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Generation of new fluorophore by Click chemistry: synthesis and properties of β-cyclodextrin substituted by 2-pyridyl triazole

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Abstract—2-Pyridyl triazole substituted β-cyclodextrins, formed by a Huisgen [2+3] cycloaddition reaction between per-(6-azido)-β-cyclodextrins and 2-ethynylpyridine, exhibited interesting solvent-polarity dependent fluorescence properties and acted as Zn^{2+}-sensitive fluorogenic chelating agents with a significant fluorescence enhancement and a large red-shift in emissions. No fluorescent emission was observed with phenyl or hydroxymethyl triazole conjugated sugar derivatives.
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Much recent effort has been devoted to the design and synthesis of chemosensors that can be used for the sensing and recognition of environmentally and biologically important molecular and ionic species. Cyclodextrins (CDs) are well-known molecular receptors with hydrophobic cavities and are readily available and very popular building blocks for functional architectures. They have been used as supramolecular mediators for the sensing of transition and alkali metal ions, amino acids and other small molecules like aromatic hydrocarbons, quinones and steroids.[1] In the course of our studies on the synthesis of sugar-derived macrocycles possessing complexing properties,[2] we were convinced that CDs substituted by nitrogen ligands would constitute valuable compounds for the complexation studies we intended to do, comforted in that way by the work of Marsura and co-workers.[1b] We thereby considered the connection of a pyridine moiety onto the small rim of β-CD (Scheme 1) using the Huisgen [2+3] cycloaddition reaction,[3] while the triazole formed in the process was envisioned to participate actively in the complexation. The Huisgen 1,3-dipolar cycloaddition can be performed under mild conditions and is compatible with many solvents and functional groups. To date, Click chemistry has been successfully used in the synthesis of new macrocyclic sugar-containing molecules,[4] oligosaccharides mimics,[5] multivalent structures,[6] polypseudorotaxane[7] or dendrimers.[8] [1,2,3]-Triazole derivatives have been used as photostabilizers and optical bleaching agents.[9] However, to the best of our knowledge, their fluorescence properties have never been reported.[10] Herein, we report the copper-mediated cycloaddition of 2-ethynylpyridine with per-(6-azido)-β-CDs for the synthesis of novel multivalent CDs, their unprecedented fluorescence properties and complexing behaviours.

Reaction of acetyl or methyl protected per-(6-azido)-β-CDs **1**[11] and **2**[12] with 2-ethynylpyridine was realized in presence of a copper(I) ion source to catalyze the [2+3] cycloaddition[13] (Scheme 1), the use of copper turnings[14] being preferred to facilitate the final purification. After one week in the presence of 17 equiv of 2-ethynylpyridine, all azides were converted into the triazole substituted adducts **3** and **4**. After filtration and evaporation of the solvents, the desired cyclodextrins were obtained as the sole products in nearly quantitative yields, although all were contaminated with substantial amounts of copper ion. Compounds **3** and **4** were then dissolved in ethyl acetate and thoroughly washed with a 5% aqueous solution of EDTA. CD **3**[15] was isolated after precipitation with Et_2O in 91% yield whereas CD **4**[16] could be purified by flash chromatography and was isolated in 62% yield. Both compounds were fully characterized by ^1H NMR, ^{13}C NMR and MALDI-TOF mass spectroscopy.

By coincidence, we found that solutions of CDs **3** and **4** were fluorescent. The photophysical study of triazoyl-substituted CDs in MeOH revealed that compounds **3** and **4** show three bands with their maxima around

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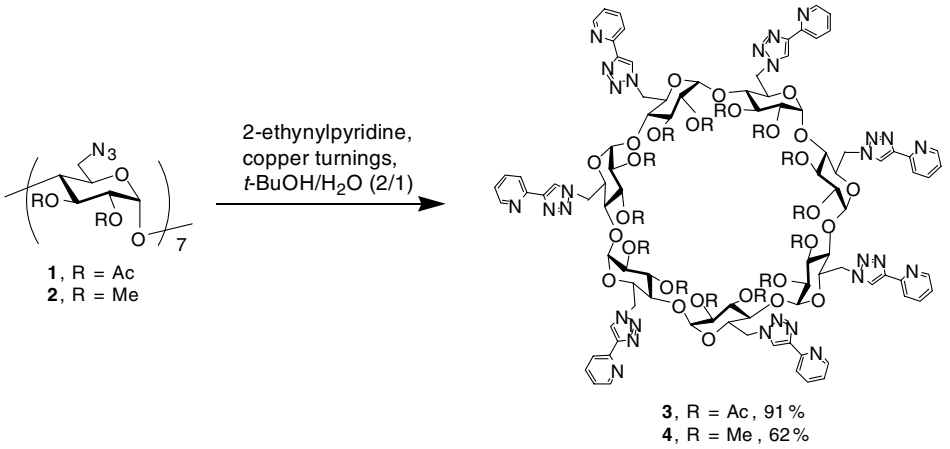


Scheme 1. Synthesis of compounds **3** and **4**.

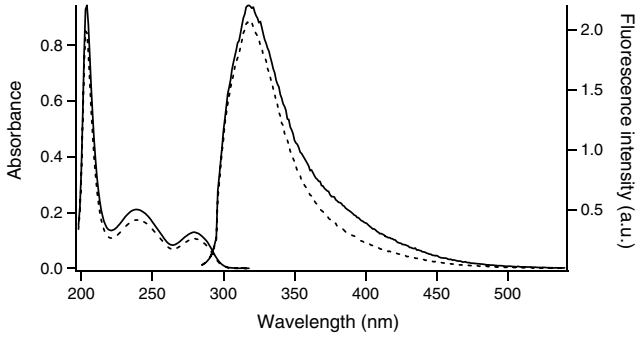


Figure 1. UV–vis and fluorescence spectra of **3** (—) and **4** (---) in MeOH (2×10^{-6} M). $\lambda_{ex} = 280$ nm.

280 nm in the UV–visible absorption spectra (Fig. 1). In the fluorescence spectra, all CDs exhibit an emission maximum at 320 nm when excited at 280 nm. The fluorescence quantum yield (Φ_F) for **3** and **4** was determined to be 0.087 and 0.086 with reference to naphthalene in nondeoxygenated cyclohexane solution, with a molar extinction coefficient of 63,636 and 59,973 mol^{-1} L cm^{-1}, respectively. More interestingly, it was found that these two compounds exhibit solvatofluorochromism.[17] As shown in Figure 2, compound **3** displayed positive solvatofluorochromism, with an increase in the fluorescence efficiency with increasing solvent-polarity (Φ_F changed from 3.8% in THF to 35.5% in CH_3CN/H_2O). A red-shift of about 60 nm is observed for the emission of **3** from 302 nm in THF to 362 nm in a mixture of acetonitrile/water.

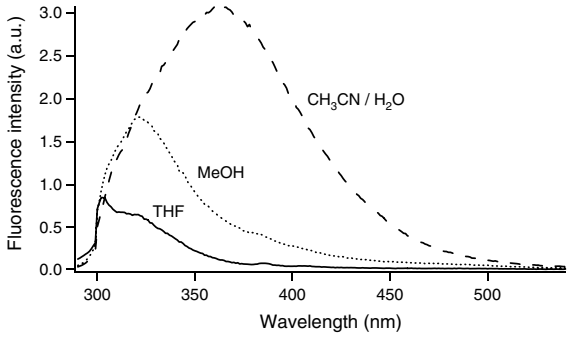
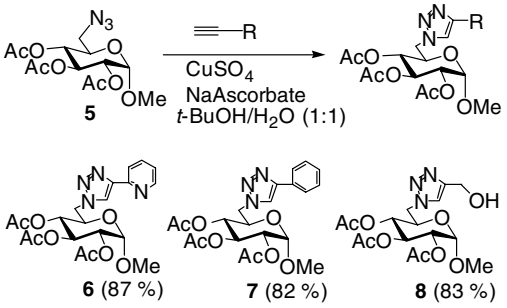


Figure 2. Emission behaviours and fluorescence spectra of **3** in THF (—-), MeOH (···) and CH_3CN/H_2O (3:7) (---). $\lambda_{ex} = 280$ nm.

To search the origin of the fluorescence properties of CDs **3** and **4**, we then decided to prepare several model triazole derivatives with methyl 2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-α-D-glucopyranoside **5**[18] (Scheme 2). 1,2,3-Triazoyl-substituted methyl glycosides **6** to **8** were then prepared by treatment of compound **5** with 1.1 equiv of either 2-ethynylpyridine, 2-ethynylbenzene or propargyl alcohol in the presence of a $CuSO_4$/ascorbate mixture in aqueous *t*-BuOH solution (1:1).

Compound **6** displayed similar fluorescence emissions as CDs **3** and **4** when irradiated at 280 nm in MeOH (Fig. 3). However, no fluorescent emission was observed with compounds **7** and **8**. These results clearly demonstrated that the fluorescence properties was exclusively originated from the conjugated triazole–pyridine system. This finding might allow the detection of triazole–pyridine conjugated biologically active compounds by fluorescence method.[19]

To get insight into the binding properties of triazoyl CDs towards metal ions, we first investigated fluorescence changes upon addition of 50 equiv perchlorate salts of selected cations to the MeOH solution of **3**. As presented in Figure 4, the fluorescence emission was slightly quenched by Mn^{2+}, Hg^{2+} and Pb^{2+} and more



Scheme 2.

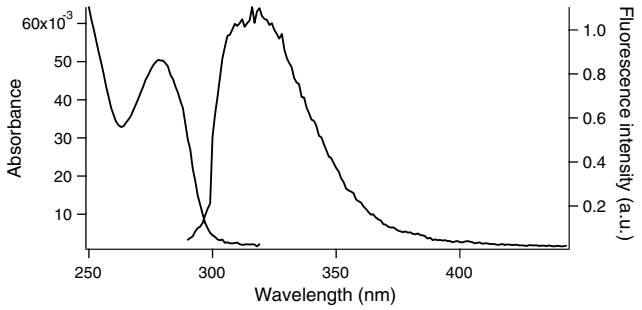


Figure 3. UV–vis and fluorescence spectra of **6** in MeOH (5.95×10^{-6} M). $\lambda_{ex} = 280$ nm.

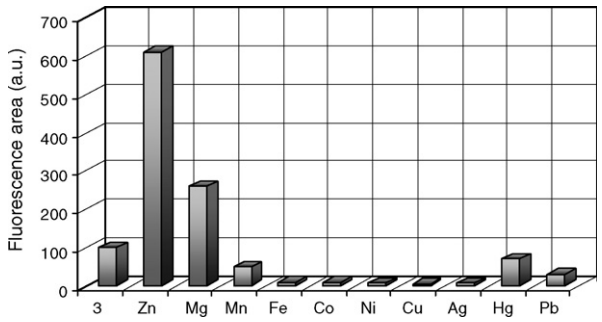


Figure 4. The fluorescence intensity change profiles of **3** (5×10^{-6} M) in MeOH with selected cations (2.5×10^{-4} M). $\lambda_{ex} = 280$ nm.

strongly quenched by Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+} and Ag^{+}. An obvious fluorescence enhancement was observed with Mg^{2+} and Zn^{2+}, with the largest fluorescence enhancement upon Zn^{2+} complexation. We then decided to realize the titration experiment by both absorption and fluorescence measurements with Zn^{2+}. As the concentration of Zn^{2+} increases, the fluorescence intensity is progressively increased (Fig. 5). The emission maximum shifted to 366 nm with a much higher fluorescence quantum yield of 60.2%. The curve was well-fitted with a 1:1 complexation equation model and provided a stability constant $\log K = 7.9 \pm 0.3$.

We have also realized the titration experiment with the model compound **6**. A similar fluorescence enhancement

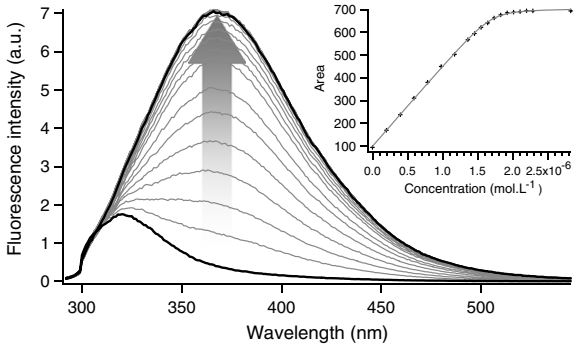


Figure 5. Fluorescence spectra obtained during the titration of **3** in MeOH (2×10^{-6} M) with $Zn(ClO_4)_2$ (from 0 to 3×10^{-6} M). $\lambda_{ex} = 280$ nm. Inset: titration curve of the integrated fluorescence as a function of Zn^{2+} concentration.

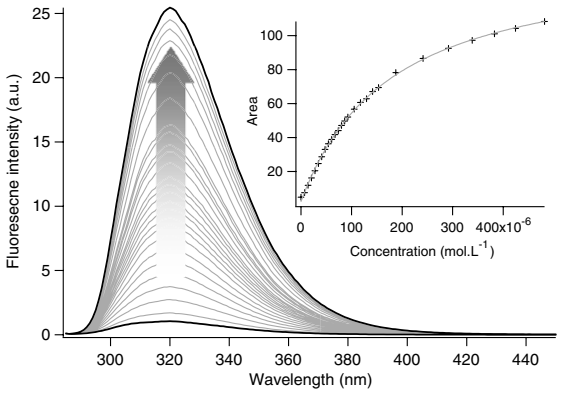


Figure 6. Fluorescence spectra obtained during the titration of **6** in MeOH (1.4×10^{-5} M) with $Zn(ClO_4)_2$ (from 0 to 4.8×10^{-4} M). $\lambda_{ex} = 280$ nm. Inset: titration curve of the integrated fluorescence as a function of Zn^{2+} concentration.

was observed with increasing concentration of Zn^{2+} (Fig. 6). The curve was well-fitted with a 1:1 complexation equation model and provided a stability constant $\log K = 3.8 \pm 0.1$. Consequently, the monovalent ligand **6** binds much weakly with the Zn^{2+} ion (ten thousand time's difference) than the multivalent cyclodextrin **3** because of loss of cooperativity.

In conclusion, we have discovered the unprecedented fluorescence properties of conjugated triazole–pyridine system during the preparation of multichromophoric triazoyl cyclodextrins using Click chemistry. Spectroscopic studies revealed that these pyridine-substituted triazoles exhibit interesting fluorescence properties (e.g., solvatofluorochromism). Fluorescence titration of CD **3** with Zn^{2+} indicated that **3** forms 1:1 complex with Zn^{2+}. A fluorescence red-shift (320–366 nm) and intensity enhancement (7-fold in quantum yield when excited at 280 nm) was observed. Consequently, the pyridine-substituted triazoles may be useful as a new type of fluorogenic chelating agents for Zn^{2+}.[20] This work may expand the application of Huisgen [2+3] cycloaddition and provide opportunities for the design of new fluorescent sensors.

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13. *General procedure for the Huisgen [2+3] cycloaddition on the CDs*: To a solution of per-acetylated or methylated 6-azido-β-cyclodextrin (0.149 mmol) in a mixture of 20 mL of *t*-BuOH and 10 mL of water, was added 2-ethynylpyridine (17 equiv, 2.54 mmol) followed by ~500 mg of clean copper turnings. After 7 days at rt, the mixture was filtrated and all volatiles removed. The green residue was dissolved in EtOAc and washed five times with a 5% solution of EDTA. The organic phases were further washed with water and then dried over $MgSO_4$. After evaporation of the solvent, the obtained yellow solid was either purified by dissolution in a minimum of CH_2Cl_2 and precipitation by slow addition of Et_2O for the compound **3** (91% yield), or subjected to chromatographic separation (Et_3N/CH_2Cl_2: 2:8 to 3:7) followed by elimination of ammonium salts (washing with water) to furnish the compound **4** (62% yield).
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15. *Spectroscopic data for compound* **3**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.48 (7H, d, $J = 4.0$ Hz), 8.30 (7H, s), 7.96 (7H, d, $J = 7.7$ Hz), 7.58 (7H, dt, $J = 1.8$, 7.7 Hz), 7.07 (7H, ddd, $J = 0.7$, 4.7, 7.3 Hz), 5.58 (7H, d, $J = 3.7$ Hz, 7 × H-1), 5.41 (7H, dd, $J = 8.1$, 9.6 Hz, 7 × H-3), 5.02 (7H, dd, $J = 2.6$, 15.1 Hz, 7 × H-6), 4.8–4.7 (14H, m, 7 × H-2,6′), 4.60 (7H, m, 7 × H-5), 3.63 (7H, dd, $J = 8.1$, 9.2 Hz, 7 × H-4), 2.05 (21H, s, 7 × Ac), 2.00 (21H, s, 7 × Ac). ^{13}C NMR (75 MHz, CD_3OD): δ 171.8, 171.3, 150.8, 150.2, 148.4, 138.7, 126.6, 124.3, 121.6, 98.0, 78.4, 71.6, 71.5, 71.3, 51.7, 21.0, 20.9. MS: (M+H) calcd for $C_{119}H_{127}N_{28}O_{42}$, 2619.87; found, 2619.91; (M+Na) calcd for $C_{119}H_{126}N_{28}O_{42}Na$, 2641.85; found, 2641.92; (M+K) calcd for $C_{119}H_{126}N_{28}O_{42}K$, 2657.82; found, 2657.88.
16. *Spectroscopic data for compound* **4**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.47–8.45 (7H, m), 8.20 (7H, s), 7.94 (7H, d, $J = 7.9$ Hz), 7.59 (7H, dt, $J = 1.8$ and 7.9 Hz), 7.06 (7H, ddd, $J = 1.0$, 4.7 and 7.3 Hz), 5.58 (7H, d, $J = 3.5$ Hz, 7 × H-1), 4.72 (14H, m, 7 × H-6, 6′), 4.36–4.33 (7H, m, 7 × H-5), 3.65 (21H, s, 7 × OMe), 3.63 (7H, t, $J = 9.5$ Hz, 7 × H-4), 3.47 (21H, s, 7 × OMe), 3.42 (7H, t, $J = 9.5$ Hz, 7 × H-3), 3.07 (7H, dd, $J = 3.5$, 9.5 Hz, 7 × H-2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 150.3, 149.5, 147.9, 136.8, 125.1, 122.7, 120.2, 98.2, 81.8, 81.4, 80.4, 69.9, 61.6, 58.9, 50.8. MS: (M+H) calcd for $C_{105}H_{127}N_{28}O_{28}$, 2228.94; found, 2228.96; (M+Na) calcd for $C_{105}H_{126}N_{28}O_{28}Na$, 2250.92; found, 2250.89; (M+K) calcd for $C_{105}H_{126}N_{28}O_{28}K$, 2266.90; found, 2266.88.
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