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Controlling the Outcome of an *N*-Alkylation Reaction by Using *N*-Oxide Functional Groups

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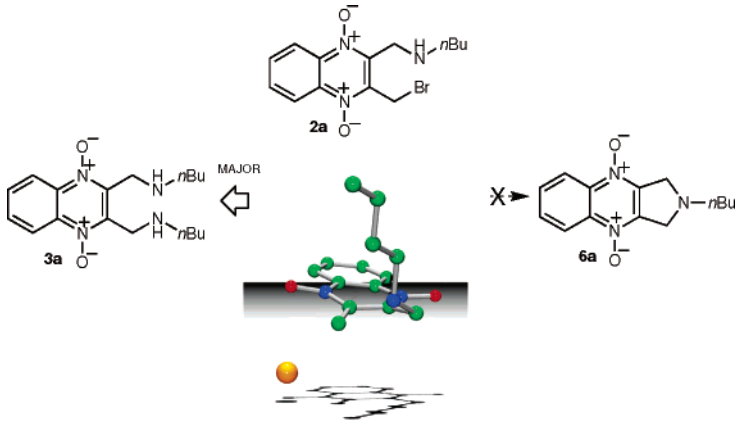

Covalent modifiers of proteins are of importance in chemical proteomics, an emerging chemical technology used to assign protein function. In this study, high-field ^{1}H NMR techniques were used to analyze the reaction of the bioactive compound, 2,3-bis(bromomethyl)quinoxaline 1,4-dioxide, with amines (a model system for proteins containing nitrogen-based nucleophiles). Unexpectedly, the results show that a double nucleophilic substitution reaction involving 2 equiv of the amine is preferred to an intramolecular cyclization pathway. A direct comparison with the reaction carried out on a substrate lacking the *N*-oxide functional groups is also provided. X-ray crystal structures and computational studies are used to rationalize the observed differences in reactivity between the two systems.

Introduction

The development of novel chemical probes that covalently modify their protein target(s) is of considerable importance in chemical proteomics.[1−4] In the majority of cases, the mechanism of covalent protein modification is obvious. However, during recent attempts to develop a novel chemical probe based on 2,3-bis(bromomethyl)-quinoxaline 1,4-dioxide (**1**), it became clear that the reaction of **1** with primary amines was more complex than first envisaged. This reaction has been the subject of previous reports,[5−7] culminating in the identification of **5** (R = CH_3) as the major compound produced on treatment of **1** with methylamine at low temperature.[6,8] This transformation was proposed to occur by the mechanism shown in path A of Scheme 1, although no evidence in support of this sequence was provided. In brief, it was proposed that two molecules of methylamine react with **1** to give **3** (R = CH_3). An intramolecular reaction

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(1) Vocadlo, D. J.; Bertozzi, C. R. *Angew. Chem., Int. Ed.* **2004**, *43*, 5338−5342.
(2) Campbell, D. A.; Szadenings, A. K. *Curr. Opin. Chem. Biol.* **2003**, *7*, 296−303.
(3) Jeffery, D. A.; Bogyo, M. *Curr. Opin. Chem. Biol.* **2003**, *14*, 87−95.
(4) Kidd, D.; Liu, Y.; Cravatt, B. F. *Biochemistry* **2001**, *40*, 4005−4015.
(5) Landquist, J. K.; Silk, J. A. *J. Chem. Soc.* **1956**, 2052−2063.
(6) Anderson, R. C.; Fleming, R. H. *Tetrahedron Lett.* **1969**, 1581−1584.
(7) Ried, W.; Grabosch J. *Chem. Ber.* **1958**, 2485−2495.
(8) Katritzky, A. R.; Lagowski, J. M. In *Chemistry of the heterocyclic N-oxides*, 1st ed.; Chapter 4, Reactions of substituents on heterocyclic *N*-oxides; Blomquist, A. T., Ed.; Academic Press: New York, 1971; p 389.

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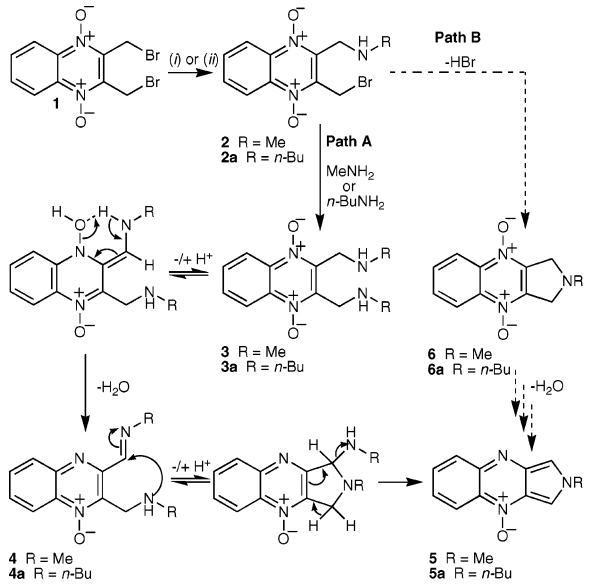
SCHEME 1. Two Possible Reaction Pathways for Conversion of Compound 1 to 5[a]



[a] Reaction conditions: (i) methylamine, −80 °C; (ii) *n*-butylamine, 25 °C.

involving the *N*-oxide functionality of the tautomer of **3** was then proposed to give the corresponding imine **4** (R = CH_3) en route to **5** (R = CH_3).

An analogue of **1** with substituents at the C(6) and C(7) positions exhibits antibacterial activity,[9,10] and **1** irreversibly inhibits host cell invasion by the parasite *Toxoplasma gondii*.[11] The mode of action of these compounds remains unassigned but almost certainly involves alkylation of nucleophilic residues present in one or more proteins. The reaction of **1** with amines is therefore of importance in explaining the details of the chemical interaction of **1** with potential protein targets. Here we present detailed ^{1}H NMR studies on the reaction of **1** with primary (and secondary) amines. During the course of these studies, several alternative mechanisms for the conversion of **1** to **5a** were considered. These included a chemically intuitive route involving the formation of **6a** through a cyclization reaction (Scheme 1, path B).[12]

Results and Discussion

Reaction of 2,3-Bis(bromomethyl)quinoxaline (7) with Primary and Secondary Amines. 2,3-Bis(bromomethyl)quinoxaline (**7**) was prepared from commercially available 1,2-diaminobenzene and 1,4-dibromobutanedione.[13,14] The reaction of **7** with *n*-butylamine was

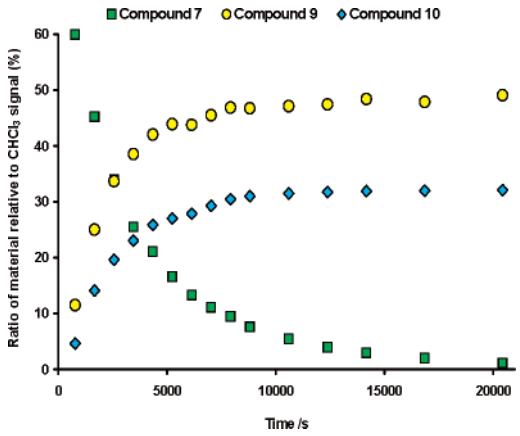
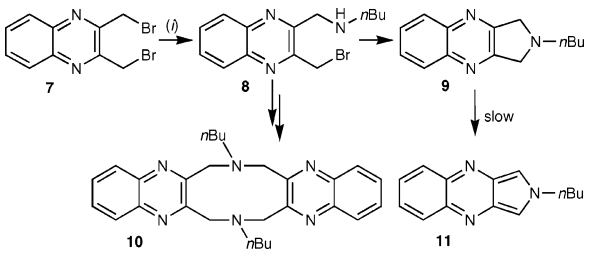


FIGURE 1. Relative abundance of compounds **7**, **9**, and **10**.[15a]

SCHEME 2. Products Formed on Reaction of 7 with *n*-Butylamine[a]



[a] Reaction conditions: (i) *n*-butylamine, 25 °C.[15a]

studied with use of 500 MHz ^{1}H NMR spectroscopy.[15a] Spectra were acquired at regular time points over a 17-h period and the signals corresponding to the starting material **7**, major reaction intermediate **8**, and products **9**, **10**, and **11** were tracked.

The ^{1}H NMR studies showed that **7** reacts with *n*-butylamine to give two major products, **9** and **10** (Scheme 2 and Figure 1).[16] The ratio of **9**:**10** after 17 h was 3:2. During the course of the reaction, minor signals corresponding to the formation of **11** (Scheme 2) were also observed due to decomposition of **9**.[17]

Compound **9** is formed by cyclization of **8** (Scheme 3) although this step must be relatively slow, resulting in the formation of **10**. ^{1}H NMR studies suggest that **10** is formed by an intermolecular reaction of **8** with **7** followed by incorporation of a second molecule of *n*-butylamine and macrocyclization.[16] As expected, repeating the reaction with a 4 mM initial concentration of **7** (a 5-fold dilution)[15a] increased the ratio of **9**:**10** to 7:2 further in

(9) Glushkov, R. G.; Vozyakova, T. I.; Adamskaya, E. V.; Aleinikova, S. A.; Radkevich, T. P.; Shepilova, L. D.; Padeiskaya, E. N.; Gus'kova, T. A. *Pharm. Chem. J.* (*Engl. Transl.*) **1994**, *28*, 17−20.

(10) Similar compounds have also been shown to exhibit antibacterial activity. McIlwain, H. *J. Chem. Soc.* **1943**, 322−325. Kim, H. K.; Miller, L. F.; Bambury, R. E.; Ritter, H. W. *J. Med. Chem.* **1977**, *20*, 557−560.

(11) Carey, K. L.; Westwood, N. J.; Mitchison, T. J.; Ward, G. E. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 7434−7438.

(12) Shields, J. E.; Bornstein, J. *Chem. Ind.* **1967**, 1404−1405.

(13) Moriconi, E. J.; Fritsch, A. J. *J. Org. Chem.* **1965**, *30*, 1542−1547.

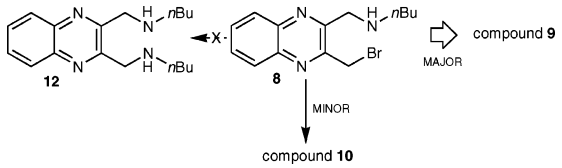
(14) Deady, L. W.; Desneves, J.; Ross, A. C. *Tetrahedron* **1993**, 9823−9828.

(15) Standard reaction conditions consisted of (a) substrate (initial concentration 20 or 4 mM) and *n*-butylamine (60 or 20 mM) in $CDCl_3$ at 25 °C studied with 500 MHz ^{1}H NMR spectroscopy, or (b) substrate (initial concentration 20 mM) and diethylamine (60 mM) in $CDCl_3$ at 25 °C, studied with 300 MHz ^{1}H NMR spectroscopy.

(16) A compound of this general structure has been previously reported as a minor component of a reaction: Kreher, R.; Use, G. *Tetrahedron Lett.* **1978**, 4671−4674. The formation of **10** probably occurs by reaction of **8** with **7**. Reaction of **8** with itself is considered unlikely, at least during the early stages of the reaction, due to the relatively low concentration of **8** present compared with **7**. A set of ^{1}H NMR signals (singlet at 4.86 ppm) corresponding to an additional minor intermediate containing a CH_2Br functional group was observed. Attempts to assign the structure of this intermediate further proved unsuccessful. An alternative route to **10** involving the reaction of **12** with **7** was ruled out due to the absence of signals corresponding to **12**.

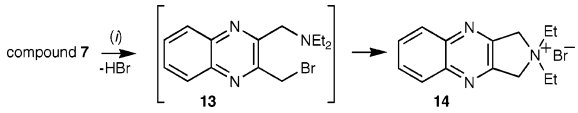
(17) Ried, W.; Grabosch J. *Chem. Ber.* **1958**, 2485−2495.

SCHEME 3. A Summary of Reaction Pathways[a] Available to Intermediate 8[15a]



[a] Evidence in support of the formation of **9** (major) and **10** (minor) was obtained. ^{1}H NMR signals corresponding to the formation of **12** were not observed.

SCHEME 4. Reaction of 7 with a Secondary Amine[a]



[a] Reaction conditions: (i) diethylamine, 25 °C.[15b] Compound **14** is assigned as the salt based on symmetrical ^{1}H NMR signals and a shift in the benzylic methylene (singlet at 5.55 ppm, cf. singlet at 4.35 ppm for the benzylic methylene in compound **17**, Scheme 6).

favor of the intramolecular reaction (cf. 3:2 at 20 mM). Interestingly, no signals corresponding to the formation of the disubstituted compound, **12** (Scheme 3), were observed. Signals corresponding to the formation of **8** were transiently observed,[18] although isolation of **8** was not possible, as predicted.

The situation is simplified further when **7** is reacted with the secondary amine, diethylamine, under analogous conditions.[15b] This results almost exclusively in the formation of **14** (>90% as judged by ^{1}H NMR analysis of the reaction mixture, 74% isolated yield, Scheme 4).[18] In contrast to the reaction of **7** with *n*-butylamine, a ^{1}H NMR spectrum of this reaction taken after 1 h shows no signals corresponding to the presence of **7** [cf. time interval at 4346 s (1.2 h) in Figure 1]. The expected increase in rate of the first nucleophilic displacement (for a secondary versus primary amine) coupled with a proposed reduction in the rate of the reaction of **13** with any remaining **7** (cf. reaction of **8** with **7**) on steric grounds probably explains the high efficiency of the conversion of **7** to **14**.

The observation that intermediates **8** and **13** prefer to undergo a cyclization reaction raises questions about the proposed mechanism of formation of **5a** from **1** (Scheme 1, path A).[6] For example, if the proposed mechanism is correct, what factors contribute to the differential reactivity of intermediate **2a** compared with intermediate **8**? It was therefore decided to study the reaction of 2,3-bis-(bromomethyl)quinoxaline 1,4-dioxide (**1**) with *n*-butylamine with the same approach.

Reaction of 2,3-Bis(bromomethyl)quinoxaline 1,4-Dioxide (1) with Primary and Secondary Amines. Compound **1** was synthesized by two different literature protocols, the more robust proving to be *N*-oxidation of **7**, using purified mCPBA.[5,19] Figure 2 shows a simplified representation of the analysis of the reaction of **1** with

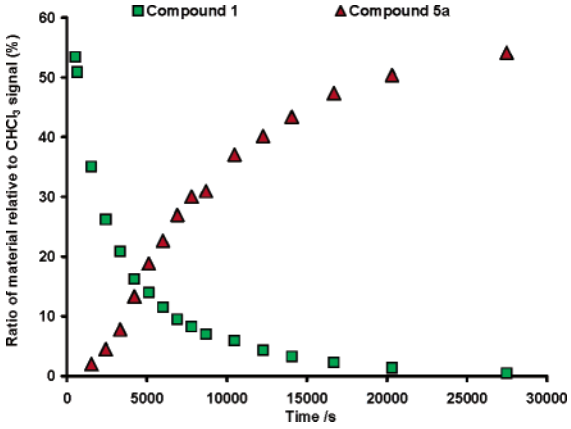


FIGURE 2. Relative abundance of **1** and **5a** following reaction of **1** with *n*-butylamine.[15a]

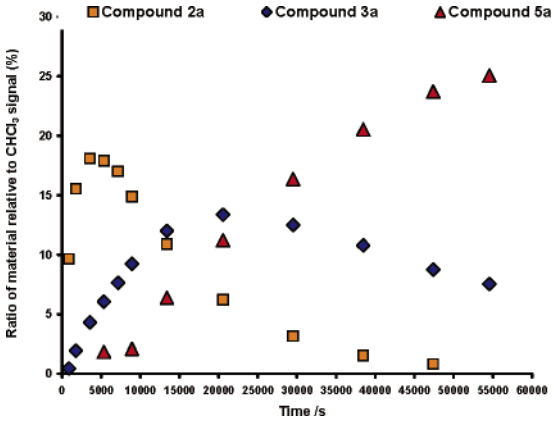


FIGURE 3. Tracking key intermediates **2a** and **3a** together with the major product **5a** from reaction of **1** with *n*-butylamine.[15a]

n-butylamine.[15a] The data show the disappearance of the ^{1}H NMR signals corresponding to **1** with concomitant formation of those corresponding to **5a**. When carried out on a preparative scale **5a** was isolated following rapid column chromatography in 90% yield.

Signals corresponding to several intermediates were also observed, but a detailed analysis became possible only when the experiment was repeated at a 5-fold dilution (Figure 3).[15a] The observed formation of signals corresponding to **2a** followed by the delayed formation of **3a** is consistent with the majority of **5a** being formed by path A in Scheme 1. The fact that the ^{1}H NMR signals corresponding to formation of **3a** increase while those corresponding to the presence of **2a** are decreasing is also supportive of the proposed reaction mechanism. Integration and summation of key ^{1}H NMR signals associated with compounds **1**, **2a**, **3a**, and **5a** at intervals throughout the time course indicate that these four compounds make up >90% of the reaction mixture at all times.[18]

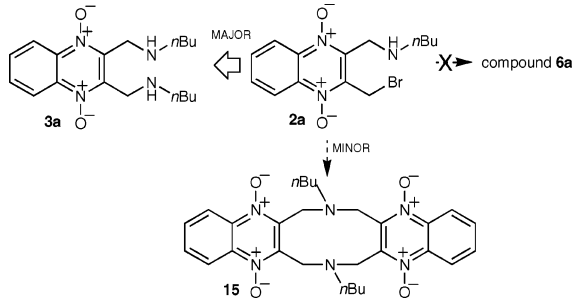
The ^{1}H NMR spectra of **2a** and **3a** differ considerably in the aromatic region, where **2a** has four signals and **3a** has two signals consistent with their respective symmetry. In addition, **2a** also has a signal at 4.99 ppm for the CH_2Br, which is absent in **3a** (singlet 4.28 ppm, benzylic methylenes).[18]

These studies therefore provide the first direct evidence for the mechanism of this reaction (Scheme 1, path A).[6]

(18) For further details including detailed spectroscopic information see the Supporting Information.

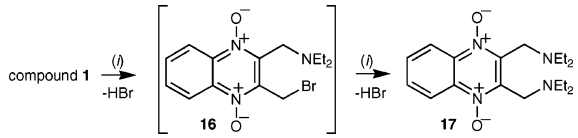
(19) Haddadin, M. J.; Samaha, M. S.; Hajj-Ubayd, A. B. *Heterocycles* **1992**, *33*, 541−544.

SCHEME 5. A Summary of Reaction Pathways[a] Available to Intermediate 2a[15a]



[a] Evidence in support of the preferential formation of **3a** was obtained. No evidence in support of the formation of **6a** was obtained (Scheme 1, path B).[18]

SCHEME 6. Reaction of 1 with a Secondary Amine[a]



[a] Reaction conditions: (i) diethylamine, 25 °C.[15b] A singlet at 4.35 ppm was observed for the benzylic methylene in compound **17** (cf. the analogous CH_2 in **14**, 5.55 ppm, Scheme 4).

They also rule out several alternative mechanisms for the transformation of **1** to **5a**. In particular, no evidence to support a reaction pathway that proceeds via intermediate **6a** was obtained (Scheme 1, path B).[18] Minor signals corresponding to the previously unreported **15** were observed (singlet, 4.71 ppm, benzylic methylenes).

Unexpectedly, these studies show that intermediate **2a** prefers to react with a second molecule of *n*-butylamine to form **3a** (Scheme 5), in contrast to the situation with intermediate **8**, which undergoes a cyclization reaction (Scheme 3). This is despite the fact that the only structural difference between these two intermediates is the presence of the *N*-oxide functionalities.[18] The reactions of **1** and **7** with a secondary amine emphasize further this differential reactivity. In contrast to **7**, which reacts to give the cyclized product **14** (Scheme 4), **1** reacts with diethylamine under analogous conditions to give the disubstituted product **17** (Scheme 6) as the only identifiable product (>90% yield as judged by ^{1}H NMR analysis of the reaction mixture).[18]

Several possible explanations were considered to rationalize the observed differences. Two of these centered on the possibility that the secondary nitrogen atom present in **2a** cannot act as a nucleophile due to either its protonation state or the fact that it is rapidly converted to the corresponding imine. The first possibility was ruled out based on the observed chemical shifts of the benzylic methylenes in **2a** (4.27 ppm for **2a**; cf. 5.55 ppm for **14**, Scheme 4), the second, by ^{1}H NMR studies that show that imine production from these types of substrates is relatively slow (data not shown).[18]

X-ray crystallographic analysis of **1** and **7** (Figure 4) showed that, in the solid state, the two bromine atoms are located almost perpendicular to the plane of the heterocyclic ring system and on opposite faces. Assuming that this is the preferred conformation in solution, the

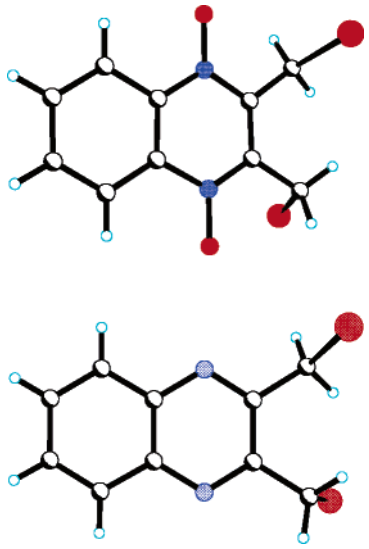
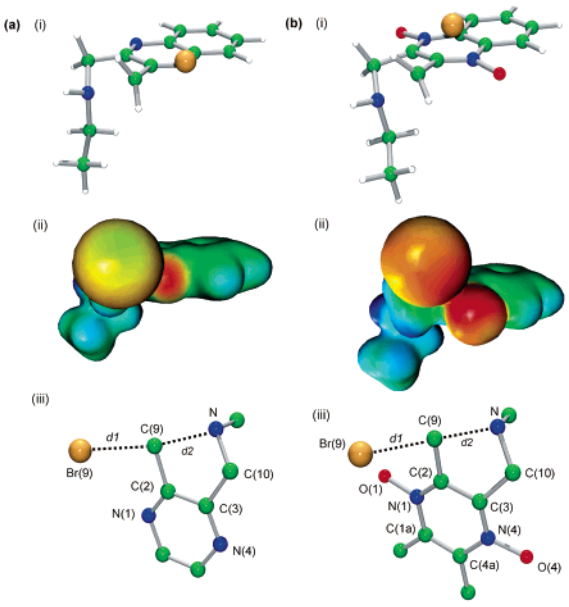


FIGURE 4. X-ray structures of compounds **1** (top) and **7** (bottom).

initially favorable S_N2 displacement would result in the formation of **2a** with both the secondary amine and the remaining bromine functionalities on the same face. A rotation about the C(2)−C(9) and/or C(3)−C(10) bond must then occur as the transition state for cyclization is approached (see Figure 5, part iii, for the atom numbering). Repetition of these ^{1}H NMR studies in d_6-DMSO showed that the major products and intermediates were as observed in $CDCl_3$ and no additional signals corresponding to the formation of **6a** were present. This indicates that hydrogen bonding interactions capable of restricting the required bond rotations are not important in determining the outcome of this reaction (data not shown).[20]

Computational studies were carried out to help visualize more effectively the relevant transition states (TS, Figure 5(i)). In both cases, a TS model consistent with an S_N2-like cyclization reaction was obtained. However, to achieve an attack angle for the approaching secondary amine of greater than 160°, significant puckering of the aromatic skeleton itself is required in **2a**‡. The aromatic backbone of **8**‡ is essentially planar. The dihedral angle Br(9)−C(9)−C(2)−N also provides a clear readout of the differences between the two systems (Figure 5(iii)). In **2a**‡, the bromine atom sits 57.8° out of the plane of the aromatic backbone, compared to only 29.6° in the case of **8**‡. The electrostatic potential surface maps for **2a**‡ and **8**‡ (Figure 5(ii)) show that the difference in these dihedral angles results from the buildup of negative charge on the bromine atom in **2a** (late transition state). As a result, the transition state for cyclization of **2a** is disfavored due to electrostatic repulsion between the *N*-oxide oxygen atom and the bromine.[21] Further evidence in support of the view that **2a**‡ is considerably more distorted than **8**‡ (and hence less energetically accessible) comes from the difference in the calculated dihedral angle N(1)−C(2)−C(3)−N(4) (−8.8 in **2a**‡ and −2.6 in **8**‡). The calculated dihedral angles C(9)−C(2)−C(3)−C(10) and N−C(9)−

(20) Hunter, C. A. *Angew. Chem., Int. Ed.* **2004**, *43*, 5310−5324.
(21) Raposo, C.; Wilcox, C. S. *Tetrahedron Lett.* **1999**, *40*, 1285−1288.



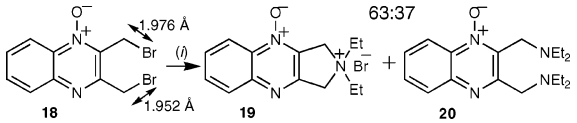
Bond length/angle	(a) **8**‡	(b) **2a**‡
Distance / Å		
d1	2.44	2.55
d2	2.08	2.11
Angle / °		
N-C(9)-Br(9) / °	167.3	159.7
Dihedral / °		
Envelope region		
C(9)-C(2)-C(3)-C(10)	-4.1	-13.1
N-C(9)-C(2)-C(3)	23.8	32.0
Br(9)-C(9)-C(2)-N	29.6	57.8
Ring system		
N(1)-C(2)-C(3)-N(4)	-2.6	-8.8
O(1)-N(1)-C(2)-C(9)	N/A	4.5
O(4)-N(4)-C(3)-C(10)	N/A	9.3

FIGURE 5. (HF/6-31G(d)), PCM Solvation model for $CHCl_3$ of (a) compound **8**‡ and (b) compound **2a**‡. In each case, part i illustrates the structure of the transition state for a cyclization reaction, part ii shows the electrostatic potential surface of the transition state (both (a) and (b) are plotted in the same scale), and part iii provides a detailed view of the transition state geometry. Values of the parameters are provided in the table in Figure 5.

C(2)−C(3) in **2a**‡ (−13.1°, 32.0°) and **8**‡ (−4.1°, 23.8°) also support the view that the envelope conformation adopted by the forming five-membered ring is more distorted in **2a**‡ than in **8**‡. Importantly, these calculations support the experimentally observed reactivity of **2a** and **8**. Invoking an unfavorable electronic interaction between the *N*-oxide oxygen atom and the developing negative charge on the bromine atom in **2a**‡ provides a concise explanation for the observed reaction path (Scheme 5). A steric clash between the *N*-oxide oxygen atom and the bromine atom in **2a**‡ presumably also contributes to the experimental outcome.

An interesting prediction arises from this mechanistic explanation. Reaction of the unsymmetrical 2,3-bis-(bromomethyl)quinoxaline 1-oxide (**18**) with diethylamine would be expected to proceed as follows: (i) initial displacement of the bromine atom adjacent to the *N*-oxide functional group leads to product **19** via cyclization and (ii) initial displacement of the alternative bromine atom leads to the disubstituted product **20**. To test this hypothesis, **18** was synthesized from **7** by mono-*N*-oxidation, using 1 equiv of *m*CPBA. ^{1}H NMR analysis of the reaction of **18** with diethylamine showed that two products **19** and **20** were formed (Scheme 7). The experimentally determined ratio of **19**:**20** was 63:37 based on ^{1}H NMR analysis of the reaction mixture. X-ray crystallographic analysis of **18** indicated that the carbon−bromine bond adjacent to the *N*-oxide functionality is the longer of the two (1.976 Å vs 1.952 Å, Scheme 7) consistent with preferential nucleophilic attack adjacent to the *N*-oxide functionality (and hence preferential formation of **19**).[18] Analogous results were obtained from the reaction of **18** with *n*-butylamine.[18]

SCHEME 7. Reaction of 18 with a Secondary Amine[a]



Reaction conditions: (i) diethylamine, 25 °C.[15b] The ratio of **19**:**20** was calculated as 63:37 in favor of the intramolecular process.

Conclusion

This study was designed to clarify the mechanism of reaction of **1** with primary amines, reflecting our interest in determining its biological mode of action and developing chemical proteomic tools based on **1**. The ^{1}H NMR studies with *n*-butylamine (a mimic of a nitrogen-bound protein nucleophile) provide the first experimental evidence in support of a reaction pathway for **1** that involves 2 equiv of the amine (Scheme 1, path A). These studies raise the intriguing possibility that two nucleophilic residues in close proximity[18] are required for irreversible modification of a protein by **1**.[22] Further biochemical studies to test this hypothesis are ongoing in our laboratory. In addition, computational techniques coupled with X-ray crystallographic studies have provided a rationalization for the differential reactivity of **1**, **7**, and **18** with amines (Schemes 3, 5, and 7). They provide a clear explanation of the influence of the *N*-oxide functional group.

Experimental Section

All commercially available substrates, reagents, and solvents were used without further purification unless otherwise stated. ^{1}H NMR spectra were recorded at 300 and 500 MHz. ^{13}C NMR spectra were recorded at either 75 or 125 MHz. Coupling constants (*J*) are given in Hz. Low- and high-resolution mass spectral analyses were recorded in either EI, CI, or ES operating in positive ion mode. Melting points are uncorrected.

(22) Several examples of small molecules that covalently modify two residues within the same protein are known. Hartman, F. C.; Wold, F. *J. Am. Chem. Soc.* **1966**, *88*, 3890−3891. Navia, M. A.; Springer, J. P.; Lin, T.-Y.; Williams, H. R.; Firestone, R. A.; Pisano, J. M.; Doherty, J. B.; Finke, P. E.; Hoogsteen, K. *Nature* **1987**, *327*, 79−82. Bode, W.; Turk, D.; Karshikov, A. *Protein Sci.* **1992**, *1*, 426−471.

(23) Hahn, W. E.; Lesiak, J. Z. *Soc. Sci. Lodz.*, *Acta Chim.* **1972**, *17*, 201−205.

2,3-Bis(bromomethyl)quinoxaline 1,4-Dioxide, 1.[5] Purified mCPBA (13.8 g, 80.0 mmol) was added to a solution of compound **7** (5.06 g, 16.0 mmol) in dry DCM (160 mL) with stirring at room temperature. After 42 h the reaction mixture was diluted with DCM (1.00 L) and washed with 10% Na_2CO_3 solution (2 × 250 mL). The organic phase was dried ($MgSO_4$) and concentrated in vacuo to give a yellow solid that was purified by flash column chromatography on silica gel (ethyl acetate:petroleum ether, 1:4) to yield a bright yellow crystalline solid (3.64 g, 10.5 mmol, 65%). Mp 178.5−179.0 °C (recrystallized from ethyl acetate) (lit.[5] mp 188.0−189.0 °C, dioxane); IR (NaCl, Nujol) 1341, 1036, 774, and 643 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.65 (m, AA′ part of the AA′XX′ system, 2H), 7.89 (m, XX′ part of the AA′XX′ system, 2H), 4.94 (s, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 139.8, 137.6, 132.5, 120.7, 20.5; MS-ES+ 373 ($[M + Na]^+$, 2 × ^{81}Br, 18%), 371 ($[M + Na]^+$, ^{79}Br + ^{81}Br, 100), 369 ($[M + Na]^+$, 2 × ^{79}Br, 20); HRMS-EI+ (*m/z*) $[M]^{+\bullet}$ calcd for $C_{10}H_8Br_2N_2O_2$ 345.8952, found 345.8949. Anal. Calcd for $C_{10}H_8Br_2N_2O_2$: C, 34.51; H, 2.32; N, 8.05. Found: C, 34.25; H, 1.95; N, 7.70. In addition compound **18** was isolated as a white crystalline solid (0.930 g, 2.8 mmol, 18%).

(3-(Bromomethyl)quinoxalin-2-ylmethyl)butylamine 1,4-Dioxide, Intermediate 2a, and Butyl(3-(butylaminomethyl)quinoxalin-2-ylmethyl)amine 1,4-Dioxide, Intermediate 3a. Prepared according to the general kinetic NMR procedure, using compound **1** with *n*-butylamine.[18] After 522 s formation of intermediate **2a** was observed in the presence of *n*-butylamine.[18] Attempts to isolate **2a** proved unsuccessful. Intermediate **2a**: ^1H NMR (500 MHz, $CDCl_3$) δ 8.67−8.60 (m, 2H), 7.90−7.82 (m, 2H), 4.99 (s, 2H), 4.26 (s, 2H), 2.77−2.67 (m, 2H), 1.54−1.48 (m, 2H), 1.39−1.33 (m, 2H), 0.92−0.89 (m, 3H). After 54 572 s intermediate **3a** was observed in the presence of **5a** and excess *n*-butylamine.[18] Attempts to isolate **3a** proved unsuccessful. Intermediate **3a**: ^1H NMR (500 MHz, $CDCl_3$) δ 8.62 (m, AA′ part of the AA′XX′ system, 2H), 7.84 (m, XX′ part of the AA′XX′ system, 2H), 4.28 (s, 4H), 2.70 (t, J = 7.2 Hz, 4H), 1.54−1.34 (m, 8H), 0.91 (t, J = 7.4 Hz, 6H).

2-Butyl-2*H*-pyrrolo[3,4-*b*]quinoxaline 4-oxide, 5a. Prepared according to the general kinetic NMR procedure, using compound **1** and *n*-butylamine.[18] Also, on preparative scale: A solution of *n*-butylamine (0.027 g, 0.369 mmol) in deuterated chloroform (3.00 mL) was added to a solution of **1** (0.040 g, 0.121 mmol) in deuterated chloroform (2.86 mL) with stirring at room temperature. After 20 h the reaction mixture was quenched with silica and the solvent removed in vacuo. Purification by flash column chromatography on silica gel (ethyl acetate:petroleum ether, 1:9 to 1:4) yielded an unstable dark red oil (0.025 g, 0.10 mmol, 90%). **5a** was observed to decompose rapidly in the absence of solvent. IR (NaCl) 1578, 1544, 1326, and 745 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.55 (d, J = 8.5 Hz, 1H), 8.00 (d, J = 8.8 Hz, 1H), 7.67 (d, J = 2.3 Hz, 1H), 7.61 (d, J = 2.3 Hz, 1H), 7.59−7.56 (m, 1H), 7.53−7.50 (m, 1H), 4.39 (t, J = 7.1 Hz, 2H), 2.02−1.98 (m, 2H), 1.46−1.34 (m, 2H), 0.97 (t, J = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 145.0, 137.9, 133.5, 130.0, 129.0, 127.9, 125.2, 118.3, 111.4, 103.6, 53.0, 33.4, 19.7, 13.4; MS-CI+ 242 ([M + 1], 100%); HRMS-EI+ (*m/z*) $[M]^{+\bullet}$ calcd for $C_{14}H_{15}N_3O$ 241.1215, found 241.1215.

2,3-Bis(bromomethyl)quinoxaline, 7.[13] A solution of 1,2-phenylenediamine (6.81 g, 63.0 mmol) in dry THF (40.0 mL) was added to 1,4-dibromo-2,3-butanedione (14.6 g, 60.0 mmol) in dry THF (80.0 mL) at 0 °C over 15 min with stirring. The reaction was warmed to room temperature and stirred for a further 17 h. After concentration in vacuo, the crude material was partitioned between 10% $NaHCO_3$ solution (150 mL) and DCM (200 mL). The organic phase was washed with brine (1 × 100 mL), dried ($MgSO_4$), and concentrated in vacuo to give a dark brown solid that was purified by flash column chromatography on silica gel (ethyl acetate:petroleum ether, 1:9) to yield a white crystalline solid (16.7 g, 52.9 mmol, 88%), mp 153.0 °C (sharp, recrystallized from ethyl acetate:petroleum ether). IR (NaCl, Nujol) 774 and 696 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.07 (m, AA′ part of the AA′XX′ system, 2H), 7.80 (m, XX′ part of the AA′XX′ system, 2H), 4.93 (s, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 150.8, 141.5, 130.9, 129.0, 30.5; MS-EI+ 318 ($[M]^{+\bullet}$, 2 × ^{81}Br, 20%), 316 ($[M]^{+\bullet}$, ^{79}Br + ^{81}Br, 40), 314 ($[M]^{+\bullet}$, 2 × ^{79}Br, 20), 237 ($[M - Br]^{+\bullet}$, ^{81}Br, 100), 235 ($[M - Br]^{+\bullet}$, ^{79}Br, 100), 156 ($[M - 2Br]^{+\bullet}$, 65); HRMS-EI+ (*m/z*) $[M]^{+\bullet}$ calcd for $C_{10}H_8N_2Br_2$ 315.9034, found 315.9035. Anal. Calcd for $C_{10}H_8N_2Br_2$: C, 38.01; H, 2.55; N, 8.87. Found: C, 37.87; H, 2.27; N, 8.72.

2-Butyl-2,3-dihydro-1*H*-pyrrolo[3,4-*b*]quinoxaline, 9,[7] and 7,16-Di-*n*-butyl[1,6]diazecino[3,4-*b*;8,9-*b*′]diquinoxaline, 10. Prepared according to the general kinetic NMR procedure, using compound **7** and *n*-butylamine.[18] Also, on a preparative scale: *n*-Butylamine (0.312 g, 4.26 mmol) was added to a solution of **7** (0.449 g, 1.42 mmol) in chloroform (40.0 mL) at room temperature under nitrogen. After 20 h the reaction mixture was concentrated in vacuo and purified by flash column chromatography on silica gel (ethyl acetate: petroleum ether, 1:3) to yield **9** as a pink solid (0.200 g, 0.881 mmol, 62%), **10** as a yellow oil (0. 0260 g, 0.0601 mmol, 8%), and **11** (trace amounts) as an unstable red oil.

Compound **9**: mp 54.5 °C dec; ^1H NMR (300 MHz, $CDCl_3$) δ 8.07−8.01 (m, AA′ part of the AA′XX′ system, 2H), 7.75−7.69 (m, XX′ part of the AA′XX′ system, 2H), 4.13 (s, 4H), 2.85 (t, J = 7.5 Hz, 2H), 1.70−1.60 (m, 2H), 1.52−1.39 (m, 2H), 0.99 (t, J = 7.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.9, 141.7, 129.1, 128.9, 57.9, 56.3, 30.3, 20.5, 14.0; MS-APCI+ 270 ($[M + 1 + CH_3CN]^+$, 20%), 269 ($[M + CH_3CN]^+$, 100), 229 ($[M + 1]^+$, 15), 228 ($[M]^+$, 90); HRMS-CI+ (*m/z*) $[M]^+$ calcd for $C_{14}H_{18}N_3$ 228.1501, found 228.1497.

Compound **10**: ^1H NMR (300 MHz, $CDCl_3$) δ 8.05−8.00 (m, AA′ part of the AA′XX′ system, 4H), 7.72−7.66 (m, XX′ part of the AA′XX′ system, 4H), 4.37 (s, 8H), 2.60 (t, J = 7.7 Hz, 4H), 1.37−1.27 (m, 4H), 1.07−0.95 (m, 4H), 0.64 (t, J = 7.3 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.5, 140.7, 129.3, 128.5, 63.1, 54.4, 28.9, 20.5, 13.7; MS-APCI+ 456 ($[M + 1]^+$, 28%), 455 ($[M]^+$, 100); HRMS-CI+ (*m/z*) $[M]^+$ calcd for $C_{28}H_{35}N_6$ 455.2923, found 455.2915.

2-Butyl-2*H*-pyrrolo[3,4-*b*]quinoxaline, 11. Crude isolation afforded a red oil: ^1H NMR (500 MHz, $CDCl_3$) δ 7.99−7.96 (m, AA′ part of the AA′XX′ system, 2H), 7.61 (s, 2H), 7.55−7.52 (m, XX′ part of the AA′XX′ system, 2H), 4.47 (t, J = 7.2 Hz, 2H), 2.03 (m, 2H), 1.65−1.20 (contains m, 2H), 0.97 (t, J = 7.4 Hz, 3H); MS-EI+ 225 ($[M]^{+\bullet}$, 33%), 183 (100), 156 (26); HRMS-EI+ (*m/z*) $[M]^{+\bullet}$ calcd for $C_{14}H_{15}N_3$ 225.1266, found 225.1276.

2,2-Diethyl-2,3-dihydro-1*H*-pyrrolo[3,4-*b*]quinoxalin-2-ium Bromide, 14.[23] Prepared according to the general kinetic NMR procedure, using compound **7** and diethylamine.[18] Reaction was scaled up 30-fold for full analysis. **14** was collected following crystallization from chloroform to yield a white crystalline solid (0.068 g, 0.32 mmoL, 74%), mp 195.5−196.0 °C dec (lit.[23] 195.0 °C); IR (KBr) 3461, 3394, 2974, 2936, 1503, 1465, 1030, and 763 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 8.10−8.04 (m, AA′ part of the AA′XX′ system, 2H), 7.93−7.87 (m, XX′ part of the AA′XX′ system, 2H), 3.80 (q, J = 7.2 Hz, 4H), 1.42 (t, J = 7.2 Hz, 6H); ^1H NMR (300 MHz, $CDCl_3$) δ 8.14−8.08 (m, AA′ part of the AA′XX′ system, 2H), 7.84−7.79 (m, XX′ part of the AA′XX′ system, 2H), 5.59 (s, 4H), 4.15 (q, J = 7.2 Hz, 4H), 1.44 (t, J = 7.2 Hz, 6H); ^{13}C NMR (75 MHz, D_2O, referenced to dioxane) δ 149.6, 142.2, 132.4, 129.0, (65.2, 64.9, 64.6, 64.3, 64.0),[18] 59.3, 8.8; MS-ES+ 228 ($[M]^+$, 100%), 229 ($[M + 1]^+$ 14); HRMS-CI+ (*m/z*) $[M]^+$ calcd for $C_{14}H_{18}N_3$ 228.1501, found 228.1497.

7,16-Di-*n*-butyl[1,6]diazecino[3,4-*b*;8,9-*b*′]diquinoxaline 5,9,14,18-Tetraoxide, 15. Prepared according to the general kinetic NMR procedure, using compound **1** and *n*-butylamine.[18] Attempts to isolate **15** by column chromatography yielded an unstable red oil (0.0015 g, 0.003 mmol, 5%). ^1H NMR (500 MHz, $CDCl_3$) δ 8.67−8.53 (m, 4H), 7.90−7.71 (m, 4H), 4.71 (s, 8H), 2.77−2.67 (m, 4H), 1.54−1.48 (m, 4H),

1.39−1.33 (m, 4H), 0.92−0.89 (m, 6H); MS-ES+ 542 ([M + 1 + Na]$^+$, 10%), 541 ([M + Na]$^+$, 100), 519 ([M + 1]$^+$, 35); HRMS-ES+ (*m/z*) [M + 1]$^+$ calcd for $C_{28}H_{35}N_6O_4$ 519.2720, found 519.2711.

(3-(Diethylaminomethyl)quinoxalin-2-ylmethyl)diethylamine 1,4-Dioxide, 17. Prepared according to the general kinetic NMR procedure, using compound **1** and diethylamine.[18] Attempts to isolate **17** on a preparative scale were unsuccessful due to rapid decomposition. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.68−8.62 (m, AA′ part of the AA′XX′ system, 2H), 7.84−7.78 (m, XX′ part of the AA′XX′ system, 2H), 4.35 (s, 4H), 2.64 (q, J = 7.1 Hz, 8H), 1.03 (t, J = 7.1 Hz, 12H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 144.1, 137.2, 131.4, 120.7, 47.8, 42.7, 12.1; MS-ES+ 355 ([M + Na]$^+$, 20%), 260 ([M − N(CH_2CH_3)$_2$]$^+$, 100), 214 (14), 198 (84); HRMS-ES+ (*m/z*) [M + 1]$^+$ calcd for $C_{18}H_{29}N_4O_2$ 333.2291, found 333.2294.

2,3-Bis(bromomethyl)quinoxaline 1-Oxide, 18.[5] Purified mCPBA (0.569 g, 3.30 mmol) was added to a solution of **7** (1.01 g, 3.20 mmol) in dry DCM (20.0 mL) at room temperature. After 20 h the reaction mixture was washed with 10% Na_2CO_3 (2 × 20 mL) and the combined aqueous washings extracted with DCM (3 × 50 mL). The combined organic phase was dried ($MgSO_4$) and concentrated in vacuo to give a pale yellow solid that was purified by flash column chromatography on silica gel (ethyl acetate:petroleum ether, 1:9 to 1:4) to yield a white crystalline solid (0.548 g, 1.65 mmol, 52%). Mp 168.5−169.0 °C (recrystallized from ethyl acetate:petroleum ether) (lit.[5] 167.0−168.0 °C, ethanol); IR (NaCl, Nujol) 1564, 1481, 1357, 1060, 769, 675, and 662 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.57 (dd, J = 8.5, 1.5 Hz, 1H), 8.54 (dd, J = 8.4, 1.5 Hz, 1H), 7.87−7.73 (m, 2H), 5.02 (s, 2H), 4.77 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.6, 143.4, 138.5, 136.3, 132.2, 130.9, 130.1, 119.2, 30.2, 20.8; MS-ES+ 357 ([M + Na]$^+$, 2 × ^{81}Br, 19%), 355 ([M + Na]$^+$, ^{79}Br + ^{81}Br, 100), 353 ([M + Na]$^+$, 2 × ^{79}Br, 23); HRMS-EI+ (*m/z*) [M]$^{+\bullet}$ calcd for $C_{10}H_8Br_2N_2O$ 329.9003, found 329.9002. Anal. Calcd for $C_{10}H_8Br_2N_2O$: C, 36.18; H, 2.43; N, 8.44. Found: C, 36.18; H, 2.07; N, 8.14. Compounds **1** (0.052 g, 0.15 mmol, 5%) and **7** (0.411 g, 1.3 mmol, 41%) were also isolated.

2,2-Diethyl-2,3-dihydro-1*H*-pyrrolo[3,4-*b*]quinoxalin-2-ium Bromide, 4-Oxide, 19, and (3-(Diethylaminomethyl)-quinoxalin-2-ylmethyl)diethylamine 4-Oxide, 20. Prepared according to the general kinetic NMR procedure, using compound **18** and diethylamine.[18] Reaction was scaled up 30-fold for full analysis. Compound **19** was collected following crystallization to yield a white crystalline solid (0.098 g, 0.30 mmol, 50%). Compound **20** was recovered from the filtrate in the presence of diethylamine.

Compound **19**: mp 203.0−204.0 °C dec; IR (KBr) 3490, 3423, 2984, 2917, 1589, 1565, 1498, 1369, 1097, and 772 cm^{-1}; ^{1}H NMR (500 MHz, D_2O) δ 8.46 (dd, J = 8.7, 1.3 Hz, 1H), 8.16 (dd, J = 8.4, 1.3 Hz, 1H), 8.01−7.92 (m, 2H), 3.81 (q, J = 7.2 Hz, 4H), 1.42 (t, J = 7.2 Hz, 6H); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.53 (m, 1H), 8.14 (m, 1H), 7.91−7.80 (m, 2H), 5.65 (s, 2H), 5.56 (s, 2H), 4.22−4.11 (m, 4H) 1.50 (t, J = 7.1 Hz, 6H); ^{13}C NMR (125 MHz, D_2O, reference to dioxane) δ 151.6 (2 × C), 146.3, 136.7, 136.3, 133.9, 132.7, 129.9, 118.2, ((66.0, 65.8, 65.7, 65.5, 65.3), (62.4, 62.2, 62.0, 61.8, 61.5)),[18] 59.8, 8.8; MS-ES+ 244 ([M]$^+$, 100%), 198 (81); HRMS-ES+ (*m/z*) [M]$^+$ calcd for $C_{14}H_{18}N_3O$ 244.1450, found 244.1456.

Compound **20**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.58 (m, 1H), 8.08 (m, 1H), 7.78−7.65 (m, 2H), 4.34 (s, 2H), 4.13 (s, 2H), 2.69−2.59 (m, 8H), 1.06−1.00 (m, 12H); MS-ES+ 339 ([M + Na]$^+$, 32%), 317 ([M + 1]$^+$, 17), 299 ([M − OH]$^+$, 24), 244 ([M]$^+$, compound **19**, 94), 228 ([M − O]$^+$, compound **19**, 100); HRMS-ES+ (*m/z*) [M + 1]$^+$ calcd for $C_{18}H_{29}N_4O$ 317.2341, found 317.2354.

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Supporting Information Available: ^{1}H and ^{13}C characterization, additional schemes, kinetic profiles, Cartesian coordinates, and total energies for the optimized structures of the calculated transition states. This material is available free of charge via the Internet at http://pubs.acs.org.

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