Supporting Information

Parallel Synthesis of Oligonucleotides Containing N-Acyl amino-LNA and Their Therapeutic Effects as Anti-microRNAs

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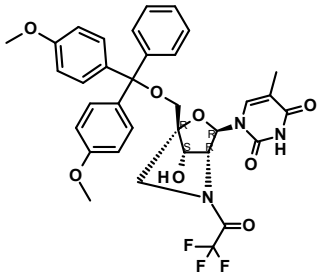
Satoshi Obika[b]

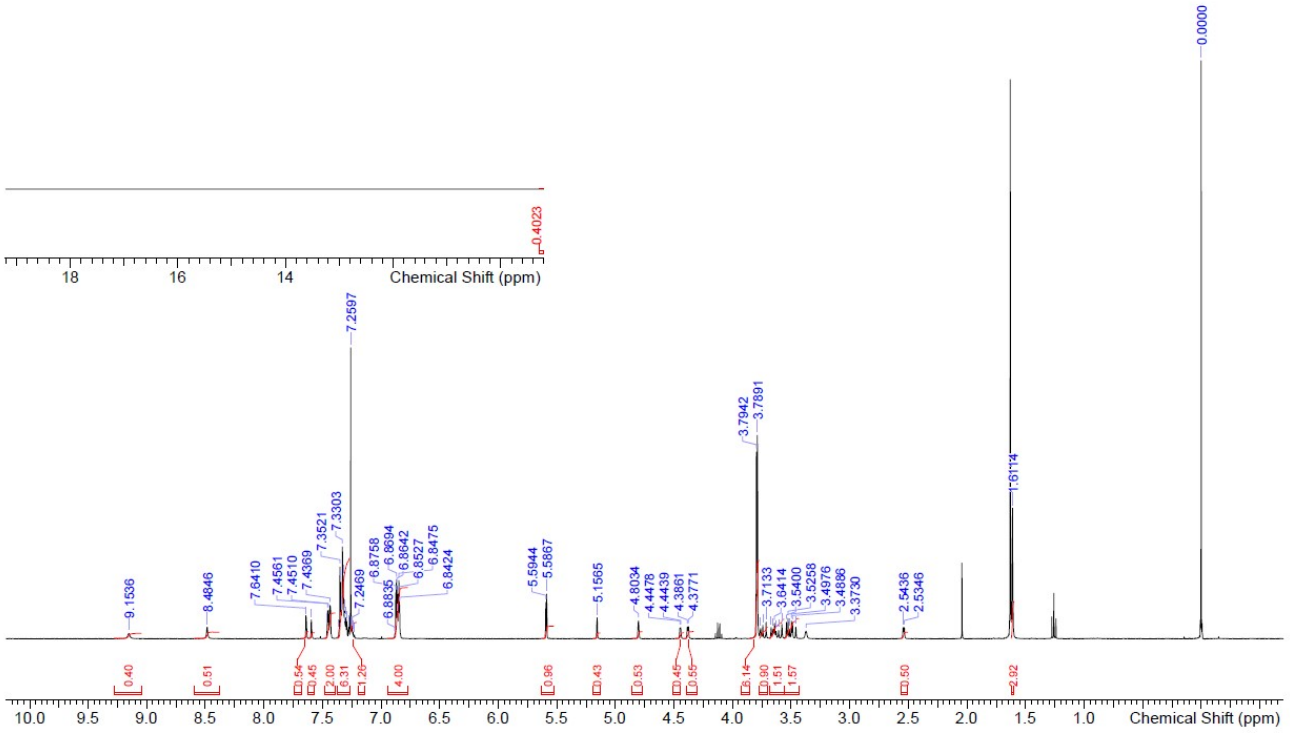
Contents

1. ^{1}H, ^{13}C and ^{31}P-NMR spectra of synthesized compounds

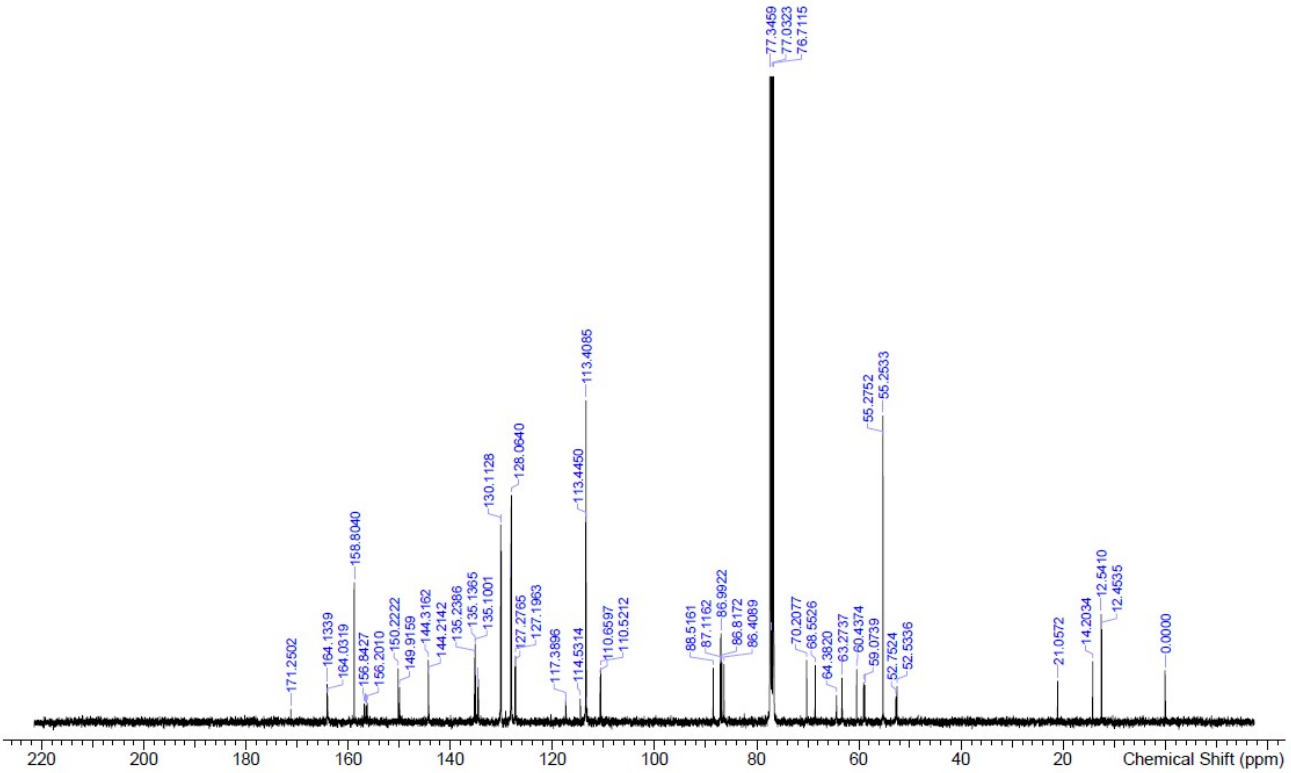
1-[(1R,3R,4R,7S)-1-[[bis(4-methoxyphenyl)-phenyl-methoxy]methyl]-7-hydroxy-5-(2,2,2-trifluoroacetyl)-2-oxa-5-azabicyclo[2.2.1]heptan-3-yl]-5-methyl-pyrimidine-2,4-dione (T-2a)



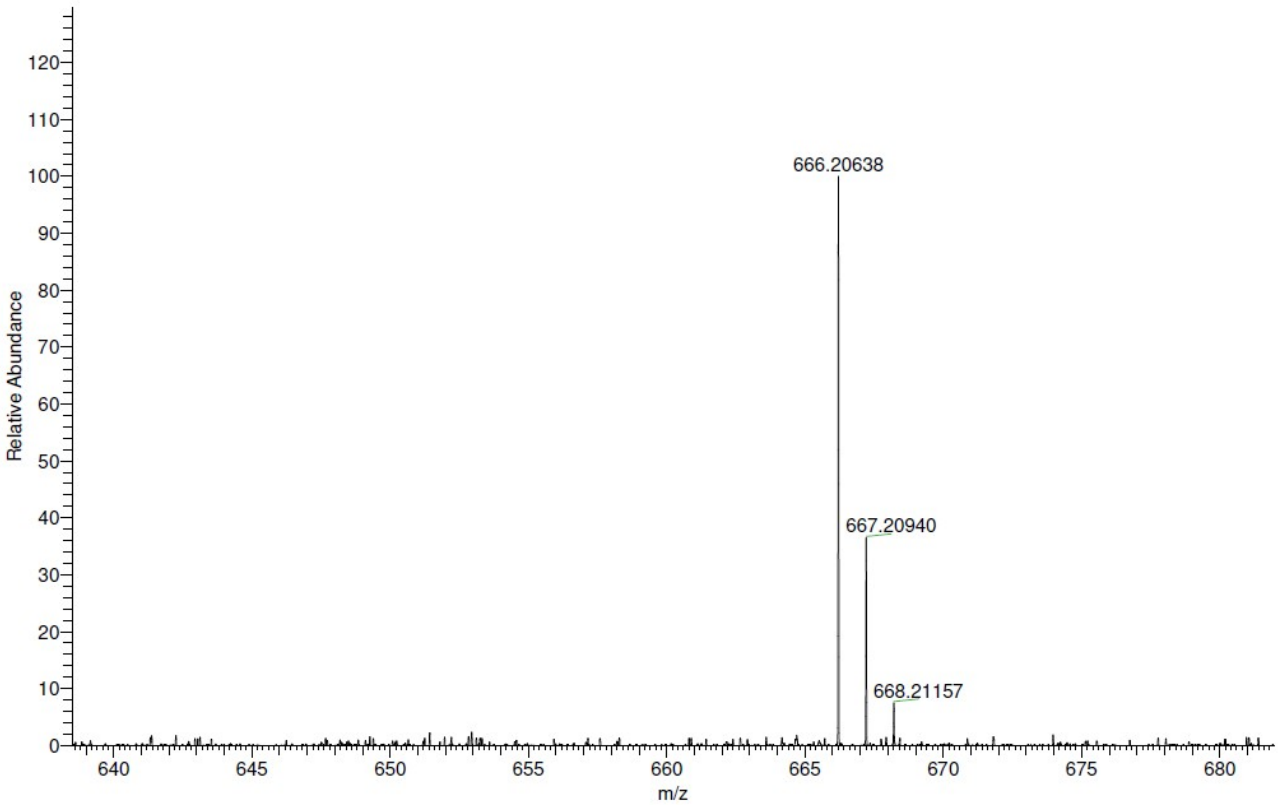
^{1}H NMR (400 MHz, CHLOROFORM-d) of **T-2a**



^{13}C NMR (101 MHz, CHLOROFORM-d) of **T-2a**



High resolution mass spectra of **T-2a**

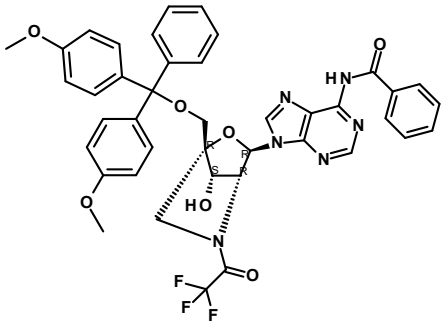


Elemental composition search on mass 666.20638

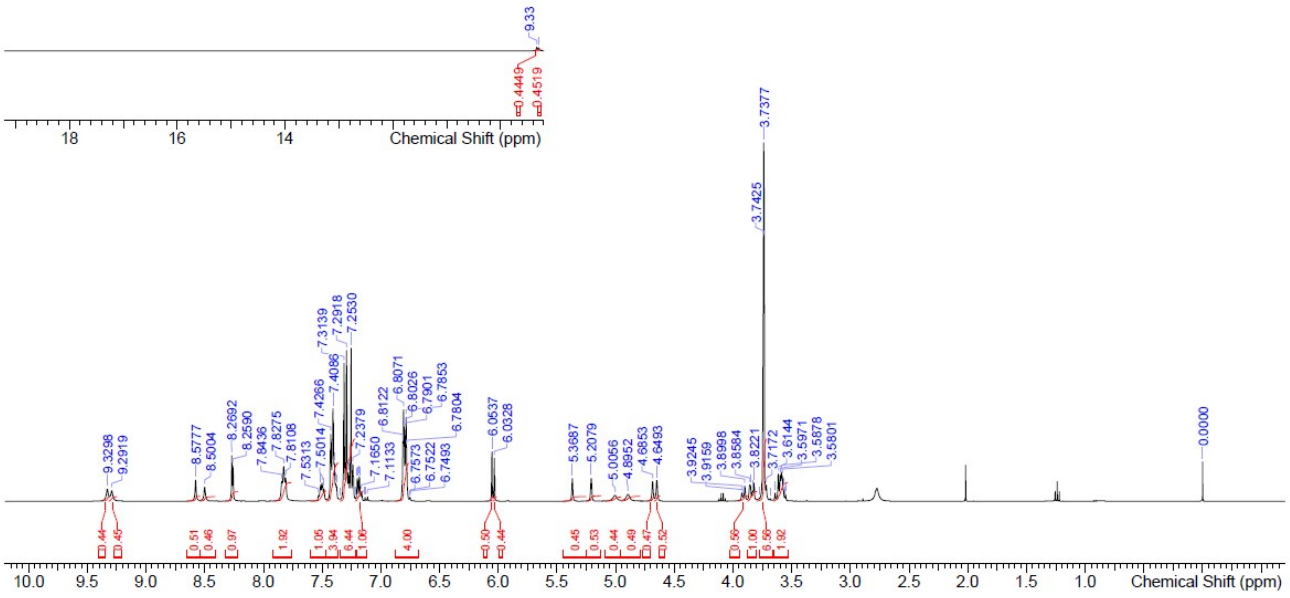
m/z= 661.20638-671.20638

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
666.20638	666.20687	-0.74	19.5	$C_{34}H_{31}O_8N_3F_3$

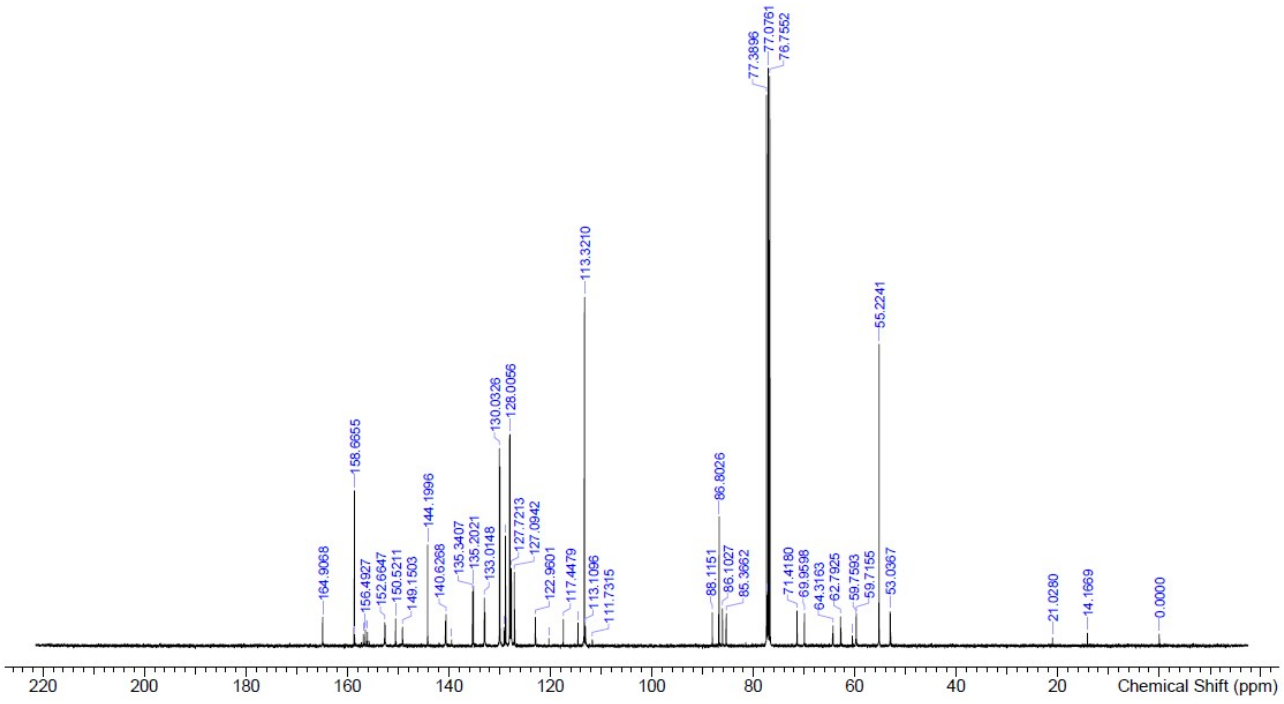
N-[9-[(1R,3R,4R,7S)-1-[[bis(4-methoxyphenyl)-phenyl-methoxy]methyl]-7-hydroxy-5-(2,2,2-trifluoroacetyl)-2-oxa-5-azabicyclo[2.2.1]heptan-3-yl]purin-6-yl]benzamide (A^{bz}-2a)



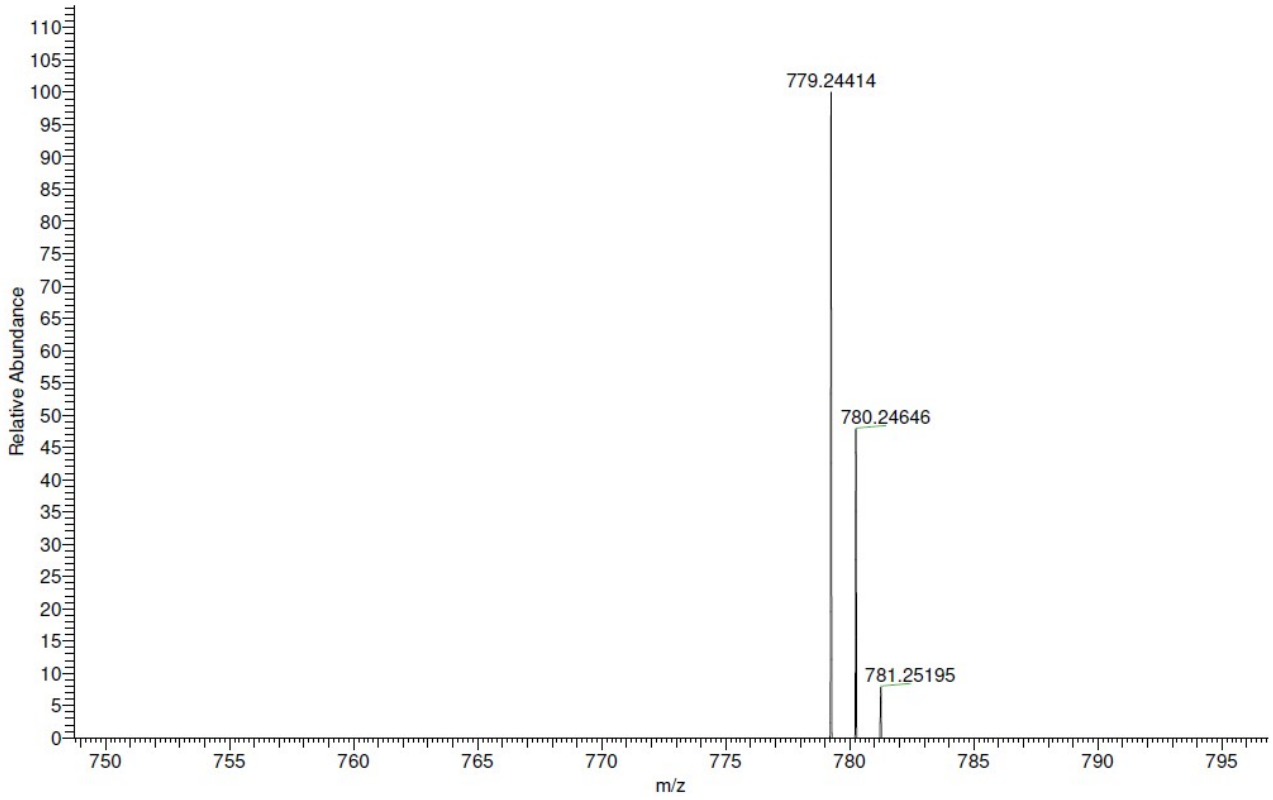
^{1}H NMR (400 MHz, CHLOROFORM-d) of **A^{bz}-2a**



^{13}C NMR (101 MHz, CHLOROFORM-d) of **A^{bz}-2a**



High resolution mass spectra of **A^{bz}-2a**

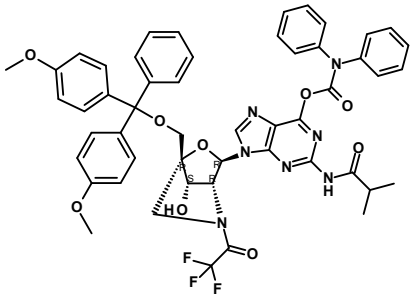


Elemental composition search on mass 779.24414

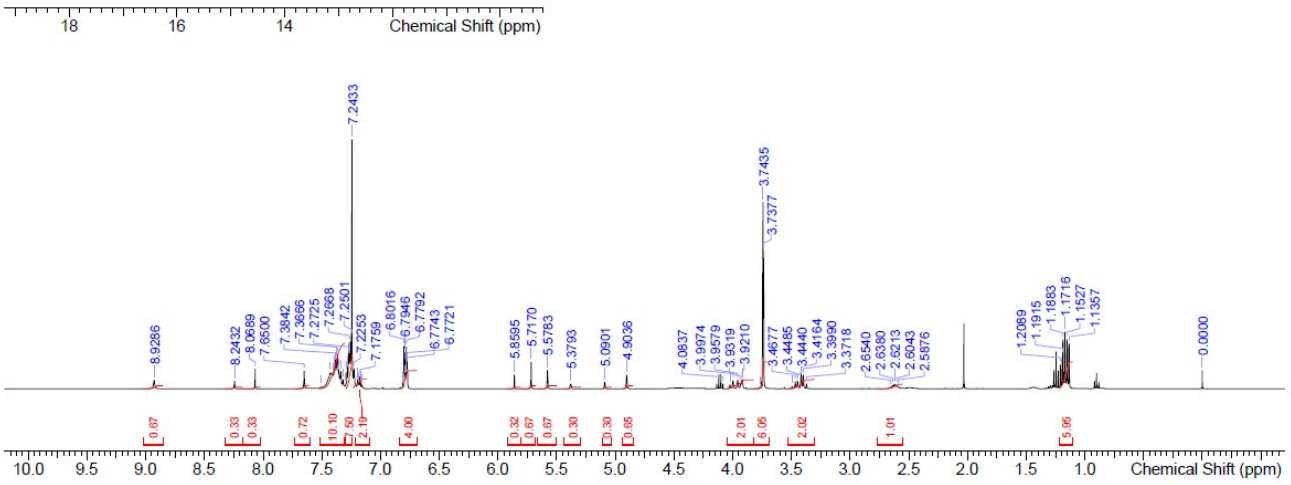
m/z= 774.24414-784.24414

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
779.24414	779.24466	-0.66	26.5	$C_{41}H_{34}O_7N_6F_3$

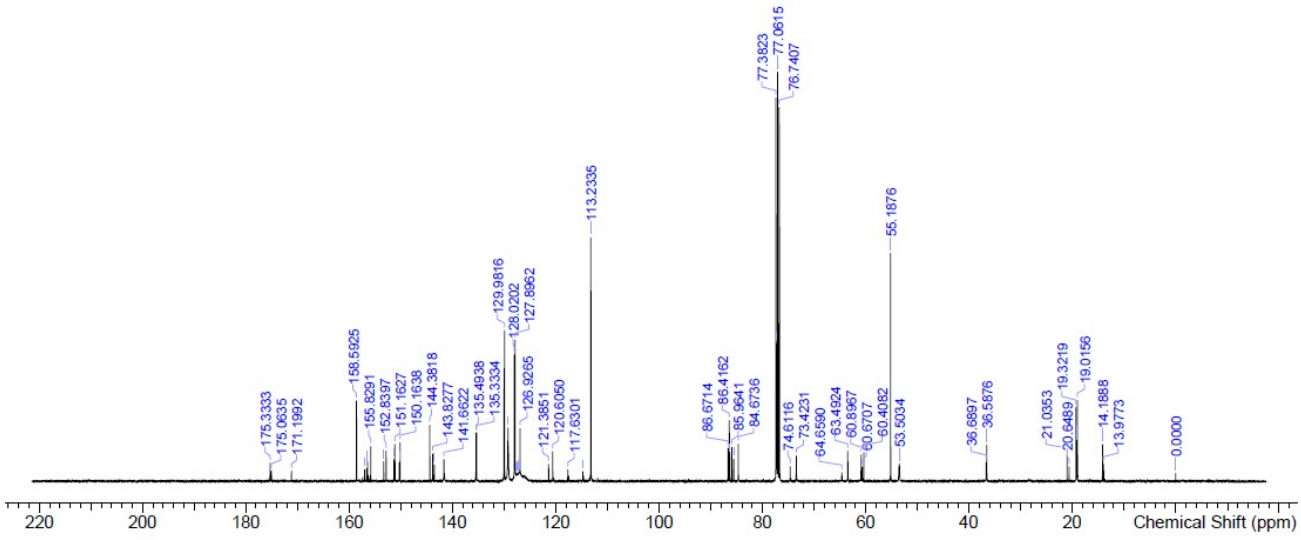
[9-[(1R,3R,4R,7S)-1-[[bis(4-methoxyphenyl)-phenyl-methoxy]methyl]-7-hydroxy-5-(2,2,2-trifluoroacetyl)-2-oxa-5-azabicyclo[2.2.1]heptan-3-yl]-2-(2-methylpropanoylamino)purin-6-yl] N,N-diphenylcarbamate ($G^{dpc, ibu}$-2a)



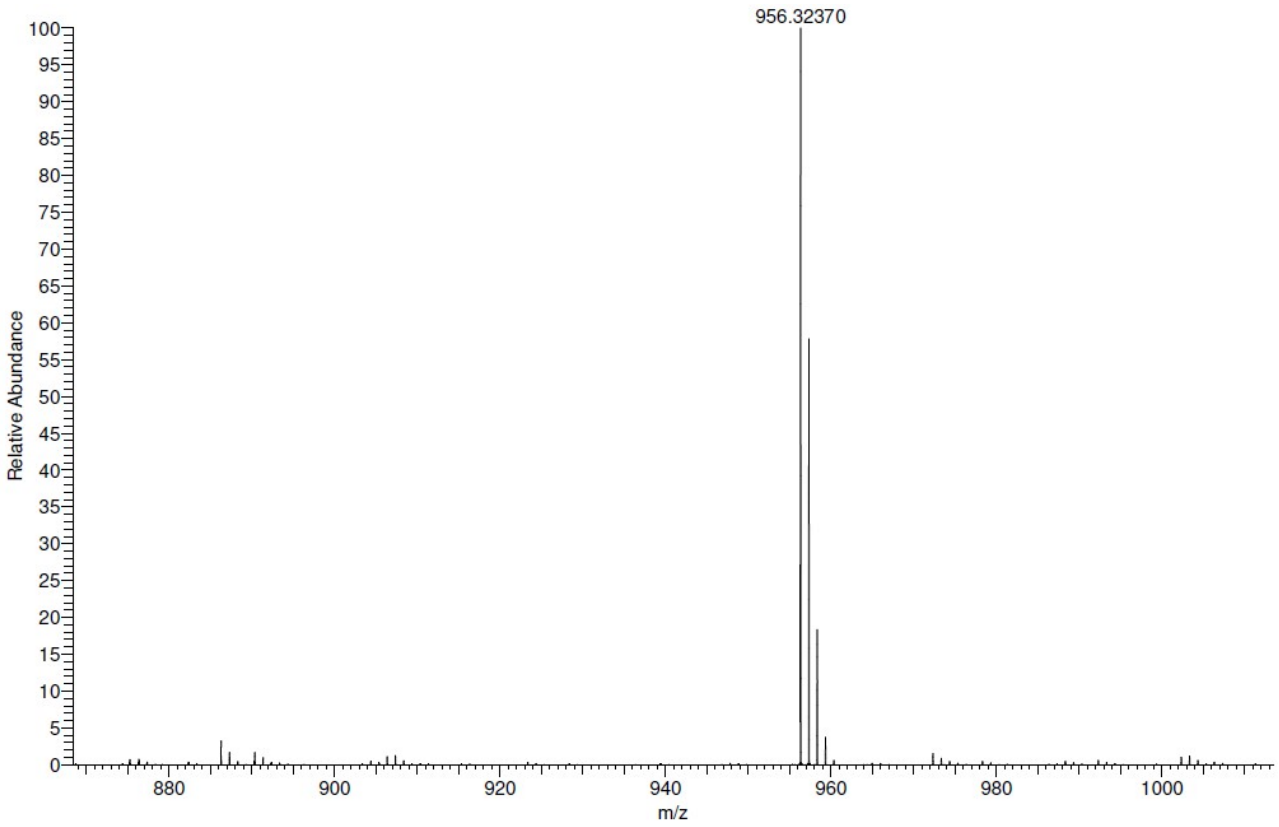
^{1}H NMR (400 MHz, CHLOROFORM-d) of **$G^{dpc, ibu}$-2a**



^{13}C NMR (101 MHz, CHLOROFORM-d) of **G$^{dpc,\ ibu}$-2a**



High resolution mass spectra of **G$^{dpc,\ ibu}$-2a**

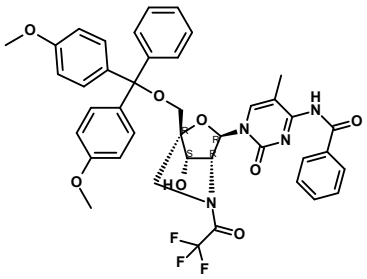


Elemental composition search on mass 956.32370

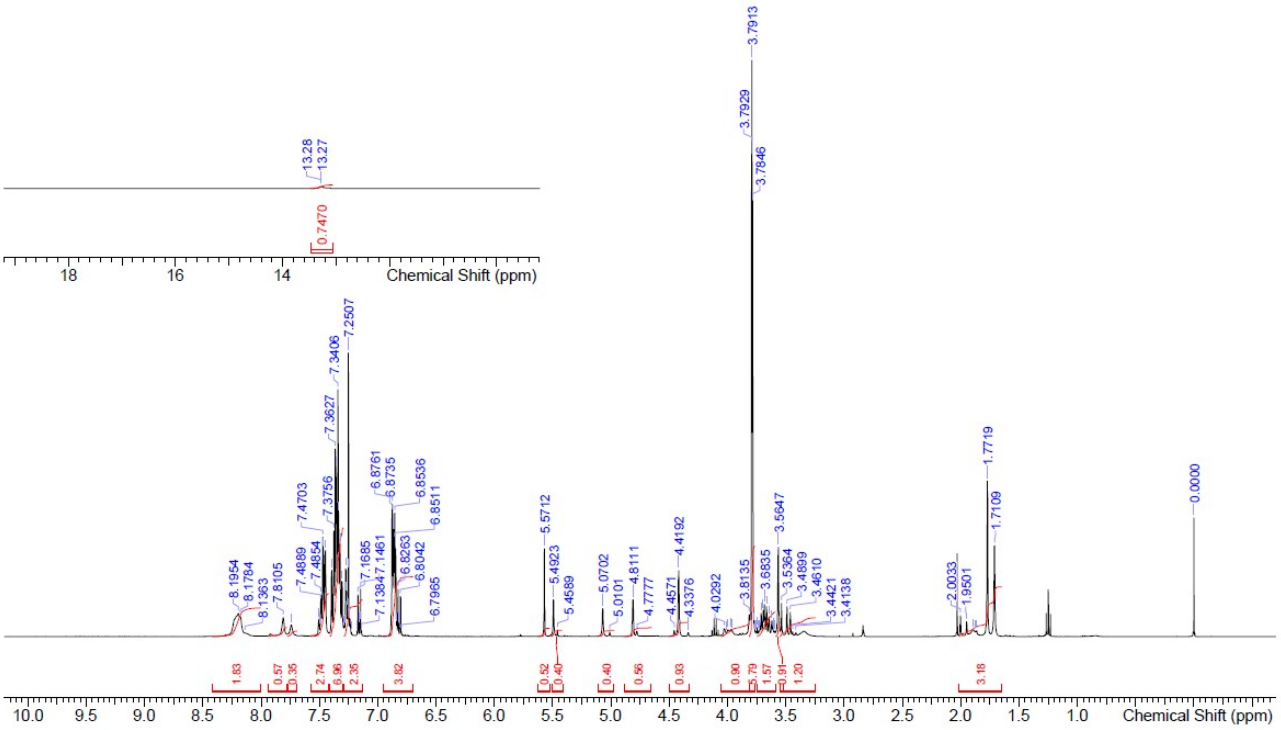
m/z= 951.32370-961.32370

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
956.32370	956.32363	0.07	31.5	$C_{51}H_{45}O_9N_7F_3$

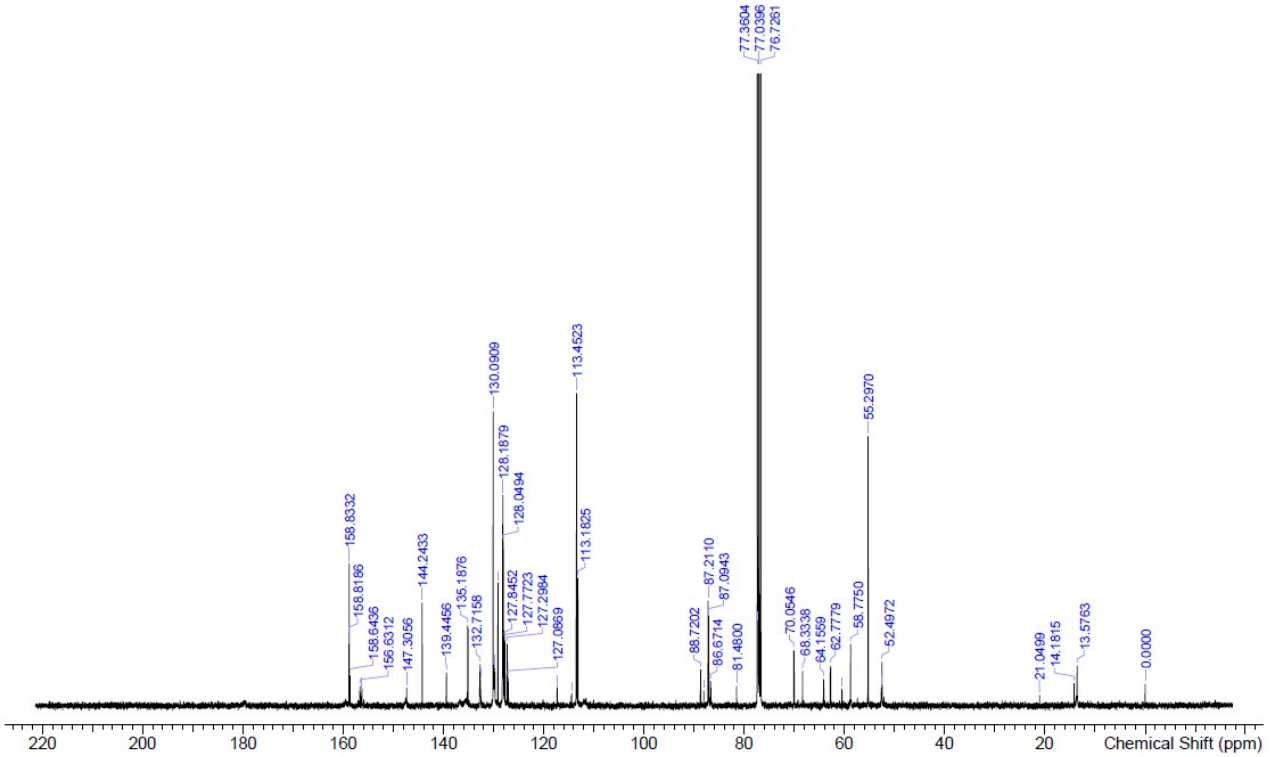
N-[1-[(1R,3R,4R,7S)-1-[[bis(4-methoxyphenyl)-phenyl-methoxy]methyl]-7-hydroxy-5-(2,2,2-trifluoroacetyl)-2-oxa-5-azabicyclo[2.2.1]heptan-3-yl]-5-methyl-2-oxo-pyrimidin-4-yl]benzamide (mC^{bz}-2a)



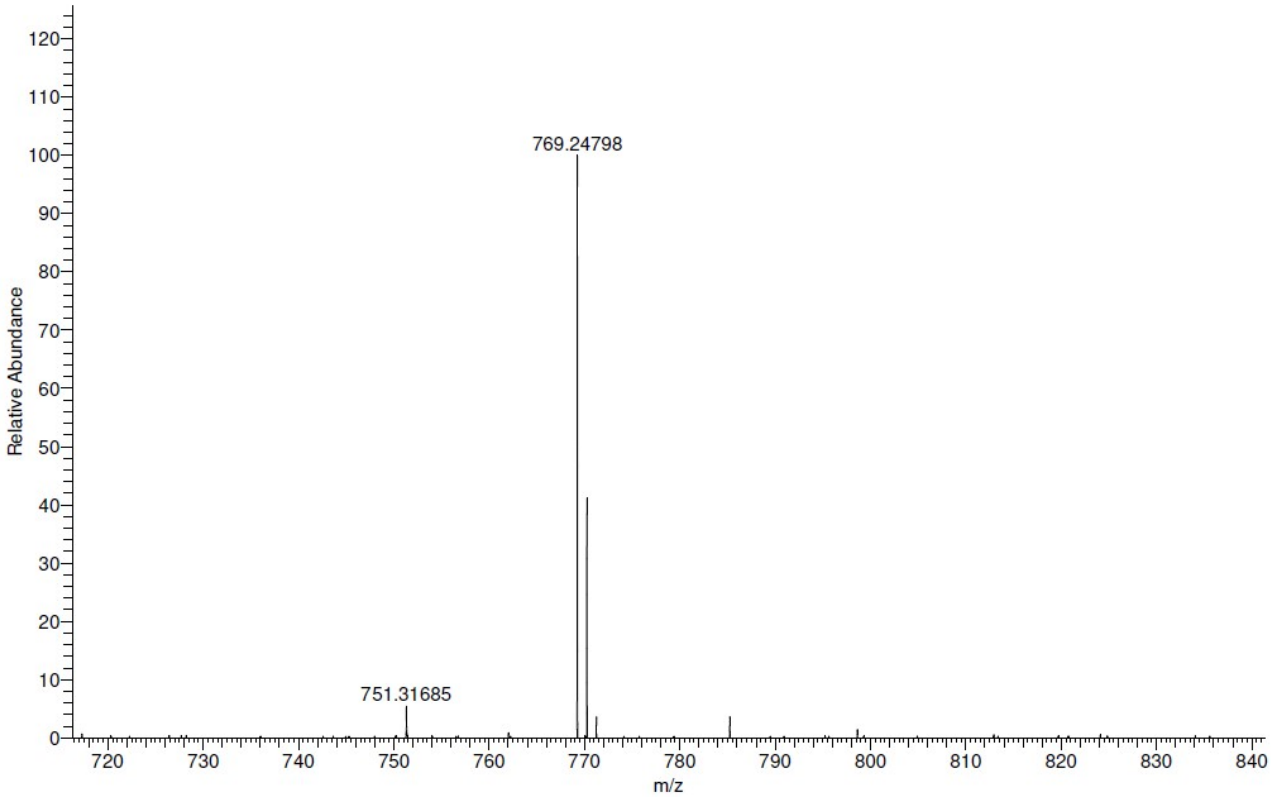
^{1}H NMR (400 MHz, CHLOROFORM-d) of **mC^{bz}-2a**



^{13}C NMR (101 MHz, CHLOROFORM-d) of **mC^{bz}-2a**



High resolution mass spectra of **mC^{bz}-2a**

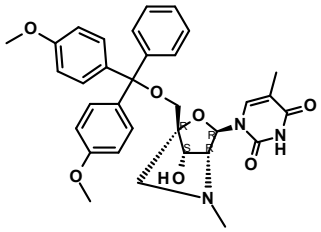


Elemental composition search on mass 769.24798

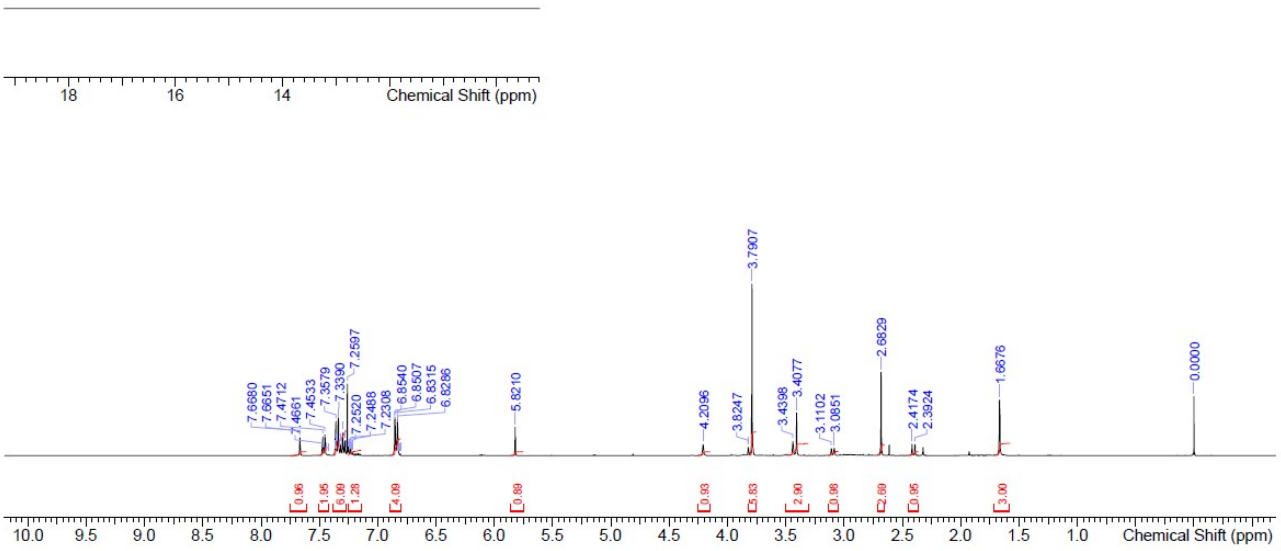
m/z= 764.24798-774.24798

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
769.24798	769.24907	-1.42	24.5	$C_{41}H_{36}O_8N_4F_3$

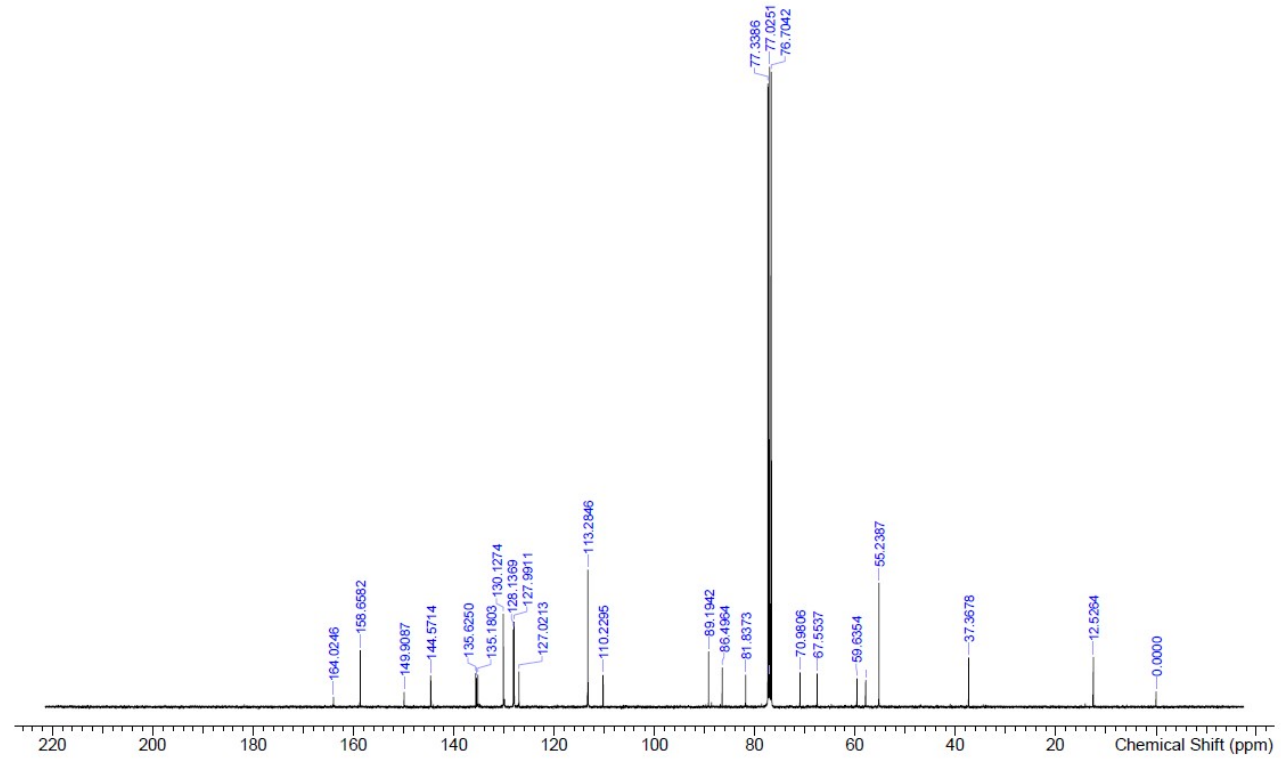
1-[(1R,3R,4R,7S)-1-[[bis(4-methoxyphenyl)-phenyl-methoxy]methyl]-7-hydroxy-5-methyl-2-oxa-5-azabicyclo[2.2.1]heptan-3-yl]-5-methyl-pyrimidine-2,4-dione (T-2b)



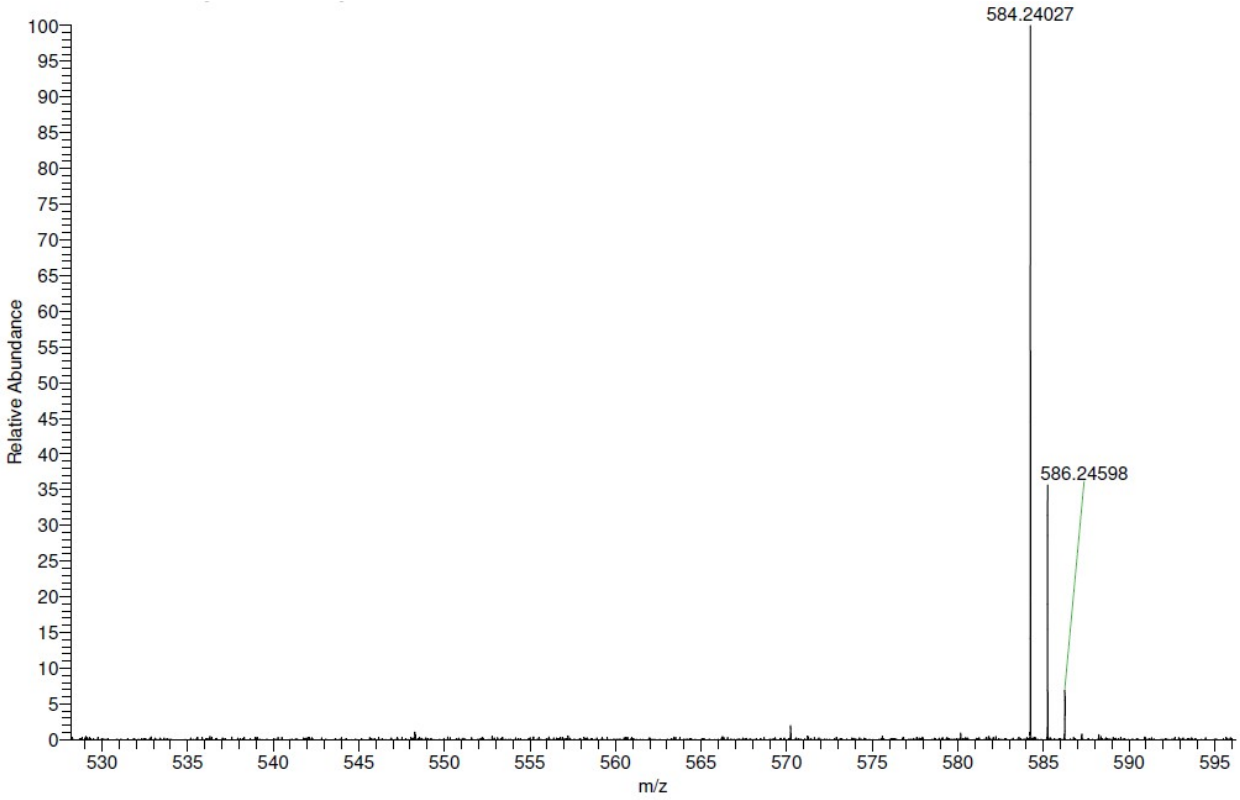
^{1}H NMR (400 MHz, CHLOROFORM-d) of **T-2b**



^{13}C NMR (101 MHz, CHLOROFORM-d) of **T-2b**



High resolution mass spectra of **T-2b**

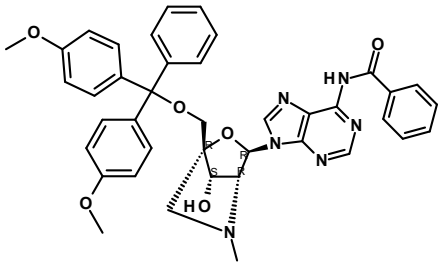


Elemental composition search on mass 584.24027

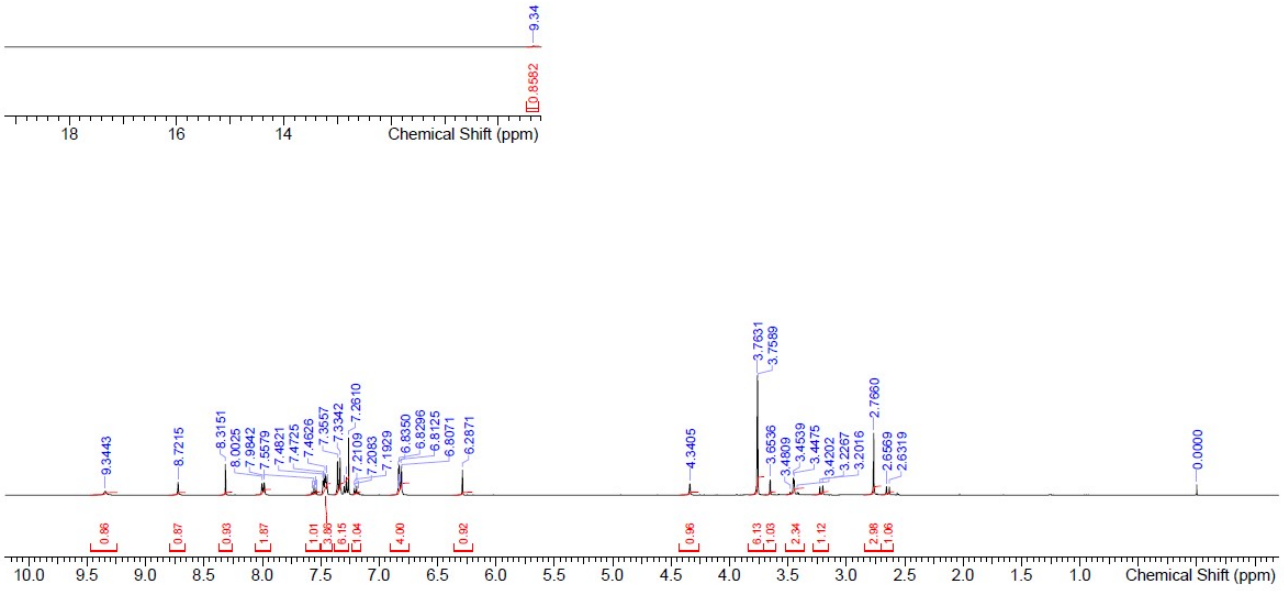
m/z= 579.24027-589.24027

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
584.24027	584.24022	0.08	18.5	$C_{33}H_{34}O_7N_3$

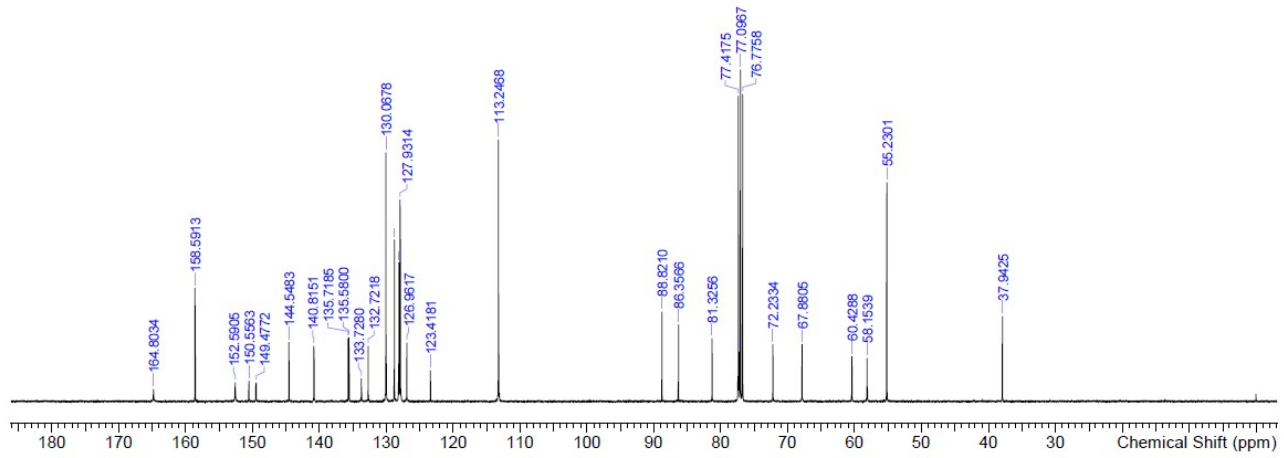
***N*-[9-[(1R,3R,4R,7S)-1-[[bis(4-methoxyphenyl)-phenyl-methoxy]methyl]-7-hydroxy-5-methyl-2-oxa-5-azabicyclo[2.2.1]heptan-3-yl]purin-6-yl]benzamide (A^{bz}-2b)**



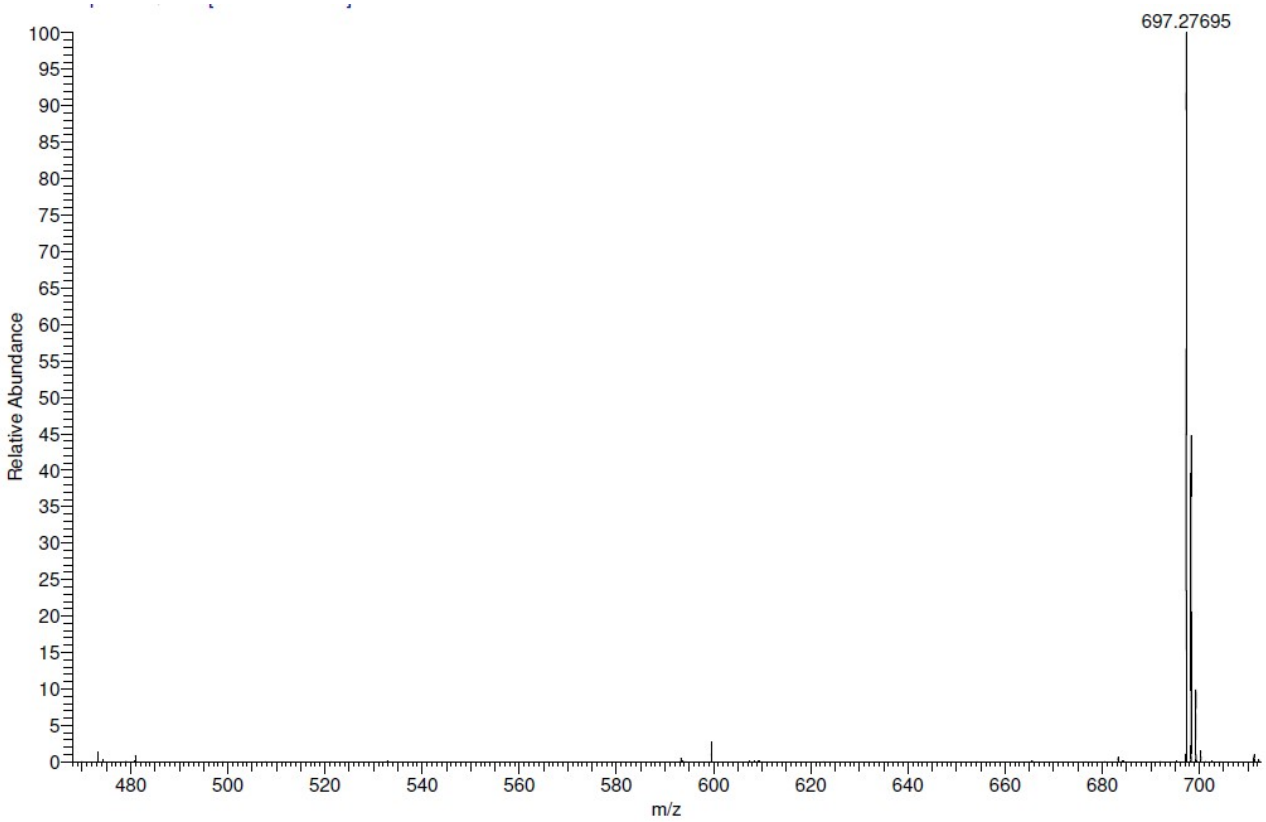
^{1}H NMR (400 MHz, CHLOROFORM-d) of **A^{bz}-2b**



^{13}C NMR (101 MHz, CHLOROFORM-d) of **A^{bz}-2b**



High resolution mass spectra of **A^{bz}-2b**

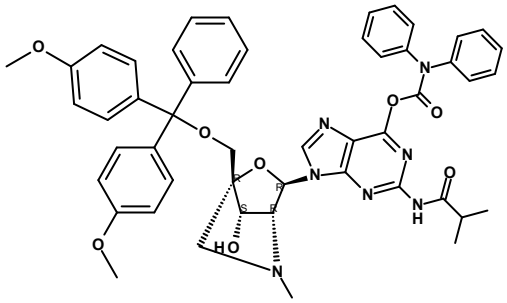


Elemental composition search on mass 697.27695

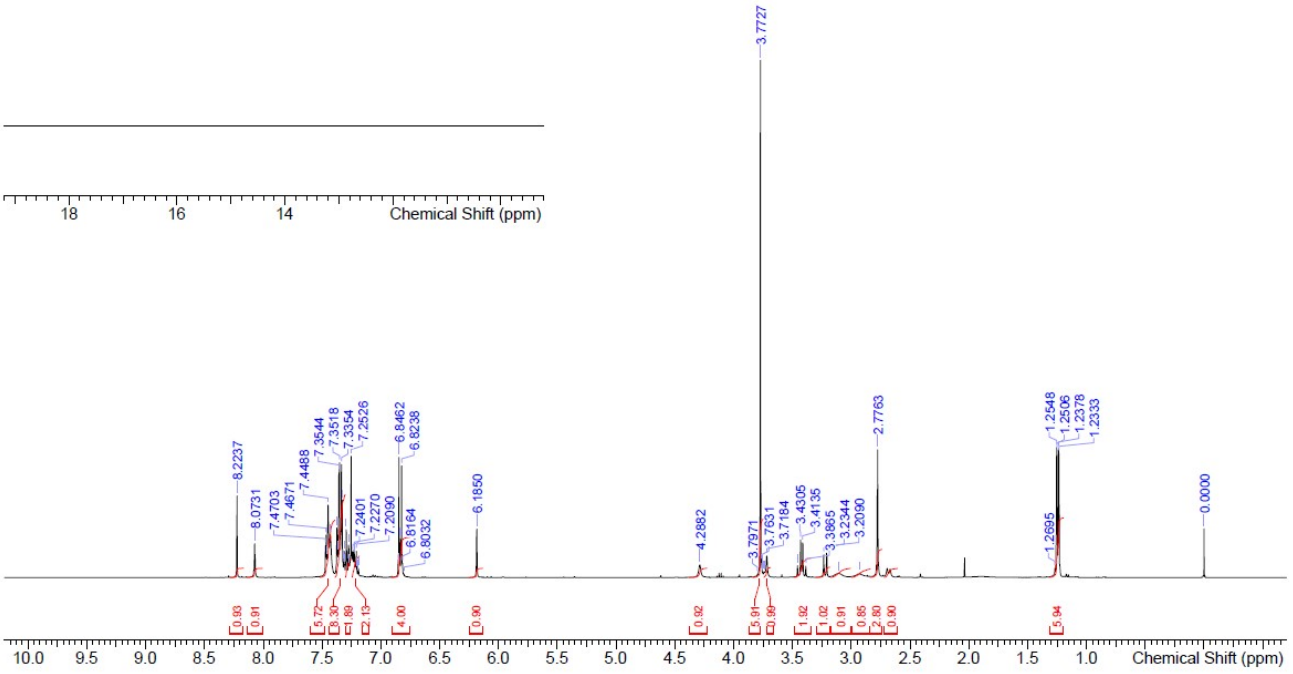
m/z= 692.27695-702.27695

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
697.27695	697.27801	-1.51	25.5	$C_{40}H_{37}O_6N_6$

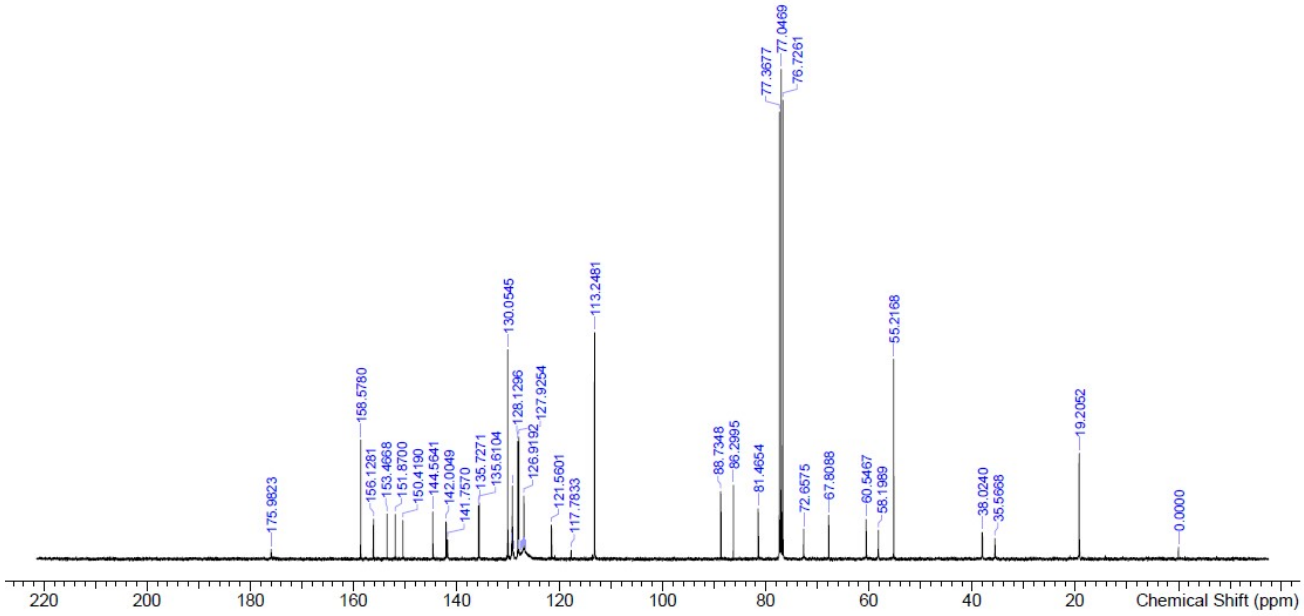
[9-[(1R,3R,4R,7S)-1-[[bis(4-methoxyphenyl)-phenyl-methoxy]methyl]-7-hydroxy-5-methyl-2-oxa-5-azabicyclo[2.2.1]heptan-3-yl]-2-(2-methylpropanoylamino)purin-6-yl] *N,N*-diphenylcarbamate ($G^{dpc,\ ibu}$-2b)



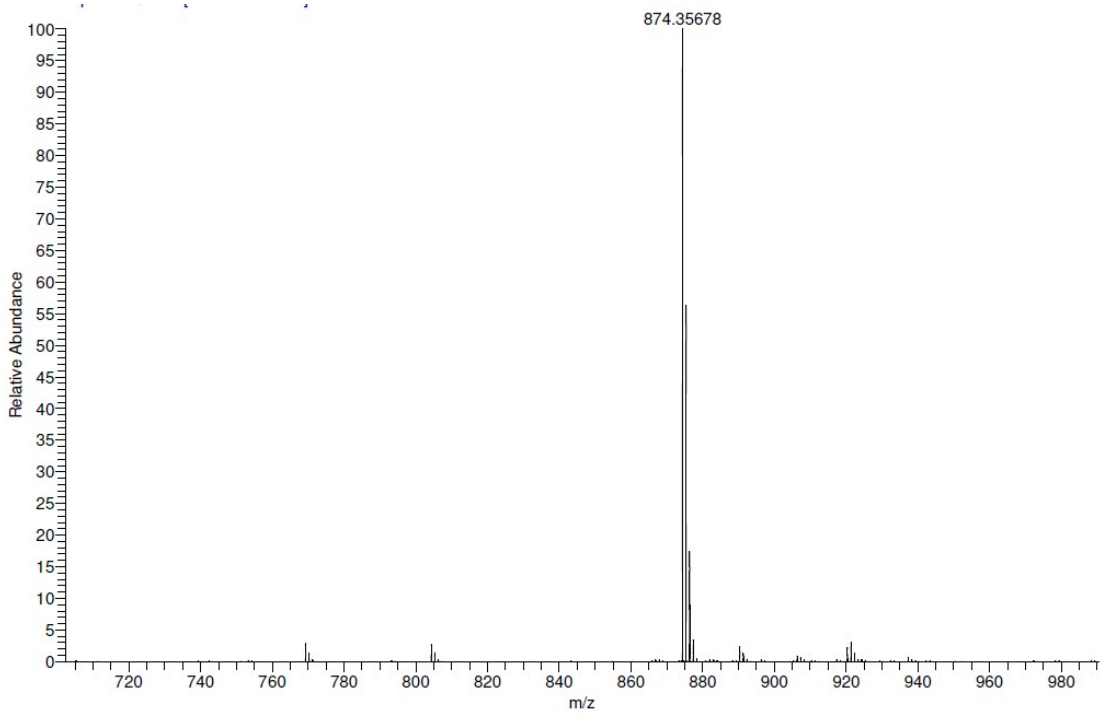
^{1}H NMR (400 MHz, CHLOROFORM-d) of **$G^{dpc,\ ibu}$-2b**



^{13}C NMR (101 MHz, CHLOROFORM-d) of **G$^{dpc,\ ibu}$-2b**



High resolution mass spectra of **G$^{dpc,\ ibu}$-2b**

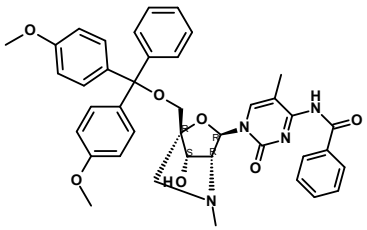


Elemental composition search on mass 874.35678

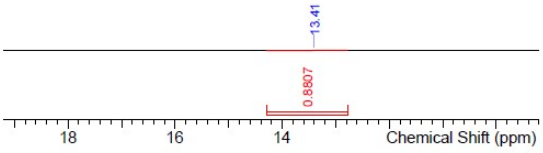
m/z= 869.35678-879.35678

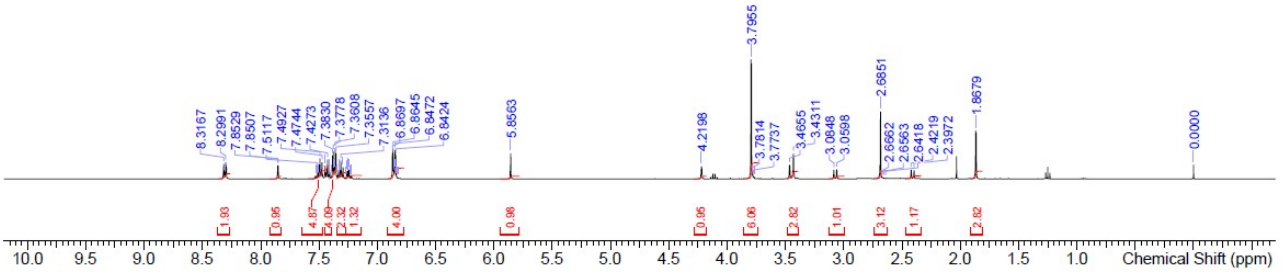
m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
874.35678	874.35698	-0.23	30.5	$C_{50}H_{48}O_8N_7$

***N*-[1-[(1R,3R,4R,7S)-1-[[bis(4-methoxyphenyl)-phenyl-methoxy]methyl]-7-hydroxy-5-methyl-2-oxa-5-azabicyclo[2.2.1]heptan-3-yl]-5-methyl-2-oxo-pyrimidin-4-yl]benzamide (mC^{bz}-2b)**

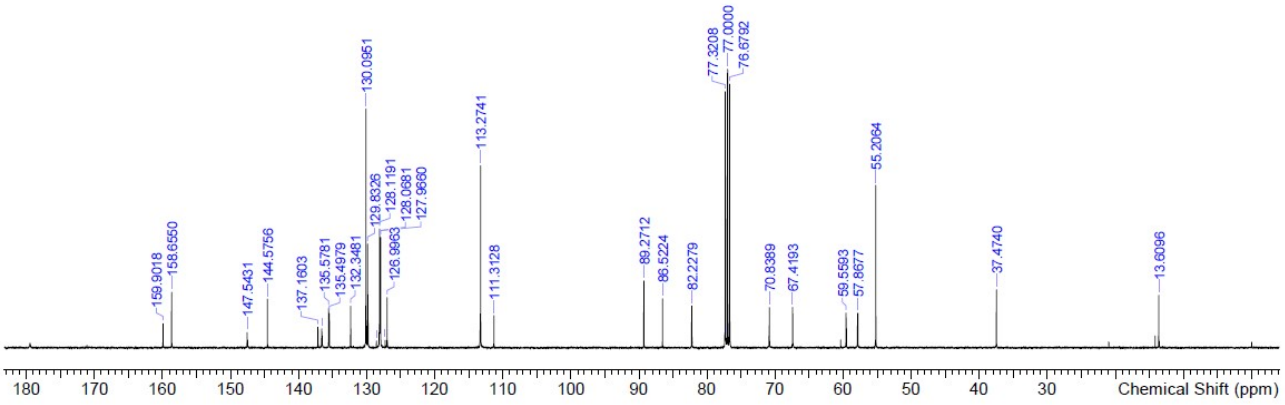


^{1}H NMR (400 MHz, CHLOROFORM-d) of **mC^{bz}-2b**

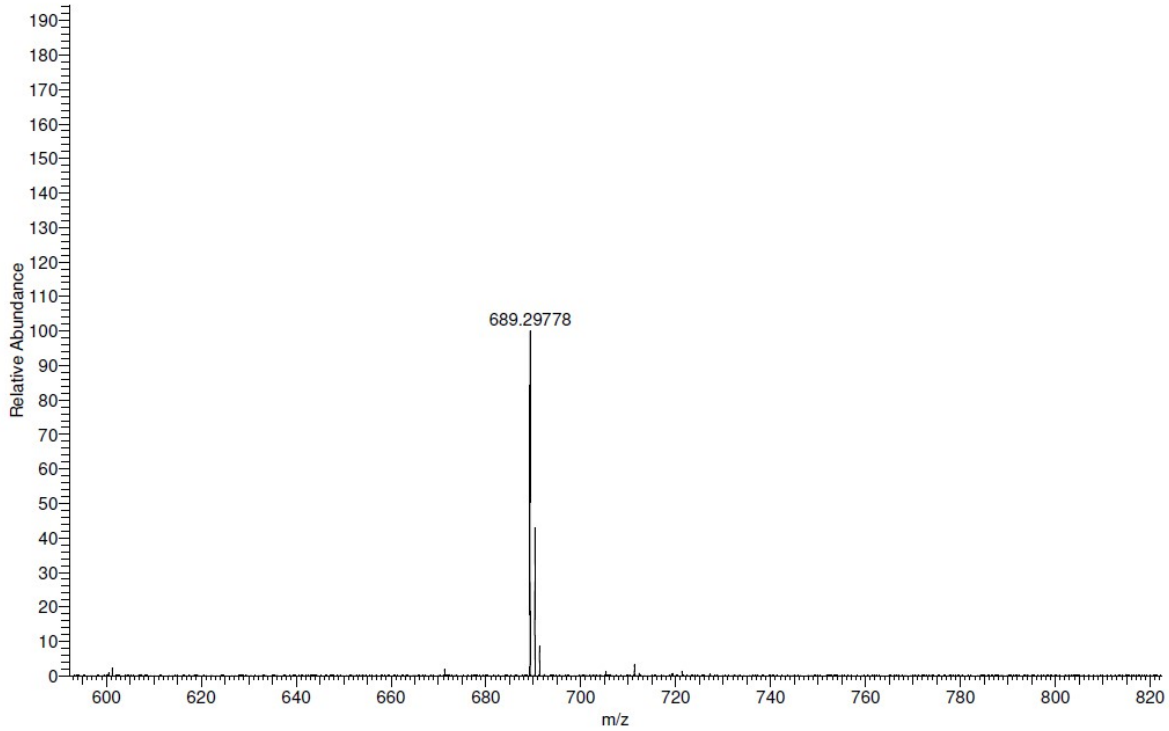




^{13}C NMR (101 MHz, CHLOROFORM-d) of **mC^{bz}-2b**



High resolution mass spectra of **mC^{bz}-2b**

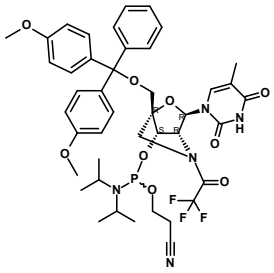


Elemental composition search on mass 689.29778

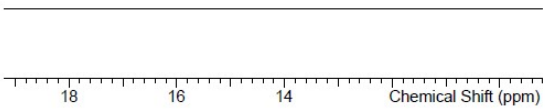
m/z= 684.29778-694.29778

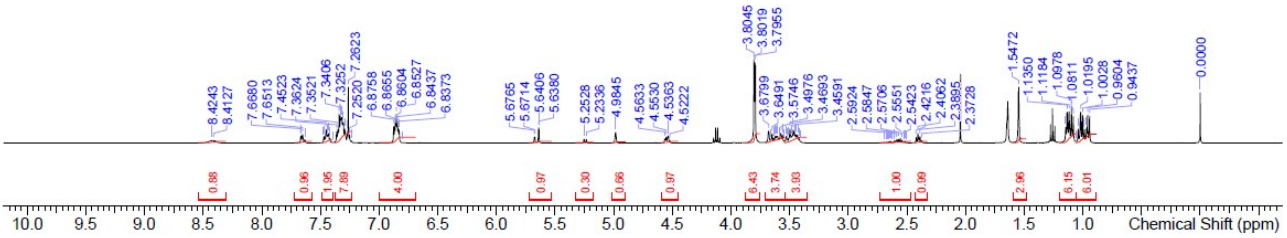
m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
689.29778	689.29698	1.17	22.5	$C_{40}H_{41}O_7N_4$

3-[[(1R,3R,4R,7S)-1-[[bis(4-methoxyphenyl)-phenyl-methoxy]methyl]-3-(5-methyl-2,4-dioxo-pyrimidin-1-yl)-5-(2,2,2-trifluoroacetyl)-2-oxa-5-azabicyclo[2.2.1]heptan-7-yl]oxy-(diisopropylamino)phosphanyl]oxypropanenitrile (T-3a)

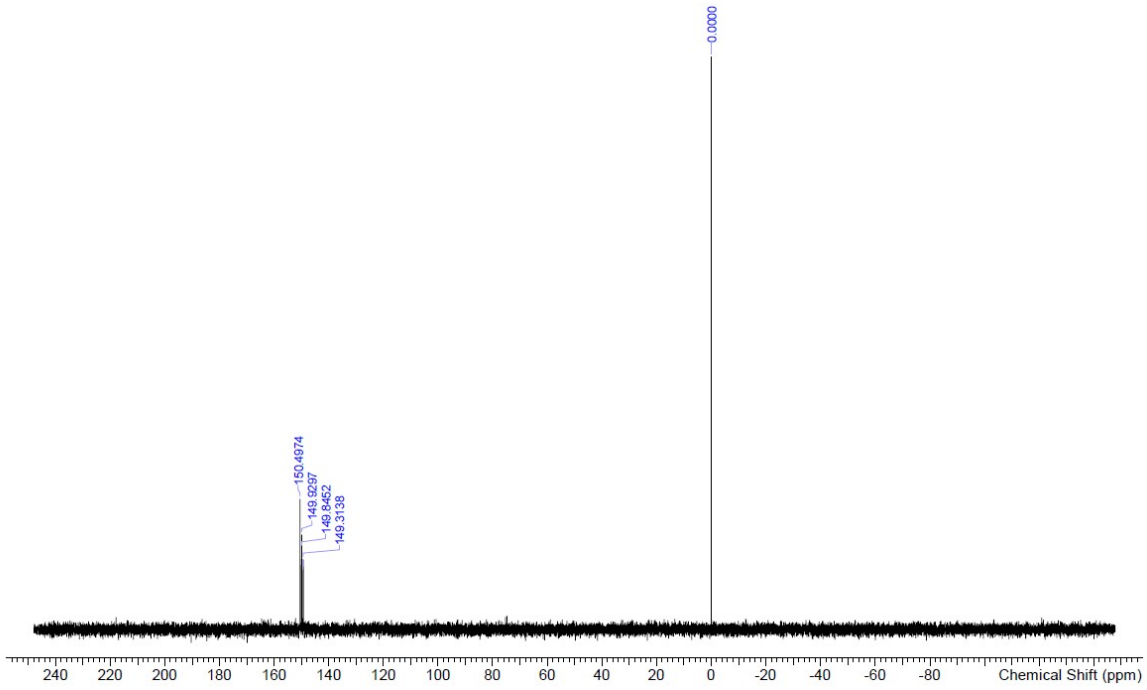


^{1}H NMR (400 MHz, CHLOROFORM-d) of **T-3a**

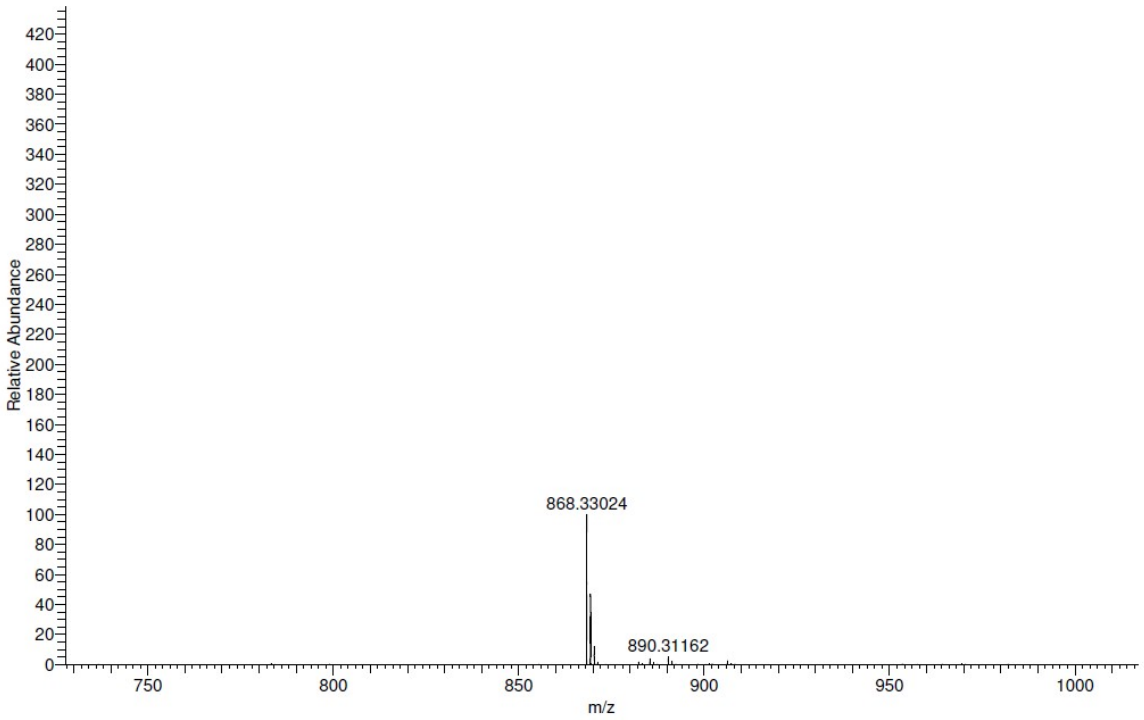




^{31}P NMR (162 MHz, CHLOROFORM-d) of **T-3a**



High resolution mass spectra of **T-3a**

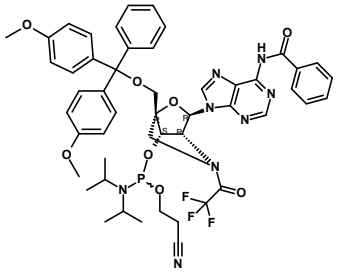


Elemental composition search on mass 868.33024

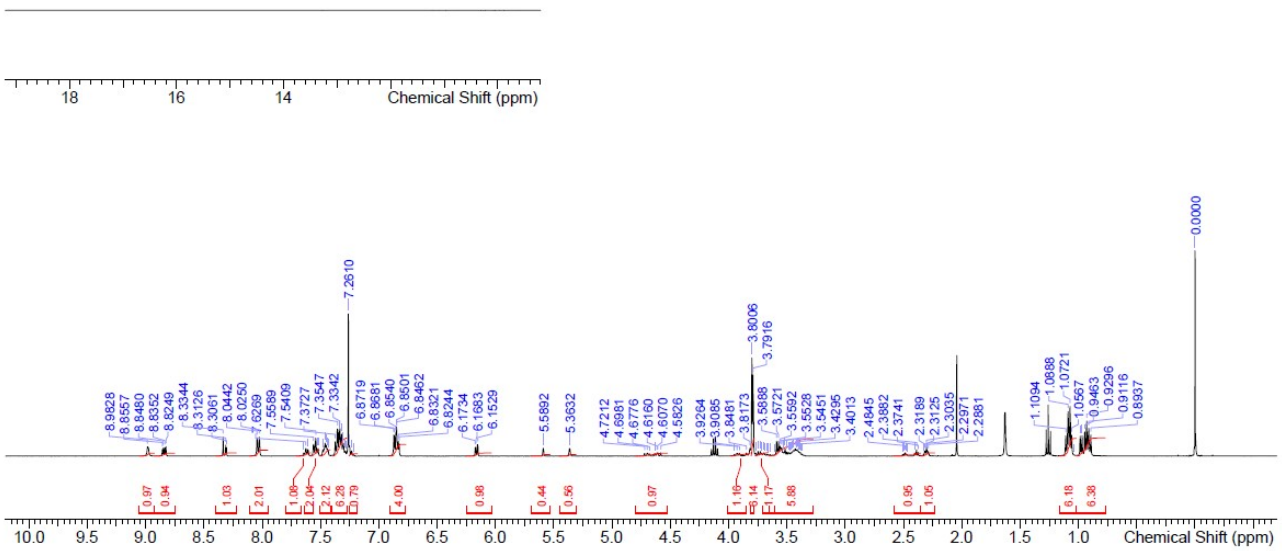
m/z= 863.33024-873.33024

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
868.33024	868.32928	1.11	20.5	$C_{43}H_{50}O_9N_5F_3P$

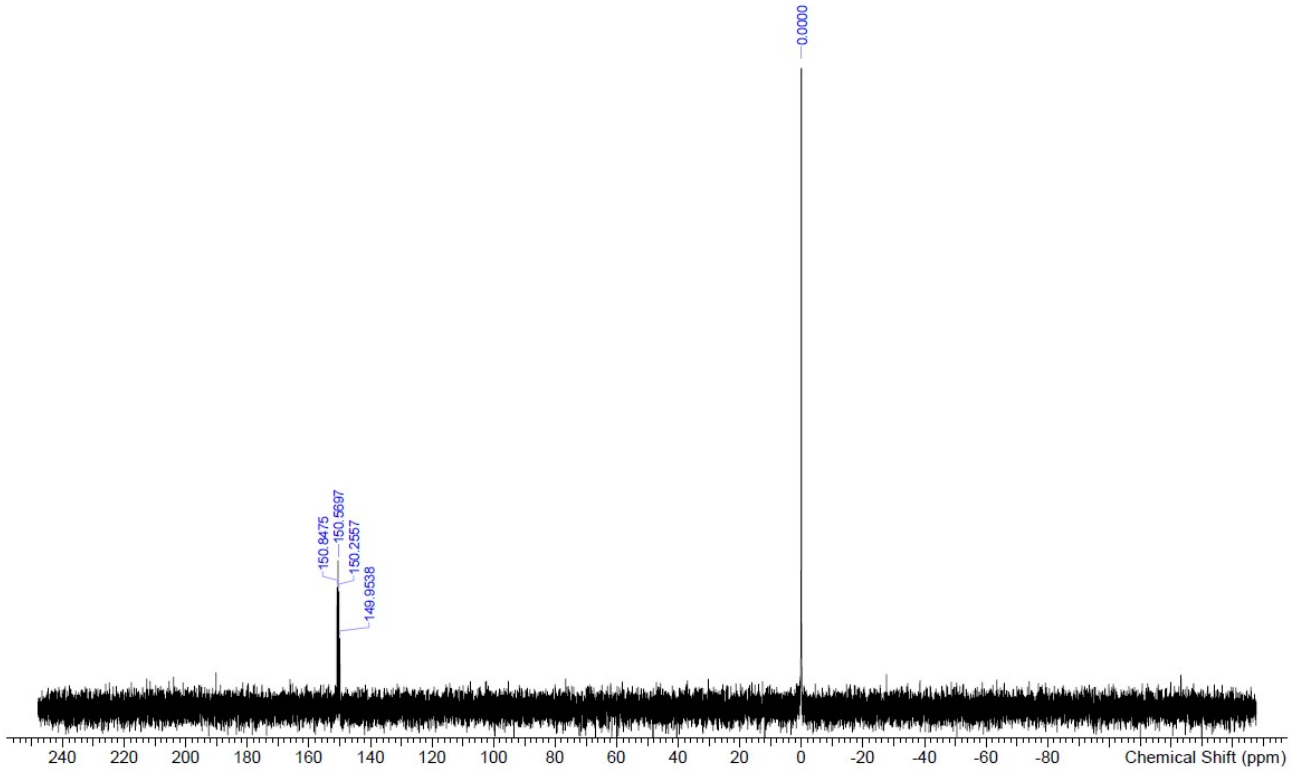
***N*-[9-[(1R,3R,4R,7S)-1-[[bis(4-methoxyphenyl)-phenyl-methoxy]methyl]-7-[2-cyanoethoxy-(diisopropylamino)phosphanyl]oxy-5-(2,2,2-trifluoroacetyl)-2-oxa-5-azabicyclo[2.2.1]heptan-3-yl]purin-6-yl]benzamide (A^{bz}-3a)**



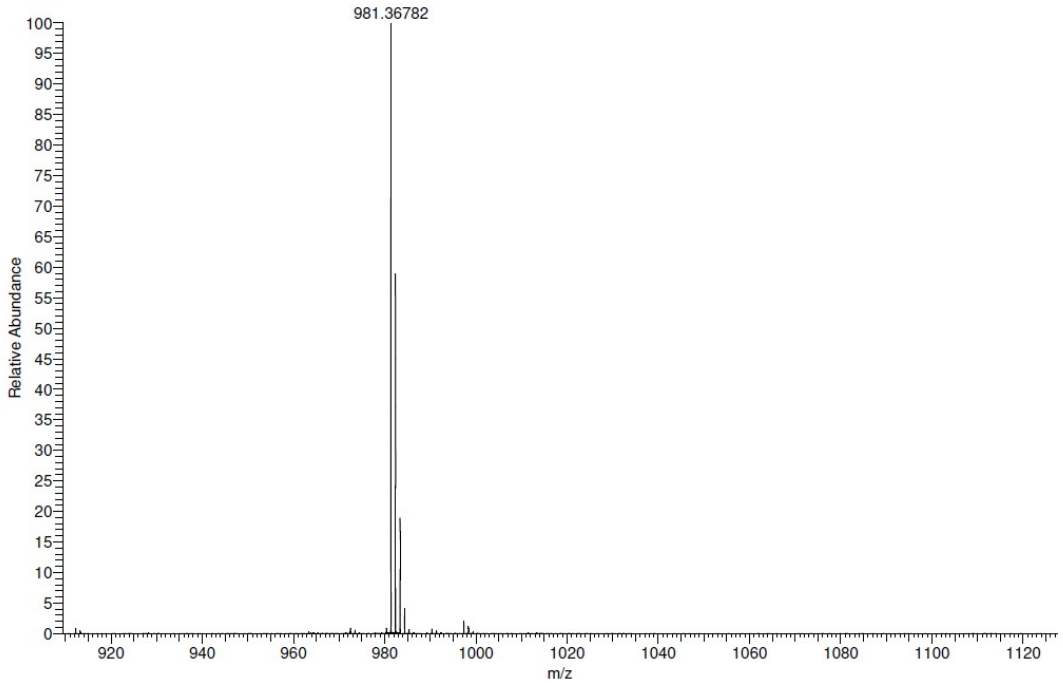
^{1}H NMR (400 MHz, CHLOROFORM-d) of **A^{bz}-3a**



^{31}P NMR (162 MHz, CHLOROFORM-d) of **A^{bz}-3a**



High resolution mass spectra of **A^{bz}-3a**

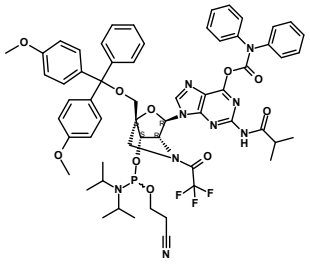


Elemental composition search on mass 981.36782

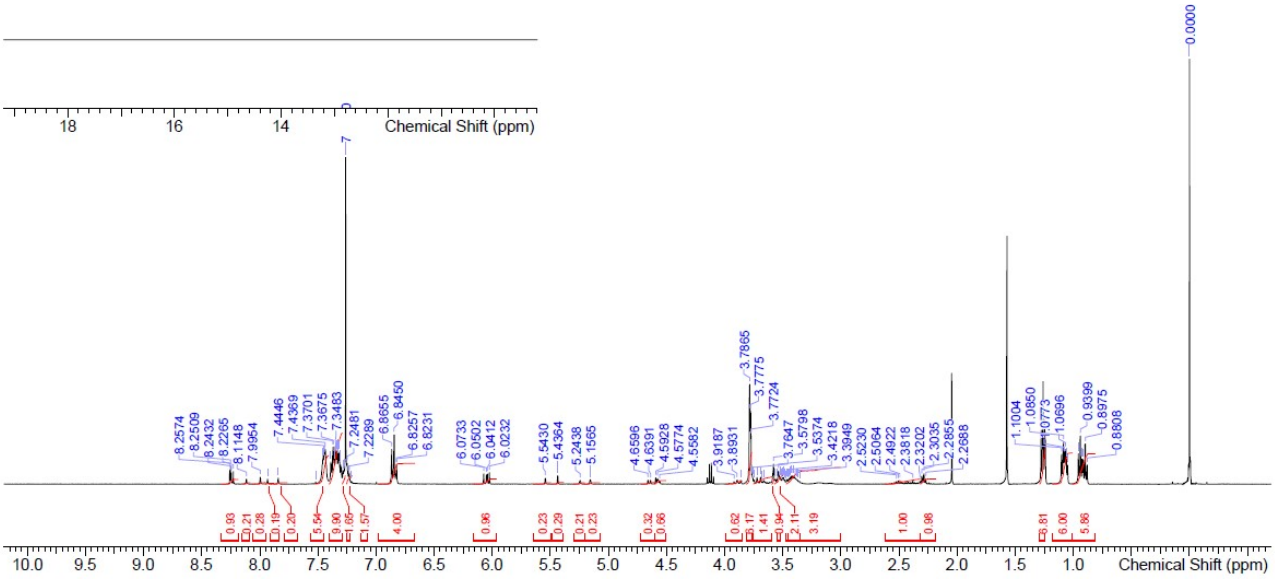
m/z= 976.36782-986.36782

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
981.36782	981.36706	0.78	27.5	$C_{50}H_{53}O_8N_8F_3P$

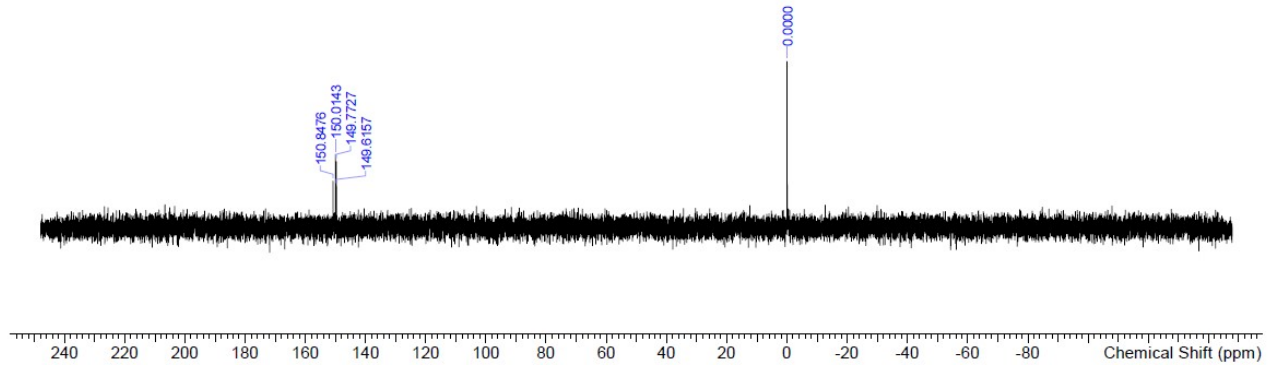
[9-[(1R,3R,4R,7S)-1-[[bis(4-methoxyphenyl)-phenyl-methoxy]methyl]-7-[2-cyanoethoxy-(diisopropylamino)phosphanyl]oxy-5-(2,2,2-trifluoroacetyl)-2-oxa-5-azabicyclo[2.2.1]heptan-3-yl]-2-(2-methylpropanoylamino)purin-6-yl] *N,N*-diphenylcarbamate ($G^{dpc,\ ibu}$-3a)



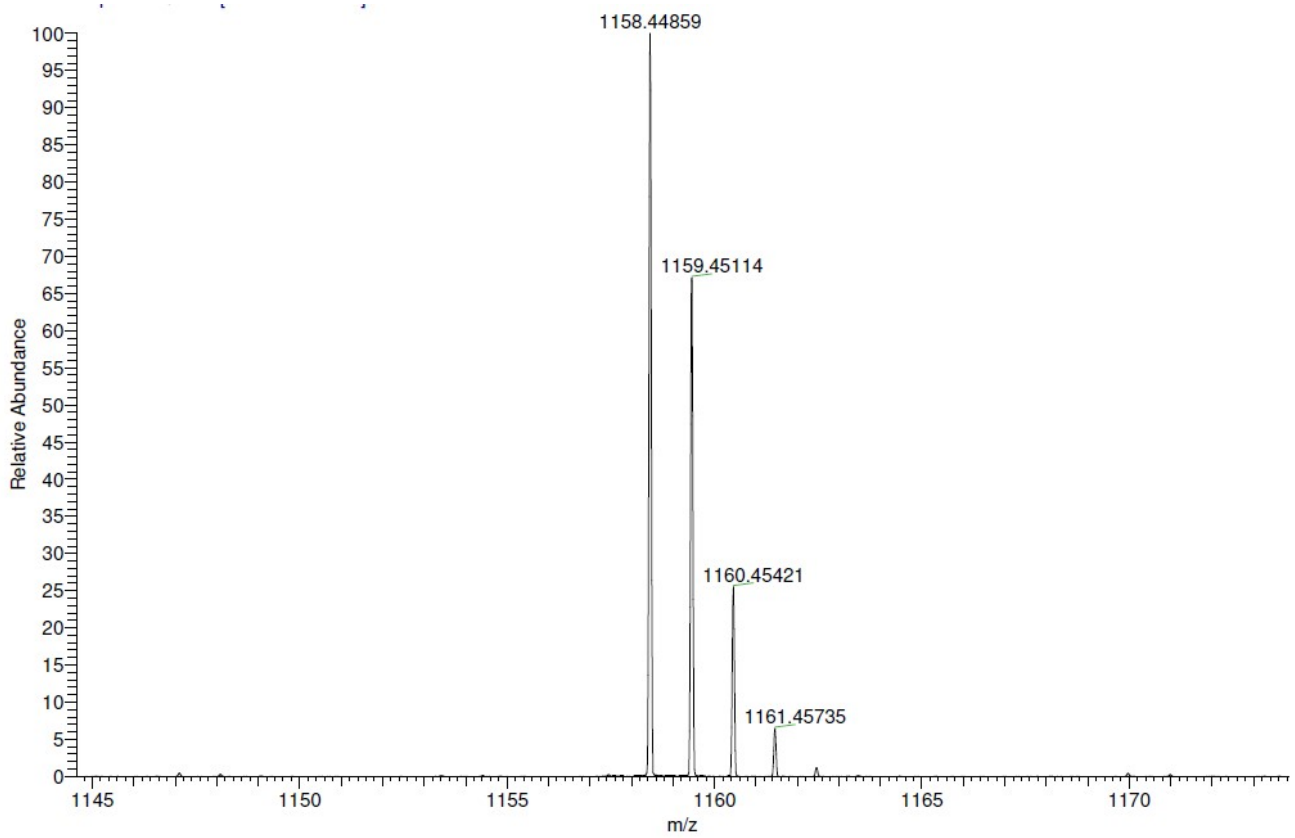
^{1}H NMR (400 MHz, CHLOROFORM-d) of **$G^{dpc,\ ibu}$-3a**



^{31}P NMR (162 MHz, CHLOROFORM-d) of **G$^{dpc,\ ibu}$-3a**



High resolution mass spectra of **G$^{dpc,\ ibu}$-3a**

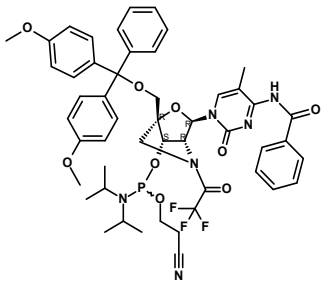


Elemental composition search on mass 1158.44859

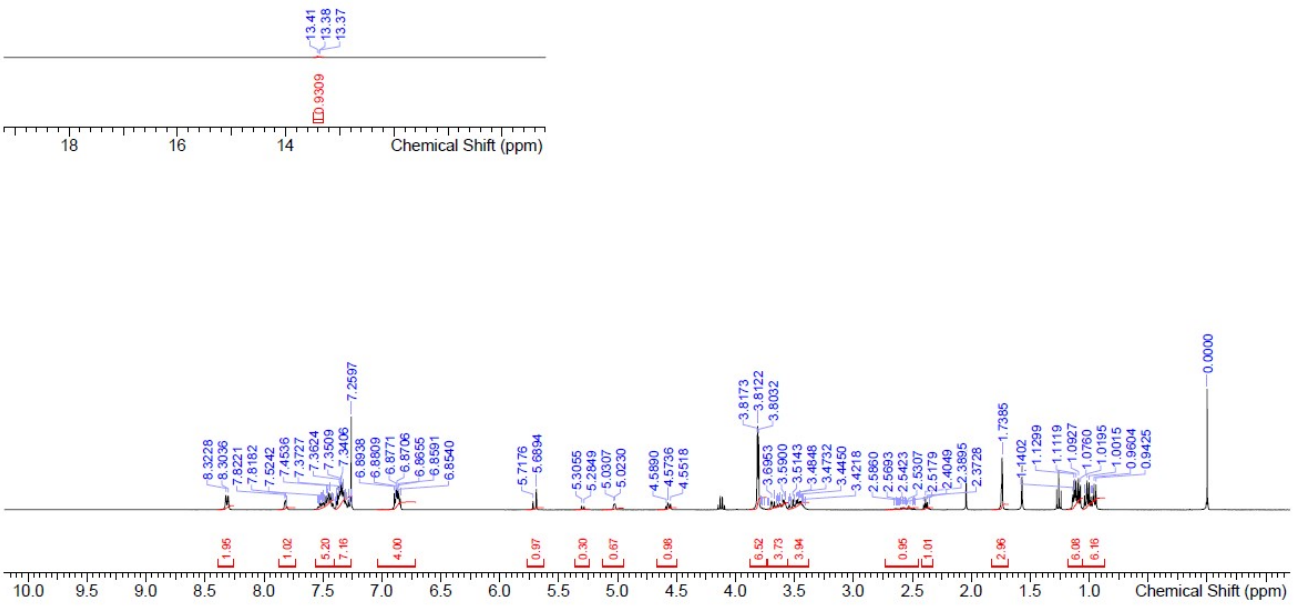
m/z= 1153.44859-1163.44859

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
1158.44859	1158.44604	2.20	32.5	$C_{60}H_{64}O_{10}N_9F_3P$

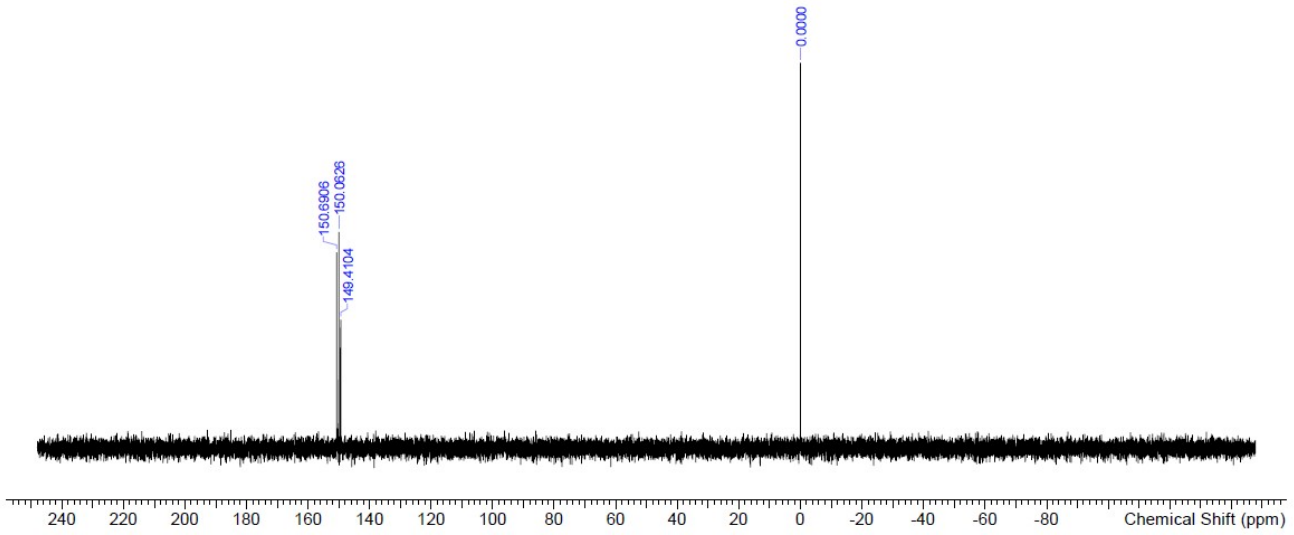
***N*-[1-[(1R,3R,4R,7S)-1-[[bis(4-methoxyphenyl)-phenyl-methoxy]methyl]-7-[2-cyanoethoxy-(diisopropylamino)phosphanyl]oxy-5-(2,2,2-trifluoroacetyl)-2-oxa-5-azabicyclo[2.2.1]heptan-3-yl]-5-methyl-2-oxo-pyrimidin-4-yl]benzamide (mC^{bz}-3a)**



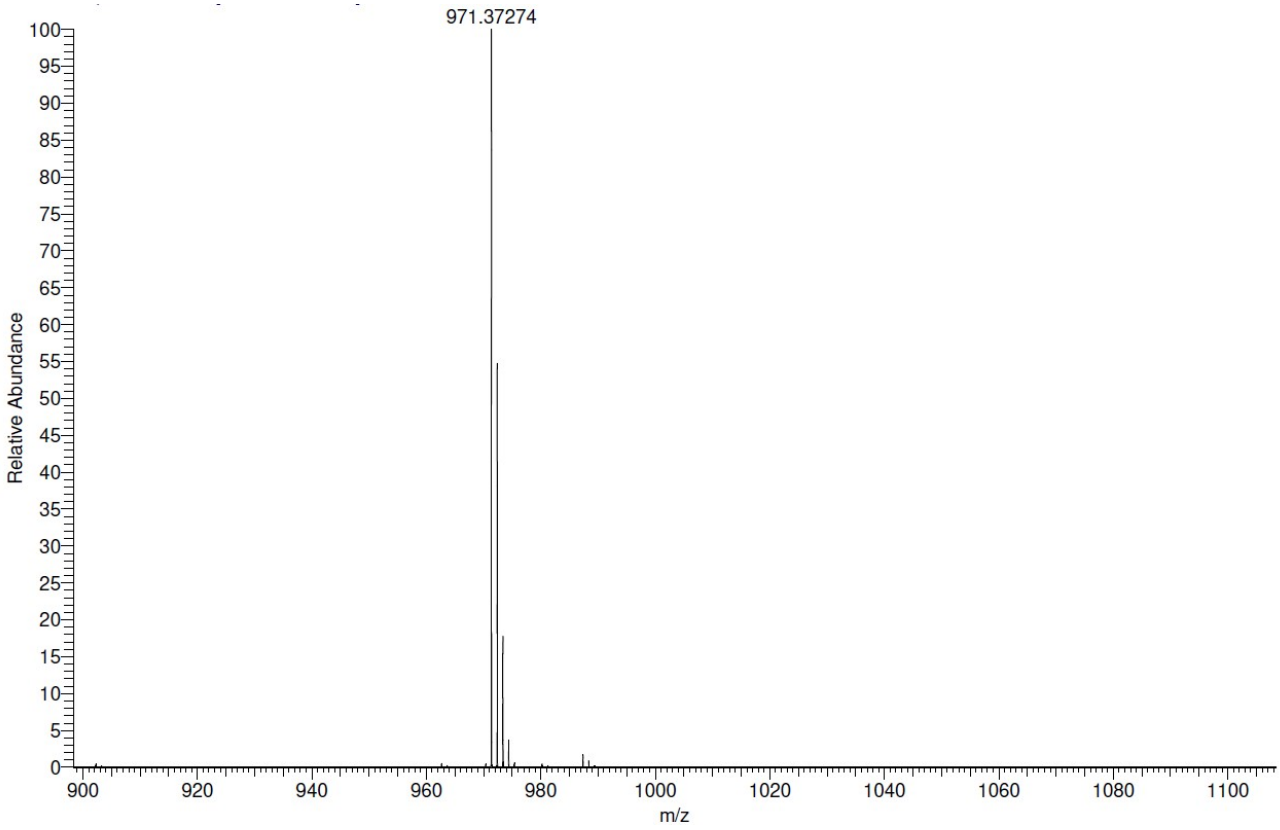
^{1}H NMR (400 MHz, CHLOROFORM-d) of **mC^{bz}-3a**



^{31}P NMR (162 MHz, CHLOROFORM-d) of **mC^{bz}-3a**



High resolution mass spectra of **mC^{bz}-3a**

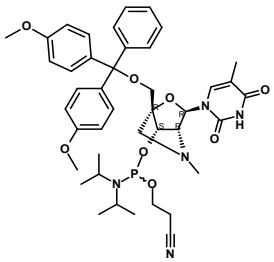


Elemental composition search on mass 971.37274

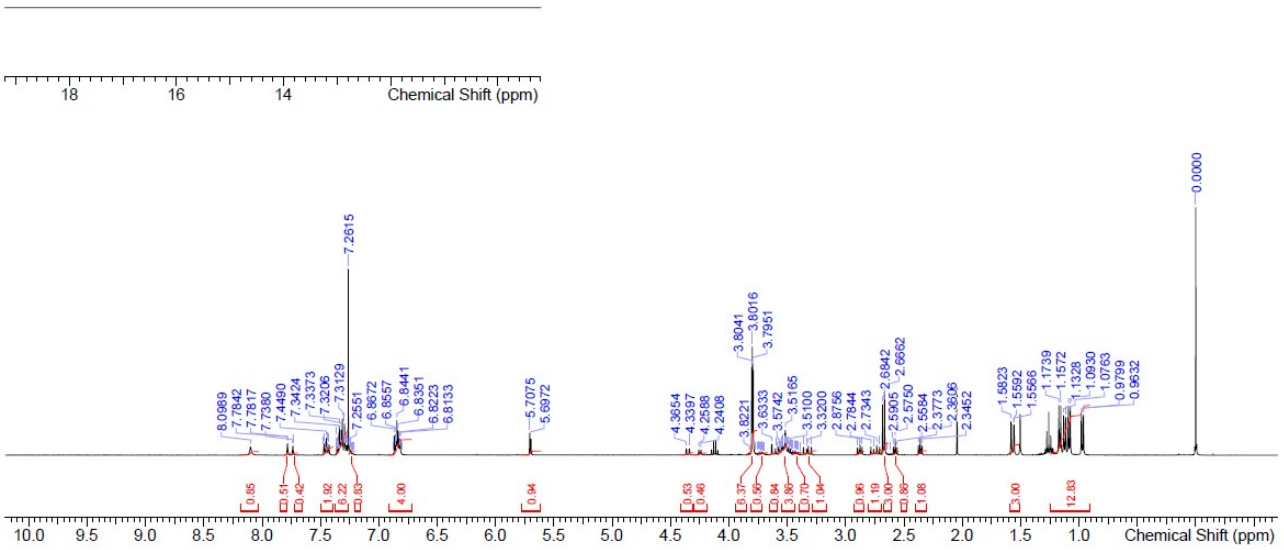
m/z= 966.37274-976.37274

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
971.37274	971.37147	1.30	25.5	$C_{50}H_{55}O_9N_6F_3P$

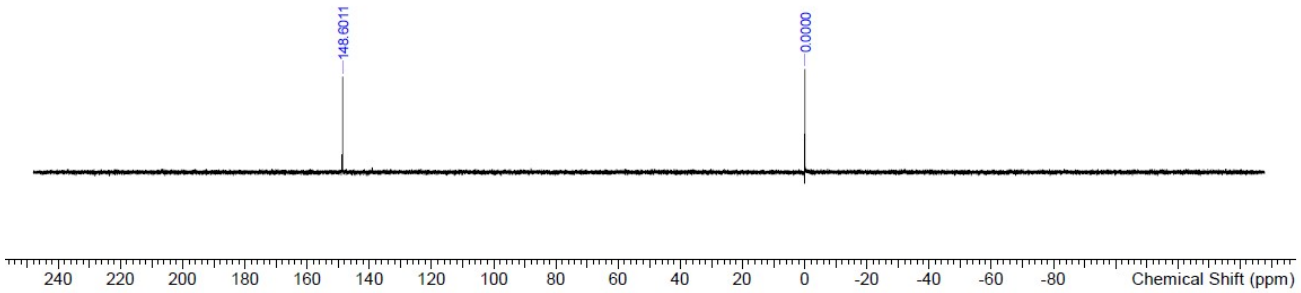
3-[[(1R,3R,4R,7S)-1-[[bis(4-methoxyphenyl)-phenyl-methoxy]methyl]-5-methyl-3-(5-methyl-2,4-dioxo-pyrimidin-1-yl)-2-oxa-5-azabicyclo[2.2.1]heptan-7-yl]oxy-(diisopropylamino)phosphanyl]oxypropanenitrile (T-3b)



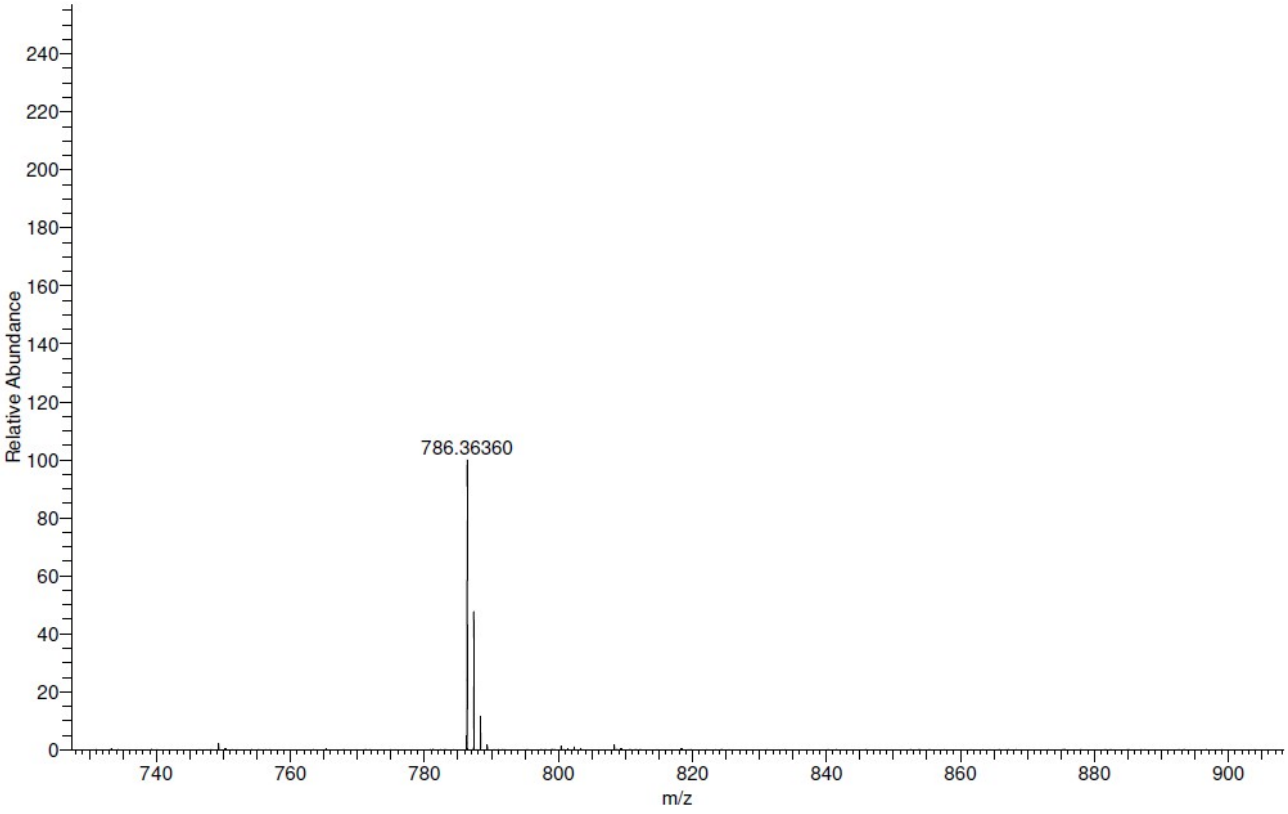
^{1}H NMR (400 MHz, CHLOROFORM-d) of **T-3b**



^{31}P NMR (162 MHz, CHLOROFORM-d) of **T-3b**



High resolution mass spectra of **T-3b**

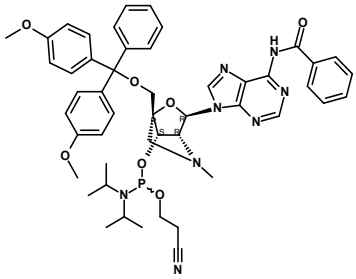


Elemental composition search on mass 786.36360

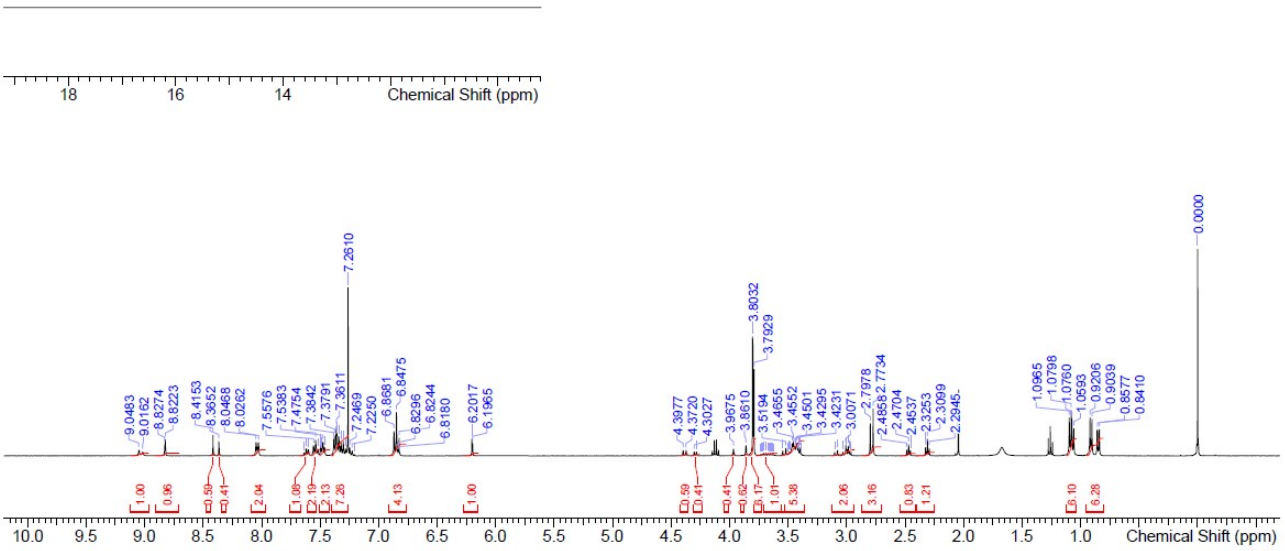
m/z= 781.36360-791.36360

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
786.36360	786.36263	1.24	19.5	$C_{42}H_{53}O_8N_5P$

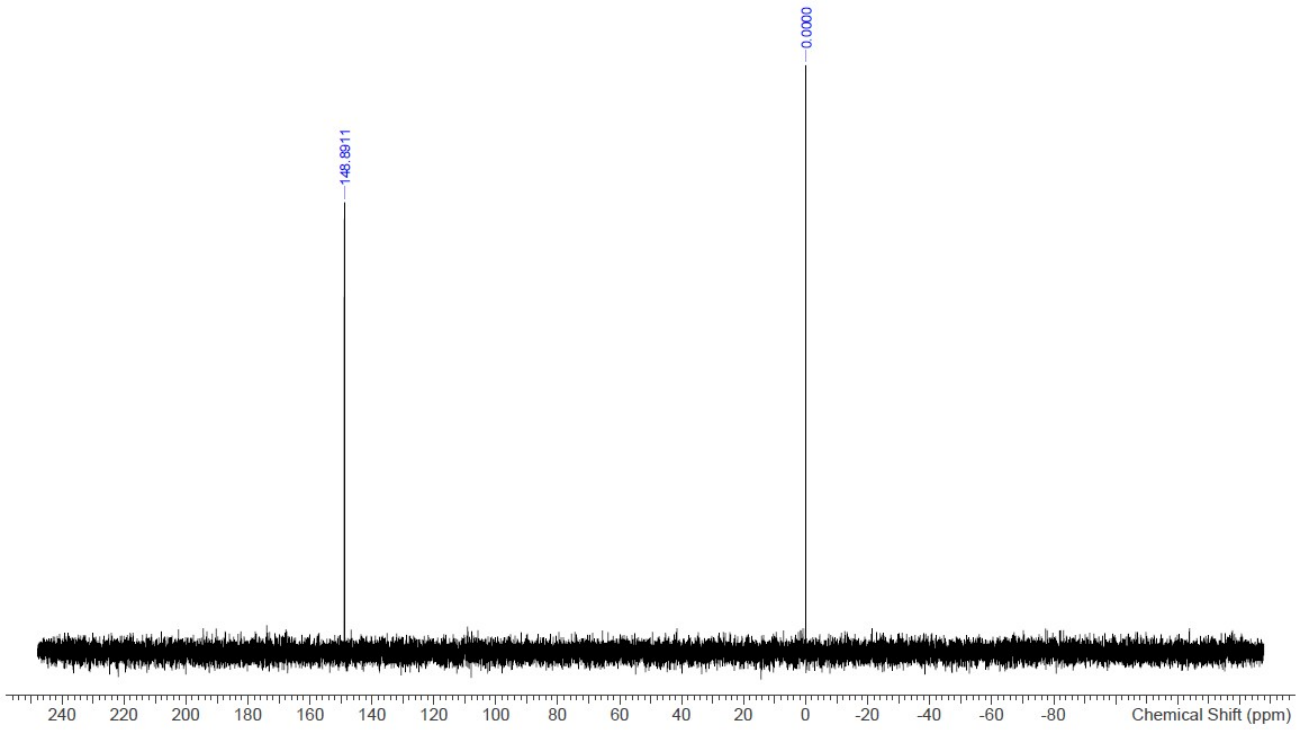
***N*-[9-[(1R,3R,4R,7S)-1-[[bis(4-methoxyphenyl)-phenyl-methoxy]methyl]-7-[2-cyanoethoxy-(diisopropylamino)phosphanyl]oxy-5-methyl-2-oxa-5-azabicyclo[2.2.1]heptan-3-yl]purin-6-yl]benzamide (A^{bz}-3b)**



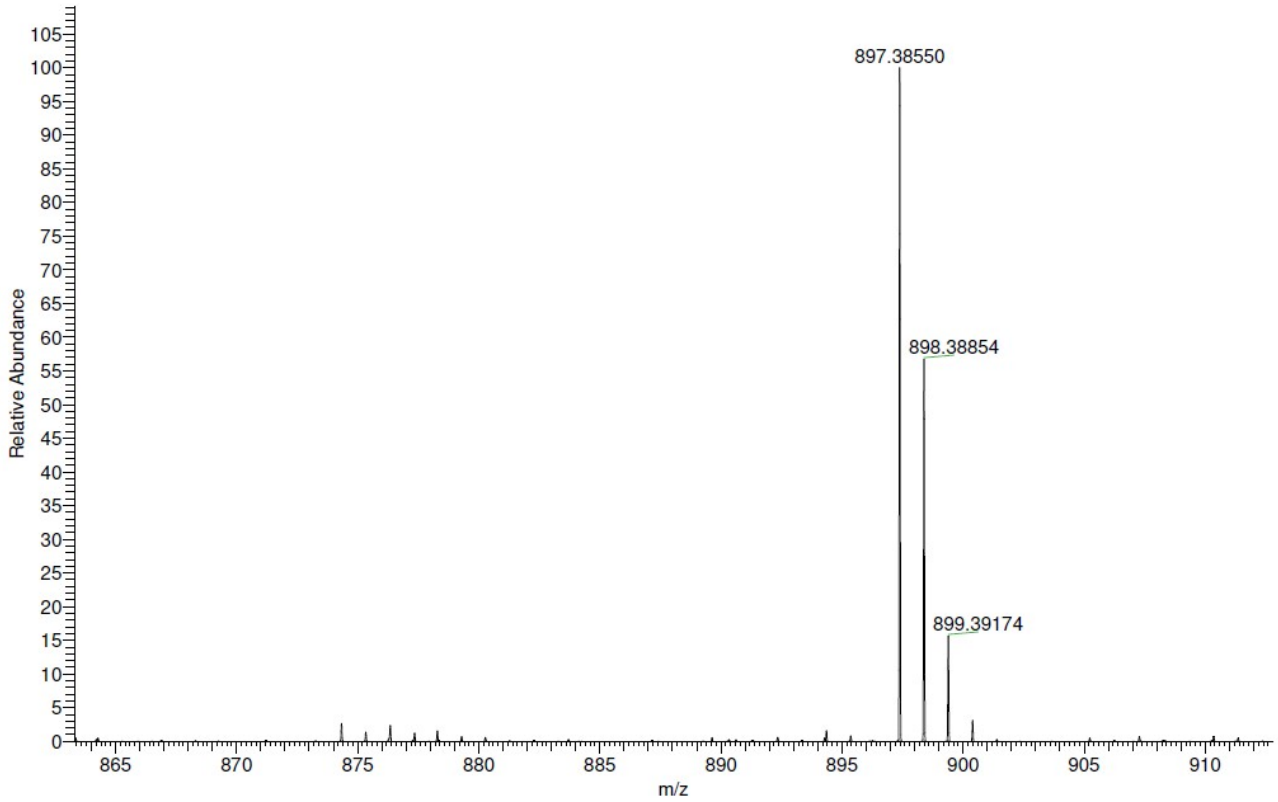
^{1}H NMR (400 MHz, CHLOROFORM-d) of **A^{bz}-3b**



^{31}P NMR (162 MHz, CHLOROFORM-d) of **A^{bz}-3b**



High resolution mass spectra of **A^{bz}-3b**

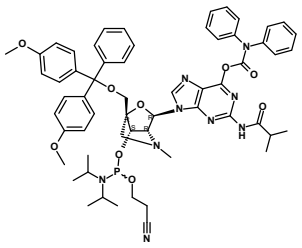


Elemental composition search on mass 897.38550

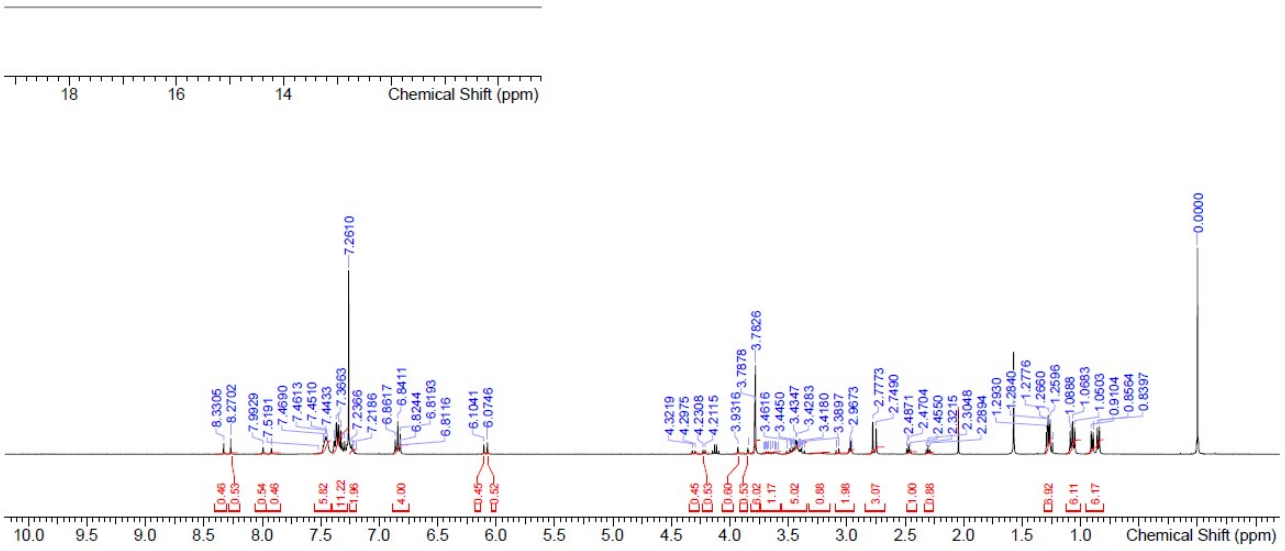
m/z= 892.38550-902.38550

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
897.38550	897.38586	-0.40	27.5	$C_{49}H_{54}O_7N_8P$

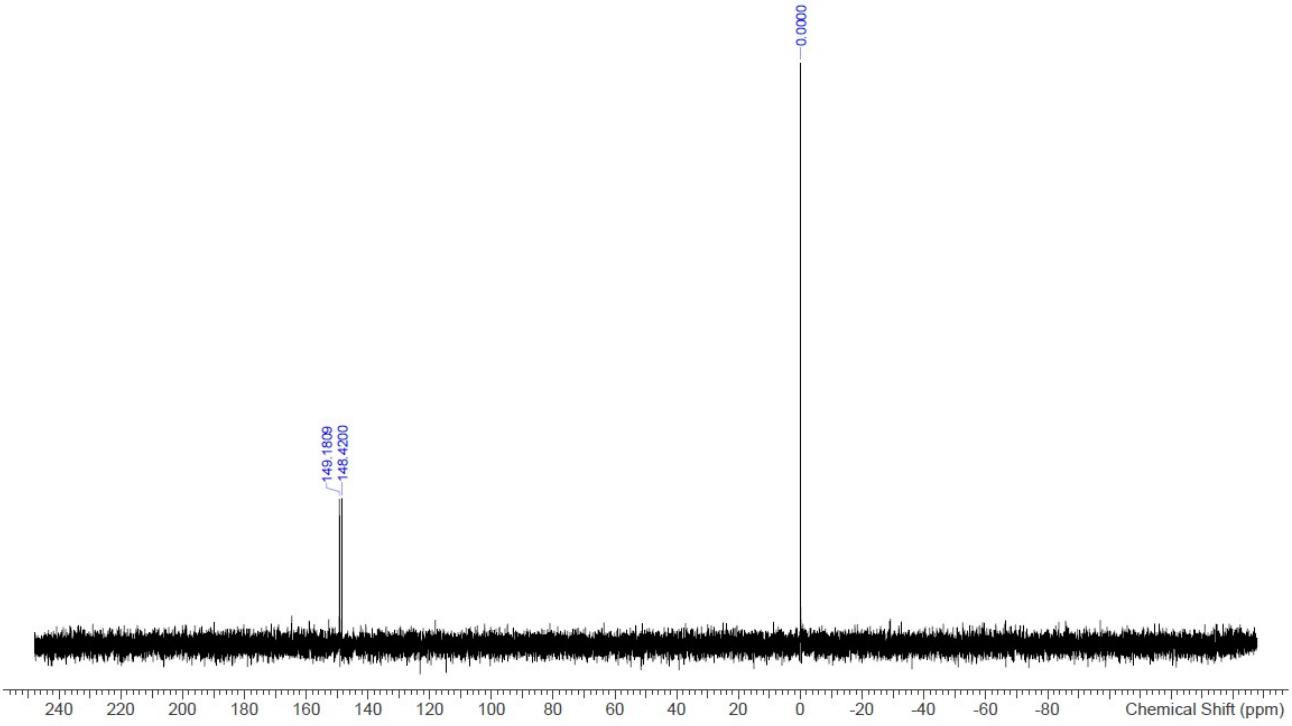
[9-[(1R,3R,4R,7S)-1-[[bis(4-methoxyphenyl)-phenyl-methoxy]methyl]-7-[2-cyanoethoxy-(diisopropylamino)phosphanyl]oxy-5-methyl-2-oxa-5-azabicyclo[2.2.1]heptan-3-yl]-2-(2-methylpropanoylamino)purin-6-yl] *N,N*-diphenylcarbamate ($G^{dpc,\ ibu}$-3b)



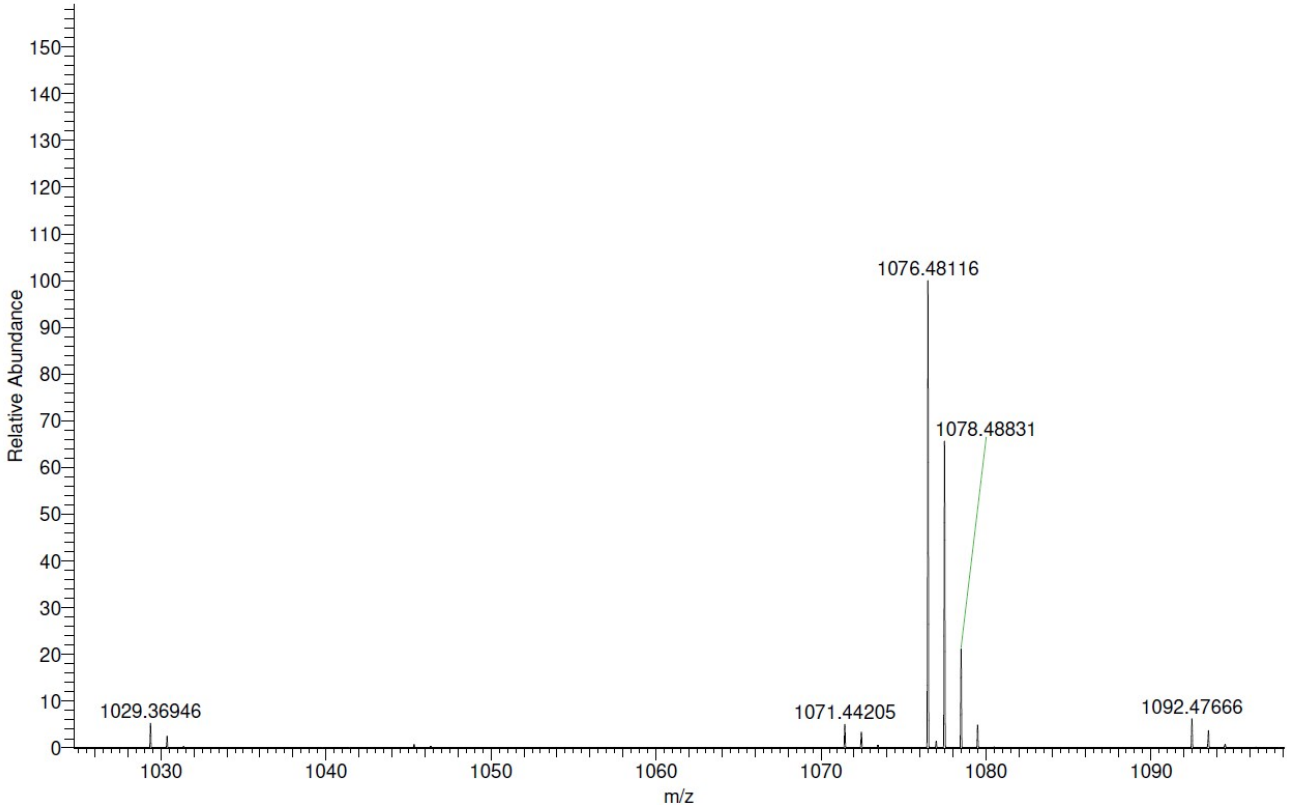
^{1}H NMR (400 MHz, CHLOROFORM-d) of **$G^{dpc,\ ibu}$-3b**



^{31}P NMR (162 MHz, CHLOROFORM-d) of **G$^{dpc,\ ibu}$-3b**



High resolution mass spectra of **G$^{dpc,\ ibu}$-3b**

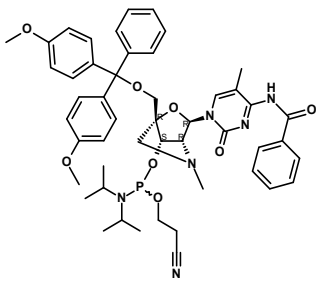


Elemental composition search on mass 1076.48116

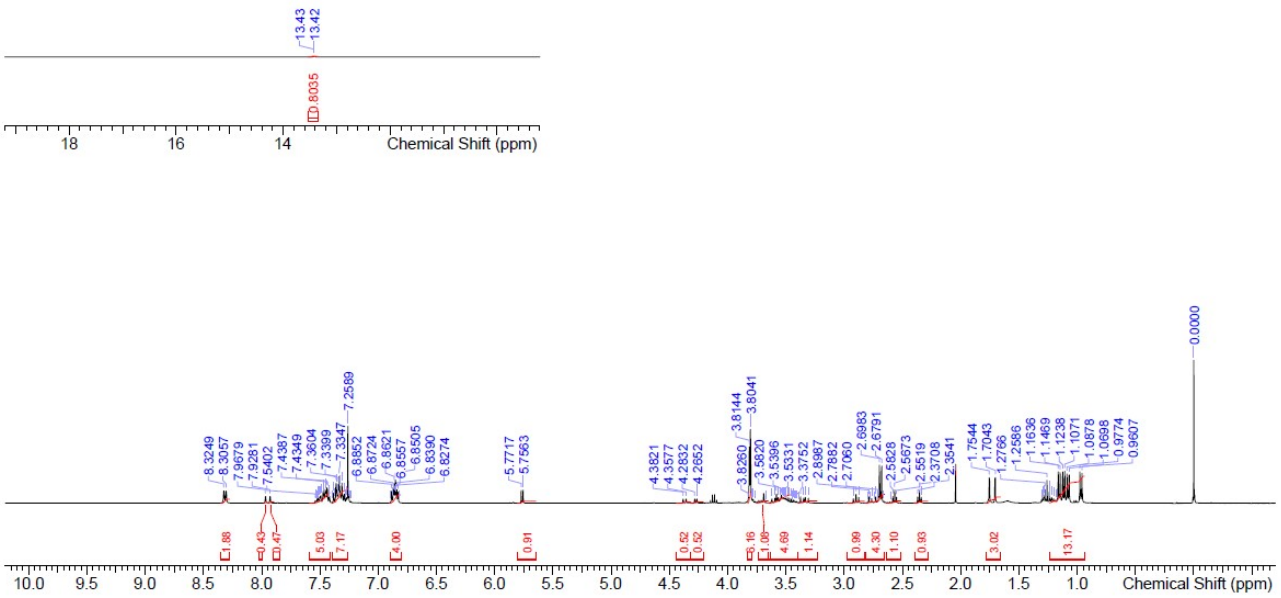
m/z= 1071.48116-1081.48116

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
1076.48116	1076.48048	0.63	31.5	$C_{59}H_{67}O_9N_9P$

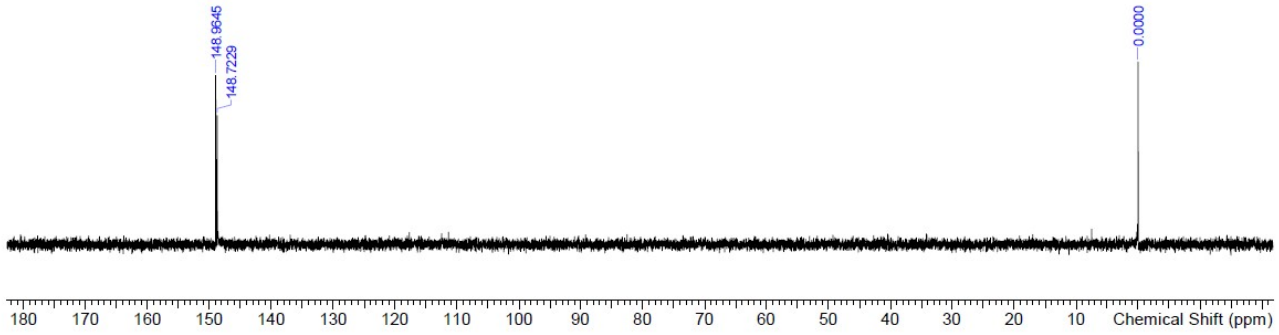
***N*-[1-[(1R,3R,4R,7S)-1-[[bis(4-methoxyphenyl)-phenyl-methoxy]methyl]-7-[2-cyanoethoxy-(diisopropylamino)phosphanyl]oxy-5-methyl-2-oxa-5-azabicyclo[2.2.1]heptan-3-yl]-5-methyl-2-oxo-pyrimidin-4-yl]benzamide (mC^{bz}-3b)**



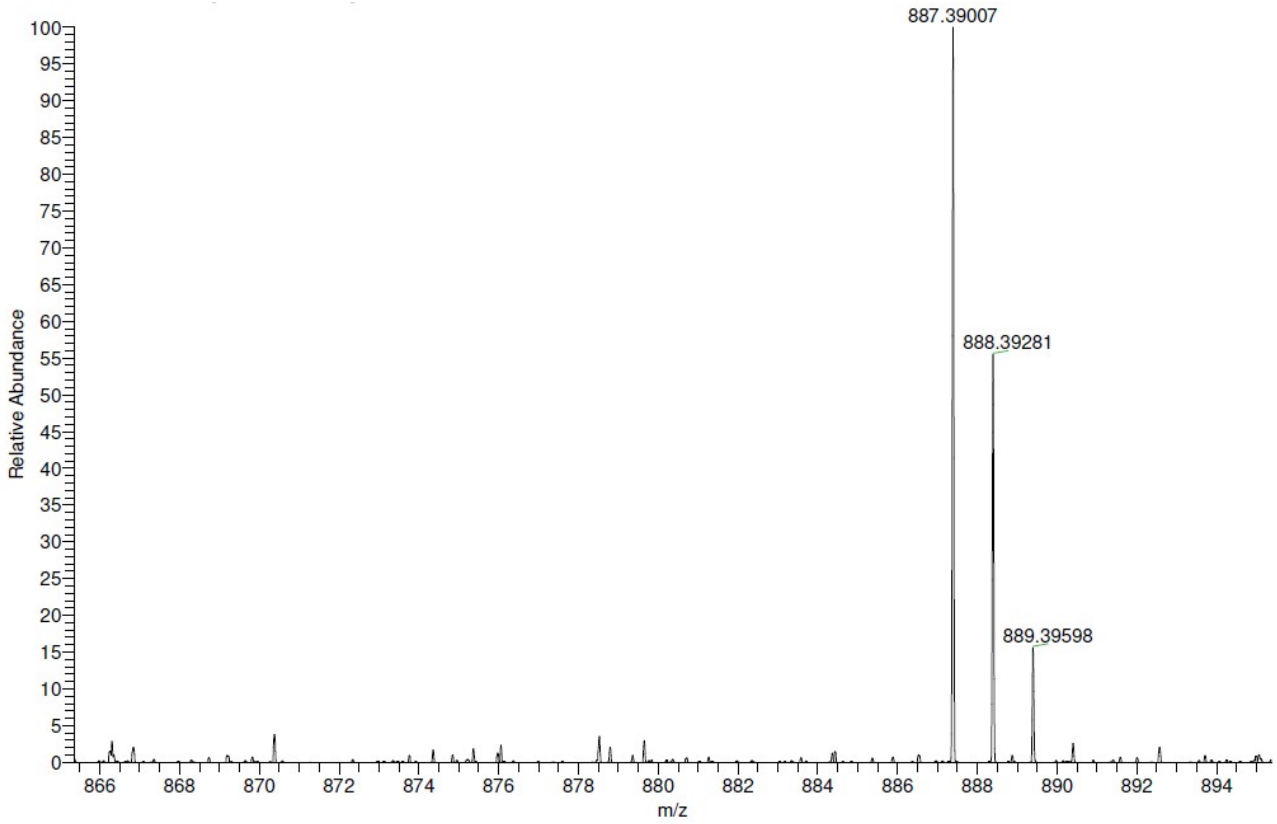
^{1}H NMR (400 MHz, CHLOROFORM-d) of **mC^{bz}-3b**



^{31}P NMR (162 MHz, CHLOROFORM-d) of **mC^{bz}-3b**



High resolution mass spectra of **mC^{bz}-3b**



Elemental composition search on mass 887.39007

m/z= 882.39007-892.39007

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
887.39007	887.39027	-0.23	25.5	$C_{49}H_{56}O_8N_6P$

Table S1. All new sequence data

SEQ ID	Sequence (5' to 3')	Modification	MS		Yield (%)	Synthesis	HPLC Purity (UV %)	HPLC Retention Time (min)
			Calcd.	Found.				
ON-1(DNA)	GTGTTTTTTGCT	DNA/all PO	3633.4	3630.9	—	solid phase	98.9	9.4
ON-2(LNA)	GTGTTTTTTGCT	LNA	3661.4	3661.4	—	solid phase	98.5	9.3
ON-1a	GTGTTTTTTGCT	ALNA[H]	3660.4	3660.5	55	solid phase	100.0	15.2
ON-1b	GTGTTTTTTGCT	ALNA[Me]	3674.4	3675.8	18	solid phase	100.0	15.3
ON-1c	GTGTTTTTTGCT	ALNA[formyl]	3688.4	3687.6	95	PEM	99.1	12.7
ON-1d	GTGTTTTTTGCT	ALNA[Ac]	3702.4	3701.6	102	PEM	100.0	15.4

ON-1e	GTGTTTTTTGCT	ALNA[Bz]	3764.5	3763.6	101	PEM	97.0	10
ON-2(LNA)	TTTTTTTTTT	LNA	3008.0	3007.9	—	solid phase	93.6	12.2
ON-2a	TTTTTTTTTT	ALNA[H]	3007.0	3006.2	20	solid phase	94.7	12.1
ON-2b	TTTTTTTTTT	ALNA[Me]	3021.0	3020.7	21	solid phase	96.6	12.2
ON-2c	TTTTTTTTTT	ALNA[formyl]	3035.0	3034.3	94	PEM	93.8	12.3
ON-2d	TTTTTTTTTT	ALNA[Ac]	3049.0	3048.4	97	PEM	95.1	14.8
ON-2e	TTTTTTTTTT	ALNA[Bz]	3111.1	3110.5	103	PEM	95.6	12.8
ON-3a	AACATCAGTCTGATAAGCT	ALNA[H]	6288.2	6287.3	22	solid phase	97.8	14.9
ON-3b	AACATCAGTCTGATAAGCT	ALNA[Me]	6386.4	6385.4	16	solid phase	98.6	15.2
ON-3d	AACATCAGTCTGATAAGCT	ALNA[Ac]	6582.4	6581.5	98	PEM	97.2	15.2
ON-4a	AACATCAGTCTGATAAGCT	ALNA[H]	6301.2	6300.7	19	solid phase	98.4	14.8
ON-4b	AACATCAGTCTGATAAGCT	ALNA[Me]	6413.4	6412.8	34	solid phase	91.9	15.1
ON-4d	AACATCAGTCTGATAAGCT	ALNA[Ac]	6637.5	6637.6	96	PEM	89.5	15.2
ON-5a	AACATCAGTCTGATAAGCT	ALNA[H]	6343.3	6342.0	20	solid phase	97.2	14.8
ON-5b	AACATCAGTCTGATAAGCT	ALNA[Me]	6455.5	6454.6	14	solid phase	98.3	15.1
ON-5d	AACATCAGTCTGATAAGCT	ALNA[Ac]	6679.6	6678.8	96	PEM	95.7	15.2
ON-6a	**A**CATCAGTCTGATAAGCT**A**	ALNA[H]/MOE	6424.4	6424.1	22	solid phase	100.0	15.3
ON-6b	**A**CATCAGTCTGATAAGCT**A**	ALNA[Me]/MOE	6494.5	6492.4	14	solid phase	100.0	18.3
ON-6c	**A**CATCAGTCTGATAAGCT**A**	ALNA[formyl]/MOE	6564.4	6563.7	97	PEM	99.1	15.5
ON-6d	**A**CATCAGTCTGATAAGCT**A**	ALNA[Ac]/MOE	6634.6	6634.3	95	PEM	100.0	18.2
ON-6e	**A**CATCAGTCTGATAAGCT**A**	ALNA[Bz]/MOE	6944.9	6939.6	99	PEM	100.0	13.8

Capital letters in the sequences refer to DNA (black), 2'-MOE (bold) and modified-amino-LNAs (underline, C denote methylcytosine). All phosphodiester linkages of **ON-3** to **ON-6** were replaced by phosphorothioate (PS) linkages.

RP-HPLC purification condition

Mobile phase A: 20 mM Hexylamine acetate in water

Mobile phase B: Acetonitrile

Gradient condition: 10-50% in 40 min

Flow rate: 4 mL/min

Column: Waters XBridge™ Oligonucleotide BEH C18 OBDTM Prep Column, 130Å 2.5 µm (10 × 50 mm)

Column oven temperature: 60 °C

Detector: UV 260 nm

RP-HPLC analysis conditions

ON-1 (DNA) and ON-1 (LNA) (purchased from GeneDesign)

Mobile phase A: 100 mM HFIP / 8mM TEA in water

Mobile phase B: Methanol

gradient condition:5-20 % (20min)

Flow rate: 1 mL/min

Column: Waters XBridge™ BEH C18 2.5 µm (4.6 × 75 mm)

Column oven temperature: 60 °C

Detector: UV 260 nm

ON1a~ON-6e

Mobile phase A: 20 mM Hexylamine acetate in water

Mobile phase B: Acetonitrile

Flow rate: 1 mL/min

Column: Waters XBridge™ BEH C18 130Å 2.5 µm (4.6 × 50 mm)

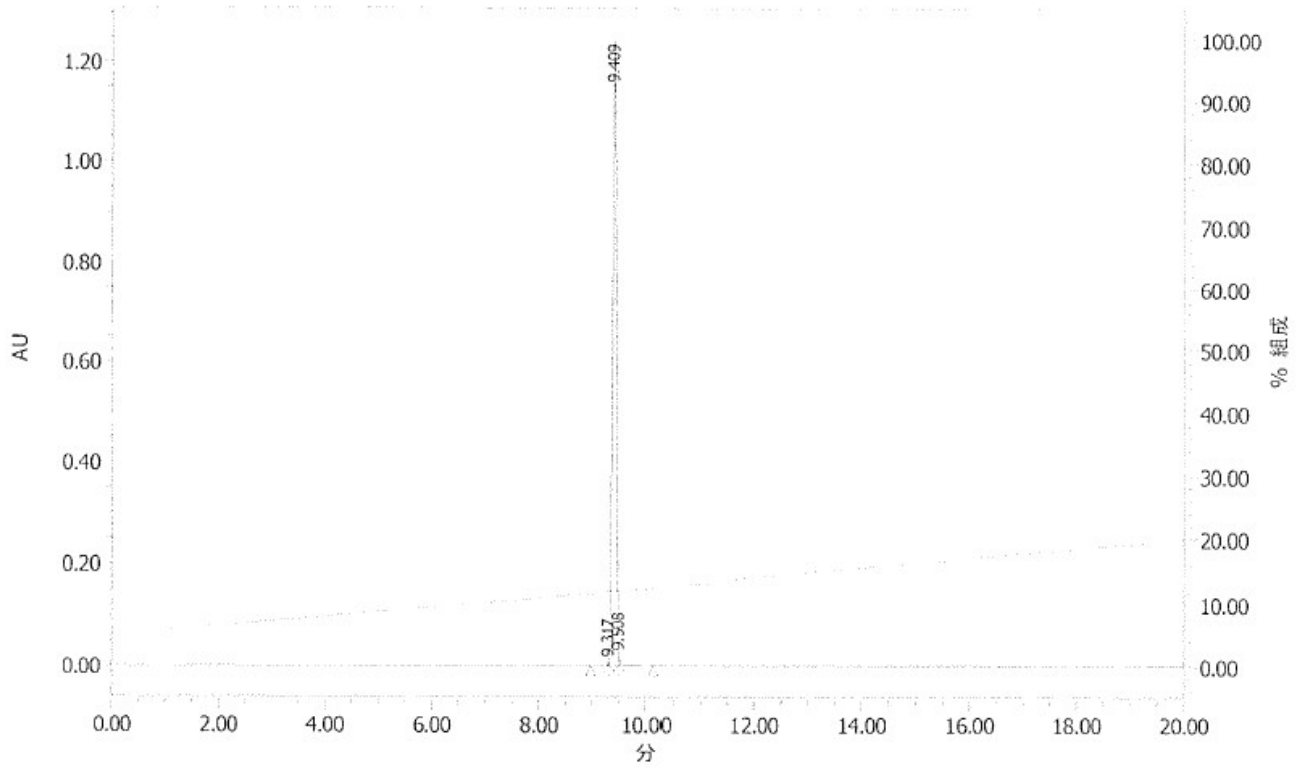
Column oven temperature: 60 °C

Detector: UV 260 nm

HPLC charts of oligonucleotides

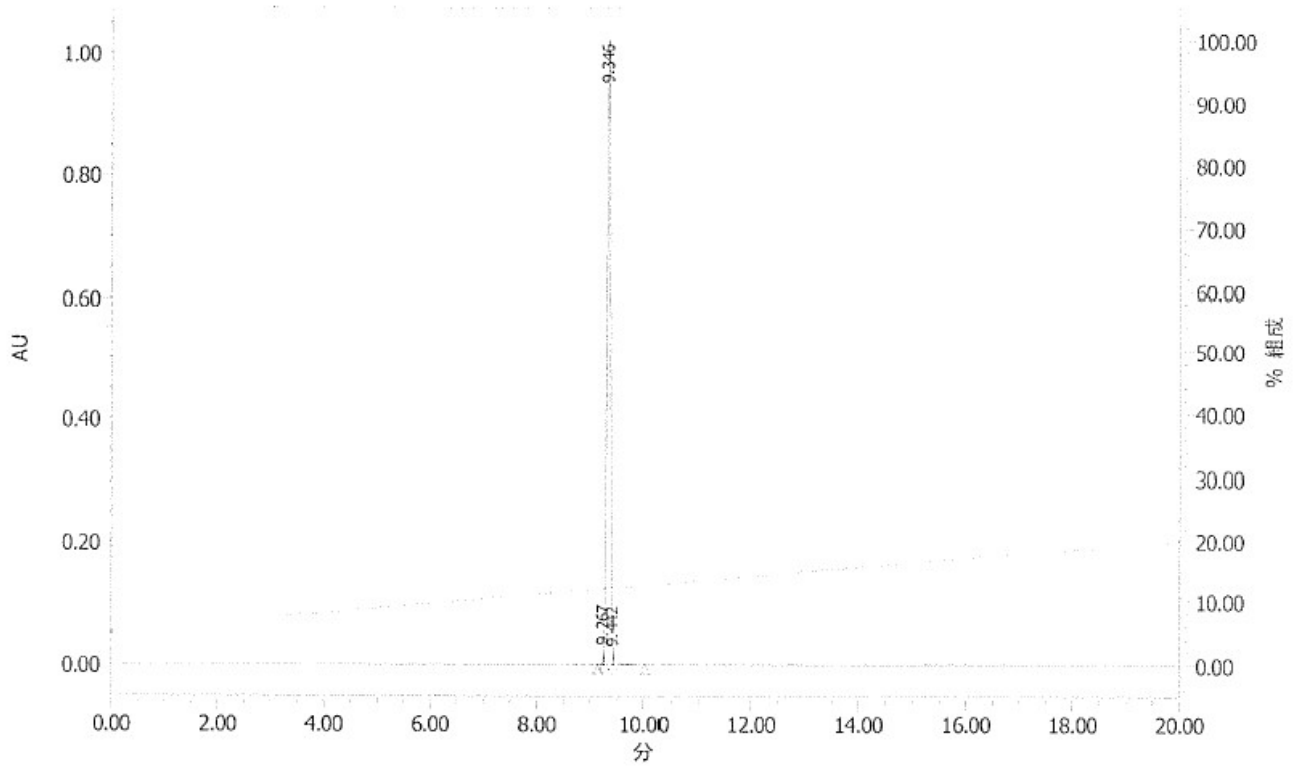
ON-1 (DNA) (purchased from GeneDesign)

HPLC (gradient condition: B% 5-20 % in 20 min)



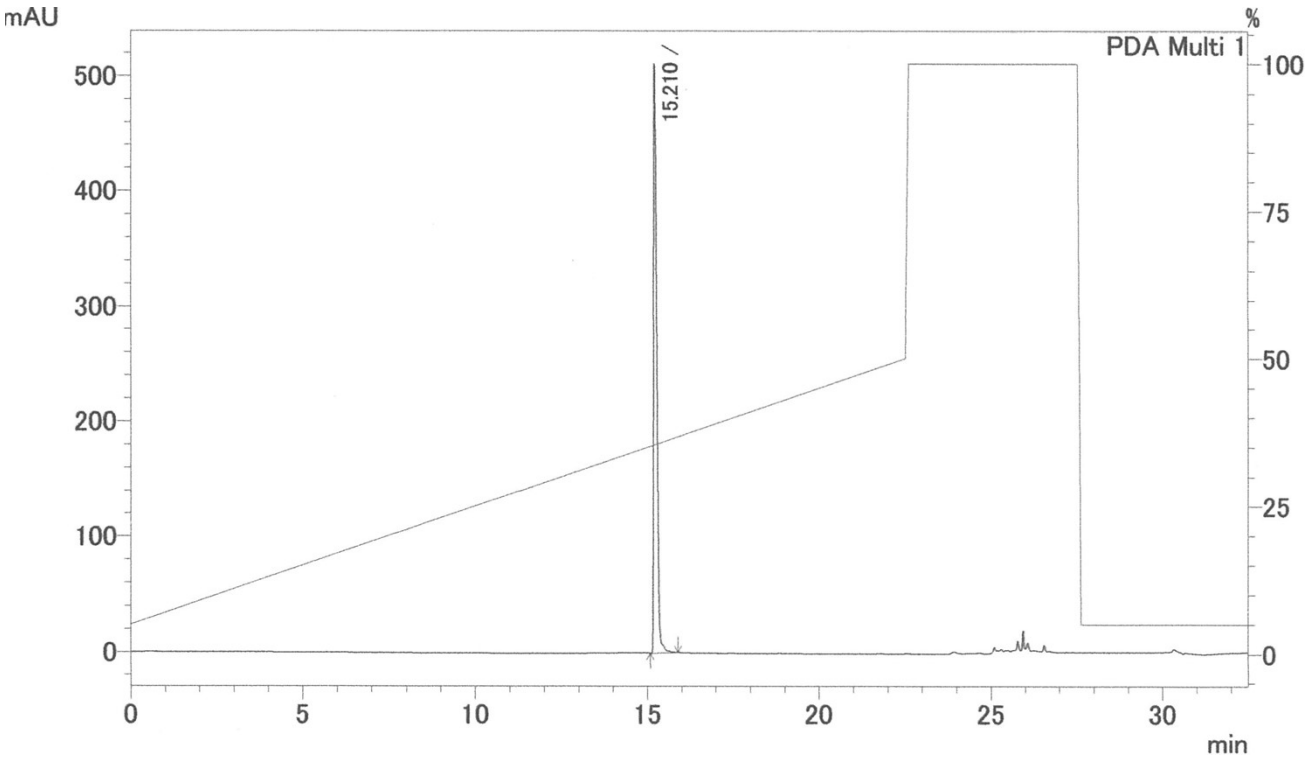
ON-1 (LNA) (purchased from GeneDesign)

HPLC (gradient condition: B% 5-20 % in 20 min)



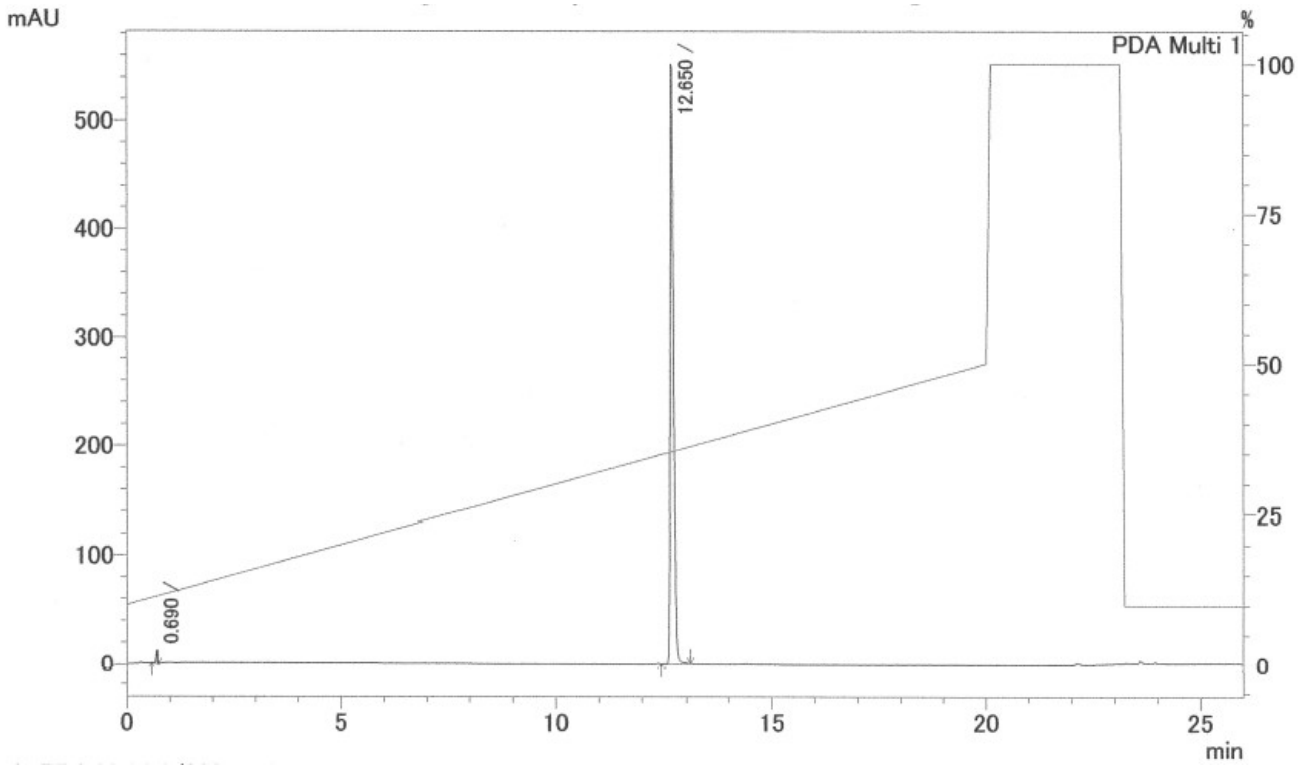
ON-1a

HPLC (gradient condition: B% 5-50 % in 22.5 min, then 100% in 5 min)



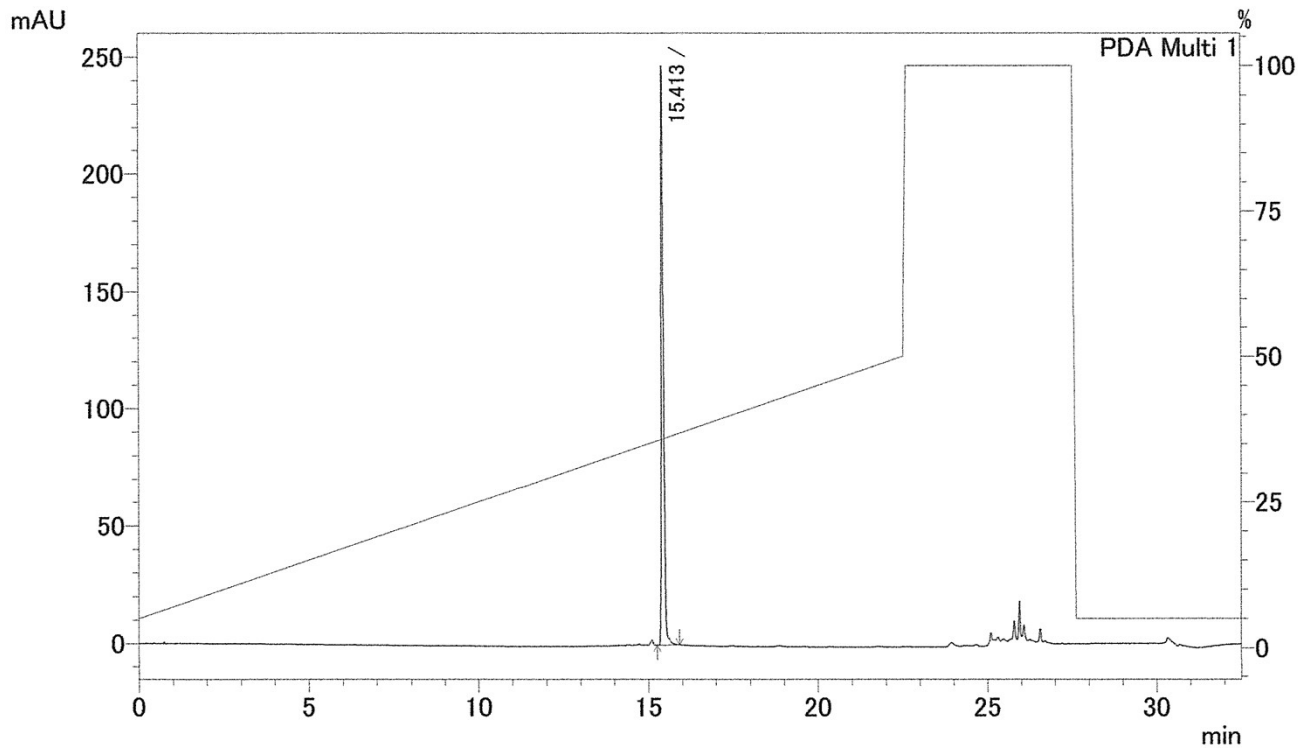
ON-1b

HPLC (gradient condition: B% 10-50 % in 20 min, then 100% in 3 min)



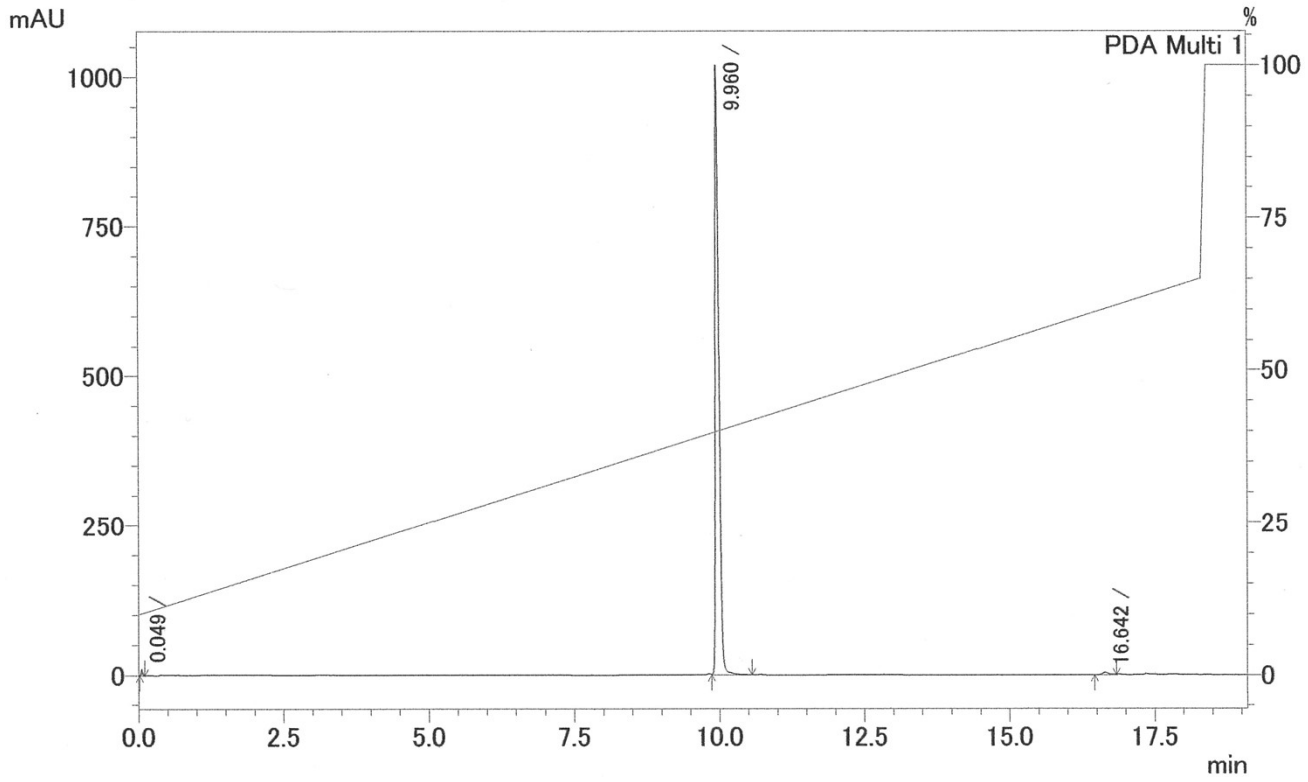
ON-1c

HPLC (gradient condition: B% 5-50 % in 22.5 min, then 100% in 5 min)



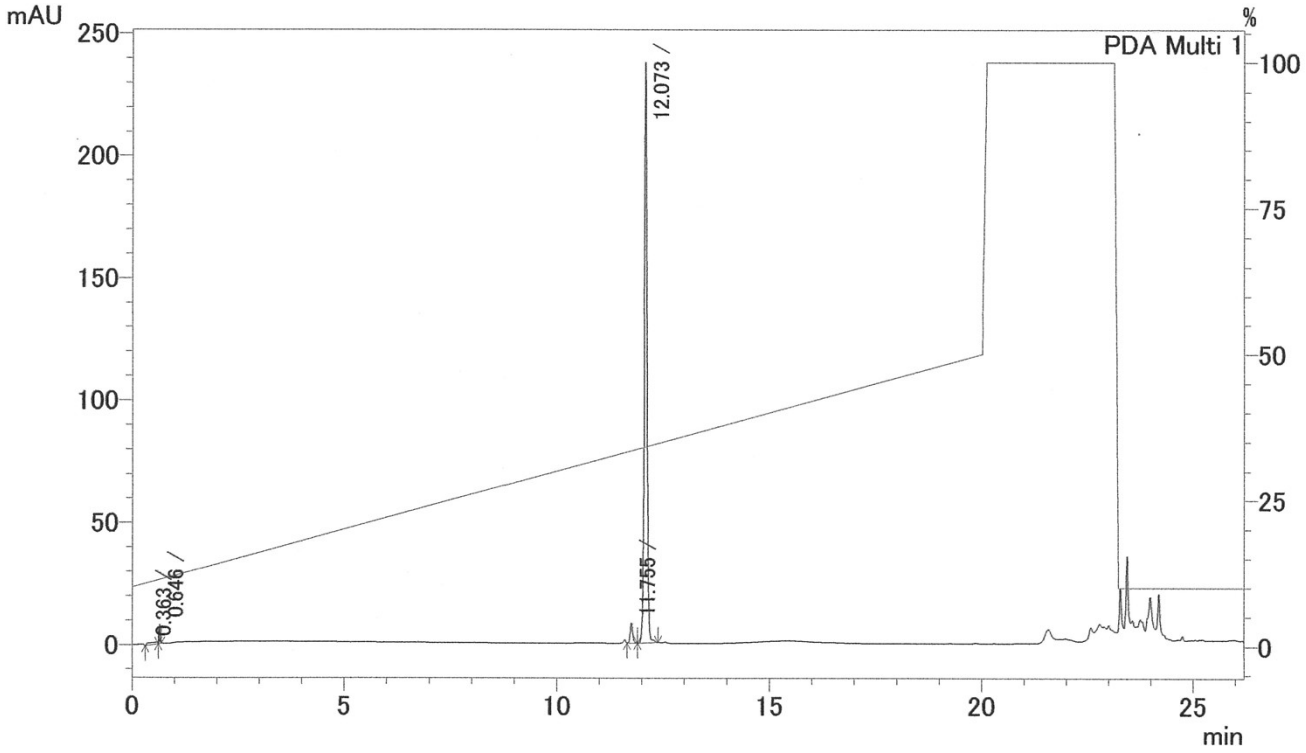
ON-1d

HPLC (gradient condition: B% 10-65 % in 18 min, then 100% in 5 min)



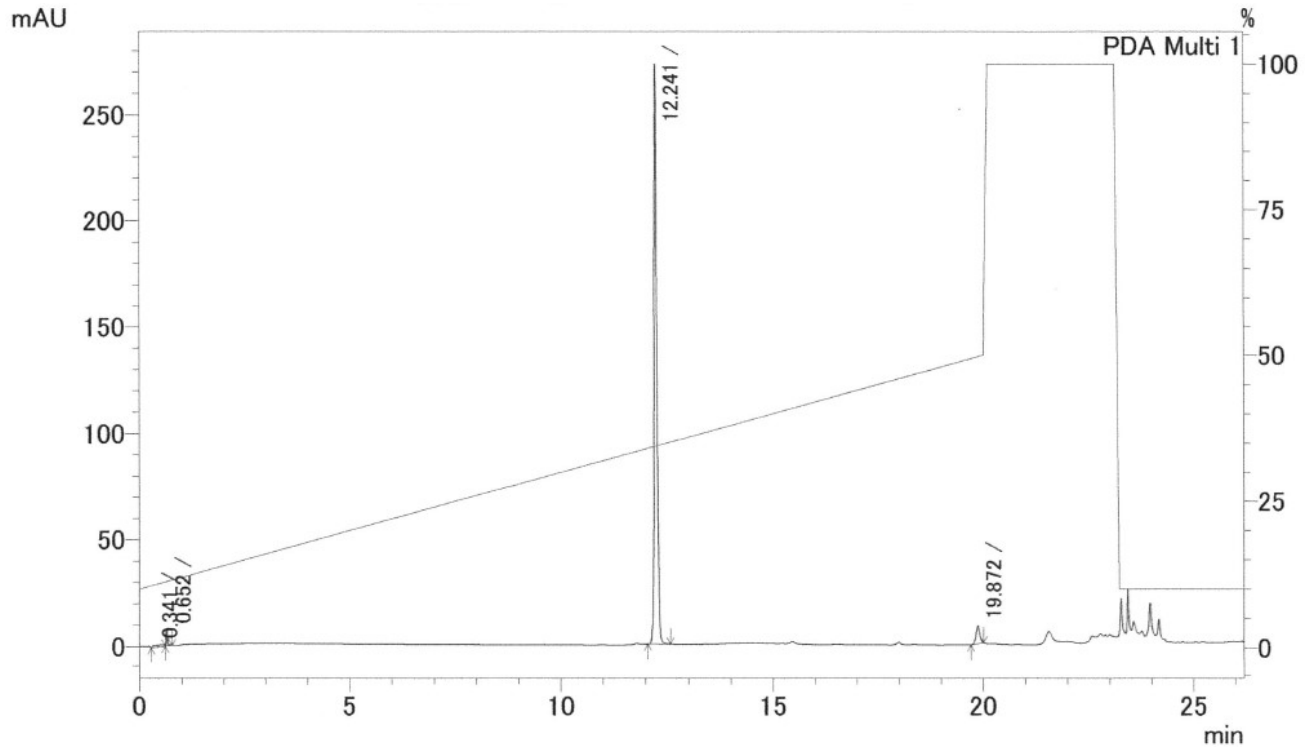
ON-1e

HPLC (gradient condition: B% 10-50 % in 20 min, then 100% in 3 min)



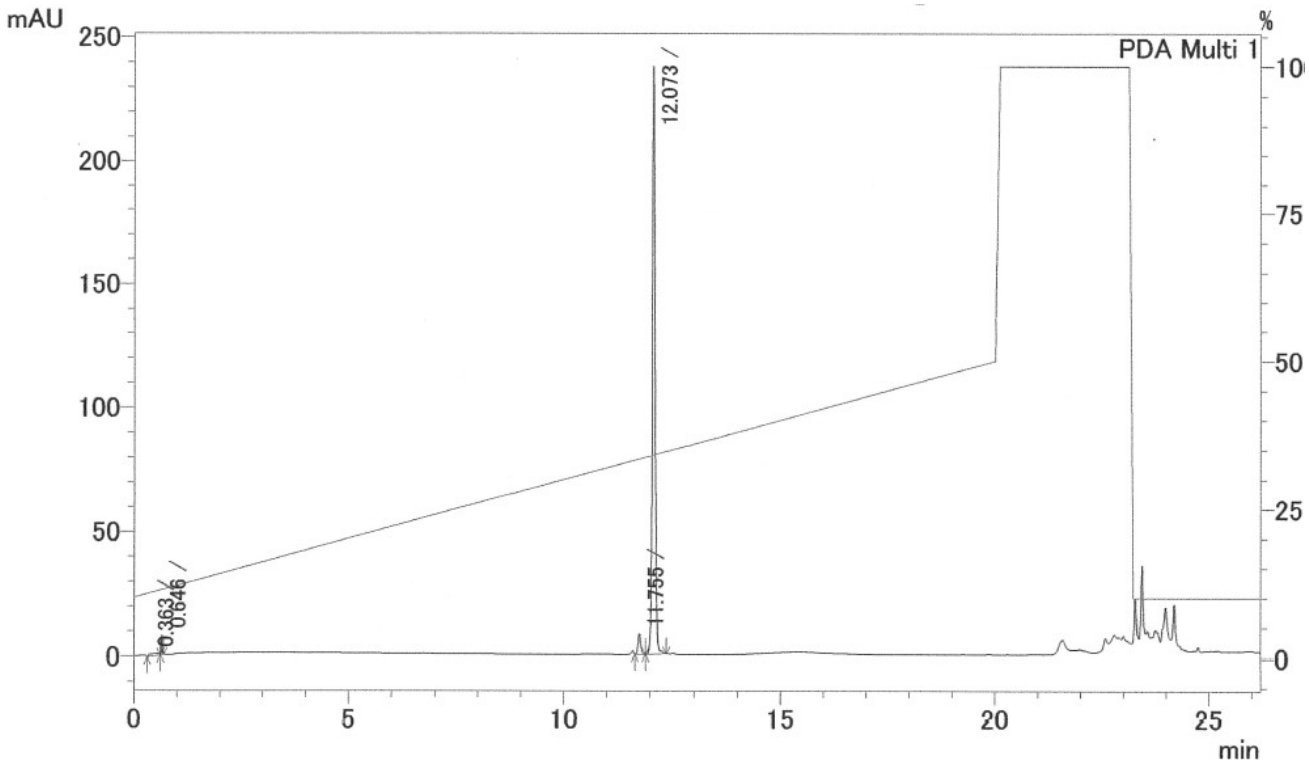
ON-2 (LNA)

HPLC (gradient condition: B% 10-50 % in 20 min, then 100% in 3 min)



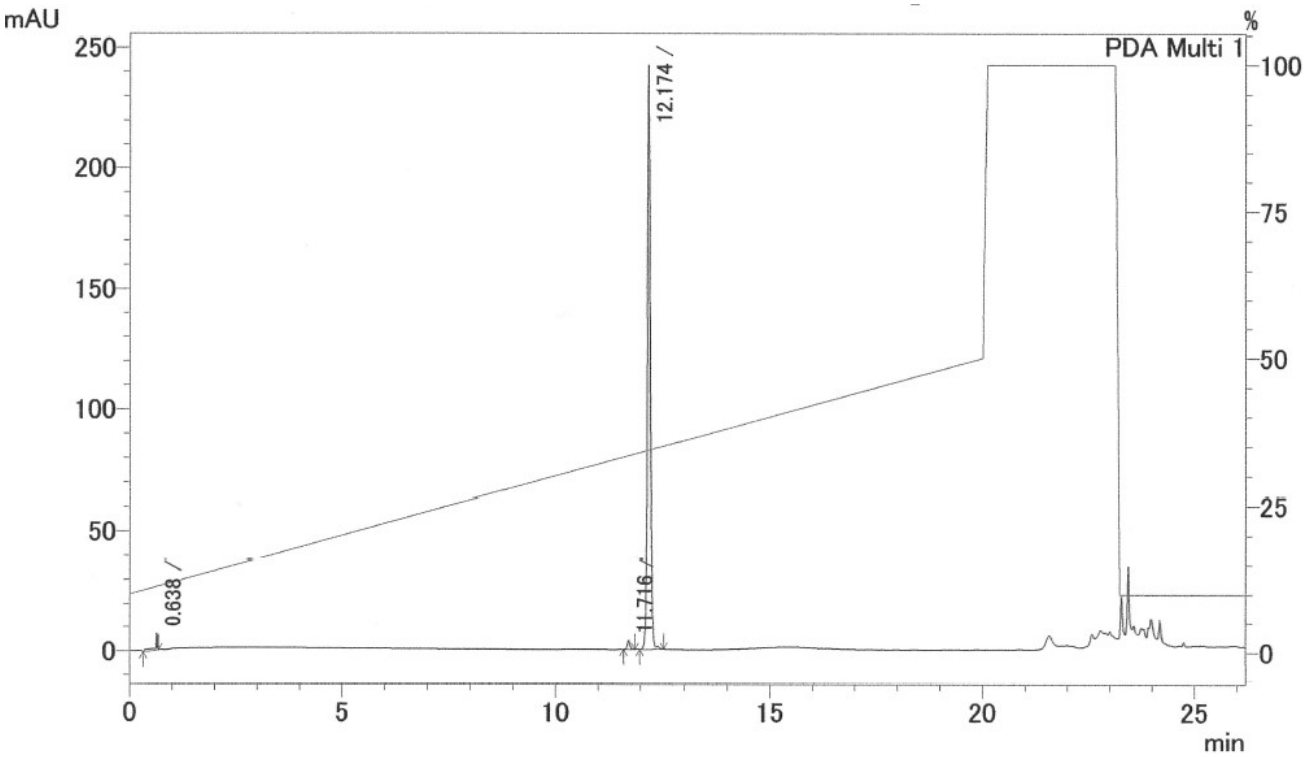
ON-2a

HPLC (gradient condition: B% 10-50 % in 20 min, then 100% in 3 min)



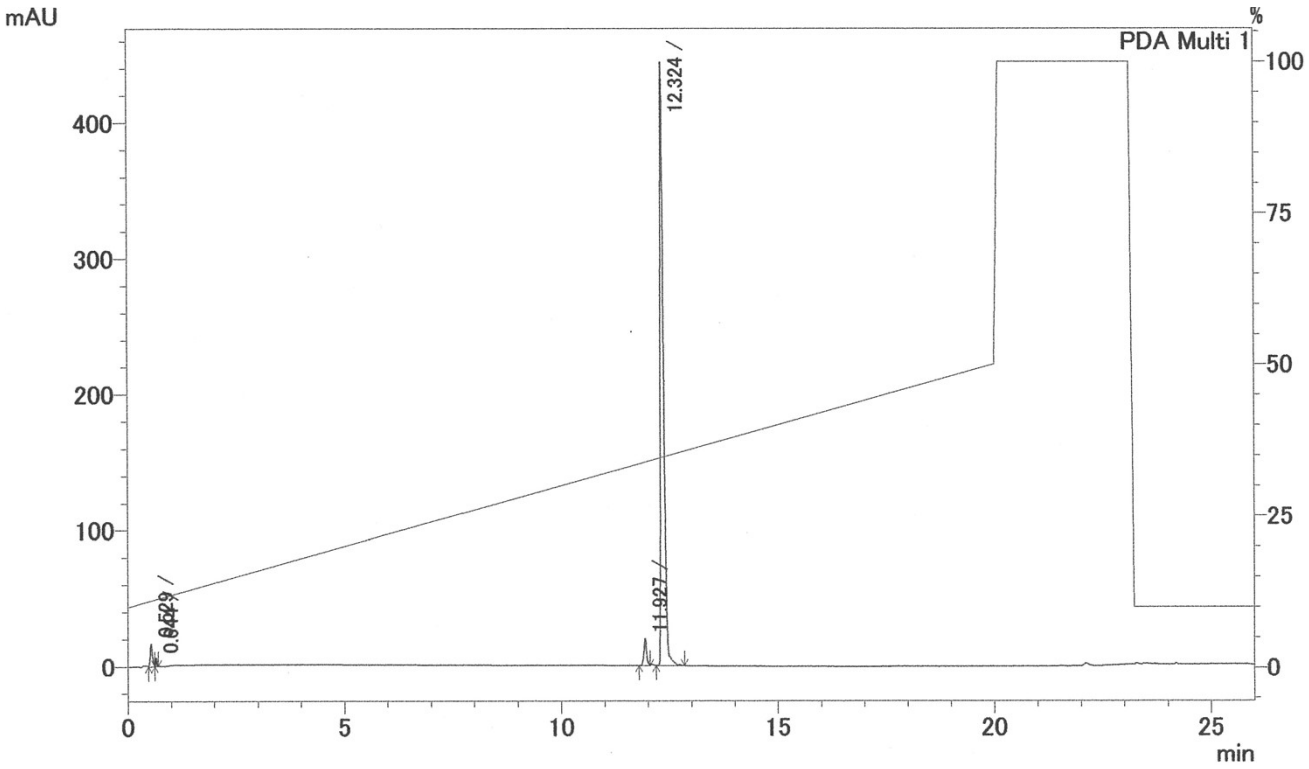
ON-2b

HPLC (gradient condition: B% 10-50 % in 20 min, then 100% in 3 min)



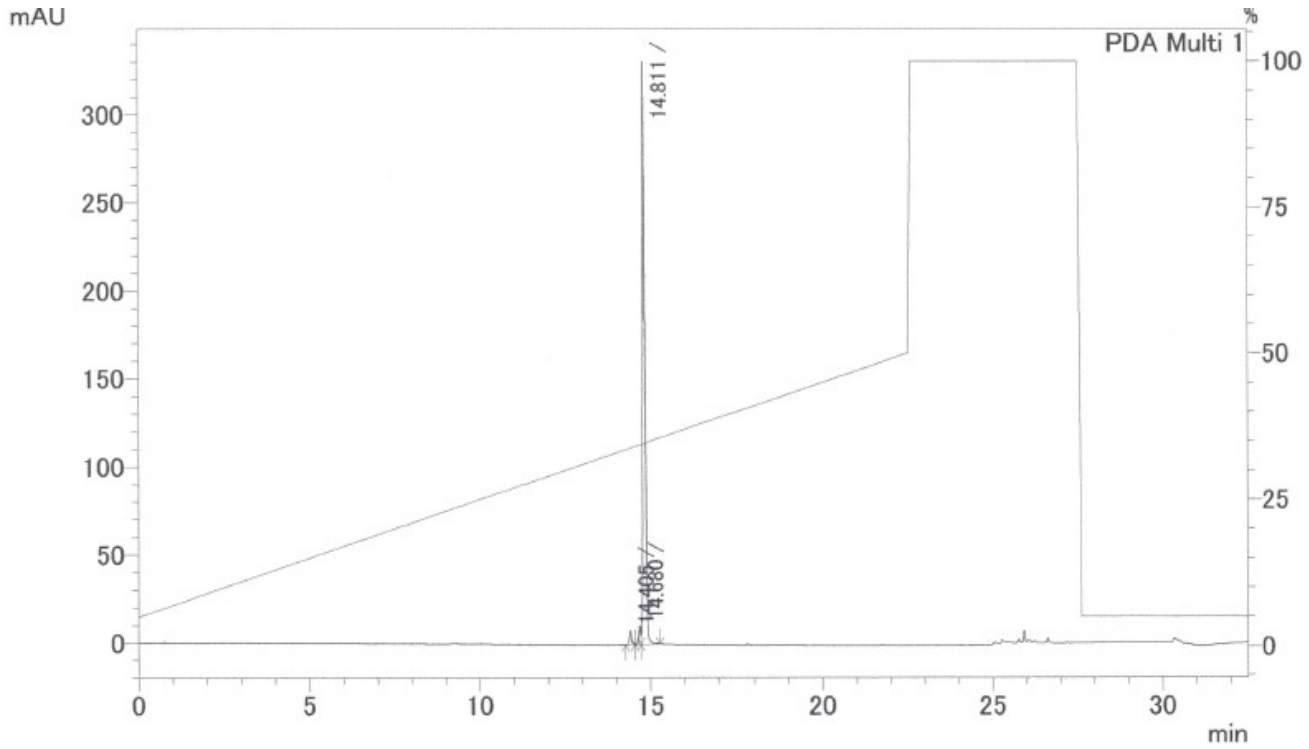
ON-2c

HPLC (gradient condition: B% 10-50 % in 20 min, then 100% in 3 min)



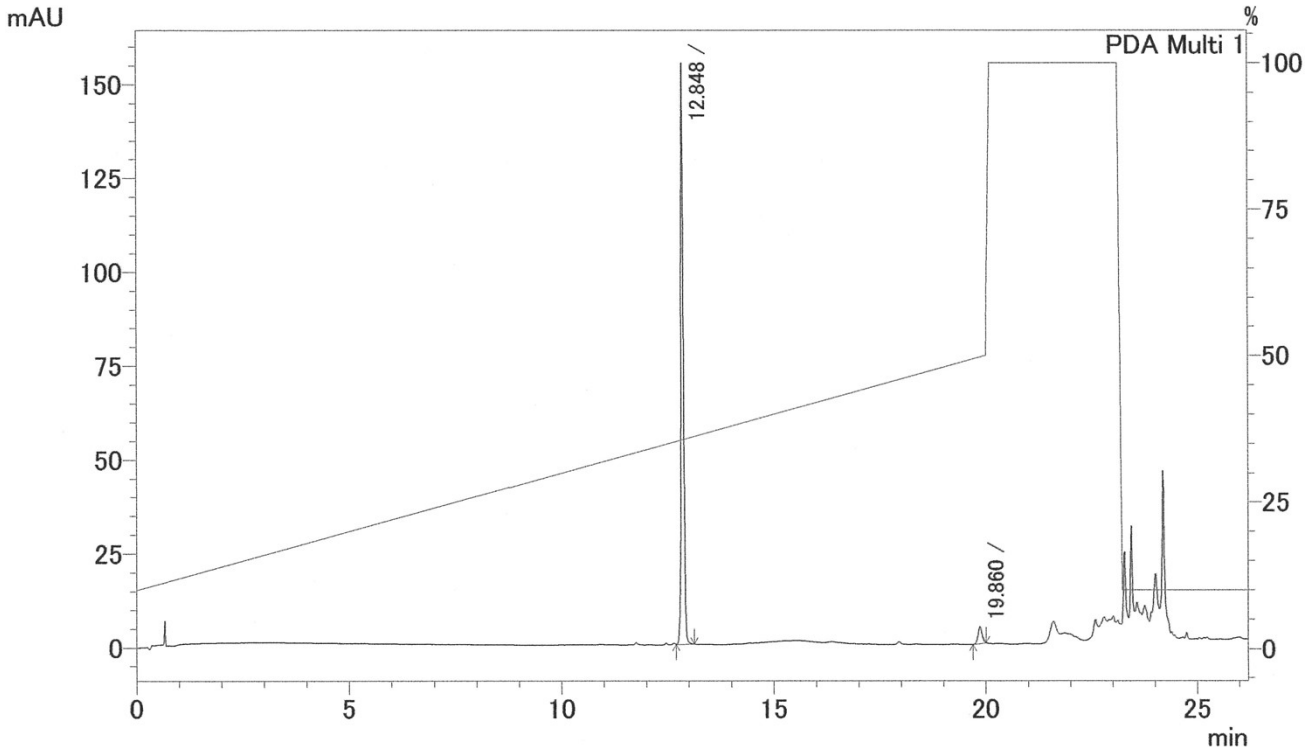
ON-2d

HPLC (gradient condition: B% 10-50 % in 20 min, then 100% in 3 min)



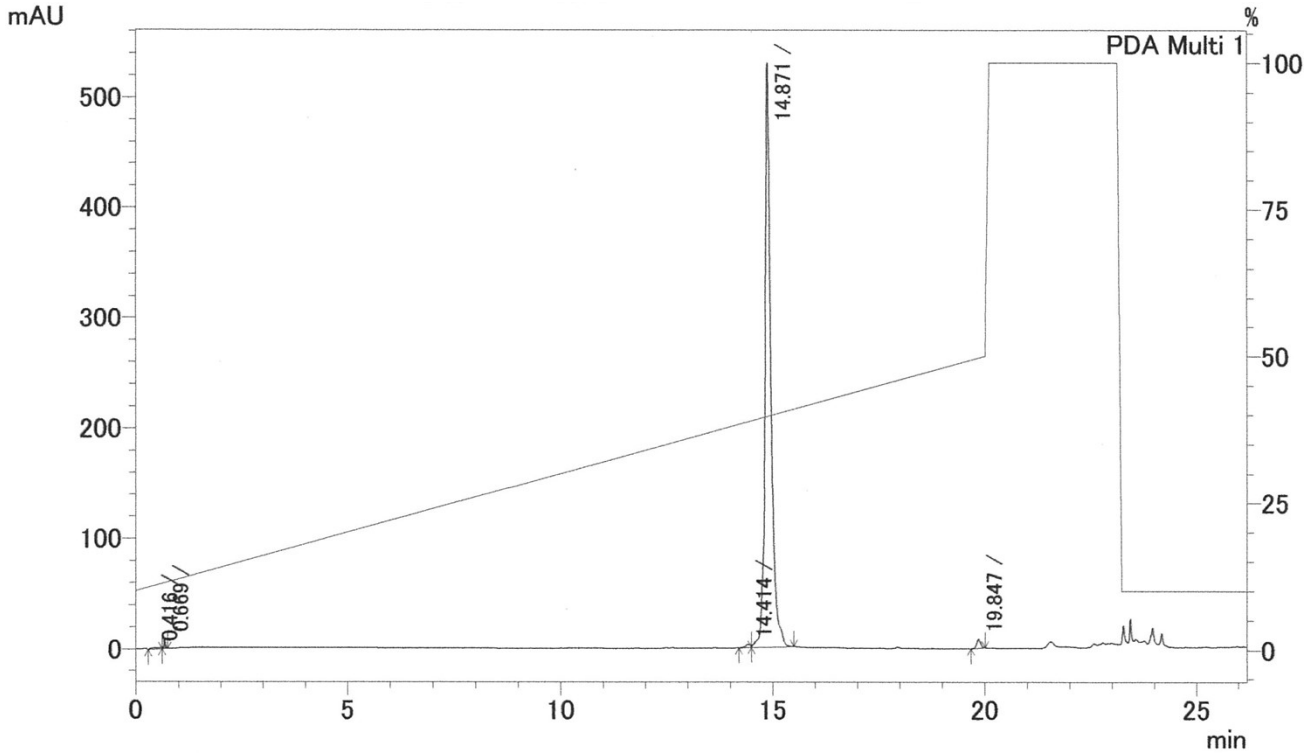
ON-2e

HPLC (gradient condition: B% 10-50 % in 20 min, then 100% in 3 min)



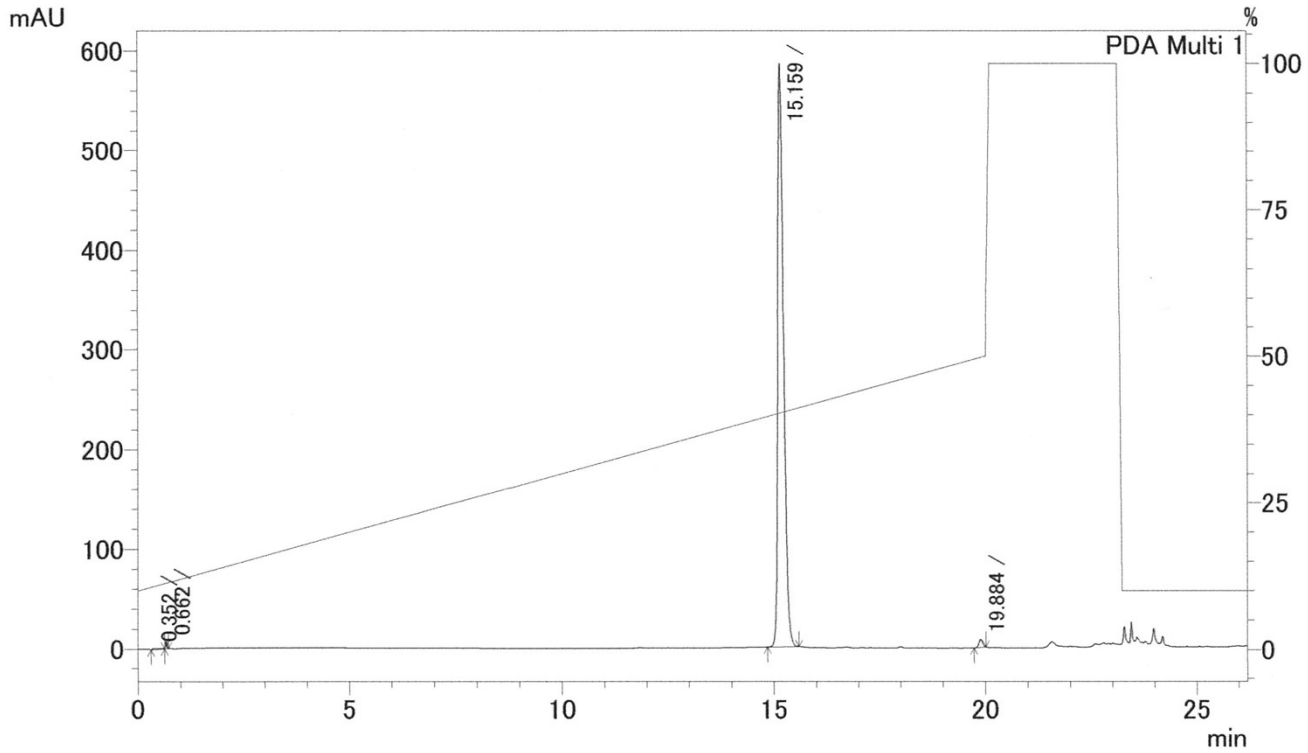
ON-3a

HPLC (gradient condition: B% 10-50 % in 20 min, then 100% in 3 min)



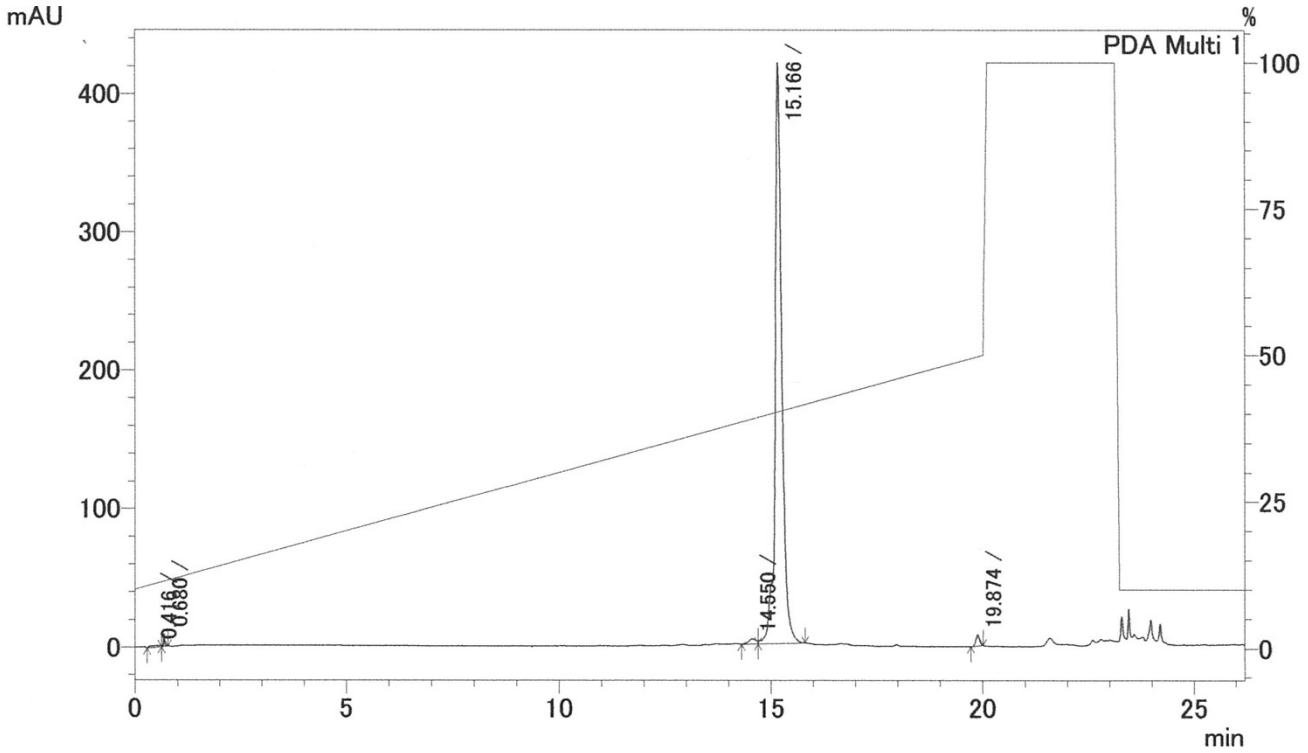
ON-3b

HPLC (gradient condition: B% 10-50 % in 20 min, then 100% in 3 min)



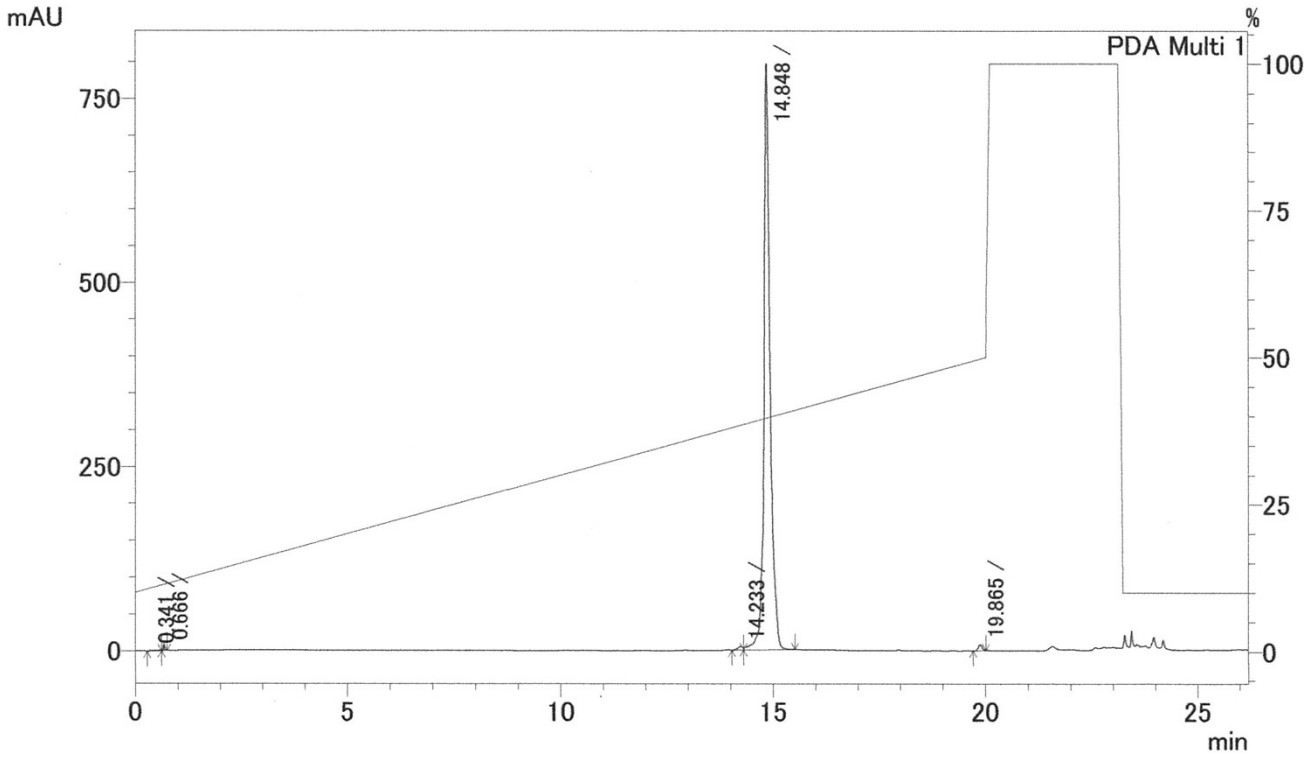
ON-3d

HPLC (gradient condition: B% 10-50 % in 20 min, then 100% in 3 min)



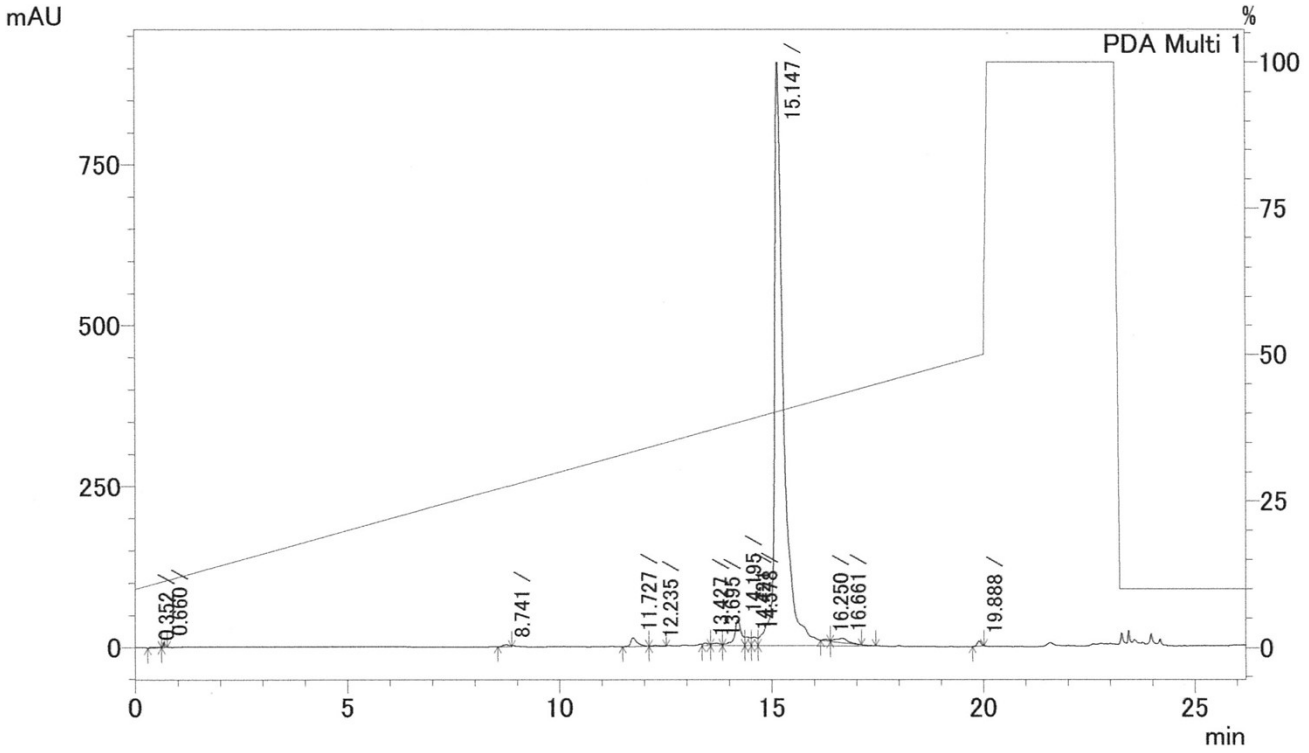
ON-4a

HPLC (gradient condition: B% 10-50 % in 20 min, then 100% in 3 min)



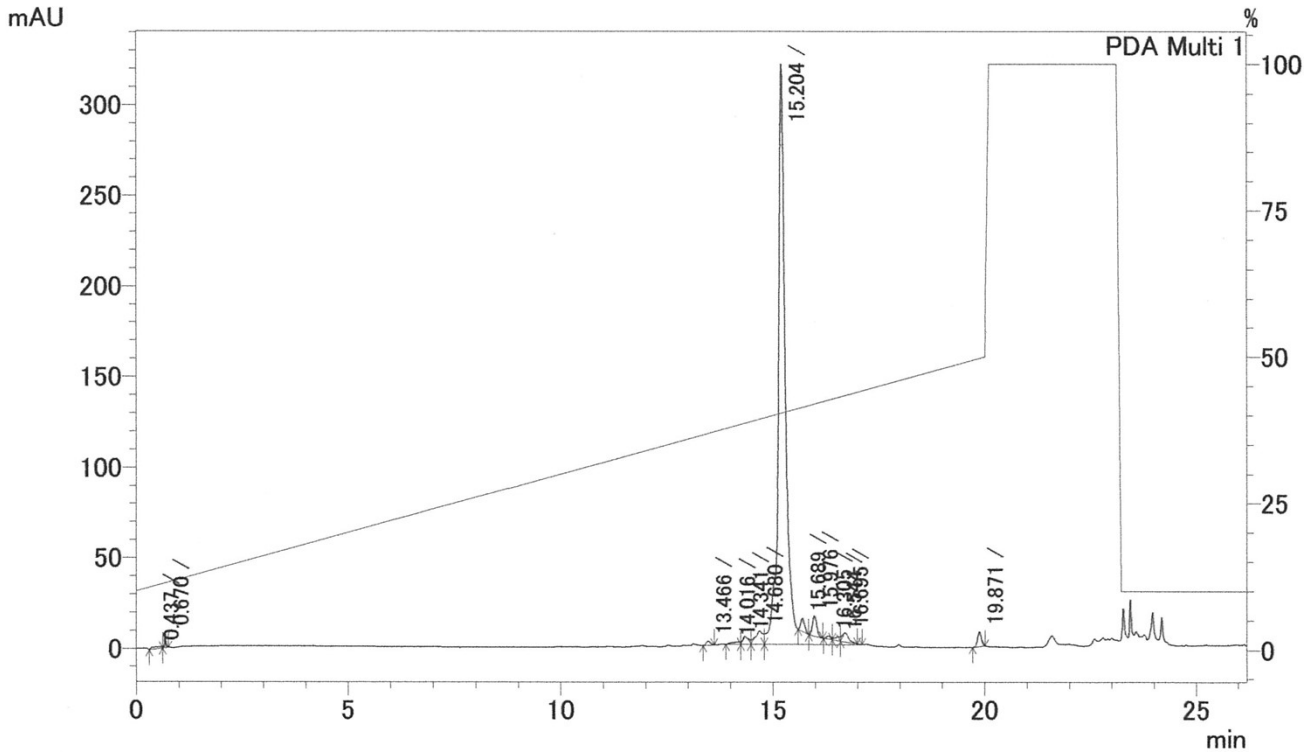
ON-4b

HPLC (gradient condition: B% 10-50 % in 20 min, then 100% in 3 min)



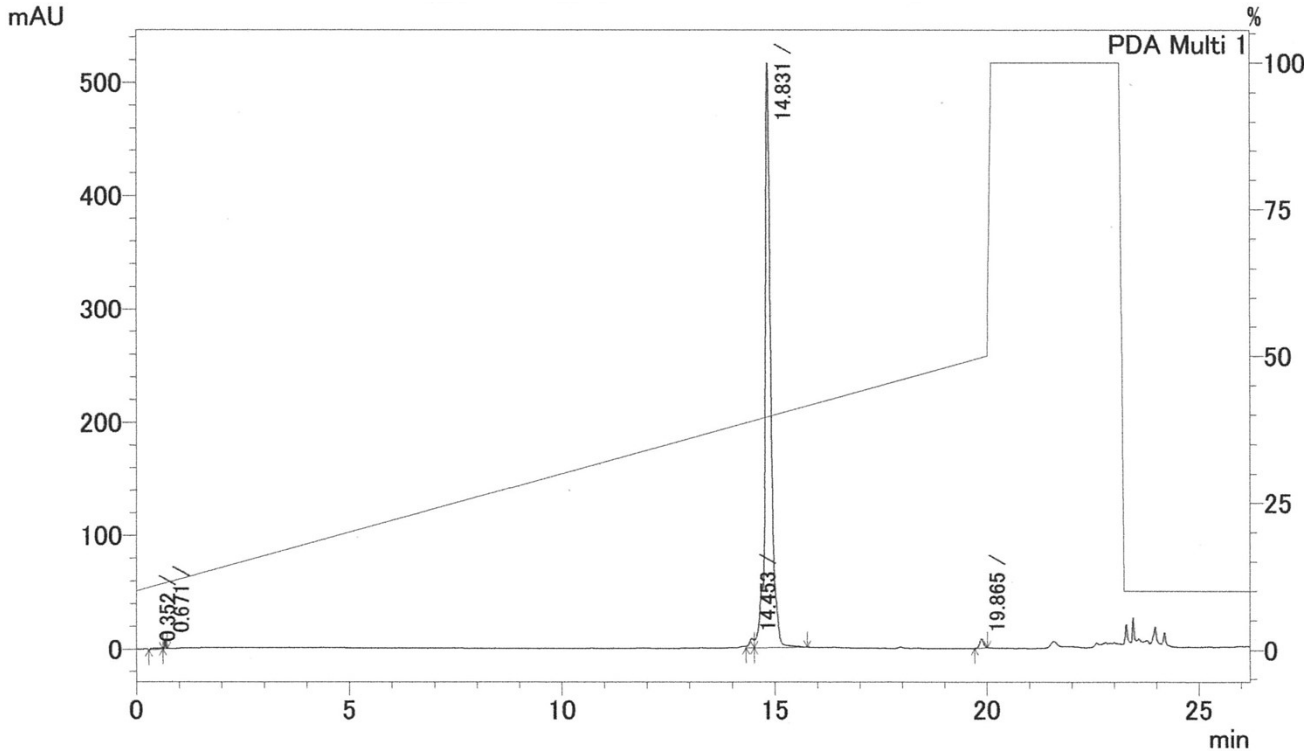
ON-4d

HPLC (gradient condition: B% 10-50 % in 20 min, then 100% in 3 min)



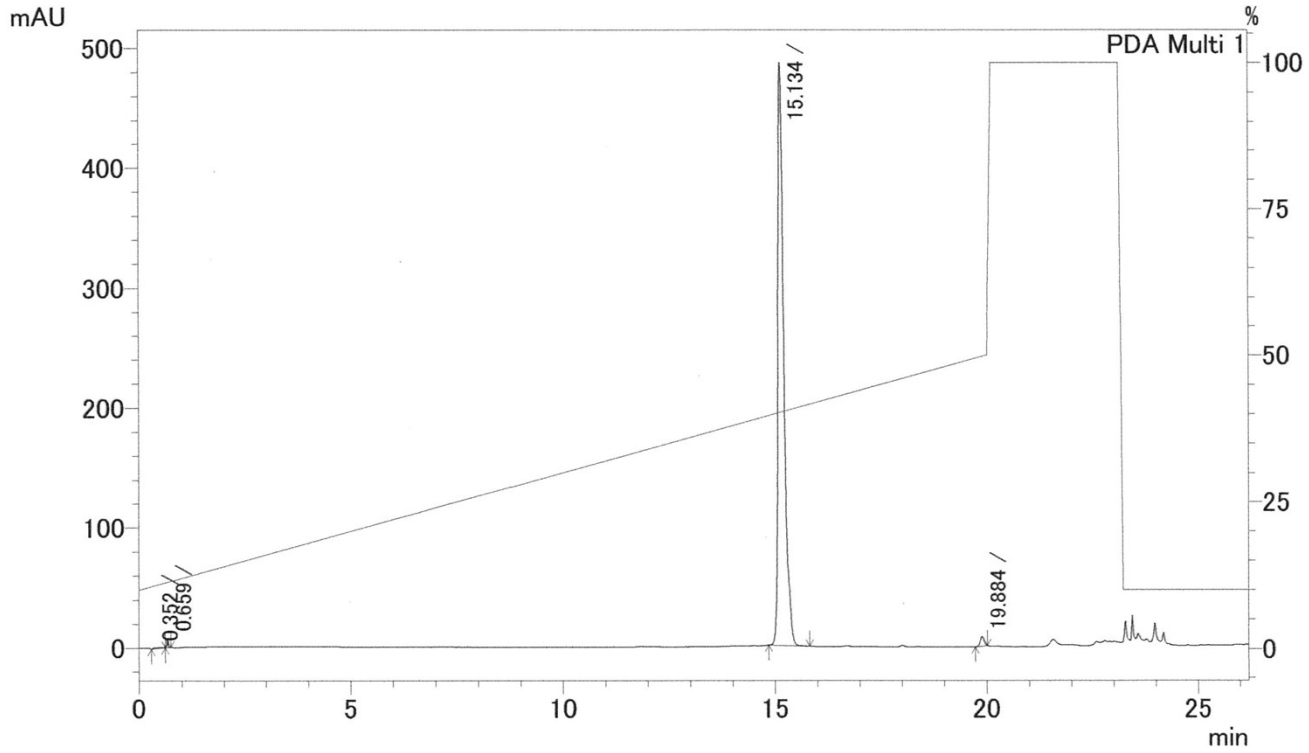
ON-5a

HPLC (gradient condition: B% 10-50 % in 20 min, then 100% in 3 min)



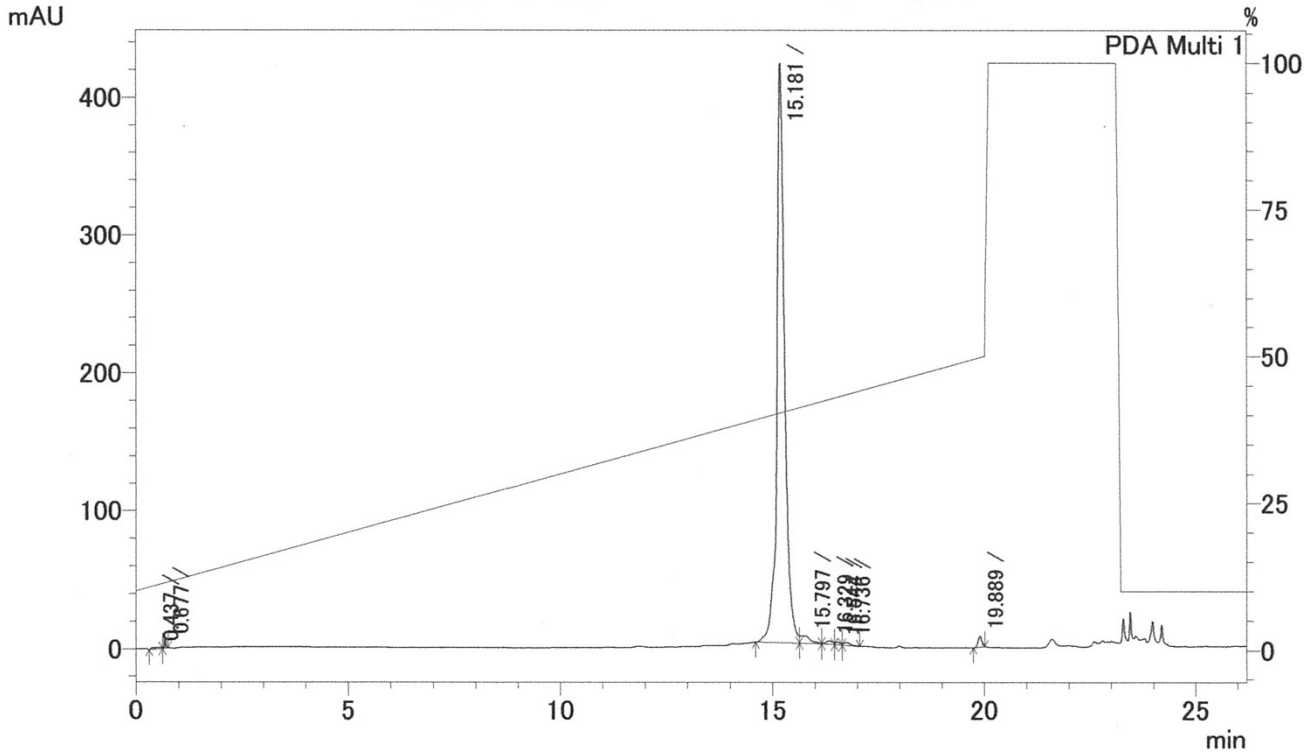
ON-5b

HPLC (gradient condition: B% 10-50 % in 20 min, then 100% in 3 min)



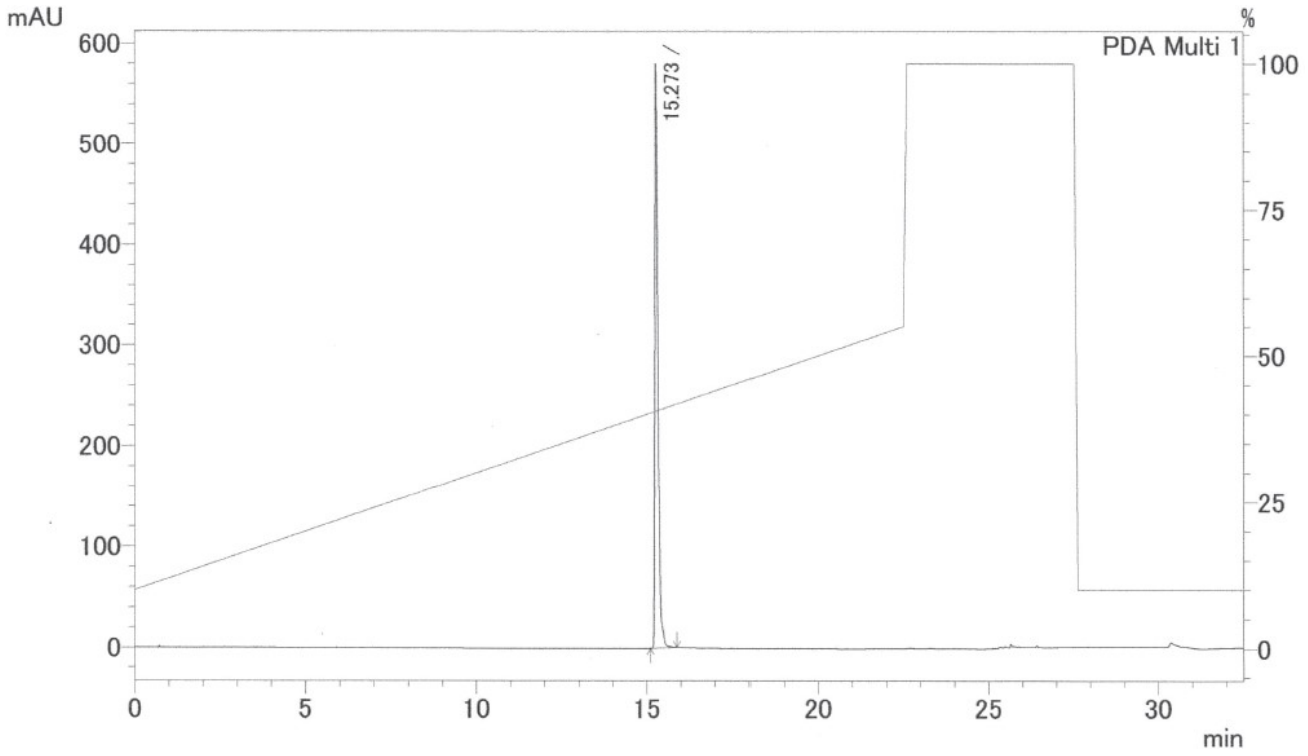
ON-5d

HPLC (gradient condition: B% 10-50 % in 20 min, then 100% in 3 min)



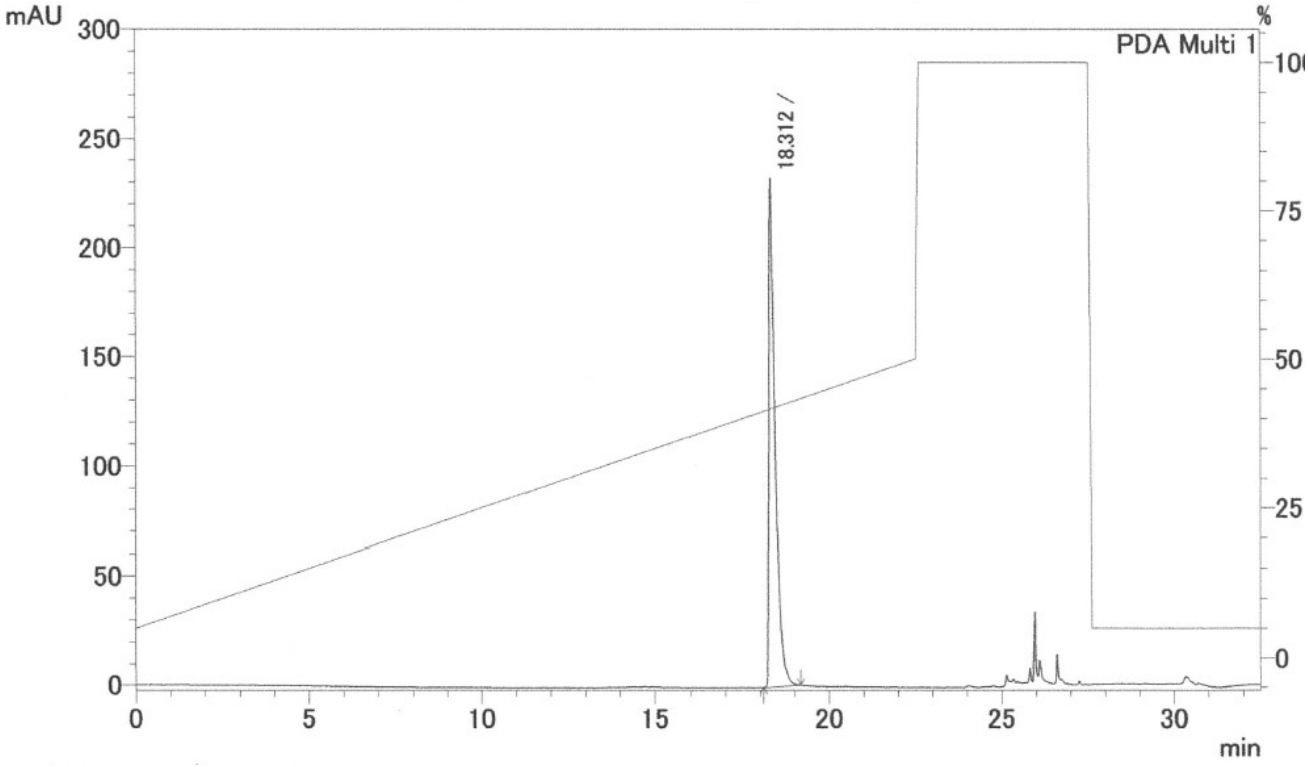
ON-6a

HPLC (gradient condition: B% 5-50 % in 22.5 min, then 100% in 5 min)



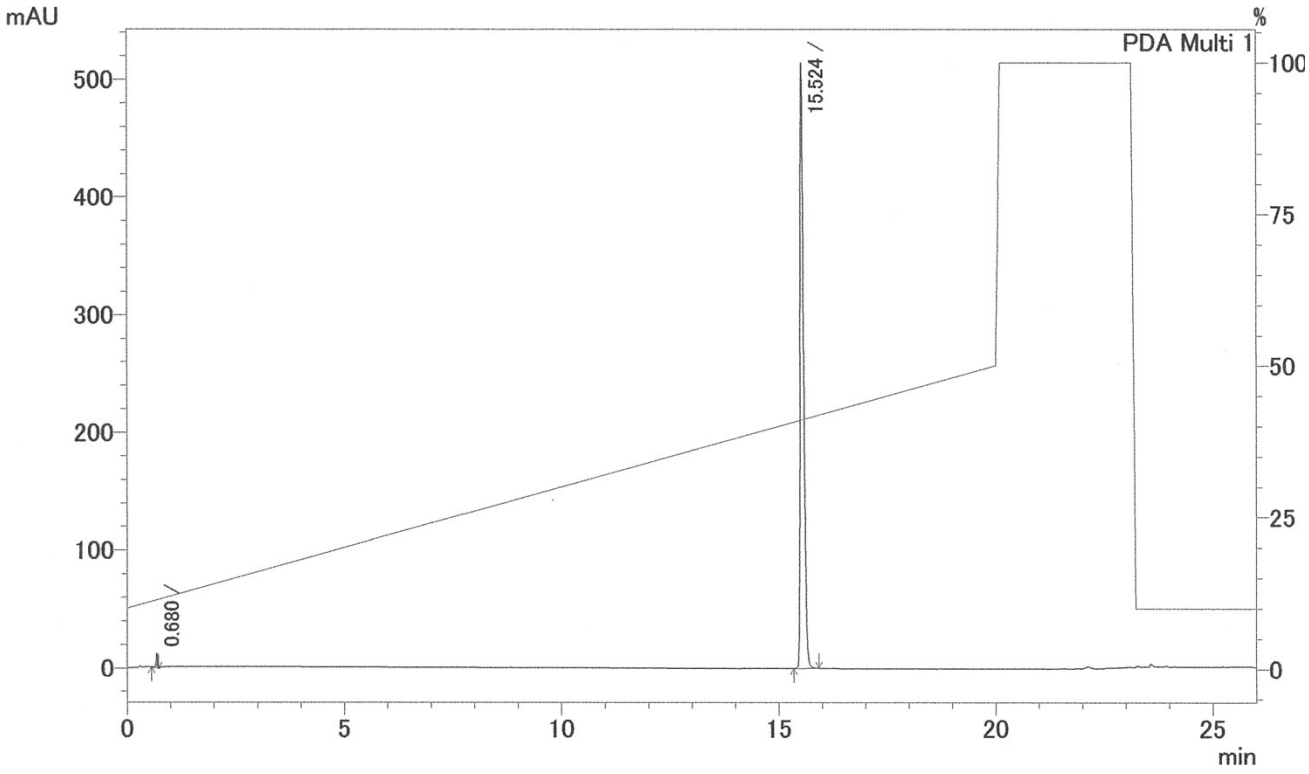
ON-6b

HPLC (gradient condition: B% 5-50 % in 22.5 min, then 100% in 5 min)



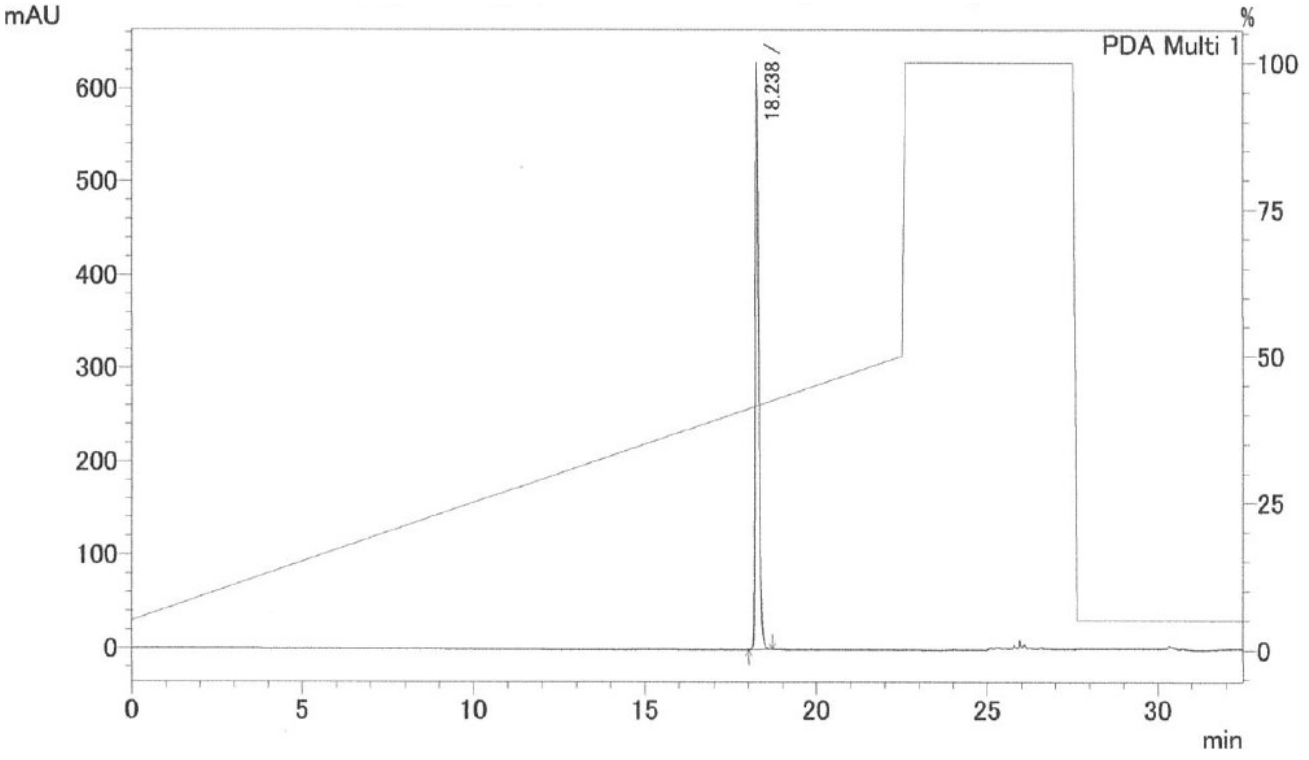
ON-6c

HPLC (gradient condition: B% 10-50 % in 20 min, then 100% in 3 min)



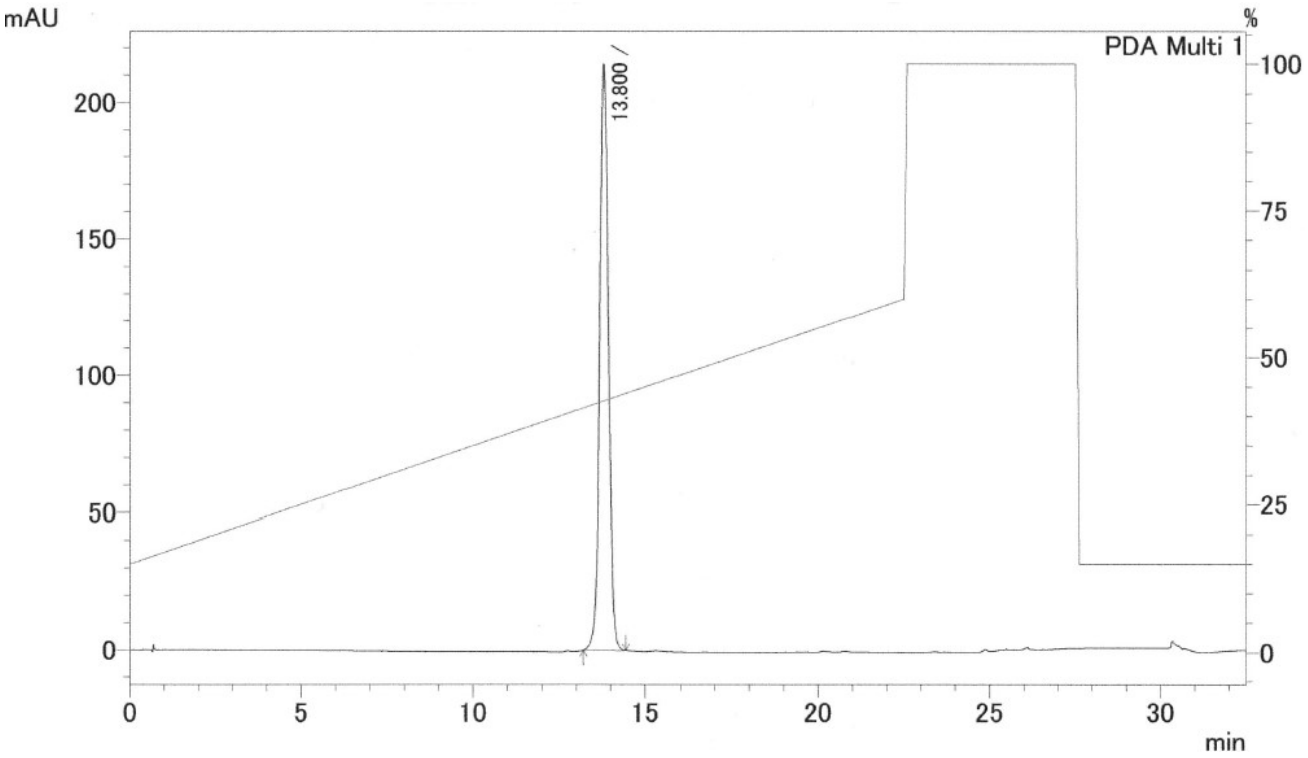
ON-6d

HPLC (gradient condition: B% 5-50 % in 22.5 min, then 100% in 5 min)

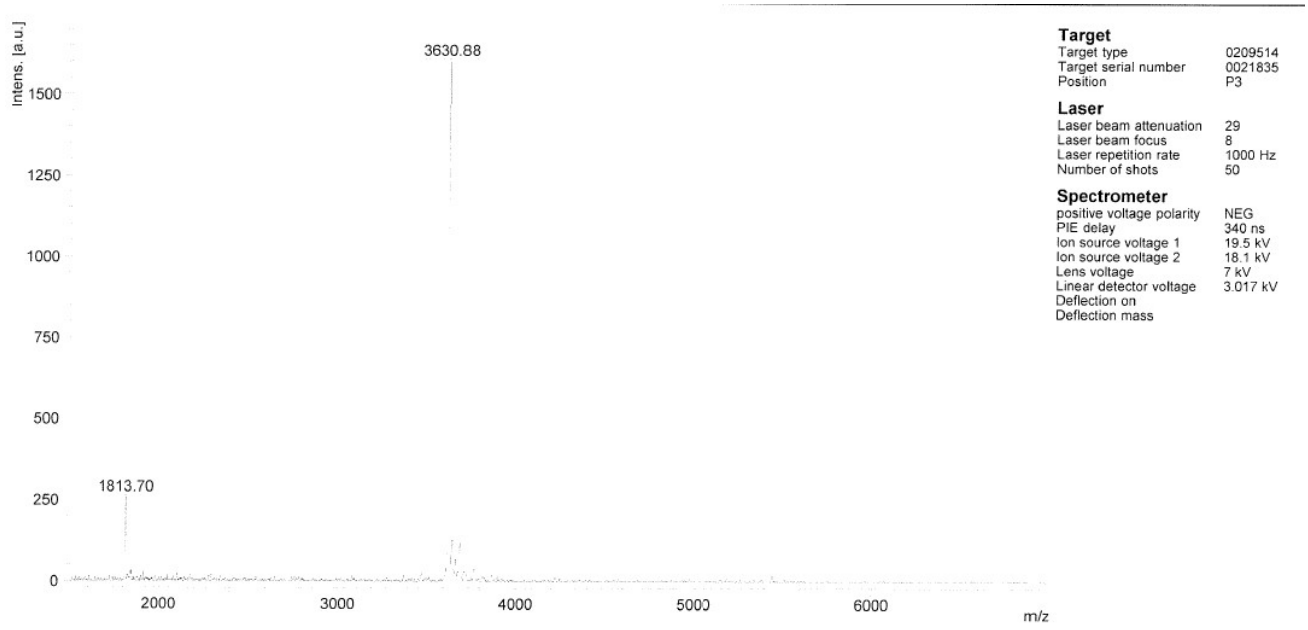


ON-6e

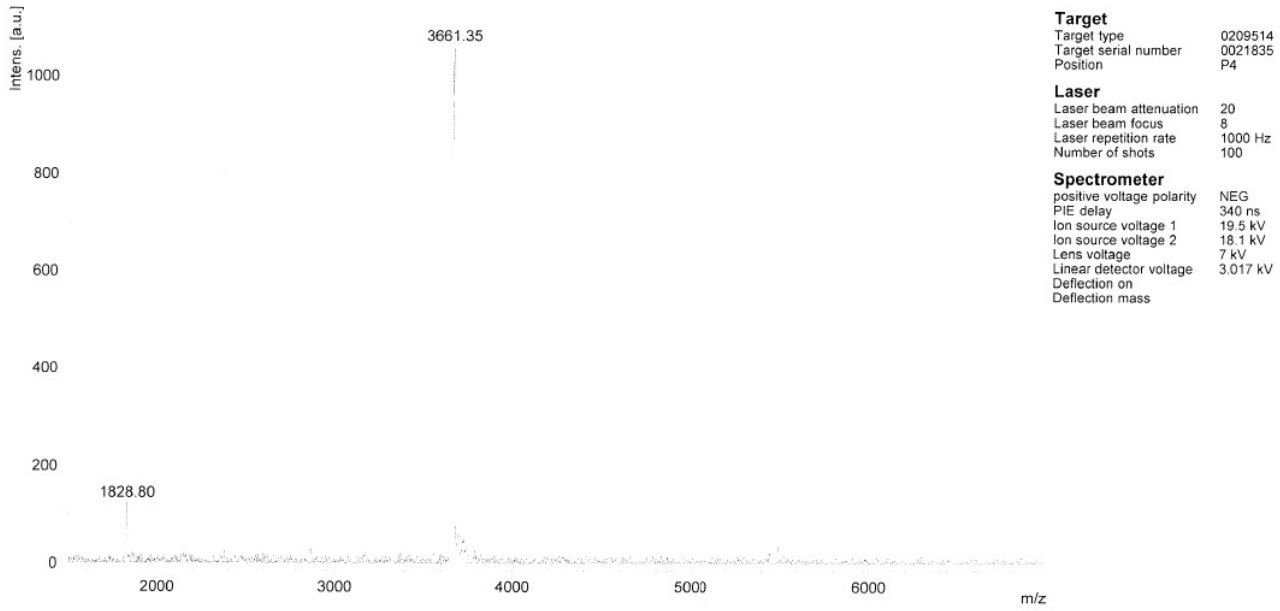
HPLC (gradient condition: B% 5-60 % in 22.5 min, then 100% in 5 min)



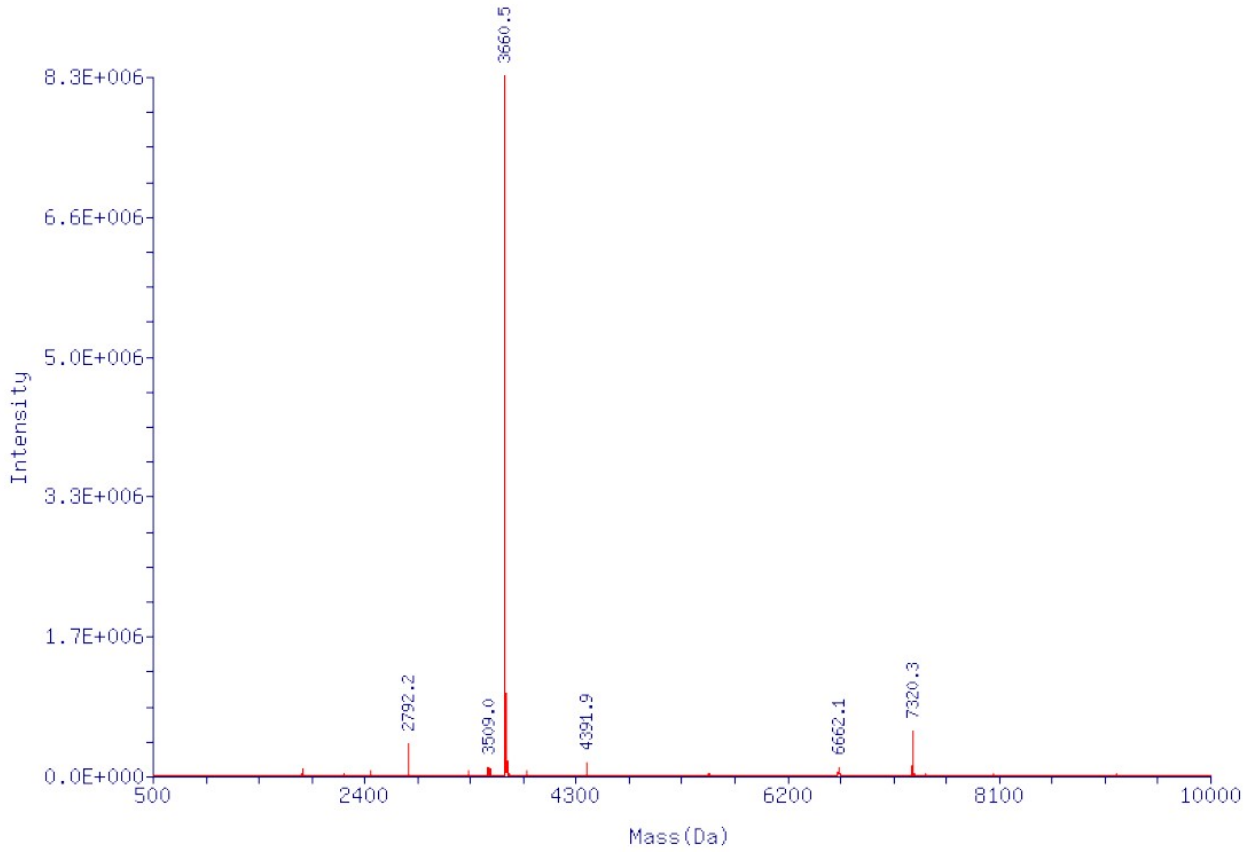
Mass chart of ON-1 (DNA)* (MALDI-TOF, Bulker) * purchased from Gene Design



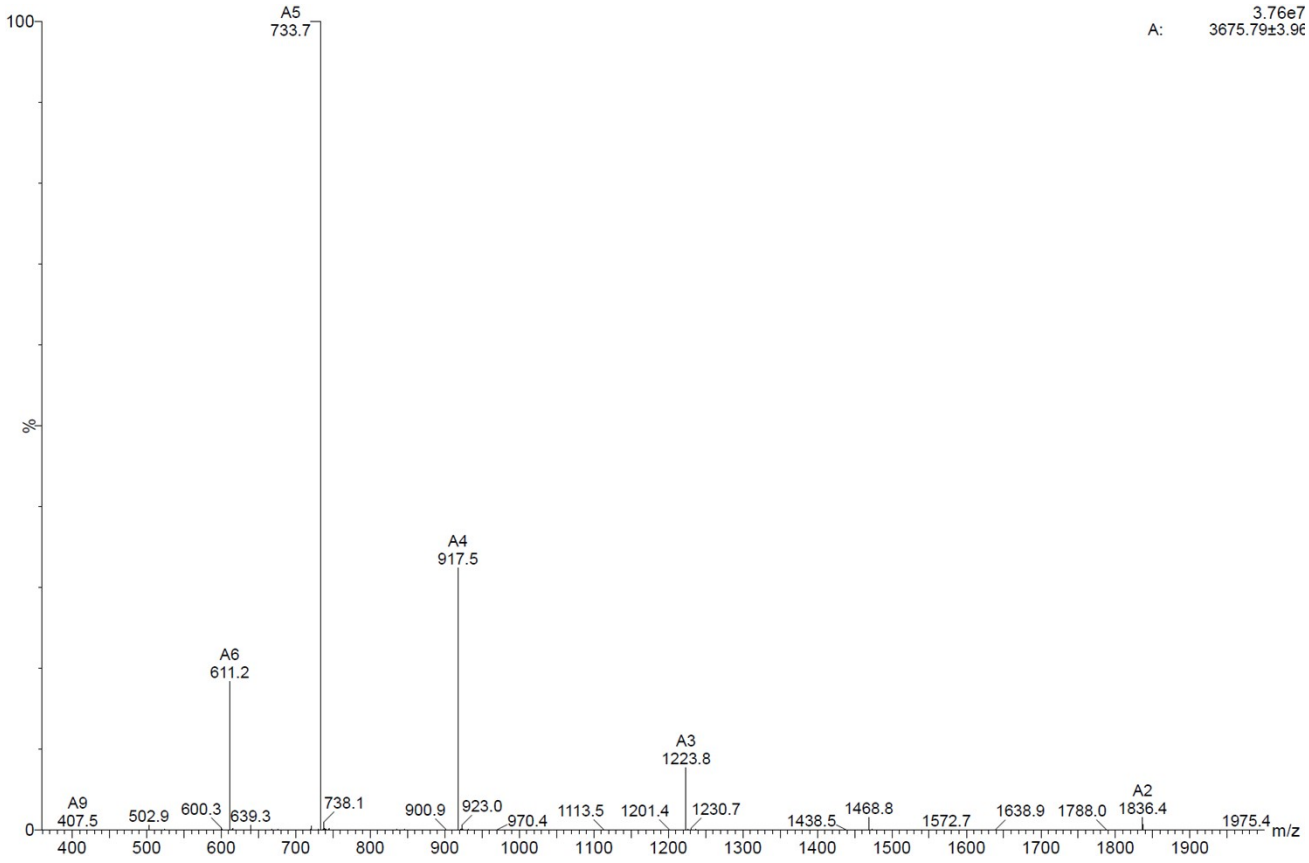
Mass chart of ON-1 (LNA)* (MALDI-TOF, Bulker) *purchased from Gene Design



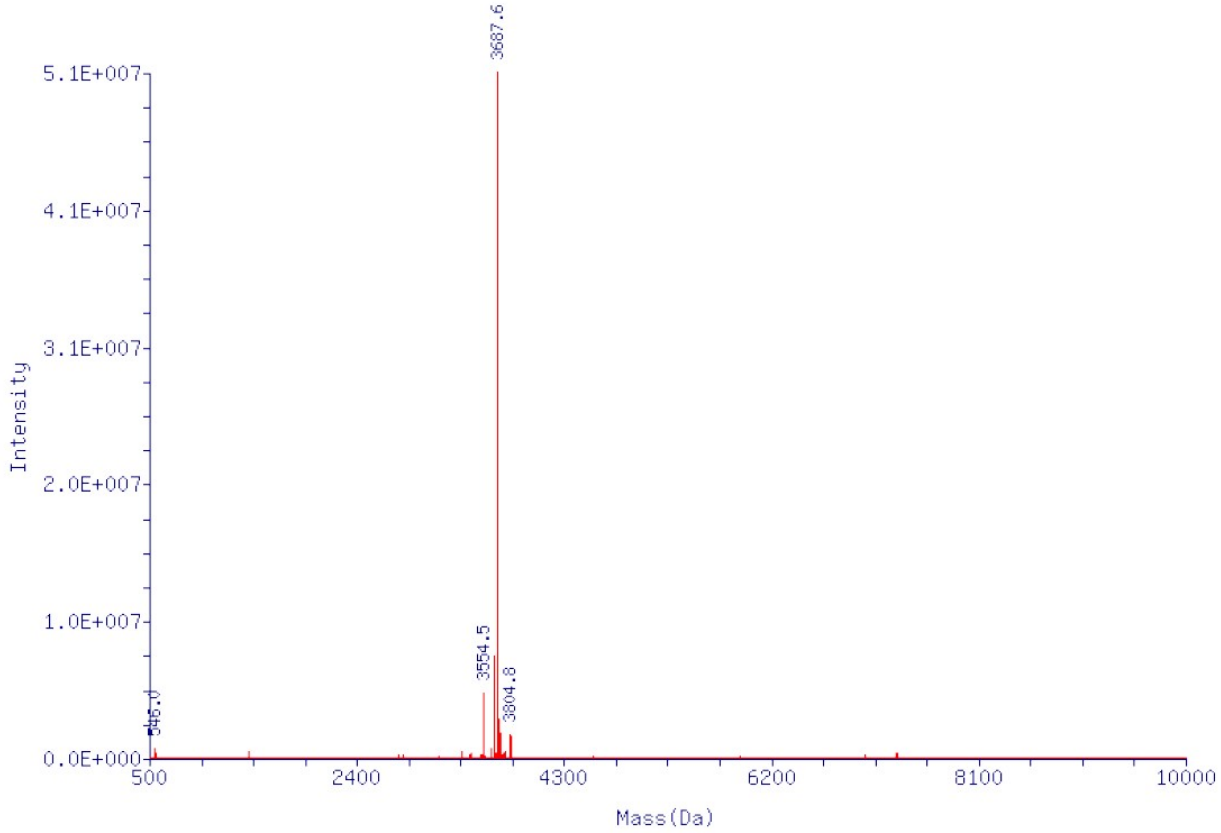
Mass chart of ON-1a (Q-Tof, Xevo G2-XS System, Waters)



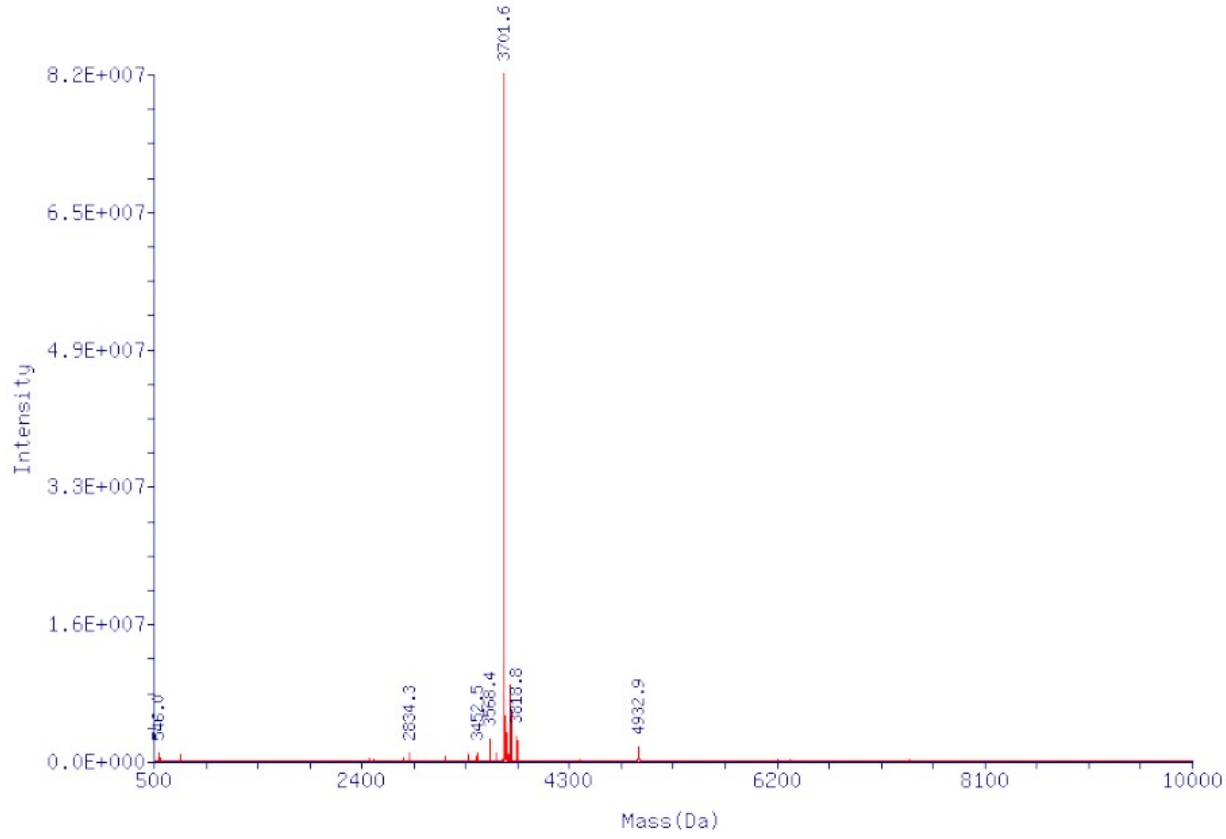
Mass chart of ON-1b (ESI, ZQ mass detector, Waters)



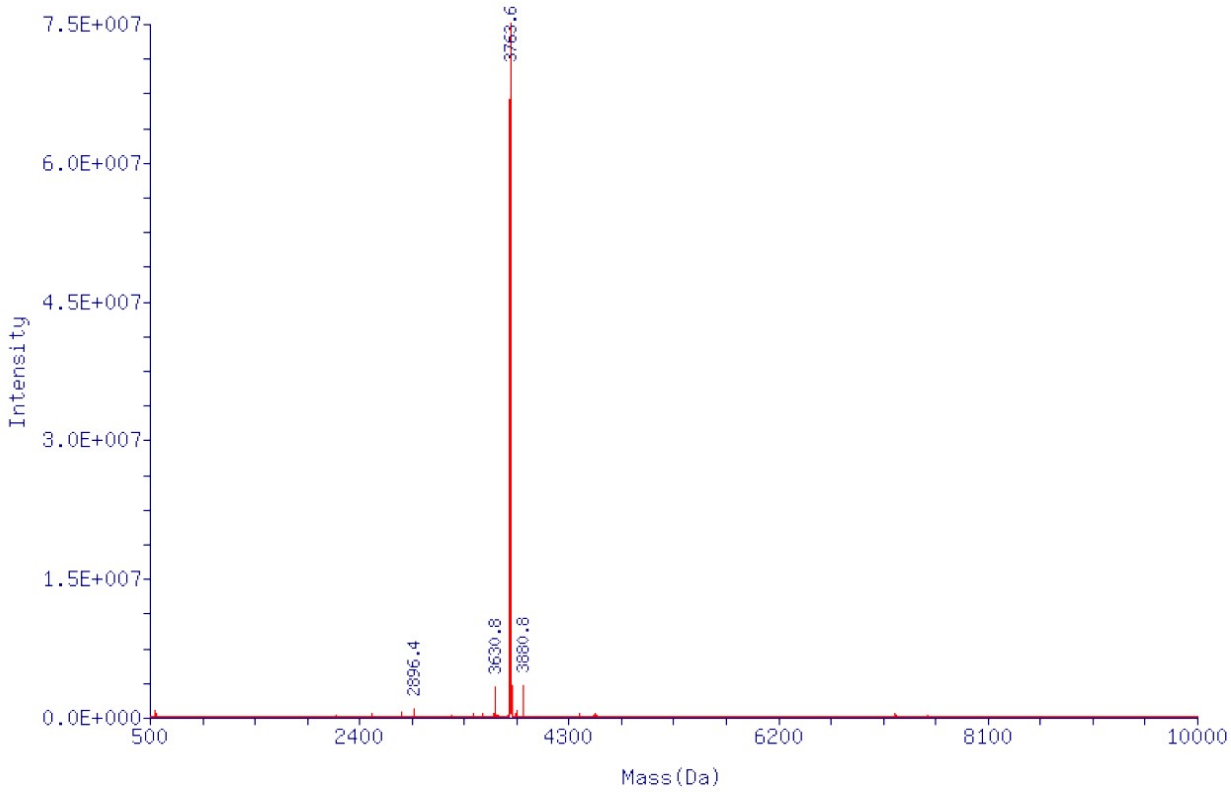
Mass chart of ON-1c (Q-Tof, Xevo G2-XS System, Waters)



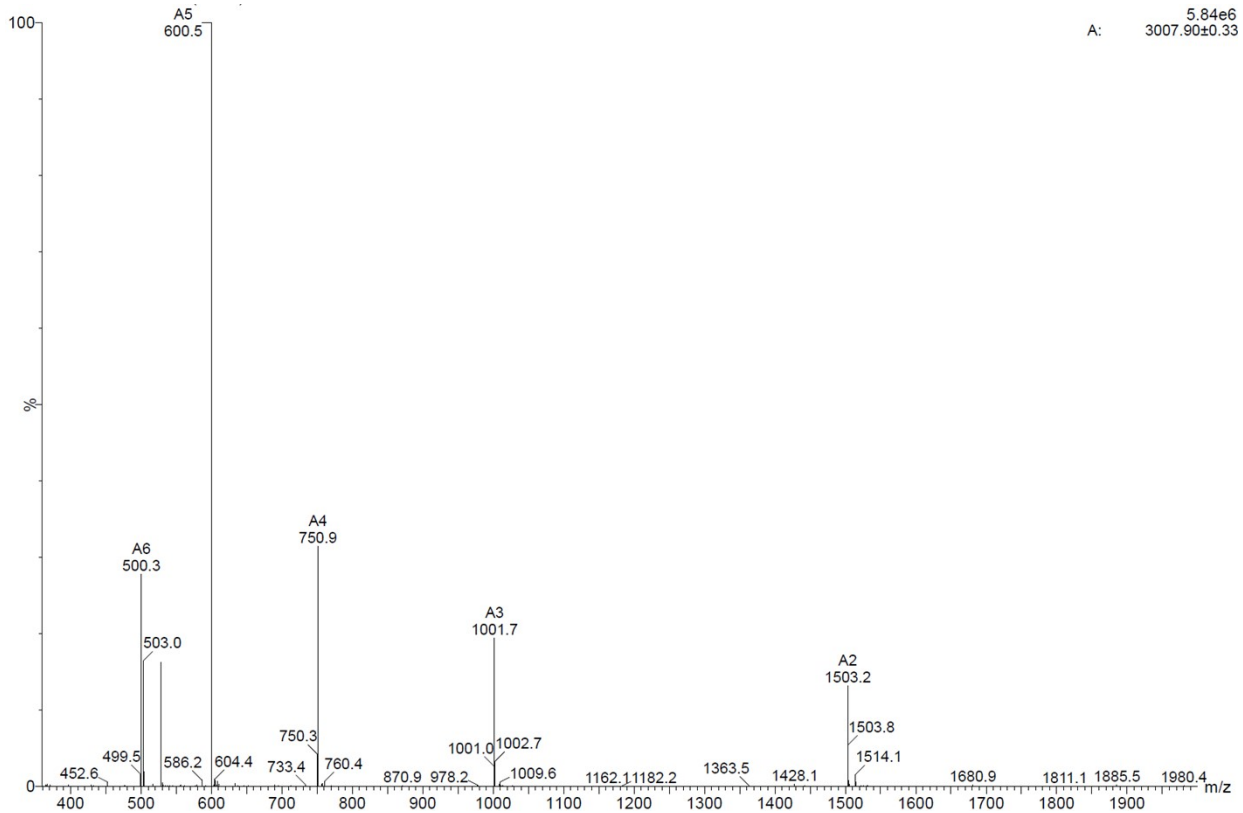
Mass chart of ON-1d (Q-Tof, Xevo G2-XS System, Waters)



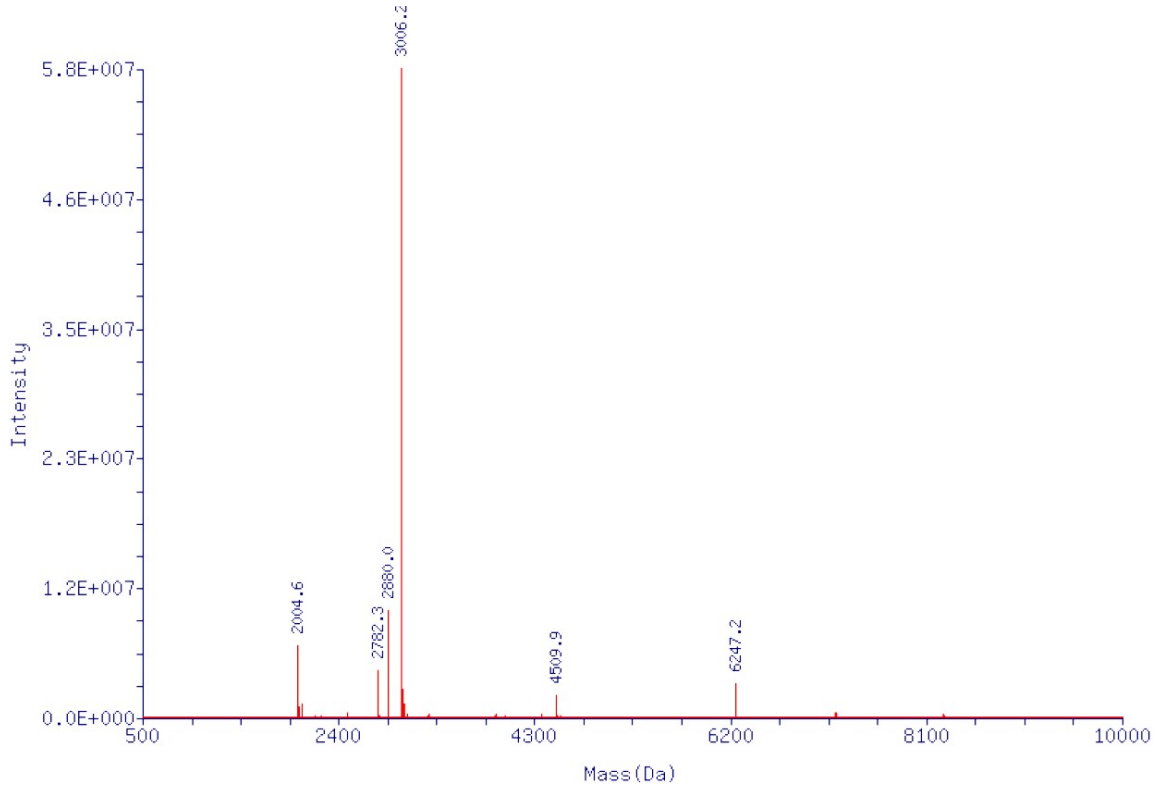
Mass chart of ON-1e (Q-Tof, Xevo G2-XS System, Waters)



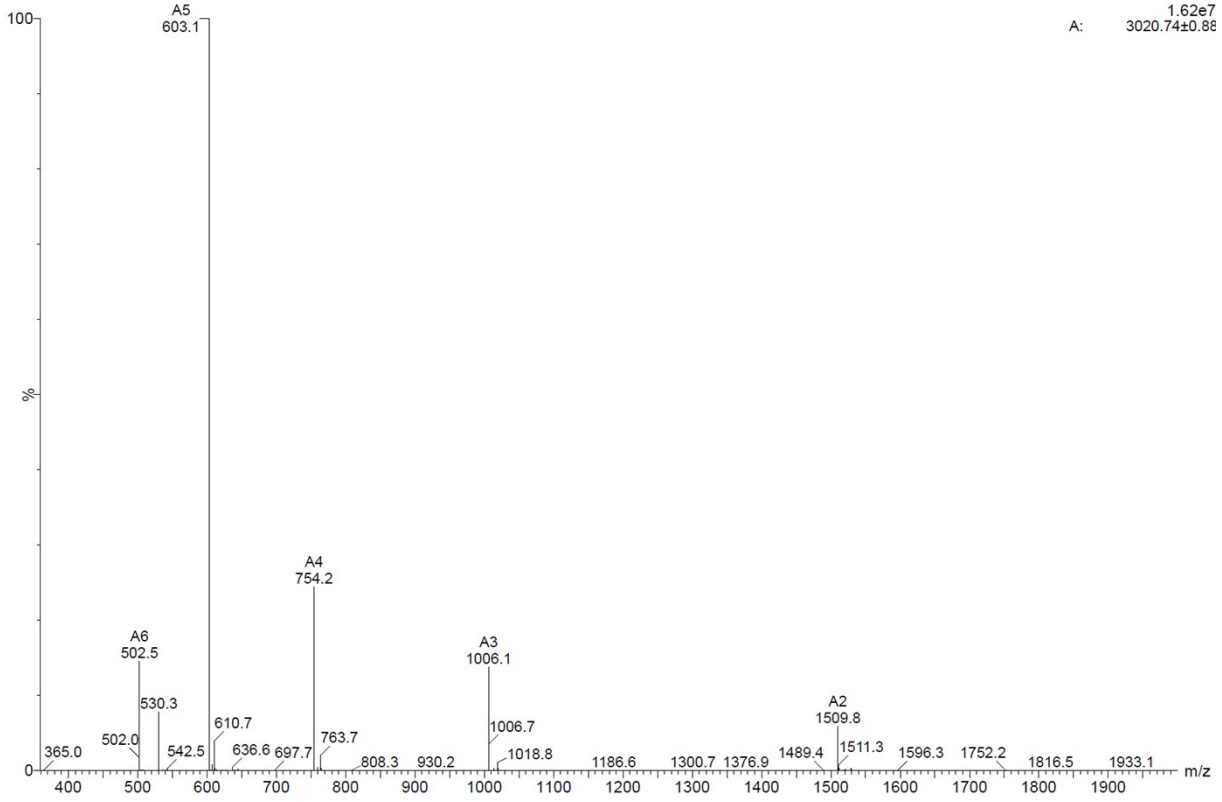
Mass chart of ON-2(LNA) (ESI, ZQ mass detector, Waters)



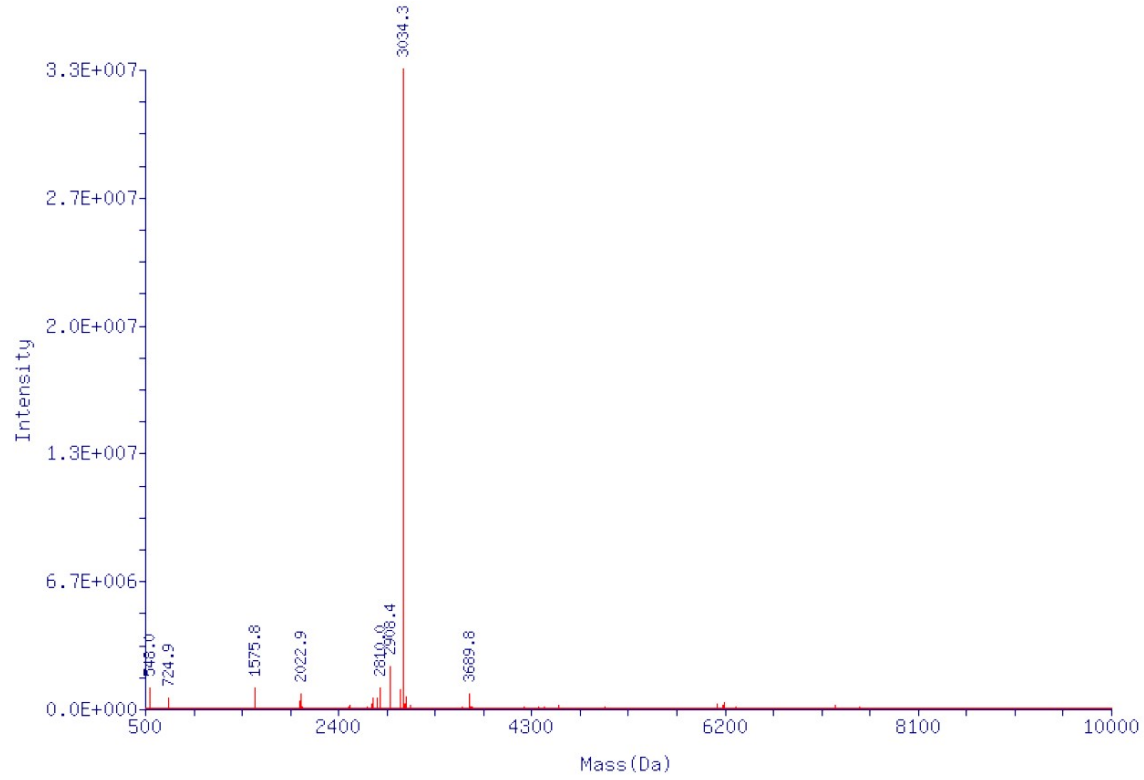
Mass chart of ON-2a (Q-Tof, Xevo G2-XS System, Waters)



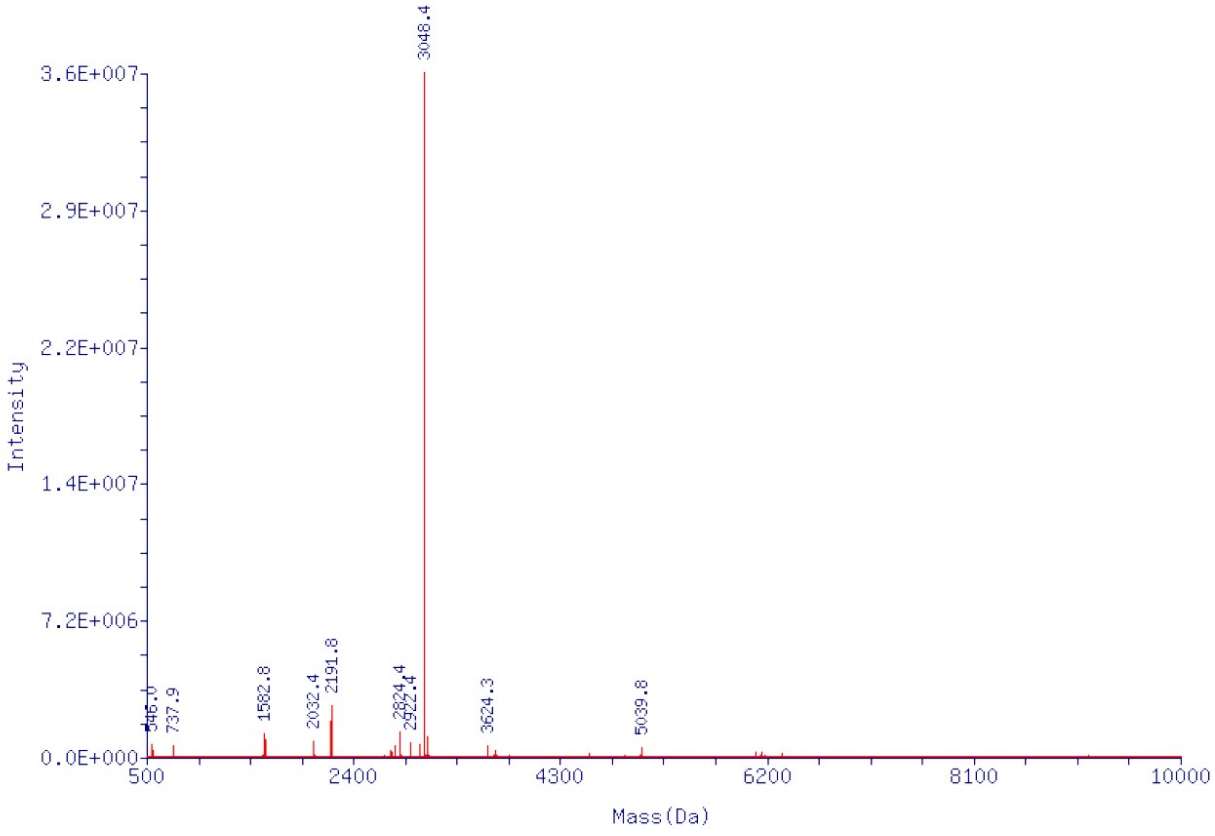
Mass chart of ON-2b (ESI, ZQ mass detector, Waters)



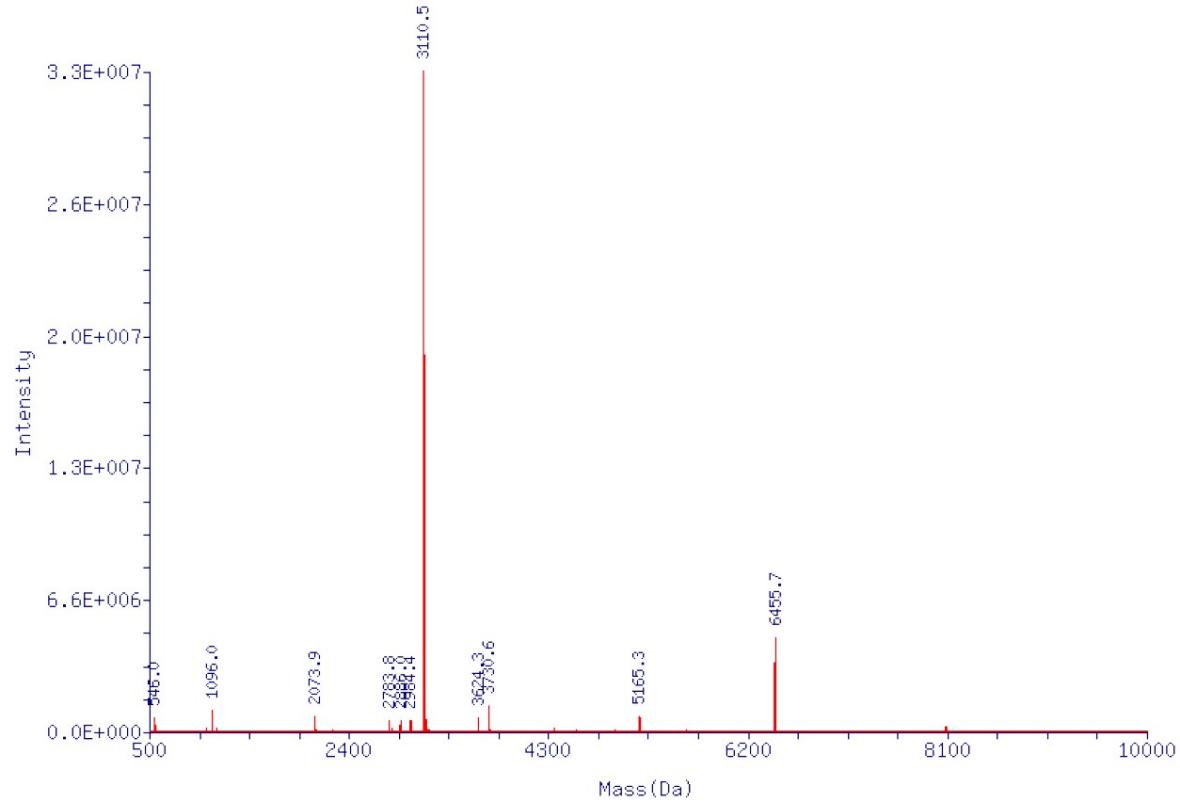
Mass chart of ON-2c (Q-Tof, Xevo G2-XS System, Waters)



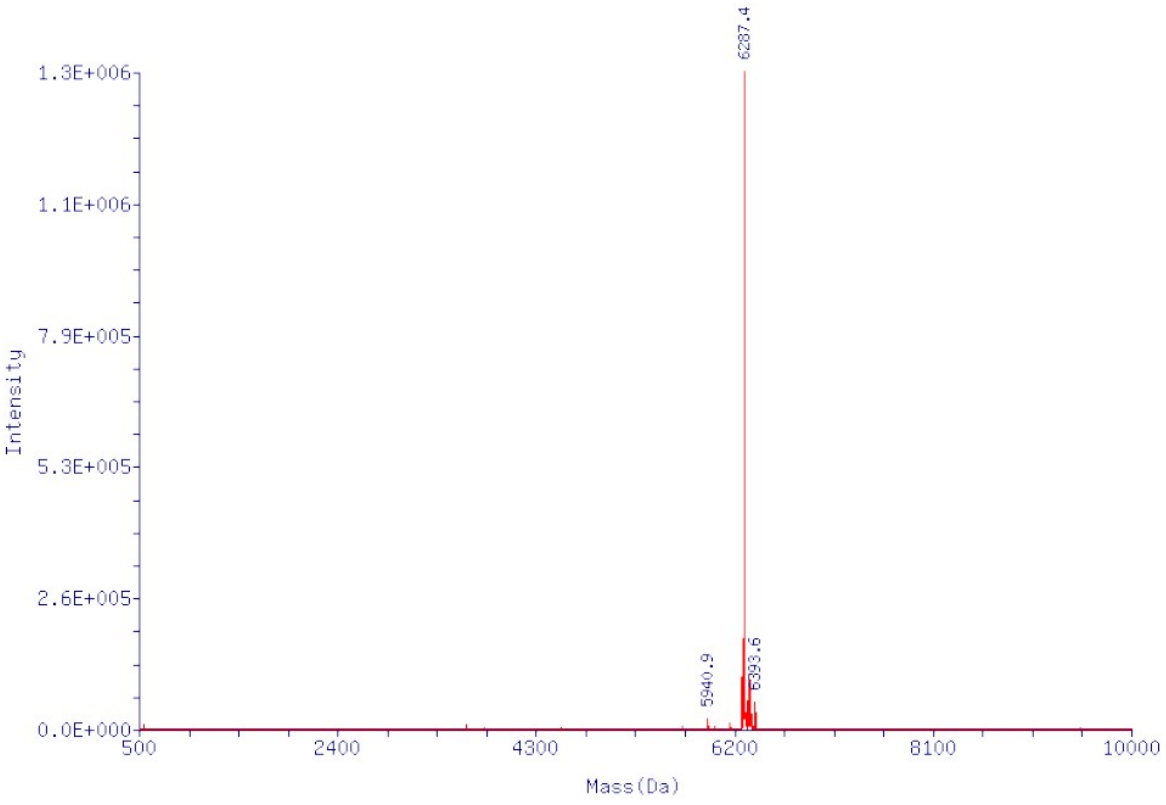
Mass chart of ON-2d (Q-Tof, Xevo G2-XS System, Waters)



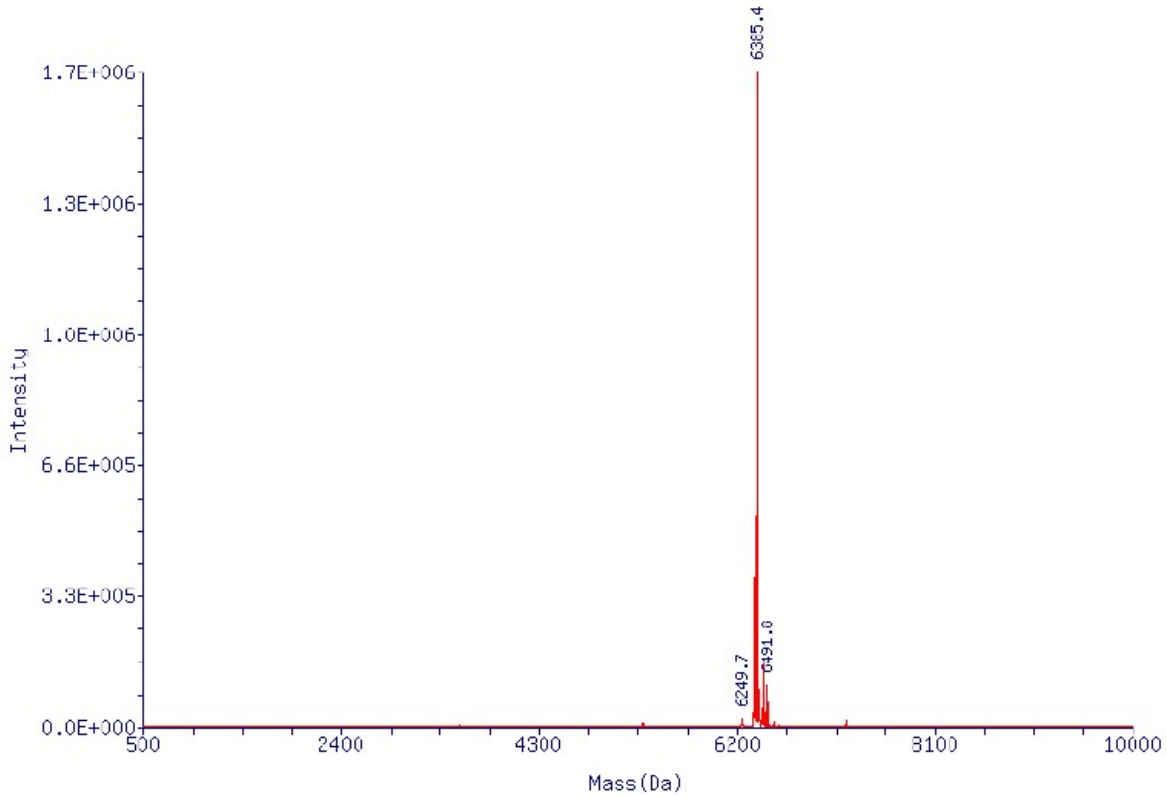
Mass chart of ON-2e (Q-Tof, Xevo G2-XS System, Waters)



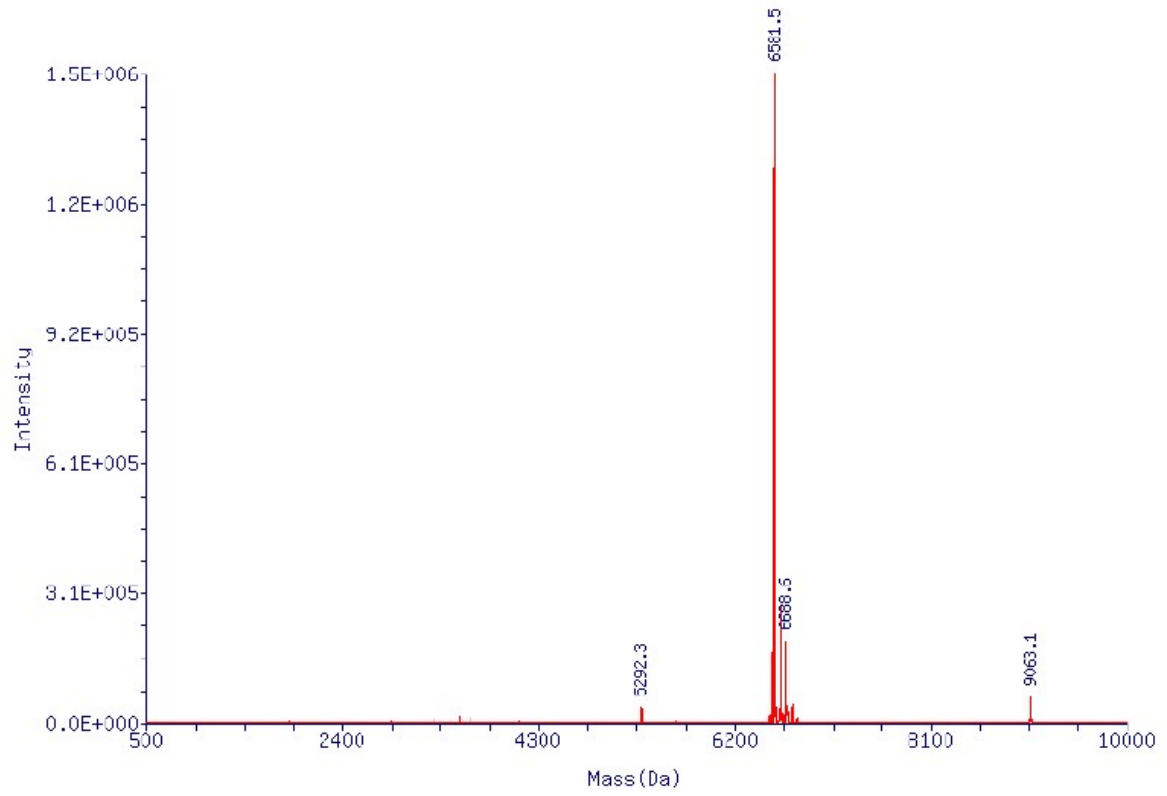
Mass chart of ON-3a (Q-Tof, Xevo G2-XS System, Waters)



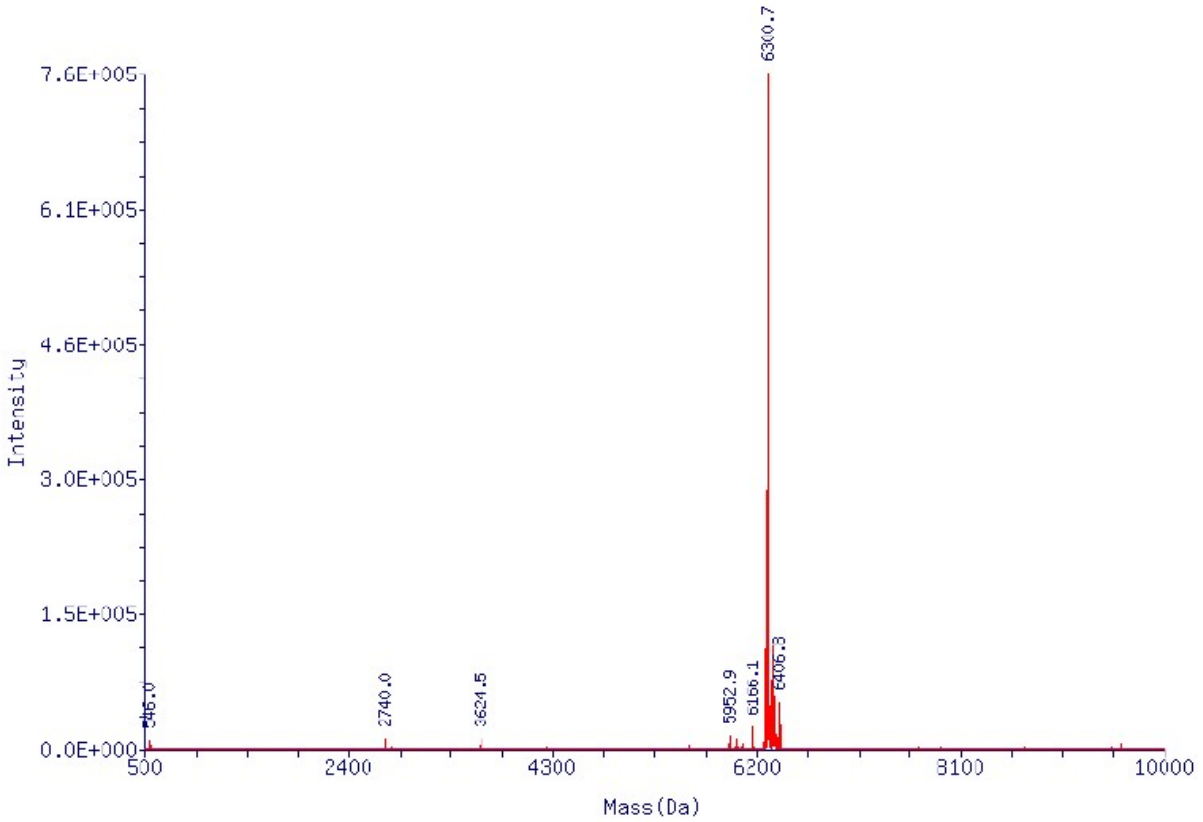
Mass chart of ON-3b (Q-Tof, Xevo G2-XS System, Waters)



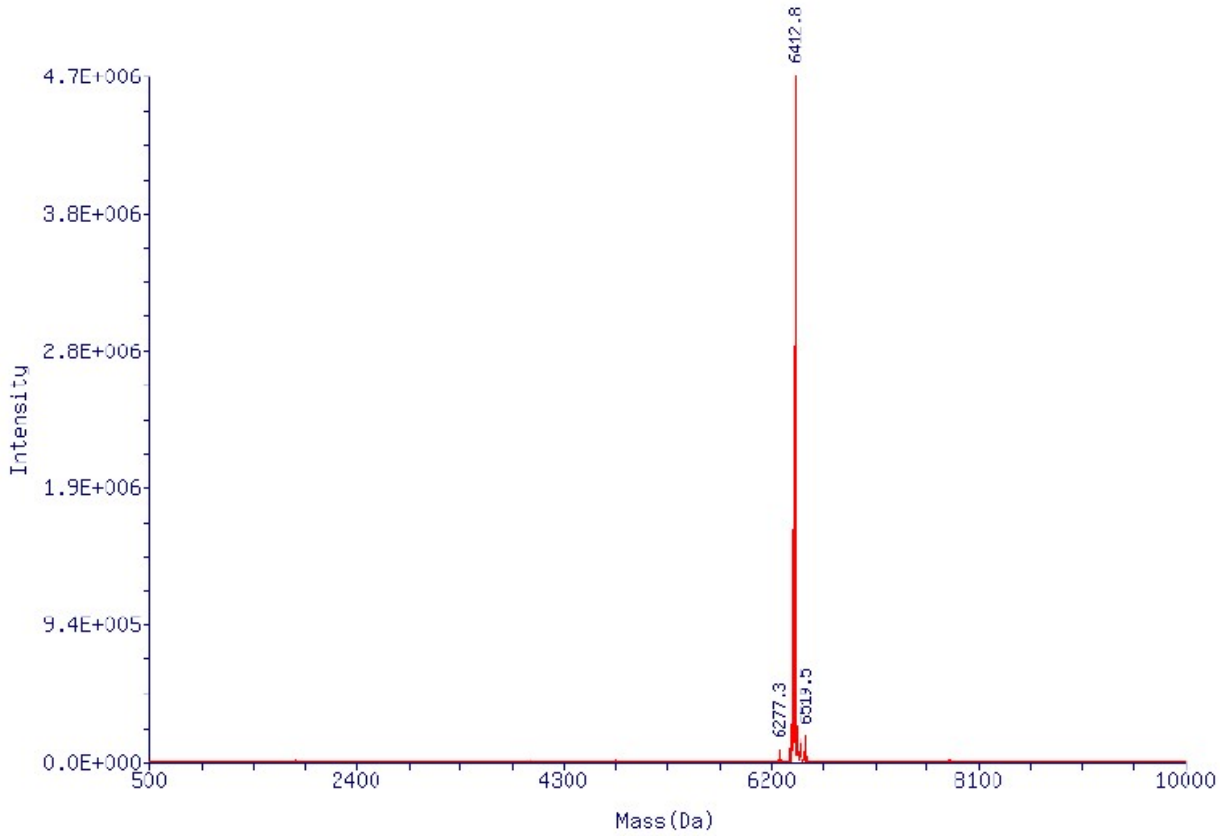
Mass chart of ON-3b (Q-Tof, Xevo G2-XS System, Waters)



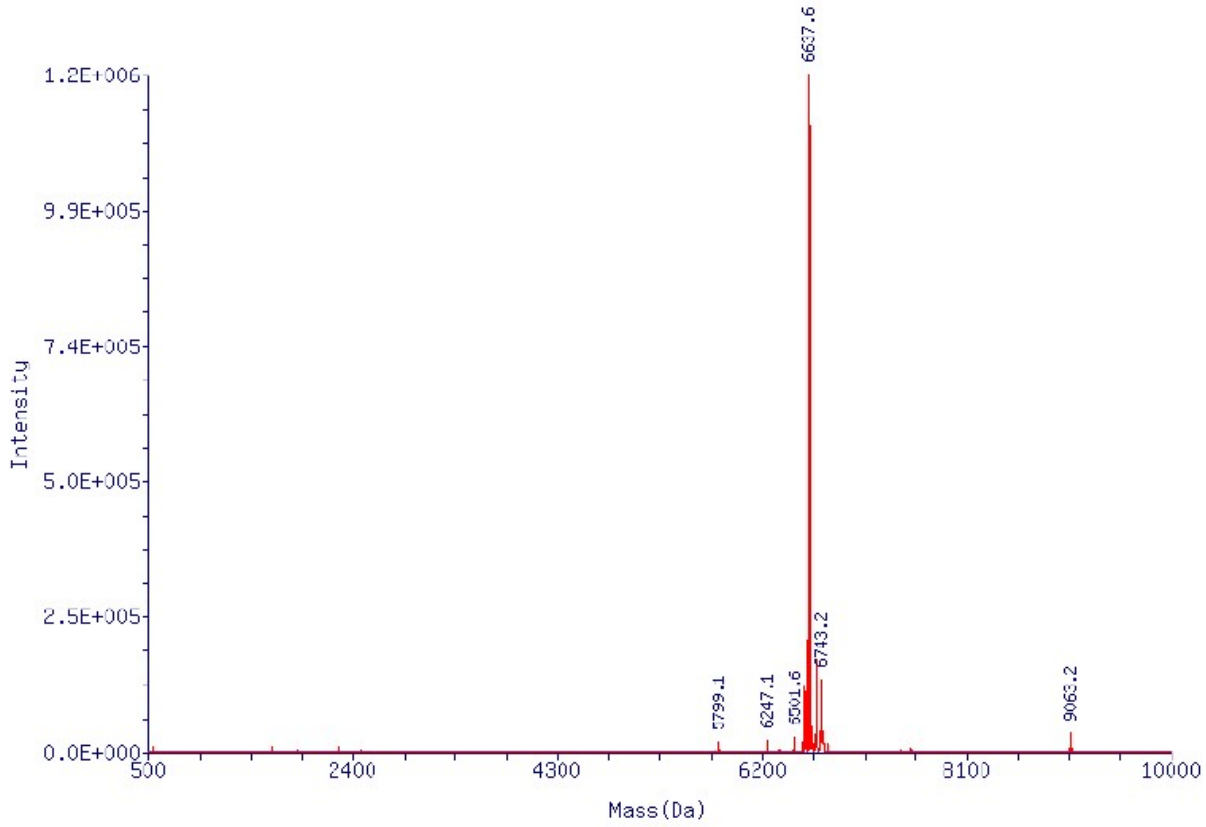
Mass chart of ON-4a (Q-Tof, Xevo G2-XS System, Waters)



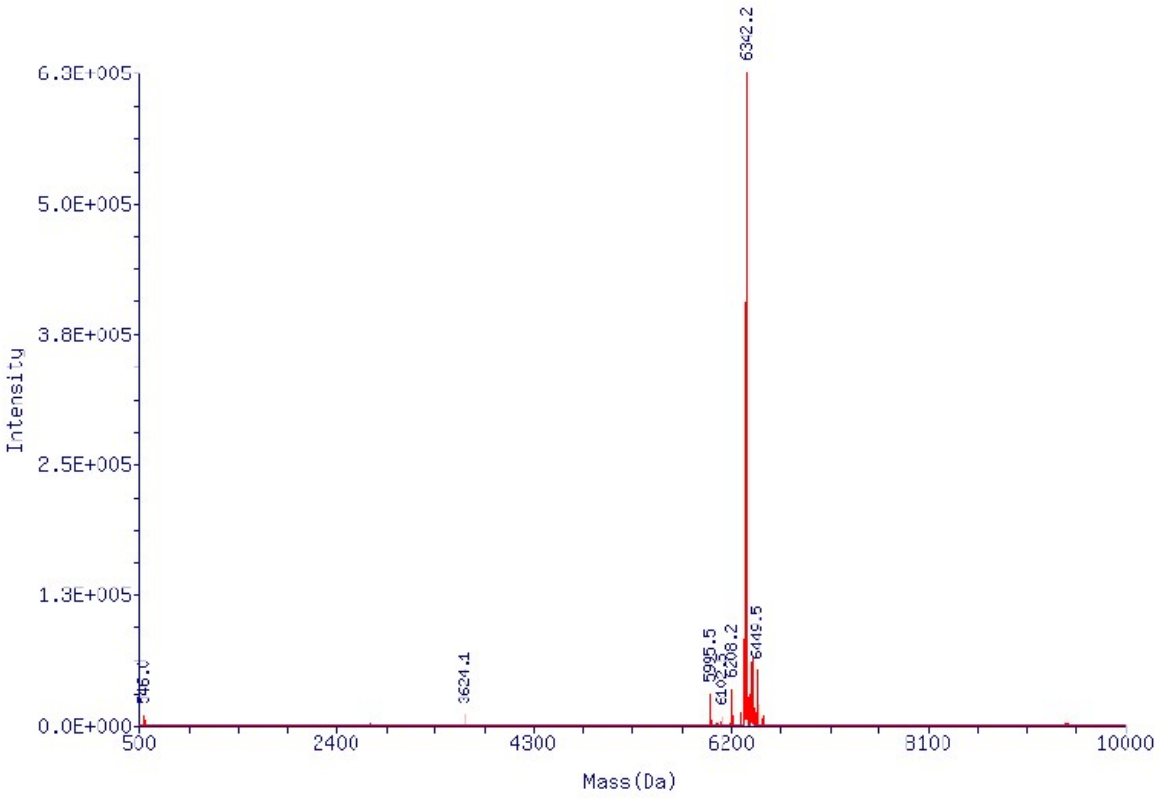
Mass chart of ON-4b (Q-Tof, Xevo G2-XS System, Waters)



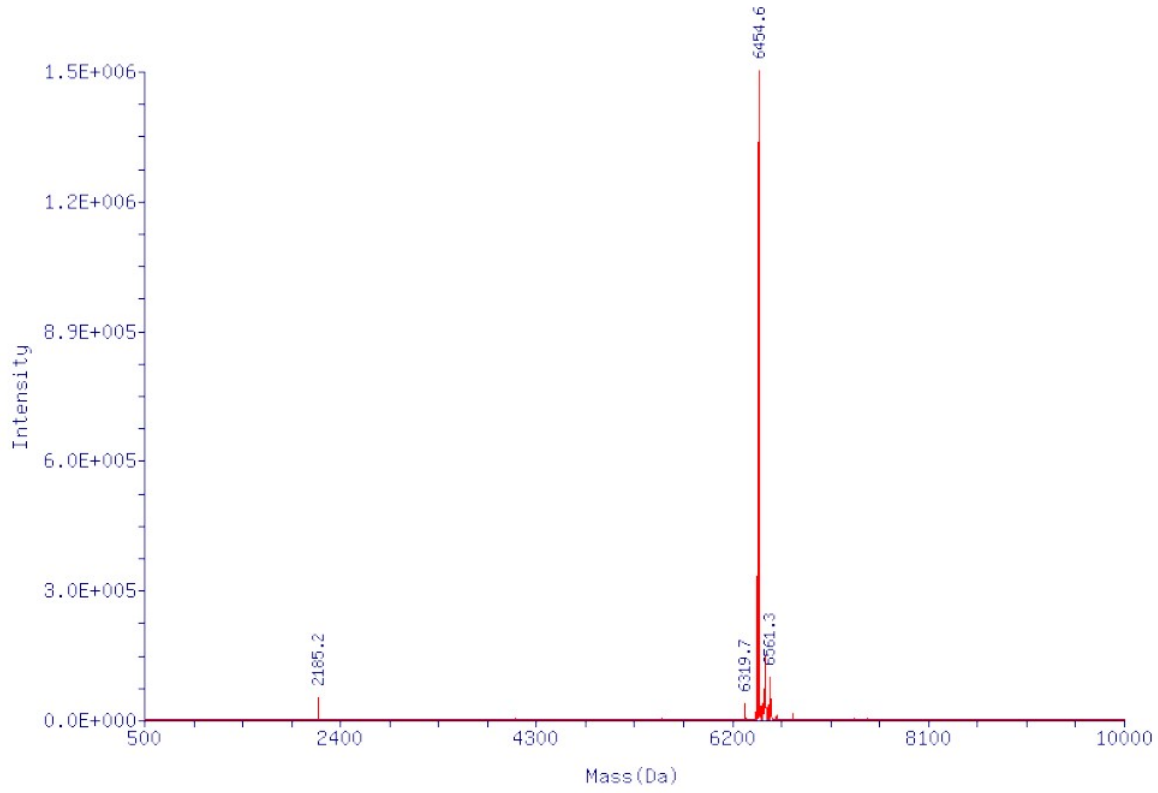
Mass chart of ON-4d (Q-Tof, Xevo G2-XS System, Waters)



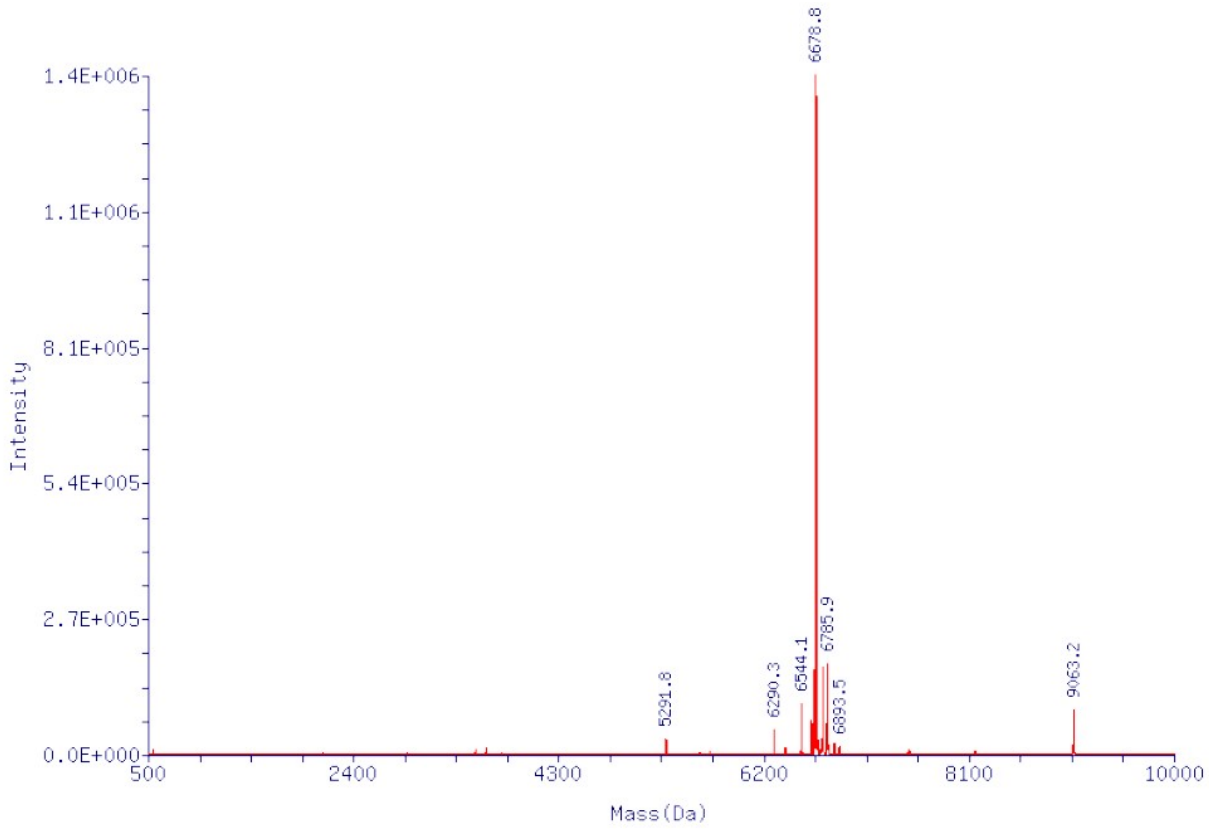
Mass chart of ON-5a (Q-Tof, Xevo G2-XS System, Waters)



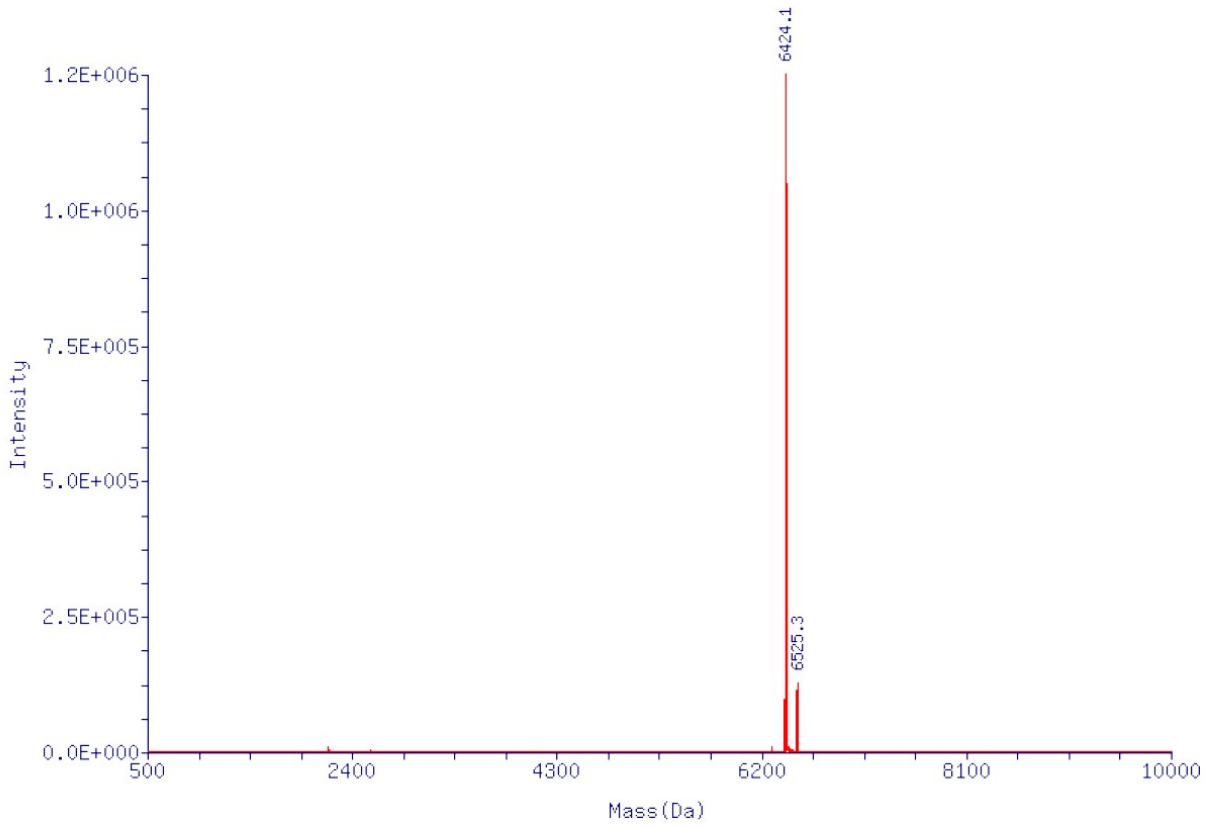
Mass chart of ON-5b (Q-Tof, Xevo G2-XS System, Waters)



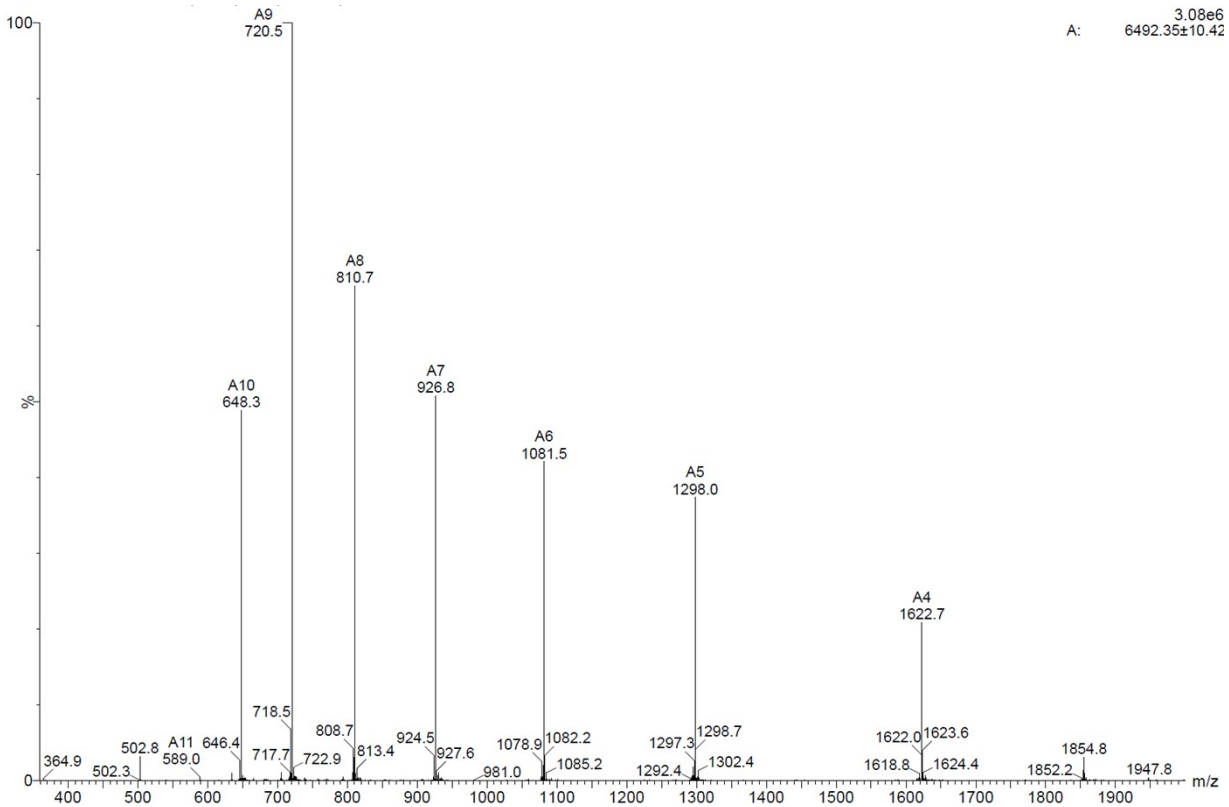
Mass chart of ON-5d (Q-Tof, Xevo G2-XS System, Waters)



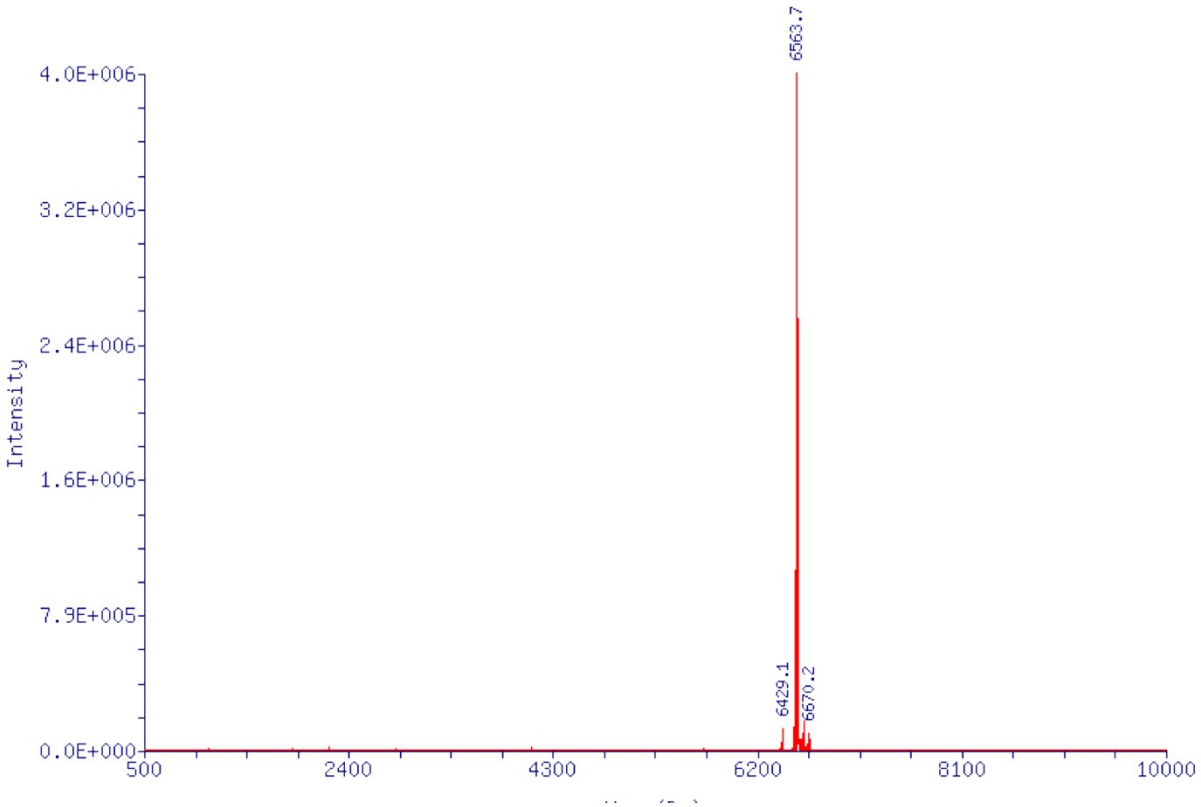
Mass chart of ON-6a (Q-Tof, Xevo G2-XS System, Waters)



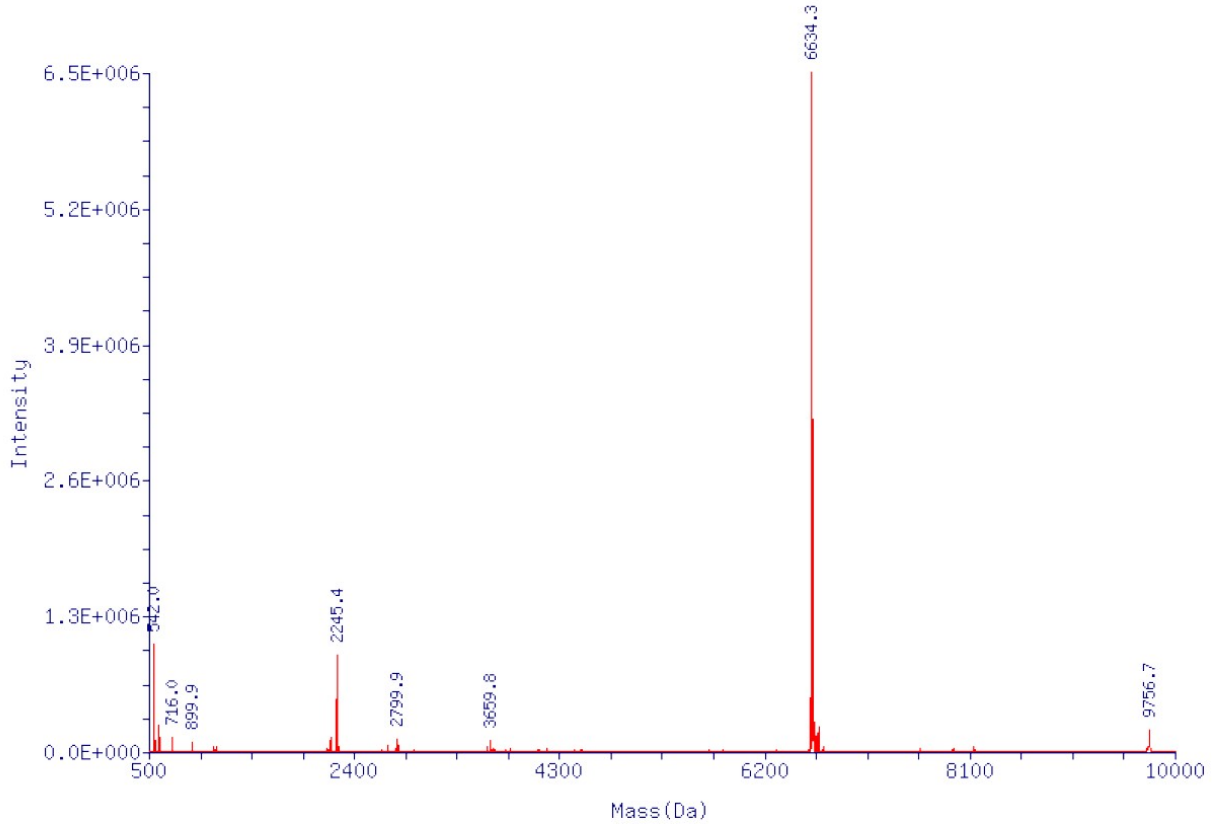
Mass chart of ON-6b (ESI, ZQ mass detector, Waters)



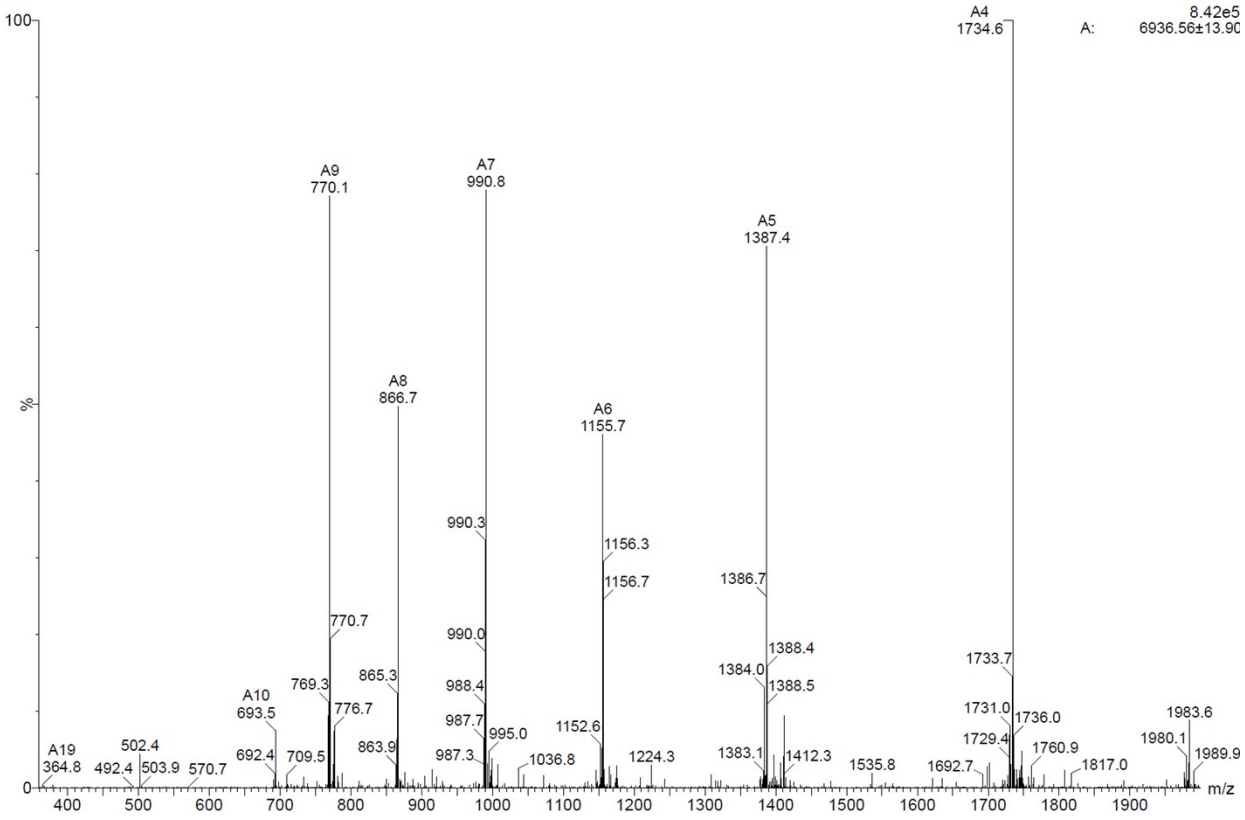
Mass chart of ON-6c (Q-Tof, Xevo G2-XS System, Waters)



Mass chart of ON-6d (Q-Tof, Xevo G2-XS System, Waters)



Mass chart of ON-6e (ESI, ZQ mass detector, Waters)



LCMS analysis condition

Waters ZQ mass detector (ESI)

Mobile phase A: 400 mM HFIP / 15mM TEA in water

Mobile phase B: Methanol

Flow rate: 0.2 mL/min

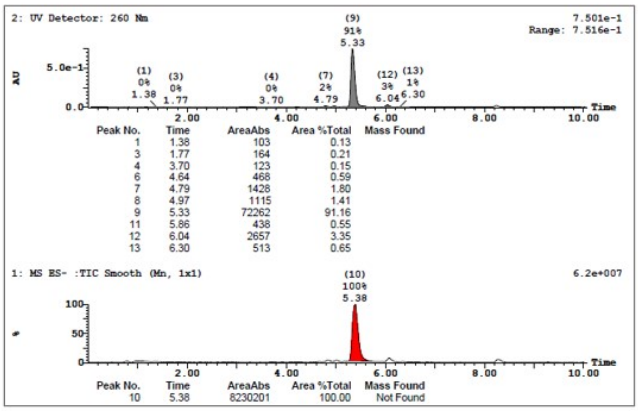
Gradient condition: 20-30 % in 2.5 min

Column: Waters XBridge™ BEH C18 1.7 µm (2.1 × 100 mm)

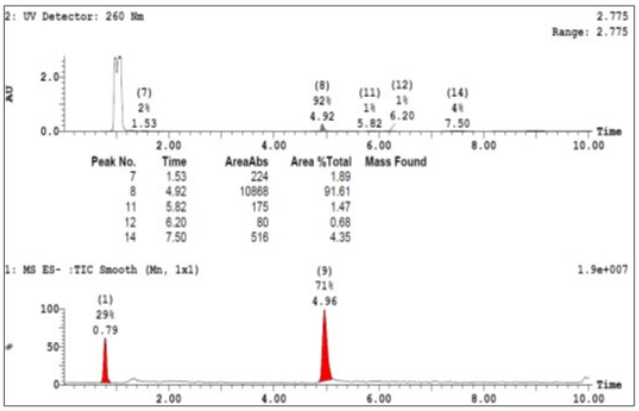
Column oven temperature: 60 °C

Detector: UV 260 nm

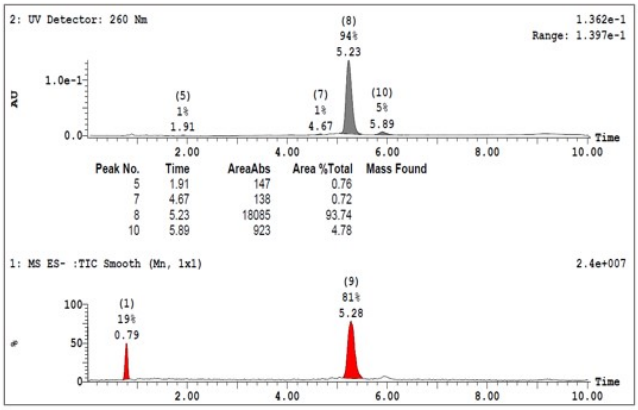
(a) T =ALNA[H], RT =5.33 min



(b) T =ALNA[formyl], RT = 4.92 min



(c) T =ALNA[Ac], RT = 5.23 min



(d) T =ALNA[Bz], RT = 5.91 min

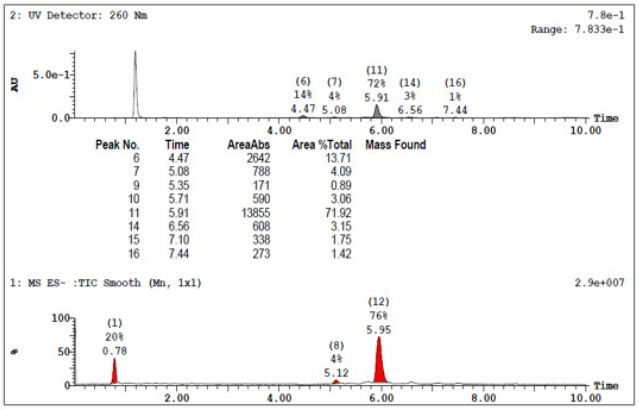


Fig. S1 Examples of profiles of LC-MS analysis. (a) LCMS analysis chart of **ON-1a** after solid-phase synthesis and HPLC purification (Sequence: 5 '- GTGTTTTTTGCT -3', Capital letters = DNA, underline T = ALNA[H]). (b) LCMS chart of reaction mixture of formylation of **ON-1a** after 2 hours. (c) LCMS chart of reaction mixture of acetylation of **ON-1a** after 2 hours. (d) LCMS chart of reaction mixture of benzoylation of **ON-1a** after 2 hours. In all charts, the retention time (RT) around 0.78 min is the effect of injection or the peak of the reaction reagent. In reactions of **b**-**d**, the starting material **ON-1a** disappeared and converged to the target compounds. All the peaks were detected at 260 nm and ESI-MS (negative mode). We measured similar data on the PEM reaction of other oligonucleotides to confirm the progress of the reaction.

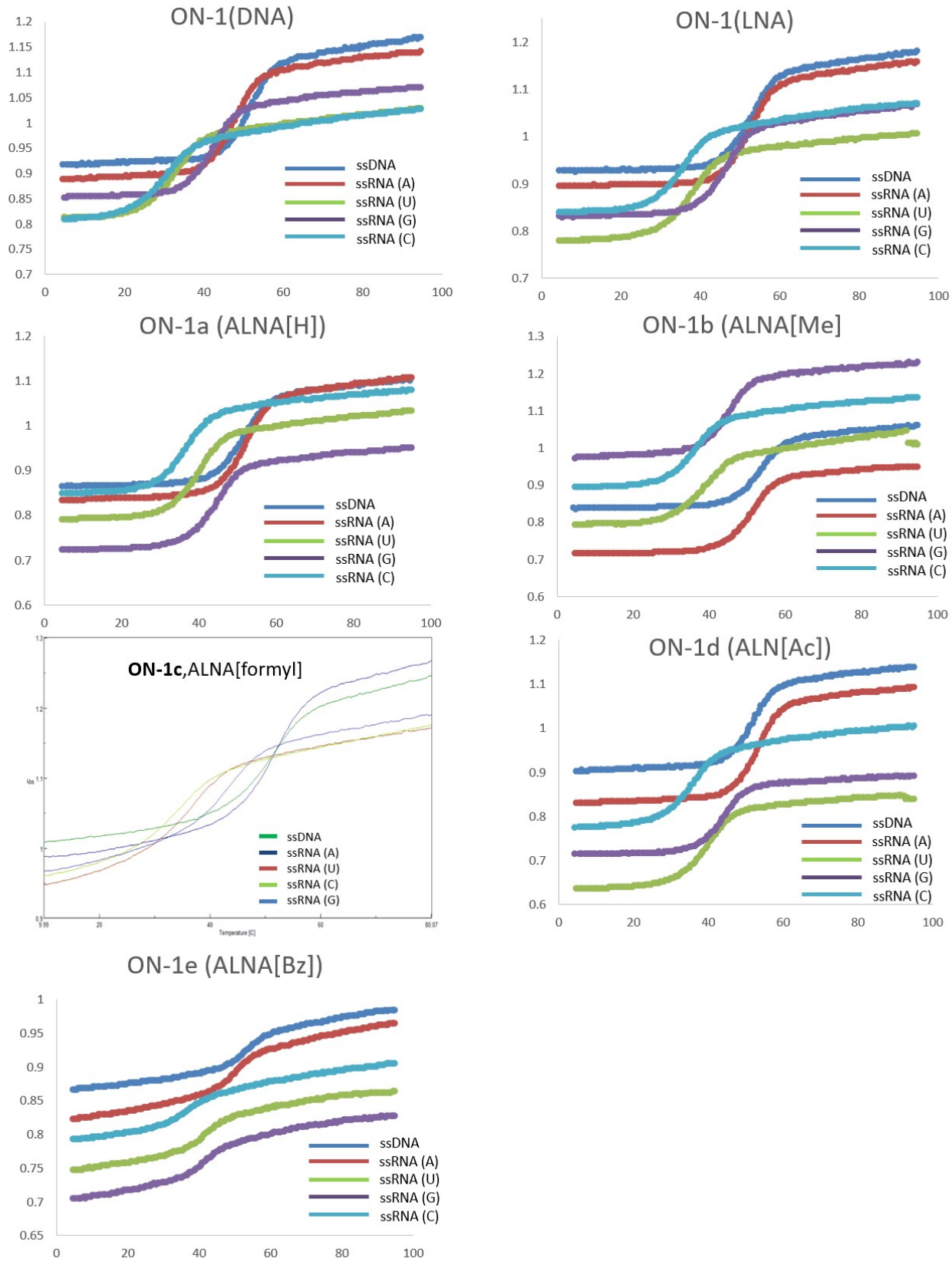


Fig. S2 Representative UV melting curves of **ON-1.**

Table. S2 T_m values were calculated as the average of three independent experiments for each sequence.

Seq No.	Ct (umol/L)	T_m [℃] toward ssRNA 5'-r(*UAGCUUAUCAGACUGAUGUUGA*)-3'	SD	SEM
ON-3d	2	65.8	0.1	0.0
ON-4d	2	69.9	0.2	0.1
ON-5d	2	73.0	0.3	0.2

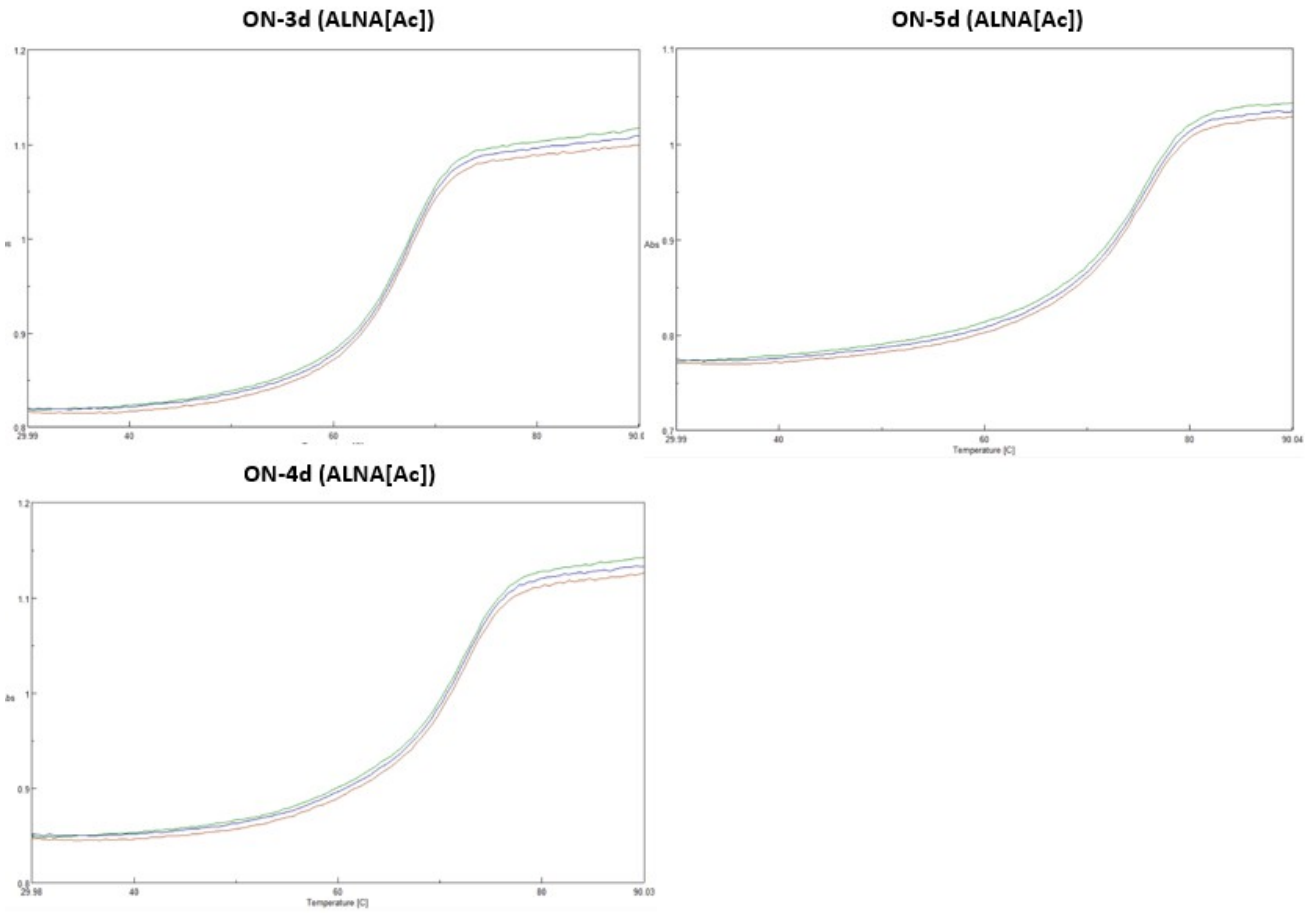


Fig. S3 Representative UV melting curves of **ON-3d**, **ON-4d** and **ON-5d**