Synthesis of a photoaffinic hepoxilin analog†

Peter M. Demin,[a,c] Dmitry M. Kochev,[a] Hélène Perrier,[b] Cecil R. Pace-Asciak[c,d] and Kasimir K. Pivnitsky*[a]

[a] *N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, 117913 Moscow, Russian Federation. Fax: +7 095 135 5328; e-mail: eicosan@glas.apc.org*
[b] *Merck Frosst Centre for Therapeutic Research, PO Box 1005, Pointe Claire-Dorval, Québec, Canada H9R 4P8*
[c] *Research Institute, Hospital for Sick Children, 555 University Avenue, Toronto, Canada M5G 1X8*
[d] *Department of Pharmacology, Faculty of Medicine, University of Toronto, Toronto, Canada M5S 1A8*

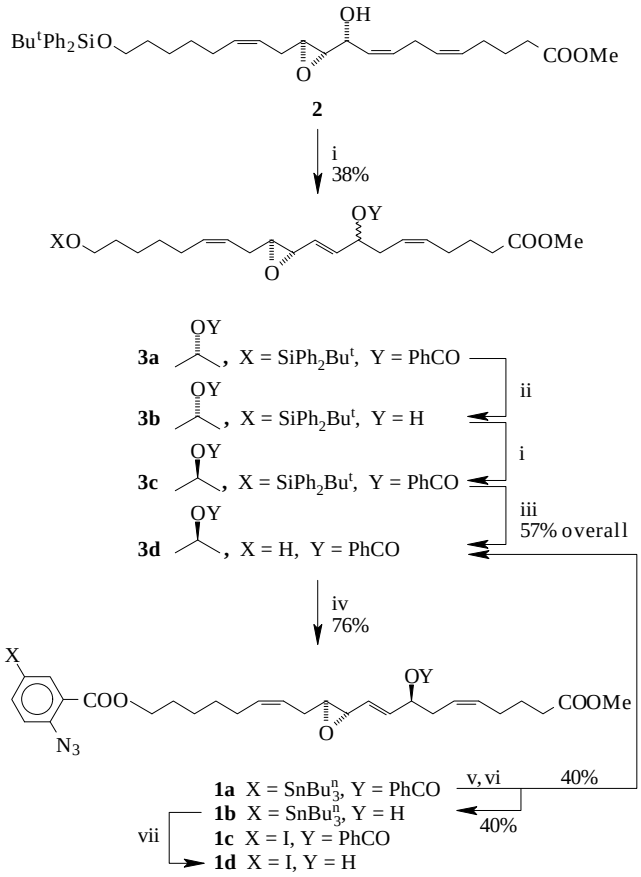
The synthesis of 20-azido(tri-*n*-butyltin)benzoate of 20-hydroxy-(8*S*)-hepoxilin A_3 (HxA_3‡) methyl ester, a tool for the labelling of proteins involved in hepoxilin metabolism, has been performed starting from a synthetic precursor of 20-hydroxy hepoxilins.

Hepoxilins, the metabolites of arachidonic acid lipoxygenase oxidation, were discovered in several mammalian tissues as well as in other natural systems.[1] The biological activity of hepoxilins is based on their ability to release intracellular calcium and to open potassium channels in the cell.[2] Recently we have employed tritium labelled hepoxilins for the determination of their specific binding sites in human neutrophils.[3,4] The results obtained indicate the existence of a putative hepoxilin receptor in human neutrophils. To continue our investigation in this area, we have now developed a method of synthesising a hepoxilin analog **1b** containing both azido- and trialkyltin functionalities at a distance from native hepoxilin functions. In the analog an azido group will serve for a photoaffinity labelling of the receptor with a covalent-binded hepoxilin, and a trialkyltin group can be employed for the introduction of radioactive ^{125}I in the label.[5,6] A general approach for the creation and use of such photoaffinity probes in the eicosanoid series has been reported.[7,8]

The (8*S*)-epimer of HxA_3 was chosen as a basic structure for the hepoxilin moiety of the analog since it revealed the maximal specificity of binding to neutrophil membranes.[4] The introduction of additional functionalities mentioned above was based on the use of an intermediate in the recently published total chemical synthesis of 20-hydroxy-hepoxilins.[9] This intermediate, 20-*tert*-butyldiphenylsilyloxy(BDPSO)-(10*R*)-HxB_3 methyl ester (ME) **2** was transformed by the Mitsunobu reaction with benzoic acid into benzoate of 20-BDPSO-(8*R*)-HxA_3 **3a** (Scheme 1). As typical for this method,[10] an accompanying product of S_N2 displacement, benzoate of 20-BDPSO-(10*S*)-HxB_3 ME, was also isolated and recycled. Selective benzoate removal from **3a** by alkaline transesterification with methanol resulted in 8-alcohol **3b** which after the second Mitsunobu reaction produced (8*S*)-epimeric benzoate **3c**. It was converted by selective deprotection of silylated hydroxyl group with fluoride ion into 8-benzoate of 20-hydroxy-(8*S*)-HxA_3 ME **3d**, a substrate for further modification by 20-esterifications.

A source of additional functionalities, 2-azido-5-(tri-*n*-butyltin)benzoic acid **4**, was prepared by a method described for a methyl homologue[7] starting from 2-amino-5-iodobenzoic acid **5**. It was transformed firstly into 2-azido-5-iodobenzoic acid **6a** and then, by Pd^0-catalysed stannylation of the corresponding methyl ester **6b** with hexa-*n*-butylditin followed by ester hydrolysis, into the target acid **4** (Scheme 2).[§,¶]

The alcohol **3d** was to be esterified with substituted benzoic acid **4** into diester **1a**. It should be noted that our initial attempts to employ for this task a variety of diimide reagents [*e.g.* dicyclohexylcarbodiimide, hydrochloride of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (DMAPEC), and corresponding methiodide] under the conditions (DMAP in anhydrous dichloromethane) described for a very similar substrate,[7] as well as under many other conditions,[††] completely failed. Using acid **6a** as a model we observed under all conditions mainly the formation of an acid **6a** anhydride, and no corresponding diester **1c** was obtained. The acid anhydride formed was found to be unreactive towards hydroxyl groups, even those of methanol. Similar results were observed with acid **4**. Paradoxically, a full conversion into diesters **1a**,**c** was achieved with both acids under the same conditions with DMAPEC but using commercial dichloromethane as received, without any additional drying. We guess that adventitious



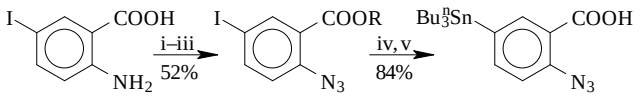
Scheme 1 *Reagents and conditions*: i, PhCOOH, DEAD, PPh_3, benzene, 20 °C, 5 min; ii, MeONa, MeOH, 20 °C, 12 h; iii, Bu^n_4NF, THF, 20 °C, 12 h; iv, **4** or **6a**, $Me_2N(CH_2)_3N{=}C{=}NEt{\cdot}HCl$, DMAP, 20 °C, 24 h; v, MeONa, MeOH, 20 °C, 3 h; vi, SP-HPLC, μPorasil 3.9×300 mm, 1.5% Pr^iOH in hexane, 2.0 ml min^{-1}; vii, NaI, $TsN(Cl)Na{\cdot}xH_2O$, 20 °C, 1.5 h.

† Part 6 of a series 'Synthetic Research of Hepoxilins'. For part 5, see ref. 11.

‡ *Trivial names and abbreviations*: HxA_3: 11(*S*),12(*S*)-epoxy-8(*R*)- and 8(*S*)-hydroxyeicosa-5(*Z*),9(*E*),14(*Z*)-trienoic acids; HxB_3: 11(*S*),12(*S*)-epoxy-10(*R*)- and 10(*S*)-hydroxyeicosa-5(*Z*),8(*Z*),14(*Z*)-trienoic acids.

§ *Physical data* for **6a**: mp 136–140 °C (decomp.), R_f 0.49 (EtOAc–hexane–AcOH, 100 : 100 : 0.1). ^1H NMR (500 MHz, $CDCl_3$) δ/ppm: 7.02 (d, 1H, *J* 8.4 Hz, H^3), 7.88 (dd, 1H, *J* 2.2 and 8.4 Hz, H^4), 8.42 (d, 1H, *J* 2.2 Hz, H^6).

For **6b**: mp 61–62 °C, R_f 0.66 (EtOAc–hexane, 1 : 9). ^1H NMR (250 MHz, $CDCl_3$) δ/ppm: 3.91 (s, 3H, COOMe), 7.15 (d, 1H, *J* 8.7 Hz, H^3), 7.80 (dd, 1H, *J* 2.2 and 8.7 Hz, H^4), 8.15 (d, 1H, *J* 2.2 Hz, H^6).



5 **6a** R = H **6b** R = Me **4**

Scheme 2 *Reagents and conditions*: i, $NaNO_2$, H^+, 0 °C, 30 min; ii, NaN_3, 0–20 °C, 2 h; iii, CH_2N_2; iv, $(Bu^n_3Sn)_2$, $Pd(PPh_3)_4$, 50 °C, 8 h; v, NaOH, MeOH–H_2O, 20 °C, 4 h.

traces of moisture in commercial reagents preferentially catalyse an acylation of the hydroxyl group, thus preventing the anhydride formation.[‡‡]

Partial alkaline hydrolysis of diester **1a** was not selective and led at 53% conversion to a mixture of equal amounts of the target 20-azido(tri-*n*-butyltin)benzoate of 20-hydroxy-(8*S*)-HxA_3 ME **1b** and 8-benzoate **3d**. After isolation by HPLC the yield of **1b** amounted to 40% on the unrecovered **1a**. The obtained compound may serve as a direct precursor in the preparation of [^{125}I]-labelled photoaffinic hepoxilin analog, 20-azidoiodobenzoate of 20-hydroxy-(8*S*)-HxA_3 ME **1d**. This was demonstrated experimentally by the electrophilic substitution of tri-*n*-butylstannyl group in **1b** with iodine using NaI in the presence of chloramine T, which smoothly gave unlabelled analog **1d**.[§§] The results of the biological testing of the hepoxilin photoaffinity probes synthesized will be reported in due course.

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References

1 C. R. Pace-Asciak, D. Reynaud and P. M. Demin, *Lipids*, 1995, **30**, 107.
2 C. R. Pace-Asciak, *Biochim. Biophys. Acta*, 1994, **1215**, 1.
3 D. Reynaud, P. Demin and C. R. Pace-Asciak, *Biochem. Biophys. Res. Commun.*, 1995, **207**, 191.
4 D. Reynaud, P. M. Demin and C. R. Pace-Asciak, *Biochem. J.*, 1996, **313**, 537.
5 G. L. Tonnesen, R. N. Hanson and D. E. Seitz, *Int. J. Appl. Radiation Isotopes*, 1981, **32**, 171.
6 L. C. Blaszczak, N. G. Halligan and D. E. Seitz, *J. Labelled Comp. Radiopharm.*, 1988, **27**, 401.
7 H. Perrier, P. Prasit and Z. Wang, *Tetrahedron Lett.*, 1994, **35**, 1501.
8 M. Abramovitz, J. Mancini, M. Cox, E. Wong, S. Charleson, H. Perrier, Z. Wang, P. Prasit, C. Richardson and P. Vickers, *FEBS Lett.*, 1993, **318**, 277.
9 P. M. Demin, T. A. Manukina, C. R. Pace-Asciak and K. K. Pivnitsky, *Mendeleev Commun.*, 1996, 130.
10 P. M. Demin, K. K. Pivnitsky, L. L. Vasiljeva and C. R. Pace-Asciak, *J. Labelled Comp. Radiopharm.*, 1994, **34**, 221.
11 L. L. Vasiljeva and K. K. Pivnitsky, *Mendeleev Commun.*, 1996, 251.

¶ *Stannylation of* **6b**. To the methyl ester **6b** (200 mg, 0.66 mmol) in dioxane (3.0 ml) was added freshly prepared $Pd(PPh_3)_4$ (15.2 mg, 2 mol%) and the mixture was purged with argon for 15 min. After addition of hexa-*n*-butylditin (1.9 g, 3.3 mmol) the darkened solution was stirred at 50 °C for 8 h under argon. TLC (EtOAc–hexane, 1 : 4) showed the formation of a less polar spot (R_f 0.63, EtOAc–hexane, 1 : 4). The deep-yellow reaction mixture was diluted with aqueous NH_4Cl, extracted with benzene, dried with Na_2SO_4 and evaporated. Subsequent purification of the residual dark oil by column chromatography (EtOAc–hexane, 1 : 9) gave the methyl ester of acid **4**, yield 260 mg (84%), yellow oil. This ester was treated with an excess of 1.5% NaOH in H_2O–MeOH (1 : 1) for 4 h at 20 °C affording the acid **4** quantitatively as a yellow oil, R_f 0.36 (EtOAc–hexane–AcOH, 33 : 64 : 0.1). ^{1}H NMR (500 MHz, $CDCl_3$) δ/ppm: 0.88, 1.09, 1.33, 1.50 (m, 27H, $SnBu^n_3$), 7.22 (d, 1H, *J* 7.8 Hz, H^3), 7.69 [br. d, 0.85H, *J* 7.8 Hz, + dd, 0.15H, *J* 7.8 and 34 (H-$^{117+119}$Sn spin-spin coupling) Hz, H^4], 8.24 [br. s, 0.85H, + dd, 0.15H, *J* 34 (H-$^{117+119}$Sn) Hz, H^6].

†† In addition to acid **6a** we also tried to use for esterification derivatives of acid **6a** obtained by ordinary methods: acid anhydride (mp 70–72 °C), *N*-hydroxysuccinimide ester (mp 153–155 °C) and amide with glycine (mp 161–163 °C), without any success as well.

‡‡ *Synthesis of* **1a**. To a solution of 8-benzoate of 20-hydroxy-(8*S*)-HxA_3 methyl ester **3d** (1.5 mg, 3.19 μmol) and of 2-azido-5-(tri-*n*-butyltin)benzoic acid **4** (5.75 mg, 12.8 μmol) in commercial dichloromethane (1.0 ml, from Caledon, Canada), DMAPEC (3.7 mg, 19.2 μmol) and DMAP (0.08 mg, 0.64 μmol) were added successively. The mixture was kept for 24 h at 20 °C, dichloromethane was concentrated to 0.1 ml and this was passed through a small capillary column of silica gel, eluent EtOAc–hexane, 1 : 2. Eluted substance was purified by preparative TLC (EtOAc–hexane, 1 : 4, R_f 0.25), giving diester **1a** as a photo-unstable crystallizing oil, yield 2.2 mg (76%).

§§ *Iodination of* **1b**. To a solution of azidotributyltinbenzoate **1b** (5 μg) in DMF (20 μl) the solutions of sodium iodide and chloramine T (each 0.1 M in phosphate buffer, pH 7.5, 10 μl) were added in succession. The yellow solution was kept for 1.5 h without stirring and worked up with 0.2 M sodium hydrogensulfite followed by extraction with EtOAc. TLC analysis showed complete conversion into azidoiodobenzoate **1d** (R_f 0.31, EtOAc–hexane, 1 : 1, whereas starting material had R_f 0.39).

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