Supporting Information

Recognition of HIV-TAR RNA using Neomycin-Benzimidazole Conjugates

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Materials and methods.

General methods. Unless otherwise specified, chemicals were purchased from Aldrich (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA) and used without further purification. Di-*tert*-butyl dicarbonate (Boc anhydride) was purchased from Advanced ChemTech (Louisville, KY). All solvents were purchased from VWR (West Chester, PA). Silica gel (32-65 μM mesh size) was purchased from Sorbtech (Atlanta, Georgia). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance (300/500 MHz) spectrometer. Chemical shift are given in ppm and are referenced to residual solvent peaks (^{1}H and ^{13}C NMR). MS (MALDI-TOF) spectra were collected using a Bruker Microflex mass spectrometer. Ultra Violet (UV) spectra were collected on a Varian (Walnut Creek CA) Cary 100 Bio UV-Vis spectrophotometer equipped with a thermoelectrically controlled 12-cell holder. FID experiments were performed on Photon Technology International (PTI) fluorimeter equipped with temperature controller.

Nucleic acids. TAR RNA (GGC GUC ACA CCU UCG GGU GAA GUC GCC) was purchased from Dharmacon RNAi Technologies (Lafayette, CO). After deprotection and subsequent lyophillization, the RNA was dissolved in water and dialyzed in sodium cacodylate buffer containing 10 mM sodium cacodylate, 0.5 mM EDTA, 100 mM KCl at pH 6.8. The concentration of the TAR RNA was determined by UV spectroscopy using extinction coefficient provided by the supplier. The TAR RNA sample used in the studies was formed by snap cooling method in which, the TAR RNA was immediately cooled on ice after being heated to 90 °C for twenty minutes. The stock solution was diluted to desired concentrations as required.

FID titration. A solution of ethidium bromide (1.25 μM, 1800 μL) was excited at 545 nm, and its fluorescence emission was monitored between 560-607 nm before and after the addition of

HIV TAR RNA. The concentration of HIV-TAR RNA was 50 nM/strand. A small fraction of ethidium bromide is bound (less than 20%) under these conditions. The ligands were titrated into the TAR-RNA solution saturated ethidium bromide until the decrease in fluorescence reaches to the minimum. IC_{50} values were determined by plotting the percent fluorescence change with concentration and then applying sigmoidal fitting to obtain the concentration required to displace 50 % of the bound intercalator. The buffer used in the experiments contained 10 mM sodium cacodylate, 0.5 mM EDTA, 100 mM KCl at pH 6.8.

tat-TAR displacement assay. The IC_{50} of each compound was calculated for each compound using a titration from 5 nM to 10 μM into 100 nM of the tat-TAR complex in 20 mM KCl, 50 mM Tris (7.4), and 0.1% Triton X-100 in 100 μL final volume. Each titration point was performed in a separate well of a 96-well black round bottom Greiner plate. The plates were read in Tecan Genios-Pro fluorescence plate reader using an excitation wavelength of 485 nm and an emission measured at 535 nm. IC_{50} was calculated by plotting the log [compound] nM vs. fluorescence. Plot was fit to a dose response curve using Origin 5.0

Equation for dose response curve fitting.

*y = A1 + (A2-A1)/(1 + 10^((LOGx0-x)*p))*

Where,

y = Fluorescence intensity; *A1* = Bottom asymptote; *A2* = Top asymptote; *x0* = IC_{50}/DC_{50}; x = concentration of ligand being added; *p* = Hill constant

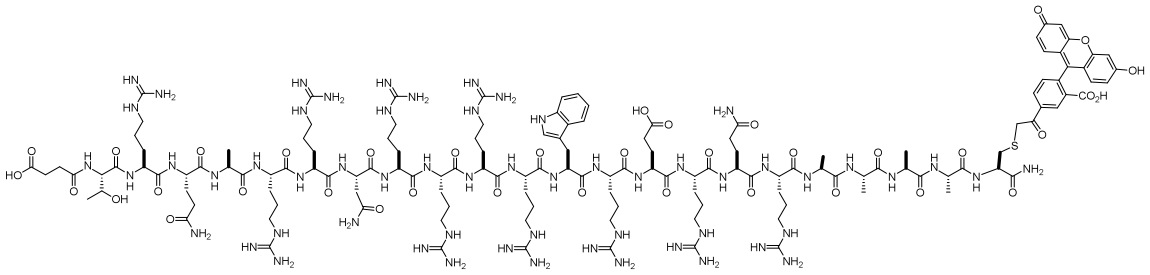
Ultra Violet (UV) thermal denaturation experiments. All UV spectra were obtained on a 12 cell holder Cary 1E UV-Vis spectrophotometer equipped with temperature controller. Quartz

cells with 1cm path length were used for all the experiments. Spectrophotometer stability and wavelength alignment were checked prior to initiation of each melting point experiment. For all experiments, the nucleic acid samples were prepared by diluting a stock sample. The melting of nucleic acid with or without the ligand was carried at a heating rate of 0.2 °C/min. Samples were brought back to 20 °C after the experiment. All UV melting experiments were monitored at 260 nm. The thermal denaturation temperatures (T_m) were obtained by drawing baselines as described by Mergny.[1] Data points were recorded every 1.0 °C. The RNA concentration was 1 μM /molecule (strand). The ligand: nucleic acid ratio was 1:1 for experiments carried out in the presence of ligands.

Circular Dichroism (CD) experiments. CD experiments performed at 20 °C using a Jasco J-810 spectropolarimeter with a thermo-electrically controlled cell holder. The CD spectra were recorded as an average of two scans. For CD titrations, small aliquots of concentrated ligand solution was serially added to the nucleic acid sample (2 μM/molecule). The experiment was performed in a buffer containing 10 mM sodium cacodylate, 0.5 mM EDTA and 100 mM KCl at pH 6.8. Following each addition of ligand, the TAR RNA-ligand complex was allowed to equilibrate for five minute before the scan was taken. The resulting spectrum was plotted for CD signal changes with respect to wavelength.

HIV-1 RRE RNA Competition Binding Assay

Before being used, HIV-1 RRE RNA (5'- GGU CUG GGC GCA GCG CAA GCU GAC GGU ACA GGC C -3', Thermo Scientific) in sterile water (10 μM batch) was heated to 95 ºC for 3 minutes and then cooled to ambient temperature over 1 hour.



Fluorescein-labeled HIV-1 Rev Peptide

The affinity of **DPA123** for HIV-1 RRE RNA was determined using fluorescence polarization-based competitive binding assay with a fluorescein-labeled HIV-1 Rev peptide. The anisotropy experiments were performed with a Spectra Max Fluorimeter (Molecular Devices) at 25 °C, with excitation and emission wavelengths of 485 and 525 nm, respectively. All samples were prepared in 96 well plates in binding buffer (30 mM HEPES, 100 mM KCl, 40 mM NaCl, 10 mM NH_4OAc, 10 mM Guanidinium Hydrochloride, 2 mM $MgCl_2$, 0.5 mM EDTA, pH 7.4) with 0.01% triton-X100 (Sigma). The IC_{50} value reported is the average of 3~5 individual measurements.

Prior to the competition experiments, the affinity of fluorescein-labeled HIV-1 Rev peptide for HIV-1 RRE RNA was determined by monitoring polarization changes of the fluorescent probe upon addition of RRE RNA. Addition of an increasing concentration (0 nM to 5000 nM) of RRE RNA to a 10 nM solution of fluorescein-labeled HIV -1 Rev peptide in binding buffer at 25 °C afforded a saturation binding curve. The saturation concentration of RRE RNA from this experiment was determined as (54 $\pm$ 14) nM.

Competition polarization assay. A solution of 100 nM RRE RNA and 10 nM fluorescein-labeled HIV-1 Rev peptide was incubated at 25 °C. After 10 min, appropriate concentrations (0 nM to 500 μM) of the DPA 123 were added. The total volume of the incubation solution was 80 μL.

After one hour, the amount of the dissociated fluorescent probe was determined by the Spectra Max fluorescence plate reader. The experimental dose-response data for DPA 123 was fit to a sigmoidal dose-response nonlinear regression model on GraphPad Prism 4.0 to afford the IC_{50} value. The IC_{50} for DPA 123 for this experiment was determined as (768 $\pm$ 309) nM.

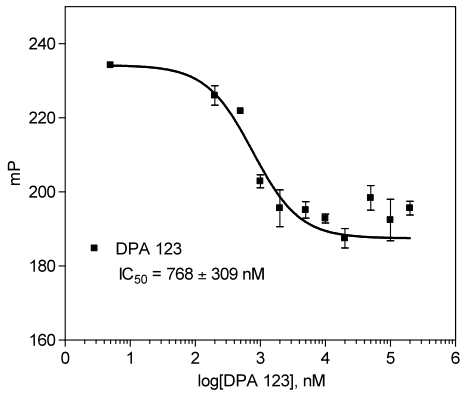
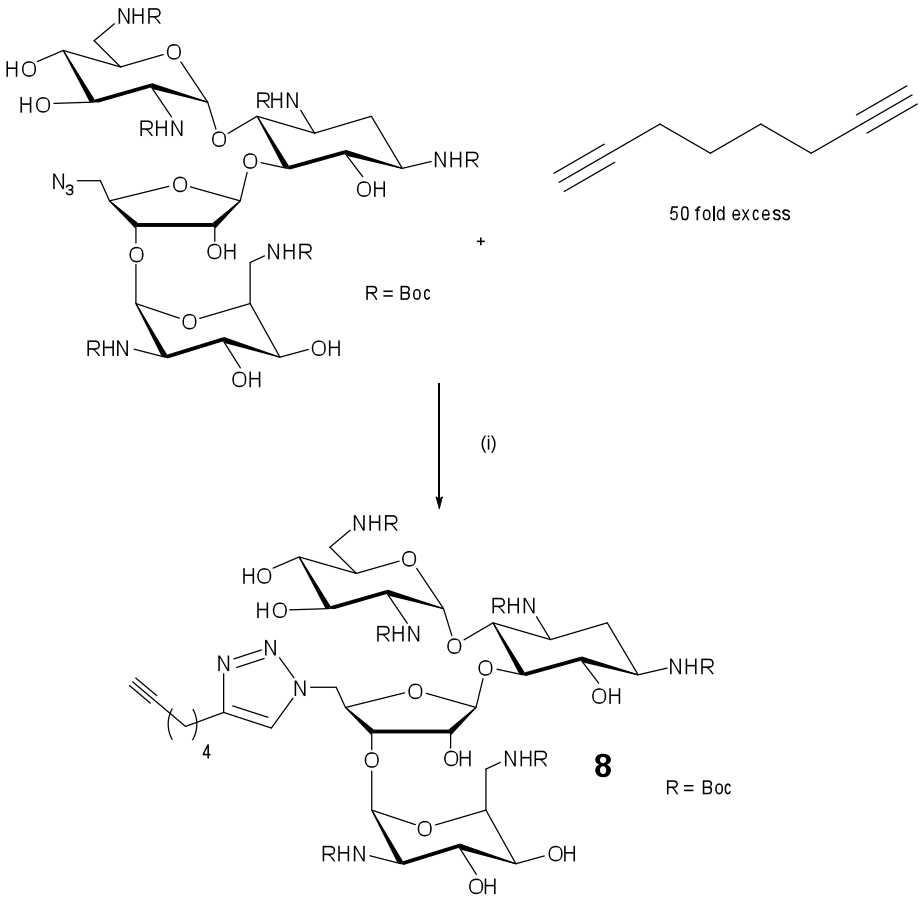
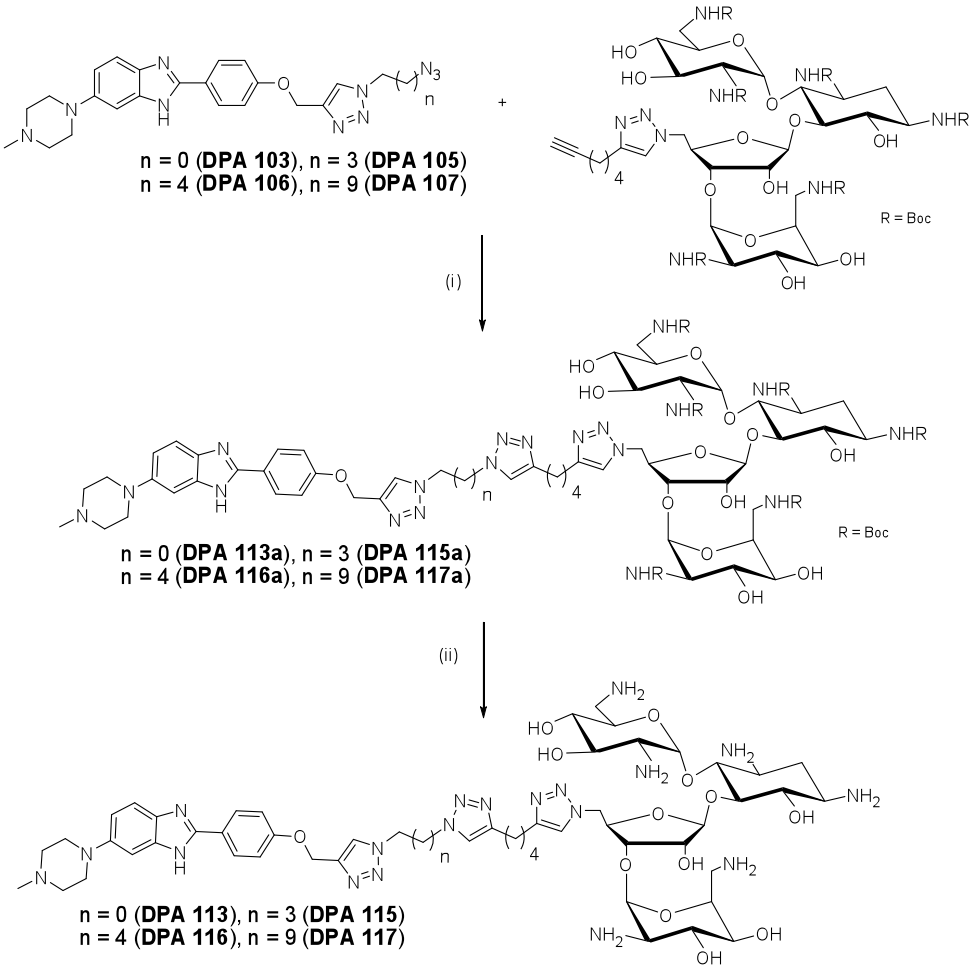


Figure S1. Competition binding of DPA 123 against saturated RRE RNA- HIV-1 Rev peptide complex.

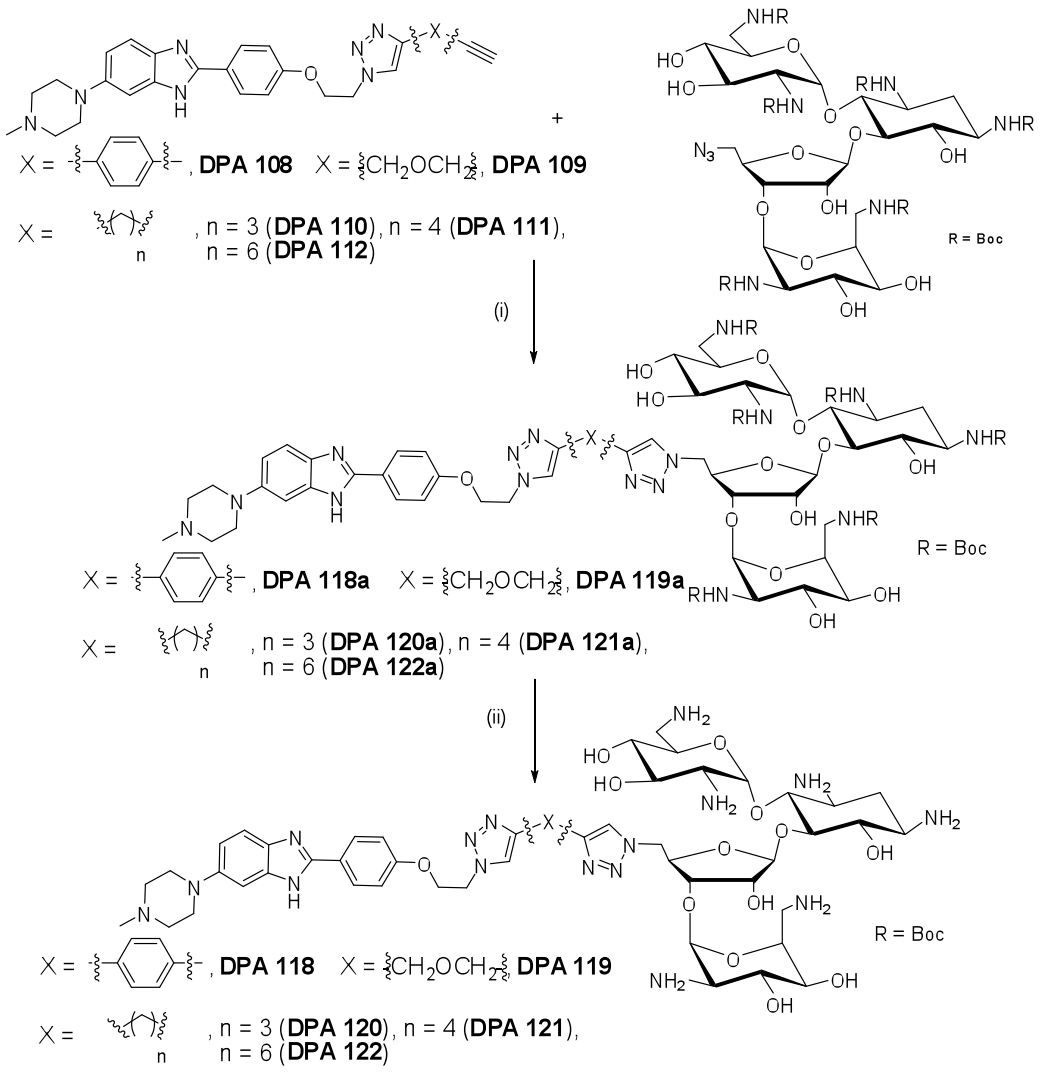
Synthesis of alkyne ended Boc protected neomycin.



Scheme S1. Reagents and conditions (i) $CuSO_4$ (0.2 equivalent), sodium ascorbate (0.4 equivalent), H_2O, C_2H_5OH, room temperature.



Scheme S2a. (i) sodium ascorbate, copper (II) sulfate, ethanol, room temperature, overnight (ii) dichloromethane, 4M HCl in 1,4 dioxane, 52-56 % cumulative yield for two steps.



Scheme S2b. (i) sodium ascorbate, copper (II) sulfate, ethanol, room temperature, overnight (ii) dichloromethane, 4M HCl in 1,4 dioxane, 66-70 % cumulative yield for two steps.

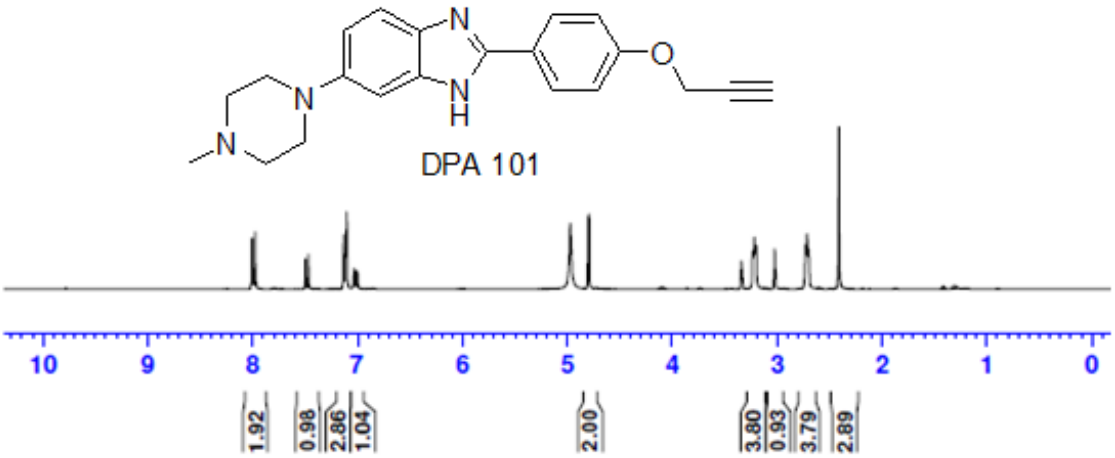
Synthesis of DPA 123.

Preparation of ***DPA 101***. To a solution of 5-(4-methylpiperazin-1-yl)-2-nitroaniline (472 mg, 2.00 mmol) in ethanol-ethyl acetate (2:1 *v/v*) mixture (20 mL), 10 % Pd-C (200 mg) was added and the mixture was hydrogenated for 4h at room temperature which afforded the corresponding diamine. Charcoal was filtered off. To this diamine, 4-(prop-2-ynyloxy) benzaldehyde (320 mg,

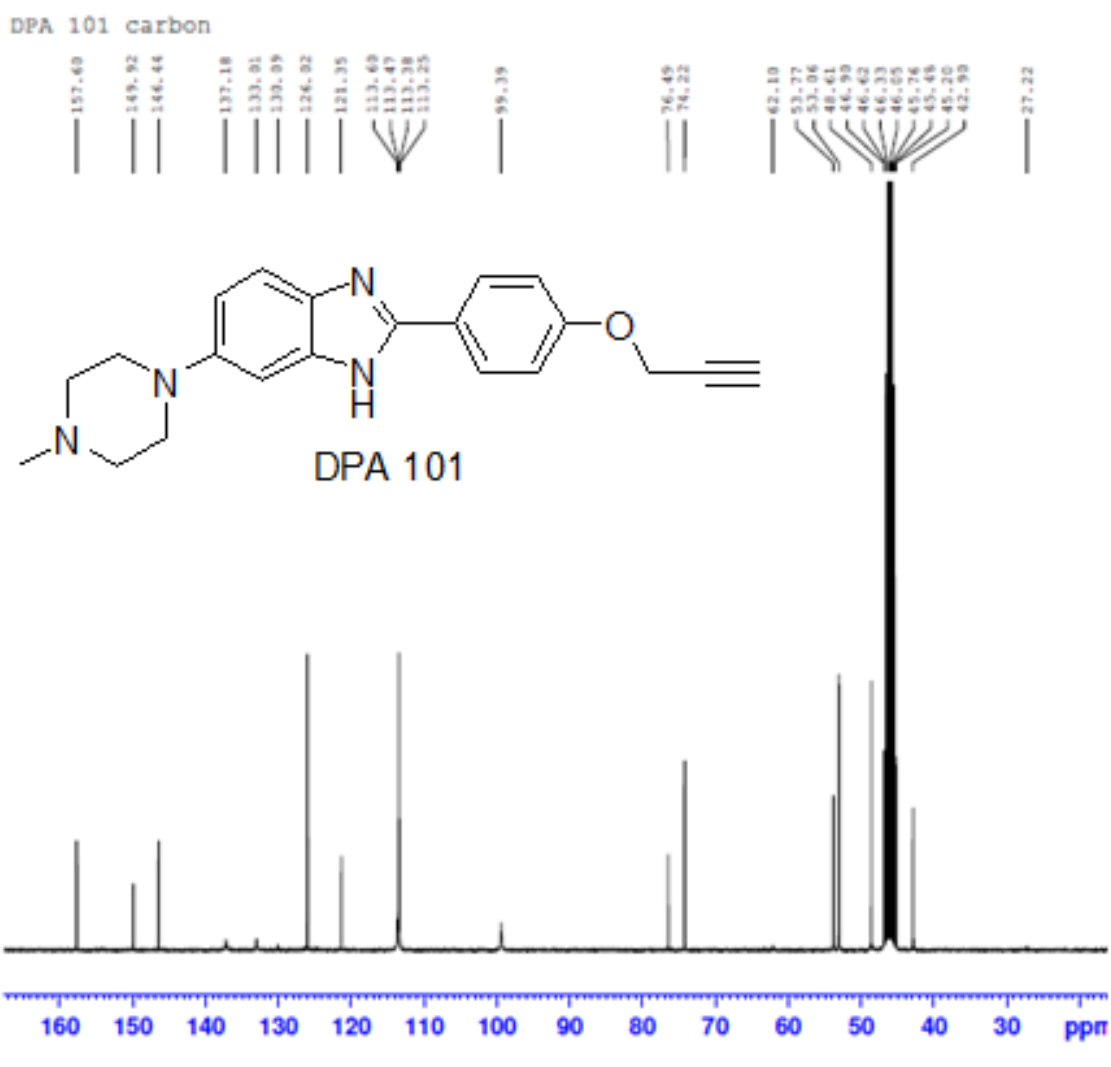
2.00 mmol) and sodium pyrosulfite (364 mg, 1.80 mmol) in water (0.5 mL) was added and the mixture was refluxed overnight. The volatiles were removed under reduced pressure. The crude mixture was purified on a silica gel column using dichloromethane-methanol as eluent to afford the desired compound as pale yellow solid (0.44 g, 65 %): R_f = 0.51 (dichloromethane-methanol 8:2 v/v); IR (KBr, cm^{-1}) 2969, 2818, 2119, 1633, 1580, 1449, 1232, 1188, 1024, 836; ^{1}H NMR (500 MHz, methanol-d_4) δ 7.99 (d, *J* = 8.87 Hz, 2H), 7.48 (d, *J* = 8.81 Hz, 1H), 7.13-7.11 (m, 3H), 7.02 (dd, J_1 = 8.89 Hz, J_2 = 2.24 Hz, 1H), 4.80 (d, *J* = 2.33 Hz, 2H), 3.22 (t, *J* = 4.57 Hz, 4H), 3.02 (t, *J* = 2.49 Hz, 1H), 2.72 (t, *J* = 4.99 Hz, 4H), 2.41 (s, 3H); ^{13}C NMR (125 MHz, methanol-d_4) δ 157.6, 149.9, 146.4, 126.0, 121.3, 113.4, 113.3, 76.4, 74.2, 53.7, 53.0, 48.6, 46.9, 42.9; MS (MALDI-TOF) *m/z* calcd for $C_{21}H_{22}N_4O$ 346.17, found 345.77 $[M]^+$.

Preparation of ***DPA 123***. To a solution of DPA 101 (12 mg, 34.7 μmol) in ethanol (1.0 mL), a solution of CuSO4 (1.59 mg, 10 mmol) and sodium ascorbate (3.96 mg, 20 mmol) in 0.5 mL water was added followed by addition of Boc protected neomycin azide (43 mg, 34.7 mmol). The reaction mixture was stirred at room temperature overnight in dark. TLC showed formation of the product. Volatiles were evaporated and the crude product was taken up in dichloromethane and then wet loaded on a silica gel column. Elution with dichloromethane-methanol (0-10 % methanol in dichloromethane) afforded the desired compound as white solid (R_f = 0.45 in dichloromethane- methanol 9:1 *v/v*). The Boc protected conjugate was taken up in dichloromethane (3 mL) and to it, 4M-HCl in 1, 4 dioxane (0.5 mL). After stirring at room temperature for 30 min, precipitation of the conjugate was induced by addition of small volumes (~1-2 mL) hexanes and ether. The precipitated product was centrifuged and the supernatant was discarded. The precipitated solid was taken up in water and lyophilized to dryness to afford the desired product as yellowish white solid (20 mg, 49 % overall yield for two steps): ^{1}H NMR (500

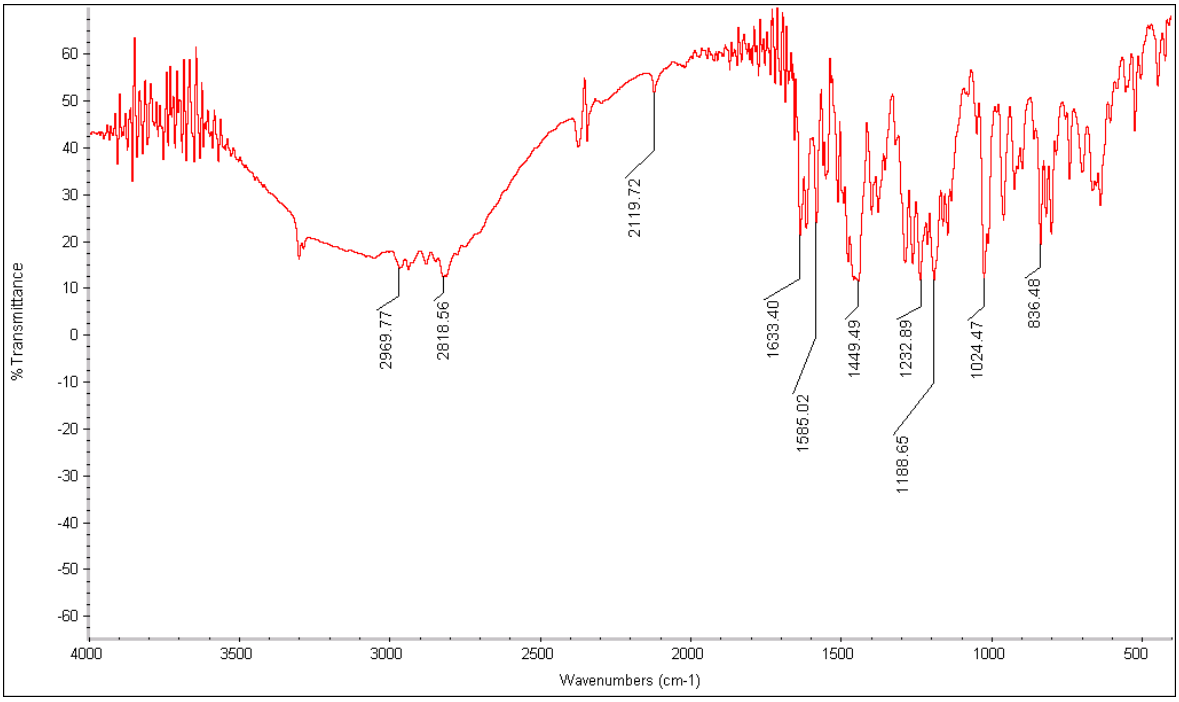
MHz, D_2O) δ 8.22 (s, 1H), 7.93 (d, *J* = 8.8 Hz, 1H), 7.61 (d, *J* = 9.6 Hz, 1H), 7.32 – 7.17 (m, 3H), 6.01 (d, *J* = 3.8 Hz, 1H), 5.35 (d, *J* = 3.0 Hz, 1H), 5.29 (s, 2H), 5.22 (s, 1H), 4.47 (m, 2H), 4.22 (s, 1H), 4.14-4.04 (m, 2H), 4.01 – 3.69 (m, 6H), 3.70 – 3.04 (m, 15H), 2.87 (s, 3H), 2.39 (d, *J* = 12.1 Hz, 1H), 1.88 – 1.74 (m, 1H); MS (MALDI-TOF) *m/z* calcd for $C_{44}H_{67}N_{13}O_{13}$ 985.48, found 1003.43 $[(M+H_2O)]^+$.



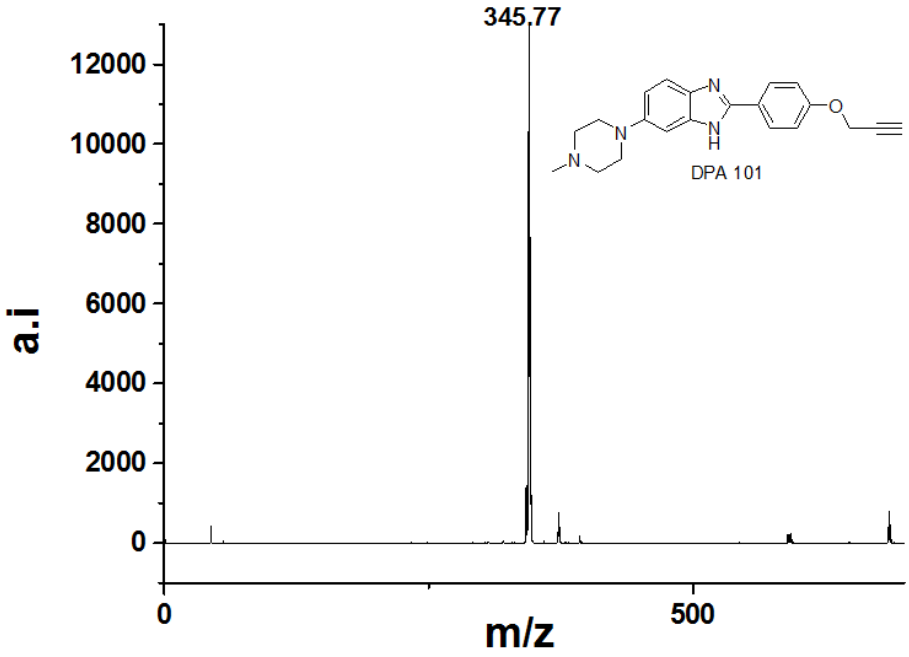
^{1}H NMR of DPA 101.



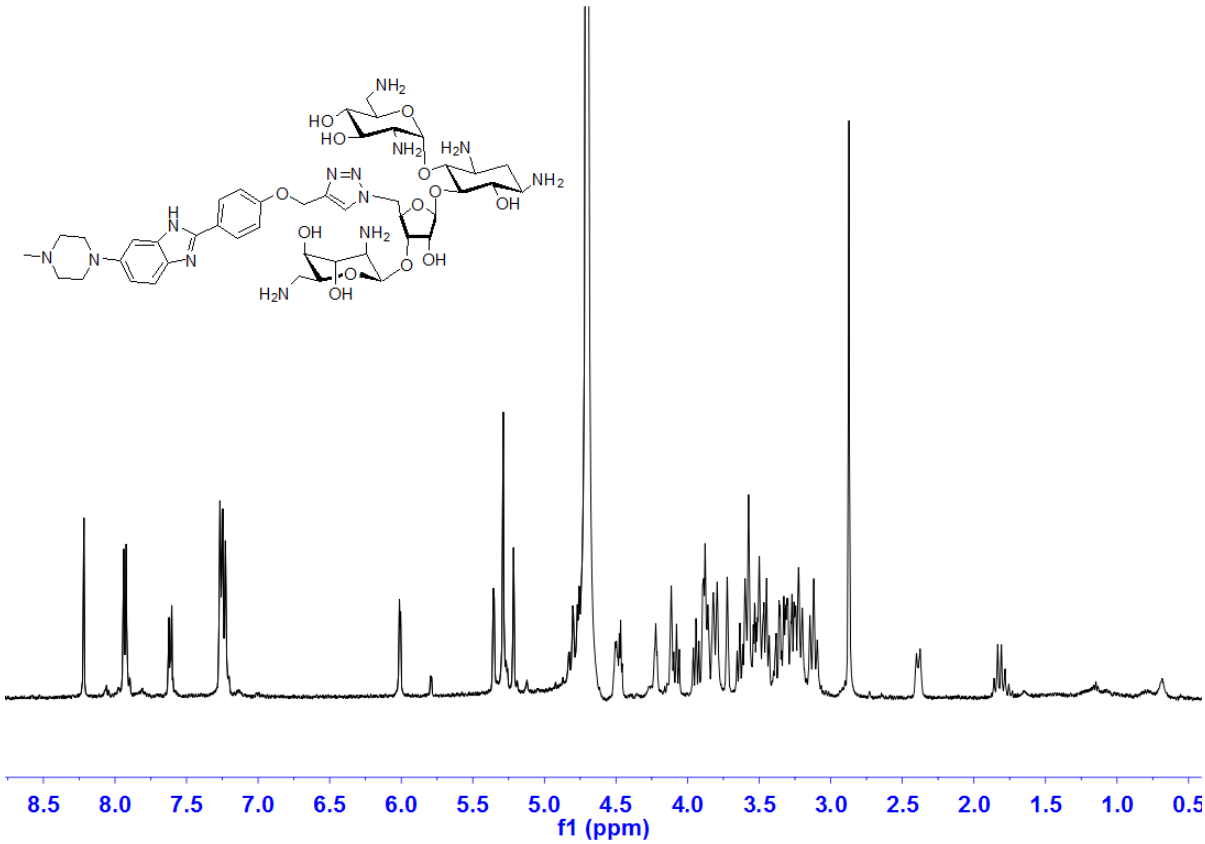
^{13}C NMR spectrum of DPA 101 .



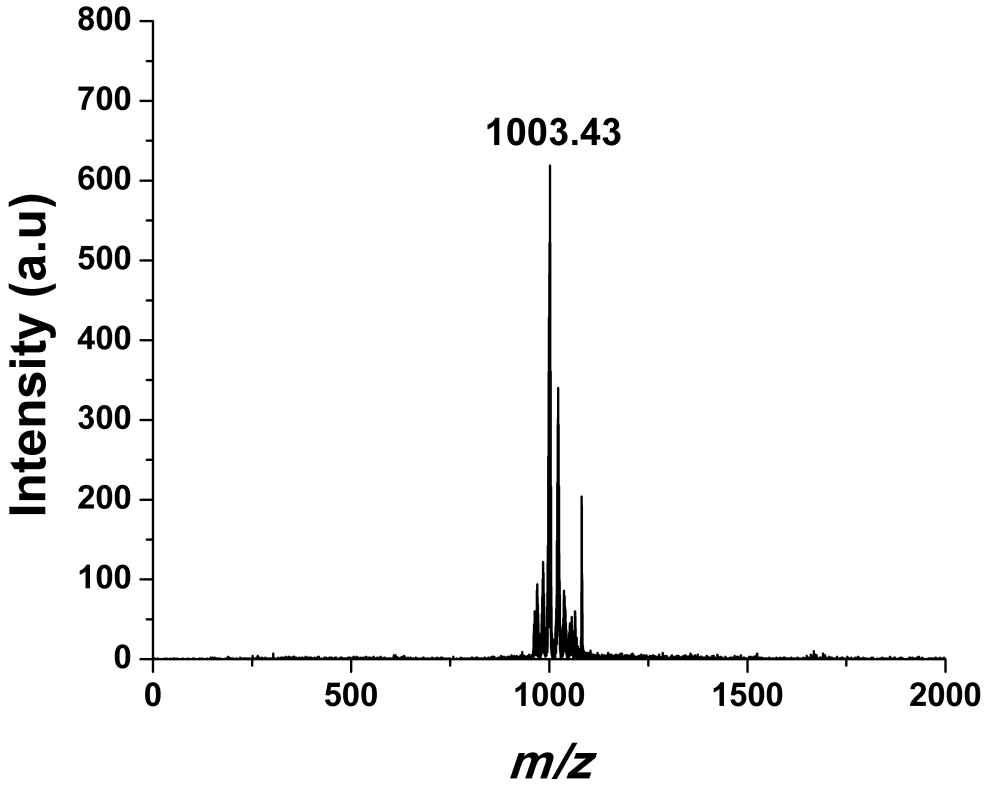
IR spectrum of DPA 101 in KBr.



MALDI-TOF spectrum of DPA 101.



^{1}H NMR spectrum of DPA 123.



MALDI-TOF of DPA 123.

Reference.

1. Mergny, J. L.; Lacroix, L. *Oligonucleotides* 2003, *13,* 515.