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An efficient regio-specific synthetic route to multiply substituted acyl-sulphated β-cyclodextrins

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Abstract—The regio-specific synthesis of a series of novel amphiphilic β-cyclodextrins is described. We are able to check the degree of sulphatation at upper rim and the degree of acylation and over-acylation at lower rim by electrospray mass spectrometry. The 6-*O*-silyl-2,3-*O*-acyl-β-cyclodextrin is synthesised in large scale by one pot reaction from β-cyclodextrin. The products are generally mixtures with varying degrees of substitution. These amphiphilic cyclodextrin form micellar aggregates.

Amphiphilic β-cyclodextrins (β-CDs), (cyclomaltoheptaoses), are of considerable interest for pharmaceutical applications in view of their capacity for self-organisation in water at physiological pH.[1–3] In previous studies such amphiphilic cyclodextrins were obtained by the introduction of lipophilic groups at the upper and/or lower rims.[4–7] For example, Tanaka et al[4] and recently Ravoo[5] have used the attachment of thio-alkyl chains at the O-6 position, while Greenhall[6] synthesised cyclodextrins per-substituted by alkyl chains at O-6 and O-2 or O-3 positions. Zhang et al[7] coupled lipophilic ester groups at the O-2 and O-3 positions. These esterified amphiphilic cyclodextrins (typically with hexanoyl chains) are capable of forming nanoparticles as aqueous dispersion in the presence of co-surfactants.[8] These systems show very high levels of encapsulation of hydrophobic guest molecules when compared to liposomes and vesicles.[8]

Polysulphated carbohydrates, such as dextran sulphate, pentosan sulphate, heparin and cyclic oligosaccharide sulphates are known to possess a wide range of biological activities, including antiviral activity.[9] Structure–activity relationship studies have shown that the antiviral activity is amplified by an increasing degree of sulphatation. However, whilst the polysulphatation of β-cyclodextrin leads to molecules showing antiviral properties and non-hematotoxic,[10] such molecules are unfortunately too water-soluble and too polar for an efficient self-organisation.[11]

In view of the above, we have studied the effect of combining the properties of the amphiphilic β-cyclodextrins with the antiviral properties generated by substituent sulphate groups on the aptitude of molecular transport and self-organisation of such new entities. In this paper we report the regio-specific synthesis and characterisation of new amphiphilic polyanionic β-cyclodextrins with different degrees of acylation and sulphatation.

The sulphated amphiphilic β-cyclodextrin derivatives **5** were synthesised from β-cyclodextrin **1** in three or four steps (Scheme 1). *O*-acylation of 6-*O*-*tert*-butyldimethylsilyl-β-cyclodextrin[7] **2** is difficult, and the difficulty lies not only in the control of the number of *O*-acylated sites but also in the reproducibility of the reaction. Zhang[7] described a mixture of *O*-acylated derivatives at O-2 and O-3 **3A** produced under forcing conditions by using 56 equiv. of hexanoyl chloride and 42 equiv. of 2,4-dimethylaminopyridine (DMAP) (entry **A**, Table 1) with a mean acylation value higher than 14. Lesieur[12] used hexanoic anhydride in similar conditions (entry **E**, Table 1) and isolated a symmetrical acylated β-cyclodextrin **3E** with only 14 secondary hydroxyl functions derivatised but the yield remains low and the degree of acylation uncertain. In view of the interest these molecules may present for pharmaceutical applications, we have studied the influence of the nature of the reagents and the quantities used (Table 1) on the number of the alkanoyl chains introduced at the lower rim of **2**. The degree of substitution is easily verified by electrospray mass spectrometry (ES-MS, positive mode). For a β-cyclodextrin protected at O-6, the max-

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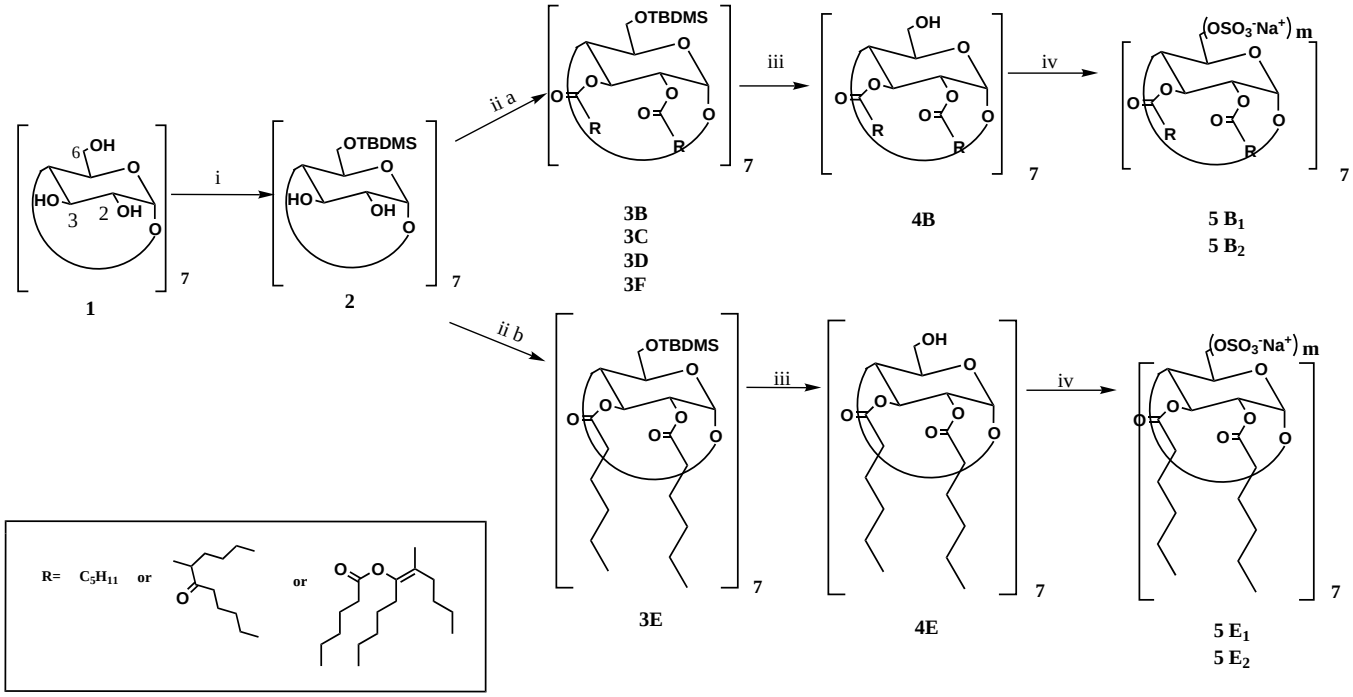

Scheme 1. Synthesis of multiply substituted acyl-sulphated β-cyclodextrins: (i) TBDMSCl, pyridine; (ii a) reaction conditions Table 1 entries **B**, **C**, **D**, **F**; (ii b) reaction conditions Table 1 entry **E**; (iii) $BF_3{\cdot}Et_2O$, $CHCl_3$; (iv) $SO_3{\cdot}Pyr$, pyridine.

Table 1. Acylation conditions of the 6-*O*-silyl-β-cyclodextrin **2**

Entry	Acylated agent (equiv.)	DMAP (equiv.)	Reaction time	Yield (%)	Alkanoyl chains number	Product
A	Hexanoyl chloride (56)	42	72 h at 60°C	30[7]	**>21**	**3A**
B	Hexanoyl chloride (28)	42	72 h at 60°C	**65**	**19–23**	**3B**
C	Hexanoyl chloride (28)	3	72 h at 60°C	**20**	**16–23**	**3C**
D	Hexanoyl chloride (28)	Without	72 h at 60°C	**12**	**17–23**	**3D**
E	Hexanoyl anhydride (56)	42	48 h at 70°C	**63**	**14**	**3E**
				33[12]	**14**	
F	Hexanoic anhydride (56)	Without	48 h at 70°C	**20**	**10–12**	**3F**

imum degree of acylation of the secondary OH is theoretically 14; it is however well known that hexanoyl chloride in presence of DMAP is capable of giving secondary reactions by self-condensation at position α of the C=O functions of acyl chains[13] and hence, the maximum substitution can reach 28 (experimentally observed). We carried out the acylation of **2** (8.1 g, 4.1 mmol) with a 65% yield (lit. 30% yield)[7] by using only 28 equiv. of hexanoyl chloride (16.2 mL, 0.11 mmol) in the presence of 42 equiv. of DMAP (21.3 g, 0.17 mmol) in dry pyridine (200 mL) (entry **B**, Table 1). Electrospray mass spectrometry clearly demonstrates that **3B**[14] is a mixture of multisubstituted derivatives resulting from coupling of 19 to 23 alkanoyl chains with an average degree of substitution of 21 (*m*/*z* 1923.1 [**3B** with 19 chains+2Na]$^{2+}$; 1972.0 [**3B** with 20 chains+2Na]$^{2+}$; 2020.5 [**3B** with 21 chains+2Na]$^{2+}$); 2069.5 [**3B** with 22 chains+2Na]$^{2+}$; 2118.6 [**3B** with 23 chains+2Na]$^{2+}$. The presence of peaks for **3B** with higher and lower degrees of substitution shows peaks spaced 98 amu apart, as expected for ($C_5H_{11}CO$). The structure of **3B** (R = double chains in O-3) is confirmed by ^{13}C NMR spectroscopy, in particular the presence of a ketone carbonyl at 206.8 ppm.[14] In order to limit and control the number of substituents, we reduced the quantity of DMAP (3 equiv.) keeping the quantity of hexanoyl chloride constant (28 equiv.) (entry **C**, Table 1) under the same conditions as before. We obtain a mixture of multisubstituted β-cyclodextrins **3C**[14] containing between 16 and 23 hexanoyl chains at O-2 and O-3, with a major product resulting from an average rate of substitution of 19 (*m*/*z* 1823.8 [**3C** with 17 chains+2Na]$^{2+}$; [1922.2 [**3C** with 19 chains+2Na]$^{2+}$; 2118.6 [**3C** with 23 chains+2Na]$^{2+}$); however the yields are low (20%). One experiment carried out without DMAP (entry **D**, Table 1), leads to the same degree of substitution, above 14, and the yield is only of 12% for **3D**. Similar experiments carried out with hexanoic anhydride (56 equiv.) and DMAP (42 equiv.) at 70°C for 48 h gave only **3E** (with 14 chains);[14] (*m*/*z* 1676.6 [**3E**+2Na]$^{2+}$); (yield 63%, lit. yield 33%[12] entry **E**, Table 1). Without DMAP (entry **F**, Table 1) 6-*O*-*tert*-butyldimethylsilyl-β-cyclodextrin **2** was condensed with hexanoic anhydride (56 equiv.) at 70°C for 48 h (yield

20%) and gave cyclodextrin derivatives **3F** incompletely substituted at O-2 and O-3.

In summary, the use of hexanoic anhydride with or without DMAP does not lead to over-acylation, whereas the corresponding acid chloride with or without DMAP always leads to a degree of acylation higher than 14. We have introduced a new technique of purification consisting of simply precipitating, from mixture of methanol–chloroform (95–5), the substituted β-cyclodextrins **3A**, **3B**, **3C**, **3D** and **3E**. The yield of the reaction is multiplied by 2 and this technique enabled us to work on a large scale on considerable quantities of silylated β-cyclodextrin **2** (20 g). In view of the success of the acylation reaction at O-2 and O-3 and the control of the degree of acylation without DMAP and with hexanoic chloride as acylating agent, we tried a one-pot reaction. To a solution of β-cyclodextrin **1** (3 g, 2.64 mmol) in dry pyridine (50 mL) is added a solution of *tert*-butyldimethylsilyl chloride (TBDMSCl) (3.3 g, 22 mmol) in 20 mL of dry pyridine at 0°C. After storing during 24 h at 20°C, acetic anhydride or hexanoyl chloride (15 mL) is added and the reaction mixture is brought to 70°C for 48 h. The reaction is stopped by pouring the mixture into water (600 mL). After extraction with dichloromethane, the solvent is eliminated and the crude oil purified by precipitation from methanol–chloroform (95–5). The product **3G**[14] with CH_3–C=O in O-2 and O-3 is obtained in 95% yield and **3D**[14] (yield 20%) with a major product resulting from an average rate of substitution of 19.

Removal of the *tert*-butyldimethylsilyl groups of **3B** and **3E** (19.8 g, 6 mmol) by boron trifluoride etherate ($BF_3{\cdot}Et_2O$; 87 mmol) in dry dichloromethane gives, respectively **4B** and **4E** (yield 95%).[15] The cyclodextrins **4B** and **4E** are sulphated under standard conditions in the presence of the sulphur trioxide–pyridine complex.[10b] We have studied the influence of the quantities used on the degree of sulphatation at O-6 position. The sulphur trioxide–pyridine complex (11.11 g, 70 mmol, 35 equiv.) was added to a solution of the β-cyclodextrin derivatives **4B** or **4E** (5 g, 2 mmol) in dry pyridine (250 mL) and stirred at 60°C for 72 h. The solvent was removed under reduced pressure and the residue was dissolved in $CHCl_3$, washed with a diluted acid solution (HCl 10%) and saturated solution of sodium hydrogenocarbonate. After evaporation of the solvent, the residue was lyophilised to give $\mathbf{5B_1}$ and $\mathbf{5E_1}$, respectively. The structures were confirmed from ^1H and ^{13}C NMR spectroscopy and electrospray mass spectroscopy.[16] The most intense peaks correspond a degree of sulphatations of 7 for $\mathbf{5B_1}$ ($m<7$ or $m=7$) with peaks for products with lower degrees of substitution spaced at 102 amu (SO_3Na). However, the pure product **4E** gives only $\mathbf{5E_1}$ ($m=7$) characterised by ES-MS m/z 1634.07 $[\mathbf{5E_1}+2\ Na]^{2+}$; 1097.22 $[\mathbf{5E_1}+3\ Na]^{3+}$.

With a view to studying the effects of the degree of sulphatation in the self-assembly properties, similar experiments were carried out with 8 equiv. (16 mmol) of sulphur trioxide–pyridine to yield $\mathbf{5B_2}$ and $\mathbf{5E_2}$[16] incompletely substituted at O-6. The mixture of derivatives shows 2 to 5 substitutions at O-6, with an average degree of 3.

In conclusion, we have synthesised a series of new amphiphilic cyclodextrins in which the upper rim is functionalised with sulphates and the lower rim with fatty acid esters. The use of hexanoyl chloride and DMAP leads to pure fully acylated product **3E** with 14 hexanoyl chains while other acylation conditions lead to mixtures with different degrees of acylation or over-acylation. Likewise, the use of 35 equiv. of sulphatation reagents leads to a pure fully sulphated compound $\mathbf{5E_1}$, $\mathbf{5B_1}$ while the use of 8 equiv. leads to mixtures with average sulphatation degrees of 4. $\mathbf{5E_1}$ is a pure fully acylated (14 chains) and sulphated (7 groups) derivative but unfortunately, separation of each compound of the mixture by HPLC or GPC is rendered impossible by the narrow polarity and mass differences of each substituent. Nevertheless, the ability of self-organisation to micellar aggregates of the mixtures compared to the pure product $\mathbf{5E_1}$ is interesting as shown by critical micellar concentration (cmc) measurements (Fig. 1).[17] Indeed, the pure derivative $\mathbf{5E_1}$ and is under-sulphated analog $\mathbf{5E_2}$ decrease the surface tension of water to 25 mN m^{-1} and possess a cmc of 345 mg mL^{-1} (10^{-4} mol l^{-1}) whilst the over acylated and fully sulphated derivative $\mathbf{5B_1}$ decrease the surface tension to 25 mN m^{-1} and possess a cmc of 45 mg mL^{-1} (10^{-6} mol l^{-1}). In each case the mixtures and the pure compounds show cmc which are effectively identical. Synthesis of these pure products or mixtures on a large scale and their high reproducibility of preparation combined with their behaviour in aqueous media confer to these system a high degree of interest for pharmaceutical applications.

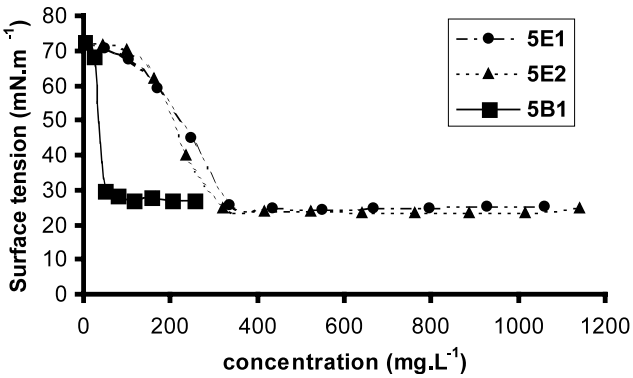

Figure 1. Plot of surface tension of acyl-sulphated β-cyclodextrin ($\mathbf{5E_1}$, $\mathbf{5E_2}$, $\mathbf{5B_1}$) solutions in pure water at 25°C as a function of concentration.

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14. Chemical and physical data for **3B** 65% yield (9 g); R_f 0.4 (*tert*-butylmethylether–heptane, 10:90); mp 225°C; $[\alpha]_D$= +69.4 (c=5.04, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm): 5.32 (bt, 7H, H3), 5.08 (bd, 7H, H1), 4.51 (bdd, 7H, H2), 3.67 (m, 28H, H5, H6, H′6, H4), 3.51 (m, -CH-), 2.49–2.37 (m, -CH_2-COO-), 1.77 (m, -CH_2-), 1.65 (m, -C$\underline{H}_2$-CH_2-COO-), 1.28 (m, -CH_2-), 0.84 (m, -CH_3, (s, C$\underline{H}_3$)$_3$-C), 0.00 (s, CH_3-Si-); ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm): 206.8 (C=O), 178.0 (COO), 173.7 (COO), 168.0 (COO), 96.5 (C1), 75.5 (C4), 72.2 (C5), 71.5–71.2 (C2. C3), 64.1 (C6), 33.9 ($\underline{CH}_2$-COO), 31.6 (-CH_2-), 29.9 ($\underline{CH}$-C=O), 28.4 ($\underline{CH}_2$-C=O), 26.3 ((CH$_3$)$_3$-$\underline{C}$), 24.8 ($\underline{CH}_2$-CH_2-COO), 24.2 (-$\underline{CH}_2$-CH_3), 23.1 (-$\underline{CH}_2$-CH_3), 18.6 (($\underline{C}H_3$)$_3$-C), 14.3 (-CH_3), −4.6 (CH_3-Si); ES-MS (+) m/z: 1923.1 [**3B** with 19 chains+2Na]$^{2+}$, 1972.0 [**3B** with 20 chains+2Na]$^{2+}$, 2020.5 [**3B** with 21 chains+2Na]$^{2+}$, 2069.5 [**3B** with 22 chains+2Na]$^{2+}$, 2118.6 [**3B** with 23 chains+2Na]$^{2+}$.
3C 20% yield (6 g); R_f 0.4 (*tert*-butylmethylether–heptane, 10:90); mp 225°C; $[\alpha]_D$= +69.4 (c=5.04, CH_2Cl_2); NMR ^{1}H ($CDCl_3$, 300 MHz): δ (ppm): 5.37 (bt, 7H, H3), 5.12 (bd, 7H, H1), 4.62 (bdd, 7H, H2), 3.70 (m, 28H, H5, H6, H′6, H4), 2.63–2.17 (m, -CH_2-COO-), 1.66 (m, -C$\underline{H}_2$-CH_2-COO-), 1.30 (m, -CH_2-), 0.88 (m, -CH_3, (s, C$\underline{H}_3$)$_3$-C), 0.03 (s, CH_3-Si-); ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm): 206.8 (C=O), 178.0 (COO), 173.7 (COO), 168.0 (COO), 96.5 (C1), 75.5 (C4), 72.2 (C5), 71.5–71.2 (C2, C3), 64.1 (C6), 33.9 ($\underline{CH}_2$-COO), 31.6 (-CH_2-), 29.9 ($\underline{CH}$-C=O), 28.4 ($\underline{CH}_2$-C=O), 26.3 ((CH$_3$)$_3$-$\underline{C}$), 24.8 ($\underline{CH}_2$-CH_2-COO), 24.2 (-$\underline{CH}_2$-CH_3), 23.1 (-$\underline{CH}_2$-CH_3), 18.6 (($\underline{C}H_3$)$_3$-C), 14.3 (-CH_3), −4.6 (CH_3-Si); ES-MS (+) m/z: 1774.9 [**3C** with 16 chains+2Na]$^{2+}$, 1823.8 [**3C** with 17 chains+2Na]$^{2+}$, 1872.9 [**3C** with 18 chains+2Na]$^{2+}$, 1922.2 [**3C** with 19 chains+2Na]$^{2+}$, 1971.3 [**3C** with 20 chains+2Na]$^{2+}$, 2020.0 [**3C** with 21 chains+2Na]$^{2+}$, 2069.5 [**3C** with 22 chains+2Na]$^{2+}$, 2118.6 [**3C** with 23 chains+2Na]$^{2+}$.
3D: 20% yield (1.7 g); R_f 0.4 (*tert*-butylmethylether–heptane, 10:90); ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm): 5.43 (bt, 7H, H3), 5.17 (bd, 7H, H1), 4.67 (bdd, 7H, H2), 3.73 (m, 28H, H5, H6, H′6, H4), 2.65–2.41 (m, -CH_2-COO-), 1.59 (m, -C$\underline{H}_2$-CH_2-COO-), 1.32 (m, -CH_2-), 0.90 (m, -CH_3, (s, C$\underline{H}_3$)$_3$-C), 0.05 (s, CH_3-Si-); ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm): 206.8 (C=O), 178.0 (COO), 173.7 (COO), 168.0 (COO), 96.5 (C1), 75.5 (C4), 72.2 (C5), 71.5–71.2 (C2, C3), 64.1 (C6), 33.9 ($\underline{CH}_2$-COO), 31.6 (-CH_2-), 29.9 ($\underline{CH}$-C=O), 28.4 ($\underline{CH}_2$-C=O), 26.3 ((CH$_3$)$_3$-$\underline{C}$), 24.8 ($\underline{CH}_2$-CH_2-COO), 24.2 (-$\underline{CH}_2$-CH_3), 23.1 (-$\underline{CH}_2$-CH_3), 18.6 (($\underline{C}H_3$)$_3$-C), 14.3 (-CH_3), −4.6 (CH_3-Si); ES-MS (+) m/z: 1775.4 [**3D** with 16 chains+2Na]$^{2+}$; 1823.8 [**3D** with 17 chains+2Na]$^{2+}$; 1873.1 [**3D** with 18 chains+2Na]$^{2+}$; 1922.2 [**3D** with 19 chains+2Na]$^{2+}$; 1971.3 [**3D** with 20 chains+2Na]$^{2+}$; 2020.3 [**3D** with 21 chains+2Na]$^{2+}$; 2069.0 [**3D** with 22 chains+2Na]$^{2+}$; 2118.0 [**3D** with 23 chains+2Na]$^{2+}$.
3E: mp 242°C; $[\alpha]_D$= +62.5 (c=5.12, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm): 5.34 (dd, 7H, J_{H3-H4}=8.3 Hz, J_{H3-H2}=10.1 Hz, H3), 5.08 (d, 7H, J=3.58 Hz, H1), 4.64 (dd, 7H, H2), 4.03 (bd, 7H, $J_{H6-H6'}$=11.5 Hz, H6), 3.81 (m, 14H, H4, H5), 3.65 (bd, 7H, H6′), 2.35 (m, 28H, CH_2COO), 1.52 (bt, 28H, $\underline{CH}_2$-CH_2COO), 1.26 (m, 56H, -(CH_2)$_2$-), 0.83 (bs, 105H, CH_3, CH_3-C), 0.00 (s, 42H, CH_3-Si); ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm): 173.9 (COO), 172.0 (COO), 96.8 (C1), 75.5 (C4), 72.2 (C5), 71.5–71.2 (C2, C3), 62.3 (C6), 34.5 ($\underline{CH}_2$-COO), 31.8 (-CH_2-), 26.3 ((CH$_3$)$_3$-$\underline{C}$), 24.8 ($\underline{CH}_2$-CH_2-COO), 22.8 (-$\underline{CH}_2$-CH_3), 18.6 (($\underline{C}H_3$)$_3$-C), 14.3 (-CH_3), −4.5 (CH_3-Si); ES-MS (+) m/z 1676.6 [**3E**+2Na]$^{2+}$.
3G: 95%yield (6.3 g); R_f 0.3 (heptane–acetone, 7:3); $[\alpha]_D$= +83 (c=1, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm): 5.35 (t, 7H, J=10.3 Hz, H3), 5.17 (d, 7H, J=3.67 Hz, H2), 4.70 (dd, 7H, H2), 4.07 (dl, 7H, $J_{H6-H6'}$=11.8 Hz, H6), 3.90 (m, 14H, H4, H5), 3.68 (dl, 7H, H6′), 2.05 (s, 42H, CH_3), 0.87 (s, 63H, CH_3-C), 0.00 (s, 42H, CH_3-Si); ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm): 171.0 (COO), 170.3 (COO), 96.5 (C1), 75.2 (C4), 71.8; 71.5, 71.2; (C2, C3, C5), 61.8 (C6), 25.8 (CH_3-$\underline{C}$), 20.8; 20.7 (2$\underline{CH}_3$-COO), 18.2 ($\underline{CH}_3$-C), −5.0 (CH_3-Si); MS-ES (+) m/z 1286.5 [M+2Na]$^{2+}$.
15. Chemical and physical data for **4B** 85% yield (7.3 g); mp: 130°C; $[\alpha]_D$= +80.5 (c=5.34, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm): 5.13 (bt, 7H, H3), 4.89 (bd, 7H, H1), 4.55 (bdd, 7H, H2), 3.78 (m, 21H, H5, H6, H′6), 3.49 (H4); 3.37 (m, -CH-), 2.22 (m, -CH_2-COO-), 1.64 (m, -CH_2-), 1.43 (m, -C$\underline{H}_2$-CH_2-COO-), 1.13 (m, -CH_2-), 0.70

(-CH_3); ES-MS (+) m/z: 1421.44 [**4B** with 17 chains+2Na]$^{2+}$, 1470.81 [**4B** with 18 chains+2Na]$^{2+}$, 1519.55 [**4B** with 19 chains+2Na]$^{2+}$, 1568.54 81 [**4B** with 20 chains+2Na]$^{2+}$, 1617.67 [**4B** with 21 chains+2Na]$^{2+}$, 1666.41 [**4B** with 22 chains+2Na]$^{2+}$, 1715.53 [**4B** with 23 chains+2Na]$^{2+}$.

4E: 99% yield (15 g); R_f 0.3 (ethyl acetate–cyclohexane–ethanol, 8:4:1); mp 207°C; $[\alpha]_D$= +90.5 (c=4.8, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm): 5.36 (t, 7H, J=9.1 Hz, H3), 5.08 (d, 7H, J=3.8 Hz, H1), 4.78 (dd, 7H, H2), 4.03 (m, 14H, H6–H5), 3.89 (dd, 7H, $J_{H6'-H5}$=5 Hz, $J_{H6'-H6}$=12 Hz, H6′), 3.75 (t, 7H, J=9 Hz, H4), 2.35 (m, 28H, CH_2COO), 1.59 (m, 28H, $\underline{CH_2}$-CH_2COO), 1.32 (m, 56H, -$(CH_2)_2$-), 0.91 (bt, 45H, CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm): 173.7 (COO), 172.2 (COO), 96.5 (C1), 77.6 (C4), 72.7 (C5), 71.0 (C2, C3), 61.6 (C6), 34.3 ($\underline{CH_2}$-COO), 31.7 (-CH_2-), 24.7 ($\underline{CH_2}$-CH_2-COO), 22.7 (-$\underline{CH_2}$-CH_3), 14.3 (-CH_3); ES-MS (+) m/z: 1277.07 [**4E** +2Na]$^{2+}$; Anal. calcd for $C_{126}H_{210}O_{49}$: C, 60.33; H, 8.38; O, 31.29. Found C, 60.63; H, 8.50; O, 30.72.

16. Chemical and physical data for **5B$_1$** 94% yield (3.3 g); mp 233°C; $[\alpha]_D$= +91.5 (c=4.48, CH_2Cl_2); ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm): 5.33 (bt, 7H, H3), 5.07 (bd, 7H, H1), 4.59 (bdd, 7H, H2), 4.05 (m, 21H, H5, H6, H′6), 3.57 (H4), 3.53 (m, -CH-), 2.50 (m, -CH_2-COO-), 1.73 (m, -CH_2-), 1.43 (m, -C$\underline{H_2}$-CH_2-COO-), 1.24 (m, -CH_2-), 0.85 (-CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz): δ (ppm): 206.3 (C=O), 173.0 (COO), 172.2 (COO), 168.2 (COO), 95.9 (C1), 74.7 (C4), 73.8.7 (C5), 71.0–69.8 (C2, C3), 65.5 (C6), 35.2 ($\underline{CH_2}$-COO), 31.5 (-CH_2-), 29.5 ($\underline{CH}$-C=O), 28.3 ($\underline{CH_2}$-C=O), 27.0 ($\underline{CH_2}$-CH_2-COO), 24.5 (-$\underline{CH_2}$-CH_3), 22.9 (-$\underline{CH_2}$-CH_3), 14.5 (-CH_3); ES-MS (–) m/z: 1182.06 [**5B$_1$** with 18 chains and 7 sulphates-3Na]$^{3-}$, 1214; 68 [**5B$_1$** with 19 chains and 7 sulphates-3Na]$^{3-}$, 1264.93 [**5B$_1$** with 20 chains and 7 sulphates-3Na]$^{3-}$, 1280.17 [**5B$_1$** with 21 chains and 7 sulphates-3Na]$^{3-}$, 1312.67 [**5B$_1$** with 22 chains and 7 sulphates-3Na]$^{3-}$, 1345.29 [**5B$_1$** with 23 chains and 7 sulphates-3Na]$^{3-}$.

5B2: 76% yield (2.6 g); mp 238°C; $[\alpha]_D$= +108.8 (c=4.96, CH_2Cl_2); ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm): 5.33 (bt, 7H, H3), 5.10 (bd, 7H, H1), 4.64 (bdd, 7H, H2), 3.85 (m, 21H, H5, H6, H′6), 3.58 (H4), 3.53 (m, -CH-), 2.50 (m, -CH_2-COO-), 1.73 (m, -CH_2-), 1.54 (m, -C$\underline{H_2}$-CH_2-COO-), 1.26 (m, -CH_2-), 0.85 (-CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz): δ (ppm): 206.3 (C=O), 173.0 (COO), 172.2 (COO), 168.2 (COO), 96.2 (C1), 75.6 (C4), 72.7 (C5), 70.9–69.9 (C2, C3), 65.4 (C6), 34.1 ($\underline{CH_2}$-COO), 31.5 (-CH_2-), 29.9 ($\underline{CH}$-C=O), 28.2 ($\underline{CH_2}$-C=O), 24.8 ($\underline{CH_2}$-CH_2-COO), 24.2 (-$\underline{CH_2}$-CH_3), 22.7 (-$\underline{CH_2}$-CH_3), 14.5 (-CH_3); ES-MS (–) m/z: 1478.52 [**5B2** with 17 chains and 2 sulphates-2Na]$^{2-}$, 1531.36 [**5B2** with 17 chains and 3 sulphates-2Na]$^{2-}$, 1580.23 [**5B2** with 18 chains and 3 sulphates-2Na]$^{2-}$, 1678.48 [**5B2** with 20 chains and 3 sulphates-2Na]$^{2-}$, 1727.86 [**5B2** with 21 chains and 3 sulphates-2Na]$^{2-}$, 1778.35 [**5B2** with 21 chains and 4 sulphates-2Na]$^{2-}$, 1878.71 [**5B2** with 22 chains and 5 sulphates-2Na]$^{2-}$, 1927.84 [**5B2** with 23 chains and 5 sulphates-2Na]$^{2-}$.

5E1: 90% yield (5 g); mp (dec) 245°C; $[\alpha]_D$= +112.8 (c=3.28, CH_2Cl_2); ^{1}H NMR (DMSO-d_6, 500 MHz): δ (ppm): 5.32 (t, 7H, J=9.46 Hz, H3), 5.12 (d, 7H, J=2.6 Hz, H1), 4.64 (dd, 7H, H2), 4.21 (dd, 7H, H6), 4.08 (dd, 7H, J=9.46 Hz, H5), 4.00 (d, 7H, H6′), 3.85 (d, 7H, J=8.83 Hz, H4), 2.37 (m, 14H, CH_2COO), 2.17 (m, 14H, CH_2COO), 1.51 (m, 28H, $\underline{CH_2}$-CH_2COO), 1.26 (m, 56H, -$(CH_2)_2$-), 0.86 (bt, 45H, CH_3); ^{13}C NMR (DMSO-d_6, 125 MHz): δ (ppm): 173.3 (COO), 172.2 (COO), 96.6 (C1), 80.05 (C4), 75.5 (C5), 71.28 (C2, C3), 65.38 (C6), 34.2 ($\underline{CH_2}$-COO), 31.7 (-CH_2-), 24.7 ($\underline{CH_2}$-CH_2-COO), 22.7 (-$\underline{CH_2}$-CH_3), 14.5 (-CH_3); ES-MS (+) m/z: 1634.07 [**5E1**+2Na]$^{2+}$, 1097.22 [**5E1**+3Na]$^{3+}$.

5E2: 92% yield (5.3 g); mp (dec) 240°C; $[\alpha]_D$= +152.9 (c=1.7, CH_2Cl_2); ^{1}H NMR (DMSO-d_6, 500 MHz): δ (ppm): 5.29 (bt, 7H, H3), 5.10 (d, 7H, H1), 4.80–4.57 (bdd, 7H, H2), 4.27–3.94 (m, 14H, H6–H5), 3.84 (dd, 7H, H4), 2.34 (m, 14H, CH_2COO), 2.18 (m, 14H, CH_2COO), 1.51 (m, 28H, $\underline{CH_2}$-CH_2COO), 1.27 (m, 56H, -$(CH_2)_2$-), 0.86 (bt, 45H, CH_3); ^{13}C NMR (DMSO-d_6, 125 MHz): δ (ppm): 173.3 (COO), 172.2 (COO), 96.6 (C1), 84.4 (C4), 75.4 (C5, C2, C3), 65.3 (C6), 34.2 ($\underline{CH_2}$-COO), 31.6 (-CH_2-), 24.7 ($\underline{CH_2}$-CH_2-COO), 22.8 (-$\underline{CH_2}$-CH_3), 14.5 (-CH_3); ES-MS (+) m/z: 1378.83 [**5E2** with 2 sulphates+2Na]$^{2+}$, 1430.16 [**5E2** with 3 sulphates+2Na]$^{2+}$, 1481.23 [**5E2** with 4 sulphates+2Na]$^{2+}$, 1531.53 [**5E2** with 5 sulphates+2Na]$^{2+}$, 1582.47 [**5E2** with 6 sulphates+2Na]$^{2+}$.

17. Measurements of surface tension were carried out on a K12 Krüss apparatus using a plate method with deionised water at surface tension of 72 mN m^{-1}.