

# Supplementary Data

## Selective Alkylation of T-T mismatched DNA using vinyl-diaminotriazine-acridine conjugate

Kazumitsu Onizuka<sup>1</sup>, Akira Usami<sup>1</sup>, Yudai Yamaoki<sup>2,3</sup>, Tomohito Kobayashi<sup>1</sup>, Madoka E. Hazemi<sup>1</sup>, Tomoko Chikuni<sup>1</sup>, Norihiro Sato<sup>1</sup>, Kaname Sasaki<sup>1</sup>, Masato Katahira<sup>2,3</sup> and Fumi Nagatsugi<sup>1,\*</sup>

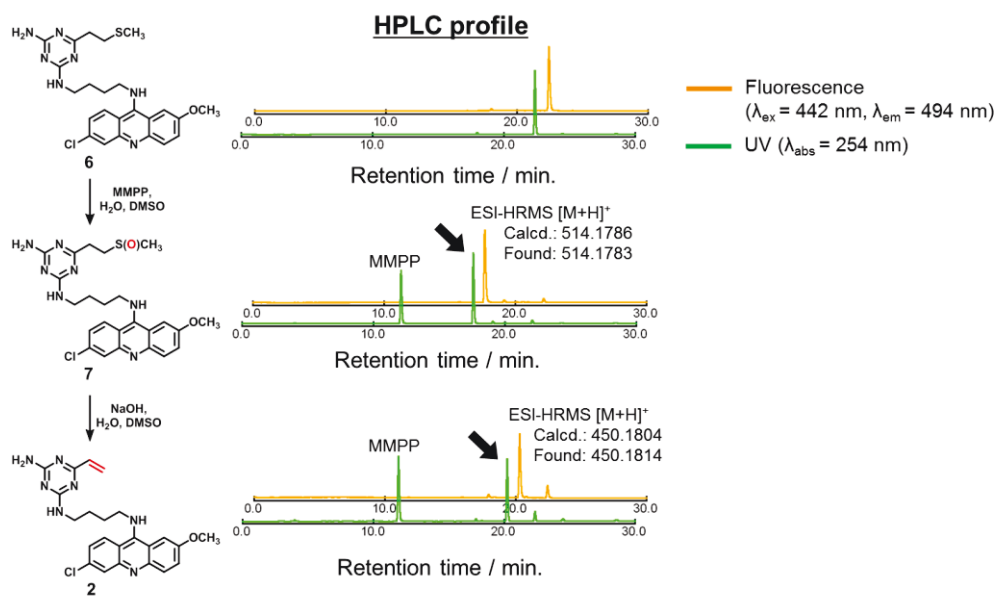
<sup>1</sup> Institute of Multidisciplinary Research for Advanced Materials, Tohoku University, 2-1-1 Katahira, Aoba-ku, Sendai, Miyagi 980-8577, Japan.

<sup>2</sup> Institute of Advanced Energy, Kyoto University, Gokasho, Uji, Kyoto 611-0011, Japan.

<sup>3</sup> Graduate School of Energy Science, Kyoto University, Gokasho, Uji, Kyoto 611-0011, Japan.

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Column: Nacalai tesque, COSMOSIL 5C<sub>18</sub>-AR-II (4.6×250 mm), Solvent A: 0.1% TFA in water, B: 0.1% TFA in CH<sub>3</sub>CN, linear gradient, B: 10% to 50%/ 30 min, flow rate: 1.0 mL/min, temperature: 40 °C.

**Figure S1. HPLC profiles of the conversion to the VDAT-acridine conjugate 2.**

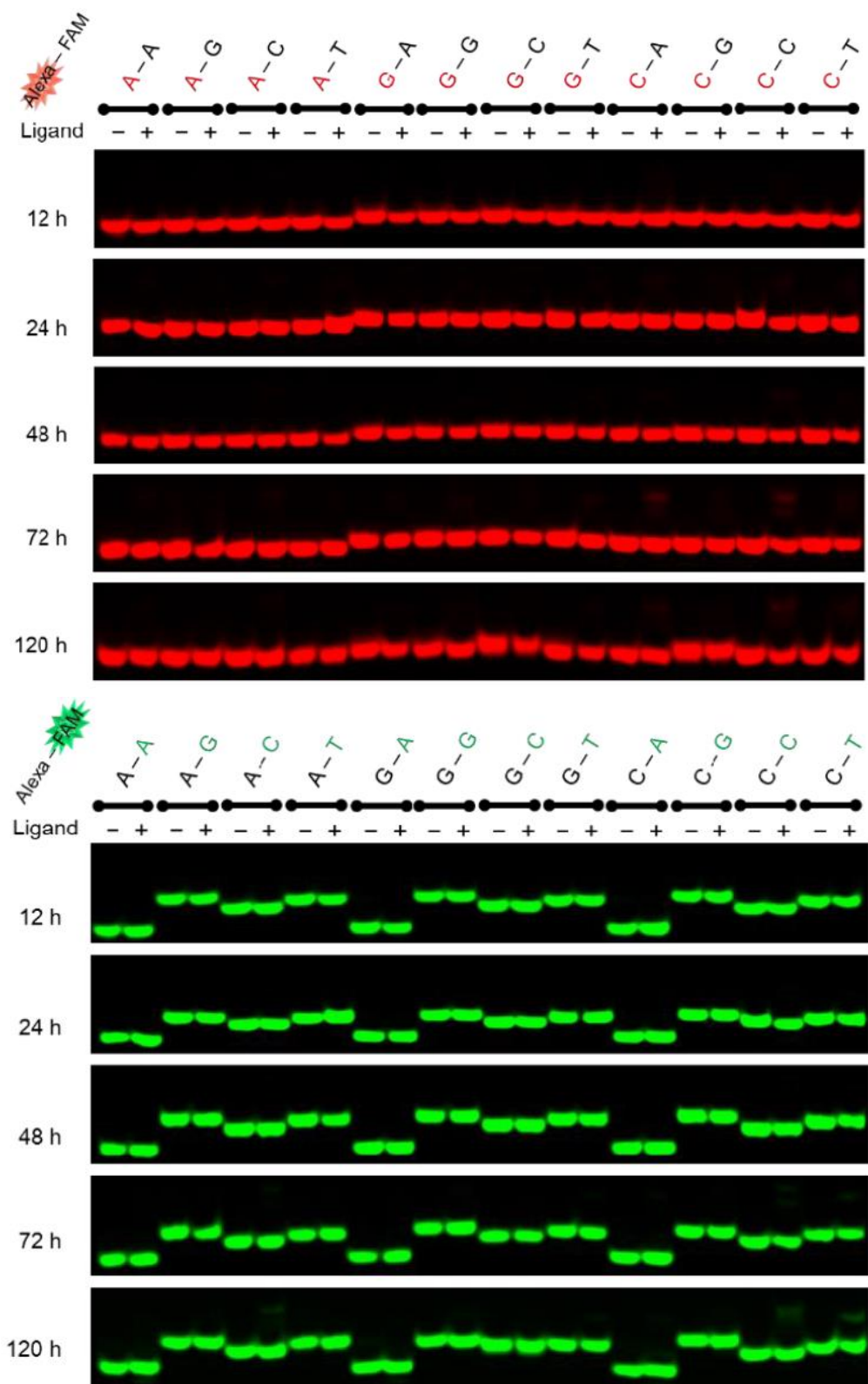
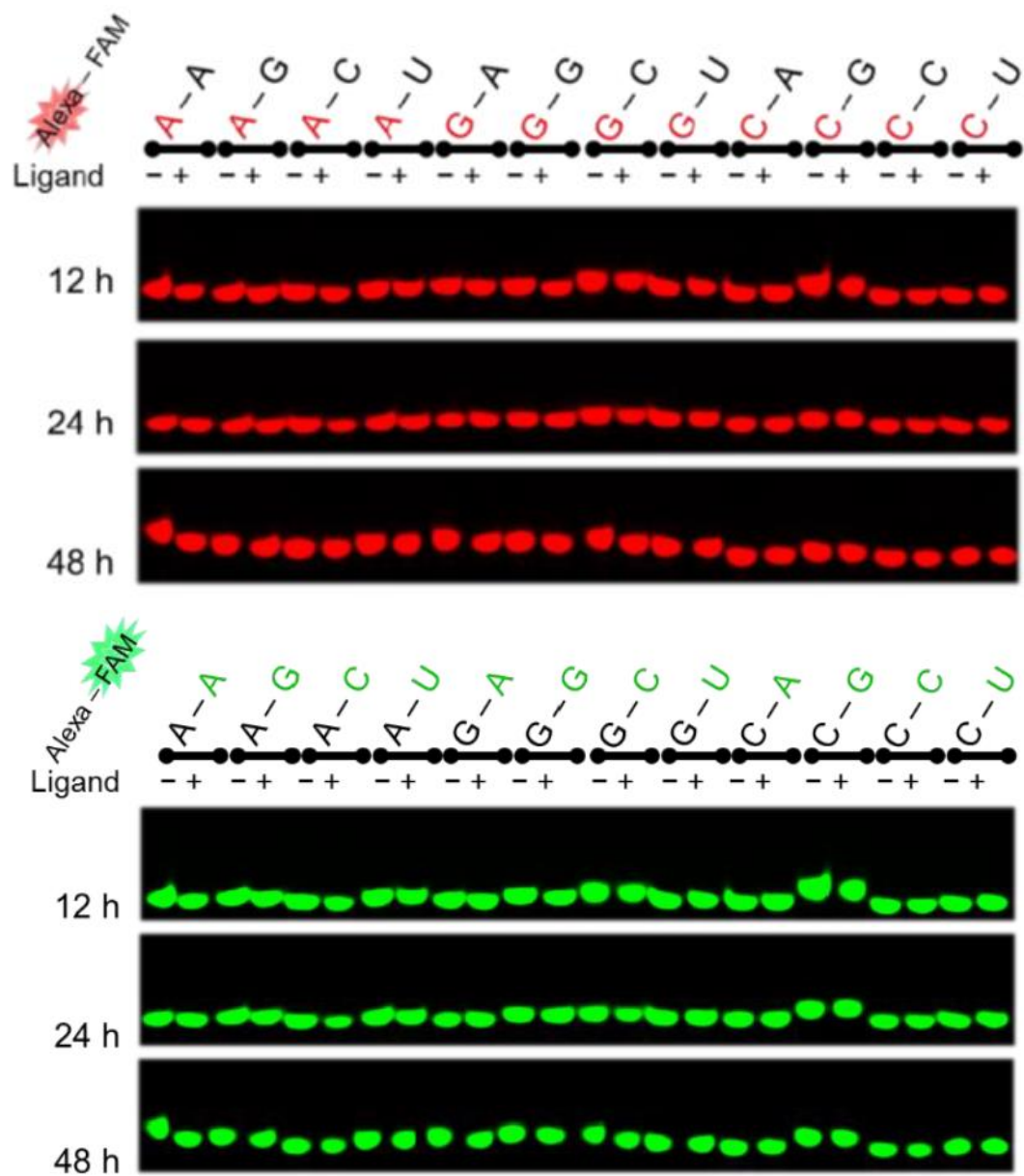
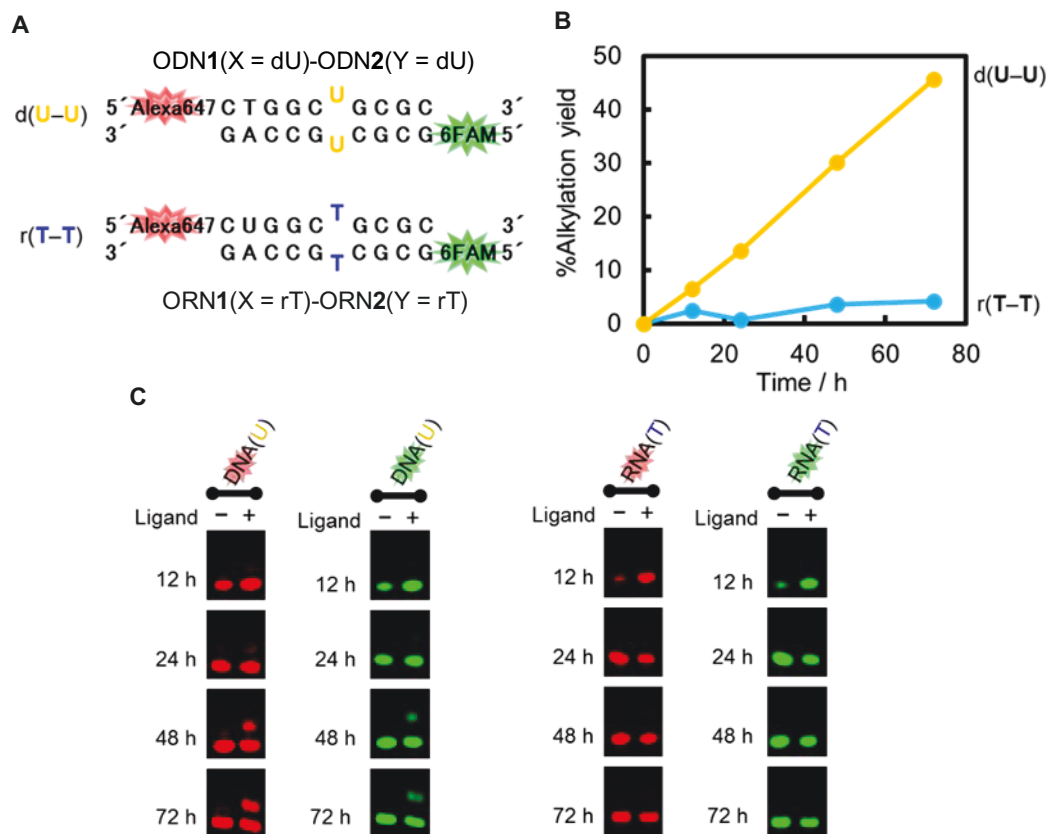


Figure S2. Gel image of the alkylation to DNA. The electrophoresis was performed on a 16% denaturing polyacrylamide gel containing 20% formamide.



**Figure S3. Gel image of the alkylation to RNA.** The electrophoresis was performed on a 16% denaturing polyacrylamide gel containing 20% formamide.

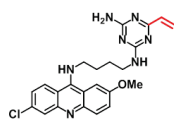


**Figure S4. Alkylation to the U-U base mismatched DNA or T-T base mismatched RNA duplex.** The reaction was carried out with ODN1(X = dU)-ODN2(Y = dU) duplex or ORN1(X = rT)-ORN2(Y = rT) (5  $\mu$ M) duplex and VAT-acridine conjugate **2** (100  $\mu$ M) in MES buffer (50 mM, pH 7.0) containing NaCl (100 mM) and 2% DMSO at 37  $^{\circ}$ C. (A) The sequence of the target duplex DNA or RNA. (B) Time course of the reaction yields. (C) Gel image of the alkylation reaction. The electrophoresis was performed on a 16% denaturing polyacrylamide gel containing 20% formamide.

ODN3 5'-FAM-CTGGCTGCGC-3'

ODN4 3'-GACCGTCGCG-5'

(20  $\mu$ M)



(100  $\mu$ M, 5 eq)

NaCl (100 mM)  
MES (50 mM, pH 7)  
37°C, 5 days

ODN5 5'-FAM-CTGGCT\*GCGC-3'

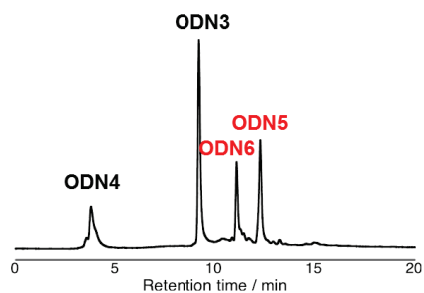
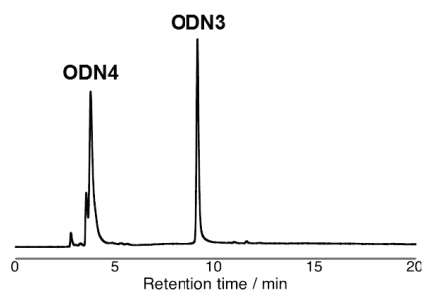
or

ODN6 3'-GACCGT\*CGCG-5'

MALDI-TOF/MS

ODN	Calcd. [M-H] <sup>-</sup>	Found
ODN5	4005.9	4006.4
ODN6	3477.2	3478.0

### HPLC profile

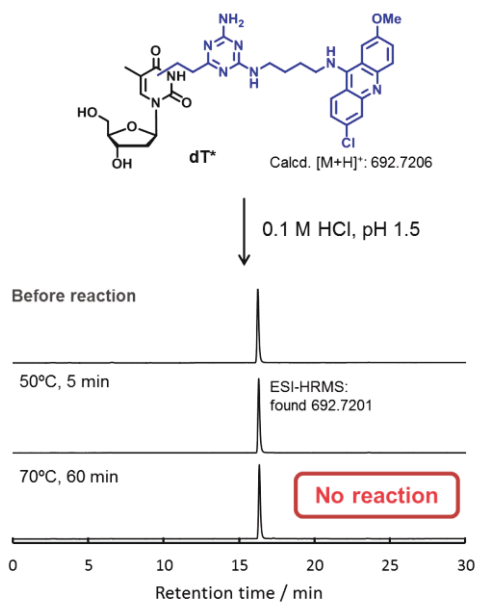
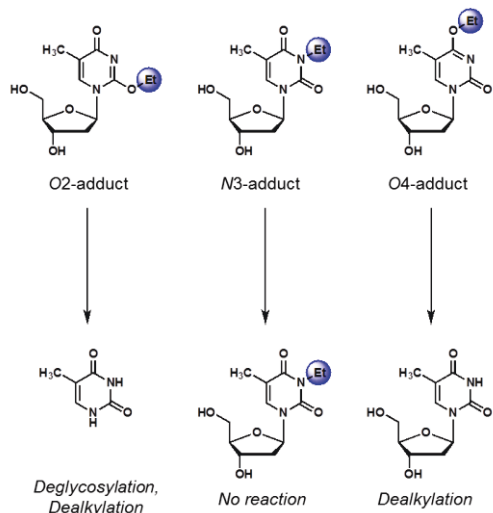


RP-HPLC conditions:

CAPCELL PAK C18 MG-II (4.0 x 250 mm), solvent A: 0.1 M TEAA, solvent B: MeCN, liner gradient: 1C to 30% B / 20 min, UV-detector:  
 $\lambda_{\text{obs}} = 254$  nm, flow rate: 1.0 mL/min, Temp.: 40°C.

**Figure S5. Synthesis and HPLC purification of the alkylated ODN5 and ODN6.**

Reactivity of ethylated thymidine under acidic conditions



RP-HPLC conditions:  
 CAPCELL PAK C18 MG-II (4.0 x 250 mm), solvent A: 0.1 M TEAA, solvent B: MeCN, liner gradient: 15 to 70% B / 30 min,  
 UV-detector:  $\lambda_{\text{abs}} = 254 \text{ nm}$ , flow rate: 1.0 mL/min, Temp.: 40°C.

Figure S6. HPLC profiles of dT\* after acid treatment.

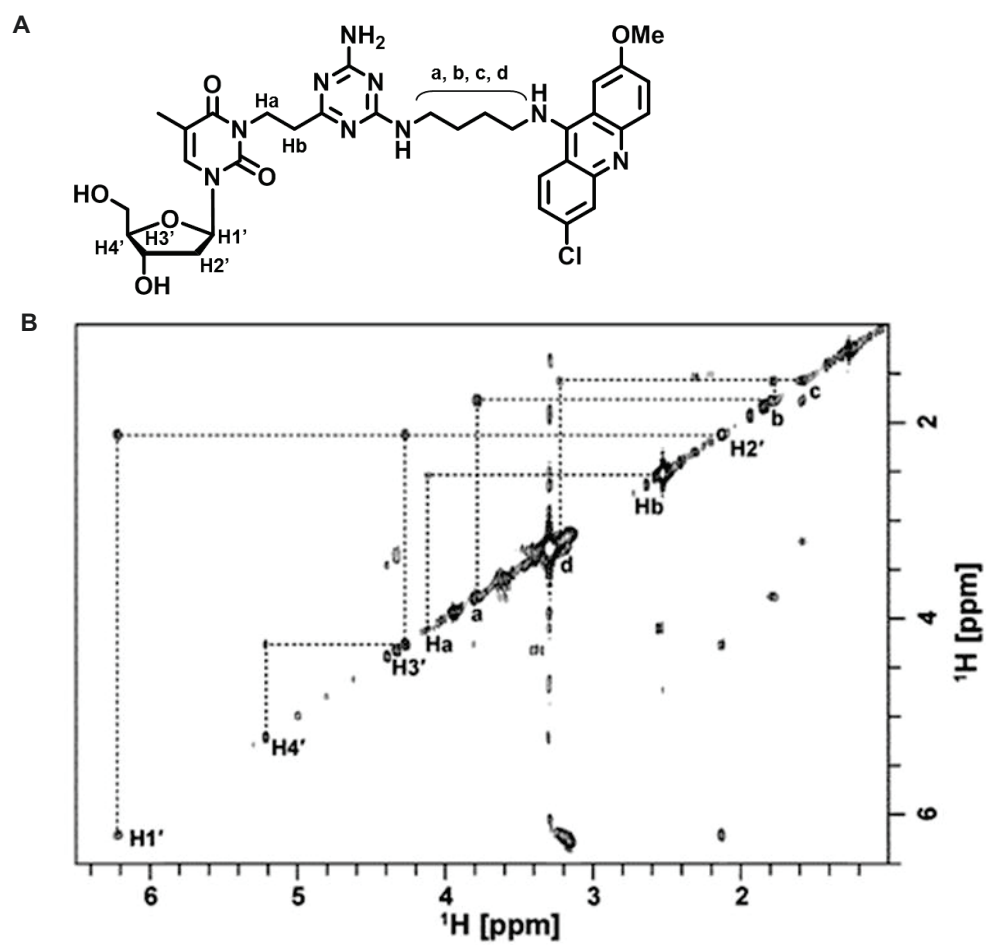
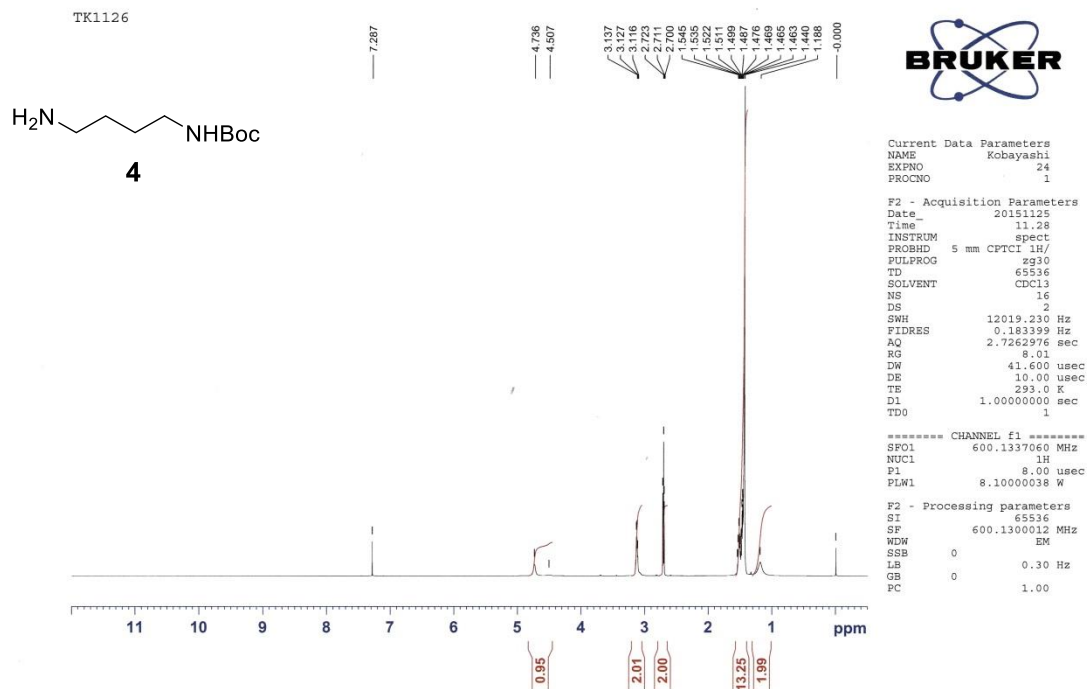


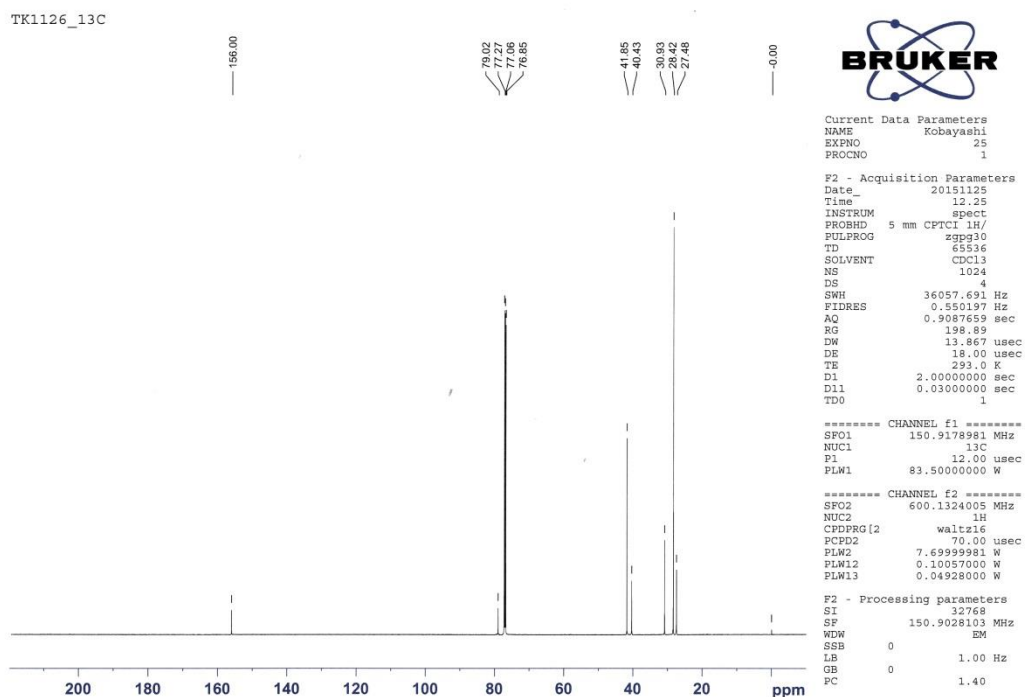
Figure S7. Chemical structure (A) and the COSY spectrum with assignments (B) of dT\*.



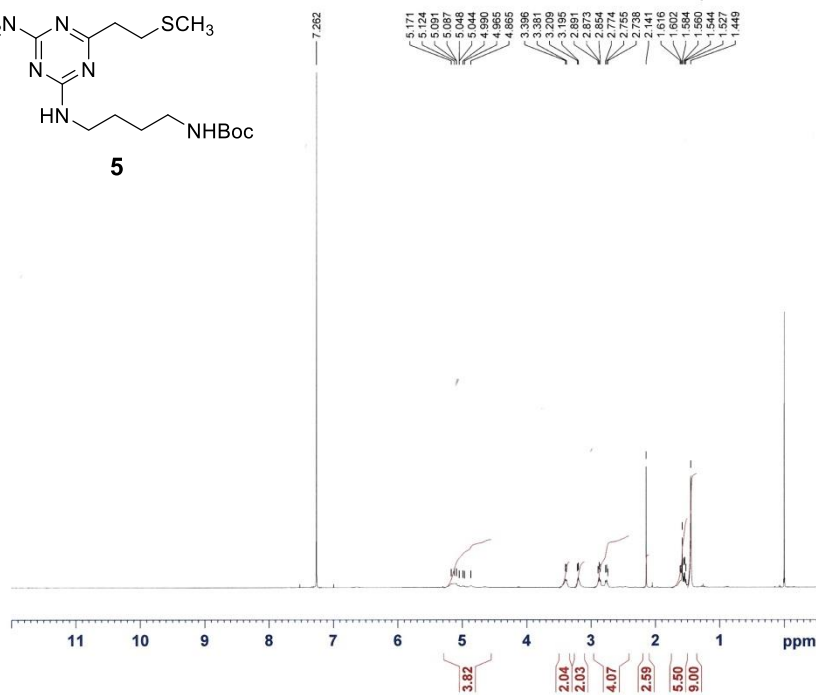
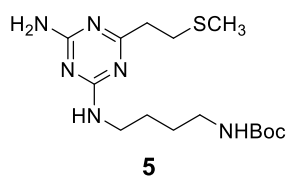
# 1H NMR



# 13C NMR



# <sup>1</sup>H NMR



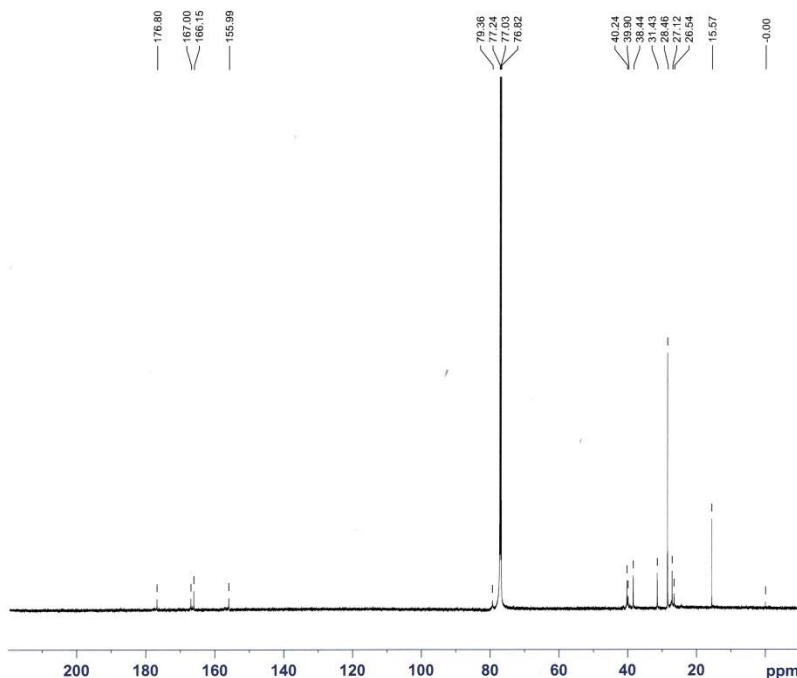
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 NS 16  
 DS 2  
 SWH 8223.685 Hz  
 FIDRES 0.125483 Hz  
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 RG 206.46  
 DW 60.800 usec  
 DE 6.50 usec  
 TE 296.1 K  
 D1 1.00000000 sec  
 TDO 1

===== CHANNEL f1 =====  
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 SFO1 400.1324710 MHz

F2 - Processing parameters  
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 GB 0  
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# <sup>13</sup>C NMR



Current Data Parameters  
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 PROCNO 1

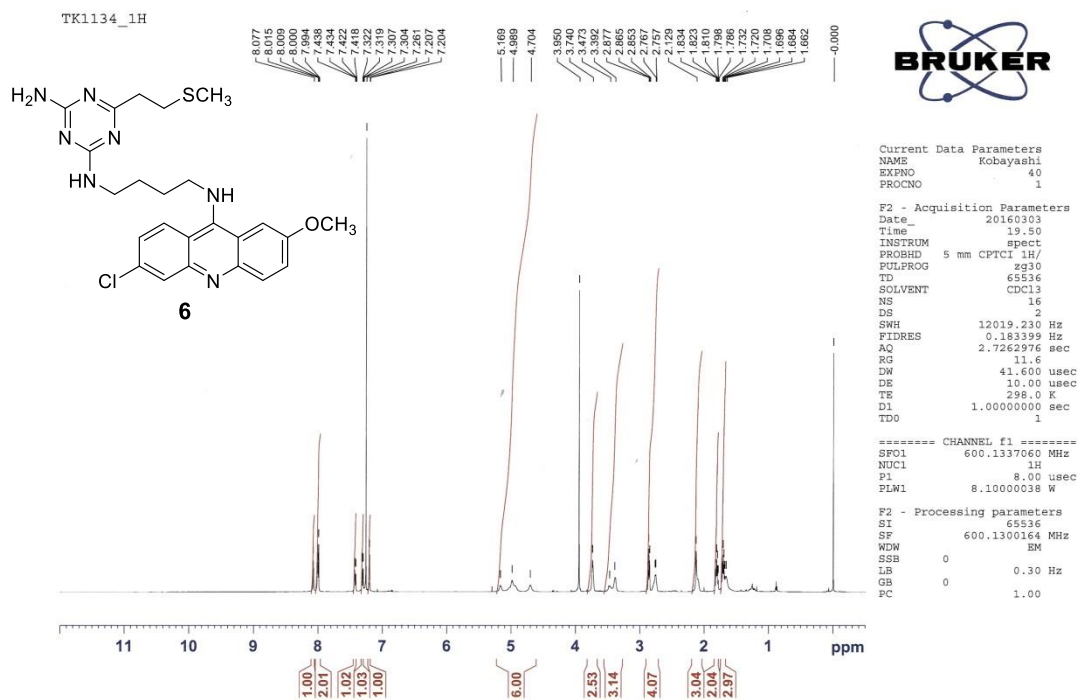
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 D1 2.00000000 sec  
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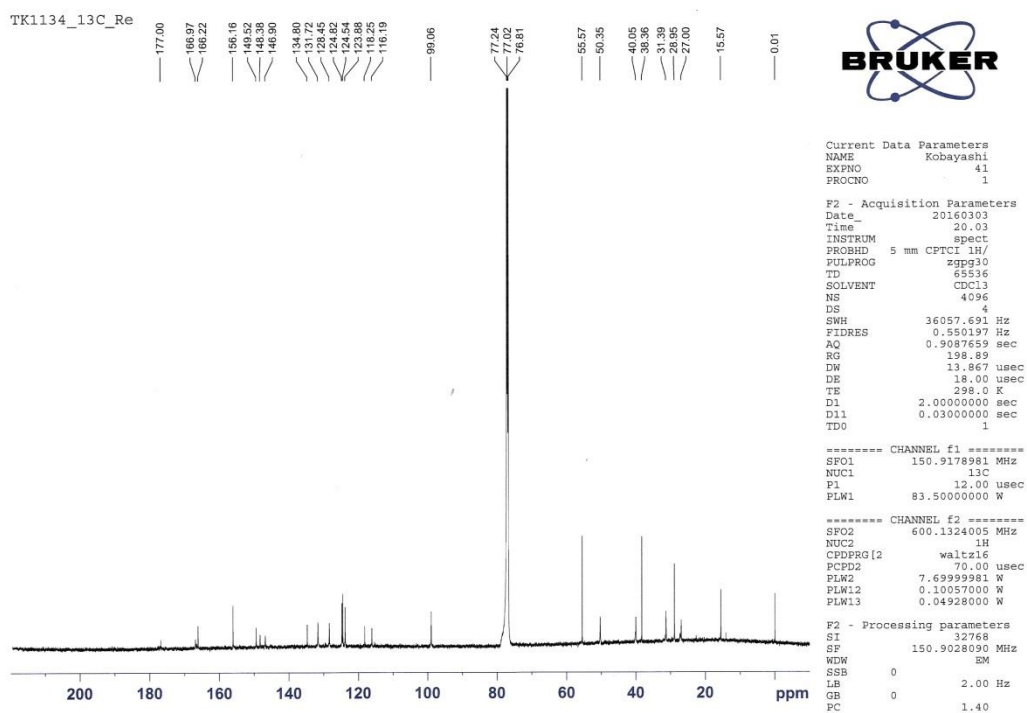
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 PLW2 7.69999981 W  
 PLW12 0.10057000 W  
 PLW13 0.04928000 W

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 GB 0  
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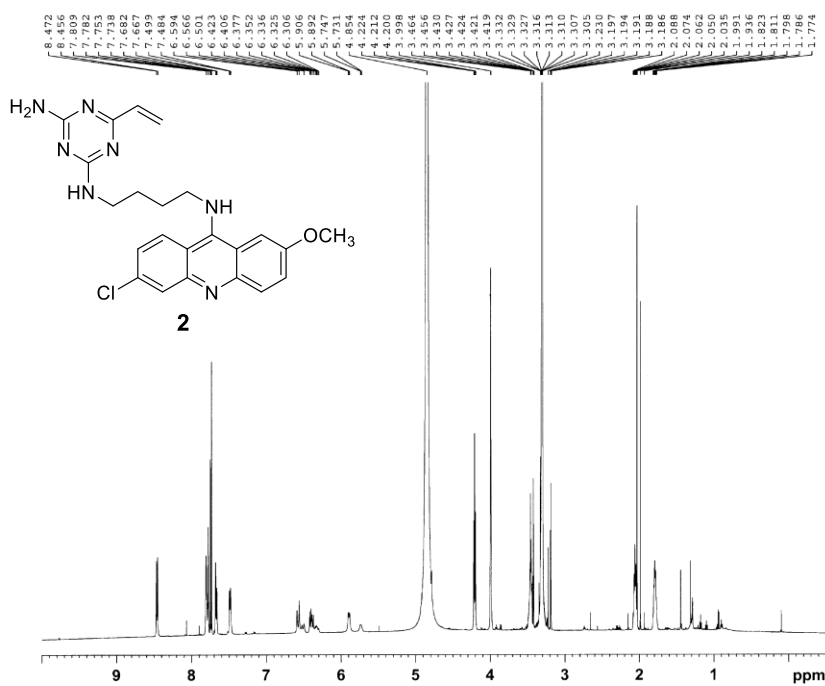
# 1H NMR



# 13C NMR



# <sup>1</sup>H NMR



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 PROCNO 4

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 SOLVENT MeOD  
 NS 1654  
 DS 2  
 SWH 12019.230 Hz  
 FIDRES 0.183399 Hz  
 AQ 2.7262976 sec  
 RG 14.68  
 DW 41.600 usec  
 DE 10.00 usec  
 TE 298.0 K  
 D1 1.00000000 sec  
 TDO 1

==== CHANNEL f1 =====  
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 P1 8.00 usec  
 PLW1 7.50000000 W

F2 - Processing parameters  
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