Chemistry of Nitrosoureas. Decomposition of Deuterated 1,3-Bis(2-chloroethyl)-1-nitrosourea

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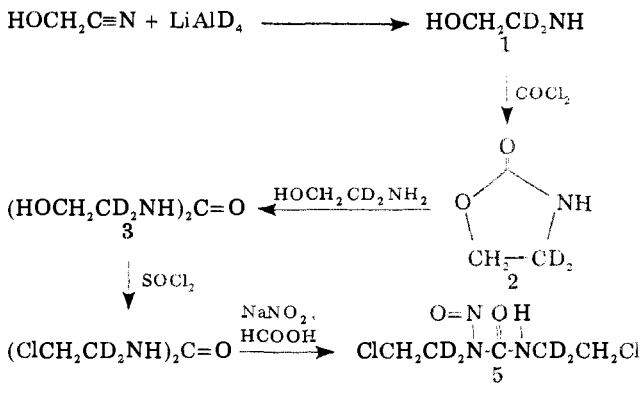
BCNU-α-d_4 [1,3-bis(2-chloro-1,1-dideuterioethyl)-1-nitrosourea] and BCNU-β-d_4 [1,3-bis(2-chloro-2,2-dideuterioethyl)-1-nitrosourea] were synthesized and decomposed in buffered (pH 7.4) water. The products were analyzed by GC–MS. The deuterium distribution in the products is inconsistent with vinylcarbonium ion or diazochloroethane intermediacy but is consistent with a 2-chloroethylcarbonium ion intermediate with some rearrangement to the 1-chloroethylcarbonium ion and the cyclic chloronium ion.

BCNU [1,3-bis(2-chloroethyl)-1-nitrosourea] is a useful agent for the treatment of certain malignant diseases. Recently, we reported that vinyl chloride, acetaldehyde, 1,2-dichloroethane, and chloroethanol were its major decomposition products in buffered aqueous solution (pH 7.4). We suggested that these arose from a chloroethylcarbonium ion or a chloroethyldiazonium ion and that alkylation by one of these species might be responsible for the antitumor activity.[1] Our product identifications have been confirmed recently and extended to other chloroethylnitrosoureas.[2,3] However, there is still some question about the mechanism of reaction of this and other clinically useful chloroethylnitrosoureas. Diazochloroethane should be considered as an intermediate because diazomethane is known to be a product from *N*-nitrosomethylurea,[4] although under very basic conditions. Montgomery and co-workers[2b,5] have proposed vinylcarbonium ions as the source of acetaldehyde and vinyl chloride. We report here the synthesis and decomposition of 1,3-bis(2-chloro-1,1-dideuterioethyl)-1-nitrosourea (BCNU-α-d_4, **5**) and 1,3-bis(2-chloro-2,2-dideuterioethyl)-1-nitrosourea (BCNU-β-d_4, **7**) in order to distinguish among the three mechanisms.

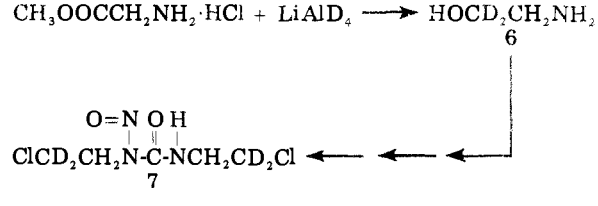
Chemistry. BCNU-α-d_4 (**5**) was synthesized as shown in Scheme I. That the deuteriums were not lost or scrambled during the synthesis was confirmed by NMR and MS. The unnitrosated urea **4** gave a MS which showed a molecular ion at 188 and a large M – 49 (CH_2Cl), but no M – 51 (CD_2Cl). The NMR ($CDCl_3$) of BCNU-α-d_4 (**5**) showed three broadened singlets (δ 7.4, 3.8, and 3.5 with relative areas 1:2:2) with no absorption at δ 4.2 for protons next to the nitroso bearing nitrogen.

BCNU-β-d_4 (**7**) was synthesized by the same route, except that the ethanolamine (2-amino-1,1-dideuterioethanol, in this case) was prepared by reducing glycine methyl ester hydrochloride (Scheme II). The spectral data again confirmed that the deuteriums were not lost or scrambled.

Scheme I



Scheme II



Results

The deuterated BCNU's were allowed to decompose at 37° in phosphate buffered (0.2 M, pH 7.4) water in a gas-tight vial and the products were analyzed by GC–MS, as previously described.[1] Each product was identified by retention time as compared to a standard, as well as by its characteristic mass spectrum. The GC–MS results are shown in Table I. Obtained from BCNU-α-d_4 were vinyl chloride containing two deuteriums, acetaldehyde containing no deuteriums, 1,2-dichloroethane containing two deuteriums, and chloroethanol containing two deuteriums predominantly on the hydroxyl bearing carbon. Obtained from BCNU-β-d_4 were vinyl chloride containing one deuterium, acetaldehyde containing a deuterium on the carbonyl, 1,2-dichloroethane containing two deuteriums, and chloroethanol containing two deuteriums predominantly on the chlorine bearing carbon. The decompositions were also done in buffer containing 10% ethanol. Under these conditions, 2-chloroethyl ethyl ether is a product. The chloroethyl ethyl ether from BCNU-α-d_4 contains two deuteriums predominantly on the oxygen bearing carbon; that from BCNU-β-d_4 contains two deuteriums predominantly on the chlorine bearing carbon.

A different deuterium isotope distribution (either number of deuteriums or position) amounting to at least 10% of any of the products should have been detectable, if it had been present. Because there is some background at most masses, smaller amounts of different distributions could go undetected. However, because there was no background at m/e 33, the peak seen here clearly indicates that chloroethanol, with two deuteriums on the hydroxyl bearing carbon, is a minor component of the chloroethanol from BCNU-β-d_4. The existence of this minor component increases our confidence in the existence of the minor components observed in the chloroethanol from BCNU-

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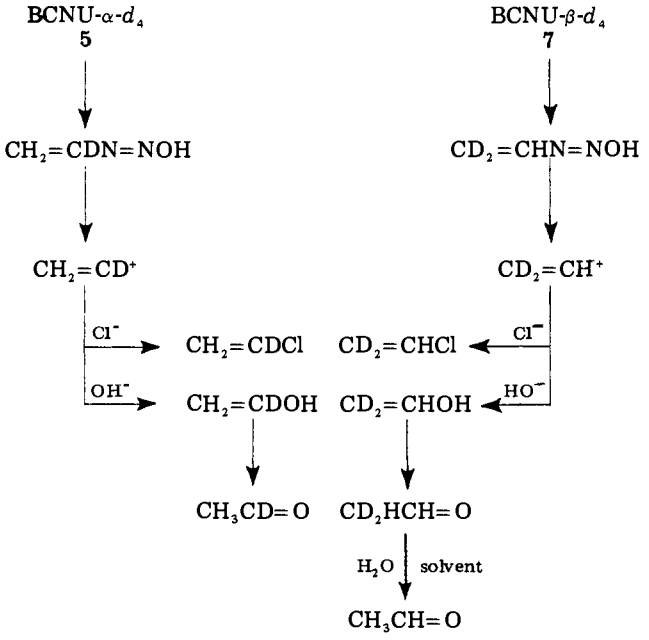
Table I. Mass Spectral Data on BCNU-α-d_4, BCNU-β-d_4, and Decomposition Products

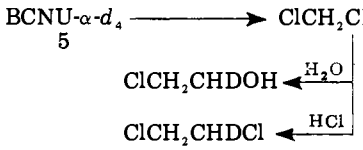
Nitrosourea	Decomposition product	m/e (fragment)
BCNU-α-d_4		217 + 219 + 221 (M^+, 4 D, 2 Cl), 168 + 170 ($M^+ - CH_2Cl$), 110 + 112 ($ClCH_2CD_2N_2OH$), 108 + 110 ($ClCH_2CD_2NHCO$), 75 ($CH_2CD_2N_2OH$), 65 + 67 ($ClCH_2CD_2$)
	Vinyl chloride	64 + 66 (M^+, 2 D, 1 Cl), 29 ($M^+ - Cl$)
	Acetaldehyde	44 (M^+, no D), 43 (CH_3CO), 29 (CHO)
	1,2-Dichloroethane[a]	100 + 102 (M^+, 2 D, 2 Cl), 64 + 66 ($M^+ - HCl$), 63 + 65 ($M^+ - DCl$), 29 (C_2HD_2)
	Chloroethanol	82 + 84 (M^+, 2 D, 1 Cl), 33 (CD_2OH), 31 (CH_2OH, ~5% of 33 peak)
	2-Chloroethyl ethyl ether	110 + 112 (M^+, 2 D, 1 Cl), 65 + 67 ($ClCH_2CD_2$), 61 ($CD_2OCH_2CH_3$), 59 ($CH_2OCH_2CH_3$, ~5% of 61 peak)
BCNU-β-d_4		217 + 219 + 221 (M^+, 4 D, 2 Cl), 166 + 168 ($M^+ - CD_2Cl$), 110 + 112 ($ClCD_2CH_2N_2OH$), 108 + 110 ($ClCD_2CH_2NHCO$), 75 ($CD_2CH_2N_2OH$), 65 + 67 ($ClCD_2CH_2$)
	Vinyl chloride	63 + 65 (M^+, 1 D, 1 Cl), 28 ($M^+ - Cl$)
	Acetaldehyde	45 (M^+, 1 D), 43 (CH_3CO), 30 (CDO)
	1,2-Dichloroethane[a]	100 + 102 (M^+, 2 D, 1 Cl), 64 + 66 ($M^+ - HCl$), 63 + 65 ($M^+ - DCl$) 29 (C_2HD_2)
	Chloroethanol	82 + 84 (M^+, 2 D, 1 Cl), 31 (CH_2OH), 33 (CD_2OH, ~5% of 31 peak)
	2-Chloroethyl ethyl ether	110 + 112 (M^+, 2 D, 1 Cl), 65 + 67 ($ClCD_2CH_2$), 59 ($CH_2OCH_2CH_3$), 61 ($CD_2OCH_2CH_3$, ~5% of 59 peak)

[a] m/e 104 was too small to detect; a large background at m/e 49 + 51 did not allow detection of the fragments expected at these masses.

Scheme III



Scheme IV



α-d_4 and in the chloroethyl ethyl ether from both BCNU-α-d_4 and BCNU-β-d_4. Vinyl chloride and dichloroethane do not fragment in a way which permits the position of the deuteriums to be determined.

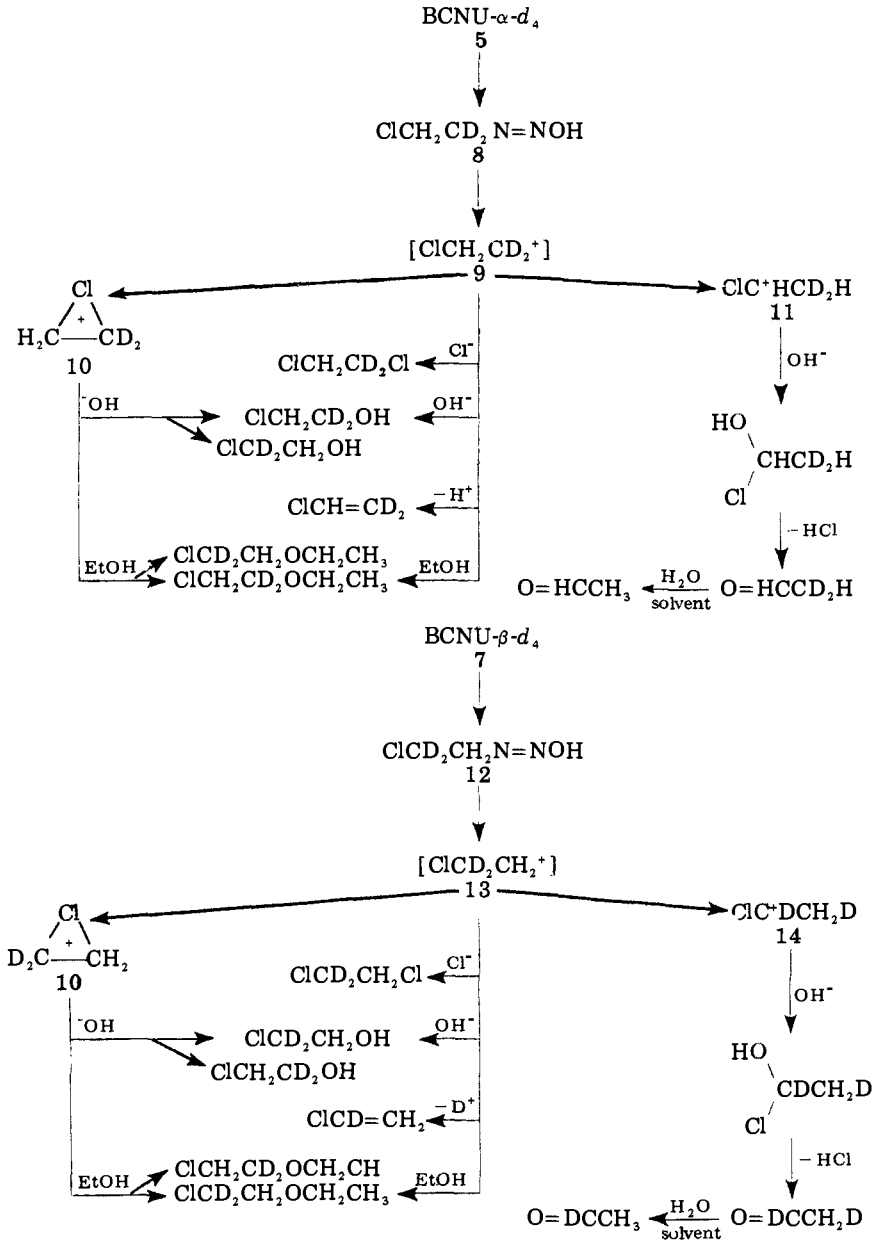
Discussion

The vinylcarbonium mechanism (Scheme III) predicts that BCNU-α-d_4 should give acetaladehyde with deuterium on the carbonyl and vinyl chloride with one deuterium, while BCNU-β-d_4 should give acetaladehyde with no deuteriums (the α-hydrogens are rapidly exchanged with the solvent) and vinyl chloride with two deuteriums. The experimental results are exactly opposite from these predictions. The diazoalkane mechanism (Scheme IV) predicts that the chloroethanol and dichloroethane from BCNU-α-d_4 should contain only one deuterium. The experimental results show that both of these products contain two deuteriums. The retention of both deuteriums is consistent with the deamination of 1,1-dideuteriobutylamine[6] and with the methylation of nucleic acids by nitrosomethyl-d_3-urea[7] and other deaminative type alkylating agents.[8] These results show that neither the vinylcarbonium ion mechanism nor the diazoalkane mechanism contributes in a quantitatively significant manner to the products.

The results are consistent with the 2-chloroethylcarbonium ion mechanism (Scheme V). In this scheme, the acetaldehyde is produced from the 1-chloroethylcarbonium ion (**11** or **14**) which is formed by a hydride shift. We have recently identified 1,1-dichloroethane as a minor product,[3] which this mechanism predicts should be formed by reaction of chloride with the 1-chloroethylcarbonium ion. The fact that in about 5% of the chloroethanol and chloroethyl ethyl ether molecules both deuteriums are shifted indicates that about 10% of these products are formed via the cyclic chloronium ion **10**.

Our results do not require that the 2-chloroethylcarbonium ion (**9** or **13**) be an intermediate. The products could arise via SN2 and E2 reactions of the proposed diazo hydroxide[9] (**8** or **12**) and rearrangements concerted with the loss of nitrogen from this intermediate bypassing the 2-chloroethylcarbonium ion. Reed and co-workers have studied the decomposition of 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea in the presence of added halide ions and found that the added halides did not increase the rate of nitrosourea decomposition but did change the products. In the presence of equal concentrations of added bromide and chloride, nearly equal amounts of 1,2-dichloroethane (increased over no added chloride) and 1-bromo-2-chloroethane were formed.[2a] These results were interpreted as indicating that the products are not arising from an SN2 attack on the nitrosourea but are generated from intermediates formed from the nitrosourea in a rate-determining reaction. Because of the lack of selectivity for nucleophiles, Reed proposed that the products came from SN1 reactions of the 2-chloroethylcarbonium ion. However, the nitrosative deamination of optically active 1-deuter-

Scheme V



iobutylamine involves predominant inversion of configuration, a fact which indicates that the decomposition of primary diazo hydroxides is an SN2 process.[6] The nitrosative deamination of 2-chloroethylamine, a reaction which should involve 2-chloroethyldiazohydroxide as an intermediate, yields the same products as the decomposition of BCNU.[1] Theoretical calculations indicate that the rearrangement of the 2-chloroethylcarbonium ion to the bridged chloronium ion and also to the 1-chloroethylcarbonium ion is exothermic and requires no activation energy[10] (i.e., the 2-chloroethylcarbonium ion is a transition state, not an intermediate). The ionization of 1-chloro-2-fluoroethane in superacidic media gives both the chloronium ion and the 1-chloroethylcarbonium ion as stable species but not the 2-chloroethylcarbonium ion.[11] Thus, a reaction which goes through a 2-chloroethylcarbonium ion would be expected to yield products predominantly from the rearranged 1-chloroethylcarbonium and cyclic chloronium ions. The major product from BCNU, 2-chloroethanol (60%), is mostly (90%) unrearranged. Therefore, the decomposition of BCNU does not appear to go through a classical SN1 reaction.

As a reasonable mechanism for the reactions of BCNU, we propose that BCNU decomposes to chloroethyldiazo hydroxide[9] in a rate-determining step catalyzed by bases and possibly some nucleophiles. The diazo hydroxide then very rapidly undergoes SN2 reactions of low activation energy and, therefore, low selectivity or undergoes rearrangement concerted with loss of nitrogen to the chloronium ion or the 1-chloroethylcarbonium ion.

There is mounting evidence that alkylation is the biologically important reaction for the antitumor effect of the chloroethylnitrosoureas.[3,12] The products of the reaction of BCNU with polycytidylic acid can be explained by a 2-chloroethyl alkylating species.[13] The results reported here show that the decomposition of BCNU does generate species capable of alkylating water, chloride, and ethanol. Of the four alkylating intermediates in Scheme V—diazo hydroxide, 2-chloroethylcarbonium ion, chloronium ion, and 1-chloroethylcarbonium ion—the first three would deliver a 2-chloroethyl group. A more exact knowledge of the mechanism of decomposition of BCNU is important to understanding the pharmacology of the therapeutically active chloroethylnitrosoureas and, in

particular, which alkylating species is(are) responsible for the antitumor effect.

Experimental Section

NMR spectra were obtained on a Varian A-60 instrument. Gas chromatography–mass spectrometry was performed on a Du Pont 491 instrument. Mass spectra of the parent BCNU's were obtained on a Du Pont CEC 21-110 instrument.

Decompositions. Deuterated BCNU (17.5 mg, 0.08 mmol) and pH 7.4, 0.2 M phosphate buffer (0.8 ml) were incubated at 37° in a gas-tight vial fitted with a Teflon lined septum for 1 week. Subsequently, methylene chloride (0.2 ml) was injected into the vial. After vigorous shaking, the organic layer was analyzed by GC–MS as previously described.

2-Amino-2,2-dideuterioethanol (1). To a stirred suspension of lithium aluminum deuteride (5.17 g, 0.12 mol) in tetrahydrofuran (180 ml, freshly distilled from $LiAlH_4$) was added a solution of glycolonitrile[14] (7.7 g, 0.135 mol) in tetrahydrofuran (80 ml) while maintaining the temperature below 10°. The mixture was then refluxed for 1 h and cooled on ice, and then saturated aqueous sodium sulfate (9 ml) was added dropwise with vigorous stirring. The solids were collected and extracted overnight in a Soxhlet with tetrahydrofuran. The tetrahydrofuran solutions were combined and the solvent was removed under vacuum. Distillation (85–90°, ~10 mm) gave a colorless liquid (4.6 g, 0.073 mol, 54%): NMR (Me_2SO-d_6) δ 3.4 (2 H, s), 2.7 (3 H, s); MS M^+ 63.

4,4-Dideuterio-2-oxazolidone (2). Phosgene (caution, poisonous gas) was slowly bubbled through a vigorously stirring mixture of **1** (3.0 g, 0.047 mol), sodium hydroxide (5.8 g, 0.145 mol, powder), sodium sulfate (10 g, anhydrous), and methylene chloride (200 ml) cooled on ice. The reaction is over when the solution remains acidic to wet litmus 10 min after the phosgene is stopped (reaction time ~0.5 h). The mixture was filtered and the solvent removed under vacuum. Chromatography (ethyl acetate on silica gel) and crystallization from benzene gave white crystals (2.55 g, 0.029 mol, 60%): mp 85–88° (lit.[15] for undeuterated, 87–89°); NMR ($CDCl_3$) δ 6.6 (1 H, br s), 4.5 (2 H, s); MS M^+ 89.

1,3-Bis(1,1-dideuterio-2-hydroxyethyl)urea (3). A solution of **1** (1.5 g, 0.024 mol) and **2** (2.5 g, 0.028 mol) in glyme (3 ml) was refluxed for 7 h (130° bath temperature). The solvent was removed under vacuum and the residue crystallized from acetone (30 ml) to give white crystals (1.85 g, 0.012 mol, 51%): mp 82–84° (lit.[16] for undeuterated, 86°); NMR (Me_2SO-d_6) δ 6.1 (2 H, s), 4.6 (2 H, s), 3.4 (4 H, s).

1,3-Bis(2-chloro-1,1-dideuterioethyl)urea (4). A solution of **3** (1.8 g, 0.012 mol) in thionyl chloride (10 ml) was refluxed for 1 h. The excess thionyl chloride was removed under vacuum. Chromatography (ethyl acetate on alumina) and crystallization from ethyl acetate gave white crystals (0.90 g, 0.0052 mol, 44%): mp 126–128° (lit.[17] for undeuterated, 127°); NMR (Me_2SO-d_6) 6.4 (2 H, s), 3.6 (4 H, s); MS M^+ 188, 190, 192; $M^+ - CH_2Cl$, 139 and 141.

1,3-Bis(2-chloro-1,1-dideuterioethyl)-1-nitrosourea (BCNU-α-d_4, 5). To a solution of **4** (188 mg, 1 mmol) in formic acid (2 ml, 90%) at 0° was added a solution of sodium nitrite (140 mg, 2 mmol) in water (1 ml). The mixture was stirred at 0° for 2 h and poured into ether, and the ether solution was extracted three times with cold water and dried with sodium sulfate. Removal of the solvent under vacuum gave a yellow oil which crystallized on standing (190 mg, 87%): mp 27–28° (lit.[18] for undeuterated, 30–32°); NMR ($CDCl_3$) δ 7.4 (1 H, s), 3.8 (2 H, s), 3.5 (2 H, s).

2-Amino-1,1-dideuterioethanol (6). To a stirred suspension of lithium aluminum deuteride (10 g, 0.24 mol) in tetrahydrofuran (250 ml, freshly distilled from $LiAlH_4$) was added slowly solid glycine methyl ester hydrochloride (22 g, 0.174 mol) while maintaining the temperature below 10°. The mixture was refluxed overnight and cooled on ice, and saturated aqueous sodium sulfate (20 ml) was added with vigorous stirring. The solids were collected and extracted in a Soxhlet for 3 days with tetrahydrofuran. The tetrahydrofuran solutions were combined and the solvent was removed under vacuum. Distillation (83–88°, ~10 mm) gave a colorless liquid (6.3 g, 0.1 mol, 57%): NMR (Me_2SO-d_6) 2.7 (3 H, s), 2.6 (2 H, s); MS M^+ 63.

5,5-Dideuterio-2-oxazolidone (15). Compound **15** was prepared from **6** following the synthesis of **2**: mp 85–87°; NMR ($CDCl_3$) δ 6.6 (1 H, br s), 3.6 (2 H, s); MS M^+ 89.

1,3-Bis(2,2-dideuterio-2-hydroxyethyl)urea (16). Compound **16** was prepared from **6** and **15** following the synthesis of **3**: mp 81–84°; NMR (Me_2SO-d_6) δ 6.1 (2 H, t), 4.6 (2 H, s), 3.1 (4 H, d).

1,3-Bis(2-chloro-2,2-dideuterioethyl)urea (17). Compound **17** was prepared from **16** following the synthesis of **4**: mp 127–128°; NMR (Me_2SO-d_6) δ 6.4 (2 H, t), 3.3 (4 H, d); MS M^+ 188, 190, and 192; $M^+ - CD_2Cl$, 137 and 139.

1,3-Bis(2-chloro-2,2-dideuterioethyl)-1-nitrosourea (BCNU-β-d_4, 7). BCNU-β-d_4 (**7**) was prepared from **17** following the synthesis of BCNU-α-d_4 (**5**): mp 28–29°; NMR ($CDCl_3$) δ 7.4 (1 H, t) 4.2 (2 H, s), 3.8 (2 H, d).

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