Dehalogenation of 1a. A mixture of **1a** (0.75 g, 2.65 mmol) and benzoic acid (1.29 g; 4 equiv) was heated (180 °C). To the melt was added copper (1.009 g; 6 equiv) slowly over 3 min with stirring. The mixture was heated for an additional 5 min and allowed to reach room temperature, the solid was digested with saturated aqueous Na_2CO_3, and the mixture was partitioned with CH_2Cl_2. The organic phase was extracted and dried and the solvent was removed in vacuo. The crude product (0.145 g; yellow oil) was purified by column chromatography (alumina, ethyl acetate) to give 3-NP (0.074 g; 22.7%): mp 43-38.5 °C (lit 39 °C);[13] ^{1}H NMR (CD_2Cl_2) 9.36 (d, J = 2.2 Hz), 8.90 (d, J = 4.3 Hz), 8.50 (dd, J = 1.6 and 7.5 Hz), 7.60 (dd, J = 8.4 and 4.7 Hz).

N-Nitration of 4 and 5. To a solution of **4** (0.82 g, 5.24 mmol) in dry CH_2Cl_2 (20 mL) was added $NO_2^+BF_4^-$ (0.827, 6.23 mmol; 1.2 equiv) under nitrogen with efficient magnetic stirring. Upon reflux (24 h), the precipitated **4b** was isolated without washing with benzene (0.466 g; 31%). ^{1}H NMR [CD_3CN, 8.63 (dd, J = 1.5 and 6 Hz), 8.46 (dt, J = 1.5 and 8.0 Hz), 8.20 (d, J = 8 Hz), 8.01 (dt, J = 1.5 and 6 Hz).

To a solution of **5** (0.85 g, 7.53 mmol) in dry CH_2Cl_2 (20 ml) was added $NO_2^+BF_4^-$ (0.5 g, 3.76 mmol; 0.5 equiv). Upon reflux (24 h) the precipitated **5b** was isolated without a benzene wash (0.228 g; 24.6%): ^{1}H NMR (CD_3CN) 8.64 (dd, J = 1.5 and 5.8 Hz), 8.58 (dt, J = 1.7 and 8 Hz), 8.07 (d, J = 8.2 Hz), 8.01 (dt, J = 1.3 and 6.8 Hz).

Attempted C-Nitration of 4 and 5. In a typical experiment, to solution of the substrate (1.88 mmol) in anhydrous acetonitrile (20 mL) prepared in the glovebox was added $NO_2^+BF_4^-$ (2 equiv) with magnetic stirring at room temperature. The reaction mixture was transferred to the hood and heated to reflux under dry nitrogen for 24 h. GC analysis of the organic phase following workup showed no C-nitration.

N- and C-Nitration of 6. To a solution of **6** (0.73 g, 7.53 mmol) in dry CH_2Cl_2 was added $NO_2^+BF_4^-$ (0.5 g, 3.76 mmol; 0.5 equiv) under dry nitrogen with magnetic stirring. Upon reflux, **6b** precipitated. The salt was thoroughly shaken with a solution of benzene/toluene (1:1) diluted in methylene chloride and vacuum dried (0.406 g; 46.9%): ^{1}H NMR (CD_3CN) 8.73 (appearance q, J = 7.6 Hz), 8.51 (d, J = 3.9 Hz), 7.91 (distorted t, J = 6.8 Hz), 7.79 (m); ^{19}F NMR -81.7 (CF) and -150 ppm (BF_4^-).

GC analysis of the toluene/benzene solution showed trace amounts of nitrotoluene isomers and nitrobenzene with a K_T/K_B = 2.25, indicative of nitration by unreacted $NO_2^+BF_4^-$. GC analysis of the organic phase, following the removal of **6b** from the reaction mixture, showed two peaks for **6** (94%) and **6a** (6%) with the latter having a longer retention time. Unreacted **6** was removed by vacuum distillation, and **6a** was isolated (0.003 g, 6%): ^{1}H NMR ($CDCl_3$) 9.08 (s), 8.66 (m), 7.25 (m).

N-Nitration of 2,6-Dimethylpyridine (Lutidine). To a solution of lutidine (0.807 g, 7.53 mmol) in 20 mL of dry CH_2Cl_2, prepared with stirring at -20 °C in the glovebox was added $NO_2^+BF_4^-$ (0.5 g, 3.76 mmol; 0.5 equiv). After 20 min, the solution was diluted with 20 mL of dry CCl_4 and cooled to -20 °C for 24 h. The resulting tan crystals were filtered in the glovebox and dried in vacuo without washing with benzene, to give **7** (0.687 g, 75%): ^{1}H NMR 8.27 (t, J = 8 Hz), 7.61 (d, J = 7.8), 2.68 (s).

N-Nitrito-2,6-dibromopyridine (8). To a solution of *N*-oxide (1.1 g, 5.93 mmol) in 20 mL of dry CH_2Cl_2 was added $NO^+BF_4^-$ (0.25 g, 2.14 mmol; 1 equiv) inside the glovebox. The solution was transferred to the fume hood and heated to reflux under dry nitrogen for 24 h. The yellow precipitate was filtered and washed with CH_2Cl_2 and the product dried in vacuo (0.162 g, 19.1%): ^{1}H NMR (CD_3CN)[14] 8.26 (q, J = 8 Hz), 8.02 (t, J = 7.8 Hz).

Transfer-Nitration. In a typical experiment the pure *N*-nitropyridinium salts (1.5 mmol) was added with vigorous magnetic stirring to a solution of benzene and toluene (1:1; 5 equiv) diluted to 1 M with dry CH_3CN under dry nitrogen. The reaction progress was monitored by GC. In cases where no reaction was observed (**1b** and **2b**) the mixture was heated under reflux (24 h). The solvent and the aromatics were distilled off to give unchanged **1b** or **2b** (NMR). A portion of the recovered **1b** was quenched with saturated aqueous bicarbonate, extracted (CH_2Cl_2), and dried ($MgSO_4$). GC analysis showed only 1.

Acknowledgment. We thank KSU for research support and the Ohio Academic Challenge Program for funds for the 300-MHz NMR. We are grateful to Dr. Norman Duffy for recording most of the NMR spectra.

Supplementary Material Available: ^{1}H NMR data for **1a**, **2a**, **1b**, **2b**, 3-NP, **3**, **3a**, **4b**, **5b**, **6**, **6a**, **6b**, **7**, **8**, ^{19}F NMR data for **6b** and **6**, and IR spectral data for **1a**, **2a**, **1b**, **2b**, 3-NP, **6**, **6b**, and **8** (27 pages). Ordering information is given on any current masthead page.

(13) Schickh, O. V.; Binz, A.; Schulz, A. *Ber.* **1936**, *69*, 2593.

(14) Unlike the nitro onium ion **1b**, the nitrito salt was totally insoluble in SO_2. In $TfOH/SO_2$ solvent (-60 °C) the spectrum consisted of a doublet at 8.26 and a broad triplet at 8.14 ppm.

Conformationally Restricted Arginine Analogues

Thomas R. Webb*,† and Charles Eigenbrot

Department of Medicinal and Biomolecular Chemistry, Genentech Inc., 460 Point San Bruno Blvd., South San Francisco, California 94080

Received October 15, 1990

We report the practical synthesis and structural characterization of a set of conformationally constrained protected arginine analogues. These enantiomerically pure analogues have the general structure **1** and are prepared in seven to eight steps from the commercially available isomers of 4-hydroxyproline. These analogues vary in side chain length and in relative and absolute stereochemistry and are suitable for the direct introduction into peptides. The resulting peptide analogues should be useful as enzymatically stable replacements for bioactive peptides and as probes for understanding the conformational aspects of protein-peptide interactions.

Introduction

The synthesis of conformationally restricted amino acid analogues which can be incorporated into peptides is of interest in the investigation of protein-peptide interaction. Although there has been significant recent interest in the synthesis of conformationally constrained amino acid analogues,[1] there has been no report of the methodical design and preparation of a series of structurally kindred

†Present address: Corvas, Inc., 3030 Science Park Road, San Diego, CA 92121.

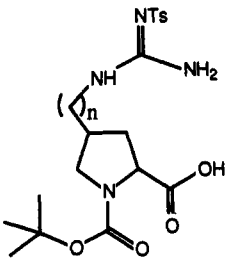
(1) For example, see: Rapoport, H.; Wolf, J.-P. *J. Org. Chem.* **1989**, *54*, 3164-3173. DiMaio, J.; Belleau, B. *J. Chem. Soc., Perkin Trans. 1* **1989**, 1687-1689.

0022-3263/91/1956-3009$02.50/0

chiral amino acid analogues, suitable for introduction into peptides. The systematic analysis of the structure–activity relationships of peptides containing such a set of related, but configurationally distinct, amino acid analogues should give insight into the bound conformations of a peptide receptor ligand, or a peptide enzyme substrate. This type of data has potential application in the design of receptor agonists/antagonists and enzyme inhibitors.

We chose to concentrate on analogues of the basic amino acids, since they can be important binding determinants in protein–peptide interactions. In this paper, we will describe the practical and efficient synthesis of a set of eight enantiomerically pure arginine analogues. These analogues were prepared using protecting groups which are suitable for the direct introduction of these modified amino acids into synthetic peptides.

In order to probe as many arginine conformations as possible, we needed a synthetic approach that was reasonably divergent. We envisioned cyclic analogues containing at least two asymmetric centers in order to modify the conformations in a systematic way. A class of starting materials which are appropriately functionalized for the preparation of such analogues are the commercially available, enantiomerically pure, isomers of 4-hydroxyproline. For these reasons we chose to prepare eight compounds of the general structure 1, using the synthetic strategy outlined in Schemes I, II, and III.



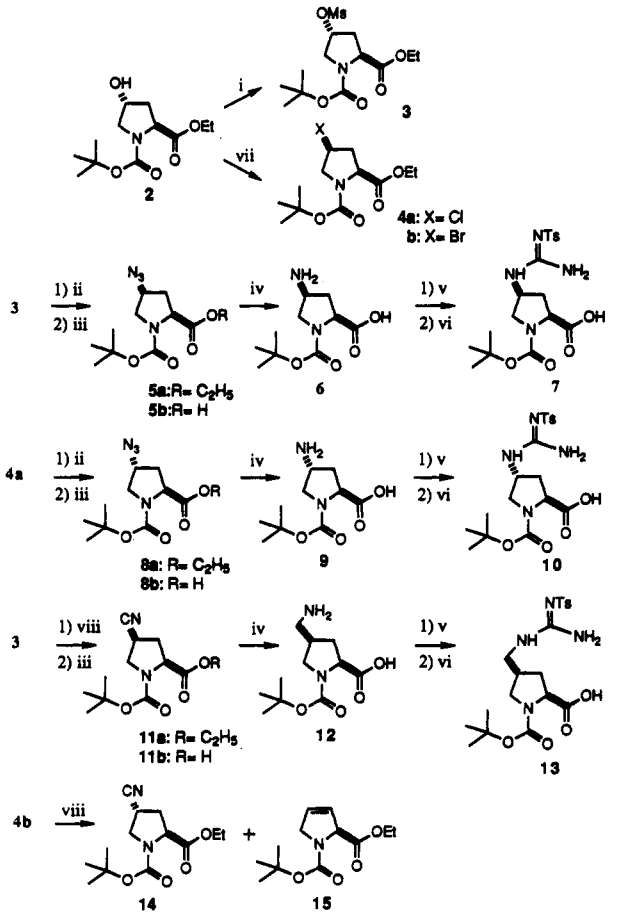
1: n= 0 or 1

Results and Discussion

Foreshortened analogues containing only two methylene groups between the α-carbon and the guanidine group were prepared from previously known derivative 2[2] (see Scheme I). This compound was converted to the known mesylate 3,[2] by treatment with mesyl chloride, or to the halogen derivatives **4a** or **4b**, with the opposite configuration at the 4-position, by reaction with a combination of triphenylphosphine and a tetrahalomethane.[3,4] This approach allowed the preparation of synthons with both possible configurations at the 4-position from the common intermediate 2, with a minimum number of steps.

The mesylate 3 was converted into the azide ester **5a**[2] with sodium azide in dimethylformamide (DMF). This ester was hydrolyzed with 1 equiv of sodium hydroxide in methanol, to give the azido acid **5b**, in good overall yield. Hydrogenation of **5b** in aqueous ethanol, with 10% palladium on carbon, gave the crystalline *tert*-butoxycarbonyl (BOC) diamino acid **6** in 84% yield. This product was then converted to the *N*-(*p*-toluenesulfonyl)guanidine derivative 7 using a known two-step procedure;[5] thus compound **6** and

Scheme I[a]



[a] (i) MsCl/py; (ii) NaN_3/DMF; (iii) NaOH/H_2O/MeOH; (iv) H_2/Pd; (v) $TsNC(SMe)_2$; (vi) $NH_3/AgNO_3$; (vii) CX_4/Ph_3P; (viii) NaCN/DMSO or *n*-Bu_4NCN/DMF, 55 °C.

S,*S*-dimethyl-*N*-[(*p*-toluenesulfonyl)imino]dithiocarboimidate were allowed to react, in the presence of base, to give an intermediate *S*-methyl-*N*-tosylisothiourea. This crude intermediate was treated with silver nitrate in the presence of anhydrous ammonia to give **7**, which was purified by crystallization. (The yield of this two step reaction was variable, depending on the amino acid substrate, giving the tosylguanidine derivatives in from 23% to 75% yield (see the Experimental Section).) If the same overall sequence of reactions was performed starting with **4a**, the derivative with the opposite configuration at the 4-position (**10**) was obtained in similar overall yield.

In an analogous fashion **3** was allowed to react with cyanide anion to give **11a**. The displacement of the mesylates using tetra-*n*-butylammonium cyanide[6] (TBACN) in DMF at 55 °C gave the best results (complete reaction in 18 h at 55 °C). The reaction with sodium cyanide in dimethyl sulfoxide was much slower giving 55% isolated yield of **11a**, with starting material isolated in 40% yield, after 55 h. The use of higher temperatures gave a mixture of epimers. The ester **11a** was converted to the crystalline cyano acid **11b** by mild alkaline hydrolysis (in 70% yield). Analysis by single-crystal X-ray diffraction confirmed the cis orientation of the cyano and carboxylate groups of **11b**, as represented in Scheme I. An ORTEP plot of the X-ray structure is displayed in Figure 1.

The cyano acid **11b** was converted into the BOC diamino acid **12** in 98% yield by catalytic hydrogenation with 10%

(2) Abraham, D. J.; Mokotoff, M.; Sheh, L.; Simmons, J. E. *J. Med. Chem.* **1983**, *26*, 549–554.
(3) Lee, J. B.; Nolan, T. J. *Can. J. Chem.* **1966**, *44*, 1331–1334.
(4) Hooz, J.; Gilani, S. S. H. *Can. J. Chem.* **1968**, *46*, 86–87.
(5) Barker, P. L.; Gendler, P. L.; Rapoport, H. *J. Org. Chem.* **1981**, *46*, 2455–2465. Bosin, T. R.; Hanson, R. N.; Rodericks, J. V.; Simpson, R. A.; Rapoport, H. *J. Org. Chem.* **1973**, *38*, 1591–6112.
(6) Kobler, H.; Schuster, K.-H.; Simshen, G. *Ann. Chem.* **1978**, 1946–1962.

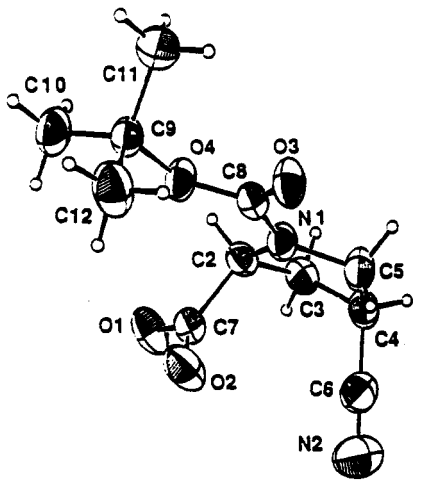
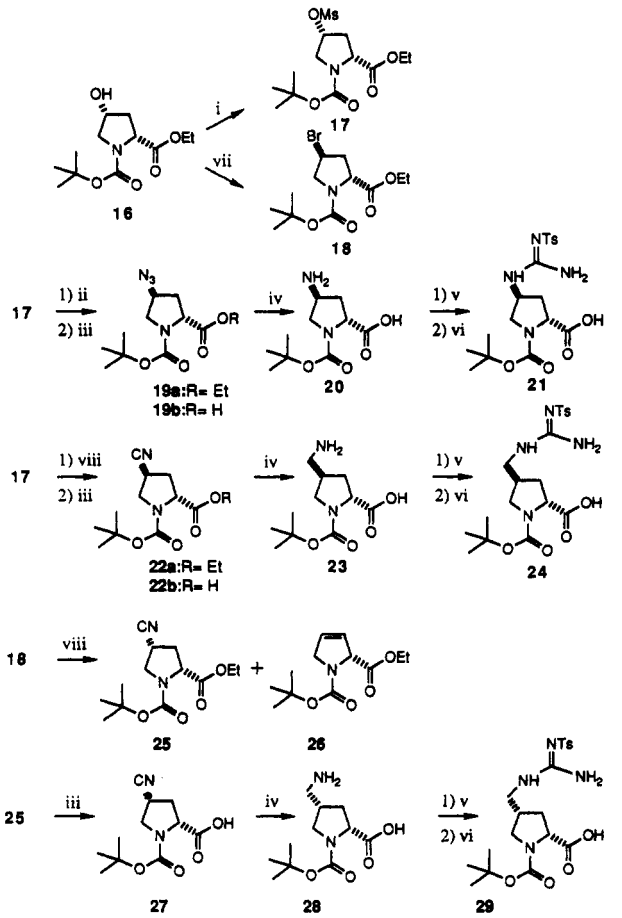


Figure 1. ORTEP drawing of **11b**, see Table I for a summary of the X-ray crystal structure data.

Scheme II[a]

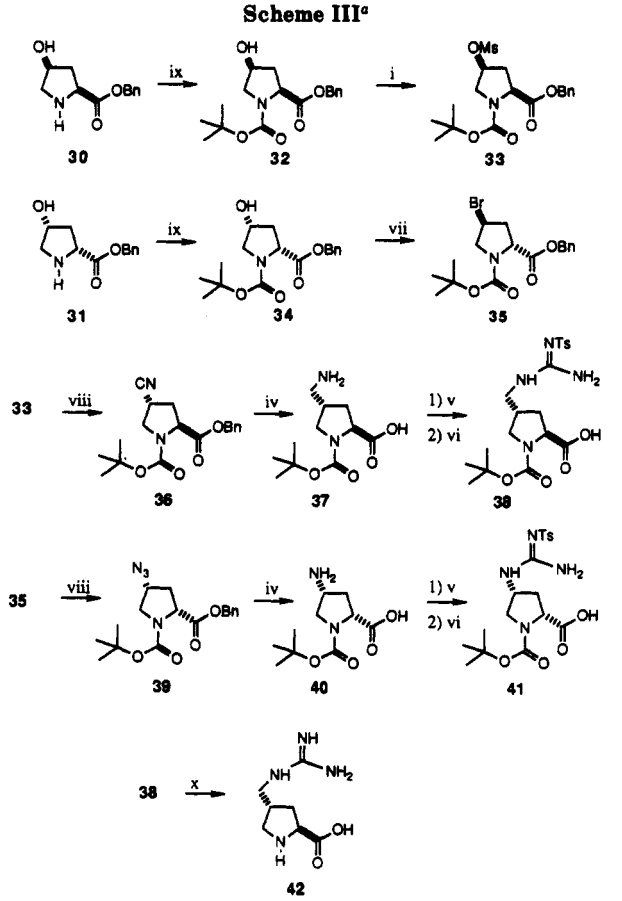


[a] (i) MsCl/py; (ii) NaN_3/DMF; (iii) NaOH/H_2O/MeOH; (iv) H_2/Pd; (v) $TsNC(SMe)_2$; (vi) $NH_3/AgNO_3$; (vii) CX_4/Ph_3P; (viii) NaCN/DMSO or n-Bu_4NCN/DMF, 55 °C.

palladium on carbon. This product was transformed into the corresponding fully protected arginine analogue **13** as for the preparation of **7**. The trans cyano ester **14** was prepared from the bromo ester **4b**, but in this case, elimination, giving olefin **15**, was a significant side reaction. The synthesis of the protected diamino acid derived from **14** was accomplished by an improved procedure which will be described in connection with Scheme III (vide infra).

The synthesis of the arginine analogues with the D configuration at the α-carbon starts with the known protected hydroxyproline derivative **16**[7] (Scheme II). This

Scheme III[a]



[a] (i) MsCl/py; (ii) NaN_3/DMF; (iii) NaOH/H_2O/MeOH; (iv) H_2/Pd; (v) $TsNC(SMe)_2$; (vi) $NH_3/AgNO_3$; (vii) CX_4/Ph_3P; (viii) NaCN/DMSO or n-Bu_4NCN/DMF, 55 °C; (ix) BOC_2O; (x) HF/anisole.

compound was converted into the mesylate **17** or the bromo derivative **18**. It was noted in this case that the isolated yield of halide, using the triphenylphosphine/tetrahalomethane procedure, varied dramatically depending on the workup. When the triphenylphosphine oxide was precipitated by the rapid addition of diethyl ether, a significant amount of the desired product was occluded within the precipitate. Slow addition of a minimal amount of diethyl ether, to precipitate most of the triphenylphosphine oxide, followed by flash chromatography, gave the pure desired product **18** in 90% yield.

The azido ester **19a** was prepared from the mesylate **17** and transformed into the foreshortened analogue **21** by repetition of the steps shown for the synthesis of **7**. Likewise **17** gave **24** by repetition of the steps used for the conversion of **3** into **13**. Treatment of the relatively sterically hindered halide **18** with TBACN in DMF gave **25** in modest but useful yield (22%). The olefin **26** was the major product in this case, but was readily separable from **25** by flash chromatography. The cyano ester **25** was then converted into **29**, as for the aforementioned analogous cases.

Though the general approach shown in Schemes I and II gave good results in most cases, a shorter route was desired. Scheme III shows a route which allows for simultaneous reduction of the nitrile to an amine and deprotection of the carboxylic acid. This route proved to have the additional advantage that, while the ethyl ester intermediates are intractable oils, many of the corre-

(7) Baker, G. L.; Fritschel, S. J.; Stille, J. R.; Stille, J. K. *J. Org. Chem.* 1981, *46*, 2954–2960.

sponding benzyl esters are highly crystalline compounds.

cis-4-Hydroxy-L-proline (see Scheme III) was transformed into the benzyl ester **31** in 94% yield by treatment with *p*-toluenesulfonic acid (TsOH) and benzyl alcohol in refluxing toluene.[8] This ester was then converted into the crystalline BOC derivative **32** in 87% yield. This latter compound was treated with mesyl chloride in pyridine to give the crude mesylate **33** in quantitative yield. This crude mesylate was then directed converted to the cyano benzyl ester **36** (in 52% yield), which was simultaneously reduced and deprotected by hydrogenation to give the amino acid **37** in 67% yield. In an analogous manner, **34** was prepared and converted via the intermediates **35** and **39** into the amino acid **40**. The free amino acids were converted into their corresponding protected guanidine derivatives **38** and **41**, respectively, as described previously.

Since the free amino acids are of interest we have also deprotected one of these derivatives with anhydrous hydrogen fluoride/anisole[9b] to give the conformationally constrained arginine analogue **42** (see Scheme III). The combination HF/anisole has been used for the high yield simultaneous removal of α-*N*-BOC and N^G-tosyl groups of arginine in protected peptides.[9b] The product **42** (as the dihydrofluoride salt) was isolated nearly pure (by analytical HPLC and NMR) after aqueous workup and extraction with ether, but could be further purified by preparative HPLC to give **42** in 55% yield with no optimization of conditions (see the Experimental Section).

Proton NMR spectroscopy was useful in confirming the relative configuration and stereoisomeric purity of the intermediates in the synthesis of these analogues. For example, the cis bromo, azido, cyano, and amino derivatives, **4b**, **5a**, **11a**, **25**, **6**, and **40** all have a significant downfield shift of a C-3 proton resonance of between 0.15 and 0.55 ppm, relative to their trans counterparts (see the experimental section). Cis amino acid **12** and **28** can be readily distinguished from the corresponding trans compounds **23** and **37** by significant differences in the ^{1}H NMR spectra (see the Experimental Section). The X-ray crystal structure of **11b** (Figure 1), in conjunction with the ^{1}H NMR data, firmly establishes the structure of all of the nitrile derivatives. The observed specific rotation data is also consistent with the proposed structures of the amino acid analogues (see the Experimental Section). Since the absolute configuration of the isomers of 4-hydroxyproline are known, and since epimers are distinguishable by NMR, the absolute configurations of the amino acid analogs are established. In all cases the displacement of the leaving group at the C-4 position by azide or cyanide results in inversion, as expected.

Summary

We have prepared a closely related set of novel conformationally restricted arginine analogues. These analogues allow for the methodical study of the relationship of arginine conformation to the biological activity of arginine containing peptides. The synthesis of these analogues has been explored and has resulted in procedures that are practical and suitable for multigram scale. The combination of amine BOC protection and guanidine tosyl protection is desirable for peptide synthesis by conventional means.[9] The analogues described in this paper are particularly suitable for introduction into peptides via solid-phase synthesis,[10] since the conditions for removal of the peptide from support (neat hydrogen fluoride) will remove all of the protecting groups from the synthetic peptide.[9,10] In addition compounds such as **12** (and its stereoisomers) can be considered conformationally constrained ornithine or lysine analogues. These compounds could be protected with, for example, the 4-chlorobenzyloxycarbonyl group[9] and incorporated into peptides in the same way as for the arginine analogues. Incorporation of the arginine analogues into peptides, and the bioactivity of such modified peptides, will be the subject of later reports.

Table I. Summary of Crystal Structure Data for 11b

formula	$C_{11}H_{16}N_2O_4$
formula weight, g/mol	240.26
Z	4 molecules/cell
crystal dimensions	0.45 × 0.30 × 0.15 mm (1) 0.30 × 0.30 × 0.15 mm (2)
crystal system	orthorhombic
space group	$P2_12_12_1$
cell parameters, Å	a, 12.988 (2); b = 14.122 (1); c = 6.871 (1)
calculated density, g/cm^3	1.266
no. of reflctns measured	7079
no. of unique reflctns	1358
no refs. used	1303 with $F_o^2 > 3\sigma(F_o^2)$
θ_{max}, deg	70.0

Experimental Section

trans-4-Hydroxy-L-proline, *cis*-4-hydroxy-L-proline, and *cis*-4-hydroxy-D-proline were all purchased from Sigma. Tetra-*n*-butylammonium cyanide and di-*tert*-butyl dicarbonate were purchased from Fluka. All other reagents were obtained from Aldrich Chemical Co. Column chromatography was performed using Merck silica gel 60 (70–230 mesh). TLC was performed on Whatman silica gel 60A MK6F plates and were developed by heating followed by spraying with ninhydrin. Optical rotation values were obtained by using a Rudolph Research Autopol II automatic polarimeter. High-resolution mass spectra were obtained on a JEOL JMS-HX110HF/HX110HF tandem MS/MS instrument using the positive ion FAB technique. Infrared spectra were obtained on a Nicolet 510 FT-IR spectrometer. ^{1}H NMR spectra were obtained on a Varian 300 MHz VXR-300S instrument, in the indicated solvent. The δ values are reported from the internal standard tetramethylsilane (0.03%) for all solvents except D_2O, which uses 3-(trimethylsilyl)propionic-*2,2,3,3-d*$_4$ acid sodium salt (0.03%) as the internal standard. Melting points were not corrected. All concentrations were performed in a rotory evaporator under reduced pressure.

General Workup: Method A. The reaction mixture was partitioned between 0.5 M citric acid and EtOAc. The aqueous layer was extracted three times with EtOAc, and the organic extracts were then combined, dried ($MgSO_4$), and concentrated under vacuum. **Method B.** The reaction mixture poured into ice–water. This was extracted with EtOAc three times, and the combined organic extracts were washed three times with water and then brine. The EtOAc extracts were combined, dried ($MgSO_4$), and concentrated. **Method C.** Method B was used, except that a wash with 5% $NaHCO_3$ replaced the water washes.

X-ray crystallographic results are summarized in Table I. Data were collected at room temperature on an Enraf-Nonius CAD4 diffractometer with graphite-monochromated Cu Kα radiation (λ = 1.541 84 Å). Lattice parameters were derived from least-squares fit of 25 centered reflection in the range of 28° < |2θ| < 42°. Two crystals were used. Small linear decay and absorption corrections were applied to the data from both crystals. After scaling, the merged data were averaged to yield 1379 unique reflections. (R_{sym} = 3.1% on intensity). Calculation were per-

(8) Zervas, L.; Winitz, M.; Greenstein, J. P. *J. Org. Chem.* **1957**, *22*, 1515–1521.

(9) (a) Bodanszky, M. *Principles of Peptide Synthesis*; Springer-Verlag: New York, 1984. (b) Yamashiro, D.; Blake, J.; Li, C. H. *J. Am. Chem. Soc.* **1972**, *94*, 2855–2859.

(10) Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149–2154.

formed on a VAX 11/785 computer using the SDP package.[11] The structure was solved by direct methods and refined to a final R factor of 4.0%. (R_W = 5.9%). Hydrogen atoms were discerned in an intermediate difference map and thenceforward included, but not refined, with temperature factors 30% higher than those of the heavy atom to which they are bonded. Final goodness of fit is 1.04. The final difference map was featureless (max peak = 0.22 $e^-/Å^3$, min peak = −0.22 $e^-/Å^3$).

(4*S*)-1-(*tert*-Butoxycarbonyl)-4-chloro-L-proline Ethyl Ester (4a). The ester alcohol **2**[2] (14 g, 54 mmol) was dissolved in 200 mL of pyridine and concentrated, the residue was dissolved in 200 mL toluene and concentrated, and this process was repeated two more times to remove *tert*-butyl alcohol and water. The residue was dissolved in a mixture of 100 mL of dichloromethane and 100 mL of carbon tetrachloride. This solution was treated with solid triphenylphosphine[3] (30 g, 114 mmol) with good stirring. Upon this addition the solution warmed slightly. This solution was allowed to stir for 2 h. After this time 10 mL of EtOH was added and the solution stirred at 22 °C for 16 h. The solution was then concd to ca. 100 mL and cooled to −20 °C to precipitate triphenylphosphine oxide. Et_2O (100 mL) was added, and the mixture was filtered and washed with 100 mL of Et_2O. The filtrate and washings were concentrated, and the residue was purified by flash chromatography (20–50% Et_2O/hexane) to give 11.4 g of **4a** (76% yield) as a colorless oil: ^1H NMR ($CDCl_3$) δ 1.28 (t, 3 H, J = 7 Hz), 1.42–1.45 (2 s, 9 H), 2.36 (m, 1 H), 2.63 (m, 1 H), 3.63 (m, 1 H), 3.95 (m, 1 H), 4.20 (q, 2 H, J = 7 Hz), 4.35 (m, 2 H).

Treatment of this oily compound with saturated anhydrous HCl in ethanol removed the t-BOC group and gave (4*S*)-4-chloro-L-proline ethyl ester hydrochloride salt, mp 132–134 °C (Et_2O/EtOH). Anal. ($C_7H_{13}NO_2Cl_2$) Calcd: C, 39.27; H, 6.12; N, 6.54. Found: C, 39.11; H, 6.09; N, 6.44.

(4*S*)-1-(*tert*-Butoxycarbonyl)-4-bromo-L-proline Ethyl Ester (4b). The ester alcohol **2**[2] (13 g, 50 mmol) was dissolved in 100 mL of dichloromethane (after concentration from pyridine and toluene as for the synthesis of **4a**), and carbon tetrabromide[4] (66 g, 200 mmol) was added. The solution was cooled in an ice bath, and triphenylphosphine (52.5 g, 200 mmol) was added with good stirring over a 10-min period. The reaction mixture warmed and turned dark red. This was allowed to stir for 5 h, 10 mL of ethanol was added, and the mixture was allowed to stir over night at 22 °C. The resulting dark solution and precipitate were then treated with 500 mL of Et_2O and filtered, and the residue was washed with 200 mL of Et_2O. The filtrate and washings were concd and purified as for **4a**, to give 8.5 g (53% yield) of pure **4b**. This product crystallized on standing to give material of mp 32–34 °C (Et_2O/hexane): ^1H NMR ($CDCl_3$) δ 1.30 (t, 3 H, J = 7 Hz), 1.43–1.47 (2 s, 9 H), 2.43 (m, 1 H), 2.83 (m, 1 H), 3.71 (m, 1 H), 4.06 (m, 1 H), 4.22 (q, 2 H, J = 7 Hz), 4.35 (m, 2 H). Anal. Calcd for $C_{12}H_{20}NO_4Br$: C, 44.73; H, 6.26; N, 4.35. Found: C, 44.42; H, 6.29; N, 4.36.

(4*S*)-1-(*tert*-Butoxycarbonyl)-4-azido-L-proline (5b). The azido ethyl ester **5a**[2] (1.80 g, 6.33 mmol) was hydrolyzed as for the synthesis of **14**. The product was purified by flash chromatography (0–10% MeOH/dichloromethane) to give 1.2 g (74% yield) of **5** as an oil: FAB MS, MH^+ calcd for $C_{10}H_{17}N_4O_4$ 257.1250, found 257.1262.

(4*S*)-1-(*tert*-Butoxycarbonyl)-4-amino-L-proline (6). Azido acid **5b** (1.10 g, 4.29 mmol) was dissolved in 100 mL of 10% water/ethanol containing 150 mg of 10% Pd/C and hydrogenated at 100 psi for 16 h in a stainless steel Parr vessel. The mixture was filtered through a pad of Celite and washed with 50 mL of 1:1 water/ethanol, and the filtrate was concentrated to dryness. This material was recrystallized from water/ethanol to give 900 mg of **6** (84% yield): mp 225–227 °C dec; ^1H NMR (D_2O) δ 1.40–1.44 (m, 9 H), 2.14 (m, 1 H), 2.68 (m, 1 H), 3.69 (m, 2 H), 3.99 (m, 1 H), 4.20 (dd, J = 9.0, 3.9 Hz, 1 H); $[\alpha]_D$ +21° (water, c = 0.24). Anal. Calcd for $C_{10}H_{18}N_2O_4 \cdot H_2O$: C, 48.36; H, 8.12; N, 11.29. Found: C, 48.54; H, 7.94; N, 11.55.

(4*S*)-1-(*tert*-Butoxycarbonyl)-4-[[[(*p*-toluenesulfonyl)-imino]aminomethyl]amino]-L-proline (7). The amino acid **6** (750 mg, 3.02 mmol) was converted to **7** using the procedure for the preparation of **13**.[5] This gave 1.15 g (74% yield) after two crystallizations from EtOAc/hexane: mp 171–172 °C; ^1H NMR ($CDCl_3$) δ 1.38–1.44 (2 s, 9 H), 2.28 (m, 2 H), 2.38 (s, 3 H), 3.45 (m, 2 H), 4.42 (m, 2 H), 5.80 (br, 1 H), 6.20 (bs, 2 H), 7.23 (d, J = 8 Hz, 2 H), 7.76 (d, J = 8 Hz, 2 H). Anal. Calcd for $C_{18}H_{26}N_4SO_6$: C, 50.69; H, 6.14; N, 13.14. Found: C, 51.04; H, 6.16; N, 12.84.

(4*R*)-1-(*tert*-Butoxycarbonyl)-4-azido-L-proline Ethyl Ester (8a). The chloro ester **4a** (5.5 g, 19.8 mmol) and NaN_3 (5.5 g, 84.6 mmol) were suspended in 200 mL of DMF, heated in a 75 °C oil bath, and stirred at this temperature for 64 h. The resulting solution was allowed to cool and then worked up using method B. This gave 4.5 g (80% crude yield) of an oil, which was used directly in the next step: ^1H NMR ($CDCl_3$) δ 1.28 (t, 3 H, J = 7 Hz), 1.41–1.46 (2 s, 9 H), 2.17 (m, 1 H), 2.32 (m, 1 H), 3.52 (m, 1 H), 3.70 (dd, 1 H, J = 6, 12 Hz), 4.18 (m, 3 H), 4.34 (m, 1 H); FAB MS MH^+ calcd for $C_{12}H_{21}N_4O_4$ 285.1563, found 285.1574.

(4*R*)-1-(*tert*-Butoxycarbonyl)-4-azido-L-proline (8b). The trans azido ester **8a** (4.45 g, 15.7 mmol) from above from hydrolyzed as for the synthesis of **11b** to give 3.1 g (77% crude yield) of **8b**, which was used directly in the next step: ^1H NMR ($CDCl_3$) δ 1.41–1.48 (2 s, 9 H), 2.24 (m, 1 H), 2.52 (m, 1 H), 3.54 (m, 1 H), 3.71 (m, 1 H), 4.17 (m, 1 H), 4.40 (m, 1 H); FAB MS MH^+ calcd for $C_{10}H_{17}N_4O_4$ 257.1250, found 257.1262.

(4*R*)-1-(*tert*-Butoxycarbonyl)-4-amino-L-proline (9). The trans azido acid **8b** (3.0 g, 11.7 mmol) was hydrogenated as for the synthesis of **6** to give 1.8 g (67% yield) of **9** after crystallization from ethanol: mp 228–229 °C dec; ^1H NMR ($CDCl_3$) δ 1.42–1.47 (2 s, 9 H), 2.29 (m, 1 H), 2.45 (m, 1 H), 3.58 (m, 1 H), 3.80 (m, 1 H), 3.99 (m, 1 H), 4.24 (m, 1 H); $[\alpha]_D$ −35° (water, c = 0.17). Anal. Calcd for $C_{10}H_{18}N_2O_4 \cdot 0.25H_2O$: C, 51.21; H, 8.37; N, 11.95. Found: C, 51.44; H, 8.33; N, 11.91.

(4*R*)-1-(*tert*-Butoxycarbonyl)-4-[[[(*p*-toluenesulfonyl)-imino]aminomethyl]amino]-L-proline (10). The amino acid **9** (1.4 g, 6.08 mmol) was converted to **10** using the procedure for the preparation of **13**. This gave 600 mg (23% yield) of **10** after crystallization from EtOAc/Et_2O/hexane: mp 190–191 °C dec; ^1H NMR (d_6-DMSO) δ 1.32–1.37 (2 s, 9 H), 2.05 (m, 2 H), 2.35 (s, 3 H), 3.04 (m, 1 H), 3.34 (bs, 2 H), 3.53 (m, 1 H), 4.11 (bs, 1 H), 6.69 (bs, 1 H), 7.29 (d, J = 8 Hz, 2 H), 7.63 (d, J = 8 Hz, 2 H). Anal. Calcd for $C_{18}H_{26}N_4SO_6$: C, 50.69; H, 6.14; N, 13.14. Found: C, 51.04; H, 6.16; N, 12.84.

(4*S*)-1-(*tert*-Butoxycarbonyl)-4-cyano-L-proline Ethyl Ester (11a). Mesylate **3**[2] (10.0 g crude, 30.7 mmol) and NaCN (15 g, 306 mmol) were stirred in 200 mL of dry DMSO. This mixture was heated in a 55 °C oil bath for 55 h, cooled to 25 °C, and worked up using method B, and the residue was purified by column chromatography (10–50% ether/hexane) to give 3.0 g of **3** and 3.0 g of the title compound (52% yield based on recovered **3**) as a colorless oil: ^1H NMR ($CDCl_3$) δ 1.30 (t, 3 H, J = 7 Hz), 1.40–1.44 (2 s, 9 H), 2.30 (m, 1 H), 2.67 (m, 1 H), 3.09 (m, 1 H), 3.67 (m, 1 H), 3.93 (m, 1 H), 4.24 (q, 2 H, J = 7 Hz), 4.34 (m, 1 H); FAB MS MH^+ calcd for $C_{13}H_{21}N_2O_4$ 269.1501, found 269.1507.

(4*S*)-1-(*tert*-Butoxycarbonyl)-4-cyano-L-proline (11b). The cyano ethyl ester **11a** from the previous experiment (2.9 g, 12.1 mmol) was dissolved in 100 mL of methanol, and 15 mL of 1 M NaOH was added over 5 min with good stirring. This was allowed to stir for 16 h at 22 °C and was then treated with 1 mL of 80% acetic acid/water. This solution was then concentrated and worked up according to method A. The residue crystallized on standing at 5 °C and was recrystallized from EtOAc/hexane to give 2.2 g (76% yield) of **11b**: mp 138–139 °C dec; IR (KBr) 2253 cm^{-1} (nitrile); ^1H NMR δ 1.40–1.46 (2 s, 9 H), 2.44 (m, 1 H), 2.70 (m, 1 H), 3.12 (m, 1 H), 3.65 (m, 1 H), 3.92 (m, 1 H), 4.36 (m, 1 H). Anal. Calcd for $C_{11}H_{16}N_2O_4$: C, 54.99; H, 6.71; N, 11.66. Found: C, 54.91; H, 6.55; N, 11.56.

(4*S*)-1-(*tert*-Butoxycarbonyl)-4-(aminomethyl)-L-proline (12). A solution of cyano acid **11b** (1.6 g, 6.67 mmol) in 100 mL of 10% water/ethanol and 500 mg of 10% Pd/C was pressurized to 450 psi in a stainless steel Parr vessel. After stirring for 16 h at 22 °C the mixture was filtered through a pad of Celite and the residue was washed with 50 mL of 1:1 ethanol/water. The filtrate was concentrated to dryness to give a white solid. This

(11) Frenz, B. A. The Enraf-Nonius CAD4 SDP-A Real Time System for Concurrent X-Ray Data Collection and Crystal Structure Determination. In *Computing in Crystallography*; Schenk, H., Olthof-Hazelkamp, R., van Konigsveld, H., Bassi, G. C., Eds.; Delft University Press: Holland, 1978.

was crystallized from water/ethanol/ether to give 1.6 g (98% yield): mp 187–189 °C dec; ^{1}H NMR (D_2O) δ 1.40–1.44 (2 s, 9 H), 1.62 (m, 1 H), 2.55 (m, 2 H), 3.13 (m, 3 H), 3.75 (m, 1 H), 4.10 (m, 1 H); $[\alpha]_D$ −57° (water, c = 0.04). Anal. Calcd for $C_{11}H_{20}N_2O_4 \cdot 0.5H_2O$: C, 52.16; H, 8.35; N, 11.06. Found: C, 52.40; H, 8.10; N, 10.77.

(4*S*)-1-(*tert*-Butoxycarbonyl)-4-[[[[(*p*-toluenesulfonyl)imino]aminomethyl]amino]methyl]-L-proline (13). Amino acid **12** (500 mg, 2.05 mmol) and *S*,*S*-dimethyl-*N*-[(*p*-toluenesulfonyl)imino]dithiocarboimidate (prepared by the method of Gompper and Haegele,[12] 600 mg, 2.18 mmol) were suspended in 12 mL of dry EtOH, and 2.00 mL of 1.00 M NaOH was added.[9] This was refluxed for 12 h, cooled to 22 °C, and treated with 0.5 mL of 80% AcOH/water. This was concentrated and worked up according to method A. This gave 1.1 g of the crude methylisothiourea derivative, which was dried by concentration from CH_3CN (3 × 100 mL). This material was dissolved in 35 mL of CH_3CN containing 0.7 mL of Et_3N. The resulting well-stirred solution was cooled in an ice bath and saturated with anhydrous ammonia. The resulting suspension was treated with a solution of $AgNO_3$ (390 mg, 2.29 mmol) in 10 mL of CH_3CN by dropwise addition over a 0.5-h period, at 5 °C (internal temperature).[9] This mixture was allowed to stir for 18 h at 22 °C and then filtered, and the residue ($AgSCH_3$) was washed with 1:1 CH_3CN/water (50 mL). The filtrate was concentrated and worked up according to method A. The residue was recrystallized from CH_2Cl_2/Et_2O/hexane to give 670 mg of **13** (73% yield). No melting was observed below 250 °C: ^{1}H NMR (d_6-DMSO) δ 1.32–1.38 (2 s, 9 H), 2.24 (m, 2 H), 2.34 (s, 3 H), 2.90 (m, 1 H), 3.09 (m, 2 H), 3.38 (m, 2 H), 4.00 (m, 1 H), 6.60 (bs, 2 H), 6.09 (bs, 1 H), 7.26 (d, J = 8 Hz, 2 H), 7.63 (d, J = 8 Hz, 2 H). Anal. Calcd for $C_{19}H_{28}N_4SO_6 \cdot 0.25EtOAc \cdot 0.25H_2O$): C, 52.05; H, 6.66; N, 11.56. Found: C, 52.70; H, 6.65; N, 11.39.

(4*R*)-1-(*tert*-Butoxycarbonyl)-4-cyano-L-proline Ethyl Ester (14). The bromo ester **4b** (7.5 g, 23.3 mmol) and tetra-*n*-butylammonium cyanide (10.5 g, 39.1 mmol) were dissolved in 70 mL of DMF. This solution was heated in a 55 °C oil bath for 18 h and then cooled to 22 °C, and this was worked up using procedure B to give 5.5 g of crude product. Analysis by TLC (40% Et_2O/hexane) and ^{1}H NMR indicated that this was a mixture of the desired cyano compound **14** and 3,4-didehydro-1-proline ethyl ester **15** in a ratio of 3:1, respectively.

(4*R*)-1-(*tert*-Butoxycarbonyl)-4-[(methylsulfonyl)oxy]-D-proline Ethyl Ester (17). *cis*-4-Hydroxy-D ethyl ester **16**[7] (16 g crude, 62 mmol) was dissolved in pyridine (200 mL) and concentrated to remove water and *tert*-butyl alcohol. The residue was dissolved in 150 mL of pyridine and stirred in an ice bath. This solution was treated with methanesulfonyl chloride (8 mL, 103 mmol) over a 30-min period and was then allowed to stir overnight at 22 °C. This solution was then cooled in an ice–acetone bath, and 50 mL of 10% water/pyridine was added over a 30-min period. This was then concentrated to a small volume and worked up according to method C. A dark oil was obtained (15 g, 72% crude) which was used directly in subsequent steps: ^{1}H NMR ($CDCl_3$) δ 1.27 (t, 3 H, J = 7 Hz), 1.42–1.46 (2 s, 9 H), 2.51 (m, 2 H), 3.01 (s, 3 H), 3.77 (m, 2 H), 4.20 (q, 2 H, J = 7 Hz), 4.43 (m, 1 H), 5.22 (m, 1 H).

(4*S*)-1-(*tert*-Butoxycarbonyl)-4-bromo-D-proline Ethyl Ester (18). *cis*-4-Hydroxy-D ethyl ester **16**[7] (8.83 g, 29.4 mmol) was dissolved in 100 mL of pyridine and concentrated, the residue was dissolved in 100 mL of toluene and concentrated, and this process was repeated two more times to remove water and *tert*-butyl alcohol. The residue was dissolved in 60 mL of dichloromethane. This solution was stirred and treated with CBr_4 (29.8 g, 89.9 mmol). The resulting solution was cooled in an ice bath, and solid triphenylphosphine (24.0 g, 91.5 mmol) was added over a 10-min period. The solution turned dark brown and after several hours a white precipitate formed. The mixture was allowed to stir for 16 h at 22 °C. After this period of time 6 mL of ethanol was added, and the mixture was stirred for 2 h. This mixture was treated with 60 mL of ether by dropwise addition over a 1-h period. The precipitate (triphenylphosphine oxide) was removed by filtration and washed with 100 mL of dichloromethane. The filtrate and washes were concentrated and purified by flash chromatography (20–40% Et_2O/hexane). This gave 8.50 g (90% yield) of **18** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.28 (t, 3 H, J = 7 Hz), 1.42–1.46 (2 s, 9 H), 2.43 (m, 1 H), 2.57 (m, 1 H), 3.8 (m, 1 H), 3.92 (m, 1 H), 4.20 (q, 2 H, J = 7 Hz), 4.46 (m, 2 H); FAB MS MH$^+$ calcd for $C_{12}H_{20}NO_4Br$ 322.0654, found 322.0654.

(4*S*)-1-(*tert*-Butoxycarbonyl)-4-azido-D-proline Ethyl Ester (19a). The crude mesylate **17** (5.5 g, 16.9 mmol) and NaN_3 (5.0 g, 77 mmol) were suspended in 200 mL of DMF. This mixture was heated in a 55 °C oil bath for 16 h and then worked up according to method B. This gave 4.5 g (93% crude yield) of the title compound as an oil, which was used directly in the subsequent steps: ^{1}H NMR ($CDCl_3$) δ 1.28 (t, 3 H, J = 7 Hz), 1.41–1.44 (2 s, 9 H), 2.17 (m, 1 H), 2.32 (m, 1 H), 3.52 (m, 1 H), 3.69 (m, 1 H), 4.18 (m, 3 H), 4.35 (m, 1 H); FAB MS MH$^+$ calcd for $C_{12}H_{21}N_4O_4$ 285.1563, found 285.1573.

(4*S*)-1-(*tert*-Butoxycarbonyl)-4-azido-D-proline (19b). The azido ethyl ester **19a** (4.45 g, 15.7 mmol) was hydrolyzed as for the synthesis of **11b** to give 2.05 g (51% crude yield) of the title compound as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.44–1.48 (2 s, 9 H), 2.24 (m, 1 H), 2.56 (m, 1 H), 3.53 (m, 1 H), 3.71 (m, 1 H), 4.17 (m, 1 H), 4.42 (m, 1 H); FAB MS MH$^+$ calcd for $C_{11}H_{17}N_4O_4$ 257.1250, found 257.1254.

(4*S*)-1-(*tert*-Butoxycarbonyl)-4-amino-D-proline (20). The trans azido acid **19b** (2.0 g, 7.81 mmol) was hydrogenated as for the synthesis of **6** to give 2.1 g (97% yield) of **20**, after crystallization from ethanol, as a white crystalline solid, which contained an equivalent of ethanol (by ^{1}H NMR): mp 225–226 °C dec; ^{1}H NMR ($CDCl_3$) δ 1.42–1.47 (2 s, 9 H), 2.28 (m, 1 H), 2.45 (m, 1 H), 3.58 (m, 1 H), 3.80 (m, 1 H), 3.99 (m, 1 H), 4.24 (m, 1 H); $[\alpha]_D$ +38° (water, c = 0.052). Anal. (after high vacuum at 55 °C for 18 h). Calcd for $C_{10}H_{18}N_2O_4 \cdot 0.5H_2O$: C, 50.19; H, 8.00; N, 11.71. Found: C, 50.48; H, 7.84; N, 11.42.

(4*S*)-1-(*tert*-Butoxycarbonyl)-4-[[[(*p*-toluenesulfonyl)imino]aminomethyl]amino]-D-proline (21). The amino acid **20** (1.5 g, 5.43 mmol) was converted to **21** using the procedure for the preparation of **13**. This gave 600 mg (26% yield) after crystallization from EtOAc/Et_2O/hexane: mp 190–191 °C dec; ^{1}H NMR (d_6-DMSO) δ 1.32–1.37 (2 s, 9H), 2.05 (m, 2 H), 2.35 (s, 3 H), 3.04 (m, 1 H), 3.34 (bs, 2 H), 3.53 (m, 1 H), 4.11 (bs, 1 H), 6.69 (bs, 1 H), 7.29 (d, J = 8 Hz, 2 Hz), 7.63 (d, J = 8 Hz, 2 H). Anal. Calcd for $C_{18}H_{26}N_4SO_6 \cdot 0.25EtOAc$: C, 50.87; H, 6.29; N, 12.49. Found: C, 50.24; H, 6.28; N, 12.02.

(4*S*)-1-(*tert*-Butoxycarbonyl)-4-cyano-D-proline Ethyl Ester (22a). Mesylate **17** (10.0 g, crude, 30.7 mmol) and tetra-*n*-butylammonium cyanide (15 g, 57 mmol) were dissolved in 100 mL of dry DMF and stirred for 20 h in a 55 °C oil bath. This was worked up as for **11a** to give 5.8 g (72% crude yield) of the title compound: ^{1}H NMR ($CDCl_3$) δ 1.27 (2 t, 3 H), 1.42–1.47 (2 s, 9 H), 2.36 (m, 1 H), 2.49 (m, 1 H), 3.66 (m, 2 H), 4.24 (q, 2 H, J = 7 Hz), 4.42 (m, 1 H).

(4*S*)-1-(*tert*-Butoxycarbonyl)-4-cyano-D-proline (22b). The cyano ethyl ester **22a** (5.0 g, 18.6 mmol) was hydrolyzed as for the synthesis of **5b** to give 4.45 g (quantitative crude yield) of **22b** as an oil: IR (KBr) 2253 cm^{-1} (nitrile); ^{1}H NMR ($CDCl_3$) δ 1.43–1.47 (2 s, 9 H), 2.52 (m, 2 H), 3.72 (m, 3 H), 4.23 (m, 1 H).

(4*S*)-1-(*tert*-Butoxycarbonyl)-4-(aminomethyl)-D-proline (23). Cyano acid **22b** (4.0 g crude, 16.6 mmol) was hydrogenated as for the synthesis of **12** to give the crude amino acid **23**. This was crystallized from water/ethanol/ether to give 1.6 g (40% yield) of pure **23**: mp 231–234 °C dec; ^{1}H NMR (D_2O) δ 1.40–1.45 (2 s, 9 H), 2.11 (m, 2 H), 2.61 (m, 1 H), 3.12 (m, 3 H), 3.73 (m, 1 H), 4.17 (dd, J = 9.0, 3.9 Hz, 1 H); $[\alpha]_D$ +16° (water, c = 0.012). Anal. Calcd for $C_{11}H_{20}N_2O_4 \cdot 0.5H_2O$: C, 53.11; H, 8.28; N, 11.25. Found: C, 53.04; H, 7.96; N, 10.11.

(4*S*)-1-(*tert*-Butoxycarbonyl)-4-[[[[(*p*-toluenesulfonyl)imino]aminomethyl]amino]methyl]-D-proline (24). Amino acid **23** (1.40 g, 5.74 mmol) was converted to **24** as for **13**. The residue was recrystallized from EtOAc/Et_2O/hexane to give 1.5 g of **24** (59% yield): mp 137–138 °C; ^{1}H NMR (d_6-DMSO) δ 1.33–1.38 (2 s, 9 H), 1.73 (m, 2 H), 2.34 (s, 3 H), 2.93 (m, 1 H), 3.07 (m, 2 H), 3.30 (m, 2 H), 3.41 (m, 1 H), 4.06 (m, 1 H), 6.60 (bs, 2 H), 6.90 (bs, 1 H), 7.26 (d, J = 8 Hz, 2 H), 7.63 (d, J = 8 Hz, 2 H). Anal. Calcd for $C_{19}H_{28}N_4SO_6 \cdot 0.25EtOAc \cdot 0.25H_2O$: C, 52.05; H, 6.66; N, 11.56. Found: C, 52.46; H, 6.67; N, 11.60.

(12) Gompper, R.; Haegele, W. *Chem. Ber.* **1966**, *99*, 2885–2899.

(4*R*)-1-(*tert*-Butoxycarbonyl)-4-cyano-D-proline Ethyl Ester (25). The bromo ester **18** (7.80 g, 24.2 mmol) and tetra-*n*-butylammonium cyanide (15.0 g, 55.9 mmol) were dissolved in 100 mL of dry DMF and stirred for 20 h in a 55 °C oil bath. This was worked up as for **11a** and purified by column chromatography (30–50% Et_2O/hexane) to give 1.44 g (22% yield) of the title compound, along with 2.64 g (45% yield) of **26**: ^1H NMR ($CDCl_3$) δ 1.29 (t, 3 H, J = 7 Hz), 1.41–1.44 (2 s, 9 H), 2.29 (m, 1 H), 2.68 (m, 1 H), 3.10 (m, 1 H), 3.68 (m, 1 H), 3.93 (m, 1 H), 4.24 (q, 2 H, J = 7 Hz), 4.34 (m, 1 H); FAB MS MH^+ calcd for $C_{13}H_{21}N_2O_4$ 269.1501, found 269.1507.

The olefin **26** was hydrolyzed with base to give a product which was identical in chromatographic and spectral properties with a standard sample (Sigma) of 1-(*tert*-butoxycarbonyl)-3,4-didehydro-L-proline.

(4*R*)-1-(*tert*-Butoxycarbonyl)-4-cyano-D-proline (27). The cyano ethyl ester **25** (1.2 g, 4.48 mmol) was hydrolyzed as for the synthesis of **11** to give 1.1 g (90% yield). The material crystallized on standing at 5 °C and was recrystallized from EtOAc/hexane to give **27**: mp 134–135 °C dec; IR (KBr) 2253 cm^{-1} (nitrile); ^1H NMR ($CDCl_3$) δ 1.40–1.46 (2 s, 9 H), 2.44 (m, 1 H), 2.70 (m, 1 H), 3.12 (m, 1 H), 3.65 (m, 1 H), 3.92 (m, 1 H), 4.36 (m, 1 H). Anal. Calcd for $C_{11}H_{16}N_2O_4$: C, 54.99; H, 6.71; N, 11.66. Found: C, 54.78; H, 6.76; N, 11.28.

(4*R*)-1-(*tert*-Butoxycarbonyl)-4-(aminomethyl)-D-proline (28). Cyano acid **27** (650 mg, 2.7 mmol) was hydrogenated as for the synthesis of **12**, and the product was crystallized from water/ethanol/ether to give 630 mg (95% yield) of the title compound: mp 187–189 °C dec; ^1H NMR (D_2O) δ 1.40–1.44 (2 s, 9 H), 1.62 (m, 1 H), 2.55 (m, 2 H), 3.13 (m, 3 H), 3.75 (m, 1 H), 4.10 (m, 1 H); $[\alpha]_D$ +51° (water, c = 0.14). Anal. Calcd for $C_{11}H_{20}N_2O_4 \cdot 0.25H_2O$: C, 53.11; H, 8.28; N, 11.25. Found: C, 53.33; H, 8.27; N, 10.73.

(4*R*)-1-(*tert*-Butoxycarbonyl)-4-[[[[(*p*-toluenesulfonyl)imino]aminomethyl]amino]methyl]-D-proline (29). Amino acid **28** (600 mg, 2.46 mmol) was converted to **29** as for **13**. The residue was recrystallized from CH_2Cl_2/Et_2O/hexane to give 550 mg of **29** (51% yield). No melting was observed below 250 °C: ^1H NMR (d_6-DMSO) δ 1.32–1.38 (2 s, 9 H), 2.25 (m, 2 H), 2.34 (s, 3 H), 2.90 (m, 1 H), 3.09 (m, 2 H), 3.37 (m, 2 H), 4.00 (m, 1 H), 6.60 (bs, 2 H), 6.90 (bs, 1 H), 7.25 (d, J = 8 Hz, 2 H), 7.62 (d, J = 8 Hz, 2 H). Anal. Calcd for $C_{19}H_{28}N_4SO_6 \cdot 0.25EtOAc \cdot 0.25H_2O$: C, 52.05; H, 6.66; N, 11.56. Found: C, 52.05; H, 6.64; N, 11.40.

(4*S*)-4-Hydroxy-L-proline Benzyl Ester *p*-Toluenesulfonyl Salt (30). A suspension of *cis*-4-hydroxy-L-proline (10 g, 76.3 mmol) in a mixture of 60 mL of benzene and 60 mL of benzyl alcohol containing *p*-toluenesulfonic acid (14.79 g, 77.5 mmol) was refluxed.[8] Water was removed by means of a Dean–Stark apparatus over 16 h, and the reddish solution was allowed to cool to ca. 22 °C. This solution was then diluted with 150 mL of dry Et_2O and allowed to set at 5 °C for 2 h. This was filtered, and the residue was washed with 150 mL of Et_2O. The residue was dried in a desiccator under vacuum to give 28.2 g (94%) of the title compound: mp 119–120 °C; ^1H NMR (D_2O) δ 2.37 (s, 3 H), 2.46 (m, 2 H), 3.39 (m, 2 H), 4.60 (m, 1 H), 4.66 (m, 1 H), 5.29 (d, J = 12 Hz, 1 H), 5.33 (d, J = 12 Hz, 1 H), 7.36 (d, J = 8 Hz, 2 H), 7.44 (s, 5 H), 7.69 (d, J = 8 Hz, 2 H). Anal. Calcd for $C_{19}H_{22}NSO_6$: C, 58.15; H, 5.65; N, 3.57. Found: C, 58.34; H, 5.86; N, 3.60.

(4*S*)-1-(*tert*-Butoxycarbonyl)-4-hydroxy-L-proline Benzyl Ester (32).[13] A suspension of **30** (9.73 g, 24.8 mmol) in 25 mL of dioxane and *N,N*-diisopropylethylamine (6 mL, 34 mmol) was treated with di-*tert*-butyl dicarbonate (8.0 g, 37 mmol) in one portion, with concomitant gas evolution.[2] This solution was allowed to stir for 0.5 h and then worked up according to method B. The residue slowly crystallized on storage at 5 °C and was recrystallized from EtOAc/hexane to give 6.9 g (87% yield) of the title compound: mp 72–73 °C; ^1H NMR ($CDCl_3$) δ 1.34–1.46 (2 s, 9 H), 2.08 (m, 1 H), 2.31 (m, 1 H), 3.21 (2 d, 1 H, J = 10 Hz), 3.61 (m, 2 H), 4.35 (m, 2 H), 5.22 (m, 2 H), 7.35 (s, 5 H). Anal. Calcd for $C_{17}H_{23}NO_5$: C, 63.54; H, 7.21; N, 4.36. Found: C, 63.71; H, 7.16; N, 4.36.

(4*S*)-1-(*tert*-Butoxycarbonyl)-4-[(methylsulfonyl)oxy]-L-proline Benzyl Ester (33). The hydroxy benzyl ester **32** (6.3, 19.7) was subjected to the procedure for the synthesis of **17** to give 8.3 g (106% crude yield) of the mesylate **33**. This crude oil was used directly in the subsequent steps: ^1H NMR ($CDCl_3$) δ 1.36–1.46 (2 s, 9 H), 2.51 (m, 2 H), 2.77–2.82 (2 s, 3 H), 3.77 (m, 2 H), 4.43–4.57 (2 dd, J = 8.7, 3.0 Hz, 1 H), 5.16 (m, 3 H), 7.35 (s, 5 H); FAB MS MH^+ calcd for $C_{18}H_{26}NSO_7$ 400.1430, found 400.1433.

(4*R*)-4-Hydroxy-D-proline Benzyl Ester *p*-Toluenesulfonyl Salt (31). *cis*-4-Hydroxy-D-proline was subjected to the procedure,[8] from above, for the preparation of **30** to give the title compound (28.0 g, 92% yield): mp 120–121 °C; ^1H NMR (D_2O) δ 2.38 (s, 3 H), 2.45 (m, 2 H), 3.42 (m, 2 H), 4.60 (m, 1 H), 4.66 (m, 1 H), 5.29 (d, J = 12 Hz, 1 H), 5.34 (d, J = 12 Hz, 1 H), 7.35 (d, J = 8 Hz, 2 H), 7.42 (s, 5 H), 7.66 (d, J = 8 Hz, 2 H). Anal. Calcd for $C_{19}H_{22}NSO_6$: C, 58.15; H, 5.65; N, 3.57. Found: C, 58.64; H, 5.75; N, 3.45.

(4*R*)-1-(*tert*-Butoxycarbonyl)-4-hydroxy-D-proline Benzyl Ester (34).[13] The D-benzyl ester **31** (6.45 g, 24.8 mmol) from above was subjected to the procedure for the synthesis **32**, to give 7.5 g (94% yield) of the title compound: mp 71–72 °C (EtOAc/hexane); ^1H NMR ($CDCl_3$) δ 1.34–1.46 (2 s, 9 H), 2.08 (m, 1 H), 2.31 (m, 1 H), 3.21 (2 d, 1 H, J = 10 Hz), 3.61 (m, 2 H), 4.32 (m, 2 H), 5.22 (m, 2 H), 7.37 (s, 5 H). Anal. Calcd for $C_{17}H_{23}NO_5$: C, 63.54; H, 7.21; N, 4.36. Found: C, 63.39; H, 7.16; N, 4.42.

(4*S*)-1-(*tert*-Butoxycarbonyl)-4-bromo-D-proline Benzyl Ester (35). The alcohol **34** (5.34 g, 16.7 mmol) and CBr_4 (16.6 g, 50 mmol) were dissolved in 100 mL of dichloromethane. This solution was treated with solid triphenylphosphine[4] (13.1 g, 50 mmol) over a 10-min period. This solution was allowed to stir for 18 h, and then 10 mL of ethanol was added. After 2 h this solution was treated with 100 mL of Et_2O by dropwise addition over a 0.5-h period. The mixture was filtered, and the filtrate and washings were then concentrated and purified as for the synthesis of **4a**. This gave 4.73 g (74% yield) of the title compound. This material crystallized on standing to give mp 87–88 °C (Et_2O/hexane): ^1H NMR ($CDCl_3$) δ 1.35–1.46 (2 s, 9 H), 2.41 (m, 1 H), 2.58 (m, 1 H), 3.90 (m, 2 H), 4.48 (m, 2 H), 5.19 (m, 2 H), 7.34 (s, 5 H). Anal. Calcd for $C_{17}H_{22}NO_4Br \cdot 0.5H_2O$: C, 51.79; H, 5.88; N, 3.55. Found: C, 51.95; H, 5.54; N, 3.70.

(4*R*)-1-(*tert*-Butoxycarbonyl)-4-cyano-L-proline Benzyl Ester (36). The benzyl mesylate **32** (8.0 g, 20 mmol) was allowed to react, and purified, according to the procedure for the synthesis of **32a** to give 3.3 g (52% yield) of the title compound. This material slowly crystallized on storage at 5 °C: mp 97–99 °C (Et_2O/hexane); ^1H NMR ($CDCl_3$) δ 1.33–1.45 (2 s, 9 H), 2.33 (m, 1 H), 2.50 (m, 1 H), 3.21 (m, 1 H), 3.62 (m, 1 H), 3.89 (m, 1 H), 4.41–4.52 (2 dd, J = 8.7, 3.0 Hz, 1 H), 5.16 (m, 2 H), 7.34 (s, 5 H). Anal. Calcd for $C_{18}H_{22}N_2O_4$: C, 65.44; H, 6.71; N, 8.48. Found: C, 65.74; H, 6.66; N, 8.47.

(4*R*)-1-(*tert*-Butoxycarbonyl)-4-(aminomethyl)-L-proline (37). Cyano benzyl ester **36** (3.0 g, 9.1 mmol) was hydrogenated as for the synthesis of **12**, and the product was crystallized from water/ethanol/ether to give 1.5 g (67% yield): mp 230–231 °C; ^1H NMR (D_2O) δ 1.40–1.45 (2 s, 9 H), 2.11 (m, 2 H), 2.61 (m, 1 H), 3.12 (m, 3 H), 3.73 (m, 1 H), 4.17 (dd, J = 9.0, 3.9 Hz, 1 H); $[\alpha]_D$ −19° (water, c = 0.012). Anal. Calcd for $C_{11}H_{20}N_2O_4 \cdot 0.5H_2O$: C, 52.16; H, 8.30; N, 11.06. Found: C, 52.36; H, 7.72; N, 10.04.

(4*R*)-1-(*tert*-Butoxycarbonyl)-4-[[[[(*p*-toluenesulfonyl)imino]aminomethyl]amino]methyl]-L-proline (38). Amino acid **37** (1.4 g, 5.7 mmol) was converted to **38** as for the synthesis of **13** to give 1.3 g (51% yield) of the title compound: mp 137–138 °C (EtOAc/Et_2O/hexane); ^1H NMR (d_6-DMSO) δ 1.34–1.40 (2 s, 9 H), 1.78 (m, 2 H), 2.36 (s, 3 H), 2.94 (m, 1 H), 3.09 (m, 2 H), 3.33 (m, 2 H), 3.41 (m, 1 H), 4.09 (m, 1 H), 6.60 (bs, 2 H), 6.90 (bs, 1 H), 7.26 (d, J = 8 Hz, 2 H), 7.63 (d, J = 8 Hz, 2 H). Anal. Calcd for $C_{19}H_{28}N_4SO_6 \cdot 0.25EtOAc \cdot 0.25H_2O$: C, 52.05; H, 6.66; N, 11.56. Found: C, 52.16; H, 6.52; N, 11.61.

(4*R*)-1-(*tert*-Butoxycarbonyl)-4-azido-D-proline Benzyl Ester (39). The bromo benzyl ester **35** (4.67 g, 12.1 mmol) and NaN_3 (4.7 g, 72.3 mmol) were suspended in 175 mL of DMF. This mixture was subjected to the procedure for the synthesis of **19a**, to give 3.73 g (89% crude yield) of a colorless oil which was used

(13) A synthesis of *N*-BOC-*trans*-4-hydroxy-L-proline benzyl ester by a different route has been published: Thottathil, J. K.; Moniot, J. L. *Tetrahedron Lett.* **1986**, *27*, 151–154.

directly in subsequent reactions: ^{1}H NMR ($CDCl_3$) δ 1.33-1.45 (2 s, 9 H), 2.18 (m, 1 H), 2.48 (m, 1 H), 3.48 (m, 1 H), 3.71 (m, 1 H), 4.14 (m, 1 H), 4.35-4.49 (2 dd, J = 8.7, 3.0 Hz, 1 H), 5.16 (m, 2 H), 7.34 (s, 5 H); FAB MS MH^+ calcd for $C_{17}H_{23}N_4O_4$ 347.1719, found 347.1726.

(4*R*)-1-(*tert*-Butoxycarbonyl)-4-amino-D-proline (40). The azido benzyl ester (2.65 g, 7.65 mmol) was hydrogenated as for the preparation of **6**. This gave 1.47 g (83% yield) of **40**, mp 263-264 °C (dec, darkening starting at 222 °C), after crystallization form water/EtOH: ^{1}H NMR (D_2O) δ 1.42-1.46 (m, 9 H), 2.12 (m, 1 H), 2.66 (m, 1 H), 3.72 (m, 2 H), 4.00 (m, 1 H), 4.18 (dd, J = 9.0, 3.9 Hz, 1 H); $[\alpha]_D$ -27° (water, c = 0.24). Anal. Calcd for $C_{10}H_{18}N_2O_4$: C, 52.16; H, 7.88; N, 12.17. Found: C, 51.97; H, 7.86; N, 12.16.

(4*R*)-1-(*tert*-Butoxycarbonyl)-4-[[[[(*p*-toluenesulfonyl)-imino]aminomethyl]amino]methyl]-D-proline (41). Amino acid **40** (1.40 g, 5.74 mmol) was converted to **41** as for **13**. The residue was recrystallized from EtOAc/Et_2O/hexane to give 1.87 g of **25** (59% yield): mp 132-133 °C; ^{1}H NMR (d_6-DMSO) δ 1.33-1.38 (2 s, 9 H), 1.74 (m, 1 H), 2.34 (s, 3 H), 2.97 (m, 1 H), 3.33 (m, 1 H), 3.63 (m, 1 H), 4.08 (m, 2 H), 6.65 (bs, 2 H), 6.93 (bs, 1 H), 7.27 (d, J = 8 Hz, 2 H), 7.64 (d, J = 8 Hz, 2 H). Anal. Calcd for $C_{19}H_{28}N_4SO_6 \cdot H_2O$: C, 49.77; H, 6.59; N, 12.22. Found: C, 50.54; H, 6.37; N, 11.39.

(4*R*)-4-(Guanidinomethyl)-L-proline (42). Tosyl derivative **38** (50 mg, 0.11 mmol) and 0.25 mL of anisole were cooled to -78 °C in a Teflon vessel, under a stream of nitrogen. Anhydrous hydrogen fluoride gas was then condensed into the vessel.[9b] The reaction was allowed to warm to -20 °C and stirred at that temperature for 30 min. The reaction was allowed to warm to 0 °C, and the HF was allowed to evaporate under a stream of nitrogen. At this point 25 mL of water was added and the resulting solution was extracted three times with 75 mL of ethyl ether. The aqueous phase was frozen and lyophilized to give the crude title compound. This material (25 mg) was >90% pure by ^{1}H NMR and analytical HPLC. The crude product was purified by using reverse-phase high-performance chromatography on a 10 μm 300 Å pore size C-18 packing. The column was eluted with a aqueous gradient of 0.1% trifluoroacetic acid going from 0% to 10% acetonitrile (containing 0.1% trifluoroacetic acid) over 20 min. Lyophilization gave the title compound as a white solid (25 mg, 55% yield): ^{1}H NMR (D_2O) δ 2.08 (m, 1 H), 2.28 (m, 1 H), 2.60 (m, 1 H), 2.94 (m, 1 H), 3.03 (m, 1 H), 3.26 (d, J = 7 Hz, 2 H), 3.63 (m, 1 H), 4.21 (dd, J = 5.7, 9.3 Hz, 1 H); ^{13}C NMR (D_2O) δ 35.9, 40.1, 45.8, 51.7, 64.4, 160.5, 177.6. Anal. Calcd for $C_7H_{14}N_4O_2 \cdot 2TFA$: C, 31.89; H, 3.89; N, 13.52. Found: C, 32.05; H, 4.34; N, 14.18.

Acknowledgment. We thank Peter L. Barker and Eugene D. Thorsett for helpful discussions and Jim Bourell and John Stults for the mass spectral measurements. We also thank Pureza Vallar for assistance with the hydrogen fluoride reaction.

Registry No. **2**, 37813-30-2; **3**, 132622-63-0; **4a**, 132622-64-1; **4a**·HCl (*N*-deprotected), 132623-02-0; **4b**, 132623-01-9; **5a**, 132623-03-1; **5b**, 132622-65-2; **6**, 132622-66-3; **7**, 132622-67-4; **8a**, 132623-04-2; **8b**, 132622-68-5; **9**, 132622-69-6; **10**, 132622-70-9; **11a**, 132623-05-3; **11b**, 132622-71-0; **12**, 132622-72-1; **13**, 132622-73-2; **14**, 132622-74-3; **15**, 83548-48-5; **16**, 77450-00-1; **17**, 132622-75-4; **18**, 132622-76-5; **19a**, 132623-06-4; **19b**, 132622-77-6; **20**, 132622-78-7; **21**, 132622-79-8; **22a**, 132623-07-5; **22b**, 132622-80-1; **23**, 132622-81-2; **24**, 132622-82-3; **25**, 132622-83-4; **26**, 132622-84-5; **27**, 132622-85-6; **28**, 132622-86-7; **29**, 132622-87-8; **30**, 132622-88-9; **30** (free acid), 618-27-9; **31**, 132622-89-0; **31** (free acid), 2584-71-6; **32**, 132622-90-3; **33**, 132622-91-4; **34**, 132622-92-5; **35**, 132622-93-6; **36**, 132622-94-7; **37**, 132622-95-8; **38**, 132622-96-9; **39**, 132622-97-0; **40**, 132622-98-1; **41**, 132622-99-2; **42**, 132623-00-8; $TsNC(SMe)_2$, 2651-15-2.

Supplementary Material Available: X-ray data for **11b** (3 pages). Ordering information is given on any current masthead page.

Kinetics of Proton Transfer of 3,5-Heptanedione, 2,6-Dimethyl-3,5-heptanedione, and Dibenzoylmethane with Amines in 50% Me_2SO-50% Water. Effect of Steric Crowding and π-Overlap on Intrinsic Rate Constants

Claude F. Bernasconi,* Douglas A. A. Ohlberg, and Michael W. Stronach

Department of Chemistry and Biochemistry of the University of California, Santa Cruz, California 95064

Received November 19, 1990

Rates of reversible deprotonation of 3,5-heptanedione (**6b**), 2,6-dimethyl-3,5-heptanedione (**6c**), and dibenzoylmethane (**6d**) by several primary aliphatic amines, by piperidine and morpholine, and by hydroxide ion (**6b** and **6d** only) have been measured in 50% Me_2SO-50% water (v/v) at 20 °C. Apparent pK_a's as well as the pK_a values of the keto and the enol forms, and the enolization equilibrium constants (K_T) were also determined. The pK_a and K_T values show the same trends observed previously in water. The intrinsic rate constants for the reactions of **6b** and **6c** with a given family of amines (primary aliphatic or secondary alicyclic) are the same and also equal to those for the reaction of acetylacetone (**6a**) with the same amines determined previously. These results indicate that steric effects play an insignificant role in the reactions of **6a**, **6b**, and **6c**. The intrinsic rate constants for the deprotonation of **6d** are approximately three fold lower than for **6a-c**. This reduction is shown not to be caused by a steric effect but by π-overlap with the phenyl groups in the enolate ion.

There has been an ongoing interest in the kinetics of proton transfers at carbon in our laboratory.[1-7] A major focus of our work has been an attempt to understand the factors that affect the intrinsic rate constants of reactions of the type shown in eq 1. The intrinsic rate constant is defined as $k_o = k_1/q = k_{-1}/p$ when $pK_a^{BH} - pK_a^{CH_2XY} +$

(1) Bernasconi, C. F.; Kanavarioti, A. *J. Org. Chem.* **1979**, *44*, 4829.
(2) Bernasconi, C. F.; Hibdon, S. A. *J. Am. Chem. Soc.* **1983**, *105*, 4343.
(3) Bernasconi, C. F.; Bunnell, R. D. *Isr. J. Chem.* **1985**, *26*, 420.
(4) Bernasconi, C. F.; Paschalis, P. *J. Am. Chem. Soc.* **1986**, *108*, 2969.
(5) (a) Bernasconi, C. F.; Terrier, F. *J. Am. Chem. Soc.* **1987**, *109*, 7115. (b) Bernasconi, C. F.; Bunnell, R. D.; Terrier, F. *J. Am. Chem. Soc.* **1988**, *110*, 6514.
(6) Bernasconi, C. F.; Kliner, D. A. V. Mullin, A. S.; Ni, J. X. *J. Org. Chem.* **1988**, *53*, 3342.
(7) Bernasconi, C. F.; Stronach, M. W. *J. Am. Chem. Soc.* **1990**, *112*, 8448.

0022-3263/91/1956-3016$02.50/0