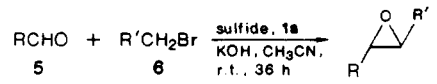
**Table II. Preparation of Optically Active Oxiranes**

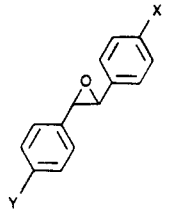



5 6

a, $C_6H_5$; b, 4-$ClC_6H_4$; c, 4-$MeC_6H_4$; d, 4-$NO_2C_6H_4$

| RCHO | $R'CH_2Br$ | yield, % | product[a] | % ee | confgn |
|---|---|---|---|---|---|
| **5b** | **6a** | 100 | **7b** | 43 | (+)-*R,R* |
| **5a** | **6b** | 114 | **7b** | 32 | |
| **5c** | **6a** | 78 | **7c** | 43 | (+)-*R,R* |
| **5a** | **6c** | 94 | **7c** | 29 | |
| **5d** | **6a** | 22 | **7d** | 15 | (+)-*R,R* |
| **5a** | **6d** | 35 | **7d** | 8 | |
| **5b** | **6c** | 90 | **8** | 30 | (+) |
| **5c** | **6b** | 81 | **8** | 21 | |
| **5b** | **6b** | 74 | **9** | 24 | (+) |

[a] [b]Determined by HPLC analysis using Chiralpack OT(+) of Daicel Chemical Ind., Ltd.




**7c**, X = H; Y = $CH_3$
**d**, X = H; Y = $NO_2$
**8**, X = $CH_3$; Y = Cl
**9**, X = Y = Cl

***exo*-2-Methoxy-10-(methylthio)bornane (1c)**: $[\alpha]_D$ = -62.2° (*c* = 1.7, $CHCl_3$); $^1H$ NMR δ 3.40 (m, 1 H), 3.25 (s, 3 H), 2.86 (d, 1 H, *J* = 12 Hz), 2.38 (d, 1 H, *J* = 12 Hz), 2.14 (s, 3 H), 1.88-1.11 (m, 7 H), 0.99 (s, 3 H), 0.84 (s, 3 H); IR (neat, $cm^{-1}$) 1100, 1310.

***endo*-2-Methoxy-10-(methylthio)bornane (2)**: $[\alpha]_D$ = +53.1° (*c* = 2.3, $CHCl_3$); $^1H$ NMR ($CDCl_3$) δ 3.80 (t, 1 H), 3.33 (s, 3 H), 2.63 (d, 2 H), 2.10 (s, 3 H), 2.02-1.04 (m, 7 H), 0.95 (d, 6 H); IR (neat, $cm^{-1}$) 1100.

**Preparation of *exo*-2-Methoxy-10-(dimethoxysulfonio)-bornane Iodide (3b).** To the sulfide **1c** (500 mg, 2.3 mmol) dissolved in $CH_2Cl_2$ (30 mL) was added MeI (2 mL). After 10 h at room temperature, the solvent was evaporated and then a small amount of anhydrous $Et_2O$ was added to the solution. Colorless crystals obtained were recrystallized from $CH_2Cl_2/Et_2O$. **3b**: yield 72.6%; mp 136.5-138 °C. $[\alpha]_D$ = -46.26° (*c* = 0.99, $CHCl_3$); $^1H$ NMR ($CDCl_3$) δ 4.00 (d, 1 H, *J* = 12 Hz), 3.52 (s, 3 H), 3.41 (m, 1 H), 3.36 (s, 3 H), 3.35 (d, 1 H, *J* = 12 Hz), 3.22 (s, 3 H), 2.06-1.13 (m, 7 H), 1.06 (s, 3 H), 1.00 (s, 3 H). Anal. Calcd for $C_{13}H_{25}IOS$: C, 43.95; H, 6.81. Found: C, 43.90; H, 7.23.

**Preparation of *exo*-2-Hydroxy-10-(dimethylsulfonio)-bornane Tetrafluoroborate (3a).** To the sulfide **1a** (398 mg, 2.0 mmol) dissolved in the presence of $AgBF_4$ (400 mg, 2.37 mmol) in $CH_2Cl_2$ (20 mL) was added MeI (150 L, 2.37 mmol) at 0 °C. After 8 h at 0 °C, the solvent was evaporated and then a small amount of anhydrous $Et_2O$ was added to the solution. Colorless crystals obtained were recrystallized from $CH_2Cl_2/Et_2O$. **3a**: yield 50%; mp 178-180 °C; $[\alpha]_D$ = -50.30° (*c* = 0.9, $CHCl_3$); $^1H$ NMR ($CDCl_3$) δ 3.95 (m, 1 H), 3.69 (d, 1 H, *J* = 14.4 Hz), 3.29 (d, 1 H, *J* = 14.4 Hz), 3.03 (s, 3 H), 2.98 (s, 3 H), 2.17-1.29 (m, 7 H), 1.11 (s, 3 H), 0.93 (s, 3 H). Anal. Calcd for $C_{12}H_{23}BF_4OS$: C, 47.69; H, 7.67. Found: C, 47.42; H, 7.80.

**Preparation of (*R,R*)-1-(*p*-Chlorophenyl)-2-phenyloxirane (7b).** Sulfide **1a** (257 mg, 1.3 mmol) and powdered KOH (250 mg) were added to $CH_3CN$ (10 mL). To this solution was added a mixture of benzyl bromide (340 μL, 2.86 mmol) and *p*-chlorobenzaldehyde (347 mg, 2.47 mmol) with vigorous stirring for 36 h. After the reaction, the product was separated by preparative liquid chromatography and its structure identified by $^1H$ NMR and elemental analysis. Yield was 235 mg based on the sulfide **1a**. **7b**: mp 91-93 °C (lit.[9] mp 100 °C); $[\alpha]_D$ = +92.5° (*c* = 0.43, EtOH); ee = 43%. The %ee was determined by HPLC analysis using Chiralpack OT(+) from Daicel Chemical Ind., Ltd. $^1H$ NMR ($CDCl_3$): δ 7.48-7.18 (m, 9 H), 3.83 (d, 2 H). Anal. Calcd for $C_{14}H_{11}ClO$: C, 72.89; H, 4.80. Found: C, 72.58; H, 4.81.

Similarly, other oxiranes were prepared and their spectra and elemental analyses are summarized as follows. **7a**: mp 69-70 °C (lit.[9] mp 69 °C); $^1H$ NMR ($CDCl_3$) δ 7.35 (s, 10 H), 3.86 (s, 2 H). Anal. Calcd for $C_{14}H_{12}O$: C, 85.68; H, 6.16. Found: C, 85.43; H, 6.11. **7c**: mp 55-56 °C (lit.[9] mp 62 °C); $^1H$ NMR ($CDCl_3$) δ 7.34-7.20 (m, 9 H), 3.83 (s, 2 H), 2.35 (s, 3 H). Anal. Calcd for $C_{15}H_{14}O$: C, 85.68; H, 6.71. Found: C, 85.59; H, 6.74. **7d**: mp 120.5-121 °C (lit.[9] mp 127 °C:); $^1H$ NMR ($CDCl_3$) δ 8.34, 7.41 (dd, 4 H, *J* = 9 Hz), 7.38 (s, 5 H), 3.97 (d, 1 H, *J* = 2 Hz), 3.86 (d, 1 H, *J* = 2 Hz). Anal. Calcd for $C_{14}H_{11}NO_3$: C, 69.70; H, 4.59; N, 5.80. Found: C, 69.61; H, 4.58; N, 5.75. **8**: mp 115-116 °C; $^1H$ NMR ($CDCl_3$) δ 7.30-7.17 (m, 8 H), 3.81 (d, 1 H, *J* = 2 Hz), 3.76 (d, 1 H, *J* = 2 Hz), 2.35 (s, 3 H). Anal. Calcd for $C_{15}H_{13}ClO$: C, 73.62; H, 5.35. Found: C, 73.51; H, 5.35. **9**: mp 96-97 °C; $^1H$ NMR ($CDCl_3$) δ 7.34 (d, 4 H, *J* = 10 Hz), 7.26 (d, 4 H, *J* = 10 Hz), 3.78 (s, 2 H). Anal. Calcd for $C_{14}H_{10}Cl_2O$: C, 63.42; H, 3.80. Found: C, 63.14; H, 3.72.

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## Enantiospecific Synthesis of L-α-Aminosuberic Acid. Synthetic Applications in Preparation of Atrial Natriuretic Factor Analogues

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*Received December 19, 1988*

L-α-Aminosuberic acid (**5c**) has been frequently utilized as a metabolically stable isostere for Cys-Cys disulfide linkage in a variety of naturally occurring cyclic hormonal peptides, such as oxytocin,[1] vasopressin,[2] somatostatin,[3] and atrial natriuretic peptides.[4] To date, there have been only two reports on the stereospecific synthesis of α-aminosuberic acid. The first method involved lengthy (18 steps) iterative extension[5a] of the side chain of L-glutamic acid via the Arndt-Eistert procedure. The second one utilized the Kolbe electrolysis[5b] of the mixture of derivatized D-glutamic and glutaric acids, upon which the desired unsymmetrical adduct (D enantiomer) was separated from the two byproduct symmetrical dimers obtained as a statistical mixture. All other syntheses[6] required resolution of racemic α-aminosuberic acid.

Herein we report on efficient and convergent synthesis of L-α-aminosuberic acid (**5c**) (Scheme I) which allows for preparation of the amino acid in an orthogonally protected form **5b**, convenient for peptide synthesis. In addition, this route provides for the novel 4,5-dehydro intermediate **4a**, which is suitable for isotopic labeling or further functionalization of the double bond.

Commercially available[7] *N*-α-t-Boc-L-aspartic acid α-(*tert*-butyl ester) (**1**) was converted to the corresponding *N*-methoxy-*N*-methylamide **2**, which was reduced with diisobutylaluminum hydride to give pure aldehyde **3** in quantitative yield. This method of obtaining α-(t-Boc-amino) aldehydes from α-(t-Boc-amino) acids has been described previously as giving products of high optical purity.[8] Subsequent Wittig condensation[9] of the aldehyde **3** with the ylide derived from (3-carboxypropyl)triphenylphosphonium bromide gave (γ,δ)-*Z*-dehydro-L-α-aminosuberic acid as the N-t-Boc, α-(*tert*-butyl ester) **4a**

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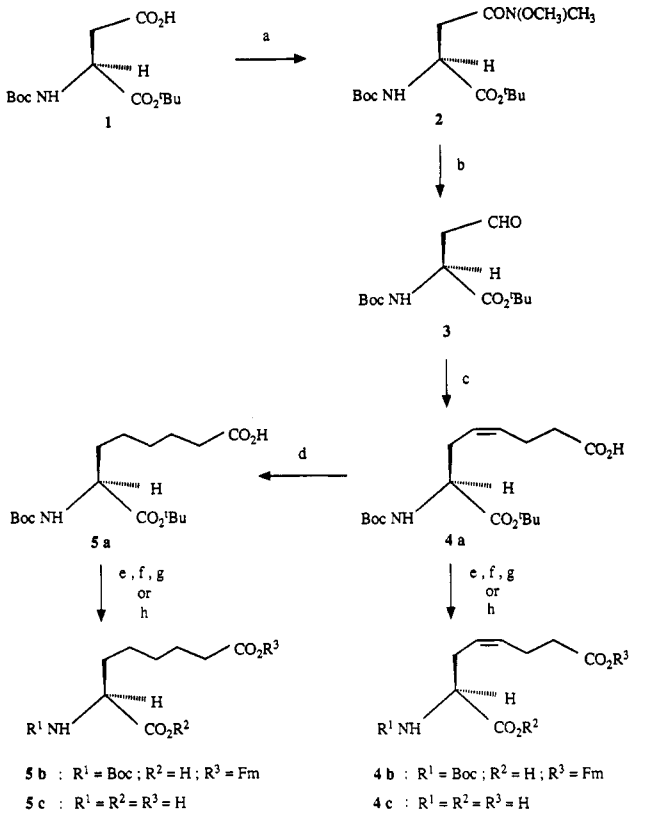
0022-3263/89/1954-4224$01.50/0 

**Scheme I**[a]




[a] (a) $Et_3N$, $CH_3ONHCH_3$·HCl, BOP·$PF_6$, $CH_2Cl_2$; (b) DIBAL, THF, −78 °C; (c) $Ph_3P^+(CH_2)_3CO_2H$·$Br^-$, LiHMDS, HMPA, THF, −78 °C/30 min, 0 °C/30 min; (d) $H_2$, 5% Pd/C, EtOH; (e) FmOH, DCC, DMAP, $CH_2Cl_2$; (f) TFA, $CH_2Cl_2$, 0 °C; (g) $(Boc)_2O$, DIPEA, dioxane/$H_2O$; (h) 6 M HCl/EtOAc.

(the *Z* configuration assignment is based on the olefinic coupling constant $J$ = 10.8 Hz in the $^1$H NMR spectrum of **4a**). Since the ylide was prepared prior to addition of the aldehyde, it is worthwhile to note that utilization of dimsyl sodium base[9a] at room temperature resulted in a racemic product mixture, whereas employment of lithium hexamethyldisilazide[9b] (LiHMDS) at −78 °C to 0 °C produced the optically pure L enantiomer **4a**. Catalytic hydrogenation of the olefinic bond of **4a** gave the desired L-α-aminosuberic acid derivative **5a**.

The optical purity was confirmed[10] at this point by hydrolysis of both protecting groups with hydrogen chloride in ethyl acetate and comparing the optical rotation of obtained L-α-aminosuberic acid hydrochloride (**5c**) with literature values.[5,6a,b]

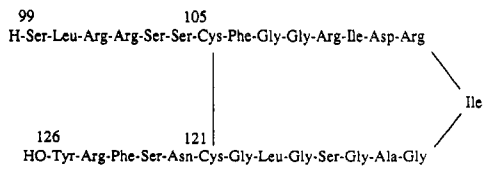
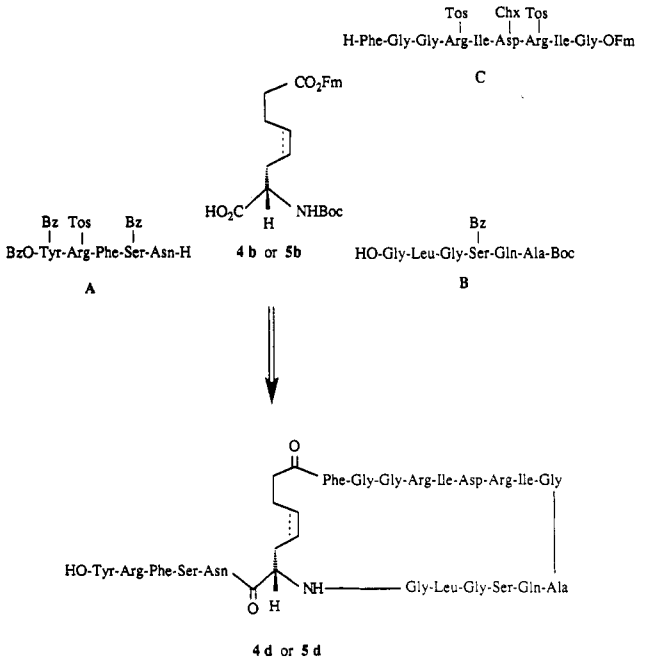



**Figure 1.**

**Scheme II**




Peptide synthesis usually requires the amino acids to be in a free α-carboxyl form with the amino group and the side-chain function derivatized by selectively removable protecting groups. Therefore, we converted the side-chain carboxyl group of **5a** into its 9-fluorenylmethyl ester (Fm) (acid stable), hydrolized both N-t-Boc and α-(*tert*-butyl ester) groups with trifluoroacetic acid, and reintroduced the N-t-Boc protecting group with di-*tert*-butyl dicarbonate to give the desired, orthogonally protected acid **5b**. Similarly, the unsaturated acid **4a** was converted to the orthogonally protected acid **4b**.

Our interest in an efficient synthesis of L-α-aminosuberic acid originated from research on the structure–activity relationship of synthetic analogues of the atrial natriuretic peptide ANF(99–126) (Figure 1). ANF(99–126) belongs to a group of peptides isolated from mammalian atria collectively named Atrial Natriuretic Factor[11] (ANF). The

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(10) Further support for the absence of epimerization during the Wittig reaction employing LiHMDS was provided by comparing the gas chromatograms of derivatives of **4a** and of the racemic products obtained when dimsyl sodium was utilized. Both Wittig products, after identical purification procedures ($^1$H NMR spectra, MS, and combustion analysis results were identical), were derivatized as trifluoroacetylamide, isopropyl diesters and were analyzed using a chiral gas chromatography column (Altech Chirasil-Val-III, 25 m × 0.25 mm, Shimatzu GC-9AM apparatus, helium carrier, split ratio 3/100, injector temperature 250 °C, column temperature 160 °C for 5 min, then continuous gradient 2 °C/min up to 185 °C, FID detector, detector temperature 300 °C). The racemic product derivatives gave two distinct peaks of equal intensity ($t$ = 18.9 min and 19.5 min) and **4a** derivative gave a single peak ($t_R$ = 19.6 min). In an analogous experiment the hydrogenated, hydrolyzed, and derivatized racemic Wittig products were compared to **5c** derivative on the same chiral GC column, resulting in two peaks from the racemate and only one from the optically active product **5c**.

peptides exhibit potent natriuretic, diuretic, and vasorelaxant[12] activity, inhibition of aldosterone secretion[13] from the adrenal gland, and lowering of plasma renin levels.[14] These activities lead to a significant decrease of blood pressure,[15] which makes ANF analogues excellent candidates for potential antihypertensive drugs.[16]

The utility of both amino acids **4b** and **5b** was shown by syntheses of corresponding ANF analogues **4d** and **5d**, according to Scheme II. The peptidic fragments A, B, and C were prepared in solution from commercially available protected N-α-t-Boc-L-amino acids, utilizing standard deprotection and coupling techniques.[17] These fragments were then coupled in solution with **4b** or **5b** in A, B, C alphabetical order, followed by a final cyclization resulting from coupling of the terminal amino acids (glycine and alanine) of the B and C chains.

The intermediate coupling and deprotection products were not purified to homogeneity. Their purity was estimated at every stage of the synthesis as the percentage of UV absorption of the main peak in the HPLC chromatogram. The fully protected, cyclic peptides were deprotected by hydrogen fluoride treatment and purified to homogeneity by reverse-phase medium pressure chromatography on an ODS column to give the final products **4d** and **5d**. The deamino-dicarba analogue **5d** has been synthesized previously by the Sakakibara[4] group, using different protection and coupling methods, but the olefinic analogue **4d** was previously unknown. The ANF analogues **4d** and **5d** were tested for biological activity and do possess antihypertensive and diuretic properties. The detailed biological profiles will be reported elsewhere (*J. Med. Chem.*, manuscript submitted).

## Experimental Section

**General.** All solvents were of commercially anhydrous grade, with the exception of hexamethylphosphoramide (HMPA), which was distilled from calcium hydride at reduced pressure. The reactions were carried out under a dry nitrogen atmosphere and were monitored by thin-layer chromatography (TLC) using E. Merck 60F-254 precoated silica (0.25 mm) on glass. Short column chromatography was performed according to the procedure described by D. F. Taber (*J. Org. Chem.* **1982**, *47*, 1351–52) using silica gel H-60770 from Fluka AG.

Medium pressure liquid chromatography was performed on a Vydac 15–20 μm octadecyl-silica gel (ODS) 2.5 × 28 cm column using continuous solvent gradient from A = 0.06% trifluoroacetic acid (TFA) solution in water to B = 65% methanol–35% A mixture; flow 5 mL/min; detector UV, λ = 277 nm.

High performance liquid chromatography (HPLC) was performed on a Whatman Partisil 10 μm ODS-3 2.2 × 50 cm column, using a continuous solvent gradient from A = 0.06% TFA/$H_2O$ to B = 50% acetonitrile–50% A mixture; flow 20 mL/min; detector UV, λ = 230 nm.

Analytical HPLC was performed on a Vydac $C_{18}$ 10 μm 0.75 × 25 cm column using solvents A = 0.06% TFA/$H_2O$ and B = acetonitrile. The following general conditions were used (unless otherwise indicated): continuous gradient from 20% B to 50% B in 20 min; flow 1.5 mL/min; detector UV, λ = 234 nm. Relative peak absorptions (%) were established on a Shimadzu C-R3A Chromatopac recorder.

$^1H$ NMR spectra were recorded on a Bruker AC-P 200 QNP high resolution FT NMR cryospectrometer (200 MHz). FAB mass spectra were obtained on a KRATOS MS-50TCT mass spectrometer provided by the Centre Régionale de Spectrométrie de Masse of the University of Montréal. Melting points were determined on a Buchi 510 melting point apapratus and are uncorrected. Optical rotations were obtained in the indicated solvent and concentration in a 1-dm cell using a Perkin-Elmer 241 polarimeter. For amino acid analysis, peptides were hydrolyzed in 6 N HCl at 110 °C for 20 h. The hydrolysates were analyzed on a Waters LC apparatus equipped with an ion exchange column, post column derivatization using *o*-phthaldehyde and a Model 420-AC fluorescence detector.

***N*-t-Boc-L-aspartic Acid 1-(*tert*-Butyl ester) *N*-Methoxy-*N*-methylamide (2).** (Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP-$PF_6$, 16.82 g, 38.03 mmol) was added to a stirred solution of *N*-t-Boc-L-aspartic acid 1-(*tert*-butyl ester) (10 g, 34.6 mmol) and triethylamine (3.9 g, 38.5 mmol) in dichloromethane (350 mL) at ambient temperature. After 5 min of stirring *O,N*-dimethylhydroxylamine hydrochloride (4 g, 39.7 mmol) was added, followed by triethylamine (3.9 g, 38.5 mmol). All solid material dissolved in 10 min and the mixture continued to stir for 2 h at ambient temperature. The reaction mixture was then washed with 1 M HCl (3 × 100 mL), $H_2O$ (1 × 100 mL), 1 M $NaHCO_3$, (2 × 100 mL), and $H_2O$ (2 × 100 mL) and was dried ($Na_2SO_4$). Concentration in vacuo gave a yellow, oily product (15.40 g). Short column chromatography (300 g of silica gel, EtOAc/hexane = 3:7) gave the title product **2** as a colorless oil (8.05 g, 70%): $[\alpha]^{27}_D$ −8.8° (*c* 1, EtOH); $^1H$ NMR (DMSO-$d_6$) δ 1.38 [s, 18 H, 2 × $C(CH_3)_3$], 2.75 (m, 2 H, $CH_2$), 3.08 (s, 3 H, $CH_3$), 3.66 (s, 3 H, $CH_3$), 4.23 [dt, 1 H, *J*(CHNH) = 8.44 Hz, *J*($CHCH_2$) = 5.98 Hz, CH], 7.02 [d, 1 H, *J*(NHCH) = 8.44 Hz, NH]. Anal. Calcd for $C_{15}H_{28}N_2O_6$: C, 54.20; H, 8.49; N, 8.43. Found: C, 53.76, H, 8.59; N, 8.21.

**1-*tert*-Butyl L-2-(t-Boc-amino)-4-oxobutanoate (3).** A solution of diisobutylaluminum hydride (DIBAL) in hexane (1 M, 30 mL, 30 mmol) was added dropwise (20 min) into a stirred solution of **2** (6.55 g, 19.7 mmol) in anhydrous THF (100 mL) at −75 °C. Upon addition the mixture continued to stir at −75 °C for 2 h. The reaction mixture was partitioned between 0.35 M $NaHSO_4$ aqueous solution (200 mL) and ether (300 mL) and the aqueous layer was extracted with ether (3 × 100 mL). The combined ethereal solutions were washed with 1 M HCl (3 × 100 mL), 1 M $NaHCO_3$ (3 × 100 mL), and brine (3 × 100 mL) and dried ($Na_2SO_4$). Concentration in vacuo gave the title product **3** (5.29 g, 98%) as a colorless oil, which solidified on standing at ambient temperature. An analytical sample was obtained by recrystallization from hexane, mp 66–67 °C dec: $[\alpha]^{25}_D$ −31.8° (*c*, 1, EtOH); $^1H$ NMR ($CDCl_3$) δ 1.44 [s, 9 H, $C(CH_3)_3$], 1.45 [s, 9 H, $C(CH_3)_3$], 2.98 (m, 2 H, $CH_2$), 4.47 (m, 1 H, CH), 5.36 [d, 1 H, *J*(NHCH) = 6.65 Hz, NH], 9.74 (s, 1 H, CHO). Anal. Calcd for $C_{13}H_{23}NO_5$: C, 57.13; H, 8.48; N, 5.12. Found: C, 57.26; H, 8.67; N, 5.05.

***N*-t-Boc-L-α-amino-γ,δ-(*Z*)-dehydrosuberic Acid, α-(*tert*-Butyl ester) (4a).** (3-Carboxypropyl)triphenylphosphonium bromide was prepared by heating to reflux a mixture of 4-bromobutyric acid (9.29 g, 54.5 mmol) and triphenylphosphine (17.5 g, 66 mmol) in toluene (200 mL) for 21 h, while removing water via a Dean–Stark adaptor. The mixture was then cooled to 0 °C and was filtered to give the product as a white solid, which was dried in vacuo at 112 °C (11.02 g, 47%).

A 1 M solution of lithium hexamethyldisilazide in THF (LiHMDS, 28.5 mL, 28.5 mmol) was added dropwise (15 min) into a stirred mixture of (3-carboxypropyl)triphenylphosphonium bromide (7.20 g, 16.75 mmol), anhydrous THF (50 mL), and anhydrous HMPA (20 mL) at 0 °C. Upon addition the orange-red mixture was stirred at 0 °C for 1 h and then was cooled to −78 °C. A solution of **3** (2.7 g, 9.88 mmol) in anhydrous THF (25 mL) was added dropwise (25 min). The resulting yellow mixture was stirred at −78 °C for 30 min and then at 0 °C for another 30 min. The reaction mixture was poured into aqueous 10% citric acid (700 mL) and was extracted with ether (5 × 100 mL). The combined ethereal extracts were washed with brine (2 × 50 mL),

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dried ($MgSO_4$), and concentrated in vacuo to give a yellow, viscous oil (5.54 g). This residue was dissolved in ethyl acetate (200 mL) and the solution was washed with 10% LiCl (3 × 50 mL), water (3 × 50 mL), and brine (2 × 50 mL). All aqueous extracts were back-extracted with ethyl acetate (1 × 20 mL). The combined organic phase was dried ($Na_2SO_4$) and concentrated in vacuo to give a yellow oil (3.84 g). Short column chromatography (300 g of silica gel; EtOAc/hexane/AcOH = 600:200:5) gave recovered starting material **3** (0.74 g, 27% recovered) and the title product **4a** as a colorless oil (1.12 g, 33% or 46% considering recovery of starting material). An analytical sample was obtianed by crystallization from hexane, mp 80–83 °C dec: $[\alpha]^{27}_D$ −4.1° (*c* 1, EtOH); $^1H$ NMR ($CDCl_3$) δ 1.43 [s, 9 H, $C(CH_3)_3$], 2.45 (m, 6 H, $CH_2$, $CH_2CH_2$), 4.25 [dt, 1 H, *J*(CHNH) = 8.18 Hz, *J*($CHCH_2$) = 5.52 Hz, CH], 5.15 [d, 1 H, *J*(NHCH) = 8.18 Hz, NH], 5.42 [m, 2 H, *J*(CH=CH) = 10.80 Hz, CH=CH], 7.40 (br, 1 H, $CO_2H$). Anal. Calcd for $C_{17}H_{29}NO_6$: C, 59.46; H, 8.51; N, 4.08. Found: C, 59.25; H, 8.58; N, 3.97.

***N*-t-Boc-L-α-amino-γ,δ-(*Z*)-dehydrosuberic Acid, 8-[(9-Fluorenyl)methyl ester] (4b).** A solution of 1,3-dicyclohexylcarbodiimide (DCC) (265 mg, 1.28 mmol) in dichloromethane (5 mL) was added to the solution of **4a** (400 mg, 1.16 mmol), 9-fluorenemethanol (250 mg, 1.27 mg, 1.27 mmol), and 4-(dimethylamino)pyridine (DMAP) (28 mg, 0.23 mmol) in dichloromethane (15 mL) at 0 °C. The mixture was stirred for 18 h, while the temperature was allowed to rise to the ambient level. The reaction mixture was filtered and the filtrate was concentrated in vacuo. The oily residue was chromatographed (short column, 30 g of silica gel, EtOAc/hexane = 1:5) to give a colorless oil (570 mg, 94%).

The above product (530 mg, 1.04 mmol) was dissolved in dichloromethane (15 mL), and the solution was cooled to 0 °C and was treated with anhydrous trifluoroacetic acid (TFA) (15 mL dropwise, 5 min). The reaction mixture was stirred for 18 h at ambient temperature and was concentrated in vacuo, and the residue was dissolved in methanol (3 × 30 mL) and concentrated in vacuo (three times) to give a yellow oily product (518 mg, 100%).

The above product (518 mg, 1.04 mmol) was dissolved in a mixture of dioxane (10 mL) and water (10 mL). The solution was cooled to 0 °C and was treated with triethylamine (0.55 mL, 3.93 mmol), followed by addition of di-*tert*-butyl dicarbonate ($Boc_2O$, 680 mg, 3.12 mmol) in dioxane (10 mL). The mixture was stirred at 0 °C for 1 h and then at ambient temperature for 18 h. The reaction mixture was then concentrated in vacuo. The residue was suspended in water (100 mL), treated with an aqueous solution of citric acid (10%, 100 mL), and extracted with ethyl acetate (4 × 30 mL). The combined organic extracts were dried ($Na_2SO_4$) and were concentrated in vacuo to give a yellow oil (530 mg). Chromatography (short column, 10 g of silica gel, EtOAc/hexane/AcOH = 300:600:4) gave the title product **4b** as a colorless, semisolid oil (380 mg, 78%): $^1H$ NMR ($CDCl_3$) δ 1.45 [s, 9 H, $C(CH_3)_3$], 2.40 (m, 4 H, $CH_2CH_2$), 2.65 (m, 2 H, $CH_2$), 4.20 [t, 1 H, *J*($CHCH_2$) = 6.89 Hz, $CH_2CH$ (fluorenyl)], 435 (m, 1 H, CHNH), 4.40 [d, 2 H, *J*($CH_2CH$) = 6.89 Hz, $CH_2CH$ (fluorenyl)], 5.25 [d, 1 H, *J*(NHCH) = 8.27 Hz, NH], 5.40 [m, 2 H, *J*(CH=CH) = 10.80 Hz, CH=CH], 7.50 (m, 8 H, Ar); FAB mass spectrum, *m/z* (rel intensity) 464 (10, M − 2), 466 (100, $M^+$), 467 (40, $MH^+$).

**L-α-Amino-γ,δ-(*Z*)-dehydrosuberic Acid Hydrochloride (4c).** A 6 M solution of HCl in EtOAc (4 mL, 24 mmol) was added to a solution of **4a** (100 mg, 0.29 mmol) in EtOAc. The mixture was stirred for 18 h at ambient temperature and then was cooled to 0 °C. Filtration gave the title product **4c** as a while solid, which was rinsed with cold EtOAc (1 mL) and hexane (20 mL) and was dried in vacuo (51 mg, 78%), mp 160–162 °C dec: $[\alpha]^{25}_D$ +14.5° (*c* 1, 6 M HCl); $^1H$ NMR (DMSO-$d_6$) δ 2.26 (m, 4 H, $CH_2CH_2$), 2.60 (m, 2 H, $CHCH_2CH$), 3.93 (m, 1 H, $H_3N^+CHCO_2H$), 5.47 [m, 2 H, *J*(CH=CH) = 10.80 Hz, CH=CH], 8.47 (br, 3 H, $^+NH_3$), 12.90 (br, 1 H, $CO_2H$). Anal. Calcd for $C_8H_{13}NO_4$·HCl: C, 42.96; H, 6.31; N, 6.26. Found: C, 42.56; H, 6.33; N, 6.15.

**Peptide Fragments A, B, and C.** The peptidic fragments A, B, and C (Scheme II) were prepared according to standard solution methods[17] from commercially available, side-chain protected *N*-α-t-Boc-L-amino acids. Dicyclohexylcarbodiimide (DCC), *N*-(ethoxycarbonyl)-2-ethoxy-1,2-dihydroquinoline (EEDQ), and diphenyl phosphorazidate (DPPA) were used as coupling agents. The carboxyl group was protected as methyl or 9-fluorenylmethyl ester (OFm, effectively removed when necessary with 10% piperidine solution in methylene chloride or *N*,*N*-dimethylformamide). The *N*-α-t-Boc group was removed when necessary with 50% trifluoroacetic acid (TFA) in methylene chloride. The fragments were characterized by TLC, HPLC, amino acid analysis (AAA), and FAB mass spectrometry. The data are presented in Table I.

**Table I**[a]

| | A | B | C |
|---|---|---|---|
| | | AAA | |
| | Asn(1.11), Ser(0.96), Phe(1.07), Arg(0.93), Tyr(0.92) | Ala(1.00), Gln(1.03), Ser(0.89), Gly(2.21), Leu(1.03) | Asp(1.01), Gly(3.16), Ile(1.90), Arg(1.83), Phe(1.10) |
| | | TLC | |
| $R_f$ (solvent) | 0.70 (I) | 0.45 (I) | 0.65 (I) |
| | | HPLC | |
| $t_R$ (solvent) | 10.9 min (II) | 10.2 min (III) | 11.5 min (II) |
| | | FABMS | |
| *m/z* | 1111 $(M + H)^+$ | 723 $(M + H)^+$ | 756 $(M + 2H)^+$ |

[a] Solvent systems: (I) *n*-BuOH/AcOH/$H_2O$ = 4/1/5 upper phase; (II) A = 0.06% TFA/$H_2O$, B = $CH_3CN$; continuous gradient 50 to 90% B in 20 min, flow 1.5 mL/min, detector UV λ = 234 nm; (III) A = 0.06% TFA/$H_2O$, B = $CH_3CN$; continuous gradient 60 to 90% B in 20 min, flow 1.5 mL/min, detector UV λ = 234 nm.

**Deamino$^{105}$-(L-α-amino-γ,δ-(*Z*)-dehydrosuberic acid)$^{105,121}$-rANF(105–126) (4d).** A solution of **4b** (350 mg, 0.75 mmol) in $CH_2Cl_2$ (20 mL) was added to a stirred mixture of the peptidic fragment A (540 mg, 0.44 mmol), 1-hydroxybenzotriazole (HOBt) (100 mg, 0.75 mmol), *N*,*N*-diisopropylethylamine (DIPEA) (193 mg, 1.5 mmol), *N*,*N*-dimethylformamide (DMF) (10 mL), and $CH_2Cl_2$ (20 mL). The mixture was cooled to 0 °C and was treated with a solution of DCC (155 mg, 0.75 mmol) in $CH_2Cl_2$ (5 mL). The reaction mixture was stirred at 0 °C for 1 h and then at ambient temperature for 18 h. Concentration in vacuo gave a semisolid material, which was partially dissolved in DMF (10 mL) and was filtered. The filtrate was concentrated in vacuo and the oily residue was triturated with EtOAc (50 mL) to give a white precipitate. The suspension was cooled to 0 °C and was filtered and the precipitate was rinsed with EtOAc (20 mL) and ether (20 mL) and dried in vacuo to give a white solid (627 mg, 91%). HPLC (general conditions) of this material indicated 88% purity.

The above product (590 mg, 0.38 mmol) was stirred in $CH_2Cl_2$ (10 mL) at 0 °C, while TFA (10 mL) was added dropwise. The reaction mixture was stirred at 0 °C for 1 h. Concentration in vacuo gave an oily residue, which upon trituration with ether and filtration gave a white solid material (600 mg, 100%). HPLC of this material indicated 82% purity.

The above product (590 mg, 0.37 mmol) and the peptidic fragment B (410 mg, 0.57 mmol) were dissolved in DMF (30 mL); the solution was cooled to 0 °C and was treated with DIPEA (170 mg, 1.31 mmol), followed by addition of diphenyl phosphorazidate (DPPA) (605 mg, 2.2 mmol). The reaction mixture was stirred at 4 °C for 8 h; then it was concentrated in vacuo to semidryness. The residue was triturated with ether, filtered, and dried to give a white solid material, which was subsequently washed with boiling ethanol (60 mL). Filtration and drying gave a white solid product (673 mg, 84%). HPLC of this material indicated 85% purity.

The above product (652 mg, 0.3 mmol) was dissolved in DMF (50 mL) with warming, and the solution was cooled to 0 °C and was treated with piperidine (10 mL, excess, dropwise). The reaction mixture was stirred at 0 °C for 1 h and was concentrated in vacuo, and the gelatinous residue was tritiurated with EtOAc (2 × 50 mL) and with a 10% AcOH solution in ethanol (3 × 50 mL). Final filtration and drying gave a white, solid product (590 mg, 99%). HPLC of this material indicated 84% purity.

The above product (570 mg, 0.28 mmol) and the peptidic fragment C 530 mg, 0.31 mmol) were dissolved in DMF (50 mL) with warming. The solution was cooled to 0 °C and treated with DIPEA (111 mg, 0.86 mmol), followed by DPPA (400 mg, 1.45 mmol). The reaction mixture was stirred at 0 °C for 16 h. HPLC of an aliquot of the reaction mixture indicated 50% conversion of the starting material. Therefore more DIPEA (111 mg, 0.85

mmol) and more DPPA (1.35 g, 4.9 mmol) were added. The reaction mixture continued to stir at 4 °C for 18 h. HPLC of an aliquot indicated 90% conversion. The reaction mixture was concentrated in vacuo. The residue was heated in methanol (50 mL) for 1 h. Filtration and washing with methanol and then with ether and drying gave a white solid material (680 mg). Additional product precipitated from the methanolic filtrate (40 mg) upon cooling to ambient temperature (total amount 720 mg, 71%). HPLC of this material indicated the presence of one major product with 50% purity.

The above product (680 mg, 0.187 mmol) was dissolved in DMF (60 mL) on heating and the solution was cooled to 0 °C. Piperidine (15 mL) was added dropwise and the mixture was stirred at 0 °C for 30 min. Concentration of the reaction mixture in vacuo, trituration of the residue with ether and then with a 10% AcOH solution in ethanol (3 × 50 mL), rinsing with ether, and drying gave a white solid material (555 mg, 88%). HPLC of this material indicated 79% purity.

The above material (550 mg, 0.16 mmol) was suspended in $CH_2Cl_2$ (40 mL) at 0 °C while TFA (20 mL) was added dropwise. The mixture was stirred at 0 °C for 30 min. The reaction mixture was then concentrated in vacuo to 2 mL. Addition of ether (50 mL) gave a white precipitate, which was filtered, rinsed with ether and hexane, and dried (540 mg, 82%). HPLC of this material indicated 75% purity.

The above product (524 mg, 0.156 mmol) was dissolved in DMF (40 mL) with warming, and the solution was cooled to ambient temperature and was added dropwise (via peristaltic pump, 30 min) into a stirred solution of DPPA (730 mg, 2.65 mmol) and DIPEA (161 mg, 1.25 mmol) in DMF (500 mL) at 0 °C. The reaction mixture was then stored at 4 °C for 18 h. Concentration in vacuo to 5 mL and dilution with EtOAc (60 mL) gave a white precipitate, which was filtered, rinsed with EtOAc and ether, and dried (490 mg, 94%). HPLC of this material indicated a single major product with 52% purity.

The above product (466 mg, 0.14 mmol) was treated with liquid hydrogen fluoride (20 mL) in the presence of anisole (2.7 mL) and 1,2-ethanedithiol (0.8 mL) at −15 °C for 1 h and then at 0 °C for 1 h. Hydrogen fluoride was removed in vacuo, and the residue was partitioned between ether (100 mL) and 10% AcOH (200 mL). The ethereal solution was further extracted with 10% AcOH (1 × 200 + 1 × 50 mL). The aqueous extracts were combined, washed with ether (5 × 50 mL), concentrated in vacuo to 100-mL volume, and lyophylized to give a white solid product (450 mg). Sonication of this material in a 0.06% TFA aqueous solution and filtration of the suspension through 45-μm membrane filter gave a clear solution of the product (HPLC indicated 47% purity).

Preparative HPLC of this solution gave the title product **4d** (140 mg, 86% HPLC purity). Further purification via ODS medium pressure column chromatography gave 50 mg of a white, solid material, which was 90.9% pure according to HPLC analysis (general conditions). TLC (nBuOH/pyridine/AcOH/$H_2O$ = 15:10:3:12) $R_f$ = 0.50 (visualized with chlorine peptide spray).[18] Amino acid analysis, expected (found) 1 Asp + 1 Asn (1.99), 2 Ser (1.81), 1 Gln (0.99), 5 Gly (5.18), 1 Ala (1.00), 2 Ile (1.90), 1 Leu (0.98), 1 Tyr (0.83), 2 Phe (2.01), 3 Arg (3.31), 2 $NH_3$ (2.33), 1 **4c** (0.97); total peptide content, 77.7 ± 1%; FAB mass spectrum, $m/z$ (rel intensity) 2325 (30, $M^+ + 2$), 2324 (70, $MH^+$), 2322 (90, $M^+ - 1$).

***N*-*t*-Boc-L-α-aminosuberic Acid, α-(*tert*-Butyl ester) (5a).** A mixture of **4a** (420 mg, 1.22 mmol) and 5% Pd/C (100 mg) in ethanol (40 mL) was shaken for 12 h in a Parr bottle charged with hydrogen at 40–50 psi. Filtration through Celite and concentration in vacuo gave a colorless oil (434 mg). The oil was dissolved in hexane (20 mL), and the solution was filtered through a 45-μm membrane and concentrated in vacuo. The residue was crystallized from hexane to give the title product **5a** as colorless crystals (376 mg, 89%), mp 81–82 °C dec: $[\alpha]^{25}_D$ = −17.0° (*c* 1, EtOH); $^1$H NMR ($CDCl_3$) δ 1.46 [s, 9 H, $C(CH_3)_3$], 1.48 [s, 9 H, $C(CH_3)_3$], 1.50 [m, 8 H, $(CH_2)_4$], 2.35 [t, 2 H, $J(CH_2CH_2)$ = 6.85 Hz, $CH_2CO_2H$], 4.16 (m, 1 H, CH), 5.05 [d, 1 H, $J$(NHCH) = 8.18 Hz, NH]. Anal. Calcd for $C_{17}H_{31}NO_6$: C, 59.11; H, 9.05; N, 4.05. Found: C, 59.04; H, 9.20; N, 3.90.

***N*-*t*-Boc-L-α-aminosuberic Acid, 8-[(9-Fluorenyl)methyl ester] (5b).** A solution of DCC (460 mg, 2.23 mmol) in dichloromethane (5 mL) was added to a solution of **5a** (700 mg, 2.03 mmol), 9-fluorenemethanol (440 mg, 2.23 mmol), and DMAP (50 mg, 0.4 mmol) in dichloromethane (20 mL) at 0 °C. The mixture was stirred for 16 h and was allowed to warm up to room temperature. The reaction mixture was filtered and the filtrate was concentrated in vacuo. The oily residue was dissolved in ethyl acetate (50 mL) and the solution was washed with 1 M HCl (4 × 30 mL), water (1 × 20 mL), 1 M $NaHCO_3$ (4 × 30 mL), water (1 × 20 mL) and brine (2 × 30 mL). The final organic solution was dried ($Na_2SO_4$) and concentrated in vacuo to give yellow oil (1.23 g). This residue was chromatographed (short column, 40 g of silica gel, hexane/ethyl acetate = 4.1) to yield a colorless oily product (1.04 g). Crystallization from hexane gave a white solid (956 mg, 90%).

The above product (920 mg, 1.76 mmol) was dissolved in dichloromethane (15 mL), and the solution was cooled to 0 °C and was treated with anhydrous TFA (dropwise; 5 min, 15 mL). The mixture was then stirred at ambient temperature for 1 h and was concentrated in vacuo and the residue was dissolved in methanol (3 × 30 mL) and concentrated in vacuo (three times). The final oily residue was triturated with ether (20 mL) to produce a precipitate, which was filtered, rinsed with ether (3 × 20 mL) and hexane (3 × 20 mL), and dried in vacuo to give a white solid (660 mg, 78%).

The above product (630 mg, 1.31 mmol) was dissolved in a mixture of dioxane (10 mL) and water (10 mL). The solution was cooled to 0 °C and was treated with triethylamine (0.55 mL, 3.93 mmol), followed by di-*tert*-butyl dicarbonate (860 mg, 3.93 mmol) in dioxane (20 mL). The mixture was stirred at 0 °C for 1 h and then at ambient temperature for 15 h. The reaction mixture was diluted with water (100 mL) and was poured into an aqueous solution of citric acid (10%, 200 mL), and the mixture was extracted with ethyl acetate (5 × 50 mL). The combined organic extracts were dried ($Na_2SO_4$) and were concentrated in vacuo to give a semisolid, oily product (1.1 g). Chromatography (short column, 50 g of silica gel, EtOAc/hexane/AcOH = 100:500:5) gave the title product as a colorless oil (488 mg, 80%): $^1$H NMR ($CDCl_3$) δ 1.50 [m, 8 H, $(CH_2)_4$], 1.45 [s, 9 H, $C(CH_3)_3$], 2.36 [t, 2 H, $J(CH_2CH_2)$ = 7.30 Hz, $CH_2CH_2CO_2$], 4.20 [t, 1 H, $J(CHCH_2)$ = 6.85 Hz, $CH_2CH$ (fluorene)], 4.30 [m, 1 H, $J$(NHCH) = 8.27 Hz, NHCH], 4.40 [d, 2 H, $J(CHCH_2)$ = 6.85 Hz, $CH_2CH$ (fluorene)], 5.00 [d, 1 H, $J$(NHCH) = 8.27 Hz, NH], 5.80 [br, 1 H, $CO_2H$, 7.52 (m, 8 H, Ar)]; FAB mass spectrum, $m/z$ (rel intensity) 465 (32, $M^+ - 21$), 467 (100, $M^+$), 4.68 (31, $MH^+$).

**L-α-Aminosuberic Acid Hydrochloride (5c).** A 6 M solution of HCl in EtOAc (4 mL, 24 mmol) was added to a solution of **5a** (100 mg, 0.29 mmol) in EtOAc. The mixture was stirred for 2.5 h at ambient temperature and then was cooled to 0 °C, while the product precipitated.

Filtration of the precipitate gave the title product **5c** as a white solid, which was rinsed with EtOAc (1 mL) at 0 °C and with hexane (20 mL) and was dried in vacuo (50 mg, 77%), mp 182–184 °C dec: $[\alpha]^{25}_D$ +21.1° (*c* 1, 6 M HCl) (lit. value[5] $[\alpha]^{25}_D$ +20.2° (*c* 0.1, 5 M HCl)); $^1$H NMR (DMSO-$d_6$) δ 1.50 [m, 8 H, $(CH_2)_4$], 2.20 [t, 2 H, $J(CH_2CH_2)$ = 6.85 Hz, $CH_2CO_2H$], 3.85 (br, 1 H, CH), 8.36 (br, 3 H, $NH_2$, $CO_2H$). Anal. Calcd for $C_8H_{15}NO_4$·HCl: C, 42.58; H, 7.15; N, 6.21. Found: C, 42.39; H, 7.18; N, 6.26.

**Deamino$^{105}$-(L-α-aminosuberic acid)$^{105,121}$-rANF(105–126) (5d).** **5d** was prepared in analogous manner to the preparation of **4d**.

Final purification of the post HF material via ODS medium pressure column chromatography gave the title product as a white, solid material (34 mg), which was 99.8% pure according to analytical HPLC (general conditions). TLC (nBuOH/pyridine/AcOH/$H_2O$ = 15:10:3:12) $R_f$ = 0.54 (visualized with chlorine peptide spray[18]). Amino acid analysis, expected (found) 1 Asp + 1 Asn (1.96), 2 Ser (1.85), 1 Gln (1.00), 5 Gly (5.14), 1 Ala (0.97), 2 Ile + **5c** (2 + 1), 1 Leu (1.01), 1 Tyr (0.94), 2 Phe (1.98), 3 Arg (3.14) 2 $NH_3$ (2.43); total peptide content 73.8 ± 2%; FAB mass spectrum, $m/z$ (rel intensity) 2327 (50, $M^+ + 2$), 2326 (99, $MH^+$), 2325 (100, $M^+$), 2324 (98, $M^+ - 1$).

**Acknowledgment.** We thank Dr. François Bruderlein for performing the GC experiments.

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