Total Synthesis of Photoactivatable Peptidoleukotriene Analogs. The Description of 7(*Z*),9(*E*)- and 7(*E*),9(*E*)-Leukotriene D_2 Aryldiazonium Derivatives as Potential Photoaffinity Probes for the Leukotriene C_4 Receptor

Philippe Klotz, Bernard Foucaud, Maurice Ph. Goeldner,* and Christian G. Hirth†

Laboratoire de Chimie Bio-organique, Faculté de Pharmacie, Université Louis Pasteur Strasbourg, B.P. 24, 67401 Illkirch Cédex, France

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The synthesis of novel photoactivatable 7*Z*,9*E* and 7*E*,9*E* dienic peptidoleukotriene derivatives has been investigated in order to design original and chemically stable photoaffinity probes for the peptidoleukotriene receptors. This new series was attained through Wittig condensation between the appropriate trans-unsaturated phosphonium ylide **8** and a previously described chiral epoxy aldehyde **7**. Nucleophilic opening of the obtained dienic epoxy esters **3a** and **3b** using a protected cysteinylglycine dipeptide **4** afforded after deprotection the dienic LTD_4 analogs **6a** and **6b**. Aryldiazonium salts **1a** and **1b** were directly obtained by reaction of the peptide N-terminal with a heterobifunctional reagent **5** containing a protected diazonium function. These salts were ultimately converted to the corresponding arylazido derivatives **2a** and **2b**. A photochemical study of these new compounds showed that only the diazonium derivatives can be selectively photoactivated at wavelengths beyond 300 nm; photoactivation of the azides is accompanied by excitation of the dienic chromophore. Preliminary binding studies revealed interesting properties for the diazonium derivatives, including a good selectivity for the LTC_4 receptor.

Introduction

Photoaffinity labeling has become an attractive methodology to characterize and identify unknown functional receptors,[1,2] allowing determination of their primary structure through appropriate cDNAs cloning and sequencing.[3] This strategy requires the knowledge of a partial amino acid sequence obtained from the irreversibly labeled receptor. Ultimately, a topographical study of a receptor binding site can be undertaken by identifying the labeled amino acid residues.[4,5]

The molecular characterization of the different leukotriene receptors and of leukotriene-metabolizing enzymes (Scheme I) should bring an interesting insight to the understanding of their biological actions.[6] This goal can theoretically be achieved through photoaffinity labeling experiments. This process requires the synthesis of a photosensitive ligand analog whose photoactivation gives rise to a reactive species producing the irreversible alkylation of the target binding site. The presence of the conjugated polyene in the leukotriene series presents additional challenges due to chemical as well as photochemical unstability. In fact, the leukotriene derivatives could be considered as photoactivatable probes per se. Direct irradiation of the trienic chromophore (λ_{max} = 270 nm) of LTE_4 around 300 nm[7,8] allowed covalent coupling to several LTE_4 binding proteins. However, this clever approach required a cryofixation of the receptor–ligand complex in liquid nitrogen, suggesting that diffusion of the reactive species from the binding site is too fast during photoactivation achieved in standard conditions. A more classical photoactivatable LTC_4 derivative incorporating a radioactive bifunctional arylazido reagent coupled to the LTC_4 tripeptide moiety has also been described.[9] This photosensitive probe has not been characterized chemically, and consequently the corresponding pharmacological evaluation could not be performed. In particular the coupling of an additional substituent to the glutathione moiety might alter the binding characteristics of the original ligand. Furthermore, isomerization and degradation processes of the polyenic structure interfere with the azido excitation of this probe at the reported irradiation wavelength (254 nm).

The ideal photoactivatable peptidoleukotriene probe should have an increased chemical stability, and photoactivation should occur at wavelengths beyond both the trienic as well as the protein chromophores (above 300 nm). Finally, the chemical modification introduced during the synthesis of the photosensitive moiety should alter the original binding characteristics as little as possible.

We report here the synthesis as well as preliminary binding studies of new, optically active, aryldiazonium- (**1a**, **1b**) and arylazido-substituted (**2a**, **2b**) dienic LTD_4

† Deceased May 28, 1992.

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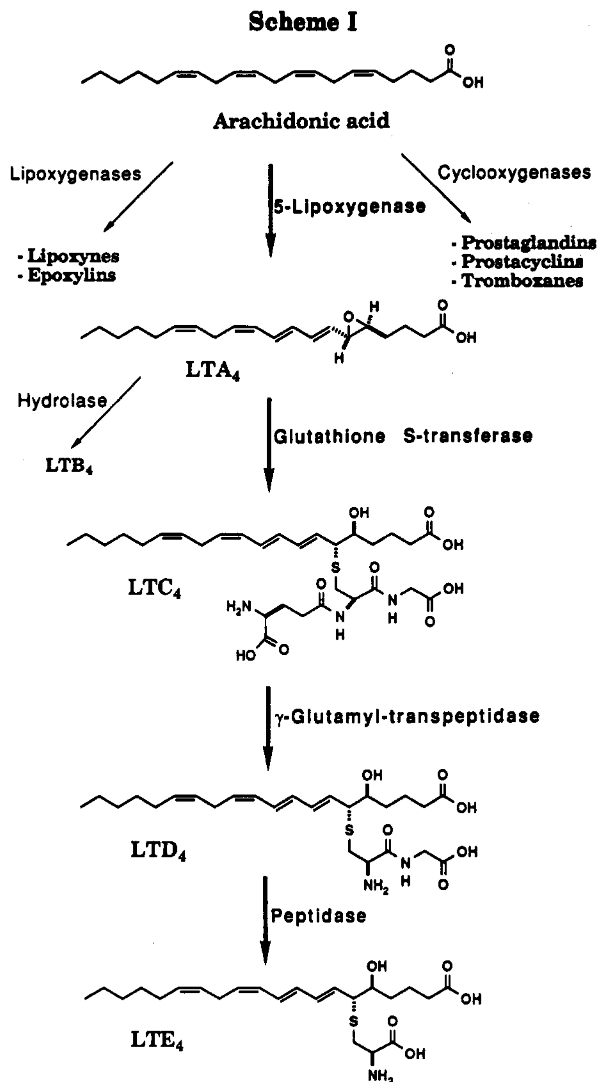
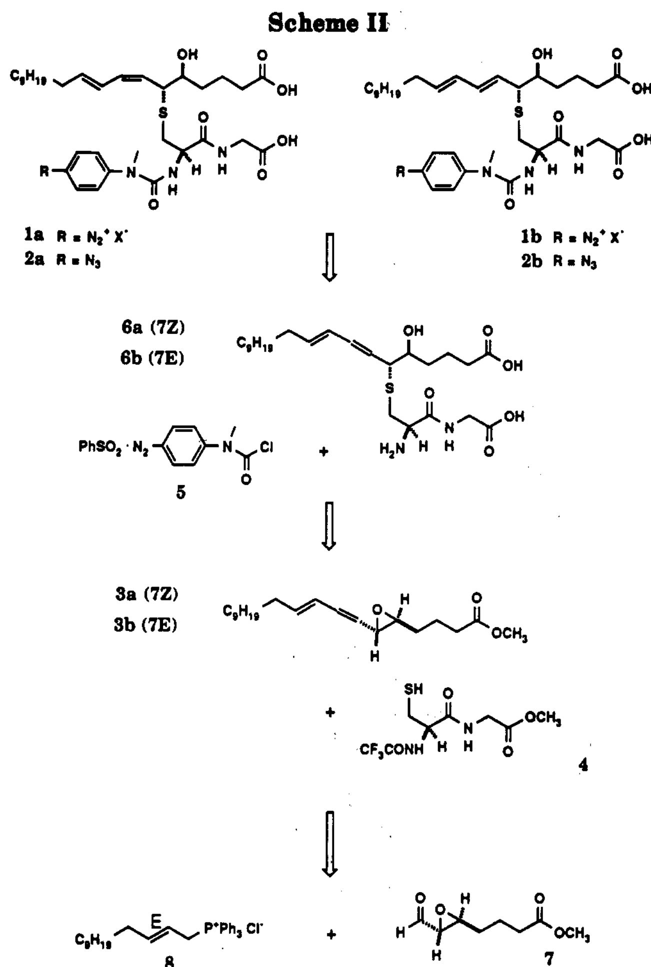
Scheme I



Scheme II



analogs. The choice of the 7Z,9E- and the 7E,9E-dienic moieties of our probes, corresponding, respectively, to the **a** and **b** series, results from the search for leukotriene analogs with increased chemical stability. The choice of aryldiazonium derivatives results from their photochemical properties, including generation of highly reactive aryl cations with a high quantum yield.[10-12] In addition, when adequately substituted, aryldiazonium species absorb strongly above 300 nm[12] and can be eventually photoactivated through an energy transfer[13] reaction leading to an increase in specificity and efficacy of the labeling reaction. Finally, the aryldiazonium function is commonly used as precursor to arylazido derivatives, which constitute alternative photoactivatable probes.

Results

Retrosynthetic Analysis. Our convergent synthetic strategy is summarized in Scheme II and is based upon the sequential coupling of the dienic epoxides **3a** and **3b**, the cysteinylglycine dipeptide **4**,[14] and the heterobifunctional reagent **5**.[15] Coupling of the dipeptide molecule to both compounds **3a** and **3b** provided the corresponding LTD_4 analogs **6a** and **6b**. The subsequent coupling of the bifunctional derivative **5** containing a protected aryldiazonium group gave access to the corresponding photoactivatable derivatives **1** and **2**.

Synthesis of the Methyl Ester of Dienic LTA_4 Analogs: Compounds 3a and 3b (Scheme III). The most convenient route to these analogs uses a Wittig condensation between Rokach's optically active epoxy aldehyde **7**[16] and the 2(E)-tridecenyltriphenylphosphonium ylide derived from **8**. The requisite phosphonium salt was synthesized in three steps: addition of the decylmagnesium bromide to acrolein followed by chlorination of the obtained allylic alcohol **10** led to a mixture of allylic chlorides **11** and **12**. Treatment of this mixture with triphenylphosphine gave exclusively the desired phosphonium salt in 59% overall yield (Scheme III). Rokach's epoxy aldehyde **7** was synthesized according to the described procedure.[16,17] The Wittig condensation of the phosphonium ylide to this aldehyde afforded a 38% yield of a mixture of the 7Z,9E- and the 7E,9E-dienes (**3a** and **3b**) in a 3/1 ratio. Attempts to increase the proportion of the trans,trans isomer (the dienic LTA_4 analog) or to isomerize the cis double bond to the corresponding trans

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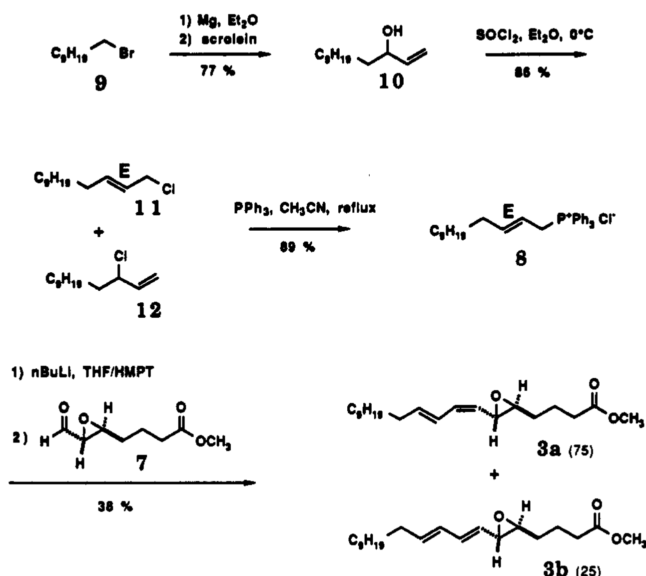
Scheme III



(**3a** into **3b**) failed in our hands. These chemicals were used as a mixture for the following reaction.

Synthesis of Dienic LTD_4 Analogs 6 and Their Photoactivatable Derivatives 1 and 2. The condensation of the protected dipeptide cysteinylglycine **4** to the mixture of dienic epoxydes (**3a** and **3b**) according to Corey's procedure[18] resulted in regio- and stereoselective opening of both epoxide isomers at the 6-position with excellent yield (87%) (Scheme IV). Simultaneous deprotection of both the methyl ester and the trifluoroacetamido group was achieved in gentle basic conditions, affording after precipitation at controlled pH a 85% yield of a 3:1 mixture of **6a** and **6b**. These isomers could be separated by HPLC and chemically characterized, allowing further biological evaluations. Both dienic compounds represent new derivatives in the peptidoleukotriene series.

The synthesis of the photoactivatable derivatives was achieved on the mixture of isomers **6a** and **6b** through use of the heterobifunctional reagent **5**. Coupling of the peptidic amino group to the chlorocarbamate moiety of the bifunctional reagent (Scheme IV) was followed by hydrolytic deprotection of the azosulfonamide to afford 69% of the diazonium salt. Although the benzenesulfinate counterion is typically exchanged for a less nucleophilic chloride anion on a Dowex resin, this method was not successful in the present case; Presumably, the presence of two carboxylate residues on both compounds **1a** and **1b** prevented their elution from this polymer. Anion exchange of sulfinate for trifluoroacetate could be achieved on a reversed-phase HPLC column, but this conversion was unfortunately incomplete. Consequently, only the isomer eluting first, the major isomer (**1a**), could be totally purified and characterized, while the later-eluting isomer (**1b**) was necessarily contaminated by **1a**. The conversion of the diazonium function to the corresponding azido was achieved on the mixture of the diazonium derivatives through treatment with sodium azide in buffered medium. The obtained azido analogs **2a** and **2b** were separated by reversed-phase HPLC and characterized.

Physicochemical Properties of the Photoactivatable Derivatives. The UV absorption spectra of the newly synthesized derivatives as well as their photodecomposition spectra are given in Figure 1. An unambiguous assignment of the different chromophores can be formulated. Absorption at 235 nm is attributed to the dienic moiety as can be seen for compounds **6a** or **6b** (Figure 1A); this chromophore overlaps substantially with the azido moiety in **2a** or **2b** (Figure 1B: λ_{max} = 245 and 243, respectively, for **2a** and **2b**). The UV spectrum of diazonium salts **1a** or **1b** shows both the dienic (λ_{max} = 235 nm) and the aromatic diazonium (λ_{max} = 360 nm) chromophores without any overlap (Figure 1C).

We also tested the chemical stability of these compounds toward buffered medium in the absence of light. As expected, the dienic LTD4 analogs **6a** or **6b** as well as the corresponding azido derivatives **2a** and **2b** are fully stable. However, the chemical stability of aryldiazonium derivatives **1a** and **1b** is very dependent on the nature and position of the aromatic substituent.[19] Para-substitution by an urea derivative[20] confers a sufficient stability for their use as photoaffinity labels; a half-life of 16 h at pH 7.4 was measured.

Preliminary Biological Evaluation of the Dienic Peptidoleukotriene Analogs. Table I summarizes the equilibrium binding characteristics of the newly synthesized dienic peptidoleukotriene analogs on lung guinea pig membranes. This biological material is suitable for binding studies on both LTC_4 and LTD_4 receptors.[21,22] The IC_{50} values have been obtained through displacement of [^{3}H] LTC_4 and [^{3}H] LTD_4 by the different ligands on these membranes, and the corresponding inhibition constant (K_i) was subsequently calculated using the Cheng–Prusoff equation.[23] It must be emphasized that the reported values are derived from single experiments.

Discussion

The natural leukotrienes are, as a general rule, rather unstable molecules which are easily isomerized and also subject to oxidation reactions. It is therefore important to synthesize chemically stabilized analogs for which the biological properties remain comparable to those of the natural products to facilitate pharmacological evaluation and exploration of structure function relationship. Simplification of the tetraene bonds to a diene represented a useful compromise between gain of stability versus loss of biological properties. This assumption was based on previous synthetic and pharmacological studies on partially reduced peptidoleukotriene derivatives.[24-26] The synthesis of the dienic 7*Z*,9*E*- and 7*E*,9*E*-LTA_2 molecules **3a** and **3b** was therefore undertaken allowing at the same time the conception of original analogs. An established synthetic strategy was used, i.e., a Wittig condensation on the optically active epoxy aldehyde **7** with the appropriate

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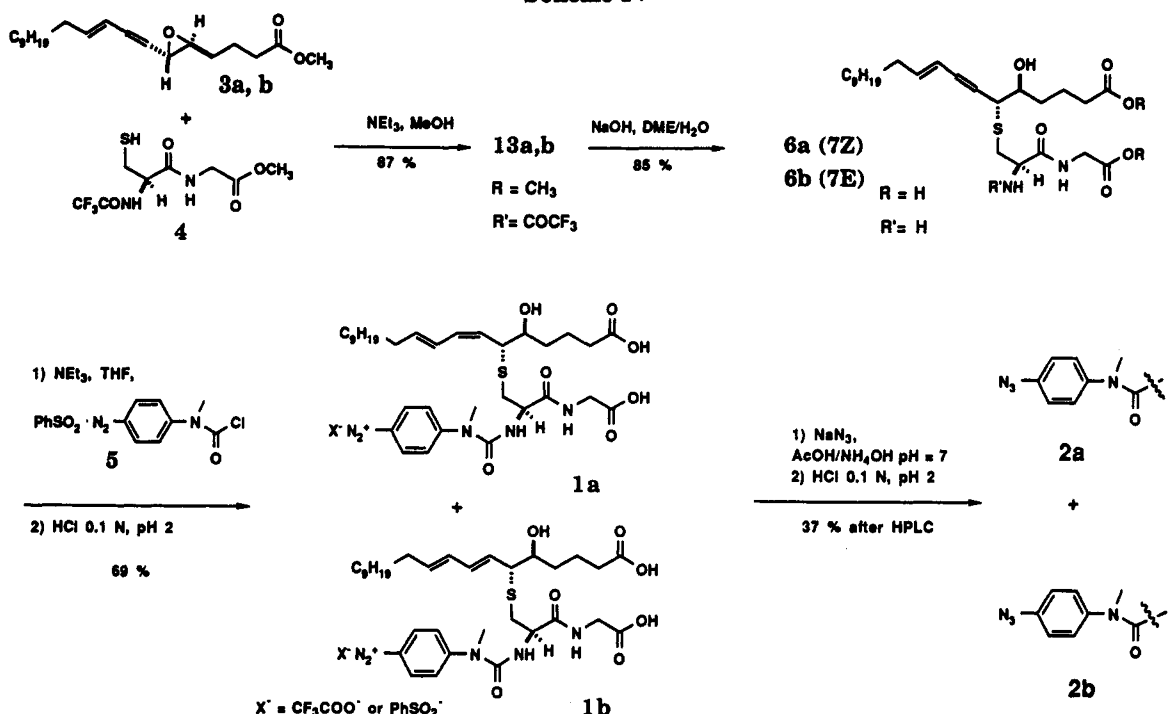
Scheme IV



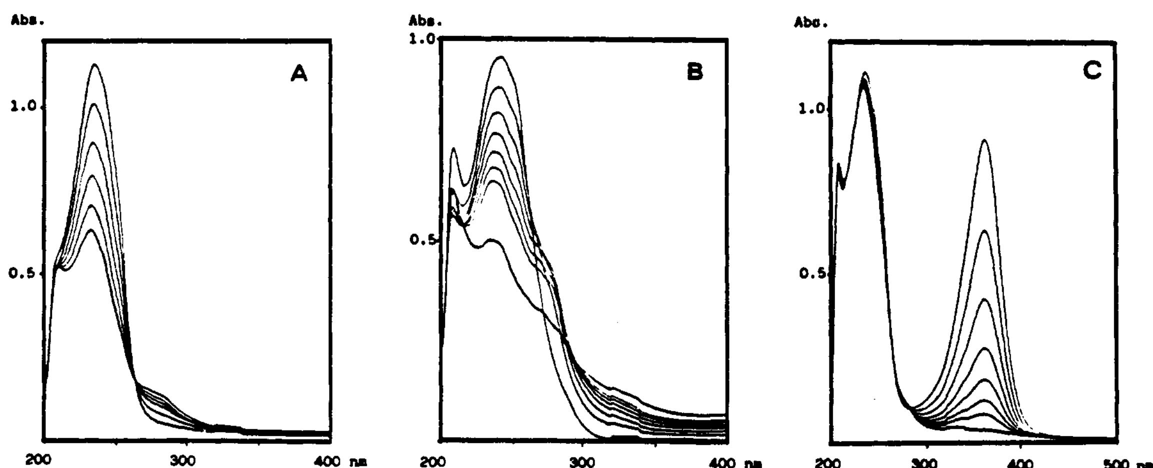


Figure 1. UV absorption spectra and photodecomposition of peptidoleukotriene derivatives: A, spectra of dienes **6a** or **6b** irradiated at 245 nm; B, spectra of the azido dienes **2a** or **2b** irradiated at 245 nm; and C, spectra of the diene diazonium salts **1a** or **1b** irradiated at 360 nm. The time interval between two spectra was 2 min in A and B and 1 min in C; the last spectra in A, B, and C (lower peak absorption) were acquired at 10, 28, and 11 min, respectively.

Table I. Binding Constants of LTC_4, LTD_4, and Peptidoleukotriene Analogs for the LTC_4 and LTD_4 Binding Sites

ligand type	ligand	K_I (μM) for the binding site of: LTC_4	LTD_4
reference	LTC_4	0.12 ± 0.005	0.03[a]
	LTD_4	4[b]	$4.6 \pm 0.4 \times 10^{-5}$
nonphotoactivatable dienes	**6a**	0.02 ± 0.001	0.002 ± 0.0002
	6b	0.045 ± 0.012	0.017 ± 0.002
aryldiazonium derivatives	**1a**	0.08 ± 0.009	8 ± 0.9
	1b	0.11 ± 0.014	40 ± 12
arylazido derivatives	**2a**	0.8 ± 0.1	2 ± 0.2
	2b	0.48 ± 0.15	2 ± 0.18

[a,b] Values are taken from refs 21 and 28, respectively.

phosphonium ylide **8**. The following steps (Scheme IV), that is coupling of the protected dipeptide and deprotection, followed described procedures and gave satisfactory results (over 75% overall yield). Both LTD_4 analogs **6a** and **6b** which are fully characterized could be tested for their binding properties. Finally, the synthesis of the photoactivatable derivatives, in particular the diazonium salts **1a** and **1b**, required an appropriate synthetic strategy which allowed introduction of the diazo group without having to perform a diazotization reaction. The use of a heterobifunctional reagent **5**, possessing a protected diazonium function as a potential photosensitive moiety, worked out successfully, thus demonstrating the usefulness of this reagent. In particular, assuming a less acidic workup, this methodology should be transposable to the natural tetraenic leukotrienes where the diazotization reaction would be even more prejudicial for the unsaturated fatty acid. In addition, the bifunctional reagent **5** is available in a tritiated state,[27] thus permitting, if desired, the synthesis of a radiolabeled probe. Noticeably, our synthetic planning implies the coupling of this reagent **5**

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at a very final stage which is a requirement for the synthesis of a radiolabeled probe.

In order to evaluate the potential of these molecules as photoaffinity probes, we tested their photochemical decomposition (Figure 1A–C). Clearly the dienic moiety is photosensitive and shows, upon irradiation at its λ_{max}, a very neat photodecomposition, as illustrated by the presence of an isobestic point for photolysis of **6a** or **6b** (Figure 1A). Although these derivatives could be used as photoaffinity probes as has been described for the natural series,[7,8] the irradiation requires very short wavelengths and the photochemical process does not necessarily lead to highly reactive species. The irradiation of either azido derivatives (**2a** or **2b**) at 245 nm (Figure 1B) shows a more complicated evolution of the photochemical reaction, and the observed pattern is consistent with a concomitant decomposition of both dienic and azido chromophores. Finally, the diazonium derivatives (**1a** or **1b**) are efficiently photodecomposed at longer wavelengths through a selective and complete loss of the diazonium chromophore with no interference from the dienic moiety (Figure 1C). Again, the presence of isobestic points during this irradiation experiment indicates the formation of a single photodecomposition product. Clearly, use of aryldiazonium compounds allows ideal photoactivation at wavelengths which neither damage the protein nor the polyenic moiety.

The chemical stability of all the synthesized derivatives, including the diazonium compounds, was sufficient to allow their study in binding experiments, and interesting preliminary results were obtained (Table I). In these experiments, the binding constants found for the reference compounds LTC_4 and LTD_4 toward their respective binding sites in lung membranes were very close to those given in the literature: 0.08 μM[28] and 5.4×10^{-5} μM,[21] respectively. Compounds **6a** and **6b** the dienic analogs of LTD_4, are satisfactory ligands for both LTC_4 and LTD_4 receptors with a slight preference for the parent (LTD_4) receptor. The replacement of the tetraene moiety in the LTD_4 molecule by dienes (molecules **6a** and **6b**) leads to a loss of affinity for the LTD4 receptor with a concomitant gain in affinity for the LTC_4 receptor. This is particularly true for **6a** which, when compared to the LTD_4 molecule, shows a 40 times loss for the LTD_4 receptor versus a 200 times gain for the LTC_4 receptor. Transforming **6a** or **6b** into the corresponding substituted aryldiazonium and arylazido derivatives (molecules **1a**, **1b**, **2a**, and **2b**) induces a loss of affinity for both receptors, but this loss is much more pronounced for the LTD4 receptor. As a consequence, these photoactivatable molecules show a better affinity for the LTC_4 receptor, and interestingly the diazonium molecules (**1a** and **1b**) are now clearly selective probes for the LTC_4 receptor (selectivity factor of 100 and 275, respectively). Apparently, besides having a dienic structure, the addition of the aromatic diazonium group to the peptidic amino moiety seems to replace in part the missing γ-glutamyl residue from glutathione in the LTC_4 molecule. The azido molecules **2a** and **2b** seem less potent and show also a much weaker degree of selectivity.

Conclusion

The total synthesis of optically active photoactivatable leukotriene derivatives is described. The convergent synthetic strategy used in its final phase the condensation of a bifunctional reagent containing a protected aryldiazonium salt with a leukotriene analog. The obtained diazonium salts could be converted to the corresponding azides. The development of such compounds was motivated by the need for true leukotriene photoaffinity probes allowing the generation of a defined reactive species. The diazonium salts **1a** or **1b** completely fulfill these requirements, generating highly reactive arylcations at wavelengths which allow efficient and selective decomposition of the diazonium chromophore without interference from the dienic system. An additional interesting feature of these *p*-urea-substituted diazonium derivatives is that they possess a chromophore which is potentially compatible with an energy-transfer-induced photoactivation through tryptophan residues (λ_{exc} approximately 295 nm),[13] a process which generally leads to improved labeling results. Finally, the diazonium salts show good binding to LTC_4 (0.1 μM) and good selectivity for LTC_4 over LTD_4. All these elements taken together strengthen the interest for these diazonium salt molecules as potential photoaffinity probes for the LTC_4 receptor.

Experimental Section

Materials and Methods. NMR coupling constants (*J*) are given in Hz. Mass spectra were provided by the Service de Spectrométrie de Masse from the Institut de Chimie de Strasbourg. Concentration (*c*) is given in g/mL for determination of specific rotation numbers. Elemental analyses were done by the Service de Microanalyses de l'Université Louis Pasteur de Strasbourg. High-pressure liquid chromatography (HPLC) was performed with a photodiode detector and a C-18 (300 × 7.5) Bondasorb column. Eluants were acetonitrile and either pH 5.6 buffered water (1 L of water containing 2 mL of AcOH and an appropriate amount of NH_4OH to bring pH at 5.6) or water with 0.08% concentrated trifluoroacetic acid (TFA).

3-Hydroxy-1-tridecene (10). To a mechanically stirred suspension of magnesium chips (2.142 g, 88.09 mmol) in dry ether (15 mL) was added 10% volume (5 mL) of a solution of bromodecane (16.59 mL, 79.96 mmol) in dry ether (50 mL) to start the reaction. A gentle boiling was maintained by slow addition of the remaining bromodecane solution. After complete addition, reflux was maintained for 15 min. After the solution was cooled at room temperature, freshly distilled acrolein (4.40 mL, 65.85 mmol) in dry ether (20 mL) was added dropwise to the reaction mixture and stirred for several hours. The flask contents were poured into 4 M NH_4Cl solution (100 mL). The crude liquid obtained after an ether extraction procedure was distilled under vacuum to afford **10** as a colorless liquid (9.99 g, 50.37 mmol, 77%): bp 95 °C (0.38 mmHg); R_f 0.65 (AcOEt/hexane, 2/8); n^{23}_D 1.4465; ^1H NMR ($CDCl_3$) δ 0.89 (t, 3 H, H_{13}, ^3J = 6.2), 1.27 (s large, 16 H, H_5 to H_{12}), 1.48 to 1.55 (m, 2 H, H_4), 4.10 (q, 1 H, H_3, J = 6.2), 5.10 (dt, 1 H, H_1, J = 1.3, ^3J = 10.5), 5.23 (dt, 1 H, H_1, J = 1.4, ^3J = 17.2), 5.88 (ddd, 1 H, H_2, ^3J = 10.5, ^3J = 17.1, ^3J = 6.2); ^{13}C NMR ($CDCl_3$) δ 13.95, 22.59, 25.28, 29.26, 29.54, 31.85, 37.04, 73.07, 114.17, 141.43; IR (neat) ν 3355, 1645, 1465, 990. Anal. Calcd for $C_{13}H_{26}O$: C, 78.72; H, 13.21. Found: C, 78.60; H, 13.24.

1-Chloro-2-tridecene (11) and 3-Chloro-1-tridecene (12). To a stirred solution of **10** (6.012 g, 30.31 mmol) in dry ether (60 mL) at 0 °C was added an ethereal solution (35 mL) of $SOCl_2$ (4.5 mL, 62.02 mmol) dropwise over a 2-h period. The reaction mixture was heated under reflux for 4 additional h and then brought to pH 8 by slow addition of a 10% aqueous $NaHCO_3$ solution. The crude product obtained after an ether extraction procedure was purified by silica gel chromatography (hexane) to afford a mixture of **11** and **12** as a colorless liquid in a 88/12 ratio (5.652 g, 26.07 mmol, 86%): bp 95 °C (0.30 mmHg); R_f 0.65 (hexane); ^1H NMR (**11**) ($CDCl_3$) δ 0.89 (t, 3 H, H_{13}, ^3J = 6.4), 1.27 (s wide, 16 H, H_5 to H_{12}), 2.06 (q, 2 H, H_4, J = 6.7), 4.05 (dd, 2 H, H_1, ^3J = 6.9, ^4J = 0.6), 5.60 (dtt, 1 H, H_2, ^3J = 6.9, ^3J = 13.7,

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^4J = 1.3), 5.79 (td, 1 H, H_3, ^3J = 6.5, ^3J = 15.1); ^1H NMR (**12**) ($CDCl_3$) δ identical to **11** except for 1.75–1.88 (m, 2 H, H_4), 4.36 (q, 1 H, H_3, J = 7.3), 5.14 (d wide, 1 H, H_1, ^3J = 10.0), 5.26 (dt, 1 H, H_1, ^3J = 16.8, J = 1.0), 5.55–5.65 (m hidden under **11** spectra, H_2); MS (70 eV) *m/e* 218 (5) [M Cl^{37}]$^+$, 216 (15) [M Cl^{35}]$^+$, 55 (100). Anal. Calcd for $C_{13}H_{25}Cl$: C, 72.02; H, 11.62. Found: C, 71.80; H, 11.76.

2(*E*)-Tridecenyltriphenylphosphonium Chloride (8). A solution of the previously obtained **11**–**12** mixture (0.501 g, 2.309 mmol) in dry acetonitrile (10 mL) and triphenylphosphine (0.485 g, 1.850 mmol) was heated to reflux for 48 h. The solvent was then removed under reduced pressure, and the hygroscopic oil was precipitated with dry ether. The phosphonium salt was triturated several times with dry ether to produce a white solid (**8**) which was rapidly filtered and dried under vacuum (0.789 g, 1.646 mmol, 89%): ^1H NMR ($CDCl_3$) δ 0.86 (t, 3 H, H_{13}, ^3J = 6.5), 1.21 (m, 16 H, H_5 to H_{12}), 1.85–1.95 (m, 2 H, H_4), 4.75 (dd, 2 H, H_1, ^2J_H-phosphore = 14.7, ^3J = 7.2), 5.15–5.34 (m, 1 H, H_3), 5.78–5.96 (m, 1 H, H_2), 7.60–7.88 (m, 15 H, Ar*H*); ^{13}C NMR ($CDCl_3$) δ 13.91, 22.52, 27.94 (d, J = 49), 28.57 (d, J = 4), 28.98 to 29.46 (5 pics), 31.76, 32.70 (d, J = 3), 113.96 (d, J = 10), 118.48 (d, J = 85), 130.20 (d, J = 12), 133.95 (d, J = 10), 134.78 (d, J = 3), 142.90 (d, J = 13).

Methyl 5(*S*),6(*S*)-Epoxy-7,9(*Z,E*)- and -7,9(*E,E*)-eicosadienoates (3a and 3b). To a stirred solution of the phosphonium salt **8** (0.265 g, 1.220 mmol) in freshly distilled THF (10 mL) and dry HMPT (1.5 mL) at −40 °C was added a 1.6 M nBuLi solution in hexane (0.77 mL, 1.232 mmol) to form the corresponding ylide. After 30 min of stirring, a solution of epoxy aldehyde **7** (0.192 g, 1.112 mmol) in freshly distilled THF (3 mL) was added dropwise. The reaction mixture was stirred for 4 h, allowing the temperature to raise slowly to 20 °C. The reaction was quenched with a 10% aqueous NH_4Cl solution (5 mL), and the residue obtained after an ether extraction procedure was purified by chromatography (ether/hexane/Et_3N (24/75/1)) on 2% Et_3N-treated silica gel to give a mixture of **3a** and **3b** as a colorless oil in a 3/1 ratio (0.143 g, 0.424 mmol, 38%): R_f 0.42 (ether/hexane/Et_3N (19/80/1)): ^1H NMR (**3a**) ($CDCl_3$) δ 0.89 (t, 3 H, H_{20}, ^3J = 6.3), 1.27 (s wide, 16 H, H_{12} to H_{19}), 1.57–1.72 (m, 2 H, H_3), 1.77–1.87 (m, 2 H, H_4), 2.08–2.19 (m, 2 H, H_{11}), 2.40 (t, 2 H, H_2, ^3J = 7.2), 2.83–2.89 (m, 1 H, H_5), 3.49 (dd, 1 H, H_6, $^3J_{6-7}$ = 9.0, $^3J_{6-5}$ = 2.1), 3.68 (s, 3 H, OCH_3), 4.93 (t, 1 H, H_7, on H_6 irradiation, the signal gave a doublet, ^3J = 10.8), 5.80 (quint, 1 H, H_{10}, J = 7.1), 6.16–6.51 (m, 2 H, H_8 + H_9); ^{13}C NMR (**3a**) ($CDCl_3$) δ 13.94, 21.27, 22.60, 29.08, 29.49, 31.29, 31.79, 32.75, 33.44, 51.31, 54.25, 59.73, 124.72, 125.15, 134.33, 138.19, 173.40; ^1H NMR (**3b**) ($CDCl_3$) δ identical to **3a** except for 3.12 (dd, 1 H, H_6, $^3J_{6-7}$ = 8.5, $^3J_{6-5}$ = 2.0), 5.38 (dd, 1 H, H_7, $^3J_{7-8}$ = 15.0, $^3J_{7-6}$ = 8.5), 5.74 (quint, 1 H, H_{10}, J = 9.5), 6.05 (dd, 1 H, H_8, $^3J_{8-7}$ = 15.0, $^3J_{8-9}$ = 10.5), 6.37–6.51 (m, 1 H, H_9); ^{13}C NMR (**3b**) ($CDCl_3$) δ identical to **3a** except for 27.75, 32.49, 37.12, 58.24, 60.07, 127.30, 128.98, 134.69, 136.23; MS (**3a** and **3b**) (70 eV) *m/e* 336 (16) [M]$^+$, 129 (100) [$C_6H_9O_3$]$^+$.

Methyl 5(*S*)-Hydroxy-6(*R*)-[*O*-methylglycinyl-*N*-(trifluoroacetyl)-L-cysteinyl]]-7,9(*Z,E*)- and -7,9(*E,E*)-eicosadienoates (13a and 13b). Compound **4** (0.110 g, 0.381 mmol) was added as a solid to a stirred N_2-saturated solution of MeOH (7 mL) containing the mixture of epoxides **3a** and **3b** (0.092 g, 0.272 mmol) and NEt_3 (10 drops). After disappearance of the starting material (TLC, 24 h), the solvent was evaporated under reduced pressure and the residue was chromatographed on a silica gel column (AcOEt/hexane (4/6)) to afford the oily compounds **13a** and **13b** in a 3/1 ratio (0.151 g, 0.241 mmol, 87%): R_f 0.50 (AcOEt/hexane (5/5)); ^1H NMR (**13a**) ($CDCl_3$) δ 0.88 (t, 3 H, H_{20}, ^3J = 6.5), 1.26 (s wide, 16 H, H_{12} to H_{19}), 1.43–1.58 (m, 2 H, H_3), 1.63–1.88 (m, 2 H, H_4), 2.13 (q, 2 H, H_{11}, J = 6.8), 2.36 (t, 2 H, H_2, ^3J = 7.1), 2.80 (dd, 1 H, SCH_2, ^2J = 14.3, ^3J = 7.1), 2.96 (dd, 1 H, SCH_2, ^2J = 14.3, ^3J = 6.7), 3.67 (s, 3 H, OCH_3), 3.78 (s, 3 H, Gly-OCH_3), 3.78 (m, 1 H, H_5), 3.98–4.15 (m, 2 H, $HNCH_2CO$), ≈4.00 (m, 1 H, H_6), 4.59 (q, 1 H, HNC*H*CO, J = 7.4), 5.33 (t, 1 H, H_7, J = 10.3), 5.75–5.95 (m, 1 H, H_{10}), 6.18–6.43 (m, 2 H, H_8 + H_9), 6.95 (t, 1 H, ^3J = 5.3, HNglycine), 7.54 (d, 1 H, ^3J = 7.6, HNcysteine); ^{13}C NMR (**13a**) ($CDCl_3$) δ 14.07, 21.06, 22.64, 29.16 to 29.58, 31.87, 32.79, 32.94, 33.58, 41.37, 48.99, 51.58, 52.54, 52.87, 72.87, 115.74 (q, J_{C-F} = 274), 123.29, 124.10, 133.00, 139.44, 157.00 (q, J_{C-F} = 38), 168.96, 169.67, 174.20; ^1H NMR (**13b**) ($CDCl_3$) δ identical to **13a** except for 5.54 (dd, 1 H, H_7, $^3J_{7-8}$ = 15.0, $^3J_{7-6}$ = 10.0), 5.75–6.40 (m, 3 H, H_8, H_9, H_{10}); ^{13}C NMR (**13b**) δ identical to **13a** except for 55.55, 125.88, 128.82, 134.94, 136.73; MS (**13a** and **13b**) (70 eV) *m/e* 624 (3) [M]$^+$, 494 (96) [M − CHOH − $(CH_2)_3$ − CO_2CH_3]$^+$ 337 (37) [M − CysGly]$^+$, 287 (38) [Cys-Gly]$^+$.

5(*S*)-Hydroxy-6(*R*)-*S*-(L-glycinylcysteinyl)-7,9(*Z,E*)-and -7,9(*E,E*)-eicosadienoic Acids (6a and 6b). To a room-temperature solution of a mixture of **13a** and **13b** (0.129 g, 0.206 mmol) in 1:1 DME/H_2O (2 mL) was added dropwise and under stirring an aqueous solution of 4 M NaOH (0.46 mL). After 24 h, the solution was concentrated to half its volume under reduced pressure without heating and transferred to a centrifuge tube. The solution was rediluted with water to the original volume and acidified slowly to pH 4 with 1 M HCl solution. The white precipitate was centrifuged. The liquid was discarded, and the solid residue was triturated with water (2 mL). After another centrifugation, the water was discarded and the solid residue dried by lyophilization. The obtained powder was subjected to several trituration–centrifugation processes, twice with ether (2 × 2 mL), and at last with an ether/$CHCl_3$ (9/1) mixture (2 mL). The obtained methanol-soluble product was then dried under vacuum to give **6a** and **6b** as a fine powder in a 3/1 ratio (0.088 g, 0.175 mmol, 85%). This mixture was then separated by reversed-phase HPLC: **6a** $[\alpha]^{20}_D$ +107° (MeOH, c = 0.003); ^1H NMR (**6a**) (MeOD) δ 0.89 (t, 3 H, H_{20}, ^3J = 6.5), 1.28 (s wide, 16 H, H_{12} to H_{19}), 1.45–1.79 (m, 4 H, H_3 + H_4), 2.13 (q, 2 H, H_{11}, J = 6.8), 2.29 (t, 2 H, H_2, ^3J = 7.0), 2.83 (dd, 1 H, SCH_2, ^2J = 14.3, ^3J = 8.4), 3.01 (dd, 1 H, SCH_2, ^2J = 14.0, ^3J = 5.5), 3.58–3.73 (m, 2 H, H_5 + H_2NCHCO), 3.87–4.02 (m, 3 H, H_6 + $HNCH_2COOH$), 5.30 (t, 1 H, H_7, J = 10.4), 5.81 (quint, 1 H, H_{10}, J = 7.0), 6.15–6.43 (m, 2 H, H_8 + H_9); MS (**6a**) FAB$^-$ (triethanolamine) *m/e* 499 (100) [M − H]$^-$; HR-SM FAB$^-$ (triethanolamine + poly(ethylene glycol) 400) for $C_{25}H_{43}N_2O_6S$ calcd 499.2842, found 499.286; UV (**6a**) (MeOH) λ_{max} = 235 nm, ϵ_{235} = 22 000; RP-HPLC (**6a**) (CH_3CN/aqueous buffr pH 5.6, 40/60), flow rate 2.2 mL/min, retention time 19.32 min; (**6b**) $[\alpha]^{20}_D$ −23° (MeOH, c = 0.0012); ^1H NMR (**6b**) (MeOD) δ identical to **6a** except for 5.53 (dd, 1 H, H_7, $^3J_{7-8}$ = 14.1, $^3J_{7-6}$ = 10.5), 5.73 (dt, 1 H, H_{10}, $^3J_{10-9}$ = 14.3, $^3J_{10-11}$ = 7.0), 6.02–6.25 (m, 2 H, H_8 + H_9); MS (**6b**) FAB$^-$ (triethanolamine) *m/e* 499 (100) [M − H]$^-$; HR-MS FAB$^-$ (triethanolamine + poly(ethylene glycol) 400) for $C_{25}H_{43}N_2O_6S$ calcd 499.2842, found 499.286; UV (**6b**) (MeOH) λ_{max} = 233 nm, ϵ_{233} = 18 000; RP-HPLC (**6b**) (CH_3CN/aqueous buffer pH 5.6, 40/60), flow rate 2.2 mL/min, retention time 25.04 min.

5(*S*)-Hydroxy-6(*R*)-[[[[*N'*-methyl[4-[(benzenesulfonyl)azo]phenyl]amino]carbonyl]glycinyl]-L-cysteinyl]-7,9(*Z,E*)-and -7,9(*E,E*)-eicosadienoic Acids 1a and 1b (X^- = $PhSO_2^-$).

5(*S*)-Hydroxy-6(*R*)-[[[[*N'*-methyl[4-(diazonium trifluoroacetyl)phenyl]amino]carbonyl]glycinyl]-L-cysteinyl]-7,9-(*Z,E*)- and -7,9(*E,E*)-eicosadienoic Acids 1a and 1b (X^- = CF_3COO^-). A solution of azosulfone **5** (0.009 g, 0.03 mmol) in dry THF (0.5 mL) was added at 0 °C and in the dark to a stirred solution of the mixture of **6a** and **6b** (0.012 g, 0.024 mmol) in dry THF (0.5 mL) containing Et_3N (0.011 mL, 0.077 mmol). The reaction was stirred for 30 min at 0 °C and 1 h at 20 °C before being filtered and transferred into a centrifuge tube. The solvent was evaporated under reduce pressure without heating and the residue triturated and centrifuged three times with ether (3 × 1 mL). The solid residue was then dissolved in a 1:1 MeOH/water solution (2 mL), and 1 M HCl (0.08 mL) was added. The isolated orange solid, obtained from an ethyl acetate extraction procedure, corresponds to the mixture of compounds **1a** and **1b** in 3/1 ratio where X^- = $PhSO_2^-$ (0.014 g, 0.017 mmol, 69%). This mixture could partially be separated by RP-HPLC: ^1H NMR (**1a, X^- = CF_3COO^-**) (MeOD) δ 0.89 (t, 3 H, H_{20}, ^3J = 6.2), 1.28 (s wide, 16 H, H_{12} to H_{19}), 1.42–1.77 (m, 4 H, H_3 + H_4), 2.10 (t wide, 2 H, H_{11}, J = 6.8), 2.27 (t, 2 H, H_2, ^3J = 7.0), 2.77 (dd, 1 H, SCH_2, ^2J = 13.9, ^3J = 10.3), 3.08 (dd, 1 H, SCH_2, ^2J = 14.0, ^3J = 4.8), 3.51 (s, 3 H, NCH_3), 3.60–3.70 (m, 1 H, H_5), 3.83–4.06 (m, 3 H, H_6 + $HNCH_2COOH$), 4.51–4.64 (m, 1 H, HNC*H*CO), 5.26 (t, 1 H, H_7, J = 10.5), 5.71–5.89 (m, 1 H, H_{10}), 6.11–6.50 (m, 2 H, H_8 + H_9), 7.83 (syst AA′XX′, part AA′, d, 2 H, Ar*H*, $^3J_{AX}$ = 10.0), 8.43 (syst AA′XX′, part XX′, d, 2 H, Ar*H*, $^3J_{XA}$ = 10.0); ^1H NMR (**1a, X^- = $PhSO_2^-$**) (MeOH) δ identical to **1a, X^- = CF_3COO^-**, except for 3.37 (s, 3 H, NCH_3), 7.60–7.96 (m, 9 H, Ar*H*); ^1H NMR (**1b, X^- = CF_3COO^-**) (MeOH) δ identical to **1a, X^- = CF_3COO^-** except for 5.40–5.57 (m, 1 H, H_7), 5.65–5.84 (m,

1 H, H_{10}), 6.00–6.28 (m, 2 H, $H_8 + H_9$); ^{1}H NMR (**1b**, **$X^- = PhSO_2^-$**) (MeOH) δ identical to **1a**, **$X^- = CF_3COO^-$**, except for 3.37 (s, 3 H, NCH_3), 5.40–5.57 (m, 1 H, H_7), 5.65–5.84 (m, 1 H, H_{10}), 6.00–6.28 (m, 2 H, $H_8 + H_9$), 7.60–7.96 (m, 9 H, ArH); MS (**1a** and **1b**, **$X^- = PhSO_2^-$**) FAB$^-$ (triethanolamine) *m/e* 802 (20), 661 (15), 632 (15) $[M - H, -C_6H_5N_2O_2S]^-$, 322 (15) $[M - H, -CysGly]^-$, 169 (100); UV (**1a** or **1b**, **$X^- = PhSO_2^-$**) (Tris HCl 50 mM, pH = 7.4 buffer), λ_{max} = 360 nm, ϵ_{360} = 23 000, $t_{1/2}$ at 10 °C ≈ 16 h; RP-HPLC (CH_3CN/water + 0.08% TFA, 50/50), flow rate 2.2 mL/min, retention times **1a**, **$X^- = CF_3COO^-$**, 17.0 min, **1a**, **$X^- = PhSO_2^-$**, 40.5 min, **1b**, **$X^- = CF_3COO^-$**, 24.5 min, **1b**, **$X^- = PhSO_2^-$**, 45.0 min.

5(*S*)-Hydroxy-6(*R*)-[[[*N*-methyl-[(4-azidophenyl)amino]-carbonyl]glycinyl]-L-cysteinyl]-7,9(*Z*,*E*)- and 7,9(*E*,*E*)-eicosadienoic Acids (2a and 2b). The mixture of compounds **1a** and **1b** ($X^- = PhSO_2^-$, 0.014 g, 0.018 mmol) was dissolved in an aqueous NH_4OAc buffer, pH 5.6 (1.3 mL) (see Materials and Methods section) containing 0.15 mL of a 5% aqueous $NaHCO_3$ solution. After the solution was cooled to 0 °C, a 2 M aqueous solution of NaN_3 (0.088 mL) was added dropwise and under stirring. The temperature was allowed to warm to 20 °C over 20 min, and the products **2a** and **2b** were precipitated by addition of 1 M HCl (0.10 mL). The precipitate was centrifuged and the solid residue subjected three times to trituration and centrifugation with water (2 mL). Finally, the product was dissolved in acetonitrile (1.6 mL) and water (0.4 mL) containing 0.08% TFA and chromatographed over an RP-HPLC column to separate **2a** and **2b** as pure compounds (0.0044 g, 0.0065 mmol, 37% overall): (**2a**) $[\alpha]^{20}_D$ +69° ($CDCl_3$, c = 0.0034); ^{1}H NMR (**2a**) (MeOD) δ 0.89 (t, 3 H, H_{20}, ^3J = 6.4), 1.28 (s large, 16 H, H_{12} to H_{19}), 1.47–1.90 (m, 4 H, $H_3 + H_4$), 2.12 (m, 2 H, H_{11}), 2.30 (t, 2 H, H_2 ^3J = 7.1), 2.70 (dd, 1 H, SCH_2, ^2J = 14.0, ^3J = 8.8), 3.02 (dd, 1 H, SCH_2, ^2J = 14.0, ^3J = 5.0), 3.60–3.70 (m, 1 H, H_5), 3.85–3.91 (m, 2 H, $HNCH_2$-COOH), 4.07 (dd, 1 H, H_6, ^3J = 10.5, ^3J = 4.3), 4.46–4.52 (m, 1 H, HNC*H*CO), 5.16 (t, 1 H, H_7, J = 10.3), 5.70–5.89 (m, 1 H, H_{10}), 6.09–6.50 (m, 2 H, $H_8 + H_9$), 7.16 (d, 2 H, Ar*H*, ^3J = 8.9), 7.44 (d, 2 H, Ar*H*, ^3J = 9.0); SM (**2a**) FAB$^-$ (poly(ethylene glycol)), *m/e* 690 (22), 673 (7) $[M - H]^-$, 655 (13) $[M - H, -H_2O]^-$, 169 (100); HR-MS for $C_{33}H_{49}N_6O_7S$ calcd 673.3383, found 673.344; UV (**2a**) (Tris HCl, 50 mM, pH = 7.4 buffer), λ_{max} = 245 nm, ϵ_{245} = 25 500; RP-HPLC (**2a**) (CH_3CN/water + 0.08% TFA, 76/24), flow rate 2.2 mL/min, retention time 15.5 min; (**2b**) $[\alpha]^{20}_D$ −60° (MeOD, c = 0.0010); ^{1}H NMR (**2b**) (MeOD) δ identical to **2a** except for 3.76–3.97 (m, 3 H, H_6 + $HNCH_2COOH$), 5.45 (dd, 1 H, H_7, $^3J_{7-8}$ = 14.0, $^3J_{7-6}$ = 9.6), 5.64–5.79 (m, 1 H, H_{10}), 5.88–6.13 (m, 2 H, $H_8 + H_9$); SM (**2b**) FAB$^-$ (poly(ethylene glycol)) *m/e* 695 (55) $[M - 2 H + Na]^-$, 673 (43) $[M - H]^-$, 169 (100); HR-MS for $C_{33}H_{49}N_6O_7S$ calcd 673.3383, found 673.343; UV (**2b**) (Tris HCl, 50 mM, pH = 7.4 buffer), λ_{max} = 243 nm, ϵ_{243} = 25500; RP-HPLC (**2b**) (CH_3CN/water + 0.08% TFA, 76/24), flow rate 2.2 mL/min, retention time 16.8 min.

Binding Assays. Guinea-pig lung membrane homogenate (~0.1 mg proteins) was incubated with 0.39 nM [^{3}H]-LTC_4 or [^{3}H]LTD_4 (128.5 Ci/mmol: NEN Research Products) for 30 min at 4 °C (LTC_4) or 40 min at 20 °C (LTD_4) in the presence of various concentrations of unlabeled leukotriene (reference compounds LTC_4 or LTD_4) or the newly synthesized leukotriene derivatives, in triplicate. The bound homogenate was then recovered on glass fiber filters by rapid filtration at 4 °C, and the filters were counted by liquid scintillation. For each point the extent of displacement was plotted as logit versus log[29] and IC50s were determined applying a linear regression analysis to this plot.

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