JOC The Journal of Organic Chemistry

Article

pubs.acs.org/joc

Tetrahydro-β-carboline-Based Spirocyclic Lactam as Type II′ β-Turn: Application to the Synthesis and Biological Evaluation of Somatostatine Mimetics

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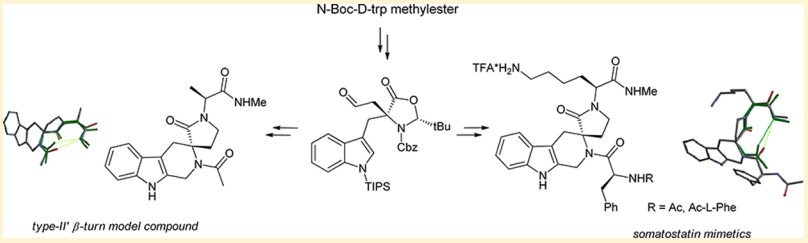
Supporting Information



ABSTRACT: The synthesis of novel spirocyclic lactams, embodying D-tryptophan (Trp) amino acid as the central core and acting as peptidomimetics, is presented. It relies on the strategic combination of Seebach's self-reproduction of chirality chemistry and Pictet−Spengler condensation as key steps. Investigation of the conformational behavior by molecular modeling, X-ray crystallography, and NMR and IR spectroscopies suggests very stable and highly predictable type II′ β-turn conformations for all compounds. Relying on this feature, we also pursued their application to two potential mimetics of the hormone somatostatin, a pharmaceutically relevant natural peptide, which contains a Trp-based type II′ β-turn pharmacophore.

INTRODUCTION

The conformation of a peptide is crucial for its biological activity. Turn structures in general have received particular attention because they play an important role in biological recognition and signal transduction processes. Many naturally occurring oligopeptides have been proposed to adopt turns in their bioactive conformation. As an example, over 100 peptide-activated G protein-coupled receptors (GPCRs) bind ligands with a turn structure.[1]

The development of privileged molecular scaffolds efficiently mimicking reverse turn motifs has attracted remarkable interest when structural constraints are exploited to increase both binding and selectivity. One of the successful approaches to restrict peptide conformation is the introduction of side-chain-restrained amino acids.[2] For instance, disubstitution in the α position of an α-amino acid leads to a conformational constraint and a stereochemically stable quaternary carbon center. In this context, spirocyclic scaffolds are able to provide, upon the attachment of appropriate functional groups, useful high-affinity ligands. Indeed, the polysubstituted central atom common to the rings of spiro compounds confers on the overall molecular framework unique 3D properties that are relevant to the field of drug discovery.[3]

In our ongoing program aimed at identifying peptidomimetic scaffolds of low molecular weight, we recently focused on tetrahydroisoquinoline-based spirocyclic lactams[4] and spiropiperidine-3,3′-oxindole scaffolds[5] as, respectively, type II′ and II β-turn inducing moieties, for which highly predictable stereostructural properties could be demonstrated by means of molecular modeling calculations and spectroscopic studies.

Since many natural peptides containing tryptophan (Trp)-based pharmacophores exhibit a wide range of important bioactivities, and so also structurally related 1,2,3,4-tetrahydro-β-carboline (THBC)-based compounds, we have recently moved our attention to conformationally constrained spirocyclic Trp analogues[6] in order to develop new reverse-turn nucleating moieties able to be inserted into pharmacologically relevant peptidomimetic compounds.

Among peptides sharing a Trp-containing β-turn motif in which the Trp residue is critical for binding, we looked at the

Received: December 27, 2012
Published: February 14, 2013

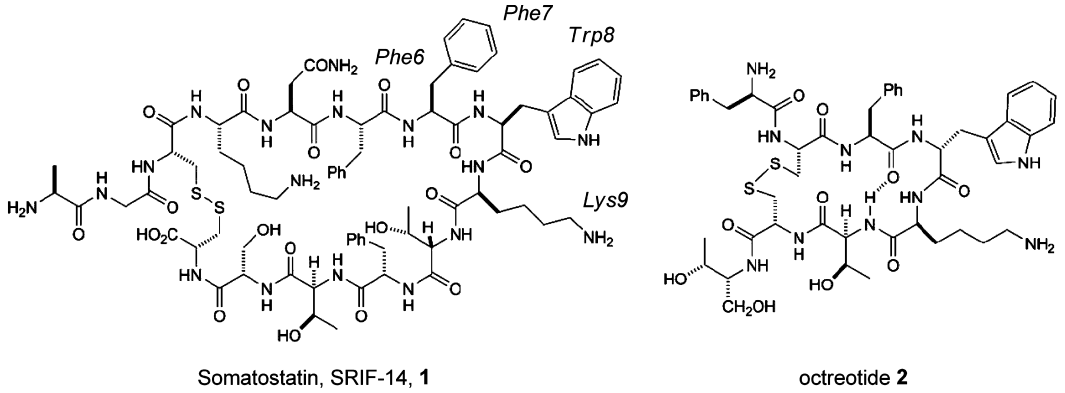


Figure 1. Molecular formulas of somatostatin (SRIF-14, **1**) and Sandostatin (octreotide, **2**).

hormone peptide somatostatin (somatotropin release-inhibiting factor, SRIF).[7] Somatostatin is normally expressed as a tetradecapeptide (SRIF-14, **1**, Figure 1) or an N-terminally extended form (SRIF-28). It appears in several organ systems, such as the central nervous system, the hypothalamo-pituitary system, the gastrointestinal tract, the exocrine and endocrine pancreas, and the immune system. In these different systems, somatostatin acts as a neuromodulator and a neurotransmitter as well as a potent inhibitor of various secretory processes and cell proliferation.[8]

Somatostatin action is mediated by specific, high-affinity somatostatin receptors located on the plasma membrane of the target cells. To date, five human somatostatin receptor subtypes (sst1, sst2, sst3, sst4, and sst5) have been cloned and characterized. These subtypes belong to a superfamily of GPCRs that can functionally couple to various intracellular effector systems.[9] The somatostatin receptors, which are overexpressed in a majority of neuroendocrine tumors, also represent the first and best example of targets for radiopeptide-based imaging and radionuclide therapy. Radiolabeled somatostatin analogues permit the localization and staging of neuroendocrine tumors that express the appropriate somatostatin receptors.[10]

The low bioavailability and poor pharmacokinetics of somatostatin have led to interest in peptidomimetic analogues which may be better drug candidates.[11] Several hexa- and octapeptide analogues have been developed, including the drug Sandostatin (octreotide **2**,[12] a cyclic octapeptide analogue) which is clinically used for the treatment of endocrine tumors and acromegaly.[13]

Somatostatin and octreotide are thought to interact with the sst1−5 receptors mainly by inserting a β-turn substructure, carrying a lysine (Lys) and a Trp side chain, into a pocket of the G protein-coupled somatostatin receptor.[14] SRIF peptidic structure−activity relationship (SAR) studies clearly indicated that the core residues Trp8 and Lys9 (numbering of the residues follows that of native SRIF) are the essential binding sites for all somatostatin receptors,[15] whereas Phe6 is specifically important for activation of subtype sst4, which has been recently recognized as an ideal therapeutic target for Alzheimer's disease.[16] With regard to the bioactive conformation of SRIF and analogues, an important step forward was taken from the finding that a type-II′ β-turn is present in the D-Trp8-SRIF series, in which D-Trp8 and Lys9 are located at the i+1 and i+2 position, respectively.[17]

We report here the preparation and structural characterization of the 1,2,3,4-tetrahydro-β-carboline (THBC)-based spirocyclic lactam **3** (Figure 2) as a type-II′ β-turn model compound, the application of its core structure to the synthesis

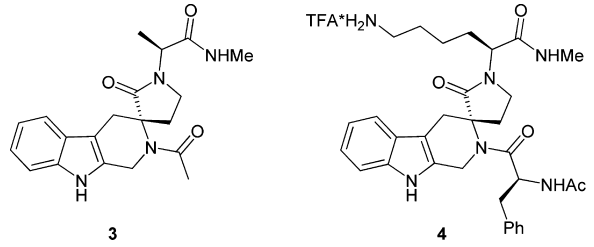


Figure 2. 1,2,3,4-Tetrahydro-β-carboline (THBC)-based spirocyclic lactam **3** and somatostatin mimetic **4**.

of the somatostatin mimetic **4** and of the closely related **21** (see later), and the result of biological evaluation of the latter compounds.

RESULTS AND DISCUSSION

In target **3**, the THBC-derived reverse turn nucleating moiety is placed at position i+1 of a model β-turn, and it enables the formation of a spirocyclic lactam bridge to the backbone nitrogen of L-Ala amino acid at the i+2 position. The resulting scaffold can so be considered a tetrapeptide Ac-Trp-Ala-NHMe analogue, and according to a preliminary computational evaluation, it promises to adopt conformations almost ideally matching the prerequisites for canonical type II′ β-turn. From MM calculations, the size of the lactam ring seems to play a crucial role in determining the favorite conformations, with the structure **3**, embodying a five-membered ring, showing the best aptitude for β-turn. The computed main geometric features of β-turns (Figure 3), such as the interatomic distance d_α ($C\alpha_1$−

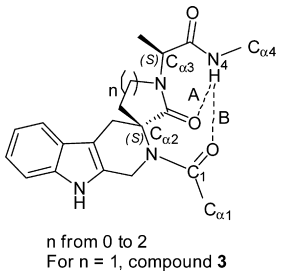


Figure 3. Parameters for the characterization of β-turn propensity of 1,2,3,4-tetrahydro-β-carboline (THBC)-based spirocyclic lactams.

dx.doi.org/10.1021/jo302737j | J. Org. Chem. 2013, 78, 2600−2610

Table 1. Results from Conformational Analysis[a]

	no. of conf < 6 (kcal/mol)	% d_α < 7 Å	% |β| < 30	% H bond β-turn (type B)	% H bond γ-turn (type A)
n = 0	15	13 (6.20 Å)	7 (51.7°)	0 (absent)	40 (present)
n = 1	13	38 (5.62 Å)	38 (−16.6°)	15 (present)	46 (absent)
n = 2	9	77 (6.27 Å)	66 (−9.9°)	0 (absent)	77 (present)

[a]Results are reported as the percentage of conformers that meet the indicated requirement. Values for the global minimum are reported in parentheses.

Table 2. Alignment Scores Values for the Superimposition of the Spirocyclic Lactams Amide Backbones (Global Minimum for n = 0, 1, and 2) with Standard Classical β-Turn Types

n =	I	I′	II	II′	III	III′	V	V′
0	0.88	<0.7	0.81	0.86	0.88	<0.7	<0.7	0.93
1	0.87	0.90	0.92	**0.97**	0.83	0.88	0.80	<0.7
2	0.84	0.86	0.88	0.94	0.82	0.84	0.75	0.78

$C\alpha_4$), the virtual torsion angle β ($C_1-C\alpha_2-C\alpha_3-N_4$), and the possible presence of the hydrogen bond $C_1O\cdots HN_4$, are reported in Table 1 for different sizes of the lactam ring.

The highest percentages of conformers satisfying the β-turn d_α and β requirements are found for the model compound with n = 2, followed by the one with n = 1 (compound **3**). The β-lactam ring (n = 0) seems to be unsuited to induce a β-turn conformation. The analysis further revealed the presence of two different H bonds, a seven-membered ring H bond (type A in Figure 3) resembling the γ-turn, and the typical 10-membered ring H bond (type B) of the β-turn. Only compound **3** is able to arrange an H bond of type B, whereas for the two other model compounds, a γ-turn around the i+1−i+3 residues is stabilized through the formation of H bond of type A.

The ability to mimic specific β-turns was also evaluated by superimposing the amide backbone of the global minima for n = 0, 1, and 2 (further optimized by DFT calculations at the B3LYP-6-31G* level) with standard classical I−V′ β-turn types. The results are reported in Table 2 as alignment scores values ranging from 0 to 1, with 1 being the perfect alignment.

The γ-lactam ring (n = 1, compound **3**) produces a very suitable mimic for a type II′ β-turn (0.97 score, see Figure 4, left), followed by the δ-lactam ring (n = 2, 0.94 score), the difference being due to the lack of the typical ten-membered ring H bond in the latter model compound.

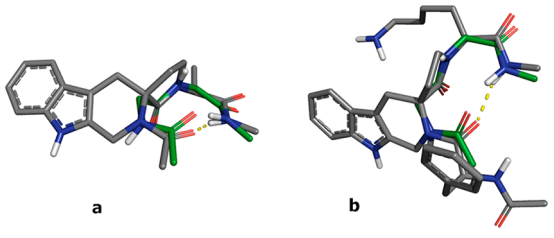


Figure 4. Superimposition of the amide backbone of the global minimum of compounds **3** (**a**, RMSD = 0.325 Å) and **4** (**b**, RMSD = 0.378 Å) with a type II′ β-turn.

Having selected structure **3**, embodying a five-membered ring, as the more promising type-II′ β-turn model compound, we also provided a computational evaluation of somatostatin mimetic **4**, which contains pharmacophore L-Lys and L-Phe amino acids. Also in this case a conformational search was performed with the same protocol. The global minimum (Figure 4, right) and its properties (see the Supporting Information) are still consistent with a β-turn of type II′.

On the basis of predictions from computational studies, we then focused our attention to the synthesis of compounds **3** and **4**, for which the common intermediate **10** was envisioned (Scheme 1). The synthesis of aldehyde **10** was carried out from N-Boc-D-tryptophan methyl ester adapting, as guideline, the protocol described from Scheidt and co-workers,[18] according to the concept of self-reproduction of chirality of Seebach.[19]

Since, in our hands, application of Seebach's chemistry proved to be rather sluggish on NH-indole-containing tryptophan, we pursued the synthesis of the suited N-TIPS-protected derivative **7** as a valuable substrate for the α-alkylation step. Starting from *N*-Boc-D-tryptophan methyl ester, introduction of the TIPS protecting group[20] afforded **5**, on which selective removal of the Boc to give primary amine **6** was cleanly and mildly effected by means of $SnCl_4$ Lewis acid.[21] The hydrolysis of methyl ester (1 M solution of LiOH) afforded *N*-TIPS-D-trp **7**, on which condensation with pivalaldehyde, followed by treatment with benzyl chloroformate, allowed us to obtain the desired oxazolidinone derivative in 76% overall yield, as the essentially unique diastereoisomer **8** (dr >99%, as from ^{1}H NMR spectroscopy). Alkylation of **8**, by reaction with KHMDS at −78 °C and allyl bromide, afforded the α-quaternary Trp-derivative as the single expected diastereoisomer **9**, whose stereochemistry was also verified by the NOESY NMR experiment (see the Supporting Information).

Because of the proven facility in the oxidation of the indole ring under ozone also at low temperature, we chose to achieve the aldehyde **10** by a two-step protocol. Treatment of **9** with OsO_4 and NMO afforded a diasteroisomeric mixture of diols which was reacted, without purification, with an aqueous solution of $NaIO_4$ in MeOH to give **10** in 90% overall yield.[22]

With the key aldehyde **10** in hands, we pursued the synthesis of the spirocyclic lactam **3** (Scheme 2).

The reductive amination of **10** with the HCl salt of L-alanine methyl ester and sodium cyanoborohydride in MeOH, followed by heating in toluene in presence of HOBT, generated the unique pyrrolidinone **11** in 70% yield. The cleavage of the Cbz protecting group by catalytic hydrogenation and of TIPS by means of a 1 M solution of TBAF in THF afforded **12** in 82% overall yield. Amine **12** went through a mild acidic (TFA 1eq) Pictet−Spengler reaction with an aqueous solution of formaldehyde in MeOH, affording carboline **13** in 66% yield. The target compound **3** was finally achieved in 71% overall yield by conversion of methyl ester **13** into methyl amide **14**, followed by acylation with acetic anhydride and crystallization from EtOAc/MeOH.

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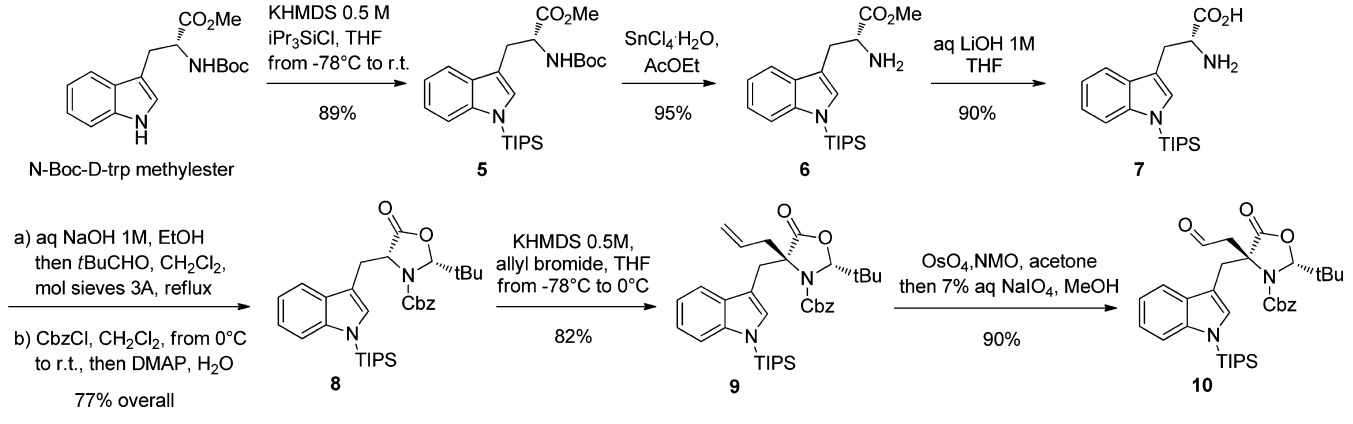
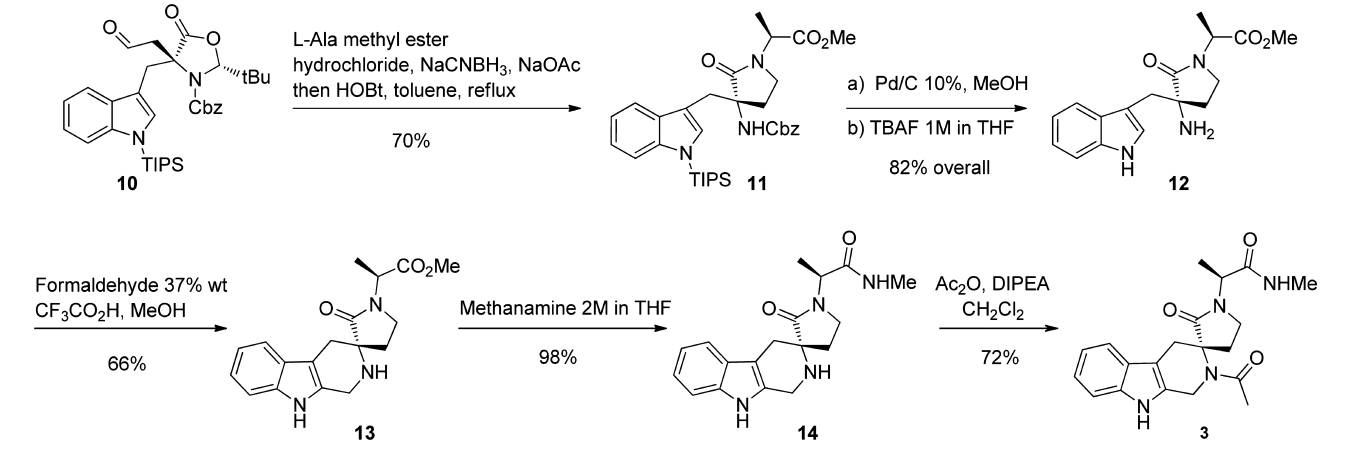
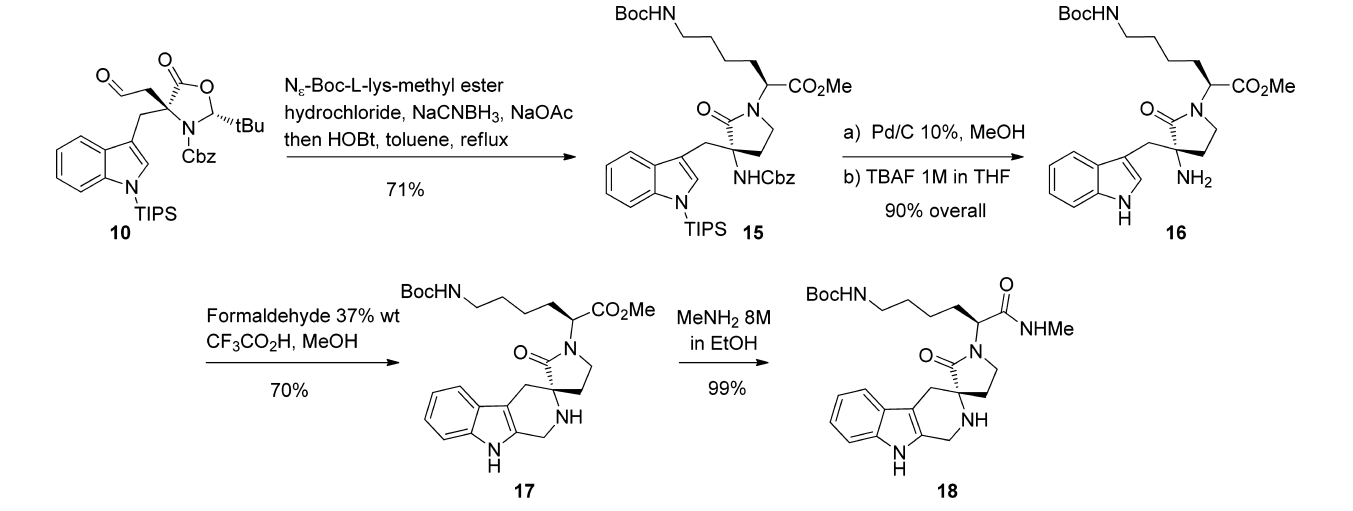
Scheme 1. Synthesis of the Aldehyde Intermediate 10



Scheme 2. Synthesis of the 1,2,3,4-Tetrahydro-β-carboline (THBC)-Based Spirocyclic Lactam 3



Scheme 3. Synthesis of the Intermediate Methyl Amide 18

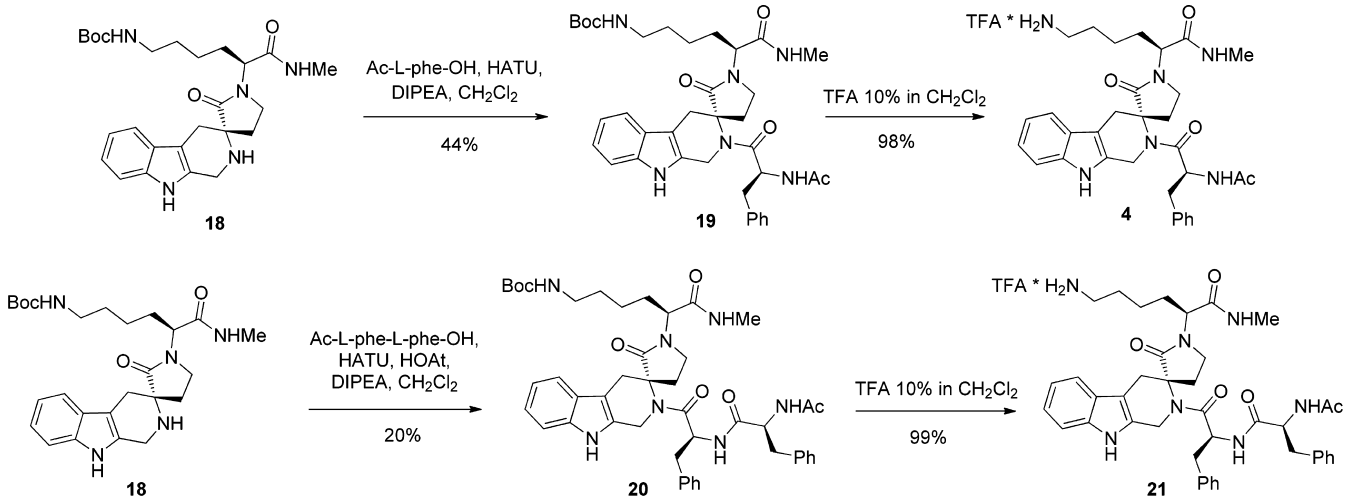


Starting from the same aldehyde **10**, reductive amination with the HCl salt of N_ε-Boc-L-lysine methyl ester gave as well the unique pyrrolidinone **15** in 71% yield (Scheme 3). The cleavage of both the Cbz and TIPS protecting groups to give amine **16**, followed by Pictet–Spengler reaction, afforded smoothly carboline **17**. Probably for a major steric congestion with respect to compound **13**, the conversion of methyl ester **17** into methyl amide **18** required a 8 M solution of NH_2Me in EtOH to afford **18** in similar yield. The compound **4** was achieved in 43% overall yield as trifluoroacetate salt by means of HATU-mediated condensation with *N*-Ac-L-phe-OH to give **19**, followed by treatment with TFA in CH_2Cl_2 (Scheme 4). Additionally, starting from **18**, HOAt/HATU-mediated condensation with *N*-Ac-L-phe-L-phe-OH, followed by treatment with TFA, allowed us to achieve (20% overall yield) the pentapeptide mimetic **21** for biological evaluation.

With model compound **3** and N_ε-protected mimic **19** in hand, we undertook a detailed conformational investigation on

dx.doi.org/10.1021/jo302737j | J. Org. Chem. 2013, 78, 2600–2610

Scheme 4. Synthesis of the Somatostatin Mimetics 4 and 21



their secondary structure in the solid state by means of X-ray crystallography for **3** and in solution by means of spectroscopic techniques for both **3** and **19**.

Crystals of **3** were obtained from a methanol/acetone solution 1:1 at room temperature as white prisms. The crystallographic structure of lactam **3** is represented in Figure 5. In order to confirm the complete stereocontrol of our synthetic approach, starting from a substrate with known chirality (*N*-Boc-D-trp methyl ester), X-ray study first highlighted the stereochemistry of the two stereocenters, which are both in the *S* configuration.

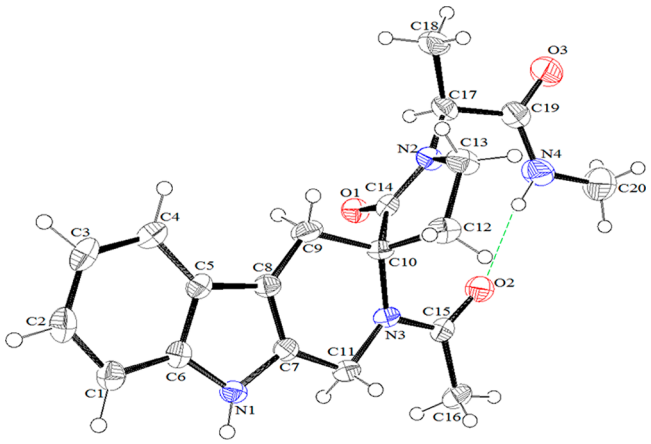


Figure 5. ORTEP[23] view of **3** showing a β-turned conformation and the relative arbitrary atom-numbering scheme (thermal ellipsoids at 40% probability). H atoms are shown as spheres of arbitrary size.

The molecule, with the exception of the pyrrolidine unit linked via a spiro ring junction to the piperidine–indole system, is almost flat due not only to the presence of fused aromatic rings (angles between the calculated mean-square planes for the three rings are in the range 1.0–5.3(1)°) but also to the occurrence of a strong intramolecular hydrogen bond between N4–H4 and O2 (ORTEP numbering, distance 2.08(1)Å, angle 172(1)°), closing a further 10-membered pseudocyclo structure. This intramolecular hydrogen bond between the CO_i oxygen atom acceptor and NH_{i+3} amide donor confers on the crystal structure a β-turn conformation, characterized by an almost ideal hydrogen bond directionality of the $_{(i)}O\cdots H-N_{(i+3)}$ angle. The values of the distance C16–C20 of 5.69(1)Å and of the virtual torsion angle β (C15···C10···C17···N4) of −20(1)° fully comply with the β-turn arrangement. A more detailed analysis of the ϕ and φ dihedral angles defines the presence of the β-turn conformation of type-II′. The values of ϕ_{i+1} = 51(1)°, φ_{i+1} = −134(1)° and ϕ_{i+2} = −105(1)°, φ_{i+1} = 31(1)° show a higher distortion with respect to the ideal values for the second couple, that could be related to the geometrical constrain of the five-membered ring (C10/C12/C13/N2/C14). This latter assumes almost an envelope conformation as described by the puckering parameters q_2 = 0.216(4) Å, ϕ^2 = −153.2(8)°,[24] with C12 forming the flap out of the best plane of the remaining four atoms by 0.347(4) Å. The pyrrolidine is nearly equatorially oriented with respect to the indole moiety (dihedral angle between the least-squares planes of 87(1)°) and the pendant residue linked to N2 is characterized by the torsion angle C13–N2–C17–C19 of 74(1)°.

These X-ray results are noteworthy, as they demonstrate that the intramolecular hydrogen bond persists also during the crystal formation when the intermolecular packing forces are crucial and, even more so, in the presence of a highly coordinating protic solvent (MeOH). Unfortunately, we were unable to obtain crystals suitable for an X-ray diffraction analysis of mimic **19**, under numerous protic solvent conditions.

As a final remark, we found a significant geometrical correspondence between the three-dimensional arrangement of the experimental structure of **3** with the modeled conformation, as provided by the rms value of 0.611 for the superimposition of the whole molecule and by a value of 0.105 Å obtained by superimposition of the backbone amide atoms in the β-turn region (see the Supporting Information).

However, since the intermolecular forces in the solid state may lead to a change in the peptidomimetic secondary structure, solution-phase studies for **3** and **19** were also performed in order to give a complete representation of their conformation and to assess the stability of the observed β-turn in a biologically meaningful medium.

After full characterization by one- and two-dimensional (COSY, HSQC) NMR analyses, an NMR study of conformational behavior was conducted. In similar constrained peptides, intramolecular hydrogen bonding provides the principal driving force for β-turn formation. In order to obtain validation about

dx.doi.org/10.1021/jo302737j | J. Org. Chem. 2013, 78, 2600–2610

the presence of such bonding, the conformational studies were carried out in a relatively nonpolar solvent (chloroform), which does not provide strong hydrogen-bonding competition. Such solvent can be considered to mimic the hydrophobic core of a protein; nevertheless, the possible influence of unpredictable factors on the conformation in biological water should be not excluded.

The involvement of the NH amide proton in intramolecular hydrogen bonding was first estimated from evaluation of its chemical shift value in $CDCl_3$ and from variable-temperature (VT) studies performed on a 2.0 mM $CDCl_3$ solution of **3** and **19**. The chemical shift of the NH amide proton ranged from 7.99 ppm (T = 238 K) to 7.80 ppm (T = 303 K) for compound **3** and from 7.82 ppm (T = 243 K) to 7.55 ppm (T = 323 K) for compound **19**. These values, in particular the two low VT coefficients (Table 3), support the involvement of the amide

Table 3. ^{1}H NMR Data for the NHMe Proton in Compounds 3 and 19[a]

compd	δ NHMe (ppm)	$\Delta\delta/\Delta$T (NHMe) (VT) (ppb/K)	$\Delta\delta$ (NHMe) (30% DMSO-d_6) (ppm)	NH/ND (NHMe) exchange (min)
3	7.69	−3.00	0.13	210
19	7.59	−3.00	0.19	300

[a]NMR experiments were performed on a 2.0 mM solution in $CDCl_3$.

proton in a hydrogen bond, in both molecules. Further, titration of the $CDCl_3$ 2.0 mM solution with up to 30% of DMSO-d_6, a strongly coordinating and hydrogen bond-acceptor solvent, produced a low variation of the NH chemical shift from 7.69 to 7.82 ppm for **3** and from 7.59 to 7.78 ppm for **19**,[25] thus highlighting the high stability of the intramolecular hydrogen bond present in both compounds.

Moreover, the rate of the H/D exchange upon addition of CD_3OD was quite low for both **3** and **19**, once again confirming the prevalence of an intramolecularly hydrogen-bonded status for the NHMe proton.

The IR[26] spectrum (2.0 mM solution $CHCl_3$) of **3** exhibited an extensive absorption band at 3355 cm^{-1} for the hydrogen-bonded NH stretch and a minor band at 3467 cm^{-1} for the non-hydrogen-bonded NH stretch, in accordance with the presence of a predominant bonded state in this molecule. The NH stretching region of the IR spectrum of **19** was complicated, due to the presence of three different NH functional groups, and did not provide useful indications about the presence of intramolecular H-bonds.

Somatostatin regulates the endocrine system and affects neurotransmission and cell proliferation via interaction with G-protein-coupled receptors. Unexpectedly, N_ε-deprotected samples **4** and **21** did not inhibit the binding of 125I-somatostatin-14 to the different human somatostatin receptors (sst1-sst5), at concentrations up to 1 μM, that we retain important for therapeutic activity. Somatostatin-28, tested in parallel as reference compound, showed complete inhibition with K_i values in the nM range, thus suggesting that further interactions would be required for affinity, besides type II′ β-turn recognition.

CONCLUSIONS

In summary, tetrahydro-β-carboline-based spirocyclic lactams have been prepared with the purpose of exploring their conformational behavior and evaluate their potential application as somatostatin mimetics. Both computational studies and spectroscopic NMR, IR, and X-ray investigations support a stable type II′ β-turn conformation for compound **3**, embodying a constrained D-Trp-Ala dipeptide analogue as the central i+1/i+2 core, with highly predictable stereostructural properties. The turn properties of **3** are efficiently transferred to the somatostatin mimic **19**, in which the pharmacophore Lys amino acid takes the place of Ala in the i+2 position. Also for **19** a type II′ β-turn conformation was predicted by computational calculations and strongly assessed with NMR experiments.

Contrary to our expectations, biological evaluation of trifluoroacetate salts **4** and **21** did not afford any acceptable level of receptor affinity. Aimed to improve these results and relying also on docking studies, a structure-based approach is now underway, to evaluate possible structural modifications of the described peptidomimetics or introduction of extra recognition features for affinity.

EXPERIMENTAL SECTION

Synthesis. *(R)-Methyl 2-((tert-Butoxycarbonyl)amino)-3-(1-(triisopropylsilyl)-1H-indol-3-yl)propanoate, **5**.* To a solution of (*R*)-methyl 2-((*tert*-butoxycarbonyl)amino)-3-(1*H*-indol-3-yl)propanoate (6.09 g, 19.13 mmol) in anhydrous THF (105 mL) cooled to −78 °C under nitrogen atmosphere was added dropwise KHMDS (1 M in THF, 24.88 mL, 24.88 mmol). After 1 h, triisopropylsilyl chloride (4.06 mL, 19.13 mmol) in THF (20 mL) was added dropwise at −78 °C, and the solution was allowed to warm to room temperature. After the solution was quenched with H_2O, THF was evaporated in vacuo, and the aqueous solution was extracted with Et_2O (3 × 30 mL). The organic layer was washed with brine, dried over Na_2SO_4, and filtered, and the solvent was removed under reduced pressure. Purification by flash column chromatography (9:1, *n*-hexane/EtOAc) afforded **5** (8.04 g, 89%) as a foam: R_f 0.61 (7:3, *n*-hexane/EtOAc); $[\alpha]^{20}_D$ −9.5 (*c* 0.40, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.51 (d, J = 7.6 Hz, 1H), 7.48 (d, J = 8.0 Hz, 1H), 7.24 (s, br, 1H), 7.21 (d, J = 8.1 Hz, 1H), 7.12 (t, J = 7.5 Hz, 1H), 7.06 (t, J = 7.4 Hz, 1H), 4.29−4.21 (m, 1H), 3.59 (s, 3H), 3.13 (dd, J = 14.5 and 5.2 Hz, 1H), 3.00 (dd, J = 14.5 and 9.5 Hz, 1H), 1.70 (ept, J = 7.4 Hz, 3H) 1.31 (s, 9H) 1.11−1.055 (m, 18H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.4, 155.8, 139.3, 136.6, 124.2, 121.4, 118.8, 118.4, 111.9, 110.2, 78.7, 55.1, 52.2, 28.6 (3C), 27.3, 18.3 (6C), 12.6 (3C); HRMS (EI) calcd for $C_{26}H_{42}N_2O_4Si$ 474.2914, found 474.2928.

*(R)-Methyl 2-Amino-3-(1-(triisopropylsilyl)-1H-indol-3-yl)-propanoate, **6**.* To a solution of **5** (5.00 g, 10.53 mmol) in EtOAc (200 mL) cooled to 0 °C was added $SnCl_4{\cdot}H_2O$ (4.94 mL, 42.13 mmol) was added. After 48 h at room temperature, the reaction mixture was poured into satd aq $NaHCO_3$ (100 mL), the layers were separated, and the aqueous layer was extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification by flash chromatography (8:2, EtOAc/ *n*-hexane) afforded **6** (3.74 g, 95% yield) as an oil: R_f 0.36 (8:2, EtOAc/*n*-hexane); $[\alpha]^{20}_D$−24.5 (*c* 0.40, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) 7.53 (d, br, J = 7.8 Hz, 1H), 7.47 (d, J = 8.1 Hz, 1H), 7.13 (s, 1H), 7.11 (m, 1H), 7.05 (m, 1H), 3.67 (m, 1H), 3.51 (s, 3H), 3.06−2.92 (m, 2H), 1.76 (d, J = 7.6 Hz, 2H), 1.70 (ept, J = 7.6 Hz, 3H), 1.08 (d, J = 7.6 Hz, 18H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.1, 141.1, 131.3, 130.0, 121.8, 119.7, 119.0, 114.1, 113.8, 55.4, 51.7, 31.2, 18.4 (6C), 12.5 (3C); HRMS (EI) calcd for $C_{21}H_{34}N_2O_2Si$ 374.2390, found 374.2385.

*(R)-2-Amino-3-(1-(triisopropylsilyl)-1H-indol-3-yl)propanoic Acid, **7**.* To a solution of **6** (7.30 g, 19.49 mmol) in THF (195 mL) was added aq LiOH 1 M (19.49 mL, 19.49 mmol). After 4 h at room temperature, the solution was acidified to pH 3 with 5% aq H_3PO_4, the THF was evaporated, and the aqueous solution was extracted with EtOAc (3 × 30 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo to afford **7** (6.36 g, 90% yield) as a foam: R_f 0.12 (8:2, EtOAc/MeOH); $[\alpha]^{20}_D$

dx.doi.org/10.1021/jo302737j | J. Org. Chem. 2013, 78, 2600−2610

+18.3 (*c* 0.30, MeOH); ^{1}H NMR (500 MHz, DMSO-d_6) 7.59 (d, br, *J* = 7.7 Hz, 1H), 7.48 (d, *J* = 8.2 Hz, 1H), 7.32 (s, 1H), 7.12 (m, 1H), 7.06 (t, br, *J* = 7.7 Hz, 1H), 3.73−2.90 (m, br, 3H), 3.45 (dd, *J* = 9.2 and 3.8 Hz, 1H), 3.33 (dd, *J* = 15.2 and 3.8 Hz, 1H), 2.95 (dd, *J* = 15.2 and 9.2 Hz, 1H), 1.71 (ept, *J* = 7.5 Hz, 3H), 1.10 (d, *J* = 7.5 Hz, 18H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.4, 141.4, 131.2, 130.5, 121.8, 119.6, 119.0, 114.1, 113.7, 54.8, 27.7, 18.5 (6C), 12.6 (3C); HRMS (EI) calcd for $C_{20}H_{32}N_2O_2Si$ 360.2233, found 360.2250.

(2R,4R)-Benzyl 2-tert-Butyl-5-oxo-4-((1-(triisopropylsilyl)-1H-indol-3-yl)methyl)oxazolidine-3-carboxylate, ***8.*** To a solution of **7** (6.17 g, 17.11 mmol) in EtOH (55 mL) was added aq NaOH 1 M (17.11 mL, 17.11 mmol). After 1 h under stirring at room temperature, the solution was concentrated in vacuo to provide a white solid. The solid was suspended in anhydrous CH_2Cl_2 (125 mL), and pivalaldehyde (2.79 mL, 25.67 mmol) was added under nitrogen atmosphere. The flask was equipped with a Soxhlet filled with molecular sieves (3 Å), and the solution was heated to reflux for 12 h. After cooling, the mixture was concentrated in vacuo to provide a yellow foam that was azeotropically dried with toluene (35 mL). The resultant solid residue was dissolved in anhydrous CH_2Cl_2 (125 mL), and benzyl chloroformate (3.18 mL, 22.25 mmol) was added at 0 °C under nitrogen atmosphere. After 48 h at 4 °C, the solution was allowed to warm to room temperature. Water (20 mL) and DMAP (6 mg) were added, and the solution was stirred for 1 h; then the layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were washed with 15% aq $NaHSO_3$ and saturated aq $NaHCO_3$, dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification by flash chromatography (95:5, *n*-hexane/EtOAc) afforded **8** (7.39 g, 77% yield) as an oil: R_f 0.61 (7:3, *n*-hexane/EtOAc); $[\alpha]^{20}_D$ −8.6 (*c* 1.25, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) 7.45 (d, *J* = 8.3 Hz, 1H), 7.43−7.32 (m, 6H), 7.15 (s, 1H), 7.07 (t, br, *J* = 7.8 Hz, 1H), 6.89 (t, br, *J* = 7.6 Hz, 1H), 5.45 (s, 1H), 5.14 (d, *J* = 12.4 Hz, 1H), 5.05 (d, *J* = 12.4 Hz, 1H), 4.64 (dd, *J* = 8.3 and 5.1 Hz, 1H), 3.30−3.17 (m, 2H), 1.67 (ept, *J* = 7.5 Hz, 3H) 1.06 (d, *J* = 7.5 Hz, 18H), 0.81 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.1, 156.4, 141.1, 136.3, 131.2, 131.1, 128.9 (3C), 128.6 (2C), 121.8, 119.7, 119.0, 114.1, 112.7, 95.6, 67.7, 57.8, 36.9, 28.4, 25.2 (3C), 18.3 (6C), 12.5 (3C); HRMS (EI) calcd for $C_{33}H_{46}N_2O_4Si$ 562.3227, found 562.3215.

(2R,4R)-Benzyl 4-Allyl-2-tert-butyl-5-oxo-4-((1-(triisopropylsilyl)-1H-indol-3-yl)methyl)oxazolidine-3-carboxylate, ***9.*** To a solution of **8** (7.30 g, 12.97 mmol) in anhydrous THF (150 mL) was added KHMDS (1 M in toluene, 18.16 mL, 18.16 mmol) dropwise at −78 °C under nitrogen atmosphere. After 1 h, allyl bromide (1.23 mL, 14.27 mmol) was added dropwise at −78 °C, and the solution was allowed to warm to −30 °C before quenching with saturated aq NH_4Cl (30 mL). At 0 °C the mixture was diluted with water (30 mL) and extracted with EtOAc (3 × 50 mL). The organic layer was washed with brine, dried over Na_2SO_4, and filtered, and the solvent was removed under reduced pressure. Purification by flash chromatography (98:2, *n*-hexane/EtOAc) afforded **9** (6.40 g, 82% yield) as an oil: R_f 0.49 (8:2, *n*-hexane/EtOAc); $[\alpha]^{20}_D$ −65.5 (*c* 0.45, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.65−7.29 (m, 7H), 7.05 (t, *J* = 7.4 Hz, 1H), 7.03−6.87 (m, 2H), 5.52 (dddd, *J* = 18.7, 10.1, 8.6, and 6.3 Hz, 1H), 5.40−5.30 (m, br, 1H), 5.28 (s, 1H), 5.28−5.19 (m, br, 1H), 5.15 (d, br, *J* = 10.1 Hz, 1H), 5.02 (dd, *J* = 18.7 and 1.5 Hz, 1H), 3.55−3.37 (m, br, 1H), 3.17−2.99 (m, br, 1H), 2.52−2.37 (m, 2H), 1.60 (ept, br, *J* = 7.5 Hz, 3H), 1.04 (d, *J* = 7.5 Hz, 18H), 0.31 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.8, 155.9, 140.8, 135.9, 132.1, 131.9, 130.9, 129.6 (2C), 129.1 (3C), 122.1, 121.8, 119.8, 119.2, 114.0, 112.2, 95.0, 69.0, 67.9, 39.5, 37.6, 32.1, 25.2 (3C), 18.3 (6C),12.5 (3C); HRMS (EI) calcd for $C_{36}H_{50}N_2O_4Si$ 602.3540, found 602.3529.

(2R,4S)-Benzyl 2-tert-Butyl-5-oxo-4-(2-oxoethyl)-4-((1-(triisopropylsilyl)-1H-indol-3-yl)methyl)oxazolidine-3-carboxylate, ***10.*** To a solution of NMO (50 wt % solution in H_2O, 3.30 mL, 15.92 mmol) in acetone/H_2O 6:1 (49 mL), OsO_4 (2 wt % solution in acetone/H_2O 1:1, 3.73 mL, 0.26 mmol) was added; the solution of **9** (3.20 g, 5.31 mmol) in acetone (90 mL) was then added, and the mixture was allowed to react for 20 h under nitrogen atmosphere. After the addition of 10% aq $NaHSO_3$ (45 mL) and 1 h under stirring, the mixture was filtered, concentrated, and extracted with EtOAc (3 × 75 mL). The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo to afford a residue which was dissolved in MeOH (90 mL). A solution of 7% aq $NaIO_4$ (16 mL, 5.57 mmol) was added, and the solution was allowed to react for 24 h at room temperature. After the addition of H_2O (15 mL), the mixture was extracted with EtOAc (3 × 50 mL), the organic layer was washed with brine, dried over Na_2SO_4, and filtered, and the solvent was removed under reduced pressure. Purification by flash chromatography (98:2, *n*-hexane/EtOAc) afforded **10** (2.89 g, 90% yield) as a brown oil: R_f 0.72 (7:3, *n*-hexane/EtOAc); $[\alpha]^{20}_D$ −38.2 (*c* 1.00, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.32 (s, br, 1H), 7.46 (d, *J* = 8.3 Hz, 1H), 7.43−7.31 (m, 6H), 7.10 (ddd, *J* = 8.3, 7.3, and 1.3 Hz, 1H), 7.06 (s, 1H), 6.99 (t, br, *J* = 7.3 Hz, 1H), 5.52 (s, 1H), 5.10 (d, *J* = 11.9 Hz, 1H), 5.03 (d, *J* = 11.9 Hz, 1H), 3.67 (d, br, *J* = 19.0 Hz, 1H), 3.33 (d, *J* = 14.9 Hz, 1H), 3.27 (d, *J* = 14.9 Hz, 1H), 3.04 (d, *J* = 19.0 Hz, 1H), 1.65 (ept, *J* = 7.5 Hz, 3H), 1.09−1.03 (m, 18H), 0.63 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 200.7, 173.4, 154.8, 140.8, 135.8, 132.1, 131.9, 129.2 (2C), 129.0 (3C), 121.9, 120.0, 119.1, 114.1, 111.3, 95.6, 67.7, 63.9, 39.8, 37.3, 33.1, 25.9 (3C), 18.3 (6C), 12.5 (3C); HRMS (EI) calcd for $C_{35}H_{48}N_2O_5Si$ 604.3333, found 604.3346.

(S)-Methyl 2-((S)-3-(((Benzyloxy)carbonyl)amino)-2-oxo-3-((1-(triisopropylsilyl)-1H-indol-3-yl)methyl)pyrrolidin-1-yl)propanoate, ***11.*** To a solution of **10** (1.26 g, 2.08 mmol), NaOAc (0.36 g, 4.37 mmol), and activated powdered 4 Å molecular sieves (2.30 g) in MeOH (45 mL) was added the hydrochloride salt of L-alanine methyl ester (0.32 g, 2.29 mmol). After 1 h, $NaCNBH_3$ (0.26 g, 4.17 mmol) was added, and the mixture was stirred at room temperature overnight. Then the suspension was filtered, and the filtrate was acidified to pH 1 with aq HCl 1 N and stirred for 15 min. The solution was basified to pH 9 with saturated aq $NaHCO_3$ and then extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo to give a colorless oil. This oil was dissolved in toluene (25 mL), and HOBt monohydrate (0.32 g, 2.08 mmol) was added. The mixture was heated at reflux for 6 h, and then it was cooled and saturated aq $NaHCO_3$ (20 mL) added. The mixture was extracted with EtOAc (3 × 30 mL), and the combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification by flash chromatography (3:1, *n*-hexane/EtOAc) afforded **11** (0.890 g, 70% yield) as a yellow oil: R_f 0.54 (3:1, *n*-hexane/EtOAc); $[\alpha]^{20}_D$ −36.7 (*c* 0.95, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.52 (m, br, 1H), 7.48 (d, br, *J* = 7.5 Hz, 1H), 7.46 (d, br, *J* = 7.6 Hz, 1H), 7.44−7.29 (m, 5H), 7.09 (ddd, *J* = 8.1, 7.1, and 1.3 Hz, 1H), 7.01 (t, br, *J* = 8.1 Hz, 1H), 5.02 (m, br, 2H), 4.25 (q, *J* = 7.3 Hz, 1H), 3.55 (s, br, 3H), 3.13 (d, *J* = 14.2 Hz, 1H), 3.08−2.98 (m, 2H), 2.47−2.27 (m, 2H), 2.20−2.09 (m, 1H), 1.74 (ept, *J* = 7.6 Hz, 3H), 1.09 (d, *J* = 7.6 Hz, 9H), 1.07 (d, *J* = 7.6 Hz, 9H), 0.19 (d, *J* = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.6, 171.8, 155.0, 140.8, 137.6, 136.3, 128.8(3C), 128.2(2C), 125.4, 121.4, 118.9, 118.6, 111.8, 107.8, 65.6, 61.6, 52.3, 49.6, 39.6, 32.7, 28.8, 18.3 (6C), 13.6, 12.6 (3C); HRMS (EI) calcd for $C_{34}H_{47}N_3O_5Si$ 605.3285, found 605.3277.

(S)-Methyl 2-((S)-3-((1H-Indol-3-yl)methyl)-3-amino-2-oxopyrrolidin-1-yl)propanoate, ***12.*** To a solution of **11** (0.850 g, 1.40 mmol) in MeOH (30 mL) was added Pd/C 10% (85 mg). After thoroughly flushing the flask with N_2, a hydrogen atmosphere was introduced. After 17 h, the reaction was filtered and the solvent was evaporated in vacuo to afford a yellow oil: R_f 0.43 (5:1, EtOAc/MeOH). The oil was dissolved in THF (15 mL), and TBAF (1 M in THF, 2.8 mL, 2.8 mmol) was added at 0 °C. After 1 h under stirring, the mixture was diluted with EtOAc (30 mL), washed with saturated aq $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification by flash chromatography (85:15, EtOAc/MeOH) afforded **12** (0.360 g, 82% yield) as a yellow oil: R_f 0.27 (85:15, EtOAc/MeOH); $[\alpha]^{20}_D$−62.3 (*c* 0.33, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.88 (m, br, 1H), 7.53 (d, *J* = 7.9 Hz, 1H), 7.32 (d, *J* = 8.1 Hz, 1H), 7.17 (d, *J* = 2.3 Hz, 1H), 7.04 (ddd, *J* = 8.1, 6.8, and 1.0 Hz, 1H), 6.95 (ddd, *J* = 8.1, 7.1, and 1.0 Hz, 1H), 4.45 (q, *J* = 7.3 Hz, 1H), 3.60 (s, 3H), 3.03 (td, *J* = 9.1 and 3.0 Hz, 1H), 2.94 (d, *J* = 13.9 Hz, 1H), 2.79 (d, *J* = 13.9 Hz, 1H), 2.47 (dt, *J* = 9.1 and 7.8 Hz, 1H), 2.12 (ddd,

dx.doi.org/10.1021/jo302737j | J. Org. Chem. 2013, 78, 2600−2610

J = 12.6, 7.8, and 3.0 Hz, 1H), 1.88 (m, br, 2H), 1.74 (ddd, J = 12.6, 9.1, and 7.8 Hz, 1H), 0.78 (d, J = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 177.8, 172.0, 136.3, 128.4, 124.8, 121.2, 118.9, 118.7, 111.7, 109.7, 60.4, 52.5, 49.6, 40.1, 34.9, 32.1, 14.0; HRMS (EI) calcd for $C_{17}H_{21}N_3O_3$ 315.1583, found 315.1565.

(S)-Methyl2-((S)-2′-Oxo-1,2,4,9-tetrahydrospiro[pyrido[3,4-b]-indole-3,3′-pyrrolidin]-1′-yl)propanoate, ***13****.* To a solution of **12** (0.435 g, 1.38 mmol) in MeOH (45 mL) were added activated powdered 4 Å molecular sieves (1.30 g), formaldehyde (37 wt % solution in H_2O, 0.123 mL, 1.66 mmol) and trifluoroacetic acid (0.11 mL, 1.38 mmol). After 7 h under stirring, molecular sieves were filtered, and the mixture was basified with saturated aq $NaHCO_3$ and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification by flash chromatography (95:5, EtOAc/MeOH) afforded **13** (0.298 g, 66% yield) as a foam: R_f 0.43 (4:1, EtOAc/MeOH); $[\alpha]^{20}_D$ +9.5 (c 0.33, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.69 (s, br, 1H), 7.35 (d, J = 7.8 Hz, 1H), 7.27 (d, J = 8.1 Hz, 1H), 7.01 (ddd, J = 8.1, 7.1, and 1.3 Hz, 1H), 6.93 (ddd, J = 7.8, 7.1, and 1.0 Hz, 1H), 4.66 (q, J = 7.3 Hz, 1H), 4.00 (d, br, J = 16.8 Hz, 1H), 3.88 (d, br, J = 16.8 Hz, 1H), 3.68 (s, 3H), 3.41−3.35 (m, 2H), 2.71−2.59 (m, 2H), 2.52−2.34 (m, br, 1H), 2.02 (dt, J = 12.4 and 7.9 Hz, 1H), 1.85 (ddd, J = 12.4, 6.9, and 5.1 Hz, 1H), 1.39 (d, J = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 175.9, 171.9, 136.3, 133.5, 127.9, 120.8, 118.6, 117.6, 111.3, 105.3, 59.1, 52.6, 49.8, 41.1, 39.2, 30.7, 28.2, 15.0; HRMS (EI) calcd for $C_{18}H_{21}N_3O_3$ 327.1583, found 327.1597.

(S)-N-Methyl-2-((S)-2′-oxo-1,2,4,9-tetrahydrospiro[pyrido[3,4-b]-indole-3,3′-pyrrolidin]-1′-yl)propanamide, ***14****.* To a solution of **13** (0.305 g 0.93 mmol) in THF (20 mL) was added methanamine (2 M in THF, 3.73 mL, 7.45 mmol). After 6 h under stirring, the mixture was concentrated in vacuo to afford pure **14** (0.300 g, 98% yield) as a yellow foam: R_f 0.31 (3:1, EtOAc/MeOH); $[\alpha]^{20}_D$ −7.5 (c 0.2 MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.69 (s, br, 1H), 7.82 (q, br, J = 4.5 Hz, 1H), 7.35 (d, br, J = 7.8 Hz, 1H), 7.27 (d, J = 7.8 Hz, 1H), 7.01 (ddd, J = 7.8, 7.3, and 1.3 Hz, 1H), 6.93 (ddd, J = 7.8, 7.3, and 1.0 Hz, 1H), 4.53 (q, J = 7.3 Hz, 1H), 4.00 (d, J = 16.9 Hz, 1H), 3.88 (d, br, J = 16.9 Hz, 1H), 3.49 (td, J = 9.3 and 3.5 Hz, 1H), 3.40−3.25 (m, br, 1H), 3.36 (dt, J = 9.3 and 7.3 Hz, 1H), 2.68 (d, br, J = 15.2 Hz, 1H), 2.62 (d, J = 15.2 Hz, 1H), 2.61 (d, J = 4.5 Hz, 3H), 2.03 (ddd, J = 12.2, 9.3, and 7.3 Hz, 1H), 1.81 (ddd, J = 12.2, 7.3, and 3.5 Hz, 1H), 1.30 (d, J = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 175.9, 171.3, 136.3, 133.5, 127.9, 120.8, 118.6, 117.6, 111.3, 105.4, 59.3, 50.2, 40.8, 39.3, 30.5, 28.1, 26.07, 15.5; HRMS (EI) calcd for $C_{18}H_{22}N_4O_2$ 326.1743, found 326.1736.

(S)-2-((S)-2-Acetyl-2′-oxo-1,2,4,9-tetrahydrospiro[pyrido[3,4-b]-indole-3,3′-pyrrolidin]-1′-yl)-N-methylpropanamide, ***3****.* To a solution of **14** (0.285 g, 0.87 mmol) in CH_2Cl_2 (13 mL) were added DIPEA (0.229 mL, 1.31 mmol) and acetic anhydride (0.124 mL, 1.31 mmol). After 16 h under stirring, the mixture was concentrated in vacuo to give a yellow foam. Crystallization (5% MeOH in EtOAc) afforded **3** (0.233 g, 72% yield) as a white solid: R_f 0.59 (3:1, EtOAc/MeOH); $[\alpha]^{20}_D$−63.2 (c 0.10, MeOH); mp 287−289 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.00 (s, br, 1H), 7.87 (q, br, J = 4.5 Hz, 1H), 7.49 (d, br, J = 7.8 Hz, 1H), 7.37 (d, br, J = 8.1 Hz, 1H), 7.09 (ddd, J = 8.1, 7.2, and 1.3 Hz, 1H), 7.00 (ddd, J = 7.8, 7.2, and 1.0 Hz, 1H), 4.91 (dd, br, J = 15.5 and 1.2 Hz, 1H), 4.73 (d, J = 15.5 Hz, 1H), 4.53 (q, J = 7.4 Hz, 1H), 3.40 (q, br, J = 8.8 Hz, 1H), 3.20 (td, J = 9.8 and 2.5 Hz, 1H), 2.99 (d, J = 14.8 Hz, 1H), 2.85 (d, br, J = 14.8 Hz, 1H), 2.67 (d, J = 4.5 Hz, 3H), 2.22 (s, 3H), 2.09 (m, 1H), 1.79 (ddd, J = 12.3, 8.8, and 2.5 Hz, 1H), 1.34 (d, J = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 173.7, 172.5, 171.0, 136.8, 130.6, 126.9, 121.5, 119.1, 118.2, 111.7, 104.3, 62.8, 50.3, 43.2, 39.8, 30.4, 29.4, 26.4, 23.6, 14.1; HRMS (EI) calcd for $C_{20}H_{24}N_4O_3$ 368.1848, found 368.1843.

(S)-Methyl 2-((S)-3-(((Benzyloxy)carbonyl)amino)-2-oxo-3-((1-(triisopropylsilyl)-1H-indol-3-yl)methyl)pyrrolidin-1-yl)-6-((tert-butoxycarbonyl)amino)hexanoate, ***15****.* To a solution of **10** (0.350 g, 0.58 mmol), NaOAc (0.099 g, 1.22 mmol), and activated powdered 4 Å molecular sieves (0.65 g) in MeOH (18 mL) was added (*S*)-methyl 2-amino-6-((*tert*-butoxycarbonyl)amino)hexanoate hydrochloride (0.189 g, 0.64 mmol). After 1 h, $NaCNBH_3$ (0.072 g, 1.16 mmol) was added, and the mixture was stirred at room temperature overnight. Then the suspension was filtered, and the filtrate was acidified to pH 4 with aq HCl 1 N and stirred for 15 min. The solution was basified to pH 9 with saturated aq $NaHCO_3$ and then extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo to give a yellow oil. The oil was dissolved in toluene (15 mL), and HOBt monohydrate (0.089 g, 0.58 mmol) was added. The mixture was heated at reflux for 6 h, and then it was cooled and saturated aq $NaHCO_3$ (10 mL) added. The mixture was extracted with EtOAc (3 × 20 mL), and the combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification by flash chromatography (8:2, *n*-hexane/EtOAc) afforded **15** (0.312 g, 71% yield) as a yellow oil: R_f 0.23 (6:4, *n*-hexane/EtOAc); $[\alpha]^{20}_D$ −23.9 (c 1.00, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.52−7.44 (m, 3H), 7.42 (s, 1H), 7.40−7.29 (m, 5H), 7.10 (t, br, J = 7.7 Hz, 1H), 7.00 (t, J = 7.5 Hz, 1H), 6.68 (m, br, 1H), 5.01 (m, br, 2H), 4.12 (dd, J = 9.3 and 6.1 Hz, 1H), 3.56 (s, br, 3H), 3.19−3.08 (m, 2H), 3.00 (d, br, J = 13.6 Hz, 1H), 2.80−2.66 (m, 2H), 2.41−2.23 (m, 3H), 1.74 (ept, J = 7.4 Hz, 3H), 1.38 (s, 9H), 1.09 (d, J = 7.4 Hz, 9H), 1.08 (d, J = 7.4 Hz, 9H), 1.15−0.88 (m, br, 3H), 0.88−0.61 (m, br, 2H), 0.02 (m, br, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.7, 171.2. 170.1, 155.0, 140.8, 137.6, 136.5, 128.8 (3C), 128.2(2C), 128.0, 122.0, 119.6, 119.0, 114.1, 111.2, 77.7, 66.6, 65.6, 54.5, 52.2, 39.9(2 C), 32.3, 29.3, 28.7 (3C), 28.6, 28.0, 23.0, 18.3 (6C), 12.4 (3C); HRMS (EI) calcd for $C_{42}H_{62}N_4O_7Si$ 762.4388, found 762.4392.

(S)-Methyl 2-((S)-3-((1H-Indol-3-yl)methyl)-3-amino-2-oxopyrrolidin-1-yl)-6-((tert-butoxycarbonyl)amino)hexanoate, ***16****.* To a solution of **15** (0.300 g, 0.39 mmol) in MeOH (14 mL) was added Pd/C 10% (30 mg). After thoroughly the flask was thoroughly with N_2, a hydrogen atmosphere was introduced. After 12 h, the reaction was filtered, and the solvent was evaporated in vacuo to afford a yellow oil: R_f 0.45 (4:1, EtOAc/MeOH). The oil was dissolved in THF (6 mL), and TBAF (1 M in THF, 0.786 mL, 0. 786 mmol) was added at 0 °C. After 1 h under stirring, the mixture was diluted with EtOAc (20 mL), washed with saturated aq $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification by flash chromatography (85:15, EtOAc/MeOH) afforded **16** (0.167 g, 90% yield) as a foam: R_f 0.32 (8:2, EtOAc/MeOH); $[\alpha]^{20}_D$ −61.4 (c 0.50, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.86 (s, br, 1H), 7.54 (d, J = 7.8 Hz, 1H), 7.32 (d, J = 7.8 Hz, 1H), 7.15 (d, J = 2.3 Hz, 1H), 7.04 (ddd, J = 7.8, 7.1, and 1.0 Hz, 1H), 6.96 (ddd, J = 7.8, 7.1, and 1.0 Hz, 1H), 6.72 (m, br, 1H), 4.37 (dd, J = 9.3, 6.1 Hz, 1H), 3.62 (s, 3H), 3.17 (td, J = 8.9 and 3.8 Hz, 1H), 2.98−2.68 (m, 5H), 2.08 (ddd, J = 12.4, 7.6, and 3.8 Hz, 1H), 2.03−1.88 (m, 2H), 1.71 (ddd, J = 15.7, 8.6, and 7.1 Hz, 1H), 1.62−1.48 (m, 1H), 1.37 (s, 9H), 1.29−1.19 (m, 3H), 1.01−0.79 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 178.0, 171.6, 156.1, 136.3, 128.5, 124.9, 121.2, 118.9, 118.8, 111.7, 109.7, 77.9, 60.6, 54.0, 52.4, 39.9 (2C), 34.3, 31.9, 29.3, 28.7 (3C), 28.1, 23.2; HRMS (EI) calcd for $C_{25}H_{36}N_4O_5$ 472.2686, found 472.2693.

(S)-Methyl 6-((tert-Butoxycarbonyl)amino)-2-((S)-2′-oxo-1,2,4,9-tetrahydrospiro[pyrido[3,4-b]indole-3,3′-pyrrolidin]-1′-yl)-hexanoate, ***17****.* To a solution of **16** (0.100 g, 0.21 mmol) in MeOH (7 mL) were added activated powdered 4 Å molecular sieves (0.200 g), formaldehyde (37 wt % solution in H_2O, 0.019 mL, 0.25 mmol), and trifluoroacetic acid (0.016 mL, 0.21 mmol). After 5 h under stirring, molecular sieves were filtered, and the mixture was basified with saturated aq $NaHCO_3$ and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification by flash chromatography (98:2, EtOAc/*n*-hexane) afforded **17** (72 mg, 70% yield) as a foam: R_f 0.53 (7:3, EtOAc/MeOH); $[\alpha]^{20}_D$ + 9.5 (c 0.33, MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.69 (s, br, 1H), 7.38 (d, br, J = 7.8 Hz, 1H), 7.27 (d, br, J = 8.1 Hz, 1H), 7.01 (t, br, J = 7.5 Hz, 1H), 6.92 (t, br, J = 7.4 Hz, 1H), 6.79 (m, br, 1H), 4.58 (dd, J = 10.5 and 5.2 Hz, 1H), 4.00 (d, J = 16.7 Hz, 1H), 3.88 (d, br, J = 16.7 Hz, 1H), 3.68 (s, 3H), 3.40 (td, J = 9.0 and 3.3 Hz, 1H), 3.36−3.26 (m, 1H), 2.95 ((m, 2H), 2.70 (d, J = 15.2 Hz, 1H), 2.63 (d, J = 15.2 Hz, 1H), 2.42 (m, br, 1H), 2.03 (dt, J = 12.4 and 7.8 Hz, 1H), 1.91−1.71 (m, 3H), 1.49−1.32 (m, 2H), 1.37 (s, 9H), 1.32−1.19 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ

dx.doi.org/10.1021/jo302737j | J. Org. Chem. 2013, 78, 2600−2610

176.4, 171.5, 156.1, 136.3, 133.5, 127.9, 120.8, 118.6, 117.7, 111.3, 105.3, 77.8, 60.2, 59.2, 53.9, 52.5, 40.9, 39.2, 30.7, 29.3, 28.7 (3C), 28.4, 28.3, 23.2; HRMS (EI) calcd for $C_{26}H_{36}N_4O_5$ 484.2686, found 484.2675.

*tert-Butyl ((S)-6-(Methylamino)-6-oxo-5-((S)-2′-oxo-1,2,4,9-tetrahydrospiro[pyrido[3,4-b]indole-3,3′-pyrrolidin]-1′-yl)hexyl)-carbamate, **18**.* To a solution of **17** (0.210 g, 0.43 mmol) in EtOH (0.5 mL), methanamine (8 M in EtOH, 3 mL, 24 mmol) was added. After 5 h under stirring, the mixture was concentrated in vacuo to afford pure **18** (0.207 g, 99% yield) as a foam: R_f 0.32 (7:3, EtOAc/MeOH); $[\alpha]^{20}_D$ +15.9 (*c* 0.57 MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.71 (s, br, 1H), 7.93 (m, br, 1H), 7.39 (d, J = 7.6 Hz, 1H), 7.28 (d, J = 7.8 Hz 1H), 7.01 (t, br, J = 7.5 Hz, 1H), 6.93 (t, br, J = 7.5 Hz, 1H), 6.78 (m, br, 1H), 4.45 (dd, *J* = 10.1 and 5.8 Hz, 1H), 4.43 (m, br, 1H), 4.02 (d, *J* = 16.8 Hz, 1H), 3.90 (d, *J* = 16.8 Hz, 1H), 3.59 (td, *J* = 9.1 and 3.2 Hz, 1H), 3.37−3.25 (m, 1H), 2.99−2.89 (m, 2H), 2.71 (d, *J* = 14.9 Hz, 1H), 2.64 (d, *J* = 14.9 Hz, 1H), 2.60 (d, *J* = 4.5 Hz, 3H), 2.02 (dt, *J* = 12.4 and 7.9 Hz, 1H), 1.86−1.58 (m, 3H), 1.47−1.34 (m, 2H), 1.37 (s, 9H), 1.31−1.08 (m, 2H); ^{13}C NMR (100 MHz $CDCl_3$) δ 176.7, 170.1, 156.1, 136.1, 131.8, 127.6, 121.8, 119.5, 117.8, 110.9, 106.2, 79.2, 59.9, 55.2, 40.9, 40.3, 39.2, 29.7, 29.5, 28.5 (3C), 28.0, 27.5, 26.3, 23.3; HRMS (EI) calcd for $C_{26}H_{37}N_5O_4$ 483.2846, found 483.2861.

*(S)-5-((S)-2-((S)-2-Acetamido-3-phenylpropanoyl)-2′-oxo-1,2,4,9-tetrahydrospiro[pyrido[3,4-b]indole-3,3′-pyrrolidin]-1′-yl)-6-(methylamino)-6-oxohexan-1-aminium 2,2,2-trifluoroacetate, **4**.* To a solution of (*S*)-2-acetamido-3-phenylpropanoic acid (50 mg, 0.24 mmol) in CH_2Cl_2 (2 mL) were added DIPEA (0.084 mL, 0.48 mmol) and HATU (92 mg, 0.24 mmol) under nitrogen atmosphere. After 1 h under stirring, a solution of **18** (0.175 g, 0.36 mmol) and DIPEA (0.084 mL, 0.48 mmol) in CH_2Cl_2 (2 mL) was added, and the mixture was stirred at room temperature for 48 h. The solution was washed with 5% aq H_3PO_4 and with saturated aq $NaHCO_3$, dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification by flash chromatography (98:2, EtOAc/MeOH) afforded **19** (71 mg, 44% yield) as a foam: R_f 0.54 (8:2, EtOAc/MeOH); $[\alpha]^{20}_D$ +22.9 (*c* 0.72, MeOH); ^{1}H NMR (400 MHz $CDCl_3$) δ 8.59 (m, br, 1H), 7.61 (m, br, 1H), 7.55 (d, *J* = 7.9 Hz, 1H), 7.42 (d, *J* = 7.3 Hz, 2H), 7.34 (d, *J* = 8.1 Hz, 1H), 7.33−7.26 (m, 2H), 7.23−7.18 (m, 2H), 7.15 (t, *J* = 7.6 Hz, 1H), 6.21 (m, br, 1H), 5.19 (dt, *J* = 7.0 and 7.3 Hz, 1H), 4.97 (d, br, *J* = 14.4 Hz, 1H), 4.73 (d, br, *J* = 9.7 Hz, 1H), 4.66 (m, br, 1H), 4.37 (d, br, *J* = 14.4 Hz, 1H), 3.27−3.08 (m, 6H), 3.03 (dd, *J* = 13.7 and 6.7 Hz, 1H), 2.86 (d, *J* = 4.7 Hz, 3H), 2.92−2.79 (m, 1H), 2.40−2.24 (m, 1H), 2.03 (s, 3H), 2.00−1.30 (m, 7H), 1.46 (s, 9H); ^{13}C NMR (100 MHz $CDCl_3$) δ 175.4, 172.3 172.0, 170.9, 156.7, 140.8, 136.4 135.8, 129.7 (2C), 128.5 (2C), 127.1, 126.5, 122.4, 120.0, 118.1, 111.2, 105.8, 80.9, 66.9, 55.0, 51.7, 42.7, 40.5, 39.8, 39.2, 30.4, 29.7, 29.6, 28.5 (3C), 26.7, 26.6, 23.6, 23.1; HRMS (EI) calcd for $C_{37}H_{48}N_6O_6$ 672.3635, found 672.3618.

To a solution of **19** (35 mg 0.052 mmol) in CH_2Cl_2 (1 mL) was added 20% TFA in CH_2Cl_2 (1 mL). After 1 h under stirring at room temperature, the mixture was concentrated in vacuo to afford **4** (35 mg, 98% yield) as a hygroscopic solid: HRMS (EI) calcd for $C_{32}H_{41}N_6O_4$ 573.3184, found 573.3167. Anal. calcd for $C_{34}H_{41}F_3N_6O_6$: C, 59.47; H, 6.02; N, 12.24. Found: C 59,58; H, 6.10; N, 12.31.

*(S)-5-((S)-2-((S)-2-((S)-2-Acetamido-3-phenylpropanamido)-3-phenylpropanoyl)-2′-oxo-1,2,4,9-tetrahydrospiro[pyrido[3,4-b]-indole-3,3′-pyrrolidin]-1′-yl)-6-(methylamino)-6-oxohexan-1-aminium 2,2,2-Trifluoroacetate **21**.* To a solution of (*S*)-2-((*S*)-2-acetamido-3-phenylpropanamido)-3-phenylpropanoic acid (44 mg, 0.12 mmol) in CH_2Cl_2 (2 mL) were added DIPEA (0.043 mL, 0.24 mmol), HATU (47 mg, 0.12 mmol), and HOAt (0.6 M in DMF, 0.207 mL, 0.12 mmol) under nitrogen atmosphere. After 1 h under stirring, a solution of **18** (90 mg, 0.19 mmol) and DIPEA (0.043 mL, 0.24 mmol) in CH_2Cl_2 (2 mL) was added. The mixture was stirred at room temperature for 48 h. The reaction mixture was washed with 5% aq H_3PO_4 and with saturated aq $NaHCO_3$, dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification by flash chromatography (98:2, EtOAc/MeOH) afforded **20** (20 mg, 20% yield) as a foam: R_f 0.46 (EtOAc); $[\alpha]^{20}_D$ +31.8 (*c* 0.50, MeOH); ^{1}H NMR (400 MHz $CDCl_3$) δ 8.94 (m, br, 0.8H), 8.84 (m, br, 0.2H), 7.74−7.63 (m, 1H), 7.61−7.49 (m 1.5H), 7.40−7.09 (m, 13.5H), 6.76 (d, br, *J* = 6.1 Hz, 0.8H), 6.67 (d, br, *J* = 6.7 Hz, 0.2H), 5.26 (dt, *J* = 6.2 and 8.8 Hz, 0.4H), 5.17−5.03 (m, 1.2H), 4.88−4.59 (m, 3H), 4.50 (d, br, *J* = 14.6 Hz, 0.4H), 4.28 (d, br, *J* = 14.6 Hz, 0.4H), 4.18 (d, br, *J* = 14.3 Hz, 0.6H), 3.32−2.93 (m, 9H), 2.8−2.79 (m, 4H), 2.31 (m, 1H), 2.03 (m, 1H), 1.96 (s, 0.1H), 1.94 (s, 1H), 1.93 (s, 1.9H), 1.89−1.80 (m, 1H), 1.74−1.21 (m, 5H), 1.45 (s, 9H); ^{13}C NMR (100 MHz $CDCl_3$) δ 174.4, 173.0, 172.7, 172.6, 171.4, 156.8, 140.4 (2C), 136.1, 136.0, 129.8 (4C), 128.6 (4C), 127.2, 127.1, 126.6, 123.6, 120.0, 118.3, 111.0, 105.8, 83.2, 66.9, 55.3, 51.7 (2C), 42.4, 41.8, 40.5, 39.2, 39.1, 30.4, 29.6 (2C), 28.5 (3C), 28.1, 26.6, 26.4, 24.0; HRMS (EI) calcd for $C_{46}H_{57}N_7O_7$ 819.4319, found 819.4342.

To a solution of **20** (20 mg 0.024 mmol) in CH_2Cl_2 (1 mL) was added 20% TFA in CH_2Cl_2 (1 mL). After 1 h under stirring, the mixture was concentrated in vacuo to afford **21** (20 mg, 99% yield) as a hygroscopic solid: HRMS (EI) calcd for $C_{41}H_{50}N_7O_5$ 720.3868, found 720.3884. Anal. Calcd for $C_{43}H_{50}F_3N_7O_7$: C, 61.93; H, 6.04; N, 11.76. Found: C, 61.99; H, 6.05; N, 11.66.

COMPUTATIONAL DETAILS

Conformational analysis was performed with the software Spartan '08[27] by means of the "conformer distribution" function using the default search method ("Systematic" or "Monte Carlo" is automatically chosen as that which leads to the smaller number of moves, depending on the number of rotatable bonds in the molecule). The MMFF force field was used for the energy minimization of the found structures. The structures were then clustered according to the default setting of the software (which consists in pruning out higher energy conformers and keeping a diverse set of the low energy conformers using the RMS torsion definition of nearness). Superimposition of the global minimum of compounds **3** and **4** with standard β-turn types was made with the alignment tool of the software and measured with the alignment score function. Images were generated with the software pyMol.[28]

ASSOCIATED CONTENT

Supporting Information

General procedures and NMR spectra for all compounds; VT NMR studies, DMSO-d_6 titrations, and IR data for compounds **3** and **19**; crystallographic data for compound **3** (CIF); computational details. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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