Supplementary Information for:

Azacrown-Attached *meta*-Ethynylpyridine Polymer:

Saccharide Recognition Regulated by Supramolecular Device

Hajime Abe,* Shunsuke Takashima, Tsuyoshi Yamamoto and Masahiko Inouye*

Graduate School of Pharmaceutical Sciences, University of Toyama, Sugitani 2630, Toyama, Toyama 930-0194, Japan

Experimental	S2
References	S 6
Figures S1–S5	S 7
¹ H NMR spectra	S10

Experimental

General. NMR spectra were recorded on a Varian Gemini 300 spectrometer using tetramethylsilane (TMS) as an internal reference. ESI–HRMS analyses were carried out on a JEOL JMS-T100LC mass spectrometer. IR, UV/Vis, and CD spectra were obtained by a JASCO spectrometers FT/IR-460plus, V-560, J-720WI, respectively. Melting points were determined with Yanako MP-500D and not corrected. GPC analyses were performed with Shodex K-802.5 and K-802 columns connected linearly and CHCl₃ was used as an eluent. THF was freshly distilled from sodium benzophenone ketyl before use. CH₂Cl₂ of anhydrous grade was purchased and used for evaluation of saccharide recognition with the polymer. 1-Aza-24-crown-8¹ and 2,6-diiodo-4-octyloxypyridine² (8) were prepared according to the procedures in the literature.

The Preparation of Polymer 2



Methyl 2,6-dibromo-4-pyridinecarboxylate.³ A mixture of citrazinic acid (5.0 g, 32 mmol), tetramethylammonium bromide (6.0 g, 38.5 mmol), and phosphoryl bromide (27.5 g, 96.5

mmol) was stirred at 140 °C for 7 days. During the stirring, the mixture became homogeneous and then gradually solidified. After cooling to 0 °C, MeOH (25 mL) was added to the mixture, and the reaction mixture was neutralized with saturated aq NaHCO₃, extracted with CH₂Cl₂ (100 mL × 3). The combined CH₂Cl₂ extract was washed with brine and dried over Na₂SO₄ and evaporated. The resulting residue was purified by silica gel column chromatography (eluent: AcOEt/hexane = 1:4) to yield methyl 2,6-dibromo-4-pyridinecarboxylate as a colorless solid (8.2 g, 86%). The ¹H NMR spectrum was identical to that reported in the literature.³

2,6-Dibromo-4-(hydroxymethyl)pyridine (3).³ To a EtOH (400 mL) solution of methyl 2,6-dibromo-4-pyridinecarboxylate (6.3 g, 21 mmol) was added NaBH₄ (4.0 g, 110 mmol) at 0 °C, and the mixture was stirred for 3 h under reflux. After cooling to room temperature, the reaction mixture was evaporated, diluted with water (320 mL), and acidified with 1 M aq HCl (76 mL). The mixture was neutralized with Na₂CO₃ and extracted with CH₂Cl₂ (100 mL × 3). The combined CH₂Cl₂ extract was dried over MgSO₄ and evaporated. The resulting colorless solid (**3**, 5.7 g, 100%) was brought to the next step without further purification. The ¹H NMR spectrum was identical to that reported in the literature.³

2,6-Dibromo-4-(bromomethyl)pyridine (4). To a 1,4-dioxane (42 mL) solution of **3** (5.1 g, 19 mmol) was added PBr₃ (6.2 g, 23 mmol) at 40 °C. The mixture was stirred for 30 min at that temperature and, after removal of a heating bath, additionally stirred for 15 h. The reaction mixture was slowly poured into saturated aq NaHCO₃ (340 mL), and the aqueous mixture was extracted with CH₂Cl₂ (200 mL × 3). The combined CH₂Cl₂ extract was dried over MgSO₄ and evaporated. The resulting residue was purified by silica gel column chromatography (eluent: CH₂Cl₂) to yield **4** as a pale brown solid (5.5 g, 88%). Mp 74–76 °C; ¹H NMR (CDCl₃, 300 Hz) δ 7.47 (s, 2 H), 4.29 (s, 2 H); ¹³C NMR (CDCl₃, 75 Hz) δ 150.6, 140.8, 126.7, 28.0; IR (KBr) ν_{max} 3060, 3020, 2961, 1579, 1530 cm⁻¹; HRMS (ESI–TOF) calcd for C₆H₃Br₃N (M + H⁺): 329.7952; found: 329.7943.

Azacrown–attached 2,6-Dibromopyridine 5. A mixture of 4 (0.23 g, 0.71 mmol), 1-aza-24-crown-8¹ (0.27 g, 0.78 mmol), and Na₂CO₃ (0.082 g, 0.78 mmol) in CH₃CN (25 mL) was stirred under reflux for 12 h. After cooling to room temperature, the reaction mixture was evaporated and purified by silica gel column chromatography (eluent: CH₂Cl₂/MeOH = 19:1) to yield 5 (0.42 g, 100%) as yellowish brown oil. ¹H NMR (CDCl₃, 300 Hz) δ 7.56 (s, 2 H), 3.78 (s, 2 H), 3.68–3.58 (m, 28 H), 2.79 (t, *J* = 5.1 Hz, 4 H); ¹³C NMR (CDCl₃, 75 Hz) δ 156.0, 140.3, 126.6, 70.7, 70.4, 69.7, 57.1, 54.0; IR (neat) ν_{max} 2866, 1575, 1532 cm⁻¹; HRMS (ESI–TOF) calcd for C₂₂H₃₇Br₂N₂O₇ (M + H⁺): 601.0949; found: 601.0920.

Azacrown–attached 2,6-Bis(TMS-ethynyl)pyridine 6. A mixture of 5 (4.8 g, 8.0 mmol), Pd(PPh₃)₄ (0.37 g, 0.32 mmol), and CuI (61 mg, 0.32 mmol) in *i*-Pr₂NH (22 mL) and THF (25 mL) was stirred for 30 min at room temperature. To this mixture was added trimethylsilylacetylene (3.15 g, 32 mmol) dropwise, and the resulting mixture was additionally stirred for 3.5 h at room temperature. The reaction mixture was then diluted with ether (50 mL) and filtered through a pad of Florisil. The filtrate was evaporated and purified by silica gel column chromatography (eluent: CH₂Cl₂/MeOH = 24:1) to yield **6** (4.1 g, 80%) as a brown oil. ¹H NMR (CDCl₃, 300 Hz) δ 7.44 (s, 2 H), 3.72–3.58 (m, 30 H), 2.80 (t, *J* = 5.6 Hz, 4 H), 0.25 (s, 18 H); ¹³C NMR (CDCl₃, 75 Hz) δ 150.3, 142.8, 126.5, 103.3, 94.5, 70.72, 70.67, 70.6, 70.5, 69.7, 57.6, 53.8, -0.3; IR (neat) v_{max} 2867, 2163, 1589, 1546 cm⁻¹; HRMS (ESI-TOF) calcd for C₃₂H₅₅N₂O₇Si₂ (M + H⁺): 635.3548; found: 635.3524.

Azacrown–attached 2,6-diethynylpyridine 7. To a THF (12 mL) solution of 6 (0.51 g, 0.81 mmol) were added tetrabutylammonium fluoride (1.0 M solution in THF, 1.7 mL, 1.7 mmol) and a few drops of water dropwise. After stirring for 1 h at room temperature, the reaction mixture was evaporated and purified by silica gel column chromatography (eluent: CH₂Cl₂ to CH₂Cl₂/MeOH = 19:1) to yield 7 (0.37 g, 92%) as a brown oil. ¹H NMR (CDCl₃, 300 Hz) δ 7.53 (s, 2 H), 3.76 (s, 2 H), 3.68–3.57 (m, 28 H), 3.14 (s, 2 H), 2.79 (t, *J* = 5.4 Hz, 4 H); ¹³C NMR (CDCl₃, 75 Hz) δ 151.0, 142.1, 126.9, 82.3, 70.7, 70.5, 69.7, 57.6, 54.0; IR (neat) v_{max} 3227, 2867, 2107, 1590, 1548 cm⁻¹; HRMS (ESI–TOF) calcd for C₂₆H₃₉N₂O₇ (M + H⁺): 491.2757; found: 491.2716.

Azacrown–attached Diiodo-co-trimer 9. A mixture of 7 (0.15 g, 0.31 mmol), 8² (0.56 g, 1.2 mmol), Pd(PPh₃)₄ (14 mg, 12 μmol), and CuI (2.3 mg, 12 μmol) in *i*-Pr₂NEt (12 mL) and THF (9 mL) was stirred for 1 day at 35 °C. After cooling to room temperature, the reaction mixture was filtered, and the filtrate was evaporated. The resulting residue was purified by silica gel column chromatography (eluent: CH₂Cl₂/MeOH = 24:1) to yield 9 (0.24 g, 68%) as a brown oil. ¹H NMR (CDCl₃, 300 Hz) δ7.68 (s, 2 H), 7.24 (d, *J* = 2.1 Hz, 2 H), 7.12 (d, *J* = 2.1 Hz, 2 H), 3.99 (t, *J* = 6.5 Hz, 4 H), 3.82 (s, 2 H), 3.70–3.58 (m, 28 H), 2.82 (t, *J* = 5.4 Hz, 4 H), 1.84–1.73 (m, 4 H), 1.50–1.23 (m, 20 H), 0.89 (t, *J* = 6.0 Hz, 6 H); ¹³C NMR (CDCl₃, 75 Hz) δ 164.1, 150.7, 142.8, 141.7, 127.0, 120.7, 117.0, 113.7, 87.6, 86.1, 70.22, 70.15, 70.1, 70.0, 69.9, 69.4, 68.1, 57.1, 53.3, 31.2, 28.61, 28.57, 28.1, 25.2, 22.1, 13.6; IR (neat) v_{max} 2925, 2856, 1575, 1531 cm⁻¹; HRMS (ESI–TOF) calcd for C₅₂H₇₅I₂N₄O₉ (M + H⁺): 1153.3623; found: 1153.3592.

Azacrown-attached Bis(TBS-ethynyl)co-trimer 10. A mixture of 9 (2.8 g, 2.4 mmol),

PdCl₂(PPh₃)₂ (84 mg, 0.12 mmol), and CuI (23 mg, 0.12 mmol) in *i*-Pr₂NH (180 mL) and THF (120 mL) was stirred for 30 min at room temperature, and then to the mixture was added *tert*-butyldimethylsilylacetylene (1.7 g, 12 mmol) dropwise. After stirring for 5.5 h at room temperature, the reaction mixture was filtered. The filtrate was evaporated and purified by silica gel column chromatography (eluent: CH₂Cl₂/MeOH = 97:3) to yield **10** (2.8 g, 100%) as a brown oil. ¹H NMR (CDCl₃, 300 Hz) δ7.64 (s, 2 H), 7.11 (d, J = 2.6 Hz, 2 H), 6.97 (d, J = 2.6 Hz, 2 H), 4.01 (t, J = 6.5 Hz, 4 H), 3.79 (s, 2 H), 3.70–3.58 (m, 28 H), 2.81 (t, J = 5.4 Hz, 4 H), 1.85–1.72 (m, 4 H), 1.50–1.24 (m, 20 H), 1.00 (s, 18 H), 0.89 (t, J = 6.8 Hz, 6 H), 0.20 (s, 12 H); ¹³C NMR (CDCl₃, 75 Hz) δ 165.0, 144.3, 143.7, 142.6, 127.5, 114.6, 113.6, 103.8, 93.2, 87.7, 87.3, 70.6, 70.5, 70.0, 68.5, 57.8, 53.8, 31.8, 29.3, 29.2, 28.8, 26.2, 25.9, 22.7, 16.8, 14.2, -4.6; IR (neat) v_{max} 2927, 2857, 2163, 1579, 1549 cm⁻¹; HRMS (ESI–TOF) calcd for C₆₈H₁₀₅N₄O₉Si₂ (M + H⁺): 1177.7420; found: 1177.7451.

Azacrown–attached Diethynyl-co-trimer 11. To a THF (108 mL) solution of 10 (2.8 g, 2.4 mmol) were added tetrabutylammonium fluoride (1.0 M solution in THF, 4.9 mL, 4.9 mmol) and a few drops of water dropwise. After stirring for 3 h at room temperature, the resulting mixture was evaporated and purified by silica gel column chromatography (eluent: CH₂Cl₂ to CH₂Cl₂/MeOH = 24:1) to yield 11 (2.1 g, 90%) as a brown oil. ¹H NMR (CDCl₃, 300 Hz) δ 7.66 (s, 2 H), 7.14 (d, J = 2.4 Hz, 2 H), 6.99 (d, J = 2.4 Hz, 2 H), 4.01 (t, J = 6.5 Hz, 4 H), 3.79 (s, 2 H), 3.71–3.58 (m, 28 H), 3.12 (s, 2 H), 2.82 (t, J = 5.4 Hz, 4 H), 1.86–1.74 (m, 4 H), 1.50–1.25 (m, 20 H), 0.89 (t, J = 6.5 Hz, 6 H); ¹³C NMR (CDCl₃, 75 Hz) δ 165.0, 151.1, 143.7, 143.3, 142.4, 127.4, 114.3, 113.8, 87.33, 87.26, 82.1, 70.7, 70.6, 70.5, 69.9, 68.5, 57.8, 53.8, 31.7, 29.14, 29.10, 28.6, 25.8, 22.6, 14.1; IR (neat) v_{max} 3226, 2926, 2857, 2109, 1580, 1550 cm⁻¹; HRMS (ESI–TOF) calcd for C₅₆H₇₇N₄O₉ (M + H⁺): 949.5691; found: 949.5669.

Azacrown–attached Co-polymer 2. A mixture of 9 (23 mg, 20 µmol), 11 (24 mg, 25 µmol), Pd(PPh₃)₄ (1.2 mg, 1.0 µmol), and CuI (0.2 mg, 1.0 µmol) in *i*-Pr₂NH (0.75 mL) and THF (0.75 mL) was stirred for 3 days at room temperature. The reaction mixture was evaporated and treated with a column of Sephadex LH-20 (eluent: CHCl₃) to yield 2 (36 mg, 76% yield by weight, $M_n = 8,800$ g/mol) as a brown oil. The mixture of the polymer was subjected to GPC (Shodex K-2002 and K-2002.5; eluent: CHCl₃), and the fraction of large molecular weight ($M_n = 11,500$ g/mol, corresponding to 37-mer) was used for evaluation of saccharide recognition. ¹H NMR (CDCl₃, 300 Hz) δ 7.67 (s, 2n H), 7.16 (s, 4n H), 4.08–3.98 (m, 4n H), 3.80 (s, 2n H), 3.72–3.54 (m, 28n H), 2.86–2.79 (m, 4n H), 1.88–1.76 (m, 4n H), 1.52–1.16 (m, 20n H), 0.94–0.84 (m, 6n H); IR (KBr) ν_{max} 2930, 1581, 1534 cm⁻¹.

Evaluation of binding affinity of 2 with glucosides. Polymer **2** $(1.0 \times 10^{-3} \text{ M}, \text{ unit concentration})$, TETA $(1.7 \times 10^{-4} \text{ M})$, and TFA $(6.7 \times 10^{-4} \text{ M})$ were dissolved in CH₂Cl₂ (commercially available anhydrous grade). This solution was titrated with a solution or solid of guest glucoside, and the observed ellipticity on CD was plotted versus the concentration of the glucoside. The formal binding constants were obtained by iterative curve-fitting measurements based on the equation⁴ and the assumption that the molecular weight of the polymer **2** was uniform in length and that the polymer and the guest associate in a 1:1 ratio.

Equation:

$$\boldsymbol{\theta}_{\text{obs}} = \frac{\boldsymbol{\theta}_{11}}{2K_{11}[\mathbf{2}]_0} \left[1 + K_{11}[\mathbf{2}]_0 + K_{11}[\mathbf{G}]_0 - \left\{ (1 + K_{11}[\mathbf{2}]_0 + K_{11}[\mathbf{G}]_0)^2 - 4K_{11}^2[\mathbf{2}]_0[\mathbf{G}]_0 \right\}^{1/2} \right]$$

 K_{11} : the 1:1 binding constant of **2** with glycosides

 $\theta_{\rm obs}$: the observed ellipticity

 θ_{11} : ellipticity of the 1:1 host-guest complex (at the saturation point)

 $[2]_0$: the total concentration of 2

[G]₀: the total concentration of guest glycoside

References

- 1) H. Mao, J. B. Thorne, J. S. Pharr and R. E. Gawley, Can. J. Chem., 2006, 84, 1273-1279.
- 2) H. Abe, H. Machiguchi, S. Matsumoto and M. Inouye, J. Org. Chem., 2008, 73, 4650-4661.
- 3) U. Lehmann, O. Henze and A. D. Schlüter, Chem. Eur. J., 1999, 5, 854-859.
- 4) K. A. Connors, Binding Constants, John Wiley & Sons, New York, 1987.



Fig. S1 The changes of (left) CD and (right) UV/Vis spectra of the mixture of 2 and β -D-Glc in the presence and absence of diethylamine (DEA) and trifluoroacetic acid (TFA). (black) only 2; (brown) 2 + β -D-Glc; (blue) 2 + β -D-Glc + DEA; (red) 2 + β -D-Glc + DEA + TFA. Conditions: 2 (1.0 × 10⁻³ M, unit conc.), β -D-Glc (2.0 × 10⁻³ M), DEA (3.3 × 10⁻⁴ M), TFA (6.7 × 10⁻⁴ M), CH₂Cl₂, 25 °C, path length = 1 mm.



Fig. S2 The changes of a UV/Vis spectrum of 2 in the presence and absence of triethylene tetramine (TETA) and trifluoroacetic acid (TFA). (black) only 2; (blue) 2 + TETA; (red) 2 + TETA + TFA. Conditions: 2 (1.0×10^{-3} M, unit conc.), *β*-D-Glc (2.0×10^{-3} M), TETA (1.7×10^{-4} M), TFA (6.7×10^{-4} M), CH₂Cl₂, 25 °C, path length = 1 mm. A different stock solution of 2 was used than other experiments.



Fig. S3 The changes of (left) CD and (right) UV/Vis spectra of the mixture of **2** and β -D-Fru in the presence and absence of triethylene tetramine (TETA) and trifluoroacetic acid (TFA). (black) only **2**; (brown) **2** + β -D-Fru; (blue) **2** + β -D-Fru + TETA; (red) **2** + β -D-Fru + TETA + TFA. Conditions: **2** (1.0 × 10⁻³ M, unit conc.), β -D-Fru (2.0 × 10⁻³ M), TETA (1.7 × 10⁻⁴ M), TFA (6.7 × 10⁻⁴ M), CH₂Cl₂, 25 °C, path length = 1 mm.



Fig. S4 The changes of (left) CD and (right) UV/Vis spectra of the mixture of 2 and β -D-Man in the presence and absence of triethylene tetramine (TETA) and trifluoroacetic acid (TFA). (black) only 2; (brown) 2 + β -D-Man; (blue) 2 + β -D-Man + TETA; (red) 2 + β -D-Man + TETA + TFA. Conditions: 2 (1.0 × 10⁻³ M, unit conc.), β -D-Man (2.0 × 10⁻³ M), TETA (1.7 × 10⁻⁴ M), TFA (6.7 × 10⁻⁴ M), CH₂Cl₂, 25 °C, path length = 1 mm.



Fig. S5 The titration curve for CDs of the mixture of 2 and β -D-Glc in the presence and absence of triethylene tetramine (TETA) and trifluoroacetic acid (TFA). β -D-Glc was used as titrant. (left) 2 + β -D-Glc, CD at 337.2 nm; (middle) 2 + TETA + β -D-Glc, CD at 340.7 nm; (right) 2 + TETA + TFA+ β -D-Glc, CD at 342.4 nm. Conditions: 2 (1.0 × 10⁻³ M, unit conc.), β -D-Glc, TETA (1.7 × 10⁻⁴ M), TFA (6.7 × 10⁻⁴ M), CH₂Cl₂, 25 °C, path length = 1 mm. Curve-fitting analyses were carried out on the assumption that the molecular weight of the polymer 2 was uniform in length and that the polymer and the guest associate in a 1:1 ratio. Deviation was observed between plots and fitted curves possibly due to the variety of the molecular length.

Supplementary Material (ESI) for Chemical Communications This journal is (c) The Royal Society of Chemistry 2009

¹H NMR spctra















