Synthesis of 9-Fluorenylmethyloxycarbonyl-Protected N-Alkyl Amino Acids by Reduction of Oxazolidinones

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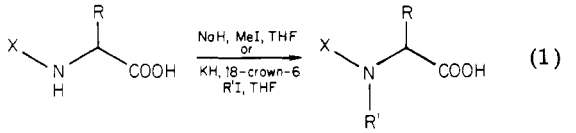
A new two-step synthesis of Fmoc-protected N-alkyl amino acids has been developed. The first step involves acid-catalyzed condensation of an Fmoc-protected amino acid with an aldehyde to form an oxazolidinone. This intermediate is then reduced with triethylsilane and trifluoroacetic acid to the N-alkylated derivative. The procedure is applicable to a variety of amino acids and aldehydes and results in less than 0.1% racemization as measured by a sensitive NMR assay which uses ^{13}C satellite signals as internal reference peaks.

N-Methyl amino acid substitutions in biologically active peptides are useful for obtaining information about backbone conformation.[1] These modifications may also lead to beneficial changes in biological properties such as enhanced potency and duration of action or conversion of an agonist to an antagonist.[2] Enhanced potencies have been observed when higher *N*-alkyl substitutions are employed.[3] Certain *N*-alkyl amino acids are biologically active in their own right.[4]

For purposes of peptide synthesis, it is desirable to have protected optically pure *N*-alkyl amino acids. The method of choice for preparing *N*-methyl amino acids protected with a base-stable group such as Boc or Cbz is the NaH–MeI procedure of Benoiton (eq 1).[5] Introduction of higher

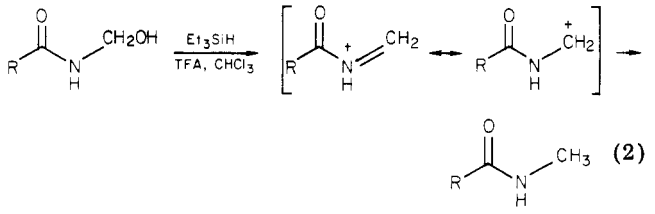


(1)

X = Boc or Cbz

N-alkyl groups by this route, however, is generally not satisfactory.[6] A recent improvement employing KH/18-crown-6 with alkyl iodides provides access to a variety of Boc *N*-alkyl amino acids, although yields appear to be low.[3,7] No general method has been reported for N-alkylation of amino acids protected with base-labile groups such as Fmoc.[17] Such a method would be useful in light of the increasing importance of Fmoc amino acids in peptide synthesis.[8]

Weinreb and co-workers have shown that simple amides can be N-methylated by reduction of their methylol derivatives with the triethylsilane–trifluoroacetic acid system (eq 2).[9] Methylols and alkylols of acyl amino acids would



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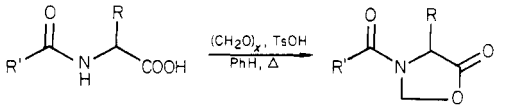
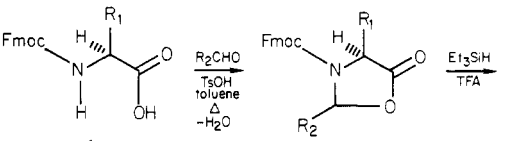


(3)

not be expected to be isolable,[10] however, making direct application of this methylation difficult. The corresponding lactone derivatives (oxazolidinones) should be stable and subject to reduction via *N*-acyl iminium ions analogous to methylols. Acid-stable acyl groups should not

Table I. Oxazolidinones and Fmoc *N*-Alkyl Amino Acids from Fmoc Amino Acids



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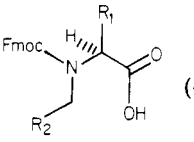
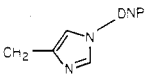


(4)

3

			yield, %	
entry	R_1	R_2	2	3
a	CH_3	H	96	98
b	CH_3	cyclohexyl	77	91
c	CH_3	CH_3	79	74
d	CH_3	CH_2Ph	30	95
e	$CH(CH_3)_2$	H	96	100
f	$(CH_2)_2SCH_3$	H	88	22
g	CH_2Ph	H	73	70
h	$(CH_2)_4NPht$	H	73	70
i	CH_2OCH_2Ph	H	98	96
j		H	37	67

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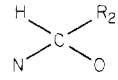
be affected by these conditions. Ben-Ishai has reported the synthesis of such oxazolidinones from acyl amino acids and paraformaldehyde in the presence of *p*-toluenesulfonic acid (eq 3).[11] This report describes the successful implementation of this chemistry resulting in a general racemization-free route from Fmoc amino acids to Fmoc *N*-alkyl amino acids by way of intermediate oxazolidinones.

Results and Discussion

The general features of this new method for N-alkylating Fmoc amino acids are illustrated in eq 4 in Table I. The often commercially available Fmoc amino acid **1** is condensed with an aldehyde in the presence of *p*-toluenesulfonic acid in refluxing toluene to furnish the oxazolidinone **2**. Yields are generally good to excellent. These oxazolidinones are stable compounds characterized by a lactone carbonyl stretch in the IR spectrum at 1800 cm^{-1} and a signal in the proton NMR spectrum in the vicinity of 5.1 ppm (see I). Treatment of the oxazolidinone with



I

excess Et_3SiH in 1:1 TFA–$CHCl_3$ results in ring opening with reduction to provide Fmoc *N*-alkyl amino acids **3** in good overall yield.

The generality of this approach is illustrated in Table I. The method works well for preparing protected *N*-methyl derivatives of a variety of amino acids and may be applied to lysine and histidine which have not been available from other general methods. A wide range of *N*-alkyl groups can also be introduced from readily available aldehydes as indicated by entries **b–d**. The only limitation to R_1 and R_2 appears to be groups which may be unstable or react by different pathways under the reaction conditions. The method in its present form is not applicable to ketones. Both benzophenone and acetophenone failed to give any desired products with Fmoc-Ala. Other acyl protecting groups stable to the reaction conditions should function as well as Fmoc. An experiment with Cbz-Gly indicates that carbobenzoxy will be compatible with the method.

Although the configuration of the new chiral center in oxazolidinones **2b–d** has not been investigated in detail, TLC usually shows evidence of two diastereomers. In the case of **2d**, an upfield shifted methyl signal at 0.5 ppm indicates that the major component is the 2*S*,4*S* isomer. For compounds **2a–c**, this signal is found in the range of 1.2–1.3 ppm. This shift is due to a shielding effect by the benzyl substituent which can only occur if the two substituents on the oxazolidinone have the cis relationship. Model building shows that this shielding effect is not likely to be due to the Fmoc group, and it has not been observed in the other compounds in this study.

Three approaches to N^{α}-methyllysine[12] have been investigated, and these serve to point out the limitations as well as the versatility of the method. As indicated by entry **h** of Table I, N^{α},N^{ϵ}-diprotected N^{α}-methyllysine can be prepared in good yield via the N^{α}-Fmoc N^{ϵ}-phthalyl derivative. This compound is adaptable to peptide synthesis since it was found that Fmoc can be removed selectively with 1:1 diisopropylamine–DMF. Piperidine, more commonly used for removal of Fmoc, also attacks phthalimide. A more versatile protecting group for the ϵ-amino group was desirable, however, and reaction of N^{α}-Fmoc-N^{ϵ}-Cbz-Lys with paraformaldehyde was studied. Unfortunately, selective formation of the desired oxazolidinone could not be accomplished. Several attempts furnished a mixture of products, indicating that masking of both hydrogens on the ϵ-amine is required. A suitably protected N^{α}-methyllysine was synthesized by the route outlined in Scheme I. α-Aminopimellic acid (**4**) was protected with FmocCl. Compound **5** was then converted to oxazolidinone **6**, thus simultaneously N^{α}-alkylating and protecting the α-carboxyl.[13] The side-chain carboxyl was then subjected to the modified Curtius rearrangement[14] in the presence of 2-chlorobenzyl alcohol to provide the fully protected N^{α}-methyllysine derivative **7**. Finally, the oxazolidinone was cleaved under the usual conditions to give the versatile intermediate **8** in 15% overall yield from **5**. This route is our preferred approach to N^{α}-methyllysine and promises to be applicable to a variety of N^{α}-alkyl N^{ω}-diamino acids.[15]

A very important feature of this synthesis of *N*-alkyl amino acids is its lack of racemization as demonstrated with the synthesis of D- and L-*N*-(9-fluorenylmethyloxycarbonyl)-*N*-methyl-*O*-benzylserine. The product *N*-methyl amino acid derivatives were converted to the corresponding methyl esters, and the optical purities were compared with those of the starting material methyl esters by proton NMR in the presence of the chiral shift reagent tris[(heptafluorobutyl)camphorato]europium(III). The methoxyl peak was used as the analytical signal, and enantiomeric separations in $CDCl_3$ of 25–39 Hz at 300 MHz were achieved in the presence of ca. 0.35–0.5 molar equiv of lanthanide.

The analytical method established that the preparation based on L-serine was appreciably better than 99.9% L. The high precision and accuracy was achieved by using the ^{13}C satellites of the methoxyl signal as internal reference peaks.[16] Since these peaks are exactly 0.55% of the parent resonance, they are ideally suited for quantitative analysis in the 0.1–1.0% range. The result thus established that any racemization that may accompany the synthetic sequence is less than the detection limit which is estimated at 0.06–0.07% (see Figure 1).

The two-step acid-catalyzed reductive alkylation procedure of eq 4 is a versatile new general method for the synthesis of *N*-alkyl amino acid derivatives. A wide variety of readily available amino acids and aldehydes may be employed. Yields are generally good, and the approach is racemization free. The method is particularly valuable for the synthesis of *N*-alkyl amino acids bearing base-labile N-protecting groups.

Experimental Section[17]

Capillary melting points were determined on a Thomas-Hoover apparatus and are uncorrected. NMR spectra were recorded on a Varian EM-390 spectrometer and are expressed in parts per million from Me_4Si as an internal standard. The shift reagent study was performed on a Varian SC-300 spectrometer. Infrared

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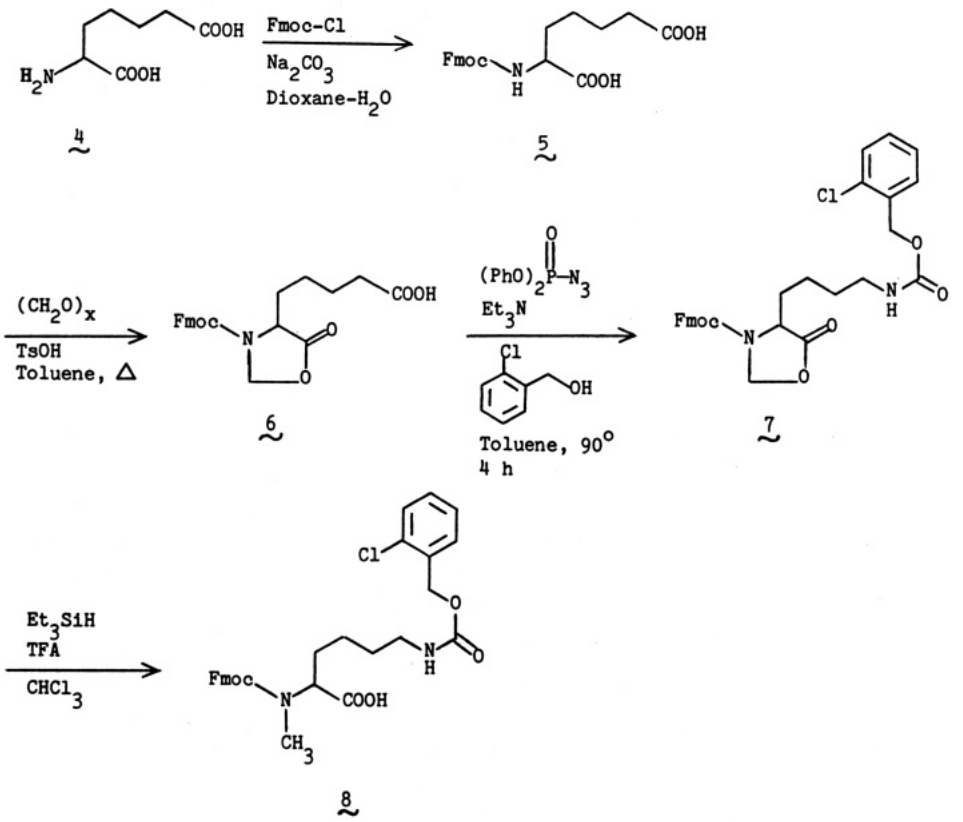
(17) Abbreviations: Fmoc, 9-fluorenylmethyloxycarbonyl; Boc, *tert*-butyloxycarbonyl; Cbz, benzyloxycarbonyl; THF, tetrahydrofuran; TFA, trifluoroacetic acid; DMF, dimethylformamide; DNP, 2,4-dinitrophenyl.

Scheme I



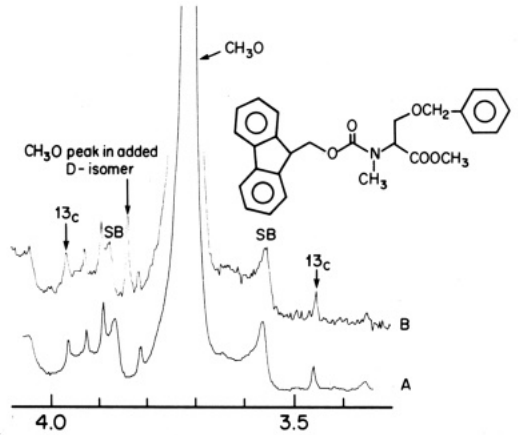


Figure 1. Optical purity determination of *N*-methylserine derivatives by using ^{13}C NMR satellite peaks: (A) 300-MHz trace of the methoxyl region of L isomer in the presence of 0.4 molar equiv of Eu(hfbc)$_3$; (B) L isomer plus a small amount of lanthanide-treated D isomer.

spectra were recorded on a Perkin-Elmer 137 spectrophotometer. Optical rotations were recorded on a Perkin-Elmer 141 polarimeter. Thin-layer chromatography (TLC) was performed on silica gel (Whatman K-1F and Analtech silica gel GF plates), and components were visualized either by ultraviolet light or by *tert*-butyl hypochlorite–KI reagents.

Two experiments which provided representative examples of oxazolidinone formation and subsequent reduction to the protected *N*-alkyl amino acid are described in detail.

(4*S*)-3-(9-Fluorenylmethyloxycarbonyl)-4-methyl-5-oxooxazolidine (2a). Fmoc-L-Ala (1.56 g, 5.0 mmol) was suspended in 100 mL of toluene, and paraformaldehyde (1 g) and *p*-toluenesulfonic acid (100 mg) were added. The mixture was refluxed for 30 min with azeotropic water removal. The solution was cooled, washed with 1 N aqueous $NaHCO_3$ (2 × 25 mL) and dried over Na_2SO_4. Concentration in vacuo gave 1.56 g (96%) of crystalline product: mp 142–144 °C; $[\alpha]^{25}_D$ +66.7° (*c* 1.0, CH_2Cl_2); IR ($CHCl_3$) 1790, 1705 cm^{-1}; NMR ($CDCl_3$) δ 1.30 (d, 3, J = 6.5 Hz), 4.07 (m, 1), 4.26 (t, 1, J = 5.5 Hz), 4.67 (d, 2, J = 5.5 Hz), 5.16 (d, 1, J = 4.5 Hz), 5.30 (d, 1, J = 4.5 Hz), 7.2–7.9 (m, 8); mass spectrum (70 eV), m/e 323 (M^+). Anal. Calcd for $C_{19}H_{17}NO_4$: C, 70.58; H, 5.30; N, 4.33. Found: C, 70.48; H, 5.38; N, 4.31.

***N*-Fmoc-*N*-methyl-L-alanine (3a).** The oxazolidinone from Fmoc-L-Ala (0.97 g, 3.0 mmol) was dissolved in 15 mL of $CHCl_3$, and 15 mL of trifluoroacetic acid and triethylsilane (1.43 mL, 1.04 g, 9.0 mmol) were added. The solution was stirred at room temperature for 22 h followed by concentration in vacuo to an oil. The oil was dissolved in CH_2Cl_2 and reconcentrated three times. The resultant oil crystallized on standing. The crystals were dissolved in ether and concentrated to a crystalline solid which was washed with 5% ether in hexane and dried: yield 0.96 g (98%); mp 154.5–155.5 °C; $[\alpha]^{25}_D$ −21.4° (*c* 1.0, CH_2Cl_2); NMR ($CDCl_3$) δ 1.43 (d, 3, J = 7.5 Hz), 2.92 (s, 3), 4.2–5.0 (m, 4), 7.2–7.8 (m, 8), 9.78 (br s); mass spectrum (70 eV), m/e 325 (M^+).

(2*RS*,4*S*)-2-Cyclohexyl-3-(9-fluorenylmethyloxycarbonyl)-4-methyl-5-oxooxazolidine (2b). The reflux period was 7 h. The product was an oil, purified by preparative silica gel TLC (95:5 $CHCl_3$–CH_3OH): $[\alpha]^{25}_D$ +22.4° (*c* 0.5, CH_2Cl_2); IR (CH_2Cl_2) 1790, 1710 cm^{-1}; NMR ($CDCl_3$) δ 1.07, 1.45, 1.65 (3 m, 11), 1.23 (d, 3, J = 7 Hz), 3.97 (m, 1), 4.21 (t, 1, J = 4.5 Hz), 4.74 (d, 2, J = 4.5 Hz), 5.10 (m, 1), 7.3–7.9 (m, 8); mass spectrum (70 eV), m/e 405 (M^+).

***N*-(Cyclohexylmethyl)-*N*-Fmoc-L-alanine (3b).** The product was purified by flash chromatography on silica gel (95:5:0.5 $CHCl_3$–CH_3OH–H_2O) to give an amorphous solid: $[\alpha]^{25}_D$ −14.2° (*c* 1.0, CH_2Cl_2); NMR ($CDCl_3$) δ 1.0–1.7 (m, 14), 2.87 (m, 2), 4.00 (q, 1, J = 7.0 Hz), 4.21 (t, 1, J = 5 Hz), 4.60 (d, 2, J = 5 Hz), 6.1 (br m, 1), 7.3–7.9 (m, 8); mass spectrum (70 eV), m/e 407 (M^+).

(2*RS*,4*S*)-2,4-Dimethyl-3-(9-fluorenylmethyloxycarbonyl)-5-oxooxazolidine (2c). The reflux period with paraldehyde was 5 h. The product was isolated as a mixture of crystals and oil. The crystalline material was characterized: $[\alpha]^{24}_D$ +32.59° (*c* 0.55, MeOH); IR ($CHCl_3$) 1790, 1710 cm^{-1}; NMR ($CDCl_3$) δ 1.27 (d, 6, J = 7 Hz), 4.00 (m, 1), 4.23 (t, 1, J = 5 Hz), 4.67 and 4.70 (2 d, 2, J = 5 Hz), 5.50 (m, 1), 7.3–7.9 (m, 8); mass spectrum (70 eV), m/e 337 (M^+).

***N*-Ethyl-*N*-Fmoc-L-alanine (3c).** The crude product was purified by silica gel chromatography ($CHCl_3$) and concentrated

in vacuo to an oil. Crystallization from ethyl acetate–hexane gave pure product: mp 178–181 °C; $[\alpha]^{24}_D$ –22.7° (*c* 0.56, MeOH); IR ($CHCl_3$) 1730 (sh), 1710 cm^{-1}; NMR ($CDCl_3$–Me_2SO-d_6) δ 1.07 (m, 3), 1.41 (d, 3, *J* = 7.5 Hz), 3.25 (m, 2), 4.36 (m, 4), 7.3–7.9 (m, 8); mass spectrum (70 eV), *m/e* 339 (M^+).

(2*S*,4*S*)-2-Benzyl-3-(9-fluorenylmethyloxycarbonyl)-4-methyl-5-oxooxazolidine (2d). The reflux period was 21 h. The product was an oil, purified by preparative silica gel TLC ($CHCl_3$): $[\alpha]^{25}_D$ –48.3° (*c* 1.0, CH_2Cl_2); IR ($CHCl_3$) 1795, 1705 cm^{-1}; NMR ($CDCl_3$) δ 0.49 (m, 3), 3.0 (br m, 2), 3.83 (m, 1), 4.25 (t, 1, *J* = 4 Hz), 4.80 (br d, 2, *J* = 4 Hz), 5.5 (m, 1), 6.9-7.9 (m, 13); mass spectrum (70 eV), *m/e* 413 (M^+).

***N*-Fmoc-*N*-(2-phenylethyl)-L-alanine (3d).** The reaction was run on crude material containing phenylacetaldehyde. Additional Et_3SiH (9.4 mL) added in increments over 138 h was required to complete the reduction. The product was purified by flash chromatography on silica gel (95:5:0.5 $CHCl_3$–MeOH–H_2O): $[\alpha]^{25}_D$ –12.4° (*c* 1.0, CH_2Cl_2); IR (CH_2Cl_2) 1700, 1610 cm^{-1}; NMR ($CDCl_3$) δ 1.31 (d, 3, *J* = 6 Hz), 2.6 (m, 2), 3.23 (m, 2), 4.21 (t, 1, *J* = 6 Hz), 4.41 (m, 1), 4.63 (d, 2, *J* = 6 Hz), 7.0–8.0 (m, 14); mass spectrum (70 eV), *m/e* 324 (M^+ – $PhCH_2$).

(4*S*)-3-(9-Fluorenylmethyloxycarbonyl)-4-isopropyl-5-oxooxazolidine (2e). The initially isolated oil crystallized: mp 70–74 °C; $[\alpha]^{25}_D$ +69.2° (*c* 1.0, CH_2Cl_2); IR ($CHCl_3$) 1800, 1710 cm^{-1}; NMR ($CDCl_3$) δ 0.8 and 0.93 (2 d, 6, *J* = 7 Hz), 2.0 (m, 1), 3.87 (m, 1), 4.26 (t, 1, *J* = 5 Hz), 4.72 (d, 2, *J* = 5 Hz), 5.05 (d, 1, *J* = 5 Hz), 5.41 (d, 1, *J* = 5 Hz), 7.3–7.9 (m, 8); mass spectrum (70 eV), *m/e* 351 (M^+).

***N*-Fmoc-*N*-methyl-L-valine (3e).** The product was isolated crystalline after evaporation of CH_2Cl_2: mp 185–187 °C; $[\alpha]^{25}_D$ –31.2° (*c* 1.0, CH_2Cl_2); IR ($CHCl_3$) 1690 cm^{-1}; NMR ($CDCl_3$) δ 0.87 and 1.0 (2 d, 6, *J* = 6 Hz), 2.2 (m, 1), 2.85 (s, 3), 4.0–4.6 (m, 4), 4.95 (br m, exchangeable with D_2O, 1), 7.2–7.8 (m, 8); mass spectrum (70 eV), *m/e* 353 (M^+).

(4*S*)-3-(9-Fluorenylmethyloxycarbonyl)-4-[2-(methylthio)ethyl]-5-oxooxazolidine (2f). The initially isolated oil crystallized: mp 74.5–77 °C; $[\alpha]^{25}_D$ –41.4° (*c* 1.0, MeOH); IR (CH_2Cl_2) 1790, 1700 cm^{-1}; NMR ($CDCl_3$) δ 1.97 (s + m, 5), 2.30 (m, 2), 4.50 (m, 1), 4.22 (t, 1, *J* = 5 Hz), 4.68 (d, 2, *J* = 5 Hz), 5.10 (d, 1, *J* = 5 Hz), 5.33 (d, 1, *J* = 5 Hz), 7.2–7.9 (m, 8); mass spectrum (70 eV), *m/e* 384 (M^+). Anal. Calcd for $C_{21}H_{21}NO_4S$: C, 65.78; H, 5.52; N, 3.65. Found: C, 65.91; H, 5.45; N, 3.88.

***N*-Fmoc-*N*-methyl-L-methionine (3f).** The reduction was run under standard conditions for 5 days, but did not go to completion. After workup, the crude product was chromatographed on silica gel (98:2 $CHCl_3$–MeOH) giving pure material: mp 142–146 °C; $[\alpha]^{25}_D$ –33.3° (*c* 1.0, CH_2Cl_2); IR ($CHCl_3$) 1695 cm^{-1}; NMR (CD_3OD) δ 2.02 and 2.04 (2 s, 3), 1.8–2.5 (m, 4), 2.75 and 2.78 (2 s, 3), 3.75 (m, 1), 4.23 (m, 1), 4.50 (d, 2, *J* = 6 Hz), 7.2–7.9 (m, 8); mass spectrum (70 eV), *m/e* 385 (M^+).

(4*S*)-4-Benzyl-3-(9-fluorenylmethyloxycarbonyl)-5-oxooxazolidine (2g). The product was purified by preparative TLC (silica gel, 95:5:0.5 $CHCl_3$–MeOH–H_2O) and isolated as an oil: IR ($CHCl_3$) 1800, 1720 cm^{-1}; NMR ($CDCl_3$) δ 2.99 (br m, 2), 4.10 (d, 2, *J* = 4 Hz), 4.36 (t, 1, *J* = 4 Hz), 4.74 (m, 2), 5.10 (d, 1, *J* = 4 Hz), 6.9–7.9 (m, 13).

***N*-Fmoc-*N*-methyl-L-phenylalanine (3g).** The product was purified by preparative TLC (silica gel, 95:5:0.5 $CHCl_3$–MeOH–H_2O) and isolated as an oil: IR ($CHCl_3$) 1690 cm^{-1}; NMR ($CDCl_3$) δ 2.78 (s, 3), 3.24 (m, 2), 4.1–5.0 (m, 4), 7.0–7.8 (m, 13). The product was also shown to be identical with a sample synthesized from *N*-methylphenylalanine and FmocCl.[18,19]

(4*S*)-3-(9-Fluorenylmethyloxycarbonyl)-4-(4-phthalylbutyl)-5-oxooxazolidine (2h). N^{α}-Fmoc-N^{ϵ}-phthalyllysine was prepared from N^{ϵ}-phthalyllysine according to standard procedures.[18] This compound was then converted to oxazolidinone **2h** according to the standard procedure with a 2.5-h reflux time. Crystallization gave pure product: mp 150–152 °C; IR ($CHCl_3$) 1800, 1775, 1700 cm^{-1}; NMR ($CDCl_3$) δ 1.2–1.7 (m, 6), 3.60 (t, 2, *J* = 7 Hz), 3.93 (m, 1), 4.20 (t, 1, *J* = 5 Hz), 4.66 (d, 2, *J* = 5 Hz), 5.03 (d, 1, *J* = 4.5 Hz), 5.20 (d, 1, *J* = 4.5 Hz), 7.2–7.9 (m, 12); mass spectrum (14 eV), *m/e* 510 (M^+).

N^{α}-Fmoc-N^{α}-methyl-N^{ϵ}-phthalyl-L-lysine (3h). The product was purified by preparative TLC (silica gel, 95:5:0.5 $CHCl_3$–MeOH–H_2O) and isolated as an amorphous foam: IR ($CHCl_3$) 1775, 1705 cm^{-1}; NMR ($CDCl_3$) δ 1.1–2.1 (m, 6), 2.84 (s, 3), 3.68 (t, 2, *J* = 7 Hz), 4.1–4.5 (m, 3), 4.79 (dd, 1, J_1 = 10 Hz, J_2 = 6 Hz), 5.23 (m, 2, exchanged by D_2O), 7.2–7.9 (m, 12).

N^{α}-Methyl-N^{ϵ}-phthalyl-L-lysine (Selective Fmoc Removal). A sample of **3h** (9.5 mg) was dissolved in 100 μL of degassed DMF, and 100 μL of diisopropylamine was added. After 2 h, the precipitated amino acid was filtered and washed with CH_2Cl_2: yield 4.2 mg (78%); IR (Nujol) 1775, 1720 cm^{-1}; NMR (deuterotrifluoroacetic acid) δ 1.83 (m, 4), 2.30 (m, 2), 3.09 (s, 3), 3.93 (m, 2), 4.1–4.35 (m, 1), 7.95 (m, 4); mass spectrum (70 eV), *m/e* 290 (M^+).

(4*S*)-4-[(Benzyloxy)methyl]-3-(9-fluorenylmethyloxycarbonyl)-5-oxooxazolidine (2i). *N*-Fmoc-*O*-benzylserine was prepared from *O*-benzylserine according to standard procedures.[18] This compound was then converted to oxazolidinone **2i** with a 1 h reflux period and isolated as an oil: $[\alpha]^{25}_D$ +93.7° (*c* 1.0, CH_2Cl_2); IR ($CHCl_3$) 1800, 1710 cm^{-1}; NMR ($CDCl_3$) δ 3.70 (m, 2), 4.20 (t, 1, *J* = 5.5 Hz), 4.40 (s, 2), 4.58 (d, 2, *J* = 5.5 Hz), 5.15 (d, 1, *J* = 4 Hz), 5.38 (d, 1, *J* = 4 Hz), 7.2–7.9 (m, 13); mass spectrum (70 eV), *m/e* 429 (M^+). Anal. Calcd for $C_{26}H_{23}NO_5$: C, 72.71; H, 5.40; N, 3.26. Found: C, 72.90; H, 5.60; N, 3.08.

The corresponding *R* enantiomer of **2i** was also prepared and had identical properties except for $[\alpha]^{25}_D$ –81.8° (*c* 1.0, CH_2Cl_2) (starting material contained ~5% *S* enantiomer).

***N*-Fmoc-*N*-methyl-*O*-benzyl-L-serine (3i).** The product was purified by flash chromatography (silica gel, 95:5 $CHCl_3$–MeOH) and isolated as an oil: $[\alpha]^{25}_D$ –6.4° (*c* 1.0, CH_2Cl_2); IR ($CHCl_3$) 3000, 1720, 1500 cm^{-1}; NMR (CD_3OD) δ 2.91 (s, 3), 3.55–3.95 (m, 2), 4.1–4.5 (m, 6), 7.0–7.9 (m, 13); mass spectrum (70 eV), *m/e* 431. The corresponding *D* enantiomer of **3i** had identical properties except for $[\alpha]^{25}_D$ +5.4° (*c* 1.0, CH_2Cl_2).

***N*-Fmoc-*N*-methyl-*O*-benzyl-L-serine Methyl Ester for Shift Reagent Study.** The acid **3i** (200 mg, 0.46 mmol) and BF_3OEt_2 (125 μL, 1.0 mmol) were dissolved in absolute methanol (2 mL) and heated at reflux for 1 h.[20] After solvent removal, the residue was treated with 5 mL of ice–water and extracted with EtOAc (5 mL). The extract was washed with water, 1 N aqueous $NaHCO_3$, and water and dried over Na_2SO_4. Solvent removal gave 190 mg of product which was purified by preparative TLC (silica gel, $CHCl_3$). The following data were recorded: $[\alpha]^{25}_D$ –10.3° (*c* 1.0, CH_2Cl_2); IR ($CHCl_3$) 3000, 1710, 1500 cm^{-1}; NMR ($CDCl_3$) δ 2.96 and 3.03 (2 s, 3), 3.63 and 3.73 (2 s, 3), 3.92 (d, 2, *J* = 6 Hz), 4.2–4.85 (m, 5), 5.03 (t, 1, *J* = 6 Hz), 7.35–7.85 (m, 13); mass spectrum (70 eV), *m/e* 445 (M^+).

The corresponding *D* enantiomer was similarly prepared and was identical except for $[\alpha]^{25}_D$ +9.9° (*c* 1.0, CH_2Cl_2).

(4*S*)-3-(9-Fluorenylmethyloxycarbonyl)-4-(N^{Im}-2,4-dinitrophenylimidazolylmethyl)-5-oxooxazolidine (2j). N^{α}-Fmoc-N^{Im}-DNP-histidine was prepared from N^{Im}-DNP-histidine according to standard procedures.[18] The reaction with paraformaldehyde was run for 21 h at reflux. Solubility problems were experienced with some starting material (~50%) sticking to the sides of the flask and being recovered. The product was substantially purified by preparative TLC (silica gel, 95:5:0.5 $CHCl_3$–MeOH–H_2O) to give an oil: IR (CH_2Cl_2) 1800, 1725, 1710, 1610, 1540, 1350, 1340 cm^{-1}; NMR (CD_2Cl_2) δ 3.0 (m, 2), 4.27 (t, 1, *J* = 5 Hz), 4.69 (m, 2), 4.85 (m, 1), 5.31 (d, 2, *J* = 3 Hz), 7.3–7.9 (m, 11), 8.60 (dd, 1, J_1 = 8 Hz, J_2 = 2 Hz), 8.89 (d, 1, *J* = 2 Hz).

N^{α}-Fmoc-N^{α}-methyl-N^{Im}-DNP-L-histidine (3j). The reduction was run for 4 days with twice the usual amount of Et_3SiH, however, it did not go to completion. After the workup, the product was purified by flash chromatography (silica gel, 90:10:1 $CHCl_3$–MeOH–H_2O) to give an oil which crystallized on trituration with CH_2Cl_2: mp 160–164 °C; $[\alpha]^{25}_D$ –15.1° (*c* 1.0, MeOH); IR (Nujol) 1725, 1675, 1605, 1530, 1455, 1365 cm^{-1}; NMR (CD_3OD) δ 2.81 and 2.95 (2 s, 3), 3.40 (m, 2), 4.18–4.40 (m, 3), 4.63 (m, 1), 7.24–7.80 (m, 9), 7.86 and 7.91 (2 d, 1, *J* = 8 Hz), 8.42 and 8.64 (2 s, 1), 8.59 and 8.73 (2 dd, 1, J_1 = 8 Hz, J_2 = 2 Hz), 9.00 and 9.04 (2 d, 1, *J* = 1 Hz), 2:1 mixture of rotomers; FAB mass spectrum, *m/e* 558 (M^+ + H).

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4-(4-*o*-Chlorobenzyloxycarbonylaminobutyl)-3-(9-fluorenylmethyloxycarbonyl)-5-oxooxazolidine (7). α-[(9-fluorenylmethyloxycarbonyl)amino]pimelic acid **5** was prepared from α-aminopimelic acid (**4**) according to standard procedures.[18] Compound **5** (10 g, 25 mmol) in toluene (485 mL) was heated at reflux with paraformaldehyde (4.85 g) and *p*-toluenesulfonic acid monohydrate (485 mg, 2.55 mmol) for 2.5 h with azeotropic water removal. Formation of a new less polar spot with concomitant disappearance of the starting material spot was observed on TLC. This observation was consistent with formation of oxazolidinone **6**.

Compound **6** was not isolated. To the above reaction solution corresponding to 25 mmol of crude **6** were added *o*-chlorobenzyl alcohol (4.0 g, 28.05 mmol), triethylamine (3.8 mL, 2.76 g, 27.3 mmol), and diphenylphosphoryl azide (5.9 mL, 7.53 g, 27.4 mmol). The resultant solution was heated at 90–95 °C for 4 h. After cooling, the reaction mixture was washed with aqueous citric acid (2 × 150 mL), 1 N aqueous $NaHCO_3$ (2 × 150 mL), water (150 mL), and saturated aqueous NaCl (150 mL) and dried (Na_2SO_4). Concentration in vacuo gave 15.45 g of crude **7**. Preparative TLC (silica gel, 99:1 $CHCl_3$–MeOH) of the concentrate from a 10-mL aliquot gave 70 mg of an oil: IR ($CHCl_3$) 1800, 1720 cm^{-1}; NMR ($CDCl_3$) δ 1.0–1.8 (m, 6), 3.12 (m, 2), 3.90 (br m, 1), 4.21 (t, 1, J = 5 Hz), 4.67 (d, 2, J = 5 Hz), 4.77 (m, 1), 5.02 (d, 1, J = 4.5 Hz), 5.23 (s, 2), 5.32 (d, 1, J = 4.5 Hz), 6.9–7.9 (m, 12).

N^{α}-Fmoc-N^{α}-methyl-N^{ϵ}-(*o*-chlorobenzyloxycarbonyl)-lysine (8). Crude oxazolidinone **7** (~13.5 g, 24.5 mmol) was dissolved in $CHCl_3$ (70 mL), and Et_3SiH (11.7 mL, 8.5 g, 73 mmol) was added. Trifluoroacetic acid (70 mL) was then added, and the resultant solution was stirred at room temperature for 18 h. Concentration in vacuo and reconcentration twice from $CHCl_3$ gave 18.5 g of yellow oil. Flash chromatography (silica gel, 120:5:0.5:0.1 $CHCl_3$–MeOH–H_2O–HOAc) followed by repeat chromatography of side cuts yielded 2.0 g (15%) of amorphous product: NMR ($CDCl_3$) δ 1.1–2.0 (m, 6), 2.83 (s, 3), 3.16 (m, 2), 4.23 (m, 1), 4.45 (d, 2, J = 5.5 Hz), 4.80 (m, 1), 5.23 (s, 2), 6.90 (m, 1), 7.2–7.9 (m, 12), 8.10 (m, 1).

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Cationic Cyclizations and Rearrangements as Models for Strobane and Hispanane Biogenesis[1]

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In contrast to methyl 15(*R*)-hydroxypimar-8(14)-en-oate (**5a**) the 15(*S*)-epimer **9a** gave a stable toluenesulfonate. Solvolysis of the latter gave, depending upon the conditions, the 15(*R*)-epimer **5a**, the methoxy derivative **9d**, or, with rearrangement, the strobane derivative **10a** and the cyclopropane resin acid ester **11a**. Treatment of methyl 15-hydroxy-16-nor-8(14)-pimaren-18-oate (**9e**) with toluenesulfonyl chloride–pyridine resulted, as in the case of **5a**, in spontaneous rearrangement to **10b** and **11b**. The difference in behavior of **5a** and **9e** on the one hand and **9a** on the other is attributed to different spatial demands in the homoallylic cations produced from the starting materials. The reactions provide laboratory analogies for postulated biogenetic pathways to diterpenoids of the strobane and hispanane families. In an effort to test an alternative proposal for biogenesis of the strobanes, the epimeric labda-8(17),13-dien-12(*S*)- and -12(*R*)-ols **22** and **23** were synthesized and subjected to reagents known to effect cationic cyclizations. These experiments failed to provide compounds with the strobane carbon skeleton. Formic acid converted **23**, but not **22**, to the tricyclic hydrocarbon **31**.

One of the possible biogenetic pathways to diterpenoids with the strobane skeleton **2** involves vinyl migration in cation A or its equivalent which is generated from an appropriate pimaradiene precursor, **1** (Scheme I).[2] A second possibility is that the strobanes are formed by direct cyclization of a labdane derivative rather than through the intermediacy of a pimarane, e.g., by cyclization (Scheme II) of a bicyclic ion D or its equivalent theoretically derivable from *cis*-biformene (**3**) or alcohol **4**.

Some years ago,[3] upon attempting to mimic the pathway sketched in Scheme I, we obtained from **1a** by way of **5a** the cyclopropane resin acid ester **7** and the strobane-like isomer **6** whose C-14 stereochemistry was opposite those of the naturally occurring strobanes (Scheme III).[4,5] In the present paper we report the biogenetic-type solvolytic rearrangement of tosylate **9c** to the strobic acid derivative **10a** and to **11a** with the correct C-14 stereochemistry as well as the facile transformation of **9e**, by way of a *primary*

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(5) Numbering in formulas **6** and **10** differs from that employed previously[3,4] and is based on the systematic name strobane for a hydrocarbon skeleton which was not known at the time commonly accepted proposals[6] for naming cyclic diterpenes were formulated. Strobic acid would thus be stroba-8(15),12-dien-18-oic acid. The discoverers[4a] originally used the systematic name abeopimarane, in terms of which strobic acid should be formulated as 14(13→15α*H*)-abeopimara-8(14),12-dien-18-oic acid, and subsequently[4b] corrected it to that of a cyclolabdane [14(*S*),17-cyclolabda-8(17),12-dien-18-oic acid]. Coates[2] has used strobane numbering. The abeopimarane or cyclolabdane nomenclature would probably be preferable to the strobane nomenclature if the actual biosynthesis route were known.

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