94. The Syntheses of Purine and Pyrimidine Secoribo-nucleosides: Acyclo-uridine Derivative of Cyclophosphamide

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The synthesis of secoribo-nucleoside analogues is described. Compounds **4** and **5** possess interesting antiviral effects *in vitro*. A procedure is also developed for the conversion of acyclo-uridine nucleoside **7** to a novel derivative of cyclophosphamide **8**.

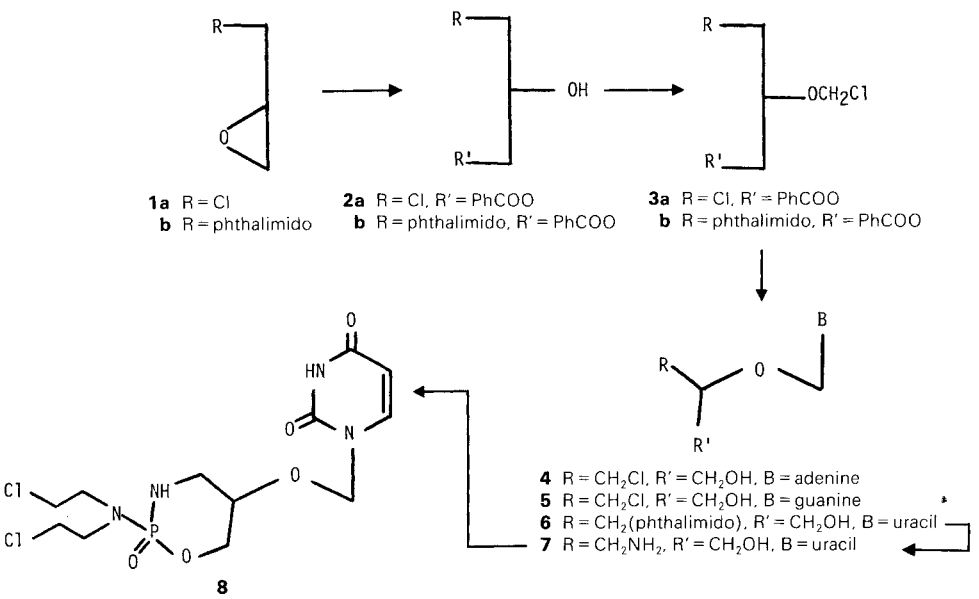
Introduction. – Nucleoside analogues have gained increasing importance through their biological activity particularly as antiviral and anticancer compounds [1]. Recently, a novel acyclic guanine nucleoside analogue, 9-{[2-hydroxy-1-(hydroxymethyl)-ethoxy]methyl}guanine (DHPG), was reported independently by several investigators [2–8] to possess antiviral activity comparable to that of 9-[(2-hydroxyethoxy)-methyl]guanine (acyclovir) [9] [10].

From another point of view, some specificity has been achieved in the case of the antitumor drug cyclophosphamide (= 2-[bis(2-chloroethyl)amino]-1,3,2λ^5-oxazaphosphorinan-2-one) [11]. This drug takes advantage of the fact that in cancer patients there is a higher dephosphorylating activity in the cancer cells than in the normal cells, and hence it will interact to a greater extent with the former.

We, therefore, began a program with the aim of synthesizing a series of purine derivatives (**4** and **5**) related to the structures of DHPG and acyclovir, thus, allowing an examination of their antiviral activities. Furthermore, we decided to prepare a new uracil acyclic nucleoside derivative **8** of cyclophosphamide. This might result in a new type of cyclophosphamide possessing interesting anticancer activity.

Results and Discussion. – The general procedure for the synthesis of **4**, **5**, and **8** is outlined in the *Scheme*. Epichlorohydrin (**1a**) was chosen as the starting material. Its reaction with potassium phthalimide gave, after 10 h, **1b** (90%). Then **1a** and **1b** were converted to **2a** [12] (95%) and **2b** (98%), respectively, with sodium benzoate in the presence of benzoic acid in DMF at 25° and reflux temperature, respectively. After treatment (7 h) of **2a,b** with 1,3,5-trioxane in the presence of HCl gas, the chloromethyl derivatives **3a,b** were obtained in mediocre yield. Then, **3a** was reacted with bis(trimethylsilyl)adenine [7] and tris(trimethylsilyl)guanine [7], after treatment of the latter with 1 equiv. of Bu_4NF [8], to give the secoribo-nucleosides **4** and **5** (*ca.* 60%), respectively. Similarly, **3b** gave **6** (80%) from bis(trimethylsilyl)uracil [7]. Presumably, the preliminary treatment with Bu_4NF removes the trimethylsilyl group from the N(9) position of bis(trimethylsilyl)adenine, tris(trimethylsilyl)guanine, and the O(2) position of bis(trimethylsilyl)uracil. The resulting highly soluble organic salts are then alkylated *in situ* by the respective chloromethyl ethers.

Scheme



It should be noted that addition of 2–3 equiv. of Bu_4NF results in complete removal of the trimethylsilyl groups from the persilylated nucleobases causing precipitation of the corresponding nucleobases and, thus, prevents the condensation with chloromethyl ethers. However, if the alkylating agent is added first or together with Bu_4NF, the yields are much lower or the reactions even fail to produce the desired compounds. Presumably, Bu_4NF undergoes competing reaction with chloromethyl ethers.

Removal of the phthalimido group from **6** was achieved in pyridine/AcOH 3:2 containing 0.5M phenylhydrazine yielding the unprotected compound **7** (68%). Finally, reaction of **7** with $POCl_3$ in dry THF in the presence of Et_3N and subsequent treatment with bis(2-chloroethyl)amine hydrochloride gave the desired compound **8** (20%).

Biological Activity. – Compounds **4**, **5**, and **7** as well as 9-(β-D-arabinofuranosyl)adenine (Ara-A) and acyclovir were tested for activity against *herpes simplex type 1 virus* (HSV-1). Most of them did not show much toxicity towards *Hela* cells [13]. The results (*Table*) show that the 50% inhibitory levels against HSV-1 range from 0.15 μg/ml for acyclovir to 17.72 μg/ml for **7**. The 50% inhibitory levels of the compounds against cell morphology is at least 97.5 μg/ml for 9-(β-D-arabinofuranosyl)adenine (Ara-A). This might show a broad safety margin for compounds **4**, **5**, and **7**.

Table. *Antiviral Activity and Selectivity of Secoribo-nucleosides in* Hela *Cell Cultures*

	ED_{50} (μg/ml)		Antiviral index (B/A)
	HSV-1 (A)	Cell morphology (B)	
4	11.36	132.00	11.61
5	0.17	155.60	915.29
7	17.72	369.00	20.82
Ara-A	14.80	97.50	6.58
Acyclovir	0.15	245.00	1633.33

Experimental Part

General. See [14].

N-*(2,3-Epoxypropyl)phthalimide* (**1b**). Epichlorohydrin (**1a**; 0.01 mol) and potassium phthalimide (0.01 mol) were suspended in dry DMF (150 ml). The mixture was stirred at 20° for 10 h. Then, it was partitioned between AcOEt (250 ml) and H_2O (250 ml). The org. layer was washed with H_2O (5 × 100 ml), dried (Na_2SO_4), filtered, and evaporated to give a syrup. Chromatography (silica gel, CH_2Cl_2) afforded **1b** as a white precipitate (90%). M.p. 100°. R_f (Et_2O) 0.72. IR (nujol): 1715*s*, 1125*s*. ^{1}H-NMR ($CDCl_3$): 2.80 (*m*, CH_2(1′)); 3.29 (br. *m*, CH(2′)); 3.19 (*m*, CH_2(3′)); 7.80 (*m*, arom. H). Anal. calc. for $C_{11}H_9NO_3$ (203.03): C 65.03, H 4.43, N 6.90; found: C 65.12, H 4.42, N 6.95.

3-Chloro-2-hydroxypropyl Benzoate (**2a**). Benzoic acid (2.44 g, 0.02 mol) and $NaHCO_3$ (0.84 g, 0.01 mol) were suspended in DMF (30 ml) containing epichlorohydrin (**1a**; 0.925 g, 0.01 mol). The mixture was stirred at 25° for 12 h and then partitioned between Et_2O and H_2O. The Et_2O layer was washed with H_2O (2 × 100 ml), dried ($MgSO_4$), and evaporated: **2a** (95%) as an oil. R_f (CH_2Cl_2) 0.17. IR (CCl_4): 3490 (br.), 1720*s*. ^{1}H-NMR ($CDCl_3$): 3.32 (*s*, OH, exchange with D_2O); 3.67 (*d*, CH_2(3)); 3.98–4.51 (*m*, CH(2)); 4.60 (*d*, CH_2(1)); 7.41–8.20 (*m*, arom. H). Anal. calc. for $C_{10}H_{11}ClO_3$ (214.52): C 56.07, H 5.14, Cl 16.59; found: C 56.13, H 5.25, Cl 16.60.

N-*[3-(Benzoyloxy)-2-hydroxypropyl]phthalimide* (**2b**). A soln. of **1b** (0.01 mol), sodium benzoate (0.01 mol), and benzoic acid (0.01 mol) in DMF (150 ml) was refluxed for 3 h. AcOEt (300 ml) was added and the soln. washed with H_2O (5 × 150 ml). The org. layer was dried (Na_2SO_4) and evaporated and the residue chromatographed (silica gel, $CHCl_3$): **2b** as white precipitate (98%). M.p. 195°. R_f (Et_2O) 0.38. IR (nujol): 3430 (br.), 1720*s*, 1770*s*. ^{1}H-NMR ($CDCl_3$): 3.68–5.05 (*m*, CH_2(1′)); 4.09 (*m*, CH(2′)); 4.48 (br. *s*, CH_2(3′)); 4.78 (br., OH, exchange with D_2O); 7.15–8.08 (*m*, arom. H). Anal. calc. for $C_{18}H_{15}NO_5$ (325.31): C 65.46, H 4.61, N 4.30; found: C 66.66, H 4.59, N 4.33.

3-Chloro-2-(chloromethoxy)propyl Benzoate (**3a**) *and 2-(Chloromethoxy)-3-(phthalimido)propyl Benzoate* (**3b**). A mixture of **2a** or **2b** (0.20 mol), 1,3,5-trioxane (0.25 mol), and CH_2Cl_2 (350 ml) was cooled in an ice-water bath, and dry HCl was bubbled through it for 7 h. Anh. $CaCl_2$ was added, and after 30 min, the mixture was filtered and the filtrate evaporated: **3a** or **3b**, resp., as an oil. Chromatography (silical gel, CH_2Cl_2) gave **3a** (*ca.* 37%) or **3b** (40%), resp.

3a: R_f (CH_2Cl_2) 0.51. ^{1}H-NMR (CCl_4): 3.71 (*d*, CH_2(3′)); 3.90–4.31 (*m*, CH(2′)); 4.73 (*d*, CH_2(1′)); 5.72 (*s*, $ClCH_2O$); 7.37 (*m*, Ph).

3b: ^{1}H-NMR (CCl_4); 4.30–4.01 (*m*, CH_2(3′)); 3.98–4.20 (*m*, CH(2′)); 4.50 (*d*, CH_2(1′)); 5.67 (*s*, $ClCH_2O$); 7.01–8.12 (*m*, arom. H).

Secoribo-nucleosides **4**, **5**, *and* **6**, *Representative Procedure.* Adenine (0.01 mol) and $(NH_4)_2SO_4$ (150 mg) were suspended in hexamethyldisilazane (HMDS, 50 ml) and refluxed for 24 h. The solvent was evaporated and the residue dissolved in THF (400 ml). Bu_4NF (0.01 mol) was dried by azeotrope distillation in benzene (500 ml) and added dropwise within 2 h at boiling temp., after the volume of the benzene soln. had been reduced to 10 ml. Then, **3a** (0.01 mol) was added dropwise within 15 min at the same temp. After 30 h, the soln. was diluted with AcOEt (600 ml) and H_2O (500 ml). The org. layer was separated, washed with H_2O (2 × 300 ml), dried (Na_2SO_4), filtered, and evaporated. Crystallization from acetone gave *9-{[2-chloro-1-(hydroxymethyl)ethoxy]methyl}adenine* (**4a**; 60%). M.p. 192°. R_f (Et_2O/MeOH 4.1) 0.33. UV (MeOH): 258 (13920). IR (nujol): 3310 (br.), 1661*s*, 1660*s*, 1090*m*. ^{1}H-NMR ((D$_6$)DMSO): 3.55 (br., OH, exchange with D_2O); 3.81 (*d*, CH_2OH, CH_2Cl); 4.25 (*m*, CHOCH$_2$N); 5.81 (*s*, CHOCH$_2$N); 7.45 (br. *s*, NH_2, exchange with D_2O); 8.31 (*s*, H–C(2)); 8.41 (*s*, H–C(8)). Anal. calc. for $C_9H_{12}ClN_5O_2$ (257.56): C 41.94, H 4.66, Cl 13.79, N 27.18; found: C 41.81, H 4.61, Cl 13.80, N 27.20.

9-{[(2-Chloro-1-(hydroxymethyl)ethoxy]methyl}guanine (**5**). Crystallization from MeOH. Yield 65%. M.p. 270° (dec.). R_f (i-PrOH/NH_4OH/H_2O 7:1:2) 0.41. UV (MeOH): 253 (12270), 270 (sh). ^{1}H-NMR ((D$_6$)DMSO/D_2O): 3.81 (*m*, $ClCH_2CH(CH_2OH)O$); 5.89 (*s*, OCH_2N); 8.31 (*s*, H–C(8)). Anal. calc. for $C_9H_{12}ClN_5O_3$ (273.53): C 39.49, H 4.39, Cl 12.98, N 25.59; found: C 39.47, H 4.40, Cl 12.87, N 25.61.

1-{{2-Hydroxy-1-[(phthalimido)methyl]ethoxy}methyl}uracil (**6**). Crystallization from Et_2O. Yield 80%. M.p. 188°. R_f (Et_2O/MeOH 9:1) 0.50. UV (EtOH): 260 (10650). IR (nujol): 3400–3500 (br.), 1680*s*, 1720*s*, 1120*s*. ^{1}H-NMR ((D$_6$)DMSO): 3.88–4.21 (*m*, (phthalimido)CH_2CH(CH_2OH)O); 5.50 (*s*, OCH_2N); 5.70 (*d*, J = 8, H–C(5)); 7.30 (*d*, J = 8, H–C(6)); 7.80 (*m*, Ph); 9.91 (br., NH). Anal. calc. for $C_{16}H_{15}N_3O_6$ (345.33): C 55.65, H 4.35, N 12.17; found: C 55.56, H 4.30, N 12.19.

1-{[2-Amino-1-(hydroxymethyl)ethoxy]methyl}uracil (**7**). To **6** (1 mmol) in pyridine (1 ml), 10 equiv. of 0.5M phenylhydrazine in pyridine/AcOH 3:2 were added. After 2 h, pentane-2,4-dione (10 equiv.) was added with cooling. Solvents were removed, and the residue was suspended in $CHCl_3$ to dissolve impurities. The precipitate was filtered and washed with $CHCl_3$: **7** (68%). Crystallization from acetone gave **7** (50%). M.p. 220°. R_f (Et_2O/MeOH 9:1) 0.28. UV (EtOH): 260 (10400). IR (nujol): 3120–3510 (br.), 1680*s*, 1720*s*, 1720*s*. ^{1}H-NMR

((D$_6$)DMSO): 3.30 (br., NH$_2$, exchange with D$_2$O); 3.09–3.42 (*m*, C*H*$_2$NH$_2$); 3.83–4.22 (*m*, CHCH$_2$OH); 5.21 (*s*, OCH$_2$N); 5.40 (*d*, *J* = 8, H–C(5)); 7.40 (*d*, *J* = 8, H–C(6)); 10.10 (br. NH, exchange with D$_2$O). Anal. calc. for C$_8$H$_{13}$N$_3$O$_4$ (215.13): C 44.65, H 6.05, N 19.53; found: C 44.76, H 6.13, N 19.62.

1-[(Cyclophosphamidoxy)methyl]uracil (= *1-{{2-[Bis(2-chloroethyl)amino]-2-oxo-1,3,2λ^5-oxazaphosphorinan-5-yl}oxymethyl}uracil;* **8**). Compound **7** (0.01 mol) was dissolved in THF (100 ml) containing Et$_3$N (0.05 mol). A soln. of POCl$_3$ (0.01 mol) in dry THF (10 ml) was added dropwise over 30 min at −5°, then stirred for 2 h at 0°. Then, bis (2-chloroethyl)amine hydrochloride (0.01 mol) was added and the mixture stirred at 0° and allowed to warm up to 25° during 13 h. The solvent was removed, the residue taken up in Et$_2$O (100 ml) and filtered. Evaporation gave a residue which was applied to a short silica gel column (12 × 2 cm). Elution with CHCl$_3$ afforded **8** (20%) as a foam. R_f (Et$_2$O/MeOH 9:1) 0.63. UV (EtOH): 262 (11020). IR (CHCl$_3$): 3300–3510 (br.), 1683*s*, 1720*s*, 1115*s*. ^{1}H-NMR (CDCl$_3$): 3.26–4.48 (*m*, 2 CH$_2$Cl, 3 CH$_2$NP, OCH$_2$CHO); 5.63 (*s*, OCH$_2$N); 5.72 (*d*, *J* = 8, H–C(5)); 6.21 (br., NHP, exchange with D$_2$O); 7.36 (*d*, *J* = 8, H–C(6)); 10.3 (br., NH, exchange with D$_2$O). Anal. calc. for C$_{12}$H$_{19}$Cl$_2$N$_4$O$_5$P (401.24): C 35.91, H 4.74, Cl 17.71, N 13.96; found: C 36.01, H 4.76, Cl 17.63, N 14.12.

REFERENCES

[1] R. T. Walker, E. de Clercq, F. Eckstein, Eds., 'Nucleoside Analogues', Plenum Press, New York, 1978.

[2] K. O. Smith, K. S. Galloway, W. L. Kennell, K. K. Ogilvie, B. K. Radatus, *Antimicrob. Agents Chemother.* **1982,** *22,* 55.

[3] W. T. Ashton, J. D. Karkas, A. K. Field, R. L. Tolman, *Biochem. Biophys. Res. Commun.* **1982,** *108,* 1716.

[4] J. C. Martin, C. A. Dvorak, D. F. Smee, T. R. Matthews, J. P. H. Verheyden, *J. Med. Chem.* **1983,** *26,* 759.

[5] D. F. Smee, J. C. Martin, J. P. H. Verheyden, T. R. Matthews, *Antimicrob. Agents Chemother.* **1983,** *23,* 679.

[6] H. J. Schaeffer, in 'Nucleosides, Nucleotides, and their Biological Applications', Eds. J. L. Rideout, D. W. Henry, and L. M. Beacham III, Academic Press, New York, 1983, pp. 1–17.

[7] G. H. Hakimelahi, A. Khalafi-Nezhad, *Helv. Chim. Acta* **1989,** *72,* 1495.

[8] G. H. Hakimelahi, A. Khalafi-Nezhad, F. Mohanazadeh, *Int. J. Chem.* **1990,** *1,* 9.

[9] H. J. Schaeffer, L. Beauchamp, P. de Miranda, G. B. Elion, D. J. Bauer, P. Collins, *Nature (London)* **1978,** *272,* 583.

[10] G. H. Hakimelahi, F. Mohanazadeh, A. Khalafi-Nezhad, M. Zakerinia, *Med. J. I. R. Iran* **1990,** to be published; G. H. Hakimelahi, Iran. Pat. 23291, Sept. 6, 1986; G. H. Hakimelahi, Iran. Pat. 28258, Jan. 21, 1990.

[11] H. Dugas, C. Penney, 'Bioorganic Chemistry, A Chemical Approach to Enzyme Action', Ed. C. R. Cantor, Springer-Verlag, Berlin, 1981, p. 36.

[12] M. F. Sorokin, L. G. Shode, A. I. Kuzmin, N. A. Novikov, *Izv. Vyssh. Uchebn. Zaved., Khim. Khim. Tekhnol.* **1984,** *27,* 658.

[13] H. Davari, Doctoral Thesis, School of Medicine, Shiraz University, Shiraz, Iran, 1988.

[14] G. H. Hakimelahi, M. Zarrinehzad, A. A. Jarrahpour, H. Sharghi, *Helv. Chim. Acta* **1987,** *70,* 219.