8.6, in a 5-mL Radiometer V531 jacketed assay vessel equilibrated at 2 °C was added buffer-diluted enzyme (25 mL). CO_2/air (5/95) was bubbled into the assay vessel at a rate of 150 mL/min. The pH stat end point was set at pH 8.3, and the volume of 0.025 N NaOH added over a 3-min period in order to maintain pH 8.3 was measured. Enzyme inhibition was measured by the addition of an inhibitor in 0.1-3.9 mL of buffer followed by the addition of enzyme and titration with NaOH. Results were expressed as the I_{50} values, which were obtained from semilog plots of percent inhibition against log concentration.

In Vitro Binding for Human Carbonic Anhydrase II. The binding of test compounds to purified human erythrocyte carbonic anhydrase II was determined by a fluorescence competition assay employing the fluorescent CA inhibitor dansylamide. This compound has been shown to produce a large increase in fluorescence upon binding to the active site of carbonic anhydrase. A fluorescence cuvette containing 1×10^{-7} M human CA II (HCA II) and 2×10^{-6} M dansylamide in pH 7.4, 0.1 ionic strength phosphate buffer was placed in the thermostated cell holder of a Perkin-Elmer MPF-44B fluorescence spectrophotometer. The temperature was maintained at 37 °C by using a constant-temperature water circulator. The excitation and emission wavelengths were set at 280 and 460 nm, respectively. Fluorescence intensities were recorded following addition, with stirring, of small, measured aliquots of a solution of the test compounds in pH 7.4 buffer. The resulting data were converted to fluorescence intensity vs compound concentration, corrected for dilution by the titrant, and fitted by nonlinear least squares to a model in which the compound and dansylamide compete for a single binding site on HCA II. The dissociation constant of the dansylamide-HCA II complex, which is needed for these calculations, was found to be 1.98×10^{-6} M under these conditions. It was found in all cases that the data fitted well to a single-site model. There was no evidence for additional, lower-affinity binding sites. All binding determinations were done a minimum of three times.

Acknowledgment. We thank Ms. Jean Kaysen for the preparation of this manuscript.

PgH_2 Analogs as Potential Antiplatelet Derivatives

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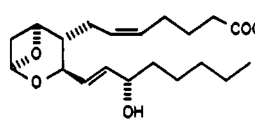
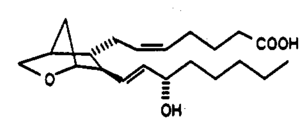
Previous observations implicating PgH_2 as a direct activator of platelets suggested that derivatives of U46619, a well-characterized TxA_2 receptor agonist having structural homology with PgH_2, might possess antiplatelet activity. The present work describes the synthesis of [1S-(1α,2β,3α,4α)]-3-[(tetrahydropyranyloxy)methyl]-2-[2-[(triphenylmethyl)oxy]ethyl]-5-oxabicyclo[2.2.1]heptane (**14**) a potentially useful intermediate for the synthesis of various epoxymethano derivatives. The latter was converted to [1S-(1α,2β(Z),3α,4α)]-7-[3-[[2-[(phenylamino)carbonyl]-hydrazino]methyl]-5-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid (**23**), an epoxymethano derivative of PgH_2 containing a hydrazide lower side chain as previously used in the TxA_2 antagonist, SQ 29,548. The intermediate **14** was also converted to [1S-(1α,2β(Z),3α,4α)]-7-[3-[(hexylamino)methyl]-5-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid (**25**) which contained a simple aza side chain as used in earlier antagonists. Derivatives **23** and **25** appeared to be specific antagonists of the human platelet TxA_2 receptor as evidenced by their inhibition of U46619 (1.5 μM) induced aggregation of human platelet rich plasma (IC_{50} = 22 and 7 μM, respectively), while having little effect on ADP (2 μM) induced aggregation at much higher concentrations. In addition, one of these derivatives, the bicycloamine **25**, was shown to compete for [^{3}H]U46619 binding to washed human platelets with an IC_{50} value of 25 μM, supporting the notion that these derivatives were acting at the thromboxane receptor. However, the potency of these derivatives was less than for previously reported TxA_2 antagonists, suggesting that simple linear combinations of functionality from molecules active at the human platelet thromboxane receptor will be of limited predictive value.

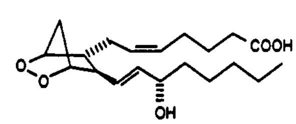
Considerable effort has been expended in an attempt to find specific TxA_2 (**1**) synthase inhibitors and TxA_2 receptor antagonists as potential antithrombotic agents. The latter work has resulted in a number of diverse structures which are reported to be specific TxA_2 antagonists.[1-9] The emphasis on TxA_2 logically stems from the original notion that TxA_2 production in the platelet was prerequisite to platelet activation by arachidonic acid. Yet evidence has indicated that PgH_2 (**2**) itself is directly capable of stimulating platelet functional change,[10] and interaction of PgH_2 with the platelet receptor appears to be coupled to calcium mobilization and thus presumably platelet functional change.[11] In addition, at least one of the best characterized TxA_2 receptor agonists, U46619 (**3**),[12,13] is closely related to PgH_2 in its structure.



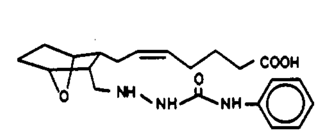
1
TXA_2



3
U46619



2
PGH_2



4
SQ29,548

On the basis of such observations, it seemed reasonable to explore compounds directly related to the structure of PgH_2 as a possible new class of antiplatelet derivatives. In the present work we describe a general synthetic scheme for preparation of side chain modified derivatives of PgH_2 which also incorporate the stable epoxymethano functionality of U46619 while retaining the natural PgH_2 chirality. It is hoped that the study of such derivatives may ultimately lead to effective antiplatelet compounds and to a better understanding of structure-activity rela-

† Department of Medicinal Chemistry and Pharmacognosy.
‡ Department of Pharmacology.

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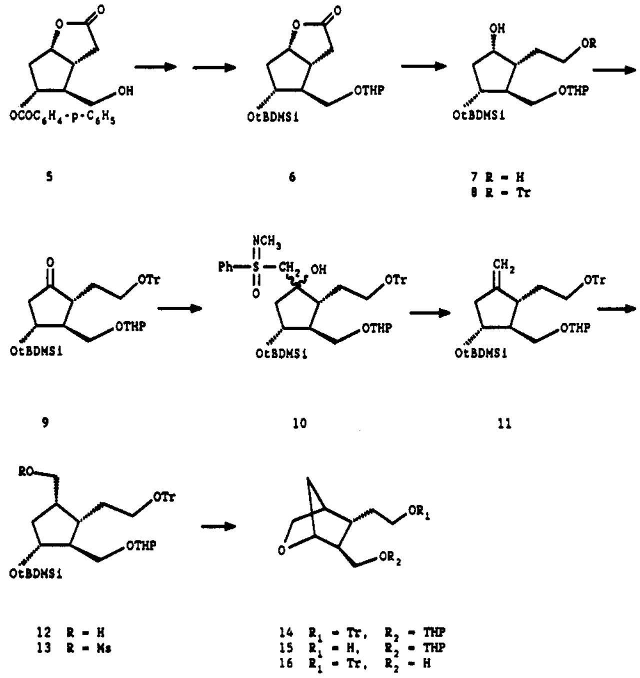
tionships for derivatives which inhibit platelet activation.

Chemistry

The present work describes the synthesis of an oxabicyclo derivative **14**, which could be used for this purpose, by virtue of the flexibility provided through selective cleavage of its protecting groups and varied side chain elaboration. In this study, the oxabicyclo derivative was converted to target derivatives **23** and **25** which were tested for antiplatelet activity. The former incorporated a hydrazide lower side chain as previously used in the potent TxA_2 antagonist, SQ 29,548 (**4**), previously prepared by Nakane and co-workers[8,9] and the latter, a simple aza side chain as used in earlier TxA_2 antagonists.[1]

The starting lactone **6** was obtained in three steps (83% overall) from the commercially available lactone 4-phenylbenzoate **5** as previously described.[14] Sodium borohydride reduction of this lactone gave the diol **7** which, after selective tritylation, was oxidized with the pyridinium dichromate–molecular sieve system[15] in dichloromethane to provide the keto derivative **9**. Using the same reaction sequence employed by Bundy in his synthesis of U46619, the ketone **9** was converted to the exocyclic methylene derivative **11** by treatment with [(*N*-methyl-*S*-phenylsulfonimidoyl)methyl]magnesium chloride[16] in tetrahydrofuran, followed by reductive elimination of the β-hydroxy sulfoximine. Hydroboration with 9-borobicyclo[3.3.2]nonane (9-BBN)[17] and subsequent oxidation afforded the primary alcohol **12**. The stereochemistry of **12** was initially assigned on mechanistic grounds (attack from the least crowded face of the molecule). Mesylation of **12** and subsequent treatment with tetrabutylammonium fluoride[18] in tetrahydrofuran furnished the desired oxabicyclo derivative **14** directly. There was no indication of the intermediate free alcohol as had been expected. Apparently, deblocking results in simultaneous cyclization in this particular molecule. The lack of any other significant product in the cyclization reaction provided further evidence that only the α-isomer was produced in the hydroboration step.



The tetrahydropyranyl group could be selectively removed with acetic acid in tetrahydrofuran/water[19] to give **16**, without indication of trityl cleavage. On the other hand, the trityl group could be selectively removed with sodium–ammonia[20] to provide **15**, which was free from products resulting from cleavage of the tetrahydropyranyl ether. The two oxabicyclo derivatives **15** and **16** were produced in good overall yield from the starting lactone

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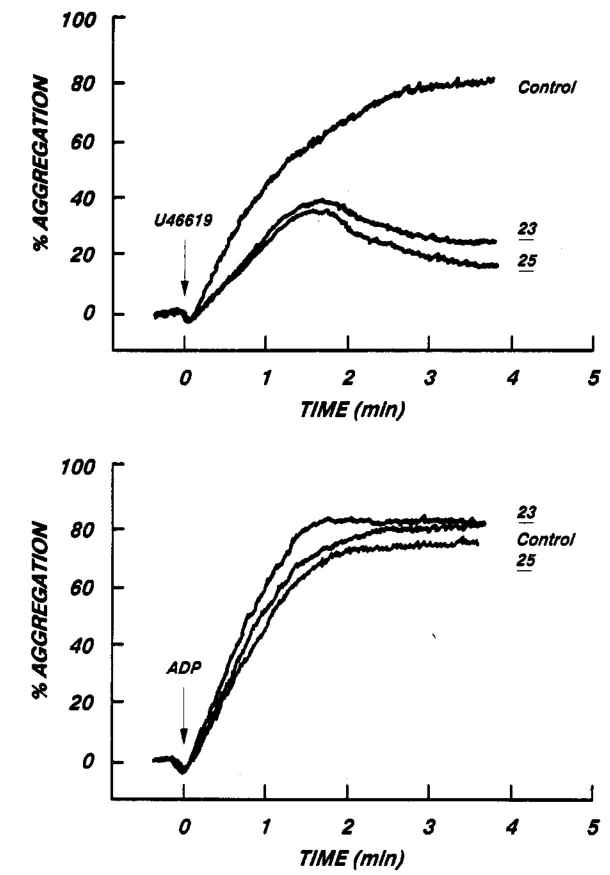
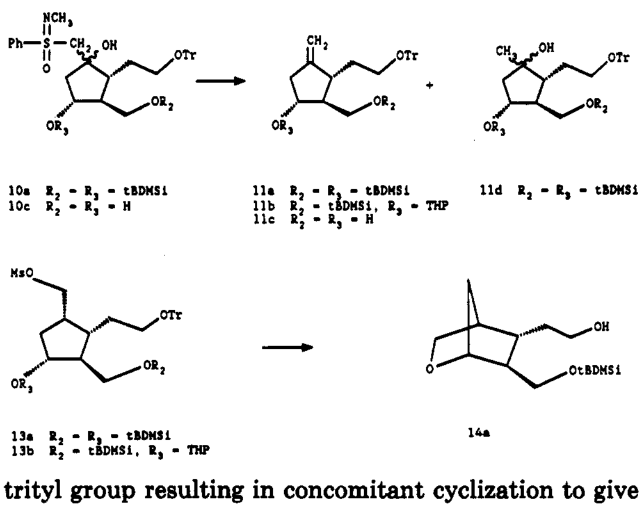


Figure 1. Effect of **23** and **25** on U46619 or ADP-induced human platelet aggregation. Platelet-rich plasma was pretreated with **23** or **25** for 1 min prior to addition of U46619 (1.5 μM) or ADP (2 μM). Upper panel: 12 μM of **23** and 90 μM of **25**. Lower panel: 90 μM of **23** or **25**. These aggregation traces are representative of three separate experiments.

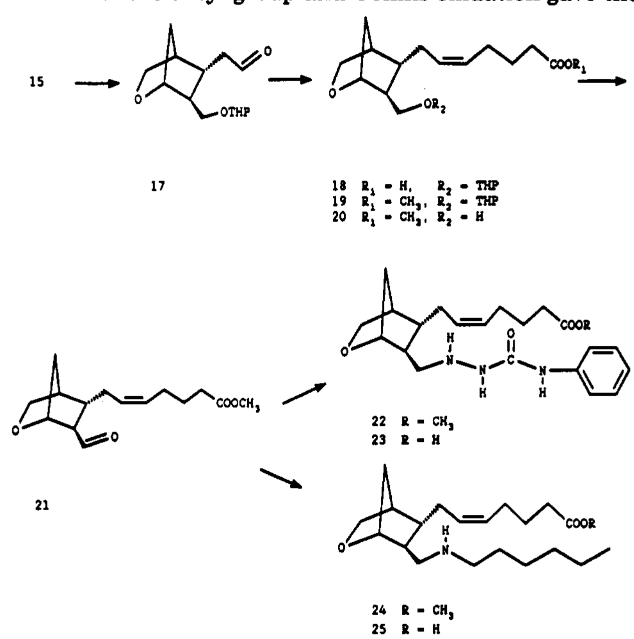
5 and should be versatile derivatives for synthesizing a variety of U46619-type analogues with retention of the PgH_2 chirality.

Preparation of the oxabicyclo derivative **14**, involved the screening of a number of protecting group combinations in order to obtain the desired selectivity. For example, with **13b**, we had hoped to selectively deblock the tetrahydropyranyl ether in the presence of the trityl group, however, due to simultaneous cleavage, we obtained a second compound in addition to the desired oxabicyclo derivative **14a**, which appeared to be the other possible bicyclo derivative. With derivative **13a**, it was possible to selectively cleave the silyl ethers in the presence of the



trityl group resulting in concomitant cyclization to give only **16**. However, the reductive elimination step in the preparation of **13a** from **10a**, gave only 60% of the desired *exo*-methylene **11a**, the remaining being the alcohol **11d**.[21] The reason why the *tert*-butyldimethylsilyl group alters the course of this reaction relative to the tetrahydropyranyl ether is not clear. However, steric phenomena are implicated by the fact that only the *exo*-methylene derivative **11c** was obtained when reductive elimination was performed on the diol **10c** formed by removing both silyl ethers.

Conversion of this central intermediate **14** to the initial targets, oxabicyclo hydrazide **23** and oxabicyclo amine **25**, was relatively straightforward. Thus, selective reductive removal of the trityl group and Collins oxidation gave the



aldehyde **17**, to which the upper side chain was added with the usual Wittig reaction. The acid **18**, resulting from the Wittig workup, was esterified and deblocked and the alcohol **20** oxidized to the corresponding aldehyde **21**. Finally, the lower side chains were conveniently introduced in a condensation/reduction reaction with sodium cyanoborohydride and 4-phenylsemicarbazide or hexylamine. The former gave hydrazino derivative **22** while the later gave the amino derivative **24**. Hydrolysis of **22** and **24** gave the target derivatives **23** and **25**, respectively. The hydrazino derivative **23** proved to be unstable in solution but stable when stored as a dry solid. The reason for this instability is not known, but is presumably due to γ-oxahydrazino orientation, as the corresponding functionality in the SQ 29,548 does not show comparable activity.

Biological Results and Discussion

The target bicyclo hydrazide **23** and amine **25** were tested for inhibition of both U46619 and ADP-induced aggregation in human platelet rich plasma (PRP) using standard methodology.[22] The bicyclo amine **25** was shown to inhibit U46619 (1.5 μM) with an IC_{50} = 22 μM and the bicyclo hydrazine **23** with an IC_{50} = 7 μM (Figure 1). Neither compound showed any measurable agonist activity

(21) Johnson and co-workers have reported[16] the Al(Hg) reduction of sulfoximines as a general reaction to produce alcohols ($PhSCH_2COH \rightarrow CH_3COH$). However, in presence of acetic acid, which potentiates elimination of water, the same reaction gives the corresponding alkene derivative ($\rightarrow CH_2{=}CH$).

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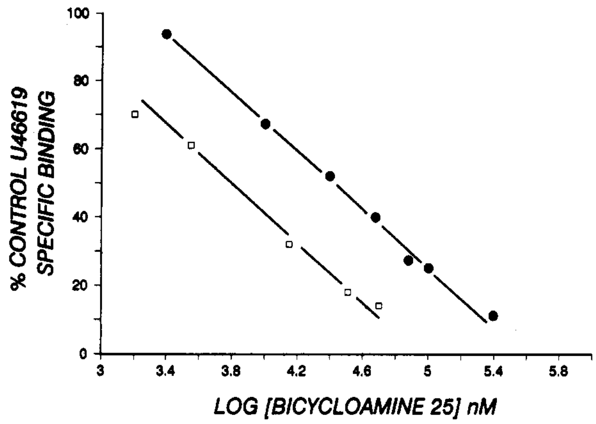

Figure 2. Inhibition of specific binding of [^{3}H]U46619 by bicycloamine **25** (●) or 13-azaprostanoic acid (□) in prostacyclin-treated human platelets. Each point is the average of two experiments performed in duplicate.

at concentrations 10-fold higher than their IC_{50} values.

That the inhibition of these derivatives is specific for the arachidonic acid pathway in the platelet is shown by the fact that neither compound showed significant inhibition of ADP (2 μM) induced aggregation. Thus, **23** or **25** in concentrations as high as 90 μM, did not show significant inhibition of ADP induced aggregation of human PRP. To further demonstrate that the pharmacological effects of the bicyclo amine **25** on platelet aggregation resulted from action at the TxA_2/PgH_2 receptor, binding studies using [^{3}H]U46619 were performed. The amine **25** was found to inhibit the specific binding of [^{3}H]U46619 in a dose-dependent manner, with the concentration for 50% inhibition being approximately 25 μM (Figure 2), which corresponds closely with the IC_{50} = 22 μM observed for platelet inhibition. For comparison, inhibition of specific [^{3}H]U46619 binding by the somewhat more active 13-azaprostanoic acid (IC_{50} = 6 μM) is also included in Figure 2.

It is instructive to compare the activity of these derivatives with previous TxA_2 antagonists. Thus, bicyclo amine **25**, containing a similar lower side chain to 13-azaprostanoic acid but with the epoxymethano functionality as a head group, did not show enhancement in activity over the simple 13-azaprostanoic acid (IC_{50} = 6 μM).[2] Similarly, the bicyclo hydrazine **23** was not nearly as active as the Squibb bicyclo derivative, SQ 29,548 (IC_{50} = 0.06 μM).[23] As has been demonstrated for the SQ derivatives, side chain stereochemistry may be important.[24] However, this can not be a universal truth, as the cis and trans isomers of 13-azaprostanoic acid have demonstrated.[1] These observations would suggest that simple linear combinations of head group, side chain stereochemistry, and lower side chain functionality is unlikely to be of predictive value for all compounds active as antagonists at the thromboxane receptor.

Experimental Section

Reactions were monitored by thin-layer chromatography (TLC), using Merck silica gel 60 F_{254} (0.2 mm) sheets. Spots were visualized by UV light, iodine vapor, or 30% aqueous sulfuric acid spray followed by charring with a heat gun. Flash column chromatography[25] was performed on silica gel 60 (230–400 mesh, Merck). All products reported were homogeneous by TLC and gave NMR spectra consistent with the assigned structures. Diastereomers caused by the tetrahydropyranyl (THP) ether protecting group showed doubling of many signals in the proton NMR. ^{1}H NMR spectra were recorded with a Varian XL-300. Chemical shifts were reported in parts per million (δ) downfield from internal $(CH_3)_4Si$ or $CHCl_3$ assigned at δ 7.24. Mass spectra were recorded on a Finnigan MAT 90 spectrometer in either chemical ionization (CI, NH_3) or electron impact (EI) modes. Melting points were measured on a Thomas–Hoover capillary melting point apparatus and are uncorrected. Solvents were routinely distilled before use: tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl, CH_2Cl_2 was distilled from P_2O_5, dimethyl sulfoxide (DMSO) and dimethylformamide (DMF) from potassium hydride. DMF, CH_2Cl_2, and DMSO were stored over activated 4-Å molecular sieves.

[1*S*-(1α,2β,3α,5α)]-2-[3-[(*tert*-Butyldimethylsilyl)oxy]-5-hydroxy-2-[(tetrahydropyranyloxy)methyl]cyclopentanyl]ethanol (7). A solution of lactone **6** (366 mg, 1 mmol) in MeOH (4 mL) and H_2O (1 mL) was treated at room temperature with sodium borohydride (190 mg, 5 mmol). After 4 h at room temperature, additional sodium borohyride (2 mmol, 76 mg) was added. The reaction mixture was then stirred for an additional 2 h, neutralized by the addition of solid $NaHSO_4$ and diluted with CH_2Cl_2. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried ($MgSO_4$), filtered, and concentrated. The residue remaining was purified by flash chromatography (EtOAc/hexane 30/70) to afford the diol derivative **7** (321 mg, 87%): R_f (EtOAc/hexane: 40/60) = 0.3.

[1*S*-(1α,2β,3α,5α)]-2-[3-[(*tert*-Butyldimethylsilyl)oxy]-5-hydroxy-2-[(tetrahydropyranyloxy)methyl]cyclopentanyl]ethanol Triphenylmethyl Ether (8). A stirred solution of diol **7** (374 mg, 1 mmol) in CH_2Cl_2 (2.5 mL) and DMF (2.5 mL) containing 4-(dimethylamino)pyridine (6.2 mg, 0.05 mmol) and triethylamine (0.335 mL, 2.4 mmol) was cooled in an ice bath. To this solution was added triphenylmethyl chloride (335 mg, 1.2 mmol) in CH_2Cl_2 (2.5 mL). The reaction mixture was stirred at 0 °C for 1 h and then allowed to warm to room temperature overnight. The reaction mixture was diluted with CH_2Cl_2, washed with water, brine, dried ($MgSO_4$), filtered, and concentrated. The crude product was purified by flash chromatography (EtOAc/hexane 10/90) to give triphenylmethyl derivative **8** (540 mg, 88%): R_f (EtOAc/hexane 10/90) = 0.4.

[1*S*-(1α,2β,3α)]-2-[3-[(*tert*-Butyldimethylsilyl)oxy]-2-[(tetrahydropyranyloxy)methyl]-5-oxocyclopentanyl]ethanol Triphenylmethyl Ether (9). To a stirred solution of triphenylmethyl derivative **8** (614 mg, 1 mmol) in CH_2Cl_2 (9 mL) containing 3-Å molecular sieves (600 mg) was added pyridinium dichromate (510 mg, 1.36 mmol). The reaction mixture was stirred at room temperature for 4 h and then poured into EtOAc. The mixture was filtered through silica gel with EtOAc/ether (50/50) and the filtrate concentrated. The crude product was purified by flash chromatography (EtOAc/hexane 10/90) to afford keto derivative **9** (424 mg, 69%): R_f (EtOAc/hexane 10/90) = 0.4.

[1*S*-(1α,2β,3α,5α,β)]-2-[3-[(*tert*-Butyldimethylsilyl)oxy]-5-hydroxy-5-[(*N*-methyl-*S*-phenylsulfonimidoyl)methyl]-2-[(tetrahydropyranyloxy)methyl]cyclopentanyl]ethanol Triphenylmethyl Ether (10). To a stirred solution of *N*-methyl-*S*-methylphenylsulfoximine (1.2 g, 8.9 mmol) in THF (17 mL), which had been cooled in an ice bath (0–5 °C), was added under nitrogen a 1 N solution of methylmagnesium chloride (2.97 mL, 8.9 mmol) in THF. Stirring was continued at 0–5 °C for 15 min. The solution of the sulfoximine anion was then added under nitrogen to a solution of keto derivative **9** (1.83 g, 3 mmol) in THF (17 mL), cooled in an acetone–dry ice bath (−78 °C). TLC analysis after 15 min showed two isomeric sulfoximine addition products. The reaction mixture was diluted with CH_2Cl_2, washed with water and brine, dried ($MgSO_4$), filtered, and concentrated. The crude product was purified by flash chromatography (EtOAc/hexane 20/80) to give the isomeric sulfoximine derivatives **10** (2.25 g,

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96%): R_f (EtOAc/hexane 30/70) = 0.5 and 0.8.

[1*S*-(1α,2β,3α,5α,β)]-2-[2-[(*tert*-Butyldimethylsilyloxy)methyl]-3-[(*tert*-butyldimethylsilyl)oxy]-5-hydroxy-5-[(*N*-methylphenylsulfonimidoyl)methyl]cyclopentanyl]ethanol Triphenylmethyl Ether (10a). The free hydroxyl of lactone **5** was protected as the tBDMSi ether and the phenylbenzoate removed by transesterification (NaOMe, in MeOH) in the usual manner. This derivative was then converted in four steps (overall yield 32%) to β-hydroxy sulfoximine derivative **10a** in the same manner as that used to convert **6** to **10**: R_f (EtOAc/hexane 20/80) = 0.6 and 0.5.

[1*S*-(1α,2β,3α)]-2-[3-[(*tert*-Butyldimethylsilyl)oxy]-5-methenyl-2-[(tetrahydropyranyloxy)methyl]cyclopentanyl]ethanol Triphenylmethyl Ether (11). To a solution of sulfoxime derivative **10** (536 mg, 0.68 mmol) in 50% aqueous acetic acid (3 mL) and THF (6 mL) was added aluminum mercury amalgam [Al(Hg), prepared from Al (6 g) and $HgCl_2$ (6 g) in H_2O (400 mL)]. The reaction mixture was maintained between 20 and 30 °C for 1 h, diluted with EtOAc, washed with H_2O, dried ($MgSO_4$), filtered, and concentrated. The residue was purified by flash chromatography using a four-step gradient going in equal increments from EtOAc/hexane (2/98) to EtOAc/hexane (4/96) to afford alkene derivative **11** (483 mg, 79%): R_f (EtOAc/hexane 4/96) = 0.3; ^{1}H NMR ($CDCl_3$) δ 7.39–7.13 (m, 15 H, trityl), 4.80 and 4.66 (s, 1 H, and s, 1 H, CH_2=C), 4.62–4.52 [m, 1 H, OCHO(THP)], 4.20–3.95 (m, 1 H, CHOtBDMSi), 3.88–3.64 (m, 2 H), 3.54–3.42 (m, 1 H), 3.34–3.16 (m, 3 H), 2.62–2.40 (m, 2 H), 2.36–2.25 (m, 1 H), 2.02–1.45 (m, 12 H), 0.91 (s, 9 H), 0.05 (s, 6 H).

[1*S*-(1α,2β,3α)]-2-[2-[[(*tert*-Butyldimethylsilyl)oxy]methyl]-3-[(*tert*-butyldimethylsilyl)oxy]-5-methenylcyclopentanyl]ethanol Triphenylmethyl Ether (11a). Hydroxy sulfoximine **10a** was treated with Al(Hg) in tetrahydrofuran, water, and acetic acid using the same methodology employed in the preparation of **11** to afford a mixture (60/40) of two compounds. One was assigned as the *exo*-methylene derivative **11a**: R_f (EtOAc/hexane 2/98) = 0.5; ^{1}H NMR ($CDCl_3$) δ 7.49–7.23 (m, 15 H, trityl), 4.81 and 4.67 (s, 1 H, and s, 1 H, CH_2=C), 4.03 (m, 1 H, CHOtBDMSi), 3.62–3.54 (m, 1 H, CH_2OtBDMSi), 3.50–3.42 (m, 1 H, CH_2OtBDMSi), 3.17 (t, J = 8, 2 H, CH_2OTr), 2.53–2.34 (m, 2 H), 2.31–2.22 (m, 1 H), 2.04–1.61 (m, 3 H), 0.89 (s, 18 H), 0.04 (s, 12 H). The second was assigned as methyl alcohol derivative **11d**:[21] presence of a methyl at 0.99 ppm, presence of OH (D_2O exchangeable, acetylation with acetic anhydride and 4-(dimethylamino)pyridine in CH_2Cl_2). The remainder of the NMR was similar to that of **10a**.

[1*S*-(1α,2β,3α)]-2-[2-[[(*tert*-Butyldimethylsilyl)oxy]methyl]-5-methenyl-3-(tetrahydropyranyloxy)cyclopentanyl]ethanol Triphenylmethyl Ether (11b). The free hydroxyl group in lactone **5** was protected as the tBDMSi ether, the phenylbenzoate removed by transesterification (NaOMe, in MeOH) and replaced with a THP group in the usual manner. This derivative, differing from the previous **6** only in the selection of protecting groups, was then converted in five steps (overall yield 31%) to *exo*-methylene derivative **11b** by the same reactions used to convert **6** to **11**: R_f (EtOAc/hexane 5/95) = 0.6; ^{1}H NMR ($CDCl_3$) δ 7.49–7.23 (m, 15 H, trityl), 4.81 (d, J = 6.5, 1 H, CH_2=C), 4.59–4.65 [m, 2 H, CH_2=C and OCHO(THP)], 4.05–3.81 (m, 2 H), 3.63–3.41 (m, 3 H), 3.21–3.11 (m, 2 H), 2.65–2.21 (m, 12 H), 0.90 (s, 9 H), 0.04 (s, 6 H).

[1*S*-(1α,2β,3α,5α)]-2-[3-[(*tert*-Butyldimethylsilyl)oxy]-5-(hydroxymethyl)-2-[(tetrahydropyranyloxy)methyl]cyclopentanyl]ethanol Triphenylmethyl Ether (12). To a cooled (0–5 °C) solution of alkene **11** (480 mg, 0.78 mmol) in THF (2.6 mL) was added a 0.5 M solution of 9-borabicyclo[3.3.1]nonane (9-BBN, 4.4 mL, 2.2 mmol) in THF under nitrogen. Stirring was continued at 0–5 °C for 4 h before adding EtOH (0.8 mL). After the reaction mixture had stirred for an additional 4 min, a 3 N solution of sodium hydroxide (1 mL) and 30% aqueous hydrogen peroxide (0.8 mL) was added simultaneously over a 3-min period. The reaction mixture was cooled intermittently in a water bath to maintain the solution temperature at 25 °C and stirring was continued for an additional 15 min. EtOAc was then added and the organic layer was washed with water and brine, dried ($MgSO_4$), filtered, and concentrated. The crude material was purified by flash chromatography (EtOAc/hexane 10/90) to give the desired alcohol **12** (350 mg, 71%): R_f (EtOAc/hexane 20/80) = 0.35; ^{1}H NMR ($CDCl_3$) δ 7.39–7.13 (m, 15 H, trityl), 4.59–4.52 [m, 1 H, OCHO(THP)], 4.21–4.12 (m, 1 H, CHOtBDMSi), 3.81–3.05 (m, 8 H), 2.01–1.45 (m, 13 H), 0.91 (s, 9 H), 0.05 (s, 6 H).

[1*S*-(1α,2β,3α,5α)]-2-[3-[(*tert*-Butyldimethylsilyl)oxy]-5-[[(methylsulfonyl)oxy]methyl]-2-[(tetrahydropyranyloxy)methyl]cyclopentanyl]ethanol Triphenylmethyl Ether (13). To a solution of alcohol **12** (350 mg, 0.56 mmol) and methanesulfonyl chloride (0.057 mL, 0.74 mmol) in CH_2Cl_2 (14 mL) was added triethylamine (0.152 mL, 1.1 mmol). The reaction mixture was stirred at room temperature for 15 min then diluted with CH_2Cl_2, washed with water, dried ($MgSO_4$), filtered, and concentrated to obtain the methylsulfonyl derivative **13** (676 mg, 98%) as a slightly yellow oil: R_f (EtOAc/hexane 20/80) = 0.35; ^{1}H NMR ($CDCl_3$) δ 7.39–7.13 (m, 15 H, trityl), 4.58–4.51 [m, 1 H, OCHO(THP)], 4.39–4.21 (m, 2 H, CH_2OMs), 4.14–4.05 (m, 1 H, CHOtBDMSi), 3.81–3.11 (m, 6 H), 2.91 (s, 3 H, mesyl), 2.01–1.45 (m, 13 H), 0.91 (s, 9 H), 0.05 (s, 6 H).

[1*S*-(1α,2β,3α,5α)]-2-[2-[[(*tert*-Butyldimethylsilyl)oxy]methyl]-3-[(*tert*-butyldimethylsilyl)oxy]-5-[[(methylsulfonyl)oxy]methyl]cyclopentanyl]ethanol Triphenylmethyl Ether (13a). *exo*-Methylene **11a** was converted to the mesyl derivative **13a** by the same procedures used to obtain **13** from **11** (overall yield 69%): R_f (EtOAc/hexane 10/90) = 0.49; ^{1}H NMR ($CDCl_3$) δ 7.49–7.23 (m, 15 H, trityl), 4.33 and 4.22 (m, 1 H, and m, 1 H, CH_2OMs), 4.14–4.08 (m, 1 H, CHOtBDMSi), 3.63–3.56 (m, 1 H, CH_2OtBDMSi), 3.44–3.38 (m, 1 H, CH_2OtBDMSi), 3.16–3.09 (m, 2 H, CH_2OTr), 2.91 (s, 3 H, mesyl), 2.32–2.22 (m, 1 H, *C*HCH_2OMs), 2.01–1.51 (m, 6 H), 0.89 (s, 18 H), 0.04 (s, 12 H).

Cyclization of 13a. As described below for the cyclization of the mesyl derivative **13**, **13a** was deblocked with tetrabutylammonium fluoride (3 mmol equiv) which directly resulted in cyclization giving a compound which was chromatographically and spectroscopically identical to the bicyclo derivative **16** as produced from **13** (88%).

[1*S*-(1α,2β,3α,5α)]-2-[2-[[(*tert*-Butyldimethylsilyl)oxy]methyl]-5-[[(methylsulfonyl)oxy]methyl]-3-(tetrahydropyranyloxy)cyclopentanyl]ethanol Triphenylmethyl Ether (13b). *exo*-Methylene **11b** was transformed to mesyl derivative **13b** in a manner similar to the conversion of **11** to **13** (overall yield 66%): R_f (EtOAc/hexane 30/70) = 0.65; ^{1}H NMR ($CDCl_3$) δ 7.49–7.23 (m, 15 H, trityl), 4.63 [s, 1 H, OCHO(THP)], 4.34–4.21 (m, 2 H, CH_2OMs), 4.16–4.04 (m, 1 H, CHOTHP), 3.91–3.79 (m, 1 H), 3.75–3.37 (m, 3 H), 3.18–3.09 (m, 2 H), 2.97 and 2.92 (s, 1.5 H, and s, 1.5 H, mesyl), 2.36–2.23 (m, 1 H, *C*HCH_2OMs), 1.96–1.35 (m, 12 H), 0.86 (s, 18 H), 0.04 (s, 12 H).

Cyclization of 13b. Mesyl **13b** was treated with either 0.1 equiv of pyridinium *p*-toluenesulfonate[26] in EtOH for 3 h (80% total yield) or 3 equiv of dimethylaluminum chloride[27] in CH_2Cl_2 for 30 min (89% total yield) to give a mixture (50/50 as estimated by NMR) of two compounds. One, **14a**, after tritylation and cleavage of the tBDMSi group was characterized chromatographically and spectroscopically as the bicyclo derivative **16**; the second is presumed to be the other possible bicyclo derivative formed by loss of the trityl group and cyclization of the upper side chain (lack of mesyl group, presence of tBDMSi determined spectroscopically and free hydroxyl as evidenced by acetylation with acetic anhydride in pyridine).

[1*S*-(1α,2β,3α,4α)]-3-[(Tetrahydropyranyloxy)methyl]-2-[2-[(triphenylmethyl)oxy]ethyl]-5-oxabicyclo[2.2.1]heptane (14). To a solution of the methanesulfonate **13** (235 mg, 0.34 mmol) in THF (5 mL) was added a 1 M solution of tetrabutylammonium fluoride (0.45 mL) in THF. The reaction mixture was stirred for 4 h at room temperature and then diluted with CH_2Cl_2, washed with water, dried ($MgSO_4$), filtered, and concentrated. The residue was purified by flash chromatography (EtOAc/hexane 20/80) to give bicyclo derivative **14** (150 mg, 89%): R_f (Et-

(26) Miyashita, N.; Yoshikoshi, A.; Grieco, P. A. Pyridinium *p*-Toluenesulfonate. A Mild and Efficient Catalyst for the Tetrahydropyranylation of Alcohols. *J. Org. Chem.* **1977**, *42*, 3772–3774.
(27) Ogawa, Y.; Shibasaki, M. Selective Removal of Tetrahydropyranyl Ethers in the Presence of *t*-Butyldimethylsilyl Ethers. *Tetrahedron Lett.* **1984**, *25*, 663–664.

OAc/hexane 20/80) = 0.30; ^{1}H NMR (CDCl$_3$) δ 7.39–7.13 (m, 15 H, trityl), 4.52–4.42 [m, 1 H, OCHO(THP)], 4.22 and 4.15 (s, 0.5 H and s, 0.5 H, CH$_2$OC*H*), 3.82–3.70 (m, 2 H), 3.54–3.29 (m, 3 H), 3.15–2.98 (m, 3 H), 2.28 (s, 1 H, C*H*CH$_2$OCH at bridge head), 2.0–1.45 (m, 12 H). Anal. ($C_{33}H_{38}O_4$) C, H.

[1*S*-(1α,2β,3α,4α)]-2-(2-Hydroxyethyl)-3-[(tetrahydropyranyloxy)methyl]-5-oxabicyclo[2.2.1]heptane (15). To a flask cooled in an acetone–dry ice bath (−78 °C) were added sodium (69 mg, 3 mmol) and ammonia gas until 10 mL of a blue solution was formed. Bicyclo derivative 14 (150 mg, 0.3 mmol) in THF (1 mL) was added and the solution was stirred at −78 °C for 15 min. Water (60 μL) was then added and the reaction mixture allowed to warm to room temperature over a period of 2 h. The residue was diluted with CH$_2$Cl$_2$, washed successively with saturated aqueous NaHSO$_4$ and NaHCO$_3$, dried (MgSO$_4$), filtered, and concentrated. The crude product was purified by flash chromatography (EtOAc/hexane 10/90) to afford 15 (64 mg, 83%): R_f (EtOAc/hexane 80/20) = 0.3; ^{1}H NMR (CDCl$_3$) δ 4.56–4.46 [m, 1 H, OCHO(THP)], 4.12 and 4.06 (s, 0.5 H and s, 0.5 H, CH$_2$OC*H*), 3.86–3.75 (m, 2 H), 3.74–3.62 (m, 3 H), 3.58–3.4 (m, 3 H), 2.35 (s, 1 H, C*H*CH$_2$OCH at bridge head), 2.01–1.45 (m, 12 H). Anal. ($C_{14}H_{14}O_4$) C, H.

[1*S*-(1α,2β,3α,4α)]-3-(Hydroxymethyl)-2-[2-[(triphenylmethyl)oxy]ethyl]-5-oxabicyclo[2.2.1]heptane (16). A solution of bicyclo derivative 14 (50 mg, 0.1 mmol) in acetic acid (0.5 mL), THF (0.375 mL), and H$_2$O (0.125 mL) was stirred at room temperature for 6 h. The reaction mixture was then diluted with CH$_2$Cl$_2$, washed twice with saturated aqueous NaHCO$_3$, once with brine and once with water, dried (MgSO$_4$), filtered, and concentrated. The crude product was purified by flash chromatography (EtOAc/hexane 30/70) to give 16 (36 mg, 87%): R_f (EtOAc/hexane 50/50) = 0.5; ^{1}H NMR (CDCl$_3$) δ 7.41–7.10 (m, 15 H, trityl), 4.15 (s, 1 H, CH$_2$OC*H*), 3.72 (d, *J* = 7.5, 1 H, C*H*$_2$OCH), 3.53–3.51 (m, 1 H, C*H*$_2$OCH), 3.35–3.05 (m, 4 H, C*H*$_2$OH and CH$_2$OTr), 2.31 (s, 1 H, C*H*CH$_2$OCH at bridge head), 1.85–1.45 (m, 6 H). Anal. ($C_{28}H_{30}O_3$) C, H.

[1*S*-(1α,2β,3α,4α)]-2-(Formylmethyl)-3-[(tetrahydropyranyloxy)methyl]-5-oxabicyclo[2.2.1]heptane (17). To a solution of the precursor alcohol 15 (680 mg, 2.66 mmol) in CH$_2$Cl$_2$ (80 mL) was added chromium oxide (1.75 g, 17.5 mmol) and pyridine (2.8 mL, 34.6 mmol). The reaction mixture was stirred for 3 h at room temperature and then poured slowly into EtOAc. The mixture was filtered through silica gel and eluted with EtOAc/ether (50/50), and the combined filtrates were concentrated to give chromatographically homogeneous aldehyde 17 (595 mg, 88%): R_f (EtOAc/hexane 80/20) = 0.55; ^{1}H NMR (CDCl$_3$) δ 9.78 (s, 1 H, CH═O), 4.55–4.45 [m, 1 H, OCHO(THP)], 4.21 and 4.15 (s, 0.5 H and s, 0.5 H, CH$_2$OC*H*), 3.82–3.62 (m, 2 H), 3.58–3.42 (m, 3 H), 3.21–3.11 (m, 1 H), 2.73–2.41 (m, 2 H, C*H*$_2$CH═O), 2.25 (s, 1 H, C*H*CH$_2$OCH at bridge head), 1.85–1.45 (m, 12 H).

Methyl [1*S*-(1α,2β(*Z*),3α,4α)]-7-[3-[(Tetrahydropyranyloxy)methyl]-5-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoate (19). A suspension of 60% sodium hydride in mineral oil (707 mg, 17.7 mmol) and (4-carboxybutyl)triphenylphosphonium bromide (4.13 g, 9.3 mmol) in freshly distilled DMSO (45 mL) was heated under nitrogen at 70 °C for 30 min. The reaction mixture (red ylide) was cooled to room temperature and then added to a solution of aldehyde 17 (676 mg, 2.7 mmol) in DMSO (5 mL). After 30 min at room temperature, the reaction was quenched by the addition of ice–water and carefully acidified to pH 5 with 0.5 N sodium bisulfate. The reaction mixture was then diluted with CH$_2$Cl$_2$, washed with brine, dried (MgSO$_4$), filtered, and concentrated. To a solution of the residual oil in MeOH (15 mL) was added ethereal diazomethane (excess). After 10 min at room temperature, the reaction mixture was concentrated and the crude methyl ester was purified by flash chromatography (EtOAc/hexane 20/80) to afford 19 (765 mg, 82%): R_f (EtOAc/hexane 30/70) = 0.3; ^{1}H NMR (CDCl$_3$) δ 5.44–5.30 (m, 2 H, CH═CH), 4.59–4.51 [m, 1 H, OCHO(THP)], 4.21–4.15 (s, 0.5 H and s, 0.5 H, CH$_2$OC*H*), 3.85–3.75 (m, 2 H), 3.65 (s, 3 H, OCH$_3$), 3.60–3.36 (m, 3 H), 3.21–3.80 (m, 0.5 H), 3.09–3.01 (m, 0.5 H), 2.30 (s, 1 H, C*H*CH$_2$OCH at bridge head), 2.27–1.41 (m, 18 H). Anal. ($C_{20}H_{32}O_5$) C, H.

Methyl [1*S*-(1α,2β(*Z*),3α,4α)]-7-[3-(Hydroxymethyl)-5-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoate (20). To a solution of methyl ester 19 (650 mg, 1.8 mmol) in THF (3 mL) and H$_2$O (10 mL) was added acetic acid (20 mL). The reaction mixture was stirred at room temperature for 3 h and then diluted with EtOAc, washed successively with brine, saturated aqueous NaHCO$_3$ (until neutral), and water, dried (MgSO$_4$), filtered, and concentrated. The residue was purified by flash chromatography (EtOAc/hexane 70/30) to give alcohol derivative 20 (440 mg, 89%): R_f (EtOAc/hexane 70/30) = 0.3; ^{1}H NMR (CDCl$_3$) δ 5.44–5.29 (m, 2 H, CH═CH), 4.21 (s, 1 H, CH$_2$OC*H*), 3.80 (d, 1 H, *J* = 7.5, C*H*$_2$OCH), 3.60 (s, 3 H, OCH$_3$), 3.61–3.53 (m, 1 H, C*H*$_2$OCH), 3.49–3.41 (m, 1 H, C*H*$_2$OH), 3.34–3.25 (m, 1 H, C*H*$_2$OH), 2.40–1.11 (m, 12 H).

Methyl [1*S*-(1α,2β(*Z*),3α,4α)]-7-[3-Formyl-5-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoate (21). The free alcohol 20 (400 mg, 1.5 mmol) was oxidized to the corresponding aldehyde in a manner essentially identical to that for the preparation of 17 to give chromatographically homogeneous 21 (358 mg, 90%): R_f (EtOAc/hexane 70/30) = 0.5; ^{1}H NMR (CDCl$_3$) δ 9.56 (s, 1 H, CH═O), 5.44–5.30 (m, 2 H, CH═CH), 4.42 (s, 1 H, CH$_2$OC*H*), 3.87 (d, 1 H, *J* = 7.5, C*H*$_2$OCH), 3.67 (s, 3 H, OCH$_3$), 3.64–3.58 (m, 1 H, C*H*$_2$OCH), 2.61–1.10 (m, 13 H). This compound was used without further purification.

Methyl [1*S*-(1α,2β(*Z*),3α,4α)]-7-[3-[[2-[(Phenylamino)carbonyl]hydrazino]methyl]-5-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoate (22). To a solution of aldehyde 21 (310 mg, 1.2 mmol) in MeOH (12 mL) containing 4-phenylsemicarbazide (117 mg, 1.2 mmol) and acetic acid (3 mL) was added NaCNBH$_3$ (294 mg, 4.7 mmol). The reaction mixture was stirred for 20 min at room temperature, and then the pH was adjusted to 1 by addition of 1 N aqueous HCl. After stirring at room temperature for an additional 30 min, the reaction mixture was diluted with EtOAc, washed with saturated aqueous NaHCO$_3$ (until neutral) and brine, dried (MgSO$_4$), filtered, and concentrated. The crude product was purified by flash chromatography (EtOAc/hexane 80/20) to give, after crystallization from CH$_2$Cl$_2$/petroleum ether, the desired hydrazino derivative 22 (314 mg, 67%): R_f (EtOAc) = 0.6; mp = 72–73 °C; ^{1}H NMR (CDCl$_3$) δ 8.05 (s, 1 H, NH, D$_2$O exchangeable), 7.41–6.95 (m, 5 H, phenyl), 6.52 (s, 1 H, NH, D$_2$O exchangeable), 5.44–5.30 (m, 2 H, CH═CH), 4.10 (s, 1 H, CH$_2$OC*H*), 3.86 (d, 1 H, *J* = 7.5, C*H*$_2$OCH), 3.59 (s, 3 H, OCH$_3$), 3.65–3.58 (m, 1 H, C*H*$_2$OCH), 2.60–1.11 (m, 15 H); CIMS, *m/e* 402 (M + 1). Anal. ($C_{22}H_{31}N_3O_4$) C, H, N.

[1*S*-(1α,2β(*Z*),3α,4β)]-7-[3-[[2-[(Phenylamino)carbonyl]hydrazino]methyl]-5-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic Acid (23). To a mixture of MeOH (2.3 mL) and 1 N aqueous potassium carbonate (0.45 mL), which was refluxed for 1 h under nitrogen in a 90 °C oil bath was added the hydrazino derivative 22 (30 mg, 0.08 mmol). The solution was refluxed for 40 min and then cooled in an ice bath and acidified to pH 4 with 1 N aqueous oxalic acid which had also been previously refluxed under nitrogen. The reaction mixture was extracted twice with EtOAc which had also been freshly distilled under nitrogen, washed twice with brine, dried (MgSO$_4$), filtered, and concentrated until 3 mL of solution remained. Hexane, freshly distilled under nitrogen, was then added causing precipitation of the acid 23 (15 mg, 51%) as a colorless solid: R_f (CH$_2$Cl$_2$/MeOH 92/8) = 0.2; mp = 132–5 °C; ^{1}H NMR (CD$_3$OD) δ 7.40–6.95 (m, 5 H, phenyl), 5.45–5.30 (m, 2 H, CH═CH), 4.21 (s, 1 H, CH$_2$OC*H*), 3.84 (d, 1 H, *J* = 7.5, C*H*$_2$OCH), 3.64–3.58 (m, 1 H, C*H*$_2$OCH), 2.60–1.11 (m, 15 H); EIMS, *m/e* 387 (M$^+$). Anal. Calcd ($C_{21}H_{29}N_3O_4$) C, 65.11; H, 7.49; N, 10.90; found C, 64.15; H, 7.40; N, 10.62. This compound was unstable in solution, as evidenced by TLC, but could be stored as a solid at 4 °C under nitrogen without significant decomposition. The compound proved to be too unstable for analyses but could be reesterified in MeOH by adding ethereal diazomethane drop by drop to afford the methyl ester 22 (52%) isolated as before by flash chromatography. The latter was chromatographically and spectroscopically identical to the precursor ester.

Methyl [1*S*-(1α,2β(*Z*),3α,4α)]-7-[3-[(Hexylamino)methyl]-5-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoate (24). To a solution of aldehyde 21 (100 mg, 0.39 mmol), hexylamine (52 μL, 0.39 mmol) and NaCNBH$_3$ (96 mg, 1.5 mmol) in anhydrous MeOH under a nitrogen atmosphere at room temperature was added dropwise glacial acetic acid to pH 6.5–7.5. The reaction mixture was stirred for 15 min and then the pH was adjusted to 1 by addition of 1 N aqueous HCl. After stirring at room temperature for 45 min, a small amount of water was added and the solution was basified by the addition of solid $NaHCO_3$. The product was extracted into EtOAc, washed with saturated NaCl solution, dried ($MgSO_4$), filtered, and concentrated. The crude product was purified by flash chromatography (CH_2Cl_2/MeOH 95/5) to give the desired amino derivative **24** (110 mg, 80%): R_f (CH_2Cl_2/MeOH 95/5) = 0.48; ^1H NMR ($CDCl_3$) δ 5.45–5.30 (m, 2 H, CH=CH), 4.21 (s, 1 H, CH_2OCH), 3.80 (d, 1 H, J = 7.5, CH_2OCH), 3.61 (s, 3 H, OCH_3), 3.58–3.52 (m, 1 H, CH_2OCH), 2.66–2.54 (m, 2 H, CH_2NH), 2.46–2.03 (m, 9 H), 1.72–0.88 (m, 18 H); CIMS m/e 352 (M + 1).

[1S-(1α,2β(Z),3α,4α)]-7-[3-[(Hexylamino)methyl]-5-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic Acid (25). The methyl ester **24** (70 mg, 0.2 mmol) was dissolved in THF (7 mL) and H_2O (1.4 mL) under a nitrogen atmosphere. A solution of 1 N LiOH (1.8 mL) was added, and the mixture was stirred at room temperature for 3.5 h. The mixture was acidified with solid $NaHSO_4$ to pH 6–6.5 and then diluted with CH_2Cl_2, washed with saturated NaCl solution, dried ($MgSO_4$), filtered, and concentrated. The crude product was chromatographed on activated neutral Al_2O_3, eluting first with MeOH and then with an 8/2 mixture of MeOH/H_2O. The MeOH/H_2O fractions were combined, concentrated (to about 4 mL), diluted with CH_2Cl_2, washed with saturated NaCl solution, dried ($MgSO_4$), filtered, and concentrated. The residue was triturated with cold petroleum ether to give acid derivative **25** (41 mg, 61%) as a white solid: R_f (CH_2Cl_2/MeOH 80/20) = 0.46; mp = 82–3 °C; ^1H NMR ($CDCl_3$) δ 5.45–5.30 (m, 2 H, CH=CH), 4.54 (s, 1 H, CH_2OCH), 3.79 (d, 1 H, J = 7.5, CH_2OCH), 3.56–3.26 (m, 1 H, CH_2OCH), 2.98–2.78 (m, 2 H, CH_2NH), 2.52–1.11 (m, 28 H); CIMS m/e 338 (M + 1). Anal. ($C_{20}H_{35}NO_3$) C, H, N.

Binding Inhibition Studies. Blood from healthy donors, who had denied having received medication for 10 days, was collected into 0.38% citrate–phosphate–dextrose–adenine buffer. Platelet rich plasma (PRP) prepared from this blood was purchased from the University of Illinois Blood Bank. [^{3}H]U46619 binding to washed human platelets was performed as previously described.[28-30] Briefly, the platelet suspensions (5–7 × 10^8 platelets/mL) were incubated 5 min with [^{3}H]U46619 (final concentration of 10 μM) in the presence of the amine **25** or 13-azaprostanoic acid at varying concentrations (2–250 μM). In order to prevent platelet activation, prostacyclin (final concentration 270 μM) was added 1 min prior to incubation. Nonspecific binding was assessed in a separate incubation in the presence of 10 μM unlabeled U46619. Specific binding was defined as total binding minus binding activity that could not be competed for by 10 μM of unlabeled U46619 and was 85% of total binding. After a 5-min incubation period, platelet suspensions were filtered rapidly under vacuum through Whatman GF/C filters and rinsed with 5 × 3 mL of ice-cold Tydrode-Hepes buffer. [^{3}H]U46619 activity on the filters was determined in a Beckman LS6800 liquid scintillation spectrometer. The results of these studies are summarized in Figure 2.

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Registry No. **5**, 31752-99-5; **6**, 142066-51-1; **7**, 142066-52-2; **8**, 142066-53-3; **9**, 142066-54-4; **10** (isomer 1), 142066-55-5; **10** (isomer 2), 142184-25-6; **10a** (isomer 1), 142066-56-6; **10a** (isomer 2), 142184-26-7; **10c**, 142066-57-7; **11**, 142066-58-8; **11a**, 142066-59-9; **11b**, 142102-19-0; **11c**, 142066-60-2; **11d**, 142066-61-3; **12**, 142066-62-4; **13**, 142066-63-5; **13a**, 142066-64-6; **13b**, 142066-65-7; **14**, 142066-66-8; **14a**, 142066-67-9; **15**, 142066-68-0; **16**, 142066-69-1; **17**, 142066-70-4; **18**, 142066-71-5; **19**, 142066-72-6; **20**, 142066-73-7; **21**, 142066-74-8; **22**, 142066-75-9; **23**, 142066-76-0; **24**, 142066-77-1; **25**, 142066-78-2; N-methyl-S-methylphenylsulfoximine, 30004-67-2; (4-carboxybutyl)triphenylphosphonium bromide, 17814-85-6; 4-phenylsemicarbazide, 537-47-3; hexylamine, 111-26-2.

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Improved Brain Delivery of AZT Using a Glycosyl Phosphotriester Prodrug

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The concentration of AZT in mice plasma and brain was measured using HPLC after an ingestion of 20 mg/kg of AZT or the molar equivalent of hexadecyl 2-(α-D-mannopyranosidyl)ethyl 3′-azido-3′-deoxy-5′-thymidinyl phosphate **3**. The results demonstrated the promising qualities of the prodrug **3** which gave AZT-5′-phosphate as the main metabolite: the total concentration of AZT derivatives detected in brain presented a peak of 156 nmol/g (5 nmol/g for AZT) at 1 h; the half-life was about 24 h (1 h for AZT) with an AUC of 4366 nmol h/g as compared to 4 nmol h/g for AZT. The lipophilic properties of **3** were confirmed by its in vitro transport of inside synaptosomes. The derivative 2-(α-D-mannopyranosidyl)ethyl 3′-azido-3′-deoxy-5′-thymidinyl phosphate (**2**) provided also a good delivery of AZT to the central nervous system, with values intermediate between those of AZT and **3**.

3′-Azido-3′-deoxythymidine (AZT)[1] remains the only clinically approved drug against HIV infection[2-4] despite its undesirable side reactions[5] and the emergence of resistant HIV variants.[6] Its serious toxicity can be limited by lower doses[7] than those previously used, but this pro-

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