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Synthesis of a New Analog of Proline

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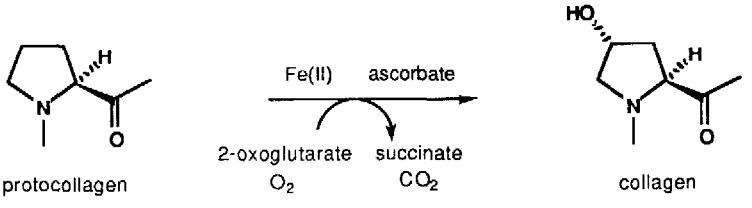
***Summary**: The synthesis of 4-azaspiro[2.4]heptanecarboxylic acid is described. This new amino acid is an analog of proline with potential application as an inhibitor and mechanistic probe of prolyl 4-hydroxylase.*

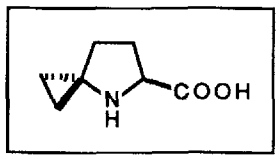
Prolyl 4-hydroxylase catalyzes the rate determining step in the biosynthesis of collagen, an abundant protein found in every tissue tested thus far.[1] The enzyme hydroxylates peptidyl proline residues of protocollagen, in a sequence selective fashion, forming *trans*-4-hydroxyprolyl residues which stabilize the triple helix of collagen via interchain hydrogen bonds.[2] Collagen-like domains are also found in other proteins, for example, the C1q subcomponent of human complement.[3] Prolyl 4-hydroxylase is a non-heme iron dioxygenase requiring 2-oxoglutarate, Fe(II), ascorbate, and molecular oxygen.[4]

Scheme I

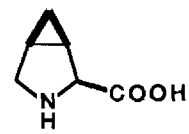


Carbon radical intermediates have been implicated in the mechanism of these catalysts.[5] However, their intermediacy has not been verified and the microscopic rate constants describing the lifetimes of the enzyme-bound transients are unknown. The free radical clock strategy[6] has been used with some success in addressing this problem in other enzymes[7] including the cytochromes P-450.[8] Due to the substrate specificity of prolyl 4-hydroxylase, application of this approach requires installation of a cyclopropane (adjacent to the proposed site of radical generation) on a substrate analog. Thus, amino acid **1** is of interest.

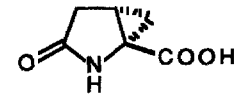
cis-3,4-Methylene-L-proline (**2**), isolated from horse chestnuts, and its *trans* isomer are known[9] A related molecule, racemic 2,3-methylenepyroglutamate (**3**), has recently been prepared by Stammer.[10] In each case the cyclopropane ring is fused to the pyrrolidine. The 4-position, critical to the proposal outlined above, is blocked in **2**. If **3** can be reduced and incorporated into protocollagen-like peptides, it might also serve as a free radical clock probe of prolyl 4-hydroxylase. The present synthesis of 4-azaspiro[2.4]heptanecarboxylic acid (**1**) is described below.



1



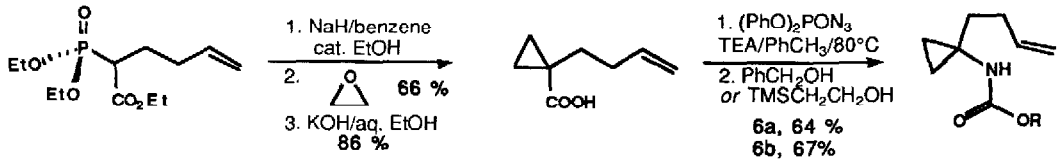
2



3

Phosphonate **4** (available by alkylation of triethyl phosphonoacetate with 4-bromo-1-butene, 84%) is converted to a cyclopropane by the homologous Horner-Emmons[11] procedure using NaH and ethylene oxide. This reaction has proved to be a flexible and efficient route to 1-substituted cyclopropanecarboxylates. Hydrolysis gives **5**, which is subjected to the Shiori modification of the Curtius rearrangement and the isocyanate intermediate is quenched with either benzyl alcohol or 2-(trimethylsilyl)ethanol to give **6a** and **6b**, respectively (yields for R=benzyl unless otherwise noted).

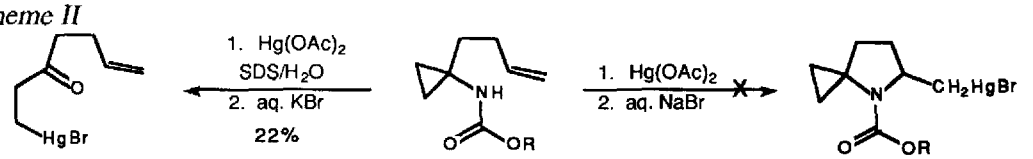
Scheme I



4 **5** **6a** R=CH_2Ph **6b** R=CH_2CH_2TMS

The original plan was to form the pyrrolidine ring at this stage using intramolecular amidomercuration.[12] The resulting alkylmercury **7** would be converted directly to the alcohol with $NaBH_4$ in the presence of molecular O_2.[13] However, the ring closure failed completely, giving a complex mixture of products. The only clean product came from $Hg(OAc)_2$ in aqueous sodium dodecyl sulfate followed by aq. KBr,[14] conditions which afforded alkylmercury **8** in 22% yield. I surmise that the nitrogen of the urethane renders the cyclopropane more nucleophilic than the olefin, leading to preferential rupture of the cyclopropane. Though ring opening of cyclopropanes by Hg(II) has been studied by Collum, Still, and Mohamadi,[15] I know of no prior examples which provide opportunity for direct competition between the two functionalities. NBS (acetone, 0°C) and related reagents effect closure of **6** to a potentially useful (though relatively unstable) 2-(bromomethyl)pyrrolidine, but never in yields >50%.

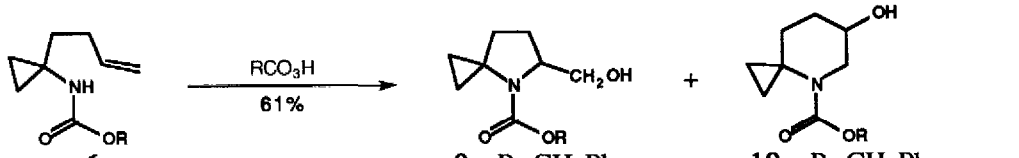
Scheme II



8 **6b** **7** R=CH_2CH_2TMS

A more convenient route involves epoxidation of olefin **6b** with MCBPA under Kishi's conditions,[16] and ensuing formation of 2-(hydroxymethyl)pyrrolidine **9b**, in addition to its isomeric 3-hydroxypiperidine **10b** (54%, 2.8:1 **9b:10b**). These isomers were distinguished by coupled ^{13}C NMR and subsequent oxidation of **9b** to acid **11b** and **10b** to the corresponding ketone. *CF_3CO_3H (61%, one isomer) was also effective. The epoxide could* be isolated by using added buffer (Na_2HPO_4) or with 3,5-dinitroperoxybenzoic acid.[17] Olefin **6** is unaffected by MMPP[18] (30 h at 80°C).

Scheme III



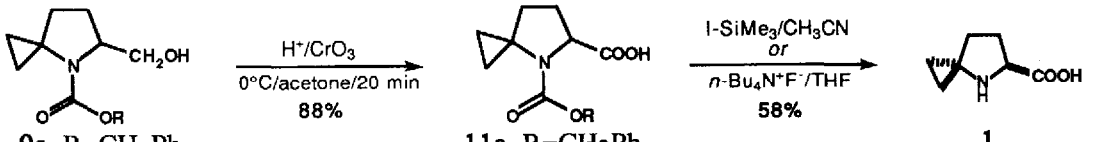
6

9a R=CH_2Ph **10a** R=CH_2Ph

9b R=CH_2CH_2TMS **10b** R=CH_2CH_2TMS

Oxidation of **9** with Jones' reagent in acetone at 0°C for 20 min gives acid **11** (88%), which is ready for assembly of the necessary (Pro-1-Gly)$_n$ peptidyl substrate for testing the enzyme (the enzyme does not act on free proline).[1] Interestingly, the rotational equilibrium (determined by ^{13}C NMR, 125 MHz, 0°C and 2D ^1H COSY in $CDCl_3$) of the Cbz group (2:1) of **11a** is shifted slightly from that exhibited by Cbz-proline under the same conditions (1:1). This suggests that with more sterically demanding acyl groups attached to this proline analog (e.g., proline), there may be a substantial shift in the equilibrium toward the *cis*-proline rotamer.[19] Thus, **1** may produce new folding patterns when incorporated into polypeptides. We intend to pursue this question by studying the coformational behavior of Pro-1-Gly; this is also of immediate importance because prolyl 4-hydroxylase is selective in its binding of substrates which assume protocollagen-like conformations.[20]

Scheme IV



9a R=CH_2Ph **11a** R=CH_2Ph **1**

9b R=CH_2CH_2TMS **11b** R=CH_2CH_2TMS

Deprotection of **11** (*n*-$Bu_4N^+F^-$ in the case of **11b**, iodotrimethylsilane with **11a**) and chromatography through Rexyn 101 (eluted with H_2O then 10% aq. NH_4OH) leads to amino acid **1**: ^1H NMR (500 MHz, D_2O, relative to TSP): δ 4.25 (1H, dd, J=7.2, 8.6 Hz), 2.51 (1H, m), 2.21 (1H, m), 2.04 (2H, m), 1.22 (1H, m), 1.13 (1H, m), 0.92 (2H, m) ppm; ^{13}C NMR (125 MHz, D_2O, dioxan reference = 67.3 ppm): δ 175.6 (s), 62.8 (d, J=148.6 Hz), 43.73 (s), 31.61 (t, J=137.1 Hz), 29.39 (t, J=138.0 Hz), 10.20 (t, J=163.7 Hz), 9.87 (t, J=163.8 Hz) ppm; IR (KBr): ν = 3112 (broad), 1638, 1400, 1320, 1090 cm^{-1}; MS (70 eV) *m/z* = 141 (6%, M^+),

96 (100%, M^+-COOH); exact mass calc. for $C_7H_{11}NO_2$ = 141.0789, observed for **1** = 141.0790.[21]

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21. All compounds gave satisfactory ^1H and ^{13}C (coupled) NMR, IR, and mass (low and high resolution) spectra.

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