**Reaction of 4 with 2-Amino-6-chloro-4-hydroxy-5-nitropyrimidine (11). Preparation of 17 and 18.** The preparation of 17, which is the 4-hydroxy analogue of 16, was carried out as described above for 15. The ketal derivative 17 was not crystalline. Upon removal of the protective group from 17 with TFA and water, 18 was obtained as a light yellow powder in 80% yield: mp 142–143 °C; UV (0.1 N NaOH) $\lambda_{max}$ 310 nm. Anal. ($C_{24}H_{30}N_6O_9S$) C, H, N, O.

**Conversion of 16 to 11-Thiohomoaminopterin (1).** The dithionite reduction of 16 and the cyclization and oxidation of the reduction product to diethyl-11-thiohomoaminopterin were carried out exactly as described for the preparation of methyl-4-amino-4-deoxy-11-thiohomopteroic acid (19). The noncrystalline diethyl ester thus obtained was hydrolyzed to 1 by using 0.33 N NaOH in acetonitrile for 4 h. The pH of the hydrolysate was adjusted to 7.3 and chromatographed over DEAE-cellulose using the NaCl gradient. The product was eluted from the column at a concentration of ~0.3 M NaCl, which was preceded by the elution of less polar minor impurities. The column effluent corresponding to the desired product was pooled and concentrated to a small volume. Acidification of this concentrate with glacial acetic acid gave a yellow precipitate, which was collected by filtration, washed several times with distilled water, and dried to obtain 1 in 50% yield based on 16. This product was identical with the glutamate conjugate obtained by the coupling of glutamate with 20 by the isobutyl chloroformate method (vide infra).

**Conversion of 20 to 11-Thiohomoaminopterin (1).** Initial attempts to convert 20 to 1 by the solid-phase procedure which was successfully used for the preparation of 10-thioaminopterin was found to be unsatisfactory due to the low yield of the final product. This was due to the instability of both the reactant (20) and product (1) under the cleavage conditions which were employed for the release of the resin-bound product. Therefore, the coupling reaction was carried out in solution using the isobutyl chloroformate method.[19,20] A solution of 342 mg (1 mmol) of 20 in a 1:1 mixture of $Me_2SO$ and THF was made by dissolving the compound first in 15 mL of dry $Me_2SO$ at 100 °C, cooling to 30 °C, and then adding 15 mL of dry THF. After cooling this solution in an ice bath, 0.14 mL (1.25 mmol) of *N*-methylmorpholine was added, which was followed by the addition of 0.13 mL (1 mmol) of freshly distilled isobutyl chloroformate. The solution was slowly allowed to warm up to 25 °C during a period of 20 min. A solution of 480 mg (2 mmol) of diethyl L-glutamate hydrochloride in 20 mL of dry $Me_2SO$ was made in a separate flask, neutralized with the addition of 0.23 mL (2 mmol) of *N*-methylmorpholine, and was added to the flask containing the mixed anhydride. After stirring for 18 h at 25 °C, most of the THF was removed by rotary evaporation under vacuum. The bright yellow solution thus obtained was diluted to 150 mL by the addition of 100 mL of distilled water and 20 mL of 1.0 N NaOH and stirred at 25 °C for 6 h to hydrolyze the ester moieties. The pH of the hydrolysate was adjusted to 7.2 and chromatographed over a DEAE-cellulose column using a linear NaCl gradient at pH 7.0 to elute the column. Two major products were eluted from the column, of which the less polar one was identified as 20 and the major one, which was obtained in 60% yield, was identified as 1. The UV spectrum of this compound was identical with that of 11-thiohomofolic acid in 0.1 NaOH but differed considerably in 0.1 N HCl: UV (0.1 N NaOH) $\lambda_{max}$ 258 nm ($\epsilon$ 32029), 372 (7139); UV (0.1 HCl) $\lambda_{max}$ 248 nm ($\epsilon$ 26944), 337 (9828); NMR (TFA) $\delta$ 1.5–2.9 (c, 4 H, glutamate), 3.55 (t, 4 H, ethylene bridge), 4.05 (t, 1 H, glutamate), 7.45, 7.8 (2 d, 4 H, aromatic), 8.78 (s, 1 H, pteridine ring). Anal. ($C_{20}H_{21}N_7O_5S \cdot 1.25H_2O$) C, H, O, S.

**Methods Used for Biological Testing.** The antitumor data on compound 1 (NSC 313384) were collected at Arthur D. Little, Inc., Boston, MA, under the auspices of the National Cancer Institute using the standard protocol for evaluating (Instruction 14) methotrexate analogues in the L-1210 leukemia test system. Dihydrofolate reductase,[23] thymidylate synthase,[24] and microbiological assays[25] were carried out as described previously.[1]

**Acknowledgment.** This investigation was supported by grants from the American Cancer Society (CH-53-B) and the National Institutes of Health (CA-27101 and CA-10914 from the National Cancer Institute). We are also indebted to Dr. Jacqueline Plowman and Robert B. Ing of the National Cancer Institute for their assistance in obtaining the antitumor data.

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# Prostaglandins and Congeners. 22.[1] Synthesis of 11-Substituted Derivatives of 11-Deoxyprostaglandins $E_1$ and $E_2$. Potential Bronchodilators

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The interesting bronchodilator activity of *l*-11-deoxy-11$\alpha$-[(2-hydroxyethyl)thio]prostaglandin $E_2$ methyl ester (3a) is described. The preparation of 3a and its analogues by Michael-type additions to various members of the PGA series or by total synthesis using the lithiocuprate conjugate addition process is also described. Structure–activity relationships in this series are discussed.

Prostaglandins of the E series are potent bronchodilators.[3] However, it also is apparent that the natural prostaglandins will not find clinical application, since they induce cough and irritation of the upper respiratory tract.

(1) For the previous paper in this series, see M. B. Floyd and M. J. Weiss, *J. Org. Chem.*, **44**, 71 (1979).

(2) Cyanamid Educational Award Recipient (1977–1979); Department of Chemistry, Princeton University, Princeton, N.J.

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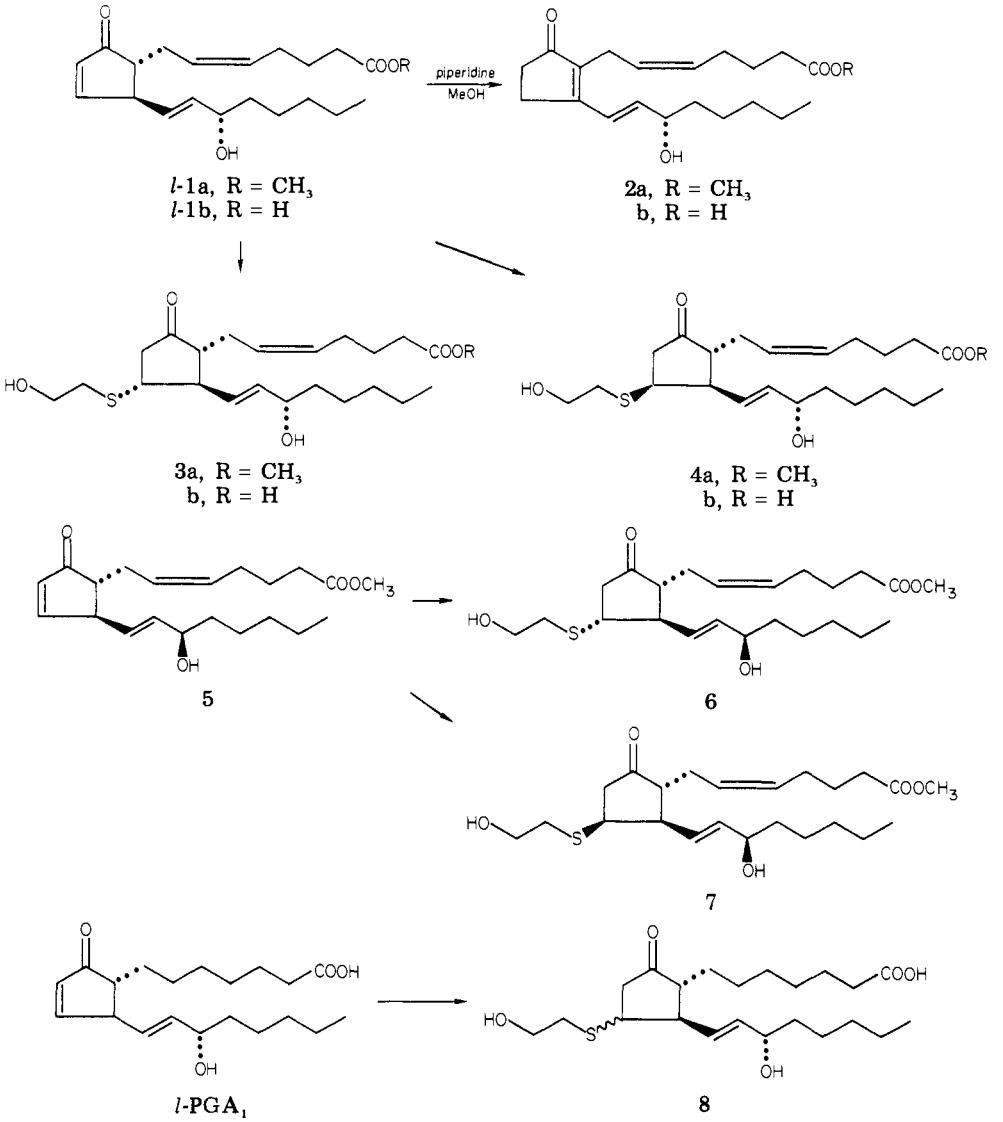
Scheme I. Preparation of 11-Deoxy-11α(β)-[(2-hydroxyethyl)thio]prostaglandin $E_1$ and -prostaglandin $E_2$




Nevertheless, because their mechanism of action is different from that of the known bronchodilators,[4] the prostaglandins do represent an important and exciting lead to further advances in bronchodilator therapy.

As a convenient and attractive approach to the exploitation of these possibilities, we have chosen to prepare a wide variety of 11-substituted derivatives of 11-deoxy-$PGE_2$ via Michael-type addition of various nucleophiles to the $\Delta^{10}$-9-keto system of *l*-$PGA_2$ (**1b**) or its methyl ester **1a**. Preliminary reports from this laboratory[5a] and elsewhere[5b] concerning this approach have appeared. We now describe our further efforts along these lines resulting in the identification of a potent prostaglandin bronchodilator and the subsequent preparation of a series of congeneric 11-deoxy-11-substituted derivatives of $PGE_1$, $PGE_2$, 15-methyl-$PGE_2$, 15-deoxy-$PGE_2$, 15-deoxy-16-hydroxy-$PGE_1$, and 15-deoxy-16-hydroxy-16-methyl-$PGE_2$.

*l*-$PGA_2$ methyl ester (**1a**)[6] and *l*-$PGA_2$ (**1b**) were obtained from the cortex of the readily available Carribean sea coral *Plexaura homomalla* (esper). The configuration at C-15 of these compounds was established by isomerization to $PGB_2$ (**2b**) or its methyl ester **2a** (Scheme I). Comparison of the optical rotation of these substances with that reported for natural $PGB_2$[7] indicated C-15 to be of the natural *S* configuration. We have found that piperidine in methanol gave preparative isomerization yields superior to those obtainable by the literature procedure[7] which utilizes aqueous sodium hydroxide.

Treatment of *l*-$PGA_2$ methyl ester (**1a**) with β-mercaptoethanol in the presence of catalytic triethylamine gave approximately a 1:1 mixture of *l*-11-deoxy-11α-[(2-hydroxyethyl)thio]-$PGE_2$ methyl ester (**3a**) and the more polar (TLC) 11β-epimer **4a**, which were separated by dry column chromatography.

Initial testing by the intravenous route of administration in the guinea pig Konzett-Rossler assay[8] of the 11α- and

(3) (a) H. Herxheimer and I. Roetscher, *Eur. J. Clin. Pharmacol.*, **3**, 123 (1971); (b) M. F. Cuthbert, *Proc. R. Soc. Med.*, **64**, 15 (1971); (c) W. J. F. Sweatman and H. O. J. Collier, *Nature (London)*, **217**, 69 (1968); (d) A. A. Mathé, in "The Prostaglandins", Vol. 3, P. W. Ramwell, Ed., Plenum Press, New York, 1977, p 169.
(4) P. Sheard, *J. Pharm. Pharmacol.*, **20**, 232 (1968).
(5) (a) C. V. Grudzinskas and M. J. Weiss, *Tetrahedron Lett.*, 141 (1973); (b) M. E. Rosenthale, A. Dervinis, and D. Strike, *Adv. Prostaglandin Thromboxane Res.*, 1, 477 (1976).
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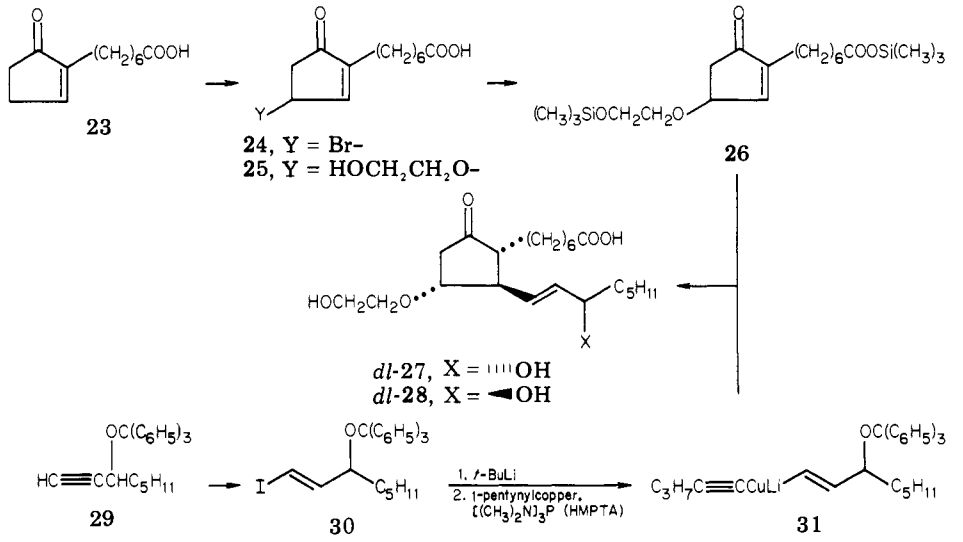
(8) F. Dessy, M. R. Maleux, and A. Cognioul, *Arch. Int. Pharmacodyn. Ther.*, **206**, 368 (1973).

**Scheme II.** Preparation of 11-*O*-(2-Hydroxyethyl)prostaglandin $E_1$




11$\beta$-isomers **3a** and **4a** indicated the 11$\alpha$-isomer **3a** to be a potent bronchodilator (approximately 15% of *l*-$PGE_2$, Table I). In addition, **3a** was an effective bronchodilator with some evidence for a prolonged duration of effect when administered as an aerosol to the pilocarpine-treated dog (see Figure 1). At the same time, no cardiovascular side effects could be noted. We therefore undertook an extensive synthetic program to identify the best member of this series for clinical investigation.

The 15-epi analogues were prepared by treatment of *l*-15-*epi*-$PGA_2$ methyl ester (**5**), obtained from *P. homomalla* (esper) gathered in Florida waters, with $\beta$-mercaptoethanol–triethylamine to provide a 1:1 mixture of *l*-11-deoxy-11$\alpha$-[(2-hydroxyethyl)thio]-15-*epi*-$PGE_2$ methyl ester (**6**) and the corresponding 11$\beta$-isomer **7**.

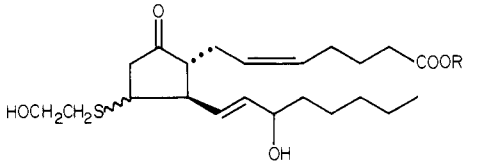
The configurational assignments at C-11 for the isomers **3a**, **4a**, **6**, and **7** are based upon the following considerations: (1) The cd curve of each isomer is negative, thereby eliminating an 8-iso structure as a possibility. (2) Treatment of either 15-nat-epimer **3a** or **4a** with excess triethylamine gave in each instance a 9:1 **3a**/**4a** equilibrium mixture. It is reasonable to assume that the all-trans 11$\alpha$-isomer **3a** predominates at equilibrium. (3) The resonance position for H-11 of the 11$\alpha$-epimers **3a** and **6** is $\delta$ 2.8–3.0, while that for the 11$\beta$-epimers **4a** and **7** is $\delta$ 3.60. In general, the downfield resonance position observed for an epimeric pair is associated with that proton occupying the position trans to an adjacent alkyl group [for example, the C-9 proton of $PGF_{2\alpha}$ (9$\beta$ H, $\delta$ 4.25) vs. $PGF_{2\beta}$ (9$\alpha$ H, $\delta$ 3.83)].[9] (4) The formation of the *dl*-11$\alpha$-epimers **3a** and **6** via a conjugate-addition reaction (see below) which is known to produce the all-trans arrangement of the 8-, 11-, and 12-substituents.

Quite unexpectedly, in the 15*S* series the 11$\beta$-isomer **4a** is less mobile (TLC, silica gel) than the corresponding 11$\alpha$-isomer **3a**, whereas in the 15*R* series the reverse relationship is observed. However, in both the 11$\alpha$ and 11$\beta$ cases, the 15*R* epimer is, as anticipated, more mobile than the corresponding 15*S* isomer (see Table I).

The 15*S* acids **3b** and **4b** were prepared by treating *l*-$PGA_2$ with $\beta$-mercaptoethanol–triethylamine under nonequilibrating conditions. Likewise, *l*-$PGA_1$ provided the 11$\alpha(\beta)$-$PGE_1$ congener **8**, which was tested as the epimeric mixture. The relative bronchodilator potencies are shown in Table I.

(9) J. F. Bagli and T. Bogri, *Tetrahedron Lett.*, 5 (1967).

Table I. Relative Bronchodilator Potency of 11-Deoxy-11-[(2-Hydroxyethyl)thio]prostaglandin $E_2$ Epimers[a]

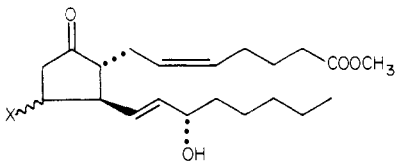


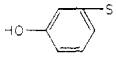

| | C-11 | C-15 | $R_f$[c] | R | rel potency in guinea pigs[b] serotonin | histamine |
|---|---|---|---|---|---|---|
| *l*-$PGE_2$ | $\alpha$ | *S* | | H | 610 | 570 |
| *l*-11-deoxy-$PGE_2$ | | *S* | | H | 112 | 92 |
| *l*-3a | $\alpha$ | *S* | 0.31 | $CH_3$ | 100 | 100 |
| *l*-4a | $\beta$ | *S* | 0.26 | $CH_3$ | 10 | 10 |
| *l*-6 | $\alpha$ | *R* | 0.36 | $CH_3$ | 22 | 19 |
| *l*-7 | $\beta$ | *R* | 0.50 | $CH_3$ | *d* | *d* |
| *l*-3b | $\alpha$ | *S* | 0.26 | H | 50 | 55 |
| *l*-4b | $\beta$ | *S* | 0.22 | H | *e* | *e* |
| *l*-8[f] | $\alpha(\beta)$ | *S* | | H | 107 | 104 |
| *dl*-3a (46) | $\alpha$ | *S* | 0.31 | $CH_3$ | 33 | 30 |
| *dl*-4a (47) | $\alpha$ | *R* | 0.36 | $CH_3$ | 8 | 6 |

[a] Measured by a modified Konzett-Rossler procedure[8] using the iv route against bronchoconstrictions induced by the iv administration of histamine and serotonin at doses (5.6–22.5 $\mu$g/kg) which produce a tracheal pressure increase of 20 to 50 cm of water. The iv injections last 12 s and are repeated every 5 min throughout the assay. (The guinea pigs are anesthetized by an interperitoneal injection of urethane and are curarized with an iv injection of gallamine.) The broncholytic activity of each compound was measured in at least four guinea pigs for each of the spasmogenic substances at each dose level. [b] Based on relative $ED_{50}$ observed over a dose range of $10^{-3}$ to $10^{-9}$ mg/kg. [c] TLC, silica gel; EtOAc-benzene-HOAc, 20:30:1; three developments. [d] Very weakly active. [e] Not tested. [f] $E_1$ series.

Variations in the structure of the C-11 sulfur substituent are summarized in Table II. All compounds were prepared by Michael-type addition of the appropriate mercaptan to *l*-$PGA_2$ methyl ester (**1a**). Also shown in the table are the Konzett–Rossler assay results. All analogues, with the exception of **13**, were tested as 11$\alpha(\beta)$ mixtures, and it is apparent that none were as effective as the 11$\alpha$-[(2-hydroxyethyl)thio] derivative **3a**.

Table II. Relative Bronchodilator Potency of 11-Deoxy-11-substituted-prostaglandin $E_2$ Derivatives[a]



| compd | X | rel potency in guinea pigs[b] sero-tonin | hista-mine |
|---|---|---|---|
| 3a (11α epimer) | $HO(CH_2)_2S$- | 100 | 100 |
| 9 | $CH_3CH_2S$- | 4 | 1 |
| 10 | $CH_3CH_2CH_2S$- | <0.2 | <0.2 |
| 11 | $HO(CH_2)_3S$- | 3 | 0.8 |
| 12 | $HOC(CH_3)HCH_2S$- | <0.2 | 2 |
| 13 (11α epimer) | $HOC(C_5H_{11})HCH_2S$- | <0.2 | <0.2 |
| 14 | $HOCH_2C(OH)HCH_2S$- | 5 | 11 |
| 15 | $HOOCCH_2S$- | 0.1 | 0.1 |
| 16 | $H_5C_2OOCCH_2S$- | <2 | 3 |
| 17 |  | 3 | 0.2 |
| 18 | $HS(CH_2)_2S$- | <0.2 | 0.4 |
| 19 | $HS(CH_2)_3S$- | 6 | 9 |
| 20 | $H_2N(CH_2)_2S$- | 0.3 | 0.3 |
| 21 | $(CH_3)_2N(CH_2)_2S$- | 10 | 14 |
| 22 | $(C_2H_5)_2N(CH_2)_2S$- | 15 | 22 |

[a, b] See footnotes *a* and *b*, Table I.

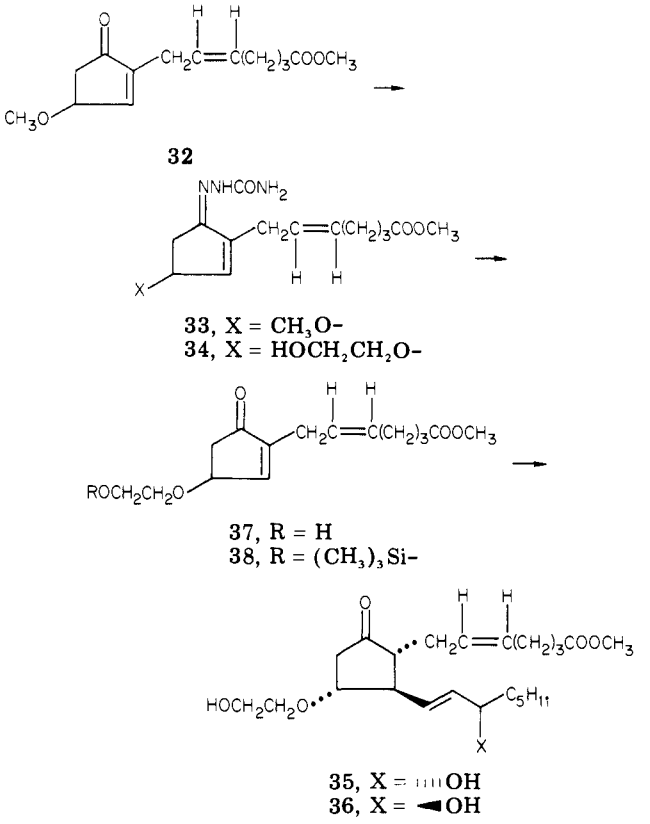
For the preparation of the 11α-(2-hydroxyethoxy) analogues, we required the correspondingly substituted cyclopentenone precursors to the $PG_1$ and $PG_2$ series, **25** and **37**, which were prepared as shown in Schemes II and III, respectively.

For the $PGE_1$ series, the requisite functionality was introduced via the 4-bromo derivative **24**, prepared by bromination of the parent cyclopentenone **23** with *N*-bromosuccinimide.[10] Solvolysis of crude **24** in ethylene glycol at room temperature was accomplished with the aid of silver fluoroborate in the presence of 2,6-lutidine to give 4-(hydroxyethoxy)cyclopentenone **25** (30% overall yield from **23**). Treatment of **25** with chlorotrimethylsilane and hexamethyldisilazane ($ClMe_3Si/Me_6DS$) in pyridine afforded the bis(trimethylsilyl) ($Me_3Si$) derivative **26** in 89% yield. Addition of cuprate **31** (generated from the 1-alkenyl iodide **30**[11]) to the blocked cyclopentenone **26** gave, after deblocking and chromatography, *dl*-11-deoxy-11α-(2-hydroxyethoxy)-$PGE_1$ (**27**) and its epimer **28** in a total yield of 31%.

Since bromination of the 4-unsubstituted cyclopentenone of the $PG_2$ series was not feasible, an alternative preparation was devised from 4-methoxycyclopentenone **32**.[12] The latter was converted to the semicarbazone **33** in an ethylene glycol solution, which then was treated with acetic acid and heated at 90 °C until exchange to the 4-(2-hydroxyethoxy)cyclopentenone semicarbazone **34** was complete.[13] The free ketone **37** was obtained in an overall yield of 37% after acid hydrolysis of crude **34** in the presence of α-ketoglutaric acid.[14] Reaction of 4-(2-hydroxyethoxy)cyclopentenone **37** with $ClMe_3Si/Me_6DS$ afforded the $Me_3Si$ derivative **38** in 94% yield. Cuprate addition, hydrolysis, and chromatography gave *dl*-11-deoxy-11α-(2-hydroxyethoxy)-$PGE_2$ methyl ester (**35**; 17%) and the more mobile (TLC, silica gel) 15-epimer **36** (23%), as well as 5% of a mixture of both epimers.

The oxy analogues **27** and **35** appeared to be significantly less potent than **3a** when examined in the Konzett–Rossler assay (Table III). Since the 11α-[(2-hydroxyethyl)thio]

Scheme III. Preparation of 11-*O*-(2-Hydroxyethyl)prostaglandin $E_2$



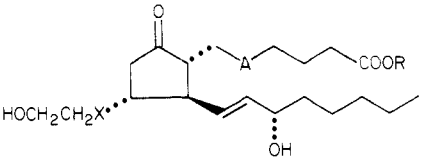
**32**

**33**, X = $CH_3O$-
**34**, X = $HOCH_2CH_2O$-

**37**, R = H
**38**, R = $(CH_3)_3Si$-

**35**, X = ⋯OH
**36**, X = ◀OH

Table III. Relative Bronchodilator Potency of 11-Deoxy-11α-(2-hydroxyethoxy)- and 11-Deoxy-11α-[(2-Hydroxyethyl)thio]prostaglandin E Analogues[a]



| compd | A | X | C-11 | R | rel potency in guinea pigs[b] sero-tonin | hista-mine |
|---|---|---|---|---|---|---|
| *l*-3a | CH=CH | S | α | $CH_3$ | 100 | 100 |
| *dl*-46 | CH=CH | S | α | $CH_3$ | 33 | 30 |
| *dl*-35 | CH=CH | O | α | $CH_3$ | 5 | 3 |
| *l*-8 | $CH_2CH_2$ | S | α(β) | H | 107 | 104 |
| *dl*-27 | $CH_2CH_2$ | O | α | H | 16 | 14 |

[a, b] See footnotes *a* and b, Table I.

(10) L. Heslinga, M. Van Gorkom, and D. A. Van Dorp, *Recl. Trav. Chim. Pays-Bas*, **87**, 1421 (1968).
(11) K. F. Bernady, J. F. Poletto, and M. J. Weiss, *Tetrahedron Lett.*, 765 (1975).
(12) M. B. Floyd, *J. Org. Chem.*, **43**, 1641 (1978).
(13) This exchange procedure was based on literature observations: W. F. McGuckin and E. C. Kendall, *J. Am. Chem. Soc.*, **74**, 5811 (1952); W. A. Woessner and R. A. Ellison, *Tetrahedron Lett.*, 3735 (1972).
(14) H. R. Harrison and E. J. Eisenbraun, *J. Org. Chem.*, **31**, 1294 (1966).

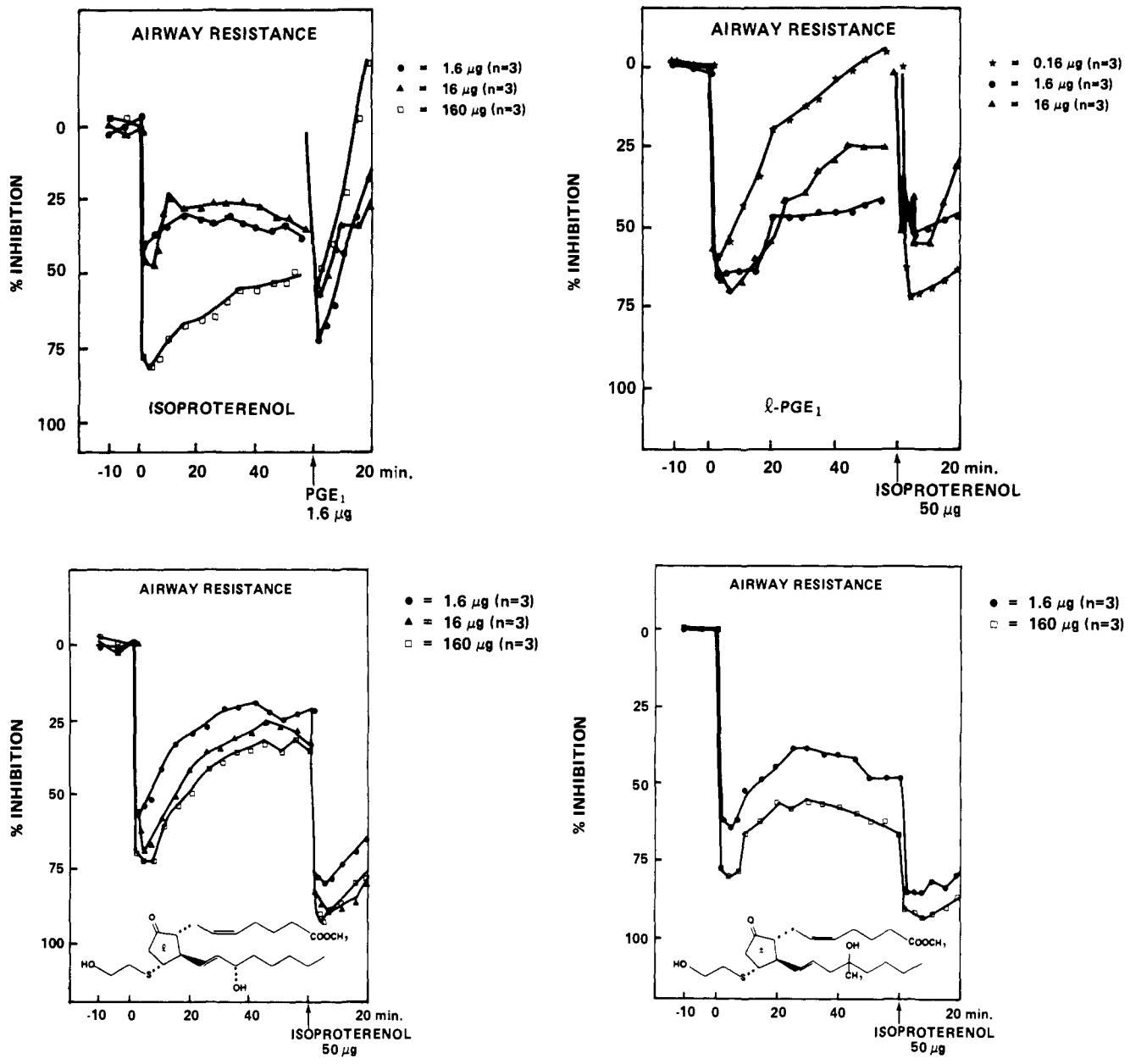



**Figure 1.** Bronchodilator activity of $PGE_1$, isoproterenol, *l*-11-deoxy-11α-[(2-hydroxyethyl)thio]prostaglandin $E_2$ methyl ester (**3a**) and *dl*-11,15-dideoxy-11α-[(2-hydroxyethyl)thio]-16-hydroxy-16-methylprostaglandin $E_2$ methyl ester (**52**) in pilocarpine-bronchoconstricted anesthetized dogs (aerosol).

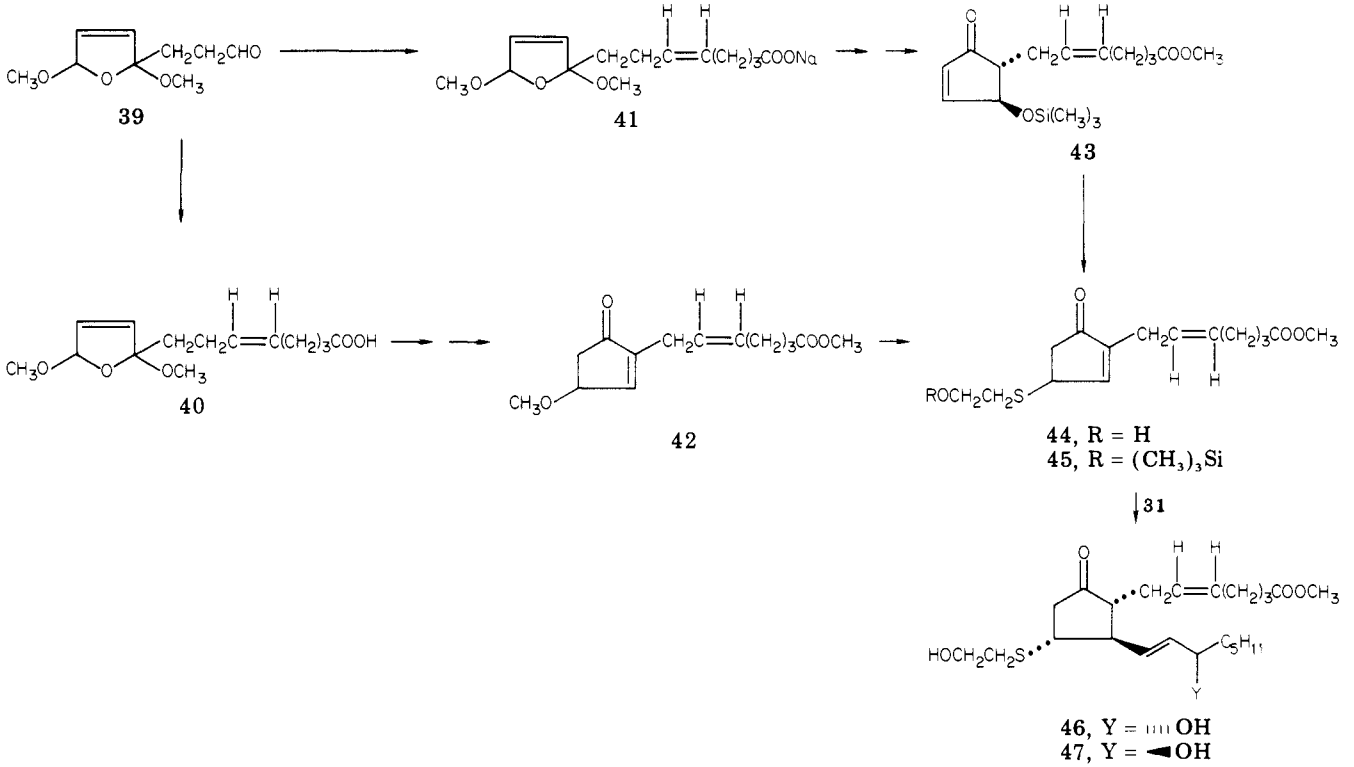
moiety apparently was the grouping with optimal activity, a total synthesis of *dl*-**3a** was undertaken. In this effort, we sought to establish a feasible synthesis independent of *l*-$PGA_2$ methyl ester (**1a**), which also could be applied to the facile preparation of additional analogues incorporating structural modifications in the β [C(13)–C(20)] side chain.

These requirements were fulfilled by a synthetic procedure involving conjugate addition of the β chain to a 4-[(2-hydroxyethyl)thio]cyclopentenone in which the α [C(1)–C(7)] chain was already positioned. The requisite cyclopentenone **45** was prepared by two routes (Scheme IV). In the first sequence, 4-methoxycyclopentenone methyl ester **42**, available as shown from aldehyde **39** in ca. 38% yield,[12] was treated with 1.05 equiv of β-mercaptoethanol and 0.5 equiv of sodium methoxide in methanol at room temperature for 50 min. After quenching with glacial acetic acid, workup and chromatography afforded the desired 4-[(2-hydroxyethyl)thio]cyclopentenone **44** in 62% yield. The hydroxyl group in **44** was protected as the $Me_3Si$ ether **45** by treatment with $ClMe_3Si/Me_6DS$ in pyridine. In the second route, the 3-[(trimethylsilyl)oxy]cyclopentenone **43**, available in 37% yield in three steps from aldehyde **39**,[12] was treated with β-mercaptoethanol under conditions virtually the same as those described above, and a 62% yield of **44** again was obtained. The identical yield observed in both cases may represent an upper limit imposed by the reaction conditions.

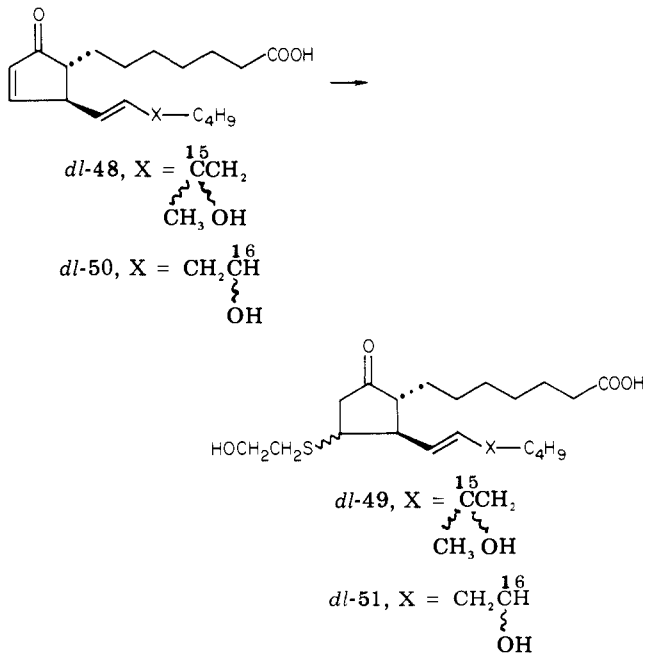
Treatment of cyclopentenone **45** with the mixed cuprate **31**, followed by deblocking with 4:2:1 HOAc–THF–$H_2O$ at 45 °C for 6 h, gave a mixture of C-15 epimers which were separated by dry column chromatography on silica gel to provide **46** and **47** in a total overall yield of 58% from cyclopentenone **45**. There was no evidence for either the formation of any PGA-type product or C-11β epimer. As anticipated, the bronchodilator potency of *dl*-**3a** (**46**) was approximately one-half that of the single antipode **3a** (Table I).

The preparation of various *dl*-11-deoxy-11α(β)-[(2-hydroxyethyl)thio] analogues with modifications at C-15 or C-16 was accomplished by either of two procedures.

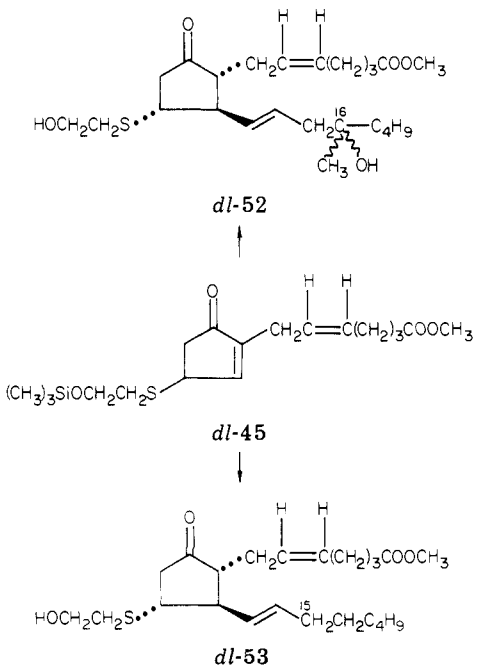
Scheme IV. Total Synthesis of 11-Deoxy-11α-[(2-hydroxyethyl)thio]prostaglandin $E_2$ Methyl Ester




*dl*-11-Deoxy-11α(β)-[(2-hydroxyethyl)thio]-15α(β)-methyl-$PGE_1$ (**49**) and *dl*-11,15-dideoxy-11α(β)-[(2-hydroxyethyl)thio]-16α(β)-hydroxy-$PGE_1$ (**51**) were obtained by addition of β-mercaptoethanol to the requisite PGA.[16]




*dl*-11,15-dideoxy-11α-[(2-hydroxyethyl)thio]-16-hydroxy-16-methyl-$PGE_2$ methyl ester (**52**) and *dl*-11,15-dideoxy-11α-[(2-hydroxyethyl)thio]-$PGE_2$ methyl ester (**53**) were prepared by conjugate addition of the appropriate 1-alkenyl lithiocuprate to the protected cyclopentenone **45**.



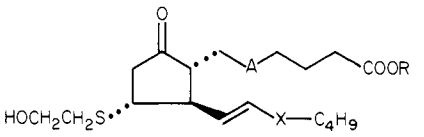

The relative bronchodilator potencies of these β-chain congeners are shown in Table IV. The only compound of high interest was the *dl*-15-deoxy-16-hydroxy-16-methyl analogue **52**, which appears to be at least as potent as the parent **3a**.

For further evaluation, **3a** and **52** were submitted to an assay[15] in which the prostaglandin is administered by aerosol to an anesthetized pilocarpine-bronchoconstricted dog ($n$ = 3–6) and the decrease in airway resistance is recorded (see Figure 1); at the same time, effects on the cardiovascular system (femoral pressure, pulmonary

(15) (a) J. Lulling, F. El Sayed, and P. Lievens, *Med. Pharmacol. Exp.*, **16**, 481 (1967); (b) J. Lulling, P. Lievens, F. El Sayed, and J. Prignot, *Arzneim.-Forsch.*, **18**, 995 (1968).

(16) For the preparation of PGA from the corresponding PGE, see E. J. Corey, N. H. Andersen, R. M. Carlson, J. Paust, E. Vedejs, I. Vlattas, and R. E. K. Winter, *J. Am. Chem. Soc.*, **90**, 3245 (1968).

Table IV. Influence of β-Chain Variation on Bronchodilator Potency[a]




| compd | X (no. of optical isomers) | A | R | rel potency in guinea pigs[b] serotonin | histamine |
|---|---|---|---|---|---|
| *l*-3a | $-\overset{15}{C}HCH_2-$ (1) <br> OH | CH=CH | $CH_3$ | 100 | 100 |
| *dl*-49 | $-\overset{15}{C}CH_2-$ [(11α(β)] (4) <br> $CH_3$ OH | $CH_2CH_2$ | H | 8 | 16 |
| *dl*-51 | $-CH_2\overset{16}{C}H-$ [11α(β)] (4) <br> OH | $CH_2CH_2$ | H | 31 | 24 |
| *dl*-52 | $-CH_2\overset{16}{C}-$ (4) <br> $CH_3$ OH | CH=CH | $CH_3$ | 118 | 109 |
| *dl*-53 | $-\overset{15}{C}H_2CH_2-$ (2) | CH=CH | $CH_3$ | 10 | 14 |

[a,b] See footnotes *a* and *b*, Table I.

pressure, heart rate) are noted. This experiment is allowed to proceed for 1 h, which permits an assessment of the compound's ability to produce a prolonged bronchodilation. In this assay, salbutamol maintains its effect for the entire hour, whereas isoproterenol and *l*-$PGE_1$ lose theirs within the first 20 min. At the conclusion of the study a standard dose of isoproterenol is administered to determine the animal's maximum capacity to respond. In this assay, both **3a** and **52** proved to be potent bronchodilators free of cardiovascular side effects at effective doses. In addition, **52** appears to produce a significantly enhanced duration of effect.

*l*-11-Deoxy-11α-[(2-hydroxyethyl)thio]-$PGE_2$ methyl ester (**3a**) was selected as a candidate for clinical investigation, and a report of this study will be forthcoming at a later date.

## Experimental Section

Infrared spectra were recorded with neat samples on a Perkin-Elmer Model 21 spectrophotometer. Proton magnetic resonance spectra were determined in $CDCl_3$ except where noted, using Varian A-60 or HA-100D spectrophotometers. Chemical shifts are given in parts per million downfield from an internal tetramethylsilane standard. Those analytical results indicated by symbol only were within ±0.4% of their calculated values. Ultraviolet spectra were obtained using a Cary recording spectrophotometer in the indicated solvent. Mass spectra were recorded on an AEI MS-9 at 70 eV. All compounds were shown to be homogeneous by thin-layer chromatography and spectroscopic analysis. Only characteristic spectral data are presented for each compound.

**Conversion of *l*-Prostaglandin $A_2$ Methyl Ester (1a) to *l*-Prostaglandin $B_2$ Methyl Ester (2a).** To a solution of 96 mg of 1a[6] in 2 mL of MeOH was added 30 μL of piperidine. After 18 h at 25 °C, the solution was concentrated to give 95 mg of an oil, which was purified by preparative TLC (EtOAc–benzene, 2:3) to give 75 mg of *l*-$PGB_2$ methyl ester (**2a**): $[\alpha]^{25}_D$ +26.5° (lit.[7a] +36°); IR 3400, 1737, 1695, 1645, 1600, 970 $cm^{-1}$; UV $\lambda_{max}$ (MeOH) 278 nm (ε 24 000); NMR δ 6.86 (d, *J* = 16 Hz, 1 H, C-13 H), 6.31 (dd, *J* = 6 and 16 Hz, 1 H, C-14 H), 5.36 (t, 2 H, C-5 and C-6 H), 4.34 (q, 1 H, C-15 H), 3.69 (s, 3 H, $COOCH_3$), 3.05 (d, 2 H, C-7 H), 2.66 (m, 2 H, C-10 H).

**Conversion of *l*-Prostaglandin $A_2$ (1b) to *l*-Prostaglandin $B_2$ (2b).** A solution of 200 mg of *l*-$PGA_2$ (**1b**) in 2 mL of MeOH was treated with 136 mg of piperidine. After 24 h, UV indicated 50% *l*-$PGB_2$ present. Additional piperidine (100 mg) and MeOH (2 mL) were added, and stirring was continued for 72 h. The solution was diluted with 30% aqueous $KH_2PO_4$ (10 mL) and extracted twice with ether to give, after concentration, 190 mg of an oil, which was purified by preparative TLC (EtOAc–benzene–HOAc, 40:60:1) to give 80 mg of *l*-$PGB_2$ (**2b**) as an oil: $[\alpha]^{25}_D$ +27° (lit.[7a] +36°); IR 3400, 1695, 1645, 1600, 970 $cm^{-1}$; UV $\lambda_{max}$ (MeOH) 278 nm (ε 26 000); NMR δ 6.85 (d, *J* = 16 Hz, 1 H, C-13 H), 6.30 (dd, *J* = 6 and 16 Hz, 1 H, C-14 H), 5.35 (t, 2 H, C-5 and C-6 H), 4.34 (q, 1 H, C-15 H), 3.09 (d, 2 H, C-7 H), 2.65 (m, 2 H, C-10 H).

**Preparation of *l*-11-Deoxy-11α-[(2-hydroxyethyl)thio]-prostaglandin $E_2$ Methyl Ester (3a) and *l*-11-Deoxy-11β-[(2-hydroxyethyl)thio]prostaglandin $E_2$ Methyl Ester (4a).** A solution of 10 g (28.7 mmol) of *l*-$PGA_2$ methyl ester (**1a**),[6] 2.24 g (28.7 mmol) of β-mercaptoethanol, and 5 drops of triethylamine ($Et_3N$) was stirred at ambient temperature under an argon atmosphere for 18 h. The resulting oil was dry column chromatographed (61 × 3 in. flat nylon column) on 1700 g of silica gel. The column was developed with EtOAc–benzene–HOAc (20:30:1), and 2000 mL of eluant was collected. The zone $R_f$ 0.19–0.28 gave 2.64 g (22%) of **3a**: IR 3400, 1740, 970 $cm^{-1}$; NMR δ 5.64 (m, 2 H, C-13 and C-14 H), 5.36 (m, 2 H, C-5 and C-6 H), 4.12 (m, 1 H, C-15 H), 3.72 (t, 2 H, $OCH_2$), 3.66 (s, 3 H, $COOCH_3$), 3.00–2.80 (m, 4 H, $-CH_2S$, C-11 and C-12 H), 2.30 (t, 2 H, C-2 H), 1.68 (m, 2 H, C-3 H), 0.88 (t, 3 H, C-20 H).

The zone $R_f$ 0.40–0.60 gave 2.46 g (20%) of **4a**: IR 3400, 1740, 970 $cm^{-1}$; NMR δ 5.84 (dd, 1 H, C-13 H, $J_{13,14}$ = 15 Hz, $J_{12,13}$ = 7 Hz), 5.64 (dd, 1 H, C-14 H, $J_{14,15}$ = 6 Hz, $J_{13,14}$ = 15 Hz), 5.37 (m, 2 H, C-5 and C-6 H), 4.14 (m, 1 H, C-15 H), 3.74 (t, 2 H, $OCH_2$), 3.67 (s, 3 H, $COOCH_3$), 3.60 (m, 1 H, C-11 H), 2.80 (m, 1 H, C-12 H), 2.76 (t, 2 H, $SCH_2$), 2.56 (d, 2 H, C-10 H, *J* = 5 Hz), 2.30 (t, 2 H, C-2 H), 1.64 (m, 2 H, C-3 H), 0.88 (t, 3 H, C-20 H). In addition, 2.83 g (23%) of a mixture of **3a** and **4a** was obtained.

**Conversion of *l*-Prostaglandin $A_2$ Methyl Ester (1a) to *l*-11-Deoxy-11α-[(2-hydroxyethyl)thio]prostaglandin $E_2$ Methyl Ester (3a) Under Equilibrating Conditions.** Treatment of 1.0 g (2.87 mmol) of *l*-$PGA_2$ methyl ester (**1a**)[6] with 0.224 g (2.87 mmol) of β-mercaptoethanol and 12 drops of $Et_3N$ for 18 h gave, after chromatography, 698 mg (56%) of **3a** and 193 mg (16%) of a mixture of **3a** and **4a**. NMR analysis indicated the mixture contained slightly more than 50% of **3a**.

**Conversion of *l*-11-Deoxy-11β-[(2-hydroxyethyl)thio]-prostaglandin $E_2$ Methyl Ester (4a) into the 11α Epimer (3a).** A mixture of 97 mg of **4a** and 1 drop of $Et_3N$ was stirred at ambient temperature for 5 days. NMR analysis indicated that more than 90% conversion to **3a** had occurred.

**General-Base-Catalyzed Michael Additon Procedure. Preparation of *l*-11-Deoxy-11α(β)-[(2,3-dihydroxypropyl)-thio]prostaglandin $E_2$ Methyl Ester (14).** A solution of 1.0 g (2.87 mmol) of *l*-$PGA_2$ methyl ester (**1a**),[6] 0.311 g (2.87 mmol) of 1,2-dihydroxypropanethiol, and 1 drop of $Et_3N$ was stirred at ambient temperature under an argon atmosphere for 18 h. The resulting oil was dry column chromatographed (1.5-in. flat nylon column) on 400 g of silica gel. The column was developed with EtOAc–benzene–HOAc (20:30:2) and then divided into 1-in. segments. Segments 28–38 furnished 200 mg (16%) of product as an oil (see Table V). In general, no attempt was made to separate the 11α and 11β epimers.

***l*-11-Deoxy-11α(β)-[[2-(dimethylamino)ethyl]thio]prostaglandin $E_2$ Methyl Ester (21).** A solution of 2 g (5.74 mmol) of *l*-$PGA_2$ methyl ester (**1a**),[6] 0.652 g (5.74 mmol) of 2-(dimethylamino)ethanethiol hydrochloride, and 0.586 g (5.74 mmol) of $Et_3N$ containing additional (1 drop) $Et_3N$ was stirred at ambient temperature under argon atmosphere for 18 h. After the addition of 10 mL of water and 10 mL of ether, the solution was stirred for several minutes when two layers formed. The ether layer was separated. The aqueous phase was neutralized with $Na_2CO_3$ and extracted several times with ether. The combined ether extracts were washed with saturated sodium chloride solution, dried, and concentrated to give 1.59 g (60%) of product as an oil. A portion (400 mg) was chromatographed on two silica gel preparative thin-layer plates (2 mm), eluting with $CHCl_3$–MeOH (50:50). The section corresponding to $R_f$ 0.27–0.64 furnished 300 mg of **21** as a pale yellow oil (see Table V).

***l*-11-Deoxy-11α(β)-[[2-(diethylamino)ethyl]thio]prostaglandin $E_2$ Methyl Ester (22).** According to the above procedure, treatment of 2 g (5.74 mmol) of *l*-$PGA_2$ methyl ester (**1a**)[6] in 0.586 g (5.74 mmol) of $Et_3N$ with 978 mg (5.7 mmol) of 2-(diethylamino)ethanethiol hydrochloride and 1 drop of triethylamine provided 1.6 g of an oil. Dry column chromatography (EtOAc–MeOH, 50:50) furnished 785 mg (30%) of yellow oil (see Table V).

**7-[3-(2-Hydroxyethoxy)-5-oxo-1-cyclopenten-1-yl]heptanoic Acid (25).** A stirred mixture of 71.8 g (342 mmol) of 7-(5-oxo-1-cyclopenten-1-yl)heptanoic acid (**23**) and 60.9 g (342 mmol) of *N*-bromosuccinimide in 1200 mL of $CCl_4$ was refluxed for 45 min. After cooling to 5 °C, the mixture was filtered and the filtrate was washed with cold water, dried over $MgSO_4$, and filtered. Evaporation at room temperature gave 101.8 g of the crude bromo acid **24**.

A stirred solution of 25.4 g of crude **24** in 430 mL of ethylene glycol was treated with 21.4 g (110 mmol) of silver fluoroborate powder, followed immediately by 11.8 g (110 mmol) of 2,6-lutidine. The resulting mixture was stirred at 25–30 °C for 2 h, diluted with water and ether, and filtered. The filtrate was saturated with solid NaCl, adjusted to pH 3 with 4 N HCl, and further extracted with ether. The extract was washed with brine, dried over $MgSO_4$, and filtered. The residue (13.9 g) obtained on concentration was subjected to chromatography on silica gel with $CHCl_3$ progressively enriched in ether (25–100%) to provide **25** as a light yellow oil (7.0 g, 30% from **23**): NMR δ 7.21 (m, 1 H, C=CH), 4.65 (m, 1 H, OCH), 3.72 (m, 4 H, $OCH_2CH_2O$); UV $\lambda_{max}$ (MeOH) 219 nm (ε 6500). Anal. ($C_{14}H_{22}O_5$) H; C: calcd, 62.20; found, 60.38.

**7-[5-Oxo-3-[2-[(trimethylsilyl)oxy]ethoxy]-1-cyclopenten-1-yl]heptanoic Acid Trimethylsilyl Ester (26).** To a stirred solution of 1.55 g (5.75 mmol) of **25** in 16 mL of pyridine was added 3.70 mL (ca. 17.5 mmol) of hexamethyldisilazane, followed by 1.85 mL (ca. 14.6 mmol) of chlorotrimethylsilane. The resulting mixture was stirred at 25 °C for 18 h. Material volatile at 30 °C was evaporated under vacuum (ca. 0.1 mm), and the residue was treated with 50 mL of petroleum ether and filtered through Celite. The filtrate was evaporated to give 2.10 g (89%) of light yellow liquid: NMR δ 0.41 (s, 9 H, $Me_3Si$ ester), 0.26 (s, 9 H, $Me_3Si$ ether). MS Calcd for $C_{20}H_{38}O_5Si_2$: 414.2257. Found: 414.2241.

***dl*-11-Deoxy-11α-(2-hydroxyethoxy)prostaglandin $E_1$ (27) and *dl*-11-Deoxy-11α-(2-hydroxyethoxy)-15-*epi*-prostaglandin $E_1$ (28).** A stirred solution of 1.64 g (3.3 mmol) of (*E*)-1-iodo-3-(triphenylmethoxy)-1-octene (**30**)[11] in 5 mL of ether was treated with 4.1 mL of 1.6 M *tert*-butyllithium in pentane, and the resulting solution was maintained at −75 °C for 80 min. The solution was treated at −75 °C with a solution prepared from 0.43 g (3.3 mmol) of copper pentyne, 1.65 mL (ca. 6.6 mmol) of tri-*n*-butylphosphine, and 5 mL of ether. The resulting solution of lithiocuprate **31** was stirred at −78 °C for 60 min and treated with a solution of cyclopentenone **26** (1.05 g, 2.54 mmol) in 5 mL of ether. The resulting dark solution was stirred at −40 °C for 60 min and −35 °C for 60 min, recooled to −78 °C, and quenched with a solution of 0.45 mL (ca. 7.5 mmol) of glacial HOAc in 5 mL of ether. The mixture was diluted with ether and poured into a stirred mixture of 35 mL of saturated $NH_4Cl$ and 1.0 mL of 4 N HCl. The ether layer was washed with brine, dried over $MgSO_4$, and concentrated. A stirred mixture of the residue (4.5 g), 50 mL of glacial HOAc, 25 mL of THF, and 12.5 mL of water was heated at 45 °C for 6.5 h. The mixture was partitioned with brine and EtOAc. The EtOAc phase was washed with brine, dried over $MgSO_4$, and concentrated. A mixture of the residue (4.4 g), 50 mL of ether, and 50 mL of 0.4 N $NaHCO_3$ was stirred vigorously for 30 min. The aqueous layer was acidified with 7.2 mL (ca. 120 mmol) of glacial HOAc and extracted with ethyl acetate. The extract was washed with brine, dried over $MgSO_4$, and concentrated. The residue (0.77 g) was subjected to dry column chromatography on 160 g of silica gel, eluting with 1% HOAc in EtOAc. The polar epimer **27** was obtained as an oil (63 mg, 6%): NMR (acetone-$d_6$) δ 5.66 (m, 2 H, CH=CH), 4.05 (m, 1 H, C-15 H), 3.90 (q, 1 H, C-11 H). The mobile epimer **28** was obtained as an oil (152 mg, 15%): NMR (acetone-$d_6$) δ 5.66 (m, 2 H, CH=CH), 4.05 (m, 1 H, C-15 H), 3.90 (q, 1 H, C-11 H). In addition fractions consisting of a mixture of the epimers, $R_f$ 0.38 and 0.50 (1% HOAc in EtOAc), were obtained (100 mg, 10%).

**(*Z*)-7-[3-(2-Hydroxyethoxy)-5-oxo-1-cyclopenten-1-yl]-5-heptenoic Acid Methyl Ester (37).** A stirred suspension of 12.6 g (50 mmol) of 4-methoxycyclopentenone **32**,[12] 5.80 g (52 mmol) of semicarbazide hydrochloride, 4.59 g (58 mmol) of pyridine, and 200 mL of ethylene glycol was stirred at 80 °C for 30 min and at 90–95 °C for 30 min. The solution of crude **33** which resulted was treated with 17.5 mL (ca. 290 mmol) of glacial HOAc and heated at 95 °C for 2 h. The solution was cooled, diluted with water, and extracted with $CHCl_3$. The extract was washed with water, dried over $MgSO_4$, and concentrated to give crude **34** as a red oil (17 g, 100%).

The semicarbazone **34** was dissolved in 150 mL of THF which contained 14.6 g (100 mmol) of 2-ketoglutaric acid and 30 mL of HCl. After 7 h at room temperature, the solution was diluted with brine and extracted with EtOAc. The extract was washed successively with saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$, and concentrated. The residue was subjected to dry column chromatography on silica gel, eluting with 1% MeOH in EtOAc. After development to end of column, the zone, $R_f$ 0.5–0.7, was eluted to provide 5.23 g (37% overall from **32**) of **37** as a light yellow oil: NMR δ 7.28 (s, 1 H, C=CH), 5.53 (m, 2 H, CH=CH), 4.67 (m, 1 H, OCH).

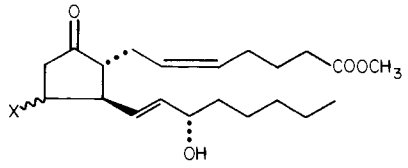
**(*Z*)-7-[5-Oxo-3-[2-[(trimethylsilyl)oxy]ethoxy]-1-cyclopenten-1-yl]-5-heptenoic Acid Methyl Ester (38).** Treatment of 4.04 g (14.3 mmol) of **37** with hexamethyldisilazane and chlorotrimethylsilane as described for the preparation of **26** gave 4.75 g (94%) of **38** as a light yellow liquid. MS Calcd for $C_{18}H_{30}O_5Si$: 354.1862. Found: 354.1868.

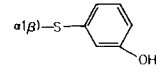
***dl*-11-Deoxy-11α-(2-hydroxyethoxy)prostaglandin $E_2$ Methyl Ester (35) and *dl*-11-Deoxy-11α-(2-hydroxyethoxy)-15-*epi*-prostaglandin $E_2$ Methyl Ester (36).** Cyclopentenone **38** (2.12 g, 6.0 mmol) was submitted to the conjugate addition reaction with lithiocuprate **31** (see preparation of **27** and **28**) and deblocked as described in the preparation of **46** and **47** (see below). The crude products were separated by dry column chromatography on silica gel with the system 30:20:1 EtOAc–benzene–HOAc. The polar epimer **35** was obtained as an oil (0.41 g, 16%): NMR δ 5.66 (m, 2 H, trans CH=CH), 5.38 (m, 2 H, cis CH=CH), 4.05 (m, 1 H, C-15 H), 3.94 (q, 1 H, C-11 H). MS Calcd for $C_{23}H_{36}O_5$: 392.2563. Found: 392.2564.

The mobile epimer **36** was obtained as an oil (0.57 g, 23%): NMR (acetone-$d_6$) δ 5.66 (m, 2 H, trans CH=CH), 5.38 (m, 2 H, cis CH=CH), 4.05 (m, 1 H, C-15 H), 3.94 (q, 1 H, C-11 H). In addition, fractions consisting of a mixture of the epimers, $R_f$ 0.15 and 0.25, were obtained (0.12 g, 5%).

**Preparation of (*Z*)-7-[3-[(2-Hydroxyethyl)thio]-5-oxo-1-cyclopenten-1-yl]-5-heptenoic Acid Methyl Ester (44). A.**

Table V. Analytical and Spectral Data for 11-Deoxy-11-substituted-prostaglandin $E_2$ Congeners




| | | | | $^1$H NMR ($CDCl_3$),[b] δ | | | | | | $H_a$ $H_b$ $HOCH_2$-$CH_2S$ | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| compd | X | formula | anal.[a] | H-13 | H-14 | H-13, H-14 | H-5, H-6 | H-15 | $OCH_3$ | $H_b$ | $H_a$ | $R_f$[c] |
| 3a | α-S($CH_2$)$_2$OH | $C_{23}H_{38}O_5S$ | C, H, S | | | 5.64 (m) | 5.36 (m) | 4.12 (m) | 3.66 (s) | 3.72 (t) | 3.00-2.80 (m)[d] | 0.30[e] |
| 4a | β-S($CH_2$)$_2$OH | $C_{23}H_{38}O_5S$ | C, H, S | 5.84 (d's)[f] | 5.64 (d's)[g] | | 5.37 (m) | 4.14 (m) | 3.67 (s) | 3.74 (t) | 2.76 (t) | 0.26[e] |
| 3b | α-S($CH_2$)$_2$OH (acid) | $C_{22}H_{36}O_5S$ | C, H, S | | | 5.66 (m) | 5.40 (m) | 4.15 (m) | | 3.73 (t) | 3.20-2.60 (m) | 0.26 |
| 4b | β-S($CH_2$)$_2$OH (acid) | $C_{22}H_{36}O_5S$ | C, H, S | 5.83 (d's)[h] | 5.36 (d's)[i] | | 5.40 (m) | 4.14 (m) | | 3.74 (t) | 2.75 (m) | 0.22 |
| 6 | α-S($CH_2$)$_2$OH (15 epi) | $C_{23}H_{38}O_5S$ | C, H, S | | | 5.74 (m) | 5.37 (m) | 4.16 (m) | 3.67 (s) | 2.74 (t) | 3.74 (t) | 0.50 |
| 7 | β-S($CH_2$)$_2$OH (15 epi) | $C_{23}H_{38}O_5S$ | C, H, S | | | 5.66 (m) | 5.38 (m) | 4.15 (m) | 3.67 (s) | 2.78 (t) | 3.73 (t) | 0.36 |
| 8 | α(β)-S($CH_2$)$_2$OH ($E_1$ acid) | $C_{22}H_{38}O_5S$ | C, H, S | | | 5.80 (m)[j] | | 4.15 (m) | | 3.78 (t) | 2.80 (m) | 0.13 |
| 9 | α(β)-S$CH_2CH_3$ | $C_{23}H_{38}O_4S$ | C, H, S | | | 5.66 (m) | 5.36 (m) | 4.14 (m) | 3.67 (s) | | 2.58 (t)[k] | 0.50[l] |
| 10 | α(β)-S($CH_2$)$_2CH_3$ | $C_{24}H_{40}O_4S$ | C, H, S | | | 5.70 (m) | 5.39 (m) | 4.15 (m) | 3.68 (s) | | 2.57 (t)[m] | 0.50[n] |
| 11 | α(β)-S($CH_2$)$_3$OH | $C_{24}H_{40}O_5S$ | C, H, S | | | 5.64 (m) | 5.36 (m) | 4.12 (m) | 3.67 (s) | | 2.72 (m) | 0.34 |
| 12 | α(β)-S$CH_2$C($CH_3$)HOH | $C_{24}H_{40}O_5S$ | H, S; C[o] | | | 5.65 (m) | 5.34 (m) | 4.19 (m) | 3.66 (s) | | 2.75 (m)[p] | 0.20<br>0.34 |
| 13 | α-S$CH_2$C($C_5H_{11}$)HOH | $C_{28}H_{48}O_5S$ | C, H; S[kk] | | | 5.60 (m) | 5.34 (m) | 4.20 (m) | 3.65 (s)[q] | | 3.00-2.60 (m) | 0.43 |
| 14 | α(β)-S$CH_2$C(OH)H$CH_2$OH | $C_{24}H_{40}O_6S$ | C, H, S | | | 5.62 (m) | 5.37 (m) | 4.20-3.70 (m)[r] | 3.66 (s) | | 3.00-2.50 (m)[s] | 0.28[t] |
| 15 | α(β)-S$CH_2$COOH | $C_{23}H_{36}O_6S$ | C, H; S[u] | | | 5.62 (m) | 5.39 (m) | 4.15 (m) | 3.75 (s)[v] | | 3.30 (m) | 0.30 |
| 16 | α(β)-S$CH_2COOC_2H_5$ | $C_{25}H_{40}O_6S$ | C, H, S | | | 5.69 (m) | 5.37 (m) | 4.12 (m) | 3.67 (s)[w] | | 3.28 (m) | 0.43 |
| 17 |  | $C_{27}H_{38}O_5S$ | C, H, S | 5.70 (d's)[x] | 5.48 (d's)[y] | | 5.31 (m) | 4.10 (m) | 3.66 (s)[z] | | | 0.55[aa] |
| 18 | α(β)-S($CH_2$)$_2$SH | $C_{23}H_{38}O_4S_2$ | C, H; S[bb] | | | 5.65 (m) | 5.35 (m) | 4.12 (m) | 3.66 (s) | | 3.00-2.40 (m)[s] | 0.67 |
| 19 | α(β)-S($CH_2$)$_3$SH | $C_{24}H_{40}O_4S_2$ | cc | | | 5.70 (m) | 5.36 (m) | 4.13 (m) | 3.66 (s) | | 3.20-2.50 (m)[s] | 0.70[dd] |
| 20 | α(β)-S($CH_2$)$_2NH_2$ | $C_{23}H_{39}NO_4S$ | ee | | | 5.50 (m) | 5.40 (m) | | 3.66 (s) | | | |
| 21 | α(β)-S($CH_2$)$_2$N($CH_3$)$_2$ | $C_{25}H_{43}NO_4S$ | C, N; S[ff] | | | 5.64 (m) | 5.38 (m) | 4.14 (m) | 3.68 (s) | | 3.68 (s)[gg] | 0.38[hh] |
| 22 | α(β)-S($CH_2$)$_2$N($C_2H_5$)$_2$ | $C_{27}H_{47}O_4NS$ | H, N, S; C[ii] | | | 5.64 (m) | 5.38 (m) | 4.08 (m) | 3.68 (s)[jj] | | | 0.50[hh] |

[a] Analyses indicated by letter only were within ±0.4% of calculated values. [b] All new compounds had infrared data consistent with the assigned structure. [c] $R_f$ values were determined in EtOAc-benzene-HOAc (20:30:1) unless otherwise noted. [d] Includes H-11, H-12. [e] EtOAc-benzene-HOAc (20:30:1), three elutions. [f] $J$ = 15 and 7 Hz. [g] $J$ = 6 and 15 Hz. [h] $J$ = 16 and 6 Hz. [i] $J$ = 6 and 16 Hz. [j] Includes OH, COOH. [k] 1.24 (t, 3 H, $CH_2CH_3$). [l] EtOAc–benzene-HOAc (20:80:2). [m] 0.98 (t, 3 H, $CH_2CH_3$). [n] EtOAc-benzene (1:4). [o] C: calcd, 65.42; found, 64.48. [p] 3.84 (m, 1 H, C*H*-0), 1.22 (t, 3 H, $CHCH_3$). [q] Includes C*H*OH. [r] Includes $CH_2OH$, CHO*H*. [s] Includes H-10, H-11. [t] EtOAc-HOAc (100:1). [u] S: calcd, 7.28; found, 7.74. MS Calcd for $C_{23}H_{34}O_5S$ (M – $H_2O$): 422.2126. Found: 422.2107. [v] 7.19 (m, 2 H, OH, COOH). [w] 4.20 (m, 2 H, $COOCH_2$–), 1.28 (t, 3 H, $OCH_2CH_3$). [x] $J$ = 15 and 6 Hz. [y] $J$ = 16 and 15 Hz. [z] 7.50-6.70 (m, 4-aromatic). [aa] EtOAc-benzene-HOAc (20:80:1). [bb] S: calcd, 14.49; found, 13.68; MS Calcd for $C_{23}H_{36}O_3S_2$(M – $H_2O$): 424.210. Found: 424.2080. [cc] MS Calcd for $C_{24}H_{38}O_3S$ (M – $H_2O$): 438.2263. Found: 438.2257. [dd] EtOAc-benzene (2:3). [ee] MS Calcd for $C_{23}H_{37}NO_3S$ (M – $H_2O$): 407.2493. Found: 407.2484. [ff] S: calcd, 7.07; found, 6.51; *m/e* 453 ($M^+$ Calcd for $C_{25}H_{43}NO_4S$: 453.2913. Found: 453.2911). [gg] 3.35 (s, 1 H, OH), 2.26 [s, 6 H, $N(CH_3)_2$]. [hh] EtOAc–MeOH (50:50). [ii] C: calcd, 67.32; found, 66.86; *m/e* 481 ($M^+$ Calcd for $C_{27}H_{47}NO_4S$: 481.3226. Found: 481.3208). [jj] 3.38 (s, 1 H, OH), 1.03 [t, 6 H, $N(CH_3CH_2)_2$]. [kk] S: calcd, 6.49; found, 5.86.

**From (*Z*)-7-(3-Methoxy-5-oxo-1-cyclopenten-1-yl)-5-heptenoic Acid Methyl Ester (42).** To a stirred solution of 7.57 g (30 mmol) of **42**[12] in 100 mL of methanol were added a solution of β-mercaptoethanol in 50 mL of methanol and then, during a period of 2 min, 15 mL of 0.1 N sodium methoxide in methanol (1.5 mmol). The solution was stirred at 25 °C for 50 min, quenched with 0.27 mL (ca. 4.5 mmol) of glacial HOAc, diluted with 150 mL of brine and 100 mL of water, and extracted with ether. The extract was washed with brine, dried over $MgSO_4$, and concentrated. The residue (10.4 g) was dissolved in 5 mL of 75:25:2 EtOAc–heptane–methanol and developed on a dry column of 465 g of Woelm Activity III silica gel. After development to end of the column, the product **44** was located (zone $R_f$ 0.43–0.64) and eluted with ethyl acetate to give 5.55 g (62%) of light yellow liquid, spectroscopically and chromatographically identical with the material prepared by method B.

**B. From (*Z*)-7-[2-Oxo-5β-[(trimethylsilyl)oxy]-3-cyclopenten-1α-yl]-5-heptenoic Acid Methyl Ester (43).** To a stirred solution of 2.25 g (7.25 mmol) of **43** in 24 mL of methanol were added a solution of 595 mg (7.6 mmol) of β-mercaptoethanol in 12 mL of methanol and then, during a period of 3 min, 3.6 mL of 0.1 N sodium methoxide in methanol (0.36 mmol). The solution was stirred at 25 °C for 45 min, then quenched with 66 mg (ca. 1.1 mmol) of glacial HOAc, and worked up with ether and brine as above to give 2.0 g of amber oil. The crude product was dissolved in 5 mL of 75:25:2 EtOAc–heptane–HOAc and developed on a dry column of 100 g of Woelm Activity III silica gel. After development to end of the column, the product **44** was located (zone $R_f$ 0.46–0.66) and eluted with ethyl acetate to give 1.33 g (62%) of light yellow liquid: NMR δ 4.03 (m, 1 H, CHS), 3.78 (t, 2 H, $J$ = 6 Hz, $CH_2O$), 3.67 (s, 3 H, $COOCH_3$), 2.75 (t, 2 H, $J$ = 6 Hz, $CH_2S$). Anal. ($C_{15}H_{22}SO_4$) C, H, S.

**(*Z*)-7-[5-Oxo-3-[[2-[(trimethylsilyl)oxy]ethyl]thio]-1-cyclopenten-1-yl]-5-heptenoic Acid Methyl Ester (45).** To a stirred, ice-cold solution of 2.98 g (10 mmol) of cyclopentenone **44** in 10 mL of pyridine was added 2.50 mL of hexamethyldisilazane, followed during a 2 min period with 1.25 mL of chlorotrimethylsilane. The resulting mixture was stirred at ambient temperature for 2.5 h, and then volatile matter was evaporated from the mixture under high vacuum at ca. 30 °C. The residue was slurried with petroleum ether and filtered through Celite. The filtrate was evaporated to give 3.75 g (ca. 100%) of light yellow liquid: TLC (1:1 heptane–EtOAc) $R_f$ 0.69; NMR δ 3.79 (t, 2 H, $J$ = 7 Hz, $CH_2O$), 0.12 [s, 9 H, $(CH_3)_3Si$].

***dl*-11-Deoxy-11α-[(2-hydroxyethyl)thio]prostaglandin $E_2$ Methyl Ester (46) and *dl*-11-Deoxy-11α-[(2-hydroxyethyl)-thio]-15-*epi*-prostaglandin $E_2$ Methyl Ester (47).** To a stirred solution of 2.48 g (5.0 mmol) of (*E*)-1-iodo-3-(triphenylmethoxy)-1-octene (**30**)[11] in 20 mL of ether at −78 °C was added 6.25 mL of 1.6 M *tert*-butyllithium in pentane during 10 min. The solution was stirred at −78 °C for 1 h, warmed to −45 °C during 15 min, and then recooled to −78 °C. A second solution was prepared from 653 mg (5.0 mmol) of copper pentyne and 2.1 mL of hexamethylphosphorous triamide, stirred at 25 °C for 20 min, diluted with 15 mL of ether, and cooled to −78 °C. To the solution of octenyllithium prepared above was added the solution of copper complex at ca. −70 °C during 10 min with stirring. The gold yellow solution was stirred at −78 °C for 60 min and then treated with a solution of 1.48 g (4.0 mmol) of cyclopentenone **45** in 10 mL of ether during 10 min. The mixture was stirred at −75 °C for 10 min, warmed to −45 °C during 10 min, and stirred at −45 to −40 °C for 2 h. The mixture was recooled to −78 °C and quenched during 1 min with a solution of 0.60 mL (ca. 10 mmol) of glacial HOAc in 10 mL of ether. The mixture was diluted with 100 mL of ether and transferred into a stirred ice-cold solution prepared from 50 mL of saturated $NH_4Cl$ and 10 mL of 4 N HCl. After phase separation, the aqueous phase was extracted with 100 mL of ether. The combined extracts were washed successively with two 20-mL portions of cold 2 N HCl, water, and brine, dried over $MgSO_4$, and concentrated to give 3.24 g of crude material. This was dissolved in a solution prepared from 32 mL of HOAc, 16 mL of THF, and 8 mL of water. The solution was stirred at 45 °C for 6 h, cooled, diluted with 80 mL of half-saturated brine, and extracted with ether. The extract was washed successively with half-saturated brine and brine, dried over magnesium sulfate, and evaporated with toluene chaser to give 3.03 g of a mixture of oil and crystalline triphenylcarbinol. The crude product was dissolved in 5 mL of 60:40:1 benzene–EtOAc–HOAc and developed on a dry column of 300 g of Woelm Activity III silica gel which had been equilibrated with 17 g of the same solvent (2.6 × 89 cm, 60:40:1 benzene–EtOAc–HOAc, 430 mL of eluant). The zone 36–46 cm from the top of the column gave 0.13 g (8%) of pure **46**: NMR δ 4.15 (C*H*OH); IR 1736 $cm^{-1}$. Anal. ($C_{23}H_{38}SO_5$) H, S; C: calcd, 64.75; found, 63.92.

The zone 46–57 cm from the top of the column gave 0.30 g (17%) of a mixture enriched in **46**; the zone 57–66 cm contained 0.40 g (24%) of a mixture enriched in **47**.

The zone 66–72 cm from the top of the column contained 0.15 g (9%) of pure **47**: TLC $R_f$ 0.36; NMR δ 4.12 (C*H*OH); IR 1736 $cm^{-1}$. Anal. ($C_{23}H_{38}SO_5$) H, S; C: calcd, 64.75; found, 64.15.

***dl*-11,15-Dideoxy-11α(β)-[(2-hydroxyethyl)thio]-16α(β)-hydroxyprostaglandin $E_1$ (51).** To a stirred solution of 189 mg (0.56 mmol) of *dl*-15-deoxy-16α(β)-hydroxy-$PGA_1$ (**50**),[16,17] 60 mg (0.77 mmol) of β-mercaptoethanol, and 1 mL of THF was added 90 mg (0.89 mmol) of triethylamine. After 3 h the reaction mixture was purified by preparative thin-layer chromatography (silica gel; 1 mm; 2:3 EtOAc–benzene + 2% acetic acid) to give 111 mg (48%) of **51** as a yellow oil: NMR δ 5.95–5.35 (m, 2 H, C-13 and C-14 H), 4.20–3.40 (m, 3 H, $CH_2OH$, C-16 H), 3.20–2.48 (m, 5 H, OH, $-CH_2S-$), 0.96 (m, 3 H, C-20 H). MS Calcd for $C_{20}H_{30}O_3$ (M − $H_2O$ − $HOCH_2CH_2SH$): 318.2195. Found: 318.2190.

***dl*-11-Deoxy-11α(β)-[(2-hydroxyethyl)thio]-15α(β)-methylprostaglandin $E_1$ (49).** By the procedure described above for **51** (sans THF) using 200 mg (0.58 mmol) of *dl*-15α(β)-methyl-$PGA_1$ (**48**),[18] 143 mg (58%) of **49** was isolated as a yellow oil: IR 3450, 1750, 1700, 975 $cm^{-1}$; NMR δ 5.68 (m, 2 H, C-13 and C-14 H), 3.75 (m, 2 H, $HOCH_2-$), 2.76 (m, 2 H, $-CH_2S$), 0.88 (t, 3 H, C-20 H). Anal. ($C_{23}H_{40}O_5S$) C, H, S.

***dl*-11,15-Dideoxy-11α-[(2-hydroxyethyl)thio]prostaglandin $E_2$ Methyl Ester (53).** A solution of 10.0 g (0.0908 mol) of 1-octyne, 60 mL of anhydrous toluene, 230 mg of azobisisobutyronitrile (AIBN), and 25 mL (0.0943 mol) of tributylstannane was refluxed under an argon atmosphere for 3 h. The reaction mixture was cooled to ambient temperature and concentrated in vacuo. The resulting yellow liquid was distilled (Kugelrohr) to give 36.29 g (99%) of (*E*/*Z*)-1-(tributylstannyl)-1-octene (**54**) as a pale yellow liquid.

To a stirred solution of 4.4 g (5.4 mmol) of **54** and 12 mL of anhydrous THF, cooled to −78 °C, was slowly added 5.2 mL (0.0114 mmol) of *n*-BuLi. After 15 min, the reaction mixture was warmed to −25 °C for 1.5 h, recooled to −78 °C, and to it was added a solution of 1.70 g (13.0 mmol) of copper(I) pentyne, 7 mL of hexamethylphosphorous triamide (HMPTA), and 20 mL of anhydrous ether. After 1 h, a solution of 2.0 g (0.54 mmol) of cyclopentenone **45** and 15 mL of anhydrous ether was added. The resulting solution was stirred at this temperature for 30 min, warmed to −30 °C for 2 h, recooled to −78 °C, and to it was added 5 mL of glacial HOAc. The reaction mixture was poured into a saturated solution of $NH_4Cl$ and was vigorously stirred for 30 min. The aqueous phase was separated and extracted with 150 mL of ether in two portions. The combined organic phases were washed with 5% HCl solution and brine, and concentrated in vacuo to give an amber oil. A solution of the oil and 140 mL of HOAc–THF–$H_2O$ (4:2:1) was stirred at ambient temperature for 1 h, diluted with toluene, and concentrated in vacuo to give 6.1 g of amber oil. This oil was applied to a silica gel dry column (3 × 55 in.; 2:3 EtOAc–benzene + 2% acetic acid; 600 mL of eluant was collected). The product **53** (951 mg, 43%) was isolated from the column at $R_f$ 0.73–0.81 as a yellow oil: IR 3550, 1740, 970 $cm^{-1}$; NMR δ 5.77–5.23 (m, 4 H, C-13 and C-14 H, C-5 and C-6 H), 3.77 (t, 2 H, $J$ = 6 Hz, $HOCH_2CH_2-$), 3.70 (s, 3 H, $-COOCH_3$), 3.10–2.56 (m, 3 H, $-CH_2S-$, C-11β H), 0.90 (t, 3 H, C-20 H). Anal. ($C_{23}H_{38}O_4S$) H, S; C: calcd, 67.27; found, 66.81.

***dl*-11,15-Dideoxy-11α-[(2-hydroxyethyl)thio]-16-hydroxy-16-methylprostaglandin $E_2$ Methyl Ester (52).** The conjugate

(17) For the preparation of *dl*-15-deoxy-16α(β)-hydroxy-$PGE_1$, see M. B. Floyd, R. E. Schaub, and M. J. Weiss, *Prostaglandins*, **10**, 289 (1975).

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addition of the cuprate derived from (*E*)-1-(tributylstannyl)-4-methyl-4-[(trimethylsilyl)oxy]-1-octene[19] (7.2 mmol) to cyclopentenone **45** (3.5 mmol) by the procedure described above for **53** furnished an amber oil. The trimethylsilyl groups of the conjugate adduct were removed (HOAc–THF–$H_2O$, 4:2:1) and the resulting brown oil was applied to a silica gel dry column (3 × 55 in.; 2:3 EtOAc–benzene + 2% acetic acid; 700 mL of eluant was collected). From the column at $R_f$ 0.35–0.45 was isolated 350 mg (23%) of **52** as a yellow oil: NMR $\delta$ 5.80–5.23 (m, 4 H, C-13 and C-14 H, C-5 and C-6 H), 3.73 (m, 2 H, $HOCH_2$), 3.70 (s, 3 H, $-COOCH_3$), 3.16–2.63 (m, 3 H, $-CH_2S-$, C-11$\beta$ H), 1.23 (s, 3 H, $-CH_3$), 0.93 (t, 3 H, C-20 H). MS Calcd for $C_{22}H_{34}O_4$ (M – $HOCH_2CH_2SH$): 362.2457. Found: 362.2450.

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# Anticandidal Activity of Pyrimidine-Peptide Conjugates

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The ability of conjugates of peptides and 5-fluorocytosine or 5-fluoroorotic acid to enter *Candida albicans* was investigated. A number of conjugates of 5-fluoroorotic acid and peptides were synthesized using 1-(ethoxycarbonyl)-2-ethoxy-1,2-dihydroquinoline as the coupling agent. Orotyl-L-leucyl-L-leucine, 5-fluoro-4-(*N*-succinamoyl-L-alanyl-L-leucine)-2(1*H*)-pyrimidinone [a 5-fluorocytosine derivative], and 5-fluoroorotyl-L-leucyl-L-leucine all inhibited the uptake of trimethionine into *C. albicans* WD 18-4. Inhibition by 5-fluoroorotyl-L-leucyl-L-leucine was competitive as judged using double-reciprocal plots. Evaluation of minimun inhibitory concentrations of peptide–5-fluorocytosine conjugates suggest that these conjugates enter *C. albicans* in the intact form. These results provide the first experimental evidence that peptides can carry pyrimidines into a eukaryote.

Many potential therapeutic agents are ineffective because of the failure of the active molecules to permeate the cell membrane. Attempts to overcome this problem include modification with lipophilic groups, attachment to macromolecules, and entrapment in liposomes. Another approach employs the use of active-transport systems to bring potentially cytotoxic drugs into cells. The advantage of this approach is that it can result in a 100- to 1000-fold concentration of the toxic agent inside the cell.

Several investigators have demonstrated that peptides can carry normally impermeant molecules into microorganisms. Fickel and Gilvarg[1] with *Escherichia coli* and Ames and co-workers[2] using *Salmonella typhimurium* showed that the metabolic intermediates homoserine phosphate and histidinol phosphate, respectively, could enter bacteria when incorporated into a peptide. Toxic amino acid analogues such as ethionine were smuggled into *S. typhimurium* as peptide residues,[2] and recently L-1-aminoethylphosphonic acid was incorporated into a peptide and brought into both Gram-negative and Gram-positive bacteria through the dipeptide transport system.[3] Although the amino acid mimetic L-1-aminoethylphosphonic acid itself was inactive, it was responsible for broad antibacterial activity when introduced as a dipeptide. Peptides have also been shown to carry a number of other unusual amino acids across the cell membrane of *C. albicans*.[4]

Previous studies from our laboratory have attempted to use peptides in the design of drugs for *Candida albicans*.[5-8] We have described the structural specificity of the peptide transport system in one strain of *Candida*[5,8] and have developed synthetic procedures to conjugate peptides to 5-fluorocytosine (5-FC).[7] Several peptide–5-fluorocytosine conjugates strongly inhibited the growth of a number of yeast strains. At the time of that report, the poor stability of these conjugates in solution prevented us from demonstrating whether they entered *C. albicans* via the peptide transport system. In this article, we extend our studies on peptide–5-fluorocytosine conjugates and report on peptide conjugates with 5-fluoroorotic acid. Using both microbiological assays and competition with the uptake of radioactive trimethionine, we provide evidence that conjugates of peptides and pyrimidines can enter yeast via the peptide transport system.

## Results

**Synthesis of Peptide–5-Fluoroorotic Acid Conjugates.** Previous attempts to prepare ester or amide derivatives of the 6-carboxyl group of orotic or fluoroorotic acid have used the corresponding acid chloride as the reactive intermediate. Many of these syntheses were conducted in nonpolar solvents such as $CHCl_3$, and in a typical procedure the orotic acid or its acid chloride and alcohol

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