Tetrahedron 65 (2009) 5343–5349

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Novel and facile solution-phase synthesis of 2,5-diketopiperazines and *O*-glycosylated analogs

Vanessa L. Campo, Maristela B. Martins, Carlos H.T.P. da Silva, Ivone Carvalho *

Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Av. do Café s/n, Ribeirão Preto 14040-930, Brazil

ARTICLE INFO

Article history:
Received 5 February 2009
Received in revised form 13 April 2009
Accepted 20 April 2009
Available online 3 May 2009

Keywords:
Diketopiperazine
Dipeptide cyclization
Glycosylated amino acid
Solution-phase synthesis

ABSTRACT

This work describes the synthesis in solution of a series of related diketopiperazines with potential biological activities: *cyclo*(L-Pro-L-Ser), *cyclo*(L-Phe-L-Ser), *cyclo*(D-Phe-L-Ser) and the corresponding glycosylated analogs of the latter, *cyclo*[D-Phe-L-Ser(αGlcNAc)] and *cyclo*[D-Phe-L-Ser(βGlcNAc)]. The synthetic approach involved coupling reactions of –OH or *O*-glycosylated serine benzyl esters with NFmoc-protected amino acids (Pro or Phe), followed by one-pot deprotection–cyclization reaction in the presence of 20% piperidine in DMF.

1. Introduction

2,5-Diketopiperazines (DKPs) are peculiar heterocyclic systems presenting spatially defined substituents around a proteolysis-resistant peptide-mimicking scaffold, characteristically derived from the cross-link between two amino acid residues via a pair of complementary amide bonds. Diketopiperazine derivatives are known by properties such as antitumor, antimicrobial, and antiviral activities, and modulation of enzymes, receptors, and biochemical mediators. These naturally occurring cyclic dipeptides are considered privileged structures for medicinal chemistry, since the DKP backbone is straightforwardly obtained and provides a stable and rigid core that can be conveniently functionalized at different positions and configurations.[1–5]

Besides, being small and fairly constrained structures with moderately polar groups like amide, particularly when functionalized with balanced hydrophilic and lipophilic residues, DKP derivatives give rise to valuable physicochemical properties for bioavailable compounds, which may include carrier pro-drugs and blood–brain barrier shuttles.[1,6] Thus, DKPs constitute a rich source of new pharmacologically active compounds that can thrust drug discovery.

Frequent positive hits in biological screens raised the interest for DKP motifs, which demands the development of efficient synthetic methods leading to diversely substituted diketopiperazines.[1] In addition to ordinary amino acid functionalities, unusual or modified side chains as well as nearly any other type of substituents of interest can be incorporated into a DKP scaffold, comprising a versatile strategy to display pharmacophoric groups in a controlled fashion and increase both selectivity and binding affinity toward biological targets.[7,8] Despite numerous derivatives from natural origin, further structural variety can be achieved by combinatorial chemistry through a number of conventional procedures that lead to head-to-tail cyclization of selected dipeptides, both in solution and solid-phase.[5,9]

Although many reports describe support-based general methods for synthesizing DKP combinatorial libraries,[10] problems with scaling up are important shortcomings[9] and the advantages of solid-phase reactions concerning to peptide chemistry do not completely apply to diketopiperazines, as the attachment of the growing chain and its cleavage from the resin introduces worthless steps for the in situ generation of only one or two peptide bonds intended for the product. Synthetic approaches in solution leading to DKPs are scarcer, except for novel strategies involving Ugi multicomponent reactions[11,12] and DKP enolates alkylation,[13,14] which might, in turn, provide poor stereocontrol because generally do not employ linear dipeptides with predefined configuration as precursors. In parallel, microwave-promoted cyclization reactions have been increasingly used with promising results.[3,9,15] Notwithstanding, comprehensive procedures in satisfactory yields for conveniently substituted DKP by homogeneous reactions are still lacking.[9]

Hence, this study exploited the synthesis in solution of a series of related diketopiperazines *cyclo*(L-Pro-L-Ser) (**1**), *cyclo*(L-Phe-L-Ser) (**2**), *cyclo*(D-Phe-L-Ser) (**3**), and the corresponding *O*-glycosylated analogs of the latter, *cyclo*[D-Phe-L-Ser(αGlcNAc)] (**4**) and *cyclo*[D-Phe-L-Ser(βGlcNAc)] (**5**) (Fig. 1).

* Corresponding author. Tel.: +55 16 36024709; fax: +55 16 36024879.
E-mail address: carronal@usp.br (I. Carvalho).

0040-4020/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2009.04.069

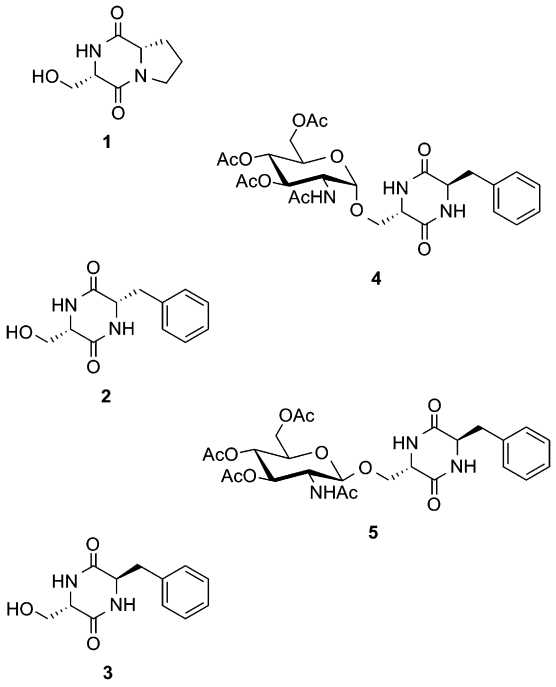


Figure 1. Diketopiperazines and *O*-glycosylated analogs prepared by solution-phase synthesis.

2. Results and discussion

Selection of amino acid residues was carefully based on chemical reactivity and equilibrium between hydrophilic and hydrophobic properties, characteristic of most drugs, and desired features in eventual hits. Residues such as proline, phenylalanine, and serine, brought together in DKPs, suitably combine affinity for aqueous and organic phases, besides belonging to representative classes of amino acids, according to side chain chemical properties. In a sense, structural variety was narrowed by the preference for residues bearing neutral character side chains under all reaction conditions of the synthetic route.

The aliphatic amino acid proline is often emphasized by the peculiarity of its secondary amino group, which induces amide bonds to adopt a cis conformation favoring intramolecular cyclization into bicyclic diketopiperazine structures.[9,10,16] Aromatic residues, including the simplest phenylalanine, are known to establish non-covalent contacts that assist molecular folding, self-assembly, and ligand–receptor interaction.[17,18] Reactive side chain functions, such as the hydroxyl group in serine, comprise versatile sites for decoration with several types of substituents of interest.[1,9] Furthermore, the interest for these amino acids is also justified by their expressive presence in biologically active diketopiperazines, either isolated from natural source or synthetically accessed.[2,19]

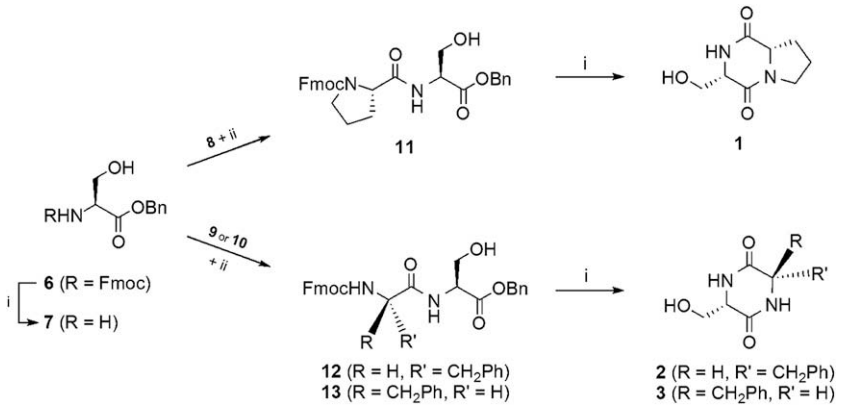
Diketopiperazines are occasionally formed as byproducts in solid-phase peptide synthesis (SPPS) after removal of the amino protecting group under basic cleavage.[20] Thus, our approach exploited this condition to extend the well-known SPPS protocols to solution-phase synthesis, employing the stable *O*Bn group for C-terminal orthogonal protection instead of the solid support. Considering the importance of the nucleophilic strength of the N-terminal nitrogen for efficient cyclization,[3] some commonly used *N*-protecting groups, such as Boc and Alloc, require further treatment with organic bases to induce cyclization after their acid cleavage.[20] Differently, Fmoc group is advantageous due to its cleavage under basic conditions that allow performing dipeptide deprotection and cyclization in a single step.

2.1. Synthesis of diketopiperazines *cyclo*(L-Pro-L-Ser) 1, *cyclo*(L-Phe-L-Ser) 2, and *cyclo*(D-Phe-L-Ser) 3

The synthesis of diketopiperazines **1**, **2**, and **3** was developed in three steps (Scheme 1), by the treatment of Fmoc–L-serine benzyl ester (**6**) with 20% piperidine in DMF, followed by coupling of the resultant L-Ser–OBn (**7**) (94%) with the amino acids Fmoc–L-proline (**8**), Fmoc–L-phenylalanine (**9**), or Fmoc-D-phenylalanine (**10**), respectively, in the presence of the coupling reagents PyBOP, HOBt, and DIEA in acetonitrile.[20] The dipeptides Fmoc–L-Pro-L-Ser–OBn (**11**), Fmoc–L-Phe-L-Ser–OBn (**12**), and Fmoc–D-Phe-L-Ser–OBn (**13**) were obtained in 80, 89, and 85% yields, respectively, and their structures were confirmed by ^{1}H NMR spectroscopy.

The dipeptides **11**, **12**, and **13** were easily cyclized in the presence of 20% piperidine in DMF. Considering that this step involves the well known *N*Fmoc deprotection followed by intramolecular cyclization, it demands longer reaction times (15–18 h) compared to the single cleavage of Fmoc group. It is suggested that, favored by the basic reaction condition, the dipeptide reacts intramolecularly by nucleophilic attack of the free amino group toward the terminal carbonyl with displacement of benzyloxy group, giving a stable six-membered ring. Nevertheless, some *N*-deprotected dipeptides may not undergo cyclization, remaining as linear byproducts.

The formed diketopiperazines **1**, **2**, and **3** were thus obtained in respective yields of 68, 57, and 53%. The absence of aromatic and alkylic hydrogens of both *N*Fmoc and particularly *O*Bn protecting groups in the ^{1}H NMR spectra of **1**, **2**, and **3** indicated the occurrence of cyclization. Subsequently, their structures were confirmed by ESI-MS, which displayed the characteristic adducts $[M+Na]^+$ 207.07 for **1** and $[M+H]^+$ 235.10 for **2** and **3**.



Scheme 1. Synthesis of diketopiperazines **1**, **2**, and **3**. Reagents and conditions: (i) 20% piperidine/DMF, rt; (ii) PyBOP, HOBt, DIEA/MeCN, rt.

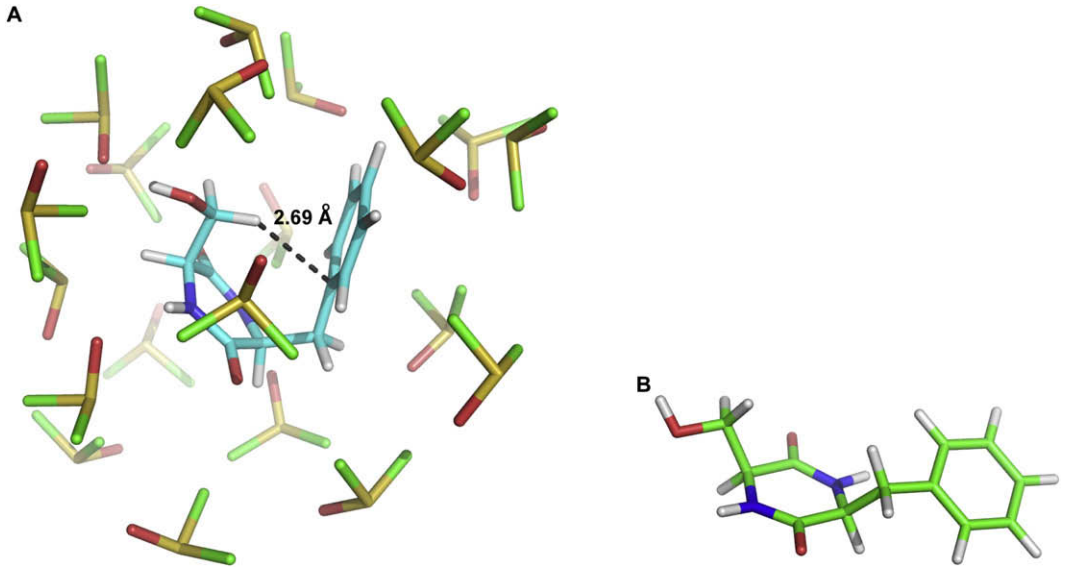


Figure 2. Structures of diketopiperazine **2** in the two main low-energy conformations. In (A), the folded conformer solvated (one layer) with DMSO, and the overall system geometry-optimized at AM1 level of calculation. After the simulation, short distance of 2.69 Å was obtained for the CH/π contact, here represented by a dashed line. Hydrogen atoms of the DMSO molecules were omitted for the sake of the clarity. In (B), the extended conformer geometry-optimized in gas phase at B3LYP/6-31G* level of calculation is shown.

The aromatic containing diketopiperazines **2** and **3** showed remarkable differences between their serine methylenic signals, due to the conformational restriction imposed by ring closure, fixing the amino acid side chains in defined cis and trans configurations, respectively. CH_2 Ser chemical shifts were δ 3.59 and 3.42 for **3**, comparable to both linear dipeptide precursors **12** (δ 3.81 and 3.69) and **13** (δ 3.83 and 3.65), whilst the corresponding diastereoisomer **2** showed δ 3.30 and 2.83 values. This considerable shielding effect observed for compound **2** indicated that, after cyclization, serine methylenic hydrogens are influenced by the paramagnetic current field of phenylalanine benzene ring, through a CH/π interaction.[21] For better understanding this different behavior at ^{1}H NMR spectra, we underwent some conformational analysis of compound **2**.

2.2. Cyclization-derived conformational effects in 2

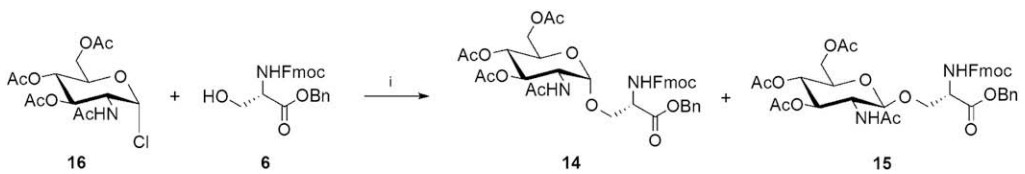
In order to investigate the energetic profiles of diketopiperazine **2** in both solvated and non-solvated conditions, this compound was initially submitted to a conformational search in gas phase. Following this approach, the MMFF molecular mechanics model implemented in the *Spartan '06* software[22] is successful in assigning low-energy conformers and in providing quantitative estimates of conformational energy differences, and it has been the method of choice for large scale conformational surveys.[23,24] Thus, two low-energy conformations, one extended and another folded, have been preferentially obtained for compound **2**. As a second step of the simulation, the folded conformer was immersed in a boundary condition containing five layers of DMSO molecules surrounding the diketopiperazine. The overall system was then energy minimized using the CVFF force field thus implemented in the *DISCOVER3* module of the *Insight II* package,[25] initially maintaining fixed compound **2** and subsequently relaxing all the solute–solvent system. From this resulting optimized system and for the sake of calculation limits, only the first layer of DMSO was energy minimized together with compound **2** using a more robust algorithm, the semi-empirical AM1 model. On the other hand, the extended conformer was fully optimized in gas phase at B3LYP/6-31G* level of calculation.[22] A short distance of 2.69 Å was obtained for the CH/π contact, thus evidenced with the simulation in explicit solvation, and whose result agrees with the expected and reported values obtained for this kind of interaction.[21]

The folded conformation of **2** led to spatial proximity of methylenic serine hydrogens toward the shielding region of the aromatic ring field of phenylalanine residue, thus explaining the observed effect.[9,14] Results of the simulations for the two low-energy conformers of diketopiperazine **2** are shown in Figure 2.

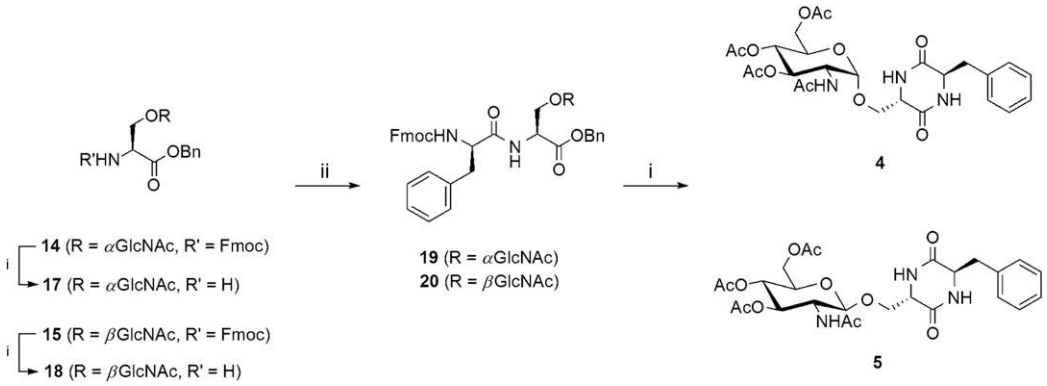
2.3. Synthesis of *O*-glycosylated diketopiperazines *cyclo*-[D-Phe-L-Ser(αGlcNAc)] 4 and *cyclo*[D-Phe-L-Ser(βGlcNAc)] 5

Glycosylated amino acids such as *O*-GlcNAc-Ser/Thr are of utmost importance considering their involvement in cellular recognition, signaling, and regulation processes.[26,27] The will to investigate their display on DKP core prompted us to use the glycosylation method previously reported by our group[28,29] to prepare the building blocks Fmoc–L-Ser(αGlcNAc)–OBn (**14**) and Fmoc–L-Ser(βGlcNAc)–OBn (**15**). As a replacement for serine amino acid **6**, these compounds could be readily employed for the synthesis of glycosylated DKPs.

The glycosylated amino acids **14** and **15** were prepared by glycosylation reaction using αGlcNAcCl (**16**) and the amino acid **6**, in the presence of $HgBr_2$ as catalyst[28,29] (Scheme 2), being obtained in 20 and 40% yields, respectively, after the separation of the anomeric



Scheme 2. Synthesis of the building blocks **14** and **15** by glycosylation reaction. Reagents and conditions: (i) $HgBr_2$, DCE, reflux 9 h.



Scheme 3. Synthesis of diketopiperazines **4** and **5**. Reagents and conditions: (i) 20% piperidine/DMF, rt; (ii) Fmoc-D-Phe-OH (**10**), PyBOP, HOBt, DIEA/MeCN, rt.

mixture by chromatographic column. The assignment of the glycosidic bond configuration of **14** and **15** was based on the coupling constants of the anomeric hydrogens in the ^{1}H NMR spectra, respectively, at δ 4.70 ($J_{1,2}$ 3.6 Hz) and δ 4.67 ($J_{1,2}$ 8.4 Hz).

The *N*Fmoc deprotection of the glycosylated amino acids **14** and **15** with 20% piperidine in DMF gave the products L-Ser(αGlcNAc)–OBn (**17**) (74%) and L-Ser(βGlcNAc)–OBn (**18**) (85%), which were subsequently treated with the amino acid **10** under coupling conditions[20] (Scheme 3), affording the glycodipeptides Fmoc–D-Phe-L-Ser(αGlcNAc)–OBn (**19**) and Fmoc–D-Phe-L-Ser(βGlcNAc)–OBn (**20**) in 75 and 68%, respectively.

The structures of the obtained glycodipeptides were confirmed by ^{1}H NMR spectra, being observed characteristic doublets at δ 4.70 ($J_{1,2}$ 3.7) and δ 4.62 ($J_{1,2}$ 7.9) relative to H-1 of **19** and **20**, respectively, signals of aromatic hydrogens (Fmoc, *O*Bn and the side chain of phenylalanine) and signals of methylenic hydrogens of phenylalanine at δ 3.11–2.94 (**19**), and at δ 3.15 and δ 2.95 (**20**).

Finally, the *N*Fmoc deprotection of the glycodipeptides **19** and **20** by treatment with 20% piperidine in DMF, as described for compounds **1**–**3**, led to the formation of the diketopiperazinic ring by intramolecular cyclization (Scheme 3). However, the cyclization was not so straightforward, as judged by TLC, requiring longer reaction times, 41 h for the α isomer and 68 h for the β isomer. The final glycosylated diketopiperazines **4** and **5** were obtained in reasonable yields, 35 and 43%, respectively. Presumably, owing to the influence of the bulky glycosidic unit in the cyclization reaction, the yields of **4** and **5** were slightly lower compared to the non-glycosylated analog **2** (53%). Although direct glycosylation of **3** could be an alternative approach to get higher yields, diketopiperazine peptide bonds could not resist to the harsh glycosylation conditions.

These final compounds were suitably characterized by means of ^{1}H NMR and ESI-MS analysis, which demonstrated, respectively, the lack of the aromatic and aliphatic hydrogens of *N*Fmoc and *O*Bn, and the presence of the characteristic adduct $[M+Na]^+$ 586.20.

2.4. Microwave-assisted cyclization reactions

An innovative approach employing microwave-assisted heating in peptide chemistry provided intermolecular coupling resulting in dimerization to symmetric diketopiperazines in higher yields (about 90%) and shorter reaction times than conventional thermal heating.[7] Other studies about the use of reactions under microwave irradiation for the synthesis of diketopiperazines derived from dipeptide esters also verified higher yields (97%) compared to classical thermal heating.[9] Therefore, based on the described advantages of reactions under microwave irradiation for synthesis of diketopiperazines, we investigated the cyclization of the corresponding protected dipeptides into DKPs **1**, **2**, and **3** using this approach.[7]

Table 1
Cyclization reactions under conventional and microwave conditions

Cyclized product	Conventional		Microwave	
	Time (h)	Yield (%)	Time (min)	Yield (%)
1	15	68	4	82
2	18	57	4	78
3	18	53	4	70
4	41	35	4	—[a]
5	68	43	4	—[a]

[a] Product not obtained.

The cyclization reactions of the dipeptide precursors in the presence of 20% piperidine in DMF were successfully carried out in open vessels under microwave irradiation (200 W, 163 °C), which led to the total consumption of the starting materials **11**, **12**, and **13** after 4 min and to the generation of the corresponding cyclized products **1**, **2**, and **3** as major products in high and satisfactory yields of 82, 78, and 70%, respectively (Table 1).

The use of reactions under microwave irradiation for obtaining the glycosylated diketopiperazines **4** and **5** was also investigated with the aim to get them in shorter periods of time and in higher yields, as described for the non-glycosylated DKPs. Nevertheless, the employment of the same microwave conditions utilized for cyclization of dipeptides **11**–**13** led to the degradation of the reaction mixtures containing the glycodipeptides **19** or **20** in 20% piperidine/DMF. This may be explained by the lability of the glycosidic bond under harsh conditions, meaning that milder microwave reaction settings have to be further investigated for the synthesis of glycosylated diketopiperazines.

3. Conclusion

In summary, we developed an alternative strategy for the synthesis of diketopiperazines by exploiting one-pot deprotection–cyclization reactions in solution-phase. Such straightforward approach proved to be suitable for both –*O*H and *O*-glycosylated amino acids, suggesting that this method can be effective for substituents of distinct nature. Furthermore, varying the amino acid units with properly protected side chain functionalities may provide DKPs containing sites for additional modification by conventional orthogonal chemistry.

4. Experimental

4.1. General

All chemicals were purchased as reagent grade and used without further purification. Solvents were dried according to standard

methods.[30] Column chromatography was performed on silica gel 60 (0.040–0.063 mm).

Microwave reactions were performed in an open vessel laboratorial oven (CEM DISCOVER). Nuclear magnetic resonance spectra were recorded on Bruker Advance DRX 300 (300 MHz), DPX 400 (400 MHz) or DPX 500 (500 MHz) spectrometers. Chemical shifts (δ) are given in parts per million downfield from tetramethylsilane. Assignments were made with the aid of HMQC and COSY experiments. Optical rotations were measured at ambient temperature on a Jasco DIP-370 digital polarimeter using a sodium lamp. Accurate mass electrospray ionization mass spectra (ESI-HRMS) were obtained using positive ionization mode on a Bruker Daltonics UltrOTOF-Q-ESI-TOF mass spectrometer.

4.2. Synthesis of diketopiperazines 1, 2, and 3

4.2.1. General procedure for NFmoc deprotection and coupling reactions

The C-terminal amino acid *N*-(9-fluorenylmethoxycarbonyl)–L-serine benzyl ester **6** (prepared from commercially available amino acid Fmoc–L-serine by treatment with cesium carbonate and benzyl bromide in DMF)[31] was treated with 20% piperidine/DMF (6 equiv) and stirred for 20 min at room temperature. After concentration in vacuo, the residue was purified by column chromatography (EtOAc/hexane 7:3 v/v; MeOH/DCM 1:9 v/v). To the obtained product L-serine benzyl ester **7**, PyBOP (1.25 equiv), HOBt (1.25 equiv), and the Fmoc-protected N-terminal amino acid (1.25 equiv) were added and dissolved in acetonitrile. The reaction started with the addition of DIEA (2.5 equiv) was allowed to stir at room temperature for periods varying from 15 to 18 h. The resulting mixture was dissolved in EtOAc, washed with dilute HCl solution, saturated aqueous Na_2CO_3 solution and brine, dried over $MgSO_4$, and concentrated in vacuo. Purification was carried out by column chromatography (EtOAc/hexane 7:3, v/v).

4.2.2. General procedure for cyclization

Method A. The protected dipeptide was treated with 20% piperidine/DMF (6 equiv) and allowed to stir at room temperature for periods varying from 15 to 18 h. After concentration in vacuo, the residue was purified by column chromatography (EtOAc/hexane 1:1 v/v, MeOH/DCM 1:9 v/v).

Method B. The solution of the protected dipeptide in 20% piperidine/DMF (6 equiv) was reacted for 4 min in an open vessel microwave oven under 200 W and at 163 °C as set temperature. The product was purified as described in Method A.

4.2.3. Specific procedures

*4.2.3.1. N-[(9H-Fluoren-9-ylmethoxy)carbonyl]-L-prolyl-L-serine benzyl ester (**11**).* Following the procedure described in Section 4.2.1, the coupling reaction of compound **7** (44 mg, 0.23 mmol), PyBOP (146 mg, 0.28 mmol), HOBt (38 mg, 0.28 mmol), and *N*-(fluoren-9-ylmethoxycarbonyl)-L-proline **8** (95 mg, 0.28 mol) was carried out for 18 h. The pure product **11** was obtained as an amorphous solid (93 mg, 0.18 mmol, 80%). R_f 0.40 [EtOAc]. $[\alpha]_D^{25}$ −29.2 (*c* 1.0, $CHCl_3$). δ_H ($CDCl_3$, 400 MHz) 7.65 (2H, d, *J* 7.3 Hz, C*H* Fmoc arom.), 7.49 (2H, dd, *J* 7.9 Hz, C*H* Fmoc arom.), 7.32–7.20 (9H, m, C*H* Bn arom., C*H* Fmoc arom.), 7.10 (1H, d, *J* 7.3 Hz, N*H* Ser), 5.09 (2H, s, CH_2Bn), 4.56 (1H, m, α-C*H* Ser), 4.31–4.21 (2H, m, CH_2 Fmoc), 4.19–4.12 (2H, m, α-C*H* Pro, C*H* Fmoc), 3.91 (1H, dd, J_1 3.4 Hz, J_2 11.5 Hz, CH_{2a} Ser), 3.85 (1H, dd, J_1 3.4 Hz, J_2 11.3 Hz, CH_{2b} Ser), 3.53–3.37 (2H, m, δ-CH_2 Pro), 2.06–1.79 (4H, m, β-CH_2 Pro, γ-CH_2 Pro). δ_C ($CDCl_3$, 100 MHz) 172.1, 170.1 (*C*O Ser, *C*O Pro), 156.1 (*C*O Fmoc), 143.9, 141.3 (*C*quat. Fmoc), 135.2 (*C*quat. Bn), 128.5, 128.1, 127.7, 127.0, 125.1, 119.9 (*C*H Fmoc arom., *C*H Bn arom.), 67.8 (*C*H_2 Fmoc), 67.4 (*C*H_2 Bn), 62.4 (*C*H_2 Ser), 60.7 (α-*C*H Pro), 55.2 (α-*C*H Ser), 47.2 (*C*H Fmoc), 47.1 (δ-*C*H_2 Pro), 29.3 (β-*C*H_2 Pro), 24.5 (γ-*C*H_2 Pro). ESI-HRMS: calculated for $C_{30}H_{30}N_2O_6Na$ $[M+Na]^+$ 537.1996, found 537.2039.

*4.2.3.2. N-[(9H-Fluoren-9-ylmethoxy)carbonyl]-L-phenylalanyl-L-serine benzyl ester (**12**).* Following the procedure described in Section 4.2.1, the coupling reaction of compound **7** (60 mg, 0.30 mmol), PyBOP (203 mg, 0.39 mmol), HOBt (53 mg, 0.39 mmol), and *N*-(fluoren-9-ylmethoxycarbonyl)-L-phenylalanine **9** (151 mg, 0.39 mmol) for 15 h afforded the product **12** as an amorphous solid (156 mg, 0.27 mmol, 89%) after purification. R_f 0.64 [EtOAc]. $[\alpha]_D^{25}$ +1.45 (*c* 1.0, $CHCl_3$). δ_H ($CDCl_3$, 400 MHz) 7.63 (2H, d, *J* 7.6 Hz, C*H* Fmoc arom.), 7.38 (2H, t, *J* 6.8 Hz, C*H* Fmoc arom.), 7.26–7.04 (14H, m, C*H* Bn arom., C*H* Phe arom., C*H* Fmoc arom.), 6.90 (1H, d, N*H* Fmoc), 6.20 (1H, d, N*H* Ser), 5.05 (2H, AB, J_{AB} 12.2 Hz, CH_2 Bn), 4.50 (1H, m, α-C*H* Ser), 4.31 (1H, m, α-C*H* Phe), 4.23–4.09 (2H, m, CH_2 Fmoc), 4.0 (1H, t, *J* 6.8 Hz, C*H* Fmoc), 3.81 (1H, dd, J_1 3.8 Hz, J_2 11.6 Hz, CH_{2a} Ser), 3.69 (1H, m, CH_{2b} Ser), 2.98 (1H, dd, J_1 5.8 Hz, J_2 13.5 Hz, CH_{2a} Phe), 2.80 (1H, dd, J_1 8.4 Hz, J_2 14 Hz, CH_{2b} Phe). δ_C ($CDCl_3$, 400 MHz) 172.1, 169.3 (*C*O Ser, *C*O Phe), 156.1 (*C*O Fmoc), 143.9, 141.3 (*C*quat. Fmoc), 135.2 (*C*quat. Bn), 129.1–127.5 (*C*H Fmoc arom., *C*H Phe arom., *C*H Bn arom.), 124.9 (*C*H Fmoc arom.), 119.7 (*C*H Fmoc arom.), 67 (*C*H_2 Fmoc, *C*H_2 Bn), 62.1 (*C*H_2 Ser), 55.9 (α-*C*H Phe), 54.8 (α-*C*H Ser), 46.8 (*C*H Fmoc), 38.1 (*C*H_2 Phe). ESI-HRMS: calculated for $C_{34}H_{32}N_2O_6Na$ $[M+Na]^+$ 587.2153, found 587.2205.

*4.2.3.3. N-[(9H-Fluoren-9-ylmethoxy)carbonyl]-D-phenylalanyl-L-serine benzyl ester (**13**).* Following the procedure described in Section 4.2.1, compound **7** (70 mg, 0.35 mmol), PyBOP (224 mg, 0.43 mmol), HOBt (58 mg, 0.43 mmol), and *N*-(fluoren-9-ylmethoxycarbonyl)-D-phenylalanine **10** (170 mg, 0.43 mmol) reacted for 15 h, being isolated the product **13** as an amorphous solid (172 mg, 0.30 mmol, 85%). R_f 0.64 [EtOAc]. $[\alpha]_D^{25}$ +14.7 (*c* 1.0, $CHCl_3$). δ_H ($CDCl_3$, 400 MHz) 7.75 (2H, d, *J* 7.6 Hz, C*H* Fmoc arom.), 7.51 (2H, t, *J* 6.8 Hz, C*H* Fmoc arom.), 7.40–7.21 (14H, m, C*H* Bn arom., C*H* Phe arom., C*H* Fmoc arom.), 6.80 (1H, d, N*H* Phe), 5.56 (1H, d, N*H* Ser), 5.13 (2H, s, CH_2 Bn), 4.61–4.27 (4H, m, α-C*H* Ser, α-C*H* Phe, CH_2 Fmoc), 4.15 (1H, t, *J* 6.8 Hz, C*H* Fmoc), 3.83 (1H, dd, J_1 3.8 Hz, J_2 11.6 Hz, CH_{2a} Ser), 3.65 (1H, m, CH_{2b} Ser), 3.07 (2H, m, CH_2 Phe). δ_C ($CDCl_3$, 100 MHz) 172.1, 169.3 (*C*O Ser, *C*O Phe), 156.1 (*C*O Fmoc), 143.9, 141.3 (*C*quat. Fmoc), 135.2 (*C*quat. Bn), 129.1–127.5 (*C*H Fmoc arom., *C*H Phe arom., *C*H Bn arom.), 124.6 (*C*H Fmoc arom.), 119.7 (*C*H Fmoc arom.), 67.5 (*C*H_2 Bn), 67.2 (*C*H_2 Fmoc), 62.4 (*C*H_2 Ser), 56.4 (α-*C*H Phe), 54.6 (α-*C*H Ser), 47.2 (*C*H Fmoc), 38.8 (*C*H_2 Phe). ESI-HRMS: calculated for $C_{34}H_{32}N_2O_6Na$ $[M+Na]^+$ 587.2153, found 587.2211.

*4.2.4. c(L-Prolyl-L-seryl) (**1**)*

Method A. Compound **11** (55 mg, 0.11 mmol) was reacted for 15 h as described in the general procedure (Section 4.2.2). The pure product **1** was obtained in 68% yield (13 mg, 0.072 mmol) as an amorphous solid.

Method B. Compound **11** (30 mg, 0.058 mol) was reacted as described in the general procedure (Section 4.2.2). The product **1** was obtained in 82% yield (8.7 mg, 0.047 mmol) after purification.

R_f 0.04 [EtOAc]. $[\alpha]_D^{25}$ −84.5 (*c* 0.94, MeOH). δ_H (CD_3OD, 300 MHz) 4.17–4.11 (1H, m, α-C*H* Pro), 4.08–4.05 (1H, m, α-C*H* Ser), 3.86–3.76 (2H, m, CH_2 Ser), 3.55–3.46 (1H, m, δ-CH_2 Pro), 3.43–3.35 (1H, m, δ-CH_2 Pro), 2.25–2.19 (1H, m, β-CH_{2a} Pro), 1.96–1.78 (3H, m, β-CH_{2b} Pro, γ-CH_2 Pro). δ_C (CD_3OD, 100 MHz) 170.5, 165 (*C*O Ser, *C*O Pro), 60.3 (*C*H_2 Ser), 58.8 (α-*C*H Pro), 56.9 (α-*C*H Ser), 44.8 (δ-*C*H_2 Pro), 28.2 (β-CH_2 Pro), 21.8 (γ-CH_2 Pro). ESI-HRMS: calculated for $C_8H_{12}N_2O_3Na\cdot[M+Na]^+$ 207.0740, found 207.0746.

*4.2.5. c(L-Phenylalanyl-L-seryl) (**2**)*

Method A. Compound **12** (50 mg, 0.080 mmol) was reacted for 18 h as described in the general procedure (Section 4.2.2). The pure product **2** was obtained in 57% yield (12 mg, 0.050 mmol) as an amorphous solid.

Method B. Compound **12** (25 mg, 0.044 mmol) was reacted as described in the general procedure (Section 4.2.2). The product **2** was obtained in 78% yield (8.0 mg, 0.034 mmol) after purification.

R_f 0.15 [MeOH/DCM: 1:9 v/v]. $[\alpha]_D^{25}$ −124.6 (*c* 0.36, MeOH). δ_H (DMSO, 400 MHz) 7.30–7.15 (5H, m, C*H* arom.), 5.00 (1H, t, *J* 5.7 Hz, O*H*), 4.05 (1H, m, C*H* Phe), 3.66 (1H, m, C*H* Ser), 3.31–3.26 (1H, m, CH_{2a} Ser), 3.10 (1H, dd, J_1 6.0 Hz, J_2 13.5 Hz CH_{2a} Phe), 2.98 (1H, dd, J_1 4.6 Hz, J_2 13.5 Hz CH_{2b} Phe), 2.83 (1H, m, dd, J_1 7.3 Hz, J_2 11.4 Hz CH_{2b} Ser). δ_C (DMSO, 100 MHz) 129.8–126.3 (*C*H arom.), 62.9 (*C*H_2 Ser), 56.9 (α-*C*H Ser), 55.2 (α-*C*H Phe), 39.6 (*C*H_2 Phe). ESI-HRMS: calculated for $C_{12}H_{15}N_2O_3\cdot[M+H]^+$ 235.1004, found 235.1083.

4.2.6. *c(D-Phenylalanyl-L-seryl) (**3**)*

Method A. Compound **13** (41 mg, 0.070 mmol) was reacted for 18 h as described in the general procedure (Section 4.2.2). The pure product **3** was obtained in 53% yield (9.1 mg, 0.040 mmol) as an amorphous solid.

Method B. Compound **13** (60 mg, 0.10 mol) was reacted as described in the general procedure (Section 4.2.2). The product **3** was obtained in 70% yield (17 mg, 0.074 mmol) after purification.

R_f 0.15 [MeOH/DCM 1:9 v/v]. $[\alpha]_D^{25}$ −117.4 (*c* 0.35, MeOH). δ_H (DMSO, 400 MHz) 7.24–7.06 (5H, m, C*H* arom.), 5.00 (1H, sl, O*H*), 4.15 (1H, m, C*H* Phe), 3.59 (1H, dd, J_1 3.0 Hz, J_2 11.6 Hz, CH_{2a} Ser), 3.42 (1H, dd, J_1 2.8 Hz, J_2 11.6 Hz, CH_{2b} Ser), 3.15 (1H, dd, J_1 3.8 Hz, J_2 13.6 Hz, CH_{2a} Phe), 3.06 (1H, m, C*H* Ser), 2.87 (1H, dd, J_1 4.8 Hz, J_2 13.6 Hz, CH_{2b} Phe). δ_C (DMSO, 100 MHz) 129.9–126.4 (*C*H arom.), 62.7 (*C*H_2 Ser), 56.3 (α-*C*H Ser), 54.9 (α-*C*H Phe), 37.6 (*C*H_2 Phe). ESI-HRMS: calculated for $C_{12}H_{15}N_2O_3\cdot[M+H]^+$ 235.1004, found 235.1083.

4.3. Synthesis of glycosylated diketopiperazines 4 and 5

4.3.1. *N-[(9H-Fluoren-9-ylmethoxy)carbonyl]-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-glucopyranosyl)-L-serine benzyl ester (**14**) and N-[(9H-fluoren-9-ylmethoxy)carbonyl]-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-L-serine benzyl ester (**15**)*[29]

A mixture of 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl chloride **16** (300 mg, 0.82 mmol) and *N*-(9-fluorenylmethoxycarbonyl)-L-serine benzyl ester **6** (171 mg, 0.41 mmol) in 1,2-dichloroethane (4.3 mL) was refluxed with mercuric bromide (400 mg, 0.82 mmol) for 9 h and followed by TLC (EtOAc/hexane 7:3, v/v). The resulting amber mixture was concentrated in vacuo and the residue was purified by a silica gel column chromatography (EtOAc/hexane 7:3, v/v). The products **14** (61 mg, 0.081 mmol, 20%) and **15** (122 mg, 0.16 mmol, 40%) were obtained as amorphous solids.

*α-Glycoside **14***. R_f 0.31 (EtOAc/hexane, 7:3 v/v). $[\alpha]_D^{25}$ +14.8 (*c* 1.0, $CHCl_3$). δ_H ($CDCl_3$, 400 MHz) 7.66 (2H, d, *J* 7.5 Hz, C*H* Fmoc arom.), 7.62 (2H, d, *J* 6.0 Hz, C*H* Fmoc arom.), 7.45–7.26 (9H, m, C*H* Fmoc arom., C*H* Bn arom.), 5.95 (1H, d, *J* 9.3 Hz, N*H*Ac), 5.85 (1H, d, *J* 9.5 Hz, N*H* Ser), 5.24–5.16 (3H, m, AB, J_{AB} 12.2 Hz, CH_2Bn, H-3), 5.08 (1H, t, $J_{3,4}$ 9.4 Hz, H-4), 4.70 (1H, d, $J_{1,2}$ 3.6 Hz, H-1), 4.60 (1H, m, C*H* Ser), 4.45 (2H, m, CH_2 Fmoc), 4.30 (1H, dt, $J_{1,2}$ 3.6 Hz, $J_{2,3}$ 9.4 Hz, H-2), 4.25 (1H, t, *J* 7.3 Hz, C*H* Fmoc), 4.20–4.06 (3H, m, CH_{2a} Ser, H-6a, H-6b), 3.88 (2H, m, CH_{2b}Ser), 3.84 (1H, m, H-5), 2.06, 2.05, 2.03, 2.02 (12H, 4s, COCH_3). δ_C ($CDCl_3$, 100 MHz) 171.5, 170.9, 170.8, 170.6 (*C*OCH_3), 169.5 (*C*OCH_2Bn), 156.7 (*C*O Fmoc), 143.6, 141.3 (*C*quat. Fmoc), 134.5 (*C*quat. Bn), 129.2, 129.1, 128.8, 128.0, 127.3, 125.3, 120.2 (*C*H Fmoc arom., *C*H Bn arom.), 99.5 (C-1), 71.4 (C-4), 68.5, 68.4 (C-3, C-5), 68.0 (*C*H_2Bn), 67.9 (*C*H_{2b} Ser), 67.7 (*C*H_2 Fmoc), 62.3 (*C*H_{2a} Ser), 62.2 (C-6), 55.0 (*C*H Ser), 52.5 (C-2), 47.3 (*C*H Fmoc), 23.3–20.8 (COCH_3). ESI-HRMS: calculated for $C_{39}H_{42}N_2O_{13}Na$ $[M+Na]^+$ 764.2579, found 764.2593.

*β-Glycoside **15***. R_f 0.25 (EtOAc/hexane, 7:3 v/v). $[\alpha]_D^{25}$ −4.6 (*c* 1.0, $CHCl_3$). δ_H ($CDCl_3$, 400 MHz) 7.77 (2H, d, *J* 7.8 Hz, C*H* Fmoc arom.), 7.64 (2H, d, *J* 7.8 Hz, C*H* Fmoc arom.), 7.40–7.26 (9H, m, C*H* Fmoc arom., C*H* Bn arom.), 5.81 (1H, d, *J* 8.7 Hz, N*H* Ser), 5.35 (1H, d, *J* 8.4 Hz, N*H*Ac), 5.25 (1H, t, $J_{3,4}$ 9.6 Hz, H-3), 5.20 (2H, AB, J_{AB} 12.2 Hz, CH_2Bn), 5.02 (1H, t, $J_{3,4}$ 10 Hz, H-4), 4.67 (1H, d, $J_{1,2}$ 8.4 Hz, H-1), 4.55–4.38 (3H, m, CH_2 Fmoc, C*H* Ser), 4.28–4.19 (3H, m, H-6a, C*H* Fmoc, CH_{2a} Ser), 4.08 (1H, dd, $J_{5,6b}$ 2.2 Hz, $J_{6a,6b}$ 12.3 Hz, H-6b), 3.85 (1H, dd, *J* 2.7, 10.8 Hz, CH_{2b} Ser), 3.70 (1H, apparent t, $J_{1,2}$, $J_{2,3}$ 10.0 Hz, H-2), 3.64–3.59 (1H, m, H-5), 2.04, 2.03, 2.02, 1.82 (12H, 4s, COCH_3). δ_C ($CDCl_3$, 100 MHz) 170.8, 170.7, 169.5, 169.4 (*C*OCH_3, *C*OCH_2Bn), 156.1 (*C*O Fmoc), 143.7; 143.6; 141.3 (*C*quat. Fmoc), 135.3 (*C*quat. Bn), 128.5, 128.4, 128.2, 127.7, 127.1, 125.1, 119.9 (*C*H Fmoc arom., *C*H Bn arom.), 100.6 (C-1), 71.9, 71.7, (C-5, C-3), 68.9; 68.3 (C-4, CH_2 Ser), 67.3 (*C*H_2Bn), 66.7 (*C*H_2 Fmoc), 61.8 (C-6), 54.6; 54.1 (C-2, CH Ser), 47.0 (*C*H Fmoc), 22.8–20.7 (COCH_3). ESI-HRMS: calculated for $C_{39}H_{42}N_2O_{13}NH_4$ $[M+NH_4]^+$ 764.3031, found 764.3025.

4.3.2. *N-[(9H-Fluoren-9-ylmethoxy)carbonyl]-D-phenylalanyl-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-glucopyranosyl)-L-serine benzyl ester (**19**)*

Compound **14** (61 mg, 0.082 mmol) was treated with 20% piperidine/DMF (0.5 mL) for removal of the *N*Fmoc group. The reaction mixture was stirred for 1 h at room temperature and then purified by column chromatography (EtOAc/hexane, 7:3 v/v; MeOH/DCM 1:9 v/v). The product (2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl)-L-serine benzyl ester **17** was obtained as a pale oil (32 mg, 0.061 mmol, 75%). PyBOP (37 mg, 0.071 mmol), HOBt (9.6 mg, 0.071 mmol), and compound **10** (27 mg, 0.070 mmol) were then added to **14** (30 mg, 0.057 mmol) and dissolved in DMF (0.8 mL), being the reaction initiated with the addition of DIEA (0.05 mL, 0.28 mmol). The mixture was allowed to stir for 20 h at room temperature and purification by column chromatography (EtOAc/hexane, 7:3 v/v) afforded the product **19** as an amorphous solid (38 mg, 0.043 mmol, 75%). R_f 0.36 [EtOAc/hexane, 7:3 v/v]. $[\alpha]_D^{25}$ −5.0 (*c* 0.36, $CHCl_3$). δ_H ($CDCl_3$, 300 MHz) 7.68 (2H, d, *J* 7.7 Hz, C*H* Fmoc arom.), 7.45–7.41 (2H, m, C*H* Fmoc arom.), 7.35–7.21 (14H, m, C*H* Fmoc arom., C*H* Phe arom., C*H* Bn arom.,), 5.84 (1H, d, *J* 8.8 Hz, N*H* Ser), 5.33 (1H, d, *J* 7.4 Hz, N*H* Phe), 5.07 (2H, s, CH_2 Bn), 5.01–4.96 (2H, m, H-3, H-4), 4.70 (1H, d, *J* 3.7 Hz, H-1), 4.44–4.34 (3H, m, H-6a, H-6b, α-C*H* Phe), 4.20–3.94 (5H, m, H-5, α-*CH* Ser, C*H* Fmoc, CH_2 Fmoc), 3.84 (1H, dd, $J_{1,2}$ 3.3 Hz, $J_{2,3}$ 10.2 Hz, H-2), 3.74–3.66 (1H, m, CH_{2a} Ser), 3.64–3.55 (1H, m, CH_{2b} Ser), 3.11–2.94 (2H, m, CH_2 Phe), 1.98, 1.97, 1.93, 1.92 (12H, 4s, COCH_3). δ_C ($CDCl_3$, 100 MHz) 129.2–127.1 (*C*H Fmoc arom., *C*H Phe arom., *C*H Bn arom.), 125.0, 119.8 (*C*H Fmoc arom.), 98.78 (C-1), 70.73 (C-3), 69.56 (C-2), 68.2 (*C*H_2 Ser), 67.9 (C-4), 67.74 (*C*H_2 Bn), 67.23 (C-6), 67.12 (C-5), 61.5 (*C*H_2 Fmoc), 56.30 (α-CH Phe), 51.8 (α-*C*H Ser), 47.0 (*C*H Fmoc), 38.37 (*C*H_2 Phe), 23.03–20.54 (COCH_3). ESI-HRMS: calculated for $C_{48}H_{52}N_3O_{14}$ $[M+H]^+$ 894.3443, found 894.3406.

4.3.3. *N-[(9H-Fluoren-9-ylmethoxy)carbonyl]-D-phenylalanyl-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-L-serine benzyl ester (**20**)*

The *N*Fmoc deprotection of the building block **15** was first realized by treatment with 20% piperidine/DMF (0.3 mL). Starting from 50 mg (0.067 mmol) of **15**, the product (2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl)-L-serine benzyl ester **18** was obtained in 85% yield (29.7 mg, 0.057 mmol) as an oil. Then, following the procedure described for compound **17**, from 28 mg (0.053 mmol) of **18**, PyBOP (37.2 mg, 0.071 mmol), HOBt (9.6 mg, 0.071 mmol), compound **10** (27.6 mg, 0.071 mmol), and DIEA (0.05 mL, 0.28 mmol), it was possible to obtain 32.5 mg (0.036 mmol, 68%) of the product **20**. R_f 0.37 [EtOAc/hexane, 7:3 v/v]. $[\alpha]_D^{25}$ −13.0 (*c* 0.4, $CHCl_3$). δ_H ($CDCl_3$, 300 MHz) 7.69 (2H, d, *J* 7.3 Hz, C*H* Fmoc arom.), 7.45–7.14 (16H, m, C*H* Fmoc arom., C*H* Bn arom., C*H* Phe arom.), 5.44 (1H, d, *J* 7.7 Hz, N*H* Phe), 5.22 (1H, t, $J_{3,4}$

9.9 Hz, H-3), 5.10 (2H, AB, J_{AB} 12.3 Hz, CH_2 Bn), 4.91 (1H, t, $J_{3,4}$ 9.6 Hz, H-4), 4.62 (1H, d, $J_{1,2}$ 7.9 Hz, H-1), 4,40 (2H, m, C*H* Fmoc, α-C*H* Phe), 4.18–3.98 (6H, m, H-5, H-6a, H-6b, α-C*H* Ser, CH_2 Fmoc), 3.77 (1H, dd, *J* 9.4, 1.5 Hz, CH_{2a} Ser), 3.53 (1H, dd, *J* 9.4, 1.5 Hz, CH_{2b} Ser), 3.39 (1H, dd, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 10.2 Hz, H-2), 3,15 (1H, dd, *J* 13.1, 5.3 Hz, CH_{2a} Phe), 2.95 (1H, dd, *J* 13.1, 7.6 Hz, CH_{2b} Phe), 1.98, 1.97, 1.92, 1.81 (12H, 4s, COCH_3). δ_C ($CDCl_3$, 100 MHz) 129.5–127.9 (*C*H Fmoc arom., *C*H Phe arom., *C*H Bn arom.), 125.1, 120.3 (*C*H Fmoc arom.), 100.9 (C-1), 71.9 (*C*H_2 Ser), 71.93 (C-3), 68.53 (C-5), 68.46 (C-4), 67.74 (*C*H_2 Bn), 67.35 (*C*H Fmoc), 62.08 (*C*H_2 Fmoc), 61.86 (C-6), 56.71 (α-*C*H Phe), 55.44 (C-2), 47.22 (α-*C*H Ser), 38.37 (*C*H_2 Phe), 23.73–20.5 (COCH_3). ESI-HRMS: calculated for $C_{48}H_{51}N_3O_{14}Na$ $[M+Na]^+$ 916.3263, found 916.3339.

4.3.4. c(D-Phenylalanyl-O-(2-acetamido-2-deoxy-3,4,6-tri-O-acetyl-α-D-glucopyranosyl)-L-seryl) (**4**)

Compound **19** (30 mg, 0.034 mmol) was treated with 20% piperidine/DMF (0.5 mL) and the reaction mixture was stirred for 41 h, being followed by TLC (EtOAc/hexane, 7:3 v/v). The mixture was then concentrated in vacuo and the residue was purified by column chromatography (EtOAc/hexane 7:3 v/v; MeOH/CH_2Cl_2 1:9 v/v). The product **4** was obtained as an amorphous solid (6.7 mg, 0.012 mmol, 35%). R_f 0.15 [EtOAc/hexane 7:3 v/v]. $[\alpha]_D^{25}$ −186.9 (*c* 0.4, MeOH). δ_H ($CDCl_3$, 300 MHz) 7.33–7.22 (5H, m, C*H* Phe arom.), 6.68 (1H, d, *J* 9.3 Hz, N*H* Phe), 5.15–5.01 (2H, m, H-3, H-4), 4.77 (1H, d, *J* 3.7 Hz, H-1), 4.32–4.27 (2H, m, H-2, α-CH Phe), 4.18–3.95 (3H, m, H-6a, H-6b, α-C*H* Ser), 3.85–3.79 (2H, m, CH_{2a} Ser), 3.58–3.50 (2H, m, H-5, CH_{2b} Ser), 3.24 (1H, dd, *J* 13.7, 3.7 Hz, CH_{2a} Phe), 3.02 (1H, dd, *J* 13.7, 7.5 Hz, CH_{2b} Phe), 2.03, 1.96, 1.93, 1.84 (12H, 4s, COCH_3). δ_C ($CDCl_3$, 75 MHz) 170.9, 169.3, 166.6 (*C*O Ser, *C*O Phe, *C*OCH_3), 129.9–128.0 (*C*H arom.), 98 (C-1), 70.7 (C-3), 68.4 (C-5), 68.3 (α-*C*H Ser), 68.15 (*C*H_2 Ser), 68 (C-4), 61.7 (C-6), 56.1 (α-*C*H Phe), 51.5 (C-2), 40.1 (β-CH_2 Phe), 22.9–20.5 (COCH_3). ESI-HRMS: calculated for $C_{26}H_{33}N_3O_{11}Na$ $[M+Na^+]$ 586.2013, found 586.2028.

4.3.5. c(D-Phenylalanyl-O-(2-acetamido-2-deoxy-3,4,6-tri-O-acetyl-β-D-glucopyranosyl)-L-seryl) (**5**)

According to the procedure described for the synthesis of the product **4**, compound **20** (30 mg, 0.033 mmol) was treated with 20% piperidine/DMF (0.5 mL) and the reaction mixture was stirred for 68 h, being followed by TLC analysis (EtOAc/hexane, 7:3 v/v). The mixture was then concentrated in vacuo and the residue was purified by column chromatography (EtOAc/hexane 7:3 v/v; MeOH/CH_2Cl_2 1:9 v/v). The product **5** was obtained as an amorphous solid (8.1 mg, 0.014 mmol, 43%). R_f 0.13 [EtOAc:hexane 7:3 v/v]. $[\alpha]_D^{25}$ −192.1 (*c* 0.4, MeOH). δ_H ($CDCl_3$, 300 MHz) 7.27–7.20 (5H, m, C*H* Phe arom.), 6.64 (1H, d, N*H* Phe), 6.41 (1H, d, *J* 9.0 Hz, N*H*Ac), 5.09–4.94 (2H, m, H-3, H-4), 4.49 (1H, d, *J* 8.5 Hz, H-1), 4.30–4.27 (1H, m, α-C*H* Phe), 4.22–3.95 (4H, m, H-2, H-6a, H-6b, α-C*H* Ser), 3.67–3.56 (3H, m, H-5, CH_2 Ser), 3.25 (1H, dd, *J* 13.8, 3.8 Hz, CH_{2a} Phe), 2.91 (1H, dd, *J* 13.8, 8.0 Hz, CH_{2b} Phe), 2.01, 1.98, 1.96, 1.93 (12H, 4s, COCH_3). δ_C ($CDCl_3$, 75 MHz) 170.9, 169.3, 166.6 (*C*O Ser, *C*O Phe, *C*OCH_3), 129.9–127.5 (*C*H arom.), 101.4 (C-1), 72.5 (C-3), 71.9 (C-5), 70.9 (α-*C*H Ser), 68.3 (C-4), 61.9 (C-6), 55.5 (*C*H_2 Ser), 55.0 (α-*C*H Phe), 53.8 (C-2), 40.9 (β-*C*H_2 Phe), 23.03–20.54 (COCH_3). ESI-HRMS: calculated for $C_{26}H_{33}N_3O_{11}Na$ $[M+Na]^+$ 586.2013, found 586.2038.

4.4. Molecular modeling procedures

Conformational search was performed on diketopiperazine **2** structure using the MONTE CARLO method with the MMFF molecular mechanics model, thus implemented in the *Spartan '06 1.1.2* software. Boundary conditions were applied to the folded conformer, which was immersed in five layers of DMSO molecules. The overall system was then energy minimized using the CVFF force field thus implemented in the *DISCOVER3* module of the *Insight II* package, initially maintaining fixed compound **2** and subsequently relaxing all the solute–solvent system. From this resulting optimized system and for the sake of calculation limits, only the first layer of DMSO was energy minimized together with compound **2** using the semi-empirical AM1 model. The molecule was then fully optimized in gas phase at B3LYP/6-31G* level of calculation. All the quantum chemical methods here used are implemented in the *Spartan '06 1.1.2* software.

Acknowledgements

Authors are thankful to Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for financial support and fellowships.

Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2009.04.069.

References and notes

1. Gellerman, G.; Hazan, E.; Brider, T.; Traube, T.; Albeck, A.; Shatzmiler, S. *Int. J. Pept. Res. Ther.* **2008**, *14*, 183.
2. Martins, M. B.; Carvalho, I. *Tetrahedron* **2007**, *63*, 9923.
3. Tullberg, M.; Grøtli, M.; Luthman, K. *J. Org. Chem.* **2007**, *72*, 195.
4. Rodionov, I. L.; Rodionova, L. N.; Baidakova, L. K.; Romashko, A. M.; Balashova, T. A.; Ivanov, V. T. *Tetrahedron* **2002**, *58*, 8515.
5. Horton, D. A.; Bourne, G. T.; Smythe, M. L. *Mol. Diversity* **2000**, *5*, 289.
6. Teixidó, M.; Zurita, E.; Malakoutikhah, M.; Tarragó, T.; Giralt, E. *J. Am. Chem. Soc.* **2007**, *129*, 11802.
7. Santagada, V.; Fiorino, F.; Perissutti, E.; Severino, B.; Terraciano, S.; Cirino, G.; Caliendo, G. *Tetrahedron Lett.* **2003**, *44*, 1145.
8. Giraud, M.; Bernad, N.; Martinez, J.; Cavelier, F. *Tetrahedron Lett.* **2001**, *42*, 1895.
9. Tullberg, M.; Grøtli, M.; Luthman, K. *Tetrahedron* **2006**, *62*, 7484.
10. Fischer, P. M. *J. Pept. Sci.* **2003**, *9*, 9.
11. Santra, S.; Andreana, P. *Org. Lett.* **2008**, *9*, 5035.
12. Marcaccini, S.; Torroba, T. *Nat. Protocols* **2007**, *2*, 632.
13. Pichowicz, M.; Simpkins, N. S.; Blake, A. J.; Wilson, C. *Tetrahedron* **2008**, *64*, 3713.
14. Bull, S. D.; Davies, S. G.; Garner, A. C.; Parkers, A. L.; Roberts, P. M.; Sellers, T. G. R.; Smith, A. D.; Tamayo, J. A.; Thomson, J. E.; Vickers, R. J. *New J. Chem.* **2007**, *31*, 486.
15. O'Neill, J. C.; Blackwell, H. E. *Comb. Chem. High Throughput Screening* **2007**, *10*, 857.
16. Santos, C.; Mateus, M. L.; Santos, A. P.; Moreira, R.; Oliveira, E.; Gomes, P. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1595.
17. Joshi, K. B.; Verma, S. *Tetrahedron Lett.* **2008**, *49*, 4231.
18. Kilian, G.; Jamie, H.; Brauns, S. C. A.; Dyason, K.; Milne, P. J. *Pharmazie* **2005**, *60*, 305.
19. Isaka, M.; Palasarn, S.; Rachtawee, P.; Vimuttipong, S.; Kongsaeree, P. *Org. Lett.* **2005**, *7*, 2257.
20. Chan, W. C.; White, P. D. *Fmoc Solid Phase Peptide Synthesis: A Practical Approach*; Oxford University Press: New York, NY, 2000.
21. Umezawa, Y.; Tsuboyama, S.; Takahashi, H.; Uzawa, J.; Nishio, M. *Tetrahedron* **1999**, *55*, 10047.
22. *Spartan '06 full v.1.1.2; Tutorial and User's Guide*; Wavefunction: Irvine, CA, USA, 2006.
23. Hehre, W. J. *A Guide to Molecular Mechanics and Quantum Chemical Calculations*; Wavefunction: Irvine, CA, 2003.
24. Leach, A. R. *Molecular Modelling: Principles and Applications*, 2nd ed.; Pearson: England, 2001.
25. *Insight II*; Accelrys: San Diego, CA, 2005.
26. Campo, V. L.; Carvalho, I. *Quim. Nova* **2008**, *31*, 1027.
27. Zachara, N. E.; Hart, G. W. *Biochim. Biophys. Acta* **2006**, *1761*, 599.
28. Campo, V. L.; Carvalho, I.; Allman, S.; Davis, B. G.; Field, R. A. *Org. Biomol. Chem.* **2007**, *5*, 2645.
29. Carvalho, I.; Scheuerl, S. L.; Kartha, K. P. R.; Field, R. A. *Carbohydr. Res.* **2003**, *338*, 1039.
30. Perrin, D. D.; Armarego, W. L. F.; Perrin, D. R. *Purification of Laboratory Chemicals*, 2nd ed.; Pergamon: New York, NY, 1980; EUA.
31. Wang, S. S.; Gisin, B. F.; Winter, D. P.; Makofske, R.; Kulesha, I. D.; Tzougraki, C.; Meienhofer, J. *J. Org. Chem.* **1977**, *42*, 1286.