Tetrahedron Letters,Vol.22,No.46,pp 4575-4578,1981 0040-4039/81/464575-04$02.00/0
Printed in Great Britain

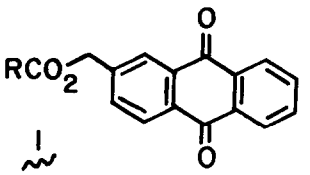
APPLICATIONS OF NEW PEPTIDE PROTECTIVE GROUPS I-- A SYNTHESIS OF BIS-S-ACETAMIDOMETHYL DIHYDROSOMATOSTATIN

D.S. Kemp*, David R. Bolin, and Marc E. Parham

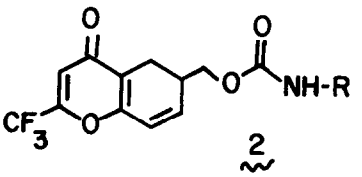
Department of Chemistry
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

Abstract: A synthesis of bis-S-Acetamidomethyl dihydrosomatostatin is reported in which the C-terminal carboxyl function is protected by the Maq ester function and the N-terminal amine as well as two ε-amino groups of lysine residues are protected as Tcroc urethanes. Clean removal of these groups is reported.

Proof of reliability for new peptide protective groups results from their trouble-free use in preparing many peptides in the medium to large size range. Recently we have described the 2-methyleneanthraquinone (Maq) ester group 1 which is cleaved reductively under a variety of mild conditions [1] and the 6-methylene-2-trifluoromethylchromone (Tcroc) urethane 2 which is cleaved by straight-chain amines such as propylamine or by hydrazine but which is inert to branched-chain primary amines.[2] We wish to report successful application of these groups to a synthesis of an S-blocked form of dihydrosomatostatin. In subsequent papers in this series, we will report syntheses of this substance that employ other protective groups which we have developed.



1



2

The disulfide-bridged tetradecapeptide somatostatin 3 is widely distributed in mammalian tissues and acts as an inhibitory factor which blocks the release of a variety of other peptide hormones including growth hormone, glucagon, and insulin.[3] A number of syntheses of somatostatin have been reported,[4] and the molecule has been the subject of much analog work directed toward discovery of therapeutic agents that can act selectively

to inhibit release of hormones such as glucagon. Such agents may be very useful in the control of diabetes. Nearly all of the reported syntheses involve as final steps S-deblocking and oxidation of a bis-S-blocked dihydrosomatostatin. For the purpose of testing new protective groups we sought to prepare the bis-S-acetamidomethyl (bis-Acm) dihydrosomatostatin, 4 which has been characterized and reported by Meienhofer and coworkers.[5]

H-Ala-Gly-Cys-Lys-Asn-Phe$_2$-Trp-Lys-Thr-Phe-Thr-Ser-Cys-OH

3 Somatostatin

Acm Acm

H-Ala-Gly-Cys-Lys-Asn-Phe$_2$-Trp-Lys-Thr-Phe-Thr-Ser-Cys-OH

4 bis-Acm dihydrosomatostatin

Building blocks

The tetrapeptides H-Asn-Phe-Phe-Trp-OH and Boc-Thr-Phe-Thr-Ser-OH were prepared by a fragment condensation (amino acid derivative → dipeptide → tetrapeptide) using the 2-ethyl-7-hydroxybenzisoxazolium cation as the amide-forming reagent.[6] Some inconvenience in the latter synthesis resulted from the water-solubility and hygroscopic character of the dipeptide H-Thr-Ser-OH. Both tetrapeptides were fully characterized and homogeneous by a variety of HPLC and TLC assays.

Double use was made of the N-hydroxysuccinimide ester of the doubly blocked lysine derivative 5. The ε-Tcroc derivative of lysine was prepared from Tcroc chloride[2] and the copper (II) complex of lysine.[7] Careful buffering was required to obtain satisfactory yields for the introduction of the α-Boc functionally in the next step, since the Tcroc group is easily cleaved at high pH in aqueous media. This is the sole limitation that we have found for this protective group in the course of the somatostatin synthesis.

H-Lys-OH —(1. Tcroc-Cl; Cu(II); 2. H_2S)→ H-Lys(Tcroc)-OH (74%) —(Tcroc di-t-butyldicarbonate; DMSO, Et_3N, 1 HR)→ Boc-Lys(Tcroc)-OH (90%) 5

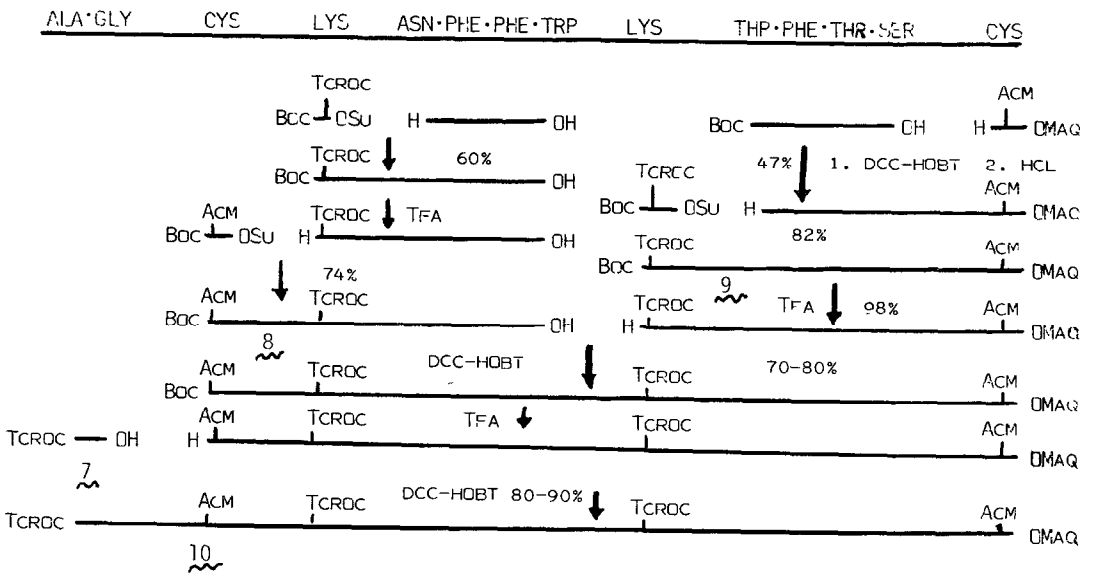
Formation of the Maq ester 6 of Acm-cysteine by the cesium salt procedure[8] is straight-forward:

Boc-Cys(Acm)-OH —(1. Cs_2CO_3; 2. Maq-Br, DMF, 24h)→ Boc-Cys(Acm)-OMaq 82% —(HCl-dioxane)→ HCl H-Cys(Acm)-OMaq 95% 6

Synthesis of 4

The synthetic sequence for the preparation of 4 is outlined in Scheme 2. The selective removal of Boc groups were carried out in the presence of anisole as scavanger for t-butyl cations and proceeded with complete retention of Tcroc and Maq groups, as

SCHEME II



expected from our earlier studies.[1,2] The dipeptide acid 7 and the two hexapeptide fragments 8 and 9 were homogeneous by TLC and HPLC criteria. These species were also characterized by their 250 MHz proton NMR spectra which showed characteristic clean doublets for the alanine and threonine methyls and clean resonances for the benzylic methylenes of the Tcroc and Maq groups. Unexpected multiplets for these resonances are often observed for inhomogeneous samples, and high field NMR thus provides a valuable criterion for product purity.[9]

The blocked tetradecapeptide 10 was purified by precipitation from DMF with acetonitrile. Though not completely free of minor impurities by HPLC, this substance showed a clean 250 MHz proton NMR spectrum for alanine and threonine resonances and for the characteristic resonances of the Acm, Maq, and Tcroc groups.

Deblocking was carried out by treatment of 10 with 25 equivalents of sodium dithionite in a bicarbonate buffer (DMF-H_2O) for 5-8 minutes at 25°C, followed by addition of propylamine (to 50% v/v) at 25°C for 35 minutes. Immediate evaporation gave a residue which gave a simple profile by HPLC (Waters μ Bondapak C_{18}, 50% MeOH/0.02M Et_3N·HOAc pH4.5, 1.0ml/min,280nm) of three peaks, two of which could be attributed to cleavage products of the protective groups. Material corresponding to the third peak was isolated in 70% yield from a single HPLC preparative run. This substance moved as a single component in all chromatographic assays and gave a satisfactory amino acid analysis.[10] It was identical to a reference sample of 4 supplied by Dr. J. Meienhofer, by HPLC, by 250 MHz ^1H NMR, and by TLC in three solvent systems.[11]

By several criteria, this synthesis has materially increased our confidence in the use of the Maq ester and Tcroc groups. The operations of Scheme 2 have provided a practical test of the stability of these groups to the acidic conditions required to

remove the t-butyl-derived protective groups as well as to the presence of free amines during normal amide-forming steps. A fore-synthesis was carried out in preparation for the reactions of Scheme 2 in which Tcroc was replaced by the familiar carbobenzoxy function. Yields and product Rf values were similar for the two syntheses, suggesting that the Tcroc group presents no unusual problems. The characteristic ^{1}H NMR resonances of Maq and Tcroc have proved valuable for preliminary product screening as well as for establishing product homogeneity. The sole restriction to the use of these groups is their lack of orthogonality. Attempts to reverse the order of deblocking reagents to first remove Tcroc from 10 results in clean conversion to a derivative of 4 which has been tentatively identified from model studies as the C-terminal N-propylamide. The electronegative carbonyls of the Maq ester function evidently render it unusually susceptible to aminolysis.

Acknowledgements

We wish to thank Dr. J. Meienhofer (Hoffmann-LaRoche) for his gift of a reference sample of 4. Financial support from the National Institutes of Health (GM 13453) is gratefully acknowledged. We wish to thank Drs. C. Hoyng, G. Hanson, S. Sofuku, and J. Samanen for technical assistance.

References

1. D. Kemp and J. Reczek, Tetrahedron Letters, 1977, 1031.
2. D. Kemp and G. Hanson, J. Org. Chem., submitted for publication.
3. P. Brazeau, W. Vale, R. Burgus, N. Ling, M. Butcher, J. Rivier, and R. Guillemin, Science, 1973, 179, 77.
4. J. Rivier, P. Brazeau, W. Vale, N. Ling, R. Burgus, C. Gilon, J. Yardley, and R. Guillemin, Compt. Rend., 1973, 276, 2737.
5. A. Felix, M. Jimenez, C-T. Wang, and J. Meienhofer, Int. J. Peptide Protein Res., 1980, 15, 342.
6. D. Kemp, S-W. Wang, Z. Bernstein, and J. Rebek, Tetrahedron, 1974, 30, 3969.
7. J.P. Greenstein and M. Winitz, "Chemistry of the Amino Acids", Vol. 2, John Wiley & Sons, 1961, p 1059.
8. Su-Sun Wang, B.F. Gisin, D.P. Winter, R. Makofske, I.D. Kulesha, C. Tzougraki, and J. Meienhofer, J. Org. Chem., 1977, 42, 1286.
9. ^{1}H NMR 250 MHz; Maq:(DMSO-d_6)δ5.35(s,2), 7.94(m,3), 8.22(m,4); Tcroc: (DMSO-d_6)δ5.17(s,2), 7.07(s,1), 7.80(d,J=8.8 Hz), 7.89(m,1,J=8.8,1.7 Hz), 8.08(s(br),1,J=1.7 Hz).
10. Amino acid analysis: Asp 0.96(1); Thr 2.01(2); Ser 1.09(1); Gly 1.04(1); Ala 1.01(1); Phe 2.87(3); Lys 2.06(2); Cys (as cysteic acid) 1.96(2).
11. Rf = 0.73 (rep 0.74[5]) in nPrOH-Pyridine-H_2O-AcOH-EtOH(5:4:6:1:4); Rf = 0.22 in nBuOH-AcOH-H_2O(4:1:5 upper phase); Rf = 0.33 in nBuOH-0.2 M AcOH-EtOH-Pyridine (4:7:1:1)[5].

(Received in USA 4 May 1981)