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Solid-phase synthesis of a type II′ β-turn peptido-mimetic library

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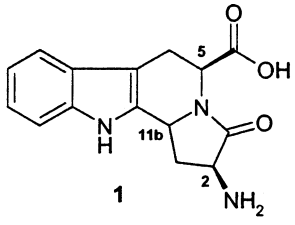
Abstract—The solid-phase synthesis of a dipeptide derived 2-amino-3-oxohexahydroindolizino[8,7-*b*]indole-5-carboxylate system (IBTM) is described. The IBTM moiety is formed via a solid-phase mediated Pictet–Spengler reaction of N-terminal tryptophan and the 4-{*N*-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl]amino}benzyl (Dmab) ester of Fmoc protected aspartic acid β-aldehyde followed by γ-lactamization. This synthesis allows the regio- and stereoselective incorporation of a dipeptide surrogate of type II′ β-turns. The procedure is easily adaptable to combinatorial synthesis and a 576-member library was synthesized.

Peptide–protein and protein–protein interactions are fundamental events that modulate diverse biological processes such as signal transduction, enzymatic specificity and immunomodulation. The energetics of these interactions are controlled by molecular recognition, a process dependent upon the spatial orientation of atoms in the interacting molecules. It is the energetic differences of these molecular interactions that can be exploited to control the outcome of a biological process. Having access to secondary structural motifs involved in various molecular recognition events should provide today's medicinal chemist with the keys to turn on or off biological processes of therapeutic interest.

β-turns are important molecular recognition sites in peptide–protein and protein–protein interactions because they allow a conformationally-defined presentation of amino acid side chains.[1] Unlike α-helices and β-sheets, β-turns possess more diversity in the number of conformations that they may adopt, making them important sites for molecular recognition. The pharmaceutical relevance of β-turn mimetics has recently been demonstrated by several high-affinity, selective peptidomimetics, including $\alpha_4\beta_1$-integrin antagonists,[2] LHRH antagonists,[3] and somatostatin antagonists.[4]

We chose to build our β-turn mimetics around the 2-amino-3-oxohexahydroindolizino[8,7-*b*]-5-carboxylate system (IBTM) **1** for two reasons.



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First, this system has previously been demonstrated to mimic a type II′-β turn[5] exemplified by the retention of biological activity when the (2*S*,5*S*,11b*R*)-IBTM scaffold was used to replace the D-Phe-Pro dipeptide portion of gramicidin S.[6] Second, this system was chosen because we felt it could be constructed from readily available amino acid precursors, making it easily accessible via solid-phase synthesis.

Our initial approach was to synthesize the Fmoc derivative of **1** in solution, then incorporate it into peptides using an automated synthesizer. However difficulties encountered in the synthesis of the necessary quantities for high-throughput solid-phase peptide synthesis led to the choice of an alternate route (Scheme 1).

It was envisioned that the synthesis of the entire library was to be carried out on solid phase. Starting with Fmoc AM-RAM-PS resin, we built out to the desired tryptophan using DIC/HOBt mediated couplings. At this point the protected amino acid aldehyde (**3**) was incorporated, which was the vehicle for the solid-phase Pictet–Spengler reaction.[7] The C-terminal protecting group (R_1) was then removed from the α-carboxylate

Keywords: β-turn mimetics; Pictet–Spengler reaction; solid-phase synthesis; IBTM.

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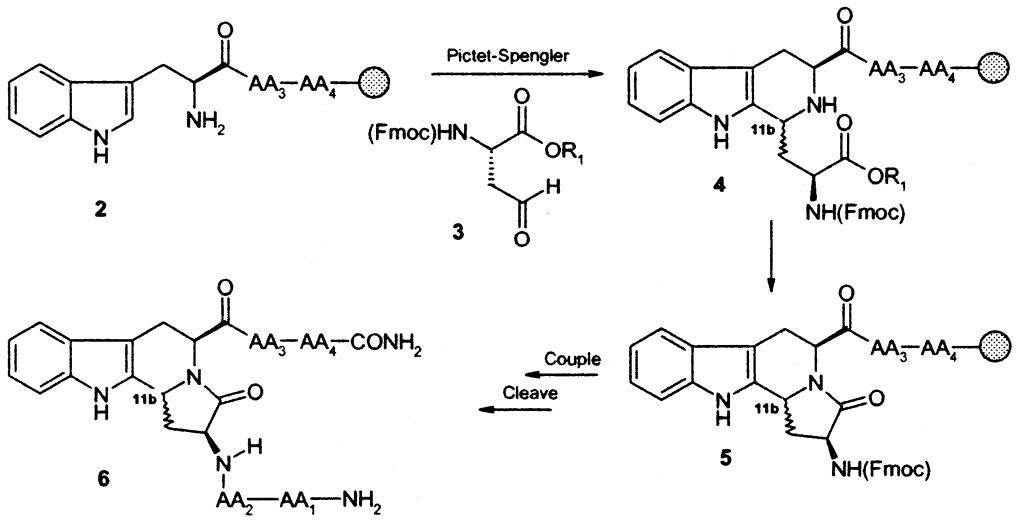


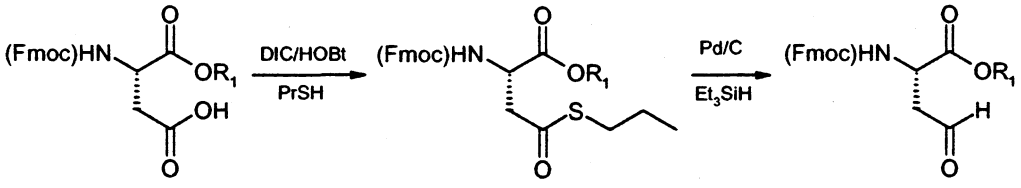
Scheme 1. General synthetic scheme for β-turn mimics.

followed by γ-lactamization. Having completed the chain elongation, the N-terminal amine was capped and the desired β-turn mimetic was cleaved from the resin.

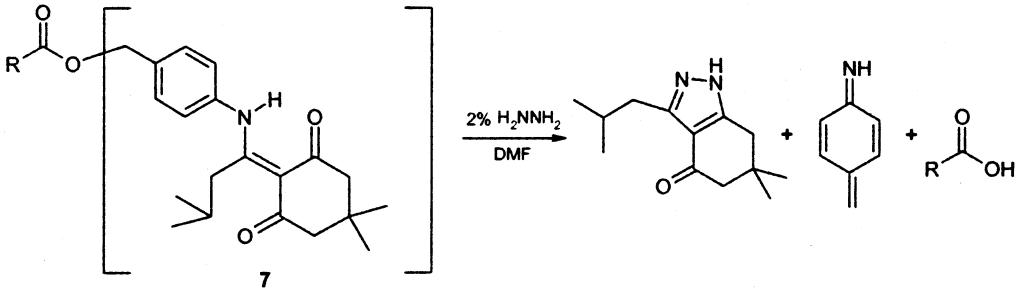
The aldehyde (**3**) could be prepared from either a homoserine or aspartic acid derivative. We chose to start from aspartic acid. Chorev and Han outline a simple procedure for transforming the β-carboxylic acid into the aldehyde (Scheme 2).[8]

With facile access to the aldehyde via the sequence of Scheme 2, identification of a protecting group (R_1) which would allow closure of the γ-lactam remained (Scheme 1; transformation of **4** to **5**). We had envisioned this happening through two steps: (1) removal of R_1 and (2) coupling of the acid to the secondary amine. Initially, we tried R_1 = benzyl which we hoped could be removed either by hydrogenolysis and then followed by lactamization, or alternatively might be displaced during γ-lactamization, thus requiring a single step. Neither procedure was successful with this compound. We next turned to the commercially available 4-{*N*-[1-(4,4-dimethyl - 2,6 - dioxocyclohexylidene) - 3 - methylbutyl]-amino}benzyl ester (Dmab) group, **7**. The Dmab group is removed in 2% hydrazine in DMF, giving the free acid and by-products that are easily washed away from the solid phase (Scheme 3).[9]

Fmoc-Asp-O-Dmab was easily transformed to the aldehyde via the protocol of Scheme 2. When we carried out the Pictet–Spengler reaction between this aldehyde and resin bound tryptophan, spontaneous γ-lactamization was usually observed under these conditions, however, with a hindered amino acid adjacent to tryptophan, closure of the γ-lactam did not reach completion. Curiously if the resin containing the incomplete



Scheme 2. Synthesis of β-aldehydic amino acid.



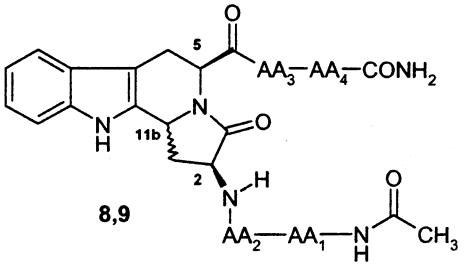
Scheme 3. Amino acid protection.

product was subjected to the Pictet–Spengler conditions for a longer time period, the γ-lactamization was not driven to completion.[10]

After chain elongation was complete, the Fmoc protecting group was removed followed by N-terminal acetylation. The resin was next subjected to hydrazine/DMF to remove any residual Dmab ester, then treated with fluoro - *N*,*N*,*N*′,*N*′ - tetramethylformamidinium hexafluorophosphate (TFFH) to finish γ-lactamization by coupling the free acid to the 2° amine via the acyl fluoride species.[11] Completion of the γ-lactam formation could not be carried out until after the chain elongation was finished due to side reactions during deprotection of the Dmab ester.

Two compounds, **8**, and **9** were synthesized for NMR spectral analysis (Table 1).[12] The peptides were cleaved from the resin and worked-up to give a crude product mixture that showed two major peaks of the same mass via LC-MS. These two peaks were due to the diastereomers generated at carbon 11b during the Pictet–Spengler reaction (Scheme 1; transformation of **2** to **4**). Purification was then achieved via reversed-phase HPLC (Vydac C_{18} 22 mm i.d.×25 cm column; linear gradient of 0.05% TFA/water and 0.05% TFA/CH_3CN).

Table 1. Examples of type II′ β-turn hexapeptides that were synthesized



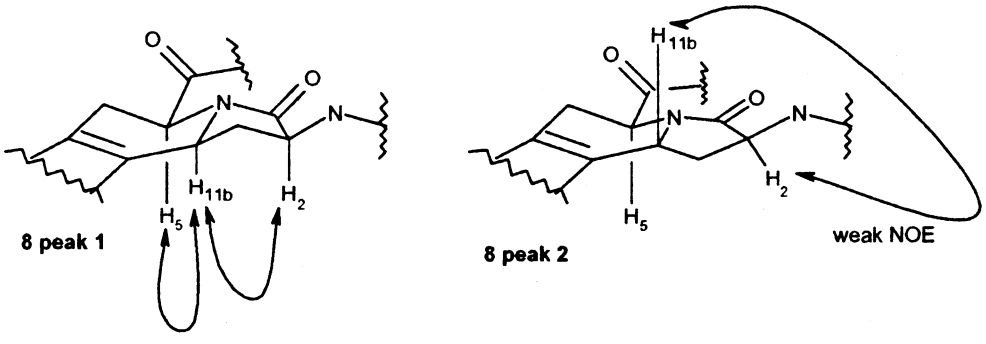
Compound	AA_1	AA_2	AA_3	AA_4
8	Asp	Phe	Nle	Leu
9	Phg	Ile	Val	Leu

Structural analysis was performed using 2D ^1H NMR on each of the purified fractions of **8** and **9**.[14] In the case of **8** a NOESY experiment indicated that for the faster eluting compound (peak 1), H_{11b} is on the same face of the molecule as H_2 and H_5 (11b = *S* configuration). For the slower eluting compound (peak 2), the NOESY cross peaks between H_{11b} and H_2 are weak, indicating that H_{11b} is on the opposite face (11b = *R* configuration) of the ring system from H_2 and H_5 (Scheme 4).

Similar results were obtained in the analysis of the purified compounds from the synthesis of **9**. The ratio of the *R* to *S* diastereomers for **8** and **9** were 78:22 and 67:33, respectively. It was shown by Andreu et al.[6] that IBTM-containing peptides with the *R* configuration at position 11b were type II′ β-turn mimetics.

Production of our β-turn mimetic libraries was carried out on a small scale in a 96-well plate (10 mg of intermediate peptide-resin per well).[13] Amino acid diversity was introduced both before and after incorporation of the IBTM moiety in the linear sequences. Plate production started with the batch-wise solid-phase synthesis of the linear sequences out to tryptophan on a Rainin Symphony® automated peptide synthesizer. The resins were then transferred to flasks for the Pictet–Spengler reaction. Resin was then transferred into Robbin's 96 well FlexChem® Multiple Synthesis Reactors, and the final two amino acids were added in a columnar and row-wise fashion to complete the sequences. The compounds were cleaved from the resin, precipitated with ether, characterized, and submitted for screening against a broad spectrum of targets.

In summary, a method has been developed for the solid-phase synthesis of constrained peptides containing the 2-amino-3-oxohexahydroindolizino[8,7-*b*]-5-carboxylate (IBTM) system, a dipeptide surrogate of type II′ β-turns. Formation of the IBTM system occurs via a solid-phase Pictet–Spengler reaction. Using the methodology developed herein, a 576-member β-turn library was synthesized in a parallel format.



Scheme 4. Key NOE's.

Acknowledgements

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10. The color of the reaction mixture was always yellowish. When a Kaiser test no longer showed blue, the resin would be removed, washed, and a small amount would be cleaved to check the molecular weight of the intermediate Pictet–Spengler product. Sometimes this would show that the γ-lactamization had not reached completion. If the resin was then resubjected to the Pictet–Spengler conditions, the reaction mixture would turn to a red color, and LC/MS would show decomposition.
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12. General procedure for the synthesis of **8** and **9**. Synthesis was carried out in a fritted 25-ml solid phase reaction vessel on Fmoc-AM RAM-polystyrene amide resin (0.65–0.76 mmol/g) (Rapp Polymere; Tubingen, Germany). Chain assembly was carried out on a 0.150 mmol scale (200 mg resin) using standard 9-fluorenyl methoxycarbonyl (Fmoc) protocols. Fmoc deprotection was carried out for 20 min with 24 ml 20% piperidine/DMF, and couplings were run for 3 h. Amino acids (1.5 mmol, 10 fold excess dissolved in 24 ml 2:1 DMF/CH_2Cl_2) were activated in situ with equimolar amounts of *N*,*N*-diisopropylcarbodiimide (DIC) and 1-hydroxy benzotriazole (HOBt). Pictet–Spengler reactions were carried out by transferring the resin to a round bottom flask containing 20 ml 1,2-dichloroethane with 5% acetic acid and gently stirring at 50°C. Reaction progress was monitored by Kaiser test. Further chain assembly was performed in a solid-phase reaction vessel as above. The N-terminus was capped with acetic anhydride in a 1:1 ratio with diisopropylethylamine (100 mM in 24 ml CH_2Cl_2). Removal of the Dmab ester and subsequent γ-lactamization was carried out with 2% hydrazine in 24 ml DMF followed by treatment with 1 equivalent of TFFH in 24 ml DMF. Resin cleavage was carried out with 20–25 mg of peptide resin in 1 mL of 92:2:2:2:2 TFA:2-methyl-5-*tert*-butylthiophenol:CH_3OH:anisole:triisopropylsilane for 2 h. The resin was then filtered off and the TFA solution concentrated to <200 μL on a rotory evaporator. The peptides were precipitated with 15 ml diethyl ether to obtain the final crude compounds.
13. Library synthesis was carried out in a 96 well 'plate' format using the same synthesis procedure as that used for **8** and **9**. Cleavage was carried out as above, except that the TFA solution was concentrated to <200 μL using a Genevac HT-4 evaporator. All products were characterized by analytical HPLC and found to have the correct molecular weight by MALDI-TOF MS within ±1 a.m.u.
14. Selected analytical data for **9** (maj. diast.): observed $(M-NH_3)^+ = 785.45$ and $(M)^+ = 768.45$ a.m.u.; calculated $(M-NH_3)^+ = 785.758$ a.m.u. ^{1}H NMR (DMSO-d_6, 500 MHz, 25°C): δ 0.57 (d, $J = 5.8$ Hz, 3H), 0.66 (d, $J = 6.8$ Hz, 3H), 0.73–0.78 (m, 6H), 0.83–0.89 (m, 6H), 1.10 (sp, $J = 6.8$ Hz, 1H), 1.26–1.32 (m, 2H), 1.33–1.40 (m, 1H), 1.44–1.53 (m, 1H), 1.67–1.76 (m, 1H), 1.94 (s, 3H), 1.99–2.07 (m, 1H), 2.28–2.36 (m, 2H), 2.86 (dd, $J_1 = 7.7$ Hz, $J_2 = 16.3$ Hz, 1H), 3.28–3.39 (m, 1H), 4.08 (q, $J = 7.3$ Hz, 1H), 4.17–4.25 (m, 2H), 4.28 (q, $J = 7.8$ Hz, 1H), 5.1 (d, $J = 6.6$ Hz, 1H), 5.34 (t br, $J = 6.6$ Hz, 1H), 5.81 (d, $J = 9.5$ Hz, 1H), 6.80 (s, 1H), 6.96 (t, $J = 7.2$ Hz, 1H), 7.05 (t, $J = 7.2$ Hz, 1H), 7.20–7.25 (m, 1H), 7.26–7.36 (m, 5H), 7.38 (d, $J = 9.9$ Hz), 8.02–8.09 (m, 2H), 8.30 (d, $J = 9.8$ Hz, 1H), 8.63 (d, $J = 9.3$ Hz, 1H), 8.94 (d, $J = 6.7$ Hz, 1H); gHSQC (multiplicity edited) (DMSO-d_6, 500 MHz, 25°C): 11.6 (CH_3), 15.5 (CH_3), 18.5 (CH_3), 20.2 (CH_3), 22.3 (CH_3), 22.8 (CH_2), 23.0 (CH_3), 23.2 (CH_3), 24.7 (CH), 25.2 (CH_2), 31.3 (CH), 32.5 (CH_2), 37.9 (CH), 41.6 (CH_2), 50.7 (CH), 51.2 (CH), 51.5 (CH), 51.6 (CH), 56.5 (CH), 57.3 (CH), 58.6 (CH), 111.8 (CH), 118.6 (CH), 119.3 (CH), 121.8 (CH), 127.4 (CH), 127.6 (CH), 128.4 (CH).