Supplementary Information (SI) for ChemComm.
This journal is © The Royal Society of Chemistry 2026

Solid-Phase Click^2 Strategy for Chromophore-DNA Conjugates and their application as Light Harvesting System

Andreas J. Schmidt,[a] Annabel M. L. Knispel[a] and Hans-Achim Wagenknecht*[a]

[a]*Karlsruhe Institute of Technology (KIT), Institute of Organic Chemistry*

Fritz-Haber-Weg 6 76131 Karlsruhe

**Corresponding author: E-mail: wagenknecht@kit.edu*

Electronic Supplementary Information

Table of content

1. Material and Methods

Unless otherwise specified, reagents and starting materials were purchased from commercial suppliers and used without further purification. Demineralized water was obtained from a Milli-Q Direct 8 desalination system (Merck). When required, reactions were carried out under an inert gas atmosphere (argon, 99.999%). For this purpose, the reaction vessels were connected to the vacuum apparatus via appropriate tubing. Before use, the glass apparatus was evacuated and refilled with argon several times until a stable pressure of $5x10^{-2}$ mbar was reached.

Compounds **1**[1]and **4**[2] (Fig. S1) were synthesized according to literature procedures. Compound **2** and **3** was purchased from commercial suppliers.

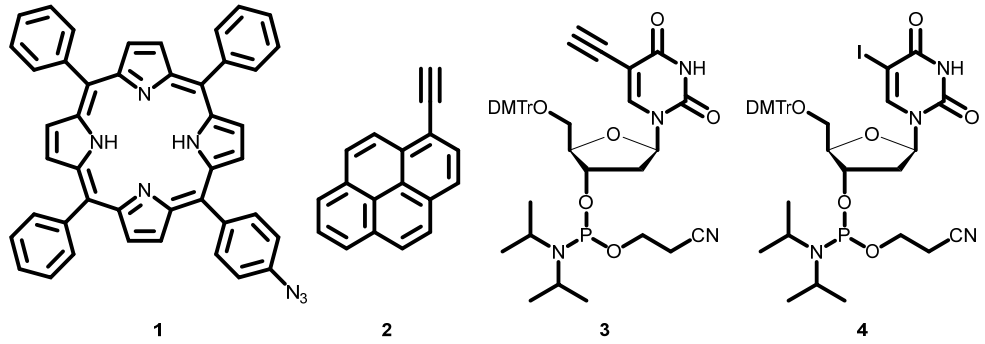

Figure S1 chemical structures of compounds **1**–**4**.

Thin-layer chromatography

Thin-layer chromatography (TLC) was performed using aluminum-backed silica gel plates from *Merck* (silica gel 60, F254, layer thickness 0.250mm).

Flash chromatography

Silica gel (40-63 µm) from *Sigma Aldrich* was used as the stationary phase. Columns were packed with solvent under positive pressure. Samples were dissolved in the eluent (wet load) or adsorbed onto silica gel (dry load) prior to application to the column.

NMR spectroscopy

NMR spectra were recorded at a Bruker Advance 400 spectrometer. Chemical shifts were reported relative to tetramethylsilane (TMS) in parts per million (ppm). Samples were dissolved in deuterated solvents (Eurisotop) and referenced to residual solvent signals.

Mass spectrometry

Oligonucleotides were analyzed by ESI mass spectrometry was on a Q Exactive Orbitrap (Thermo Fisher Scientific).

2. DNA synthesis and on bead *click* procedure

A DNA/RNA synthesizer H6 (K&A Laborgeräte GbR) was used for the synthesis of the oligonucleotides. Controlled pore glass (CPG, 500 Å, 1 µmol loading) served as the solid phase. Phosphoramidites and CPG columns were purchased from *Sigma Aldrich*, *Chem Genes* and *Glen Research*. Phosphoramidites were dissolved in dry acetonitrile and adjusted to a final concentration of 0.1 M. Oligonucleotide synthesis was carried out in DMTr-On mode (1mmol) according to standardized coupling protocols.

First *click* on bead

Modified oligonucleotides (**DNA1'** or **DNA3'**) were transferred to 1.5 mL microcentrifuge tubes. 100 µL azidoporphyrin **1** (100 mM, in DMSO), 50 µL sodium ascorbate (1000 mM, in H_2O), 400 µL TBTA (27.1 mM, in DMSO/t-BuOH = 3:1) and 200 µL tetrakis(acetonitrile)copper(I)hexafluorophosphate (142 mM, in DMSO/t-BuOH = 3:1) were added sequentially. The reaction mixture was homogenized, heated to 40°C for 30 min and subsequently incubated overnight at room temperature with shaking. The suspension was centrifuged and the supernatant removed. The CPG was then washed three times with 1 mL each of DMSO, DCM, EtOH and demineralised water, followed by drying under vacuum.

***In situ* azide formation and *click* on bead**

To introduce the first (**DNA2'**) or second label (**DNA3'**), the azide group of AdU was generated *in situ* from the corresponding IdU precursor. Freshly prepared sodium azide solution (1 mL, 200 mM, in DMSO) was added to **DNA2'** or **DNA3'** and the reaction mixture was heated at 55°C for 1h. After centrifugation, the supernatant was discarded and the CPG was washed four times with 1 mL each of DMSO and MeCN. 300 µL of ethynylpyrene **2** (50 mM, in DMSO), 50 µL sodium ascorbate (1000 mM, in H_2O), 400 µL TBTA (27.1 mM, in DMSO/t-BuOH = 3:1) and 200 µL tetrakis(acetonitrile)-copper(I) hexafluorophosphate (142 mM, in DMSO/t-BuOH = 3:1) were then added. The suspension was incubated at room temperature overnight. Following centrifugation (5 min at 13 000 rpm) the supernatant was carefully removed. The CPG was washed three times with 1 mL DMSO and DCM. Additional washing steps were performed using 500 µL sodium acetate (100 mM, in H_2O) and 300 µL sodium EDTA (300 mM, in H_2O), followed by demineralized water and EtOH.

DNA deprotection and purification

Oligonucleotides (**DNA1**-**DNA3**) were cleaved from CPG and deprotected by treatment with concentrated ammonia solution for 18h at 55°C. After cleavage, the crude solution was processed using a Glen-Pak™ DNA purification cartridge (Glen Research). The pre-purified oligonucleotide was evaporated to dryness, dissolved in 300 µL water and purified by high-performance liquid chromatography (HPLC) using a Thermo Scientific Dionex UltiMate 3000. A VDSphere OptiBio PUR 300 C18-SE-column (250 x 10 mm) was employed. The mobile phase consisted of 50 mM ammonium acetate buffer (pH 6.5) as solvent A and acetonitrile as solvent B. Elution was carried out using a linear gradient, starting from 0% B and increasing to 50% B over 25 minutes, followed by an isocratic hold for 20 min. The flow rate was maintained at 2.5 mL/min, and the column oven temperature was set to 60°C.

Detection was performed using a diode array detector (DAD) at λ = 260 nm and absorption maxima of the respective dye: For porphyrin **1**, bands at λ = 419 nm and λ = 516 nm; for pyrene **2**, the visible maximum at λ = 350 nm. Target products were collected in fractions and identified by ESI mass spectrometry.

Analytical RP-HPLC was performed to assess purity using a VDSphere OptiBio PUR 300 C18 SE column (250 mm x 4.6 mm, VDS Optilab) at a flow rate of 1.15 mL/min and 60°C, applying the same gradient conditions.

3. Optical spectroscopy

Sample preparation

To hybridize **DNA1**-**DNA3** (2.5 µM, 1.00 eq.) with a complementary counterstrand (2.75 µM, 1.10 eq.), samples were heated in a sodium phosphate buffer solution (10mM, pH 7.0) and a sodium chloride solution (250mM) for 10 min at 95°C. Duplex formation was achieved by slow cooling to room temperature. Single-stranded samples were prepared under identical conditions but in the absence of the complementary strand.

UV/Vis absorption spectroscopy

The absorption spectra were recorded on a Cary3500 spectrometer (Agilent) equipped with a Peltier element. Measurements were performed using 10 mm quartz cuvettes (Starna) with a volume of 1 mL at 20°C.

Steady-state and time-resolved Fluorescence spectroscopy

Fluorescence spectra were recorded on a Fluoromax-4 spectrofluorometer (HORIBA SCIENTIFIC) with a Peltier element at 20°C. The spectrometer was calibrated using the Raman scattering of water as a reference to ensure accurate wavelength calibration. Measurement parameters were as follows: integration time: 0.1 s; step size: 1.0 nm; increment time: 0.2 s; excitation wavelength: 352 nm. The slit width was 3.0 nm.

CD spectroscopy

Circular dichroism (CD) spectroscopy was recorded on a JASCO J-810 spectropolarimeter with a PTC-423S Peltier element and an AC 200 thermostat (Thermo Scientific). Data were acquired using SpectraManager software with constant acquisition parameters (scanning speed: 100 nm/min, accumulations: 4, D.I.T.: 4s, bandwidth: 1 nm). Ozone formation was suppressed by continuously flushing the sample chamber with nitrogen gas (N_2, 99.999%).

4. Additional Information

4.1 NMR and ESI-mass spectra

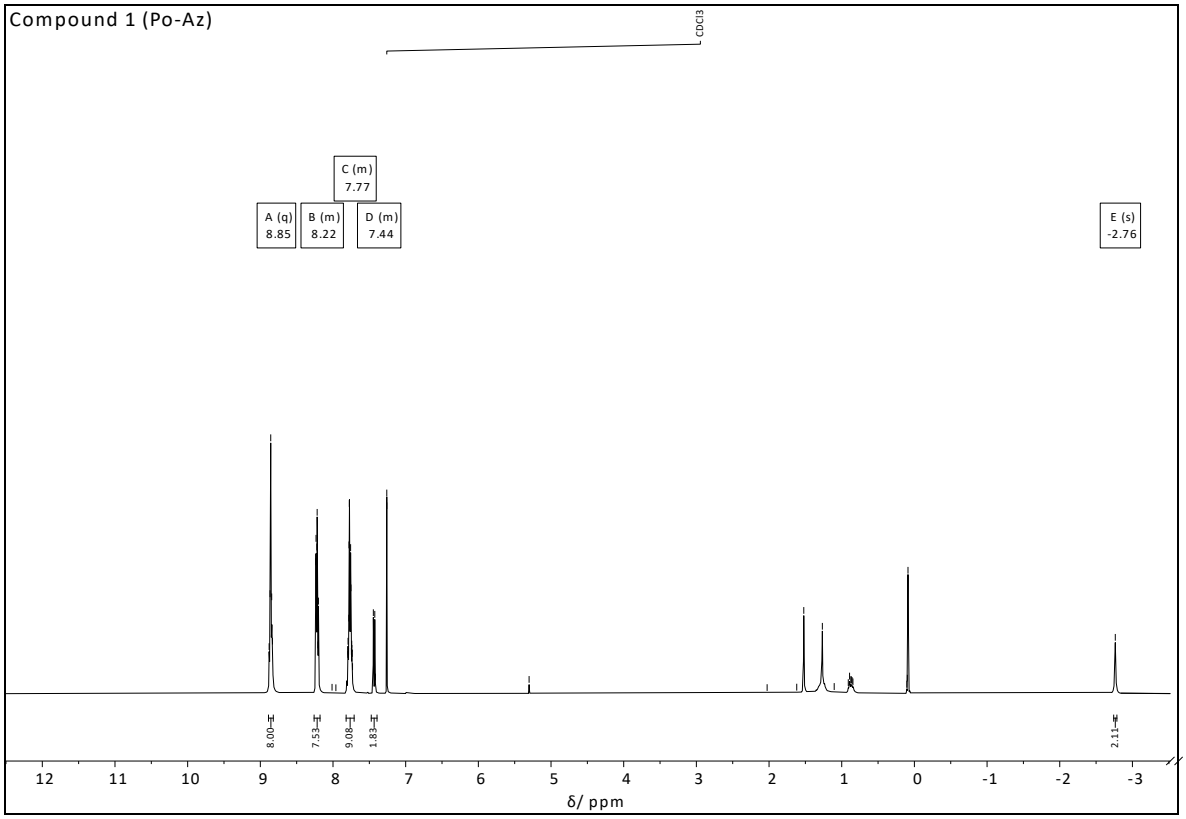

Figure S2 ^{1}H-NMR spectrum (400 MHz, $CDCl_3$-d_1) of **1**.

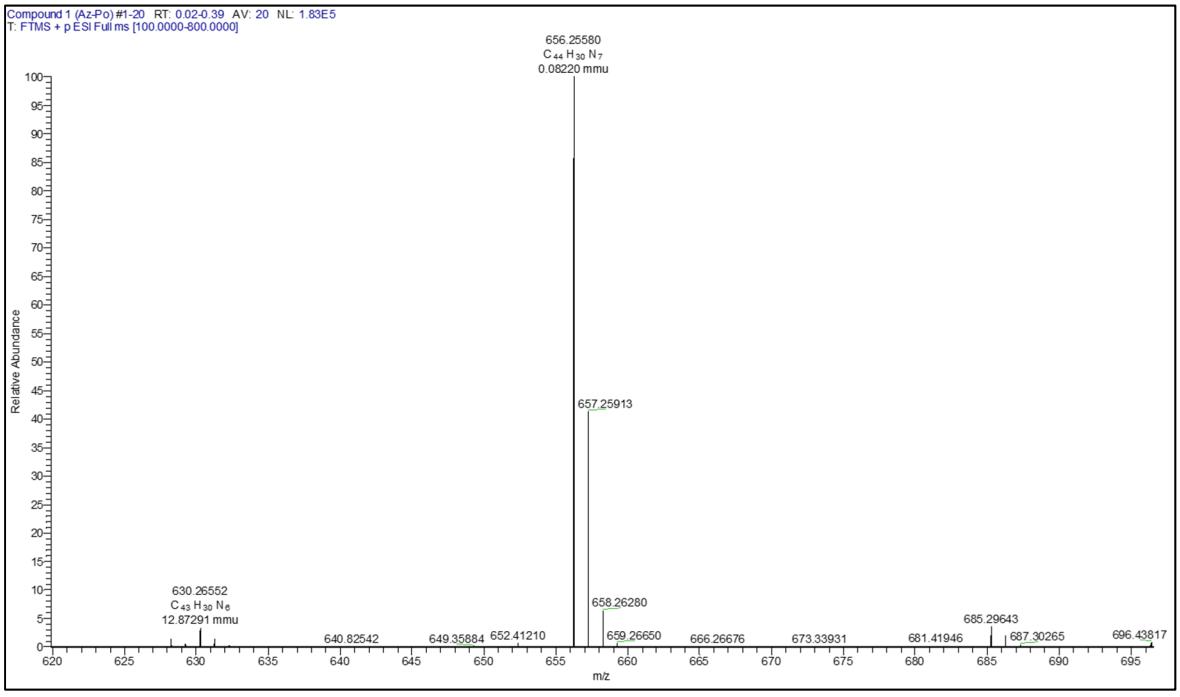

Figure S3 HR-MS (ESI) of **1** (calculated for $C_{44}H_{29}N_7$: 655.248 g·mol^{-1}; found: 656.256 g·mol^{-1} [M+H].

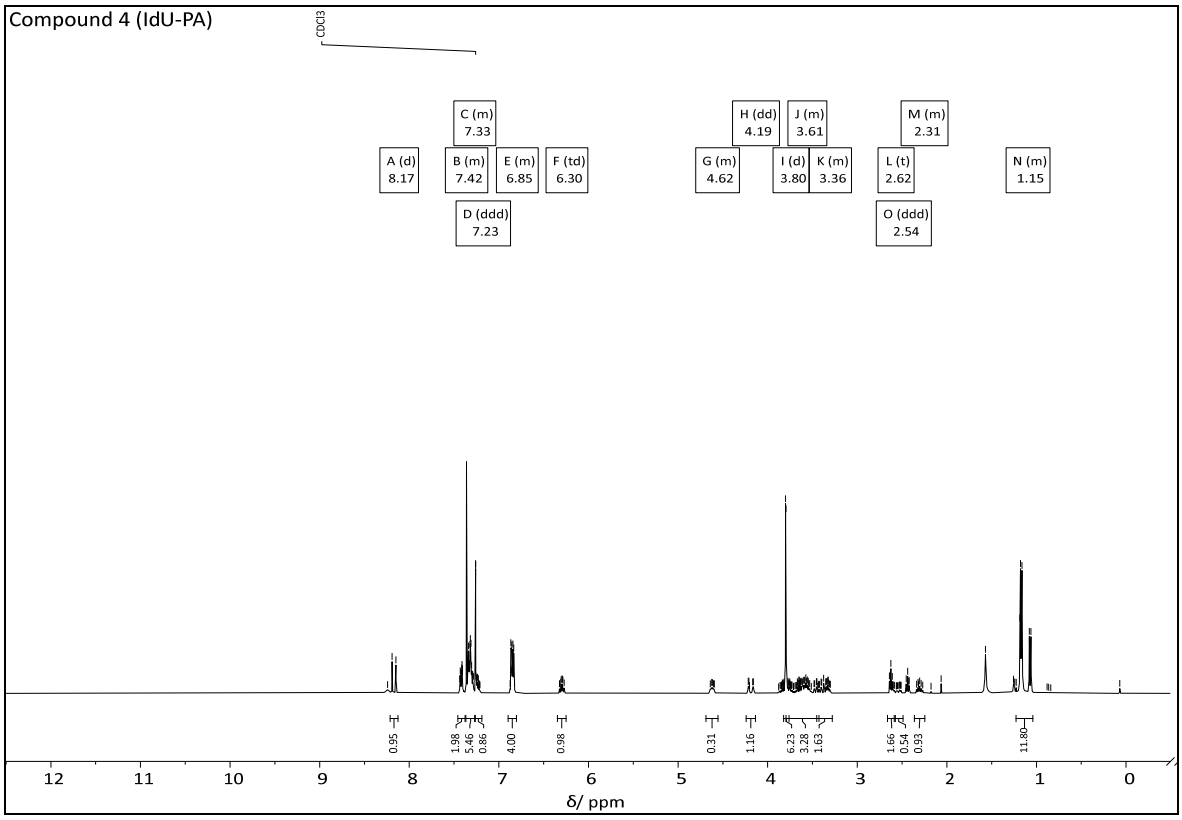

Figure S4 ^{1}H-NMR spectrum (400 MHz, $CDCl_3$-d_1) of **4**.

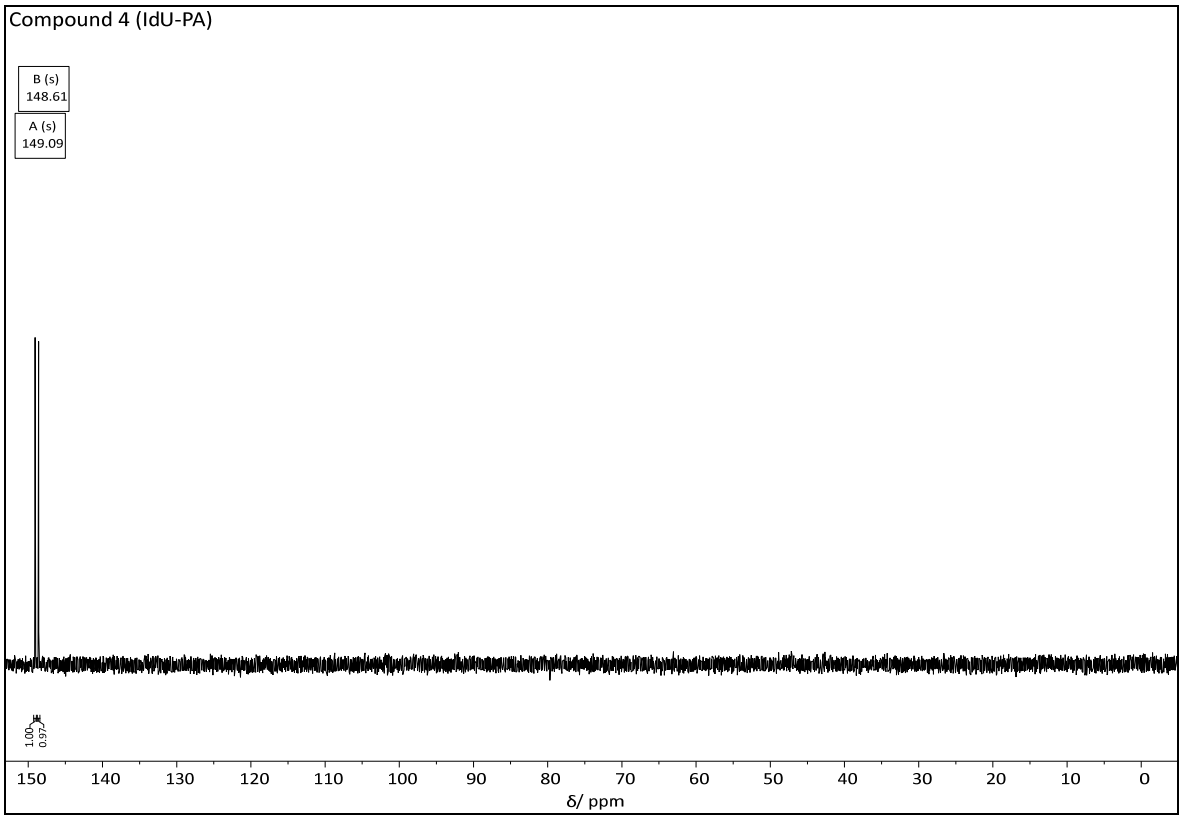

Figure S5 ^{31}P-NMR spectrum (162 MHz, $CDCl_3$-d_1) of **4**.

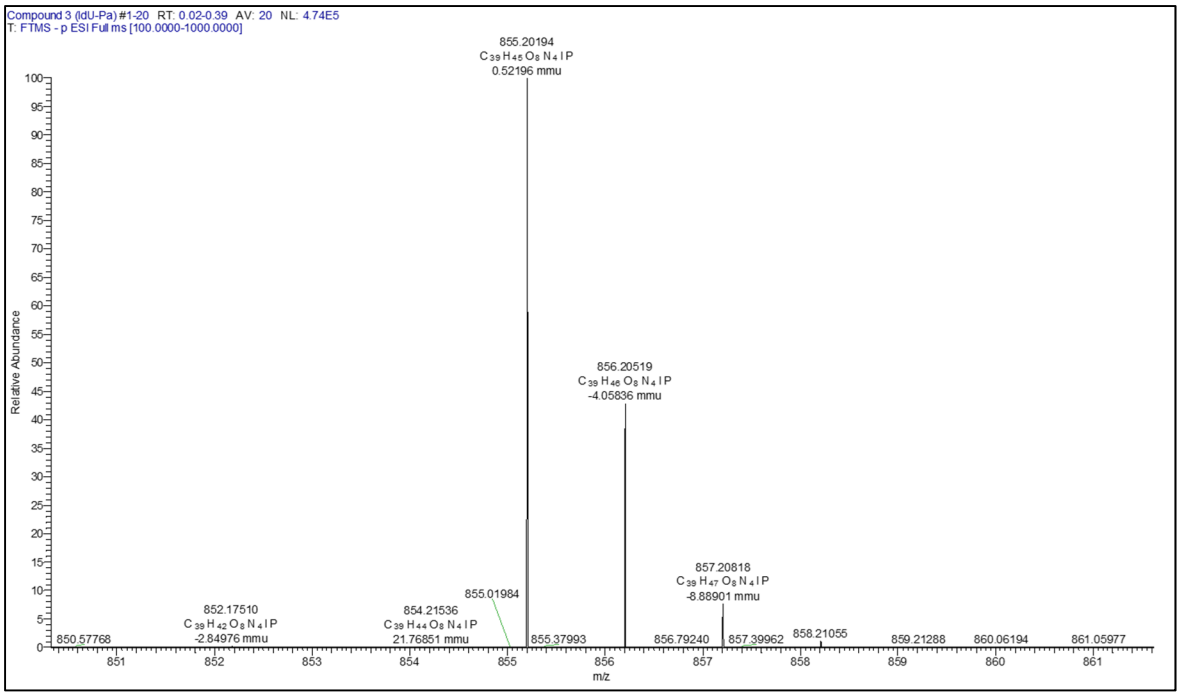

Figure S6 HR-MS (ESI) of **4** (calculated for $C_{39}H_{46}IN_4O_8P$: 856.210 g·mol^{-1}; found: 855.202 g·mol^{-1} [M-H]; 856.206 g·mol^{-1} [M].

4.2 ESI-MS analysis of modified DNA

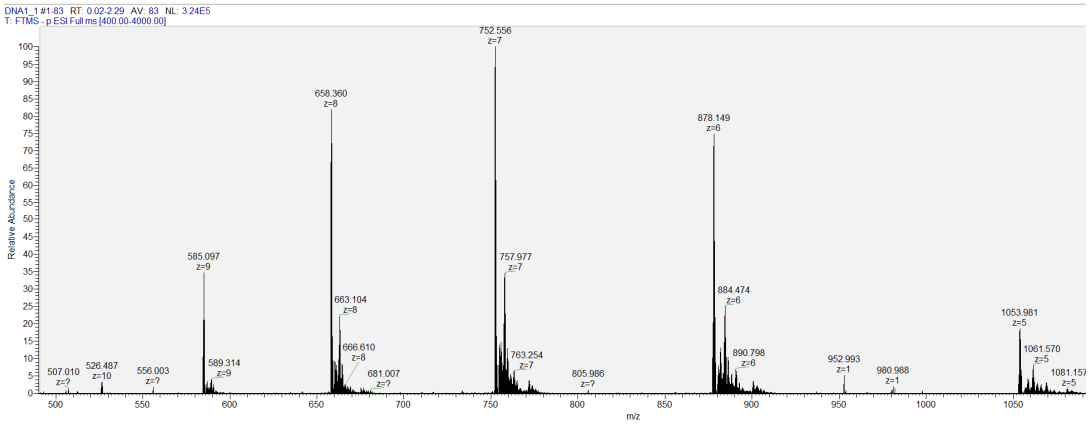

Figure S7 HR-MS (ESI) of **DNA1** (calculated for M_{DNA1} = $C_{193}H_{217}N_{54}O_{93}P_{14}$: 5212.02 g·mol^{-1}; $M_{DNA1\text{-}2H+Cu}$ = $C_{193}H_{215}CuN_{54}O_{93}P_{14}$: 5272.94 g·mol^{-1}; found: 5274.95 g·mol^{-1} deconvoluted from [$M_{DNA1\text{-}2H+Cu}$-9H]$^{-9}$, 5274.94 g·mol^{-1} [$M_{DNA1\text{-}2H+Cu}$-8H]$^{-8}$, 5274.95 g·mol^{-1} [$M_{DNA1\text{-}2H+Cu}$-7H]$^{-7}$, 5274.94 g·mol^{-1} [$M_{DNA1\text{-}2H+Cu}$-6H]$^{-6}$).*

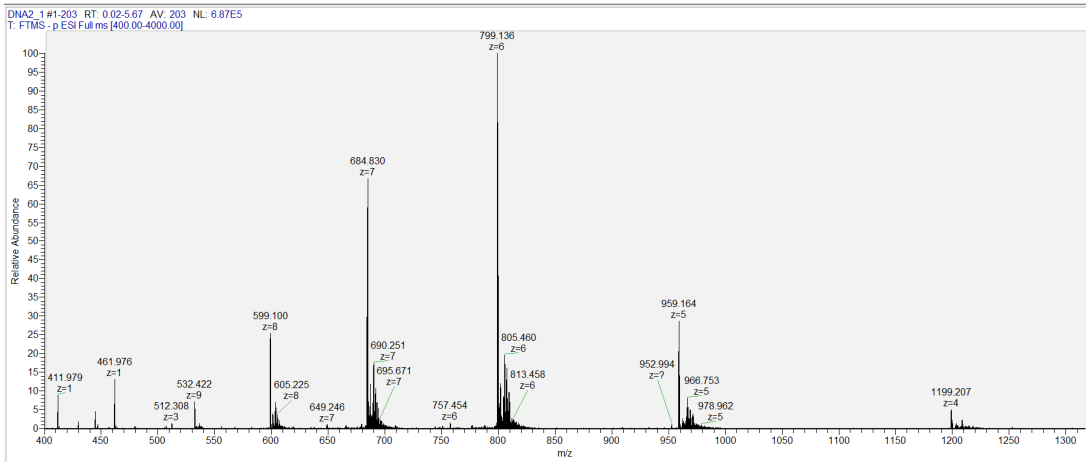

Figure S8 HR-MS (ESI) of **DNA2** (calculated for M_{DNA2} = $C_{165}H_{197}N_{50}O_{93}P_{14}$: 4799.86 g·mol^{-1}; found: 4800.86 g·mol^{-1} deconvoluted from [M_{DNA2}-8H]$^{-8}$, 4800.87 g·mol^{-1} deconvoluted from [M_{DNA2}-7H]$^{-7}$, 4800.86 g·mol^{-1} deconvoluted from [M_{DNA2}-6H]$^{-6}$, 4800.86 g·mol^{-1} deconvoluted from [M_{DNA2}-5H]$^{-5}$).*

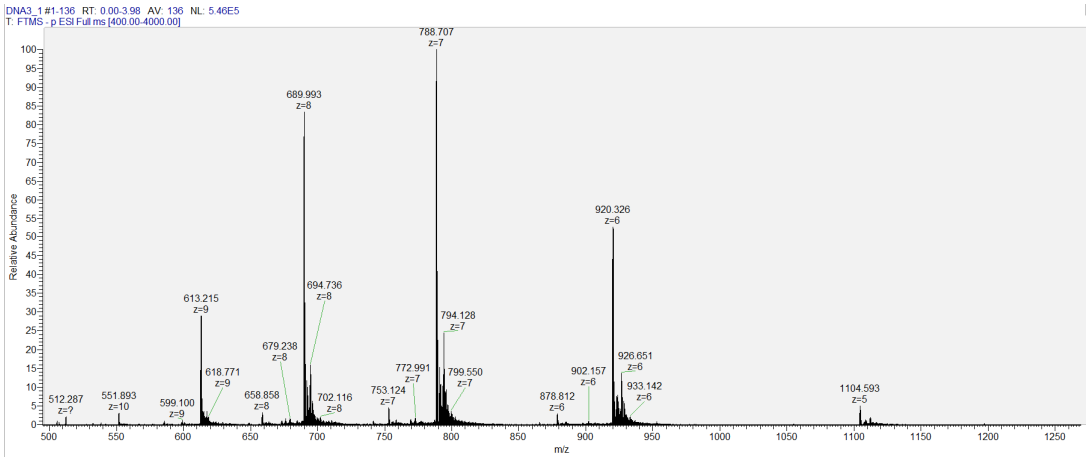

Figure S9 HR-MS (ESI) of **DNA3** (calculated for M_{DNA3} = $C_{210}H_{224}CuN_{57}O_{93}P_{14}$: 5465.09 g·mol^{-1}; $M_{DNA3\text{-}2H+Cu}$ = $C_{210}H_{222}CuN_{57}O_{93}P_{14}$: 5526.00 g·mol^{-1}; found: 5528.01 g·mol^{-1} deconvoluted from [$M_{DNA3\text{-}2H+Cu}$-9H]$^{-9}$, 5528.01 g·mol^{-1} deconvoluted from [$M_{DNA3\text{-}2H+Cu}$-8H]$^{-8}$, 5528.01 g·mol^{-1} deconvoluted from [$M_{DNA3\text{-}2H+Cu}$-7H]$^{-7}$, 5528.00 g·mol^{-1} deconvoluted from [$M_{DNA3\text{-}2H+Cu}$-6H]$^{-6}$).*

* All found values refer to the most abundant mass (m/z), accounting for the isotopic distribution according to the molecular sum formula.

4.3 Analytical HPLC of modified DNA

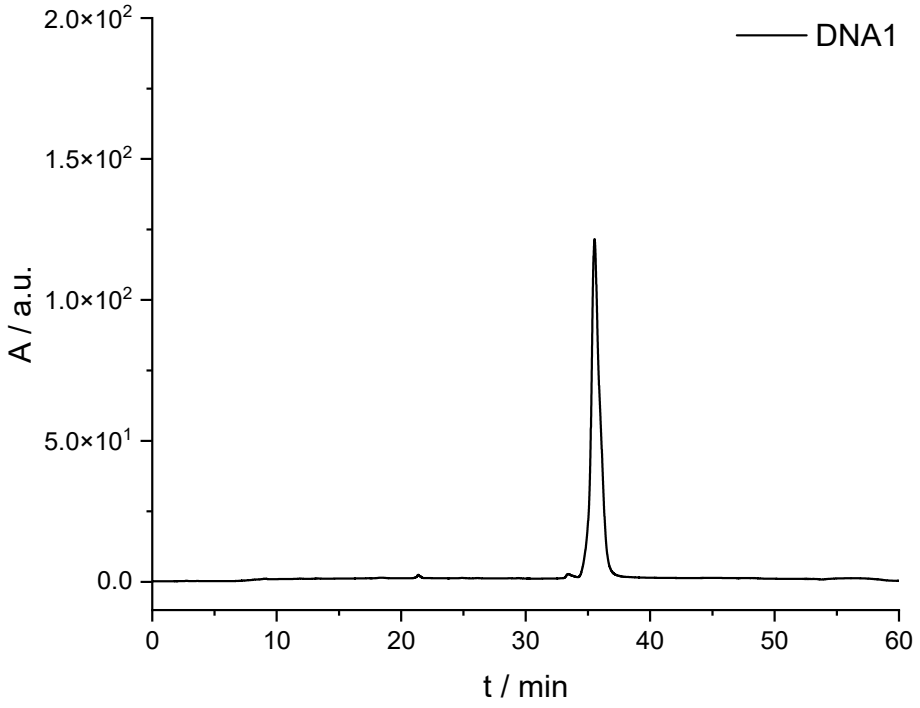

Figure S10 HPLC analysis of **DNA1** (λ=260 nm).

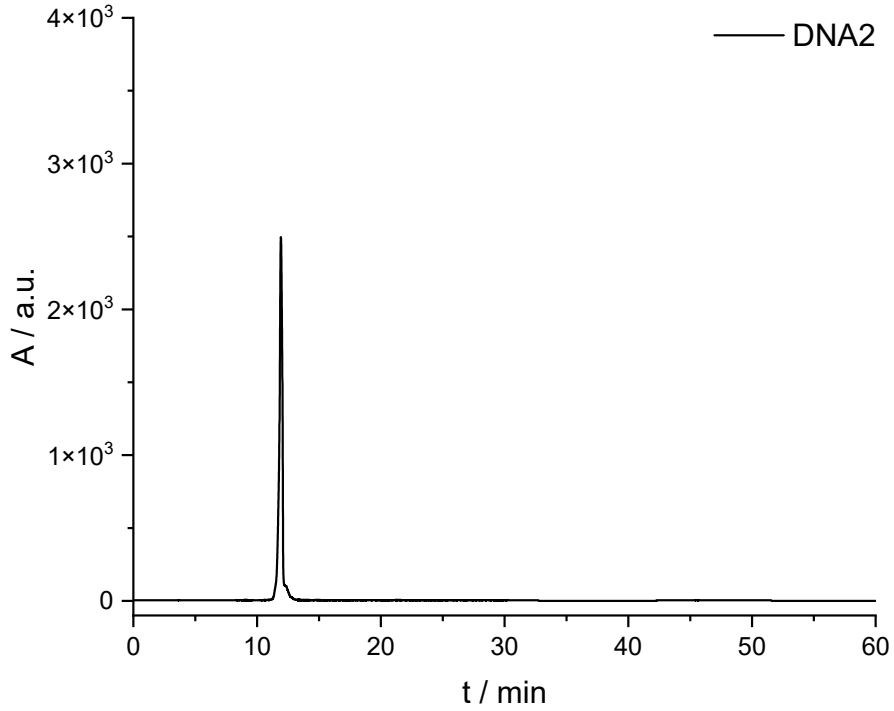

Figure S11 HPLC-analysis of **DNA2** (λ=260nm).

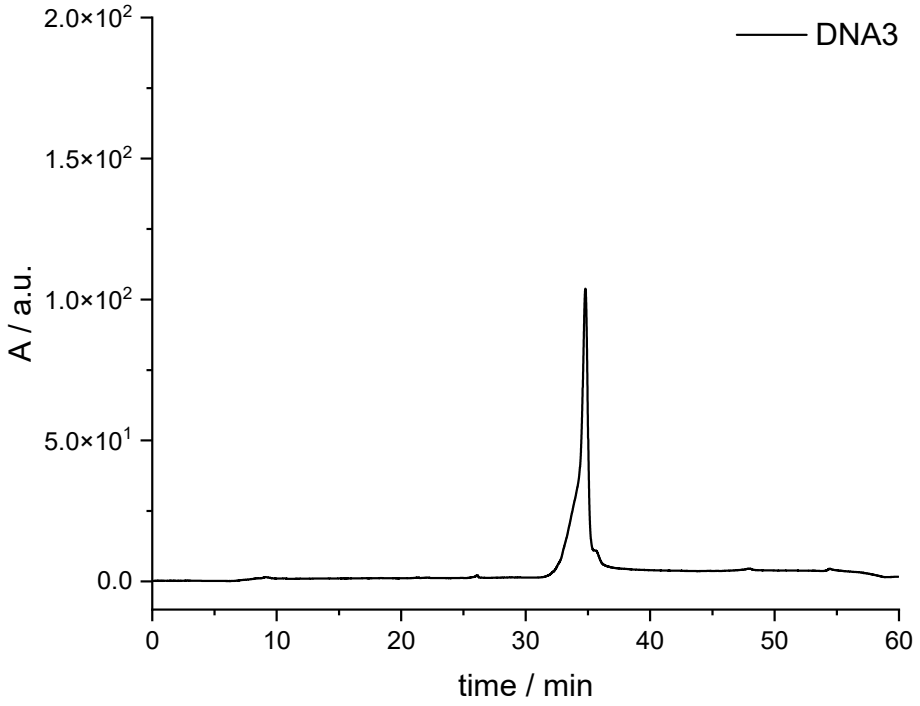

Figure S12 HPLC analysis of **DNA3** (λ=260nm).

5. References

1. K. Flavin, M. N. Chaur, L. Echegoyen and S. Giordani, *Org. Lett.*, 2010, **12**, 840-843.
2. M. B. Walunj and S. G. Srivatsan, *Bioconjug Chem.*, 2020, **31**, 2513-2521.