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Fmoc-protected Tropane-based Amino Acids for Peptide Structure-Function Studies.

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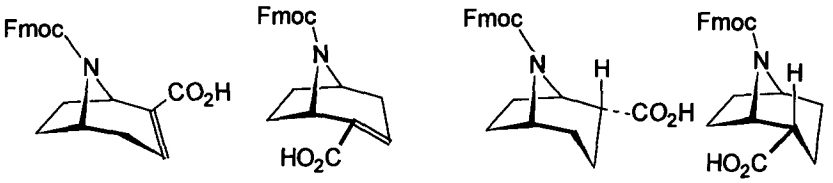
Abstract: Cyclic amino acids derived from the tropane alkaloid nucleus have been prepared and incorporated into synthetic peptides. These conformationally constrained β-amino acids hold considerable potential for use in the development of novel, synthetic analogues of biologically active peptides. © 1997 Elsevier Science Ltd.

The design of novel, readily available amino acids for use in inducing conformational constraint in peptides and pseudopeptides has been identified as an important goal in peptidomimetic research.[1] Elaborate computational strategies leading to the design novel peptidomimetics either *de novo* or by database searching have been reported.[2] Conversely, analysis of libraries of candidates containing commercially available amino acids requires the use of rapid, multiple screening techniques.[3] To date however, only a relatively small number of peptidomimetics which induce specific biologically active conformations have been identified.[4]

We have approached this problem with a desire to utilize the massive natural product literature to generate potential peptidomimetic groups which can be readily and inexpensively synthesized, and which possess simple, rigid chemically inert structures appropriate to solid phase peptide synthesis schemes.[5] To this end, we have focussed upon the tropane alkaloid family, which includes such members as cocaine, scopolamine and atropine. Importantly, the chemistry of these alkaloids has been extensively studied both in the natural product and medicinal chemistry literature.[6]

Here we report on the synthesis of racemic and optically pure Fmoc-protected derivatives of norecgonidine (**1**) and nortropan-2α-carboxylic acid (**2**) and demonstrate the incorporation of these amino

acids into solid phase peptide synthesis protocols. The variation of chirality and unsaturation allows the generation of distinct constrained structural templates from a common precursor which may prove useful in peptide structure activity studies. (Figure 1).



(R)-1 (S)-1 (R)-2 (S)-2

Figure 1

N-Boc-protected tropane amino acids have beeen recently described by Rapoport's group in the synthesis of ferruginine enantiomers, utilizing the iminium ion cyclisation strategy previously applied to anatoxin-a and epibatidine.[7] The synthetic strategy follows that developed by Carroll and co-workers in the development of cocaine analogues and isomers (Scheme 1).[8] In the synthesis of racemic amino acids, the precursor (RS)-2-carbomethoxytropinone (RS)-**3** is obtained either by Willstätter condensation of acetonedicarboxylic acid monomethyl ester, methylamine and succindialdehyde or by condensation of 3-tropinone with dimethylcarbonate.[8,9] Low temperature sodium borohydride reduction of (RS)-**3** yields predominantly the corresponding allopseudoecgonine methyl ester,[9] (RS)-**4**, which in turn undergoes dehydration with phosphorus oxychloride to yield (RS)-methylecgonidine, (RS)-**5**, in 50% yield.[10]

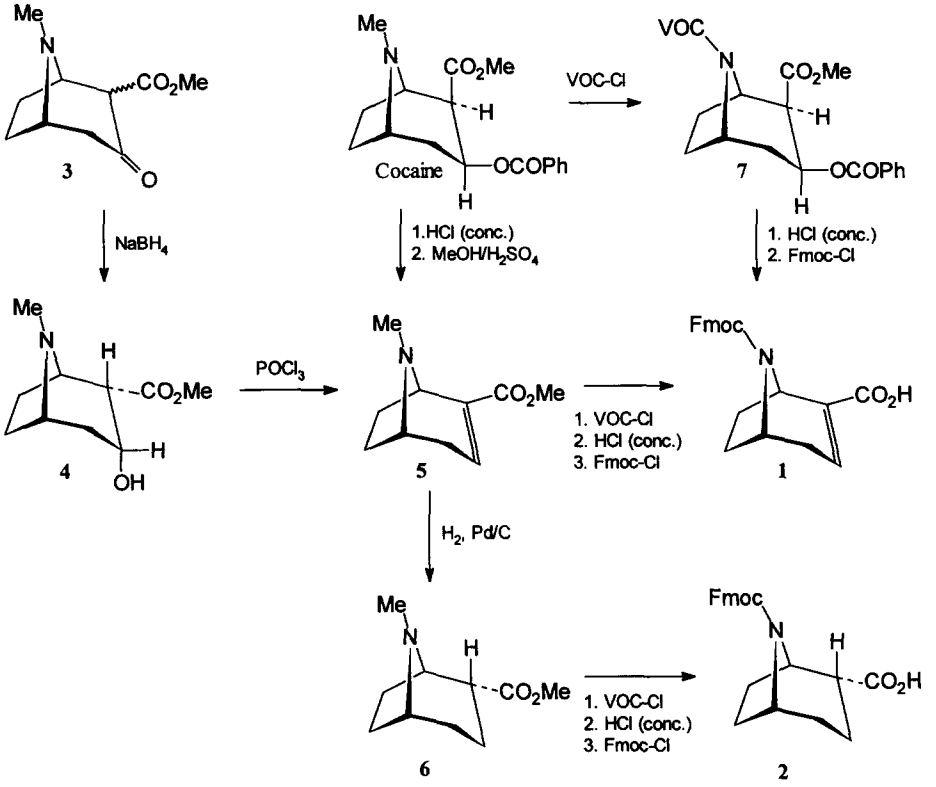
The elaboration of the precursor, (RS)-**5**, to the target Fmoc protected amino acids involves demethylation with vinyl chloroformate followed by hydrolysis of the resulting vinyl carbamate and methyl ester. Amino protection with fluorenylmethylchloroformate yields the unsaturated target, Fmoc-norecginidine [(RS)-Fmoc-Nec, (RS)-**1**] in 44% yield.[11] Hydrogenation of (RS)-**5** yielded the predominantly the α isomer of (RS)-methyl tropane-2-carboxylate, (RS)-**6**.[12] As above, demethylation with vinyl chloroformate and hydrolysis followed by Fmoc-protection as above yielded the pure (RS)-Fmoc-nortropane-2α-carboxylic acid [(RS)-Fmoc-Ntc$^{2\alpha}$, (RS)-**2**] after chromatography.[11]

In order to obtain optically pure materials, resolution of (RS)-2-carbomethoxy-3-tropinone, (RS)-**3** according to Carroll et al,[13] using (+)- and (-)-tartaric acid yields optically pure (R)-2-carbomethoxy-3-tropinone, (R)-**3**, and (S)-2-carbomethoxy-3-tropinone, (S)-**3** respectively, which can be carried through the synthesis as above. Natural (R)-cocaine can be utilized as a precursor to prepare (R)-**1** or (R)-**2**, either by intial hydrolysis/esterification to yield (R)-**5** or by intial demethylation via carbamate (R)-**7**.

In summary, the elaboration of the synthetic strategies pioneered by others in the synthesis of tropane derivatives has been utilized in this investigation for the synthesis of racemic and optically pure Fmoc-amino acids from a single precursor.

These tropane amino acids have been incorporated into synthetic peptides using routine solid phase peptide synthesis protocols without any apparent difficulty. For example, in the synthesis of H-Leu-Ser-Arg-Leu-Phe-**(RS)-Ntc$^{2\alpha}$**-Gly-Ala-$CONH_2$, **8**, a derivative of a human growth hormone fragment, hGH(6-13)[14], coupling of (RS)-**2**, to H-Gly-Ala-Rink Resin using a twofold excess of (**1**) and HBTU was complete within

30 minutes, and subsequent deprotection and coupling of Fmoc-Phe also presented no difficulty.[11] Subsequent syntheses have used the amino acid as the limiting reagent to preserve material but these couplings have still proceeded to near completion.



Scheme 1

Potential application to the synthesis of novel oligotropanes has been demonstrated by the synthesis of trimeric tropanes, (RS)-Ntc$^{2\alpha}$-(RS)-Ntc$^{2\alpha}$-(RS)-Ntc$^{2\alpha}$-$CONH_2$ (**9**), a mixture of eight diastereomers (Figure 2a) , as well as the synthesis of the single compound, (R)-Ntc$^{2\alpha}$-(R)-Ntc$^{2\alpha}$-(R)-Ntc$^{2\alpha}$-$CONH_2$ (Figure 2b).[11] The conformational heterogeneity of these isomers is well demonstrated by RP-HPLC. The potential applications to combinatorial style synthesis using these isomers are also apparent.

We anticipate that the rigidity of these amino acids should significantly influence the conformation of the resultant peptide, and that they may stabilize quite specific conformational motifs. Also, these amino acids can be further elaborated and modified with respect to substitution, chirality, homologation and ring size further extending the applications of these amino acids.

In summary, a new group of Fmoc-protected amino acids based upon the tropane nucleus has been prepared. The semi-rigid nature of these amino acids make them excellent candidates for examining the conformational requirements of peptides in structure activity studies and they appear well suited to applications in peptide combinatorial libraries.

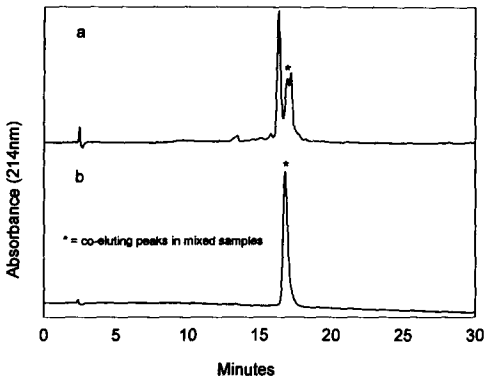


Figure 2

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11. Selected data for compounds **1**, **2**, **8** and **9**.
1: MS (IS) m/z 375.2 (M+H), ^{1}H NMR (300 MHz, $CDCl_3$) δ (rotamers) 1.5-2.3 (m, 5H), 2.4 & 2.9 (br d, 1H) 4.3-4.6 (m, 2H,) 4.15 (t, 1H), 4.91 (d, J = 5.5Hz, 1H), 6.78 & 6.87 (br s, 1H, C=CH), 7.2-7.4 (m, 4H, ArH), 7.56 (d, J = 7.4Hz, 2H, ArH), 7.72 (d, J = 9.5Hz, 2H, ArH)
2: MS (FAB) m/z 378 (M+H) ^{1}H NMR (300 MHz, $CDCl_3$) δ (rotamers) 1.4-2.0 (m, 5H), 2.45 & 2.75 (m, 1H) 4.0-4.3 (m, H), 4.3-4.6 (m, H),7.2-7.5 (m, 4H, ArH), 7.5-7.8 (m, 4H)
8: MS (FAB) m/z 899 (M+H)
9: MS (IS) m/z 430.4 (M+H)
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