The 9-Fluorenylmethyloxycarbonyl Family of Base-Sensitive Amino-Protecting Groups

LOUIS A. CARPINO

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

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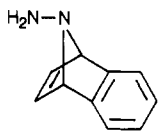
Several years ago we presented an Account of our work on the acid-sensitive *t*-BOC amino-protecting group and also described the beginnings of a parallel search for a spectrum of base-labile protective functions.[1] During these early studies one group emerged, the 9-fluorenylmethyloxycarbonyl (FMOC) function,[2,3] which appeared to be especially promising and which in fact in the intervening years has become widely adopted, particularly in peptide synthesis. Applications have been described to both solution-[4] and solid-phase[5] syntheses with results in the latter case being particularly impressive.[6] Use can be made of the ultraviolet absorption of the fluorene chromophore for easy control of the deblocking process, with automated instrumentation being available or under development.[9] Recently, fluorescence detection of the FMOC chromophore has led to a sensitive new technique for amino acid analysis based on derivatization via 9-fluorenylmethyloxycarbonyl chloride and subsequent separation on an ordinary reversed-phase HPLC column.[10]

Due to space limitations, this Account cannot deal with equally interesting base-sensitive protecting groups devised in other laboratories. Here we limit ourselves to a brief description of the FMOC function, its genesis and broad synthetic utility, the chemical implications of its introduction and removal, and finally its central role in inspiring the development of a numerous progeny. We also describe how selected members of the FMOC family have made possible some new or improved strategies in peptide synthesis.

Our work on both the *t*-BOC and FMOC groups originated in a search for amino-protecting groups capable of being utilized in the synthesis of sensitive nitrogen compounds of exceptional theoretical or synthetic interest. An example of a synthetic target which served as an early inducement to the development of protecting groups of the FMOC type is the thermally unstable, acid-sensitive hydrazine, 7-amino-7-azabenzonorbornadiene (1). A brief description of the



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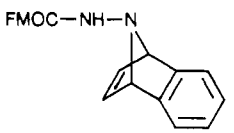

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synthesis of 1 has been presented.[11] Amination of the corresponding secondary amine by means of *O*-mesitylenesulfonylhydroxylamine gave the very labile 1, which was best isolated and stored as the FMOC derivative 2. Free hydrazine 1 could be generated as needed by mild deblocking of 2 at 0 °C by means of diethylamine in a 50–50 mixture of methylene dichloride and acetonitrile. This example also illustrates other useful properties of the FMOC function, some of the advantages of which are set out in Table I. In the case of 2, volatile reagents and solvents are required in order to allow their subsequent removal without de-

Table I.
Advantages of the FMOC Protecting Group

(1) exceptional acid stability
(2) rapid nonhydrolytic deblocking by simple amines
(3) compatibility with *tert*-butyl- and benzyl-based systems
(4) direct liberation of protected amine in free base form
(5) facile UV or fluorescence monitoring of deblocking
(6) manipulation of properties of byproduct adducts with cyclic secondary amines

Louis A. Carpino is Professor of Chemistry at the University of Massachusetts. He is the author of a previous account (**1973**, *6*, 191) which contains a brief biographical note.

(1) Carpino, L. A. *Acc. Chem. Res.* **1973**, *6*, 191.

(2) (a) Carpino, L. A.; Han, G. Y. *J. Am. Chem. Soc.* **1970**, *92*, 5748. (b) Carpino, L. A.; Han, G. Y. *J. Org. Chem.* **1972**, *37*, 3404; **1973**, *38*, 4218.

(3) Abbreviations of the new urethane-type protecting groups described in this Account are based on patterns previously established for the *t*-BOC and FMOC systems: IMOC, 1-indenylmethyloxycarbonyl; CLIMOC, 2-chloro-1-indenylmethyloxycarbonyl; BIMOC, 1-benzo[*f*]-indenylmethyloxycarbonyl; DBD–TMOC, 2,7-di-*tert*-butyl-10,10,10,10-tetrahydro-10,10-dioxothioxanthen-9-ylmethyloxycarbonyl.

(4) For a few selected references, see: (a) Bodanszky, A.; Bodanszky, M.; Chandramouli, N.; Kwei, J. Z.; Martinez, J.; Tolle, J. C. *J. Org. Chem.* **1980**, *45*, 72. (b) Bodanszky, M.; Bodanszky, A.; Tolle, J. C.; Bednarek, M. A. In *Chemical Synthesis and Sequencing of Peptides and Proteins*; Liu, T.-Y., Schechter, A. N., Heinrikson, R. L., Condliffe, P. G., Eds.; Elsevier: Amsterdam, 1981; p 163. (c) Kisfaludy, L.; Schön, I. *Synthesis* **1983**, 325. (d) Ten Kortenaar, P. B. W.; Krüse, J.; Hemminga, M. A.; Tesser, G. I. *Int. J. Pept. Protein Res.* **1986**, *27*, 401. (e) Toth, G. K.; Penke, B.; Zarandi, M.; Kovacs, K. *Int. J. Pept. Protein Res.* **1985**, *26*, 630. (h) Kessler, H.; Kuhn, M.; Loschner, T. *Liebigs Ann. Chem.* **1986**, *1*, 21.

(5) Only a few selected references can be given here: (a) Meienhofer, J. M.; Waki, M.; Heimer, E. P.; Lambros, T. J.; Makofske, R. C.; Chang, C.-D. *Int. J. Pept. Protein Res.* **1979**, *13*, 35. (b) Atherton, E.; Gait, M. J.; Sheppard, R. C.; Williams, B. J. *Bioorg. Chem.* **1979**, *8*, 351. (c) Atherton, E.; Sheppard, R. C.; Ward, P. *J. Chem. Soc.* **1985**, 2065. (d) Scanlon, D. B.; Eefting, M. A.; Lloyd, C. J.; Burgess, A. W.; Simpson, R. J. *J. Chem. Soc., Chem. Commun.* **1987**, 516.

(6) In sequential peptide syntheses premature deblocking by amino components inherent in the coupling process could be a potential source of contamination by incorrect sequences. Careful studies of syntheses carried out in solution via active esters have demonstrated that while such deblocking reactions occur, under the ordinary conditions of peptide synthesis they can be maintained at a low level.[7] Should it be necessary, coupling reactions can be carried out in the presence of weak acids, which in fact were the conditions deliberately chosen[2b] for the very first couplings involving FMOC amino acids. In solid-phase syntheses, such premature deblocking is unlikely.[8]

(7) Bodanszky, M.; Deshmane, S. S.; Martinez, J. M. *J. Org. Chem.* **1979**, *44*, 1622.

(8) Atherton, E.; Logan, C. J.; Sheppard, R. C. *J. Chem. Soc., Perkin Trans. 1* **1981**, 538.

(9) Jonczyk, A.; Meienhofer, J. In *Peptides. Proceedings of the 8th American Peptide Symposium*; Hruby, V. J., Rich, D. H., Eds.; Pierce Chemical Co.: Rockford, IL, 1983; p 73. (b) Eberle, A. N.; Atherton, E.; Dryland, A.; Sheppard, R. C. *J. Chem. Soc., Perkin Trans. 1* **1986**, 361. (c) Dryland, A.; Sheppard, R. C. *J. Chem. Soc., Perkin Trans. 1* **1986**, 125.

(10) (a) Einarsson, S.; Folestad, S.; Josefsson, B.; Lagerkvist, S. *Anal. Chem.* **1986**, *58*, 1638. (b) Cunico, R.; Mayer, A. G.; Wehr, C. T.; Sheehan, T. L. *Biochromatography* **1986**, *1*, 6. (b) Betner, I.; Földi, P. *Chromatographia* **1986**, *22*, 381. (c) Näsholm, T.; Sandberg, G.; Ericsson, A. *J. Chromatogr.* **1987**, *396*, 225.

(11) Carpino, L. A.; Padykula, R. E. *J. Chem. Soc., Chem. Commun.* **1986**, 747.

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struction of free hydrazine 1. The deblocking rate is enhanced in the presence of the polar solvent acetonitrile, allowing for the use of low temperatures. As a volatile, relatively hindered secondary amine, diethylamine was chosen as deblocking agent since it gives no adduct with dibenzofulvene (DBF, 10), thus facilitating the eventual low-temperature chromatographic separation of basic hydrazine 1 and neutral hydrocarbon 10.

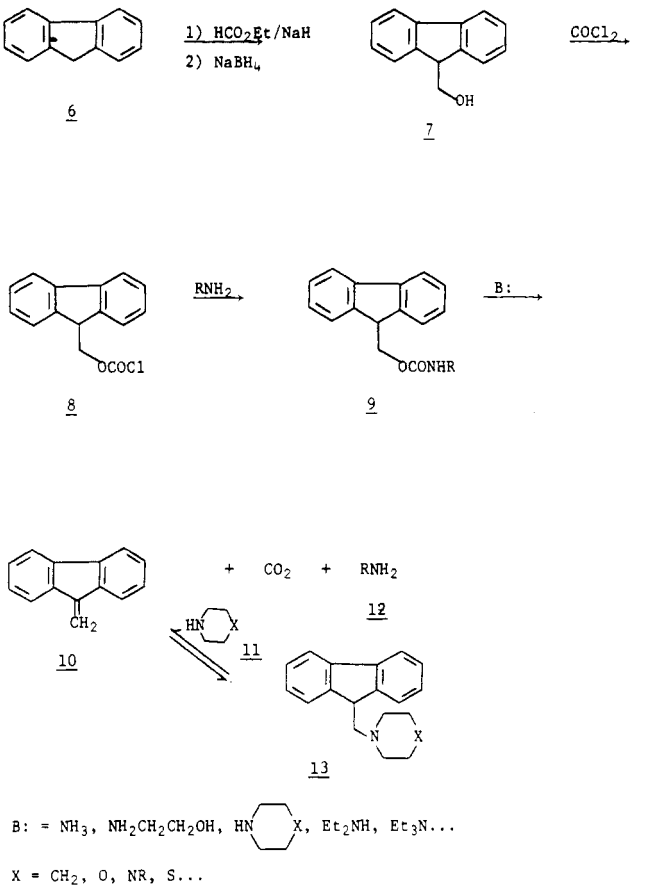
The FMOC system grew out of general studies on the use of β-elimination processes (eq 1) for amino pro-

$$\underset{3}{ZCH_2CH_2OCONHR} \xrightarrow{B:} \underset{4}{ZCH{=}CH_2} + \underset{5}{OCONHR^-} \quad (1)$$

tection.[12] In the earliest studies substrates 3 bearing an electron-withdrawing group, Z, capable of strong activation of an adjacent C–H bond, were examined. Our work began with urethanes of β-nitroethanol (3, Z = NO_2). The first practical system (3, Z = Ts) was described by Kader and Stirling.[16] This system, in its more hydrophilic incarnation (MSC, 3, Z = CH_3SO_2),[17] is often used where high solubility in aqueous or partially aqueous media is desired.

At about the time it became clear that urethanes of β-nitroethanol would not provide adequate protection for practical use, we learned by chance[18] from Professor A. Ceccon in 1968 of the work of his group[19] on the unusually facile elimination of thiofluorenone from 9-fluorenyl isothiocyanates under conditions where benzhydryl isothiocyanates are stable. These results inspired an examination of urethane derivatives of the long-known alcohol 7. As it turned out, such urethanes were shown to be deblocked slowly in liquid ammonia or more rapidly by simple aliphatic amines under anhydrous conditions. Scheme I outlines a simple route[20] to the key alcohol 7[21,22] and important aspects of the deblocking process. The free amine 12 is formed directly in contrast to the carbamate salts obtained in the presence of more basic reagents, as under the normal deblocking technique recommended for the MSC function.[23] In the protection step, reaction of an amine with stable chloroformate 8 proceeds without premature deblocking. In the case of amino acids, protection via

Scheme I



6 7 8 9 10 11 12 13

the chloroformate has lately been superseded by use of several chloroformate-derived active esters or the corresponding azidoformate in order to avoid the complicating formation of dipeptides or higher amino acid oligomers.[24] Alternatively, the amino acid is converted in situ to the corresponding trimethylsilyl ester and in this form safely acylated by means of 8.[25]

Deblocking proceeds readily by means of a variety of 1°, 2°, and some 3° amines, most rapidly via unhindered cyclic secondary amines such as piperidine. The use of anhydrous organic bases in deblocking reactions represented a departure from previous approaches and has been an important factor in the widespread adoption of the FMOC function. The exact choice of deblocking amine depends on the nature of the system at hand. For instance, the volatility of diethylamine, mentioned above, or dimethylamine is also of advantage in peptide syntheses carried out in solution.[4] The bulky character of diisopropylamine can be used to advantage in effecting selective deblocking of FMOC protection in the presence of phthalimido residues which are sensitive to piperidine.[26] Consideration must also be given to possible subsequent reactions of the deblocking amine with the initially formed byproduct, dibenzofulvene (10). This unusual hydrocarbon bears a highly electrophilic double bond, the reactivity of which has its origin in the pronounced stabilization of negative charge in the aromatic cyclopentadienide anion.[27]

(12) Although not recognized as such, among the first examples of the β-elimination process now commonly used for amino and carboxyl protection must have been the observations of Chattaway[13] that β-nitroethyl acetates reacted with ammonia to give not the expected acetamide but rather ammonium acetate. Crowley[14] showed that analogous urethanes underwent deblocking by anhydrous ammonia in benzene solution. The deliberate exploitation of such reactions for carboxyl protection began with the work of Rydon.[15]

(13) (a) Chattaway, F. D. *J. Chem. Soc.* **1936**, 355. (b) Chattaway, F. D.; Drewitt, J. G. N.; Parkes, G. D. *J. Chem. Soc.* **1936**, 1530.

(14) Crowley, P. J. M.S. Thesis, University of Massachusetts, Amherst, MA, 1958.

(15) (a) Crane, C. W.; Rydon, H. N. *J. Chem. Soc.* **1947**, 766. (b) Mamalis, P.; Rydon, H. N. *J. Chem. Soc.* **1955**, 1049.

(16) Kader, A. T.; Stirling, C. J. M. *J. Chem. Soc.* **1964**, 258.

(17) Tesser, G. I.; Balvert-Geers, I. C. *Int. J. Pept. Protein Res.* **1975**, *7*, 295.

(18) During the task of helping to translate a manuscript for publication[19a] from "Brokish" into English.

(19) Compare: (a) Ceccon, A.; Miotti, U.; Tonellato, U.; Padovan, M. *J. Chem. Soc. B* **1969**, 1084. (b) Miotti, U.; Sinico, A.; Ceccon, A. *J. Chem. Soc., Chem. Commun.* **1968**, 724.

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

(21) Brown, W. G.; Bluestein, B. *J. Am. Chem. Soc.* **1940**, *62*, 3256; **1943**, *65*, 1082.

(22) As a dramatic illustration of the unusual nature of 9-fluorenylmethanol, the alcohol itself undergoes facile base-catalyzed elimination. See: (a) More O'Ferrall, R. A.; Slae, S. *J. Chem. Soc. B* **1970**, 260. (b) More O'Ferrall, R. A. *J. Chem. Soc. B* **1970**, 268, 274.

(23) Tesser, G. I.; Boon, P. J. *Recl. Trav. Chim. Pays-Bas* **1980**, *99*, 289.

(24) For a recent reference with citations to earlier work, see: Henklein, P.; Heyne, H.-U.; Halatsch, W.-R.; Niedrich, H. *Synthesis* **1987**, 166.

(25) Bolin, D. Eur Patent EP-129075, Dec 27, 1984; *Chem. Abstr.* **1985**, *102*, 204290.

(26) Freidinger, R. M.; Hinkle, J. S.; Perlow, D. S.; Arison, B. H. *J. Org. Chem.* **1983**, *48*, 77.

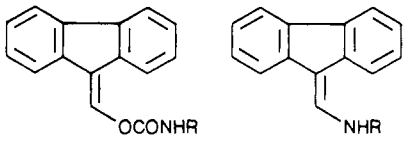
Selectivity in adduct formation is still not completely understood. Both intrinsic basicity/nucleophilicity and steric factors are clearly important. Thus, while piperazine and its mono-*N*-methyl and *N*-phenyl derivatives as well as piperidine, *cis*-3,5-dimethylpiperidine, and morpholine undergo facile adduct formation, the less basic amino acid derivative isonipecotic acid and the highly basic but sterically congested 2,6-dimethyl- and 2,2,6,6-tetramethylpiperidines do not. Among primary amines, *n*-propylamine and (aminomethyl)-cyclohexane react readily whereas ethanolamine, ethylenediamine, and 2-methoxyethylamine require large excesses of amine or long times to induce extensive adduct formation.

Dibenzofulvene (**10**) is not an easily handled molecule, and its synthesis in a state of high purity requires extraordinary precautions.[28] In the free state under ordinary conditions DBF undergoes rapid polymerization. In solution it is more stable yet still subject to unpredictable conversion to polymer and/or oligomers. Reaction mixtures containing DBF may slowly precipitate the highly insoluble polymer or alternately set in the form of unworkable gels. Thus, the unforeseen formation of adducts such as **13** proved to be a fortunate circumstance, allowing control of the undesirable properties of DBF.[29] Deliberate manipulation of the nature of the byproduct to vary its solubility under specific conditions has added to the versatility of the FMOC protective system, as will be apparent later in this discussion.

Closer examination of the process of adduct formation revealed the existence of an equilibrium[30] between DBF and the deblocking amine, a fact first noted accidentally when an analytically pure sample of the piperidine adduct **13** (X = CH_2) was noted by TLC analysis to slough off small quantities of DBF on standing in solution. The position of equilibrium depends on the particular solvent as well as the identity of the amine. For studies of these equilibria DBF was generated in solution by deblocking of 9-fluorenylmethyl carbamate by the various amines in question. In a typical case in deuteriochloroform, with 10 mol equiv of piperidine at a concentration of 0.5 M urethane, deblocking was complete in 7 h with equilibrium being established only after 6 days with 23% DBF remaining unreacted (77% scavenging). In DMSO-d_6 both reactions were accelerated with deblocking complete in 5 min and scavenging within 1 h at an equilibrium position involving 88% adduct formation. With the less basic morpholine equilibrium was reached more slowly (~3 days) but scavenging was more complete (95–100%). Curiously, in methylene dichloride deblocking and scavenging were also far more rapid than in the superficially analogous solvent chloroform. Special effects were also noted in the case of piperazine derivatives. In DMSO the adducts, whether from piperazine itself or its mono-*N*-methyl or *N*-phenyl derivatives, were very insoluble and precipitated from solution, thereby shifting the equilibrium toward complete scavenging within a relatively short period. NMR examination showed, however, that even prior to precipitation nearly complete scavenging had occurred.

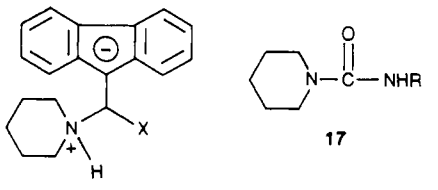
Revelation of these equilibria has raised the possibility of using the 9-fluorenylmethyl (FM) group generally as a base-sensitive analogue of the acid-sensitive trityl function. In fact, treatment of **13** (X = NMe) with 10 mol equiv of piperidine in DMF at room temperature effects conversion to the corresponding piperidine adduct within several hours. Similarly, in DMF an excess of *N*-methylpiperazine displaces piperidine from its adduct. These reactions are slower in chloroform or methylene dichloride and occur only in the direction of displacement of the weaker base (*N*-methylpiperazine). A practical example involving the similar but clearly more facile displacement of an amide-type leaving group has been described by Hoyng.[31,32] Such FM derivatives are also deblocked by catalytic hydrogenolysis.

The base-induced deblocking of the FM group relies on the presence of the 9-fluorenyl proton as is also the case for the FMOC system. Consider the result of introducing unsaturation into FMOC or FM systems, e.g., generation of structures **14** and **15**. Both of these



14 **15**

compounds (R = C_6H_5) were reported many years ago[33,34] although their reactivity was not studied. In view of the nature of the reactions of dibenzofulvene, described above, would an amine, e.g., piperidine, be expected to add to the terminal position to give an adduct such as **16** and thereby cause deblocking? As



16 (X = OCONHR, NHR) **17**

might have been predicted, a more facile competing reaction occurs in the case of **14** with conversion to urea **17** (R = C_6H_5). Here **14** exhibits reactivity typical of a vinyl ester. In the case of **15** no such competing reactions are possible. On the other hand, the N atom of such simple enamines is "protected" in the same sense as the carbonyl analogues (compare **15a** and **18**) and to the extent that such enamine resonance is important, the addition of piperidine to give a species such

(27) Bordwell, F. G.; Drucker, G. E.; Fried, H. E. *J. Org. Chem.* **1981**, *46*, 632.

(28) (a) Neuenschwander, M.; Vögeli, R.; Fahrni, H.-P.; Lehmann, H.; Ruder, J.-P. *Helv. Chim. Acta* **1977**, *60*, 1073. (b) Kice, J. L., *J. Am. Chem. Soc.* **1958**, *80*, 348.

(29) The first description of the addition of cyclic secondary amines to a DBF derivative appears to be that of Quelet and Matarasso-Tchiroukhine, who noted the addition of piperidine and morpholine to 2,3,6,7-tetramethoxydibenzofulvene as well as the lack of reaction with diethylamine. See: (a) Matarasso-Tchiroukhine, E. *C R. Seances Acad. Sci. Ser. C* **1959**, *248*, 2015. (b) Matarasso-Tchiroukhine, E. *Ann. Chim. (Paris)* **1958**, *3*(13), 405. Quelet, R.; Matarasso-Tchiroukhine, E. *C. R Seances Acad. Sci., Ser. C* **1958**, *246*, 1227.

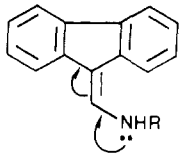
(30) Carpino, L. A.; Mansour, E. M. E.; Knapczyk, J. *J. Org. Chem.* **1983**, *48*, 666.

(31) Hoyng, C. F.; Patel, A. D. *Tetrahedron Lett.* **1980**, 4795; *J. Chem. Soc., Chem. Commun.* **1981**, 491.

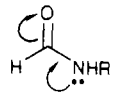
(32) The FM group has also been recommended for carboxyl and thiol protection: See: (a) Bednarek, M.; Bodanszky, M. *Int. J. Pept. Protein Res.* **1983**, *21*, 196. (b) Kessler, H.; Siegmeier, R. *Tetrahedron Lett.* **1983**, 281. (c) Bodanszky, M. *Int. J. Pept. Protein Res.* **1982**, *20*, 434.

(33) Wislicenus, W.; Waldmuller, M. *Ber.* **1909**, 42, 785.

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



15a



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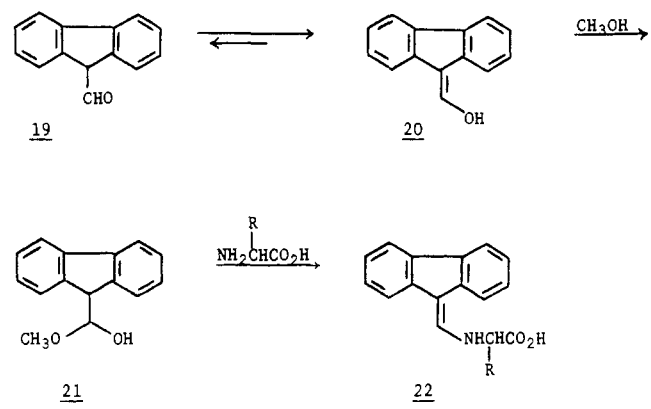
as **16** would be hindered. In fact, when **15** (R = *p*-ClC_6H_4) is let stand in DMF for 3 days in the presence of excess piperidine or even refluxed for 15 h in pure piperidine, no reaction occurs and the enamine is recovered unchanged. Degradation occurs in refluxing pyrrolidine with the formation of fluorene. This is understandable on the basis of proton transfer in **16** followed by ejection of fluorenide anion. However, deblocking can readily be achieved by mild acid hydrolysis (HCl/dioxane/H_2O)[35] or catalytic hydrogenolysis.

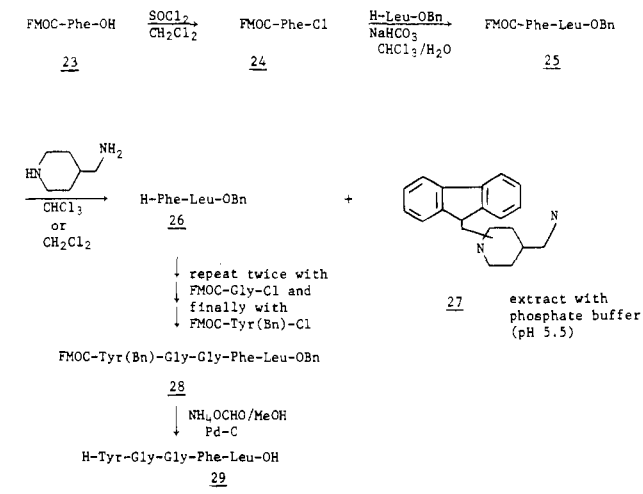
Infrared and NMR data confirm the enamine structure **15** as opposed to the isomeric imine alternative. In view of the expected conjugative effects (**15a**), attention was directed toward the question of whether these new $\overline{FM}$[36] derivatives had any potential for the α- or side chain protection of amino acids. $\overline{FM}$ amino acid derivatives are generally available by condensation with fluorene-9-aldehyde (**19**) obtained by interrupting the previously described route to key alcohol **7** at the aldehyde stage (Scheme II). Aldehyde **19** exists in equilibrium with, but mainly as, the vinyl alcohol **20**.[33,37] It is best stored as the crystalline hemiacetal **21**. Reaction of the free aldehyde/vinyl alcohol or, more conveniently, the hemiacetal with amino acids or their esters gives the $\overline{FM}$ derivatives, e.g. **22**. Upon storage some $\overline{FM}$ derivatives have been noted to undergo slow air oxidation as evidenced by the gradual development of a yellow color due to formation of the corresponding fluorenone.[38] Whether this will interfere with their use as amine protectants remains to be determined.

As noted previously, deliberate design of DBF adducts might facilitate their eventual separation from a desired deblocked amine. An illustration of the practical use of this concept comes from a newly developed, exceptionally rapid sequential solution synthesis of peptides in which coupling and deblocking reactions occur in a water-immiscible solvent and all byproducts are removed by aqueous extractions with retention of the growing peptide in the organic phase.[39] Scheme III outlines the method, the first example of which involved the synthesis of leucine-enkephalin. Taking advantage of the pronounced stability of the FMOC group toward acidic reagents, one can obtain FMOC amino acid chlorides by reaction of the protected amino acid with thionyl chloride under ordinary conditions. Such amino acid chlorides are crystalline, shelf-stable

Scheme II



Scheme III



reagents which promise to become general-purpose coupling reagents of choice. Reaction with an amino acid or peptide ester in a two-phase system in the presence of aqueous bicarbonate gives within a few minutes the appropriate coupling product. Excess acid chloride is removed by reaction with 4-(aminomethyl)piperidine (4-AMP) which simultaneously effects deblocking of the desired protected peptide and any FMOC-bearing byproducts. Within 15–45 min, deblocking is complete and washing with water removes excess 4-AMP. The adduct **27** (possibly a mixture) remains in the organic phase; however, its basicity is sufficiently different from that of the growing amino peptide ester that it can be selectively removed along with byproducts derived from excess acid chloride by extraction with a phosphate buffer of pH 5.5. Rapid chain extension is possible by this method on a multigram scale. Model studies on the coupling of the acid chloride of FMOC phenylalanine with leucine methyl ester showed no detectable racemization by ^1H NMR and HPLC analysis (<0.1% by the latter technique[40]). Short segments related to substance P bearing up to seven amino acid units have also been synthesized in one operation by this approach.[41]

Protected pentapeptide **28** was converted to leucine-enkephalin (**29**) with removal of all protecting

(35) Compare: (a) Dane, E.; Drees, F.; Konrad, P.; Dockner, T. *Angew. Chem.* **1962**, *74*, 873. (b) Dane, E.; Dockner, T. *Angew. Chem.* **1964**, *76*, 342.

(36) The symbol $\overline{FM}$ will be used for the 9-fluorenylmethylene group with the unsaturation being indicated by the extra bond drawn over the FM symbol (**15**, $\overline{FM}$–NHR).

(37) Compare: Curtin, D. Y.; Kampmeier, J. A.; O'Conner, B. R. *J. Am. Chem. Soc.* **1965**, *87*, 863.

(38) Tien, J.-H.; Chao, H.-G., University of Massachusetts, unpublished studies. Compare: Taylor, R. J. *Chem. Res., Synop.* **1987**, 178.

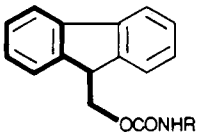
(39) Carpino, L. A.; Cohen, B. J.; Stephens, K. E.; Sadat-Aalaee, S. Y.; Tien, J.-H.; Langridge, D. C. *J. Org. Chem.* **1986**, *51*, 3732. In our laboratory we informally refer to this technique as the FAACST method.

(40) For basic elements of the racemization test, see: Carpino, L. A.; Rice, N. W.; Mansour, E. M. E.; Triolo, S. *J. Org. Chem.* **1984**, *49*, 836.

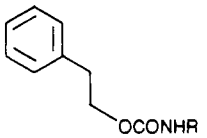
(41) (a) Beyermann, M.; Bienert, M.; Repke, H.; Carpino, L. A. *Abstracts of Papers*, 19th European Peptide Symposium, Porto Carras, Greece, 1986. (b) Beyermann, M.; Granitza, D.; Bienert, M.; Mehlis, B.; Niedrich, H.; Carpino, L. A. *Abstracts of Papers*, 10th American Peptide Symposium, St. Louis, MO, 1987.

groups in a single step by catalytic transfer hydrogenolysis with ammonium formate in the presence of palladium carbon catalysts.[42] The catalytic hydrogenolysis of the FMOC function is now a well-established process for which a short historical note is in order. In the first announcement[2] of the utility of the FMOC group, one of the advantages cited was the stability toward hydrogenolysis under conditions which caused deblocking of Z- and other O-benzyl functions. Synthetic use has been made of this stability toward hydrogenolysis in the synthesis of certain side-chain-bearing FMOC amino acids.[43] It was therefore at first surprising when both Bodansky[44] and Sheppard[45] described the instability of FMOC derivatives toward hydrogenation over palladium catalysts. Reinvestigation showed that the catalysts used in our initial studies were of low catalytic activity. Results with newly purchased, more active catalysts are in agreement with the work of the later investigators. Comparison showed, however, that FMOC systems are deblocked at far lower rates than O-benzyl-based systems, and it is to be expected that in many cases, similar to those already reported,[43] conditions can be found to allow good selectivity.

Since initial experiments confirmed our preconceived notion that, as aryl*ethyl* rather than aryl*methyl* derivatives, FMOC systems would not be expected to be deblocked by hydrogenolysis, we did not examine extensively a wide variety of catalysts and reaction conditions. A mechanistic rationale for the facile hydrogenolysis of FMOC systems is not yet obvious. Reflection (compare **9a**) suggested, however, that susceptibility to catalytic hydrogenolysis might well be shared by the parent β-phenylethyl system **30**. This was in-



9a



30

deed found to be the case, and a thorough study of the β-phenylethyloxycarbonyl ("homobenzyloxycarbonyl", hZ) has demonstrated its general susceptibility to catalytic deblocking. Why had this simple reaction not previously been noted? A possible explanation may lie in the fact that about 20 years ago the β-phenylethyl ester of glycine was reported by Zervas and co-workers[46] to be stable to catalytic hydrogenolysis. In the study cited, deblocking of the *N*-benzyloxycarbonyl derivative of the phenacyl ester of glycine to free glycine was accompaned by reduction of the phenacyl function to the β-phenylethyl function which survived further treatment (eq 2).

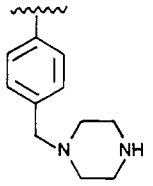
$$\underset{\mathbf{31}}{\text{Z-NHCH}_2\text{COOCH}_2\text{COC}_6\text{H}_5} \xrightarrow{\text{H}_2/\text{Pd-C}} \underset{\mathbf{32}}{\text{NH}_2\text{CH}_2\text{CO}_2\text{H}}\ (75\%) + \underset{\mathbf{33}}{\text{NH}_2\text{CH}_2\text{COOCH}_2\text{CH}_2\text{C}_6\text{H}_5}\ (25\%) \quad (2)$$

In a rerun of the FMOC tale we now find that while β-phenylethyl *esters* are indeed less susceptible than β-phenylethyl *carbamates*, they can be deblocked under appropriate conditions (freshly precipitated catalysts, high catalyst/substrate ratios, warming and/or sonication, etc.). An example is outlined in eq 3. Some

$$\underset{\mathbf{34}}{\text{hZ-Gly-Gly-OCH}_2\text{CH}_2\text{C}_6\text{H}_5} \xrightarrow[\text{Pd-C}\ \text{Pd(OAc)}_2]{\text{NH}_4\text{OCHO/MeOH}} \underset{\mathbf{35}}{\text{H-Gly-Gly-OH}} + \text{CO}_2 + \underset{\mathbf{36}}{\text{C}_6\text{H}_5\text{CH}_2\text{CH}_3} \quad (3)$$

selectivity is, however, possible depending on catalytic conditions as described in a preliminary report on the utilization of the homobenzyloxycarbonyl group.[47] For example, in a Parr apparatus over Pd-C catalyst hZ-Gly-$OCH_2C_6H_5$ suffers loss only of the benzyl ester function whereas with catalytic transfer hydrogenolysis (Pd-C/$Pd(OAc)_2$/NH_4OCHO) both urethane and ester groups are removed.

Returning to the broad topic of the synthetic utility of base-sensitive protecting groups, we consider another possible method of taking advantage of the formation of adducts between DBF and cyclic secondary amines, namely, by fixing the latter onto a polymeric support. Such reagents, in addition to effecting deblocking, could act to scavenge DBF from the reaction mixture. Several such piperazine-based reagents have been synthesized by using both polystyrene[48] and silica[30] supports. Examples include **37** and **38**. Initial studies with these



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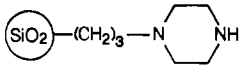


38

materials were carried out prior to our knowledge of the equilibria described above, and it is not now surprising that scavenging was incomplete. Depending on the solvent used, even in the best cases, 10–15% of DBF remained in solution at equilibrium. Such reagents were first examined in attempts to develop a continuous "inverse Merrifield" synthesis of peptides in which two polymeric reagents are used in tandem, one to provide transfer of an FMOC amino acid to a growing peptide ester held in solution and the second to effect cleavage of the FMOC group prior to further chain extension. While not a new concept, previous attempts to effect such syntheses cleanly have not succeeded.[49] Our first attempts involving FMOC protection also failed due to

(42) Anwer, M. K.; Spatola, A. F. *Synthesis* **1980**, 929; *Tetrahedron Lett.* **1981**, 4369.

(43) (a) Chang, C.-D.; Waki, M.; Ahmad, M.; Meienhofer, J.; Lundell, E. O.; Haug, J. D. *Int. J. Pept. Protein Res.* **1980**, *15*, 59. (b) Almquist, R. G.; Christie, P. H.; Chao, W.-R.; Johnson, H. L. *J. Pharm. Sci.* **1983**, *72*, 63. (c) For particularly striking examples of selectivity between FMOC systems and benzyl esters using catalytic transfer hydrogenolysis, see: Paulsen, H.; Schultz, M. *Liebigs Ann. Chem.* **1986**, 1435; Kelly, R. C.; Gebhard, I.; Wichienski, N. *J. Org. Chem.* **1986**, *51*, 4590.

(44) Martinez, J.; Tolle, J. C.; Bodanszky, M. *J. Org. Chem.* **1979**, *44*, 3596.

(45) Atherton, E.; Bury, C.; Sheppard, R.; Williams, B. J. *Tetrahedron Lett.* **1979**, 3041.

(46) Taylor-Papadimitriou, J.; Yovanidis, C.; Paganou, A.; Zervas, L. *J. Chem. Soc. C* **1967**, 1830.

(47) Carpino, L. A.; Tunga, A. *J. Org. Chem.* **1986**, *51*, 1930.

(48) Carpino, L. A.; Mansour, E. M. E.; Cheng, C.-H.; Williams, J. R.; MacDonald, R.; Knapczyk, J.; Carman, M.; Lopusinski, A. *J. Org. Chem.* **1983**, *48*, 661.

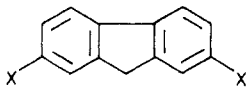
(49) For reviews, see: (a) Fridkin, M. In *Peptides. Analysis. Synthesis. Biology*; Gross, E., Meienhofer, J., Eds.; Academic: New York, 1980; Vol. 2, p 333. (b) Patchornik, A.; Cohen, B. J. In *Perspectives in Peptide Chemistry*; Eberle, A., Geiger, R., Wieland, T., Eds.; Karger: Basel, Switzerland, 1981; p 118.

Table II.
Reaction X-MOC-NHC$_6$H$_4$Cl-*p*/C$_5$H$_{11}$N/CH$_2$Cl$_2$

	FMOC	IMOC	CLIMOC
k_{rel} (deblocking)	1	10	200
k_{rel} (scavenging)	1	250	5000

these incomplete scavenging processes.

Since numerous attempts to improve the scavenging efficiency of the polymeric reagents failed, a second approach involving changes in the protecting group was examined. Since the ease of both deblocking and scavenging should correlate with the acidity of the fluorene element built into the urethane system, a number of more acidic fluorene derivatives were examined.[50] Appropriate urethanes derived from the readily available 2,7-dichloro- and 2,7-dibromofluorenes **39** were, as expected, more rapidly deblocked than the unsubstituted analogue, but unexpectedly the scavenging step was no more complete.[20] A switch to indene derivatives



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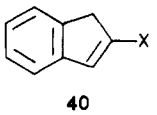
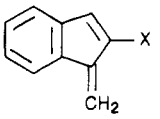
X = H, pK_a = 22.6
X = Br, pK_a = 17.9



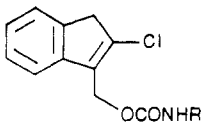
40

X = H, pK_a = 20.1



41

was more satisfactory, the greater acidity of **40** assurring higher deblocking rates and greater reactivity at the exocyclic methylene function of the intermediate monobenzofulvenes **41** relative to DBF making for more complete scavenging. Simple IMOC[3] derivatives obtained from **40** (X = H) showed the desired properties except for crystallinity. Many amino acids protected by this function were obtained only as oils which were difficult to purify.[51] The corresponding CLIMOC[3] and BIMOC[3] derivatives **42** and **43** overcame this problem.



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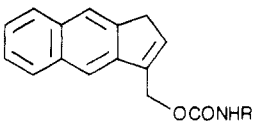
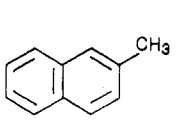


43

With the CLIMOC system scavenging could be followed by disappearance of the yellow color due to **41** (X = Cl). Appropriate comparisons of deblocking and scavenging reactions are shown in Table II. Somewhat less reactive than the CLIMOC derivatives were the FMOC-isomeric BIMOC urethanes **43** derived from benzo[*f*]-indene (**46**), which was synthesized from 2-methyl-



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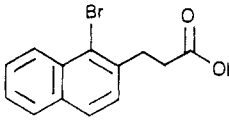

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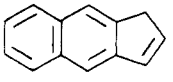
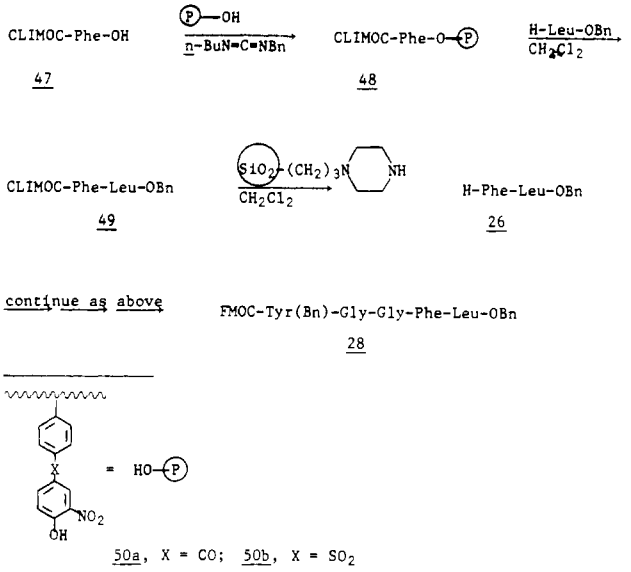


46

naphthalene (**44**).[52] A key element of the synthesis involved protection of the 1-position of **44** by bromine substitution so as to guarantee Friedel–Crafts cyclization of intermediate **45** to give the linear polycyclic ketone[53] which was converted to **46** by standard techniques.[54]

(50) For pK_a data of substituted fluorenes, see: Bordwell, F. G.; McCollum, G. J. *J. Org. Chem.* **1976**, *41*, 2391.
(51) Cohen, B. J., University of Massachusetts, unpublished studies.
(52) Lin, Y.-Z. Ph.D. Thesis, University of Massachusetts, 1987.
(53) Mayer, F.; Sieglitz, A. *Chem. Ber.* **1922**, *55*, 1835.
(54) Compare Brown, H. C.; Krishnamurthy, S. *J. Org. Chem.* **1969**, *34*, 3918.

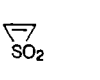
Scheme IV



A simple inverse Merrifield synthesis of leucine-enkephalin, using CLIMOC protection was demonstrated, as outlined in Scheme IV.

First, the CLIMOC derivative of phenylalanine is loaded onto an active phenolic polymer **50**. For example, a sample of **50a** (1.8 mequiv of OH/g) gave active ester **48** (0.6 mequiv of protected amino acid/g). Using a 2 M excess of **48**, transfer of protected amino acid was achieved in 95% yield within 30 min by stirring with a methylene dichloride solution of leucine benzyl ester. The CLIMOC group was then removed by reaction for 30–45 min with silica-based piperazine reagent **38** bearing 10 mol equiv of piperazino residues per mole of CLIMOC derivative. Alternating use of **38** with the glycine and FMOC tyrosine analogues of **48** eventually gave **28**.[55] The final amino acid was added as the FMOC derivative in order to simplify comparison with the material previously obtained by the sequential solution route described above. CLIMOC-protected amino derivatives could not be used in DMF, being somewhat unstable in this more useful solvent. On the other hand, BIMOC derivatives were stable indefinitely in DMF and a comparable synthesis of leucine-enkephalin could be carried out in DMF via BIMOC protection. Extension of these inverse Merrifield techniques to the synthesis of longer and/or more complex peptides as well as to column operation seems feasible.

A second approach to the development of base-sensitive protecting groups more labile than the FMOC function grew out of some unrelated work[56] dealing with the fundamental question of the electronic character of the theoretically interesting thiirene dioxides **51**.



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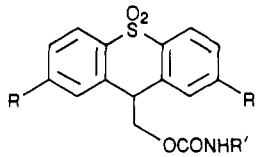
52

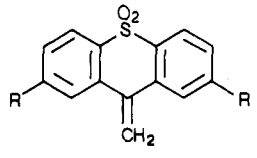
This work led to conjectures on the high stability to be expected of analogous 6-π-electron anions **52**. Literature confirmation[57] of these speculations induced us to

(55) Sequential assembly of the CLIMOC–tetrapeptide in 74% overall yield required about 6 h. The tyrosine unit was added separately.
(56) (a) Carpino, L. A.; McAdams III, L. V. *J. Am. Chem. Soc.* **1965**, *87*, 5804. (b) Carpino, L. A.; McAdams III, L. V.; Rynbrandt, R. H.; Spiewak, J. W. *J. Am. Chem. Soc.* **1971**, *93*, 476.

test urethanes of structure **53** (R = H). In fact, such



53



54

carbamates are deblocked readily by pyridine whereas FMOC derivatives are stable in this solvent over a period of several days. More interestingly, these urethanes underwent deblocking simply by warming to 40–45° in the dipolar aprotic solvent DMSO. In nonpolar solvents such as benzene, tetrahydrofuran, and chloroform or hydroxylic solvents such as methanol and ethanol, the compounds were stable. The mechanistic implications of this finding are not yet clear although elimination may be viewed as a supereffective "acetate pyrolysis"[58] via a transition state somehow stabilized by dipolar aprotic solvents.

Urethane sulfones of structure **53** (R = H) proved to be exceptionally insoluble in most solvents and therefore difficult to handle in a practical way. As derivatives of potentially[59] greater solubility the 2,7-di-*tert*-butyl derivatives **53** (R = CMe_3) were examined. As expected, these DBD–TMOC[3] derivatives proved to be readily soluble in common solvents and underwent the same solvolytic deblocking reaction in DMSO as previously noted for the unsubstituted derivatives. More remarkable was the fact that during this process the methylene sulfone byproduct **54** (R = CMe_3) precipitated completely from solution. The marked insolubility of **54** (R = CMe_3) in moist DMSO (ca. 1% H_2O) relative to the ready solubility of **53** (R = CMe_3) even in nonpolar solvents is of significant practical interest. If a DBD–TMOC protected peptide is used, the filtrate, following removal of **54** (R = CMe_3), contains a free peptide amine ready for further chain extension.[60]

In a number of the systems described above, the problem of separating the deblocking byproducts from the desired amine has arisen. In the case of the classic *t*-BOC system, the problem does not arise since all byproducts are volatile. Indeed, this is one of the great advantages of *t*-BOC protection. Can a comparable process be developed for a deblocking scheme based on β-elimination? One partially successful approach involved the specific base fluoride ion, with attack at silicon and concomitant fragmentation of a β-trimethylsilylethyl urethane **55** (eq 4).[61] For facile reactivity only so-called "naked fluoride" reagents are effective. Total volatility of *all* reagents involved, e.g., the deblocking base as well, is still not possible via β-elimination by any published technique.

$$\underset{\mathbf{55}}{Me_3SiCH_2CH_2OCONHR} \xrightarrow{F^-} Me_3SiF\uparrow + CH_2{=}CH_2\uparrow + OCONHR^- \xrightarrow{H^+} CO_2\uparrow + RNH_2 \quad (4)$$

An exceptional group of co-workers, cited in the references, have contributed handsomely to the chemistry described in these pages. Especially to be noted were the efforts of Dr. Grace Han, who was the first to experience the exciting deblocking chemistry of FMOC urethanes, and Dr. Beri Cohen, whose fine experimental hand and creative spirit did much to initiate the work on both the FAACST synthesis and the CLIMOC-based inverse Merrifield approach to peptide synthesis. We acknowledge also the warm hospitality of Professor H. Niedrich and Dr. M. Bienert and their colleagues at the Institut für Wirkstofforschung, Berlin, DDR, where this paper was written, and where Dr. M. Beyermann's experimental skill converted the skeleton of our FAACST synthesis into a reliable synthetic process. Financial support has been generously supplied by the National Institutes of Health, the U.S. National Academy of Sciences, and the Akademie der Wissenschaften, DDR. We also thank the Humboldt Universität, Berlin DDR, and the Technische Universität and Freie Universität, Berlin, BRD, for use of library facilities.

(57) (a) Bradamante, S.; Maiorana, S.; Mangia, A.; Pagani, G. *J. Chem. Soc. B* **1971**, 74. (b) Gaviraghi, G.; Pagani, G. *J. Chem. Soc., Perkin Trans. 2* **1973**, 50.

(58) DePuy, C. H.; King, R. W. *Chem. Rev.* **1960**, *60*, 431.

(59) Compare Voelter, W.; Müller, J. *Liebigs Ann. Chem.* **1983**, 248.

(60) Segev, D.; Gao, H.-S., University of Massachusetts, unpublished studies.

(61) Carpino, L. A.; Tsao, J.-H.; Ringsdorf, H.; Fell, E.; Hettrich, G. *J. Chem. Soc., Chem. Commun.* **1978**, 358.