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A Facile Synthesis of 3-Substituted Pipecolic Acids, Chimeric Amino Acids

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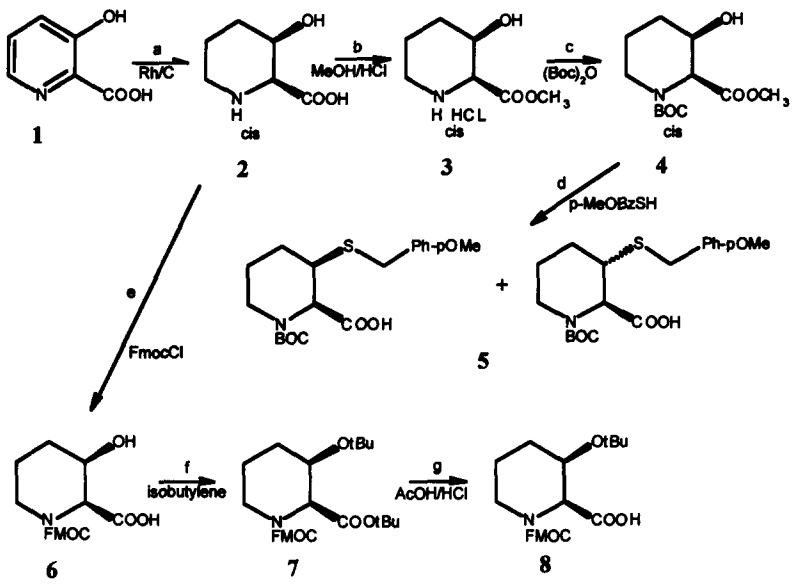
Abstract: Three protected 3-substituted pipecolic acid analogs were prepared as constrained chimeric amino acid building blocks. The concise synthetic route enables the synthesis of other derivatives with sidechain functionality of amino acids as well. © 1997 Elsevier Science Ltd.

Pipecolic acid, the next higher homolog of proline, has generated considerable attention as a proline analog. The compound not only can serve as an analog of proline, but it has utility in the area of constrained amino acids, especially in combination with proline of opposite chirality in the N-terminal position. Substitution of the six-membered ring by any sidechain moiety found in natural amino acids yields a constrained, chimeric amino acid. By holding the sidechain and the backbone in a limited number of conformations, active analogs of biologically active peptides containing this unusual amino acid can provide valuable insights into the conformational requirements of ligand binding.[1] Computational studies carried out in our laboratory indicate that pipecolic acid shows similar, if not a higher, propensity to occupy the i+2 position in reverse turns compared to proline.[2] Thus, incorporation of carefully designed pipecolic acid analogs as building blocks in peptides at appropriate positions can trigger the formation of turns while retaining the sidechain functionality for important molecular recognition.

Several routes have recently been published for the synthesis of pipecolic acid[3] and its derivatives.[4] Preparation of 3-, 4-, 5-, and 6-oxo derivatives has also been reported.[5] However, our search in the literature has revealed no methods for the synthesis of 3-analogs having polar functionality and appropriately protected for solid phase peptide synthesis. Dictated by an ongoing project on the development of constrained amino acids possessing high β-turn propensity, a concise route to 3-carboxy, hydroxy and mercapto pipecolic acid analogs, suitably protected for solid phase synthesis, has been developed.[6] The devised method provides synthetic avenues to other 3-substituted derivatives as well.

The synthesis of the protected 3-hydroxy and 3-mercapto analogs was envisioned starting from the commercially available 3-hydroxypyridinecarboxylic acid (**1**). Reduction of the pyridine ring and esterification of the resultant racemic piperidinecarboxylic acid **2** were carried out as reported.[5a] Protection of the amine in DMF/triethylamine (2 h at 60 °C) provided **4** after column chromatography. The free hydroxyl group was to be masked as a benzylether to give a key intermediate of the desired BocPip(OBz), however, this functionality proved to be extremely labile when treated with bases and gave the 2,3-dehydroanalogs as side products. All attempts to circumvent this undesired elimination were fruitless, thus we sought an alternate route to a



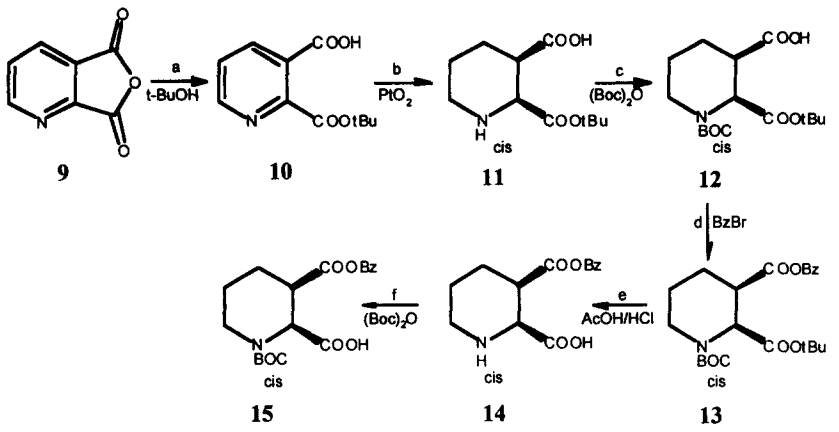
a, Rh/C (5%), NH_4OH, H_2 (50 psi, 24 h); b, MeOH, HCl (60 °C, 12 h); c, $(Boc)_2O$, DMF/TEA (60 °C, 2 h); d, 1, CH_3SO_2Cl/TEA (1h, 0 °C) 2, p-MeOBzSH, DMF/ NaH (r.t., 12 h); e, FmocCl, Na_2CO_3/H_2O (r.t., 5 h); f, cc.H_2SO_4/isobutylene (r.t., 96 h); g, CH_2Cl_2, AcOH/HCl(g) (5 °C, 2 h)

Scheme 1

derivative suitably protected for solid phase synthesis. The same problem prevented application of the well-documented Mitsunobu-procedure for the introduction of the thiol moiety through esterification with thiol acetic acid followed by hydrolysis.[7] Preparation of a triflate intermediate was also unsuccessful due to elimination to 2,3-dehydropipecolic acid (70% yield). Finally, mesylation with methanesulfonyl chloride (1.1 eq.) in chilled chloroform/TEA yielded the desired active ester, which could be converted to **5** in 55% overall yield from **4** (excess p-MeOBzSH, DMF/NaH, overnight at r.t.). The concomitant hydrolysis (thiolates have been shown to cleave esters) was completed by addition of NaOH-solution (2 h at 40 °C, acidification, extraction with ethyl acetate). An approximately 50-50% mixture of cis and trans products was isolated indicating an elimination-addition mechanism instead of Sn_2-substitution, which is consistent with the ease of elimination observed with all of the intermediates throughout these syntheses. The two pairs of diastereomers were conveniently separated by flash chromatography (ethyl acetate:acetic acid=50:1)

An alternate route to **8** involved the use of the Fmoc strategy, which permits the use of acidic conditions. Thus, protection with FmocCl under the standard conditions was followed by t-butylation of both hydroxyls with isobutylene/cc.H_2SO_4 (96 h at r.t. in a pressure bottle). Selective deprotection of the acid was possible by dropwise treatment of a solution of the ester in methylene chloride with acetic acid saturated with HCl gas (2 h at 5 °C, extraction with Na_2CO_3, flash chromatography).

Major goals in the synthesis of the 3-carboxyl analog were to distinguish between the 2- and 3- positions and to achieve stereoselectivity to reduce the number of isomer products. Reduction of pyridine dicarboxylic acids are not entirely selective, giving rise to a small, but troublesome, amount of trans product beside the



a, t-BuOH (75 °C, 36 h); b, PtO_2/EtOH (50 psi, 20 h); c, $(Boc)_2O$, dioxane (100 °C, 1 h); d, DMF/NaH, BzBr (r.t., 15 h); e, AcOH/HCl(g) (15 °C, 1 h); f, $(Boc)_2O/Na_2CO_3$ (r.t., 4 h)

Scheme2.

major cis piperidinedicarboxylic acids. Thus, we took advantage of the selective ring opening of quinolinic anhydride during alcoholysis to give monoester **10** (36 h at 75 °C), which can be reduced with PtO_2 in ethanol (50 psi, 20 h) to the corresponding cis piperidine derivative **11**.[8] Protection of the amine with $(Boc)_2O$ in dioxane (100 °C for 1h, concentration), was followed by that of the 3-carboxyl group with benzyl bromide (NaH/DMF at r.t. for 15 h, extraction). The acid sensitive groups of **13** were removed in acetic acid presaturated with HCl (1 h at 15 °C) and then the amine was reprotected with $(Boc)_2O$ using the standard method to give the suitably protected **15** in 56% overall yield from **10**. It is noteworthy that only the final compound **15** of the 5-step scheme had to be purified. One can easily envision the easy access of other carboxylic acid derivatives starting from **12** or the mesylate of **4** is likely to serve as an excellent intermediate for the synthesis of other 3-position analogs as well.

The stereochemistry of compounds **8** and **15** was established by ^1H NMR. The 3-proton shows a large axial-axial coupling to the 4-protons and a small coupling to the 2-proton. This could only take place if the 3-H is in an axial and the 2-H is in an equatorial position indicating a cis stereochemistry. The identity of the two isomers of **5** was determined as follows. The 3-proton belonging to the trans isomer, assigned based on COSY and TOCSY spectra, is shifted downfield by 0.76 ppm compared to that of the cis isomer (3.46 vs. 2.70 ppm in $CDCl_3$). Molecular modeling indicates that this is due to the magnetic anisotropy effect of the 2-carboxylic group on the 3-proton in the trans isomer (O-H distance<3 Å). The modeling also reveals that the Boc protected pipecolic acids can exist only in a conformation in which the 2-COOH is axial due to steric interference with the bulky Boc group. The coupling constant between the 2-H and the 3-H is less than 4.5 Hz in both isomers confirming the axial orientation for the 2-carboxylic groups. High field NMR spectra show, however, 3-H as a broad multiplet for the cis isomer with 31 Hz linewidth. This is the result of large coupling constants between the axial 3-H and the axial 4-H overlapped for the two rotational isomers derived from slow rotation of the Boc-carbamate. The band of the same proton for the cis optical isomer is only 14 Hz wide and is a combination of two sharp peaks with very small couplings, suggesting an equatorial 3-proton.

Compounds **5**, **8**, and **15** were obtained as racemic mixtures which can be applied as building blocks in solid phase peptide synthesis[9] without resolution of the enantiomers. Separation of the resultant diastereomeric peptides with reversed-phase HPLC techniques has been well documented.[10] Incorporation of the new constrained amino acids **5**, **8**, and **15** into biologically active peptides, where reverse-turn recognition is suspected, is currently underway in our laboratory and will be reported elsewhere. The use of these compounds in combinatorial synthesis as a probe of conformational preference should prove of considerable value in determining the receptor-bound conformation of peptides.

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