The 9-Fluorenylmethoxycarbonyl (Fmoc) Group for the Protection of Amino Functions of Cytidine, Adenosine, Guanosine and Their 2'-Deoxysugar Derivatives

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In the chemical synthesis of DNA and RNA fragments, it is desirable [1] to protect the exocyclic amino functions of cytosine, adenine and guanine residues in view of their susceptibility to attack by electrophiles such as phosphorylating agents. Khorana and his coworkers [2] in their phosphodiester approach have introduced *N*-acyl groups to protect all three base residues and, subsequently, these acyl groups have also been used in the phosphotriester approach.[1] The *N*-acyl groups on cytosine, adenine and guanine residues are relatively stable both under neutral and acidic conditions; however, their rates of removal, under an alkaline condition, are clearly dependent upon the nature of base residues.[1,3] Thus, the removal of *N*-benzoyl groups is complete at room temperature with 5 M aqueous NH_3 in dioxane (1:1; v/v) in 385, 1410 and 4350 min from the corresponding 2'-deoxyribofuranosyl derivatives of cytosine, adenine and guanine residues, respectively.[3] The above periods of deprotection seem to be too long,[4] unless it is carried out at *ca.* 50 °C,[5,6] in view of the fact that the chemical synthesis of a fully protected 10–14 units long DNA sequence on the solid support takes only a day or so.[6] To circumvent this problem, especially in the case of guanine residues, acetyl and isobutyryl groups have been proposed (see Ref. 1). Several other protective groups have also been proposed for this purpose.[4]

Here we report the preparation and properties of 9-fluorenylmethoxycarbonyl-(Fmoc), as in *2* to *7*, as an exocyclic amino protecting group for cytosine, adenine and guanine residues of their corresponding 2'-deoxyribo- and ribonucleosides. The Fmoc group was first introduced [7] for the protection of an α-amino function (pK_a *ca.* 9.7) of an amino acid and it was removable with the help of liquid ammonia in 10–12 h at room temperature. The chemistry of its deprotection centers 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 as shown in Scheme 1. We reasoned that, in such a β-elimination reaction, the pK_a of the base as a leaving group should have a significant effect on the overall rate of removal of the Fmoc group under the influence of either a nucleophilic or non-nucleophilic basic condition. Thus, it should be expected that the Fmoc group from the corresponding derivatives of cytidine, adenosine, guanosine and their 2'-deoxysugar analogs with pK_a [10] of *ca.* 4.2(4.3), 3.55(3.8) and 2.1(2.4) respectively, should be removable with greater ease. This has led us to prepare the Fmoc derivatives of cytidine, adenosine and guanosine and their corresponding 2'-deoxyribose derivatives, as in *2* to *7*, from their respective parent nucleosides through a "one-pot" synthesis in 88.5, 81, 70, 90, 58 and 70 % yields, respectively, as crystalline compounds. All new compounds have been characterized by UV, ^1H NMR and element analysis. The general procedure for such a "one-pot" synthesis involves trimethylsilylation of a particular nucleoside in dry pyridine solution which is followed by the addition of 9-fluorenylmethyl chloroformate (Fmoc-Cl), 1.2 equiv. with respect to the nucleoside, and then hydrolysis.[9] Thus, Table 1 records some physical and spectroscopic properties of compounds *2* to *7* in support of their chemical structures. We then proceeded to explore the relative rates of removal of these Fmoc groups from the substrates *2* to *7*. Table 2 records the relative rates of removal of the Fmoc group from the corresponding Fmoc

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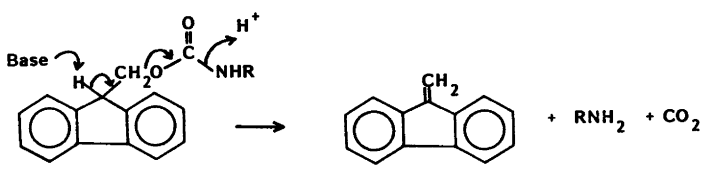


Scheme 1.

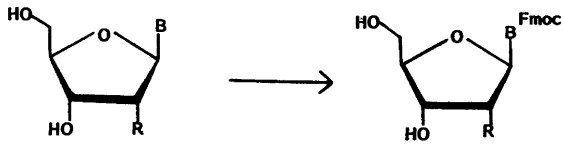
0302-4369/83 $2.50

Table 1. The physical and the spectroscopic properties of Fmoc derivatives of cytidine, adenosine, guanosine and their 2′-deoxy analogs.

Compound	M.p. (°C)	Crystallization media	UV absorption properties in ethanol (nm)	^{1}H NMR absorptions in DMSO-d_6+D_2O (δ scale)
2	145	ethanol	λ_{max} (pH 2): 258, 266, 292 (sh.) 301 (sh.); (pH 7): 256, 264, 290 (sh.), 300 (sh.); (pH 13): 259, 266, 293 (sh.), 301 (sh.).	8.71 (d, 4 Hz, 1H); 8.32 (d, 4 Hz, 1H); 7.83 (m, 4H); 7.4 (m, 4H); 6.28 (s, 1H), 4.4 (m, 3H); 3.97 (m, 2H); 3.66 (m, 2H).
3	138–140	toluene	λ_{max} (pH 2): 266, 290 (sh.), 302 (sh.) (pH 7): 266, 290 (sh.), 303 (sh.) (pH 13): 266, 296 and 302.	8.71 (s, 1H); 8.69 (s, 1H); 7.77 (m, 4H), 7.36 (m, 4H); 6.05 (d, 8.5 Hz, 1H); 4.66 (m, 1H);4.4 (m, 4H); 3.82 (m, 2H).
4	167–170	ethanol	λ_{max} (pH 2): 266, 290 (sh.), 301 (sh.) (pH 7): 261, 290 (sh.), 300 (sh.) (pH 13):267, 290 (sh.), 302 (sh.).	8.26 (s, 1H); 7.87 (m, 4H); 7.40 (m, 4H); 5.84 (d, 5 Hz, 1H); 4.46 (m, 3H); 4.19 (m, 1H); 3.91 (m, 1H); 3.66 (m, 2H).
5	145	ethanol–$CHCl_3$ 1:1 v/v	λ_{max} (pH 2): 256, 265, 293 (sh.) 301 (sh.); (pH 7): 256, 264, 292 (sh.), 301 (sh.); (pH 13): 256, 266, 292 (sh.), 300 (sh.).	8.32 (d, 10 Hz, 1H); 7.83 (m, 4H); 7.40 (m, 4H); 6.98 (d, 10 Hz, 1H); 6.12 (t, 8 Hz, 1H); 4.34 (m, 3H); 3.84 (m, 2H); 3.62 (m, 2H).
6	128–130	toluene	λ_{max} (pH 2): 266, 287 (sh.), 302 (sh.); (pH 7): 266, 288 (sh.), 302 (sh.); (pH 13): 266, 292 (sh.), 298 (sh.).	8.69 (s, 1H); 8.14 (s, 1H); 7.69 (m, 4H); 7.36 (m, 4H); 6.38 (t, 10 Hz, 1H); 4.68 (m, 3H); 4.18 (m, 2H); 3.89 (m, 2H).
7	155	toluene	λ_{max} (pH 2): 263, 290 (sh.), 302 (sh.); (pH 7): 261, 290 (sh.), 300 (sh.); (pH 13): 266, 291 (sh.), 301 (sh.).	8.26 (s, 1H); 7.85 (m, 4H); 7.43 (m, 4H); 6.25 (t, 8.5 Hz, 1H); 4.49 (m, 3H); 3.88 (m, 4H).

Table 2. The relative rates of removal of Fmoc group from the corresponding derivatives of cytidine, adenosine, guanosine and their 2′-deoxyribonucleoside analogs.

Fmoc derivatives of nucleosides	Reagents for the removal of the Fmoc group		
	Condition A aq. NH_3 (d 0.85)–pyridine 1:1 (v/v) at 20 °C		Condition B Et_3N (20 eq.) in dry pyridine (10 ml/mmol)
	$t_{1/2}$ (min)	t_∞ (min)	$t_{1/2}$ (min)
2	17	110	180
3	22	150	180
4	120	–	65
5	16	110	330
6	20	130	300
7	105	–	60



General Formula: (1)
R = H or OH
B = 1-Cytosinyl;
9-Adeninyl;
9-Guaninyl;

(2); R= OH; B= 1-Cytosinyl;
(3); R= OH; B= 9-Adeninyl;
(4); R= OH; B= 9-Guaninyl;
(5); R= H ; B= 1-Cytosinyl;
(6); R= H ; B= 9-Adeninyl;
(7); R= H ; B= 9-Guaninyl;

protected derivatives and the data illustrate the pronounced effect of the pK_a of the leaving group on the fragmentation reaction shown in Scheme 1. Thus it is interesting to note that the removal of the Fmoc group, using condition B, from the corresponding derivatives of guanosine and its 2'-deoxy analog. Table 2; compounds: *4* and *7* respectively, is faster than the corresponding cytidine and adenosine derivatives; this is understandably due to the exclusive operation of a β-elimination pathway (Scheme 1). This observation is particularly relevant in the light of the fact that the relative rate of removal of a particular *N*-acyl group, under a usual alkaline conditions (condition: A in Table 2), from the base residues follows the order: guanosine<adenosine<cytidine.

It should also be added that, despite the desired stability of the Fmoc group both under neutral and acidic conditions, it could be completely deprotected within 40 min at room temperature when the compounds *2* to *7* in pyridine (1 part) were treated with liquid ammonia (*d* 0.88) (9 parts, v/v).

We then examined the effect of the Fmoc as 6-amino protecting group of 2'-deoxyadenosine (*6*) on the cleavage of the glycosidic bond (depurination) [1] in view of the recent report in literature [8] which suggests that 6-*N*-benzoyl-2'-deoxyadenosine and 6-*N*-phthaloyl-2'-deoxydenosine have half-lives of depurination of 30 and 120 min, respectively, in 80 % aqueous acetic acid at 30 °C. Under a similar acidic condition the glycosidic bond of the 6-*N*-Fmoc-2'-deoxyadenosine (*6*) was found to be more stable with a half-life of *ca.* 180 min. Thus, it is anticipated that the employment of a building block like *6* in the chemical synthesis of a DNA segment should reduce the formation of the by-products due to depurination during acid hydrolyses steps.

Further work is now in progress in this laboratory on the application of these exocylic amino protected building blocks in DNA and RNA chemistry.

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