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Synthesis of model compounds for the investigation of biomarker's thermal behaviour

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The model compounds, which are used to investigate the organic structure and thermal behaviour of fossil fuels, have been synthesized starting from 4-hydroxybenzyl thiocholesterol, 4-hydroxybenzyl cholesterol and 4-hydroxybenzyl cholestanol.

Keywords: Fossil fuels, thermal behaviour, biomarker, cholesterol, cholestanol.

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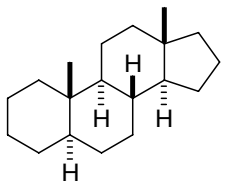
Biomarkers are organic compounds detected in geosphere derived from living organisms whose basic carbon skeleton has survived processes of diagenesis and thermal maturation. Biomarkers can yield valuable information about the thermal maturity of sediments and petroleum. Their distribution is often exploited in exploration applications for identifying the source rock of particular oil and for correlating crude oils derived from the same source. The most commonly studied biomarkers are the cyclic alkanes; the hopanes and steranes, which were derived from natural compounds, hopanoids and steroids, respectively, are ubiquitous components of crude oil, kerosene and coal[1,2].

The sterol precursors of the tetracyclic steranes are widely distributed in nature and the most commonly encountered steranes are those with carbon number $C_{27,}$ C_{28} and C_{29} although variable amounts of C_{21} and C_{22} isomers with shorter alkyl side chains are often present (**Figure 1**). The biologically synthesized configurations of their precursors are not the most thermally stable and configurational isomerisation is observed at certain chiral centers as maturation proceeds.

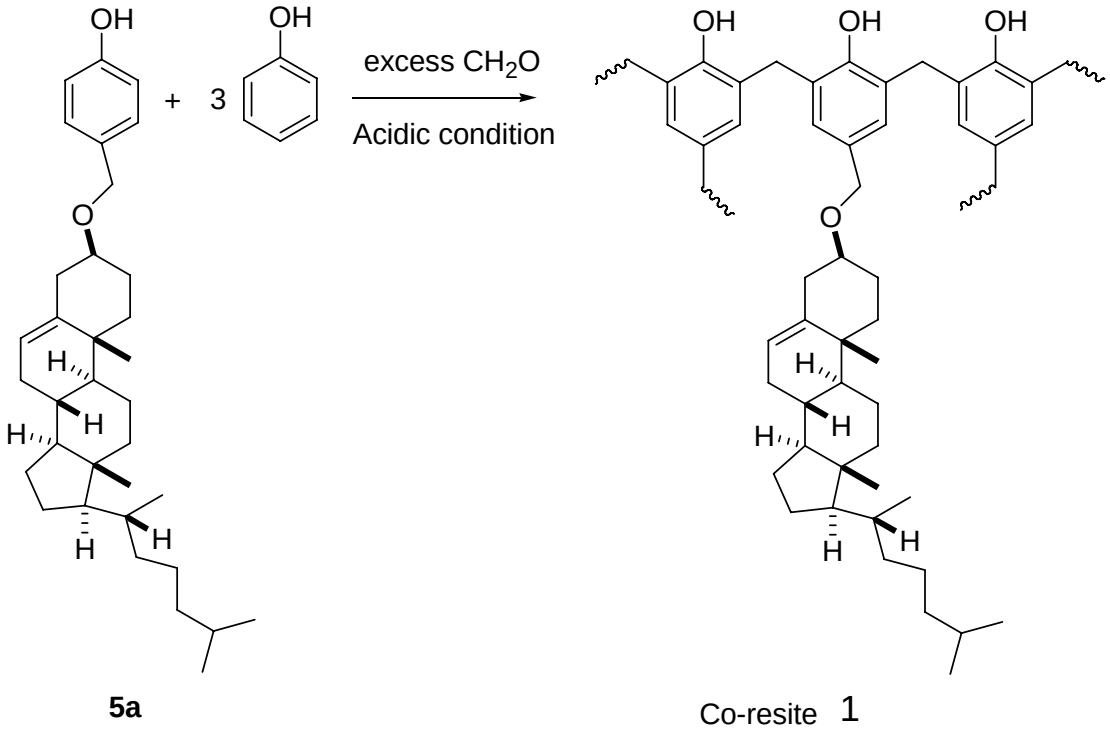
Previous studies have established that in lignite, kerosene and high-volatile bituminous coal, hopanes and steranes can be incorporated into the polymeric backbone via functional groups present in the original biolipids while retaining their less thermodynamically stable biological configurations. Thus, it appears that compounds covalently bound to the macromolecular network may be less sensitive to thermal alterations. Therefore, in order to investigate the behaviour of covalently-bound biomarkers, model substrates which are representative of biomarker entities are required. Model substrates should have a single aromatic ring which allows incorporating the substrates into a macromolecular framework, which does not soften at the elevated temperatures under hydropyrolysis conditions. In this work, phenol-formaldehyde resins were chosen as macromolecular network, because they are more similar to natural geo-macromolecules than other synthetic polymers and have the ability not to soften at elevated temperatures[3]. Steroids, which have 4-hydroxybenzyl group, were synthesised and reacted with formaldehyde to produce the macromolecule, co-resite **1** (**Scheme I**).

In the second step of this study, the synthesized co-resite will be initially pyrolysed under different conditions to determine how the distribution and the yield of pyrolysed products varied. Uncatalysed and



Sterane

Figure 1



5a Co-resite 1

Scheme I

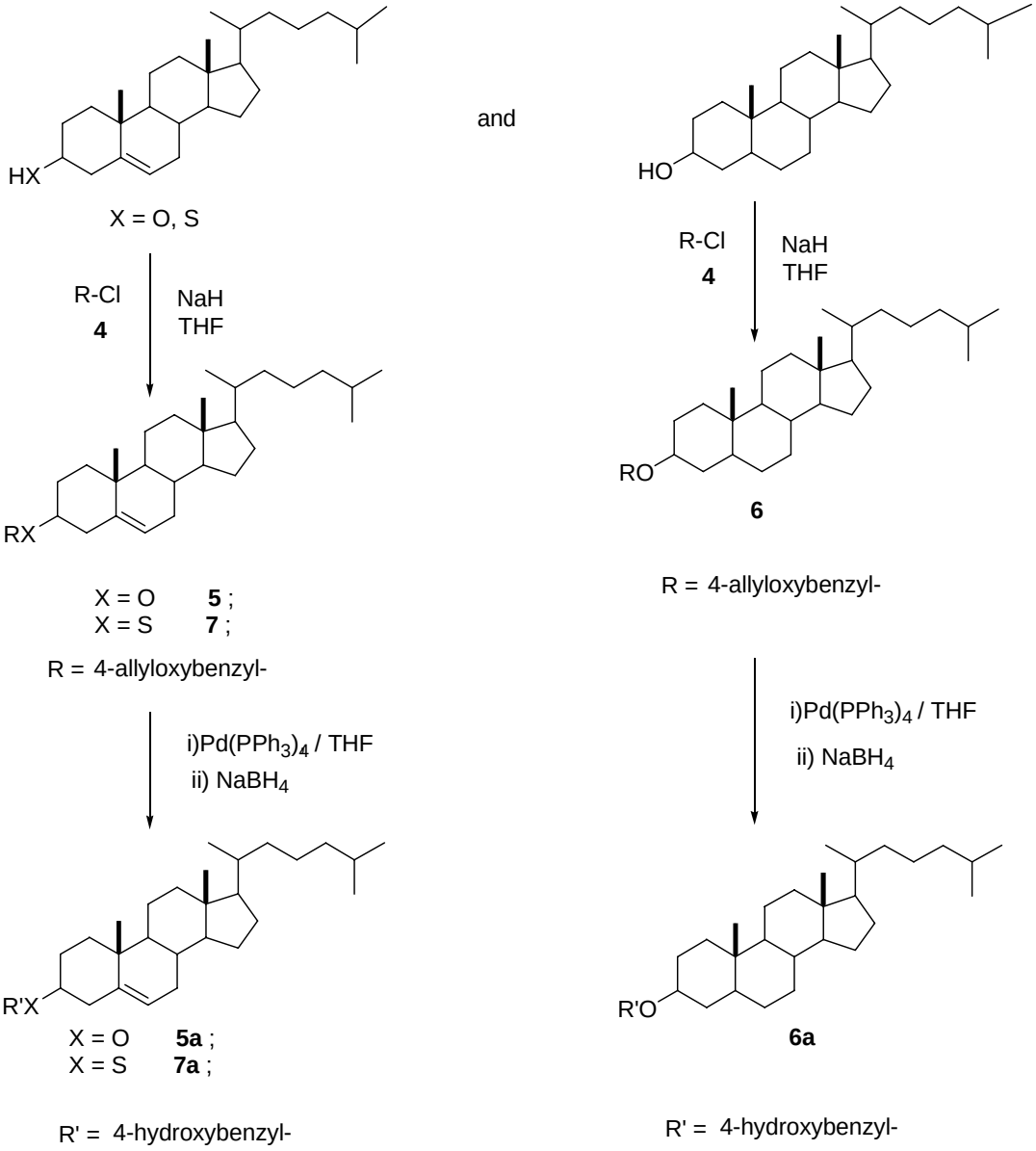
catalysed hydropyrolysis products will be compared with the normal nitrogen pyrolysis products to determine the degree of distribution and isomerisation occurring in each pyrolysis technique.

Results and Discussion

In order to synthesize model substrates, which have 4-hydroxybenzyl moiety, we started with comercially available chlorosteroids. First, chlorocholesterol was reacted with 4-hydroxybenzyl alcohol using two reagents namely, sodium hydride and copper acetylacetonate. However, the poor yields of the reactions discouraged us for further study and the reaction pathway was changed. As starting material, 3-hydroxy cholesterol was chosen instead of chlorocholesterol and this was reacted with 4-hydroxybenzyl chloride using again the two reagents mentioned before. However, the desired product could not be obtained, because 4-hydroxybenzyl chloride preferred to react with itself and gave the self polymerization product. In order to prevent self polymerization, we wanted to protect the phenolic hydroxy group by converting it to an ether. The most important factor was choosing the type of the ether group, because the target steroid ethers would have two types of ether functions, benzylic and phenolic ethers. The phenolic ether group should be selectively removed in the presence of the benzylic ether bond. Numerous reagents for the protection and deprotection reactions have been examined. However, none appeared suitable except the Beugelmans method[7]. Beugelmans and coworkers[7] found that the combination of a catalytic amount of $Pd(PPh_3)_4$ and $NaBH_4$ can selectively cleave the allylic ether in the presence of benzylic ether.

In order to prepare 4-allyloxybenzyl chloride, an allylation reaction was conducted on 4-hydroxybenzaldehyde. 4-Allyloxybenzaldehyde was reduced by $NaBH_4$ to 4-allyloxybenzyl alcohol and finally, 4-allyloxybenzyl alcohol was chlorinated by using $SOCl_2$ (ref. 6). The sodium salt of the steroid was formed with NaH in super dried THF and reacted with the synthesized 4-allyloxybenzyl chloride. Then, the cleavage reaction of the allyl ether bond was realized selectively by using $Pd(PPh_3)_4/NaBH_4$ catalyst system. The reaction pathway is summarized in **Scheme II**.

In order to investigate their thermal behaviour under pyrolysis conditions, the synthesised **5a**, **6a** and **7a** were reacted with formaldehyde to prepare their co-resites. However, only **5a** has been used to prepare co-resite **1** by using phenol as the second component.



Scheme II

A mole ratio of 3:1 (phenol-*p*-hydroxybenzyl cholesteryl ether) was adopted in order to achieve high degree of crosslinking structure. The structure of co-resite was analyzed with solid-state ^{13}C NMR. The peaks lined at 68-73 belong to the CH_2-O- and –O-CH carbons. These signals indicated the presence of cholesteryl fragment in the resin structure.

Experimental Section

Melting points were determined on an Electrothermal 9100 capillary melting point apparatus. Boiling and melting points are given in Celsius (°C). The ^1H and ^{13}C NMR spectra were recorded on a Bruker 250 MHz instrument in $CDCl_3$, using TMS as internal standard (chemical shifts in δ, ppm). Solid-state ^{13}C NMR spectra of co-resite were obtained using a Bruker MSL 100 instrument operating at a frequency of 25 MHz for carbon. The standard cross polarization-magic angle spinning technique was used, the contact time being 1 ms with a recycle delay of 1.5 s between successive contacts. The samples (250 mg, < 250 μ) were packed inside a 7 mm diameter zirconia rotor for analysis. Spectroscopic data which are identical to the literature

values are not indicated. Mass spectra were obtained on Varian MAT 711 spectrometer at 70 eV electron impact at University of Nottingham. Reactions were monitored using Merck TLC aluminum sheets (Kieselgel 60 HF_{254}).

4-Allyloxybenzaldehyde[6] 2. Dried K_2CO_3 (1.63 g, 11.8 mmoles) was added to a solution of 4-hydroxybenzaldehyde (1.44g, 11.8 mmoles) in CH_3OH (20 mL) under nitrogen atmosphere. After stirring for 10 min, the solution of allyl bromide (1.43 g, 11.8 mmoles) in CH_3OH (10 mL) was added and the mixture stirred at room temperature for 4 hr. The mixture was diluted with water (200 mL) and extracted with diethyl ether. The organic layer was dried with Na_2SO_4 and distilled under vacuum, yield 1.81 g (95%), b.p. 85 °C/15 mmHg; ^{1}H NMR (250 MHz,): δ 9.88 (s, 1H), 7.21-6.86 (dd, 4H, *J* = 8 Hz), 6.02 (m, 1H, *J* = 5,2 Hz), 5.40 (d, 1H, *J* = 10 Hz), 5.22 (d, 1H, *J* = 10 Hz), 4.48 (d, 2H, *J* = 8 Hz); MS (FAB): m/z (%)162 (M^+, 80).

4-Allyloxybenzyl alcohol[6] 3. 4-Allyloxybenzaldehyde **2** (0.61 g, 3.78 mmoles) was dissolved in dry THF (50 mL) and $NaBH_4$ (0.14g, 3.78 mmoles) was slowly added to this solution. After stirring overnight, 10% HCl was added. Most of the THF was removed under reduced pressure; the residue was extracted with ethyl acetate and dried over Na_2SO_4. The solvent was removed under reduced pressure and the product obtained was distilled under vacuum, yield 0.60g (98%), b.p. 65°C/10 mmHg; ^{1}H NMR (250 MHz): δ 7.24 (d, 2H, *J* = 8.6 Hz, ArH), 6.9 (d, 2H, *J* = 8.5 Hz, ArH), 6.0 (m, 1H, allylic CH), 5.36 (dd, 1H, J_{trans} = 17.3 Hz, *J* = 1.4 Hz, 2H, allylic CH_2), 5.28 (dd, 1H, J_{cis} = 11.6 Hz, *J* = 1.2 Hz, allylic CH_2), 4.56 (s, 2H, CH_2OH), 4.5 (d, 2H, *J* = 5.2 Hz, OCH_2), 2.26 (s, 1H, OH); ^{13}C NMR (62.5 MHz): δ 157.3, 133.2, 132.5, 128.4 (2C,Ar), 117.2 (2C,Ar), 115.9, 74.2, 68.7; MS (FAB): m/z (%) 164 (M^+, 92).

4-Allyloxybenzyl chloride[6] 4. 4-Allyloxybenzylalcohol **3** (0.6 g, 3.64 mmoles) was chlorinated using excess of thionyl chloride to give **4**, yield 0.66 g (100%), b.p. 110°C/7 mmHg; ^{1}H NMR (250 MHz): δ 7.3 (d, 2H, *J* = 8.4 Hz, ArH), 6.9 (d, 2H, *J* = 8.4 Hz, ArH), 5.98 (m, 1, CH), 5.41 (dd, 1H, J_{trans} = 17.1, *J* = 1.4 Hz, 2H, allylic CH_2), 5.28 (dd, 1, J_{cis} = 11.6, *J* = 1.2 Hz, allylic CH_2), 4.5 (s, 2H, CH_2), 4.42 (d, 2H, *J* = 5.1 Hz); ^{13}C NMR (65 MHz): δ 157.3, 132.5, 131. 8, 129.9 (2C,Ar), 117.4, 117.2 (2C, Ar), 68.7, 47.0; MS (FAB): m/z (%) 182 (M^+, 55).

4-Allyloxybenzyl cholesteryl ether 5. NaH (0.24 g, 10 mmoles) was added to a solution of cholesterol (3.86 g, 10 mmoles) in dry THF (20 mL) under nitrogen atmosphere. After stirring for 10 min, a solution of 4-allyloxybenzyl chloride (1.82 g, 10 mmoles) in THF (10 mL) was added and the mixture stirred for 4 hr at room temperature. The reaction mixture was poured into water and after cooling extracted with diethyl ether (3 times). The organic layer was washed with Na_2CO_3 solution and brine (3 times), dried over Na_2SO_4, filtered and concentrated *in vacuo*. 4-Allyloxy benzyl cholesteryl ether was recovered as a yellowish solid after column chromatography (hexane-EtOAc 70:30), yield 2.93g (55%), m.p. 126-28°C; ^{1}H NMR (250 MHz): δ 7.25 (d, 2H, *J* = 8.5 Hz, ArH), 6.8 (d, 2H, *J* = 8.5 Hz, ArH), 6.0 (m, 1H, allylic CH), 5.45 (bd, 1H, *J* = 6.4 Hz, H-5), 5.34 (d, 1H, J_{trans} = 13.2 Hz, allylic CH_2), 5.26 (d, 1H, J_{cis} = 10.8 Hz, allylic CH_2), 4.52 (d, 2H, *J* = 5.0 Hz, OCH_2), 4.49 (s, 2H, benzylic CH_2), 3.3 (m, 1H, H-3), 2.4-0.6 (other cholesteryl H)[12]; ^{13}C NMR (62.5 MHz): δ 157.3, 145.1, 133.2, 132.5, 129.6, 128.6 (2C, Ar) 117.2 (2C, Ar), 115.6, 82.3, 74.1, 71.4 and 19-68.7 (other cholesteryl carbons)[12]; MS: m/z (%) 532 (M^+, 22), 385 (70), 369 (30), 355 (12), 301 (15), 276 (60), 230 (16), 215 (30), 147 (32), 106 (40), 91 (35), 77 (20) . Anal.Calcd for $C_{37}H_{56}O_2$: C, 83.39; H, 10.59. Found: C, 83.52; H, 10.47 %.

4-Allyloxybenzyl cholestanyl ether 6. The reaction was carried out with the same method reported for compound **5**. 4-Allyloxy benzyl cholestanyl ether was recovered as a bright yellowish solid after column chromatography (hexane-EtOAc; 80:20), yield 3.47g (65%), m.p. 122-23°C; ^{1}H NMR (250 MHz): δ 7.24 (d, 2H, *J* = 8.5 Hz, ArH), 6.89 (d, 2H, *J* = 8.5 Hz, ArH), 6.0 (m, 1H, allylic CH), 5.33 (d, 1H, J_{trans} = 13.4, allylic CH_2), 5.26 (d, 1H, J_{cis} = 11.6 Hz, allylic CH_2), 4.51 (d, 2H, *J* = 5.3 Hz, OCH_2), 4.42 (s, 2H, benzylic CH_2), 3.26 (m, 1H, H-3) 0.6-2.4 (cholestanyl protons); ^{13}C NMR (62.5 MHz): δ 157.0, 132.5, 133.4, 129.6, 128.6 (2C, Ar), 117.2 (2C, Ar), 115.6, 82.3, 74.1, 71.1 and 68.7-19.0 (other cholestanyl carbons); MS: m/z (%) 534 (M^+, 15), 387 (48), 371 (37), 357 (39), 303 (40), 278 (60), 232 (26), 217 (50), 149 (15), 147 (32), 106 (80), 91 (30), 77 (15). Anal.Calcd for $C_{37}H_{58}O_2$: C, 83.08; H, 10.93. Found: C, 83.17; H, 10.81 %.

4-Allyloxybenzyl thiocholesteryl ether 7. The reaction was carried out with thio cholesterol[8,9] by using the same method as employed for compound **5**.

Product was recovered as a yellowish-white solid after column chromatography (hexane-EtOAc; 70:30), yield 3.40g (62%), m.p. 120-21°C; ^{1}H NMR (250 MHz): δ 7.2 (d, 2H, *J* = 8.2 Hz, ArH), 6.85 (d, 2H, *J* = 8.0 Hz, ArH), 6.02 (m, 1H, allylic CH), 5.44-5.27 (m, 3H, olefinic protons), 4.51 (s, 2H, CH_2O), 3.72 (s, 2H, CH_2S), 2.46(m, 1H, S-CH), 2.27-0.67(other cholesteryl protons); ^{13}C NMR (62.5 MHz): δ 158.2, 143.4, 133.4, 132.6, 130.8, 127.3 (2C, Ar) 117.2 (2C, Ar), 114.3, 82.3, and peaks between 68.7-19 are for CH_2S, CHS and other cholesteryl carbons; MS: m/z (%) 548 (M^+, 21), 401 (43), 369 (73), 355 (22), 301 (10), 276 (45), 230 (19), 215 (31), 147 (38), 106 (21), 91 (65), 77 (34) . Anal. Calcd for $C_{37}H_{56}OS$: C,80.95; H,10.28;S, 5.84. Found: C, 81.23; H, 10.02; S,5.93 %.

4-Hydroxybenzyl cholesteryl ether 5a. Tetrakis (triphenylphosphine) palladium, $Pd(PPh_3)_4$ (1.6 mg, 1.2×10^{-3} mmole) was added to a solution of 4-allyl-oxybenzyl cholesteryl ether (0.36 g, 0.69 mmole) in dry THF (20 mL) under nitrogen atmosphere. The yellowish solution was stirred for 10 min; then $NaBH_4$ (0.04 g, 1 mmole) was added and the reaction was stirred further for 1 hr at ambient temperature. The mixture was cooled and excess amount of $NaBH_4$ was destroyed with 1 N HCl solution. The neutralized mixture was extracted with diethyl ether (3 times). The organic layer was washed with Na_2CO_3 solution and brine (3 times). The organic phase was dried over Na_2SO_4, filtered and concentrated *in vacuo*. 4-Hydroxy benzyl cholesteryl ether was recovered as a yellowish solid after column chromatography (hexane-EtOAc; 70:30), yield 0.35 (98%); m.p. 138-39°C; ^{1}H NMR (250 MHz): δ 7.25 (d, 2H, *J* = 8.5 Hz,ArH), 6.9 (d, 2H, *J* = 8.6 Hz, ArH), 5.43 (m, 1H, olefinic), 4.8 (s, 1H, OH), 4.46 (s, 2H, CH_2), 3.23 (m, 1H, OCH) 2.4-0.6 (cholesteryl protons); ^{13}C NMR (62.5 MHz): δ 156.1, 145.0, 130.2, 130.0 (2C, Ar), 115.3, 114.0 (2C, Ar), 77.2, 71.5 and 57-11.8 peaks for other cholesteryl carbons; MS: m/z (%) 492 (M^+, 32), 385 (91), 369 (52), 355 (20), 301 (13), 276 (43), 230 (28), 215 (44), 147 (24), 107 (61), 91 (38), 76 (30) . Anal. Calcd for $C_{34}H_{52}O_2$: C,82.86; H, 10.63. Found: C, 82.98; H, 10.55 %.

4-Hydroxybenzyl cholestanyl ether 6a. The reaction was carried out by the same method described for compound **5a**. 4-Hydroxy benzyl cholestanyl ether was recovered as a bright-yellowish solid after column chromatography (hexane-EtOAc; 80:20), yield 0.29g (88%); m.p. 131-32°C; ^{1}H NMR (250 MHz): δ 7.25 (d, 2H, *J* = 8.3 Hz, ArH), 6.9 (d, 2H, *J* = 8.4 Hz, ArH), 4.91 (s, 1H, OH), 4.51 (s, 2H, CH_2O), 3.9 (m, 1H, OCH), 2.4-0.6 (cholestanyl protons); ^{13}C NMR (62.5 MHz): δ 158.2, 130.2, 129.7 (2C, Ar), 115.3 (2C, Ar), 77.9, 71.8, 56-19 (tare for typical cholestanyl carbons) MS: m/z (%) = 494 (M^+, 12), 387 (78), 371 (23), 357 (32), 303 (15), 278 (37), 232 (26), 217 (35),149 (13), 147 (35), 107 (46), 90 (65), 76 (23) . Anal. Calcd for $C_{34}H_{54}O_2$: C, 82.53; H, 11.00. Found: C, 82.68; H, 10.84 %.

4-Hydroxybenzyl thiocholesteryl ether 7a. Compound **7a** was prepared by the procedure described for compound **5a** and was obtained as a yellowish-white solid after column chromatography (hexane-EtOAc; 70:30), yield 0.32g (92%); m.p. 128-29°C; ^{1}H NMR (250 MHz): δ 7.2 (d, 2H, *J* = 8.4 Hz, ArH), 6.8 (d, 2H, *J* = 8.6 Hz, ArH), 5.3 (m, 1H, olefinic H), 5.0 (s, 1H, OH), 3.7 (s, 2H, CH_2), 2.5 (m, 1H, SCH), 2.4-0.6 (cholesteryl protons); ^{13}C NMR (62.5 MHz): δ 155.0, 143.4, 130.4, 127.1(2C, Ar), 116.6, 114.3 (2C, Ar), 47.1, 45.4, 43.4 and 40.3-18.2 (other cholesteryl carbons); MS: m/z (%) = 508 (M^+, 11), 401 (63), 369 (93), 355 (42), 301 (10), 276 (48), 230 (10), 215 (42), 147 (46), 107 (35), 90 (54), 77 (32) . Anal. Calcd for $C_{34}H_{52}OS$: C,80.25; H,10.30; S,6.30. Found: C, 80.16; H,10.38; S, 6.35 %.

Synthesis of the co-resite. The co-resite was prepared using a total phenolic compounds (phenol + *p*-hydroxybenzyl cholesteryl ether) to formaldehyde mole ratio of 1: 2.5, following established procedure[10,11]. Hydrogen chloride (36%) was used as a catalyst in the condensation reaction at a mole ratio of 0.1 with respect to phenolic compounds. The phenolic compounds (phenol + *p*-hydroxy benzyl cholesteryl ether) and formalin (37% v/v formaldehyde) were mixed in a 250 mL three-neck round bottom flask and stirred until all the phenolic compounds were in solution form. The mixture was then cooled in an ice-bath for about 10 min before hydrogen chloride (0.06 mole) was added dropwise to the reaction mixture. The mixture was heated at 70°C for 30 min. After cooling, the mixture was poured into water. The resinous material was filtered and washed with water twice. The co-resite was dried in oven overnight.

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