Interaction with Hydrogen Donors. The lifetime of $Np\dot{C}H_2$* was determined in two excellent hydrogen donors, tri-*n*-butylstannane and 1,4-cyclohexadiene. The lifetime in 1,4-cyclohexadiene was ~30 ns which is only slightly shorter than that measured in other solvents and corresponds to a rate constant of ca. 5×10^5 M^{-1} s^{-1}, though this value is subject to rather large errors due to a small difference between lifetimes (see Table I). In tri-*n*-butylstannane the lifetime was ~14 ns, which gives a rate constant of ca. 1×10^7 M^{-1} s^{-1}.
for the excited radical. Furthermore, the insensitivity of its lifetime to solvent and temperature and its rapid reaction with oxygen (with ground-state recovery) indicate that the intermolecular reactivity of Np$\dot{C}$H* parallels that observed for excited diphenylmethyl radicals. It would appear that $Np\dot{C}H_2$* has predominantly excited state properties, rather than enhanced free radical properties. Obviously, more studies are required to establish whether this is a general characteristic of excited free radicals.

It is interesting to note the similarity between the spectra of the excited 1-naphthylmethyl radical and triplet naphthalenes. This may be a further reflection of the excited-state-like properties of excited radicals.

Conclusion

1-Naphthylmethyl radicals generated from various sources show a sharp absorption maximum at 365 nm. The radicals were produced by direct photolysis of 1-(chloro- or bromomethyl)-naphthalene at 308 nm and were then excited with the pulses from a nitrogen laser (337.1 nm) leading to the expected fluorescence at 590 nm. The absorption spectrum of $Np\dot{C}H_2$* shows λ_{max} ~430 nm; its lifetime is ~35 ns in several hydrocarbon, alcohol, or aromatic solvents but much shorter in carbon tetrachloride, where its decay involves Cl-atom abstraction.

Reaction of Np$\dot{C}$H* with certain amines and with methyl viologen indicates enhanced electron donor/acceptor properties

Acknowledgment. Thanks are due to Professor M. A. Fox and Dr. D. Meisel for preprints of unpublished materials and to Mr. S. E. Sugamori for his technical assistance.

Registry No. 1, 17288-12-9; chlorine, 7782-50-5; hydrogen, 1333-74-0; 1-naphthylmethyl radical, 7419-60-5; oxygen, 7782-44-7; methyl viologen, 1910-42-5; carbon tetrachloride, 56-23-5; 1-(chloromethyl)-naphthalene, 86-52-2; 1-(bromomethyl)naphthalene, 3163-27-7; 1,3-di-(1-naphthyl)-2-propanone, 51042-38-7; ethyl 1-naphthylacetate, 2122-70-5; 1-naphthylacetonitrile, 132-75-2; 2,3-di(1-naphthyl)-3-oxo-1-propanoic acid, 98577-45-8; 1-naphthyldiazomethane, 10378-55-9; di-*tert*-butyl peroxide, 110-05-4; 1-methylnaphthalene, 90-12-0.

Synthesis of Thromboxane A_2

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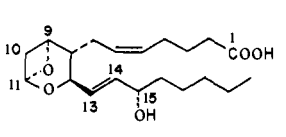
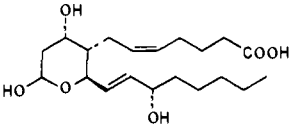
Contribution from the Department of Chemistry, Columbia University, New York, New York 10027. *Received February 19, 1985. Revised Manuscript Received April 18, 1985*

Abstract: Thromboxane B_2 (TXB_2) was converted by (1) 1,15-macrolactonization, (2) C11 dehydration, and (3) C10,C11 bromohydrin formation to 10-bromo-1,15-anhydrothromboxane B_2. On treatment with $EtO_2CN{=}NCO_2Et/(MeO)_3P$ in methylene chloride, the bicyclic oxetane formed and was debrominated with tin hydride. The product, 1,15-anhydrothromboxane A_2, was characterized by high field NMR spectroscopy and single-crystal X-ray crystallography. Saponification of that material gave the proposed Samuelsson bicyclic oxetane structure of thromboxane A_2 (TXA_2) which was shown by a variety of biological assays to be indistinguishable from natural platelet-derived TXA_2.

We recently described a method for the preparation of the putative thromboxane A_2 (TXA_2) nucleus, an acid-labile 2,6-dioxabicyclo[3.1.1]heptane,[1] and now report application of the method to the synthesis of TXA_2 itself. TXA_2 is an unstable substance ($T_{1/2}$(37 °C) = 32 s in aqueous Krebs medium at pH 7.4) which is derived from the prostaglandin endoperoxide PGH_2 and which is an important blood platelet aggregation factor.[2] Although TXA_2 has not been previously isolated and characterized, its structure was proposed as **1** on the basis of its lability in neutral aqueous media, isotope incorporation experiments, and the isolation of various TXB_2-like addition products. In this paper, we describe



1, TXA_2



TXB_2

the preparation of structure **1** and comparisons of its biological properties with those of natural, platelet-derived TXA_2.

Our synthesis began with commercially available TXB_2 which was esterified (CH_2N_2 and Et_2O), peracetylated (Ac_2O and pyridine), and selectively deacetylated (catalytic KOMe and MeOH, −15 °C) at the more reactive C11 anomeric center to **3** in 78% overall yield. Dehydration to the unstable enol ether (MsCl, Et_3N, and CH_2Cl_2), deacetylation (K_2CO_3 and MeOH, 25 °C), and immediate bromohydrin formation (NBS, H_2O, and THF) led to 10-bromo-TXB_2 methyl ester **4** in 51% yield. Cyclization to the oxetane proceeded as in the model study leading to **2**[1] by using a modified Mitsunobu reaction[3] ($(MeO)_3P$, $EtO_2CN{=}NCO_2Et$ (DEAD), and CH_2Cl_2, 25 °C, 30 min) to

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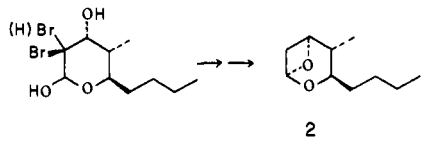

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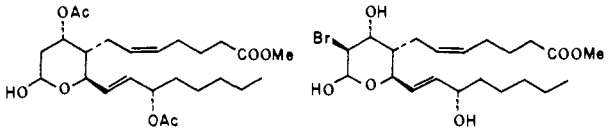

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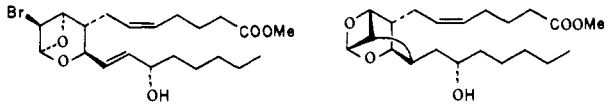


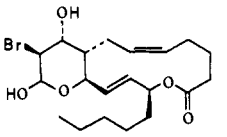
5 6

provide **5** in 20% isolated yield. Although the yield was not high, **5** was well separated from the byproducts by TLC and was easily isolated in high purity by silica gel chromatography. In addition to approximately 25% starting material, other byproducts of the cyclization included small amounts of enol ether products of simple and reductive elimination and a number of uncharacterized adducts with DEAD. Attempts to hydrolyze the DEAD adducts back to **4** were unsuccessful. The product **5** displayed ^1H and ^{13}C NMR ring system resonances which closely matched those of the model bicyclic bromooxetane system described previously[1] including the characteristic long-range C9–C11 proton–proton coupling having J = 3.9 Hz (see Experimental Section).

In contrast to the straightforward reductive behavior of the model which led from bromooxetane to **2**, however, free-radical debromination (neat Bu_3SnH, Ph_3SnH, or Bu_2SnH_2, sunlamp irradiation) of **5** did not lead to substantial quantities of the simple reduction product but instead to a material in which the C13,C14 olefin had been lost. We have not fully characterized this undesired reduction product, but the ^1H NMR and MS are compatible with the radical cyclization product **6**.

To prevent interaction of the intermediate C_{10} free radical with the C13,C14 olefinic linkage, the olefin in principle could be protected, shifted to C14,C15, or somehow oriented away from C10. This last strategy is particularly appealing since it would require the least number of chemical transformations on the C12 sidechain and since any such chemistry would have to be compatible with the sensitive bicyclic oxetane nucleus after debromination. To conformationally restrict the approach of the intermediate C10 radical to the C13,C14 olefin, we decided to anchor the C12 allylic alcohol side chain to the C1 carboxyl by formation of the 1,15-macrolactone.[4] Thus, TXB_2 was converted to a 3.6:1 mixture of 1,15- and 1,9-macrolides via the thiopyridyl ester[5] (**1**, PyrSCOCl, Et_3N, and Et_2O/CH_2Cl_2, 0 °C; 2.5 mM in $PhCH_3$, 110 °C, 13 h), and the major 1,15-macrolide was isolated by silica gel chromatography in 65% yield. The minor 1,9-macrolide (18% yield) could be recycled with partial decomposition by saponification with $LiOH/THF/H_2O$ back to TXB_2.

Dehydration at C11 was smoothly effected with the Mukaiyama reagent 2-chloro-1-methylpyridinium iodide[6] (Et_3N and CH_3CN, reflux), and immediate bromohydrin formation (NBS, Et_2O, H_2O) gave 10-bromo-1,15-anhydro-TXB_2 (**7**) in 65% yield. A simple but less attractive alternative to the sequential macrolactonization and dehydration was direct treatment of TXB_2 with the Mukaiyama reagent to yield the same 10,11-enol ether 1,15-macrolide intermediate prepared above but in lesser purity and in ≤30% yield. We should mention that dehydration of unprotected 2-deoxy-



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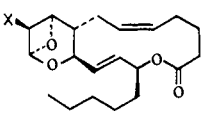

8a X = Br
b X = H

pyranoses with the Mukaiyama reagent to unprotected glycals seems to be a unique transformation of some generality which could be of value in other synthetic endeavors.

Modified Mitsunobu cyclization as in the methyl ester series provided the 10-bromo-TXA_2 derivative **8a** in 21% isolated yield at 81% conversion. A substantial effort was made to improve the yield of this key cyclization, and variations in the solvent, the electrophilic component, and the phosphorus component were investigated. Among solvents yields were best in CH_2Cl_2, but Et_2O and $CHCl_3$ were almost as effective. The usual $EtO_2CN{=}NCO_2Et$ (DEAD) electrophile was more effective than the corresponding diisopropyl or di-*tert*-butyl esters (10–15% yields), and the related bis(*N*,*N*-dimethylazocarboxamide) and *N*-phenyltriazolinedione afforded only 5–10% of the desired bromooxetane. The cyclization reaction was quite sensitive to the structure of the phosphorus component, and more than 20 phosphines, phosphites, phosphonites, and phosphinites were evaluated. Relatively nucleophilic phosphines such as Bu_3P and Ph_3P gave substantial reductive elimination to the debrominated enol ether while more highly substituted phosphites (e.g., $(i\text{-}PrO)_3P$ and $(PhO)_3P$) reacted only sluggishly. Triethyl phosphite and $(MeO)_2PPh$ proved comparable to trimethyl phosphite in the cyclization but showed no clear advantages. More classical base-promoted 1,3-eliminations of intermediates having a leaving group (e.g., trifluoroacetate, mesylate, and triflate) at the anomeric C11 center provided only traces of the desired oxetane. With the optimal reagents, DEAD and $(MeO)_3P$ in CH_2Cl_2, yields turned out to be the best when large excesses of reagents were avoided and when the azo compound and the phosphite were premixed before addition to the cyclization precursor. Although a more effective cyclization would be quite valuable, the phosphite Mitsunobu cyclization is both simple and reproducible and the oxetane product **8a** is well-resolved from the byproducts and is thus easily isolated by silica gel chromatography.

With the C12 side chain conformationally anchored by the 1,15-macrolactonic linkage, bromooxetane **8a** cleanly yielded the desired 1,15-anhydro-TXA_2 (**8b**) on reduction with tributyltin hydride. No byproducts in which the C13,C14 olefin had been lost could be found. Since **8b** was unstable to chromatography and thus could not be entirely freed of Bu_3SnBr and excess Bu_3SnH, it was most readily prepared in high purity when using a polymer-bound tin hydride[7] suspended in pentane (catalytic AIBN, 1–2 h, 15 °C, sunlamp irradiation) and could be isolated as a crystalline solid by filtration of the reagent and solvent evaporation (85% yield; mp(pentane) 81–82 °C). The ^1H and the ^{13}C NMR resonances of the bicyclic oxetane nucleus corresponded to those of the previously prepared model system **2**. The X-ray crystal structure[8] (below) of **8b** confirms both the structural assignment as the novel bicyclic oxetane structure of 1,15-anhydro-TXA_2 and, at least in the crystal, the anticipated large spatial separation of C10 and C13.

A low resolution conformational analysis of compound **8b** (C16–C20 replaced by a methyl) was conducted with molecular mechanics by using the MM2 force field and starting from 104 macrocycle geometries generated at 60° dihedral angle resolution. This analysis showed the 13-membered macrolide to be quite flexible with more than a dozen different minimum energy conformations being found within 3 kcal/mol of the ground state. The majority of the conformers had C10 oriented close to the nodal plane of the C13,C14 olefin and thus could not easily lead to the

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(5) Corey, E. J.; Nicolaou, K. C. *J. Am. Chem. Soc.* **1974**, *96*, 5614–5616. Corey, E. J.; Clark, D. A. *Tetrahedron Lett.* **1979**, 2875–2878.

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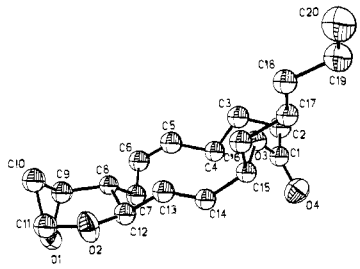
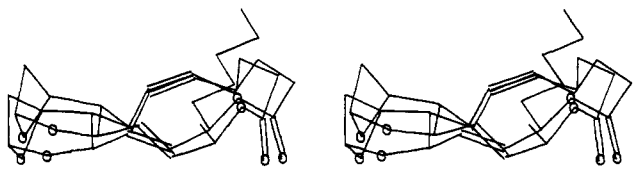
(8) Chiang, M., to be reported in detail elsewhere.



cyclization product observed in the methyl ester series described above. Two conformers (ca. 1.0 and 2.5 kcal/mol above the ground state) did have C10 and the C13,C14 olefin approximately aligned for cyclization but with C10 and C13 rather widely separated (4.0 and 4.1 Å). The lowest energy conformation found was qualitatively the same as that found in the crystal. Least-squares superimposition of the macrocycle atoms for the X-ray structure and the lowest energy molecular mechanics conformer gave an RMS deviation of 0.40 Å, and a stereoscopic superimposition of the two structures is shown below.



When **8b** is dissolved in 1:1 CD_3OD/D_2O containing 10 equiv of NaOD (30 min, 22 °C), the macrolactone is saponified without loss of the bicyclic oxetane ^1H NMR resonances to yield the sodium salt of **1**. We estimate the purity of the salt thus prepared to be approximately 80%. **8b** may also be opened in THF or Et_2O with Me_3SiOK[9] to yield the potassium salt of **1** (^1H NMR (THF-d_8) δ 5.87 (2 H, br t, $J \sim 9$ Hz, H13 and H14), 5.73 (1 H, t, $J \sim 4$ Hz, H11), 5.45–5.70 (2 H, m, H5 and H6), 4.72 (1 H, dd, $J \sim 4$, 6 Hz, H9), 4.53 (1 H, br t, $J \sim 6$ Hz, H12), 4.18 (1 H, br q, $J \sim 6$ Hz, H15), 3.22 (1 H, m, H10a)) which may be isolated with partial decomposition as an amorphous solid after solvent removal. Whereas **8b** is biologically inactive, the methanol/water saponification solution shows marked activity which peaks 30 min after mixing and which reproduces the activities of natural platelet-derived TXA_2 in a variety of assays. Thus, in experiments conducted by Dr. Frank Fitzpatrick and Dr. Stuart Bunting of the Upjohn Company, the sodium and potassium salts of synthetic **1** were found to be indistinguishable from natural TXA_2 by their comparative potency in aggregating human platelets but not neutrophils, their constriction of vascular tissue including rabbit aorta, pulmonary, mesenteric and celiac arteries, their stability in plasma at 37 °C (half-life in Krebs medium at 37 °C = 30 s leading to TXB_2), and their resistance to inhibition by selective thromboxane synthetase or cyclooxygenase inhibitors.[10]

While TXA_2 is rapidly hydrolyzed to TXB_2 in aqueous high salt, neutral pH buffers, its stability is appreciably enhanced at high pH. We were able to store the salts of TXA_2 for more than 1 week at −20 °C either as isolated solids or in basic methanol or tetrahydrofuran solution. In aprotic solvents such as deuteriochloroform, the free acid could be generated by washing the sodium or potassium salt of **1** with aqueous sodium dihydrogen phosphate but it underwent elimination to the corresponding enol ether over the course of several hours.

These results give very strong support to the Samuelsson oxetane structure for TXA_2 as well as providing the first source of isolated TXA_2 for biological studies. The synthesis should also lend itself to the preparation of radiolabeled TXA_2 by substitution of tin tritiide for tin hydride in the final reduction step. In closing, we should note that while this synthesis yields the previously proposed TXA_2 structure which is shown to be experimentally indistinguishable from natural TXA_2, it does not conclusively prove the Samuelsson structure to be TXA_2. Although the biological profile and stability of **1** make the Samuelsson proposition the most likely, our results would also be compatible with in vivo conversion of **1**, perhaps by addition of a biological nucleophile at C11, to a derivative which is the endogenous TXA_2 and which is biologically synthesized from PGH_2 without the intervention of **1**.

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(10) Bhagwat, S. S.; Hamann, P. R.; Still, W. C.; Bunting, S.; Fitzpatrick, F. A. *Nature (London)* **1985**, *315*, 511–513.

Experimental Section

Thromboxane B_2 Methyl Ester 9,15-Diacetate (3). To 190 mg (0.49 mmol) of thromboxane B_2 methyl ester (Upjohn Co.) in 1.4 mL of pyridine at 0 °C was added 0.7 mL (7.5 mmol, 15 equiv) of acetic anhydride. The ice bath was allowed to melt, and the reaction mixture was stirred at room temperature for 18 h. The reaction mixture was diluted with 1:1 ether/petroleum ether and washed twice with 5% aqueous HCl, once with water, and once with saturated aqueous $NaHCO_3$. After drying over $MgSO_4$, the product was isolated by solvent evaporation and flash chromatography (silica gel, step gradient from 20% to 50% ethyl acetate in petroleum ether) to yield 225 mg (90%) of the desired triacetate and 12 mg (5%) of the 11,15-diacetate: ^1H NMR ($CDCl_3$) δ 5.93 (1 H, dd, J = 3, 10 Hz), 5.73 (1 H, dd, J = 6, 16 Hz), 5.60 (1 H, dd, J = 7, 16 Hz), 5.40 (1 H, m), 5.3–5.15 (3 H, m), 4.16 (1 H, dd, J = 8, 11 Hz), 3.65 (3 H, s), 2.26 (2 H, t, J = 7 Hz), 2.10 (3 H, s), 2.09 (3 H, s) 2.02 (3 H, s), 2.1–1.2 (17 H, m), 0.86 (3 H, br t, J = 7 Hz); ^{13}C NMR ($CDCl_3$) δ 173.58, 169.99, 169.74, 168.99, 133.10, 130.91, 129.18, 126.37, 90.46, 76.59, 73.22, 69.30, 51.25, 42.87, 34.55, 34.05, 33.14, 31.28, 26.31, 24.60, 24.43, 24.01, 22.25, 20.96, 20.95, 20.94, 13.90; IR ($CDCl_3$) cm^{-1} 1733, 1436, 1245, 1050; MS (NH_3-CI), m/e 528 (m + NH_4), 468 (M − CH_2CO), 451 (M − CH_3CO_2); TLC (silica gel, 25% ethyl acetate/pentane) R_f = 0.16–0.21.

To 225 mg (0.44 mmol) of the above triacetate in 5 mL of anhydrous MeOH at −15 °C was added 0.05 mL (0.08 mmol) of a 1.65 M (saturated) solution of potassium *tert*-butoxide in tetrahydrofuran. The reaction mixture was allowed to warm to 0 °C over 30 min, diluted with 1:1 ether/petroleum ether, and washed 3 times with water. After drying over $MgSO_4$, filtering, and concentrating, the crude product was chromatographed on silica gel (step gradient from 25% ethyl acetate in petroleum ether to neat ethyl acetate) to yield 180 mg (87%) of the 9,15-diacetate **3** as a mixture of anomers and 23 mg (ca. 10%) of a mixture of other acetates: ^1H NMR ($CDCl_3$) (major anomer) δ 5.78 (1 H, dd, J = 6.5, 15.5 Hz), 5.63 (1 H, dd, J = 6.5, 15.5 Hz), 5.40, 5.25 (2 H, m), 5.25 (1 H, m), 5.17 (1 H, m), 5.02 (1 H, m), 4.08 (1 H, dd, J = 8, 11 Hz), 3.64 (3 H, s), 2.90 (1 H, d, J = 7 Hz), 2.30 (2 H, br t, J = 7 Hz), 2.08 (3 H, s), 2.04 (3 H, s), 2.2–1.2 (17 H), 0.86 (3 H, br t, J = 7 Hz); ^{13}C NMR ($CDCl_3$) (major anomer) δ 173.80, 170.17, 169.88, 132.78, 130.78, 130.07, 126.64, 92.21, 75.92, 73.46, 69.01, 51.38, 43.14, 37.21, 34.12, 33.25, 31.39, 26.39, 24.71, 24.51, 24.17, 22.36, 21.09, 20.99, 13.85; IR (neat) cm^{-1} 3420 (br), 1735, 1435, 1242, 1042, 1020, 920; MS (CI, NH_3), m/e 486 (M + NH_4), 469 (M + 1), 451 (M − OH); HRMS calcd for $C_{25}H_{39}O_7$ (M − OH) 451.2696, found 451.2650; TLC (silica gel, 50% ethyl acetate/pentane) R_f = 0.31–0.40.

10-Bromothromboxane B_2 Methyl Ester (4). To 44 mg (0.094 mmol) of 9,15 diacetate **3** in 1 mL of methylene chloride at 0 °C was added 0.13 mL (0.94 mmol, 10 equiv) of triethylamine and 0.008 mL (0.1 mmol, 1.05 equiv) methane sulfonyl chloride. The reaction was allowed to warm to room temperature for one hour before concentrating to about 0.1 mL. The concentrate was then transferred to the top of a chromatography column containing silica gel slurry-packed with solvent containing 1% triethylamine and eluted with 20% ethyl acetate in petroleum ether. After removing the solvent and pumping to dryness, the chromatographed product was taken up in 2 mL dry MeOH, cooled to 0 °C and treated with excess solid K_2CO_3. The reaction mixture was warmed to room temperature and stirred for one hour, diluted with 1:1 ether/petroleum ether and washed three times with water. After drying over $MgSO_4$, filtering and evaporating, the crude acid-sensitive enol ether was obtained as a thick oil (20 mg, 58%). Pure material could be obtained by flash chromatography with 25% ethyl acetate in petroleum ether but the lability of the product made direct conversion to the bromohydrin below preferable: ^1H NMR ($CDCl_3$) δ 6.47 (1 H, d, J = 6 Hz), 5.88 (1 H, dd, J = 7, 16 Hz), 5.71 (1 H, dd, J = 8, 16 Hz), 5.46–5.39 (2 H, m), 4.99 (1 H, t, J = 6 Hz), 4.21–4.11 (2 H, m), 4.01 (1 H, m), 3.65 (3 H, s), 2.32 (2 H, t, J = 7 Hz), 2.2–1.2 (17 H, m), 0.86 (3 H, br t, J = 7 Hz); ^{13}C NMR ($CDCl_3$) δ 174.26, 146.04, 138.37, 130.59, 127.91, 127.78, 102.86, 75.59, 72.05, 60.19, 51.60, 43.68, 37.01, 33.01, 31.71, 26.58, 25.12, 27.21, 24.25, 22.58, 14.01; IR (neat) cm^{-1} 3447 (v br), 1740, 1643, 1232, 1021; TLC (silica gel, 25% ethyl acetate/pentane) R_f = 0.13.

To 3.4 mg (0.0093 mmol) of crude enol ether in 0.5 mL of tetrahydrofuran and 0.1 mL of water at 0 °C was added 1.7 mg (0.01 mmol) of powdered *N*-bromosuccinimide. After 10 min, the reaction was diluted with ether, washed twice with saturated aqueous $NaHCO_3$, dried over

$MgSO_4$, filtered, concentrated, and flash-chromatographed on silica gel (step gradient from 25% to 50% ethyl acetate in petroleum ether) to give 3.8 mg (88%) of an anomeric mixture of bromohydrins **4** as a thick oil: ^{1}H NMR ($CDCl_3$) δ 5.9–5.6 (2 H, m), 5.45–5.25 (3 H, m), 5.11 (0.5 H, d, J = 10 Hz), 5.02 (0.5 H, dd, J = 12, 2 Hz), 4.45–4.05 (5 H), 3.65 (3 H, s), 3.55 (0.5 H, d, J = 6 Hz), 3.40 (0.5 H, d, J = 12 Hz), 2.4–1.2 (17 H), 0.68 (3 H, br t, J = 7 Hz); ^{13}C NMR ($CDCl_3$) δ 175.16, 138.84, 138.74, 131.22, 130.99, 128.82, 128.43, 127.10, 127.39, 89.52, 94.80, 72.36, 76.64, 69.87, 72.27, 69.61, 69.22, 57.57, 47.75, 51.87, 38.25, 36.76, 33.22, 31.64, 26.48, 25.12, 24.70, 24.53, 23.98, 22.55, 13.96; IR ($CDCl_3$) cm^{-1} 3600, 3465 (br), 1720, 1438, 1229, 1155, 1097, 1046, 975; MS (CI–NH_3), m/e 480, 482 (M + NH_4), 462, 464 (M + 1), 445, 447 (M + 1 – OH); TLC (silica gel, 50% ethyl acetate/pentane) R_f = 0.31–0.36.

10-Bromothromboxane A_2 Methyl Ester (5). To a solution of 0.049 mL (0.41 mmol) of distilled trimethyl phosphite in 1 mL of dry methylene chloride (0 °C, nitrogen) was added 0.049 mL (0.31 mmol) of distilled diethyl azodicarboxylate (DEAD). The solution was stirred for 5 min at 25 °C and then a 0.10-mL aliquot (1.5 equiv of $(MeO)_3P$, 1.1 equiv of DEAD) was added to a well-stirred 0 °C solution of 12 mg (0.026 mmol) of 10-bromothromboxane B_2 methyl ester (**4**) in 0.5 mL of dry methylene chloride under nitrogen. The reaction mixture was stirred for 30 min at 25 °C, and then most of the volatile material was removed under vacuum. The crude product was immediately flash-chromatographed on slurry-packed silica gel with 20% tetrahydrofuran-/2% triethylamine in petroleum ether. The bromooxetane product **5** was isolated as a colorless oil (2.5 mg, 20%): ^{1}H NMR ($CDCl_3$) δ 5.95 (1 H, dd, J = 15.7, 7.7 Hz), 5.83 (1 H, dd, J = 15.7, 5.6 Hz), 5.60 (1 H, dd, J = 3.9, 3.3 Hz), 5.22–5.55 (2 H, m), 4.92 (1 H, dd, J = 5.9, 3.3 Hz), 4.74 (1 H, dd, J = 5.9, 3.9 Hz), 4.21 (1 H, m), 3.67 (3 H, s), 2.31 (2 H, m), 1.2–2.3 (15 H), 0.87 (3 H, t, J = 6.7 Hz); ^{13}C NMR (C_6D_6) δ 173.64, 138.16, 132.35, 128.18, 127.10, 106.13, 85.73, 77.41, 72.27, 51.47, 46.88, 42.17, 37.93, 33.66, 32.50, 27.82, 27.12, 25.88, 25.28, 23.39, 14.93; IR (neat) cm^{-1} 3312 (br), 2933, 2859, 1720, 1378, 1248, 1181, 1096, 848; MS (CI–NH_3), m/e 462, 464 (M + NH_4), 444, 446 (M + NH_4 – H_2O); HRMS calcd for $C_{21}H_{33}{}^{79}BrO_5$ 445.1589, found 445.1571; TLC (silica gel, 20% tetrahydrofuran/pentane) R_f = 0.27.

10-Bromo-1,15-Anhydrothromboxane B_2 (7). To 1.00 g (2.70 mmol) of thromboxane B_2 (Upjohn Co.) in 50 mL of ether was added 0.56 mL (4.05 mmol, 1.5 equiv) of triethylamine (mixture was nonhomogeneous) and then 3.6 mL (2.97 mmol, 1.1 equiv) of an 0.82 M solution of 2-thiopyridyl chloroformate[6] in methylene chloride. After 90 min of rapid stirring, the reaction mixture was transferred with ether directly to the top of a 2.7 cm × 15 cm silica gel chromatography column which had been slurry-packed with 1% triethylamine in ethyl acetate. The column was eluted rapidly with a step gradient of 0–10% acetone in ethyl acetate to yield the oily yellow thiopyridyl ester as a 4:1 mixture of anomers. Flushing the column with 30% methanol in ethyl acetate yielded 2–5% recovered thromboxane B_2: ^{1}H NMR ($CDCl_3$) δ (major anomer) 8.59 (1 H, m), 7.72 (1 H, dt, J = 2, 8 Hz), 7.53 (1 H, d, J = 8 Hz), 7.29 (1 H, dd, J = 6, 8 Hz), 5.8–5.5 (2 H, m), 5.4–5.2 (4 H, m), 4.5–4.0 (4 H, m), 2.70 (2 H, m), 2.25–1.2 (18 H, m), 0.86 (3 H, br t, J = 7 Hz); ^{13}C NMR (CDCl3) δ (major anomer) 196.43, 150.84, 150.12, 138.13, 137.38, 130.28, 130.15, 129.34, 128.24, 123.63, 92.37, 72.14, 69.22, 64.68, 44.44, 43.05, 36.76, 36.21, 31.57, 25.80, 25.18, 25.02, 24.89, 22.46, 13.90; IR (neat) cm^{-1} 3380 (v br), 1710, 1570, 1450, 1420, 1100, 1020, 970; MS (CI–NH_3), m/e 464 (M + 1), 446 (M – OH), 428 (M – OH – H_2O); TLC (silica gel, ethyl acetate) R_f = 0.19–0.30.

The thiopyridyl ester was azeotropically dried by stripping twice with 50 mL of freshly distilled toluene under nitrogen at reduced pressure in a 1-L flask. The flask was then fitted with a reflux condenser and a vacuum/argon line and evacuated to about 0.05 torr for 1 h. After venting with argon, 500 mL of toluene (freshly distilled from Na/Ph_2CO) was added to give a 5 mM solution, and the reaction mixture was refluxed for 13 h. The solvent was removed and the crude product was transferred to a separatory funnel with anhydrous ether. The material was then washed twice with 5% NaOH and once with brine, dried over $MgSO_4$, and stripped. Flash chromatography on a 2.7 cm × 15 cm silica gel column with a step gradient of 50% ethyl acetate in petroleum ether to pure ethyl acetate gave 618 mg (65%) of the crystalline 1,15-anhydro-TXB_2 as a 5:1 mixture of anomers and 174 mg (18%) of impure 1,9-anhydro-TXB_2.

Major macrolide (1,15-anhydro-TXB_2): ^{1}H NMR ($CDCl_3$) δ (major anomer) 6.08 (1 H, dd, J = 6, 16 Hz), 5.95 (1 H, dd, J = 7, 16 Hz), 5.57–5.13 (4 H, m), 4.34 (1 H, dd, J = 7, 11 Hz), 4.1–4.0 (1 H, m), 3.82 (1 H, d, J = 6 Hz), 2.43–2.32 (2 H, m), 2.3–1.2 (18 H, m), 0.86 (3 H, br t, J = 7 Hz); ^{13}C NMR ($CDCl_3$) δ (major anomer) 173.44, 133.88, 132.90, 129.08, 128.46, 92.63, 72.20, 67.04, 65.59, 48.43, 35.85, 34.20, 33.97, 31.41, 25.67, 25.54, 25.15, 23.63, 22.39, 13.93; IR ($CDCl_3$) cm^{-1} 3590, 3510 (br), 1721, 1435, 1246, 1103, 885; MS (CI–CH_4), m/e 353 (M + 1), 335 (M + 1 – H_2O), 317 (M + 1 – $2H_2O$); HRMS calcd for $C_{20}H_{33}O_5$ (M + 1) 353.2327, found 353.2286; TLC (silica gel, ethyl acetate) R_f = 0.45–0.53; mp (from ethyl acetate/pentane) 137 °C.

Minor macrolide (1,9-anhydro-TXB_2): ^{1}H NMR ($CDCl_3$) δ 5.82–5.78 (1 H, m), 5.72–5.60 (1 H, m), 5.45–5.20 (3 H, m), 5.0 (0.5 H, br d, J = 10 Hz), 4.9 (1 H, br s), 4.45–4.35 (0.5 H, m), 4.15–3.95 (2.5 H, m), 2.4–1.2 (20 H, m), 0.86 (3 H, br t, J = 7 Hz); ^{13}C NMR ($CDCl_3$) δ (major anomer) 174.28, 138.07, 132.08, 128.32, 125.94, 92.54, 72.24, 72.16, 69.70, 39.95, 36.76, 35.58, 33.55, 31.57, 29.49, 25.87, 25.44, 25.04, 22.47, 13.90; IR ($CDCl_3$) cm^{-1} 3592, 3440 (v br), 1733, 1450, 1149, 1088, 1035; MS (CI–NH_3), m/e 370 (M + NH_4), 352 (M^+), 335 (M – OH), 317 (M – OH – H_2O); TLC (silica gel, ethyl acetate) R_f = 0.27–0.35.

In a 100-mL round-bottom flask were placed 618 mg (1.75 mmol) of 1,15-anhydro-TXB_2 (above), 1.35 g (5.3 mmol, 3 equiv) of 2-chloro-1-methylpyridinium iodide (Aldrich), and a magnetic stirring bar. A reflux condenser was attached, and the apparatus was evacuated to about 0.05 torr for 60 min. After venting with argon, 50 mL (0.035 M) of acetonitrile (freshly distilled from CaH_2) and 1.5 mL (11 mmol, 6 equiv) of triethylamine were added. After refluxing for 90 min, the solvent was removed on a rotary evaporator to give the crude enol ether: ^{1}H NMR ($CDCl_3$) δ 6.46 (1 H, d, J = 5.9 Hz), 6.04–5.94 (2 H, m), 5.50 (1 H, m), 5.44–5.29 (2 H, m), 5.02 (1 H, t, J = 5.9 Hz), 4.13 (1 H, dd, J = 5.3, 11.2 Hz), 4.01 (1 H, dd, J = 3.6, 5.6 Hz), 2.43–2.30 (2 H, m), 2.29–1.20 (16 H, m), 0.87 (3 H, br t, J = 7 Hz); TLC (silica gel, 25% ethyl acetate/pentane) R_f = 0.38.

The crude enol ether was immediately taken up in ether, washed twice with saturated aqueous $NaHCO_3$, filtered through Na_2SO_4, stripped to a volume of about 10 mL, and transferred to a 100-mL round-bottom flask with ether (total volume ca. 50 mL). Aqueous $NaHCO_3$ (4 mL of a 2% solution) was added and the solution was cooled to 0 °C. Powdered *N*-bromosuccinimide (312 mg, 1.75 mmol) was added to the well-stirred solution. After 15 min, TLC indicated the complete consumption of starting material. The aqueous phase was then removed, and the ethereal phase was washed 3 times with saturated aqueous $NaHCO_3$, dried over $MgSO_4$, and concentrated. The crude product was flash chromatographed on a 2.7 cm × 15 cm silica gel column with a gradient of 20%–30% ethyl acetate in petroleum ether to yield 496 mg (65%) of oily **7** as a 1:1 mixture of anomers: ^{1}H NMR ($CDCl_3$) δ 6.20–5.85 (2 H, m), 5.5–5.2 (4 H, m), 4.95 (0.5 H, d, J = 11 Hz), 4.40–4.05 (3.5 H, m), 3.34 (0.5 H, d, J = 8 Hz), 3.27 (0.5 H, d, J = 12 Hz), 2.45–1.20 (17 H), 0.86 (3 H, br t, J = 7 Hz); ^{13}C NMR ($CDCl_3$) δ 173.66, 134.31, 132.26, 131.86, 129.34, 129.23, 127.69, 127.99, 94.75, 89.21, 74.68, 70.51, 72.28, 70.00, 67.20, 57.25, 46.98, 41.63, 33.95, 33.82, 33.74, 31.30, 25.47, 25.41, 25.34, 25.12, 22.39, 20.19, 22.31, 13.95; IR ($CDCl_3$) cm^{-1} 3510, 3600 (br), 1720, 1248, 1101, 1041; MS (CI–CH_4), m/e 431, 433 (M + 1), 415, 413 (M – OH), 397, 395 (M – OH – H_2O), 333 (M – Br – H_2O); HRMS calcd for $C_{20}H_{32}{}^{81}BrO_5$ (M + 1) 433.1412, found 433.1384; TLC (silica gel, 25% ethyl acetate/pentane) R_f = 0.14–0.20.

10-Bromo-1,15-anhydrothromboxane A_2 (8a). To a stirred solution of 0.535 mL (4.5 mmol) of freshly distilled trimethyl phosphite in 10 mL of dry methylene chloride (0 °C, nitrogen) was added 0.520 mL (3.3 mmol) of freshly distilled diethyl azodicarboxylate. The ice bath was removed, and the solution was stirred for an additional 5 min. The resulting pale-yellow solution was added to a well-stirred solution of 650 mg (1.5 mmol) of **7** in 20 mL of dry methylene chloride at 0 °C under nitrogen. The ice bath was removed, and the reaction mixture was stirred for 35 min at 25 °C. The mixture was then concentrated to ca. 4 mL and applied to a 2 cm × 25 cm slurry-packed column of silica gel and rapidly eluted with 2% triethylamine/5% tetrahydrofuran in petroleum ether to give 108 mg (17.5%) of the pure bromooxetane **8a**. The lower R_f byproducts were eluted from the column with ethyl acetate and rechromatographed with 20% ethyl acetate in petroleum ether to provide a recovery of 120 mg (18.5%) of starting material. The yield of **8a** based on consumed material is 21%: ^{1}H NMR ($CDCl_3$) δ 6.34 (1 H, dd, J = 16.1, 5.9 Hz), 6.15 (1 H, dd, J = 16.1, 8.6 Hz), 5.59 (1 H, dd, J = 3.9, 3.3 Hz), 5.20–5.55 (2 H, m), 5.17 (1 H, m), 4.93 (1 H, dd, J = 5.9, 3.3 Hz), 4.83 (1 H, dd, J = 5.9, 3.9 Hz), 4.13 (1 H, t, J = 8.2 Hz), 2.39 (2 H, ddd, J = 12.5, 7.9, 2.6 Hz), 1.2–2.2 (15 H, m), 0.88 (3 H, t, J = 6.3 Hz); ^{13}C NMR ($CDCl_3$) δ 172.89, 133.60, 132.81, 131.71, 127.11, 106.09, 85.68, 77.08, 71.51, 47.09, 45.50, 35.06, 32.92, 32.15, 26.80, 26.61, 26.34, 25.54, 23.24, 14.58; IR (neat) cm^{-1} 3010, 2955, 2932, 1727, 1242, 1074, 846; MS (CI–CH_4), m/e 413, 415 (M + 1), 333 (M – Br); HRMS calcd for $C_{20}H_{29}{}^{81}BrO_4$ 414.1227; found 414.1209; TLC (silica gel, 5% tetrahydrofuran/pentane) R_f = 0.30.

1,15-Anhydrothromboxane A_2 (8b). To a solution of 73 mg (0.176 mmol) **8a** in 5 mL of dry pentane under argon was added 1.75 g (ca. 10 equiv of SnH) of polymer-bound tin hydride[7] and 3 mg of recrystallized AIBN. The mixture was cooled under flowing tap water and stirred while irradiating with a 275-W sunlamp. After approximately 2 h, TLC showed consumption of starting material and the polymer was removed

by filtration. Solvent removal at reduced pressure gave **8b** (50 mg, 85%) as a crystalline solid. Recrystallization from pentane gave the X-ray sample[8] (mp 81-82 °C). The product was not stable to silica gel chromatography and gave almost complete hydrolysis to 1,15-anhydrothromboxane B_2 on attempted analytical or preparative chromatography: ^{1}H NMR (C_6D_6) δ 6.29 (1 H, dd, J = 16.1, 6.3 Hz), 6.10 (1 H, dd, J = 16.1, 8.6 Hz), 5.60 (1 H, dd, J = 3.9, 3.3 Hz), 5.37-5.52 (2 H, m), 5.36 (1 H, m), 4.50 (1 H, t, J = 8.2 Hz), 4.48 (1 H, dd, J = 6.5, 3.9 Hz), 2.73 (1 H, ddd, J = 10.3, 6.5, 3.6 Hz), 1.2-2.4 (17 H), 1.38 (1 H, d, J = 10.3 Hz), 0.95 (3 H, t, J = 6.7 Hz); ^{13}C NMR (C_6D_6) δ 172.71, 134.99, 132.20, 130.96, 128.17, 106.11, 82.19, 77.46, 71.53, 51.12, 42.28, 35.14, 32.92, 32.23, 26.79, 26.67, 26.29, 25.00, 23.21, 14.58; IR (CH_2Cl_2) cm^{-1} 3010, 2959, 2931, 1730, 1243, 1111, 1032; MS (CI-CH_4), m/e 335 (M + 1), 317 (M + 1 - H_2O); HRMS calcd for $C_{20}H_{31}O_4$ (M + 1) 335.2222, found 335.2192; TLC dec to 1,15-anhydro-TXB_2.

Sodium and Potassium Thromboxane A_2 (1). A. Hydrolysis in Methanol/Water. To a vial containing 1 mg (0.003 mmol) of **8a** in 0.050 mL of methyl-d_4 alcohol was immediately added 0.025 mL of a 0.12 M solution of NaOH(D) in (D)H_2O under nitrogen. After stirring at 25 °C for 30 min, ^{1}H NMR and biological assay showed the saponification to sodium TXA_2 to be complete. Such solutions were diluted and used for biological testing and could be stored for at least a week of -20 °C without significant loss in biological activity.

B. Aprotic Hydrolysis with Me_3SiOK. To 5 mg (0.015 mmol) of **8a** was added 0.10 mL (0.03 mmol) of a 0.3 M solution of Me_3SiOK (Petrarch) in anhydrous tetrahydrofuran (distilled from Ph_2CO/Na) or ether at 25 °C under argon. The solution slowly turned yellow, and the reaction proceeded to completion over the course of 5 h. The tetrahydrofuran or ether saponification solutions were used after dilution for biological testing. Saponification for NMR analysis was carried out in dry THF-d_8.

In a few instances, the methanol/water procedure gave substantial decomposition of the product sodium TXA_2 during the saponification. The Me_3SiOK/THF hydrolysis procedure on the other hand is highly reproducible and is thus preferred: ^{1}H NMR THF-d_8) δ (potassium salt) 5.87 (2 H, br t, J = 9 Hz, H13, H14), 5.73 (1 H, t, J = 4 Hz, H11), 5.45-5.70 (2 H, m, H5, H6), 4.72 (1 H, dd, J = 4, 6 Hz, H9), 4.53 (1 H, br t, J = 6 Hz, H12), 4.18 (1 H, br q, J = 6 Hz, H15), 3.22 (1 H, m, H10a), 1.4-2.5 (18 H, m), 1.05 (3 H, br t, H20). All peaks were broadened (w/2 ca. 2 Hz) possibly due to slow exchange of the salt aggregates. Further physical characterization was not possible due to the lability of the TXA_2 salts.

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Mechanism of the Reaction between *cis*-[$PtCl_2(NH_3)_2$] and DNA in Vitro†

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Abstract: Products of the reaction between *cis*-[$PtCl_2(NH_3)_2$] and salmon sperm DNA in vitro have been purified. These adducts were compared with synthesized model compounds of known structures and identified as *cis*-[$Pt(NH_3)_2(Gua)_2]^{2+}$, *cis*-[$Pt(NH_3)_2(Gua)(Ade)]^{2+}$, and *cis*-[$Pt(NH_3)_2(Gua)(H_2O)]^{2+}$. Kinetics of the reaction indicate that the platinum compound binds initially at the N(7) position of Gua, the majority of these monofunctional lesions rapidly chelate to another purine base (preferentially but not exclusively Gua), and the remaining monofunctional lesions react slowly, primarily with an Ade base.

There is good evidence that the fixation of *cis*-[$PtCl_2(NH_3)_2$] (*cis*-DDP)[3] on DNA is the cellular event responsible for the antitumor activity of this drug.[1] However, the trans isomer which is not antitumoral[2,3] also enters the cell and covalently binds to DNA.[4-6] Several studies have quantitated the chemical and biological effects of the DNA damage caused by these compounds. When equal amounts of *cis*- or *trans*-DDP fixed on the DNA are compared, lesions formed by *cis*-DDP are more toxic[4-6] and more mutagenic.[5] They also inhibit DNA synthesis[6-8] and undergo DNA repair[6,9] to a greater extent than DNA lesions formed by *trans*-DDP. Physical chemical studies indicate that *cis*-DDP and *trans*-DDP bind differently to DNA in vitro. Their effects on the secondary structure and the stability of DNA have been compared when 5-50 platinum atoms are bound per 1000 nucleotides. Under these conditions, both isomers form interstrand cross-links, shorten the DNA, and prevent the intercalation of ethidium bromide. However *cis*-DDP destabilizes the DNA while *trans*-DDP stabilizes the polymer, and only the cis isomer causes an increase in the circular dichroism spectrum of DNA at these levels of DNA binding (ref 10, and references therein). The structures of the platinum-DNA adducts which are responsible for the different biochemical and physical chemical effects of these compounds have not yet been determined.

Evidence has accumulated for several years that *cis*-DDP binds to the N(7) position of guanine (Gua) bases in oligonucleotides (ref 11 and 12 and references therein), but platinum-containing adducts have only recently been isolated from DNA, and their quantitation is an active area of research. We have previously developed a method to separate platinum-DNA adducts from

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