Organic & Biomolecular Chemistry

Dynamic Article Links

Cite this: *Org. Biomol. Chem.*, 2012, **10**, 555

www.rsc.org/obc

PAPER

Discovery of a sensitive Cu(II)-cyanide "off–on" sensor based on new *C*-glycosyl triazolyl bis-amino acid scaffold†

Yan-Hui Tang,[a] Yi Qu,[a] Zhuo Song,[a] Xiao-Peng He,*[a,b] Juan Xie,[b] Jianli Hua*[a] and Guo-Rong Chen*[a]

Received 24th July 2011, Accepted 19th October 2011
DOI: 10.1039/c1ob06242e

A new functional glycosyl peptidomimetic, featuring a *C*-glucosyl 1,4-dimethoxynaphthalene backbone in conjugation with two triazolyl phenylalanine moieties on its adjacent C3,4-positions, was readily synthesized *via* click chemistry. Primary optical measurements indicated that the fluorescence of the ester form of this probe (**4**) could be selectively quenched by Pb^{2+}. In contrast, the fluorescence intensity of its analog **5** with released carboxylic groups was uniquely diminished by Cu^{2+} with remarkably enhanced sensitivity and selectivity. Moreover, subsequent addition of cyanide to the methanol solution of the resulting Cu^{2+}-**5** complex induced its fluorescence recovery with a nanomolar detection limit, which was two orders of magnitude smaller than the regulated concentration limit of CN^- in drinking water. This suggests the promising applicability of *C*-glycosyl bis-triazolyl amino acid scaffold in the future design and exploration of sensitive "off–on" Cu(II)-cyanide chemosensors.

Introduction

Cyanide is acutely toxic toward both environmental and biological systems,[1–3] therefore considerable efforts have been devoted to the development of effective chemosensors.[4–9] Among the various intelligent strategies in designing cyanide sensors, the use of Cu(II)-compound complexes for sensing CN^-, which takes advantage of the high affinity between copper and cyanide,[10] has emerged as a novel effective approach.[11,12] The general rationale behind this strategy relies on cyanide induced fluorescence recovery of a chemosensor that has previously been fluorescently deactivated through complexation with Cu(II). However, since the regulated concentration of cyanide in drinking water is extraordinarily low due to its extreme toxicity,[3] the majority of currently developed probes encounter the main drawback of unsatisfactory detection limits. As a consequence, further exploration of new chemical entities that are highly sensitive to both copper(II) and cyanide is fairly desirable.

Development of fluorescent compounds using small-molecule sugar units as the core scaffold is a rarely explored but promising alternative for the discovery of potent chemosensors as naturally occurring sugars possess substantial merits such as well-defined stereo-structures, low toxicity and high bioavailability.[13] In using Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC)[14] which is the best paradigm of click chemistry,[15,16] we have recently synthesized a series of bidentate glycomimetics that have bis-triazolyl functional groups on two different sites of a monosaccharide.[17] Some of these compounds bearing fluorophores were identified as new selective metal chelators.

With continued interest in developing sugar-based fluorescent probes, we report herein our new discovery of a bis-triazolyl *C*-glycosyl amino acid derivative that may potentially serve as an "off–on" Cu(II)–cyanide sensor with desirable sensitivity, however with moderate selectivity over phosphates. Enlightened by the structural feature of our previously synthesized *C*-glycosyl 1,4-dimethoxynaphthalene that embodies a free sugar moiety and a naphthalene group (**1**, Fig. 1), two phenylalaninyl fragments were further introduced onto the adjacent C3,4-position of the glycosyl scaffold *via* CuAAC for embracing ions. The fluorescence of the ester form of this probe was identified to be selectively quenched by Pb^{2+}, whereas that of its carboxylic acid-exposed analog was uniquely diminished by Cu^{2+} with more enhanced sensitivity. Subsequent addition of CN^- to this Cu^{2+}-probe complex induced its fluorescence recovery with a nanomolar detection limit.

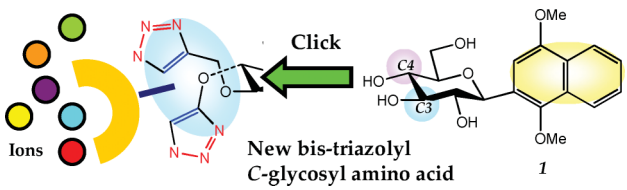


Fig. 1 Construction of bis-triazolyl *C*-glycosyl amino acid derivatives as new ion chemosensors.

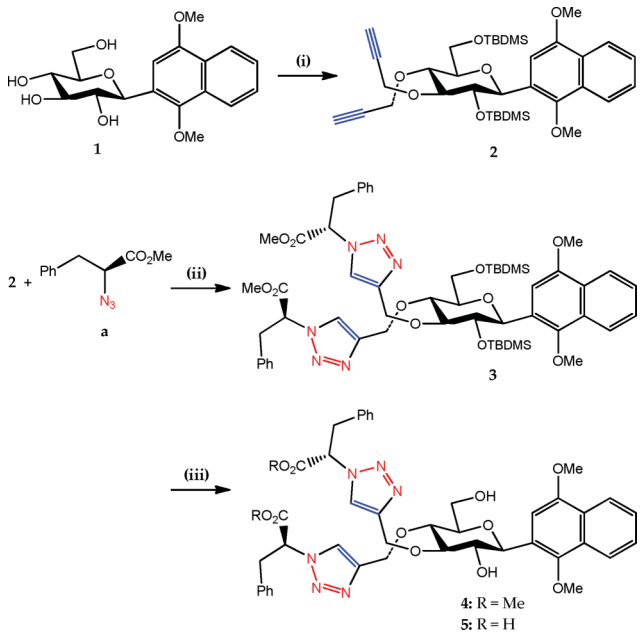
[a]*Key Laboratory for Advanced Materials and Institute of Fine Chemicals, East China University of Science and Technology, 130 Meilong Road, Shanghai, 200237, PR China. E-mail: frogmaster_eminem@yahoo.com.cn, jlhua@ecust.edu.cn, mrs_guorongchen@ecust.edu.cn; Fax: +86-21-64252758; Tel: +86-21-64253016*
[b]*PPSM, Institut d'Alembert, ENS de Cachan, CNRS UMR 8531, 61 Avenue du Pt Wilson, F-94235, Cachan, France*

† Electronic supplementary information (ESI) available: Fig. S1–S3, ^1H and ^{13}C NMR copies of compounds **3**–**5**. See DOI: 10.1039/c1ob06242e

Results and discussion

Synthesis

Using our previously prepared aryl *C*-glucoside **1**[18] as the starting material, 3,4-di-*O*-propynyl-2,6-*O*-TBDMS-*C*-glucosyl 1,4-dimethoxynaphthalene **2** was readily synthesized *via* selective 2,6-silylation by TBDMSCl, and then *O*-alkylation in the presence of propargyl bromide and NaH.[17c] A dual click reaction between this di-alkynyl *C*-glycoside and an azido phenylalanine (**a**)[19] promoted by excessive Na ascorbate and $CuSO_4 \cdot 5H_2O$[17c] sequentially led to the bis-triazolyl amino acid-*C*-glucoside conjugate **3** in a moderate yield of 65%. Desilylation of compound **3** by AcCl gave the desired compound in its ester form (**4**). Subsequent removal of the methyl esters in the presence of LiOH eventually resulted in the formation of carboxylic acid-exposed glycosyl bis-amino acid **5** in quantitative yield (Scheme 1).



Scheme 1 Reagents and conditions: (i) TBDMSCl, DMAP, pyridine; then propargyl bromide, NaH, DMF; (ii) Na ascorbate, $CuSO_4 \cdot 5H_2O$, CH_2Cl_2/H_2O; (iii) AcCl, MeOH, then LiOH, MeOH.

Optical study

The metal cation sensitivity of the ester-form of probe **4** in methanol was first studied *via* fluorescence spectroscopy by excitation at 242 nm, shown in Fig. 2. Upon addition of 10 equiv. of various selected metal cations (100 μM) including Ba^{2+}, Mn^{2+}, Co^{2+}, Zn^{2+}, Al^{3+}, Ni^{2+}, Cd^{2+}, Ag^{+}, Hg^{2+}, Fe^{2+}, Cu^{2+} and Pb^{2+} to the methanol solution of **4** (10 μM), its fluorescence intensity altered accordingly (Fig. 2a). We observed that Pb^{2+} could more compellingly quench the fluorescence of compound **4** by a quenching rate ($[F_0 - F]/F_0$, where F_0 is the original fluorescence intensity and F is the varied fluorescence intensity in the presence of the metal) of about 50% compared with other competing cations. However, we also noticed that the presence of Cu^{2+} and Ag^{+} may lead to the fluorescence quenching of **4** by a relatively smaller rate of 20% and 15%, respectively. This should be

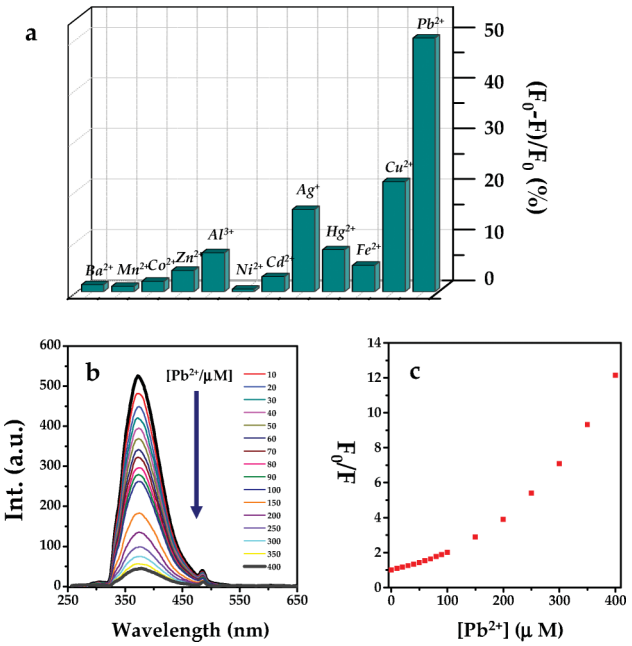


Fig. 2 (a) Fluorescence quenching rate in the presence of 100 μM of various metal cations; (b) Fluorescence spectrum in the presence of various concentrations (10 to 400 μM) of Pb^{2+}; (c) Stern–Volmer plot in the presence of various concentrations (0 to 400 μM) of Pb^{2+} of compound **4** (10 μM) in methanol (λ_{ex} = 242 nm).

ascribable to the broad-range binding ability of the N-rich triazole moiety with various metal cations.[20]

A fluorescence titration experiment of **4** in the presence of Pb^{2+} with varied concentrations was then performed, shown in Fig. 2b and Fig. 2c. By excitation at 242 nm, a narrow emission band of probe **4** (10 μM in methanol) ranging from 300 to 500 nm was observed (Fig. 2b, top curve). Clearly, the gradual increase of Pb^{2+} from 10 μM to 400 μM induced the gradual decrease of fluorescence intensity of **4**, while the quenching plateau could be reached in the presence of 40 equiv. Pb^{2+}. The detection limit of this probe for Pb^{2+} in methanol was calculated to be 0.29 μM (Fig. S1a, ESI†).[12d]

Having preliminarily determined the metal ion sensitivity of the ester-form of the designed probe, we sequentially attempted to study the fluorescence change of its analog **5** with exposed carboxylic acid groups in the presence of the same selection of cations as described above (excitation at 242 nm). To our surprise, by simply releasing the carboxylic acid groups, the resulting sensitivity as well as specificity of probe **5** was significantly varied. Fig. 3a depicts the specificity of **5** (10 μM in methanol) toward various metal cations, which reveals that this probe is sensitive to binding with Cu^{2+} as the presence of only 1 equiv. of Cu^{2+} may afford a fluorescence quenching rate of approximately 75%. In the meanwhile, whereas **5** gave almost no response to the addition of Ba^{2+}, Mg^{2+} and Cd^{2+}, the presence of other competing cations such as Co^{2+}, Ni^{2+}, Fe^{2+} and Pb^{2+} resulted in its slight fluorescence decrease by a rate of less than 20%.

It is interesting that the removal of the methyl ester group of compound **4** has caused its remarkable change in binding preference from Pb^{2+} to Cu^{2+}, which is most likely ascribable to the specific coordination of Cu^{2+} with the released carboxylic acid groups.[11d] Furthermore, by comparing with several

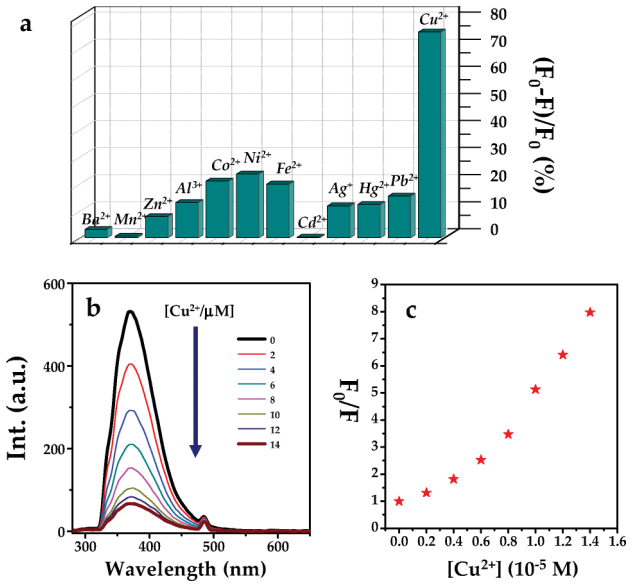


Fig. 3 (a) Fluorescence quenching rate in the presence of various metal cations (10 μM); (b) Fluorescence spectrum in the presence of various concentrations (0 to 14 μM) of Cu^{2+}; (c) Stern–Volmer plot in the presence of various concentrations (0 to 14 μM) of Cu^{2+} of compound **5** (10 μM) in methanol (λ_{ex} = 242 nm).

former reports in which the developed fluorescent probes were generally equally sensitive to Cu^{2+} and another competing cation,[21] compound **5** takes advantage of its more distinct selectivity. Meanwhile, the sensitivity of probe **5** is significantly enhanced (*vs.* **4**) with a low detection limit of 14 nM (Fig. S1b, ESI†). This value is comparable to recent work wherein heterocyclic mono-amino acid frameworks were developed as Cu^{2+} chelators.[21a] However, those chelators showed unsatisfactory selectivity for Cu^{2+} over Co^{2+}, Ni^{2+} and especially Zn^{2+}, which means that the bis-triazolyl amino acid system constructed herein on a sugar scaffold is advantageous for achieving improved metal-sensing selectivity with simultaneously comparable sensitivity.

The fluorescence spectrum excited at 242 nm of compound **5** shown in Fig. 3b similarly interprets a narrow emission band from 300 to 500 nm (top curve). Upon addition of Cu^{2+} with gradually increased concentration (0–14 μM) to the methanol solution of **5** (10 μM), the corresponding fluorescence intensity gradually decreased till quenching plateau at the highest concentration, shown in Fig. 3b and 3c. To test the potential practicality of **5**, a fluorescence titration experiment in the presence of various concentrations of Cu^{2+} was further performed in pure water. As shown in Fig. S2a (ESI†), the emission band ranging from 300 to 500 nm of **5** was equally observed by excitation at 242 nm in water, while the gradual addition of Cu^{2+} to this aqueous solution led to the gradual decrease in fluorescence intensity. Eventually, the presence of 50 equiv. of Cu^{2+} caused a quenching rate of about 50%. The corresponding detection limit calculated through Fig. S2b (ESI†) was assigned to 44 μM. Despite the diminished sensitivity, probe **5** has proven useful in the direct sensing of Cu^{2+} in pure water, unravelling the applicability of glycosyl bis-triazolyl amino acid framework for the development of water soluble chemosensors.

The quantum yields (Φ_F) of **4** and **5** in methanol were assigned to 0.278 and 0.279, respectively, using L-tryptophan (Φ_F = 0.13 in water) as the reference.

The strategy for the sensing of cyanides using fluorescently deactivated Cu^{2+}-sensor complexes has been well established in the literature.[11,12] Hence, we further examined whether the successive addition of CN^- to the methanol solution of **5**-Cu^{2+} (10 μM) complex would result in any variations.

As shown in Fig. 4a, the fluorescence of **5**-Cu^{2+} complex could be renewed with different fluorescence recovery ratios ([FRR] = $[F - F_{min}]/[F_{max} - F_{min}]$, where F_{max} is the fluorescent intensity of **5** without Cu^{2+}, F_{min} is the fluorescent intensity of **5**-Cu^{2+}, F is the fluorescent intensity of **5**-Cu^{2+} with different concentrations of CN^-) in terms of the nature of the anion added (30 μM). The presence of F^-, Br^-, Cl^-, ClO_4^- and NO_3^- induced almost no fluorescence change, while the presence of I^-, AcO^- and HSO_4^- weakly enhanced its fluorescence intensity. In contrast, whereas $H_2PO_4^-$ induced a half fluorescence recovery rate of the complex, the presence of CN^- increased its fluorescence intensity with a recovery rate of 65%. A detailed fluorescence titration test shown in Fig. 4b and 4c further implies that with increased concentration of CN^-, the fluorescence intensity of **5** can regress gradually. The presence of 10 equiv. CN^- nearly rendered its full fluorescence recovery.

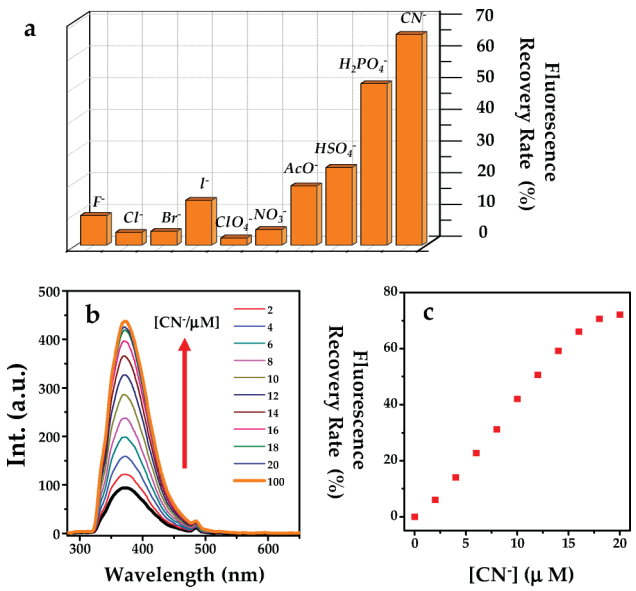


Fig. 4 (a) Fluorescence recovery rate in the presence of TBA (tetrabutylammonium) salts of various anions (30 μM); (b) Fluorescence spectrum in the presence of various concentrations (0 to 100 μM) of CN^-; (c) Fluorescence recovery plot in the presence of various concentrations (0 to 20 μM) of CN^- of compound **5** (10 μM) in methanol (λ_{ex} = 242 nm).

Such a phenomenon is most likely caused by the association of CN^- with Cu^{2+} which then positively sets free the original fluorescence of probe **5** (Fig. 4, top curve).[11d] However, **5** may similarly be isolated by $H_2PO_4^-$ that is also known to have high binding affinity with Cu^{2+}. We subsequently examined the impact of several other anions including CO_3^{2-} and the phosphates such as $H_2PO_4^-$, HPO_4^{2-} and PO_4^{3-} toward the fluorescence change of **5**-Cu^{2+} (10 μM) in aqueous media (MeOH/water = 9 : 1, v/v), shown in Fig. S3 (ESI†).

The fluorescence of **5** could similarly be recovered by CN^- (30 μM) in this new aqueous ambiance by 65%, while the presence of excessive CO_3^{2-} only resulted in a recovery rate of 30%. Since phosphates exist extensively in biological systems, an excess of, respectively, $H_2PO_4^-$, HPO_4^{2-} and PO_4^{3-} were added to the solution, which led to maximal renewed fluorescence of **5** by a rate of approximately 60%. Although the selectivity of **5**-Cu^{2+} between phosphates and cyanide is not adequate, there is a clear tendency that this complex has a recognition preference to the latter. Future efforts could be made to achieve better specificity for CN^- over other anions by appropriate structural modifications. Additionally, this probe is remarkably more selective to cyanide over F^- compared with previously developed amide-type cyanide sensors, probably due to the lack of NH protons sensitive to halogens.[12d]

Notably, the detection limit of complex **5**-Cu^{2+} is assigned to a favorable value of 37.5 nM (Fig. S1c, ESI†), which is two orders of magnitude lower than the regulated concentration of cyanide in drinking water (around 200 nM).[3] This could be a substantial merit for the further exploration of sensitive cyanide sensors.

Conclusions

To summarize, a unique *C*-glycosyl bis-triazolyl amino acid derivative was readily synthesized by taking advantage of the modular and selective CuAAC as the key step. This fluorescent molecule with exposed di-carboxylic acid functional groups was subsequently identified as a new "off–on" Cu(II)-cyanide sensor with a satisfactorily nanomolar detection limit, albeit with moderate selectivity over phosphates. Our study would therefore set out a unique basis for the future design and development of sensitive and potentially biocompatible cyanide sensors based on the double "clicked" *C*-glycosyl bis-amino acids. Programs related to this subject are currently underway.

Experimental section

General techniques and materials

All chemicals and reagents as well as $Ba(OAc)_2$, $Mn(OAc)_2$, $Co(OAc)_2$, $ZnSO_4$, $Al(NO_3)_3$, $Ni(OAc)_2$, $Cd(NO_3)$, $AgNO_3$, $Hg(OAc)_2$, $FeSO_4$, $CuSO_4$ and $Pb(OAc)_2$, and all K and TBA salts used for fluorescence studies are of high commercially available grade. ^1H and ^{13}C NMR spectra were recorded on a Jeol-400 MHz spectrometer in $CDCl_3$ or CD_3OD solutions. Analytical thin-layer chromatography was performed on E. Merck aluminium precoated plates of Silica Gel 60F-254 with detection by UV and by spraying with 6 N H_2SO_4 and heating at 300 °C. High resolution mass spectra (HRMS) were recorded on an MA1212 instrument using standard conditions (ESI, 70 eV). Optical rotations were measured using a Perkin–Elmer 241 polarimeter at room temperature and 10 cm/1 mL cell. All UV-Vis absorption spectra were measured on a Varian Cary 500 UV-Vis spectrophotometer and fluorescence spectra measured on a Varian Cary Eclipse Fluorescence spectrophotometer.

Synthesis of 2,6-di-*O*-TBDMS-3,4-di-*O*-{1-[1(*S*)-methoxycarbonyl-2-phenylethyl]-4-methyl-1*H*-1,2,3-triazol-4-yl}-β-D-glucopyranosyl 1,4-dimethoxynaphthalene (3). To a soln. of alkyne **2** (257 mg, 0.4 mmol) and azide **a** (202 mg, 1.0 mmol) in a solvent mixture of CH_2Cl_2 (5 mL) and H_2O (5 mL), were added sodium ascorbate (1073 mg, 5.4 mmol) and $CuSO_4 \cdot 5H_2O$ (867 mg, 3.5 mmol). This mixture was stirred at rt for 12 h, and was then diluted directly with CH_2Cl_2 and washed with brine and deionized water. The combined organic layer was dried over $MgSO_4$, filtered and then concentrated under vacuum. The resulting residue was purified by column chromatography (petroleum ether/EtOAc = 3 : 1) to afford compound **3** as a white solid (189 mg, 65.2%). ^1H NMR (400 MHz, $CDCl_3$): δ = 8.21 (dd, J = 1.6, 6.8 Hz, 1H), 8.01 (dd, J = 1.6, 7.2 Hz, 1H), 7.56 (s, 1H), 7.52–7.44 (m, 2H), 7.27–7.21 (m, 3H), 7.20–7.18 (m, 3H), 7.04 (dd, J = 2.4, 7.6 Hz, 1H), 6.94 (dd, J = 2.0, 7.2 Hz, 1H), 6.86 (s, 1H), 6.80 (s, 1H), 5.56 (dd, J = 6.8, 8.4 Hz, 1H), 5.13 (dd, J = 7.2, 8.0 Hz, 1H), 4.94 (d, J = 11.2 Hz, 1H), 4.88 (d, J = 11.6 Hz, 1H), 4.58 (d, J = 11.6 Hz, 1H), 3.97 (s, 3H), 3.94 (dd, J = 3.6, 12.4 Hz, 1H), 3.88 (d, J = 8.4 Hz, 1H), 3.86–3.82 (m, 2H), 3.82 (s, 3H), 3.74 (s, 3H), 3.71–3.63 (m, 2H), 3.58 (s, 3H), 3.52 (dd, J = 6.8, 14.4 Hz, 2H), 3.47–3.39 (m, 2H), 3.31 (dd, J = 6.8, 14.0 Hz, 1H), 3.31 (dd, J = 8.4, 14.0 Hz, 1H), 0.92 (s, 9H), 0.88 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H), 0.04 (s, 3H), 0.02 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 168.8, 168.4, 152.1, 147.8, 145.7, 145.2, 134.9, 134.8, 129.0, 128.9, 128.7, 128.3, 127.6, 127.4, 127.2, 126.7, 126.5, 125.8, 122.6, 122.3, 122.2, 122.1, 104.1, 83.9, 80.4, 79.0, 78.7, 75.1, 66.5, 66.4, 64.0, 63.7, 63.3, 62.5, 55.7, 53.1, 52.9, 39.0, 38.5, 26.2, 26.1, 18.4, 18.1, –3.8, –3.9, –4.7, –5.0.

Synthesis of 3,4-di-*O*-{1-[1(*S*)-methoxycarbonyl-2-phenylethyl]-4-methyl-1*H*-1,2,3-triazol-4-yl}-β-D-glucopyranosyl 1,4-dimethoxynaphthalene (4). To a soln. of **3** (189 mg, 0.2 mmol) in MeOH (5 mL) was added AcCl (30 μL, 0.3 mmol), stirring at rt for 12 h. The solvent was removed under vacuum and the residue was then diluted with EtOAc and washed successively with sat. $NaHCO_3$ and brine. The combined organic layer was dried over $MgSO_4$, filtered and then concentrated under vacuum. The resulting residue was purified by column chromatography (petroleum ether/EtOAc = 1 : 1) to afford compound **4** as a white solid (136 mg, 91.5%). $[\alpha]_D^{25}$ = +14 (c = 0.02, MeOH); UV-vis (MeOH) λ_{max}: 211, 242, 301, 313, 327 nm; ^1H NMR (400 MHz, $CDCl_3$): δ = 8.23 (dd, J = 0.8, 7.6 Hz, 1H), 8.01 (dd, J = 1.2, 7.6 Hz, 1H), 7.63 (s, 1H), 7.56–7.47 (m, 2H), 7.28–7.25 (m, 1H), 7.24–7.17 (m, 5H), 7.06 (s, 1H), 7.05 (dd, J = 1.6, 7.6 Hz, 2H), 6.94 (dd, J = 1.6, 7.6 Hz, 2H), 6.75 (s, 1H), 5.56 (dd, J = 6.8, 8.8 Hz, 1H), 5.34 (dd, J = 6.4, 8.8 Hz, 1H), 5.03 (d, J = 13.2 Hz, 1H), 4.99 (d, J = 12.8 Hz, 1H), 4.88 (d, J = 9.6 Hz, 1H), 4.27 (d, J = 13.2 Hz, 1H), 4.21 (d, J = 13.6 Hz, 1H), 4.02 (d, J = 8.8 Hz, 1H), 3.97 (s, 3H), 3.89 (s, 3H), 3.80 (dd, J = 2.8, 12.4 Hz, 1H), 3.77–3.72 (m, 2H), 3.74 (s, 3H), 3.70–3.65 (m, 1H), 3.66 (s, 3H), 3.55–3.51 (m, 1H), 3.50 (d, J = 6.4 Hz, 1H), 3.45 (dd, J = 8.4, 14.0 Hz, 1H), 3.45 (dd, J = 6.4, 14.4 Hz, 1H), 3.24 (dd, J = 8.8, 14.4 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 168.7, 168.4, 152.4, 148.2, 145.6, 145.3, 134.8, 134.7, 129.0, 128.9, 128.8, 128.4, 127.7, 127.6, 126.9, 126.8, 126.4, 126.1, 122.6, 122.4, 122.0, 101.8, 83.7, 79.4, 79.1, 78.7, 74.9, 65.4, 65.2, 64.2, 64.0, 63.4, 62.4, 55.9, 53.2, 53.1, 38.9, 38.7. HRESIMS m/z: $[M + H]^+$ calcd. for $C_{42}H_{45}N_6O_{11}$: 837.3459; found: 837.3458.

Synthesis of 3,4-di-*O*-{1-[1(*S*)-carboxy-2-phenylethyl]-4-methyl-1*H*-1,2,3-triazol-4-yl}-β-D-glucopyranosyl 1,4-dimethoxynaphthalene (5). To a soln. of **4** (65 mg, 0.08 mmol) in a solvent mixture of MeOH (3 mL) and H_2O (3 mL) was added LiOH (6 mg, 0.2 mmol),

stirring at rt for 3 h. H^+ resin was then added for neutralization and the mixture was filtered. The filtrate was concentrated under vacuum to give the pure product **5** as a white solid (62.7 mg, quantitative). $[\alpha]_D^{25} = +5$ ($c = 0.02$, MeOH); UV-vis (MeOH) λ_{max}: 211, 242, 301, 313, 327 nm; ^{1}H NMR (400 MHz, CD_3OD): $\delta = 8.20$ (d, $J = 8.4$ Hz, 1H), 8.04–8.00 (m, 2H), 7.55–7.45 (m, 2H), 7.21–7.05 (m, 8H), 6.98 (brs, 1H), 6.93 (dd, $J = 2.0$, 6.0 Hz, 2H), 6.80 (dd, $J = 2.0$, 5.6 Hz, 1H), 5.55 (dd, $J = 3.6$, 10.4 Hz, 1H), 5.24–5.17 (m, 1H), 5.00 (d, $J = 12.0$ Hz, 1H), 4.87–4.81 (m, 2H), 4.61–4.46 (m, 1H), 4.18 (dd, $J = 3.2$, 12.4 Hz, 1H), 4.01–3.94 (m, 3H), 3.86 (t, $J = 8.4$ Hz, 1H), 3.79 (s, 3H), 3.72 (dd, $J = 7.2$, 13.6 Hz, 1H), 3.69–3.59 (m, 3H), 3.55 (dd, $J = 2.4$, 9.2 Hz, 1H), 3.49 (dd, $J = 3.2$, 14.4 Hz, 1H), 3.45–3.43 (m, 1H), 3.41 (dd, $J = 4.8$, 14.0 Hz, 1H), 3.32 (brs, 2H). ^{13}C NMR (100 MHz, CD_3OD): $\delta = 170.9$, 170.4, 152.1, 148.0, 144.4, 143.3, 136.4, 136.1, 128.6, 128.5, 128.2, 128.1, 127.1, 127.0, 125.6, 124.1, 124.0, 123.1, 123.0, 122.2, 122.1, 102.3, 83.1, 82.5, 80.1, 78.9, 78.2, 77.9, 74.8, 65.6, 65.1, 62.6, 61.8, 55.0, 38.0, 37.7. HRESIMS m/z: $[M + H]^+$ calcd. for $C_{42}H_{49}N_6O_{11}$: 809.3146; found: 809.3146.

Fluorescence measurements

The fluorescence measurements were carried out on a Varian Cary Eclipse Fluorescence spectrophotometer by using a path length of 10 mm and excitation at 242 nm by scanning the emission spectra between 250 and 650 nm. The bandwidth for both excitation and emission spectra was 5 nm.

Preparation of sample solutions for the evaluation of ion specificity. Stock solutions of 0.01 M of $Hg(OAc)_2$, $Ba(OAc)_2$, $Mn(OAc)_2$, $Co(OAc)_2$, $Ni(OAc)_2$, $Cu(OAc)_2$, $Zn(SO_4)_2$, $Cd(NO_3)_2$, $Pb(OAc)_2$, $FeSO_4$ and $AgNO_3$ were prepared in de-ionized water. Stock solutions of 0.01 M of F^-, Br^-, Cl^-, I^-, ClO_4^-, NO_3^-, AcO^-, HSO_4^-, $H_2PO_4^-$, CN^- were prepared in acetonitrile and stock solutions of sensors (2×10^{-4} M) **4** and **5** prepared in methanol or in water or methanol/water (90 : 1, v/v). Stock solutions of 0.01 M of CO_3^{2-} and excessive $H_2PO_4^-$, HPO_4^{2-} and PO_4^{3-} were prepared in water. For the selectivity experiment of sensor **4**, test solutions were prepared by adding 30 μL of each metal stock to 3 mL test solution of sensor **4** ([**4**] = 10 μM). For the selectivity experiment of sensor **5**, test solutions were prepared by adding 3 μL of each of metal stock to 3 mL test solution of sensor **5** ([**5**] = 10 μM). For the selectivity experiment of sensor Cu^{2+}-**5**, test solutions were prepared by adding 3 μL of Cu^{2+} stock to 3 mL test solution of sensor **5** ([**5**] = 10 μM). Then, 9 μL of different anion stock were added in the test solutions.

Preparation of stock solutions and samples for the titration experiments. Stock solutions of 0.01 M of $Pb(OAc)_2$, $CuSO_4$ and TBACN in de-ionized water or acetonitrile were prepared. For the titration experiment of sensor **4** with Pb^{2+}, to 3 mL test solution of sensor **4** ([**4**] = 10 μM) was added 0–120 μL of $Pb(OAc)_2$ solution. For the titration experiment of sensor **5** with Cu^{2+}, to 3 mL test solution of sensor **5** ([**5**] = 10 μM) was added 0–4.2 μL of $CuSO_4$ solution. For the titration experiment of sensor Cu^{2+}-**5** with CN^-, to 3 mL of test solution of sensor Cu^{2+}-**5** ([Cu^{2+}-**5**] = 10 μM) was added 0–30 μL of TBACN solution.

Acknowledgements

Generous funding was provided by the Natural Science Foundation of China (Grant No. 21176076), Shanghai Science and Technology Community (No. 10410702700) and the Fundamental Research Funds for the Central Universities (No. WK1013002). X.-P. H. also gratefully acknowledges the French Embassy in Beijing, PR China for a co-tutored doctoral fellowship.

Notes and references

1 K. W. Kulig, *Cyanide Toxicity*, U. S. Department of Health and Human Services, Atlanta, GA, 1991.
2 S. I. Baskin and T. G. Brewer, *Medical Aspects of Chemical and Biological Warfare*, ed. F. Sidell, E. T. Takafuji and D. R. Franz, TMM Publication, Washington, DC, 1997, ch. 10, pp. 271–286.
3 *Guidelines for Drinking-Water Quality*, World Health Organization, Geneva, 1996.
4 (*a*) Y.-H. Kim and J.-I. Hong, *Chem. Commun.*, 2002, 512; (*b*) P. Anzenbacher, Jr, D. S. Tyson, K. Jursiková and F. N. Castellano, *J. Am. Chem. Soc.*, 2002, **124**, 6232; (*c*) C.-F. Chow, M. H. W. Lam and W.-Y. Wong, *Inorg. Chem.*, 2004, **43**, 8387.
5 (*a*) R. Badugu, J. R. Lakowicz and C. D. Geddes, *J. Am. Chem. Soc.*, 2005, **127**, 3635; (*b*) J. V. Ros-Lis, R. Martínez-Máñez and J. Soto, *Chem. Commun.*, 2005, 5260.
6 (*a*) W. J. Jin, M. T. Fernández-Argüelles, J. M. Costa-Fernández, R. Pereiro and A. Sanz-Medel, *Chem. Commun.*, 2005, 883; (*b*) S.-S. Sun and A. J. Lees, *Chem. Commun.*, 2000, 1687; (*c*) H. Miyaji and J. L. Sessler, *Angew. Chem., Int. Ed.*, 2001, **40**, 154.
7 (*a*) M. Tomasulo, S. Sortino, A. J. P. White and F. M. Raymo, *J. Org. Chem.*, 2006, **71**, 744; (*b*) J. Ren, W. Zhu and H. Tian, *Talanta*, 2008, **75**, 760.
8 (*a*) F. García, J. M. García, B. García-Acosta, R. Martínez-Máñez, F. Sancenón and J. Soto, *Chem. Commun.*, 2005, 2790; (*b*) J. V. Ros-Lis, R. Martínez-Máñez and J. Soto, *Chem. Commun.*, 2002, 2248; (*c*) Y. M. Chung, B. Raman, D.-S. Kim and K. H. Ahn, *Chem. Commun.*, 2006, 186; (*d*) Y. Chung, H. Lee and K. H. Ahn, *J. Org. Chem.*, 2006, **71**, 9470–9474; (*e*) Y.-K. Yang and J. Tae, *Org. Lett.*, 2006, **8**, 5721; (*f*) K.-S. Lee, H.-J. Kim, I. Shin and J.-I. Hong, *Org. Lett.*, 2008, **10**, 49; (*g*) S. K. Kwon, S. Kou, H. N. Kim, X. Chen, H. Hwang, S.-W. Nam, S. H. Kim, K. M. K. Swamy, S. Park and J. Yoon, *Tetrahedron Lett.*, 2008, **49**, 4102.
9 (*a*) V. Ganesh, M. P. C. Sanz and J. C. Mareque-Rivas, *Chem. Commun.*, 2007, 5010; (*b*) Q. Zeng, P. Cao, Z. Li, J. Qin and B. Z. Tang, *Chem. Commun.*, 2008, 1094; (*c*) X. Lou, L. Zhang, J. Qin and Z. Li, *Chem. Commun.*, 2008, 5848.
10 K. Kumia, D. E. Giles, P. M. May, P. Singh and G. T. Hefter, *Talanta*, 1996, **43**, 2045.
11 (*a*) V. Ganesh, M. P. C. Sanz and J. C. Mareque-Rivas, *Chem. Commun.*, 2007, 5010; (*b*) Q. Zeng, P. Cai, Z. Li, J. Qin and B. Z. Tang, *Chem. Commun.*, 2008, 1094; (*c*) X. Lou, L. Zhang, J. Qin and Z. Li, *Chem. Commun.*, 2008, 5848; (*d*) X. Lou, J. Qin and Z. Li, *Analyst*, 2009, **134**, 2071.
12 (*a*) S.-Y. Chung, S.-W. Nam, J. Lim, S. Park and J. Yoon, *Chem. Commun.*, 2009, 2866; (*b*) R. Guliyev, O. Buyukcakir, F. Sozmen and O. A. Bozdemir, *Tetrahedron Lett.*, 2009, **50**, 5139; (*c*) P. Kaur, S. Kaur and K. Singh, *Inorg. Chem. Commun.*, 2009, **12**, 978; (*d*) R. Kumar, V. Bhalla and M. Kumar, *Tetrahedron*, 2008, **64**, 8095.
13 (*a*) H. Yuasa, N. Miyagawa, M. Nakatani, M. Izumi and H. Hashimoto, *Org. Biomol. Chem.*, 2004, **2**, 3548; (*b*) H. Yuasa, N. Fujii and S. Yamazaki, *Org. Biomol. Chem.*, 2007, **5**, 2920; (*c*) H. Yuasa, N. Miyagawa, T. Izumi, M. Nakatani, M. Izumi and H. Hashimoto, *Org. Lett.*, 2004, **6**, 1489; (*d*) S. Qu, Z. Lin, C. Duan, H. Zhang and Z. Bai, *Chem. Commun.*, 2006, 4392; (*e*) N. K. Singhal, B. Ramanujam, V. Mariappanadar and C. P. Rao, *Org. Lett.*, 2006, **8**, 3525; (*f*) N. K. Singhal, A. Mitra, G. Rajsekhar, M. M. Shaikh, S. Kumar, P. Guionneau and C. P. Rao, *Dalton Trans.*, 2009, 8432; (*g*) J. Xie, M. Ménand, S. Maisonneuve and R. Métivier, *J. Org. Chem.*, 2007, **72**, 5980.
14 (*a*) V. V. Rostovtsev, L. G. Green, V. V. Fokin and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2002, **41**, 2596; (*b*) C. W. Tornøe, C. Christensen and M. Meldal, *J. Org. Chem.*, 2002, **67**, 3057.

15 H. C. Kolb, M. G. Finn and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2001, **40**, 2004.
16 (*a*) K. A. Kalesh, H. Shi, J. Ge and S. Q. Yao, *Org. Biomol. Chem.*, 2010, **8**, 1749; (*b*) A. Dondoni, *Org. Biomol. Chem.*, 2010, **8**, 3366; (*c*) D. Deniaud, K. Julienne and S. G. Gouin, *Org. Biomol. Chem.*, 2011, **9**, 966; (*d*) A. J. McCaroll, C. S. Matthews, G. Wells, T. D. Bradshaw and M. F. G. Stevens, *Org. Biomol. Chem.*, 2010, **8**, 2078; (*e*) J. van Ameijde, A. J. Poot, L. T. M. van Wandelen, A. E. M. Wammes, R. Ruijtenbeek, D. T. S. Rijkers and R. M. J. Liskamp, *Org. Biomol. Chem.*, 2010, **8**, 1629; (*f*) J. E. Moses, D. J. Ritson, F. Zhang, C. M. Lombardo, S. Haider, N. Oldham and S. Neidle, *Org. Biomol. Chem.*, 2010, **8**, 2926; (*g*) A. Rolfe, G. H. Lushington and P. R. Hanson, *Org. Biomol. Chem.*, 2010, **8**, 2198.
17 (*a*) Y.-J. Zhang, X.-P. He, M. Hu, Z. Li, X.-X. Shi and G.-R. Chen, *Dyes Pigm.*, 2011, **88**, 391; (*b*) Z. Song, X.-P. He, X.-P. Jin, L.-X. Gao, L. Sheng, Y.-B. Zhou, J. Li and G.-R. Chen, *Tetrahedron Lett.*, 2011, **52**, 894; (*c*) X.-P. He, C. Li, X.-P. Jin, Z. Song, H.-L. Zhang, C.-J. Zhu, Q. Shen, W. Zhang, L. Sheng, X.-X. Shi, Y. Tang, J. Li, G.-R. Chen and J. Xie, *New J. Chem.*, 2011, **35**, 622; (*d*) X.-P. He, Z. Song, Z.-Z. Wang, X.-X. Shi, K. Chen and G.-R. Chen, *Tetrahedron*, 2011, **67**, 3343.
18 L. Lin, X.-P. He, Q. Xu, G.-R. Chen and J. Xie, *Carbohydr. Res.*, 2008, **343**, 773.
19 (*a*) J.-W. Yang, X.-P. He, C. Li, L.-X. Gao, L. Sheng, J. Xie, X.-X. Shi, Y. Tang, J. Li and G.-R. Chen, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 1092; (*b*) X.-P. He, Q. Deng, L.-X. Gao, C. Li, W. Zhang, Y.-B. Zhou, Y. Tang, X.-X. Shi, J. Xie, J. Li, G.-R. Chen and K. Chen, *Bioorg. Med. Chem.*, 2011, **19**, 3892.
20 D. M. Nguyen, A. Frazer, L. Rodriguez and K. D. Belfield, *Chem. Mater.*, 2010, **22**, 3472.
21 (*a*) C. I. C. Esteves, M. Manuela, M. Raposo and S. P. G. Costa, *Tetrahedron*, 2010, **66**, 7479; (*b*) Y. H. Lau, J. R. Price, M. H. Todd and P. J. Rutledge, *Chem.–Eur. J.*, 2011, **17**, 2850; (*c*) E. Tamanini, A. Katewa, L. M. Sedger, M. H. Todd and M. Watkinson, *Inorg. Chem.*, 2009, **48**, 319.

