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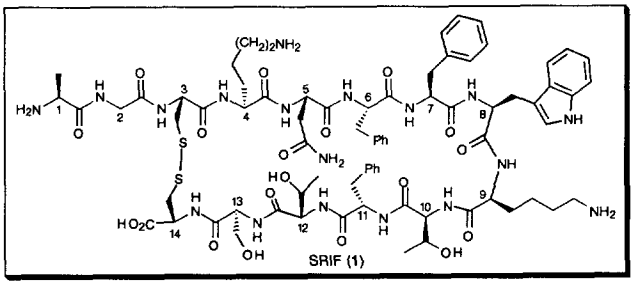
THE FIRST SYNTHESIS OF A TRICYCLIC HOMODETIC PEPTIDE EMPLOYING COORDINATED ORTHOGONAL PROTECTION

Ralph Hirschmann,[†*] Wenqing Yao,[†] Byron Arison,[‡*] Laurie Maechler,[§*]
Avery Rosegay,[‡*] Paul A. Sprengeler,[†] and Amos B. Smith, III[†*]

[†] *Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19104*
[‡] *Merck Research Laboratories, Rahway, New Jersey 07065*
[§] *Panlabs Inc., 11804 North Creek Parkway South, Bothell, Washington 98011*

Abstract: In an effort to cause significant conformational distortion and thereby possibly generate a pure SRIF antagonist, we undertook the synthesis of **2**, the first homodetic tricyclic peptide. This required five dimensional orthogonal amino protection and three carboxyl protecting groups to allow the selective closure of the three rings and to differentiate the ε-amino groups of the two lysines. We also exploited acid catalyzed removal from the resin to achieve selective deprotection of a side chain carboxyl moiety simultaneously with its partner amino group for subsequent cyclization.

The peptide hormone somatostatin (SRIF) **1**, a tetradecapeptide containing a ring of twelve amino acids, which inhibits the release of several hormones and neurotransmitters including growth hormone, insulin, and glucagon, was first isolated, characterized and synthesized by Brazeau et al. in 1973.[1] A solution conformation incorporating a γ-turn was subsequently proposed by Holladay and Puett[2] based on CD spectra. Veber and his collaborators[3,4] presented experimental support for a β-turn involving amino acids 7-10, generating an antiparallel β-pleated sheet as the bioactive conformation of SRIF at its receptor.



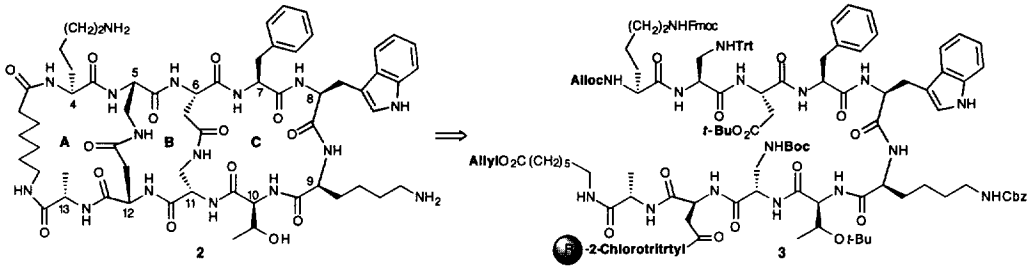
The synthesis of a pure SRIF antagonist remains a desirable goal for both biological and conformational considerations.[5] We therefore undertook the design and synthesis of homodetic[6] tricyclic **2** (Scheme 1), incorporating significant conformational distortion, possibly leading to an SRIF antagonist. The synthetic objective presented a significant chemical challenge, since to our knowledge no tricyclic homodetic peptide has heretofore been prepared.

Cyclopeptides of either natural or synthetic origin have been of great interest,[7] because the introduction of conformational constraints into biologically active peptides *via* cyclization can provide important information about their bioactive conformations.[3,8] In elucidating the bioactive conformation of SRIF, the Merck group synthesized three potent heterodetic[6] bicyclic peptides, incorporating 12/8, 12/6 and 8/6-sized rings.[3,4] Methods have been developed for the synthesis of monocyclic, homodetic peptides either in solution or on solid support.[9] The construction of tricyclic homodetic peptides, however, remains a challenge in that multiple orthogonal[10] protection and deprotection tactics are required. This task is even more complex in the case of **2**, given the presence of two lysine ε-amino groups which we wished to be able to deprotect selectively.[11]

Our synthetic strategy was designed to permit sequential selective deprotection of a carboxyl group simultaneously with its partner amino group for iterative ring constructions. To this end, we needed three different amine/carboxyl protecting group partners; we chose *t*-Boc/*t*-Butyl, Alloc/Allyl, and finally trityl/2-chlorotrityl solid support (Scheme 1). In

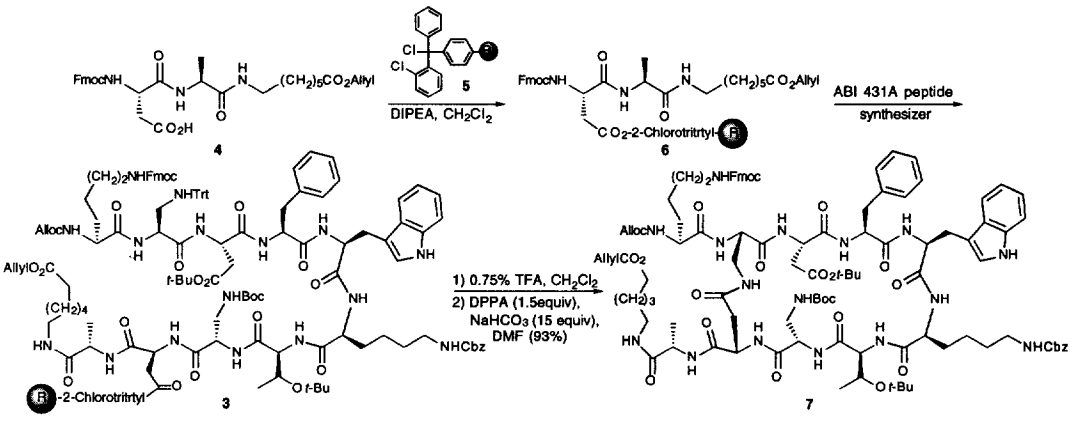
addition, to incorporate the capability to deprotect either of the two ε-amino groups selectively,[10] five dimensional orthogonal amino protection was required. The strategy therefore involved the following sequential operations: (1) side chain anchoring of partially protected tripeptide **4** to a polymeric solid support via a β-aspartyl ester linkage; (2) stepwise solid phase assembly of the linear sequence using Fmoc chemistry; (3) treatment with dilute TFA (0.75%), effecting cleavage of the linear peptide from the 2-chlorotrityl solid support, generating the unprotected Asp^{12} side chain, and also removing the trityl protecting group from the Dpr^5 β-amino group; (4) high-dilution DPPA mediated cyclization of the Asp^{12} and Dpr^5 side chains allowing formation of the first ring which embodies the B and C rings of **2**; (5) selective removal of the protecting groups of the Lys^4 α-amino group and the Aha (7-aminoheptanoic acid) carboxyl moiety (Alloc and Allyl, respectively) using palladium, followed by formation of ring A; (6) TFA mediated removal of the *t*-Boc/*t*-Butyl ester of the Dpr^{11} and Asp^6, respectively, followed by formation of the B and C rings; and (7) stepwise removal of the ε-amino protecting groups of Lys^4 (Fmoc) and Lys^9 (Cbz) using piperidine and Pd black respectively, affording **2**.

Scheme 1



As our point of departure, tripeptide **4**[12] was prepared using standard peptide chemistry and then coupled to a polystyrene solid support by esterification with 2-chlorotrityl chloride resin **5**[13] to afford **6.** Compound **3** was then synthesized on an ABI 431A peptide synthesizer using Fmoc chemistry[14] along with HBTU/DIPEA coupling reagents as shown in Scheme 2. Treatment of **3** with a solution of 0.25% TFA in CH_2Cl_2 for 30 min effected cleavage from the solid support; the resulting product, however, was found to be still partially tritylated at position 4 (SRIF numbering). Further treatment with 0.75% TFA in CH_2Cl_2 solution provided a homogeneous product as shown by HPLC and NMR. The analytical RP-HPLC of

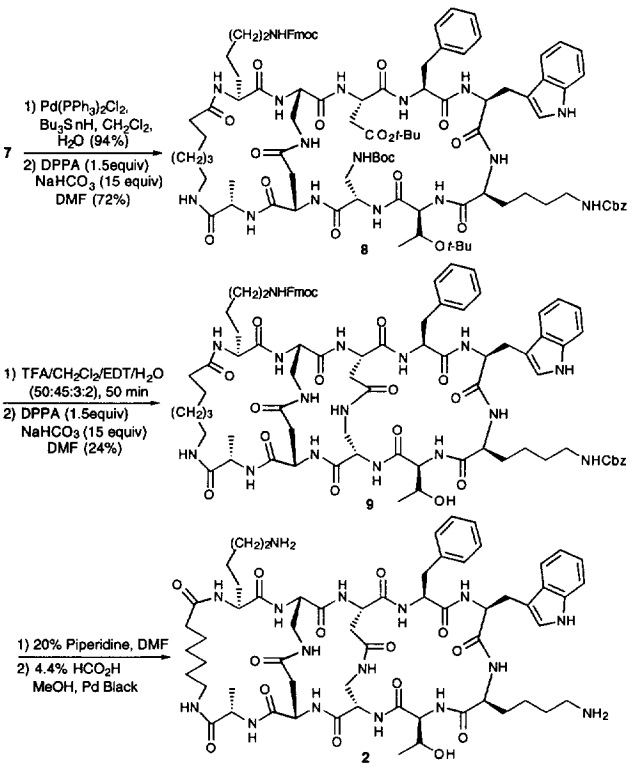
Scheme 2



the crude material indicated a purity greater than 95%; this material was used directly for the formation of the first ring (embodying the B and C rings of **2**) via treatment with diphenylphosphoryl azide (DPPA, 1.5 equiv)[15,16] and solid sodium bicarbonate (15 equiv) in anhydrous DMF (final concentration ca. 0.008 M) at 4 °C. The cyclization was shown by analytical RP-HPLC to be complete in less than 24 hours. The monocyclic **7** was isolated in 93% yield after purification by flash chromatography.

With **7** in hand, deprotection of the Alloc and Allyl groups was accomplished simultaneously in 94% yield (Scheme 3) using $Pd(PPh_3)_2Cl_2$ as catalyst and Bu_3SnH as the hydride source. Cyclization under our standard DPPA conditions provided **8** in 72% yield after silica gel chromatography. The simultaneous removal of Boc and *t*-Bu groups from **8** was then readily achieved by the action of 50% TFA in CH_2Cl_2 with 2% water and 3% 1,2-dithioethane as ion scavenger. The free carboxyl was activated for cyclization by DPPA under dilute conditions; however, in this case the desired product **9** was formed in only 24% yield, with higher molecular weight compounds representing the major byproducts. A possible explanation for the lower yield in this case may be that formation of two highly constrained bridges makes **9** both difficult to form and susceptible to decomposition via imide formation and subsequent cleavage of the bridge. Support for this interpretation was provided by the observation that treatment of **9** under mild conditions (20% piperidine in DMF) gave a complex mixture of products containing the desired product plus a wide range of higher molecular weight material as shown by HPLC and mass spectrometry. Nevertheless, the desired product could be purified by RP-HPLC and subjected to the final Cbz removal via hydrogenation to afford a mixture containing the desired product **2**.

Scheme 3



The crude material was purified by preparative RP-HPLC (C18 column). Analytical RP-HPLC employing a C18 column in conjunction with two different linear gradients and one isocratic solvent system in all three cases resulted in a symmetric peak, suggesting homogeneity. However, ^1H NMR analysis clearly indicated the presence of a significant impurity which had initially been thought to be due to a minor conformational isomer of **2** (vide infra). Assignment of the resonances of the ^1H spectrum of **2** showed, however, that the peaks of the minor component were due to an impurity. Analytical RP-HPLC using a C4 column (Vydac) enabled us to remove a 10-14% impurity. A second purification using a semipreparative C4 column afforded **2** which was shown to be a pure entity by 500-MHz ^1H NMR and high resolution FAB mass spectrometry. These results serve as a reminder that, despite the widely accepted notion in peptide chemistry, single symmetric peaks by analytical HPLC in more than two different gradient solvent systems do not assure purity.

The tricyclic peptide **2** failed to completely inhibit ^{125}I-labeled somatostatin binding to somatostatin receptors from mouse anterior pituitary AtT-20 cell membranes. Maximal inhibition of 50% (three experiments) was observed at 1 μM, with no additional dose-related inhibiton up to 10 μM, the highest concentration tested. A more detailed discussion of the structural and biological studies of **2**, as well as its purification will be published elsewhere.

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