by the method of Shibko et al.[20]

Enzymatic Assays. In vitro effects of test compounds on fatty acid synthesis (AcCoA synthetase enzymatic activity) were tested at 2.4 μmol concentrations by the method of Goodridge.[21] Compound 11 was also tested at 8.6 μmol for its effects on in vitro cholesterol synthesis by using the radioactive incorporation of [^{14}C]acetate into cholesterol as described by Haven et al.[22] and extracted according to the method of Wada et al.[23]

Acute Toxicity Studies. LD_{50} studies in CF_1 male mice (≃30 g) were carried out by the method of Litchfield and Wilcoxon[24] over the range of 50 μg to 2 g/kg for compound 11.

Uterotropic activity of compound 11 was determined in ovariectomized immature Holtzman female rats (≃50 g). Ovaries were removed by the method of Emmens.[25] Three days after operating, animals were dosed at 20 (mg/kg)/day ip for the next 3 days. 17-β-Ethinylestradiol at 10 (μg/kg)/day was used as a standard. On the 4th day, the animals were sacrificed, and the uteri were removed, trimmed, and weighed.[25] The uteri for the control was 68 ± 8 mg, for the ethinylestradiol standard 184 ± 9 mg, and after treatment with compound 11, 52 ± 7 mg.

In Tables I–VII, the probable significant level (p) was determined between experimental groups and the control by the Student's t test; the number of animals per group is expressed as N; the mean of the percentage of the control and the standard deviation are expressed as $\bar{x}$ ± SD.

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Synthesis and Biological Evaluation of 9,11-Azo-13-oxa-15-hydroxyprostanoic Acid, a Potent Inhibitor of Platelet Aggregation

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The synthesis of a prostaglandin endoperoxide analogue, 9,11-azo-13-oxa-15-hydroxyprostanoic acid (AOHP), is described. AOHP was found to block effectively both the thromboxane synthetase and the PGH_2/TxA_2 receptors in human platelets. It inhibits the platelet aggregation induced by arachidonic acid, 9,11-methanoepoxy-PGH_2, PGH_2, and TxA_2 but does not affect the ADP-induced aggregation in aspirinated platelet-rich plasma. Some of the intermediates for the synthesis of AOHP also are effective in inhibiting platelet aggregation.

Over the past several years prostaglandins have received considerable attention with regard to their divergent effects on platelet aggregation.[2,3] The greatest attention has been focused on the physiologic antagonists thromboxane A_2

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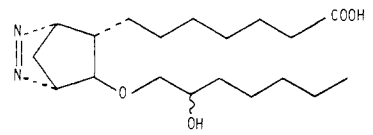
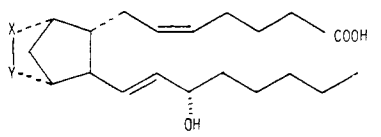
(TxA_2)[4] and prostacyclin (PGI_2).[5] The former is a potent inducer and the latter a potent inhibitor of platelet aggregation. Both TxA_2 and PGI_2 are produced from arachidonic acid via a prostaglandin endoperoxide intermediate (PGH_2), but their sites of biosynthesis differ.[6] Platelets have the capacity to produce TxA_2, while vascular tissue possesses the capacity to form PGI_2. It is believed that PGI_2 limits adhesion of platelets to the inner surface of blood vessels and thereby impedes the development of thrombosis.[7]

Since the balance between PGI_2 and TxA_2 appears to determine the extent of platelet aggregation,[8] compounds which selectively inhibit TxA_2 synthetase or block TxA_2 receptors might prove to be effective in the treatment of thrombosis. As a part of our effort to develop effective antithrombotic agents, we report on the synthesis of a prostaglandin analogue, 9,11-azo-13-oxa-15-hydroxyprostanoic acid (1, AOHP). AOHP has been found to be both a TxA_2 synthetase inhibitor and a TxA_2/PGH_2 receptor antagonist.

Design Considerations. The design rationale for the target compound, AOHP (1), was based in part on the



1, (AOHP)



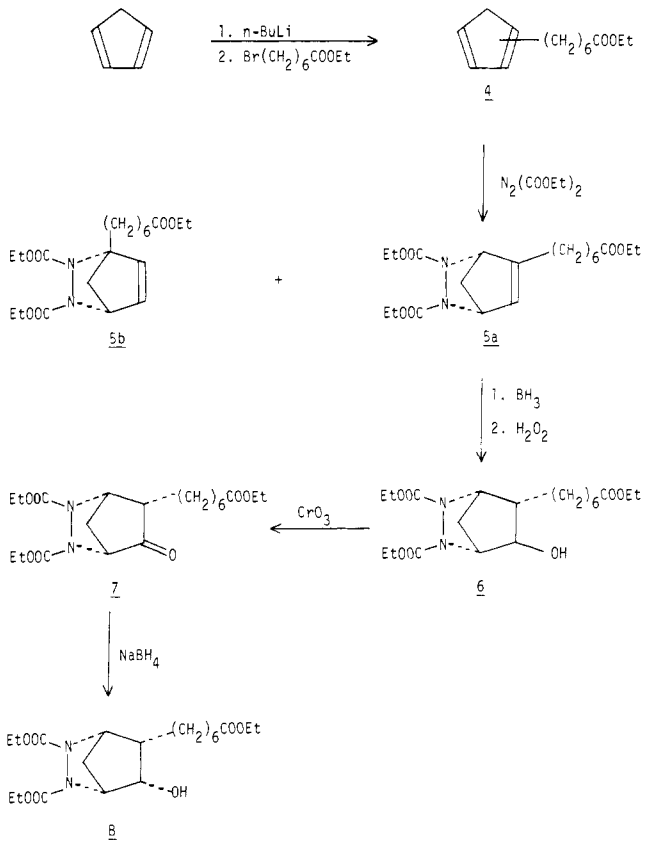
2, X-Y = O-O
3a, X-Y = N=N
3b, X = CH_2; Y = O

stereoelectronic similarity of its diazabicyclo[2.2.1]heptane system with that of the endoperoxide bicyclic system of PGH_2 (2). The fact the 9,11-azo-PGH_2 (**3a**) is a potent stimulator of platelet aggregation[9] and also is an inhibitor of thromboxane synthetase in microsomal preparations[10] illustrates that the azo group is neither detrimental to PGH_2/TxA_2 receptor binding nor to binding to the active site of the enzyme. However, because endoperoxide analogues **3a** and **3b** behave as agonists, our approach involved the incorporation of modified side chains into the molecule in an effort to eliminate the agonist component without diminishing affinity for the receptor or the enzyme.

The selection of the carboxyhexyl side chain in 1 was based on the finding that a C-5 double bond is an absolute requirement for aggregatory activity.[11] Thus, PGH_1 not only fails to stimulate platelet aggregation,[11] but it also inhibits thromboxane synthetase.[12] These findings suggested that a saturated side chain would be desirable for receptor antagonistic activity in platelets.

Still another consideration in the design of 1 involved our desire to protect it from metabolic degradation. Thus, it was contemplated that substitution of the C-13 double bond with an oxygen–carbon bond would make AOHP less susceptible to oxidation of the hydroxy group by the 15-hydroxy dehydrogenase.[13] Additionally, the oxygen electron density might crudely simulate that of a double bond and contribute to the binding at the platelet receptor and at the thromboxane synthetase active site.

Scheme I



Chemistry. The route (Scheme I) employed for the elaboration of the key intermediate **6** used in the synthesis of the target compound 1 involved the construction of Diels–Alder adduct **5a**. The diene **4** for the Diels–Alder reaction was prepared by condensing lithium cyclocopentadienide with ethyl 7-bromoheptanoate. This product **4** consisted of an equal mixture of the 2- and 3-alkylated cyclopentadiene, as suggested from prior studies.[14] Reaction of **4** with diethyl azodicarboxylate afforded the Diels–Alder adducts **5a** and **5b** in a 1:1 ratio. These two adducts presumably were derived from 3- and 2-(ethoxycarbonylhexyl)cyclopentadiene, respectively.

Although the desired regioisomer **5a** could be separated from the undesired adduct **5b** through a laborious chromatographic procedure involving silica gel impregnated with silver nitrate, it was discovered that **5a** could be purified more easily by selective thermal destruction of **5b** followed by silica gel chromatography. The decomposition products of **5b**, which appeared to be polymeric material, were easily retained on the column.

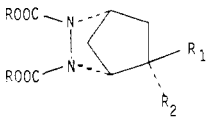
The regioisomers **5a** and **5b** were easily distinguishable by NMR. The desired intermediate **5a** exhibits an NMR spectrum which contains a single olefinic proton resonance, while the isomer **5b** with the bridgehead chain possesses two olefinic protons.

The next step in the sequence (Scheme I) involved the hydroboration of **5a** with diborane, followed by H_2O_2 oxidation. This procedure was selected because it was anticipated that the regioselectivity and stereoselectivity of these reactions would lead to the formation of an intermediate, **6**, whose relative stereochemistry would correspond to that of PGH_2 (2). Thus, it was expected that hydroboration of **5a** would proceed with cis addition of hydrogen and boron to the exo face[15] of the bicyclic system in an anti-Markovnikov manner.[16] Moreover, since oxidation of the intermediate organoborane is known to proceed with retention of configuration,[17,18] the resulting

OH group should have a cis relationship to the added hydrogen.

Support for the stereochemistry of **6** was obtained from chemical and spectroscopic studies. Oxidation of **6** to the corresponding ketone **7**, followed by $NaBH_4$ reduction, afforded the endo alcohol **8**. Formation of similar endo alcohols has been reported[19,20] in the hydride reduction of the ketonic group in other bicyclo[2.2.1]heptanes and apparently is the preferred stereoisomer when the bridge position is unsubstituted.

The NMR chemical-shift values of the carbinol methine proton (Table I) of **6**, **8**, and model compounds **9**–**12**[19,20]



9, R = Me; R_1 = OH; R_2 = H

10, R = Me; R_1 = H; R_2 = OH

11, R = Et; R_1 = OH; R_2 = H

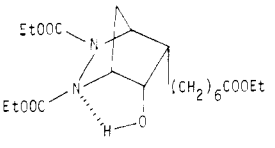
12, R = Et; R_1 = H; R_2 = OH

prepared by procedures identical with those described above are consistent with the proposed assignment. It can be noted that the methine proton resonance in the endo alcohols absorbs at higher field when compared to that of the endo alcohols. Similar absorption characteristics have been reported[21] in norborneol systems. The much larger chemical-shift difference between the methine protons of **6** and **8** is due to the shielding of the methine proton in **6** by the alkyl chain to which it bears a cis relationship.

In order to corroborate the stereochemical assignment, hydrogen-bonding studies of **6** and **8** were carried out using infrared spectroscopy. Of these stereoisomers, only the endo alcohol **8** should be capable of forming an intramolecular hydrogen bond, as illustrated in **13**. There is precedent[22] for this type of bonding in related azanorbornanes.

At a concentration of 0.025 M in CCl_4, the exo alcohol **6** displayed a strong, broad absorption at 3480 cm^{-1} (intermolecular hydrogen bonding) and a weak, narrow band at 3620 cm^{-1} (free OH).[23] When diluted to 0.00125 M, the 3480-cm^{-1} absorption was reduced almost to base line, and the free OH was the only peak remaining. These results compare quite well with those obtained from comparable studies conducted on *N*-benzyl-*exo*-6-hydroxy-2-azabicyclo[2.2.1]heptane.[22]

The endo alcohol **8** (0.025 M) possesses two overlapping absorptions, one at 3480 cm^{-1} (intermolecular hydrogen bonding) and another, more intense absorption at 3520 cm^{-1}. On dilution to 0.00125 M, the 3480-cm^{-1} absorption disappeared completely and the 3520-cm^{-1} band (which shifted to 3530 cm^{-1}) became the most intense peak in the OH region. These results suggest that the 3530-cm^{-1} absorption originates from intramolecular hydrogen bonding, as illustrated in **13**. Hydrogen bonding between



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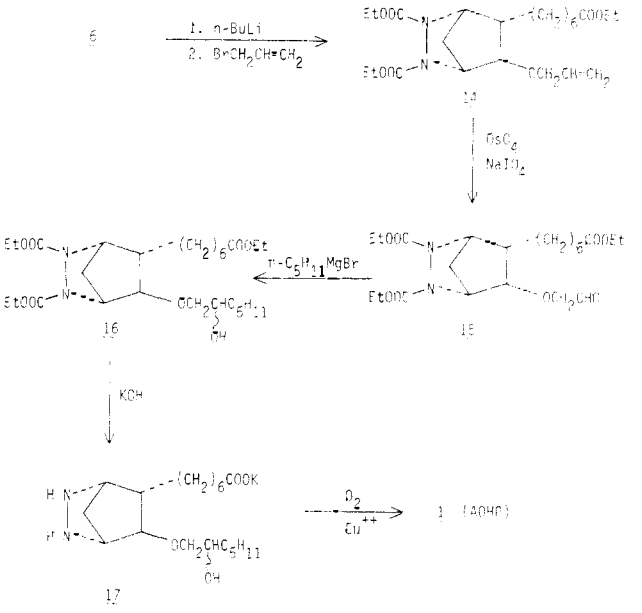
the endo OH and the carbamate carbonyl is less likely because the partial sp^2 character of the carbamate group prevents the ether or carbonyl oxygen from assuming a conformation which would allow it to come within bonding distance with the OH.[24]

Table I. Chemical-Shift Values (δ, $CDCl_3$) of the Carbinol Methine Proton of Hydroxy-2,3-diazabicyclo[2.2.1]heptane Derivatives

exo alcohol	δ	endo alcohol	δ
6	3.50	8	4.32
9	4.1[a]	10	4.5[a]
11	4.00	12	4.25

[a] Reported values (ref 15) in CCl_4.

Scheme II



With sufficient evidence to support the stereochemistry of **6**, we then proceeded to transform this intermediate to the target compound, AOHP (**1**). An obvious approach was to condense **6** with an electrophile which would lead directly to the desired skeleton. In this regard, a number of unsuccessful attempts were made to condense **6** (as the Li salt) with 1,2-epoxyheptane, 1-bromo-2-(1-ethoxyethoxy)heptane, and bromoheptan-2-one. Even under forcing conditions, there was no evidence for the formation of the desired compounds. On the other hand, bromoacetaldehyde dimethyl acetal, allyl bromide, and 1-bromo-2-(1-ethoxyethoxy)ethane afforded products in reasonable yield.

The fact that the electrophiles which condensed with **6** all are less bulky than their unreactive counterparts suggests that the alkoxide oxygen of **6** is sufficiently sterically hindered to prevent nucleophilic attack. This was illustrated by the remarkable difference in reactivity between the dimethyl acetal and diethyl acetal derivatives of bromoacetaldehyde; the former reacted smoothly with **6**, while the latter was unreactive under identical conditions.

In view of the failure to obtain AOHP (**1**) by utilizing an electrophile with the requisite number of carbons, a more circuitous route was followed (Scheme II). The allyl ether condensation product **14** arising from the reaction of **6** with allyl bromide was transformed to the aldehyde **15** in quantitative yield. In this connection, it is noteworthy that the identical aldehyde **15** was produced in poor yield from the dimethyl acetal derivative arising from the reaction between **6** and bromoacetaldehyde dimethyl acetal; this was apparently due to the instability of the aldehyde during the hydrolysis of the acetal condensation

Table II. Action of Endoperoxide Analogues[a] on Human Platelet-Rich Plasma

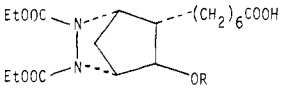
	% inhibn of platelet aggregation[b]			
no.	8.2×10^{-4} M arachidonic acid	9.5×10^{-7} M 3b	1.4×10^{-6} M PGH_2	2×10^{-6} M ADP[c]
1 (AOHP)	100	100	100	0
18	0	20		
19	0	21		
20	0	88	100	0
21	0	20		

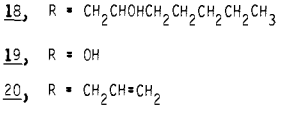
[a] None of the analogues induced platelet aggregation at concentrations of 10^{-4} or 10^{-6} M. [b] The base line (0% aggregation) of the recording was established with PRP and the scale adjusted to maximum deflection (100%) at the optical density of plasma which had been treated with the aggregating agent alone. Percent inhibition was obtained by measuring the differences in scale between the aggregation obtained in the presence of a potential inhibitor (10^{-4} M) and 100% aggregation. The difference is expressed as a percentage of the latter. The endoperoxide analogues were incubated with PRP for 1.5 min prior to the addition of the aggregating agents. Each test represents the average of two determinations. [c] Conducted in aspirinated PRP.

product. The remainder of the chain was assembled by allowing **15** to react with *n*-pentylmagnesium bromide. The product, **16**, of this reaction possesses an additional chiral center at C-15. Although thin-layer chromatography shows a single spot, it is possible that **16** may be a mixture of unresolved C-15 epimers.

Hydrolysis of the carbamate and ester groups in **16** was accomplished by heating with KOH in ethylene glycol. The hydrazo intermediate **17** then was converted to AOHP (**1**) by air oxidation in the presence of Cu(II) catalyst.[25]

Some of the intermediates (**6**, **14**, and **16**) in Schemes I and II were deblocked to produce compounds **18**–**21**.

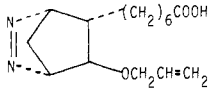




18, R = $CH_2CHOHCH_2CH_2CH_2CH_2CH_3$

19, R = OH

20, R = $CH_2CH{=}CH_2$



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These also were screened for biological activity on platelets.

Biological Results and Discussion. Since it is known that most endoperoxide analogues[26-29] are potent aggregating agents, preliminary screening was performed to determine the ability of AOHP and its congeners to induce platelet aggregation. At a concentration of 10^{-4} M, none of the analogues showed any aggregatory activity on platelet-rich plasma (PRP). Since prostaglandin E_2 can inhibit aggregation at high concentration but enhance aggregation at low concentration,[30] these compounds also were examined at 10^{-6} M in PRP. All compounds (**1** and **18**–**21**) were found to be inactive in this test.

In view of the inability of these congeners to promote platelet aggregation, experiments were carried out to determine whether they inhibit this process (Table II). At a concentration of 10^{-4} M, only AOHP (**1**) inhibits the platelet aggregation induced by arachidonic acid. This suggests that the action of AOHP might be due to the inhibition of prostaglandin-related events. Additionally, at concentrations which were not effective in blocking the action of arachidonic acid, AOHP could delay the onset of aggregation. The IC_{50} value of AOHP which inhibits aggregation induced by 8.3×10^{-4} M arachidonic acid in PRP was found to be 3.1×10^{-5} M ($N = 2$).

Table III. Effect of AOHP on Arachidonic Acid Metabolism in Washed Platelets as Measured[a] by Computer-Assisted GC-MS

	HETE, nmol	HHT, nmol	TxB_2, nmol
washed platelets (1 mL) + 82 nmol of arachidonic acid	23	8	14
washed platelets (1 mL) + 82 nmol of arachidonic acid + 10^{-4} M AOHP	27	3	0

[a] Ricinoleic acid was added as an internal standard. Data represent single determination.

In order to examine the affect of AOHP on prostaglandin biosynthesis, arachidonic acid was incubated with washed human platelets in the presence and absence of the inhibitor. The metabolites were esterified with diazomethane, silylated, and quantitated by gas chromatography–mass spectrometry selected ion monitoring. As shown in Table III, no thromboxane B_2 (the stable metabolite of TxA_2) was formed in the presence of 10^{-4} M AOHP. The formation of 12-L-hydroxy-5,8,10-heptadecatrienoic acid (HHT) also was partially inhibited by AOHP. However, the amount of 12-L-hydroxy-5,8,-10,14-eicosatetraenoic (HETE) acid remained almost unchanged, which indicates that AOHP does not inhibit platelet lipoxygenase. These results suggest that AOHP is able to inhibit thromboxane synthetase in intact platelets. Indeed, preliminary studies utilizing human platelet microsomes indicate that the IC_{50} value for AOHP inhibition of the conversion of [1-^{14}C]PGH_2 to [1-^{14}C]TxA_2 is in the range of 10^{-6} M.

The analogues also were evaluated against the aggregation induced by the endoperoxide analogue, 9,11-methanoepoxy-PGH_2 (**3b**; Table II). Since **3b** is stable, its ability to induce platelet aggregation is very likely related to the interaction with receptors which trigger this process. All analogues with a carboxyhexyl side chain inhibit the aggregation induced by **3b** to varying degrees. The two most potent inhibitors are AOHP (**1**) and **20**, which produced 100 and 88% inhibition, respectively, at a concentration of 10^{-4} M. AOHP was found to inhibit effectively the aggregation induced by **3b** (8.5×10^{-7} M) with an approximate IC_{50} value of 5.5×10^{-6} M ($N = 2$). Compound **20** also inhibited the action of **3b** (8.5×10^{-7} M) with a higher IC_{50} value of 3.6×10^{-5} M ($N = 1$). Thus, it appears that AOHP inhibits platelet aggregation by both inhibition of thromboxane synthetase and receptor blockade. The receptors which are blocked may be those which recognize the endoperoxides or TxA_2 or both.

Experiments also were performed to evaluate the antagonistic activity of AOHP on TxA_2-induced aggregation. Exogenous TxA_2 can be prepared by incubation of PGH_2 with fresh human platelet microsomes.[31] Thus, PGH_2 was incubated with human platelet microsomes for 15 s, followed by the addition of aspirinated PRP. Aggregation obtained by this means is due mainly to the effect of

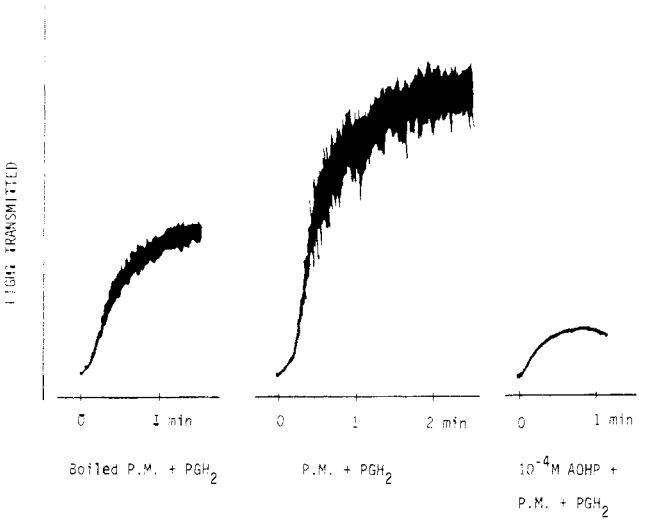

Figure 1. Inhibition of TxA_2-induced aggregation by AOHP in aspirinated human PRP. Thromboxane A_2 was generated by incubating PGH_2 (1.4 nmol) with human platelet microsomes (P.M.) (0.1 mL, 0.19 mg of protein) at 37.5 °C for 15 s. Aspirinated PRP (0.9 mL) was added and the aggregation was monitored. For the inhibition experiment, AOHP (10^{-4} M) was incubated with the aspirinated PRP for 1.5 min prior to the addition to the PGH_2-microsomal mixture.

TxA_2.[32] The inhibitor AOHP was incubated with the aspirinated PRP for 1.5 min prior to the addition to the PGH_2-microsomal mixture. As shown in Figure 1, with a concentration of 10^{-4} M AOHP, the TxA_2-induced aggregation was inhibited effectively. This, therefore, corroborates the above studies which suggest that AOHP is a PGH_2/TxA_2 receptor blocker, as well as a thromboxane synthetase inhibitor.

AOHP and **20** also inhibit PGH_2-induced platelet aggregation (Table II). Since PGH_2 can be transformed to the highly potent aggregating agent, TxA_2, the antiaggregatory effect of the inhibitors might be the result of blockage of the PGH_2/TxA_2 receptors and/or inhibition of thromboxane synthetase.

AOHP and **20** also were evaluated against the aggregation induced by ADP in aspirinated PRP (Table II). It is noteworthy that neither of these compounds inhibits ADP-induced aggregation, suggesting that they are acting specifically on prostaglandin-related events.

Conclusions. The ability of AOHP (**1**) to inhibit thromboxane synthetase indicates that the stereoelectronic similarity between its bicyclic system and that of PGH_2 confers considerable recognition for the active site of this enzyme.

That AOHP also behaves as a PGH_2/TxA_2 receptor blocker suggests that the absence of intrinsic activity is largely due to the nature of the side chains. Thus, all stable endoperoxide analogues which stimulate platelet aggregation contain side chains identical with that of PGH_2. On the other hand, it appears that the constitution of the bicyclic moiety is less critical, inasmuch as a change in the size of the bicyclic system[26] or its heteroatoms[27-29] does not destroy agonist activity.

The results of this study indicate the feasibility of developing antithrombotic agents which block both thromboxane synthetase and PGH_2/TxA_2 receptors by the appropriate combination of the bicyclic system and side chains.

Experimental Section

Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. Elemental analyses were performed with a Perkin-Elmer Model 237B grating spectrophotometer as a liquid film or KBr disk unless otherwise stated. Ultraviolet (UV) spectra were recorded in 95% ethanol with a Beckman DG-GT grating spectrophotometer. NMR data (δ) were recorded with Varian Models A-60D or T-60 spectrometers in $CDCl_3$ or CCl_4 using tetramethylsilane as the internal standard. Mass spectral analyses were performed with a Hitachi Perkin-Elmer Model RMU-6D spectrometer by the Mass Spectrometry Service Laboratory, School of Chemistry, University of Minnesota.

Ethyl 7-Bromoheptanoate. 7-Bromoheptanoic acid (146 g, 0.063 mol; Sapon Organic Chemicals, Bloomsburg, N.J.) was added to a mixture of 300 mL of absolute ethanol and 500 mL of benzene containing concentrated H_2SO_4 (3 mL). The reaction mixture was heated to reflux, and the benzene–ethanol–water azeotrope (~750 mL) was distilled to 66 °C. The remaining solvent then was removed in vacuo. The resulting oil was partitioned between 500 mL of diethyl ether and 100 mL of water. The ether layer was washed with 100 mL of a saturated $NaHCO_3$ solution, followed by 100 mL of water, dried over anhydrous $MgSO_4$, and distilled in vacuo. The product (156 g, 90%) was obtained as a colorless liquid: bp 42 °C (0.2 mmHg), lit.[33] bp 112 °C (5 mmHg); IR (liquid film) 1740 cm^{-1} (ester C=O); NMR (CCl_4) δ 1.25 (t, 3 H, $COOCH_2CH_3$), 3.4 (t, 2 H, $BrCH_2$–), 4.12 (q, 2 H, $COOCH_2CH_3$).

6-(Ethoxycarbonyl)hexylcyclopentadiene (4). The preparation of this compound is a modified version of the procedure of Sih.[34] A solution of methyllithium in ether (333 mL, 2 M, 0.66 mol, Ventron Corp., Danvers, Mass.) was added under nitrogen to a magnetically stirred solution of freshly distilled cyclopentadiene (60 mL, 0.68 mol) in 550 mL of dry tetrahydrofuran with cooling in ice. To the resulting suspension, ethyl 7-bromoheptanoate (120 g, 0.54 mol) was added over a period of 0.5 h. The mixture was allowed to warm to room temperature and stirred for 3 h. The resulting solution was poured into 500 mL of water and extracted with 2 × 1000 mL of ether. The ether extract was washed with saturated brine and with water and dried (anhydrous $MgSO_4$), and the solvent was removed in vacuo at ambient temperature. This procedure gave **4** (88 g, 82.6%) as an oil: IR (liquid film) 3000–3150 (C=C), 1740 cm^{-1} (C=O); NMR (CCl_4) δ 5.85–6.5 (m, 3 H, olefinic protons); UV (EtOH, 1-cm cell) λ_{max} 243.5 nm. Distillation of this product under high vacuum gave an analytically pure sample, bp 95–98 °C (0.2 mmHg). Anal. ($C_{14}H_{22}O_2$) C, H.

Diethyl 5-[6-(Ethoxycarbonyl)hexyl]-2,3-diazabicyclo-[2.2.1]hept-5-ene-2,3-dicarboxylate (5a). To a 20-mL ether solution of **4** (1.65 g, 7.4 mmol) diethyl azodicarboxylate (1.3 g, 7.5 mmol) in 3 mL of ether was slowly added with ice cooling. After the addition was completed, the reaction mixture was allowed to warm to 25 °C and stirred for 1 h. The crude reaction product obtained after removal of solvent contained an equal amount of **5a** and its isomer **5b** as estimated from NMR analysis: NMR (CCl_4) δ 4.98 (br s, 2 H, bridgehead protons of **5a**), 5.18 (br s, 1 H, bridgehead proton of **5b**), 6.03 (br s, 1 H, olefinic proton of **5a**), 6.3–6.54 (m, 2 H, olefinic protons of **5b**). The crude product was dissolved in toluene and refluxed for 9 h in order to selectively destroy the isomer **5b**. The toluene was removed in vacuo and the oil was purified on a 50-g silica gel column which was deactivated with 1% of water. Pure isomer **5a** (1.4 g, 46%) was eluted with $CHCl_3$: IR (liquid film) 1720 (carbamate C=O), 1740 cm^{-1} (ester C=O); NMR (CCl_4) δ 1.1–1.34 (2 t, 9 H, $2NCOOCH_2CH_3$, $-COOCH_2CH_3$), 1.34–1.9 [m, 8 H, $CH_2(CH_2)_4CH_2$], 1.65 (br s, 2 H, C-7 bridge protons), 2.05–2.4 (m, 4 H, $CH_2(CH_2)_4CH_2COOEt$), 3.86–4.3 (2 q, 6 H, $2NCOOCH_2CH_3$, $COOCH_2CH_3$), 4.9 (br s, 2 H, bridgehead proton), 5.98 (br s, 1 H, olefinic proton); MS (70 eV) m/e 396 (M^+). Anal. ($C_{20}H_{32}N_2O_6$) C, H, N.

Diethyl (±)-5-*endo*-[6-(Ethoxycarbonyl)hexyl]-6-*exo*-hydroxy-2,3-diazabicyclo[2.2.1]heptane-2,3-dicarboxylate (6). A solution of 39.6 g (0.1 mol) of **5a** in 400 mL of THF was added to 100 mL of 1.0 M diborane (0.1 mol) in THF (Aldrich Chemical Co., Milwaukee, Wis.) at 0 °C. After the reaction mixture was stirred at 0 °C for 2.5–3 h, water was added cautiously to hydrolyze the excess diborane. The mixture was oxidized by the slow addition of a cold solution of 30% H_2O_2 (16 mL) in 6 N NaOH (22 mL). The oxidant was added at such a rate that the reaction temperature was kept at 0 °C. Oxidation was accomplished by stirring at 0 °C for 2 h. The THF layer was removed, filtered,

and dried (anhydrous $MgSO_4$). After removal of solvent under reduced pressure, the residual oil was chromatographed on silica gel (400 g, 5 × 60 cm) using EtOH–EtOAc–petroleum ether (30–60 °C)–CH_2Cl_2 (0.5:2:3:1) to give pure **6** (28 g, 70%) as a colorless oil: TLC R_f 0.74 (silica gel, 5% *i*-PrOH in Et_2O); IR (liquid film) 3475 (OH), 1720 (carbamate C=O), 1740 cm^{-1} (ester C=O); NMR (CCl_4) δ 1.1–2.1 [m, 22 H, $2NCOOCH_2CH_3$, $COCH_2CH_3$, $-CH(CH_2)_5CH_2COO$], 1.67 (2 H, C-7 bridge protons), 2.1–2.4 (t, 2 H, $-CH_2COOEt$), 3.5 (br s, 1 H, CHOH), 3.75–4.4 (m, 8 H, $2NCOOCH_2CH_3$, $COOCH_2CH_3$, C-4 bridgehead proton, OH), 4.5 (br s, 1 H, C-1 bridgehead proton); MS (70 eV) *m/e* 414 (M^+). Anal. ($C_{20}H_{34}N_2O_7$) C, H, N.

Diethyl (±)-5-*endo*-[6-(Ethoxycarbonyl)hexyl]-6-*exo*-(2-propenyloxy)-2,3-diazabicyclo[2.2.1]heptane-2,3-dicarboxylate (14). Compound **6** (207 mg, 0.5 mmol) was dissolved in 2 mL of dry HMPT (dried by refluxing over CaO for 24 h and then distilling over Na). To this mixture was added 0.25 mL of 2.0 M *n*-BuLi in hexane (0.5 mmol) at 0 °C. After stirring for 5 min, 75 mg (0.7 mmol) of allyl bromide was introduced and stirring was continued for 1 h at 0 °C. The product was partitioned between 50 mL of water and 2 × 100 mL of ether. The ether layer was removed in vacuo to give an oil, which was chromatographed on silica gel (10 g, 1.3 × 30 cm) using 0.5:2:3:1 of EtOH–EtOAc–petroleum ether (30–60 °C)–CH_2Cl_2, to afford 110 mg (50%) of **14**. An analytical sample was prepared by rechromatographing the essentially pure **14** on a silica gel column (10 g, 1.3 × 30 cm) using CH_2Cl_2 with a gradient increase of methanol: IR (liquid film) 3080 (C=CH_2), 1650 cm^{-1} (C=C); NMR (CCl_4) δ 3.18 (br s, 1 H, >CHOCH$_2$), 3.9–4.4 (m, 9 H, $2NCOOCH_2CH_3$, $COOCH_2CH_3$, $OCH_2CH{=}CH_2$, bridgehead proton at C-4), 4.52 (br s, 1 H, C-1 bridgehead proton), 5.02–5.9 (m, 3 H, CH=CH_2); MS (70 eV) *m/e* 454 (M^+). Anal. ($C_{23}H_{38}N_2O_7$) C, H, N.

Diethyl (±)-5-*endo*-[6-(Ethoxycarbonyl)hexyl]-6-*exo*-(formylmethoxy)-2,3-diazabicyclo[2.2.1]heptane-2,3-dicarboxylate (15). To a cooled mixture (0 °C) containing **14** (0.541 g, 1.2 mmol) in 15 mL of ether and 8 mL of water was added, with vigorous stirring, a 4% aqueous solution of osmium tetroxide (1.53 mL). After 5 min, sodium periodate (1.05 g, 6.3 mmol) was added in divided amounts to the reaction mixture. The mixture was allowed to warm to 25 °C and it was stirred vigorously for 2 h. The product was partitioned between water (50 mL) and ether (2 × 100 mL), the organic phase was dried (anhydrous $MgSO_4$), and the solvent was removed in vacuo at 0 °C. The oily product **15** (0.51 g, 94%) was essentially pure by NMR analysis. This compound decomposed when chromatographed with silica gel: IR (CCl_4) 2675–2800 cm^{-1} (CHO); NMR (CCl_4) δ 3.9–4.4 (m, 9 H, OCH_2CHO, $3COCH_2CH_3$, C-4 bridgehead proton), 4.55 (br s, 1 H, C-1 bridgehead proton), 9.82 (s, 1 H, CHO). Compound **15** was used without further purification for the synthesis of **16**.

Diethyl (±)-5-*endo*-[6-(Ethoxycarbonyl)hexyl]-6-*exo*-(2-hydroxyheptoxy)-2,3-diazabicyclo[2.2.1]heptane-2,3-dicarboxylate (16). Compound **15** (1.63 g, 3.6 mmol) was azeotroped with benzene (30 mL) to remove all the water, dissolved in anhydrous ether (19 mL), and cooled to −78 °C in a dry ice–acetone bath. After stirring under nitrogen for 5 min, 1.54 mL of a 2.8 M ethereal *n*-pentylmagnesium bromide (4.3 mmol) was added dropwise. The dry ice–acetone bath then was removed and the reaction mixture was allowed to warm to 0 °C over a period of 10 min. Water (50 mL) was added and the product was extracted with 2 × 200 mL of ether. The ether layer was separated and the solvent removed in vacuo. The residual oil was purified by eluting on silica gel (50 g, 2.3 × 35 cm) with ether–acetone (9:1) to give **16** as a colorless oil (0.52 g) and 0.47 g of starting material **15** (65% conversion). Further purification of **16** was carried out by column chromatography on silica gel (10 g, 1.3 × 30 cm) using 10% *i*-PrOH in petroleum ether (30–60 °C): IR (liquid film) 3490 cm^{-1} (OH); NMR (CCl_4) δ 0.7–1.6 [m, 31 H, OH, $3COOCH_2CH_3$, $(CH_2)_5CH_2COO$, C_5H_{11}] 3.2 (m, 1 H, C-6 methine proton), 3.2–3.9 (m, 3 H, OCH_2CHOH). MS (70 eV, high resolution) *m/e* calcd M^+ for $C_{27}H_{48}O_8N_2$, 528.3410; found, 528.3395. Anal. ($C_{27}H_{48}N_2O_8$) C, H, N.

(±)-5-*endo*-(6-Carboxyhexyl)-6-*exo*-(2-hydroxyheptoxy)-2,3-diazabicyclo[2.2.1]hept-2-ene (1). A mixture of **16** (0.22 g, 0.4 mmol) and ethylene glycol (8 mL) which contained 0.3 g (5.4 mmol) of KOH was heated at 115 °C for 4.5 h. The reaction mixture then was cooled to 0 °C and neutralized to pH 7 with 10% HCl. About 5 mL of a pH 7 phosphate buffer was added and the mixture was adjusted to a homogeneous solution by the addition of 80 mL of MeOH–H_2O (1:2). After the addition of 0.5 g of cupric acetate to catalyze the oxidation, air was bubbled into the solution maintained at 0 °C. The mixture was stirred at 0 °C for 1 h and then at 25 °C for 2 h. After removing the methanol in vacuo, the reaction mixture was acidified with 10% HCl to pH 1 and the product was partitioned between water (50 mL) and ether (2 × 200 mL). The ether layer was dried (anhydrous $MgSO_4$) and the solvent removed in vacuo to give an oil, which was chromatographed on silica gel (10 g, column size 1.3 × 30 cm) using Et_2O containing 4% MeOH and 0.5% HOAc. The purified product **1** was obtained as a colorless oil (0.54 g, 38%). An analytical sample was obtained by rechromatographing **1** on silica gel (10 g, 1.3 × 30 cm) using CH_2Cl_2 containing 10% of *i*-PrOH and 0.5% of acetic acid as eluant: IR (liquid film) 3400 (OH), 3000–2400 (COOH), 1710 (carboxylic C=O), 1490 cm^{-1} (N=N); NMR ($CDCl_3$) δ 0.8–1.9 [m, 23 H, $CH(CH_2)_5CH_2$, 2 C-7 bridge protons, C_5H_{22}], 2.05–2.45 (m, 2 H, CH_2COOH), 3.1–4.0 (m, 3 H, OCH_2CHOH), 4.86 (br s, 1 H, C-4 bridgehead proton), 5.06 (br s, 1 H, C-1 bridgehead proton), 6.83 (br s, 2 H, OH, COOH); MS (20 eV) *m/e* 326 ($M^+ - N_2$), 297 ($M^+ - C_5H_{11}$), 280 ($M^+ - N_2$ – HCOOH). Anal. ($C_{19}H_{34}N_2O_4$) C, H, N.

Diethyl (±)-5-*endo*-[6-(Ethoxycarbonyl)hexyl]-6-oxo-2,3-diazabicyclo[2.2.1]heptane-2,3-dicarboxylate (7). A solution of **6** (297 mg, 0.72 mmol) in CH_2Cl_2 (3 mL) was added to a mixture CrO_3–pyridine–CH_2Cl_2 which was prepared by dissolving 340 mg of dry pyridine and 600 mg (6 mmol) of CrO_3 in 10 mL of CH_2Cl_2. After stirring at 25 °C for 10 min, ether (200 mL) was added, the reaction mixture was filtered, and the ether was removed in vacuo. The oily residue was redissolved in ether (20 mL) and filtered to eliminate the last trace of insoluble chromium salt. Removal of ether afforded an oil, **7** (150 mg, 50%), which was utilized in the preparation of alcohol **8** without further purification: IR (liquid film) 1760 cm^{-1} (bicyclic ketone); NMR (CCl_4) δ 1.85 (s, 2 H, 2 C-7 bridgehead protons), 1.96–2.33 [m, 3 H, CH_2COO, $CH(CH_2)_6$], 4.7 (br s, 1 H, C-1 bridgehead proton).

Diethyl (±)-5-*endo*-[6-(Ethoxycarbonyl)hexyl]-6-*endo*-hydroxy-2,3-diazabicyclo[2.2.1]heptane-2,3-dicarboxylate (8). Ketone **7** (150 mg, 0.36 mmol) was dissolved in 80:20 EtOH–H_2O (10 mL) and cooled to 0 °C. To this was added with stirring a solution of $NaBH_4$ (30 mg, 0.8 mmol) in 80:20 EtOH–H_2O (2 mL). Stirring was continued for 0.5 h at 0 °C, and the reaction then was quenched by adding dilute HCl until no further generation of hydrogen occurred. Ether (200 mL) and 5 g of anhydrous $MgSO_4$ was added into the reaction mixture. The ether solution was filtered and the solvent removed in vacuo to give a colorless oil, **8** (89 mg, 30% yield from **6**). This product was purified by column chromatography on silica gel (10 g, 1.3 × 30 cm) using 5% *i*-PrOH in Et_2O. An analytically pure product was obtained by rechromatography on silica gel (10 g, 1.3 × 30 cm) and eluted with ether. Product **8**, R_f 0.56 (silica gel, 5% *i*-PrOH in Et_2O), decomposes on prolonged chromatography: IR (liquid film) 3475 cm^{-1} (OH); NMR (CCl_4) δ 3.75–4.2 (m, 7 H, C-4, bridgehead proton, $2NCOOCH_2CH_3$, $COOCH_2CH_3$), 4.32 (br s, 2 H, C-1 bridgehead and C-6 methine protons). MS (70 eV, high resolution) *m/e* calcd M^+ for $C_{20}H_{34}N_2O_7$, 414.2365; found, 414.2368. Anal. ($C_{20}H_{34}N_2O_7$) C, H, N.

Diethyl (±)-5-*endo*-(6-Carbonylhexyl)-6-*exo*-(2-hydroxyheptoxy)-2,3-diazabicyclo[2.2.1]heptane-2,3-dicarboxylate (18). To a solution of **16** (0.2 g, 0.4 mmol) in 50 mL of THF–MeOH–H_2O (3:1:1) was added 0.04 g (1 mmol) of NaOH. After the mixture was stirred for 12 h at 25 °C, the organic solvent was removed in vacuo. The aqueous phase was acidified (10% HCl) and extracted with ether (2 × 200 mL). The ether layer was dried (anhydrous $MgSO_4$), the solvent was removed, and the residue was purified by chromatography on silica gel (10 g, 1.3 × 30 cm) using 20% *i*-PrOH in petroleum ether (30–60 °C) containing 0.5% acetic acid. The yield of product **18** was 0.19 g (90%): IR (liquid film): 3000–2400 cm^{-1} (COOH); NMR (CCl_4) δ 3.9–4.4 (m, 5 H, $2COOCH_2CH_3$, C-1 bridgehead proton), 6.2 (br s, 2 H, OH, COOH); MS (70 eV) *m/e* 500 (M^+). Anal. ($C_{25}H_{44}N_2O_8$) C, H, N.

Diethyl (±)-5-*endo*-(6-Carboxyhexyl)-6-*exo*-hydroxy-2,3-diazabicyclo[2.2.1]heptane-2,3-dicarboxylate (19). Compound **6** (300 mg, 0.73 mmol) was subjected to base hydrolysis in the same manner as described for the preparation of **18** from **16**. The product was chromatographed on silica gel (10 g, 1.3 × 30 cm) using 10% MeOH in Et_2O containing 0.5% HOAc to give **19** (252 mg, 90%): IR (liquid film) 3400–2400 cm^{-1} (OH, COOH); NMR (CCl_4) δ 3.5 (br s, 1 H, C-6 methine proton), 4.0–4.35 (m, 5 H, 2NCOOCH_2CH$_3$, C-4 bridgehead proton), 4.48 (br s, 1 H, C-1 bridgehead proton), 6.25 (br s, 2 H, COOH, OH); MS (70 eV) *m/e* 386 (M^+). Anal. ($C_{18}H_{30}N_2O_7$) C, H, N.

Diethyl (±)-5-*endo*-(6-Carboxyhexyl)-6-*exo*-(2-propenyloxy)-2,3-diazabicyclo[2.2.1]heptane-2,3-dicarboxylate (20). Compound **20** was obtained from **14** (300 mg, 0.66 mmol) by base hydrolysis in the same manner as described in the preparation of **18** from **16**, except the amount of NaOH used was increased to 4 equiv. The product was obtained (225 mg, 80%) by first chromatographing on silica gel (10 g, 1.3 × 30 cm) with ether containing 0.5% HOAc followed by rechromatography on silica gel (10 g, 1.3 × 30 cm) with ether–CH_2Cl_2 (7:3) containing 0.5% HOAc: IR (liquid film) 3400–2400 cm^{-1} (COOH); NMR (CCl_4) δ 3.1 (s, 1 H, C-6 methine proton), 3.95–4.2 (m, 7 H, 2COOCH_2CH$_3$, C-4 bridgehead proton, OCH_2CH=C), 4.95–6.0 (m, 3 H, CH=CH_2); MS (70 eV) *m/e* 426 (M^+). Anal. ($C_{21}H_{34}N_2O_7$) C, H, N.

(±)-5-*endo*-(6-Carboxyhexyl)-6-*exo*-(2-propenyloxy)-2,3-diazabicyclo[2.2.1]hept-2-ene (21). Compound **21** was obtained from **14** (2.3 g, 5 mmol) by base hydrolysis and oxidation in the same manner as described in the preparation of **1** from **16**. The crude product was chromatographed on silica gel (50 g, 2.3 × 35 cm) using 20% *i*-PrOH in petroleum ether (30–60 °C) with 0.5% HOAc to afford **21** (0.674 g, 49%). A second chromatography using the same conditions afforded an analytically pure sample: IR (liquid film) 3000–2400 (COOH), 1650 (C=C), 1490 cm^{-1} (N=N); NMR (CCl_4) δ 2.72 (br s, 1 H, C*H*OCH$_2$), 3.8–4.1 (m, 2 H, OCH_2C=C), 4.8–6.0 (m, 5 H, CH=CH_2, two bridgehead protons); MS (70 eV) *m/e* 252 ($M^+ - N_2$). Anal. ($C_{15}H_{24}N_2O_3$) C, H, N.

Hydrogen-Bonding Studies of 6 and 8. Solutions (0.025, 0.005, and 0.00125 M) of **6** and **8** in spectral grade carbon tetrachloride were prepared and placed in 1.0-cm cells, and the infrared spectra were recorded between 4000 and 3000 cm^{-1} using a Beckman IR-9 infrared spectrophotometer.

Platelet-Aggregation Studies. Human platelet-rich plasma (PRP) was prepared according to Gerrard et al.[35] and incubated with the potential inhibitor for 1.5 min at 37.5 °C before the addition of an aggregating agent. Aggregation or inhibition of aggregation was monitored for 5 min on a Payton dual-channel aggregometer.

For TxA_2-induced aggregation, the TxA_2 was generated by mixing suspended human platelet microsomes (0.1 mL, 1.9 mg of protein/mL) with 10^{-4} M PGH_2 at 37.5 °C. After stirring for 15 s, aspirinated PRP (100 mg of aspirin/mL, 0.9 mL) was added and the aggregation was monitored for 5 min. Boiled microsomes were used for control experiments.

Metabolism Studies of Arachidonic Acid by Platelets. A solution of arachidonic acid (0.1 mL, 0.82 mM) was added to a suspension of washed human platelets[35] (1.9 mL, 2×10^9 platelets/mL) which had been preincubated with 10^{-4} M AOHP for 1.5 min. The metabolites were isolated by extraction with EtOAc and esterified by diazamethane.[35] Prior to GC–MS analysis, the sample was silylated by treatment with *N,N*-bis(trimethylsilyl)trifluoroacetamide in dry pyridine. The column used for GC was a 3% OV-17 (6 ft) with the temperature programmed to increase 0.6 °C/min from 200 to 280 °C. The mass units monitored were: 187 for ricinoleic acid, 225 for HHT, 295 for HETE, and 256 for TxB_2. Controls were performed in the absence of AOHP and on AOHP itself. The presence of AOHP did not affect the intensity of the measured mass units.

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