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BROMONITRILE OXIDE [3+2] CYCLOADDITIONS IN WATER[1]

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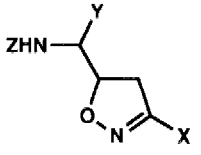
Key Words: Acivicin; transglutaminase; cysteine; 4,5-dihydroisoxazole; dibromoformaldoxime.

Abstract: *Bromonitrile oxide can be generated homogeneously in water at acidic pH, allowing efficient [3+2] cycloaddition with water soluble olefins and acetylenes. Allylammonium salts react with high regioselectivity and without the need for N-group protection.*

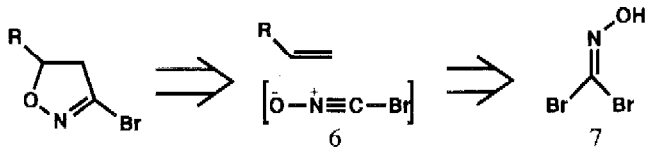
The discovery of the amino-acid antibiotic Acivicin **1** has stimulated considerable interest in the synthesis and pharmacology of the 3-chloro-4,5-dihydroisoxazole (3-Cl-IO) ring system.[2] Acivicin mimics glutamine and irreversibly inactivates anthranilate synthetase by covalent modification of an active-site cysteine residue.[3] The thiol is believed to react with the electrophilic imino-chloride moiety in the 3-Cl-IO ring. Castelhano appended (S)-5-aminomethyl-3-chloro-IO (**S**)-**2**, the decarboxylated analogue of **1**, to the C-terminus of various amino-acid and peptide fragments and thereby discovered RS-10025 **4**, a topically active inhibitor of another cysteine amidotransferase, epidermal transglutaminase.[4] The 3-bromo-IO analogue, RS-10823 **5**, was found to be even more active. The potential of these agents for the treatment of human acne[5] prompted studies in this laboratory to develop a simplified chemical synthesis of **4** and **5**, and particularly of the 3-halo-IO "warheads".



	X	Y	Z
1	Cl	CO_2H	H
2	Cl	H	H
3	Br	H	H
4	Cl	H	N-Cbz-L-Tyr
5	Br	H	N-Cbz-L-Tyr

A general synthetic approach to the 5-substituted-3-bromo-IO ring system is via [3+2] cycloaddition of bromonitrile oxide (BrCNO **6**) with a terminal olefin. The proclivity of BrCNO towards competitive self-condensation requires that it be slowly generated in the presence of excess dipolarophile. This has typically been accomplished by slow alkaline decomposition of readily-available crystalline dibromoformaldoxime **7**,[6] in a biphasic (aqueous/organic) reaction mixture. When olefinic amines are the substrates a further complication has been held[7] to be the need for protection of the amino function with an acyl or bulky alkyl group. Protecting groups like Cbz,[4,8] Boc,[9] dichloroacetyl,[7] trichloroacetyl,[6d] phthaloyl,[10] and trityl[11] have historically been employed. We reasoned that if BrCNO could be generated homogeneously in water under acidic conditions, then allylic amines could be utilized without the need for inefficient group protection.[12] The amines would be effectively neutralized by protonation at low pH. Initial studies focused on using aqueous silver nitrate[10] to

dehydrohalogenate **7** in the presence of an allylamine nitrate salt. Good yields of [3+2] cycloadducts were obtained but an even simpler protocol was discovered which circumvented the high cost of silver.



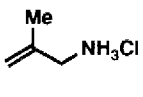
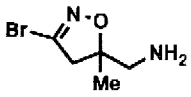
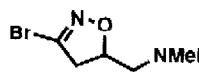
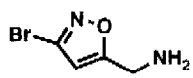
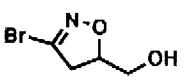
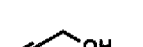
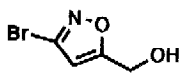
Dibromoformaldoxime **7** was found to be very soluble in water (>3M) and surprisingly acidic (a saturated solution displays pH ~2). When small portions of 2M aq. potassium bicarbonate were added to a solution of **7**, the pH transiently rose to 3-5, the characteristic gummy yellow by-products of BrCNO oligimerization came out of solution, and the pH dropped back to 2 within minutes. When **7** was decomposed in this way in the presence of a moderate excess (1.5 eq) of allylamine hydrochloride (pKa~10), the BrCNO was efficiently trapped by the olefin giving 5-aminomethyl-3-bromo-IO **3** in >95% yield (Table 1).[13] The product was obtained as a pale yellow oil of sufficient purity for subsequent chemical transformations and was fully characterized as its crystalline hydrobromide salt.

An analogous synthesis of the 3-chloro-IO **2** was initially foiled by the extremely rapid self-condensation of chloronitrile oxide, at the expense of [3+2] cycloadduct.[10,14] However, it was found that **2** could be easily prepared from **3** in one pot by saturation of the aqueous BrCNO reaction mixture with lithium chloride (25 eq) and catalytic HCl (2 eq). This exceptionally mild halogen metathesis was monitored by derivatization of the compounds **2** and **3** as their acetamides (acetic anhydride, sodium carbonate) and analysis by reverse-phase HPLC (ODS-2, 5% acetonitrile in 0.15 M $NH_4H_2PO_4$, 225 nm). The conversion of **3** into **2** was found to proceed smoothly in 30-60 minutes at room temperature. Remarkably, the equilibrium position for the conversion was not the statistical 27Cl:2Br, but a much more favorable 99.6Cl:0.4Br, probably as a consequence of the greater carbon-chlorine bond strength. After filtration and alkaline work-up, **2** was obtained as an oil in 96% crude yield and fully characterized as its hydrochloride salt.[15] It is noteworthy that lithium plays a crucial role in this reaction since neither sodium or potassium chloride effected the metathesis under these conditions.[16]

To complete the synthesis of **4** and **5** the crude heterocycles **2** and **3** were resolved with (+)-mandelic acid[4] (70% yield) and coupled with N-Cbz-L-tyrosine by an economical mixed anhydride method (iBuOCOCl, NMM, THF, 5°C; KOH, MeOH; 75% yield).[17] The products derived were identical by all spectral and chromatographic criteria to authentic samples prepared by published procedures.[4]

To demonstrate the scope of the aqueous BrCNO cycloaddition, a variety of water-soluble olefins and acetylenes were condensed under the standard conditions (Table 1), with uniformly high yields for the olefins. Work-up and purification was quite easy since any BrCNO polymer formed precipitated out and was easily removed by filtration. This is a major practical improvement over heterogenous conditions where the polymer typically stays dissolved in the organic layer with the product.

Table 1. Aqueous Bromonitrile Oxide Cycloadditions.

Dipolarophile	Product	Regio-selectivity[a]	Yield[b] (Yield, mp°C)[c]	Analysis
	**3**	(>98:2)	96% (76%, 123-6°)[e]	For: $C_4H_8Br_2N_2O$ Calcd: C 18.48; H 3.10; N 10.78. Fnd: C 18.54; H 3.10; N 11.17
	**4**[d]	(>98:2)	96% (82%, 144-7°)[f]	For: $C_4HCl_2N_2O$ Calcd: C 28.09; H 4.71; N 16.38. Fnd: C 28.11; H 4.79; N 16.75.
		(95:5)	75% (67%, 104-7°)[e]	For: $C_5H_{10}Br_2N_2O$ Calcd: C 21.92; H 3.68; N 10.23. Fnd: C 21.93; H 3.60; N 10.35.
		(>98:2)	80% (72%, 104-6°)[e]	For: $C_5H_{10}Br_2N_2O$ Calcd: C 21.92; H 3.68; N 10.23. Fnd: C 21.68; H 3.57 N 10.09.
		ND	51% (28%, 215°d)[e]	For: $C_4H_6Br_2N_2O$ Calcd: C 18.63; H 2.34; N 10.86. Fnd: C 18.37; H 2.15; N 10.68.
		(91:9)[8]	90%[g]	For: $C_4H_6BrNO_2$ Calcd: C 26.69; H 3.36; N 7.78. Fnd: C 26.79; H 3.44; N 7.85.
		(93:7)[18]	80%[g]	For: $C_4H_4BrNO_2$ Calcd: C 26.99; H 2.27; N 7.86. Fnd: C 27.14, H 2.26, N 8.19.

a) Estimated by 300 MHz ^{1}H-NMR. Minor product is 4-substituted isomer. b) Crude product, based on **7**, with 1.5 eq. dipolarophile. c) Analytically pure crystalline derivative. d) After LiCl treatment, see text. e) Hydrobromide salt. f) Hydrochloride salt. g) Pure oily product by silica gel chromatography.

The perturbation of the charged amino group enhances the regioselectivity of this [3+2] cycloaddition. De Amici reported[8] formation of 4-9% of 4-substituted-IO regioisomer on cycloaddition of BrCNO with N-Boc-allylamine and similar neutral terminal olefins under heterogeneous conditions. Homogeneous condensation with allylammonium chloride gives <2% regioisomer.

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References and Notes

1. Contribution #850 from the Institute of Organic Chemistry, Syntex Research, Palo Alto, California.
2. a) Earhart, R. H.; Neil, G. L. *Adv. Enzyme Regulation* **1985**, *24*, 179-205; b) Prajda, N. *ibid.*, 207- 223.
3. Tso, J Y.; Bower, S. G.; Zalkin, H. *J. Biological Chem.* **1980**, *255*, 6734-6738.
4. Castelhano, A. L.; Billedeau, R.; Pliura, D. H.; Bonaventura, B.J.; Krantz, A. *Bioorganic Chemistry* **1988**, *16*, 335.
5. De Young, L.; Ballaron, S.; Epstein, W. *J. Investigative Dermatology* **1984**, *82*, 275-279.
6. The yield for the standard literature preparation of **7**, from glyoxylic acid oxime, is increased by buffering the reaction mixture. Buffering allows consumption of the full theoretical charge of bromine. See: a) De Paolini, I. *Gazz. Chim. Ital* **1930**, *60*, 704, b) Wade, P.A.; Bereznak, J.F.; Palfey, B.A.; Carroll, P.J.; Dailey, W.P.; Sivasubramanian, S. *J. Org. Chem.* **1989**, *55*, 3045, c) Hsu, A. *European Pat. App.* **1987**, 232012; *CA* **1987**, *108(1)*, 5580x, d) Vyas, D.M.; Chiang, Y.; Doyle, T.W. *Tetrahedron Lett.* **1984**, *25*, 487. **Dibromoformaldoxime (7).** A solution of 50% glyoxylic acid (304 g, 2.05 mol), and hydroxylamine hydrochloride (143 g, 2.05 mol) in water (0.5 L) was stirred for 1 hour at room temperature. The acidic solution was cooled in ice and brought to pH 7 by slow addition of 50% aq. NaOH (328 g, 4.10 mol) at a rate that kept the temperature below 25°C. Sodium phosphate monobasic (492 g, 4.10 mol) was added followed by methylene chloride (50 mL) as surfactant to control foaming. The solution was maintained at 10-15°C by ice-water cooling as neat bromine (211 mL, 4.10 mol) was added dropwise over 2-3 hours. After 12 hours additional stirring, traces of residual bromine color were decomposed with a few drops of sodium bisulfite solution leaving a snow white slurry. The mixture was extracted with methylene chloride (2x1 L). After drying over sodium sulfate, the extracts were evaporated carefully to prevent sublimation (35°C, 100 torr) to yield 324 g (78% yield) of **7** as shiny white crystals of analytic purity; mp 63-65°C (lit.[b] mp 70-71°C, lit.[d] mp 65-66°C); ^{13}C NMR (90 MHz, $CDCl_3$) d 100.26; Anal. Calcd. for $CHBr_2NO$: C 5.92; H, 0.50; N, 6.90; Br, 78.79. Found: C, 6.05; H, 0.54; N, 6.96; Br, 78.61.
7. Chiarino, D.; Napoletano, M.; Sala, A. *Tetrahedron Lett.*.**1986**, *27*, 3181.
8. De Amici, M.; De Micheli, C.; Misani, V. *Tetrahedron* **1990**, *46*, 1975.
9. a) Caldirola, P.; De Amici, M.; De Micheli, C. *Tetrahedron Lett.* **1986**, *27*, 4651, b) Nishi, T.; Morisawa, Y. *Heterocycles* **1989**, *29*, 1835-1842.
10. Wade, P. A.; Pillay, M. K.; Singh, S. M. *Tetrahedron Lett.* **1982**, *23*, 4563-4566.
11. Arzeno, H. B.; Robinson , J., *unpublished results from this laboratory.*
12. An early synthesis of **1** supported this postulate: Hagedorn III, A. A.; Miller, B. J.; Nagy, J. O. *Tetrahedron Lett.* **1980**, *21*, 229-230.
13. **General Procedure for [3+2] Cycloadditions; (S,R)-3-Bromo-5-aminomethyl-4,5-dihydroisoxazole (3).** Dibromoformaldoxime (331.00g, 1.63 moles) was added to a room temperature solution of allylamine HCl (228.88g, 2.45 moles) in water (1.1 L). The mixture was stirred at room temperature until all of the solid had dissolved. A solution of $KHCO_3$ (196.07g, 1.96 moles) in water (0.8 L) was added dropwise over a 6 hour period while stirring at room temperature. 50% KOH (238.00g, 2.13 moles) was added and the aqueous mixture was filtered through a medium frit and subjected to continuous liquid-liquid extraction with dichloromethane (1.8 L). After 16 cycles, the extracts were dried with $MgSO_4$, filtered, and evaporated under reduced pressure to yield 280.09g (96%) of crude **3**. A 5g sample of the crude material was dissolved in 2-propanol (16 mL) and treated with 2N HBr in ethanol (14 mL). After filtration and drying, 5,49g (76%) of (S,R)-**3** hydrobromide salt was obtained. 1H NMR (300 MHz, DMSO-d_6), ∂=8.12 (NH), 4,94 (m, 1H), 3.54 (dd, J=10.7, 17.6 Hz, 1H), 3.21 (dd, J=7.3, 17.7 Hz, 1H), 3.08 (m, 2H).
14. Baldwin, J. E., Hoskins, C.; Kruse, L. *J. Chem. Soc. Chemical Communications* **1976**, 795-796.
15. **(S,R)-3-Chloro-5-aminomethyl-4,5-dihydroisoxazole (2).** The cycloaddition reaction mixture containing **3** (or isolated **3** in 1.8 L water) was cooled in ice and treated with conc. HCl (271.95 ml, 3.26 moles). Solid LiCl (1727.39g, 40.75 moles) was added in portions at a rate that kept the temperature below 35°C. After the addition was completed, the temperature of the thick, saturated mixture was adjusted to 25°C and stirring was continued for 1 hour. The mixture was cooled on ice before 50% KOH (548.76g, 4.89 moles) and water (1.5 L) were added. The salty aqueous mixture was filtered through a medium frit and subjected to continuous liquid-liquid extraction with dichloromethane (3.6 L). After 16 cycles, the extracts were dried with $MgSO_4$ and filtered. Evaporation under reduced pressure yielded 210.54g (96%) of **2**. A 5g sample of the crude material was dissolved in 2-propanol (17.5 mL) and treated with 3N HCl in ethanol (12.5 mL). After filtration and drying, 5.55g (82%) of (S,R)-**2** hydrochloride salt was obtained. 1H NMR (300MHz, DMSO-d_6), ∂=8.40 (NH), 5.06 (m, 1H), 3.49 (dd, J=10.7, 17.6 Hz, 1H), 3.24 (dd, J=7.5, 17.6 Hz, 1H), 3.07 (d, J=6.2, 2H).
16. Calcium and magnesium chlorides do effect the metathesis but give intractable metal hydroxides on alkaline work-up.
17. Chen, F. M. F.; Lee, Y.; Steinauer, R.; Benoiton, N. L. *Can J. Chem.* **1987**, *65*, 613-618.
18. Chiarino, D.; Napoletano, M.; Sala, A. *J. Heterocyclic Chem.* **1987**, *24*, 43-46.
19. During the reviewing process it was pointed out that another example of aqueous BrCNO cycloaddition with an unprotected olefinic amine, specifically allyl glycine, had previously been reported; Castelhano, A. L.; Pliura, D. H.; Venuti, M. C. *US Patent* **1990** #4,970,297.

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