approximately 1 h under a maintained resting tension of 0.5 g prior to drug testing. Isometric measurements were made with a Grass FT03C force-displacement transducer and recorded on a Grass Model 79D Polygraph as changes in grams of force.

Results were expressed as either $-\log IC_{50}$ or pK_B values. The former represents the –log of that concentration of antagonist that caused a submaximal LTD_4-induced contractions of guinea pig ileum to be reduced by 50% whereas the latter is –log of that antagonist concentration producing a twofold rightward shift of the LTD_4 concentration–response curve.[17] These values were similar for a particular compound and for all intents and purposes were interchangeable. $-\log IC_{50}$ was generally obtained with two, three, or four concentrations of an experimental compound on a single ileum. The extrapolated antagonist concentration that produced 50% inhibition of the LTD_4 responses was calculated by using linear regression. Thus, the larger the $-\log IC_{50}$ value assigned to a compound, the more potent it was as an LTD_4 antagonist relative to other members of the chemical series. This assumes that the investigational compound did not exert significant nonspecific depression of the bioassay tissue. All compounds were therefore examined as antagonists of contractions induced by bradykinin. Those agents that similarly reduced the responsiveness of the ileum to LTD_4 and to bradykinin were considered to be nonspecific smooth muscle depressants and were eliminated from consideration. pK_B values were more rigorously obtained, and this type of analysis was reserved for those compounds with a higher degree of interest.

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Registry No. 1, 15184-99-3; **2**, 97582-36-0; **3**, 97581-90-3; 4, 97582-07-5; **5**, 107223-29-0; **6**, 107223-30-3; **7**, 107223-31-4; **8**, 107223-32-5; **9**, 107223-33-6; **10**, 107223-34-7; 11, 107223-35-8; **12**, 91339-33-2; **13**, 97582-09-7; 14, 97582-08-6; **15**, 107223-36-9; **16**, 97582-10-0; 17, 61186-04-7; **18**, 97582-16-6; **19**, 97582-17-7; **20**, 97582-18-8; **21**, 107223-37-0; **22**, 97582-19-9; **23**, 107223-38-1; **24**, 97582-23-5; **25**, 97582-24-6; **26**, 97582-22-4; **26** (free base), 97582-21-3; **27**, 107223-39-2; **28**, 97582-27-9; **29**, 107223-40-5; **30**, 97582-30-4; **31**, 97582-31-5; **32**, 97582-29-1; **33**, 107223-41-6; **34**, 97582-32-6; **35**, 107223-42-7; **36**, 97582-37-1; **37**, 97582-38-2; **38**, 107223-43-8; **39**, 97582-41-7; **40**, 107223-44-9; **41**, 97603-23-1; **42**, 97581-95-8; **43**, 97581-67-4; **44**, 107223-45-0; **45**, 97581-68-5; **46**, 97581-58-3; **47**, 107223-46-1; **48**, 107223-47-2; **49**, 97581-59-4; **50**, 97581-96-9; **51**, 97581-60-7; **52**, 97581-97-0; **53**, 107223-48-3; **54**, 107223-49-4; **55**, 97581-66-3; **56**, 97581-63-0; **57**, 97581-62-9; **58**, 97581-91-4; **59**, 107223-50-7; **60**, 107223-51-8; **61**, 107223-52-9; **62**, 97581-65-2; **63**, 107223-53-0; **64**, 97581-76-5; **65**, 97581-99-2; **66**, 97581-81-2; **67**, 97581-69-6; **68**, 97581-70-9; **69**, 107223-54-1; **70**, 107223-55-2; **71**, 97581-72-1; **72**, 97582-01-9; **73**, 97581-73-2; **74**, 97582-02-0; **75**, 107223-56-3; **76**, 107223-57-4; **77**, 97581-80-1; **78**, 97581-77-6; **79**, 97581-75-4; **80**, 97581-92-5; **81**, 107223-58-5; **82**, 107223-59-6; **83**, 107223-60-9; **84**, 97581-79-8; **85**, 107223-61-0; **86**, 107223-62-1; **87**, 107223-63-2; **88**, 107223-64-3; **89**, 107223-65-4; **90**, 107223-66-5; **91**, 107223-67-6; **92**, 107223-70-1; **93**, 107223-71-2; **94**, 107223-72-3; **95**, 107223-73-4; **96**, 107223-74-5; **97**, 107223-77-8; **97** (free base), 97582-35-9; **98**, 97582-40-6; **99**, 107244-53-1; **100**, 107223-75-6; **101**, 107223-76-7; **102**, 107223-78-9; VI (X = O, Y = 4-CH_2), 14191-95-8; VI (X = O, Y = 4-CH_2CH_2), 17362-17-3; VI (X = O, Y = 3-CH_2), 25263-44-9; VI (X = O, Y = 2-CH_2), 14714-50-2; VI (X = O, Y = 4-$CH_2CH_2CH_2$), 107223-68-7; VI (X = O, Y = 4-$CHCH_3$), 21850-61-3; VI (X = O, Y = 4-OCH_2), 96562-56-0; VI (X = O, Y = 3-OCH_2), 107223-69-8; VI (X = O, Y = 4-CH=CH), 82575-52-8; VI (X = NH, Y = 4-CH_2), 3544-25-0; VI (X = S, Y = 4-CH_2), 36801-01-1; *n*-anisaldehyde, 591-31-1; ethyl chloroformate, 541-41-3; 4-cyanophenyl, 767-00-0; (4-acetyl-3-hydroxy-2-propylphenyl)methanethiol, 97582-45-1; α-bromo-*p*-tolunitrile, 17201-43-3; methanesulfonyl chloride, 124-63-0; tri-*n*-butyltin azide, 17846-68-3; pivaloyl chloride, 3282-30-2; 2-nitropropane (sodium salt), 34537-87-6; propionyl chloride, 79-03-8; 2,4-dihydroxy-3-propylacetophenone, 40786-69-4.

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Preparation and Biological Evaluation of a Potential Photoaffinity Label for the Prostaglandin H_2/Thromboxane A_2 Receptor[1]

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Two aromatic azides (**24** and **26**) were prepared as potential photoaffinity probes for the PGH_2/TXA_2 receptor. The compounds are based on the well-characterized PGH_2/TXA_2 receptor antagonist 13-azaprostanoic acid, with the terminus of its lower side chain replaced with phenoxy (**24**) or benzyl (**26**) azide functionality. The two compounds were shown to irreversibly inhibit platelet function after photolysis and resuspension. However, of the two aromatic azides, only the benzyl derivative **26** appeared to be selective for the prostaglandin pathway. The latter compound was also prepared as the aromatic ^{125}I (**29**) derivative, which may ultimately prove useful as a labeled probe for the identification and isolation of the putative TXA_2/PGH_2 receptor.

Research on the role of prostaglandins in platelet aggregation has provided us with a better understanding of cardiovascular disease states and a rationale for the design of drugs potentially useful in the treatment of thrombotic conditions such as myocardial infarction, stroke, and pulmonary embolism.[2] In recent years significant progress has been made in understanding the inhibitory mechanisms of the prostaglandins PGI_2, PGE_1, and PGD_2 on platelet function. With use of labeled derivatives, it has been possible to show that these particular prostaglandins are powerful adenylate cyclase stimulators that mediate their effects by presumably binding to receptors on the platelet membrane.[3-8]

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In contrast, interaction of the stimulatory arachidonic acid metabolites, i.e., thromboxane A_2 (TXA_2) and prostaglandin H_2 (PGH_2) with the platelet are not so well characterized. In part this is due to the highly unstable nature of these molecules. PGH_2 is known to have a half-life of about 5 min in aqueous media[9] whereas TXA_2 has been judged to have a half-life of about 32 s, as determined by bioassay.[10] The recent synthesis of TXA_2 by Still and co-workers[11] and the availability of PGH_2[12,13] should provide answers to many of the questions surrounding the relative biological role of these molecules in the platelet and other tissues.

However, because of the lability of these structures, localization, characterization, and isolation of the putative receptor(s) at which these unstable prostaglandins act require synthesis of stable analogues that either mimic or antagonize these natural prostaglandins at the receptor level. Recently, the syntheses of various chemical compounds that directly antagonize the action of TXA_2/PGH_2 have been reported.[14-21] Thus, various agents are currently being employed as reversible pharmacological probes to characterize the molecular interaction of agonists or antagonists with the cellular receptor. In this connection, we have previously shown that one such compound, 13-azaprostanoic acid,[16,22] binds to the platelet membrane in a specific, saturable, and reversible manner.[23] These findings provided direct evidence for the existence of a discrete TXA_2/PGH_2 binding component in human platelets. Comparable results have since been reported with radiolabeled derivatives of pinane thromboxane[24] and the TXA_2/PGH_2 mimetics U44069[25] or U46619.[26] In a preliminary report, a tritium-labeled 13-azaprostanoic acid[27] has also been used in identification of the platelet TXA_2/PGH_2 receptor during its partial purification.[28] It remains to be seen if these reversible antagonists will be useful in further characterization and possible isolation of the putative receptor(s) responsible for the TXA_2 and PGH_2 activities in the platelet and other tissues.

Receptor isolation can be greatly facilitated by a ligand that remains associated with the receptor during isolation. Consequently, the usefulness of reversible ligands is a function of the dissociation constant of the ligand for the receptor under the conditions of purification employed. In the past, problems of dissociation have been circumvented by the use of a covalent link between the ligand and receptor, generated via a chemically reactive moiety (affinity label) or a photoactivable moiety (photoaffinity label) built into a known agonist or antagonist for the given receptor.[29] We chose the latter approach in our search for a derivative that might be useful in the identification and isolation of the putative TXA_2/PGH_2 receptor.

Of the aforementioned compounds that have been synthesized and shown to be platelet PGH_2/TXA_2 agonists or antagonists, the well-characterized 13-azaprostanoic acid possesses the type of specificity necessary for a successful photoaffinity probe. The molecule inhibits TXA_2 activation of both the platelet and the vasculature but does not interfere with the metabolism of arachidonic acid.[16,22,30,31] Inhibition of arachidonic acid induced aggregation by the azaprostanoids exhibits stereospecificity,[16] structural specificity,[16] and reversibility.[23] Moreover, 13-azaprostanoic acid does not cause cAMP accumulation nor is it involved in the stimulation of adenylate cyclase by PGE_1 or PGI_2.[32] Finally 13-azaprostanoic acid specifically binds to the TXA_2/PGH_2 platelet receptor in isolated platelet membranes and intact human platelets.[23,26] This apparent biological specificity for the putative TXA_2/PGH_2 receptor, coupled with its chemical stability and ease of synthesis, makes 13-azaprostanoic acid a logical starting point for preparation of an affinity label.

We reasoned that replacement of the terminal five carbons of the lower side chain in the parent 13-azaprostanoic acid structure might not be detrimental to biological activity. This was based largely on previous work showing that replacement of five of the carbons on the lower side chain of $PGF_{2\alpha}$ with a phenoxide moiety resulted in retention of prostaglandin-like activity.[33] Such a derivative would then provide us with the aromatic functionality necessary to introduce a photolabile group (N_3) and radiolabeled (^{125}I) functionality by standard procedures.

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Table I. Structure Summary of Derivatives Prepared

1, R = C_2H_5
2, R = CH_3
3, X = CH_2O, R′ = H
4, X = CH_2O, R′ = NO_2
5, X = CH_2, R′ = NO_2

compd	stereo	substituent X	R	R′	R″	% yield from keto ester 1 or 2
6	cis	CH_2O	C_2H_5	H	H	34
7	trans	CH_2O	C_2H_5	H	H	42
8	cis	CH_2O	C_2H_5	NO_2	H	29
9	trans	CH_2O	C_2H_5	NO_2	H	36
10	cis	CH_2	CH_3	NO_2	H	36
11	trans	CH_2	CH_3	NO_2	H	38
12	cis	CH_2O	H	H	H	28
13	trans	CH_2O	H	H	H	29
14	cis	CH_2O	H	NO_2	H	24
15	trans	CH_2O	H	NO_2	H	28
16	cis	CH_2	H	NO_2	H	32
17	trans	CH_2	H	NO_2	H	36
18	trans	CH_2O	C_2H_5	NH_2	H	25
19	cis	CH_2	CH_3	NH_2	H	35
20	trans	CH_2	CH_3	NH_2	H	37
21	trans	CH_2O	H	NH_2	H	19
22	cis	CH_2	H	NH_2	H	30
23	trans	CH_2	H	NH_2	H	30
24	trans	CH_2O	H	N_3	H	16
25	cis	CH_2	H	N_3	H	19
26	trans	CH_2	H	N_3	H	20
27	trans	CH_2	H	NH_2	^{127}I	15
28	trans	CH_2	H	N_3	^{127}I	14
29	trans	CH_2	H	N_3	^{125}I	

The present paper describes the synthesis and preliminary biological examination of two such potential photoaffinity ligands for the putative PGH_2/TXA_2 receptor(s).

Chemistry

The general approach to the synthesis of derivatives presented in this paper is outlined in the reaction given in Table I. A given lower side chain (**3**, **4**, or **5**) was condensed with one of the keto esters[34] **1** or **2** to produce an imine, which was subsequently reduced with $NaBH_4$ in situ to give the five membered ring cis and trans isomers, which were separated by chromatography. Conventional aromatic functional-group chemistry was then used to transform these precursors into the various derivatives studied (Table I).

In order to determine what effect replacing part of the lower side chain of 13-azaprostanoic acid with an aromatic group would have on the PGH_2/TXA_2 inhibitory properties of 13-azaprostanoic acid, phenoxy derivatives **12** and **13** were prepared. The encouraging biology for these derivatives, discussed below, prompted further synthesis and evaluation of the corresponding photoactivable derivatives.

Initially, we chose as our model the phenoxy azide **24**, which was to be examined for specific irreversible inhibition of platelet aggregation before labeling with iodine. The nitrophenoxy derivative **4**, which was used as the starting lower side chain for this molecule, was prepared from commercially available 1-bromopropylamine, first protected as the carbamate. Subsequent displacement of the halogen with the anion of *p*-nitrophenol and removal of the blocking group gave 1-amino-3-(4′-nitrophenoxy)propane (**4**) in 60–67% overall yield.[35]

Condensation of this nitro lower side chain **4** with the keto ester **1** and reduction with sodium borohydride gave the cis derivatives (**8** and **9**) of ethyl 17,18,19,20-tetranor-16-(4′-nitrophenoxy)-13-azaprostanoate after chromatographic separation. Only the trans isomer, possessing the natural prostaglandin stereochemistry, was carried through to the aromatic azide stage. This was accomplished by catalytic reduction of **9** to the aromatic amine **18**, which was diazotized and converted to the aromatic azide by treatment with sodium azide. Subsequent hydrolysis of the ester afforded **24**.

Both the cis and trans nitro (**8** and **9**) and the trans amino (**18**) derivatives were also hydrolyzed to their corresponding acids for biological testing. As discussed in the following biological section, these three derivatives showed antiplatelet activity comparable to that of the parent 13-azaprostanoic acid. In addition, after photolysis, the azide **24** showed characteristics of irreversible inhibition. However, under more careful examination this platelet inactivation did not appear specific. In addition, exhaustive attempts to iodinate either the azide **24** or its ester or the precursor amine **18** or its ester were unsuccessful.

We felt that the lability of the phenoxide bond might be responsible for the failure of these derivatives to undergo iodination. Consequently, the corresponding methylene derivatives **25** and **26** were prepared. The starting lower side chain in this series was prepared from phenylpropyl bromide, which was first nitrated[36] and then converted to the desired amine via a standard Gabriel reaction.[37] Subsequent chemistry analogous to that for the phenoxy series provided the corresponding trans methylene series: nitro **17**, amine **23**, and azido **26** derivatives. Because of the encouraging biology obtained on this series, the corresponding cis isomers, **16**, **22**, and **25**,

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Table II. Platelet Inhibitory Activity Remaining after Treatment with Azides, Photolysis, and Resuspension

compound	% inhibn relative to 13-azaprostanoic acid[a] ADP	U46619 (*N*)
phenoxy azide **24**	50 ± 9	38 ± 9 (5)
phenyl azide **26**	19 ± 6	63 ± 6 (8)
iodo azide **28**	15 ± 11	81 ± 3 (5)

[a] 13-Azaprostanoic acid, or one of the three derivatives listed, was added to platelet-rich plasma at the minimum concentration necessary to produce 100% inhibition of 3 μM U46619-induced aggregation. The incubates were photolyzed (30 min) with a mercury light. The platelets were then centrifuged and the platelet pellet resuspended in Tris·HCl buffer (pH 7.4). Platelet aggregation of the resuspended cells was induced by addition of ADP (10 μM) or U46619 (0.5 μM). The values presented were calculated by the following relationship: percent inhibition relative to 13-azaprostanoic acid = [1 − (residual platelet activity with given azide/the residual platelet activity with 13-azaprostanoic acid)] × 100.

were also isolated and tested for their antiplatelet activity.

Attempts to prepare the target iodo azide **28** by direct iodination of either the free acid **26** or its ester met with failure under a variety of conditions. However, the desired iodo azide could be prepared by introducing the iodine at the amino acid stage to give the iodo amine **27**, which was subsequently diazotized and converted to the azide. Moreover, this approach proved to be successful on a very small scale and, when coupled with TLC separation, was successfully used to prepare the corresponding ^{125}I-labeled aromatic azide **29**. The labeled material was found to cochromatograph with the authentic, unlabeled iodo azide **28** both in adsorption chromatography (TLC) and reversed-phase modes (HPLC).

Biology

Both model aromatic derivatives **12** and **13**, all of the nitro derivatives **14–17**, the corresponding amines **21–23**, and corresponding azides **24–26** inhibited U46619 (a direct TXA_2 agonist,[38] 3 μM) induced aggregation in human platelets and were of comparable activity (ED_{50} = 5–20 μM) to the parent 13-azaprostanoic acid (ED_{50} = 8 μM). The target iodo azide **28** was found to be somewhat more effective (ED_{50} = 2 μM) than the parent 13-azaprostanoic acid in its ability to inhibit U46619-induced aggregation in human platelet rich plasma (PRP). On the basis of these encouraging results, we attempted to determine if the observed inhibition was irreversible after photolysis and whether the inhibitory properties were specific for the prostaglandin pathway.

A photoaffinity label directed specifically at the platelet TXA_2 receptor should, after photolysis and washing, inhibit U46619-induced aggregation (a direct TXA_2 receptor agonist) but not ADP-induced aggregation, which causes platelet activation by a prostaglandin-independent pathway. Table II summarizes the results of such a study. In these experiments 13-azaprostanoic acid, or one of the three derivatives studied, was added to PRP at the minimum concentration necessary to produce 100% inhibition of U46619. The platelets were then centrifuged and resuspended in buffer to reduce the amount of noncovalently linked drug. The platelet resuspension was then challenged with ADP or U46619.

As may be seen, all of the azides show the ability to inhibit U46619 after photolysis and resuspension. However, the phenoxy azide **24** shows no difference in its ability to inhibit U46619 relative to ADP. Consequently, we believe that this derivative is acting nonspecifically to generally depress platelet function. On the other hand, both the phenyl azide **26** and iodophenyl azide **28** show a clear ability to inhibit U46619 after photolysis and resuspension, while showing little inhibitory activity toward ADP. We believe these agents are acting irreversibly and specifically on the prostaglandin pathway in the platelet, presumably at the platelet TXA_2/PGH_2 receptor level.

This preliminary conclusion is supported by recent binding studies in whole platelets.[39,40] The iodo azide **28** was found to have a K_i of 290 nM for inhibition of [^{3}H]-U46619 binding in dark incubated platelets. Platelets incubated in the dark with iodo azide **28** were also compared with those incubated with **28** under photolysis conditions. Both incubates were washed several times with buffer, and their ability to bind U46619 was evaluated. It was found that iodo azide **28** significantly inhibited (38 ± 8%, $p < 0.02$, $n = 6$) UV-irridiated platelets relative to dark incubated platelets after washing, suggesting specific, irreversible inhibition of U46619 binding after photoactivation.

On the basis of these observations, we believe that the 13-azaprostanoic acid based iodo azide **29** is acting as a photoaffinity label for the TXA_2/PGH_2 receptor in the platelet and could ultimately be useful in the identification and isolation of TXA_2 receptor protein(s) in various tissues.

Experimental Section

All melting points were determined in open capillary tubes on a Thomas-Hoover apparatus and are uncorrected. IR spectra were obtained on a Nicolet MX-1 FT infrared spectrometer. Spectra were obtained as neat films on NaCl disks or as KBr pellets. Varian XL300 and Nicolet NT360 spectrometers were used to obtain the 300- and 360-MHz spectra, respectively. All carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra were obtained with a 5-mm probe on the NT-360 instrument operating at 90 MHz. Conventional heteronuclear and homonuclear 2D techniques were used, in part, to assign resonances. Chemical shifts for both proton (^{1}H NMR) and ^{13}C NMR are reported in parts per million (ppm, δ) downfield from the internal standard tetramethylsilane (Me_4Si). Both high- and low-resolution electron-impact mass spectra (EIMS) were obtained with a Varian MAT-112S double-focusing instrument operating at an ionizing potential of 70 eV. Chemical-ionization mass spectra (CIMS) were obtained with a Finnigan MAT 4510 mass spectrometer with an INCOS data system. Generally, direct chemical ionization (DCI) was used for solid and nonvolatile samples. Ammonia was used as a reagent gas (0.4 mmHg, 50 °C). Thin-layer chromatography (TLC) was performed on Merck silica gel $60F_{254}$ (0.2 mm, precoated on TLC aluminium sheet), and spots were visualized by UV light, iodine vapor, and nitroprusside–acetaldehyde spray reagent,[41] when appropriate. Silica gel (Sigma Type H, 10–40 μm) was used for medium-pressure chromatography. All HPLC was performed with Waters (Milford, MA) Model 510 pumps and Model 680 gradient controller and Rheodyne Model 7125 injector (100-μL loop). An IBM-ODS (4.5 mm × 25 cm) reversed-phase column eluted at 1 mL/min (CH_3OH–H_2O (4:6) with 0.05 M NH_4OAc isocratic for 10 min, linear gradient to 100% CH_3OH from 10 min to 33 min) was used for all HPLC analyses. Elemental analyses were performed by Micro-Tech Laboratories Inc., Skokie, IL.

Chemistry. *dl*-2-*epi*-16-Phenoxy-17,18,19,20-tetranor-13-azaprostanoic Acid (12). To a solution of 2-(6′-carbethoxyhexyl)cyclopentanone[34] (1; 8.0 g, 0.033 mol) in absolute ethanol (150 mL) was added 4 A molecular seive (10 g). The reaction mixture was stirred and 1-amino-3-phenoxypropane (3; 8 g, 0.049

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mol) was added. The solution was stirred under a nitrogen atmosphere at room temperature for 48 h. After cooling (ice–water), $NaBH_4$ (1.3 g, 0.034 mol, 98%) was added and the mixture stirred for an additional 30 min. The excess sodium borohydride was decomposed by the addition of acetone (2 mL). The molecular sieve was filtered and washed with ethanol (100 mL). After removal of the solvents, the residue remaining was dissolved in dichloromethane (300 mL), washed with water (2 × 50 mL) and brine (2 × 50 mL), and dried ($MgSO_4$). Removal of the solvent gave a clear oil (15 g). Medium-pressure chromatography of this oil on silica gel (400 g, 10–40 μm, absolute ether) gave **6** (4.2 g, 34 %) as a clear oil: TLC R_f 0.065 (Et_2O–NH_4OH, 99:1); ^{1}H NMR ($CDCl_3$, 360 MHz) δ 7.29–7.24 (m, 2 H, Ar-XX′), 6.95–6.88 (m, 3 H, Ar-AA′B), 4.12 (q, 2 H, OCH_2CH_3), 3.02–2.96 (m, 1 H, CHNH), 2.27 (t, 2 H, $CH_2COOCH_2CH_3$); CIMS, 376 (M + 1).

The cis amino ester **6** (4 g, 0.01 mol) was refluxed with aqueous NaOH (2.5%, 120 mL) until a homogeneous solution was obtained (6 h). After cooling, the solution was acidified by the dropwise addition of 5% aqueous HCl and then made strongly basic by addition of excess concentrated NH_4OH. The solution was gently heated to expel excess NH_3 until it became neutral to pH paper. The solution was cooled and the crude amino acid was collected by filtration, washed with water, and air-dried to give **12** (3.1 g, 84%), mp 131–134 °C, as a white crystalline solid. Two recrystallizations from ethanol gave the analytical sample: mp 133–134 °C; ^{1}H NMR (CD_3OD, 360 MHz) δ 7.30–7.25 (m, 2 H, Ar-XX′), 6.96–6.91 (m, 3 H, Ar-AA′B), 3.51–3.30 (m, 1 H, CHNH), 2.19–2.12 (m, 2 H, CH_2COOH overlapping CH_2OAr and $NHCH_2$); CIMS, 348 (M + 1). Anal. $C_{21}H_{33}NO_3$ (C, H, N).

***dl*-16-Phenoxy-17,18,19,20-tetranor-13-azaprostanoic Acid (13).** Further elution of the column used to isolate the corresponding cis isomer **6** gave several fractions which were a mixture of the cis and trans isomers (1 g, 8%) and then pure ethyl *trans*-phenoxyprostanoate **7** (5.2 g, 42%): TLC R_f 0.45 (Et_2O–NH_4OH, 99:1); ^{1}H NMR ($CDCl_3$, 360 MHz) δ 7.3–7.2 (m, 2 H, Ar-XX′), 6.95–6.88 (m, 3 H, Ar-AA′B), 4.12 (q, 2 H, OCH_2CH_3), 2.70–2.61 (m, 1 H, CHNH), 2.27 (t, 2 H, CH_2CO); CIMS, 376 (M + 1).

The trans amino ester **7** (2.0 g, 0.005 mol) was hydrolyzed and brought to its isoelectric point in the same manner as for the cis isomer to give white crystalline **13** (1.75 g). Recrystallization (2×) from EtOH gave the analytical sample (1.4 g, 76%): mp 156–156.5 °C; ^{1}H NMR ($CDCl_3$, 360 MHz) δ 7.30–7.25 (m, 2 H, Ar-XX′), 6.96–6.91 (m, 3 H, Ar-AA′B), 3.30–3.20 (m, 1 H, CHNH), 2.21–2.12 (m, CH_2COOH overlapping CH_2OAr); CIMS, 348 (M + 1). Anal. $C_{21}H_{33}NO_3$ (C, H, N).

***dl*-12-*epi*-16-(4′-Nitrophenoxy)-17,18,19,20-tetranor-13-azaprostanoic Acid (14).** Synthesis of the nitro derivative **8** was carried out in essentially the same manner as that for the parent acid **12**. (*p*-Nitrophenoxy)propylamine (13.6 g, 0.07 mol) was condensed with 2-(6′-carbethoxyhexyl)cyclopentanone (12.0 g, 0.05 mol) in ethanol. In situ reduction with $NaBH_4$ (2.26 g, 0.06 mol), workup, and medium-pressure chromatography (400 g, Et_2O), as for the parent **6**, gave the ethyl *cis*-(nitrophenoxy)-prostanoate **8** (6.0 g, 29%): TLC R_f 0.40 (Et_2O–CH_3OH, 80:20); ^{1}H NMR ($CDCl_3$, 360 MHz) δ 8.25–8.10 (m, 2 H, Ar-XX′), 7.05–6.9 (m, 2 H, Ar-AA′), 4.125 (q, 2 H, OCH_2CH_3), 3.15–2.95 (m, 1 H, CHNH), 2.274 (t, 2 H, $CH_2COC_2H_5$); CIMS, 421 (M + 1).

Hydrolysis of this ester (1 g, 0.002 mol) and workup as for the parent **12** gave the desired nitrophenoxy prostanoid **14** as a slightly yellow crystalline material (0.8 g, 86%, mp 150–152 °C). Two crystallizations from ethanol gave the analytical sample (0.72 g, 77%): mp 151–152 °C; ^{1}H NMR (CD_3COOD, 300 MHz) δ 8.24–8.16 (m, 2 H, Ar-XX′), 7.10–7.0 (m, 2 H, Ar-AA′), 3.68–3.58 (m, 1 H, CHNH), 2.36 (t, 2 H, CH_2COOH); CIMS, 393 (M + 1). Anal. $C_{21}H_{32}N_2O_5$ (C, H, N).

***dl*-16-(4′-Nitrophenoxy)-17,18,19,20-tetranor-13-azaprostanoic Acid (15).** Further elution of the column used to isolate the corresponding cis isomer **8** gave several fractions which were a mixture of the cis and trans isomers (**8** and **9**, 1.2 g, 6%) and then pure ethyl *trans*-(nitrophenoxy)prostanoate **9** (7.5 g, 36%): TLC R_f 0.31 (Et_2O–CH_3OH, 80:20); ^{1}H NMR ($CDCl_3$, 360 MHz) δ 8.25–8.10 (m, 2 H, Ar-XX′), 7.1–6.9 (m, 2 H, Ar-AA′), 2.63–2.55 (m, 1 H, CHNH), 2.00 (t, 2 H, $CH_2COOC_2H_5$).

The trans amino ester **9** (0.2 g, 0.005 mol) was hydrolyzed and worked up in the same manner as the cis isomer to give yellow crystalline **15** (0.173 g). Two recrystallizations from ethanol gave the analytical sample (0.145 g, 78%): mp 181–182 °C; ^{1}H NMR (CD_3COOD, 300 MHz) δ 8.24–8.17 (m, 2 H, Ar-XX′), 7.10–7.02 (m, 2 H, Ar-AA′), 3.40–3.20 (m, 1 H CHNH overlapping $NHCH_2$), 2.36 (t, 2 H, CH_2COOH); CIMS, 393 (M + 1). Anal. $C_{21}H_{32}N_2O_5$ (C, H, N).

***dl*-16-(4′-Aminophenoxy)-17,18,19,20-tetranor-13-azaprostanoic Acid (21).** The trans phenoxynitro derivative **9** (2.0 g, 0.0047 mol) was hydrogenated in ethanol (100 mL) over Pd/C (10%, 200 mg) with a Parr hydrogenation apparatus (35 psi) for 10 h. The reaction mixture was filtered through Celite and the filtrate concentrated under vacuum to give a clear oil which showed a single spot on TLC (R_f 0.41, Et_2O–CH_3OH–NH_4OH, 80:20:0.2) with a small amount of impurity at the top of the plate. Purification was achieved by medium-pressure chromatography (100 g, Et_2O–petroleum ether, 80:20) to give chromatographically homogeneous **18** (1.65 g, 89%): ^{1}H NMR ($CDCl_3$, 360 MHz) δ 6.75–6.72 (m, 2 H, Ar-BB′), 6.65–6.61 (m, 2 H, Ar-AA′), 4.12 (q, 2 H, OCH_2CH_3), 3.05–2.90 (m, 1 H, CHNH), 2.27 (t, 2 H, $CH_2COOC_2H_5$); CIMS, 391 (M + 1).

The aromatic amino ester **18** (1 g, 0.0025 mol) was hydrolyzed in the same manner as described for the preparation of **12** to give the crystalline aromatic amino acid **21** (0.75 g). Two crystallizations from ethanol gave the analytical sample (0.70 g, 76%): mp 164–165 °C; ^{1}H NMR (CD_3COOD, 360 MHz) δ 7.43–7.40 (m, 2 H, Ar-XX′), 7.00–6.98 (m, 2 H, Ar-AA′), 3.34–3.27 (m, CHNH, overlapping $NHCH_2$), 2.35 (t, 2 H, CH_2COOH); CIMS, 363 (M + 1). Anal. ($C_{21}H_{34}N_2O_3$) C, H, N.

***dl*-16-(4′-Azidophenoxy)-17,18,19,20-tetranor-13-azaprostanoic Acid (24).** The aromatic amino ester **18** (420 mg, 0.001 mol) was dissolved in acetic acid (3 M, 700 mL) and treated with sodium nitrite (630 mg, 9.1 mmol/30 mL of H_2O) at room temperature. After 15 min of stirring, sodium azide (630 mg, 9.69 mmol/30 mL of H_2O) was added dropwise. Following an additional 30 min of stirring, the reaction mixture was stopped by neutralization with concentrated NH_4OH and extracted with $CHCl_3$ (3 × 100 mL) and washed with H_2O (2 × 25 mL), and the organic layer was dried ($MgSO_4$). Removal of the solvents gave the ester of **24** as a dark yellow oil. The intermediate ester of **24** (360 mg, 80%) was purified by medium-pressure chromatography (80 g, Et_2O): TLC R_f 0.27 (CH_3OH–Et_2O, 10:90); IR (neat) 2112 cm^{-1} (N_3); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.00–6.80 (m, 4 H, Ar-AA′BB′), 4.12 (q, 2 H, OCH_2CH_3), 2.81–2.74 (m, 1 H, CHNH), 2.27 (t, 2 H, $CH_2COOC_2H_5$); IR (KBr) 2115 cm^{-1} (N_3), CIMS, 417 (M + 1).

The hydrolysis of ester **24** (200 mg, 0.48 mmol) in the usual manner and crystallization from CH_3OH (2×) gave the analytical sample **24** (150 mg, 81%): mp 151–152 °C dec; IR (KBr) 2114 cm^{-1} (N_3); ^{1}H NMR (CD_3COOD, 360 MHz) δ 6.98–6.91 (m, 4 H, Ar-AA′BB′), 3.33–3.28 (m, CHNH, overlapping OCH_2), 2.35 (t, 2 H, CH_2COOH); CIMS, 389 (M + 1). Anal. ($C_{21}H_{32}N_4O_3$) C, H, N.

***dl*-12-*epi*-16-(4′-Nitrophenyl)-17,18,19,20-tetranor-13-azaprostanoic Acid (16).** To a solution of 2-(6′-carbomethoxyhexyl)cyclopentanone (11.3 g, 0.05 mol) in absolute CH_3OH (200 mL) was added 4 A molecular sieve (20 g) and 3-(*p*-nitrophenyl)propylamine[36] (**5**; 13.5 g, 0.075 mol). The resulting mixture was stirred under a nitrogen atmosphere for 24 h. After cooling (ice bath), $NaBH_4$ (2.27 g, 0.059 mol) was added and the mixture stirred for an additional 30 min. Excess $NaBH_4$ was decomposed by the addition of acetone (2 mL). The reaction mixture was filtered, and the solvents were removed in vacuo. The residue remaining was dissolved in Et_2O (400 mL), washed with water (3 × 25 mL) and brine (2 × 25 mL), and dried ($MgSO_4$), and the solvent was removed in vacuo. The yellow oil (24 g) that remained was subjected to medium-pressure chromatography (500 g, Et_2O) to give the cis nitro ester **10** (7.0 g, 36%) as a light yellow oil: TLC R_f 0.63 (Et_2O–CH_3OH–NH_4OH, 60:40:0.07); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.20–8.10 (m, 2 H, Ar-XX′), 7.45–7.30 (m, 2 H, Ar-AA′), 3.687 (s, 3 H, $COOCH_3$), 3.05–2.90 (m, 1 H, CHNH), 2.33 (t, 2 H, CH_2COOCH_3); ^{13}C NMR ($CDCl_3$, 360 MHz) δ 172.41 (C=O), 59.92 ($COOCH_3$), 49.67 (C_{12}), 41.18 (C_8); CIMS, 391 (M + 1).

The cis nitro ester **10** (2.0 g, 5 mmol) was refluxed with aqueous NaOH (2.5%, 60 mL) until a homogeneous solution was obtained (4 h). The usual workup and recrystallization (1×, CH_3OH) gave the analytical sample of **16** (1.72 g, 90%): mp 160–161 °C; ^{1}H

NMR (CD_3COOD, 300 MHz) δ 8.20–8.10 (m, 2 H, Ar-XX'), 7.50–7.35 (m, 2 H, Ar-AA'), 3.63–3.52 (m, 1 H, C*H*NH), 2.36 (t, 2 H, CH_2COOH); CIMS, 377 (M + 1). Anal. ($C_{21}H_{32}N_2O_4$) C, H, N.

***dl*-16-(4'-Nitrophenyl)-17,18,19,20-tetranor-13-azaprostanoic Acid (17).** Further elution of the column used to isolate the cis nitro ester 10 gave a mixture of cis and trans isomers (10 and 11, 1 g, 5%) and then pure trans nitro ester 11 (7.5 g, 38%): TLC R_f 0.52 (Et_2O–CH_3OH–NH_4OH, 60:40:0.07); ^1H NMR ($CDCl_3$, 300 MHz) δ 8.20–8.13 (m, 2 H, Ar-XX'), 7.40–7.30 (m, 2 H, Ar-AA'), 3.66 (s, 3 H, $COOCH_3$), 2.86–2.70 (m, 1 H, C*H*NH), 2.33 (t, 2 H, CH_2COOCH_3); ^{13}C NMR ($CDCl_3$, 360 MHz) δ 174.07 (C=O), 65.53 ($COOCH_3$), 51.34 (C_{12}), 46.38 (C_8); CIMS 391 (M + 1).

The trans ester 11 (2.0 g, 5 mmol) was hydrolyzed and brought to its isoelectric point as previously described to give the free acid 17, which was recrystallized (2×) from CH_3OH (1.79 g, 93%): mp 163–163.5 °C; ^1H NMR (CD_3COOD, 300 MHz) δ 8.20–8.10 (m, 2 H, Ar-XX'), 7.48–7.38 (m, 2 H, Ar-AA'), 3.29–3.19 (m, 1 H, C*H*NH), 2.36 (t, 2 H, CH_2COOH); CIMS, 377 (M + 1). Anal. ($C_{21}H_{32}N_2O_4$) C, H, N.

***dl*-12-*epi*-16-(4'-Aminophenyl)-17,18,19,20-tetranor-13-azaprostanoic Acid (22).** The cis nitro ester 10 (5 g, 12 mmol) in CH_3OH (200 mL) was hydrogenated in a Parr apparatus over Pd/C (10%, 200 mg, 35 psi) overnight at room temperature. Filtration (Celite) and removal of the solvent in vacuo gave crude amino ester 19 as a colored oil. The residue was purified by medium-pressure chromatography (200 g, Et_2O–hexane, 80:20) to give the amino ester 19 as a colorless oil (4.2 g, 97%): TLC R_f 0.52 (Et_2O–CH_3OH–NH_4OH, 60:40:0.07); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.22–7.12 (m, 2 H, Ar-XX'), 7.0–6.9 (m, 2 H, Ar-AA'), 3.66 (s, 3 H, $COOCH_3$), 3.00–2.90 (m, 1 H, C*H*NH), 2.30 (t, 2 H, CH_2COOCH_3); CIMS 361 (M + 1).

The cis amino ester 19 (1.0 g, 2.7 mmol) was hydrolyzed and brought to its isoelectric point as previously described to give the amino acid 22 as a white crystalline solid (0.82 g, 86%, mp 179–181 °C). Two recrystallizations from CH_3OH gave the analytically pure sample: mp 180–181 °C; ^1H NMR (CD_3OD, 300 MHz) δ 7.01–6.92 (m, 2 H, Ar-XX'), 6.73–6.64 (m, 2 H, Ar-AA'), 3.47–3.35 (m, 1 H, C*H*NH), 2.15 (t, 2 H, CH_2COOH); CIMS, 347 (M + 1). Anal. ($C_{21}H_{34}N_2O_2$) C, H, N.

***dl*-16-(4'-Aminophenyl)-17,18,19,20-tetranor-13-azaprostanoic Acid (23).** The trans amino ester 20 was prepared from trans nitro ester 11 (5.5 g, 13 mmol) by the same procedure as described for its cis counterpart 22. The product was purified by medium-pressure chromatography (Et_2O–hexane, 80:20) to give 20 (4.8 g, 96%) as a colorless oil: TLC R_f 0.33 (Et_2O–CH_3OH–NH_4OH, 60:40:0.07); ^1H NMR ($CDCl_3$, 300 MHz) δ 6.99–6.92 (m, 2 H, Ar-XX'), 6.65–6.58 (m, 2 H, Ar-AA'), 3.66 (s, 3 H, $COOCH_3$), 2.70–2.50 (m, 1 H, C*H*NH overlapping CH_2Ar and $NHCH_2$), 2.30 (t, 2 H, CH_2COOCH_3); ^{13}C NMR ($CDCl_3$, 360 MHz) δ 174.12 (C=O), 65.41 ($COOCH_3$), 51.35 (C_{12}), 46.04 (C_8); CIMS, 361 (M + 1).

The trans ester 20 (5 g, 13 mmol) was hydrolyzed and brought to its isoelectric point in the usual manner to give the crude amino acid 23 (4.2 g). Two crystallizations from CH_3OH gave 23 as the analytical sample (3.84 g, 80%): mp 187–187.5 °C; ^1H NMR (CD_3COOD, 300 MHz) δ 7.24–7.16 (m, 2 H, Ar-XX'), 7.01–6.93 (m, 2 H, Ar-AA'), 3.26–3.16 (m, 1 H, C*H*NH), 2.36 (t, 2 H, CH_2COOH); CIMS, 347 (M + 1). Anal. ($C_{21}H_{34}N_2O_2$) C, H, N.

***dl*-12-*epi*-16-(4'-Azidophenyl)-17,18,19,20-tetranor-13-azaprostanoic Acid (25).** To a solution of cis amino ester 19 (600 mg, 1 mmol), dissolved in a mixture of acetic acid (100 mL) and water (400 mL), was added dropwise a solution of sodium nitrite (900 mg, 1.3 mmol) in water (10 mL). The reaction mixture was stirred for 30 min at room temperature and then treated with a solution of sodium azide (900 mg, 1.38 mmol) in water (10 mL) and allowed to stir for an additional 30 min. The light yellow solution was neutralized with NH_4OH and extracted with CH_2Cl_2 (3 × 50 mL). The organic layer was washed with H_2O (2 × 15 mL) and dried ($MgSO_4$). The solvents were removed in vacuo, and the residue (600 mg) was chromatographed (80 g of silica gel, Et_2O) to give the chromatographically homogeneous (TLC) ester of the desired azide 25 (520 mg, 82%): TLC R_f 0.29 (Et_2O); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.22–7.12 (m, 2 H, Ar-XX'), 7.0–6.9 (m, 2 H, Ar-AA'), 3.66 (s, 3 H, $COOCH_3$), 3.0–2.9 (m, 1 H, C*H*NH), 2.30 (t, 2 H, CH_2COOCH_3); CIMS, 387 (M + 1).

The methyl ester of 25 (500 mg, 0.9 mmol) was hydrolyzed as previously described to give the corresponding crude cis azide acid 25 (400 mg). The analytical sample was obtained after two recrystallizations from CH_3OH (395 mg, 82%): mp 154–155 °C dec; IR (Nujol) 2112 cm^{-1} (N_3); ^1H NMR (CD_3COOD, 300 MHz) δ 7.27–7.17 (m, 2 H, Ar-XX'), 7.03–6.93 (m, 2 H, Ar-AA'), 3.63–3.50 (m, 1 H, C*H*NH), 2.36 (t, 2 H, CH_2COOCH_3); CIMS, 373 (M + 1). Anal. ($C_{21}H_{32}N_4O_2$) C, H, N.

***dl*-16-(4'-Azidophenyl)-17,18,19,20-tetranor-13-azaprostanoic Acid (26).** The methyl ester of the trans derivative 26 (600 mg, 1.66 mmol) was prepared by the same procedure as for the ester of the cis azide 25. Chromatography (80 g, Et_2O) gave chromatographically homogeneous methyl ester of 26 (540 mg, 84%): TLC R_f 0.21 (Et_2O); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.22–7.12 (m, 2 H, Ar-XX'), 7.00–6.90 (m, 2 H, Ar-AA'), 3.65 (s, 3 H, $COOCH_3$), 2.8–2.5 (m, 1 H, C*H*NH overlapping CH_2-Ar and $NHCH_2$), 2.30 (t, 2 H, CH_2COOCH_3); CIMS, 387 (M + 1).

The methyl ester of 26 (540 mg, 1.39 mmol) was hydrolyzed as previously described to give the corresponding crude cis azide acid 26 (450 mg). The analytical sample was prepared by recrystallization (2×) from CH_3OH (437 mg, 84%): mp 121–122 °C dec; IR (Nujol) 2110 cm^{-1} (N_3); ^1H NMR (CD_3COOD, 300 MHz) δ 7.42–7.33 (m, 2 H, Ar-BB'), 7.33–7.24 (m, 2 H, Ar-AA'), 3.40–3.17 (m, 1 H, C*H*NH), 2.37 (t, 2 H, CH_2COOH); CIMS, 373 (M + 1). Anal. ($C_{21}H_{32}N_4O_2$) C, H, N.

***dl*-16-(4'-Azido-3-iodophenyl)-17,18,19,20-tetranor-13-azaprostanoic Acid (28).** To a solution of trans amino acid 23 (40 mg, 0.115 mmol) in CH_3OH (10 mL) was added aqueous sodium acetate buffer (0.174 M, 100 mL), followed by sodium iodide (50 mg, 0.333 mmol) in the same buffer (1.5 mL) and Chloramine-T (85 mg, 0.373 mmol) in the same buffer (1.5 mL). An orange solid appeared immediately. The reaction mixture was quenched after 10 min by the addition of sodium metabisulfite (100 mg, 0.526 mmol) in the same buffer (2.5 mL) and then allowed to stir an additional 5 min. The solid was filtered, washed with water, and dried (vacuum pump). The iodinated amine 27 (27 mg, 50%) was isolated by preparative TLC ($CHCl_3$–CH_3OH, 40:15): mp 110–111 °C; TLC ($CHCl_3$–CH_3OH, 40:15) R_f 0.37; HPLC t_R = 27.82 min; ^1H NMR (CD_3OD, 300 MHz) δ 7.49 (d, 1 H, $H_{6'}$, J = 2.1 Hz), 7.01 (dd, 1 H, $H_{2'}$, J_{meta} = 2.1 Hz and J_{ortho} = 8.4 Hz), 6.77 (d, 1 H, $H_{3'}$, J_{ortho} = 8.4 Hz), 3.10–3.22 (m, 1 H, C*H*NH), 2.18 (t, 2 H, CH_2COOH); CIMS 473 (M + 1); EIMS calcd for M^+ of $C_{21}H_{33}N_2O_2I$/found = 472.1576/472.1518. Analysis showed 2.3% residue which we presume is due to inorganics extracted (CH_3OH) from the TLC plate. This material was used in the next step without further purification.

The iodo amine 27 (3 mg, 6.3 μmol) was dissolved in acetic acid (3 M, 10 mL) and treated at room temperature with sodium nitrite (3 mg, 43 μmol in 0.5 mL of H_2O). After 15 min, sodium azide (3 mg 46 μmol in 0.5 mL, of H_2O) was added and the reaction mixture stirred for an additional 15 min, before removal of the solvent with a vacuum pump. The iodinated azide 28 (3 mg, 97%) was isolated by preparative TLC ($CHCl_3$–CH_3OH, 40:15): mp 109–110 °C dec; IR (Nujol) 2112 cm^{-1} (N_3); TLC R_f 0.41 ($CHCl_3$–CH_3OH, 40:15); HPLC t_R 29.41 min; ^1H NMR δ (CD_3OD, 300 MHz) 7.73 (d, 1 H, $H_{2'}$, J_{meta} = 2.4 Hz), 7.34 (dd, 1 H, $H_{6'}$, J_{meta} = 2.4 Hz, J_{ortho} = 7.2 Hz), 7.20 (d, 1 H, $H_{5'}$, J_{ortho} = 4.2 Hz), 3.08–3.20 (m, 1 H, C*H*NH), 2.16 (t, 2 H, CH_2COOH); CIMS 499 (M + 1); EIMS 498 (M^+), 470 ($M^+ - N_2$). Anal. ($C_{21}H_{31}IN_4O_2$) C, H, N.

***dl*-16-(4'-Azido-3'-[^{125}I]iodophenyl)-17,18,19,20-tetranor-13-azaprostanoic Acid (29).** To a small glass vial fitted with a septum (all additions were made by injection through the septum) was added trans amino acid 23 (0.3 μg, 0.867 nmol) in CH_3OH (1.5 μL) and then sodium acetate buffer (all reference to buffer in this experimental refers to 1.43 g of sodium acetate in 100 mL of distilled water, pH 7.9). After cooling (ice bath), 0.4 mCi of $Na^{125}I$ was added, followed by NaI (1.5 μg, 0.01 μmol) in buffer (1.2 μL) and Chloramine-T (*N*-chloro-*p*-toluenesulfonamide, sodium salt, 2.52 μg, 8.9 nmol) in buffer (1.2 μL). The reaction mixture was allowed to stand for 5 min before addition of sodium metabisulfite (3 μg, 15.8 nmol) in buffer (1.2 μL). This mixture was allowed to stand for an additional 5 min and then diluted with CH_3OH (75 μL). The contents of the vial were removed via syringe and applied to approximately half of a

standard TLC plate (20 × 6 cm). An analogous reaction, using ^{127}I, was conducted with all reagents and solvents at 3 times higher concentration. The contents of this reaction were applied to the second half of the same TLC plate. The plate was developed for about 10 cm (CH_3OH–$CHCl_3$, 15:35) and then air-dried in a hood. The TLC plate was cut in half and the ^{127}I side used to mark (visualized under UV light, TLC R_f 0.41, CH_3OH–$CHCl_3$, 15:35) the expected band for the ^{125}I amine on the remaining half. The zone corresponding to the ^{125}I amine, so identified, was scrapped into a 10-mL vial containing CH_3OH (5 mL). The mixture was vortexed for 1 min and then centrifuged. The organic layer, containing the labeled amine, was removed with a pipet and retained. This procedure was repeated twice more, the organic layers were combined, and the solvent was removed under a stream of nitrogen. The combined CH_3OH extracts was found to contain 60% of the originally added $Na^{125}I$.

The ^{125}I amine was dissolved in aqueous acetic acid (100 μL, AcOH–H_2O, 2:8, v/v). After cooling (ice bath), $NaNO_2$ (0.3 μg, 4.3 nmol) in water (2 μL) was added and the reaction mixture allowed to stand for 5 min, before the addition of sodium azide (0.3 μg, 4.6 nmol) in water (2 μL). After being allowed to stand an additional 5 min, the entire reaction mixture was applied to a standard TLC plate (20 × 6 cm). The plate was developed for about 10 cm (CH_3OH–$CHCl_3$, 15:35) and then air-dried in a hood. The ^{125}I azide (TLC R_f 0.52, CH_3OH–$CHCl_3$, 15:35) was isolated in the same manner as its precursor amine. A portion of the product so obtained was diluted with 12 μg of the fully characterized unlabeled **28** and subjected to the usual reversed-phase HPLC analysis. Of the applied radioactivity, 87% was recovered in the eluate and 99.6% of the recovered radioactivity resided in the UV-absorbing peak corresponding to the iodo azide **28** (t_R = 29.4 min).

Biology. Inhibition of U46619 Activity. Platelet aggregation was studied by the turbidometric method of Born[42] at 37 °C over a 3-min time course. Citrated human platelet-rich plasma from normal, healthy donors who denied receiving any medication for 10 days was purchased from a commercial blood bank. The plasma was centrifuged at 164*g* to remove any remaining red blood cells and maintained at 25 °C until the experiments were performed. The antagonists were dissolved in 50% ethanol/normal saline and then added as 10-μL aliquots per 1 mL of platelet-rich plasma to give the specified concentration 3 min before being challenged with U46619 (3 μM). Inhibition was measured as a percentage of control bloods that had not received any drug. The ED_{50} values were estimated from three to four concentrations of drug that gave inhibition in the range of 10–90%.

Dark and Light Inhibition Studies of Aromatic Azides. 13-Azaprostanoic acid, or one of the three derivatives listed in Table II, was added to platelet-rich plasma at the minimum concentration necessary to produce 100% inhibition of 3 μM U46619-induced aggregation. The incubates were photolyzed (30 min) with a HBD100W OSRAM mercury light. This extended photolysis time was necessary because of the high UV absorption by the plasma. The platelets were then centrifuged and the platelet pellet resuspended in Tris·HCl buffer (pH 7.4). Platelet aggregation of the resuspended cells was induced by addition of ADP (10 μM) or U46619 (0.5 μM). The results are presented in Table II, where the values were calculated by the following relationship: percent inhibition relative to 13-azaprostanoic acid = [1 – (residual platelet activity with given azide/the residual platelet activity with 13-azaprostanoic acid)] × 100.

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Notes

Synthesis of 5-β-D-Ribofuranosylnicotinamide and Its *N*-Methyl Derivative. The Isosteric and Isoelectronic Analogues of Nicotinamide Nucleoside[1]

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The pyridine *C*-nucleosides 5-β-D-ribofuranosylnicotinamide and its *N*-methylpyridinium derivative (1 and 2), which are isosteric and isoelectronic, respectively, to nicotinamide nucleoside were synthesized. Condensation of 3-bromo-5-lithiopyridine with 2,4:3,5-di-*O*-benzylidene-D-aldehydoribose (**7**) afforded an allo/altro mixture of the corresponding bromopyridine derivatives, which were converted into nicotinamide *C*-nucleoside precursors **10**. Mesylation of the hydroxyl group of **10** followed by acid hydrolysis of the product afforded the anomeric nicotinamide *C*-nucleosides. The β anomer 1 was separated and treated with MeI to give 2.

The pyridine *C*-nucleosides 1 and 2 (Chart I), which are isosteric and isoelectronic, respectively, to nicotinamide nucleoside, may be converted biologically into the corresponding NAD analogues **3** and **4** and exert interesting biological activities. The noncharged NAD isostere **3** may inhibit the NAD-dependent enzyme IMP-dehydrogenase and may induce cytotoxicity by blocking the de novo GMP synthesis. The isoelectronic analogue of NAD (**4**) should be capable of participating in the same enzymic oxidoreduction process(es) as NAD. Both **3** and **4** certainly cannot serve as ADP-ribose donors and may inhibit ADP-ribosylation, which is important in protein synthesis regulation[2-4] and in the DNA repair process.[5-8] Actually, the

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