(29) Reduction of **23** with AlD_2Cl afforded **28a** containing the isotopic label only at C_1, α to the silicon atom (^1H NMR analysis). Structural assignment to this alcohol was further secured by its reaction with sodium hydride in tetrahydrofuran to give 4-*tert*-butylcyclohexene-*1-d* (>92.5% d_1 by mass spectroscopy). Accordingly, the stronger Lewis acid character of AlH_2Cl and AlD_2Cl does not appear to bring into play a new mechanism such as initial electrophilic α C–O fission with concomitant intramolecular 1,2-hydride shift and subsequent normal reduction of 3-*tert*-butylcyclohexanone. The possible intervention of a pentacoordinated silicon hydride intermediate[30] which provides directionality to this epoxide-opening process has been neither established nor disproven.

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Peptide-Gap Inhibitors.[2] 2. Stereoselective Synthesis of Enantiomeric Dipeptide Analogues of Glycylleucine Which Contain Methylene Thioether Groups Substituted for Peptide Linkages

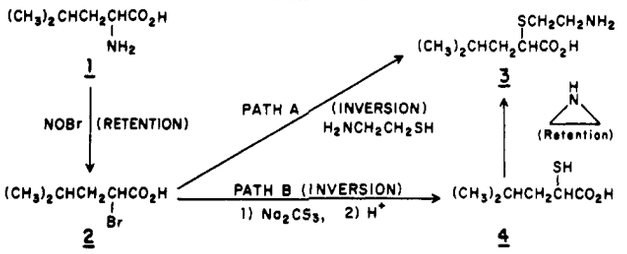
Summary: Stereoselective syntheses of (*R*)- and (*S*)-(*S*-cysteaminyl)-4-methylpentanoic acids have been developed which involve: (a) displacement of bromide in the appropriate enantiomers of 2-bromo-4-methylpentanoic acid by cysteamine; or (b) displacement of bromide of these substrates with thiocarbonate followed by aminoethylation of the resulting 2-mercapto-4-methylpentanoic acids.

Sir: Recently we reported from this laboratory that the dipeptide analogue *S*-2-(*S*-cysteaminyl)-4-methylpentanoic acid (**3**) binds several times more tightly to aminopeptidase M than the natural substrate glycyl-L-leucine.[1] **3** is a member of a class of peptide analogues which contain methylene thioether groups substituted for the peptide bond atoms. Model building as well as enzymatic studies indicate that such analogues may satisfy binding requirements while being resistant to enzymatic hydrolysis.[1] It is, therefore, clear that practical routes to such analogues would permit access to a broad new avenue for study and modulation of biological control. Accordingly, the purpose of this communication[2] is to describe for the first time two complementary synthetic paths which provide a general basis for gram scale preparation of peptide analogues of Gly–X, where X may be a variety of amino acids. The syntheses described are for the instances where X is L- or D-Leu.

Scheme I summarizes the routes. Treatment of D-leucine (**1**) (16.4 g, 125 mmol) with nitrosyl bromide,[3] followed by distillation of the crude product, gave 15.1 g (77.4 mmol) of purified (*R*)-2-bromo-4-methylpentanoic acid (**2**)[4]: bp 97–98 °C (0.25 mm); $[\alpha]_D^{22}$ +38.2 ± 1.8° (*c* 2, methanol) [lit. (*S* isomer),[4a] $[\alpha]_D^{27}$ −34° (methanol)]. The (*R*)-bromo acid (5.3 g, 27 mmol) was dissolved in 530 mL of nitrogen-purged 0.5 M $NaHCO_3$ and a threefold molar excess of 2-mercaptoethylamine hydrochloride (9.2 g, 81 mmol) was added (path A, Scheme I). The reaction vessel was flushed with nitrogen for 1 h and then sealed. After standing 24 h at room temperature, automatic amino acid chromatography revealed that 70% of **2** had been converted to **3**.

The solution was acidified with 6 N HCl and extracted twice

Scheme I



with ether. The ether extracts were discarded. The aqueous portion was neutralized with 2 N NaOH and diluted to 2 L with deionized water. This solution was desalted on a 5.5 × 30 cm column of Dowex 2-X8 resin according to Dréze et al.[5] The fractions from the 1 M acetic acid wash containing **3** were pooled and evaporated to dryness under reduced pressure. The residue was triturated with 20 mL of acetone (discarded) and then crystallized from 47.5% ethanol to give white needles (yield: 2.34 g, 12.1 mmol, 44.5%): mp 205–210 °C dec. For analytical purposes, **3** was subjected to gel filtration on Sephadex G-15 using 0.1 M acetic acid as eluent and was further crystallized: anal ($C_8H_{17}NO_2S$) C, H, N, S; mol wt 191, QM^+ (*m/e*) 192; $[\alpha]_D^{21}$ −23.2 ± 1.2° (*c* 2, H_2O); ^1H NMR (90 MHz, 2 N DCl in D_2O) multiplets centered at δ 0.902 (6 H), 1.622 (3 H), 2.919 (2 H), 3.221 (2 H), 3.475 (1 H) ppm (DSS as standard); TLC R_f 0.48 (1-butanol–acetic acid–H_2O, 12:3:5, silica gel). **3** is resistant to 6 N HCl hydrolysis (24 h, 110 °C, 91% recovery) and elutes near the position of arginine during automatic amino acid chromatography of the single column type[6] (ninhydrin constant, 0.82 times that of leucine).

Alternatively, the same enantiomer of **3** may be prepared in two steps (path B) from the bromo acid **2**. Bromide was displaced with trithiocarbonate and the resulting thioester was decomposed with acid.[7] The product was extracted with ether and the ether extract was dried over sodium sulfate. Removal of the ether at reduced pressure left an oil which was subjected to vacuum distillation to give purified (*S*)-2-mercapto-4-methylpentanoic acid (**4**): $[\alpha]_D^{22}$ −23.8 ± 1.8° (*c* 1.8, ether) [lit[4b] $[\alpha]_D^{20}$ −15.6° (ether)]. Treatment of **4** with ethylene imine[8] gave crude **3**. Purification by Dowex 2-X8 column chromatography and recrystallization from 47.5% ethanol gave a 56% yield of **3** based on **4** or an overall yield from D-leucine of 34%, $[\alpha]_D^{22}$ −16.3° (*c* 1, H_2O). This compound was otherwise indistinguishable in all respects from that prepared by path A.

When L-leucine was substituted for D-leucine in path A the enantiomeric (*R*) form of **3** was obtained: $[\alpha]_D^{22}$ +24.1 ± 1° (*c* 2, H_2O).

The assignment of the *S* configuration to the product (**3**) derived from D-leucine, which exhibits a negative rotation at 589 nm, is supported by the following. (1) Substitution of 2-bromo acids branched at C-4 with nitrogen as the nucleophilic atom produces inversion of configuration.[9] Accordingly, treatment with mercaptoethylamine, where displacement by sulfur predominates over nitrogen, would also be expected to cause inversion as would trithiocarbonate. Aminoethylation of the mercapto acid **4** would not be expected to alter the configuration. The properties of **3** obtained by paths A and B are indistinguishable with the exception of the specific rotation. The greater magnitude of the rotation of **3** obtained from the single step synthesis suggests that this product is of higher optical purity. (2) Brewster's studies[10] suggest that the (*S*)-2-mercapto-3-methylpentanoic acid would have a negative rotation at the sodium D line as would the final product **3**. (3) The conclusion that both paths involve a predominant overall inversion of configuration is consistent with the observed binding patterns of the *S* and *R* analogues with aminopeptidase M. This enzyme is known to cleave only those

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peptides which contain L-amino acid residues.[11] As reported earlier the *S* form of **3** competitively inhibits aminopeptidase M with a K_I of 1.2 mM.[1] More recent studies have shown that the *R* form of **3** also inhibits competitively, but with a K_I of 9.2 mM, reflecting an eightfold preference for the *S* form. The corresponding constants for Gly-L-Leu and Gly-D-Leu were found to be 4.8 and 24 mM, respectively.[12]

When considering the preparation of other Gly–X analogues, it appears that if the displacement of bromide from the 2-bromo acid precursor is facile, then the preferred nucleophile is 2-mercaptoethylamine. However, in some instances the more powerful nucleophile, trithiocarbonate, may be required. The combination of the two synthetic approaches provides the basis for preparation of Gly–X analogues of substantial biochemical interest.

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(2) The term "peptide-gap inhibitor" (alternatively "pseudopeptide") is used to describe compounds whose structures are identical with dipeptides with the exception that the atoms of the peptide linkage (–CONH–) have been replaced by a methylene thioether linkage (–CH_2S–). Thus, any peptide containing this alteration exhibits a "gap" in its peptide backbone at the position of replacement. The support of this research by Grant EY 00969 from the National Eye Institute is gratefully acknowledged. Thanks are due to Dr. Arno F. Spatola of the Department of Chemistry for determination of the NMR spectra reported in this communication.

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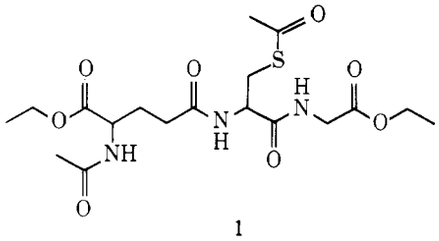
Thiazoles from Cysteinyl Peptides

Summary: Certain thiazoles are obtained via dehydrative cyclization of the corresponding cysteinyl peptides and oxidation of the resulting thiazolines with NiO_2; the biomimetic syntheses of two natural products are reported, as is the potential of NiO_2 as an oxidant for other partially reduced heterocycles.

Sir: Thiazolines and thiazoles are structural components of a number of peptide-derived natural products; among these are the antibiotics siomycin,[1] thiostrepton[2] and micrococcin P,[3] the antitumor antibiotics phleomycin[4] and bleomycin[4] (elaborated by *Streptomyces verticillus*), and Jadot's novel dicarboxylic amino acid (**4**),[5] isolated from the mushroom *Xerocomus subtomentosus*. Several lines of evidence suggest that the biosyntheses of these natural products proceed via the dehydrative cyclization of the corresponding cysteinyl peptides and subsequent oxidation to thiazoles.[6] The facility with which polypeptides may now be assembled makes the biomimetic preparation of peptide-derived thiazoles an attractive synthetic approach; the cysteinyl peptide → thiazoline → thiazole transformation has also been of interest as a possible peptide sequencing tool.[7] However, in spite of the potential utility of such transformations, and the likelihood that biosynthesis proceeds in this fashion, attempted chemical syntheses of all but the simplest thiazoles have failed during dehydrative cyclization[8] or subsequent dehydrogenation.[9] Our interest in the total synthesis of the thiazole-containing antibiotic bleomycin prompted us to reinvestigate the conversion of cysteinyl peptides to their corresponding thiazoles. We report herein the realization of this transformation in a synthetically useful fashion.

Cyclization of glutathione to the corresponding thiazoline was first reported by Calvin,[10] who observed its formation in strong mineral acid by monitoring changes in the ultraviolet spectrum of the reaction mixture. This observation has been verified by others, but it has not been possible to isolate the product.[11] Indeed, Hirotsu et al.[12] reported that their "attempt to secure a pure thiazoline compound by dehydration of *N*-acylglutathione dibenzyl ester in nonaqueous acidic medium ... failed". In spite of the reported experimental difficulties, we observed that the slow addition of anhydrous hydrogen chloride to *N*,*S*-diacetylglutathione diethyl ester (**1**)[13] in a 5% ethanolic chloroform solution over a period of 24 h effected its cyclization to thiazoline **2**. Treatment of the reaction mixture with solid sodium bicarbonate, followed by filtration, concentration of the filtrate, and trituration of the residue with benzene afforded the thiazoline as a white solid in 70% yield. The proton NMR spectrum of thiazoline **2** included signals characteristic[14] of Δ^2-thiazolines at δ 3.61 (d, 2, J = 9.5 Hz) and 5.07 (t, 1, J = 9.5 Hz) and the UV spectrum had the expected[12] λ_{max} (1:1 C_2H_5OH–HCl) 267 nm (ϵ 5400); $[\alpha]^{25}_D$ +40° (*c* 2.0, $CHCl_3$). Dehydrative cyclization of several cysteinyl peptides not requiring prior ethanolysis of S-protecting groups has been accomplished conveniently in chloroform solution;[6,15] the choice of protecting groups was important in such cases, since better yields were generally obtained when the desired thiazolinium chlorides were insoluble in the reaction medium.



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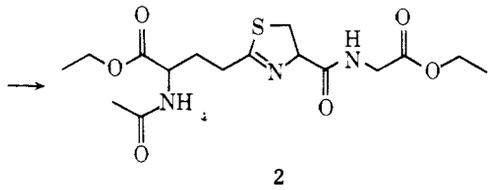

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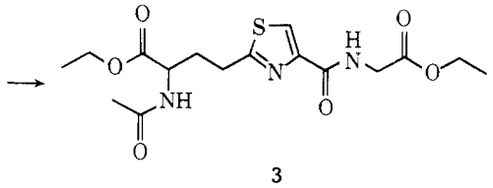

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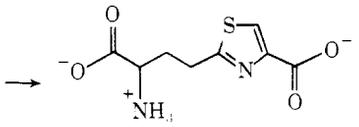

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