Since HRP binds a large number of aromatic substrates,[11,18] it should be possible to gain insight into which substrates can alter its heme–CO recombination kinetics at room temperature. Thus, picosecond studies using different aromatic donors are planned, and these investigations should yield information complementary to any future results on mutant peroxidases.

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((9-Fluorenylmethyl)oxy)carbonyl (FMOC) Amino Acid Fluorides. Convenient New Peptide Coupling Reagents Applicable to the FMOC/*tert*-Butyl Strategy for Solution and Solid-Phase Syntheses

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Recently FMOC amino acid chlorides were found to be stable highly reactive reagents for peptide-bond formation[1] which, in view of their favorable properties, were adopted as key intermediates in a new technique for the rapid, continuous synthesis of short peptide segments.[2] Applications to solid-phase syntheses have also been described.[3] Unfortunately, the impossibility of obtaining stable FMOC amino acid chlorides from trifunctional amino acids bearing side-chain protection incorporating the *tert*-butyl moiety (*t*-BOC, *tert*-butyl esters, *tert*-butyl ethers, etc.) seriously limits the applicability of this class of coupling agents. For example, attempts to obtain FMOC-aspartic acid chloride β-*tert*-butyl ester from **1** (n = 1) gave only the cyclic anhydride **3** by loss of *tert*-butyl chloride from the presumed intermediate acid chloride **2**. The same reaction occurred even in the case of the less sensitive β-1-adamantyl ester[4] related to **1**. Similarly stable, crystalline FMOC amino acid chlorides are not obtainable from glutamic acid γ-*tert*-butyl ester **1** (n = 2), ε-BOC-lysine, and the *O*-*tert*-butyl ethers of serine, threonine, and tyrosine.

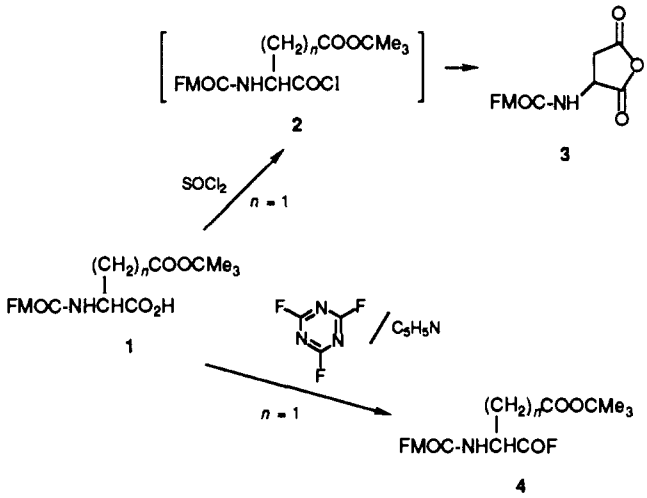

Table I. FMOC Amino Acid Fluorides[a,b]

compound	yield (%)	mp (°C)	optical rotation, $[\alpha]_D$, t (°C)
FMOC-Gly-F	80.5	140–141	
FMOC-Ala-F	75.4	111–112	+3.6° (*c* 0.5, EtOAc), 23
FMOC-Val-F	70.2	113–114	+10.7° (*c* 1, CH_2Cl_2), 24
FMOC-Leu-F	75.2	95–96	−7.9° (*c* 1, EtOAc), 23
FMOC-D-Leu-F	75.0	96–97	+7.6° (*c* 1, EtOAc), 23
FMOC-Ile-F	73.3	115–116	+15.6° (*c* 0.5, EtOAc), 23
FMOC-Pro-F	78.2	88–89	−28.6° (*c* 0.5, EtOAc), 24
FMOC-Phe-F	63.9	118–120	+35.5° (*c* 1, CH_2Cl_2), 24
FMOC-Trp-F[c]	70.7	125–128	−5.2° (*c* 1, EtOAc), 24
FMOC-Ser(*t*-Bu)-F[c]	72.7	89–91	+28.8° (*c* 0.5, EtOAc), 26
FMOC-Thr(*t*-Bu)-F[c]	72.6	53–55	+12.3° (*c* 0.4, EtOAc), 27
FMOC-Lys(BOC)-F[c]	80.0	128–130	−2.2° (*c* 0.5, CH_2Cl_2), 24
FMOC-Asp(O-*t*-Bu)-F[c]	67.8	74–75	+4.0° (*c* 0.5, EtOAc), 23
FMOC-Met-F	72.0	137–139	−12.9° (*c* 0.55, EtOAc), 25
FMOC-Glu(O-*t*-Bu)-F[c]	71.5	80–82	−11.2° (*c* 0.5, EtOAc), 25
FMOC-Tyr(*t*-Bu)-F[c]	67.4	97–99	+33.0° (*c* 0.5, CH_2Cl_2), 25
FMOC-Phg-F	75.0	144–146	+97.5° (*c* 0.5, CH_2Cl_2), 25

[a] All reactions involving the synthesis of cyanuric fluoride and the derived FMOC amino acid fluorides were carried out in ordinary glass vessels without any evidence of etching, etc. For a representative example of conversion to the acid fluoride, a solution of 0.339 g (1 mmol) of FMOC-Val-OH in 5 mL of CH_2Cl_2 was refluxed under N_2 with 1.08 g (8 mmol,[d] 700 μL, d = 1.6) of cyanuric fluoride and 81 μL (1 mmol) of pyridine for 2 h. The mixture, from which a water-soluble white precipitate had settled, was extracted with two 15-mL portions of ice water. Removal of solvent from the dry ($MgSO_4$) organic layer gave a white solid, which was recrystallized from CH_2Cl_2/hexane to give the pure acid fluoride. Detection of residual acid by TLC or analysis of fluoride content by HPLC was carried out as described earlier[1] after addition to dry MeOH except that it was necessary to wait for 15–300 min to allow time for complete conversion to methyl ester. For example, a solution analyzing initially for 82.7% FMOC-Val-F (as Me ester) came to complete conversion after 5 h with a measured content of 98.2% FMOC-Val-OMe and 0.9% FMOC-Val-OH. Esterification of the residual FMOC amino acid in the resulting methanolic HF solution did not occur, in contrast to the analogous acid chloride case. [b] In all cases, elemental analyses for C, H, and N agreed with theoretical values (±0.3%). [c] In these cases, the reaction mixtures were stirred at room temperature for 0.5–1.5 h. [d] More recently it was found convenient to use only 2 mmol of cyanuric fluoride with stirring at room temperature for 3 h for all fluorides listed in the table.

The long-known marked stability of *tert*-butyl fluoroformate relative to that of the corresponding chloro analogue[5,6] prompted an examination of FMOC amino acid fluorides. Our expectations were fully realized, and the remarkable reagents obtained have proved to be an exceptionally useful category of peptide coupling agents.

With the readily available reagent cyanuric fluoride,[7] aspartic acid ester **1** (n = 1) gave the stable, crystalline acid fluoride **4** (n = 1). Similarly other acid-sensitive protected amino acids (e.g., ε-BOC-FMOC-lysine, FMOC-glutamic acid γ-*tert*-butyl ester, the *O*-*tert*-butyl ethers of serine, threonine, and tyrosine, and unprotected tryptophan) were converted to the corresponding FMOC amino acid fluorides without difficulty. Table I collects the fluorides so far synthesized along with key physical properties.[8]

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(8) The infrared and especially the ^{13}C NMR data for model compounds FMOC-Val-Cl and FMOC-Val-F provide clear evidence for the simple acid halide structure of these compounds: (a) IR (KBr) 1790 (ClC=O), 1843 cm^{-1} (FC=O); (b) ^{13}C NMR ($CDCl_3$) 174.6 (ClC=O), 161.9 ppm (d, J_{CF} = 285.5 Hz, FC=O). For the acid fluoride, the expected carbon–fluorine coupling was also observed for the α-carbon atom: 58.2 ppm (d, J_{CF} = 38.5 Hz). The ^{13}C NMR position of the carbonyl group in the oxazolone[9] [mp 82–84 °C or 90–92 °C, α^{23}_D −29.6° (*c* 0.5, CH_2Cl_2)] derived from FMOC-Val-OH (obtained in an analytically pure state) is 175.3 ppm.[10] H–F coupling is also observed for the α-CH unit in the ^1H NMR spectrum (DMF-d_7) of FMOC-Phg-F (δ 5.7, dd).

(9) Compare: Paquet, A.; Chen, F. M. F.; Benoiton, N. L. *Can. J. Chem.* **1984**, *62*, 1335. Paquet et al. obtained a crude sample of this compound contaminated with 15% of dibenzofulvene.

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Apart from the stability of these *tert*-butyl side chain protected derivatives, it is the special character of the acyl fluoride function itself that is likely to assure the widespread applicability of this general class of reagents. Thus, due to the nature of the C–F bond, acyl fluorides are of greater stability than the corresponding chlorides toward neutral oxygen nucleophiles such as water or methanol yet appear to be of equal or nearly equal reactivity toward anionic nucleophiles and amines.[11] Application of FMOC amino acid fluorides in the FMOC/polyamine rapid segment synthesis is possible with little if any difference in the time required for completion of the coupling step between the fluorides and chlorides.[12] More importantly a striking qualitative difference between the two classes of compounds toward tertiary amines allows direct application of the fluorides to solid-phase peptide coupling reactions. In the case of FMOC amino acid chlorides, necessary basic co-reagents (DIEA, NMM, pyridine, etc.) cause immediate conversion to the corresponding oxazolones, which are more sluggish in their further conversion to the desired acylation product than the acid chlorides themselves.[13] For solid-phase reactions, maximum speed in the coupling step can be achieved by prior conversion of the acid chloride to an active ester (e.g., the HOBt ester obtained via a 1:1 mixture of DIEA and HOBt).[3] In remarkable contrast, FMOC amino acid fluorides are stable to these same tertiary bases,[14] which then serve to catalyze the direct acylation step and bind the liberated acid.[15] Model racemization studies[2,3] showed no significant loss of chirality in either solution or solid-phase syntheses.

(10) These data exclude from consideration any question of an oxazolone hydrohalide structure for these compounds. Compare: Carter, H. E.; Hinman, J. W. *J. Biol. Chem.* **1949**, *178*, 403. Carter and Hinman discuss such structures in the case of *N*-acyl amino acid halides. See also: Ronwin, E. *Can. J. Chem.* **1957**, *35*, 1031.

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(12) As an example, the protected heptapeptide i was obtained in the normal manner by using TAEA[2b] except that FMOC-Asp($OCMe_3$)-F and FMOC-Ser(CMe_3)-F were used along with FMOC-Val-Cl and FMOC-Leu-Cl. Following flash chromatography on silica gel using $CHCl_3$/MeOH/HOAc (90/10/1), the pure protected heptapeptide was obtained in analytically pure form (33%) as white crystals, mp 245 °C dec, α^{23}_D −19.4° (*c* 0.18, DMF); MS/FAB 1255 (MH^+), calcd 1253.8 (M). TAEA deblocking of the FMOC group followed by 10% *m*-cresol in TFA/CH_2Cl_2 (1:1) for 2 h gave in 54.5% yield the free heptapeptide TFA salt, mp 210–220 °C dec; MS/FAB 808.4437 (MH^+), calcd MH^+ 808.4457. Amino acid analysis (48-h hydrolysis): Asp, 0.97 (1); Ser, 1.02 (1); Val, 1.72 (2); Leu, 2.05 (2); Tyr, 0.95 (1).

FMOC-Val-Asp($OCMe_3$)-Val-Leu-Leu-Ser(CMe_3)-Tyr(CMe_3)-$OCMe_3$
i

(13) For peptide coupling reactions in solution, this problem is avoided by slow addition of the FMOC amino acid chloride to a solution of the amino acid ester and the base taken as HCl acceptor. Direct reaction with the acid chloride competes favorably with oxazolone formation. Similarly, under two-phase conditions with $NaHCO_3$ or Na_2CO_3 in the aqueous phase, the desired direct reaction occurs quickly in the organic phase. If the amino acid ester is omitted, oxazolone builds up slowly (ca. 10 min) in the organic phase.

(14) For a recent citation to the similar stability of alkyl fluoroformates toward solvents such as DMSO relative to the analogous chloroformates, see: Dang, V. A.; Olofson, R. A. *J. Org. Chem.* **1990**, *55*, 1851.

(15) As a first model to confirm direct acylation via FMOC amino acid fluorides, prothrombin (1–9) Ii was assembled manually on a batch synthesizer in DMF solution using 1 g of a TFA-sensitive polyamide resin bearing 0.1 mequiv/g of FMOC-valine. Glu, Leu, Phe, Gly, Lys, and Ala were incorporated as the FMOC amino acid fluorides (4 equiv of acid fluoride, 0.08 M in DMF, 4 equiv of DIEA) and Asn as pentafluorophenyl ester. Deblocking was carried out for 4 min twice with 20% piperidine in DMF. All washing steps involved DMF. Resin samples were removed after each 10-min coupling period and tested by the ninhydrin method. All couplings were complete by this time except for the Phe-to-Leu coupling, which was allowed to proceed for 25 min. No couplings were repeated. Final deblocking and removal from the resin (950 mg; at each coupling stage, 5–10 mg of resin was lost due to the ninhydrin tests) was achieved with 30 mL of TFA containing 5% H_2O and 5% *m*-cresol at 20 °C for 2 h to give 74 mg of peptide ii as the TFA salt, MS/FAB 1006 (MH^+), calcd 1005 (M), identified by coelution with an authentic sample.

H-Ala-Asn-Lys-Gly-Phe-Leu-Glu-Glu-Val-OH
ii

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Supplementary Material Available: HPLC trace of crude prothrombin ii (1 page). Ordering information is given on any current masthead page.

Biosynthesis of 3,6-Dideoxyhexoses: Stereochemical Analysis of the Deprotonation Catalyzed by the Pyridoxamine 5′-Phosphate Dependent Enzyme CDP-4-keto-6-deoxy-D-glucose-3-dehydrase Isolated from *Yersinia pseudotuberculosis*

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The 3,6-dideoxyhexoses are found in the O-specific side chains of cell wall lipopolysaccharides of a number of Gram-negative bacteria, where they have been identified as the dominant antigenic determinants.[1] Studies of the biosynthesis of CDP-ascarylose (**1**), the 3,6-dideoxy-L-*arabino*-hexopyranose derived from CDP-4-keto-6-deoxy-D-glucose (**2**), have shown that the C–O bond cleavage at C-3 is catalyzed by CDP-4-keto-6-deoxy-D-glucose-3-dehydrase (E_1), a pyridoxamine 5′-phosphate (PMP) dependent enzyme.[2] The proposed mechanism of this enzyme-catalyzed reaction involves the coupling of the coenzyme with the C-4 keto group of the substrate (**2**) to form a Schiff base (**3**) followed by a C-4′ proton abstraction from the resulting adduct (**3**) that triggers the expulsion of the C-3 hydroxy group (Scheme I).[2c,d] This enzymatic process is unique since it represents the only PMP-dependent catalysis that is not a transamination reaction. Although a reductive step catalyzed by an NAD(P)H-dependent reductase (E_3) has been shown to constitute the second phase of C-3 deoxygenation (Scheme I),[2a,d,3] the putative $\Delta^{3,4}$-glucoseen intermediate (**4**) has never been isolated or characterized.[2] In an attempt to explore the mechanism of this deoxygenation in detail, we have recently isolated an "E_1 equivalent" from *Yersinia pseudotuberculosis*[4] and determined the stereospecificity of its mediated deprotonation from the PMP–substrate adduct (**3**). Summarized in this paper are the results of this stereochemical

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(4) The original E_1 was isolated from *Pasturella pseudotuberculosis*,[2a] which is now classified as *Yersinia pseudotuberculosis*. However, the strains used in Strominger's studies and our current research are different. This recently purified enzyme consists of a single polypeptide chain with a molecular mass of 49 000 daltons.

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