## Supporting Information

# Riboflavin-catalyzed templated reaction to translate nucleic acid cues into signals of rhodamine derivatives

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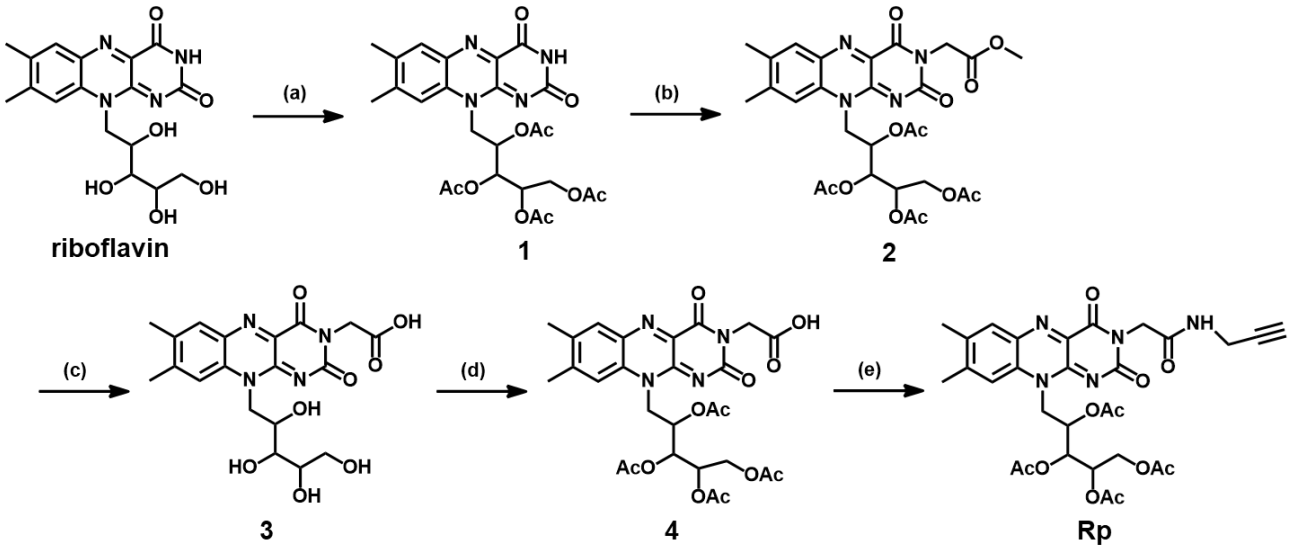
# 1. Materials and Methods

All reagents and solvents were purchased from commercial sources (TCI, Samchun, Daejung, Sigma-Aldrich, and Alfa) and used without further purification. Silica gel plates (Merck Plastikfolien or Merck Alufolien; 20 cm × 20 cm; thickness: 0.2 mm) were used to monitor the progress of the reactions. For purification, column chromatography or reverse-phase high-pressure liquid chromatography (HPLC) was performed on a silica gel (Silica Flash, 230–400 mesh). To identify the synthesized compound, $^{1}H$ and $^{13}C$ NMR spectra were recorded using a Bruker AVANCE III 400 MHz or AVANCE 500 MHz NMR spectrometer (at Chungbuk National University). The mass values (m/z) of the synthesized small molecules (rhodamine and riboflavin derivatives) were measured using a maXis 4G spectrometer (scan mode: m/z 50–1000). All natural and modified oligonucleotides in this study were purchased from Bioneer (Daejeon, South Korea). The mass values of the synthesized oligodeoxynucleotides were measured through matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS, Axima LNR MALDI-TOF mass spectrometer, Shimadzu) at Bioneer.

## 1.1 Synthesis of propargylated riboflavin tetraacetate (Rp)

**Scheme S1** Synthesis of **Rp**




(a) Riboflavin (1 eq.), acetic acid (90 eq.), acetic anhydride (30 eq.), perchloric acid (0.7 eq.), 100 ℃, 12 h., quant.; (b) methyl bromoacetate (4 eq.), potassium carbonate (6 eq.), r.t., 2 days, 92%; (c) 2M HCl (40 eq.), 100 ℃, 6 h., quant.; (d) acetic acid (90 eq.), acetic anhydride (30 eq.), perchloric acid (0.7 eq), 25 ℃, 12h., quant.; (e) HATU (1.1 eq.), DIPEA (4 eq.), 2.6-lutidine (4 eq.), propargylamine (2 eq.), DMF, 25 ℃, 12 h, 22%.

**Synthesis of compound 4**

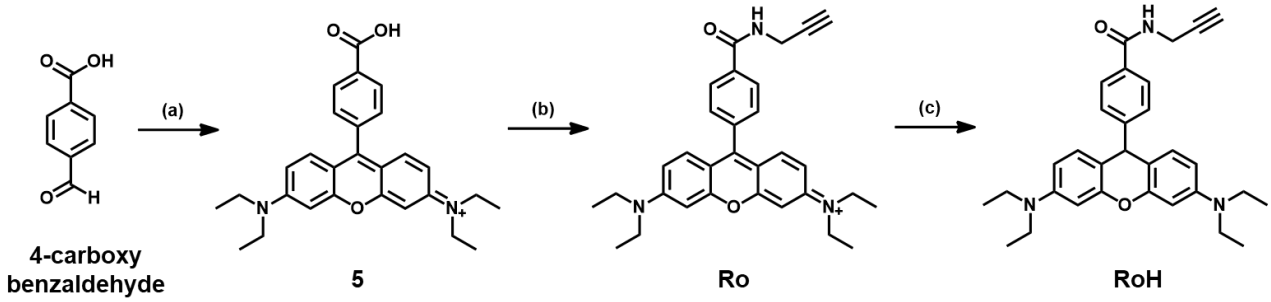
Compounds **1**, **2**, and **3** were synthesized by adapting a procedure from a previous report.[1] Compound **3** (0.1446 g, 16 mmol) was dissolved in 1.7 mL of acetic acid (90.0 eq., 30 mmol). Perchloric acid (0.7 eq., 0.01 mL, 0.23 mmol) was added to the reaction mixture, followed by the addition of 0.85 mL of acetic anhydride. The mixture was stirred overnight at 25 ℃. The mixture was diluted with water and extracted using ethyl acetate (EA). The organic phase was dried over anhydrous sodium sulfate and filtered. The organic layer was evaporated and purified via silica gel column chromatography (dichloromethane (DCM):methanol (MeOH) = 5:1) to yield a yellow solid (284 mg, quant.). $^{1}$H NMR (500 MHz, methanol-$d_4$) δ 7.91 (s, 1H), 7.83 (s, 1H), 5.66 (m, 1H), 5.56 (m, 1H), 5.43 (m, 2H), 5.06 (br, 1H), 4.58 (s, 2H), 4.48 (dd, 1H), 4.25 (q, 1H), 2.59 (s, 3H), 2.45 (s, 3H), 2.22 (s, 3H), 2.21 (s, 3H), 2.03 (s, 3H), 1.95 (s, 3H); $^{13}$C NMR (126 MHz, methanol-$d_4$) δ 170.98, 170.49, 170.14, 160.24, 155.87, 149.45, 147.94, 137.12, 135.60, 134.58, 131.67, 131.30, 116.30, 70.22, 69.36, 61.62, 44.36, 19.99, 19.97, 19.69, 19.64, 19.36, 19.23, 19.01, 17.95; ESI HRMS: observed at m/z = 625.1758 $[M+Na]^+$, calc. for $C_{27}H_{30}N_4O_{12}$ = 602.1860.

**Synthesis of Rp**

N,N-diisopropylethylamine (DIPEA; 4.0 eq., 0.33 mL, 1.9 mmol), 2.6-lutidine (4.0 eq., 0.22 mL, 1.9 mmol), and HATU (1.1 eq., 0.2 g, 0.52 mmol) were added to a solution of compound **4** (0.2841 g, 0.47 mmol) and 5.5 mL of dimethyl formamide (DMF). The reaction mixture was stirred at 25 ℃ for 20 min. Subsequently, propargylamine (2.0 eq., 0.07 ml, 1 mmol) was added to the mixture and stirred overnight at 25 ℃. The aqueous layer was extracted using EA; then, the organic phase was washed with brine and dried over anhydrous sodium sulfate. The organic layer was evaporated and purified via silica gel column chromatography (EA:hexane = from 2:1 to 10:1) to yield a yellow solid (65 mg, 22%). $^{1}$H NMR (500 MHz, chloroform-*d*) δ 8.02 (s, 1H), 7.57 (s, 1H), 6.31 (t, 1H), 5.64 (dt, 1H), 5.40 (m, 3H), 4.79 – 4.70 (m, 3H), 4.41 (dd, 1H), 4.24 (dd, 1H), 4.06 (dt, 2H), 2.55 (s, 3H), 2.43 (s, 3H), 2.25 (s, 3H), 2.19 (s, 3H), 2.06 (s, 3H), 1.77 (s, 3H); $^{13}$C NMR (126 MHz, chloroform-*d*) δ 170.69, 170.35, 166.52, 159.61, 154.64, 136.94, 134.89, 133.02, 115.49, 79.21, 71.91, 70.39, 69.06, 61.86, 44.66, 44.45, 29.71, 29.41, 21.49, 21.06, 20.82, 20.73, 20.40, 19.47, 14.13; ESI HRMS: observed at m/z = 662.2081 $[M+Na]^+$, calc. for $C_{30}H_{33}N_5O_{11}$ = 639.2177.

## 1.2 Synthesis and properties of rosamine (Ro) and dihydrorosamine (RoH)

**Scheme S2** Synthesis of **Ro** and **RoH**




(a) i) 3-diethylaminophenol (2 eq.), $H_2O{\cdot}H_2SO_4$, 120 ℃, 18 h. ii) $Fe_2(SO_4)_3$ (7.2 eq.), 90 ℃, 4h. iii) 0 ℃, 40% KOH, 6%; (b) DCC (4 eq.), *N*-hydroxysuccinimide (12 eq.), propargylamine (6 eq.), DMF, 25 ℃, 12 h., 83%. (c) $NaBH_4$, 25 ℃, 95%.

**Synthesis of Ro**

Compound **5** was synthesized by following a procedure from a previous report.[2] *N*,*N*'-dicyclohexylcarbodiimide (DCC) (4.0 eq., 0.35 g, 1.68 mmol) and *N*'-hydroxysuccinimide (NHS; 12.0 eq., 0.58 g, 5.04 mmol) were added to a solution of compound **5** (0.19 g, 0.42 mmol) in 10 ml of DMF. The reaction mixture was stirred at 25 ℃ for 20 min. Subsequently, propargylamine (6.0 eq., 0.16 ml, 2.52 mmol) was added to the mixture and stirred overnight at 25 ℃. The aqueous layer was extracted using DCM; then, the organic phase was washed with brine and dried over anhydrous sodium sulfate. The organic layer was evaporated and purified via silica gel column chromatography (DCM:MeOH = from 40:1 to 10:1) to yield **Ro** as a dark-purple solid (0.17 g, 83%). $^1$H NMR (500 MHz, Methanol-$d_4$) δ 8.16 (d, 2H), 7.62 (d, 2H), 7.36 (d, 2H), 7.12 (dd, 2H), 7.02 (d, 2H), 4.25 (d, 2H), 3.71 (q, 8H), 2.69 (t, 1H), 1.34 (t, 12H); $^{13}$C NMR (126 MHz, methanol-$d_4$) δ 167.15, 158.10, 156.11, 155.82, 135.46, 135.43, 131.46, 129.72, 127.63, 114.33, 112.90, 96.15, 79.33, 73.12, 70.94, 60.15, 53.52, 51.37, 45.56, 32.44, 31.37, 30.50, 28.73, 24.94, 24.86, 24.66, 22.33, 19.53, 13.13, 13.09, 11.51; ESI HRMS: observed at m/z = 480.2646 $[M]^+$, calc. for $C_{31}H_{34}N_3O_2^+$ = 480.2646.

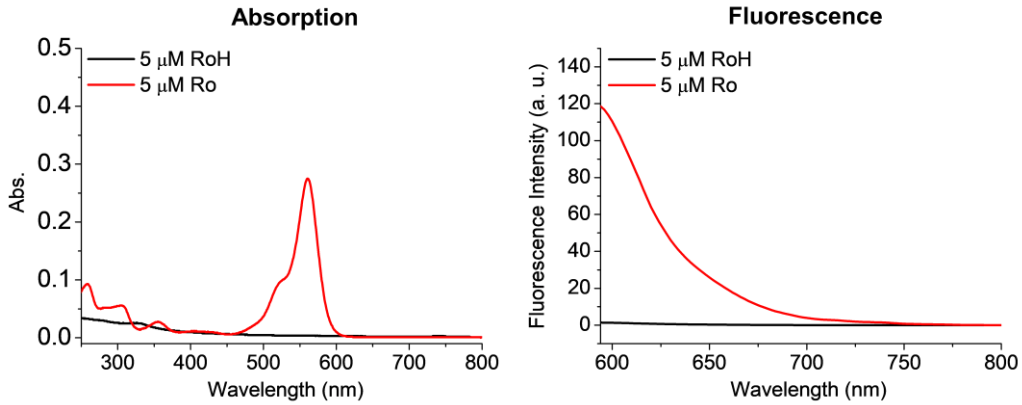
**Synthesis of RoH**

An excess of $NaBH_4$ (1 mg) was added to 40-mM **Ro** in 440 μL of DMSO:MeOH (2:3). When the sample became transparent, it was transferred to a microtube. The solution was centrifugated and purified through HPLC (Agilent 1260 with an Agilent ZORBAX 300SB-C18). Conditions: A = 0.01% trifluoroacetic acid (TFA) in deionized (DI) water; B = 0.01% TFA in acetonitrile; A:B ratio = 100:0 at 0 min, 30:70 at 25 min, 0:100 at 28 min, 100:0 at 29 min, and 100:0 at 30 min; RT. The flow rate was maintained at 2.5 mL/min.

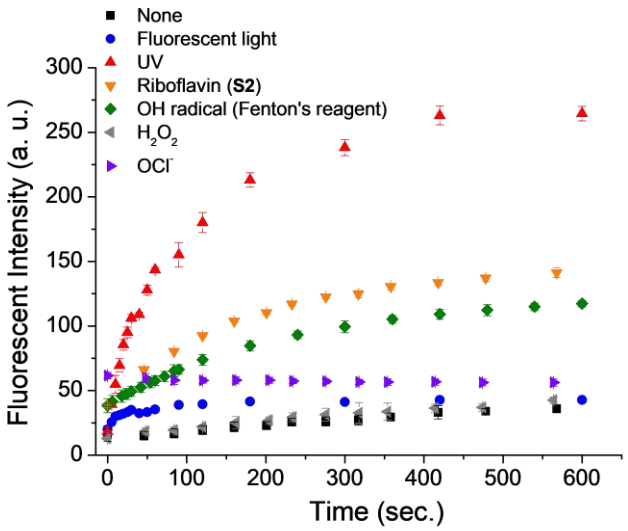
The detection wavelength was 260 and 553 nm (for monitoring the remaining **Ro**). The injection volume was 100 µL. The lyophilization of purified samples affords reduced rosamine (**RoH**; 8.1 mg, 95%). $^1$H NMR (500 MHz, methanol-$d_4$) δ 7.76 – 7.72 (m, 2H), 7.32 – 7.27 (m, 2H), 7.19 – 6.65 (br, 7H), 4.13 (d, 2H), 3.54 (dd, $J$ = 26.8, 8H), 2.59 (t, 1H), 1.16 (t, 12H); ESI HRMS: observed at m/z = 482.2802 $[M+H]^+$, calc. for $C_{31}H_{35}N_3O_2$ = 481.2729.

**Properties of Ro and RoH**

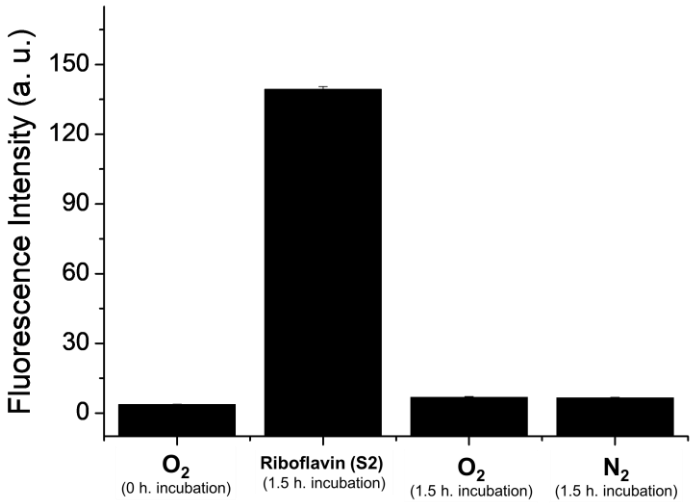
**1. Absorption and fluorescence spectra of fresh RoH and Ro in $H_2O$.**



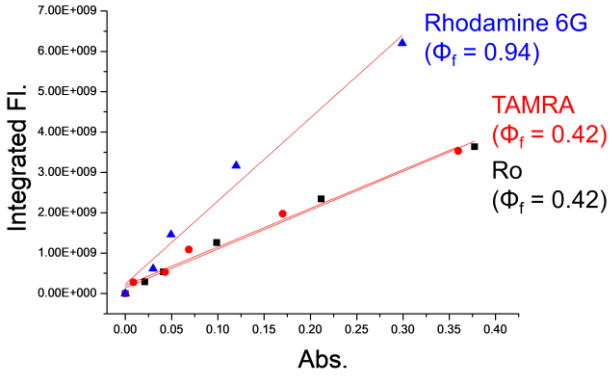

**2. Reactivity of RoH to lights or reactive oxygen species (ROS)**. Fluorescence change of 200 nM oligodeoxynucleotide **S1** (containing **RoH**) was monitored in the presence of fluorescent light, UV, **S2** (having riboflavin), or 10 µM ROS in $H_2O$. The signal was increased by UV and some ROS, including OH radical and $OCl^-$.



**3. Reactivity of RoH to oxygen molecules**. Fluorescence of 200 nM oligodeoxynucleotide **S1** (containing **RoH**) was measured after 1.5 h of incubation with $O_2$, $N_2$, or **S2** (riboflavin). The result showed that **RoH** is not oxidized by $O_2$.




**4. Quantum yields ($\Phi_f$) of DNA-conjugated Ro and TAMRA in phosphate buffered saline (PBS)**. To measure fluorescence quantum yields of Ro and TAMRA on a DNA strand, absorbance and fluorescence of **S1ox** or **S5ox** solution were measured in 1 × PBS buffer at 25 ℃. The fluorescence quantum yields were determined by general calculation methods using rhodamine 6G ($\Phi_f$ = 0.94 in ethanol) as a standard.[3]



### 1.3 Preparation of oligodeoxynucleotides

For conjugation between azide-modified oligodeoxynucleotide and **Rp** or **Ro**, 25 μL of 200-μM azide-modified oligodeoxynucleotide, 10 μL of pH 7.0 2 M triethylammonium acetate buffer, 45 μL of DMSO, 3 μL of 10 mM alkyne compounds in DMSO, 10 μL of 5 mM sodium ascorbate in water, and 5 μL of a 10 mM copper(II) tris(benzyltriazolylmethyl)amine (TBTA) complex in aqueous 55% DMSO were mixed in a microtube. The reaction mixture was incubated at room temperature (RT) overnight. Purification of conjugated oligodeoxynucleotide was conducted using an Agilent ZORBAX 300SB-C18 column through HPLC (Agilent 1260). Conditions: A = pH 7.2 triethylammonium acetate (TEAA) buffer, B = 0.01% TFA in acetonitrile; A:B ratio = 100:0 at 0 min, 30:70 at 28 min, 0:100 at 29 min, 100:0 at 30 min; RT. The flow rate was kept at 2.5 mL/min. The detection wavelength was 260 and 553 nm for rosamine, 445 nm for riboflavin, 557 nm for tetramethylrhodamine (TAMRA), 525 nm for rhodamine 6G, and 585 nm for Texas Red). The injection volume was 100 μL. The lyophilized oligodeoxynucleotides were identified via MALDI-TOF MS using 3-hydroxypyridine-2-carboxylic acid as the matrix.

To reduce rhodamine derivatives on DNA, 1 mg of $NaBH_4$ (excess) was added to a mixture of 10 μL of 500-μM rhodamine-modified oligodeoxynucleotides and 40 μL of 0.2 M pH 7 sodium phosphate buffer, and the solution was gently mixed. Once the pink color of rhodamine vanished, the reaction mixture was directly injected into the HPLC for purification (under general conditions mentioned above). The detection wavelength was 260 nm. The lyophilized reduced products were identified by MALDI-TOF MS.

For the quantification of oligodeoxynucleotides, UV absorbances of oligodeoxynucleotide samples at 260 nm were measured. The extinction coefficients of A, T, G, and C at 260 nm used were 13700, 6600, 11700, and 8800 $M^{-1}$, respectively.

List of oligodeoxynucleotides used in this study

| Name | Sequence (5′ to 3′) | Expected mass (m/z) | Observed mass (m/z) |
|---|---|---|---|
| S1 | **RoH**-TAG CAT CAT ACA GGC | 5330.2109 | 5330.4862 |
| S1ox | **Ro**-TAG CAT CAT ACA GGC | 5329.1929 | 5330.5562 |
| S2 | GCC TGT ATG ATG CTA-**Rp** | 5540.1390 | 5540.0825 |
| S3 | GCC TGT ATG ATG CTA | 4580.7964 | 4580.4958 |
| S4 | **Rhodamine 6G**-TAG CAT CAT ACA GGC | 5180.0738 | 5179.6431 |
| S4ox | **Rhodamine 6G (ox)**-TAG CAT CAT ACA GGC | 5178.0582 | 5178.2241 |
| S5 | **TAMRA**-TAG CAT CAT ACA GGC | 5136.0112 | 5135.8191 |
| S5ox | **TAMRA (ox)**-TAG CAT CAT ACA GGC | 5135.0034 | 5134.74 |
| S6 | **Texas red**-TAG CAT CAT ACA GGC | 5441.1231 | 5440.6562 |
| S6ox | **Texas red (ox)**-TAG CAT CAT ACA GGC | 5440.1153 | 5437.59 |
| S7 | **Rp**-AGA CAG TGT TA | 4307.9487 | 4307.6411 |
| S8 | CCA TCT TTA CC-**RoH** | 4026.9733 | 4026.3672 |
| miR-141 DNA | TAA CAC TGT CTG GTA AAG ATG G | 6795.1779 | 6795.4062 |
| M1 | TAA CAC TAT CTG GTA AAG ATG G | 6779.1830 | 6779.4612 |
| M3 | TTA CAC TAT CTG GTG AAG ATG G | 6786.1644 | 6786.5556 |
| miR-126 DNA | TCG TAC CGT GAG TAA TAA TGC | 6442.1142 | 6442.7359 |
| miR-34 DNA | TGG CAG TGT CTT AGC TGG TTG T | 6800.1331 | 6800.1616 |

### 1.4 DFT calculations

We first optimized the molecular geometries of the reactants and products in Figure S1 using the Gaussian09 software.[4] Then, the Gibbs free energies, which include the solvation and intramolecular components, were computed. All DFT calculations were performed at the B3LYP/6-31G(2df,p) level of theory. The solvent effect due to the water solvent was considered in all DFT calculations using the SCRF theory.[5]

### 1.5 Absorbance recovery of RoH by Rf (for Fig. 2)

In a quartz UV cuvette, 5 μL of 10-mM **RoH** in DMSO and 975 μL of water were mixed. The absorbance of the starting solution was measured. Next, 20 μL of 10-mM **Rf** in DMSO was added to initiate the reaction,

and the absorbance change was measured. To obtain normalized absorption spectra, a 50-μM **RoH**+**Rf** solution (1 mL) incubated for 96 h in the dark was used. The spectrum of **Ro**+**Rf** was obtained from a mixture of 5-μM **Ro** and 50-μM **Rf** for comparison.

### 1.6 Absorbance recovery by riboflavin (Rf) or dihydroriboflavin ($RfH_2$) (for Fig. S2)

In a quartz UV cuvette containing 1.2 mL of 3.6-mM **Rf** in DCM (or pure DCM), 0.2 mL of 0.5-M KCl-saturated sodium phosphate buffer (pH 8) containing 432-mM sodium dithionite (20 eq., $Na_2S_2O_4$) was added. In the case of control samples, a KCl-saturated sodium phosphate buffer containing $Na_2S_2O_4$ was added. The two-phase solution was vigorously shaken to obtain an emulsion and then separated again by centrifugation (15 min) into DCM and the aqueous solution to remove all $Na_2S_2O_4$ from the DCM solution that includes **$RfH_2$**. The formation of **$RfH_2$** was verified through UV spectra and $^1$H-NMR spectroscopy.[6,7] Next, **RoH** (10 mM, 60 μL) or **Ro** (2 mM, 120 μL) was directly injected using a syringe into the DCM layer. The mixture was gently mixed and then changes in the visible light absorbance of **Ro** were measured using a UV–vis spectrophotometer (SpectraMax M2) to evaluate the reaction progress.

### 1.7 Evaluation of templated reactions (for Fig. 3, S3, and 4)

To evaluate the templated reaction, 50 μL of pH 7.4 4 × PBS buffer (Tablet, Sigma-Aldrich), DI water, and oligodeoxynucleotides modified with a reduced rhodamine derivative were mixed well in a 96-well microtiter plate (Thermo Fisher, Nunc™, round bottom), followed by the addition of a stock solution of **S2** to afford a final oligodeoxynucleotide concentration of 200 nM. In the case of templated reactions using $MgCl_2$, 50 μL of a 40- or 200-mM $MgCl_2$ solution in DI water was added to the mixture to obtain a final concentration of 10 or 50 mM $MgCl_2$, respectively. The fluorescence enhancement was directly measured using microplate readers (SpectraMax M2) in the kinetic mode at 25 ℃ or 37 ℃. Parameters: $\lambda_{exc}$: 553 nm, cutoff: 590 nm, $\lambda_{emi}$: 600 nm for rosamine; $\lambda_{exc}$: 557 nm, cutoff: 590 nm, $\lambda_{emi}$: 592 nm for TAMRA; $\lambda_{exc}$: 525 nm, cutoff: 550 nm, $\lambda_{emi}$: 560 nm for rhodamine 6G; $\lambda_{exc}$: 585 nm, cutoff: 610 nm, $\lambda_{emi}$: 620 nm for Texas Red; PMT gain: medium; 6 flashed/read; shake off; carriage speed normal; column priority. The experiments were conducted in triplicate. Further, 100% yield of the templated reaction was estimated using the fluorescence intensity of 200-nM-oxidized **S1**, **S4**, **S5**, or **S6** hybridized with **S2**.

### 1.8 MiR-141 DNA and miR-141 detection (for Fig. 5 and S6)

First, the reaction solution containing 200-nM **S8**, 20-nM **S7** (200-nM **S7** for miR-141 detection), and different concentrations of miR-141 DNA or miR-141 were prepared in pH 7.4 1 × PBS buffer. Afterward, the fluorescence of the reaction mixture was immediately measured in the kinetic mode (every 5 min) at 37 ℃. The experiments were conducted in triplicate. Other control sequences were tested in the same way.

Parameters: $\lambda_{exc}$: 553 nm, cutoff: 590 nm, $\lambda_{emi}$: 600 nm; PMT gain: medium; 6 flashed/read; shake off; carriage speed normal; column priority.

To calculate signal amplification, 100% yield of each reaction was estimated using the fluorescence intensity of a 200-μL sample containing 200-nM-oxidized **S8**, 20-nM **S7**, and the same amount of miR-141 DNA.

### 1.9 Gel shift assay (for Fig. 6)

To evaluate antibody selectivity, 4 μM of reduced TAMRA-modified oligodeoxynucleotide (**S5**) in water and 4 μM of fresh anti-rhodamine antibody (**Ab**, Rockland, 200-301-246, mouse monoclonal) in pH 7.4 1 × PBS buffer were prepared. In microtubes, the stock solutions were mixed to obtain a total of 9 μL of 1-μM samples (comprising **S5**, **Ab**, or **S5**+**Ab**) in pH 7.4 1 × PBS buffer. The samples were incubated in the dark at 4 ℃ for 30 min. After incubation, 1 μL of glycerol was added, and the prepared final samples were analyzed by 10% nondenaturing polyacrylamide gel electrophoresis (PAGE). Conditions: 100 V, 30–35 min. Gel electrophoresis images were captured through GelDoc Go (Bio-Rad) using the SYBR Gold® method to visualize TAMRA. Visualization of **S5** was achieved by UV irradiation, which changes **S5** into fluorescent **S5ox**, after running gels.

For the templated reaction signaling antibody, 13.5 μL samples of 1 μM **S5** and **S5**+**S2** in pH 7.4 1 × PBS buffer were prepared from stock solutions. The samples were incubated for 0–3 h. (for **S5**, 3 h of incubation). Then, the resulting samples were mixed with fresh **Ab** and further incubated in the dark at 4 ℃ for 20 min. After adding 1.5 μL of glycerol, the final samples were analyzed by 10% nondenaturing PAGE. Conditions: 100 V, 30–35 min.

## 2. Supplementary figures

**Figure S1:** A hypothetical redox reaction was used for the reaction free energy calculation. The Gibbs free energies for the reaction of RoH, Rhodamine 6G, TAMRA, and Texas Red, computed at the B3LYP/6-31(2df,p) level of theory, were −41.87, −37.50, −37.89, −46.29 kcal/mol at 298.15 K, respectively. Refer to Section 4 for DFT-optimized molecular geometries.

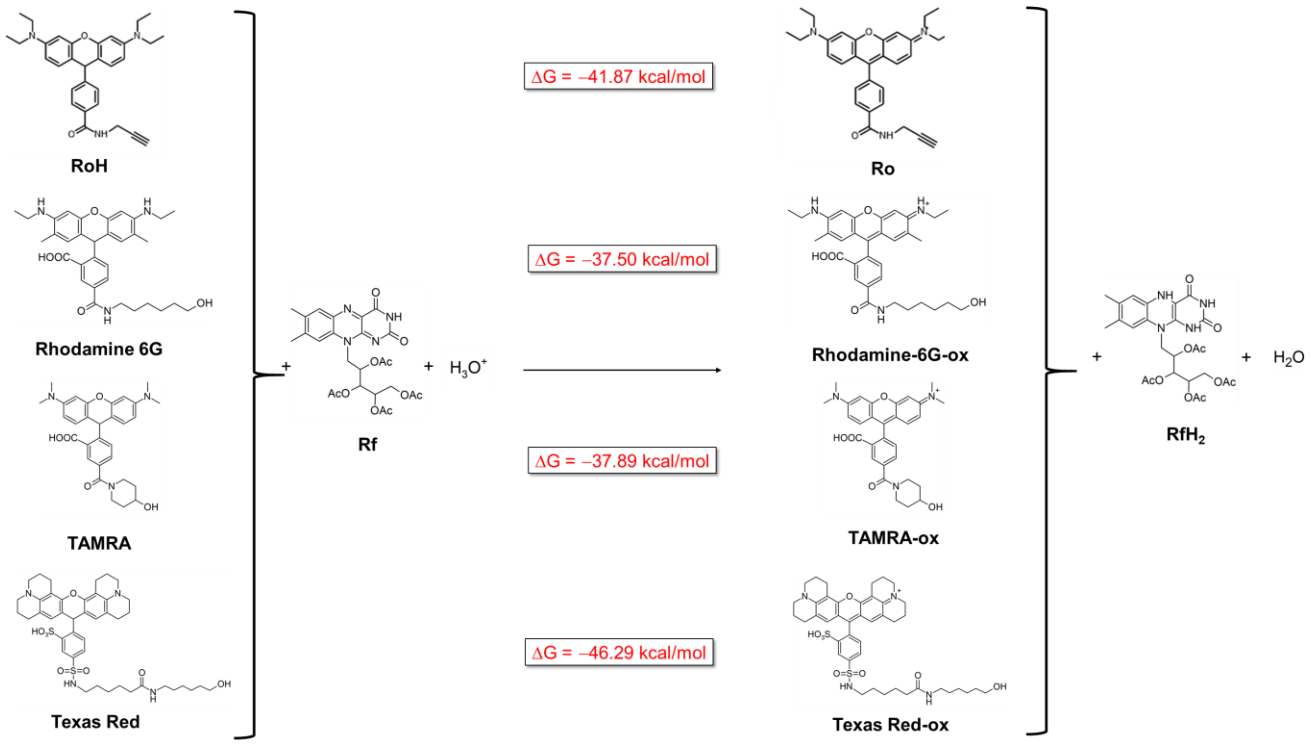
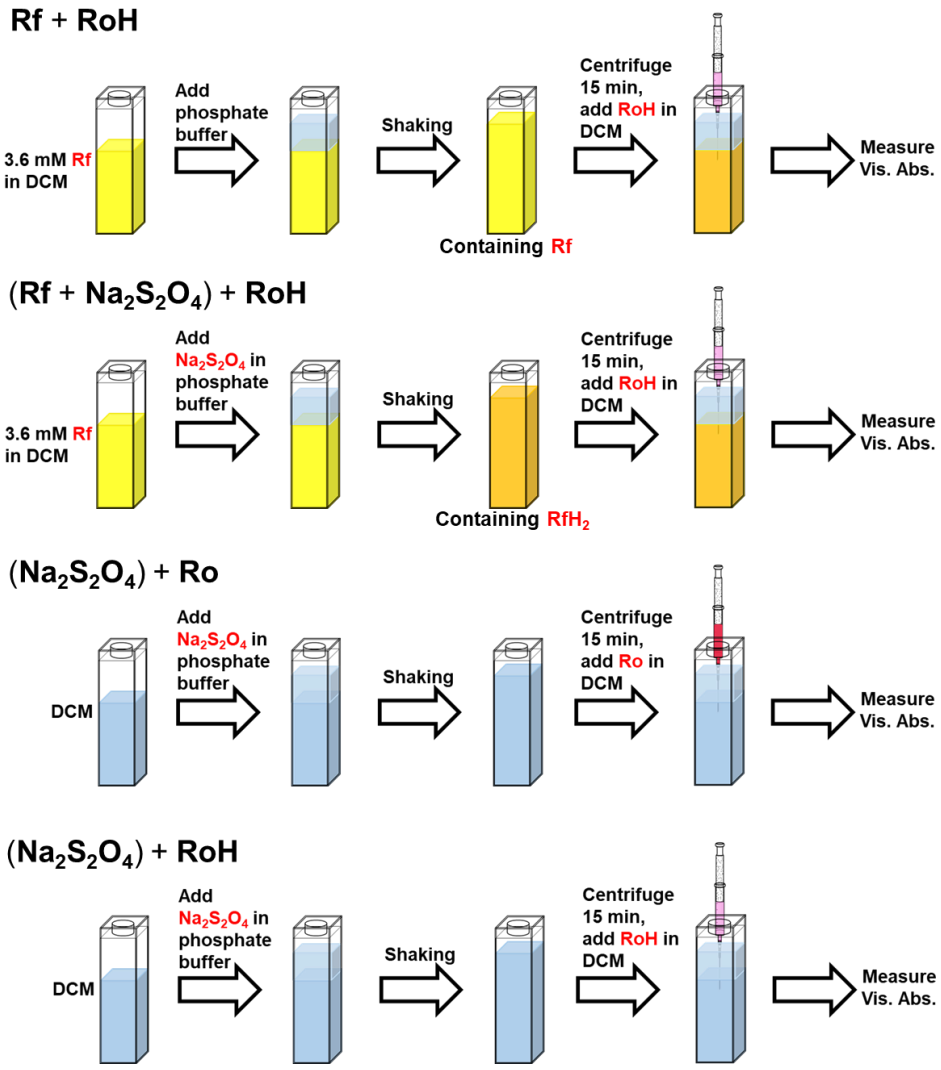


**Figure S2:** Visible light absorbance recovery by riboflavin (**Rf**) or dihydroriboflavin (**$RfH_2$**). The experimental procedures are shown in the scheme below. **$RfH_2$** was obtained from **Rf** by vigorously shaking the aqueous-phase-containing 432-mM sodium dithionite ($Na_2S_2O_4$), saturated KCl, and 0.5-M sodium phosphate buffer (pH 8). The resulting emulsion was separated by centrifugation into DCM and the aqueous phase to remove all remaining $Na_2S_2O_4$ from the DCM layer. Next, **RoH** or **Ro** was directly injected using a syringe into the DCM layer. The mixture was gently mixed and placed in a UV–Vis spectrophotometer to monitor changes in the absorbance of **RoH** or **Ro**. The results showed that (a) **Rf** oxidizes **RoH** to generate **Ro** and (b) **$RfH_2$** could not oxidize **RoH**. (c, d) Remaining $Na_2S_2O_4$ in the DCM layer after centrifugation has a negligible effect on the stability of **RoH** or **Ro**. These results indicate that the oxidized form of riboflavin (**Rf**) is a crucial component for the recovery of rosamine. 3.6-mM **Rf** in 1.2 mL of DCM, 0.2 mL of 432-mM $Na_2S_2O_4$ in buffer, 60 µL of 10-mM **RoH** in DCM, and 120 µL of 2-mM **Ro** in DCM were used for the preparation of each mixture.

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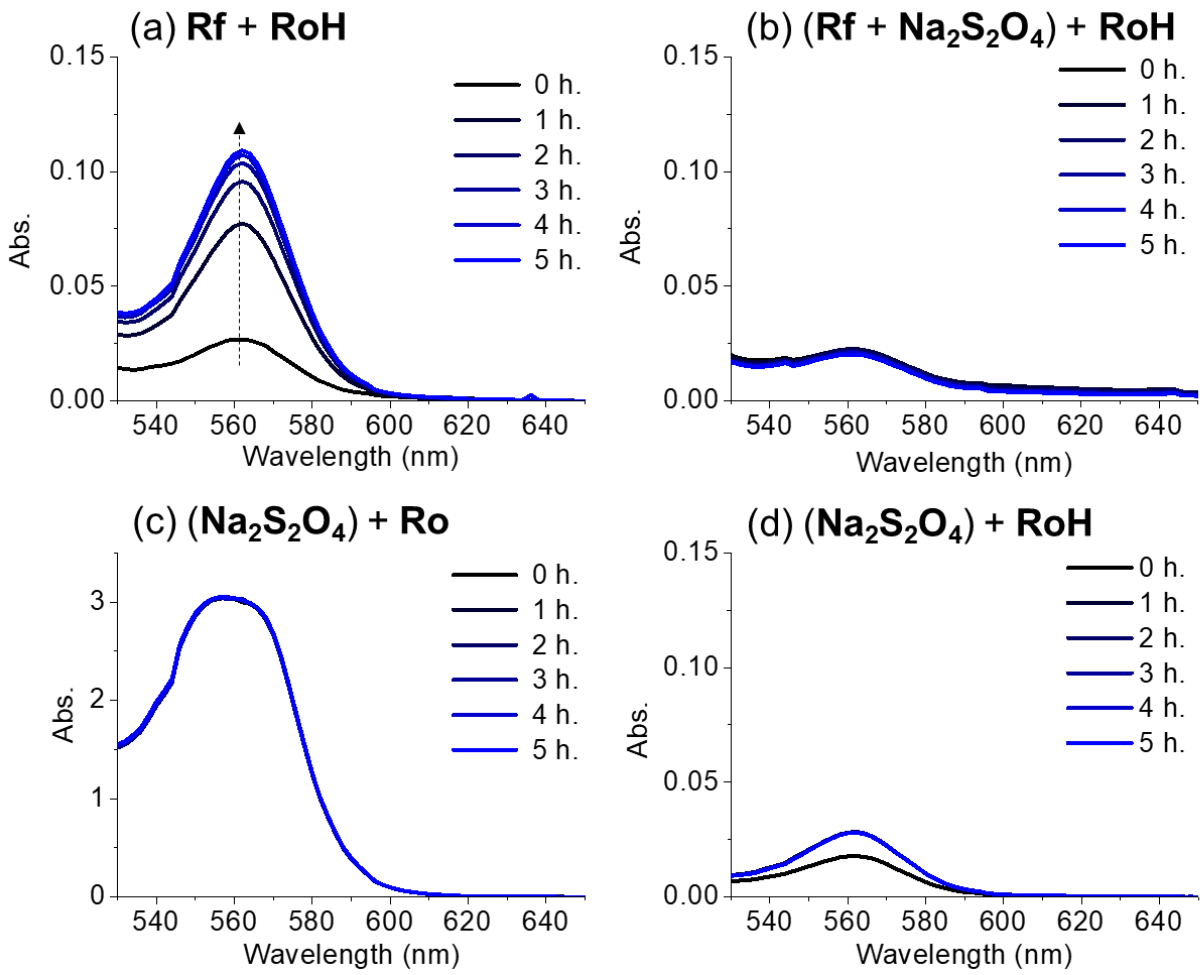

(a) Rf + RoH
(b) (Rf + Na2S2O4) + RoH
(c) (Na2S2O4) + Ro
(d) (Na2S2O4) + RoH
Abs.
Wavelength (nm)
0 h.
1 h.
2 h.
3 h.
4 h.
5 h.

**Figure S3:** Riboflavin-catalyzed templated oxidation under different conditions. The graphs show that the rate of the template reaction did not further improve by changing the (a) concentration of $MgCl_2$ or (b) temperature (25 ℃ and 37 ℃). Conditions: 200-nM **S1** and **S2** in pH 7.4 1 × PBS, no light, total volume: 200 μL.

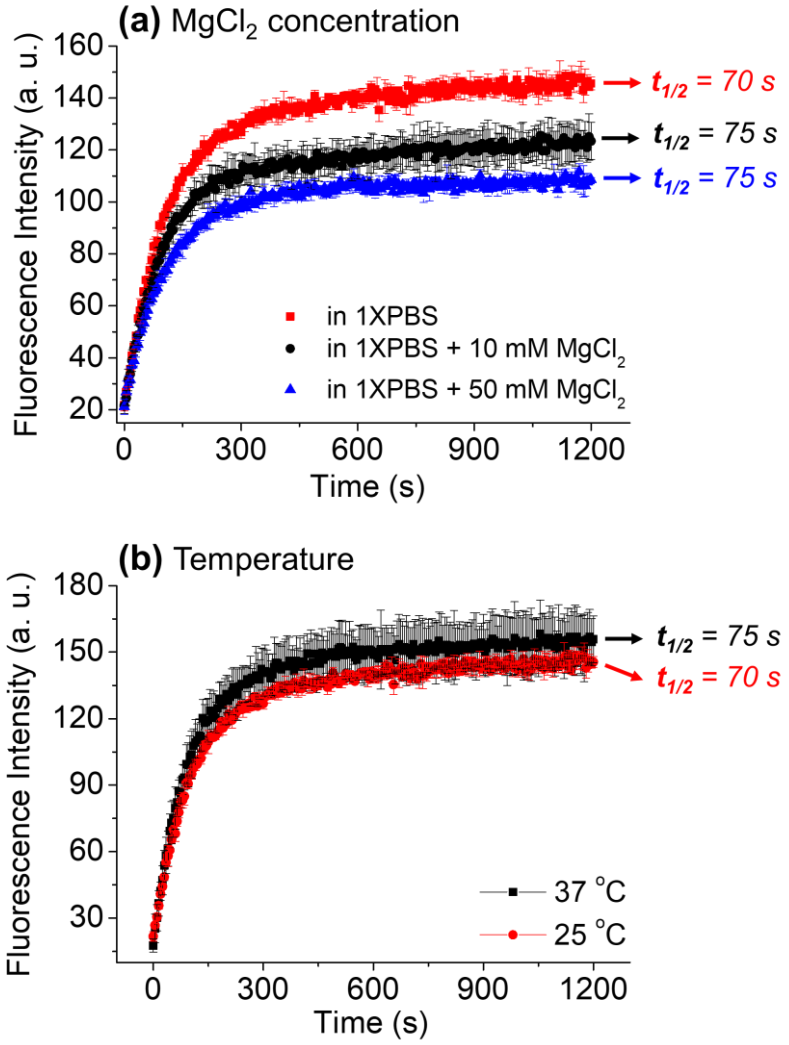


**Figure S4:** Detection of miR-141 DNA by using riboflavin-catalyzed templated oxidation at 25 ℃. Compared with the reaction at 37 ℃, the templated reaction at 25 ℃ showed very low fluorescence enhancement even in the presence of 200-nM miR-141 DNA. This result was explained through stable hybridization between the 11-mer probe (**S8**) and target miR-141 DNA at 25 ℃. As the fluorescence of **S8ox** is quenched by riboflavin upon hybridization (2.3-fold decrease), at 25 ℃, a high concentration of miR-141 DNA yields a low signal gain. By increasing the reaction temperature to 37 ℃, where the dissociation fraction of the DNA duplex increases, the turnover rate and fluorescence intensity improved (Fig. 5). Conditions: 200-nM **S8**, 20-nM **S7**, 1–200-nM miR-141 DNA, pH 7.4 1 × PBS buffer, no light, 25 ℃, 0.05% Tween-20, total volume: 200 μL.

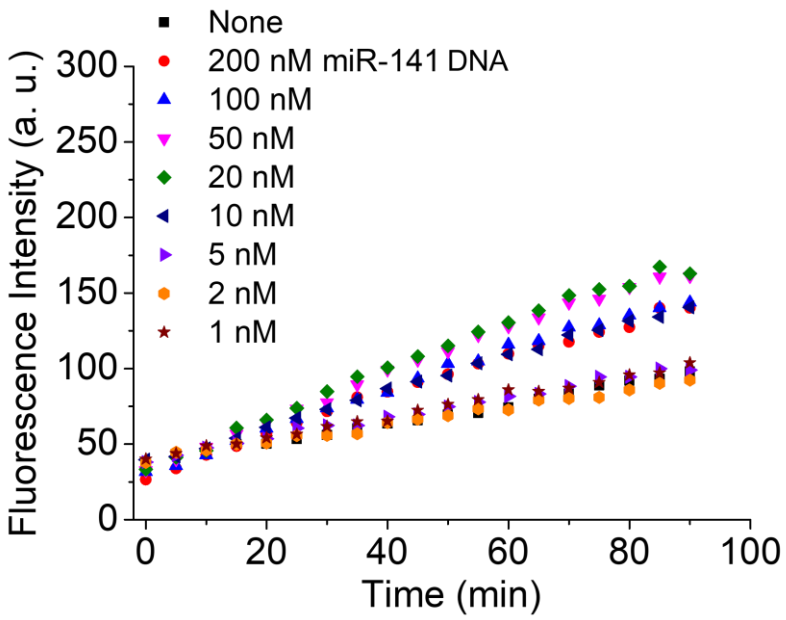


**Figure S5:** Sequence selectivity of the designed nucleic acid sensor. This sensor showed strong target (miR-141 DNA) selectivity over other control sequences. Signal enhancement was not observed even in a single-mismatched sequence (**M1**). The result indicates that the highly sequence-selective sensor was designed by riboflavin-catalyzed templated oxidation. Conditions: 200-nM **S8**, 20-nM **S7**, 20-nM targets, pH 7.4 1 × PBS, no light, 37 ℃, total volume: 200 μL.

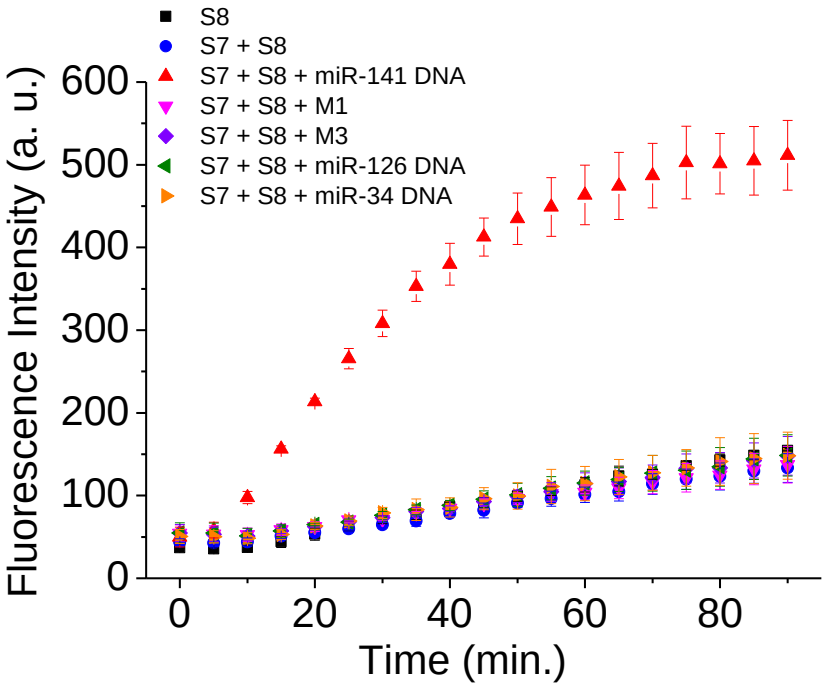


**Figure S6:** Detection of miR-141 by using the rosamine-signaling templated reaction. 200-fmol target miR-141 could be detected by the system. Conditions: 200-nM **S8**, 200-nM **S7**, 1–50-nM miR-141, pH 7.4 1 × PBS buffer, 37 °C, total volume: 200 μL.

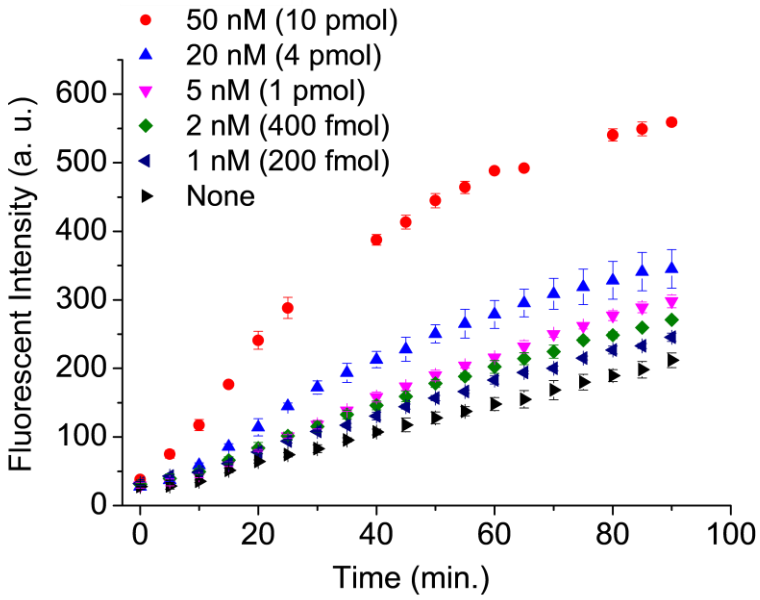
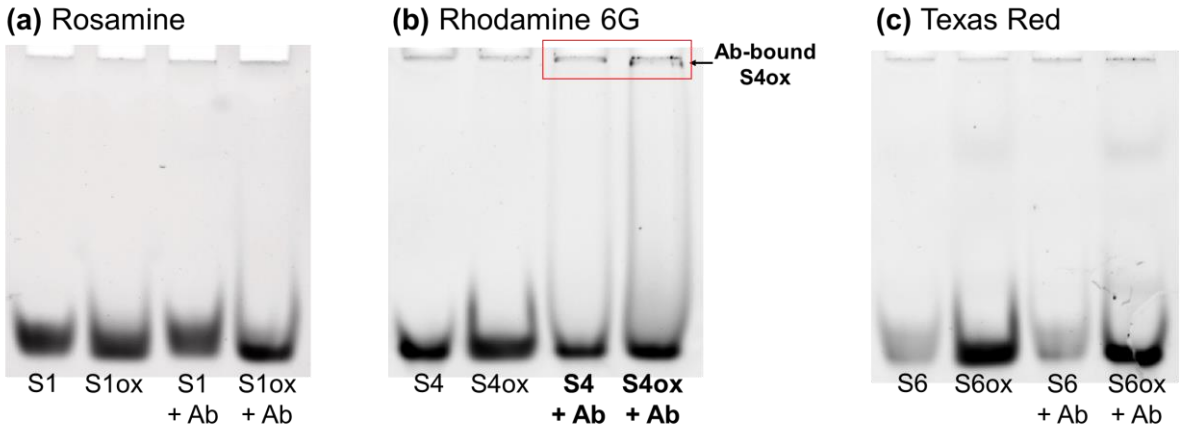


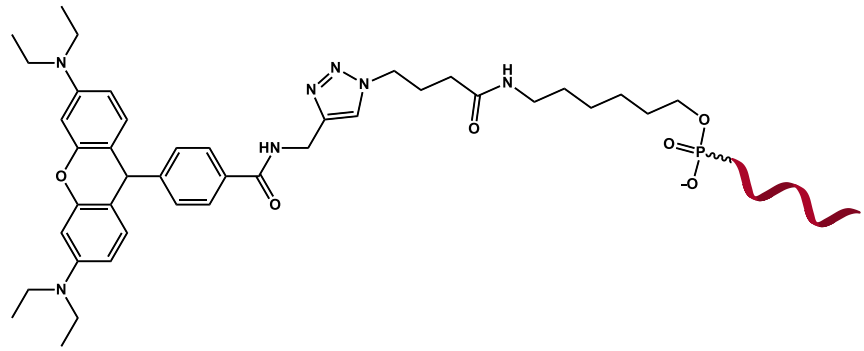
**Figure S7:** Gel shift assay for evaluating the binding affinity of antirhodamine antibody (**Ab**) to the reduced or original form of (a) rosamine, (b) rhodamine 6G, and (c) Texas Red. **Ab** showed moderate binding affinity to **S4** and **S4ox**, containing reduced and original rhodamine 6G, respectively. In the case of rosamine and Texas Red, antibody binding was not observed. Conditions: 10% nondenaturing polyacrylamide gel, 1-μM samples in pH 7.4 1 × PBS buffer. The oligodeoxynucleotides were visualized using the fluorescence intensity of rhodamine derivatives. Visualization of **S1**, **S4**, and **S6** was achieved by UV irradiation, which changes each rhodamine derivative into a fluorescent form, after running gels.

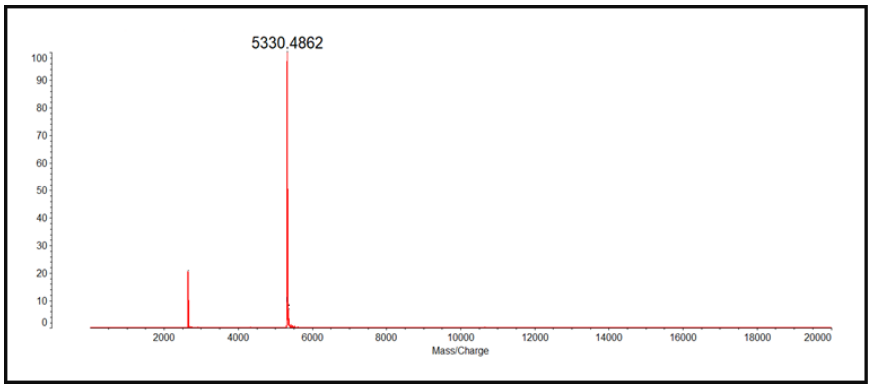


# 3. Mass and NMR charts

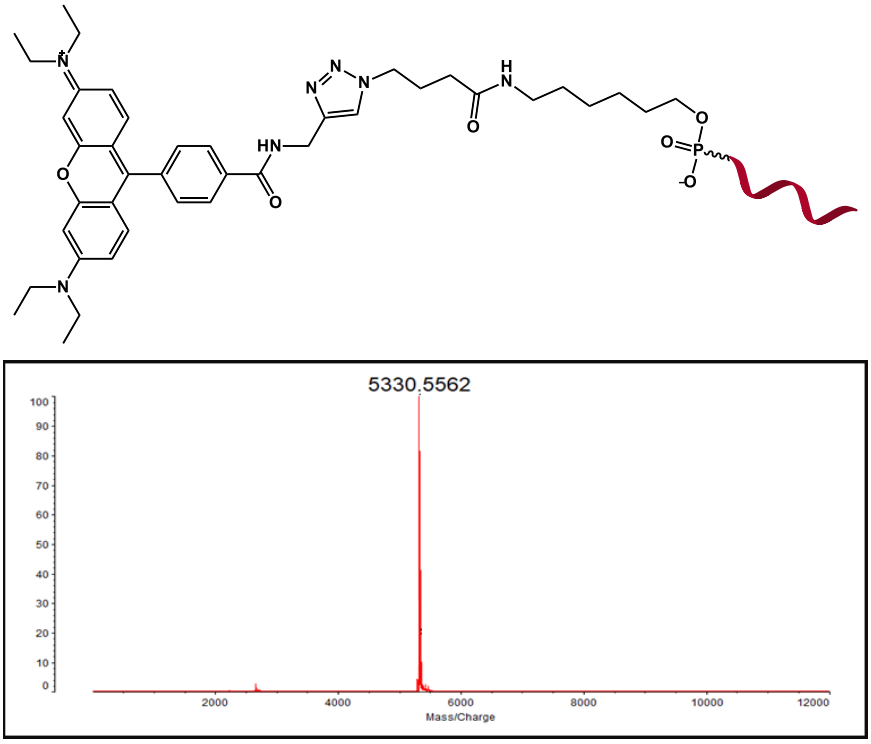
## 3.1 MALDI-TOF spectra of the synthesized DNAs

**S1** (**RoH**-TAG CAT CAT ACA GGC); Exact Mass for $C_{187}H_{238}N_{65}O_{92}P_{15}$: 5330.2109, MALDI-TOF m/z found: 5330.4862

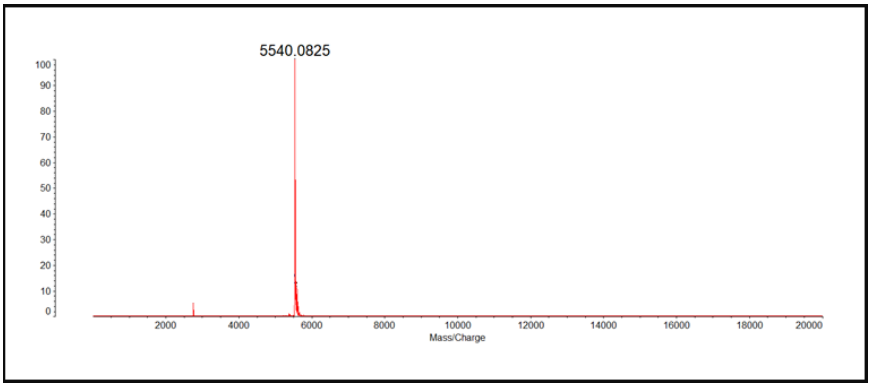




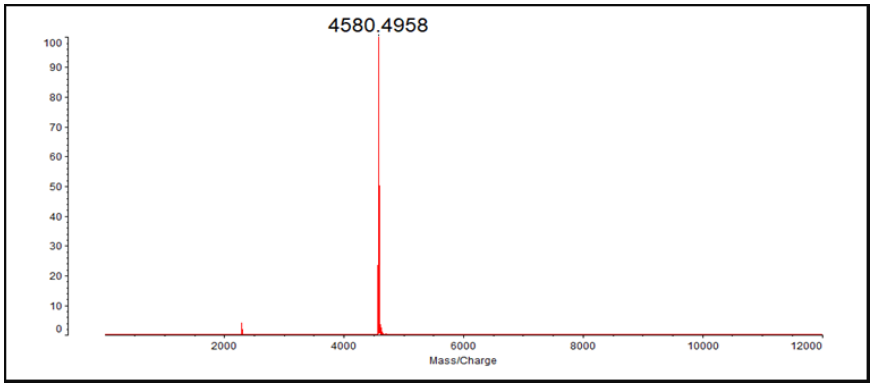
**S1ox** (**Ro**-TAG CAT CAT ACA GGC); Exact Mass for $C_{187}H_{237}N_{65}O_{92}P_{15}$: 5329.1929, MALDI-TOF m/z found: 5330.5562



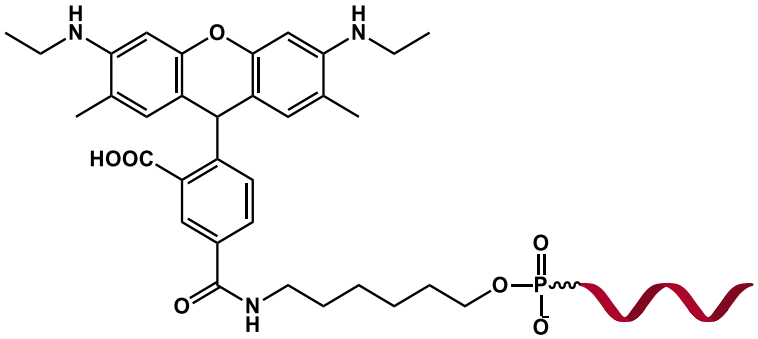

**S2** (GCC TGT ATG ATG CTA-**Rp**); Exact Mass for $C_{188}H_{240}N_{63}O_{106}P_{15}$: 5540.1393, MALDI-TOF m/z found: 5540.0825

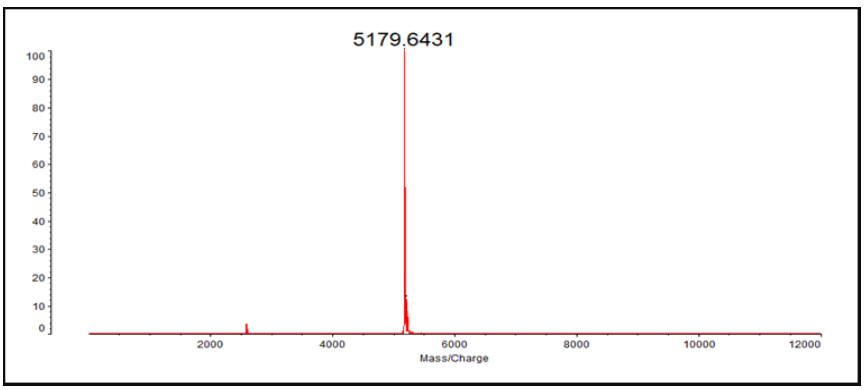


**S3** (GCC TGT ATG ATG CTA); Exact Mass for $C_{147}H_{186}N_{54}O_{90}P_{14}$: 4580.7964, MALDI-TOF m/z found: 4580.4958

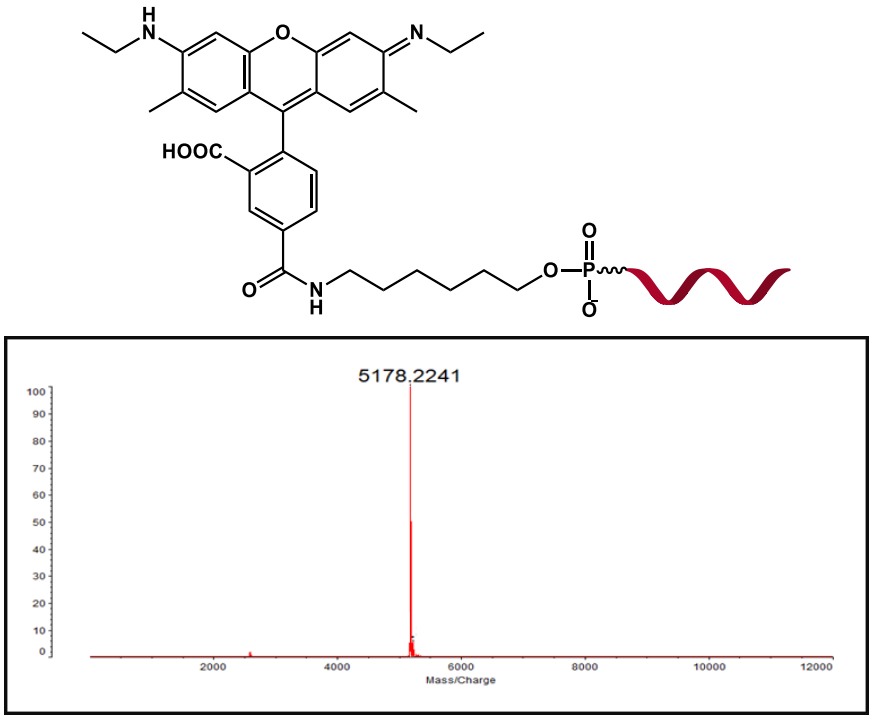



**S4** (**Rhodamine G6**-TAG CAT CAT ACA GGC); Exact Mass for $C_{179}H_{224}N_{61}O_{93}P_{15}$: 5180.0738, MALDI-TOF m/z found: 5179.6431



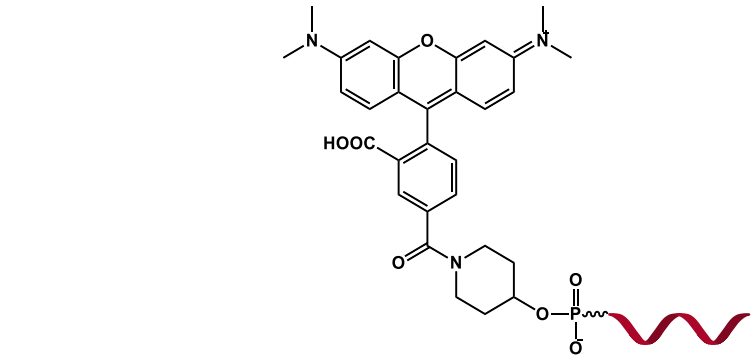



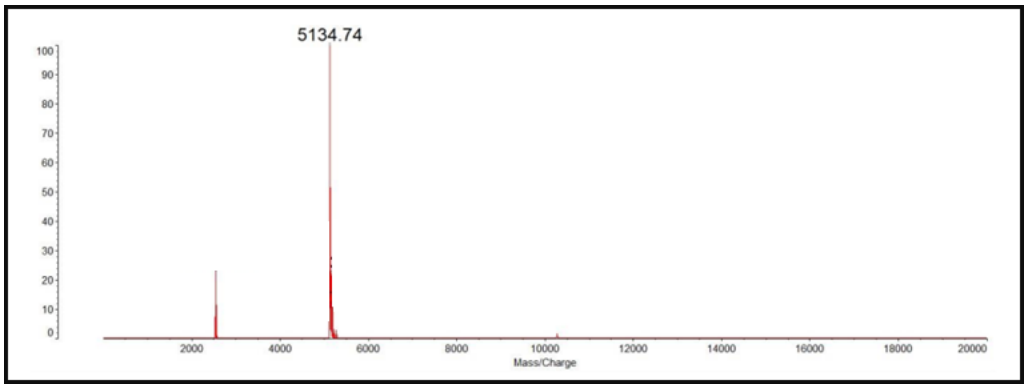
**S4ox** (**Rhodamine G6 (ox)**-TAG CAT CAT ACA GGC); Exact Mass for $C_{179}H_{222}N_{61}O_{93}P_{15}$: 5178.0582, MALDI-TOF m/z found: 5178.2241



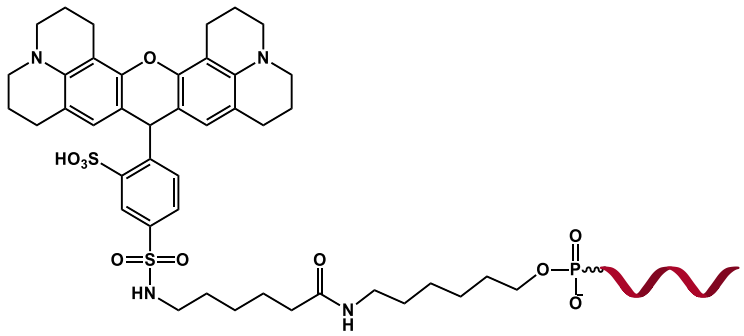

**S5** (**TAMRA**-TAG CAT CAT ACA GGC); Exact Mass for $C_{176}H_{216}N_{61}O_{93}P_{15}$: 5136.0112, MALDI-TOF m/z found: 5135.8191

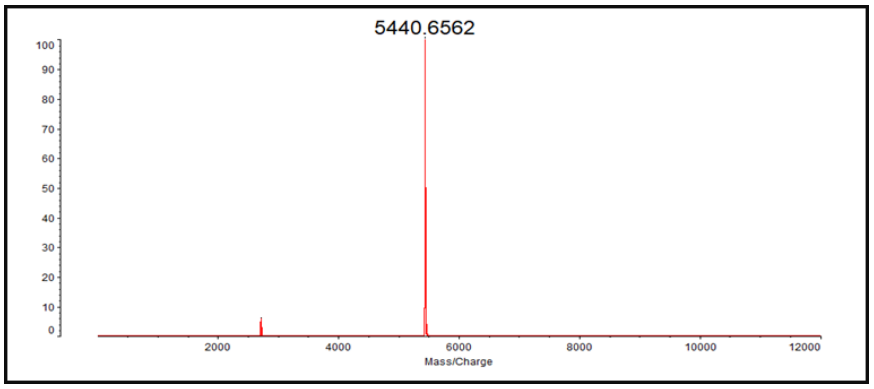
**S5ox** (**TAMRA (ox)**-TAG CAT CAT ACA GGC); Exact Mass for $C_{176}H_{215}N_{61}O_{93}P_{15}$: 5135.0034, MALDI-TOF m/z found: 5134.74



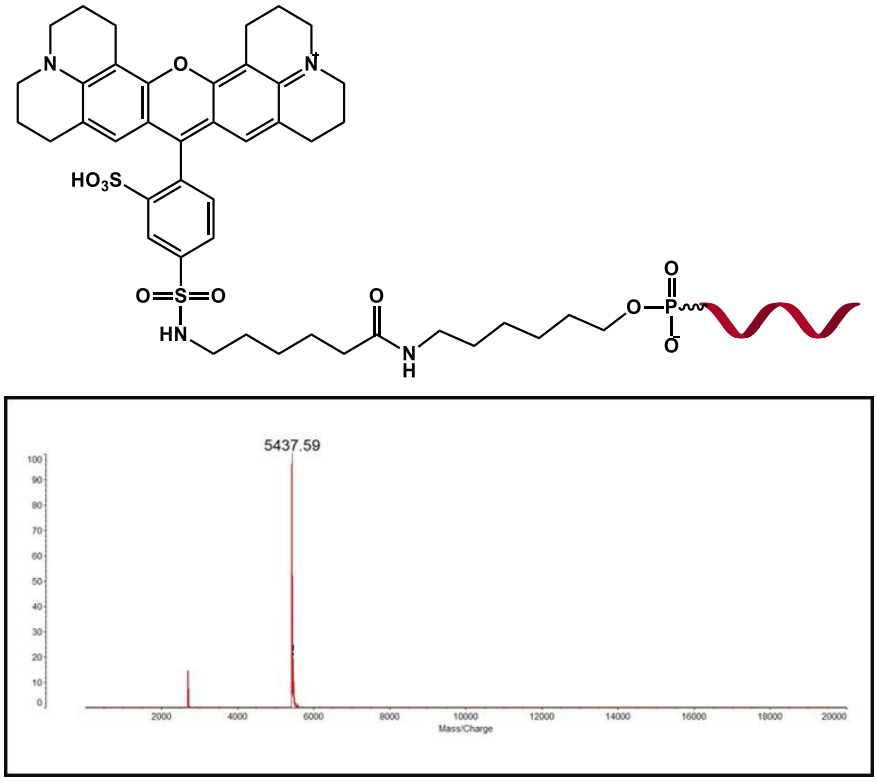




**S6** (**Texas red**-TAG CAT CAT ACA GGC); Exact Mass for $C_{189}H_{239}N_{62}O_{96}P_{15}S_2$: 5441.1231, MALDI-TOF m/z found: 5440.6562

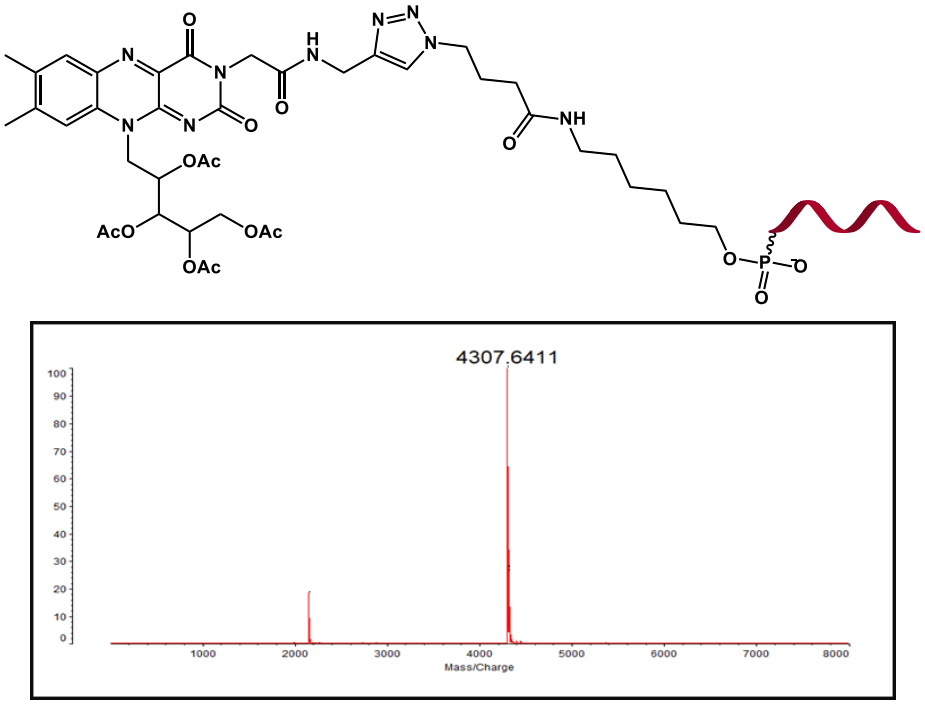


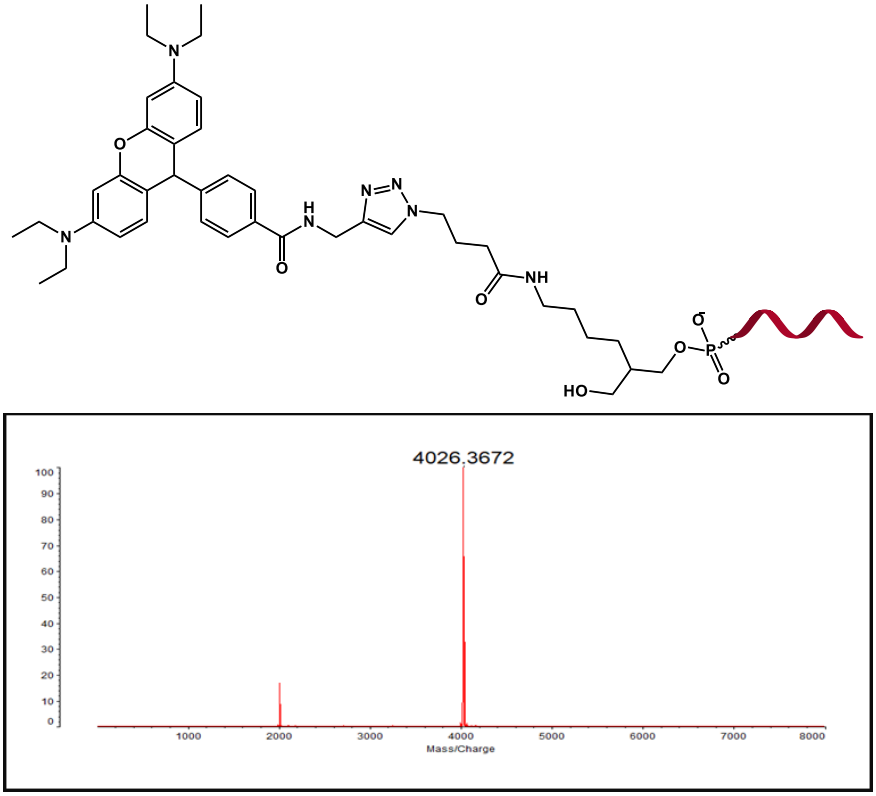
**S6ox** (**Texas red (ox)**-TAG CAT CAT ACA GGC); Exact Mass for $C_{189}H_{238}N_{62}O_{96}P_{15}S_2$: 5440.1153, MALDI-TOF m/z found: 5437.59



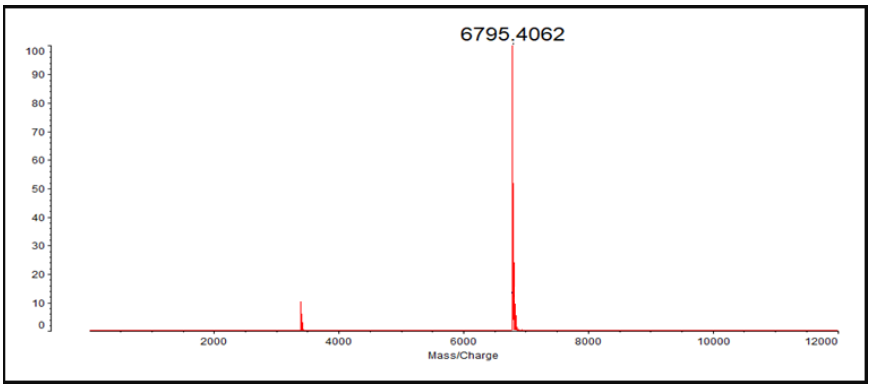

**S7** (**Rp**-AGA CAG TGT TA); Exact Mass for $C_{149}H_{188}N_{53}O_{78}P_{11}$: 4307.9487, MALDI-TOF m/z found: 4307.6411



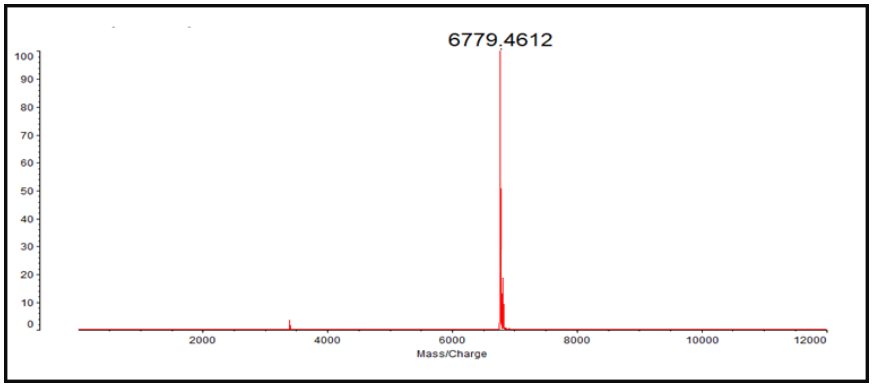
**S8** (CCA TCT TTA CC-**RoH**); Exact Mass for $C_{147}H_{193}N_{40}O_{73}P_{11}$: 4026.9733, MALDI-TOF m/z found: 4026.3672



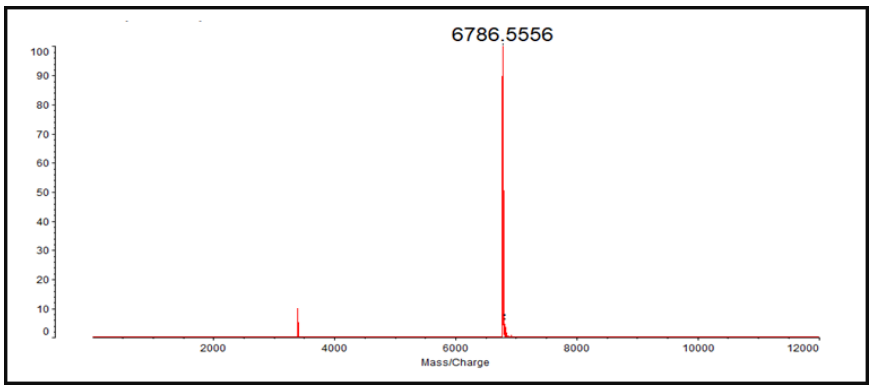

**miR-141 DNA** (TAA CAC TGT CTG GTA AAG ATG G); Exact Mass for $C_{217}H_{271}N_{86}O_{129}P_{21}$: 6795.1779, MALDI-TOF m/z found: 6795.4062



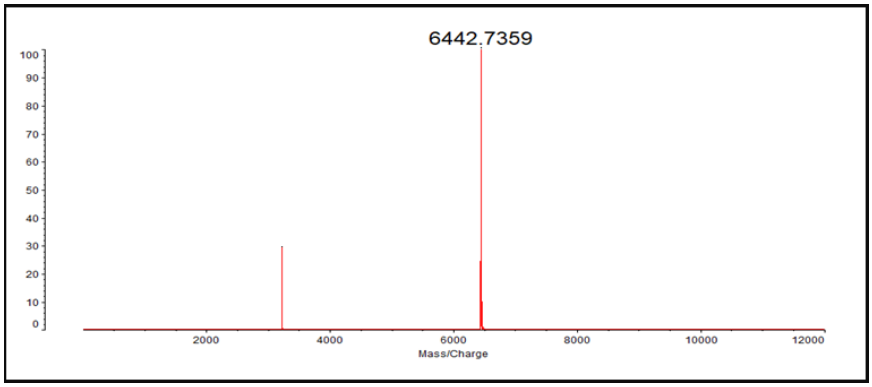
**M1** (TAA CAC TAT CTG GTA AAG ATG G); Exact Mass for $C_{217}H_{271}N_{86}O_{128}P_{21}$: 6779.1830, MALDI-TOF m/z found: 6779.4612



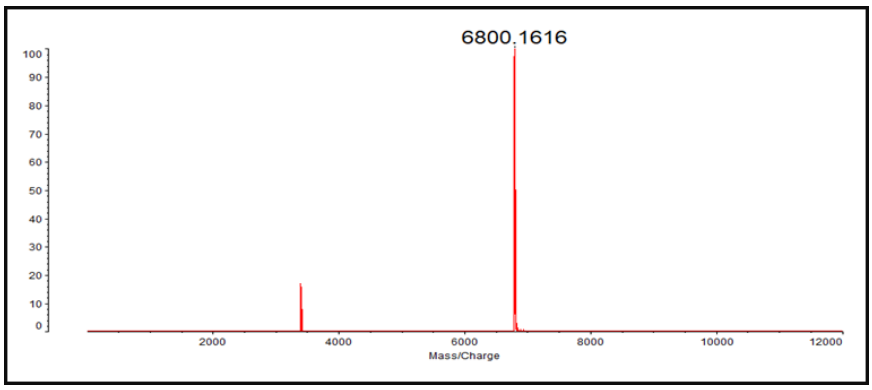

**M3** (TTA CAC TAT CTG GTG AAG ATG G); Exact Mass for $C_{217}H_{272}N_{83}O_{131}P_{21}$: 6786.1644, MALDI-TOF m/z found: 6786.5556



**miR-126 DNA** (TCG TAC CGT GAG TAA TAA TGC); Exact Mass for $C_{206}H_{259}N_{79}O_{124}P_{20}$: 6442.1142, MALDI-TOF m/z found: 6442.7359

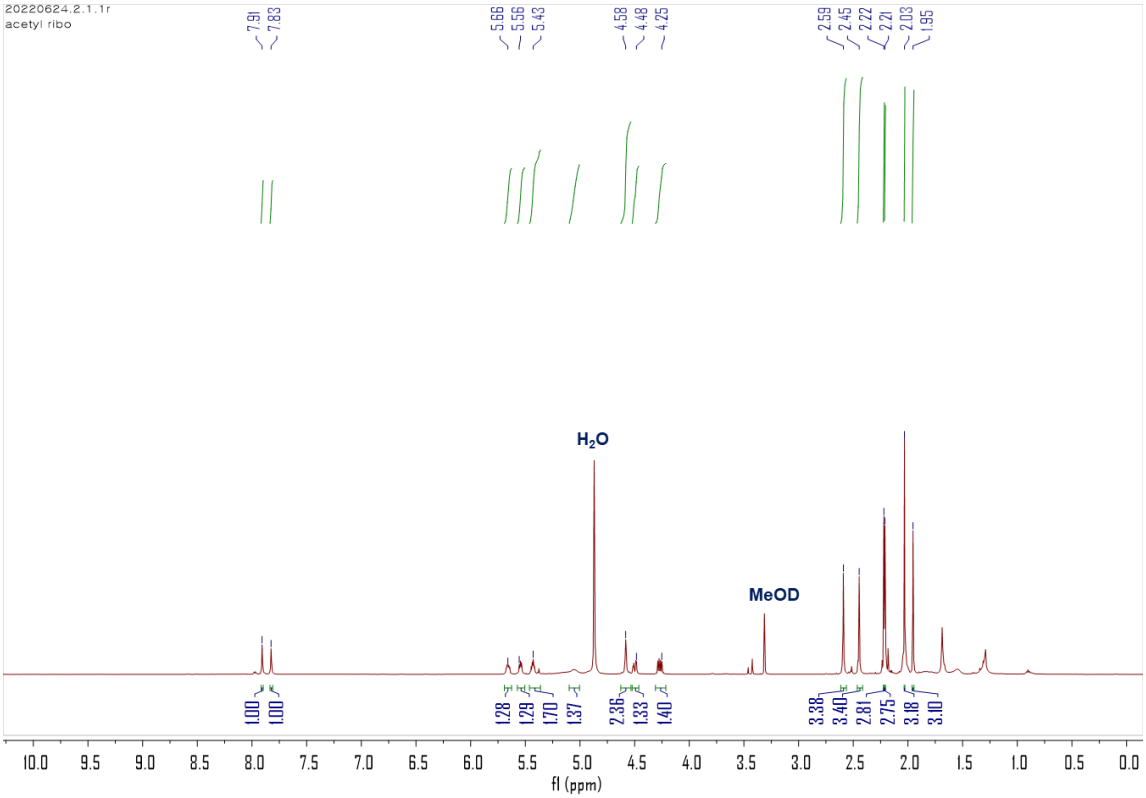


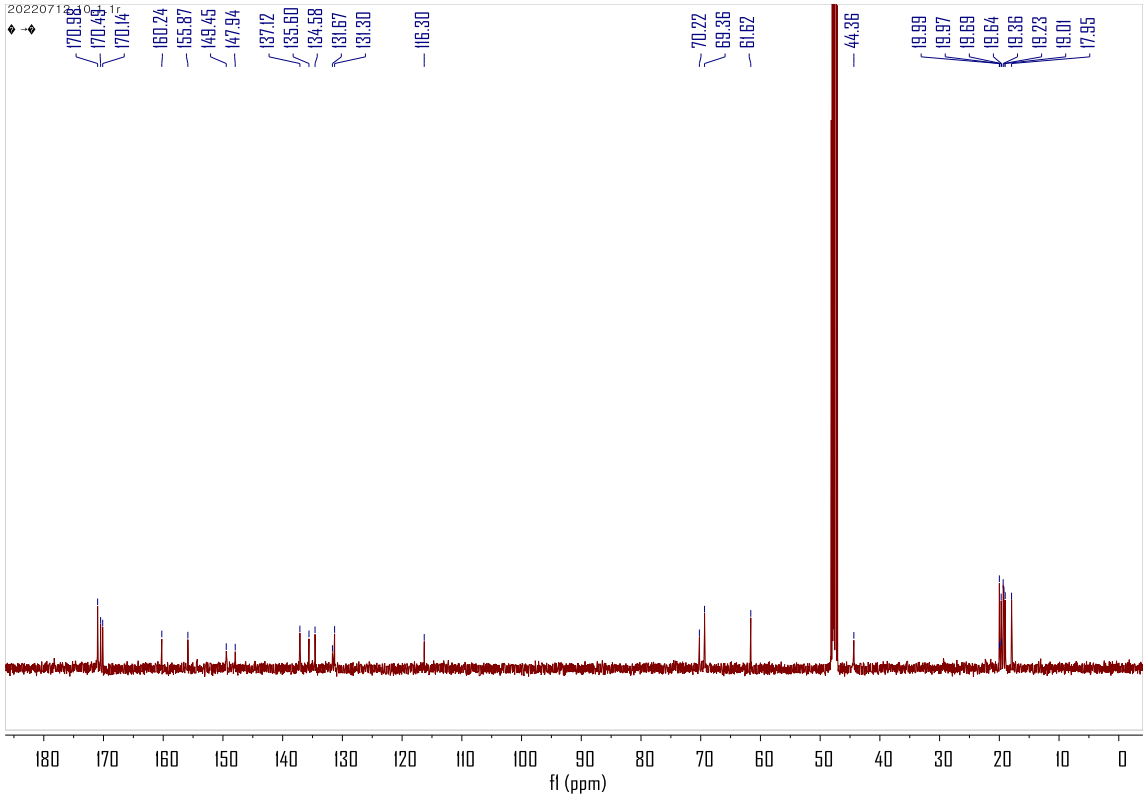

**miR-34 DNA** (TGG CAG TGT CTT AGC TGG TTG T); Exact Mass for $C_{217}H_{274}N_{77}O_{137}P_{21}$: 6800.1331, MALDI-TOF m/z found: 6800.1616



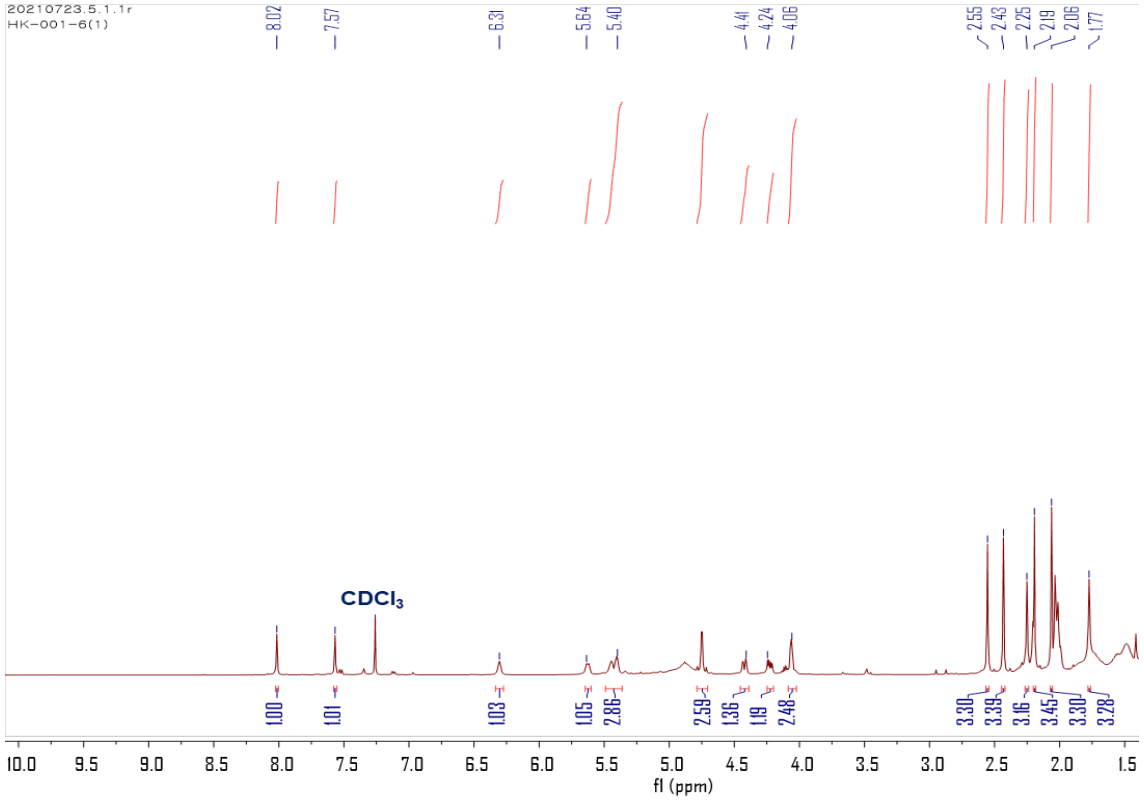
## 3.2 NMR spectra of compounds

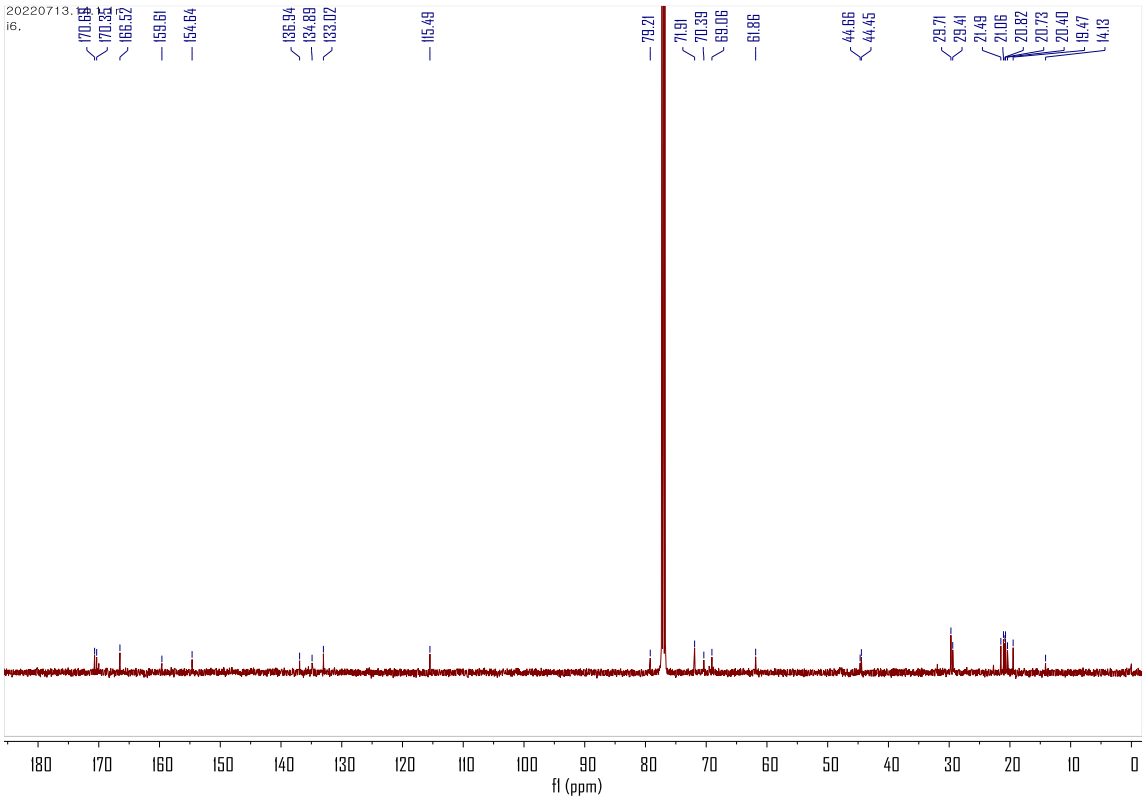
$^{1}H$ and $^{13}C$ NMR of compound **4**



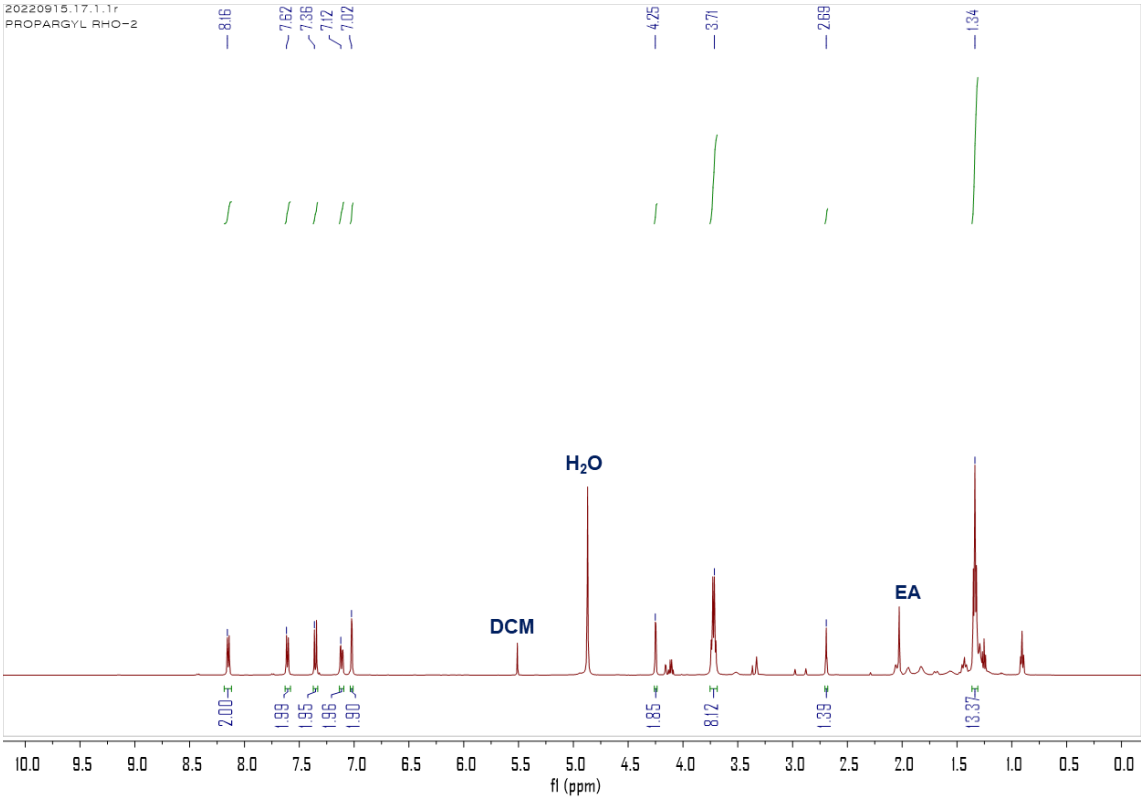



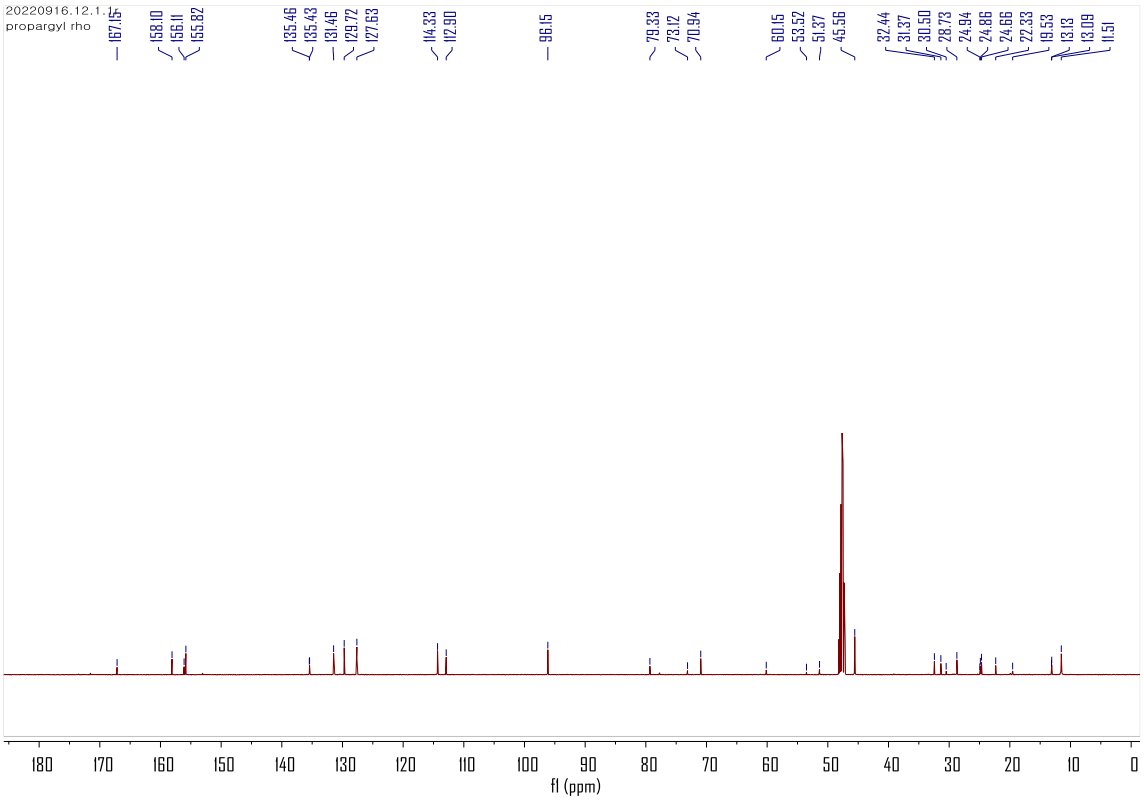
$^{1}H$ and $^{13}C$ NMR of **Rp**



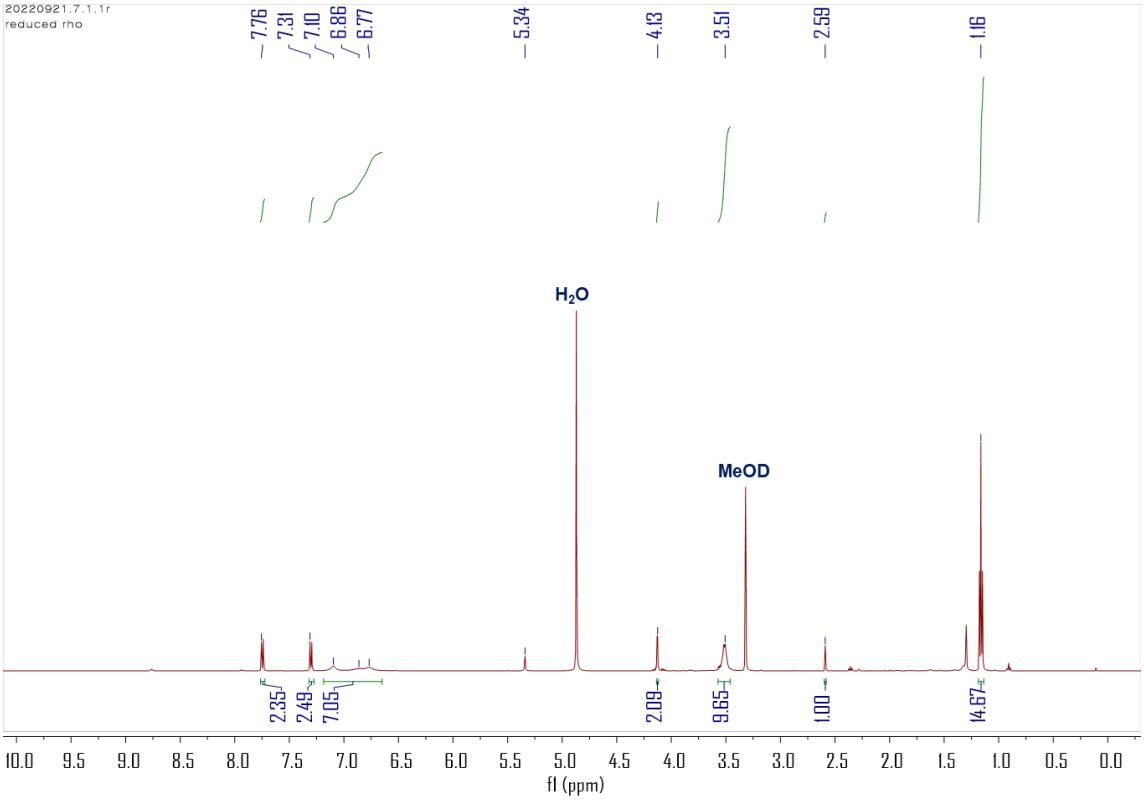



$^{1}H$ and $^{13}C$ NMR of **Ro**



$^{1}$H of **RoH**


20220921.7.1.1r
reduced rho
7.76
7.31
7.10
6.86
6.77
5.34
4.13
3.51
2.59
1.16
H2O
MeOD
2.35
2.49
7.05
2.09
9.65
1.00
14.67
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

## 4. DFT optimized molecular geometries

Optimized geometry (in Å) and absolute energy (in $E_h$) at the B3LYP/6-31G(2df,p) level of theory for **RoH**

| | | | |
|---|---|---|---|
| O | -3.992640 | -3.804112 | -2.633747 |
| O | 1.995120 | 1.313799 | -0.127595 |
| N | -5.617351 | -3.213806 | -1.194475 |
| N | -0.398649 | 5.420107 | 0.077761 |
| N | 5.073774 | -2.342769 | -0.166412 |
| C | -6.964210 | -3.539825 | 2.034187 |
| C | -6.658044 | -2.993425 | 1.007497 |
| C | -6.294464 | -2.333218 | -0.249772 |
| C | -4.323112 | -3.180385 | -1.628948 |
| C | -3.252167 | -2.368304 | 0.538985 |
| C | -2.224803 | -1.684975 | 1.188842 |
| C | -3.312136 | -2.388023 | -0.856140 |
| C | -2.316218 | -1.720302 | -1.583201 |
| C | -1.310242 | -1.025507 | -0.930790 |
| C | -1.246810 | -0.998061 | 0.469137 |
| C | -1.851896 | 7.370022 | -0.544119 |
| C | 1.273994 | 6.348084 | -1.571997 |
| C | -1.586025 | 6.204137 | 0.412261 |
| C | 0.851175 | 6.161363 | -0.109727 |
| C | -1.300482 | 2.022430 | 1.292406 |
| C | -1.387415 | 3.383372 | 1.044680 |
| C | -0.344208 | 4.064681 | 0.372516 |
| C | 0.776211 | 3.294758 | 0.006608 |
| C | 0.837819 | 1.931876 | 0.281399 |
| C | -0.197171 | 1.250254 | 0.919906 |

| | | | |
|---|---|---|---|
| C | -0.131299 | -0.241505 | 1.193131 |
| C | 1.240165 | -0.785062 | 0.836275 |
| C | 2.205648 | -0.002277 | 0.208014 |
| C | 1.605165 | -2.107298 | 1.111954 |
| C | 2.846784 | -2.625000 | 0.787645 |
| C | 3.460440 | -0.497523 | -0.143886 |
| C | 3.822452 | -1.824734 | 0.143469 |
| C | 5.754918 | -4.595512 | 0.754775 |
| C | 5.200468 | -3.782145 | -0.420506 |
| C | 6.094893 | -1.476520 | -0.752106 |
| C | 7.530375 | -1.911302 | -0.448072 |
| H | -7.233863 | -4.021382 | 2.943498 |
| H | -3.987438 | -2.905444 | 1.127910 |
| H | -2.185118 | -1.690993 | 2.273823 |
| H | -2.345653 | -1.757410 | -2.666196 |
| H | -0.555858 | -0.503462 | -1.510906 |
| H | -2.256733 | 3.914672 | 1.404634 |
| H | -2.123618 | 1.535984 | 1.808608 |
| H | 1.617333 | 3.728662 | -0.515934 |
| H | 0.883897 | -2.752692 | 1.605562 |
| H | 3.070284 | -3.648332 | 1.056980 |
| H | 4.122335 | 0.179598 | -0.664829 |
| H | -6.210890 | -3.805783 | -1.759279 |
| H | -5.655070 | -1.471109 | -0.046846 |
| H | -7.290000 | -1.941439 | -0.726910 |
| H | -0.299850 | -0.403814 | 2.266603 |
| H | -1.965706 | 7.014345 | -1.572547 |
| H | -2.781093 | 7.866581 | -0.248375 |
| H | -1.058553 | 8.121676 | -0.524705 |

| | | | |
|---|---|---|---|
| H | -2.451960 | 5.543276 | 0.366306 |
| H | -1.527996 | 6.584503 | 1.445842 |
| H | 0.525915 | 6.916060 | -2.130893 |
| H | 2.223157 | 6.892379 | -1.620169 |
| H | 1.408032 | 5.386007 | -2.074770 |
| H | 0.737149 | 7.141902 | 0.364135 |
| H | 1.648550 | 5.653713 | 0.443435 |
| H | 5.122610 | -4.494973 | 1.641724 |
| H | 5.799257 | -5.656606 | 0.486877 |
| H | 6.762905 | -4.269873 | 1.024175 |
| H | 4.221042 | -4.175376 | -0.710667 |
| H | 5.846647 | -3.917318 | -1.294680 |
| H | 5.962136 | -0.475824 | -0.337495 |
| H | 5.961322 | -1.390082 | -1.843844 |
| H | 7.706667 | -1.953281 | 0.630858 |
| H | 7.776200 | -2.886435 | -0.876173 |
| H | 8.220884 | -1.180392 | -0.879680 |

Energy (including solvation free energy) = -1517.13896763

Absolute Gibbs Free Energy = -1516.613570

Optimized geometry (in Å) and absolute energy (in $E_h$) at the B3LYP/6-31G(2df,p) level of theory for

**Rhodamin 6G**

| | | | |
|---|---|---|---|
| O | 10.748376 | 1.149744 | 0.900032 |
| O | 1.947049 | -3.790574 | -2.891133 |
| O | 1.232016 | -2.269577 | 2.981958 |

| | | | |
|---|---|---|---|
| O | -0.931340 | -1.711334 | 3.068076 |
| O | -3.443661 | 1.579949 | -0.997054 |
| N | 3.568384 | -3.404623 | -1.368682 |
| N | -0.810670 | 5.555337 | -0.844891 |
| N | -6.915623 | -1.688160 | -0.788929 |
| C | 9.961416 | 0.443029 | -0.053631 |
| C | 8.624982 | -0.035965 | 0.511449 |
| C | 7.806031 | -0.843177 | -0.501571 |
| C | 6.461643 | -1.323950 | 0.055191 |
| C | 5.647500 | -2.130604 | -0.961673 |
| C | 4.310633 | -2.614051 | -0.390241 |
| C | 2.313710 | -3.230439 | -1.859366 |
| C | 0.079569 | -1.862757 | 2.413499 |
| C | 0.490935 | -1.532854 | -1.833699 |
| C | 1.353599 | -2.357381 | -1.102716 |
| C | 1.205213 | -2.412110 | 0.278008 |
| C | -0.453296 | -0.757570 | -1.183249 |
| C | 0.215624 | -1.666173 | 0.940461 |
| C | -0.622493 | -0.802324 | 0.206982 |
| C | -0.674643 | 7.999322 | -0.593627 |
| C | -0.935094 | 6.670769 | 0.108782 |
| C | -9.296663 | -2.245633 | -0.494718 |
| C | -8.034484 | -1.704702 | 0.168720 |
| C | -5.366845 | -3.340449 | 1.099897 |
| C | 1.115948 | 4.310391 | 1.015351 |
| C | -0.388169 | 2.302030 | 0.897591 |
| C | -0.130181 | 3.617938 | 0.521569 |
| C | -1.030889 | 4.241467 | -0.374207 |

| | | | |
|---|---|---|---|
| C | -2.125688 | 3.515314 | -0.849904 |
| C | -3.632755 | -1.529691 | 0.944508 |
| C | -4.888607 | -2.007886 | 0.578157 |
| C | -5.654885 | -1.240863 | -0.330182 |
| C | -5.124635 | -0.047379 | -0.826313 |
| C | -3.858596 | 0.388804 | -0.447625 |
| C | -3.084452 | -0.342620 | 0.449895 |
| C | -1.685910 | 0.112387 | 0.835424 |
| C | -1.473209 | 1.558778 | 0.425060 |
| C | -2.336352 | 2.195215 | -0.463069 |
| H | 1.045337 | -2.424747 | 3.920920 |
| H | 10.256872 | 1.939792 | 1.149964 |
| H | 10.569041 | -0.416384 | -0.359638 |
| H | 9.790237 | 1.054807 | -0.953280 |
| H | 8.044047 | 0.837089 | 0.841130 |
| H | 8.818265 | -0.640446 | 1.406715 |
| H | 8.391378 | -1.711406 | -0.833809 |
| H | 7.629265 | -0.233240 | -1.397970 |
| H | 5.874298 | -0.457047 | 0.386702 |
| H | 6.636755 | -1.935931 | 0.950289 |
| H | 6.233061 | -2.998983 | -1.292703 |
| H | 5.453795 | -1.523802 | -1.854917 |
| H | 3.700562 | -1.757017 | -0.100285 |
| H | 4.488769 | -3.208261 | 0.515766 |
| H | 4.118343 | -4.043680 | -1.928912 |
| H | 1.834983 | -3.068209 | 0.863844 |
| H | -1.085813 | -0.097272 | -1.766408 |
| H | 0.575838 | -1.504003 | -2.913914 |

| | | | |
|---|---|---|---|
| H | -1.588864 | 0.039107 | 1.917725 |
| H | -1.396220 | 8.166143 | -1.401402 |
| H | -0.767145 | 8.829011 | 0.112969 |
| H | 0.330098 | 8.020806 | -1.026437 |
| H | -1.932058 | 6.684337 | 0.578194 |
| H | -0.210071 | 6.531432 | 0.913381 |
| H | -1.420051 | 5.737989 | -1.635222 |
| H | -9.137939 | -3.264283 | -0.861295 |
| H | -10.126843 | -2.257349 | 0.217404 |
| H | -9.594866 | -1.621546 | -1.344855 |
| H | -7.763196 | -2.338182 | 1.015963 |
| H | -8.226248 | -0.698233 | 0.574841 |
| H | -7.193406 | -1.143696 | -1.598964 |
| H | -4.518148 | -3.965444 | 1.390957 |
| H | -6.010006 | -3.240478 | 1.982946 |
| H | -5.945519 | -3.872177 | 0.338279 |
| H | 0.928824 | 4.939280 | 1.894282 |
| H | 1.874249 | 3.576521 | 1.301905 |
| H | 1.535417 | 4.957390 | 0.238762 |
| H | -3.031330 | -2.114425 | 1.634173 |
| H | -5.701997 | 0.566514 | -1.510043 |
| H | -2.838113 | 3.977419 | -1.525724 |
| H | 0.310515 | 1.813864 | 1.572642 |

Energy (including solvation free energy) = -1822.77861503

Absolute Gibbs Free Energy = -1822.168054

Optimized geometry (in Å) and absolute energy (in $E_h$) at the B3LYP/6-31G(2df,p) level of theory for

**TAMRA**

| | | | |
|---|---|---|---|
| O | 0.321366 | -1.364086 | 3.199766 |
| O | 8.447080 | 0.132273 | 0.983701 |
| O | 3.694261 | -1.652601 | -2.616409 |
| O | 2.175618 | -0.175773 | 3.296979 |
| O | -3.699038 | 0.363781 | -0.714189 |
| N | 4.916410 | -0.249260 | -1.308247 |
| N | -3.522737 | 5.117243 | -0.503107 |
| N | -4.927816 | -4.234631 | -0.557815 |
| C | 7.261940 | -0.882551 | -0.911861 |
| C | 7.443369 | 0.338605 | -0.003228 |
| C | 6.100700 | 0.751226 | 0.612663 |
| C | 5.038482 | 0.948459 | -0.475245 |
| C | 6.167852 | -0.642738 | -1.959699 |
| C | 3.751781 | -0.845780 | -1.689391 |
| C | 1.152141 | -0.719178 | 2.603103 |
| C | 0.116464 | -0.087335 | -0.997911 |
| C | 1.323761 | -0.269791 | -1.652146 |
| C | 2.490015 | -0.536807 | -0.927148 |
| C | 2.397835 | -0.643222 | 0.456879 |
| C | 1.168712 | -0.497760 | 1.120765 |
| C | 0.000211 | -0.207770 | 0.392407 |
| C | -3.032686 | 6.332315 | 0.123721 |
| C | -4.737514 | 5.178955 | -1.295457 |
| C | -4.829398 | -5.541128 | 0.069330 |
| C | -6.117716 | -3.922885 | -1.329070 |

| | | | |
|---|---|---|---|
| C | -4.275414 | -1.894794 | -0.600421 |
| C | -4.093052 | -3.214988 | -0.148379 |
| C | -3.018488 | -3.450705 | 0.742943 |
| C | -2.172137 | -2.419452 | 1.115794 |
| C | -1.424624 | 2.553346 | 1.139090 |
| C | -1.927742 | 3.793265 | 0.781454 |
| C | -3.024183 | 3.893723 | -0.109277 |
| C | -3.586414 | 2.690652 | -0.575496 |
| C | -3.407809 | -0.881115 | -0.204490 |
| C | -2.331684 | -1.109526 | 0.652149 |
| C | -1.375896 | 0.005125 | 1.040663 |
| C | -1.959415 | 1.352995 | 0.661278 |
| C | -3.056003 | 1.462203 | -0.192897 |
| H | 2.702381 | 0.405471 | 2.732374 |
| H | 8.162820 | -0.601537 | 1.541706 |
| H | 7.818864 | 1.173550 | -0.609621 |
| H | 5.972158 | -1.537406 | -2.547687 |
| H | 6.479668 | 0.152686 | -2.651757 |
| H | 8.205944 | -1.117377 | -1.413410 |
| H | 6.988524 | -1.750634 | -0.297421 |
| H | 6.219931 | 1.677888 | 1.182481 |
| H | 5.770320 | -0.028607 | 1.311694 |
| H | 5.331800 | 1.789106 | -1.121415 |
| H | 4.072370 | 1.200856 | -0.044074 |
| H | -0.768987 | 0.148131 | -1.578471 |
| H | 1.373868 | -0.208676 | -2.733322 |
| H | 3.283597 | -0.899120 | 1.029141 |
| H | -1.238283 | -0.022373 | 2.122973 |

| | | | |
|---|---|---|---|
| H | -3.530501 | 7.191134 | -0.326605 |
| H | -3.222142 | 6.355491 | 1.206920 |
| H | -1.954363 | 6.453766 | -0.031311 |
| H | -5.606430 | 4.754095 | -0.771064 |
| H | -4.958205 | 6.219948 | -1.531568 |
| H | -4.621685 | 4.639678 | -2.242964 |
| H | -5.041812 | -5.511713 | 1.148449 |
| H | -5.545509 | -6.215971 | -0.399902 |
| H | -3.830785 | -5.971811 | -0.064832 |
| H | -6.633349 | -4.849601 | -1.581376 |
| H | -6.820484 | -3.276554 | -0.781873 |
| H | -5.860474 | -3.418954 | -2.267889 |
| H | -4.445904 | 2.680020 | -1.230944 |
| H | -0.576981 | 2.511024 | 1.817882 |
| H | -1.470633 | 4.682639 | 1.192449 |
| H | -1.352482 | -2.623299 | 1.797414 |
| H | -2.845268 | -4.439744 | 1.144065 |
| H | -5.091776 | -1.624379 | -1.255382 |

Energy (including solvation free energy) = -1703.59699294

Absolute Gibbs Free Energy = -1703.082214

Optimized geometry (in Å) and absolute energy (in $E_h$) at the B3LYP/6-31G(2df,p) level of theory for **Texas Red**

| | | | |
|---|---|---|---|
| S | 3.089671 | 0.226312 | -2.343123 |

| | | | |
|---|---|---|---|
| S | -0.514578 | 4.080286 | -1.355036 |
| O | 5.561108 | -1.295265 | -0.131464 |
| O | 2.875119 | 0.954681 | -3.566739 |
| O | 2.723674 | -1.166086 | -2.280707 |
| O | 4.637320 | 0.383925 | -2.045307 |
| O | 0.055295 | 4.529017 | -2.604975 |
| O | -0.815823 | 5.013521 | -0.293315 |
| O | -9.383794 | 1.517869 | -1.046038 |
| O | -18.065867 | -1.385374 | 1.561305 |
| N | 3.431902 | -5.589050 | -0.341277 |
| N | 8.695285 | 1.895371 | 1.600424 |
| N | -1.923719 | 3.329212 | -1.745507 |
| N | -9.425183 | -0.491862 | 0.008603 |
| C | 4.441575 | -2.079329 | 0.162911 |
| C | 3.317142 | -1.502766 | 0.736447 |
| C | 3.301432 | -0.045694 | 1.146439 |
| C | 4.718408 | 0.499364 | 1.247339 |
| C | 5.786100 | -0.163305 | 0.655966 |
| C | 4.525614 | -3.420558 | -0.202507 |
| C | 3.406550 | -4.252254 | 0.040034 |
| C | 2.251915 | -3.698571 | 0.653030 |
| C | 2.236058 | -2.350106 | 0.977192 |
| C | 4.705500 | -6.208700 | -0.677542 |
| C | 5.534104 | -5.283591 | -1.561111 |
| C | 5.789508 | -3.959094 | -0.840085 |
| C | 2.391121 | -6.494517 | 0.126001 |
| C | 1.024342 | -5.819050 | 0.090440 |
| C | 1.053243 | -4.569287 | 0.970000 |

| | | | |
|---|---|---|---|
| C | 5.025887 | 1.654451 | 1.966883 |
| C | 6.316784 | 2.149770 | 2.079626 |
| C | 7.386688 | 1.449435 | 1.463766 |
| C | 7.108940 | 0.267041 | 0.736735 |
| C | 6.571667 | 3.435129 | 2.839830 |
| C | 8.023698 | 3.527962 | 3.309052 |
| C | 8.954739 | 3.223169 | 2.140254 |
| C | 8.208016 | -0.518247 | 0.052726 |
| C | 9.584903 | -0.164451 | 0.616577 |
| C | 9.728313 | 1.348760 | 0.732591 |
| C | 2.355683 | 0.899113 | 0.371611 |
| C | 1.521897 | 1.678715 | 1.187816 |
| C | 1.390610 | 2.105644 | -1.526877 |
| C | 0.592052 | 2.857272 | -0.672249 |
| C | 0.651334 | 2.644003 | 0.698380 |
| C | 2.256573 | 1.133506 | -1.024189 |
| C | -2.779545 | 2.748523 | -0.696698 |
| C | -4.157899 | 2.421259 | -1.271443 |
| C | -5.103901 | 1.831113 | -0.220262 |
| C | -6.478947 | 1.486485 | -0.799739 |
| C | -7.441150 | 0.904526 | 0.242851 |
| C | -8.837443 | 0.682590 | -0.329556 |
| C | -10.774451 | -0.858605 | -0.394662 |
| C | -11.822974 | -0.624435 | 0.700024 |
| C | -13.230821 | -1.031781 | 0.254134 |
| C | -14.297085 | -0.799779 | 1.330241 |
| C | -15.704540 | -1.208723 | 0.882692 |
| C | -16.764456 | -0.961320 | 1.955139 |

| | | | |
|---|---|---|---|
| H | 4.992102 | -0.368456 | -1.512468 |
| H | -16.526711 | -1.536388 | 2.857531 |
| H | -16.767173 | 0.102283 | 2.240962 |
| H | -14.298091 | 0.261037 | 1.615860 |
| H | -14.025719 | -1.359168 | 2.236049 |
| H | -18.320295 | -0.859780 | 0.794970 |
| H | -13.501670 | -0.471783 | -0.651463 |
| H | -13.229541 | -2.092400 | -0.032537 |
| H | 1.342680 | -1.939375 | 1.441668 |
| H | 4.491921 | -7.146803 | -1.199238 |
| H | 5.274458 | -6.469841 | 0.232133 |
| H | 4.988482 | -5.104074 | -2.494433 |
| H | 6.480273 | -5.766698 | -1.821534 |
| H | 6.201441 | -3.222692 | -1.535683 |
| H | 6.558555 | -4.102177 | -0.068176 |
| H | 2.603346 | -6.852133 | 1.149014 |
| H | 2.400868 | -7.374687 | -0.525201 |
| H | 0.258115 | -6.520053 | 0.434049 |
| H | 0.784965 | -5.548826 | -0.944519 |
| H | 1.087093 | -4.876210 | 2.024835 |
| H | 0.132073 | -3.988929 | 0.852208 |
| H | 4.221010 | 2.201585 | 2.450788 |
| H | 5.883197 | 3.507437 | 3.688338 |
| H | 6.349394 | 4.295549 | 2.193196 |
| H | 8.209169 | 2.805882 | 4.112556 |
| H | 8.242551 | 4.524355 | 3.703687 |
| H | 9.999704 | 3.249942 | 2.465711 |
| H | 8.842613 | 3.998487 | 1.362216 |

| | | | |
|---|---|---|---|
| H | 8.190413 | -0.313251 | -1.026819 |
| H | 8.015522 | -1.590033 | 0.153397 |
| H | 10.375638 | -0.564564 | -0.024534 |
| H | 9.710838 | -0.609055 | 1.610368 |
| H | 9.690154 | 1.808314 | -0.270633 |
| H | 10.699091 | 1.609610 | 1.165299 |
| H | 2.891781 | -0.023778 | 2.162121 |
| H | 0.042193 | 3.230150 | 1.374943 |
| H | 1.564665 | 1.524478 | 2.260144 |
| H | 1.351644 | 2.277385 | -2.593064 |
| H | -1.808882 | 2.744937 | -2.567795 |
| H | -2.865473 | 3.492731 | 0.098639 |
| H | -2.324964 | 1.845730 | -0.265715 |
| H | -4.044048 | 1.710136 | -2.100341 |
| H | -4.591405 | 3.333863 | -1.697043 |
| H | -5.223640 | 2.545350 | 0.605423 |
| H | -4.651139 | 0.930372 | 0.215353 |
| H | -6.358932 | 0.769333 | -1.622175 |
| H | -6.938804 | 2.380571 | -1.233614 |
| H | -7.545291 | 1.610639 | 1.077446 |
| H | -7.039452 | -0.023089 | 0.664502 |
| H | -8.928228 | -1.118187 | 0.621862 |
| H | -10.772699 | -1.913256 | -0.692313 |
| H | -11.013219 | -0.261804 | -1.278283 |
| H | -11.811056 | 0.436562 | 0.979457 |
| H | -11.540574 | -1.188663 | 1.598489 |
| H | -15.719841 | -2.272121 | 0.611963 |
| H | -15.977964 | -0.650021 | -0.023676 |

Energy (including solvation free energy) = -3289.69017447

Absolute Gibbs Free Energy = -3288.839291

Optimized geometry (in Å) and absolute energy (in $E_h$) at the B3LYP/6-31G(2df,p) level of theory for **Rf**

| | | | |
|---|---|---|---|
| O | 5.240940 | 2.714366 | -0.619717 |
| O | 5.052782 | -1.623499 | 0.930913 |
| O | 2.075150 | -2.938635 | 0.360022 |
| O | 0.881224 | 0.093849 | 2.431894 |
| O | 4.324060 | 1.467592 | 1.032679 |
| O | 4.022768 | -0.633264 | -0.828840 |
| O | 1.575240 | -1.716809 | -1.490284 |
| O | -0.100518 | -1.039221 | 0.745872 |
| O | -0.602394 | 4.783604 | -0.995345 |
| O | -4.867531 | 4.173282 | 0.425554 |
| N | -1.287974 | 2.612692 | -0.860824 |
| N | -2.722201 | 4.432342 | -0.289223 |
| N | -2.083284 | 0.448764 | -0.717871 |
| N | -4.555193 | 1.395846 | 0.179655 |
| C | 6.513611 | 2.233492 | 1.380541 |
| C | 5.319350 | 2.187788 | 0.463783 |
| C | 6.053819 | -1.725174 | -1.267999 |
| C | 5.018797 | -1.345264 | -0.241594 |
| C | 1.631369 | -4.043620 | -1.740707 |
| C | 1.790178 | -2.869048 | -0.810277 |
| C | -0.242392 | -2.002528 | 2.882999 |

| | | | |
|---|---|---|---|
| C | 0.249224 | -0.858973 | 2.042437 |
| C | 3.106795 | 1.293335 | 0.295525 |
| C | 2.920492 | -0.195001 | -0.012363 |
| C | 1.657207 | -0.415783 | -0.872796 |
| C | 0.310857 | -0.056693 | -0.213794 |
| C | -0.793807 | 0.012989 | -1.275521 |
| C | -1.470535 | 3.967386 | -0.737795 |
| C | -3.822076 | 3.680314 | 0.047276 |
| C | -2.278043 | 1.801909 | -0.569477 |
| C | -3.587005 | 2.215659 | -0.103881 |
| C | -2.930275 | -1.847257 | -0.474578 |
| C | -3.084355 | -0.456386 | -0.390973 |
| C | -4.333786 | 0.059891 | 0.047650 |
| C | -5.380492 | -0.831350 | 0.360927 |
| C | -3.758719 | -4.194834 | -0.267202 |
| C | -3.972017 | -2.709139 | -0.161457 |
| C | -5.232569 | -2.196539 | 0.261272 |
| C | -6.370043 | -3.123937 | 0.599065 |
| H | 6.828503 | 1.217129 | 1.630993 |
| H | 7.328066 | 2.771784 | 0.897957 |
| H | 6.242445 | 2.731707 | 2.315684 |
| H | 6.444235 | -0.827102 | -1.754038 |
| H | 6.863878 | -2.271620 | -0.787128 |
| H | 5.596192 | -2.344526 | -2.044489 |
| H | 1.812758 | -4.968165 | -1.194610 |
| H | 0.622521 | -4.051156 | -2.162471 |
| H | 2.333845 | -3.956538 | -2.573866 |
| H | -1.302560 | -2.186133 | 2.694128 |
| H | 0.307061 | -2.904136 | 2.595833 |

| | | | |
|---|---|---|---|
| H | -0.073932 | -1.786353 | 3.936896 |
| H | -2.814276 | 5.437569 | -0.211234 |
| H | -1.980965 | -2.277792 | -0.756033 |
| H | -6.316415 | -0.392352 | 0.688502 |
| H | 3.150097 | 1.884552 | -0.620803 |
| H | 2.299678 | 1.643927 | 0.939424 |
| H | 2.912744 | -0.774914 | 0.905032 |
| H | 1.759689 | 0.245682 | -1.739496 |
| H | 0.393049 | 0.913887 | 0.275428 |
| H | -0.926926 | -0.947095 | -1.766794 |
| H | -0.507167 | 0.752165 | -2.022333 |
| H | -4.462294 | -4.642674 | -0.978193 |
| H | -2.744670 | -4.429592 | -0.595764 |
| H | -3.931240 | -4.688013 | 0.696024 |
| H | -6.644065 | -3.749633 | -0.257770 |
| H | -6.102768 | -3.805251 | 1.414639 |
| H | -7.254186 | -2.560389 | 0.903974 |

Energy (including solvation free energy) = -1941.04457122

Absolute Gibbs Free Energy = -1940.601521

Optimized geometry (in Å) and absolute energy (in $E_h$) at the B3LYP/6-31G(2df,p) level of theory for **Ro**

| | | | |
|---|---|---|---|
| O | -2.515213 | 0.993372 | -0.069969 |
| O | 5.464141 | -3.207137 | 1.854069 |
| N | -0.690516 | 5.349935 | -0.456946 |

| | | | |
|---|---|---|---|
| N | -4.799242 | -3.141978 | 0.296126 |
| N | 6.069045 | -2.805940 | -0.284130 |
| C | -2.033078 | 5.929141 | -0.601473 |
| C | -2.758942 | 6.185131 | 0.723970 |
| C | -6.485485 | -2.187574 | -1.318459 |
| C | -0.517177 | 4.017084 | -0.290485 |
| C | 0.796257 | 3.445373 | -0.178003 |
| C | -6.096038 | -2.469131 | 0.136897 |
| C | 0.447660 | 6.281479 | -0.459375 |
| C | -1.613729 | 3.123577 | -0.238187 |
| C | 0.980339 | 2.101765 | -0.031819 |
| C | 0.977600 | 6.633695 | 0.934691 |
| C | -1.404774 | 1.771116 | -0.090033 |
| C | -0.110865 | 1.190638 | 0.034581 |
| C | -3.622172 | -1.038396 | 0.084889 |
| C | -2.429061 | -0.352171 | 0.072865 |
| C | -3.639583 | -2.443648 | 0.254294 |
| C | -2.369166 | -3.099004 | 0.399308 |
| C | -1.161972 | -0.987891 | 0.203244 |
| C | -1.198261 | -2.399558 | 0.380619 |
| C | 0.008668 | -0.205075 | 0.180889 |
| C | -4.561553 | -5.381261 | -0.842564 |
| C | -4.814467 | -4.604111 | 0.453746 |
| C | 1.816530 | -1.710344 | -0.681060 |
| C | 1.346582 | -0.839498 | 0.308804 |
| C | 3.076425 | -2.289683 | -0.569386 |
| C | 2.153440 | -0.579281 | 1.425148 |
| C | 3.879192 | -2.028646 | 0.544755 |
| C | 3.396729 | -1.184608 | 1.549220 |

| | | | |
|---|---|---|---|
| C | 6.162786 | -1.936898 | -1.457151 |
| C | 7.272519 | -0.986624 | -1.361494 |
| C | 5.200227 | -2.709054 | 0.765814 |
| C | 8.196434 | -0.221609 | -1.278826 |
| H | 1.667988 | 4.080982 | -0.217258 |
| H | 1.988341 | 1.713059 | 0.032857 |
| H | -2.635953 | 3.463015 | -0.307521 |
| H | -3.754542 | 6.590443 | 0.520124 |
| H | -1.918010 | 6.868556 | -1.145747 |
| H | -2.629239 | 5.275911 | -1.244250 |
| H | -5.742545 | -1.561225 | -1.819540 |
| H | -7.445772 | -1.663904 | -1.343194 |
| H | -6.084503 | -1.541873 | 0.716462 |
| H | 0.110130 | 7.188790 | -0.963425 |
| H | -2.216769 | 6.908631 | 1.338235 |
| H | -2.877425 | 5.265133 | 1.302770 |
| H | 0.206950 | 7.118723 | 1.539161 |
| H | -6.588888 | -3.115990 | -1.885961 |
| H | -4.527132 | -0.462788 | -0.036161 |
| H | -2.325053 | -4.169521 | 0.531352 |
| H | -0.265088 | -2.932615 | 0.508183 |
| H | 1.821467 | 7.323471 | 0.839041 |
| H | -5.330687 | -5.166043 | -1.588767 |
| H | -6.847220 | -3.109747 | 0.602595 |
| H | 1.200837 | -1.925557 | -1.546867 |
| H | 1.247127 | 5.869354 | -1.080919 |
| H | -3.587782 | -5.135263 | -1.274640 |
| H | 1.322954 | 5.745501 | 1.470647 |
| H | -5.794560 | -4.867885 | 0.855401 |

| | | | |
|---|---|---|---|
| H | -4.580373 | -6.454887 | -0.632744 |
| H | -4.087358 | -4.887814 | 1.218958 |
| H | 3.419797 | -2.968018 | -1.342392 |
| H | 1.794654 | 0.081604 | 2.206024 |
| H | 6.282529 | -2.553164 | -2.355381 |
| H | 5.222655 | -1.395327 | -1.571656 |
| H | 4.004513 | -1.005895 | 2.428357 |
| H | 9.009042 | 0.460937 | -1.207245 |
| H | 6.923091 | -3.290210 | -0.037166 |

Energy (including solvation free energy) = -1516.40782841

Absolute Gibbs Free Energy = -1515.890837

Optimized geometry (in Å) and absolute energy (in $E_h$) at the B3LYP/6-31G(2df,p) level of theory for

**Rhodamin 6G-ox**

| | | | |
|---|---|---|---|
| O | 10.450418 | 1.348233 | 0.974623 |
| O | 2.073180 | -4.741768 | -2.044261 |
| O | 0.444890 | -1.745293 | 2.956326 |
| O | -1.140587 | -0.274996 | 2.366085 |
| O | -3.748716 | 2.088912 | -0.123913 |
| N | 3.537547 | -3.883078 | -0.553860 |
| N | -0.732343 | 5.654778 | -0.333324 |
| N | -7.196917 | -1.060777 | 0.152127 |
| C | 9.763220 | 0.385384 | 0.181695 |
| C | 8.391447 | 0.017580 | 0.745415 |

| | | | |
|---|---|---|---|
| C | 7.674740 | -1.055312 | -0.081015 |
| C | 6.290292 | -1.412997 | 0.470423 |
| C | 5.574719 | -2.489275 | -0.352031 |
| C | 4.190546 | -2.825191 | 0.212887 |
| C | 2.353749 | -3.878476 | -1.215846 |
| C | -0.300355 | -1.088196 | 2.050416 |
| C | 0.630942 | -2.247244 | -1.964480 |
| C | 1.350351 | -2.800407 | -0.902890 |
| C | 1.032516 | -2.414630 | 0.396943 |
| C | -0.353139 | -1.296121 | -1.729863 |
| C | 0.027403 | -1.473693 | 0.648906 |
| C | -0.669077 | -0.893750 | -0.428392 |
| C | 0.008969 | 7.978091 | -0.295807 |
| C | 0.448090 | 6.517724 | -0.251653 |
| C | -9.443472 | -1.863708 | 0.664499 |
| C | -7.976721 | -2.250880 | 0.502414 |
| C | -5.235619 | -3.396848 | -0.150015 |
| C | 1.669396 | 3.751813 | -0.580283 |
| C | -0.090782 | 2.020590 | -0.402970 |
| C | 0.218526 | 3.357850 | -0.424371 |
| C | -0.867466 | 4.308448 | -0.326147 |
| C | -2.190146 | 3.816107 | -0.232211 |
| C | -3.557100 | -1.575444 | -0.174708 |
| C | -4.879985 | -1.929806 | -0.078151 |
| C | -5.860477 | -0.873471 | 0.036210 |
| C | -5.417008 | 0.468346 | -0.001034 |
| C | -4.083494 | 0.774927 | -0.108501 |
| C | -3.088692 | -0.235400 | -0.187852 |

| | | | |
|---|---|---|---|
| C | -1.736406 | 0.139589 | -0.269416 |
| C | -1.410063 | 1.506120 | -0.299961 |
| C | -2.450881 | 2.469113 | -0.220387 |
| H | -7.727365 | -0.203837 | 0.215273 |
| H | 0.169989 | -1.437582 | 3.833666 |
| H | 9.921724 | 2.153670 | 0.971256 |
| H | 10.410878 | -0.498716 | 0.162359 |
| H | 9.659975 | 0.733326 | -0.858148 |
| H | 7.770337 | 0.923662 | 0.787904 |
| H | 8.516954 | -0.325265 | 1.780273 |
| H | 8.295066 | -1.961164 | -0.119478 |
| H | 7.572931 | -0.710678 | -1.119094 |
| H | 5.668850 | -0.507814 | 0.504403 |
| H | 6.389928 | -1.754893 | 1.509403 |
| H | -2.820807 | -2.365963 | -0.260287 |
| H | 6.186275 | -3.401225 | -0.375357 |
| H | 5.464070 | -2.158394 | -1.392083 |
| H | 3.558490 | -1.936302 | 0.189586 |
| H | 4.282824 | -3.131411 | 1.262991 |
| H | 4.122424 | -4.669333 | -0.808436 |
| H | 1.550107 | -2.856364 | 1.237602 |
| H | -0.890888 | -0.860293 | -2.564111 |
| H | 0.848737 | -2.568057 | -2.976275 |
| H | -0.650582 | 8.217297 | 0.544675 |
| H | 0.884629 | 8.628227 | -0.234781 |
| H | -0.519549 | 8.200590 | -1.227835 |
| H | 0.994949 | 6.325046 | 0.677771 |
| H | 1.123303 | 6.314447 | -1.084830 |

| | | | |
|---|---|---|---|
| H | -1.603238 | 6.157550 | -0.241968 |
| H | -9.842258 | -1.441360 | -0.263049 |
| H | -10.032156 | -2.748318 | 0.917793 |
| H | -9.571803 | -1.128947 | 1.465822 |
| H | -7.884221 | -3.010108 | -0.276994 |
| H | -7.603795 | -2.683368 | 1.437085 |
| H | -4.325563 | -3.986735 | -0.276473 |
| H | -5.738583 | -3.757283 | 0.750245 |
| H | -5.886908 | -3.611924 | -1.002612 |
| H | 2.030099 | 4.370082 | 0.244827 |
| H | 2.288522 | 2.853040 | -0.612260 |
| H | 1.844275 | 4.302176 | -1.509479 |
| H | -6.133463 | 1.278436 | 0.072900 |
| H | -3.021446 | 4.508261 | -0.161592 |
| H | 0.721659 | 1.307345 | -0.477120 |

Energy (including solvation free energy) = -1822.03739110

Absolute Gibbs Free Energy = -1821.438363

Optimized geometry (in Å) and absolute energy (in $E_h$) at the B3LYP/6-31G(2df,p) level of theory for **TAMRA-ox**

| | | | |
|---|---|---|---|
| O | -0.518359 | -0.089663 | 2.445423 |
| O | 8.167846 | 0.955704 | 1.573411 |
| O | 4.278438 | -2.858728 | -1.451774 |

| | | | |
|---|---|---|---|
| O | 1.481842 | 0.799907 | 2.730258 |
| O | -4.085153 | 0.567242 | -0.053511 |
| N | 5.208817 | -0.874919 | -0.830252 |
| N | -3.194168 | 5.204260 | -0.365550 |
| N | -5.429503 | -3.959540 | 0.254608 |
| C | 7.408441 | -0.983353 | 0.271745 |
| C | 7.397145 | 0.538382 | 0.453194 |
| C | 5.955034 | 1.056949 | 0.515791 |
| C | 5.149652 | 0.582437 | -0.699630 |
| C | 6.569979 | -1.407101 | -0.940464 |
| C | 4.152712 | -1.695013 | -1.076822 |
| C | 0.578066 | 0.128731 | 1.990013 |
| C | 0.483655 | -0.963428 | -1.638995 |
| C | 1.809029 | -1.320541 | -1.856202 |
| C | 2.757274 | -1.168358 | -0.844476 |
| C | 2.346910 | -0.671514 | 0.392066 |
| C | 1.013174 | -0.324402 | 0.629241 |
| C | 0.063448 | -0.473701 | -0.399352 |
| C | -2.263048 | 6.320107 | -0.519729 |
| C | -4.620600 | 5.488769 | -0.241463 |
| C | -5.118011 | -5.387024 | 0.289398 |
| C | -6.825003 | -3.540338 | 0.343458 |
| C | -4.741495 | -1.654933 | 0.097532 |
| C | -4.448831 | -3.037620 | 0.143076 |
| C | -3.070661 | -3.431708 | 0.070935 |
| C | -2.076566 | -2.505672 | -0.051048 |
| C | -0.897545 | 2.346137 | -0.441456 |
| C | -1.352168 | 3.632073 | -0.468829 |

| | | | |
|---|---|---|---|
| C | -2.750807 | 3.928653 | -0.344234 |
| C | -3.644339 | 2.841609 | -0.205883 |
| C | -3.721534 | -0.738514 | -0.025213 |
| C | -2.350713 | -1.110846 | -0.115867 |
| C | -1.374446 | -0.107428 | -0.238440 |
| C | -1.777455 | 1.239076 | -0.283851 |
| C | -3.162534 | 1.552167 | -0.180064 |
| H | 2.262863 | 1.035148 | 2.212041 |
| H | -5.753377 | -1.282540 | 0.156035 |
| H | -2.807864 | -4.478563 | 0.118430 |
| H | -1.046840 | -2.836997 | -0.097857 |
| H | 7.770472 | 0.570941 | 2.363755 |
| H | 7.894275 | 0.997109 | -0.411677 |
| H | 6.499717 | -2.490137 | -1.021978 |
| H | 7.029012 | -1.029005 | -1.864926 |
| H | 8.436726 | -1.336391 | 0.146574 |
| H | 7.002798 | -1.458366 | 1.174854 |
| H | 5.951926 | 2.150586 | 0.552279 |
| H | 5.480437 | 0.692237 | 1.436495 |
| H | 5.570772 | 1.027278 | -1.613023 |
| H | 4.113177 | 0.907370 | -0.636707 |
| H | -0.239466 | -1.069285 | -2.439495 |
| H | 2.109453 | -1.727476 | -2.814572 |
| H | 3.070708 | -0.607533 | 1.197491 |
| H | -2.825682 | 7.251389 | -0.507302 |
| H | -1.536079 | 6.348003 | 0.298520 |
| H | -1.721227 | 6.259974 | -1.469454 |
| H | -5.020277 | 5.098883 | 0.700897 |

| | | | |
|---|---|---|---|
| H | -4.771402 | 6.566151 | -0.257622 |
| H | -5.186785 | 5.047436 | -1.069213 |
| H | -4.510706 | -5.642792 | 1.164153 |
| H | -6.048286 | -5.949018 | 0.343503 |
| H | -4.582923 | -5.698839 | -0.613275 |
| H | -7.455747 | -4.420842 | 0.446562 |
| H | -6.989040 | -2.895313 | 1.213399 |
| H | -7.133723 | -2.996898 | -0.556467 |
| H | -4.711063 | 2.986268 | -0.121370 |
| H | 0.163713 | 2.159182 | -0.547945 |
| H | -0.641082 | 4.436156 | -0.590796 |

Energy (including solvation free energy) = -1702.85878771

Absolute Gibbs Free Energy = -1702.353139

Optimized geometry (in Å) and absolute energy (in $E_h$) at the B3LYP/6-31G(2df,p) level of theory for **Texas Red-ox**

| | | | |
|---|---|---|---|
| S | 3.647888 | 1.522523 | -2.714798 |
| S | -0.294323 | 4.545561 | -0.496990 |
| O | 5.303458 | -2.243891 | 0.572818 |
| O | 3.228073 | 2.398250 | -3.777074 |
| O | 3.765937 | 0.108191 | -2.928312 |
| O | 5.100584 | 1.972598 | -2.218149 |
| O | 0.221709 | 5.433920 | -1.511943 |
| O | -0.567618 | 5.012993 | 0.842086 |

| | | | |
|---|---|---|---|
| O | -9.144720 | 2.013910 | -0.522300 |
| O | -17.471662 | -2.013806 | 2.227870 |
| N | 2.160420 | -5.372192 | -1.099124 |
| N | 8.673299 | 0.540997 | 2.400746 |
| N | -1.696928 | 3.936996 | -1.090337 |
| N | -9.214864 | -0.247406 | -0.316153 |
| C | 4.079465 | -2.479374 | 0.033458 |
| C | 3.190959 | -1.397435 | -0.214089 |
| C | 3.611095 | -0.091257 | 0.083389 |
| C | 4.876094 | 0.124761 | 0.652658 |
| C | 5.719712 | -0.994188 | 0.900560 |
| C | 3.777906 | -3.797837 | -0.250655 |
| C | 2.500497 | -4.093221 | -0.801298 |
| C | 1.564408 | -3.019993 | -1.043490 |
| C | 1.927498 | -1.733125 | -0.766854 |
| C | 3.025242 | -6.493094 | -0.721480 |
| C | 4.497180 | -6.120747 | -0.836305 |
| C | 4.790551 | -4.887841 | 0.016804 |
| C | 0.839801 | -5.733969 | -1.622613 |
| C | 0.220916 | -4.603411 | -2.432325 |
| C | 0.189203 | -3.335291 | -1.582395 |
| C | 5.391269 | 1.395719 | 1.022811 |
| C | 6.628676 | 1.555126 | 1.576595 |
| C | 7.452558 | 0.395573 | 1.829155 |
| C | 6.971259 | -0.896136 | 1.477830 |
| C | 7.153414 | 2.931936 | 1.908869 |
| C | 8.145026 | 2.865989 | 3.068217 |
| C | 9.232425 | 1.852986 | 2.740578 |

| | | | |
|---|---|---|---|
| C | 7.793825 | -2.138411 | 1.734268 |
| C | 8.834514 | -1.884216 | 2.823510 |
| C | 9.589458 | -0.595442 | 2.528626 |
| C | 2.677296 | 1.059743 | -0.120853 |
| C | 1.822991 | 1.393294 | 0.934378 |
| C | 1.708803 | 2.891848 | -1.408466 |
| C | 0.869230 | 3.193028 | -0.340485 |
| C | 0.926297 | 2.452869 | 0.836449 |
| C | 2.606800 | 1.834285 | -1.294256 |
| C | -2.509682 | 2.998173 | -0.297970 |
| C | -3.928029 | 2.921271 | -0.863687 |
| C | -4.810664 | 1.949320 | -0.073382 |
| C | -6.241964 | 1.877059 | -0.613200 |
| C | -7.122234 | 0.898211 | 0.171545 |
| C | -8.584666 | 0.952646 | -0.259740 |
| C | -10.633872 | -0.400948 | -0.599641 |
| C | -11.458207 | -0.748478 | 0.645822 |
| C | -12.947763 | -0.927540 | 0.335802 |
| C | -13.779271 | -1.283548 | 1.572977 |
| C | -15.270895 | -1.461427 | 1.270221 |
| C | -16.091267 | -1.808029 | 2.511811 |
| H | 5.195431 | 2.936654 | -2.287278 |
| H | 1.219241 | -0.936912 | -0.964685 |
| H | -15.735011 | -2.749068 | 2.946483 |
| H | -15.959443 | -1.027609 | 3.277725 |
| H | -13.651634 | -0.499654 | 2.332035 |
| H | -13.385629 | -2.207029 | 2.019345 |
| H | -17.820397 | -1.184906 | 1.882027 |

| | | | |
|---|---|---|---|
| H | -13.340269 | -0.004879 | -0.113009 |
| H | -13.071110 | -1.712649 | -0.422729 |
| H | 2.786247 | -7.329329 | -1.382516 |
| H | 2.788135 | -6.811712 | 0.303990 |
| H | 4.735895 | -5.918045 | -1.886048 |
| H | 5.110757 | -6.966787 | -0.516458 |
| H | 5.797509 | -4.513757 | -0.183593 |
| H | 4.771854 | -5.158628 | 1.081034 |
| H | 0.177925 | -6.013973 | -0.789948 |
| H | 0.968736 | -6.624861 | -2.242966 |
| H | -0.786613 | -4.893834 | -2.740825 |
| H | 0.810851 | -4.436408 | -3.340306 |
| H | -0.505520 | -3.483963 | -0.744322 |
| H | -0.188308 | -2.482375 | -2.153083 |
| H | 4.781364 | 2.274466 | 0.849573 |
| H | 6.318382 | 3.600521 | 2.135606 |
| H | 7.659789 | 3.350655 | 1.028335 |
| H | 7.631871 | 2.568499 | 3.989392 |
| H | 8.605872 | 3.840943 | 3.245966 |
| H | 9.895904 | 1.711217 | 3.597701 |
| H | 9.853440 | 2.214534 | 1.908020 |
| H | 8.293986 | -2.449842 | 0.807463 |
| H | 7.136401 | -2.964314 | 2.016791 |
| H | 9.543155 | -2.714228 | 2.881553 |
| H | 8.345247 | -1.798691 | 3.799889 |
| H | 10.179756 | -0.697834 | 1.606587 |
| H | 10.288420 | -0.364025 | 3.335501 |
| H | 0.288896 | 2.707524 | 1.673712 |

| | | | |
|---|---|---|---|
| H | 1.868505 | 0.814018 | 1.849053 |
| H | 1.675255 | 3.476933 | -2.317284 |
| H | -1.612129 | 3.703204 | -2.074550 |
| H | -2.531427 | 3.376309 | 0.726686 |
| H | -2.054101 | 1.998675 | -0.279939 |
| H | -3.883520 | 2.605274 | -1.914199 |
| H | -4.369468 | 3.924672 | -0.854494 |
| H | -4.835537 | 2.255058 | 0.981333 |
| H | -4.358361 | 0.948736 | -0.092179 |
| H | -6.221644 | 1.581096 | -1.670054 |
| H | -6.707974 | 2.867250 | -0.577499 |
| H | -7.097152 | 1.160838 | 1.237979 |
| H | -6.731426 | -0.122013 | 0.093294 |
| H | -8.694285 | -1.072487 | -0.062726 |
| H | -10.761064 | -1.178763 | -1.361963 |
| H | -10.970690 | 0.545588 | -1.028255 |
| H | -11.324054 | 0.045546 | 1.391309 |
| H | -11.062348 | -1.668908 | 1.095185 |
| H | -15.412595 | -2.252502 | 0.522880 |
| H | -15.668850 | -0.536931 | 0.828289 |

Energy (including solvation free energy) = -3288.96179478

Absolute Gibbs Free Energy = -3288.123704

Optimized geometry (in Å) and absolute energy (in $E_h$) at the B3LYP/6-31G(2df,p) level of theory for **$RfH_2$**

| | | | |
|---|---|---|---|
| O | -1.301551 | 4.969159 | -0.659319 |
| O | -5.620427 | 3.489327 | -0.264366 |
| O | 1.672445 | -1.304416 | -1.781276 |
| O | 2.028086 | -2.843045 | -0.146651 |
| O | 4.138417 | -0.343543 | -0.982854 |
| O | 0.277252 | -0.713794 | 0.901706 |
| O | 0.245079 | 0.999287 | 2.378896 |
| O | 5.138438 | -1.756616 | 0.479762 |
| O | 5.476136 | 2.698719 | -0.146065 |
| O | 4.459987 | 1.289816 | 1.305746 |
| N | -1.706397 | 2.723796 | -0.606705 |
| N | -2.043826 | 0.369259 | -0.527181 |
| N | -3.457421 | 4.218275 | -0.450303 |
| N | -4.824249 | 0.806874 | -0.470848 |
| C | 1.696809 | -3.553407 | -2.432063 |
| C | -2.099647 | 4.052258 | -0.580643 |
| C | -0.754059 | 0.083302 | -1.151299 |
| C | -2.567993 | 1.648339 | -0.527440 |
| C | -4.428318 | 3.210046 | -0.371707 |
| C | -3.909990 | 1.877450 | -0.443775 |
| C | -2.943190 | -0.702838 | -0.188603 |
| C | -4.328770 | -0.460890 | -0.146993 |
| C | -2.480535 | -1.981475 | 0.105103 |
| C | -5.187494 | -1.507179 | 0.178029 |
| C | -3.345394 | -3.039541 | 0.414030 |
| C | -4.724961 | -2.796481 | 0.452609 |

| | | | |
|---|---|---|---|
| C | -2.781594 | -4.409414 | 0.702312 |
| C | -5.703376 | -3.895305 | 0.784605 |
| C | 1.822790 | -2.564839 | -1.301331 |
| C | 1.772116 | -0.125373 | -0.954935 |
| C | 0.464155 | 0.176641 | -0.204833 |
| C | 6.157029 | -1.322983 | -1.671022 |
| C | 3.043769 | -0.087144 | -0.084578 |
| C | 5.118574 | -1.196529 | -0.587575 |
| C | 0.143095 | -0.181070 | 2.145429 |
| C | 3.248361 | 1.296421 | 0.539537 |
| C | -0.142348 | -1.262217 | 3.148496 |
| C | 5.505484 | 2.023426 | 0.853943 |
| C | 6.682444 | 1.871993 | 1.781229 |
| H | -0.709261 | 2.594343 | -0.657016 |
| H | -5.728827 | 1.052508 | -0.090637 |
| H | 0.725299 | -3.441472 | -2.920817 |
| H | -0.591250 | 0.773819 | -1.986073 |
| H | -0.807543 | -0.915465 | -1.583293 |
| H | 2.465915 | -3.357363 | -3.184066 |
| H | -3.154498 | -5.158858 | -0.006547 |
| H | -5.627593 | -4.729186 | 0.076714 |
| H | -3.789982 | 5.172485 | -0.416330 |
| H | 1.804566 | -4.565447 | -2.044606 |
| H | -1.416310 | -2.172502 | 0.114982 |
| H | -6.254705 | -1.305540 | 0.200603 |
| H | -1.690506 | -4.406788 | 0.640239 |
| H | 1.874929 | 0.665477 | -1.705395 |
| H | -6.732294 | -3.528207 | 0.762950 |
| H | 5.690589 | -1.668348 | -2.597453 |

| | | | |
|---|---|---|---|
| H | -3.059552 | -4.761920 | 1.702880 |
| H | 0.544241 | 1.187102 | 0.201834 |
| H | -5.516305 | -4.314049 | 1.780608 |
| H | 6.599594 | -0.343503 | -1.872799 |
| H | 3.308984 | 2.067509 | -0.230759 |
| H | 3.029776 | -0.856982 | 0.682529 |
| H | 6.930548 | -2.022676 | -1.357997 |
| H | 2.442769 | 1.525456 | 1.239484 |
| H | -1.130840 | -1.686243 | 2.947244 |
| H | -0.116922 | -0.847379 | 4.154926 |
| H | 0.587567 | -2.068882 | 3.047709 |
| H | 6.944421 | 0.815078 | 1.878022 |
| H | 7.530581 | 2.435261 | 1.394761 |
| H | 6.417229 | 2.236058 | 2.777782 |

Energy (including solvation free energy) = -1942.24564501

Absolute Gibbs Free Energy = -1941.781488

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