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Synthesis of 19-[(2-Azido-5-iodo)-benzoyloxy]-LTA$_4$ and Enzymatic Conversion to the LTC$_4$ Analogue

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Abstract—New photoaffinity probes based on C-19 position of leukotriene A$_4$ has been synthesized from 19-hydroxy-LTA$_4$ methyl ester. Enzymatic conversion into the LTC$_4$ analogue yielded a potential tool for the study of *cys*-LT$_2$ receptors.

Since the discovery of the leukotrienes[1] (LTs) there has been intensive effort to synthesize these products and analogues.[2] The cysteinyl leukotrienes C$_4$, D$_4$ and E$_4$ (*cys*-LTs) are sequential metabolites of leukotriene A$_4$ (LTA$_4$), itself produced by action of 5-lipoxygenase (5-LO) and five lipoxygenase activating protein (FLAP) on arachidonic acid.[3,4] They are among the most potent bronchoconstricting agents and active on isolated human airway cells as well as in vivo.[5]

Biological activities of *cys*-LTs are mediated through at least two specific membrane receptors in guinea-pig. They were classified[6] as *cys*-LT$_1$ and *cys*-LT$_2$.

We are particulary interested in the characterization of the human *cys*-LT$_2$ receptor through photoaffinity labelling and we became interested in 19-[(2-azido-5-iodo)-benzoyloxy]-LTA$_4$ methyl ester **1a** and 19-[(2-azido-5-trimethylstannyl)-benzoyloxy]-LTA$_4$ methyl ester **1b** which were obtained according to the strategy described in Scheme 1.

Chemistry

The synthesis of epoxydienal **2** was carried out as described in the literature[7,8] and the preparation of 2-azido-5-iodobenzoic acid **4a** and 2-azido-5 trimethylstannylbenzoic acid **4b** were carried out as described by Perrier et al.[9]

The phosphonium salt **3** was prepared by a new strategy (Scheme 2), avoiding any protection and deprotection steps as we had described in our previous papers.[10]

The first 6 steps leading to ketonenol **6** were achieved according to our procedure.[10] The iodo derivative **7** was obtained in 85% yield with PPh_3, I_2, imidazole in xylene at 80 °C.[11] Ketone **7** after treatment by sodium borohydride in dry ethanol gave the racemic alcohol **8** in 80% yield and transformed into the white crystalline phosphonium salt **3** with PPh_3 in toluene in 85% yield. Finally, we have used different silyl protective groups as Me_3SiCl, Et_3SiCl and *n*-$PrMe_2SiCl$ for protection of the hydroxy function at C8 and obtained the best result with the *n*-$PrMe_2SiCl$ in presence of DBU in dry CH_2Cl_2 affording the protected phosphonium salt **9**[12] in 84% yield.

Because of the lack of reactivity of hydroxy-phosphonium salt **3**, the protected leukotriene A$_4$ **10**[13] was obtained by a Wittig reaction between epoxydienal **2** and phosphonium salt **9** (3 equiv) in presence of *n*-butyllithium (3 equiv), in a mixture THF:HMPA (5:1) at −78 °C for 15 min in only 55% yield. Deprotection of *n*-propyldimethylsilyl ether was carried out using tetrabutylammonium fluoride in THF at room temperature to afford 19-hydroxy-LTA$_4$ methyl ester **10** in 85% yield (Scheme 3).

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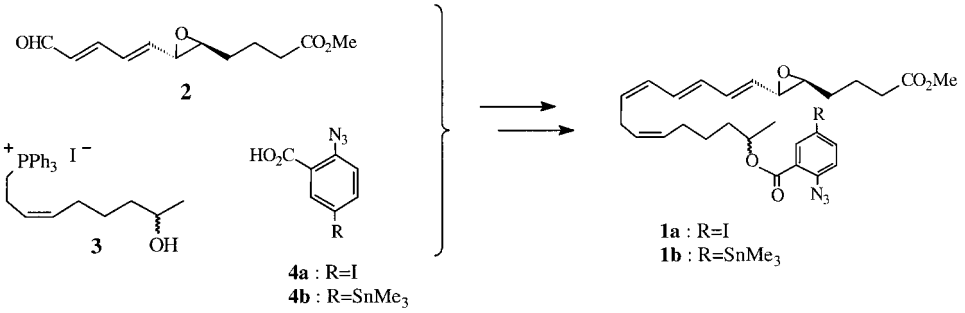
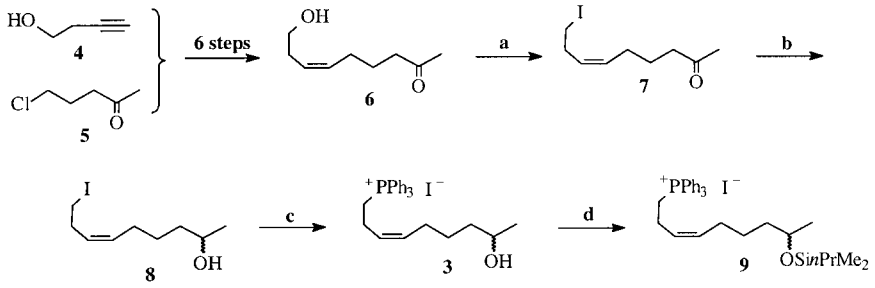
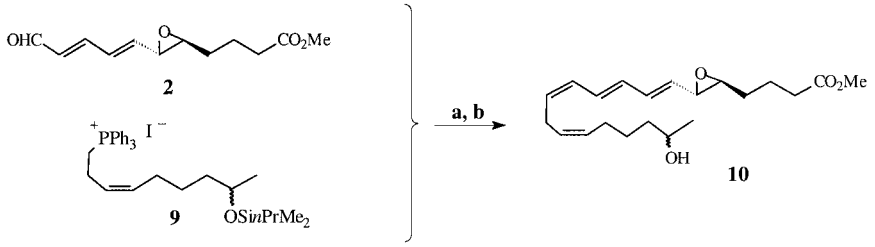


Scheme 1.



Scheme 2. Reagents: (a) PPh_3/I_2/imiadazole/xylene 80 °C 15 min. 85%, (b) $NaBH_4$/MeOH 80%, (c) PPh_3/toluene 90 °C 24 h. 85%, (d) *n*-$PrMe_2SiCl$/DBU/CH_2Cl_2, 84%.



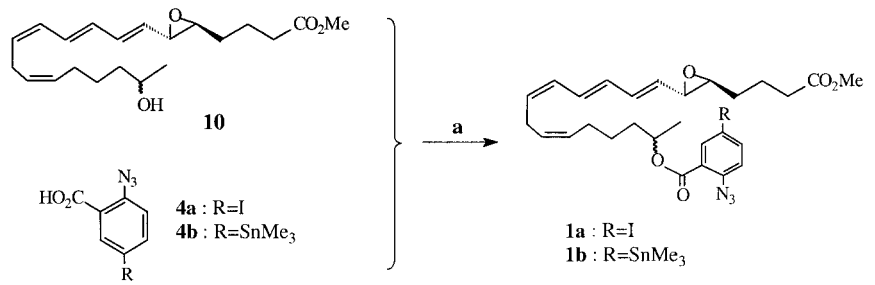
Scheme 3. Reagents: (a) *n*-BuLi/THF/HMPA, −78 °C, 15 min, 55%, (b) *n*-Bu_4NF/THF, 85%.

Finally, the aromatic portion of the molecules **1a** and **1b**, containing the photoactivable azido group and potential iodo label was introduced by the coupling of the three fragments **10**, **4a** or **4b** in presence of DCC, DMAP in THF in 59% yield for 19-[(2-azido-5-iodo)-benzoyloxy]-LTA_4 methyl ester **1a**[14] and 55% for 19-[(2-azido-5-trimethylstannyl)-benzoyloxy]-LTA_4 methyl ester **1b**[15] (Scheme 4).

It is important to notice that the 2-azido-5-trimethylstannylbenzoic acid is an efficient way for introducing a radioactive iodine through the oxidation of a stannyl derivative with radioactive sodium iodide and chloramine-T as previously described in the literature.[9]

Biological Results and Discussion

Conversion into the LTC_4 analogue was obtained, as previously described by using human platelets as a source of LTC_4-synthase.[16] Briefly, washed human platelets were prepared according to Patscheke.[17] Free acid



Scheme 4. Reagents: (a) DCC:DMAP:THF, 59% for **1a** and 55% for **1b**.

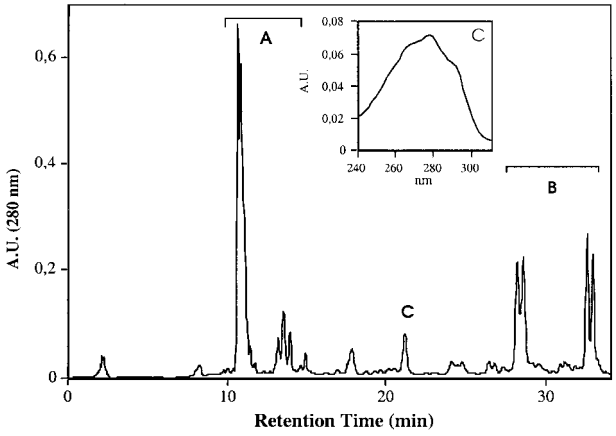


Figure 1. UV absorbance profile at 280 nm from the RP-HPLC of human platelets incubated with **1a**.

of compound **1a** was obtained through base catalysed hydrolysis of corresponding methyl esther (ME) and was added to human platelets (3×10^9 cells in 6 ml) at the final concentration of 10 μM.

LTA_4-derived metabolites were analysed by RP-HPLC coupled to a diode-array UV detector as previously described[18] (Fig. 1) and effluent was directly interfaced into the electrospray source of a Finnigan LC-Q Ion Trap mass spectrometer. UV (210–340 nm) and negative ion mass spectra (200–1200 *m*/*z*) were collected every 4 s. Hydrolysis of methyl ester resulted in substantial loss of 2-azido-5-iodobenzoic acid, as confirmed in separate experiments (data not shown), resulting in the formation of 19-OH-LTA_4. According to this fact, the family of early eluting peaks (Fig. 1, group A), represents the compounds arising from non enzymatic epoxide opening of 19-OH-LTA_4, as indicated by RP-HPLC retention time, UV spectra and molecular weight determination. The second group of peaks (Fig. 1 group B), eluting at much later retention times, represents the non enzymatic degradation products of 2-azido-5-iodobenzoyl analogue of LTA_4. A relevant peak (Fig. 1, peak C) eluting earlier than group B, according with the presence of a polar glutathione moiety, showed UV spectrum (λ_{max} 279 nm, asymmetric shoulders at 268 and 290 nm) and molecular weight (*m*/*z* 911, corresponding to a MW of 912) compatible with the proposed structure of 19[(2-azido-5-iodo)benzoyloxy]LTC_4.

Conclusion

We have described the first syntheses of 19-[(2-azido-5-iodo)-benzoyloxy]-LTA4 methyl ester **1a** and 19-[(2-azido-5-trimethylstannyl)-benzoyloxy]-LTA4 methyl ester **1b**. Compound **1a** could be enzymatically converted into the LTC_4 analogue, suggesting that the substituents at C-19 do not interfere with the specific recognition of the 5,6 epoxide by the enzyme LTC_4 synthase. This compound, bearing a photo-sensitive group at the ω-1 position may represent a useful tool for characterization and identification of high-affinity binding site *cys*-LT_2 receptor.[19] Such studies are currently being carried out in our laboratories.

Acknowledgements

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References and Notes

1. Borgeat, P.; Samuelsson, B. *J. Biol. Chem.* **1979**, *254*, 2643.
2. Delorme, D.; Guindon, Y.; Lau, C. K.; Zamboni, R. *J. Org. Chem.* **1988**, *53*, 267; Tolstikov, G. A.; Miftakhov, M. S.; Tolstikov, A. G. *Tetrahedron Lett.* **1985**, *26*, 3867; Rokach, J.; Guindon, Y.; Young, R. N.; Adams, J.; Atkinson, J. G. In *Synthesis of Natural Products*; Apsimon, J.; Ed.; Wiley-Interscience: New York, 1988, Vol. 7, pp 141–273.
3. Dixon, R. A. F.; Diehl, R. E.; Opas, E.; Rands, E.; Vickers, J. F.; Evans, J. F. *Nature* **1990**, *343*, 282.
4. Samuelsson, B. *Adv. Prostaglandin, Thromboxane, and Leukotriene Res.* **1983**, *11*, 1.
5. Dahlen, S. E.; Hedqvist, P.; Hammarström, S.; Samuelsson, B. *Nature* **1980**, 288, 484; Dahlen, S. E. in *Asthma: Basic mechanisms and clinical management*; Barnes, P. J.; Roger, I. W.; Thomson, N. C. Eds.; Academic Press, London, **1988**, pp 213–230.
6. Watson, S.; Girdlestone, D. NC-IUPHAR, 1996 TiPS receptor and ion chanel nomenclature supplement; *Trends Pharmacol. Sci.* 1995 (suppl.), 45.
7. Rokach, J.; Zamboni, R.; Lau, C. K.; Guindon, Y. *Tetrahedron Lett.* **1981**, *22*, 2759.
8. Ernest, J.; Main, A. J.; Menasse, R. *Tetrahedron Lett.* **1982**, *23*, 167.
9. Perrier, H.; Prasit, P.; Wang, Z. *Tetrahedron Lett.* **1993**, *35*, 1501.
10. Pastouret, A.; Vidal, J. P.; Durand, T.; Girard, J. P.; Rossi, J. C. *Bull. Soc. Chim. Fr.* **1993**, *130*, 206; Garcia, M.; Durand, T.; Vidal, A.; Vidal, J. P.; Rossi, J. C.; Kuklev, D; Serkov, I.; Bezuglov, V. *Bull. Soc. Chim. Fr.* **1997**, *134*, 451
11. Rondot, B.; Durand, T.; Rossi, J. C.; Rolin, P. *Carbohydr. Res.* **1994**, *621*, 149.
12. Compound **9**: ^{1}H NMR (360 MHz, $CDCl_3$): 0.022, 0.027 (s, 6H, $SiCH_3$), 0.48–0.56 (m, 2H, $SiCH_2CH_2CH_3$), 0.91 (t, 3H, $SiCH_2CH_2CH_3$, *J* = 7.3 Hz), 1.05 (d, 3H, 9-H, *J* = 6.9 Hz), 1.31 (t, 2H, $SiCH_2CH_2CH_3$), 1.24 (m, 2H, 7-H); 1.29 (m, 2H, 6-H), 1.70–1.80 (m, 2H, 5-H), 2.35–2.49 (m, 2H, 2-H), 3.62–3.77 (m, 1H, 8-H), 3.73–3.86 (m, 2H, 1-H), 5.37 (m, 1H, 4-H), 5.56 (m, 1H, 3-H), 7.66-7,87 (m, H_{arom}). ^{13}C NMR (90 MHz, $CDCl_3$): −1,29 ($SiCH_3$), 20.3 (C-2), 23.2, 23.8 (C-1. $J_{P\text{-}Cl}$ = 48.3 Hz), 16.7 ($SiCH_2CH_2CH_3$), 18.1 ($SiCH_2CH_2CH_3$), 19.3 ($SiCH_2CH_2$ CH_3), 23.9 (C-9), 25.6 (C-6), 27.3 (C-5), 39.2 (C-7), 68.3 (C-8), 117.6, 118.6 ($C_{IVarom.}$ $J_{P\text{-}C}$ = 85.6 Hz), 125.9, 126.0 (C-3, $J_{P\text{-}C3}$ = 13.3 Hz), 132.5 (C-4),130.4, 130.6, 133.7, 133.8, 135.1 ($C_{arom.}$). ^{31}P NMR (81 MHz, $CDCl_3$): +24,71.
13. Compound **10**: U.V. (nm. cyclohexane:ethyl acetate: Et_3N) = λ_{max}: 278. shoulders 268 and 290 nm. ^{1}H NMR (360 MHz, $CDCl_3$): 1.15 (d, *J* = 4.14 Hz, 3H, 20-H), 1.36–1.49 (m, 4H, 17-H, 18-H), 1.52–1.67 (m, 2H, 4-H), 1.70–1.80 (m, 2H, 3-H), 2.05 (q, *J* = 6.4 Hz, 2H, 16-H), 2.33 (t, *J* = 7.37 Hz, 2H, 2-H), 2.79–2.85 (m, 1H, 5-H), 2.90 (t, *J* = 6.95 Hz, 2H, 13-H), 3.10 (dd, *J* = 2.04 Hz, *J* = 7.96 Hz, 1H, 6-H), 3.62 (s, 3H, OCH_3), 3.66–3.77 (m, 1H, 19-H), 5.33–5.42 (m, 4H, 7-H, 12-H, 14-H, 15-H), 5.97 (t, *J* = 10.93, 1H, 11-H), 6.15 (dd, *J* = 10.8 Hz, *J* = 14.82 Hz, 1H, 9-H), 6.39–6.53 (m, 2H, 8-H, 10-H). ^{13}C NMR (90 MHz, $CDCl_3$): 21.3 (C-3), 23.5 (C-20), 25.7 (C-17),

26.2 (C-13), 27.1 (C-16), 31.3 (C-4), 33.5 (C-2), 38.8 (C-18), 51.5 (OCH_3), 58.3 (C-6), 60.5 (C-5), 67.9 (C-19), 127.3 (C-14), 128.3 (C-11), 128.8 (C-10), 130.0 (C-7), 130.4 (C-15), 131.4 (C-9), 131.5 (C-12), 134.5 (C-8), 173.6 (C-1).

14. Compound **1a**: UV (nm. cyclohexane:ethyl acetate: Et_3N) = λ_{max}: 264, shoulders: 275 and 288 nm. ^{1}H NMR (360 MHz. $CDCl_3$): 0.83–0.92 (m, 3H, 20-H), 1.30–1.45 (m, 4H, 17-H, 18-H), 1.52–1.84 (m, 4H, 3-H, 4-H), 2.35 (t, J = 7.35 Hz, 2H, 2-H), 2.81–2.84 (m, 1H, 5-H), 2.92 (t, J = 7.20 Hz, 2H, 13-H), 3.11 (dd, J = 2.05 Hz, J = 7.81 Hz, 1H, 6-H), 3.65 (s, 3H, 28-H), 5.11–5.14 (m, 1H, 19-H), 5.36–5.46 (m, 4H, 7-H, 12-H, 14-H, 15-H), 6.00 (t, J = 11.01 Hz, 1H, 11-H), 6.17 (dd, J = 10.83 Hz, J = 14.83 Hz, 1H, 9-H), 6.41–6.55 (m. 2H, 8-H, 10-H), 6.96 (d, Jo = 8.42 Hz, 1H, 3′-H), 7.77 (dd, Jm = 1.98 Hz, 1H, 4′-H), 8.07 (d, 1H, 6′-H). ^{13}C NMR (90 MHz, $CDCl_3$): 21.3 (C-3), 19.97 (C-20), 25.4 (C-17), 26.2 (C-13), 26.9 (C-16), 31.3 (C-4), 33.5 (C-2), 35.4 (C-18), 51.5 (OCH_3), 58.3 (C-6), 60.5 (C-5), 72.6 (C-19), 87.4 (C-5′), 121.7 (C-3′), 125.0 (C-3′), 127.7 (C-14), 128.4 (C-11), 128.8 (C-10), 130.1 (C-7), 130.4 (C-15), 131.4 (C-9), 131.5 (C-12), 134.5 (C-8), 140.0 (C-2′), 140.0 (C-6′), 141.5 (C-4′), 163.3 (CO), 173.4 (C-1).

15. Compound **1b**: UV (nm. cyclohexane:ethyl acetate: Et_3N) = λ_{max}: 264. shoulders 275 and 288 nm. ^{1}H NMR (360 MHz, $CDCl_3$): 0.29 (s, 9H, $SnMe_3$), 1.34 (d, 3H, 20-H, $J_{20\text{-}19}$ = 6.3 Hz); 1.43–1.70 (m, 5H, 4-H 17-H 18-H); 1.70–1.82 (m, 2H, 3-H), 2.07–2.16 (m, 2H, 16-H), 2.35 (t, 2H, 2-H, $J_{2\text{-}3}$ = 7.2 Hz), 2.81–2.84 (m, 1H, 5-H); 2.92 (t, 2H, 13-H); 3.11 (dd, 1H, 6-H, $J_{6\text{-}5}$ = 2.0 Hz, $J_{6\text{-}7}$ = 8.0 Hz), 3.65 (s, 3H, OMe), 5.10–5.50 (m, 1H, 19-H), 5.32–5.49 (m, 4H, 7-H 12-H 14-H 15-H); 5.99 (t, 1H, 11-H, J = 11.0 Hz), 6.17 (dd, 1H, 9-H, $J_{9\text{-}8}$ = 10.8 Hz, $J_{9\text{-}10}$ = 14.8 Hz), 6.41–6.56 (m, 2H, 8-H 10-H), 7.17 (d, 1H, 3′-H, $J_{3'\text{-}4'}$ = 7.8 Hz), 7.58 (dd, 1H, 4′-H, $J_{4'\text{-}6'}$ = 1.2 Hz), 7.84 (d, 1H, 6′-H). ^{13}C NMR (90 MHz, $CDCl_3$): -9.4 ($SnMe_3$), 20.0 (C-20), 21.30 (C-3), 24.55 (C-17), 26.27 (C-13), 26.98 (C-16), 31.35 (C-4), 33.54 (C-4), 35.53 (C-18), 51.55 (OMe), 58.31 (C-6), 60.49 (C-5), 72.06 (C-19), 118.44 (C-3′), 119.25 (C′1), 127.58 (C-14), 128.39 (C-11), 128.82 (C-10), 130.01 (C-7), 130.02 (C-15), 130.70 (C-9), 131.47 (C-12), 134.55 (C-8), 138.24 (C-2′, C-6′), 138.46 (C-5′), 140.07 (C-4′), 165.68 (CO), 173.60 (C-1).

16. Sala, A.; Folco, G.; Henson, P. M.; Murphy, R. C. *Biochem. Pharmacol.* **1997**, *53*, 905.

17. Patscheke, H. *Haemostasis* **1980**, *10*, 14.

18. Sala, A.; Bolla, M.; Zarini, S.; Müller-Peddinghaus, R.; Folco, G. *J. Biol. Chem.* **1996**, *271*, 17945.

19. Priè, S.; Guillemette, G.; Boulay, G.; Borgeat, P.; Sirois, P. *J. Pharmacol. Exp. Ther.* **1995**, *275*, 312.