Tetrahedron Letters,Vol.29,No.34,pp 4341-4344,1988 0040-4039/88 $3.00 + .00
Printed in Great Britain Pergamon Press plc

APPLICATION OF THE N^G-(2,2,5,7,8-PENTAMETHYLCHROMAN-6-SULPHONYL) DERIVATIVE OF FMOC-ARGININE TO PEPTIDE SYNTHESIS

J. Green. O.M. Ogunjobi, R. Ramage* and A.S.J. Stewart

Department of Chemistry, University of Edinburgh,
West Mains Road, Edinburgh, EH9 3JJ

S. McCurdy and R. Noble

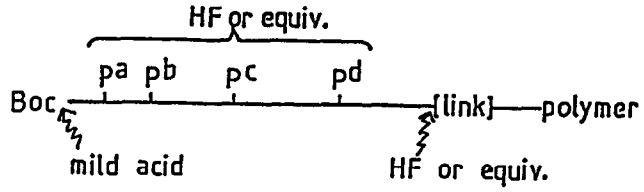
Applied Biosystems Inc., 850 Lincoln Centre Drive,
Foster City, California 94404, USA

Summary. The trifluoroacetic acid labile pentamethylchromanylsulphonyl protecting group for the guanidino group of arginine has been used in conjunction with the base-labile Fmoc N^{α} protecting group for the synthesis of arginine-containing peptides.

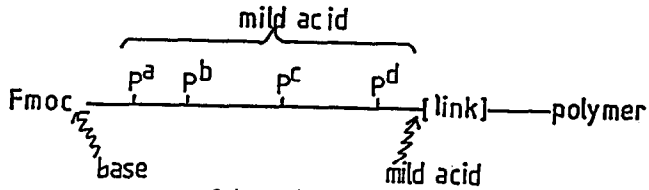
At the present time there are two main strategies for synthesis of peptides by the Merrifield Solid Phase Method, the first of which is essentially that due to Merrifield[1] where the repetitive N^{α} protection/deprotection processes depend upon mild acid cleavage (trifluoroacetic acid, TFA) of the N^{α}-t butyloxycarbonyl group (Boc). A consequence of this is that the side chain protection protocol and peptide-linker attachment to the resin rely upon more acid-stable groups which require strong acid (HF or CF_3SO_3H) for final liberation of the peptide product (Scheme 1). The other approach to Solid Phase Synthesis of Peptides (SPPS) utilises the base-labile 9-fluorenylmethyloxycarbonyl group (Fmoc) for N^{α} protection which was designed by Carpino[2] and applied elegantly independently, by Meienhofer[3] and Sheppard[4] to SPPS. This strategy involves the use of side chain protecting groups which may be cleaved by mild acid (TFA) (Scheme 2). Furthermore recent work on the design of linkers, which release the final peptide product by mild acid[5] or fluoride ion,[6] adds a further dimension to the N^{α}-base labile strategy which will undoubtedly be of great value in future synthesis of large, protected peptide fragments.

The majority of α-amino acid side chain protecting groups utilised in the Fmoc strategy for SPPS are easily deprotected with 95% TFA in 1.5 hours at room temperature. One exception is the relative difficulty with which the guanidine side chain functionality of arginine (Arg) is deprotected by TFA when employing the 4-methoxy-2,3,6-trimethylbenzenesulphonyl (Mtr) derivative.[7] This is especially problematic when multi-arginine residues are present in the target peptide. It is therefore most important that a

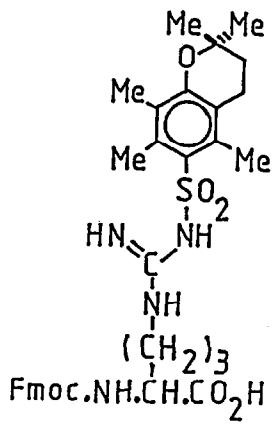
TFA-labile guanidino protecting group is designed for the crucial Arg residues in order to derive most benefit from the Fmoc strategy of SPPS.



Scheme 1



Scheme 2



1

Consideration of a series of structures of arylsulphonyl derivatives of Arg, with respect to acid lability, showed clearly the effects of substituents on the benzene ring of N^G-Arg-sulphonamides in controlling the acid lability of such systems. This led us to develop[8] the N^G-(2,2,5,7,8-pentamethylchroman-6-sulphonyl, Pmc) derivative as a TFA-labile protecting group for Arg. The initial study involved Z.Arg(Pmc).OH in the synthesis of C-terminal fragments of ubiquitin and we have subsequently extended this to Fmoc.Arg(Pmc).OH (1) for use in SPPS.

Z.Arg(Pmc).OH[¶] was hydrogenolysed (Pd/C in methanol) to afford H.Arg(Pmc).OH which was converted to Fmoc.Arg(Pmc).OH (1)[9] using 9-fluorenylmethyl succinimidyl carbonate[10] using Na_2CO_3 in aqueous DMF. We have utilised Fmoc.Arg(Pmc).OH (1) in the synthesis of the peptides (2-7) illustrated using the TFA labile Wang linker[5] on polystyrene resin. The Fmoc.Arg(Pmc).OH unit could be incorporated efficiently into the peptide synthesis (ninhydrin test and amino acid analysis) using the symmetrical

anhydride or DCCI/HOBt procedures employing Applied Biosystems Models 430 and 431A Peptide Synthesisers. On completion of the syntheses using Fmoc α-amino acids the side chain protection *and* cleavage from the resin was achieved in 1.5 hours using 95% TFA, 3% anisole, 1% ethanedithiol and 1% ethyl methyl sulphide. The cleavage mixture was filtered, evaporated *in vacuo* with the addition of CH_2Cl_2 to aid the removal of TFA, precipitated with ether, then subjected to G25 or G50 gel filtration followed by preparative HPLC.

H.Ser.Tyr.Ser.Met.Glu.His.Phe.Arg.Trp.Gly.OH
2 ACTH (1-10)

H.Arg.Gln.Gln.Gly.Glu.Ser.Asn.Gln.Glu.Arg.Gly.Ala.Arg.Ala.Arg.Leu.OH
3 GRF (29-44)

H.Ala.Glu.Lys.Lys.Asp.Glu.Gly.Pro.Tyr.Arg.Met.Glu.His.Phe.Arg.Trp.Gly.
Ser.Pro.Lys.Asp.OH
4 β-MSH ($desPro^{19}$)

Glp.Met.Ser.Tyr.Gly.Tyr.Asp.Glu.Lys.Ser.Ala.Gly.Val.Ser.Val.Pro.Gly.
Pro.Met.Gly.Pro.Ser.Gly.Pro.Arg.Gly.Leu.Hyp.Gly.OH
5 Nα1(I) Collagen Telopeptide (extended with 1-13 rat collagen)

H.Gly.Ala.Hyp.Gly.Ala.Asp.Gly.Pro.Ala.Gly.Ala.Hyp.Gly.Thr.Pro.Gly.Pro.
Gln.Gly.Ile.Ala.Gly.Gln.Arg.Gly.Val.Val.Gly.Leu.Hyp.Gly.Gln.Arg.Gly.OH
6 Collagen α1(I) (757-790)

H.Lys.Gln.Leu.Glu.Asp.Gly.Arg.Thr.Leu.Ser.Asp.Tyr.Asn.Ile.Gln.Lys.Glu.
Ser.Thr.Leu.His.Leu.Val.Leu.Arg.Leu.Arg.Gly.Gly.OH
7 Ubiquitin (48-76)

It was found that in peptides having more than one Arg residue the deprotection proceeded normally, although the presence of tryptophan (Trp) could cause problems associated with undesired trapping of the intermediate formed during the Pmc deprotection,[11] in an analogous fashion to the Mtr deprotection,[12] hence care must be taken to have scavengers present. Comparative studies using Mtr protected Arg showed the peptide products to have residual Mtr protection after TFA treatment under conditions which completely cleave Pmc groups.

Acknowledgment

We thank Applied Biosystems Inc, SERC, Merck, Sharp & Dohme and Wendstone Chemicals for financial support and S. Orszulik (Wendstone), K. Shaw and B. Whigham for invaluable technical support.

References and Notes

¶ All compounds quoted in the text were analytically pure with physical data consistent with the structures given.

1. R.B. Merrifield, *J. Am. Chem. Soc.*, 85, 2149 (1963).
2. L.A. Carpino and G.Y. Han, *J. Org. Chem.*, 37, 3404 (1972).
3. C.D. Chang and J. Meienhofer, *Int. J. Pept. Prot. Res.*, 11, 246 (1978).
4. E. Atherton, D. Harkiss and R.C. Sheppard, *Bioorganic Chem.*, 8, 351 (1979).
5. S.S. Wang, *J. Am. Chem. Soc.*, 95, 1328 (1973); R.C. Sheppard and B.J. Williams, *Int. J. Pept. Prot. Res.*, 15, 451 (1982).
6. D.G. Mullen and G. Barany, *Tetrahedron Lett.*, 28, 491 (1987); R. Ramage, C.A. Barron, S. Bielecki and D.W. Thomas, *Tetrahedron lett.*, 28, 4105 (1987).
7. M. Fujino, M. Wakimasu and C. Kitada, *Chem. Pharm. Bull.*, 29, 2825 (1981).
8. R. Ramage and J. Green, *Tetrahedron Lett.*, 28, 2287 (1987).
9. Patent applied for.
10. L. Lapatsanis, G. Milias, K. Froussion and M. Kolovos, *Synthesis*, 671 (1983).
11. B. Riniker, Ciba-Geigy, Basle; private communication.
12. P. Sieber, *Tetrahedron Lett.*, 28, 1637 (1987).

(Received in UK 17 June 1988)