Synthesis and Binding Affinities of Novel Spirocyclic Lactam Peptidomimetics of Somatostatin

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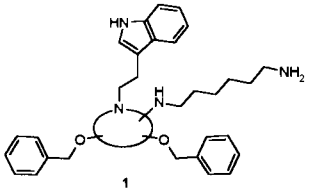
(Received May 11, 1998; CL-980366)

Synthesis of novel spirocyclic lactam peptidomimetics of the binding domain of somatostatin from commercially available D, L-methionine is described.

As part of our ongoing programme towards the development of peptide-based analogs of neuropeptides, we have recently reported the design, synthesis and biological evaluation of non-peptide mimics of somatostatin within sugar-based (**1**)[1] or 3-substituted-proline-based (**2**)[2] series.



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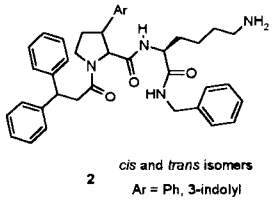

2

cis and *trans* isomers
Ar = Ph, 3-indolyl

In this communication, we report the synthesis and binding affinities of novel β-turn peptidomimetics **3** (Scheme 1) of the tetrapeptide sequence Phe[7]-Trp[8]-Lys[9]-Thr[10] of somatostatin (SRIF-14) which is the most relevant for biological activity.[3]

Starting from 3D structures of low-energy conformations of the somatostatin analogue Sandostatin®,[4a] and non-peptide mimics of somatostatin described,[4b] we designed the ambiscalemic compounds **3**,[5] using spirocyclic lactam as scaffold, as potential non-peptide mimics of Somatostatin. In these compounds, the phenyl moiety borne by the proline cycle, the *N*-aminobutyl group and the two phenylalkyl chains are expected to be in an adequate spatial orientation,[6] and to retain key electronic features. They could be superimposed onto respectively the D-Trp[4'], Lys[5'], Phe[3'] and Thr[6'] moieties of Sandostatin®.

As outlined in Scheme 1, our synthesis was initiated from the commercially available D, L-methionine **4,** the thiomethyl group serving as a latent leaving group for the intramolecular displacement reaction. Thus, esterification of **4** followed by *N*-acetylation under standard reaction conditions gave **5**[7] in 95% overall yield. Then, condensation-cyclization reaction of **5** with *trans*-cinnamaldehyde in presence of NaH as base gave the Michael adduct **6** with 59% yield. C-5 deoxygenation is readily effected in a single step in quantitative yield using Et_3SiH/CF_3CO_2H leading to **7** with a predominance of *trans* isomer (**7** *cis/trans*: 2/8 ratio). Diastereoselective saponification (NaOH 1N) cleanly removed the minor *cis* isomer as ester **9** (22%) and gave the *trans*-proline carboxylic acid derivative **8** as the major compound isolated with 57% yield. Then, *N*-deprotection of **8**, followed by successive condensation of 3,3-diphenylpropionyl chloride and N-ε-CBz-L-Lysine methyl ester[8] under standard conditions gave the amides **12** as a mixture of diastereomers (form A / form B : 1/1 ratio) in 47% overall yield. At this point, two synthetic ways were possible to obtain the expected precursor compounds **13 form A** and **13 form B**. The first approach (Pathway A) was based on a previous separation of **12** (form A / form B : 1/1 ratio) by flash chromatography affording pure **12 form A** and **12 form B** with 25% and 12% yields respectively. Then, methylation on sulfur with an excess of iodomethane was followed by base treatment (K_2CO_3) to induce a ring closure to give γ-lactams **13 form A** and **13 form B** with 45% and 36% yield respectively. The second approach (Pathway B) required the direct spirolactamization of the mixture of diastereomers **12** under the same experimental reaction conditions as above, followed by separation of the two isomers by flash chromatography on silicagel resulting in pure **13 form A** and **13 form B** with respectively 25% and 12% yields. The methyl esters **13** were then treated with aqueous NaOH (0.5 N) given the corresponding carboxylic acids (61% and 81% yields respectively). Coupling of these acids to benzylamine using HOBt/EDCI as coupling agents furnished **14 form A** and **14 form B** with 79% and 44% yields respectively. Finally, the benzyl carbamates **14** were hydrogenolyzed (cyclohexene/Pd/C) to give the expected *trans*-spirocyclic γ-lactams **3 form A** and **3 form B** with 57% and 36.5% yields respectively.

Binding assays[9] have shown weak affinities for the proline derivatives **2** and for the spirocyclic lactam derivatives **3** for somatostatin receptors *versus* SRIF-14 itself (Table). These results suggest that the orientations of the side chains are quite similar whatever the geometry (forms A *versus* forms B) and the nature of the scaffold used (**2** *versus* **3**). These results, and those obtained using other non-peptidic derivatives (*e.g.* sugar-based or benzodiazepinone derivatives) exhibiting affinities for the SRIF-14 receptors, strongly support the validity of the concept of the utilisation of non-peptide scaffold in the synthesis of non-peptide molecules as peptide analogues. In addition, the incorporation of these novel spirocyclic lactams into other pharmaceutically relevant target molecules may be of interest.

We wish to express our many thanks to J-C. Szmigel, M. Vuilhorgne and co-workers for analytical assistance.

References and Notes

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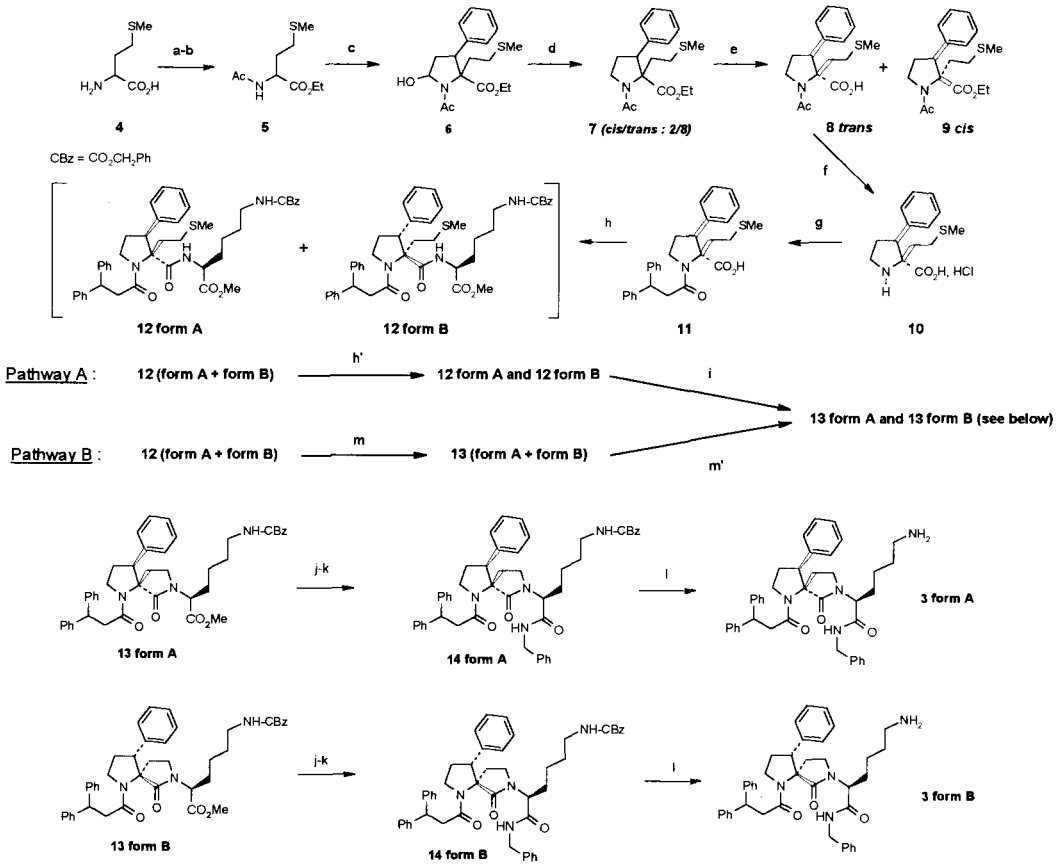
4 For stable cyclic-peptide somatostatin analogues, see: a) Z.



Scheme 1. Synthesis of spirocyclic lactams **3.**

a) 4, EtOH, HCl_g, 0 °C to rt, 3.5 h, 100% **b)** MeCOCl, TEA, CH_2Cl_2, 25 °C, 12 h, 95% **c) 5** (1eq.), NaH (1eq.), THF, rt, 1.5 h then PhCH=CHCHO (1eq.), THF, 0 °C to rt, 12 h, two isomers 6/4 (^{1}H-NMR, 300MHz, DMSO-d_6), 59% **d) 6** (1eq.), Et_3SiH (1.5eq.), CF_3CO_2H, 10 °C, 1 h, two isomers 8/2 (^{1}H-NMR, 300MHz, DMSO-d_6), 100% **e) 7** (1eq.), NaOH (1N, 2eq.), EtOH, reflux, 72 h, **8**: 57%, **9**: 22% **f)** HCl (6N), AcOH, 100 °C, 24 h, 86% **g) 10** (1eq.), Ph_2CHCH_2COCl (1eq.), Et_3N (2eq.), CH_2Cl_2, rt, 12 h, 64%. **h) 11** (1eq.), H_2N-L-Lys(ε-CBz)-CO_2Me (1eq.), EDCI (1eq.), HOBT (1eq.), TEA (1eq.), CH_2Cl_2, rt, 12 h followed by flash cromatography on silica gel (AcOEt), 85% (**12 form A / 12 form B** : 1/1, ^{1}H-NMR, 250MHz, DMSO-d_6) **h')** flash chromatography on silica gel (AcOEt/cyclohexane 3/7), **12 form A**: 25%, **12 form B**: 12%. **i) 12 form A** or **12 form B** (1eq.), MeI (120 eq.), rt, 24 h then evaporated to dryness, then K_2CO_3 (3eq.), THF, rt, 16 h, **13 form A**: 45%, **13 form B**: 36% **j) 13 form A** or **13 form B**, NaOH (0.5N), THF, rt, 12 h, 61-81% **k)** $PhCH_2NH_2$ (1eq.), EDCI (1eq.), HOBt (1eq.), CH_2Cl_2, rt, 24 h then flash chromatography on silica gel (AcOEt/cyclohexane 8/2), **14 form A**: 79%, **14 form B**: 44%. **l) 14 form A** or **14 form B**, cyclohexene, Pd/C (10%), MeOH, reflux, 16 h, **3 form A**: 57%, **3 form B**: 36.5% **m) 12** (mixture 1/1) (1eq.), MeI (120 eq.), rt, 24 h then evaporated to dryness followed K_2CO_3 (3eq.), THF, rt, 16 h, (**13**: 1/1, ^{1}H-NMR, 300MHz, DMSO-d_6) **m')** flash chromatography on silica gel (AcOEt/cyclohexane 3/7), **13 form A**: 25%, **13 form B**: 12%.

compound	SRIF-14	2 (Ar = Ph)				3	
		cis A	cis B	trans A	trans B	trans A	trans B
IC_{50} (μM)	0.0002	20	22	11	22	11	15

Table . Binding affinities of **2, 3** and **SRIF-14** for somatostatin receptors on membranes of rat cerebral cortex (3-[^{125}I]-Tyr^{11}-SRIF-14)

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9 Binding assays were performed as reported by Srikant et al. [C. B. Srikant and Y. C. Patel, *Proc. Natl. Acad. Sci. USA*, **78**, 3930 (1981)]. Data obtained with different concentrations of derivatives and SRIF-14 were used to generate inhibition curves. The calculated IC50 values which are the mean of at least three determinations each with six concentrations of the test compound in triplicate.