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Mechanism of Thio Acid/Azide Amidation

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Abstract: A combined experimental and computational mechanistic study of amide formation from thio acids and azides is described. The data support two distinct mechanistic pathways dependent on the electronic character of the azide component. Relatively electron-rich azides undergo bimolecular coupling with thiocarboxylates via an anion-accelerated [3+2] cycloaddition to give a thiatriazoline. Highly electron-poor azides couple via bimolecular union of the terminal nitrogen of the azide with sulfur of the thiocarboxylate to give a linear adduct. Cyclization of this intermediate gives a thiatriazoline. Decomposition to amide is found to proceed via retro-[3+2] cycloaddition of the neutral thiatriazoline intermediates. Computational analysis (DFT, 6-31+G(d)) identified pathways by which both classes of azide undergo [3+2] cycloaddition with thio acid to give thiatriazoline intermediates, although these paths are higher in energy than the thiocarboxylate amidations. These studies also establish that the reaction profile of electron-poor azides is attributable to a prior capture mechanism followed by intramolecular acylation.

Introduction

It is of considerable interest to develop new amidation reactions that can accommodate a broader range of substrates than conventional amidation and that are compatible with a wider range of reaction conditions, including in vitro and in vivo studies.[1,2] Systematic mechanistic and methodological investigations have shown that chemoselectivity, solvent compatibility, and rate of intermolecular coupling limit conventional intermolecular amidation. One alternative, and the basis for most new methods, is to apply a nonacylation reaction to bring the amine, or amine equivalent, and the acyl group into proximity.[3] To be broadly effective, the nonacylating reaction must be chemically orthogonal to the functionality of the coupling partners and the solvent. This prior-capture strategy has the potential to facilitate amide bond formation by rendering the amidation reaction intramolecular. If the prior-capture reaction is faster than conventional intermolecular amidation and leads to a favorable geometry for a subsequent intramolecular amidation,[4] then the overall rate of amide formation by prior-capture may be significantly faster than conventional amidation. Native peptide ligation,[5] a powerful amidation reaction, is chemoselective, can be performed on unprotected peptide segments, and is effective in water. This and related methods rely on amino acid side-chain functionality to facilitate the prior-capture step.[6] Recent reports suggest that methods for the chemical synthesis of fully elaborated post-translationally modified protein targets may soon be within reach.[7,8] Among the

(1) Wieland, T., Bodanszky, M.; *World of Peptides: A Brief History of Peptide Chemistry*; Springer-Verlag: New York, 1991.

(2) (a) Prescher, J. A.; Bertozzi, C. R. *Nat. Chem. Biol.* **2005**, *1*, 13. (b) Saxon, E.; Bertozzi, C. R. *Science* **2000**, *287*, 2007. (c) Kochendoerfer, G. G.; Kent, S. B. H. *Curr. Opin. Chem. Biol.* **1999**, *3*, 665. (d) Tam, J. P.; Yu, Q.; Miao, Z. *Biopolymers* **1999**, *51*, 311. (e) Humphrey, J. M.; Chamberlin. R. *Chem. Rev.* **1997**, *97*, 2243. (f) Kohn, M.; Breinbauer, R. *Angew. Chem., Int. Ed.* **2004**, *43*, 3106. (g) See also: Brase, S.; Gil, C.; Knepper, K.; Zimmermann, V. *Angew. Chem., Int. Ed.* **2005**, *44*, 5188.

(3) Prior Capture: (a) Wieland, T.; Bokelmann, E.; Bauer, L.; Lang, H.; Lau, H.; Schafer, W. *Liebigs Ann.* **1953**, *583*, 129. (b) Brenner, M.; Zimmerman, J. P.; Wehrmüller, J.; Quitt, P., Hardtmann, A.; Snieider, W.; Beglinger, U. *Helv. Chim. Acta* **1957**, *40*, 1497. (c) Kemp, D. S. *Biopolymers* **1981**, *20*, 1793.

(4) Avoidance of steric congestion in the transition state has been shown to be critically important for prior-capture strategies, see (a) Kemp, D. S.; Carey, R. I. *J. Org. Chem.* **1993**, *58*, 2216, reviewed in: (b) Coltart, D. M. *Tetrahedron* **2000**, *56*, 3449.

(5) *C*-terminal thio ester peptide segments couple with *N*-terminal cysteine peptide segments to form an amide. Thus, trans-thioesterification is a suitably fast prior-capture reaction. Intramolecular *S*- to *N*-acyl transfer via a five-membered transition state constitutes a geometry that is not overly congested. Consequently, the chemical synthesis of proteins and protein-like compounds of approximately one hundred residues is feasible, see: (a) Dawson, P. E.; Muir, T. W.; Clark-Lewis, I.; Kent, S. B. *Science* **1994**, *266*, 776. (b) Muir, T.; Dawson, P. E.; Kent, S. B. H. *Methods Enzymol.* **1997**, *289*, 266. See also, ref 2c. Compare with: (c) Gutte, B.; Merrifield, R. B. *J. Am. Chem. Soc.* **1969**, *91*, 501. (d) Denkewalter, R. G.; Veber, D. F.; Holly, F. W.; Hirschmann, R. *J. Am. Chem. Soc.* **1969**, *91*, 502. (e) Yajima, H.; Fujii, N. *J. Chem. Soc., Chem. Commun.* **1980**, *3*, 115.

(6) Alternatives to cysteine include: (a) Offer, J.; Dawson, P. E. *Org. Lett.* **2000**, *2*, 23. (b) Hacking, T. M.; Griffin, J. H.; Dawson, P. E. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 10068. (c) Canne, L. E.; Bark, S. J.; Kent, S. B. H. *J. Am. Chem. Soc.* **1996**, *118*, 5891. Alternatives to native amide linkages have been explored and reviewed, see ref 2d and (d) Liu, C.-F.; Tam, J. P. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 6584. See also: (e) Tolbert, T. J.; Wong, C. H. *J. Am. Chem. Soc.* **2000**, *122*, 5421. (f) Hohsaka, T.; Ashizuka, Y.; Sasaki, H.; Murakami, H.; Sisido, M. *J. Am. Chem. Soc.* **1999**, *121*, 12194. (g) Cornish, V. W.; Mendel, D.; Schultz, P. G. *Angew. Chem., Int. Ed. Eng.* **1995**, *34*, 621. See also: (h) Leleu, S.; Penhoat, M.; Bouet, A.; Dupas, G.; Papamicaël, C.; Marsais, F.; and Levacher, V. *J. Am. Chem. Soc.* **2005**, *127*, 15668.

(7) (a) Nilsson, B. L.; Hondal, R. J.; Soellner, M. B.; Raines, R. T.; *J. Am. Chem. Soc.* **2003**, *125*, 5268. (b) Galonic, D. P.; Van der Donk, W. A.; Gin, D. Y. *J. Am. Chem. Soc.* **2004**, *126*, 12712. (c) Miller, S. J.; Dudkin, V. Y.; Lyon, G. J.; Muir, T. W.; Danishefsky, S. J. *Angew. Chem., Int. Ed. Engl.* **2003**, *42*, 431.

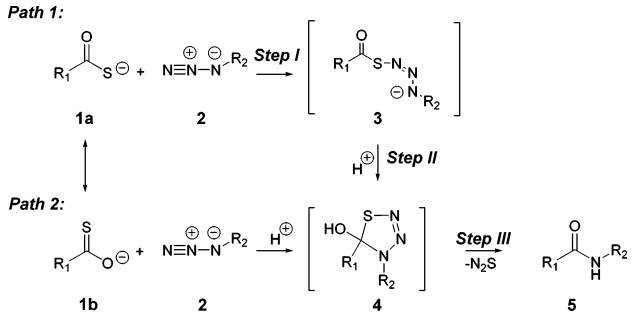
(8) (a) Nilsson, B. L.; Kiessling, L. L.; Raines, R. T. *Org. Lett.* **2000**, *2*, 1939. (b) Nilsson, B. L.; Kiessling, L. L.; Raines, R. T. *Org. Lett.* **2001**, *3*, 9. (c) Saxon, E.; Armstrong, J. I.; Bertozzi, C. R. *Org, Lett.* **2000**, *2*, 2141. (d) Merkx, R.; Rijkers, D. T. S.; Kemmink, J.; Liskamp, R. M. J.; *Tetrahedron Lett.* **2003**, *44*, 4515. (e) David, O.; Meester W. J. N.; Bieraugel, H.; Schoemaker, H. E.; Hiemstra, H.; van Maarseveen, J. H. *Angew. Chem., Int. Ed. Engl.* **2003**, *42*, 4373.

most impressive displays of the advances in this area, the Staudinger ligation has been combined with metabolic engineering to effect post-translational-like modification of a cell-surface protein in an animal model.[9] These advances constitute milestone achievements and are suggestive of the benefits that would accrue if additional methodologies were available. Ideal methods would impose minimal structural requirements upon the coupling partners, and the full range of amide target systems, peptide, non-peptide, and hybrids, would be accessible using reactions that are rapid, selective and produce innocuous byproducts.[10]

Our research toward the development of new reactions that enable the synthesis of complex amides led us to reevaluate the acetylation of alkyl azides with thioacetic acid. This reaction was first noted in the literature in 1980.[11] Subsequently, thioacetic acid was shown to be generally useful for the conversion of organic azides to the corresponding acetamide products when applied as solvent or cosolvent.[12] It was proposed that adventitious hydrogen sulfide reduces the azide to the corresponding amine, which undergoes rapid acetylation with thioacetic acid to regenerate hydrogen sulfide and to allow the cycle to be repeated. Hence, thioacetic acid-induced formation of amides from azides was postulated to be a very rapid, but otherwise conventional, nucleophilic acyl substitution reaction. A few observations inconsistent with this mechanism have been noted,[13] but perhaps since the reaction appeared to be a conventional amidation, no mechanistic studies had been disclosed.

We, however, demonstrated that the reaction of thio acids and azides must proceed through a mechanism different than that originally suggested.[14] Azide substrates participate in the reaction, whereas the corresponding amines fail to couple: hence amines are not intermediates. Interestingly, the reaction is accelerated by the presence of base. Unlike other amidations, the thio acid/azide coupling can be used to readily fashion amides from electron-deficient azides and hence from nonnucleophilic amine equivalents.[15] Indeed, a variety of thio acids, including *N*-protected α-amino thio acids, chemoselectively couple with organic azides bearing electron-withdrawing or electron-donating functionality. For example, *N*-acyl sulfonamides, *N*-aryl amides, enamides, unprotected hydroxy amides, and *β-N*-amidoglycosides are readily prepared in solvents ranging from chloroform to water and give rise to amides

Scheme 1. Proposed Mechanistic Framework for Thio Acid/Azide Amidation



ranging from sensitive alkyl amides to safety-catch linkers and fluorescently labeled amino acid derivatives.[16] The reaction also represents a traceless ligation method, since as with conventional amidation, the substrate requirements are minimal, the reaction can be fast, and only the native amide function remains after coupling.[17] Hence the thio acid/azide amidation appears to be a potentially useful synthetic method.

The transformation, however, was not well understood on a mechanistic level. We set out to gain mechanistic insight into the coupling of thio acids with electron-poor and electron-rich azides in order to better understand the reaction, further improve the methodology, and facilitate the discovery of mechanistically related transformations. Two frameworks consistent with the available data are outlined below in Scheme 1, and were advanced as models of the reaction mechanism. The proposals, *Path 1* and *Path 2*, have in common the involvement of a thiatriazoline (**4**) as opposed to an amine intermediate. They differ in the exact timing of the bond formation process.[18] While *Path 2* forms a thiatriazoline in a single [3+2] cycloaddition step, the first step of *Path 1* is intermolecular coupling between the sulfur of the thio acid and the terminal nitrogen of the azide and is reminiscent of a prior-capture reaction. The second step of *Path 1* is analogous to the *S*- to *N*-acyl transfer[3] of the most powerful protein ligation strategies advanced to date and proceeds via a five-membered transition state. Thiatriazoline **4** is proposed as the key intermediate for both paths, which could decompose either stepwise or in a single retro-[3+2] reaction to give the amide and the observed nitrogen and sulfur products. Protonation in this base-promoted reaction may occur either before or after thiatriazoline conversion to amide and is represented as occurring prior to amide formation. Herein, we disclose experimental and computational data consistent with *Path 1* for electron-deficient azides and *Path 2* for electron-rich azides.

Results

Kinetic studies on both classes of azide are summarized in Table 1. We consider benzene sulfonyl azide representative of

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(10) For example, Click Reactions: (a) Lewis, W. G.; Green, L. G.; Grynszpan, F.; Radic, Z.; Carlier, P. R.; Taylor, P.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 1053. (b) Demko, Z. P.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2110. (c) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004.
(11) Hakimelahi, G. H.; Just, G. *Tetrahedron Lett.* **1980**, *21*, 2119.
(12) (a) Rosen, T.; Lico, I. M.; Chu, T. W. *J. Org. Chem.* **1988**, *53*, 1580. (b) Rakotomanomana, N.; Pavia, J.-M. L. A. A. *Carbohydr. Res.* **1990**, *197*, 318.
(13) (a) Paulsen, H.; Bielfeldt, T.; Peters, S.; Bock, K. *Liebigs Ann. Chem.* **1994**, 369. (b) Elofsson, M.; Salvador, L. A.; Kihlberg, J. *Tetrahedron* **1997**, *53*, 369. (c) Marcaurelle, L. A.: Bertozzi, C. R. *J. Am. Chem. Soc.* **2001**, *123*, 1587. (d) Chou, S.-S. P.; Chow, T. J.; Hsu, C.-H.; Yang, C.; Long, S.-H.; Lin, C.-D. *J. Chem. Soc., Perkin Trans. 1* **1997**, 1691. (e) McKervey, M. A.; Sullivan, B. O.; Myers, P. L.; Green, R. H. *J. Chem. Soc., Chem. Commun.* **1993**, 94. (f) Yamashiro, D.; Blake, J. *Int. J. Pept. Prot. Res.* **1981**, *18*, 383.
(14) Shangguan, N.; Katukojvala, S.; Greenberg, R.; Williams, L. J. *J. Am. Chem. Soc.* **2003**, *125*, 7754.
(15) Amidation of electron-deficient azides via the Staudinger reaction appears to be very slow compared to electron-rich azides, opposite the trend observed for the thio acid/azide amidation, see: Lin, F. L.; Hoyt, H. M.; van Halbeek, H.; Bergman, R. G.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2005**, *127*, 2686.
(16) (a) Wu, X.; Hu, L. *Tetrahedron Lett.* **2005**, *46*, 8401. (b) Knapp, S.; Darout, E. *Org. Lett.* **2005**, *7*, 203. (c) Fazio, F.; Wong, C.-H. *Tetrahedron Lett.* **2003**, *44*, 9083. (d) Zhu, X.; Pachamuthu, K.; Schmidt, R. R. *Org. Lett.* **2004**, *6*, 1083.
(17) (a) Merkx, R.; Brouwer, A. J.; Rijkers, D. T. S.; Liskamp, R. M. J. *Org. Lett.* **2005**, *7*, 1125. (b) Barlett, K. N.; Kolakowski, R.; Katukojvala, S.; Williams, L. J. *Org. Lett.* **2006**, *8*, 823.
(18) A third path not shown in Scheme 1 can be advanced: Formation of the carbon nitrogen bond in *Path 2* could be faster than formation of the sulfur nitrogen bond. The Schmidt amidation is mechanistically analogous to this proposal; however, since azides bearing electron-withdrawing groups react with thio acids more rapidly than azides bearing electron-donating functionality, it seems unlikely that this path represents a generally relevant mechanism. If, however, the mechanism were substrate dependent, this possibility should not be ignored and might be promoted by a suitable additive, including metals or acid.

Table 1. Amidation Activation Parameters with Thiobenzoic Acid and Azide[a]

azide	E_a (kcal mol^{-1})	$\Delta H^{\ddagger}$ (kcal mol^{-1})	$\Delta S^{\ddagger}$ (eu)
$PhSO_2N_3$	11.3 ± 0.1[b]	10.7 ± 0.1	-32 ± 0.2
BnN_3	11.7 ± 0.2	11.0 ± 0.2	-45 ± 0.2
R = SO_2Ph	$k_{obs} = 5.7 \times 10^{-3}$ M^{-1} s$^{-1} \pm 0.1$	@ 21 °C	
R = CH_2Ph	$k_{obs} = 4.5 \times 10^{-6}$ M^{-1} s$^{-1} \pm 0.1$	@ 21 °C	

[a] Activation parameters determined by variable temperature pseudo first-order kinetics and fit to the model: rate = k_{obs}[PhCOSH][N_3R]. Arrhenius plot correlation coefficients ≥ 0.999. [b] σ for the activation parameters estimated via least-squares regression method of the corresponding Arrhenius plots.[20] For experimental details and plots, see Supporting Information.

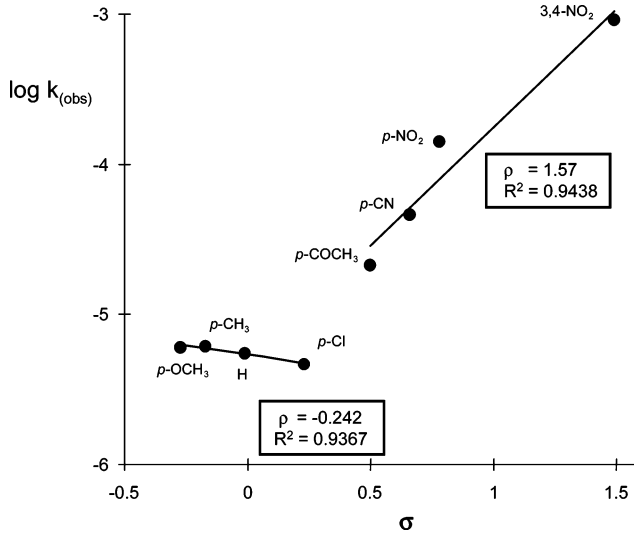
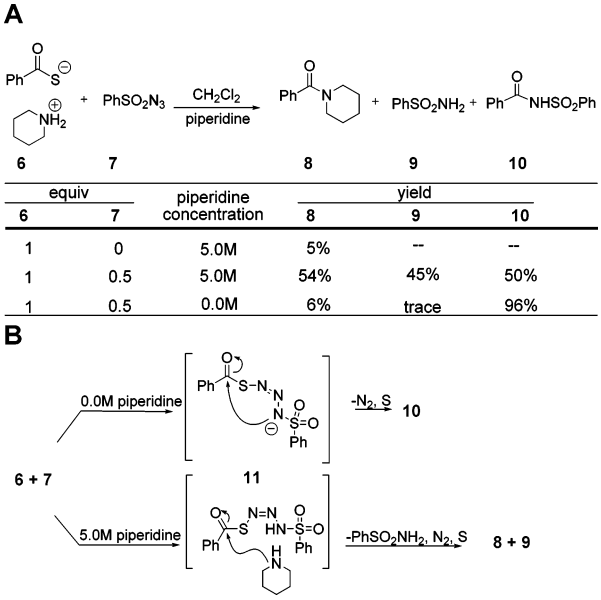


Figure 1. Hammett correlation of phenyl azides. Rate determined by monitoring UV disappearance of thiobenzoic acid. Rate is weakly dependent on $\sigma < 0.3$. Rate increases markedly with increasing $\sigma > 0.4$. For experimental details, plots, and error analysis see Supporting Information.

electron-deficient azides and benzyl azide representative of electron-rich azides.[19] While electron-deficient azides react much more rapidly with thio acids than electron-rich azides, both classes of reaction are accelerated by addition of base. In the reaction of thiobenzoic acid and sulfonyl azides, 1 equiv. of lutidine increased the reaction rate by a factor of 80; similar rate acceleration was observed for benzyl azide. The reactions are first order in thio acid and first order in azide. Variable temperature kinetic analysis revealed similar activation parameters for the two classes of azide, with benzyl azide having higher enthalpies and entropies of activation.

We also conducted a Hammett correlation study with substituted phenyl azides. As shown in Figure 1, there is a distinct break in the σ plot. Two lines model the data well, with $\sigma < 0.3$ substituents fitted to $\rho = -0.242$ ($R^2 = 0.9367$) and $\sigma > 0.4$ fitted to $\rho = 1.57$ ($R^2 = 0.9438$). Within the limit $\sigma < 0.3$, the $\rho = -0.242$ plot is indicative of a reaction pathway that is relatively insensitive to azide electronics, whereas the pathway represented by $\rho = 1.57$ is highly sensitive, within the limit $\sigma > 0.4$, to the electronic properties of the azide. *Taken together, the nonlinear Hammett correlation provides strong evidence that the mechanism for electron-poor azides is different from the mechanism for relatively electron-rich azides*.

Scheme 2. Interception of a Linear Intermediate



equiv 6	equiv 7	piperidine concentration	yield 8	yield 9	yield 10
1	0	5.0M	5%	--	--
1	0.5	5.0M	54%	45%	50%
1	0.5	0.0M	6%	trace	96%

To gain further insight, we sought to intercept the proposed intermediate of type **3**. If *Path 1* represents the relevant mechanism, electron-deficient azides should undergo bimolecular coupling, *step I*, faster than electron-rich azides; however, intramolecular cyclization, *step II*, should be comparatively slow. The linear intermediate might be relatively long-lived for electron-deficient azides, and thus it might be possible to intercept **3** with a good nucleophile. For example, thio acid coupling with a sulfonazide in the presence of an amine nucleophile might give amine-derived acylation products in addition to, or instead of, the expected *N*-acyl sulfonamide. *Indeed, the linear intermediate could be intercepted, as described below and shown in Scheme 2*.

When benzene sulfonazide (**7**) was added to a solution of piperidinium thiobenzoate (**6**) in methylene chloride that did not have excess piperidine, *N*-benzoyl sulfonamide (**10**) was produced in near quantitative yield in 30 min, as expected (Scheme 2A). Trace amounts of amide **8** and benzene sulfonamide (**9**) were measurable, but insignificant. In contrast, when benzene sulfonazide (**7**) was added to piperidinium thiobenzoate (**6**) in the presence of excess piperidine, amide **8** was produced in 54% yield.[21] In addition to molecular sulfur and nitrogen, the reaction also gave benzene sulfonamide in 45% yield and produced *N*-benzoyl sulfonamide in only 50% yield.[22] It is important to note that amine salts of thiobenzoic acid are relatively stable in solution and that piperidinium thiobenzoate (**6**) in methylene chloride gave only trace amounts of amide product **8** over the course of 30 min. Similar results were noted

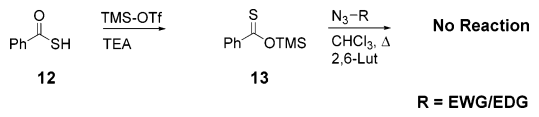
(19) Benzyl azide represents alkyl azides in general, the amide products of which are typical of most amidation reactions. The amidation products of sulfonyl azides, *N*-acyl sulfonamides, are also useful intermediates in medicinal chemistry as well as in solution and solid support-based safety-catch chemistry. See: (a) Kenner, G. W. *Chem. Commun.* **1971**, 636. (b) Backes, B. J.; Ellman, J. A. *J. Org. Chem.* **1999**, *64*, 2322. (c) Shin, Y.; Winans, K. A.; Backes, B. J.; Kent, S. B. H.; Ellman, J. A.; Bertozzi, C. R. *J. Am. Chem. Soc.* **1999**, *121*, 11684. (d) Ingenito, R.; Dreznjak, D.; Guffler, S.; Wenschuh, H.; *Org. Lett.* **2002**, *4*, 1187. (e) Mclean, D.; Hale, R.; Chen, M. *Org. Lett.* **2001**, *3*, 2977. (f) Abbate, F.; Supuran, C. T.; Scozzafava, A.; Orioli, P.; Stubbs M. T.; Klebe, G. A.; Jones, B. R. *J. Med. Chem.* **2002**, *45*, 3583. See also ref 17.

(20) *Data Reduction and Error Analysis for the Physical Sciences*; Beving, P. R.; McGraw-Hill: New York, 1969.

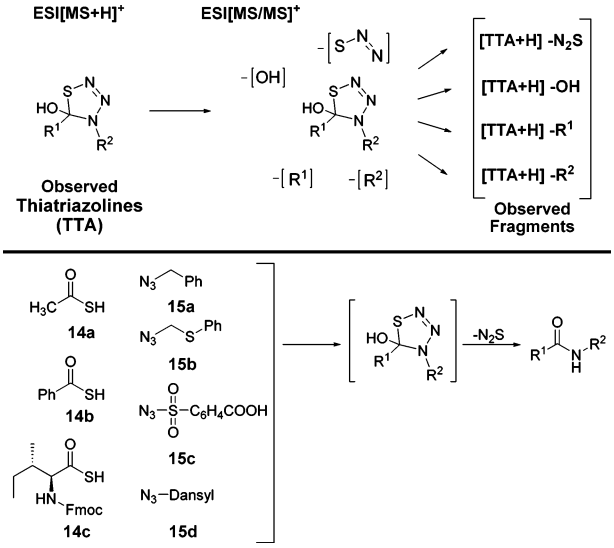
(21) (a) For further experimental data, procedures, and characterization see Supporting Information. (b) Related observations were noted for the reaction of dithio acids with azides. See: (c) Kolakowski, R. V.; Shangguan, N.; Williams, L. J.*Tetrahedron Lett.* **2006**, *47*, 1163.

(22) Similar results were noted for related couplings of electron deficient azides (e.g. 4-nitrophenyl azide), but not for electron-rich azides (data not shown).

Scheme 3. Silyl Thiono Ester Reaction with Organic Azides.



Scheme 4. ESI Analysis of Thiatriazolines.



for **6** in 5.0 M piperidine. Therefore, the formation of amide **8** is induced by addition of benzene sulfonazide. The simplest interpretation of these results invokes **11** as an active ester-like species formed in solution in the course of the thio acid/azide amidation with electron-deficient azides (Scheme 2B).

In light of the known propensity of thionoesters to couple with organic azides at elevated temperatures (vide infra), we examined the viability of *Path 2* under our more mild conditions. If *Path 2* represents the primary mechanistic route to amide product, conditions that favor the thiono form of a thio acid could accelerate the reaction. Addition of trimethylsilyl triflate to thiobenzoic acid and triethylamine or lutidine effected complete conversion to trimethylsilyl thionobenzoate (**13**, Scheme 3).[23] Subsequent treatment of **13** with benzyl azide or benzene sulfonazide under our original amidation conditions gave only trace amounts of the corresponding amide products.[24] Importantly, if trimethylsilyl triflate is omitted from these reactions, the amide products are obtained in excellent yield.[14] These data indicate that under these conditions either minimal steric interactions or the retention of the anionic character of **1**, or both, are required for amidation.

Careful monitoring of thio acid/azide reactions by standard spectroscopic techniques yielded no measurable intermediates; however, several intriguing observations were documented with electrospray mass spectrometry (Scheme 4). Reaction mixtures of thio acids (**14a**−**c**) and electron-rich or electron-poor azides (**15a**−**d**) gave ionic adducts corresponding to a 1:1 ratio of thio acid:azide. ESI−MS/MS revealed that these species fragment uniformly. Specifically, the loss of fragments corresponding to

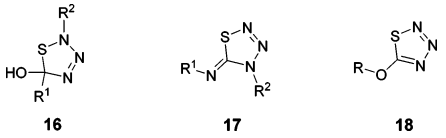


Figure 2. Related heterocycles.

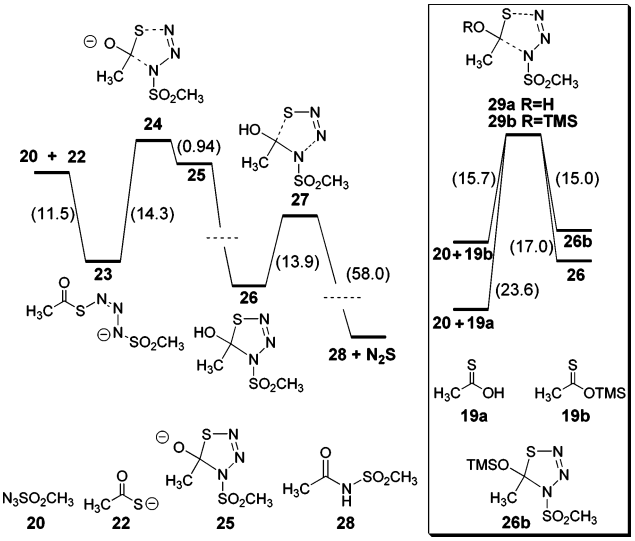


Figure 3. Relative enthalpies (kcal/mol) for methane sulfonazide reactions at 298 K in simulated acetone.

R^1, R^2, OH, and N_2S were readily apparent for each set of coupling partners, regardless of the nature of the azide or complexity of the thio acid. *These data are inconsistent with linear structures of type **3** and isomeric thiatriazoline **16** (Figure 2) but are consistent with a cyclic intermediate corresponding to a thiatriazoline of type **4**.*

We also examined the potential energy surfaces of the reactions of thioacetate ion and thioacetic acid with methane sulfonazide and methyl azide using Density Functional (DFT) calculations.[25] The mechanism was found to be dependent on the electronic character of the azide and protonation state of the thio acid. As outlined in Scheme 1, *Path 1* is found to be favored for the reaction with methane sulfonazide and thioacetate. *Path 2* is favored for the reaction of methyl azide and thioacetate. In contrast, *Path 2* is preferred for both azides when the coupling partner is thioacetic acid. Figures 3 and 4 show computed stationary points for these reactions.

In Figure 3 we compare the reactions of electron-deficient methane sulfonazide (**20**) with thioacetate (**22**) and thioacetic acid (depicted as thiono tautomer **19a**, inset). Both systems produce thiatriazoline **26** and subsequently undergo retro-[3+2] cycloaddition to produce nitrous sulfide and amide **28**. The detailed pathways are found to be quite different, however.

(23) For synthesis and characterization of trimethylsilyl thionobenzoate, see: (a) Kato, S.; Wataru, A.; Mizuta, M.; Ishii, Y. *Bull. Chem. Soc. Jpn.* **1973**, *46*, 244. See also: (b) Perlmutter, P.; Rose, M.; Vounatos, F. *Eur. J. Org. Chem.* **2003**. (c) Shalaby, M. A. and Rapoport, H. *J. Org. Chem.* **1999**, *64*, 1065.

(24) If the thio acid is not distilled or sparged with nitrogen prior to reaction, false coupling results may be observed, which we have found are an indication of the presence of impurities and/or incomplete formation of the thionoester.

(25) (a) All structures were fully optimized by analytical gradient methods using the Gaussian 98 suites, Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A. Jr.; Stratmann, R. E.; Burant, J. C.; et al., *Gaussian 98*, Revision A.9; Gaussian, Inc.: Pittsburgh, PA, 1998. (b) Density functional (DFT) calculations used the exchange potentials of: Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648−5652. (c) the correlation functional of: Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785, and the 6-31+G(d) basis set for C, H, N and O augmented with the LANL2DZ set for calculations involving sulfur and silicon. Frequencies were computed by analytical methods. All stationary points gave rise to the correct number of imaginary frequencies. Reported energy differences are based on single point PCM calculations in a continuum medium with dielectric 20.7 (acetone) and were corrected for zero point energies and thermal effects at 298.15 K (obtained from the results in vacuo).

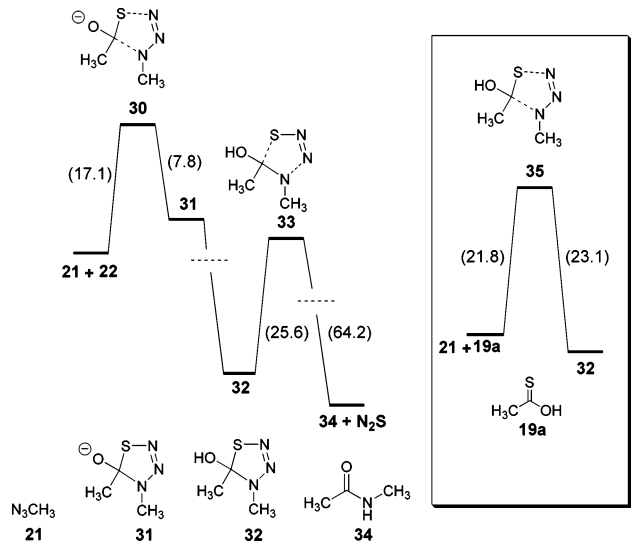


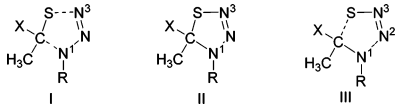
Figure 4. Relative enthalpies (kcal/mol) for methyl azide reactions at 298.15 K in simulated acetone.

Thioacetate (**22**) adds to methane sulfonazide (**20**) to form a stable linear adduct **23**, which is 11.5 kcal/mol lower in energy.[26] Transition structure **24** connects this intermediate with anionic thiatriazoline **25** as shown by intrinsic reaction coordinate (IRC) calculations. At this stage, protonation of the unstable anion **25** leads to intermediate **26**, which ultimately produces **28** and nitrous sulfide via retro-[3+2] cycloaddition transition structure **27**. In contrast, thio acetic acid (**19a**) undergoes direct [3+2] cycloaddition with methane sulfonazide (**20**) via transition structure **29a** to form thiatriazoline **26** (see inset), a path that poses a significantly higher barrier compared to **24**. This pathway is supported by IRC calculations, which link **29a** to **19a** and **20** and to thiatriazoline **26**.

The reaction of **20** with the related *O*-trimethylsilyl acetate (**19b**) was also studied computationally (Figure 3, inset). The activation barrier computed for the concerted cycloaddition step to produce silyl thiatriazoline **26b** was found to be 15.7 kcal/mol, significantly lower than that found for thioacetic acid (23.6 kcal/mol). As before, thiatriazolines **26** and **26b** were shown to undergo retro-[3+2] cycloadditions with barriers of 13.9 kcal/mol (**26**) and 15.8 kcal/mol (**26b**) (not shown).

Figure 4 describes the related chemistry of reactions between methyl azide with thioacetic acid and thioacetate. Examination of the coupling of thioacetate (**22**) with comparatively electron-rich methyl azide (**21**) shows that the reactants undergo [3+2] cycloaddition via transition structure **30** to form anionic thiatriazoline **31**. Although a stable linear adduct was found (not shown), IRC calculations link transition structure **30** with **31** along with methyl azide (**21**) and thioacetate (**22**). As expected, protonation of **31** is highly favorable and gives thiatriazoline **32**, which undergoes retro-[3+2] cycloaddition via transition structure **33** to produce amide (**34**) and nitrous sulfide.[27] The coupling of thioacetic acid (**19a**) with methyl azide produces **32** directly by way of transition structure **35** (Figure 4 inset), albeit with a higher activation energy (21.8 kcal/mol) than observed for the anionic counterpart (17.1 kcal/mol). Importantly, IRC calculations also established connectivity between **33** and components **32**, **34** and nitrous sulfide.

Table 2. Calculated Structures for Cycloaddition TS (I), Thiatriazoline (II), and Retrocycloaddition TS (III) (Å)



R, X	C–N^1	S–N^3	C–N^1	S–N^3	N^1-N^2	C–S
1. CH_3, O^-	2.29	1.94	1.55	1.77		
2. CH_3, OH	2.09	2.43	1.46	1.78	1.67	2.46
3. CH_3SO_2, O^-	1.73	1.77	1.61	1.76		
4. CH_3SO_2 ,OH	2.20	2.21	1.50	1.88	1.91	2.22

Table 2 presents key atomic distances computed for the stationary states discussed above. For the alkyl azide (entries 1 and 2), the [3+2] cycloaddition transition states (I) are not symmetric, and the protonation state of the thio acid dictates the relative rate of bond formation. Thus, C−N^1 bond formation is less advanced (2.29 Å) than S−N^3 bond formation for the thiocarboxylate (entry 1). The reverse is observed for the higher energy thio acid transition state (entry 2). Comparison of the C−N^1 and S−N^3 distances of the thiatriazoline-forming transition structure for entry 1 and 3 reveals the distinct mechanistic differences between electron-deficient azide (entry 3) and the electron-rich azide (entry 1) when coupling with thioacetate. For the sulfonyl azide (entry 3), the S−N^3 bond is already established (1.77 Å) and consequently does not change significantly during C−N^1 bond formation. In contrast, the C−N^1 and S−N^3 distances for entry 2 and entry 4 are similar for the transition structures leading to thiatriazoline and are reflective of analogous [3+2] cycloadditions. As expected, the C−N^1 distances of the anionic thiatriazolines (II) are greater than the C−N^1 distances of the neutral analogues (compare entries 1 and 3 with entries 2 and 4). As with the [3+2] cycloaddition, the [3+2] cycloreversion transition (III) structures are highly asymmetric. This is most pronounced for the methyl azide-derived substrate (entry 2). In this transition structure, the C−S bond is very long and weak (2.46 Å), whereas the N^1−N^2 bond is still very substantial. Similar though less pronounced trends are observed for the sulfonyl azide-derived substrate.

Discussion

The above data are in accord with two related but distinctly different mechanisms. The mechanisms differ primarily in the manner and rate with which the common thiatriazoline intermediate forms.

The stoichiometry of the transition states, as shown by kinetic analysis, consists of one molecule of the anion of the thio acid and one molecule of the azide, regardless of the electronic character of the azide. Amidation for the sulfonazide is fast ($k_{obs} = 5.7 \times 10^{-3}$ M^{-1} s$^{-1} \pm 0.1$, 21 °C), whereas the reaction rate for the alkyl azide is comparatively slow. *Thus thio acid/azide amidation strongly complements Staudinger amidation, which is slow for electron-deficient azides and fast ($k_{obs} = 2.5 \times 10^{-3}$ $M^{-1}s^{-1} \pm 0.2$, 20−21 °C) for electron-rich azides.*[15]

The enthalpy of activation ($\Delta H^{\ddagger}$) for the more polarized electron-deficient benzene sulfonazide is smaller than that of benzyl azide. Alone this small difference cannot rule out either pathway. The entropy of activation ($\Delta S^{\ddagger}$) is large and negative

(26) A protonated form of this adduct was computed to have a *higher* energy than that of **19a** + **20**. This adduct is not involved in the formation of **26** as shown by IRC calculations.

(27) Anionic transition states related to **33** and leading to **34** were not found. This does not preclude retro-[3+2] nor stepwise decomposition pathways of such anionic intermediates.

for both azides, although it is significantly larger in magnitude for the electron-rich benzyl azide. This accounts for the significant differences in rates for the two substrates. It is difficult to further interpret $\Delta S^{\ddagger}$ with certainty. For the coupling of thiobenzoic acid and benzyl azide, the significantly larger $\Delta S^{\ddagger}$ may indicate that electron-rich azides couple via a more entropically demanding bimolecular cycloaddition transition state (*Path 2)*,[28] a rationale that is supported by our computational studies (vide infra). Thus, the difference in rate of amidation for the two classes of azides is due primarily to differences in the entropies of activation and is reflective of the fundamentally different mechanisms represented in Scheme 1, *Path 1* and *Path 2*.

The Hammett correlation study of subsituted phenyl azides (Figure 1) is indicative of two mechanisms and is not attributable to a change in rate determining step (e.g. a switch from *Step I* to *Step II* in *Path 1*, Scheme 1). Crossover from one mechanism to the other occurs near $\sigma \sim 0.35$. Thus electron-rich azides and modestly electron-poor azides follow one reaction path, whereas highly electron-deficient azides follow a separate reaction path. The reaction mechanism for comparatively electron-rich azides is otherwise relatively insensitive to azide electronics ($\rho = -0.242$), findings consistent with a cycloaddition reaction pathway. In contrast, the reaction mechanism for electron-poor azides is highly sensitive to azide electronics ($\rho = 1.57$), findings consistent with a stepwise reaction pathway that proceeds by way of an anionic intermediate that can be stabilized by the electron-withdrawing substituent of the azide.

The chemical trapping experiments (Scheme 2) strongly support the presence of a linear intermediate for sulfonazides, and by analogy, other fast-reacting electron-deficient azides. At high concentrations of piperidine, benzene sulfonazide induces the rapid conversion of the piperidinium thiobenzoate salt to the piperidine-derived benzamide. These findings are readily rationalized by considering an acyclic precursor with active ester character, such as **11** (compare with **3**). Thus, at high concentrations the amine nucleophile intercepts the intermediate in an intermolecular reaction with formation of benzene sulfonamide, nitrogen and sulfur, whereas under dilute conditions intramolecular cyclization dominates ultimately leading to the *N*-acyl sulfonamide product.[29] In principle, the failure to intercept the corresponding intermediate of benzyl azide could imply that the linear intermediate formed from benzyl azide cyclizes faster than piperidine interception. The Hammett correlation study provides strong evidence an alternate mechanism is operative and that the amidation of electron-rich azides may not proceed through a linear intermediate.

Thiatriazolines appear to be intermediates for both classes of azide. ESI has been applied to detect transient species in the Wittig, Mitsunobo, and Staundinger reactions, as well as related tetrahedral intermediates **4**.[30] For the full range of thio acids and azides studied, ESI reveals the presence of a 1:1 ratio of adducts derived from the thio acid and the azide coupling partner. The fragmentation pattern of these adducts includes loss of subsituents associated with the parent thio acid and azide, as well as loss of hydroxyl and nitrous sulfide (see Scheme 2). It is difficult to establish unequivocally that ionic species detected in electrospray ionization mass spectrometry experiments are present as neutral, relevant intermediates in the reaction; nevertheless, the fragmentation pattern rules out isomeric structures such as **3** and **16** and is only consistent with thiatriazolines of type **4**.[31]

Since ethyl thiono esters and methyl dithio esters are known to react with alkyl azides at elevated temperatures (110 °C) to form imidates and thioimidates,[32,33] it seemed reasonable that such intermediates might be relevant to the thio acid/azide amidation. Under the mild reaction conditions of our amidation, however, neither class of azide reacted with silyl thionoesters. The steric, electronic, and conformational properties of the silyl thionoesters are significantly different from thiocarboxylates, and consequently, this finding is difficult to interpret. In agreement with the DFT calculations discussed below, the [3+2] cycloaddition of neutral coupling partners would appear to require higher temperatures than the coupling of thiocarboxylates.

The available experimental data on known thiatriazolines and thiatriazoles suggest likely routes by which thiatriazolines of type **4** form amides. Thiatriazolines of type **17** (Figure 2) have been demonstrated to decompose stepwise, first expunging molecular nitrogen and ultimately losing sulfur. The corresponding reactive intermediates have been intercepted.[34] In contrast to **17**, which houses an exocyclic electron-withdrawing group, thiatriazoles **18** bear an exocyclic electron-donating group. This type of heterocycle loses nitrous sulfide (N_2S), in what is probably a single step process, which rapidly decomposes to nitrogen and sulfur.[35] Nitrous sulfide generated from thiatriazoles has been characterized and used to deliver sulfur to strained alkenes generating thiiranes.[36] While ESI does not provide insight to the nature of the bond breaking process, data summarized in Scheme 4 show that thiatriazolines formed from thio acids and azides can lose a fragment corresponding to nitrous sulfide.[37]

The DFT calculations also support the experimental conclusions in that two discrete reaction paths have been identified and that the mechanism is dictated by the electronic properties of the azide. The electron-deficient azide reacts via a stepwise mechanism[38] to form a thiatriazoline intermediate. Thus the anion of the thio acid adds to the polarized azide terminus. This

(28) Interpretation of the data is complicated by the sensitivity of $\Delta S^{\ddagger}$ to reaction solvent. *Organic Reactions: Equilibria, Kinetics and Mechanism*; Csizmadia, I. G., Ruff, F., Eds.; Elsevier: New York, 1994.

(29) These experiments do not directly establish that compounds of type **3** are on the reaction path, since they do not address the important issue of the nature of the equilibrium between intermediates of type **3** and **4**. However, given the computational findings, **3** does indeed appear to be a relevant intermediate. See also ref 21c.

(30) (a) Wilson, S. R.; Perez, J.; Pasternak, A. *J. Am. Chem. Soc*. **1993**, *115*, 1994. (b) Merkx, R.; Rijkeers, D. T. S.; Kemmink, J.; Liskamp, R. M. J. *Tetrahedron Lett.* **2003**, *44*, 4515. See also: (c) Guo, H.; Qian, R.; Liao, Y.; Ma, S.; Guo, Y. *J. Am. Chem. Soc.* **2005**, *127*, 13060.

(31) Although **3**, if present, could cyclize under these conditions to give the observed thiatriazoline **4** and is not distinguishable via the ESI−MS experiment.

(32) Mlostoñ, G.; Romañski, R.; Heimgartner, H. *Polish J. Chem.* **2001**, *75*, 975.

(33) The DFT calculations reported here strongly implicate Path 2 as the likely mechanism by which the putative thiatriazolines of ref 32 lead to the observed imidates. The amidation reactions in ref 16a and 16b likely follow reaction mechanisms analogous to those outlined here as well.

(34) (a) Loock, E. V.; Vandensavel, J.-M.; L'Abbe, G.; Smets, G. *J. Org. Chem*. **1973**, *38*, 2916. (b) L'Abbe, G.; Verhelst, G.; Yu, C.-C.; Toppet. S. *J. Org. Chem.* **1975**, *40*, 1728. (c) L'Abbe, G.; Brems, P.; Albrecht, E. *J. Heterocyclic Chem*. **1990**, *27*, 1059. Metal analogues are also known, see: (d) Fruhauf, H.-W *Chem. Rev.* **1997**, *97*, 523.

(35) Wentrup, C.; Kambouris, P. *Chem. Rev.* **1991**, *91*, 363.

(36) Adam, W.; Bargon, R. M. *Eur. J. Org. Chem.* **2001**, 1959.

(37) To date, capture of nitrous sulfide or S_1 according to the procedure of Adam (see ref 36) has failed to provide unequivocal experimental evidence that nitrous sulfide is being generated under the reaction conditions.

(38) Related to diazotransfer, see: Regitz, M. *Synthesis* **1972**, 351.

establishes the S−N connectivity and generates a stabilized aza-anion (**23**, Figure 3). This species cyclizes to form the C−N bond leading to **25**. There is virtually no barrier to ring opening and reversion to the linear intermediate.[39] Protonation of this anion, however, prevents reversion to the linear intermediate and leads to the relatively stable, neutral thiatriazoline **26**. In contrast to thio acetate, thioacetic acid (**19a**) was found to undergo a one-step cycloaddition with methyl sulfonazide to form **26** directly. While both routes converge on the same intermediate, the reaction barrier for the one-step process is significantly higher in comparison to the anion (Figure 3). Intermediate **26** undergoes a 3+2 cycloreversion to form the enol form of the product amide plus nitrous sulfide, which decomposes to the observed nitrogen and sulfur.[35]

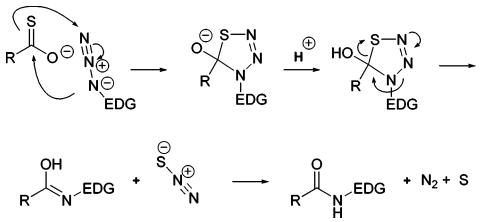
Electron-rich alkyl azide participates in a concerted process for both the thiocarboxylate and the thio acid to give the thiatriazoline in a single step. However, the reaction proceeds via a lower activation barrier for the thiocarboxylate (17.1 kcal/mol vs 21.8 kcal/mol, see Figure 4). Thus for both the electron-rich and the electron-poor azides, the anionic reactions are accelerated relative to the neutral reactions.

The computed activation energy difference between reactions of methyl sulfonazide and methyl azide with thioacetic acid (1.8 kcal/mol) is somewhat larger than the experimentally determined value (0.35 kcal/mol). There are two likely causes for this difference. First, the single point PCM computations only approximate the kinetic medium; and second, the use of methyl groups in the calculations in place of phenyl and benzyl used in the experiments ignores steric interactions that may be relevant. This minor difference aside, the studies indicate that the thio acid/azide coupling of azides bearing electron-donating substituents constitutes an anion-accelerated [3+2] cycloaddition process that results in the formation of a stable thiatriazoline.[40] This comparatively more sterically demanding transition state is consistent with our experimental findings and the trend wherein primary azides react more rapidly than secondary azides and much more rapidly than tertiary azides. The anionic thiatriazoline **31** is significantly more stable than **25** and is not as prone to C−N cleavage and conversion to a linear intermediate. Protonation of **31** and retro-[3+2] reaction gives the amide product as well as nitrous sulfide in a manner completely analogous to the pathway outlined for the thio acid reaction with electron-deficient azides.

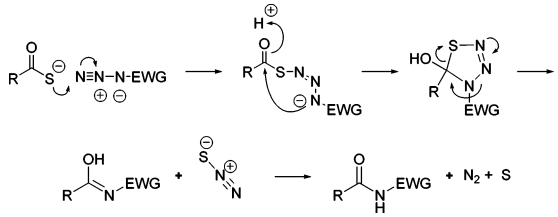
Summary

The experimental results from kinetic studies, Hammett correlation, trapping experiments, and ESI−MS/MS data coupled with computational analysis uniquely support our contention

Scheme 5. Mechanism of Amidation: Electron-Rich Azide



Scheme 6. Mechanism of Amidation: Electron-Deficient Azide



that the reaction pathway for relatively electron-rich azides is different from the pathway for highly electron-deficient azides. The mechanisms shown above summarize our current understanding of these reactions.

For relatively electron-rich azides (Scheme 5), the nitrogen−sulfur and nitrogen−carbon connectivity of the thiatriazoline intermediate is formed in a single step by anion-accelerated [3+2] cycloaddition. Subsequent protonation and loss of nitrous sulfide via retro-[3+2] cycloaddition gives the amide product.

Highly electron-poor azides first form the nitrogen−sulfur bond to give a linear intermediate (Scheme 6). In a separate step, formation of the nitrogen−carbon bond and protonation gives the thiatriazoline intermediate. Retro-[3+2] cycloaddition gives the amide product. This mechanism is in complete accord with the prior capture concept.[3]

The mechanistic data provide a basis for understanding the thio acid/azide amidation and explain why this reaction approaches the ideal click-type reaction profile for highly electron-poor azide substrates. Such azides form the desired amide products in high yield under dilute or concentrated solutions at room temperature. The coupling is effective in solvents ranging from organic to aqueous. Nearly equimolar quantities of the coupling partners provide high yield of the desired product. These observations are directly attributable to the efficient, chemoselective capture of the thiocarboxylate by the polarized azide followed by efficient intramolecular nucleophilic addition. Molecular nitrogen and sulfur, both of which are innocuous, form as the only identifiable byproducts by spontaneous and exothermic decomposition of the thiatriazoline. Virtually all of these characteristics are retained in the amidation of electron-rich azides. However, since the azide is not highly polarized, the thiatriazoline is formed directly and extended heating of more concentrated reaction mixtures is required to induce satisfactory reaction rates.

Since our original disclosure on the thio acid/azide amidation as a method for complex amide synthesis and as a potential ligation strategy, several advances and applications of the reaction have appeared, including selenium variants[16a,16b] and metal promoted couplings.[16c] New applications include the preparation of neoglycopeptides,[16d] thioamides,[21c] *N*-acyl sulfonamides and their conjugates,[14,17b] including the promising *N*-acyl β-substituted aminoethane sulfonamides.[17a]

(39) This finding is analogous to the very low barrier to hydroxide addition/chloride elimination from acyl chlorides [(a) Fox, J. M.; Dmitrenko, O.; Liao, L.-A.; Bach, R. D. *J. Org. Chem.* **2004**, *69*, 7317]. It is noteworthy in this regard that the pK_a of protonated **23** may well be near that of an *N*-acyl sulfonamide (p$K_a \approx 2.5$, see ref 19a) and the pK_a of thiatriazoline **26** near other tetrahedral intermediates [(b) p$K_a \approx 12$, see: Deslongchamps, P. *Tetrahedron*, **1975**, *31*, 2463. (c) Perrin, C. L.; Nunez, O. *J. Am. Chem. Soc.* **1986**, *108*, 5997; and (d) Perrin, *Acc. Chem. Res.* **2002**, *35*, 28] and hence represents a Ka difference of approximately 10 orders of magnitude. This difference in anion stability anticipates the small reaction barrier separating **25** from **23**.

(40) Analogous anion accelerated [3+2] cycloadditions, though known, appear to be very rare (for an example, see: (a) Shindo, M., Kotaro, I.; Tsuchiya, C.; Shishido, K. *Org. Lett.* **2002**, *4*, 3119) and models for predicting the effect of an exo-anion on a [3+2] cycloaddition do not appear to be developed (see for example: (b) Carpenter, B. K. *Tetrahedron* **1978**, *34*, 1877).

Thio acid/azide amidation provides an alternative chemoselective strategy to amide synthesis that complements the Staudinger amidation method, as it is especially effective for electron-deficient azide partners and effective over a range of solvents, including water. This report lays a mechanistic foundation for amide synthesis via thio acid/azide amidation and should enable hypothesis-driven approaches to other amide forming reactions and methodological improvements. Further investigations are ongoing and will be reported in due course.

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Supporting Information Available: Complete ref 25a, experimental procedures and computational and experimental data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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