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NEW PROTECTIVE GROUPS FOR PEPTIDE SYNTHESIS--III THE MAQ ESTER GROUP MILD REDUCTIVE CLEAVAGE OF 2-ACYLOXYMETHYLENEANTHRAQUINONES

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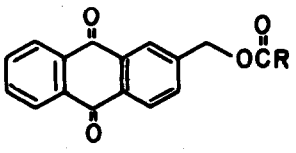
As part of our long range program for developing a compatible set of new reagents for peptide synthesis,[1] we wish to report the preparation and uses of a new carboxyl protective group, the Maq esters. Esters of the 2-oxymethyleneanthraquinone (Maq) function are stable to the conventional operations of peptide synthesis but are cleaved in high yield by a variety of selective, mild reducing agents.

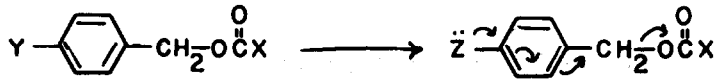
Maq Ester I



Although many reliable and versatile amino and carboxyl protective groups are available,[2] they nevertheless fall short of meeting the most stringent requirements of peptide synthesis. Ideally, a new protective group should meet four conditions. It must be inert to the functional groups of peptides and to their reaction and isolation procedures. It should facilitate, not hinder isolation and purification. It must be removable quantitatively under very mild reaction conditions. Finally, it should remain intact during the procedures used to remove other protective groups. In a preliminary study involving two small model peptides, we have found the Maq esters to meet these conditions.

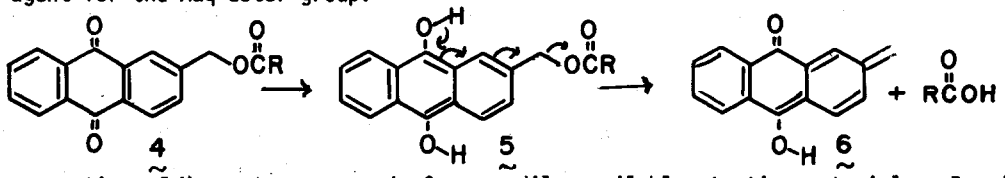
In designing the Maq esters, we have relied on a general principle. It is well known that the acid or neutral cleavage of benzyl esters or urethanes is retarded by electron-withdrawing substituents but accelerated by many orders of magnitude by electron-donating groups. In the accompanying scheme, any reagent or process that can convert an electron-withdrawing substituent

Y, into an electron-donating substituent, Z, thus has the potential for generating a new protective group. The phenyl moiety can be viewed as a chemical transducer which both transmits the electronic change Y → Z and isolates this change from peptide-derived functional groups that may enter into undesired reactions with neighboring reactive sites. New reagents of this structural class should therefore share the trouble-free characteristics of the exhaustively studied benzyl esters and benzyl urethanes while possessing novel susceptibility to cleavage.



2 NON LABILE X = R, NHR LABILE 3

A Maq ester, in its quinone form, is thus expected to be resistant to alkyl-oxygen ester cleavage; in its hydroquinone form cleavage is expected to be rapid. Perhaps the mildest and most specific of all quinone reducing agents is the corresponding hydroquinone, reduction occurring by electron transfer in a disproportionation reaction through the semiquinone intermediate. We therefore expected to be able to use 9,10-dihydroxyanthracene as an exceedingly selective cleavage agent for the Maq ester group.



4 5 6

The preparation of Maq esters proceeds from readily available starting materials. Bromination of 2-methylanthraquinone, followed by hydrolysis, generates 2-hydroxymethylanthraquinone,[3] which can be conveniently converted into Maq esters by reaction with a carboxylic acid in the presence of equivalents of dicyclohexylcarbodiimide and hydroxybenzotriazole.

Based on our experience with the syntheses outlined below, we conclude that no undesired reactivity is observed with Maq derivatives under normal peptide coupling, deblocking, or workup conditions.[4] Several convenient features have attended work with Maq esters. Their UV chromophore allows very sensitive detection on fluorescent thin layer media. Maq esters of simple amino acid and peptide derivatives are usually highly crystalline and readily soluble in the most useful organic solvents, such as dichloromethane, ethyl acetate, and DMF. Strikingly, the hydrochloride salts of several amino acid Maq esters are freely soluble in dichloromethane and chloroform. The important question of the effect of the Maq ester group on the solubility of much larger peptide fragments has yet to be addressed.

We have observed ready, selective cleavage of the Maq ester of carbobenzoxyglycine under four generally applicable conditions: reaction with sodium dithionite[5] (5 eq., 8 hr, 100%)[6] in dioxane-water, pH 7-8; photolysis[7] in isopropanol containing N-methylmorpholine (4 eq., 4 hr, 350nm, 99%); reaction with 9-hydroxyanthrone[8] (4 eq., 5 hr, 99%) in DMF solution containing triethylamine (2 eq.); and stirring with excess polystyrene resin functionalized with 9,10-dihydroxyanthracene residues (1.5 hr, 100%).

Reducing resin is conveniently prepared by Friedel-Crafts alkylation of polystyrene by 2-bromomethylanthraquinone ($AlCl_3$, CH_2Cl_2, reflux 2 hours), followed by workup and reduction with

sodium borohydride (EtOH-H_2O, pH 13).[9] Resin has been recovered and regenerated repeatedly without loss of capacity.

With the exception of the photochemical conditions, in which several products are formed, the above deprotection experiments are observed to yield 2-methylanthraquinone quantitatively, even in the presence of the efficient trapping agent, bisulfite. From this we conclude that the xylylidene derivative 6 that must be initially formed by fragmentation of the hydroquinone 5, must rapidly tautomerize without reacting with nucleophiles at its electrophilic methylene site. Thus, Maq ester cleavage does not require trapping agents.[1]

Two tests of the synthetic utility of the Maq group have been carried out; synthesis of the blocked methionine enkephalin derivative, BocTyrGlyGlyPheMetOMaq,[10] 8, and the blocked angiotensin analog, CbzArg(NO_2)ValTyrIleHisProAlaOMaq,[1] 9. Thus, 8 was prepared from BocTyrGlyOH and HGlyPheMetOMaq in 72% yield[11] by the DCC-HOBT procedure. The tripeptide ester was generated by the following reaction sequence: BocMetOH → BocMetOMaq[12] (DCC-HOBT, 70-80%, DMF, m.p. 116-118°) → HMetOMaq·HCl (HCl-CH_2Cl_2, 82%) → BocGlyPheMetOMaq[12] (DCC-HOBT, 80%, DMF, m.p. 114°) → HGlyPheMetOMaq·HCl (HCl-dioxane, 84%). Treatment of 8 with reducing resin and triethylamine generated BocTyrGlyGlyPheMetOH in 92% yield.[13] A quantitative yield was observed with the dithionite procedure.

Coupling of CbzArg(NO_2)ValTyrN_3 with HIleHisProAlaOMaq resulted in the isolation of 62% of 9.[11] Yields for couplings of the azide with analogous fragments bearing other ester protective groups lie in the range of 60-70%. The tetrapeptide ester was prepared by the sequence: BocAlaOH → BocAlaOMaq[12] (DCC-HOBT, 70-75%, m.p. 160-161°) → HAlaOMaq·HCl (HCl-CH_2Cl_2, 80%) → BocProAlaOMaq[12] (DCC-HOBT, 76%, m.p. 140°) → HProAlaOMaq·TFA (TFA, 96%) + ZIleHisN_3 → ZIleHisProAlaOMaq[12] (98% crude, 58% after purification, m.p. 125°) → HIleHisProAlaOMaq·2HBr (sat. HBr/AcOH, 1 hr., 84%). Hydrogenation of 9 (Pd, 10 hr.) gave 99% crude deblocked heptapeptide[1] and 78% after gel filtration.[13]

The Maq group is recovered unchanged from treatment with trifluoroacetic acid (1 hr, 20°), HCl in CH_2Cl_2 (1 hr, 20°), or triethylamine in water-dioxane (2 eq., 24 hr.). The Maq benzoate ester reacts with saturated HBr in acetic acid with a half-time of 65 hours (Bzl $t_{\frac{1}{2}}$ < 15 min.). The Maq group is cleaved by catalytic hydrogenation, or by the special reducing conditions described above. It is thus complementary to the Bpoc, Boc, Cbz, and t-butyl ester functions, as well as to the Bic and Dobz urethanes. We will report on more definitive tests of the Maq group on its urethane equivalent, subsequently.

ACKNOWLEDGEMENTS

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REFERENCES

1. D.S. Kemp, D. Roberts, C. Hoyng, J. Grattan, F. Vellaccio, J. Reczek, "New Approaches to Peptide Synthesis," Peptides: Chemistry, Structure and Biology, Ann Arbor Science Publisher,

Inc., Ann Arbor, Michigan, 1975; D.S. Kemp, C. Hoyng, Tet. Letters, 52, 4625 (1975); D.S. Kemp, D.C. Roberts, Tet. Letters, 52, 4629 (1975).

2. L.A. Carpino, Accts. of Chem. Res., 6, 191 (1973). For general reviews, see "Methoden der Organishen Chemie (Houben Weyl)," Vol. 15/1, E. Wunsch, ed., 6. Thieme, Stuttgart, 1974; also, see yearly reviews in "Amino Acids, Peptides, and Proteins," Vols. 1-6, The Chemical Society, London.
3. Andre Etienne, G. Izoret, Air Liquide, Fr. 1,317,258 Feb. 8, 1963, Appl. Dec. 21, 1961. In our procedure, 2-bromomethylanthraquinone was obtained by slow addition of bromine to a hot solution of 2-methylanthraquinone in CCl_4 while irradiating with light. Cooling of the solution precipitates out product which is recrystallized from ethyl acetate, m.p. 198-201°.
4. Maq esters and 2-hydroxymethylanthraquinone are observed to discolor if exposed to light for prolonged periods. We have also observed discoloration of the corresponding urethanes in DMSO in the presence of strong bases (TMG or alkoxides). As a routine precaution, manipulation of Maq esters in alcoholic solvents have been carried out in foil-wrapped glassware.
5. E. Grandmougin, Chem. Ber., 57, 3561 (1906).
6. Unless otherwise specified, deprotection yields were obtained by isotopic dilution. Minimum reaction times required for complete deprotection by dithionite or 9-hydroxyanthrone have not been determined. All deprotections were done under nitrogen.
7. K. Meyer, Monatshefte fur Chemie, 39, 249 (1916).
8. K. Meyer, Justus Liebig's Annalen der Chemie, 379, 37 (1911).
9. Resin is washed well with aqueous acid, to remove excess hydride; it then gives a negative boron flame test. The formation of 2-methylanthraquinone by treatment of Maq esters with 9-hydroxyanthrone establishes the feasibility of the reaction scheme: 4 → 5 → 6. On this basis we regard reduction by traces of residual hydridic boron as unlikely.
10. J. Hughes, et al., Nature, 577, 258 (1975).
11. Isolated yield.
12. Characterized by satisfactory elemental analysis.
13. Identical by TLC and Sephadex G-25 trace to samples made by conventional methods.