Investigation of the Reaction between Amino Acids or Amino Acid Esters and 9-Formylfluorene and Its Equivalents. Possible Utility of the Derived Enamines as Amino Group Protectants[1,2]

Louis A. Carpino,* Hann Guang Chao, and Jien-Heh Tien

Department of Chemistry, University of Massachusetts, Amherst, Massachusetts 01003

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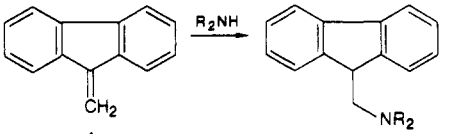
Treatment of 9-(hydroxymethylene)fluorene/9-formylfluorene (storable as the hemiacetal with methanol, **7**) with amino acids and amino acid esters yields the corresponding enamines **8**, which may be considered to be hydrocarbon analogues of *N*-formyl amino acid derivatives. Attempted coupling of the free acids **8** (R′ = H) with amino acid esters failed, suggesting insufficient reduction in basicity of the amino group due to the enamine residue. The introduction of electron-withdrawing substituents into the fluorene ring decreases the basicity sufficiently to allow normal peptide coupling reactions, as for example with the 2,7-dichloro analogues derived from **17**. Thus phenylalanine derivative **18** treated with leucine methyl ester and DCC gave dipeptide **19**. The DC-FM-bar group could be removed by catalytic transfer hydrogenolysis. Mild acid hydrolysis represents a second general deblocking technique for the FM-bar function. It was demonstrated in a model study involving the highly sensitive amino acid α-phenylglycine that the FM-bar protecting group was less prone to cause racemization than the benzyloxycarbonyl function. It was demonstrated that the simple pentapeptide leucine enkephalin **29** could be synthesized using α-DC-FM-bar protection along with *tert*-butyl-based side chain protecting groups.

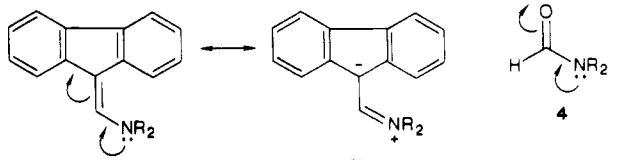
During our studies on the FMOC amino-protecting group,[3] we became intrigued by the unusual properties of the deblocking byproduct, DBF **1**. This unusual olefin undergoes ready addition of primary and secondary amines to give adducts **2** by reactions that presumably have their origin in the special aromatic character of the cyclopentadiene anion (eq 1). DBF can thus be looked upon



(1)

1 **2**

as an all-carbon analogue of formaldehyde, suggesting also that substitution of one of the exo methylene hydrogen atoms by an amino function, as in **3**, should result in enamines of unique properties. To the extent that resonance interaction symbolized by structure **3b** is important these enamines could well be expected to show properties analogous to those of the corresponding formamide derivatives **4**. If the effect is pronounced and if formation of the enamine is easily reversed or the amino function otherwise easily liberated such enamines might be considered to be "protected" forms of the amine.



3a **3b** **4**

Should this be realized for the α-protection of amino acids at least one advantage of such a system might be a lesser tendency toward racemization during coupling reactions than for the corresponding amide or urethane analogues in view of (a) the lesser inductive effects which might contribute to α-hydrogen abstraction and (b) the impossibility of formation of any cyclic intermediate related to the oxazolones[4] which are thought to contribute to the racemization of *N*-acyl amino acids. Enamines bearing strongly electron-withdrawing carbonyl[5] or nitro[6] functions have long been known as amino-protecting groups, although there has been little study to date of their relative susceptibility toward racemization.[7]

In this paper we describe the use of appropriately substituted 9-fluorenylmethylene protectants and demonstrate that under the normal conditions of peptide coupling the system is indeed less readily racemized than comparable urethane-based systems.

The older literature contains a few examples of the synthesis of such enamines, e.g., the aniline derivative **6** (Ar = C_6H_5), a bright yellow compound obtained by reaction of 9-(hydroxymethylene)fluorene **5** with aniline.[8] Although **6** (Ar = C_6H_5) appears to be stable indefinitely, the few aliphatic[9] derivatives described in the literature appear to be somewhat sensitive to hydrolysis and/or spontaneous air oxidation.

(1) Dedicated, with good wishes for his busy and productive days ahead in the field of peptide chemistry, to Professor Haruaki Yajima on the occasion of his retirement from Kyoto University.

(2) A number of abbreviations are used in this paper. Those for natural amino acids and nomenclature for peptide structures follow the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature (*J. Biol. Chem.* **1971**, *247*, 997). Other abbreviations are as follows: TFA = trifluoroacetic acid, Phg = α-phenylglycine, FMOC = 9-fluorenylmethyloxycarbonyl, DCC = dicyclohexylcarbodiimide, MCPBA = m-chloroperbenzoic acid, EDCI = 1-ethyl-3-[3-(dimethylamino)propyl]-carbodiimide, HOBt = N-hydroxybenzotriazole, DMF = dimethylformamide, DBF = dibenzofulvene, NMP = N-methylpiperidine, $\overline{\text{FM}}$ = FM-bar = 9-fluorenemethylenyl (the bar over the symbol for the 9-fluorenylmethyl group refers to the extra bond present due to unsaturation), DC-$\overline{\text{FM}}$ = 2,7-dichloro-$\overline{\text{FM}}$, $\overline{\text{DTM}}$ = 10,10-dioxythioxanthene-9-methylenyl, DIB-$\overline{\text{FM}}$ = 2,7-diisobutyryl-$\overline{\text{FM}}$.

(3) Carpino, L. A. *Acc. Chem. Res.* **1987**, *20*, 401.

(4) For reviews, see: (a) Benoiton, N. L. In *The Peptides*; Gross, E., Meienhofer, J., Eds.; Academic Press: New York, 1983; Vol. 5, 217. (b) Kemp, D. S. In *The Peptides*; Gross, E., Meienhofer, J., Eds.; Academic Press: New York, 1979; Vol. 1, p 315.

(5) (a) Review: Halpern, B. In *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins*; Weinstein, B., Ed.; Dekker: New York, 1981; Vol. 5, p. 95. (b) Dane, E.; Drees, F.; Konrad, P.; Dockner, T. *Angew. Chem.* **1964**, *74*, 873. (c) Dane, E.; Dockner, T. *Angew. Chem.* **1964**, *76*, 342. (d) Chiba, T.; Sakaki, J.; Kaneko, C. *Peptide Chemistry 1984*; Izumiya, N., Ed.; Protein Research Foundation: Osaka, 1985; p 79. (e) Chiba, T.; Sakaki, J.; Kaneko, C. *Yakugaku Zasshi* **1986**, *106*, 154.

(6) Southwick, P. L.; Dufresne, R. F.; Lindsey, J. J. *J. Org. Chem.* **1974**, *39*, 3351.

(7) For recent examples demonstrating the relative chiral stability of analogous enamines, see: (a) Kemp, D. S.; Carter, J. S. *J. Org. Chem.* **1989**, *54*, 109. (b) Sauvé, G.; Mansour, T. S.; Lachance, P.; Belleau, B. *Tetrahedron Lett.* **1988**, 2295. (c) Sauvé, G.; Le Berre, N.; Zacharie, B. *Tetrahedron Lett.* **1988**, 2299.

(8) Wislicenus, W.; Russ, K. *Ber.* **1910**, *43*, 2719.

(9) (a) Miller, F. D.; Wagner, E. C. *J. Org. Chem.* **1951**, *16*, 279. (b) Weingarten, H.; Edelmann, N. K. *J. Org. Chem.* **1967**, *32*, 3293. (c) Von, I.; Wagner, E. C. *J. Org. Chem.* **1944**, *9*, 157.

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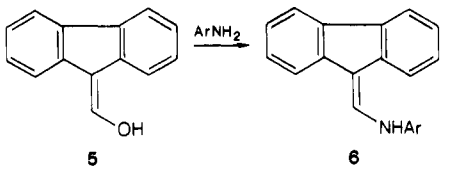
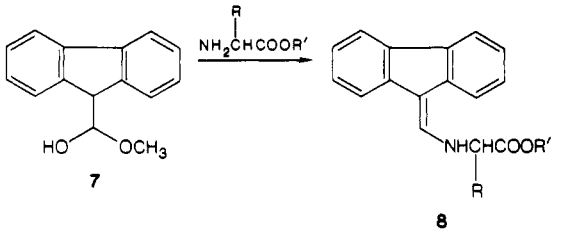


(2)

5 6

With this background we examined the reaction of 5[10] with amino acids and amino acid esters. Since 5 has been available only as a difficulty handled syrupy mass, we first examined its reaction with methanol. This gave a crystalline adduct, which is believed to be the hemiacetal 7, a compound that can be stored indefinitely in a refrigerator, although in the open at room temperature it reverts readily to the aldehyde/vinyl alcohol. Hemiacetal 7 underwent reaction with glycine or phenylalanine or their simple esters to give enamines 8. The free acids 8 (R = H, $C_6H_5CH_2$; R′ = H) were stable enough to isolate and handle although on long storage in the open or at room temperature slow degradation due to spontaneous air oxidation occurred.[11] Such degradation is evidenced by development of a yellow surface color due to formation of fluorenone. The esters, as opposed to the free acids, were significantly more stable toward such air oxidation, and the preferred method of obtaining the free acids involved saponification of the methyl or ethyl esters as needed. The sensitivity of these FM-bar amino acids may be related to the fact that acid-catalyzed hydrolysis of the enamine function occurs readily. Indeed treatment with mild acid represents a simple deblocking technique. A second general deblocking procedure involves catalytic hydrogenolysis (see below). Although not likely to be of such general utility as these two methods, deblocking also occurs upon treatment of the enamine with bromine (titration!) in ether which leads to immediate precipitation of the hydrobromide of the deprotected amine (eq 4).[13]



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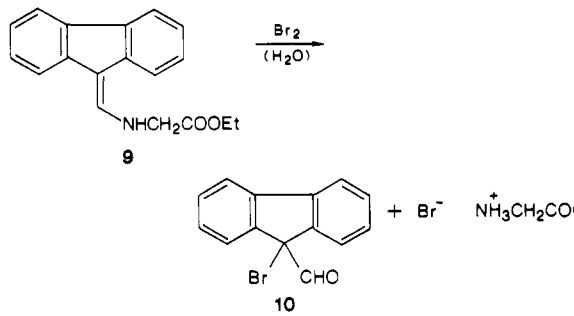


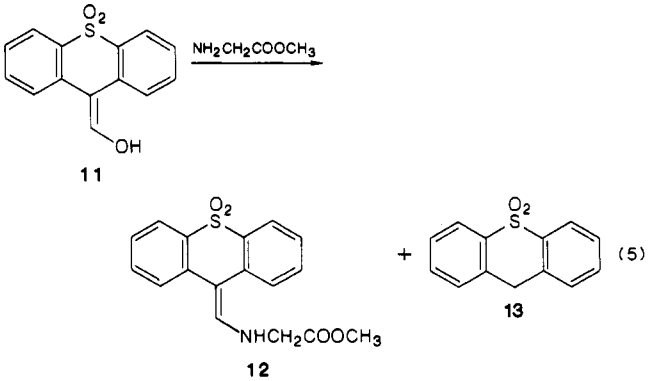
(4)

9 10

Attempts to carry out a coupling reaction between the FM-bar derivative of phenylalanine and leucine methyl ester via DCC or EDIC in ethyl acetate/methylene dichloride or tetrahydrofuran gave none of the desired dipeptide. No well-defined product could be isolated from such reaction mixtures. Conceivably the amino function of 8 (R = $CH_2C_6H_5$, R′ = H) is not sufficiently reduced in basicity to allow simple, directed coupling processes.

In order to determine whether simple steric or inductive effects might affect the properties of these FM-bar derivatives we synthesized the appropriate amino acids derived from 2,7-di-*tert*-butyl-[14] and 2,7-dichlorofluorene.[15] 2,7-Di-*tert*-butylfluorene has previously been prepared by treatment of fluorene with *tert*-butyl chloride in the presence of aluminum chloride.[14a] In our hands this method was unsatisfactory although we obtained excellent results by use of the milder Friedel–Crafts catalyst $FeCl_3$. Conversion of 2,7-di-*tert*-butylfluorene to its 9-formyl derivative was best carried out with potassium hydride. The initial reaction product was difficult to characterize due to its sensitivity toward air oxidation. Reaction with methanol appeared to give the corresponding methyl vinyl ether rather than the hemiacetal. Without isolation the crude enol ether was characterized by condensation with *p*-chloroaniline. Ethyl glycinate gave a low yield (29%) of the FM-bar derivative although the free acid could not be obtained either by direct condensation with glycine or saponification of the ethyl ester. These attempted reactions were always accompanied by the formation of large amounts of 2,7-di-*tert*-butylfluorenone showing that the 2,7-di-*tert*-butyl system is significantly more sensitive toward air oxidation than the unsubstituted analogue and thus of little practical utility.

These results contrasted with those observed for systems bearing electron-withdrawing moieties. Enamines (such as 12) derived from thioxanthene sulfone[16] appeared to be indefinitely stable toward air oxidation. Unfortunately, however, conversion of vinyl alcohol 11 to amino acid ester 12 was accompanied by retro aldol cleavage to give thioxanthene sulfone 13 as the major product. Analogous facile retro aldol reactions were also observed with the isomeric sulfone 14.[17] Introduction of electron-with drawing substituents into the fluorene nucleus of 5 was also examined. The 1-chloro compound 15 was unique among the compounds synthesized in the course of this work in adopting the 9-formylfluorene structure shown rather than the tautomeric vinyl alcohol structure. This is clearly



(5)

11 12 13

(10) We represent 5 in the vinyl alcohol form as shown on the basis of IR and ^1H NMR studies. Extensive studies of the tautomeric equilibrium between 5 and the corresponding aldehyde form have been carried out by More O'Ferrall: (a) Harcourt, M. P.; More O'Ferrall, R. A. *Bull. Chim. Soc. Fr.* **1988**, 407. (b) Harcourt, M. P.; More O'Ferrall, R. A. *J. Chem. Soc., Chem. Commun.* **1987**, 822, 823.

(11) Sensitivity toward air oxidation is clearly related to the basicity of the amine from which the enamine is derived. Thus the amino acid derivatives are intermediate in stability between simple aliphatic derivatives[9] and the corresponding amides (FM-NHCOR, R = CH_3, C_6H_5), which appear to be stable indefinitely.[12]

(12) Eiden, F.; Nagar, B. S. *Arch. Pharm.* **1963**, *296*, 548.

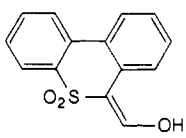
(13) Compare ref 9c and Halpern, B.; James, L. B. *Aust. J. Chem.* **1968**, *18*, 417.

(14) (a) Bruch, M.; Grosse, M.; Rewicki, D. *Liebigs Ann. Chem.* **1976**, 74. (b) Kajigaeshi, S.; Kadowaki, J.; Nishida, A.; Fujisaki, S. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 97.

(15) Carpino, L. A. *J. Org. Chem.* **1980**, *45*, 4250.

(16) Bugakova, L. P.; Rozantsev, E. G. *Sintez i Issled. Effektivn. Stabilizatorov dlya Polymern. Materialov*, Sb., Voronezh, **1964**, 211; *Chem. Abstr.* **1966**, *65*, 12162g.

(17) Schank, K.; Werner, F. *Justus Liebigs Ann. Chem.* **1983**, 1739.



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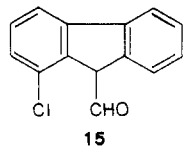

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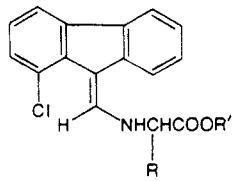
revealed in the ^{1}H NMR spectrum, which shows two sets of doublets at δ 4.8 and 9.2 for the 9- and formyl protons respectively. The infrared spectrum shows no –OH absorption. Presumably due to the bulky 1-substituent the planar vinyl alcohol is destabilized relative to **15** in which the formyl group can rotate out of the plane of the aromatic ring and the adjacent olefinic linkage. On the other hand amino acid derivatives obtained from **15** show the normal enamine structure **16** rather than the corresponding imine possibly because resonance interaction involving the donation of electrons by nitrogen, relative to oxygen, outweighs any destabilizing steric effects. Since the



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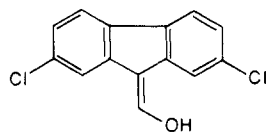
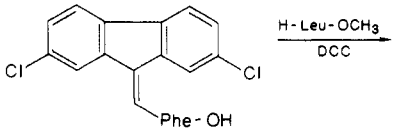


17

synthesis of 1-substituted fluorene derivatives is tedious, it was more convenient to examine the readily available 2,7-disubstituted fluorenes. Among the compounds examined were the dibromo, dichloro, difluoro, and bis(isobutyryl) derivatives. None gave enamines that were completely stable toward air oxidation, but the best combination of properties and ease of synthesis was found in the case of the 2,7-dichloro compound. The precursor vinyl alcohol **17** was readily available on a large scale, could be stored as such in a refrigerator for extended periods, and reacted readily with free amino acids or their esters to give protected derivatives which, while not totally stable toward air oxidation, were sufficiently stable for normal use and additional evaluation. Examples of some of the compounds obtained are collected in Table I as well as in the Experimental Section.

Coupling of DC-FM-bar amino acids by means of DCC, mixed anhydride or active ester techniques took place without difficulty. An example is shown in eq 6, which served also to demonstrate that no significant racemization occurred during the coupling step. Upon deblocking by



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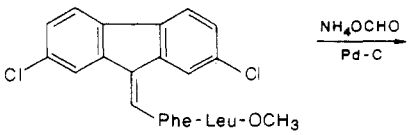

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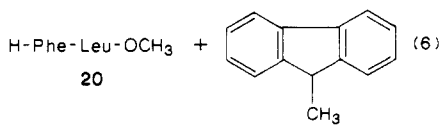
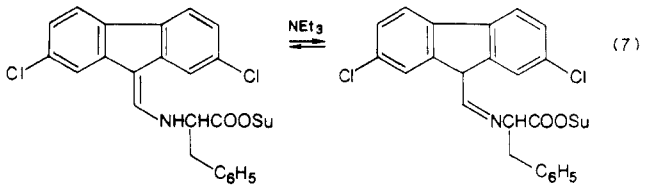


20 21 (6)

catalytic transfer hydrogenolysis with ammonium formate it may be noted that the byproduct 9-methylfluorene **21** has also suffered loss of the two chloro substituents. Benzoylation of crude **20** followed by HPLC analysis[18] demonstrated the chiral integrity of the product (<0.1% of DL-C_6H_5CO-Phe-Leu-OCH_3 formed).

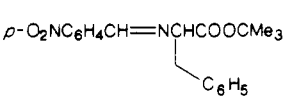
In line with our initial interests in the FM-bar system, we then shifted to more demanding tests of optical stability. Some years ago Anderson and co-workers[19] carried out extensive studies of the optical stability of *p*-nitrophenyl and *N*-hydroxysuccinimide esters of amino acids toward triethylamine. By the drop in optical rotation of such solutions it was shown that the ease of racemization for several protected amino acid active esters followed the order BOC < Z < Pht. We confirmed this order, but an extension of the method to the DC-FM-bar system gave inconsistent results, possibly due to the effect of air oxidation, the extent of which may have been influenced by the presence of the organic base.

The test was modified by treating a series of protected phenylalanine *N*-hydroxysuccinimide esters with triethylamine in methylene dichloride for a specified period of time and then adding alanine methyl ester, thus quenching the reaction via conversion to the dipeptide. The resulting crude dipeptide was examined by ^{1}H NMR analysis,[20] the ratio of DL- and LL-diastereomers being determined at 200 MHz using the differing *C*-methyl doublets arising from the alanine residue. Although "quenching" is not instantaneous, the occurrence of some racemization following addition of the amino acid ester is not expected to interfere with the comparisons studied. The results are collected in Table II. Again the same order (BOC < Z < Pht) was observed for the three well-known protectants, but we were surprised to find that the DC-FM-bar derivative had undergone racemization faster than the three other derivatives. One can rationalize these results if one assumes that under the conditions chosen base-catalyzed isomerization of the enamine to the corresponding imine might occur (eq 7). An open-chain ana-



22 23 (7)

logue of an oxazolone, a species often implicated in the racemization of *N*-acyl amino acid derivatives, **23** might be expected to suffer ready α-hydrogen abstraction with consequent racemization. Siemion and Wilschowitz[21] reported many years ago that the analogous *p*-nitrobenzaldimine of *tert*-butyl phenylalanine **24** lost 75% of its



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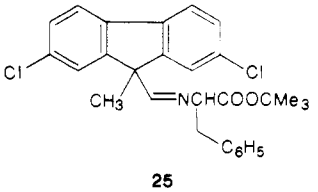

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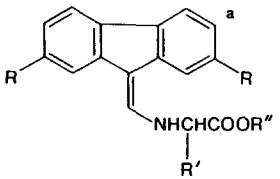
optical activity upon standing in THF or $CHCl_3$ with 3 equiv of triethylamine for 48 h. The pronounced inductive

(18) For the method used see Carpino, L. A.; Rice, N. W.; Mansour, E. M. E.; Triolo, S. A. *J. Org. Chem.* **1984**, *49*, 836.
(19) Anderson, G. W.; Callahan, F. M.; Zimmerman, J. E. *Acta Chim. Hung.* **1965**, *44*, 51.
(20) (a) Halpern, B.; Chew, L. F.; Weinstein, B. *J. Am. Chem. Soc.* **1967**, *89*, 501. (b) Halpern, B.; Nitecki, D. E.; Weinstein, B. *Tetrahedron Lett.* **1967**, 3075. (c) Weinstein, B.; Pritchard, A. E. *J. Chem. Soc., Perkin Trans. 1* **1972**, 1015.
(21) Siemion, Z. I.; Wilschowitz, L. Z. *Z. Naturforsch.* **1971**, *26B*, 726.

Table I. Characterization of FM Derivatives



R	R′	R″	yield %	mp, °C (recrys solv)	α_D, deg (T, °C)	^{1}H NMR, δ[b]	mol formula	analytical data calc/found C	H	N
H	H	Et	70	109–10 (EtOH)	–	1.2 (t, 3, CH_3), 3.75–4.15 (m, 4, CH_2), 6.72 (s, 1, CH=), 6.9–7.7 (m, 8, aryl)	$C_{18}H_{17}O_2N$	77.39 77.42	6.14 6.36	5.01 4.95
H	H	H	36	179–81 (EtOAc–hexane)	–	3.9 (d, 2, CH_2), 6.7 (s, 1, CH=), 6.9–7.7 (m, 8, aryl)[c]	$C_{16}H_{13}O_2N$	76.48 76.33	5.21 5.31	5.57 5.40
Cl	H	H	65	150(dec) (EtOAc–Skelly B)	–	4.2 (d, 2, CH_2), 6.9–8.0 (m, 8, aryl, CH=, NH)	$C_{16}H_{11}Cl_2NO_2$	60.02 60.17	3.46 3.58	4.38 4.38
Cl	CH_3	H	74.3	122(dec) (EtOAc–Skelly B)	+35.7 (27) (c = 0.5, EtOAc)	1.65 (d, 3, CH_3CH), 4.15 (m, 1, $CHCH_3$), 7.1–8.1 (m, aryl, CH=, NH)[d]	$C_{17}H_{13}Cl_2NO_2$	61.09 60.93	3.92 4.13	4.19 4.14
Cl	C_6H_5	H	71	150(dec) (EtOAc–Skelly B)	–86.3 (28) (c = 0.3, THF)	5.26 (d, 1, CHC_6H_5), 6.8–7.9 (m, 13, aryl, CH=, NH)[d]	$C_{22}H_{15}Cl_2NO_2$	66.68 66.53	3.82 3.82	3.53 3.58
F	Bn	H	29.1	186(dec) (acetone–water)	–136.0 (27) (c = 0.2, acetone)	3.2–3.5 (m, 2, $CH_2C_6H_5$), 4.1 (m, 1, $CHCH_2$), 6.4 (m, 1, NH), 6.8–7.9 (m, 12, aryl, CH=)[e]	$C_{23}H_{17}F_2NO_2$	73.20 73.43	4.54 4.76	3.71 3.96
Br	Bn	H	81	192(dec) (acetone–water)	–	3.2 (m, 2, $CH_2C_6H_5$), 4.4 (m, 1, $CHCH_2$), 6.8–8.0 (m, 13, aryl, CH=, NH)	$C_{23}H_{17}Br_2NO_2$	55.33 55.40	3.43 3.43	2.81 2.80
Cl	Bn	H	80.5	203(acetone–water)	–127.1 (25) (c = 0.7, acetone)	3.3 (m, 2, $CH_2C_6H_5$), 4.4 (m, 1, $CHCH_2$), 6.8–8.0 (m, 13, aryl, CH=, NH)[d]	$C_{23}H_{17}Cl_2NO_2$	67.33 67.17	4.18 4.30	3.41 3.43
Cl	Bn[f]	H	76.6	202(acetone–water)	+126.9 (23) (c = 0.7, acetone)	3.35 (m, 2, $CH_2C_6H_5$), 4.4 (m, 1, $CHCH_2$), 6.8–8.0 (m, 13, aryl, CH=, NH)[d]	$C_{23}H_{17}Cl_2NO_2$			
Cl	$CH_2C_6H_4$-OCMe$_2$-p	H	76	185 (Skelly B–EtOAc, 9:1)	–169.25 (21) (c = 0.4, acetone)	1.35 (s, 9, CMe_3), 3.05–3.2 (m, 2, $CH_2C_6H_5$), 4.2–4.3 (m, 1, $CHCH_2$), 6.1–6.2 (m, 1, NH), 6.9–7.8 (m, 11, aryl, vinyl)	$C_{27}H_{21}Cl_2NO_3$	67.22 67.10	5.22 5.07	2.90 2.84
Cl	Bn	CMe_3	80.6	159–60 (Skelly B–acetone, 9:1)	–78.5 (27) (c = 1, EtOAc)	1.4 (s, 9, CMe_3), 3.1–3.3 (m, 2, CH_2C_{H5}), 4.2 (m, 1, $CHCH_2$), 5.7 (m, 1 NH), 6.9–7.7 (m, 12, aryl, vinyl)	$C_{27}H_{25}Cl_2NO_2$	69.53 69.75	5.40 5.49	3.00 3.02
Cl	Me	C_6H_4-NO$_2$-p[g]	75.6	185–6 (EtOAc–Skelly B, 2:3)	+25 (25) (c = 0.4, dioxane)	1.85 (d, 3, CH_3CH), 4.55–4.7 (m, 1, $CHCH_3$), 791–8.4 (m, 12, aryl, NH, vinyl)[d]	$C_{23}H_{16}Cl_2N_2O_4$	60.67 60.47	3.54 3.43	6.15 6.07
Cl	Bn	C_6H_4-NO$_2$-p[g]	79	213–5 (EtOH–acetone, 3:1)	–48.5 (25) (c = 1, dioxane)	3.4 (d, 2, $CH_2C_6H_5$), 4.6 (m, 1, $CHCH_2$), 6.8 (m, 1, NH), 7.0–8.3 (m, 16, aryl, vinyl)[c]	$C_{29}H_{20}Cl_2N_2O_4$	65.54 65.87	3.79 3.90	5.27 5.21
Cl	Me	Su[g]	70.2	210–212 (EtOAc–Skelly B, 1:3)	+4.9 (23) (c = 2, dioxane)	1.85 (d, 3, $CHCH_3$), 2.9 (s, 4, CH_2CH_2), 4.7–4.8 (m, 1, $CHCH_3$), 7.1–8.2 (m, 8, aryl, NH, vinyl)[d]	$C_{21}H_{16}Cl_2N_2O_4$	58.48 58.51	3.74 3.82	6.50 6.47
Cl	Bn	Su[g]	78.7	204–5 (EtOAc–Skelly B, 1:3)	–172.4 (23) (c = 1, EtOAc)	2.9 (s, 4, CH_2CH_2), 3.5 (d, 2, $CH_2C_6H_4$), 4.7 (m, 1, $CHCH_2$), 5.2 (m, 1 NH), 7.0–8.0 (m, 12, aryl, vinyl)[d]	$C_{27}H_{20}Cl_2N_2O_4$	63.97 64.07	3.97 4.04	5.52 5.45
Me_2CHCO	Bn	CMe_3	81.1	146–7 (EtOAc–Skelly B, 1:3)	–81.4 (26) (c = 1, CH_2Cl_2)	1.38 (d, 12, two $CHMe_2$), 1.54 (s, 9, CMe_3), 3.2 (m, 2, $CH_2C_6H_5$), 3.7 (m, 2 $CHMe_2$), 4.3 (m, 1 $CHCH_2$), 6.0 (m, 1 NH), 7.1–7.4 (7, 5, aryl, 7.7–8.3 (m, 7, aryl, CH=)	$C_{35}H_{39}NO_4$	78.18 78.26	7.31 7.19	2.60 2.58

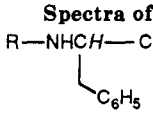
[a] General techniques and other specific examples are given in the Experimental Section. [b] In $CDCl_3$ unless otherwise indicated. [c] Spectrum taken in DMSO-d_6. [d] Spectrum taken in $CDCl_3$–DMSO-d_6. [e] Spectrum taken in acetone-d_6. [f] From D-phenylalanine. [g] All active esters listed in this table were obtained from the corresponding acid and either p-nitrophenol or N-hydroxysuccinimide via DCC coupling according to standard techniques.

Table II. Treatment of R-Phe-OSu with Triethylamine Followed by Conversion to R-Phe-Ala-OMe and ^{1}H NMR Analysis for DL-Diastereomer[a]

	amount of DL form,[b] %			
time, h	R = BOC	R = Z	R = Pht[c]	R = DC-$\overline{FM}$
0	<1, <1	<1, <1	<1, <1	<1, <1
1	18.6, 16.7	25.0, 26.8	31.3, 32.9	50.0, 50.0
2	29.5, 31.2	37.7, 33.3	47.2, 45.8	50.0, 50.0
4	38.5, 37.5	50.0, 50.0	50.0, 50.0	50.0, 50.0

[a] A 0.1 M solution of active ester in CH_2Cl_2 was pretreated with NEt_3 for the time indicated prior to effecting a coupling reaction with H-Ala-OMe. [b] Figures given are for two independent runs in each case. Sensitivity ±1%. [c] 5% (w/w) tris[3-(trifluoromethylhydroxymethylene)-(+)-camphorato]europium(III) added in order to distinguish the diastereomers.

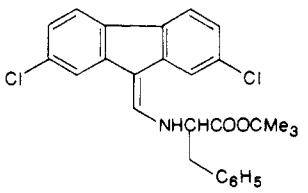
Table III. Chemical Shift of the CH Proton in the ^{1}H NMR Spectra of



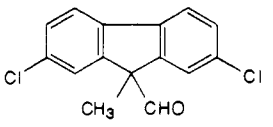
R	R′	CH, δ (ppm)
$\overline{FM}$	CH_3	4.15
DC-$\overline{FM}$	CMe_3	4.2
DC-$\overline{FM}$	H	4.4
DC-$\overline{FM}$	ONP	4.6
DC-$\overline{FM}$	OSu	4.7
HCO	ONP	5.1
Pht	OSu	5.5

effects of the *N*-hydroxysuccinimide moiety undoubtedly contribute to the reactivity difference between **22** (**23**) and the simple *tert*-butyl ester **24**. A possible rough measure of such inductive effects may be the chemical shift of the α-proton in the ^{1}H NMR spectrum. Some values for derivatives obtained in the course of this work are collected in Table III.

These tests were extended to simple nonactivated esters such as **25** and **26**, the former of which is locked in an imine structure. Although it was not possible to synthesize a pure sample of imine **25**, an appropriate test sample was obtained as a 3/2 mixture of **25** and the corresponding precursor aldehyde, 2,7-dichloro-9-methyl-9-formylfluorene **27**. The 3/2 mixture of **25** and **27** as well as enamine **26**



26



27

were treated with triethylamine in chloroform for a specified period of time, the reaction quenched, the protecting group removed, and the resulting amino acid ester treated with FMOC-α-phenylglycine chloride. The resulting crude dipeptides were examined for contamination of the LL by the LD diastereomer according to an NMR method previously described.[22] For FMOC-Phg-Phe-$OCMe_3$[23] the LL (DD) diastereomer shows its *tert*-butyl peak at δ 1.32; the LD (DL) isomer at 1.43. Results are presented in Table IV. Clearly imine **25** leads to more racemization than the simple enamine **26**, which might undergo slow tautomerism to the corresponding imine.

Table IV. Base-Catalyzed Racemization of 25 and 26 via Triethylamine[a]

	amt LD-FMOC-Phg-AA-$OCMe_3$,[b] %	
time, h	**25**	**26**
0	<1, <1	<1, <1
8	4.1, 4.9	<1, <1
24	10.9, 11.3	4.3, 4.8

[a] The enamine or aldimine ester mixture was treated with 3 equiv of NEt_3 in $CHCl_3$ for the times indicated, and the reaction mixture was worked up as described in the Experimental Section. [b] Amount given is for two individual runs; sensitivity ±1%.

Table V. Racemization during the Mixed Anhydride Coupling of Protected α-Phenylglycine with Alanine Methyl Ester[a,b]

activation time, min	amt DL diastereomer, %		
	base	Z	DC-$\overline{FM}$
10	2 equiv of NEt_3	35.0, 33.7	5.7, 6.4
6	1.5 equiv of NMP	25.0, 29.0	4.7, 5.0

[a] A 0.1 M solution of the protected α-phenylglycine in 5 mL of THF was treated with the base indicated and 1 equiv of isobutyl chloroformate and stirred for the time shown prior to the addition of H-Ala-OMe·HCl. See the Experimental Section. [b] The figures given are for two independent runs; sensitivity ±1%.

Tests such as these are somewhat artificial and may not be pertinent to the actual conditions of peptide synthesis. In order to compare coupling reactions for DC-FM-bar and urethane protectants peptide-bond formation by the mixed anhydride method[24] was examined. For the DC-FM-bar and benzyloxycarbonyl derivatives of phenylalanine no significant amount of the DL diastereomer (<1%) could be detected when carrying out the reaction using long activation times (10–15 min) at either 0 °C or room temperature in the presence of 2 equiv of triethylamine. With the more sensitive amino acid α-phenylglycine, analogous reaction conditions led to significantly greater racemization in the case of urethane protection. For the results see Table V.

Having thus demonstrated the low tendency toward racemization of the DC-FM-bar function and in spite of the fact that this group may not be the optimum system among this family of protectants, the synthesis of a simple model peptide, leucine enkephalin **29**, was examined. All

$$\text{DC-}\overline{\text{FM}}\text{-Tyr(CMe}_3\text{)-Gly-Gly-Phe-Leu-OCMe}_3 \xrightarrow{\text{TFA}} \underset{\textbf{28}}{} \underset{\textbf{29}}{\text{H-Tyr-Gly-Gly-Phe-Leu-OH}} \quad (8)$$

coupling reactions were carried out under normal conditions with DCC in the absence of any additive. Had an additive such as *N*-hydroxybenzotriazole been used in the coupling steps yields might have been higher,[25] but it was considered more significant to demonstrate lack of racemization in the absence of additive. Deblocking of the intermediate di-, tri-, and tetrapeptides was effected by catalytic hydrogenolysis. With the C-terminal leucine and the N-terminal tyrosine units protected by *tert*-butyl ester and ether functions, respectively, the final deblocking by TFA removed all protecting groups including the last DC-FM-bar function (eq 8). The resulting free pentapeptide **29** was shown to be identical with an authentic

(22) Carpino, L. A. *J. Org. Chem.* **1988**, *53*, 875.
(23) All four diastereomers of this set of protected dipeptide esters have been isolated and characterized according to the methods of ref 22. The results will be published separately.
(24) Meienhofer, J. In *The Peptides*; Gross, E., Meienhofer, J., Eds.; Academic Press: New York, 1979; Vol. 1, p 263.
(25) Compare: Bodanszky, M.; Martinez, J. In *The Peptides*; Gross, E., Meienhofer, J., Eds.; Academic Press, New York, 1983; Vol. 5, p 191.

sample according to TLC, HPLC, melting point, and optical rotation. The ^1H NMR spectrum agreed with data previously described, and the high-resolution FAB mass spectrum gave the appropriate molecular ions for both the protected and free peptide. Hydrolysis of the free peptide followed by derivitization of the constituent amino acids and GC analysis on a chiral column confirmed the lack of any significant racemization, although small amounts of diastereomeric impurities might have been removed at each coupling step since in this first model synthesis the di-, tri-, and tetrapeptide intermediates were recrystallized or chromatographed before proceeding to the next step. During the synthesis no particular difficulty arose because of air oxidation of the DC-FM-bar unit although some yellow color developed at certain stages of the process. As had been observed with the simple amino acid derivatives, DC-FM-bar peptide esters, as opposed to free acids, were relatively stable toward hydrolysis and/or air oxidation.

In conclusion, evidence is presented that certain substituted 9-fluorenylmethylene groups represent novel forms of α-protection for amino acids which, under conditions of peptide synthesis, are more protective toward racemization than a common urethane function. Whether analogous protection of side chain amino or amide functions is also possible or advantageous remains to be seen. Indeed in the case of certain amino acids there is currently a greater need for such new and improved side chain amino or amide protectants.

Experimental Section

Instrumentation and General Procedures. Melting points and boiling points were uncorrected. Infrared spectra were determined on Perkin-Elmer Model 237B or 1420 spectrometers with polystyrene as reference and ^1H NMR spectra on Perkin-Elmer R-12 (60 MHz) or Varian XL-200 (200 MHz) or XL-300 (300 MHz) instruments with Me_4Si as internal standard. Optical rotations were obtained with a Rudolph Autopol III digital polarimeter using a 10-cm quartz cell. Elemental analyses were carried out by the University of Massachusetts Microanalytical Laboratory under the direction of Greg Dabkowski. GC data were obtained on a Perkin-Elmer Model Sigma-2000 instrument using a flame ionization detector and a Perkin-Elmer LC-100 integrator. A 25-m Chirasil-Val-L capillary column obtained from Chrompak, Inc., was used to resolve derivatized enantiomeric amino acid esters. HPLC data were obtained on an automated Waters system with the following components: 721 System Controller, M730 Data Module, U6K injector, 710B Wisp, 510 and 6000A pumps, Z-module radial compression unit, and 441 absorbance detector. Mass spectral data were obtained on a Kratos MS 25 instrument by the fast atom bombardment technique.

9-Formylfluorene Methyl Hemiacetal (7). This preparation involves modifications of reported techniques.[9c,15] A mixture of 83 g of fluorene, 65 g of 50% NaH-in-oil dispersion, and 90 mL of ethyl formate in 500 mL of dry ether was brought to reflux in a 2-L, three-neck round-bottomed flask. The reaction mixture was watched carefully and as soon as the refluxing became vigorous the heating mantle was moved away from the flask in order to maintain a gentle reflux rate. If the reaction became too vigorous, the heating mantle was removed and cooling water used to moderate the reaction. After the reaction mixture had been refluxed for 7–10 h the heating mantle was removed and the mixture was treated, slowly at first, with 750 mL of water with stirring. If too little water is used three layers result. The ether layer was separated and discarded, and the aqueous layer was washed with 200 mL of ligroin (bp 30–60 °C) in order to remove any remaining mineral oil and unreacted fluorene. The aqueous solution was cooled in an ice bath and acidified with a solution prepared from 200 mL of water and 50 mL of concentrated sulfuric acid. The oil which separated was extracted with three 125-mL portions of ether and the combined organic extracts washed with two 150-mL portions of water and 150 mL of 1 M $NaHCO_3$ solution. The solution was dried over $MgSO_4$, the solvent was removed with a rotary evaporator, and the thick brown residue was dissolved in 300 mL of warm methanol. After storage in a refrigerator or freezer for 1–2 days a mass of shimmering white crystals separated. Filtration and collection of additional crops gave 79.2 g (70%) of the hemiacetal. Although a convenient storage form of 9-formylfluorene, this compound was too unstable to be purified for analysis. In a melting point capillary it softened and melted indistinctly from about 80 to 97 °C. The IR spectrum showed a strong hydroxyl but no carbonyl or enol ether absorption. Upon standing in the open, carbonyl adsorption developed as methanol was lost to the atmosphere. If, on the scale given, the brown residue was distilled rather than added to methanol, there was obtained 71.5 g (73.7%) of the aldehyde/vinyl alcohol as a golden-yellow syrup, bp 205–208 °C (25 mm). This material could be used in the preparation of derivatives, although the hemiacetal was more convenient to handle.

$\overline{FM}$-Phe-OMe. A mixture of 4.3 g of H-Phe-OMe·HCl and 2.8 mL of NEt_3 in 50 mL of CH_2Cl_2 was treated with 5.42 g of hemiacetal **7**. A complete solution resulted upon addition of the hemiacetal. After 5–6 h the solution was washed with two 50-mL portions of water, dried ($MgSO_4$), and evaporated to give a yellow solid (4.7 g), which was recrystallized from 75 mL of ethanol containing 10–20% of nitroethane to give 3.5 g (82%) of the ester as light yellow crystals: mp 145.5–147 °C; ^1H NMR ($CDCl_3$) δ 3.15 (d, 2, CH_2), 3.75 (s, 3, CH_3O), 4.1–4.5 (m, 1, CH), 5.5 (m, 1, NH), 6.86–7.9 (m, 14, aryl, CH=). Anal. Calcd for $C_{24}H_{21}O_2N$: C, 81.10; H, 5.96; N, 3.94. Found: C, 81.01; H, 6.16; N, 3.92.

$\overline{FM}$-Phe-OH. A mixture of 4.53 g of **7**, 3.3 g of phenylalanine, 1.3 g of NaOH, and 50 mL of MeOH was heated to reflux with stirring under N_2 for 3 h. After filtration to remove a trace of insoluble material, the MeOH was removed on a rotary evaporator, and 100 mL of water was added. The solution was extracted with several 50-mL portions of ether to remove the unreacted aldehyde, cooled, and acidified with 0.2 N HCl to pH ca. 4. The white precipitate was extracted from the aqueous mixture with 100 mL of cold EtOAc. The extracts were washed with two 100-mL portions of cold saturated NaCl solution, dried over $MgSO_4$ in the cold, evaporated to a volume of about 15 mL, cooled, and the precipitate collected. Additional crops were obtained by repeating this concentration procedure. There was obtained 5.86 g (85%) of the crude acid, mp 130–135 °C dec. The acid could be recrystallized from EtOAc–hexane with a recovery of 60%: mp 132–8 °C dec; α^{24}_D = −125.7° (c = 1.291, MeOH); ^1H NMR ($CDCl_3$–CD_3SOCD_3) δ 3.3 (d, 2, CH_2), 4.2–4.6 (m, 1, CH), 7.1–8.1 (m, 14, aryl, CH=). Anal. Calcd for $C_{23}H_{19}O_2N$: C, 80.91; H, 5.61; N, 4.10. Found: C, 80.58; H, 5.80; N, 4.06.

$\overline{FM}$-Gly-OH. A suspension of 0.56 g of $\overline{FM}$-Gly-OEt in 10 mL of water containing 0.4 g of NaOH was stirred and refluxed until the solid dissolved completely (30 min). The solution was cooled, filtered to remove a trace of precipitate, extracted with ether to remove unreacted ester, and acidified to pH 3 with 0.2 N HCl. The resulting precipitate was extracted into two 30-mL portions of ethyl acetate, the extracts were washed with two 30-mL portions of saturated NaCl solution, dried ($MgSO_4$), and concentrated without the application of heat, and the solution was treated with hexane to precipitate 0.28 g (55.7%) of the acid, mp 179–81 °C. The ^1H NMR and IR data agreed with data obtained for the sample obtained by direct condensation of **7** with glycine (Table I).

2,7-Di-*tert*-butylfluorene. To a solution of 50 g of fluorene and 100 mL of *tert*-butyl chloride in 500 mL of CH_2Cl_2 under an atmosphere of N_2 was added in small portions anhydrous $FeCl_3$ at a rate to maintain steady evolution of HCl. Over a period of 2.5 h a total of 6 g of $FeCl_3$ was used. When the reaction was finished addition of the catalyst no longer caused HCl evolution. The reaction mixture was washed with five 200-mL portions of 10% hydrochloric acid and two 200-mL portions of water and dried over $MgSO_4$. Removal of solvent and passage of the crude product through a column of basic alumina with elution by hexane served to remove residual $FeCl_3$. There was obtained 60 g (72%) of the hydrocarbon as white crystals, mp 122 °C (lit.[14a] mp 122 °C).

Formylation of 2,7-Di-*tert*-butylfluorene. To a suspension of 7.3 g of 36% KH-in-oil dispersion in 50 mL of dry ether was added dropwise under N_2 a solution of 4 g of 2,7-di-*tert*-butylfluorene in 100 mL of ether. A small evolution of H_2 occurred.

Immediately after addition of the hydrocarbon was complete, 10 mL of ethyl formate was added dropwise at a rate to maintain a moderate evolution of H_2. A precipitate separated, and the mixture was stirred overnight and the ether evaporated. The precipitated solid was washed with pentane to remove any excess hydrocarbon, and the residue was added to 100 mL of ice water and 150 mL of CH_2Cl_2. After acidification with 20% H_2SO_4 the organic layer was collected and washed successively with 1 M $NaHCO_3$ solution and water and then dried over $MgSO_4$. Evaporation gave a light yellow semisolid, presumably the expected aldehyde, which was recrystallized from methanol to give 3.4 g of a yellow solid, mp 103.5–105 °C, which appeared from the ^1H NMR (two *tert*-butyl peaks) and IR (no C=O) spectra to be the methyl ether of 2,7-di-*tert*-butyl-9-hydroxymethylene fluorene. On standing overnight in the open partial conversion to the 9-formyl derivative appeared to occur (^1H NMR, IR). This material was very sensitive to air oxidation and was not purified. It was characterized by its reaction with *p*-chloroaniline: upon stirring a solution of 2.7 g of the crude vinyl ether with 1.07 g of *p*-chloroaniline in 25 mL of CH_2Cl_2 and 25 mL of hexane for 2 days at room temperature there was obtained 2.23 g (64%) of **2,7-di-*tert*-butyl-9-[[(*p*-chlorophenyl)amino]methylene]-fluorene** as a yellow solid, mp 224–225 °C. Recrystallization from MeOH–acetone (3:1) gave yellow crystals, mp 243–244 °C. Anal. Calcd for $C_{28}H_{30}ClN$: C, 80.84; H, 7.27; Cl, 8.52; N, 3.37. Found: C, 80.76; H, 7.05; Cl, 8.35; N, 3.33.

2,7-Dichloro-9-(hydroxymethylene)fluorene (17). A solution of 77.6 mmol of EtOK, freshly prepared from 3 g of potassium metal and 9 mL of dry EtOH in 60 mL of dry ether was cooled to 0 °C under nitrogen. To the solution was added 11.75 g (50 mmol) of 2,7-dichlorofluorene[15] portionwise. After addition was complete, a solution of 5 mL of dry ethyl formate in 20 mL of dry ether was added dropwise through a dropping funnel. The mixture was refluxed for 3 h, allowed to cool to room temperature, and then poured into 150 mL of ice–water. The aqueous layer was extracted with Skelly B (3 × 50 mL), and the extracts were discarded. The aqueous layer was acidified with 5% H_2SO_4 to Congo Red, and the precipitated solid was dissolved by the addition of 200 mL of EtOAc. The solution was washed with saturated NaCl solution, dried over $MgSO_4$, and the solvent removed in vacuo with the aid of a water aspirator to give a yellow solid. Recrystallization from ether–Skelly B (1:4) gave 12.1 g (92.0%) of the vinyl alcohol, mp 152–155 °C dec, which was stored under nitrogen in a refrigerator. For characterization the vinyl alcohol was converted to its **benzoyl derivative**.

To a solution of 0.526 g (2 mmol) of the vinyl alcohol in 10 mL of 1% NaOH solution was added 0.34 g (2.4 mmol) of benzoyl chloride with vigorous stirring. The mixture was stirred at room temperature for 15 min. The white precipitate was extracted into EtOAc, and the solution was washed with 10% $NaHCO_3$, 5% HCl, and finally H_2O. Drying over $MgSO_4$ and removal of solvent in vacuo with the aid of a water aspirator gave 0.612 g (90.0%) of the *O*-benzoyl derivative as a white solid. Recrystallization from benzene–acetone (10:1) gave the ester as white needles: mp 218–219 °C; ^1H NMR (DMSO-d_6) δ 7.3–8.8 (m, vinyl + aryl); IR (KBr) 1740 cm^{-1} (C=O). Anal. Calcd for $C_{21}H_{12}Cl_2O_2$: C, 68.68; H, 3.30; Cl, 19.31. Found: C, 68.58; H, 3.21; Cl, 19.25.

DC-$\overline{\text{FM}}$-*p*-chloroaniline. A solution of 0.526 g (2 mmol) of DC-$\overline{\text{FM}}$-OH and 0.255 g (2 mmol) of *p*-chloroaniline in 40 mL of EtOAc was refluxed for 16 h, allowed to cool to room temperature, and washed three times each with 3 N HCl, 5% $NaHCO_3$, and H_2O. Drying over $MgSO_4$ and removal of solvent in vacuo with a water aspirator gave a yellow solid, which was recrystallized twice from MeOH–acetone (3:1) to give 0.589 g (80.5%) of the enamine: mp 222–227 °C dec; ^1H NMR (DMSO-d_6) δ 7.2–8.3 (m, 11, aryl, CH=), 9.15 (d, 1, NH); IR (KBr) 3450 (NH), 1675 cm^{-1} (C=C). Anal. Calcd for $C_{20}H_{12}Cl_3N$: C, 64.45; H, 3.25; N, 3.76. Found: C, 64.76; H, 3.49; N, 3.80.

General Procedure for the Preparation of 2,7-Dichloro-9-fluorenylmethylene Derivatives of Amino Acids (DC-$\overline{\text{FM}}$ Derivatives). To a clear solution of 10 mmol of amino acid and 11 mmol of NaOH in 50 mL of MeOH was added 10 mmol of DC-$\overline{\text{FM}}$-OH. The mixture was refluxed for 4–6 h and allowed to cool to room temperature, and the solvent was removed in vacuo with the aid of a water aspirator. The residue was dissolved in 150 mL of H_2O, and the aqueous layer was extracted with EtOAc (3 × 20 mL) to remove unreacted starting material. The aqueous solution was acidified with 5% H_2SO_4 to Congo Red. The protected amino acid which had precipitated was dissolved in EtOAc, and the solution was washed with saturated NaCl solution. Drying over $MgSO_4$ and solvent removal in vacuo with the aid of a water aspirator gave the protected amino acid, which was recrystallized from an appropriate solvent. Specific examples are given in Table I.

DC-$\overline{\text{FM}}$-Phg-Ala-OMe. A solution of 0.396 g (1 mmol) of DC-$\overline{\text{FM}}$-Phg-OH, 0.14 g (1 mmol) of H-Ala-OMe·HCl, 0.14 mL (1 mmol) of NEt_3, and 0.248 g (1 mmol) of EEDQ in a mixture of 5 mL of CH_3CN and 3 mL of DMF was kept in a refrigerator (−5 °C) overnight and then at room temperature with stirring for 4 h. The mixture was diluted with 20 mL of EtOAc and washed with 10% $NaHCO_3$, 5% HCl, and H_2O. After drying ($MgSO_4$) and removal of solvent, the residue was chromatographed (2 × 70 cm; 50 g of silica gel) with elution by 20% acetone/Skelly B. After collecting the desired fraction and evaporating the solvent the residual solid was recrystallized from $CHCl_3$–hexane (1:3) to give 0.306 g (65.0%) of the protected dipeptide: mp 195 °C dec; $[\alpha]^{27}_D$ = −55° (*c* = 0.4, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.45 (d, 3, $CHCH_3$), 3.7 (s, 3, OCH_3), 4.6 (m, 1, $CHCH_3$), 5.0 (d, 1, CHC_6H_5), 6.15 (d, 1, NH), 6.8 (dd, 1, NH), 7.0–7.8 (m, 12, aryl, CH=); IR (KBr) 3400, 3300 (NH), 1740, 1640 cm^{-1} (C=O). Anal. Calcd for $C_{26}H_{22}Cl_2N_2O_3$: C, 64.87; H, 4.61; N, 5.82. Found: C, 64.82; H, 4.66; N, 5.75.

***N*-Formyl-Phe-ON*p* from *N*-Formylphenylalanine.** A solution of 0.597 g (3 mmol) of For-Phe-OH[26] and 0.44 g (3 mmol) of *p*-nitrophenol in 15 mL of THF was cooled to 0 °C, and 0.63 g (3 mmol) of DCC was added. The mixture was stirred at 0 °C for 3 h and then at room temperature for 2 h. After filtration the residue was recrystallized from MeOH and then from EtOAc–Skelly B (1:3) to give 0.74 g (81.0%) of the *p*-nitrophenyl ester: mp 138–139 °C; $[\alpha]^{23}_D$ −24.0° (*c* = 0.2, dioxane); ^1H NMR ($CDCl_3$) δ 3.2 (d, 2, $CH_2C_6H_5$), 5.1 (q, 1, CH), 6.6 (d, 1, NH), 7.1–8.3 (m, 10, aryl, CHO); IR (KBr) 3280 (NH), 1760, 1660 cm^{-1} (C=O). Anal. Calcd for $C_{16}H_{14}N_2O_5$: C, 61.14; H, 4.49; N, 8.92. Found: C, 61.13; H, 4.36; N, 8.84.

***N*-Formyl-Phe-ON*p* by Oxidation of DC-$\overline{\text{FM}}$-Phe-ON*p*.** A solution of 0.531 g (1 mmol) of DC-$\overline{\text{FM}}$-Phe-ON*p* in 25 mL of THF was stirred at room temperature for 2 days. The original enamine was still present according to TLC analysis. Therefore 0.173 g (1 mmol) of MCPBA was introduced after which the starting material disappeared within 1 h. After removal of solvent the residue was chromatographed (2 × 56 cm; 50 g of silica gel) and eluted with 40% Skelly B–EtOAc to give 0.305 g (97.0%) of the formyl derivative, mp 136–138 °C; $[\alpha]^{25}_D$ = −24.2° (*c* = 0.2, dioxane); identified (NMR and IR) by spectral comparison with an authentic sample prepared as described above.

DC-$\overline{\text{FM}}$-Phe-Ala-OMe and DC-$\overline{\text{FM}}$-D-Phe-Ala-OMe via the DCC Method. A solution of 0.53 g (1.3 mmol) of DC-$\overline{\text{FM}}$-Phe-OH, 0.182 g (1.3 mmol) of H-Ala-OMe·HCl, and 0.182 mL (1.3 mmol) of NEt_3 in 15 mL of THF was cooled to 0 °C under nitrogen, and 0.269 g of DCC was added with stirring. The resulting solution was kept in a refrigerator overnight and then at room temperature with stirring for 3 h. After filtration and removal of solvent, the residue was dissolved in 15 mL of EtOAc and the solution was washed with 10% $NaHCO_3$, 5% HCl, and H_2O. Drying over $MgSO_4$ and removal of solvent gave a yellow solid, which was recrystallized from MeOH–$MeNO_2$ (9:1) to give 0.32 g (50.0%) of the protected dipeptide: mp 241–243 °C; $[\alpha]^{27}_D$ = −101° (*c* = 0.4, EtOAc); ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 1.42 (d, 3, $CHCH_3$), 3.1–3.4 (m, 2, $CH_2C_6H_5$), 3.72 (s, 3, OCH_3), 4.3 (m, 1, $CHCH_2$), 4.6 (m, 1, $CHCH_3$), 6.9–7.9 (m, 13, aryl, CH=, NH), 8.5 (d, 1, NH); IR (KBr) 3400, 3300 (NH), 1725, 1640 cm^{-1} (C=O). Anal. Calcd for $C_{27}H_{24}Cl_2N_2O_3$: C, 65.46; H, 4.88; N, 5.66. Found: C, 65.27; H, 4.98; N, 5.65.

The corresponding DL diastereomer was prepared from DC-$\overline{\text{FM}}$-D-Phe-OH following the procedure described above. Recrystallization from EtOAc–Skelly B (1:2) gave 0.4 g (56.0%) of the protected dipeptide: mp 219–220 °C; $[\alpha]^{25}_D$ = +86.75° (*c* =

(26) Snoke, J. E.; Neurath, H. *J. Biol. Chem.* **1949**, *181*, 780.

0.8, EtOAc); ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ 1.36 (d, 3, $CHCH_3$), 3.1–3.4 (m, 2, $CH_2C_6H_5$), 3.74 (s, 3, OCH_3), 4.3 (m, 1, CH_2CH), 4.6 (m, 1, $CHCH_3$), 6.6 (m, 1, NH), 7.0–8.2 (m, 13, aryl, CH=, NH); IR (KBr) 3410, 3300 (NH), 1730, 1600 cm^{-1} (C=O). Anal. Calcd for $C_{27}H_{24}Cl_2N_2O_3$: C, 65.46; H, 4.88; N, 5.66. Found: C, 65.43; H, 4.96; N, 5.65.

DC-$\overline{FM}$-Phe-Ala-OMe via the Mixed Carbonic Anhydride Method. A solution of 0.82 g (2 mmol) of DC-$\overline{FM}$-Phe-OH, 0.28 g (2 mmol) of NEt_3, and 0.2 mL (2 mmol) of ethyl chloroformate in 20 mL of $CHCl_3$ was cooled to −15 °C under nitrogen with stirring for 10 min. To the solution was added 0.28 g (2 mmol) of H-Ala-OMe·HCl followed by 0.28 mL (2 mmol) of NEt_3. The mixture was stirred at −15 °C for 1 h and at room temperature for 1 h. After washing with 10% $NaHCO_3$, 5% HCl, and H_2O, drying over $MgSO_4$, and removal of solvent in vacuo with a water aspirator there was obtained a yellow solid, which was recrystallized from EtOAc–Skelly B (1:2) to give 0.62 g (63.0%) of the protected dipeptide, mp 240–242 °C. All physical properties and spectral data agreed with analogous data obtained for the sample prepared via the DCC coupling technique.

NMR-Based Racemization Test. Coupling of DC-$\overline{FM}$-Phe-OH with H-Ala-OMe via DCC or MA Methods. The crude material from either the DCC or MA methods described directly above was loaded onto a column (2 × 51 cm; 50 g of silica gel) and eluted with 30% EtOAc in Skelly B. TLC analysis showed that both diastereomers had the same R_f value. Desired fractions were collected and examined by ^{1}H NMR analysis for contamination of the LL diastereomer (methyl peak at δ 1.42) by the DL diastereomer (methyl peak at δ 1.36). Duplicate runs for both the DCC and MA methods showed no detectable amount (<1%) of the DL diastereomer (recovery of pure LL diastereomer was 78.0–81.0%).

DC-$\overline{FM}$-Ala-Phe-OMe. A solution of 0.398 g (1 mmol) of DC-$\overline{FM}$-Ala-OH, 0.221 g (1 mmol) of H-Phe-OMe·HCl, 0.140 mL (1 mmol) of NEt_3, and 0.253 g (1 mmol) of EEDQ in 10 mL of CH_2Cl_2 was stirred at 0 °C under nitrogen for 4 h and at room temperature for 14 h. The mixture was diluted with 20 mL of ether and washed with 10% $HaHCO_3$, 5% HCl, and H_2O. Drying over $MgSO_4$ and removal of solvent in vacuo with the aid of a water aspirator gave a yellow solid. Recrystallization from 40% acetone in Skelly B gave 0.388 g (79.0%) of the protected dipeptide; mp 222.5 °C dec; $[\alpha]^{21}_D$ = +92.6° (c = 0.5, acetone); ^{1}H NMR (DMSO-d_6) δ 1.6 (d, 3, CH_3CH), 3.0–3.2 (m, 2, $CH_2C_6H_5$), 3.7 (s, 3, OCH_3), 4.2 (m, 1, $CHCH_2$), 4.9 (m, 1, $CHCH_3$), 6.8–8.2 (m, 14, aryl, CH=, 2 NH); IR (KBr) 3400, 3280 (NH), 1760, 1670 cm^{-1} (C=O). Anal. Calcd for $C_{27}H_{24}Cl_2N_2O_3$: C, 65.46; H, 4.88; N, 5.66. Found: C, 65.20; H, 4.99; N, 5.50.

DC-$\overline{FM}$-Phe-Leu-OMe. To a solution of 0.84 g (2.23 mmol) of DC-$\overline{FM}$-Phe-OH, 0.44 g (2.23 mmol) of H-Leu-OMe·HCl, 0.31 mL (2.23 mmol) of NEt_3, and 0.331 g (2.23 mmol) of N-hydroxybenzotriazole hydrate in a mixture of 15 mL of EtOAc and 2 mL of DMF was added 0.48 g (2.23 mmol) of DCC at 0 °C under nitrogen. The mixture was stirred at 0 °C for 3 h and at room temperature for 14 h. Dicyclohexyl urea and NEt_3·HCl were removed by filtration, and the solution was washed with 10% $NaHCO_3$, 5% HCl, and H_2O. Drying over $MgSO_4$ and removal of solvent in vacuo with the aid of a water aspirator gave a yellow solid, which was recrystallized from EtOAc–Skelly B (1:4) to give 0.91 g (74.0%) of the protected dipeptide: mp 201–202 °C; $[\alpha]^{23}_D$ = −69.8° (c = 1, EtOAc); ^{1}H NMR ($CDCl_3$) δ 0.9 (dd, 6, $CHMe_2$), 1.4–1.8 (m, 3, $CHCH_2$), 3.1–3.4 (m, 2, $CH_2C_6H_5$), 3.72 (s, 3, OCH_3), 4.2 (m, 1, $CHCH_2$), 4.7 (m, 1, $CHCH_2C_6H_5$), 5.5 (m, 1, NH), 6.5 (d, 1, NH), 7.0–7.8 (m, 12, aryl, CH=); IR (KBr) 3400, 3300 (NH), 1740, 1640 cm^{-1} (C=O). Anal. Calcd for $C_{30}H_{30}Cl_2N_2O_3$: C, 67.04; H, 5.63; N, 5.21. Found: C, 67.08; H, 5.54; N, 5.07.

HPLC-Based Racemization Test. Coupling of DC-$\overline{FM}$-Phe-OH with H-Leu-OMe via the DCC Technique. To a solution of 0.377 g (1 mmol) of DC-$\overline{FM}$-Phe-OH, 0.18 g (1 mmol) of H-Leu-OMe·HCl, and 0.14 mL (1 mmol) of NEt_3 in a mixture of 7 mL of EtOAc and 1 mL of DMF was added 0.206 g (1 mmol) of DCC at 0 °C under nitrogen. The resulting solution was stirred at 0 °C for 3 h and at room temperature for 14 h. Dicyclohexyl urea and NEt_3·HCl were removed by filtration, and the filtrate was washed with 10% $NaHCO_3$, 5% HCl, and H_2O. Drying over $MgSO_4$ and removal of solvent in vacuo with the aid of a water aspirator gave a yellow solid, which, without any purification, was dissolved in a mixture of 5 mL of MeOH and 3 mL of EtOAc. To the solution was added 50 mg of 10% Pd–C and 50 mg of $Pd(OAc)_2$ followed by 0.5 g of NH_4OCHO. The mixture was stirred at room temperature until the protected dipeptide spot disappeared as monitored by TLC (ca. 7 h). Filtration and removal of solvent gave a yellow oil, which was dissolved in 10 mL of CH_2Cl_2. To the solution was added 0.58 mL (5 mmol) of benzoyl chloride with vigorous stirring, followed by 10 mL of 10% $NaHCO_3$ solution. The resulting solution was stirred at 0 °C for 30 min, the organic layer was collected, and 1.1 mL (10 mmol) of N-methylpiperazine was added followed by stirring at room temperature for 30 min. The resulting mixture was diluted with 30 mL of ether, washed with 5% HCl, 10% $NaHCO_3$, and H_2O, and dried over $MgSO_4$, and the solvent was removed in vacuo with the aid of a water aspirator to give the crude benzoyl dipeptide which was examined by HPLC analysis. Triplicate runs from both the DCC and MA methods showed no detectable amount of the DL isomer (<0.1%).[18]

2,7-Difluoro-9-(hydroxymethylene)fluorene. This compound was prepared following the procedure described for the dichloro system from 1.01 g (5 mmol) of 2,7-difluorofluorene[27] to give 1.11 g (96.5%) of the vinyl alcohol, mp 121–123 °C. Due to the same oxidation problem mentioned for the dichloro system, the vinyl alcohol was characterized as its ***O*-benzoyl derivative.** After recrystallization from benzene–hexane (1:2) there was obtained 0.28 g (84.0%) of the benzoate: mp 180–181 °C; ^{1}H NMR ($CDCl_3$) δ 7.0–8.6 (m, aryl, CH=); IR (KBr) 1745 cm^{-1} (C=O). Anal. Calcd for $C_{21}H_{12}F_2O_2$: C, 75.44; H, 3.62; F, 11.37. Found: C, 75.61; H, 3.82; F, 11.30.

2,7-Diisobutyrylfluorene. A suspension of 8.3 g (50 mmol) of fluorene and 14.63 g (110 mmol) of anhydrous $AlCl_3$ in 100 mL of CS_2 was cooled to 0 °C. To the suspension was added 11.77 g (110 mmol) of isobutyryl chloride dropwise through a dropping funnel. After addition was complete, the resulting solution was stirred at room temperature for 1 h, refluxed for 3 h, allowed to cool to room temperature, and poured into 500 g of crushed ice. There was added 50 mL of 6 N HCl to decompose the $AlCl_3$. After extracting with $CHCl_3$ (3 × 100 mL), the organic solution was washed with 200 mL of 15% NaOH solution followed by saturated NaCl solution. An emulsion which was difficult to break formed upon addition of the NaOH. Drying over $MgSO_4$ and removal of solvent gave a brown residue which was passed through a short alumina column (elution by ether) and then Kugelrohred to give a yellow solid. After recrystallization from EtOH followed by acetone–hexane (1:4), there was obtained 5.6 g (36.0%) of the diketone as a white solid: mp 127–128 °C; ^{1}H NMR ($CDCl_3$) δ 1.2 (d, 12, 2 $CHMe_2$), 3.8 (m, 2, 2 CH), 4.03 (s, 2, CH_2), 7.9–8.3 (m, 6, aryl); IR (KBr) 1670 cm^{-1} (C=O). Anal. Calcd for $C_{21}H_{22}O_2$: C, 82.32; H, 7.14. Found: C, 82.09; H, 7.11.

2,7-Diisobutyryl-9-(hydroxymethylene)fluorene. A solution of EtONa, freshly prepared from 0.4 g of NaH and 1 mL of EtOH in 20 mL of dry ether, was cooled to 0 °C under nitrogen. To the solution was added 1.53 g (5 mmol) of 2,7-diisobutyrylfluorene portionwise. After stirring for 10 min, 1 mL of ethyl formate in 5 mL of dry ether was added through a dropping funnel. The resulting solution was refluxed for 3 h, allowed to cool to room temperature, and poured into 30 mL of ice–water. The aqueous solution was extracted with Skelly B (3 × 30 mL) and then acidified with 5% H_2SO_4 to Congo Red. The precipitate was dissolved in 50 mL of EtOAc and washed with saturated NaCl solution. After drying ($MgSO_4$) and removal of solvent, the residue was recrystallized from acetone–hexane (1:4) to give 1.17 g (70.0%) of the vinyl alcohol: mp 220 °C dec; ^{1}H NMR ($CDCl_3$) δ 1.2 (d, 12, 2 $CHMe_2$), 3.8 (m, 2, 2 CH), 7.7–8.8 (m, 7, aryl, CH=); IR (KBr) 3600–3000 br (OH), 1680 cm^{-1} (C=O). Anal. Calcd for $C_{22}H_{22}O_3$: C, 79.01; H, 6.63. Found: C, 78.88; H, 6.54.

9-(Hydroxymethylene)thioxanthene 10,10-Dioxide (11). To a solution of 4.6 g (20 mmol) of thioxanthene 10,10-dioxide in 80 mL of dry ether was added 1.92 g (40 mmol) of 50% NaH in oil under nitrogen. After stirring for 15 min, a solution of 3 mL of ethyl formate in 20 mL of ether was added dropwise through a

(27) Berkovic, S. *Isr. J. Chem.* **1963**, *1*, 1.

dropping funnel. After addition was complete the mixture was refluxed for 6–8 h and allowed to cool to room temperature, and small chips of ice were added to decompose excess NaH. The resulting solution was poured into 100 mL of ice–water and extracted with Skelly B (3 × 50 mL). The aqueous solution was acidified to Congo Red with 5% H_2SO_4, and the precipitate was dissolved in 100 mL of EtOAc and washed with saturated NaCl solution. After drying over $MgSO_4$ and removal of solvent, the residue was recrystallized from EtOAc–Skelly B (1:1) to give 3.33 g (64.6%) of the vinyl alcohol as a white solid: mp 204–206 °C; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ 7.2–8.1 (m, 9, aryl, CH=), 11 (s, 1, OH); IR (KBr) 3600–3300 (OH), 1600 (C=C), 1280, 1160 cm^{-1} (SO_2). Anal. Calcd for $C_{14}H_{10}O_3S$: C, 65.10; H, 3.90; S, 12.41. Found: C, 65.33; H, 4.02; S, 12.23.

DTM-*p*-chloroaniline. A solution of 0.516 g (2 mmol) of DTM-OH and 0.256 g (2 mmol) of *p*-chloroaniline in 20 mL of EtOAc was refluxed for 2 h, and the reaction mixture worked up as given for the corresponding DC-FM-bar derivative to give 0.47 g (64.0%) of the pure enamine as a yellow solid: mp 280 °C dec; ^{1}H NMR ($CDCl_3$) δ 7.3–8.2 (m, 13, aryl, CH=), 9.5 (d, 1, NH); IR (KBr) 3400 (NH), 1640 cm^{-1} (C=C). Anal. Calcd for $C_{20}H_{14}ClNO_2S$: C, 65.30; H, 3.84; N, 3.81. Found: C, 65.36; H, 3.68; N, 3.73.

DTM-OTs. To a solution of 1.04 g (4 mmol) of DTM-OH and 0.85 g (4.5 mmol) of TsCl in 20 mL of acetone was added dropwise 3 mL of 5% NaOH solution, which induced a color change from yellow to red. After TsCl disappeared (TLC), solvent was removed, the residue was dissolved in CH_2Cl_2, and the solution was washed with saturated NaCl solution. After drying over $MgSO_4$ removal of solvent in vacuo gave a red solid, which was recrystallized from MeOH–acetone (2:1) to give 0.77 g (64.0%) of the tosylate as a white solid: mp 180–181 °C; ^{1}H NMR ($CDCl_3$) δ 2.3 (s, 3, CH_3), 7.15–8.2 (m, 13, aryl, CH=); IR (KBr) 1650 (C=C), 1300, 1180 cm^{-1} (SO_2). Anal. Calcd for $C_{21}H_{16}O_5S_2$: C, 61.15; H, 3.91; S, 15.55. Found: C, 61.11; H, 3.69; S, 15.56.

DTM-Gly-OMe (12). A solution of 0.26 g (1 mmol) of DTM-OH and 0.133 g (1.5 mmol) of H-Gly-OMe in 10 mL of EtOAc was refluxed for 6 h, cooled, and washed in order with 5% HCl, 10% $NaHCO_3$, and H_2O. After drying over $MgSO_4$ and removal of solvent, the residue was recrystallized from $MeNO_2$–MeOH (1:3) to give 0.185 g (56.0%) of the ester: mp 216–217 °C; ^{1}H NMR ($CDCl_3$) δ 3.8 (s, 3, OCH_3), 4.05 (d, 2, CH_2), 5.6 (m, 1, NH), 6.9 (d, 1, vinyl), 7.3–8.2 (m, 8, aryl); IR (KBr) 3420 (NH), 1740 cm^{-1} (C=O). Anal. Calcd for $C_{17}H_{15}NO_4S$: C, 61.99; H, 4.59; N, 4.25. Found: C, 62.09; H, 4.42; N, 4.51.

1-Chlorofluorene. A suspension of 7.83 g (37.7 mmol) of fluorene-1-carboxylic acid in 50 mL of thionyl chloride was refluxed for 2 h and allowed to cool to room temperature, and solvent was removed in vacuo with the aid of a vacuum pump to give a yellow solid, which was recrystallized from Skelly B to give 8.11 g (95.5%) of the acid chloride, mp 110–112 °C (lit.[28a] mp 112–113 °C). The acid chloride was mixed with 150 mg of $[(C_6H_5)_3P]_3$-Rh(III)Cl and heated to 270 °C in a sand bath for 5 min, allowed to cool to room temperature, and extracted with hot Skelly B (3 × 200 mL). After removal of solvent, the residue was purified by Kugelrohr distillation, and 5.88 g (78.5%) of the pure chloro compound was obtained: mp 66–68 °C (lit.[29] mp 69–70 °C); ^{1}H NMR ($CDCl_3$) δ 3.95 (s, 2, CH_2), 7.3–7.8 (m, 7, aryl).

1-Fluoro-9-(hydroxymethylene)fluorene. This compound was prepared from 1-fluorofluorene[28b,c] following the procedure described for the 2,7-dichlorofluorene system. The crude product was used for the subsequent reactions without further purification. Because of the problem of air oxidation it was characterized as its ***p*-chloroaniline derivative**, which was obtained (55%) in the usual way: mp 178.5–180 °C; ^{1}H NMR ($CDCl_3$) δ 7.0–8.5 (m, aryl, CH=, NH); IR (KBr) 3450 (NH), 1670 cm^{-1} (C=C). Anal. Calcd for $C_{20}H_{13}ClFN$: C, 74.65; H, 4.07; N, 4.35. Found: C, 74.76; H, 4.40; N, 4.49.

1-Fluoro-FM-Gly-OMe. A solution of 0.55 g (2.6 mmol) of crude 1-fluoro-FM-OH, 0.4 g (3.17 mmol) of H-Gly-OMe·HCl, and 0.444 mL (3.17 mmol) of NEt_3 in 20 mL of $CHCl_3$ was refluxed overnight under nitrogen, and the solution was worked up as given for FM-Phe-OMe. Recrystallization from EtOAc–hexane (1:5) gave 0.41 g (44.9%) of the ester as a white solid: mp 149–150 °C; ^{1}H NMR ($CDCl_3$) δ 3.81 (s, 3, OCH_3), 4.13 (d, 2, CH_2), 6.6 (m, 1, NH), 7.0–7.9 (m, 8, aryl, CH=); IR (KBr) 3410 (NH), 1740 (C=O), 1640 cm^{-1} (C=C). Anal. Calcd for $C_{17}H_{14}FNO_2$: C, 72.07; H, 4.98; N, 4.94. Found: C, 71.98; H, 4.74; N, 4.88.

1-Chloro-9-formylfluorene (15). This compound was prepared following the procedure described for the 2,7-dichlorofluorene system from 2.1 g of 1-chlorofluorene. There was obtained 2.22 g (97.0%) of the crude aldehyde as a yellow oil, which was chromatographed (4.4 × 70 cm; 150 g of silica gel; R_f = 0.2) with elution by 10% EtOAc in Skelly B to give the pure aldehyde as a white solid, which immediately upon isolation was spontaneously reconverted to a yellow oil, ^{1}H NMR ($CDCl_3$) δ 4.8 (d, 1, CH), 7.1–7.9 (m, 7, aryl), 9.2 (d, 1, CHO). In most cases the crude oil was used for subsequent reactions without further purification and because of the oxidation problem it was characterized as its ***p*-chloroaniline derivative**, which was obtained (24%) in the usual way: mp 156 °C dec; ^{1}H NMR ($CDCl_3$) δ 7.0–8.0 (m, 12, aryl), 8.7 (d, 1, NH); IR (KBr) 3440 (NH), 1640 cm^{-1} (C=C). Anal. Calcd for $C_{20}H_{13}Cl_2N$: C, 71.02; H, 3.87; N, 4.14. Found: C, 70.76; H, 3.74; N, 4.08.

1-Chloro-FM-Phe-OMe. A solution of 0.94 g (4.12 mmol) of 1-chloro-9-formylfluorene, 0.974 g (4.53 mmol) of H-Phe-OMe·HCl, and 0.634 mL (4.53 mmol) of NEt_3 was refluxed under nitrogen overnight, allowed to cool to room temperature, and washed with 10% $NaHCO_3$, 5% HCl, and H_2O. After drying over $MgSO_4$ and removal of solvent in vacuo with the aid of a water aspirator, there was obtained a yellow solid which was recrystallized from EtOAc–hexane (1:5) to give 1.1 g (62.3%) of the pure amino acid ester: mp 147–148 °C; $[\alpha]^{25}_D$ = −95.7° (*c* = 0.7, THF); ^{1}H NMR ($CDCl_3$) δ 3.15–3.35 (m, 2, $CH_2C_6H_5$), 3.8 (s, 3, OCH_3), 4.4 (m, 1, CH), 5.8 (m, 1, NH), 7.1–8.3 (m, 13, aryl, CH=); IR (KBr) 3450 (NH), 1730 (C=O), 1620 cm^{-1} (C=C). Anal. Calcd for $C_{24}H_{20}ClNO_2$: C, 73.93; H, 5.17; N, 3.59. Found: C, 73.81; H, 5.20; N, 3.67.

General Procedure for the Preparation of *N*-Hydroxysuccinimide Esters. A solution of 8 mmol of the protected amino acid, 1.02 g (8.8 mmol) of *N*-hydroxysuccinimide, and 1.85 g (8.8 mmol) of DCC in 40 mL of THF was kept in a freezer (−20 °C) overnight and at room temperature with stirring for 3 h. After filtration and removal of solvent, the residue was recrystallized from an appropriate solvent. Specific details for the Pht derivative are given below. Characterization data for other new compounds are given in Table I. Melting points and specific rotation data for the BOC and Z derivatives agreed with literature data.[30]

Pht-Phe-OSu. This compound was recrystallized from *i*-PrOH–Et_2O–Skelly B (1:1:4) to give 2.1 g (67.0%) of the *N*-hydroxysuccinimide ester: mp 123 °C dec; $[\alpha]^{23}_D$ = −156.4° (*c* = 1, EtOAc); ^{1}H NMR ($CDCl_3$) δ 2.9 (s, 4, CH_2CH_2), 3.6–3.8 (m, 2, $CH_2C_6H_5$), 5.5 (m, 1, CH), 7.2 (s, 5, aryl), 7.7–7.9 (m, 2, aryl); IR (KBr) 3300 br (NH), 1820, 1780, 1720 cm^{-1} (C=O). Anal. Calcd for $C_{21}H_{16}N_2O_6$: C, 64.28; H, 4.11; N, 7.14. Found: C, 63.99; H, 4.28; N, 7.00.

General Procedure for the Preparation of Model Protected Dipeptides. A solution of 2 mmol of the protected amino acid, 2 mmol of amino acid ester salt, 2 mmol of NEt_3, and 2 mmol of EEDQ in 15 mL of CH_2Cl_2, or in the case of Pht derivatives, THF, was kept in a refrigerator (−5 °C) overnight and at room temperature with stirring for 4 h. The mixture was diluted with 30 mL of ether and washed with 10% $NaHCO_3$, 10% citric acid, and H_2O. After drying ($MgSO_4$) and removal of solvent, the residue was recrystallized from an appropriate solvent. Specific details are given in Table VI for new derivatives.

General Procedure for NMR-Based Racemization Tests via Active Esters. A solution of 0.25 mmol of protected *N*-hydroxysuccinimide ester and 1 equiv of NEt_3 in 3 mL of CH_2Cl_2 was stirred for the specific period of time indicated followed by

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Table VI. Characterization of Protected Dipeptides, X-AA$_1$-AA$_2$-OMe[a]

X	AA$_1$	AA$_2$	yield, %	mp, °C (recrys solv)	α_D, deg (T, °C)	^{1}H NMR, δ[b]	mol formula	analytical data calc/found C	H	N
Z	D-Phe	Ala	84	135–135.5 (EtOAc–Skelly B, 1:5)	−15.0 (24) (c = 0.4, MeOH)	1.25 (d, 3, CHCH_3), 3.06 (m, 2, CH_2C$_6$H$_5$), 3.69 (s, 3, OCH$_3$), 4.5 (m, 2, CH, CH), 5.1 (s, 2, OCH$_2$), 5.4 (br, 1, NH), 6.3 (d, 1, NH), 7.1–7.4 (m, 10, aryl)	$C_{21}H_{24}N_2O_5$	65.61 65.51	6.29 6.32	7.29 7.14
BOC	D-Phe	Ala	84	89–91 (EtOAc–Skelly B, 1:4)	+5.81 (21) (c = 0.5, CHCl$_3$)	1.25 (d, 3, CHCH_3), 1.41 (s, 9, CMe$_3$), 3.05 (d, 2, CH_2C$_6$H$_5$), 3.72 (s, 3, OMe), 4.35–4.6 (m, 2, CH, CH), 5.1 (br, 1, NH), 6.3 (d, 1, NH), 7.15–7.4 (m, 5, aryl)	$C_{18}H_{26}N_2O_5$	61.69 61.92	7.48 7.46	8.00 7.95
Pht	Phe	Ala	84	148–50 (EtOAc–Skelly B, 1:3)	+120.6 (24) (c = 0.6, MeOH)	1.4–1.5 (d, 3, CHCH_3), 3.55 (d, 2, CH_2C$_6$H$_5$), 3.71 (s, 3, OMe), 4.6 (m, 1, CHCH$_3$), 5.2 (t, 1, CHCH$_2$), 6.7 (d, 1, NH), 7.2 (s, 5, aryl), 7.7–7.9 (m, 4, aryl)	$C_{21}H_{20}N_2O_5$	66.30 66.24	5.30 5.13	7.30 7.22
Pht	D-Phe	Ala	88	132 (EtOAc–Skelly B, 1:3)	−152 (24) (c = 0.5, MeOH)	1.4–1.5 (d, 3, CHCH_3), 3.55 (d, 2, CH_2C$_6$H$_5$), 3.71 (s, 3, OMe), 4.6 (m, 1, CHCH$_3$), 5.2 (t, 1, CHCH$_2$), 6.7 (d, 1, NH), 7.2–7.5 (s, 5, aryl), 7.7–7.9 (m, 4, aryl)	$C_{21}H_{20}N_2O_5$	66.30 66.31	5.30 5.38	7.30 7.32
Z	Phg	Ala	80	179–81 (95% EtOH)	+59.4 (24) (c = 0.5, EtOAc)	1.4 (d, 3, CHCH_3), 3.66 (s, 3 OMe), 4.6 (m, 1, CHCH$_3$), 5.1 (q, 2, OCH$_2$), 5.3 (m, 1, CHC$_6$H$_5$), 6.1 (d, 1, NH), 6.4 (d, 1, NH), 7.3–7.5 (m, 10, aryl)	$C_{20}H_{22}N_2O_5$	64.85 64.81	5.99 5.91	7.56 7.50
Z	D-Phg	Ala	74	182–3 (CHCl$_3$–Skelly B, 1:4)	−79.4 (24) (c = 0.5, EtOAc)	1.3 (d, 3, CHCH_3), 3.72 (s, 3, OMe), 4.1 (m, 1, CHCH$_3$), 5.0–5.3 (m, 3, CHC$_6$H$_5$, CH$_2$O), 6.0 (b s, 1, NH), 6.3 (d, 1, NH), 7.2–7.4 (m, 10, aryl)	$C_{20}H_{22}N_2O_5$	64.85 64.98	5.99 5.84	7.56 7.52

[a] For the general procedure see the Experimental Section. [b] In CDCl$_3$ solution.

addition of 0.25 mmol of an appropriate amino ester. The resulting solution was stirred for 3 h and washed with 5% HCl, 10% NaHCO$_3$, and H$_2$O. After drying over MgSO$_4$ and removal of solvent in vacuo, the residue was examined by NMR analysis. Results are collected in Table II.

General Procedure for NMR-Based Racemization Tests via Mixed Anhydrides. A solution of 0.5 mmol of protected amino acid, 1 equiv of isobutyl chloroformate, and base (where indicated) in 5 mL of THF at 0 °C was stirred for a specified period of time. To the mixture was added 1 equiv of H-Ala-OMe·HCl followed by 1 equiv of base. After stirring for 1 h the organic solution was washed with 5% HCl, 10% NaHCO$_3$, and H$_2$O. After drying over MgSO$_4$ and removal of solvent the crude dipeptide was examined by NMR analysis. Results are collected in Table V.

2,7-Dichloro-9-formyl-9-methylfluorene. A solution of 4.15 g (15.8 mmol) of 2,7-dichloro-9-(hydroxymethylene)fluorene, 4 mL (64 mmol) of MeI and 0.384 g (16 mmol) of NaH in 60 mL of dry THF was refluxed for 3 h, allowed to cool to room temperature, diluted with 100 mL of ether, and washed with saturated NaCl solution. Drying over MgSO$_4$ and removal of solvent gave a brown oil, which slowly solidified on standing. ^{1}H NMR analysis of the crude material showed three different methyl peaks in the ratio 1:3:9. The crude aldehyde was chromatographed (5.6 × 70 cm; 300 g of silica gel) with elution by 10% EtOAc in Skelly B. The first fraction gave 0.27 g of a white solid, which after recrystallization from MeOH melted at 128 °C. This material was identified as **2,7-dichloro-9-methylfluorene**: ^{1}H NMR (CDCl$_3$) δ 1.55 (d, 3, CH$_3$), 3.9 (q, 1, CH), 7.3–7.7 (m, 6, aryl); IR (KBr) 1450, 1270, 1160, 1070 cm^{-1}. Anal. Calcd for C$_{14}$H$_{10}$Cl$_2$: C, 67.49; H, 4.05. Found: C, 67.48; H, 4.24.

From the third fraction there was obtained 2.94 g (67.6%) of the C-methylated compound. After two recrystallizations from Skelly B, the analytical sample melted at 134–135 °C: ^{1}H NMR (CDCl$_3$) δ 1.66 (s, 3, CH$_3$), 7.38–7.67 (m, 6, aryl), 9.1 (s, 1, CHO); IR (KBr) 1725 cm^{-1} (C=O). Anal. Calcd for C$_{15}$H$_{10}$Cl$_2$O: C, 65.00; H, 3.64; Cl, 25.59. Found: C, 65.07; H, 3.74; Cl, 25.63.

2,7-Dichloro-9-methyl-9-formylfluorene *tert*-Butyl Phenylalanine Aldimine (25). A solution of 0.442 g (2 mmol) of phenylalanine *tert*-butyl ester and 0.277 g (1 mmol) of 2,7-dichloro-9-formyl-9-methylfluorene was refluxed under a Dean–Stark trap for 3 h. After removal of solvent the residue was chromatographed (2 × 70 cm; 50 g of silica gel) with elution by 5% EtOAc in Skelly B. After collecting the desired fraction and removal of solvent there was obtained 0.3 g of an oil, which, according to NMR analysis, consisted of a mixture of the starting aldehyde and the aldimine in the ratio 1:1.5. The yield of aldimine was 45.6%. No method was found to obtain the imine free from contamination by the precursor aldehyde, and therefore the mixture was used without further purification in the following racemization test.

Racemization Test. Effect of Triethylamine on *tert*-Butyl Phenylalanine Aldimine (26). A solution of 100 mg of the above oily mixture which contained 73 mg (0.152 mmol) of the aldimine *tert*-butyl ester in 7 mL of CHCl$_3$ was treated with 0.64 mL (0.456 mmol) of NEt$_3$, and the solution was stirred for 8 or 24 h. In each case after removal of solvent the residue was dissolved in 10 mL of ether and 10 mL of 5% HCl. After stirring at room temperature for 3 h, the aqueous layer was neutralized with 1% NaOH solution and extracted with ether three times. The combined ether solution was washed with 10% NaHCO$_3$ and saturated NaCl solution. After drying over MgSO$_4$ and removal of solvent the residue was dissolved in 5 mL of CH$_2$Cl$_2$ and 5 mL of 10% NaHCO$_3$ and cooled to 0 °C. To the solution was added 65 mg (0.167 mmol) of FMOC-Phg-Cl.[22] After stirring at 0 °C for 10 min and at room temperature for 10 min, the organic layer was diluted with 20 mL of ether and washed with 10% citric acid and H$_2$O. After drying (MgSO$_4$) and removal of solvent the residue was purified by preparative TLC collecting in one fraction the mixture of LL and LD diastereomeric FMOC-α-phenylglycine phenylalanine *tert*-butyl esters. The ratio of diastereomers was determined by ^{1}H NMR analysis at 200 MHz. For the results see Table IV.

Racemization Test. Effect of Triethylamine on DC-FM-Phe-*O*-*t*-Bu (26). A solution of 70 mg (0.15 mmol) of DC-FM-Phe-*O*-*t*-Bu and 0.63 mL (0.45 mmol) of NEt$_3$ in 7 mL of CHCl$_3$ was stirred for 24 h. After removal of solvent the *tert*-butyl ester was quantitatively recovered, mp 157–159 °C; $[\alpha]^{24}_D$ = −70.1° (c = 0.7, EtOAc), the pure sample had mp 158–160 °C; $[\alpha]^{28}_D$ = −78.5° (c = 1, EtOAc). The recovered *tert*-butyl ester

was dissolved in a mixture of 5 mL of MeOH and 2 mL of EtOAc. To the solution was added 20 mg of 10% Pd–C and 20 mg of $Pd(OAc)_2$ followed by 100 mg of ammonium formate. After stirring at room temperature for 5 h, the catalyst was filtered and the solvent was removed to give an oil, which was dissolved in 20 mL of ether and washed with saturated NaCl solution to remove ammonium formate. Coupling with FMOC-Phg-Cl[22] was carried out via the two-phase method given above, and preparative TLC was used to collect the set of LL and LD diastereomers in a single fraction, which was then examined by ^1H NMR analysis (200 MHz). A second run was continued for a period of 8 h. For the results see Table IV.

DC-$\overline{FM}$-Phe-Leu-*O*-*t*-Bu. A solution of 2.4 g (6.37 mmol) of DC-$\overline{FM}$-Phe-OH, 1.7 g (7.58 mmol) of H-Leu-*O*-*t*-Bu·HCl and 1.06 mL (7.58 mmol) of NEt_3 in a mixture of 5 mL of DMF and 25 mL of CH_2Cl_2 was cooled to 0 °C, and 1.38 g (6.7 mmol) of DCC was added. The resulting solution was kept in a freezer (−20 °C) overnight and then at room temperature for 4 h. After filtration the filtrate was diluted with 50 mL of EtOAc and washed with 10% citric acid, 10% $NaHCO_3$, and H_2O. After drying over $MgSO_4$ and removal of solvent the residue was recrystallized from EtOAc–hexane (1:3) to give 3.4 g (77.4%) of the protected dipeptide: mp 190 °C dec; $[\alpha]^{22}_D = -70.1°$ (c = 1, EtOAc); ^1H NMR ($CDCl_3$) δ 1.0 (t, 6, $CHMe_2$), 1.4–1.8 (m, 12, CMe_3, $CHCH_2$), 3.4 (m, 2, $CH_2C_6H_5$), 4.3 (m, 1, $CHCH_2$), 4.6 (m, 1, $CHCH_2C_6H_5$), 5.5 (m, 1, NH), 6.5 (d, 1, NH), 7.1–7.8 (m, 12, aryl, CH=); IR (KBr) 3400–3300 br (NH), 1720 cm^{-1} (C=O). Anal. Calcd for $C_{33}H_{36}Cl_2N_2O_3$: C, 68.39; H, 6.26; N, 4.83. Found: C, 68.51; H, 6.18; N, 4.72.

DC-$\overline{FM}$-Gly-Phe-Leu-*O*-*t*-Bu. To a solution of 2.316 g (4 mmol) of DC-$\overline{FM}$-Phe-Leu-*O*-*t*-Bu in a mixture of 20 mL of EtOAc and 20 mL of MeOH was added 300 mg of 10% Pd–C, 300 mg of $Pd(OAc)_2$, and 1 g of ammonium formate. After the mixture was stirred at room temperature for 6 h the catalyst was filtered and the solvent removed. The residue was dissolved in 20 mL of EtOAc, washed with saturated NaCl solution to remove excess ammonium formate, and then dried ($MgSO_4$). To the solution there was added 1.408 g (4.4 mmol) of DC-$\overline{FM}$-Gly-OH in 20 mL of EtOAc in one portion followed by 0.906 g (4.4 mmol) of DCC. The mixture was kept in a freezer (−20 °C) overnight and then at room temperature with stirring for 4 h. DCU was filtered, and the filtrate was washed with 10% $NaHCO_3$, 10% citric acid, and H_2O. After drying over $MgSO_4$ and removal of solvent, the residue was recrystallized from EtOAc–Skelly B (1:2) to give 1.83 g (72.0%) of the tripeptide: mp 215 °C dec; $[\alpha]^{22}_D = -3.34°$ (c = 0.6, DMF); ^1H NMR ($CDCl_3$) δ 0.9 (t, 6, $CHMe_2$), 1.4–1.8 (m, 12, CMe_3, $CHCH_2$), 3.1 (m, 2, $CH_2C_6H_5$), 4.0 (d, 2, CH_2NH), 4.4 (m, 1, $CHCH_2$), 4.8 (m, 1, $CHCH_2C_6H_5$), 5.8 (m, 1, NH), 6.4 (d, 1, NH), 6.9–7.8 (m, 13, aryl, NH, CH=); IR (KBr) 3400–3270 br (NH), 1730, 1640 cm^{-1} (C=O). Anal. Calcd for $C_{35}H_{39}Cl_2N_3O_4$: C, 66.03; H, 6.18; N, 6.60. Found: C, 66.23; H, 6.21; N, 6.45.

DC-$\overline{FM}$-Gly-Gly-Phe-Leu-*O*-*t*-Bu. To a solution of 1.59 g (2.5 mmol) of DC-$\overline{FM}$-Gly-Phe-Leu-*O*-*t*-Bu in a mixture of 20 mL of EtOH and 20 mL of DMF was added 200 mg of 10% Pd–C, 200 mg of $Pd(OAc)_2$, and 800 mg of ammonium formate. After the mixture was stirred at room temperature for 7 h the catalyst was filtered, and the solvent was removed in vacuo with the aid of a water aspirator. The residue was dissolved in 30 mL of EtOAc and washed with saturated NaCl solution. After drying over $MgSO_4$ and removal of solvent there was obtained the free tripeptide ester as a white solid, which was dissolved in a mixture of 10 mL of EtOAc and 15 mL of DMF. To the solution was added 0.88 g (2.75 mmol) of DC-$\overline{FM}$-Gly-OH followed by 0.566 g (2.75 mmol) of DCC. The resulting solution was kept in a refrigerator (−5 °C) overnight and then at room temperature for 4 h. After filtration and removal of solvent, the residue was chromatographed (4.4 × 68 cm, 150 g of silica gel) with elution by 70% EtOAc/Skelly B to give 1.40 g (81.0%) of the tetrapeptide. The analytical sample was recrystallized from EtOAc–hexane (1:1): mp 215 °C dec; $[\alpha]^{22}_D = -4.1°$ (c = 1, DMF); ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 0.9 (t, 6, $CHMe_2$), 1.4–1.8 (m, 12, CMe_3, $CHCH_2$), 2.9–3.2 (m, 2, $CH_2C_6H_5$), 4.1 (b s, 4, 2 CH_2NH), 4.4 (m, 1, $CHCH_2$), 4.7–4.8 (m, 1, $CHCH_2C_6H_5$), 7.0–8.0 (m, 16, aryl, 4 NH, CH=); IR (KBr) 3400–3300 br (NH), 1760, 1650 cm^{-1} (C=O). Anal. Calcd for $C_{37}H_{42}Cl_2N_4O_5$: C, 64.06; H, 6.01; N, 8.08. Found: C, 64.32; H, 6.00; N, 8.33.

DC-$\overline{FM}$-Tyr(*t*-Bu)-Gly-Gly-Phe-Leu-*O*-*t*-Bu (28). To a solution of 1.04 g (1.5 mmol) of DC-$\overline{FM}$-Gly-Gly-Phe-Leu-*O*-*t*-Bu in a mixture of 5 mL of DMF and 10 mL of MeOH were added 200 mg of 10% Pd–C, 200 mg of $Pd(OAc)_2$, and finally 600 mg of ammonium formate. After the mixture was stirred at room temperature for 7 h the catalyst was filtered and the solvent was removed. The residue was dissolved in 30 mL of EtOAc and washed with saturated NaCl solution. After drying ($MgSO_4$) and removal of solvent the free tetrapeptide was dissolved in a mixture of 5 mL of DMF and 5 mL of EtOAc. To the solution was added 0.8 g (1.66 mmol) of DC-$\overline{FM}$-Tyr(*t*-Bu)-OH followed by 0.31 g (1.5 mmol) of DCC. The resulting solution was kept in a refrigerator (−5 °C) overnight and then at room temperature with stirring for 4 h. After filtration the filtrate was diluted with 20 mL of EtOAc and washed with 10% $NaHCO_3$, 10% citric acid, and H_2O. After drying ($MgSO_4$) and removal of solvent the residue was chromatographed (4.4 × 68 cm; 100 g of silica gel) with elution by 25% $CH_3CN/CHCl_3$ to give 1.08 g (79.0%) of the pentapeptide. The analytical sample was recrystallized from $CHCl_3$–Skelly B (1:3): mp 147 °C dec; $[\alpha]^{23}_D = -50.3°$ (c = 0.5, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 0.8–1.0 (dd, 6, $CHMe_2$), 1.2–1.9 (m, 21, 2 CMe_3, $CHCH_2$), 2.9–3.4 (m, 4, $CH_2C_6H_5$, $CH_2C_6H_4O$), 4.0–4.4 (m, 5, 2 CH_2NH, CH), 4.7 (b s, 1, CH), 5.4 (b s, 1, CH), 6.5 (b s, 1, NH), 6.8–8.4 (m, 20, aryl, 4 NH, CH=); IR (KBr) 3400–3000 br (NH), 1720–1600 br cm^{-1} (C=O); MS (FAB) m/e (relative intensity) 912 (42, M + H), 856 (58), 799 (58), 562 (31), 521 (48), 380 (100). Anal. Calcd for $C_{50}H_{59}Cl_2N_5O_7$: C, 65.78; H, 6.57; N, 7.67. Found: C, 65.55; H, 6.42; N, 7.56.

Leucine Enkephalin (29). A solution of 0.912 g (1 mmol) of pentapeptide **28** and 3.25 g (30 mmol) of anisole in 5 mL of TFA–CH_2Cl_2 (1:1) was stirred overnight. After removal of solvent in vacuo the residue was dissolved in 20 mL of 1 N NaOH solution and extracted with ether (3 × 30 mL). The aqueous solution was adjusted to pH 5.6–6.0 by the addition of phosphate buffer, and the free pentapeptide which slowly precipitated was collected, washed, and air-dried. After recrystallization from MeOH–ether there was obtained 0.395 g (71.0%) of the pentapeptide as a white solid: mp 158–160 °C (lit.[31] mp 158 °C and lit.[32] mp 206 °C); $[\alpha]^{24}_D = -28.7°$ (c = 1, DMF) (lit.[33] $[\alpha]^{23}_D = -26.1°$ (c = 1, DMF)); ^1H NMR (DMSO-d_6) δ 0.9 (m, 6, $CHMe_2$), 1.45–1.72 (m, 3, CH_2CH), 2.62, 2.8, 3.1 (m, 4, CH_2 (Tyr and Phe)), 3.6 (m, 1, CH (Tyr)), 3.7 (m, 4, 2 CH_2 (Gly)), 4.1 (m, 1, CH (Leu)), 4.45 (m, 1, CH (Phe)), 6.7, 7.0 (d, 4, aryl (Tyr)), 7.2 (m, 1, NH (Tyr)), 7.25 (m, 5, aryl (Phe)), 7.95 (m, 2, 2 NH (Gly_3 + Leu)), 8.2 (d, 1, NH (Phe)), 8.45 (b s, 1, NH (Gly)); MS (FAB) m/e (relative intensity) 556 (100, M + H), 379 (50). The NMR spectrum was superimposable on that recorded by Garbay–Jaureguiberry and co-workers.[34] Chemical shift assignments are based on comparisons with the earlier data.

Examination of the Chiral Purity of Optically Active Amino Acids Incorporated into Leucine Enkephalin via the DC-$\overline{FM}$ Technique. Two milligrams of a sample of leucine enkephalin synthesized as described above was treated with 2 mL of 6 N HCl solution at 110 °C for 18–24 h. All glassware used was preextracted with 6 N HCl overnight.[35] The reaction mixture was evaporated by a stream of N_2, and the resulting residue was treated with 1 mL of 1.5 N HCl–isopropyl alcohol, prepared from acetyl chloride and isopropyl alcohol, for 1 h. The mixture was again evaporated to dryness by means of a stream of N_2, and the resulting residue was treated with a solution of 0.15 mL of pentafluoropropionic anhydride in 0.75 mL of EtOAc at 110 °C for 20 min. The mixture was evaporated by a stream of N_2, the residue was dissolved in 0.5 mL of CH_2Cl_2, and the resulting solution was used for GC analysis[36] with a chiral column (Chirasil

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Val-L, column no. 314734, Chrompak, Inc.).

Blank tests were carried out in the same way with exactly the same samples of amino acids or esters that were used as precursors for the pentapeptide synthesis. This included H-Gly-OH, H-Phe-OH, H-Tyr-OH, and H-Leu-OH (2 mg of each). Results for the three chiral amino acids as percent of D form were 0.69 (Phe), 1.01 (Tyr), and 0.60 (Leu). Analogous results for the blanks were 0.83 (Phe), 0.89 (Tyr), and 0.63 (Leu). The figures given are the averages for three independent determinations.

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New Mycotoxins from *Fusarium sambucinum*

Dale R. Sanson, David G. Corley, Charles L. Barnes, Scott Searles, Elmer O. Schlemper, and Michael S. Tempesta*

Department of Chemistry, University of Missouri, Columbia, Missouri 65211

George E. Rottinghaus

Veterinary Medical Diagnostic Laboratory, University of Missouri, Columbia, Missouri 65211

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Isolation, characterization, and bioassay results of three new trichothecene mycotoxins, 3-ketoapotrichothecene (**6**), FS-3 (**9**), and FS-4 (**11**), as well as a new bisaboline, 4,5,10,11-tetrahydroxybisaboline (**14**), from *Fusarium sambucinum* are presented. 3,15-Diacetoxyscirpenol (3,15-DAS) (**1**), FS-1 (**10**), and 3α- and 3β-hydroxyapotrichothecenes (**7**, **8**) are reported for the first time from *F. sambucinum*. The previously reported 4,15-diacetoxyscirpenol (4,15-DAS) (**2**) (anguidine), 4-monoacetoxyscirpenol (4-MAS) (**3**), 15-MAS (**4**), 3,4,15-triacetoxyscirpenol (TAS) (**5**), neosolaniol, sambucoin, and scirpenetriol were also found. The solid-state structure of DAS (**2**) (anguidine) obtained from single-crystal X-ray analysis is reported.

The trichothecene mycotoxins found in *Fusarium*-damaged grains[1,2] are responsible for alimentary toxic aleukia (ATA), skin inflammation, vomiting, and death in humans and farm animals. Our recent studies of *Fusarium sporotrichioides*, which produces large amounts of T-2 toxin, revealed a number of new trichothecenes.[3-7] *Fusarium sambucinum*, which produces DAS (4,15-diacetoxyscripenol), also known as anguidine, another potent toxin, in significant quantity, as well sambucinol, sambucoin,[8] and sambucinic acid,[9] was cultured and followed by chromatographic workup procedures previously described.[7] We now report the structure elucidation, spectral data, and preliminary bioassay results of a new 4,5,10,11-tetrahydroxybisaboline; three unusual modified trichothecenes 3-ketoapotrichothecene, FS-3, FS-4; as well as the isolation of three known metabolites 3,15-DAS, FS-1, 3α- and 3β-hydroxyapotrichothecenes not reported previously from *F. sambucinum*. Sambucoin, scirpenetriol, neosolaniol, 4-MAS, 15-MAS, and 3,4,15-triacetoxyscirpenol have been found in *F. sambucinum* by others.[8,10-12] To our knowledge, this is the first report of a bisaboline produced by a fungus.

A large-scale workup of the culture filtrate used a modified method of Burmeister.[13] Approximately 400 jars were harvested in batches of 100–200 jars over a period of 1 year. The corn grits were extracted with $CHCl_3$–Me_2CO (85:15) (400 mL/jar) by blending at high speed until homogenized. The fungal–solvent mixture was allowed to stand overnight and suction filtered. The solid residue was reextracted with Me_2CO, suction filtered, autoclaved, and discarded. The Me_2CO extract was combined with the $CHCl_3$–Me_2CO (85:15) extract and concentrated under vacuum. The dark-red oil (~0.5 L/200 jars) was jubjected to a hexane drip to remove the nonpolar constituents. This was achieved by dripping the oil into a stirring solution of hexane–Me_2CO (85:15) (ca. 50 mL oil/2 L solution) and allowing to stand for 24 h. The solvent was decanted and concentrated under vacuum.

The majority of DAS (**2**) was removed from the oil by crystallization from Me_2CO–hexane. Approximately 100 g of **2** was obtained from the 400 jars (0.25%). Multiple runs of 2-g aliquots of the remaining red oil were flash chromatographed.[14] A gradient solvent system was used employing toluene; toluene–Me_2CO (4:1), (2:1), (1:1); Me_2CO; and 1:1 Me_2CO–MeOH. Each of the six resultant fractions were further separated by flash chromatography. Oil from each fraction was applied to a 4 × 45 cm flash

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