Pergamon

Tetrahedron Letters, Vol. 38, No. 35, pp. 6123-6126, 1997
© 1997 Elsevier Science Ltd
All rights reserved. Printed in Great Britain
0040-4039/97 \$17.00 + 0.00

PII: S0040-4039(97)01388-9

A NEW METHOD FOR OLIGONUCLEOTIDE DERIVATIZATION OF THE 3' OR 5'- TERMINI WITH A CPG-SUPPORT CARRYING THE NATURAL PRODUCT ISOARGENTATIN-D

Paul Gaytán,[¥]* Jorge Yañez[¥], Xavier Soberón[¥] and Roberto Martínez[#]

[¥]Instituto de Biotecnología, Universidad Nacional Autónoma de México, Apartado Postal 510-3, Cuernavaca, Morelos 62271, México.

[#]Instituto de Química, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, Coyoacán 04510, México D.F, México.

Abstract: Oligonucleotides were modified at their 5' or 3'-termini using a CPG-support carrying isoargentatin-D, taking advantage of suitable commercial phosphoramidites to perform automated oligonucleotide synthesis in the 3'→5' or 5'→3' direction. © 1997 Elsevier Science Ltd.

It is well known that attachment of lipophilic molecules to oligonucleotides (ODNs) increases their cellular uptake[1]. However due to the additional purification steps, non-nucleosidic phosphoramidites are more difficult to prepare than the corresponding modified supports. Also, the phosphoramidite is used much less efficiently than the support because of the large excess used in solid phase-synthesis. Moreover, both phosphoramidite and support are necessary when chemical derivatization of the 5' or 3'-termini is accomplished in solid-phase automated synthesis in 3'→5' direction as usual[2]. These considerations are especially important when only small quantities of rare substances are available. As part of a program directed toward the synthesis of modified oligonucleotides for antisense and antigene applications, we believed that the use of a derivatized modified support along with the regular 3' or 5'-cyanoethylphosphoramidites[3] would provide a flexible method to produce either 3' or 5' modification of oligonucleotides.

In this report, we describe the synthesis of Isoargentatin-D-modified support (**4**) and its evaluation in automated synthesis of oligonucleotides using two pentathymidylic acids as models. Reduction of isoargentatin-B (**1**) -a cholesteryl-like product obtained in high proportions from the Mexican rubber plant Parthenium argentatum (guayule)[4,5]- with $NaBH_4$ in THF gave the mixture of isoargentatin-D epimers (**2**). This mixture was treated with 1.5 equivalents of 4,4'-dimethoxytrityl chloride (DMT-Cl) in pyridine for 24 hours to afford a mixture of 3-DMT-isoargentatin-D epimers **3a** and **3b** in proportions 1.0:2.7 respectively. This mixture was then subjected to reverse-phase HPLC purification[6] and gave retention times (t_{RS}) of 11.61 min assigned to the equatorial isomer **3a**, and 13.74 min assigned to the axial isomer **3b** after ^1H NMR analysis[7].

Using the axial isomer **3a** as a representative example, LCAA-CPG support was derivatized with **3a** through an oxalyl linkage following the conditions of Letsinger[8] to give the modified solid support **4** with a

loading of 3.8 µmol/g. This low loading reflects the steric hindrance of tertiary hydroxyl group used as the point of attachment. Some attempts to succinylate **3a** under different conditions[9,10] were unsuccessful.

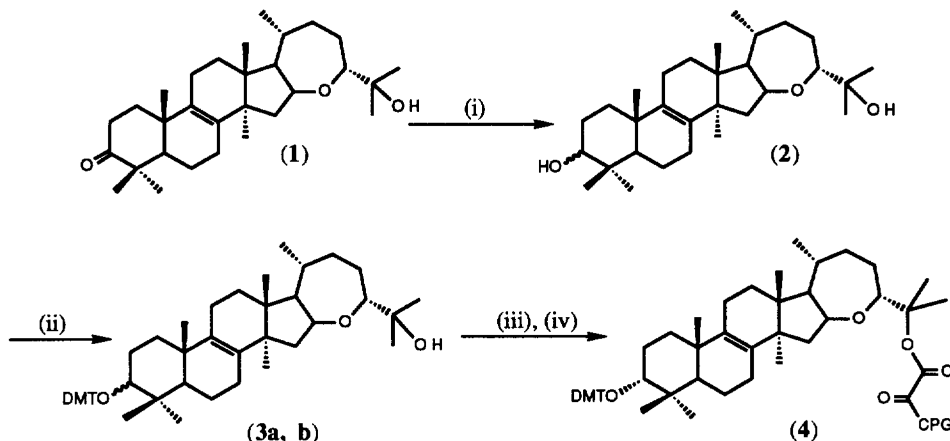

Figure 1. Derivatization of the CPG-support with isoargentatin-D. (i) $NaBH_4$/THF, (ii) DMT-Cl/py, (iii) oxalyl chloride / 1,2,4-triazol / acetonitrile / py, (iv) LCAA-CPG

The modified support **4** was evaluated in automated synthesis of oligonucleotides of two pentathymidylic acids employing either 5'-dimethoxytrityl-thymidine-3'-ß-cyanoethyl-N,N-diisopropylamino-phosphoramidite, to perform the synthesis in 3'→5' direction[11], or 3'-dimethoxytrityl-thymidine-5'-ß-cyanoethyl-N,N-diisopropylamino-phosphoramidite, to perform the synthesis in 5'→3' direction[12], according to a 40 nmol standard protocol from Applied Biosystems.

Treatment of the solid supported oligonucleotides with NH_3 (aq) at room temperature for 2 h enabled removal of the protecting groups and detachment of the oligonucleotide product from the solid support. Oligonucleotides were resuspended in 0.2 M triethylammonium hydrogencarbonate buffer at pH 7.5 to avoid loss of DMT group. Additionally, we synthesized two hexathymidylic acids in both directions under normal conditions to employ them as reference in the reversed-phase HPLC[13] and PAGE[14] analysis; In this way, all four oligonucleotides would bear five phosphate groups and similar molecular weights as shown in table 1. All them were left in their DMT-on mode.

In order to demonstrate the lipophilic nature of the dimethoxytrityl and isoargentatin-D molecules, a portion of each oligonucleotide was detritylated with 80% acetic acid. The 5'-DMT-hexathymidylic acid (**5**) was subjected to reverse-phase HPLC analysis and gave retention time of 28.35 min: elimination of the hydrophobic DMT group afforded compound **6** with a retention time of 8.99 min (Δt_R =19.36 min, table 1). On the other hand, the 5'-isoargentatin-pentathymidylic-3'-DMT acid (**7**) and 5'-DMT-pentathymidylic-3'-isoargentatine acid (**9**) showed highest t_R and lowest mobility in PAGE because they have two lipophilic molecules per oligonucleotide. Elimination of the DMT group from **7** and **9** acids gave **8** and **10** acids whose t_R was lowered

5.47 and 6.27 min, respectively. This little Δt_R confirmed the presence of the lipophilic isoargentatine-D molecule.

Table 1. Physical behaviour in RP-HPLC[13] and PAGE[14] of the oligonucleotides synthesized in this work.

Compound	Molecular weight	$t_R{}^a$ (min)	$R_m{}^b$
(**5**) 5' DMT-$(Tp)_5$T 3'	2149	28.35	0.822
(**6**) 5' $(Tp)_5$T 3'	1847	8.99	0.934
(**7**) 5' Arg-$(pT)_5$-DMT 3'	2363	37.46	0.702
(**8**) 5' Arg-$(pT)_5$ 3'	2061	31.99	0.784
(**9**) 5' DMT-$(Tp)_5$-Arg 3'	2363	38.17	0.714
(**10**) 5' $(Tp)_5$-Arg 3'	2061	31.90	0.808
(**11**) 5' $(Tp)_5$T-DMT 3'	2149	24.08	0.804

a. t_R = retention time

b. R_m = Relation of mobilities with respect to bromophenol blue.

In this way, we have set up a new approach for oligonucleotide derivatization at the 3' or 5'-terminus through the employment of only one modified CPG-support. The important point here is to select the appropriate DMT-nucleoside-phosphoramidites to perform the oligonucleotide synthesis either in 3'→5' or 5'→3' direction. Although not reported here, it is evident that both kinds of derivatizations can also be accomplished with only one appropriately modified phosphoramidite.

Acknowledgments. We thank Eugenio López and Isabel Chávez for helpful advice on ODN synthesis and NMR analysis and Dr. Hugh Mackie from Glen Research Co. for careful reviewing of the manuscript.

REFERENCES AND NOTES

1. Boutorin, A.S.; Gus'kova, L. V.; Ivanova, E. M.; Kobetz, N. D.; Zarytova, V. F.; Ryte, A. S.; Yurchenko, L. V.; Vlassov, V. V.; *FEBS Lett.* **1989**, *254*, 129-132.

2. Beaucage, S. L.; Radhakrishnan, P. I.; *Tetrahedron* **1993**, *49*, 1925-1963.

3. Reagents available from Glen Research Corporation. 44901 Falcon Place, Sterling, VA 20166, USA.

4. Romo de Vivar, A.; Martínez-Vazquez, M.; Matsubara, C.; Pérez-Sánchez, G.; Joseph-Nathan, P.; *Phytochemistry* **1990**, *29*, 915-918.

5. Isoargentin-B was obtained by hexane elution of a silicagel column chromatography previously charged with

the acetonic extract of guayule.

6. For this analysis we used an analytical (4.6 x 250 mm) ODS column from Vydac and an isocratic run during 5 min of buffer A followed of a gradient from 0% to 30% of buffer B in 20min. The flow was setup at 1.2 ml/min. Buffer A was acetonitrile and buffer B was methanol.

7. ^{1}H NMR analysis was performed at 300 MHz in a Varian Unity 300. The main difference between both epimers was found in the signals corresponding to H-3.

8. Alul, R. H.; Singman, C. H.; Zhang, G.; Letsinger, R. L.; *Nucleic Acids. Res.* **1991**, *19*, 1527-1532.

9. Sharma, P.; Sharma, A. K.; Malhotra, V. P.; Gupta, K. C.; *Nucleic Acids. Res.* **1992**, *20*, 4100.

10. Atkinson, T.; Smith, M.; Solid-phase Synthesis of Oligodeoxyribonucleotides by the Phosphite-triester Method. In Oligonucleotide Synthesis: a practical approach; Gait, M. J. Ed.; IRL press: Oxford, 1984; pp. 47-48.

11. Average coupling step with this phosphoramidite was 97%.

12. Average coupling step with this phosphoramidite was 98.5%.

13. Oligonucleotides were analyzed on an analytical (4.6 x 150 mm) ODS Spherisorb column from Beckman, with a flow of 1 ml/min. Buffer A was 0.1M triethylammonium acetate pH 7.0 and buffer B was acetonitrile. The run was carried out under next conditions: gradient of buffer B from 7% to 27% in 16 min, followed by an isocratic of 27% of buffer B during 8 min and a gradient of buffer B from 27% to 40% in 5 min.

14. PAGE analysis was performed in an analytical "tall mighty small" camera from Hoefer Scientific Instruments, employing a 30% polyacrylamide / 5M urea gel.

(*Received in USA* 30 *April* 1997; *revised* 25 *June* 1997; *accepted* 2 *July* 1997)