Synthesis and LTB_4 Receptor Antagonist Activities of the Naturally Occurring LTB_4 Receptor Antagonist Leucettamine A and Related Analogues

Jeffrey C. Boehm,*,† John G. Gleason,† Israil Pendrak,† Henry M. Sarau,‡ Dulcie B. Schmidt,‡ James J. Foley,‡ and William D. Kingsbury†

Departments of Medicinal Chemistry and Pharmacology, Smithkline Beecham Pharmaceuticals, P.O. Box 1539, King of Prussia, Pennsylvania 19406-0939

Received March 5, 1993•

The isolation and structure determination of the naturally occurring LTB_4 receptor antagonist Leucettamine A (1) was recently reported.[1] Herein we describe the synthesis of this natural product, the preparation of several analogues, and their effectiveness as antagonists of [^{3}H]LTB_4 binding to intact human U-937 cells. Total synthesis of Leucettamine A (1) is achieved by a convergent route which takes advantage of the elements of symmetry within the molecule. Syntheses of analogues of 1, which lacked the same degree of symmetry, are achieved by a different approach starting from α-amino acids. The natural product 1 inhibits [^{3}H]LTB_4 binding to its receptors on intact human U-937 cells with a $K_i = 3.5 \pm 0.8$ μM and is devoid of measurable agonist activity at the concentrations tested. 2-Amino imidazole analogues of 1 lacking the dioxolane groups were prepared. Generally these are significantly less potent than 1. However, one (**26**), designed on the basis of a putative structural overlay with LTB_4, demonstrated potency comparable to that of the natural product ($K_i = 2.4 \pm 0.2$ μM).

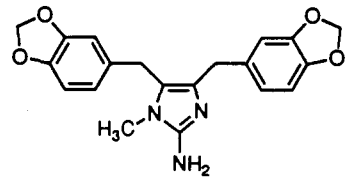
Introduction

5(*S*),12(*R*)-Dihydroxy-6,14-*cis*-8,10-*trans*-eicosatetraenoic acid (LTB_4)is one of the family of leukotrienes derived from the release of arachidonic acid from cellular membrane lipids and subsequent metabolism by 5-lipoxygenase. *In vitro* studies with tissue and whole cells as well as *in vivo* studies with animal models indicate that LTB_4 is an inflammatory mediator acting through specific receptors on polymorphonuclear leukocyte (PMN) membranes.[2a] *In vitro*, LTB_4 stimulates PMN chemotaxis, aggregation, degranulation, superoxide generation, and adhesion.[2] LTB_4 has been shown to be present in physiologically relevant concentrations at inflammatory sites associated with several disease states including asthma, psoriasis, gout, reperfusion injury, and inflammatory bowel disease.[2] A number of LTB_4 receptor antagonists (for example SC-41930, LY 223982, and U-75302) have been reported to exhibit sub-micromolar IC_{50}s for inhibition of LTB_4 binding to human neutrophils.[3] We have initiated studies aimed at identifying potent, novel, and selective LTB_4 receptor antagonists which should be useful as tools for further evaluation of the role that LTB_4 plays in human disease.

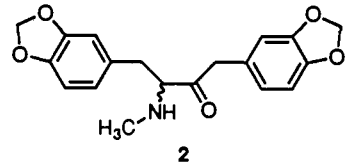
Screening marine natural products utilizing [^{3}H]-LTB_4 competive binding to human U-937 cells[4] resulted in the isolation and identification of the natural LTB_4 receptor antagonist Leucettamine A (1) from the Paluan marine sponge *Leucetta microraphis*.[1] We report here the synthesis of 1 and related analogues and the effectiveness of these compounds as LTB_4 receptor antagonists *in vitro*.

Chemistry

α-Amino ketones are known to be useful precursors to 2-amino imidazoles through their reaction with cyanamide under mildly acidic conditions (pH 4–5).[5] On the basis of this chemistry, a convergent synthesis of Leucettamine A (1) was chosen in which the key intermediate would be the amino ketone **2**. This approach, which is generally



Leucettamine A (1)



2

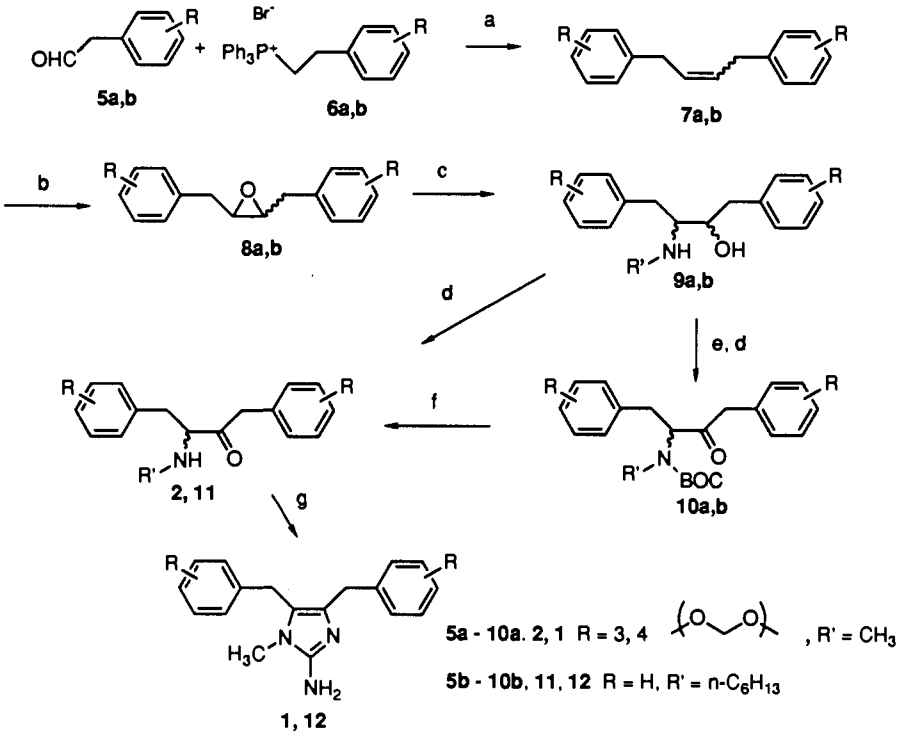
applicable to the synthesis of 2-aminoimidazoles with identical substituents at C-4 and C-5, is depicted in Scheme I. For the synthesis of 1 where R is 3,4-methylenedioxy, the aldehyde **5a** and phosphonium salt **6a** were prepared in a straightforward manner from 3,4-(methylenedioxy)-phenylacetic acid in two and three steps, respectively (see the Experimental Section). Wittig reaction of **5a** and **6a** gave **7a** as a mixture of *E* and *Z* olefins.[6] Although **7** and subsequent intermediates were isolated as isomeric mixtures, the stereochemical differences are lost upon imidazole formation. Epoxidation of **7a** with MCPBA afforded a modest yield (47%) of **8a**. In the case of **7b**, when the phenyl rings were unsubstituted, the epoxidation proceeded in 96% yield, thereby implicating the participation of the electron rich aromatic rings of **7a** in side reactions with the electrophilic oxygen of MCPBA.[7,8] Opening of the epoxides with a primary amine at temperatures above 100 °C afforded the amino alcohols **9a,b**.

Repeated attempts to oxidize the electron rich bis-dioxolane **9a** directly to the amino ketone **2** and then to cyclize to the target molecule resulted in very low yields of 1. The α-amino ketone was subsequently found to be unstable as the free base, decomposing to a nonpolar product during isolation. Alternatively, blocking of the

† Department of Medicinal Chemistry.
‡ Department of Pharmacology.
• Abstract published in *Advance ACS Abstracts*, September 15, 1993.

0022-2623/93/1836-3333$04.00/0

Scheme I[a]



[a] Conditions: (a) NaH, DMSO; (b) MCPBA; (c) R'NH$_2$, >100 °C; (d) Jones or PDC; (e) BOC_2O; (f) HCl, Et_2O; (g) H_2NCN, pH 4.5.

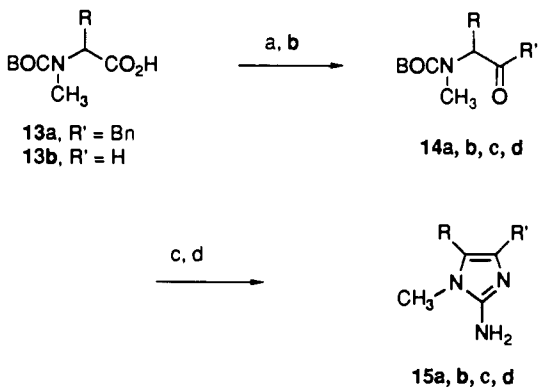
amino function of **9a** with di-*tert*-butyl dicarbonate led to the N-blocked amino alcohol. PDC oxidation produced the stable, t-BOC amino ketone **10a**.[9,10] Treatment of **10a** with ethereal HCl (25 °C, 1 h) afforded **2** as the crystalline hydrochloride. Adjustment of the pH to 4.5 and immediate cyanamide treatment[5] gave the 2-aminoimidazole **1** (49% yield from **9a**) which was spectroscopically, chromatographically, and biologically identical to natural Leucettamine A.[1]

This modified approach to 2-aminoimidazoles, through BOC-protected α-amino ketones, is generally applicable, and in addition to Leucettamine A, the 2-aminoimidazoles **12** and **15a–d**, were prepared in this way. The modified procedure minimizes the potential difficulties resulting from the reactivity of the α-amino ketone intermediates.

When both the substituents on C-4 and C-5 of the imidazole analogues were identical, the strategy of obtaining the requisite α-BOC-amino ketones from symmetrical epoxides, as in the synthesis of **1**, proved to be effective. Thus, the *N*-hexyl-BOC-amino ketone **10b** (Scheme I) was prepared from the symmetrical epoxide **8b** by reaction with refluxing hexylamine, protection of the amino group with BOC_2O, and subsequent PDC oxidation. Conversion of **10b** to 2-aminoimidazole **12** was effected by the procedure described above for the conversion of **10a** to **1**.

When the substituents on C-4 and C-5 of the imidazole analogues were different (**15b–d**), or when the appropriate starting materials (α-amino acids and Grignard reagents; vide infra) were commercially available (**15a**), a second approach was used. In these cases (Scheme II) the protected α-amino ketones **14a–d** were prepared from α-amino acids[11] by methodology for ketone synthesis originally described by Weinreb, and later applied to carbamate-protected α-amino acids by Rapoport and others.[12] Conversion of the α-BOC-amino ketones to the 2-aminoimidazoles **15a–d** then proceeded as described for the preparation of **1**.

Scheme II[a]



14a, **15a**, R = R' = Bn

14b, **15b**, R = H, R' = Bn

14c, **15c**, R = Bn, R' = m-$MeOC_6H_4CH_2$-

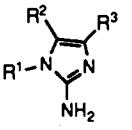
14d, **15d**, R = Bn, R' = p-TBDPSO$(CH_2)_4C_6H_4CH_2$-

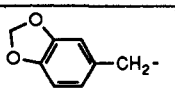
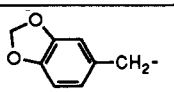
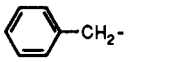
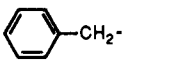
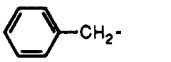
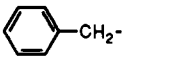
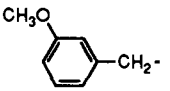
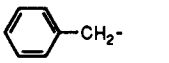
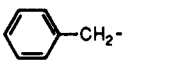
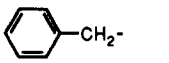
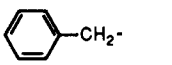
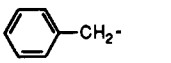
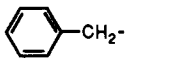
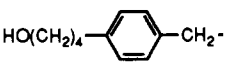
[a] Conditions: (a) $HNCH_3(OCH_3)$, i-BuOCOCl; (b) R''MgCl; (c) HCl/Et_2O; (d) H_2NCN, pH 4–5, 90 °C.

The Grignard reagent required to synthesize **15d** was prepared from the benzylic chloride precursor **18**. This in turn was obtained by a Suzuki coupling[13] of 4-[(di-*tert*-butylphenylsilyl)oxy]-1-butene (**16b**) and 4-bromobenzyl alcohol and subsequent conversion of the resulting alcohol to the chloride (Scheme III). Compound **26** (Table I) was obtained from **15d** by desilylation with TBAF.

Scheme IV illustrates a third, concise approach to α-amino ketones, which was applied to the preparation of the α-amino ketone **23**.[14–16] The unprotected α-amino ketone **23** was used directly to synthesize imidazole **24** in low yield. Imidazole **25** was prepared as previously described.[5]

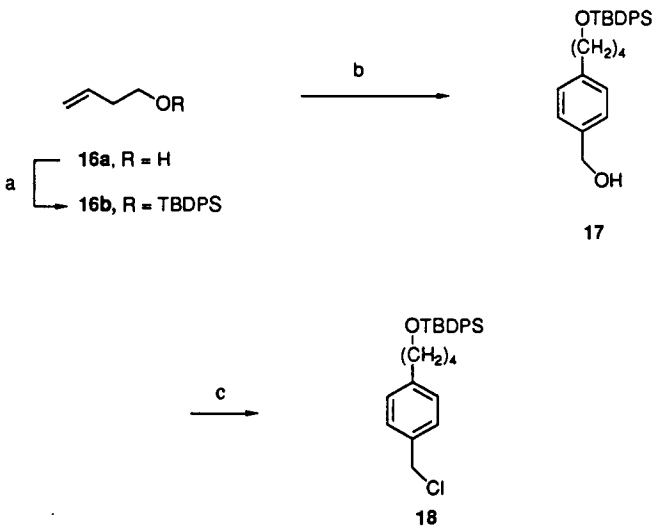
Table I. Binding of 2-Aminoimidazoles to LTB$_4$ Receptors



compd	R^1	R^2	R^3	whole cell (U-937) IC$_{50}$, μM	whole cell (U-937) K_i, μM
1[a]	CH_3			5.2 ± 1.1	3.5 ± 0.8
15a[a]	CH_3			30.0 ± 2.0	19.0 ± 1.2
15b[b]	CH_3	H		>100	
15c[a]	CH_3			40	27
12[a]	n-C_6H_{13}			>100	
24[a]	H			13	9
25[a]	H	CH_3		>100	
26[a]	CH_3			3.8 ± 0.3	2.4 ± 0.2

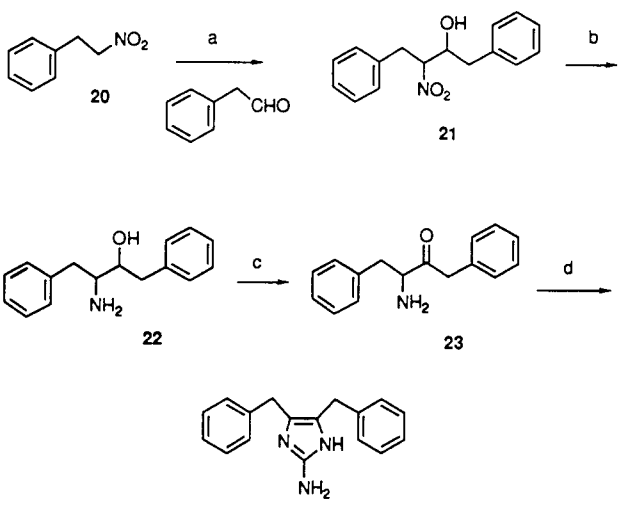
[a] Satisfactory C, H, N analyses were obtained. [b] Satisfactory C, H, N analyses were not obtained. Satisfactory ^{13}C and ^1H NMR and mass spectra were obtained.

Scheme III[a]



[a] Conditions: (a) TBDPS-Cl, imidazole (95%); (b) (i) 9-BBN, (ii) *p*-bromobenzyl alcohol, $PdCl_2$, dppf, DMF, K_2CO_3 (62%); (c) $SOCl_2$, pyridine (61%).

Scheme IV[a]



[a] Conditions: (a) KF, Al_2O_3, CH_2Cl_2; (b) PtO_2, H_2, EtOH; (c) CrO_3/acetone; (d) $NCNH_2$, H_2O, pH 4.5, 90 °C.

Biological Results and Discussion

Leucettamine A (1) binds to the LTB$_4$ receptor on intact differentiated U-937 cells with a K_i of 3.5 ± 0.5 μM in competitive binding assays with [^{3}H]LTB$_4$ (Table I).[17] In additional studies, compound 1 demonstrated moderate binding affinity to membrane preparations of U-937 cells with a K_i of 1.8 ± 0.3 μM. In comparison, the structurally unrelated LTB$_4$ receptor antagonists LY223982 and SC 41930 had K_is of 0.36 ± 0.05 and 0.22 ± 0.06 μM, respectively, in similar U-937 membrane binding assays. LTB$_4$ induces an intracellular calcium transient in fura 2 containing human PMNs which is readily blocked by LTB$_4$ receptor antagonists.[18] Although the receptors on PMNs and U-937 cells have been shown, in binding studies, to be identical,[4] the LTB$_4$-induced Ca^{2+} mobilization response was much greater in PMNs than in U-937 cells. For this reason PMNs were used to evaluate agonist activity of the compounds. Leucettamine A (1) did not induce significant calcium mobilization up to 33 μM and therefore does not appear to be an agonist on human PMNs. Higher concentrations of 1 (up to 100 μM) resulted in fluorescent interference such that quantitation was not feasible. However, even at 100 μM, no Ca^{2+} response was observed. This compound effectively antagonized LTB$_4$ induced calcium mobilization in a concentration dependent manner, with an IC$_{50}$ of 5.2 ± 0.8 μM (Figure 1). This data suggests that 1 is an antagonist acting at the LTB$_4$ receptor.

Several analogues related to 1 were prepared and evaluated for LTB$_4$ receptor antagonist activity (Table I).

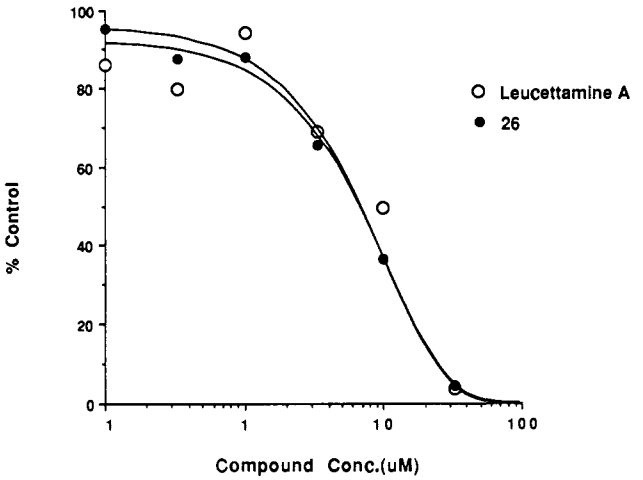
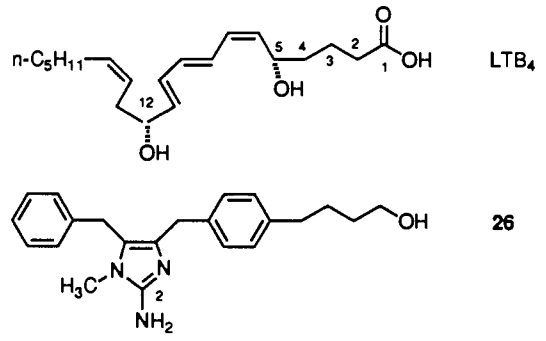


Figure 1. Effects of Leucettamine A and **26** on LTB_4-induced calcium mobilization. Fura 2 loaded human PMNs treated with varying concentrations of Leucettamine A (O) or **26** (●) for 1 min at 37 °C before the addition of 0.1 nM LTB_4. The maximum $[Ca^{2+}]i$ concentration achieved was calculated and compared with the LTB_4 response in the absence of antagonist (100%). Presented is a typical experiment of three where each point is the mean of duplicate determinations.

Chart I



In general, SAR revealed that the dioxolane groups are important for receptor binding (compare **1** with **15a**). The *N*-methyl group may not be required for binding (compare **15a** with **24**), but a bulky alkyl group on nitrogen, such as the *n*-hexyl group (compound **12**), resulted in a loss of potency. Replacement of the 5-benzyl group with a hydrogen or methyl (**15b**, **25**) led to a significant loss in LTB_4 receptor binding affinity.

In addition, **26** was prepared in an attempt to test the hypothesis that the 2-amino group of this analogue might mimic the LTB_4 12-hydroxyl and that the hydroxyl side chain of **26** would mimic the 1-carboxyl group of LTB_4 (Chart I). We were uncertain as to whether or not this change would improve affinity for the LTB_4 receptors since the previously described anologues of **1**, which lacked the dioxolane functional groups, were all less active than **1**. We observed that **26** was comparable to **1** in its affinity for the LTB_4 receptors in intact U-937 cells. The ability of **26** to induce Ca^{2+} mobilization in PMNs was also examined, and analogous to Leucettamine A, **26** did not exhibit detectable agonist properties up to 100 μM. The concentration dependent inhibition of the LTB_4-induced calcium transient by **26** (IC_{50} = 6.2 μM) and Leucettamine A is depicted in Figure 1.

Additional modifications to the 2-aminoimidazole series of LTB_4 receptor antagonists to more closely mimic the structure of LTB_4 can be envisioned. However, since we had concurrently developed several structurally unrelated, more promising leads,[19] additional work on the series of LTB_4 receptor antagonists related to Leucettamine A was not pursued.

In summary, Leucettamine A (**1**), a marine natural product possessing LTB_4 receptor antagonist properties, was synthesized in eight steps in 9.4% overall yield. Synthetic Leucettamine A antagonized $[^3H]LTB_4$ binding to U-937 cells, inhibited LTB_4 induced Ca^{2+} mobilization in U-937 cells and human PMNs, and was devoid of measurable agonist properties at the concentrations tested. Synthetic methods for the preparation of analogues were developed, and a variety of analogues which lacked the 1,3-dioxolane moieties of **1** were prepared. Generally, these compounds exhibited significantly weaker binding to LTB_4 receptors on intact U-937 cells. However, one compound, **26**, designed in an attempt to mimic some of the structural features of LTB_4, bound to LTB_4 receptors on intact U-937 cells equally as well as the natural product. While this series is not of interest for further development, it represents a novel class of LTB_4 receptor antagonists derived from natural sources.

Experimental Section

General Procedures. ^1H NMR spectra were recorded on a Bruker WM-250 (250 MHz) instrument in $CDCl_3$ unless otherwise noted and with tetramethylsilane as internal standard. Elemental analyses were performed in the Analytical and Physical Chemistry Department of SmithKline Beecham. Mass spectra were obtained by the Physical and Structural Chemistry Department of SmithKline Beecham. Analytical thin-layer chromatography (TLC) was carried out using Merck silica gel 60 F-254 glass-backed plates or Whatman KC 18 F reversed-phase RP-18 glass-backed plates with the solvent systems indicated. Reversed-phase medium-pressure liquid chromatography (MPLC) (P < 100 psi, 10 mL/min) used Beckman glass columns packed with Whatman Partisil 40 ODS3 (25–40 μm). Flash chromatogtaphy utilized Merck silica gel 60 (230–400 mesh).

THF was distilled from Na^0/benzophenone ketyl. Where other anhydrous solvents were required, Aldrich anhydrous solvents were used. Reactions requiring anhydrous and/or O_2 free conditions were conducted in flame-dried glassware under argon.

Compound **25** was prepared as previously described.[5]

3,4-(Methylenedioxy)phenethyl Alcohol (4). To a solution of 2,3-(methylenedioxy)phenylacetic acid (**3**) (54.0 g, 0.30 mol) dissolved in THF (300 mL) and cooled to 10–15 °C was added dropwise 10 M borane dimethyl sulfide complex (33 mL, 0.33 mol).[20,21] The reaction mixture was allowed to warm to 25°C, and the temperature was maintained at 25–30 °C for $1^1/_4$ h by cooling with an ice bath as necessary. (*Caution*: an exotherm occurs after an induction period.) The reaction mixture was then cooled to 10 °C, and CH_3OH (100 mL) was added. After being allowed to stand for 30 min at 23 °C, the reaction mixture was combined with EtOAc (1 L), and the resulting mixture was washed with H_2O (300 mL), 5% $NaHCO_3$ (2 × 300 mL), and saturated aqueous NaCl (300 mL) and dried (Na_2SO_4). The solvent was removed *in vacuo* to yield 50.4 g (100% yield) of **4**. A portion of this material (20 g) was purified by flash chromatography (0–20% EtOAc/hexanes) to afford 15.45 g of **4** as an amorphous gum. ^1H NMR : 2.58 (s, 1H), 2.62 (t, 2H), 3.62 (t, 2H), 5.76 (s, 4H), 6.53 (dd, 1H), 6.58 (d, 1H), 6.62 (d, 1H). MS (DCI, CH_4) (*m/e*): 166 (M^+).

3,4-(Methylenedioxy)phenylacetaldehyde (5a). To a solution of pyridinium chlorochromate (0.33 g, 1.5 mmol) in CH_2Cl_2 (2.0 mL)[22,23] was added **4** (0.150 g, 0.90 mmol) in CH_2Cl_2 (2.0 mL). After being stirred at 23 °C for 2 h, the reaction mixture was filtered through florisil, and the pad was washed with Et_2O (3 × 20 mL). The combined filtrates were concentrated *in vacuo*, and the residue was purified by flash chromatography (0–10 % EtOAc / hexanes) to afford 65.3 mg (44%) of **5a** as an amorphous gum. ^1H NMR ($CDCl_3$): 3.58 (d, 2H), 5.94 (s, 2H), 6.66 (dd, 1H), 6.67 (d, 1H), 6.79 (d, 1H), 9.68 (t, 1H). MS (DCI, CH_4) (*m/e*): 165 (MH^+).

3,4-(Methylenedioxy)phenethyl Bromide. To **4** (15.0 g, 0.10 mol) and CBr_4 (41.4 g, 0.125 mol) in CH_2Cl_2 (150 mL) at 10 °C

was added slowly in small portions PPh_3 (39.2 g, 0.15 mol).[25] The temperature was maintained below 10 °C during the addition, and the resulting mixture was then allowed to warm to 23 °C, stirred for 30 min, and filtered. The filtrate was concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 and purified by flash chromatography (0–4% EtOAc/hexanes) to afford 21.51 g (94% yield) of 3,4-(methylenedioxy)phenethyl bromide as a colorless oil. ^1H NMR: 3.06 (t, 2H), 3.50 (t, 2H), 5.92 (s, 2H), 6.65 (d, 1H), 6.68 (s, 1H), 6.75 (d, 1H). MS (DCI, CH_4) (*m/e*): 229 (MH^+).

[3,4-(Methylenedioxy)phenethyl]triphenylphosphonium Bromide (6a). 3,4-(Methylenedioxy)phenethyl bromide (14.35 g, 62.7 mmol), CH_3CN (450 mL), and PPh_3 (32.75 g, 0.125 mol) were refluxed for 42 h[26] and cooled to 23 °C, the solvent was removed in vacuo, and the residue was dissolved in CH_2Cl_2 (150 mL) and purified by preparative HPLC (Whatman Mag 40 column packed with flash silica) (0–8% CH_3OH/CH_2Cl_2) to afford 25.29 g (88%) of **6a** as a white, hydroscopic foam. ^1H NMR: 3.00 (m, 2H), 4.02 (m, 2H), 5.85 (s, 2H), 6.63 (d, 1H), 6.73 (dd, 1H), 6.77 (d, 1H), 7.65–7.9 (m, 15H).

(*E*)- and (*Z*)-1,4-Bis[3,4-(methylenedioxy)phenyl]-2-butene (7a). A mineral oil suspension of sodium hydride (0.998 g, 22.7 mmol) was washed with hexanes, and DMSO (10 mL) was added. The mixture was heated to 70 °C for 45 min and then cooled to 25 °C. A solution of **6** (11.15 g, 22.7 mmol) in DMSO (20 mL) was added dropwise, maintaining the temperature between 25 and 30 °C with cooling on an ice bath as necessary. The resulting red solution was stirred for 30 min, and **5** (3.1 g, 18.9 mmol) in DMSO (15 mL) was added dropwise, maintaining the temperature between 25 and 30 °C by cooling with a cold-water bath as necessary. After 30 min, the mixture was poured into ice water and extracted with EtOAc (5 × 200 mL). The combined organic extracts were washed with H_2O (200 mL) and saturated aqueous NaCl (100 mL), dried (Na_2SO_4), and concentrated *in vacuo*, and the residue was purified by flash chromatography (0–1% EtOAc/hexanes) to afford 3.15 g (56% yield) of **7a** as a waxy solid. ^1H NMR: 3.4 and 3.55 (2 dd, 4H), 5.5–6.5 (m, 2H), 5.9 (2 s, 4H), 6.6–6.9 (m, 6H). MS (DCI, CH_4) (*m/e*): 297 (MH^+).

***cis*- and *trans*-1,4-Bis[3,4-(methylenedioxy)phenyl]-2,3-epoxybutane (8a).** To **7a** (0.50 g, 1.69 mmol) in 1:1 hexanes/CH_2Cl_2 (20 mL) was added at 0 °C *m*-chloroperoxybenzoic acid (0.53 g, 2.53 mmol). The reaction mixture was stirred for 5 h at 23 °C, hexanes (100 mL) were added, and the solution was filtered. The filtrate was purified by flash chromatography (0–4% EtOAc/hexanes). The main fraction was dissolved in CH_2Cl_2 (25 mL), washed with 5% $NaHCO_3$ and saturated aqueous NaCl (15 mL), and dried (Na_2SO_4). The solvent was removed *in vacuo* to afford 0.27 g (47% yield) of **8a** as a waxy solid. ^1H NMR: 2.88 (m, 4H), 3.20 (m, 2H), 5.95 (s, 4H), 6.72–6.78 (m, 6H). MS (DCI, CH_4) (*m/e*): 313 (MH^+)

3-(Methylamino)-1,4-bis[3,4-(methylenedioxy)phenyl]-2-butanol (9a). A solution of **8a** (265 mg, 0.85 mmol) in toluene (5 mL) was placed in a pressure vessel. Methylamine (25 mL) was condensed into the vessel, and the mixture was heated at 130 °C and 300 psi. After 38 h, the vessel was cooled on a dry ice–acetone bath and vented. The solution was concentrated *in vacuo* and purified by flash chromatography (0–2% CH_3OH/0.2% Et_3N/CH_2Cl_2) to provide 244 mg (84%) of **9a** as a white solid. ^1H NMR: 2.40 (s, 3H), 2.55–2.9 (m, 5H), 3.15 (br s, 2H), 3.6 (m, 1H), 5.9 and 5.95 (2 s, 4H), 6.55–6.8 (m, 6H). MS (DCI, CH_4) (*m/e*): 344 (MH^+).

3-[*N*-Methyl-*N*-(*tert*-butoxycarbonyl)amino]-1,4-bis[3,4-(methylenedioxy)phenyl]-2-butanol. A mixture of **9a** (171 mg, 0.50 mmol) in 2:1 THF/H_2O (6 mL), $NaHCO_3$ (92 mg, 1.10 mmol), and di-*tert*-butyl dicarbonate (120 mg, 0.55 mmol) was stirred at 23 °C for $2^1/_2$ h, concentrated under reduced pressure, dissolved in CH_2Cl_2, washed with aqueous $NaHCO_3$ and saturated aqueous NaCl, and dried (Na_2SO_4). The solvent was removed *in vacuo* and the residue triturated several times with hot hexanes. The combined organic washes were concentrated under reduced pressure to give 239 mg (100% yield) of 3-[*N*-methyl-*N*-(*tert*-butoxycarbonyl)amino]-1,4-bis[3,4-(methylenedioxy)phenyl]-2-butanol as a white solid. ^1H NMR: 1.3 (br s, 1H), 1.45 (s, 9H), 2.6 (br s, 3H), 2.65–3.15 (m, 4H), 3.85 (m, 1H), 5.9 (br s, 4H), 6.7 (m, 6H). MS (DCI, CH_4) (*m/e*): 444 (MH^+).

3-[*N*-Methyl-*N*-(*tert*-butoxycarbonyl)amino]-1,4-bis[3,4-(methylenedioxy)phenyl]-2-butanone (10a). To 3-[*N*-methyl-*N*-(*tert*-butoxycarbonyl)amino]-1,4-bis[3,4-(methylenedioxy)phenyl]-2-butanol (232 mg, 0.52 mmol) in 20% DMF/CH_2Cl_2 (10 mL) was added pyridinium dichromate (390 mg, 1.04 mmol).[27] After being stirred at 23 °C for 20 h, the reaction mixture was diluted with 20% CH_3OH/CH_2Cl_2 (50 mL) and filtered through florisil. The filtrate was concentrated *in vacuo*, and the residue was purified by flash chromatography (0–16% EtOAc/hexanes) to afford 128 mg (56% yield) of **10a** as a gum which was a mixture of carbamate conformational isomers by ^1H NMR. ^1H NMR ($CDCl_3$): 1.35 and 1.45 (2 s, 9H), 2.55 and 2.65 (2 s, 3H), 2.75 (m, 1H), 3.10 (m, 1H), 3.62 (dd, 2H), 4.35 and 4.7 (2 dd, 1H), 5.90 and 5.95 (2 s, 4H), 6.5–6.8 (m, 6H). MS (DCI, CH_4) (*m/e*): 441 (M^+), 442 (MH^+).

2-Amino-4,5-bis[[3,4-(methylenedioxy)phenyl]methyl]-1-methylimidazole (1). To **10a** (45 mg, 0.10 mmol) in Et_2O was added HCl saturated Et_2O (2 mL). The resulting mixture was allowed to stand at 23 °C for 1 h, and the Et_2O was decanted. The remaining white solid was washed with Et_2O (2 mL) and dissolved in H_2O (2 mL), and the pH was adjusted to 4.5 with 0.01 N NaOH. Cyanamide (90 mg, 2.14 mmol) was added, the solution was heated at 90 °C for 30 min and concentrated *in vacuo*, and the residue was purified by flash chromatography (CH_2Cl_2/0–1% Et_3N/0–2% CH_3OH) to provide 40 mg (89% yield) of 1. Crystals were obtained from CH_3OH/H_2O. Mp: 125–175 °C dec. ^1H NMR (acetone-d_6): 3.31 (s, 3H), 3.86 (s, 2H), 4.02 (s, 2H), 4.63 (br s, 2H), 5.90 and 5.92 (2 s, 4H), 6.5–6.85 (m, 6H). MS (DCI, NH_3) (*m/e*): 366 (MH^+). Anal. ($C_{20}H_{19}N_3O_4$) C, H, N.

(*E*)- and (*Z*)-1,4-Diphenyl-2-butene (7b). Phenethyltriphenylphosphonium bromide (0.65 g, 1.5 mmol) and phenylacetaldehyde (120 mg, 1.0 mmol) were reacted as described for the preparation of **7a** to afford, after flash chromatography in hexanes, 104 mg (54%) of **7b** as an oil. ^1H NMR: 3.50 (d, 4H), 5.70 (m, 2H), 7.2 (m, 10 H). MS (DP, CH_4) (*m/e*): 208 (M^+).

***cis*- and *trans*-2,3-Epoxy-1,4-diphenylbutane (8b).** To **7b** (7.28 g, 35.0 mmol) in 1:1 CH_2Cl_2/hexanes (150 mL) was added *m*-chloroperoxybenzoic acid (80–85%) (14.56 g, 70 mmol), and the resulting mixture was stirred overnight at 23 °C. The mixture was filtered, and the solid was washed several times with hexanes. The filtrate and washings were combined and concentrated *in vacuo*, triturated with hexanes, and filtered once more. The filtrate was purified by flash chromatography (0–2% EtOAc/hexanes) to afford 7.59 g (97% yield) of **8b** as a waxy solid. ^1H NMR: 3.0 (m, 4H), 3.25 (m, 2H), 7.3 (m, 10H). MS (DP, CH_4) (*m/e*): 225 (MH^+).

3-(*N*-*n*-Hexylamino)-1,4-diphenyl-2-butanol (9b). A solution of **8b** (200 mg, 0.89 mmol) in *n*-hexylamine (20 mL) was refluxed for 48 h. The hexylamine was removed *in vacuo*, and the residue was purified by flash chromatography (0–1% CH_3OH/0–0.25% Et_3N) to afford 143 mg (49% yield) of **9b** as a gum. ^1H NMR: 0.8 (t, 3H), 1.2 (m, 8H), 2.4–2.9 (m, 7H), 3.5 (m, 1H), 7.15 (m, 10 H).

3-[*N*-*n*-Hexyl-*N*-(*tert*-butoxycarbonyl)amino]-1,4-diphenyl-2-butanol. To **9b** (106 mg, 0.33 mmol) in 3:1 THF/H_2O (8 mL) were added di-*tert*-butyl dicarbonate (78 mg, 0.36 mmol) and $NaHCO_3$ (61 mg, 0.73 mmol). After being stirred at 23 °C for 2 days, the solution was concentrated *in vacuo*, and the residue was partitioned between H_2O (10 mL) and EtOAc (3 × 20 mL). The combined organic extracts were washed with saturated aqueous NaCl (20 mL), dried (Na_2SO_4), and concentrated *in vacuo*, and the residue was purified by flash chromatography (2–8% EtOAc/hexanes) to afford 103 mg (76% yield) of 3-[*N*-*n*-hexyl-*N*-(*tert*-butoxycarbonyl)amino]-1,4-diphenyl-2-butanol as a waxy solid. ^1H NMR: 0.8 (t, 3H), 1.0–1.8 (m, 8H), 1.40 (s, 9H), 2.65 (br d, 2H), 2.8 - 3.1 (m, 4H), 3.8 (m, 1H), 7.15 (m, 10H).

3-[*N*-*n*-Hexyl-*N*-(*tert*-butoxycarbonyl)amino]-1,4-diphenyl-2-butanone (10b). To a solution of 3-[*N*-*n*-hexyl-*N*-(*tert*-butoxycarbonyl)amino]-1,4-diphenyl-2-butanol (100 mg, 0.25 mmol) in 20% DMF/CH_2Cl_2 (5 mL) was added pyridinium dichromate (188 mg, 0.5 mmol). After the mixture was stirred at 23 °C for 4 h, CH_2Cl_2 (10 mL) was added and the solution filtered through florisil. The florisil was washed with CH_2Cl_2 (3 × 15 mL), and the combined filtrates were concentrated *in vacuo* and purified by flash chromatography (10% EtOAc/hexanes) to afford 49 mg (50% yield) of **10b** as a gum which was a mixture of carbamate conformational isomers by ^1H NMR. ^1H NMR:

0.8 (t, 3H), 1.0–1.3 (m, 8H), 1.34 and 1.40 (2 s, 9H), 2.55 (m, 1H), 2.8–3.1 (m, 2H), 3.2 (m, 1H), 3.65 (dd, 2H), 3.70 (s, 2H), 3.95 and 4.30 (2 m, 1H), 7.0–7.3 (m, 10H).

N-BOCSta N-Methoxy-N-methylamide. *N,O*-Dimethylhydroxylamine hydrochloride (0.98, 10 mmol) was acylated with BOCSta (1.98 g, 10 mmol) by a mixed anhydride procedure[12e] to afford 2.00 g (92%) of BOCSta *N*-methoxy-*N*-methylamide as an oil. ^{1}H NMR: 1.47 (s, 9H), 2.93 (s, 3H), 3.19 (s, 3H), 3.71 (s, 3H), 4.11 (m, 2H).

N-BOC-N-methylPhe N-methoxy-N-methylamide. *N,O*-Dimethylhydroxylamine hydrochloride (0.49 g, 5.0 mmol) was acylated with *N*-BOC-*N*-methylPhe (5 mmol obtained from the commercially available dicyclohexylamine salt by neutralization) by a mixed anhydride procedure[12e] to afford 1.40 g (87%) of *N*-BOC-*N*-methylPhe *N*-methoxy-*N*-methylamide as a white solid after flash chromatography (0–10% EtOAc/hexanes). ^{1}H NMR: 1.35, 1.49 (2 s, 9H), 2.87 (s, 3H), 2.91–3.25 (m, 5H), 3.61, 3.65 (2 s, 2H), 5.13, 5.52 (2 m, 1H), 7.2 (m, 5H).

3-[N-Methyl-N-(*tert*-butoxycarbonyl)amino]-1,4-diphenyl-2-butanone (14a). *N*-BOC-*N*-methylPhe *N*-methoxy-*N*-methylamide (0.688 g, 2.0 mmol) was reacted with 2.0 M benzylmagnesium chloride in THF (1.1 mL, 2.2 mmol) by the procedure of Weinreb[12a] to afford 0.508 g (72%) of **14a** as a gum after flash chromatography (0–10% EtOAc/hexanes). ^{1}H NMR: 1.32, 1.42 (2 s, 9H), 2.49, 2.58 (2 s, 3H), 2.83, 3.17 (2 m, 2H), 3.74 (m, 2H), 4.40, 4.74 (2 m, 1H), 7.27 (m, 10H).

1-[N-Methyl-N-(*tert*-butoxycarbonyl)amino]-3-phenyl-2-propanone (14b). BOCSta *N*-methoxy-*N*-methylamide (0.218 g, 1.0 mmol) was reacted with 2.0 M benzylmagnesium chloride in THF (0.55 mL, 1.1 mmol) by the procedure of Weinreb[12a] to afford 0.138 g (52%) of **14b** as a white solid after flash chromatography (0–10% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$) ^{1}H NMR ($CDCl_3$) 1.35, 1.46 (2 s, 9H), 2.81 (s, 3H), 3.67 (s, 2H), 4.01 (m, 2H), 7.26 (m, 5H).

3-[N-Methyl-N-(*tert*-butoxycarbonyl)amino]-1-(3-methoxyphenyl)-4-phenyl-2-butanone (14c). Mg (1.0 g, 42 mmol), THF (2 mL), and EtI (30 μL), were refluxed for 15 min, and then 3-methoxybenzyl chloride (1.56 g, 10 mmol) in THF (10 mL) was added dropwise over 1 h to the refluxing mixture. Refluxing was continued for 1 h, and the mixture was cooled to 23 °C. The Grignard reagent was immediately reacted with *N*-BOC-*N*-methylPhe *N*-methoxy-*N*-methyamide (1.57 g, 4.88 mmol) by the procedure of Weinreb[12a] to afford 0.80 g (43%) of **14c** as a white solid after flash chromatography (0–20% EtOAc/hexanes). ^{1}H NMR: 1.33, 1.42 (2 s,9H), 2.49, 2.59 (2 s, 3H), 2.86, 3.15 (2 m, 2H), 3.71 (m, 2H), 3.79 (s, 3H), 4.38, 4.73 (2m,1H), 6.73 (m, 2H), 7.18 (m, 7H).

4-[(*tert*-Butyldiphenylsilyl)oxy]-1-butene (16b) was prepared from 3-buten-1-ol (7.1 g, 0.1 mol), imidazole (14.96 g, 0.22 mol), and *tert*-butyldiphenylsilyl chloride in DMF (250 mL) by the procedure of Hannesian[28] and purified by filtration through a silica gel column (hexanes) to afford 29.5 g (95%) of **16b** as an oil. ^{1}H NMR: 1.07 (s, 9H), 2.29 (m, 2H), 3.72 (d, 2H, J = 3.5 Hz), 5.01(m, 2H), 5.65 (m, 1H), 7.3 (m, 6H), 7.77 (m, 4H).

4-[4-[(*tert*-Butyldiphenylsilyl)oxy]butyl]benzyl Alcohol (17). A solution of **16b** (6.84 g, 22 mmol) and THF (10 mL) was cooled to 0 °C, 0.5 M 9-BBN in THF (44 mL, 22 mmol) was added, and the resulting mixture was warmed to 23 °C and stirred for 5 h. To the resulting solution was added 4-bromobenzyl alcohol (3.74 g, 20 mmol), $PdCl_2$·dppf[29] (440 mg, 0.6 mmol), K_2CO_3 (5.52 g, 40 mmol), and DMF (100 mL), and the reaction mixture was heated to 55 °C for 14 h. The reaction mixture was cooled, the DMF was evaporated *in vacuo*, and the residue was dissolved in CH_2Cl_2 (300 mL) and washed with H_2O (2 × 100 mL). The combined H_2O was extracted with additional CH_2Cl_2 (2 × 150-mL). The combined CH_2Cl_2 extracts were washed with H_2O (2 × 100 mL) and saturated aqueous NaCl (50 mL), dried (Na_2SO_4), and purified by flash chromatography (0–5% EtOAC/hexanes) to afford 5.2 g (62%) of **17** as an oil. ^{1}H NMR: 1.04 (s, 9H), 1.65 (m, 4H), 2.60 (t, 2H, J = 7.5 Hz), 3.67 (t, 2H, J = 6.0 Hz), 4.66 (d, 2H, J = 5.8 Hz), 7.2–7.8 (m, 14H). MS (DCI, NH_3) (*m/e*): 418 (M + NH_4^+ – H_2O).

4-[4-[(*tert*-Butyldiphenylsilyl)oxy]butyl]benzyl Chloride (18). A mixture of **17** (1.25 g, 3.0 mmol), $SOCl_2$ (326 μL, 4.5 mmol), pyridine (363 μL, 4.5 mmol), and toluene (10 mL) was combined and heated to 70 °C for 13 h. The dark mixture was partitioned between H_2O (20 mL) and EtOAc (3 × 30 mL). The combined EtOAc was washed with H_2O (2 × 20 mL) and saturated aqueous NaCl (20 mL), dried (Na_2SO_4), concentrated, and purified by flash chromatography (0–2% EtOAc/hexanes) to afford 0.80 g (61%) of **18** as a gum. ^{1}H NMR: 1.05 (s, 9H), 1.65 (m, 4H), 2.59 (t, 2H, J = 7.7 Hz), 3.67 (t, 2H, J = 6.1 Hz), 4.54 (s, 2H), 7.12 (d, 2H, J = 8.0 Hz), 7.27 (d, 2H, J = 8.0 Hz), 7.3 (m, 6H), 7.6 (m, 4H). MS (DCI, isobutane) (*m/e*): 437 (MH^+).

3-[N-Methyl-N-(*tert*-butoxycarbonyl)amino]-1-[4-[1-[(*tert*-butyldiphenylsilyl)oxy]butyl]phenyl]-4-phenyl-2-butanone (14d). A reaction flask containing Mg (200 mg, 8.0 mmol) was flame dried *in vacuo* and cooled to 23 °C. THF (2 mL) and EtI (30 μL) were added, the mixture was refluxed for 15 min, and then **18** (0.80 g, 1.83 mmol) in THF (10 mL) was added to the refluxing THF solution via syringe pump over 3 h. Refluxing was continued for 1 h, and the mixture was cooled to 23 °C. The resulting Grignard reagent was immediately reacted with *N*-BOC-*N*-methylPhe *N*-methoxy-*N*-methylamide (0.65 g, 2.0 mmol) by the method of Weinreb[12a] to afford 0.89 g (73%) of **14d** as a gum after flash chromatography (0–4% EtOAc/hexanes). ^{1}H NMR: 1.04 (s, 9H), 1.32, 1.42 (2 s, 9H), 1.60 (m, 4H), 2.48, 2.58 (2 s, 3H), 2.55 (m, 2H) 2.81, 3.14 (2 m, 2H), 3.65 (m, 4H), 4.35, 4.71 (2 m, 1H), 6.80–7.80 (m, 14H). MS (DCI, NH_3) (*m/e*): 664 (MH^+).

General Procedure for the Preparation of 2-Aminoimidazoles 12 and 15a–d from Boc-Protected Amino Ketones. The BOC amino ketones **10b**, **14a–d** (0.1 mmol) were dissolved in HCl saturated Et_2O (2 mL) and allowed to stand at 23 °C for 2 h. The resulting gel like solids were filtered and washed with Et_2O and dissolved or suspended in H_2O (10 mL). Cyanamide (100 mg, 2.4 mmol) was added, the pH was carefully adjusted to 4.5 with 0.1 and 0.01 N NaOH, and the mixture was heated to 90 °C. The pH was maintained at 4.5 by addition of dilute NaOH or HCl if necessary. Purification was achieved either by reverse phase MPLC (for **15b–d** and **26**), eluting with 50–100% aqueous methanol and 0.05% TFA, or by silica gel flash chromatography (for **12**, **15a**), eluting with CH_2Cl_2 containing 0–4% CH_3OH and 0–0.2% Et_3N.

1-Methyl-2-amino-4,5-bis(phenylmethyl)imidazole (15a). Yield 33%. ^{1}H NMR ($CDCl_3$) (acetone-d_6): 2.98 (s, 3H), 3.74 (s, 2H), 3.80 (s, 2H), 4.02 (br s, 2H), 6.9–7.2 (m, 10H). MS (DCI, NH_3) (*m/e*): 278 (MH^+). Mp: 125–162 °C dec. Anal. ($C_{18}H_{19}N_3 \cdot 1.5H_2O \cdot 0.33CF_3CO_2H$) C, H, N.

1-Methyl-2-amino-4-(phenylmethyl)imidazole (15b). Yield 40%. The product decomposes on standing at 23 °C. Conversion of freshly chromatographed **15b** to the HCl salt by addition of ethereal HCl, concentration, dissolving in H_2O, and lyophilization afforded the more stable, hydroscopic salt. Mp: 59–60 °C. ^{1}H NMR: 3.34 (s, 3H), 3.71 (s, 2H), 5.66 (br s, 2H), 6.00 (s, 1H), 7.27 (m, 5H). MS (DCI, NH_3) (*m/e*): 188 (MH^+). Anal. ($C_{11}H_{13}N_3 \cdot 3.25H_2O \cdot 0.25$ HCl) C, H; N: calcd, 16.48; found, 14.48.

1-Methyl-2-amino-4-[(3′-methoxyphenyl)methyl]-5-(phenylmethyl)imidazole (15c). Yield 40%. Mp: 147–148 °C. ^{1}H NMR ($CDCl_3/CD_3OD$) 3.09 (s, 3H), 3.77 (s, 3H), 3.82 (s, 2H), 3.88 (s, 2H), 6.7–7.4 (m, 9H). MS (DCI, NH_3) (*m/e*): 308 (MH^+). Anal. ($C_{19}H_{21}N_3O \cdot {}^1/_8H_2O \cdot CF_3CO_2H$) C, H, N.

1-Methyl-2-amino-4-[[4′-[4″-[(*tert*-butyldiphenylsilyl)-oxy]butyl]phenyl]methyl]-5-(phenylmethyl)imidazole (15d). Yield 46%. ^{1}H NMR: 1.04 (s, 9H), 1.60 (m, 4H), 2.55 (t, J = 7.5 Hz, 2H), 3.17 (s, 3H), 3.60 (t, J = 5.8 Hz, 2H), 3.83 (s, 2H), 5.31 (br s, 2H), 7.10–7.77 (m, 19H). MS (DCI, NH_3) (*m/e*): 588 (MH^+).

1-Hexyl-2-amino-4,5-bis(phenylmethyl)imidazole (12). Yield 37%. ^{1}H NMR: 0.75 (t, 3H), 0.9–1.4 (m, 8H), 3.37 (t, 2H), 3.73 (s, 2H), 3.78 (s, 2H), 5.7 (very br s, 2H), 6.9–7.3 (m, 10H). MS (DCI, NH_3) (*m/e*): 348 (MH^+). Anal. ($C_{23}H_{29}N_3 \cdot {}^1/_4H_2O$) C, H, N.

1-Methyl-2-amino-4-[[4′-(4″-hydroxybutyl)phenyl]methyl]-5-(phenylmethyl)imidazlole (26). A solution of **15d** (200 mg, 0.34 mmol), THF (4 mL), and 1 M tetrabutylammonium fluoride in THF (1 mL, 1 mmol) was stirred at 23 °C for 1 h. The THF was evaporated, and the residue was injected onto a 15-mm × 250-mm reverse-phase MPLC column in 25% aqueous MeOH containing 0.05% TFA. Elution with 25–100% MeOH containing 0.05% TFA gave 35.6 mg (30%) of **26**. ^{1}H NMR: 1.55 (m, 4H), 2.60 (t, 2H), 3.08 (s, 3H), 3.63 (t, 2H), 3.82 (s, 2H), 3.86 (s, 2H), 6.95–7.4 (m, 9H). MS (DCI, NH_3) (*m/e*): 350 (MH^+). Mp: 170 °C. Anal. ($C_{23}H_{29}N_3O \cdot {}^1/_4H_2O \cdot CF_3CO_2H$) C, H, N.

1-Nitro-2-phenylethane (20). A solution of phenethylamine (2.07 mL, 2.0 g, 16.5 mmol) in $CHCl_3$ (20 mL) was added over a

30-min period to a refluxing solution of *m*-chloroperoxybenzoic acid (28 g, 0.115 mol) in $CHCl_3$ (200 mL). After 3 h the solutionn was cooled, washed 10% aqueous Na_2SO_3 (2 × 25 mL), saturated aqueous $KHCO_3$ (2 × 25 mL), and once with H_2O (25 mL), dried (Na_2SO_4), concentrated, and purified by flash chromatography (hexanes) affording 750 mg (30%) of **20**. ^1H NMR: 3.3 (t, 2H), 4.6 (t, 2H), 7.2 (m, 5H).

3-Nitro-1,4-diphenyl-2-butanol (21). To ice-cold (neat) **20** (750 mg, 5.0 mmol) was added phenylacetaldehyde (0.58 mL, 592 mg, 4.9 mmol). The resulting mixture was stirred for 2 min at 0 °C, and then alumina-supported KF (129 mg, 0.9 mmol) was added. The resulting solution was warmed to 23 °C. After 5 h, CH_2Cl_2 (30 mL) was added, the mixture was filtered, and the filtrate was purified by flash chromatography (5% EtOAc/hexane) to afford 600 mg (44%) of **21**. ^1H NMR: 2.37 and 2.40 (s, 1H), 2.89 (2 dd, 2H), 3.31 (2 dd, 2H), 4.12 (m, 1H), 4.72 (m, 1H), 7.14–7.34 (m, 10 H). MS (DCI, NH_3) (*m/e*): 225 (MH^+).

3-Amino-1,4-diphenyl-2-butanol (22). A mixture of **21** (60 mg, 0.22 mmol), EtOH (5 mL), and PtO_2 (10 mg) was shaken on a Parr hydrogenator at 50 psi of H_2 for 3 h. Filtration, followed by flash chromatography (5% CH_3OH/CH_2Cl_2) afforded 50 mg (94% yield) of **22**. ^1H NMR: 1.95 (br s, 3H), 2.35–3.15 (m, 5H), 3.8 (m, 1H), 7.25 (m, 10H).

2-Amino-4,5-bis(phenylmethyl)imidazole (24). To a solution of **22** (230 mg, 0.95 mmol) in acetone (25 mL) at 0 °C was added Jones reagent (0.9 mL), and the mixture was stirred at 0 °C for 1 h. Excess reagent was destroyed by the addition of 2-propanol (2 mL). Aqueous NaOH (25 mL) was added, and the mixture extracted with CH_2Cl_2. The organic extracts were washed with 5% aqueous NaOH and saturated aqueous NaCl, dried ($MgSO_4$), and concentrated to afford 150 mg of crude **23** which was immediately suspended in H_2O (2 mL), and 1 N HCl (2 mL) was added. The mixture was filtered, and the filtrate was treated with cyanamide (150 mg, 3.57 mmol), adjusted to pH 4.5 by the addition of 0.1 N NaOH, heated at 80–90 °C for 1 h, cooled to 23 °C, poured into 5% aqueous NaOH, and extracted with EtOAc. The organic extract was washed with H_2O and saturated aqueous NaCl, dried (Na_2SO_4), concentrated, and flash chromatographed eluting with 20% CH_3OH/ CH_2Cl_2 to afford 15 mg (7% yield from **22**) of **24**. ^1H NMR: 3.70 (s, 4H), 7.10 (m, 10H). MS (DCI, NH_3) (*m/e*): 264 (MH^+). Anal. ($C_{17}H_{17}N_3 \cdot {}^3/_4H_2O$) C, H, N.

LTB_4 Antagonist Assay Methods. [3H]LTB_4 Binding Assays. [^{3}H]LTB_4 with specific activity of 140–210 Ci/mmol was obtained from New England Nuclear (Boston, MA). Unlabeled LTB_4 was synthesized by the Medicinal Chemistry Prep Group at SmithKline Beecham. U-937 cells were obtained from the American Type Culture Collection and grown in RPMI-1640 media supplemented with 10% (v/v) heat-inactivated fetal calf serum in spinner culture in a humidified environment of 5% CO_2, 95% air at 37 °C. U-937 cells were differentiated with 1.3% DMSO for 3–4 days, grown to a density of 10^6 cells/mL, and then harvested by centrifugation. Cells were washed with 50 mM Tris, pH 7.4, containing 1 mM EDTA (buffer A). Washed cells were utilized for whole-cell binding or for the preparation of membranes. To prepare membranes the washed cells were suspended in buffer A at 5 x 10^7 cells/mL and disrupted by nitrogen cavitation at 750 psi for 10 min at 0 °C. Broken cells were centrifuged at 1000*g* for 10 min, and the supernatant was centrifuged at 50000*g* for 30 min. The pellet was washed twice with buffer A. The membrane pellet was resuspended in 50 mM Tris, pH 7.4, at 3 mg of protein/mL, and aliquots were rapidly frozen and stored at −80 °C. Protein concentration was determined by the Bradford[30] method with bovine serum albumin (BSA) as the standard.

Human peripheral blood from healthy aspirin-free donors was phlebotimized into sterile heparinized syringes. PMNs were isolated by standard Ficoll- Hypaque centrifugation, dextran 70 sedimentation, and hypotonic lysis procedures.[31] Cell preparations were >90% neutrophils and >95% viable.

Test compounds were evaluated for the ability to compete with [^{3}H]LTB_4 for receptors on intact U-937 cells and membranes prepared from these cells utilizing methods described previously.[4,32,33] Equilibrium binding for membrane preparations was performed at 25 °C for 30 min in 50 mM Tris, pH 7.4, containing 10 mM $CaCl_2$, 10 mM $MgCl_2$, 40–50 μg of membrane protein, and 0.2 nM [^{3}H]LTB_4. Total and nonspecific binding of [^{3}H]LTB_4 were determined in the absence and presence of 1 μM unlabeled LTB_4, respectively. For radioligand competition experiments, increasing concentrations of LTB_4 (0.1–10 nM) or test compound (0.1–300 μM) were included. Unbound radioligand and competing compounds were separated from membrane-bound ligand by vacuum filtration through Whatman GF/C filters. Membrane-bound radioactivity was determined by liquid scintillation spectrometry. The percent inhibition of specific [^{3}H]LTB_4 binding was determined for each concentration, and the IC_{50} was defined as the concentration of test compound required to inhibit 50% of the specific [^{3}H]LTB_4 binding. Each concentration response curve for test compounds was run in duplicate, and each compound was tested at five to eight concentrations in at least two assays. Values presented are the mean IC_{50} and K_i values. The K_i value was determined from the IC_{50} value as described by Cheng and Prusoff.[34]

[^{3}H]LTB_4 competition binding experiments with intact U-937 cells was essentially as described.[33] Washed U-937 cells (10^6 cells) were incubated in Hanks balanced salt solution (HBSS) with 0.1% ovalbumin and 0.2 nM [^{3}H]LTB_4 for 20 min at 0 °C in a total volume of 500 μL. All other conditions and procedures were as described for membranes. The precision of measurments can be assessed by standard error presented for compounds that were tested in three or more separate assays.

LTB_4-Induced Calcium Mobilization. The functional assay used to determine agonist/antagonist activity of test compounds was LTB_4-induced Ca^{2+} mobilization in human PMNs.[35] The $[Ca^{2+}]_i$ was estimated with the calcium fluorescent probe fura 2.[36] Isolated PMNs were suspended in Krebs Ringer Hensilet buffer at 2 x 10^6 cells/mL containing 0.1% BSA, 1.1 mM $MgCl_2$, and 5 mM HEPES, pH 7.4 (buffer B). The diacetoxymethoxy ester of fura 2 (fura 2/AM) was added at a concentration of 2 μM and incubated for 45 min at 37°C. Cells were centrifuged at 225*g* for 10 min and resuspended at 2 × 10^6 cells/mL in buffer B and incubated an additional 20 min to allow complete hydrolysis of the entrapped ester. Cells were centrifuged as above and resuspended at 10^6 cells/mL in buffer B containing 1 mM $CaCl_2$ and maintained at room temperature until used in the fluorescent assay which was performed within 3 h.

The fluorescence of fura 2 containing PMNs was measured with a fluorometer designed by the Johnson Foundation Biomedical Instrumentation Group. The fluorometer was equipped with a temperature control and a magnetic stirrer under the cuvette holder. Wavelengths were set at 340 nm (10-nm band width) for excitation and 510 nm (20-nm band width) for emission. All experiments were performed at 37°C with constant stirring. For compound studies fura 2 loaded cells were centrifuged and resuspended in buffer B with 1 mM $CaCl_2$ minus BSA at 10^6 cells/mL, and 2 mL aliquotted into a cuvette and warmed in a water bath at 37 °C for 5 min. The cuvette was transferred to the fluorometer, and fluorescence was recorded for 15 s to ensure a stable baseline before addition of compound, LTB_4,or vehicle. Fluorescence was recorded continuously for up to 3 min after addition of compounds to monitor agonist activity. None of the compounds tested induced calcium mobilization, i.e., they were without agonist activity. For antagonist studies varying concentrations of antagonists were added to the fura 2 loaded cells and monitored for 1 min to ensure that there was no change in baseline fluorescence. LTB_4 (0.1 nM) was then added, and the maximal $[Ca^{2+}]$/fura 2 fluorescence was determined. The $[Ca^{2+}]_i$ was calculated as previously described,[34] using the following formula:

$$[Ca^{2+}]_i = 224\ (\text{nM})\ \frac{F - F_{min}}{F_{max} - F}$$

The percent of maximal LTB_4-induced $[Ca^{2+}]_i$ was determined for each concentration of compound, and the IC_{50} was defined as the concentration of test compound that inhibits 50% of the maximal LTB_4 response. Dose–response curves (five to six concentrations) were run for interesting compounds in three assays. The precision of measurements can be assessed by the standard error reported for the compounds tested.

References

(1) Chan, G. W.; Mong, S.; Hemling, M.; Freyer, A. J.; Offen, P. H.; Debrosse, C. W.; Sarau, H. M.; Wesley, J. W. New Leukotriene B_4 Receptor Antagonist: Leucettamine A And Related Imidazole Alkaloids From The Marine Sponge *Leucetta Microraphis*. *J. Nat. Prod.* **1993**, *56*, 116–121.

(2) (a) Brain, S. D.; Williams, T. J. Leukotrienes and Inflammation. *Pharm. Ther.* **1990**, *46*, 57–66. (b) Sammuelson, B.; Dahlen, S.-E.; Lindgren, J. A.; Rouzer, C. A.; Serhan, C. N. Leukotrienes and Lipoxins: Structure, Biosynthesis and Biological Effects. *Science* **1987**, *237*, 1171–1176. (c) Lee, J. B.; Katayama, S. Prostaglandins, Thromboxanes and Leukotrienes. In *Textbook of Endrocrinology, 7th ed.;* Wilson, J. D.; Foster, D. W. Eds.; W. B. Saunders Co.: Philadelphia, 1987; pp 1345–1362. (d) Bray, M. A. Leukotrienes in Inflammation. *Agents Actions* **1986**, *19*, 87–89. (e) Thorsen, S. Leukotriene B_4, a Mediator of Inflammation? *Scand. J. Rheumatol.* **1986**, *15*, 225–236. (f) Ford-Hutchinson, A. W. Leukotrienes: Their Formation and Role as Inflammatory Mediators. *Fed. Proc.* **1985**, *44*, 25–29.

(3) Kingsbury, W.; Daines, R.; Gleason, J. Leukotriene Receptors. In *Comprehensive Medicinal Chemistry*; Hansch, C.; Sammes, P. G., Taylor, J. B., Emmett, J. C., Eds.; Pergamon Press: New York, 1990; Vol. 3, pp 763–796.

(4) (a) Bomalaski, J. S.; Mong, S. Binding of Leukotriene B_4 and Analogs to Human Polymorphonuclear Leukocyte Membrane Receptors. *Prostaglandins* **1987**, *33*, 855–867. (b) Sarau, H. M.; Mong, S. Co-Expression Of Leukotriene B_4 And Leukotriene D_4 Receptors On Human Monocytic U-937 Cells. *Adv. Prostaglandin Thromboxane Leukotriene Res.* **1989**, *19*, 180–186.

(5) Lancini, G. C.; Lazzari, E. A New Synthesis of Alkyl and Aryl 2-Aminoimidazoles. *J. Heterocycl. Chem.* **1966**, *3*, 152–154.

(6) Greenwald, R.; Chaykovsky, M.; Corey, E. J. The Wittig Reaction Using Methylsulfinyl Carbanion-Dimethyl Sulfoxide. *J. Org. Chem.*, **1963**, *28*, 1128–1129.

(7) Related to the observed reactivity of **7a** with MCPBA, Baeyer–Villiger oxidation of electron-rich aryl ketones is also known to be fraught with difficulties (see ref 8).

(8) Boger, D. L.; Coleman, R. S. Benzylic Hydroperoxide Rearrangement: Observations on a Viable and Convenient Alternative to the Baeyer -Villiger Rearrangement. *J. Org. Chem.* **1986**, *51*, 5436–5439.

(9) Stable *N*-carbobenzyloxy protected α-amino ketones have been prepared by a different route. Deblocking with HBr/HOAc gave the HBr salt without significant decomposition (see ref 10).

(10) Fittkau, S.; Jahreis, G.; Peters, K. α-Aminoketones—A Contribution to the Synthesis of Optically Active Derivatives of Aminoacids and Peptides. *J. Prakt. Chem.* **1986**, *328*, 529–538.

(11) Since the target 2-aminoimidazoles are achiral, the α-amino acid precursors may be racemic.

(12) (a) Nahm, S.; Weinreb, S. M. N-Methoxy-N-Methylamides as Effective Acylating Agents. *Tetrahedron Lett.* **1981**, *22*, 3815–3818. (b) Cupps, T. L.; Boutin, R. H.; Rapoport, H. α-Amino Acids as Chiral Adducts for Asymmetric Products. The Synthesis of α'-Amino-α,β-ynones. *J. Org. Chem.* **1985**, *50*, 3972–3979. (c) Boutin, R. H.; Rapoport, H. α-Amino Acid Derivatives as Chiral Educts for Asymmetric Products. Synthesis of Sphingosine from α'-Amino-α,β-ynones. *J. Org. Chem.* **1986**, *51*, 5320–5327. (d) Yuan, W.; Munoz, B.; Wong, C.-H.; Haeggestrom, J. Z.; Wetterholm, A.; Samuelsson, B. Development of Selective Tight Binding Inhibitors of Leukotriene A_4 Hydrolase. *J. Med. Chem.* **1993**, *36*, 211–220. (e) Goel, O. P.; Krolls, U.; Keston, S. N-tert-Butoxycarbonyl-L-Leucinal. *Org. Synth.* **1988**, *67*, 69–75.

(13) Miyaura, N.; Ishiyama, T.; Sasaki, H.; Isikawa, M.; Satoh, M.; Suzuki, A. Palladium-Catalyzed Inter- and Intramolecular Cross-Coupling Reactions of B-Alkyl-9-borobicyclo[3.3.1]nonane Derivatives with 1-Halo-1-alkenes or Haloarenes. Syntheses of Functionalized Alkenes, Arenes, and Cycloalkenes via a Hydroboration-Coupling Sequence. *J. Am. Chem. Soc.* **1989**, *111*, 314–321.

(14) 1-Nitro-2-phenylethane, **20**, was easily prepared by oxidation of phenethylamine with *m*-chloroperoxybenzoic acid. This procedure is shorter and more convenient than that reported previously (see ref 16).

(15) Azipurua, J. M.; Olabide, M.; Palomo, C. Reduction of α,β-unsaturated nitro compounds with Tributyltin Hydride. *Tetrahedron Lett.* **1987**, *28*, 5365–5366.

(16) Melot, J. M.; Texier-Boullet, F.; Foucaud, A. Preparation and Oxidation of α-Nitroalcohols with Supported Reagents. *Tetrahedron Lett.* **1986**, *27*, 493–496.

(17) Initial *in vitro* studies utilized human PMNs from freshly drawn peripheral blood. However, the [^{3}H]LTB_4 binding results described herein were obtained using intact human U-937 cells, a human monocyte/macrophage cell line. This was done because of the biohazard associated with human PMN preparations. Since the LTB_4 receptor on U-937 cells has been demonstrated to be identical to the receptor on PMNs,[4] the U-937 cells provide a safer, more stable assay. The binding affinities of the active compounds (K_i < 50 μm) were always confirmed using intact human PMNs, and in each case both cellular assays gave comparable results.

(18) Mong, S.; Votta, B.; Sarau, H. M.; Foley, J. J.; Schmidt, D.; Carte, B. K.; Poehland, B.; Westley, J. 15-Acetoxy-Furodysinin Lactone, Isolated From A Marine Sponge *Dysidea*, Sp. Is A Potent Agonist To Human Leukotriene B_4 Receptor. *J. Prostaglandins* **1990**, *39*, 89–97.

(19) (a) Kingsbury, W. D.; Pendrak, I.; Leber, J. D.; Boehm, J. C.; Mallet, B.; Sarau, H. M.; Foley, J. L.; Schmidt, D. B.; Daines, R. A. Synthesis Of Structural Analogs Of Leukotriene B_4 And Their Receptor Binding Activity. *J. Med. Chem.*, accompanying paper in this issue. (b) Daines, R. A., Chambers, P. A.; Pendrak, I. P.; Jakas, D. J.; Sarau, H. M.; Foley, J. J.; Schmidt, D. B.; Kingsbury, W. D. Trisubstituted Pyridine Leukotriene B_4 Receptor Antagonists. Synthesis And Structure - Activity Relationships. *J. Med. Chem.*, accompanying paper in this issue.

(20) Lane, C. F.; Myatt, H. L.; Daniels, J.; Hopps, H. Organic Synthesis Using Borane - Methyl Sulfide II. Reduction of Aromatic Carboxylic Acids in the Presence of Trimethyl Borate. *J. Org. Chem.* **1974**, *39*, 3052–3054.

(21) The use of BH_3·DMS permitted the reduction of **3** to be run in a relatively concentrated solution compared to BH_3·THF. This was an advantage for the large-scale reduction.

(22) Corey, E. J.; Suggs, J. W. Pyridinium Chlorochromate. An Efficient Reagent for Oxidation of Primary and Secondary Alcohols to Carbonyl Compounds. *Tetrahedron Lett.* **1975**, *16*, 2647–2650.

(23) The PCC oxidation of **4** gave **5** which was isolated by direct chromatography of the crude reaction without an aqueous workup. A higher yield of **6** was achieved by oxidation with SO_3/pyridine/DMSO (71%). (See ref 24.) Unfortunately the aldehyde obtained in this way failed to undergo a successful Wittig reaction. We attribute this to the hydration of the aldehyde during the aqueous workup.

(24) Parikh, J. R.; von E. Doering, W. Sulfur Trioxide in the Oxidation of Alcohols by Dimethyl Sulfoxide. *J. Am. Chem. Soc.* **1967**, *89*, 5505–5507.

(25) Kocienski, P. J.; Cernigliaro, G.; Feldstein, G. A Synthesis of (+−)-Methyl n-Tetradeca-*trans*-2,4,5-trienoate, an Allenic Ester Produced by the Male Dried Bean Beetle Acanthoscelides obtectus (Say). *J. Org. Chem.* **1977**, *42*, 353–355.

(26) Guindon, Y.; Zamboni, R.; Lau, C.-K.; Rokach, J. Stereospecific Synthesis of Leukotriene B_4 (LTB_4). *Tetrahedron Lett.* **1982**, *23*, 739–742.

(27) Corey, E. J.; Schmidt, G. Useful Procedures for Oxidation of Alcohols Involving Pyridinium Dichromate in Aprotic Media. *Tetrahedron Lett.* **1979**, *20*, 399–402.

(28) Hanessian, S.; Lavallee, P. The Preparation and Synthetic Utility of tert-Butyldiphenylsilyl Ethers. *Can. J. Chem.* **1975**, *63*, 2975–2977.

(29) Hayashi, T.; Konishi, M.; Kobou, Y.; Kumada, M.; Higuchi, T.; Hirotsu, K. Dichloro[1,1′-bis(diphenylphosphino)ferocene]-palladium(II): An Effective Catalyst for Cross-Coupling of Secondary and Primary Alkyl Grignard and Alkylzinc Reagents with Organic Halides. *J. Am. Chem. Soc.* **1984**, *106*, 158–163.

(30) Bradford, M. A Rapid And Sensitive Method For The Quantitation Of Microgram Quantities Of Protein Utilizing The Principle Of Protein Dye Binding. *Anal. Biochem.* **1976**, *72*, 248–254.

(31) Boyum, A. Isolation Of Mononuclear Cells And Granulocytes From Human Blood. *Scan. J. Clin. Lab Invest.* **1968**, *21* (Supp 97), 77–89.

(32) Winkler, J. D., Sarau, H. M.; Foley, J. J.; Mong, S.; Crooke, S. Leukotriene B_4- Induced Homologous Desensitization Of Calcium Mobilization And Phosphoinositide Metabolism In U-937 Cells. *J. Pharm. Exp. Ther.* **1988**, *246*, 204–210.

(33) Goldman, D. W.; Goetzl, E. J. Specific Binding Of Leukotriene B_4 To Receptors On Human Polymorphonuclear Leukocytes. *J Immunol.* **1982**, *129*, 1600–1600.

(34) Cheng, Y.-C.; Prusoff, W. H. Relationship Between The Inhibition Constant (K_i) And The Concentration Of Inhibitor Which Cause 50 Percent Inhibition (Ic_{50}) Of An Enzymatic Reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.

(35) Goldman, D. W.; Gifford, L. A.; Olson, D. M.; Goetzl, E. J. Transduction By Leukotriene B_4 Receptors Of Increases In Cytosolic Calcium In Human Polymorphonuclear Leukocytes *J. Immunol.* **1985**, *135*, 525–530.

(36) Grynkiewicz, G.; Poenie, M.; Tsein, R. Y. A New Generation Of Ca^{2+} Indicators With Greatly Improved Fluorescence Properties. *J. Biol. Chem.* **1985**, *260*, 3440–3450.