Pergamon

Tetrahedron Letters, Vol. 36, No. 37, pp. 6651-6654, 1995
Elsevier Science Ltd
Printed in Great Britain
0040-4039/95 $9.50+0.00

0040-4039(95)01395-4

Tricyclic "Oxadiazaphosphorine Oxide" Guanosines A New Strategy To Functionalize Guanosine at the Exocyclic Amino Group

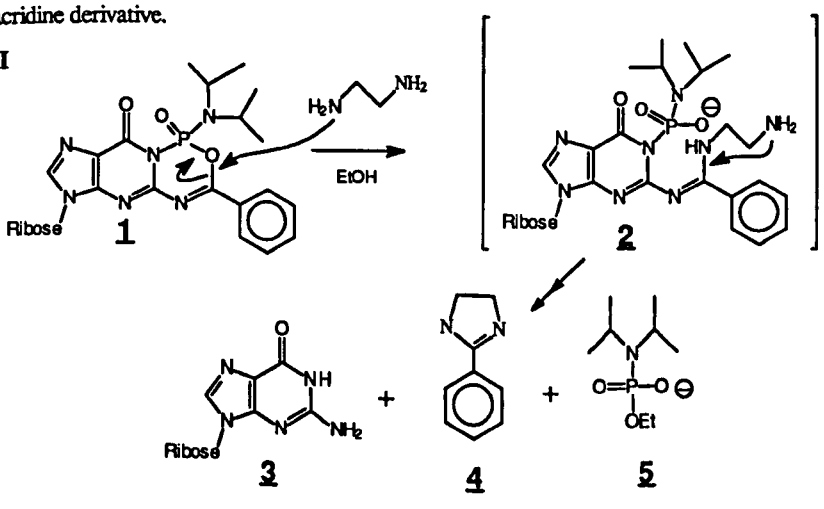
Sébastien Robidoux, Yunkui Guo and Masad J. Damha*
Department of Chemistry, McGill University
Montreal, Quebec, Canada H3A 2K6

Abstract: A simple procedure is described for the preparation of modified guanosines via an oxadiazaphosphorine oxide guanosine derivative. This nucleoside has been successfuly used in the synthesis of guanosine containing $-(CH_2)_2OH$, $-(CH_2)_6NH_2$, $-NHNH_2$ "handles", or a tethered acridine moiety at the 2-position.

The synthesis of oligonucleotides conjugates has been a very powerful tool to study the physical and biochemical properties of nucleic acids.[1,2] This is due to the inherent specificity of the nucleic acid portion of the bioconjugate and the almost infinite effects of the different molecules that can be coupled to the oligonucleotides. Conventionally, oligonucleotide conjugates have been prepared by chemical assembly of the oligomer using modified nucleosides or 5'-phosphitylating reagents bearing the conjugate.[3,4] In a few cases, the conjugate has been introduced only after the oligomer has been completely assembled.[5,6] This latter approach is particularly attractive as it avoids the multi-step synthesis of the nucleoside conjugate and is, in principle, applicable to the synthesis of more complex and base sensitive bioconjugate structures.

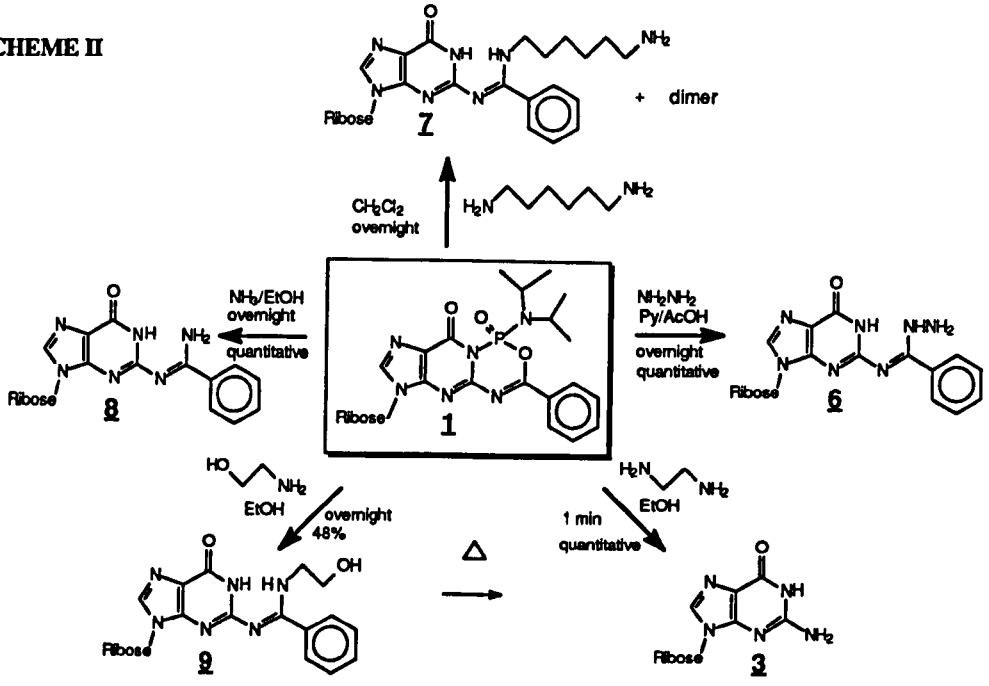
Recently, we reported the synthesis of novel tricyclic guanosine derivatives containing an oxadiazaphosphorine ring and demonstrated their usefulness as "protected" guanosines in the solid-phase synthesis of oligoribonucleotides.[7] The strength of these derivatives is their stability to the normal conditions of DNA/RNA synthesis, yet their exceptionally rapid and clean transformation into guanosine **3** (ethylenediamine/ethanol 1:4, r.t., 1 min) (Scheme I). We now describe the reactions of these derivatives with other amino nucleophiles including an alkylamine acridine derivative.

SCHEME I



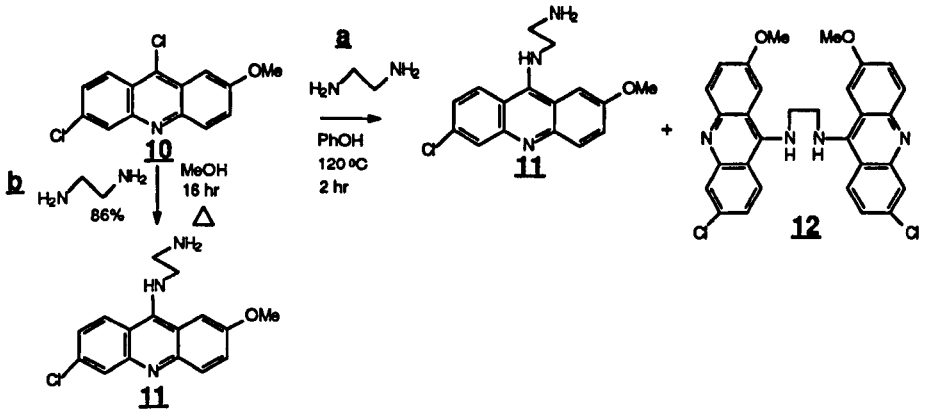
The reactions investigated are summarized in **Scheme II**. The key tricyclic precursor **1** was prepared in two steps from 5',3',2'-tri-O-t-BDMSi-N^2-benzoylguanosine according to our procedure (94% overall).[7] Treatment of **1** with either hydrazine, hexanediamine, ammonia or ethanolamine produced moderate to high yields of guanosine derivatives **6**, **7**, **8** and **9**, respectively. In each case, the adducts formed could be isolated and characterized (Table I). Thus, the only requirement in these reactions was to have a $-NH_2$ nucleophile to open the phosphorine ring. Upon standing and/or heating, compound **9** converted cleanly to 5',3',2'-tri-O-t-butyldimethylsilylguanosine.

SCHEME II



We then investigated whether our model tricyclic compound could be coupled to a polycyclic aromatic molecule of potential interest. Acridine was chosen because of its ready availability and current use as intercalating agent in "antisense" and "antigene" strategies.[8,9] In order to provide an anchor for attachment onto our guanine derivative, acridine was derivatized with an ethylenediamine linker (i.e., **11**) according to the procedure of Hélène and co-workers.[10] Some difficulties were encountered during this synthesis. In our hands, the reaction of 6,9-dichloro-2-methoxyacridine (**10**) with ethylenediamine gave, in addition to **11**, **12** and other side-products (tlc analysis) (**Scheme IIIa**). Moreover, removal of phenol and separation of these products was very tedious and time consuming. When acridine **10** was treated with a large excess of ethylenediamine in refluxing methanol, the desired

SCHEME III



acridine **11** was produced, with no other side products (**Scheme IIIb**). Acridine **11** was purified by partially removing methanol from the reaction mixture and precipitating the compound by addition of water (84% yield). The target guanosine-acridine bioconjugate **13** was obtained by treating **1** with 1.25 equivalents of **11** in dichloromethane and methanol (9:1 v/v) for 60 min at room temperature (**Scheme IV**). Purification by column chromatography (gradient 100 % CH_2Cl_2 to CH_2Cl_2:MeOH 9.5:0.5) afforded **13** in 40% yield (unoptimized); the structure of which was established by FAB-MS and spectroscopic means (**Table I**).

The chemistry described here represents an attractive strategy for the preparation of novel nucleosides. It is also amenable to the synthesis of oligonucleotides that can be conjugated after synthesis with a wide variety of biologically important molecules. This work is in progress.

SCHEME IV

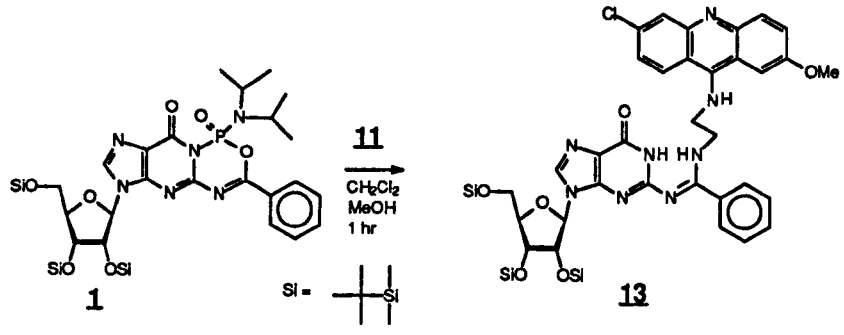


TABLE I. Physical properties of guanosine derivatives

Compound	R_f(TLC)	UV (EtOH, λ max)	FAB-MS[i] MH^+ $B+2H^+$
1	0.73[a]	358, 345, 264, 208	875, 401
3	0.03[b]	256, 278sh	626, 152
6	0.47[c]	268sh, 248sh	744, 270
7	0.38[d]	234, 272sh, 300	828, 354
8	0.32[e]	321, 242	729, 255
9	0.32[f]	247, 308, 268sh	773, 299
10	0.93[g]	405, 386, 353, 336, 321, 264	
11	0.36[h]	415, 359, 342, 326, 268, 228	302
13	0.27[g]	415, 359, 341, 269, 226	1013, 539

a) ether:CH_2Cl_2 1:3; b) ether:CH_2Cl_2 3:7; c) EtOAc; d) CH_2Cl_2:MeOH:Et_3N 8:1:1;
e) ether : CH_2Cl_2 1:1; f) MeOH : EtOAc 7 : 93; g) CH_2Cl_2 : MeOH 9 : 1;
h) CH_2Cl_2 : MeOH : Et_3N 8 : 2 : 1; i) Glycerol matrix, except for **13** (NBA matrix).

Acknowledgements. We gratefully acknowledge financial support from the National Research Council of Canada (NSERCC) and Les Fonds pour la Formation de Chercheurs et l'Aide à la Recherche (FCAR), and a FCAR and NSERC post-graduate scholarships to S.R.

References

1. Mergny, J. L.; Boutorine, A. S.; Garestier, T.; Belloc, F.; Rougee, M.; Bulychev, N. V.; Koshkin, A. A.; Bourson, J.; Lebedev, A. V.; Valeur, B.,Thuong, N. T. and Hélène, C. *Nucl. Acids Res.* **1994**, *22*, 920-928.
2. Singh, M. P.; Hill, G. C.; Peoc'h, D.; Rayner, B.; Imbach, J. L.; Lown, J. W. *Biochemistry* **1994**, *33*, 10271-10285.
3. Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1993**, *46*, 10441-10488.
4. Manoharan, M.; Tivel, K. L.; Andrade, L. K.; Cook, P. D. *Tetrahedron Lett.* **1995**, *36*, 3647-3650.
5. Harris, C. M.; Zhou, L.; Strand, E. A.; Harris, T. M. *J. Am. Chem. Soc.* **1991**, *113*, 4328-4329.
6. Zheng, Q.; Xu, Y.Z.; Swann, P.F. *Nucleosides & Nucleotides* **1995**, *14*, 939-942, and references therein.
7. Damha, M.J.; Guo, Y.; Zabarylo, S.V.; Ganeshan, K.; Giannaris, P.A. *Tetrahedron Lett.* **1992**, *33*, 6739-6742.
8. Toulmé, J.-J.; Krisch, H. M.; Loreau, N.; Thuong, N.T.; Hélène, C. *Proc.Natl.Acad.Sci.USA* **1986**, *83*, 1227-1231.
9. Durand, M., Maurizot, J.C., Asseline, U., Thuong, N.T., Hélène, C. *Bioconjugate Chem.* **1993**, *4*, 206-211.
10. Asseline, U.; Thuong, N.T.; Hélène, C. *Nucleosides & Nucleotides* **1986**, *5*, 45-63.

(*Received in USA* 30 *June* 1995; *revised* 18 *July* 1995; *accepted* 20 *July* 1995)