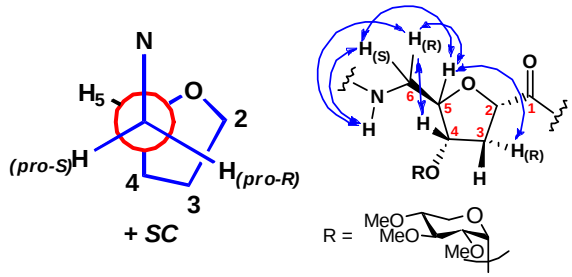


Figure 6. Newman projection and nOe representation for **3**.

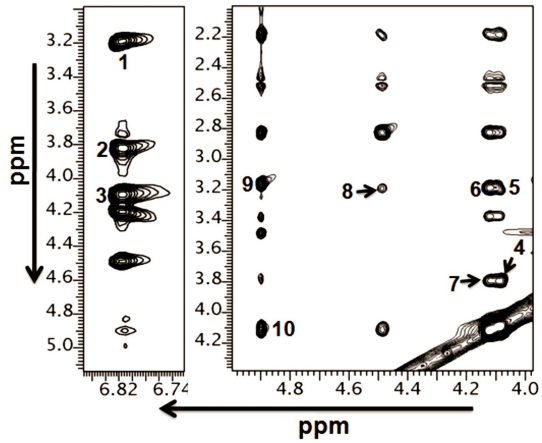


Figure 7. 2D-ROESY expansion and nOe representations for **3**; characteristic nOes between NH↔C6H$_{(pro\text{-}R)}$, NH↔C6H$_{(pro\text{-}S)}$, NH↔C5H, C5H↔C6H$_{(pro\text{-}R)}$, C5H↔C6H$_{(pro\text{-}R)}$, C4H↔C6H$_{(pro\text{-}R)}$, C5H↔C6H$_{(pro\text{-}S)}$, C2H↔C6H$_{(pro\text{-}R)}$, SugC1H↔C6H$_{(pro\text{-}R)}$, SugC1H↔C5H are marked as **1-10**, respectively.

Aggregation studies carried out as a function of concentration from 0.6 mM to 20 mM revealed the chemical shift changes of the amide protons in both the glycopeptides **3** and **4** as 0.019 ppm and 0.024 ppm, respectively. These observations further suggested that the molecules were self-aggregating and the amide protons were involved in intermolecular H-bonding.[16]

4. Molecular Dynamics

Energy minimization and simulated molecular dynamics (MD) calculations were performed on Discovery studio 3.0 version,[17a] using CHARMm force field[17b] with default parameters throughout the simulation with the aid of distance dependent dielectric constant with ε = 4.81 (dielectric constant for $CDCl_3$). Distance restraints used in the MD were calculated from the volume integrals of the cross peaks in the ROESY spectra with the use of two-spin approximation with a reference distance of 1.80 Å for the geminal protons. Force constants of 10 K cal/Å, 5 K cal/Å were employed for distance and torsional restraints respectively. Minimization was done with steepest descent algorithm followed by conjugate gradient methods for maximum 1000 iterations each iterations or RMS deviation of 0.001 Kcal /mol, whichever was earlier. The molecules were initially equilibrated for 5 pS and then subjected to 1 nS production run. Starting from 50 K, they were heated to 300 K in five steps, increasing the temperature 50 K at each step. 20 structures were stored from the production run and were again energy minimized with the above-mentioned protocol. Superposition was done on to the average structure of these minimized structures. The RMSD values calculated with all atoms and heavy atoms were 1.018 Å, 0.784 Å and 1.65 Å, 1.28 Å for compounds **1** and **2**, respectively. Likewise, for the cyclic trimers, the RMSD values calculated for all atoms and heavy atoms are 1.45 Å, 1.12 Å and 1.52 Å, 1.26 Å for compounds **3** and **4**, respectively. Similar coupling constant values and nOes observed in the cyclic dimers **1**,**2** and the cyclic trimers **3**,**4** confirm analogous backbone conformations among these cyclic glycopeptides irrespective of the anomeric configurations of the attached sugars (Figure 8).

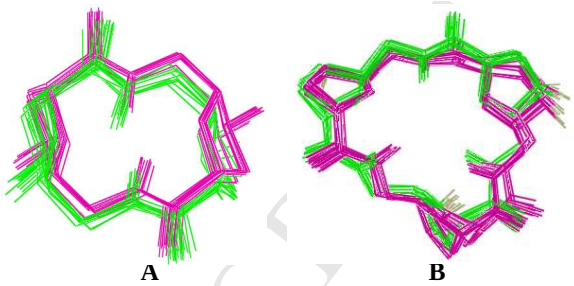


Figure 8. 15 superimposed structures of (A) cyclic dimers **1** (purple) and **2** (green); (B) cyclic trimers **3** (green) and **4** (purple); after superposition the attached sugars were removed for clarity.

5. Antimicrobial activity

The synthesized glycopeptides **1-4** and their unglycosylated congeners **25**[18] and **26**[18] (Figure 9) were tested for their antimicrobial activities against 4 bacterial strains and 6 fungal strains, of which many were multidrug-resistant strains.[16] For the purpose of comparison, gentamycin and norfloxacin were employed as positive controls for antibacterial activity and amphotericin-B and fluconazole were employed as positive controls for antifungal activity.

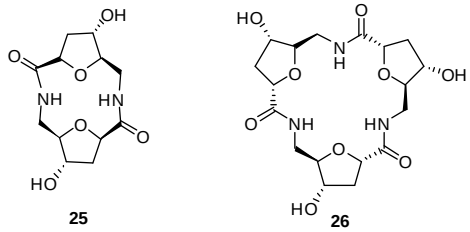


Figure 9. Non-glycosylated precursors **25** and **26**.

While the cyclic non-glycosylated homooligomers **25** and **26** did not show any antimicrobial or antifungal activity at all (both showing MICs > 100 µM against all strains), their glycosylated counterparts **1-4** showed relatively better activity. The cyclic peptides **1-3** {MIC, **1** (100 µM), **2** (50 µM) and **3** (50 µM)} showed moderate activity only against bacterium *E. coli*, but remained inactive against the rest of the bacterial strains. All the cyclic peptides {MIC, **1** (100 µM), **2** (50-100 µM), **3** (100 µM) and **4** (100 µM)} showed activity against fungus strains *S. schenckii*, *T. mentagrophytes*, *A. fumigatus*, except *C. parapsilosis*. The data suggested that the cyclic glycopeptides **1-3** displayed moderate antimicrobial activities only against *E. coli*, but all of them showed moderate antifungal activities against three out of four strains.

6. Conclusion

The glycosylated furanoid sugar amino acid based cyclic homooligomers constitute a new class of novel molecular scaffolds that displayed interesting 3D structures in $CDCl_3$. Conformational studies of cyclodimeric compounds **1** and **2** with

2*R* and 5*R* stereocenters revealed that the C2H and CO atoms were placed on same side of the ring while the NHs were pointing to other side. The dihedral angle ψ noticed between NH-CO-C2H-O was ≈ 50˚. Both the CO and NH were arranged perpendicular to the ring plane. Conformational studies of cyclic trimers **3** and **4** with 2*S* and 5*R* stereocenters in $CDCl_3$ showed that the C2H and CO were positioned outside the plane of the ring similar to the cyclic dimer and NHs pointed inside the ring. The dihedral angle ψ noticed between NH-CO-C2H-O was ≈ 15˚.

That the cyclic glycopeptides showed better activity than their non-glycosylated precursors lends support to the plausible role of the attached sugars on modulating their antimicrobial and antifungal activities. But the manner in which *O*-glycosylation has influenced the activity and up to what extent the impact of such influence can be generated *via* implementing variable sugar moieties on different SAA backbones is yet to be ascertained. Besides, the conformational studies carried out here will also be useful in developing new SAA based scaffolds amenable to glycosylation for further enhancement of their biological, pharmacological and pharmacokinetic properties. NMR analysis suggested that these cyclic glycopeptides have well defined structures in solution that are independent of the anomeric configurations of the attached sugar and can serve as simple, and yet versatile, tool to investigate many molecular recognition processes of biological significance, especially taking advantage of the unlimited structural diversities accessible for the appended carbohydrates. Further work is under progress.

7. Experimental section

7.1. General

All the reactions were carried out under an inert atmosphere in oven-dried glassware using dry solvents, unless otherwise stated. All chemicals purchased from commercial suppliers were used as received unless otherwise stated. Reactions and chromatography fractions were monitored by silica gel 60 F-254 glass TLC plates and visualized using UV light, 7% ethanolic phosphomolybdic acid-heat or 2.5% ethanolic anisaldehyde (with 1% AcOH and 3.3% conc.H_2SO_4)-heat as developing agents. Flash column chromatography was performed with 100-200 mesh silica gel and yields refer to chromatographically and spectroscopically pure compounds. All NMR spectra were recorded in $CDCl_3$ on 300, 400 and 500 MHz instruments at 300 K and are calibrated to chloroform solvent residual peak (7.26 ppm) as a reference for the proton NMR spectra and deuterated chloroform peak (77.16 ppm) as a reference for carbon NMR spectra. Multiplicities are abbreviated as: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplate. All IR data were recorded as neat liquid or KBr pellets. HRMS spectra were taken under ESI-Q-TOF conditions. Optical rotations were measured using sodium (589 nm, D line) lamp and are reported as follows: $[\alpha]_D^{25}$ [*c* (g/100 mL), $CHCl_3$].

7.2. Synthesis of compound 12

To a stirred solution of **10** (2.33 g, 8.02 mmol) in dry CH_2Cl_2 (24 mL) was slowly added BCl_3 solution (24 mL, 24 mmol, 1.0 M in CH_2Cl_2) at 0 °C and stirring was continued for 10 min at the same temperature. The reaction mixture was quenched at 0 °C by slow addition of saturated aqueous $NaHCO_3$ solution (50 mL) and extraction was done with EtOAc (2 x 200 mL). The combined organic extracts were washed with water (60 mL), brine (50 mL), dried (Na_2SO_4), filtered and concentrated in *vacuo* to give the crude product, which was purified by flash chromatography (30% EtOAc/Hexane) to give the title compound **12** (1.40 g, 87%) as a yellow oil; R_f (50% EtOAc /hexane) 0.5; $[\alpha]_D^{27}$ +73.1 (*c* 0.60, $CHCl_3$); ν_{max} (neat liquid) 3409, 3019, 2105, 1740, 1641, 1403, 1215, 1109, 928, 758, 669 cm^{-1}; δH (300 MHz, $CDCl_3$) 4.69 (1H, t, *J* 7.7 Hz), 4.37 - 4.30 (1H, m), 4.08 - 3.99 (1H, m), 3.76 (3H, s), 3.51 - 3.36 (2H, m), 2.27 (2H, dd, *J* 7.7, 4.8 Hz), 2.18 (1H, br); δC (100 MHz, $CDCl_3$) 172.6, 85.9, 76.7, 73.4, 52.7, 52.4, 38.7; HRMS: $[M+Na]^+$, found 224.0642. $C_7H_{11}N_3O_4Na$ requires 224.0642.

7.3. Synthesis of compound 13

Compound **13** was synthesized from **11** (2.50 g, 8.58 mmol) following the same procedure described above for the synthesis of **12** with the same reagents under identical reaction conditions to give the title compound **13** (1.45 g, 84%) as a yellow oil; R_f (50% EtOAc/hexane) 0.45; $[\alpha]_D^{28}$ +88.5 (*c* 0.44, $CHCl_3$); ν_{max} (neat liquid) 3409, 3019, 2399, 1644, 1403, 1215, 1108, 928, 758, 669 cm^{-1}; δH (300 MHz, $CDCl_3$) 4.68 (1H, dd, *J* 8.9, 2.9 Hz), 4.32 - 4.22 (2H, m), 3.78 (3H, s), 3.48 (1H, dd, *J* 12.9, 4.1 Hz), 3.31 (1H, dd, *J* 12.9, 4.1 Hz), 2.93 (1H, br), 2.56 - 2.43 (1H, m), 2.23 - 2.12 (1H, m); δC (100 MHz, $CDCl_3$) 174.5, 86.5, 77.0, 73.7, 52.7, 52.6, 38.8; HRMS: $[M+Na]^+$, found 224.0647. $C_7H_{11}N_3O_4Na$ requires 224.0642.

7.4. Synthesis of compounds 15 and 16

Compound **12** (48 mg, 0.24 mmol) was dissolved in dry CH_3CN (3 mL) and cooled to 0 °C under Argon atmosphere. Then the solution of the glycosyl donor **14** (75 mg, 0.36 mmol) in CH_3CN (2 mL) was added by cannulation followed by drop wise addition of $BF_3{\cdot}Et_2O$ (45 µL, 0.36 mmol) at the same temperature. The reaction mixture was stirred at room temperature for 2 h and then quenched by saturated aqueous $NaHCO_3$ solution (5 mL) and extraction was done with EtOAc (2 x 20 mL). The combined organic extracts were washed with water (5 mL), brine (5 mL), dried (Na_2SO_4), filtered and concentrated in *vacuo* to give the crude product, which was purified by flash chromatography (20% EtOAc/Hexane) to give the title compounds **15** (48 mg, 53%) and **16** (32 mg, 36%) as yellow oils in a ratio of 1.5:1 (**15**:**16**) with overall 89% yield; Data for **15**: R_f (40% EtOAc/hexane) 0.45; $[\alpha]_D^{28}$ +42.6 (*c* 0.32, $CHCl_3$); ν_{max} (neat liquid) 3410, 3019, 2399, 2105, 1644, 1403, 1215, 1107, 929, 758, 669 cm^{-1}; δH (300 MHz, $CDCl_3$) 4.88 (1H, d, *J* 3.3 Hz), 4.68 (1H, t, *J* 7.6 Hz), 4.30 - 4.15 (2H, m), 3.76 (3H, s), 3.71 (1H, dd, *J* 10.7, 5.0 Hz), 3.60 (3H, s), 3.49 (3H, s), 3.47 (3H, s), 3.46 – 3.42 (2H, m), 3.42 – 3.33 (2H, m), 3.30 – 3.20 (1H, m), 3.14 (1H, dd, *J* 9.3, 3.3 Hz), 2.41 – 2.19 (2H, m); δC (100 MHz, $CDCl_3$) 172.2, 95.9, 83.9, 83.3, 81.3, 79.8, 77.8, 61.0, 60.2, 59.2, 59.1, 52.7, 52.5. 35.8; HRMS: $[M+Na]^+$, found 398.1525. $C_{15}H_{25}N_3O_8Na$ requires 398.1539.

Data for **16**: R_f (40% EtOAc/hexane) 0.5; $[\alpha]_D^{29}$ +7.17 (*c* 0.36, $CHCl_3$); ν_{max} (neat liquid) 3409, 3019, 2399, 1644, 1403, 1215, 1107, 928, 758, 669 cm^{-1}; δH (300 MHz, $CDCl_3$) 4.65 (1H, t, *J* 7.9 Hz), 4.29 - 4.13 (3H, m), 3.96 (1H, dd, *J* 11.4, 4.8 Hz), 3.76 (3H, s), 3.60 (3H, s), 3.53 (3H, s), 3.46 (3H, s), 3.45 - 3.38 (2H, m), 3.30 - 3.18 (1H, m), 3.16 - 3.03 (2H, m), 2.94 (1H, dd, *J* 8.7, 7.6 Hz), 2.52 - 2.39 (1H, m), 2.33 - 2.19 (1H, m); δC (100 MHz, $CDCl_3$) 172.3, 103.7, 85.3, 84.1, 83.4, 80.8, 79.3, 63.6, 60.9, 60.8, 58.9, 52.8, 52.4, 37.3; HRMS: $[M+Na]^+$, found 398.1518. $C_{15}H_{25}N_3O_8Na$ requires 398.1539.

7.5. Synthesis of compounds 17 and 18

Compounds **17** and **18** were synthesized from **13** (74 mg, 0.37 mmol) and **14** (115 mg, 0.56 mmol), respectively following the same procedure described above for the synthesis of **15** and **16** with the same reagents under identical reaction conditions to give the title compounds **17** (69 mg, 50%) and **18** (45 mg, 34%) as

yellow oils in a ratio of 1.5:1 (**17**:**18**) with overall 84% yield; Data for **17**: R_f (40% EtOAc/hexane) 0.3; $[\alpha]_D^{28}$ +121.5 (*c* 0.30, $CHCl_3$); ν_{max} (neat liquid) 3409, 3019, 2106, 1641, 1402, 1385, 1215, 1160, 1097, 758, 668 cm^{-1}; δH (300 MHz, $CDCl_3$) 4.85 (1H, d, *J* 3.3 Hz), 4.68 (1H, dd, *J* 8.4, 4.2 Hz), 4.40-4.32 (1H, m), 4.26 - 4.19 (1H, m), 3.77 (3H, s), 3.71 (1H, dd, *J* 10.7, 5.3 Hz), 3.57 (3H, s), 3.55 – 3.51 (1H, m), 3.46 (3H, s), 3.43 (3H, s), 3.37 – 3.26 (3H, m), 3.25 – 3.16 (1H, m), 3.09 (1H, dd, *J* 9.4, 3.5 Hz), 2.55 – 2.41 (1H, m), 2.30 (1H, dt, *J* 13.3, 3.9 Hz); δC (100 MHz, $CDCl_3$) 172.8, 95.8, 83.4, 82.1, 81.3, 79.6, 77.4, 76.6, 60.9, 60.2, 59.1, 58.8, 52.4, 52.2, 36.3; HRMS: $[M+Na]^+$, found 398.1532. $C_{15}H_{25}N_3O_8Na$ requires 398.1539.

Data for **18**: R_f (40% EtOAc/hexane) 0.4; $[\alpha]_D^{25}$ +34.0 (*c* 0.34, $CHCl_3$); ν_{max} (neat liquid) 3408, 3019, 2105, 1639, 1402, 1385, 1215, 1158, 1071, 758, 669 cm^{-1}; δH (300 MHz, $CDCl_3$) 4.65 (1H, dd, *J* 8.6, 4.6 Hz), 4.34 - 4.27 (1H, m), 4.25 - 4.17 (2H, m), 3.95 (1H, dd, *J* 11.4, 4.8 Hz), 3.74 (3H, s), 3.58 (3H, s), 3.57 – 3.53 (1H, m), 3.51 (3H, s), 3.45 (3H, s), 3.32 (1H, dd, *J* 13.2, 3.8 Hz), 3.26 - 3.17 (1H, m), 3.15 - 3.03 (2H, m), 2.88 (1H, dd, *J* 8.7, 7.6 Hz), 2.63 - 2.50 (1H, m), 2.36 (1H, dt, *J* 13.6, 4.2 Hz); δC (100 MHz, $CDCl_3$) 172.8, 103.9, 85.2, 83.5, 83.4, 80.0, 79.4, 76.6, 63.4, 60.8, 60.7, 58.8, 52.3, 37.7; HRMS: $[M+Na]^+$, found 398.1536. $C_{15}H_{25}N_3O_8Na$ requires 398.1539.

7.6. Synthesis of compound 19

To a stirred solution of compound **15** (250 mg, 0.67 mmol), $(Boc)_2O$ (0.31 mL, 1.34 mmol) and NEt_3 (0.23 mL, 1.68 mmol) in MeOH (10 mL) was added 10% Pd-C (100 mg) and subjected to hydrogenation condition using a hydrogen filled balloon for 5 h. The reaction mixture was filtered through a short pad of Celite and further washed with EtOAc (3 x 30 mL). The combined organic extracts were concentrated in *vacuo* to give the crude product, which was purified by flash chromatography (35% EtOAc/Hexane) to give the title compound **19** (205 mg, 68%) as a colourless oil; R_f (55% EtOAc/hexane) 0.45; $[\alpha]_D^{28}$ +18.9 (*c* 0.24, $CHCl_3$); ν_{max} (neat liquid) 3425, 2925, 1637, 1398, 1219, 1098, 769 cm^{-1}; δH (300 MHz, $CDCl_3$) 5.27 (1H, br), 4.88 (1H, d, *J* 3.3 Hz), 4.65 (1H, t, *J* 7.8 Hz), 4.26 - 4.12 (2H, m), 3.76 (3H, s), 3.71 (1H, dd, *J* 11.0, 5.5 Hz), 3.60 (3H, s), 3.48 (3H, s), 3.46 (3H, s), 3.43 – 3.30 (4H, m), 3.28 – 3.18 (1H, m), 3.13 (1H, dd, *J* 9.5, 3.5 Hz), 2.44 – 2.30 (1H, m), 2.24 - 2.14 (1H, m), 1.43 (9H, s); δC (100 MHz, $CDCl_3$) 173.0, 156.1, 95.7, 84.0, 82.1, 81.4, 79.7, 76.4, 60.9, 60.2, 58.7, 52.4, 42.4, 36.2, 28.5; HRMS: $[M+Na]^+$, found 472.2185. $C_{20}H_{35}NO_{10}Na$ requires 472.2159.

7.7. Synthesis of compound 20

Compound **20** was synthesized from **16** (200 mg, 0.53 mmol) following the same procedure described above for the synthesis of **19** with the same reagents under identical reaction conditions to give the title compound **20** (167 mg, 70%) as a colourless oil; R_f (55% EtOAc/hexane) 0.5; $[\alpha]_D^{27}$ +2.14 (*c* 0.28, $CHCl_3$); ν_{max} (neat liquid) 3425, 3021, 2925, 1637, 1399, 1216, 1091, 768, 670 cm^{-1}; δH (300 MHz, $CDCl_3$) 5.30 (1H, br), 4.63 (1H, t, *J* 7.7 Hz), 4.31 - 4.19 (2H, m), 4.17 - 4.09 (1H, m), 3.94 (1H, dd, *J* 11.4, 4.7 Hz), 3.76 (3H, s), 3.59 (3H, s), 3.53 (3H, s), 3.46 (3H, s), 3.41 - 3.34 (1H, m), 3.32 – 3.18 (2H, m), 3.16 - 3.03 (2H, m), 2.93 (1H, dd, *J* 8.6, 7.6 Hz), 2.53 - 2.41 (1H, m), 2.26 - 2.12 (1H, m), 1.44 (9H, s); δC (100 MHz, $CDCl_3$) 173.4, 156.3, 103.5, 85.3, 84.5, 83.5, 80.3, 79.4, 76.5, 63.5, 60.9, 60.7, 58.9, 52.4, 42.5, 37.7, 28.5; HRMS: $[M+Na]^+$, found 472.2141. $C_{20}H_{35}NO_{10}Na$ requires 472.2159.

7.8. Synthesis of compound 21

Compound **21** was synthesized from **17** (285 mg, 0.76 mmol) following the same procedure described above for the synthesis of **20** with the same reagents under identical reaction conditions to give the title compound **21** (236 mg, 69%) as a colourless oil; R_f (60% EtOAc/hexane) 0.45; $[\alpha]_D^{30}$ +64.5 (*c* 0.34, $CHCl_3$); ν_{max} (neat liquid) 3410, 3019, 2399, 1640, 1385, 1215, 1159, 1097, 758, 669 cm^{-1}; δH (300 MHz, $CDCl_3$) 4.86 (1H, d, *J* 3.3 Hz), 4.82 (1H, br), 4.62 (1H, dd, *J* 8.4, 4.2 Hz), 4.30 - 4.22 (1H, m), 4.19 - 4.11 (1H, m), 3.76 (3H, s), 3.71 (1H, dd, *J* 10.7, 5.4 Hz), 3.57 (3H, s), 3.45 (3H, s), 3.43 (3H, s), 3.38 – 3.16 (5H, m), 3.08 (1H, dd, *J* 9.3, 3.4 Hz), 2.46 – 2.26 (2H, m), 1.43 (9H, s); δC (100 MHz, $CDCl_3$) 172.9, 156.0, 95.5, 83.9, 81.9, 81.2, 79.6, 76.2, 60.7, 60.0, 58.7, 58.5, 52.1, 42.3, 36.1, 28.3; HRMS: $[M+H]^+$, found 450.2344. $C_{20}H_{36}NO_{10}$ requires 450.2339.

7.9. Synthesis of compound 22

Compound **22** was synthesized from **18** (229 mg, 0.61 mmol) following the same procedure described above for the synthesis of **21** with the same reagents under identical reaction conditions to give the title compound **22** (175 mg, 64%) as a yellow oil; R_f (60% EtOAc/hexane) 0.5; $[\alpha]_D^{29}$ -7.9 (*c* 0.70, $CHCl_3$); ν_{max} (neat liquid) 3409, 3019, 1643, 1402, 1385, 1215, 1157, 1069, 769, 669 cm^{-1}; δH (300 MHz, $CDCl_3$) 4.83 (1H, br), 4.58 (1H, dd, *J* 8.6, 4.6 Hz), 4.27 (1H, d, *J* 7.4 Hz), 4.22 - 4.09 (2H, m), 3.93 (1H, dd, *J* 11.4, 4.8 Hz), 3.73 (3H, s), 3.59 (3H, s), 3.52 (3H, s), 3.45 (3H, s), 3.39 - 3.27 (2H, m), 3.26 - 3.17 (1H, m), 3.15 - 3.05 (2H, m), 2.88 (1H, dd, *J* 8.5, 7.4 Hz), 2.56 - 2.43 (1H, m), 2.35 (1H, dt, *J* 13.6, 4.1 Hz), 1.44 (9H, s); δC (75 MHz, $CDCl_3$) 172.9, 156.1, 103.6, 85.1, 83.9, 83.4, 80.0, 79.4, 76.3, 63.4, 60.8, 60.7, 58.8, 52.2, 42.4, 37.5, 28.5; HRMS: $[M+H]^+$, found 450.2342. $C_{20}H_{36}NO_{10}$ requires 450.2339.

7.10. Synthesis of compound 23

To a solution of **19** (290 mg, 0.65 mmol) in THF:MeOH:H_2O (3:1:1, 5 mL) at 0 °C was added $LiOH \cdot H_2O$ (164 mg, 3.9 mmol) and the mixture was stirred at room temperature for 1 h. The reaction mixture was then acidified to pH 2 with aqueous 1 N HCl. The reaction mixture was extracted with EtOAc (2 x 100 mL) and the combined organic extracts were washed with water (50 mL), brine (30 mL), dried (Na_2SO_4), filtered and concentrated in *vacuo* to obtain the acid.

To a solution of **19** (306 mg, 0.68 mmol) in dry CH_2Cl_2 (10 mL) at 0 °C was added TFA (3.3 mL) and the mixture was stirred for 2 h at room temperature. The reaction mixture was then concentrated and co-evaporated using dry CH_2Cl_2 in *vacuo* to obtain the TFA-salt.

To a stirred solution of the acid in dry CH_2Cl_2 (5 mL) at 0 °C was added HOBt (132 mg, 0.98 mmol) and EDCI (188 mg, 0.98 mmol). The mixture was stirred for another 30 min at room temperature followed by addition of a solution of TFA-salt in dry CH_2Cl_2 (5 mL) by cannulation and slow addition of DIPEA (0.57 mL, 3.25 mmol) at 0 °C. After stirring for 12 h at room temperature, the reaction mixture was quenched with aqueous 1 N HCl (5 mL) and the solvent was evaporated and extraction was done with $CHCl_3$ (3 x 50 mL). The combined organic extracts were washed with aqueous 1 N HCl (2 x 10 mL), saturated aqueous $NaHCO_3$ solution (2 x 10 mL), water (40 mL), brine (30 mL), dried (Na_2SO_4), filtered and concentrated in *vacuo* to give the crude product, which was purified by flash chromatography (2% MeOH/$CHCl_3$) to give the title compound **23** (358 mg, 72%) as a yellow oil; R_f (4% MeOH/EtOAc) 0.3; $[\alpha]_D^{26}$ +97.2 (*c* 0.20, $CHCl_3$); ν_{max} (neat liquid) 3409, 1643, 1402, 1385, 1216, 1157, 1069, 770, 668 cm^{-1}; δH (400 MHz, $CDCl_3$) 7.52 (1H, br), 5.46 (1H, br), 4.90 (1H, d, *J* 3.6 Hz), 4.87 (1H, d, *J* 3.6 Hz), 4.67 (1H, t, *J* 7.6 Hz), 4.57 (1H, dd, J 9.1, 7.1 Hz), 4.28 - 4.17 (2H, m), 4.16 – 4.09 (2H, m), 3.77 (3H, s), 3.71 (1H, dd, *J* 10.8, 5.7 Hz), 3.60 (3H, s), 3.59 (3H, s), 3.51 – 3.48 (2H, m), 3.48 (3H, s), 3.47

(3H, s), 3.46 (3H, s), 3.45 (3H, s), 3.43 - 3.34 (6H, m), 3.28 - 3.19 (3H, m), 3.17 - 3.09 (2H, m), 2.47 - 2.35 (2H, m), 2.25 - 2.12 (1H, m), 2.09 - 1.98 (1H, m), 1.43 (9H, s); δC (100 MHz, $CDCl_3$) 174.2, 172.3, 170.5, 156.4, 96.2, 95.3, 84.6, 82.3, 81.3, 79.8, 78.0, 75.9, 75.8, 61.0, 60.2, 60.1, 59.1, 59.0, 58.9, 52.7, 52.6, 43.1, 40.2, 36.2, 28.5; HRMS: $[M+H]^+$, found 767.3798. $C_{34}H_{59}N_2O_{17}$ requires 767.3814.

7.11. Synthesis of compound 1

To a stirred solution of **23** (200 mg, 0.26 mmol) in THF:MeOH:H_2O (3:1:1, 5 mL) at 0 °C was added LiOH·H_2O (66 mg, 1.56 mmol) and the mixture was stirred at room temperature for 1 h. The reaction mixture was then acidified to pH 2 with aqueous 1 N HCl. The reaction mixture was extracted with EtOAc (2 x 50 mL) and the combined organic extracts were washed with water (20 mL), brine (20 mL), dried (Na_2SO_4), filtered and concentrated in *vacuo* to obtain the acid. The acid was again dissolved in dry CH_2Cl_2 (5 mL) followed by slow addition of TFA (1.7 mL) at 0 °C and the mixture was stirred for 2 h at room temperature. The reaction mixture was then concentrated and co-evaporated using dry CH_2Cl_2 in *vacuo* to obtain the TFA-salt.

To a stirred solution of the TFA-salt in dry CH_3CN (25 mL, 5 x 10^{-3} M dilution) at 0 °C was added FDPP (300 mg, 0.78 mmol) and stirred for 30 min at room temperature followed by the slow addition of DIPEA (0.32 mL, 1.82 mmol) at 0 °C. After stirring for 72 h at room temperature, the solvent was evaporated and extraction was done with $CHCl_3$ (3 x 50 mL). The combined organic extracts were washed with aqueous 10% NaOH solution (2 x 10 mL), water (20 mL), brine (20 mL), dried (Na_2SO_4), filtered and concentrated in *vacuo* to give the crude product, which was purified by flash chromatography (5% MeOH/$CHCl_3$) to give the title compound **1** (44 mg, 54%) as a yellow oil; R_f (12% MeOH/EtOAc) 0.5; $[\alpha]_D^{30}$ +2.73 (*c* 0.22, $CHCl_3$); ν_{max} (neat liquid) 3415, 3019, 2400, 1637, 1384, 1215, 1084, 758, 669 cm^{-1}; δH (500 MHz, $CDCl_3$) 7.05 (1H, dd, *J* 10.0, 2.8 Hz), 5.00 (1H, d, *J* 3.7 Hz), 4.57 - 4.52 (1H, m), 4.20 (1H, dd, *J* 14.6, 10.2 Hz), 4.08 (1H, dd, *J* 8.2, 4.0 Hz), 3.86 - 3.81 (1H, m), 3.73 (1H, dd, *J* 11.5, 5.8 Hz), 3.63 – 3.59 (1H, m), 3.60 (3H, s), 3.47 (3H, s), 3.46 (3H, s), 3.40 (1H, t, *J* 9.2 Hz), 3.26 - 3.17 (2H, m), 3.15 (1H, dd, *J* 9.7, 3.7 Hz), 2.79 – 2.74 (1H, m), 2.24 (1H, dt, *J* 12.3, 9.8 Hz); δC (125 MHz, $CDCl_3$) 172.5, 95.6, 83.4, 82.2, 81.4, 79.8, 77.5, 73.7, 61.0, 60.3, 59.0, 58.9, 37.5, 34.6; HRMS: $[M+Na]^+$, found 657.2824. $C_{28}H_{46}N_2O_{14}Na$ requires 657.2847.

7.12. Synthesis of compound 24

Compound **24** was synthesized from **20** (513 mg, 1.14 mmol) following the same procedure described above for the synthesis of **23** with the same reagents under identical reaction conditions to give the title compound **24** (602 mg, 69%) as a yellow oil; R_f (4% MeOH/EtOAc) 0.5; $[\alpha]_D^{29}$ -29.5 (*c* 0.32, $CHCl_3$); ν_{max} (neat liquid) 3411, 3019, 2399, 1643, 1402, 1385, 1215, 1158, 1069, 928, 757, 669 cm^{-1}; δH (300 MHz, $CDCl_3$) 7.54 (1H, br), 5.49 (1H, br), 4.64 (1H, t, *J* 7.5 Hz), 4.54 (1H, t, *J* 7.5 Hz), 4.32 - 4.05 (6H, m), 4.00 - 3.88 (2H, m), 3.76 (3H, s), 3.58 (6H, s), 3.53 (3H, s), 3.51 (3H, s), 3.45 (6H, s), 3.40 - 3.17 (6H, m), 3.14 - 3.03 (4H, m), 2.97 - 2.88 (2H, m), 2.58 - 2.44 (2H, m), 2.22 - 2.00 (2H, m), 1.42 (9H, s); δC (100 MHz, $CDCl_3$) 172.8, 156.0, 103.6, 103.3, 85.0, 84.9, 83.8, 83.4, 83.3, 83.1, 80.0, 79.8, 79.4, 79.3, 77.7, 76.2, 63.2, 60.7, 60.6, 58.7, 58.6, 52.1, 40.3, 37.6, 37.0, 28.4; HRMS: $[(M-Boc)+H]^+$, found 667.3280. $C_{29}H_{51}N_2O_{15}$ requires 667.3289.

7.13. Synthesis of compound 2

Compound **2** was synthesized from **24** (135 mg, 0.18 mmol) following the same procedure described above for the synthesis of **1** with the same reagents under identical reaction conditions to give the title compound **2** (29 mg, 52%) as a yellow oil; R_f (10% MeOH/EtOAc) 0.5; $[\alpha]_D^{30}$ -71.9 (*c* 0.14, $CHCl_3$); ν_{max} (neat liquid) 3412, 3019, 2399, 1637, 1523, 1475, 1421, 1384, 1215, 1083, 928, 757, 669, 627 cm^{-1}; δH (500 MHz, $CDCl_3$) 7.04 (1H, dd, *J* 10.2, 2.6 Hz), 4.55 - 4.48 (2H, m), 4.13 (1H, dd, *J* 14.9, 10.2 Hz), 3.99 - 3.89 (2H, m), 3.74 - 3.63 (1H, m), 3.60, (3H, s), 3.56 (3H, s), 3.45 (3H, s), 3.26 – 3.14 (4H, m), 2.96 (1H, dd, *J* 8.7, 7.5 Hz), 2.75 - 2.66 (1H, m), 2.37 - 2.26 (1H, m); δC (125 MHz, $CDCl_3$) 172.8, 104.0, 84.8, 83.4, 83.1, 79.5, 77.7, 76.8, 63.2, 60.8, 60.4, 58.7, 37.4, 36.4; HRMS: $[M+Na]^+$, found 657.2822. $C_{28}H_{46}N_2O_{14}Na$ requires 657.2847.

7.14. Synthesis of compound 3

Compound **3** was synthesized from **21** (327 mg, 0.72 mmol) following the same procedure described above for the synthesis of **1** and **2** with the same reagents under identical reaction conditions to give the title compound **3** (73 mg, 32%) as a yellow oil; R_f (20% MeOH/EtOAc) 0.5; $[\alpha]_D^{30}$ +57.9 (*c* 0.18, $CHCl_3$); ν_{max} (neat liquid) 3424, 3019, 2399, 1637, 1403, 1215, 1084, 928, 758, 669 cm^{-1}; δH (500 MHz, $CDCl_3$) 6.81 (1H, dd, *J* 6.4, 2.7 Hz), 4.89 (1H, d, *J* 3.2 Hz), 4.48 (1H, t, *J* 8.3 Hz), 4.14 - 4.06 (2H, m), 3.84 - 3.71 (2H, m), 3.61 (3H, s), 3.48 (3H, s), 3.46 (3H, s), 3.42 - 3.32 (2H, m), 3.28 - 3.11 (3H, m), 2.85 – 2.78 (1H, m), 2.24 - 2.13 (1H, m); δC (125 MHz, $CDCl_3$) 171.5, 95.8, 82.2, 81.3, 81.2, 79.7, 61.1, 60.4, 59.0, 58.9, 42.2, 34.9; HRMS: $[M+Na]^+$, found 974.4316. $C_{42}H_{69}N_3O_{21}Na$ requires 974.4321.

7.15. Synthesis of compound 4

Compound **4** was synthesized from **22** (290 mg, 0.65 mmol) following the same procedure described above for the synthesis of **3** with the same reagents under identical reaction conditions to give the title compound **4** (59 mg, 29%) as a yellow oil; R_f (20% MeOH/EtOAc) 0.3; $[\alpha]_D^{30}$ -7.9 (*c* 0.38, $CHCl_3$); ν_{max} (neat liquid) 3430, 3019, 2400, 1637, 1403, 1215, 1084, 928, 758, 669 cm^{-1}; δH (500 MHz, $CDCl_3$) 6.79 (1H, dd, *J* 6.8, 2.1 Hz), 4.45 (1H, t, *J* 8.4 Hz), 4.25 (1H, d, *J* 7.3 Hz), 4.01 (1H, q, *J* 7.6 Hz), 3.97 – 3.92 (2H, m), 3.83 (1H, ddd, *J* 13.6, 7.2, 2.1 Hz), 3.58 (3H, s), 3.52 (3H, s), 3.45 (3H, s), 3.25 - 3.20 (1H, m), 3.17 - 3.05 (3H, m), 2.94 (1H, dd, *J* 8.8, 7.3 Hz), 2.75 (1H, dt, *J* 13.3, 7.5 Hz), 2.28 (1H, dt, *J* 13.3, 8.6 Hz); δC (100 MHz, $CDCl_3$) 171.6, 104.1, 85.0, 83.2, 81.4, 81.1, 79.3, 77.0, 63.3, 60.8, 60.7, 58.8, 42.3, 36.6; HRMS: $[M+Na]^+$, found 974.4287. $C_{42}H_{69}N_3O_{21}Na$ requires 974.4321.

8. Antimicrobial activity: Material and methods

In vitro antimicrobial activities of compounds were tested against 6 medically important fungi; *Candida albicans* (*Ca,* patient isolate), *Cryptococcus neoformans* (*Cn,* patient isolate), *Sporothrix schenckii* (*Ss*, patient isolate), *Trichophyton mentagrophytes* (*Tm*, patient isolate), *Aspergillus fumigatus* (*Af*, patient isolate) *and C. parapsilosis* (*Cp*, ATCC-22019) and 4 bacteria *viz*, *Escherichia coli* (*Ec*, ATCC 9637), *Pseudomonas aeruginosa* (*Psu*, ATCC BAA-427), *Staphyllococcus aureus* (*Sa*, ATCC 25923), *Klebsiella pneumoniae* (*Kpn*, ATCC 27736). The susceptibility testing was performed by Standard Broth Microdilution method as per National Committee for Clinical Laboratory Standards (now CLSI)[19] guidelines using RPMI 1640 medium buffered with MOPS [3-(*N*-morpholino) propanesulphonic acid] for fungi and Mueller Hinton Broth (Difco) for bacteria in 96 well microtitre plates. The maximum concentration of the test peptides tested was 100µg/ml and the inoculums load in each test well was in the range of 1-5×10^3

cells. The plates were incubated for 24-48 h for yeasts, 72-96 h for mycelial fungi at 35 ºC and 24 h for bacteria at 37 ºC and read visually as well as spectrophotometerically (Spectra max) at 492 nm for determination of minimal inhibitory concentrations (MIC).[16]

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Supplementary data

Experimental details, NMR spectral data, chemical shift tables, restraints used in MD calculations and antibacterial-antifungal data are provided.

9. References and note

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18. Compounds **25** was synthesized by intramolecular cyclization (following the same method used for making **1** from **23**) of the linear dimer of *O*-benzylated SAA monomer derived from **10** by selective hydrogenation of azide using 10% Pd-C as catalyst and EtOAc as solvent followed by Boc-protection. The cyclized product (66% yield in three steps from the *N*-Boc protected dimeric methyl ester) was hydrogenated using 10% Pd-C as catalyst and MeOH as solvent to furnish **25** in 74% yield after chromatographic purification. Data for **25**: R_f (15% MeOH/EtOAc) 0.5; $[\alpha]_D^{27}$ -21.6 (*c* 0.40, MeOH); ν_{max} (KBr) 3429, 3018, 2399, 2128, 1637, 1404, 1215, 1052, 1027, 1009, 928, 819, 757, 668, 624 cm^{-1}; δH (300 MHz, DMSO-d_6) 8.23 (1H, br), 5.23 (1H, br), 4.36 (1H, d, *J* 8.6 Hz,), 3.85 (1H, dd, *J* 13.3, 10.2 Hz), 3.68 - 3.60 (1H, m), 3.58 - 3.39 (1H, m), 3.06 - 2.91 (1H, m), 2.42 - 2.28 (1H, m), 2.04 - 1.07 (1H, m); δC (100 MHz, DMSO-d_6) 172.5, 84.5, 76.6, 68.2, 37.3, 36.5; HRMS: $[M+Na]^+$, found 309.1043. $C_{12}H_{18}N_2O_6Na$ requires 309.1063.
Synthesis of **26** commenced from **11** which on selective hydrogenation of azide using 10% Pd-C as catalyst and EtOAc as solvent followed by Boc-protection gave the requisite monomer Boc-SAA(OBn)-OMe. Cyclotrimerisation of the monomer, following the same protocol used for getting **3** from **21**, furnished the *O*-Bn protected cyclotrimer *cyclo*-$[SAA(OBn)]_3$ in 54% yield in three steps, which on hydrogenation using 10% Pd-C as catalyst and MeOH as solvent furnished **26** in 71% yield after chromatographic purification. Data for **26**: R_f (30% MeOH/EtOAc) 0.45; $[\alpha]_D^{27}$ +58.9 (*c* 0.35, MeOH); ν_{max} (KBr) 3426, 3018, 2257, 2129, 1644, 1384, 1215, 1050, 1026, 1007, 821, 757, 668 cm^{-1}; δH (300 MHz, DMSO-d_6) 8.27 (1H, br), 5.31 (1H, d, *J* 5.4 Hz), 4.41 - 4.32 (1H, m), 3.87 - 3.68 (2H, m), 3.63 - 3.54 (1H, m), 2.92 - 2.79 (1H, m), 1.86 - 1.74 (1H, m); δC (100 MHz, DMSO-d_6) 172.4, 82.3, 75.7, 72.4, 41.7, 37.3; HRMS: $[M+Na]^+$, found 452.1623. $C_{18}H_{27}N_3O_9Na$ requires 452.1645.
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