New Family of Base- and Nucleophile-Sensitive Amino-Protecting Groups. A Michael-Acceptor-Based Deblocking Process. Practical Utilization of the 1,1-Dioxobenzo[*b*]thiophene-2-ylmethyloxycarbonyl (Bsmoc)† Group

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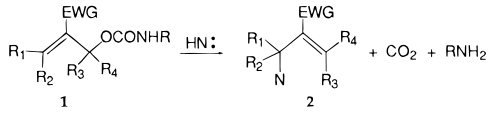
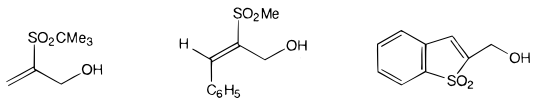
Received April 30, 1997

Base-sensitive amino-protecting groups such as Fmoc and related functions owe their reactivity to facile β-elimination processes. A recent attempt to apply Fmoc protection to an inverse Merrifield synthesis of peptide segments foundered when resins bearing cyclic secondary amino functions were found to be inefficient scavenging agents for the β-elimination byproduct dibenzofulvene (DBF).[1] These disappointing results triggered a search for a fundamentally different type of amino-protecting group in which the deblocking event is simultaneously a scavenging event. Appropriate positioning of a urethane function relative to a Michael acceptor accomplished the desired result (eq 1).[2]



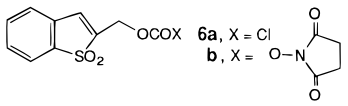
1 2 (1)

As expected for a Michael-type addition process, significant rate variation accompanied changes in the nature of the electron-withdrawing group (EWG), the presence or absence of substituents at the β-position, and the chemical nature and/or steric constraints built into the deblocking agent. For model substrates **1** ($R_1 = R_2 = R_3 = R_4 = H$; $R = -C_6H_4Cl$-*p*) the following order of reactivity toward a number of cyclic secondary amines (piperidine, morpholine, etc.) was established for various EWGs: $C_6H_5SO_2$ > Me_3CSO_2 > EtOCO > C_6H_5SO > *p*-$O_2NC_6H_4-$. In view of its lack of significant alternate reactivity, its position at the head of this series, and the ready availability of key precursors, the sulfone unit, built into an allylic alcohol **3**, was selected for initial evaluation.[3] Although **3** could be

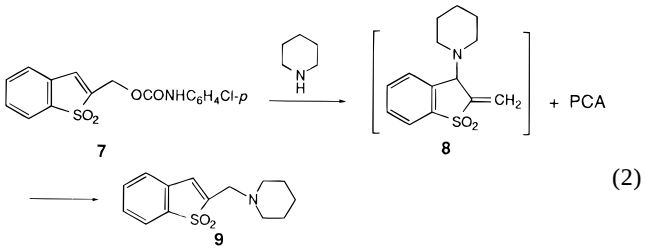


3 4 5

converted to the appropriate protected 2-*tert*-butylsulfonyl-2-propenoxycarbonyl (Bspoc−) amino acids which were successfully used in the assembly of model peptides, the protected substrates were in some cases subject to premature or competitive deblocking.[4,5] All such problems were eliminated by switching to a Michael acceptor bearing a β-substitutent such as one based on alcohols **4** or **5**. While the β-substitutent protects the system from premature deblocking, reactivity is still many times greater than for the Fmoc system and, in addition, the presence of the styrene chromophore [for **9** (MeOH), λ_{max} 310.5 nm (ϵ 3048)] allows for facile UV detection, tracking, and quantitation.[6,7] Alcohol **4** was obtained by hydrolysis of the allyl bromide, whereas **5** was obtained from commercially available benzo[*b*]thiophene by hydroxymethylation (*n*-BuLi/$(CH_2O)_n$; 80%) followed by peracid oxidation (80%). Because of its desirable properties and greater availability, most work to date has concentrated on the 1,1-dioxobenzo[*b*]thiophene-2-ylmethyloxycarbonyl (Bsmoc) group derived from **5**. Acylation of amino acids could be effected by the Bolin method[8] via chloroformate **6a** or via the *N*-hydroxysuccinimide ester **6b**.[9]



NMR studies in $CDCl_3$ clearly reveal the high reactivity of the Bsmoc residue and provide evidence for a reactive intermediate generated by initial Michael-like attack by piperidine. Thus treatment of **7** with 2 equiv of piperidine is followed by



(2)

complete liberation of *p*-chloroaniline (PCA) within 3−5 min and concomitant formation of a labile intermediate whose ^{1}H NMR spectrum is consistent with structure **8**. Adduct **8** decays over the next 8−10 min to give the final stable deblocking product **9**.

† Common name: benzo[*b*]thiophenesulfone-2-methyloxycarbonyl.

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(1) More appropriate were indene-derived Fmoc-analogous functions, yet even in these cases, small amounts of the analogous fulvene byproducts remained unscavenged. See: Carpino, L. A.; Cohen, B. J.; Lin, Y.-Z.; Stephens, K. E., Jr.; Triolo, S. A. *J. Org. Chem.* **1990**, *55*, 251.

(2) Previously, related structural elements have been built into substrates studied in connection with protein cross-linking and so-called multiple coupling agents. See: (a) Mitra, S.; Lawton, R. G. *J. Am. Chem. Soc.* **1979**, *101*, 3097. (b) Seebach, D.; Knochel, P. *Helv. Chim. Acta* **1984**, *67*, 261. (c) Knochel, P.; Normant, J. F. *Tetrahedron Lett.* **1985**, 425.

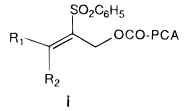
(3) Alcohol **3** was obtained by hydrolysis of the corresponding bromide[2c] in wet methanol in the presence of NaOCHO according to a reported method (Harris, M.; Bull, M. J. *Synth. Commun.* **1985**, *15*, 1225).

(4) Potential problems associated with premature deblocking of the Fmoc group have been evaluated previously. See: Bodanszky, M.; Deshmane, S. S.; Martinez, J. *J. Org. Chem.* **1979**, *44*, 1622.

(5) For example, in the acylation of H-Phe-OCMe$_3$ with Bspoc-Phe-Cl, the desired dipeptide (94%) was accompanied by the ester (2%) formed by competing attack at the olefinic residue. No comparable premature deblocking occurred in the case of the Bsmoc group.

(6) Comparison of deblocking rates under identical conditions by NMR analysis in $CDCl_3$ for Bspoc-PCA and Bsmoc-PCA using 3 equiv of amine showed the following relative rates (time in minutes for complete deblocking/scavenging). Piperidine: Bspoc, <5 min; Bsmoc, <5 min. Morpholine: Bspoc, <5 min; Bsmoc, 45 min. *cis*-2,6-Dimethylpiperidine: Bspoc, 45 min; Bsmoc, no reaction. Comparison of the deblocking of Bsmoc-PCA with that of Fmoc-PCA showed the former underwent complete deblocking/scavenging with 2 equiv of piperidine in $CDCl_3$ within 40 min, whereas the latter required over 300 min for deblocking and no scavenging had occurred at this point.

(7) For a series of four 2-phenylsulfonylpropenoxycarbonyl systems **i** differently substituted at the β-position complete deblocking by morpholine in $CDCl_3$ solution (0.02 M) gave the results indicated (time in minutes for complete reaction).



i

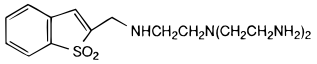
Cpd	Time
(a) $R_1 = R_2 = H$	<5
(b) $R_1 = C_6H_5$, $R_2 = H$(Z)	18
(c) $R_1 = H$, $R_2 = C_6H_5$(E)	38
(d) $R_1 = R_2 = C_6H_5$	no reaction

(8) Bolin, D. R.; Syturu, I.; Humeic, F.; Meienhofer, J. *Int. J. Pept. Protein Res.* **1983**, *33*, 353.

(9) Compare: Paquet, A. *Can. J. Chem.* **1982**, *60*, 976.

S0002-7863(97)01369-3 CCC: $14.00

Most common amino acids have been converted to their Bsmoc derivatives[10] and used in peptide assembly. Generally the corresponding acid fluorides or in situ activation via ammonium (guanidinium, formamidinium) or phosphonium salts were used for the coupling step. Both solution and solid phase syntheses were executed. Because of its hydrophilic character, the Bsmoc residue made possible a unique simplification of the recently described technique for the rapid solution synthesis of short peptide segments based on Fmoc chemistry.[11] With the latter, the deblocking/scavenging step is carried out with 4-(aminomethyl)piperidine or preferably tris(2-aminoethyl)amine (TAEA) with buffer extractions at pH 5.5 being used to remove the byproduct Fm adduct. By substituting the Bsmoc for the Fmoc group, it is possible to dispense with the phosphate buffer and simply wash with water or saturated sodium chloride solution since the TAEA adduct **10** is highly soluble in water.

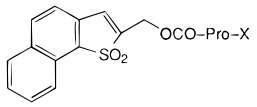


10

In addition, the amount of excess TAEA used could be reduced with consequent reduction in loss of material during aqueous washings. Coupling could be carried out under two-phase ($DCM/H_2O/NaHCO_3$) or one-phase (DCM/DIEA) conditions. In the latter case TAEA is added directly to the reaction mixture once coupling is over (TLC). Ionic and highly polar byproducts from the coupling step are then washed out along with adduct **10**. This technique was followed in the synthesis of several short peptides including Bsmoc-Tyr(*t*-Bu)-Ile-Asp(O-*t*-Bu)-Gly-O-*t*-Bu (**11**) (87%), Bsmoc-Tyr(*t*-Bu)-Gly-Gly-Phe-Leu-O-*t*-Bu (**12**) (49%), Bsmoc-Phe-Phe-Val-Gly-Leu-Met-OBn (**13**) (37%), and Fmoc-Ile-Thr(*t*-Bu)-Arg(Pbf)-Gln(Trt)-Arg(Pbf)-Tyr(*t*-Bu)-ODcpm[12] (**14**) (40.3%) (Dcpm = dicyclopropylmethyl). Cycle times were approximately 1 h. Classic methods of solution synthesis may also benefit from a change to Bsmoc chemistry since the corresponding Fmoc-based syntheses are sometimes compromised by the unpredictable tendency of DBF to undergo polymerization.

Bsmoc amino acids were also used in standard solid-phase syntheses, using acid fluoride, HBTU,[13] or HATU[13] coupling techniques. Examples include the acyl carrier protein fragment 65−74 (ACP decapeptide) magainin-II amide and alamethicin amide. The deblocking step was routinely carried out with 2, 3, or 5% piperidine rather than the 20% piperidine commonly used for Fmoc removal, thereby allowing reduction or elimination of base-catalyzed side reactions. Thus, in the assembly of Bz-Val-Lys(BOC)-β-Asp(α-O-*t*-Bu)-Gly-Tyr(*t*-Bu)-Ile-OH (**15**) according to the normal Fmoc protocol (20% piperidine/7 min), succinimide formation occurred due to the presence of the sensitive Asp-Gly sequence to the extent of 11.6%.[14] Under normal Bsmoc conditions (2% piperidine/7 min) 4.8% of the imide was observed. An additional advantage of Bsmoc over Fmoc assembly was noted in the case of pentapeptide H-Tyr-Aib-Aib-Phe-Leu-OH (**16**),[15] where the difficult Aib−Aib coupling was favored by about 13% in the Bsmoc case (ratio **16**/des-Aib-**16** of 54/46 vs 41/59 for the Fmoc case).

In a few cases among the Bsmoc-protected proteinogenic amino acids and their acid fluorides, the products were oily or amorphous materials not obtainable in crystalline form. For such systems, it appeared generally possible to obtain crystalline intermediates by substitution of the Bsmoc function by an equivalent residue of higher molecular weight. Thus neither Bsmoc-Pro-OH nor Bsmoc-Pro-F could be obtained in solid form, whereas the related 1,1-dioxonaphtho[1,2-*b*]thiophene-2-ylmethyloxycarbonyl (Nsmoc) derivatives **17** are nicely



17a, X = OH
b, X = F

Bsmoc−(Leu)$_3$-OFm
18

crystalline. The Bsmoc and Nsmoc derivatives are deblocked at comparable rates.

In view of the high base-sensitivity of the Bsmoc residue, it was possible to deblock this system selectively in the presence of Fmoc or Fm protection. As an example, the tripeptide **18** could be assembled from Bsmoc-Leu-OFm by treatment with 2% TAEA/DCM followed by coupling with Bsmoc-Leu-F and subsequent repetition of the same procedure. On the other hand, precisely the opposite selectivity could be achieved upon deblocking via 10% *N*-methylcyclohexylamine or diisopropylamine in DCM. Such selective removal of the Fm residue, which might at first seem surprising, is illustrative of the importance of steric factors in additions to a Michael acceptor.[16]

Acknowledgment. We are indebted to the National Science Foundation (NSF CHE-9003192), the National Institutes of Health (GM-09706), and Research Corporation Technologies for support of this work. The National Science Foundation is also thanked for grants used to purchase the high-field NMR spectrometers used in this research.

Supporting Information Available: Experimental procedures and characterizing spectral data for the utilization of Bsmoc protection and HPLC and MS data for Bsmoc-derived peptides (38 pages). See any current masthead page for ordering and Internet access instructions.

JA9713690

(10) Bsmoc amino acids are available from Oryza Laboratories, Inc., Chelmsford, MA 01824.

(11) For a review and earlier references, see: Carpino, L. A.; Beyermann, M.; Wenschuh, H.; Bienert, M. *Acc. Chem. Res.* **1996**, *29*, 268.

(12) For the corresponding Fmoc-based synthesis (20.9%), see: Carpino, L. A.; Chao, H.-G.; Ghassemi, S.; Mansour, E. M. E.; Riemer, C.; Warrass, R.; Sadat-Aalaee, D.; Truran, G. A.; Imazumi, H.; El-Faham, A.; Ionescu, D.; Ismail, M.; Kowaleski, T. L.; Han, C.-H.; Wenschuh, H.; Beyermann, M.; Bienert, M.; Shroff, H.; Albericio, F.; Triolo, S. A.; Sole, N. A.; Kates, S. A. *J. Org. Chem.* **1995**, *60*, 7718. The reported yields are calculated on the basis of the fully protected hexapeptide. In both syntheses the final step involved coupling of Fmoc-Ile-F so that the same product would result. The increased yield in the Bsmoc case is attributed mainly to the need to use less TAEA which results in less loss of the growing peptide into the aqueous phase during the extraction process. Yields reported for peptides **11**−**13** may not be representative since these syntheses were carried out with a greater excess of TAEA than needed.

(13) HATU = *N*-[[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-yl]methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide. HBTU = *N*-[(1*H*-benzotriazol-1-yl)(dimethylamino)methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide. For a description of the current view of the structure of these coupling reagents, see: Carpino, L. A.; El-Faham, A.; Albericio, F. *J. Org. Chem.* **1995**, *60*, 3561.

(14) The high sensitivity of model hexapeptide **15** toward base-catalyzed cyclization has been described previously: Lauer, J.; Fields, C. G.; Fields, G. B. *Lett. Pept. Sci.* **1994**, *1*, 197.

(15) Carpino, L. A.; El-Faham, A.; Minor, C. A.; Albericio, F. *J. Chem. Soc., Chem. Commun.* **1994**, 201.

(16) The sensitivity of the Bspoc and Bsmoc residues was also examined under conditions commonly used for the deblocking of other standard amino protecting groups. Stability of the Bspoc group was shown toward either neat TFA or saturated HCl in HOAc for periods up to 24 h at room temperature. Degradation however occurred in saturated HBr in HOAc. In dimethylamine-free DMF no reaction occurred for the Bsmoc residue up to at least 24 h. Under the conditions of catalytic hydrogenolysis (H_2/Pd−C) the Bspoc function is, as expected, subject to rapid deblocking. Both *tert*-butyl 2-propenyl sulfone and *tert*-butyl isopropyl sulfone were observed as byproducts, the latter on extended treatment. For Bsmoc derivatives no reaction has yet been observed on attempted catalytic hydrogenolysis.[17] Bsmoc derivatives were also found to be stable toward tertiary amines (pyridine, DIEA) as well as HOBt/DIEA (1:1) for at least 24 h. On the other hand, base-catalysed mercaptan deblocking of the Bsmoc group occurs readily. Thus treatment of the stable solution of **7** and 1 equiv of α-toluenethiol in MeOH-d_4 with 5−10 mol % of DIEA causes rapid deblocking.

(17) Negative reactions under conditions of catalytic hydrogenolysis must be interpreted with caution. See: Carpino, L. A.; Tunga, A. *J. Org. Chem.* **1986**, *51*, 1930.