Tetrahedron Vol. 49, No. 29, pp 6375-6380, 1993
Printed in Great Britain

0040-4020/93 $6.00+.00

First Asymmetric Synthesis of Carnosadine[1]

David J. Aitken, Dominique Guillaume and Henri-Philippe Husson*

Laboratoire de Chimie Thérapeutique, URA 1310 du CNRS, Faculté des Sciences Pharmaceutiques et Biologiques, 4 Avenue de l'Observatoire, 75270 Paris cédex 06, France.

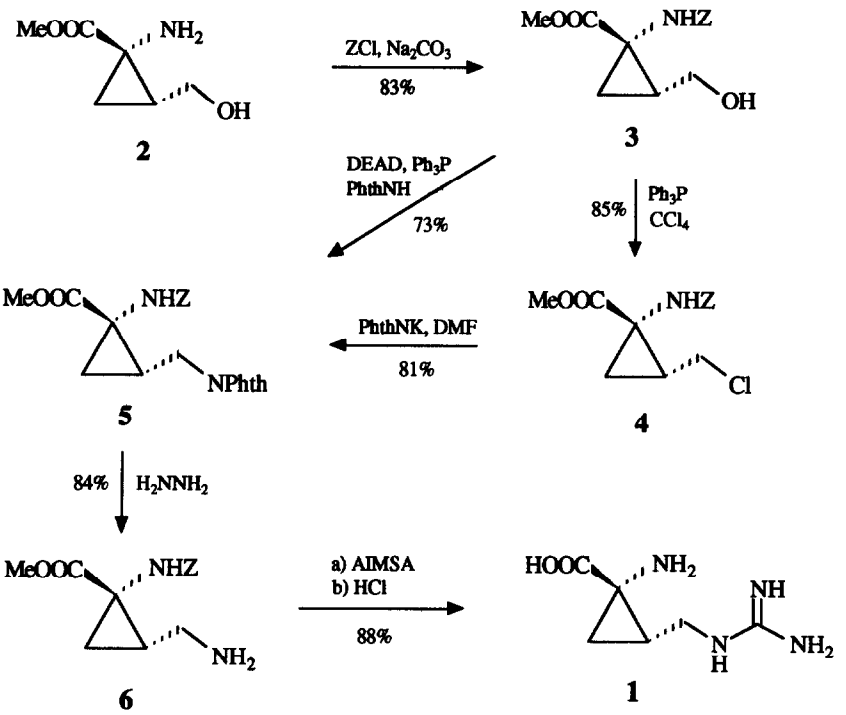
(*Received in Belgium* 19 *November* 1992; *accepted* 8 *April* 1993)

Abstract: The first asymmetric synthesis of the naturally-occurring cyclopropane amino acid carnosadine, **1**, has been carried out. Starting from the previously available chiral intermediate **2**, appropriate amine protection and side-chain modifications permitted introduction of the key guanidyl substituent in a short and efficient synthesis to give the title compound in five steps and 45% overall yield.

Carnosadine **1** was first isolated in 1984 from the red marine alga *Grataloupia carnosa* by Shiba and coworkers,[2] who deduced its structure as 1-amino-2-guanidinomethyl-1-cyclopropanecarboxylic acid.[3] Shortly after,[4] the same research group described a 14-step synthesis of carnosadine starting from benzoylglycine, with an overall yield of 5%. The strategy incorporated a resolution procedure which allowed assignment of the absolute configuration of the natural product as 1*S*, 2*S*.

As a derivative of 1-amino-1-cyclopropanecarboxylic acid (ACC), carnosadine is one of the few naturally-occurring members[5] of this novel class of α-amino acids which have attracted special attention recently on account of their fascinating biological activities.[6,7] Substituted ACCs present a challenge to synthetic chemists due to the requirement for control of stereochemistry at the substituted cyclopropane ring. A variety of different synthetic approaches have been explored,[6] but only a small number have been adapted for asymmetric synthesis.[8] Shiba's preparation[4] of carnosadine remains the only one to appear in the literature to date.

Recently we described an asymmetric synthesis of both *cis*- and *trans*-2-hydroxymethyl-ACC (2,3-methanohomoserine) in optically pure form *via* the CN(*R*,*S*) method, in which the key step was formation of the appropriately functionalized cyclopropane by double alkylation of a chiral aminonitrile synthon with epibromohydrin.[9] This synthetic methodology has been adapted for an even simpler synthesis of the *cis* and *trans* racemates of the same amino acid.[10] One of the intermediates in the asymmetric synthesis was the methyl ester **2**, which can be prepared on gram-scale. The facile chemical transformation of the primary hydroxyl group into a variety of other functional groups led us to suggest that **2** could provide an entry to other 2-substituted ACCs in stereochemically pure form. In this paper we describe the practical and efficient preparation of (1*S*,2*S*)-carnosadine from chiral ester **2** in 5 steps and 45% overall yield. This straightforward synthesis (Scheme) involves protection of the amine function, appropriate side chain interconversion, and finally deprotection.



Scheme

The primary amine of **2** was protected as a carbamate by reaction with benzyl chloroformate in aqueous sodium carbonate solution without hydrolysis of the ester, to give derivative **3** in 83% yield. Side chain manipulation was then undertaken by refluxing **3** with triphenylphosphine in carbon tetrachloride in an inert atmosphere for 72 hours which gave a high conversion (85%) into the chloride **4**. The remaining unreacted alcohol was recovered by chromatography. Direct nucleophilic displacement of halide by guanidine represented the most direct synthetic route, and there was some literature precedent for this type of transformation.[11] However, treatment of a methanolic solution of **4** with freshly prepared aqueous guanidine solution resulted only in hydrolysis of the ester. We therefore followed a more conventional strategy for construction of the guanidyl function, *via* the corresponding amine.[12]

The side-chain nitrogen was introduced either by reaction of **3** with phthalimide in a Mitsunobu procedure[13] or by heating chloride **4** with potassium phthalimide in DMF. These reactions furnished phthalimido derivative **5** in 73% and 81% yield, respectively. While the Mitsunobu reaction had the advantage of producing **5** directly, care was required to avoid contamination by the diethyl hydrazinedicarboxylate by-product, and the yield was only marginally better than that for the two-step procedure *via* chloride **4** which involved clean reactions. Selective deprotection of the side-chain nitrogen was achieved by hydrazinolysis to give amine **6**. Purification of this compound was initially hampered by the protecting groups' sensitivity to acid and base, but it was found that formation of the hydroacetate salt permitted separation from the phthalhydrazine by-product, and by subsequent basification and reextraction

into organic solution the amine was obtained in 84% yield. Indeed, the free base form of **6** was necessary for efficient grafting of the guanidyl group in the next step.

We investigated several guanidylating agents, including 3,5-dimethylpyrazole-1-carboxamidine nitrate[14] and *S*-methylisothiourea sulfate,[15] but best results were obtained with aminoiminomethanesulfonic acid (AIMSA), which has recently been introduced by several research groups.[16] When the hydroacetate of **6** was treated with AIMSA in the presence of aqueous alkali, the starting material was recovered unreacted, even after prolonged reaction times. However by stirring the free amine **6** with an equimolar amount of AIMSA in methanol under mild and neutral conditions, guanidine formation was complete after 3 hours. It was essential to ensure that no amine **6** remained in the mixture, since the final deprotection step was carried out without isolation of the guanidylated intermediate. ^{13}C NMR spectroscopy in CD_3OD was diagnostic in this respect, for the conversion of amine into guanidine resulted not only in the appearance of a new signal at 158.3 ppm but also in an upfield shift of the cyclopropane C-2 from 31.3 to 27.4 ppm. All other carbon resonances, including that of C-4 which bears the nitrogen function, remained virtually unchanged. The guanidyl derivative was deprotected by refluxing in strong acid, and final purification was carried out by elution from a conventional ion-exchange column, to give carnosadine ($[\alpha]_D$ –20.0° as the dihydrochloride) in 88% yield for the last two steps.[17] Enantiomeric purity was verified by analysis on a chiral hplc column and was found to be >99%.

Since the starting ester **2** possesses a 1*S*,2*R* absolute configuration, and none of the chemical transformations carried out involve the chiral centers of any intermediates, a 1*S*,2*S* configuration of the carnosadine prepared by this method is guaranteed. Samples thus obtained exhibited identical spectroscopic data to those published[2,18] for natural carnosadine dihydrochloride and had the same optical rotation (lit.[4] $[\alpha]_D$ –20.0°), confirming stereochemical assignment. We are currently investigating the application of stereochemically pure 2-hydroxymethyl-ACC derivatives to the asymmetric synthesis of other cyclopropane amino acids.

EXPERIMENTAL SECTION

General. ^1H and ^{13}C NMR spectra were recorded at 300 MHz and 75 MHz respectively. FTIR spectra were recorded in chloroform solution. Analytical TLC was performed on silica gel F-254 plates and components visualized using ethanolic phosphomolybdic acid solution. Flash chromatography was carried out on 230-400 mesh silica gel using 50:50 hexane-EtOAc as eluent. R_f values are given for the same solvent system unless otherwise stated. Compound **2**[9] and AIMSA[16c] were obtained as previously reported. Triphenylphosphine was recrystallized from hexane, phthalimide was recrystallized from EtOH and potassium phthalimide was purified as previously described.[19] Carbon tetrachloride, THF and DMF were dried and redistilled under nitrogen before use. All other reagents and solvents were used directly as supplied commercially.

(1*S*,2*R*)-1-Benzyloxycarbonylamino-2-hydroxymethyl-1-cyclopropanecarboxylic acid methyl ester, 3 Benzyl chloroformate (0.95 mL, 6.65 mmol) was added dropwise to a vigorously stirred suspension of **2** (0.75 g, 5.17 mmol) in 1 M aqueous Na_2CO_3 solution (12 mL) at 0 °C. The mixture was stirred continually at 0 °C for 4 h then extracted with EtOAc (3 x 15 mL). Combined extracts were dried over $MgSO_4$ and

evaporated to leave an orange oil. Flash chromatography gave the title compound (1.20 g, 83%) as a clear oil: R_f 0.31; $[\alpha]^{23}_D$ –43.7° (*c* 1.00, $CHCl_3$); FTIR 3425 br, 1728, 1602 m cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.89 (dd, 1 H, *J* = 5.1 and 7.4 Hz), 1.68 (dd, 1 H, *J* = 5.1 and 9.9 Hz), 2.47 (m, 1 H), 2.85 (br s, 1 H), 3.25 (t, 1 H, *J* = 11.8 Hz), 3.73 (s, 3 H), 4.05 (dd, 1 H, *J* = 12.1 and 3.4 Hz), 5.23 (t, 2 H, coalesced *AB* system, apparent *J* = 12.0 Hz), 5.53 (s, 1 H), 7.42 (s, 5 H); ^{13}C NMR ($CDCl_3$) δ 19.8 (t), 31.0 (d), 38.7 (s), 52.6 (q), 61.4 (t), 67.5 (t), 128.1, 128.3, 128.6 (each d), 136.2, 158.4, 172.6 (each s); Anal. Calcd for $C_{14}H_{17}NO_5$: C, 60.21; H, 6.14; N, 5.02. Found: C, 60.27; H, 6.39; N, 4.84.

(1*S*,2*R*)-1-Benzyloxycarbonylamino-2-chloromethyl-1-cyclopropanecarboxylic acid methyl ester, 4 A mixture of **3** (1.20 g, 4.30 mmol), triphenylphosphine (1.35 g, 5.15 mmol) and carbon tetrachloride (150 mL) was heated under reflux under N_2 for 72 h, then the solvent was evaporated. The residual solids were subjected to flash chromatography to give unreacted **3** (0.16 g, 13%) and the title compound (1.09 g, 85%) as a clear oil which crystallized on standing: R_f 0.65; mp 77-80 °C; $[\alpha]^{23}_D$ +37.8° (*c* 1.00, $CHCl_3$); FTIR 3418, 1724, 1602 m cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.26 (m, 1 H), 1.92 (dd, 1 H, *J* = 7.0 and 8.5 Hz), 2.21 (m, 1 H), 3.47 (t, 1 H, *J* = 10.9 Hz), 3.71 (s, 3 H), 3.80 (dd, 1 H, *J* = 6.1 and 11.4 Hz), 5.17 (2d, 2 H, partially resolved *AB* system, apparent *J* = 12.0 Hz), 5.95 (br s, 1 H), 7.37 (s, 5 H); ^{13}C NMR ($CDCl_3$) δ 23.6 (t), 29.7 (d), 40.0 (s), 44.1 (t), 52.9 (q), 67.3 (t), 128.2, 128.4, 128.6 (each d), 136.1, 157.1, 172.3 (each s); Anal. Calcd for $C_{14}H_{16}ClNO_4$: C, 56.48; H, 5.42; N, 4.70; Cl, 11.91. Found: C, 56.42; H, 5.34; N, 4.48; Cl, 11.84.

(1*S*,2*S*)-1-Benzyloxycarbonylamino-2-(*N*-phthalimido)methyl-1-cyclopropanecarboxylic acid methyl ester, 5 *METHOD A:* Potassium phthalimide (0.70 g, 3.78 mmol) was added to a solution of **4** (1.03 g, 3.46 mmol) in DMF (5 mL) under N_2, and the mixture was heated at 100 °C for 3 h. After cooling, chloroform (15 mL) was added and the mixture poured into water (30 mL). The organic layer was collected and the aqueous phase washed with more chloroform (2 x 10 mL). Combined extracts were dried over $MgSO_4$ and evaporated to leave a brown oil. Flash chromatography gave the title compound (1.15 g, 81%) as a viscous oil which crystallized on standing: R_f 0.50; mp 152-155 °C; $[\alpha]^{23}_D$ +78.6° (*c* 0.79, $CHCl_3$); FTIR 3359 m, 1772 m, 1714, 1600 m, cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.19 (dd, 1 H, *J* = 4.8 and 6.1 Hz), 1.85-1.95 (m, 2 H), 3.46 (dd, 1 H, *J* = 9.3 and 14.6 Hz), 3.66 (s, 3 H), 4.24 (dd, 1 H, *J* = 2.6 and 14.6 Hz), 5.20 (2d, 2 H, partially resolved *AB* system, apparent *J* = 12.3 Hz), 6.84 (br s, 1 H), 7.33-7.45 (m, 5 H), 7.75 (dd, 2 H, *J* = 3.1 and 5.4 Hz), 7.87 (dd, 2 H, *J* = 3.1 and 5.4 Hz); ^{13}C NMR ($CDCl_3$) δ 21.0 (t), 27.2 (d), 36.4 (t), 38.4 (s), 52.5 (q), 66.9 (t), 123.5, 128.0, 128.5 (each d), 132.0 (s), 134.3 (d), 136.6, 157.2, 168.4, 172.5 (each s). Anal. Calcd for $C_{22}H_{20}N_2O_6$: C, 64.70; H, 4.94; N, 6.86. Found C, 64.58; H, 4.80; N, 6.79.

METHOD B: A solution of **3** (1.50 g, 5.37 mmol), triphenylphosphine (1.41 g, 5.38 mmol) and phthalimide 0.79 g, 5.37 mmol) in THF (15 mL) under N_2 was treated dropwise with diethyl azodicarboxylate (0.85 mL, 5.40 mmol). A slight exotherm ensued, and the mixture was stirred at rt overnight, then evaporated *in vacuo* to leave an orange oil. Flash chromatography gave **5** contaminated by an approximately equimolar quantity of diethyl hydrazinedicarboxylate, as estimated from the ^1H NMR spectrum. Further flash chromatography using 5:95 EtOAc-CH_2Cl_2 as eluent gave the pure title compound (1.60 g, 73%) exhibiting physicochemical data identical with those of the product from Method A.

(1*S*,2*S*)-2-Aminomethyl-1-benzyloxycarbonylamino-1-cyclopropanecarboxylic acid methyl ester, 6
Hydrazine hydrate (0.35 mL, 6.99 mmol) was added to a solution of **5** (1.00 g, 2.45 mmol) in MeOH (7.5 mL) and chloroform (7.5 mL) and the mixture stirred at rt for 24 h. A solution of acetic acid (4 mL) in MeOH (12 mL) was then added, the mixture was stirred for 5 min, then set aside at -20 °C for 3 h. The white solids were removed by filtration and the filtrate evaporated. A 20% solution of acetic acid in water (40 mL) was added to the residue, the resulting solids were removed by filtration and the filtrate was evaporated to leave the hydroacetate salt as a pale yellow syrup which could not be crystallized. 1 M aqueous Na_2CO_3 solution (10 mL) was added and the resulting suspension extracted with EtOAc (4 x 25 mL). Combined extracts were dried over $MgSO_4$ and evaporated to give the title compound (0.57 g, 84%) as a pale yellow oil: R_f (90:8:2 EtOAc:MeOH:NH_4OH) 0.34; $[\alpha]^{27}_D$ –29.3° (*c* 1.17, MeOH); FTIR 3428 m, 3383 w, 3308 w, 1726, 1600 m cm^{-1}; ^1H NMR (CD_3OD) δ 1.02 (dd, 1 H, *J* = 5.1 and 7.4 Hz), 1.63 (dd, 1 H, *J* = 5.1 and 9.5 Hz), 2.06 (m, 1 H), 2.59 (dd, 1 H, *J* = 13.6 and 8.9 Hz), 2.92 (dd, 1 H, *J* = 13.6 and 4.7 Hz), 3.75 (s, 3 H), 5.21 (s, 2 H), 7.42 (s, 5 H); ^{13}C NMR (CD_3OD) δ 21.2 (t), 31.3 (d), 39.3 (s), 41.4 (t), 53.0 (q), 67.6 (t), 128.6, 129.0, 129.4 (each d), 138.0, 159.7, 174.5 (each s).

(1*S*,2*S*)-Carnosadine, 1
A solution of **6** (0.55 g, 1.98 mmol) in MeOH (5 mL) was treated with AIMSA (0.25 g, 2.01 mmol) and the mixture was stirred at rt for 3 h then evaporated. The residual syrup displayed the following ^{13}C NMR data: (CD_3OD) δ 21.2 (t), 27.4 (d), 39.3 (s), 41.3 (t), 53.2 (q), 67.8 (t), 128.6, 129.0, 129.4 (each d), 137.8, 158.3, 159.7, 174.0 (each s). The syrup was treated with 5 M HCl (25 mL) and heated under reflux for 5 h then evaporated to dryness. The residue was applied to a column of Dowex 50X8-100 ion exchange resin (20 mm x 10 cm) and eluted with 1 M NH_4OH. Sakaguchi-positive[20] fractions were combined and evaporated to give carnosadine (0.30 g, 88%) as an off-white solid[17] which was converted to its dihydrochloride; $[\alpha]^{27}_D$ –20.0° (*c* 1.85, 1 M HCl). FAB-MS (glycerol matrix), ^1H and ^{13}C NMR spectra were identical with published data.[2,18] The dihydrochloride was twice precipitated from EtOH solution by slow addition of chloroform, to give an amorphous white powder: Anal. Calcd for $C_6H_{12}N_4O_2 \cdot 2HCl$: C, 29.40; H, 5.76; N, 22.86. Found: C, 29.33; H, 5.42; N, 22.61. Hplc analysis on a CHIRALPAK WM column (mobile phase: 0.25 mM aqueous $CuSO_4$) showed only one enantiomer present; racemic material displayed two peaks with complete base-line resolution.

Acknowledgments. We are indebted to Dr J. Kalbfleisch and the J. T. Baker Chiral Separation Service, Deventer, The Netherlands, for the chiral hplc analysis, and we thank Dr F. Libot for recording the FAB mass spectrum. This work was supported by SERC-NATO and CNRS.

REFERENCES AND NOTES

1. Asymmetric Synthesis by the CN(*R,S*) Method; Part 27. For Part 26, see: Yue, C.; Royer, J.; Husson, H.-P. *J. Org. Chem.* **1992**, *57*, 4211.

2. Wakamiya, T.; Nakamoto, H.; Shiba, T. *Tetrahedron Lett.* **1984**, *25*, 4411.

3. In this paper we have retained the cyclopropane amino acid nomenclature which considers compounds like carnosadine as derivatives of 1-amino-1-cyclopropanecarboxylic acid (ACC) bearing a C-2 substituent. An alternative approach is to regard such compounds as 2,3-methano derivatives

of α-amino acids, but this system is convenient only when a common (*e.g.* proteinogenic) α-amino acid residue is present, and results in a different cyclopropane ring numbering system.

4. Wakamiya, T.; Oda, Y.; Fujita, H.; Shiba, T. *Tetrahedron Lett.* **1986**, *27*, 2143.

5. Only three other naturally-occurring ACCs have been discovered so far; (a) ACC itself: Burroughs, L.F. *Nature* **1957**, *179*, 360. Vahatolo, M.L.; Virtanen, A.I. *Acta Chem. Scand.* **1957**, *11*, 741. (b) Coronamic acid: Ichihara, A.; Shiraishi, K.; Sato, H.; Sakamura, S.; Nishiyama, K.; Sakai, R.; Furusaki, A.; Matsumoto, T. *J. Am. Chem. Soc.* **1977**, *99*, 636. (c) Norcoronamic acid: Mitchell, R.E.*Phytochemistry* **1985**, *24*, 1485. In addition, *N*-methyl norcoronamic acid has been found in a *Streptomyces* antibiotic: Rance, M.J.; Ruddock, J.C.; Pacey, M.S.; Cullen, W.P.; Huang, L.H.; Jefferson, M.T.; Whipple, E.B.; Maeda, H.; Tone, J. *J. Antibiot.* **1989**, *42*, 206. Like carnosadine, the other natural ACC derivatives have well-defined stereochemical configurations.

6. Reviews: (a) Stammer, C.H. *Tetrahedron* **1990**, *46*, 2231. (b) Alami, A.; Calmes, M.; Daunis, J.; Jacquier, R. *Bull. Soc. Chim. Fr.* **1993**, *130*, 5.

7. For leading references, see: (a) Kemp, A.; Tedford, C.M.; Suckling, C.J. *Bioorg. Med. Chem. Lett.* **1991**, *1*, 557. (b) Slama, J.T.; Satsangi, R.K.; Simmons, A.; Lynch, V.; Bolger, R.E.; Suttie, J. *J. Med. Chem.* **1990**, *33*, 824. (c) Zhu, Y.-F.; Yamazaki, T.; Tsang, J.W.; Lok, S.; Goodman, M. *J. Org. Chem.* **1992**, *57*, 1074. (d) Ahmad, S.; Phillips, R.S.; Stammer, C.H. *J. Med. Chem.* **1992**, *35*, 1410. (e) Matsui, S.; Srivastava, V.P.; Holt, E.M.; Taylor, E.W.; Stammer, C.H. *Int. J. Peptide Protein Res.* **1991**, *37*, 306. (f) Mapelli, C.; Elrod, L.F.; Switzer, F.L.; Stammer, C.H.; Holt, E.M. *Biopolymers* **1989**, *28*, 123. (g) Pirrung, M.C.; Dunlap, S.E.; Trinks, U.P. *Helv. Chim. Acta* **1989**, *72*, 1301.

8. For a recent paper dealing with asymmetric synthesis of ACCs which provides detailed leading references, see: Williams, R.M.; Fegley, G.J. *J. Am. Chem. Soc.* **1991**, *113*, 8796.

9. Aitken, D.J.; Royer, J.; Husson, H.-P. *J. Org. Chem.* **1990**, *55*, 2814.

10. Guillaume, D.; Aitken, D.J.; Husson, H.-P. *Synlett* **1991**, 747.

11. Hebrand, P.; Olomucki, M. *Bull. Soc. Chim. Fr.* **1970**, 1938, and references therein. See also: Monro, A.M. *Chem. Ind.* **1964**, 1806.

12. Yamamoto, Y.; Kojima, S. In *The Chemistry of Amidines and Imidates*; Patai, S., Rappoport, Z., Eds.; Wiley: New York, 1991; Vol. 2; pp 485-526.

13. (a) Mitsunobu, O. *Synthesis* **1981**, 1. (b) Wada, M.; Sano, T.; Mitsunobu, O. *Bull. Chem. Soc. Japan* **1973**, *46*, 2833.

14. Bannard, R.A.B.; Casselman, A.A.; Cockburn, W.F.; Brown, G.M. *Can. J. Chem.* **1958**, *36*, 1541.

15. Shildreck, P.R.; Windus, W. *Organic Syntheses*; Wiley: New York, 1943; Vol. 2, p 411.

16. (a) Miller, A.E.; Bischoff, J.J. *Synthesis* **1986**, 777. (b) Maryanoff, C.A.; Stanzione, R.C.; Plampin, J.M.; Mills, J.E. *J. Org. Chem.* **1986**, *51*, 1882. (c) Kim, K.; Lin, Y.-T.; Mosher, H.S. *Tetrahedron Lett.* **1988**, *29*, 3183.

17. Incomplete guanidylation of **6** leads eventually to the formation of carnosadine contaminated with 2-aminomethyl ACC, and separation of these two basic amino acids is a less trivial affair than simple purification of the title compound.

18. The chemical shift of one of the proton signals was quoted erroneously in reference 2. See erratum: *Tetrahedron Lett.* **1985**, *26*, 2138, and note 2 in reference 4.

19. Bornstein, J.; Drummond, P.E.; Bedell, S.F. *Organic Syntheses*; Wiley: New York, 1963; Vol. 4, p 810, note 2.

20. Acher, R.; Crocker, C. *Biochim. Biophys. Acta* **1952**, *9*, 704.