# (E)-3-[[[[6-(2-Carboxyethenyl)-5-[[8-(4-methoxyphenyl)octyl]oxy]-2-pyridinyl]-methyl]thio]methyl]benzoic Acid: A Novel High-Affinity Leukotriene $B_4$ Receptor Antagonist

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Leukotriene $B_4$ (I, $LTB_4$; Chart I), a product of 5-lipoxygenase-catalyzed oxygenation of arachidonic acid, has been postulated to be a mediator of a variety of inflammatory diseases.[1,2] The pharmacological effects of extracellular $LTB_4$ are mediated through its interaction with specific cell surface receptors. These receptors are widely distributed and have been characterized on PMNs, monocytes, lymphocytes, mast cells, smooth muscle cells, and endothelial cells.[3] Therefore, the availability of potent and selective $LTB_4$ receptor antagonists should prove useful in elucidating the role of $LTB_4$ in a variety of human inflammatory diseases. We report herein the synthesis and biological activity of a novel, potent $LTB_4$ receptor antagonist.

In recent years, the efforts of several labs have been directed toward the identification of selective $LTB_4$ receptor antagonists. Eli Lilly has reported LY 223982 (1) as having an $IC_{50}$ of 12 nM in binding studies using human PMNs.[4] Ono reported that ONO-4057 (2) has a $K_i$ of 4 nM for $LTB_4$ receptors on human PMNs[5] and Searle reported that SC 41930 (3) exhibits an $IC_{50}$ of 300 nM for $LTB_4$ receptors on human PMNs.[6] More recently, Rhone-Poulenc Rorer reported that RG 14893 (4) has a $K_i$ of 2 nM on human PMNs.[7] In this communication we report that (E)-3-[[[[6-(2-carboxyethenyl)-5-[[8-(4-methoxyphenyl)octyl]oxy]-2-pyridinyl]methyl]thio]methyl]-benzoic acid (5, SB 201993) is a novel, competitive $LTB_4$ receptor antagonist with a $K_i$ of 7.1 nM on human PMNs.

The $LTB_4$ receptor antagonist program was initiated with the objective to discover novel anti-inflammatory agents. The program quickly produced compounds with high receptor affinity; however, the initial compounds exhibited partial agonist activity. In the course of this work, the trisubstituted pyridine 6 was identified as a $LTB_4$ receptor antagonist with a $K_i$ of 280 nM for the human PMN receptor. However, the potency of this compound was not considered adequate for effective *in vivo* anti-inflammatory activity. In order to improve the receptor binding affinity within this series, a structure-activity investigation was undertaken that led to the discovery of 5. The SAR results indicated that (a) both carboxylic acid groups were required for maximum potency, (b) a lipid tail was essential, and (c) the spacing between the two carboxylic acids was critical, as was the relationship between the benzoic acid moiety and the pyridine ring.[8] In contrast to the findings of others, the SAR indicated that the methoxy substituent on the phenyl ring of the tail did not play a major role in the *in vitro* binding affinity.[4] In addition, there was considerable latitude in the requirements for the length of the lipid tail.

The synthetic route used in the preparation of 5 is shown in Schemes I–III. The lipid tail fragment 11 and the mercaptan head group 14 were prepared according to Schemes I and II, respectively. Commercially available 3-octyn-1-ol (7) was rearranged to the terminal alkyne using potassium aminopropylamide (KAPA).[9] Protection of the alcohol as the silyl ether and palladium-catalyzed coupling with 4-iodoanisole provided alkyne 9.[10] Reduction of the triple bond, fluoride-induced liberation of the primary alcohol, and conversion to the primary iodide provided fragment 11. Reaction of 3-bromomethylbenzoate (12, Scheme II) with thiourea provided the isothiuronium salt 13. Hydrolysis of 13 followed by re-esterification afforded the benzyl mercaptan 14.

The synthesis of 5 is shown in Scheme III. 2,6-Lutidine-$\alpha^2$,3-diol (15) was oxidized with $MnO_2$ and the resulting hydroxy aldehyde 16 was alkylated with iodide 11, providing aldehyde 17. Reaction of 17 with methyl (triphenylphosphoranylidene)acetate afforded acrylate 18, which was converted to pyridine *N*-oxide 19 (MCPBA) followed by TFAA rearrangement to give hydroxymethyl pyridine 20.[11] Treatment of 20 with $SOCl_2$ provided the chloromethyl pyridine 21 as a hydrochloride salt. The synthesis was completed by reaction of chloride 21 with mercaptan 14, followed by ester hydrolysis to provide diacid 5 as a white crystalline solid.[12] The synthesis of 5 proceeds in 13 linear steps in 25% overall yield starting from 3-octyn-1-ol (7).

The $LTB_4$ receptor affinity of 5 was determined by evaluating its ability to compete with the binding of [$^3$H]-$LTB_4$ to receptors on intact human PMNs.[13] Compound 5 was found to competitively antagonize the binding of 0.2 nM [$^3$H]$LTB_4$ to the receptor with a $K_i$ of 7.1 ± 1.1 nM (mean ± SEM for five experiments). In addition, 5 was evaluated in a human PMN functional assay where the effect of 5 on $LTB_4$-induced calcium mobilization was determined.[14] This assay provides information concerning the potency of the compound and also assesses its agonist/antagonist activity. The $IC_{50}$ for 5 in this assay, using 1 nM $LTB_4$, was determined to be 131 ± 23 nM (mean ± SEM for six experiments) with no demonstration of agonist activity up to 10 μM. Furthermore, $LTB_4$ induces degranulation of human PMNs by activation of low-affinity receptors (i.e., receptors requiring higher concentration of $LTB_4$ for a response). Neutrophil degranulation was measured by quantitating the release of myeloperoxidase (MPO) induced by 100 nM $LTB_4$.[15] Compound 5 inhibited $LTB_4$-induced degranulation in a concentration-dependent manner with an $IC_{50}$ of 268 ± 14 nM (mean ± SEM for four experiments).

In order to demonstrate oral $LTB_4$ receptor antagonist activity, 5 was evaluated in the $LTB_4$-induced peritonitis assay in the mouse.[16] In this assay, 5 produced dose-related inhibition of $LTB_4$-induced (250 ng/mL) PMN infiltration with an $ED_{50}$ of 7.1 mg/kg, po. The ability of 5 to act as

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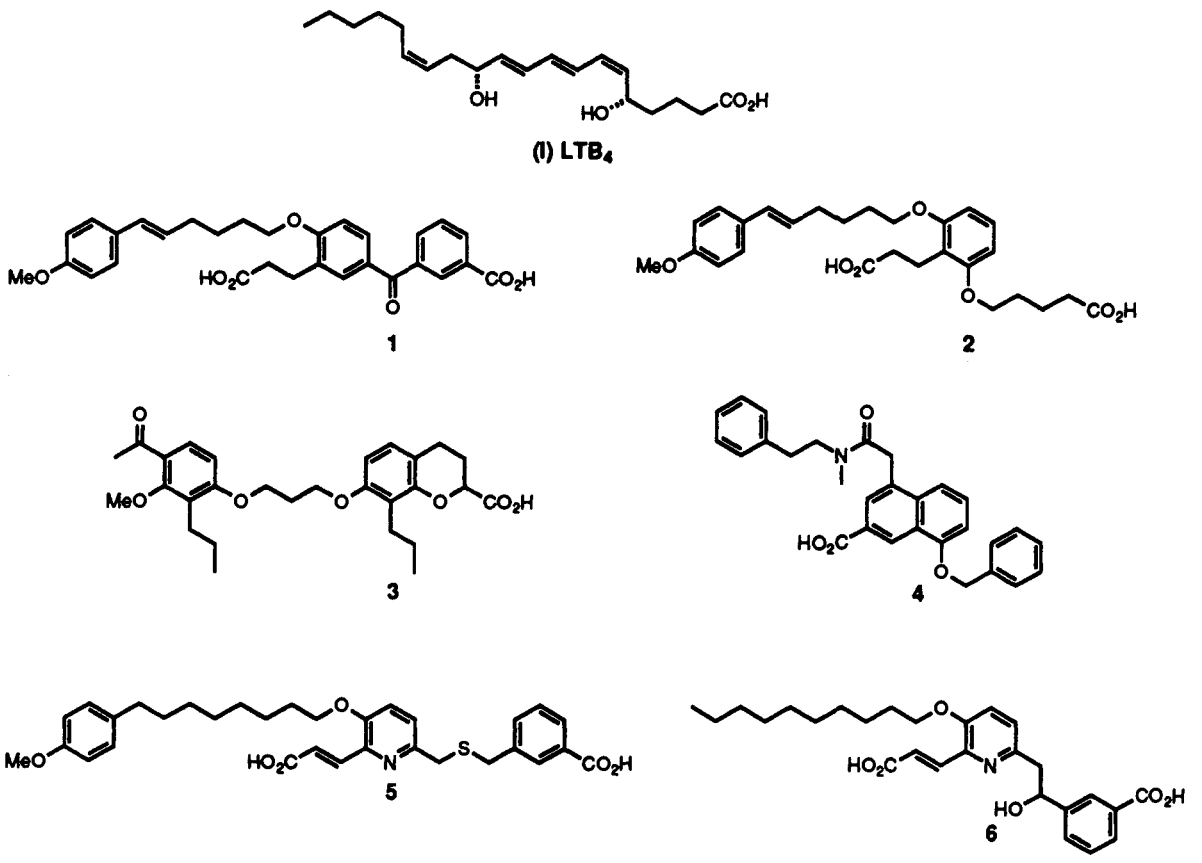
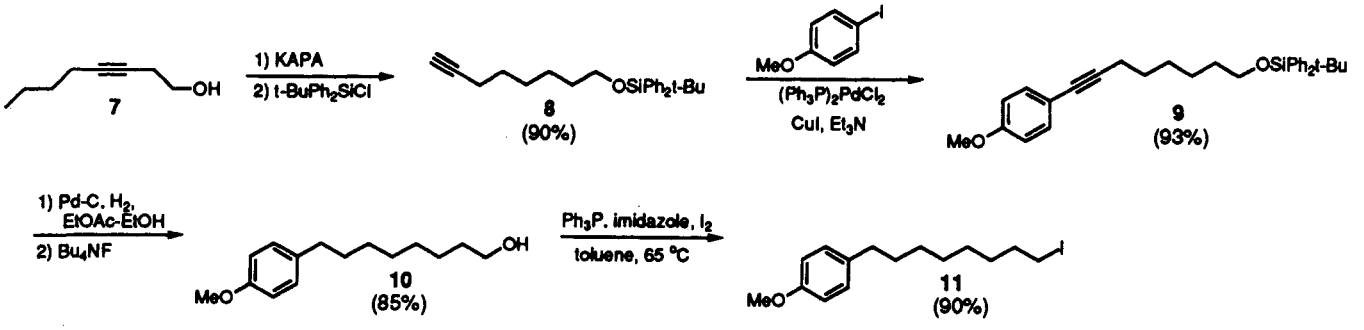
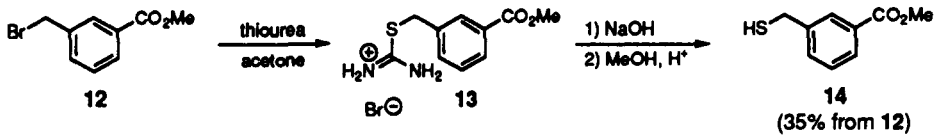
**Chart I**




**Scheme I.** Synthesis of the Lipid Tail



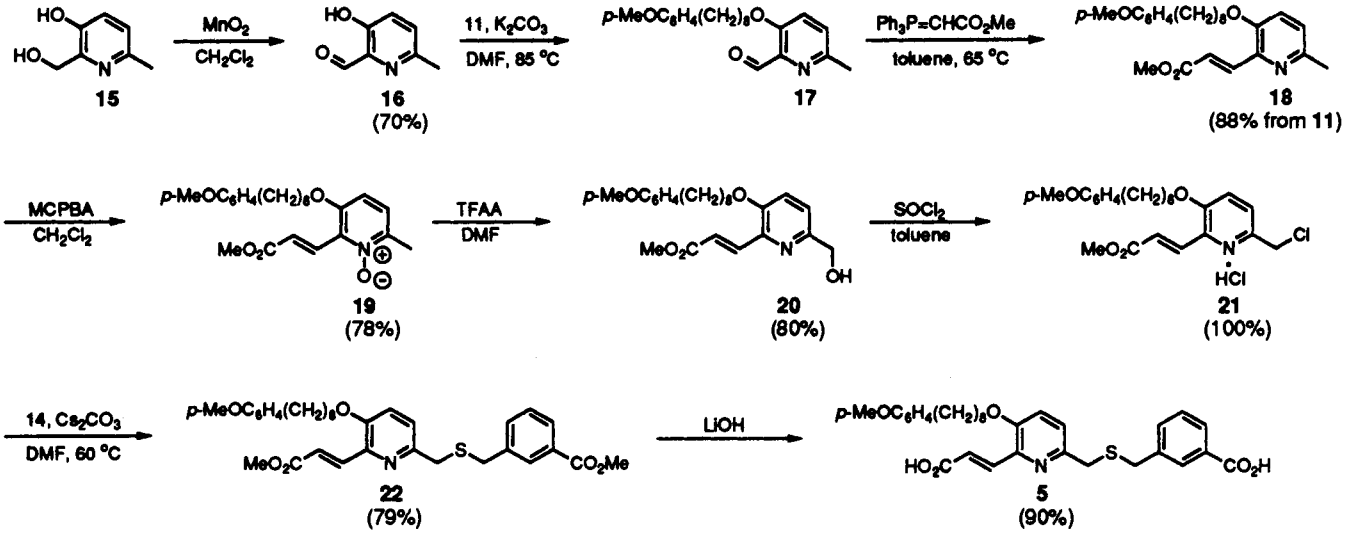

**Scheme II.** Synthesis of the Benzyl Mercaptan




a topical anti-inflammatory agent was also evaluated in the mouse. The topical application of $LTB_4$ to a mouse ear results in an infiltration of PMNs as indicated by an increase in MPO levels. Topical application of compound 5 (1 mg/ear) to the mouse ear, prior to treatment with $LTB_4$, resulted in a 92% reduction of PMN infiltration. A similar model using arachidonic acid (AA) applied to the mouse ear was also utilized. AA-induced inflammation involves both the fluid and the cellular phases of the inflammatory response.[17] Topical application of 5 immediately after administration of 1 mg of AA produced $ED_{50}$ values of 0.39 mg/ear for neutrophil infiltration and 0.58 mg/ear for the edematous response. In addition to demonstrating dose-related anti-inflammatory activity in this model, a single dose (1 mg/ear) of compound 5, applied topically, displayed significant inhibitory activity 24 h following application of arachidonic acid.

$LTB_4$ is proposed to be an important mediator in many inflammatory disease states. However, its specific role in human inflammatory diseases has yet to be established and only a few potent $LTB_4$ receptor antagonists have been reported in the literature. In the present study, diacid 5 was shown to be a potent $LTB_4$ receptor antagonist both *in vitro* and *in vivo* and as such will be an important tool in elucidating the role of $LTB_4$ in human diseases. Details of the pharmacology and structure–activity relationships that led to the discovery of this novel compound will be described in future publications.

**Scheme III.** Synthesis of Compound 5

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(12) mp 113–114 °C; $^1$H NMR (250 MHz, $CDCl_3$) $\delta$ 8.23 (d, $J$ = 15.7 Hz, 1H, vinyl), 8.11 (s, 1H, aryl), 7.99 (d, $J$ = 7.6 Hz, 1H, aryl), 7.68 (d, $J$ = 7.6 H, 1H, aryl), 7.42 (m, 2H, aryl), 7.25 (d, $J$ = 15.7 Hz, 1H, vinyl), 7.20 (d, $J$ = 9 Hz, 1H, aryl), 7.08 (d, $J$ = 8.5 Hz, 2H, aryl), 6.78 (d, $J$ = 8.5 Hz, 2H, aryl), 4.01 (t, $J$ = 6.5 Hz, 2H, $OCH_2$), 3.76 (s, 3H, $OCH_3$), 3.55 and 3.51 (singlets, 4H total, $-CH_2SCH_2-$), 2.55 (t, $J$ = 7.6 Hz, 2H, benzylic), 1.85 (m, 2H, $CH_2$), 1.59 (m, 2H, $CH_2$), 1.52 (m, 2H, $CH_2$), 1.38 (m, 6H, aliphatic): HRFAB-MS: $m/z$ 564.2410 (M + H).

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