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# Nitroxide–fluorophore double probes: a potential tool for studying membrane heterogeneity by ESR and fluorescence<sup>†</sup>

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A serious drawback of ESR, particularly in its application to cells, is the lack of information on the location of spin probes in the system. In order to realize real time tracking, a spin probe was combined with a fluorophore in a new kind of nitroxide–fluorophore double probe which, in addition to information about lipid dynamics, enables visualization by fluorescence microscopy. The two sets of probes synthesized are based on an amino-alkyne-functionalized sugar that serves as a central polar group and as a linker between the 7-nitrobenz-2-oxa-1,3-diazol-4-yl (NBD) fluorophore and the derivative of the spin labelled fatty acid. In this setting, the location of the fluorophore is restricted to the water–lipid interface, while the nitroxide is located deep in the lipid bilayer. Preliminary tests on cells show preferential localization of both probes in the plasma membrane, with a relatively slow redistribution to other membranes of the cell. We believe that such double probes would be particularly useful for studies of plasma membrane heterogeneity and associated cellular processes.

### Introduction

Lipid-dependent plasma membrane heterogeneity is now widely accepted as a requirement for the normal function of biological membranes.<sup>1</sup> Certain important cellular processes are associated with this heterogeneity including cell signalling, membrane trafficking, pathogen invasion, neurodegenerative diseases and angiogenesis.<sup>2-6</sup> Microdomains of ordered lipids, the so-called "lipid rafts", are believed to be platforms for performing these biological functions.<sup>7</sup> However, despite the need to determine the exact molecular basis of these membrane organizations, progress in this field is slow, mostly reflecting the technical and experimental difficulties faced when studying lipid rafts *in vivo.*<sup>8</sup>

Electron spin resonance (ESR) is commonly employed to study the membrane physical properties that reflect its lateral inhomogeneity. Owing to the unique time scale of ESR, which spans almost all the motional range that occurs in membranes, ESR spectroscopy is particularly useful for studying lipid dynamics.<sup>9</sup> Since membranes do not possess paramagnetic moieties, ESR investigation depends on spin probes (Fig. 1) inserted into the membrane bilayer, that report on the properties of their immediate surroundings.<sup>10</sup> The resulting ESR spectra of model or biological membranes are complex to analyze but, together with simulation methods, much information on membrane dynamics and structure can be obtained.<sup>11</sup> This methodology works well with simple membrane systems like liposomes, and can be applied to more complex systems like cells, however, in the latter case specificity is a major problem. Cells, unlike liposomes, comprise many membranes of very different lipid and protein compositions that in turn yield different spectra.12 Because spin probes introduced into the plasma membrane of a cell rapidly undergo spontaneous or catalyzed flipflop, followed by redistribution to other intracellular membranes, the resulting ESR spectra comprise the superimposition of spectra of several membranes.<sup>13</sup> This can be partially circumvented by working with plasma membrane vesicles that lack cytosolic organelles.<sup>14</sup> Another way is to label the cells at lower temperatures, where redistribution to other membranes is much slower, followed by measurements at physiological temperature.<sup>13-15</sup> However, the redistribution increases with acquisition time, thus decreasing the specificity of labelling. This lack of definition of spin probe location in the system can be a serious drawback for the use of ESR on cells.

One possible solution is to couple ESR with fluorescence microscopy, a technique that provides real time visualization together with high sensitivity. In order to realize this approach, a double probe, containing nitroxide and fluorophore moieties in the same molecule, is necessary. However, implementation of this idea is hampered by the fact that nitroxides are strong quenchers of fluorescence.<sup>16</sup> This phenomenon is exploited by "profluorescent nitroxides", which are double probes in which the nitroxide serves as a 'molecular switch' that turns on fluorescence intensity by its conversion to a diamagnetic moiety by reduction or oxidation.<sup>17</sup> Profluorescent nitroxides have found application as sensors of cationic metals,<sup>18</sup> free radical generation in polymers,<sup>19–21</sup> ROS generation in cigarette smoke,<sup>22</sup> cellular redox environment,<sup>23</sup>

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Fig. 1 Examples of a) fluorescent probe, b) spin probe, c) double nitroxide-fluorophore probe.

nitric oxide,<sup>24,25</sup> singlet oxygen,<sup>26,27</sup> thiyl,<sup>28</sup> superoxide<sup>29</sup> and hydroxyl radicals,<sup>30</sup> as well as various antioxidants.<sup>31–33</sup> In a similar manner, quantum dots have been utilized in the place of the organic fluorophore, raising new possibilities for future applications.<sup>34,35</sup>

We present the design and synthesis of double nitroxidefluorophore probes that, after labelling the sample, enable detection by both ESR and fluorescence microscopy. Most importantly, this does not require any modification of the nitroxide moiety, as is the case with profluorescent nitroxides. To overcome the problem of quenching we designed a class of probes in which the fluorophore and nitroxide moieties are as far apart as possible, since contact between the two is a precondition for quenching.<sup>16</sup> To minimize this interaction a hydrophilic sugar was inserted between the lipophilic chain bearing the nitroxide and the fluorophore. Additionally, the fluorophore was tightly bound to the hydrophilic sugar moiety to prevent it from penetrating through the water-lipid interface into the interior of the lipophilic membrane and coming into contact with the spin labelled fatty acid derivative located there. Also, quenching was reduced by choosing a derivative of the spin labelled fatty acid in which the nitroxide is located close to the end of the alkyl chain and near the bilayer centre.<sup>36</sup> In the set of double probes presented here, a nitroxide reports on the lipid dynamics inside the membrane bilayer, while the fluorophore indicates the distribution of the probe among the membranes of the cell, as well as its lateral distribution in the membrane. With proper design and a suitable distance between the fluorophore and the nitroxide group we have combined two powerful and independent techniques that had previously been mutually exclusive. The described approach could be a powerful new tool for research into membrane heterogeneity. The synthesis of two double probes and their basic characterization are described.

### **Results and discussion**

An amino-alkyne-functionalized sugar served as a central polar group linking a 7-nitrobenz-2-oxa-1,3-diazol-4-yl (NBD) fluo-rophore and the derivative of the spin labelled fatty acid. The NBD

Spin labelled azido derivatives were prepared from spin labelled fatty acid methyl ester  $1,^{38}$  which was first reduced to the alcohol 2 (Scheme 1.).<sup>39</sup> Alcohol 2 was treated with mesylchloride to obtain a mesylated derivative which yielded azide 3 after treatment with NaN<sub>3</sub> in DMF at elevated temperature.<sup>40</sup> Similarly, azide 5 was obtained after treating 1-bromotetradecane 4 with NaN<sub>3</sub> in DMF.<sup>41</sup>



Scheme 1 Reagents and conditions: (i) LiAlH<sub>4</sub>, diethyl ether, 0 °C, 73%; (ii) MsCl, Et<sub>3</sub>N, DCM, 0 °C; (iii) NaN<sub>3</sub>, DMF, 55 °C (78% (over two steps) for **3** and 94% for **5**).

Synthesis of the glycosyl core for the first pair of compounds that would bear the NBD fluorophore in position 2 (Scheme 2.) proceeded from readily obtainable D-glucosamine chloride to compound **6** in four steps which are described elsewhere.<sup>42</sup> Compound **6** was reacted with propargyl alcohol in the presence of BF<sub>3</sub>·Et<sub>2</sub>O as an activator, to afford propargyl glycoside 7.<sup>43</sup> Prolonged reaction times (3 days) were necessary, as alkyne **7** could not be separated from starting compound **6** and so complete conversion was required, despite the fact that longer reaction times lowered the yield. Nonetheless the reaction afforded the propargyl moiety of alkyne **7** in  $\beta$  conformation only, showing high stereoselectivity in this case.



Scheme 2 Reagents and conditions: (i) propargyl alcohol,  $BF_3\cdot Et_2O,$  DCM, 0 °C, 63%.

D-Galactose served as starting material for the synthesis of the glycosyl core of the second pair of compounds that would bear the NBD fluorophore in position 6 (Scheme 3.). To introduce the precursor of the amino functionality, D-galactose was converted to azide **8** in three previously described steps.<sup>44</sup> Azide **8** was next reduced to the amine by  $H_2$  in the presence of Pd/C, followed by addition of trifluoroacetanhydride that afforded



Scheme 3 Reagents and conditions:  $H_2$ , Pd/C, THF, RT; (ii) ( $CF_3CO$ )<sub>2</sub>O,  $Et_3N$ , THF, 0 °C, 90% (over two steps); (iii) TFA, AcOH,  $H_2O$ , 50 °C; (iv) Ac<sub>2</sub>O, Py, 0 °C, 55% (over two steps); (v) BnNH<sub>2</sub>, THF, RT, 72%; (vi) CCl<sub>3</sub>CN, DBU, 0 °C, 57%, (vii) propargyl alcohol, TMSOTf, DCM, -30 °C, 63%.

trifluoroacetamide 9, both steps being conducted in one pot. The switch from acetonide to acetate protecting groups was accomplished by treatment of compound 9 with aqueous trifluoric acid, that released hydroxyl groups,<sup>45</sup> and subsequent introduction of acetates with acetic anhydride that afforded compound 10, again both steps being conducted in one pot. Under conditions of acid hydrolysis of acetonides, the trifluoroacetamide functionality proved to be only moderately stable, the yield being considerably lower in the case of longer reaction times. To form a glycosidic bond a similar strategy was employed to that used previously for

compound 7 but, to our surprise, the reaction between compound 10 and propargyl alcohol in the presence of  $BF_3 \cdot Et_2O$  led to an anomeric mixture of  $\alpha$  and  $\beta$  propargyl glycoside 13, the  $\beta$  anomer being the predominant one. To overcome this problem a new approach to glycoside formation *via* trichloroacetimidate was applied. In order to do so, the anomeric acetate group of compound 10 was selectively removed with benzylamine to give compound 11.<sup>46</sup> The free anomeric hydroxyl group of compound 11 was coupled with trichloroacetonitrile in the presence of DBU as a base, to yield trichloroacetimidate 12 in  $\alpha$  conformation only.<sup>46</sup> Glycoside formation from trichloroacetimidate 12 and propargyl alcohol with TMSOTf as an activator went smoothly, affording alkyne 13 in good yield and high stereoselectivity towards the  $\beta$  product.<sup>47</sup>

Click reactions between azides 3 and 5 and alkynes 7 and 13 went smoothly, yielding compounds 14a, 14b and 17a, 17b (Scheme 4).48 The best results were obtained when CuBr, in the presence of N-methylmorpholine, was used as catalyst. Under these conditions, the reaction proceeded to completion in just 30 min at room temperature. The trifluoroacetamide and acetate protecting groups were removed in two consecutive steps, yielding 15a, 15b and 18a, 18b. In the first step, acetate groups were removed in the presence of catalytic amounts of NaOMe in MeOH.<sup>49</sup> To our surprise, after addition of aqueous NaOH to the reaction mixture, only partial cleavage of trifluoroacetamide was observed. In contrast, complete cleavage of trifluoroacetamide was observed if the solvent was removed prior to the addition of aqueous NaOH.<sup>50</sup> Finally, amines 15a, 15b and 18a, 18b were reacted with fluorogenic benzofurazan reagent (NBD-Cl) to afford a set of double probes 16a, 19a and its derivates 16b, 19b without the spin label.51

Absorption and emission fluorescence spectra of fluorescent and double probes were recorded in methanol (Fig. 2). When the nitroxide moiety of the double probes was reduced, fluorescence intensity significantly increased. However, the increase of



Scheme 4 Reagents and conditions: (i) CuBr, NMM, EtOAc, RT, 63% for 14a, 78% for 14b; 63% for 17a, 90% for 17b (ii) NaOMe, MeOH, RT; (iii) NaOH<sub>(aq)</sub>, 77% for 15a, 78% for 15b, 31% for 18a, 53% for 18b (over two steps); (iv) NBD-Cl,  $K_2CO_3$ , THF, 0 °C, 59% for 16a, 43% for 16b, 13% for 19a, 24% for 19b.



**Fig. 2** Normalised absorption and fluorescence emission spectra shown together with fluorescence emission spectrum after reduction of the nitroxide of double probe **16a**. Fluorescence emission spectra were recorded with  $10^{-7}$  M solutions of **16a** in methanol ( $\lambda_{ex} = 450$  nm). Absorption spectrum was recorded with  $5 \times 10^{-5}$  M solution of **16a** in methanol. For absorption and fluorescence emission spectra of other probes see ESI.†

fluorescence intensity is not as great as it was reported in the literature for double probes, where nitroxide is in close proximity of the fluorophore.<sup>19-21</sup>

The exact degree of quenching present when the probe is in the membrane compared to the case when the probe is in the solution, is hard to establish due to the sensitivity of the NBD fluorescence to the environment and insolubility of the probes in water. Nevertheless, the influence of the double probe's design on quenching of the fluorescence was studied by comparing the fluorescence intensity of probes **16a** and **19a** incorporated into the liposomes before and after reduction of nitroxide (Fig. 3). An increase in fluorescence was observed after reduction of the nitroxide group with probes **16a** and **19a**, which means that some quenching persists even when the probe is incorporated into the membrane. The increase of fluorescence after reduction of nitroxide is more pronounced with **19a**. In the case of double probe



Fig. 3 Fluorescence emission spectra of double probe 16a incorporated into the liposomes before and after reduction of the nitroxide. Probe concentration was  $10^{-7}$  M ( $\lambda_{ex} = 470$  nm). For fluorescence spectra of other probes see the ESI.<sup>†</sup>

**19a** the lipophilic NBD fluorophore has more conformational freedom and can easily move into the interior of the lipophilic membrane. This can explain the higher degree of quenching observed with **19a** in comparison to more tightly bound NBD fluorophore of double probe **16a**.

Fluorescence and ESR properties of the probes were further studied on MCF-7 cells. For fluorescence microscopy, the probes were dissolved in DMSO for addition to the medium, since they were not sufficiently soluble in buffer. Labelling of the membrane was almost instantaneous, lag times arising mainly from the preparation of the optical equipment after adding the probes. Fluorescence microscopy revealed that all probes concentrated specifically in the plasma membrane, with little redistribution to intracellular membranes even after one hour of incubation (Chart 1.). The preferential localization in the plasma membrane should make these probes particularly interesting for research of lipid dynamics and changes that occur during certain cell events *e. g.* cell signalling.



**Chart 1** Confocal fluorescence images (upper row) and corresponding bright field images (lower row) of MCF-7 cells labelled with **16a** (A), **16b** (B), **19a** (C) and **19b** (D). Images were taken 5 min after addition of probes dissolved in DMSO.

ESR spectra of MCF-7 cells labelled with double probes 16a and 19a were recorded (Fig. 4.). The ESR signal-to-noise ratio was satisfactory up to 30 min after labelling. Afterwards the signal was lost due to the reduction of the nitroxide group. In order to assess the influence of the fluorophore and sugar moiety on the mobility and the ESR signature of the probes, ESR spectra of liposomes labelled with 1, 16a and 19a were recorded (see ESI†). The recorded spectra are very similar, therefore we believe that the fluorophore and the sugar moiety do not have any significant



Fig. 4 ESR spectra of MCF-7 cells labelled with double probes 16a and 19a.

impact on the motion of the doxyl group on the fatty acid chain. No other particular differences regarding fluorescence or ESR properties were observed, either between double probes 16a and 19a or in comparison with their non-paramagnetic analogues 16b and 19b.

### Conclusions

To summarize, we have succeeded in synthesizing new double nitroxide-fluorophore probes 16a and 19a, based around a hydrophilic amino sugar that connects an NBD fluorophore and a spin labelled fatty acid derivative. Both probes and their nonparamagnetic analogues were tested on the MCF-7 cell line by ESR spectroscopy and fluorescence microscopy, providing, for the first time, ESR spectra with corresponding information of the probe's distribution in the cells. Furthermore, early testing on MFC-7 cells showed that all the probes concentrated specifically in the plasma membrane with little redistribution to intracellular membranes. Based on these encouraging results, we believe that these double nitroxide-fluorophore probes will open up new possibilities for studying plasma membrane heterogeneity.

Human breast adenocarcinoma cell line MCF-7 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (FSC) and antibiotics: 100 U mL<sup>-1</sup> penicillin and 100 µg mL<sup>-1</sup> streptomycin (all from Gibco). The cells were incubated at 37 °C in a humidified 5% CO<sub>2</sub> atmosphere.

For fluorescence microscopy observation, cells were plated on glass-bottom cell culture dishes (Lab-Tek(tm) Chambered Coverglass) for one day. 0.25 µL 10<sup>-5</sup> M of fluorescence probe 16a or 19a in DMSO was added to 30000 cells/well at room temperature and observed with an inverted Nikon TE-2000 E fluorescence microscope, equipped with a confocal unit Carv II (BD Biosciences) and a Rolera-MGi camera. The samples were excited from 430 nm to 490 nm and emission was observed from 506 nm to 594 nm.

### Electron spin resonance measurements

For ESR measurements cells were spin labelled with double probes 16a and 19a. A thin film of the probe was prepared on the walls of a glass tube by rotary evaporation of a methanol solution of the probe (12  $\mu$ L for **16a** and 48  $\mu$ L for **19a**). 6 ± 2 million cells (in the case of 19a 8  $\pm$  2 million cells) were then mixed with 1.5  $\mu$ L of medium without serum, placed into the tube and vortexed for 10 min at room temperature, then centrifuged for 2 min at 1500 rpm. Samples were transferred into a glass capillary for ESR measurements which were performed on an X-band ESR spectrometer Bruker ESP 300 at room temperature. Spectrometer settings were: microwave power 10 mW, modulation amplitude 0.1 mT, frequency of modulation 100 kHz and 9 scans for each spectrum.

### General methods

Chemicals from Sigma-Aldrich and Acros were used without further purification. All reactions were performed under argon at-

Experimental Fluorescence microscopy

as the internal standards. In the case of compounds 15b and 19b <sup>13</sup>C NMR spectra were additionally recorded at 353 K, in order to confirm assignation of signals, since some were broad when recorded at 302 K (see ESI<sup>†</sup>). Optical rotation was measured using a Perkin-Elmer 241 MC polarimeter at 589 nm (sodium D-line) and the  $[\alpha]_D$  values are quoted in units  $10^{-1} \text{ deg cm}^2 \text{ g}^{-1}$ . Microanalysis was performed on a Perkin-Elmer C, H, N analyzer 240C. Mass spectra were recorded using a VG-Analytical Q-TOF Premier mass spectrometer. Fluorescence spectra were measured with Perkin-Elmer LS 55 fluorescence spectrophotometer with a 10 mm cuvette. The excitation and emission wavelength bandpasses were both set at 10 nm. Absorption spectra were measured with Varian Cary 50 UV-Vis spectrophotometer. 2-(12-Hydroxydodecyl)-4,4-dimethyl-2-propyloxazolidin-3-oxyl (2). Spin-labelled fatty acid methyl ester 1 (690 mg, 1.86 mmol) was dissolved in dry diethyl ether (20 mL) and added dropwise to a suspension of LiAlH<sub>4</sub> (141 mg, 2 equiv, 3.72 mmol) in dry diethyl ether (20 mL) cooled to 0 °C on an ice bath. The temperature was allowed to reach ambient and stirring was continued for 2 h. A saturated solution of NaHCO<sub>3</sub> (250 µL) was added to the reaction mixture and stirring was continued for 15 min. The reaction mixture was then filtered and the solvent removed under reduced pressure. The crude product was purified by flash chromatography (ethyl acetate-hexane, 1:3) to give the desired product (461 mg, 1.35 mmol, 73%) as an orange oil. IR (KBr, cm<sup>-1</sup>): 3448, 2927, 2854, 1465, 1363, 1260, 1054. HRMS (ESI), m/z calcd for C<sub>20</sub>H<sub>42</sub>NO<sub>3</sub> 344.3165 (M-H<sub>2</sub>)<sup>+</sup>, found 344.3179.

mosphere unless otherwise stated. Analytical TLC was performed

on Merck silica gel (60 F<sub>254</sub>) plates (0.25 mm) and visualized with ultraviolet light and detected with 20% sulfuric acid in

ethanol. Flash chromatography was performed on an Isolera

One flash purification system from Biotage using KP-Sil SNAP cartridges. Melting points were determined on a Reichert hot

stage microscope and are uncorrected. IR spectra were obtained on a Perkin-Elmer FT-IR System Spectrum BX. <sup>1</sup>H and <sup>13</sup>C

NMR spectra were recorded on a Brucker AVANCE DPX300

spectrometer in CDCl<sub>3</sub>, DMSO-d<sub>6</sub>, MeOH-d<sub>4</sub>, acetone-d<sub>6</sub> and

pyridine-d<sub>5</sub> solution, with TMS or residual undeuterated solvent

2-(12-Azidododecyl)-4,4-dimethyl-2-propyloxazolidin-3-oxyl (3). Alcohol 2 (355 mg, 1.04 mmol) was dissolved in dry dichloromethane (20 mL) and cooled to 0 °C on an ice bath. Methanesulfonyl chloride (82 µL, 1.05 equiv, 1.09 mmol) was added to the solution and stirring continued for 30 min. The reaction mixture was washed with water (50 mL), saturated solution of NaHCO<sub>3</sub> (40 mL), 10% citronic acid (40 mL) and brine (40 mL), dried with Na<sub>2</sub>SO<sub>4</sub> and the solvent evaporated under reduced pressure. To the oily residue were added sodium azide (340 mg, 5 equiv, 5.2 mmol) and dimethylformamide (5 mL) and the obtained suspension was stirred for 4 h at 55 °C. The reaction mixture was diluted with ethyl acetate (50 mL) and washed with water  $(3 \times 50 \text{ mL})$ , brine (40 mL), dried with Na<sub>2</sub>SO<sub>4</sub> and the solvent evaporated under reduced pressure to give the desired product (281 mg, 0.76 mmol, 78%) as an orange oil. For analytical purposes azide 3 was purified using silica gel chromatography (ethyl acetate-hexane, 1:4). IR (NaCl, cm<sup>-1</sup>): 2927, 2854, 2095, 1464, 1362, 1259, 1053. HRMS (ESI), m/z

Microanalysis calcd for C<sub>20</sub>H<sub>40</sub>NO<sub>3</sub>·0.17 H<sub>2</sub>O (%): C 69.52, H

11.77, N 4.05; found C 69.30, H 12.13, N 4.04.

calcd for  $C_{20}H_{39}N_4O_2Na$  390.2971 (M–Na)<sup>+</sup>, found 390.2970. Microanalysis calcd for  $C_{20}H_{39}N_4O_2\cdot 0.1$  EtOAc + 0.1 hexane (%): C 65.52, H 10.79, N 14.55; found C 65.52, H 10.76, N 14.50.

**1-Azidotetradecane** (5). 1-Bromotetradecane **4** (3 g, 10.8 mmol) and sodium azide (3.52 g, 5 equiv, 54.1 mmol) were suspended in dimethylformamide (10 mL) and the obtained suspension stirred for 6 h at 60 °C. The reaction mixture was diluted with ethyl acetate (50 mL) and washed with water (3 × 50 mL), brine (40 mL), dried with Na<sub>2</sub>SO<sub>4</sub> and the solvent evaporated under reduced pressure to give the desired product (2.44 g, 10.2 mmol, 94%) as a colourless liquid. IR (KBr, cm<sup>-1</sup>): 3370, 2922, 2857, 2094, 1877, 1595, 1460, 1258. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta$  (ppm) 3.24 (t, 2H, J = 6.9 Hz, CH<sub>2</sub>) 1.59 (m, 2H, CH<sub>2</sub>), 1.40–1.00 (m, 22H, 11 × CH<sub>2</sub>), 0.88 (t, 3H, J = 6.9 Hz, CH<sub>3</sub>). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz):  $\delta$  (ppm) 51.44, 31.91, 29.67, 29.65, 29.62, 29.54, 29.48, 29.35, 29.15, 28.84, 26.71, 22.66, 14.02. MS (ESI), m/z: 214.3 (MH-28)<sup>+</sup>.

(2R,3S,4R,5R,6R)-2-(Acetoxymethyl)-6-(prop-2-yn-1-yloxy)-5-(2,2,2-trifluoroacetamido)tetrahydro-2H-pyran-3,4-diyl diacetate (7). Compound 6 (2.5 g, 5.64 mmol) and propargyl alcohol (1.64 mL, 5 equiv, 28.2 mmol) were dissolved in anhydrous dichloromethane (5 mL) and the solution cooled to 0 °C on an ice bath. BF<sub>3</sub>·Et<sub>2</sub>O (3.6 mL, 5 equiv, 28.2 mmol) was added dropwise and the reaction mixture was allowed to reach room temperature while stirring was continued for 72 h. The reaction mixture was then diluted with ethyl acetate (50 mL) and poured into saturated solution of NaHCO<sub>3</sub> (50 mL). After gas stopped evolving, the organic layer was separated, washed with brine and dried with Na<sub>2</sub>SO<sub>4</sub>. The solvent was evaporated under reduced pressure and the crude product purified by flash chromatography (ethyl acetate-hexane, 1:2) to give the desired product (1.55 g, 3.53 mmol, 63%) as a colourless solid. Mp 175-177 °C. IR (KBr, cm<sup>-1</sup>): 3315, 3270, 3112, 2975, 1749, 1714, 1562, 1379, 1233, 1181, 1075, 1048, 885, 680.  $[\alpha]_D$  –38.9 (*c* 0.23, MeOH). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta$  (ppm) 6.53 (d, 1H, J = 8.7 Hz, NH), 5.32 (dd, 1H, J = 10.5, 9.3 Hz, H-3), 5.12 (dd, 1H, J = 9.9, 9.3 Hz, H-4), 4.88 (d, 1H, J = 8.4 Hz, H-1), 4.38 (d, 2H, J = 2.2 Hz,  $CH_2-C \equiv$ ), 4.29 (dd, 1H, J = 12.6, 4.8 Hz,  $H_{ab}$ -6), 4.16 (dd, 1H, J = 12.6, 2.7 Hz, H<sub>ab</sub>-6'), 4.08–3.98 (m, 1H, H-2), 3.79–3.73 (m, 1H, H-5), 2.47 (t, 1H, J = 2.2 Hz,  $\equiv$ CH), 2.10 (s, 3H, CH<sub>3</sub>), 2.04 (s, 3H, CH<sub>3</sub>), 2.03 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz): δ (ppm) 171.14, 170.69, 169.21, 158.20, 157.70, 157.20, 156.70, 121.29, 171.45, 131.65, 101.13, 97.65, 78.02, 75.59, 72.01, 71.84, 68.47, 61.90, 56.00, 54.32, 20.64, 20.52, 20.33. HRMS (ESI), m/z calcd for  $C_{17}H_{20}F_3NO_9Na$  462.0988 (M + Na)<sup>+</sup>, found 462.0973. Microanalysis calcd for  $C_{17}H_{20}F_3NO_9$  (%): C 46.47, H 4.59, N 3.19; found C 46.67, H 4.46, N 3.16.

2,2,2-Trifluoro-*N*-(((3aR,5R,5aS,8aS,8bR)-2,2,7,7-tetramethyltetrahydro-3aH-bis([1,3]dioxolo)[4,5-b:4',5'-d]pyran-5-yl)methyl) acetamide (9). Azide 8 (4.0 g, 13.8 mmol) was dissolved in anhydrous tetrahydrofuran (50 mL) and argon was bubbled through the solution for 10 min. Pd/C (250 mg, 6.25%) was added and the suspension stirred under hydrogen for 48 h. The suspension was cooled to 0 °C on ice while argon was bubbled through. Triethylamine (2.9 mL, 1.5 equiv, 20.7 mmol) was added to the suspension followed by dropwise addition of trifluoroacetic anhydride (2.1 ml, 1.1 equiv, 15.2 mmol). After 2 h of stirring Pd/C was filtered off and the solvent evaporated under reduced pressure. The residue was dissolved in ethyl acetate and washed with water (50 mL), saturated solution of NaHCO<sub>3</sub> (50 mL), 1 M HCl (50 mL) and brine (50 mL), dried with Na<sub>2</sub>SO<sub>4</sub> and the solvent evaporated under reduced pressure. The crude product was purified by flash chromatography (ethyl acetate-hexane, 1:3 to 1:1) to give the desired product (4.5 g, 12.5 mmol, 90%) as a colourless solid. Mp 93-95 °C. IR (KBr, cm<sup>-1</sup>): 3587, 3342, 2999, 1718, 1560, 1459, 1382, 1255, 1215, 1185, 1156, 1112, 1068, 1017, 925, 899, 878, 858, 767, 729, 685. [α]<sub>D</sub> 9.6 (*c* 0.20, MeOH). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta$  (ppm) 7.08 (bs, 1H, NH), 5.47 (d, 1H, J = 5.0 Hz, H-1), 4.59 (dd, 1H, J = 7.8, 2.4 Hz, H-3), 4.29 (dd, 1H, J = 5.0, 2.4 Hz, H-2), 4.20 (dd, 1H, J = 7.8, 1.8 Hz, H-4), 3.93–3.88 (m, 1H, H-5), 3.82–3.74 (m, 1H, H-6), 3.37–3.28 (m, 1H, H-6'), 1.44 (s, 3H, CH<sub>3</sub>), 1.41 (s, 3H, CH<sub>3</sub>), 1.30 (s, 3H, CH<sub>3</sub>), 1.28 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz): δ (ppm) 158.53, 158.04, 157.55, 157.06, 121.93, 118.12, 114.31, 110.50, 110.08, 109.34, 96.60, 72.37, 71.21, 70.85, 65.57, 41.04, 26.25, 26.16, 25.23, 24.53. HRMS (ESI), m/z calcd for C<sub>14</sub>H<sub>21</sub>F<sub>3</sub>NO<sub>6</sub> 356.1312 (M + H)<sup>+</sup>, found 356.1315. Microanalysis calcd for C<sub>14</sub>H<sub>20</sub>F<sub>3</sub>NO<sub>6</sub>·0.5EtOAc (%): C 48.12, H 6.06, N 3.51; found C 48.35, H 6.13, N 3.61.

(3R,4S,5S,6R)-6-((2,2,2-Trifluoroacetamido)methyl)tetrahydro-2H-pyran-2,3,4,5-tetrayl tetraacetate (10). Compound 9 (4.18 g, 11.7 mmol) was dissolved in methanol (20 mL). Trifluoroacetic acid (5 mL) and water (10 mL) were added and the reaction mixture was stirred at 50 °C for 15 h. Solvents were removed under reduced pressure; removing traces of water from the residue by toluene  $(2 \times 10 \text{ mL})$  co-evaporation under reduced pressure. Residue was dissolved in pyridine (18 mL) and the solution was cooled on an ice bath to 0 °C. Acetic anhydride (7.2 mL, 5.5 equiv, 65.2 mmol) was added dropwise to the solution and stirring was continued for 24 h while the temperature was allowed to reach room temperature. 10 mL of methanol was added to the reaction mixture and, after 10 min, the solvent was evaporated under reduced pressure. The residue was dissolved in ethyl acetate and washed with water (50 mL), saturated solution of NaHCO<sub>3</sub> (50 mL), 1 M HCl (50 mL) and brine (50 mL), dried with Na<sub>2</sub>SO<sub>4</sub> and the solvent evaporated under reduced pressure. The crude product was purified by flash chromatography (ethyl acetatehexane, 1:3 to 1:1) to give the desired product (2.6 g, 6.4 mmol, 55%) as a viscous oil. IR (KBr, cm<sup>-1</sup>): 3422, 1752, 1569, 1375, 1225, 1069, 932, 727, 600. HRMS (ESI), m/z calcd for  $C_{16}H_{20}F_3NO_{10}Na$ 466.0937 (M + Na)<sup>+</sup>, found 466.0940. Microanalysis calcd for C<sub>16</sub>H<sub>20</sub>F<sub>3</sub>NO<sub>10</sub>·0.2EtOAc (%): C 43.78, H 4.72, N 3.04; found C 43.67, H 4.79, N 3.00.

(3*R*,4*S*,5*S*,6*R*) - 2 - Hydroxy - 6 - ((2, 2, 2 - trifluoroacetamido)methyl)tetrahydro - 2*H* - pyran - 3, 4, 5 - triyl triacetate (11). Compound 10 (733 mg, 1.65 mmol) was dissolved in tetrahydrofuran (5 mL) followed by the addition of benzylamine (191  $\mu$ L, 1.05 equiv, 1.73 mmol). Reaction mixture was stirred at room temperature for 72 h. The solvent was evaporated under reduced pressure and the residue dissolved in ethyl acetate (30 ml), washed with saturated solution of 1 M HCl (2×30 mL), NaHCO<sub>3</sub> (30 mL), and brine (50 mL). After drying with Na<sub>2</sub>SO<sub>4</sub> the solvent was evaporated under reduced pressure and the crude product purified by flash chromatography (ethyl acetate–hexane, 1 : 1) to give the desired product (480 mg, 1.2 mmol, 72%) as a white foam. Mp 68–70 °C. IR (KBr, cm<sup>-1</sup>): 3447, 1751, 1560, 1438, 1375, 1228, 1158, 1062, 728, 600. HRMS (ESI), m/z calcd for C<sub>14</sub>H<sub>18</sub>F<sub>3</sub>NO<sub>9</sub>Na 424.0831 (M + Na)<sup>+</sup>, found 424.0834. Microanalysis calcd for C<sub>14</sub>H<sub>18</sub>F<sub>3</sub>NO<sub>9</sub> (%): C 41.90, H 4.52, N 3.49; found C 41.98, H 4.58, N 3.36.

(3R,4S,5S,6R)-2-(2,2,2-Trichloro-1-iminoethoxy)-6-((2,2,2-trifluoroacetamido)methyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (12). Compound 11 was dissolved in dry dichloromethane (50 mL) and cooled to 0 °C on an ice bath. 1,8-Diazabicyclo[5.4.0]undec-7-ene (160 µL, 0.33 equiv, 1.1 mmol) was added followed by trichloroacetonitrile (510 µL, 1.5 equiv, 5 mmol). The temperature was allowed to reach ambient temperature and stirring was continued for 2 h. The reaction mixture was adsorbed on silica gel, dried and purified by flash chromatography (ethyl acetate-hexane, 1:10 to 1:2) to give the desired product (1.0 g, 1.9 mmol, 57%) as a white foam. Mp 50-55 °C. IR (KBr, cm<sup>-1</sup>): 3447, 2931, 1735, 1376, 1228, 1159, 1064, 833.  $[\alpha]_{\rm D}$  143 (c 0.26, CH<sub>2</sub>Cl<sub>2</sub>). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz): δ (ppm) 8.69 (s, 1H, = NH), 6.72 (bs, 1H, NH), 6.55 (d, 1H, J = 3.4 Hz, H-1), 5.52 (dd, 1H, J = 3.0, 0.9 Hz, H-4), 5.42 (dd, 1H, J = 10.8, 3.0 Hz, H-3), 5.35 (dd, 1H, J = 10.8, 3.4 Hz, H-2), 4.33–4.29 (m, 1H, H-5), 3.65– 3.56 (m, 1H, H-6), 3.39–3.29 (m, 1H, H-6'), 2.19 (s, 3H, CH<sub>3</sub>), 2.04 (s, 3H, CH<sub>3</sub>), 2.02 (s, 3H, CH<sub>3</sub>).<sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz): δ (ppm) 170.32, 170.10, 169.76, 161.02, 158.00, 157.53, 157.03, 156.54, 121.29, 117.51, 113.70, 109.22, 93.30, 30.57, 69.09, 68.32, 67.40, 66.81, 39.51, 20.58, 20.53, 20.50. HRMS (ESI), m/z calcd for  $C_{16}H_{18}F_3N_2O_9Cl_3Na$  566.9928 (M + Na)<sup>+</sup>, found 566.9930. Microanalysis calcd for C<sub>16</sub>H<sub>18</sub>Cl<sub>3</sub>F<sub>3</sub>N<sub>2</sub>O<sub>9</sub>·0.33 H<sub>2</sub>O (%): C 34.83, H 3.41, N 5.08; found C 34.76, H 3.39, N 4.93.

(2R,3R,4S,5S,6R)-2-(Prop-2-yn-1-yloxy)-6-((2,2,2-trifluoroacetamido)methyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (13). Trichloroacetimidate 12 (1.01 g, 1.85 mmol) and propargyl alcohol (1.14 mL, 10 equiv, 19.5 mmol) were dissolved in dry dichloromethane (10 mL). Molecular sieves (1 g) were added and the solution cooled to -30 °C. Trimethylsilyl triflate (85  $\mu$ L, 0.25 equiv, 0.47 mmol) was added to the solution, the temperature of which rose to -15 °C in a period of 30 min. A saturated solution of NaHCO<sub>3</sub> (500  $\mu$ L) was added to the reaction mixture and the solvent removed under reduced pressure. The crude product was purified by flash chromatography (ethyl acetate-hexane, 1:9 to 1:1) to give the desired product (510 mg, 1.16 mmol, 63%) as a white foam. Mp 40-42 °C. IR (KBr, cm<sup>-1</sup>): 3362, 2926, 1750, 1559, 1372, 1223, 1156, 1073, 905, 727. [α]<sub>D</sub> 4.2 (*c* 0.29, MeOH). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta$  (ppm) 6.85 (bs, 1H, NH), 5.33 (dd, 1H, J = 3.3 Hz, H-4), 5.26 (dd, 1H, J = 10.3, 8.0 Hz, H-2), 5.09 (dd, 1H, J = 10.3, 3.3 Hz, H-3), 4.76 (d, 1H, J = 8.0 Hz, H-1), 4.40–4.39 (m, 2H, CH<sub>2</sub>–C=), 3.87 (dt, 1H, J = 6.9 Hz, H-5), 3.65-3.56 (m, 1H, H-6), 3.49-3.40 (m, 1H, H-6'), 2.50 (t, 1H, J = 2.1 Hz, CH), 2.09 (s, 3H, CH<sub>3</sub>), 2.06 (s, 3H, CH<sub>3</sub>), 2.03 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz):  $\delta$  (ppm) 170.64, 169.90, 169.55, 158.07, 157.58, 157.08, 156.59, 121.29, 117.47, 113.67, 109.86, 98.93, 77.99, 75.42, 70.61, 70.49, 68.42, 67.82, 56.13, 39.16, 20.52, 20.39, 20.29. HRMS (ESI), m/z calcd for  $C_{17}H_{19}F_3NO_9$  438.1012 (M – H)<sup>-</sup>, found 438.1020. Microanalysis calcd for C<sub>17</sub>H<sub>20</sub>F<sub>3</sub>NO<sub>9</sub>·0.2 H<sub>2</sub>O (%): C 45.24, H 4.76, N 3.10; found C 45.11, H 4.59, N 3.06.

General procedure for a click reaction. Alkyne (1 mmol), azide (1 mmol) and *N*-methylmorpholine (103  $\mu$ L, 1 mmol) were

dissolved in ethyl acetate (5 mL). CuBr (7 mg, 1,1 equiv, mmol) was added and the reaction mixture stirred for 30 min at room temperature. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography.

(2*R*,3*S*,4*R*,5*R*,6*R*)-2-(Acetoxymethyl)-6-((1-(12-(3-oxyl-4,4dimethyl-2-propyloxazolidin-2-yl)dodecyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-5-(2,2,2-trifluoroacetamido)tetrahydro-2*H*-pyran-3,4diyl diacetate (14a). The reaction between alkyne 7 and azide 3 was carried out according to the general procedure for click reaction. Removal of the solvent under reduced pressure and purification of the crude product by flash chromatography (ethyl acetate–hexane, 2 : 3 to 3:2) yielded the desired product (63%) as an orange viscous oil. IR (KBr, cm<sup>-1</sup>): 3448, 2929, 1751, 1643, 1567, 1466, 1378, 1233, 1182, 1047.  $[\alpha]_D$  –9.9 (*c* 0.15, MeOH). HRMS (ESI), *m*/*z* calcd for C<sub>37</sub>H<sub>60</sub>F<sub>3</sub>N<sub>5</sub>O<sub>11</sub> 807.4241 (M + H)<sup>+</sup>, found 807.4252. Microanalysis calcd for C<sub>37</sub>H<sub>59</sub>F<sub>3</sub>N<sub>5</sub>O<sub>11</sub> (%): C 55.08, H 7.37, N 8.68; found C 55.01, H 7.22, N 8.36.

(2R,3S,4R,5R,6R)-2-(Acetoxymethyl)-6-((1-tetradecyl-1H-1,2, 3-triazol-4-yl)methoxy)-5-(2,2,2-trifluoroacetamido)tetrahydro-2H-pyran-3,4-diyl diacetate (14b). The reaction between alkyne 7 and azide 5 was carried out according to general procedure for click reaction. Removal of solvent under reduced pressure and purification of the crude product by flash chromatography (ethyl acetate-hexane, 1:1) yielded the desired product (78%) as a colourless solid. Mp 129-131 °C. IR (KBr, cm<sup>-1</sup>): 3344, 2918, 2850, 1754, 1713, 1558, 1471, 1379, 1228, 1181, 1119, 1076, 1049, 884, 782, 664, 604.  $[\alpha]_{\rm D}$  –13.5 (c 0.22, MeOH). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta$  (ppm) 7.47 (s, 1H, H-Ar), 7.20 (d, 1H, J = 8.7 Hz, NH), 5.24 (dd, 1H,  $J_{1,2} = 9.6$  Hz, H-3), 5.13 (dd, 1H,  $J_{1,2} = 9.6$ Hz, H-4), 4.95 (d, 1H, J = 8.4 Hz, H-1), 4.91 (d, 1H, J = 12.6Hz, OCH<sub>ab</sub>-Ar), 4.81 (d, 1H, J = 12.6 Hz, OCH<sub>ab</sub>-Ar), 4.32 (dd, 1H, J = 10.0, 1.8 Hz, H<sub>ab</sub>-6), 4.29(t, 2H, J = 4.5 Hz, CH<sub>2</sub>-Ar), 4.17 (dd, 1H, J = 10.0, 2.4 Hz,  $H_{ab}$ -6'), 4.15–4.06 (m, 1H, H-2), 3.79–3.73 (m, 1H, H-5), 2.10 (s, 3H, CH<sub>3</sub>), 2.03 (s, 3H, CH<sub>3</sub>), 2.02 (s, 3H, CH<sub>3</sub>), 1.95–1.80 (m, 2H, CH<sub>2</sub>), 1.38–1.16 (m, 22H, 11  $\times$  $CH_2$ ), 0.88 (t, 3H, J = 6.6 Hz,  $CH_3$ ). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz): δ (ppm) 171.07, 170.50, 169.23, 157.90, 157.41, 156.92, 156.42, 144.06, 122.74, 121.37, 117.55, 113.73, 109.91, 99.63, 72.25, 71.72, 68.35, 62.57, 61.91, 54.27, 50.33, 31.74, 30.02, 29.50, 29.43, 29.43, 29.38, 29.21, 29.17, 28.87, 26.34, 22.50, 20.52, 20.33, 20.24, 13.92. HRMS (ESI), m/z calcd for  $C_{31}H_{50}F_3N_4O_9$  679.3530 (M + H)<sup>+</sup>, found 679.3556. Microanalysis calcd for C<sub>31</sub>H<sub>49</sub>F<sub>3</sub>N<sub>4</sub>O<sub>9</sub> (%): C 54.86, H 7.28, N 8.25; found C 54.93, H 7.39, N 8.18.

General procedure for one pot removal of acetate and trifluoroacetamide protective groups. The product of the click reaction (0.16 mmol) was dissolved in dry methanol (2 mL). Sodium methoxide solution in methanol (20  $\mu$ L, 30%) was added and stirring continued for 1 h at room temperature. Solvent was evaporated under reduced pressure and NaOH solution (2 mL, 1 M) was added to the residue. After approximately 1 h a white precipitate was formed. A small volume of methanol (~2 mL) was added to wash unreacted compound from the wall of the flask and stirring was continued for 30 min.

(2*R*,3*S*,4*R*,5*R*,6*R*)-5-Amino-6-((1-(12-(3-oxyl-4,4-dimethyl-2propyloxazolidin-2-yl)dodecyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-2-(hydroxymethyl)tetrahydro-2*H*-pyran-3,4-diol (15a). Reaction was carried out according to the general procedure for one pot removal of acetate and trifluoroacetamide protective groups, starting from compound **14a**. After reaction proceeded to completion, the reaction mixture was adsorbed on silica gel followed by flash chromatography (dichloromethane–methanol–ammonia<sub>(aq)</sub>, 12:2:1), yielding the desired product (77%) as an orange viscous oil. IR (KBr, cm<sup>-1</sup>): 3422, 2926, 2854, 1639, 1458, 1382, 1260, 1081, 1054, 618.  $[\alpha]_D$  –18.8 (*c* 0.19, MeOH). HRMS (ESI), *m/z* calcd for C<sub>29</sub>H<sub>55</sub>N<sub>5</sub>O<sub>7</sub> 585.4101 (M + H)<sup>+</sup>, found 585.4113. Microanalysis calcd for C<sub>29</sub>H<sub>54</sub>N<sub>5</sub>O<sub>7</sub>·MeOH (%): C 58.42, H 9.48, N 11.35; found C 58.31, H 9.82, N 11.70.

(2R, 3S, 4R, 5R, 6R) - 5 - Amino - 2-(hydroxymethyl) - 6 - ((1-tetradecyl-1H-1,2,3-triazol-4-yl)methoxy)tetrahydro-2H-pyran-3,4diol (15b). Reaction was carried out according to the general procedure for one pot removal of acetate and trifluoroacetamide protective groups, starting from compound 14b. After the reaction proceeded to completion, the reaction mixture was adsorbed on silica gel followed by flash chromatography (dichloromethanemethanol-ammonia<sub>(aq)</sub>, 12:2:1) that yielded the desired product (78%) as a colourless solid. Mp 118-120 °C. IR (KBr, cm<sup>-1</sup>): 3360, 2920, 2849, 1609, 1466, 1376, 1224, 1054. [α]<sub>D</sub> 22.6 (*c* 0.24, MeOH). <sup>1</sup>H NMR (Pyr-d<sub>5</sub>, 300 MHz): δ (ppm) 7.60 (s, 1H, H-Ar), 5.35 (d, 1H, J = 12.3 Hz, OCH<sub>ab</sub>-Ar), 5.12 (d, 1H, J = 12.3 Hz, OCH<sub>ab</sub>-Ar), 4.92 (d, 1H, J = 8.6 Hz, H-1), 4.54 (dd, 1H, J = 12.1, 2.4Hz,  $H_{ab}$ -6), 4.38 (dd, 1H, J = 12.1, 5.4 Hz,  $H_{ab}$ -6'), 4.34 (t, 2H, J = 7.2 Hz, CH<sub>2</sub>-Ar), 4.21 (dd, 1H,  $J_{1,2} = 8.6$  Hz, H-4), 4.02 (dd, 1H,  $J_{1,2} = 8.6$  Hz, H-3), 3.96–3.90 (m, 1H, H-5), 3.32 (dd, 1H,  $J_{1,2} = 8.6$  Hz, H-2), 1.84–1.79 (m, 2H, CH<sub>2</sub>), 1.30–1.10 (m, 22H,  $11 \times CH_2$ ), 0.88 (t, 3H, J = 6.3 Hz, CH<sub>3</sub>. <sup>13</sup>C NMR (CD<sub>3</sub>OD, 75 MHz): δ (ppm) 145.45, 125.35, 103.40, 78.32, 77.46, 71.90, 62.88, 58.28, 51.44, 33.09, 31.30, 30.80, 30.77, 30.74, 30.67, 30.57, 30.48, 30.12, 27.53, 23.75, 14.47. HRMS (ESI), m/z calcd for  $C_{23}H_{45}N_4O_5$  457.3390 (M + H)<sup>+</sup>, found 457.3399. Microanalysis calcd for C<sub>23</sub>H<sub>44</sub>N<sub>4</sub>O<sub>5</sub>·0.33H<sub>2</sub>O (%): C 59.71, H 9.73, N 12.11; found C 59.70, H 9.79, N 11.99.

General procedure for reaction with 4-chloro-7-nitrobenzofurazan (NBD-Cl). Free amine (1.0 mmol) and  $K_2CO_3$  (140 mg, 2.5 equiv, 2.5 mmol) were suspended in methanol (3 mL) and the reaction mixture cooled to 0 °C on an ice bath. To the suspension was added in small portions NBD-Cl (360 mg, 1.8 equiv, 1.8 mmol) and the reaction mixture was allowed to reach room temperature while stirring was continued for 12 h. Solvent was evaporated under reduced pressure and the crude product was purified by flash chromatography.

(2R, 3S, 4R, 5R, 6R)-6-((1-(12-(3-Oxyl-4,4-dimethyl-2-propyloxazolidin-2-yl)dodecyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-2-(hydroxymethyl)-5-<math>((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-tetrahydro-2*H*-pyran-3,4-diol (16a). Reaction was carried out according to the general procedure for reaction with NBD-Cl, starting from amine 15a. Flash chromatography (dichloromethane– methanol, 1:0 to 15:1) of the crude product yielded the desired compound (59%) as an orange solid. Mp 71–73 °C. IR (KBr, cm<sup>-1</sup>): 3849, 3741, 2926, 2849, 1699, 1649, 1539, 1075, 826. [ $\alpha$ ]<sub>D</sub> 41.8 (c 0.14, MeOH). HRMS (ESI), m/z calcd for C<sub>35</sub>H<sub>56</sub>N<sub>8</sub>O<sub>10</sub> 748.4114 (M + H)<sup>+</sup>, found 748.4127. Microanalysis calcd for C<sub>35</sub>H<sub>55</sub>N<sub>8</sub>O<sub>10</sub>·MeOH (%): C 55.44, H 7.63, N 14.37; found C 55.12, H 7.51, N 14.11.

(2R,3S,4R,5R,6R)-2-(Hydroxymethyl)-5-((7-nitrobenzo[c][1,2, 5]oxadiazol-4-yl)amino)-6-((1-tetradecyl-1H-1,2,3-triazol-4-yl)methoxy)tetrahydro-2H-pyran-3,4-diol (16b). Reaction was carried out according to the general procedure for reaction with NBD-Cl, starting from amine 15b. Flash chromatography (dichloromethane-methanol, 96:4 to 92:8) of the crude product yielded the desired compound (43%) as an orange solid. Mp 110-112 °C. IR (KBr, cm<sup>-1</sup>): 3392, 2924, 2853, 1585, 1498, 1310, 1075, 1034, 902.  $[\alpha]_{D}$  44.9 (c 0.21, MeOH). <sup>1</sup>H NMR (acetone-d<sub>6</sub>, 300 MHz):  $\delta$  (ppm) 8.47 (d, 1H, J = 9.0 Hz, H-Ar), 8.04 (s, 1H, NH), 7.71 (s, 1H, H-Ar), 6.65 (d, 1H, J = 9.0 Hz, H-Ar), 4.92 (d, 1H, J = 8.1 Hz, H-1), 4.91 (s, 1H, OH), 4.88 (d, 1H, J = 12.9 Hz,  $OCH_{ab}$ -Ar), 4.73 (d, 1H, J = 12.9 Hz,  $OCH_{ab}$ -Ar), 4.59 (s, 1H, OH), 4.27 - 4.22 (m, 2H, CH<sub>2</sub>-Ar), 4.10-3.85 (m, 4H, H-2, H-3,  $H_{ab}$ -6, OH), 3.78 (dd, 1H, J = 10.5, 5.4 Hz,  $H_{ab}$ -6'), 3.58 (dd, 1H,  $J_{1,2} = 8.4$  Hz, H-4), 3.51–3.45 (m, 1H, H-5), 1.79–1.73 (m, 2H,  $CH_2$ ), 1.40–1.15 (m, 22H, 11× $CH_2$ ), 0.88 (t, 3H, J = 6.6 Hz,  $CH_3$ . <sup>13</sup>C NMR (acetone-d<sub>6</sub>, 75 MHz):  $\delta$  (ppm) 147.66, 146.56, 145.97, 145.80, 138.65, 125.04, 124.38, 103.04, 102.56, 78.78, 77.18, 73.11, 64.36, 63.79, 62.59, 51.48, 33.60, 31.37, 31.31, 31.25, 31.13, 31.04, 30.62, 30.61, 28.06, 24.29, 15.33. HRMS (ESI), m/z calcd for  $C_{29}H_{46}N_7O_8$  620.3408 (M + H)<sup>+</sup>, found 620.3394. Microanalysis calcd for C<sub>29</sub>H<sub>45</sub>N<sub>7</sub>O<sub>8</sub>·MeOH (%): C 55.29, H 7.58, N 15.04; found C 55.03, H 7.32, N 15.16.

(2*R*,3*R*,4*S*,5*S*,6*R*) - 2 - ((1 - (12 - (3 - Oxyl - 4,4 - dimethyl - 2-propyloxazolidin-2-yl)dodecyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-6-((2, 2,2-trifluoroacetamido)methyl)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (17a). The reaction between alkyne 13 and azide 3 was carried out according to the general procedure for click reaction. Removal of solvent under reduced pressure and purification of the crude product by flash chromatography (ethyl acetate–hexane, 2 : 3 to 3 : 2) yielded the desired product (63%) as an orange viscous oil. IR (KBr, cm<sup>-1</sup>): 3422, 2932, 2857, 1752, 1560, 1370, 1222, 1159, 1058, 905, 727. [*α*]<sub>D</sub> –6.8 (*c* 0.18, MeOH). HRMS (ESI), *m/z* calcd for C<sub>37</sub>H<sub>60</sub>F<sub>3</sub>N<sub>5</sub>O<sub>11</sub> 807.4241 (M + H)<sup>+</sup>, found 807.4258. Microanalysis calcd for C<sub>37</sub>H<sub>59</sub>F<sub>3</sub>N<sub>5</sub>O<sub>11</sub> (%): C 55.08, H 7.37, N 8.68; found C 54.80, H 7.18, N 8.44.

(2R,3R,4S,5S,6R)-2-((1-Tetradecyl-1H-1,2,3-triazol-4-yl)methoxy)-6-((2,2,2-trifluoroacetamido)methyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (17b). The reaction between alkyne 13 and azide 5 was carried out according to general procedure for click reaction. Removal of solvent under reduced pressure and purification of the crude product by flash chromatography (ethyl acetate-hexane, 1:1) yielded the desired product (90%) as a colourless viscous oil. IR (KBr, cm<sup>-1</sup>): 3422, 2927, 2856, 1752, 1568, 1458, 1375, 1223, 1159, 1074, 905, 726.  $[\alpha]_D$  5.1 (c 0.24, MeOH). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta$  (ppm) 7.94 (t, 1H, J = 5.1 Hz, NH) 7.52 (s, 1H, H-Ar), 5.33 (dd, 1H, J = 3.3, 0.6 Hz, H-4), 5.23 (dd, 1H, J = 10.3, 8.0 Hz, H-2), 5.01 (dd, 1H, J = 10.3, 3.3 Hz, H-3), 4.90 (d, 1H, J = 12.9 Hz, OCH<sub>ab</sub>-Ar), 4.82 (d, 1H, J = $12.9 \text{ Hz}, \text{OCH}_{ab}\text{-Ar}$ , 4.58 (d, 1H, J = 8.0 Hz, H-1), 4.34 (t, 2H, J = 8.0 Hz)7.2 Hz, CH<sub>2</sub>-Ar), 3.84-3.79 (m, 1H, H-5), 3.63-3.54 (m, 1H, H-6), 3.45–3.38 (m, 1H, H-6'), 2.16 (s, 3H, CH<sub>3</sub>), 2.02 (s, 3H, CH<sub>3</sub>), 1.98 (s, 3H, CH<sub>3</sub>), 1.94–1.86 (m, 2H, CH<sub>2</sub>), 1.35–1.22 (m, 22H,  $11 \times CH_2$ , 0.88 (t, 3H, J = 6.6 Hz, CH<sub>3</sub>). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz): δ (ppm) 170.49, 769.87, 169.46, 158.40, 157.91, 157.41, 156.92, 143.51, 122.97, 121.48, 171.67, 113.86, 110.05, 100.26, 70.92, 70.677, 68.78, 67.98, 62.37, 50.37, 39.57, 31.80, 30.15, 29.55,

29.52, 29.48, 29.41, 29.28, 29.22, 28.88, 26.40, 22.56, 20.61, 20.49, 20.40, 13.38. HRMS (ESI), m/z calcd for  $C_{31}H_{50}F_3N_4O_9$  679.3530 (M + H)<sup>+</sup>, found 679.3525.

(2*R*,3*S*,4*S*,5*R*,6*R*)-2-(aminomethyl)-6-((1-(12-(3-oxyl-4,4dimethyl-2-propyloxazolidin-2-yl)dodecyl)-1*H*-1,2,3-triazol-4-yl)methoxy)tetrahydro-2*H*-pyran-3,4,5-triol (18a). Reaction was carried out according to the general procedure for one pot removal of acetate and trifluoroacetamide protective groups starting from compound 17a. After reaction proceeded to completion, the reaction mixture was adsorbed on silica gel followed by flash chromatography (dichloromethane–methanol–ammonia<sub>(aq)</sub>, 8:2:1), yielding the desired product (31%) as an orange viscous oil. IR (KBr, cm<sup>-1</sup>): 3431, 2927, 2854, 1672, 1456, 1378, 1204, 1135, 1062. [ $\alpha$ ]<sub>D</sub> 1.2 (*c* 0.14, MeOH). HRMS (ESI), *m/z* calcd for C<sub>29</sub>H<sub>55</sub>N<sub>5</sub>O<sub>7</sub> 585.4101 (M + H)<sup>+</sup>, found 585.4108. Microanalysis calcd for C<sub>29</sub>H<sub>54</sub>N<sub>5</sub>O<sub>7</sub>·2H<sub>2</sub>O (%): C 56.11, H 9.42, N 11.28; found C 56.22, H 9.12, N 11.01.

(2R,3S,4S,5R,6R)-2-(Aminomethyl)-6-((1-tetradecyl-1H-1,2,3-triazol-4-yl)methoxy)tetrahydro-2*H*-pyran-3,4,5-triol (18b). Reaction was carried out according to the general procedure for one pot removal of acetate and trifluoroacetamide protective groups starting from compound 17b. After reaction proceeded to completion, the reaction mixture was adsorbed on silica gel followed by flash chromatography (dichloromethane-methanol- $\operatorname{ammonia}_{(aq)}, 8:2:1$ ), yielding the desired product (53%) as a white solid. Mp 109-112 °C. IR (KBr, cm<sup>-1</sup>): 3426, 2922, 2853, 1606, 1340, 1064.  $[\alpha]_{\rm D}$  -4.7 (c 0.28, MeOH). <sup>1</sup>H NMR (Pyr-d<sub>5</sub>, 300 MHz):  $\delta$  (ppm) 8.12 (s, 1H, H-Ar), 5.39 (d, 1H, J = 12.0 Hz,  $OCH_{ab}$ -Ar), 5.19 (d, 1H, J = 12.0 Hz,  $OCH_{ab}$ -Ar), 4.98 (d, 1H, J = 8.0 Hz, H-1), 4.49 (dd, 1H, J = 9.3, 8.0 Hz, H-2), 4.40 (dd, 1H, J = 3.1 Hz, H-4), 4.32 (t, 2H, J = 7.2 Hz, CH<sub>2</sub>-Ar), 4.13 (dd, 1H, J = 9.3, 3.1 Hz, H-3), 3.97–3.87 (m, 1H, H-5), 3.81–3.24 (m, 2H, H-6), 1.89–1.71 (m, 2H, CH<sub>2</sub>), 1.40–1.07 (m, 22H, 11×CH<sub>2</sub>), 0.88 (t, 3H, J = 6.0 Hz, CH<sub>3</sub>). <sup>13</sup>C NMR (Pyr-d<sub>5</sub>, 75 MHz):  $\delta$  (ppm) 146.90, 125.67, 106.04, 76.82, 73.89, 72.62, 64.56, 51.86, 44.93, 33.79, 32.21, 31.61, 31.58, 31.51, 31.35, 31.27, 30.94, 28.39, 24.60, 15.95. HRMS (ESI), m/z calcd for  $C_{23}H_{45}N_4O_5$  457.3390 (M + H)<sup>+</sup>, found 457.3382. Microanalysis calcd for C<sub>23</sub>H<sub>44</sub>N<sub>4</sub>O<sub>5</sub>·2H<sub>2</sub>O (%): C 56.07, H 9.82, N 11.37; found C 56.09, H 9.80, N 11.16.

(2R, 3R, 4S, 5R, 6R)-2-((1-(12-(3-Oxyl-4, 4-dimethyl-2-propyloxazolidin-2-yl)dodecyl)-1*H*-1,2,3-triazol-4-yl)methoxy)-6-(((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)methyl)tetrahydro-2*H*-pyran-3,4,5-triol (19a). Reaction was carried out according to the general procedure for reaction with NBD-Cl, starting from amine 18a. Flash chromatography (dichloromethane–methanol, 96:4 to 92:8) of the crude product yielded the desired compound (13%) as an orange solid. Mp 80–82 °C. IR (KBr, cm<sup>-1</sup>): 3422, 2931, 2857, 1752, 1560, 1458, 1370, 1222, 1159, 1058, 905, 827, 726. [ $\alpha$ ]<sub>D</sub> 28.4 (*c* 0.31, MeOH). HRMS (ESI), *m/z* calcd for C<sub>35</sub>H<sub>56</sub>N<sub>8</sub>O<sub>10</sub> 748.4119 (M + H)<sup>+</sup>, found 748.4141. Microanalysis calcd for C<sub>35</sub>H<sub>55</sub>N<sub>8</sub>O<sub>10</sub> (%): C 56.21, H 7.41, N 14.98; found C 56.50, H 7.64, N 15.37.

(2*R*,3*R*,4*S*,5*R*,6*R*)-2-(((7-Nitrobenzo]c][1,2,5]oxadiazol-4-yl)amino)methyl) - 6 - ((1 - tetradecyl - 1*H* - 1, 2, 3 - triazol - 4 - yl)methoxy)tetrahydro-2*H*-pyran-3,4,5-triol (19b). Reaction was carried out according to the general procedure for reaction with NBD-Cl, starting from amine 18b. Flash chromatography (dichloromethane-methanol, 96:4 to 92:8) of the crude product yielded the desired compound (24%) as an orange solid. Mp 99-101 °C. IR (KBr, cm<sup>-1</sup>): 3392, 2926, 28.57, 2094, 1593, 1305, 1037, 824.  $[\alpha]_{D}$  44.2 (*c* 0.095, MeOH).<sup>1</sup>H NMR (acetone-d<sub>6</sub>, 300 MHz):  $\delta$  (ppm) 8.55 (d, 1H, J = 8.8 Hz, H-Ar), 7.88 (s, 1H, H-Ar), 6.61 (d, 1H, J = 8.8 Hz, H-Ar), 4.83 (d, 1H, J = 12.3 Hz, OCH<sub>ab</sub>-Ar), 4.67 (d, 1H, J = 12.3 Hz, OCH<sub>ab</sub>-Ar), 4.40 (d, 1H, J = 7.2Hz, H-1), 4.35 (t, 2H, J = 7.2 Hz, CH<sub>2</sub>-Ar), 4.05–3.97 (m, 3H, H-5, H-6), 3.98 (dd, 1H, J = 2.9 Hz, H-4), 3.64–3.57 (m, 1H, H-2), 3.56 (dd, 1H, J = 9.3, 2.9 Hz. H-3), 1.88–1.81 (m, 2H, CH<sub>2</sub>), 1.40–1.00 (m, 22H, 11 × CH<sub>2</sub>), 0.86 (t, 3H, J = 6.9 Hz, CH<sub>3</sub>). <sup>13</sup>C NMR (Acetone-d<sub>6</sub>, 75 MHz): δ (ppm) 146.45, 146.11, 145.96, 138.92, 125.18, 124.17, 104.51, 101.27, 75.26, 74.54, 72.74, 71.34, 63.52, 51.63, 46.63, 33.54, 31.92, 31.30, 31.29, 31.26, 31.21, 30.97, 30.66, 28.07, 24.23, 15.29. HRMS (ESI), m/z calcd for C<sub>29</sub>H<sub>46</sub>N<sub>7</sub>O<sub>8</sub> 620.3408 (M + H)<sup>+</sup>, found 620.3431. Microanalysis calcd for C<sub>29</sub>H<sub>45</sub>N<sub>7</sub>O<sub>10</sub>·0.66MeOH (%): C 55.58, H 7.49, N 15.29; found C 55.38, H 7.43, N 15.32.

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### Notes and references

- 1 K. Jacobson, O. G. Mouritsen and R. G. W. Anderson, Nat. Cell Biol., 2007, 9, 7–14.
- 2 K. Simons and D. Toomre, Nat. Rev. Mol. Cell Biol., 2000, 1, 31-39.
- 3 S. Manes, G. del Real and A. C. Martinez, *Nat. Rev. Immunol.*, 2003, 3, 557–568.
- 4 J. S. Wadia, M. Schaller, R. A. Williamson and S. F. Dowdy, *PLoS One*, 2008, **3**, e3314.
- 5 H. Cheng, K. S. Vetrivel, P. Gong, X. Meckler, A. Parent and G. Thinakaran, *Nat Clin Pract Neurol*, 2007, **3**, 374–382.
- 6 J. Oshikawa, N. Urao, H. W. Kim, N. Kaplan, M. Razvi, R. McKinney, L. B. Poole, T. Fukai and M. Ushio-Fukai, *PLoS One*, 2010, 5, e10189.
- 7 D. Lingwood and K. Simons, Science, 2010, 327, 46-50.
- 8 H. T. He and D. Marguet, EMBO Rep., 2008, 9, 525-530.
- 9 B. Dzikovski, J. H. Freed and T. P. Begley, *Membrane Fluidity*, John Wiley & Sons, Inc., 2007.
- 10 G. I. Likhtenshtein, J. Yamauchi, S. Nakatsuji, A. I. Smirnov and R. Tamura, *Nitroxide Spin Probes for Studies of Molecular Dynamics and Microstructure*, Wiley-VCH Verlag GmbH & Co. KGaA, 2008.
- 11 M. A. Hemminga and L. J. Berliner, ESR spectroscopy in membrane biophysics, Springer, New York, 2007.
- 12 G. van Meer, D. R. Voelker and G. W. Feigenson, Nat. Rev. Mol. Cell Biol., 2008, 9, 112–124.
- 13 T. Pomorski, P. Muller, B. Zimmermann, K. Burger, P. F. Devaux and A. Herrmann, J. Cell Sci., 1996, 109, 687–698.
- 14 A. Gidwani, D. Holowka and B. Baird, *Biochemistry*, 2001, 40, 12422– 12429.
- 15 M. Ge, A. Gidwani, H. A. Brown, D. Holowka, B. Baird and J. H. Freed, *Biophys. J.*, 2003, 85, 1278–1288.
- 16 J. R. Lakowicz, *Principles of fluorescence spectroscopy*, 3rd edn, Springer, New York, 2006, pp. 278–351.
- 17 N. V. Blough and D. J. Simpson, J. Am. Chem. Soc., 1988, 110, 1915– 1917.
- 18 V. Y. Nagy, I. y. M. Bystryak, A. I. Kotel'nikov, G. I. Likhtenshtein, O. M. Petrukhin, Y. A. Zolotov and L. B. Volodarskii, *Analyst*, 1990, **115**, 839–841.
- 19 J. P. Blinco, J. C. McMurtrie and S. E. Bottle, *Eur. J. Org. Chem.*, 2007, 28, 4638–4641.
- 20 C. Coenjarts, O. García, L. Llauger, J. Palfreyman, A. L. Vinette and J. C. Scaiano, J. Am. Chem. Soc., 2002, 125, 620–621.

- 22 B. Miljevic, K. E. Fairfull-Smith, S. E. Bottle and Z. D. Ristovski, *Atmos. Environ.*, 2010, 44, 2224–2230.
- 23 B. J. Morrow, D. J. Keddie, N. Gueven, M. F. Lavin and S. E. Bottle, *Free Radical Biol. Med.*, 2010, **49**, 67–76.
- 24 P. Meineke, U. Rauen, H. de Groot, H. G. Korth and R. Sustmann, *Chem.-Eur. J.*, 1999, 5, 1738–1747.
- 25 P. M. Düppe, P. M. Talbierski, F. S. Hornig, U. Rauen, H. G. Korth, T. Wille, R. Boese, T. Omlor, H. de Groot and R. Sustmann, *Chem.-Eur. J.*, 2010, **16**, 11121–11132.
- 26 É. Hideg, T. Kálai, P. B. Kós, K. Asada and K. Hideg, *Photochem. Photobiol.*, 2006, 82, 1211–1218.
- 27 P. Bilski, K. Hideg, T. Kálai, M. A. Bilska and C. F. Chignell, *Free Radical Biol. Med.*, 2003, **34**, 489–495.
- 28 G. G. Borisenko, I. Martin, Q. Zhao, A. A. Amoscato and V. E. Kagan, J. Am. Chem. Soc., 2004, 126, 9221–9232.
- 29 N. Medvedeva, V. V. Martin, A. L. Weis and G. I. Likhtenshten, J. Photochem. Photobiol., A, 2004, 163, 45–51.
- 30 X.-F. Yang and X.-Q. Guo, Anal. Chim. Acta, 2001, 434, 169-177.
- 31 C. Aliaga, J. M. Juárez-Ruiz, J. C. Scaiano and A. Aspée, Org. Lett., 2008, 10, 2147–2150.
- 32 Y. Tang, F. He, M. Yu, S. Wang, Y. Li and D. Zhu, *Chem. Mater.*, 2006, 18, 3605–3610.
- 33 E. Lozinsky, V. V. Martin, T. A. Berezina, A. I. Shames, A. L. Weis and G. I. Likhtenshtein, J. Biochem. Biophys. Methods, 1999, 38, 29– 42.
- 34 C. Tansakul, E. Lilie, E. D. Walter, F. Rivera, A. Wolcott, J. Z. Zhang, G. L. Millhauser and R. Braslau, J. Phys. Chem. C Nanomater Interfaces, 2010, 114, 7793–7805.
- 35 W. Chen, X. Wang, X. Tu, D. Pei, Y. Zhao and X. Guo, *Small*, 2008, 4, 759–764.

- 36 J. Mravljak, J. Konc, M. Hodoscek, T. Solmajer and S. Pecar, J. Phys. Chem. B, 2006, 110, 25559–25561.
- 37 H. C. Kolb, M. G. Finn and K. B. Sharpless, Angew. Chem., Int. Ed., 2001, 40, 2004–2021.
- 38 S. Pecar, B. Sorg, M. Schara and E. Hecker, *Chem. Phys. Lipids*, 1984, 35, 151–159.
- 39 J. Mravljak and S. Pecar, Synth. Commun., 2004, 34, 3763-3771.
- 40 S. Amel Diab, A. Hienzch, C. Lebargy, S. Guillarme, E. Pfund and T. Lequeux, Org. Biomol. Chem., 2009, 7, 4481–4490.
- 41 L. Yu, X. Li, J. Dong, X. Zhang, Z. Guo and X. Liang, Anal. Methods, 2010, 2, 1667–1670.
- 42 H. Myszka, D. Bednarczyk, M. Najder and W. Kaca, *Carbohydr. Res.*, 2003, 338, 133–141.
- 43 H. N. Lin and C. T. Walsh, J. Am. Chem. Soc., 2004, 126, 13998– 14003.
- 44 V. L. Campo, I. Carvalho, C. H. T. P. Da Silva, S. Schenkman, L. Hill, S. A. Nepogodiev and R. A. Field, *Chem. Sci.*, 2010, 1, 507–514.
- 45 O. Monasson, M. Ginisty, J. Mravljak, G. Bertho, C. Gravier-Pelletier and Y. Le Merrer, *Tetrahedron: Asymmetry*, 2009, 20, 2320–2330.
- 46 H.-S. Oh, R. Xuan and H.-Y. Kang, Org. Biomol. Chem., 2009, 7, 4458–4463.
- 47 R. Fauré, T. C. Shiao, D. Lagnoux, D. Giguère and R. Roy, Org. Biomol. Chem., 2007, 5, 2704–2708.
- 48 C. W. Tornoe, C. Christensen and M. Meldal, J. Org. Chem., 2002, 67, 3057–3064.
- 49 A. Babic, S. Gobec, C. Gravier-Pelletier, Y. Le Merrer and S. Pecar, *Tetrahedron*, 2008, 64, 9093–9100.
- 50 A. Makino, K. Kurosaki, M. Ohmae and S. Kobayashi, *Biomacro-molecules*, 2006, 7, 950–957.
- 51 R. A. Vishwakarma, S. Vehring, A. Mehta, A. Sinha, T. Pomorski, A. Herrmann and A. K. Menon, Org. Biomol. Chem., 2005, 3, 1275–1283.