# Synthesis and biocompatibility evaluation of partially fluorinated pyridinium bromides

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Although cationic surfactants are of general interest for a variety of consumer and biomedical applications, only a limited number of partially fluorinated, single-tailed, cationic surfactants have been synthesized. To study the potential usefulness of fluorinated cationic surfactants for these applications we synthesized a series of partially fluorinated pyridinium bromide surfactants. Three 10-perfluoroalkyldecyl pyridinium surfactants were synthesized by coupling a perfluoroalkyl iodide with 9-decene-1-yl acetate using an AIBN mediated radical reaction. The resulting 9-iodo-10-perfluoroalkyldec-1-yl acetates were deiodinated using HI-Zn-EtOH and hydrolyzed using KOH-EtOH to yield the corresponding 10-perfluoroalkyldecanol. The partially fluorinated alcohol was converted into the bromide using Br<sub>2</sub>-PPh<sub>3</sub>. Alkylation of excess pyridine with the bromides gave the desired 10-perfluoroalkyldecyl pyridinium bromides in good yields. Three 10perfluoroalkylundecyl surfactants were synthesized using a similar approach with 10-undecenoic acid methyl ester as starting material. Based on an initial in vitro toxicity assessment, the toxicity of the partially fluorinated pyridinium surfactants was slightly lower or comparable to benzalkonium chloride, a typically cationic surfactant (with  $IC_{50}$ s of tested compounds ranging from 5 to 15  $\mu$ M). An increase in the length and/or the degree of fluorination of the hydrophobic tail correlated with a mild decrease of cytotoxicity and haemolytic activity. Partially fluorinated pyridinium surfactants may, therefore, be useful for biomedical applications such as components for novel gene and drug delivery systems.

# Introduction

Fluorinated surfactants are highly surface active, display weak intermolecular interactions, and typically have only low acute toxicity, properties which make them highly suitable for a large number of technical and, potentially, biomedical applications.<sup>1</sup> Because of these unique properties there is, for example, considerable interest in fluorinated cationic surfactants as templates for the synthesis of ordered porous ceramics.<sup>2-7</sup> Other ongoing research is focusing on the use of fluorinated surfactants in biomedical applications.<sup>8,9</sup> Single tailed partially fluorinated surfactants with a variety of nonionic or zwitterionic head groups, for example carnitine,10 morpholinophosphate,<sup>11</sup> phosphocholine,<sup>12</sup> pyridinium,<sup>2–7</sup> and carbohydrate<sup>13–16</sup> head groups, have been synthesized. In contrast to their hydrocarbon counterparts, many partially fluorinated surfactants, especially surfactants with a high degree of fluorination, display low or moderate toxicity in in vitro and in vivo studies and are, thus, considered to be biocompatible.

Although hydrocarbon cationic surfactants such as benzalkonium chloride (BAC) and hexadecylpyridinium chloride are of considerable interest for a broad range of consumer and biomedical applications (for example, antimicrobial agents in mouthwashes,<sup>17,18</sup> ophthalmic and contact lens solutions<sup>18</sup> and novel catanionic gene or drug delivery systems<sup>19-21</sup>) only a small number of fluorinated cationic surfactants have been synthesized and their biocompatibility assessed. More work is, therefore, needed to determine how the biocompatibility of a partially fluorinated, cationic surfactant is different from its hydrocarbon analogue and how structural features, for example the chain-length and the degree of fluorination of the hydrophobic tail, influence a surfactant's biocompatibility. The aim of this work was to develop an efficient synthetic route to partially fluorinated pyridinium surfactants and to perform an initial toxicity assessment to test their potential usefulness for novel drug delivery systems and other biomedical applications.

# **Results and discussion**

### Synthesis

Most partially fluorinated compounds are not available from commercial sources. One crucial step in the synthesis of any partially fluorinated surfactants is, therefore, the synthesis of a functionalized partially fluorinated alkyl chain. Such partially

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Scheme 1 a. CH<sub>3</sub>OH, PTSA, toluene,  $\Delta$ ; b. R<sub>F</sub>I, AIBN,  $\Delta$ ; c. HI (55%), Zn, C<sub>2</sub>H<sub>5</sub>OH,  $\Delta$ ; d. (i) CH<sub>3</sub>OH, KOH,  $\Delta$ ; (ii) LiAlH<sub>4</sub>, anhydrous ether, ambient temperature; e. LiAlH<sub>4</sub>, anhydrous ether, ambient temperature; f. PPh<sub>3</sub>, Br<sub>2</sub>, DCM, ambient temperature; g. pyridine,  $\Delta$ .

fluorinated alkyl moieties are typically synthesized by the coupling of a perfluorinated iodide with an  $\omega$ -unsaturated hydrocarbon precursor in an AIBN mediated radical reaction.<sup>22–25</sup> We have utilized this approach to synthesize a series of terminally fluorinated pyridinium bromides.

As shown in Schemes 1 and 2, the iodo methyl esters **5a–c** or iodoacetates **6a–c** were synthesized using this reaction from undecenoic methyl ester **3** or decenol acetate **4**, respectively. Addition of AIBN in multiple portions was a key factor in this reaction. The secondary iodides **5a–c** or **6a–c** were deiodinated using HI–Zn–EtOH without further purification. Hydroiodic acid and zinc were chosen because both reagents are easy to handle and less toxic compared to tributyltin hydride,<sup>24</sup> which is typically employed in similar deiodination reactions.

As shown in Scheme 1, the series of methyl esters 7a-c was converted into the corresponding alcohols 9a-c by two different routes. Initially the esters 7a-c were converted into the corresponding,  $R_F$ -substituted (*i.e.* partially fluorinated) undecanoic acids<sup>22</sup> which were subsequently reduced to the alcohols 9a-c with lithium aluminium hydride in anhydrous ether (reaction condition **d** in Scheme 1). This approach was chosen because the partially fluorinated acids can be more easily purified by recrystallization from hexanes compared to structurally related partially fluorinated alcohols,<sup>22,23</sup> thus allowing one to perform the synthesis on a large (100 gram) scale. To further improve the overall yield of the alcohols we optimized the direct reduction of the methyl ester 7a-c with lithium aluminium hydride (reaction condition **e** in Scheme 1). The direct reduction of the esters 7a-c increased the yield of



Scheme 2 a. DMAP, AcCl, pyridine, DCM; b.  $R_FI$ , AIBN,  $\Delta$ ; c. HI (55%), Zn,  $C_2H_5OH$ ,  $\Delta$ ; d. CH<sub>3</sub>OH, KOH,  $\Delta$ ; e. PPh<sub>3</sub>, Br<sub>2</sub>, DCM, ambient temperature; f. pyridine,  $\Delta$ .

the alcohols **9a–c** from approximately 60% to 70%. In the case of the acetate series, the alcohols **10a–c** were obtained by hydrolysis of the acetate followed by recrystallization from hexane.<sup>23</sup> Typical overall yields of the alcohols **10a–c** were 65-85%.

The alcohols **9a–c** and **10a–c** were brominated using triphenylphosphine and bromine similar to the procedure reported by Naud and co-workers.<sup>24</sup> Bromination of the alcohols **9a–c** and **10a–c** with HBr/H<sub>2</sub>SO<sub>4</sub> also yielded the desired partially fluorinated bromides, but this approach was abandoned because the purification of the bromides is tedious and the yields are low. In the final step of the synthesis excess pyridine and the respective bromides were heated under reflux to yield the corresponding pyridinium bromides **13a–c** and **14a–c**.<sup>26</sup>

The <sup>1</sup>H NMR spectra of most of the pyridinium bromides showed one singlet around  $\delta = 3.1$  ppm which was not related to any group in the compound. The chemical shift of this peak was not consistent, thus suggesting that, like their hydrocarbon analogues, several of the partially fluorinated pyridinium salts form stable monohydrates. This interpretation of the <sup>1</sup>H NMR spectra was further confirmed by D<sub>2</sub>O exchange experiments. In addition, the results from the combustion analysis experiments confirm the presence of one water molecule in the pyridinium salts **13a** and **14a–c**.

### Cytotoxicity and haemolytic assessments

Despite their importance for a variety of consumer and biomedical applications, only limited information about the toxicity of cationic surfactants, especially fluorinated cationic surfactants, is available.<sup>18</sup> An initial cytotoxicity assessment of all six pyridinium surfactants was therefore performed using the A2780 ovarian carcinoma cell line.<sup>27</sup> The IC<sub>50</sub> values of the pyridinium salts and several other surfactants are summarized in Tables 1 and 2, respectively. The partially fluorinated pyridinium surfactants (13a-c and 14a-c) were highly toxic in the A2780 cancer cell line with  $IC_{50}$  values ranging from 5 to 15  $\mu$ M. These IC<sub>50</sub> values are comparable to IC<sub>50</sub> values of typical anticancer agents, e.g. platinum complexes.<sup>27</sup> The most toxic compounds 13a and 14a were also bactericidal against Salmonella typhimurium (results not shown). Overall, the low IC<sub>50</sub> values of **13a-c** and **14a-c** are in agreement with a previous report that highly fluorinated 1H,1H,2H, 2H,3H,3H-perfluoroundecyltrialkylammonium salts are toxic in Namalya lymphoblastoid cells with IC<sub>50</sub>s below 0.16 mM.<sup>28</sup>

The short-chain pyridinium salts **13a** and **14a** were the most toxic surfactants in this series with toxicities comparable with BAC. Similar results were obtained with several other cell lines (data not shown). The increase in the length of the partially fluorinated tail caused a mild decrease in cytotoxicity, thus suggesting that the toxicity of cationic surfactants can be reduced by introducing a certain degree of fluorination. This hypothesis is further supported by the observation that the partially fluorinated pyridinium surfactant **14a** was less toxic compared to its hydrocarbon analogue tetradecylpyridinium bromide. This hydrocarbon surfactant was the most toxic compound investigated in our study. 1H, 1H, 2H, 2H-perfluorododecylpyridinium chloride, a highly fluorinated pyridinium surfactant with a slightly shorter hydrophobic tail compared

Table 1Haemolytic and cytotoxicity assessment of the partially fluorinated pyridinium bromides 14a-c and 15a-c and related cationicfluorocarbon and hydrocarbon surfactants in the A2780 cell line

Compound	A2780 cell line IC <sub>50</sub> [µM]	Haemolysis PBS [µM]	Haemolysis PBS + 20% FBS [µM]
$N^{t}$ (CH <sub>2</sub> ) <sub>11</sub> (CF <sub>2</sub> ) <sub>3</sub> CF <sub>3</sub> Br <sup>-</sup> 13a	7.97	62.5	250
$N^{+}$ (CH <sub>2</sub> ) <sub>11</sub> (CF <sub>2</sub> ) <sub>5</sub> CF <sub>3</sub> Br <sup>*</sup> 13b	11.1	62.5	250
$N^{i} - (CH_2)_{11}(CF_2)_7CF_3$ Br <sup>7</sup> 13c	14.7	62.5	125
$N^{i}$ (CH <sub>2</sub> ) <sub>10</sub> (CF <sub>2</sub> ) <sub>3</sub> CF <sub>3</sub> Br 14a	4.9	62.5	250
N <sup>4</sup> (CH <sub>2</sub> ) <sub>10</sub> (CF <sub>2</sub> ) <sub>5</sub> CF <sub>3</sub> Br <sup>7</sup> 14b	9.3	62.5	125
$N^{i}$ (CH <sub>2</sub> ) <sub>10</sub> (CF <sub>2</sub> ) <sub>7</sub> CF <sub>3</sub> Br 14c	11.7	62.5	125
N <sup>+</sup> (CH <sub>2</sub> ) <sub>2</sub> (CF <sub>2</sub> ) <sub>9</sub> CF <sub>3</sub> CI <sup>+</sup> (1H,1H,2H,2H-perfluorododecyl pyridinium chloride)	31.4	250	> 500
N*-(CH <sub>2</sub> ) <sub>13</sub> CH <sub>3</sub> Br (Tetradecylpyridinum bromide monohydrate)	2.3	62.5	250
$(R = C_8H_{17} \text{ to } C_1BH_{37})$ (Benzalkonium chloride)	5.3	125	400

to **14a**, was the least toxic cationic surfactant, an observation that also suggests that an increased degree of fluorination may decrease the cytotoxicity of pyridinium surfactants.

In agreement with previous studies,<sup>18</sup> the partially fluorinated cationic surfactants 13a-c and 14a-c (Table 1) were much more toxic compared to typical anionic surfactants and several non-ionic surfactants with a hydrocarbon tail (Table 2). Some non-ionic surfactants, *e.g.* Triton X-100 or Nonidet P40, were also toxic towards A2780 cells in a range comparable to that of the partially fluorinated pyridinium surfactants. The mechanisms of the relatively high toxicity of cationic surfactants in general and of the partially fluorinated surfactants 13a-c and 14a-c in particular most likely involve a disruption of the integrity of the cell membrane. This hypothesis is supported by the fact that the partially fluorinated surfactants are equally toxic in several cell lines and in bacteria, *e.g. Salmonella typhimurium* (data not shown).

Because of the potential biomedical use of the partially fluorinated surfactants **13a–c** and **14a–c** we also investigated their haemolytic activity on rabbit red blood cells in both PBS

and PBS containing 20% fetal bovine serum. As with the cytotoxicity, it is well established that the in vitro haemolytic activity of fluorinated surfactants on red blood cells dramatically decreases with increasing degree of fluorination and increasing length of the fluorinated hydrophobic tail.<sup>11–14,29–31</sup> In contrast, the haemolytic activity of hydrocarbon surfactants typically increases with increasing chain length. The pyridinium surfactants showed significantly stronger haemolytic activity than BAC (Table 1) and the anionic and nonionic surfactants (Table 2) investigated. This observation is in agreement with the strong haemolytic activity reported for highly fluorinated trialkylammonium salts.35 The lowest concentration of the pyridinium surfactants at which haemolytic activity was observed did not appear to depend on the degree of fluorination and/or the length of the hydrophobic tail. One exception was the highly fluorinated 1H,1H,2H,2H-perfluorododecylpyridinium chloride which exhibited haemolytic activity at concentrations comparable to several anionic and nonionic surfactants, thus suggesting that, in contrast to the cytotoxicity studies, a significant degree of fluorination may

Table 2	Haemolytic and cytotoxicity assessment of typical anionic and non-ionic surfactants in the A2780 cell line	
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Compound	A2780 cell line IC <sub>50</sub> [µM]	Haemolysis PBS [µM]	Haemolysis PBS + 20% FBS [µM]
(Sodium dodecylsulphate)	294	250	> 1000
HO <sup>11</sup> (Solium domuchalata)	243	>1000	> 1000
(sodum deoxycholate) $NaO_2C$ ( $\alpha$ -tocopheryl succinate)	56	300	> 500
$H_3C(CH_2)_7$ $S_{1_{1_1}}$ $O_{1_1}$ $O_{1_$	680	500	> 500
$H_{3C(CH_{2})_{11}} \longrightarrow 0$ $H_{n}$ (Brij 35)	68	125	> 1000
(Triton X-100)	22.3	830	> 1000
$H(OC_{2}H_{4})_{W}O = O(C_{2}H_{4}O)_{X}H$ $O(C_{2}H_{4}O)_{Y}H = O(C_{2}H_{4}O)_{Y}H$ $O(C_{2}H_{4}O)_{Z}COC_{11}H_{23}$ (Tween 20)	260	>1000	> 1000
(Nonidet P40)	11.2	400	830

be necessary before a protective effect against haemolysis can be observed.

therefore, be useful for biomedical applications such as components for novel gene and drug delivery systems.

# Conclusion

These findings suggest that partial fluorination of the hydrophobic tail of pyridinium and other cationic surfactants may reduce their toxicity and haemolytic activity. This observation is comparable to single tailed non-ionic and zwitterionic surfactants, where partial fluorination of the hydrophobic tail results in a reduction of the toxicity compared to the hydrocarbon analogue. Despite their cytotoxicity, cationic surfactant-lipids are used for the construction of synthetic delivery systems, *e.g.* liposomes, for transfection of cells by DNA/ RNA constructs.<sup>32</sup> Fluorinated, cationic surfactants may,

# Experimental

Perfluorinated iodides were purchased from Oakwood Chemical Co. (West Columbia, South Carolina, USA) and used as received. Long-chain hydrocarbon starting materials were purchased from TCI Chemicals (Portland, Oregon, USA). Anhydrous solvents were purchased from Fisher Scientific (Fairlawn, New Jersey, USA). All the <sup>1</sup>H NMR spectra were recorded in CDCl<sub>3</sub> on a multinuclear Bruker Avance 300 Digital NMR spectrometer at 297 K; all chemical shifts are reported in parts per million (ppm) relative to internal tetra-methylsilane (Me<sub>4</sub>Si), with the residual solvent proton

resonance as internal standard. Coupling constants are given in hertz (Hz). <sup>19</sup>F chemical shifts were determined using CFCl<sub>3</sub> as internal standard. Melting points were determined using a Thermal Analysis 2920 Differential Scanning Instrument. Thermograms were recorded using a heating rate of  $20^{\circ}$  min<sup>-1</sup> from 20 to 230 °C and the temperature maxima of the main phase transitions after one DSC run were determined.<sup>22</sup> Mass spectra on non-ionic intermediates were measured at the University of Iowa Mass Spectrometry Facility using a Voyager GC-MS instrument operated at 70 eV in the electron impact mode. The reported fragment peaks correspond to the most abundant ions, in addition to the parent ion(s). Mass spectra of pyridinium salts were recorded at the University of Kentucky Mass Spectrometry Facility using a Bruker Autoflex time-of-flight mass spectrometer equipped with MALDI. Microanalytical data were obtained from Atlantic Micro Lab Microanalysis Service (Atlanta, Georgia, USA). IR spectra were measured with a ThermoNicolet Nexus 470 FT-IR spectrophotometer.

#### AIBN mediated coupling reaction (general procedure)

The radical addition of  $R_F I$  to undecenoic methyl ester  $3^{33}$  or decenol acetate 4<sup>34</sup> in the presence of AIBN was performed following literature procedures.<sup>23,35,36</sup> The alkenoic acid methyl ester 3 (98 g, 0.5 mol) or alkenol acetate 4 (91 g, 0.5 mol) and a perfluorinated iodide  $R_F I$  ( $R_F = C_4 F_9$ ,  $C_6 F_{13}$ ,  $C_8F_{17}$ , 0.55 mol) were placed in a 500 ml round bottomed flask equipped with a reflux condenser. The radical initiator AIBN (2 mol%) was added. The reaction mixture was heated to 90 °C under stirring. After 30 min, the flask was cooled to room temperature, and another portion of AIBN (2 mol%) was added. The stirred solution was again heated to 90 °C, and the entire process was repeated at least two additional times; 3 h were allowed to pass after the final addition of AIBN. The progress of the reaction was monitored by GC and <sup>1</sup>H NMR spectroscopy of small aliquots collected between AIBN additions. The flask was cooled to room temperature, and the crude iodo methyl esters 5a-c or iodoacetates 6a-c were used in the next step without further purification.

# Terminally perfluorinated esters 7a–c and acetates 8a–c (general procedure)

The crude secondary iodides **5a–c** or **6a–c** were dissolved in 150 ml of  $C_2H_5OH$  and hydroiodic acid (non-stabilized, 55%, 35 ml, 1.5 mol) was added. Zinc dust (97 g, 1.5 mol) was added very slowly at 0 °C. The mixture was refluxed for 6 h and then filtered. The filtrate was evaporated to dryness and re-dissolved in diethyl ether (*ca.* 200 ml). The solution was washed with water (2 × 100 ml), saturated aqueous NaHCO<sub>3</sub> solution (1 × 100 ml), and brine (1 × 100 ml), and then dried over MgSO<sub>4</sub>. Removal of the solvent afforded the crude terminally fluorinated ester **7a–c** or acetate **8a–c**. The complete conversion was confirmed by <sup>1</sup>H NMR spectroscopy and GC.

# Hydrolysis of 7a-c and 8a-c (general procedure)

The partially fluorinated esters **7a–c** or acetates **8a–c** were dissolved in 150 ml of  $C_2H_5OH$ . To this KOH (57 g, 1.5 mol) was added slowly. The reaction mixture was refluxed for six

hours under stirring. After complete conversion it was cooled to 0 °C and water was added dropwise. The mixture was acidified with 2N H<sub>2</sub>SO<sub>4</sub> and extracted with diethyl ether (3 × 100 ml). The solution was washed with water (2 × 100 ml), saturated aqueous NaHCO<sub>3</sub> solution (1 × 100 ml), and brine (1 × 100 ml), and then dried over MgSO<sub>4</sub>. Removal of the solvent by rotary evaporation afforded the crude acids or alcohols which were purified by recrystallization with hexane.<sup>22,23</sup> The analytical data of the acids and alcohol **10b** are in agreement with previously published data.<sup>23</sup>

**11,11,12,12,13,13,14,14,14-Nonafluorotetradecanol (10a).** <sup>1</sup>H NMR δ/ppm 1.34 (m, 12H,  $-(CH_2)_{6}$ -), 1.57 (m, 4H, R<sub>F</sub>CH<sub>2</sub>CH<sub>2</sub>- and  $-CH_2$ CH<sub>2</sub>OH), 1.9–2.4 (m, 2H + OH, R<sub>F</sub>CH<sub>2</sub>CH<sub>2</sub>-), 3.66 (t, J = 6.6 Hz, 2H,  $-CH_2CH_2OH$ ). <sup>13</sup>C NMR δ/ppm 20.1 (t, J = 4.0 Hz), 25.7, 29.1, 29.2, 29.3, 29.4, 29.5, 30.8 (t, J = 22.0 Hz), 32.7, 62.9. <sup>19</sup>F NMR δ/ppm -81.6 (CF<sub>3</sub>), -115.1, -124.9, -126.6. GC/MS m/z (relative intensity, %) 358 (M - H<sub>2</sub>0, 5), 330 (30), 302 (20), 288 (30). IR(KBr)  $\nu$ /cm<sup>-1</sup> 3346 (OH), 2930, 2858, 1235, 1133, 1117, 722.

**11,11,12,12,13,13,14,14,15,15,16,16,17,17,18,18,18-Heptadecafluorooctadecanol (10c).** <sup>1</sup>H NMR  $\delta$ /ppm 1.35 (m, 12H, –(CH<sub>2</sub>)<sub>6</sub>–), 1.59 (m, 4H, R<sub>F</sub>CH<sub>2</sub>CH<sub>2</sub>– and –CH<sub>2</sub>CH<sub>2</sub>OH), 1.88 (br s, 1H, OH), 2.08 (m, 2H, R<sub>F</sub>CH<sub>2</sub>CH<sub>2</sub>–), 3.68 (t, *J* = 6.7 Hz, 2H, –CH<sub>2</sub>CH<sub>2</sub>OH). <sup>13</sup>C NMR  $\delta$ /ppm 20.0 (t, *J* = 3.8 Hz), 25.7, 29.1, 29.2, 29.3, 29.4, 29.5, 30.8 (t, *J* = 22.5 Hz), 32.7, 62.9. <sup>19</sup>F NMR  $\delta$ /ppm –81.5 (CF<sub>3</sub>), –115.1, –122.3 (3 × CF<sub>2</sub>), –123.3, –124.1, –126.7. GC/MS *m*/*z* (relative intensity, %) 558 (M – H<sub>2</sub>O, 2) 530 (10), 502 (5), 488 (9). IR(KBr)  $\nu$ /cm<sup>-1</sup> 3322 (OH), 2933, 2855, 1242, 1205, 1150, 1117, 661.

# Reduction of partially fluorinated acids and esters with LiAlH<sub>4</sub> (general procedure)

The partially fluorinated acids or esters (0.15 mol) were dissolved in 50-80 ml of THF and added over 5 min to a slurry of LiAlH<sub>4</sub> (3.8 g, 0.1 mol) in 10 ml THF at 0 °C under nitrogen. The mixture was allowed to come to room temperature and was stirred overnight. After complete conversion it was cooled to 0 °C and water was added dropwise until the evolution of hydrogen ceased. The mixture was acidified with 2N HCl and extracted with diethyl ether (3  $\times$  100 ml). The solution was washed with water (2  $\times$  100 ml), saturated aqueous NaHCO<sub>3</sub> solution (1  $\times$  100 ml), and brine (1  $\times$  100 ml), and then dried over MgSO<sub>4</sub>. Removal of the solvent by rotary evaporation afforded the crude alcohols (9a-c), which were purified by chromatography on silica gel (hexane-ethyl acetate = 9:1) or recrystallization with hexane. Typical yields ranged from 80-85%. The analytical data of 9a-c are in agreement with previously published data.<sup>24</sup>

# Terminally perfluorinated bromides 11a-c and 12a-c (general procedure)

Triphenylphosphine (24.2 g, 0.13 mol) and bromine (7 ml, 0.13 mol) were dissolved in 50 ml of anhydrous methylene chloride at 0 °C. After 30 min, a DCM solution of the alcohol (**9a–c** or **10a–c**, 0.1 mol in 25 ml of DCM) was added, and the solution was stirred at room temperature for 15 h. The solution was poured into ice water (300 ml). The product was extracted with

diethyl ether (4 × 100 ml). The solution was washed with water (2 × 100 ml), saturated aqueous NaHCO<sub>3</sub> solution (1 × 100 ml), and brine (1 × 100 ml), and then dried over MgSO<sub>4</sub>. Removal of the solvent by rotary evaporation afforded the crude bromide (**11a–c**, **12a–c**), which was purified by chromatography on silica gel (hexane–ethyl acetate = 9:1) or recrystallization with hexane.<sup>22,23</sup> Yields were typically 70–75%. The analytical data of **11a–c** are in agreement with previously published data.<sup>24</sup>

**11,11,12,12,13,13,14,14,14-Nonafluorotetradecyl bromide (12a).** <sup>1</sup>H NMR  $\delta$ /ppm 1.24 (m, 12H,  $-(CH_2)_6-)$ , 1.53 (m, 2H, R<sub>F</sub>CH<sub>2</sub>CH<sub>2</sub>-), 1.79 (m, 2H,  $-CH_2$ CH<sub>2</sub>Br), 1.98 (m, 2H, R<sub>F</sub>CH<sub>2</sub>CH<sub>2</sub>-), 3.34 (t, J = 6.9 Hz, 2H,  $-CH_2$ CH<sub>2</sub>Br). <sup>13</sup>C NMR  $\delta$ /ppm 20.2 (t, J = 4.0 Hz), 28.3, 28.9, 29.2, 29.3, 29.4, 29.5, 30.9 (t, J = 22.0 Hz), 33.0, 33.9. <sup>19</sup>F NMR  $\delta$ /ppm -81.6 (CF<sub>3</sub>), -115.1, -125.1, -126.6. GC/MS m/z (relative intensity, %) 438 (M - 1, < 1), 317 (23), 303 (30), 151 (12), 137 (100), 135 (96). IR(KBr)  $\nu/$ cm<sup>-1</sup> 2930, 2854, 1283, 1236, 1134.

**11,11,12,12,13,13,14,14,15,15,16,16,16-Heptadecafluorohexadecyl bromide (12b).** <sup>1</sup>H NMR  $\delta$ /ppm 1.3 (m, 12H, –(CH<sub>2</sub>)<sub>6</sub>–), 1.56 (m, 2H, R<sub>F</sub>CH<sub>2</sub>CH<sub>2</sub>–), 1.83 (m, 2H, –CH<sub>2</sub>CH<sub>2</sub>Br), 2.02 (m, 2H, R<sub>F</sub>CH<sub>2</sub>CH<sub>2</sub>–), 3.38 (t, J = 6.8 Hz, 2H, –CH<sub>2</sub>CH<sub>2</sub>Br). <sup>13</sup>C NMR  $\delta$ /ppm 20.7 (t, J = 4.0 Hz), 28.1, 28.7, 29.0, 29.2, 29.3, 29.3, 30.9 (t, J = 22.2 Hz), 32.8, 34.0. <sup>19</sup>F NMR  $\delta$ /ppm –81.3 (CF<sub>3</sub>), –114.9, –122.5, –123.4, –124.1, –126.7. GC/MS *m*/*z* (relative intensity, %) 538 (M – 1, <1), 417 (14), 403 (20), 151 (12), 137 (100), 135 (95). IR(KBr)  $\nu$ /cm<sup>-1</sup> 2932, 2858, 1241, 1207, 1145.

**11,11,12,12,13,13,14,14,15,15,16,16,17,17,18,18,18-Heptadecafluorooctadecyl bromide (12c).** <sup>1</sup>H NMR  $\delta$ /ppm 1.32 (m, 12H, -(CH<sub>2</sub>)<sub>6</sub>-), 1.60 (m, 2H, R<sub>F</sub>CH<sub>2</sub>CH<sub>2</sub>-), 1.86 (m, 2H, -CH<sub>2</sub>CH<sub>2</sub>Br), 2.07 (m, 2H, R<sub>F</sub>CH<sub>2</sub>CH<sub>2</sub>-), 3.40 (t, *J* = 6.8 Hz, 2H, -CH<sub>2</sub>CH<sub>2</sub>Br). <sup>13</sup>C NMR  $\delta$ /ppm 20.3 (t, *J* = 4.0 Hz), 28.4, 28.9, 29.3, 29.4, 29.5, 29.5, 31.1 (t, *J* = 21.9 Hz), 33.1, 33.9. <sup>19</sup>F NMR  $\delta$ /ppm -81.4 (CF<sub>3</sub>), -114.9, -122.4 (3 × CF<sub>2</sub>), -123.3, -124.1, -126.7. GC/MS *m*/*z* (relative intensity, %) 638 (M – 1, <1), 517 (13), 503 (20), 151 (10), 137 (100), 135 (96). IR(KBr)  $\nu$ /cm<sup>-1</sup> 2937, 2854, 1246, 1205, 1151.

### General procedure for pyridinium bromides 13a-c and 14a-c<sup>26</sup>

Bromides (**12a–c**, **13a–c**, 0.1 mol) and anhydrous pyridine (41 ml, 0.5 mol) were refluxed for 10 hours. A white precipitate was obtained after addition of ether to the cold reaction mixture. The filtrate was washed thoroughly with ether and recrystallized twice from acetone. Typical yields were 80–85% ( $py = H_5C_5N^+$ ).

**12,12,13,13,14,14,15,15,15-Nonafluoropentadecylpyridinium bromide monohydrate (13a).** Mp 25.5 °C (minor phase transition), 45.0 °C. <sup>1</sup>H NMR  $\delta$ /ppm 1.2–1.5 (m, 14H, –(CH<sub>2</sub>)<sub>7</sub>–), 1.57 (m, 2H, R<sub>F</sub>CH<sub>2</sub>CH<sub>2</sub>–), 1.9–2.1 (m, 4H, R<sub>F</sub>CH<sub>2</sub>CH<sub>2</sub>– and –CH<sub>2</sub>CH<sub>2</sub>–py), 3.2 (br s, OH), 4.98 (t, J = 7.5 Hz, 2H, –CH<sub>2</sub>CH<sub>2</sub>–py), 8.24 (t, J = 6.3 Hz, 2H, H-3,5), 8.63 (t, J =7.8 Hz, 1H, H-4), 9.50 (d, J = 5.7 Hz, 2H, H-2,6). <sup>13</sup>C NMR  $\delta$ /ppm 19.91 (t, J = 3.7 Hz), 25.93, 28.90, 28.93, 29.02, 29.04, 29.17, 29.24, 30.67 (t, J = 21.8 Hz), 31.82, 61.93, 128.41, 145.00, 145.11. <sup>19</sup>F NMR  $\delta$ /ppm –81.6 (CF<sub>3</sub>), –115.1, -125.0, -126.6. MS m/z (calcd) 452 (452). IR(KBr)  $\nu/cm^{-1}$  2928, 2856, 1233, 1133. Anal. calcd for C<sub>20</sub>H<sub>27</sub>F<sub>9</sub>NBr · H<sub>2</sub>O: C 43.63; H 5.27; N 2.54. Found: C 43.45; H 5.27; N 2.50%.

**12,12,13,13,14,14,15,15,16,16,17,17,17-Pentadecafluoroheptadecylpyridinium bromide (13b).** Mp 61.0 °C, 75.6 °C. <sup>1</sup>H NMR δ/ppm 1.2–1.6 (m, 14H,  $-(CH_2)_{7-}$ ), 1.57 (m, 2H, R<sub>F</sub>CH<sub>2</sub>CH<sub>2</sub>–), 1.9–2.1 (m, 4H, R<sub>F</sub>CH<sub>2</sub>CH<sub>2</sub>– and  $-CH_2$ CH<sub>2</sub>–py), 5.05 (t, J = 7.2 Hz, 2H,  $-CH_2$ CH<sub>2</sub>–py), 8.24 (t, J = 6.6 Hz, 2H, H-3,5), 8.62 (t, J = 7.8 Hz, 1H, H-4), 9.70 (d, J = 5.7 Hz, 2H, H-2,6). <sup>13</sup>C NMR δ/ppm 19.89 (t, J = 3.6 Hz), 25.89, 28.88, 28.90, 28.99, 29.11, 29.13, 29.20, 30.66 (t, J = 21.8 Hz), 31.94, 61.77, 128.41, 145.14 (2 × C). <sup>19</sup>F NMR δ/ppm -81.7 (CF<sub>3</sub>), -115.2, -122.8, -123.6, -124.4, -126.9. MS m/z (calcd) 552 (552). IR(KBr)  $\nu$ /cm<sup>-1</sup> 2925, 2855, 1239, 1205, 1144, 1123. Anal. calcd for C<sub>22</sub>H<sub>27</sub>F<sub>13</sub>NBr: C 41.77; H 4.27; N 2.21. Found: C 41.64; H 4.29; N 2.07%.

12,12,13,13,14,14,15,15,16,16,17,17,18,18,19,19,19-Heptadecafluorononadecylpyridinium bromide (13c). Mp 78.1 °C. <sup>1</sup>H NMR  $\delta$ /ppm 1.2–1.6 (m, 14H, –(CH<sub>2</sub>)<sub>7</sub>–), 1.62 (m, 2H,  $R_FCH_2CH_2-$ ), 1.9–2.1 (m, 4H,  $R_F C H_2 C H_2$ and  $-CH_2CH_2-py$ ), 5.07 (t, J = 7.2 Hz, 2H,  $-CH_2CH_2-py$ ), 8.22 (t, J = 6.6 Hz, 2H, H-3,5), 8.59 (t, J = 7.8 Hz, 1H, H-4), 9.63 (d, J = 5.4 Hz, 2H, H-2,6). <sup>13</sup>C NMR  $\delta$ /ppm 20.20 (t, J = 3.6 Hz), 26.19, 29.19, 29.31, 29.42 (2 × C), 29.50 (2 × C), 30.68 (t, J = 22.4 Hz), 32.21, 62.19, 127.63, 145.30, 145.36. <sup>19</sup>F NMR  $\delta$ /ppm -81.2 (CF<sub>3</sub>), -114.8, -122.3 (3 × CF<sub>2</sub>), -123.1, -124.0, -126.6. MS m/z (calcd) 652 (652). IR(KBr)  $\nu/cm^{-1}$ 2922, 2854, 1245, 1204, 1147, 1115. Anal. calcd for C<sub>24</sub>H<sub>27</sub>F<sub>17</sub>NBr: C 39.36; H 3.68; N 1.91. Found: C 39.39; H 3.66; N 1.80%.

**11,11,12,12,13,13,14,14,14-Nonafluorotetradecylpyridinium bromide monohydrate (14a).** Mp—broad phase transition at ambient temperature; determination of the onset of this transition was not possible. <sup>1</sup>H NMR δ/ppm 1.2–1.5 (m, 12H, –(CH<sub>2</sub>)<sub>6</sub>–), 1.60 (m, 2H, R<sub>F</sub>CH<sub>2</sub>CH<sub>2</sub>–), 1.9–2.1 (m, 4H, R<sub>F</sub>CH<sub>2</sub>CH<sub>2</sub>– and –CH<sub>2</sub>CH<sub>2</sub>–py), 3.1 (br s, OH), 5.00 (t, J =7.2 Hz, 2H, –CH<sub>2</sub>CH<sub>2</sub>–py), 8.25 (t, J = 6.6 Hz, 2H, H-3,5), 8.63 (t, J = 7.8 Hz, 1H, H-4), 9.51 (d, J = 6.0 Hz, 2H, H-2,6). <sup>13</sup>C NMR δ/ppm 20.00 (t, J = 3.6 Hz), 26.02, 28.97, 28.99, 29.11, 29.18, 29.20, 30.67 (t, J = 22.4 Hz), 31.95, 62.05, 128.58, 145.07, 145.32. <sup>19</sup>F NMR δ/ppm –81.6 (CF<sub>3</sub>), –115.1, –125.0, –126.6. MS m/z (calcd) 438 (438). IR(KBr)  $\nu/cm^{-1}$ 2930, 2852, 1234, 1214, 1194, 1133. Anal. calcd for C<sub>19</sub>H<sub>25</sub>F<sub>9</sub>NBr·H<sub>2</sub>O: C 42.53; H 4.85; N 2.61. Found: C 42.51; H 4.96; N 2.67%.

**11,11,12,12,13,13,14,14,15,15,16,16,16-Pentadecafluorohexadecylpyridinium bromide monohydrate (14b).** Mp 54.9 °C, 73.0 °C. <sup>1</sup>H NMR  $\delta$ /ppm 1.2–1.5 (m, 12H, –(CH<sub>2</sub>)<sub>6</sub>–), 1.60 (m, 2H, R<sub>F</sub>CH<sub>2</sub>CH<sub>2</sub>–), 1.9–2.1 (m, 4H, R<sub>F</sub>CH<sub>2</sub>CH<sub>2</sub>– and –CH<sub>2</sub>CH<sub>2</sub>–py), 2.5 (br s, OH), 4.99 (t, J = 7.6 Hz, 2H, –CH<sub>2</sub>CH<sub>2</sub>–py), 8.20 (t, J = 6.6 Hz, 2H, H-3,5), 8.57 (t, J =7.8 Hz, 1H, H-4), 9.52 (d, J = 5.6 Hz, 2H, H-2,6). <sup>13</sup>C NMR  $\delta$ /ppm 19.91 (t, J = 3.6 Hz), 25.89, 28.87 (2 × C), 29.00, 29.06, 29.08, 30.67 (t, J = 22.2 Hz), 31.85, 61.93, 128.41, 145.00, 145.11. <sup>19</sup>F NMR  $\delta$ /ppm –81.5 (CF<sub>3</sub>), –115.0, –122.6, –123.6, –124.2, –126.8. MS m/z (calcd) 538 (538). IR(KBr)  $\nu/\text{cm}^{-1}$  2922, 2843, 1253, 1217, 1175, 1146. Anal. calcd for C<sub>21</sub>H<sub>25</sub>F<sub>13</sub>NBr · H<sub>2</sub>O: C 39.62; H 4.25; N 2.20. Found: C 39.95; H 4.18; N 2.24%.

11,11,12,12,13,13,14,14,15,15,16,16,17,17,18,18,18-Heptadecafluorooctadecylpyridinium bromide monohydrate (14c). Mp 68.7 °C. <sup>1</sup>H NMR δ/ppm 1.2–1.6 (m, 12H,  $-(CH_2)_6-$ ), 1.58 (m, 2H, R<sub>F</sub>CH<sub>2</sub>CH<sub>2</sub>--), 1.9–2.1 (m, 4H, R<sub>F</sub>CH<sub>2</sub>CH<sub>2</sub>- and  $-CH_2$ CH<sub>2</sub>-py), 2.7 (br s, OH), 5.01 (t, J = 7.6 Hz, 2H,  $-CH_2$ CH<sub>2</sub>-py), 8.21 (t, J = 6.6 Hz, 2H, H-3,5), 8.59 (t, J =7.8 Hz, 1H, H-4), 9.53 (d, J = 5.6 Hz, 2H, H-2,6). <sup>13</sup>C NMR  $\delta$ /ppm 20.15 (t, J = 3.6 Hz), 26.15, 29.13 (2 × C), 29.27, 29.33, 29.35, 30.90 (t, J = 21.75 Hz), 32.12, 62.14, 128.68, 145.43, 145.58. <sup>19</sup>F NMR  $\delta$ /ppm -81.4 (CF<sub>3</sub>), -115.0, -122.5 (3 × CF<sub>2</sub>), -123.1, -124.1, -126.8. MS *m*/*z* (calcd) 638 (638). IR(KBr)  $\nu$ /cm<sup>-1</sup> 2921, 2853, 1253, 1209, 1169, 1139. Anal. calcd for C<sub>23</sub>H<sub>25</sub>F<sub>17</sub>NBr · H<sub>2</sub>O: C 37.50; H 3.6; N 1.90. Found: C 37.49; H 3.60; N 1.88%.

# Assessment of cytotoxicity

#### Cancer cell lines

The A2780 ovarian carcinoma cell line (ECACC) was selected from a panel of cancer cell lines used for testing in our laboratory.<sup>27</sup> The cell line was grown in RPMI 1640 medium (Sigma, Czech Republic) supplemented with 10% of fetal calf serum (Gibco, Czech Republic), 40 IU of insulin per 100 ml of medium, 50 µg ml<sup>-1</sup> penicillin, 50 µg ml<sup>-1</sup> streptomycin, 100 µg ml<sup>-1</sup> neomycin, and 300 µg ml<sup>-1</sup> L-glutamine as reported previously.<sup>27</sup> Cultures were maintained in a humidified incubator at 37 °C and 5% CO<sub>2</sub>.

### MTT-based cytotoxicity test

The MTT assay<sup>37,38</sup> was used to assess the cytotoxicity of the pyridinium salts in cells in the exponential growth phase. In short, cells were seeded on 96-well flat-bottom microplates at the density  $2.5-3.0 \times 10^4$  per ml, 100 µl per well, and allowed to grow for 16 to 24 hours in culture medium. The pyridinium salts dissolved in PBS (total volume of 20 µl) were added to wells and the cytotoxic effect was evaluated after 24 hours of exposure over a concentration range from 0.3 µM to 250 µM using the MTT assay. Benzalkonium chloride (BAC) (Sigma-Aldrich), a cationic surfactant employed in cosmetics as a foaming and cleaning agent and bactericide, was used as a reference compound.

MTT (Sigma Chemical Co., Czech Republic) was dissolved in PBS at a concentration of 5 mg ml<sup>-1</sup> 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 iEMS MF (Labsystems, Turku, Finland) at 540 nm and the IC<sub>50</sub> (*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 IC<sub>50</sub> values were calculated using GraphPad PRISM V.3.00 (GraphPad Software Inc., San Diego, CA).

The results from the MTT assay were further confirmed by Hoffman modulation contrast and fluorescent microscopy (epifluorescent inverted microscope T200, Nikon, Japan) exposing morphological changes of the cells treated with various pyridinium salts. Propidium iodide and YO-PRO-1 (Molecular Probes, Oregon, USA), were used to distinguish dead or apoptotic cells from vital living ones (data not shown).<sup>39</sup>

### 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. Concentration ranges of tested compounds were from 1  $\mu$ M to 1 mM. The released haemo-globin was quantified using a well-plate spectrophotometer reader iEMS MF (Labsystems, Turku, Finland) at 540 nm after two hours incubation of red cells with a particular compound at 37 °C. Data were expressed as the lowest concentration of surfactants causing haemolysis.

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### References

- 1 E. Kissa, *Fluorinated surfactants and repellents*, Marcel Dekker, New York, 2001, vol. 97.
- 2 G. Osei-Prempeh, H.-J. Lehmler, B. L. Knutson and S. E. Rankin, *Microporous Mesoporous Mater.*, 2005, 85, 16.
- 3 B. Tan, H.-J. Lehmler, S. M. Vyas, B. L. Knutson and S. E. Rankin, *Chem. Mater.*, 2005, **17**, 916.
- 4 S. E. Rankin, B. Tan, H.-J. Lehmler, K. P. Hindman and B. L. Knutson, *Microporous Mesoporous Mater.*, 2004, 73, 197.
- 5 B. Tan, A. Dozier, H.-J. Lehmler, B. L. Knutson and S. E. Rankin, *Langmuir*, 2004, **20**, 6981.
- 6 B. Tan, H.-J. Lehmler, S. M. Vyas, B. L. Knutson and S. E. Rankin, *Nanotechnology*, 2005, 16, S502.
- 7 K. Ghosh, H.-J. Lehmler, S. E. Rankin and B. L. Knutson, *Langmuir*, 2005, **21**, 6145.
- 8 H.-J. Lehmler, P. M. Bummer and M. Jay, *CHEMTECH*, 1999, **29**, 7.
- 9 J. G. Riess, J. Fluorine Chem., 2002, 114, 119.
- 10 J. B. Nivet, M. Le Blanc and J. G. Riess, *Eur. J. Med. Chem.*, 1991, 26, 953.
- 11 V. M. Sadtler, F. Jeanneaux, M. P. Krafft, J. Rabal and J. G. Riess, *New J. Chem.*, 1998, **22**, 609.
- 12 M.-P. Krafft, J.-P. Rolland, P. Vierling and J. G. Riess, *New J. Chem.*, 1990, 14, 869.
- 13 A. Milius, J. Greiner and J. G. Riess, New J. Chem., 1991, 15, 337.
- 14 L. Zarif, J. Greiner, S. Pace and J. G. Riess, J. Med. Chem., 1990, 33, 1262.
- 15 L. Zarif, J. Greiner and J. G. Riess, J. Fluorine Chem., 1989, 44, 73.

- 16 J. Greiner, A. Milius and J. G. Riess, *Tetrahedron Lett.*, 1988, 29, 2193.
- 17 F.-A. Pitten and A. Kramer, Arzneim.-Forsch., 2001, 51, 588.
- 18 H. P. Drobeck, Current topics on the toxicity of cationic surfactants, in *Cationic Surfactants (Surfactant Science Series)*, ed. J. Cross and E. J. Singer, Marcel Dekker, New York, 1994, vol. 53, p. 61.
- 19 J.-H. S. Kuo, M.-S. Jan, C.-H. Chang, H.-W. Chiu and C.-T. Li, *Colloids Surf.*, B, 2005, 41, 189.
- 20 T. Bramer, M. Paulsson, K. Edwards and K. Edsman, *Pharm. Res.*, 2003, **20**, 1661.
- 21 C. Tondre and C. Caillet, *Adv. Colloid Interface Sci.*, 2001, **93**, 115. 22 H.-J. Lehmler, M. O. Oyewumi, M. Jay and P. M. Bummer, *J.*
- *Fluorine Chem.*, 2001, **107**, 141. 23 H.-J. Lehmler and P. M. Bummer, *J. Fluorine Chem.*, 2002, **117**, 17
- 24 C. Naud, P. Calas, H. Blancou and A. Commeyras, J. Fluorine Chem., 2000, 104, 173.
- 25 M. Graupe, T. Koini, V. Y. Wang, G. M. Nassif, R. Colorado, Jr, R. J. Villazana, H. Dong, Y. F. Miura, O. E. Shmakova and T. R. Lee, J. Fluorine Chem., 1999, 93, 107.
- 26 T. Asakawa, H. Hisamatsu and S. Miyagishi, *Langmuir*, 1995, 11, 478.

- 27 J. Turanek, A. Kasna, D. Zaluska, J. Neca, V. Kvardova, P. Knotigova, V. Horvath, L. Sindlerova, A. Kozubik, P. Sova, A. Kroutil, F. Zak and A. Mistr, *Anti-Cancer Drugs*, 2004, **15**, 537.
- 28 J. B. Nivet, R. Bernelin, M. Le Blanc and J. G. Riess, *Eur. J. Med. Chem.*, 1992, 27, 891.
- 29 J. G. Riess, S. Pace and L. Zarif, Adv. Mater., 1991, 3, 249.
- 30 F. Guillod, J. Greiner and J. G. Riess, *Chem. Phys. Lipids*, 1995, 78, 149.
- 31 M. P. Krafft, P. Vierling and J. G. Riess, *Eur. J. Med. Chem.*, 1991, 26, 545.
- 32 D. D. Lasic, *Liposomes in Gene Delivery*, CRC Press, Boca Raton, 1997.
- 33 S. Ravi, D. Padmanabhan and V. R. Mamdapur, J. Indian Inst. Sci., 2001, 81, 299.
- 34 K. E. Murphy and A. H. Hoveyda, J. Am. Chem. Soc., 2003, 125, 4690.
- 35 N. O. Brace, J. Chem. Soc., 1962, 4491.
- 36 N. O. Brace, J. Fluorine Chem., 1982, 20, 313.
- 37 T. Mosmann, J. Immunol. Methods, 1983, 65, 55.
- 38 U. Bank, D. Reinhold and S. Ansorge, *Allerg. Immunol.*, 1991, 37, 119.
- 39 T. Idziorek, J. Estaquier, F. De Bels and J.-C. Ameisen, J. Immunol. Methods, 1995, 185, 249.