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Cysteine Derivatives with Reactive Groups as Potential Antitumor Agents

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Cysteine derivatives having diazo ketone and chloro ketone functions have been prepared. In order to effect adequate protection for modifying the carboxyl group, cysteine was converted to a thiazolidine derivative I, which was then converted to the *N*-acetyl derivative VII. The active ester method or activation with DCC yielded the diazo ketone derivative VIII. Similar treatment of the parent compound I with DCC led to a self-condensation reaction giving a diketopiperazine VI. The diazo ketone derivative VIII has been used in preparing α-chloro ketone derivative X and a homologue of cysteine. Deblocking N-acetylated thiazolidine derivatives with various reagents did not proceed satisfactorily. Interaction of the N-acetylated blocked ester XII with trifluoroacetic acid opened the thiazolidine ring to give the N-acetylated blocked diester XIII and other products. The chloro ketone derivative X was found to have a moderate inhibitory activity against mouse mammary adenocarcinoma in cell culture. *S*-(1-Adamantyl)-L-cysteine was prepared and was found to be inactive.

Diazo ketone or diazo ester analogues of amino acids include well-known antibiotics, such as DON (6-diazo-5-oxo-L-norleucine) and azaserine, which have been found to possess antitumor activity. Azaserine was shown to inhibit specifically a glutamine transferase involved in purine biosynthesis and was considered by Baker to be the first example of a classical-type antimetabolite causing specific active-site-directed irreversible inhibition.[1] One of the effects that have been shown for DON is inhibition of the biosynthesis of D-glucosamine by interfering with formation of the latter compound from fructose 6-phosphate. In this step, glutamine is the amino-group donor, and thus DON is considered to be a glutamine antagonist.[2] Analogues of other amino acids having similar groups may have interesting biological properties, particularly with respect to their possible antiproliferative action. For instance, it has been found that certain cancer cells have an absolute requirement for cysteine.[3] A derivative of cysteine, *S*-trityl-L-cysteine, was found to have antileukemic activity.[4,5] These findings gave impetus for our studies of cysteine analogues, although other nonessential amino acids, such as L-serine, were also found to be required for growth of neoplastic cells.[6-9]

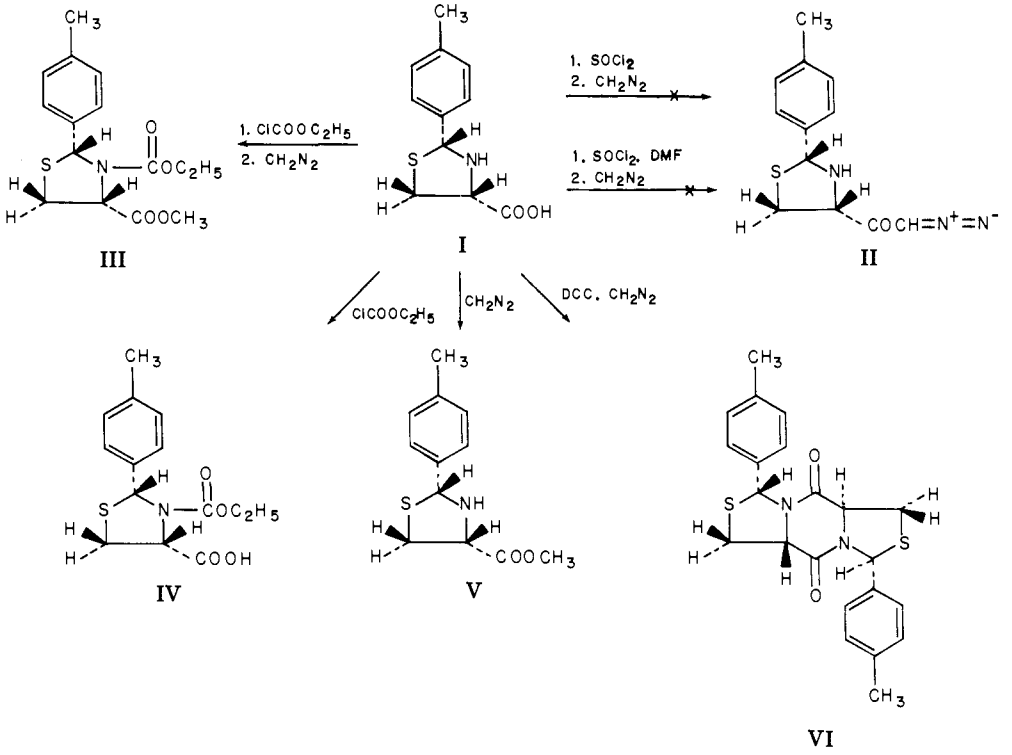
The main purpose of this study was to synthetize diazo ketone derivatives of cysteine. We also considered them as potential intermediates for the synthesis of homologues and halo ketones related to this amino acid.

We considered both the blocked and the deblocked compounds of potential biological interest, particularly since some highly substituted cysteine derivatives have been shown to be active,[4,5] and since partial or complete deblocking could be accomplished in vivo. Aldehydes are known to block the vicinal SH and NH_2 functions of cysteine, and being readily prepared by condensing cysteine with appropriate aldehydes to form 2-substituted thiazolidine-4-carboxylic acids, they appeared attractive for this purpose. Thus 2-phenylthiazolidine-4-carboxylic acid, 2-(*p*-nitrophenyl)thiazolidine-4-carboxylic acid, 2-(*p*-anisyl)thiazolidine-4-carboxylic acid, and 2-(*p*-tolyl)-thiazolidine-4-carboxylic acid were synthesized from L-cysteine by using the appropriate aromatic aldehydes. Initial study indicated that these compounds can be cleaved by methanolic hydrazine solution, giving substituted phenylhydrazones and cystine. The formation of cystine can be avoided by adding β-mercaptoethanol to the reaction mixture, and cysteine is recovered.

On the basis of its stability and the results of cleavage studies, we selected 2-(*p*-tolyl)-(*R*)-thiazolidine-4-carboxylic acid (I, Scheme I) for further modification. Initially, we attempted to prepare the diazo ketone II from I via the acid chloride. Nevertheless, even using a mild method (DMF and $SOCl_2$),[10] the acid chloride synthesis was not successful. King and his co-workers[11] have also been unsuccessful in making the acid chloride from thiazolidine-4-carboxylic acid by the conventional method. Next the mixed anhydride procedure was tried with ethyl chloroformate and diazomethane.[12] This resulted in preferential N-acylation, giving III. When the diazomethane was omitted from the reaction mixture, *N*-(ethoxycarbonyl)-2-(*p*-tolyl)-(*R*)-thiazolidine-4-carboxylic acid (IV) was obtained. Treatment of I with ethereal diazomethane gave the expected methyl ester V. Application of the dicyclohexylcarbodiimide method for the synthesis of diazo ketones[13] yielded the biomolecular cyclic amide VI as the only identifiable product. Treatment of I with DCC alone also gave VI along with unidentified products.

In order to avoid complications, the NH group in I had to be blocked by acetylation of I to VII (Scheme II). The configuration of C-2 in this key intermediate has been

Scheme I



determined by x-ray crystallography as being cis to the 4-carboxyl group, and the compound was found to exist as two stable conformers, as determined by both NMR spectroscopy and x-ray crystallography.[14] Once the NH group was acetylated, preparation of the diazo ketone derivative VIII, either by reaction with DCC or by the mixed anhydride method, was readily achieved. An analogous method was successfully applied to *N*-acetyl-(*R*)-thiazolidine-4-carboxylic acid to give the diazo ketone IX.

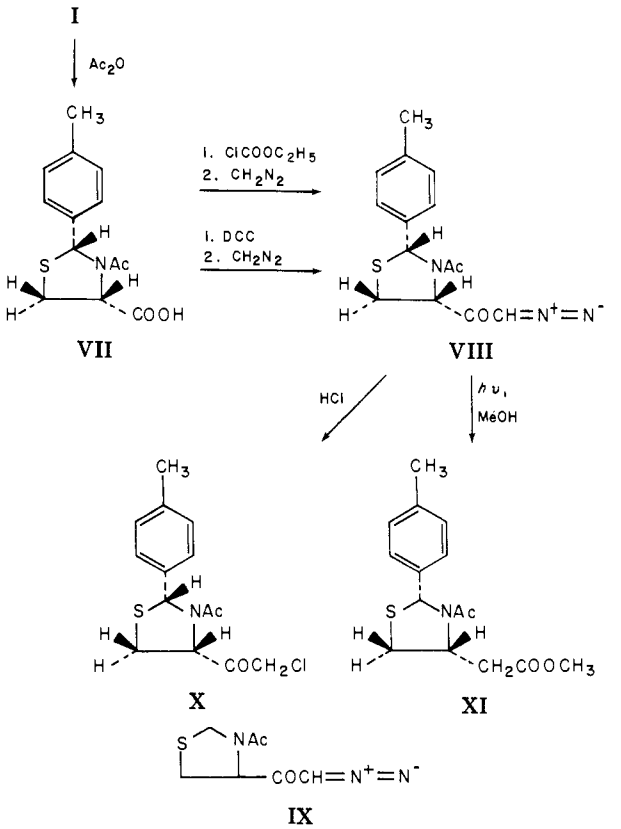
Whereas simple thiazolidine derivatives, such as I, can be deblocked with hydrazine, all attempts to deblock VII and VIII, such as treatment with methanolic hydrazine, $Et_4N^+OH^-$, or Girard's reagent T, were unsuccessful, indicating that N-acetylation stabilizes the ring structure. To overcome the deblocking problems, we have replaced the *N*-acetyl group in VII with the more readily hydrolyzable trifluoroacetyl group. The new intermediate, however, failed to give the diazo ketone by any of the methods already described. Since only intractable mixtures could be obtained, we conclude that the trifluoroacetyl group does not offer adequate protection.

Treatment of the diazo ketone VIII with ethereal HCl gave the expected chloro ketone X. Acid hydrolysis of the latter did not yield the expected deblocked compound.

Application of the Wolff rearrangement to VIII, using silver oxide in methanol, gave a mixture of compounds and not the expected higher homologous ester. Recently it has been reported that silver forms a complex with the sulfur of the thiazolidine ring, and formation of this complex may result in opening the ring, thus complicating the reaction.[15] Photochemical rearrangement of VIII, however, gave the expected higher homologous ester, methyl *N*-acetyl-2-(*p*-tolyl)-(*R*)-thiazolidine-4-acetate (XI), as an oil.

We have also investigated hydrolysis of the thiazolidine derivative XII with trifluoroacetic acid, which was found to be an excellent deblocking agent, particularly for benzyl groups.[16] This investigation was partly stimulated by the reports made by Goodman and his co-workers,[17] who observed changes in proton resonance on the α, β, and γ

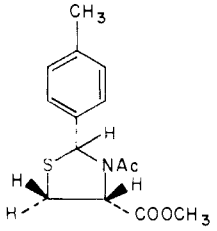
Scheme II



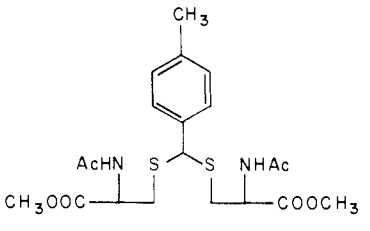
carbons in methyl (*R*)-thiazolidene-4-carboxylate on changing the solvent from $CDCl_3$ to CF_3COOH. These changes were thought to be caused by conformational effects, but the possibility of a permanent chemical change was not considered.[17] When we dissolved XII in trifluoroacetic acid, evaporated the solvent, and redetermined the NMR spectrum of XII in $CDCl_3$, the spectrum was different from that of the original sample. It was obvious

that a permanent chemical change had occurred. TLC (silica gel) of the solution shows three uv-absorbing spots, which were separated by column chromatography.

The first fraction, corresponding to the high R_f spot on TLC, has been identified as being due to *p*-toluic acid, probably formed from *p*-tolualdehyde by aerial oxidation. This assumption has been confirmed by the isolation of *p*-tolualdehyde 2,4-dinitrophenylhydrazone on adding 2,4-dinitrophenylhydrazine to the trifluoroacetic acid solution of XII.



XII



XIII

The fraction corresponding to the middle spot was found to be a mixture of the starting material and an unidentified compound. The latter could not be isolated by either column or gas chromatography. The material corresponding to the lower spot could be isolated, and the structure was shown to be that of XIII by its NMR spectrum and elemental analysis. The structure of the compound was confirmed by an alternative preparation in which *N*-acetylcysteine was allowed to react with *p*-tolualdehyde in methanolic HCl. Thus traces of water in trifluoroacetic acid are capable of hydrolyzing the tolyl group, but the reaction is complicated by recombination of the *N*-acetylcysteine derivative with the hydrolysate to give XIII, as well as an unidentified by-product.

Although we have not been able to develop a deblocking procedure for the N-acetylated thiazolidine derivatives, the possibility cannot be excluded that deblocking could occur in vivo in the cell or that the fully substituted amino acid derivatives could exhibit biological activity per se. This possibility led us to synthesize DL-penicillamine analogues of I and VII to increase further the hydrophobic nature of cysteine derivatives.

Compounds synthesized in this study, as well as *S*-trityl-L-cysteine, were tested as inhibitors of the growth of mouse mammary adenocarcinoma (TA3) cells grown in cell culture. *N*-Acetyl-2-(*p*-tolyl)-4-(α-chloroacetyl)-(*R*)-thiazolidine (X) was the most active inhibitor in the series, with an ID_{50} of 3.2×10^{-5} M. The unsubstituted thiazolidine derivative I was found to promote growth at 10^{-4} M, and its N-acetylation to VII or N-trifluoroacetylation abolished all growth-promoting activity at the same concentration. Compounds VII and VIII, and DL-penicillamine analogues of I and VII, were not active at 10^{-4} M.

In contrast, *S*-tritylcysteine inhibited TA3 cells at an ID_{50} of 6.3×10^{-7} M. In order to test whether the activity of this compound is due to the hydrophobic character of the trityl group, *S*-(1-adamantyl)-L-cysteine was prepared. The synthesis proceeded satisfactorily by condensing 1-bromoadamantane with L-cysteine or by condensing 1-adamantanol with L-cysteine in the presence of BF_3 etherate. The relative insolubility of the compound precluded its being tested at a higher concentration than 3×10^{-5} M, at which concentration it was found to be inactive.

Experimental Section

Melting points (uncorrected) were determined by the capillary method. Ultraviolet spectra were determined with a Cary Model 14 spectrophotometer. Infrared spectra were determined with a Perkin-Elmer 457 spectrophotometer, and NMR spectra were obtained with a Varian A-60A instrument; positions of peaks are expressed in δ (ppm) from Me_4Si or from sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) or dioxane. Optical rotation was measured with a Perkin-Elmer 141 polarimeter. Thin-layer chromatograms were run on Merck HF-254 silica gel plates, and spots on chromatograms were detected by their ultraviolet absorption or by spraying with ninhydrin reagent.

The mouse mammary adenocarcinoma cells (TA3) were grown in stationary tube culture in RPMI medium 1640 containing 10% horse serum. An inoculum of 50 000 cells in 1 ml of medium was supplemented with 1 ml of medium containing the compound to be tested. The tubes were incubated in an upright position for 3 days, and growth was estimated by protein assay. Growth in controls varied from six- to tenfold. Every concentration was tested in five tubes each. For compounds found inhibitory, the tests were repeated at least twice. Variation between different tests was within ±10% for the 50% inhibitory concentration.

2-(*p*-Tolyl)-(*R*)-thiazolidine-4-carboxylic Acid (I). A mixture of cysteine hydrochloride monohydrate (5 g, 28.47 mmol) and potassium acetate (3 g, 30.61 mmol) was dissolved in aqueous alcohol (40%, 140 ml). To the clear solution, *p*-tolualdehyde (4.5 ml, 37.5 mmol) was added drop by drop, with stirring. After stirring for 10 min, the solution became cloudy, and a white precipitate started to separate out. The reaction mixture was stirred at room temperature for 48 h and was then filtered. The precipitate was washed with ether and dried: yield 5.66 g (88.9%). The compound was crystallized from methanol: mp 169–170° dec; NMR (Me_2SO-d_6) δ 1.90 (s, CH_3 aromatic), 2.82 (m, $5CH_2$), 3.90 (m, 4CH), 5.13, 5.32 (s, 2CH), 6.92 (m, aromatic protons), 6.18 (b, NH); ir ν_{max}^{KBr} 2740, 2620, 2470 ($^+NH_3$), 1562 cm^{-1} (C=O). Anal. ($C_{11}H_{13}NO_2S$) C, H, N.

Attempted Synthesis of 2-(*p*-Tolyl)-4-diazoacetyl-(*R*)-thiazolidine (II). Formation of Methyl *N*-Ethoxycarbonyl-2-(*p*-tolyl)-(*R*)-thiazolidine-4-carboxylate (III). Triethylamine (1.16 ml, 11.6 mmol) was added slowly, with stirring, to an ice-cold suspension of 2-(*p*-tolyl)-(*R*)-thiazolidine-4-carboxylic acid (1 g, 4.5 mmol) in dry THF (30 ml), and a clear solution was obtained. The solution was evaporated in vacuo, and the residue was dissolved in dry THF (20 ml). The resulting solution was cooled in an ice–salt mixture at −10°, and ethyl chloroformate (0.5 ml, 5.3 mmol) was added drop by drop, with stirring. After a white precipitate separated out, stirring was continued for 20 min. The precipitate (triethylamine hydrochloride) was removed by filtration, and the filtrate was added to an ethereal solution of diazomethane (0.6 g, 14.3 mmol, from 4.3 g of Diazald). The reaction mixture was kept at 0° for 3 h and then at room temperature overnight. The excess of diazomethane was removed by passing N_2 through. The product was distilled with a Kugelrohr apparatus: bp 145–150° (bath temperature) (0.05 mm); yield 1.1 g (79%); ir ν_{max}^{KBr} 1748, 1650 cm^{-1} (C=O); NMR ($CDCl_3$) δ 1.13 (t, CH_2CH_3, J = 7 Hz), 2.37 (s, aromatic CH_3), 3.33 (d, $5CH_2$, J = 7 Hz), 3.87 (s, ester CH_3), 4.95 (t, 4CH, J = 7 Hz), 6.22 (s, 2CH), 7.42 (q, aromatic H's, J = 7 Hz). Anal. ($C_{15}H_{19}NO_4S$) C, H, N.

Attempted Synthesis of 2-(*p*-Tolyl)-4-diazoacetyl-(*R*)-thiazolidine (II). Formation of 2-(*p*-Tolyl)-(*R*)-thiazolidine-4-carboxylic Acid Biomolecular Cyclic Amide (VI). 2-(*p*-Tolyl)-(*R*)-thiazolidine-4-carboxylic acid (1 g, 4.5 mmol) was added slowly to a solution of dicyclohexylcarbodiimide (1 g, 4.0 mmol) in dry THF (15 ml, dried over $LiAlH_4$ and freshly distilled). The suspension was stirred at room temperature for 4 h and then was added slowly, with stirring, to an ethereal solution of diazomethane (2.4 g, 57.4 mmol, from 17.2 g of Diazald). The yellowish diazomethane solution was left at 0° for 2 h and then at room temperature overnight. The excess of diazomethane was removed by passing N_2 through at 40°. The colorless residue was taken up in ether, when a white precipitate (dicyclohexylurea) separated out; this was removed by filtration. On concentration of the filtrate, more dicyclohexylurea separated out. The oily residue, obtained after complete removal of dicyclohexylurea, was dissolved in a minimum amount of ether. When the solution was kept in a refrigerator, white crystalline material separated out. The product was filtered, washed with cold ether, and dried: yield 0.63 g (68%); mp 164–165°; ir ν_{max}^{KBr} 1675, 1660, 1625 cm^{-1} (C=O); NMR ($CDCl_3$) δ 2.33 (s, aromatic CH_3), 3.49 (d, $5CH_2$,

J = 7 Hz), 4.70 (t, 4CH, J = 7 Hz), 6.52 (s, 2CH), 7.33 (z, aromatic H's, J = 8 Hz); $[\alpha]^{26}D$ −276.2° (MeOH, c 0.29). Anal. ($C_{22}H_{22}N_2S_2O_2$) C, H, N.

Methyl 2-(*p*-Tolyl)-(*R*)-thiazolidine-4-carboxylate (V). 2-(*p*-Tolyl)-(*R*)-thiazolidine-4-carboxylic acid (1 g, 4.5 mmol) was dissolved in dry DMF (10 ml) and was added slowly to an ethereal solution of diazomethane (1.2 g, 28.6 mmol, from 8.6 g of Diazald), cooled in ice. The resulting solution was kept at room temperature overnight, and excess diazomethane was removed by passing N_2 through at 40° and then evaporating the reaction mixture completely in vacuo. The mass spectrum of the oil showed a molecular ion peak at 237: NMR ($CDCl_3$) δ 2.35 (s, aromatic CH_3), 3.24 (m, $5CH_2$), 3.78 (s, ester CH_3), 4.60 (m, 4CH), 5.42, 5.57, 5.82 (s, 2CH), 7.36 (m, aromatic H's). At least two different conformers are present in the $CDCl_3$ solution.

***N*-Ethoxycarbonyl-2-(*p*-tolyl)-(*R*)-thiazolidine-4-carboxylic Acid (IV).** The triethylamine salt of 2-(*p*-tolyl)-(*R*)-thiazolidine-4-carboxylic acid (1 g, 4.5 mmol), prepared as previously described, was dissolved in dry THF (20 ml). The solution was cooled in an ice–salt mixture to −10°, and ethyl chloroformate (1 ml, 10.6 mmol) was added until the solution became turbid. A white precipitate (triethylamine hydrochloride) separated out. The mixture was stirred for 15 min and then the precipitate was removed by filtration. The filtrate was evaporated in vacuo, and an oily gum was obtained. The NMR spectral pattern indicates the expected compound: NMR ($CDCl_3$) δ 1.12 (t, CH_2CH_3, J = 7 Hz), 4.96 (t, 4CH, J = 7 Hz), 6.18 (s, 2CH), 7.38 (q, aromatic H's, J = 8 Hz).

***N*-Acetyl-2-(*p*-tolyl)-(*R*)-thiazolidine-4-carboxylic Acid (VII).** Acetic anhydride (6 ml) was added slowly, with stirring, to a suspension of 2-(*p*-tolyl)-(*R*)-thiazolidine-4-carboxylic acid (2.4 g, 10.76 mmol) in water (6 ml), and the suspension became gummy. This reaction mixture was heated on a steam bath for 1 h and a clear solution was obtained. The solution was cooled in ice and was then evaporated in vacuo. Water (25 ml) was added to the residue. On thorough mixing, a white precipitate separated out. The mixture was cooled in ice and filtered, and the precipitate was washed with ice-cold water, dried, and weighed: yield 2.58 g (90.2%). The product was crystallized from alcohol: mp 177–178°; NMR ($CDCl_3$) δ 2.03 (s, CH_3 acetyl), 2.35 (s, CH_3 aromatic), 3.36 (d, $5CH_2$, J = 7 Hz), 5.12 (t, 4CH, J = 7 Hz), 7.40 (q, aromatic protons, J = 8 Hz), 11.55 (s, COOH); ir ν_{max}^{KBr} 1725 (C=O carboxylic acid), 1615 cm^{-1} (C=O amide); $[\alpha]^{24}D$ +76.6° ($CHCl_3$, c 1.03); $[\alpha]^{22}D$ +134° (MeOH, c 1.04), uv λ_{max}^{MeOH} 258 nm (ϵ 1.4 × 10^{-3}). Anal. ($C_{13}H_{15}NO_3S$) C, H, N.

Methyl *N*-Acetyl-2-(*p*-tolyl)-(*R*)-thiazolidine-4-carboxylate (XII). A solution of *N*-acetyl-2-(*p*-tolyl)-(*R*)-thiazolidine-4-carboxylic acid (4 g, 15.09 mmol) in anhydrous dimethylformamide (10 ml, dried over CaH_2 and distilled) was added slowly to an ice-cold ethereal solution of diazomethane (prepared from 21.5 g of Diazald). The resulting solution was left at 0° for 1 h and then at room temperature overnight. The excess of diazomethane was removed by passing N_2 through the solution. The residual liquid was evaporated in vacuo, and the residue was crystallized from ether: yield 3.9 g (92.6%); mp 89–90°; NMR ($CDCl_3$) δ 1.97 (s, CH_3 acetyl), 2.38 (s, CH_3 aromatic), 3.88 (s, CH_3 ester), 3.29 (d, $5CH_2$, J = 7 Hz), 5.05 (t, 4CH, J = 7 Hz), 6.13 (s, 2CH), 7.48 (q, aromatic protons, J = 8 Hz); ir ν_{max}^{KBr} 1650 (C=O amide), 1748 cm^{-1} (C=O ester); $[\alpha]^{24}D$ +98.2° ($CHCl_3$, c 1.06). Anal. ($C_{14}H_{17}NO_3S$) C, H, N.

***N*-Trifluoroacetyl-2-(*p*-tolyl)-(*R*)-thiazolidine-4-carboxylic Acid.** Trifluoroacetic anhydride (2 ml, 22.4 mmol) was added drop by drop, with stirring, to a suspension of 2-(*p*-tolyl)-(*R*)-thiazolidine-4-carboxylic acid (1.1 g, 4.9 mmol) in a 1:2 mixture of ethyl acetate and chloroform (60 ml), which was cooled in ice. The reaction mixture was stirred at 0° for 1 h and a clear solution was obtained. The solution was washed with ice-cold water (20 ml × 2) and dried over Drierite. The drying agent was removed by filtration, and the filtrate was evaporated in vacuo. The oily gum that was obtained was crystallized from a mixture of ethyl acetate and petroleum ether: yield 1.2 g (76%); mp 171–172°; ir ν_{max}^{KBr} 1743, 1675 (C=O), 1185, 1154 cm^{-1} (CF_3); NMR ($CDCl_3$) δ 2.37 (s, aromatic CH_3), 3.42–3.58 (b, $5CH_2$), 5.40 (b, 4CH), 6.52 (b, 2CH), 7.22 (aromatic H's), 11.37 (4COOH); $[\alpha]^{22}D$ −248° (MeOH, c 1.03). Anal. ($C_{13}H_{12}NO_3F_3S$) C, H, N.

***N*-Acetyl-2-(*p*-tolyl)-4-diazoacetyl-(*R*)-thiazolidine (VIII).**

A. Mixed Carboxylic–Carbonic Anhydride Method. Triethylamine (0.26 ml, 2.6 mmol) was added drop by drop, with stirring, to a solution of *N*-acetyl-2-(*p*-tolyl)-(*R*)-thiazolidine-4-carboxylic acid (0.5 g, 1.9 mmol) in dry THF (10 ml) cooled in a mixture of dry ice and acetone to −10°. The reaction mixture was stirred for 10 min. Ethyl chloroformate (0.19 ml, 2.0 mmol) was added drop by drop, and a white precipitate (triethylamine hydrochloride) separated out. The reaction mixture was stirred at −10° for 10 min, the precipitate was removed by filtration, and the filtrate was added to an ethereal solution of diazomethane (1.20 g, 28.6 mmol, prepared form 8.6 g of Diazald) cooled in ice. The solution was left at 0° for 3 h and then at room temperature overnight. Excess diazomethane was removed by passing N_2 through, and the residue was dried in vacuo. The residue was dissolved in ether, when a gummy reddish material separated out. The ether solution was decanted and was evaporated in vacuo. The yellowish crystalline material that separated out on evaporation was filtered off, washed with ether, and dried: yield 0.39 g (71%); mp 131–132°; ir ν_{max}^{KBr} 2122 (diazo), 1630 cm^{-1} (C=O); NMR ($CDCl_3$) δ 2.02 (s, *N*-acetyl CH_3), 2.38 (s, aromatic CH_3), 3.39 (d, $5CH_2$, J = 7 Hz), 5.72 (s, CH diazoacetyl), 6.12 (s, 2CH), 7.45 (2, aromatic H's, J = 8 Hz). Anal. ($C_{14}H_{15}N_3O_2S$) C, H, N.

B. DCC Method. *N*-Acetyl-2-(*p*-tolyl)-(*R*)-thiazolidine-4-carboxylic acid (0.5 g, 1.9 mmol) was added, with stirring, to a solution of dicyclohexylcarbodiimide (0.39, 1.9 mmol) in dry THF (10 ml). The reaction mixture was stirred at room temperature for 4 h and then was added slowly, with stirring, to an ethereal solution of diazomethane (1.20 g, 28.6 mmol, prepared from 8.6 g of Diazald), cooled in ice. The yellowish diazomethane solution was left at 0° for 2 h and then at room temperature overnight. The excess of diazomethane was removed by passing a stream of N_2 through, and the residue was dried in vacuo. The residue was taken up in ether (35 ml), and the dicyclohexylurea that precipitated out was removed by filtration. The filtrate was washed with water, dried ($MgSO_4$), and evaporated in vacuo. The residue, on TLC, showed a major spot and a few minor spots. The fraction corresponding to the major spot was isolated by preparative TLC (1:1 benzene–ether) and crystallized from ether: yield 0.26 g (48%); mp 131–132°. The product is identical with that obtained by method A on the basis of mixture melting point and ir and NMR spectra.

***N*-Acetyl-2-(*p*-tolyl)-4-(α-chloroacetyl)-(*R*)-thiazolidine (X).** Ethereal HCl was added drop by drop, with stirring, to a solution of *N*-acetyl-2-(*p*-tolyl)-4-diazoacetyl-(*R*)-thiazolidine (200 mg, 0.69 mmol) in dry THF (5 ml), cooled in ice. The yellow diazo ketone solution lost its color and became acidic. The colorless solution was evaporated in vacuo. On addition of cold ether (5 ml), a crystalline material separated out. This was filtered, washed with petroleum ether, and dried. The compound gave a positive Baker's test:[18] yield 0.18 g (87%); mp 109–110°; ir ν_{max}^{KBr} 1738, 1640 cm^{-1} (C=O); NMR ($CDCl_3$) δ 1.95 (s, acetyl CH_3), 2.38 (s, aromatic CH_3), 3.27 (d, $5CH_2$, J = 8 Hz), 4.43, 4.50 (s, α-CH_2), 5.12 (t, 4CH, J = 8 Hz), 6.10 (s, 2CH), 7.57 (q, aromatic H's, J = 9 Hz). Anal. ($C_{14}H_{15}NO_2SCl$) C, H, N.

Photolysis of *N*-Acetyl-2-(*p*-tolyl)-4-(α-diazoacetyl)-(*R*)-thiazolidine (VIII). Formation of Methyl *N*-Acetyl-2-(*p*-tolyl)-(*R*)-thiazolidine-4-acetate (XI). N_2 was passed through a solution of *N*-acetyl-2-(*p*-tolyl)-4-(α-diazoacetyl)-(*R*)-thiazolidine (0.2 g, 0.69 mmol) in dry methanol (100 ml) for 1 h. Then the solution, water cooled, was irradiated under N_2 with a uv light (a 100-W Hanovia high-pressure mercury lamp in an immersion well equipped with a Pyrex filter) for 24 h. TLC of the reaction (1:1 benzene–ether) showed one major spot and five minor spots, including the starting material. The fraction corresponding to the major spot was isolated as an oil by column chromatography (24 × 0.75 in., Mallinckrodt silicAR, CC7, 200–325 mesh, 1:1 benzene–ether as eluent) and was identified as methyl *N*-acetyl-2-(*p*-tolyl)thiazolidine-4-acetate: NMR ($CDCl_3$) δ 1.90 (s, *N*-acetyl CH_3), 2.35 (s, aromatic CH_3), 3.11 (m, $5CH_2$, α^4-CH_2), 3.68 (s, ester CH_3), 4.97 (m, 4CH), 6.02 (s, 2CH), 7.25 (aromatic H's).

***N*-Acetyl-(*R*)-thiazolidine-4-carboxylic acid** was prepared by treating (*R*)-thiazolidine-4-carboxylic acid (10 g, 75.2 mmol) with acetic anhydride (30 ml, 319.7 mmol) in water (30 ml) according to the procedure described for the preparation of

N-acetyl-2-(*p*-tolyl)-thiazolidine-4-carboxylic acid (VII): yield 12.4 g (94%); mp 145–146° (lit. 145° dec,[19] 143.5–144.5°[20]); NMR (Me_2SO) δ 2.03, 2.10 (s, acetyl CH_3), 3.28 (m, $5CH_2$), 4.53 (m, $2CH_2$), 5.06 (m, 4CH). In Me_2SO-d_6 the existence of two conformers is apparent: ir ν_{max}^{KBr} 3400, 2870, 2500 (carboxylic OH), 1720 (C=O, acid), 1590 (C=O, amide).

***N*-Trifluoroacetyl-(*R*)-thiazolidine-4-carboxylic acid** was prepared by treating (*R*)-thiazolidine-4-carboxylic acid (2 g, 15 mmol) with trifluoroacetic anhydride (4 ml, 28.4 mmol) in a 1:2 mixture of ethyl acetate and chloroform (90 ml), as described for the preparation of *N*-trifluoroacetyl-2-(*p*-tolyl)-(*R*)-thiazolidine-4-carboxylic acid. It was crystallized from a mixture of petroleum ether and ether: yield 2.8 g (81%); mp 60–61°; ir ν_{max}^{KBr} 1736, 1676 (C=O), 1205, 1152 cm^{-1} (CF_3); NMR ($CDCl_3$) δ 3.38 (d, $5CH_2$, J = 7 Hz), 4.83 (s, $2CH_2$), 5.18 (t, 4CH, J = 7 Hz), 11.58 (s, COOH). The presence of a small amount of another conformer is apparent from the NMR spectrum. Anal. ($C_6H_6NO_3SF_3$) C, H, N.

***N*-Acetyl-4-diazoacetyl-(*R*)-thiazolidine (IX).** Triethylamine (1.6 ml, 11.6 mmol) was added slowly with stirring to a solution of *N*-acetyl-(*R*)-thiazolidine-4-carboxylic acid (2 g, 15 mmol) in dry THF (40 ml), cooled in a mixture of dry ice and acetone to −10°. The reaction mixture was stirred for 1.2 h while ethyl chloroformate (1.2 ml, 12.6 mmol) was added drop by drop. A white precipitate of triethylamine hydrochloride separated out, and stirring was continued for 1 h. Then the white precipitate was removed by filtration, and the filtrate was added, with stirring, to an ice-cold ethereal solution of diazomethane (2.4 g, 57.2 mmol, prepared from 17.2 g of Diazald). The reaction mixture was kept at 0° for 3 h and then at room temperature overnight and was finally worked up as in the case of *N*-acetyl-2-(*p*-tolyl)-4-diazoacetyl-(*R*)-thiazolidine. The yellowish gummy residue, on TLC (EtAc), showed one major spot and a few minor spots. The fraction corresponding to the major spot was isolated by preparative TLC and was crystallized from a mixture of ether and petroleum ether: yield 1.2 g (53%); mp 70–71°; ir ν_{max}^{KBr} 2106 (diazo), 1628 cm^{-1} (C=O); NMR ($CDCl_3$) δ 2.20 (acetyl CH_3), 3.34 ($5CH_2$), 4.68 ($2CH_2$), 5.09 (4CH), 5.73 (α-CH). The presence of at least two conformers is apparent. Anal. ($C_7H_9N_3O_2S$) C, H, N.

Methyl *N*-Acetylthiazolidine-4-carboxylate. A solution of *N*-acetylthiazolidine-4-carboxylic acid (6 g, 34.3 mmol) in methanol (25 ml) was added slowly to an ethereal solution of diazomethane (4.2 g, 100 mmol, from 30 g of Diazald), and the reaction mixture was worked up as in the preparation of methyl *N*-acetyl-2-(*p*-tolyl)-(*R*)-thiazolidine-4-carboxylate. The product was a liquid: bp 65–70° (bath temperature, 0.1 mm, with Aldrich Kugelrohr apparatus); yield 0.58 g (90%); ir ν_{max}^{neat} 1742 (C=O ester), 1655 cm^{-1} (C=O amide); NMR ($CDCl_3$) δ 2.17 (acetyl CH_3), 3.36 ($5CH_2$), 3.85 (ester CH_3), 4.70 ($2CH_2$), 5.17 (4CH). The presence of more than one conformer is apparent from the NMR spectra. Anal. ($C_7H_{11}NO_3S$) C, H, N.

5,5-Dimethyl-2-(*p*-tolyl)thiazolidine-4-carboxylic Acid. *p*-Tolualdehyde (1 ml, 8.5 mmol) was added slowly, with stirring, to a solution of DL-penicillamine (1 g, 6.7 mmol) in aqueous alcohol (30 ml, 40%), and stirring was continued for 18 h. The resulting thick white precipitate was filtered, washed with cold alcohol followed by ether, and crystallized from an alcohol–ether mixture: yield 1.38 g (82%); mp 114–115°; ir ν_{max}^{KBr} 3460 (NH), 1694, 1618, 1580 cm^{-1} (C=O); NMR ($CDCl_3$) δ 1.38, 1.68 (s, $5CH_3$), 2.35 (s, aromatic CH_3), 3.89, 4.08 (s, 4CH), 5.73, 5.87 (s, 2CH), 7.35 (q, aromatic H's, J = 8 Hz), 8.95 (exchangeable H). The presence of two distinct molecular species is apparent from the NMR spectral pattern. Anal. ($C_{13}H_{17}NO_2S \cdot 0.5H_2O$) C, H, N.

***N*-Acetyl-2-(*p*-tolyl)-5,5-dimethylthiazolidine-4-carboxylic Acid.** Acetic anhydride (5 ml, 53.4 mmol) was added slowly to a suspension of 5,5-dimethyl-2-(*p*-tolyl)-thiazolidine-4-carboxylic acid (1.45 g, 5.78 mmol) in water (5 ml) and heated on a steam bath for 1.5 h. The reaction mixture was evaporated in vacuo, and water (10 ml) was added and evaporated. The crystalline residue was taken up in water, and the resulting suspension was filtered. The solid material was washed with cold water and dried: yield 1.36 g (80%). The product was crystallized from alcohol: mp 208–209°; ir ν_{max}^{KBr} 1730 cm^{-1} (C=O amide); NMR ($CDCl_3$) δ 1.41, 1.65 (s, $5CH_3$), 1.93 (s, *N*-acetyl CH_3), 2.35 (s, aromatic CH_3), 4.80 (s, 4CH), 6.18 (s, 2CH), 7.47 (q, aromatic H's, J = 8 Hz), 10.20 (s, COOH). Anal. ($C_{15}H_{19}NO_3S$) C, H, N.

Reaction of Methyl *N*-Acetyl-2-(*p*-tolyl)-(*R*)-thiazolidine-4-carboxylate (XII) with Trifluoroacetic Acid. Trifluoroacetic acid (5 ml) was added to methyl *N*-acetyl-2-(*p*-tolyl)thiazolidine-4-carboxylate (1 g), and the mixture was shaken, giving a clear solution. The solution was evaporated in vacuo at room temperature and the residue was dissolved in ether (50 ml). The ether solution was washed with water and then was evaporated. An oil was obtained. On TLC (1:1 benzene–ether), the oil showed three spots (R_f 0.03, 0.33, 0.87). It was chromatographed on a silica gel column (40 × 60 cm, 30 g of Mallinckrodt SilicAR CC-7, 200–325 mesh), with 1:1 benzene–ether as eluent. The fraction corresponding to R_f 0.87 was isolated and was identified as *p*-toluic acid. The fraction corresponding to R_f 0.33 was isolated as an oil, from which starting material could be isolated by crystallization from alcohol. The presence of unidentified material is evident from the NMR spectrum of the mother liquor. After isolation of the two identified fractions, the column was eluted with methanol, the latter was evaporated, the residue was dissolved in ethyl acetate, and the solution was filtered to remove traces of silica gel. The filtrate was evaporated in vacuo. Crystalline material separated out, and it was filtered off, washed with cold ether, and dried: mp 149–150°; NMR ($CDCl_3$) δ 2.00, 2.05 (s, $2CH_3$ acetyl, 6), 2.33 (s, aromatic CH_3, 3), 3.01 (m, $5CH_2$, 4), 5.02 (5 CH_3 ester, 6), 4.85 (t, 4CH, J = 6 Hz, 2), 5.02 (s, 2CH, 1), 6.68, 6.79 (d, NH), 7.22 (q, aromatic H's, J = 8 Hz, 4); ir ν_{max}^{KBr} 3310 (NH), 1745 (C=O ester), 1675, 1572 cm^{-1} (C=O ester), absence of SH peak at 2600–2550 cm^{-1}.

The compound is evidently XIII, the dimethyl ester of *p*-methylbenzylidene-*S*,*S*′-bis(*N*-acetylcysteine). Anal. ($C_{20}H_{28}N_2O_6S_2$) C, H, N. The structure of compound XIII was indicated by the following synthesis. *N*-Acetyl-L-cysteine (1 g) and *p*-tolualdehyde (0.4 g) were dissolved in methanol (25 ml). The reaction mixture was saturated with hydrogen chloride, heated to 70–75° for 10 min, and left standing at room temperature for 4 days. The solution was evaporated in vacuo at room temperature and the residue was dissolved in alcohol-free chloroform (100 ml). The chloroform solution was washed with water, sodium bicarbonate solution (1%, 30 ml), and water and was dried (Drierite). The chloroform extract was evaporated, and the residue was crystallized from methanol: yield 0.6 g (43%); mp 149°. The compound was identical in all respects with that isolated from the interaction of trifluoroacetic acid with XII.

***S*-(1-Adamantyl)-L-cysteine. Method A. From 1-Bromoadamantane.** A mixture of cysteine hydrochloride monohydrate (2 g) and 1-bromoadamantane (2 g) in glacial acetic (10 ml) was heated at 115° with stirring for 16 h. The reaction mixture was evaporated in vacuo at room temperature, and the residue was taken up in water (30 ml). The resulting suspension was filtered and washed with ether. The solid material was next suspended in sodium acetate solution (10%, 10 ml), and the suspension was filtered. The solid material was then washed with water, dried, and crystallized from alcohol: yield 2.18 (74%); mp 214–215° dec; TLC (1:1 MeOH–$CHCl_3$) shows one ninhydrin-positive spot (R_f 0.7); ir ν_{max}^{KBr} 2900 ($-NH_3^+$), 1600 cm^{-1} (C=O carboxylate); NMR (in hexafluoroacetone deuteriohydrate) δ 1.76–2.66 (br, $C_{10}H_{15}$). Anal. ($C_{13}H_{21}NO_2S \cdot 0.5H_2O$) C, H, N.

Method B. From 1-Adamantanol. Boron trifluoride etherate (1.5 ml) was added, with stirring, to a suspension of cysteine hydrochloride (1.1 g) and 1-adamantanol (1.1 g) in glacial acetic acid (5 ml). The suspension was stirred at room temperature for a week, and a clear solution was obtained. The solution was evaporated in vacuo, and the residue was worked up as in method A: yield 0.88 g (50%); mp 215–216°. TLC and the ir spectrum were identical with those of the product obtained by method A, and no depression of mixture melting point was observed.

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Synthesis and Biological Evaluation of ω-Homologues of Prostaglandin E_1

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The synthesis and biological activities of some compounds with novel modifications of the ω side chain of prostaglandin E_1 (PGE_1) are described. The preparation of (±)-ω-Me-PGE_1 (**3**), (±)-ω-Et-PGE_1 (**4**), (±)-ω-Pr-PGE_1 (**5**), and (±)-ω-Bu-PGE_1 (**6**) is outlined. The compounds were evaluated for in vitro smooth muscle stimulating activity on isolated gerbil colon preparations, for hypotensive action in anesthetized rats, and for gastric antisecretory effects in histamine-stimulated Heidenhain pouch dogs. Structural changes in the ω position of the noncarboxyl side chain of PGE_1 influenced the biological potency of the resulting compound when compared to the reference standard PGE_1 (**2**). The homologues demonstrated interesting separation of biological activities; for example, **4** showed potent hypotensive activity (84% of PGE_1) but, unlike PGE_1, it showed very low smooth muscle stimulating activity. Compound **3** possessed the same order of potency as **2** in the gastric antisecretory assay.

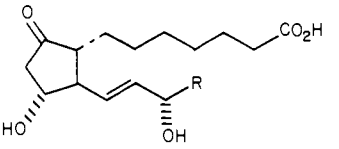
Simple chemical modification of a hormonal substance, or a drug, profoundly modifies its biological activity. A number of currently known drugs are either methyl homologues or desmethyl analogues of known compounds. Examples are methyl-Dopa, epinephrine, norgestrel, and most oral contraceptives chemically defined as 19-norprogestins. Such changes in biological activity may sometimes be predicted based on the inhibition of metabolic degradation.

Labhsetwar[1] showed that a C-22 derivative of $PGF_{2\alpha}$ was 20 times more potent than the parent $PGF_{2\alpha}$ in terminating early pregnancy in hamsters when administered orally. Beerthuis et al.[2] prepared (Scheme I) ω-nor-PGE_1, PGE_1, and ω-homo-PGE_1 by biosynthesis from the corresponding unsaturated fatty acids and determined some aspects of structure and activity relationships (SAR) for these compounds on platelet aggregation, isolated guinea pig ileum, and hypotensive effects in the anesthetized rat.

To elucidate more salient SAR in the ω-homo series, we have synthesized (±)-ω-Me-PGE_1 (**3**), (±)-ω-Et-PGE_1 (**4**), (±)-ω-Pr-PGE_1 (**5**), and (±)-ω-Bu-PGE_1 (**6**) and tested[3] their activities on the gerbil colon, blood pressure of the anesthetized rat, and gastric secretion in the dog. The chemical structures are shown in Scheme I.

Chemistry. The synthesis of (±)-**3**, **-4**, **-5**, and **-6** is outlined in Scheme II. The starting aldehyde tetrahydropyranyl ether (**7**) prepared by a previously published procedure[4] was condensed with an appropriate phosphorane (**8**) to give 15-dehydro-ω-homo-PGE_1 tetrahydropyranyl ethers (**9**). Reduction of **9** with thexyl tetrahydrolimonyl borohydride followed by acid hydrolysis

Scheme I. Chemical Structures of ω-Homologues of PGE_1



1, R = n-C_4H_9
2, R = n-C_5H_{11}
3, R = n-C_6H_{13}
4, R = n-C_7H_{15}
5, R = n-C_8H_{17}
6, R = n-C_9H_{19}

Table I. Properties of Phosphoranes $(C_6H_5)_3P{=}CHCOR$

R	Mp, °C (recrystn solvent)	Formula	Analyses
n-C_6H_{13}	86 (benzene–Skelly B)	$C_{26}H_{29}OP$	C, H
n-C_7H_{15}[a]	83 (Skelly B)	$C_{27}H_{31}OP$	C, H
n-C_8H_{17}[b]	202 (EtOAc)	$C_{28}H_{34}OPCl$	C, H
n-C_9H_{19}	78 (Skelly B)	$C_{29}H_{35}OP$	C, H

[a] See ref 4. [b] Characterized as hydrochloride.

produced the (±)-ω-homologues of PGE_1 (**3–6**). The phosphoranes (**8**) required for the synthesis were prepared by the imidazolide procedure.[5] Their physical and chemical properties are summarized in Table I.

Compounds **3–6** were pure crystalline substances and each exhibited a single spot on a thin-layer plate [silica gel, benzene–ethyl acetate–acetic acid (25:25:1), sprayed with ethanolic phosphomolybdic acid]. That they have "natural" configurations was confirmed by comparison of