Supplementary Material (ESI) for Chemical Communications

Supporting Information

Design, Synthesis and Evaluation of Hypoxia-Activated Pro-Oligonucleotides

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Experimental

5′-O-dimethoxytrityl thymidine 3′-O-[(5-nitro-2-furyl)methyl *N*,*N*-diisopropylphosphoramidite] (2a)

5′-O-dimethoxytrityl thymidine (544.6 mg, 1 mmol) was dissolved in dry CH_2Cl_2 (10 mL), then bis (*N*,*N*-diisopropylamino) chlorophosphine (320.8 mg, 1.2 mmol) and *N*,*N*-diisopropylethylamine (241 μL, 1.5 mmol) were added. The reaction mixture was stirred for 30 min at room temperature under argon. 5-nitro-2-hydroxymethylfuran (171.7 mg, 1.2 mmol) and 4,5-dicyanoimidazole (118.1 mg, 1 mmol) were added, and the reaction mixture was stirred for 1 h at room temperature under argon. CH_2Cl_2 (10 mL) was added and the reaction mixture was washed by 5% $NaHCO_3$ (10 mL) and brine (10 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered and evaporated. The residue was purified by column chromatography (n-hexane: ethyl acetate = 1:1, with 1% triethylamine) to afford **2a** (555.4 mg, 0.68 mmol, 68%).

^{31}P NMR(300 MHz, $CDCl_3$): 149.7, 150.2 (two isomers, 1:1)

ESI-MS: $[M+Na]^+$ m/z Calcd for $C_{42}H_{49}N_4NaO_{11}P$: 839.3033, Found: 839.3033

5′-O-dimethoxytrityl thymidine 3′-O-[(5-nitro-2-thienyl)methyl N,N-diisopropylphosphoramidite] (2b)

5′-O-dimethoxytrityl thymidine (544.6 mg, 1 mmol) was dissolved in dry CH_2Cl_2 (10 mL), then bis (*N*,*N*-diisopropylamino) chlorophosphine (320.8 mg, 1.2 mmol) and *N*,*N*-diisopropylethylamine (241 μL, 1.5 mmol) were added. The reaction mixture was stirred for 30 min at room temperature under argon. 5-nitro-2-hydroxymethylthiophene (191.0 mg, 1.2 mmol) and 4,5-dicyanoimidazole (118.1 mg, 1 mmol) were added, and the reaction mixture was stirred for 1 h at room temperature under argon. CH_2Cl_2 (10 mL) was added and the reaction mixture was washed by 5% $NaHCO_3$ (10 mL) and brine (10 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered and evaporated. The residue was purified by column chromatography (n-hexane: ethyl acetate = 1:1, with 1% triethylamine) to afford **2b** (708.0 mg, 0.85 mmol, 85%).

^{31}P NMR(300 MHz, $CDCl_3$): 149.9, 150.1 (two isomers, 1:1)

ESI-MS: $[M+Na]^+$ m/z Calcd for $C_{42}H_{49}N_4NaO_{10}PS$: 855.2805, Found: 8559.2813

Synthesis, Deprotection and Purification of Oligonucleotides

Oligonucleotides were synthesized on ABI 3900 DNA Synthesizer at 0.1 μmol scale, using phosphoramidite approach. 0.1 M solution of phosphoramidate **2a** or **2b** in anhydrous acetonitrile was used for synthesis of modified oligonucleotides, and 5-ethylthio-1*H*-tetrazole (ETT) was used as an activator. When phosphoramidate **2a** or **2b** was incorporated into oligonucleotides, two portions of phosphoramidate solutions were delivered followed by 5 min coupling time. Oligonucleotides with modifications in the 3'-terminal ends were synthesized using universal CPG.. Oligonucleotides were removed from CPG using aqueous ammonia at 55 □ for 12 h, and the protecting groups was removed at the same time. Crude oligonucleotides were purified by HPLC (LiChrosphere 100, RP-18, 4.6×250 mm, flow 1 ml/min, eluent A: water, eluent B: acetonitrile, gradient started from A:B=100:0, ended with A:B=30:70 over 40 min). The isolated yield of modified oligonucleotides was approximately 30%. Oligoucleotides were analyzed by HPLC and MALDI-TOF mass spectroscopy.

Hydrolysis with E.Coli Nitroreductase

The oligonucleotide (0.25 OD)was incubated with 1 mM NADPH at 37 □ in 10 mM sodium phosphate buffer (pH 7.0) in a total volume of 250 μL. The reaction was initiated by addition of 1.8 μg of E.Coli nitroreductase. The solution was vortex-mixed and then was incubated at 37 □ in air or in N_2 for 12 h. Aliquots (5 μL) were taken at various times, and quenched by acetonitrile. Degradation of oligonucleotides was analyzed by HPLC.

HPLC condition: Agilent Zorbax oligo, 6.2 × 80 mm, flow 1 ml/min, eluent A: water, eluent B: acetonitrile, gradient started from A:B=100:0, ended with A:B=30:70 over 40 min).

CEM cell extracts preparation

CEM cells in logarithmic growth were separated from their culture medium by centrifugation (10^4 g, 4 min, 4 □). The residue was resuspended in 2 mL of buffer (10 mM Tris HCl, 140 mM KCl, pH 7.4) and sonicated. The lysate was centrifuged (10^5 g, 1 h, 4□) to remove membranes, organelles and chromatin. The upper layer was filtered (0.22 μm Millex GV, Millipore) and stored in a sterile container at -80□.

Hydrolysis in CEM cell extract

The oligo (0.25 OD)was incubated in CEM cell extract (250 μL) at 37 □ (in air or N_2). Aliquots (5 μL) were taken at various times, and quenched by acetonitrile. Degradation of oligonucleotides was analyzed by HPLC.

HPLC condition: Agilent Zorbax oligo, 6.2×80mm, flow 1 ml/min, eluent A: water, eluent B: acetonitrile, gradient started from A:B=100:0, ended with A:B=30:70 over 40 min).

Hydrolysis with snake venom phosphodiesterase

The oligo (0.25 OD)was incubated with snake venom phosphodiesterase (0.06 U) in 0.01 M $MgCl_2$, 0.1 M Tris–HCl (pH 7.3) in a total volume of 250 μL at 37 □. Aliquots (5 μL) were taken at various times, and quenched by acetonitrile. Degradation of oligonucleotides was analyzed by HPLC.

HPLC condition: Agilent Zorbax oligo, 6.2×80mm, flow 1 ml/min, eluent A: water, eluent B: acetonitrile, gradient started from A:B=100:0, ended with A:B=30:70 over 40 min).

Hydrolysis with DNase I

The oligo (0.25 OD)was incubated with DNase I (20 U) in 0.01 M $MgCl_2$, 0.1 M Tris–HCl (pH 7.5) in a total volume of 250 μL at 37 □. Aliquots (5 μL) were taken at various times, and quenched by acetonitrile. Degradation of oligonucleotides was analyzed by HPLC.

HPLC condition: Agilent Zorbax oligo, 6.2×80mm, flow 1 ml/min, eluent A: water, eluent B: acetonitrile, gradient started from A:B=100:0, ended with A:B=30:70 over 40 min).

Hydrolysis in human serum

The oligo (0.25 OD) was incubated in human serum (250 μl) at 37 □. Aliquots (5 μL) were taken at various times, and quenched by acetonitrile. Degradation of oligonucleotides was analyzed by HPLC.

HPLC condition: Agilent Zorbax oligo, 6.2×80mm, flow 1 ml/min, eluent A: water, eluent B: acetonitrile, gradient started from A:B=100:0, ended with A:B=30:70 over 40 min).

Cell culture

Hela cells were routinely maintained in DMEM high-glucose medium supplemented with 10% heat-inactived fetal bovine serum, 100 units/ml penicillin and 100 μg/ml streptomycin in a humidified atmosphere at 37℃ in 5% CO_2. One day before transfection, 1×10^5 cells were plated in a 24-well plate. Cells were cultured in 100 μL fresh Opti-MEMI medium 30 min before transfection.

Confocal microscopic analysis

Appropriate amount of oligonucleotide were diluted in 10 μL TE, then diluted to 100 μL in Opti-MEMI medium. Remove Opti- MEMI medium from well and add diluted oligonucleotide to each well containing cells and incubate at 37℃ in 5% CO_2. After incubation, remove medium and wash cells with PBS for 5 times, and confocal microscopic analysis were carried out on a Leica TCS SP2 model.

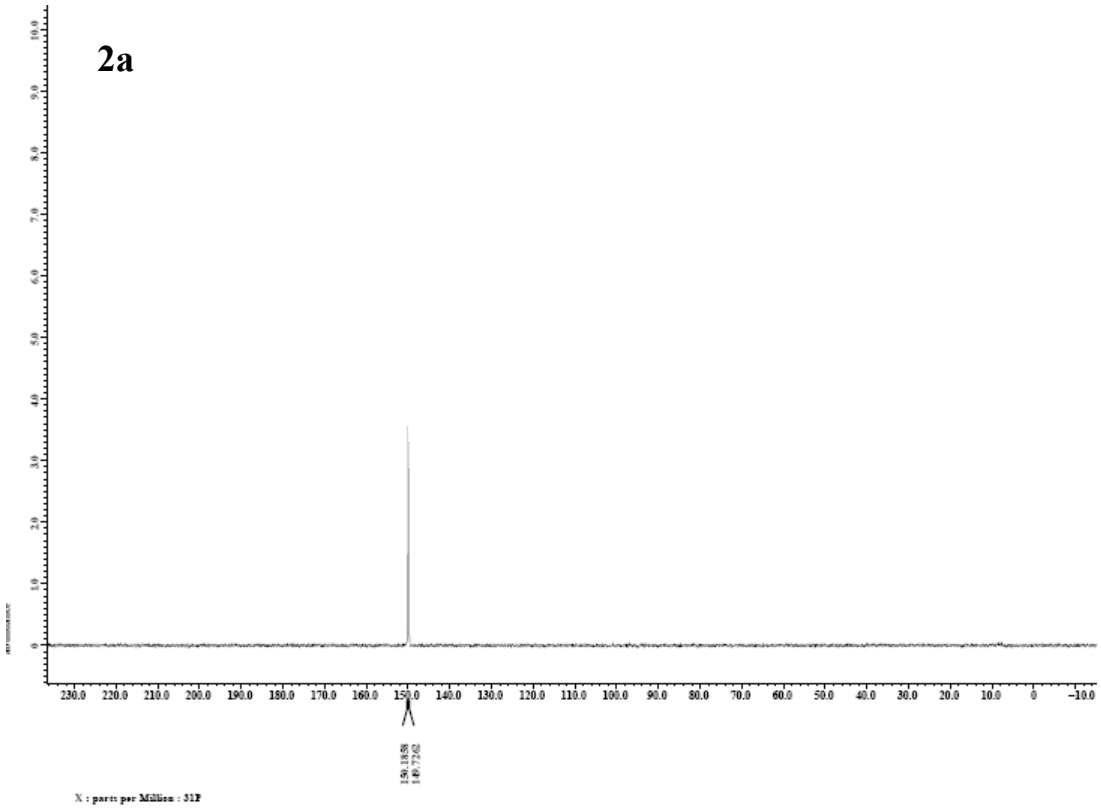
Table S1. Half-lives of pro-oligos **3a** and **3b** degraded by E. Coli nitroreductase and CEM cell extracts in N_2 and in air, respectively.

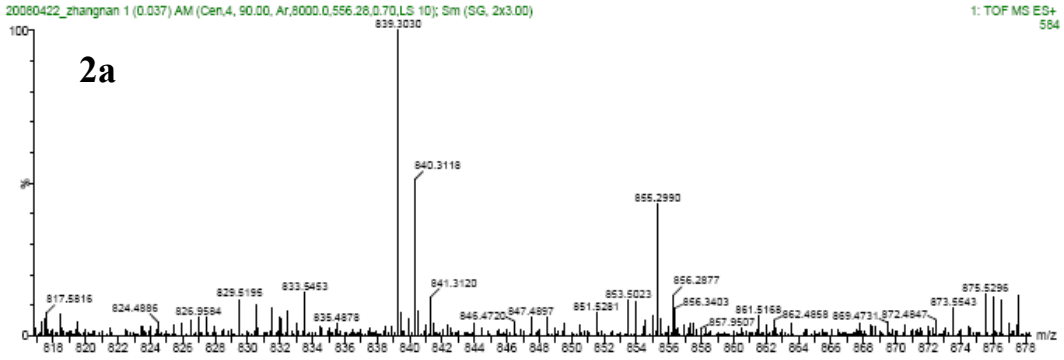
Pro-Oligo	$t_{1/2}$ / h			
	E. Coli nitroreductase		CEM Cell extract	
	N_2	air	N_2	air
3a	3.1	9.2	---	---
3b	4.3	8.8	---	---
4a	---	---	3.6	9.2
4b	---	---	4.2	9.8

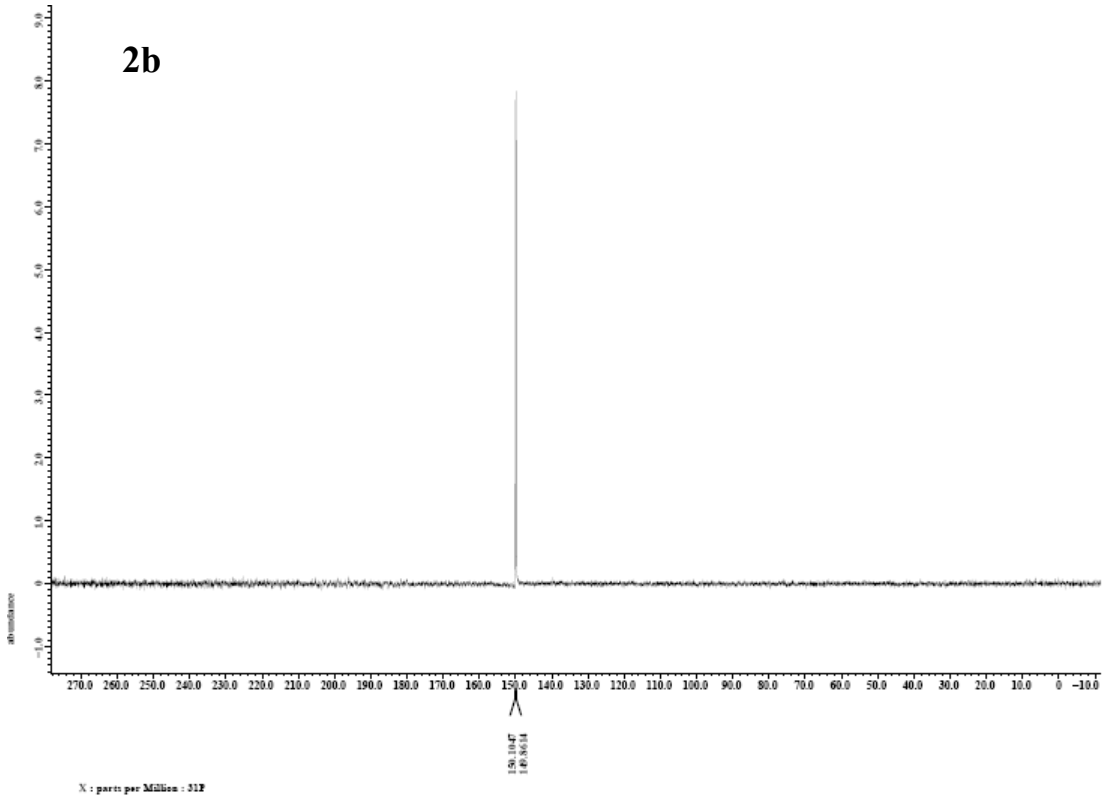
Table S2. Half-lives of pro-oligo **4a** and **4b** and oligo **5** incubated with nucleases and in biological media.

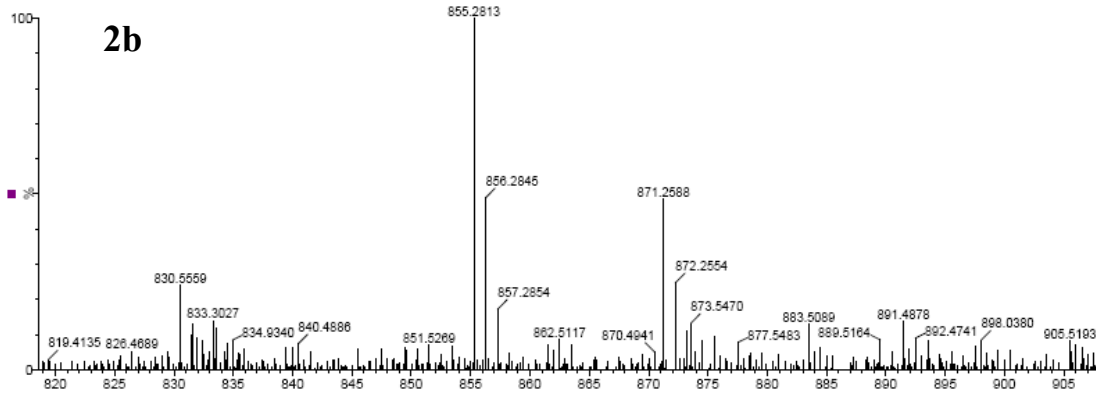
Compound	$t_{1/2}$ / h		
	SVPED	DNase I	Serum
4a	8.0	8.9	8.2
4b	10.4	10.9	8.5
5	0.30	0.35	0.2

Mass and NMR spectrum.






2b
abundance
X : parts per Million : 31P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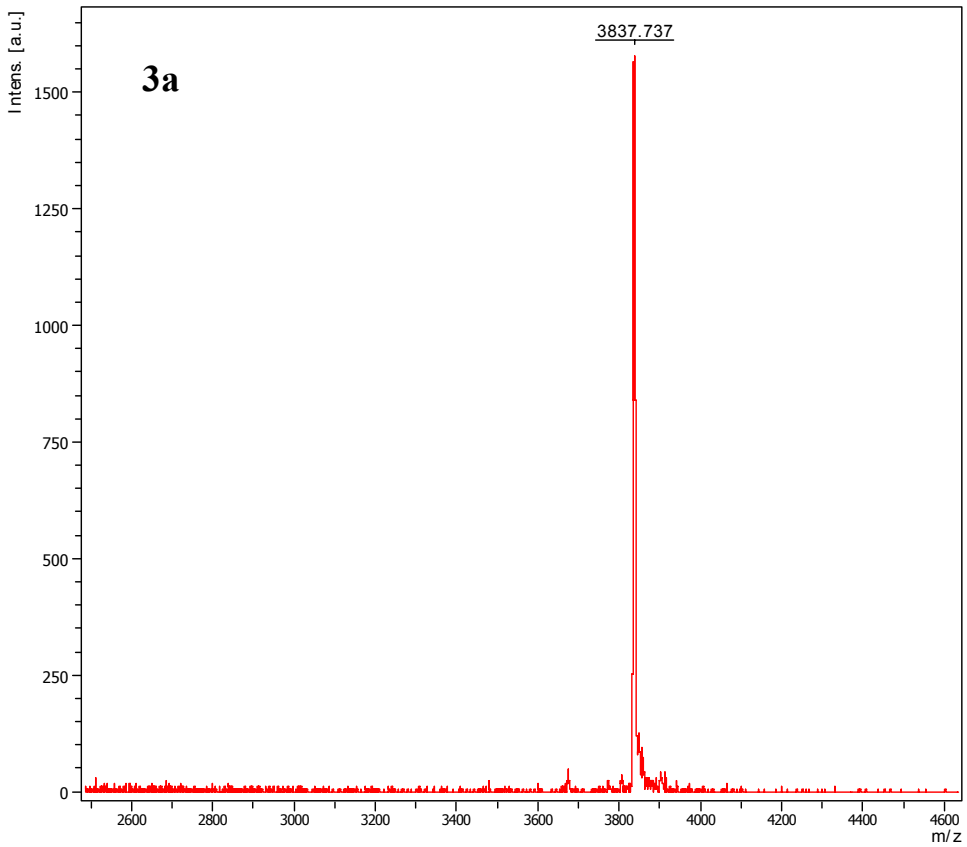
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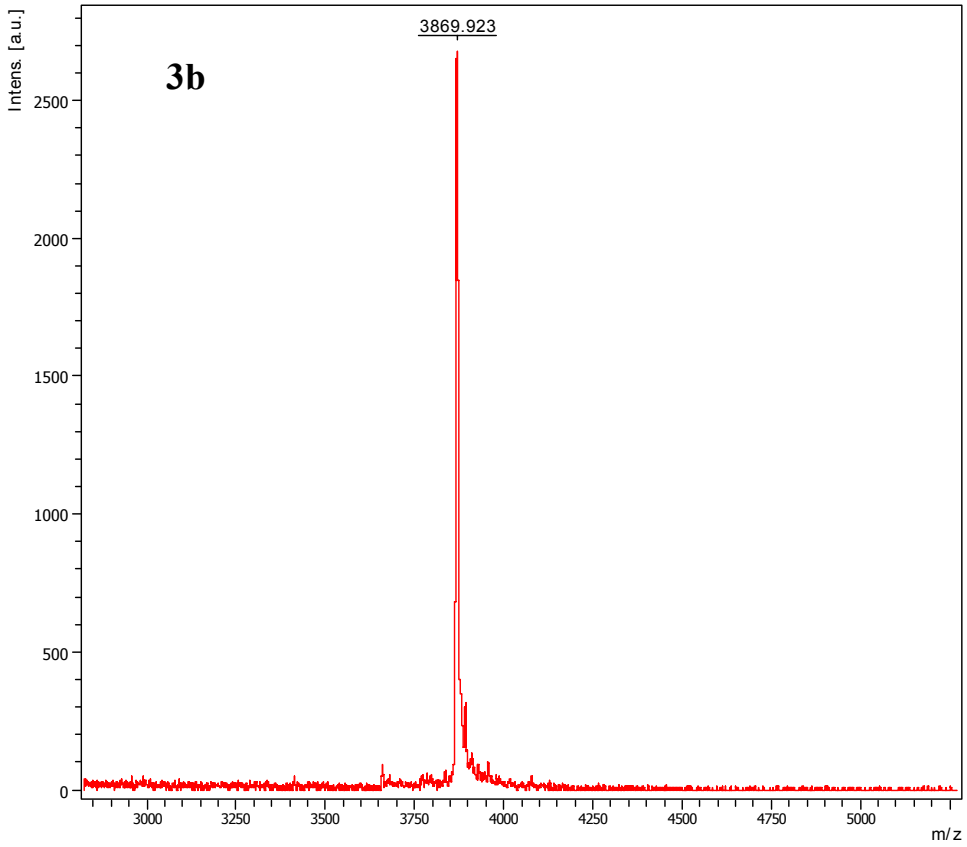
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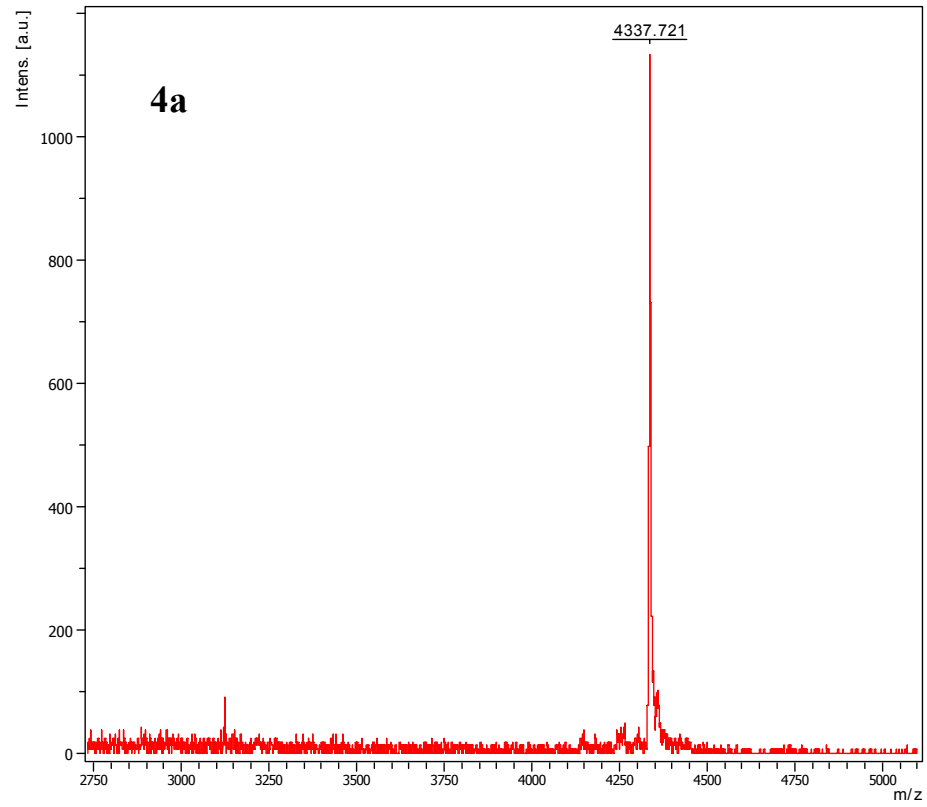
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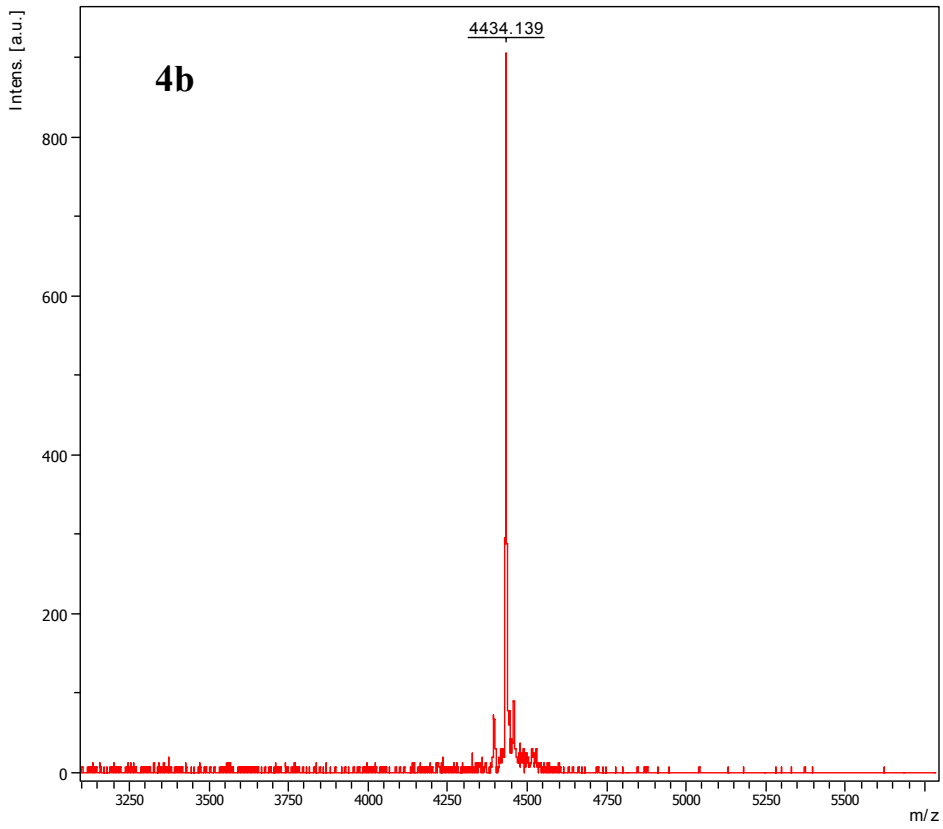
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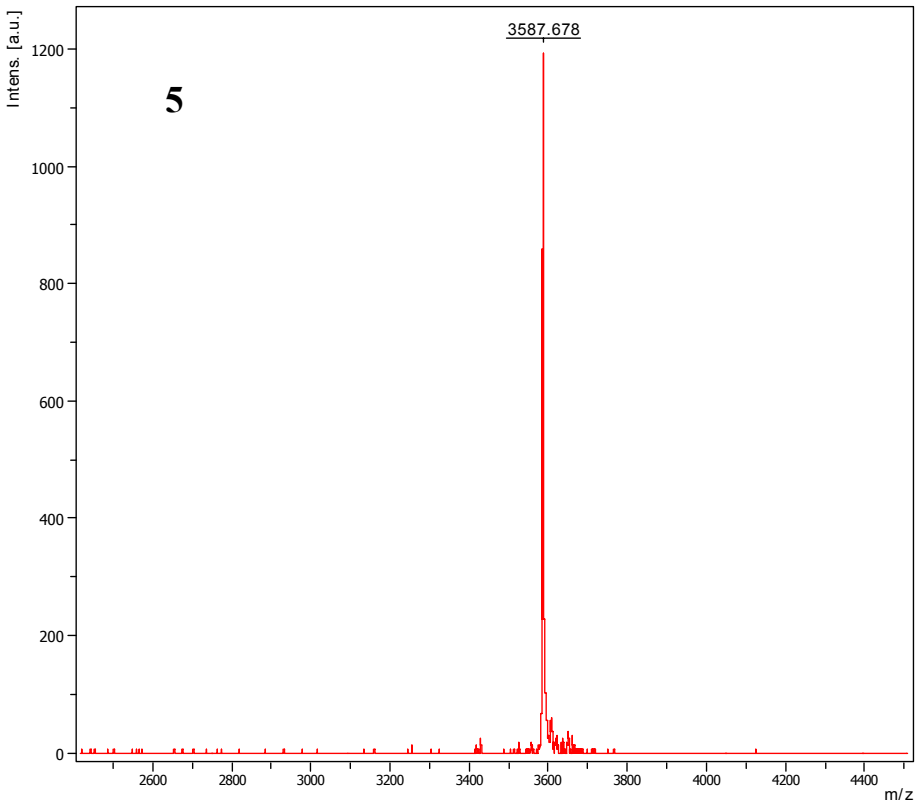
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