[CONTRIBUTION FROM LIFE SCIENCES DIVISION, STANFORD RESEARCH INSTITUTE]

Potential Anticancer Agents.[1] LXVIII. Synthesis of Alkylating Agents Derived from 3-Amino-3-deoxy-D-allose

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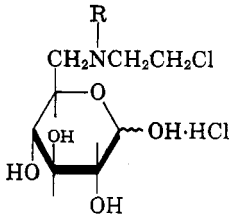
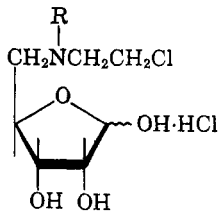
The synthesis, from 3-amino-3-deoxy-1,2:5,6-di-*O*-isopropylidene-D-allofuranose, of 3-[bis(2-chloroethyl)amino]-3-deoxy-D-allose hydrochloride and of 3-[(2-chloroethyl)ethylamino]-3-deoxy-D-allose hydrochloride is described and some preliminary biological results are presented.

Previous papers in this series on alkylating agents derived from carbohydrates described the synthesis of a group of one-armed mustards and the bis-mustard of 6-amino-6-deoxy-D-glucose[4,5] as well as the bis-mustard and ethyl one-armed mustard of 5-amino-5-deoxy-D-ribose.[5] The standard three-tumor screen[6] indicated that anticancer activity was limited to the one-armed mustards (I, II, and IV) and the bis-mustards (III and V).

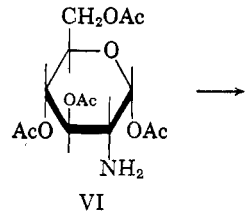


I. R = CH_3
II. R = C_2H_5
III. R = CH_2CH_2Cl

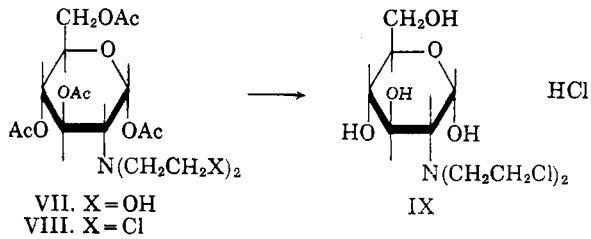


IV. R = C_2H_5
V. R = CH_2CH_2Cl

It was of interest to prepare other sugar mustards in which the alkylating group was attached to a secondary carbon of the sugar. Vargha and co-workers[6] have previously described the synthesis of the bis-mustard of glucosamine (IX) in which the mustard moiety is carried on a secondary sugar carbon. Compound IX was prepared from 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranose (VI) via conventional hydroxyethylation to VII, followed by chlorination of VII and deblocking of the resulting VIII to give the free sugar mustard (IX). This mustard (IX) was reported[6] to be quite toxic but to possess some activity against the Guerin tumor. In view of these biological results, it seemed especially important to prepare both the bis-mustard and a one-armed mustard of amino sugars where the amino group was attached to a secondary carbon.



VI



VII. X = OH
VIII. X = Cl

IX

The ready availability of 3-amino-3-deoxy-1,2:5,6-di-*O*-isopropylidene-D-allofuranose (X)[7] made this compound an attractive starting material for the preparation of both the bis-mustard and the corresponding one-armed mustard of 3-amino-3-deoxy-D-allose. The use of X had an added advantage over an acetylated amine such as VI in that the isopropylidene blocking groups of X obviated the complications of an O→N migration of the blocking group,[8] while still possessing the necessary acid lability to permit easy removal by acid hydrolysis.

Reaction of 3-amino-3-deoxy-1,2:5,6-di-*O*-isopropylidene-D-allofuranose (X) with ethylene oxide in 1,2-dimethoxyethane gave crude 3-deoxy-3-bis(2-hydroxyethyl)amino-1,2:5,6-di-*O*-isopropyli-

(1) This work was carried out under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Public Health Service, Contract No. SA-43-ph-1892. The opinions expressed in this paper are those of the authors and are not necessarily those of the Cancer Chemotherapy National Service Center. For the preceding paper in this series, see R. H. Iwamoto, E. M. Acton, B. R. Baker, and L. Goodman, *Chem. & Ind. (London)*, 1404 (1961).

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(4) E. J. Reist, R. R. Spencer, and B. R. Baker, *J. Am. Chem. Soc.*, **82**, 2025 (1960).

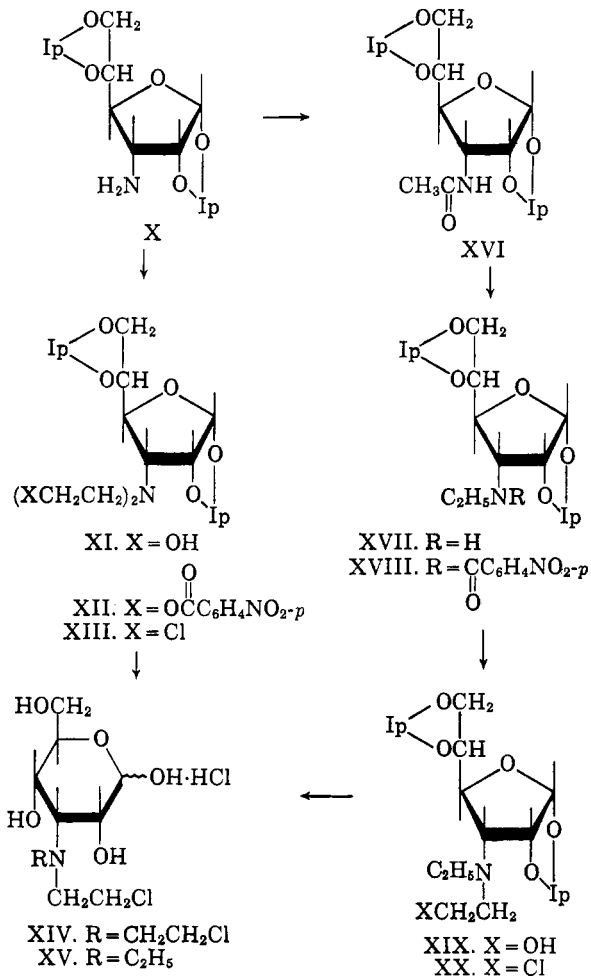
(5) E. J. Reist, R. R. Spencer, M. E. Wain, I. G. Junga, L. Goodman, and B. R. Baker, *J. Org. Chem.*, **26**, 2821 (1961).

(6) L. Vargha, Ö. Fehér, and S. Lendvai, *J. Chem. Soc.*, 810 (1957).

(7) K. Freudenberg and F. Brauns, *Ber.*, **55**, 3233 (1922); M. L. Wolfrom, F. Shafizadeh, and R. K. Armstrong, *J. Am. Chem. Soc.*, **80**, 4885 (1958).

(8) A. B. Foster and D. Horton in *Advances in Carbohydrate Chemistry*, **Vol. XIV**, Academic Press, Inc., New York, N. Y., 1959, p. 213.

dene-D-allofuranose (XI) as a sirup[9] that failed to crystallize. Purification of XI was effected by the conversion of the crude product to a crystalline di-*O*-*p*-nitrobenzoate (XII). The saponification of XII with sodium hydroxide in 50% methanol gave XI as a colorless sirup which crystallized when triturated with *n*-pentane. Recrystallization from *n*-pentane gave a 55% over-all yield of XI from the amine (X).



Treatment of crystalline XI with thionyl chloride in dichloromethane gave the blocked bis-mustard (XIII) as a foam, which was immediately hydrolyzed with aqueous hydrochloric acid to give the bis-mustard (XIV) as a colorless, amorphous solid that was homogeneous on paper chromatography[10] and was analytically pure.

The synthesis of 3-deoxy-3-ethylamino-1,2:5,6-di-*O*-isopropylidene-D-allofuranose (XVII), a key intermediate for the synthesis of the one-armed mustard (XV), was accomplished in 100% crude yield by the reduction of the corresponding amide (XVI)[12] with lithium aluminum hydride. Although XVII could not be crystallized or obtained analytically pure, it was characterized as the crystalline *p*-nitrobenzamide (XVIII). Treatment of crude XVII with ethylene oxide in 1,2-dimethoxyethane at 170° for 90 hours gave, after one distillation, a 63% yield of the hydroxyethylamine (XIX). The distillate was recrystallized from Skellysolve B to give crystalline 3-deoxy-3-[ethyl(2-hydroxyethyl)amino]-1,2:5,6-di-*O*-isopropylidene-D-allofuranose (XIX) in 31% yield from XVII.

Treatment of XIX with thionyl chloride afforded the blocked mustard (XX) as an amorphous solid. The crude product (XX) was hydrolyzed with 6*N* aqueous hydrochloric acid to give the one-armed mustard (XV) as a colorless, amorphous solid which was homogeneous on paper chromatography[10] and was analytically pure.

Biological results.[13] The two mustards (XIV and XV) were evaluated against Sarcoma 180, Adenocarcinoma 755, and Leukemia L-1210, and preliminary data are available. The bis-mustard (XIV), was considerably more toxic than the one-armed mustard (XV). Both were inactive against Sarcoma 180 and Adenocarcinoma 755, and were active against Leukemia L-1210.

EXPERIMENTAL[14]

3-Deoxy-3-bis(hydroxyethyl)amino-1,2:5,6-di-O-isopropylidene-D-allofuranose (XI). A solution of 6.0 g. (23.1 mmoles) of the amine (X) and 12 ml. of ethylene oxide in 24 ml. of 1,2-dimethoxyethane was heated in a Parr bomb for 72 hr. in an oil bath at 145°. At the end of this time, the cooled reaction mixture was partitioned between 50 ml. each of chloroform and water. The water layer was extracted with three 10-ml. portions of chloroform. The combined chloroform extracts were dried over magnesium sulfate, then evaporated to dryness *in vacuo* to give 5.35 g. (67%) of crude XI as a tan sirup; $\lambda_{max(\mu)}^{film}$ 2.90 (OH), 7.24 (CH_3).

Acetylation of an aliquot of crude XI with acetic anhydride in pyridine gave a sirup which contained no *N*-acetate, as shown by the infrared spectrum.[9]

A solution of 4.94 g. (14.2 mmoles) of the above crude XI

(9) The completeness of the reaction with ethylene oxide could be easily determined by acetylation of an aliquot of the hydroxyethylation product, followed by infrared examination of the resulting acetate. Complete hydroxyethylation is evidenced by the absence of infrared absorption at 6.0 μ, assignable to an *N*-acetate carbonyl. See E. J. Reist, H. P. Hamlow, I. G. Junga, R. M. Silverstein, and B. R. Baker, *J. Org. Chem.*, **25**, 1455 (1960).

(10) The paper chromatograms were run by the descending technique on Whatman No. 1 paper, using the solvent systems *n*-butyl alcohol–acetic acid–water (4:1:5) (solvent A) and isopropyl alcohol–2*N* aqueous hydrochloric acid (65:35) (solvent B). Spots were detected by an aniline citrate or potassium periodate cuprate (KPR)[11] spray. Glucose was used as a standard and spot locations were expressed as R_g units with glucose at R_g 1.00.

(11) T. G. Bonner, *Chem. & Ind. (London)*, 345 (1960).

(12) R. U. Lemieux and P. Chu, *J. Am. Chem. Soc.*, **80**, 4745 (1958); B. Coxon and L. Hough, *J. Chem. Soc.*, 1643 (1961).

(13) These tests were performed by the Biology Department at this Institute under contract with the Cancer Chemotherapy National Service Center.

(14) Melting points were taken on a Fisher-Johns apparatus and are uncorrected. Optical rotations were measured with a Standard Polarimeter model D attachment to the Beckman DU spectrophotometer calibrated with standard sucrose solution.

in 30 ml. of dry pyridine was treated with 9.0 g. (48.7 mmoles) of *p*-nitrobenzoyl chloride. The reaction was stirred at room temperature for 20 hr., then poured slowly with stirring into 300 ml. of saturated aqueous sodium bicarbonate solution. The bicarbonate solution was extracted with four 20-ml. portions of chloroform. The combined chloroform extracts were evaporated to dryness *in vacuo* and the gummy residue was triturated with 50 ml. of cold methanol to give 6.81 g. of XII as a white solid, m.p. 135–138°. Recrystallization from 30 ml. of chloroform-methanol (1:2) gave 6.20 g. (45% yield from X) of XII, m.p. 134.5–135.5°.

The analytical sample from a previous run had m.p. 135.0–135.5°; $[\alpha]_D^{24}$ +45° (1% in chloroform); λ_{max}^{Nujol} 5.80 (C=O), 6.52, 7.40 (NO_2).

Anal. Calcd. for $C_{30}H_{35}N_3O_{13}$: C, 55.8; H, 5.43; N, 6.52. Found: C, 55.3; H, 5.58; N, 6.43.

A suspension of 6.20 g. (9.65 mmoles) of the di-*p*-nitrobenzoate (XII) in 20 ml. of methanol and 20 ml. of 10% aqueous sodium hydroxide was heated on a steam bath for 1½ hr., then cooled, diluted with an additional 20 ml. of water, and extracted with five 10-ml. portions of chloroform. The combined chloroform extracts were washed with 10 ml. of water, dried over magnesium sulfate, then evaporated to dryness *in vacuo* to give 3.24 g. of XI as a clear sirup that crystallized on trituration with 25 ml. of *n*-pentane. Recrystallization from *n*-pentane gave the analytical sample of XI, m.p. 87–88°, $[\alpha]_D^{24}$ +122° (1% in chloroform), λ_{max}^{Nujol} 2.90 (OH).

Anal. Calcd. for $C_{16}H_{29}NO_7$: C, 55.3; H, 8.41; N, 4.03. Found: C, 55.2; H, 7.95; N, 4.09.

A subsequent hydroxyethylation of the amine (X) gave a 55% over-all yield of purified XI via the *p*-nitrobenzoate (XII).

3-Bis(chloroethyl)amino-3-deoxy-D-allose hydrochloride (XIV). To a solution of 3.6 g. (10.4 mmoles) of purified XI in 20 ml. of dry dichloromethane was added 6.0 ml. of thionyl chloride. The reaction mixture was heated at reflux for 1½ hr., then cooled, diluted with an equal volume of dry dichloromethane, and added dropwise with vigorous stirring to 150 ml. of saturated aqueous sodium bicarbonate, care being taken to prevent the formation of any local acidity. After the addition was complete, the layers were separated and the aqueous phase was washed with an additional 20 ml. of dichloromethane. The combined organic extracts were dried over magnesium sulfate, then evaporated to dryness, to give 4.1 g. of XIII as a yellow foam which was essentially free of OH according to the infrared spectrum.

A solution of 4.1 g. of the blocked mustard (XIII) in 15 ml. of 16*N* aqueous hydrochloric acid was heated on the steam bath for 1½ hr. The acid solution was decolorized with Norit, then lyophylized to yield 1.45 g. of the bis-mustard (XIV) as a colorless foam that was homogeneous on paper chromatography,[10] with R_g 1.02 in solvent A.

Anal. Calcd. for $C_{10}H_{19}Cl_2NO_5 \cdot HCl \cdot H_2O$: C, 33.5; H, 6.16; Cl, 29.6.; N, 3.91. Found: C, 33.7; H, 5.84; Cl, 29.9; N, 3.87.

3-Deoxy-3-ethylamino-1,2:5,6-di-O-isopropylidene-D-allofuranose (XVII). A mixture of 2.0 g. of powdered lithium aluminum hydride in 50 ml. of anhydrous ether was cooled to 0° in an ice bath and 5.0 g. (16.6 mmoles) of 3-acetamido-3-deoxy-1,2:5,6-di-*O*-isopropylidene-D-allofuranose (XVI)[13] was added with good stirring over a period of 20 min. After the addition was complete, the reaction was heated at reflux for 18 hr. The excess lithium aluminum hydride was decomposed by the cautious addition of 15 ml. of water, followed by 15 ml. of 10% aqueous sodium hydroxide. The ether extract was decanted and the residual white sludge was extracted with three 10-ml. portions of ether. The combined ether extracts were washed with 10 ml. of water, then dried over magnesium sulfate and evaporated to dryness *in vacuo* to yield 4.88 g. (100%) of XVII as a colorless sirup; $\lambda_{max,\mu}^{film}$ 3.04, 6.75 (NH), 7.25 (CH_3); there was no *N*-acetate absorption at 6.0 μ. Evaporative distillation at 60–65° (0.1 mm.) of a 500-mg. sample of XVII prepared as above gave 300 mg. of a colorless sirup.

Anal. Calcd. for $C_{14}H_{25}NO_5$: C, 58.5; H, 8.77; N, 4.87. Found: C, 57.4; H, 8.63; N, 5.06.

Hydrolysis of an aliquot of XVII with 6 *N* hydrochloric acid yielded a foam that contained no 3-amino-3-deoxy-D-allose according to paper chromatography.

A crystalline *N*-*p*-nitrobenzoate (XVIII), m.p. 134–139°, was prepared in 54% yield from crude XVII. Recrystallization from methanol gave the analytical sample, which had m.p. 143.5–144.0°; $[\alpha]_D^{25}$ +60° (1% in chloroform); $\lambda_{max,\mu}^{Nujol}$ 6.08 (C=O), 6.52, 7.40 (NO_2).

Anal. Calcd. for $C_{21}H_{29}N_3O_8$: C, 57.8; H, 6.43; N, 6.43. Found: C, 58.0; H, 6.43; N, 6.42.

3-Deoxy-3-[ethyl(2-hydroxyethyl)amino]-1,2:5,6-di-O-isopropylidene-D-allofuranose (XIX). A solution of 20.0 g. (0.69 mole) of crude XVII in 80 ml. of 1,2-dimethoxyethane and 40 ml. of distilled ethylene oxide was heated at 170° in a Parr bomb for 90 hr. The reaction mixture was diluted with 40 ml. of chloroform, then washed with two 40-ml. portions of water. The water washes were extracted with two 20-ml. portions of chloroform. The combined chloroform extracts were dried over magnesium sulfate, then evaporated to dryness *in vacuo* to give 24.1 g. of crude XIX as a dark sirup. Acetylation of an aliquot of XIX gave a sirup that was free of *N*-acetate according to infrared spectroscopy. Distillation of XIX at 107–110° (0.007 mm.) gave 14.4 g. (63%) of a pale yellow sirup that slowly crystallized. Recrystallization of this distillate from Skellysolve B gave 7.08 g. (31% yield from XVII) of XIX, m.p. 44.5–45.0°, $[\alpha]_D^{19}$ +119° (1% in chloroform), $\lambda_{max,\mu}^{Nujol}$ 2.93 (OH).

Anal. Calcd. for $C_{16}H_{29}NO_6$: C, 58.0; H, 8.82; N, 4.23. Found: C, 58.3; H, 8.82; N, 4.22.

3-[(2-Chloroethyl)ethylamino]-3-deoxy-D-allose hydrochloride (XV). A solution of 3.0 g. (9.05 mmoles) of XIX in 50 ml. of dry dichloromethane and 3.0 ml. of thionyl chloride was heated on the steam bath for 1½ hr. The reaction mixture was cooled, then diluted with 50 ml. of dry dichloromethane and added dropwise with stirring to 300 ml. of saturated aqueous sodium bicarbonate. The organic layer was separated and washed with water, then dried over magnesium sulfate and evaporated to dryness *in vacuo* to yield 3.16 g. (100%) of XX as a yellow sirup. The infrared spectrum of crude XX was essentially free of hydroxyl absorption near 3.0 μ.

A solution of 3.1 g. (9.05 mmoles) of crude XX in 10 ml. of 6*N* aqueous hydrochloric acid was left at room temperature for 1½ hr., then heated on the steam bath for 1 hr. After treatment with Norit, the reaction was lyophilized to give 1.85 g. (67%) of the one-armed mustard (XV) as a colorless foam that was homogeneous on paper chromatography,[10] with R_g 1.15 in solvent A and R_g 2.10 in solvent B.

Anal. Calcd. for $C_{10}H_{20}ClNO_5 \cdot 1^1/_8 HCl \cdot H_2O$: C, 36.5; H, 7.04; Cl, 22.9; N, 4.26. Found: C, 36.5; H, 7.05; Cl, 22.9; N, 4.18.

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