A general and versatile molecular design for host molecules working in water: a duplex-based potassium sensor consisting of three functional regions

Kazuhisa Fujimoto,*[a] Yu Muto[a] and Masahiko Inouye*[a,b]

[a] Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University.
[b] PRESTO, Japan Science and Technology Agency (JST).

Supplementary Information

General Methods and Materials. High-resolution mass spectra were obtained by the ESI-TOF method. ^{1}H and ^{13}C NMR spectra were recorded at 500 and 125 MHz, respectively. Phosphoramidite **2** and succinimidyl ester **4** were prepared according to our previously reported procedure.[1]

Phosphoramidite 3. To a CH_3CN (3 mL) solution of 1*H*-tetrazole (51 mg, 0.74 mmol) and 2-cyanoethyl tetraisopropyl phosphorodiamidite (295 mg, 0.98 mmol) was added a CH_3CN solution (7 mL) of 4-(3-hydroxypropyl)benzo-15-crown-5 ether[2] (160 mg, 0.49 mmol) dropwise at 0 °C. After the solution had been stirred at room temperature for 1 h, the solvent was removed by a rotary evaporator. The residue was poured into an $NaHCO_3$ aqueous solution and extracted with ethyl acetate. The ethyl acetate extract was evaporated by a rotary evaporator and chromatographed (silica gel pre-treated with hexane:Et_3N = 9:1; eluent, hexane:AcOEt = 2:1) to give **3**: yield 62% (160 mg); oil; IR (KBr) 2964, 1514, 1455, 1362, 1262, 1131, 1046 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.18 (m, 12 H), 1.88 (m, 2 H), 2.62 (m, 2 H), 2.64 (t, J = 6.8 Hz, 2 H), 3.56–3.68 (m, 4 H), 3.75 (s, 8 H), 3.77–3.88 (m, 2 H), 3.90 (q, J = 4.5 Hz, 4 H), 4.11 (m, 4 H), 6.71 (m, 2 H), 6.78 (d, J = 8.5 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 20.3, 20.4, 24.52, 24.58, 24.64, 29.9, 31.7, 32.88, 32.94, 42.9, 43.0, 58.2, 58.3, 62.7, 62.8, 69.0, 69.3, 69.6, 69.7, 70.50, 70.53, 71.0, 114.4, 114.6, 117.7, 120.9, 135.1, 147.2, 149.0; HRMS (ESI) *m/z* calcd for $C_{26}H_{43}N_2NaO_7P$ ($[M+Na]^+$) 549.2706, found 549.2671.

Succinimidyl Ester 5. A CH_3CN (2 mL) solution of 4-(2-carboxyethyl)benzo-15-crown-5 ether[3] (50 mg, 0.15 mmol), pyridine (12.5 μL), and *N*,*N'*-disuccinimidyl carbonate (75 mg, 0.29 mmol) was stirred at 55 °C for 2.5 h. After removal of the solvent by a rotary evaporator, the residue was poured into a

saturated NaCl aqueous solution and extracted with ethyl acetate. The ethyl acetate extract was evaporated to give crude **5**. This compound was used in the next reaction without further purification. Purification for obtaining the physical data of **5** was carried out by reverse phase HPLC on a COSMOSIL 5C18-AR-II column (20 × 250 mm; nakalai tesque, eluent, MeOH): yield 92% (60 mg); oil; IR (KBr) 2871, 1809, 1780, 1737, 1514, 1429, 1361, 1263, 1206, 1130, 1069 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.86 (br s, 4 H), 2.90 (t, J = 7.5 Hz, 2 H), 3.00 (t, J = 7.5 Hz, 2 H), 3.78 (s, 8 H), 3.92 (q, J = 4.8 Hz, 4 H), 4.14 (m, 4 H), 6.76 (m, 2 H), 6.83 (d, J = 8.5 Hz, 1 H); ^{13}C NMR ($CDCl_3$) δ 25.4, 29.9, 32.7, 68.7, 69.0, 69.4, 69.5, 70.31, 70.34, 70.86, 70.87, 114.0, 114.2, 120.7, 132.2, 147.7, 149.0, 167.8, 169.1; HRMS (ESI) *m/z* calcd for $C_{21}H_{27}NNaO_9$ ($[M+Na]^+$) 460.1584, found 460.1593.

Monomer units 1a and 1b. A connection of the 5′ end of each single-stranded 7-mer DNA possessing a C3-alkylamino linker (3′ end) with **2** and **3** was performed by using an Applied Biosystems 392 DNA/RNA synthesiser. The 5′-modified DNAs were further tethered to **5** and **4** at the amino terminal of the 3′-linker to give **1a** and **1b**, respectively. These monomer units **1a** and **1b** were purified by reverse phase HPLC on a CHEMCOBOND 5-ODS-H column (10 × 150 mm; Chemco SCIENTIFIC.,LTD., elution with 5 mM ammonium formate, linear gradient over 30 min from 0% to 40% acetonitrile for **1a** (from 0% to 60% acetnitrile for **1b**) at a flow rate of 1.5 mL/min). **1a**: MALDI-MS *m/z* calcd for $C_{107}H_{132}N_{29}O_{51}P_8$ ($[M+H]^+$) 2886.65, Found 2886.93. **1b**: MALDI-MS *m/z* calcd for $C_{107}H_{133}N_{26}O_{53}P_8$ ($[M+H]^+$) 2877.64, Found 2877.98.

Reference monomers 1a′ and 1b′. These reference monomers were prepared in a manner similar to those described for **1a** and **1b**. In reverse phase HPLC purification, these acetonitrile linear gradients for **1a′** and **1b′** were 10–45% and 0–40%, respectively. **1a′**: MALDI-MS *m/z* calcd for $C_{87}H_{102}N_{28}O_{42}P_7$ ($[M+H]^+$) 2427.49, Found 2428.23. **1b′**: MALDI-MS *m/z* calcd for $C_{90}H_{108}N_{26}O_{45}P_7$ ($[M+H]^+$) 2489.51, Found 2489.57.

Fluorescence Titration. To a 200 nM solution of **1a** and **1b** (**1a′** and **1b′**) containing 20 mM Tris-HCl buffer (pH 8.0) was added alkali-metal chloride (5–100 mM) at 25 °C. Each mixture was stirred at 25 °C until no more change of the fluorescence spectra occurred (ca. 10 min). The excitation wavelength was 350 nm, and the fluorescence spectra were recorded from 300 to 650 nm at every addition of alkali-metal chloride.

Determination of T_m. Melting curves were measured at 260 nm in the temperature range from 0 to 60 °C. The solution for the measurements contained **1** or **1′** (1 μM) and 20 mM Tris-HCl (pH 8.0) in the presence or absence of alkali-metal chloride (100 mM).

(1) K. Fujimoto, H. Shimizu, and M. Inouye, *J. Org. Chem.*, 2004, **69**, 3271.

(2) K. Kikukawa, A. Abe, F. Wada, and T. Matsuda, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 961.

(3) L. Yi, Z. Zhuangyu, and H. Hongwen, *Synth. Commun.*, 1995, **25**, 595.

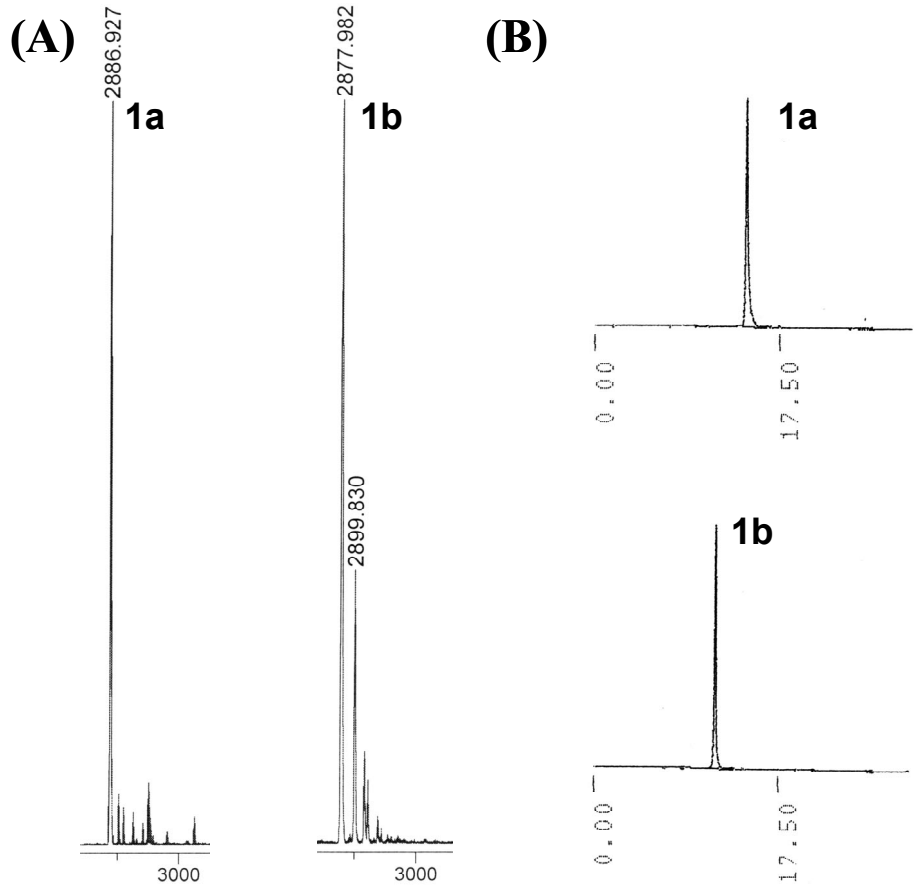

Fig. S1 (A) MALDI-MS spectra and (B) HPLC profiles of **1a** and **1b**.