Design, synthesis and biological evaluation of carbohydrate-functionalized cyclodextrins and liposomes for hepatocyte-specific targeting†

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Targeting glycan-binding receptors is an attractive strategy for cell-specific drug and gene delivery. The C-type lectin asialoglycoprotein receptor (ASGPR) is particularly suitable for liver-specific delivery due to its exclusive expression by parenchymal hepatocytes. In this study, we designed and developed an efficient synthesis of carbohydrate-functionalized β-cyclodextrins (βCDs) and liposomes for hepatocyte-specific delivery. For targeting of ASGPR, rhodamine B-loaded βCDs were functionalized with glycodendrimers. Liposomes were equipped with synthetic glycolipids containing a terminal D-GalNAc residue to mediate binding to ASGPR. Uptake studies in the human hepatocellular carcinoma cell line HepG2 demonstrated that βCDs and liposomes displaying terminal D-Gal/D-GalNAc residues were preferentially endocytosed. In contrast, uptake of βCDs and liposomes with terminal D-Man or D-GlcNAc residues was markedly reduced. The D-Gal/D-GalNAc-functionalized βCDs and liposomes presented here enable hepatocyte-specific targeting. Gal-functionalized βCDs are efficient molecular carriers to deliver doxorubicin *in vitro* into hepatocytes and induce apoptosis.

Introduction

Carbohydrate-based therapies hold great promise and glycan-binding receptors have been targeted for cell-specific drug and gene delivery.[1–3] Specific binding of isolated and synthetic carbohydrates to endogeneous lectins on cell surfaces resulted in cell-specific binding and uptake.[4] However, due to the relatively low affinity of proteins to their carbohydrate ligands multivalent interactions are often required to exert biological effects. Recent reports showed that lectins can be targeted by either multivalent or chemically-modified ligands.[5–7] Different strategies have been employed to achieve multivalency such as the use of glyconanoparticles functionalized with sialyl-Lewis^x for selectin targeting,[8] polymers displaying multiple sugar residues[9] or cross-linked carbohydrate antigens[10] for siglec CD22 targeting, and glycodendrimers for high-affinity binding to the C-type lectin DC-SIGN.[11] Glycodendrimers represent an excellent tool to exploit multivalency for targeting purposes,[12,13] but degradable dendrimers have also been used in the field of controlled drug delivery systems.[14–16]

The asialoglycoprotein receptor (ASGPR) is a C-type lectin that is a particularly attractive target for liver-specific drug delivery since it is expressed exclusively on parenchymal hepatocytes.[17] The main function of ASGPR is to maintain homeostasis of serum glycoprotein levels by binding and uptake of desialylated (galactosyl-terminated) glycoproteins.[18] Human ASGPR consists of two subunits, H1 and H2. The H1 subunit mediates Ca^{2+}-dependent D-Gal/D-GalNAc recognition whereas the H2 subunit is responsible for functional configuration.[19] Recent studies describe the use of natural ASGPR ligands or synthetic ligands with galactosylated cholesterol, glycolipids, or polymers.[20–22] Specific targeting of hepatocytes has enabled gene delivery,[23] for example using galactosylated DNA lipid nanocapsules as the vector.[24] Binding of D-Gal and D-GalNAc to ASGPR strongly depends on the valency of the ligands with a binding hierarchy increasing from monoantennary to tetraantennary ligands.[25] It is also known that ASGPR exhibits a 10–50 fold higher affinity to D-GalNAc compared to D-Gal residues.[26] Furthermore, the distance between the terminal D-Gal/D-GalNAc residues is important for optimal spatial orientation resulting in maximal binding to the heterooligomeric ASGPR.[27]

We previously reported the use of carbohydrate-capped quantum dots (QDs) for selective targeting of ASGPR on hepatocytes *in vitro* and *in vivo*.[28] Intravenous injection of D-Gal/D-galactosamine-functionalized QDs into mice led to specific sequestration in the liver indicating that specific targeting of hepatocytes by multivalent Gal-terminated ligands is possible. While QDs represent an excellent tool for imaging purposes due to high quantum yield and non-bleachable fluorescence, they are not suitable for targeted drug and gene delivery due to their inherent toxicity. Herein, we report the design and synthesis of β-cyclodextrins (βCDs) functionalized with Gal dendrimers and liposomes that displayed D-GalNAc-terminated lipids to target ASGPR by exploiting multivalent D-Gal/D-GalNAc interactions. Both βCDs and glycoliposomes were equipped with a fluorescent dye to enable detection of hepatocyte binding and uptake *in vitro*. Our results indicate that the constructed βCDs and glycoliposomes displaying multiple D-Gal/D-GalNAc residues for

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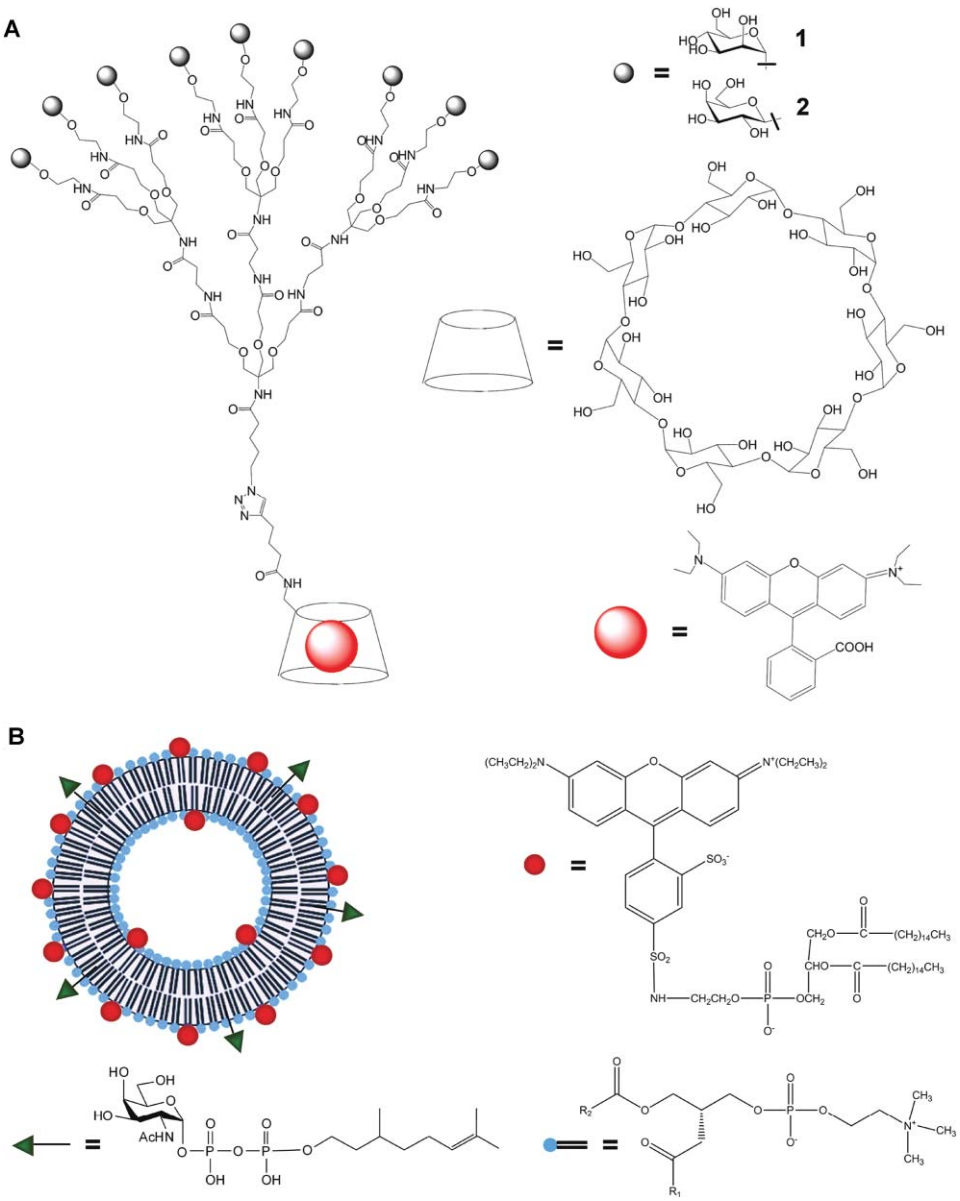


Fig. 1 Schematic representation of the delivery systems used in this study. A, βCD glycodendrimer consisting of βCD (cylinder), the encaged fluorescent dye rhodamine B (red ball), and terminal D-Man **1** or D-Gal **2** residues. B, Glycoliposomes consisting of L-α-phosphatidylcholine (blue), the fluorescent lipid rhodamine-DHPE (red ball), and synthetic glycolipids containing either a terminal GalNAc or GlcNAc residue (green arrow).

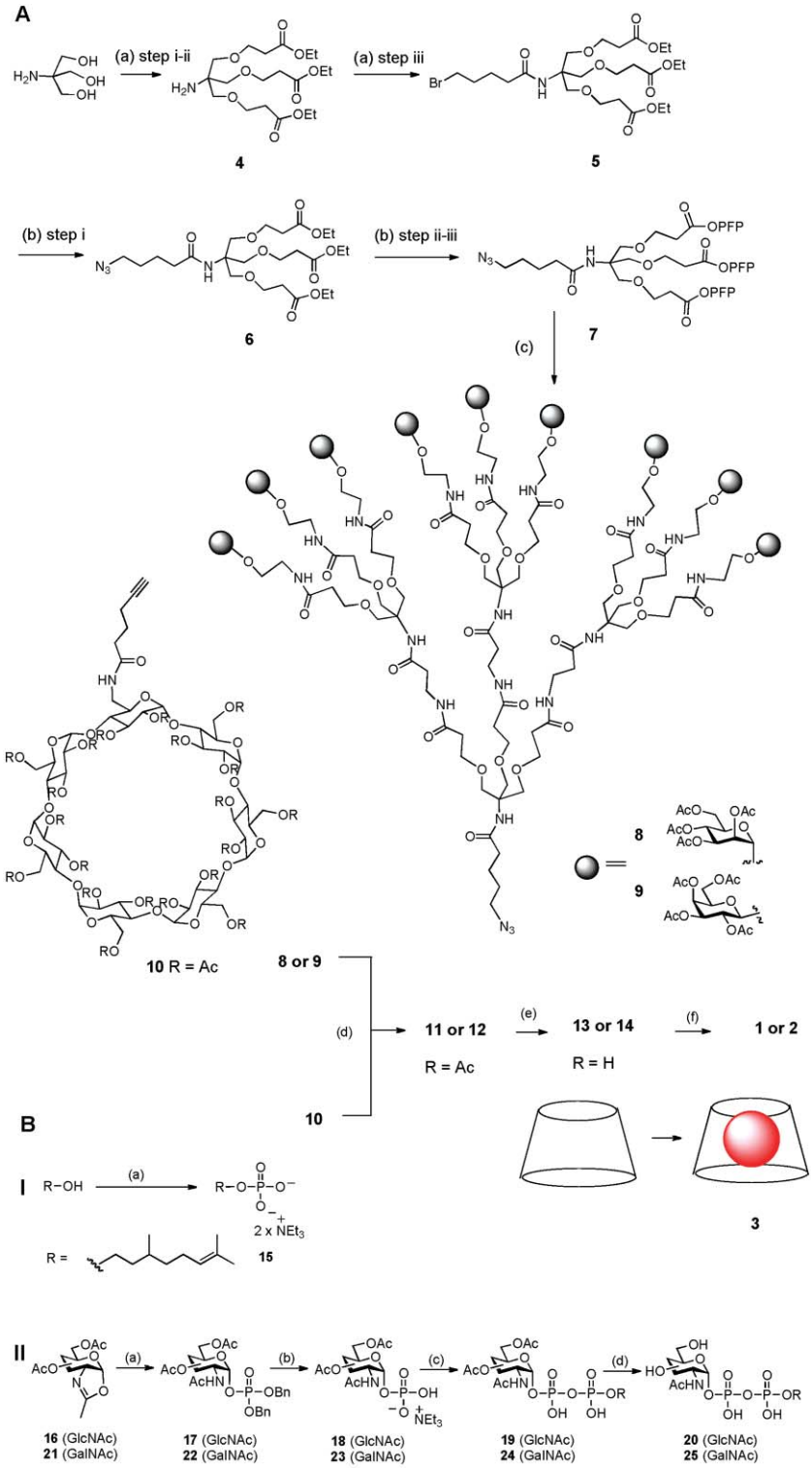
multivalent binding to ASGPR are suitable hepatocyte-specific targeting systems.

Results and discussion

The delivery systems evaluated in this study are shown in Fig. 1. We used (1) a β-cyclodextrin (βCD)- and (2) liposome-based systems where drugs or siRNAs may be encaged, and (2) a liposome-based system. For detection of βCD uptake into HepG2 cells, a βCD inclusion complex equipped with the fluorescent dye rhodamine B (RhB) was assembled. Previous studies indicate that the formation of a 1 : 1 inclusion complex of RhD with βCD may be suitable for delivery strategies such as the one proposed here.[29] Additionally, βCDs were equipped with a glycodendrimer displaying either D-Gal residues to induce ASGPR-mediated endocytosis into HepG2 cells or D-Man as control (Fig. 1A). For the liposome-based delivery system, a standard method for preparation of liposomes was employed.[30] Liposomes were composed of L-α-phosphatidylcholine and the integration of the fluorescent lipid rhodamine-DHPE into the liposome membrane made the detection of liposome uptake into HepG2 cells possible. For hepatocyte-specific delivery glycolipids displaying a terminal D-GalNAc residue (for targeting ASGPR) or D-GlcNAc (specificity control) were synthesized (Fig. 1B).

Design and synthesis of targeting systems

Glycodendrimer-functionalized βCDs were prepared using Cu(I)-catalyzed [2+3] cycloaddition between tripodal sugar azides and a βCD bearing an alkyne reactive moiety (Scheme 1A).[31,32]



Scheme 1 Schematic representation of βCD glycodendrimer and glycolipid synthesis. A, Synthesis of rhodamine B-labeled βCD **3** and its functionalization with glycodendrimers. Reagents and conditions: (a) (i) acrylonitrile/NaOH (40%); (ii) conc. HCl/EtOH, 51%; (iii) 5-bromovaleric acid, DIC, HOBT, DCM, 69%; (b) (i) NaN_3, DMF, 72%; (ii) NaOH/MeOH; (iii) pentafluorophenol, DIC, HOBT, DCM, 86% (2 steps); (c) tripod-Man or tripod-Gal, DCM, TEA, 52–59%; (d) compound **8** or **9**, $CuSO_4$, ascorbic acid, THF–H_2O (1 : 1), 82–84%; (e) NaOMe/MeOH 77–78%; (f) compound **11** or **12** or βCD, rhodamine B, H_2O. B, Synthesis of glycolipids **20** and **25**. Reagents and conditions: (I) (a) $POCl_3$, hexane, Et_3N, 2 h, RT; then acetone : H_2O : Et_3N (85 : 10 : 5), overnight, 80% (2 steps). (II) (a) Dibenzylphosphate, 1,2-dichloroethane, 41–69%; (b) H_2, Pd/C, MeOH, TEA, 88–100%; (c) 1,1′-carbonyldiimidazole (CDI), **15**, DMF–MeOH, 72 h, 40–66%; (d) NaOMe, DCM–MeOH, 30 min, 100%.

Tripodal sugar-azides were prepared by coupling tris(hydroxylmethyl)aminomethane with acrylonitrile, followed by treatment with HCl in ethanol and amide bond formation with 5-bromovaleric acid yielded 69% of **5**. Bromide substitution with azide, saponification and further esterification with pentafluorophenol afforded **7** in 86% yield. The pentafluorophenol ester **7** was further reacted with tripod-Man or tripod-Gal to obtain azide dendrons **8** or **9** (Scheme 1, ESI†). Dendrons **8**, **9** were coupled with stoichiometric amounts of alkynyl substituted βCD **10** by Cu(I)-catalyzed [2+3] cycloaddition and deacetylated to yield 77–78% of dendrimer **13** or **14** respectively (Scheme 2, ESI†). Finally, functionalized βCD-inclusion complexes **1–3** of dendrimers **13**, **14** and βCD with rhodamine B (RhB) were prepared (Scheme 3, ESI†) and used for uptake studies in HepG2 cells. Inclusion complexation of RhB and dendrimer (**13**, **14** and βCD) was verified by ^{1}H NOESY experiments (see Fig. 22–24 in the ESI†). An equimolar mixture of dendrimer **13** and RhB displayed a clear nuclear Overhauser effect (NOE) correction between the –CH proton of βCD and methyl protons of the diethylamino groups of RhB and the aromatic protein of the diethylaminophenyl group of RhB. Similar results were also observed with **2** and **3**. Inclusion of RhB and **13** showed a slight increase in quantum yield, indicating the encapsulation of the fluorescent probe by the dendrimer.

Glycolipids **20** and **25** were chosen for incorporation into liposomes and the synthetic approach selected for their synthesis is shown in Fig. 2B. To increase targeting specificity, glycolipids were equipped with terminal D-GalNAc residues since ASGPR displays a preference for D-GalNAc over D-Gal.[26] Sugar oxazolines **16** and **21** were synthesized according to literature procedures[33,34] and were converted to the corresponding α-glycosyl dibenzylphosphate derivatives **17** and **22** in moderate yield (**17**: 41%, **22**: 69%). Hydrogenolytic deprotection produced the corresponding α-glycosyl phosphates **18** and **23** in excellent yields. Lipid monophosphate **15**, that was generated by treating citronellol with phosphorus oxychloride was activated using 1,1′-carbonyldiimidazole (CDI) and subsequently coupled to the α-glycosyl phosphates **18** and **23** to produce **19** and **24** in moderate yields (**19**: 40%, **24**: 66%).[35] Finally, removal of the acetate groups by treatment with sodium methoxide in a mixture of DCM–methanol[36] yielded the desired deprotected α-diphosphate glycolipids **20** and **25**. We chose citronellol, a short, saturated isoprenol lipid, due to difficulties in handling long lipid chains and instability of allylic pyrophosphates. Glycoliposomes were prepared by dissolving L-α-phosphatidylcholine, the fluorescent lipid rhodamine-DHPE and glycolipid **20** (terminal D-GlcNAc, control) or glycolipid **25** (terminal D-GalNAc) in ethanol, evaporating the ethanol and dissolving the lipid film in PBS buffer. To ensure that the liposomes were comparable in shape and size, liposomes were extruded through a 100 nm polycarbonate membrane. TEM measurements of the liposome preparations were performed after staining them with an aqueous solution of uranyl acetate and indicated that the liposomes had a size of about 100 nm or less, spherical shape and exhibited a high degree of monodispersity (Fig. 2B).

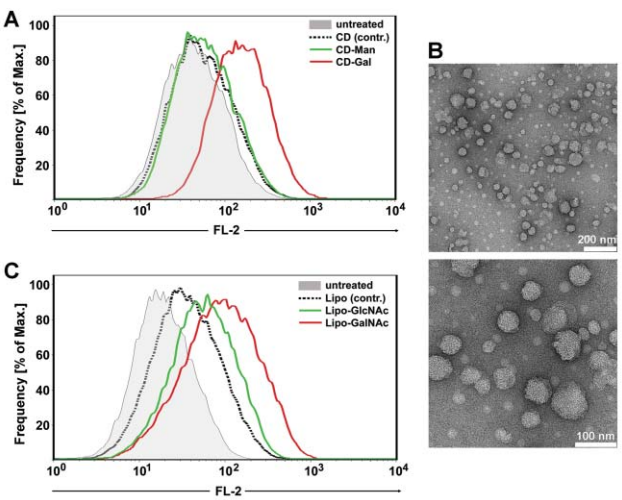


Fig. 2 Targeting of the hepatocellular carcinoma cell line HepG2 with βCD glycodendrimers and glycoliposomes. A, HepG2 cells were left untreated (grey area), incubated with 250 μmol rhodamine B-labeled βCD (dotted line, negative control) or labeled βCD glycodendrimers containing terminal D-Man (green line, specificity control) or D-Gal residues (red line). Uptake was measured by flow cytometry. Specific uptake of βCD displaying a D-Gal dendrimer by HepG2 cells was observed. B, Typical TEM images of glycoliposomes used in this study. C, HepG2 cells were left untreated (gray area), incubated with rhodamine-DHPE-labeled liposomes (dotted line, negative control) or labeled liposomes containing glycolipids with terminal D-GlcNAc (green line, specificity control) or D-GalNAc residues (red line). Uptake was measured by flow cytometry. Preferential uptake of liposomes functionalized with the D-GalNAc glycolipid was observed.

Hepatocyte-specificity studies

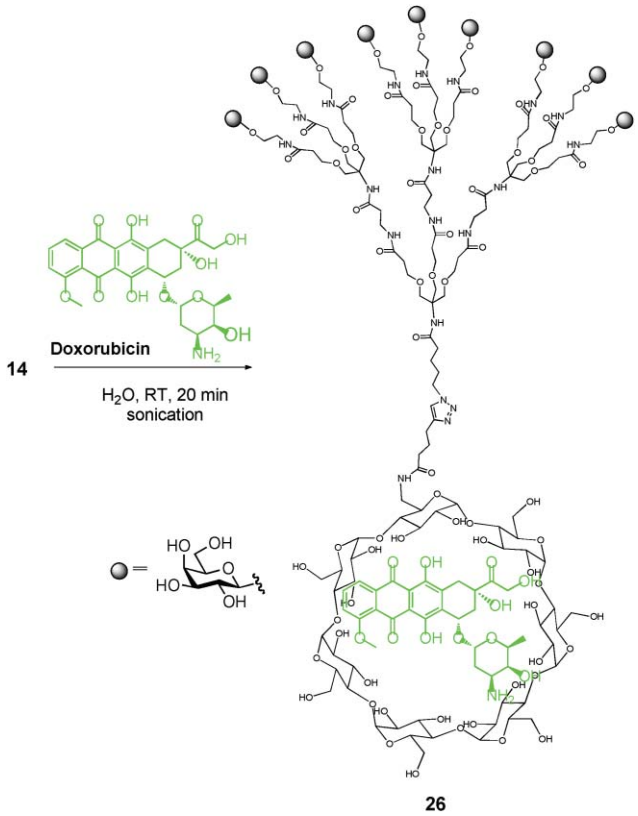
To analyze ASGPR-mediated uptake of βCD functionalized with glycodendrimers, the hepatocellular carcinoma cell line HepG2 was incubated with **1**, **2** and **3**. The HepG2 cell line has been previously employed to investigate ASGPR-mediated uptake of galactosylated polymers and liposomes.[37–39] Uptake by HepG2 cells was analyzed by flow cytometry to enable a quantitative analysis of targeting specificity. As expected, the incubation of HepG2 cells with non-functionalized rhodamine B-labeled βCD did not induce uptake (Fig. 2A). βCD displaying a Man dendron was used as a specificity control since ASGPR exhibits specificity for galactosyl-terminated structures. Indeed, only marginal unspecific uptake of Man-functionalized βCD was observed (Fig. 2A). In contrast, functionalization of βCD with Gal dendrons dramatically increased the uptake by HepG2 cells indicating receptor-mediated endocytosis. Recently, we have targeted hepatocytes *in vitro* and *in vivo* through ASGPR-mediated uptake of D-Gal-capped quantum dots.[28] Knock-down of ASGPR by siRNA led to a reduction in D-Gal QDs uptake indicating specificity of the hepatocyte targeting. While the use of QDs as drug/gene delivery system is limited, βCD have been synthesized and successfully used as carriers.[40–44]

Glycoliposomes were assembled from L-α-phosphatidylcholine, the respective glycolipid **20** or **25** and rhodamine-DHPE. D-GlcNAc-functionalized liposomes were used as specificity control. In contrast to the βCD glycodendrimers where almost no unspecific uptake was observed, background fusion of control liposomes with HepG2 cells was detected to a limited extent (Fig. 2C). However, preferential uptake of D-GalNAc-capped liposomes was observed again indicating specificity of D-GalNAc-mediated hepatocyte targeting. Liposome-based delivery systems

are widely used since liposomes can be loaded with drugs or siRNAs and thus may be used as efficient carrier systems. Consistently, recent studies have focused on liposomal delivery systems for targeting ASGPR.[20,21]

In vitro evaluation of doxorubicin-loaded βCD-Gal 26

βCD functionalized systems have been widely explored as molecular vectors for gene[45–47] and drug delivery.[48] To evaluate the ability of our Gal functionalized βCD systems to deliver pharmacologically active species into hepatocytes, βCD-Gal **14** was loaded with doxorubicin, a potent chemotherapy drug used in cancer treatment.[49] This system was chosen as a model because the physico-chemical properties of inclusion complexes of anthracycline antibiotics and βCDs are well described.[50] An equimolar inclusion complex of βCD-Gal **14** and doxorubicin hydrochloride was prepared over 20 min with sonication and using water as the solvent yielding doxorubicin-loaded βCD-Gal **26** (Scheme 2). Cross peaks between H-3 and H-5 of βCD (~3.75 ppm) and protons in the aromatic region of doxorubicin (~7.60 ppm) were detected by nuclear overhauser effect spectroscopy (NOESY), indicating inclusion of doxorubicin into βCD-Gal **14** (Fig. S31, ESI†).[51] The potential of this system to induce apoptosis in HepG2 cells was evaluated. Cells were left untreated or were treated with doxorubicin only, βCD-Gal **14** or doxorubicin-loaded βCD-Gal **26** for 24 h. The highest degree of apoptosis was observed in HepG2 cells incubated with doxorubicin-loaded βCD-Gal **26** whereas βCD-Gal **14** alone induced no apoptosis (Fig. 3). This result encourages the use of Gal functionalized βCDs as a drug-delivery system.



Scheme 2 Synthesis of doxorubicin-loaded βCD-Gal **26**.

Conclusions

In summary, we have developed a practical synthesis of delivery systems based on synthetic carbohydrates for multivalent receptor interactions that enable targeting of ASGPR expressed by hepatocytes. The Gal functionalized βCD-based system is a molecular carrier for doxorubicin inducing a high degree of apoptosis *in vitro*. The use of liposomal-based systems in hepatocyte-specific gene delivery and Gal functionalized βCD-based systems for drug-delivery is currently under investigation in our laboratory.

Experimental

Synthesis of dendrons 8 and 9 as well as βCD 10

Compound **4**, tripod-Man, tripod-Gal, βCD-amine were synthesized according to published procedures.[52,53]

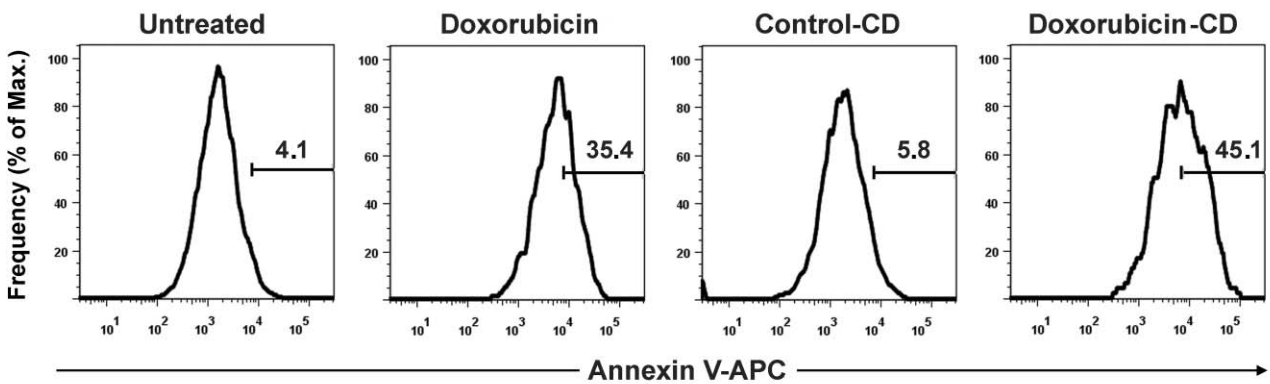


Fig. 3 Induction of apoptosis in HepG2 cells treated with doxorubicin-loaded βCD-Gal **26**. Cells were left untreated or were treated with doxorubicin only, βCD-Gal **14** or doxorubicin-loaded βCD-Gal **26** for 24 h. Apoptosis was detected by Annexin-V-APC staining and subsequent measurement by flow cytometry. Bars indicate the frequency of apoptotic cells present in the whole HepG2 cell population. The highest degree of apoptosis was observed in HepG2 cells incubated with doxorubicin-loaded βCD-Gal **26** whereas βCD-Gal **14** alone induced no apoptosis. Data are representative of three independent experiments.

General procedure A: synthesis of sugar-nonapods

The Boc-protected tripod-sugar (4 eq) was dissolved in 10 mL dichloromethane/trifluoroacetic acid (3 : 1) and stirred at room temperature for 1 h. The solvent was evaporated under reduced pressure and the resulting oil was dissolved in anhydrous dichloromethane (20 mL). To this mixture was added *tert*-butoxycarbonyl-3-{*N*-{tris[3-[pentafluorophenylcarboxyl-ethoxy)methyl]}methylamine}-3-β-alanine (1 eq), adjusted to pH 8 with triethylamine (TEA) and the mixture was stirred at room temperature for 12 h. The solvent was evaporated *in vacuo* and purified by flash silica column chromatography.

N-{Tris[3-[ethylcarboxyl-ethoxy)methyl]}methylamide}-5-bromo pentamide 5

To a solution of *N*-{tris[(3-[ethylcarboxyl-ethoxy)methyl]}-methylamine (5 g, 11.8 mmol) and 5-bromovaleric acid (2.1 g, 11.8 mmol) in dichloromethane (20 mL) at 0 °C, were added *N*,*N*′-diisopropylcarbodiimide (DIC) (2.25 ml, 12.2 mmol) and 1-hydroxybenzotriazole (0.15 g, 1.18 mmol) The reaction mixture was stirred at room temperature for 12 h and concentrated *in vacuo*. The crude residue was purified by flash silica column chromatography to yield **5** (4.8 g, 69%). R_f 0.5 (CH_2Cl_2–MeOH, 98 : 2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.14 (q, J = 7.2 Hz, 6H), 3.67 (br. s, 11H), 3.41 (t, J = 6.6 Hz, 2H), 2.52 (t, J = 6.0 Hz, 6H), 2.17 (t, J = 6.6 Hz, 2H), 1.9–1.7 (m, 4H), 1.6–1.4 (m, 2H), 1.24 (t, J = 6.9 Hz, 9H), ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.4, 171.4, 69.5, 66.5, 60.7, 60.3, 35.2, 33.4, 33.3, 24.5, 14.1, FTIR($CHCl_3$): 3390, 2982, 2873, 1734, 1726, 1643, 1521 cm^{-1}. HRMS (MALDI-ToF) (m/z) calcd. for $C_{24}H_{42}BrNO_{10}Na$ 606.1884, found: 606.1878.

N-{Tris[3-[ethylcarboxyl-ethoxy)methyl]}methylamide}-5-azido pentamide 6

5 (3 g, 5.14 mmol) and sodium azide (1.34 g, 20.58 mmol) in anhydrous DMF (20 mL) were mixed and stirred at 70 °C for 24 h. The crude residue was dissolved in 20 mL water and extracted with 40 mL ethyl acetate. The organic layer was washed five times with 20 mL water and concentrated *in vacuo*. The crude product was purified by flash silica column chromatography to yield **6** (2.35 g, 72%). R_f 0.5 (CH_2Cl_2–MeOH, 98 : 3); ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.14 (q, J = 7.2 Hz, 6H), 3.69 (br. s, 11H), 3.31 (t, J = 6.6 Hz, 2H), 2.52 (t, J = 6.0 Hz, 6H), 2.18 (t, J = 6.6 Hz, 2H), 1.71–1.62 (m, 6H), 1.27 (t, J = 6.9 Hz, 9H), ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.4, 171.5, 69.1, 66.6, 60.3, 51.1, 36.2, 35.3, 28.2, 32.6, 20.8, 14.1, FTIR($CHCl_3$): 3390, 2982, 2873, 1734, 1726, 1643, 1521 cm^{-1}. HRMS (MALDI-ToF) (m/z) calcd. for $C_{24}H_{42}N_4O_{10}Na$ 569.2793, found: 569.2802.

N-{Tris[3-[pentafluorophenylcarboxyl-ethoxy)methyl]}methylamide}-5-azido pentamide 7

6 (2.5 g, 3.57 mmol) was dissolved in ethanol (20 mL) and sodium hydroxide solution (aqueous, 1 N, 2 mL) was added and the mixture was stirred at room temperature for 2 h, concentrated in *vacuo*, the adjusted to pH 5 with hydrochloric acid (aqueous 1 N, 3 mL) and extracted with ethyl acetate. The organic layer was dried with sodium sulfate and concentrated to dryness under reduced pressure. The residue was dissolved in dichloromethane (10 mL) and 2,3,4,5,6-pentafluorophenol (4.2 g, 17.8 mmol) was added. After cooling to 0 °C, *N*,*N*′-diisopropylcarbodiimide (DIC) (3.95 mL, 19.9 mmol) was added and the reaction mixture was stirred for 12 h at room temperature. The reaction mixture was concentrated *in vacuo* and purified by silica column flash chromatography to afford **7** (3.7 g, 86%). R_f 0.6 (CH_2Cl_2–EtOH, 88 : 12); ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.83 (br. s, 12H), 3.24 (t, J = 6.6 Hz, 2H), 2.92 (t, J = 6.0 Hz, 7H), 2.17 (t, J = 6.6 Hz, 2H), 1.71–1.62 (m, 6H), ^{13}C NMR (75 MHz, $CDCl_3$): δ 167.2, 69.9, 65.9, 59.6, 36.6, 34.1, 28.2, 24.2. FTIR($CHCl_3$): 3688, 3385, 1749, 1658, 1522, 1359 cm^{-1}. HRMS (MALDI-ToF) (m/z) calcd. for $C_{36}H_{27}F_{15}N_4O_{10}Na$ 983.1385, found: 983.1388.

3-{Tris[3-carboxylethoxy]methyl]3′-{tris-[2-ethoxy-2,3,4,6-tetra-*O*-acetyl-α-D-manno pyranoside-ethoxy]methyl]methylamide}-5-azido pentamide 8

Using general procedure A, *tert*-butoxycarbonyl-3-{tris[3-[2-ethoxy-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside-ethoxy]-methylamide}-3-β-alanine (0.3 g, 0.18 mmol) and **7** (44 mg, 0.045 mmol), followed by purification using flash chromatography yielded **8** (0.21 g, 59%). $[\alpha]_D^{r.t}$ = +19.4 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz,$CDCl_3$): δ 7.27 (br, 1H), 5.27 5.2 (m, 27H), 4.75 (s, 9H), 4.15 (dd, J = 9.0 Hz, 9 H), 4.05 (br, 9H), 3.81–3.24 (m, 127H), 3.24 (br, 8H), 2.47 (br, 32H), 2.13 (s, 27H), 2.06 (s, 27H), 2.04 (s, 27H), 1.9 (s, 27H), 1.7–1.5 (m, 6H), ^{13}C-NMR (75 MHz, $CDCl_3$): δ 173.92, 172.2, 171.4, 171.3, 98.7, 70.7, 70.6, 70.4, 69.8, 68.5, 67.6, 67.0, 63.4, 54.6, 39.0, 37.2, 30.5, 20.6, FTIR($CHCl_3$): 3332, 2734, 1745,1365, cm^{-1}; HRMS-MALDI (m/z): [M+ Na]$^+$ Calcd for $C_{210}H_{315}N_{19}O_{118}Na$ 5013.913; Found : 5013.945.

3-{Tris[3-carboxyl ethoxy]methyl] 3′-{tris[2′-ethoxy-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside-ethoxy]methyl] methylamide}-5-azido pentamide 9

Using general procedure A, *tert*-butoxycarbonyl-3-{tris[3-[2-ethoxy-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside-ethoxy]-methyl] methylamide}-3-β-alanine (0.45 g, 0.27 mmol) and **7**, followed by purification using flash silica column chromatography and CH_2Cl_2–CH_3OH (12–13%) as the eluent yielded 0.21 g (52%) of **9**. R_f 0.55 (CH_2Cl_2–MeOH = 90 : 10); $[\alpha]_D^{rt}$ = +0.8 (c = 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, MeOD): δ 5.37 (d, J = 3.3 Hz, 9H), 5.22–5.12 (m, 27H), 4.99 (d, J = 4.2 Hz, 9H), 4.51–4.45 (m, 2H), 4.14 (s, 16H), 3.95–3.92 (m, 9H), 3.84–3.78 (m, 9H), 3.66–3.56 (m, 74H), 3.41–3.36 (m, 39H), 2.41 (t, J = 5.4 Hz, 36H), 2.14 (s, 27H), 2.04 (s, 27H), 2.02 (s, 27H), 1.96 (s, 27H), 1.9–1.7 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ171.3, 170.2, 170.0, 169.8, 169.6, 101.2, 70.7, 68.9, 67.4, 67.3, 67.0, 61.2, 39.3, 36.4, 28.9, 20.9. FTIR($CHCl_3$): 3385, 3019, 1749, 1658, 1522, 1232, 1205 cm^{-1}; HRMS-MALDI (m/z): [M+ Na]$^+$ Calcd for $C_{210}H_{315}N_{19}O_{118}Na$ 5013.913; Found : 5013.911.

Mono-(2,3-di-*O*-acetyl-6-deoxy-6-(5-hexynyl)-amido)-hexakis-(2,3,6-tri-*O*-acetyl)-βCD 10

To a solution of βCD-amine (0.36 g, 0.18 mmol) in dichloromethane (2 mL) under argon were added 5-hexynoic acid (40 mg, 0.36 mmol, 2 eq), HOBt monohydrate (61 mg, 0.40 mmol, 2.2 eq) and DIPC (61 mg, 0.40 mmol, 2.2 eq). The reaction was allowed to stir for 12 h at room temperature. The

solvent was evaporated and the residue was purified by column chromatography on silica gel (EtOAc) to give a mixture of **2** and diisopropyl urea. This mixture was purified by LH-20 column (1 : 1 DCM–MeOH) to give 309 mg of **2** (82%) as an amorphous solid. R_f : 0.32 (EtOAc). $[\alpha]_D^{20}$ +96 (C = 2.3, DCM). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.04 (t, J = 6.2 Hz, 1H), 5.34–5.24 (m, 6H), 5.17 (dd, J = 6.8, 8.8 Hz, 1H), 5.14–4.97 (m, 7H), 4.85–4.73 (m, 7H), 4.60–4.05 (m, 20H), 3.99 (ddd, J = 9.2, 6.1, 3.1 Hz, 1H), 3.76–3.58 (m, 6H), 3.48 (dd, J = 9.2, 7.0 Hz, 1H), 2.33 (m, 2H), 2.25–2.21 (m, 2H), 2.13–1.99 (m, 60H), 1.98 (t, J = 2.6 Hz, 1H), 1.90–1.78 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 172–169, 97.2–96.3, 78.1–76.2, 71.0–70.6, 70.4–70.0, 69.5–69.3, 69.1 5, 62.7–62.4, 34.7, 24.0, 20.8–20.6, 17.7. FT-IR = 2901, 2848, 2264, 1709 cm^{-1} MS ESI$^+$ HRMS m/z $[M+Na]^+$ calcd for $C_{88}H_{117}NO_{55}Na$ 2091.6315, found 2091.6302.

Synthesis of complexes 1–3

General procedure B. [2+3] Cycloaddition. The azide-nonapod (1 eq) and acetylene-βCD (1 eq) was dissolved in 10 mL tetrahydrofuran–water (2 : 1). To this mixture, copper sulfate (2 eq) and ascorbic acid (2 eq) were added and stirred at room temperature for 12 h. The solvent was evaporated *in vacuo* and purified by flash silica column chromatography.

General procedure C. Synthesis of fluorescent-sugar complex. CD-glycodendrimer (1 eq) and sodium methoxide (10 eq) were dissolved in methanol (10 mL) and stirred at room temperature for 12 h. The solvent was then evaporated *in vacuo*, the residue was washed with Amberline-H$^+$ resin and re-dissolved in water and dialyzed against water using 500 molecular weight cut-off resin. After two days of dialysis, the sample was lyophilized.

Peracetylated βCD-Man dendrimer 11

Using general procedure B, **8** (70 mg, 0.014 mmol), **10** (40 mg, 0.019 mmol), ascorbic acid (5 mg, 0.028 mmol) and copper sulfate (5 mg, 0.031 mmol), followed by purification using flash silica column chromatography and CH_2Cl_2–CH_3OH (13–17%) as the eluent yielded 81 mg (82%) of **11**. R_f 0.5 (CH_2Cl_2 : MeOH = 90 : 10); $[\alpha]_D^{rt}$ = +35.7 (c = 1.0, $CHCl_3$); ^{1}H NMR (700 MHz, MeOD): δ 7.64 (s, 1H), 5.44–5.24 (m, 14H), 5.26–5.05 (m, 41H), 4.85 (d, J = 9.1 Hz, 7H), 4.73 (d, J = 6.8 hz, 7H), 4.51 (d, J = 9.0 Hz, 4H), 4.32 (t, J = 3.1 Hz, 6H), 4.25(t, J = 3.5 Hz, 6H), 4.18 (brs, 38H), 3.81 (s, 12H), 3.74–3.51 (m, 62 H), 3.23 (t, J = 5.0 Hz, 28H), 2.47 (br, 32H), 2.13–1.99 (m, 168H), 1.8–1.57 (m, 6H); ^{13}C NMR (175 MHz, MeOD): δ 173.2; 171.3, 170.5, 170.2, 98.5, 97.6, 77.9, 72.3–70.1, 69.4, 68.5, 67.6, 67.0, 63.4, 61.4, 54.6, 39,6, 36.5, 28.9, 20.5, FTIR($CHCl_3$): 3332, 2734, 1745, 1365, cm^{-1}.

Peracetylated βCD-Gal dendrimer 12

Using general procedure B, **9** (40 mg, 0.008 mmol), **10** (17 mg, 0.008 mmol), ascorbic acid (3.5 mg, 0.02 mmol) and copper sulfate (3 mg, 0.02 mmol), followed by purification using flash silica column chromatography and CH_2Cl_2–CH_3OH (13–17%) as the eluent yielded 48 mg (84%) of **12**. R_f 0.5 (CH_2Cl_2 : MeOH = 88 : 12); $[\alpha]_D^{rt}$ = +22.7 (c = 1.0, $CHCl_3$); ^{1}H NMR (700 MHz, MeOD): δ 5.44–5.24 (m, 14H), 5.36–5.25 (m, 34H), 5.23 (s, 7H), 4.75–4.56 (m, 7H), 4.45–4.41 (m, 14H), 4.51 (d, J = 9.0 Hz, 4H), 4.32–4.03 (m, 21H), 3.87–3.77 (m, 24H), 3.74–3.51 (m, 71H), 3.23 (m, 28H), 2.47 (br, 32H), 2.13–1.99 (m, 168H), 1.74–1.57 (m, 6H). ^{13}C NMR (175 MHz, MeOD): δ 173.5; 171.1, 170.3, 170.2, 102.3, 98.5, 77.5, 70.9–65.2, 63.4, 61.4, 54.6, 38,6, 36.5, 28.9, 24.5, 14.2 FTIR($CHCl_3$): 3362, 2711, 1745, 1365, cm^{-1}.

βCD-Man dendrimer 13

Using general procedure C, **11** (60 mg, 8.5 µmol) and sodium methoxide (4.6 mg, 85 µmol) gave 31 mg (78%) of **13**. $[\alpha]_D^{rt}$ = −12.3 (c = 1.0, H_2O); ^{1}H NMR (700 MHz, D_2O): δ 4.95 (s, 7H), 4.65 (s, 9H), 3.81–3.55 (m, 106H), 3.46–3.30 (m, 7H), 2.47–2.39 (m, 14H); 1.9–1.43 (m, 12H);^{13}C NMR (125 MHz, CD_3OD): δ 181.1, 174.4, 104.3, 100.2, 82.3, 73.8, 73.5, 71.8, 70.8, 68.5, 67.6, 66.6, 62.4, 61.4, 39.1, 36.8, 17.3. ESI-MS (m/z): $[M]^+$ Calcd for $C_{186}H_{319}N_{20}O_{117}$ 4704.595; Found 2375.731 $[M+Na/2]^+$.

βCD-Gal dendrimer 14

Using general procedure C, **12** (65 mg, 9.2 µmol) and sodium methoxide (5 mg, 92µ mol) gave 34 mg (77%) of **14**. $[\alpha]_D^{rt}$ = −34.8 (c = 1.0, H_2O); ^{1}H NMR (700 MHz, MeOD): δ 4.95 (s, 7H), 4.43 (s, 9H), 4.11–3.35 (m, 102H), 2.47–2.35 (m, 14H), 1.9–1.43 (m, 12H);^{13}C NMR (175 MHz, CD_3OD): δ 181.1, 174.4, 171.7, 104.3, 97.2, 78.3, 76.2, 75.8, 74.2, 73.8, 73.5, 71.8, 68.5, 67.6, 66.6, 61.2, 39.1, 36.8, 17.3. ESI-MS (m/z): $[M]^+$ Calcd for $C_{186}H_{320}N_{20}O_{117}$ 4704.595; Found 2375.731 $[M+Na/2]^+$.

Synthesis of 1–3

A solution of 1eq of βCD-glycodendrimer **13**, **14** and 1 eq of rhodamine B in 1 mL distilled water was prepared and sonicated for 10 min. Complexation was established by fluorescent and ^{1}H-NOESY experiment.

Synthesis of glycolipids 20 and 25

Citronellylphosphate 15. Citronellol (0.29 mL, 1.60 mmol) was dissolved in hexane (5 mL) and triethylamine (0.22 mL, 1.60 mmol) was added. The mixture was stirred under argon and cooled to 0 °C. Phosphorus oxychloride (0.15 mL, 1.60 mmol) was added dropwise to the above solution and the resulting mixture allowed to warm to room temperature. The reaction was stirred for a 2 h period. The reaction was quenched by the addition of a acetone : water : triethylamine (85 : 10 : 5) mixture and stirred overnight. The solution was concentrated *in vacuo*, diluted with toluene (10 mL) and washed with water (2 × 6 mL). The organic layer was concentrated *in vacuo* to afford **15** (300 mg, 80%) as a white foam; FTIR (KBr disc) 1259, 1066, 1017 cm^{-1}; ^{1}H NMR (400 MHz, MeOD) δ 5.12 (d, J = 7.1 Hz, 1H), 4.05–3.92 (m, 2H), 2.08–1.96 (m, 2H), 1.68 (s, 3H), 1.62 (s, 3H), 1.52–1.15 (m, 5H), 0.94 (d, J = 6.6 Hz, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 131.1, 124.8, 64.6, 37.7, 37.3, 29.3, 25.5, 25.0, 18.8, 16.8; ^{31}P-NMR (162 MHz, MeOD) δ 0.29; m/z (ESI) 235 $[M - H]^-$; HRMS (ESI) Calcd. For $C_{10}H_{20}O_4P$ $[M - H]^-$ 235.1094. Found: 235.1091.

2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranose 1-phosphate, monotriethyl-ammonium salt 18. A catalytic amount of palladium on activated charcoal was added to a stirred solution of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranose 1-dibenzyl-phosphate[54] **17** (122 mg, 0.20 mmol) in anhydrous methanol (4 mL). The resulting solution was degassed and

purged with hydrogen gas, then left to stir under an atmosphere of hydrogen. After a 3 h period, TLC (diethyl ether–methanol, 98 : 2) indicated the formation of a product (R_f 0) with complete consumption of starting material (R_f 0.3). The solution was filtered through Celite®. Triethylamine (1 eq) was added and the resulting solution stirred for further 30 min. The reaction mixture was concentrated *in vacuo* to afford **18** (110 mg, 100%) as a white amorphous solid; $[\alpha]_D^{25}$ +28.2 (*c*, 1 in MeOH); FTIR (KBr disc) 3447, 1749, 1240, 1033 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.48 (br d, J = 9.3 Hz, 1H), 5.49 (dd, J = 2.9, 7.4 Hz, 1H), 5.27 (dd, J = 9.9, 10.1 Hz, 1H), 5.15 (d, J = 9.9 Hz, 1H), 4.33 (ddd, J = 2.2, 7.6, 9.8 Hz, 1H), 4.25 (br d, J = 10.0 Hz, 1H), 4.16 (dd, J = 3.1, 12.0 Hz, 1H), 4.09 (dd, J = 1.7, 12.2 Hz, 1H), 2.95 (q, J = 6.9 Hz, 6H), 2.04 (s, 3H), 1.97 (s, 3H), 1.96 (s, 3H), 1.93 (s, 3H), 1.25 (d, J = 6.8 Hz, 9H), ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 171.0, 170.9, 170.8, 169.4, 91.5, 71.7, 68.3, 68.2, 61.8, 52.4, 45.5, 23.1, 20.8, 20.7, 20.6, 9.0; ^{31}P-NMR (162 MHz, $CDCl_3$) δ –10.0; *m/z* (ESI) 426 $[M - H]^-$; HRMS (ESI) Calcd. For $C_{14}H_{21}NO_{12}P$ $[M - H]^-$ 426.0796. Found: 426.0795.

P^1-Citronellyl P^2-[2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*α*-D-glucopyranosyl] diphosphate 19. To a suspension of **15** (83 mg, 0.35 mmol, dried over P_2O_5 overnight) in anhydrous DMF (2.5 mL) was added 1,1′-carbonyldiimidazole (CDI, 0.28 g, 1.77 mmol) in one portion. After stirring at room temperature for 2 h, anhydrous methanol (60 µL) was added and the reaction stirred for another 1 h to destroy excess CDI. Methanol was removed under reduced pressure at room temperature. In a separate flask, **18** (150 mg, 0.35 mmol, dried over P_2O_5 overnight) was dissolved in anhydrous DMF (2.5 mL), and transferred to the above flask. The resulting mixture was stirred at room temperature under an argon atmosphere for 72 h. The reaction mixture was concentrated *in vacuo*, and the residue purified over a C-18 column (5–100% gradient, water–acetonitrile) to afford **19** (87 mg, 40%) as a foam; $[\alpha]_D^{22}$ +8.4 (*c*, 1 in $CHCl_3$); FTIR (thin film) 3447, 1748, 1232, 1063 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$: MeOD; 3 : 97) δ 6.11 (br d, J = 9.4 Hz, 1H), 5.66 (dd, J = 2.7, 7.4 Hz, 1H), 5.23 (d, J = 10.1 Hz, 1H), 5.17 (d, J = 9.7 Hz, 1H), 5.04 (d, J = 7.1 Hz, 1H), 4.40 (d, J = 9.9 Hz, 1H), 4.24 (br d, J = 9.9 Hz, 1H), 4.19–4.09 (m, 2H), 4.02–3.97 (m, 2H), 2.07 (s, 3H), 2.02 (s, 3H), 1.99 (s, 3H), 1.98 (s, 3H), 1.94–1.86 (m, 2H), 1.71–1.66 (m, 2H), 1.64 (s, 3H), 1.56 (s, 3H), 1.45–1.38 (m, 1H), 1.31–1.25 (m, 1H), 1.16–1.09 (m,1H) 0.85 (d, J = 6.6 Hz, 3H), ^{13}C NMR (100.6 MHz, $CDCl_3$: MeOD; 3 : 97) δ 171.4, 170.9, 170.8, 169.3, 131.2, 124.5, 94.7, 71.0, 68.8, 67.8, 64.9, 61.4, 52.3, 37.9, 37.1, 29.2, 25.7, 25.4, 22.8, 20.8, 20.7, 20.6, 19.1, 17.6; ^{31}P NMR (162 MHz, $CDCl_3$: MeOD; 3 : 97) δ –12.6 (d), –9.9 (d); *m/z* (ESI) 644 $[M - H]^-$; HRMS (ESI) Calcd. For $C_{24}H_{41}NNaO_{15}P_2$ $[M+Na]^+$ 668.1844. Found: 668.1836.

P^1-Citronellyl P^2-[2-acetamido-2-deoxy-*α*-D-glucopyranosyl] diphosphate 20. To a stirred solution of **19** (25 mg, 39 µmol) in anhydrous DCM (3 mL) at room temperature was added a solution of sodium methoxide (1 mL of a 110 mg mL^{-1} solution in methanol). After 30 min, cation exchange resin (DOWEX 50WX8-200, pyridinium form, 300 mg) was added and the mixture was stirred for 10 min. The resin was then filtered and washed with chloroform–methanol (2/1, v/v). The filtrate was concentrated *in vacuo* to afford **20** (20 mg, 100%) as a foam; $[\alpha]_D^{22}$ +10.3 (*c*, 1 in MeOH); FTIR (thin film) 3356, 1135 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$: MeOD; 3 : 97) δ 5.57 (dd, J = 3.2, 7.4 Hz, 1H); 5.11 (d, J = 7.1 Hz, 1H), 4.09–4.04 (m, 2H), 4.00 (d, J = 2.8, 10.4 Hz, 1H), 3.93 (ddd, J = 9.9, 2.1, 5.1 Hz, 1H), 3.84 (dd, J = 2.3, 11.9 Hz, 1H), 3.74 (d, J = 9.7 Hz, 1H), 3.70 (dd, J = 5.3, 11.9 Hz, 1H), 3.41 (d, J = 9.5 Hz, 1H), 2.17 (s, 3H), 2.00–1.94 (m, 2H), 1.75–1.69 (m, 2H), 1.68 (s, 3H), 1.61 (s, 3H), 1.48–1.41 (m, 1H), 1.39–1.32 (m, 1H), 1.30–1.23 (m, 1H), 0.92 (d, J = 6.6 Hz, 3H), ^{13}C NMR (100.6 MHz, $CDCl_3$: MeOD; 3 : 97) δ 174.0, 131.9, 125.9, 96.4, 75.0, 73.3, 72.0, 65.6, 62.8, 55.5, 38.7, 38.4, 30.5, 26.5, 25.9, 23.0, 19.8, 17.6; ^{31}P NMR (162 MHz, $CDCl_3$: MeOD; 3 : 97) δ –12.6 (d), –10.3 (d); *m/z* (ESI) 518 $[M - H]^-$; HRMS (ESI) Calcd. For $C_{18}H_{34}NO_{12}P_2$ $[M - H]^-$ 518.1551. Found: 518.1548.

2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*α*-D-galactopyranose 1-phosphate, monotriethyl-ammonium salt 23. A catalytic amount of palladium on activated charcoal was added to a stirred solution of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*α*-D-galactopyranose 1-dibenzylphosphate[55] **22** (0.47 g, 7.74 mmol) in anhydrous methanol (10 mL). The resulting solution was degassed and purged with hydrogen gas, then left to stir under an atmosphere of hydrogen. After 2 h, TLC (diethyl ether–methanol, 98 : 2) indicated the formation of a product (R_f 0) with complete consumption of starting material (R_f 0.2). The solution was filtered through Celite®. Triethylamine (1 eq) was added and the resulting solution stirred for a further 30 min period. The reaction mixture was concentrated *in vacuo* to afford **23** (0.41 g, 88%) as a white amorphous solid; FTIR (KBr disc) 3446, 1747, 1242, 1031 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.13 (br d, J = 9.2 Hz, 1H), 5.56 (dd, J = 3.1, 7.4 Hz, 1H), 5.40 (br d, J = 2.1 Hz,1H), 5.24 (dd, J = 3.2, 11.2 Hz, 1H), 4.60 (ddd, J = 1.4, 9.4, 10.8 Hz, 1H), 4.49 (br, J = 6.5 Hz, 1H), 4.16 (dd, J = 7.5, 11.2 Hz, 1H), 4.02 (dd, J = 5.9, 10.9 Hz, 1H), 2.83 (q, J = 7.3 Hz, 6H), 2.14 (s, 3H), 2.00 (s, 3H), 1.96 (s, 3H), 1.95 (s, 3H), 1.19 (br, J = 7.3 Hz, 9H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 171.2, 170.6, 170.5, 170.4, 94.2, 68.6, 67.5, 67.1, 61.5, 48.0, 45.7, 23.2, 20.8, 20.7, 20.6, 9.6; δ_P ^{31} (162 MHz, $CDCl_3$) –0.29; *m/z* (ESI) 426 $[M - H]^-$; HRMS (ESI) Calcd. For $C_{14}H_{21}NO_{12}P$ $[M - H]^-$ 426.0796. Found: 426.0807.

P^1-Citronellyl P^2-[2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*α*-D-galactopyranosyl] diphosphate 24. To a suspension of **15** (0.28 g, 1.16 mmol, dried over P_2O_5 overnight) in anhydrous DMF (7 mL) was added 1,1′-carbonyldiimidazole (CDI, 0.78 g, 4.85 mmol) in one portion. After stirring at room temperature for 3 hs, anhydrous methanol (150 µL) was added and the reaction was stirred for another 1 h to destroy the excess CDI. Methanol was removed under reduced pressure at room temperature. In a separate flask, **23** (0.41 g, 9.69 mmol, dried over P_2O_5 overnight) was dissolved in anhydrous DMF (7 mL), and transferred to the above flask. The resulting mixture was stirred at room temperature under an argon atmosphere for 72 h. The reaction mixture was concentrated *in vacuo*, and the residue purified over a C-18 column (5–100% gradient, water–acetonitrile) to afford **24** (0.42 g, 66%) as a foam; $[\alpha]_D^{22}$ +5.1 (c, 1 in $CHCl_3$); FTIR (thin film) 3445, 1746, 1229, 1067 cm^{-1}; ^1H -NMR (500 MHz, $CDCl_3$: MeOD; 3 : 97) δ 5.57 (dd, J = 3.2, 7.4 Hz, 1H), 5.32 (br d, J = 2.5 Hz, 1H), 5.10 (dd, J = 3.1, 11.3 Hz, 1H), 5.00 (br.d, J = 7.1 Hz, 1H), 4.43–4.50 (m, 2H), 4.19–4.09 (m, 1H), 4.09 (dd, J = 8.0, 10.8 Hz, 1H), 4.01–3.91 (m, 2H), 2.04 (s, 3H), 1.91 (s, 3H), 1.83 (s, 3H), 1.90 (s, 3H), 1.62–1.57 (m, 2H), 1.50 (m, 2H), 1.45 (s, 3H), 1.46 (s, 3H), 1.32–1.38 (m, 1H), 1.29–1.21 (m, 1H), 1.10–1.02 (m, 1H), 0.81

(d, J = 6.6 Hz, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 174.2, 172.3, 172.1, 171.8, 131.9, 125.8, 96.4, 70.2, 68.9, 68.5, 65.7, 62.4, 49.3, 39.0, 38.3, 30.4, 26.5, 25.9, 22.8, 20.8, 20.7, 20.6, 19.8, 17.8; ^{31}P NMR (202 MHz, $CDCl_3$: MeOD; 3 : 97) δ –13.0 (d), –10.3 (d); m/z (ESI) 644 [M – H]$^-$; HRMS (ESI) Calcd. For $C_{24}H_{41}NNaO_{15}P_2$ [M+Na]$^+$ 668.1844. Found: 668.1844.

P^1-Citronellyl P^2-[2-acetamido-2-deoxy-α-D-galactopyranosyl] diphosphate 25. To a stirred solution of **24** (0.27 g, 0.43 mmol) in anhydrous DCM (25 mL) at room temperature was added a solution of sodium methoxide (3 mL of a 150 mg mL^{-1} solution in methanol). After 30 min, cation exchange resin (DOWEX 50WX8-200, pyridinium form) was added and the mixture stirred for 10 min. The resin was then filtered and washed with chloroform–methanol (2/1, v/v). The filtrate was concentrated *in vacuo* to afford **25** (0.27 g, 100%) as a foam; $[\alpha]_D^{22}$ +8.3 (*c*, 1 in MeOH); FTIR (thin film) 3352, 1131 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$: MeOD; 3 : 97) δ 5.62 (br s,1H), 5.07 (d, J = 7.2 Hz, 1H), 4.38 (br d, J = 6.3 Hz, 1H), 4.18–3.46 (m, 7H), 2.04 (s, 3H), 1.93–1.99 (m, 2H), 1.66 (m, 2H), 1.59, 1.55 (s, 6H), 1.38–1.32 (m, 1H), 1.39–1.29 (m, 1H), 1.13 (m, 1H), 0.91 (d, J = 6.3 Hz, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 174.6, 130.6, 124.4, 96.4, 72.3, 70.9, 69.0, 68.4, 61.6, 45.6, 36.9, 31.6, 29.0, 25.1, 24.6, 22.3, 22.1, 21.6;^{31}P NMR (162 MHz, $CDCl_3$: MeOD; 3 : 97) δ_P –12.8 (d), –10.1 (d); m/z (ESI) 518 [M – H]$^-$; HRMS (ESI) Calcd. For $C_{18}H_{34}NO_{12}P_2$ [M – H]$^-$ 518.1551. Found: 518.1556.

Preparation of liposomes

L-α-Phosphatidylcholine (18 mM) and glycolipid **20** (specificity control) or glycolipid **25** (4 mM or 10 mM) were added as solids to a 10 mL round bottomed flask. A solution of rhodamine DHPE (0.5 mM) in ethanol (0.25 mL) was added. The lipids were dissolved in ethanol (2.75 mL) and stirred at room temperature for 2 h. The reaction mixture was concentrated *in vacuo* and placed under high vacuum for additional 2 h. The lipid film was dissolved in sodium phosphate buffer (pH 7, 50 mM, 0.5 mL) and submitted to extrusion through the Avanti® Extruder (100 nm polycarbonate membrane) 11 times. The glycoliposomes were stored after preparation at 4 °C until further use.

Transmission electron microscopy (TEM) measurements

After extrusion, a drop of a 50 mM solution of glycolyposomes was diluted with ultrapure water (1 : 10 v/v) and deposited on the carbon membrane of a copper grid. The grids were stained subsequently with a 1% aqueous solution of uranyl acetate for 30 s at room temperature. After drying, the grid was transferred to a transmission electron microscope (*EM 912 Omega/Carl-Zeiss Oberkochen*, *Germany*), and TEM measurements were performed.

Uptake studies in HepG2 cells

Human hepatocellular carcinoma cell line, HepG2, was plated in 6-well plates and cultivated in D-MEM medium supplemented with 10% fetal calf serum (FCS) and 1% penicillin–streptomycin. When cells were 70% confluent, different concentrations (10 µmol, 50 µmol, and 250 µmol) of CD **1**, **2** or **3** were added to the cells. After incubation times between one and six hours, cells were washed with phosphate buffered saline (PBS). The cells were then collected with PBS containing 1% FCS by shearing force. Uptake of βCD was measured by flow cytometry using a FACSCanto™ II flow cytometer (Becton Dickinson, Franklin Lakes, NJ). Excitation wavelength in the FACS experiments was 540 nm, which is the excitation wavelength of Rhodamine B. Cells were gated on living cells and fluorescence channel FL-2 (PE-A) was used to detect HepG2 cells that had endocytosed RhB-labeled βCD molecules. All data were analyzed with the FlowJo software (Tree Star, Inc., Ashland, OR).

For analysis of liposome uptake by the HepG2 cell line, cells were incubated with liposomes consisting of L-α-phosphatidylcholine and rhodamine-DHPE (negative control) or liposomes displaying additionally glycolipid **20** (GlcNAc, specificity control) or **25** (GalNAc) on their surface. After incubation times between one to six hours, cells were washed with PBS and collected from the plate. Detection of liposome uptake was performed as described above.

Synthesis of doxorubicin-loaded βCD-Gal 26

A solution of βCD-glycodendrimer **14** (1 eq) and doxorubicin-HCl (1 eq) in 1 mL distilled water was prepared and sonicated for 20 min. The complexation was established by ^{1}H-NOESY (see the ESI for full details†).

Apoptosis studies in HepG2 cells

For analysis of apoptosis, HepG2 cells were incubated with doxorubicin-loaded βCD-Gal **26** for 24 h at concentrations of 1 µg ml^{-1}, 5 µg ml^{-1}, and 10 µg ml^{-1} of the incorporated drug. The following controls were used: untreated cells (negative control), cells incubated with doxorubicin alone (positive control), and cells treated with βCD-Gal **14** only. The concentrations for doxorubicin and empty βCD-Gal **14** were the same as those of doxorubicin-loaded βCD-Gal **26**. After incubation, the adherent cells were detached from the growth surface using Trypsin/EDTA (Pan Biotech, Germany). The staining procedure was performed according to the protocol in the Annexin V-APC Apoptosis Detection Kit purchased from eBioscience (Frankfurt, Germany). Detection of apoptopic cells was done by flow cytometry as described above. Cells were gated on living cells. Additionally, fluorescence channel FL-3 (APC) was used to detect apoptotic cells.

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