make the hydrocarbon moiety appear more reactive by comparison. Since D-ring cleavage was enhanced in the mass spectrum of **1a** relative to **1d**, it may well be more useful to study this cleavage process in fluoro- or nitroxy-substituted cholestanes rather than in unfunctionalized cholestane; this might permit the determination of the identity of the enhanced product ion which currently cannot be adduced by deuterium labeling[4] by enhancing subsequent daughter ions.

Since excitation energy is not localized in a carbonium ion, fragmentations are more likely to occur in the region where the positive charge is *localized* since it is this region where the critical energy for bond cleavage is lowest. Thus, if one is able to determine the movement of positive charge in a gas-phase carbonium ion, the succession of fragmentations can be predicted from the principles one has learned about carbonium ion solution chemistry. Vicinal charge triggered losses of HNO_3 and HOAc have parallel mechanisms but differ in ionization energies so that more HOAc elimination occurs via direct initial ionization of the corresponding acetate group.

Extrusion of $NO_2\cdot$ radical from nitrate esters is similar to extrusion of Ac· radical from acetate esters and is preceded by transfer of the hydrogen atom geminal with the alkoxy-containing C–O bond system to a remote electron-deficient site. The former is more facile since it also can occur from an even-electron carbonium ion system to regenerate an odd-electron ion radical system.

Oxidative elimination of HNO_2 involves transfer of the hydrogen atom geminal with the alkoxy-containing C–O bond system to the departing N–O system. Ejection of HNO_3, HNO_2, and $NO_2\cdot$ from the nitroxy function group is preferred to loss of $NO_3\cdot$ and NO· radicals. Scheme IV summarizes the fragments ejected from acetate esters vs. nitrate esters. Finally, these processes appear to be electron impact induced; however, thermal reactions on the sample probe cannot unequivocally be excluded.

Experimental Section

All mass spectra were obtained with a Nuclide 12-90-G single-focusing instrument having a resolution capability of 10 000. Spectra were obtained at ionization voltages of 12 and 70 eV and accelerating voltages of 4–6 kV. The inlet source temperature was 180 °C and the probe temperature ranged between 50 and 100 °C. Successive scans were taken as the temperature increased to verify spectral alterations resulting from decomposition. The dinitrates exhibited some darkening and the trinitrates exhibited significant darkening of the unvolatilized residue remaining in the quartz crucible after acquisition of the spectra. The deuterated nitrate esters **1b** and **1c** were synthesized by reducing 5α-cholestan-3-one with $NaBD_4$ and reacting the isolated deuterated analogues of 3α- and 3β-hydroxy-5α-cholestane with fuming HNO_3 in Ac_2O and $CHCl_3$. The syntheses of all the compounds have been described.[7,8,9]

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Registry No. **1a**, 71883-62-0; **1b**, 71837-72-4; **2a**, 64219-17-6; **2b**, 14942-96-2; **2c**, 71837-73-5; **2d**, 14942-97-3; **3a**, 64219-18-7; **4a**, 64219-19-8; **4b**, 64219-22-3; **5a**, 63533-75-5; **5b**, 63533-73-3; **5c**, 64934-52-7; **6a**, 71837-74-6; **6b**, 64934-54-9; **6c**, 65254-33-3; **7a**, 63533-79-9; **7b**, 63533-84-6; **8a**, 63533-91-5; **8b**, 63533-92-6; **8c**, 63533-97-1.

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Hexafluoro-2-propyl Esters in Peptide Synthesis[1a]

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The use of hexafluoro-2-propanol and its esters for peptide coupling reactions was investigated. Hexafluoro-2-propyl esters of N-protected amino acids and peptides may be prepared without racemization by carbodiimide-mediated coupling of the carboxyl component with hexafluoro-2-propanol (HFP). In peptide coupling reactions HFP esters are about 10^3 times less reactive than the corresponding *p*-nitrophenyl esters. In HFP, which is a powerful peptide solvent, more reactive acyl components are converted to HFP esters under base catalysis. Coupling in HFP is much slower than in dimethoxyethane or dimethylformamide. HFP esters are stable to conditions for removal of benzyloxycarbonyl protecting groups.

In the fragment condensation steps of a large peptide synthesis or in polymerization of peptides to make sequence polymers, solubility is often a problem. The dipolar aprotic solvents in common use, dimethylformamide (DMF) and dimethyl sulfoxide (Me_2SO), are difficult to purify and difficult to remove, and they do not always have sufficient solvent power. A different class of powerful peptide solvents are the fluorinated alcohols, particularly trifluoroethanol (TFE) and hexafluoro-2-propanol (HFP). In contrast to DMF and Me_2SO, they are low boiling (TFE, 74 °C; HFP, 59 °C) and weakly nucleophilic,[2] but like DMF and Me_2SO they are miscible with water and most organic solvents. They have been used in optical and magnetic resonance spectroscopic studies of peptides, but their reported use in synthesis has been limited. TFE has been employed as a solvent in acidolytic deblocking procedures[3,4] and as a cosolvent in the coupling step of sol-

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Table I. Second-Order Rate Constants (L mol^{-1} h^{-1}) for Peptide Bond Formation from Hexafluoro-2-propyl Esters (25 °C)[a]

	amino component				
		H-Ala-OMe			
ester	H-Gly-OBzl DME[b]	DME	DMF	H-Leu-OMe DME	H-Val-OMe DME
(Z)-Gly-OHFP	0.62	0.53	0.32	0.52	0.40
(Z)-Ala-OHFP	0.32	0.16	0.13	0.11	0.036
(Z)-Phe-OHFP	0.075[c]	0.10	0.09	0.05	0.014
Boc-Val-OHFP	0.0035	0.0023	0.007	0.0018	0.0006

[a] Rates in DME were obtained under pseudo-first-order conditions by using the first 50% of the reaction at 0.15 M fluoro ester and 0.60 M amino component; the reaction progress was measured by using the decrease in fluoro ester carbonyl absorption near 1780 cm^{-1}. Rates in DMF were obtained under second-order conditions, the initial concentration of each component was 0.5 M, and the reaction progress was measured by titration. [b] Solvent abbreviations; DME, 1,2-dimethoxyethane; DMF, dimethylformamide. [c] Complex formation between reactants indicated by an unusual precipitate formation.

id-phase peptide synthesis.[5] Waki and Meienhofer have shown that TFE and HFP are suitable solvents for the four-component condensation peptide synthesis.[6] We have lately been examining the further utility of HFP for peptide work.

When a protic solvent, such as HFP, is used as a solvent for peptide coupling (amide formation), solvolysis of the active carboxyl component is a potential side reaction. However, because of the electronegativity of trifluoromethyl groups (HFP has pK_A = 9.3[7]), it may be expected that the HFP solvolysis products, the hexafluoro-2-propyl esters, might themselves be mild acylating agents. We therefore investigated their preparation and coupling with amino compounds and also their stability to the conditions for removal of benzyloxycarbonyl groups.

Results

Preparation and Reactivity of Hexafluoro-2-propyl Esters. Hexafluoro-2-propyl esters of amino acid and peptide derivatives were prepared in good yield by carbodiimide-mediated coupling with the fluoro alcohol in dioxane or dimethoxyethane. In the present work we prepared crystalline HFP esters of (Z)-L-Phe and Boc-L-Val, using *N,N'*-dicyclohexylcarbodiimide as the condensing agent. These esters were recrystallized from isopropyl alcohol without transesterification. Liquid esters were obtained from (Z)-L-Ala and (Z)-Gly. In these cases it proved advantageous to use a water-soluble carbodiimide, since the side products urea and isourea could then be removed from the organic-soluble esters by extraction into water.

Yields of HFP esters were poorer when HFP alone was used as the solvent for ester formation, owing to an irreversible competing reaction that consumes the carbodiimide by direct reaction with HFP. From reaction of HFP and *N,N'*-dicyclohexylcarbodiimide alone we isolated *O*-(hexafluoro-2-propyl)isourea.

Attempts were made to produce perfluoro-*tert*-butyl esters by reaction of the much more acidic (pK_a = 5.2) perfluoro-*tert*-butyl alcohol[8] with N-protected amino acids and dicyclohexylcarbodiimide. This combination did not yield fluoro esters. The crystalline product isolated in good yield from (Z)-Phe-OH in DME proved to contain no fluorine and to be the known symmetrical anhydride ((Z)-L-Phe)$_2$O,[9] identified by proton and carbon-13 spectra, elemental analysis, and melting point. Similar experiments, using the more acid-sensitive Boc-Val-OH, yielded only valine oligomers.

The HFP esters of the amino acid derivatives were characterized by ester carbonyl absorption near 1780 cm^{-1} and by the septet magnetic resonance of the hexafluoro-2-propyl proton, which appears near 5.7 ppm, well separated from the corresponding resonance near 4.3 ppm of the alcohol itself.

The crystalline peptide esters (Z)-L-Ala-L-Phe-OHFP and (Z)-L-Ala-D-Phe-OHFP were also obtained by carbodiimide condensation. The epimeric Ala-Phe derivatives could be distinguished by their alanine methyl proton chemical shifts. It was established by 360-MHz proton magnetic resonance that in each of the isolated crystalline esters there was less than 0.5% of the other epimer, i.e., that racemization does not occur in the esterification process.

As might have been anticipated, it was not possible to prepare the hexafluoro-2-propyl esters from methyl esters by acid-catalyzed transesterification in HFP: N-blocked amino acid derivatives were recovered unchanged. As described in a subsequent section, however, HFP esters may form on storage of other active acyl derivatives in HFP.

The reactivity of the four N-blocked amino acid HFP esters with a set of α-amino esters in dimethoxyethane (DME) was measured. With L-alanine methyl ester, the reaction was also followed in dimethylformamide and rates similar to those in DME were observed. The results are presented as approximate second-order rate constants in Table I. These measurements showed that the HFP esters are about 1000 times less reactive than *p*-nitrophenyl esters in DMF in the experiments reported by Kemp.[10] As with the *p*-nitrophenyl esters, the coupling rate is strongly affected by the steric requirements of the side chains. The rate is much more sensitive to chain bulk in the blocked HFP esters than to side chain bulk in the amino esters. Addition of catalytic amounts of acetic acid, imidazole, or 1-hydroxybenzotriazole had little effect on reaction rate.

A series of experiments were performed to determine the extent of racemization in aminolysis of HFP esters. When (benzyloxycarbonyl)-L-phenylalanine HFP ester was allowed to react with L-alanine methyl ester hydrochloride and 1 equiv of *N*-methylmorpholine in DMF, crystalline (Z)-L-Phe-L-Ala-OMe was isolated in 73% yield, identical with the product obtained with the corresponding hydroxysuccinimide ester. No D,L isomer was detected upon examination of the alanine methyl proton resonances. The

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detection limit is about 1% in our 80-MHz FT spectra in this case. When the corresponding experiment was carried out with the HFP ester prepared from acetyl-L-phenylalanine, however, the proton NMR spectrum of the product showed it to be a 1:1 mixture of L,L and D,L dipeptides.[11] In another study, (*Z*)-L-Ala-L-Phe-OHFP in DMF (optically pure by the NMR criterion) was allowed to react with L-phenylalanine ester hydrochloride plus (in parallel experiments) 0.9 or 1.1 equiv of added tertiary amine. When the total tripeptide product was examined for optical purity by proton resonance it was found in both cases to be an approximately 2:1 mixture of L,L,L and L,D,L isomers. The coupling is extremely slow, requiring about 1 week at room temperature, so that the unreacted ester remains in a basic environment for a long period; possibly racemization would be less severe in a less sterically inhibited coupling.

Finally, experiments with protected HFP esters have shown that the HFP ester function is stable to commonly employed conditions for acidolytic and hydrogenolytic cleavage of the benzyloxycarbonyl group.

Fluoro Alcohols as Solvents for Peptide Bond Formation. NMR studies of an *N*-hydroxysuccinimide ester dissolved in HFP showed that transesterification occurs only slowly in the absence of catalyzing bases. Transesterification is competitive with peptide bond formation, however, under the conditions of peptide coupling. The reaction of 1 M (*Z*)-L-Phe-O-*N*-Su and 1 M H-L-Ala-OMe in HFP, allowed to proceed overnight at room temperature in HFP, yielded 38% isolated (*Z*)-L-Phe-OHFP and 42% isolated dipeptide. Presumably complete conversion to dipeptide would have occurred eventually as the less reactive HFP ester combined with the remaining amino component.

Analogously, an experiment in which (*Z*)-Gly-$NHNH_2$ was diazotized in trifluoroethanol (TFE) and the resulting azide was allowed to react with H-Ala-OMe at 0.1 M in TFE yielded, after 2 days at −15 °C, a 4:1 mixture of (*Z*)-Gly-OTFE and (*Z*)-Gly-L-Ala-OMe according to NMR analysis of the neutral reaction product.

The use of the hexafluoro-2-propyl esters in hexafluoro-2-propanol was also examined. This acidic solvent, coordinating with the amino component, might be expected to have an inhibiting effect on the already slow reaction of HFP esters, and this was found to be the case. The condensation of (*Z*)-Gly-OHFP with H-L-Ala-OMe proceeded with a second-order rate constant of 0.002 L mol^{-1} h^{-1} in HFP at 25 °C. This corresponds to a half-time of 10 days for initially 1 M reactant and is to be compared with a value of 0.53 L mol^{-1} h^{-1} for reaction in DME. At 45 °C the rate constant is 0.025 L mol^{-1} h^{-1}, a half-time of 20 h.

Preliminary experiments to examine the feasibility of peptide polymerization via HFP esters were carried out on (*Z*)-Pro-Gly-Gly-OHFP. This blocked ester was hydrogenated in HFP over Pd/C to free the N terminus. Films cast on salt plates from the centrifuged hydrogenolysis solution and stored in a dry atmosphere showed a continuous decrease in the ratio of peptide ester carbonyl absorption at 1780 cm^{-1} to the amide I band at 1650 cm^{-1}. The solution in HFP itself, concentrated to about 0.3 M and stored at 50 °C, showed a continuous decrease in the intensity of the magnetic resonance of the hexafluoro-2-propyl proton of the ester, and films cast of aliquots showed parallel changes in the infrared absorptions. The condensation proceeded on a time scale of 3–5 days. Further experimentation in this area is under way.

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Experimental Section

Methods. NMR spectra were recorded at 60 MHz with a Varian T-60 spectrometer or at 80 MHz with a Varian CFT-20; chemical shifts are reported in parts per million (δ) from tetramethylsilane. Infrared spectra were obtained by using a Perkin-Elmer Model 257 spectrometer. Mass spectra were determined on a Varian MAT CH-7 instrument, using the direct inlet.

Thin-layer chromatograms utilized Quantum Q-6 silica-gel-coated glass plates. Microanalyses were carried out by Micro-Tech Laboratories, Inc. All melting points were corrected.

N,N-Dimethylformamide (DMF) was purified and dried by distillation from ethylene–maleic anhydride copolymer (Monsanto EMA 11), and *N*-methylmorpholine was freed of secondary amine by distillation from phenyl isocyanate. 2,2,2-Trifluoroethanol (TFE) was purchased from Aldrich Chemical Co., 1,1,1,3,3,3-hexafluoro-2-propanol (HFP) from Pierce Chemical Co., and perfluoro-*tert*-butyl alcohol (FTB) from PCR Research Chemical, Inc. Dicyclohexylcarbodiimide (DCC) was obtained from Aldrich. Amino acid derivatives were obtained from either Aldrich, Pierce, or Bachem.

***N*-(Benzyloxycarbonyl)-L-phenylalanine Hexafluoroisopropyl Ester.** To a stirred solution of 3.75 g of *N*-(benzyloxycarbonyl)-L-phenylalanine (12.5 mmol) in 15 mL of dry dimethoxyethane (DME) at 0 °C was added 4.2 mL of HFP (40 mmol), followed by 2.8 g of melted DCC (13.6 mmol). Stirring was continued for ca. 20 min, by which time a solid mass of dicyclohexylurea (DCU) had formed. The mixture was stored overnight at 4 °C and then filtered. The filtrate was evaporated under vacuum to yield 5.75 g of an oil that solidified to a waxy solid with a melting point of 75–77 °C. Recrystallization from isopropyl alcohol yielded 4.62 g of white solid (10.3 mmol, 83%) in several crops: mp 80–81.5 °C; NMR ($CDCl_3$) δ 3.17 (m, 2 H), 4.72 (m, 1 H), 5.07 (s, 2 H), 5.73 (septet, 1 H, J = 6 Hz), 7.30 (m, 10 H); IR ($CHCl_3$) 1730 (s), 1790 (s) cm^{-1}; mass spectrum m/e (relative intensity) 449 (M^+, 0.5), 358 ($M-C_7H_7$, 1), 314 ($M-C_8H_7O_2$, 0.5), 254 ($M-C_2HF_6O$, 1), 91 (C_7H_7, 100).

Anal. Calcd for $C_{20}H_{17}F_6NO_4$: C, 53.45; H, 3.82; F, 25.37. Found: C, 53.67; H, 3.85; F, 25.43.

***N*-(*tert*-Butoxycarbonyl)-L-valine Hexafluoro-2-propyl Ester.** Boc-L-Val-OHFP was prepared from 2.17 g of Boc-Val-OH (10 mmol), 2.1 mL of HFP (20 mmol), and 2.06 g of DDC (11 mmol) in 10 mL of DME. The crude product (2.80 g, 7.6 mmol, 76%) had a melting point of 85–88 °C; recrystallization from ca. 5 mL of isopropyl alcohol yielded 2.24 g (6.1 mmol, 61%) of fine white needles: mp 89–90 °C; NMR ($CDCl_3$) δ 1.00 (m, 6 H), 1.48 (s, 9 H), 1.83 (m, 1 H), 4.95 (d, 1 H), 4.80 (septet, 1 H, J = 6 Hz); IR (DME) 1715 (s), 1775 (s) cm^{-1}.

Anal. Calcd for $C_{13}H_{19}F_6NO_4$: C, 42.50; H, 5.22; 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide 31.03. Found: C, 42.84; H, 5.28; F, 30.89.

***N*-(Benzyloxycarbonyl)glycine Hexafluoro-2-propyl Ester.** This ester was prepared utilizing the water-soluble carbodiimide 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide methyl-*p*-toluenesulfonate (CMC). A 1.05-g portion of (*Z*)-Gly-OH (5.0 mmol) was dissolved in 14 mL of dry dioxane in a 25-mL Erlenmeyer flask, and the solution was cooled. To this was added 2.32 g of CMC (5.4 mmol) and 4 mL of HFP (3 mmol). The solution was allowed to stand at 25 °C overnight and then filtered. The filtrate was concentrated by rotary evaporation to a viscous oil, which was taken up in 40 mL of ethyl acetate and extracted successively with 40 mL of water, 40 mL of water, 40 mL of 10% aqueous sodium bicarbonate, and 40 mL of water. The organic layer was dried ($MgSO_4$) and concentrated at reduced pressure to an oil which was distilled in a short-path apparatus at 100 °C (0.1 mmHg) to yield 1.1 g (3.0 mmol, 60%) of (*Z*)-L-Gly-OHFP as a colorless oil: NMR ($CDCl_3$) δ 3.95 (d, 2 H), 5.05 (s, 2 H), 5.80 (septet, 1 H, J = 6 Hz), 5.93 (m, 1 H), 7.27 (s, 5 H); IR (film) 1715 (s), 1795 (s) cm^{-1}. The product was chromatographically homogeneous (R_f 0.67 with chloroform–methanol (60:1)).

Anal. Calcd for $C_{13}H_{11}F_6NO_4$: C, 43.47; H, 3.09; F, 31.73. Found: C, 43.89; H, 3.18; F, 31.50.

***N*-(Benzyloxycarbonyl)-L-alanine Hexafluoro-2-propyl Ester.** This ester was prepared in the same manner as the glycine analogue, using the water-soluble carbodiimide. The product was

an oil that was not induced to crystallize and was therefore subjected to short-path vacuum distillation at about 100 °C (0.1 mmHg): NMR ($CDCl_3$) δ 1.40 (d, 3 H), 4.45 (m, 1 H), 5.06 (s, 2 H), 5.75 (septet, 1 H), ~5.8 (d, 1 H), 7.26 (s, 5 H); IR (film) 1720 (s), 1785 (s) cm^{-1}. The product was homogeneous on TLC (R_f 0.58 with chloroform–methanol (60:1)).

Anal. Calcd for $C_{14}H_{13}F_6NO_4$: C, 45.05; H, 3.51; F, 30.54. Found: C, 45.13; H, 3.40; F, 30.56.

***N*-(Benzyloxycarbonyl)-L-alanyl-L(and D)-phenylalanine Hexafluoro-2-propyl Esters.** (*Z*)-L-Ala-L-Phe-OH (612 mg, 1.65 mmol) was dissolved in 2.5 mL of dry DME containing 0.55 g (ca. 0.35 mL, 3.3 mmol) of HFP. The solution was chilled to 0 °C before 408 mg (2.0 mmol) of DCC in 0.8 mL of DME was added. It was stirred at 0 °C overnight, and precipitated dicyclohexylurea was then removed. The filtrate was evaporated to dryness under vacuum and the residue was taken up in dichloromethane from which more urea separated. This solvent was evaporated to give crystalline ester which was crystallized twice from isopropyl alcohol: yield 616 mg (72%); mp 118–120 °C. An analytical sample was dried at 0.1 torr, room temperature, for 30 h.

Anal. Calcd for $C_{23}H_{22}F_6N_2O_5$: C, 53.08; H, 4.26; F, 21.90; N, 5.38. Found: C, 53.16; H, 4.31; F, 21.03; N, 5.35.

A similar preparation but on a smaller scale and starting with (*Z*)-L-Ala-D-Phe-OH[12] was carried out to obtain a sample of the L,D HFP ester for NMR comparison. This was crystallized to chromatographic homogeneity but was not subjected to elemental analysis. Both diastereoisomers had the appropriate NMR spectra ($CDCl_3$) and showed clean methyl doublets, that of the L,L isomer at 1.28_2 ppm and that of the D,L isomer at 1.25_8 ppm, readily distinguishable in 360-MHz spectra. From these spectra it was estimated that each contained less than 0.5% of the other, indicating negligible racemization in the specification procedure.

***N,N'*-Dicyclyhexyl-*O*-(1,1,1,3,3,3-hexafluoro-2-propyl)-isourea.** Dicyclohexylcarbodiimide (941 mg, 4.57 mmol) and HFP (909 mg, 5.41 mmol) were mixed. Reaction was complete after storage overnight at room temperature, as judged by complete disappearance of the 2100-cm^{-1} absorption of the carbodiimide. The product was distilled at 0.02 mmHg, using a bath temperature of 95–100 °C, to obtain 1.14 g (66%) of a chromatographically homogeneous and analytically pure sample: ^1H NMR (Me_2SO-d_6) δ 1.72–2.6 (m, 20 H), 3.19 (m, 1 H), 3.96 (m, 1 H), 6.45 (d, J = 8 Hz), 7.35 (septet, 1 H); ^{13}C NMR δ 149.9, 121.6 (q, J = 236 Hz), 66.3 (septet, J = 34 Hz), 53.9, 50.7, 34.2, 33.8, 25.9, 25.5, 24.8, 24.4; IR (neat) 1710 (s) cm^{-1}, negligible absorption between 1500 and 1700 cm^{-1}; mass spectrum m/e 374 (M^+, 21), 331 (M^+– HNCO, 40), 249 ($C_6H_{11}NHCH(CF_3)_2^+$, 38), 211 ($(CF_3)_2CHOC(NH_2)_2^+$, 32), 98 ($C_6H_{11}NH^+$, 100).

Anal. Calcd for $C_{16}H_{24}F_6N_2O$: C, 51.34; H, 6.46; F, 30.45. Found: C, 51.54; H, 6.41; F, 30.06.

Identification as the isourea is supported by the absence of amide I and II absorption in the infrared spectrum between 1500 and 1700 cm^{-1} and by the chemical shift of the central carbon at 149.9 ppm, outside the 160–165-ppm range reported[13] for urea carbonyls.

Peptide Coupling Using Hexafluoroisopropyl Esters. *N*-(Benzyloxycarbonyl)-L-phenylalanyl-L-alanine Methyl Ester. A solution of 0.140 g of alanine methyl ester hydrochloride (1.0 mmol) in 1.0 mL of DMF was cooled and treated with 0.11 mL of *N*-methylmorpholine (1.0 mmol). To the resulting solution was added 0.475 g of (*Z*)-L-Phe-OHFP (1.05 mmol); the reaction mixture was stirred overnight at 25 °C and then diluted with 15 mL of ethyl acetate and 10 mL of water. The organic layer was treated with 0.2 mL of *N,N*-dimethylethylenediamine for ca. 20 min and next extracted successively with 10 mL of 1 M aqueous HCl, 10 mL of water, 10 mL of 10% aqueous Na_2CO_3, and 10 mL of water. The dried ($MgSO_4$) organic solution was evaporated to yield 0.28 g of (*Z*)-L-Phe-L-Ala-OMe (0.73 mmol, 73%), mp 122–125 °C (lit.[14] mp 130–131 °C), spectrally identical with the authentic dipeptide prepared by coupling using the *N*-hydroxysuccinimide ester of (benzyloxycarbonyl)-L-phenylalanine. The NMR spectrum in $CDCl_3$ of the dipeptide prepared from the HFP ester displayed only a single methyl doublet at δ 1.30, indicative of pure L,L diastereomer (less than 3% D,L isomer estimated from 60-MHz spectra). The diastereomer, prepared from (*Z*)-D-Phe-O-*N*-Su, displayed a methyl doublet at δ 1.20.[15]

Racemization Test. *N*-(Benzyloxycarbonyl)-L-alanyl-L-phenylalanyl-L-phenylalanine Methyl Ester. (*Z*)-L-Ala-L-Phe-OHFP (156 mg, 0.3 mmol) and L-phenylalanine methyl ester hydrochloride (65 mg, 0.3 mmol) were combined in 0.5 mL of DMF. To the solution was added 0.030 mL (0.27 mmol) of *N*-methylmorpholine. A precipitate of the amine hydrochloride soon formed, and the mixture was stirred at room temperature for 1 week before the solvent was removed at reduced pressure and the residue triturated with water. The solid residue (150 mg) showed on thin-layer chromatography both unreacted dipeptide HFP ester and tripeptide. It was dissolved in ethyl acetate and treated with *N,N*-dimethylethylenediamine, then washed with 1 N HCl, 1 N $NaHCO_3$, and water, dried, and evaporated to give 91 mg of product free of HFP ester (63% based on available amino component). Before recrystallization, part of this product was taken up in $CDCl_3$ for NMR analysis. The remainder was recrystallized twice from methanol to give an analytical sample, mp 185–187 °C (dried at 0.1 torr, room temperature, 30 h).

Anal. Calcd for $C_{30}H_{33}N_3O_6$: C, 67.78; H, 6.26; N, 7.90. Found: C, 67.66; H, 6.50; N, 7.80.

The methyl proton region of the unrecrystallized product exhibited two doublet resonances at 1.16 and 1.12 ppm in the ratio of approximately 2:1, indicating extensive racemization. When the reaction was repeated with a 10% excess of *N*-methylmorpholine, rather than a 10% deficiency, the result was the same.

Rate Measurements. In Dimethoxyethane. Reaction mixtures were prepared to be 0.60 M in fluoro ester and 0.15 M in amino ester hydrochloride plus 1 equiv of *N*-methylmorpholine. They were maintained in a bath at 25 °C, and reaction progress was followed by the loss in intensity of the fluoro ester carbonyl stretching absorption near 1790 cm^{-1}. Because *N*-methylmorpholine hydrochloride was present as a precipitate in the reacting mixtures, they were centrifuged before the supernatant was sampled for infrared measurement. Pseudo-first-order rate constants were obtained by linear least-squares treatment of points taken oven at least 1 half-life of the fluoro ester.

In Dimethylformamide. Reaction mixtures were prepared to be 0.50 M in both fluoro ester and amino ester; the latter were liberated from the hydrochlorides by 1 equiv of added *N*-methylmorpholine. The concentration of unacylated amine as a function of time was determined by titration of aliquots with 0.1 M hydrochloric acid. Second-order rate constants were derived from least-squares analysis of plots of reciprocal amine concentration vs. time.

In Hexafluoro-2-propanol. A solution of 0.14 g of H-L-Ala-OMe·HCl (1 mmol) in 0.5 mL of HFP was cooled to 0 °C and mixed with 0.11 mL of *N*-methylmorpholine. To this solution was added 0.36 g of (*Z*)-Gly-OHFP (1 mmol) and the mixture was sonicated to effect complete solution. The viscous solution was transferred to an NMR sample tube, the proton spectrum was measured, and the tube was stored in a thermostated bath at 25 or 45 °C, from which it was withdrawn intermittently for measurement of the NMR spectrum. The progress of the reaction was followed by observing the disappearance of the HFP ester methine septet at δ 5.70, the disappearance of the alanine methyl ester doublet at δ 1.37, and/or the appearance of the dipeptide alanine methyl doublet at δ 1.23. At the end of the reaction the dipeptide derivative was obtained in 60% isolated yield. Its melting point of 91–92 °C (from ether) was considerably higher than the 64–65 °C earlier reported (from ethyl acetate–petroleum ether)[16] but was the same as that of a sample prepared by reaction of *N*-(benzyloxycarbonyl)glycine *N*-hydroxysuccinide ester with L-alanine methyl ester in DME. A mixture melting point confirmed the identity of the two samples: NMR ($CDCl_3$) δ 1.36 (d,

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3 H), 3.70 (s, 3 H), 3.87 (d, 2 H), 4.53 (quintet, 1 H), 5.10 (s, 2 H), 6.00 (t, 1 H), 7.10 (d, 1 H), 7.30 (s, 5 H); IR ($CHCl_3$) 1510 (s), 1680 (s), 1720 (sh), 1740 (s) cm^{-1}.

L-Phenylalanine Hexafluoro-2-propyl Ester Hydrobromide. A 10-mL Erlenmeyer flask equipped with a Teflon-coated magnetic stirring bar and protected with a drying tube was charged with 0.225 g of (*Z*)-L-Phe-OHFP (0.5 mmol) and 3 mL of 33% hydrobromic acid in glacial acetic acid. This mixture was stirred for ca. 20 min, at which time evolution of CO_2 was no longer evident. The solution was concentrated under vacuum at room temperature, and the residue was triturated with dry ether to precipitate a white solid, H-Phe-OHFP·HBr, 163 mg (0.42 mmol, 83%): NMR (1:2 $CDCl_3$–Me_2SO-d_6) δ 3.30 (m, 2 H), 4.68 (m, 1 H), 6.80 (septet, 1 H, J = 6 Hz), 7.33 (s, 5 H); IR (KBr) 1785 (s) cm^{-1}. An analytical sample was recrystallized from HFP–ether, mp 215–225 °C dec.

Anal. Calcd for $C_{12}H_{12}BrF_6NO_2$: C, 36.38; H, 3.05; F, 28.78; Br, 20.17. Found: C, 36.39; H, 2.93; F, 28.96; Br, 20.38.

Hydrogenolysis of (*Z*)-L-Phe-OHFP. A 15-mL centrifuge tube equipped with a Teflon-coated magnetic stirring bar and sealed with a rubber septum was charged with 0.67 g of (*Z*)-L-Phe-OHFP, 0.07 g of 5% palladium-on-carbon, 4 mL of glacial acetic acid, and 0.2 mL of concentrated HCl. Hydrogen was bubbled through the stirred mixture for 5 h. After centrifugation, the supernatant was diluted with anhydrous ether and the precipitate was collected by filtration and triturated and washed further with ether. A 0.39-g (75%) yield of H-Phe-OHFP·HCl as a white powder which afforded a single, ninhydrin-positive spot on TLC was obtained. NMR and IR spectra were consistent with the structure of the expected product: NMR (Me_2SO-d_6) 3.30 (m, 2 H), 4.60 (m, 1 H), 6.93 (septet, 1 H, J = 6 Hz), 7.50 (s, 5 H); IR (KBr) 1780 cm^{-1}, no absorption near 1700 cm^{-1}.

Conclusions

Reactions of amino acid and peptide hexafluoro-2-propyl esters with carboxyl-protected amino acid derivatives in hexafluoro-2-propanol are considerably slower than might be desired. However, the system may be of use in special cases such as oligopeptide polymerization, where solubility is a more important consideration than time. Because there are no facile side reactions to destroy the active ester itself, the use of elevated temperatures (up to the boiling point of 59 °C) may be a feasible method for overcoming the intrinsic unreactivity so that the advantages of a powerful and volatile solvent and a volatile coupling coproduct may still be enjoyed. Racemization does occur with chiral α-acylamino HFP esters in the presence of amines.

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Registry No. (*Z*)-L-Phe-OHFP, 71785-36-9; (*Z*)-L-Phe-OH, 1161-13-3; HFP, 920-66-1; Boc-L-Val-OHFP, 71785-37-0; Boc-L-Val-OH, 13734-41-3; (*Z*)-Gly-OHFP, 71785-38-1; (*Z*)-Gly-OH, 1138-80-3; (*Z*)-L-Ala-OHFP, 71785-39-2; (*Z*)-L-Ala-L-Phe-OH, 2768-53-8; (*Z*)-Ala-L-Phe-OHFP, 71807-15-3; (*Z*)-L-Ala-D-OHFP, 71785-40-5; (*Z*)-L-Ala-D-Phe-OH, 17461-43-7; *N,N'*-dicyclohexyl-*O*-(1,1,1,3,3,3-hexafluoro-2-propyl)isourea, 71785-41-6; (*Z*)-L-Phe-L-Ala-OMe, 25422-44-0; L-Ala-OMe·HCl, 2491-20-5; (*Z*)-D-Phe-O-*N*-Su, 71785-36-9; (*Z*)-D-Phe-L-Ala-OMe, 3397-36-2; (*Z*)-Ala-L?-Phe-L-Phe-OMe, 71785-42-7; (*Z*)-Gly-OH *N*-hydroxysuccinimide ester, 2899-60-7; L-Ala-OMe, 10065-72-2; H-L-Phe-OHFP·HBr, 71785-43-8; H-L-Phe-OHFP·HCl, 71785-44-9; L-Phe-OMe·HCl, 7524-50-7; (*Z*)-L-Phe-OH *N*-hydroxysuccinimide ester, 3397-32-8; (*Z*)-D-Phe-D-Ala-OMe, 71785-45-0; (*Z*)-Gly-L-Ala-OMe, 16816-28-7.

Photolysis of Sodium Arenesulfonates in Aqueous Solution: Desulfonylation and Desulfonation[1,2]

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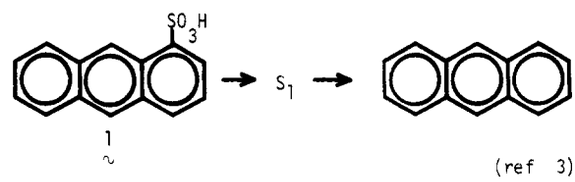
Photolysis of sodium anthracene-9-sulfonate (**3a**) gave 9,10-anthraquinone (**7a**) via the 9-anthranol anion (**6a**) by desulfonylation ($-SO_2$) and anthracene (**4a**) by desulfonation ($-SO_3$) in aqueous solution; in contrast, both sodium naphthalene-1- and mesitylenesulfonates (**3b** and **3c**) gave mainly desulfonation products with a trace of desulfonylation products.

Limited information is available on the relationship between the photochemical behavior and electronic configuration (n–π^* or π–π^*) of the excited states of the arenesulfonates.[3-7] Studzinskii et al. photolyzed in acid aqueous media anthracene-1-sulfonic acid (1) and observed its desulfonation via the lower singlet state (S_1).[3] Properties of the S_1 state of reactive 1- and unreactive 2-anthracenesulfonic acids were compared.[3] They also investigated the photolysis of reduced anthraquinonesulfonic



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