Supporting Information

Novel Propargyl-linked Bisubstrate Analogs as Tight-binding Inhibitors for Nicotinamide N-Methyltransferase

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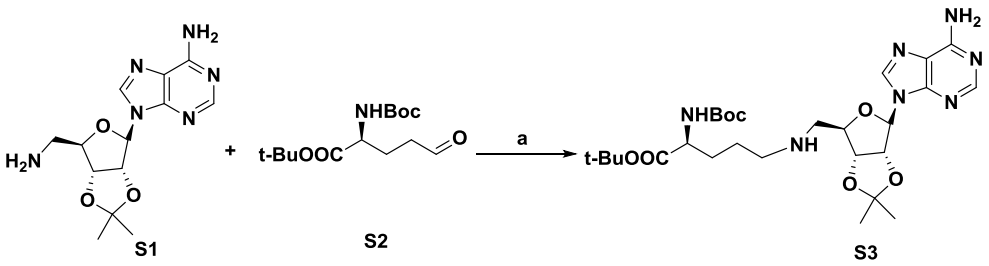
General procedures for the synthesis of **S3**

Scheme S1. Synthetic route of S3.[a]

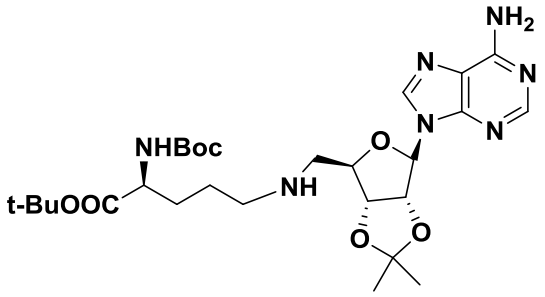


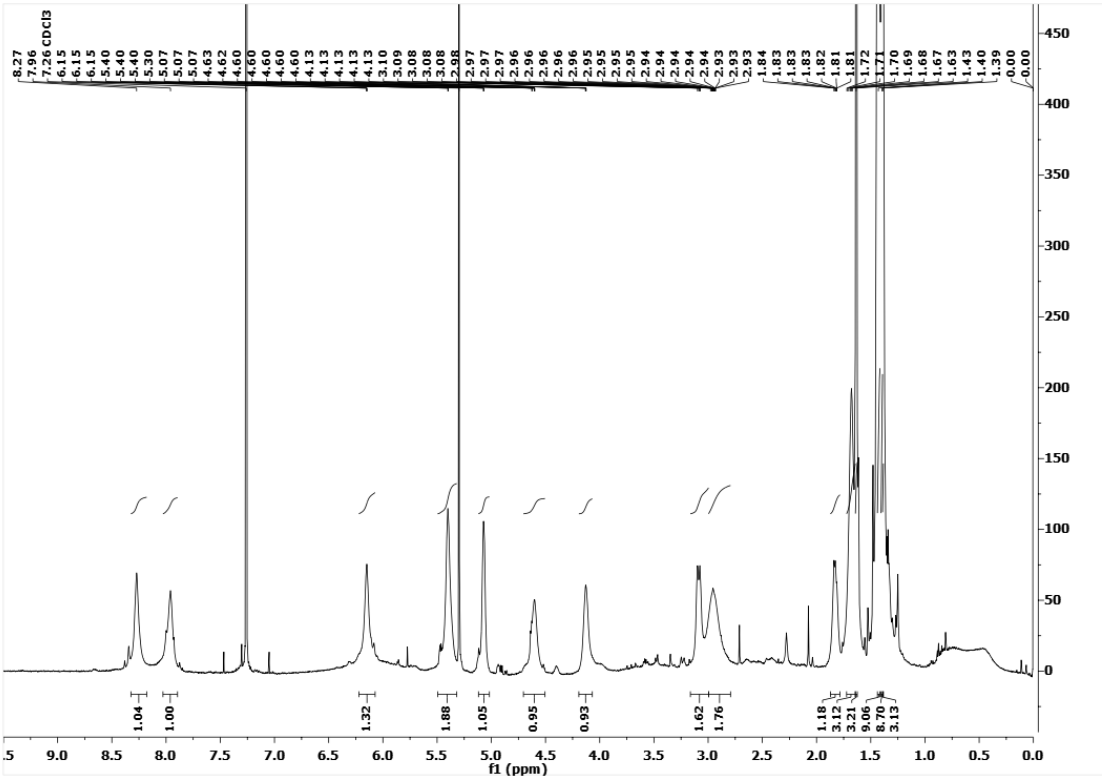
[a]Reagents and conditions: (a) $NaBH_3CN$, MeOH, r.t., 64%.

Tert-butyl (S)-5-((((3aR,4R,6S,6aR)-6-(6-amino-9H-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)amino)-2-((tert-butoxycarbonyl)amino)pentanoate (**S3**). To a solution of 9-((3aR,4S,6R,6aR)-6-(aminomethyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-9H-purin-6-amine **(S1)**[1] (0.33 g, 1.0 mmol) in methanol, tert-butyl (S)-2-((tert-butoxycarbonyl)amino)-5-oxopentanoate (**S2**)[2] (0.4 g, 1.4 mmol) was added in to the reaction. The mixture stirred for 30min at r.t.. Then $NaBH_3CN$ (94 mg, 1.5 mmol) was add to the reaction. After 6 h, the solvent was removed in vacuum and the residue was purified by column chromatography (CH_2Cl_2:MeOH = 25: 1) to get 0.4 g product (64%). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.27 (s, 1H), 7.96 (s, 1H), 6.22 – 6.07 (m, 1H), 5.50 – 5.32 (m, 2H), 5.12 – 5.02 (m, 1H), 4.70 – 4.50 (m, 1H), 4.19 – 4.07 (m, 1H), 3.16 – 3.00 (m, 2H), 3.00 – 2.79 (m, 2H), 1.87 – 1.78 (m, 1H), 1.72-1.65 (m, 3H), 1.63 (s, 3H), 1.43 (s, 9H), 1.40 (s, 9H), 1.39 (s, 3H).

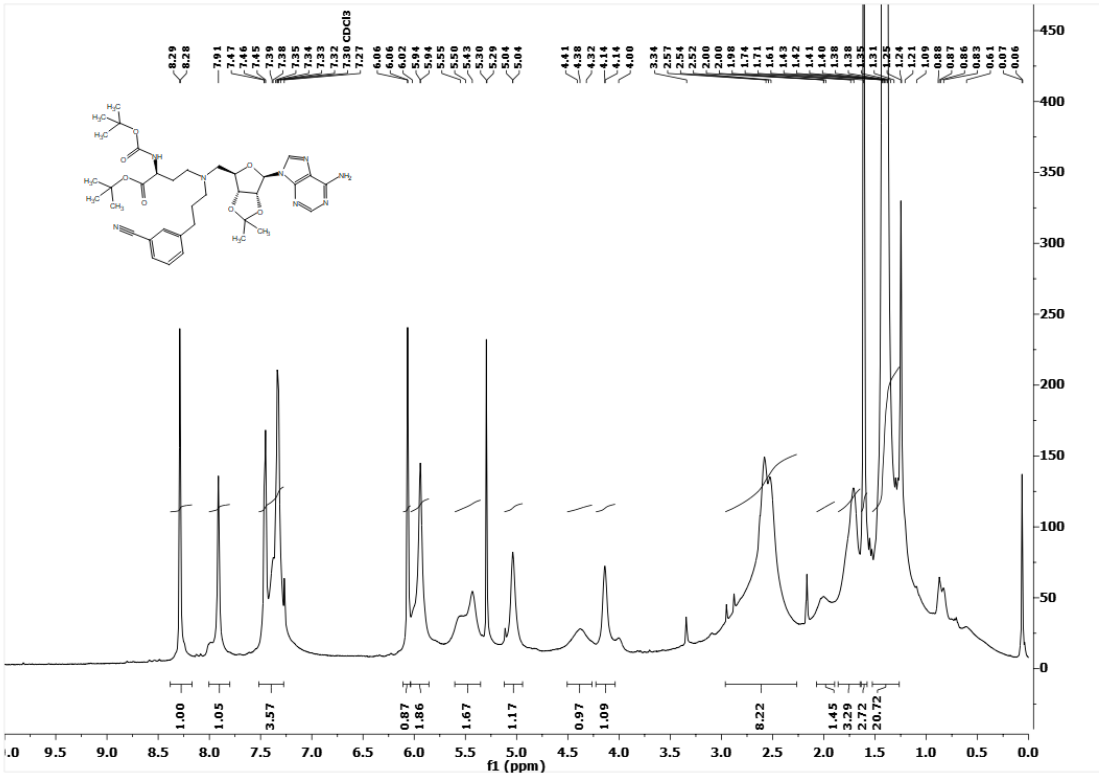
NMR spectra of compound **S3, 6a-j, 9a-d**, and **12a-b**

HNMR of **S3**





HNMR of **6a**



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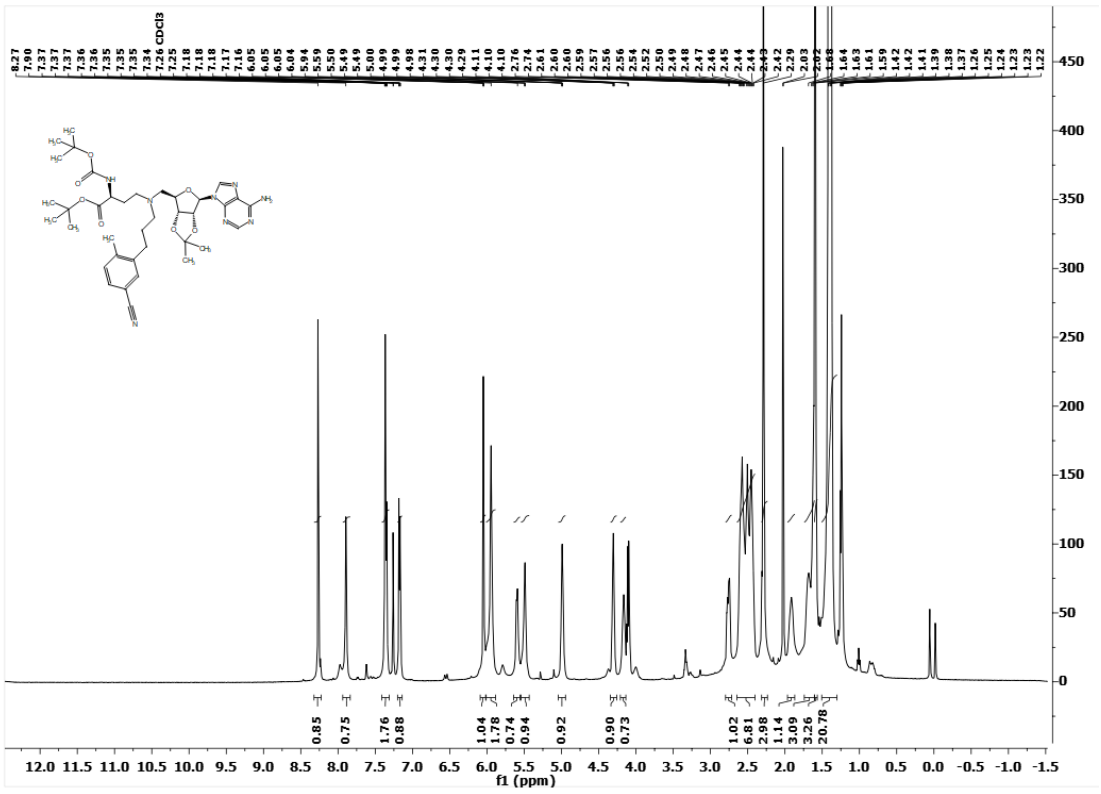

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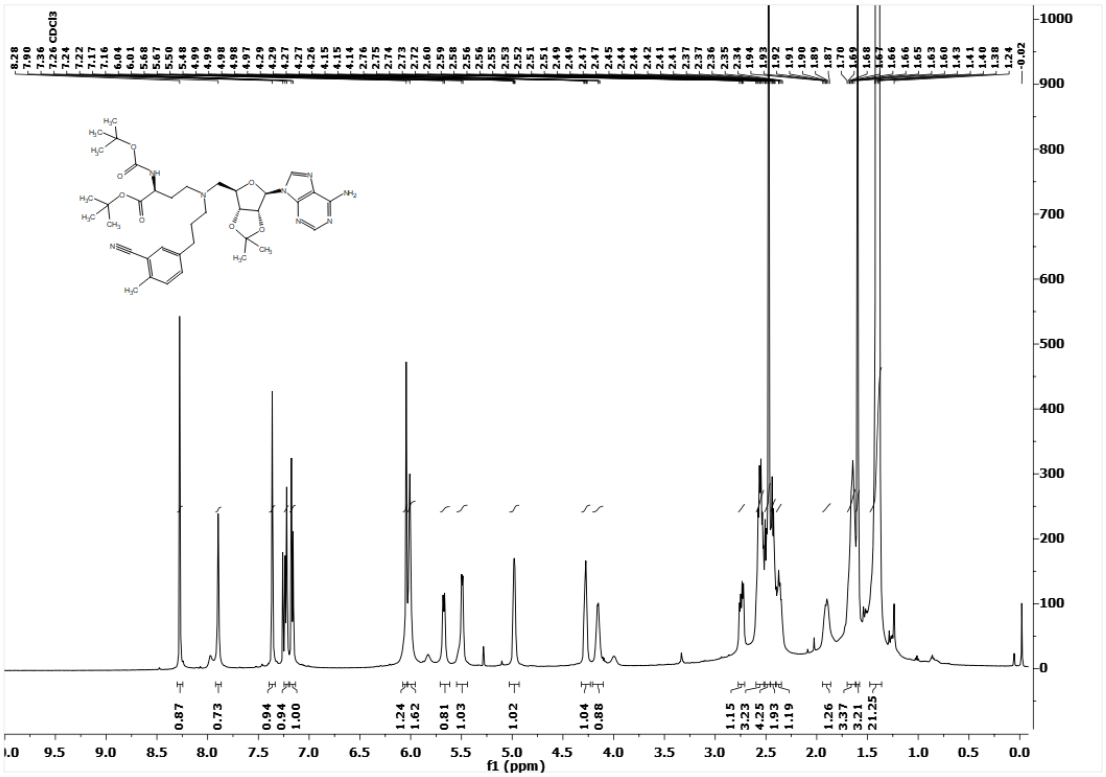

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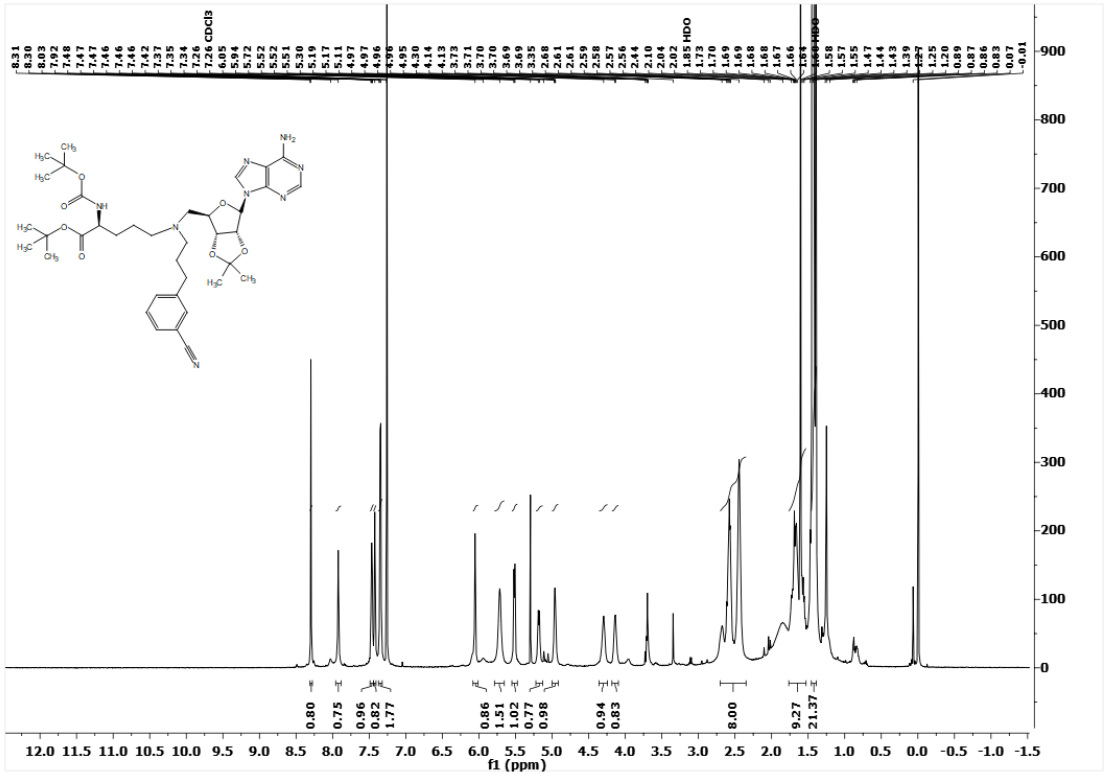
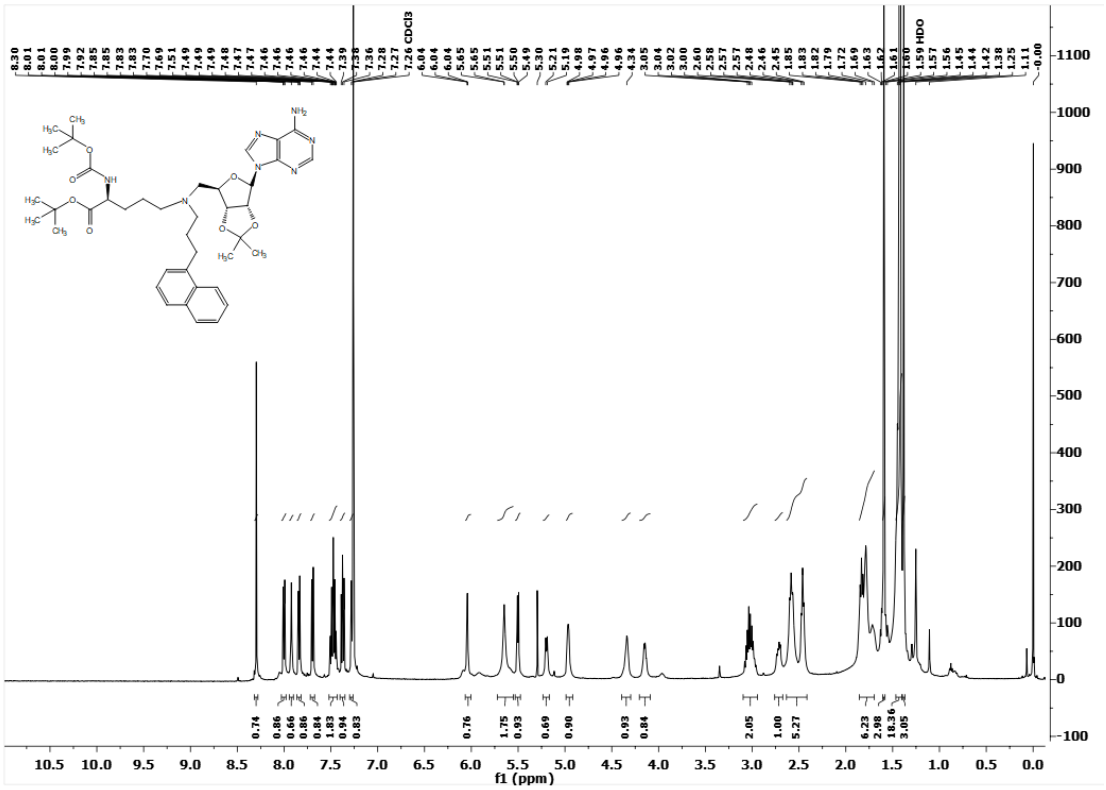


HNMR of **6e**



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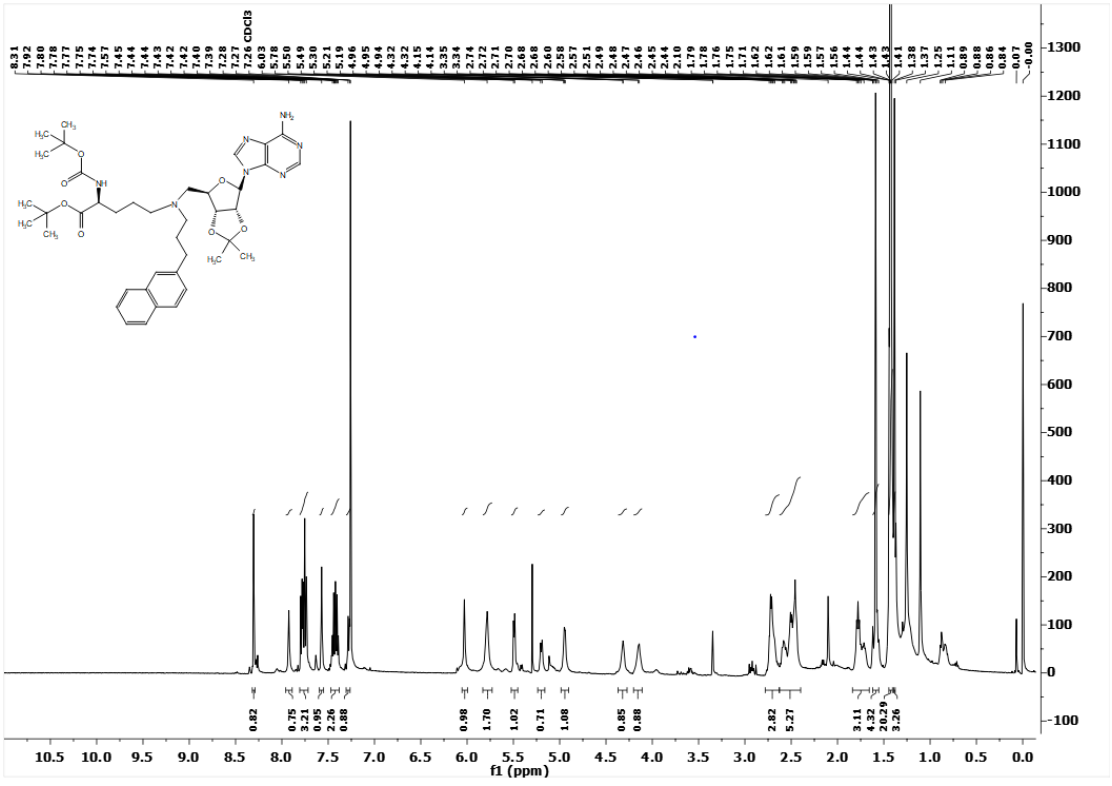

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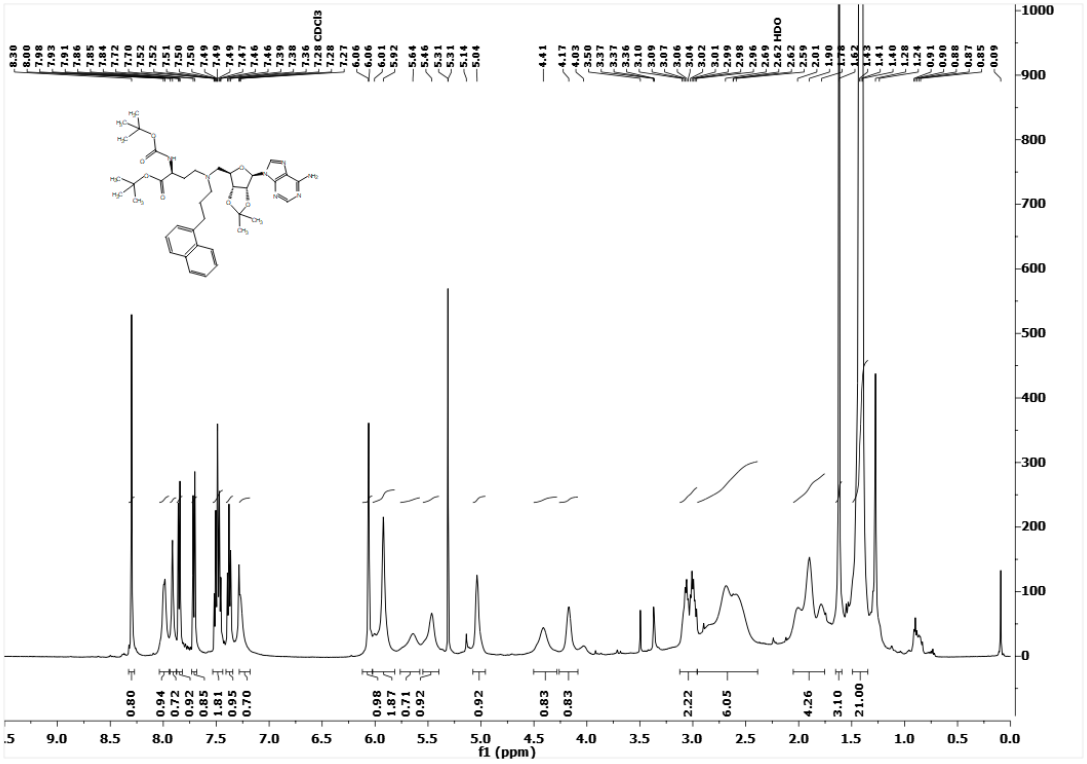

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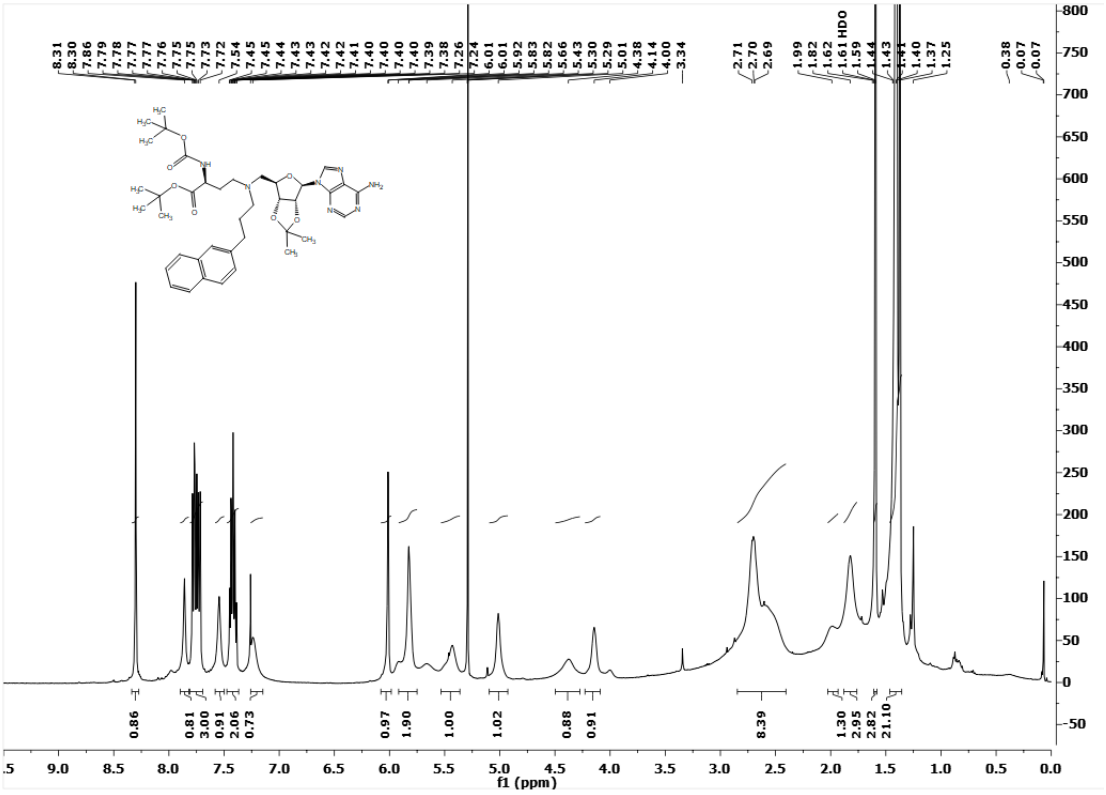

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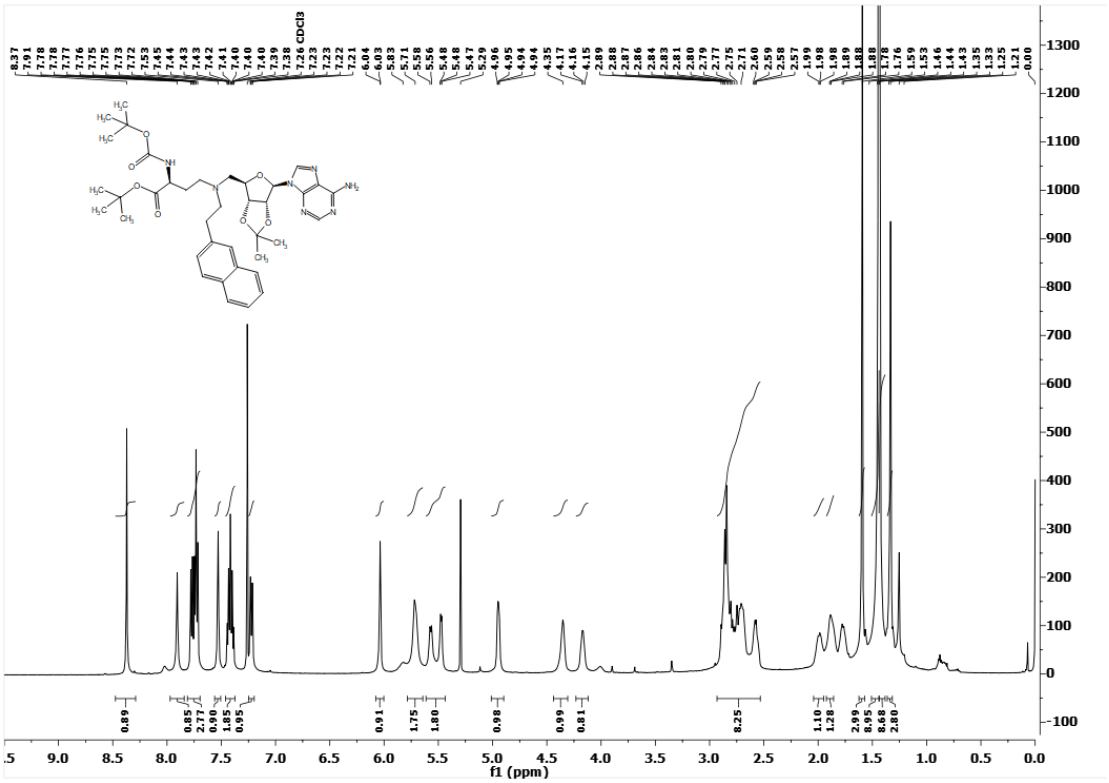

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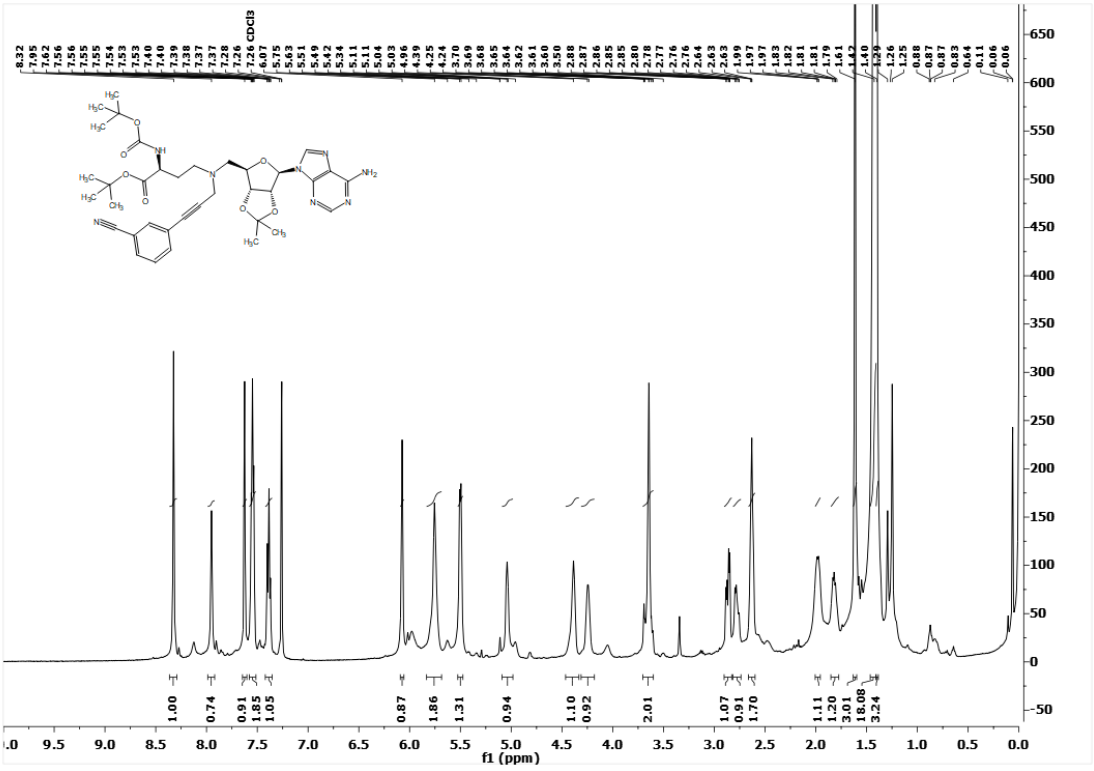

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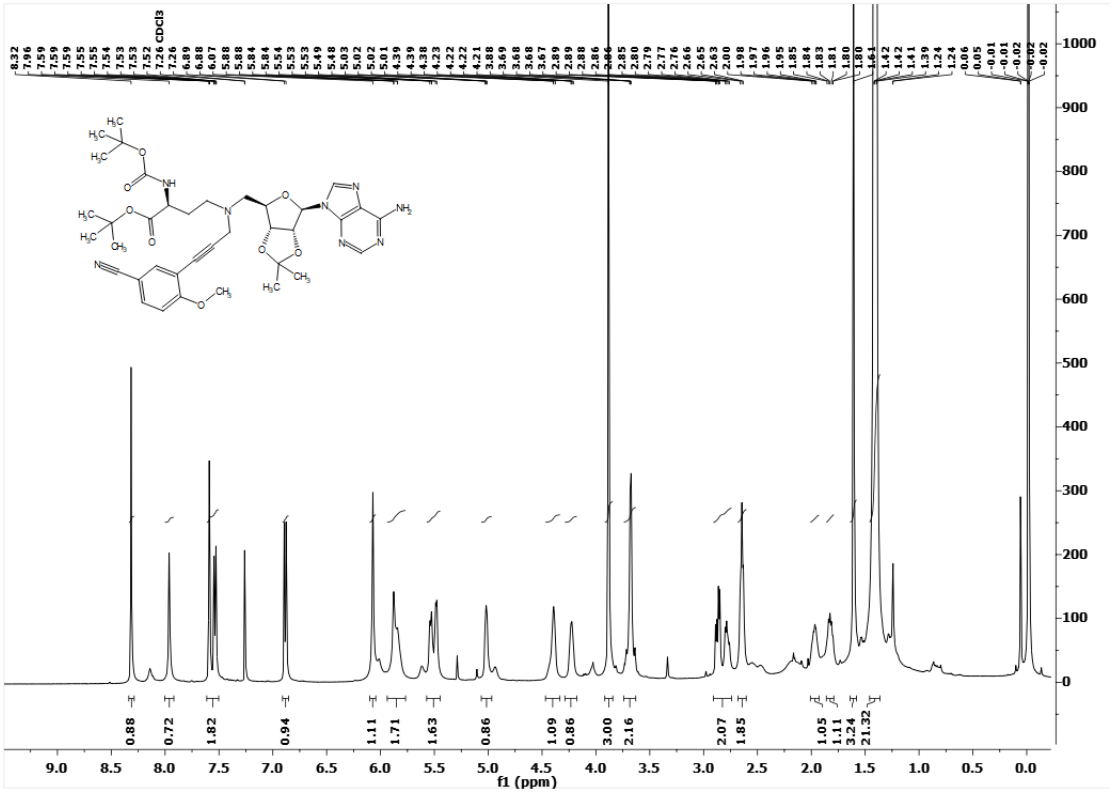

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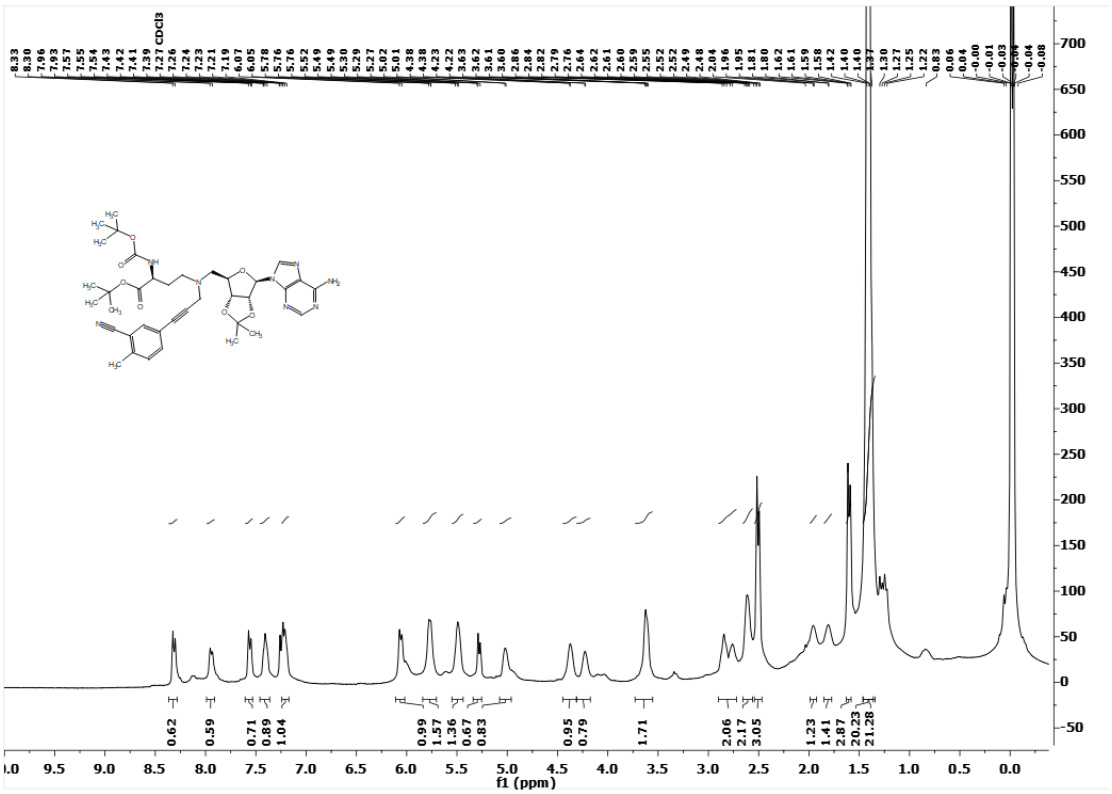

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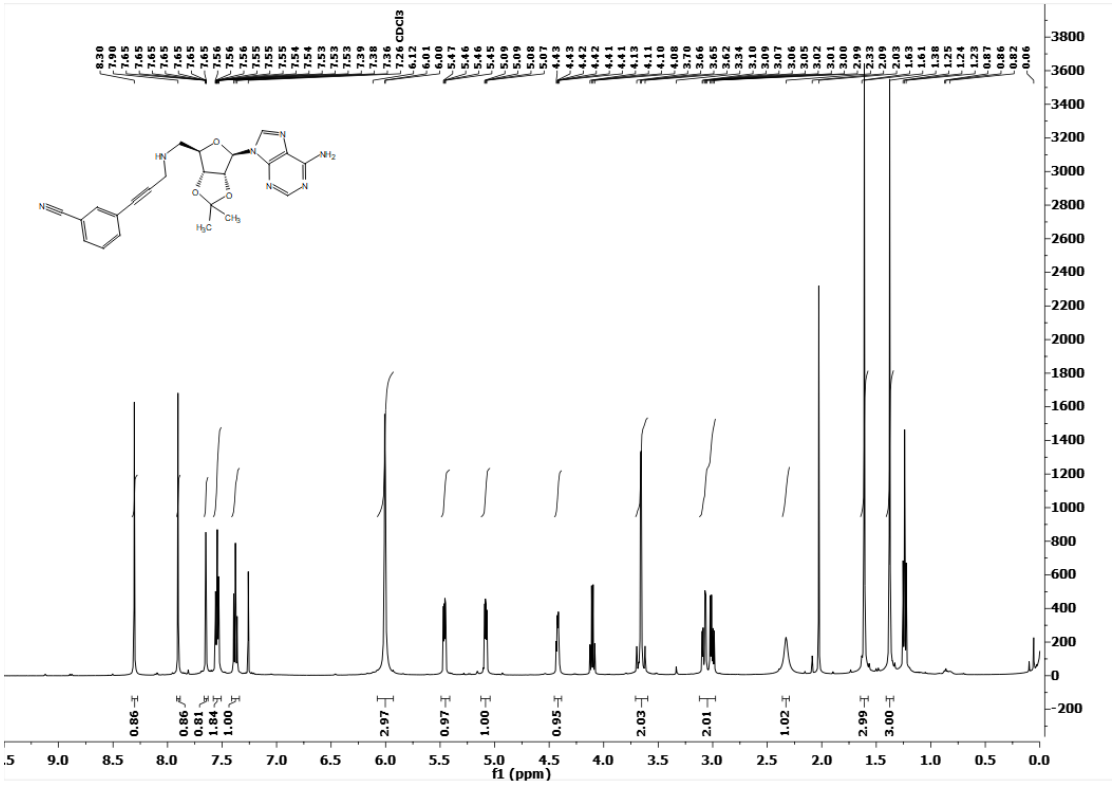

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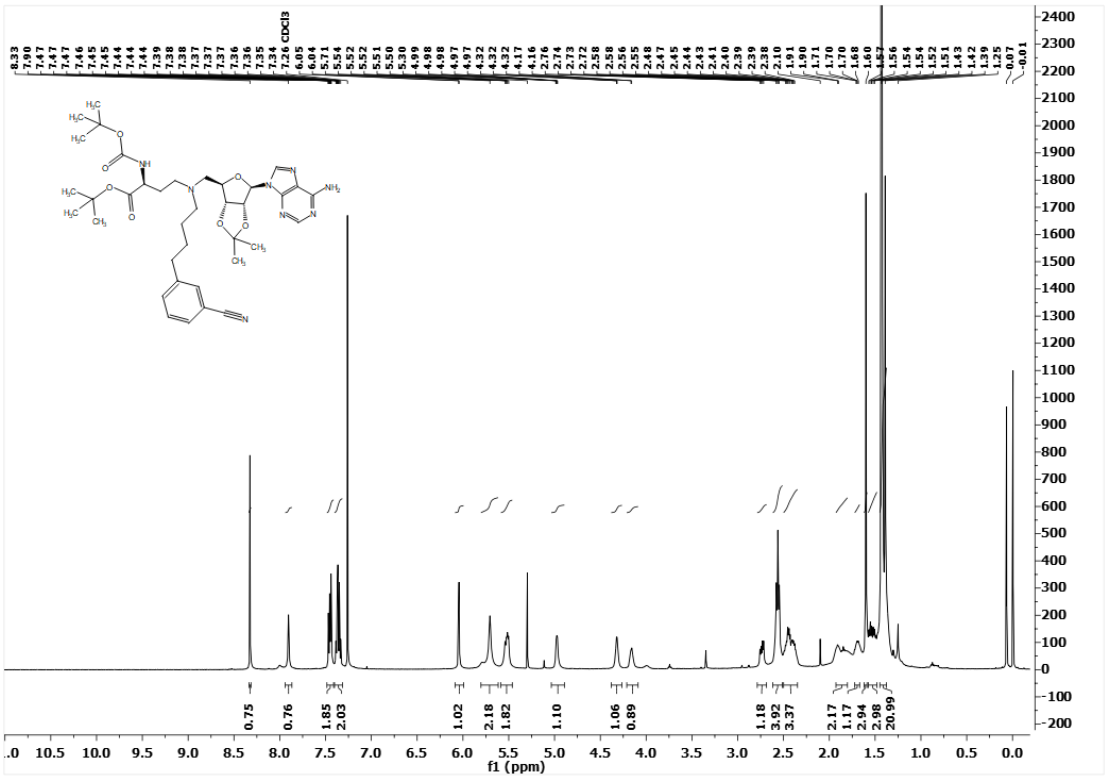

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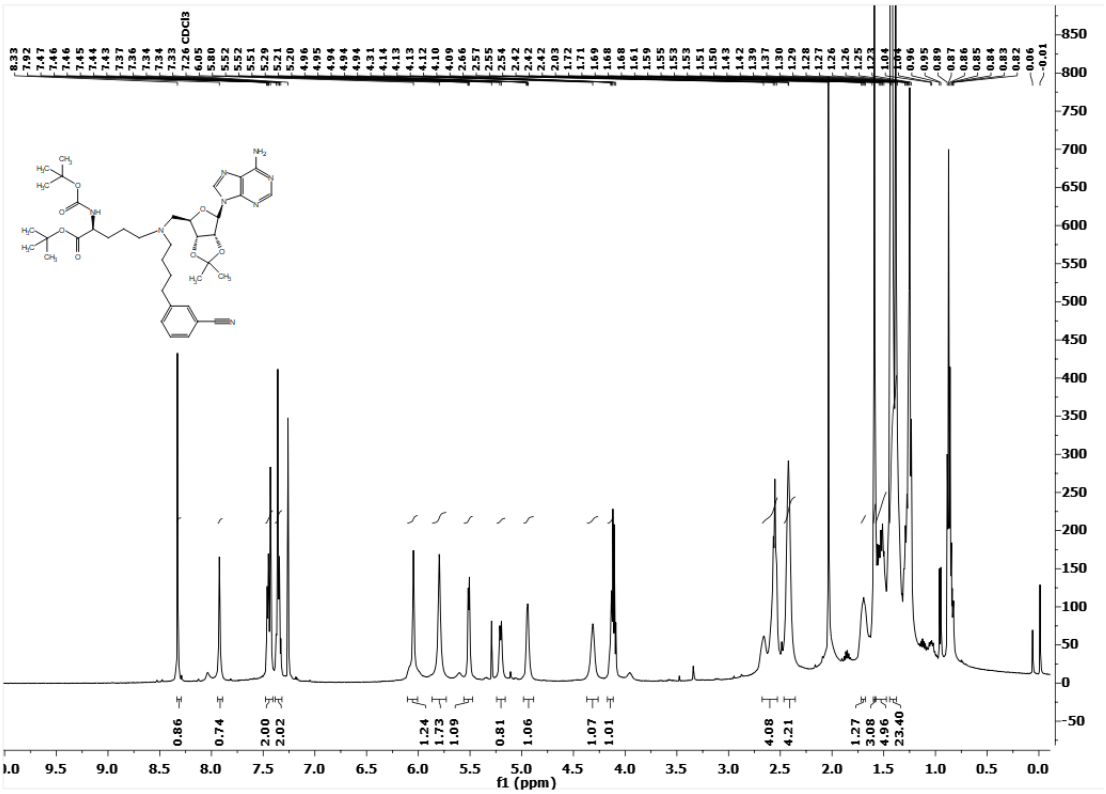
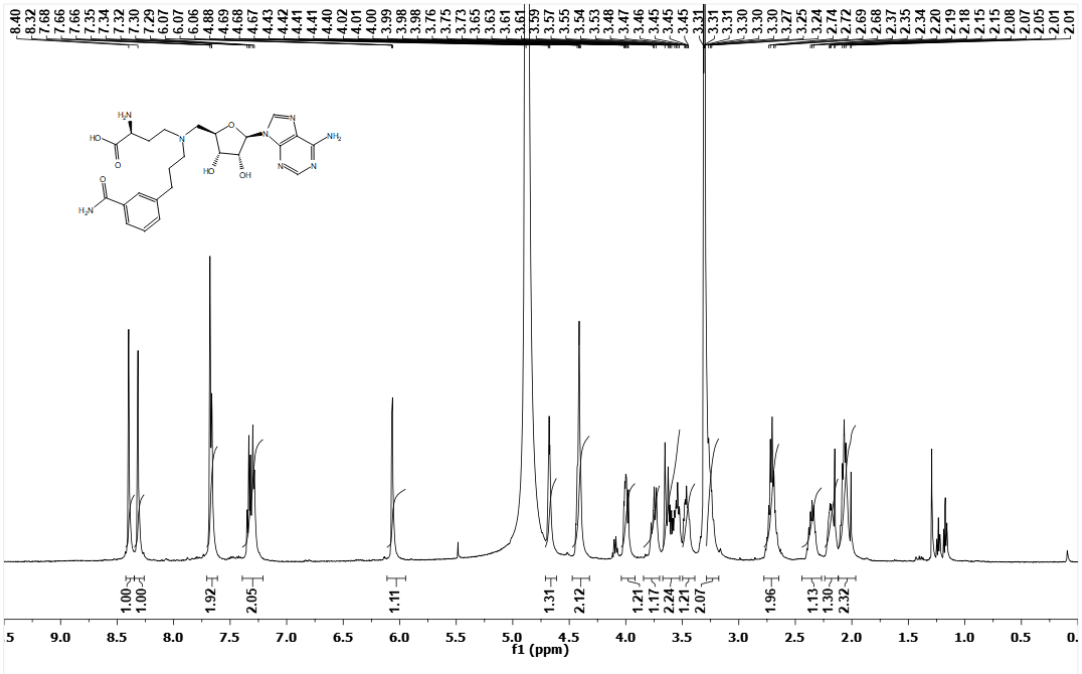


NMR, MALDI-MS and HPLC spectra of compound **1a-j, 1a*, 2a-d, 2a*** and **3a-b**

NMR, MALDI-MS and HPLC spectra of compound **1a**

HNMR



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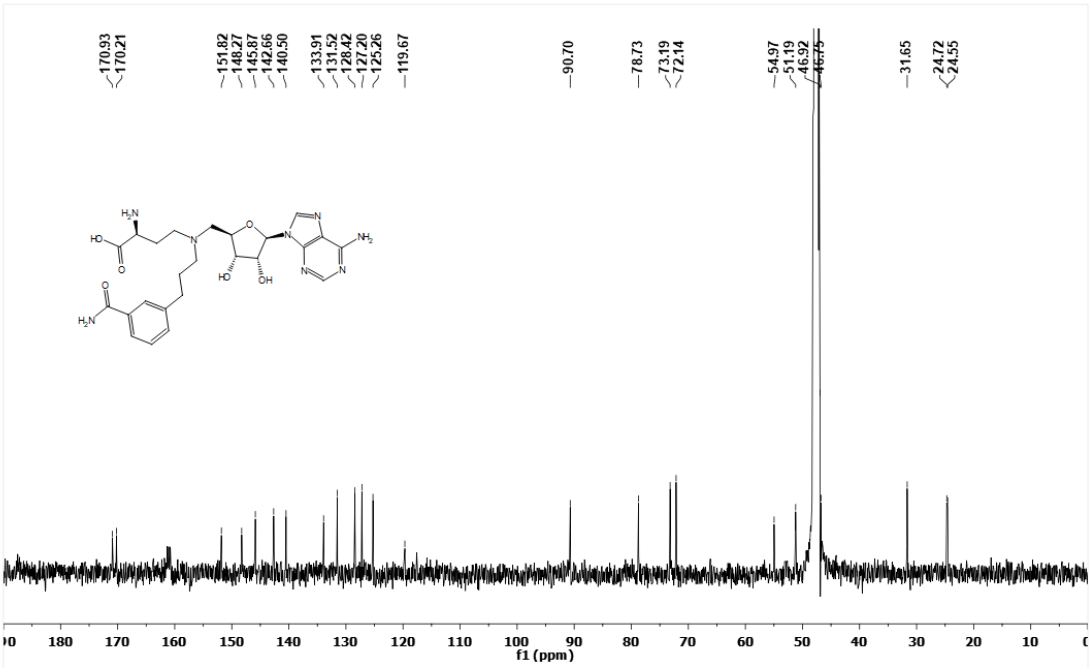
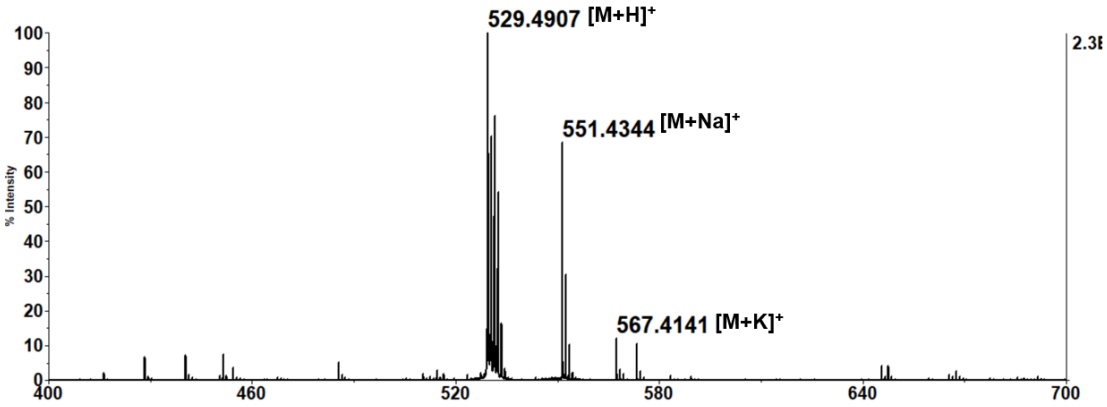


MALDI-MS



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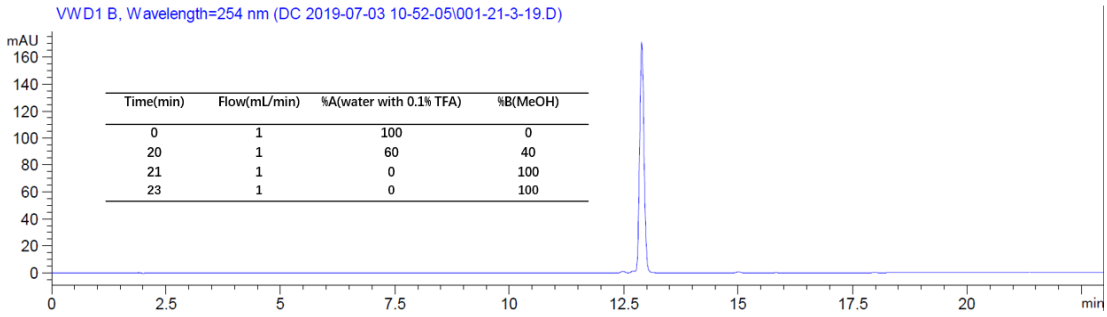
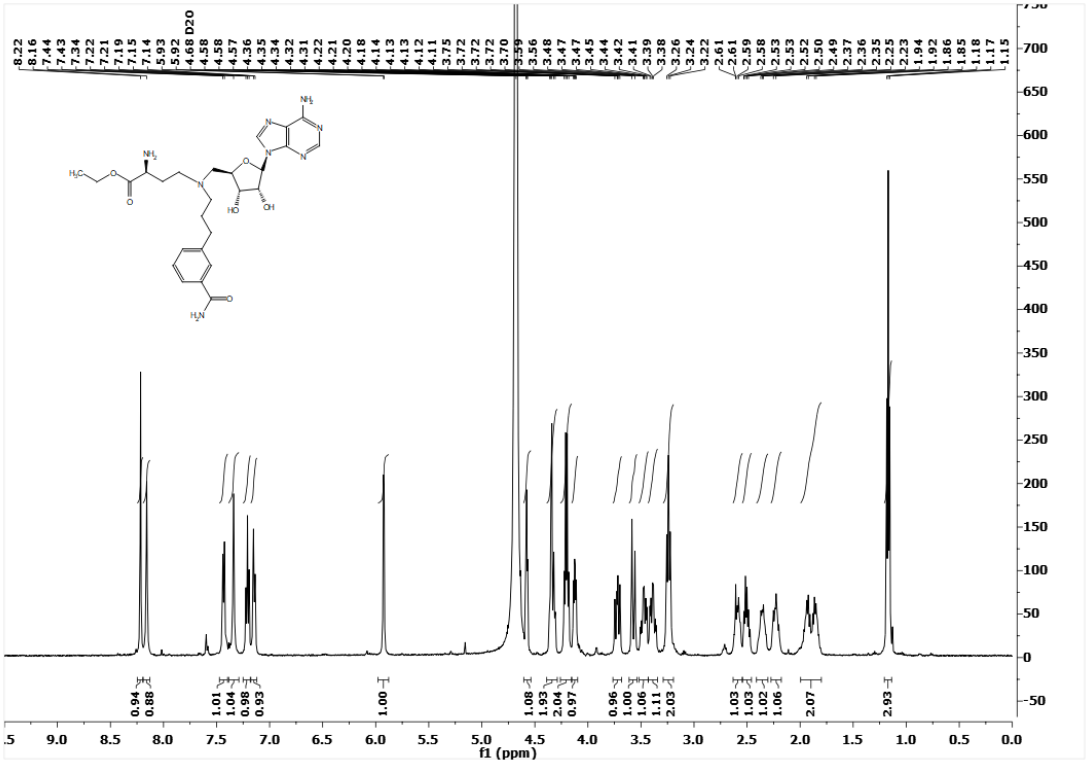


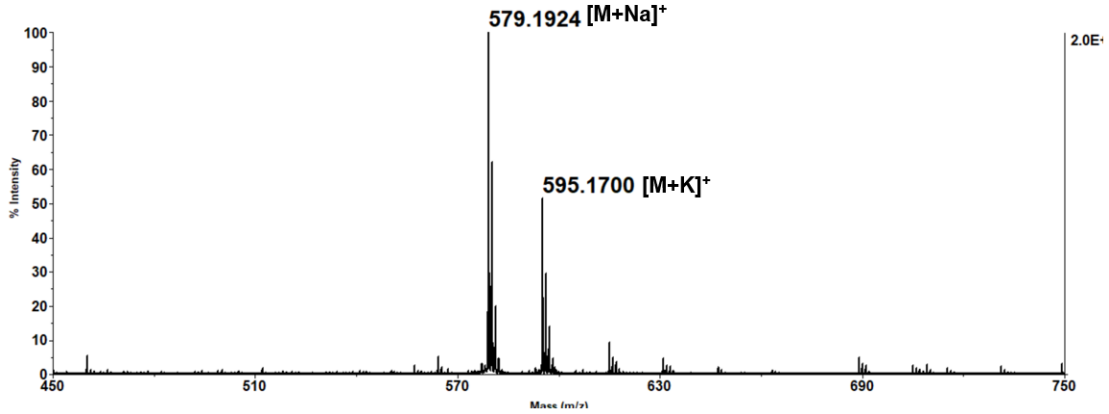
Time(min)	Flow(mL/min)	%A(water with 0.1% TFA)	%B(MeOH)
0	1	100	0
20	1	60	40
21	1	0	100
23	1	0	100

NMR, MALDI-MS and HPLC spectra of compound **1a***

H-NMR



MALDI-MS



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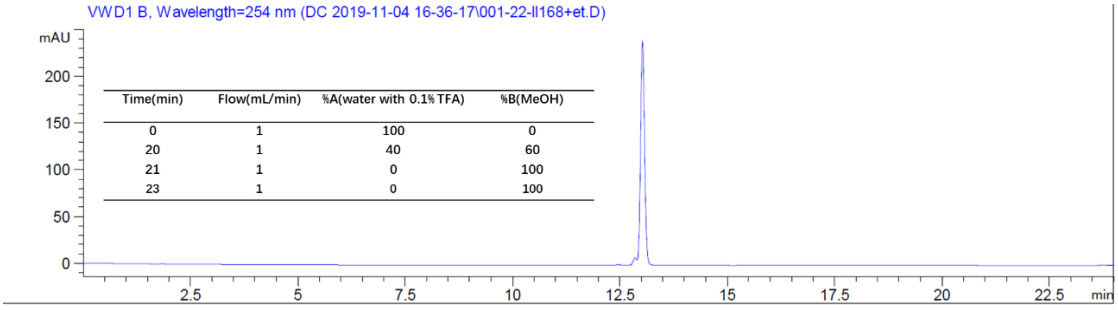

Time(min)	Flow(mL/min)	%A(water with 0.1% TFA)	%B(MeOH)
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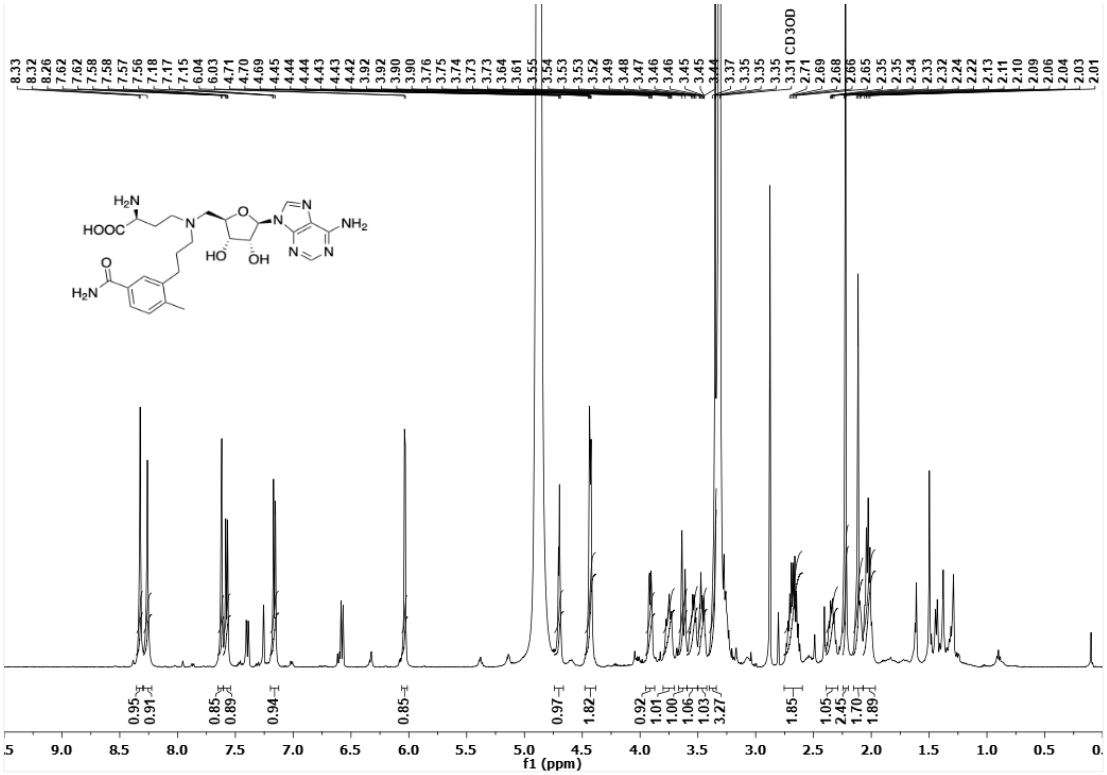
21	1	0	100
23	1	0	100

NMR, MALDI-MS and HPLC spectra of compound **1b**

HNMR



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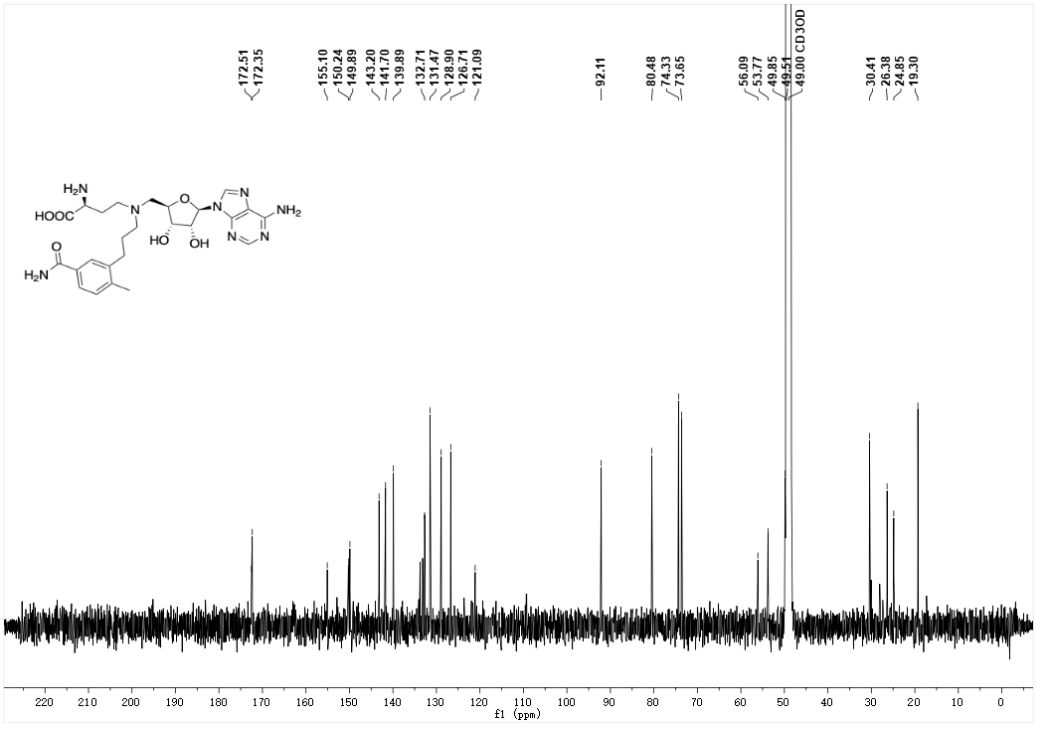
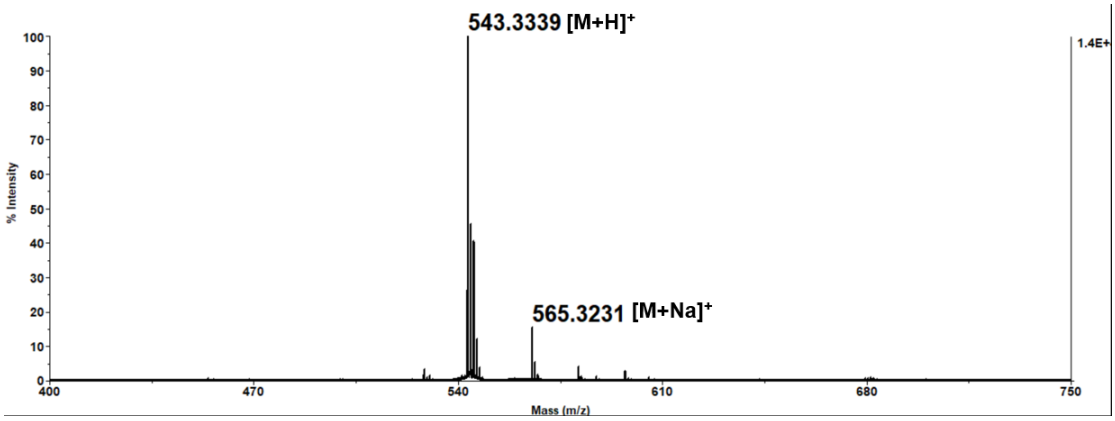


MALDI-MS



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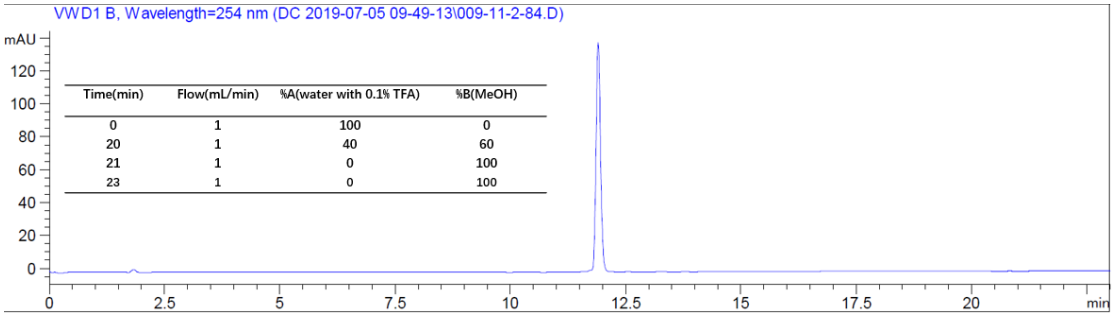

Time(min)	Flow(mL/min)	%A(water with 0.1% TFA)	%B(MeOH)
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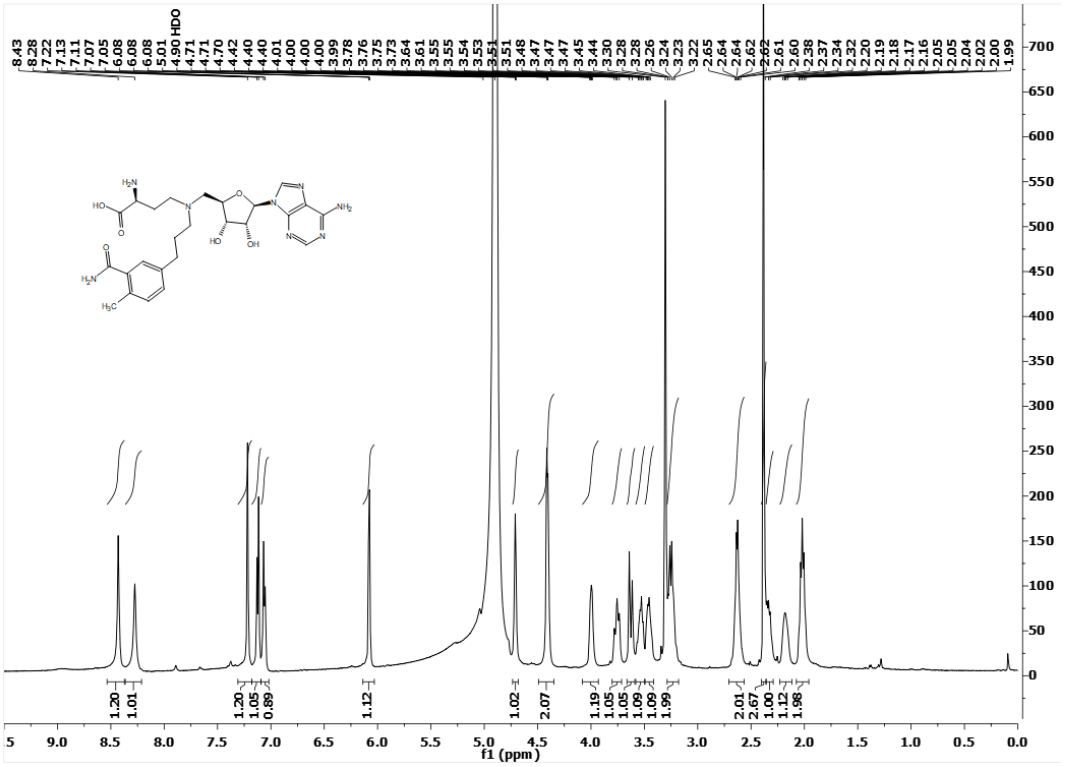
21	1	0	100
23	1	0	100

NMR, MALDI-MS and HPLC spectra of compound **1c**

HNMR



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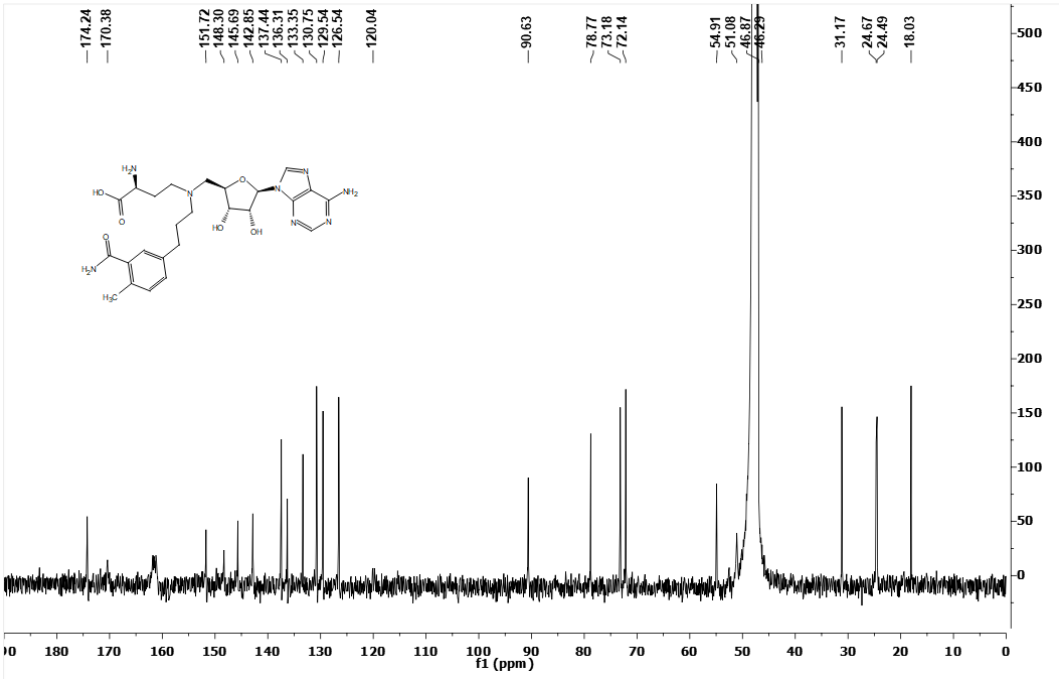

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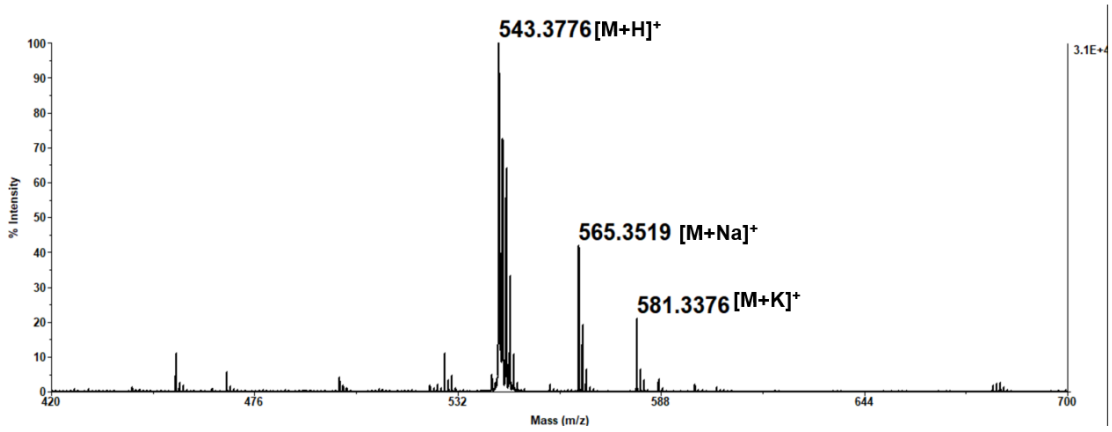

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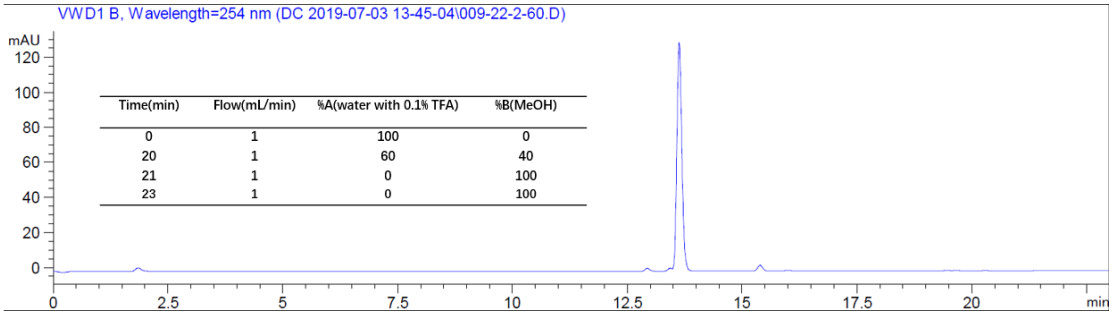

Time(min)	Flow(mL/min)	%A(water with 0.1% TFA)	%B(MeOH)
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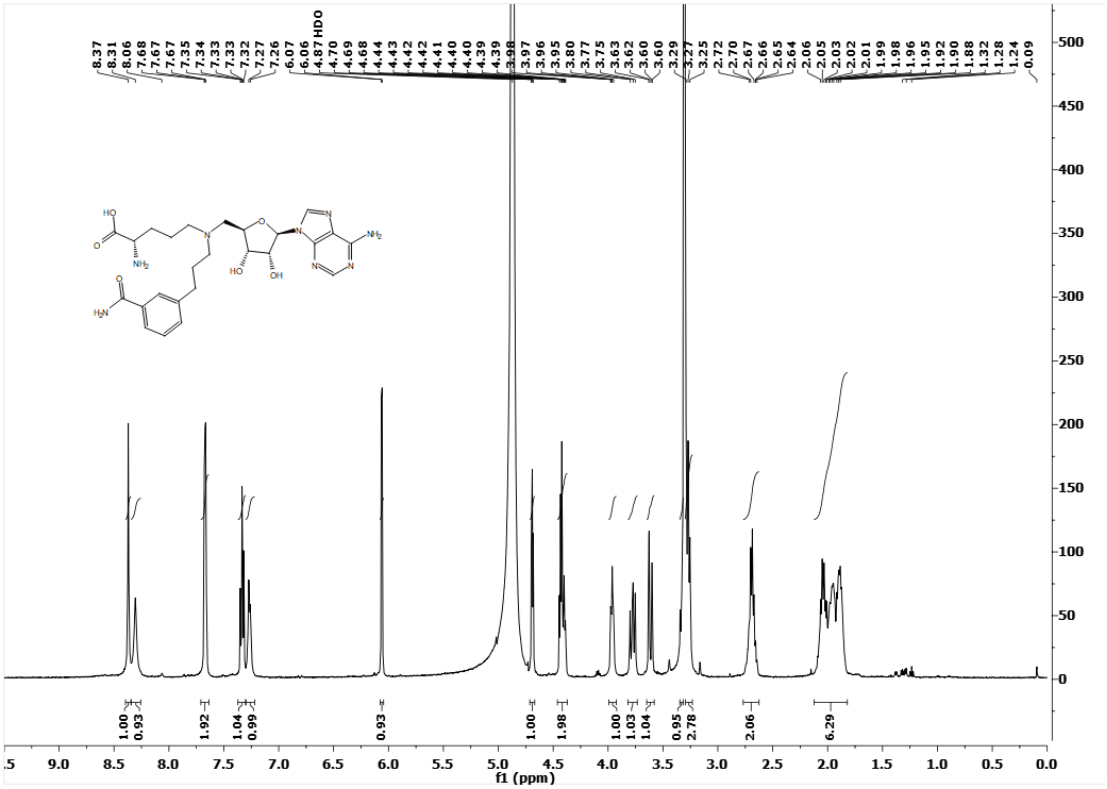
NMR, MALDI-MS and HPLC spectra of compound **1d**

HNMR



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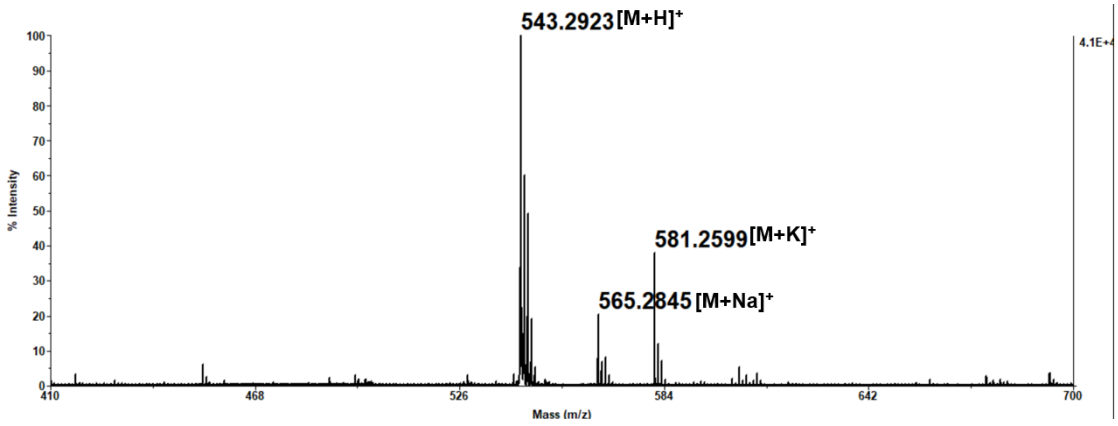

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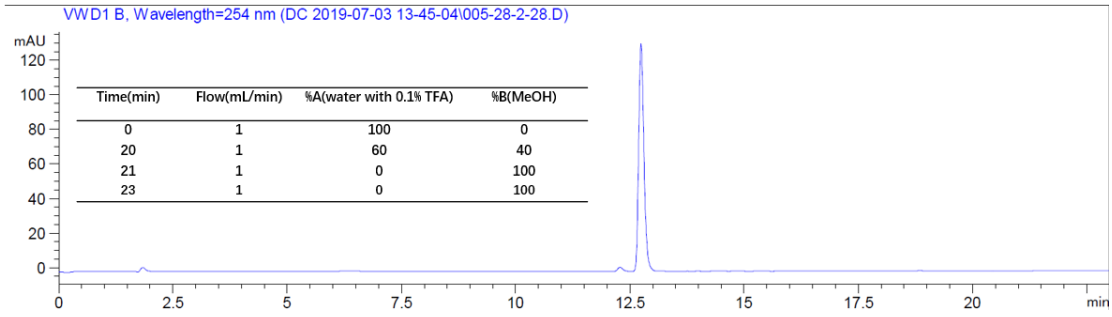

Time(min)	Flow(mL/min)	%A(water with 0.1% TFA)	%B(MeOH)
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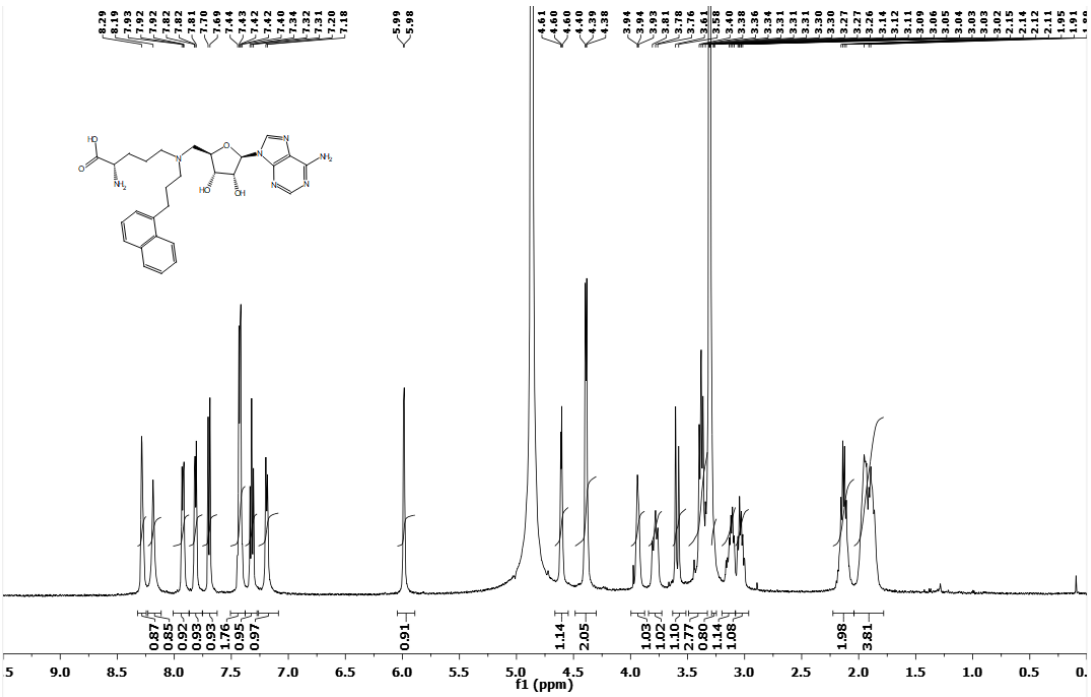
NMR, MALDI-MS and HPLC spectra of compound **1e**

HNMR



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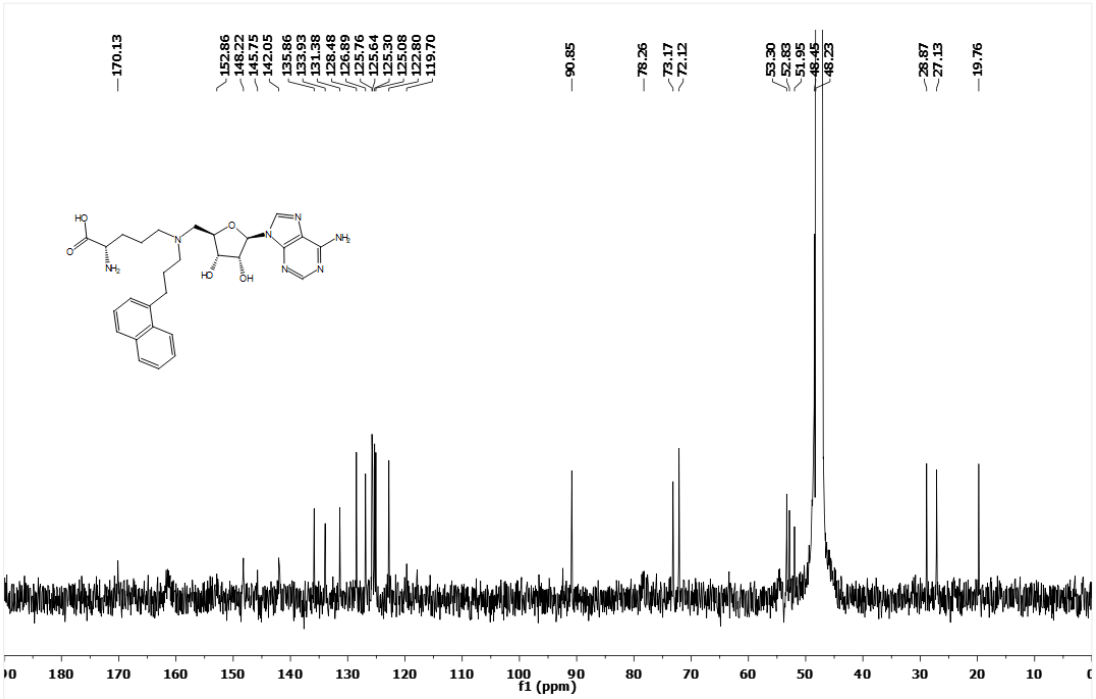

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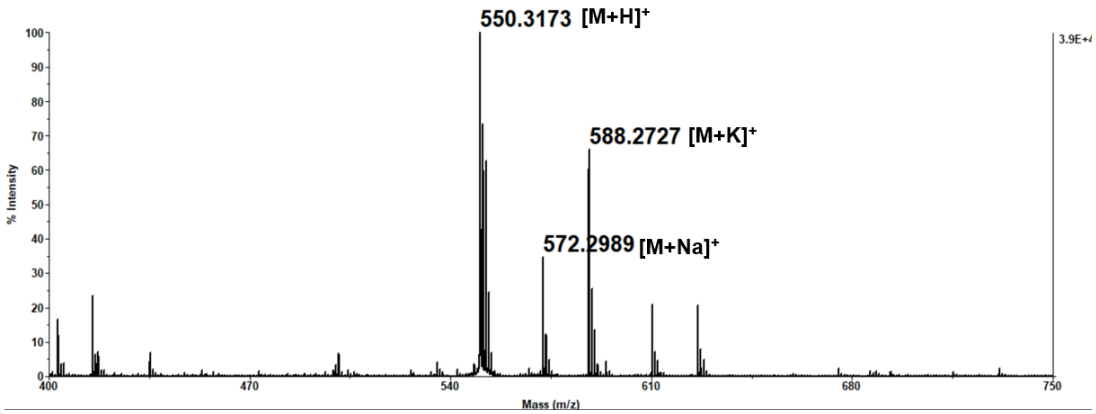

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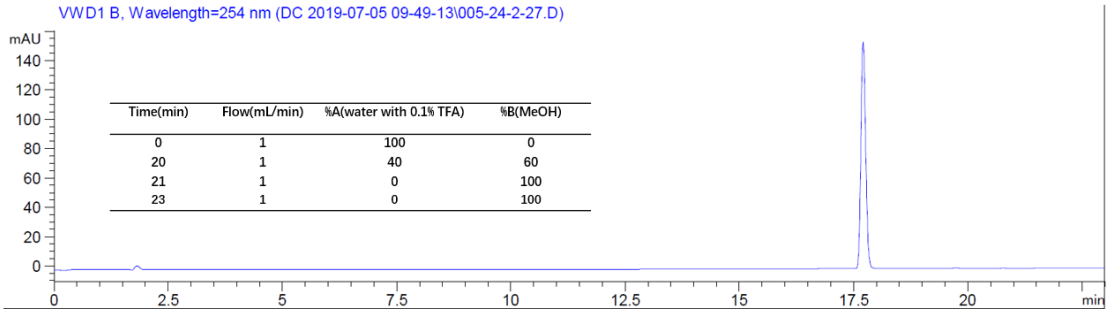
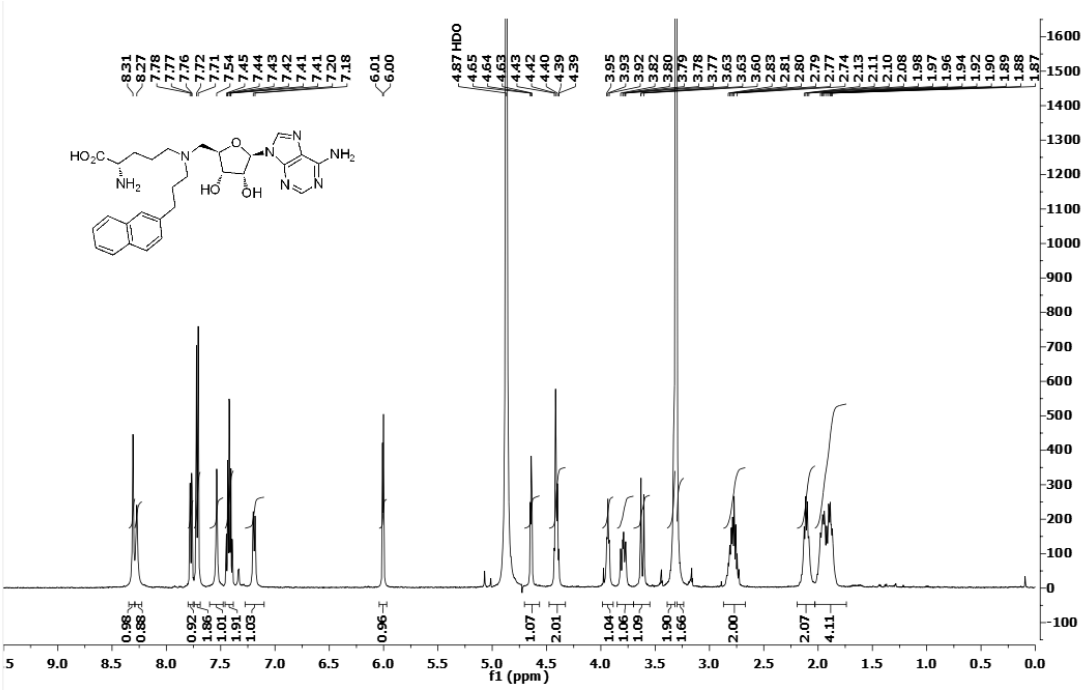


Time(min)	Flow(mL/min)	%A(water with 0.1% TFA)	%B(MeOH)
0	1	100	0
20	1	40	60
21	1	0	100
23	1	0	100

NMR, MALDI-MS and HPLC spectra of compound **1f**

HNMR



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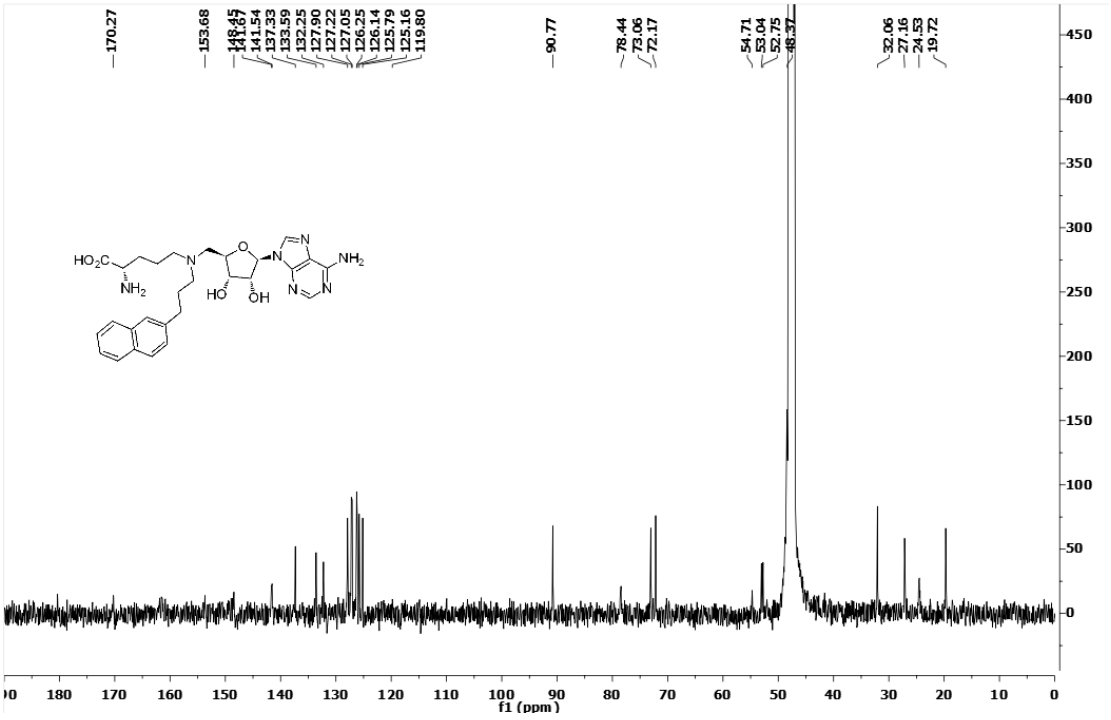
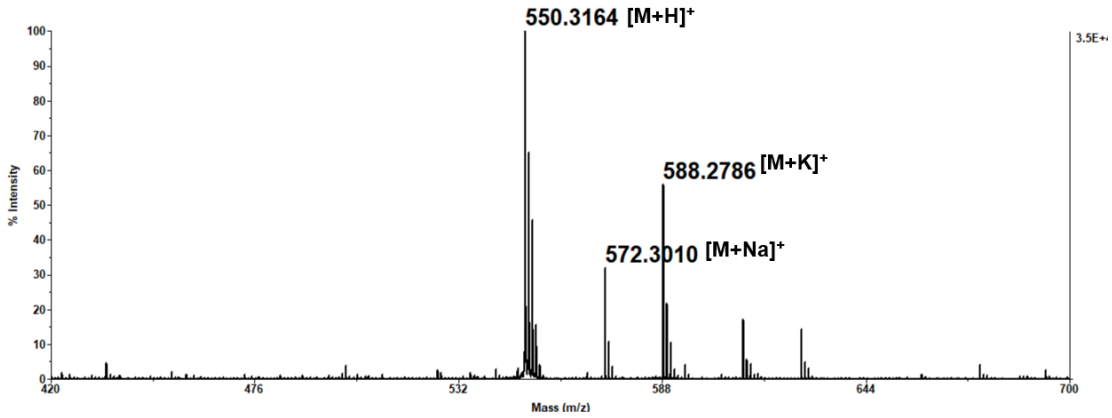


MALDI-MS



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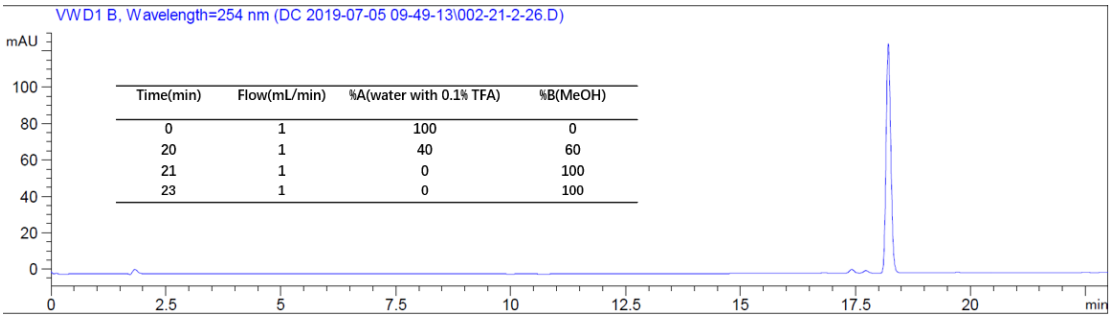

Time(min)	Flow(mL/min)	%A(water with 0.1% TFA)	%B(MeOH)
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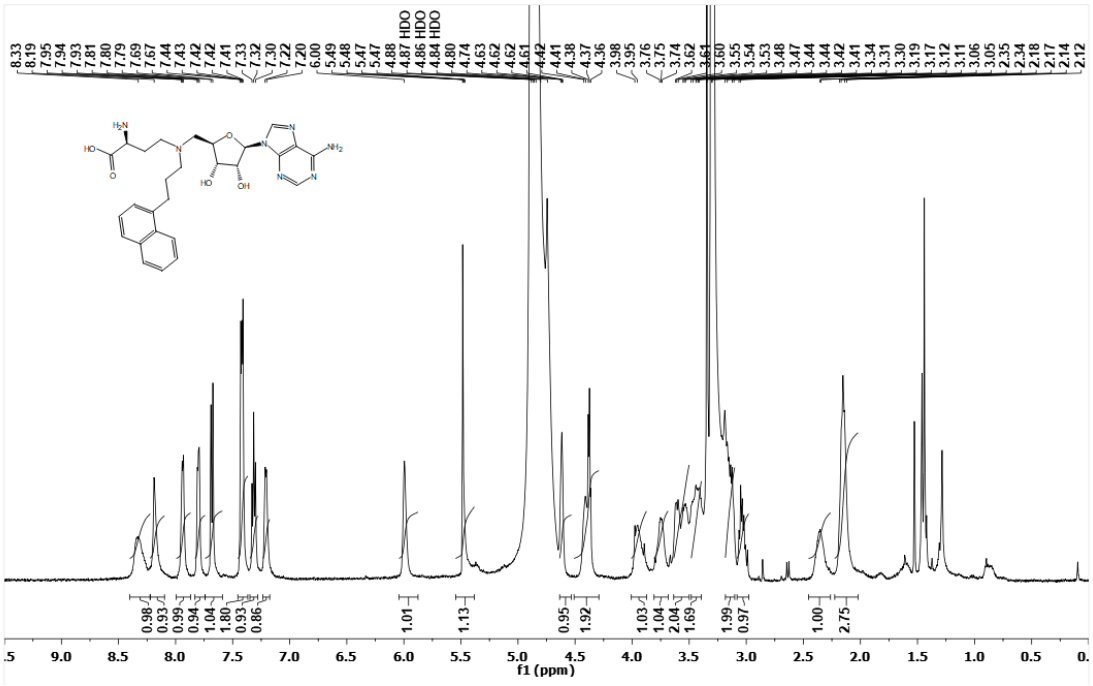
20	1	40	60
21	1	0	100
23	1	0	100

NMR, MALDI-MS and HPLC spectra of compound **1g**

HNMR



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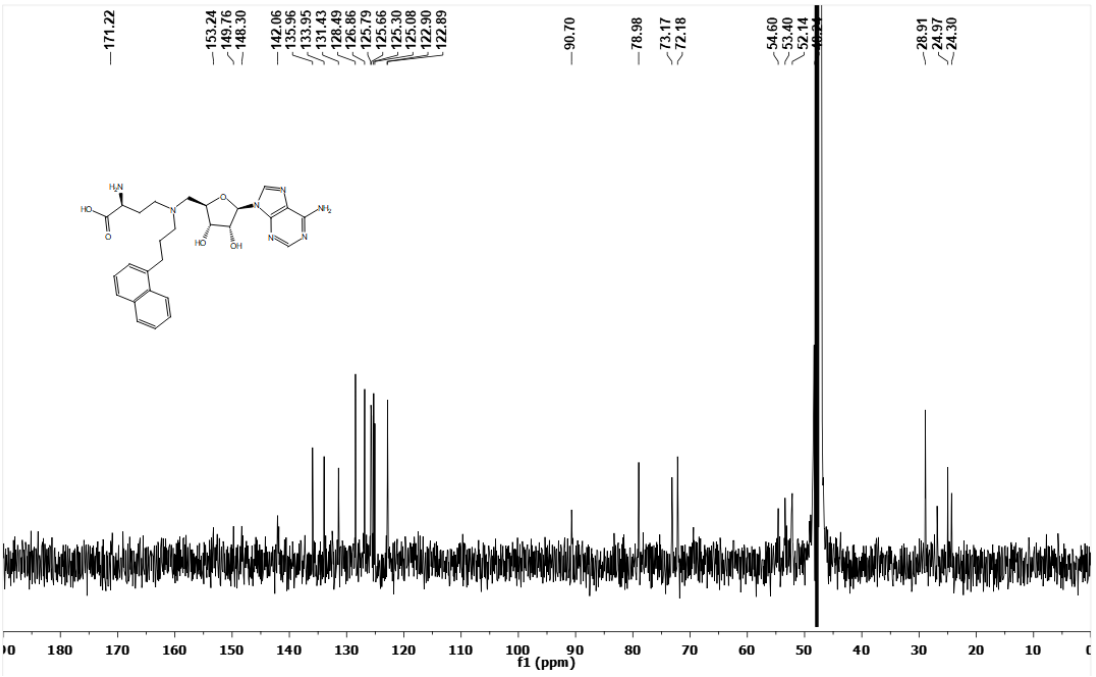
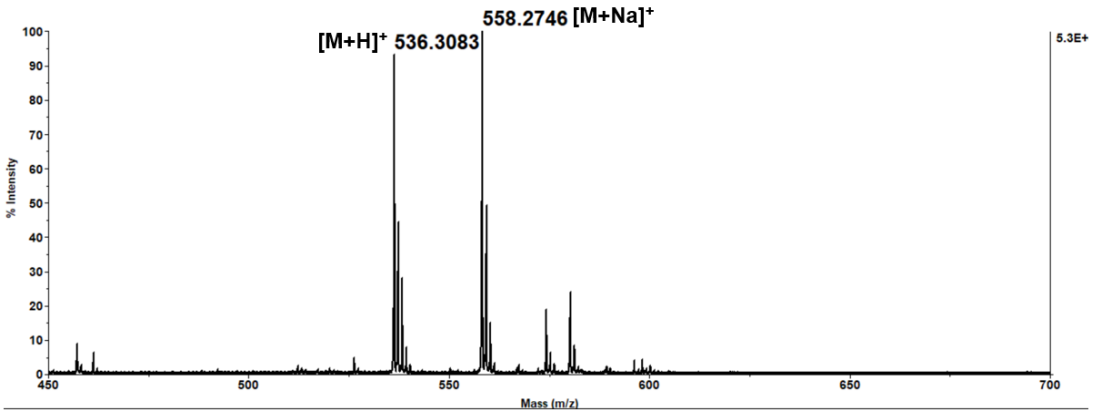


MALDI-MS



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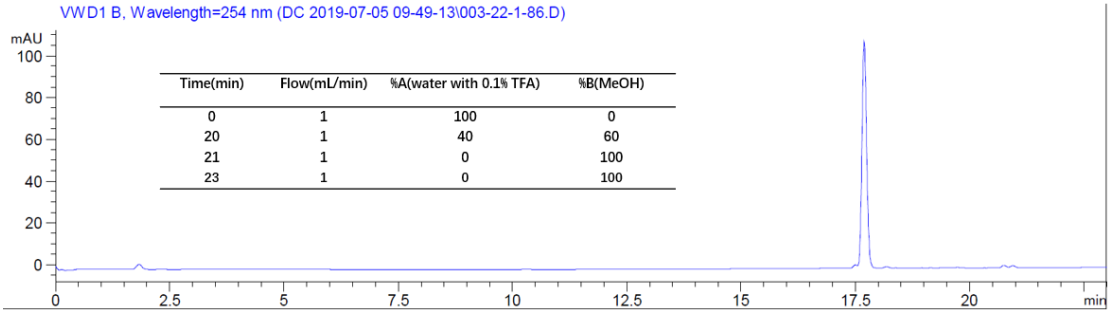
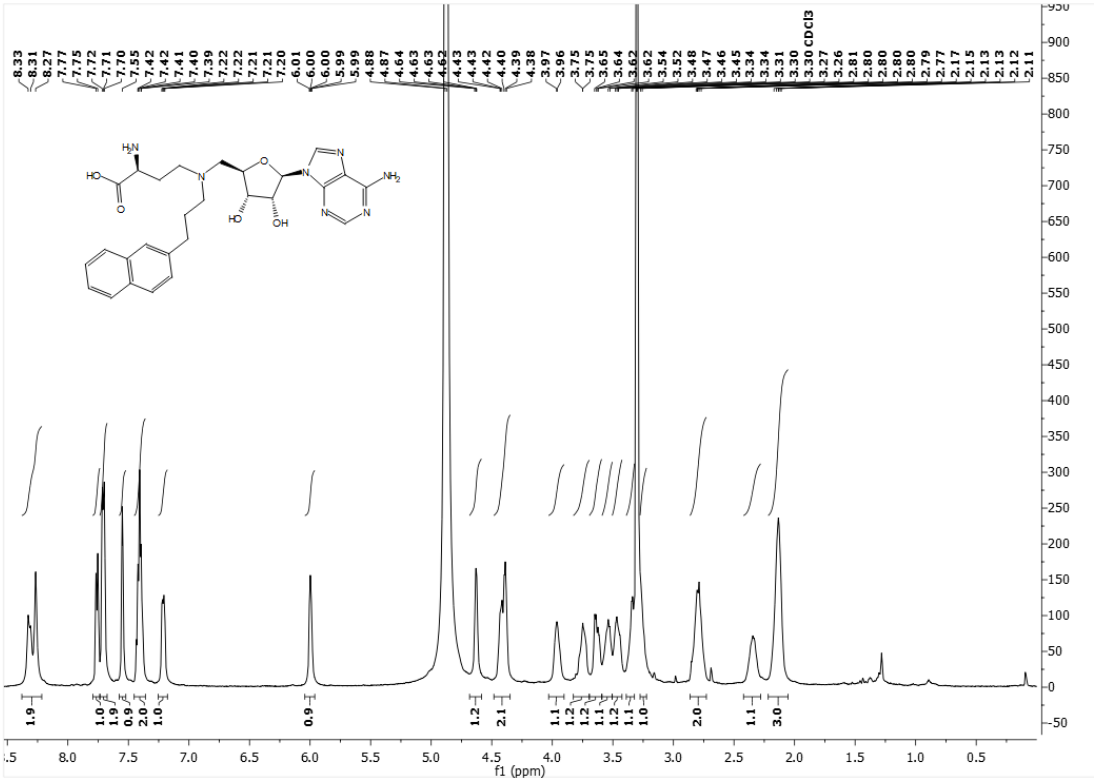


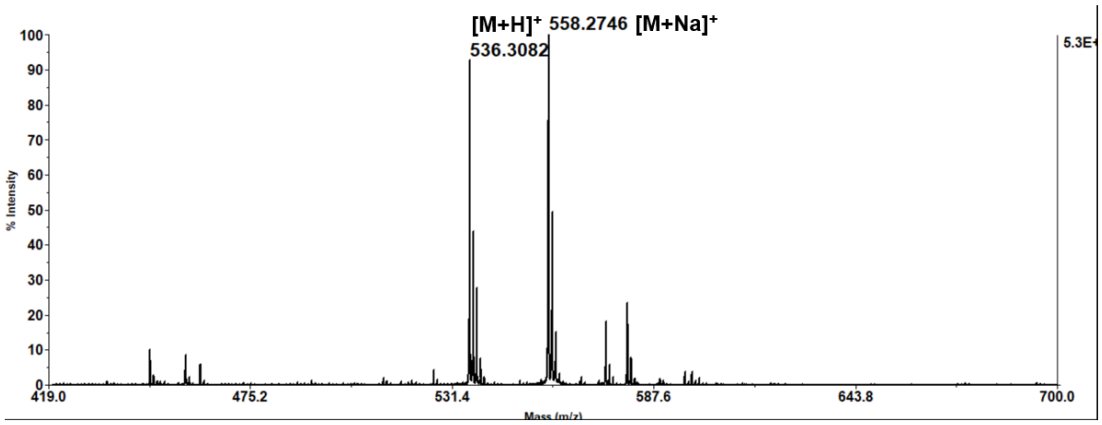
Time(min)	Flow(mL/min)	%A(water with 0.1% TFA)	%B(MeOH)
0	1	100	0
20	1	40	60
21	1	0	100
23	1	0	100

NMR, MALDI-MS and HPLC spectra of compound **1h**

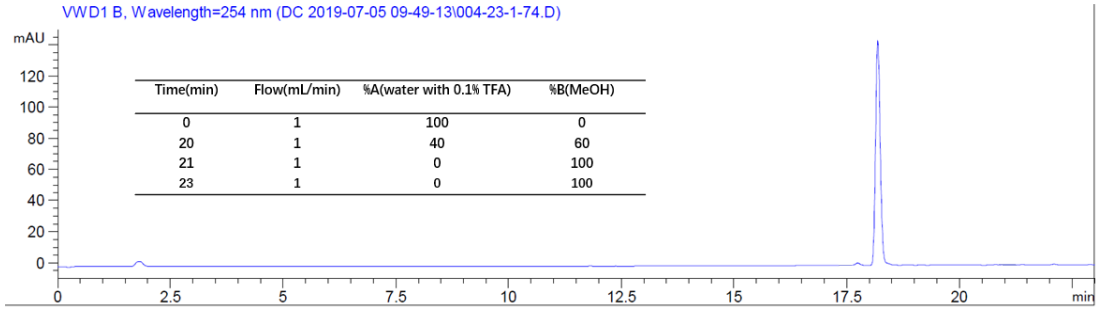
HNMR



MALDI-MS



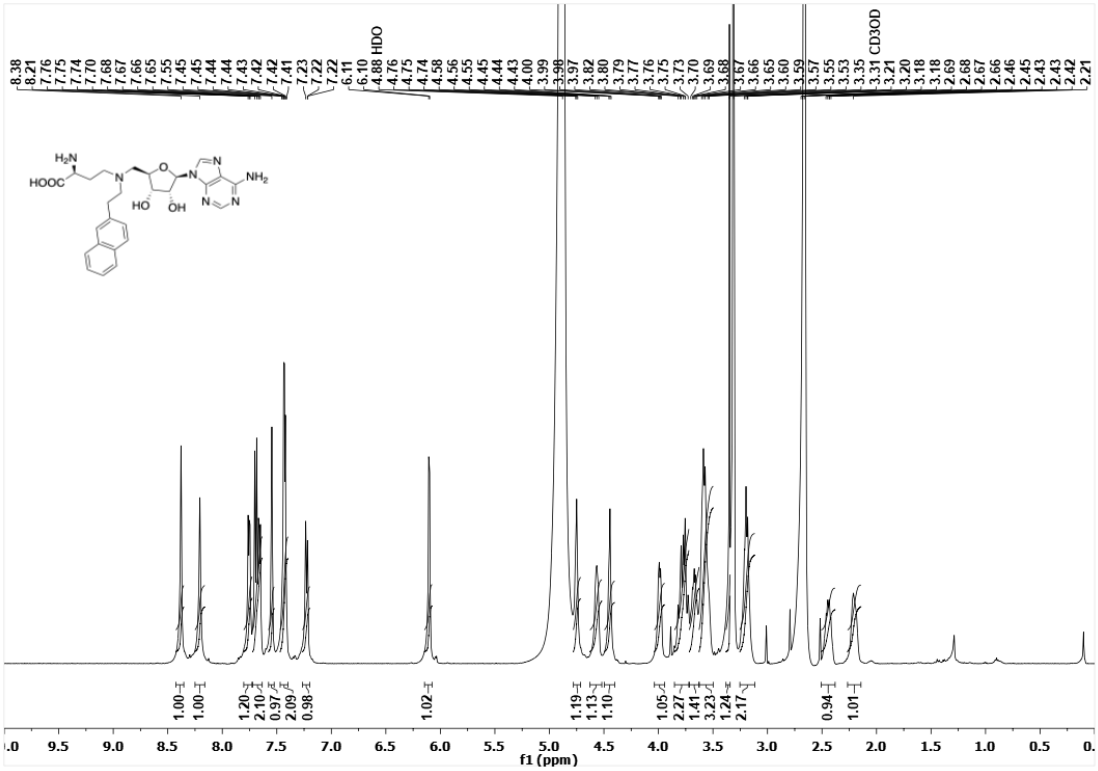
HPLC



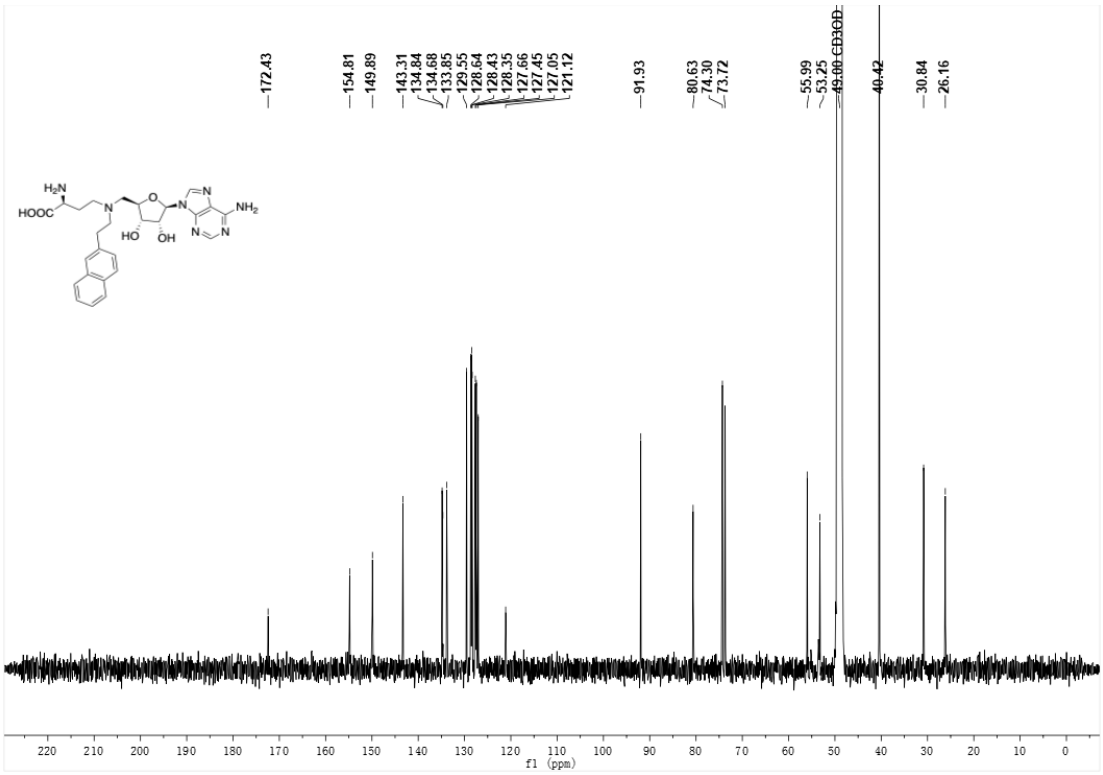
Time(min)	Flow(mL/min)	%A(water with 0.1% TFA)	%B(MeOH)
0	1	100	0
20	1	40	60
21	1	0	100
23	1	0	100

NMR, MALDI-MS and HPLC spectra of compound **1i**

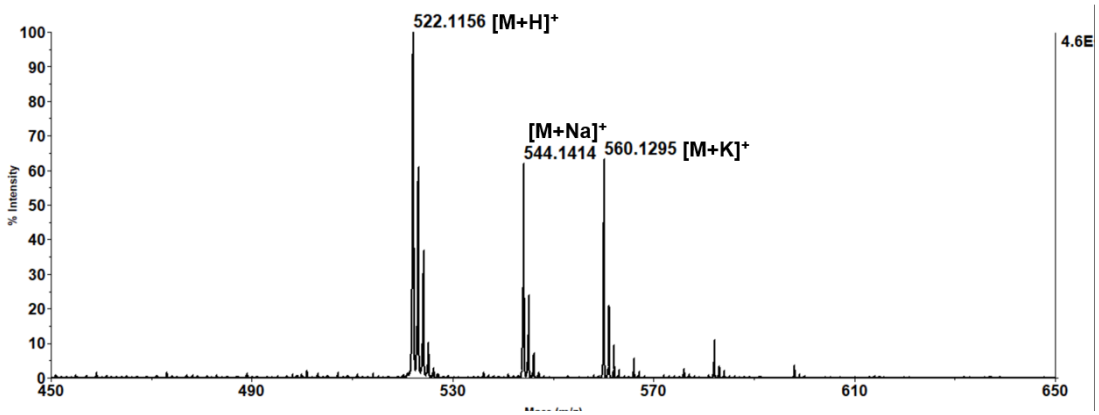
HNMR



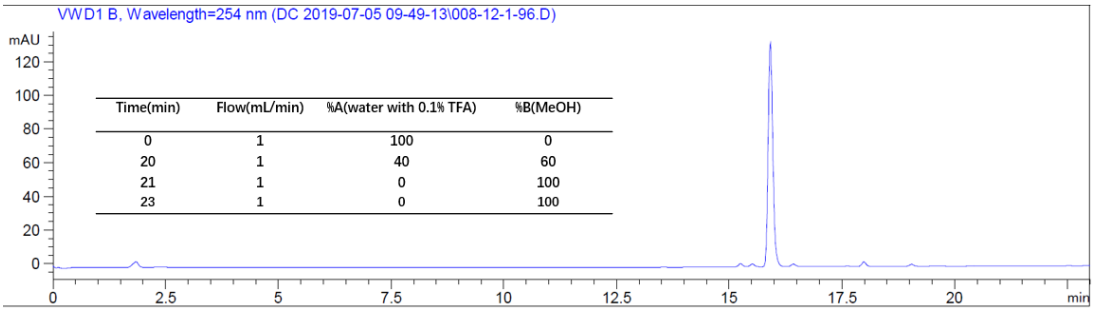
CNMR



MALDI-MS



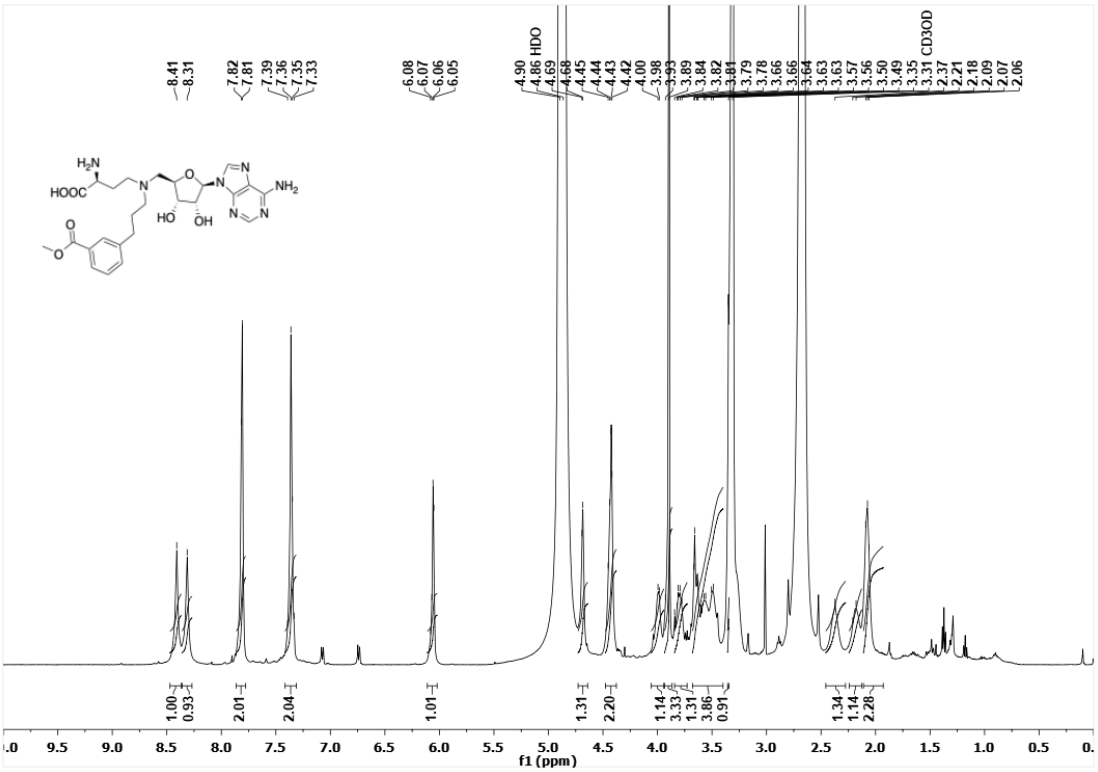
HPLC



Time(min)	Flow(mL/min)	%A(water with 0.1% TFA)	%B(MeOH)
0	1	100	0
20	1	40	60
21	1	0	100
23	1	0	100

NMR, MALDI-MS and HPLC spectra of compound **1j**

HNMR



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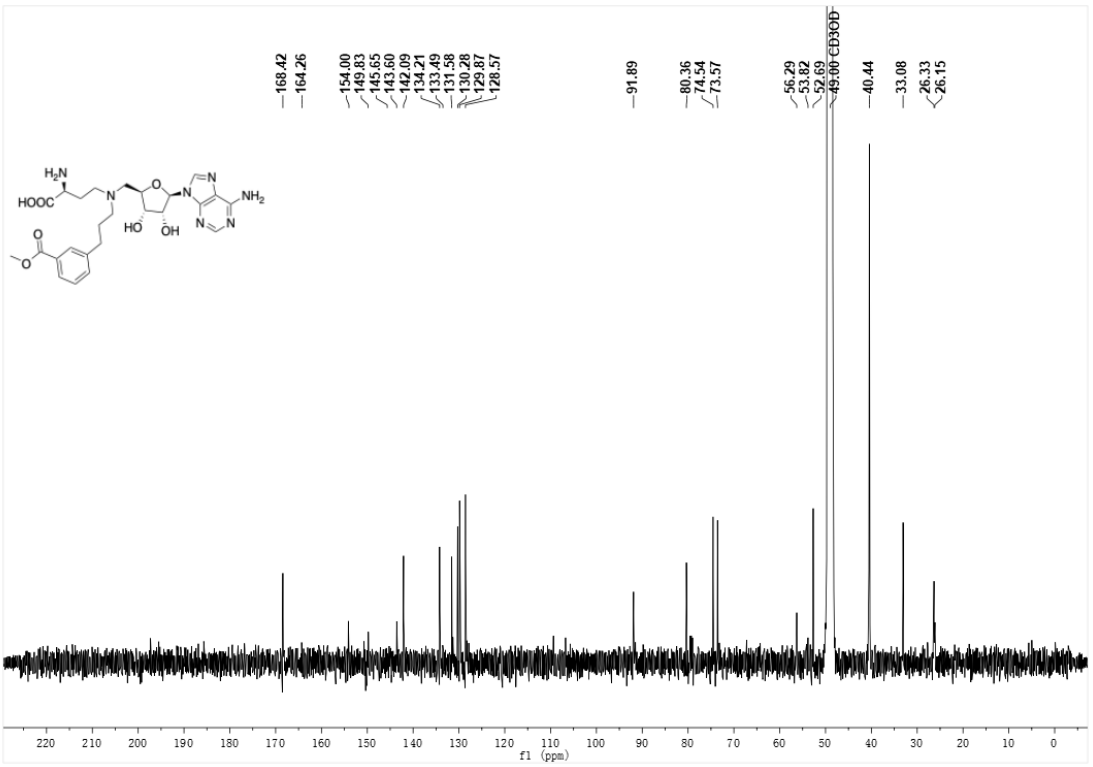
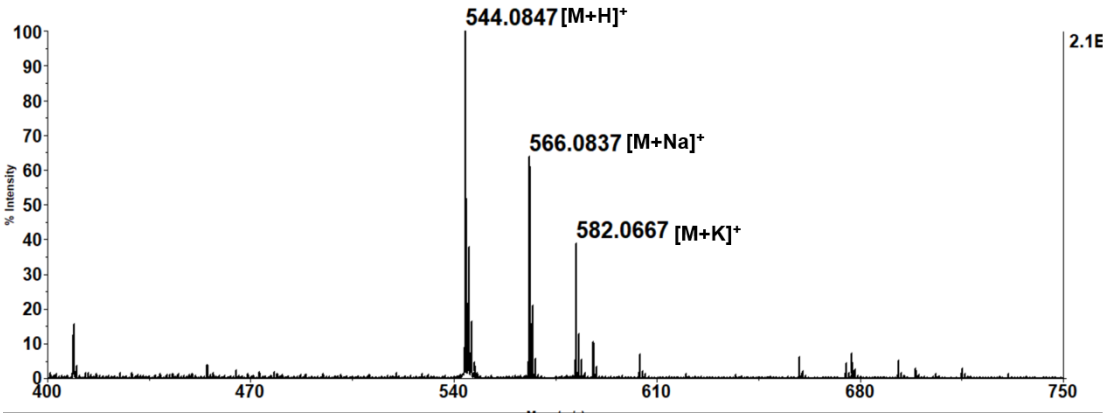


MALDI-MS



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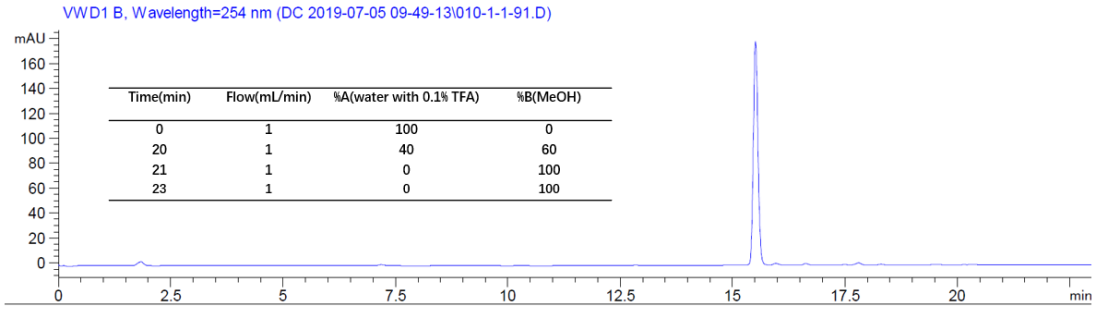
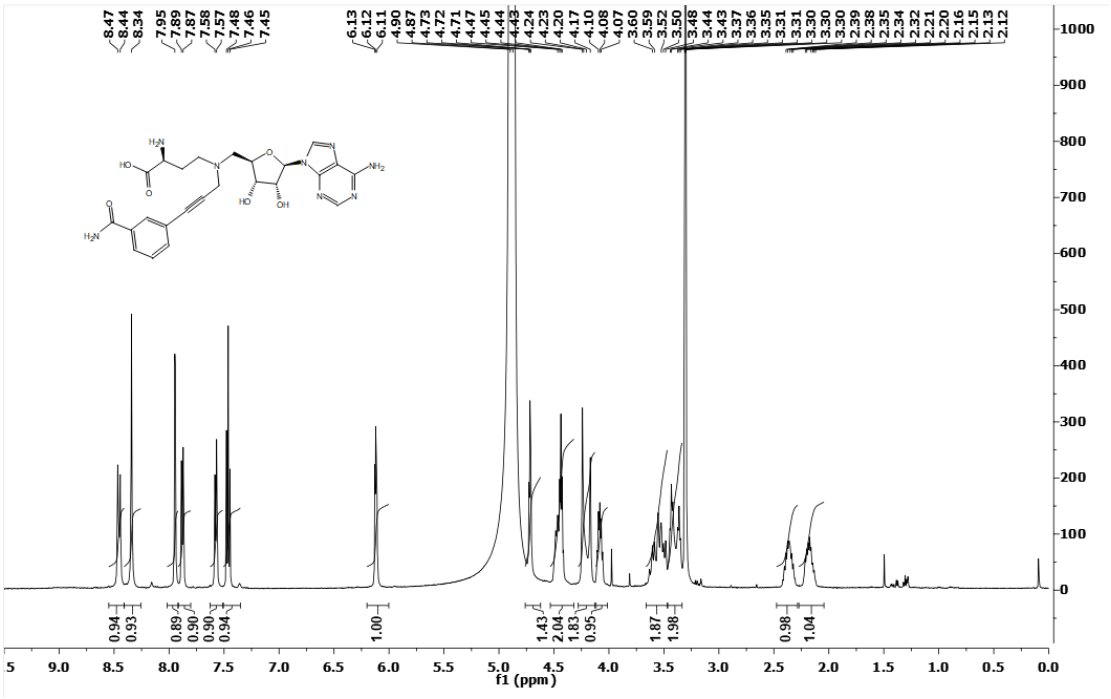


Time(min)	Flow(mL/min)	%A(water with 0.1% TFA)	%B(MeOH)
0	1	100	0
20	1	40	60
21	1	0	100
23	1	0	100

NMR, MALDI-MS and HPLC spectra of compound **2a**

HNMR



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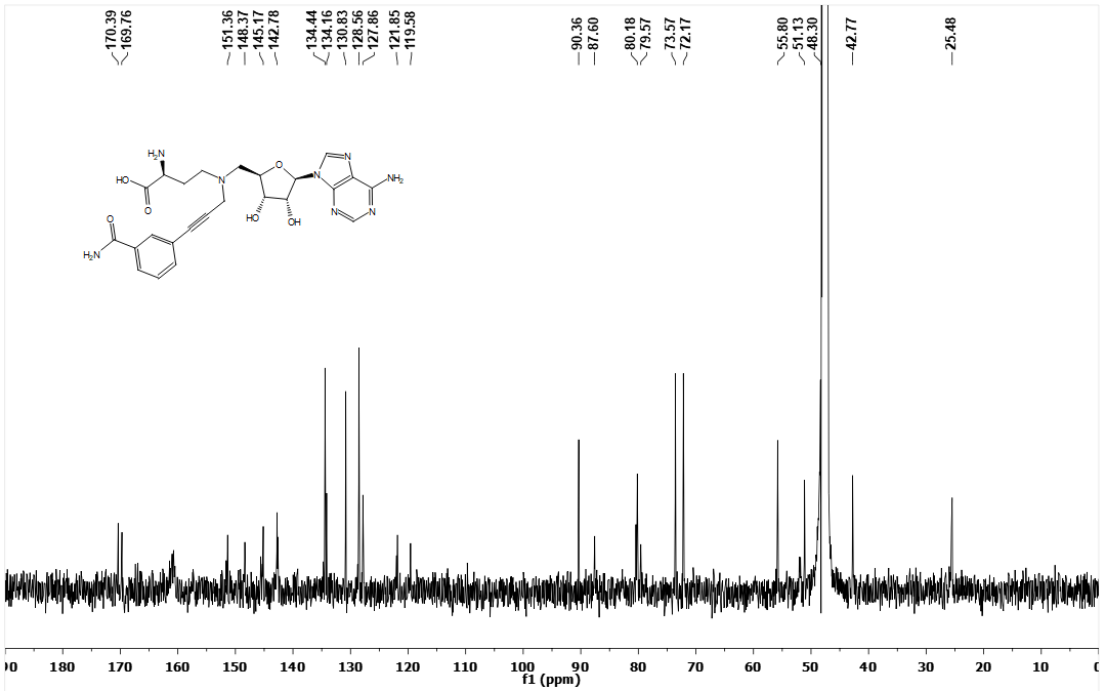
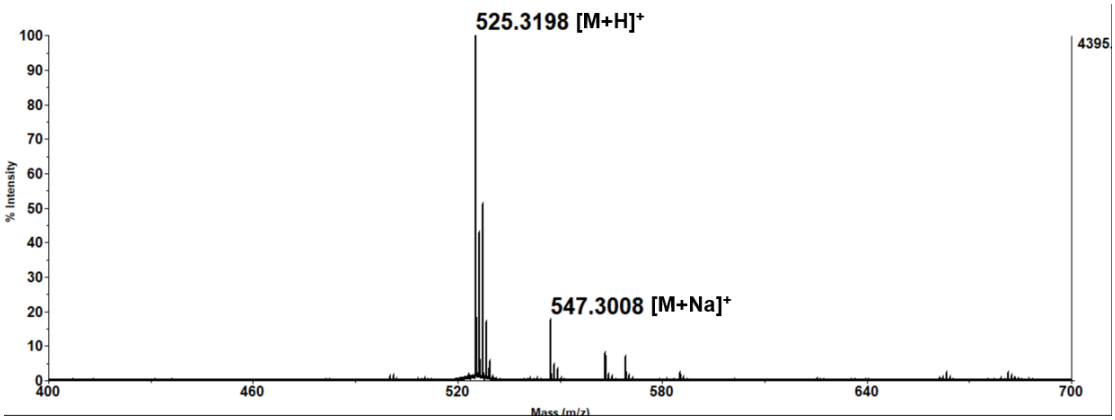


MALDI-MS



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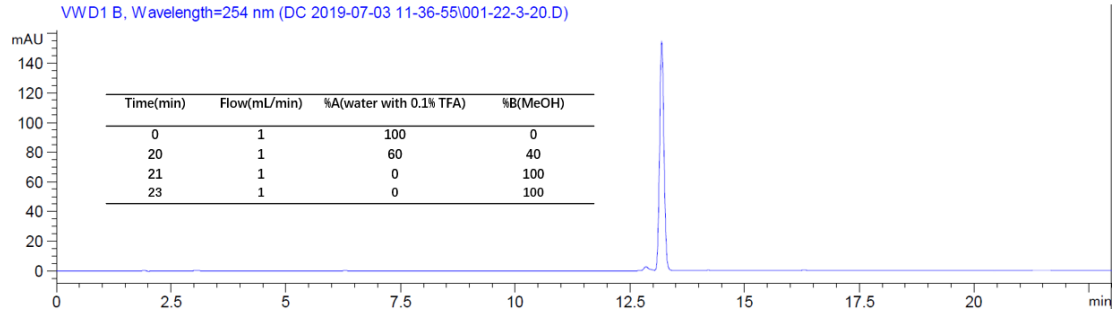
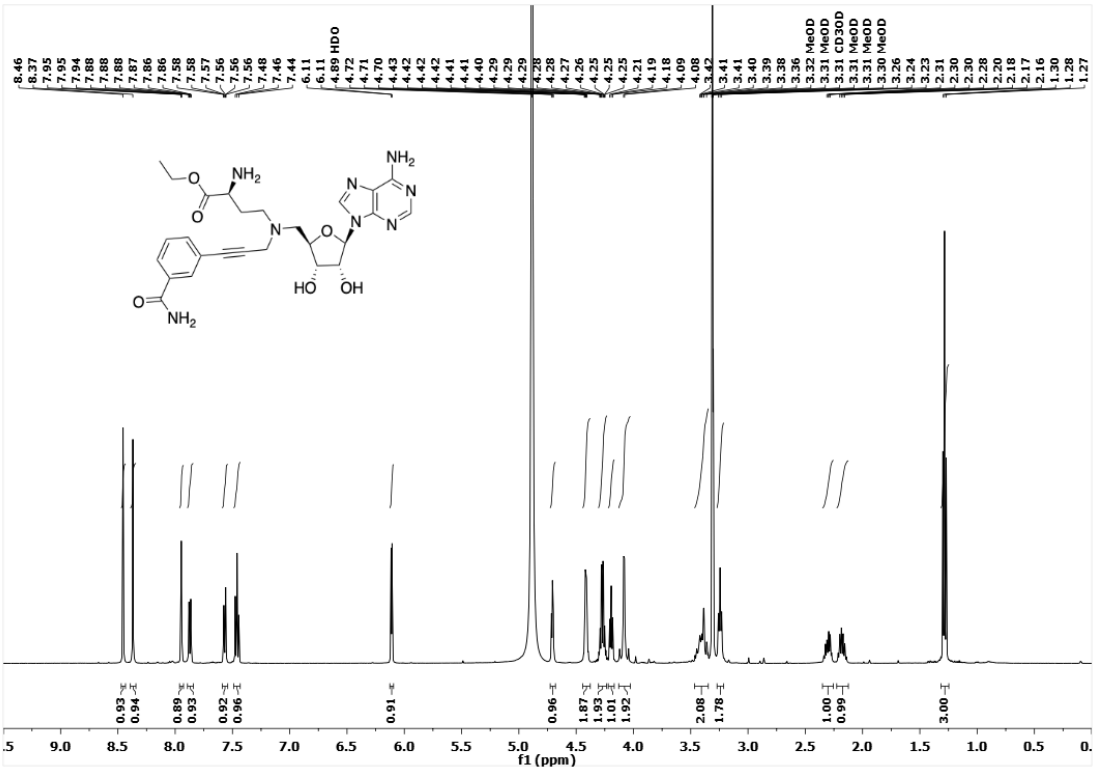


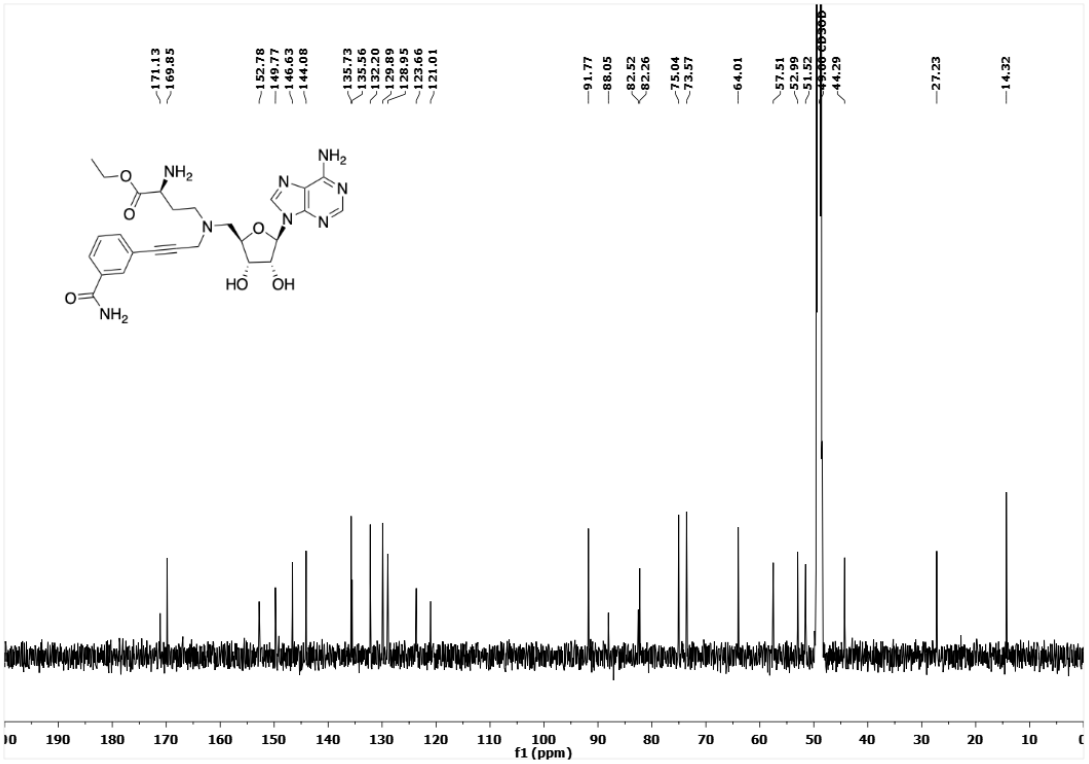
Time(min)	Flow(mL/min)	%A(water with 0.1% TFA)	%B(MeOH)
0	1	100	0
20	1	60	40
21	1	0	100
23	1	0	100

NMR, MALDI-MS and HPLC spectra of compound **2a***

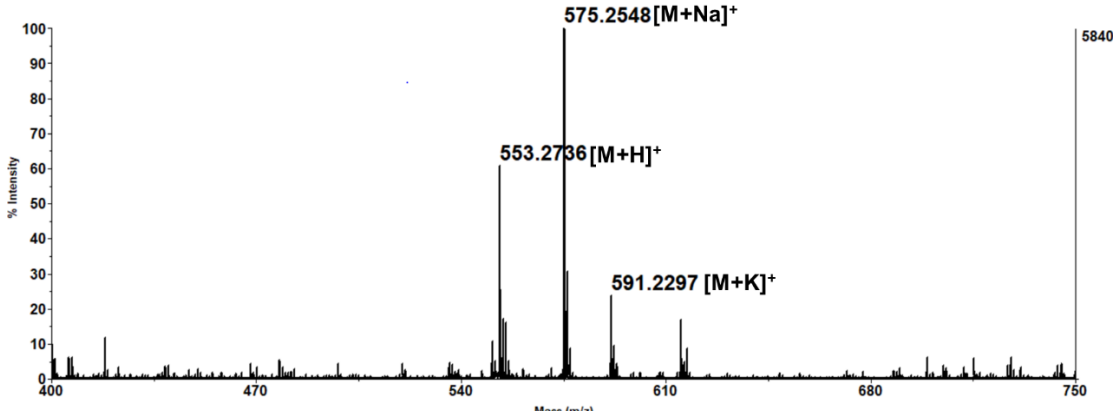
H-NMR



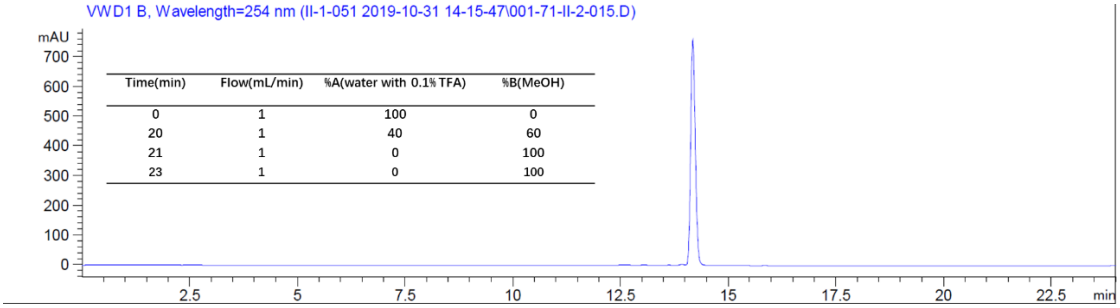
C-NMR



MALDI-MS



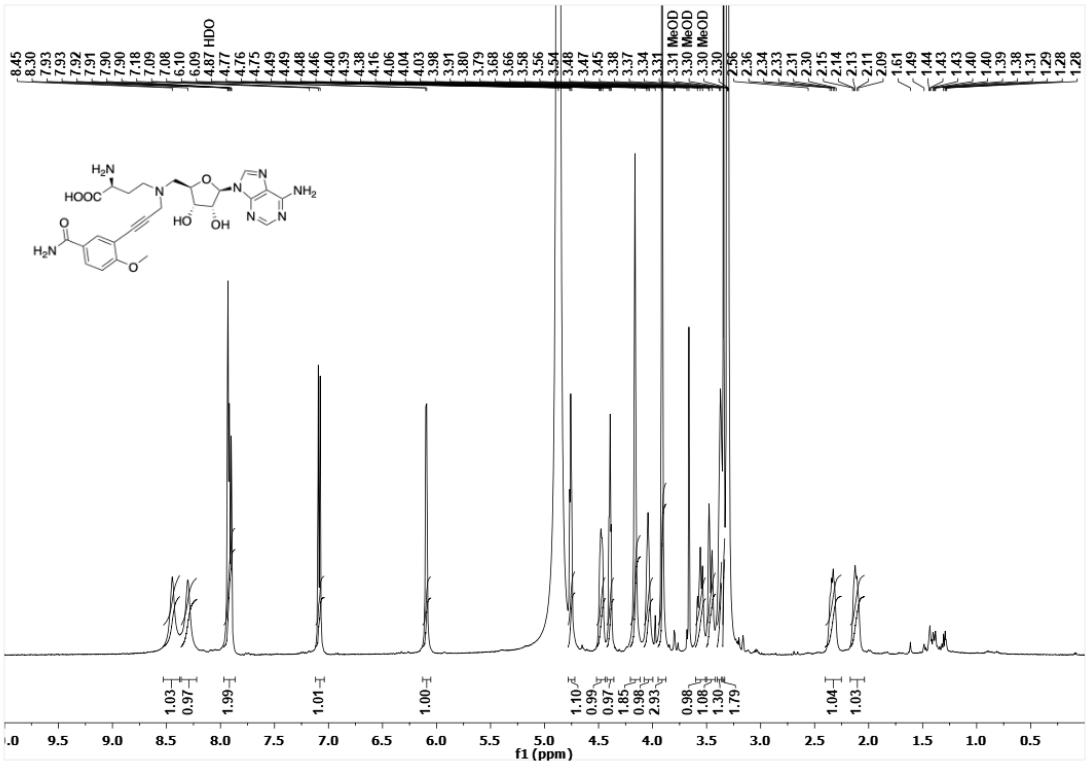
HPLC



Time(min)	Flow(mL/min)	%A(water with 0.1% TFA)	%B(MeOH)
0	1	100	0
20	1	40	60
21	1	0	100
23	1	0	100

NMR, MALDI-MS and HPLC spectra of compound **2b**

HNMR



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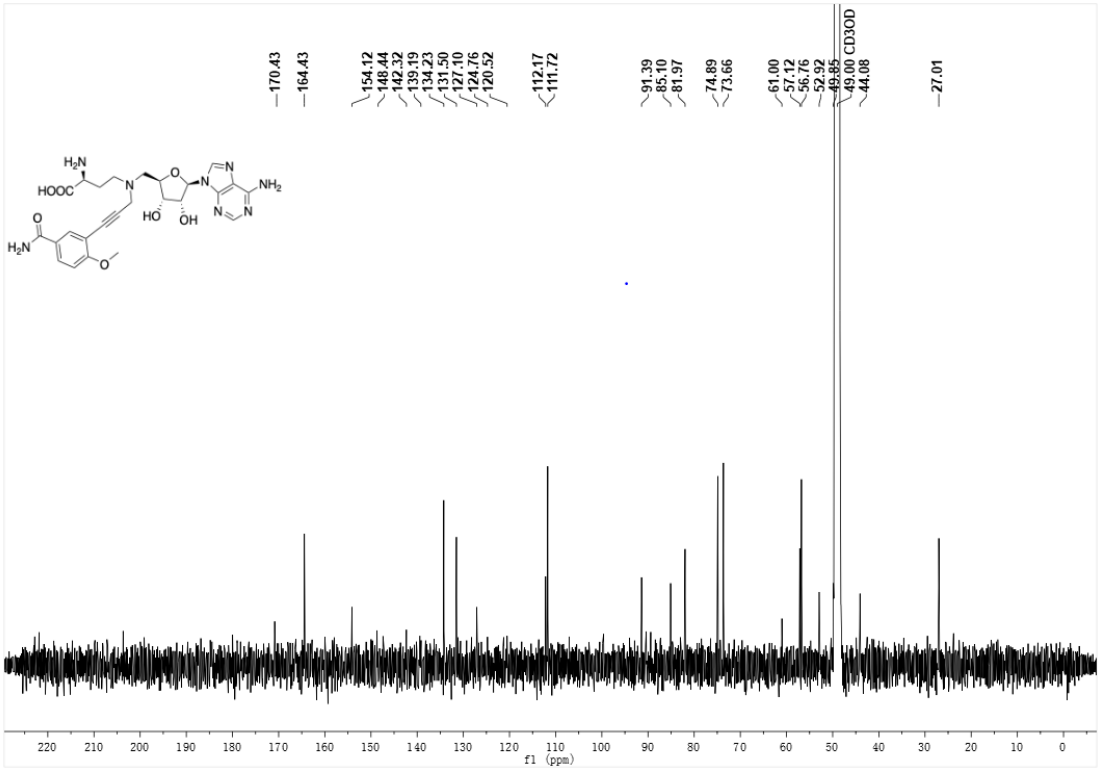
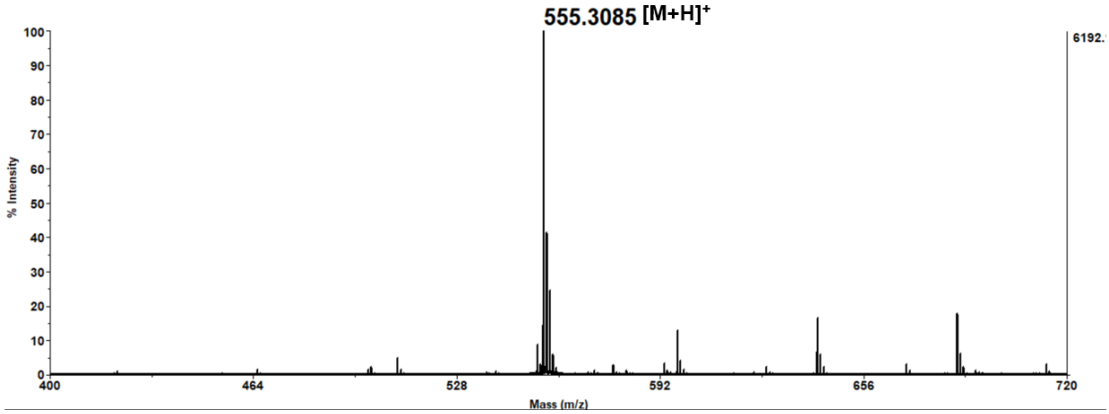


MALDI-MS



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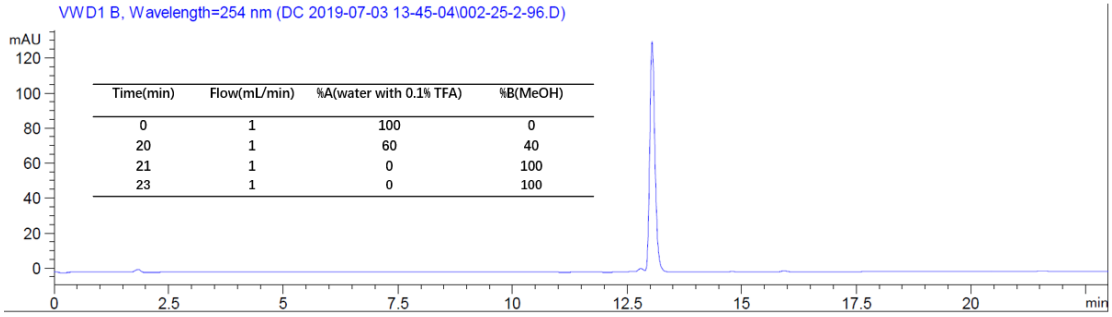
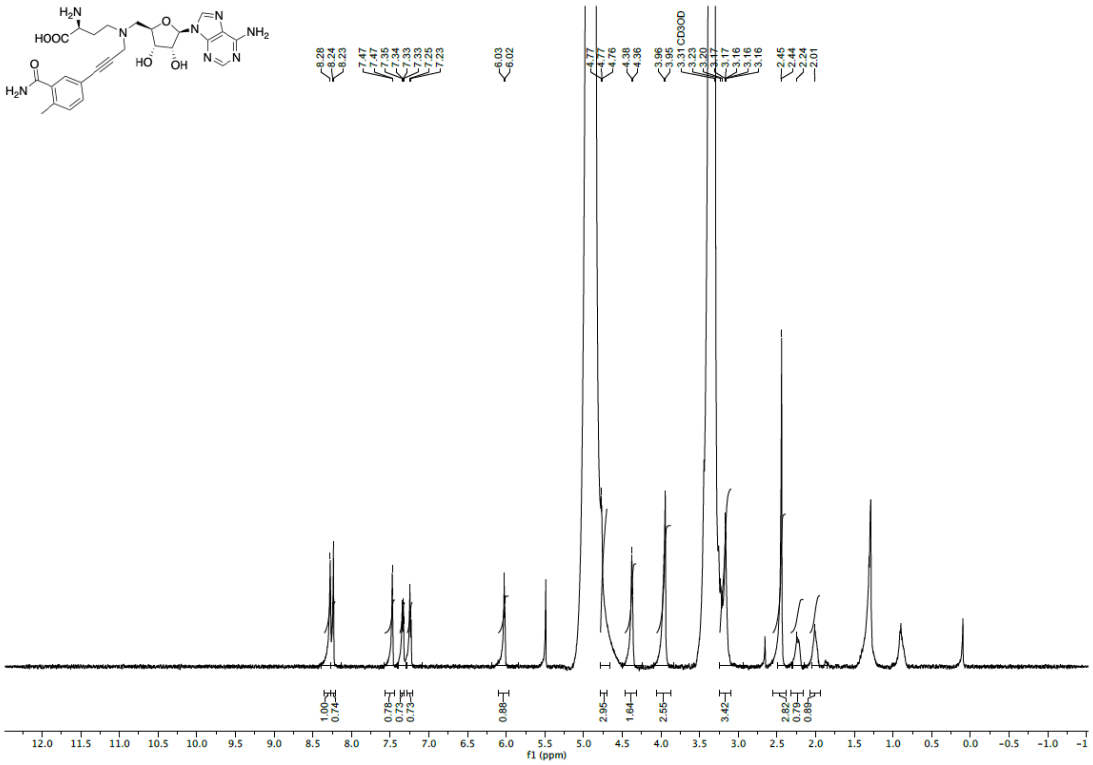


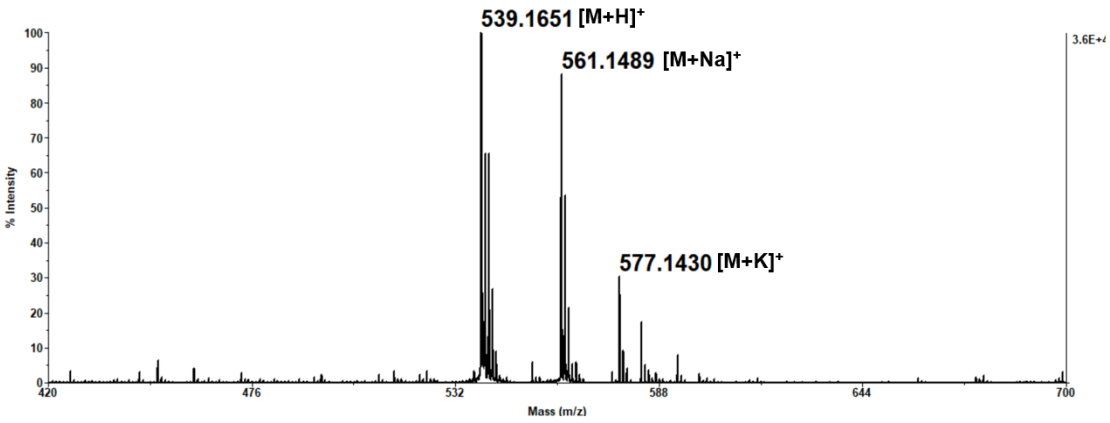
Time(min)	Flow(mL/min)	%A(water with 0.1% TFA)	%B(MeOH)
0	1	100	0
20	1	60	40
21	1	0	100
23	1	0	100

NMR, MALDI-MS and HPLC spectra of compound **2c**

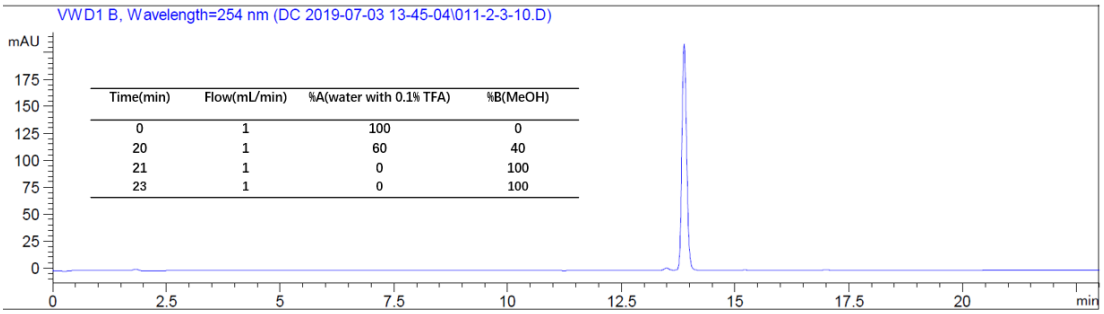
HNMR



MALDI-MS



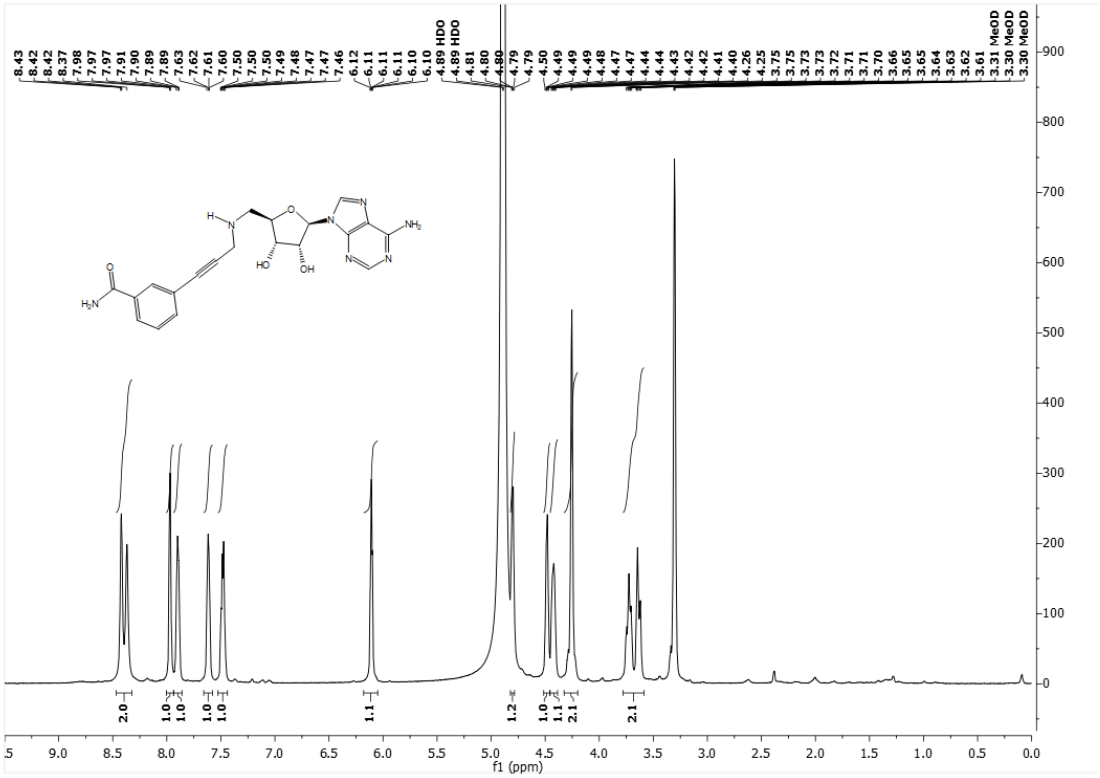
HPLC



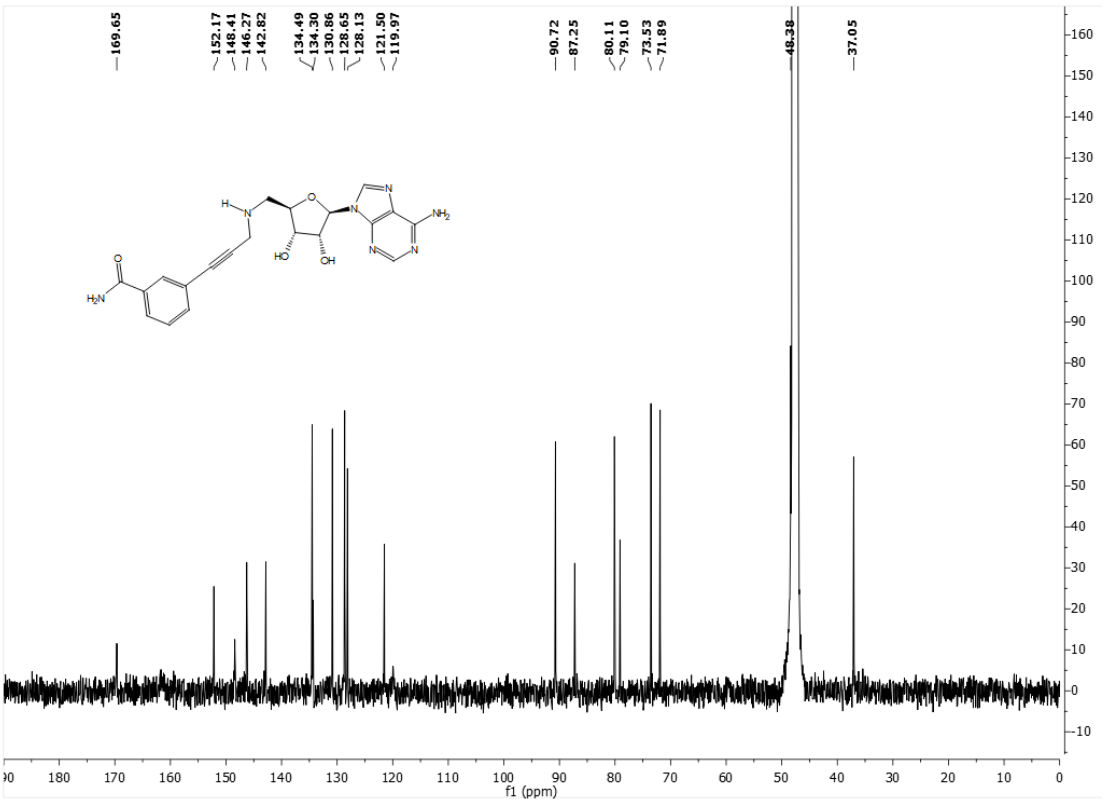
Time(min)	Flow(mL/min)	%A(water with 0.1% TFA)	%B(MeOH)
0	1	100	0
20	1	60	40
21	1	0	100
23	1	0	100

NMR, MALDI-MS and HPLC spectra of compound **2d**

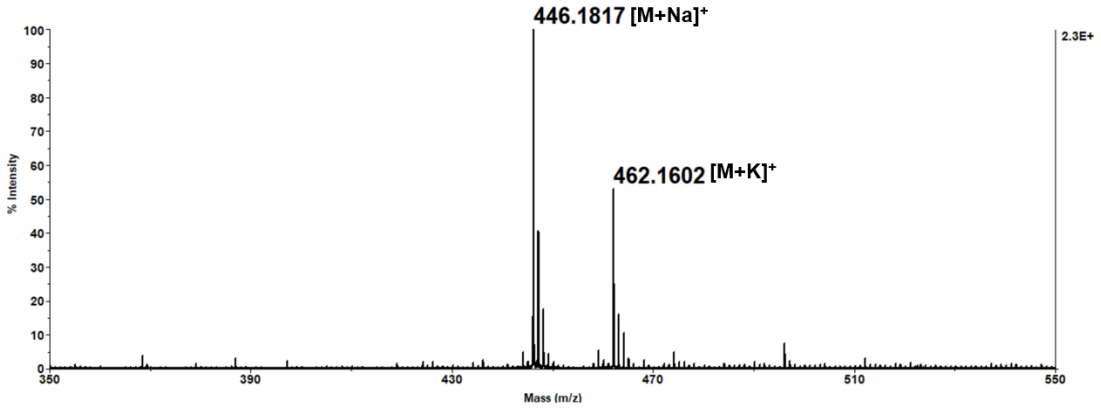
HNMR



CNMR



MALDI-MS



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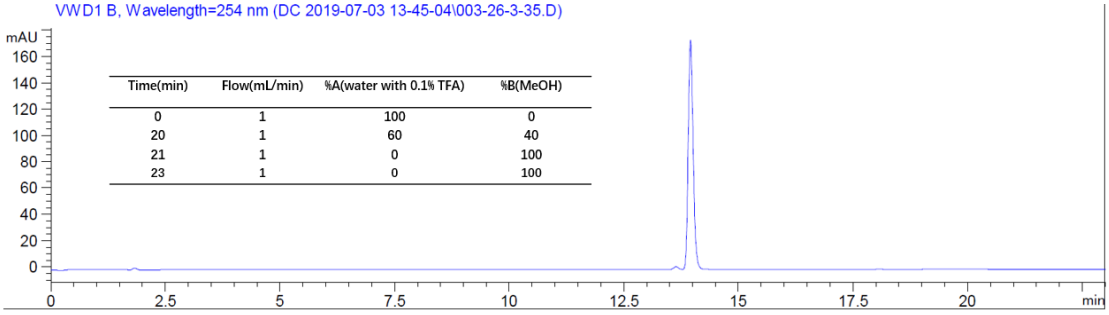
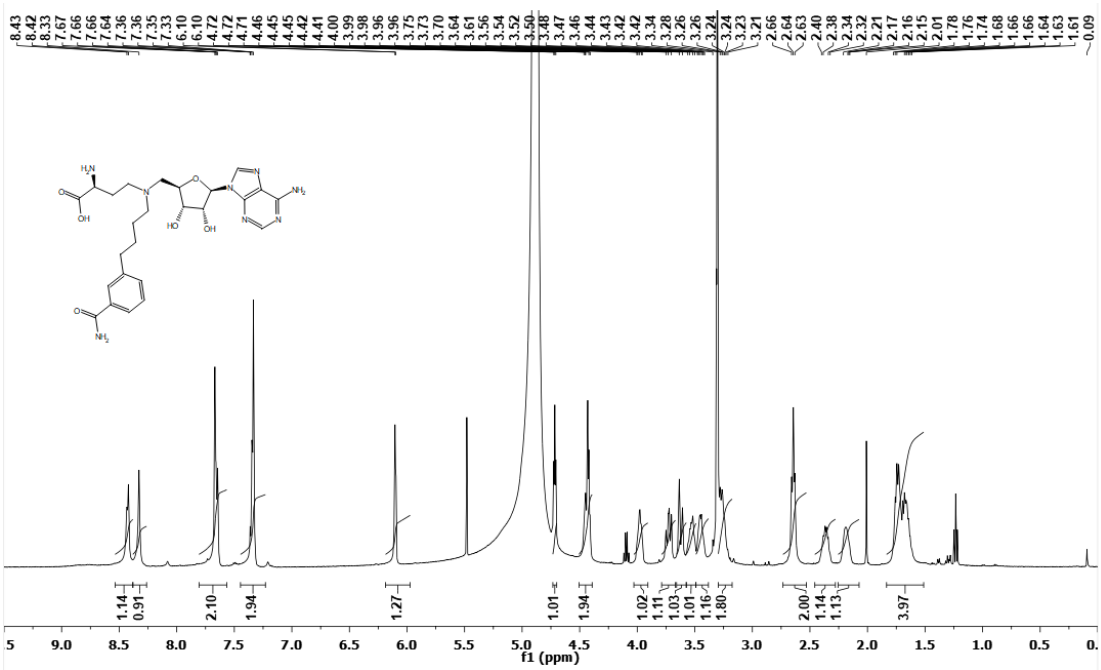


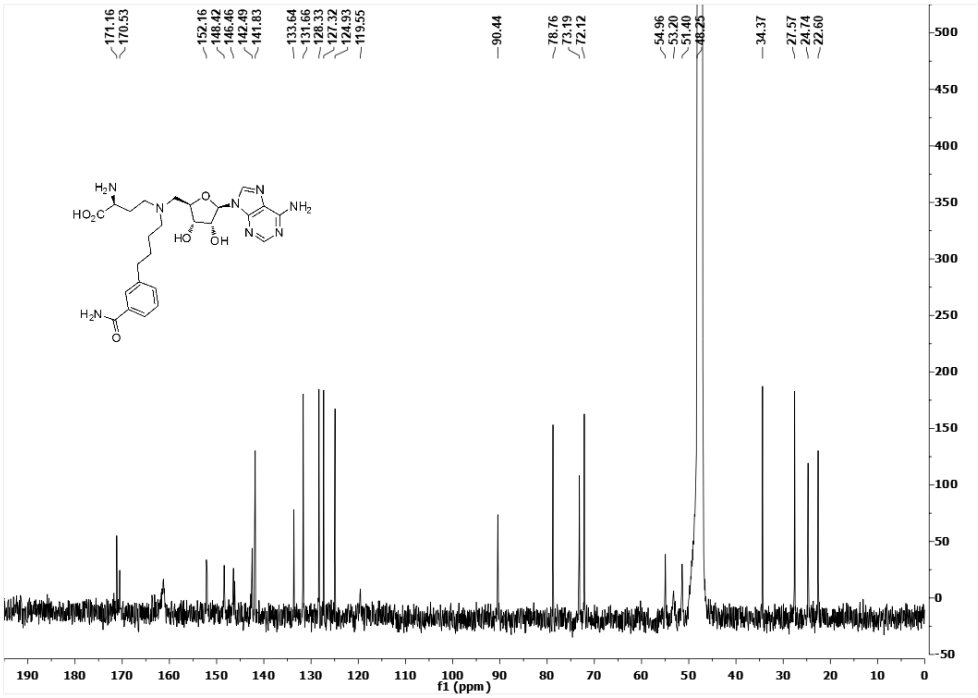
Time(min)	Flow(mL/min)	%A(water with 0.1% TFA)	%B(MeOH)
0	1	100	0
20	1	60	40
21	1	0	100
23	1	0	100

NMR, MALDI-MS and HPLC spectra of compound **3a**

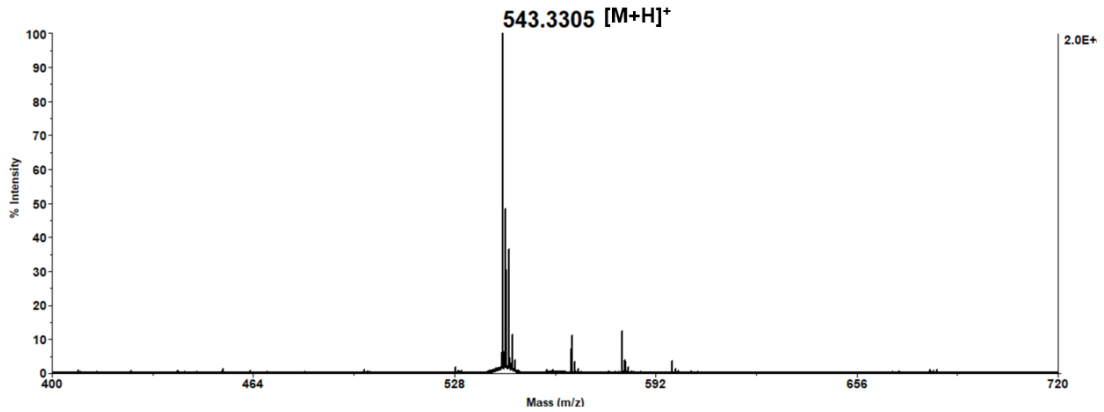
HNMR



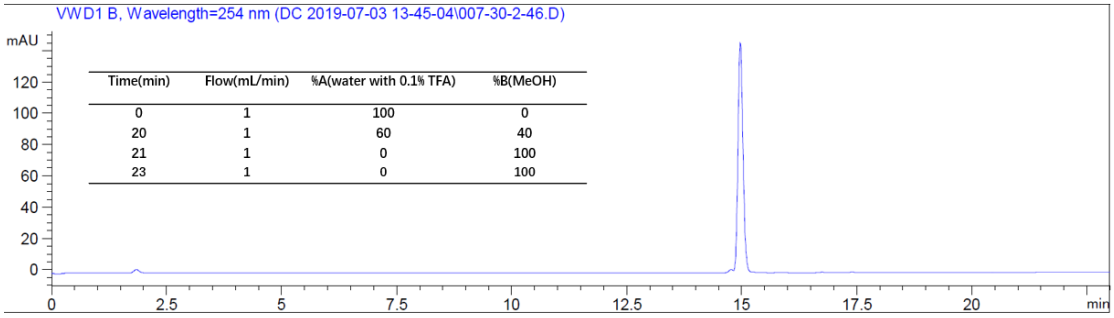
CNMR



MALDI-MS



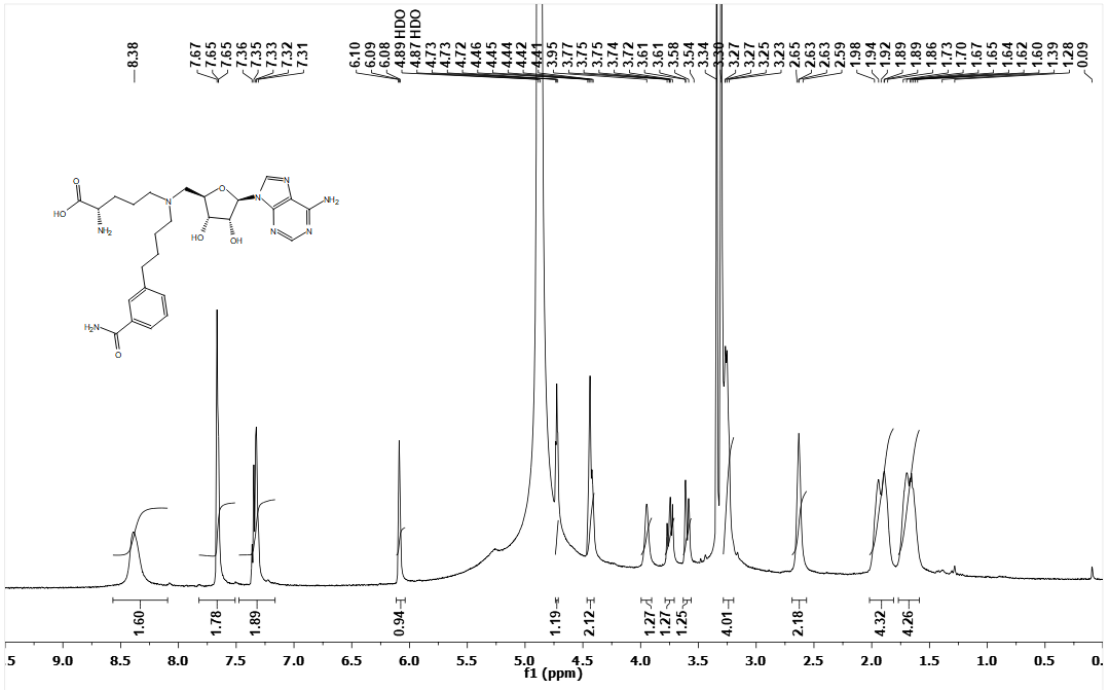
HPLC



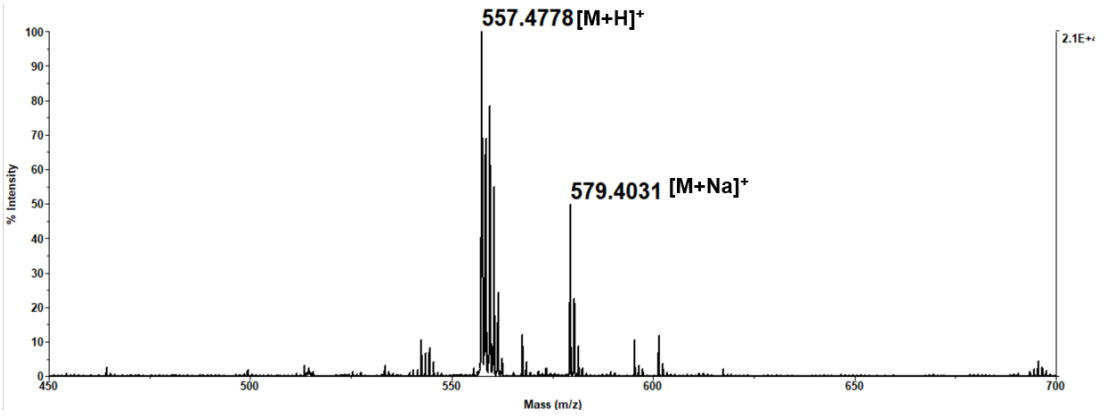
Time(min)	Flow(mL/min)	%A(water with 0.1% TFA)	%B(MeOH)
0	1	100	0
20	1	60	40
21	1	0	100
23	1	0	100

NMR, MALDI-MS and HPLC spectra of compound **3b**

HNMR



MALDI-MS



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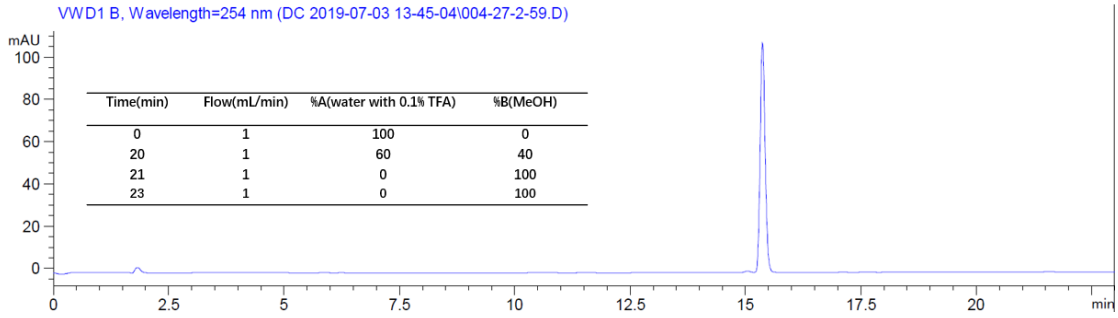
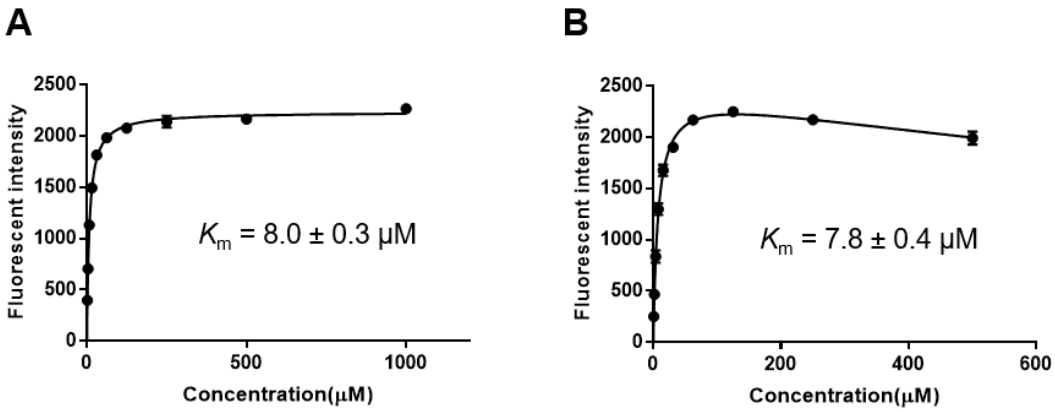
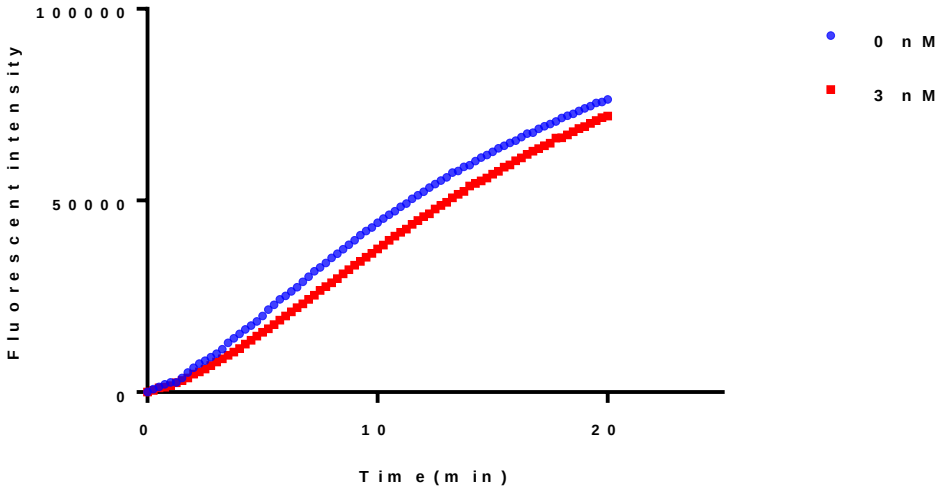


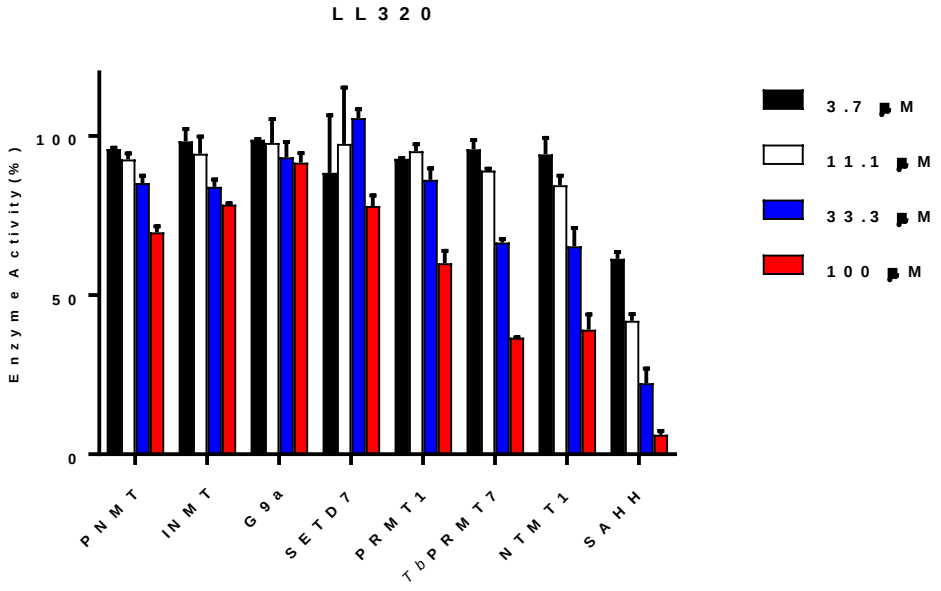
Time(min)	Flow(mL/min)	%A(water with 0.1% TFA)	%B(MeOH)
0	1	100	0
20	1	60	40
21	1	0	100
23	1	0	100



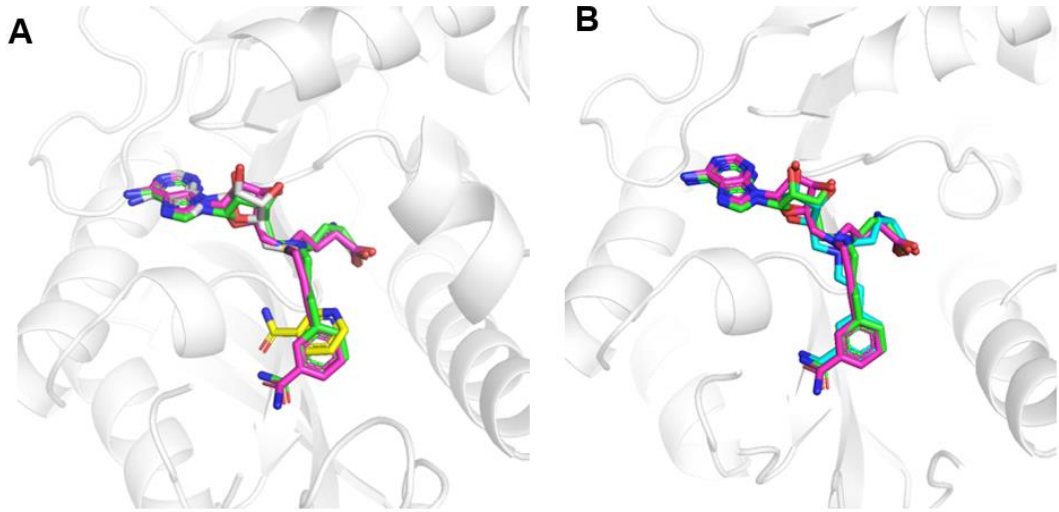
Supporting Figure S1. Kinetic parameter determination for NNMT. K_m values were determined for (A) nicotinamide at fixed SAM concentration (100 μM) and (B) for SAM at fixed concentration of nicotinamide (100 μM). Data were obtained in duplicate.



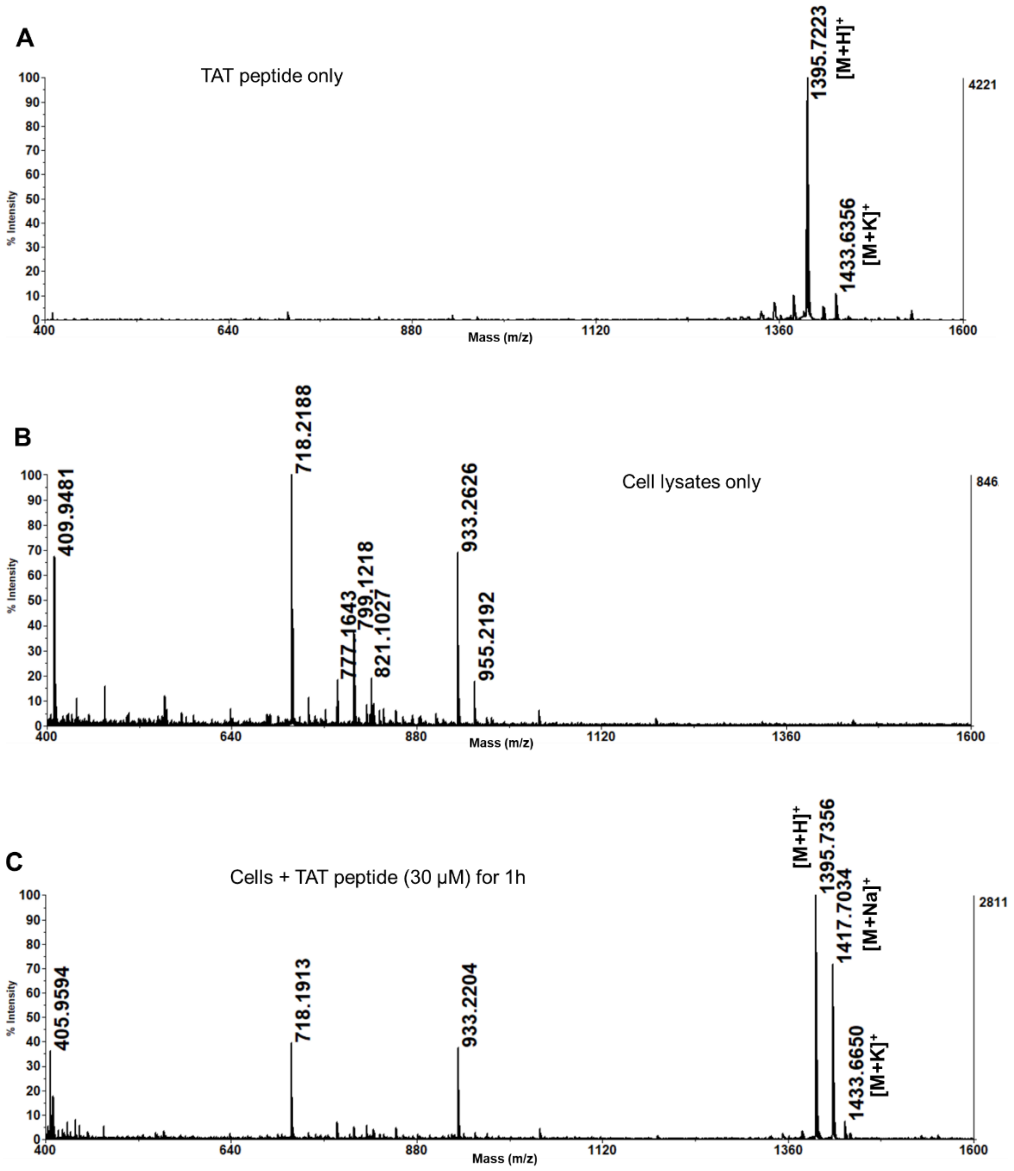
Supporting Figure S2. Rapid dilution assay for compound **LL320**



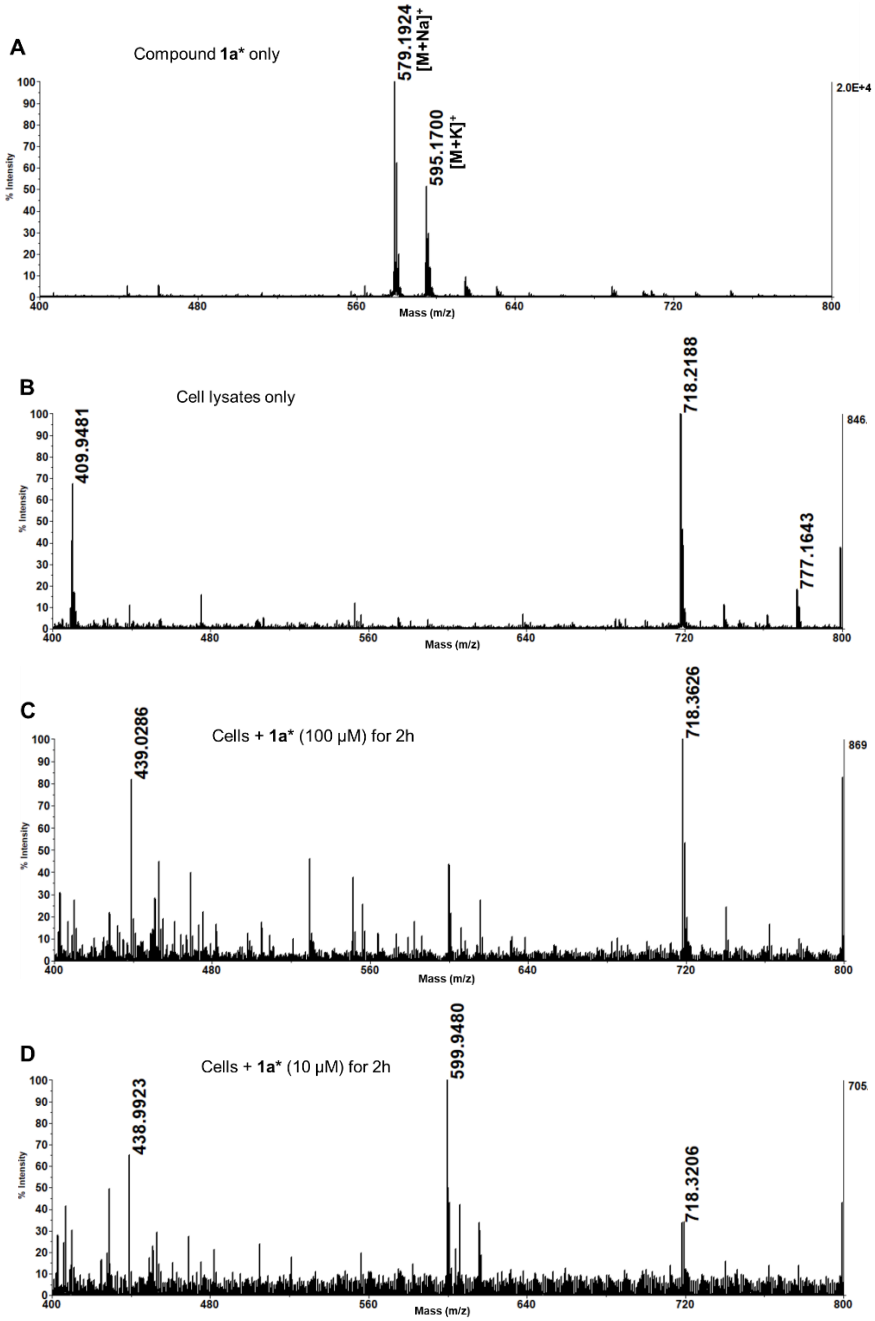
Supporting Figure S3. The selectivity study of LL320



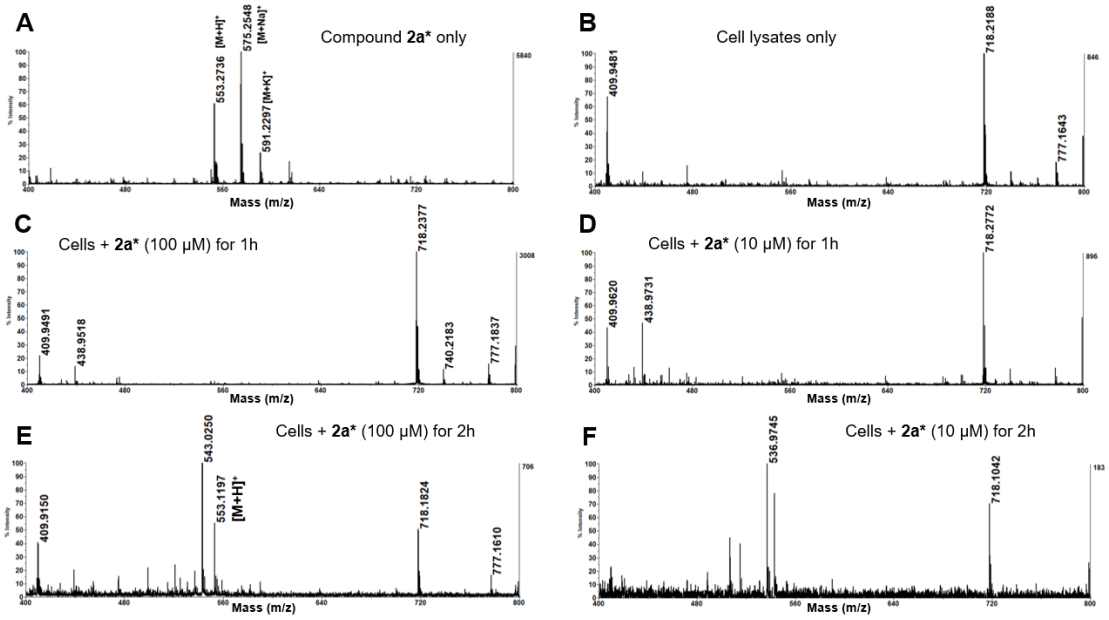
Supporting Figure S4. Superimposed co-crystal structures. (A) Overlay co-crystal structures NNMT (gray carton)- LL319 (green stick) (PDB ID: 6PVE), NNMT-LL320 (pink stick) (PDB ID: 6PVS) and NNMT-SAH (gray stick)-nicotinamide (yellow stick) (PDB ID: 3ROD); (B) Overlay co-crystal structures NNMT (gray carton)- LL319 (green stick) (PDB ID: 6PVE), NNMT-LL320 (pink stick) (PDB ID: 6PVS) and NNMT- MS2756 (blue stick) (PDB ID: 6CHH).



Supporting Figure S5. Cell permeability evaluation of TAT peptide as positive control. (A) MALDI-MS for TAT peptide (GRKKRRQRRR-NH_2) only; (B) MALDI-MS for cell lysates only; (C) MALDI-MS for incubation of 30 μM TAT peptide with HCT116 cells for 1h.



Supporting Figure S6. Cell permeability evaluation of **1a***. (A) MALDI-MS for compound **1a*** only; (B) MALDI-MS for cell lysates only; (C) and (D) MALDI-MS for incubation of 100 µM and 10 µM **1a*** with HCT116 cells for 2h, respectively. [**1a***+H]$^+$ = 557.3, [**1a***+Na]$^+$ = 579.3, [**1a***+K]$^+$ = 595.2, [**1a***-Et+H]$^+$ = 529.3, [**1a***-Et+Na]$^+$ = 551.2, [**1a***-Et+K]$^+$ = 567.2.



Supporting Figure S7. Cell permeability evaluation of **2a***. (A) MALDI-MS for compound **2a*** only; (B) MALDI-MS for cell lysates only; (C) and (D) MALDI-MS for incubation of 100 μM and 10 μM **2a*** with HCT116 cells for 1h, respectively; (E) and (F) MALDI-MS for incubation of 100 μM and 10 μM **2a*** with HCT116 cells for 2h, respectively. [**2a***+H]$^+$ = 553.3, [**2a***+Na]$^+$ = 575.2, [**2a***+K]$^+$ = 591.2, [**2a***-Et+H]$^+$ = 525.2, [**2a***-Et+Na]$^+$ = 547.2, [**2a***-Et+K]$^+$ = 563.2.

Supplementary Table S1. Crystallography data and refinement statistics (PDB ID: 6PVE and 6PVS)

Data Collection	**NNMT/LL319**	**NNMT/LL320**
λ (Å)	1.0332	1.0332
Space group	P1	P1
a, b, c (Å)	45.90, 62.36, 107.86	46.23, 62.53, 108.36
α, β, γ (º)	91.78, 97.61, 111.56	82.72, 82.48, 68.34
Resolution (Å)*	50 – 2.30 (2.38 – 2.30)	50.90 – 2.57 (2.71 – 2.57)
Completeness (%)*	98.3 (99.1)	88.4 (94.7)
Redundancy*	2.8 (2.8)	1.8 (1.8)
R_{sym} †*	0.22 (0.75)	0.16 (1.0)
I / σ (I)*	7.64 (1.6)	1.9 (0.6)
$CC_{1/2}$	0.95 (0.46)	0.98 (0.40)
Refinement		
Resolution (Å)	2.30	2.57
No. reflections	48089	31186
$R^{§}/R_{free}$¶	0.20/0.24	0.23/0.29
r.m.s. deviations		
Bonds (Å)	0.008	0.008
Angles (º)	1.35	1.31
No. Protein atoms	8172	8144
No. Ligand atoms	152	152
No. Waters	500	106
B-factors (Å^2)		
Wilson B	20.28	42.12
Protein	25.01	44.84
Ligands	16.59	39.83
Waters	26.36	37.69
Ramachandran Analysis¥		
Favored (%)	97.61	96.08
Allowed (%)	2.20	3.53
Outliers (%)	0.19	0.38
PDB code	6PVE	6PVS

† $R_{sym} = \Sigma_{hkl,j} (|I_{hkl}-\langle I_{hkl}\rangle|) / \Sigma_{hkl,j} I_{hkl}$, where $\langle I_{hkl}\rangle$ is the average intensity for a set of j symmetry related reflections and I_{hkl} is the value of the intensity for a single reflection within a set of symmetry-related reflections.

§ R factor $= \Sigma_{hkl} (||F_o| - |F_c||) / \Sigma_{hkl}|F_o|$ where F_o is the observed structure factor amplitude and F_c is the calculated structure factor amplitude.

¶ $R_{free} = \Sigma_{hkl,T} (||F_o| - |F_c||) / \Sigma_{hkl,T}|F_o|$, where a test set, T (5% of the data), is omitted from the

refinement.

¥ Performed using Molprobity within PHENIX.

* Indicates statistics for last resolution shell shown in parenthesis.

Reference

(1) Zhang, G.; Richardson, S. L.; Mao, Y.; Huang, R. Design, Synthesis, and Kinetic Analysis of Potent Protein N-Terminal Methyltransferase 1 Inhibitors. *Org. Biomol. Chem.* **2015**, *13* (14), 4149–4154.

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