Isophosphoramide Mustard and Its Mechanism of Bisalkylation

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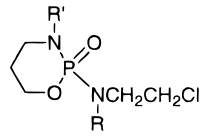
To investigate the mechanism(s) of bisalkylation by isophosphoramide mustard (IPM), IPM-$\beta,\beta,\beta',\beta'$-$d_4$ was synthesized and the products of its reaction with thiosulfate (at pD 7.0) were analyzed by NMR. By both ^{1}H and ^{13}C NMR, the distribution of deuterium in the products was consistent with bisalkylation through sequential aziridinyl intermediates [(NCH_2CD_2S):(NCD_2CH_2S) = 53:47]. Under the given reaction conditions, label scrambling as a result of thiosulfate acting as a leaving group was ruled out through control experiments. The data gave a calculated kinetic isotope effect of 0.97 per deuterium. For the initial aziridine species formed from IPM, ab initio quantum chemical calculations gave a hybridization value of $sp^{2.4-2.5}$ for each of the C−H bonds of the reaction centers, and this correlated with the observed inverse isotope effect. Other structure and bond order data were also determined for this aziridine intermediate and related compounds.

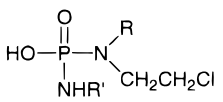
Introduction

The clinically useful anticancer agents ifosfamide (**1**) and cyclophosphamide (**2**) metabolize through similar pathways to give isophosphoramide mustard (**3**) and phosphoramide mustard (**4**), respectively.[1−3] It has been



1: R = H, R' = CH_2CH_2Cl

2: R = CH_2CH_2Cl, R' = H



3: R = H, R' = CH_2CH_2Cl

4: R = CH_2CH_2Cl, R' = H

shown that **4** alkylates nucleophiles (e.g., DNA) through the consecutive formation of two aziridinyl ion intermediates,[4] and it is generally accepted that **3** reacts in a similar manner, as shown in Scheme 1. Although this has not been verified, there is indirect evidence to support this conclusion. Aziridinyl intermediates (**5**, **7**) have been identified (^{1}H, ^{15}N, and ^{31}P NMR) in aqueous solutions of **3**,[5−7] and one study, albeit limited, has shown that the half-life of **3** is unaffected by the addition of nucleophiles to the reaction solution.[8] On the other hand, these data do not unequivocally rule out some contribution from competing alkylation mechanisms involving intermolecular displacement of chloride from **3** (direct conversion of **3** → **6**) or intermediate **6** (direct conversion of **6** → **8**). The reported half-lives for **3** (under apparently similar reaction conditions) do vary considerably in the literature,[8−10] but regardless of exact reactivity values, it has been shown consistently that the lifetime of **3** is much longer than that of **4**. Thus, **3** could be more susceptible to competing intermolecular reactions, especially in the presence of strong nucleophiles. For meaningful comparisons of the in vivo activities of **3** and **4** as well as that of **1** and **2**, it is necessary to understand the metabolisms of each. Differences between **3** and **4** in alkylation kinetics and mechanism would be expected to impact on the efficiency of DNA cross-linking. Thus, to obtain direct evidence for the mechanism(s) of bisalkylation by **3**, **3**-$\beta,\beta,\beta',\beta'$-$d_4$ was synthesized and the products of its reaction with thiosulfate (as a model nucleophile) were analyzed by multinuclear NMR.

Experimental Section

All nonaqueous reactions were carried out under N_2 or using a drying tube. Flash chromatography used 230−400 mesh silica gel (Merck). ^{1}H (400 MHz), ^{13}C (100 MHz), and ^{31}P (162 MHz) NMR spectra were recorded on a Varian Inova-400 spectrometer. ^{1}H and ^{13}C chemical shifts were referenced to TMS ($CDCl_3$), TSP (D_2O), or DMSO-d_5 (2.49 ppm). For NMR samples in $CDCl_3$, DMSO-d_6, or D_2O, ^{31}P chemical shifts were referenced externally to capillary inserts of 25% H_3PO_4 in $CDCl_3$, DMSO-d_6, or D_2O, respectively. Values of pD correspond to the observed readings on an Orion model 720A pH meter.[11] Unlabeled **3** was a gift from the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute.

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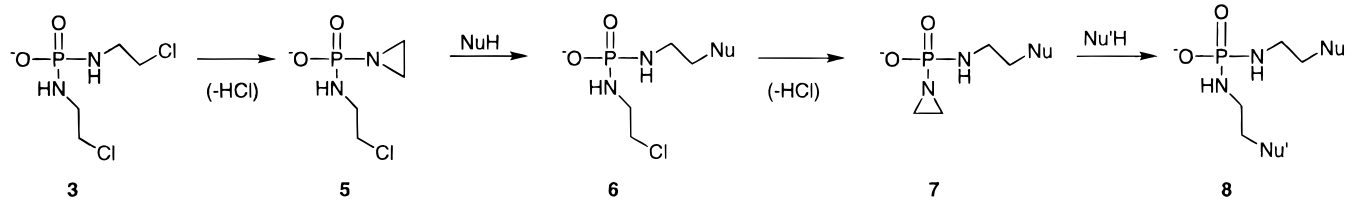
Scheme 1



2-Amino-2,2-dideuterioethanol Hydrochloride (10). Compound **10** was made using modifications to a literature procedure for ^{15}N-labeled material.[12] In solid form, glycine methyl ester hydrochloride (**9**, 18 mmol, 2.28 g, Aldrich Chemical Co.) was added slowly to a suspension of $LiAlD_4$ (24 mmol, 1.05 g, 98 atom % D, 95% chemical purity, Aldrich Chemical Co.) in THF (42 mL, dried, distilled). After being stirred for 2 h at room temperature, the reaction was quenched by the sequential, dropwise addition of 50% H_2O/THF (5.9 mL) and 40% NaOH (1.9 mL). The mixture was filtered and concentrated at reduced pressure. The residue was dissolved in CH_2Cl_2, dried (Na_2SO_4), filtered, and concentrated to give the product as an oil [0.33 g; R_f 0.09 (saturated NH_3/CH_3OH/CH_2Cl_2 (1:4)]. Soxhlet extraction (THF, overnight) of the solids from the reaction mixture provided additional product (0.57 g). The combined product (0.90 g) was dissolved in CH_2Cl_2 (25 mL), and HCl was bubbled through this for several minutes. Evaporation of solvent gave a residue that was then dissolved in saturated HCl/CH_3OH (10 mL). The CH_3OH (and trace H_2O) was removed azeotropically with CH_3CN at reduced pressure. At no stage was **10** obtained as a solid, but rather it was isolated as a gummy semisolid that was used without further purification (1.30 g, ca. 13 mmol, ca. 72% yield).

2-Chloro-2,2-dideuterioethylamine Hydrochloride (11). Using **10** as starting material, **11** was made in 65% yield using a procedure reported previously for ^{15}N labeled product.[12] There was one modification: the reaction time was increased from 24 to 50 h.

***N,N′*-Bis(2-chloro-2,2-dideuterioethyl)phosphorodiamidic Acid Phenylmethyl Ester (12).** Using **11** as starting material, **12** was made as previously described for ^{15}N-labeled product.[13] The crude product was purified by flash chromatography (3.5 × 15 cm column, 100 mL silica gel) using ethyl acetate with increasing (0, 1, and 5%) amounts of ethanol as eluent. Benzyl ester **12** was obtained as an oil [2.0 mmol, 0.63 g, 47% yield, R_f 0.55 (5% EtOH/EtOAc)]. ^{1}H NMR ($CDCl_3$) δ 7.45−7.23 (m, 5H), 5.02 (d, $^3J_{HP}$ = 8 Hz, 2H), 3.33−3.12 (br, 2H), 3.23 (d, $^3J_{HP}$ = 10 Hz, 4H); ^{13}C NMR ($CDCl_3$) δ 136.7 (d, $^3J_{CP}$ = 6 Hz), 128.8, 128.5, 128.0, 67.36 (d, $^2J_{CP}$ = 5 Hz), 42.93; ^{31}P NMR ($CDCl_3$) δ 14.9.

Palladium Black. With modifications to a procedure by Wieland,[14] $PdCl_2$ (28 mmol, 5 g) was dissolved in ∼20 mL of 5 M HCl, and the resultant mixture was filtered into H_2O (300 mL at 90 °C). Throughout the following additions, the solution was stirred vigorously, and the temperature was maintained at 90 °C. Formic acid (0.9 mL) was added slowly, and the reaction mixture was then made strongly basic (pH ∼12−14, pH paper) with 5 M KOH (∼50 mL). The pH of the solution was then lowered to ∼8 (pH paper) by the dropwise addition of formic acid. The mixture was then cooled to ambient temperature, and the liquids were removed by decanting. The Pd black precipitant was washed thoroughly with deionized water followed by CH_3OH, and it then was stored under CH_3OH (drying causes the catalyst to lose activity). By this method, Pd black was synthesized in approximately quantitative yield, and this amount was enough to make three or four 1 × 8 cm columns (and each column could be used several times).

***N,N′*-Bis(2-chloro-2,2-dideuterioethyl)phosphorodiamidic Acid (3-*β,β,β′,β′-d*$_4$).** The following catalytic transfer hydrogenation was carried out with modifications to a literature procedure for the debenzylation of esters.[15] Pd black (as a slurry in methanol) was added to a glass column (1 cm width) to a height of 8 cm. The methanol was then removed by washing the column with ethanol. Benzyl ester **12** (1.8 mmol, 0.58 g) was dissolved in a solution of 5.9% 1,4-cyclohexadiene in ethanol (67 mL), and this was passed through the column of Pd black catalyst at a flow rate of 2.3 mL/min. The collected solution was concentrated on a rotary evaporator at ambient temperature. The residual solid was washed with CH_2Cl_2 and dried (vacuum desiccator) to give the product as a white solid (0.32 g). The combined organic washes were centrifuged, and the solids were washed with minimal CH_2Cl_2 to provide additional product (24 mg) and a total yield of 82% [1.5 mmol, 0.34 g, mp 113−115 °C (compared with 110−114 °C for unlabeled **3**)]. In DMSO-d_6: ^{1}H NMR δ 3.00 (d, $^3J_{HP}$ = 12 Hz); ^{31}P NMR δ 13.2. In ∼0.1 M phosphate in D_2O, pD ∼7: ^{1}H NMR δ 3.14 (d, $^3J_{HP}$ = 10 Hz); ^{13}C NMR δ 45.86; ^{31}P NMR δ 13.5. Anal. Calcd for $C_4H_7D_4Cl_2N_2O_2P$: C, 21.35; H + D, 4.94 (in the elemental analysis, all D atoms were detected as if they were H; Atlantic Microlabs, Inc.); N, 12.45. Found: C, 22.19; H + D, 4.90; N, 12.05.

Thiosulfate Trapping Reaction. With minor modifications, deuterium-labeled **3** was reacted with thiosulfate as previously described for unlabeled material.[8] In short, **3**-*β,β,β′,β′-d*$_4$ (0.5 mmol, 117 mg) was dissolved in a solution (2 mL) of $Na_2S_2O_3$ (2.7 mmol, 673 mg of the pentahydrate) in 1 M phosphate/D_2O (with TSP) at pD 7.0.[11] The pD was readjusted to 7.0, and the reaction mixture was then heated at 37 °C for 22 h. Over this time period, the pD was checked periodically and adjusted as necessary. The final product was analyzed in situ by NMR at ambient probe temperature (the isolation and characterization of unlabeled product was described previously[8]). In 1 M phosphate/D_2O, pD 7.0: ^{1}H NMR δ 3.32 (s, 0.47 H, CH_2S), 3.27 (d, $^3J_{HP}$ = 10 Hz, 0.53 H, NCH_2); ^{13}C NMR δ 43.10 (s, 0.53 C, NCH_2), 38.84 (d, $^3J_{CP}$ = 8 Hz, 0.47 C, CH_2S); ^{31}P NMR δ 14.18, 14.17.

Control Experiments. A. Thiosulfate and 3. The thiosulfate trapping reaction described above was repeated exactly except unlabeled **3** (0.5 mmol, 115 mg) and 0.5 M phosphate/D_2O were used. The ^{31}P NMR (25 °C) spectrum of the reaction mixture after 21 h at 37 °C displayed one signal (other than that for phosphate): δ 14.2 (**8**, Nu = Nu′ = $S_2O_3^-$).

B. Thiocyanate and 3. Procedure A was repeated exactly as above but using NaSCN (2.7 mmol, 222 mg) in place of thiosulfate. ^{31}P NMR (25 °C) of the reaction mixture after 21 h displayed a major product signal at δ 13.6 (77% of the total P intensity, excluding phosphate). In addition to the signal for thiocyanate (δ 135.9), the ^{13}C NMR (internal TSP reference) displayed only three resonances: δ 118.1, 43.69, and 38.22 (d, $^3J_{CP}$ = 7 Hz). On the basis of much literature precedence,[6−8,16,17] these spectral data were consistent with the bisthiocyanate adduct **8**, Nu = Nu′ = SCN.

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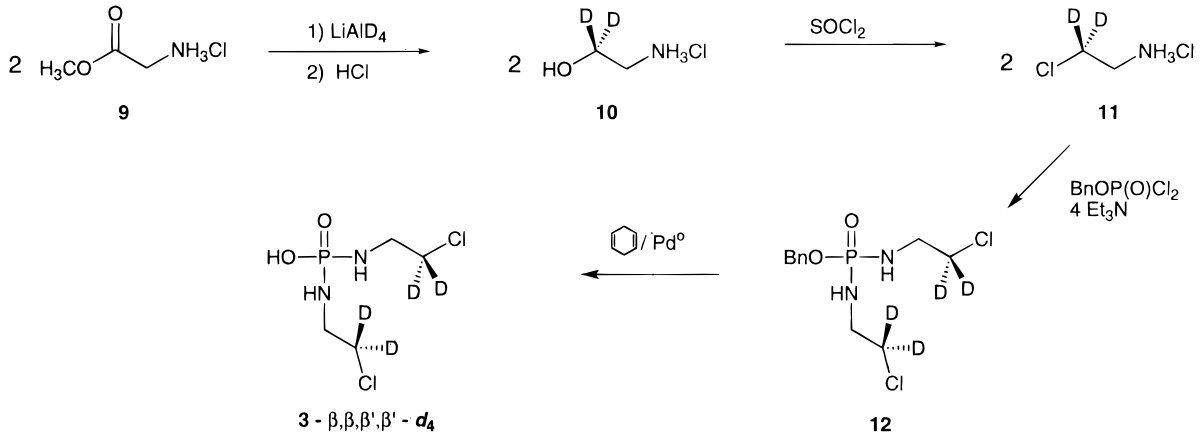
Table 1. HF/6-31G*-Optimized Bond Lengths and Angles, and HF/6-31G NBO Bond Hybridizations for the Ethylene Fragment within Each Given Species**

	C−C (Å)	C−H (Å)	H−C−H (deg)	C−C σ bond (hybridization)	C−H bond (hybridization)
5[a](anionic; P−O^-)	1.475	1.076−1.080	114.7−115.4	$sp^{3.4}$	$sp^{2.4-2.5}$
5[a](neutral; P−OH)	1.473	1.075−1.076	115.4−115.5	$sp^{3.3}$	$sp^{2.4}$
ethane	1.528	1.086	107.7	$sp^{2.6}$	$sp^{3.2}$
ethene[b]	1.317	1.078	116.4	$sp^{1.5}$	$sp^{2.3}$
aziridine	1.472	1.076−1.078	114.4	$sp^{3.3}$	$sp^{2.4}$
cyclopropane	1.498	1.076	114.0	$sp^{3.6}$	$sp^{2.6}$
$H_2NCH_2CH_2SH$[c]	1.532	1.083	108.4	$sp^{2.5}$	$sp^{3.0}$

[a] Calculations refer to the H_2C-CH_2 moiety in the aziridinyl ring for unlabeled material. [b] The C−C π bond is formed purely from carbon p atomic orbitals. [c] Hybridization calculated for the C_1−H bonds in 2-aminoethanethiol.

Scheme 2



C. Thiosulfate, Thiocyanate, and 3. Procedure A was repeated exactly but using both $Na_2S_2O_3 \cdot 5H_2O$ (2.7 mmol) and NaSCN (2.7 mmol). ^{31}P NMR (25 °C) of the reaction mixture after 21 h displayed signals at δ 14.3 (80% of products), 14.0 (14% of products), and 13.8 (1% of products). These chemical shift values were consistent[6−8,16] with the bisthiosulfate adduct (δ 14.3; **8**, Nu = Nu′ = $S_2O_3^-$), the mixed adduct (δ 14.0; **8**, Nu = $S_2O_3^-$, Nu′ = SCN), and the bisthiocyanate adduct (δ 13.8; 8, Nu = Nu′ = SCN).

D. Exchange Reaction. After the initial NMR analysis of the reaction mixture from procedure A was complete, NaSCN (255 mg) was added. The new NMR sample then consisted of 0.5 mmol of bisthiosulfate adduct **8** (Nu = Nu′ = $S_2O_3^-$), 1.7 mmol of thiosulfate, and 3.2 mmol of thiocyanate. The solution (pD adjusted to 7.3) was heated at 37 °C for 24 h. By ^{31}P NMR, the sample was unchanged.

Quantum Chemical Calculations. The structures of the compounds listed in Table 1 were fully optimized at the Hartree−Fock level of theory with a 6-31G* basis set.[18] Natural bond orbital analysis (NBO)[19,20] was performed at these optimized geometries using the Hartree−Fock 6-31G** wave function. NBO analysis is a procedure for transforming the highly delocalized molecular orbitals calculated in the Hartree−Fock procedure to a unique set of localized bonding and lone-pair orbitals. The NBOs between each pair of bonded atoms are composed of combinations of s, p, and d atomic orbitals on the two atoms, so that the hybridization of each atom's bonding orbital can be directly calculated. Note that if an atom is bonded to nonequivalent ligands, each bond will have a different hybridization. The resulting hybridizations are typically in good agreement with chemical intuition, qualitative rules involving the degree of s and p character in bonding orbitals (such as Bent's Rule[21]), and other quantum chemical methods for calculating hybridization. All calculations were done using Gaussian 94.[22]

Results and Discussion

NMR Studies. Compound **3**-$\beta,\beta,\beta',\beta'$-$d_4$ was synthesized from its precursor benzyl ester **12** as shown in Scheme 2. Conversions of such esters to acids **3** and **4** were reported previously using catalytic hydrogenation with H_2; however, the yields and product purity varied considerably.[12,13,23−25] Catalytic transfer hydrogenation[15] using 1,4-cyclohexadiene as the hydrogen donor was found to effect the desired reaction in a cleaner and more facile manner.

As a measure of labeling integrity, a comparison was made of ^1H and ^{13}C NMR spectra taken of **3** and **3**-$\beta,\beta,\beta',\beta'$-$d_4$ under the same conditions (D_2O/TSP, pD ~7). In the sample of unlabeled material, resonances for

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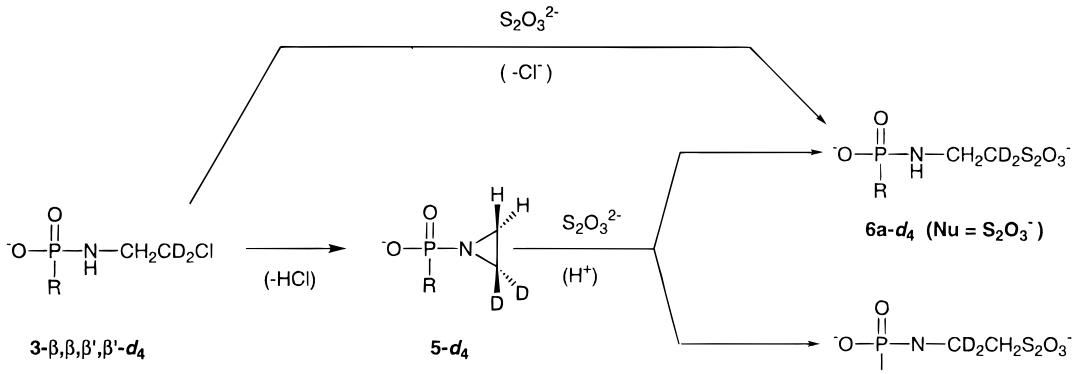
Scheme 3[a]



[a] R = $NHCH_2CD_2Cl$.

CH_2Cl were observed for the protons at 3.65 ppm (t, $^3J_{HH}$ = 6 Hz) and for the carbon at 48.76 ppm (d, $^3J_{CP}$ = 7 Hz). For the deuterated material, no similar proton or carbon resonances consistent with CHDCl could be detected (limits of detection by ^{1}H NMR ~3%).

The consequences of competing alkylation mechanisms on isotope positioning are illustrated in Scheme 3. Direct reaction of a nucleophile (e.g., thiosulfate) with **3**-$\beta,\beta,\beta',\beta'$-$d_4$ would provide a monoalkylation product with deuterium maintained in the β position of the reactant ethylene moiety (**6a**-d_4). Reaction of **6a**-d_4 with a second equivalent of nucleophile by the same mechanism would provide the same result: maintenance of deuterium in the β position with the final product of bisalkylation being **8**-$\beta,\beta,\beta',\beta'$-$d_4$ (Nu = Nu′ = $S_2O_3^-$). Alternatively, alkylation through aziridinyl intermediate **5**-d_4 (the nitrogen is not protonated at neutral pH[5]) would result in a near equal mixture of **6a**-d_4 and **6b**-d_4 (assuming minimal isotope effects). Formation and subsequent reaction of a second aziridine from **6a/b**-d_4 would give three isomers of **8**-d_4 (Nu = Nu′ = $S_2O_3^-$): (a) $\alpha,\alpha,\alpha',\alpha'$-$d_4$ (~25% of product mixture); (b) $\beta,\beta,\beta',\beta'$-$d_4$ (~25% of product mixture); and (c) $\alpha,\alpha,\beta',\beta'$-$d_4$ (~50% of product mixture). Considered as a whole, the final product mixture would reflect a nearly equal distribution of deuterium between the α/α' and β/β' positions of the ethylene moieties.

Compound **3**-$\beta,\beta,\beta',\beta'$-$d_4$ was reacted with 5 molar equiv of thiosulfate at pD 7.0[11] as previously described for unlabeled material.[8] After sufficient time was allowed for the bisalkylation reaction to be complete, the product mixture was analyzed in situ by ^{1}H, ^{13}C, and ^{31}P NMR. By both proton and carbon NMR, the ratio of NCH_2CD_2S to NCD_2CH_2S was found to be 53:47. This distribution of deuterium was consistent with bisalkylation through sequential aziridinyl intermediates and was in accord with the expected effects of the isotopic labeling. Using the relative integrated signal intensities as a measure of k_H/k_D, the kinetic isotope effect was 0.97 per deuterium [$(47/53)^{1/4}$]. This value, uncorrected for any concurrent β effect, was in the appropriate range for an α secondary isotope effect (0.7−1.5).[26] That the value was near unity was indicative of an S_N2 reaction.[27] The apparent, slight preference for substitution at the labeled reaction center was consistent with an inverse kinetic isotope effect (see Quantum Chemical Calculations below).

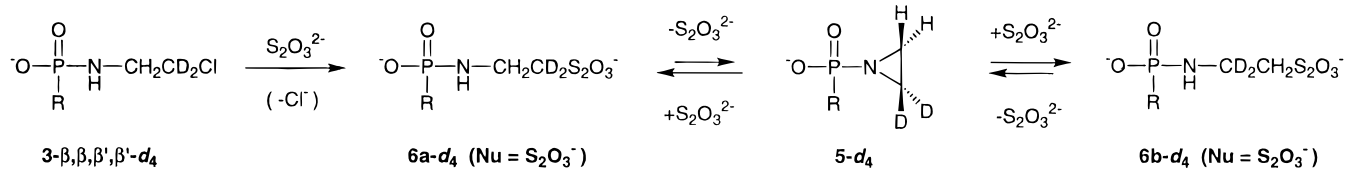
While the observed isotope effect is likely to arise primarily from an α contribution (when the reaction center in **5**-d_4 is the aziridinyl CD_2), an additional β effect may result when the nucleophilic attack is at the aziridinyl CH_2 in **5**-d_4. This effect is generally attributed to hyperconjugation and would require that the reaction center acquire substantial cationic character during the transition state.[26]

In the ^{31}P NMR spectrum of the bisalkylation product mixture, two resonances could be distinguished at δ 14.18 and 14.17 with relative peak heights of 52 and 48, respectively. While it was interesting that more than one signal was detected, no weight was given to these data in the determination of the k_H/k_D value. The signals were overlapped (so that peak height measurements were not necessarily an accurate reflection of signal intensity), the observed resonances were not assigned to a particular product, and most significantly, the spectrum did not allow for the detection of three discrete resonances. Complete reaction of **3**-$\beta,\beta,\beta',\beta'$-$d_4$ with thiosulfate through aziridinyl intermediates gives three isomers of **8**-d_4 (Nu = Nu′ = $S_2O_3^-$), as described above.

In the review of this paper, it was pointed out that the observed label scrambling could be accounted for by a sequence such as that shown in Scheme 4, whereby aziridine formation occurs through loss of thiosulfate rather than chloride. To resolve this ambiguity, it was necessary to establish the stability of the thiosulfate linkage under the conditions of the experiment. It was reasoned that if alkylation of thiosulfate were reversible then the presence of a second, strong nucleophile in solution would provide for a competitive alkylation reaction and the formation of additional products. Using unlabeled materials and excess thiosulfate, bisthiosulfate adduct **8** (Nu = Nu′ = $S_2O_3^-$) was generated in situ, and as expected, the ^{31}P NMR spectrum of this solution exhibited a single product resonance (see Control Experiments in the Experimental Section for details). Sodium thiocyanate (in a quantity twice that of residual thiosulfate) was then added to this sample, and after 24 h at 37 °C, the ^{31}P NMR spectrum was unchanged. To establish the competitive reactivity of the chosen nucleophiles, **3** was reacted with an equal mixture of thiosulfate and thiocyanate, and after 21 h at 37 °C, ^{31}P NMR resonances were observed for adducts derived from both nucleo-

(26) Carey, F. A.; Sundberg, R. J. *Advanced Organic Chemistry, Part A: Structure and Mechanism*, 2nd ed.; Plenum Press: New York, 1984; pp 190−194.

Scheme 4[a]



[a] R = $NHCH_2CD_2Cl$.

philes. While thiosulfate was the stronger nucleophile (resulting in a higher yield of thiosulfonated adducts), thiocyanate also formed adducts that were clearly visible by NMR. On the basis of these control experiments, it was concluded that the thiosulfate alkylations were not reversible and that label scrambling as a result of the reactions shown in Scheme 4 was not applicable *under the reaction conditions of our experiments*.

Quantum Chemical Calculations. Inverse kinetic isotope effects[26,27] are associated with a change in C−H bond hybridization from sp^2 to sp^3. Relative to acyclic analogues, the constraints of three-membered rings are known to result in C−H bonds with greater s character.[27] It was of interest to verify this for **5** due to the fact that the electronic natures of **5** and related species do not always follow intuition.[5,28]

For unlabeled **5** and related compounds, selected structural and bond-order data from ab initio quantum chemical calculations are given in Table 1 (full bond length and angle data for the compounds listed in Table 1 are available as Supporting Information). By way of comparison, the bond lengths and angles given by these calculations for ethane and ethene are within 2% of traditional literature values.[29] For considerations of the electronic nature of **5** at physiological pH, the structural data for the anionic form (as depicted in Schemes 2 and 3) are most relevant. For comparative purposes, the same structural data calculated for the neutral P−OH form of **5** are included in Table 1.

The bond lengths and bond angles in Table 1 indicate that the ethylene segment of the aziridinyl ring in **5** is stucturally between that in ethene and ethane. Comparisons of the groups of interest show that the C−H bond lengths and the H−C−H bond angles in anionic **5** are similar to those in ethene, while the C−C bond length in **5** is closer to that in ethane. That the C−C bond of the aziridinyl ring is somewhat shorter than that in ethane is a result of ring strain rather than enhanced double-bond character.

For comparison, the same set of calculations was performed on aziridine and cyclopropane. The C−H and C−C bonds in aziridine were found to have structural properties and bond hybridizations nearly identical to those of the corresponding moieties in anionic **5**. Not surprisingly, the structure of the CH_2CH_2 group in unlabeled **5** (and aziridine) was very similar to that of cyclopropane.

The bonding molecular orbital hybridizations given in Table 1 do not correspond exactly to the idealized integer s−p ratios, but instead, they give a more accurate description of how the hybridization adjusts to the electronegativity and orientation of the bonded atoms.[19] Thus, in the strained aziridinyl ring of anionic **5**, the C−H bonds are found to have a hybridization value of $sp^{2.4-2.5}$. As a model for the change in hybridization upon ring opening (e.g., **5** → **6**), the hybridization of the C_1-H bonds in 2-aminoethanethiol was calculated, and the value was found to be $sp^{3.0}$. The inverse kinetic isotope effect observed for bisalkylation by **3**-*β*,*β*,*β*′,*β*′-d_4 was, therefore, consistent with a change in hybridization of $sp^{2.4-2.5}$ to sp ∼3.0 for the C−H(D) bonding orbitals at the reaction centers.

Conclusion

The results of the isotope-labeling experiments provided direct evidence for the intermediacy of aziridinyl moieties in the mechanism of bisalkylation by **3** at pD 7, as illustrated in Scheme 1. Even in the presence of an excess of a strong nucleophile (thiosulfate), there were no data to support any significant contribution from a mechanism involving direct nucleophilic displacement of chloride from **3** or acyclic intermediate **6**. Under the given conditions, label scrambling as a result of thiosulfate acting as a leaving group was ruled out through control experiments. In general, of course, the reversibility of any of the alkylation steps with reformation of an aziridinyl intermediate would be dependent on the nucleophile (Scheme 1, **6** → **5** and **8** → **7**).

The distribution of NCH_2CD_2S to NCD_2CH_2S in the product mixture was consistent with an α secondary kinetic isotope effect for an S_N2 reaction. The inverse kinetic isotope effect correlated with considerations of hybridization changes during the reaction sequence (e.g., **5** → **6**) and the calculation of $sp^{2.4-2.5}$ hybridization for each C−H(D) bonding orbital at the reaction centers in **5**.

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Supporting Information Available: A listing of the full structures (HF/6-31G* optimized) in Cartesian coordinates is available for all species given in Table 1 (3 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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