PREPARATION OF NOVEL CYCLOPHOSPHAMIDE DERIVATIVES OF SUGARS

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Abstract-----Novel cyclophosphamide derivatives bearing a sugar structure were prepared by the treatment of the amino sugars from D-glucose and D-xylose with bis-(2-chloroethyl)aminophosphoric dichloride as well as with phosphoryl chloride and nucleophiles.

Cyclophosphamides, which are widely used as one of alkylation type anti-cancer agents,[1] have previously been prepared *via* cyclo-condensation of amino alcohols derived from amino acids with substituted phosphoramidodichloridate.[2] In this communication some amino sugars were used as the starting materials to synthesize cyclophosphamide derivatives of sugars, because sugars are expected to excert biological activities by their affinity to organs.[3]

D-Glucose and D-xylose were employed as sugar starting materials in this study. These sugars were converted into amino sugars. For example, 1,2-*O*-isopropylidene-5-*O*-(*p*-toluenesulfonyl)-α-D-xylofuranose (1X,[4] 2.3 g, 5.9 mmol) was treated with NaN_3 (0.54 g, 8.3 mmol) in DMF (5 ml) for 4 h at 80℃ to afford the azide (1.3 g, 82%). The azide was treated with Bu_3SnH (1.7 g, 6.0 mmol) in dry toluene (10 ml) for 5 h under reflux to give 5-amino-5-deoxy-1,2-*O*-isopropylidene-α-D-xylofuranose (2XH, 1.1 g, 90%); ir (neat): ν=3360 cm^{-1} (OH, NH_2); ^1H nmr ($CDCl_3$/TMS): δ=1.25, 1.42 (6H, 2s, CMe_2), 3.2-3.6 (2H, m, H-5,5'), 3.7-4.5 (5H, m, H-3,4, NH_2, OH), 4.57 (1H, d, *J*=3.8 Hz, H-2), 5.95 (1H, d, *J*=3.8 Hz, H-1); ms (70 eV): m/z=189 (M^+). 6-Amino glucose derivatives (2GHa) (59% yield) and (2GHb) (43%) were prepared similarly from 1,2-*O*-isopropylidene-3-*O*-methyl- and 3-*O*-methyl-1,2-*O*-isopropylidene-6-*O*-(*p*-toluenesulfonyl)-α-D-glucofuranoses (1Ga and b), respectively.[5] A solution of 1X (6.7 g, 19 mmol) and benzylamine (10 ml, 92 mmol) in ethanol (30 ml) was

refluxed for 6 h. Concentration and chromatography (eluent: EtOAc/n-hexane=1/1) on silica gel of the reaction product gave 5-benzylamino derivative (2XB); yield 1.9 g (34%); ir (neat): ν=3360 cm^{-1} (OH, NH); ^1H nmr ($CDCl_3$/TMS): δ=1.30, 1.45 (6H, 2s, CMe_2), 2.8-3.9 (4H, m, H-3,4,5,5'), 4.1-5.4 (5H, m, H-2, CH_2Ph, NH, OH), 5.91 (1H, d, J=3.4 Hz, H-1), 7.1-7.4 (5H, m, Ph); ms (70 eV): m/z=279 (M^+). 6-Benzylamino glucose derivatives (2GBa) (26%) and (2GBb) (22%) were similarly prepared by the above method.[6]

Amino alcohols (2G and 2X) were converted into cyclophosphamide derivatives by the treatment with bis-(2-chloroethyl)aminophosphoric dichloride.[7] A dioxane (10 ml) solution of amino sugar (2XH) (0.38 g, 2.1 mmol), bis-(2-chloroethyl)aminophosphoric dichloride (0.58 g, 2.3 mmol), and triethylamine (0.5 ml, 3.6 mmol) was stirred for 4 h at room temperature. Evaporation of the solvent followed by work-up afforded diastereomeric crude product (3XH), (yield 0.26 g, 66%). Compounds (3GH and 3GB) and (3XB) were similarly prepared by the above method (Table 1 and Figure 1).

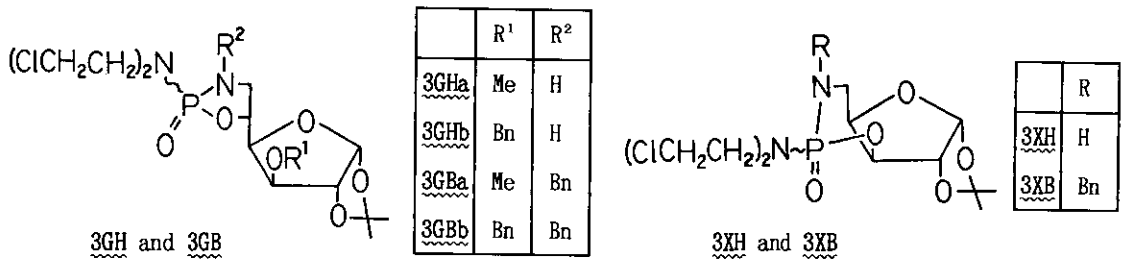

Figure 1. Cyclophosphamides (3G) and (3X) prepared from amino sugars (2G) and (2X).

^1H Nmr and ir spectral data of these derivatives shown in Table 1 clearly show the formation of the cyclophosphamide ring. The ^1H nmr spectrum shows that these compounds include the mixture of diastereomers judging from a pair of doublets for the proton on the C-1 (H-1). The evidence shows that both *R*- and *S*-stereoisomers on the phosphorus atom were produced during the present reaction. Diastereomers of cyclophosphamides (3GH and 3XH) could not be separated by column chromatography on silica gel, however, they suffered a decomposition during the procedure by the acidity of silica gel. On the other hand, protected cyclophosphamides (3GB and 3XB) have no active hydrogen on the nitrogen atom of cyclophosphamide ring and then they were so stable as not to be decomposed by silica gel (EtOAc/n-hexane=2/1).

For the stereoselective synthesis of cyclophosphamides, a method using phosphoryl chloride was attempted as shown in Figure 2. The chlorine on phosphorus atom in the first formed cyclophosphamide ring was subsequently attacked by a nucleophile as follows: A solution of phosphoryl chloride

Table 1. Cyclophosphamide derivatives (3G) and (3X).

Product	Yield/%	^{1}H Nmr ($CDCl_3$/TMS)	Ir (neat)/cm^{-1}	Ms/m/z
3GHa	35	1.25, 1.45(6H, 2s, CMe_2), 2.9(1H, bs, NH), 3.1-4.0(14H, m, H-6, 6', NH, OMe, $2CH_2CH_2Cl$), 4.1-4.7(4H, m, H-2, 3, 4, 5), 5.80, 5.90 (1H, 2d, J=3.8 Hz, H-1)	3330(NH) 1260(P=O) 1095(P-O-C)	418(M^+)
3GHb	28	1.35, 1.55(6H, 2s, CMe_2), 2.8-3.9(11H, m, H-6, 6', NH, $2CH_2CH_2Cl$), 4.0-4.5(3H, m, H-3, 4, 5), 4.6-4.9(3H, m, H-2, CH_2Ph), 5.85, 5.95 (1H, 2d, J=4.0 Hz, H-1), 7.3-7.5(5H, m, Ph)	3250(NH) 1230(P=O) 1090(P-O-C)	494(M^+)
3GBa	20	1.32, 1.48(6H, 2s, CMe_2), 2.8-3.9(13H, m, H-6, 6', OMe, $2CH_2CH_2Cl$), 4.0-4.9(6H, m, H-2, 3, 4, 5, CH_2Ph), 5.84(1H, d, J=3.8 Hz, H-1), 6.9-7.6(5H, m, Ph)	1240(P=O) 1080(P-O-C)	508(M^+)
3GBb	42	1.28, 1.45(6H, 2s, CMe_2), 2.9-3.8(10H, m, H-6, 6', $2CH_2CH_2Cl$), 3.8-4.9(8H, m, H-2, 3, 4, 5, $2CH_2Ph$), 5.71, 5.80(1H, 2d, J=4.0 Hz, H-1), 6.9-7.4(10H, m, 2Ph)	1250(P=O) 1080 (P-O-C)	584(M^+)
3XH	35	1.20, 1.40(6H, 2s, CMe_2), 2.8-4.0(11H, m, H-5, 5', NH, $2CH_2CH_2Cl$), 4.35(1H, d, J=3.6 Hz, H-4), 4.65(1H, d, J=3.6 Hz, H-3), 4.80(1H, d, J=3.6 Hz, H-2), 5.95, 6.15(1H, 2d, J=3.6 Hz, H-1)	3250(NH) 1225(P=O) 1040(P-O-C)	374(M^+)
3XB	48	1.25, 1.37(6H, 2s, CMe_2), 3.0-4.0(12H, m, H-5, 5', CH_2Ph, $2CH_2CH_2$-Cl), 4.0-4.7(2H, m, H-3, 4), 4.80(1H, d, J=3.6 Hz, H-2), 6.15 (1H, d, J=3.6 Hz, H-1), 7.0-7.4(5H, m, Ph)	1220(P=O) 1045(P-O-C)	464(M^+)

(0.6 ml, 6.4 mmol) in CH_2Cl_2 (5 ml) was added portionwisely to a cold CH_2Cl_2 solution (5 ml) of amino sugar (2XB, 1.6 g, 5.7 mmol) and triethylamine (3 ml, 22 mmol), and the mixture was stirred for 2 h at room temperature. Work-up and purification of the product by chromatography on silica gel (EtOAc/n-hexane=2/1) afforded 4XB, being obtained as a sole diastereomer; yield 0.90 g (44%); ir (neat): ν=1030 (P-O-C), 1250 cm^{-1} (P=O); ^{1}H nmr ($CDCl_3$/TMS): δ= 1.30, 1.40 (6H, 2s, CMe_2), 3.0-3.4 (2H, m, H-5, 5'), 3.5-5.0 (5H, m, H-2, 3, 4, CH_2Ph), 5.97 (1H, d, J=3.4 Hz, H-1), 7.0-7.4 (5H, m, Ph); ms (70 eV): m/z=359 (M^+), 361 (M^+ + 2). A solution of 4XB (0.28 g, 0.78 mmmol) and sodium methoxide (0.1 g, 1.9 mmol) in MeOH (85 ml) was refluxed for 6 h. Work-up and purification of the puroduct by chromatography on silica gel (EtOAc) afforded 1,2-*O*-isopropylidene-α-D-xylofuranose cyclic-3(*O*),5(*N*)-(methylphosphamidate), 5XBa; yield: 0.096 g (35%). In the case where diethylamine (2.0 ml, 19 mmol) was used as the nucleophile in CCl_4 (5 ml), 4XB (0.17 g, 0.47 mmol) afforded 5XBb (0.08 g, 43%) (Table 2 and Figure 2).

According to previous reports on cyclophosphamide syntheses[8] and on stereochemistry about the nucleophilic attack proceeding with inversion of the configuration,[9] as well as to the present result where only one spot was observed in the each tlc analysis of the product, the *R*-stereoisomer on the phosphorus atom in the ring of reaction products (4XB) and (5XBa, b) might be prepared as the major product in the present reaction.

Studies on the conformation of these new cyclophosphamide derivatives are in progress.

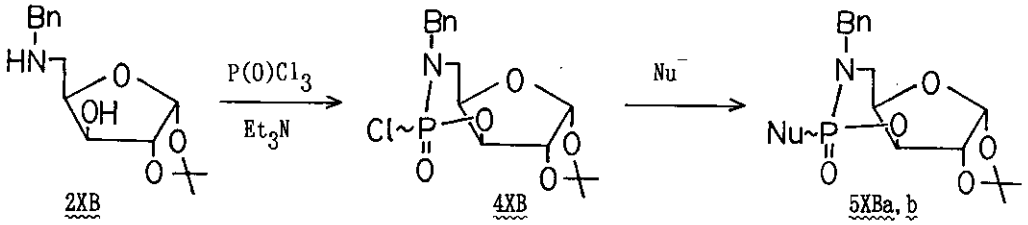

Figure 2. Preparation of cyclophosphamides (4XB) and (5XB) by an action of phosphoryl chloride and nucleophiles.

Table 2. Cyclophosphamide derivatives (5XBa and b).

Product	Nu	Yield/%	^{1}H Nmr ($CDCl_3$/TMS)	Ir (neat)/cm^{-1}	Ms/m/z
5XBa	Et_2N	35	1.05(6H, t, J=7.8 Hz, $2CH_2CH_3$), 1.29, 1.42(6H, 2s, CMe_2), 2.4-3.5(6H, m, H-5, 5', $2CH_2CH_3$), 3.6-4.5(3H, m, H-4, CH_2Ph), 4.55(1H, d, J=4.0 Hz, H-3), 4.71-4.90(1H, m, H-2), 5.91(1H, d, J=4.0 Hz, H-1), 7.0-7.5(5H, m, Ph)	1230(P=O) 1040(P-O-C)	396(M^+)
5XBb	MeO	43	1.30, 1.43(6H, 2s, CMe_2), 3.0-3.5(2H, m, H-5, 5'), 3.70(3H, d, J=12 Hz, OMe), 4.0-4.9(5H, m, H-2, 3, 4, CH_2Ph), 5.94(1H, d, J=3.4 Hz, H-1), 6.9-7.5(5H, m, Ph)	1250(P=O) 1030(P-O-C)	355(M^+)

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2. T. Sato, H. Ueda, K. Nakagawa, and N. Bodor, *J. Org. Chem.*, 1983, 48, 98.
3. K. Hatanaka, *Chemistry and Chemical Industry*, 1988, 42, 1404.
4. Compound numbers with G and X mean glucose and xylose derivatives, respectively, and those with H and B mean unprotected and protected (by benzyl) amino derivatives, respectively.
5. Data of 2GHa: Ir (neat): ν=3350 cm^{-1} (OH, NH_2); ^{1}H nmr ($CDCl_3$/TMS): δ=1.30, 1.47 (6H, 2s, CMe_2), 1.8-2.3 (3H, m, NH_2, OH), 2.9-4.3 (5H, m, H-3, 4, 5, 6, 6'), 3.42 (3H, s, OMe), 4.56 (1H, d, J=3.5 Hz, H-2), 5.77 (1H, d, J=3.5 Hz, H-1); ms (70 eV): m/z=233 (M^+). Data of 2GHb: Ir (neat): 3380 cm^{-1} (OH, NH_2); ^{1}H nmr ($CDCl_3$/TMS): δ=1.30, 1.47 (6H, 2s, CMe_2), 1.8-2.2 (3H, m, NH_2, OH), 2.90 (2H, d, J=3.5 Hz, H-6, 6'), 3.7-4.3 (3H, m, H-3, 4, 5), 4.4-4.7 (3H, m, H-2, CH_2Ph); ms (70 eV): m/z=309 (M^+).
6. Data of 2GBa: Ir (neat): ν=3310 cm^{-1} (OH, NH_2); ^{1}H nmr ($CDCl_3$/TMS): δ=1.24, 1.40 (6H, 2s, CMe_2), 2.1-2.5 (2H, bs, NH, OH), 2.6-2.9 (2H, m, H-6, 6'), 3.28 (3H, s, OMe), 3.5-4.1 (5H, m, H-3, 4, 5, CH_2Ph), 4.40 (1H, d, J=3.5 Hz, H-2), 5.67 (1H, d, J=3.5 Hz, H-1), 6.9-7.3(5H, m, Ph); ms (70eV): m/z=323 (M^+). Data of 2GBb: Ir (neat): ν=3345 cm^{-1} (OH, NH_2); ^{1}H nmr ($CDCl_3$/TMS): δ=1.30, 1.47 (6H, 2s, CMe_2), 2.7-3.0 (2H, m, H-6, 6'), 3.78, 3.82 (4H, 2s, $2CH_2Ph$), 4.0-4.8 (6H, m, H-2, 3, 4, 5, NH, OH), 5.88 (1H, d, J=3.5 Hz, H-1), 7.0-7.5 (10H, 2m, 2Ph); ms (70 eV): m/z=399 (M^+).
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