N-Trityl- and *N*-Phenylfluorenyl-*N*-carboxyanhydrides and Their Use in Dipeptide Synthesis

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Introduction

The *N*-carboxyanhydrides (Leuchs' anhydrides, NCAs) at one time appeared to be the panacea for peptide synthesis. They are easily prepared in one step with negligible waste stream, they couple rapidly at room temperature with nitrogen nucleophiles, and the coupling byproduct is carbon dioxide. Their value, however, was quickly adumbrated. The same quality that made them attractive, their extreme reactivity, limited their use, with one notable exception, the synthesis of ribonuclease.[1] Accompanying this reactivity is an inherent instability and propensity for polymerization, as well as some loss in enantiomeric integrity.

In attempting to overcome these deficiencies, the nitrogen of the NCA was additionally substituted with tosyl[2] and nitrophenylsulfonyl groups[3] with little improvement. The most promising results were achieved recently with an alkoxycarbonyl group added to the nitrogen, forming the urethane-protected-NCA (UNCA).[4] While this modification does overcome their instability and tendency to polymerize, there still remains a significant loss of enantiomeric purity during peptide synthesis[5] and some instability to base.[6]

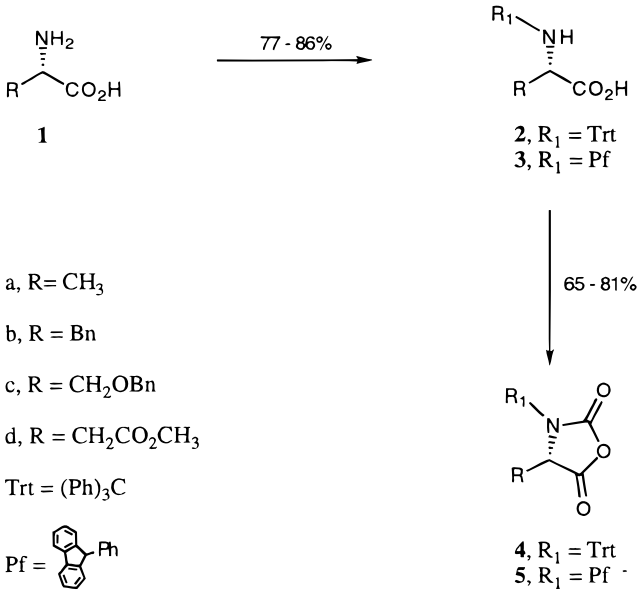
We have addressed these problems by preparing the *N*-trityl-NCAs (TNCAs) and the *N*-phenylfluorenyl-NCAs (PFNCAs). Our intent was to increase stability and, primarily, to inhibit any epimerization, a well-established effect of substitution of a trityl or phenylfluorenyl residue on the α-amino group.[7]

Results and Discussion

Only one previous claim for synthesis of an *N*-trityl-NCA was found. At a meeting in 1962 the preparation was reported of the TNCAs of glycine and alanine; the process failed with other amino acids.[8]

We now report the synthesis and use in peptide formation of four *N*-trityl or *N*-phenylfluorenyl NCAs: those of alanine, phenylalanine, *O*-benzylserine, and the *β*-methyl ester of aspartic acid. These four amino acids

Scheme 1. Synthesis of *N*-Trityl- and *N*-Phenylfluorenyl-*N*-carboxy Anhydrides (NCAs)



were chosen as representative of a range of enantiomeric stabilities,[5] with the introduction of some additional functionality as well. For example, *O*-benzylserine and the *β*-ester of aspartic acid were included because of their established epimerization when used as UNCAs.[5]

Shown in Scheme 1 is the preparation of the *N*-trityl (Trt) derivatives, which is uneventful and follows reported procedures,[9] and the preparation of the *N*-phenylfluorenyl (Pf) derivatives, which proceeded following our general procedure.[10] In all examples high yields were readily obtained.

To convert these derivatives to the corresponding NCAs, we have used triphosgene in some cases and phosgene in others. The yields varied from 65% to 81%, and both TNCAs and PFNCAs were easily isolated. They are relatively stable (several months at room temperature), crystalline compounds.

We then proceeded to use these TNCAs and PFNCAs for dipeptide formation. The objective, of course, was to use the mildest conditions commensurate with acceptable yields and the absence of epimerization. Various conditions, with and without catalysts, were explored. The best reaction conditions we have found are summarized in Table 1, entries 1−7. These are simply to heat the reaction mixture in THF at 40 °C or at reflux. Yields are generally 80−90%. In each example, dipeptide formation took place with retention of complete enantiomeric integrity. Analysis was by HPLC of the dipeptide using standards prepared from DL-amino acids. Our limits of detection are 0.5% of epimerization.

Since it might be advantageous to shorten the time and lower the temperature, we examined alternative conditions recently reported[11] in dipeptide formation with some limited NCAs. These reports describe the use of KCN or NaN_3 in DMF at room temperature for relatively shorter times.

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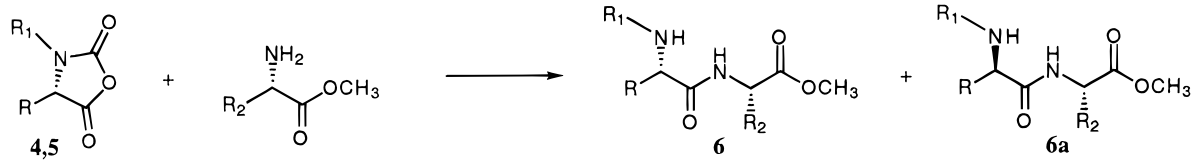
Table 1. Dipeptide Formation from TNCAs and PFNCAs



entry	R	R_1	R_2	conditions solvent/ temp, °C/time, h	additive	yield, %	enantiomeric ratio, **6**/**6a**
1	CH_3	Trt	Bn	THF/Δ_x/6		81	100
2	Bn	Trt	CH_3	THF/Δ_x/7		72	100
3	$BnOCH_2$	Trt	Bn	THF/40/20		89	100
4	CH_3	Pf	Bn	THF/Δ_x/6		82	100
5	$BnOCH_2$	Pf	Bn	THF/40/12		91	100
6	$CH_3O_2CCH_2$	Trt	Bn	THF/Δ_x/9		94	100
7	$CH_3O_2CCH_2$	Pf	Bn	THF/Δ_x/9		92	100
8	CH_3	Trt	Bn	DMF/rt/3	KCN	87	36/64
9	Bn	Trt	CH_3	DMF/rt/3	KCN	73	23/77
10	CH_3	Pf	Bn	DMF/rt/3	KCN	73	87/13
11	CH_3	Trt	Bn	DMF/rt/3	NaN_3	86	91/9
12	$BnOCH_2$	Trt	Bn	DMF/rt/15	NaN_3	86	50/50
13	CH_3	Pf	Bn	DMF/rt/3	NaN_3	91	100
14	$BnOCH_2$	Pf	Bn	DMF/rt/9	NaN_3	91	83/17
15	$CH_3O_2CCH_2$	Trt	Bn	DMF/rt/12	NaN_3	92	81/19
16	$CH_3O_2CCH_2$	Pf	Bn	DMF/rt/12	NaN_3	86	98/2
17	$CH_3O_2CCH_2$	Trt	Bn	DMF/rt/15	NaF	88	97/3

[a] Reflux is designated as Δ_x. [b] Enantiomeric ratio was determined by HPLC; 100 indicates no detectable epimer.

When we applied the KCN catalysis procedure to our TNCAs and PFNCAs, we found significant epimerization in all cases (Table 1). Thus, even the *N*-trityl and *N*-Pf groups could not suppress epimerization. Similarly, catalysis by NaN_3 led to significant epimerization, with one exception, the PFNCA of alanine, entry 13. Also, the PFNCA of aspartic acid β-methyl ester, entry 16, showed only 2% epimerization. The results in entries 13 and 16 are not surprising since they involve the most protective nitrogen substituent, the Pf group, and the two amino acids (of the four) less susceptible to epimerization. Of interest is the one example of catalysis with NaF in which only 3% of epimerization was found with the TNCA of aspartic acid β-methyl ester, entry 17.

As one would have predicted, epimerization during these couplings took place in the NCA component and not in the coupling companion amino acid. Proof of this conclusion was obtained by preparing authentic samples of the epimeric dipeptides. These controls permitted establishment of identity and limits of detection by HPLC. All the dipeptides were easily isolated, crystalline compounds; no diketopiperazine formation was detected. These dipeptides have been completely stable on storage at room temperature for 6 months.

Conclusions

We have prepared the *N*-trityl-NCA and *N*-phenylfluorenyl-NCA derivatives of four representative amino acids. In all cases, these TNCAs and PFNCAs are crystalline solids, readily prepared in good yields, and are relatively stable on storage. When heated in THF in the presence of a companion amino acid, they are converted in good yield to dipeptides with complete enantiomeric integrity. These dipeptides are crystalline solids and are stable on storage at room temperature.

Experimental Section

General Methods. Melting points were determined on a capillary apparatus and are uncorrected. THF and Et_2O were distilled from Na−benzophenone ketyl under nitrogen; $CHCl_3$ was distilled from P_2O_5; DMF was dried over molecular sieves (3 Å), and TrtCl was recrystallized from isooctane. Petroleum ether and hexane were used from the supplier without further purification. All final organic solutions were dried over Na_2SO_4 before evaporation. NMR spectra were taken in $CDCl_3$ and are referenced to TMS. ^{1}H-coupling constants, *J*, are reported in Hz. Column chromatography was performed using 230−400 mesh silica gel. HPLC analyses were conducted on a 4.6 × 250 mm 5 μm Si normal-phase silica column, monitoring at 254 nm. Elemental analyses were obtained from the Microanalytical Laboratory, Department of Chemistry, University of California, Berkeley.

Synthesis of *N*-Triphenylmethyl (Trityl) Derivatives of L-Alanine (1a) and L-Phenylalanine (1b). To a solution of trityl chloride (30.67 g, 110 mmol) in $CHCl_3$/DMF (350 mL, 2/1) was added the amino acid (50 mmol), the mixture was vigorously stirred for 3 h, triethylamine (20.2 g, 200 mmol) in $CHCl_3$/DMF (50 mL, 2/1) was added slowly over a period of 1 h, and the mixture was stirred for another 2 h. After addition of methanol (250 mL), the reaction mixture was heated at 50−55 °C for 2 h, the solvent was evaporated, and the residue was distributed between Et_2O (500 mL) and 10% aqueous citric acid (100 mL). The organic layer was washed with 10% aqueous citric acid (2 × 100 mL) and H_2O (3 × 100 mL), dried, and evaporated, and the residue was chromatographed (hexane/EtOAc, 6/4) to afford the *N*-tritylamino acid.

***N*-Trityl-L-alanine (2a):** 78% yield; **2a**·$HNEt_2$ mp 154−155 °C (lit.[13] mp 157 °C); ^{1}H NMR δ 1.23 (d, J = 7.1, 3H), 3.43 (q, J = 7.1, 1H), 7.20−7.30 (m, 10H), 7.39−7.41 (m, 5H).

***N*-Trityl-L-phenylalanine (2b):** eluting solvent, hexane/EtOAc, 7/3; 87% yield; **2b**·$HNEt_2$ mp 148−150 °C (lit.[13] mp 150−151 °C); ^{1}H NMR δ 2.08 (dd, J = 13.4, 5.8, 1H), 2.89 (dd, J = 13.4, 6.7, 1H), 3.56 (dd, J = 6.7, 5.8), 7.09−7.36 (m, 20H).

Synthesis of *N*-Trityl Derivatives of *O*-Benzyl-L-serine (1c) and L-Aspartic Acid β-Methyl Ester (1d). To a solution of the amino acid (12 mmol) in $CHCl_3$/CH_3CN (60 mL, 5/1) was added chlorotrimethylsilane (1.52 mL, 12 mmol), and the

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mixture was vigorously stirred for 3 h at room temperature. Triethylamine (3.68 mL, 26.4 mmol) was then added slowly to maintain a gentle reflux, and the mixture was stirred for 15 min after which TrtCl (4.01 g, 14.4 mmol) in 30 mL of $CHCl_3$ was added.

The mixture was vigorously stirred for 3 h at room temperature; methanol (2.4 mL, 60 mmol) was added, and the mixture was stirred for an additional 30 min. The mixture was evaporated, and the residue was partitioned between ether (200 mL) and aqueous 5% citric acid (60 mL). The aqueous layer was extracted with ether (2 × 50 mL), the combined organic solution was dried, filtered, and evaporated, and the residue was chromatographed.

***O*-Benzyl-*N*-trityl-L-serine (2c):** eluting solvent, hexane/EtOAc, 8/2, then hexane/THF, 6/4; 82% yield; mp 180−181 °C; ^{1}H NMR δ 2.33 (dd, J = 9.1, 4.0, 1H), 3.59 (dd, J = 9.1, 2.5, 1H), 3.49−3.51 (m, 1H), 4.21 (d, J = 12.0, 1H), 4.32 (d, J = 12.0, 1H), 7.17−7.28 (m, 20H). Anal. Calcd for $C_{29}H_{27}NO_3$: C, 79.6; H, 6.2; N, 3.2. Found: C, 79.7; H, 6.5; N, 3.4.

***N*-Trityl-L-aspartic acid *β*-methyl ester (2d):** from L-aspartic acid β-methyl ester;[12] after addition of TrtCl, the reaction mixture was stirred for 5 h; eluting solvent, hexane/EtOAc, 7/3, then THF; 85% yield; mp 191−192 °C; ^{1}H NMR δ 1.22 (dd, J = 17.5, 5.1, 1H), 2.68 (dd, J = 17.5, 3.3, 1H), 3.59 (s, 3H), 3.60−3.61 (m, 1H), 7.23−7.45 (m, 15H). Anal. Calcd for $C_{24}H_{23}NO_4$: C, 74.0; H, 6.0; N, 3.6. Found: C, 73.9; H, 6.2; N, 3.4.

***N*-(9-Phenyl-9-fluorenyl)-L-alanine (3a).** To a solution of L-alanine (**1a**, 4.45 g, 50 mmol) in $CHCl_3$/DMF (150 mL, 5/1) was added chlorotrimethylsilane (6.35 mL, 50 mmol), and the mixture was heated at reflux for 2 h with vigorous stirring. The mixture was cooled to room temperature under a stream of nitrogen, triethylamine (15.3 mL, 110 mmol) was slowly added to maintain a gentle reflux, and the mixture was stirred for 15 min after which $Pb(NO_3)_2$ (10.03 g, 33.3 mmol) was added, followed by the addition of 9-bromo-9-phenylfluorene (19.3 g, 60 mmol) in 60 mL of $CHCl_3$. The mixture was vigorously stirred for 48 h at room temperature, methanol (5.1 mL, 125 mmol) was then added, and the mixture was stirred for an additional 30 min. The mixture was filtered, the filter cake was washed with $CHCl_3$ (3 × 20 mL), and the dark orange filtrate was evaporated to a residue that was partitioned between ether (300 mL) and aqueous 5% citric acid (300 mL). The aqueous layer was extracted with ether (4 × 100 mL), and the combined organic solution was extracted with 1 M NaOH (100 mL). The aqueous solution was washed with 100 mL of ether and cooled to 0 °C with stirring, and the pH was adjusted to 7 by the dropwise addition of glacial acetic acid. The mixture, containing an off-white precipitate, was extracted with 2-propanol/$CHCl_3$ (1/3, 5 × 100 mL). The combined organic solution was washed with 100 mL of brine, dried, filtered, and evaporated to a light yellow foam to afford **3a** (12.67 g, 77%): mp 153−155 °C (lit.[10] mp 158−161 °C); ^{1}H NMR δ 1.00 (d, J = 7.1, 3H), 2.69 (q, J = 7.1, 1H), 7.07−7.40 (m, 11H), 7.64−7.70 (m, 2H).

***O*-Benzyl-*N*-(9-phenyl-9-fluorenyl)-L-serine (3c).** To a solution of L-serine (**1c**, 2.34 g, 12 mmol) in $CHCl_3$/DMF (60 mL, 5/1) was added chlorotrimethylsilane (1.52 mL, 12 mmol), and the mixture was vigorously stirred for 3 h at room temperature. Triethylamine (3.68 mL, 26.4 mmol) was then added slowly to maintain a gentle reflux, and the mixture was stirred for 15 min after which $Pb(NO_3)_2$ (2.66 g, 8.0 mmol) was added, followed by the addition of 9-bromo-9-phenylfluorene (4.36 g, 14.4 mmol) in 30 mL of $CHCl_3$. The mixture was vigorously stirred for 48 h at room temperature, methanol (1.2 mL, 30 mmol) was then added, and the mixture was stirred for an additional 30 min. The mixture was filtered, the filter cake was washed with $CHCl_3$ (2 × 50 mL), and the dark orange filtrate was evaporated to a residue that was partitioned between ether (200 mL) and aqueous 5% citric acid (60 mL). The aqueous layer was extracted with ether (2 × 50 mL), the combined organic solution was dried, filtered, and evaporated, and the residue was chromatographed (hexane/EtOAc, 8/2; hexane/THF, 6/4) to afford **3c** (4.23 g, 81%) as a white solid: mp 137−138 °C; ^{1}H NMR δ 2.64−2.66 (m, 1H), 2.94 (dd, J = 9.2, 4.1, 1H), 3.66 (dd, J = 9.2, 2.2, 1H), 4.28 (d, J = 12.0, 1H), 4.47 (d, J = 12.0, 1H), 6.93−7.71 (m, 18H). Anal. Calcd for $C_{29}H_{25}NO_3$: C, 80.0; H, 5.8; N, 3.2. Found: C, 79.7; H, 6.0; N, 3.1.

***N*-(9-Phenyl-9-fluorenyl)-L-aspartic Acid *β*-Methyl Ester (3d).** To a solution of l-aspartic acid β-methyl ester (**1d**, 0.44 g, 3 mmol) in $CHCl_3$/CH_3CN (40 mL, 3/1) was added chlorotrimethylsilane (0.38 mL, 3 mmol), and the mixture was vigorously stirred for 4 h at room temperature. Triethylamine (0.92 mL, 6.6 mmol) was added slowly to maintain a gentle reflux, and the mixture was stirred for 15 min after which $Pb(NO_3)_2$ (0.67 g, 2.0 mmol) was added followed by 9-bromo-9-phenylfluorene (1.16 g, 3.6 mmol) in 10 mL of $CHCl_3$. The mixture was vigorously stirred for 72 h at room temperature, methanol (0.3 mL, 7.5 mmol) was then added, and the mixture was stirred for an additional 30 min. The mixture was filtered, the filter cake was washed with $CHCl_3$ (2 × 20 mL), and the dark orange filtrate was evaporated to a residue that was partitioned between ether (100 mL) and 5% aqueous citric acid (50 mL). The aqueous layer was extracted with ether (2 × 50 mL), and the combined organic solution was washed with brine (20 mL), dried, and filtered. Evaporation and chromatography of the residue (hexane/EtOAc, 6/4; THF) gave **3d** (0.95 g, 82%) as a white solid: mp 163−164 °C (lit.[12] mp 160−161 °C); ^{1}H NMR δ 1.95 (dd, J = 17.2, 4.8, 1H), 2.76 (dd, J = 17.2, 3.8, 1H), 2.86−2.88 (m, 1H), 3.65 (s, 3H), 7.22−7.76 (m, 13H).

Synthesis of *N*-Trityl- and *N*-Phenylfluorenyl(Pf)-*N*-carboxyanhydrides (NCAs). Procedure A. To a solution of triphosgene (1.51 g, 5.6 mmol) in EtOAc (280 mL) was added the *N*-tritylamino acid (14 mmol). 1-Ethylpiperidine (1.74 g, 15.4 mmol) in EtOAc (20 mL) was added dropwise to the solution over a period of 40 min, and the mixture was stirred for another 2 h. The reaction mixture was filtered and evaporated, and the residue was chromatographed through a short column (EtOAc). Evaporation and crystallization of the residue gave the *N*-trt-NCA.

***N*-Trityl-L-alanine NCA (4a):** crystallized from EtOAc/petroleum ether; 71% yield; 205−206 °C (lit.[8] mp 208−210 °C); $[\alpha]^{21}_D$ +34.4° (*c* 0.5, EtOAc) [lit.[8] $[\alpha]^{20}_D$ +34.7° (*c* 0.5, EtOAc)]; ^{1}H NMR δ 0.94 (d, J = 6.8, 3H), 4.51 (q, J = 6.8, 1H), 7.22−7.25 (m, 5H), 7.34−7.44 (m, 10H); ^{13}C NMR δ18.8, 57.7, 74.7, 128.0, 128.1, 129.7, 140.9, 150.9, 169.7. Anal. Calcd for $C_{23}H_{19}NO_3$: C, 77.3; H, 5.4; N, 3.9. Found: C, 77.2; H, 5.4; N, 3.5.

***N*-Trityl-L-phenylalanine NCA (4b):** eluting solvent, hexane/Et_2O, 3/7; crystallized from Et_2O/hexane; 68% yield; mp 92−95 °C; $[\alpha]^{23}_D$ +53.3° (*c* 1.0, EtOAc); ^{1}H NMR δ (dd, J = 14.3, 8.3, 1H), 2.88 (dd, J = 14.3, 2.8, 1H), 4.46 (dd, J = 8.3, 2.8, 1H), 6.84−6.85 (m, 2H), 7.20−7.37 (m, 18H); ^{13}C NMR δ 38.0, 62.4, 75.6, 127.7, 128.1, 128.2, 128.6, 129.8, 130.0, 133.2, 140.9, 151.5, 168.4. Anal. Calcd for $C_{29}H_{23}NO_3$: C, 80.3, H, 5.4; N, 3.2. Found: C, 80.3; H, 5.6; N, 3.0.

Procedure B. To a solution of the *N*-trityl or *N*-Pf amino acid (1.0 mmol) in 1,4-dioxane (26 mL) was added phosgene (0.20 g, 2.0 mmol) in benzene (0.8 mL) followed by the dropwise addition of 1-ethylpiperidine (0.453 g, 4.0 mmol) in 1,4-dioxane (4 mL), and the mixture was stirred for another 3 h at room temperature. The reaction mixture was passed through a short column of SiO_2, with 1,4-dioxane/hexane, 7/3, and rapidly eluted with an additional 100 mL of 1,4-dioxane/hexane, 7/3. The organic solution was evaporated, and the residue was recrystallized to give the NCA.

***N*-Pf-L-alanine NCA (5a):** crystallized from the THF/hexane; 81% yield; mp 107−108 °C; $[\alpha]^{21}_D$ +636° (*c* 0.5, $CHCl_3$); ^{1}H NMR δ 0.75 (d, J = 6.9, 3H), 4.40 (q, J = 6.9, 1H), 7.20−7.42 (m, 9H), 7.49−7.53 (m, 1H), 7.70−7.76 (m, 2H), 7.97 (d, J = 7.5, 1H); ^{13}C NMR δ 17.4, 57.6, 72.1, 120.2, 120.6, 124.6, 125.6, 127.1, 127.8, 128.3, 128.7, 129.1, 129.7, 129.8, 139.8, 139.9, 140.1, 143.9, 147.3, 151.3, 169.3. Anal. Calcd for $C_{23}H_{17}NO_3$: C, 77.7; H, 4.8; N, 3.9. Found: C, 77.5; H, 5.0; N, 4.1.

***O*-Benzyl-*N*-Pf-L-serine NCA (5c):** 74% yield; crystallized from THF/hexane; mp 86−87 °C; $[\alpha]^{22}_D$ +450° (*c* 1.0, $CHCl_3$); ^{1}H NMR δ 2.56 (dd J = 10.2, 2.2, 1H), 3.32 (dd, J = 10.2, 1.8, 1H), 3.59 (d, J = 12.1, 1H), 3.71 (d, J = 12.1, 1H), 4.38−4.39 (m, 1H), 6.91−6.93 (m, 2H), 7.21−7.50 (m, 13H), 7.69−7.78 (m, 2H), 7.97 (d, J = 7.7, 1H); ^{13}C NMR δ 62.6, 66.6, 72.1, 72.6, 120.0, 120.7, 124.7, 125.8, 127.1, 127.5, 127.9, 128.2, 128.4, 128.7, 129.2, 129.6, 129.7, 136.8, 139.6, 140.4, 144.1, 146.8, 152.0, 167.4. Anal. Calcd for $C_{30}H_{23}NO_4$: C, 78.1; H, 5.0; N, 3.0. Found: C, 77.8; H, 5.3; N, 3.2.

***O*-Benzyl-*N*-trityl-L-serine NCA (4c):** initial eluting solvent, EtOAc/hexane, 1/1, followed by 1,4-dioxane/hexane, 7/3; crystal-

lized from THF/hexane; 66% yield; mp 163−164 °C; $[\alpha]^{22}_D$ +14.3° (*c* 1.5, $CHCl_3$); ^1H NMR δ 2.08 (dd, J = 10.2, 1.2, 1H), 3.41 (dd, J = 10.2, 1.2, 1H), 4.27 (d, J = 11.7, 1H), 4.41 (d, J = 11.7, 1H), 4.48−4.50 (m, 1H), 7.25−7.50 (m, 20H); ^{13}C NMR δ 62.8, 67.3, 73.5, 74.9, 127.8, 127.9, 128.0, 128.1, 128.5, 129.9, 136.5, 144.1, 151.4, 167.9. Anal. Calcd for $C_{30}H_{25}NO_4$: C, 77.7; H, 5.4; N, 3.0. Found: C, 77.5; H, 5.6.; N, 2.9.

***N*-Trityl-L-aspartic acid *β*-methyl ester NCA (4d):** eluting solvent, EtOAc/hexane, 1/1; crystallized from EtOAc/hexane; 71% yield; mp 156−157 °C; $[\alpha]^{22}_D$ +52.8° (*c* 1.0, $CHCl_3$); ^1H NMR δ 1.56 (dd, J = 17.8, 5.2, 1H), 2.54 (dd, J = 17.8, 2.7, 1H), 3.72 (s, 3H), 4.56 (dd, J = 5.2, 2.7, 1H), 7.21−7.37 (m, 15H); ^{13}C NMR δ 35.3, 52.4, 58.2, 75.2, 128.1, 128.2, 129.7, 140.6, 151.7, 168.5, 169.2. Anal. Calcd for $C_{25}H_{21}NO_5$: C, 72.3; H, 5.1; N, 3.4. Found: C, 72.0; H, 5.2; N, 3.4.

***N*-Pf-L-aspartic acid *β*-methyl ester NCA (5d):** eluting solvent, EtOAc/hexane, 6/4; crystallized from EtOAc/hexane; yield, 75%; mp 193−194 °C; $[\alpha]^{22}_D$ +585° (*c* 1.0, $CHCl)_3$; ^1H NMR δ 1.69 (dd, J = 18.1, 4.6, 1H), 2.48 (dd, J = 18.1, 2.5, 1H), 3.23 (s, 3H), 4.49 (dd, J = 4.6, 2.5, 1H), 7.20−7.95 (m, 13H); ^{13}C NMR δ 35.8, 52.0, 57.8, 72.1, 120.4, 120.9, 124.6, 125.7, 127.9, 128.0, 128.5, 128.7, 129.1, 129.8, 130.0, 139.7, 139.8, 140.1, 143.7, 146.2, 151.8, 168.3, 168.4. Anal. Calcd for $C_{25}H_{19}NO_5$: C, 72.6; H, 4.6; N, 3.4. Found: C, 72.8; H, 4.7; N, 3.4.

General Procedure for Dipeptide Formation from *N*-Trityl- and *N*-Phenylfluorenyl-NCAs. In all reactions the concentration of NCA was approximately 0.1 M and the conditions of solvent, temperature, and time were those specified in Table 1. The stoichiometry of the amino ester component was 120 mol %. Times were those determined for the disappearance of the NCA as monitored by TLC. When additives were used, 100 mol % was added. Isolation from those reactions conducted in THF consisted in evaporation of the solvent and chromatography of the residue. Isolation from those reactions conducted in DMF consisted in adding EtOAc (twice the reaction volume), washing with H_2O (3×, each wash equal to the reaction volume), drying, and evaporating. The residue was then chromatographed and analyzed by HPLC on 5 μm silica. Numbers in front of headings refer to entry numbers in Table 1.

1. *N*-Trityl-L-Ala-L-Phe-OMe: eluting solvent, hexane/EtOAc, 7/3; 81% yield; HPLC (EtOAc/ hexane, 1/9, 2 mL/min) t_R 26.6 min; mp 162−164 °C; $[\alpha]^{22}_D$ −22.1° (*c* 0.75, EtOAc); ^1H NMR δ 0.84 (d, J = 7.1, 3H), 2.03 (d, J = 5.2, 1H), 3.10 (d, J = 5.7, 2H), 3.26 (dq, J = 5.2, 7.1, 1H), 3.72 (s, 3H), 4.56 (dt, J = 5.7, 7.4, 1H), 7.10−7.11 (m, 2H), 7.18−7.36 (m, 18H), 7.69 (d, J = 7.4, 1H); ^{13}C NMR δ 21.2, 38.0, 52.1, 52.6, 53.8, 71.9, 126.7, 217.0, 127.9, 128.5, 128.7, 129.3, 136.0, 145.5, 171.6, 175.3. Anal. Calcd for $C_{32}H_{32}N_2O_3$: C, 78.0; H, 6.5; N, 5.7. Found: C, 77.7; H, 6.5; N, 5.5.

2. *N*-Trityl-L-Phe-L-Ala-OMe: eluting solvent, hexane/EtOAc, 7/3; 72% yield; HPLC (EtOAc/hexane, 1/9, 2 mL/min) t_R 22.4 min; mp 75−77 °C; $[\alpha]^{22}_D$ +18.3 (*c* 0.3, EtOAc); ^1H NMR δ 1.24 (d, J = 7.2, 3H), 2.06 (dd, J = 31.5, 5.5, 1H), 2.53 (d, J = 6.0, 1H), 2.71 (dd, J = 31.5, 5.5, 1H), 3.50 (d, J = 6.0, 5.5, 5.5, 1H), 3.71 (s, 3H), 4.23 (dq, J = 6.0, 7.2, 1H), 6.98−7.00 (m, 2H), 7.16−7.37 (m, 19H); ^{13}C NMR δ 18.6, 39.4, 47.8, 52.3, 58.7, 71.8, 126.7, 126.8, 128.0, 128.5, 128.8, 129.9, 136.6, 145.6, 173.0, 173.7. Anal. Calcd for $C_{32}H_{32}N_2O_3$: C, 78.0; H, 6.6; N, 5.7. Found: C, 77.8; H, 6.8; N, 5.5.

3. *O*-Benzyl-*N*-trityl-L-Ser-L-Phe-OMe: eluting solvent, hexane/EtOAc, 7.5/2.5; 89% yield; HPLC (EtOAc/hexane, 1/9, 1.5 mL/min) t_R 29.0 min; mp 47−50 °C; $[\alpha]^{22}_D$ −14.3° (*c*1.0, $CHCl_3$); ^1H NMR δ 1.87 (dd, J = 8.8, 3.9, 1H), 2.91 (d, J = 7.8, 1H), 3.04 (dd, J = 31.7, 5.7, 1H), 3.25−3.31 (m, 2H), 3.56 (dd, J = 8.8, 2.1, 1H), 3.76 (s, 3H), 4.03 (d, J = 11.6, 1H), 4.22 (d, J = 11.6, 1H), 4.99 (ddd, J = 8.7, 5.7, 4.4, 1H), 7.11−7.41 (m, 25H), 8.34 (d, J = 8.7, 1H); ^{13}C NMR δ 38.1, 52.2, 52.5, 57.7, 69.9, 71.6, 73.2, 126.6, 127.0, 127.6, 127.7, 128.0, 128.3, 128.4, 128.5, 129.5, 135.7, 137.9, 145.8, 171.6, 172.7. Anal. Calcd for $C_{39}H_{38}N_2O_4$: C, 78.2; H, 6.4; N, 4.7. Found: C, 78.2; H, 6.7; N, 4.7.

4. *N*-(9-Phenyl-9-fluorenyl)-L-Ala-L-Phe-OMe: eluting solvent, hexane/EtOAc, 7/3; 82% yield; HPLC (EtOAc/hexane, 1/9, 1.5 mL/min) t_R 56.6 min, mp 139 °C; ^1H NMR δ 1.03 (d, J = 7.1, 3H), 2.21 (br s, 1H), 2,51 (q, J = 7.1, 1H), 3.08 (dd, J = 13.7, 5.9, 1H), 3.14 (dd, J = 13.7, 5.1, 1H), 3.75 (s, 3H), 4.71 (ddd, J = 7.8, 5.9, 5.1, 1H), 7.02−7.48 (m, 15H), 7.61 (d, J = 7.5, 1H), 7.71 (d, J = 7.5, 1H), 7.96 (d, J = 7.8, 1H); ^{13}C NMR δ 21.4, 38.1, 52.2, 52.4, 73.1, 120.0, 120.1, 124.4, 125.9, 149.2, 171.9, 174.9. Anal. Calcd for $C_{32}H_{30}N_2O_3$: C, 78.3; H, 6.2; N, 5.7. Found: C, 78.1; H, 6.2; N, 5.6.

5. *O*-Benzyl-*N*-(9-phenyl-9-fluorenyl)-L-Ser-L-Phe-OMe: eluting solvent, hexane/EtOAc, 7.5/2.5; 91% yield; HPLC (EtOAc/hexane, 1.5/8.5, 1.5 mL/min) t_R 21.0 min; mp 48−50 °C; $[\alpha]^{22}_D$ +175.6° (*c* 1.5, $CHCl_3$); ^1H NMR ($CHCl_3$ + 1 drop of D_2O) δ 2.53 (dd, J = 4.3, 2.5, 1H), 2.93 (dd, J = 9.0, 4.3, 1H), 3.07 (dd, J = 13.6, 5.7, 1H), 3.20 (dd, J = 13.6, 4.4, 1H), 3.70 (dd, J = 9.0, 2.5, 1H), 3.74 (s, 3H), 4.24 (d, J = 11.8, 1H), 4.43 (d, J = 11.8, 1H), 4.85 (ddd, J = 8.8, 5.7, 4.4, 1H), 7.00−7.38 (m, 20H), 7.59 (d, J = 7.5, 1H), 7.67 (d, J = 7.5, 1H), 8.43 (d, J = 8.4, 1H); ^{13}C NMR δ 38.1, 52.1, 52.7, 56.2, 70.1, 72.6, 73.0, 119.9, 120.0, 124.7, 125.7, 125.9, 127.0, 127.2, 127.8, 127.9, 128.0, 128.3, 128.4, 128.5, 129.6, 135.8, 137.9, 139.8, 141.1, 144.1, 147.8, 149.6, 171.6, 172.4. Anal. Calcd for $C_{39}H_{36}N_2O_4$: C, 78.5; H, 6.1; N, 4.7. Found: C, 78.4; H, 6.3; N, 4.5.

6. *N*-Trityl-*β*-OMe-L-Asp-L-Phe-OMe: eluting solvent, hexane/EtOAc, 7.5/2.5; 94% yield; HPLC (EtOAc/hexane, 1.5/8.5, 2.0 mL/min) t_R 18.8 min; mp 59−61 °C; $[\alpha]^{22}_D$ −28.2° (*c* 1.0, $CHCl_3$; ^1H NMR δ 0.88 (dd, J = 17.5, 5.5, 1H), 2.64 (dd, J = 17.5, 3.0, 1H), 3.04 (dd, J = 13.7, 5.5, 1H), 3.22 (d, J = 9.7, 1H), 3.32 (d, J = 13.7, 4.7, 1H), 3.42 (ddd, J = 9.7, 5.5, 3.0, 1H), 3.51 (s, 3H), 3.76 (s, 3H), 5.04 (ddd, J = 9.2, 5.5, 4.7, 1H), 7.16−7.46 (m, 20H), 8.47 (d, J = 9.2, 1H); ^{13}C NMR δ 34.2, 38.2, 51.4, 52.2, 52.6, 54.1, 71.4, 126.7, 127.1, 128.2, 128.5, 128.8, 129.5, 135.8, 145.8, 171.6, 172.8, 172.9. Anal. Calcd for $C_{34}H_{34}N_2O_5$: C, 74.2; H, 6.2; N, 5.1. Found: C, 73.9; H, 6.5; N, 4.9.

7. *N*-(9-Phenyl-9-fluorenyl)-*β*-OMe-L-Asp-L-Phe-OMe: eluting solvent, hexane/EtOAc, 7.5/2.5; 92% yield; HPLC (EtOAc/hexane, 1.5/8.5, 20 mL/min) t_R 21.0 min; mp 53−55 °C; $[\alpha]^{22}_D$ +171° (*c* 1.0, $CHCl_3$); ^1H NMR δ 1.85(dd, J = 18.0, 5.8, 1H), 2.76−2.81 (m, 2H), 3.04 (dd, J = 13.6, 5.6, 1H), 3.24 (dd, J = 13.6, 4.4, 1H), 3.52 (d, J = 8.7, 1H), 3.61 (s, 3H), 3.73 (s, 3H), 4.87 (ddd, J = 9.0, 5.6, 4.4, 1H), 7.07−7.43 (m, 16H), 7.62 (d, J = 7.5, 1H), 7.72 (d, J = 7.5, 1H), 8.47 (d, J = 9.2, 1H); ^{13}C NMR δ 35.1, 38.3, 51.6, 52.1, 52.7, 53.0, 72.6, 120.0, 120.4, 124.6, 125.5, 125.9, 127.1, 127.3, 128.0, 128.2, 128.4, 128.6, 128.7, 128.8, 129.6, 135.9, 140.8, 144.1, 147.8, 150.1, 171.6, 172.6, 172.7. Anal. Calcd for $C_{34}H_{32}N_2O_5$: C, 74.4; H, 5.9; N, 5.1. Found: C, 74.5; H, 6.1; N, 5.0.

8. *N*-Trityl-L- and -D-Ala-L-Phe-OMe: eluting solvent, hexane/EtOAc, 7/3; 87% yield; HPLC (EtOAc/hexane, 1/9, 2 mL/min) t_R 25.8 min for L,L, t_R 32.8 min for D,L; ^1H NMR δ 0.84 (d, J = 7.1), (d, J = 7.0), 2.03 (d, J = 5.2), 2.28 (d, J = 5.8), 2.81 (dd, J = 13.8, 5.7), 3.02 (dd, J = 13.8, 6.1), 3.10 (d, J = 5.7), 3.25−3.28 (m), 3.69 (s), 3.72 (s), 4.50−4.57 (m), 7.05−7.36 (m) 7.69 (d, J = 7.4).

9. *N*-Trityl-L- and -D-Phe-L-Ala-OMe: eluting solvent, hexane/EtOAc, 7/3; 73% yield; HPLC EtOAc/hexane, 1/9, 2 mL/min) t_R 22.4 min for L,L, t_R 19.0 min for D,L; ^1H NMR δ 0.99 (d, J = 7.1), 1.24 (d, J = 7.2), 2.06 (dd J = 13.5, 5.5), 2.53 (d, J = 6.0), 2.57 (dd J = 13.5, 6.6), 2.71 (dd J = 13.5, 5.5), 2.80 (d, J = 5.8), 2.96 (dd, J = 13.5, 6.2), 3.43 (ddd, J = 6.6, 6.2, 5.8), 3.50 (ddd, J = 6.0, 6.5, 5.5), 3.70 (s), 3.71 (s), 4.02 (dq, J = 5.8, 7.1), 4.32 (dq, J = 6.0, 7.2), 6.48 (d, J = 7.0), 6.98−7.37 (m).

10. *N*-(9-Phenyl-9-fluorenyl)-L- and -D-Ala-L-Phe-OMe: eluting solvent, hexane/EtOAc, 7.3; 73% yield; HPLC (EtOAc/hexane, 1/9, 1.5 mL/min) t_R 55.4 min for L,L; t_R 75.4 min for D,L; ^1H NMR δ 1.03 (d, J = 7.1), 1.05 (d, J = 6.0), 2.20 (br s, 1H), 2.48−2.53 (m, 1H), 3.04−3.17 (m, 2H), 3.71 (s), 3.76 (s), 4.51−4.54 (m), 5.25−5.40 (ddd, J = 7.8, 5.9, 5.1), 6.70−7.43 (m 15H), 7.59−7.72 (m, 2H), 7.96 (d, J = 7.8, 1H).

11. *N*-Trityl-L- and -D-Ala-L-Phe-OMe: eluting solvent, hexane/EtOAc, 7.3; 86% yield; HPLC (EtOAc/hexane, 1/9, 2 mL/min) t_R 25.8 min for L,L; t_R 32.8 min for D,L.

12. *O*-Benzyl-*N*-trityl-L- and -D-Ser-L-Phe-OMe: eluting solvent, hexane/EtOAc, 8/2; 86% yield; HPLC (EtOAc/hexane, 1/9, 1.5 mL/min) t_R 25.0 min for D,L, t_R 29.2 min for L,L; ^1H NMR δ 1.87 (dd, J = 8.8, 3.9), 2.26 (dd, J = 9.1, 4.7), 2.90−3.34 (m, 4H), 3.48 (dd, J = 9.1, 3.2), 3.56 (dd, J = 8.8, 2.1), 3.66 (s), 3.76 (s), 4.03 (d, J = 11.6), 4.16 (d, J = 12.1), 4.22 (d, J = 11.6), 4.23 (d, J = 12.1), 4.69−4.74 (m), 4.99 (ddd, J = 9.0, 5.7, 4.4), 7.11−7.38 (m, 24H), 7.76 (d, J = 7.5), 8.34 (d, J = 8.7).

13. *N*-(9-Phenyl-9-fluorenyl)-L-Ala-L-Phe-OMe, prepared in DMF in the presence of NaN_3, was identical with the product of entry 4.

14. *O*-Benzyl-*N*-(9-phenyl-9-fluorenyl)-L- and -D-Ser-L-Phe-OMe: eluting solvent, hexane/EtOAc, 7.5/2.5; 91% yield; HPLC (EtOAc/hexane, 1.5/8.5, 1.5 mL/min) t_R 17.8 min for d,L; t_R 21.6 min for L,L; ^{1}H NMR δ 2.54 (br s, 1H), 2.88 (dd, J = 9.1, 4.4), 2.93 (dd, J = 9.0, 4.3), 3.05−3.22 (m, 3H), 3.60−3.75 (m, 4H), 3.70 (s), 3.74 (s), 4.20 (d, J = 12.1), 4.25 (d, J = 11.8), 4.35 (d, J = 12.1), 4.43 (d, J = 11.8), 4.61−4.66 (m), 4.86 (ddd, J = 8.8, 5.7, 4.4), 6.76−7.38 (m, 20H), 7.58−7.68 (m, 2H), 8.07 (d, J = 8.0), 8.43 (d, J = 8.4).

15. *N*-Trityl-*β*-OMe-L- and -D-Asp-L-Phe-OMe: eluting solvent, hexane/EtOAc, 7.5/2.5; 92% yield; HPLC (EtOAc/hexane, 1/5, 8.5, 2.0 mL/min) t_R 19.4 min for L,L; t_R 27,2 min for D,L; ^{1}H NMR δ 0.88 (dd, J = 17.5, 5.5), 1.10 (dd, J = 16.9, 3.2), 2.51 (dd, J = 16.9, 3.2), 2.64 (d, J = 17.5, 3.0), 3.04 (dd, J = 13.7, 5.5), 3.18−3.32 (m), 3.30−3.35 (m), 3.39−3.44 (m), 3.51 (s), 3.52 (s), 3.75 (s), 3.76 (s), 4.75−4.80 (m), 5.04 (ddd, J = 9.2, 5.5, 4.7), 7.17−7.42 (m), 8.02 (d, J = 7.2), 8.47 (d, J = 9.2).

16. *N*-(9-Phenyl-9-fluorenyl)-*β*-OMe-L- and -D-Asp-L-Phe-OMe: eluting solvent, hexane/ EtOAc, 7.5/2.5; 86% yield; HPLC (EtOAc/hexane, 1.5/8.5, 2.0 mL/min) t_R 21.0 min for l,L; t_R 31.4 min for D,L; ^{1}H NMR δ 1.85 (dd, J = 18.0, 5.8, 1H), 2.76−2.81 (m, 2H), 3.04 (dd, J = 13.6, 5.6, 1H), 3.24 (dd, J = 13.6, 4.4, 1H), 3.52 (d, J = 8.7, 1H), 3.61 (s, 3H), 3.73 (s, 3H), 4.87 (ddd, J = 9.0, 5.6, 4.4, 1H), 7.07−7.43 (m, 16H), 7.62 (d, J = 7.5, 1H), 7.72 (d, J = 7.5, 1H), 8.47 (d, J = 9.2, 1H).

17. *N*-Trityl-*β*-OMe-L- and -D-Asp-L-Phe-OMe, prepared in the presence of NaF, were the same as the products of entry 15, except that the ratio of diastereomers was different.

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