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Total synthesis of 20-hydroxy-hepoxilins, new metabolites of the hepoxilin family

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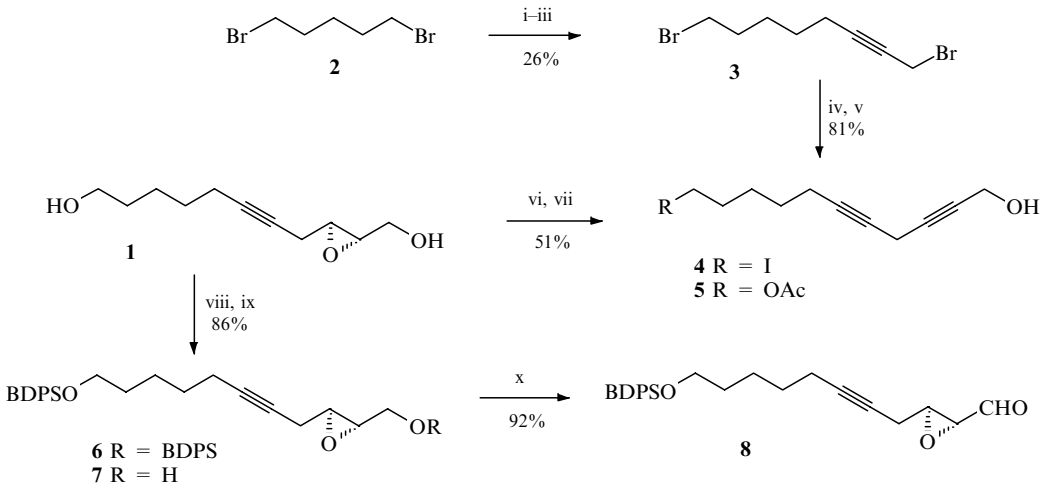
A newly described enzymatically formed ω-hydroxy metabolite of hepoxilin A_3 (HxA_3) has been prepared *via* total synthesis involving polyacetylenic intermediates and Mitsunobu rearrangement of the intermediate putative ω-hydroxy metabolite of hepoxilin B_3.

We have recently observed that HxA_3 is metabolised by intact human neutrophils through a new pathway involving ω-oxidation.[1] The metabolite was shown to be ω-hydroxy-HxA_3, demonstrating that the intact human neutrophils lack metabolism of hepoxilins *via* the epoxide hydrolase pathway that was identified previously in other tissues for the metabolism of hepoxilins.[2,3] We describe herein the total chemical synthesis of 20-hydroxy-HxA_3 which is identical to the metabolite formed by human neutrophils. This metabolite is biologically active on human neutrophils causing the release of intracellular calcium and is able to bind competitively to the putative hepoxilin receptor in these cells.[1]

(2*S*,3*S*)-Epoxy-1,11-dihydroxyundec-5-yne **1** served as a key synthon. The diol **1** was obtained starting from 1,5-dibromopentane **2**. Its condensation with 1 equiv. of lithio-1-tetrahydropyranyloxyprop-2-yne in DMSO followed by OTHP→OH→Br exchange (see Scheme 1) produced the dibromide **3** in 26% overall yield. The condensation of dibromide **3** with propargyl alcohol under the conditions described[4] proceeded selectively at the 1-position of the molecule; in this reaction the aliphatic bromine exchanges with iodine, thus producing cleanly (yield 85%) the diacetylenic hydroxyiodide **4**. The future 20-hydroxyl functionality was created *in situ* by exchange of the iodide **4** with an acetate ion affording the acetate **5** in 81% overall yield. Reduction of the propargylic triple bond with $LiAlH_4$ accompanied by simultaneous acetate reduction was followed by Sharpless asymmetric epoxidation of the formed allylic *trans*-double bond and led to the key synthon **1** in 51% overall yield. The enantiomeric purity of synthon **1** was increased up to ee >95% by crystallisation, mp 24–26 °C (EtOAc) and confirmed by HPLC analysis of its mono-*tert*-butyldiphenylsilyl (BDPS) ether **7** on a chiral stationary phase.†

Several approaches were tested to differentiate the two hydroxyl groups of the diol **1** and to prepare the desired corresponding 1-aldehyde **8**. Oxidation under mild conditions



Scheme 1 *Reagents and conditions*: i, $HC{\equiv}CCH_2OTHP$, Bu^nLi, DMSO, 20 °C, 3 h; ii, IR-120 (H^+), MeOH, 20 °C, 3 h; iii, PBr_3, Et_2O, 20 °C, 1 h; iv, $HC{\equiv}CCH_2OH$, CuI, NaI, K_2CO_3, 20 °C, 20 h; v, AcOK, DMF, 50 °C, 4 h; vi, $LiAlH_4$, Et_2O, reflux, 9 h; vii, Bu^tOOH, $Ti(OPr^i)_4$, (+)-DET, MS 4 A, CH_2Cl_2, −20 °C, 10 d; viii, BDPSCl, ImH, DMF, 60°C, 1 h; ix, Bu^n_4NF, C_6H_6–THF, 5 : 1, 20 °C, 4 h; x, $Py_2H_2Cr_2O_7$, MS 4 A, CH_2Cl_2, 20 °C, 2 h.

using pyridinium dichromate produced, with high regioselectivity, an undesired 11-aldehyde. Silylation using an equimolar amount of (BDPS)chloride in the presence of pyridine led to a mixture consisting of the starting diol **1**, its BDPS bis-ether **6** and a mixture of the BDPS mono-ether **7** and its regioisomer, from which the required ether **7** was separated with a yield of 15% only. However, partial desilylation of the bis-ether **6**, (quantitatively produced with an excess of BDPSCl)[5] was regiospecific. Bis-ether **6** on treatment with an equimolar amount of Bu^n_4NF in benzene–THF produced, in 55% conversion, the single mono-ether **7** (isolated in 91% yield based on consumed **6**) and trace amounts of the diol **1**. The unconverted bis-ether **6** was recovered and recycled.‡

Condensation of aldehyde **8**, which was obtained by oxidation of alcohol **7** with pyridinium dichromate, with the Li-derivative of propargyl chloride led to the diacetylenic chloride **9a**,**b** isolated as a diastereomeric mixture (1 : 1.5) (see Scheme 2). Condensation of the latter (without separation) with methyl hexynoate[4] gave the triacetylenic precursor of 20-hydroxy-HxB_3 **10a**,**b**. Lindlar hydrogenation, monitored by RP-HPLC,§ afforded the epimeric 20-BDPSO-HxB_3 methyl esters **11a**,**b** with 92% selectivity. Epimers **11a** and **11b** were separated and their relative configuration was assigned from NMR data.¶ After removal of the BDPS group with Bu^n_4NF the two 10-epimeric 20-hydroxy-HxB_3 methyl esters **12a**,**b** were obtained and then subjected to Mitsunobu inversion/rearrangement followed by debenzoylation. As expected,[6]

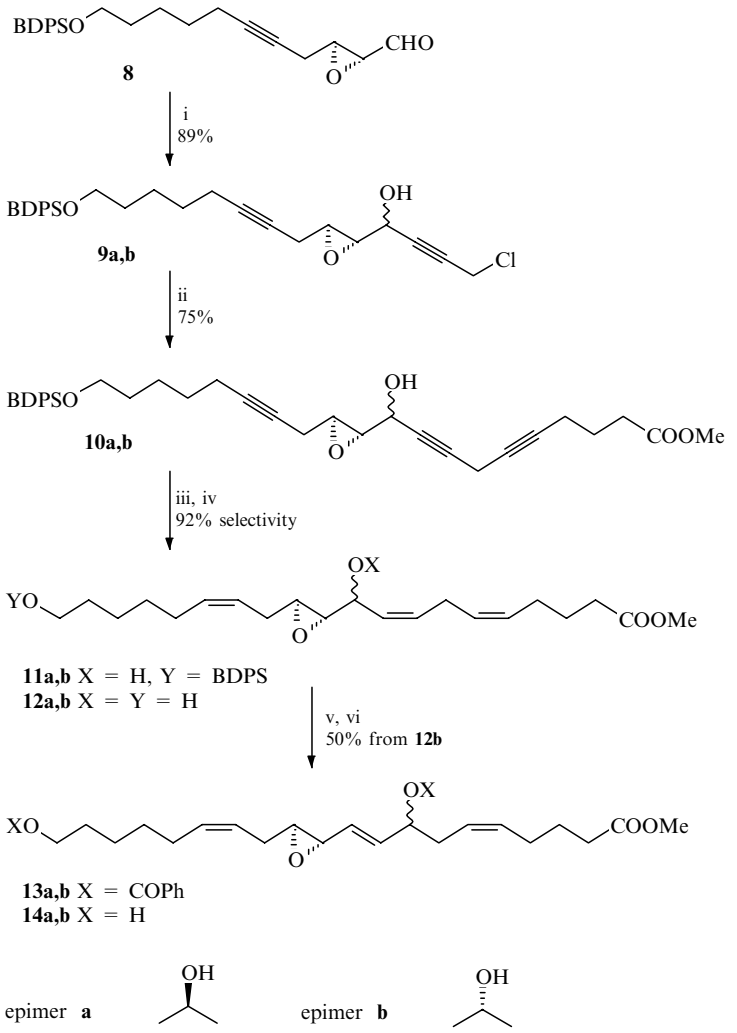
† Column Chiracel OD [cellulose tris(2,4-dimethylphenylcarbamate) on silica], 4.6×250 mm, eluent 1.0% Pr^iOH in hexane, 1 ml min^{-1}, UV detector at 220 nm: a single peak at 12 min.

‡ *Synthesis of* **7**. A solution of BDPS bis-ether **6** (0.74 mmol) and Bu^n_4NF (1.1 mmol as a 1 mol dm^{-3} solution in THF) in C_6H_6–THF (5 : 1, 10 ml) was stirred for 4 h at 20 °C, then diluted with water (100 ml) and extracted with EtOAc. The resultant mixture was separated by flash chromatography, giving 50% of the mono-ether **7** and 45% of the recovered bis-ether **6**. For **7** ^{1}H NMR spectrum (500 MHz, $CDCl_3$) δ(ppm): 1.05 (s, 9H, Si-CMe_3), 1.45 and 1.55 [2×m, 6H, H_2-(8+9+10)], 2.13 (m, 2H, H-7), 2.49 and 2.60 (2×br.d, 2H, *J* 15 Hz, H_2-4), 3.11 (m, 2H, H-(2+3)), 3.65 (overlapping t and br.signal, 3H, *J* 6.41 Hz, H_2-11 and H′-1), 3.92 (br.d, 1H, *J* 13 Hz, H″-1), 7.40 and 7.67 (2×m, 10H, $SiPh_2$). The structure of BDPS mono-ether **7** was established by the oxidation into the corresponding aldehyde **8**. Its ^{1}H NMR spectrum (500 MHz) showed a doublet at 9.05 ppm (*J* 5.99 Hz, CHO), whereas the signal for its regioisomer (obtained by partial direct silylation and oxidation) showed a triplet at 9.76 ppm (*J* 1.71 Hz, CHO).

§ Column Nova-Pak RP C18, 3.9×300 mm, eluent MeCN–H_2O, 80:20, 1.5 ml min^{-1}, UV detector at 220 nm, retention times: **10a** and **10b** 16.0 min, **11a** 30.9 min, **11b** 30.2 min.

¶ ^{1}H NMR spectra (500 MHz, $CDCl_3$) δ/(ppm): **11a**: 1.02 (s, 9H, $SiCMe_3$), 1.34 [m, 6H, H_2-(17+18+19)], 1.70 (quintet, 2H, *J* 7.26 Hz, H_2-3), 2.02 and 2.10 [m, 4H, H_2-(4+16)], 2.26 and 2.39 (2×m, 2H, H_2-13), 2.32 (t, 2H, *J* 7.26 Hz, H_2-2), 2.80 and 2.92 (2×m, 2H, H_2-7), 2.84 (dd, 1H, *J* 2.35 and 3.20 Hz, H-11), 3.04 (dt, 1H, *J* 2.35 and 7.69 Hz, H-12), 3.65 (t, 2H, *J* 6.62 Hz, H_2-20), 3.67 (s, 3H, OMe), 4.67 (br.d, 1H, *J* 8.55 Hz, H-10), 5.37, 5.51 and 5.60 (3×m, 6H, olefinic H), 7.40 and 7.66 (2×m, 10H, $SiPh_2$).
11b: 1.02 (s, 9H, $SiCMe_3$), 1.34 [m, 6H, H_2-(17+18+19)], 1.69 (quintet, 2H, *J* 7.48 Hz, H_2-3), 2.00 and 2.09 [2×m, 4H, H_2-(4+16)], 2.28 and 2.40 (2×m, 2H, H_2-13), 2.31 (t, 2H, *J* 7.48 Hz, H_2-2) 2.80 and 2.92 (2×m, 2H, H_2-7), 2.84 (dd, 1H, *J* 2.35 and 4.91 Hz, H-11), 2.97 (dt, 1H, *J* 2.35 and 5.55 Hz, H-12), 3.65 (t, 2H, *J* 6.41 Hz, H_2-20), 3.67 (s, 3H, OMe), 4.33 (ddd, 1H, *J* 5.02, 7.90 and 8.76 Hz, H-10), 5.37, 5.51 and 5.58 (3×m, 6H, olefinic H), 7.40 and 7.66 (2×m, 10H, $SiPh_2$).
Configurations of **11a**,**b** at C-10 were deduced from data of H-10 signals, in agreement with our previous publication.[7]

* *Synthesis of* **14**. To a solution of a mixture of 13 μmol of **12b** in 1 ml of dry THF, PhCOOH, diethyl azodicarboxylate (DEAD) and then PPh_3 (52 mmol of each) were added at 20 °C. After 5 min of stirring the reaction mixture was analysed by TLC showing the complete conversion into dibenzoates. The reaction was quenched by the addition of 100 μl of MeOH and the solvent was evaporated to dryness. The benzoate group was removed by addition of 25% MeONa in MeOH (400 μl, 20 °C, 3 h). The reaction mixture was freed of the reagents on a 20×20 cm TLC plate with silica gel and EtOAc–hexane (2 : 1) as the developing solvent. The separation was performed by RP-HPLC (column Nova-Pak RP C18, 3.9×300 mm, eluent MeCN–H_2O, 60:40, UV detector at 210 nm) resulting in isolation of **12a** and 20-hydroxy-HxA_3 methyl esters **14a**,**b** in yields of 50%. ^{1}H NMR spectrum (500 MHz, $CDCl_3$) δ(ppm) for **14a**,**b**: 1.38 [m, 6H, H_2-(17+18+19)], 1.71 (quintet, 2H, *J* 7.47 Hz, H_2-3), 2.06 and 2.10 [2×m, 4H, H_2-(4+16)], 2.30 and 2.40 (2×m, 2H, H_2-13), 2.32 (t, 2H, *J* 7.47 Hz, H_2-2), 2.88 (dt, 1H, *J* 2.14 and 5.34 Hz, H-12), 3.16 (dd, 1H, *J* 2.14 and 7.69 Hz, H-11), 3.64 (t, 2H, *J* 6.41 Hz, H_2-20), 3.67 (s, 3H, OMe), 4.18 (br.q, 1H, *J* 5.77 Hz, H-8), 5.38 and 5.54 (2×m, 4H, olefinic H), 5.47 (ddd, 1H, *J* 1.28, 7.69 and 15.49 Hz, H-10), 5.96 (dd, 1H, *J* 5.77 and 15.49 Hz, H-9). The presence of much smaller amounts of a second 8-epimer is manifested only by a very small additional splitting ($\Delta\delta$ −0.006 ppm) of the H-9 signal. Mass-spectrum (EI mode, trimethylsilyl bis-ether, in the course of chromatography on a 30 m capillary column HP-1) relative intensity: 369 [(C-8−C-20)$^+$, 13.8], 279 [(369−TMSOH)$^+$, 4.1], 243 [(C-1−C-8)$^+$, 2.4], 103 [(C-20)OTMS$^+$, 100%].



Scheme 2 *Reagents and conditions*: i, $HC{\equiv}CCH_2Cl$, Bu^nLi, Et_2O, $-70\,^\circ C$, 15 min; ii, $HC{\equiv}C(CH_2)_3COOMe$, CuI, NaI, K_2CO_3, 20 °C, 12 h; iii, H_2, Lindlar, quinoline, 20 °C, 60 min; iv, Bu^n_4NF, THF, 20 °C, 3 h; v, PhCOOH, DEAD, PPh_3, THF, 20 °C, 5 min; vi, MeONa, MeOH, 20 °C, 3 h.

(10*R*)-epimer **12b** was transformed with partial allylic rearrangement into a 1 : 1 mixture of the pair of 20-hydroxy-HxA_3 methyl esters **14a**,**b** and 20-hydroxy-HxB_3 **12a**, whereas for the (10*S*)-epimer **12a** gave **12b** exclusively. The two 8-epimeric 20-hydroxy-HxA_3 methyl esters **14a**,**b** as well as the intermediate dibenzoates **13a**,**b** were indistinguishable both chromatographically and spectrometrically.*

The chromatographic and spectral data obtained for the synthetic sample of 20-hydroxy-HxA_3 methyl ester **14** were identical to those of the methylated biological sample of ω-hydroxy metabolite of HxA_3 prepared by incubation of HxA_3 methyl ester with human neutrophils.[1] The results of the biological testing of these synthetic hepoxilin metabolites will be reported in due course.

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