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PAPER

Sugar-bearing tetraphenylethylene: novel fluorescent probe for studies of carbohydrate–protein interaction based on aggregation-induced emission†

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Neutral sugar-bearing tetraphenylethenes (TPE) are designed and prepared as "turn-on" luminescent sensors for lectins and glycosidases based on aggregation-induced emission. Through aggregation derived from carbohydrate–lectin binding, multivalent mannosyl-bearing TPE shows a good selectivity and sensitivity to Con A by switching on the fluorescence of water-soluble tetraphenylethylene-based glyco-conjugates in aqueous solution. Meanwhile, cellobiosyl-bearing TPE can be used to investigate enzymatic hydrolysis based on emission enhancing by glycosidase-induced aggregation.

Introduction

Carbohydrate-mediated biological interactions play a crucial role in numerous biological processes such as cell growth, recognition and differentiation, cancer metastasis, inflammation, and bacterial and viral infection.[1] As a model system of natural carbohydrates, synthetic glyco-conjugates have demonstrated to be an important well-defined tool for investigating carbohydrate-based biological events. In particular, fluorescent glyco-conjugates which possess both fluorescent dye and reporting carbohydrate ligands are attractively used in carbohydrate–protein interaction studies and biosensor applications because of their intrinsic optical properties, high sensitivities to minor stimuli, and good biocompatibilities.[2] Furthermore, fluorescence-based assays are convenient, cost-effective, and easily scaled-up to a high-throughput screening format.

However, when traditional dyes are dispersed in aqueous media or interacted with biomacromolecules, aggregation-induced fluorescence quenching often takes place, which results in drastically negative effects on efficiencies and sensitivities of biosensors or bioprobes.[3] To overcome this problem, recently, molecules with aggregation-induced emission characteristics have been developed and drawn much attention, due to their enhanced emission in aggregate form or solid-state.[4] The aggregation-induced emission (AIE) effect can greatly improve the fluorescence quantum yields of the molecules by up to three orders of magnitude, enhancing the photoluminescence (PL) intensity from faint luminophores into strong emitters.[5] Especially, since Tang's group reported the AIE feature of tetraphenylethylene (TPE)-based luminophors,[6] TPE-based AIE active materials have already shown practical applications in OLEDs,[6] chem-sensors,[5,7] and bio-probes,[8] due to their efficient preparation and facile functionalization.

Various functional groups were introduced to the tetraphenylethylene scaffold for potential sensing purposes. For example, TPE motif containing charged groups such as a sulfonate group or an ammonium group was used in the establishment of a fluorometric assay method for acetylcholinesterase or studies of a label-free DNA assay system.[8b,8c] Glucosamine hydrochloride-functionalized TPE developed by our group was used for fluorometric detection of alkaline phosphatase.[8e] However, the presence of ionic groups in TPE might cause some interfering responses due to nonspecific electrostatic interactions in a complicated biological environment, resulting in negative effects on sensitivity and selectivity.[9] Therefore, it is important to explore neutral TPE-based bio-probes or bio-sensors with well-defined structures to investigate biological interactions. Recently, neutral sugar-substituted tetraphenylethenes were designed as "turn-on" luminescent sensors for lectins and influenza virus.[10] To extend the potential applications of the TPE-based AIE active probes in biomacromolecule detections, herein, neutral water-soluble TPE-based artificial glycoclusters were designed and employed for studies of carbohydrate–protein interaction. In addition, a novel method to detect glycosidases based on the AIE effect was also developed. Integration of sugar moieties with TPE can not only improve water-solubility and bio-compatibility of the dyes, but also provide neutral ligands to bind specific proteins. As illustrated schematically in Scheme 1, carbohydrate-bearing TPE nearly shows no luminescence in diluted aqueous solution

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† Electronic supplementary information (ESI) available: ^{1}H NMR, ^{13}C NMR and MS spectra of the key compounds; fluorescence spectra of **CTPE-1** in the absence and presence of different proteins; fluorescence spectra of **CTPE-4** in the absence and presence of β-glucosidase. See DOI: 10.1039/c0ob00680g

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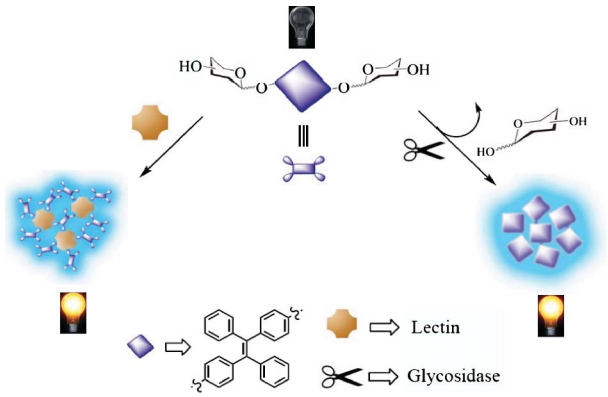

Scheme 1 Illustration of studies of carbohydrate–protein interaction using TPE-based artificial glyco-conjugates as fluorescent probes based on the AIE feature.

when existing in the molecular state. However, aggregation derived from specific carbohydrate–lectin binding or selective glycosidase-induced hydrolysis can "turn on" the photoluminescence due to the TPE moiety with the AIE feature, which can be used to study carbohydrate–protein interactions. Based on the rationale aforementioned, a multivalent mannosyl-bearing TPE was designed, which is expected to show high photoluminescence in the presence of Con A through aggregation derived from selective mannose–Con A binding. Meanwhile, cellobiosyl-bearing TPE, as a substrate for β-glucosidase, can be hydrolyzed by the enzyme in aqueous buffer solution and lose glucose units to give a fluorescent suspension due to the formation of a water-insoluble aggregate. Thus, it can be used to investigate enzymatic hydrolysis based on emission enhancing by glycosidase-induced aggregation.

Results and discussion

Design and synthesis of TPE-based glyco-cojugates

It's well-known that the affinity and specificity of carbohydrate-mediated biological interactions strongly depend on multivalency due to the so-called glycoside cluster effect.[11] Therefore, TPE-based multivalent glyco-conjugate, **CTPE-1**, was synthesized according to the synthetic route shown in Scheme 2. Treatment of known TPE derivative **1** with excess NaN_3 in DMF gave azido-functionalized TPE **2** quantitatively. Cu(I)-catalyzed "click" ligation[12] between azido-attached mannoside **4** derived from mannose derivative **3** and the known tetrakis(2-propynyloxymethyl)methane **5** furnished propargyl-bearing mannopyranoside cluster **6**, which was reacted with **2** through cycloaddition reaction again to afford peracetylated TPE-based multivalent glyco-conjugate **7**. After global deacetylation under Zemplén condition in NaOMe/MeOH, the desired glycocluster **CTPE-1** was obtained in an excellent yield. To investigate the significance of multivalency, as a control study, TPE derivative **CTPE-2** containing two mannopyranosyl moieties was also obtained according to a similar method from propargyl-attached TPE **8**. Glycosyl-bearing luminophores are often used to assess glycosidase capability. Therefore, cellobiosyl- or lactosyl-attached TPEs (**CTPE-3** or **CTPE-4**) were synthesized from propargyl glycosides (**10** or **11**) through "click" ligation and Zemplén deacetylation to detect specific glycosidase based on the AIE effect. All the synthetic glyco-conjugates are soluble in aqueous solution. All the synthetic TPE-based glyco-conjugates are soluble in aqueous solution and well characterized by ^1H NMR, ^{13}C NMR, IR, and MS (in the ESI†).

Aggregation derived from carbohydrate–lectin binding

In order to study carbohydrate–protein interaction using the synthetic water-soluble TPE-based glyco-conjugates, Concanavalin A (Con A), a member of the lectin family, was chosen as a target protein since it is a well-known α-mannose- or α-glucose-binding protein, which exists predominantly as a tetramer of four identical subunits and possesses four binding sites to interact with four α-mannose or α-glucose units simultaneously.[13] In dilute aqueous solution, as expected, **CTPE-1** nearly does not show any luminescence. After treatment with Con A (30 μM), the aqueous mixture is highly emissive, due to the formation of the **CTPE-1**–Con A ensemble. From the molecular solution in water to the aggregating state with Con A, the fluorescence intensity of **CTPE-1** at 469 nm increases by 11-fold (Fig. 1). The non-emissive nature of the molecular state and emissive nature of the aggregates were clearly indicated in the photographs given in the insets in Fig. 1. To evaluate the influence of the glycoside cluster effect, **CTPE-2** was also treated with Con A (30 μM) under the same conditions. In presence of Con A (30 μM), the fluorescence intensity of **CTPE-2** at 469 nm only increased by 3-fold compared to that in the absence of Con A, which means the TPE motif containing the glycocluster residue possesses a higher affinity to lectin. Fluorescence spectra of **CTPE-1** [20 μM in PBS (10 mM) buffer solution, pH = 7.6] in the presence of Con A at different concentrations were shown in Fig. 2, indicating that the emission enhancement of **CTPE-1** is observed at a concentration of Con A as low as 1.0 μM. To investigate the selectivity of fluorometric detection to Con A, **CTPE-1** was also treated by BSA protein under the same conditions and no significant change in photoluminescence intensity was observed (Fig. S1 in the ESI†).

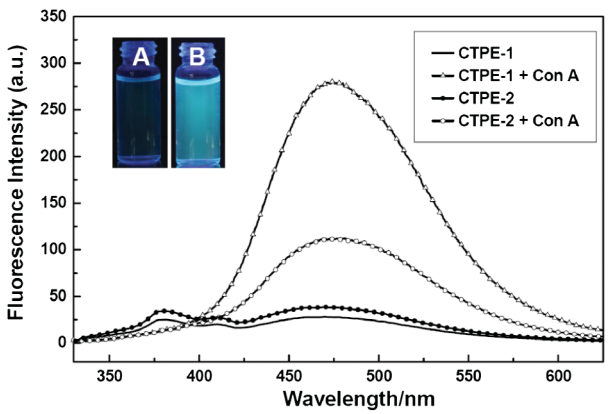

Fig. 1 Fluorescence spectra of **CTPE-1** and **CTPE-2** [20 μM in PBS (10 mM) buffer solution, pH = 7.6] in the absence and presence of Con A (30 μM); the insets display the photos of the corresponding buffer solutions of **CTPE-1** (20 μM) in the absence (A) and presence (B) of Con A (30 μM) under UV light (365 nm) illumination.

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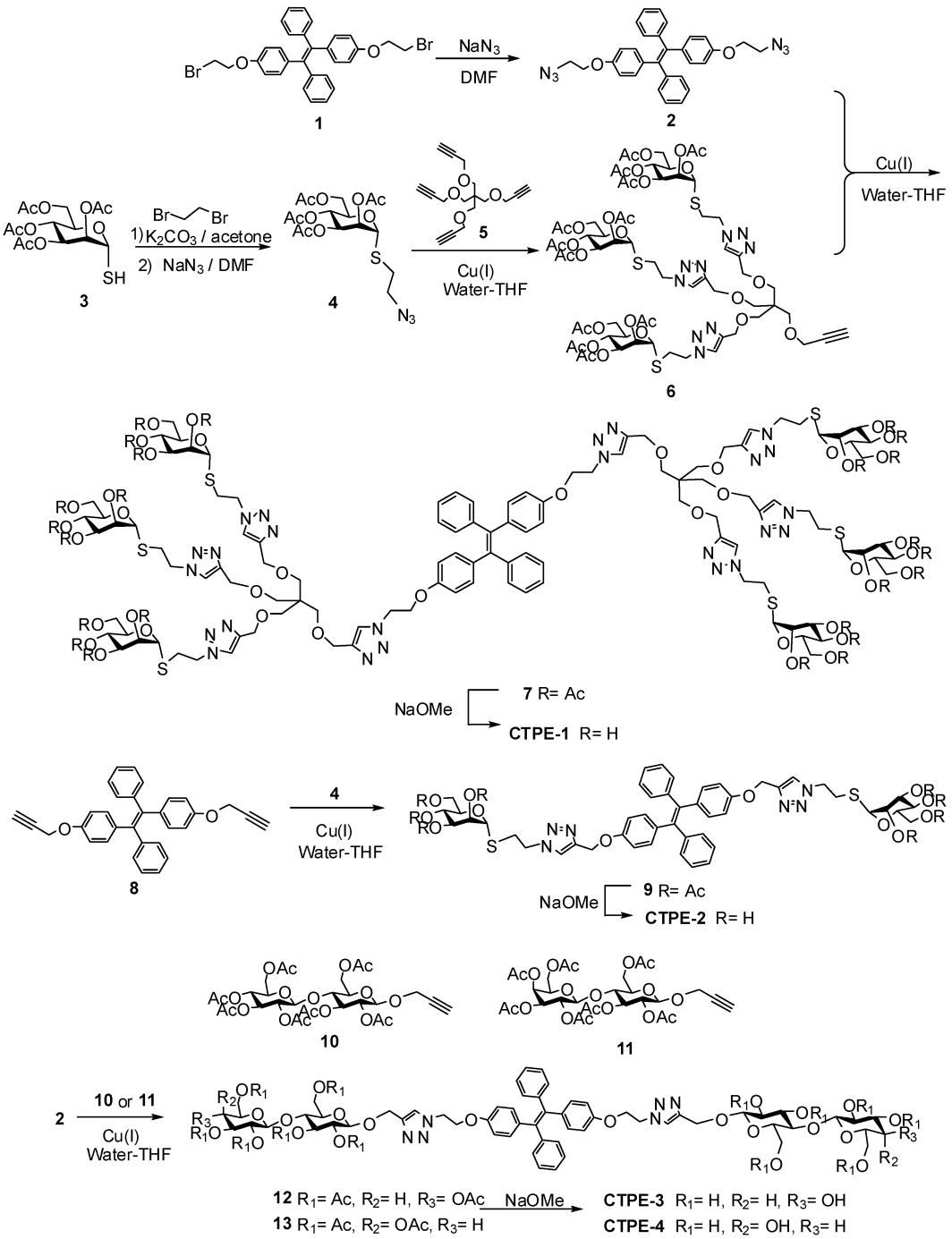

Scheme 2 Synthetic route to TPE-based glyco-conjugates.

Aggregation derived from glycosidase-induced hydrolysis

β-Glucosidase with specificity for a variety of β-D-glycoside substrates catalyzes the hydrolysis of terminal non-reducing residues in β-D-glucosides with release of glucose.[14] β-Cellobiosyl-carrying TPE (**CTPE-3**), as a certain substrate for β-glucosidase, can be hydrolyzed by the enzyme in aqueous buffer solution and lose glucose units one by one. Therefore, it can be expected that the water-soluble **CTPE-3** will be transformed gradually into water-insoluble TPE derivatives after enzymatic hydrolysis. In that case, the fluorescence intensity of the TPE moiety with AIE property will significantly increase due to glycosidase-induced aggregation and the purpose to detect the glycosidase can be achieved. As shown in Fig. 3, **CTPE-3** (20 μM) displays a weak fluorescence in aqueous buffer solution. After being treated with β-glucosidase (1.5 U), the resulting mixture became turbid (Fig. S2 in the ESI†), which indicates the formation of the aggregate. Fluorescence intensity of the resulting suspension at 460 nm increases by 9-fold compared to **CTPE-3** in absence of β-glucosidase. Photographs of solutions of **CTPE-3** and the resulting suspension in buffer

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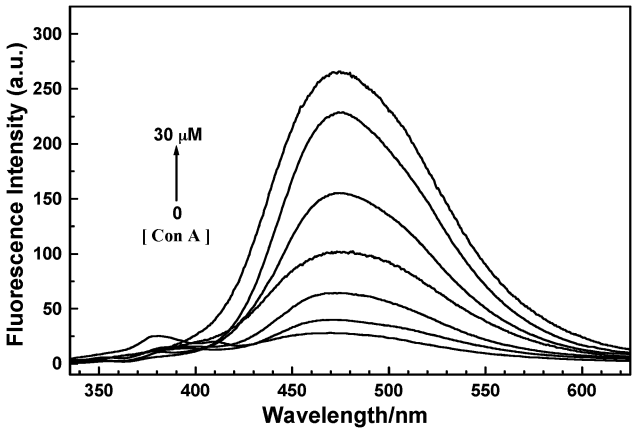

Fig. 2 Fluorescence spectra of **CTPE-1** [20 μM in PBS (10 mM) buffer solution, pH = 7.6] in the presence of Con A at different concentrations: Con A concentrations (μM) from the bottom upwards are 0, 5, 10, 15, 20, 25, and 30.

taken under illumination of a UV lamp are displayed in the insets in Fig. 3. The enzymatic hydrolysis process can be further confirmed by ESI-MS. Fig. 4 shows the mass spectrum of a **CTPE-3** sample digested by β-glucosidase for 1 h. The ion at *m/z* 1264.64 corresponds to $[CTPE+H]^+$. The signal peaks at *m/z* 1102.82, 939.33, 775.85, and 614.54 indicate the glucose losses, which clearly shows that the glucose residues in **CTPE-3** are released one by one. To investigate the selectivity of fluorometric detection to β-glucosidase, lactosyl-carrying TPE (**CTPE-4**) was also treated by β-glucosidase under the same conditions and no significant change of photoluminescence intensity was observed (Fig. S3 in the ESI†). The terminal non-reducing sugar residue in **CTPE-4** is β-galactose, which can not be easily hydrolyzed by β-glucosidase. It can be inferred from these data that functionalization of TPE with certain glycosyl residues provided a unique platform for selective glycosidase detection.

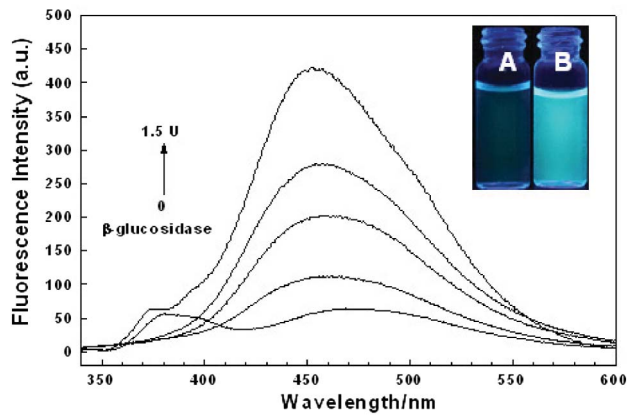

Fig. 3 Fluorescence spectra of **CTPE-3** [20 μM in citric acid–Na_2HPO_4 buffer solutions, pH = 5.8] in the absence and presence of β-glucosidase: β-glucosidase from the bottom upwards are 0, 0.5, 1.0, 1.2, and 1.5 U; the inset displays the photos of the corresponding buffer solutions of **CTPE-3** (20 μM) in the absence (A) and presence (B) of β-glucosidase (1.5 U) under UV light (365 nm) illumination.

Conclusions

In summary, four TPE-based glyco-conjugates were prepared through Cu(I)-catalyzed "click" ligation and used for studies of carbohydrate–protein interaction and related biosensing. Significant fluorescence change of water-soluble glyco-conjugates can be observed based on aggregation derived from specific carbohydrate–lectin binding or selective glycosidase-induced hydrolysis in aqueous solution, due to the TPE moiety with an AIE feature. We believe the method inspired by this novel concept to investigate carbohydrate-mediated biological interaction shows promising applications in biomacromolecule detections and glycobiology studies.

Experimental

Materials and characterization

All chemical reagents were commercially available and used as received unless otherwise stated. Ultra-pure water was purified with a Millipore purification system (Milli-Q water). Con A and β-glucosidase were purchased from Sigma-Aldrich Co. TPE derivatives (**1**, **8**)[3c,8e] and sugar derivatives (**3**, **10**, **11**)[15] were prepared according to reported methods.

The ^1H and ^{13}C NMR spectra were recorded on a Bruker DMX400 NMR spectrometer. Mass spectra were recorded with a Thermo LCQ Deca XP MAX mass spectrometer using the ESI(+) technique. The optical rotations were measured with a JASCO DIP-1000 digital polarimeter. Infrared (IR) spectra were recorded in KBr pellets using a Spectrum One Fourier transform IR spectrometer (Perkin Elmer Instruments Co. Ltd). Ultraviolet-visible (UV-vis) spectra were measured using a Perkin-Elmer Lambda 950 UV-vis-NIR spectrophotometer and quartz cells with 1 cm path length. The fluorescence spectra were measured in a conventional cell with 1 cm path length using a Perkin-Elmer LS55 spectrophotometer.

Azido-functionalized TPE 2

To a mixture of compound **1** (90 mg, 0.16 mmol) and NH_4Cl (17 mg, 0.31 mmol) in DMF solution (5 mL) was added NaN_3 (60 mg, 0.93 mmol). The resulting mixture was stirred at 80 °C for 12 h. The reaction mixture was then diluted with water (20 mL) and was further extracted with ethyl acetate (2 × 25 mL). The combined organic phase was washed with water (2 × 20 mL) and dried over anhydrous Na_2SO_4. After evaporation at diminished pressure, the desired product **2** was obtained as an off-yellow oil (72 mg, 95%). ^1H NMR (400 MHz, $CDCl_3$): δ 7.12–7.07 (m, 6H), 7.04–6.99 (m, 4H), 6.96–6.90 (m, 4H), 6.67–6.62 (m, 4H), 4.25–4.19 (m, 4H), 3.62–3.57 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.6, 144.1, 139.9, 137.2, 132.7, 131.5, 127.8, 126.4, 113.9, 67.8, 58.6. ESI(+)-MS: calcd. for $C_{30}H_{26}N_6O_2$: 502.21 [M]; found 503.29 $[M+H]^+$. IR (KBr): 2930, 2855, 2105, 1640, 1464, 1403, 1256, 800 cm^{-1}.

Thio-mannopyranosyl derivative 4

A mixture of **3** (2.12 g, 5.82 mmol) and potassium carbonate (930 mg, 6.73 mmol) in acetone–water (2 : 1, 24 mL) was stirred under nitrogen. Then, 1,2-dibromoethane (6.3 mL, 72.7 mmol)

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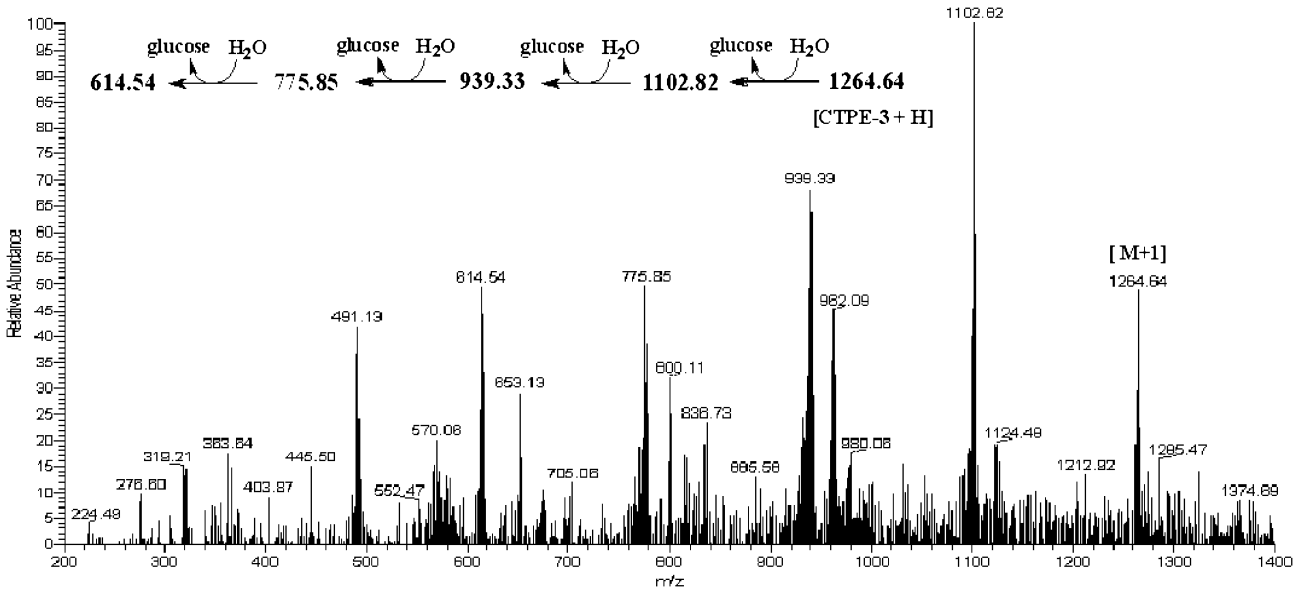

Fig. 4 Mass spectrum of **CTPE-3** solution digested by β-glucosidase for 1 h.

was added and the reaction was stirred at room temperature for 3 h. The mixture was extracted twice with CH_2Cl_2 (50 mL). The combined organic layer was dried with anhydrous Na_2SO_4 and concentrated to give a yellow oil. The residue was purified by a silica gel chromatography using petroleum ether and ethyl acetate (3 : 1) as eluent to give the bromide intermediate as a yellow oily liquid in 68% yield (1.86 g). $[\alpha]_D^{25}$ +65 (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.19 (s, 1H), 5.16 (s, 1H), 5.09 (t, J = 6.8 Hz, 1H), 5.03 (dd, J = 2.0, 6.4 Hz, 1H), 4.23 (t, J = 4.8 Hz, 1H), 4.11 (dd, J = 4.4, 8.0 Hz, 1H), 3.95 (d, J = 3.6 Hz, 1H), 3.50–3.46 (m, 1H), 3.39–3.34 (m, 1H), 2.99–2.78 (m, 2H), 1.99 (s, 3H), 1.94 (s, 3H), 1.90 (s, 3H), 1.82 (s, 3H). To a mixture of the bromide intermediate (470 mg, 1.00 mmol) and ammonium chloride (55 mg, 1.0 mmol) in dried DMF was added sodium azide (200 mg, 3.0 mmol). The resulting mixture was stirred at 80 °C for 7 h. The reaction mixture was poured into water (20 mL) and extracted twice with ethyl acetate (50 mL). The combined organic layer was washed once by water (20 mL). After separation, the organic layer was dried over Na_2SO_4 and concentrated to give a yellow oil. The residue was purified by a silica gel chromatography using petroleum ether and ethyl acetate (3 : 1) as eluent to give thio-mannopyranosyl derivative **4** as a yellow oily liquid (424.76 mg, 98%). $[\alpha]_D^{25}$ +56 (*c* 1, $CHCl_3$);^{1}H NMR (400 MHz, $CDCl_3$): δ 5.23–5.20 (m, 2H), 5.17 (t, J = 10.0 Hz, 1H), 5.09 (dd, J = 2.8, 10.0 Hz, 1H), 4.28–4.23 (m, 1H), 4.17 (dd, J = 6.0, 12.4 Hz, 1H), 3.98 (dd, J = 2.0, 12.0 Hz, 1H), 3.49–3.36 (m, 2H), 2.81–2.74 (m, 1H), 2.72–2.65 (m, 1H), 2.04 (s, 3H), 1.97, (s, 3H), 1.93 (s, 3H), 1.86 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.2, 169.6, 169.5, 169.4, 82.7, 70.5, 69.0, 65.9, 62.2, 50.6, 30.5, 20.6, 20.4, 20.3. ESI(+)-MS: calcd. for $C_{16}H_{23}N_3O_9S$: 433.12 [M]; found 456.08 [M+Na]$^+$. IR (KBr): 2935, 2860, 2100, 1750, 1450, 1250, 1080, 610 cm^{-1}.

Mannosyl cluster 6

To the mixture of thio-mannopyranosyl derivative **4** (454.5 mg, 1.05 mol) and **5** (145.3 mg, 0.50 mol) in THF–water (2 : 1, 45 mL) were added ascorbate sodium (30 mg) and $CuSO_4$ (20 mg) as catalyst. The mixture was refluxed for 1.5 h. The reaction mixture was cooled to room temperature and then extracted with ethyl acetate (2 × 30 mL). The organic layer was combined and concentrated at diminished pressure. The residue was purified by silica gel chromatography (ethyl acetate) to furnish **6** (166.6 mg, 30%). $[\alpha]_D^{25}$ +140 (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.67 (s, 3H), 5.28–5.20 (m, 9H), 5.14 (dd, J = 2.8, 10.0 Hz, 3H), 4.60–4.57 (m, 7H), 4.55 (s, 6H), 4.29 (d, J = 8.4 Hz, 3H), 4.25 (d, J = 6.4 Hz, 3H), 4.08 (t, J = 8.4 Hz, 6H), 4.03 (d, J = 6.0 Hz, 6H), 3.18–3.08 (m, 6H), 2.42 (s, 1H), 2.12 (s, 9H), 2.02 (s, 9H), 2.00 (s, 9H), 1.94 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6, 169.9, 169.7, 167.7, 145.6, 123.0, 82.9, 82.0, 74.4, 71.8, 70.8, 69.5, 69.3, 69.1, 66.1, 65.9, 64.9, 62.4, 60.4, 58.7, 58.2, 49.5, 21.1, 20.9, 20.6, 19.2. ESI(+)-MS: calcd. for $C_{65}H_{89}N_9O_{31}S_3$: 1588.64 [M]; found 1611.70 [M+Na]$^+$. IR (KBr): 2928, 2855, 2100, 1750, 1464, 1403, 1240, 1080, 650 cm^{-1}.

Peracetylated TPE-based mannopyranosyl cluster 7

Glyco-cluster **6** (88.9 mg, 55.95 μmol) and compounds **2** (15.3 mg, 30.4 μmol) in THF–water (2 : 1, 15 mL) was added a freshly prepared aqueous ascorbate sodium (30 mg) and $CuSO_4$ (20 mg). The mixture was stirred at 80 °C for 9 h. The resulting mixture was extracted twice with ethyl acetate (50 mL). The combined organic layer was dried over Na_2SO_4 and concentrated to give a yellow oil. Silica gel chromatography (ethyl acetate : methanol = 20 : 1) gives compound **7** in a yield of 52% (53.2 mg). $[\alpha]_D^{25}$ +185 (*c* 0.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.71 (br, 8H), 7.09–7.04 (m, 6H), 6.99–6.94 (m, 4H), 6.92–6.87 (m, 4H), 6.62–6.57 (m, 4H), 5.31 (s, 10H), 5.28 (d, J = 9.6 Hz, 6H), 5.18 (dd, J = 2.4, 10.0 Hz, 6H), 4.71 (br, 6H), 4.62–4.58 (m, 12H), 4.55 (br, 18H), 4.35–4.26 (m, 18H), 4.13–4.06 (m, 18H), 3.22–3.10 (m, 12H), 2.14 (s, 18H), 2.05 (s, 18H), 2.04 (s, 18H), 1.97 (s, 18H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6, 169.9, 169.8, 167.8, 156.4, 145.6, 145.2, 139.8, 137.3, 132.7,

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132.5, 131.4, 131.0, 128.9, 127.8, 124.0, 123.1, 113.9, 113.7, 82.9, 71.9, 70.8, 69.5, 69.4, 69.2, 66.2, 65.0, 62.5, 58.5, 49.8, 45.3, 21.0, 20.8, 20.7, 19.3. ESI(+)-MS: calcd. for $C_{160}H_{204}N_{24}O_{64}S_6$: 3679.18 [M]; found 3702.31 $[M+Na]^+$. IR (KBr): 2930, 2850, 1640, 1465, 1403, 1250, 1080, 650 cm^{-1}.

CTPE-1

To a solution of **7** (36 mg, 9.78 μmol) in methanol (5 mL) was added a 1 M solution of NaOMe in methanol until the pH value reached 11. The reaction was stirred for 24 h at room temperature, and then, the resulting mixture was neutralized by acidic resin. After filtration, the filtrate was concentrated to furnish **CTPE-1** as a white solid (24.56 mg, 95%). $[\alpha]_D^{25}$ +163 (*c* 0.2, H_2O); ^{1}H-NMR (400 MHz, d_6-DMSO): δ 8.21 (br, 8H), 7.42–7.38 (m, 6H), 7.25–7.18 (m, 8H), 6.72–6.69 (m, 4H), 5.78 (s, 16H), 5.22 (s, 6H), 5.02 (s, 16H), 4.70–4.60 (m, 12H), 4.59–4.49 (m, 12H), 3.78–3.60 (m, 20H), 3.48–3.40 (m, 10H), 3.39–3.37 (m, 14H), 3.34–3.30 (m, 4H), 3.20–3.07 (m, 20H). ^{13}C-NMR (100 MHz, d_6-DMSO): δ 156.3, 145.8, 145.1, 139.7, 137.5, 132.6, 132.4, 131.5, 131.0, 128.8, 127.8, 124.1, 123.1, 113.8, 113.6, 82.9, 71.6, 70.8, 69.7, 69.5, 69.1, 62.5, 60.9, 59.1, 53.5, 49.8, 45.2. ESI(+)-MS: calcd. for $C_{112}H_{156}N_{24}O_{40}S_6$: 2670.96 [M]; found 2694.79 $[M+Na]^+$. IR (KBr): 3400, 2928, 2857, 1634, 1464, 1403, 1085, 799 cm^{-1}. Elem. anal. calcd: C, 50.36; H, 5.89; found: C, 50.42; H, 5.92.

Peracetylated TPE-based mannopyranosyl derivative 9

To the mixture of **8** (95 mg, 0.21 mol) and mannosyl derivative **4** (202 mg, 0.46 mol) in THF–water (2 : 1, 10 mL) were added ascorbate sodium (15 mg) and $CuSO_4$ (10 mg) as catalyst. After refluxing for 8 h, the reaction mixture was cooled to room temperature and then extracted with ethyl acetate (2 × 35 mL). The combined organic layer was concentrated and then purified by silica gel chromatography (petroleum ether : ethyl acetate = 1 : 2) to yield **9** (270.8 mg, 96%) as a foamy solid. $[\alpha]_D^{25}$ +110 (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.68 (s, 2H), 7.08–6.97 (m, 10H), 6.91 (d, *J* = 8.4 Hz, 4H), 6.70 (d, *J* = 8.8 Hz, 4H), 5.29 (d, *J* = 3.2 Hz, 2H), 5.24 (s, 2H), 5.17 (dd, *J* = 3.6, 10.0 Hz, 2H), 5.10 (d, *J* = 11.6 Hz, 4H), 4.65–4.53 (m, 4H), 4.32–4.26 (m, 4H), 4.12–4.06 (m, 4H), 3.21–3.07 (m, 4H), 2.14 (s, 6H), 2.03 (s, 6H), 2.01 (s, 6H), 1.96 (s, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 170.0, 169.8, 169.7, 156.7, 144.0, 139.8, 137.0, 132.6, 131.4, 127.7, 127.6, 126.3, 113.9, 82.9, 70.7, 69.5, 69.2, 66.1, 62.5, 61.8, 60.4, 49.7, 20.9, 20.7, 20.6, 18.5. ESI(+)-MS: calcd. for $C_{64}H_{70}N_6O_{20}S_2$: 1307.40 [M]; found 1308.55 $[M+H]^+$. IR (KBr): 2935, 2850, 1643, 1465, 1400, 1240, 1080, 660 cm^{-1}.

CTPE-2

To a solution of compound **9** (100 mg, 76 μmol) in methanol (15 mL), 1 M sodium methoxide in methanol was added dropwise until pH = 11. The reaction mixture was stirred at room temperature overnight. After neutralization with Amberlite IR120 acidic resin and filtration, the solvent was removed under reduced pressure to give the desired product **CTPE-2** (65 mg, 90%). $[\alpha]_D^{25}$ +105 (*c* 0.5, DMF); ^{1}H NMR (400 MHz, d_6-DMSO): δ 8.25 (s, 2H), 7.18–7.05 (m, 6H), 6.99–6.94 (m, 4H), 6.90 (d, *J* = 8.8 Hz, 4H), 6.83 (d, *J* = 8.8 Hz, 4H), 5.25 (s, 2H), 5.06 (s, 4H), 5.04 (d, *J* = 9.3 Hz, 2H), 4.87 (br, 2H), 4.77(br, 2H), 4.70–4.63 (m, 4H), 4.61–3.56 (m, 2H), 3.72 (d, *J* = 11.6 Hz, 2H), 3.69 (s, 2H), 3.65 (d, *J* = 6.8 Hz, 2H), 3.47 (dd, *J* = 7.2, 11.6 Hz, 2H), 3.41–3.37 (m, 4H), 3.09 (t, *J* = 6.8 Hz, 4H). ^{13}C NMR (100 MHz, d_6-DMSO): δ 156.6, 143.7, 142.4, 139.3, 136.0, 132.0, 130.8, 128.7, 127.8, 124.8, 114.0, 85.4, 74.8, 71.7, 71.5, 67.3, 61.2, 56.0, 52.6, 49.2. ESI(+)-MS: calcd. for $C_{48}H_{54}N_6O_{12}S_2$: 971.11 [M]; found 994.75 $[M+Na]^+$. IR (KBr): 3400, 2934, 2843, 1640, 1464, 1400, 1075, 810 cm^{-1}. Elem. anal. calcd: C, 59.37; H, 5.60; found: C, 59.30; H, 5.64.

TPE-based peracetylated β-cellobioside 12

To a solution of peracetylated β-cellobiose (0.68 g, 1.0 mmol) in 5 mL of anhydrous dichloromethane were added boron trifluoride diethyl etherate (0.85 g, 3.0 mmol) and propargyl alcohol (0.07 g, 1.2 mmol). The mixture was stirred for 2 h at room temperature, and then diluted with dichloromethane (30 mL) and the organic phase was washed with brine (60 mL) and dried over anhydrous Na_2SO_4. The solvent was removed to give peracetylated propargyl β-cellobioside **10** as a white powder (0.54 g, 80%). The crude product was used without further purification. To a mixture of **2** (53 mg, 0.11 mmol) and **10** (178 mg, 0.26 mmol) in H_2O–THF (1 : 1, 10 mL) were added freshly prepared aqueous 1.0 M sodium ascorbate (150 μL, 0.15 mmol) and $CuSO_4$ (12 mg, 0.075 mmol). The heterogeneous mixture was stirred vigorously in a dark room at 50–60 °C until the complete consumption of the reactants based on TLC analyses. After removal of THF under reduced pressure, water (20 mL) was added and the product was extracted with EtOAc (3 × 25 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The crude product was subjected to column chromatography (ethyl acetate : petroleum ether = 5 : 1) to give **12** as a foamy solid (162 mg, 80%). $[\alpha]_D^{25}$ −31 (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.69 (s, 2H), 7.07–7.03 (m, 6H), 6.96–6.93 (m, 4H), 6.90 (d, *J* = 8.4 Hz, 4H), 6.59 (d, *J* = 8.8 Hz, 4H), 5.17–5.10 (m, 4H), 5.05 (t, *J* = 8.8 Hz, 2H), 4.92–4.87 (m, 6H), 4.77 (d, *J* = 12.8 Hz, 2H), 4.71 (t, *J* = 10.0 Hz, 4H), 4.62 (d, *J* = 8.0 Hz, 2H), 4.54–4.50 (m, 4H), 4.35 (dd, *J* = 4.4, 8.4 Hz, 2H), 4.27 (t, *J* = 6.0 Hz, 4H), 4.13–4.07 (m, 2H), 4.02 (dd, *J* = 2.0, 10.4 Hz, 2H), 3.78 (t, *J* = 19.2 Hz, 2H), 3.70–3.60 (m, 4H), 2.10 (s, 6H), 2.06 (s, 6H), 2.01 (s, 6H), 1.99 (s, 12H), 1.96 (s, 6H), 1.89 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6, 170.4, 170.3, 169.8, 169.7, 169.4, 169.1, 156.2, 144.2, 143.8, 137.4, 132.7, 131.3, 127.7, 126.4, 124.0, 113.8, 100.8, 99.7, 76.4, 73.0, 72.9, 72.5, 72.0, 71.6, 71.5, 67.8, 66.2, 62.9, 61.8, 61.6, 58.4, 49.9, 20.9, 20.7, 20.6, 18.5. ESI(+)-MS: calcd. for $C_{88}H_{102}N_6O_{38}$: 1850.62 [M]; found 1873.50 $[M+Na]^+$. IR (KBr): 2925, 2850, 1644, 1465, 1408, 1250, 1080, 660 cm^{-1}.

TPE-based peracetylated β-lactoside 13

TPE-based peracetylated β-lactoside **13** was obtained as a foamy solid (158 mg, 78%) from peracetylated β-lactose (0.68 g, 1.0 mmol) according to the same synthetic method for **12**. $[\alpha]_D^{25}$ −52 (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.67 (s, 2H), 7.10–7.02 (m 6H), 7.00–6.97 (m, 4H), 6.89 (d, *J* = 8.8 Hz, 4H), 6.57 (d, *J* = 8.8 Hz, 4H), 5.33 (d, *J* = 2.8 Hz, 2H), 5.18 (t,

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J = 18.8 Hz, 4H), 5.09 (dd, J = 8.0, 10.4 Hz, 2H), 4.94 (dd, J = 3.2, 10.4 Hz, 2H), 4.88 (d, J = 10.8 Hz, 4H), 4.77 (d, J = 12.8 Hz, 2H), 4.69 (t, J = 5.6 Hz, 4H), 4.62 (d, J = 8.0 Hz, 2H), 4.47 (d, J = 8.0 Hz, 2H), 4.25 (t, J = 4.4 Hz, 4H), 4.11–4.06 (m, 8H), 3.86 (t, J = 13.6 Hz, 2H), 3.79 (t, J = 18.8 Hz, 2H), 3.64–3.60 (m, 2H), 2.14 (s, 6H), 2.10 (s, 6H), 2.03 (bs, 18H), 1.95 (s, 6H), 1.89 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 170.3, 170.2, 169.8, 169.7, 169.2, 156.2, 144.2, 143.9, 139.7, 137.4, 132.7, 131.3, 131.0, 128.9, 127.8, 126.5, 124.0, 113.7, 101.1, 99.7, 76.2, 72.9, 72.8, 71.9, 71.6, 71.5, 71.1, 69.1, 66.7, 66.2, 62.9, 61.9, 60.8, 58.4, 49.9, 21.0, 20.9, 20.7, 20.6, 19.2, 18.5. ESI(+)-MS: calcd. for $C_{88}H_{102}N_6O_{38}$: 1850.62 [M]; found 1873.71 $[M+Na]^+$. IR (KBr): 2926, 2850, 1645, 1463, 1405, 1250, 1080, 660 cm^{-1}.

CTPE-3

To a solution of TPE-based peracetylated β-cellobioside **12** (100 mg, 0.054 mmol) in dry CH_2Cl_2 (5 mL) and MeOH (5 mL) was added 1.0 M CH_3ONa/CH_3OH solution dropwise. The pH was adjusted to 11 and stirred at room temperature for 4 h, and then, the resulting mixture was neutralized to 7.0 using acidic resin. After filtration, the filtrate was collected and concentrated under reduced pressure to give **CTPE-3** as a foamy solid (65 mg, 95%). $[\alpha]_D^{25}$ −25 (c 0.2, DMF); ^{1}H NMR (400 MHz, d_6-DMSO): δ 8.19 (s, 2H), 7.11–7.05 (m, 6H), 6.94–6.90 (m, 4H), 6.85–6.80 (m, 4H), 6.72–6.66 (m, 4H), 5.71 (br, 14H), 4.85 (d, J = 12.0 Hz, 2H), 4.71 (br, 4H), 4.63 (d, J = 12.0 Hz, 2H), 4.34–4.26 (m, 8H), 3.79 (d, J = 11.2 Hz, 2H), 3.69–3.62 (m, 4H), 3.45–3.38 (m, 4H), 3.31 (s, 4H), 3.21–3.15 (m, 4H), 3.08–2.97 (m, 6H). ^{13}C NMR (100 MHz, d_6-DMSO): δ 156.3, 143.7, 139.4, 136.3, 132.0, 130.8, 127.9, 126.4, 124.9, 114.0, 113.9, 103.3, 101.9, 80.6, 76.9, 76.5, 75.1, 73.4, 73.2, 70.1, 66.0, 61.6, 61.0, 60.5, 56.1, 49.2. ESI(+)-MS: calcd. for $C_{60}H_{74}N_6O_{24}$: 1263.26 [M]; found 1264.95 $[M+H]^+$. IR (KBr): 3400, 2930, 2845, 1640, 1463, 1403, 1075, 790 cm^{-1}. Elem. anal. calcd: C, 57.05; H, 5.90; found: C, 57.10; H, 5.93.

CTPE-4

CTPE-4 was obtained as a foamy solid (44 mg, 65%) from TPE-based peracetylated β-lactoside **13** (0.68 g, 1.0 mmol) according to the same synthetic method for **CTPE-3**. $[\alpha]_D^{25}$ −46 (c 0.2, DMF); ^{1}H NMR (400 MHz, d_6-DMSO): δ 8.18 (s, 2H), 7.13–7.07 (m, 6H), 6.95–6.90 (m, 4H), 6.86–6.80 (m, 4H), 6.70–6.66 (m, 4H), 4.85 (d, J = 12.0 Hz, 2H), 4.70 (br, 18H), 4.62 (d, J = 12.0 Hz, 2H), 4.34–4.21 (m, 8H), 3.81 (bs, 4H), 3.64 (s, 4H), 3.49–3.41 (m, 8H), 3.32 (s, 8H), 3.04 (br, 2H). ^{13}C NMR (100 MHz, d_6-DMSO): δ 156.2, 143.8, 143.7, 139.3, 136.2, 132.0, 131.7, 130.8, 128.8, 127.9, 126.5, 124.8, 113.9, 113.8, 103.9, 101.8, 80.6, 75.6, 75.0, 74.9, 73.3, 73.1, 70.7, 68.0, 66.0, 61.7, 60.5, 60.3, 56.1, 49.1. ESI(+)-MS: calcd. for $C_{60}H_{74}N_6O_{24}$: 1263.26 [M]; found 1264.91 $[M+H]^+$. IR (KBr): 3400, 2930, 2843, 1640, 1465, 1406, 1070, 790 cm^{-1}. Elem. anal. calcd: C, 57.05; H, 5.90; found: C, 57.09; H, 5.88.

Studies of carbohydrate–lectin interaction based on spectrofluorometric titration

A solution of **CTPE-1** was prepared in PBS (10 mM) buffer solution (pH = 7.6) containing 0.1 mM $CaCl_2$ and 0.1 mM $MnCl_2$. Aliquots of lectin in the same buffer were added to the solution. The final concentration of **CTPE-1** is 20 μM. After each addition, the sample was allowed to equilibrate for 2 h prior to recording a spectrum. Additions of lectin were continued until no significant change in the fluorescence signal was observed. The excitation wavelength was 360 nm and the emission scan ranged from 330 nm to 650 nm.

Studies of glycosidase-induced hydrolysis based on fluorescence spectrum

A solution of **CTPE-3** was prepared in citric acid–Na_2HPO_4 buffer solutions (pH = 5.8). Aliquots of β-glucosidase in the same buffer were added to the solution. The final concentration of **CTPE-3** is 20 μM. After each addition, the sample was allowed to equilibrate for 3 h prior to recording a spectrum. The excitation wavelength was 360 nm and the emission scan ranged from 330 nm to 650 nm.

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