# The Fluoren-9-ylmethoxycarbonyl Group for the Protection of Hydroxy-groups; Its Application in the Synthesis of an Octathymidylic Acid Fragment

Carlo Gioeli and Jyoti B. Chattopadhyaya*

*Department of Microbiology, Box 581, Biomedical Center, Uppsala University, S-751 23 Uppsala, Sweden*

The fluoren-9-ylmethoxycarbonyl (Fmoc) group may be used to protect hydroxy-groups in conjunction with a variety of acid- and base-labile protecting groups; the Fmoc group may be conveniently removed by the action of triethylamine (10 equiv.) in dry pyridine solution within 2 h at 20 °C while other base-labile protecting groups remained intact.

Carpino and Han[1] introduced the fluoren-9-ylmethoxycarbonyl (Fmoc) group for the protection of amino-functions in 1970, and it has been used subsequently[2] in oligopeptide synthesis. The chemistry of its deprotection centres on the acidic nature of the proton on the β-carbon atom, and hence upon its abstraction by base; the Fmoc group fragments *via* β-elimination liberating the amine (Scheme 1).[1] The Fmoc group [*e.g.* in (1)] is normally deprotected by one of the following basic reagents:[3] aqueous ammonia, piperidine, morpholine, or ethanolamine. Any of these reagents can also act as a good nucleophile and can participate in a substitution reaction. We consequently expected that if the Fmoc group were used for the hitherto unreported protection of hydroxy-groups and used in conjunction with another protecting group containing an ester or a carbonate linkage, it

(1) Q = NHR
(2) Q = OR
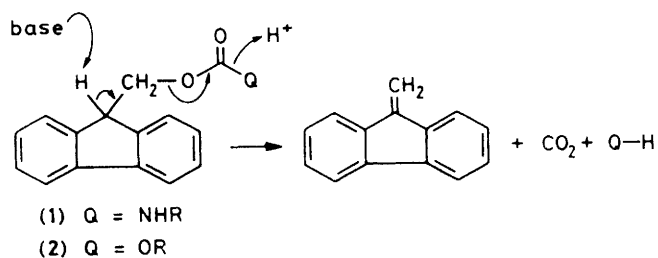
Scheme 1

should be conveniently removable by the action of a non-nucleophilic tertiary base.[4] We now report that this is indeed

R^{1}O $\diagdown$ O $\diagup$ R^2
OR^3

Px = [9-phenylxanthen-9-yl structure]
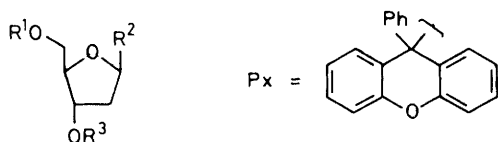
(3) R^1 = Px, R^2 = Thy, R^3 = H
(4) R^1 = o-Br$_2$CHC$_6$H$_4$C(:O)–, R^2 = 4-N-benzoylcytosin-1-yl, R^3 = H
(5) R^1 = H, R^2 = Thy, R^3 = tetrahydropyran-2-yl
(6) R^1 = H, R^2 = Thy, R^3 = Me$_3$SiCH$_2$CH$_2$–
(7) R^1 = H, R^2 = Thy, R^3 = MeC(:O)CH$_2$CH$_2$C(:O)–
(8) R^1 = R^3 = H, R^2 = Thy

**Table 1.** Preparation of Fmoc derivatives and recovery yields.

Substrate	% Yield[a]		
	(A)	(B)	(C)
(3)	91.0	97.0	87.0
(4)	93.0	38.0	83.0
(5)	96.0	85.0	88.0
(6)	88.0	89.0	79.0
(7)	96.0	95.0	77.0
(8)	81.0[b]	––	96.0[c]
Cholesterol	84.0		93.0

[a] (A) = formation of Fmoc derivative; (B) = selective deprotection of other groups; (C) = selective deprotection of Fmoc group. [b] 5′-OH could be selectively acylated to obtain 5′-O-Fmoc-thymidine. [c] Isolated as the 3′,5′-di-O-benzoate.

possible in the case of the fluoren-9-ylmethyl carbonate linkage [$e.g.$ (2)], which can be fragmented in the usual way to generate the starting alcohol quantitatively within 2 h at 20 °C by the action of a volatile, non-nucleophilic, tertiary base such as triethylamine (10 equiv.) in dry pyridine solution (10 ml/mmol; $t_{1/2}$ $ca.$ 20 min).

Table 1 shows the variety of substrates, containing different protecting groups, whose Fmoc derivatives have been prepared,† together with yields for the formation of the Fmoc derivatives,† for the recovery of substrate† after the removal of the Fmoc group in the presence of other protecting groups, and for recovery of the hydroxy-component containing the Fmoc group† after deprotection of the corresponding acid- or base-labile protecting groups from the Fmoc derivatives. These examples clearly establish the synthetic applicability of the Fmoc group in conjunction with acid-labile groups such as 9-phenylxanthen-9-yl (Px, pixyl)[5] and tetrahydropyran-2-yl,[6] and base-labile groups such as 2-dibromomethylbenzoyl,[7] 2-trimethylsilylethoxycarbonyl,[8] (TMSEC), and levulinyl.[9]

Finally, the application of the Fmoc group as a 5′-hydroxy-protecting group in DNA synthesis was investigated by the chemical synthesis of a octathymidylic acid fragment using a reported strategy.[10] Thus the dimer (9) was synthesized in 88% yield starting from 5′-O-Fmoc-thymidine following our procedure.[11] The 3′-hydroxy-function of (9) was then blocked with the pixyl group[5] to provide (10) quantitatively. The 5′-hydroxy-dimer (11) was then prepared in 95% isolated yield (powder) by removal of the Fmoc group with Et$_3$N (15 equiv.) in dry pyridine solution within 1.5 h at 20 °C. The 5′-

R^{2}O $\diagdown$ O $\diagup$ Thy
o-ClC$_6$H$_4$O—P(=O)—O—[...Thy]
OR^1
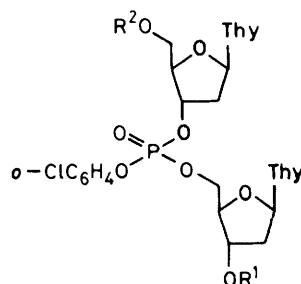
(9) R^1 = H, R^2 = Fmoc
(10) R^1 = Px, R^2 = Fmoc
(11) R^1 = Px, R^2 = H
(12) R^1 = o-ClC$_6$H$_4$O—P(=O)—O$^-$Et$_3$NH$^+$, R^2 = Fmoc
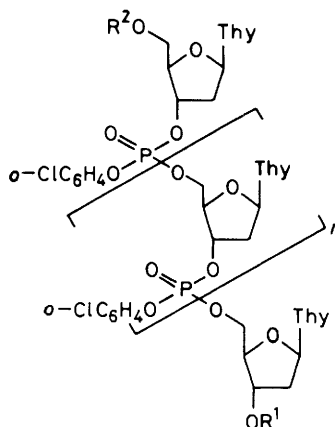
(13) n = 2, R^1 = Px, R^2 = Fmoc
(14) n = 2, R^1 = o-ClC$_6$H$_4$O—P(=O)—O$^-$Et$_3$NH$^+$, R^2 = Fmoc
(15) n = 2, R^1 = Px, R^2 = H
(16) n = 6, R^1 = Px, R^2 = Fmoc

protected dimer (12), which was prepared as in ref. 11 was then condensed in the usual way[10,11] in the presence of 1-mesityl-sulphonyl-3-nitro-1,2,4-triazole[12] to provide the fully protected tetramer (13) in 86% yield (powder). Removal of the Fmoc group under the above conditions provided the 5′-hydroxy-tetramer (15) in 91% yield within 3.5 h, and the pure 5′-protected phosphodiester component (14) following the literature procedure.[10,11] They were coupled[10] to give the fully protected octamer (16) in 73% yield. The Fmoc group could also be conveniently removed, under the above conditions, from the octamer (16) to generate the 5′-hydroxy-component in 89% yield. The chromatographic separation of the 5′-hydroxy-components from the fully protected 5′-Fmoc derivatives of di-, tetra-, and octa-thymidylic acids was facilitated by the significant differences in their physical properties. A portion of the fully protected octamer (16) was deprotected under the usual conditions.[11] The total deprotected material in the aqueous phase was then examined by h.p.l.c. and the main peak contained more than 96% of the optical density at 260 nm (overall yield: 45.8%) which was characterized[11] as octathymidylic acid by Maxam–Gilbert

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† The Fmoc derivatives were conveniently prepared by treating a pyridine solution (10 ml/mmol) of the substrate at 20 °C with fluoren-9-ylmethyl chloroformate (1.2 equiv.) for 40 min followed by standard work-up. All products have been characterized by ^{1}H n.m.r., i.r., and mass spectrometry and microanalyses.

sequencing of ^{32}P labelled material. The synthetic octathy-midylic acid also contained exclusive 3′ → 5′ linkages as shown by its complete digestion with *C. Adamanteus* snake venom phosphodiestrase.

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