2-Azabicyclo[2.2.1]heptane-3-carboxylic Acid - A Bicyclic Proline

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Diels-Alder reaction of cyclopentadiene and methyl *N*-carbobenzyloxy-2-iminoacetate generated *in situ* from methyl 2-chloro-*N*-carbobenzyloxyglycinate by triethylamine gave the *N*-carbobenzyloxy unsaturated bicyclic proline ester. This was converted in two steps to 2-azabicylo[2.2.1]heptane-3-carboxylic acid. In contrast to *N*-carbobenzyloxy-L-proline methyl ester, the corresponding bicyclic proline ester was resistant to hydrolysis catalyzed by carboxypeptidase Y.

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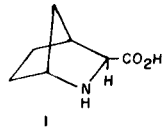
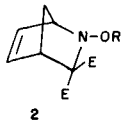
Proline is an interesting amino acid because when incorporated into a peptide it imparts conformational constraints into the molecule by limiting many of the bond angles and fixing the relationship of the side chain carbons (*i.e.*, the carbons of the pyrrolidine ring) to the backbone [1]. However, even in acyl prolines some conformational flexibility remains, and we thought it would be of interest to prepare an even more conformationally constrained bicyclic proline analog such as **1**. Biologically active molecules incorporating **1** rather than proline might have increased potency due to either a better fit to a receptor or a smaller proportion of the molecule being in conformations which do not bind. They might have increased enzymatic stability if for the reverse of the above reasons they did not fit the active site of a degrative enzyme. Finally, they might exhibit increased specificity for a particular receptor [1].



1

The syntheses of bicyclic compounds structurally related to **1** have been approached by the imino Diels-Alder reaction [2]. Imine dienophiles require the presence of strong electron withdrawing groups or must be converted to iminium groups by protonation or alkylation in order to participate in a Diels-Alder reaction.



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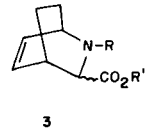

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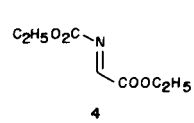

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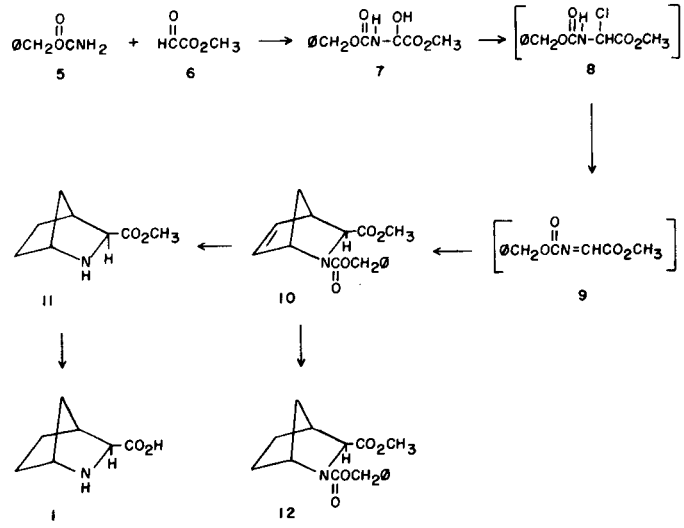
Compounds such as **2** (R = $SO_2C_6H_4CH_3$, SO_2CH_3, COAr; E = CN, CO_2Et, $CONH_2$) were prepared by reacting the isolable isonitroso malonate derivatives with cyclopentadiene [3,4]. Conversion of **2** to the secondary amine is difficult because of facile rearrangement reactions [5], but since the completion of our work this has been achieved to give azaprostaglandin synthons [6,7].

Compounds such as **3** have been prepared by reaction of cyclohexadiene with **4** which was generated *in situ* by treating ethyl *N*-carboethoxy-2-methoxyglycinate with boron trifluoride etherate [8,9]. In our hands attempts to react cyclopentadiene with **4** generated in this way failed due to the boron trifluoride catalyzed polymerization of the cyclopentadiene. This suggested that a similar reaction with **4** generated in the absence of a Lewis acid might give the desired 2-azabicyclo[2.2.1]heptane ring system.

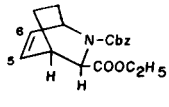


5 **6** **7** **8** **9** **10** **11** **1** **12**

Reaction of benzyl carbamate (**5**) with freshly distilled methyl glyoxalate (**6**) in acetone-ether gave methyl 2-hydroxy-*N*-carbobenzyloxyglycinate (**7**) in 41% yield along with a bis adduct carbomethoxymethylene bis-benzylcarbamate. Treatment of **7** with thionyl chloride and a catalytic amount of pyridine in refluxing methylene chloride gave methyl 2-chloro-*N*-carbobenzyloxyglycinate **8** [10] which was used without purification. Treatment of a mixture of **8** and freshly cracked cyclopentadiene in methylene chloride with triethylamine gave 2-carbobenzyloxy-3-carbomethoxy-2-azabicyclo[2.2.1]hept-5-ene **10** *via* the acylimine **9** in 56% yield.

The stereochemistry of **10** was determined by comparison of its nmr spectrum with that of the known *exo* and *en-*

do isomers **13** and **14** in the corresponding 2-azabicyclo-[2.2.2]cyclohexene series.



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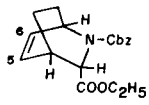

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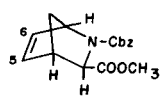

10

In the *exo* isomer **13** the H_5 and H_6 signals are both at δ 6.46. In the *endo* isomer **14** the H_5 and H_6 peaks are δ 6.15 and 6.46, the peak at H_5 being shifted downfield due to deshielding by the ester carbonyl group. In **10**, the H_5 and H_6 peaks are both found at δ 6.40 suggesting that the carbomethoxy group is *exo*. The same preference was observed in acid catalyzed reactions of *E*-imines and dienes. However, under the conditions described in this paper, the imine is not protonated. This might be rationalized by assuming that steric factors make the *E*-form of **9** favored over the *Z*-form, and that an *N*-acyl group on an imino dienophile generally has stronger *endo* directing ability relative to a competing *C*-acyl group [11].

Hydrogenation of **10** over palladium-on-carbon reduced the double bond and removed the amine protecting group to give the desired amino ester **11** in 85% yield. Hydrolysis of **11** with 6*N* hydrochloric acid gave the amino acid **1** in 92% yield.

In an attempt to obtain the enantiomers of **1**, the *N*-carbobenzyloxy derivative **12** was treated with carboxypeptidase Y at *p*H 7 and 8, but no ester hydrolysis occurred. Under these conditions *N*-acetyl-L-tyrosine ethyl ester underwent a rapid hydrolysis and *N*-carbobenzyloxy-L-proline methyl ester hydrolyzed slowly. A similar experiment with α-chymotrypsin failed to hydrolyze **12**. Carboxypeptidase Y was chosen for this attempt because it has been reported to release most amino acid residues, including proline, from the carboxy terminal of peptides [12]. Thus the bicyclic proline ester does have enhanced stability to at least this enzymatic reaction.

After completion of our work an alternative approach to the imino Diels-Alder was described [13] in which the acyl imine **9** was generated by an aza-Wittig between an acyl phosphineimine and methyl glyoxalate. The phosphineimine was generated by reaction of benzyl chloroformate and trimethylsilylazide to give benzyl azidoformate which was then reacted *in situ* with triphenylphosphine.

EXPERIMENTAL

Melting points were uncorrected. Infrared spectra were taken on a Perkin Elmer 683 spectrophotometer. The ^{1}H-nmr spectra were recorded on a Varian EM 390 spectrometer with DSS or TMS as internal standard (δ = 0 ppm). Mass spectra were taken on a Finnigan Model 3600 CC/mass spectrometer. A *p*H-stat Radiometer (SBR2c/ABUlb/TTAB) was used for the enzymatic experiments.

Methyl 2-Hydroxy-*N*-carbobenzyloxy-glycinate (**7**).

Benzyl carbamate [14a] (**5**, 137 g, 0.91 mole) was added to a stirred mixture of freshly distilled methylglyoxylate [14b] (**6**, 96 g, 1.09 mole) and anhydrous ether (1.2 *l*) under a nitrogen atmosphere. The mixture was reduced in volume by distilling 300 ml of ether which was then replaced with 300 ml of dry acetone. The mixture was refluxed for 24 hours, cooled in an ice-bath, and concentrated under reduced pressure to 700 ml. After chilling in an ice-bath, **7** (90 g, 41%), mp 101-103° was isolated as a colorless crystalline solid. The product was recrystallized from toluene for analysis, mp 100-102°; nmr (deuteriochloroform): δ 3.72 (s, CH_3), δ 5.44 (d, CH), δ 5.12 (s, CH_2Ph), δ 7.30 (s, aromatic).

Anal. Calcd. for $C_{11}H_{13}NO_5$: C, 55.23; H, 5.48; N, 5.86. Found: C, 55.28; H, 5.51; N, 5.78.

2-Carbobenzyloxy-3-carbomethoxy-2-azabicyclo[2.2.1]hept-5-ene (**10**).

A mixture of **7** (40.62 g, 0.178 mole), thionyl chloride (13 ml, 0.178 mole), pyridine (0.4 ml) and dry methylene chloride (200 ml) was refluxed for 1 hour, cooled, filtered and evaporated under reduced pressure (water aspirator) then at 0.1 mm Hg to remove traces of thionyl chloride. The resulting chloro intermediate **8** [15] and freshly cracked cyclopentanediene [16] (24.0 g, 0.363 mole) were dissolved in dry methylene chloride (200 ml), stirred, and cooled to 0°. Triethylamine (21.8 g, 0.363 mole) was added slowly (0.5 hour) and the resulting suspension stirred for 18 hours at 25°, then evaporated under reduced pressure. The residue was partitioned in a mixture of 300 ml of 2:1 ether and 10% hydrochloric acid. The organic phase was washed successively with 10% hydrochloric acid (100 ml), water (100 ml), saturated sodium bicarbonate solution (100 ml), and finally with saturated brine (100 ml), dried (sodium sulfate) filtered and evaporated under reduced pressure to yield 40.0 g of crude syrupy product. The crude product was chromatographed on silica gel using petroleum ether (bp 35-60°)-ethyl acetate (2:1) to give purified **10** (28.51 g, 56%) as a syrup, m/e = 287; nmr (deuteriochloroform): δ 4.83 (s, H_1), δ 3.58 (s, H_3), δ 3.33 (s, H_4), δ 6.40 (s, H_5, H_6), δ 2.00 (d, H_{7a}), δ 1.50 (d, H_{7b}), δ 3.70 (s, CH_3), 5.10 (s, CH_2Ph), δ 7.30 (s, aromatic). This product was used in the next reaction without further purification.

3-Carbomethoxy-2-azabicyclo[2.2.1]heptane Hydrochloride (**11**).

A solution of the heptene ester (**10**, 9.08 g, 0.0316 mole) in ethyl acetate (150 ml) and 10% palladium-on-carbon (0.90 g) was hydrogenated on a Parr shaker for 20 hours. The catalyst was removed by filtration, and the filtrate was evaporated under reduced pressure to give 4.79 g (98%) of the viscous free base. The free base was dissolved in ether (100 ml) and made acidic with ethereal hydrogen chloride. The resulting colorless crystalline salt was collected by filtration and washed with ether to give 5.37 g of **11**, 85% yield, mp 163-165°.

Anal. Calcd. for $C_8H_{13}NO_2$·HCl: C, 50.14; H, 7.36; N, 7.31. Found: C, 50.06; H, 7.70; N, 7.29.

2-Azabicyclo[2.2.1]heptane-3-carboxylic Acid (**1**).

A solution of **11** (1.00 g, 0.00522 mole) in 6*N* hydrochloric acid (20 ml) was heated on a steam bath for 18 hours. The mixture was evaporated to dryness under reduced pressure. The last trace of water was azeotroped with ethanol, and then with toluene. The residue was triturated with acetone to give 0.86 g of solid product **1** in 92% yield, mp 185-189°. A sample was recrystallized from 2-propanol-acetone, mp 191-193°.

Anal. Calcd. for $C_7H_{11}NO_2$·HCl: C, 47.33; H, 6.81; N, 7.89. Found: C, 47.59; H, 6.85; N, 8.00.

2-Carbobenzyloxy-3-carbomethoxy-2-azabicyclo[2.2.1]heptane (**12**).

A mixture of **10** (5.27 g, 0.0183 mole) and 0.5 g 10% palladium-on-carbon in ethyl acetate (100 ml) was hydrogenated on a Parr shaker for 15 minutes. The catalyst was removed by filtration and the filtrate was washed with two 25 ml portions of 10% hydrochloric acid, followed by saturated aqueous sodium bicarbonate, water and saturated brine. It was dried (sodium sulfate) and then concentrated under reduced pressure to give **12** (3.21 g, 61%) as a clear yellow liquid; nmr (deuteriochloroform): δ 4.40 (d, H), δ 3.88 (d, H_3), δ 2.71 (s, H_{7a}), δ 1.25-2.10 (m, H_5, H_6, H_{7b}), δ 3.70 (d, CH_3), δ 5.12 (s, CH_2Ph), δ 7.20 (d, aromatic). This product was used in

the next experiment without further purification.

Attempted Enzymatic Resolution of **12** with Carboxypeptidase Y [17a].

A mixture of 2 ml of 12.5 m*M* solution of *N*-acetyl-L-tyrosine ethyl ester [18a] in water and 0.2 ml of 1.25 *M* potassium chloride was equilibrated at 25° for 5 minutes under a nitrogen atmosphere and the *p*H then adjusted to 8.0 with 0.01 *N* sodium hydroxide. To initiate the reaction 200 $\mu\ell$ of a solution of 25 μg/ml of carboxypeptidase Y [18b] in water was added, and the *p*H kept at 8.0 by titration with 0.01 *N* sodium hydroxide using a *p*H stat. After addition of enzyme a rapid consumption of titrant indicated that as expected under these conditions high enzymatic activity was present. A similar experiment using a 100 m*M* solution in 1:9 methanol water of *N*-carbobenzyloxy-L-proline methyl ester [19] at *p*H 7 or at *p*H 8 gave a slow consumption of titrant indicating that carboxypeptidase Y could use this proline ester as a substrate, but that the proline ester was a poorer substrate than the tyrosine ester. When **12** was used as a substrate under identical conditions (*p*H 7 or 8), no consumption of titrant was observed indicating that the bicyclic proline ester was not a substrate for this enzyme.

Attempted Enzymatic Resolution of **12** with α-Chymotripsin [17b].

A similar set of experiments was carried out using α-chymotrypsin [18b]. With this enzyme at *p*H 7.85 a rapid hydrolysis of *N*-acetyl-L-tyrosine ethyl ester occurred, but no hydrolysis of either *N*-carbobenzyloxy-L-proline methyl ester or **12** was observed.

REFERENCES AND NOTES

[1a] V. J. Hruby, *Life Sci.*, **31**, 189 (1982); [b] G. E. Schulz and R. H. Schriner, "Principles of Protein Structure", Springer-Verlag, New York, 1979, p 11.

[2] For an excellent review see S. M. Weinreb and J. I. Levin, *Heterocycles*, **12**, 949 (1979).

[3] J.-M. Biehler and J.-P. Fleury, *J. Heterocyclic Chem.*, **8**, 431 (1971).

[4] J.-M. Biehler, J. Perchois and J.-P. Fleury, *Bull. Soc. Chim. France*, 2711 (1971).

[5] J.-M. Biehler and J.-P. Fleury, *Tetrahedron*, **27**, 3171 (1971).

[6] A. Basco, S. Bennetti, P. G. Baraldi, F. Moroder, G. P. Pollini and D. Simoni, *Ann. Chem.*, 960 (1982).

[7] C. Morin and D. Blondet, *Tetrahedron Letters*, 3681 (1982).

[8] G. R. Krow, C. Johnson, and M. Boyle, *Tetrahedron Letters*, 1971 (1978).

[9] A. J. G. Baxter and A. B. Holmes, *J. Chem. Soc., Perkin Trans. I*, 2343 (1977).

[10] Z. Bernstein and D. Ben-Ishai, *Tetrahedron*, **33**, 881 (1977).

[11] B. Nader, T. R. Bailey, R. W. Franck and S. M. Weinreb, *J. Am. Chem. Soc.*, **103**, 7573 (1981).

[12] R. Hayashi, Y. Bai and T. Hata, *J. Biochem.*, **77**, 69 (1975).

[13] M. E. Jung, K. Shishido, L. Light and L. Davis, *Tetrahedron Letters*, 4607 (1981).

[14a] Compound **5** was purchased from Aldrich Chemical Co; [b] Compound **6** was obtained from American Hoechst Corp. It was also prepared by the procedure of T. R. Kelley, T. E. Schmidt and J. G. Haggerty, *Synthesis*, 544 (1972).

[15] The method of H. Ulrich, B. Tucker and A. A. R. Sayigh, *J. Org. Chem.*, **33**, 2887 (1968), was adopted for the synthesis of **8**.

[16] R. B. Moffett, "Organic Syntheses", Coll Vol IV, John Wiley and Sons, Inc, New York, NY, 1963, p 238.

[17a] R. Hayashi in "Methods of Enzymology", Vol 45, L. Lorond, ed, Academic Press, Inc, New York, NY, 1976, p 570; [b] M. Laskoski, *ibid.*, Vol 2, S. P. Colowick and N. O. Kaplan, eds, Academic Press, Inc, New York, NY, 1955, pp 23 and 36.

[18a] Purchased from Aldrich Chemical Co; [b] Purchased from Sigma Chemical Co.

[19] Prepared by the usual procedure from L-proline methylester (purchased from Vega Biochemicals).