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The 2,2,4,6,7-Pentamethyldihydrobenzofuran-5-sulfonyl Group (Pbf) as Arginine Side Chain Protectant[1]

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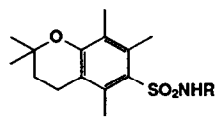
Abstract: The 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl residue (Pbf) is more easily deblocked by trifluoroacetic acid (TFA) than the corresponding Pmc analog and can be used efficiently in the synthesis of arginine-containing peptides.

With the recent development of the TFA-sensitive 2,2,5,7,8-pentamethylchroman-6-sulfonyl group (Pmc) in which the key structural element is the chroman unit pictured in **1**, Ramage and coworkers[2] made a definitive contribution to the problem of handling arginine in 9-fluorenylmethyloxycarbonyl (FMOC)-based solid phase peptide synthesis by recasting the alkoxy residue of the widely used 4-methoxy-2,3,6-trimethylbenzenesulfonyl (Mtr) group[3] (see structure **2**) in the form of a cyclic ether. No rationale for choosing the 6-ring chroman over



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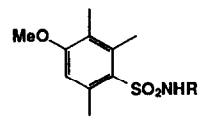
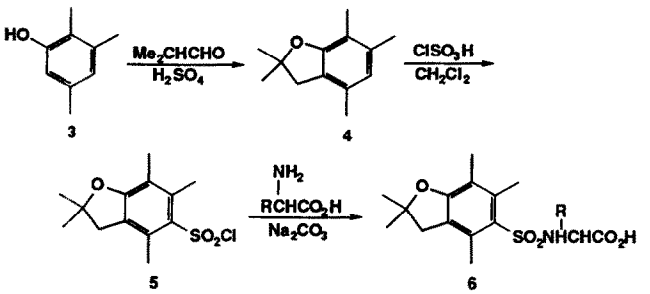


2

the more rigid 5-ring analog was presented, although a subsequent paper,[3] which appeared after the present study was begun, described extensive X-ray crystallographic comparisons on model compounds as the deciding factor. A more recent report[5] from another laboratory described an unsuccessful search for additional acid lability by further manipulation of the 6-ring motif. In the middle fifties Baddeley[6] established experimentally that benzannelated 5-ring cyclic ethers are more electron-donating than the 6-ring analogs, and on this basis we initiated a study of the dihydrobenzofuran counterparts of the Pmc system in order to compare TFA cleavage rates.

At the time this work was begun, it was not related to arginine side chain protection but was rather part of a search for a TFA-sensitive arenesulfonyl amino acid protectant which would make possible the synthesis of stable protected amino acid chlorides.[7,8] Although a selection of such acid chlorides derived from the Pmc residue was readily synthesized, it was surprising to find that the Pmc function could not be removed from the α-position by means of neat TFA at room temperature over a period of several hours. While deblocking could be achieved in the presence of 10% dimethyl sulfide or thioanisole, these conditions do not leave benzyl-based

protectants unaffected.[2,4] In order to explore the implications of Baddeley's work, the 5-ring analogs were next investigated. Synthesis of the precursor cyclic ether **4** was easily carried out by modification of the method of



Martini, Franke and Singerman.[9] Chlorosulfonation of **4** gave Pbf-Cl **5** in 51% yield and the α-protected amino acid derivatives **6** were obtained normally. The new sulfonyl function was again found not to be deblocked by TFA. Since the related tosyl function is stable to liquid HF when situated at the α-position but readily deblocked by the same reagent when found on the side chain of arginine,[10] the corresponding guanidino derivatives bearing Pmc and Pbf groups were compared. The synthesis of FMOC-Arg(Pbf)-OH was similar to that described for the Pmc analog.[2,4] Removal of the Pbf function was faster than that of the corresponding Pmc system at temperatures between ambient and 37°C by factors of 1.2 - 1.4 for TFA/H_2O (95/5; 80/20). Rough kinetic studies[11] made use of HPLC techniques to follow the disappearance of Z-Arg(Pmc)-OH or Z-Arg(Pbf)-OH into Z-Arg-OH.

Similar reactivity enhancements were observed in the case of solid phase syntheses of arginine-containing peptides. For example, assembly of model hexapeptide **7**[12] via FMOC-Arg(Pbf)-OH or FMOC-Arg(Pmc)-OH

Ac-Trp-Arg-Arg-Arg-Arg-Val-OH

7

H-Gly-Arg-Ala-Asp-Ser-Pro-Lys-NH_2

8

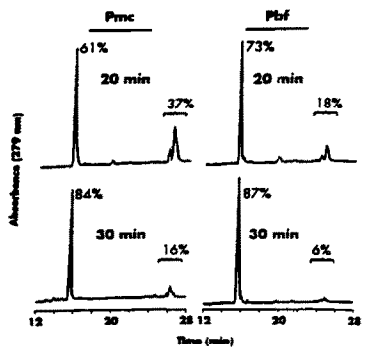


Fig. I Comparison of crude peptide **7** released from the resin during deblocking of X = Pmc or Pbf in the case of Ac-Trp(BOC)-Arg(X)-Arg(X)-Arg(X)-Arg(X)-Val-PAC-PEG-PS by Reagent R

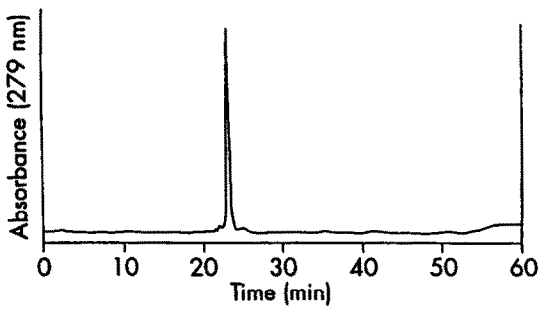


Fig. II. HPLC chromatogram of crude peptide **13** after 2-h cleavage with Reagent R Reversed phase C-18 column Elution with a linear gradient over 30 min of 0.1% TFA in CH_3CN and 0 1% TFA in H_2O from 1 9 to 1:1, flow rate 1.0 mL/min

showed that if the deblocking reaction using Reagent R[13] is quenched after 20 and 30 min, significantly more undeblocked and/or partially deblocked material remained in the latter case. This is illustrated in Fig. I for syntheses carried out with tryptophan introduced via FMOC-Trp(BOC)-OH.[14] For the 7-mer **8** half-lives for deblocking of the Pbf and Pmc runs were 8 and 13 min respectively.

Other peptides which were assembled in good yield and purity by automated solid phase techniques using Pbf protection for arginine include **9-13**. A typical example of the quality of the crude peptide is shown in Fig. II for the 17-mer **13**.[18]

H-Arg-Lys-Asp-Val-Tyr-NH_2
9[15]

H-Tyr-Arg-Gln-Arg-Tyr-NH_2
10[16]

H-Tyr-Gly-Lys-Arg-NH_2
11[16]

H-Gly-Asn-Arg-Val-Arg-Arg-Ser-Val-Gly-Ser-Ser-Leu-Lys-Cys-NH_2
12[17]

H-Tyr-Pro-Ser-Lys-Aca-Arg-His-Tyr-Ile-Asn-Leu-Ile-Thr-Arg-Gln-Arg-Tyr-NH_2
13[16]

ACKNOWLEDGMENT

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REFERENCES AND NOTES

1. All new compounds were characterized on the basis of elemental analyses (± 0.3% C, H, N) and consistent IR and ^{1}H NMR spectral data. Crude peptides were examined by HPLC (e. g., Fig. II in the case of **13**). All peptides showed the expected amino acid analysis and molecular weights as determined by the matrix-assisted laser desorption technique.
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7. The Mtr group has previously been used for α-protection but is known to be stable to TFA. See Wakimasu, M.; Kitada, C.; Fujino, M. *Chem. Pharm Bull.* **1982**, *30*, 2766.
8. *t*-Butyloxycarbonyl (BOC) amino acid chlorides are unstable at ordinary temperatures, being converted to the corresponding Leuchs anhydrides [Wilder, R.; Mobashery, S. *J. Org. Chem.* **1992**, *57*, 2755]

Subsequently, with the synthesis of BOC amino acid fluorides, the immediate need for such a TFA-sensitive α-arenesulfonyl residue disappeared. See Carpino, L. A.; Mansour, E. M. E.; Sadat-Aalaee, D. *J Org. Chem.* **1991**, *56*, 2611.

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11. Reaction conditions: 16 mg of the appropriate Pmc or Pbf derivative was treated with TFA/H_2O (80/20) at 37°C. At regular intervals aliquots were diluted four fold with H_2O and analyzed by HPLC. Mobile phase: H_2O-CH_3CN (A-B) containing 0.1% TFA. Gradient: 13% B to 50% B, 15 min; 50% B, 13 min Flow: 3 mL/min. The average of two runs was used for comparison. A preparative run in the Pbf case led to the isolation of Z-Arg-OH•TFA in 89% yield. In an NMR experiment at 37°C in TFA-d Z-Arg(Pbf)-OH underwent complete deblocking within 15 min whereas reaction with Z-Arg-(Pmc)-OH was still incomplete after 30 min.
12. Compare Rinıker, B.; Hartmann, A., in *Peptides. Chemistry, Structure, and Biology. Proc. of the 11th Am Pept. Symp.* Rivier, J. E.; Marshall, G. R., Eds.; ESCOM, Leiden, 1990, p. 950.
13. Reagent R contains CF_3CO_2H/thioanisole/1,2-ethanedithiol/anisole (90/5/3/2). See Albericio, F ; Kneib-Cordonier, N.; Biancalana, S.; Gera, L.; Masada, R. I.; Hudson, D.; Barany, G. *J. Org. Chem.* **1990**, *55*, 3730.
14. Franzén, H.; Grehn, L.; Ragnarsson, U. *J. Chem. Soc., Chem. Commun.* **1984**, 1699; (b) White, P., in *Peptides. Proceedings of the 12th American Peptide Symposium*, Smith, J. A., Rivier, J. E , Eds., ESCOM, Leiden, 1992, p. 537. This derivative avoids arenesulfonation of tryptophan.
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18. All peptides were synthesized via FMOC/*t*-Bu chemistry on a Millipore 9050-plus automated peptide synthesizer. Acylation was carried out with 5 eqs of the FMOC amino acid, preactivated with PyBOP/HOBt/DIEA or with Pfp or Dhbt esters/HOBt, using 60-min coupling times. FMOC deblocking was carried out with 2% DBU/2% piperidine in DMF for 6 min. Treatment with Reagent R for 2 h was used to deblock and remove the peptide from the resin. Crude peptides were examined by HPLC (e.g. Fig. II in the case of **13**).

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