Pergamon

Tetrahedron Letters, Vol. 37, No. 29, pp. 5123-5126, 1996

0040-4039/96 $15.00 + 0.00

PII: S0040-4039(96)01065-9

Preferential Phosphorylation at the Primary Alcohol of Non-Protected Thymidine or Carbohydrates.

Laurent Knerr, Xavier Pannecoucke, Gaby Schmitt, and Bang Luu*

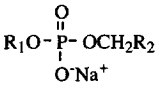
Laboratoire de Chimie Organique des Substances Naturelles, associé au CNRS, Université Louis Pasteur, Centre de Neurochimie, 5, rue Blaise Pascal - 67084 Strasbourg (France)

Abstract : A preferential phosphorylation at the primary alcohol of non-protected thymidine or carbohydrates by the hydrogen phosphonate method is described. Thus, 6-(mannopyrannosyl), 6-(glucopyrannosyl) or 5'-(thymidinyl) phosphodiesters were obtained in 35% to 80% yields.

Biological substances such as phospholipids, oligonucleotides or carbohydrate phosphates often present in their structure a phosphorylated primary alcohol function. Therefore, selective phosphorylation at the desired position is a challenge for organic chemists. [1-4] Recently, many phosphate prodrugs of biologically active compounds have been synthesized, owing to the improvement of hydrosolubility and bioavailability, faciliting *in vivo* studies. [5-9]

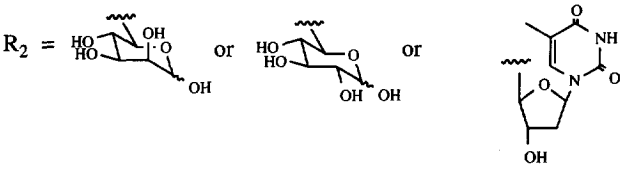
In our research program on oxygenated derivatives of oxysterols, we are interested in the preparation of prodrugs of several lipophilic compounds. We have already shown that the linkage of an oxysterol of biological interest with a carbohydrate or a nucleotide via a phosphodiester bond permits an increase of the water solubility and/or a targeting of the drug to a specific organ. [10-11]

However the use of protected nucleosides or carbohydrates often gives undesirable side reactions, during the phosphorylation reaction itself or the removal of the corresponding protective groups. [1-2,12-14] We have, therefore developed a straightforward method for the synthesis of phosphodiesters of type **1**, using the hydrogen phosphonate methodology. [15]

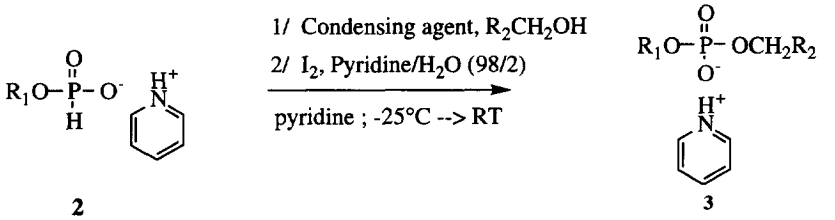


1

R_1 = cyclic or linear alkyl chain



These syntheses require only two steps and because high regioselectivity can be achieved in the second coupling step, protection of the carbohydrate or nucleoside is not needed.



2 **3**

The choice of the hydrogen phosphonate method was made as its mild conditions are compatible with most organic fonctions. Furthermore intermediates of type **2** are stable and easy to handle in contrast with phosphoramidite equivalents. [11]

Following the procedure of van Boom and coworkers [15], compounds of type **2** were obtained in very good yields, even for less reactive long chain alcohols such as the neurotrophic hexacosanol. [16] Slow addition (15 to 20 min) of a solution of the corresponding alcohol (1eq.) in dioxane/pyridine (4/1) to 2-chloro-1,3,2-

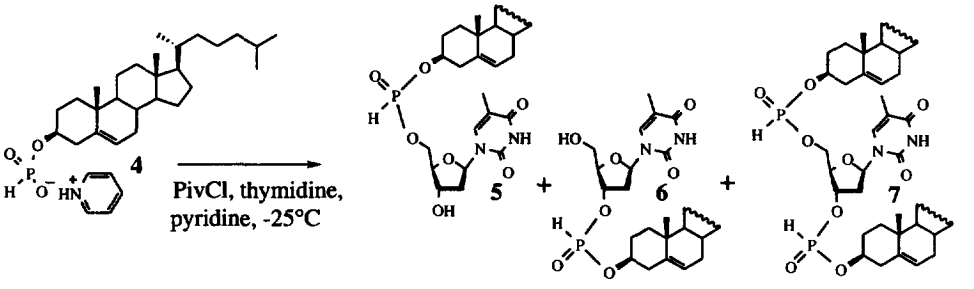
benzodioxaphosphorin-4-one (1.2 eq.) in dioxane afforded, after hydrolysis and chromatography on silicic acid [17] (Sigma), the desired H-phosphonate. Yields are indicated in Table 1.

Table 1 : Synthesis of H-phosphonates monoesters of type **2**

R_1OH	Yield (%)[a]	^{31}P NMR δ (ppm) ; J_{P-H} (Hz)
Cholesterol	95	4.04 ; 660
Cholestanol	92	4.45 ; 662
Menthol	97	4.49 ; 674
Decanol	88	6.58 ; 671
Hexadecanol	81	6.19 ; 693
Hexacosanol [b]	83 [c]	6.34 ; 680

[a] Isolated yields ; [b] hexacosanol was added in chloroform/pyridine (4/1) ; [c] reaction time 8 hours

Cholesterol hydrogen phosphonate **4** was used as a model compound to determine the best conditions for the second reaction, using pivaloyl chloride (PivCl) as the condensing agent and thymidine as the second alcohol (R_2CH_2OH) . The condensing agent (3 eq.) was added over 10 min to a solution of **4** (1 eq.) and thymidine in pyridine at -25°C and stirred for 20 min. Results are plotted in Table 2. [18]

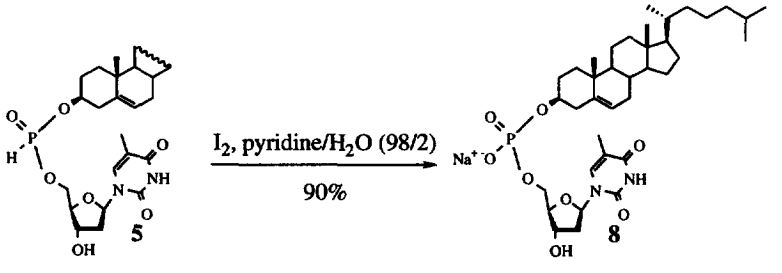


Removal of the solvents and chromatography on silicic acid afforded the desired compound **5** and two minor by-products **6** and **7** corresponding to phosphorylation and bis phosphorylation of the secondary alcohol Conditions and yields are indicated in Table 2.

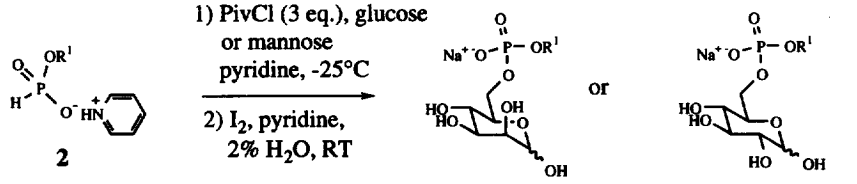
Table 2 : Study of the coupling of **4** with thymidine

	Yields (%)		
Thymidine	5	6	7
1 eq.	45	12	13
2 eq.	70	17	5
3 eq.	87	8	1

Oxidation of compound **5** with iodine in pyridine/water (98/2, v/v) [15], afforded **8** in 90% yield.



We have extended the scope of this method by using other compounds of type **2** and mannose or glucose as the second alcohol. However in these cases it was not possible to isolate the corresponding hydrogen-phosphonate diester, owing to their instability on the chromatographic support. Thus, after 20 min reaction at -25°C, iodine (1 eq.) in pyridine/water (98/2, v/v) was added to the reaction mixture and stirred for 2 h at room temperature. After work-up and chromatography on silicic acid and Dowex AG 50W-X8 (Na^+ form) compounds of type **9** and **10** were obtained (Table 3).[18]



2 **9** **10**

The highest and most reproducible yields were obtained using 5 eq. of the carbohydrate and 3 eq. of PivCl. Studies of other more selective condensing agents are in progress. 6-O-Pivaloyl mannose or glucose was the major side product. For linear alcohols, yields decrease rapidly when the length of the alkyl chains used increases and despite our efforts a prodrug of the neurotrophic hexacosanol could not be obtained.

The hydrogen-phosphonate method has already been shown to be a mild and chemoselective [18] method of phosphorylation of functionalized molecules. We wished here to demonstrate that it also permits the preferential phosphorylation of the primary alcohol site of some unprotected carbohydrates or nucleosides. Yields from 35 to 80% can be achieved, depending on the reactivity of the hydrogen-phosphonate monoester used.

Table 3 : Synthesis of H-phosphonate diesters

	R^1	R^2CH_2OH	Yield (%)
9a	3β(Cholesteryl)	Mannose 1 eq.	35
9a	3β(Cholesteryl)	Mannose 2eq.	45
9a	3β(Cholesteryl)	Mannose 5 eq	45
10a	3β(Cholesteryl)	Glucose 5 eq.	45
9b	3β(Cholestanyl)	Mannose 5 eq	41
10b	3β(Cholestanyl)	Glucose 5 eq	65
9c	Menthyl	Mannose 5 eq	58
10c	Menthyl	Glucose 5 eq	39
9d	Benzyl	Mannose 5 eq	70
9e	Decyl	Mannose 5 eq	41
9f	Hexadecyl	Mannose 5 eq	35
9g	Hexacosyl [a]	Mannose 5 eq	No reaction

[a] reaction at RT.

References :

1. Mitsunobu, O. Kato, K. and Kimura, J. *J. Am. Chem. Soc.* **1969**, *91*, 6510-6511.
2. Mitsunobu, O. *Synthesis* **1981**, 1-28.
3. Follmann, H. *Tetrahedron Lett.* **1967**, 2113-2116.
4. Yoshikawa, M.; Kato, T. and Takenishi, T. *Tetrahedron Lett.* **1967**, 5065-5068.
5. Roche, E.B. "Design of Biopharmaceutical Properties through Prodrugs and Analogs" *American Pharmaceutical Association.* ; Washington DC., USA **1977**, 375-376.
6. Meier, C. ; Neumann, J.M. ; André, F. ; Hénin, Y. and Huyn-Dinh, T. *J.Org.Chem.* **1992**, *57*, 7300-7308.
7. Balagopala, M.I. ; Ollapally ; A.P. and Lee, H.J. *Cell. Mol. Biol.* **1995**, *41*, 51-57.

8. Meier, C. *Angew. Chem. int. Ed. Engl.* **1996**, *35* , 71-73.
9. Morin, G.T and Smith, B.D. *Tetrahedron Lett.* **1996**, *37*, 3101-3104.
10 Ji, Y.H.; Moog, C.; Schmitt, G.; Bischoff P. and Luu, B. *J. Med. Chem.* **1990**, *33*, 2264-2270.
11. Pannecoucke, X.; Schmitt, G. and Luu B. *Tetrahedron* **1994**, *50*, 6569-6578.
12. Rammler, D.H. and Khorana, H.G. *J. Am. Chem. Soc.* **1962**, *84*, 3112-3122.
13. Reese, C.B. and Trentham, D.R. *Tetrahedron Lett.* **1965**, 2459-2462 and 2467-2470.
14. Söll, D. and Khorana, H.G. *J. Am. Chem. Soc.* **1965**, *87*, 360-367.
15. Stawinski, J. Some Aspects of H-Phosphonate Chemistry In *Handbook of Organophosphorus Chemistry* ; R. Engel Ed, Marcel Dekker, Inc. ; New York, **1992**, pp 377-434 and references cited therein.
16. Borg, J.; Toazara, J.; Hietter, H.; Henry, M.; Schmitt, G. and Luu, B. *FEBS Lett.* **1987**, *213*, 406-410.
17. Hirsch, J.; Ahrens, E.H., Jr. *J. Biol. Chem.*, **1958**, *233*, 311-318.
18. Selected data :

(8) $[\alpha]_D$ = + 1 (c =1, DMSO). **^{1}H-NMR** (DMSO-d_6) : 0.63 (s, 3H, CH_3-18); 0.83 (d, 6H, J = 6.5 Hz, CH_3-26 and 27); 0.88 (d, J = 6.5 Hz, 3H, CH_3-21); 0.92 (s, 3H, CH_3-19); 1.79 (s, 3H, CH_3nuc); 3.68-3.84 (m, 4H, J = 8 Hz, H-3, H-4'nuc, 2 H-5'nuc); 4.28 (b, 1H, H-3'nuc); 5.21 (s, 1H, H-6); 5.71 (b, 1H, OH-3'nuc); 6.19 (t, 1H, J = 6.6 Hz, H-1'nuc); 7.80 (s, 1H, H-6nuc). **^{13}C-NMR** (DMSO-d_6) : 11.60 (C-18); 12.11 (CH_3 nuc); 18.48 (C-21); 19.00 (C-19); 22.31 (C-26); 22.58 (C-27); 55.51 (C-14); 56.08 (C-17); 64.24 (C-5'nuc); 70.87 (C-3'nuc); 73.15 (C-3); 83.61 (C-4'nuc); 86.12 (C-1'nuc); 109.64 (C-5nuc); 120.68 (C-6); 136.34 (C-5); 140.77 (C-6nuc); 150.49 (C-2nuc); 163.76 (C-4nuc). **^{31}P-NMR** (DMSO-d_6) : - 0.98. **FAB-MS** negative (matrix: NBA): 711.2 [M-H ; 17]; 689.2 [M - Na; 100]; 563.2 [M - thymine - Na; 18]; 487.0 [M - thymidine; 10]; 465.2 [487.0 - Na + H; 50]; 321.0 [M - Steroid - Na + H; 8]. **Anal.** Calc. for C_{37} H_{58} O_8 N_2 P Na, 3 H_2O (766.8) C : 57.95, H : 8.41. Found C : 57.95, H : 8.39.

(9a) $[\alpha]_D$ = - 26 (c = 1, DMSO). **^{1}H-NMR** (DMSO-d_6) : 0.62 (s, 3H, CH_3-18); 0.83 (d, 6H, J = 6.5 Hz, CH_3-26 and 27); 0.88 (d, J = 6.4 Hz, 3H, CH_3-21); 0.96 (s, 3H, CH_3-19); 3.51-3.90 (m, 6H, H-3, H-6'a, H-5', H-4', H-3', H-2'); 4.17 (d, 1H, J = 8 Hz, H-6'b); 4.51-4.62 (m, 3H, OH-4', OH-3', OH-2'); 4.85 (b, 1H, H-1'α); 5.30 (b, 1H, H-6); 6.29 (b, 1H, OH-1'). **^{13}C-NMR** (DMSO-d_6) : 11.66 (C-18); 18.59 (C-21 et C-19); 22.32 (C-26); 22.59 (C-27); 55.00 (C-14); 55.89 (C-17); 64.98 (C-6'); 65.93 (C-5'); 66.47 (C-4'); 70.01 (C-2'); 71.26 (C-3'); 76.88 (C-3); 94.12 (C-1'); 121.47 (C-6); 140.73 (C-5). **^{31}P-NMR** (DMSO-d_6) : 0.97. **FAB-MS** negative (matrix: NBA): 627.2 [M - Na; 100]; 507.1 [10]; 465.2 [M - sugar - Na^+ + H; 30]; 281.1 [M - Steroid; 35],. **Anal.** Calc. for C_{33} H_{56} O_9 Na P, 3 H_2O (704.8) C : 56.27, H : 8.87. Found C : 56.24, H : 8.65.

(9d) $[\alpha]_D$ = + 10 (c = 1, DMSO). **^{1}H-NMR** (DMSO-d_6) : 3.57-3.93 (m, 5H, H-6'a, H-5', H-4', H-3', H-2'); 4.24 (b, 1H, H-6'b); 4.42 (b, 2H, CH2-benzyl); 4.43-4.61 (m, 3H, OH-4', OH-3', OH-2'); 4.84 (b, 1H, H-1'α); 6.26 (b, 1H, OH-1'); 7.30 (m, 5H, H-aromatic). **^{13}C-NMR** (DMSO-d_6) : 65.17 (C-6'); 65.86 (C-5'); 66.38 (C-4'); 70.14 (C-2'); 71.79 (C-3'); 72.95 (CH2-benzyl); 94.72 (C-1'); 127.41-128.22 (CH-benzyl); 138.39 (C-benzyl). **^{31}P-NMR** (DMSO-d_6) : 1.19. **FAB-MS** negative (matrix: NBA): 349.1 [M - Na; 100]; 281.1 [M - Steroid; 50]; 187.0 [M - sugar - Na^+ + H; 40]. **Anal.** Calc. for C_{13} H_{18} O_9 Na P, 3 H_2O (426.24) C : 36.63, H : 5.68. Found C : 36.77, H : 5.67.

(10a) $[\alpha]_D$ = + 2 (c = 1, DMSO). **^{1}H-NMR** (DMSO-d_6) : 0.64 (s, 3H, CH_3-18); 0.84 (d, 6H, J = 6.5 Hz, CH_3-26 and 27); 0.89 (d, J = 6.4 Hz, 3H, CH_3-21); 0.98 (s, 3H, CH_3-19); 3.47-4.01 (m, 6H, H-3, H-6'a, H-5', H-4', H-3', H-2'); 4.24 (d, 1H, J = 8 Hz, H-6'b); 4.51-4.62 (m, 3H, OH-4', OH-3', OH-2'); 4.88 (b, 1H, H-1'α); 5.28 (b, 1H, H-6); 6.57 (b, 1H, OH-1'). **^{13}C-NMR** (DMSO-d_6) : 11.62 (C-18); 18.50 (C-21); 19.02 (C-19); 22.34 (C-26); 22.61 (C-27); 55.51 (C-14); 56.10 (C-17); 63.73 (C-6'); 65.93 (C-5'); 66.47 (C-4'); 70.01 (C-2'); 72.48 (C-3'); 75.80 (C-3); 97.11 (C-1'); 120.84 (C-6); 141.22 (C-5). **^{31}P-NMR** (DMSO-d_6) : 1.04. **FAB-MS** negative (matrix: NBA): 627.2 [M - Na; 100]; 507.1 [15]; 465.2 [M - sugar - Na^+ + H; 35]; 281.1 [M - Steroid; 20],. **Anal.** Calc. for C_{33} H_{56} O_9 Na P, 3 H_2O (704.8) C : 56.27, H : 8.87. Found C : 56.36, H : 9.00.

(*Received in France* 14 *May* 1996; *accepted* 31 *May* 1996)