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Synthesis and structural study of cyclic 5-aminovaleric acid-linked β-Ala–β-Ala dipeptides ☆

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ABSTRACT

5-Aminovaleric acid and ornithine were evaluated as linkers for the cyclization of β-dipeptides. Two linked examples of β-Ala-β-Ala were prepared by standard coupling methods and their conformations probed by NMR, CD, and computational means. The data suggest that these non- or monosubstituted versions of the target compounds are flexible in solution.

β-Peptides have captured the imagination of a wide range of researchers, from those interested in basic principles of macromolecular design to nanotechnologists.[1] For medicinal chemists, β-amino acids hold promise as components of potential therapeutic agents because they provide a diversity of shape and functionality similar to that found in standard peptides.[2] In addition, β-peptides may be less likely to undergo deleterious metabolism. Cyclization is a useful strategy for the discovery of β-peptide-based therapeutics for the same reason as in α-peptides: to bias compound conformation in a given (hopefully bioactive) conformation.[3]

The use of unsubstituted[4] and substituted[5] versions of ε-aminocaproic acid (Aca) as a linker in cyclic peptide derivates has become well established as a means of constraining a dipeptide into a β-turn-like conformation. In some cases, control over the conformation of the mimetic (e.g., type I vs type II β-turns) may be exerted by either the dipeptide or the Aca linker portion of the molecule. Given the success of the Aca cyclization strategy in conferring β-turn conformations onto standard dipeptides, we decided to pursue the synthesis and evaluation of two cyclic versions of β-Ala–β-Ala using a related strategy (Fig. 1).

Molecular modeling[7] carried out in a series of α,ω-amino acids ($n = 1–3$, Fig. 1) combined with β-Ala–β-Ala suggested that the optimal spacer length in structures of type **2** would be $n = 2$

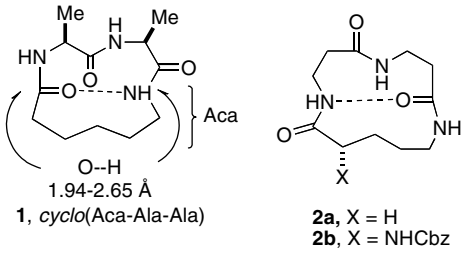

Figure 1. Structures of an Aca-cyclized Ala–Ala dipeptide (**1**) and the two cyclized β-Ala–β-Ala-containing structures targeted in this study (**2** and **3**). Note the C–N distance in **1**, which complies with one standard definition of a β-turn.[6]

(Fig. 2). These studies also suggested that a turn structure including the depicted intramolecular NH–OC hydrogen bond would also be favored for these structures. Thus, for **2a**, an energy minimum

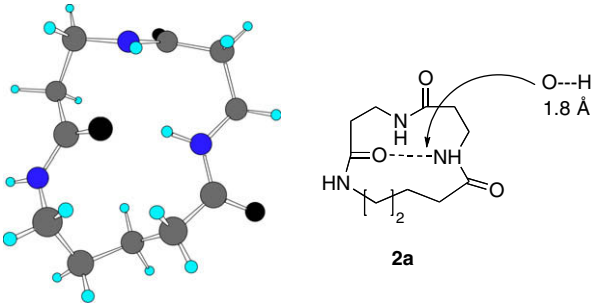

Figure 2. Energy minimum found for **2a** by molecular modeling.

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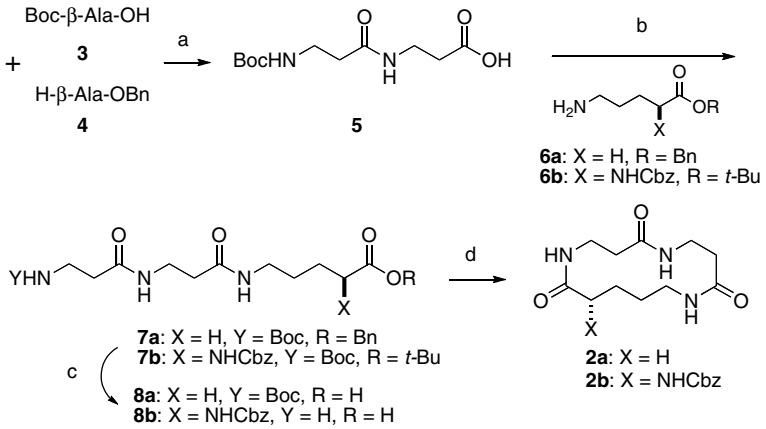

Scheme 1. Synthesis of cyclic peptides **2**. Reagents and conditions: (a) i—EDC, HOBt, NEt_3, 81%; ii—Pd/C, H_2, 91%; (b) **7a**: **6a** HCl, EDC, HOBt, NEt_3, 81%; **7b**: **6b**, EDC, HOBt, NEt_3, 57%; (c) **8a**: Pd/C, H_2, trifluoroethanol, 96%; **8b**: TFA, CH_2Cl_2, quant. (crude); (d) **2a**: i—EDC, C_6F_5OH 53%; ii—TFA, CH_2Cl_2; iii—DIPEA, 43% (2 steps); **2b**: $(EtO)_2P(O)CN$, NEt_3, 27%.

was found in which NH–OC of the C- and N-terminal part of the β-Ala–β-Ala fragment was aligned with a distance of 1.8 Å. Shorter ($n = 1$) or longer ($n = 3$; i.e., Aca) spacers resulted in minimized conformations that did not reside in β-turn-like structures, and furthermore lacked hydrogen bonding between the NH and CO fragments.

Consequently, 5-aminovaleric acid (Ava) and ornithine were chosen as spacers to bridge the β-Ala–β-Ala dipeptide. *N*-Boc-β-Ala–β-Ala-OH **5**, readily prepared from *N*-Boc-β-Ala-OH (**3**) and H-β-Ala-OBn (**4**) by standard peptide coupling followed by hydrogenation of the benzylic ester, served as a convenient starting material for the envisaged peptides **2** (Scheme 1). Elongation of **5** by coupling with **6a** or **6b** again followed by deprotection gave rise to **8**. Solubility problems of **7a** called for its hydrogenation in an alcoholic solvent: while methanol resulted in some transesterification of **7a** to the corresponding methyl ester, the use of trifluoroethanol cleanly allowed deprotection of the benzyl ester to give **8a** in almost quantitative yield. For the final cyclization, activation of **8a** as its pentafluorophenyl ester turned out to be necessary because the acid was not soluble in standard peptide coupling solvents. N-deprotection of this ester then led to the cyclized peptide **2a**. The amine-containing analog **8b** could be directly cyclized by activation with diethylcyanophosphonate, yielding **2b**. The moderate yields in which **2** was obtained in the final cyclization were a result of the very low solubility of the products, making purification a laborious task. In both cases, sufficient quantities of peptides were made available for standard characterization.[8]

The structural analysis of **2a** and **2b** was hampered by solubility concerns. For example, it proved necessary to obtain IR spectra in pyridine at relatively high concentrations (ca. 5 mmol). Under these conditions, N–H stretching bands were observed at 3272 cm^{-1} for **2a** and 3276 cm^{-1} and 3296 cm^{-1} for **2b**. Although values of less than 3400 cm^{-1} are generally considered consistent with internal hydrogen bond formation under standard conditions (typically 1 mmol in DCM),[9] we hesitate to read too much into these results due to the differences in solvent and concentration. However, it is not unreasonable to suggest that the hydrogen bonding pattern indicated in Figure 2 is at least possible. In addition, ROESY NMR studies were done on **2a** and **2b**. These results were ambiguous, suggesting that these macrocycles have a highly dynamic structure. Only a few significant crosspeaks were observed between distant protons (defined as 1,6 relationship or larger). There were a greater number of these peaks in the ROESY of **2b**, suggesting a greater population in this compound, possibly due to the conformational bias of NHZ substituent. One approach that will be taken in future work will be to add further substituents and other constraints to try to add more of a bias to the macrocycles.

Compound **2b** was also subjected to circular dichroism spectroscopy in methanol solution (Fig. 3a). The resulting spectrum, as well as that of the β-peptide-derived macrocycle **9** reported by Seebach (Fig. 3b, spectrum not shown),[10] was similar to that expected for a random coil. The structural differences between these different systems preclude any meaningful interpretation of this result at the present time.

In conclusion, the present work demonstrates the synthetic feasibility of cyclizing β-dipeptides with Ava linkers in a manner similar to our previously published use of Aca linkers with α-dipeptides. The resulting compounds are likely to take up turn conformations due to the simple fact that they are cyclic, but preliminary data are most consistent with a fairly extensive range of possible interconverting conformations for the simple substitution types shown. Future work will add substituents and further constraints[11] in attempts to obtain more highly ordered and possible biologically interesting structures.

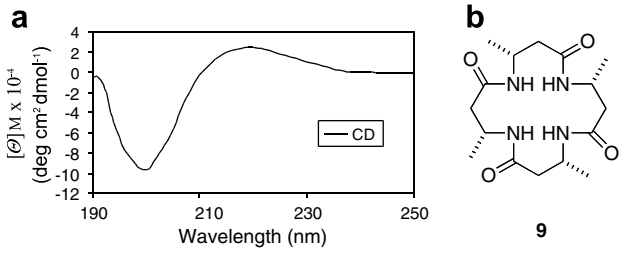

Figure 3. (a) CD spectrum of **2b** taken in methanol. (b) Cyclic β-peptide reported by Seebach.[10]

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7. Modeling was carried out using the Sybyl platform equipped with the Tripos force field, Gasteiger Hückel Charge, and setting $e = 1$.
8. *Compound* ***2a***: IR (Teflon® film) 3291, 3087, 1724, 1654, 1561, 1441 cm^{-1}; ^1H NMR (400 MHz, MeOH-d_4) δ 1.49–1.55 (m, 2H, 4-H), 1.64 (quin, $J = 7.0$ Hz, 2H, 3-H), 2.20 (t, $J = 6.9$ Hz, 2H, 2-H), 2.37 (t, $J = 5.7$ Hz, 2H, 7/10-H), 2.46 (t, $J = 5.9$ Hz, 2H, 7/10-H), 3.25 (t, $J = 5.6$ Hz, 2H, 5-H), 3.46 (t, $J = 5.7$ Hz, 2H, 8/11-H), 3.50 (t, $J = 5.9$ Hz, 2H, 8/11-H); ^{13}C NMR (100.6 MHz, MeOH-d_4) 24.0 (4-C), 28.8 (3-C), 35.0 (CH_2), 35.2 (CH_2), 35.4 (CH_2), 35.7 (CH_2), 36.1 (CH_2), 37.7 (CH_2), 172.6 (CO), 172.9 (CO), 175.6 (CO). MS (DCI–NH_3): m/z (%) = 242 (60) [MH^+], 207 (45), 143 (70), 60 (100). $C_{11}H_{20}N_3O_3$ (POS FAB VSCAN): 242.1505 (cal), 242.1501 (found).
Compound ***2b***: [α] −38 (MeOH, *c* 0.06). IR (Teflon® film) 3301, 3083, 2925, 1684, 1649, 1571, 1431 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) 1.37–1.50 (m, 4H, 3-H and 4-H), 2.03–2.09 (m, 1H, 7/10-H), 2.13–2.19 (m, 1H, 7/10-H), 2.20–2.26 (m, 1H, 7/10-H), 2.42–2.48 (m, 1H, 7/10-H), 2.97–3.18 (m, 4H, 5/8/11-H), 3.45–3.60 (m, 2H, 5/8/11-H), 3.99–4.04 (m, 1H, 2-H), 5.00 (s, 2H, CH_2Ph), 7.23 (d, $J = 7.9$ Hz, 1H, NHCbz), 7.31–7.39 (m, 6H, Ph, NH), 7.78 (t, $J = 5.7$ Hz, 1H, NH), 7.85 (t, $J = 5.3$ Hz, 1H, NH). MS (DCI–NH_3): m/z (%) = 391 (60) [MH^+], 283 (100), 225 (20). $C_{19}H_{27}N_4O_5$ (POS FAB VSCAN): 391.1981 (cal), 391.1967 (found).
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