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SYNTHESIS OF ANALOGUES OF THE ANTITUMOR ANTIBIOTIC AT-125 (ACIVICIN)

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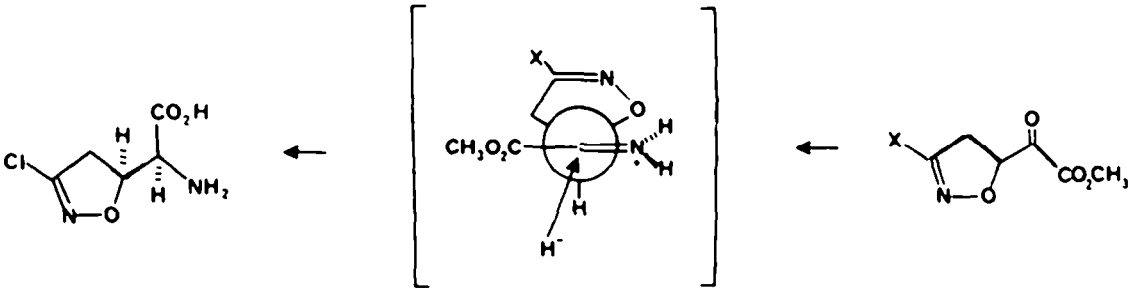
Abstract—Several haloisoxazoline analogues of α-amino-3-chloro-4,5-dihydro-5-isoxazole acetic acid (1, AT-125) were prepared by means of 1,3-dipolar cycloaddition methodology. The chemistry of these compounds is discussed, and a possible biological mode of action of AT-125 presented.

IN 1973 Martin *et al.*,[1] reported the isolation and structure of a novel antimetabolite (*αS, 5S*) α - amino - 3 - chloro - 4,5 - dihydro - 5 - isoxazole acetic acid (**1**, AT-125, Acivicin). This unusual amino acid, isolated from fermentation broths of *Streptomyces sviceus*, displayed significant activity against a number of tumors in experimental animals and is currently being developed by the U.S. National Cancer Institute for clinical evaluation.[2] To date, three total syntheses have appeared[3] as well as certain analogues.[4]

The synthetic strategy we adopted is based on the empirical prediction (Cram's rule[5]) that reductive amination of α-keto acid **3** should proceed stereo-selectively to afford the desired *erythro*-diastereomer (*cf.* **2**). The requisite α-keto acid (**3**) could, in principle, be prepared by oxidation of the corresponding α-hydroxyester **4**, or by hydrolysis of ketal **5** or thioketal **6**. It had been reported previously[4b] that cycloaddition of chloronitrile oxide, generated *in situ* from phosgene oxime (**7a**), to styrene gave a poor yield (6%) of adduct **8a**. We observed equally disappointing yields under homogeneous conditions. However, when a two phase ($Et_2O/H_2O/Na_2CO_3$) system was employed in the **7a** to **8a** transformation the yield was improved to 32%. Furthermore, under these conditions, bromonitrile oxide and *p*-toluenesulfonylnitrile oxide, generated from the corresponding bromides, afforded even higher yields, 67 and 48%, of cycloadducts **8b** and **8c**, respectively.

The cycloaddition of chloronitrile oxide to vinyl glycolic acid (**9**), followed by esterification, afforded α-hydroxyester **4** as a mixture of diastereomers in only 11.5% overall yield. Even more disappointing was our inability to oxidize this alcohol to α-ketoester **3a** under a variety of conditions. Although the precise reasons for this lack of reactivity remain obscure, steric factors are certainly involved; conversion of the alcohol to the corresponding tosylate required stirring with tosyl chloride in pyridine for several days at room temperature.

The second route to α-ketoester **3** required ketal **14**. Treatment of dichloroacetic acid with $NaOCH_3$ followed



1: AT - 125 (Acivicin)

2

3a: X = Cl
3b: X = Br

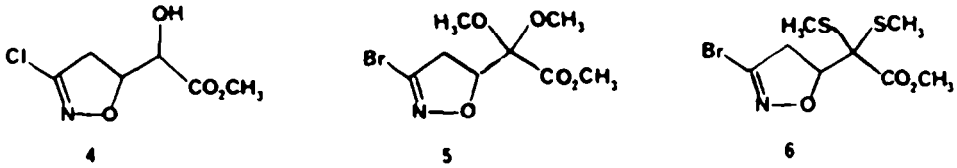


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Fig. 1.

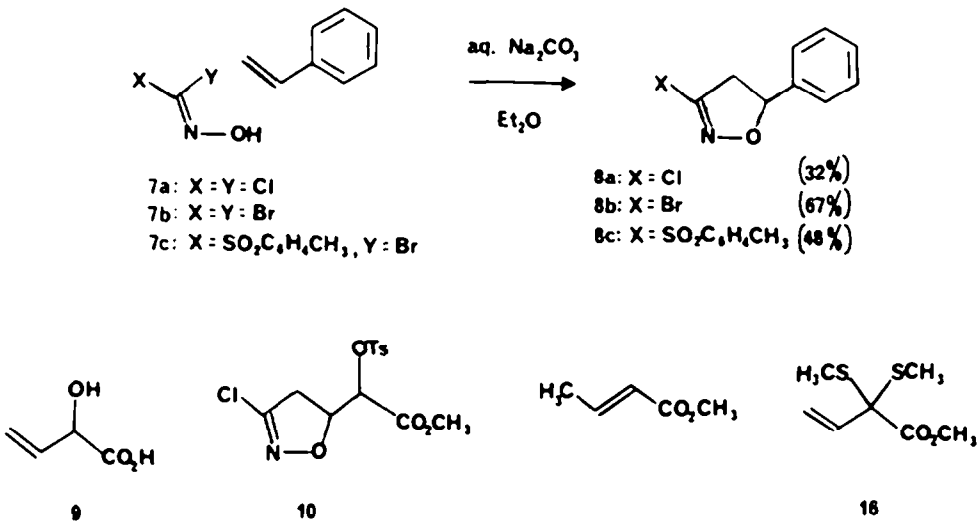

Fig. 2.

by esterification afforded methyl glyoxylate dimethyl acetal[6] (11; Fig. 3). Deprotonation followed by conjugate addition[7] to phenyl vinyl sulfoxide[7,8a] provided 13 in 38% yield after chromatography. Sulfoxide elimination[8b] at 170° in the presence of $CaCO_3$ gave 14 in yields ranging from 15–30%. In view of the low yields observed in the cycloaddition of chloronitrile oxide to vinyl glycolic acid we employed dibromoformoxime (7b) for subsequent studies. This decision was justifiable since it has been shown[4a] that bromo AT-125 is nearly as active as the natural product. Reaction of 14 with excess dibromoformoxime produced isoxazoline 5 (66%). Attempts to selectively hydrolyze ketal 5 to α-ketoester 3b resulted in either no reaction, partial degradation to unidentifiable products, or complete degradation, depending on the conditions employed. It would appear that the most reactive site in 5 is the imino bromide. Hydrolysis of this moiety to produce the corresponding isoxazolidone nucleus would be expected to have disasterous consequences since it is known[9] that a closely related substance, tricholomic acid (15) decomposes even at room temperature in dilute aqueous hydrochloric acid.

Thioketal 16 was prepared from methyl crotonate by exhaustive thiolation[10] with methyl methanethiosulfonate employing lithium diisopropyl amide as the base. Smooth cycloaddition to 16 was observed using the two-phase procedure described above to afford 6 in 65% yield after chromatography. Even this substance failed to undergo

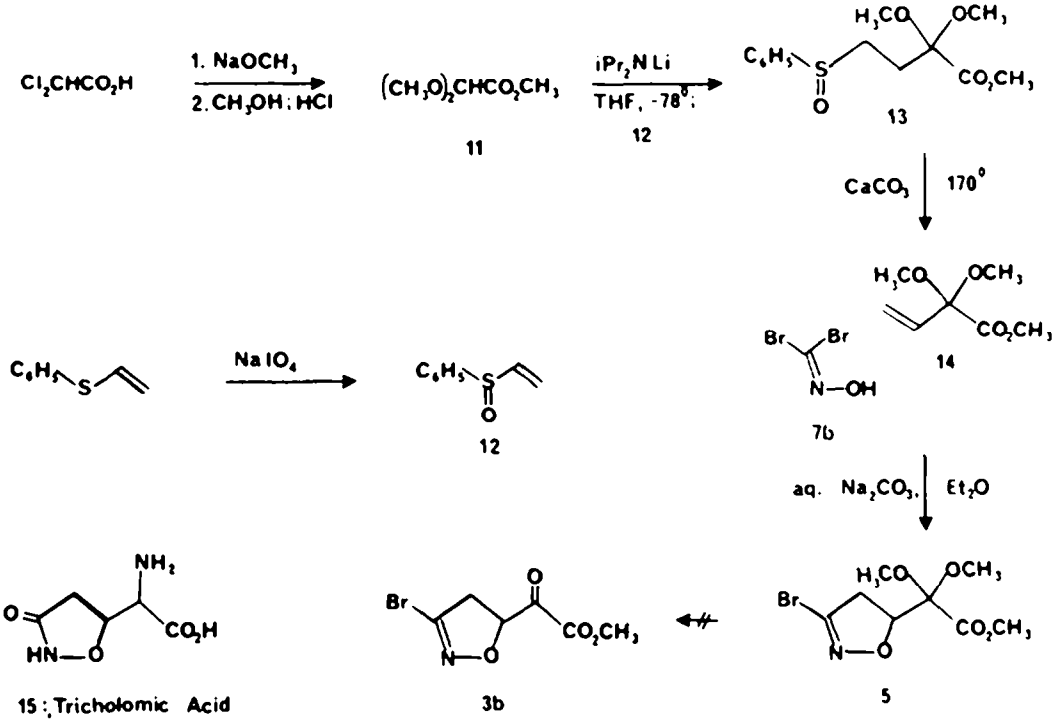

Fig. 3.

hydrolysis to the desired α-ketoester **3b** under conditions which one might have expected to be selective for sulfur. For example, NBS in $MeCN/H_2O$ or NCS and $AgNO_3$ in $MeCN/H_2O$ led to total destruction. The employment of MeI in acetone/H_2O at reflux or dimethyl sulfate in $MeCN/H_2O$ at 80° resulted in no reaction.

Although this study failed to provide a new total synthesis of AT-125, it did produce a number of closely related structures for biological testing. Isoxazolines **5**, **6**, and **10** were evaluated[11] in the *in vitro* murine L1210 leukemia tube dilution assay with a 3-day incubation of each compound with the cells. Each analogue was tested over a range of concentrations to a maximum of 10 μg/mL. AT-125 was included as a positive control. The results are expressed in Table 1 as ID_{50} and ID_{90} values, which are drug concentrations required to inhibit the growth of cells by 50 and 90% respectively. Of the three substances tested, isoxazoline **10** is clearly the most interesting. Although not nearly as potent as AT-125 itself this substance does show activity. When one takes into account further that **10** is actually a mixture of all four possible diastereomers this activity could prove to be a significant lead.

Table 1.

Compound	ID_{50}(μg/mL)	ID_{90}(μg/mL)
AT-125 (**1**)	0.08	0.16
10	3.00	8.1
5	> 10.00	> 10.00
6	> 10.00	> 10.00

Studies on the mode of action of AT-125 at the molecular level have shown[2] that it inhibits CTP synthetase as well as other enzymes which catalyze the transfer of the amino group of L-glutamine. It also causes effects on ribonucleotide pools which are consistent with inhibition of this enzyme and with the inhibition of another L-glutamine-dependent enzyme, XMP aminase. During the course of the present investigation, we became impressed by the fact that AT-125 incorporates certain structural features in common with several other substances (**15**, **17–19**) known to be specific inhibitors of pyridoxal dependent enzymes. Thus, cycloserine (**18**),[12] O-carbamoyl-D-serine (**17a**), O-acetyl-D-serine (**17b**), and β-halo-D-serines

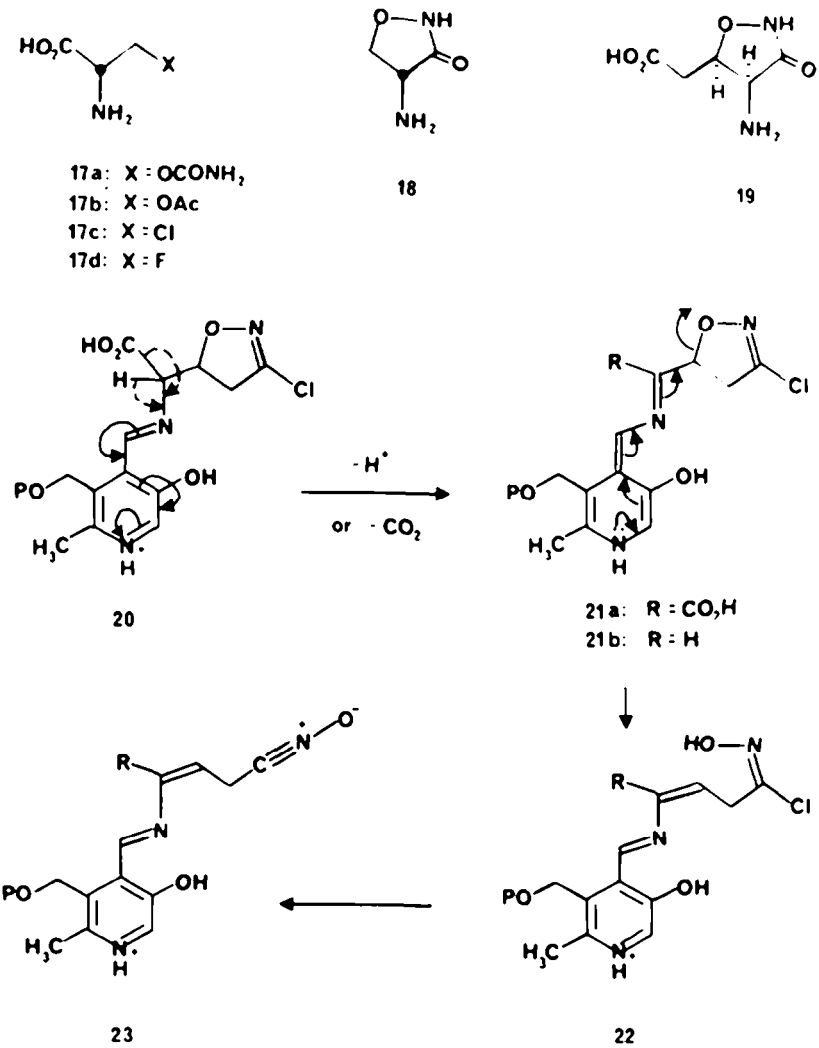

Fig. 4.

(**17c, d**)[13] are all α-amino acids which form Schiff bases with enzyme-bound pyridoxal phosphate. Subsequent β-elimination produces an acrylic acid derivative which is captured by nucleophilic sites on the enzyme. It is not unreasonable to suggest that AT-125 and another structurally related substance, *cis*-4-aminoisoxazolidin-3-one-5-acetic acid (**19**),[14] may form suicide inhibitors in a similar fashion. Thus, as shown in Fig. 4, Schiff base formation (**20**) with enzyme-bound pyridoxal phosphate provides an electrophilic site (the imino chloride) for acylation of the enzyme. Alternatively, deprotonation (or decarboxylation) of **20** produces an unstable intermediate (**21a** or **21b**) which could β-eliminate the isoxazolidine oxygen to afford hydroxamoyl chloride **22**. The latter intermediate possesses two electrophilic sites for nucelophilic capture, namely, the acrylic acid moiety and/or the hydroxamoyl chloride. The latter functional group could also undergo 1,3-elimination of hydrogen chloride to form the highly reactive nitrile oxide (**23**). It will be interesting to see, as knowledge about the mode of action of AT-125 at the molecular level emerges, if the above suggestions have, in fact, any validity.

EXPERIMENTAL

IR spectra were recorded on a Beckmann IR 4210 IR spectrophotometer. NMR spectra were taken on Varian T-60 and Bruker WP200 spectrometers, in dil $CDCl_3$ solns by using TMS as internal standard. Mass spectra were determined on an AEI-MS9 mass spectrometer. M.pts and bpts are uncorrected. MeOH was distilled from Mg turnings, THF from soldium-benzophenone, and diisopropylamine from calcium hydride. All chromatography was performed in open columns employing Baker analyzed reagent silica gel, 60-200 Mesh.

p-Toluenesulfonyl formoxamoyl bromide (**7c**). Prepared according to the procedure of Wade[15] from *p*-toluenesulfonyl nitromethane,[16] yield: 23%, mp 126.5–127.5°. IR (KBr) 3260 cm^{-1} (s), 1585 cm^{-1} (m), 667 cm^{-1} (s); ^1H NMR (200 MHz) δ8.81 (broad s, 1H), 7.89 (d, J = 8.3 Hz, 2H), 7.40 (d, J = 8.3 Hz, 2H), 2.48 (s, 3H); mass spectrum, exact mass (M^+-HBr), calcd: 197.0147, found: 197.0145.

3 - Chloro - 4,5 - dihydro - 5 - phenyl isoxazole (**8a**). To a vigorously stirred mixture **7a**[17] (1.0093 g, 0.010 mol), styrene (3.9443 g, 0.038 mol, 3.8 equiv), and ether (25 mL), a soln of Na_2CO_3 (0.9323 g, 0.009 mol) in water (15 mL) was added dropwise over a period of 26 hr.

The ether layer was separated, the aqueous layer extracted twice with ether, the ether layers combined, washed once with water, dried ($MgSO_4$), filtered, and solvent removed on the rotary evaporator. The residue was chromatographed (5:95 EtOAc: cyclohexane), affording a yellow oil: 0.5609 g, 32.02%; IR (neat) 3055 cm^{-1} (w), 3025 cm^{-1} (w), 1585 cm^{-1} (m), 760 cm^{-1} (s); ^1H NMR (200 MHz) δ7.36 (broad s, 5H), 5.68–5.78 (dd, 1H), 3.49–3.63 (dd, 1H), 3.09–3.22 (dd, 1H); mass spectrum, exact mass (M^+), Calc. 181.0294, Found: 181.0298.

3 - Bromo - 4,5 - dihydro - 5 - phenyl - isoxazole (**8b**). To a vigorously stirred mixture of styrene (1.6742 g, 0.016 mol, 3 eq.), Na_2CO_3 (1.0697 g, 0.010 mol, 2 eq.), ethyl ether (10 mL) and water (10 mL), a soln of **7b**,[18] (1.0120 g, 0.005 mol) in water (20 mL) was added over a period of 30 hr. The mixture was worked up as for **8a**, and provided an oil: 0.7950 g, 66.7%; IR (neat) 3057 cm^{-1} (w), 3028 cm^{-1} (w), 1577 cm^{-1} (w), 760 cm^{-1} (s); ^1H NMR (200 MHz) δ7.35 (broad s, 5H), 5.60–5.70 (overlapping dd, 1H), 3.53–3.67 (dd, 1H), 3.13–3.26 (dd, 1H); mass spectrum, exact mass (M^+), Calc. 224.9789, Found: 224.9790.

3 - p - Toluenesulfonyl - 4,5 - dihydro - 5 - phenyl - isoxazole (**8c**). Prepared according to the procedure for **8b**, from styrene (0.4221 g, 0.004 mol), Na_2CO_3 (0.1062 g, 0.001 mol) and **7c**, (0.2796 g, 0.001 mol), yield: 47.9%. A similar experiment employing a 50-fold excess of styrene afforded the dihydroisoxazole in 55.6% yield, mp: 91–92°, IR (KBr) 3059 cm^{-1} (w), 1585 cm^{-1} (m), 1335 cm^{-1} (s), 1170 cm^{-1} (s), 759 cm^{-1} (s), 675 cm^{-1} (s); ^1H NMR (200 MHz) δ7.91 (d, J = 8.3 Hz, 2H), 7.23–7.43 (m, 7H), 5.74–5.84 (dd, 1H), 3.66–3.80 (dd, 1H), 3.26–3.40 (dd, 1H), 2.48 (s, 3H); mass spectrum, exact mass (M^+), Calc. 301.0773, Found: 301.0783.

α - Hydroxy - 3 - chloro - 4,5 - dihydro - 5 - isoxazole acetic acid methyl ester (**4**, *mixture of diastereomers*). A soln of **7a**[17] (16.71 g, 0.15 mol) in water (50 mL) was added over a period of 2 days to a rapidly stirred soln of vinyl glycolic acid[19] (3.0297 g, 0.03 mol) and Na_2CO_3aq (1M, 350 mL), the soln extracted with ether, the ether discarded, and the aqueous soln acidified to pH = 1 (H_2SO_4). Ether extraction, drying ($MgSO_4$), filtration, and solvent removal provided an oil which was dissolved in dry MeOH (200 mL) containing 35 drops of conc H_2SO_4. Stirring for 2 days was followed by basification to pH = 9 (Na_2CO_3), solvent removal, partitioning between ether and water, drying the organic phase ($MgSO_4$), filtering, solvent removal and chromatography (30:70 EtOAc:hexane) to afford a clear oil which solidified upon standing: 0.6457 g, 11.5%; IR (neat) 3460 cm^{-1} (broad, m), 2845 cm^{-1} (w), 1755 cm^{-1} and 1730 cm^{-1} (s), 1592 cm^{-1} (w), 1110 cm^{-1} (s); ^1H NMR ($CDCl_3$-D_2O, 60 MHz) δ5.03–5.44 (m, 2H), 4.63 and 4.40 (each a broad doublet, combined integral = 2H), 4.02 and 3.98 (each a singlet, combined integral = 6H), 3.29–3.58 (unsymmetrical dd, 4H); mass spectrum *m/e* 193 (M^+, parent).

Methyl - 2,2 - dimethoxy - 4 - phenyl sulfinyl butanoate (**13**). To a stirring soln of lithium diisopropylamide (0.022 mol) in THF (40 mL) at −78° was added dropwise **11**,[5] (2.6883 g, 0.020 mol) in THF (10 mL) under N_2. The soln was stirred at −78° for 15 min, then **12**[7,8a] (3.0387 g, 0.020 mol) in THF (10 mL) added dropwise at −78°, and after 15 min quenched with excess (3–5 mL) AcOH. After warming to room temp, the mixture was poured into sat NH_4Claq, extracted with ether, the ether layers combined and washed twice with sat $NaHCO_3$aq. Drying ($MgSO_4$), filtering, and solvent removal followed by chromatography (65:35 EtOAc: hexane) provided a yellow oil: 2.1948 g, 38.4%; IR (neat) 3050 cm^{-1} (w), 2831 cm^{-1} (w), 1755 cm^{-1} (s), 1170 cm^{-1} (s), 1050 cm^{-1} (s); ^1H NMR (200 MHz) δ7.49–7.62 (m, 5H), 3.75 (s, 3H), 3.24 (s, 3H), 3.20 (s, 3H), 2.80–2.94 (m, 1H), 2.57–2.72 (m, 1H), 2.26–2.41 (m, 1H), 2.01–2.11 (m, 1H); mass spectrum, exact mass, (M^+–C_6H_5SO) Calc. 161.0814, Found: 161.0812.

Methyl - 2,2 - dimethoxy - but - 3 - en - oate (**14**). Compound **13** was distilled at approx. 20 torr in the presence of $CaCO_3$ (oil bath ~ 170°) to provide a yellow oil which was chromatographed (25:75 EtOAc:hexane) to afford **14** in yields of 15–30%. IR (neat) 2815 cm^{-1} (w), 1755 cm^{-1} (s), 1103 cm^{-1} (s); ^1H NMR (200 MHz) δ5.45–5.77 (m, 3H), 3.80 (s, 3H), 3.29 (s, 6H); mass spectrum *m/e* 129 (M^+–OCH_3).

α,α - Dimethyl - 3 - bromo - 4,5 - dihydro - 5 - isoxazole acetic acid methyl ester (**5**). Prepared according to the procedure for **8b** from **14**, (1.2967 g, 0.008 mol), Na_2CO_3 (4.3025 g, 0.041 mol), water (40 mL) and ether (20 mL); and **7b**[18], (7.5266 g, 0.0403 mol) in ether (110 mL). Addition was carried out over a period of 4 days. (**5**): 1.4155 g, 65.66%; IR (neat) 2840 cm^{-1} (w), 1750 cm^{-1} (s), 1586 cm^{-1} (w), 1180 cm^{-1} (s); ^1H NMR (200 MHz) δ4.93–5.02 (dd, 1H), 3.81 (s, 3H), 3.55–3.67 (dd, 1H), 3.44 (s, 3H), 3.36 (s, 3H), 3.21–3.35 (dd, 1H); mass spectrum *m/e* 222 (M^+–CO_2CH_3).

Methyl - 2,2 - di(methylthio) - but - 3 - en - oate (**16**). To a soln of lithium diisopropylamide (0.01 mol) in THF (15 mL) at −78° was added hexamethylphosphortriamide (2.0 mL, 1.1 eq.), the soln stirred for 40 min, and then methyl crotonate (1.0007 g, 0.01 mol) in THF (5 mL) added dropwise, and the soln stirred for 30 min at −78°. Methyl methanethiosulfonate[20] (1.3273 g, 0.011 mol) in THF (5 mL) was then added, the soln stirred at −78° for 30 min, the cooling bath removed, and the stirred gelatinous mixture allowed to warm to room temp. The mixture was poured onto sat NH_4Claq, and the product isolated by ether extraction. The ether was washed twice with water, dried ($MgSO_4$), filtered, solvent removed, and chromatographed (5:95 EtOAc:hexane) affording a yellow oil: 0.2950 g, 15.4%; IR (neat) 1733 cm^{-1} (s), 1625 cm^{-1} (w), 1239 cm^{-1} (s); ^1H NMR (200 MHz) δ5.90–6.04 (dd, 1H), 5.36–5.47 (m, 2H), 3.80 (s, 3H), 2.04 (s, 6H); mass spectrum, exact mass (M^+) Calc. 192.0279, Found: 192.0271.

α,α - Di(thiomethyl) - 3 - bromo - 4,5 - dihydro - 5 - isoxazole

acetic acid methyl ester (**6**). Prepared according to the procedure for **8b** from **16**, (2.4782 g, 0.013 mol), Na_2CO_3 (6.8409 g, 0.0645 mol), water (70 mL) and ether (10 mL); and **7b**[18], (12.2605 g, 0.066 mol) in ether (195 mL). Addition was performed over a period of 5 days. (**6**): 2.6893 g, 66.35%; IR (neat) 1725 cm^{-1} (s), 1605 cm^{-1} (w); ^1H NMR (200 MHz) δ5.32–5.41 (dd, 1H), 3.85 (s, 3H), 3.57–3.69 (dd, 1H), 3.26–3.41 (dd, 1H), 2.24 (s, 3H), 2.22 (s, 3H); mass spectrum *m/e* 313 (M^+, parent).

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