Tetrahedron Letters No. 10, pp 883 - 886. 0040-4039/79/0301-0883$02.00/0

SYNTHESIS OF ACTIVATED CYCLOPHOSPHAMIDE DERIVATIVES BEARING FUNCTIONAL GROUPS

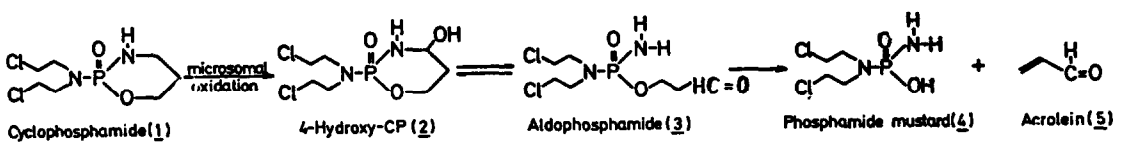
Takasni Hirano, Wolfgang Klesse, Joerg H. Heusinger, Gabriele Lambert and Helmut Ringsdorf

Institute of Organic Chemistry, University of Mainz, J.J.-Becher-Weg 18-20, D-6500 Mainz, West-Germany

This paper describes the synthesis of stabilized 4-hydroxy-cyclophosphamide derivatives with functional groups for the further modification of these active anti-tumor agents, especially for their fixation to polymeric carriers.

One of the serious short comings of most anti-tumor agents used in cancer chemotherapy is the fact that they are cell cycle specific and thus not only active against tumors with a high rate of growth, but also against normal cells, especially rapidly growing ones, e.g. of the bone marrow and the gastro-intradermal epithelium [1]. Thus to increase the specificity of anti-tumor drugs the role of drug delivery systems in cancer chemotherapy has become of interest during the last years. Attempts with natural [2] and synthetic polymers [3], along with tumor cell-specific immunoglobulins [4] and liposomes [5], as a carrier system have been described. For cell-specific drugs of low molecular weight the problem and the selectivity of alkylating cytostatics has been recently discussed by Brock and Hohorst based on the studies on cyclophosphamide [6], one of the most widely used anti-tumor agents [7] together with melphalan, chloro-ambucil, methotrexate and 5-fluorouracil.

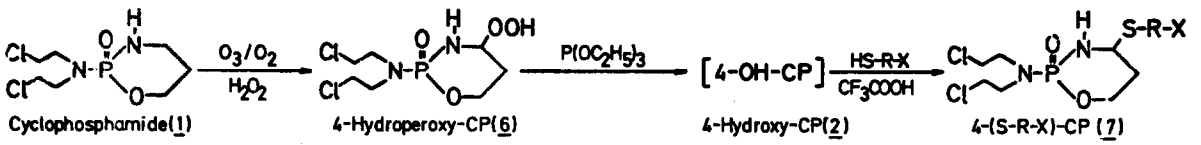
To use cyclophosphamide in a drug delivery system suitable cyclophosphamide derivatives bearing functional groups should be synthesized. But in such a synthesis the metabolism of the drug must be kept in mind, because the pharmacological activities might be lost by the modification. Cyclophosphamide is active against tumor cells only in vivo [8], while other alkylating anti-tumor agents such as melphalan and chloroambucil are active both in vivo and in vitro. The initial activation step in the metabolism of cyclophosphamide (1) is the hydroxylation by the liver microsomal oxidase at the C-4 position to give 4-hydroxycyclophosphamide (2) as the first metabolite [9], which is active both in vivo and in vitro.



4-Hydroxycyclophosphamide (2) is in equilibrium with aldophosphamide (3) and these tautomers are unstable under physiological conditions to decompose into phosphamide mustard (4) and acrolein (5), the former metabolite is most likely responsible for the alkylating and cytotoxic action of cyclophosphamide [6].

Because the drug in a carrier system should be active directly after its release in tumor tissue, it is necessary to use the primary activated metabolite of cyclophosphamide, 4-hydroxycyclophosphamide (2). 4-Hydroxycyclophosphamide was synthesized for the first time by Takamizawa via 4-hydroperoxycyclophosphamide (6) [10]. The stabilization of 4-hydroxycyclophosphamide by the reaction with mercaptans forming corresponding 4-alkyl-sulfido-cyclophosphamide was reported by Peter [11]. These derivatives are stable at room temperature but found to undergo reversible hydrolysis to 4-hydroxycyclophosphamide and mercaptans under physiological conditions [11]. The 4-alkyl-sulfido-cyclophosphamide showed nearly the same cytotoxic activity as cyclophosphamide against Yoshida ascites tumor cells [11].

In the present work the synthesis of activated derivatives of cyclophosphamide bearing functional groups is described. 4 Hydroperoxycyclophosphamide(6) was synthesized by the direct ozonization of cyclophosphamide according to the method of Peter [11].



Cyclophosphamide(1) 4-Hydroperoxy-CP(6) 4-Hydroxy-CP(2) 4-(S-R-X)-CP (7)

-R-X: $-(CH_2)_n-COOH$ n=1,2,3,10
$-CH_2-C_6H_4-COOH$
$-(CH_2)_n-OH$ n=3,4,6
$-(CH_2)_n-COOC_2H_5$ n=1,2
$-CH_2CH_2O-CH_2CH_2-OH$

After reducing 4-Hydroperoxycyclophosphamide (6) with triethyl phosphite, the resulting 4-hydroxycyclophosphamide (2) was treated with mercaptoalkane bearing functional groups (HS-R-X, X=COOH, OH and $COOC_2H_5$) in the presence of trifluoroacetic acid. The prepared derivatives are summarized in Table 1 and the synthesis of 4-(S-hexane-6-ol)-sulfidocyclophosphamide (7) (in Table 1 (7)-h) is described below. (7)-h has been proved to be highly cancerotoxic against Yoshida ascites tumor in rat [12]. *)

*) Anti-Tumor Activity 12.5 mg/kg 5/6 cure (i.p. injection)
25.0 mg/kg 6/6 cure
50.0 mg/kg 6/6 cure
Tumor; Yoshida Ascites Tumor AH13 in Rat

The 4-(S-alkyl)-sulfidocyclophosphamide should be exist a pair of disastereo-isomers and these are observed by following the reaction by means of thin layer chromatography. On the other hand only one isomer was isolated as a stable derivative. In the case of 4-(S-alkyl)-sulfidoisophosphamide two stereo-isomers were isolated [13]. The stereochemistry of the isolated 4-(S-alkyl)-sulfidocyclophosphamide may be elucidated to be less hindered axial as already discussed by Peter on the basis of NMR measurement [14].

The fixation of these activated derivatives of cyclophosphamide bearing functional groups to polymeric carriers and tumor-cell specific immuno-globulins will be described elsewhere [15].

Synthesis of 4-(S-hexane-1-ol)-cyclophosphamide. To a stirred solution of 4-hydroperoxycyclophosphamide(879 mg,3 mmol) in 10 ml of methylene chloride was added triethylphosphite (0.50 g, 3 mmol) in 3 ml of methylene chloride at 0°C. After stirring for 2 h at 0°C, a cold solution of 6-mercaptohexane-1-ol (0.39 ml, 3 mmol) in 2 ml of methylene chloride followed by 0.34 g of trifluoroacetic acid (1,3 mmol) in 3 ml of methylene chloride were added and the solution stirred for 2 h at 0°C. The solution was concentrated in vacuo below 4°C to give an oily residue. To the residue were added 2 ml of methylene chloride, 10 ml of diethyl ether and a small amount of petroleum ether until a slight turbidity appeared. After standing for 12 h at -25°C the white crystals deposited were filtered and washed with cold diethyl ether (yield 56.5 %). Recrystallization from methylene chloride at 0°C gave white crystals (mp 67.5-69°C). The purity of the product was tested by thinlayer chromatography in ethyl acetate at room temperature (R_f=0.19). The NBP (p-nitro-benzyl-pyridine) spray test was used for the detection of alkylating activity and the iodine-sodium azide spray test for the detection of sulfur.

IR (KBr disk) max cm^{-1}, 3260(-OH),3200(-NH),1210,990,975,945,750; NMR (60MHz in DMSO-d^6, TMS as internal standard) ppm; 1.36(m),1.7-2.2(m),2.37-2.76(m,C_4-S-CH_2- and DMSO), 3.0-3.7(m,N-CH_2CH_2-Cl),4.1(t,-OH,J_{CH_2-OH}=4.8Hz),4.82-5.00(m,C_4-H), 5.48 (d of d,J_{p-NH}=21.6,J_{C_4-H-NH}=4.5, determined after D_2O exchange) Anal. cald. for $C_{13}H_{27}N_2O_3SPCl_2$: C, 39.69, H, 6.92, N, 7.12, Cl, 18.03, found: C, 39.58, H, 6.97, N, 7.02, Cl, 17.86, FD-MS m/e = 394/393.

Literature

1. D. Gericke and M. Schorr, "Arzneimittel; Entwicklung Wirkung und Darstellung", Verlag Chemie, Weinheim/Bergstr., West-Germany, Vol. 5, Teil 2, 345 (1972)

2. (a) A. Trouet, D.D. Campeneere and C.De Duve, Nature, New Biology, 239, 110 (1972)
   (b) A. Bernstein, E. Hurwitz, R. Maron, M. Sela and M. Wilchek, J. Natl. Cancer Inst., 60, 379 (1978)

3. H. Ringsdorf, J.Polym.Soc., Sym. 51, 135 (1975)

4. (a) E. Hurwitz, R. Levy, R. Maron, M. Wilchek, R. Arnon and M. Sela, Cancer Res. 35, 1175 (1975)
(b) G.F. Rowland, G.J. O'Neill and D.A.L. Davies, Nature 255, 488 (1975)
(c) G.F. Rowland, Europ. J. Cancer 13, 1403 (1977)

5. (a) R.L. Juliano, "Membrane and Neoplasia; New Approaches and Strategies", Alan R. Liss Inc., NY, 21 (1976)
(b) G. Gregoriadis, Nature 265, 407 (1977)

6. N. Brock and H.-J. Hohorst, Z. Krebsforsch. 88, 185 (1977)

7. L.H. Schmidt, R. Fradkin, R. Sullivan and A. Flowers, Cancer Chemother. Rep. (Suppl.2), 1(1965)

8. N. Brock and H.-J. Hohorst, Arz.Forsch.(Drug-Res.) 13, 1021 (1963)

9. (a) H.-J. Hohorst, A. Ziemann and N. Brock, Arz.Forsch.(Drug-Res.) 21, 1254 (1971)
(b) T.A. Connors, P.J. Cox, P.B. Farmer, A.B. Foster and M. Jarman, Biochem. Pharmacol. 23, 115 (1974)
(c) D.L. Hill, W.R. Laster and R.F. Struck, Cancer Res. 32, 658 (1972)

10. A. Takamizawa, S. Matsumoto, T. Iwata, K. Katagiri, Y. Tochino and K. Yamaguchi, J.Am.Chem.Soc. 95, 985 (1973)

11. G. Peter, T. Wagner and H.-J. Hohorst, Cancer Treat.Rep. 60, 429 (1976)

12. N. Brock, Asta-Werke Bielefeld, W.-Germany - private communication

13. A. Takamizawa, S. Matsumoto, T. Iwata and I. Makino, Chem.Pharm. Bull. 25, 1877 (1977)

14. G. Peter, dissertation, University of Frankfurt/M., 135 (1977)

15. T. Hirano, H. Ringsdorf, submitted for "Makromol.Chemie", Nov. 1978

Table 1:

4-(S-Sulfidoalkyl)-cyclophosphamide derivatives (7)

Substituent -R-X	Yield (%)	R_f value	mp (°C)	Elemental Analysis (calcd. / found)
a. $-CH_2-COOH$	86.0	0.45	123-125	C;30.78, H;4.88, N;7.98, Cl;20.19 30.67 4.91 7.87 19.71
b. $-(CH_2)_2-COOH$	58.0	0.00	119-120	C;32.89, H;5.24, N;7.67, Cl;19.41 32.67 5.00 7.50
c. $-(CH_2)_3-COOH$	57.0	0.00	111-120	C;34.84, H;5.58, N;7.39, Cl;18.70 34.69 5.36 7.44
d. $-(CH_2)_{10}-COOH$	75.0	0.00	112.113	C;45.28, H;7.39, N;5.87, Cl;14.85 44.86 7.49 5.88
e. $-CH_2-C_6H_4-COOH$	67.0	0.00	134-136	C;42.17, H;4.95, N;6.59, Cl;16.59 42.17 4.87 6.48
f. $-(CH_2)_3-OH$	37.5	0.12	79-81	C;34.19, H;6.03, N;7.98, Cl;20.19 33.93 5.96 7.81 19.82
g. $-(CH_2)_4-OH$	43.3	0.10	65-65.5	C;36.17, H;6.35, N;7.67, Cl;19.41 36.16 6.45 7.87 19.42
h. $-(CH_2)_6-OH$	56.5	0.19	67.5-69	C;39.69, H;6.92, N;7.12, Cl;18.03 39.58 6.97 7.02 17.86
i. $-CH_2-COOC_2H_5$	57.3	0.44	71-71.5	C;34.83, H;5.58, N;7.39, Cl;18.69 34.73 5.70 7.34 18.40
j. $-(CH_2)_2-COOC_2H_5$	63.2	0.56	68-69	C;36.65, H;5.89, N;7.12, Cl;18.03 36.51 5.72 7.02 18.34
k. $-(CH_2)_2-O-(CH_2)_2-OH$	53.8	0.18	67-68	C;34.65, H;6.08, N;7.35, Cl;18.41 34.53 6.14 7.48 18.60

R_f; thin layer chromatography in ethylacetat at rt, mp; uncorrected,

(Received in UK 20 November 1978)