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Research Article

Preparation of isotopically labelled benzophenone-containing lipid analogues

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Summary

Methods have been developed for isotopic labelling of the benzophenone-containing cholesterol analogue **1** and the fatty acid analogue **2** by use of deuteriated sodium borohydride derivatives. These procedures avoid the commercial catalytic tritiation which had previously been used to obtain ^{3}H-**1** and ^{3}H-**2**. Copyright © 2005 John Wiley & Sons, Ltd.

Key Words: benzophenones; cholesterol; fatty acids; deuteriation; borohydrides

Introduction

We have been preparing benzophenone-containing analogues of sterols[1] and phospholipids[2] for use as photoaffinity labelling agents in studies of cellular cholesterol efflux and HDL formation.[3–5] In particular, the cholesterol analogue **1**, known as FCBP,[3] and the fatty acid analogue **2** (Figure 1),[2] were required in radioactive form for use as biochemical research tools. This has been accomplished to date by commercial catalytic tritiation of the brominated *i*-steroid derivative **3**[1] and unsaturated acid **4**[2], respectively. However, these catalytic tritiations have been somewhat capricious, requiring carefully controlled conditions to avoid undesired reduction of the benzophenone carbonyl group; they are also expensive. Therefore, we wished to developed alternative procedures for isotopic labelling of **1** and **2** that could be conducted in our own laboratories. This paper describes such procedures, employing deuteriated borohydride derivatives.

Results and discussion

The method adopted for labelling of FCBP (**1**) was an adaptation of that of Malik *et al.*[6] for tritiation of cholesterol via mild pyridinium chlorochromate

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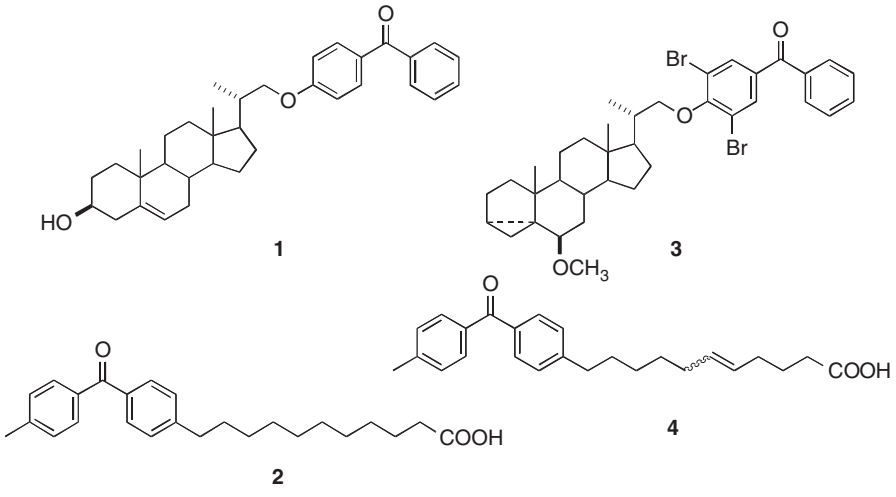
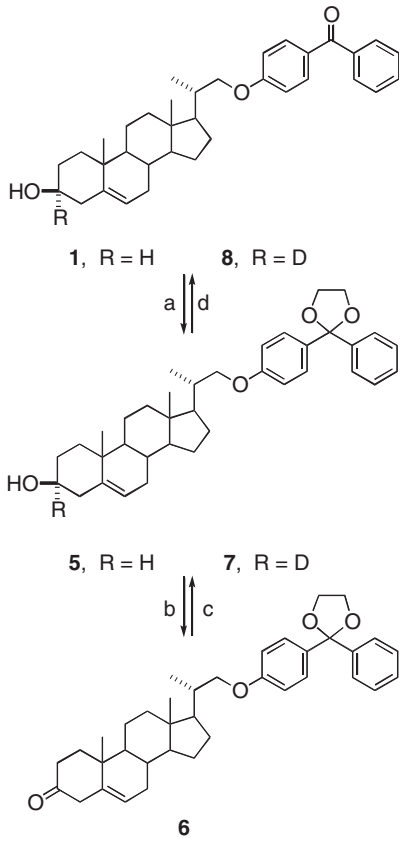


Figure 1. Structures of photoactivable lipid analogues and tritiatable derivatives

(PCC) oxidation to 5-cholesten-3-one followed by reduction with tritiated sodium borohydride (NaB^3H_4). In applying this approach to **1**, of course, it was necessary initially to protect the benzophenone carbonyl group, which was accomplished by treatment of **1** with acidic ethylene glycol to afford ethylene ketal **5** (Scheme 1). Oxidation of **5** with PCC according to the procedure of Parish *et al.*[7] then afforded unconjugated ketone **6**. In order to establish the validity of this approach for introduction of isotopic labelling, NaB^2H_4 was used to reduce **6** back to 3α-^{2}H-**5** (**7**), which was converted without purification to 3α-^{2}H-**1** (**8**) by hydrolysis in aqueous acidic acetone. The ^{1}H NMR spectra of **1** and the **8** thus produced are shown in Figure 2, clearly demonstrating essentially complete absence of ^{1}H at the 3α position of **8**. The single absorbance at δ 3.55 in the ^{2}H NMR spectrum of **8** and the essentially complete disappearance of a peak at δ 72.0 in the ^{1}H-decoupled ^{13}C NMR spectrum of **8** confirmed its 3α deuteriation. The appearance of ^{13}C NMR signals for carbon atoms bonded to ^{2}H depends on experimental conditions such as spin-lattice relaxation time, and can vary from a distinct multiplet to no detectable signal; e.g. see, Cunnane *et al.*[8]

As an alternative to commercial catalytic tritiation of unsaturated acid **4** for radiolabelling of **2**, we decided to investigate benzylic bromination followed by chemical reduction. This approach was initially explored with the 2:1 mixture of monobromo **9** and dibromo **10** produced by treatment of ester **11**[2] with 1.5 equivalents of NBS in CCl_4 in the presence of benzoyl peroxide (Scheme 2), since we were ultimately interested in radiolabelling phosphatidylcholine analogues containing acid **2** esterified in the C1 or C2 acyl chain.[2] When the



Scheme 1. (a) $HOCH_2CH_2OH$, PTSA, PhH, Δ; (b) PCC, $CaCO_3$, 4 Å molecular sieves, CH_2Cl_2, rt, 30 min; (c) NaB^2H_4, CH_3OH, rt, 1 h; and (d) 9:1 acetone:H_2O, PPTS, Δ

mixture of **9** plus **10** was treated with NaB^2H_3CN in HMPA at 90°C for 48 h, according to the procedure of Hutchins *et al.*,[9] the ^1H NMR spectrum of the product showed complete disappearance of the peaks at δ 4.98 and 4.54 characteristic of the protons on the brominated positions of **9** and **10**, and the ^2H NMR spectrum of the product showed peaks at δ 2.69 and 2.46, indicating clearly the formation of a mixture of **12** and **13**. This conclusion was confirmed by comparison of the ^{13}C NMR spectra of **12** plus **13** vs **11**, which showed that the singlets at δ 36.2 and 31.4 in **11**, had, in **12** plus **13**, essentially disappeared and diminished in intensity, respectively.

The reaction of acid **2** itself with NBS under the same conditions likewise produced a ca 2:1 mixture of **14** and **15**. Debromination of **14** plus **15** was initially, but unrewardingly, attempted with zinc in acetic acid.[10] Reduction of **14** plus **15** with NaB^2H_3CN, however, again proved successful, resulting in a

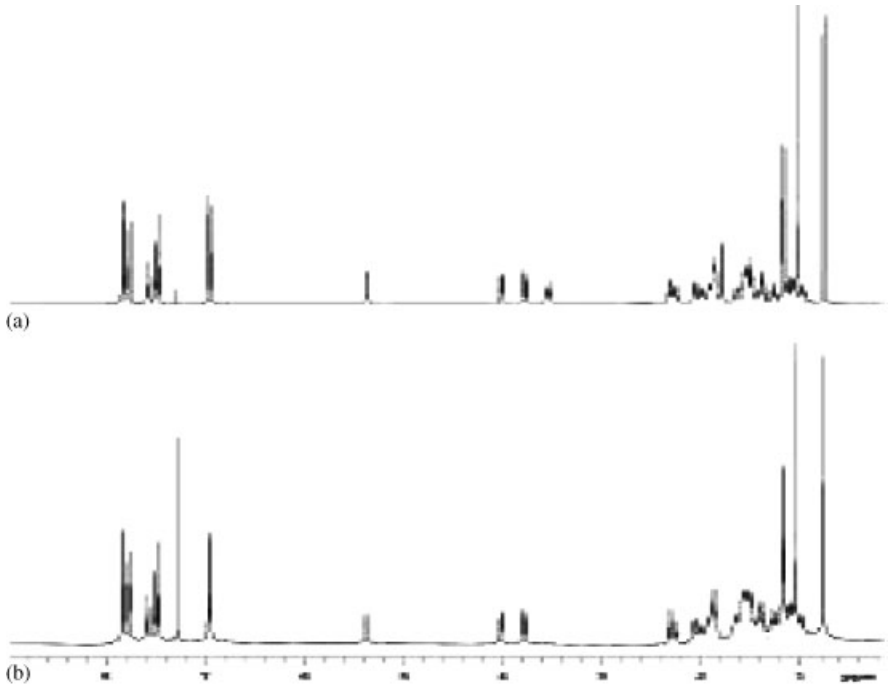
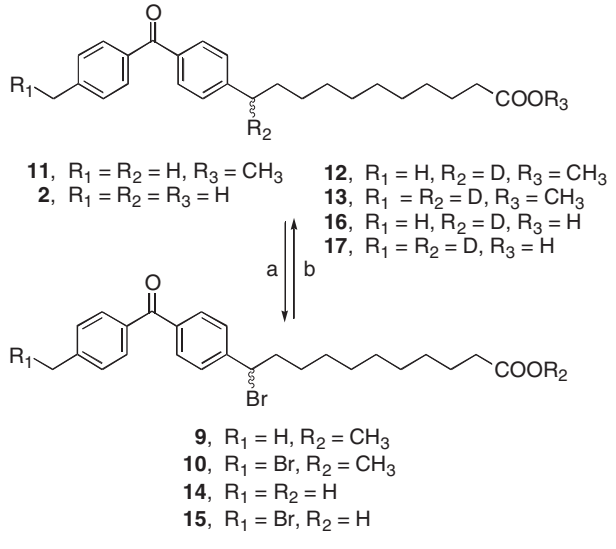


Figure 2. ^{1}H NMR spectra of: (a) FCBP (1) and (b) 3α-^{2}H-1 (8) showing disappearance of peak for 3α-^{1}H at δ 3.55



Scheme 2. (a) NBS, CCl_4, $(PhCO)_2O_2$; (b) NaB^2H_3CN, HMPA, 90°C, 48 h

moderate yield of a clean mixture of **16** and **17**, which showed changes in its ^{1}H, ^{2}H, and ^{13}C NMR spectra completely analogous to those observed for **12** plus **13**.

Experimental

General methods

^{1}H NMR and ^{13}C NMR spectra were taken at 300 MHz in $CDCl_3$ unless otherwise indicated. Flash column chromatography was carried out on EM Reagent silica gel 60 (230–400 mesh). Thin layer chromatography (TLC) was performed on polyester sheets precoated with silica gel 60 F-254. All plates were visualized by a UV_{254} light source or staining with 5% phosphomolybdic acid in ethanol. Na_2SO_4 was used as a drying agent unless otherwise noted. Methylene chloride was distilled from CaH_2. Reagents were obtained from Aldrich, and were used without further purification unless otherwise noted.

*22-(p-2-Phenyl-[1,3]dioxolan-2-ylphenoxy)-23,24-bisnorcholan-5-en-3β-ol (**5**)*

To a solution of 200 mg (0.39 mmol) of **1** and 0.11 ml (1.95 mmol) of ethylene glycol in 5 ml of dry benzene was added 4 mg (0.02 mmol) of *p*-toluenesulphonic acid (PTSA). The mixture was heated at reflux overnight, cooled, diluted with 80 ml of EtOAc, washed with 2×15 ml of saturated $NaHCO_3$ and 2×10 ml of brine, dried, filtered, and evaporated to give 243 mg of residue which was chromatographed on 8 g of silica gel with 9:1 to 3:1 hexane:EtOAc to give 150 mg (69%) of **5** as a colourless oil: ^{1}H NMR (300 MHz) δ 7.53 (m, 2H), 7.36 (m, 5H), 6.84 (m, 2H), 5.38 (s, 1H), 4.06 (m, 4H), 3.92 (dd, $J = 9.0$, 3.0 Hz, 1H), 3.65 (dd, $J = 9.0$, 7.0 Hz, 1H), 3.55 (m, 1H), 2.27-0.91 (m, 22H), 1.12 (d, $J = 6.6$ Hz, 3H), 1.04 (s, 3H), 0.74 (s, 3H); ^{13}C NMR (75 MHz) δ 159.5, 142.5, 141.1, 134.2, 132.9, 128.4, 128.3, 126.5, 121.8, 114.3, 109.7, 73.2, 71.9, 65.1, 56.7, 53.0, 50.4, 42.8, 42.5, 39.9, 37.6, 36.8, 36.7, 32.2, 32.1, 31.9, 28.1, 24.7, 21.4, 19.7, 17.7, 12.2. Analytically Calculated for $C_{37}H_{48}O_4$: C, 79.82; H, 8.69. Found: C, 79.60; H, 8.63.

*22-(p-2-Phenyl-[1,3]dioxolan-2-ylphenoxy)-23,24-bisnorcholan-5-en-3β-one (**6**)*

According to a procedure by Parish *et al.*,[7] a mixture of 22.7 mg (0.045 mmol) of **5**, 19 mg (0.15 mmol) of $CaCO_3$ and 4 mg of 4 Å molecular sieves in 4 ml of dry CH_2Cl_2 was stirred at room temperature for 15 min. Then 33 mg (0.15 mmol) of PCC was added. The mixture was stirred at room temperature for 30 min, diluted with 3 ml of brine, and extracted with 3×40 ml of ether. The combined organic layers were passed through a short pad of $MgSO_4$, and evaporation of the filtrate gave 32 mg of residue which was chromatographed on 3 g of silica gel with 15:1 to 4:1 hexane:EtOAc to give 17 mg (75%) of **6** as a wax-like solid, plus 4.5 mg of overoxidized product: ^{1}H NMR (500 MHz) δ 7.52 (m, 2H), 7.40 (m, 2H), 7.32 (m, 3H), 6.84 (m, 2H), 5.36 (br, 1H), 4.10 (m, 4H), 3.92 (dd, $J = 9.0$, 3.0 Hz, 1H), 3.66 (dd, $J = 9.0$, 7.0 Hz, 1H), 3.30 (dd, $J = 16.5$, 2.5 Hz, 1H), 2.85 (dd, $J = 16.5$, 2.5 Hz, 1H), 2.50 (m, 1H), 2.34

(m, 1H), 2.08 (m, 3H), 1.88 (m, 1H), 1.65-0.86 (m, 13H), 1.22 (s, 3H), 1.15 (d, $J = 6.6$ Hz, 3H), 0.77 (s, 3H); ^{13}C NMR (125 MHz) δ 210.6, 159.5, 142.5, 138.8, 134.2, 128.3, 128.2, 127.8, 126.5, 123.1, 114.2, 109.7, 73.2, 65.1, 56.5, 52.9, 49.4, 48.6, 42.8, 39.7, 37.9, 37.1, 36.7, 32.2, 32.0, 29.9, 28.0, 24.6, 21.6, 19.4, 17.7, 12.2.

Conversion of **6** *to* **8**

To a solution of 35 mg (0.063 mmol) of **6** in 2 ml of MeOH was added 8 mg (0.189 mmol) of NaB^2H_4. The mixture was stirred at room temperature for 1 h, diluted with 2 ml of brine, extracted with 3×30 ml of CH_2Cl_2, washed with 2×5 ml of brine, dried over Na_2SO_4, filtered, evaporated to give 36 mg of crude **7**. This material was dissolved in 10 ml of 9:1 acetone:H_2O solution and treated with 46 mg (0.185 mmol) of pyridium toluenesulphonate (PPTS). The resulting mixture was heated at reflux overnight, cooled, evaporated, diluted with 50 ml of CH_2Cl_2, and washed with 2×5 ml of sat. $NaHCO_3$ solution and 5 ml of brine. The organic layer was separated and evaporated to give 39 mg of residue which was chromatographed on 3 g of silica gel with 4:1 hexane:EtOAc to give 23 mg (72%) of **8** as a colourless solid: ^{1}H NMR (500 MHz) δ 7.84-7.76 (m, 4H), 7.57 (m, 1H), 7.49 (m, 2H), 6.95 (m, 2H), 5.38 (br, 1H), 4.01 (dd, $J = 9.0$, 3.0 Hz, 1H), 3.76 (dd, $J = 9.0$, 7.0 Hz, 1H), 2.33-0.97 (m, 22H), 1.16 (d, $J = 6.5$ Hz, 3H), 1.05 (s, 3H), 0.78 (s, 3H); ^{2}H NMR δ 3.55; ^{13}C NMR δ 195.8, 163.4, 141.0, 138.5, 132.8, 132.0, 130.0, 129.9, 128.3, 121.8, 114.2, 73.5, 56.6, 52.8, 50.3, 42.8, 42.5, 39.8, 37.5, 36.7, 36.6, 32.1, 32.1, 31.8, 28.0, 24.6, 21.3, 19.6, 17.6, 12.2.

*Bromination of methyl 11-[4-(4-methylbenzoyl)-phenyl]-undecanoate (***11***) to* **9** *plus* **10**

To a solution of 316 mg (0.8 mmol) of **11** in 30 ml of CCl_4 was added 216 mg (1.21 mmol) of NBS and 37 mg (0.15 mmol) of benzoyl peroxide. The mixture was heated at reflux for 14 h, cooled, filtered, and evaporated to give 820 mg of residue which was chromatographed on 5 g of silica gel with 25:1 to 20:1 hexane:EtOAc to give 200 mg (53%) of **9** and 109 mg (25%) of **10** as colourless oils. Compound **9**: ^{1}H NMR δ 7.74 (m, 4H), 7.49 (m, 2H), 7.28 (m, 2H), 4.98 (t, $J = 7.5$ Hz, 1H), 3.67 (s, 3H), 2.45 (s, 3H), 2.30 (t, $J = 7.5$ Hz, 2H), 2.30 (m, 1H), 2.18 (m, 1H), 1.62 (m, 2H), 1.28 (br, 12H); ^{13}C NMR δ 196.0, 174.5, 146.6, 143.6, 137.9, 134.9, 130.6, 130.5, 130.0, 127.4, 54.6, 51.7, 40.0, 34.3, 29.5, 29.4, 29.3, 29.1, 28.4, 25.2, 21.9. Compound **10**: ^{1}H NMR δ 7.79 (m, 4H), 7.51 (m, 4H), 4.98 (t, $J = 7.5$ Hz, 1H), 4.54 (s, 2H), 3.67 (s, 3H), 2.30 (t, $J = 7.5$ Hz, 2H), 2.30 (m, 1H), 2.09 (m, 1H), 1.60 (m, 2H), 1.28 (br, 12H); ^{13}C NMR δ 195.5, 174.5, 147.1, 142.5, 137.5, 137.3, 130.8, 130.7, 129.2, 127.6, 54.4, 51.7, 40.0, 34.3, 32.5, 29.5, 29.4, 29.3, 29.1, 28.4, 25.2.

Reduction of **9** *plus* **10** *to* **12** *plus* **13**

According to the procedure of Hutchins *et al.*,[9] to 34 mg of a 2:1 mixture of **9** and **10** in 1 ml of dry HMPA was added 45 mg (0.72 mmol) of NaB^2H_3CN. The mixture was heated at 90°C for 48 h, cooled, and chromatographed directly on 5 g of silica gel with 30:1 to 20:1 hexane:EtOAc to give 11 mg (42%) of a colourless oily mixture of **12** and **13**. ^{1}H NMR δ 7.73 (m, 4H), 7.28 (m, 4H), 3.68 (s, 3H), 2.69 (t, $J = 7.6$ Hz, 1H), 2.45 (s, 2.7H), 2.31 (t, $J = 7.6$ Hz, 2H), 1.65 (m, 4H), 1.30 (s, 12H); ^{2}H NMR δ 2.69, 2.46; ^{13}C NMR δ 196.6, 174.6, 148.1, 143.1, 135.6, 135.4, 130.4, 129.1, 128.5, 51.7, 34.3, 31.4, 29.72, 29.70, 29.67, 29.5, 29.49, 29.4, 25.2, 21.9.

*Bromination of 11-[4-(4-methylbenzoyl)-phenyl]-undecanoic acid (***2***) to* **14** *plus* **15**

To a solution of 200 mg (0.53 mmol) of **2** in 20 ml of CCl_4 was added 140 mg (0.79 mmol) of NBS and 26 mg (0.11 mmol) of benzoyl peroxide. The mixture was heated at reflux for 18 h, cooled, filtered, and evaporated to give 400 mg of residue which was chromatographed on 5 g of silica gel with 4:1 to 2:1 hexane:EtOAc to give 244 mg of light-yellowish oily ca 2:1 mixture of **14** and **15**. Rechromatography on 15 g of silica gel effected separation to give pure **14** and **15**: Compound **14**: ^{1}H NMR δ 7.76 (m, 4H), 7.49 (m, 2H), 7.28 (m, 2H), 4.98 (t, $J = 7.5$ Hz, 1H), 2.45 (s, 3H), 2.33 (t, $J = 7.5$ Hz, 2H), 2.29 (m, 1H), 2.18 (m, 1H), 1.63 (m, 2H), 1.28 (br, 12H); ^{13}C NMR δ 196.0, 180.1, 146.6, 143.7, 137.9, 134.9, 130.6, 130.5, 130.0, 127.4, 54.6, 40.0, 34.2, 29.5, 29.48, 29.4, 29.2, 29.1, 28.4, 24.9, 21.9. Compound **15**: ^{1}H NMR δ 7.78 (m, 4H), 7.51 (m, 4H), 4.98 (t, $J = 7.5$ Hz, 1H), 4.55 (s, 2H), 2.30 (t, $J = 7.5$ Hz, 2H), 2.26 (m, 1H), 2.17 (m, 1H), 1.63 (m, 2H), 1.29 (br, 12H); ^{13}C NMR δ 195.5, 179.8, 147.1, 142.5, 137.5, 137.3, 130.8, 130.7, 129.2, 127.6, 54.4, 40.0, 34.2, 32.5, 29.5, 29.4, 29.3, 29.2, 29.1, 28.4, 25.2.

Reconversion of **14** *and* **15** *to* **16** *and* **17**

To a mixture of 42 mg of **14** and **15** in 0.8 ml of dry HMPA was added 70 mg (1.06 mmol) of NaB^2H_3CN. The mixture was heated at 80°C for 48 h, cooled, and chromatographed directly on 3 g of silica gel with 4:1 to 3:1 hexane:EtOAc to give 15 mg (45%) of a colourless oily mixture of **15** and **16**. ^{1}H NMR δ 7.74 (m, 4H), 7.28 (m, 4H), 2.69 (m, 1H), 2.45 (br, 2.7H), 2.37 (t, $J = 7.2$ Hz, 2H), 1.65 (m, 4H), 1.32 (s, 12H); ^{1}H NMR δ 2.69, 2.45; ^{13}C NMR δ 196.6, 179.1, 148.1, 143.2, 135.6, 135.4, 130.5, 130.5, 129.1, 128.5, 34.1, 31.4, 29.7, 29.6, 29.6, 29.5, 29.4, 29.3, 24.9, 21.9.

Conclusion

Convenient, reliable procedures for isotopic labelling of photoactivable cholesterol analogue **1** and fatty acid analogue **2** have been developed, using

NaB^2H_4 and NaB^2H_3CN, respectively, that will be useful as less expensive alternatives to commercial catalytic tritiation for introduction of radiolabels into these biochemical research tools.

Acknowledgements

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