Supporting Information

Solid-Supported DNA for Asymmetric Synthesis: a Stepping Stone toward Practical Applications

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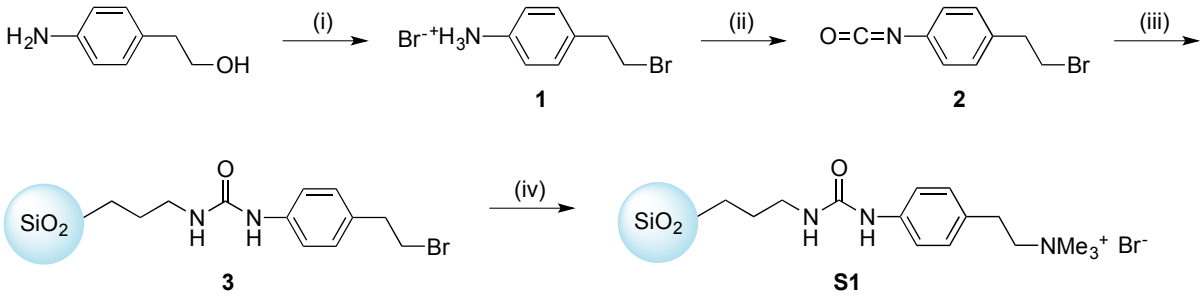
Materials

3-aminopropyl-functionalized silica gel (40-63 μm, ~1 mmol/g NH_2 loading), 3-(trimethylammonium)propyl-functionalized silica gel, carbonate (200-400 mesh, 0.8 mmol/g loading) (**S2**), copper(II) nitrate hydrate, 4,4'-dimethyl-2,2'dipyridyl (dmbpy) were purchased from Sigma-Aldrich Chemicals Co. (Milwaukee, WI) and used as received. Silica Gel 60 N (spherical, neutral, 40-50 μm) (**S3**) was purchased from Kanto Chemical Co. and used as received. 2-azachalcone (**4**) was prepared by following the literature procedures. [ref] Cyclopentadiene (**5**) was freshly prepared from its dimer prior to use. Dicyclopentadiene was obtained from Wako Chemicals. All other chemicals and solvents were purchased from Sigma-Aldrich Chemicals Co., Wako Pure Chemical Ind. Ltd., TCI, or Kanto Chemical Co. Inc. and used without further purification. Copper(II) complexes with the 4,4'-dimethyl-2,2'-bipyridine (dmbpy) ligand and MOPS solution were prepared using the procedures reported by Feringa and Roelfes. [ref] Salmon testes DNA and synthetic oligonucleotides were obtained from Sigma-Aldrich Chemicals Co. Water was deionized (specific resistance of $\geq$ 18.0 M□ cm at 25^{o}C) by a Milli-Q system (Millipore Corp.).

Methods

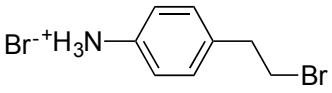
NMR spectra were obtained on a JEOL JNM ECA-600 spectrometer operating at 600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR in $CDCl_3$ unless otherwise noted. Flash column chromatography was performed employing Silica Gel 60 (70–230 mesh, Merck Chemicals). Silica-gel preparative thin-layer chromatography (PTLC) was performed using plates from Silica gel 70 PF_{254} (Wako Pure Chemical Ind. Ltd.). Enantiomeric excess (*ee*) determinations were performed by HPLC analysis (Chiralcel OD-H) using UV-detection. DNA concentrations were measured by Nanodrop ND-1000 spectrophotometer. Rotary mixing of reaction suspension was performed by Intelli-Mixer RM-2 (Elmi).

Synthetic routes for ammonium-modified silica gels



Reagents and conditions: (i) 48% HBr, reflux; (ii) triphosgene, sat. $NaHCO_3$ solution, dichloromethane, 0 °C; (iii) 3-aminopropyl-functionalized silica gel, dichloromethane, rt; (iv) NMe_3/methanol solution, ethanol, 50 °C

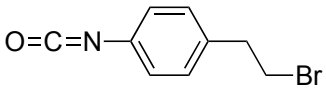
Analytical Data for new substrates:



4-(2-bromoethyl)anilinium bromide (**1**)

To a 48% HBr solution was added 4-(2-hydroxymethyl)aniline (240 mg, 1.75 mmol) and the resulting mixture was heated under reflux for 4 hours. After cooled to room temperature, the reaction mixture was cooled further to 0 °C and collected precipitates as a white solid. This solid was readily used for a following reaction.

^{1}H NMR (DMSO-*d*6): δ 7.41 (d, $^3J_{HH}$ = 8.8 Hz, 2H), 7.34 (d, $^3J_{HH}$ = 8.1 Hz, 2H), 4.47 (br, 3H(NH_3^+)), 3.74 (t, $^3J_{HH}$ = 6.8 Hz, 2H), 3.15 (t, $^3J_{HH}$ = 6.8 Hz, 2H). ^{13}C NMR ($CDCl_3$): d 139.40, 130.16, 129.75, 123.33, 37.58, 34.49. HRMS (ESI-TOF) cald for $C_8H_{11}NBr$ ($M\text{-}Br^-$) 200.0075, found 199.9952.

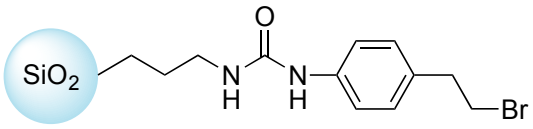


4-(2-bromoethyl)phenyl-isocyanate (**2**)

The whole amount of 4-(2-bromoethyl)anilinium bromide (**1**) was added to a bilayer solution of dichloromethane (4 mL) and saturated $NaHCO_3$aq (4 mL) and the resulting mixture was cooled to 0 °C and then added triphosgene (114 mg, 0.39 mmol) and stirred for 40 minutes at 0 °C. After cooled to room temperature, the reaction mixture was extracted with dichloromethane. The organic layer was dried over $MgSO_4$, filtered, and concentrated under

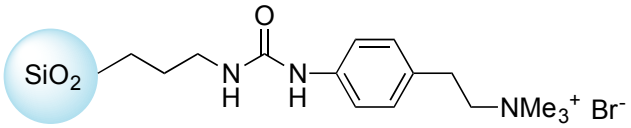
vacuum. The obtained yellow oil (224 mg, 1 mmol, 57% yield among two steps) was readily used for the following reaction.

^{1}H NMR ($CDCl_3$): δ 7.17 (d, $^{3}J_{HH}$ = 8.2 Hz, 2H), 7.05 (d, $^{3}J_{HH}$ = 8.2 Hz, 2H), 3.55 (t, $^{3}J_{HH}$ = 7.5 Hz, 2H), 3.14 (t, $^{3}J_{HH}$ = 7.5 Hz, 2H). ^{13}C NMR ($CDCl_3$): d 136.49, 132.18, 129.83, 124.87, 119.65, 38.62, 32.69. HRMS (ESI-TOF) cald for $C_8H_{11}NBr$ ($M+3H^{+}-CO^{2-}$) 200.0075, found 199.9986.



3-((4-((2-bromo)ethyl)phenyl)ureido)propyl-functionalized silica gel (**3**)

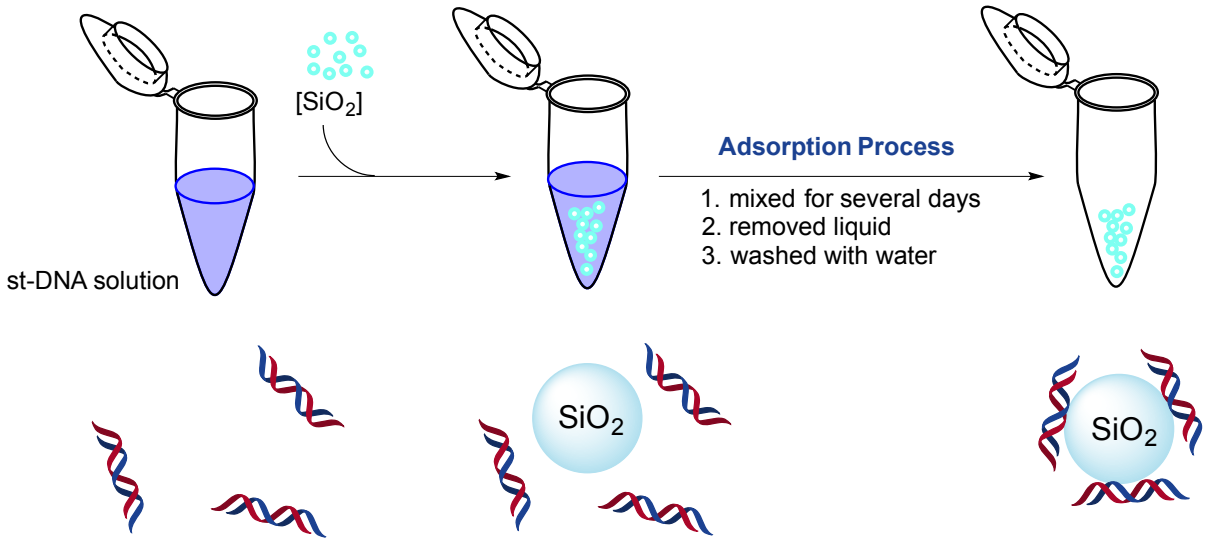
224 mg of **2** was added to dichloromethane (20 mL) and 3-aminopropyl-functionalized silica gel (800 mg, 40-63 μm, 1 mmol/g ammonium loading) was subsequently added. The resulting suspension was vigorously stirred for 20 hours at room temperature, and then the solid was filtered and washed with dichloromethane. The obtained solid was readily used for the following reaction.



3-((4-((2-trimethylammonium)ethyl)phenyl)ureido)propyl-functionalized silica gel, bromide (**S1**)

The whole amount of **3** and ethanol (2mL) was added to a trimethylamine-in-methanol solution (3.2 mol/L, 7 mL). The resulting suspension was vigorously stirred for 3 days at 50 ^{o}C, then the solid was filtered and washed with methanol, acetone, water, then dried under reduced pressure to obtain **S1** (914 mg).

Adsorption of DNA to ammonium-functionalized silica gels



To st-DNA solution (2 mg of st-DNA in 1 mL of water), 50 mg of **S1** was added. After well homogenizing, the suspension was set to rotary mixer and mixed by continuous rotation at 5 °C. Proceeding of adsorption was monitored by measurement of the decreasing of the absorbance intensity at 260 nm in supernatant liquid. After the absorbance intensity became lower than 1/10 of the original solution, mixing was stopped and supernatant liquid was removed. Remaining powder was washed with water (1 mL, two times), and then dried under reduced pressure. **st-DNA/S1** powder was obtained as a pale ivory solid.

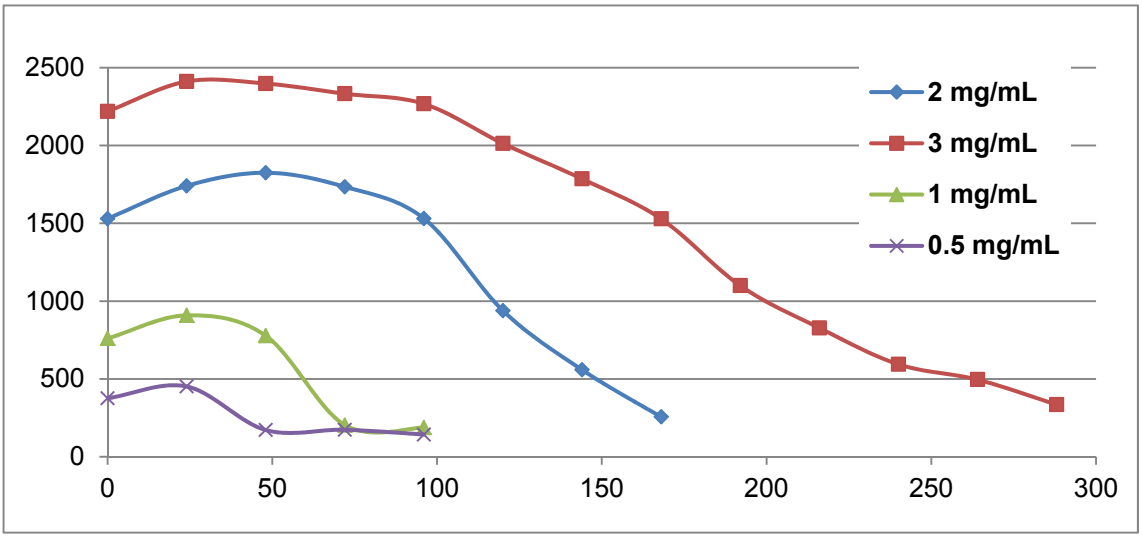


Figure S1. DNA condensation monitoring for the mixture of **S1** and various condensation of stDNA solutions. To st-DNA solution (2 mg of st-DNA in 1 mL of water), 50 mg of **S1** was added and mixed. The values of DNA concentration were calculated by the UV absorbance at 260 nm.

Asymmetric Diels-Alder reactions

A catalytic suspension for the Diels-Alder reaction was prepared by mixing the Cu(dmbpy) complex with **st-DNA/S1** in buffer solution. For example, in Table 2, 50 mg of **st-DNA/S1** was added to 240 µL of 30 mM MOPS solution (pH 6.5) followed by mixing with 120 µL of a 2.7 mM solution of Cu(dmbpy) complex (33 mol%). The catalytic suspension was prepared and stored 1.5 h in advance at 5 °C before its use. To **st-DNA/S1** suspension with Cu(dmbpy), an aliquot of a stock solution of 2-azachalcone **4** in CH_3CN (2 µL of a 0.5 M solution, final concentration was 2.78 mM) was added and the reaction mixture was cooled to 5 °C. The reaction was started by addition of cyclopentadiene (2 µL, final concentration was 66 mM) and mixed by continuous rotation for 3 days. The product was extracted with Et_2O three times and concentrated under reduced pressure.

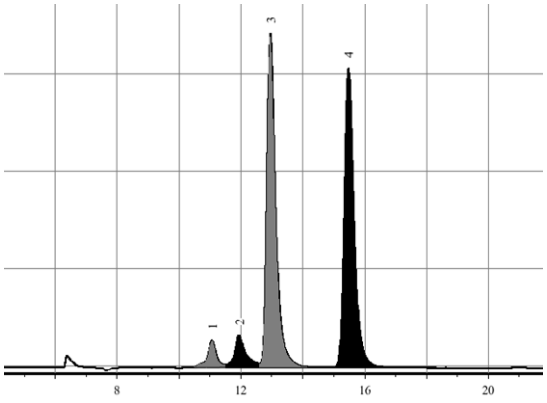
The *ee* of **6** was determined on a Daicel Chiralcel OD-H column with hexane/2-propanol = 95/5, flow = 0.5 mL/min, λ = 254 nm. Retention times: 13.0 min [minor enantiomer], 15.5 min [major enantiomer] (retention time for each enantiomers of **7**: 11.1 min and 12.0 min, for **4**: 17.7 min).

Conversions were calculated using the formula:

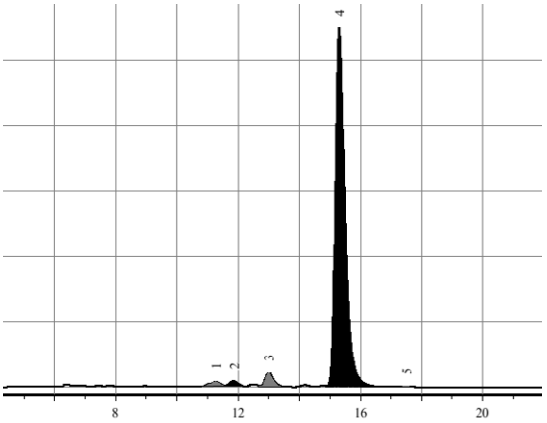
$$\text{Conversion (\%)} = \frac{A_{\text{Norm}}(P)}{\frac{A_{\text{Norm}}(S)}{c} + A_{\text{Norm}}(P)} \cdot 100\ \%$$

Where $A_{Norm}(P)$ is the total peak area of the product of the reaction (peak 1+2+3+4), $A_{Norm}(S)$ is the peak area of the starting material (peak 5) and *c* is the correction factor determined to be 1.39 from other calibrating measurements.

Where peak 1, 2 are both enantiomer of *exo*-products ((*R,S*)-**7**), and peak 3, 4 are both enantiomer of *endo*-products ((*R,S*)-**6**).



Peak	Fill	t_R (min)	t_S (min)	t_E (min)	A_{Norm}
1		11.06	10.04	11.51	3.79
2		11.93	11.51	12.56	5.56
3		12.95	12.56	14.81	45.69
4		15.47	14.91	16.61	44.96
					100.00



Peak	Fill	t_R (min)	t_S (min)	t_E (min)	A_{Norm}
1		11.27	10.61	11.56	1.35
2		11.84	11.56	12.23	1.17
3		13.00	12.68	13.76	3.14
4		15.30	14.88	17.13	94.12
5		17.48	17.13	19.33	0.21
					100.00

General procedure for Recycling

Solid-supported DNA was salvaged from reaction mixture by following process: **St-DNA/S1** powder was filtered from the aqueous reaction suspension and then washed with 1 mL of water, 1 mL of methanol and 1 mL of EtOAc. The obtained solid was dried under reduced pressure, then used for next reaction cycle. (Fresh buffer solution and Cu(dmbpy) was added for the next reaction.)