Synthesis and biocompatibility evaluation of fluorinated, single-tailed glucopyranoside surfactants†

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Partially fluorinated non-ionic surfactants are of interest for a range of biomedical applications, such as the pulmonary administration of drugs using reverse water-in-perfluorocarbon microemulsions. We herein report the synthesis and characterization of a series of partially fluorinated β-D-glucopyranoside surfactants from the respective alcohols and peracetylated β-D-glucopyranoside using $BF_3{\cdot}Et_2O$ as catalyst. The surfactant packing parameter of the fluorinated surfactants ranged from 0.472 to 0.534 (MOPAC calculations) or 0.562 to 0.585 (calculated from literature values), which is comparable to surfactants with a similar partially fluorinated tail. Based on an initial biocompatibility assessment, the β-D-glucopyranoside surfactants have low toxicities in the B16F10 mouse melanoma cell line and comparatively low haemolytic activities towards rabbit red blood cells. The fluorinated surfactants appear to be less toxic towards cells in culture and to have a lower haemolytic activity compared to their hydrocarbon analogs. Furthermore, an increasing degree of fluorination appears to reduce both the cytotoxicity and the haemolytic activity. Similar structure–activity relationships have been reported for other partially fluorinated surfactants. Overall, these findings suggest that the surfactants may be useful for biomedical applications, such as novel drug delivery systems.

Introduction

Surfactants with a partially fluorinated tail of the general structure $(CH_2)_mC_nF_{2n+1}$ have unique physicochemical properties, such as high surface activity and weak molecular interactions.[1] Furthermore, perfluorinated tails are larger and stiffer compared to hydrocarbon tails. For example, the limiting molecular areas of perfluorinated and hydrocarbon chains at the air–water interface are 30 and 20 $Å^2$, respectively.[2,3] This difference in physicochemical properties and molecular geometry allows (partially) fluorinated surfactants to self-assemble in supramolecular structures that are distinctively different from those observed for their hydrocarbon analogues.[4] They tend to self-assemble more easily and to form better organized and more stable systems than their hydrocarbon counterparts.[5]

Partially fluorinated surfactants are of interest for biomedical applications, such as pulmonary drug delivery,[6,7] because of these unique properties. A variety of single tailed partially fluorinated surfactants with different headgroups, for example carnitine,[8] (di-)morpholinophosphate,[9,10] phosphocholine,[11] pyridinium[12] and carbohydrate[5,13] headgroups, have been synthesized, and their biocompatibility has been assessed using *in vitro* and *in vivo* approaches. There is evidence that partially fluorinated surfactants in general display low to moderate acute toxicity compared to their hydrocarbon analogues.[5] Partially fluorinated surfactants with a perfluorooctyl or perfluorodecyl group in the hydrophobic tail have particularly low toxicities. In addition, the haemolytic activity of partially fluorinated surfactants is low despite their high surface activity.[5]

Some fluorinated surfactants form stable, reverse water-in-perfluorocarbon (micro-)emulsions.[14–18] These emulsions are of particular interest for the perfluorocarbon-assisted pulmonary administration of drugs[6,7] because perfluorocarbon-insoluble drugs can be administered by dissolving them in the aqueous core of the reverse (micro-)emulsion. For example, some dimorpholinophosphate surfactants form reverse (micro-) emulsions that are highly fluid and have a water content of up to 30 vol% at a surfactant concentration of 2% (w/v).[19] In addition, these reverse (micro-)emulsions appear to be biocompatible *in vitro* and *in vivo*.[19,20] However, all other reverse water-in-perfluorocarbon (micro-)emulsions investigated to date are based on perfluorinated surfactants (*e.g.*, poly(oxyethylene) derivatives) that are not biocompatible.[15–18] Therefore, there is considerable need for novel partially fluorinated surfactants that are biocompatible and have the potential to form stable, reverse water-in-perfluorocarbon (micro-)emulsions.

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Building on earlier studies with partially fluorinated carbohydrate surfactants,[5] the present study describes the synthesis and characterization of a series of partially fluorinated β-D-glucopyranoside surfactants. In addition, this study evaluates their toxicity in a cell culture model as well as their haemolytic activity in comparison to the analogous hydrocarbon surfactants.

Results and discussion

Surfactant packing parameters of β-D-glucopyranosides

Although it is difficult to predict if a partially fluorinated surfactant forms reverse water-in-perfluorocarbon microemulsions,[5] the molecular geometry of a surfactant is one important determinant of the self-assembled structures formed by a surfactant. One possible way to describe the molecular geometry of a surfactant is the surfactant packing parameter $P = v/(a_0 l_c)$ (where v is the volume of the hydrophobic chain, a_0 is the area of the polar head group and l_c is the length of the hydrophobic chain).[4,21] To guide us with the selection of promising target molecules, we calculated the surfactant packing parameter for several β-D-glucopyranosides using either MOPAC or published values for v, a_0 and l_c.[4,22] In addition, we calculated the surfactant packing parameters of two dimorpholinophosphate surfactants which have been shown to form stable, reverse water-in-perfluorocarbon (micro-)emulsions.[14,19] The results of these calculations are shown in Table 1.

The volumes, tail lengths and surfactant parameters calculated with the two different approaches differed significantly from each other. Similarly, Giulieri and Krafft reported significant differences between published and experimental values for v and l_c.[4] Despite these discrepancies, the calculated surfactant packing parameters allowed a comparison of the molecular geometry of glucopyranoside and dimorpholinophosphate surfactants and the identification of trends. Independent of the approach used, the surfactant packing parameters of both types of surfactants were similar, and near the packing parameter value that would be associated with ideal cylindrical micelles ($P = 0.5$).[21] For the MOPAC results, P for the glucopyranosides was slightly larger than the P for dimorpholinophosphate surfactants, while the converse was suggested from the experimental data due to the smaller experimental dimorpholinophosphate headgroup area.[4] Furthermore, P of the partially fluorinated glucopyranoside surfactants increased slightly with increasing degree of fluorination, whereas P for the hydrocarbon glucopyranoside surfactants did not change with increasing chain length. Overall, the surfactant packing parameters shown in Table 1 suggest that partially fluorinated glucopyranoside and dimorpholinophosphate surfactants have a similar molecular geometry. Therefore, glucopyranosides **8a–f** were synthesized for further studies because they may also form reverse water-in-perfluorocarbon microemulsions.

Synthesis

The synthesis of several fluorinated β-D-glucopyranosides has been reported in the literature.[23–25] The hydrocarbon spacer between the glucose moiety and the terminal perfluoroalkyl group of these surfactants is short, with two,[23] three[26–28] or six[24,25] methylene groups. To the best of our knowledge, glucopyranoside surfactants with longer hydrocarbon spacers have not been synthesized previously. The following section describes the synthesis of the partially fluorinated β-D-glucopyranosides **8a–f**, which were selected based on their surfactant packing parameters.

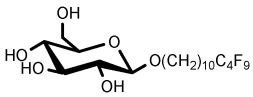
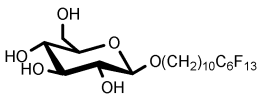
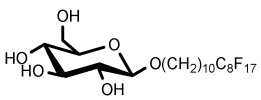
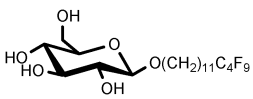
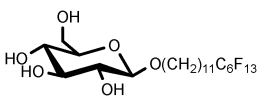
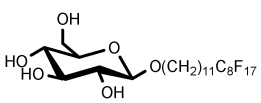
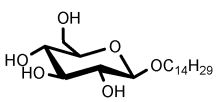
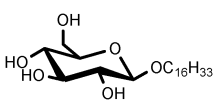
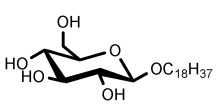
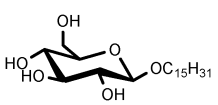
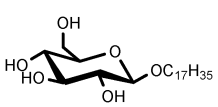
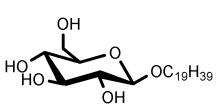
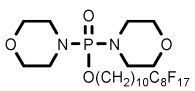
First, a series of partially fluorinated alcohols with the general structure $C_mF_{2m+1}(CH_2)_nOH$ (m = 4, 6 and 8, n = 10 and 11) were synthesized from decenol (**1**) or 10-undecenoic acid (**2**), respectively.[12] As shown in Scheme 1, the two starting materials were converted into the corresponding acetate or methyl ester, and the hydrocarbon precursor was coupled with a perfluorinated iodide in an AIBN mediated radical reaction.[29–32] The resulting secondary iodides were deiodinated with HI/Zn/EtOH and converted into the respective alcohol **3** either by saponification or by reduction with LAH.

Subsequently, the fluorinated alcohols **3a–f** and the corresponding hydrocarbon analogues **4a–f** were reacted with peracetylated β-D-glucopyranoside (**5**) to obtain the peracetylated glucopyranosides **6a–f** and **7a–f**. This glycosylation reaction can be performed using a number of Lewis acids, for example $SnCl_4$,[33,34] $ZnCl_2$[35] or $BF_3{\cdot}Et_2O$[24,36–39] as catalysts. Initial experiments showed that reaction of the fluorinated alcohols **3** with **5** in the presence of $ZnCl_2$ or $AlCl_3$ required long reaction times and high reaction temperatures, which led to the formation of mixtures of the α- and β-anomers of the desired peracetylated glucopyranosides **6a–f**. In contrast, $BF_3{\cdot}Et_2O$ gave the desired β-anomers of the glucopyranosides **6a–f** and **7a–f** in 40–55% yield when low reaction temperatures (<5 °C) and short reaction times (<3 h) were employed. The glycosylation products obtained under these conditions typically contained traces of α-anomer (<1%) as determined by ^{1}H NMR. This small amount of the α-anomer was considered acceptable for the intended application of these compounds as surfactants in biomedical applications. The thermodynamically more stable α-anomers were obtained when the reaction temperature was raised to 30 °C and the reaction time was extended to 10 h (data not shown). No other products, for example 2-, 3-, 4- or 6-(*F*-)alkyl glucopyranosides, were formed according to TLC and ^{1}H NMR analysis.

In the final step of the synthesis, the (*F*-)alkylated peracetylated β-D-glucopyranosides **6a–f** and **7a–f** were deacetylated using NaOMe in absolute methanol, followed by neutralization with Dowex® 50W×8-100 ion exchange resin.[38] The crude product was purified by column chromatography on silica gel followed by recrystallization from acetone. The pure glucopyranosides **8a–f** and **9a–f** were obtained with yields ranging from 92 to 98%.

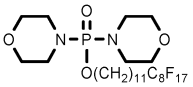
In a preliminary study we also investigated a different route to the desired glucopyranosides to improve the overall yields of the synthesis. Analogous to published syntheses that employ bromo-sugars and an alcohol as starting materials,[40,41] we reacted 2,3,4,6-tetraacetyl-α-D-glycopyranosyl bromide (**10**) with a partially fluorinated alcohol in the presence of Ag_2CO_3 in DCM at 0 °C (Scheme 2). However, instead of the desired glucopyranoside **6e**, we obtained the fluorinated

Table 1 Surfactant packing parameter P^a of the glucopyranosides **8a–f** and their hydrocarbon analogues **9a–f**

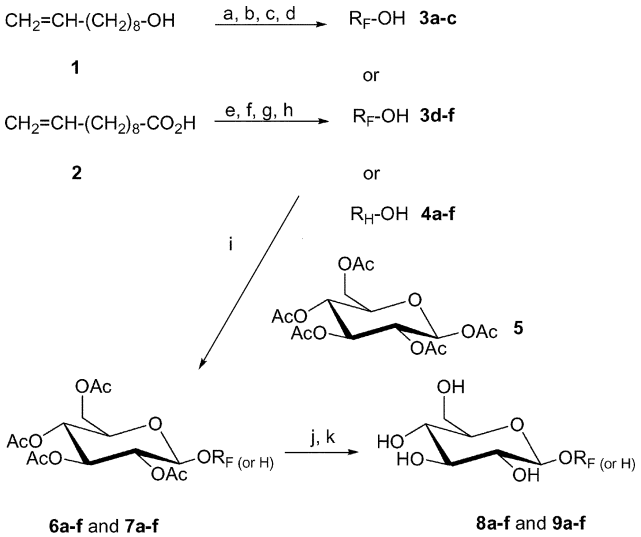
		MOPAC calculation results				
Entry	Structure	Area, a_0/Å^2	Volume, v^b/Å^3	Length, $l_c{}^c$/Å	P	P^d
8a		41	357	18.5	0.472	0.566
8b		41	426	21.1	0.493	0.579
8c		41	495	23.7	0.510	0.589
8d		41	394	19.7	0.488	0.562
8e		41	469	22.3	0.513	0.575
8f		41	545	24.9	0.534	0.585
9a		41	307	18.2	0.410	0.488
9b		41	350	20.7	0.413	0.490
9c		41	394	23.2	0.415	0.492
9d		41	332	19.5	0.415	0.489
9e		41	376	21.9	0.418	0.491
9f		41	420	24.4	0.419	0.492
10a		50	495	23.7	0.419	0.618

(*continued on next page*)

Table 1 (*continued*)

Entry	Structure	MOPAC calculation results				
		Area, a_0/Å^2	Volume, v^b/Å^3	Length, $l_c{}^c$/Å	P	P^d
10b		50	545	24.9	0.438	0.614

[a] Surfactant packing parameter $P = v/(a_0 l_c)$, where a_0 is the area of the polar head group, v is the volume of the hydrophobic chain, and l_c is the length of the hydrophobic chain.[4,21] [b] The volume v (Å^3) from MOPAC correlations was calculated as $22.1n_H + 37.7n_F$ (n_H odd) or $21.9n_H + 34.5n_F$ (n_H even), where n_H = the number of hydrogenated and n_F = the number of fluorinated carbons. [c] The length l_c (Å) from MOPAC correlations was calculated as $0.86 + 1.24n_H + 1.3n_F$. [d] Surfactant packing parameter P calculated using published values for a_0, v and l_c.[4,22]



(A) Even numbered tail

3, 6 or 8	-R_F	4, 7 or 9	-R_H
a	-$(C)_{10}C_4F_9$	a	-$C_{14}H_{29}$
b	-$(C)_{10}C_6F_{13}$	b	-$C_{16}H_{33}$
c	-$(C)_{10}C_8F_{17}$	c	-$C_{18}H_{37}$

(B) Odd numbered tail

3, 6 or 8	-R_F	4, 7 or 9	-R_H
d	-$(C)_{11}C_4F_9$	d	-$C_{15}H_{31}$
e	-$(C)_{11}C_6F_{13}$	e	-$C_{17}H_{35}$
f	-$(C)_{11}C_8F_{17}$	f	-$C_{19}H_{39}$

Scheme 1 Synthesis of perfluoroalkyl and alkyl β-D-glucopyranosides. *Reagents and conditions*: (a) DMAP, Ac–Cl, pyridine, DCM; (b) $F(CF_2)_mI$ (m = 4, 6 or 8), AIBN; (c). HI (55%), Zn, C_2H_5OH; (d) CH_3OH, KOH; (e) CH_3OH, PTSA, toluene; (f) $F(CF_2)_mI$ (m = 4, 6 or 8), AIBN; (g) HI (55%), Zn, C_2H_5OH; (h) $LiAlH_4$, anhydrous ether, ambient temperature; (i) BF_3/OEt_2(48%), anhydrous DCM, 0 °C to ambient temperature, 3 h; (j) MeONa/MeOH, 0 °C to ambient temperature; (k) Dowex® 50W×8-100 ion-exchange resin.

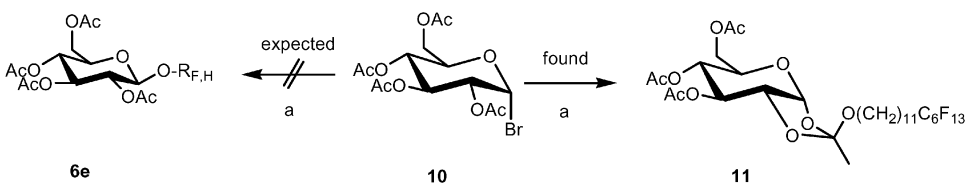
1,2-orthoacetate **11** in 80% yield. Based on the chemical shifts of the H-1′ proton at 5.70 ppm, the 1,2-orthoacetate **11** is the *exo* isomer (*exo*: δ 5.72 ppm *vs*. *endo*: δ 5.59 ppm[42]). Similarly, Tsui and Gorin have reported the formation of 1,2-orthoacetates as side products (21–34% yield) under comparable reaction conditions.[42] The lower yield in that study may be due to the shorter chain length of the alcohols employed (≤C-8).

Melting points of (*F*-)alkyl glucopyranosides

Glucopyranosides are compounds that form both thermotropic liquid crystalline phases upon heating and lyotropic liquid crystalline phases upon addition of solvents such as water.[43–45] As a result of this complex phase behaviour, a range of melting points has been reported in the literature for alkyl β-D-glucopyranosides. To investigate the thermotropic properties of the partially fluorinated glucopyranosides, the phase transitions of the glucopyranosides **8a–f** and **9a–f** were investigated using differential scanning calorimetry (DSC). The phase transitions determined by DSC were in agreement with the melting points measured with a MelTemp apparatus.

As shown in Fig. 1, both hydrocarbon and fluorocarbon glucopyranosides displayed at least two phase transitions. The main phase transitions were observed at temperatures ranging from 69 to 116 °C. A minor phase transition observed at temperatures from 145 to 190 °C corresponds to the clearing temperatures reported for long-chain hydrocarbon glucopyranosides[43,44] and probably represents a transition from an anisotropic liquid crystalline phase to an isotropic liquid phase.

While the phase transition temperatures for the even numbered glucopyranosides **8a–b** and **9a–b** were almost the same, the respective phase transition temperatures for the highly fluorinated glucopyranoside **8c** were clearly higher compared to the corresponding hydrocarbon analogue **9c** (Fig. 1(A)). Similarly, the partially fluorinated glucopyranosides with an odd number of carbon atoms in the hydrophobic tail (**8d–f**)



Scheme 2 Synthesis of orthoester **11**. *Reagents and conditions*: (a) **3e**, Ag_2CO_3, DCM, 0 °C to ambient temperature.

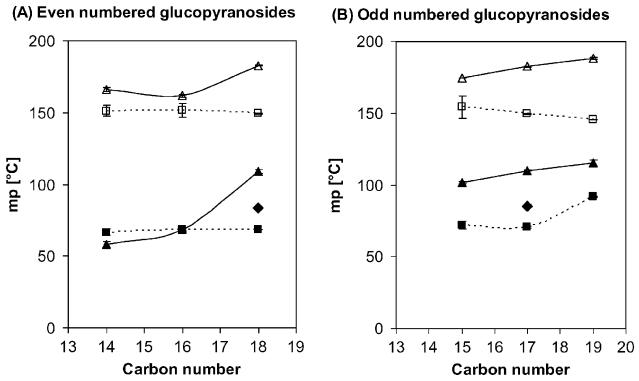


Fig. 1 Comparison of the phase transition maxima of perfluoroalkyl and alkyl β-D-glucopyranosides with an (A) even (**8a–c** and **9a–c**) and (B) odd number (**8d–f** and **9d–f**) of carbon atoms in the hydrophobic tail (▲ and △ = fluoroalkyl; ■ and □ = alkyl; closed symbols = major phase transition; open symbols = minor phase transition; ◆ represents the maximum of a second phase transition observed for alkyl glucopyranosides **9c** and **9e**). Values shown are the experimental mean ± one standard deviation of at least three DSC experiments.

displayed higher phase transition temperatures compared to their hydrocarbon analogues **9d–f** (Fig. 1(B)). In comparison, the melting points of the partially fluorinated β-D-glucopyranosides **8a–f** are lower compared to fluorinated glucopyranosides with a propylene hydrocarbon spacer ($-(CH_2)_3C_mF_{2m+1}$, m = 4, 6 and 8).[26,28]

These differences in the phase transition temperatures between hydrocarbon and fluorocarbon β-D-glucopyranosides are a result of the perfluoroalkyl terminus in the hydrophobic tail. Due to the larger steric demand of the fluorine atom, perfluoroalkyl chains are more rigid, which allows a more efficient packing in the solid state. As a consequence, the melting point of the glucopyranosides **8c–f** was higher compared to the corresponding hydrocarbon analogues **9c–f**. Similar trends in phase transition temperatures have been reported for other partially fluorinated compounds, such as partially fluorinated, long-chain carboxylic acids with the general structure $F_{2m+1}C_m(CH_2)_{10}COOH$ (m = 4, 6 and 8)[29] or fluorinated β-D-glucopyranosides with a short hydrocarbon spacer.[26,28]

Biological studies

An initial cytotoxicity assessment of the glucopyranoside surfactants **8a–f** and **9a–f** was performed using the B16F10 mouse melanoma cell line.[46] Haemolytic activities, both in the presence and absence of 20% serum, were determined using rabbit red blood cells.[12] Octylthioglucoside was used as a positive control for the cytotoxicity studies and the determination of haemolytic activities. Cytotoxicities and haemolytic activities, expressed as EC_{50} values, are summarized in Table 2 for all compounds under investigation. Representative cytotoxicity curves for surfactants **8c**, **8e** and **9e** are presented in Fig. 2.

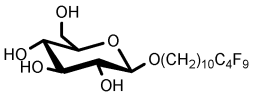
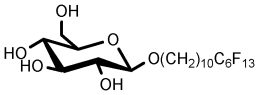
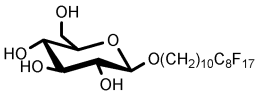
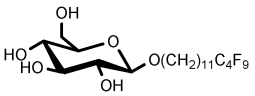
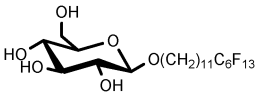
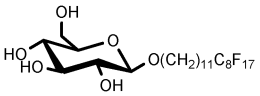
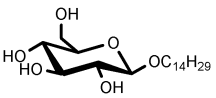
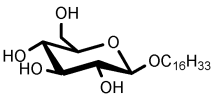
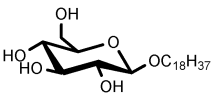
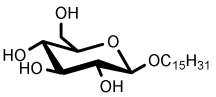
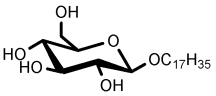
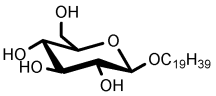
The partially fluorinated glucopyranosides **8a–b** and **8d–e** were moderately toxic, with EC_{50} values ranging from 179 to 311 μM. These EC_{50} values are comparable to the EC_{50} values of the analogous hydrocarbon derivatives **9a–d**, which ranged from 190 to 600 μM. This is in contrast to the positive control, octylthioglucoside (OTG), which showed significant cytotoxicity over the same concentration range. Similarly, partially fluorinated surfactants derived from maltose,[47] mannitol,[48] sorbitan,[48] sucrose,[49] trehalose,[49] and xylitol[50] also display moderate-to-low toxicity in cells in culture, with EC_{50} typically >100 μM. In contrast, glucopyranosides with shorter fluorinated chains (≤ 12 carbon atoms) appear to be more cytotoxic than glucopyranosides **8a–f**.[25] For example, 7,7,8,8,9,9,10,10,11,11,12,12,12-tridecafluorododecyl-β-D-glucopyranoside was cytotoxic at concentrations of 50 μM.

The two glucopyranosides **8c** and **8f** with the perfluorooctyl terminus were even less toxic than **8a–b** and **8d–e**, with EC_{50} values >1.5 mM. Similarly, the cytotoxicity of fluorinated pyridinium surfactants has been shown to decrease with an increasing degree of fluorination.[12] One possible explanation for this decrease in cytotoxicity is a lower cellular uptake due to the hydrophobic and lipophobic perfluoroalkyl terminus of the hydrophobic tail. This interpretation is supported by a recent study that showed no cellular uptake of a highly fluorinated galactopyranoside in the B16 melanoma cell line.[25] However, there is also evidence that fluorinated surfactants readily partition into model membranes.[2,51–53] and, thus, should be able to enter cells in culture. Therefore, cellular uptake studies are necessary to determine if the glucopyranosides **8c** and **8f** can indeed partition though cell membranes.

The results from the MTT assay were further confirmed by Hoffman modulation contrast microscopy (Fig. 3). In agreement with the results form the MTT assay, treatment of B16F10 cells with high concentrations of surfactant **8c** (24 h, 1.5 mM) surfactant neither altered cell growth nor induced morphological changes in comparison to the control (Fig. 3(A) and (B)). In contrast, surfactants **8e** and **9e** induced retardation in cell proliferation and caused an abnormal prolongated morphology of the cells at a concentration close to the EC_{50} (Fig. 3(C) and (D)). In addition, both surfactants induced apoptosis in a significant number of cells. No viable cells were observed at high concentrations (1.5 mM) of **8e** and **9e** (Fig. 3(E) and (F)). Only necrotic cells and cellular debris were evident at these high concentrations. Fluorescence staining with PI and YO-PRO-1 showed only a small portion of B16F10 cells in early or late stage of apoptosis in control cells (Fig. 3(G)) but significant amount of cells treated with **8e** at concentration close to the EC_{50} were in various stages of apoptosis (see Fig. 3(H)).

In addition to the cytotoxicity experiments, the haemolytic activity of all surfactants was assessed using rabbit red blood cells.[12] The glucopyranosides **8a–f** and **9a–f** were haemolytic at low millimolar concentrations. The haemolytic activity decreased for both groups of surfactants in the presence of serum, with EC_{50} values >15 mM for **8a–f** in the presence of serum and >5 mM for **9a–f** in the absence of serum. Furthermore, the hydrocarbon glucopyranosides **9a–f** had a higher haemolytic activity compared to their fluorinated analogues **8a–f**. Similar observations have been reported for various other fluorinated surfactants.[5,12,47–50] In addition, the haemolytic activity decreased for structurally related glucopyranosides (*i.e.*, **8a–d** with ten and **8d–f** with eleven methylene groups in the hydrocarbon spacer) with increasing

Table 2 Assessment of cytotoxicity and haemolytic activity of partially fluorinated glucopyranosides and their hydrocarbon analogues in the B16F10 cell line.[12,46]

Entry	Structure $R_{(F \text{ or } H)}$-β-D-Glu	Cytoxicity, EC_{50}/μM	Haemolytic activity, EC_{50}[a]/mM	
			Without serum	20% Serum
Fluorocarbon surfactants				
8a		194	>20 (10%)	>20 (10%)
8b		311	>20 (25%)	20
8c		>1500	>20	>20
8d		190	15	20
8e		179	>20	>20
8f		>1500	>20	>20
Hydrocarbon surfactants				
9a		250	10	15
9b		300	>20 (30%)	>20
9c		190	>20 (10%)	>20
9d		230	5	15
9e		230	15	>20 (5%)
9f		600	>20	>20
OTG	Octylthioglucoside	20	0.5	3

[a] Data in parenthesis represent the percentage haemolysis at a 20 mM concentration of the respective glucopyranoside.

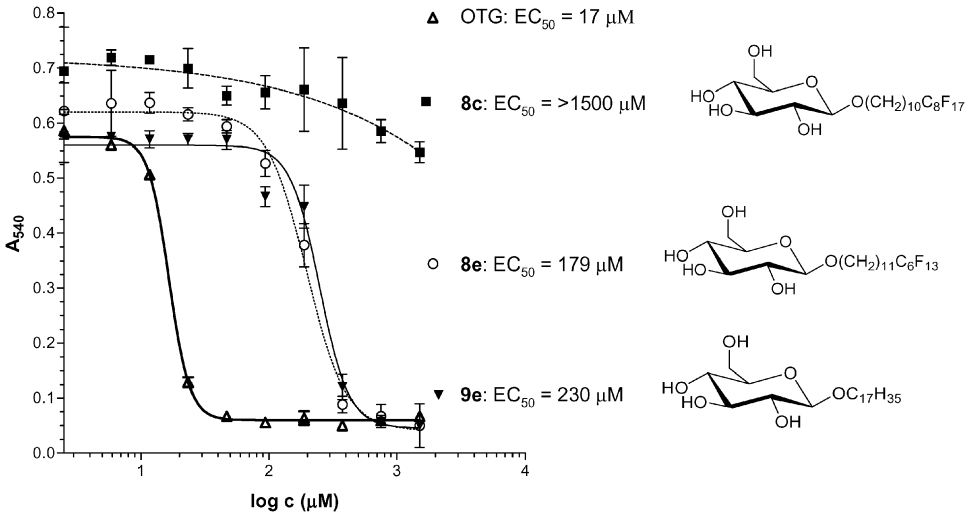


Fig. 2 Cytotoxic effect of glucopyranosides **8c**, **8e** and **9e** in the B16F10 mouse melanoma cell line. B16F10 cells were exposed for 24 h to the respective glucopyranoside at the concentrations shown and assessed for MTT activity as described in the Experimental section. A representative hydrocarbon surfactant, octylthioglucoside (OTG), is shown for comparison.

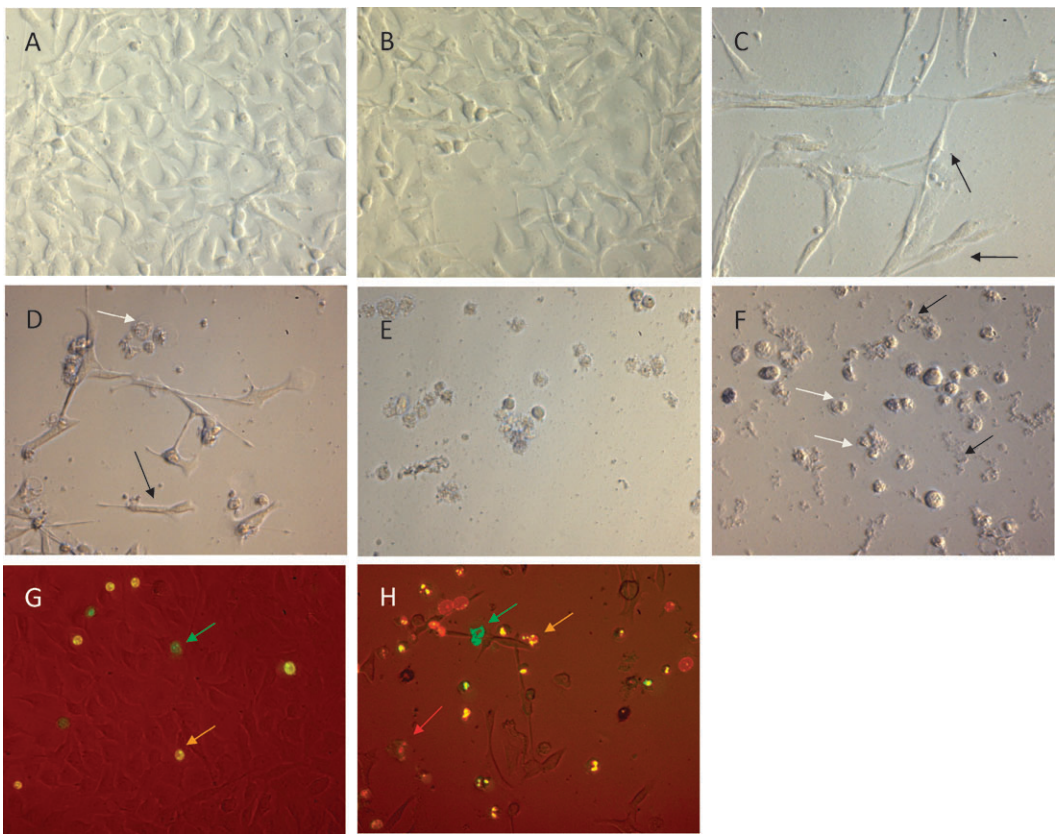


Fig. 3 Cytotoxic effect of glucopyranosides **8c**, **8e** and **9e** in the B16F10 mouse melanoma cell line. The B16F10 cells were treated for 24 h with (A) the vehicle (PBS + 1% DMSO) or with glucopyranoside (B) **8c** (1.5 mM), (C) **8e** (250 μM, black arrows indicate abnormal spindle-shaped cells), (D) **9e** (250 μM, black arrow indicate abnormal spindle-shaped cell, white arrow indicate typical necrotic cell), (E) **8e** (1.5 mM) or (F) **9e** (1.5 mM, black arrows indicate cellular debris, white arrows indicate necrotic cells). The fluorescent markers Yo-Pro-1 (green) and PI (red/yellow) were used for the visualization of early apoptotic changes and post-apoptotic secondary necrosis in B16F10 cells treated for 24 h with (G) vehicle (green arrows indicate early stage of apoptosis, orange arrows indicate late stage of apoptosis) or (H) **8e** (250 μM, green arrows indicate early stage of apoptosis, orange arrows indicate late stage of apoptosis, red arrows indicate secondary necrosis). The cells were observed under an epifluorescent microscope.

length of the perfluoroalkyl terminus. This anti-haemolytic effect of an increasing degree of fluorination has been reported previously for a number of structurally diverse surfactants.[5,12,47–50]

Conclusions

A series of β-D-glucopyranoside surfactants with (F-)alkyl chain lengths ranging from 14 to 19 carbon atoms was synthesized in good yields from the respective alcohols and peracetylated β-D-glucopyranoside (**5**) using $BF_3{\cdot}Et_2O$ as catalyst. Similar to other partially fluorinated surfactants, an increasing degree of fluorination reduced their toxicity and haemolytic activity. Because of their biocompatibility as well as their molecular geometry (*i.e.*, the surfactant packing parameter), the partially fluorinated glucopyranoside surfactants **8a–e** are of particular interest for biomedical applications, such as the pulmonary administration of drugs using reverse water-in-perfluorocarbon (micro-)emulsions.[6,7]

Experimental

Calculation of surfactant packing parameters of (*F*-)alkyl β-D-glucopyranosides

The geometry of each molecule was optimized using MOPAC as implemented in Chem3D Ultra 9.0 (CambridgeSoft).[54] Molecular volumes were calculated as COSMO volumes, and tail lengths were measured directly from the optimized geometry as the length from the O atom to the terminal atom in the tail. Tail length and volume correlations were developed for hydrocarbon and fluorocarbon surfactants in the series. Areas were determined by determining volumes for a series of at least three surfactants with the same head group and using a linear correlation between tail length and volume to determine the head volume (from the intercept). The area was then calculated assuming a spherical head geometry (which may not be reasonable for dimorpholinophosphinate surfactants). The alternative calculations were based on experimental areas and correlations between carbon number and bond lengths and tail volumes.[4,22]

Synthesis of (*F*-)alkyl β-D-glucopyranosides

The long-chain hydrocarbon starting materials **1** and **2** were purchased from TCI Chemicals (Portland, Oregon, USA). Pentaacetyl-β-D-glucopyranose (**5**), the long chain alkyl alcohols **4a–f** and anhydrous dichloromethane were obtained from Fisher Scientific (Fairlawn, New Jersey, USA). Perfluorinated iodides were purchased from Oakwood Chemical Co. (West Columbia, South Carolina, USA). The ^1H and ^{13}C NMR spectra were recorded on a multinuclear Bruker Avance 300 or Bruker DRX 400 Digital NMR Bruker spectrometers at ambient temperature. Due to their poor solubility, NMR spectra of long-chain glucopyranosides were recorder at 318 K. All ^1H and ^{13}C chemical shifts are reported in parts per million (ppm) relative to internal tetramethylsilane. ^{13}C signals of the glucose moiety were assigned as described previously.[36] ^{19}F spectra were recorded using the Bruker Avance 300 with $CFCl_3$ as internal standard. The mass spectra were measured at the University of Iowa Mass Spectrometry Facility. High-resolution mass spectra (HR-MS) were measured using an Autospec ESI-MS instrument and ESI mass spectra were recorded using a ThermoFinnigan LCQ Deca mass spectrometer. Elemental analyses were obtained from Atlantic Micro Lab Microanalysis Service (Atlanta, Georgia, USA). Melting points were determined using a MelTemp apparatus and are uncorrected. In addition, the maxima of the major phase transitions of all glucopyranosides was determined using differential scanning calorimetry (DSC) from 0 to 200 °C with a 10 °C min^{-1} ramp.[29,45] All reactions were monitored by thin layer chromatography, followed by visualization with anisaldehyde–H_2SO_4.[55] The detailed characterization of representative compounds with odd and even numbered tails is shown in the text below (the detailed characterization of the remaining compounds is available as ESI†).

General procedure for the synthesis of perfluoroalkyl and alkyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosides (6a–f and 7a–f)[38]

Boron trifluoride diethyl ether complex (10.0 mmol, 48% w/w) in 5 mL dry dichloromethane was added dropwise to a solution of pentaacetyl-β-D-glucopyranose **5** (5.0 mmol) and the corresponding alcohol **3a–f** or **4a–f** (6.0 mmol) in 15 mL dry dichloromethane at 0 °C. The reaction mixture was stirred at 0 °C for 2 h and allowed to slowly warm to ambient temperature. Dichloromethane (20 mL) was added and the mixture was washed with saturated $NaHCO_3$ solution (3 × 15 mL) and brine (2 × 15 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, and the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel with hexane and ethyl acetate as eluent (hexane–ethyl acetate = 3 : 1). The product was obtained as a white solid or a yellowish viscous liquid with moderate yields ranging from 46 to 55%.

Perfluoroalkyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosides

11,11,12,12,13,13,14,14,14-Nonafluorotetradecyl-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (6a). Viscous liquid; yield, 55%; ^1H NMR (300 MHz, $CDCl_3$): δ/ppm 1.1–1.3 (m, 12H, 6 × CH_2), 1.45 (m, 4H, H-2′ and H-9′), 1.8–2.0 (m, 14H, 4 × CH_3CO and H-10′), 3.35 (m, 1H, H-1′a), 3.59 (m, 1H, H-5), 3.76 (m, 1H, H-1′b), 4.00 (d, 1H, $J_{6a,6b}$ = 12.0 Hz, H-6a), 4.16 (dd, 1H, $J_{6a,6b}$ = 12.0 Hz, $J_{5,6b}$ = 4.7 Hz, H-6b), 4.39 (d, 1H, J = 8.0 Hz, H-1), 4.84 (dd, 1H, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 9.5 Hz, H-2), 4.96 ("t", 1H, J ~ 9.9 Hz, H-4), 5.09 ("t", 1H, J ~ 9.5 Hz, H-3). ^{13}C NMR (75 MHz, $CDCl_3$): δ/ppm 20.0 (*C*-9′), 20.5 (4 × CH_3CO), 25.8 (*C*-3′), 29.0–29.4 (*C*-2′, *C*-4′ to *C*-8′), 30.7 (t, J = 21 Hz, *C*-10′), 62.0 (C-6), 68.5 (C-4), 70.0 (C-1′), 71.3 (C-2), 71.7 (C-5), 72.8 (C-3), 100.8 (C-1), 169.1, 169.4, 170.2, 170.5 (4 × $COCH_3$). ^{19}F NMR (282 MHz, $CDCl_3$): δ/ppm −81.6 (CF_3), −115.1 (CF_2), −124.9 (CF_2), −126.6 (CF_2). Positive-ion ESI-MS peak at m/z 729 ($[M + Na]^+$).

12,12,13,13,14,14,15,15,15-Nonafluoropentadecyl-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (6d). Viscous liquid; yield, 51%; ^1H NMR (300 MHz, $CDCl_3$): δ/ppm 1.1–1.3 (m, 14H, 7 × CH_2), 1.44 (m, 4H, H-2′ and H-10′), 1.8–2.0 (m, 14H, 4 × CH_3CO and H-11′), 3.34 (m, 1H, H-1′a), 3.60 (ddd, 1H,

$J_{4,5}$ = 9.8 Hz, $J_{5,6a}$ = 2.4 Hz, $J_{5,6b}$ = 4.5, H-5), 3.74 (m, 1H, H-1′b), 4.10 (dd, 1H, $J_{6a,6b}$ = 12.2 Hz, $J_{5,6a}$ = 2.3 Hz, H-6a), 4.15 (dd, 1H, $J_{6a,6b}$ = 12.2 Hz, $J_{5,6b}$ = 4.7 Hz, H-6b), 4.38 (d, 1H, J = 8.0 Hz, H-1), 4.84 (“t”, 1H, $J \sim$ 8.4 Hz, H-2), 4.95 (dd, 1H, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 9.5 Hz, H-4), 5.08 (“t”, 1H, $J \sim$ 9.4 Hz, H-3). ^{13}C NMR (75 MHz, $CDCl_3$): δ/ppm 20.0 (*C*-10′), 20.4 (4 × *C*H_3CO), 25.7 (*C*-3′), 29.0–29.5 (C-2′, C-4′ to C-9′), 30.6 (t, J = 22 Hz, C-11′), 61.9 (C-6), 68.5 (C-4), 70.0 (C-1′), 71.3 (C-2), 71.7 (C-5), 72.8 (C-3), 100.8 (C-1), 169.0, 169.3, 170.1, 170.5 (4 × *C*OCH$_3$). ^{19}F NMR (282 MHz, $CDCl_3$): δ/ppm −81.6 (CF_3), −115.1 (CF_2), −124.9 (CF_2), −126.6 (CF_2). Positive-ion ESI-MS peak at m/z 743 ([M + Na]$^+$).

Alkyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosides

Tetradecyl-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (7a)[38,56]. White solid; mp 63–64 °C (lit.: 57–84 °C[56,57]); yield, 51%; ^{1}H NMR (300 MHz, $CDCl_3$): δ/ppm 0.88 (t, 3H, J = 7.0 Hz, H-14′), 1.1–1.4 (m, 22H, 11 × CH_2), 1.56 (m, 2H, H-2′), 1.9–2.1 (4 × s, 12H, 4 × CH_3CO), 3.48 (dt, 1H, $J_{1'a,1'b}$ = 9.6 Hz, $J_{1'a,2'}$ = 6.6 Hz, H-1′a), 3.69 (ddd, 1H, $J_{4,5}$ = 9.8 Hz, $J_{5,6a}$ = 2.5 Hz, $J_{5,6b}$ = 4.7, H-5), 3.86 (dt, 1H, $J_{1'a,1'b}$ = 9.6 Hz, $J_{1'a,2'}$ = 6.6 Hz, H-1′b), 4.13 (dd, 1H, $J_{6a,6b}$ = 12.2 Hz, $J_{6a,5}$ = 2.5 Hz, H-6a), 4.26 (dd, 1H, $J_{6a,6b}$ = 12.2 Hz, $J_{5,6b}$ = 4.7 Hz, H-6b), 4.48 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.98 (dd, 1H, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 9.5 Hz, H-2), 5.09 (“t”, 1H, $J \sim$ 9.6 Hz, H-4), 5.21 (“t”, 1H, $J \sim$ 9.4 Hz, H-3). ^{13}C NMR (75 MHz, $CDCl_3$): δ/ppm 14.3 (*C*-14′), 20.8–20.9 (4 × *C*H_3CO), 22.9 (*C*-13′), 26.1 (*C*-3′), 29.5–29.9 (C-2′, C-4′ to C-11′), 32.1 (*C*-12′), 62.2 (C-6), 68.7 (C-4), 70.4 (C-1′), 71.6 (C-2), 71.9 (C-5), 73.0 (C-3), 101.0 (C-1), 169.4, 169.6, 170.5, 170.9 (4×*C*OCH$_3$). Positive-ion ESI-MS peak at m/z 567 ([M + Na]$^+$).

Pentadecyl-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (7d)[6]. White solid; mp 68–69 °C; yield, 53%; ^{1}H NMR (300 MHz, $CDCl_3$): δ/ppm 0.88 (t, 3H, J = 7.0 Hz, H-15′), 1.1–1.4 (m, 24H, 12 × CH_2), 1.56 (m, 2H, H-2′), 2.0–2.1 (4 × s, 12H, 4 × CH_3CO), 3.47 (dt, 1H, $J_{1'a,1'b}$ = 9.7 Hz, $J_{1'a,2'}$ = 6.7 Hz, H-1′a), 3.69 (ddd, 1H, $J_{4,5}$ = 9.9 Hz, $J_{5,6a}$ = 2.5 Hz, $J_{5,6b}$ = 4.7, H-5), 3.86 (dt, 1H, $J_{1'a,1'b}$ = 9.7 Hz, $J_{1'a,2'}$ = 6.4 Hz, H-1′b), 4.13 (dd, 1H, $J_{6a,6b}$ = 12.3 Hz, $J_{6a,5}$ = 2.5 Hz, H-6a), 4.26 (dd,1H, $J_{6a,6b}$ = 12.3 Hz, $J_{5,6b}$ = 4.6 Hz, H-6b), 4.48 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1), 4.98 (dd, 1H, $J_{1,2}$ = 7.9 Hz, $J_{2,3}$ = 9.6 Hz, H-2), 5.08 (“t”, 1H, $J \sim$ 9.7 Hz, H-4), 5.21 (“t”, 1H, $J \sim$ 9.4 Hz, H-3). ^{13}C NMR (75 MHz, $CDCl_3$): δ/ppm 14.3 (*C*-15′), 20.8–20.9 (4 × *C*H_3CO), 22.9 (*C*-14′), 26.0 (*C*-3′), 29.5–29.9 (C-2′, C-4′ to C-12′), 32.2 (*C*-13′), 62.3 (C-6), 68.7 (C-4), 70.5 (C-1′), 71.6 (C-2), 72.0 (C-5), 73.1 (C-3), 101.1 (C-1), 169.5, 169.6, 170.5, 170.8 (4 × *C*OCH$_3$). Positive-ion ESI-MS peak at m/z 581 ([M + Na]$^+$).

General procedure for the synthesis of perfluoroalkyl and alkyl β-D-glucopyranosides (8a–f and 9a–f)[8]. A solution of sodium methoxide (5 mmol) in methanol (5 mL) was added dropwise to a solution of the respective tetraacetylated glucopyranoside **6a–f** or **7a–f** (2 mmol) in methanol (10 mL) and the mixture was stirred at ambient temperature for 1 h. The reaction mixture was neutralized by addition of Dowex® 50W×8-100 ion exchange resin (2.0 g). The ion exchange resin was filtered off and the solvent was removed under reduced pressure. The crude product was purified by recrystallization from acetone or column chromatography on silica gel (eluent: DCM–MeOH = 8 : 1), followed by recrystallization from acetone, to give the products as white solids in high yields ranging from 92 to 98%.

Perfluoroalkyl β-D-glucopyranosides

11,11,12,12,13,13,14,14,14-Nonafluorotetradecyl-β-D-glucopyranoside (8a). White solid; mp 59–61 °C; mp (DSC) 58.43 ± 1.76 °C, 166.19 ± 1.51 °C; yield, 95%; ^{1}H NMR (300 MHz, CD_3OD): δ/ppm 1.3–1.5 (m, 12H, 6 × CH_2), 1.6–1.7 (m, 4H, H-2′ and H-9′), 2.18 (m, 2H, H-10′), 3.22 (“t”, 1H, $J \sim$ 8.3 Hz, H-2), 3.3–3.4 (m, 3H, H-3, H-4 and H-5), 3.57 (m, 1H, H-1′a), 3.70 (dd, 1H, $J_{6a,6b}$ = 11.9 Hz, $J_{5,6b}$ = 5.1 Hz, H-6a), 3.8–4.0 (m, 2H, H-6b and H-1′b), 4.29 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1). ^{13}C NMR (75 MHz, CD_3OD) δ/ppm 21.3 (*C*-9′), 27.1 (*C*-3′), 30.2–30.9 (*C*-2′, *C*-4′ to *C*-8′), 31.7 (t, J = 22 Hz, *C*-10′), 62.8 (C-6), 70.9 (*C*-1′), 71.8 (C-4), 75.1 (C-2), 78.0 (C-5), 78.1 (C-3), 104.4 (C-1). ^{19}F NMR (282 MHz, CD_3OD) δ/ppm −81.0 (CF_3), −114.1 (CF_2), −124.0 (CF_2), −125.7 (CF_2). Anal. Calc. for $C_{20}H_{31}F_9O_6$: C 44.61, H 5.80. Found: C 44.39, H 5.68%. HR-MS of [M + Na]$^+$ m/z: Calc. 561.1875, Found. 561.1880.

12,12,13,13,14,14,15,15,15-Nonafluoropentadecyl-β-D-glucopyranoside (8d). White solid; mp 98–99 °C; mp (DSC) 101.57 ± 0.88 °C, 174.57 ± 0.07 °C; yield, 98%; ^{1}H NMR (400 MHz, CD_3OD): δ/ppm 1.3–1.4 (m, 14H, 7 × CH_2), 1.6–1.7 (m, 4H, H-2′ and H-10′), 2.18 (m, 2H, H-11′), 3.22 (“t”, 1H, $J \sim$ 8.0 Hz, H-2), 3.3–3.4 (m, 3H, H-3, H-4 and H-5), 3.58 (m, 1H, H-1′a), 3.70 (dd, 1H, $J_{6a,6b}$ = 11.9 Hz, $J_{5,6b}$ = 5.3 Hz, H-6a), 3.8–4.0 (m, 2H, H-6b and H-1′b), 4.29 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1). ^{13}C NMR (100 MHz, CD_3OD) δ/ppm 21.3 (C-10′), 27.1 (C-3′), 30.2–30.9 (C-2′, C-4′ to C-9′), 31.8 (t, J = 22 Hz, *C*-11′), 62.9 (C-6), 70.9 (C-1′), 71.8 (C-4), 75.2 (C-2), 77.9 (C-5), 78.3 (C-3), 104.4 (C-1). ^{19}F NMR (282 MHz, CD_3OD) δ/ppm −81.0 (CF_3), −114.1 (CF_2), −124.0 (CF_2), −125.7 (CF_2). Anal. Calc. for $C_{21}H_{33}F_9O_6$: C 45.65, H 6.02. Found: C 44.97, H 6.11%. HR-MS of [M + Na]$^+$ m/z: Calc. 575.2031, Found. 575.2041.

Alkyl β-D-glucopyranosides

Tetradecyl-β-D-glucopyranoside (9a)[38,44]. White powder; mp 69–71 °C (lit.: 64.8 °C[44]); mp (DSC) 66.96 ± 2.52 °C, 151.47 ± 3.97 °C; yield, 95%; ^{1}H NMR (300 MHz, CD_3OD): δ/ppm 0.90 (m, 3H, J = 6.9 Hz, H-14′), 1.3–1.4 (m, 22H, 11 × CH_2), 1.63 (m, 2H, H-2′), 3.31 (“t”, 1H, $J \sim$ 8.9 Hz, H-2), 3.2–3.4 (m, 3H, H-3, 4 and 5, overlapped with residue proton of CD_3OD), 3.57 (m, 1H, H-1′a), 3.71 (dd, 1H, $J_{6a,6b}$ = 12.0 Hz, $J_{5,6a}$ = 5.0 Hz, H-6a), 3.8–4.0 (m, 2H, H-6b and H-1′b), 4.28 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1). ^{13}C NMR (75 MHz, CD_3OD) δ/ppm 14.4 (C-14′), 23.7 (C-13′), 27.1 (C-3′), 30.4–30.8 (C-2′, C-4′ to C-11′), 33.0 (C-12′), 62.9 (C-6), 71.0 (C-1′), 71.8 (C-4), 75.2 (C-2), 77.9 (C-5), 78.2 (C-3), 104.4 (C-1). Anal. Calc. for $C_{20}H_{40}O_6$: C 63.80, H 10.71. Found: C 63.97, H 10.67%. HR-MS of [M + Na]$^+$ m/z: Calc. 399.2723, Found. 399.2735.

Pentadecyl-β-D-glucopyranoside (9d). White powder; mp 75–77 °C; mp (DSC): 72.27 ± 2.85 °C, 154.32 ± 7.84 °C; yield, 95%; ^{1}H NMR (300 MHz, CD_3OD): δ/ppm 0.90 (m, 3H, J = 7.0 Hz, H-15′), 1.2–1.4 (m, 24H, 12 × CH_2), 1.63 (m, 2H, H-2′), 3.19 ("t", 1H, $J \sim$ 8.9 Hz, H-2), 3.2–3.4 (m, 3H, H-3, 4 and 5, overlapped with residue proton of CD_3OD), 3.57 (m, 1H, H-1′a), 3.71 (dd, 1H, $J_{6a,6b}$ = 12.0 Hz, $J_{5,6a}$ = 5.2 Hz, H-6a), 3.8–4.0 (m, 2H, H-6b and H-1′b), 4.27 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1). ^{13}C NMR (75 MHz, CD_3OD) δ/ppm 14.4 (C-15′), 23.7 (C-14′), 27.1 (C-3′), 30.4–30.8 (C-2′, C-4′ to C-12′), 33.0 (C-13′), 62.9 (C-6), 71.0 (C-1′), 71.8 (C-4), 75.2 (C-2), 77.8 (C-5), 78.2 (C-3), 104.4 (C-1). Anal. Calc. for $C_{21}H_{42}O_6$: C 64.58, H 10.84. Found: C 64.06, H 10.64%. HR-MS of $[M + Na]^+$ m/z: Calc. 413.2879. Found. 413.2870.

Synthesis of orthoester 11

Ag_2CO_3 (5 mmol) was added to a solution of 2,3,4,6-tetra-acetyl-α-D-glycopyranosyl bromide (**10**)[58] in anhydrous DCM (10 mL) at 0 °C under a nitrogen atmosphere. The mixture was stirred for 10 min and alcohol **3e** (5 mmol) in anhydrous DCM (10 mL) was added slowly. After 3 h, additional DCM (20 mL) was added, the reaction mixture was filtered and the solvent removed under reduced pressure. The yellowish residue was purified by column chromatograph on silica gel with hexane and ethyl acetate (3 : 1, v/v) as eluent.

3,4,6-Tri-*O*-acetyl-1,2-[1-(12,12,13,13,14,14,15,15,16,16,17,17,-17-tridecafluoroheptadecyloxy)ethylidene]-α-D-glucopyranose). White powder; mp 82–83 °C; yield, 80%; ^{1}H NMR (300 MHz, $CDCl_3$): δ/ppm 1.27 (m, 14H, 7 × CH_2), 1.56 (m, 4H, H-2′ and H-10′), 1.71 (s, 3H, CH_3), 2.04–2.11 (m, 11H, 3 × CH_3CO and H-11′), 3.46 (t, 2H, J = 6.6 Hz, H-1′), 3.96 (m, 1H, H-5), 4.19 (m, 2H, H-2 and H-6aHH), 4.30 (m, 1H, H-6b), 4.90 (m, 1H, H-4), 5.17 ("t", 1H, J = 2.7 Hz, H-3), 5.70 (d, 1H, J = 5.2 Hz, H-1). ^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm 20.3 (C-3′), 20.9–20.1 (3 × CH$_3$CO and CH$_3$), 26.3 (C-10′), 29.3–29.9 (C-2′, C-4′ to C-9′), 31.1 (t, J = 22 Hz, C-11′), 63.3 (C-6), 63.9 (C-1′), 67.1 (C-5), 68.4 (C-4), 70.3 (C-3), 73.2 (C-2), 97.1 (C-1), 121.5 (=C(CH$_3$)OR), 169.4, 169.9, 170.9 (3 × COCH$_3$). ^{19}F NMR (282 MHz, $CDCl_3$): δ/ppm −81.3 (CF_3), −114.9 (CF_2), −122.5 (CF_2), −123.4 (CF_2), −124.1 (CF_2), −126.7 (CF_2). Anal. Calc. for $C_{31}H_{41}F_{13}O_{10}$: C 45.37, H 5.04. Found: C 45.40, H 5.05%.

Assessment of cytotoxicity

Cancer cell line. The B16F10 mouse melanoma cell line (ECACC) was selected from a panel of cancer cell lines used for testing in our laboratory. The cell line was grown in D-MEM medium (Sigma, Czech Republic) supplemented with 10% of fetal calf serum (Gibco, Czech Republic), 50 mg L^{-1} penicillin, 50 mg L^{-1} streptomycin, 100 mg L^{-1} neomycin, and 300 mg L^{-1} L-glutamine as reported previously.[12,46] Cultures were maintained in a humidified incubator (Shellab, Sheldon, OR, USA) at 37 °C and 5% CO_2.

MTT-based cytotoxicity test. The MTT assay[59,60] was used to assess the cytotoxicity of the glucopyranosides in cells in the exponential growth phase. In short, cells were seeded on 96-well flat-bottom microplates at the density 2.5–3.0 × 10^4 mL^{-1}, 100 μL per well, and allowed to grow for 16–24 h in culture medium. The tested compounds were first dissolved in DMSO (Sigma, Czech Republic) and than in sterile PBS. Final concentrations of DMSO in samples were below 1%. PBS with DMSO (1 and 5%) served as control. No cytotoxicity of 1% DMSO in PBS was observed. Glucopyranosides dissolved in sterile PBS (total volume of 20 μL) were added to each well and the cytotoxic effect was evaluated after 24 h of exposure over a concentration range from 6 μM to 1.5 mM using the MTT assay. Octylthioglucoside (Roche) was used as a positive control.

MTT (Sigma Chemical Co., Czech Republic) was dissolved in PBS at a concentration of 5 mg mL^{-1} and sterilized by filtration. MTT solution was added into all wells of 96-well flat-bottom microplates with cells in a dose of 20 μL per well. The plates were incubated for 3 h. To enhance the dissolution of dark-purple crystals of formazan, 110 μL of 10% SDS in PBS (final pH 5.5) were added to all wells. The microtitre plates were stored in a light-tight box at room temperature, evaluated on the next day using a well-plate spectrophotometer reader EL 800 (BioTek, USA) at 540 nm and the EC_{50} (*i.e.* the molar concentration which produces 50% of the maximum possible inhibitory response) values were calculated from the dose response curves. All experiments were performed in triplicate and EC_{50} values were calculated using GraphPad PRISM V.4.00 (GraphPad Software Inc., San Diego, CA).

The results from the MTT assay were further confirmed by Hoffman modulation contrast microscopy (epifluorescent inverted microscope T200, Nikon, Japan) exposing morphological changes of the cells treated with various surfactants. Propidium iodide (PI) and YO-PRO-1 (Molecular Probes, Oregon, USA) were used to distinguish dead or apoptotic cells from vital living ones.[61]

Haemolytic activity. Rabbit red blood cells (2% in PBS) were used to perform standard haemolytic tests in both PBS and PBS containing 20% fetal bovine serum (Gibco). The surfactants were tested in concentration range extending from 40 μM to 20 mM. The tested compounds were dissolved in DMSO and added into PBS. Maximal final concentration of DMSO in PBS was 5% for 20 mM concentration of tested compound. This concentration of DMSO did not cause any haemolysis in control red blood cells. After 2 h incubation of red cells with a particular compound at 37 °C, the released haemoglobin was separated from red blood cells by centrifugation (700 g, 10 min, three washes) and quantified using a well-plate spectrophotometer reader EL 800 (BioTek, USA) at 540 nm. Data are expressed as the lowest concentration of surfactant causing 50% haemolysis.

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