Tetrahedron 65 (2009) 1389–1396

Contents lists available at ScienceDirect

Tetrahedron

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

Synthesis of orthogonally protected optically pure ketopiperazine, diketopiperazine, ketodiazepane, and 3-aminopyrrolidone building blocks for peptidomimetic combinatorial chemistry

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ARTICLE INFO

Article history:
Received 27 August 2008
Received in revised form 23 November 2008
Accepted 11 December 2008
Available online 24 December 2008

Keywords:
'Around-the-scaffold' drug optimization
Orthogonal protection
Boc/Fmoc strategy
Reductive alkylation
Solid Phase Organic Chemistry (SPOC)

ABSTRACT

A simple and convenient synthesis of orthogonally protected multi-tethered, optically pure 2-ketopiperazine, diketopiperazine, 2-ketodiazepane and 3-aminopyrrolidone scaffolds for Fmoc combinatorial chemistry has been developed. It utilizes accessible chiral amino acid precursors, sequentially applying reductive alkylation, dipeptide coupling and regioselective ring formation as key steps. These scaffolds are expansion of our 'pool of privileged building blocks' and can introduce valuable drug-like properties in three independent directions to any medicinally relevant piperazine-, diazepane- and pyrrolidone-based motif by 'around-the-scaffold' drug optimization. The synthetic routes reported in this work are general and applicable for the preparation of a diverse library of scaffolds, controlling chirality, arm position and length as well as the nature of functional moieties at the arms for further diversification in three independent directions. In addition, these building blocks have a wide application scope in managing fast and efficient multi-cyclic optimization processes in the combinatorial chemistry and drug design fields.

1. Introduction

Piperazines, diazepanes, and their keto analogs are amongst the most important backbones in today's drug discovery. In recent years combinatorial chemistry around these core structures has become an important tool for generation of new lead structures in drug discovery processes and for providing access to diverse chemical entities with novel structures and properties.[1] These templates are therefore defined in medicinal chemistry as 'privileged scaffolds'—molecular backbones with versatile binding properties representing a frequently occurring binding motif, and providing potent and selective ligands for a wide range of biological targets.[2]

The high number of positive hits revealed in biological screens with the above mentioned scaffolds urged chemists to develop different synthetic methods that allow fast and efficient building of these heterocyclic systems on solid support as well as by homogenous chemistry.[2,3] Yamamoto and coworkers have described the utilization of 3,4,5-trifluorobenzeneboronic acid as an active amidation catalyst in the formation of chiral but symmetrical diketopiperazines (DKPs),[3c] while Brase and coworkers have employed $MeOPCl_2$ in a stoichiometric amount as a coupling reagent in the synthesis of symmetrical and non-symmetrical chiral DKPs containing five-membered ring amino acids.[3d] Other approaches rely on stereospecific alkylation of DKP lithium enolates.[3e,f] This particular method involves necessary bulky groups on the DKP structure, which restricts the fictionalization of the scaffold. However, the majority of the methodologies are not stereospecific and complex mixtures of stereoisomers are generated.[4]

One of the most fascinating methods is multicomponent reactions (MCRs) that seem to be particularly well suited to assemble piperazines, introducing extremely high diversity 'around the scaffold'.[4] On the other hand, it, too, is not stereospecific with regard to the carbons in the piperazine template, and therefore it generates mixtures of stereoisomers.

We have recently introduced a new strategy to overcome this obstacle and to prepare the 'privilege' backbone in optically pure form, bearing various tethers with orthogonally protected groups, which could be further manipulated via Solid Phase Organic Chemistry (SPOC).[5] Such an approach preserves the important chiral centers and the related diversity in potential hits. It was applied in a facile and convenient synthesis of ketopiperazine and

Abbreviations: Alloc, allyloxycarbonyl; Boc, *tert*-butyloxycarbonyl; Cbz, benzyloxycarbonyl; DCM, dichloromethane; DKP, diketopiperazine; Fmoc, 9-fluorenylmethoxycarbonyl; NMM, *N*-methyl morpholine; PE, petrol ether; SPOC, Solid Phase Organic Chemistry.

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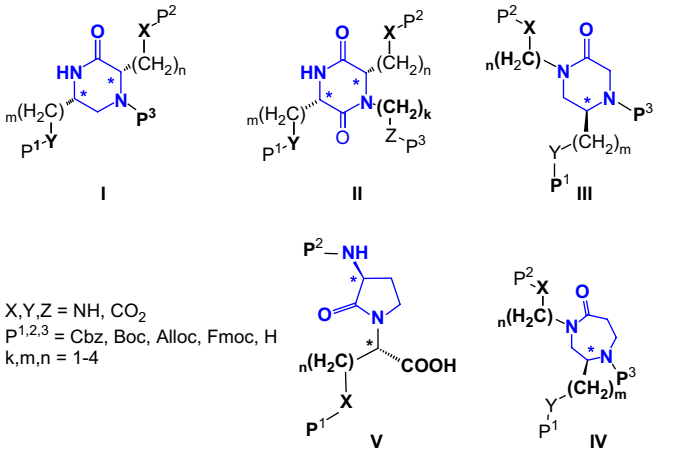
doi:10.1016/j.tet.2008.12.046



Scheme 1. General structures of orthogonally protected optically pure keto- and diketopiperazine, 2-ketodiazepane, and 3-aminopyrrolidone building blocks.

DKP scaffolds of types **I** and **II** (Scheme 1) tethered with orthogonally protected arms ready for Fmoc and Boc SPOC 'around the scaffold'.

In this article, we report on the expansion of our repertoire of 'privileged' core structures, describing novel syntheses of 2-ketopiperazine, 2-ketodiazepane, and 3-aminopyrrolidone scaffolds of the general structures **III**, **IV**, and **V**, respectively (Scheme 1), and the preparation of additional DKP and 2-ketopiperazine scaffolds of types **I** and **II**. Noteworthy, **I** differs from **III** by positioning of the arms at the piperazine template, further emphasizing the versatility of our approach for diversification of the chosen core. These chiral building blocks can be applied in 'around-the-scaffold' modification strategy and efficient multi-cyclic optimization processes by SPOC in Fmoc (Fmoc/Alloc/Cbz protection) mode, introducing valuable physico-chemical properties in three independent diversity points and yielding compounds amenable to the Lipinski rule of five and ADMETox requirements. Being small and relatively constrained scaffolds with three hydrophilic groups like amine and carboxyl, structures **III**–**V**, in the same manner as their antecedents **I** and **II**, comprise a favorable source for generation of new bioavailable compounds.[6] Furthermore, being chiral and controllable in the length and the nature of the side arms, our scaffolds will likely yield piperazine, diazepane, and pyrrolidone libraries with high-resolution coverage of medicinal space around these privileged motifs.

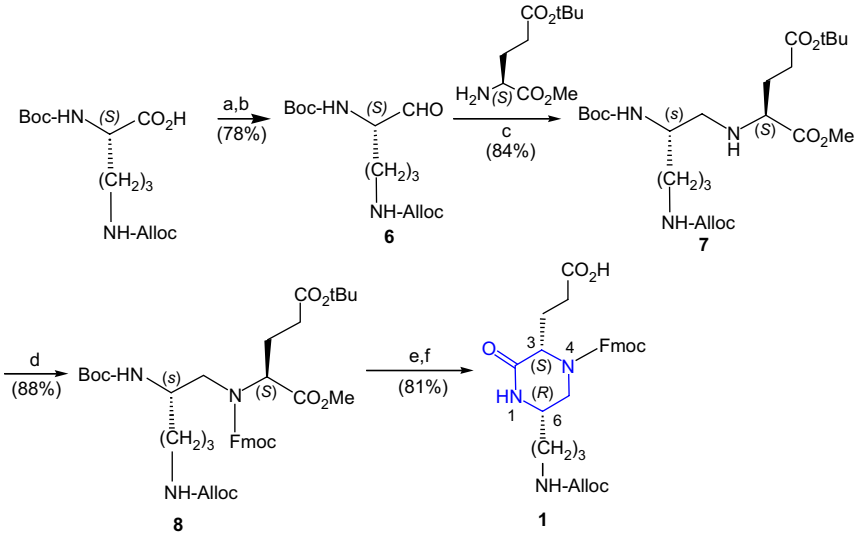
The methodology presents some unique features: we describe representative syntheses of several scaffolds, demonstrating the potential of our approach to diversify the piperazine, diazepane, and pyrrolidone templates; the ring formation process can provide unusual macrocyclic systems; the tethered amines are orthogonally protected while a carboxylic group is used without protection, ready for loading on the resin for further SPOC manipulations.

2. Results and discussion

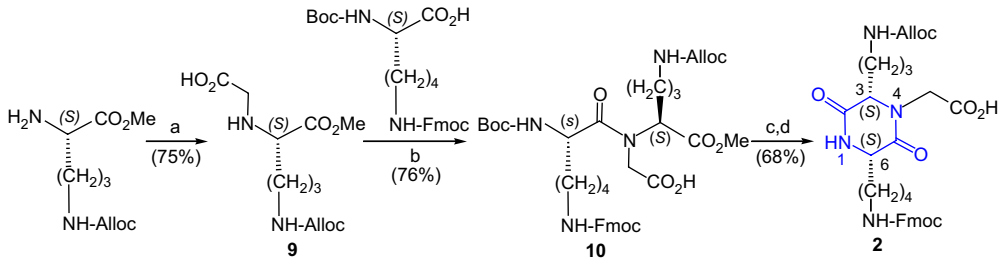
2.1. Optically pure Alloc/Fmoc orthogonally protected ketopiperazine 1

Our preliminary studies on the synthesis of 2-ketopiperazine and DKP building blocks [5] encouraged us to prepare additional Fmoc/Alloc protected analogs of this type under optimized conditions. For 2-ketopiperazines, we successfully employed a simple reductive alkylation reaction[7] between the optically pure orthogonally protected ornithinal **6** and optically pure (*S*)-glutamic acid diester (Scheme 2) to furnish the reduced dipeptide intermediate **7** (Scheme 2) for Fmoc chemistry.

In light of this need, we initially synthesized ornithinal **6** carrying α-Boc and ω-Alloc orthogonal protecting groups (Scheme 2), starting from the commercially available (*S*)-ornithine counterpart. Thus, Boc-L-Orn(Alloc)-OH was converted into the corresponding hydroxamate (Weinreb amide) by reaction with *N*,*O*-dimethylhydroxylamine hydrochloride[8] in the presence of ^{i}BuOCOCl/NMM. Subsequent reduction of the Weinreb amide with $LiAlH_4$ at rt in dry THF[9] led to the formation of aldehyde **6** (78% yield for the two steps). (*S*)-Glutamic acid diester was then reductively alkylated with **6** in methanol containing 1% acetic acid in the presence of $NaBCNH_3$[7,10] to form pseudodipeptide **7** (84% yield). The pseudodipeptide, with the reduced amide bond [CH_2NH], was protected by Fmoc-Cl in DCM in the presence of TEA, yielding fully protected pseudodipeptide **8** (Scheme 2),[11] which after acidic ^{t}Bu/Boc removal was submitted to cyclization toward the ketopiperazine structure. Thus, the deprotected dipeptide intermediate from **8** was cyclized under optimized diketopiperazine cyclization conditions by reflux in 2-butanol[12] in the presence of 2 equiv of NMM in order to release the α-amine from its TFA salt. Apparently, the absence of toluene



Scheme 2. Synthesis of optically pure Alloc/Fmoc orthogonally protected ketopiperazine **1**. (a) NH(OMe)Me, ^{i}BuOCOCl, NMM, THF, rt, 18 h; (b) $LiAlH_4$, THF, rt; (c) $NaBH_3CN$, AcOH, MeOH, 1:99, rt; (d) Fmoc-Cl, TEA, DCM, 0 °C to rt, overnight; (e) TFA, DCM, 1:1, 0 °C to rt, 2 h; (f) TEA, 2-butanol, reflux, 18 h.



Scheme 3. Synthesis of optically pure Alloc/Fmoc orthogonally protected diketopiperazine **2**. (a) $HCOCO_2H$, $NaBH_3CN$, AcOH, MeOH, 1:99, rt, 24 h; (b) HATU, TEA, DMF, 60 °C, 6 h; (c) 4 N HCl in dioxane, 0 °C, 24 h; (d) NMM, 2-butanol, reflux, 10 h.

afforded ketopiperazine building block **1** in better yields (81%) after purification by flash chromatography (5% MeOH/EtOAc).[5,13,14]

2.2. Fmoc/Alloc orthogonally protected optically pure diketopiperazine (DKP) 2

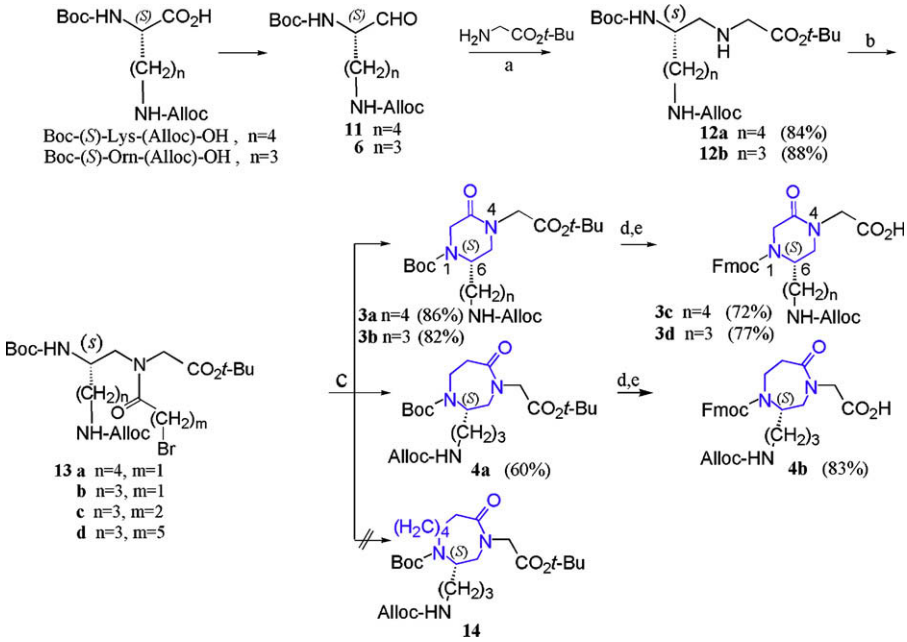
Advancing to our goal, namely the formation of a pool of scaffolds for 'around-the-scaffold' combinatorial chemistry, we prepared a non-symmetrical diketopiperazine (DKP) scaffold, similarly amenable to our library generation concept. The synthetic route is simple and applicable for the synthesis of chiral DKPs with various arm positioning, length, and nature of functional moieties at the end of the arms. In this particular case our chiral DKP building block **2** bears AllocNH propyl (generated from Orn), FmocNH butyl (generated from Lys), and methylene carboxylic acid (generated from glyoxylic acid) arms.

Analogous to ketopiperazines, the most convenient mode to initiate the synthesis was a straightforward reductive alkylation of accessible precursor H-(*S*)-Orn(Alloc)-OMe[15] with glyoxylic acid, which constructed a side chain bearing a carboxyl functionality in the final DKP scaffold. Thus, the N_α-carboxymethyl ω-protected ornithine **9** (Scheme 3) was prepared from these precursors using $NaCNBH_3$ in 1% of AcOH in methanol in 75% yield (estimated by HPLC). Compound **9**, with secondary amine, was then subjected to the difficult coupling with Boc-(*S*)-Lys(Fmoc)-OH. Upon heating for 6 h at 60 °C with HATU in DMF,[5,16] dipeptide **10** was afforded in 58% (Scheme 3). Subsequently, the obtained dipeptide was deprotected using 4 N HCl in dioxane, and after evaporation of the solvent the resulted hydrochloride was subjected to the ring closure attempts without further purification.

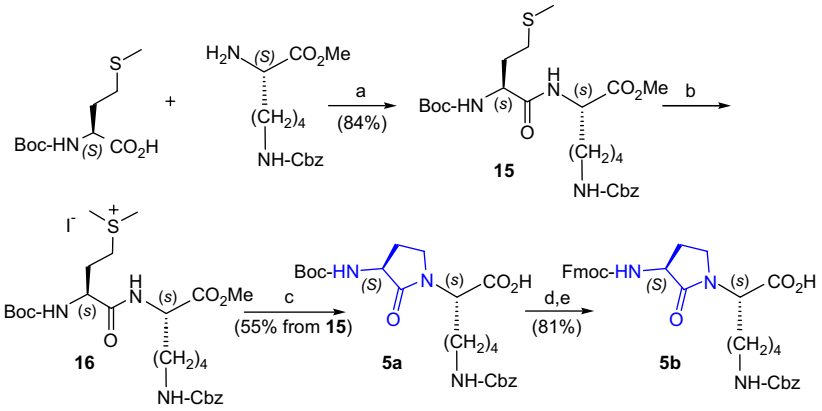
Noteworthy, **10** already possesses the required orthogonal protection before the final cyclization to the corresponding 2,5-diketopiperazine, significantly simplifying the entire synthetic route. Finally, after refluxing in 2-butanol in the presence of an equivalent amount of NMM, the crude desired diketopiperazine was obtained. Due to its inability to precipitate upon continuous cooling, the crude compound was purified by flash chromatography (5% MeOH/EtOAc), to give pure **2** in 68% yield.[17] A small amount of a DKP byproduct, without the 6-ω-Fmoc protection, was also isolated (^{i}PrOH/AcOH/H_2O (90:8:2), 7%). This side reaction was previously observed[5] and attributed to the rather vigorous conditions for obtaining the Fmoc protected DKPs. Due to the insignificant yield of the byproduct in this particular case, it was not subjected to Fmoc reprotection.

2.3. Optically pure Fmoc/Alloc protected ketopiperazines 3 and ketodiazepane 4

Our approach for the synthesis of 2-ketopiperazines **3a**,**b** and 2-ketodiazepane **4** building blocks is also based on a simple reductive alkylation reaction between the optically pure orthogonally protected lysinal **11** or ornitinal **6** and glycine *tert*-butyl ester (Scheme 4). This convenient and useful reaction is a key step in the synthesis of almost all of our intermediates toward the



Scheme 4. Synthesis of Alloc/Boc and Alloc/Fmoc orthogonally protected ketopiperazines **3** and ketodiazepane **4**. (a) $Na(AcO)_3BH$, DCE, 4 Å MS, N_2, 0 °C, overnight; (b) $ClCO(CH_2)_mBr$, aq EtOAC, $NaHCO_3$, 0 °C, 20 min; (c) $CsCO_3$, DMF, 65 °C, 2 h; (d) TFA/DCM, 1 h; (e) Fmoc-Cl, DIPA, DCM, 1:1, 0 °C, overnight.



Scheme 5. Synthesis of Cbz/Boc and CBz/Fmoc orthogonally protected aminopyrrolidone **5**. (a) DIC, HOBT; (b) CH_3I; (c) NaH, DMF/DCM 1:1; (d) HCO_2H, 98%; (e) Fmoc-Cl, DIEA, DCM.

'privileged scaffolds', nicely reflected in the yields of the final compounds.

Lysinal **11** was synthesized similar to **6** (Scheme 2) starting from the commercially available doubly protected (*S*)-lysine.[5] Glycine *tert*-butyl ester was then reductively alkylated with **11** and **6** in dichloroethane in the presence of $NaB(OAc)_3H$[7,10] to form dipeptides **12a** (84% yield) and **12b** (88% yield) correspondingly. Other reductive conditions like $NaBH_3CN$ in 1% AcOH/MeOH led to lower yields. The obtained pseudodipeptides with the reduced amide bond [CH_2NH] were acylated by 2-bromo acetyl, 3-bromo propionyl, and 6-bromo hexanoyl chlorides in ethyl acetate/$NaHCO_3$ (aq), yielding the corresponding **13a–d** (Scheme 4),[11] which were subjected to the cyclization reactions toward the ketopiperazine and related structures. Despite the presence of two alkylating moieties on **13** (NHBoc and NHAlloc), we anticipated to obtain regioselective cyclization toward the thermodynamically more favorable smaller ring, namely through the NHBoc. Indeed, after utilizing several alkylating conditions (heating **13** with Cs_2CO_3 in DMF as the best choice), regioselectivity was obtained for **13a–c**, yielding the desired Boc/Alloc protected ketopiperazine **3a**,**b** and ketodiazepane **4a** in reasonable yields (86% for **3a**, 82% for **3b**, and 60% for **4a**). All attempts to cyclize **13d** into 10-membered 1,4-diazecan-5-one analog (**14**) were unsuccessful, probably due to thermodynamic restrictions to form large rings.

Noteworthy, scaffolds **3a**,**b** and **4a** bear Boc/Alloc protection, suitable for Boc chemistry. Thus, we decided to also prepare the corresponding scaffolds with orthogonal protection adapted to Fmoc chemistry, namely Fmoc on the secondary ring amine and Alloc on the side chain primary amines (Scheme 4). This would demonstrate the versatility of protecting strategies that can be applied in our structures using the above synthetic method. Standard Boc/^{t}Bu removal from **3a**,**b** and **4a** (1:1 mixture of TFA in DCM, 30 min at 0 °C, then 30 min at rt) led to reddish intermediates, which were used in the next step without purification. Finally, the above intermediaries were Fmoc reprotected (Fmoc-Cl, in DCM, overnight in the presence of diisopropylethyl amine), yielding the desired ketopiperazines **3c**,**d** and ketodiazepane **4b** scaffolds in reasonable yields (72%, 77%, and 83%, respectively) after flash chromatography purification (5% MeOH/EtOAc).

2.4. Fmoc/Cbz orthogonally protected optically pure 2-aminopyrrolidone scaffold 5

The pyrrolidone (2-oxopyrrolidine) family has been the subject of research for more than three decades. Experimental and clinical studies first focused on their so-called nootropic effects, later came the possibilities for neuroprotection after stroke and their use as antiepileptic agents.[18] This information encouraged us to develop synthesis for aminopyrrolidone building blocks suitable for Fmoc combinatorial chemistry.

An intramolecular cyclative alkylation route, similar to that used for the synthesis of **3** and **4**, was applied to the synthesis of the 2-aminopyrrolidone scaffold **5** (Scheme 5). Protected methionine dipeptide **15** was chosen as a starting material since the methylthio function can potentially be converted into a leaving group. This dipeptide was prepared by a standard method from (*S*)-Boc-methionine and (*S*)-lysine-(Cbz) methyl ester in optically pure form (84% yield). Sulfonium salt **16** was formed by alkylation with methyl iodide, and cyclization to the corresponding γ-lactam **5a** was induced by sodium hydride in 1:l DMF/methylene chloride in 55% yield from **15**.[4] Other bases like lithium diisopropylamide (LDA) and dimethyaminopyridine (DMAP) in THF were not effective.[19] Competitive amide O-alkylation under these conditions was not observed by NMR spectroscopy and HPLC. Interestingly, under these conditions of the cyclization, the methyl ester is almost completely hydrolyzed yielding the free acid **5a** as the major isolated product. This change is comparable to the rate of disappearance of a transient TLC spot of higher R_f observed in the cyclization reactions. The source of this ester cleavage was previously investigated and addressed to the presence of Na_2O contamination in the commercial NaH.[19] In fact, acid **5a** is already Boc/Cbz orthogonally protected 2-aminopyrrolidone scaffolds suitable for Boc SPOC. In the next step we converted **5a** to the corresponding Fmoc analog. Thus, 2-aminopyrrolidone **5a** was deprotected using formic acid for 2 h, and after evaporation of the solvent the resulted residue was subjected to Fmoc protection as for **1** and **2** without further purification. The crude product was purified by flash chromatography (1–5% MeOH/EtOAc), to give pure **5b** in 81% yield as Fmoc/Cbz orthogonally protected 2-aminopyrrolidone building block applicable in Fmoc SPOC.

Development of the general synthetic approaches for protectable tethering of core structures with various functional arms at various positions as well as controlling stereochemistry is a valuable capability for drug design by combinatorial chemistry. The work presented in this paper contributes to this endeavor. Attempts to facilitate cyclization into other ring systems and optimization of the reaction conditions, including microwave assisted chemistry,[20] are in progress.

3. Conclusions

In this paper we have introduced a simple and convenient preparation of orthogonally protected multi-tethered, optically

pure ketopiperazine, DKP, 2-ketodiazepane, and 3-amino-pyrrolidone structural elements for Boc and Fmoc combinatorial chemistry.

These medicinal cores of the general structures **I–V** are expansion of our 'pool of privileged building blocks' useful for 'around-the-scaffold' modification strategy by SPOC, introducing valuable physico-chemical properties at three independent diversity points.

The key step of the synthesis involves standard reductive alkylation between optically pure amino acid synthons that bear suitable protecting groups for Boc solid phase synthetic methodologies. Subsequent ring closure in the intermediate compounds, followed by Boc to Fmoc change of protection, afforded the desired scaffolds for Fmoc SPOC. Being optically pure, relatively constrained and controllable in stereochemistry and the length and the nature of the side arms, our scaffolds generate piperazine, diazepane, and pyrrolidone-based libraries with high-resolution coverage of medicinal space around the chosen motif, improving the selectivity properties of revealed hits. In addition, our approach enables to manage fast and efficient multi-cyclic optimization processes around 'privileged' templates for the discovery of novel peptidomimetic bioactive compounds.

4. Experimental section

4.1. General

Analytical HPLC was performed on a 250×4.2 mm Lichroprep RP-18 column from Merck, with a 1 mL/min flow and detection at 214 nm. The eluents were triply distilled water and HPLC-grade CH_3CN (containing 0.1% TFA) or MeOH. Optical rotations were recorded at 25 °C in a 10-cm length cell and $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2/g. The concentration of all the samples was 0.5%. Mass spectra were measured in the positive and negative modes using a quadrupole mass spectrometer equipped with an electrospray ionization source and cross-flow inlet. ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively, in $CDCl_3$, unless otherwise indicated. Assignments in the final products were supported by 2D COSY, TOCSY, NOESY, ROESY, HMBC, and HMQC spectroscopy. All chemical shifts are reported with respect to TMS. Chromatography was carried out by standard flash chromatography and TLC on silica gel (Merck 7735).

4.1.1. Boc-(S)-Orn-(Alloc)-H (**6**)

Compound **9** was prepared by optimized literature procedure:[5] to a solution of commercial Boc-(*S*)-Orn-(Alloc)-OH (3.53 g, 12 mmol) in DCM (50 mL) was added *N*-methyl morpholine (2.6 mL, 24 mmol). The mixture was cooled to −15 °C, and isobutyl chloroformate (1.81 g, 12 mmol) was added. After 15 min at this temperature, *N*,*O*-dimethylhydroxylamine hydrochloride (1.02 g, 14 mmol) was added. The mixture was stirred at −15 °C for 1 h, allowed to warm to rt, and stirred for additional 3 h. The reaction mixture was poured into H_2O (40 mL), and the aqueous phase was extracted with DCM (2×40 mL). The combined organic extracts were dried over Na_2SO_4 and the solvent was removed in vacuo to give crude hydroxamate, which after purification by flash chromatography (50% EtOAc/PE) yielded pure intermediate hydroxamate (3.80 g, 90%) as a colorless oil. R_f=0.35 (EtOAc/PE, 1:1); MS *m*/*z* 382 (MNa^+, 100); ^{1}H NMR δ 5.91 (m, 1H), 5.36 (dd, *J*=10, 2 Hz, 1H), 5.20 (dd, *J*=16, 2 Hz, 1H), 4.48 (d, *J*=5 Hz, 2H), 4.35–4.32 (m, 2H), 3.62 (s, 3H), 2.75 (s, 3H), 2.21–2.18 (m, 2H), 1.9–1.5 (m, 2H), 1.42 (s, 9H), 1.10 (m, 2H). To the solution of the hydroxamate (2.57 g, 7 mmol) in THF (100 mL) $LiAlH_4$ (0.53 g, 14 mmol) was added in several portions and the reaction mixture was stirred under N_2 for 40 min in an ice bath. A solution of $KHSO_4$ (1.35 g) in 30 mL H_2O was added, and THF was removed under reduced pressure. The residue was extracted with ether (4×40 mL). The combined organic layers were washed with 1 N HCl solution (3×40 mL), satd $NaHCO_3$ (40 mL), and brine (40 mL), and then dried ($MgSO_4$). Evaporation of the solvent gave crude aldehyde **4** as colorless oil (1.58 g, 88% yield), which was used in the next step without further purification. R_f=0.75 (EtOAc/PE, 1:1); MS *m*/*z* 323 (MNa^+, 100); ν_{max} (KBr): 1710, 1655, 1280 cm^{-1}; ^{1}H NMR δ 9.05 (br s, 1H), 5.75 (m, 1H), 5.40 (dd, *J*=10, 2 Hz, 1H), 5.15 (dd, *J*=16, 2 Hz, 1H), 4.52 (d, *J*=5 Hz, 2H), 4.17–4.14 (m, 2H), 2.29–2.26 (m, 2H), 1.9–1.5 (m, 2H), 1.42 (s, 9H), 1.15–1.12 (m, 2H).

4.1.2. Boc-(S)-Orn-(Alloc)-(S)-Glu-(^{t}Bu)-OMe pseudodipeptide diester (**7**)

H-(*S*)-Glu(^{t}Bu)-OMe (1.04 g, 5 mmol) was added to 30 mL of MeOH/AcOH (99:1) until the solution became clear. Then aldehyde **6** (1.42 g, 5 mmol) was added in 10 mL of MeOH/AcOH (99:1), the reaction mixture was stirred for 1 h at rt, followed by portion wise addition of $NaCNBH_3$ (0.39 g, 6.5 mmol). When TLC showed complete conversion of the starting materials, the solvent was evaporated in vacuo and the residue was used in the next step without any purification. The yield of conversion was estimated by calculation of the area under a peak by HPLC (1.38 g, 84% yield). $[\alpha]_D^{20}$ +23 (*c* 1.6, $CHCl_3$); MS *m*/*z* 502 (M^+, 100); ν_{max} (KBr): 1670, 1650, 1320, 1210 cm^{-1}; ^{1}H NMR δ 5.72 (m, 1H), 5.33 (dd, *J*=10, 2 Hz, 1H), 5.22 (dd, *J*=16, 2 Hz, 1H), 4.33 (d, *J*=5 Hz, 2H), 4.26–4.22 (m, 2H), 3.61 (s, 3H), 2.94–2.91 (m, 4H), 2.23–2.18 (m, 4H), 1.9–1.5 (m, 8H), 1.50 (s, 9H), 1.46 (s, 9H), 1.17–1.14 (m, 2H).

4.1.3. Fmoc protected Boc-(S)-Orn (Alloc)-(S)-Glu(^{t}Bu)-OMe pseudodipeptide (**8**)

Compound **7** (1.32 g, 2.65 mmol) was taken in 30 mL DCM. The reaction mixture was stirred for a few minutes, cooled, and TEA (1.5 mL, 15 mmol) was added, followed by addition of Fmoc chloride (0.59 g, 2.65 mmol). After stirring overnight, DCM (40 mL) was added and the organic phase was washed sequentially twice with cold 0.5 N HCl and brine, and dried over Na_2SO_4. After filtration, the solvent was evaporated and the residue was chromatographed (EtOAc/PE, 1:1) to afford 1.82 g (88% yield) of pure **8** as a colorless oil. R_f=0.70 (EtOAc/PE, 1:2.5), $[\alpha]_D^{20}$ +19 (*c* 1.7, $CHCl_3$); HRMS *m*/*z* 624.3783 (100%) (MH^+−Boc, calculated 723.3731 for $C_{39}H_{53}N_3O_{10}$), ν_{max} (KBr): 1685, 1660, 1220, 1070 cm^{-1}; ^{1}H NMR δ 7.80 (d, *J*=8 Hz, 2H), 7.63 (d, *J*=8 Hz, 2H), 7.44 (t, *J*=8 Hz, 2H), 7.40 (t, *J*=8 Hz, 2H), 5.75 (m, 1H), 5.39 (dd, *J*=10, 2 Hz, 1H), 5.22 (dd, *J*=16, 2 Hz, 1H), 4.52–4.49 (m, 2H), 4.45 (d, *J*=5 Hz, 2H) 4.35 (t, *J*=6 Hz, 1H), 4.03–3.98 (m, 2H), 3.84 (s, 3H), 2.95–2.90 (m, 4H), 2.24–2.19 (m, 4H), 1.9–1.5 (m, 8H), 1.55 (s, 9H), 1.42 (s, 9H), 1.13–1.10 (m, 2H). ^{13}C NMR δ 179.1, 170.3, 168.5, 166.2, 157.0, 155.4, 143.7, 141.8, 131.8, 127.3, 127.3, 124.2, 118.3, 118.3, 78.9, 77.3, 67.5, 67.2, 65.9, 59.4, 47.3, 44.0, 42.5, 40.3, 33.2, 29.4, 29.0, 28.2, 23.6, 21.9.

4.1.4. Fmoc/Alloc orthogonally protected 2-ketopiperazine carboxylic acid (**1**)

Compound **8** (1.16 g, 1.36 mmol) was carefully dissolved in ice bath cooled TFA in DCM (1:1, 30 mL). The reaction mixture was left to warm to rt and after 2 h the solvent was removed by repeated evaporation with DCM (50 mL, three times) in vacuum. To the resulting viscous residue were added 2-butanol (40 mL) and NMM (0.14 mL, 1.36 mmol). The resulting reaction mixture was refluxed overnight. The solvent was evaporated to afford an oily residue, which after purification gave 0.77 g (81%) of **1** as a colorless oil; $[\alpha]_D^{20}$ +30 (*c* 1.7, $CHCl_3$); HRMS *m*/*z* 521.2478 (MH^+, calculated 521.2162 for $C_{30}H_{35}N_3O_7$); ν_{max} (KBr): 3500–3100 (br s), 1700, 1650, 1430, 1120 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 7.61 (d, *J*=8 Hz, 2H), 7.54 (d, *J*=8 Hz, 2H), 7.36 (t, *J*=8 Hz, 2H), 7.22 (t, *J*=8 Hz, 2H), 6.30 (m, 1H), 5.30 (dd, *J*=12, 10 Hz, 1H), 5.24 (br d, 1H), 4.47 (s, 2H), 4.23 (t, *J*=6 Hz, 1H), 4.15–4.12 (m, 2H), 2.68–2.63 (m, 4H), 2.14–2.09 (m,

4H), 1.8–1.4 (m, 8H), 1.28–1.25 (m, 2H); ^{13}C NMR δ 175.7, 168.3, 168.0, 165.2, 154.3, 142.3, 141.4, 131.2, 129.3, 126.3, 125.2, 117.5, 117.0, 67.0, 66.5, 65.1, 46.7, 44.3, 42.7, 33.9, 28.4, 28.0, 24.6, 22.9.

4.1.5. *N-(Methylenecarboxy)-(S)-Orn-(Alloc)-OMe (**9**)*

Compound **9** (colorless oil) was prepared from commercial H-(*S*)-Orn-(Alloc)-OMe (2.44 g, 10 mmol) and glyoxylic acid monohydrate (0.92 g, 10 mmol) by the same procedure as for **7** in 75% yield (HPLC conversion). The residue was used in the next step without any purification. MS *m*/*z* 289 (M^+, 100); ν_{max} (KBr): 1690, 1680; 1645, 1310 cm^{-1}; ^{1}H NMR δ 6.34 (m, 1H), 5.38 (dd, *J*=12, 10 Hz, 1H), 5.18 (br d, 1H), 4.14–4.11 (m, 2H), 3.98 (s, 3H), 2.99 (br s, 2H), 2.39–2.35 (m, 2H), 1.8–1.4 (m, 3H), 1.27–1.25 (m, 2H).

4.1.6. *N$^{\alpha}$-Boc-(S)-Lys-(Fmoc)-N$^{(\prime)\alpha}$-(CH$_2$CO$_2$H)-(S)-Orn-(Alloc)-OMe (**10**)*

Compound **10** was synthesized from **9** (4.06 g, 14 mmol) and commercial Boc-(*S*)-Lys-(Fmoc)-OH (6.52 g, 14 mmol) in DMF by heating at 60 °C with HATU (6.35 g, 16.8 mmol) and DIEA (5.7 mL, 50 mmol) for 6 h. After evaporation of the solvent, the residue was taken in DCM (100 mL) and washed twice with 1 N citric acid and brine. Purification by flash chromatography (EtOAc) gave pure **10** (7.15 g, yield 76%) as colorless oil. R_f=0.35 (EtOAc) $[\alpha]_D^{20}$ +12 (*c* 1.0, $CHCl_3$); HRMS *m*/*z* 737.3445 (M^-, calculated 738.3476 for $C_{38}H_{50}N_4O_{11}$); ν_{max} (KBr): 1705, 1670, 1640, 1150 cm^{-1}; ^{1}H NMR δ 7.83 (d, *J*=8 Hz, 2H), 7.63 (d, *J*=8 Hz, 2H), 7.63–7.48 (m, 9H), 6.26 (m, 1H), 5.42 (br d, 1H), 5.27 (br d, 1H), 4.80 (s, 2H), 4.56 (s, 2H), 4.28–4.22 (m, 4H), 3.94 (s, 3H), 2.97–2.88 (m, 6H), 2.26–2.19 (m, 4H), 1.9–1.5 (m, 10H), 1.5 (s, 9H), 1.32–1.14 (m, 8H).

4.1.7. *Fmoc/Alloc orthogonally protected 2,5-DKP carboxylic acid (**2**)*

Compound **10** (1 g, 1.38 mmol) was subjected to Boc removal in 4 N HCl dioxane (40 mL) at 0 °C for 24 h, and then the solvent and the excess HCl were removed by repeated evaporation with dioxane (3×20 mL). The resulting hydrochloride was cyclized into the corresponding DKP carboxylic acid **2** in the same manner as for **1**, yielding after chromatography (EtOAc) 0.56 g (68% yield) of colorless oil. R_f=0.65 (5% MeOH/EtOAc), $[\alpha]_D^{20}$ +38 (*c* 1.9, $CHCl_3$); HRMS *m*/*z* 593.2840 (MH^+, calculated 593.2413 for $C_{31}H_{36}N_4O_8$); ν_{max} (KBr): 3500–3100 (br s), 1695, 1660, 1640, 1270, 1090 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 7.54 (d, *J*=8 Hz, 2H), 7.43 (d, *J*=8 Hz, 2H), 7.40 (t, *J*=8 Hz, 2H), 7.20 (t, *J*=8 Hz, 2H), 5.98 (m, 1H), 5.55 (dd, *J*=10, 12 Hz, 2H), 5.25 (br d, 2H), 5.11 (s, 2H), 4.40 (br d, 2H), 4.22 (t, *J*=6 Hz, 1H), 3.88 (br s, 2H), 3.66 (m, 1H), 2.77–2.70 (m, 4H), 1.5–1.2 (m, 10H); ^{13}C NMR δ 179.8, 167.1, 165.5, 164.6, 153.2, 143.5, 140.8, 132.0, 125.7, 126.4, 123.5, 119.0, 118.0, 67.0, 64.8, 63.0, 47.0, 46.5, 43.0, 41.9, 40.6, 33.2, 30.2, 27.3, 23.6.

4.1.8. *Boc-(S)-Lys-(Alloc)-Gly-O-^{t}Bu pseudodipeptide ester (**12a**)*

Lysinal **11** (1.61 g, 5 mmol) and Gly-*O*-^{t}Bu free base (0.65 g, 5 mmol) were added under N_2 atmosphere to 30 mL of dry dichloroethane (DCE) in the presence of activated 4 Å molecular sieves and were stirred for 1 h at 0 °C. Then $NaBH(AcO)_3$ (1.42 g, 7 mmol) was added and the reaction mixture was stirred overnight at 0 °C. The solvent was evaporated in vacuo and the oily residue was used in the next step without any purification. The yield was estimated by HPLC (1.90 g, 84% yield). $[\alpha]_D^{20}$ +14 (*c* 2.1, $CHCl_3$); MS *m*/*z* 430 (M^+, 50), 330 (M^+−Boc, 100); ν_{max} (KBr): 1650; 1630, 1115 cm^{-1}; ^{1}H NMR δ 5.70 (m, 1H), 5.37 (dd, *J*=10, 2 Hz, 1H), 5.23 (dd, *J*=16, 2 Hz, 1H), 4.33 (d, *J*=5 Hz, 2H), 4.23–4.20 (m, 2H), 2.93–2.88 (m, 4H), 2.12–2.09 (m, 2H), 1.9–1.5 (m, 4H), 1.51 (s, 9H), 1.48 (s, 9H), 1.17–1.14 (m, 2H).

4.1.9. *Boc-(S)-Orn-(Alloc)-Gly-O-^{t}Bu pseudodipeptide ester (**12b**)*

Compound **12b** was prepared in the same manner as **12a** from the corresponding ornithinal **6**. The yield was estimated by HPLC (2.03 g, colorless oil, 88% yield). $[\alpha]_D^{20}$ +16 (*c* 1.5, $CHCl_3$); MS *m*/*z* 416 (M^+, 60), 316 (M^+−Boc, 100); ν_{max} (KBr): 1650, 1640, 1575, 1020 cm^{-1}; ^{1}H NMR δ 5.70 (m, 1H), 5.37 (dd, *J*=10, 2 Hz, 1H), 5.23 (dd, *J*=16, 2 Hz, 1H), 4.33 (d, *J*=5 Hz, 2H), 4.23–4.19 (m, 2H), 2.91–2.86 (m, 4H), 2.11–2.08 (m, 2H), 1.9–1.5 (m, 4H), 1.51 (s, 9H), 1.48 (s, 9H), 1.18–1.15 (m, 2H).

4.1.10. *Boc-(S)-Lys-(Alloc)-(NHCOCH$_2$Br)-Gly-O-^{t}Bu pseudodipeptide ester (**13a**)*

A solution of 2-bromo acetyl bromide (1.2 g, 6 mmol) in EtOAc (10 mL) was added dropwise at 0 °C to a stirring mixture of **12a** (2.15 g, 5 mmol) in EtOAc and 1 N $NaHCO_3$ (4:1, 50 mL). After 2 h at 0 °C, the mixture was diluted with EtOAc (50 mL) and saturated solution of $NaHCO_3$ (50 mL) was added. The organic phase was collected and washed with H_2O (20 mL) and brine (20 mL), dried over Na_2SO_4, and concentrated. The crude product was filtered over a silica gel column to afford compound **12a** (colorless oil), which was used without further purification. R_f=0.60 (EtOAc). HRMS *m*/*z* 550.205 (100.0%), 552.204 (90%) (MH^+, calculated 550.2050 and 552.2029 for $C_{23}H_{40}BrN_3O_7$); ν_{max} (KBr): 1660, 1650, 1320, 1015 cm^{-1}; ^{1}H NMR δ 5.90 (m, 1H), 5.46 (dd, *J*=10, 2 Hz, 1H), 5.34 (dd, *J*=16, 2 Hz, 1H), 4.45 (s, 2H), 4.26 (d, *J*=5 Hz, 2H), 4.12–4.09 (m, 2H), 2.88–2.83 (m, 4H), 2.22–2.18 (m, 4H), 1.9–1.5 (m, 6H), 1.45 (s, 9H), 1.40 (s, 9H).

4.1.11. *Boc-(S)-Orn-(Alloc)-(NHCOCH$_2$Br) Gly-O-^{t}Bu pseudodipeptide ester (**13b**)*

Compound **13b** (colorless oil) was prepared in the same manner as **13a** from the corresponding **12b** and 2-bromo acetyl bromide. R_f=0.60 (EtOAc). HRMS *m*/*z* 536.187 (75.0%), 538.189 (73%) (MH^+, calculated 536.1893 and 538.1893 for $C_{22}H_{38}BrN_3O_7$); ν_{max} (KBr): 1660, 1340, 1030 cm^{-1}; ^{1}H NMR δ 5.90 (m, 1H), 5.45 (dd, *J*=10, 2 Hz, 1H), 5.35 (dd, *J*=16, 2 Hz, 1H), 4.50 (s, 2H), 4.23 (d, *J*=5 Hz, 2H), 4.12–4.09 (m, 2H), 2.75–2.68 (m, 4H), 2.17–2.14 (m, 4H), 1.9–1.5 (m, 4H), 1.47 (s, 9H), 1.40 (s, 9H).

4.1.12. *Boc-(S)-Orn-(Alloc)-(NHCOCH$_2$CH$_2$Br)-Gly-O-^{t}Bu pseudodipeptide ester (**13c**)*

Compound **13c** (colorless oil) was prepared in the same manner as **13a** from the corresponding **12b** and 3-bromo propionyl chloride. R_f=0.60 (EtOAc). HRMS *m*/*z* 550.206 (50.0%), 552.203 (46%) (MH^+, calculated 550.2050 and 552.2029 for $C_{23}H_{40}BrN_3O_7$); ν_{max} (KBr): 1660, 1640, 1355, 1085 cm^{-1}; ^{1}H NMR δ 5.90 (m, 1H), 5.45 (dd, *J*=10, 2 Hz, 1H), 5.35 (dd, *J*=16, 2 Hz, 1H), 4.23 (d, *J*=5 Hz, 2H), 4.11–4.09 (m, 2H), 3.62–3.59 (m, 2H), 2.67–2.63 (m, 4H), 2.26–2.18 (m, 6H), 1.9–1.5 (m, 4H), 1.45 (s, 9H), 1.42 (s, 9H).

4.1.13. *Boc-(S)-Orn-(Alloc)-(NHCO(CH$_2$)$_5$Br)-Gly-O-^{t}Bu pseudodipeptide ester (**13d**)*

Compound **13d** (colorless oil) was prepared in the same manner as **13a** from the corresponding **12b** and 6-bromo hexanoyl chloride. R_f=0.60 (EtOAc). HRMS *m*/*z* 592.253 (90.0%), 594.251 (85%) (MH^+, calculated 592.253 and 594.250 for $C_{26}H_{46}BrN_3O_7$); ν_{max} (KBr): 1650, 1490, 1305, 1035 cm^{-1}; ^{1}H NMR δ 5.95 (m, 1H), 5.45 (dd, *J*=10, 2 Hz, 1H), 5.35 (dd, *J*=16, 2 Hz, 1H), 4.32 (d, *J*=5 Hz, 2H), 4.12–4.08 (m, 2H), 3.54–3.51 (m, 2H), 2.66–2.60 (m, 4H), 2.28–2.20 (m, 6H), 1.9–1.5 (m, 10H), 1.50 (s, 9H), 1.40 (s, 9H).

4.2. General procedure for ring closure of 13a–c to 3a,b and 4a

Compound **13a**, **13b** or **13c** (5 mmol) was dissolved in 30 mL of dry DMF. Cs_2CO_3 (10 mmol) was added and the reaction mixture was heated to 65 °C under N_2 atmosphere with vigorous stirring. After 2 h at this temperature, the solvent was evaporated, the residue was taken into DCM (100 mL), washed twice with 1 N citric acid (50 mL) and brine (50 mL), and dried over Na_2SO_4. After

filtration, the solvent was evaporated and the oily residue was purified by chromatography (EtOAc/PE, 1:1) to give the desired product.

Compound **3a:** 0.67 g colorless oil (86% yield), R_f=0.8 (EtOAc/PE 1:1), $[\alpha]_D^{20}$ +23 (*c* 1.8, $CHCl_3$); HRMS *m*/*z* 470.5798 (MH^+, calculated 470.5717 for $C_{23}H_{39}N_3O_7$); ν_{max} (KBr): 1670, 1650, 1600, 1235, 1015 cm^{-1}; ^1H NMR δ 5.92 (m, 1H), 5.38 (dd, *J*=12, 10 Hz, 2H), 5.20 (dd, *J*=12, 10 Hz, 2H), 5.11 (br s, 2H), 4.40 (br d, 2H), 3.88 (br s, 2H), 3.66 (m, 1H), 2.71–2.67 (m, 2H), 1.8–1.2 (m, 24H).

Compound **3b:** 0.59 g colorless oil (82% yield), R_f=0.8 (EtOAc/PE 1:1); $[\alpha]_D^{20}$ +25 (*c* 2.0, $CHCl_3$); HRMS *m*/*z* 456.5501 (MH^+, calculated 456.5451 for $C_{22}H_{37}N_3O_7$); ν_{max} (KBr): 1670, 1640, 1310 cm^{-1}; ^1H NMR δ 5.98 (m, 1H), 5.36 (br d, 2H), 5.22 (br d, 2H), 5.10 (br s, 2H), 4.45 (br d, 2H), 3.80 (br s, 2H), 3.70 (m, 1H), 2.61–2.57 (m, 2H), 1.8–1.2 (m, 22H).

Compound **4a:** 0.42 g colorless oil (60% yield), R_f=0.8 (EtOAc/PE, 1:1), $[\alpha]_D^{20}$ +14 (*c* 2.2, $CHCl_3$); HRMS *m*/*z* 470.5786 (MH^+, calculated 470.5717 for $C_{23}H_{39}N_3O_7$); ν_{max} (KBr): 1670, 1650, 1170 cm^{-1}; ^1H NMR δ 5.95 (m, 1H), 5.40 (dd, *J*=12,10 Hz, 2H), 5.20 (br d, 2H), 5.11 (br s, 2H), 4.40 (br d, 2H), 3.88 (br s, 2H), 3.66 (m, 1H), 2.73–2.70 (m, 2H), 2.19–2.15 (m, 2H), 1.8–1.2 (m, 22H).

4.3. General procedure for the synthesis of 3c,d and 4b

Compound **3a**, **3b** or **4a** (1.36 mmol) was carefully dissolved in ice bath cooled TFA in DCM (1:1, 30 mL). The reaction mixture was left to warm to rt and after 2 h the solvent was removed by repeated evaporation with DCM (50 mL, three times) in vacuum giving viscous reddish oil, which was used in the next step without further purification. In the next step the reddish oil was taken into 50 mL of DCM and 10 mmol of DIEA was added at 0 °C. Fmoc-Cl (1.2 mmol) was added in small portions and the reaction mixture was left overnight at rt. Then, additional 50 mL DCM was added and the organic phase was washed twice with 1 N citric acid, satd $NaHCO_3$, and brine. After drying over Na_2SO_4 and subsequent filtration, the solvent was evaporated and the final crude product was purified by flash chromatography.

*4.3.1. (S)-2-(5-(Allyloxycarbonylamino)butyl)-4-(2-fluorenyl-2-oxoethyl)-5-oxopiperazine-1-carboxylic acid (**3c**)*

Colorless oil (0.65 g, 72% yield), R_f=0.35 (10% MeOH/EtOAc), $[\alpha]_D^{20}$ +17 (*c* 1.9, $CHCl_3$); HRMS *m*/*z* 536.2780 (MH^+, calculated 536.2319 for $C_{29}H_{33}N_3O_7$); ν_{max} (KBr): 3500–3100 (br s), 1685, 1650, 1100 cm^{-1}; ^1H NMR δ 7.80 (d, *J*=8 Hz, 2H), 7.60 (br d, 2H), 7.42 (t, *J*=8 Hz, 2H), 7.30 (br t, 2H), 5.95 (m, 1H), 5.38 (br d, 2H), 5.20 (br d, 2H), 5.00 (s, 2H), 4.60–4.20 (m, 10H), 3.88 (br s, 2H), 3.69–3.64 (m, 3H), 3.20–3.17 (m, 2H), 1.5–1.2 (m, 8H); ^{13}C NMR δ 174.0, 165.3, 160.8, 162.1, 142.5, 141.2, 131.0, 128.2, 125.0, 120.5, 120.0, 117.0, 66.0, 65.1, 64.2, 48.7, 46.9, 42.5,, 42.0, 41.0, 30.4, 29.9, 20.5.

*4.3.2. (S)-2-(5-(Allyloxycarbonylamino)propyl)-4-(2-fluorenyl-2-oxoethyl)-5-oxopiperazine-1-carboxylic acid (**3d**)*

Colorless oil (0.68 g, 77% yield), R_f=0.35 (10% MeOH/EtOAc), $[\alpha]_D^{20}$ +18 (*c* 1.2, $CHCl_3$); HRMS *m*/*z* 521.2452 (MH^+, calculated 521.2162 for $C_{28}H_{31}N_3O_7$), ν_{max} (KBr): 3500–3100 (br s), 1685, 1655, 1205 cm^{-1}; ^1H NMR δ 7.70 (br d, 2H), 7.55 (br d, 2H), 7.40 (br t, 2H), 7.28 (br t, 2H), 5.90 (m, 1H), 5.30 (br d, 2H), 5.23 (br d, 2H), 5.05 (s, 2H), 4.60–4.20 (m, 10H), 3.82 (br s, 2H), 3.57–3.53 (m, 3H), 3.11–3.08 (m, 2H), 1.5–1.2 (m, 6H); ^{13}C NMR δ 175.1, 163.3, 162.8, 161.1, 143.2, 140.2, 130.2, 127.8, 124.0, 122.3, 121.2, 118.6, 65.0, 64.5, 63.1, 45.2, 45.0, 44.7, 43.6, 42.0, 28.5, 22.7.

*4.3.3. (S)-2-(5-(Allyloxycarbonylamino)propyl)-4-(2-fluorenyl-2-oxoethyl)-5-oxodiazepane-1-carboxylic acid (**4b**)*

Colorless oil (0.71 g, 83% yield), R_f=0.40 (3% MeOH/AtOAc), $[\alpha]_D^{20}$ +12 (*c* 1.8, $CHCl_3$); HRMS *m*/*z* 536.2643 (MH^+, calculated 536.2319 for $C_{29}H_{33}N_3O_7$); ν_{max} (KBr): 3500–3100 (br s), 1680, 1640, 1110 cm^{-1}; ^1H NMR δ 7.78 (d, *J*=8 Hz, 2H), 7.57 (d, *J*=8 Hz, 2H), 7.35 (t, *J*=8 Hz, 2H), 7.26 (t, *J*=8 Hz, 2H), 5.90 (m, 1H), 5.60 (br s, 1H), 5.33 (br d, 2H), 5.20 (br d, 2H), 4.56 (br d, 2H), 4.40 (m, 1H), 4.20–4.17 (m, 2H), 3.90 (br s, 1H), 3.66 (m, 1H), 3.21–3.17 (m, 2H), 1.5–1.2 (m, 8H); ^{13}C NMR δ 175.4, 163.0, 162.2, 160.5, 141.4, 140.7, 133.3, 126.4, 124.0, 122.2, 121.1, 118.4, 66.0, 65.5, 63.0, 62.3, 45.8, 45.2, 43.7, 41.3, 40.9, 31.6, 19.8.

*4.3.4. Boc-(S)-Met-(S)-Lys-(Cbz)-OMe (**15**)*

(*S*)-Lysine-(Cbz)-methyl ester hydrochloride (1 g, 3 mmol), HOBT (0.4 g, 3 mmol), *tert*-butyloxycarbonyl-(*S*)-methionine (0.8 g, 3 mmol), and *N*,*N*-diisopropylethylamine (1.05 mL, 6 mmol) were dissolved in dry THF (15 mL), the solution was cooled in an ice-water bath, and diisopropylcarbodiimide (0.4 g, 3.15 mmol) was added. Stirring was continued for 1 h at 0 °C and an additional hour at rt. The solvent was evaporated in vacuo. A mixture of EtOAc (15 mL) and satd $NaHCO_3$ (7.5 mL) was added to the residue and the organic phase was sequentially extracted with 10% citric acid in water, satd $NaHCO_3$, and water (7.5 mL each). The solution was dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The residue was triturated with hexane, filtered, washed with hexane, and dried. The crude dipeptide derivative (1.80 g,) was purified by chromatography on basic alumina (EtOAc) (1.46 g, white solid, 84% yield). ν_{max} (KBr): 1670, 1650, 1420, 1090 cm^{-1}; ^1H NMR δ 8.89 (br d, 1H), 7.85 (s, 5H), 5.11 (s, 2H), 3.71 (s, 3H), 3.25–3.22 (m, 2H), 2.5 (t, *J*=6 Hz, 2H), 2.1–2.3 (m, 5H), 2.1–1.1 (m, 8H), 1.42 (s, 9H).

*4.3.5. Boc-Met-Lys-(Cbz)-methyl ester methylsulfonium iodide (**16**)*

Boc-Met-Lys(Cbz)-OMe (**15**, 18.7 g) was dissolved in CH_3I (60 mL) and stirred at rt for 3 days. Concentration in vacuo gave an amorphous yellowish solid (19.1 g, 95% yield). ν_{max} (KBr): 1675, 1650, 1420, 1375, 1200, 1065 cm^{-1}; ^1H NMR δ 8.89 (d, *J*=7 Hz, 1H), 7.8 (s, 5H), 6.03 (d, *J*=7 Hz, 1H), 5.37 (m, 1H), 5.11 (s, 2H), 4.7–4.3 (m, 2H), 3.71 (s, 3H), 3.3–3.0 (m, 2H), 3.27–3.24 (m, 3H), 3.24–3.21 (m, 2H), 3.1 (s, 3H), 2.1–1.1 (m, 8H), 1.42 (s, 9H).

*4.3.6. (S)-3-[(tert-Butoxycarbonyl)amino]-2-oxo-1-pyrrolidine-(S)-6-[(benzyloxycarbonyl)amino]-2-heptanoic acid (**5a**)*

Sulfonium salt **16** (10 g, 15.3 mmol) was dissolved in 300 mL of 1:l DMF–CH_2C1_2 under N_2 and cooled to 0 °C. NaH (1.5 g of a 50% mineral oil suspension, 31.5 mmol) was added at once, and the mixture was stirred at 0 °C for 2.5 h. Ethyl acetate (100 mL) followed by water (24 mL) was added, and the resultant solution was left overnight at rt. The solution was concentrated in vacuo to a small volume and partitioned between water (50 mL) and CH_2Cl_2 (50 mL). The phases were separated, and the aqueous phase was acidified to pH 4 with 0.5 M citric acid. Continuous extraction with CH_2Cl_2 followed by concentration in vacuo gave crystalline off-white product (4.6 g, 58% yield): mp 137–139 °C. Recrystallization from EtOAc gave 4 g of white crystals: mp 141.5–143 °C; $[\alpha]_D^{20}$ +21 (*c* 2.5, $CHCl_3$); MS *m*/*z* 477 (M^+−Boc, 100); ν_{max} (KBr): 1670, 1660,1385, 1225 cm^{-1}; ^1H NMR δ 7.8 (s, 5H), 5.11 (s, 2H), 4.54 (dd, *J*=11, 6 Hz, 1H), 4.3 (br t, *J*=9 Hz, 1H), 3.5–3.2 (m, 2H), 3.1 (t, *J*=6 Hz, 2H), 2.1–1.1 (m, 8H), 1.42 (s, 9H).

*4.3.7. (S)-2-((S)-3-(((9H-Fluoren-9-yl)methoxy)carbonylamino)-2-oxopyrrolidin-1-yl)-6-(benzyloxycarbonylamino)-2-methylhexanoic acid (**5b**)*

Compound **5a** (4 g, 8.2 mmol) was dissolved in 98% HCO_2H (50 mL) and the solution was kept at rt for 2 h. After removal of the excess formic acid in vacuo, the residue was dissolved in 50 mL of DCM at 0 °C, then diisopropylethylamine (8.5 g=10.2 mL, 65.6 mmol) and Fmoc chloride (2.12 gr=8.2 mmol) were added, and the resultant solution was left overnight with vigorous stirring. The organic layer was extracted with 1 N HCl and then twice with brine. The solution was dried over anhydrous Na_2SO_4 filtered and

evaporated to dryness in vacuo. The oily residue was crystallized from EtOAc affording 5.4 g (81% yield) of white crystals. $[\alpha]_D^{20}$ +24 (*c* 1.4, $CHCl_3$); HRMS *m*/*z* 586.2530 (MH^+, calculated 586.2475 for $C_{33}H_{35}N_3O_7$); ν_{max} (KBr): 1680, 1660, 1015 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.8–7.2 (m, 13H), 5.11 (s, 2H), 4.54 (dd, *J*=11, 6 Hz, 1H), 4.3 (br t, *J*=9 Hz, 1H), 3.5–3.2 (m, 2H), 3.1 (t, *J*=6 Hz, 2H), 2.1–1.1 (m, 8H); ^{13}C NMR δ 176.2, 171.0, 153.9, 152.3, 138.2, 130.3, 127.4, 126.8, 126.0, 118.0, 117.6, 67.0, 66.5, 65.4, 44.2, 43.1, 41.1, 34.5, 28.9, 27.2, 21.5.

Acknowledgements

We wish to thank Mr. Vladimir Gaisin for analytical assistance.

Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2008.12.046.

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