

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

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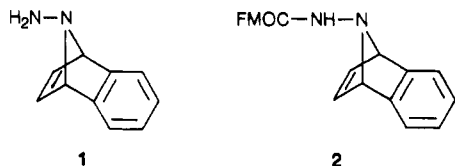
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Several years ago we presented an Account of our work on the acid-sensitive *t*-BOC amino-protecting group and also described the beginnings of a parallel search for a spectrum of base-labile protective functions.¹ During these early studies one group 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-phase⁵ 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



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

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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

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-

- (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-benzof[*l*]indenylmethyloxycarbonyl; DBD-TMOC, 2,7-di-*tert*-butyl-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. (f) 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) Joczzyk, 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. (c) Betner, I.; Földi, P. *Chromatography* 1986, 22, 381. (d) 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.

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 equilibrium³⁰ between DBF and the deblocking amine, a fact first noted accidentally when an analytically pure sample of the piperidine adduct 13 (X = CH₂) 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.

(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.

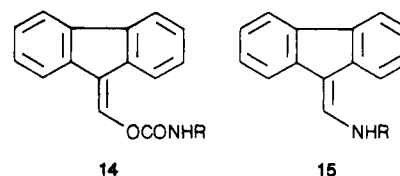
(29) The first description of the addition of cyclic secondary amines to a DBF derivative appears to be that of Quelet and Matarasso-Tchiroukhine, who noted the addition of piperidine and morpholine to 2,3,6,7-tetramethoxydibenzofulvene as well as the lack of reaction with diethylamine. See: (a) Matarasso-Tchiroukhine, 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.

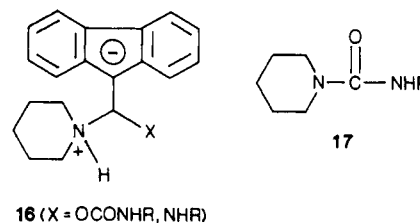
Special effects were also noted in the case of piperazine derivatives. In DMSO the adducts, whether from piperazine itself or its mono-*N*-methyl or *N*-phenyl derivatives, were very insoluble and precipitated from solution, thereby shifting the equilibrium toward complete scavenging within a relatively 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



compounds (R = C₆H₅) 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



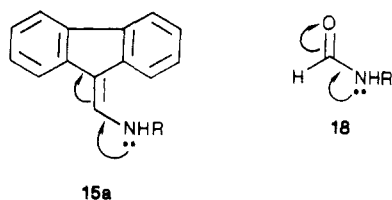
might have been predicted, a more facile competing reaction occurs in the case of 14 with conversion to urea 17 (R = C₆H₅). Here 14 exhibits reactivity typical of a vinyl ester. In the case of 15 no such competing reactions are possible. On the other hand, the N atom of such simple enamines is "protected" 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

(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.; Siegmeyer, 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.



as **16** would be hindered. In fact, when **15** ($R = p\text{-ClC}_6\text{H}_4^-$) 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 fluorenyl anion. However, deblocking can readily be achieved by mild acid hydrolysis ($\text{HCl}/\text{dioxane}/\text{H}_2\text{O}$)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 $\overline{\text{FM}}$ ³⁶ derivatives had any potential for the α - or side chain protection of amino acids. $\overline{\text{FM}}$ 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 $\overline{\text{FM}}$ derivatives, e.g. **22**. Upon storage some $\overline{\text{FM}}$ 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

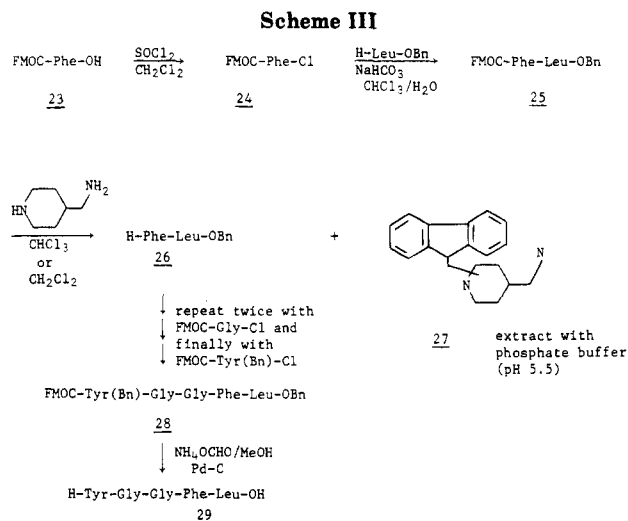
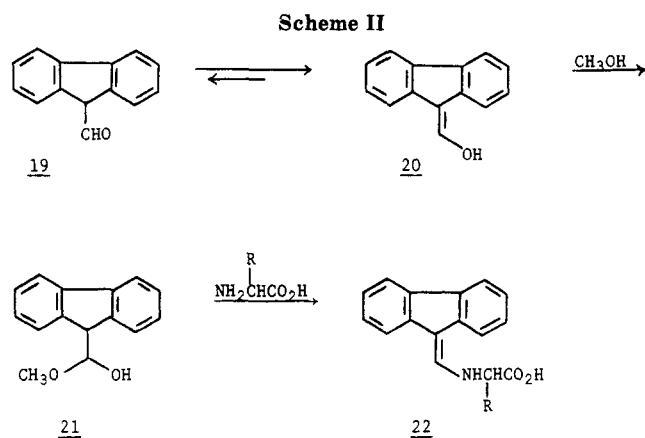
(35) Compare: (a) Dane, E.; Drees, F.; Konrad, P.; Dockner, T. *Angew. Chem.* **1962**, *74*, 873. (b) Dane, E.; Dockner, T. *Angew. Chem.* **1964**, *76*, 342.

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

(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-Aalae, 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.



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 technique⁴⁰). Short segments related to substance P bearing up to seven amino acid units have also been synthesized in one operation by this approach.⁴¹

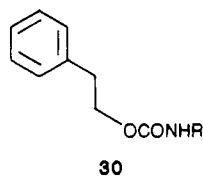
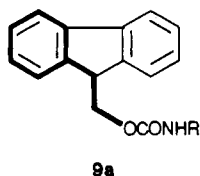
Protected pentapeptide **28** was converted to leucine-enkephalin (**29**) with removal of all protecting

(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.; Granitz, D.; Bienert, M.; Mehlig, 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 announcement² 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 Bodansky⁴⁴ and Sheppard⁴⁵ 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 arylethyl rather than arylmethyl 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-



deed found to be the case, and a thorough study of the β -phenylethoxycarbonyl ("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-workers⁴⁶ 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 accompanied by reduction of the phenacyl function to the β -phenylethyl function which survived further treatment (eq 2).

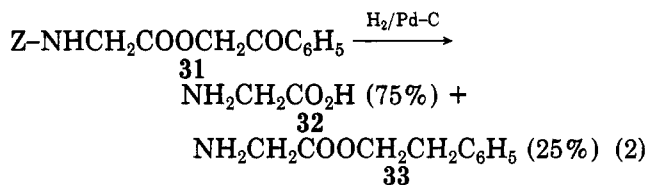
(42) Anwer, M. K.; Spatola, A. F. *Synthesis* 1980, 929; *Tetrahedron Lett.* 1981, 4369.

(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.; Wichienki, N. *J. Org. Chem.* 1986, 51, 4590.

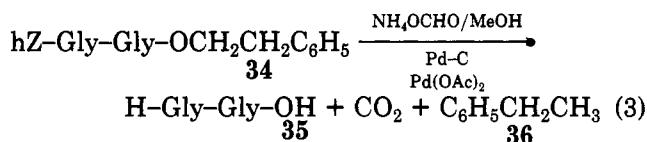
(44) Martinez, J.; Tolle, J. C.; Bodanszky, M. *J. Org. Chem.* 1979, 44, 3596.

(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.

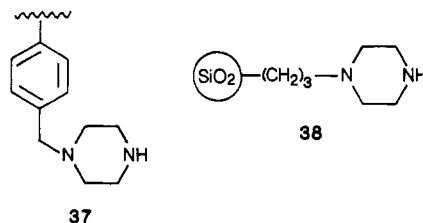


In a rerun of the Fmoc tale we now find that while β -phenylethyl esters are indeed less susceptible than β -phenylethyl carbamates, they can be deblocked under appropriate conditions (freshly precipitated catalysts, high catalyst/substrate ratios, warming and/or sonication, etc.). An example is outlined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



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-OCH₂C₆H₅ suffers loss only of the benzyl ester function whereas with catalytic transfer hydrogenolysis (Pd-C/Pd(OAc)₂/NH₄OCHO) 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 polystyrene⁴⁸ and silica³⁰ supports. Examples include 37 and 38. Initial studies with these



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

(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.

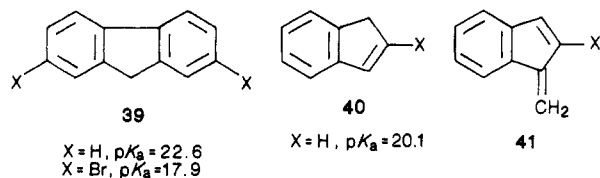
(49) For reviews, see: (a) Fridkin, M. In *Peptides. Analysis. Synthesis. Biology*; Gross, E., Meienhofer, J., Eds.; Academic: New York, 1980; Vol. 2, p 333. (b) Patchornik, A.; Cohen, B. J. In *Perspectives in Peptide Chemistry*; Eberle, A., Geiger, R., Wieland, T., Eds.; Karger: Basel, Switzerland,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

Table II.
Reaction X-MOC-NHC₆H₄Cl-*p*/C₉H₁₁N/CH₂Cl₂

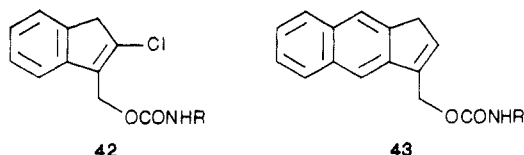
	FMOC	IMOC	CLIMOC
k_{rel} (deblocking)	1	10	200
k_{rel} (scavenging)	1	250	5000

these incomplete scavenging processes.

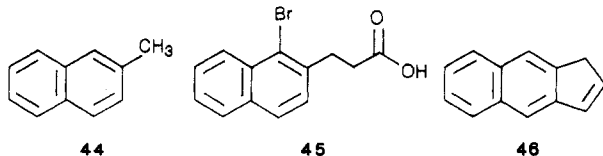
Since numerous attempts to improve the scavenging efficiency of the polymeric reagents failed, a second approach involving changes in the protecting group was examined. Since the ease of both deblocking and scavenging should correlate with the acidity of the fluorene element built into the urethane system, 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



was more satisfactory, the greater acidity of **40** assuring 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 IMOC³ 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 CLIMOC³ and BIMOC³ derivatives **42** and **43** overcame this problem.



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-



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 ketone⁵³ 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.

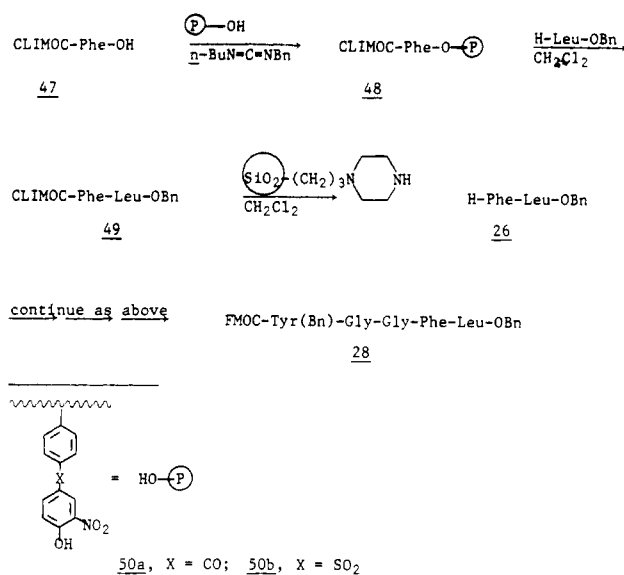
(51) Cohen, B. J., University of Massachusetts, unpublished studies.

(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 **48** 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 work⁵⁶ dealing with the fundamental question of the electronic character of the theoretically interesting thiirene dioxides **51**.

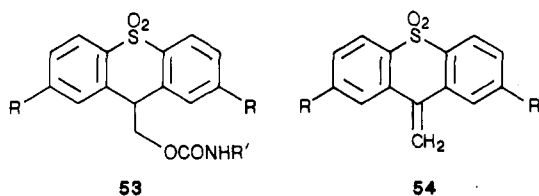


This work led to conjectures on the high stability to be expected of analogous 6- π -electron anions **52**. Literature confirmation⁵⁷ of these speculations induced us to

(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

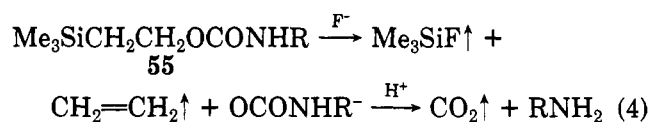


carbamates are deblocked readily by pyridine whereas FMOC derivatives are stable in this solvent over a period of several days. More interestingly, these urethanes underwent deblocking simply by warming to 40–45° in the dipolar aprotic solvent DMSO. In nonpolar solvents such as benzene, tetrahydrofuran, 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 potentially⁵⁹ greater solubility the 2,7-di-*tert*-butyl derivatives **53** (R = CMe₃) were examined. As expected, these DBD-TMOC³ 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 = CMe₃) precipitated completely from solution. The marked insolubility of **54** (R = CMe₃) in moist DMSO (ca. 1% H₂O) relative to the ready solubility of **53** (R = CMe₃) even in nonpolar solvents is of significant practical interest. If a DBD-TMOC protected peptide is used, the filtrate, following removal of **54** (R = CMe₃), 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.



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. Beyersmann'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 und 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.