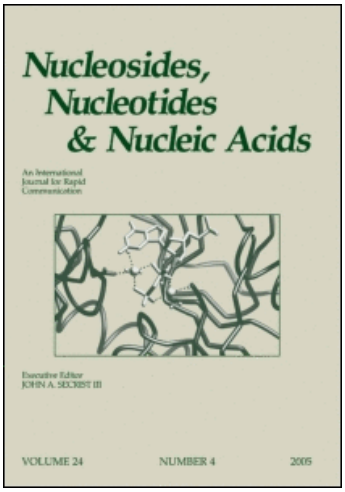

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General Synthesis of 2'(3')-O-Aminoacyl Oligoribonucleotides Related to the 3'-Terminus of aa-tRNA

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GENERAL SYNTHESIS OF 2'(3')-O-AMINOACYL OLIGORIBONUCLEOTIDES RELATED TO THE 3'-TERMINUS OF aa-tRNA

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Abstract: A general method is presented for the synthesis of the title compounds using phosphotriester methodology and employing a unique combination of protecting groups, including the double protection of the guanosine aglycon.

Previous work from our laboratory has described a new methodology for the synthesis of 2'(3')-O-aminoacyl oligonucleotides[1,2]. The latter are models of the aa-tRNA 3'-terminus and as such are important tools for mechanistic studies in protein biosynthesis.[3]

The key feature of our methodology is a system for blocking the nucleoside functionalities: 9-fluorenylmethyloxycarbonyl groups for aglycon amino groups; a phenyl group for O^4-protection of uracil; a dimethoxytrityl group for 5'-hydroxy group; 4-methoxytetrahydropyranyl groups for 2'-hydroxy groups; and a (2-(4-biphenylyl)-isopropyloxy)carbonyl group for protection of the α-amino group of the amino acid. The protected oligonucleotides containing all 4 common bases were synthesized in a stepwise fashion using benzotriazolyl phosphotriester methodology.[4]

Although we have been able to synthesize several guanosine containing sequences using synthons protected only on N^2 of its aglycon (Figure 1., e.g. **1**), we have obtained rather modest yields of protected oligonucleotides.[2] In fact, if compound **2** is treated with an excess of phosphorylating reagent **3**, and the reaction mixture is analyzed by TLC, a base line material is detected, indicating phosphorylation on the unprotected O^6 of guanine in agreement with Reese and Richards [5], but in variance with de Vroom et al.[6]

Therefore, we have investigated the O^6-protection of guanosine which would be suitable, when combined with the N^2-Fmoc group, for the synthesis of 2'(3')-O-aminoacyl oligonucleotides. Needless to say, all protecting groups have to be removable from the newly formed 2'(3')-O-aminoacyl oligonucleotide molecules with retention of the unstable 2'(3')-O-aminoacyl bond. This fact *per se* precludes the use of several O^6-guanosine protecting groups described in the literature.[7]

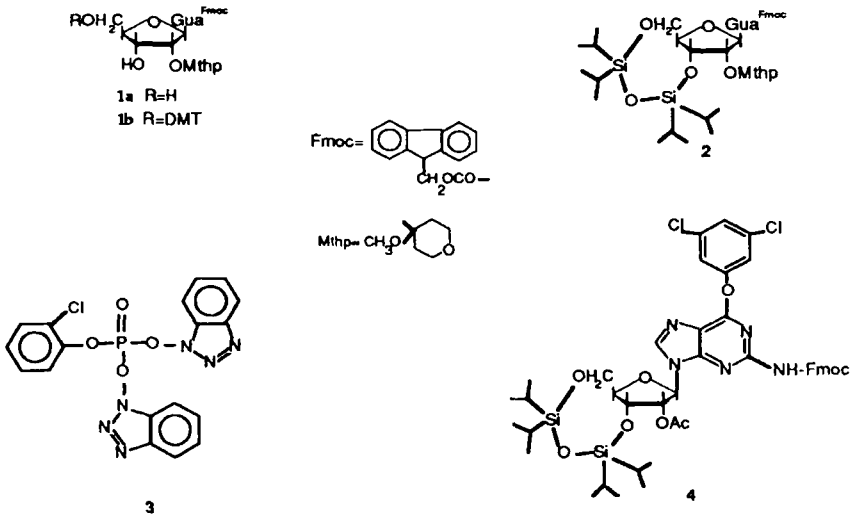
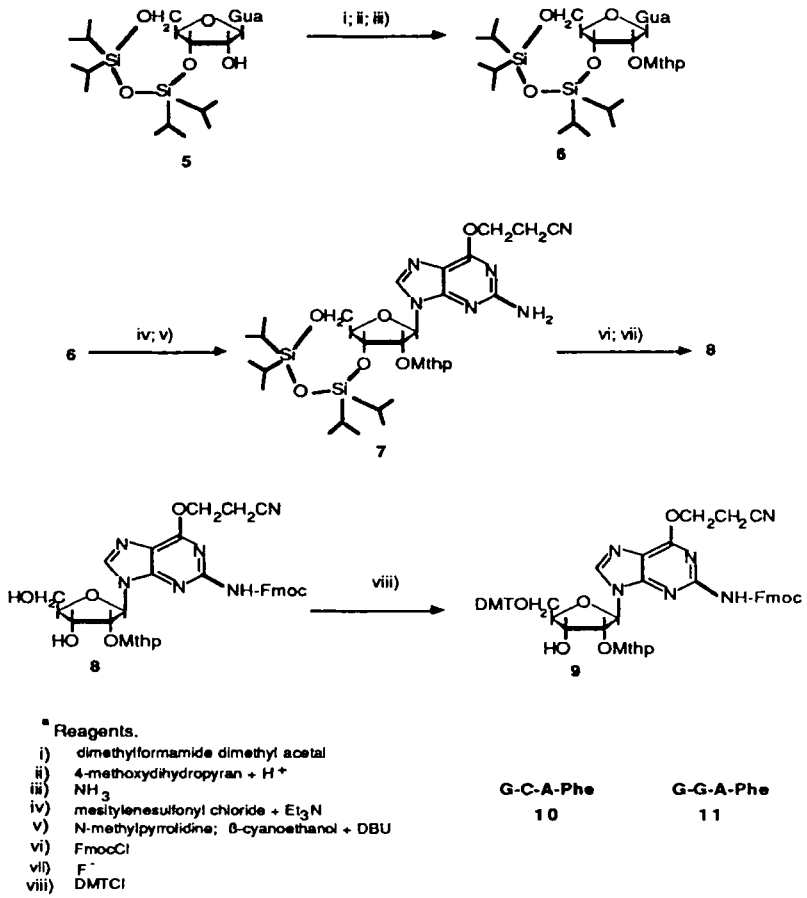


FIGURE 1.

First, we studied an application of the 3,5-dichlorophenyl group for the protection of the O^6-guanosine functionality, since this group can be easily removed *via* the oximate[8] treatment employed for the removal of the 2-chlorophenyl and N-Fmoc groups.[1] However, we found that the reaction of the model compound **4** with dry oximate/TMG reagent[1] does not lead to removal of the 3,5-dichlorophenyl group as expected on the basis of a previous report.[8] It appears that removal of the phenol-type protecting groups from the O^6 of guanosine with oximate/TMG requires the substitution of the N^2 with a group which *is not removable* by oximate treatment - e.g. phenylacetyl group as in cf[8]. It is clear then that this route is not applicable to the synthesis of our target compounds.

We have thus searched for another O^6- blocking group which would be easily removable with the oximate/TMG reagent and found that the O^6-(β-cyanoethyl) group[9] fulfills our requirements perfectly. We discovered that the latter group is removable from the O^6 of guanine *via* brief treatment with oximate/TMG, regardless of whether the N^2 functionality is protected or unprotected. Thus, synthons **8** and **9** were synthesized as shown in Scheme 1. The β-cyanoethyl group was introduced *via* displacement reactions according to Reese and Skone[8] and Gaffney and Jones[9] (entry **6** to **7**). In contrast to the introduction of the Fmoc group to a O^6-unprotected guanosine intermediate (cf[2]), the introduction of the Fmoc group to **7** proceeds in a rapid fashion and in an excellent yield to form **8**.



[a] Reagents.

i) dimethylformamide dimethyl acetal
ii) 4-methoxydihydropyran + H^+
iii) NH_3
iv) mesitylenesulfonyl chloride + Et_3N
v) N-methylpyrrolidine; β-cyanoethanol + DBU
vi) FmocCl
vii) F^-
viii) DMTCl

G-C-A-Phe	G-G-A-Phe
10	11

SCHEME 1.[a]

To illustrate the use of our blocking scheme, two 2'(3')-O-phenylalanyl trinucleotides - G-C-A-Phe (10) and G-G-A-Phe (11) - were synthesized using synthons **8** and **9** and analogous cytidine and adenosine components[1] applying a stepwise benzotriazolyl phosphotriester approach.[4] As expected, the yields of the protected di- and trinucleotides were higher than those without the O^6-protection of guanosine, and the reaction mixtures were completely free of side products. The protected G-C-A and G-G-A were amino-acylated on the free 3'-OH with BPOC-Phe and (mesitylenesulfonyl)tetrazole (cf[1,2]), and the resulting intermediates were deblocked in two steps (oximate/TMG treatment and hydrolysis with dilute formic acid) to generate sequences **10** and **11**.

Both oligonucleotides were characterized by routine analytical means, including electrophoresis, TLC, UV spectroscopy and enzymatic hydrolysis with T_1 RNAse or RNAse

A and *Crotalus durissus* snake venom diesterase to the expected products without detecting the unnatural phosphodiester linkages .

The methodology is now applicable for the synthesis of longer 2'(3')-O-aminoacyl oligoribonucleotide fragments of aa-tRNA.

Acknowledgements: This research was supported by the NIH Training Grant T-32-CA-09531 and by Institutional Grant to the Michigan Cancer Foundation from the United Foundation of Greater Detroit.

REFERENCES AND FOOTNOTES

For abbreviations used see: *Handbook of Biochemistry,* Sober, H.A., Ed.; CRC Press, Cleveland OH, Sections A and B. Other abbreviations: aa-tRNA, aminoacyl transfer ribonucleic acid; Gua^{Fmoc}, N^6-((9-fluorenylmethyloxy)carbonyl)guanine-9-yl; BPOC, 2-(4-biphenylyl)isopropyl-oxycarbonyl; BT, benzotriazolyl; G-C-A-Phe, guanylyl(3'-5')-cytidylyl(3'-5')-2'(3')-0-(*L*-phenyl-alanyl)adenosine; 2Cl-Ph, 2-chlorophenyl; DMT, 4,4'-dimethoxytrityl; Fmoc, 9-fluorenylmethyl-oxycarbonyl; MST, (mesitylenesulfonyl) tetrazole; Mthp,4-methoxytetrahydropyran-4-yl; TMG, N^T,N^1,N^3,N^3-tetramethylguanidine.

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