Supplementary Material (ESI) for Chemical Communications


# Supplementary Information Accompanying

"Modular Assembly of Novel DNA-based catalysts"

by

Núria Sancho Oltra and Gerard Roelfes

## Experimental and synthetic procedures

### Synthesis

### General remarks

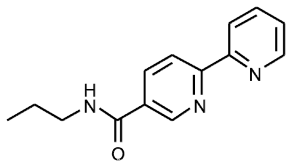
RP-HPLC analysis were performed on a Shimadzu LC-10AD VP, Waters Xterra MS C18 column (3.0 x 150 mm, particle size 3.5 μm) using a gradient of $CH_3CN$/TEAA buffer 50 mM pH 7; gradient: 05/95 0 to 10 min, to 35/65 at 60 min, to 70/30 at 65 min. Flow: 0.5mL/min. MALDI-TOF measurements were done on a Voyager-DE Pro apparatus. (Matrix: 20 μL of a solution of 2, 4, 6- Trihydroxyacetophenone 0.5 M in ethanol + 10 μL of a solution of ammonium citrate dibasic 0.1 M in Milli Q water + 2 μL sample solution in Milli Q water).

Aza-chalcone (**2**) was prepared following published procedures.[1]

**2,2'-bipyridine-5-carboxylic acid.** Synthesized following published procedures.[2, 3]

**N-hydroxysuccinimide 2,2'-bipyridine-5-carboxylate (1).** 314 mg (1.57 mmol) of 2,2'-bipyridine-5-carboxylic acid was dissolved in 4 mL of thionyl chloride and a catalytic amount of dimethylformamide under inert atmosphere and the mixture was heated under reflux for 4 hours. The thionyl chloride was evaporated under reduced pressure and the residue was dried under vacuum for several hours. Subsequently, 15 mL of dry $CH_2Cl_2$ and 181 mg (1.57 mmol) of N-hydroxysuccinimide were added and the reaction mixture was cooled in an ice bath. While stirring, 0.4 mL of triethylamine was added dropwise. The mixture was stirred overnight at room temperature under nitrogen atmosphere. The solvent was evaporated and the product was purified by column chromatography (silica, EtOAc/$CH_2Cl_2$ 1:1 v/v) to yield the product (286 mg, 61 %) as a white solid. m.p. = 180.0-181.8 °C.
$^1$H-NMR ($CDCl_3$, 300 MHz) δ 9.37 (s, 1H), 8.75 (d,1H, J = 4.39 Hz), 8.64 (d, 1H, J = 8.42 Hz), 8.56-8.49 (m, 2H), 7.91 (t, 1H, J = 7.8), 7.43 (t, 1H, J = 7.5), 2.94 (s, 4H); $^{13}$C-NMR (101MHz, $CDCl_3$) δ 169.2, 161.1, 154.5, 151.3, 149.4, 139.0, 137.7, 125.3, 122.6, 121.4, 121.1, 25.9 (a signal of carbonyl is missing due to low intensity); MS (CI): 298 (M+1)

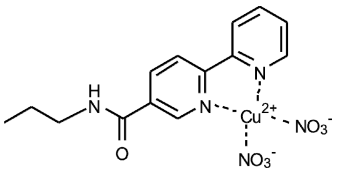
**N-propyl-2,2'-bipyridine-5-carboxamide.**




18 mg (0.06 mmol) of **1** was dissolved in dry $CH_2Cl_2$. Two drops of triethylamine were added and the mixture was stirred for 1.5 h at room temperature. The solvent was evaporated and the product was purified by column chromatography (silica, EtOAc/$CH_2Cl_2$ 1:1 v/v) to yield the product as a white solid (11.5 mg, 79 %).m.p. = 139.6-140.4 °C.
$^1$H-NMR ($CDCl_3$, 400MHz) δ 9.04 (s, 1H), 8.71 (d, 1H, J= 4.03 Hz), 8.46 (m, 2H), 8.20 (dd, 1H, J = 8.3 Hz, J = 2.4 Hz), 7.85 (t, 1H, J = 7.9), 7.35 (t, 1H, J = 6.2 Hz), 6.17 (bs, 1H), 3.48 (q, 2H, J

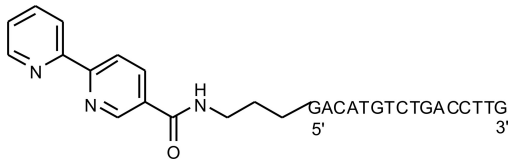
= 7.0 Hz), 1.68 (m, 2H), 1.02 (t, 3H, J = 7.5 Hz); $^{13}$C-NMR (50 MHz, $CDCl_3$) δ 158.3, 151.0, 147.8, 142.0, 140.3, 129.8, 128.4, 122.6, 117.0, 114.3, 113.4, 34.6, 15.6, 4.1.

**[Cu(N-propyl-2,2'-bipyridine-5-carboxamide) $(NO_3)_2$].**

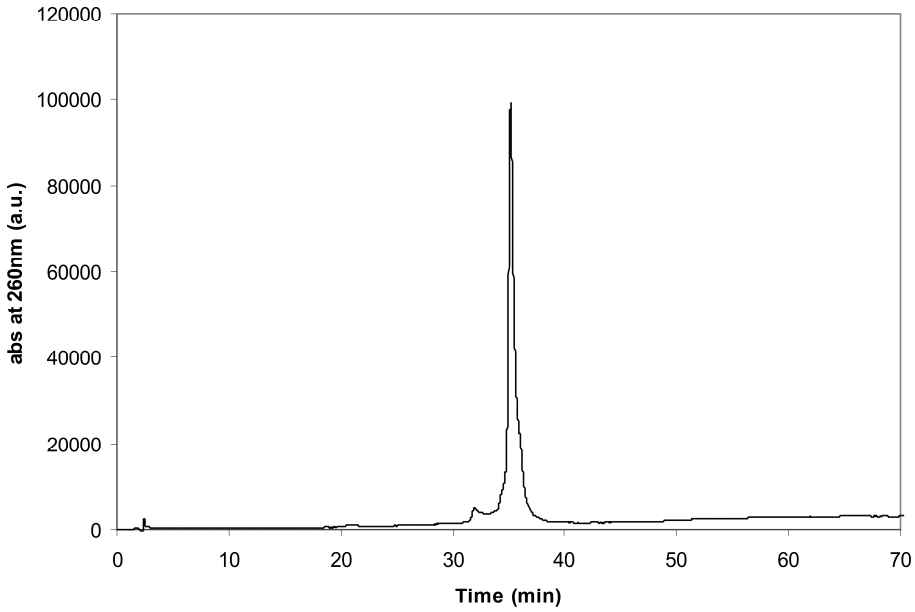



2,2'-bipyridine-5-propylamide (9.7 mg, 0.04 mmol) was added to a $Cu(NO_3)_2 \cdot 3H_2O$ (16.51 mg, 0.068 mmol) solution in ethanol (1.5 mL). The complex was crystallized by vapour diffusion of ethylacetate. After standing for 3 nights, the resulting blue needles were filtrated, washed with ethanol and dried under vacuum at 40 °C. Yield: 10 mg (0.023 mmol, 58 %). Anal. Calcd for $C_{14}H_{15}CuN_5O_7$: C, 39.21 H, 3.53 N, 16.33. Found: C, 38.99 H, 3.48 N, 16.06.

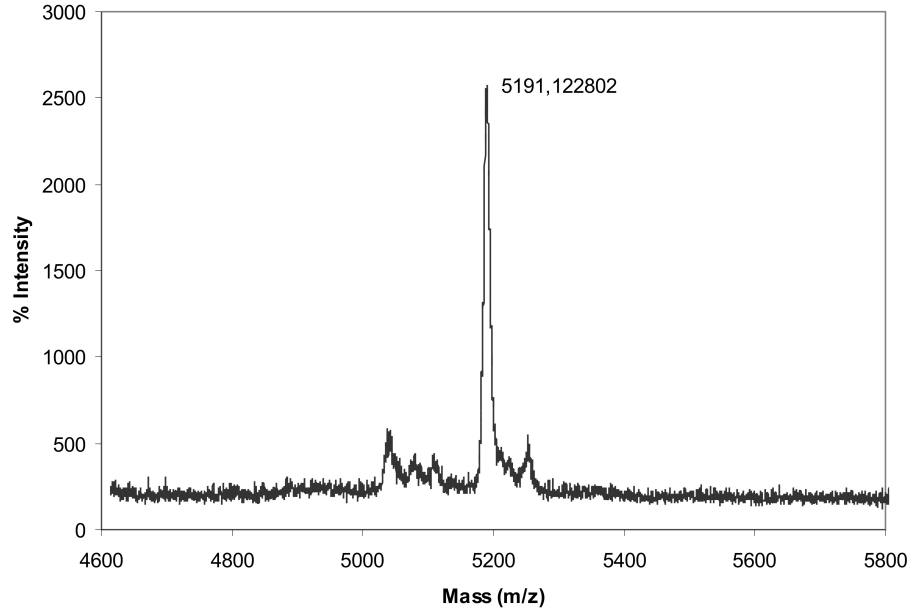
**Synthesis of bipyridine-DNA conjugates, representative procedure.** 250 µL of a stock solution of aminomodified-oligonucleotide (200 µM in $H_2O$) was mixed with 200 µL of Phosphate buffer (200 mM pH 7.2) and 50 µL of dimethylformamide. To this solution, 100 µL of a stock solution of N-hydroxysuccinimide 2,2'-bipyridine-5-carboxylate (20 mg/mL in dimethylformamide) was added in fractions of 25 µL over a period of 4 hours with continuous shaking. The mixture was shaken overnight and the coupled product was purified by size exclusion chromatography (Sephadex$^{TM}$ G-25 DNA Grade, Triethylammonium acetate 50 mM pH 7). The products were analyzed by rp-HPLC and MALDI-TOF.

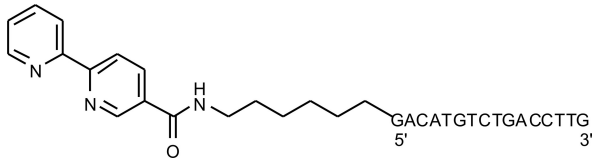



rp-HPLC



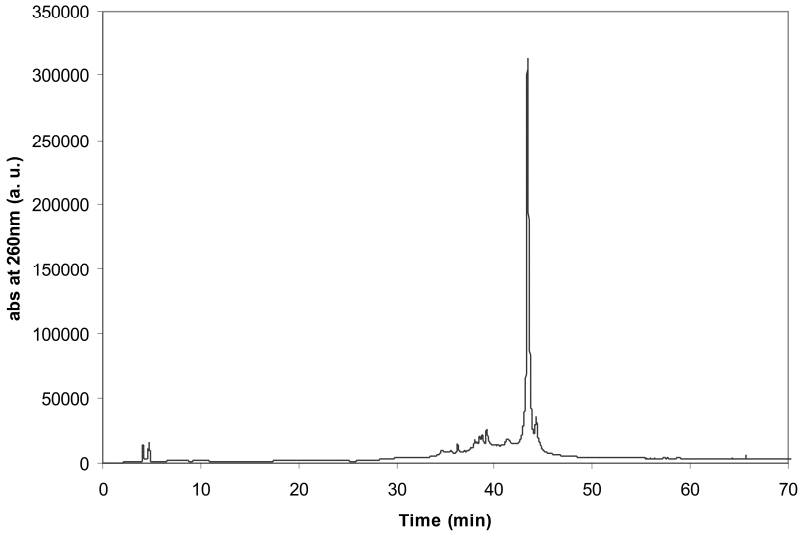
MALDI-TOF (calcd (m/z) = 5191.42)

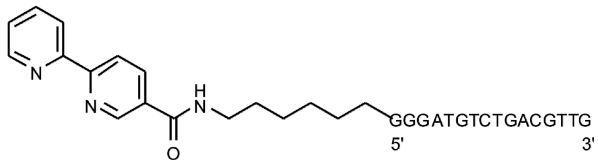



m/z (MALDI-TOF) = 5229.08 (calcd (m/z) = 5233.5)

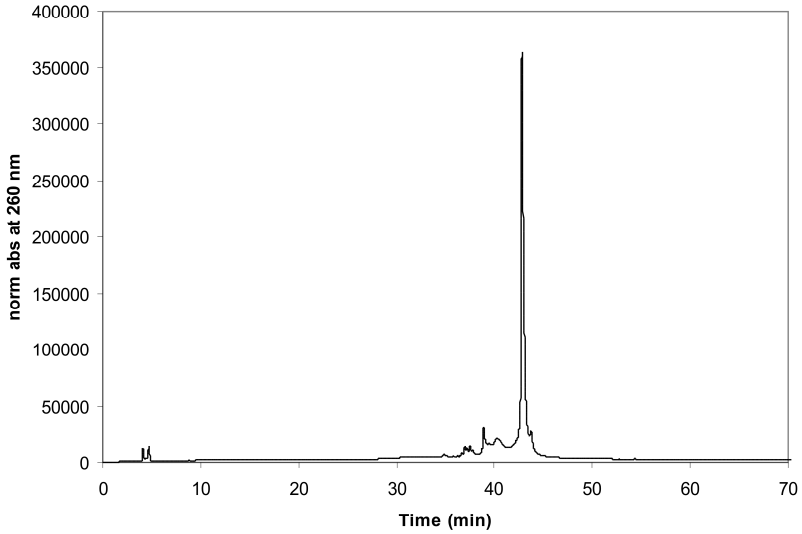
rp-HPLC

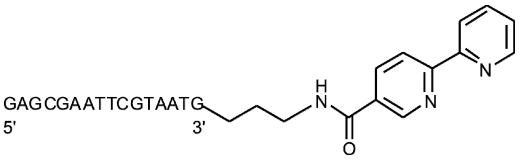





m/z (MALDI-TOF) = 5337.50 (calcd (m/z) = 5329.55)

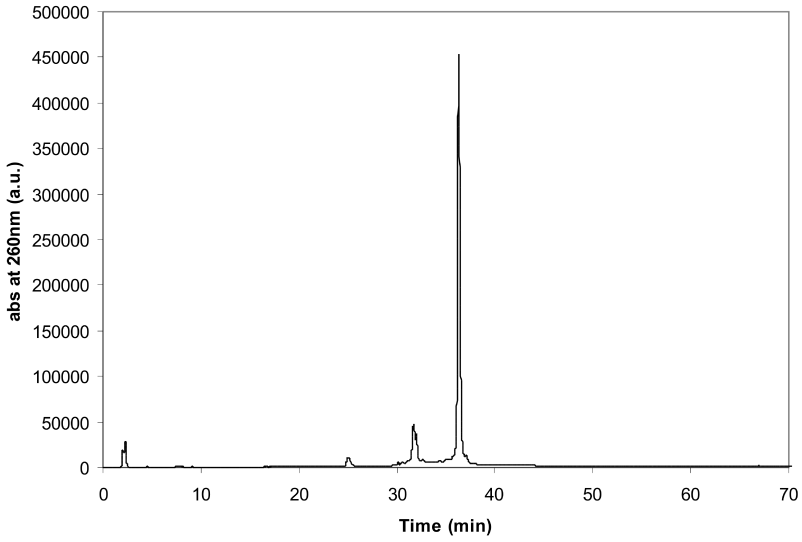
rp-HPLC




m/z (MALDI-TOF) = 5270.70 (calcd (m/z) = 5264.49)

rp-HPLC



**General procedure for the assembly of the catalyst and the catalytic Diels-Alder reactions.**

The DNA-based catalysts were assembled by mixing of aqueous solutions containing 0.04 μmol of the oligonucleotides ON1, ON2 and the template olignucleotide. After freeze-drying the mixture of oligonucleotides 300 μL of a 0.1 mM solution of $Cu(NO_3)_2 \cdot 3H_2O$ in MOPS buffer (20mM pH 6.5) was added and the solution was warmed to 70 ºC for 3 minutes and then cooled down slowly to room temperature. Prior to use the solution was kept at 5 ºC for 1.5 h.
To the catalyst solution was added 3 μL (0.296 μmol) of a stock solution 100 mM of aza-chalcone in acetonitrile. The reaction was started by the addition of 0.8 μL (9.8 μmol) of cyclopentadiene. After mixing by continuous inversion for 3 days at 5 ºC the mixture was extracted with diethyl ether. The conversion and enantiomeric excess were determined by chiral HPLC. HPLC conditions: Daicel chiralcel-ODH column, heptane/*i*PrOH 99:1, 0.5 mL/min. Retention times: 14.1, 15.9 (*exo* isomer), 18.5, 22.9 (*endo* isomer), 29.78 (starting material).

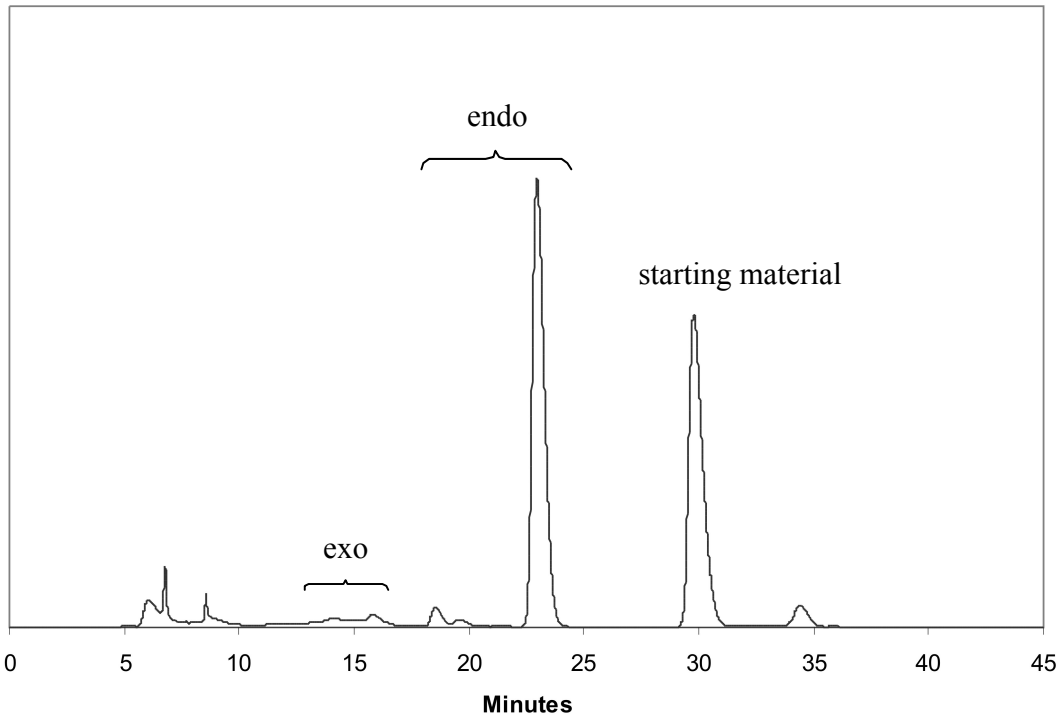



**Figure S1.** HPLC trace of the crude product **4** of the Diels-Alder reaction catalyzed by DNA-based catalyst (Table 1, entry 8).

Conversions were calculated using the formula:

$$conv.(\%) = \frac{area\ P}{\frac{area\ S}{c} + area\ P} \cdot 100\ \%$$

Where *area P* is the total peak area of the product of the reaction, *area S* is the peak area of the starting material and *c* is the correction factor determined to be 1.21 from a calibration curve.

**Table S1.** Control experiments of the Diels-Alder reaction of **2** with **3** catalyzed by DNA-based catalysts.[a, b, c]

| Entry | Catalyst (ON1, ON2 3'→ 5'/ template 5'→ 3') | Linker | Conversion (%)[d] | Ee (%)[e] |
|---|---|---|---|---|
| 1 | GTTCCAGTCTGTACAG ACCATGCTTAAGCGAG<br>CAAGGTCAGACATGTCATGGTACGAATTCGCTC | | 94 | 7 (-) |
| 2 | GTTCCAGTCTGTACAG**A**CCATGCTTAAGCGAG<br>CAAGGTCAGACATGTCTGGTACGAATTCGCTC | (a) | 51 | 69 (+) |
| 3 | GTTCCAGTCTGTACAGACCATGCTTAAGCGAG<br>CAAGGTCAGACATGTCTGGTACGAATTCGCTC | | 89 | 8 (-) |
| 4 | GTTCCAGTCTGTACAGGTAATGCTTAAGCGAG<br>CAAGGTCAGACATGTCCATTACGAATTCGCTC | | 94 | 32 (-) |
| 5[f] | GTTCCAGTCTGTACA**G**GTAATGCTTAAGCGAG<br>CAAGGTCAGACATGTCCATTACGAATTCGCTC | | 88 | 0 |
| 6 | GTTCCAGTCTGTACA**G** | (a) | 11 | 25 (+) |
| 7[g] | GTTCCAGTCTGTACAGGTAATGCTTAAGCGAG<br>CAAGGTCAGACATGTCCATTACGAATTCGCTC | | 46 | 15 (-) |

[a] All experiments were carried out with 0.13 mM oligonucleotides, 0.1 mM $Cu(NO_3)_2$, 1 mM aza-chalcone and 33 mM cyclopentadiene in MOPS buffer (20 mM pH 6.5) for 3 days at 5 ºC, unless noted otherwise. [b] Bold letters indicate the position where the metal complex is attached. [c] Results correspond to the average of at least two experiments. [d] Determined by chiral HPLC. Errors in the range of ±6 %. [e] Determined by chiral HPLC. For the *endo* isomer. Errors in the range of ±4 %. [f] Aminohexyl is used as the linker and the coordinating group. [g] No $Cu(NO_3)_2$ present.

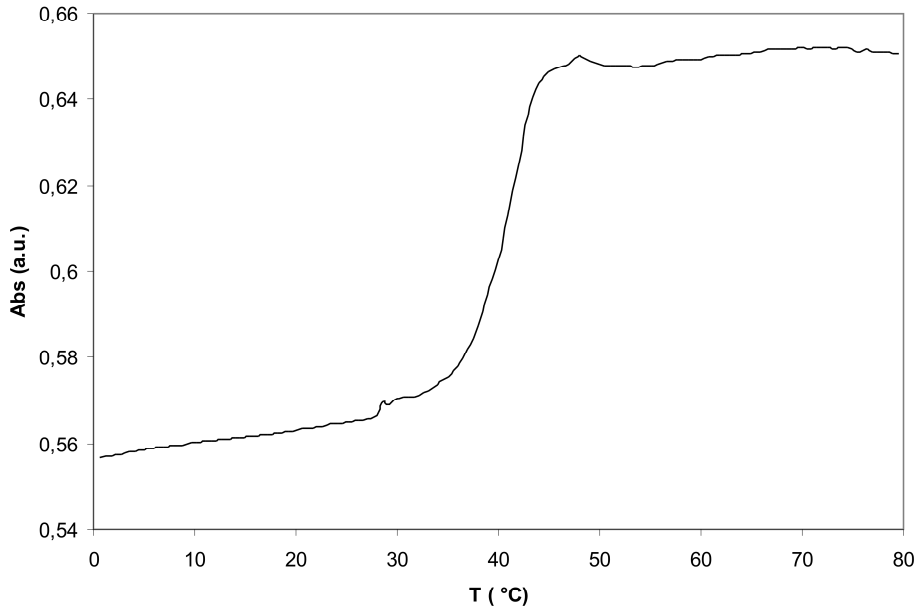



**Figure S2.** Melting curve of DNA duplex (Table 1, entry 9) (no copper (II) present).

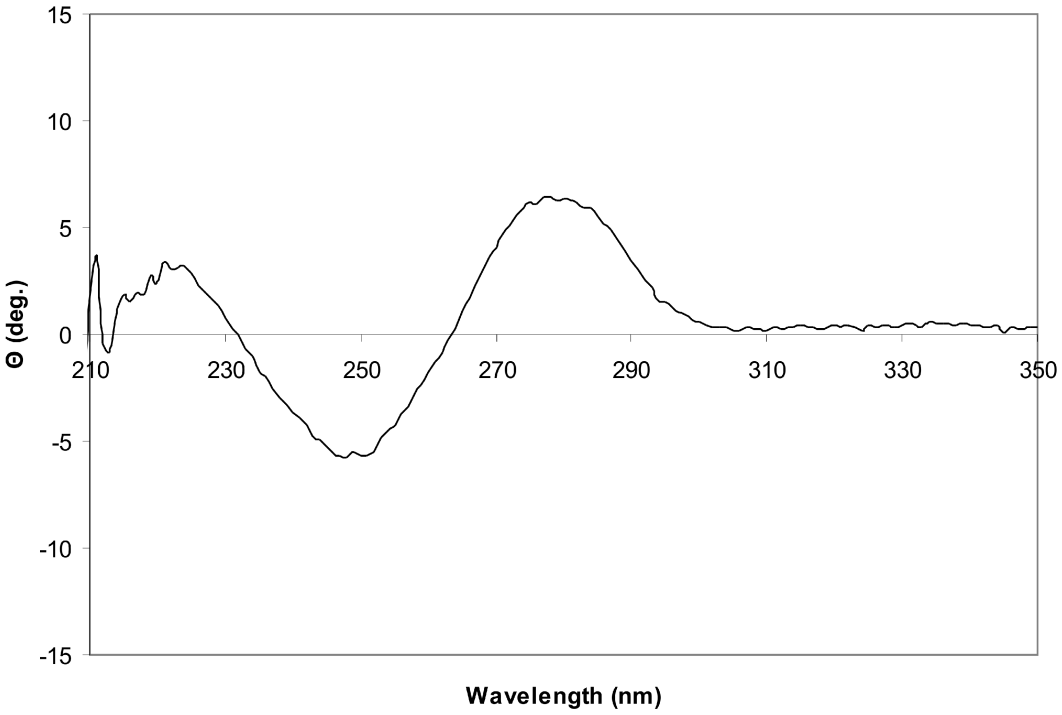



**Figure S3.** CD spectrum of DNA-based catalyst (Table 1, entry 8) (no copper (II) present).

## Reference list

1. S. Otto, J. B. F. N. Engberts, *J. Am. Chem. Soc.*, 1999, **121**, 6798-6806
2. F. Krohnke, K. F. Gross, *Chemische Berichte-Recueil*, 1959, **92**, 22-36
3. H. Liu, S. Kasahara and Y. Yoshikawa, *J. Coord. Chem.*, 2005, **58**, 1249-1259