Supporting Information

for

Shaping bioinspired photo-responsive microstructures by the light-driven modulation of selective interactions.

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GENERAL METHODS

NMR

^{1}H and ^{13}C NMR spectra were recorded at room temperature on a Bruker AC-200 (200 MHz) instrument using the partially deuterated solvent as the internal reference. Chemical shifts (δ) are expressed in ppm. The multiplicity of a signal is indicated as: br - broad, s - singlet, d - doublet, t - triplet, m - multiplet, etc.

Mass Spectrometry

ESI-MS experiments were performed using an ESI-ToF Mariner^{TM} Biospectrometry^{TM} Workstation of Applied Biosystems by flow injection analysis using MeOH as the mobile phase. High-resolution mass spectra were obtained by electrospray ionization on a Perseptive Biosystem Mariner ESI-ToF spectrometer (Foster City, CA). An 1 x 10^{-9} M solution of neurotensin, angiotensin I, and bradykinin in an 1:1 CH_3CN/H_2O mixture, containing 1% formic acid, was used for calibration.

Transmission electron microscopy (TEM)

Samples were analyzed on a Jeol 300PX instrument. Samples were prepared immediately before used. A small drop of solutions was floated on a glow discharged carbon coated grid and excess was removed by #50 hardened Whatman filter paper. For the samples with negative staining, the grid was then floated on 2% uranyl acetate solution for 10 seconds, and the excess was removed by #50 hardened Whatman filter paper.

Scanning electron microscopy (SEM)

A Carl Zeiss Merlin field emission scanning electron microscope operating at 5kV accelerating voltage was used. A small drop of the milk-like aqueous suspension was placed on a microscope glass cover slip and allowed to dry overnight.

FT-IR absorption

FT-IR absorption spectra were recorded with a Perkin-Elmer 1720X spectrophotometer.

UV-Vis Absorption Spectroscopy

The UV-Vis absorption spectra were recorded using a Varian Cary 5000 UV-Vis-NIR spectrophotometer. A 1-cm path length quartz cell was used.

Fluorescence Emission Spectroscopy

The fluorescence spectra were measured upon excitation at different wavelengths using a Varian Cary Eclipse Fluorimeter. A 1 cm path length quartz cell was used. The samples prepared for UV-

Vis were used to collect the fluorescence data.

SYNTHESES AND CHARACTERIZATIONS

General

1-hydroxy-7-aza-1,2,3-benzotriazole (HOAt) was purchased from GL Biochem (Shanghai) Ltd. N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC·HCl) and dimethylsulfoxide-d_6 were obtained from Iris Biotech (Germany). *N*,*N*-Diisopropylethylamine (DIPEA) was purchased from Fluka (Switzerland). Triethylamine (TEA), 1,5-Diazabicyclo[5.4.0]undec-5-ene (DBU), bromoacetic acid, ethyl bromoacetate, 4-(phenylazo)aniline, 5-(4-aminophenyl)-10,15,20-triphenyl porphyrin, tetrachloroauric acid and lipoic acid were obtained from Sigma-Aldrich. The deuterated solvent $CDCl_3$ was purchased from Euriso-Top (France).
All other chemicals and solvents were Sigma-Aldrich, Fluka or Acros products and used as provided without further purifications.

Synthesis of thymine-1-acetic acid

Thymine (4 g, 31.7 mmol) was dissolved in a solution of KOH (6.82 g, 121 mmol) in 20 ml of water. The solution was warmed at 40°C and a solution of bromoacetic acid (6.25 g, 45 mmol) dissolved in 10 ml of water was added in 30 minutes. The reaction was stirred for another 30 minutes at this temperature. The solution was cooled at room temperature and the pH was adjusted to 5.5 with conc. HCl. The reaction was cooled for 2 h in the refrigerator. After filtration of the precipitate formed, the solution was adjusted to pH 2 with conc. HCl and it was put in the freezer for 2 h. The product was filtrated, washed with water and dried (4.7 g, 85% yield). White solid.

Melting point: 252-255°C

^{1}H NMR: (200 MHz, DMSO-d_6, Me_4Si): δ 1.75 (s, 3H, CH_3), 4.36 (s, 2H, CH_2), 7.49 (s, 1H, CH), 11.33 (s, 1H, NH), 13.09 (s, 1H, OH).

MS (ESI-TOF): [M] calc. = 184.1494 m/z; $[M+H]^+$ found = 185.0567 m/z; [2M] calc. = 368.2988 m/z; $[2M+H]^+$ found = 369.1056 m/z.

IR (KBr): 3178, 3073, 3026, 2962, 1739, 1706, 1664, 1633 cm^{-1}.

Synthesis of ethyl adenine-9-acetate

Adenine (4 g, 30 mmol) was suspended in dry DMF (60 ml) and NaH (0.82, 34 mmol, washed with petroleum ether) was added. The reaction was stirred for 2 h at room temperature. After this time, ethyl bromoacetate (6.64 ml, 60 mmol) was added dropwise in 3 h and the solution was stirred for another 3 h. the solvent was removed by evaporation *in vacuo*. The remaining oil was shaken with water and the resulting white precipitate was isolated by filtration, washed with water and dried (3.7 g, 56% yield).

Melting point: 227-229°C

^{1}H NMR: (200 MHz, $CDCl_3$, Me_4Si): δ 1.21 (t, 3H, CH_3), 4.15 (q, 2H, CH_2), 5.06 (s, 2H, CH_2), 7.25 (s, 2H, NH_2), 8.11 (d, 2H, CH).

MS (ESI-TOF): [M] calc. = 221.2159 m/z; $[M+H]^+$ found = 222.2057 m/z.

IR (KBr): 3103, 2924, 1741, 1671, 1604, 1582 cm^{-1}.

Synthesis of thymine-4-phenilazoanilide (2)

Thymine-1-acetic acid (0.5 g, 2.7 mmol) was dissolved in dry DMF and activated with HOAt (0.36 g, 2.7 mmol) and EDC·HCl (0.52 g, 2.7 mmol). The solution was cooled with an ice/water bath and stirred for 30 minutes. Separately, 4-(phenylazo)aniline (1.33 g, 6.7 mmol) was dissolved in dry DMF and it was added to the active ester. Triethylamine (376 μl) was added until basic pH. The reaction was stirred for 1 h. Subsequently, the solvent was concentrated by evaporation *in vacuo* and the product was isolated by mean of chromatographic column (eluent CH_2Cl_2:CH_3OH 9:1).

^{1}H NMR (cis): (200 MHz, DMSO-d_6, Me_4Si): δ 1.76 (s, 3H, CH_3), 4.46 (s, 2H, CH_2), 6.80-6.86 (m, 4H, CH azo), 7.78-7.97 (m, 5H, CH azo), 10.38 (s, 1H, CH), 11.32 (s, 1H, NH); (trans) (200 MHz, DMSO-d_6, Me_4Si): δ 1.78 (s, 3H, CH_3), 4.55 (s, 2H, CH_2), 7.43-7.65 (m, 4H, CH azo), 7.73-7.97 (m, 5H, CH azo), 10.62 (s, 1H, CH), 11.32 (s, 1H, NH).

MS (ESI-TOF): [M] calc. = 363.3699 m/z; $[M+H]^+$ found = 364.1413 m/z.

IR (KBr): 3291, 1692, 1668, 1598, 1551 cm^{-1}.

Synthesis of thymine-(aminophenyl) porphyrin

Thymine-1-acetic acid (0.1 g, 0.54 mmol) was dissolved in dry DMF and activated with HOAt (0.073 g, 0.54 mmol) and EDC·HCl (0.104 g, 0.54 mmol) and the solution was stirred for 30 minutes. 5-(4-aminophenyl)-10,15,20-triphenyl porphyrin (0.07 g, 0.11 mmol) was added at the solution and the pH was adjusted to basicity with TEA (100 μl). The reaction was stirred for another 3 h, then water was added to the solution. The resulting precipitate was isolated by mean of a spin-dryer and dried (0.08 g, 90% yield).

^{1}H NMR: (200 MHz, DMSO-d_6, Me_4Si): δ 1.22 (s, 2H, NH pyrrole), 1.84 (s, 3H, CH_3), 4.69 (s, 2H, CH_2), 7.64 – 8.83 (m, 27H, porphyrinic ring), 10.74 (s, 1H, NH), 11.41 (s, 1H, NH).

MS (ESI-TOF): [M] calc. = 795.8844 m/z; $[M+H]^+$ found = 796.3231 m/z.

IR (KBr): 3314, 1702, 1675 cm^{-1}.

Synthesis of metallated thymine-(aminophenyl) porphyrin (1)

Thymine-(aminophenyl) porphyrin (0.035 g, 0.044 mmol) was dissolved in $CHCl_3$ (10 ml). Subsequently, a saturated solution of zinc acetate in CH_3OH (4 ml) was added to the solution of thymine-(aminophenyl) porphyrin and the mixture was refluxed for 1 h. The excess of acetate was removed by treatment with water (3v), then the solution was dried over Na_2SO_4, filtered and evaporated under reduced pressure (0.034 g, 90% yield).

MS (ESI-TOF): [M] calc. = 858.3913 m/z; $[M+H]^+$ found = 859.2334 m/z.

Synthesis of adenine-9-ethylenamide amine

Adenine-9-acetate (0.3 g, 1.35 mmol) was dissolved in ethylenediamine (10 ml, 149 mmol) and DBU was added as catalyst. The reaction was stirred for 2 h at 50°C, then it was allowed to cool the solution at room temperature. The product was precipitated by adding ethyl ether, filtered, washed with ethyl ether and dried (0.278 g, 87% yield).

^{1}H NMR: (200 MHz, DMSO-d_6, Me_4Si): δ 2.61 (m, 3H), 3.07 (m, 3H), 4.82 (s, 2H, CH_2), 7.18 (s, 2H, NH_2), 8.08 (d, 2H, CH), 8.24 (s, 1H, NH).

IR (KBr): 3356, 3265, 3098, 1669, 1602 cm^{-1}.

Synthesis of 4

Lipoic acid (0.206 g, 1 mmol) was activated in dry DMF (10 ml) by adding HOAt (0.136 g, 1 mmol) and EDC·HCl (0.192 g, 1 mmol). The mixture was stirred for 30 minutes, then adenine-9-ethylenamide amine (0.235 g, 1 mmol) was added to the solution. DIPEA (200 µl) was added until basic pH and the reaction was stirred overnight. The solvent was concentrated under reduced pressure. The residue was shaken with CH_3CN resulting in precipitation. The solid recovered after filtration was dried obtaining 0.28 g of product as a solid (66% yield).

^{1}H NMR: (200 MHz, DMSO-d_6, Me_4Si): δ 1.25-1.75 (m, 5H, CH_2 lipoic ac.), 1.75-1.98 (m, 1H, CH_2 lipoic ac.), 2.01 (m, 2H, CH_2 lipoic ac.), 2.42 (m, 1H, CH_2 lipoic ac.), 3.13 (m, 5H, CH_2 lipoic ac.), 3.60 (m, 1H, CH_2 lipoic ac.), 4.80 (s, 2H, CH_2), 7.19 (s, 2H, NH_2), 7.82 (s, 1H, NH), 8.09 (d, 2H, CH), 8.31 (s, 1H, NH).

IR (KBr): 3297, 3122, 2933, 1665, 1643, 1605, 1560 cm^{-1}.

Synthesis of adenine-capped gold nanoparticles

4 (0.105 g, 0.35 mmol) was dissolved in THF (5 ml) and cooled with an ice/water bath. Separately, tetrachloroauric acid (0.069 g, 0.177 mmol) was dissolved in THF (5 ml), cooled and added dropwise to the solution of **4**. The reaction was stirred overnight to allow the complexation. $NaBH_4$ (0.07 g, 17 mmol) dissolved in water (2 ml) and cooled was added quickly and the solution. After 48 h of aging, the gold nanoparticles were recovered by filtration, washed with CH_3OH and dried.

IR (KBr): 3355, 3095, 2929, 1646 cm^{-1}.

Synthesis of Carbon Quantum Dots (CQDs)

Chlorohydrated Arginine (10.2 g, 48.4 mmol) and ethylendiamine (3.56 ml, 53.3 mmol) are introduced in a 100 or 250 ml beaker containing 26.6 ml of ultrapure water. The solution was stirred at r.t. until complete dissolution of reactants and then it was irradiated in a domestic microwave oven for 4 minutes at 1000 W. During this lapse of time, white-gray aqueous vapor came out from the vents of the oven. A porous black-reddish solid was obtained and it was washed in a gooch filter with 4x20 ml aliquots of acetonitrile and 4x20 ml of diethyl ether. The solid was dried for several minutes in air and dissolved in the minimum volume of ultrapure water to obtain a dark and turbid solution, which is filtered through a cellulose syringe filter (0.45 µm cut-off). The purified

solution was freezed using a bath of acetone and solid carbon dioxide (T ≈ -78 °C) and then dried under vacuum through a lyophilization process, which brought to a brownish final compound.

Synthesis of thymine doped CQDs (3)

Thymine-1-acetic acid (0.590 g, 3.2 mmol) was dissolved in dry DMF (2.5 ml) and activated with HOAt (0.48 g, 3.8 mmol) and EDC·HCl (0.288 g, 1.62 mmol) and the solution was stirred for 15 minutes with an ice/water bath. Separately, CQDs (0.28 g) were dissolved in dry DMF (1 ml) and the solution was stirred for 15 minutes. This solution was added to the active ester and pH was adjust to basicity with TEA (0.984 ml). After 10 minutes the ice/water bath was removed and the reaction was stirred overnight. The product was precipitated with CH_3CN and washed with CH_2Cl_2.

Synthesis of functionalized polymer based on 2,4,6-triallyloxy-1,3,5-triazine and 2,2'-(ethylendioxy)diethanthiol

2,4,6-triallyloxy-1,3,5-triazine (1.82 g, 7.3 mmol) and 2,2'-(ethylendioxy)diethanthiol (2 g, 11 mmol) were stirred in presence of 2,2'-dimethoxy-2-phenylacetophenone (DMPA, 0.40 g, 1.5 mmol) as photoiniziator for the polymerization. The solution was irradiated at 365 nm in to the appropriate mold and the cross-linker polymer was obtained as a rubber-like material.

Self-assembly procedures

(i) Thymine self-recognition experiments were performed as follow: a lyophilized samples of **1** and **2** (in its *trans* form) were dissolved in a THF solutions to the final concentration of 2 mM. To these solutions water was slowly added to the final concentration of 1 mM for **1** and 0.5 mM for **2**. The solutions were allowed to stay at room temperature to the open air thus to let part of THF mixture evaporate. During this time, formation of crystals was observed. Crystals from **2** (in its *cis* form) were obtained after a prior isomerization of **2** run directly in the THF solution. Organized microstructures from **3** were obtained starting from a lyophilized sample that was dissolved in pure water to the final concentration of 3 mM. The solvent was allowed to slowly evaporate over a flat area vessel.

(ii) Adenine-thymine recognition experiments were performed as follow: a 0.6 mM stock solution of adenine-capped GNPs was prepared starting from 100 mg of lyophilized sample dissolved in 5 ml in water. ***(a)*** **1**/adenine-capped GNPs monomeric nanosystems: to a 200 μl of adenine-capped

GNPs stock solution (corresponding to 4 μmol of ligands), **1** was slowly added from a its solution prepared dissolving 3.2 mg (4 μmol) in 2 ml of THF. After an appropriate mixing time the solution was examined by DLS and TEM. ***(b)*** **1**/adenine-capped GNPs large aggregates: Similar conditions were adopted for the formation of large aggregates, with the experimental difference that **1** was added from a 10 to 20 times concentrated THF solution. Similar experimental conditions were adopted for the formation of **2**/adenine-capped GNPs microstructures.

(iii) **3**/adenine-capped GNPs nanosystems: to a 400 μl of adenine-capped GNPs stock solution (corresponding to 8 μmol of adenine ligands), **3** was slowly added from a its solution prepared dissolving 9.5 mg (corresponding to 32 μmol of thymine ligands) in 2 ml of water at pH 8. The resulting mixture was allowed to equilibrate for 1 hr. Successively, a slow addition of THF to the mixture allowed the formation of a nanostructurated material.

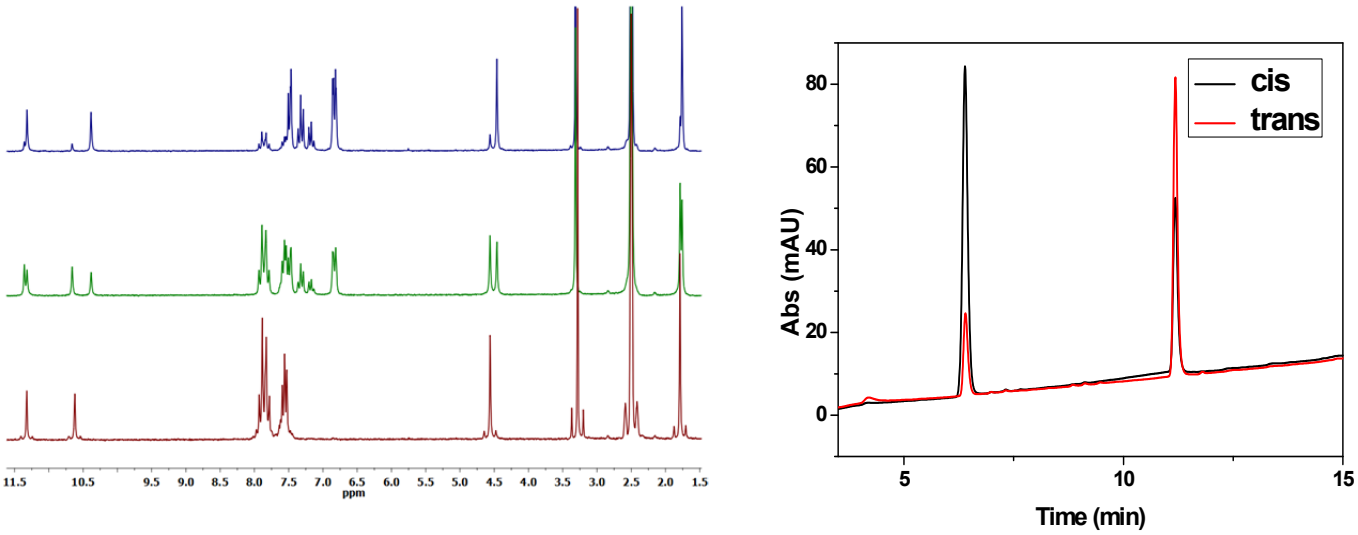


Fig. S1 Left: ^{1}H-NMR spectra of **2** after different times of irradiation at 350 nm (*cis* form in blue, *trans* form in red, and *cis*/*trans* mixture in green). Right: HPLC chromatogram of **2** showing interconversion between *trans* form (red line) and *cis* form (black line).

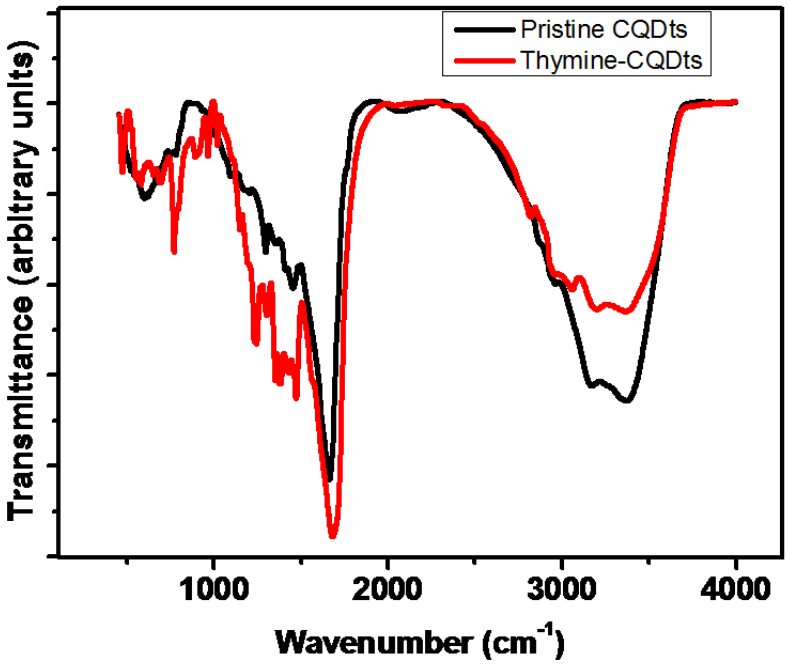
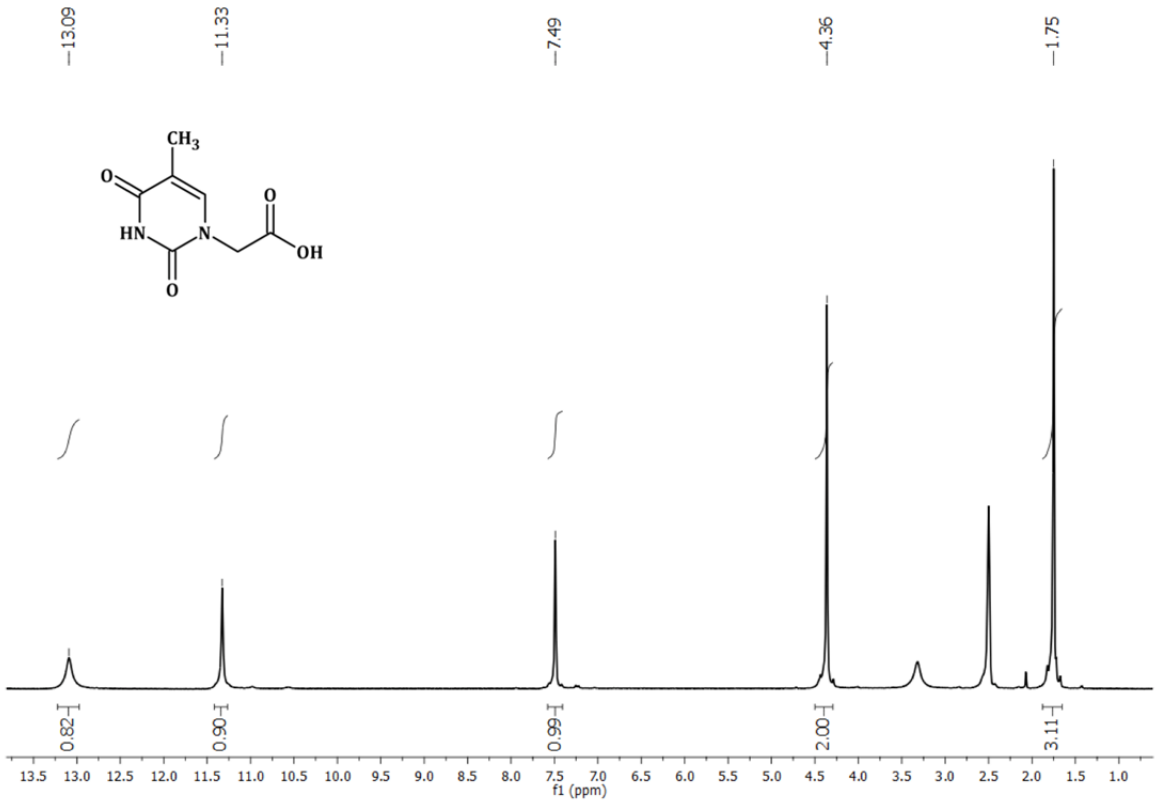


Fig. S2 Solid-state FT-IR absorption spectra recorded for **3** (red line) and its pristine CQDs precursor (black line).



S3 [1]H NMR spectrum of thymine-1-acetic acid.

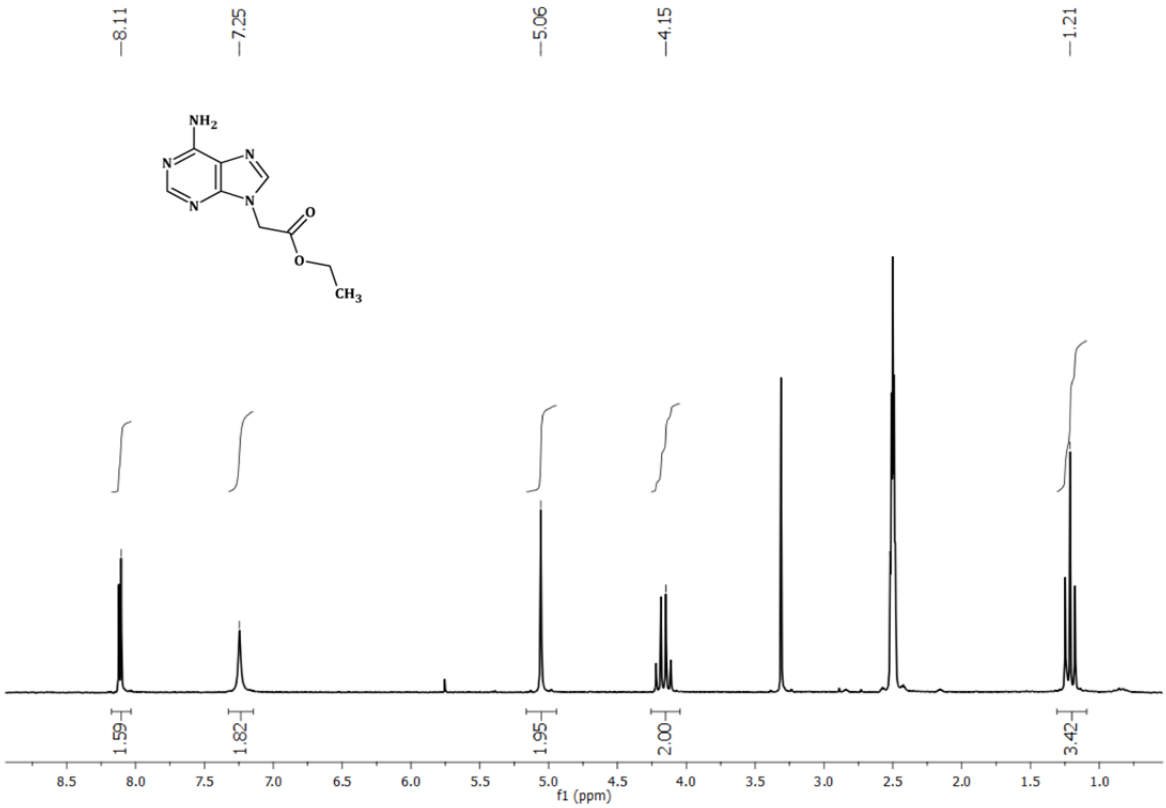


Fig. S4 [1]H NMR spectrum of ethyl adenine-9-acetate.

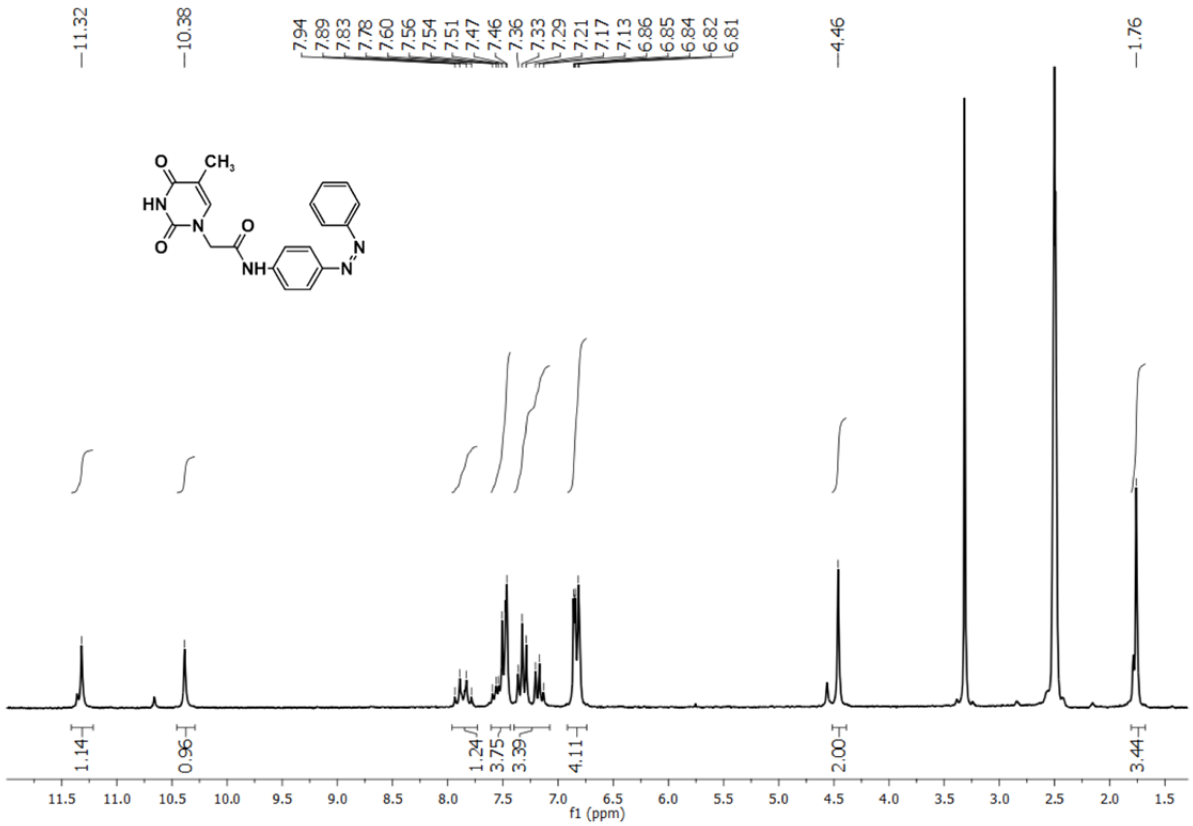


Fig. S5 ^{1}H NMR spectrum of thymine-4-phenilazoanilide (*cis* form).

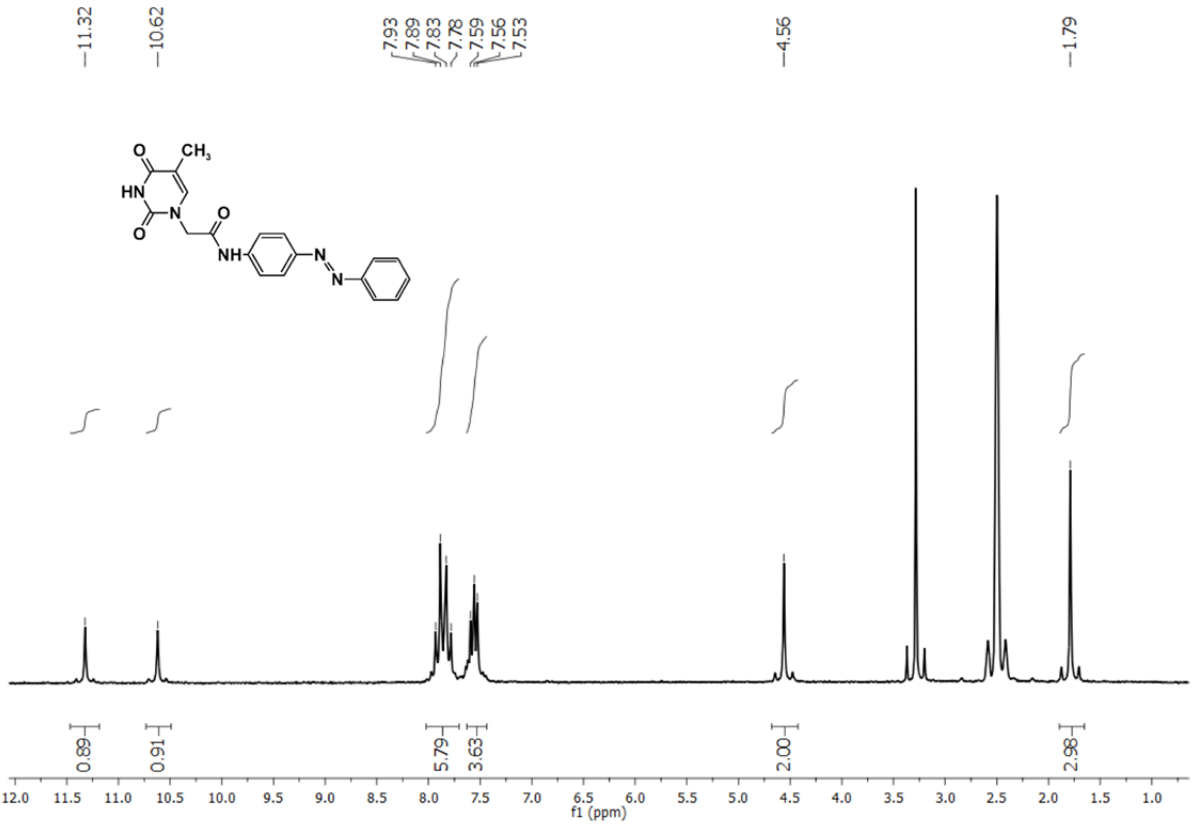


Fig. S6 ^{1}H NMR spectrum of thymine-4-phenilazoanilide (*trans* form).

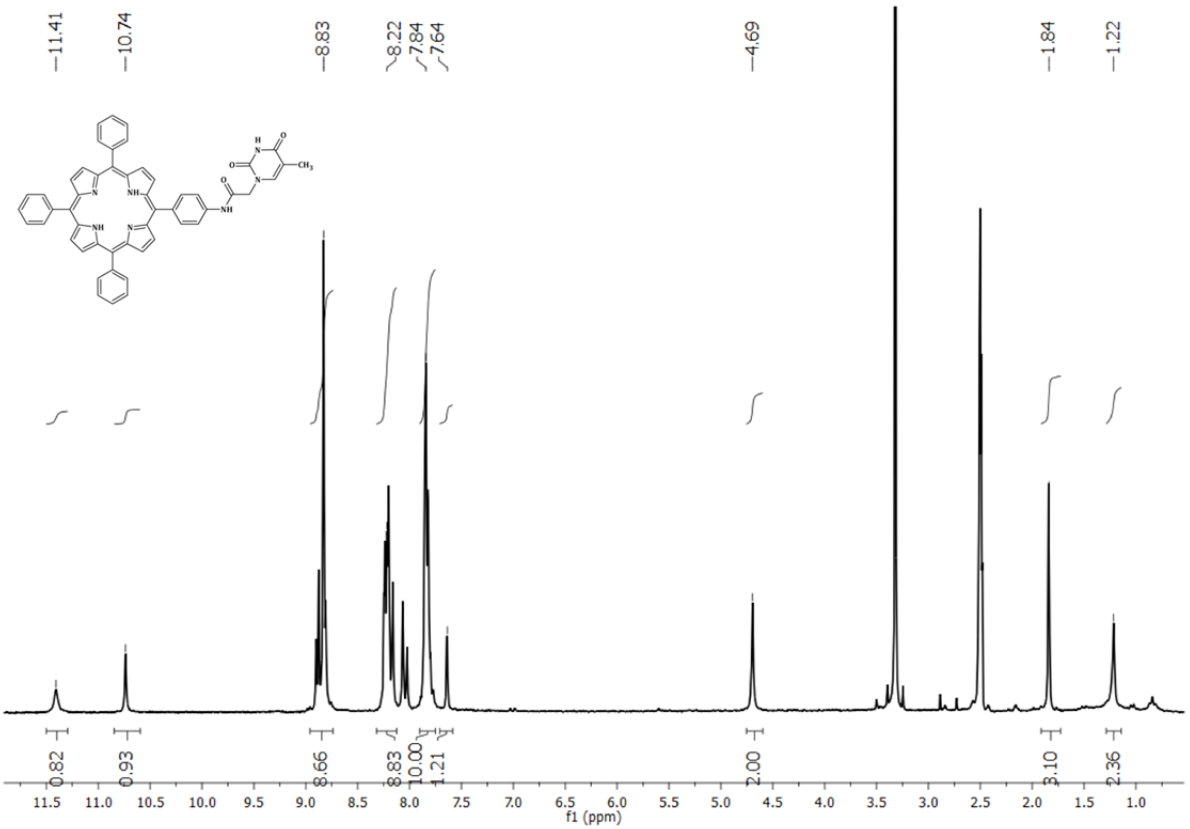


Fig. S7 ^{1}H NMR spectrum of thymine-(aminophenyl) porphyrin.

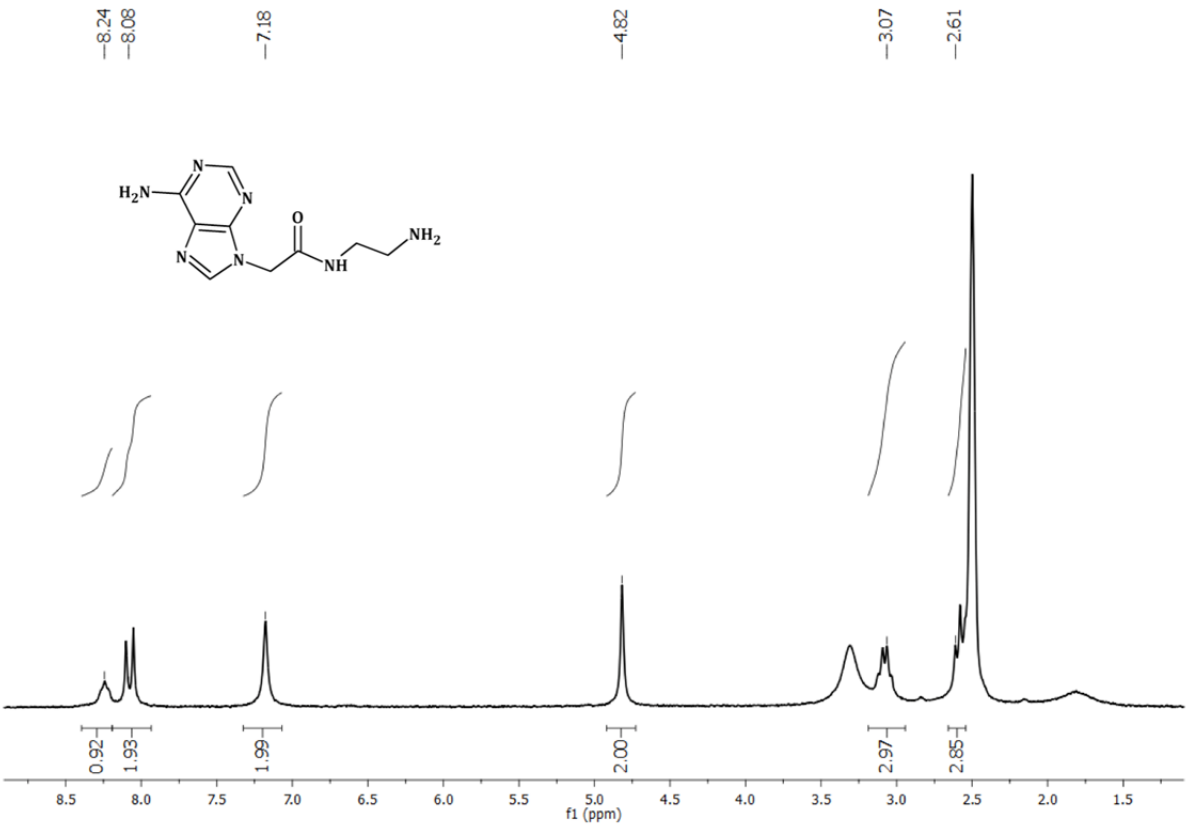


Fig. S8 ^{1}H NMR spectrum of adenine-9-ethylenamide amine.

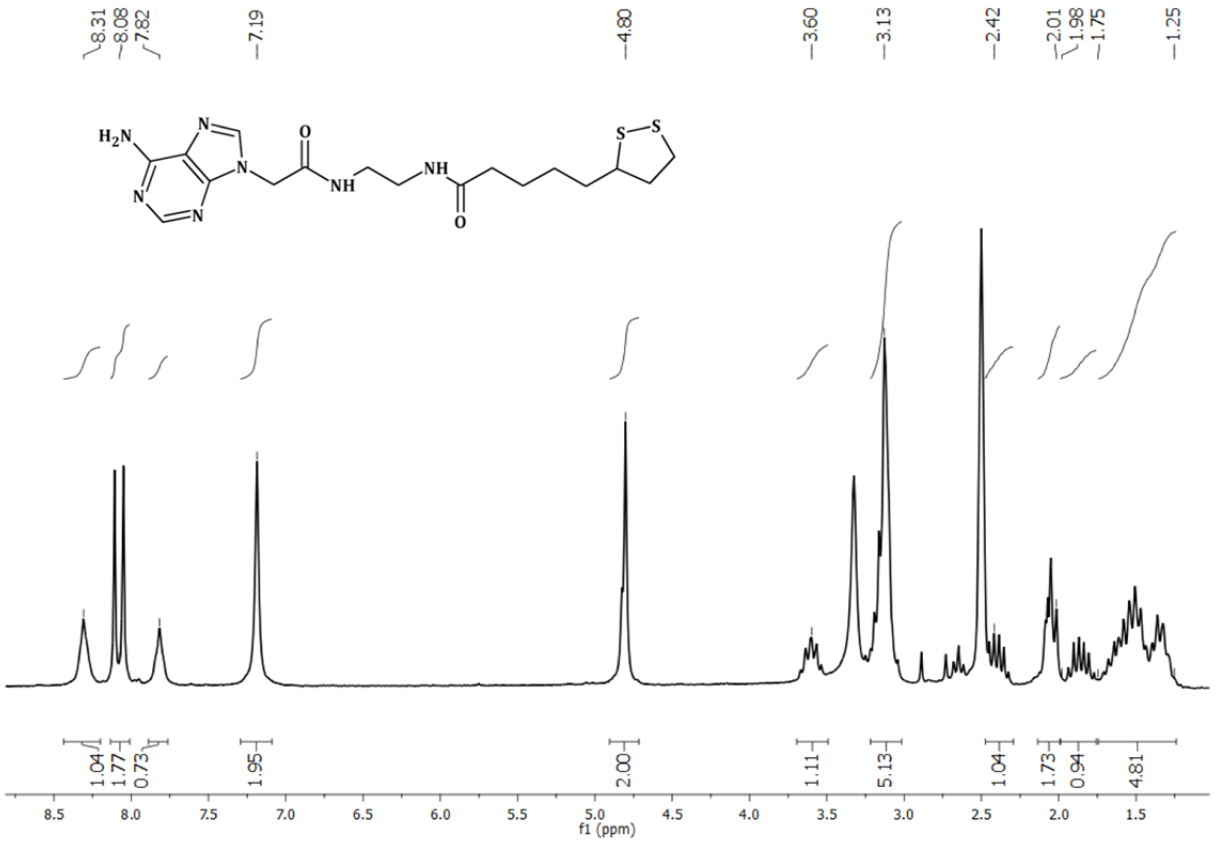


Fig. S9 ^{1}H NMR spectrum of **4**.

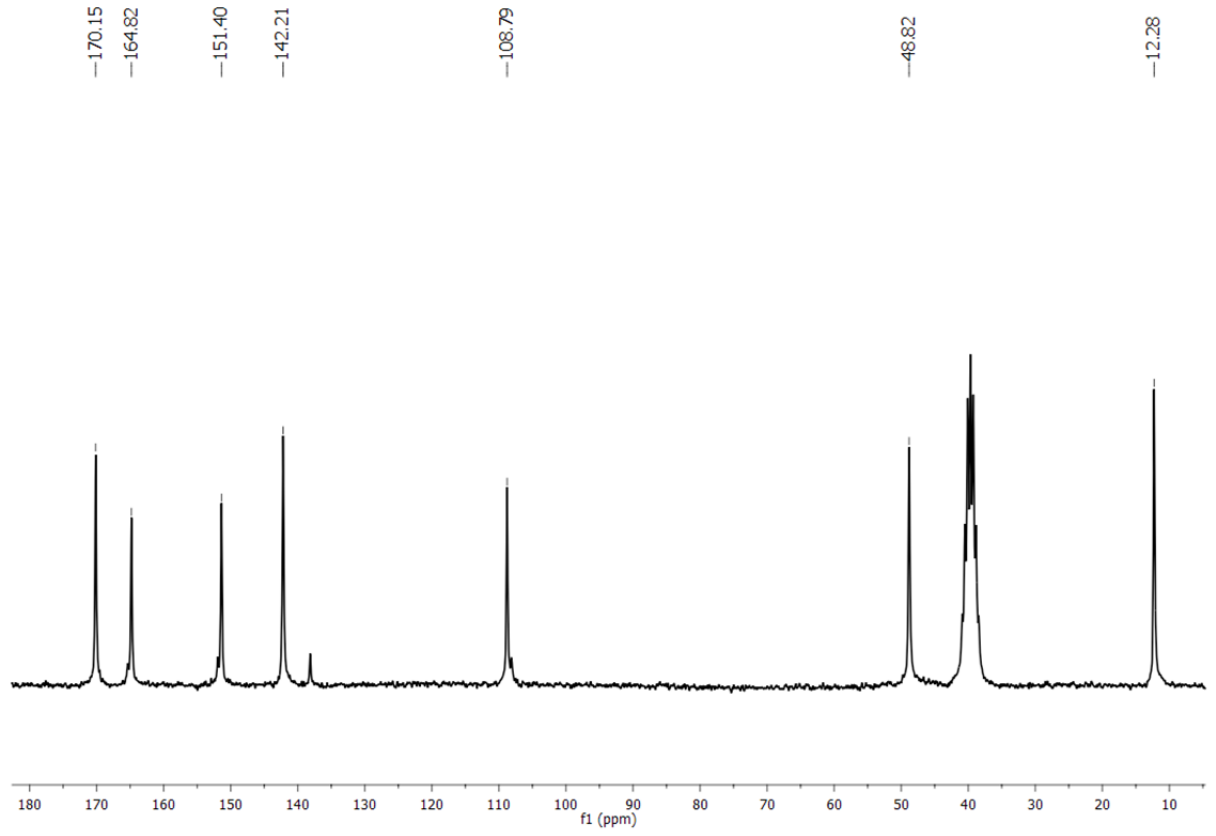


Fig. S10 ^{13}C NMR spectrum of thymine-1-acetic acid.

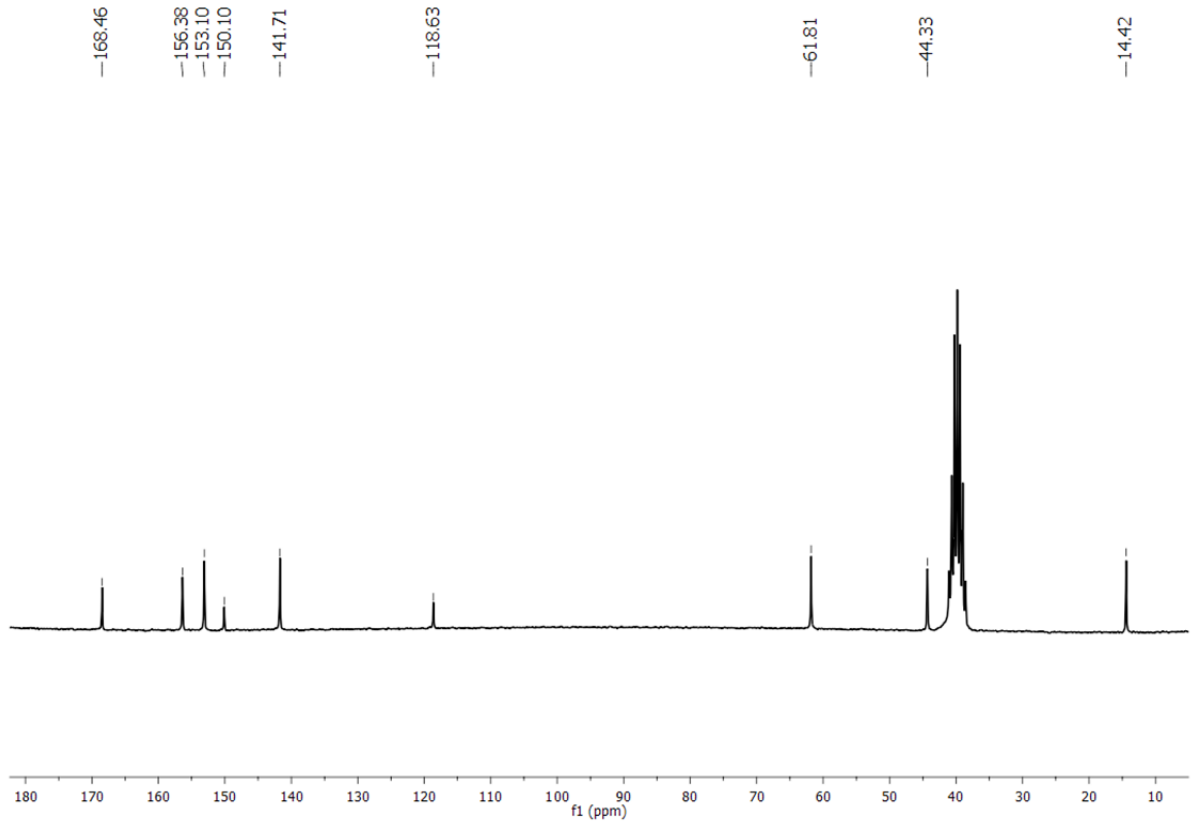


Fig. S11 ^{13}C NMR spectrum of ethyl adenine-9-acetate.

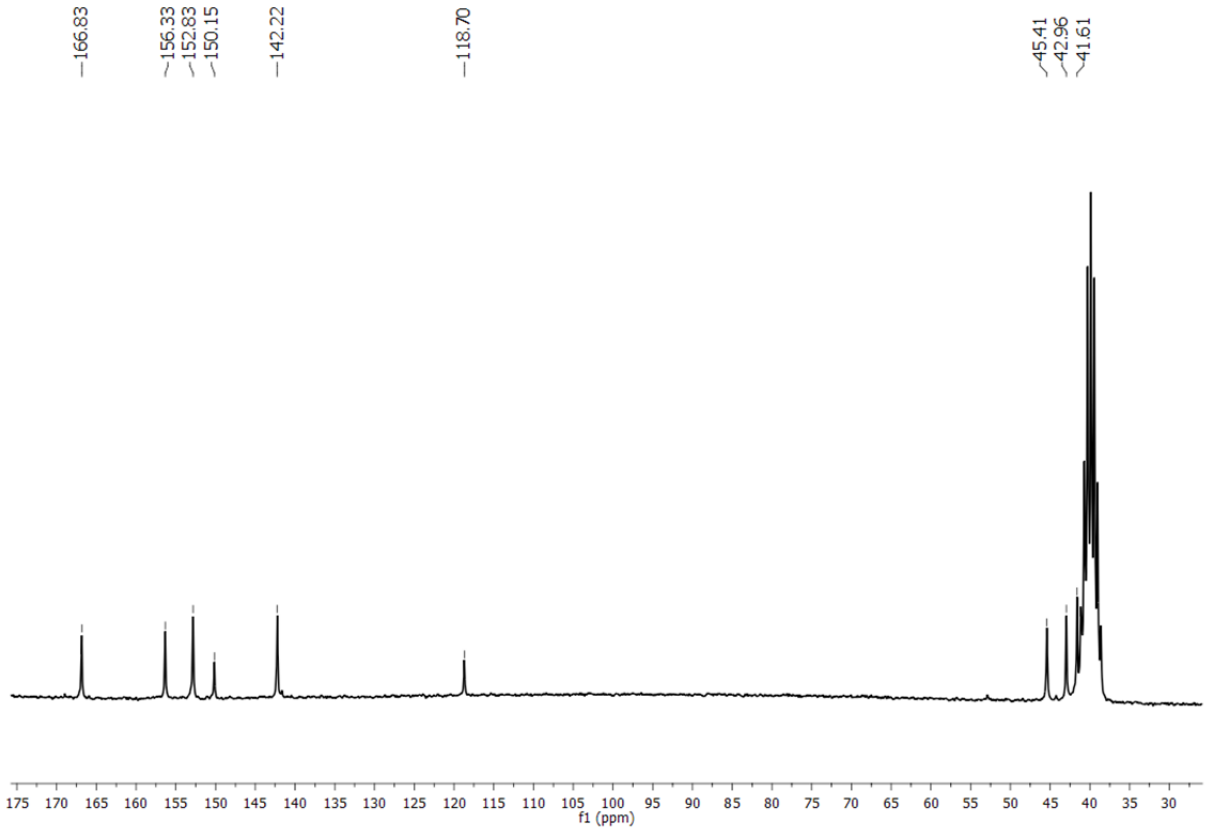


Fig. S12 ^{13}C NMR spectrum of ethyl adenine-9-ethylenamide amine.

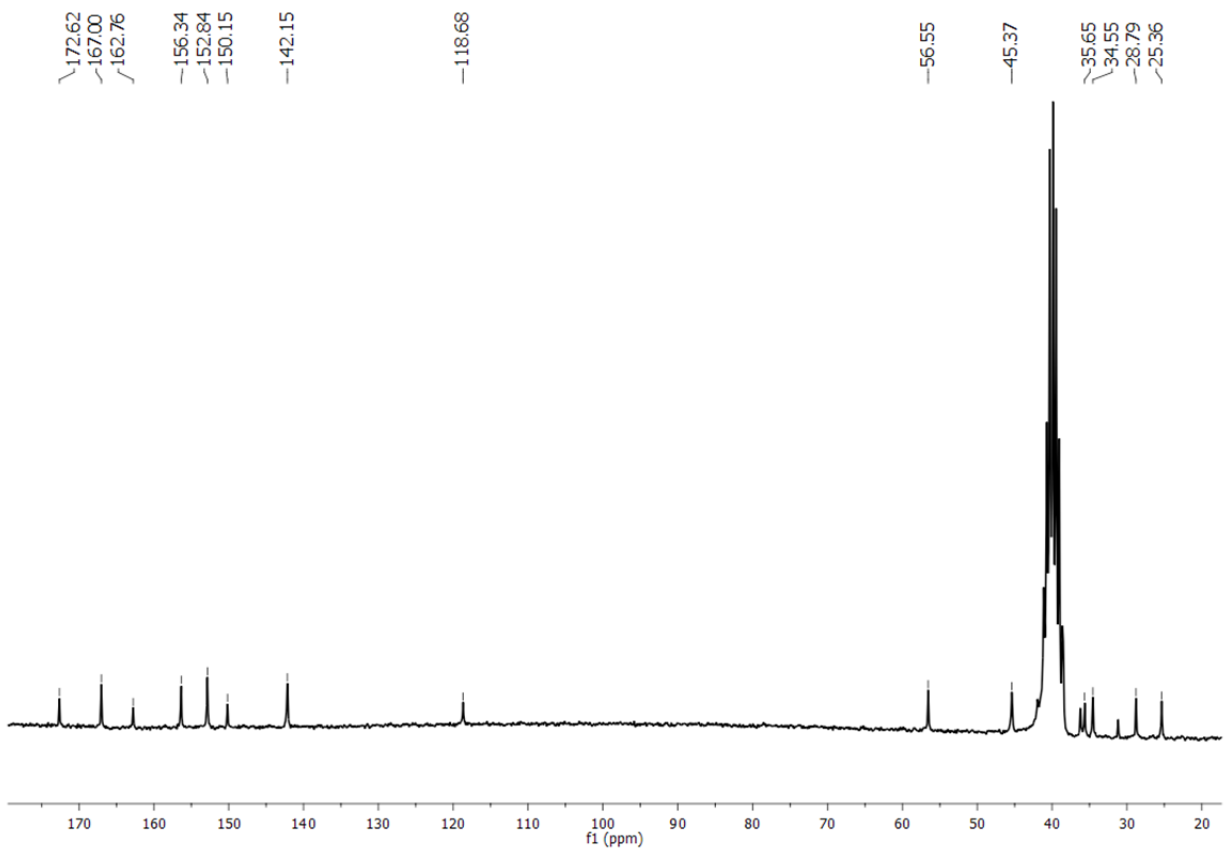


Fig. S13 ^{13}C NMR spectrum of ethyl **4**.