iScience, Volume *23*

Supplemental Information

Solution-Phase DNA-Compatible Pictet-Spengler Reaction Aided by Machine Learning Building Block Filtering

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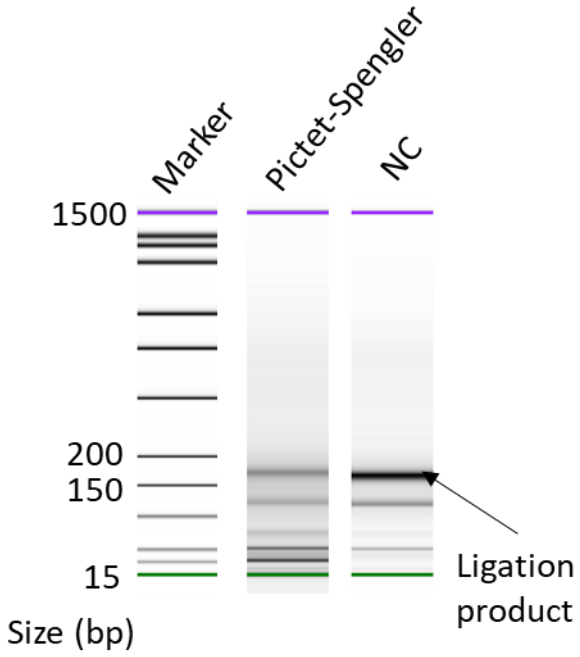
Supporting Information

Content

Figures

Figure S1 Capillary gel electrophoresis result of ligations, related to Figure 6.



Size(bp)	Pictet-Spengler		NC	
	Conc. [ng/µl]	Molarity [nmol/l]	Conc. [ng/µl]	Molarity [nmol/l]
15	4.2	424.2	4.2	424.2
171	**11.49**	**101**	**60.5**	**535.5**
1500	2.1	2.1	2.1	2.1

Fig. S1 Capillary gel electrophoresis result of ligations

Figure S2 qPCR data summary of the concentration check group, related to Figure 6.

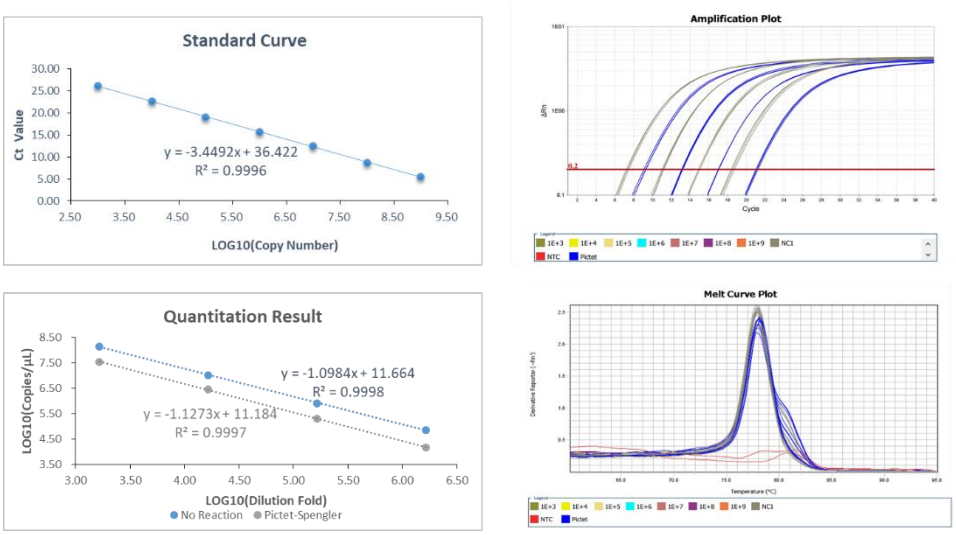
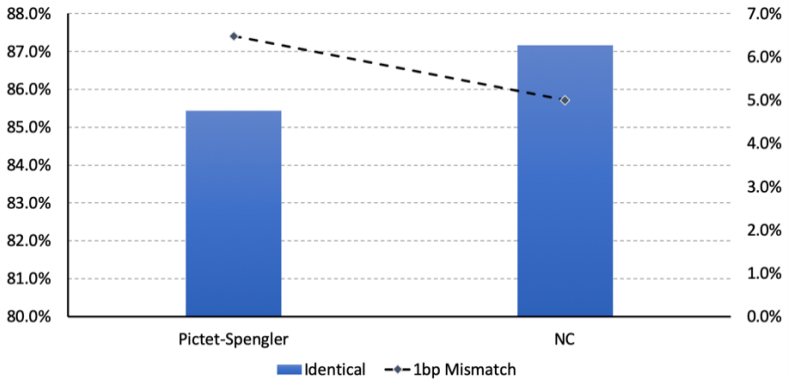


Fig. S2 qPCR data summary of the concentration check group

Figure S3 Statistics of next-generation sequencing results. The left Y-axis is the fraction of identical reads from perfect match, while the right Y-axis is the fraction of 1bp mismatch, related to Figure 6.



Condition	sequenced reads	perfect match	1bp mismatch
Pictet-Spengler	141,183,054	120,623,714	9,151,620
NC	121,974,103	106,321,624	6,097,462

Fig. S3 Statistics of next-generation sequencing results. The left Y-axis is the fraction of identical reads from perfect match, while the right Y-axis is the fraction of 1bp mismatch.

Figure S4, Trace and Mass of 3a, related to Figure 3.

Following **General Procedure 1**
Purity: >99.00%
Exact mass: 5414.97
Triply charged mass [M-3]/3, calculated: 1803.99; observed:1804.0

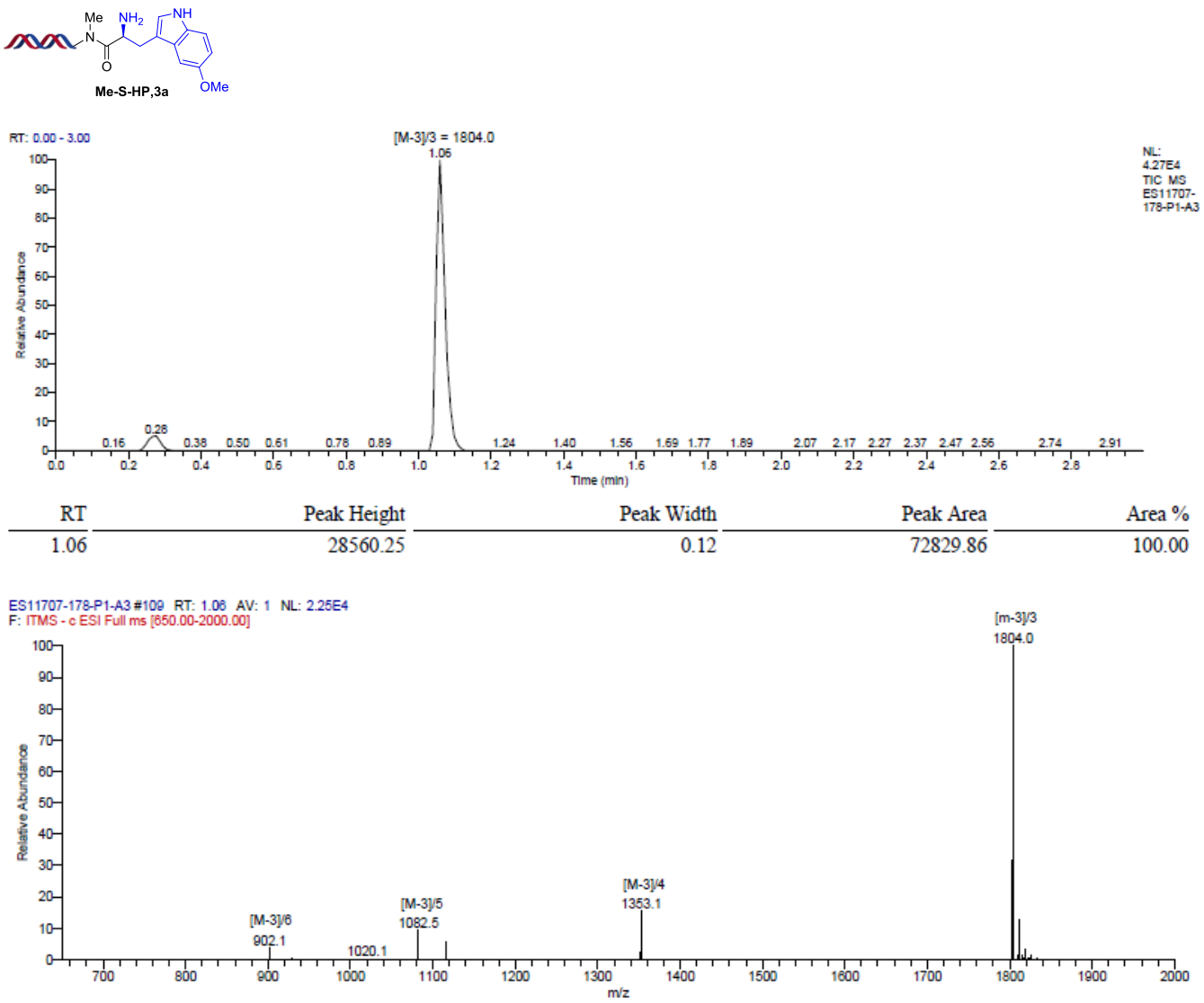
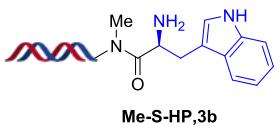
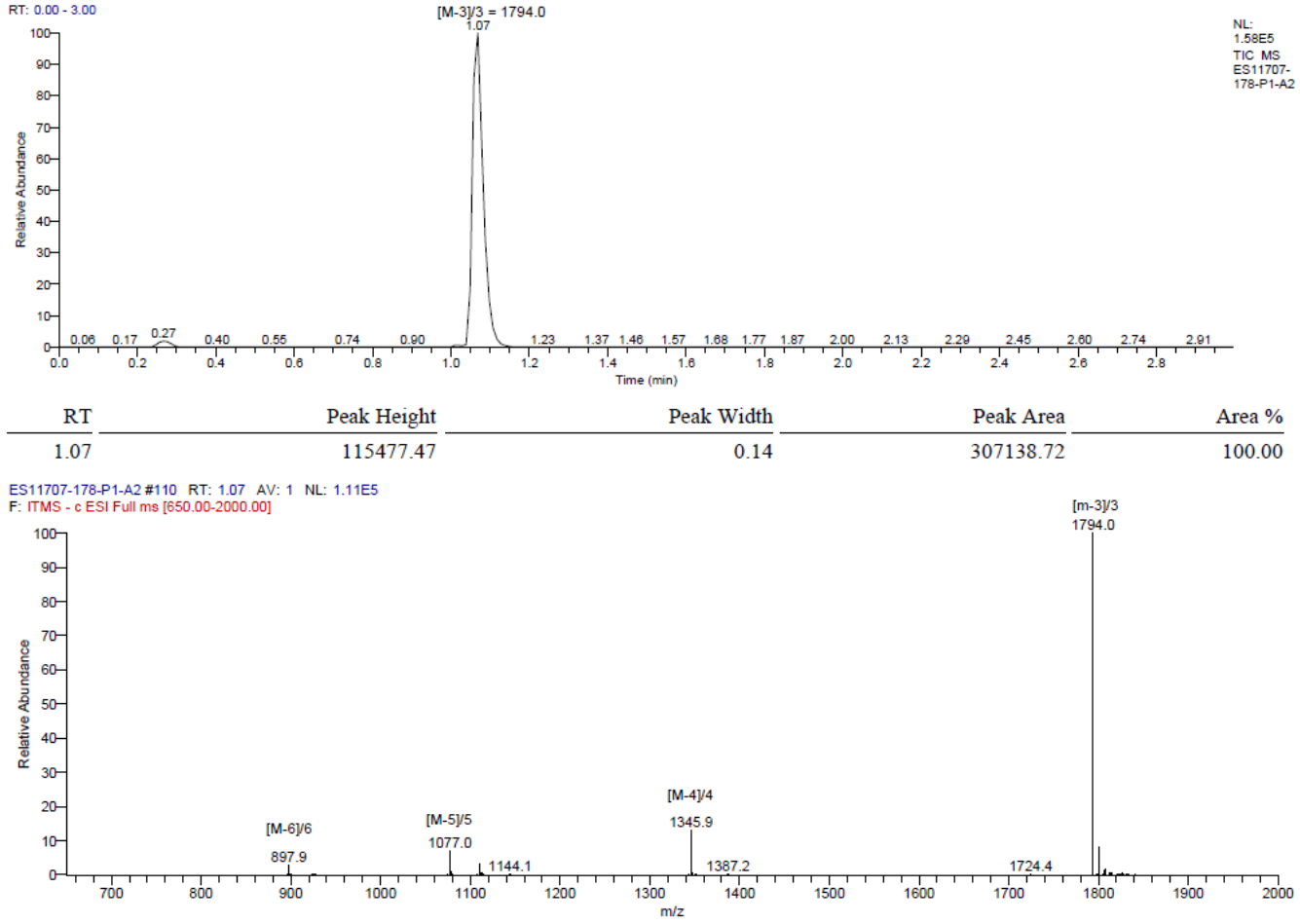


Fig. S4. LC trace and mass of **3a**.

Figure S5, Trace and Mass of 3b, related to Figure 3.

Following **General Procedure 1**
Purity: >99.00%
Exact mass: 5384.95
Triply charged mass [M-3]/3, calculated: 1793.98; observed:1794.0





RT	Peak Height	Peak Width	Peak Area	Area %
1.07	115477.47	0.14	307138.72	100.00

Fig. S5. LC trace and mass of **3b**.

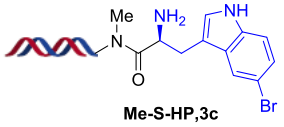
Figure S6, Trace and Mass of 3c, related to Figure 3.

Following **General Procedure 1**

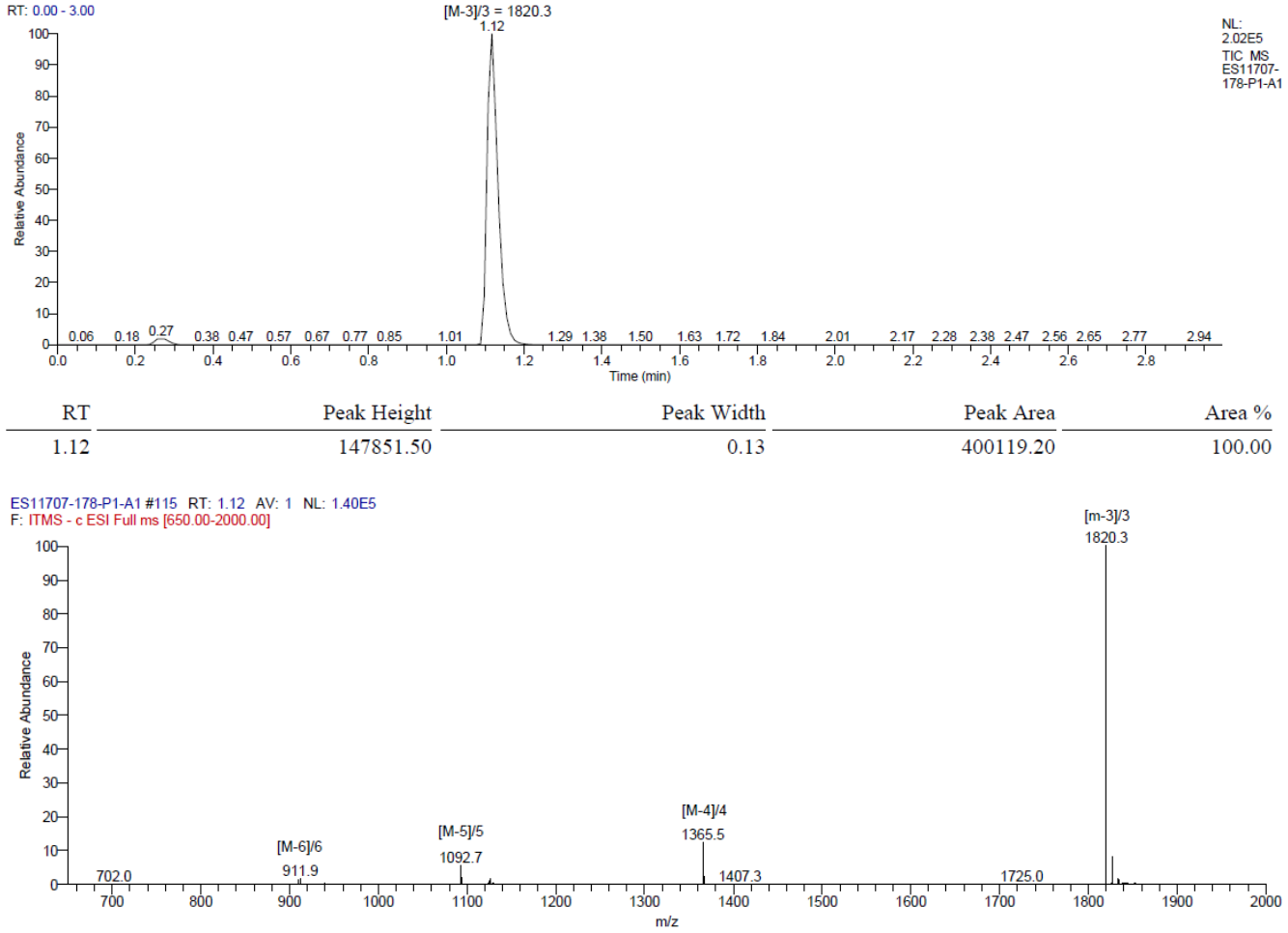
Purity: >99.00%

Exact mass: 5463.84

Triply charged mass [M-3]/3, calculated: 1820.28; observed:1820.3



Me-S-HP,3c

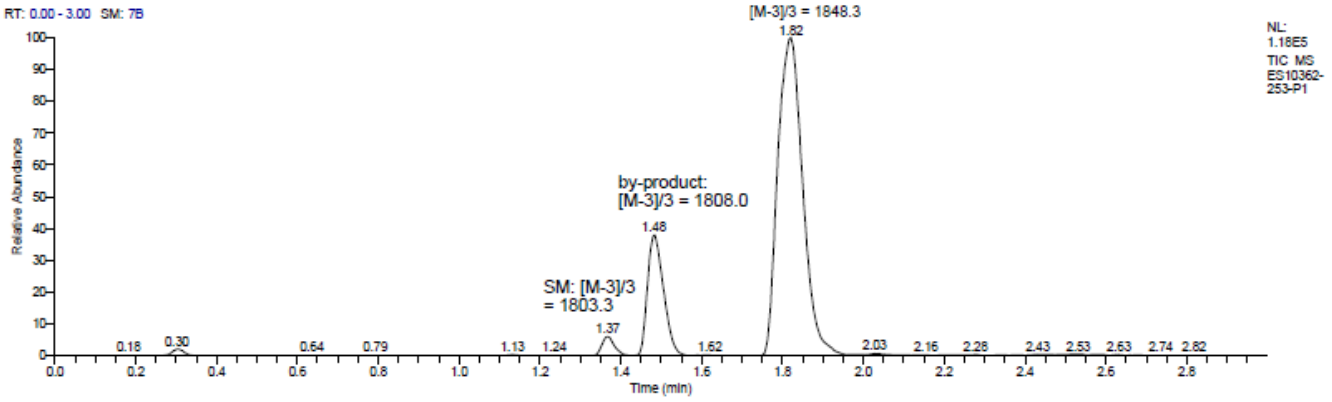


RT	Peak Height	Peak Width	Peak Area	Area %
1.12	147851.50	0.13	400119.20	100.00

Fig. S6. LC trace and mass of **3c**.

Figure S7, Trace and Mass of 4a, related to Figure 3.

Following **General Procedure 2**
Percent conversion: 78.54%
Exact mass: 5548.09
Triply charged mass [M-3]/3, calculated: 1848.36; observed:1848.3



RT	Peak Height	Peak Width	Peak Area	Area %
1.37	7522.86	0.10	14770.99	2.30
1.48	45900.65	0.14	122931.83	19.16
1.82	119469.26	0.22	503847.65	78.54

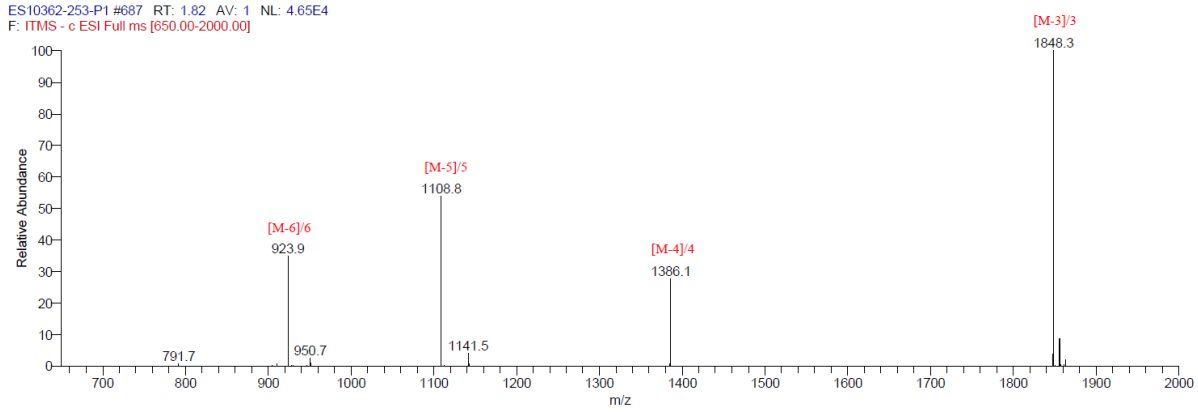


Fig. S7. LC trace and mass of **4a**.

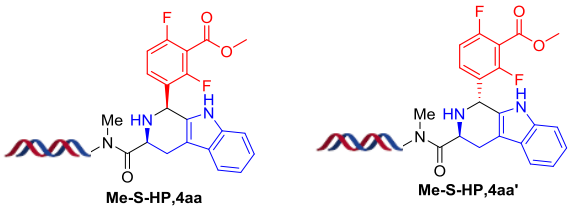
Figure S8, Trace and Mass of 4aa and 4aa', related to Figure 3.

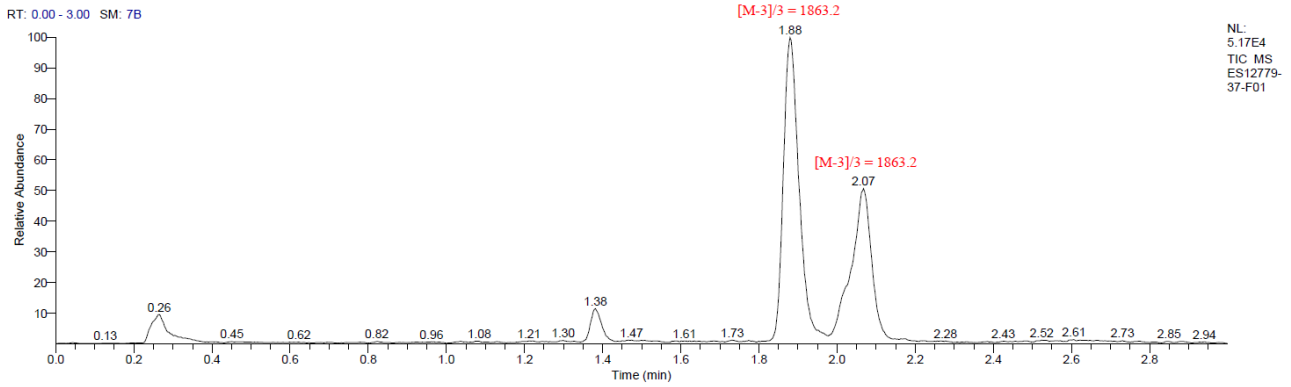
Following **General Procedure 2**

Percent conversion: 56.77%& 38.02%, totally 94.79%

Exact mass: 5596.84

Triply charged mass [M-3]/3, calculated: 1864.61; observed:1863.2&1863.2





RT	Peak Height	Peak Width	Peak Area	Area %
1.38	5996.54	0.20	13113.83	5.21
1.88	53406.53	0.15	142914.51	56.77
2.07	26972.42	0.22	95694.35	38.02

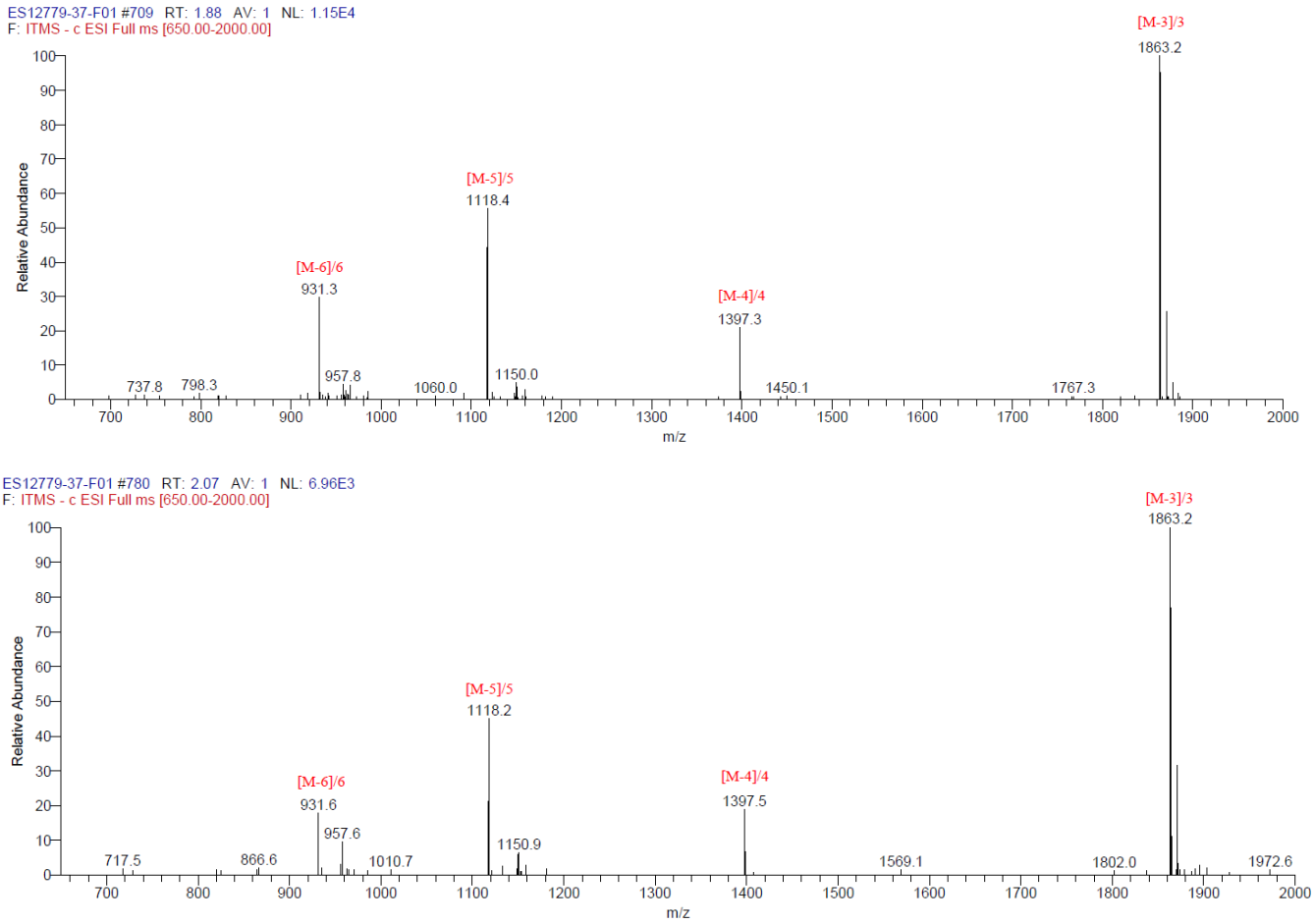


Fig. S8. LC trace and mass of **4aa and 4aa'**.

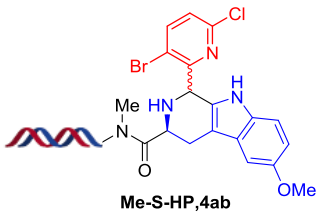
Figure S8, Trace and Mass of 4ab, related to Figure 3.

Following **General Procedure 2**

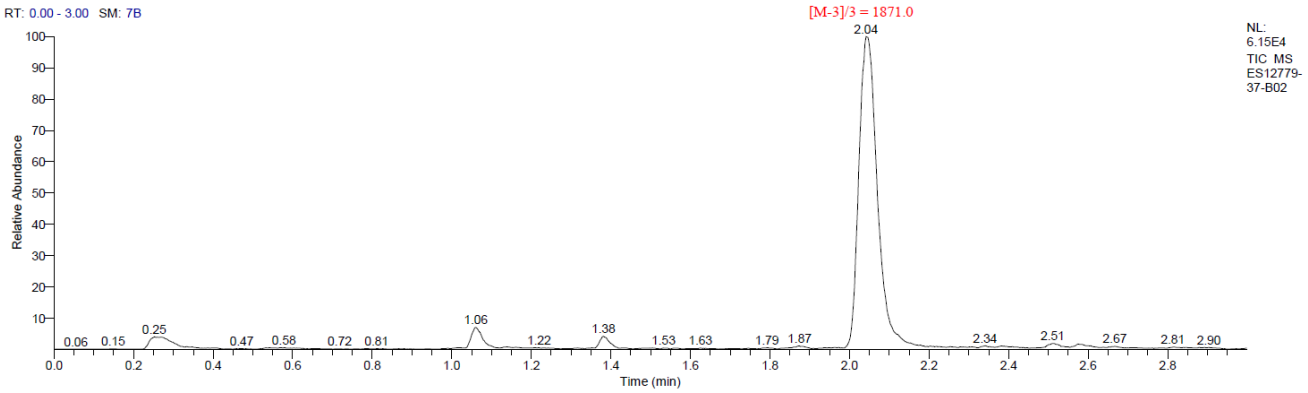
Percent conversion: 91.52%

Exact mass: 5617.42

Triply charged mass [M-3]/3, calculated: 1871.47; observed:1871.0



Me-S-HP,4ab



RT	Peak Height	Peak Width	Peak Area	Area %
1.06	4230.85	0.28	10472.04	4.84
1.38	2757.17	0.10	4460.32	2.06
2.05	62186.68	0.24	197967.26	91.52
2.51	981.50	0.08	2021.16	0.93
2.58	993.09	0.05	1397.73	0.65

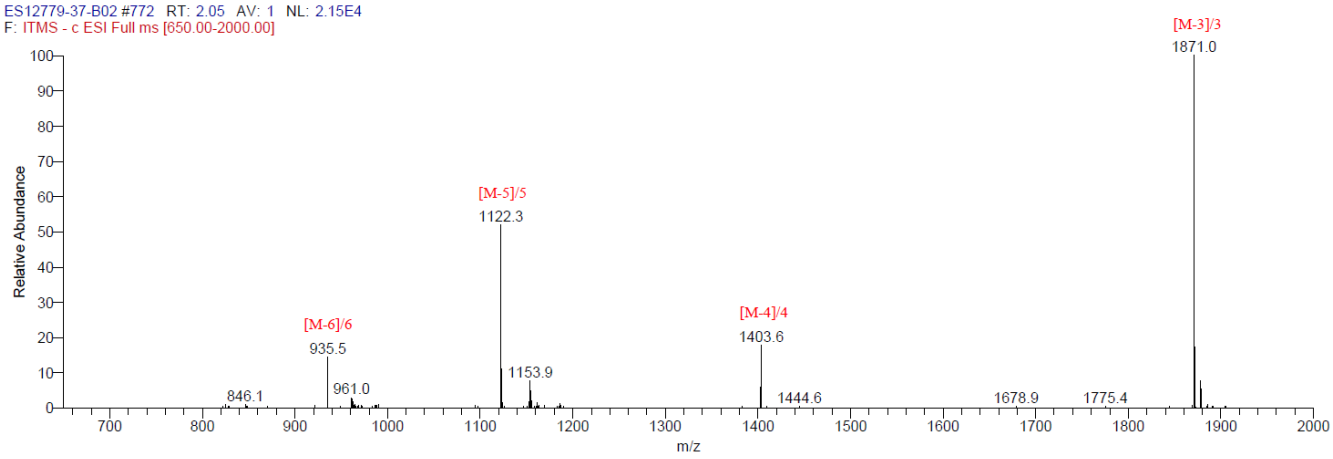


Fig. S9. LC trace and mass of **4ab**

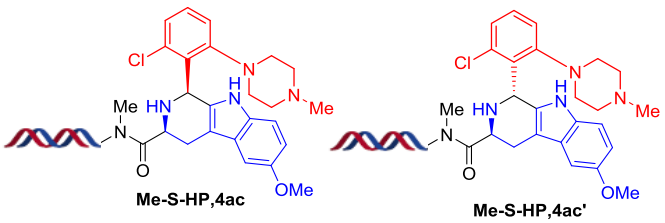
Figure S10, Trace and Mass of 4ac and 4ac', related to Figure 3.

Following **General Procedure 2**

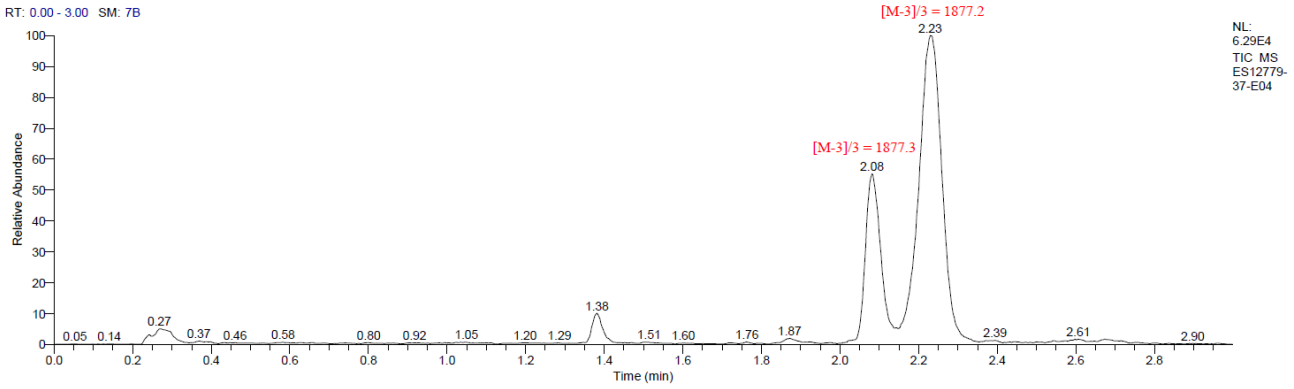
Percent conversion: 25.47% & 69.54%, totally 95.01%

Exact mass: 5635.69

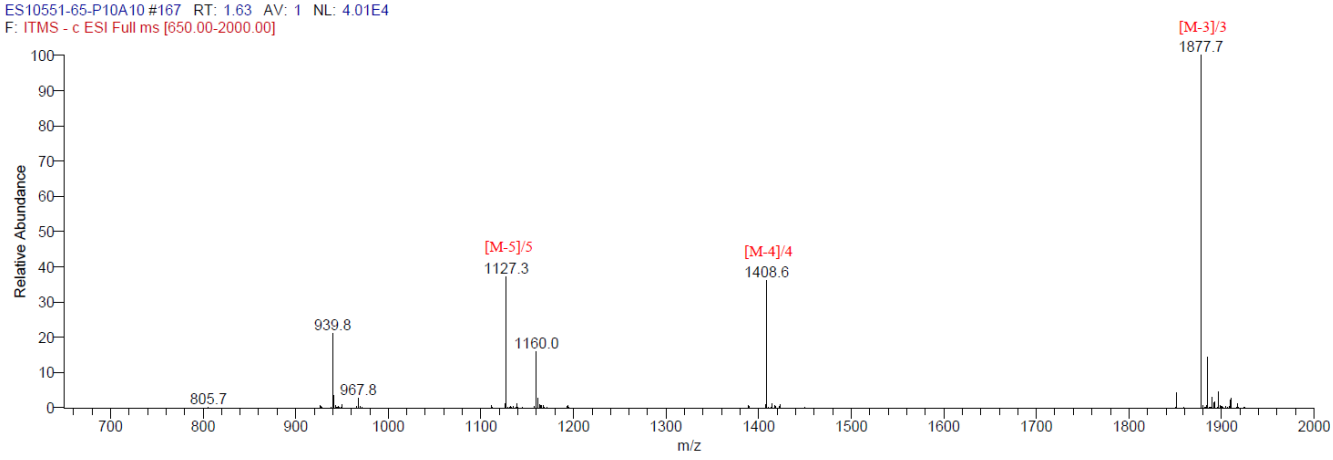
Triply charged mass [M-3]/3, calculated: 1877.56; observed:1877.3&1877.2



Me-S-HP,4ac Me-S-HP,4ac'



RT	Peak Height	Peak Width	Peak Area	Area %
1.38	6465.13	0.13	12013.33	3.26
1.87	1201.29	0.12	3413.85	0.93
2.08	35553.80	0.11	93989.70	25.47
2.24	62824.80	0.20	256594.28	69.54
2.67	856.85	0.10	2970.67	0.81



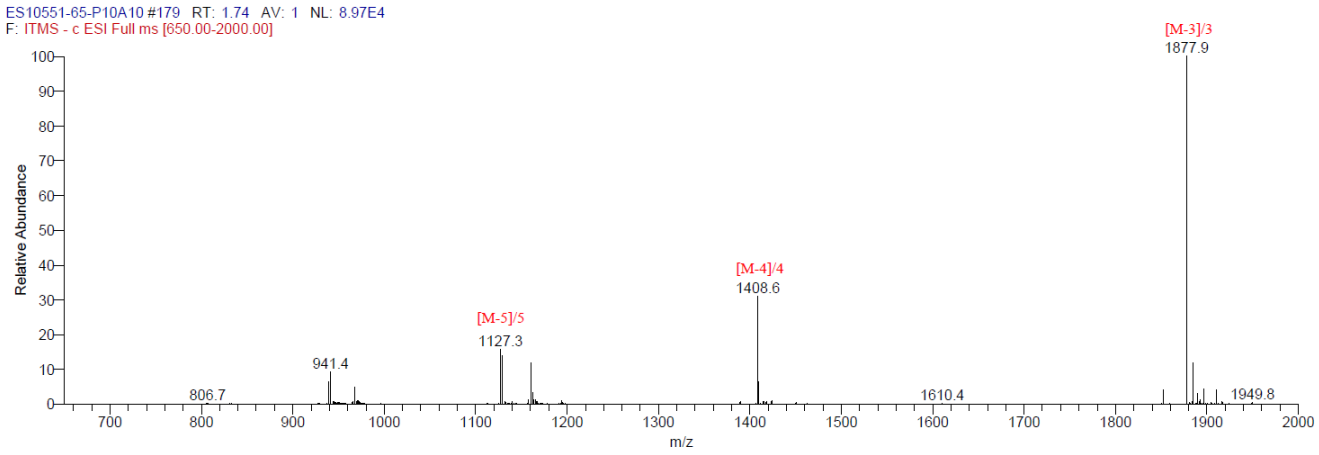
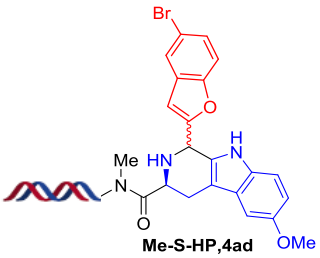


Fig. S10. LC trace and mass of **4ac and 4ac'**

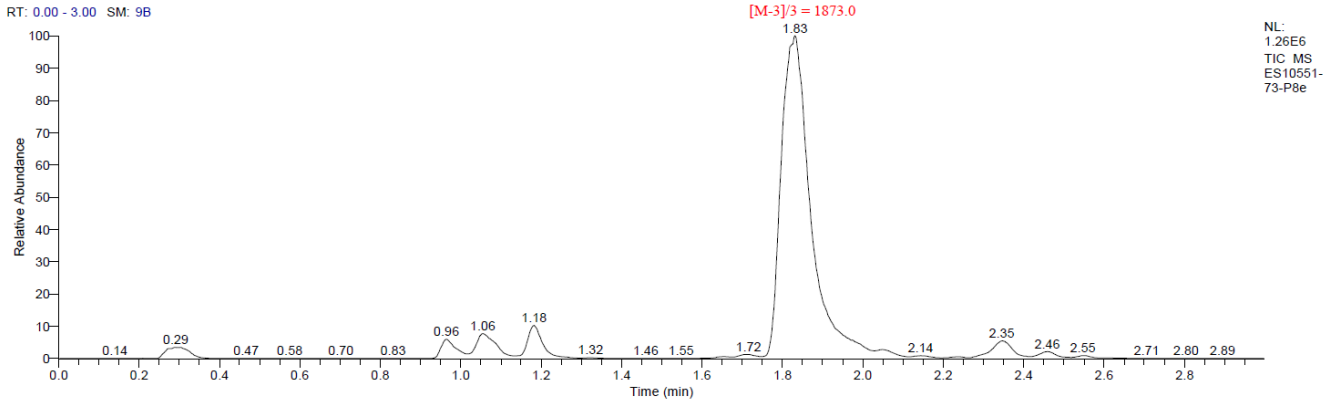
Figure S11, Trace and Mass of 4ad, related to Figure 3.

Following **General Procedure 2**
Percent conversion: 85.01%
Exact mass: 5622.01

Triply charged mass [M-3]/3, calculated: 1873.0; observed:1873.0



Me-S-HP,4ad



RT	Peak Height	Peak Width	Peak Area	Area %
0.96	84020.35	0.16	203731.35	2.63
1.06	109847.24	0.11	340623.44	4.39
1.18	145603.79	0.26	364129.24	4.69
1.83	1307271.23	0.36	6596635.55	85.01
2.34	70043.20	0.16	254270.02	3.28

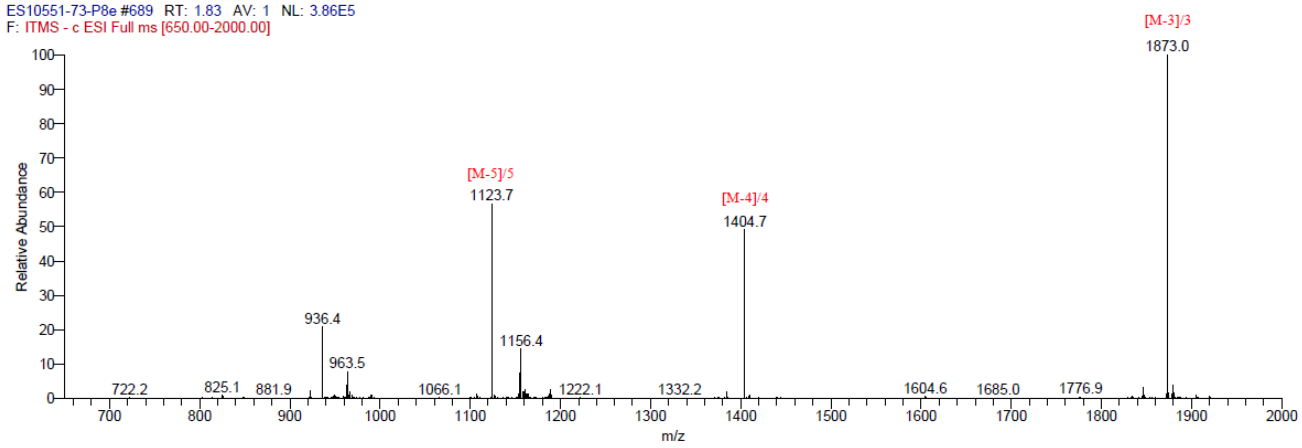


Fig. S11. LC trace and mass of **4ad**

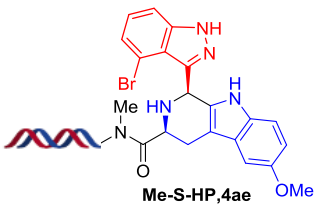
Figure S12, Trace and Mass of 4ae and 4ae', related to Figure 3.

Following **General Procedure 2**

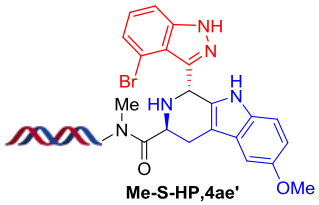
Percent conversion: 50.58% & 39.12%, totally 89.70%

Exact mass: 5622.02

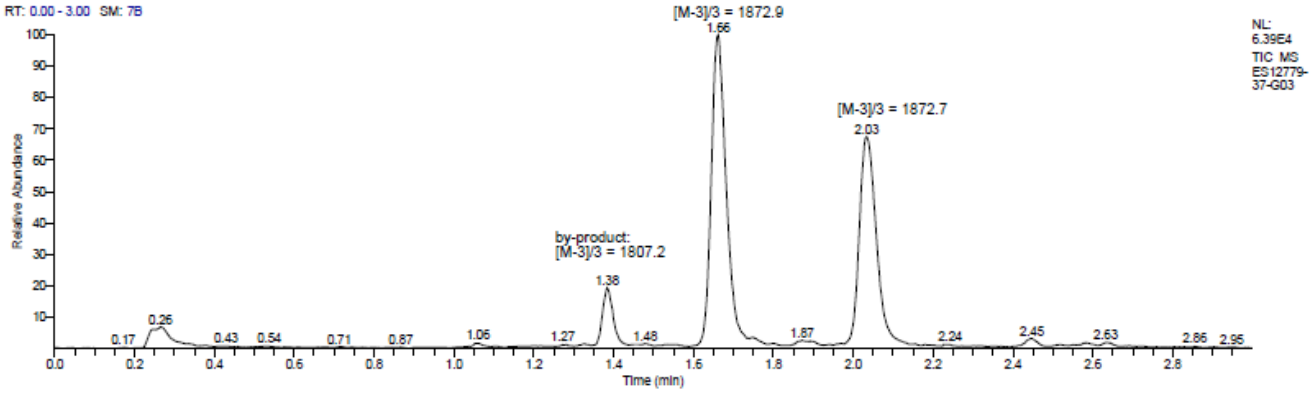
Triply charged mass [M-3]/3, calculated: 1873.01; observed:1872.9&1872.7



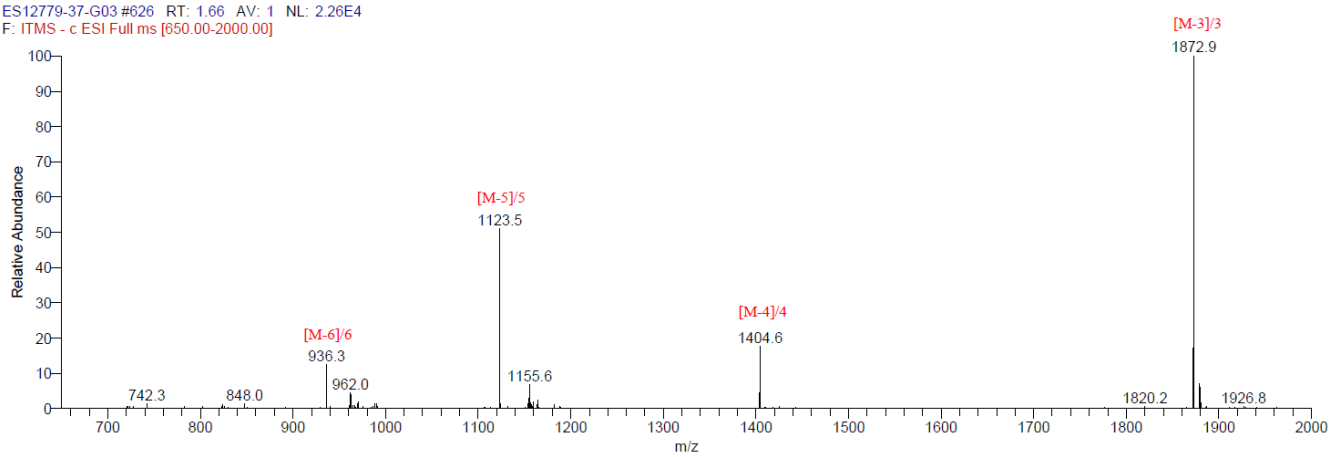
Me-S-HP,4ae



Me-S-HP,4ae'



RT	Peak Height	Peak Width	Peak Area	Area %
1.38	12397.34	0.31	26670.70	7.96
1.66	66787.65	0.25	169467.46	50.58
1.90	1294.92	0.08	3906.43	1.17
2.04	43767.81	0.29	131090.13	39.12
2.44	1862.73	0.09	3925.55	1.17



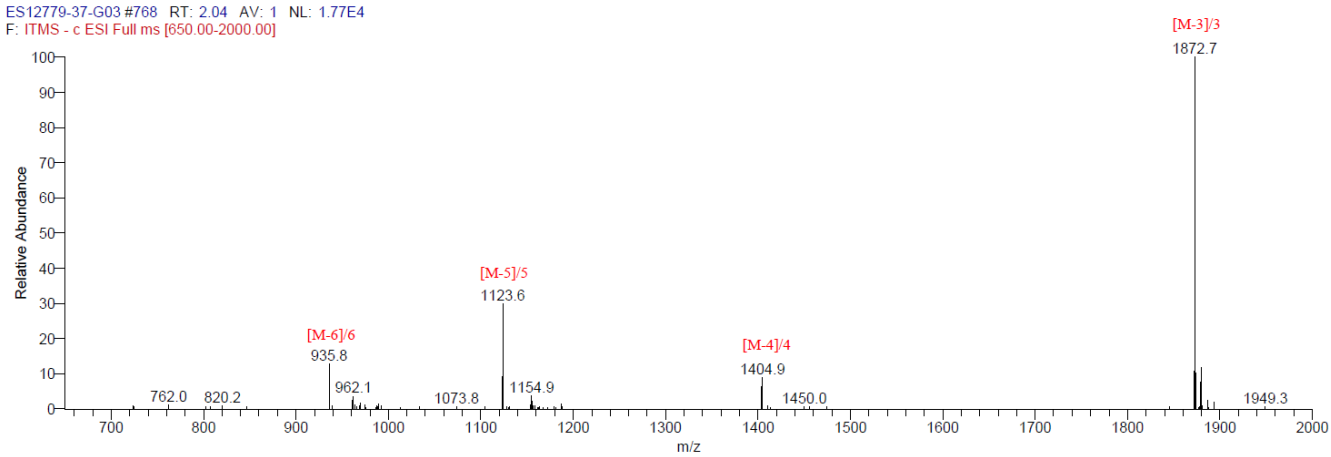
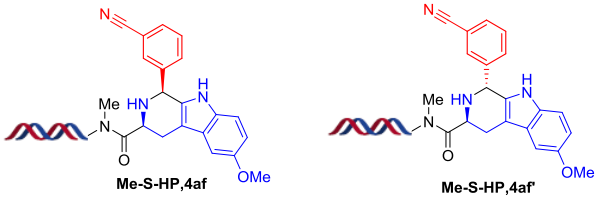
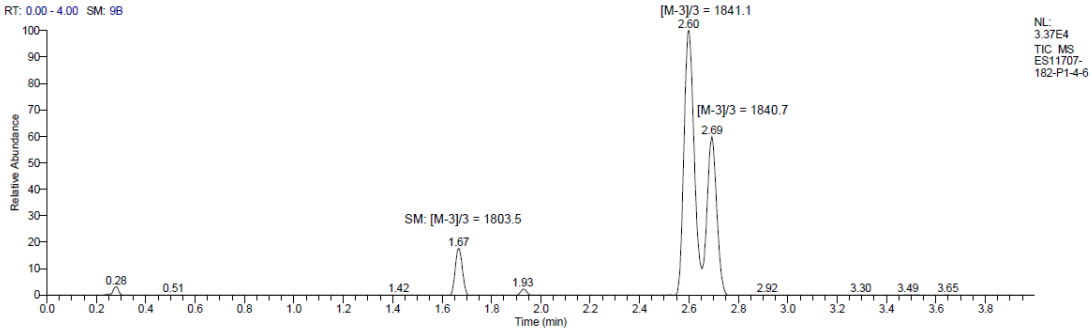


Fig. S12. LC trace and mass of **4ae and 4ae'**

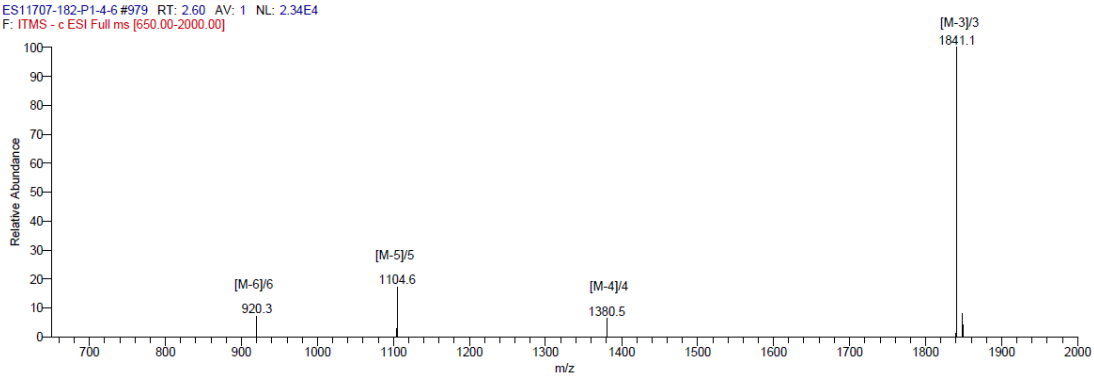
Figure S13, Trace and Mass of 4af and 4af', related to Figure 3.

Following **General Procedure 2**
Percent conversion: 59.17% & 33.65%, totally 92.82%
Exact mass: 5528.10
Triply charged mass [M-3]/3, calculated: 1841.7; observed: 1841.1&1840.7





RT	Peak Height	Peak Width	Peak Area	Area %
1.67	6701.44	0.06	11699.80	7.18
2.60	35171.56	0.10	96409.10	59.17
2.69	21512.70	0.09	54830.57	33.65



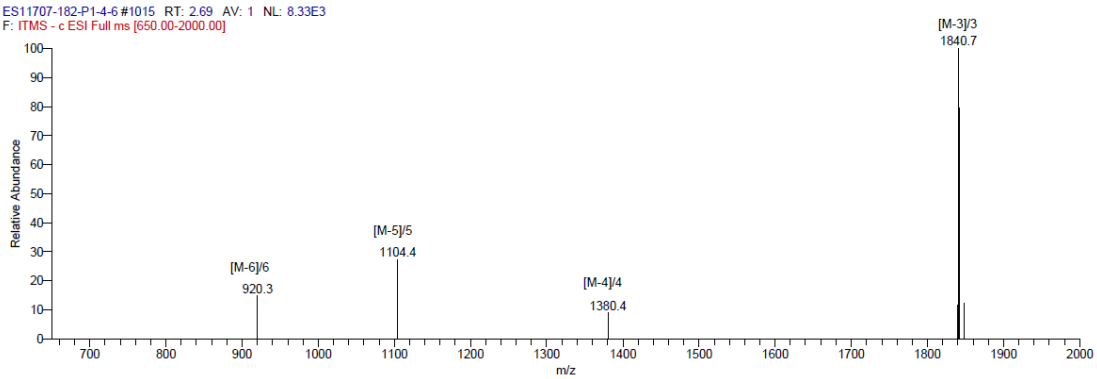
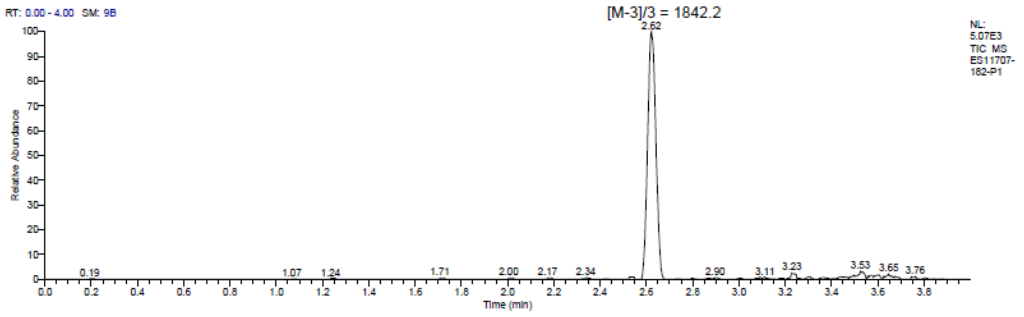


Fig. S13. LC trace and mass of **4af and 4af'**

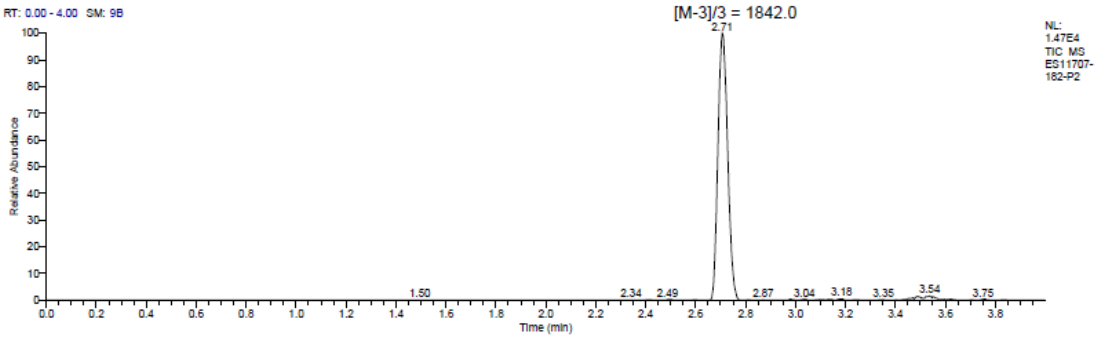
Figure S14, 4af and 4af' were separated by HPLC, related to Figure 3.

Retain time = 2.62



RT	Peak Height	Peak Width	Peak Area	Area %
2.62	5672.18	0.08	12528.58	98.89
3.24	256.21	0.02	140.09	1.11

Retain time = 2.71



RT	Peak Height	Peak Width	Peak Area	Area %
2.70	15284.30	0.10	39618.11	100.00

Fig. S14. LC trace of **4af and 4af'**

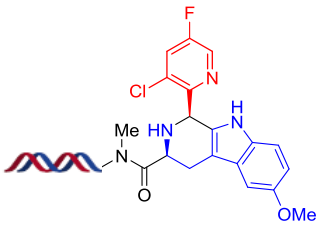
Figure S15, Trace and Mass of 4ag and 4ag', related to Figure 3.

Following **General Procedure 2**

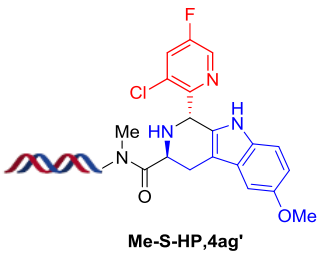
Percent conversion: 64.78% and 30.30%, totally 95.08%

Exact mass: 5617.94

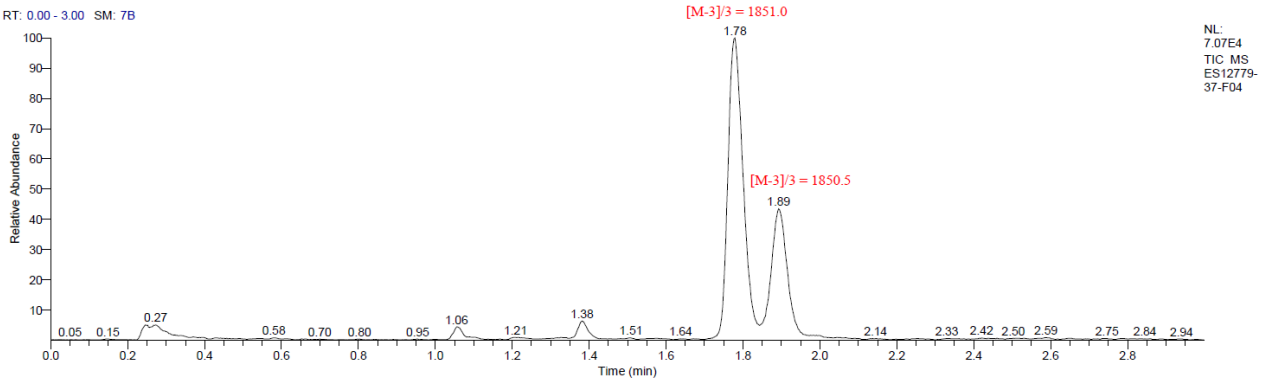
Triply charged mass [M-3]/3, calculated: 1871.66; observed:1871.70



Me-S-HP,4ag



Me-S-HP,4ag'



RT	Peak Height	Peak Width	Peak Area	Area %
1.06	3419.32	0.06	5250.78	1.76
1.10	596.77	0.04	805.29	0.27
1.38	4666.00	0.21	9396.50	3.15
1.78	74009.31	0.14	193113.16	64.78
1.89	31004.09	0.17	89520.43	30.03

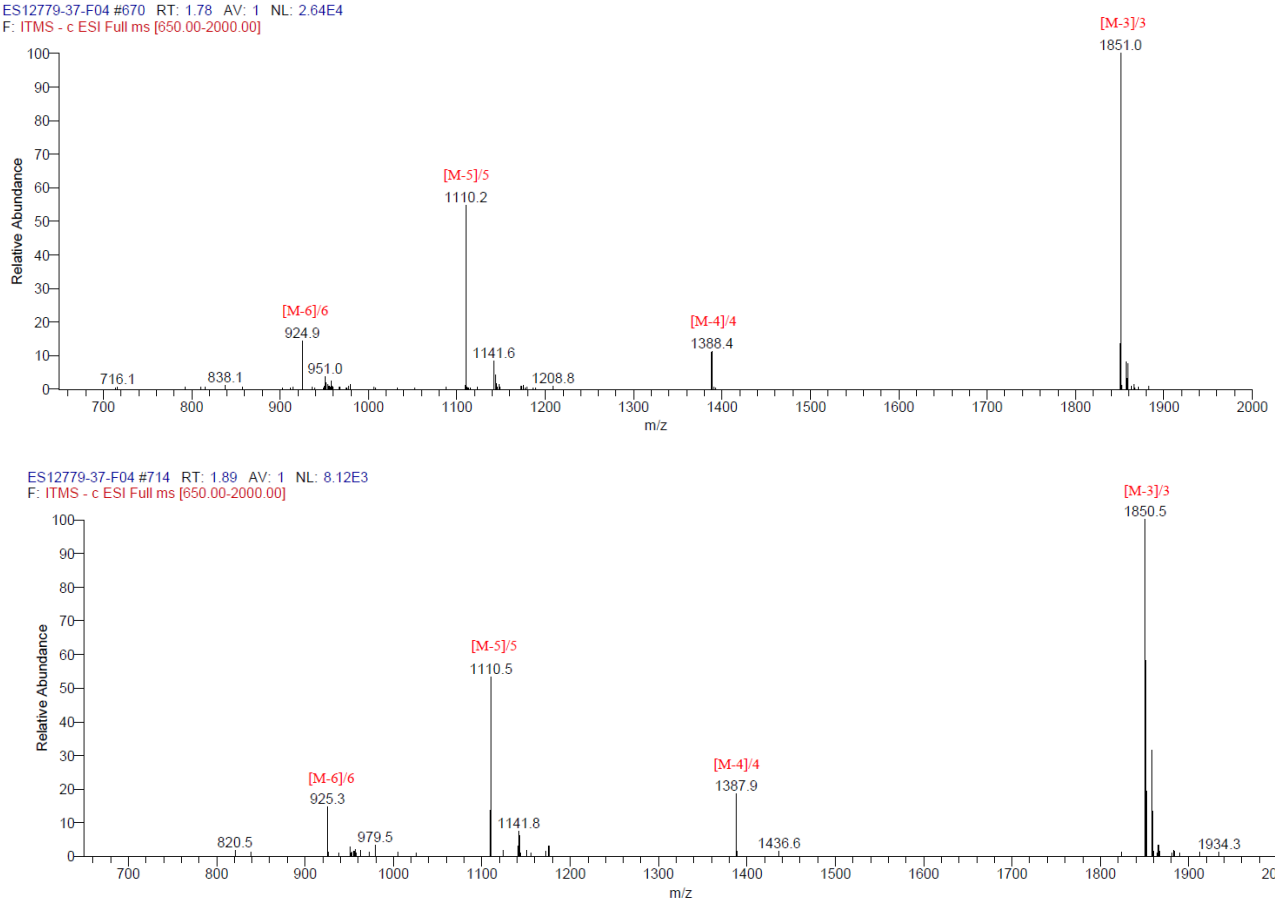
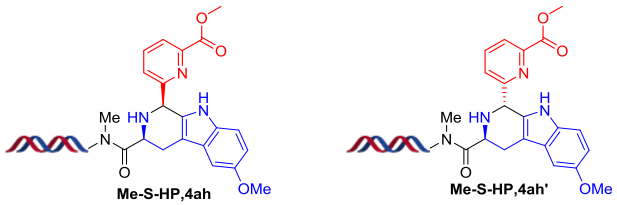
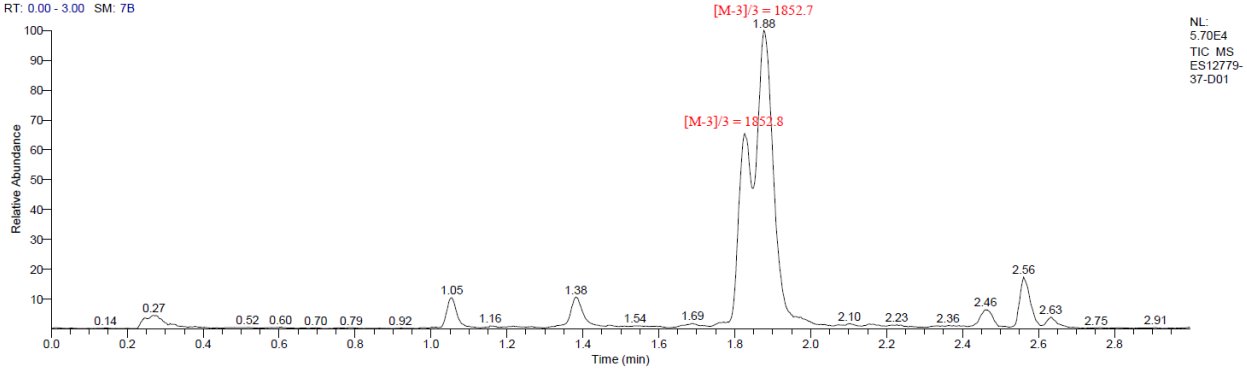


Fig. S15. LC trace and mass of **4ag and 4ag'**

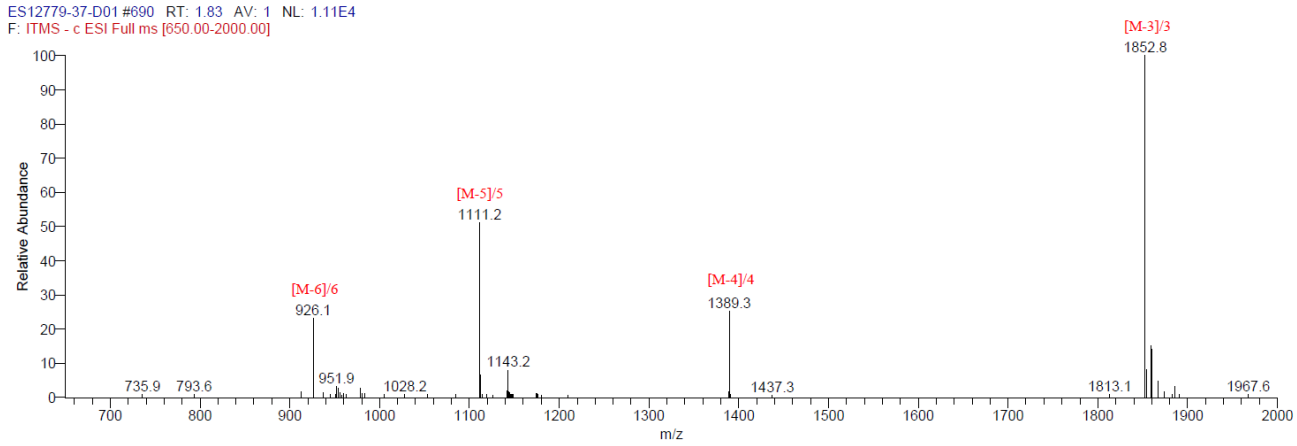
Figure S16, Trace and Mass of 4ah and 4ah', related to Figure 3.

Following **General Procedure 2**
Percent conversion: 25.95% & 54.87%, totally 80.82%
Exact mass: 5562.12
Triply charged mass [M-3]/3, calculated: 1853.04; observed:1852.8&1852.7





RT	Peak Height	Peak Width	Peak Area	Area %
1.05	6239.64	0.13	10935.10	3.45
1.38	6209.66	0.31	17139.13	5.41
1.83	38485.46	0.07	82142.41	25.95
1.88	58533.04	0.20	173670.18	54.87
2.47	3452.89	0.22	9999.99	3.16
2.56	9969.86	0.08	18715.71	5.91
2.63	2129.45	0.09	3930.05	1.24



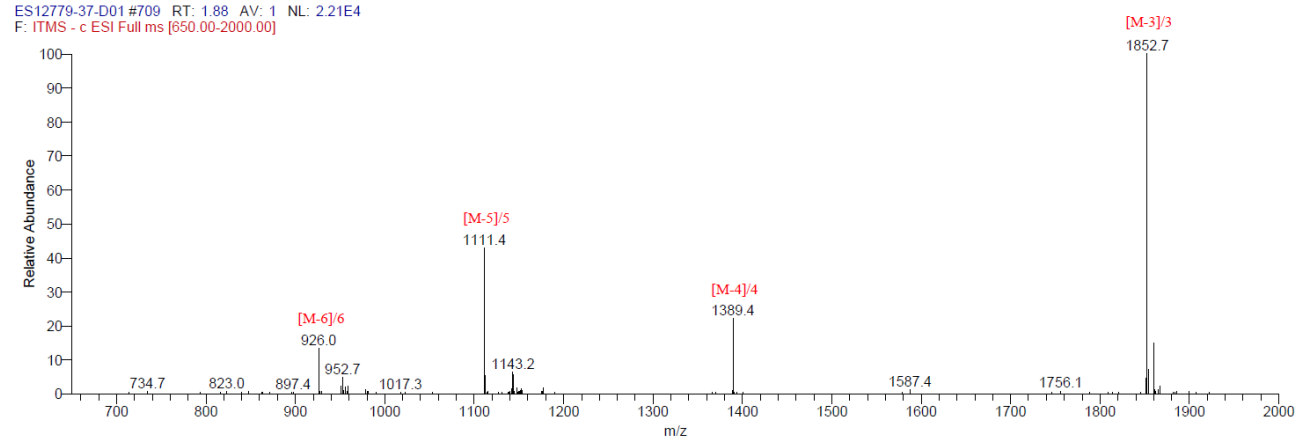
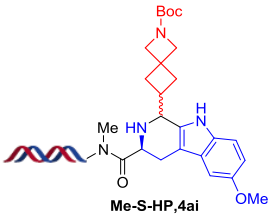
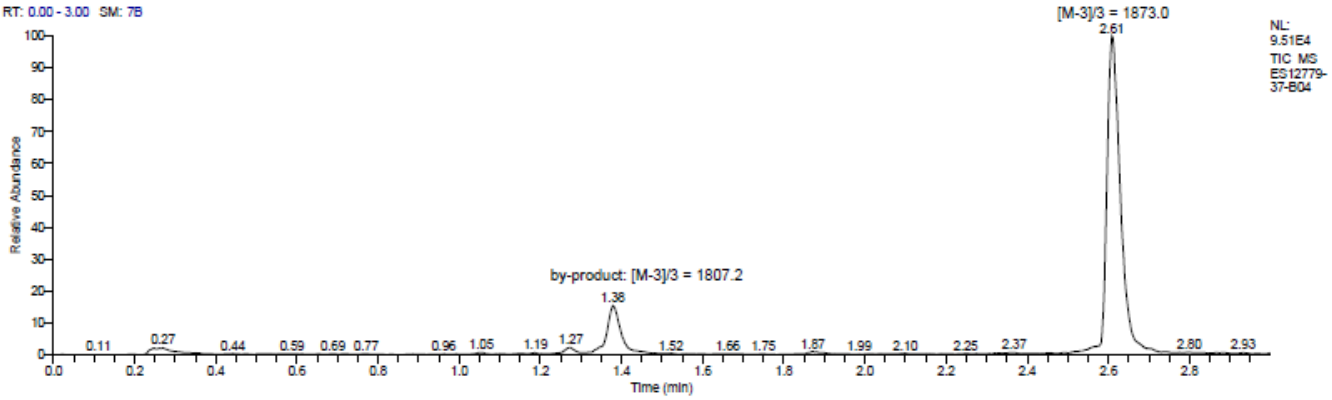


Fig. S16. LC trace and mass of **4ah and 4ah'**

Figure S17, Trace and Mass of 4ai, related to Figure 3.

Following **General Procedure 2**
Percent conversion: 82.58%
Exact mass: 5622.26
Triply charged mass [M-3]/3, calculated: 1873.09; observed:1873.0





RT	Peak Height	Peak Width	Peak Area	Area %
1.27	2212.28	0.10	4354.08	1.59
1.38	15302.15	0.23	37225.31	13.62
1.87	947.79	0.05	1204.93	0.44
2.61	99230.41	0.28	225662.29	82.58
2.79	739.04	0.22	4823.96	1.77

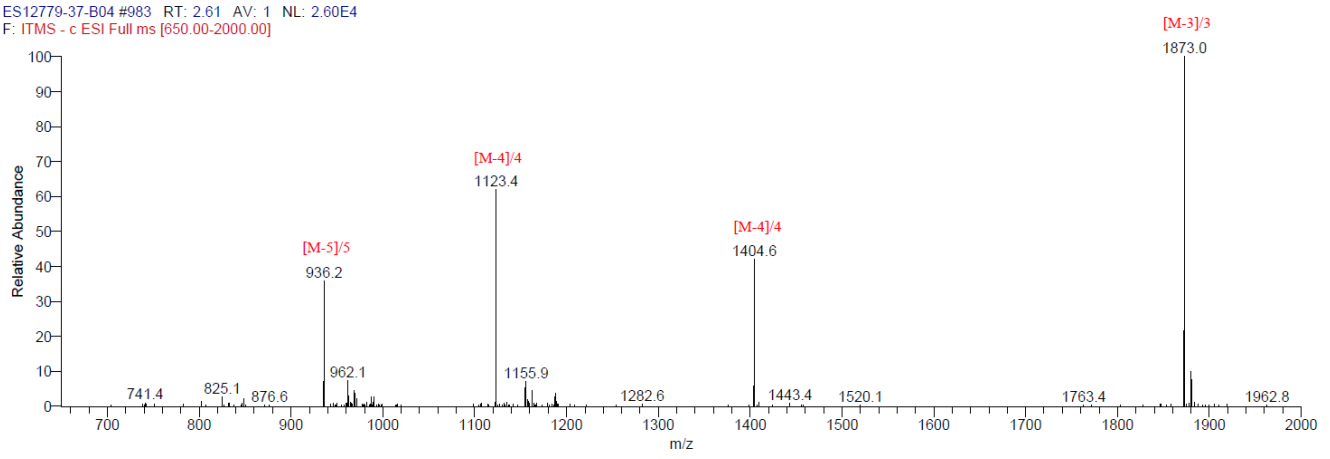
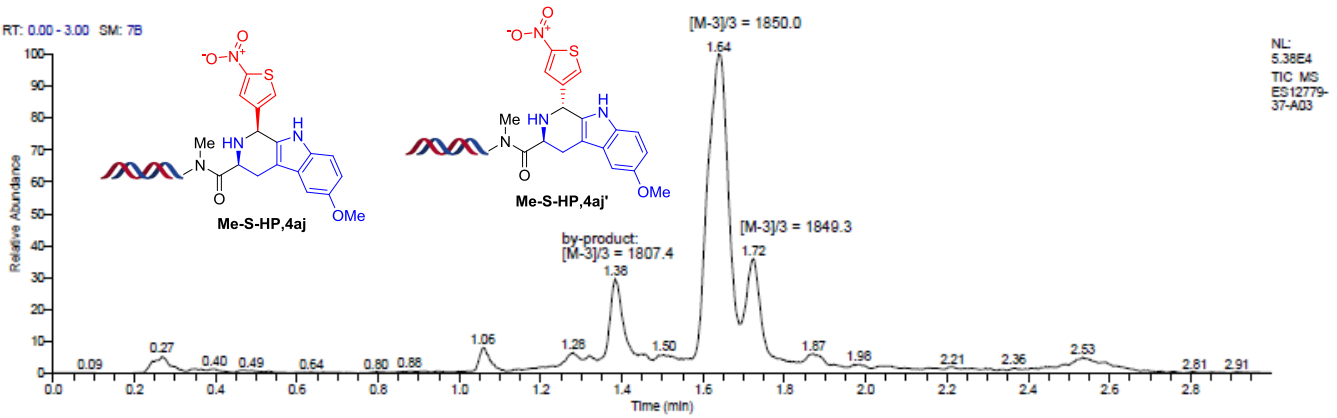


Fig. S17. LC trace and mass of **4ai**

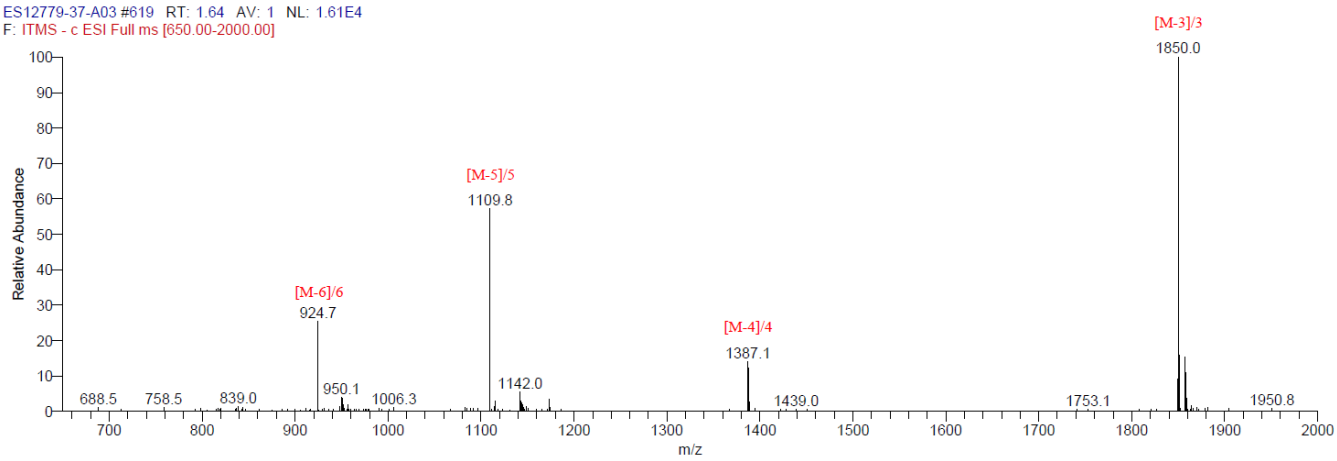
Figure S18, Trace and Mass of 4aj and 4aj', related to Figure 3.

Following **General Procedure 2**
Percent conversion: 63.28% & 17.23%, totally 80.51%
Exact mass: 5554.12

Triply charged mass [M-3]/3, calculated: 1850.37; observed:1850.0&1849.3



RT	Peak Height	Peak Width	Peak Area	Area %
1.38	15190.19	0.14	35388.36	12.28
1.64	54024.18	0.11	182279.76	63.28
1.72	18594.61	0.14	49628.25	17.23
1.87	2265.47	0.13	7698.09	2.67
2.54	2290.97	0.23	13079.71	4.54



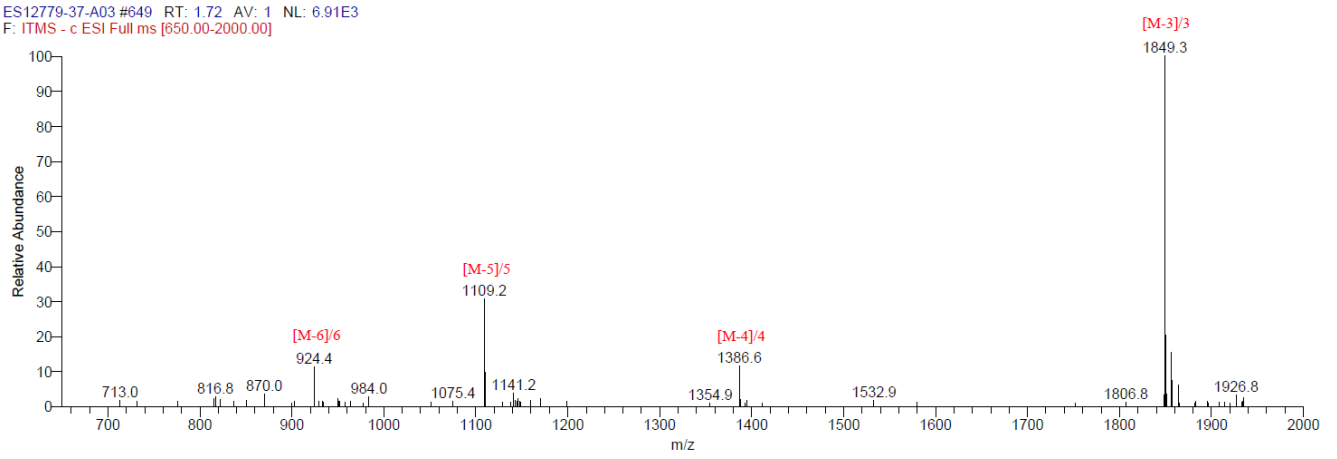
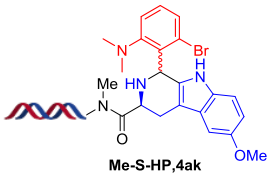


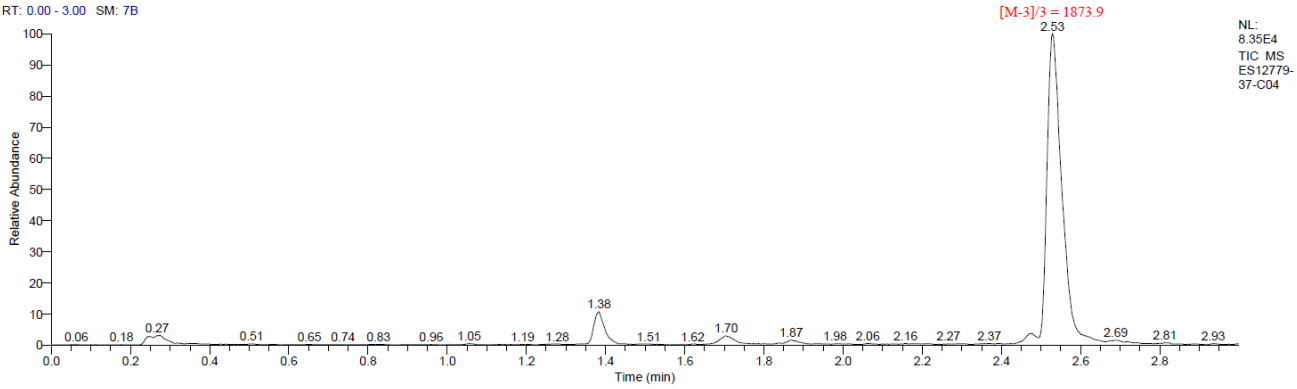
Fig. S18. LC trace and mass of **4aj and 4aj'**

Figure S19, Trace and Mass of 4ak, related to Figure 3.

Following **General Procedure 2**
Percent conversion: 85.85%
Exact mass: 5625.06
Triply charged mass [M-3]/3, calculated: 1874.02; observed:1873.9



Me-S-HP,4ak



RT	Peak Height	Peak Width	Peak Area	Area %
1.38	9513.96	0.32	19463.58	7.72
1.71	2433.60	0.18	7122.57	2.82
1.87	1181.36	0.15	2965.93	1.18
2.47	3095.64	0.08	6136.89	2.43
2.53	84558.80	0.16	216499.12	85.85

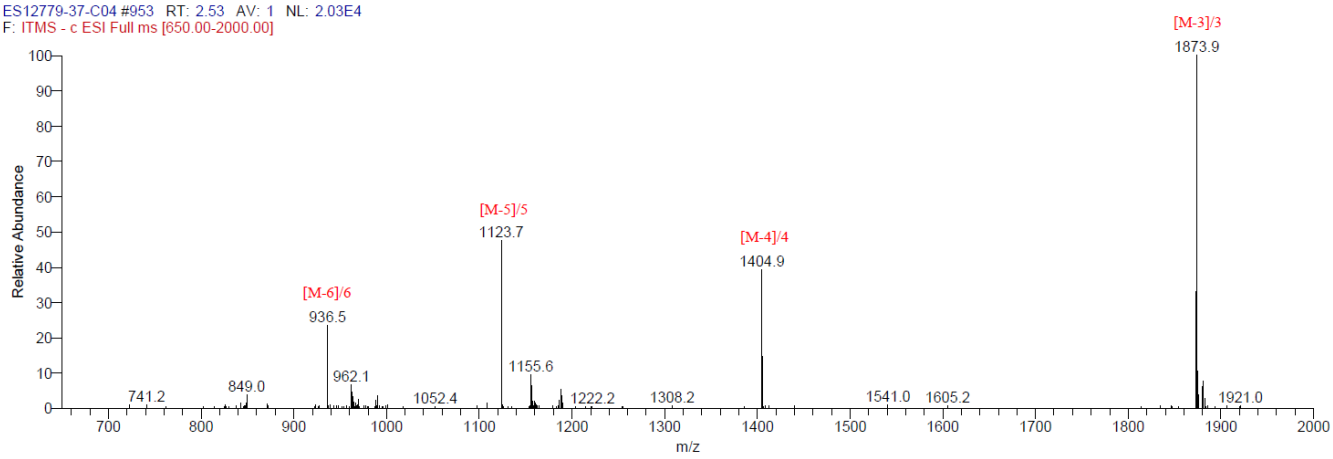
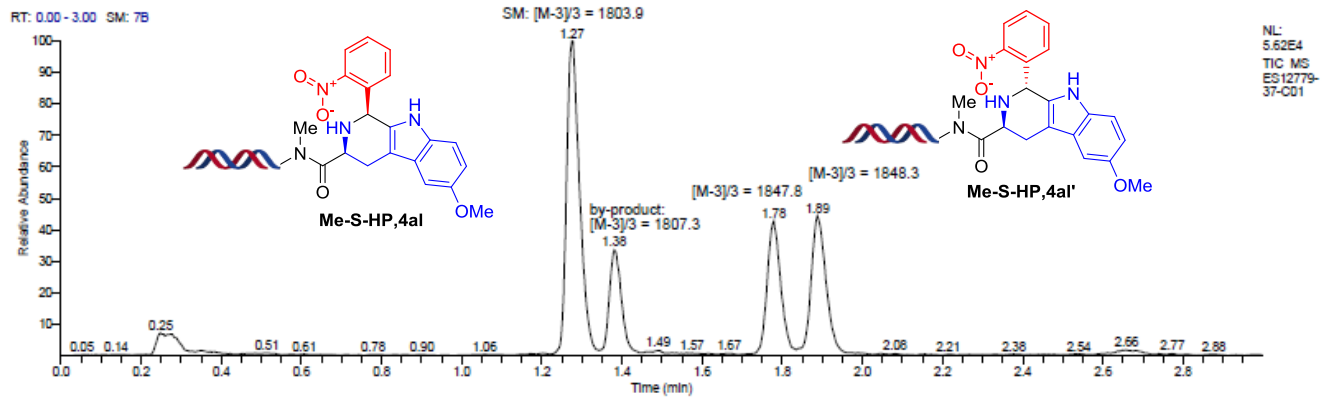


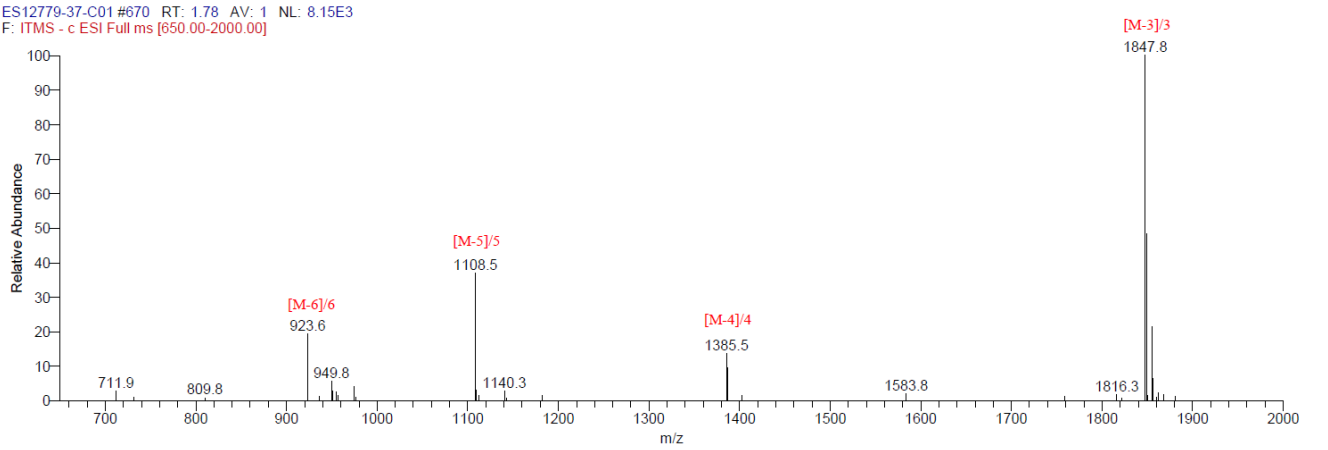
Fig. S19. LC trace and mass of **4ak**

Figure S20, Trace and Mass of 4al and 4al', related to Figure 3.

Following **General Procedure 2**
Yield: 19.15% & 22.35%, totally 41.50%
Exact mass: 5548.09
Triply charged mass [M-3]/3, calculated: 1848.36; observed:1847.8&1848.3



RT	Peak Height	Peak Width	Peak Area	Area %
1.27	60115.72	0.12	126688.35	43.65
1.38	18959.38	0.10	39168.61	13.50
1.78	24337.33	0.11	55580.53	19.15
1.89	25076.06	0.18	64867.29	22.35
2.66	1005.22	0.14	3903.55	1.35



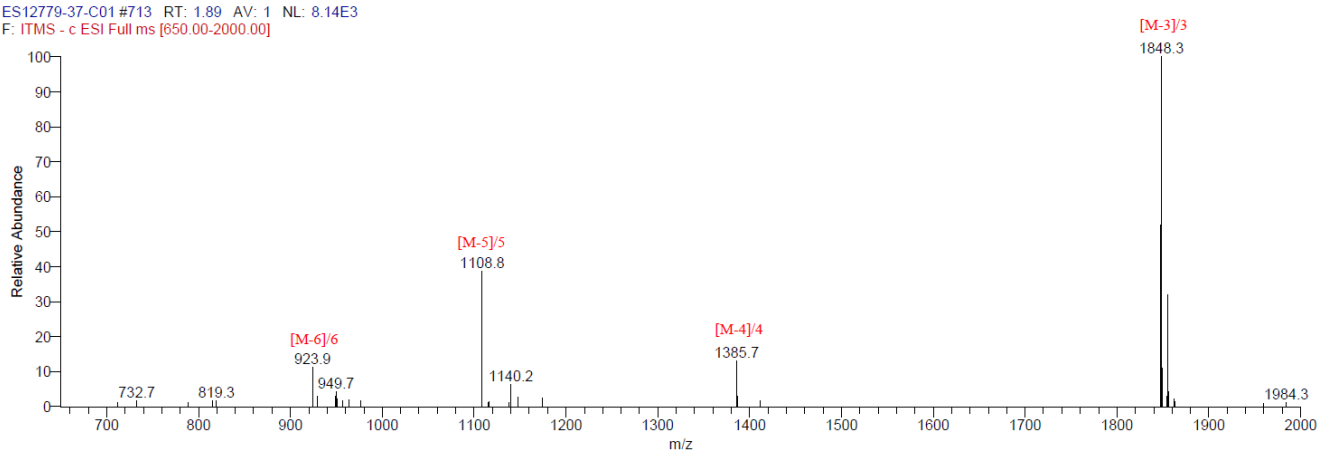
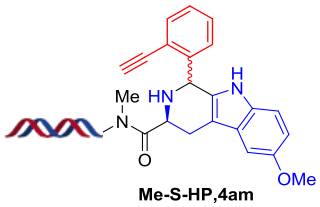


Fig. S20. LC trace and mass of **4al and 4al'**

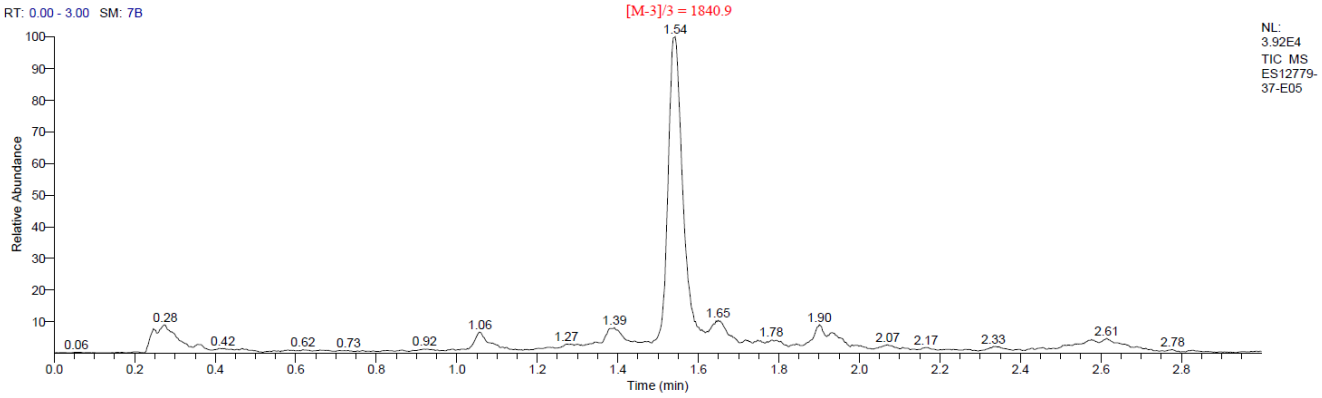
Figure S21, Trace and Mass of 4am, related to Figure 3.

Following **General Procedure 2**
Percent conversion: 74.53%
Exact mass: 5527.12

Triply charged mass [M-3]/3, calculated: 1841.37; observed:1840.9



Me-S-HP,4am



RT	Peak Height	Peak Width	Peak Area	Area %
1.38	2736.38	0.10	8895.09	6.48
1.54	40191.83	0.16	102327.31	74.53
1.65	3401.66	0.08	10085.29	7.35
1.90	2992.37	0.15	9393.42	6.84
2.58	1405.86	0.18	6599.88	4.81

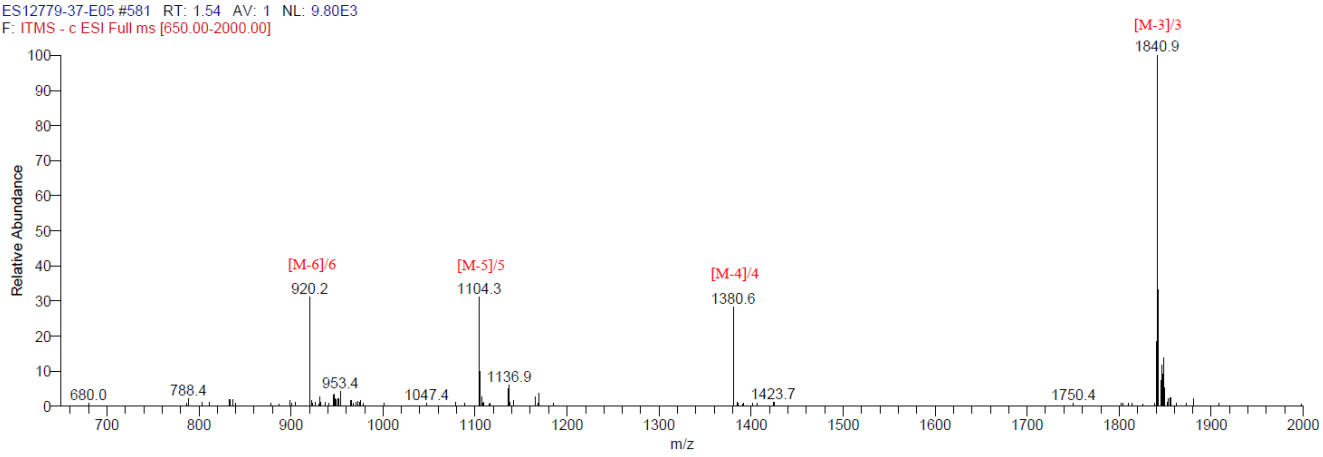
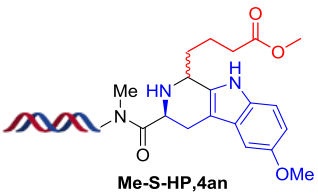


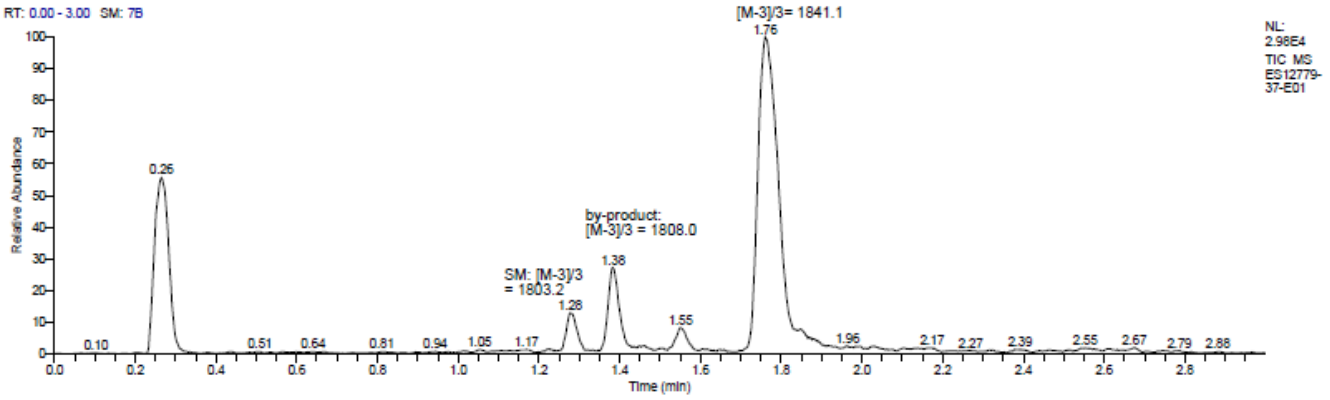
Fig. S21. LC trace and mass of **4am**

Figure S22, Trace and Mass of 4an, related to Figure 3.

Following **General Procedure 2**
Percent conversion: 77.61%
Exact mass: 5527.11
Triply charged mass [M-3]/3, calculated: 1841.37; observed:1841.1



Me-S-HP,4an



RT	Peak Height	Peak Width	Peak Area	Area %
1.28	4151.99	0.14	7376.16	5.36
1.38	8840.46	0.10	15881.45	11.54
1.55	2499.27	0.14	5172.97	3.76
1.77	29575.75	0.21	106807.96	77.61
1.92	659.57	0.10	2388.20	1.74

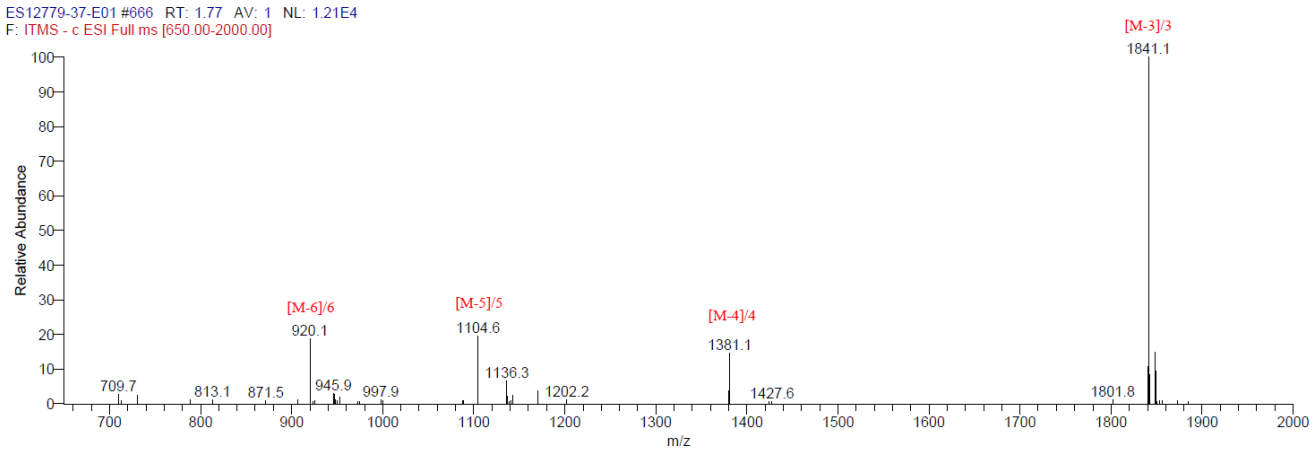
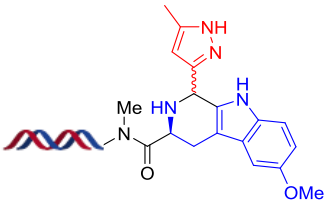


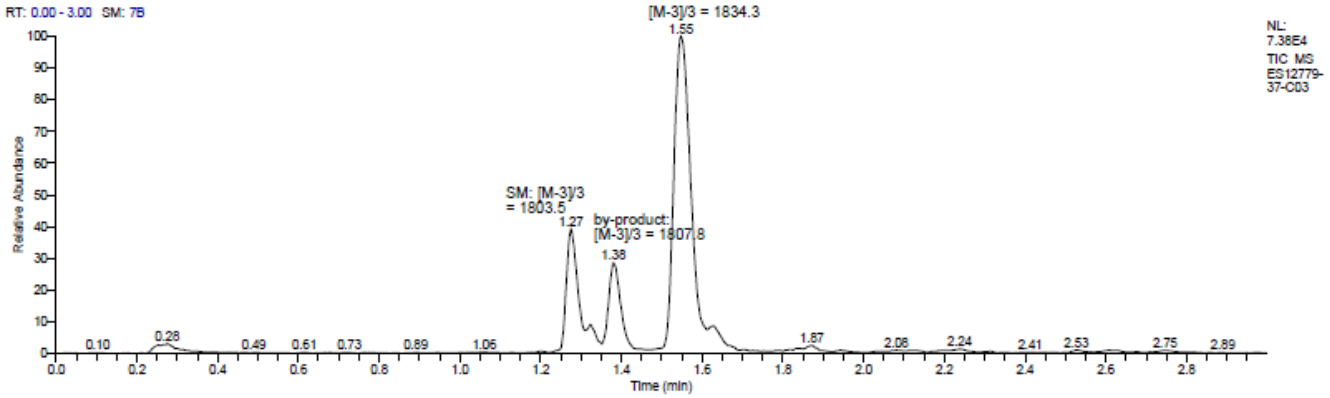
Fig. S22. LC trace and mass of **4an**

Figure S23, Trace and Mass of 4ao, related to Figure 3.

Following **General Procedure 2**
Percent conversion: 65.33%
Exact mass: 5506.81
Triply charged mass [M-3]/3, calculated: 1834.60; observed:1834.3



Me-S-HP,4ao



RT	Peak Height	Peak Width	Peak Area	Area %
1.27	30103.49	0.13	66503.50	19.08
1.38	22332.29	0.13	45980.14	13.19
1.55	75894.09	0.26	227705.91	65.33
1.88	1451.28	0.12	4269.15	1.22
2.24	863.62	0.16	4082.19	1.17

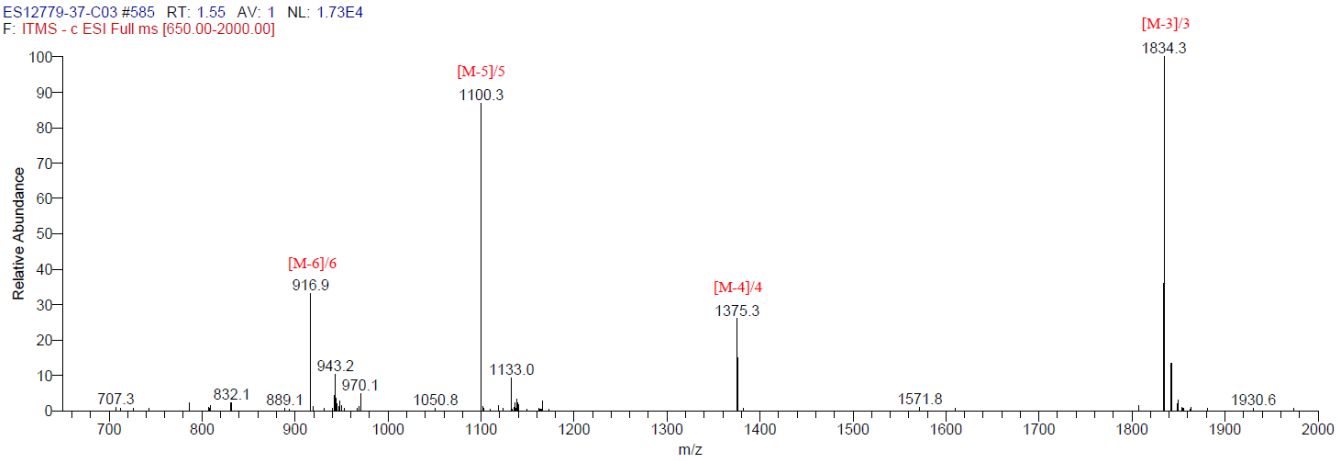


Fig. S23. LC trace and mass of **4ao**

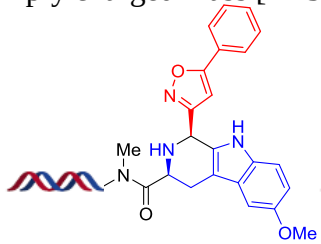
Figure S24, Trace and Mass of 4ap and 4ap', related to Figure 3.

Following **General Procedure 2**

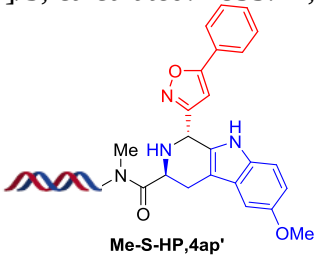
Percent conversion: 53.85% & 27.93%, totally 81.78%

Exact mass: 5570.14

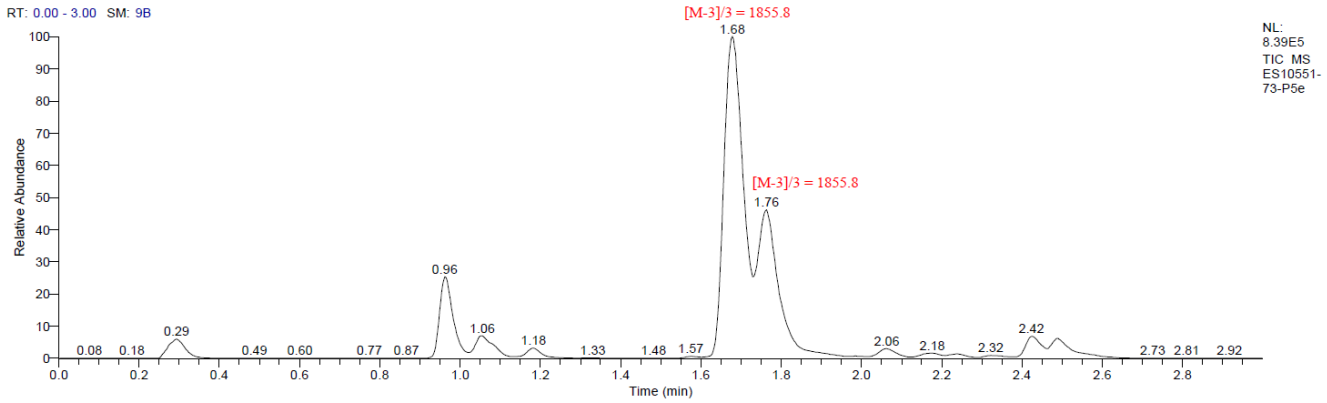
Triply charged mass [M-3]/3, calculated: 1855.71; observed:1855.8



Me-S-HP,4ap



Me-S-HP,4ap'



RT	Peak Height	Peak Width	Peak Area	Area %
0.96	244725.74	0.13	502628.70	9.67
1.05	67679.69	0.10	193983.67	3.73
1.68	867050.88	0.13	2799586.85	53.85
1.76	401511.75	0.29	1451920.10	27.93
2.42	57372.51	0.17	250393.95	4.82

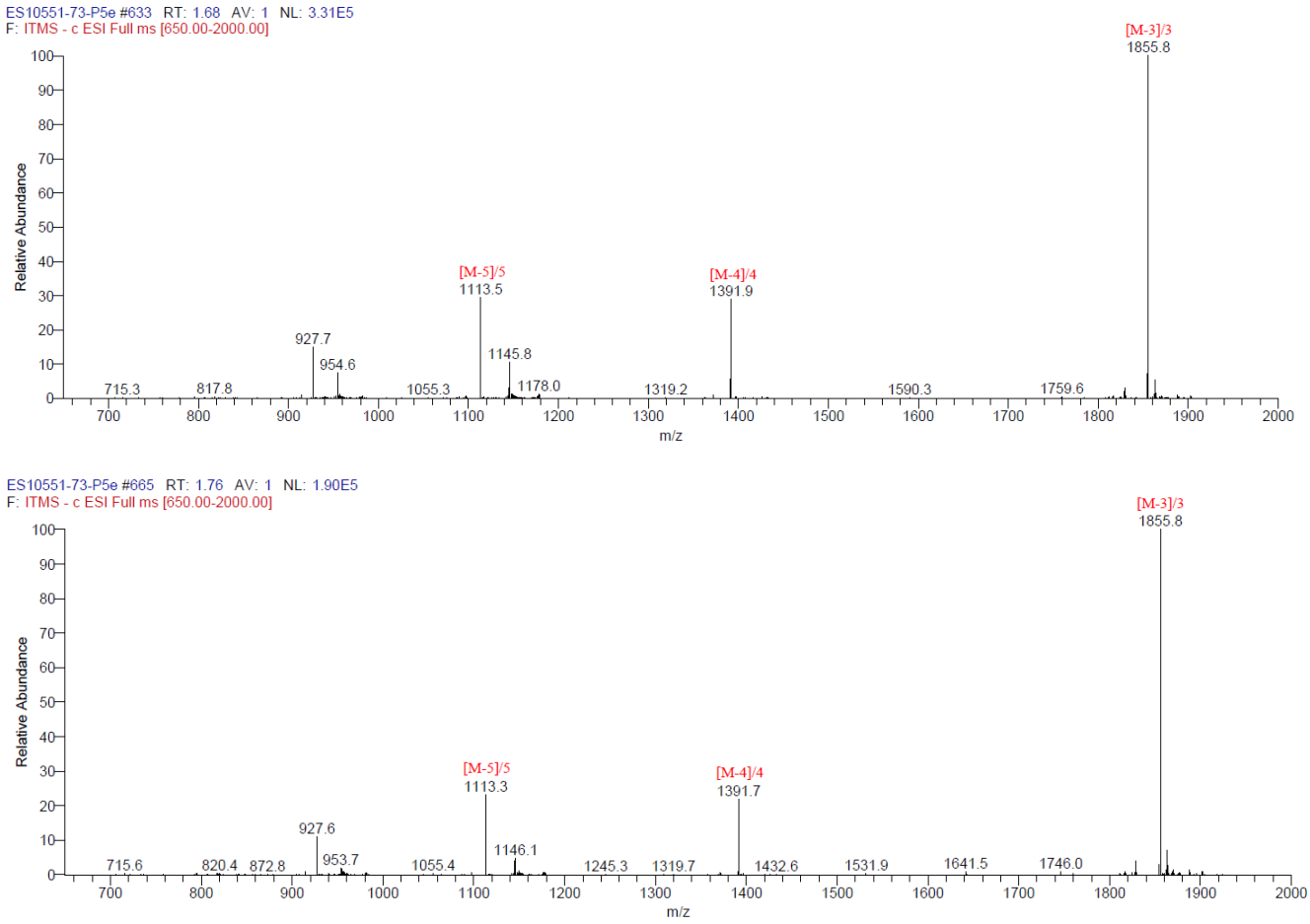


Fig. S24. LC trace and mass of **4ap and 4ap'**

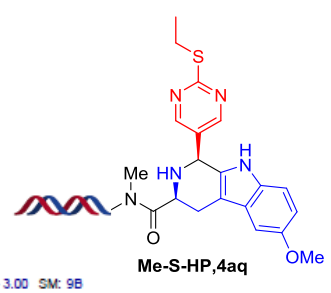
Figure S25, Trace and Mass of 4aq and 4aq', related to Figure 3.

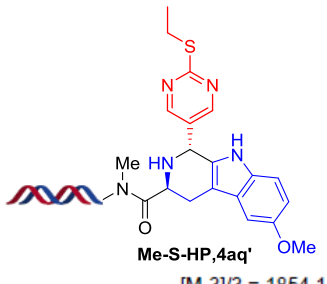
Following **General Procedure 2**

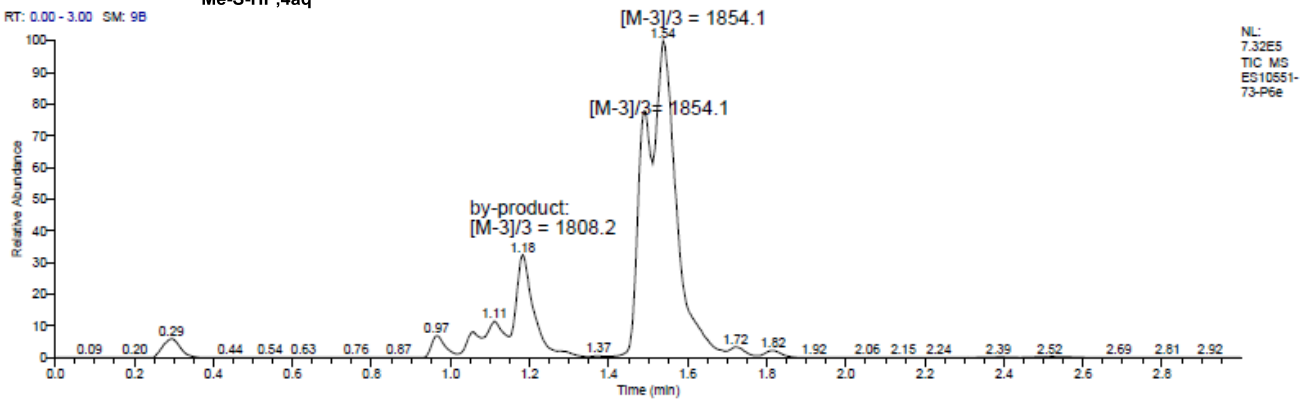
Percent conversion: 23.84% & 53.99%, totally 77.83%

Exact mass: 5565.19

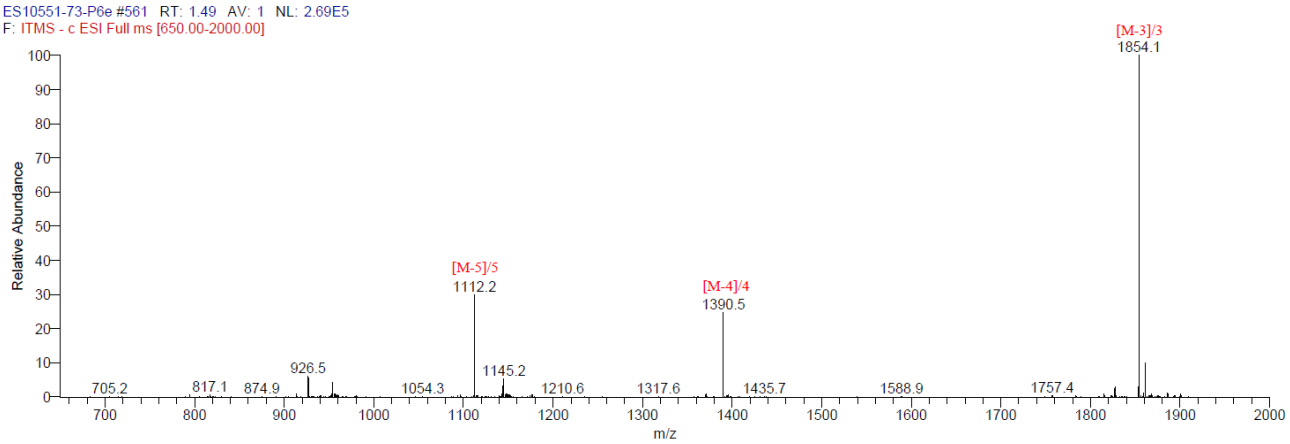
Triply charged mass [M-3]/3, calculated: 1854.06; observed:1854.1







RT	Peak Height	Peak Width	Peak Area	Area %
0.97	58516.51	0.15	129060.76	2.43
1.11	90505.79	0.08	298007.28	5.61
1.18	255398.20	0.19	749718.65	14.12
1.49	612901.23	0.11	1265538.53	23.84
1.54	749602.53	0.27	2866059.89	53.99



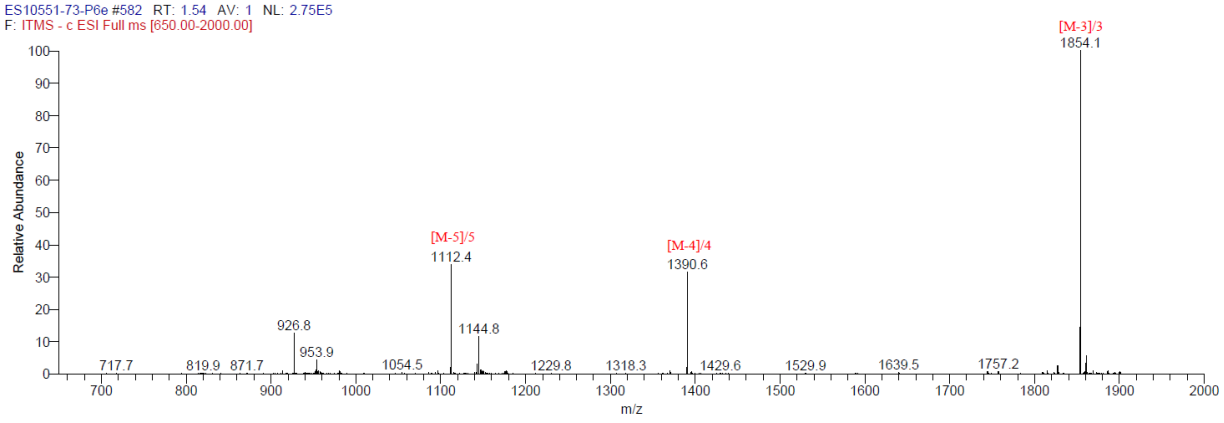
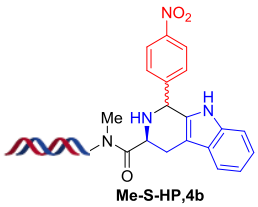


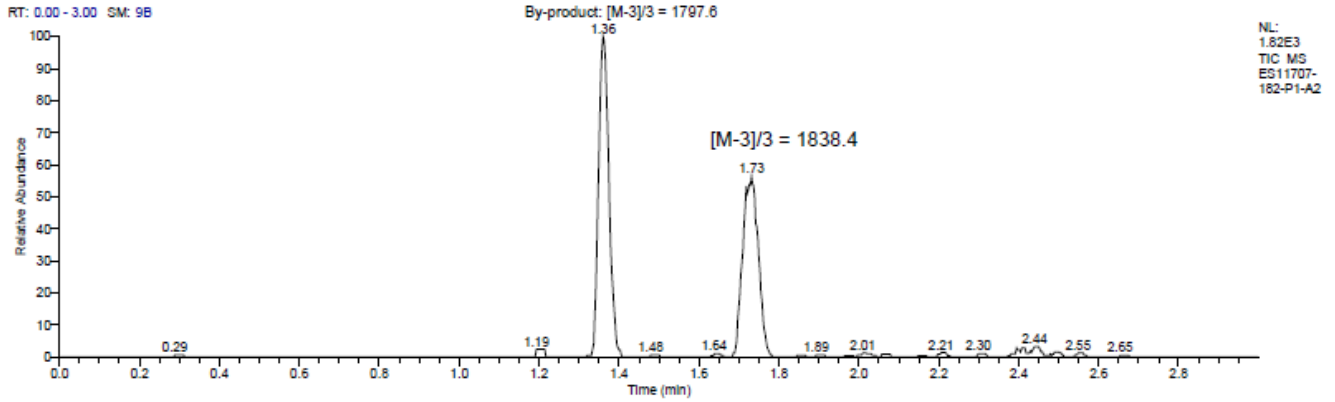
Fig. S25. LC trace and mass of **4aq and 4aq'**

Figure S26, Trace and Mass of 4b, related to Figure 5.

Following **General Procedure 2**
Percent conversion: 45.97%
Exact mass: 5518.07
Triply charged mass [M-3]/3, calculated: 1838.36; observed:1838.4



Me-S-HP,4b



RT	Peak Height	Peak Width	Peak Area	Area %
1.36	2199.16	0.10	3286.43	54.03
1.73	1145.73	0.11	2796.18	45.97

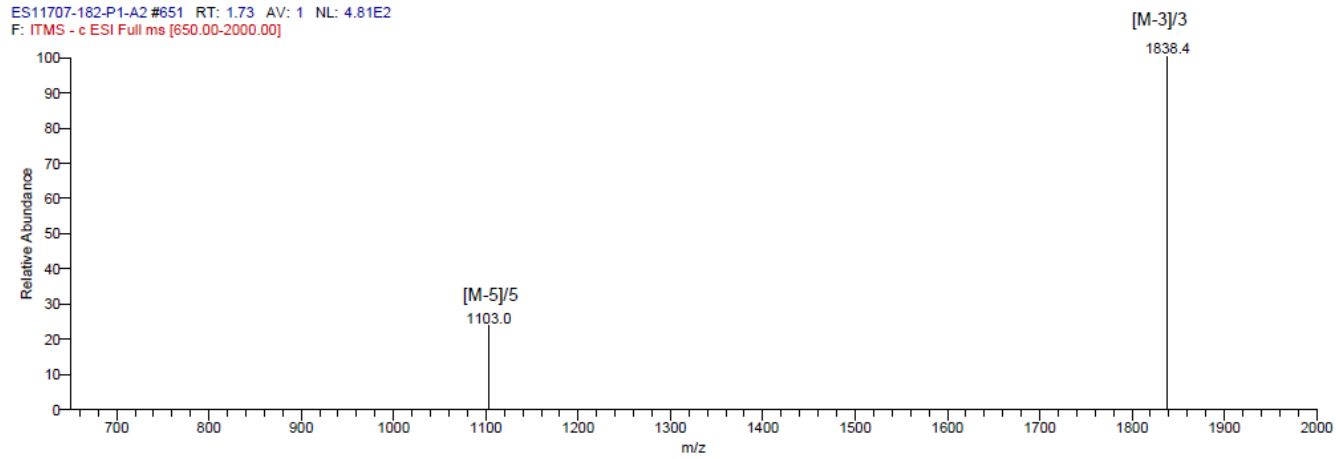
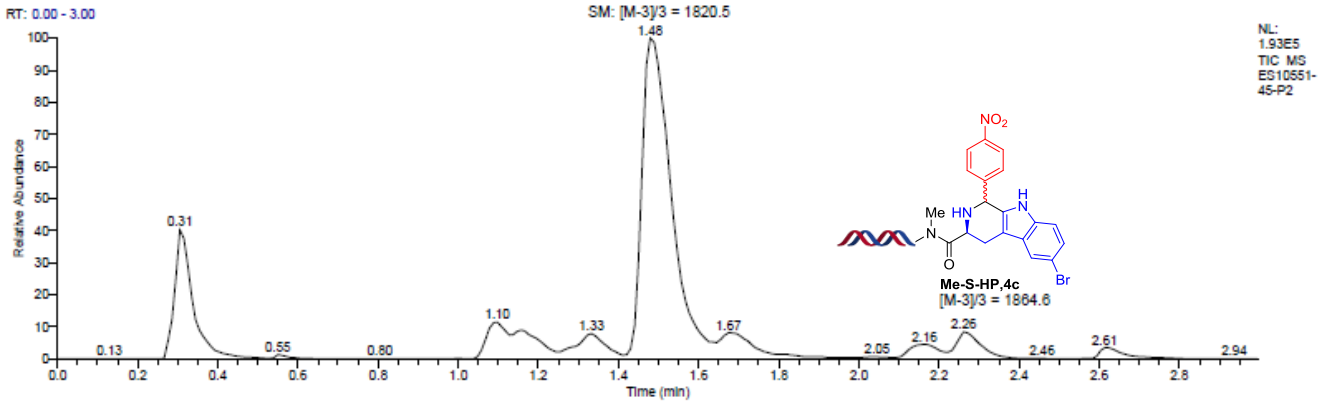


Fig. S26. LC trace and mass of **4b**.

Figure S27, Trace and Mass of 4c, related to Figure 5.

Following **General Procedure 2**
Percent conversion: 5.16%
Exact mass: 5596.96

Triply charged mass [M-3]/3, calculated: 1864.65; observed:1864.6



RT	Peak Height	Peak Width	Peak Area	Area %
1.10	20412.54	0.12	82941.93	5.68
1.16	16491.42	0.11	76927.03	5.27
1.33	13968.92	0.17	83428.48	5.71
1.49	181670.50	0.22	987404.57	67.62
1.68	15236.92	0.33	110163.62	7.54
2.16	8545.71	0.14	43981.62	3.01
2.27	14607.89	0.29	75282.50	5.16

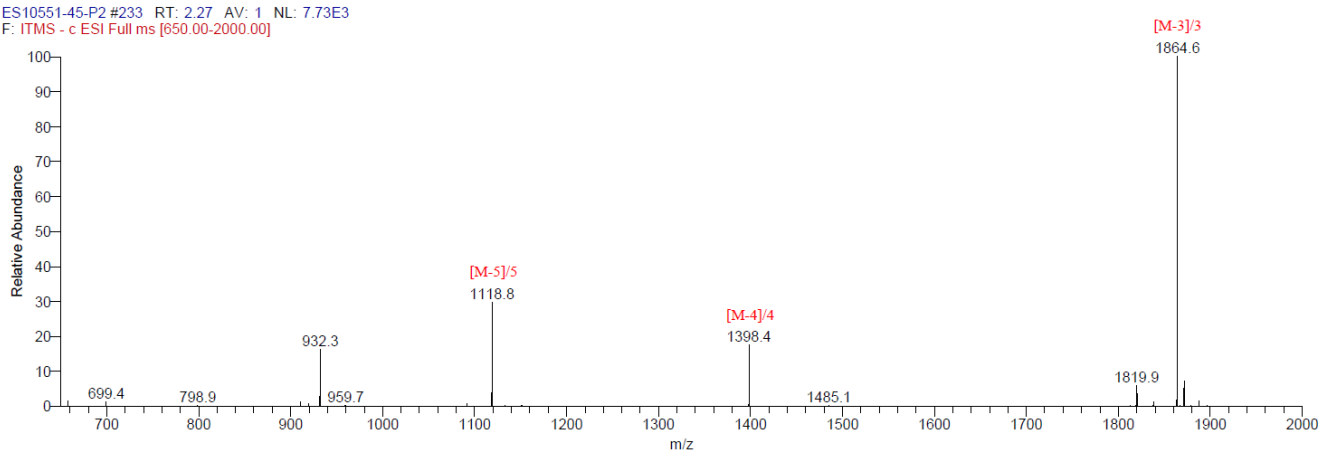
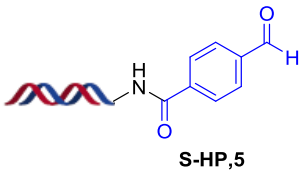
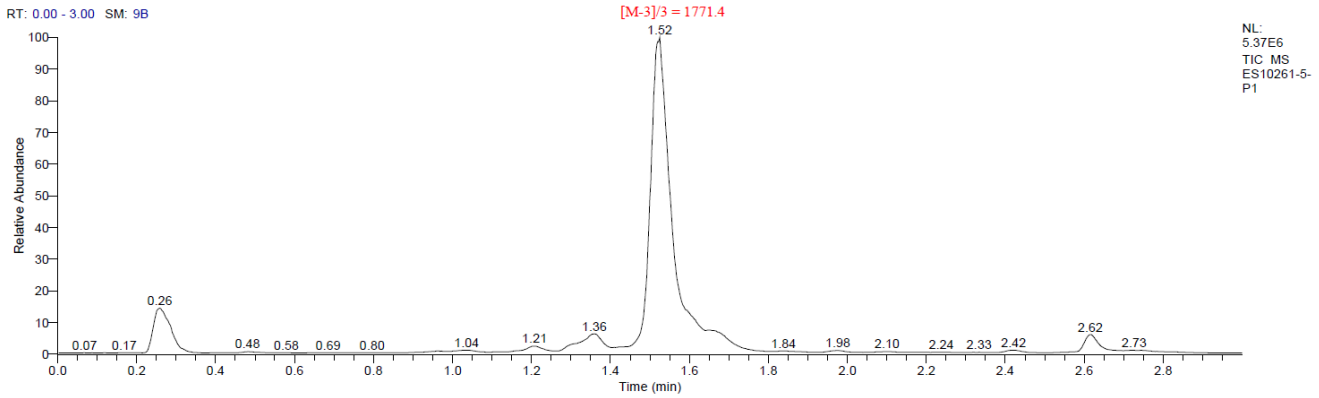


Fig. S27. LC trace and mass of **4c**.

Figure S28, Trace and Mass of 5, related to Figure 5.

Following **General Procedure 1**
Purity: 90.83%
Exact mass: 5316.61
Triply charged mass [M-3]/3, calculated: 1771.2; observed:1771.4





RT	Peak Height	Peak Width	Peak Area	Area %
1.03	41986.16	0.10	148617.42	0.75
1.21	100941.19	0.13	278799.36	1.40
1.36	248195.90	0.10	608614.27	3.05
1.52	5604412.58	0.22	18100890.92	90.83
2.61	325012.89	0.18	791440.10	3.97

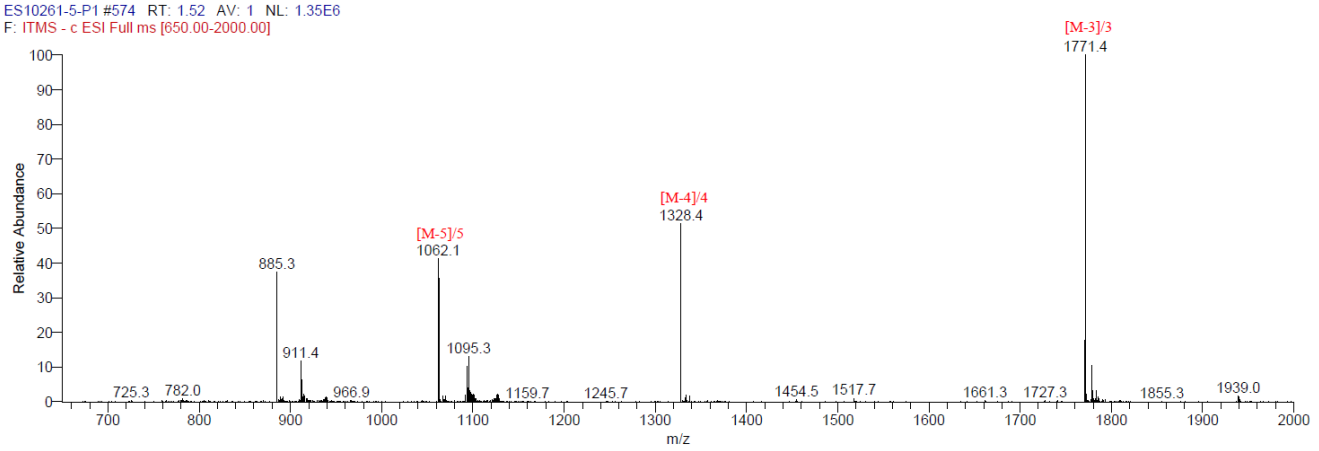
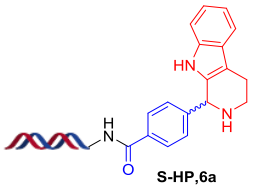


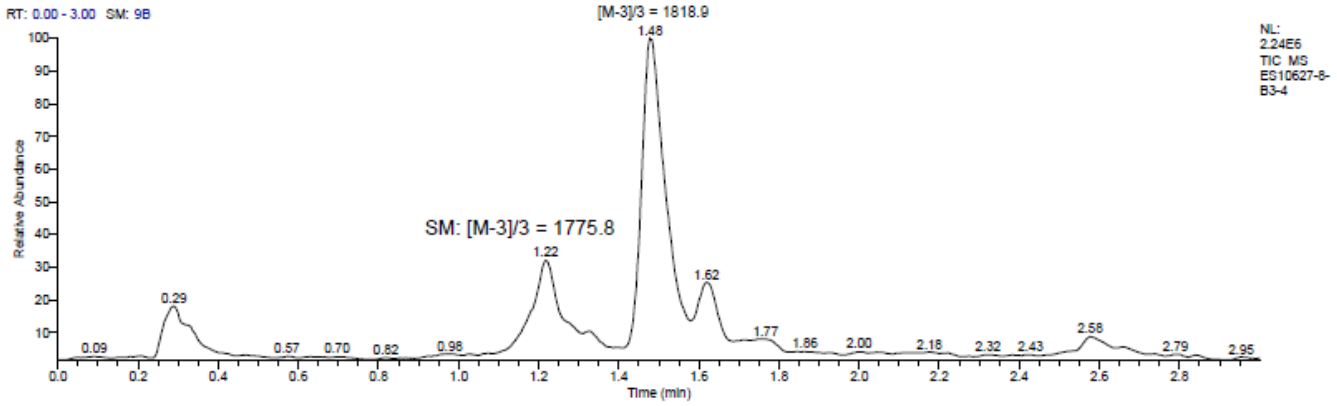
Fig. S28. LC trace and mass of **5.**

Figure S29, Trace and Mass of 6a, related to Figure 5.

Following **General Procedure 3**
Percent conversion: 64.42%
Exact mass: 5458.79
Triply charged mass [M-3]/3, calculated: 1818.60; observed:1818.9



S-HP,6a



RT	Peak Height	Peak Width	Peak Area	Area %
1.22	641282.46	0.21	2933774.86	21.80
1.33	120328.96	0.07	283924.03	2.11
1.48	2209307.88	0.17	8670371.09	64.42
1.62	449223.63	0.10	1397600.11	10.38
1.71	30393.19	0.10	172945.78	1.29

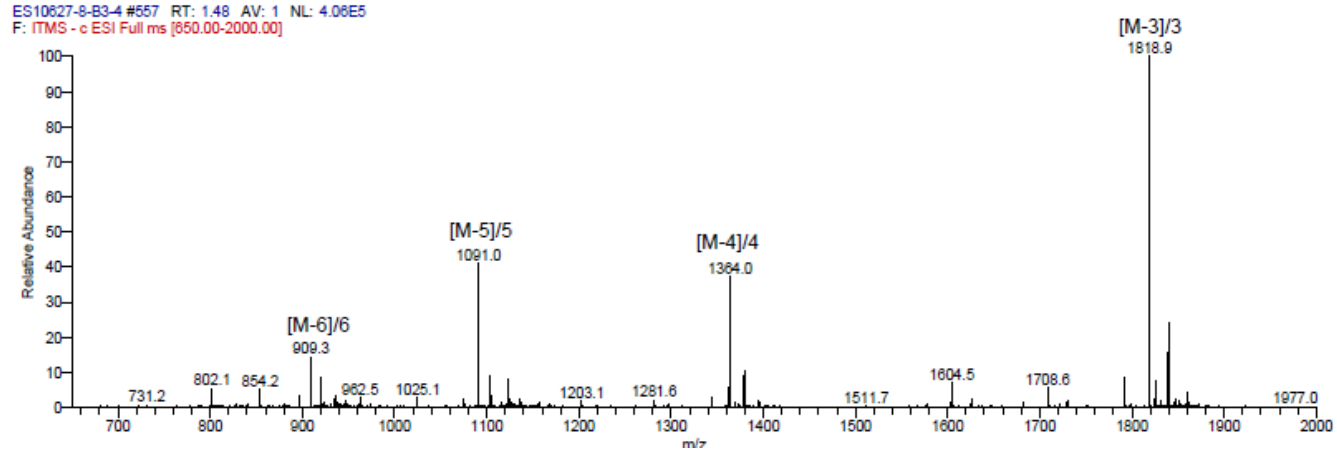
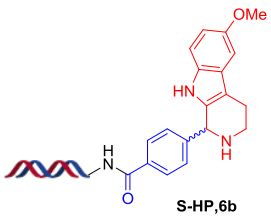


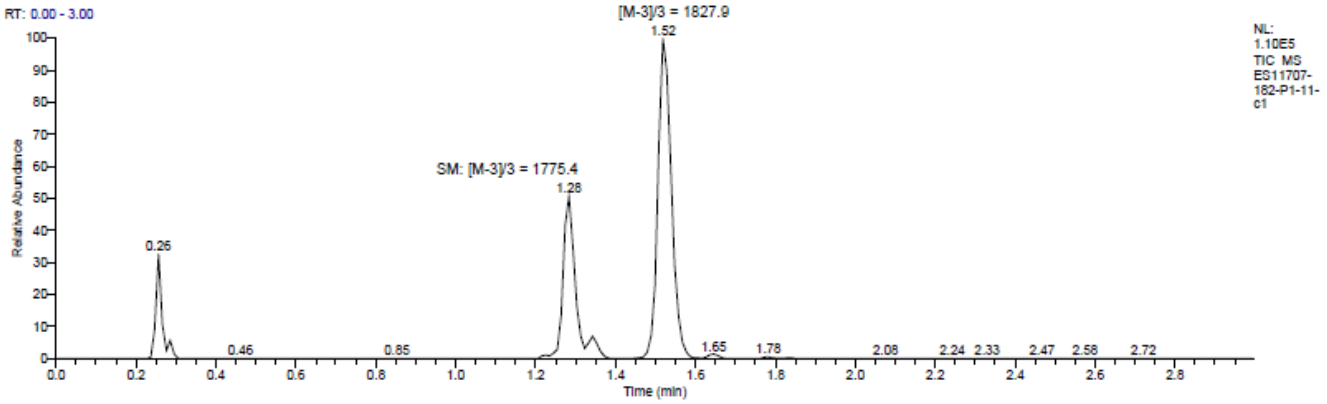
Fig. S29. LC trace and mass of **6a**

Figure S30, Trace and Mass of 6b, related to Figure 5.

Following **General Procedure 3**
Percent conversion: 66.67%
Exact mass: 5488.89
Triply charged mass [M-3]/3, calculated:1828.63; observed:1827.90



S-HP,6b



RT	Peak Height	Peak Width	Peak Area	Area %
1.28	40881.23	0.23	125893.36	32.48
1.52	84858.02	0.17	258459.05	66.67
1.65	1054.33	0.07	2445.48	0.63
1.78	372.92	0.08	861.06	0.22

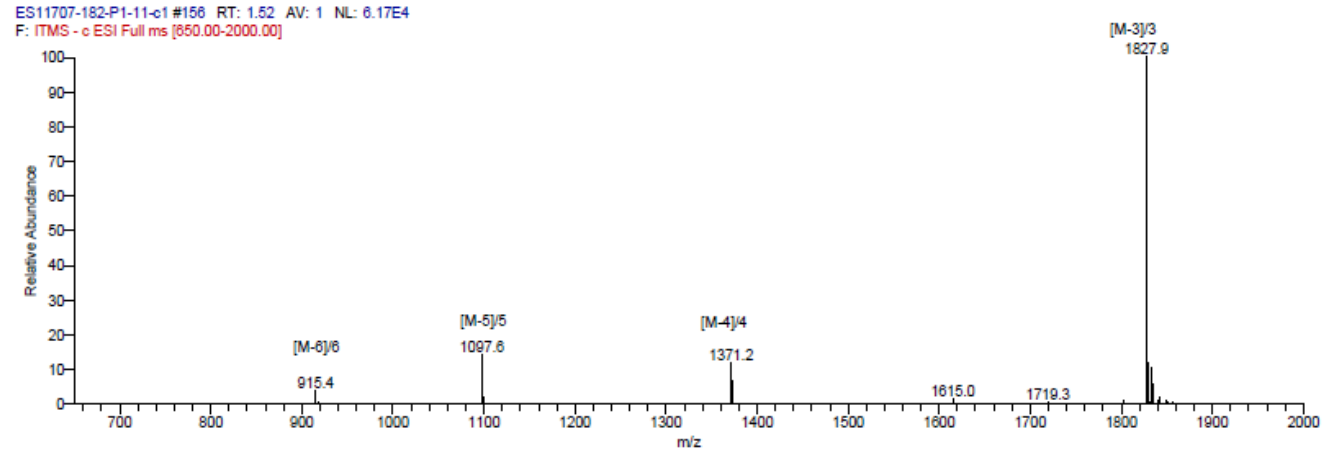
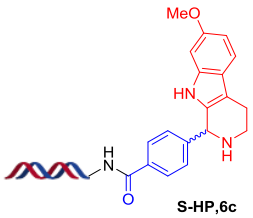


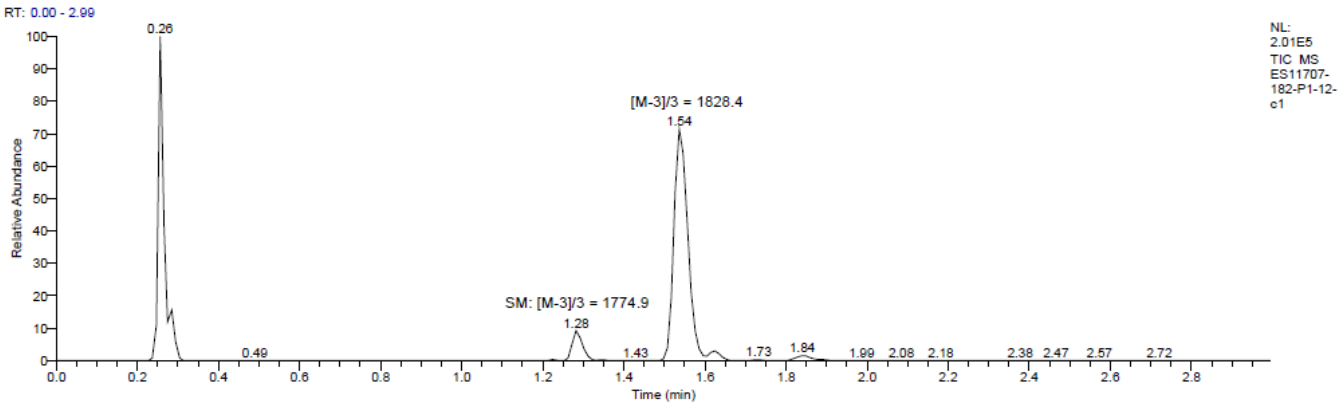
Fig. S30. LC trace and mass of **6b**

Figure S31, Trace and Mass of 6c, related to Figure 5.

Following **General Procedure 3**
Percent conversion: 89.64%
Exact mass: 5488.86
Triply charged mass [M-3]/3, calculated:1828.62; observed:1828.40



S-HP,6c



RT	Peak Height	Peak Width	Peak Area	Area %
1.22	303.99	0.04	437.66	0.11
1.28	12591.48	0.12	32813.77	8.31
1.54	112961.87	0.22	353818.78	89.64
1.73	426.03	0.06	479.66	0.12
1.84	2384.38	0.10	7163.25	1.81

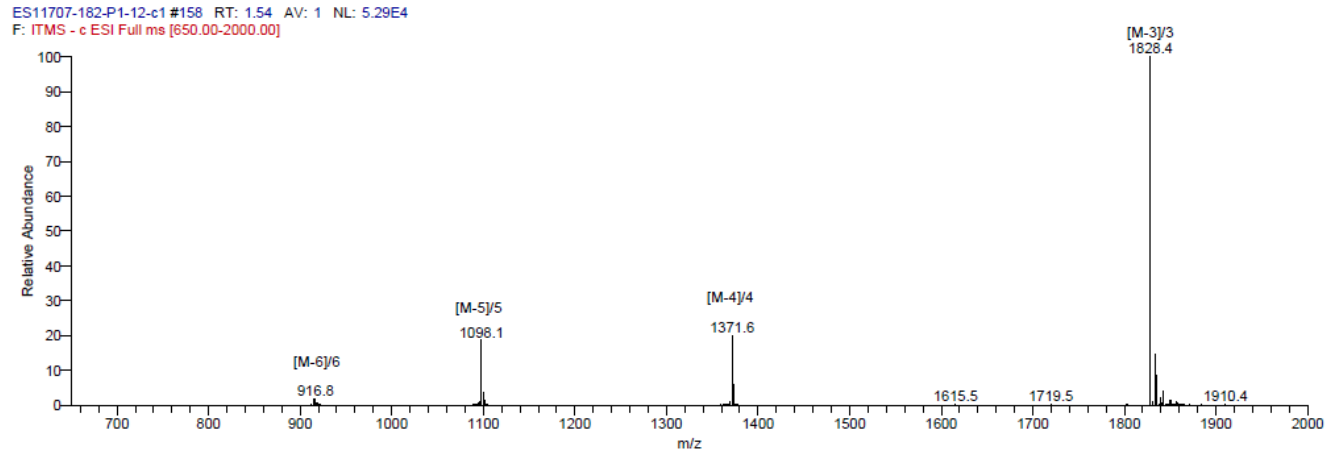
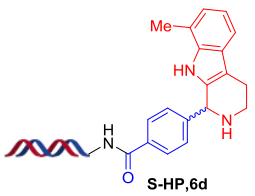
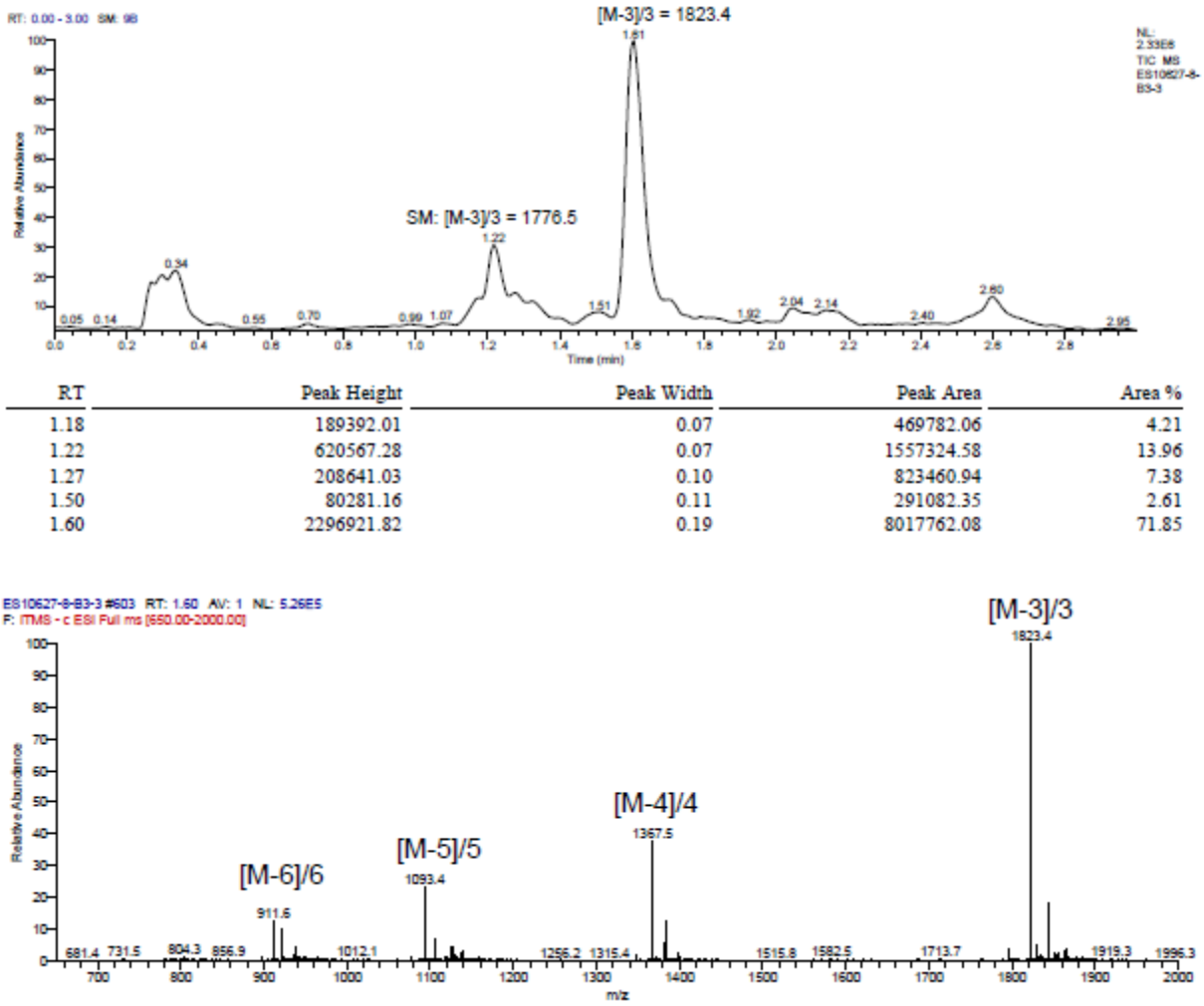


Fig. S31. LC trace and mass of **6c**

Figure S32, Trace and Mass of 6d, related to Figure 5.

Following **General Procedure 3**
Percent conversion: 71.85%
Exact mass: 5472.86
Triply charged mass [M-3]/3, calculated: 1823.86; observed:1823.4



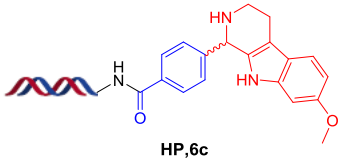


RT	Peak Height	Peak Width	Peak Area	Area %
1.18	189392.01	0.07	469782.06	4.21
1.22	620567.28	0.07	1557324.58	13.96
1.27	208641.03	0.10	823460.94	7.38
1.50	80281.16	0.11	291082.35	2.61
1.60	2296921.82	0.19	8017762.08	71.85

Fig. S32. LC trace and mass of **6d**

Figure S33, Mass Spectrum of 6c, related to Figure 5.

Following **General Procedure 3**
Percent conversion: 83.33%
Exact mass: 5241.59, observed mass:5241.95



HP,6c

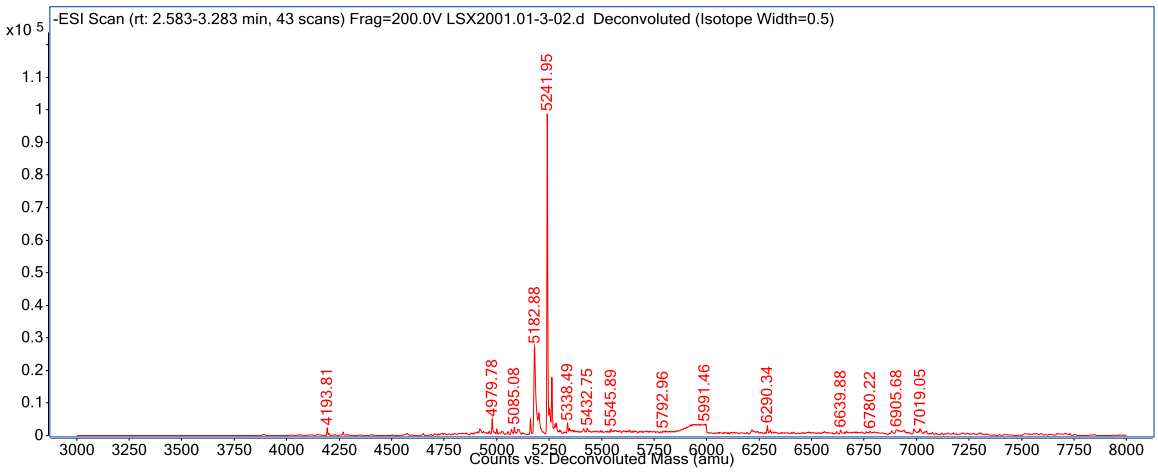
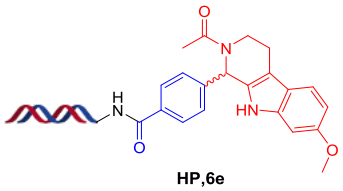


Fig. S33. Deconvoluted mass of **6c**

Figure S34, Mass Spectrum of 6e, related to Figure 5.

Following **General Procedure 4**
Percent conversion: 61.02%
Exact mass: 5283.62, observed mass:5284.02



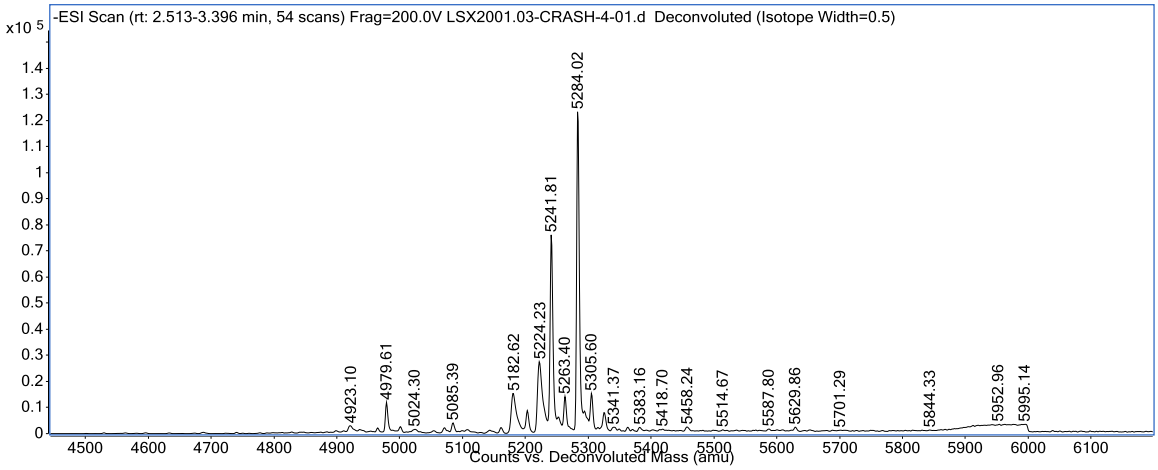


Fig. S34. Deconvoluted mass of **6e**

Figure S35, Mass Spectrum of 6f related to Figure 5.

Following **General Procedure 4**
Percent conversion: 84.06%
Exact mass: 5331.74, observed mass:5332.14

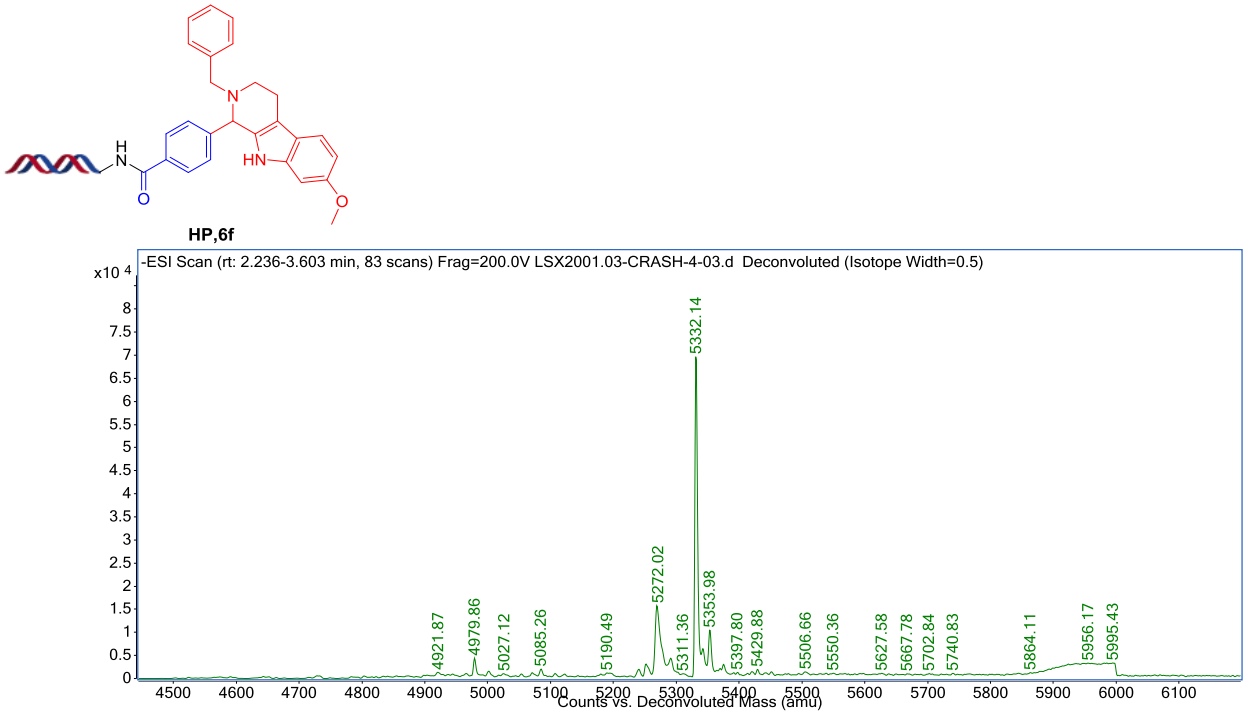


Fig. S35. Deconvoluted mass of **6f**

Figure S36, Mass Spectrum of 7a, related to Figure 5.

Percent conversion: 78%
Exact mass: 5229.57
Observed: 5230.09

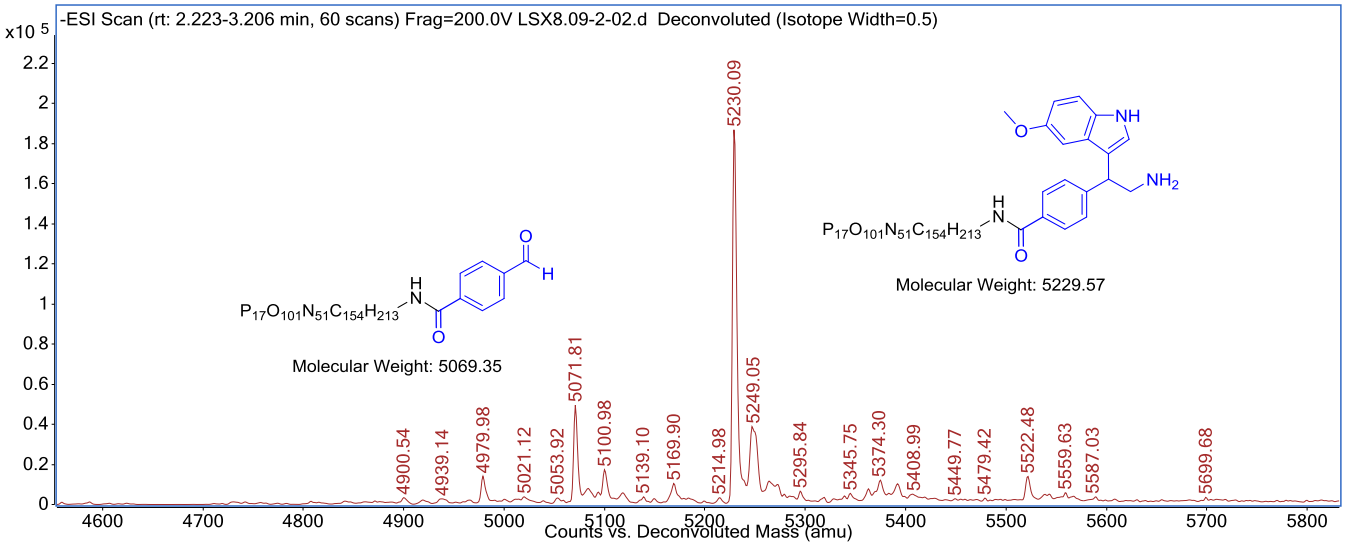


Fig.S36. Deconvoluted mass of **7a**

Figure S37, Mass Spectrum of 7b, related to Figure 5.

Percent conversion: 74.19%
Exact mass: 5229.57
Observed: 5230.13

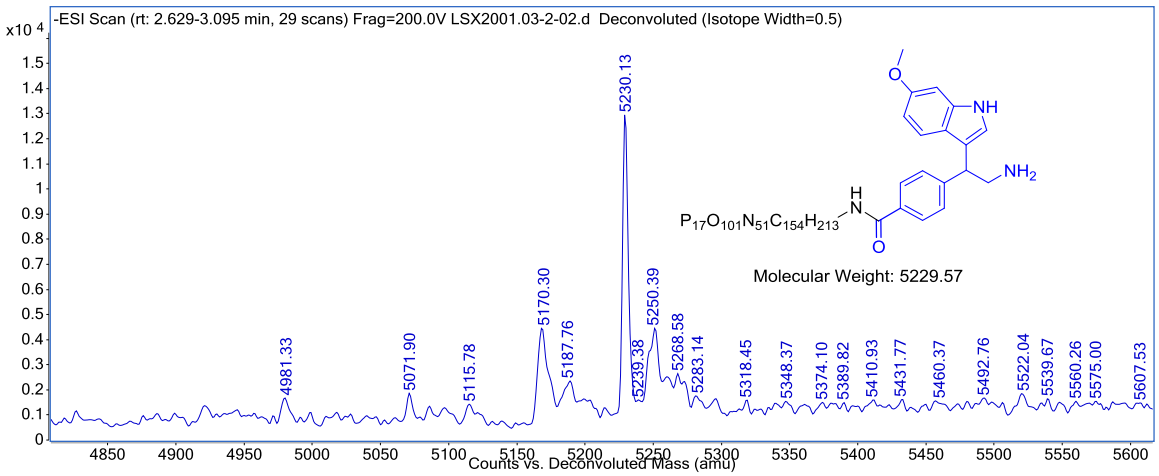


Fig. S37. Deconvoluted mass of **7b**

Figure S38, Mass Spectrum of 7c, related to Figure 5.

Percent conversion: 75.41%
Exact mass: 5259.59
Observed: 5260.14

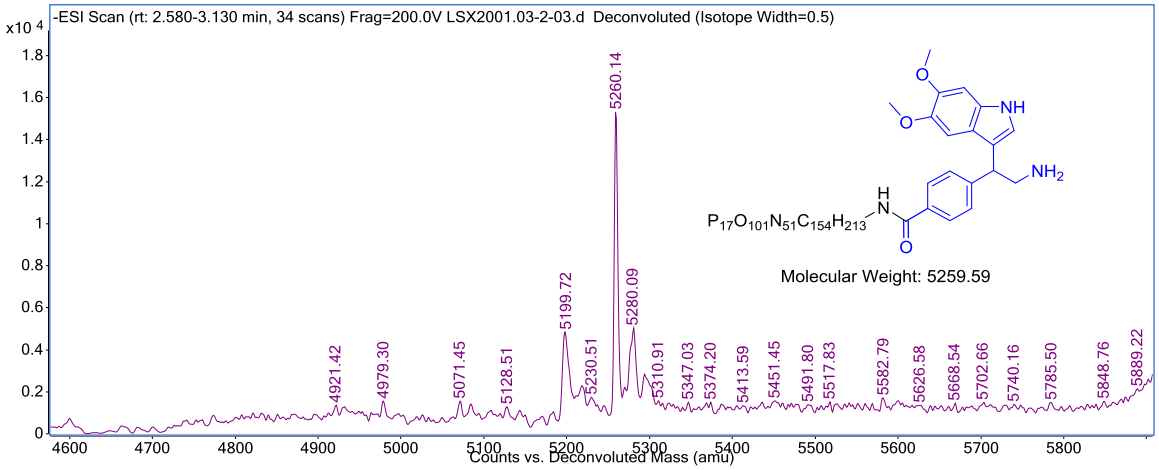


Fig. S38. Deconvoluted mass of **7c**

Figure S39, Mass Spectrum of 8a, related to Figure 5.

Percent conversion: 50%
Exact mass: 5362.67
Observed: 5362.87

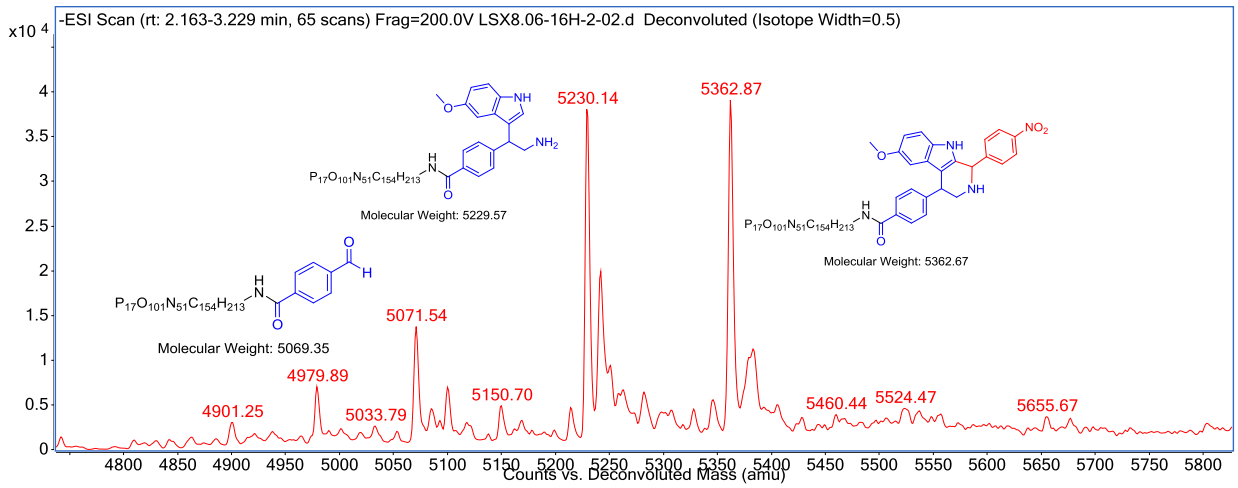


Fig. S39. Deconvoluted mass of **8a**

Figure S40, Mass Spectrum of 8b, related to Figure 5.

Percent conversion: 80.52%
Exact mass: 5476.46
Observed: 5477.12

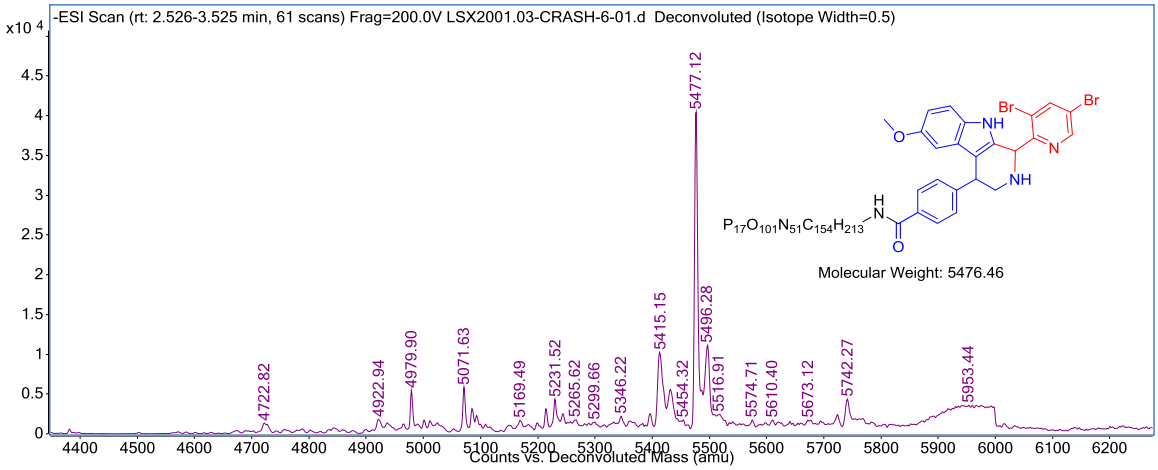


Fig. S40. Deconvoluted mass of **8b**

Figure S41, Mass Spectrum of 8b', related to Figure 5.

Percent conversion: 72.53%
Exact mass: 5580.57
Observed: 5581.35

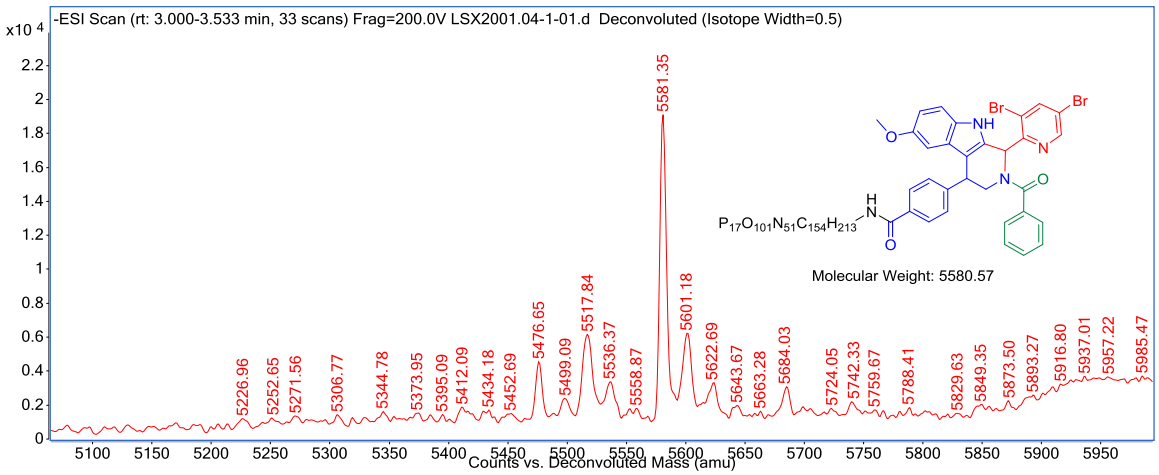


Fig. S41. Deconvoluted mass of **8b'**

Figure S42, Mass Spectrum of 8c, related to Figure 5.

Percent conversion: 80.52%
Exact mass: 5385.73
Observed: 5387.27

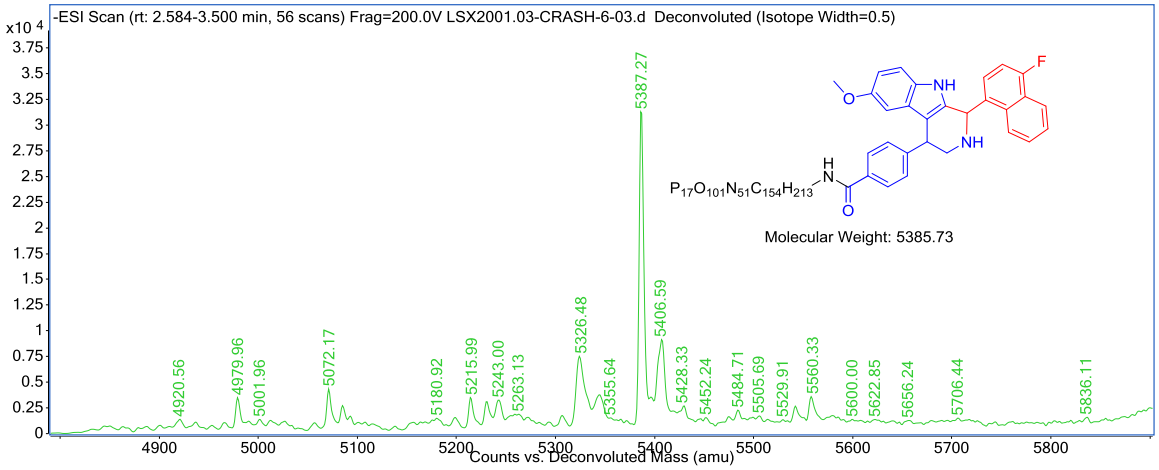


Fig. S42. Deconvoluted mass of **8c**

Figure S43, Mass Spectrum of 8c', related to Figure 5.

Percent conversion: 74.70%
Exact mass: 5489.84
Observed: 5491.59

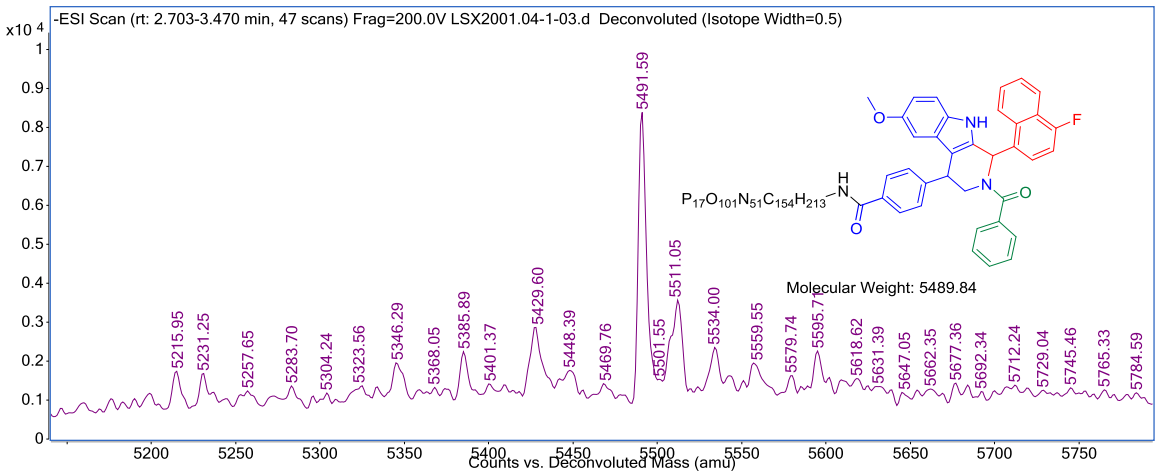


Fig. S43. Deconvoluted mass of **8c'**

Figure S44, Mass Spectrum of 8d, related to Figure 5.

Percent conversion: 66.99%
Exact mass: 5354.65
Observed: 5355.15

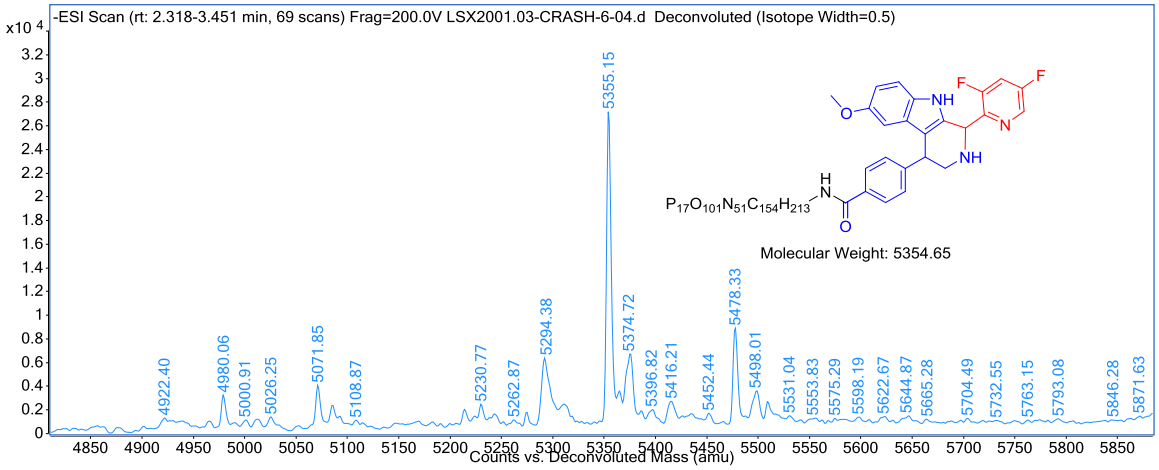


Fig. S44. Deconvoluted mass of **8d**

Figure S45, Mass Spectrum of 8d', related to Figure 5.

Percent conversion: 52.94%
Exact mass: 5458.75
Observed: 5459.34

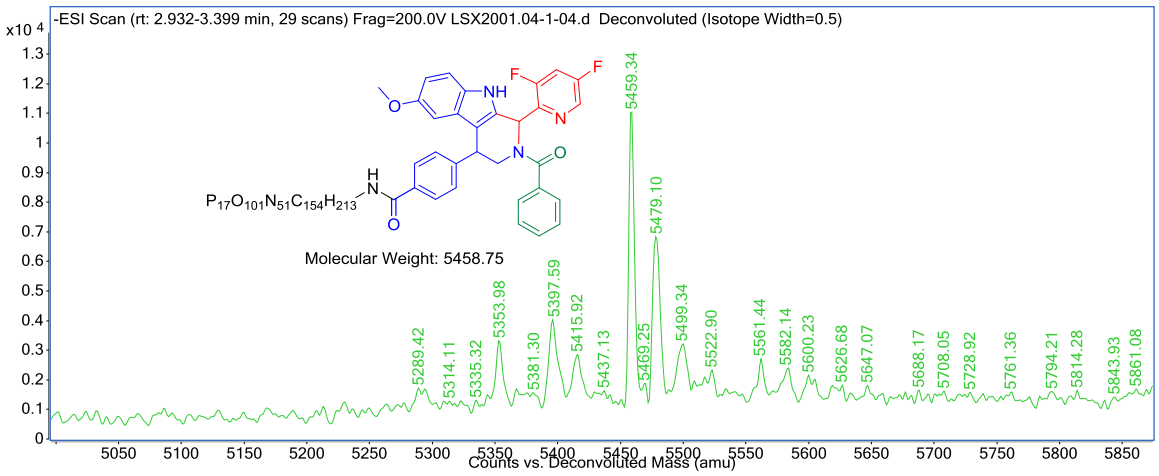


Fig. S45. Deconvoluted mass of **8d'**

Figure S46, Mass Spectrum of 8e, related to Figure 5.

Percent conversion: 43.95%
Exact mass: 5371.10
Observed: 5371.62

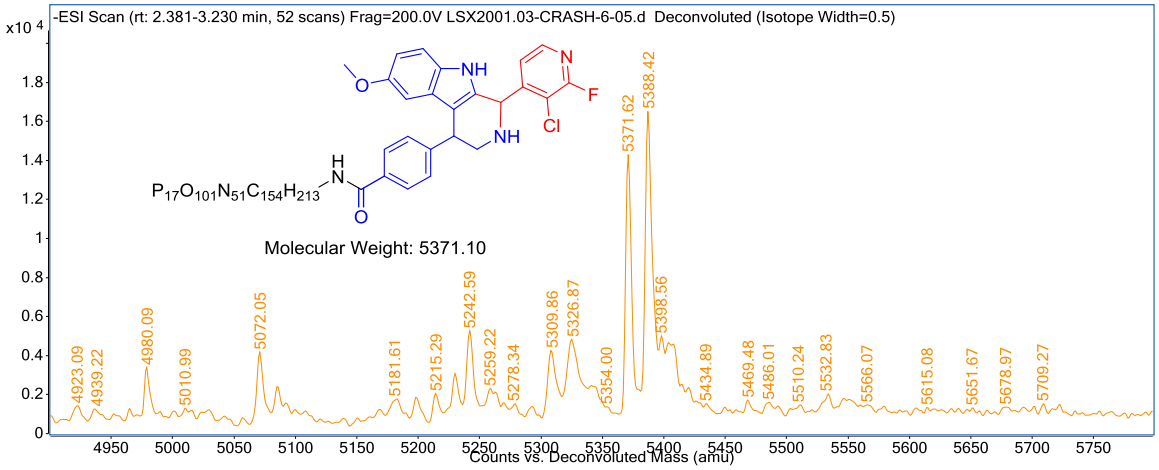


Fig. S46. Deconvoluted mass of **8e**

Figure S47, Mass Spectrum of 8f, related to Figure 5.

Percent conversion: 66.99%
Exact mass: 5414.81
Observed: 5414.79

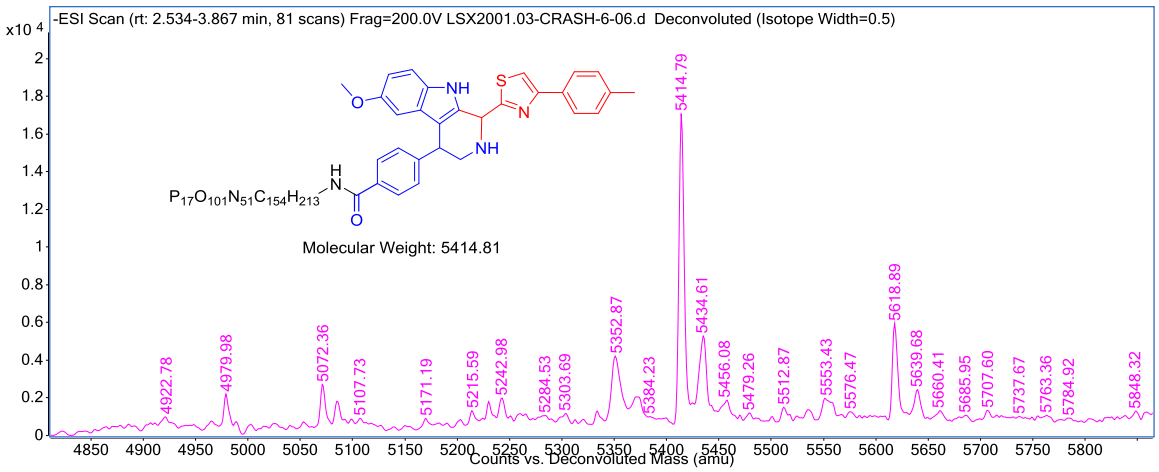


Fig. S47. Deconvoluted mass of **8f**

Figure S48, Mass Spectrum of 8f', related to Figure 5.

Percent conversion: 66.30%
Exact mass: 5504.94
Observed: 5505.49

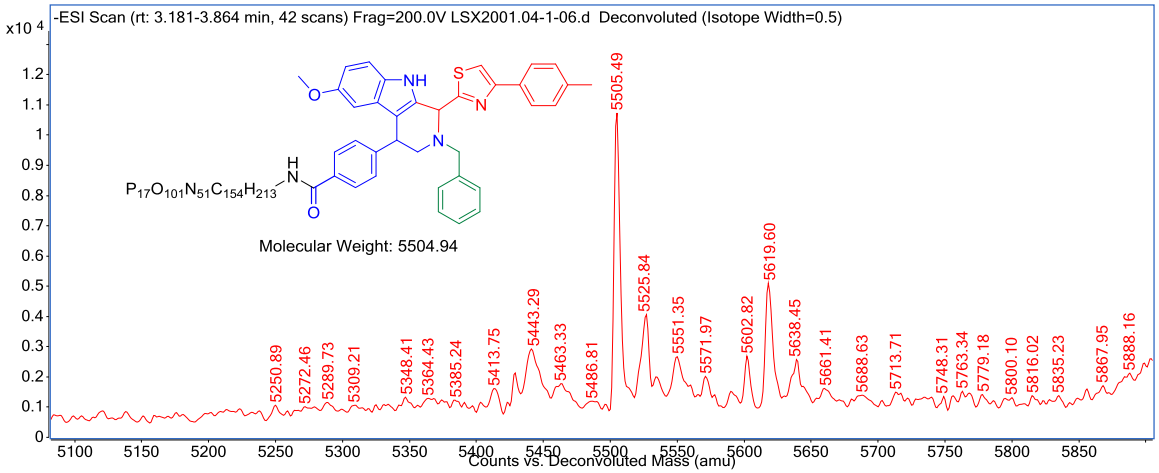


Fig. S48. Deconvoluted mass of **8f'**

Figure S49, Mass Spectrum of 8g, related to Figure 5.

Percent conversion: 75.96%
Exact mass: 5371.10
Observed: 5371.59

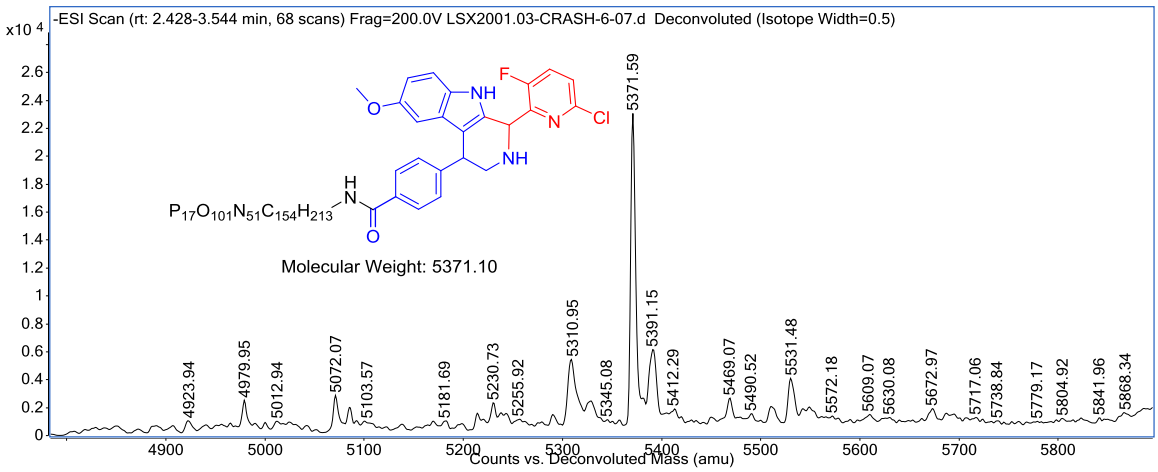


Fig. S49. Deconvoluted mass of **8g**

Figure S50, Mass Spectrum of 8g', related to Figure 5.

Percent conversion: 83.56%
Exact mass: 5461.22
Observed: 5461.86

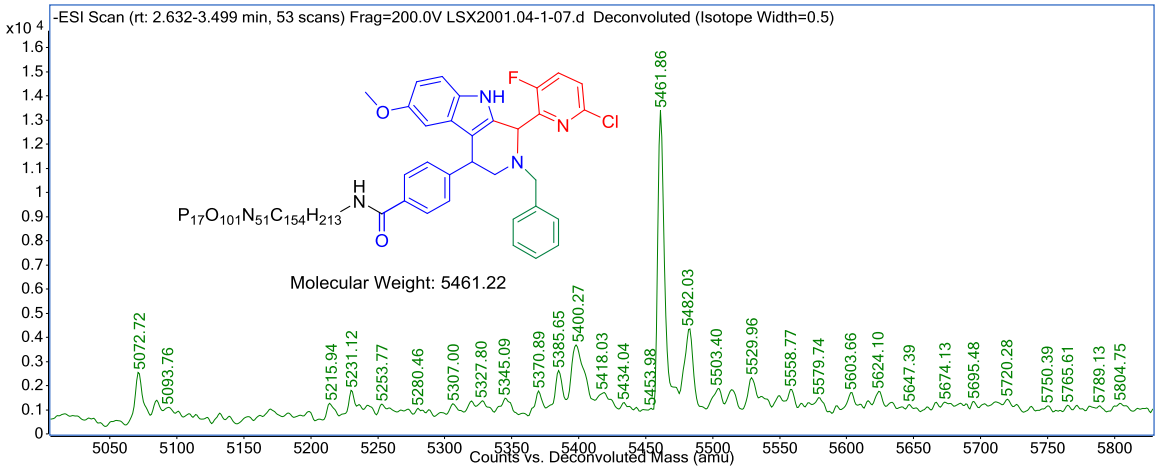


Fig. S50. Deconvoluted mass of **8g'**

Figure S51, Mass Spectrum of 8h, related to Figure 5.

Percent conversion: 73.58%
Exact mass: 5358.69
Observed: 5359.30

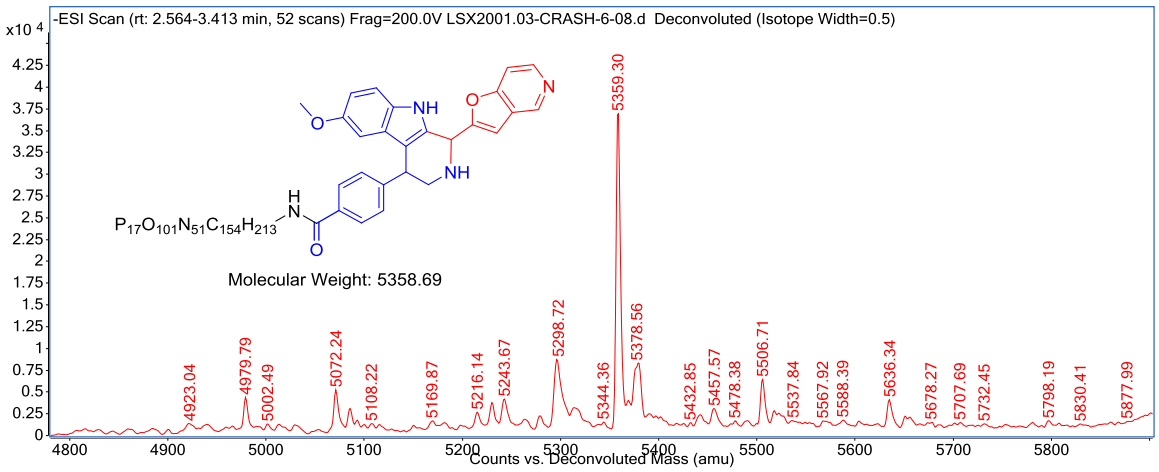


Fig. S51. Deconvoluted mass of **8h**

Figure S52, Mass Spectrum of 8h', related to Figure 5.

Percent conversion: 70.45%
Exact mass: 5448.81
Observed: 5449.44

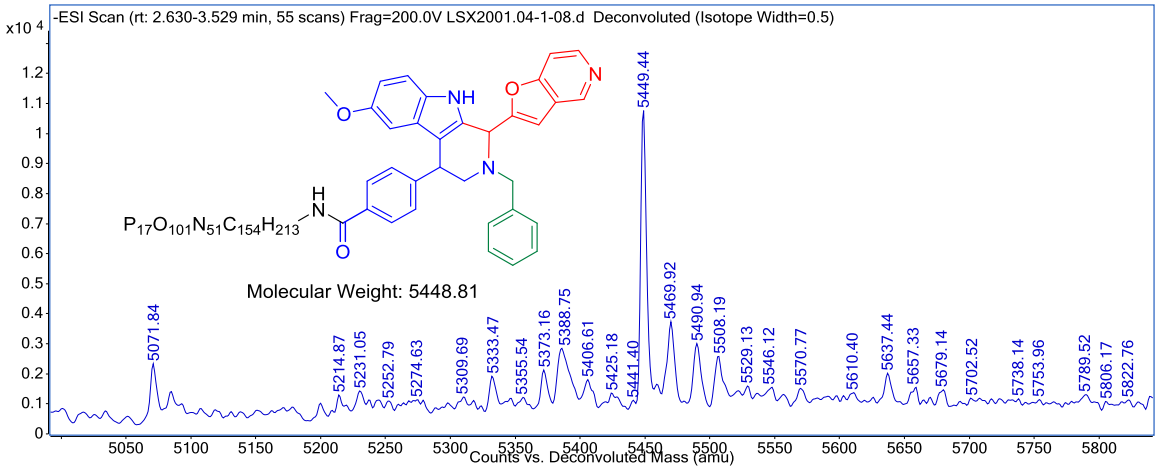


Fig. S52. Deconvoluted mass of **8h'**

Figure S53, Mass Spectrum of 8i, related to Figure 5.

Percent conversion: 54.66%
Exact mass: 5380.67
Observed: 5380.94

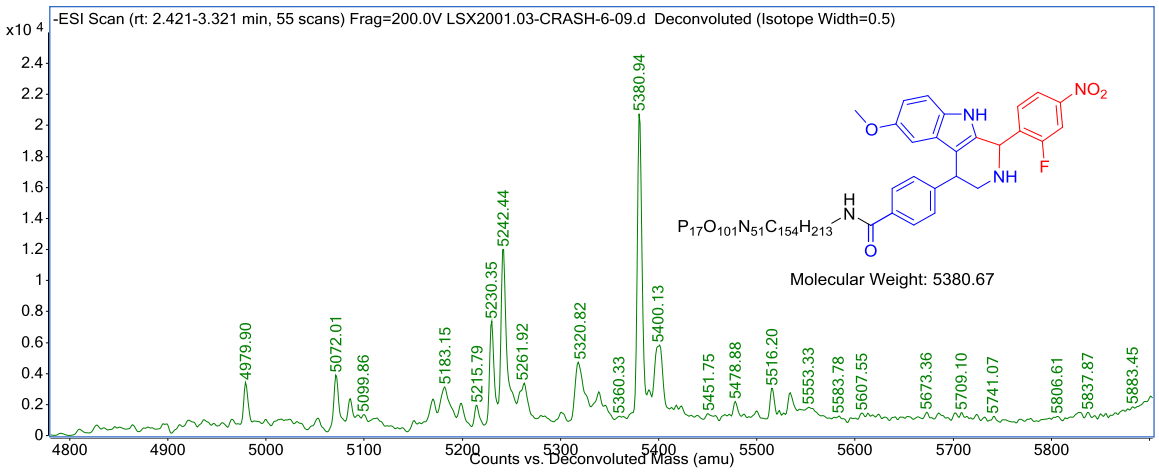


Fig. S53. Deconvoluted mass of **8i**

Figure S54, Mass Spectrum of 8i', related to Figure 5.

Percent conversion: 54.78%
Exact mass: 5470.79
Observed: 5471.55

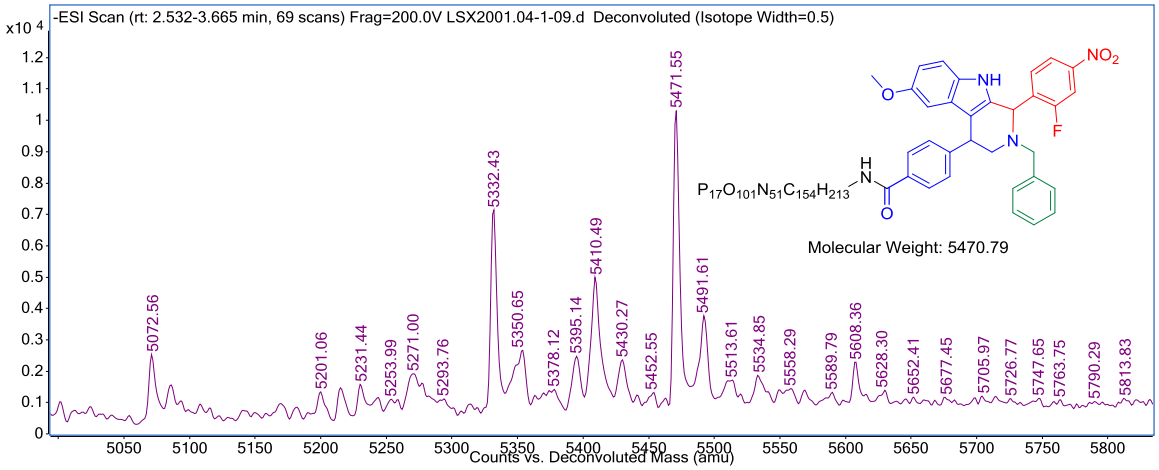


Fig. S54. Deconvoluted mass of **8i'**

Figure S55, Mass Spectrum of j, related to Figure 5.

Percent conversion: 69.07%
Exact mass: 5346.72
Observed: 5347.18

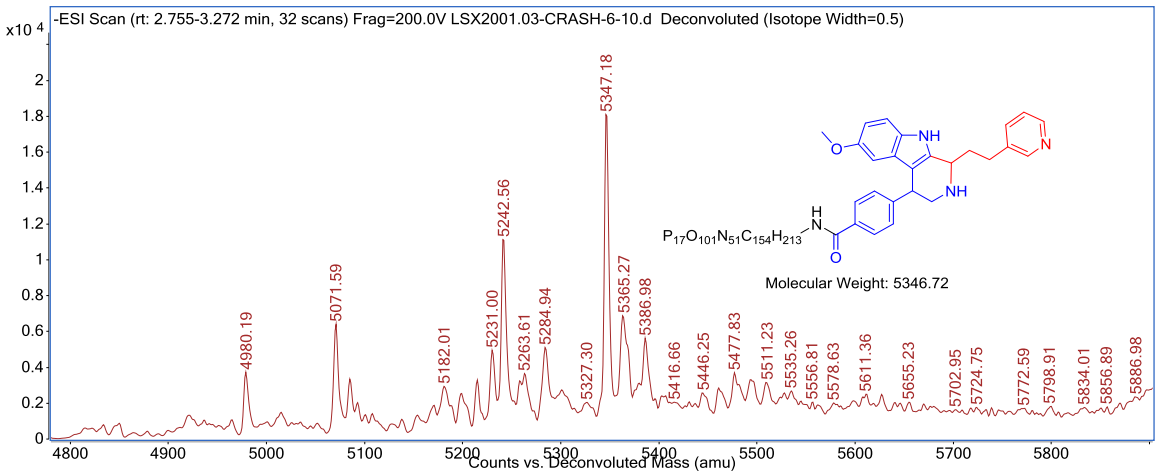


Fig. S55. Deconvoluted mass of **8j**

Figure S56, Mass Spectrum of 8k, related to Figure 5.

Percent conversion: 75.81%
Exact mass: 5362.67
Observed: 5361.87

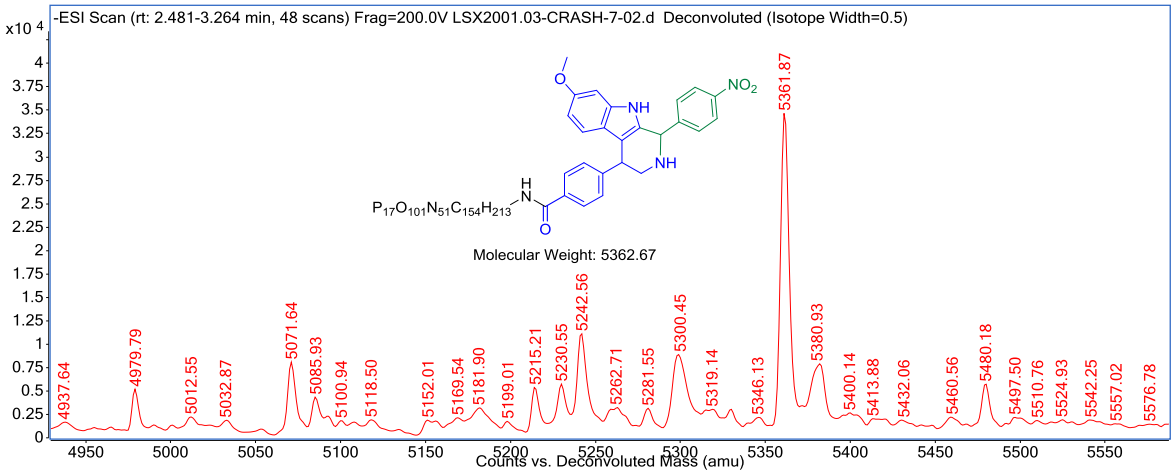


Fig. S56. Deconvoluted mass of **8k**

Figure S57, Mass Spectrum of 8l, related to Figure 5.

Percent conversion: 81.48%
Exact mass: 5392.70
Observed: 5391.74

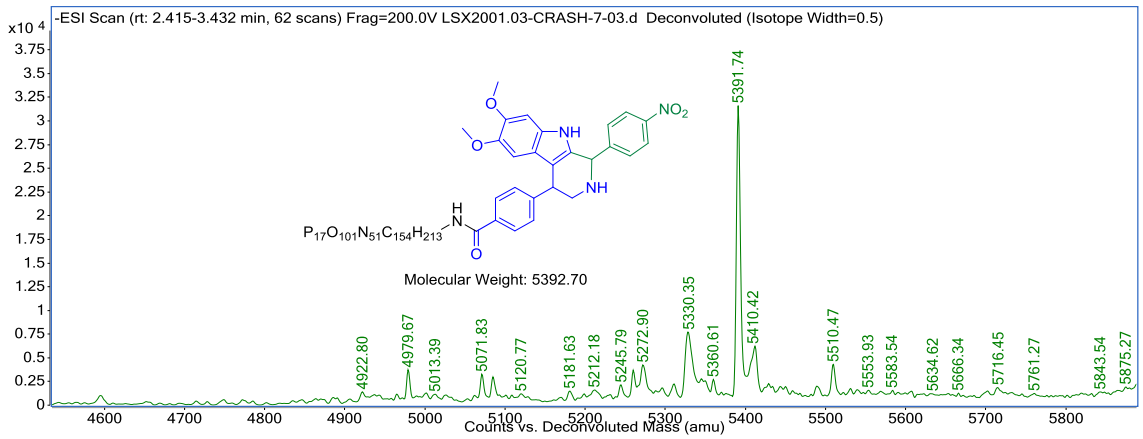


Fig. S57. Deconvoluted mass of **8l**

Figure S58, Mass Spectrum of 3a, related to Figure 3.

Exact mass: 5414.79
Observed: 5415.15

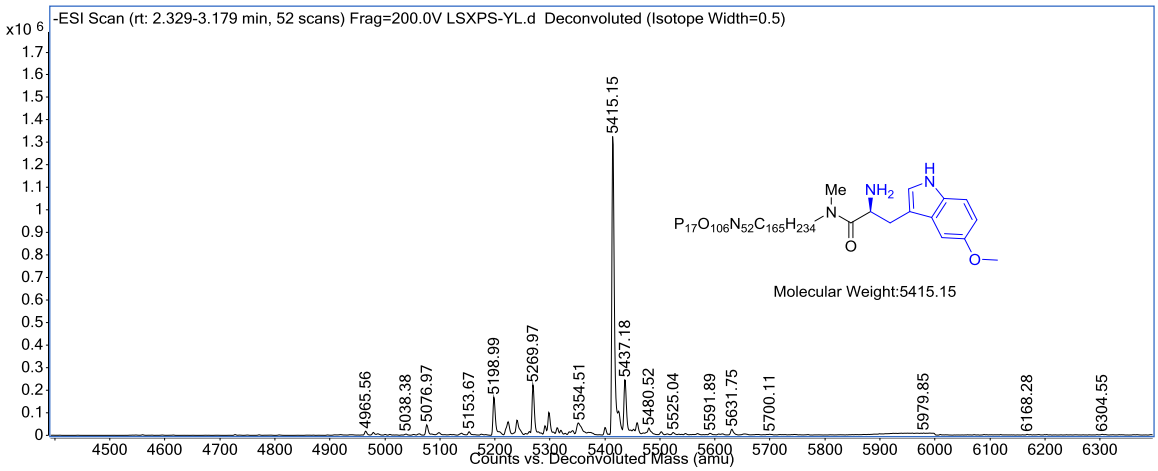


Fig. S58. Deconvoluted mass of on-DNA product **3a**

Figure S59, Mass Spectrum of 4ea, related to Figure 4.

Percent conversion: 83.84%
Exact mass: 5545.90
Observed: 5546.20

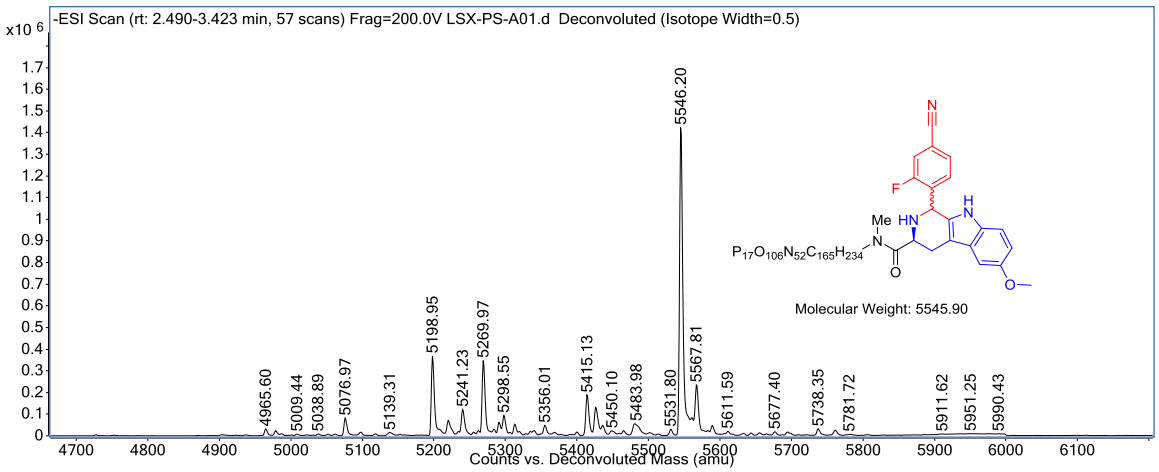


Fig. S59. Deconvoluted mass of **4ea**

Figure S60, Mass Spectrum of 4eb, related to Figure 4.

Percent conversion: 88.04%
Exact mass:5620.45
Observed: 5620.75

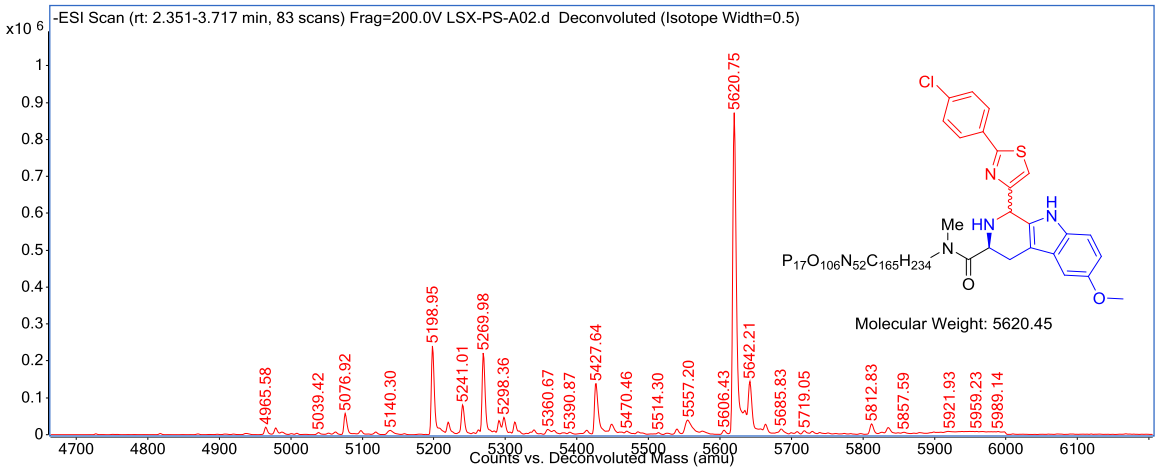


Fig. S60. Deconvoluted mass of **4eb**

Figure S61, Mass Spectrum of 4ec, related to Figure 4.

Percent conversion: 84.47%
Exact mass: 5542.92
Observed: 5542.90

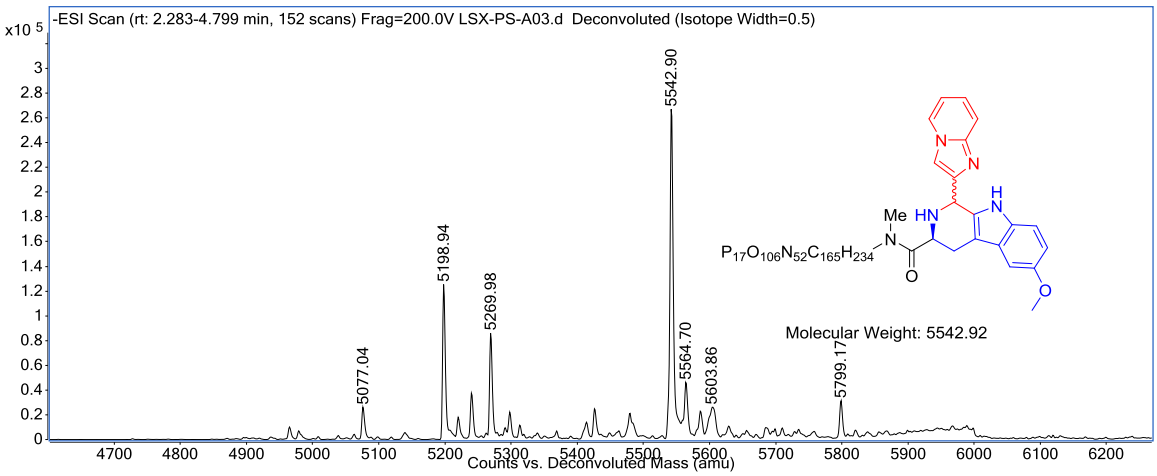


Fig. S61. Deconvoluted mass **4ec**

Figure S62, Mass Spectrum of 4ed, related to Figure 4.

Percent conversion: 91.51%
Exact mass: 5661.68
Observed: 5662.16

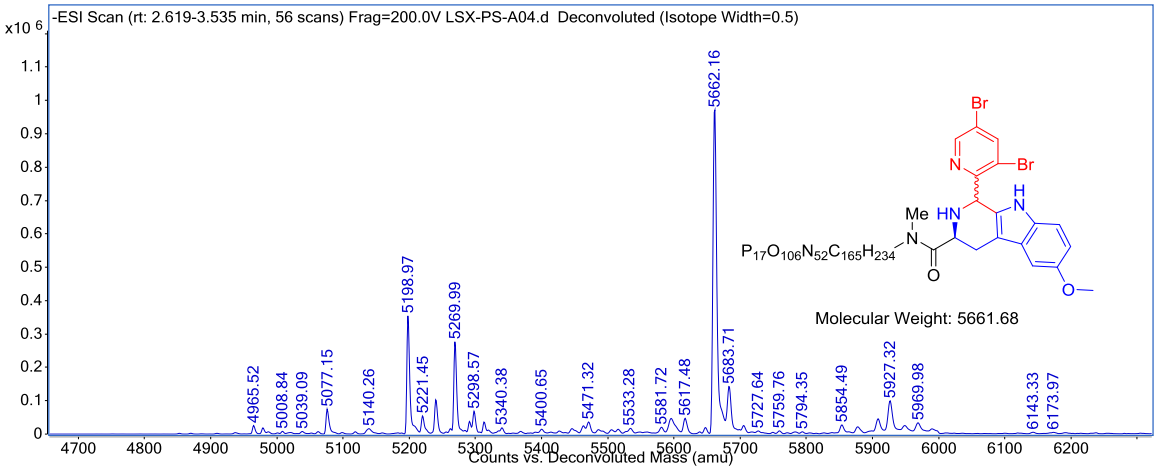


Fig. S62. Deconvoluted mass of **4ed**

Figure S63, Mass Spectrum of 4ee, related to Figure 4.

Percent conversion: 77.91%
Exact mass: 5604.00
Observed: 5604.31

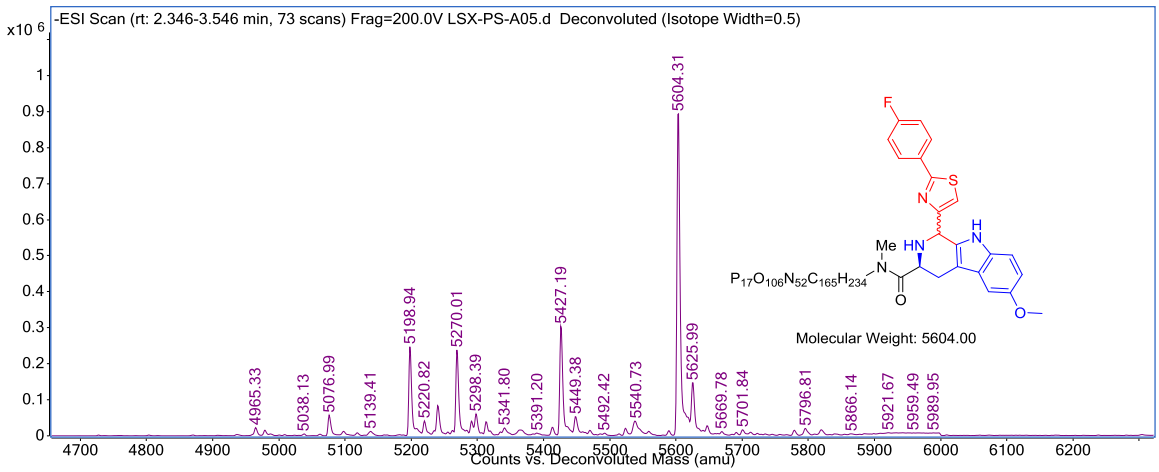


Fig. S63. Deconvoluted mass of **4ee**

Figure S64, Mass Spectrum of 4ef, related to Figure 4.

Percent conversion: 35.04%
Exact mass: 5572.77
Observed: 5573.14

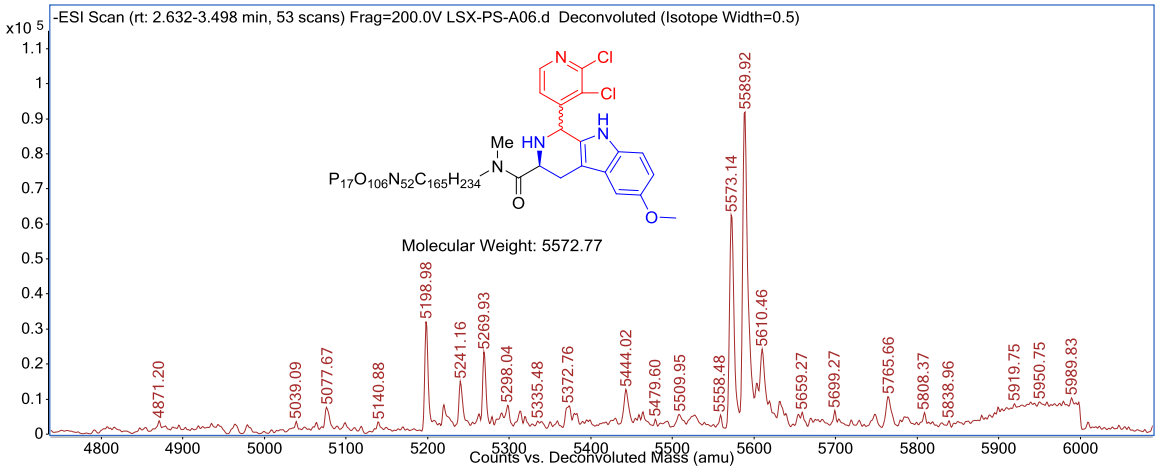


Fig. S64. Deconvoluted mass of **4ef**

Figure S65, Mass Spectrum of 4eg, related to Figure 4.

Percent conversion: 87.51%
Exact mass: 5592.03
Observed: 5592.36

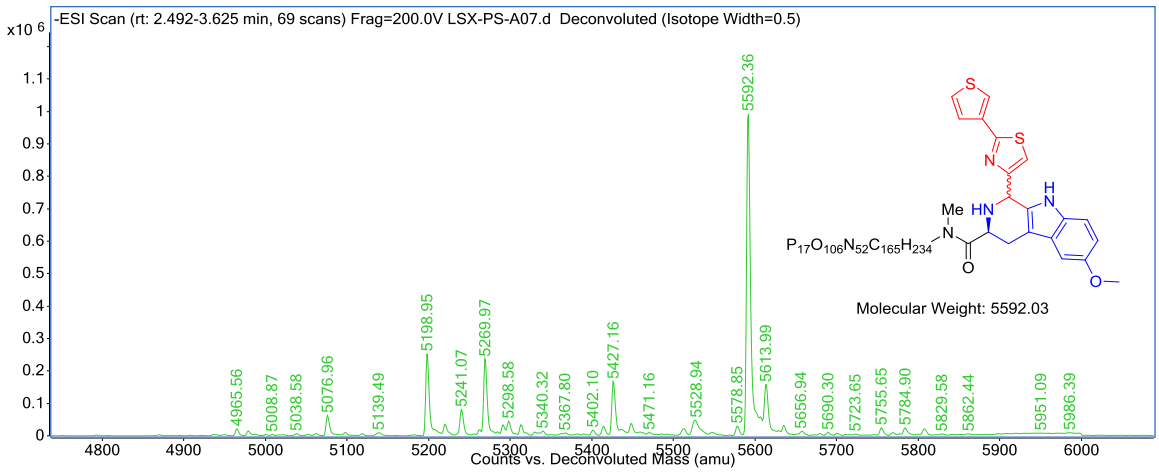


Fig. S65. Deconvoluted mass of **4eg**

Figure S66, Mass Spectrum of 4eh, related to Figure 4.

Percent conversion: 98%
Exact mass: 5577.91
Observed: 5578.28

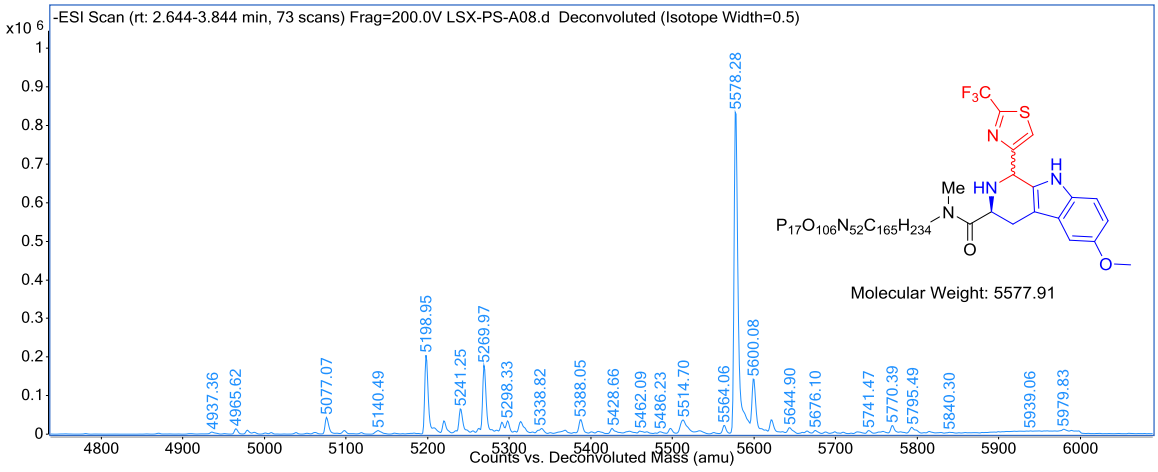


Fig. S66. Deconvoluted mass of **4eh**

Figure S67, Mass Spectrum of 4ei, related to Figure 4.

Percent conversion: 30.22%
Exact mass: 5617.22
Observed: 5617.60

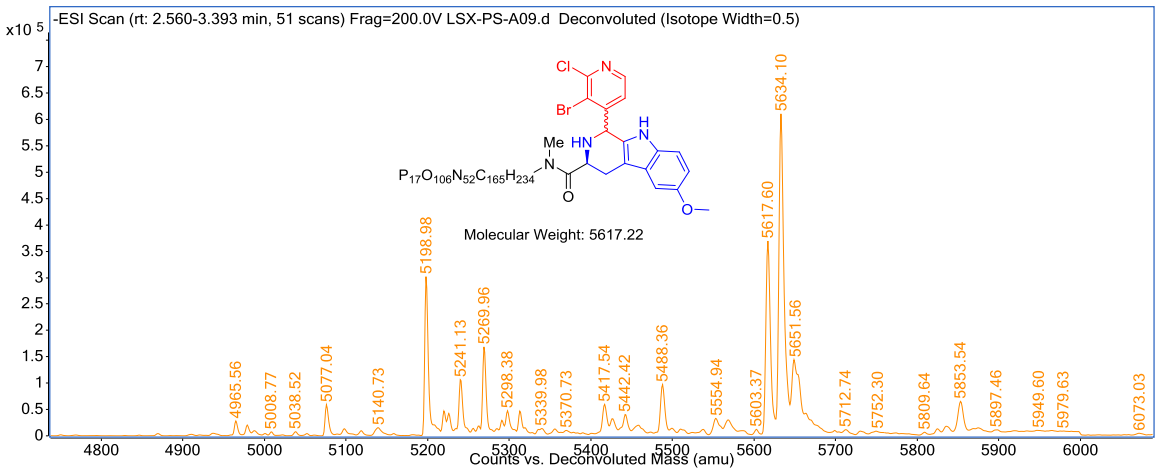


Fig. S67. Deconvoluted mass of **4ei**

Figure S68, Mass Spectrum of 4ej, related to Figure 4.

Percent conversion: 98%
Exact mass: 5571.94
Observed: 5572.25

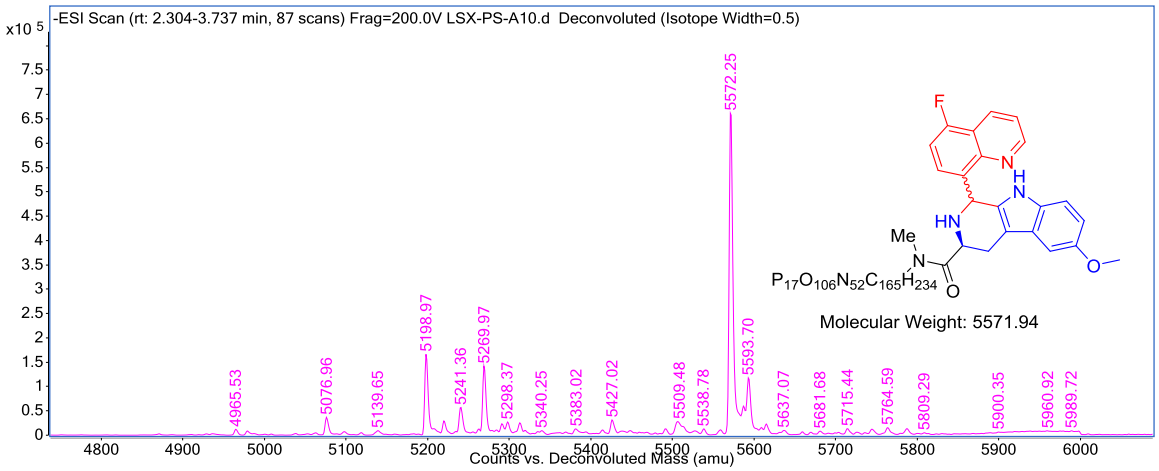


Fig. S68. Deconvoluted mass of **4ej**

Figure S69, Mass Spectrum of 4ek, related to Figure 4.

Percent conversion:98%
Exact mass: 5539.87
Observed: 5540.21

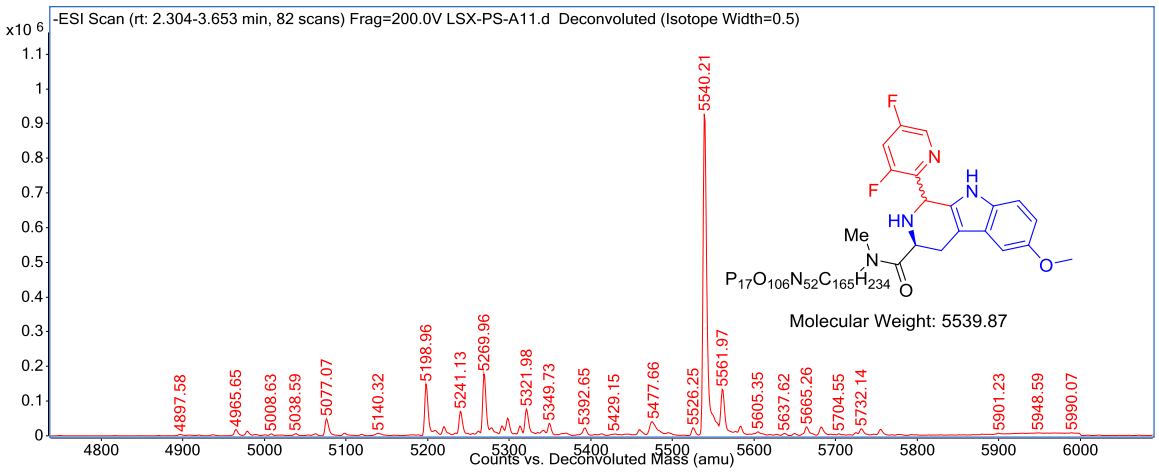


Fig. S69. Deconvoluted mass of **4ek**

Figure S70, Mass Spectrum of 4el, related to Figure 4.

Percent conversion: 82.34%
Exact mass: 5556.95
Observed: 5557.30

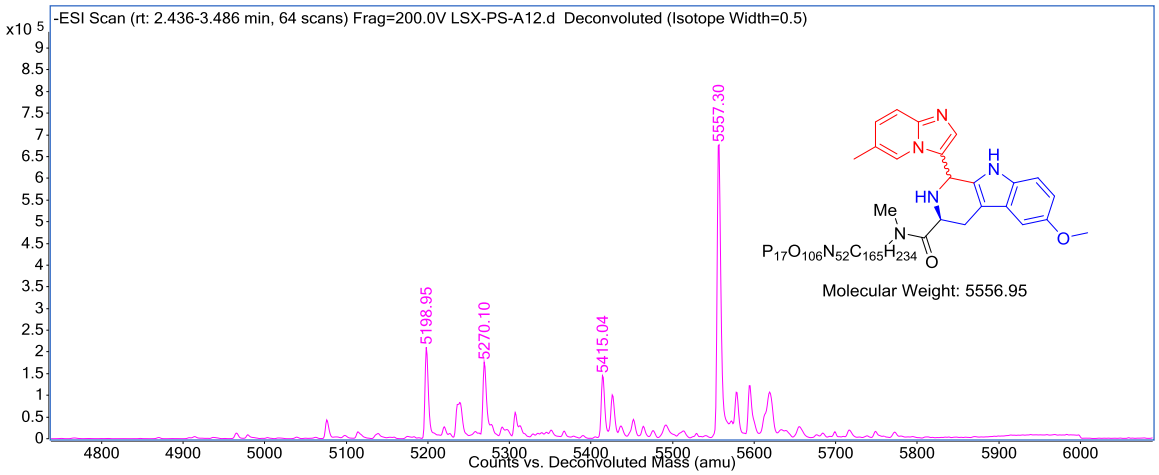


Fig. S70. Deconvoluted mass of **4el**

Figure S71, Mass Spectrum of 4em, related to Figure 4.

Percent conversion: 91.69%
Exact mass: 5531.94
Observed: 5532.25

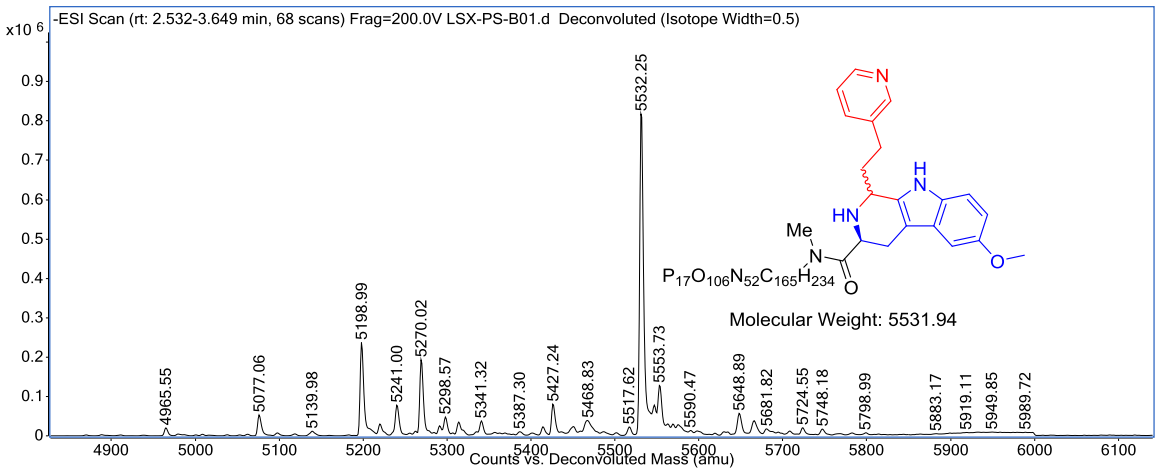


Fig. S71. Deconvoluted mass of **4em**

Figure S72, Mass Spectrum of 4en, related to Figure 4.

Percent conversion: 39.19%
Exact mass: 5544.35
Observed: 5544.35

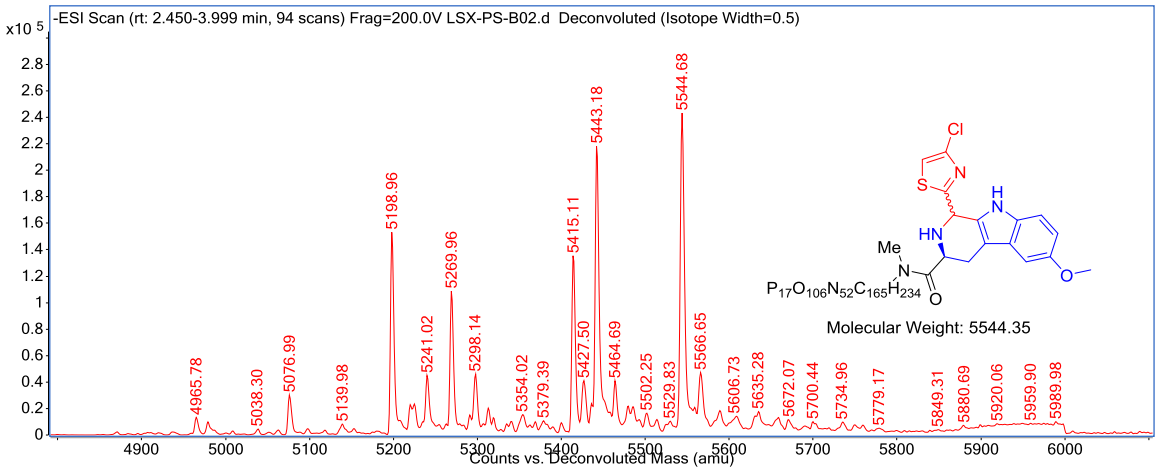


Fig. S72. Deconvoluted mass of **4en**

Figure S73, Mass Spectrum of 4eo, related to Figure 4.

Percent conversion: 88.90%
Exact mass: 5583.88
Observed: 5582.50

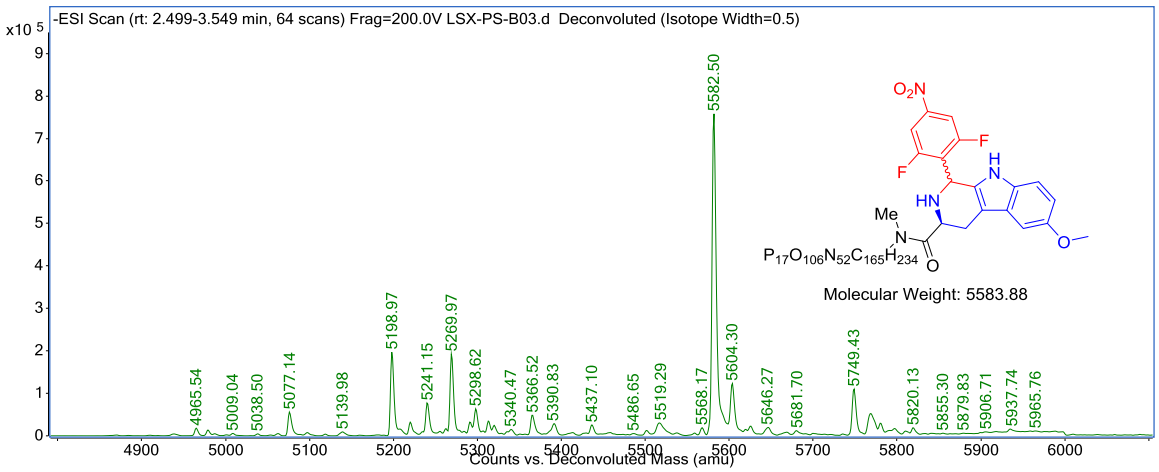


Fig. S73. Deconvoluted mass of **4eo**

Figure S74, Mass Spectrum of 4ep, related to Figure 4.

Percent conversion: 95.00%
Exact mass: 5565.89
Observed: 5566.22

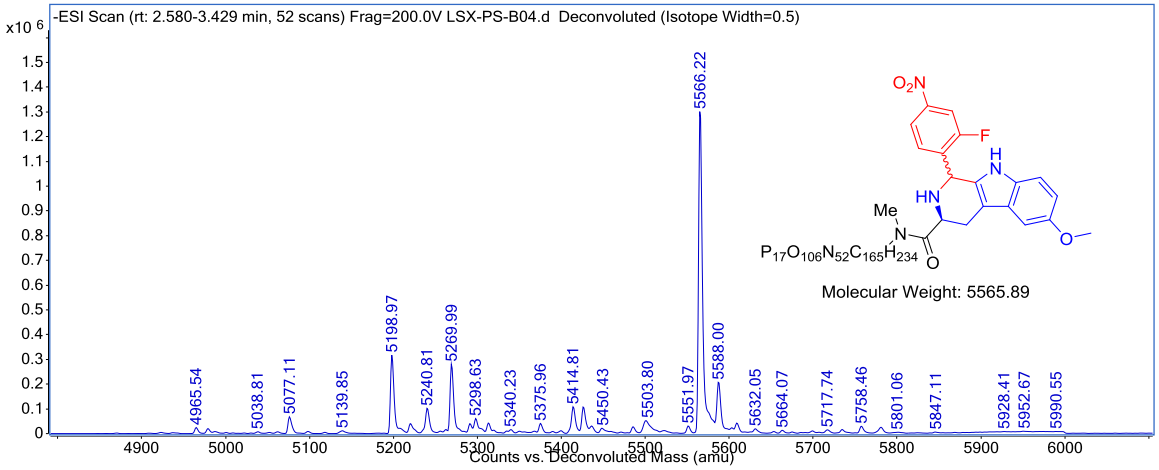


Fig. 74. Deconvoluted mass of **4ep**

Figure S75, Mass Spectrum of 4eq, related to Figure 4.

Percent conversion: 83.67%
Exact mass: 5542.92
Observed: 5543.01

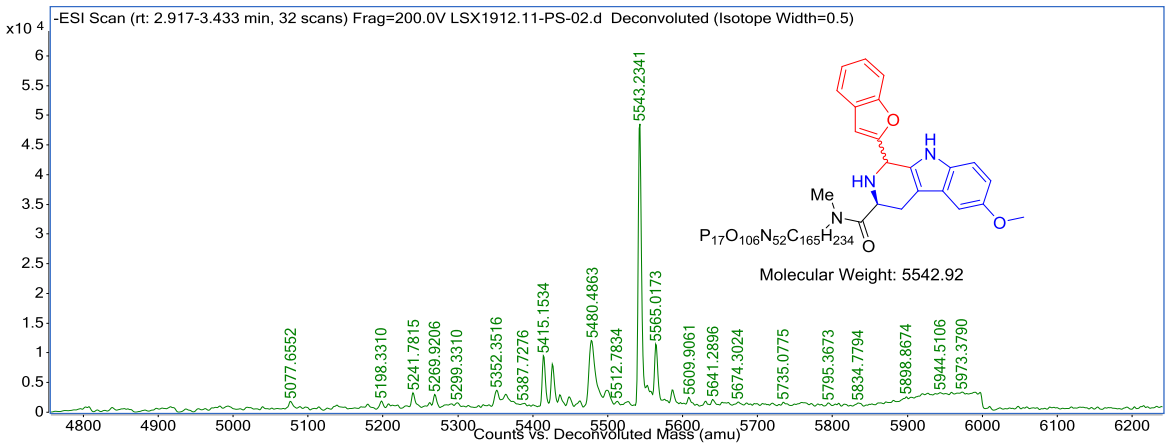


Fig. S75. Deconvoluted mass of **4eq**

Figure S76, Mass Spectrum of 4er, related to Figure 4.

Percent conversion: 83.50%
Exact mass: 5588.80
Observed: 5588.73

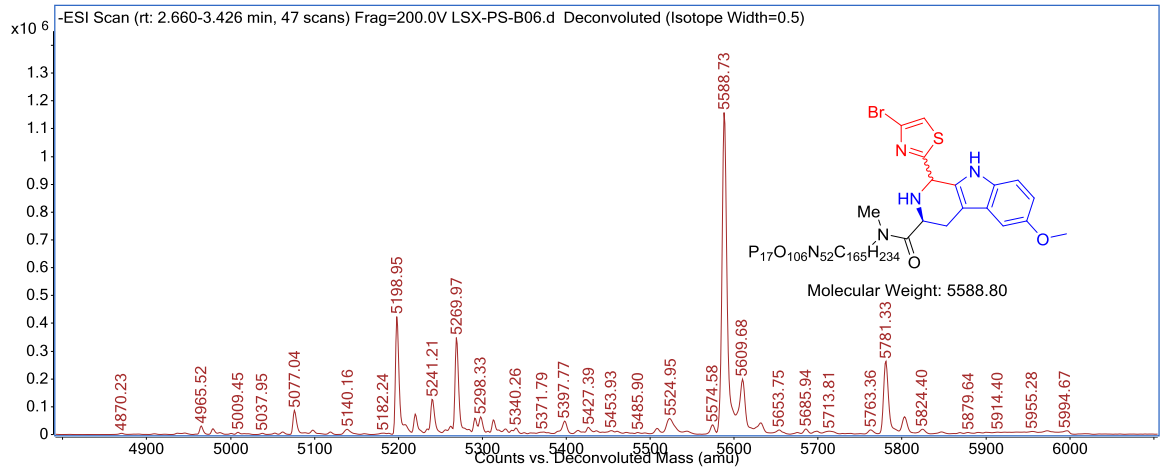


Fig. S76. Deconvoluted mass of **4er**

Figure S77, Mass Spectrum of 4es, related to Figure 4.

Percent conversion: 27.74%
Exact mass: 5616.03
Observed: 5616.45

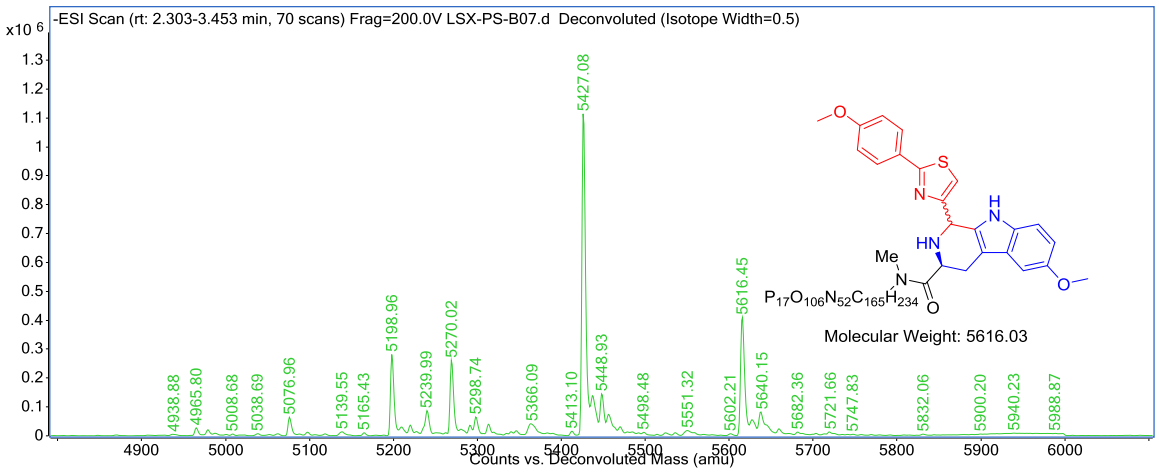


Fig. S77. Deconvoluted mass of **4es**

Figure S78, Mass Spectrum of 4et, related to Figure 4.

Percent conversion: 76.96%
Exact mass: 5592.89
Observed: 5593.27

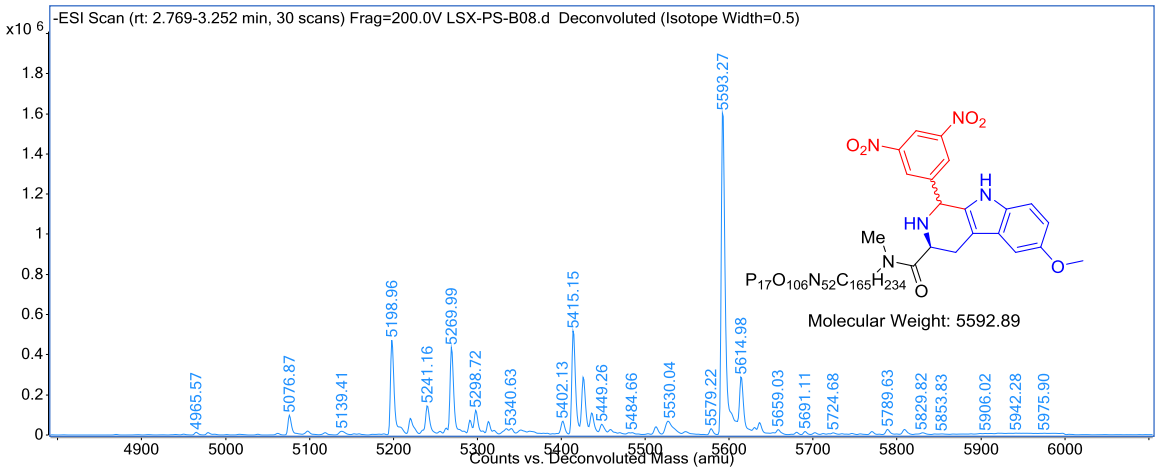


Fig. S78. Deconvoluted mass of **4et**

Figure S79, Mass Spectrum of 4eu, related to Figure 4.

Percent conversion: 98%
Exact mass: 5543.91
Observed: 5593.27

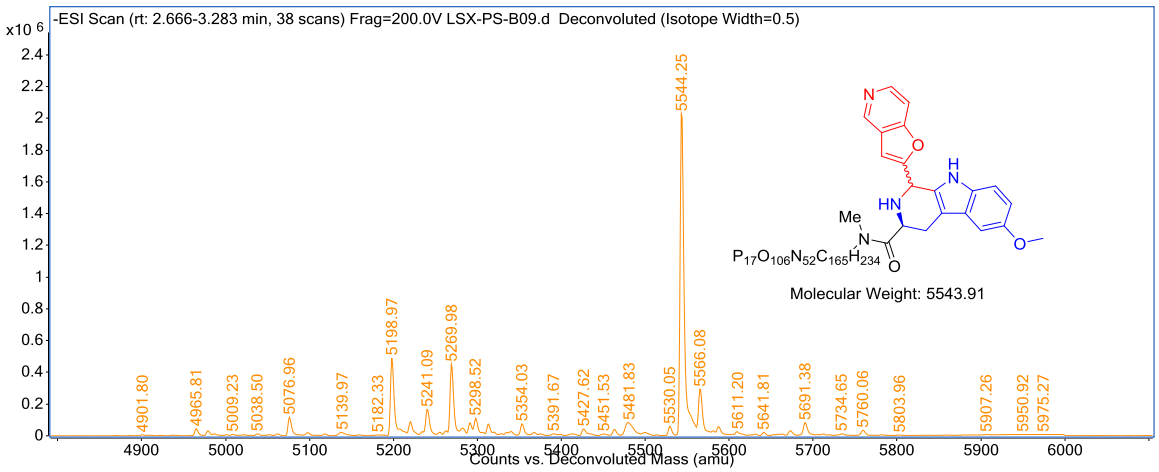


Fig. S79. Deconvoluted mass of **4eu**

Figure S80, Mass Spectrum of 4ev, related to Figure 4.

Percent conversion: 98%
Exact mass: 5556.32
Observed: 5556.68

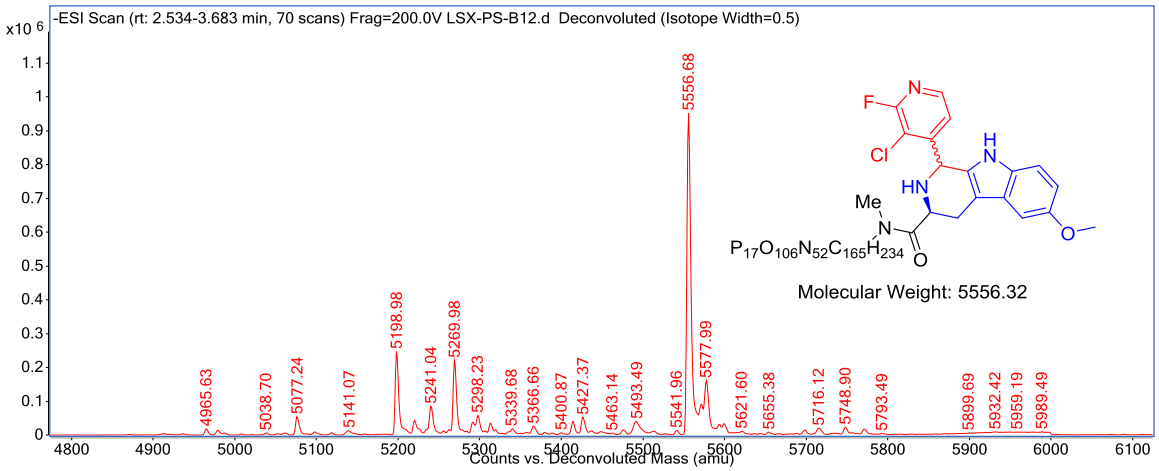


Fig. S80. Deconvoluted mass of **4ev**

Figure S81, Mass Spectrum of 4ew, related to Figure 4.

Percent conversion: 68.87%
Exact mass: 5617.22
Observed: 5617.64

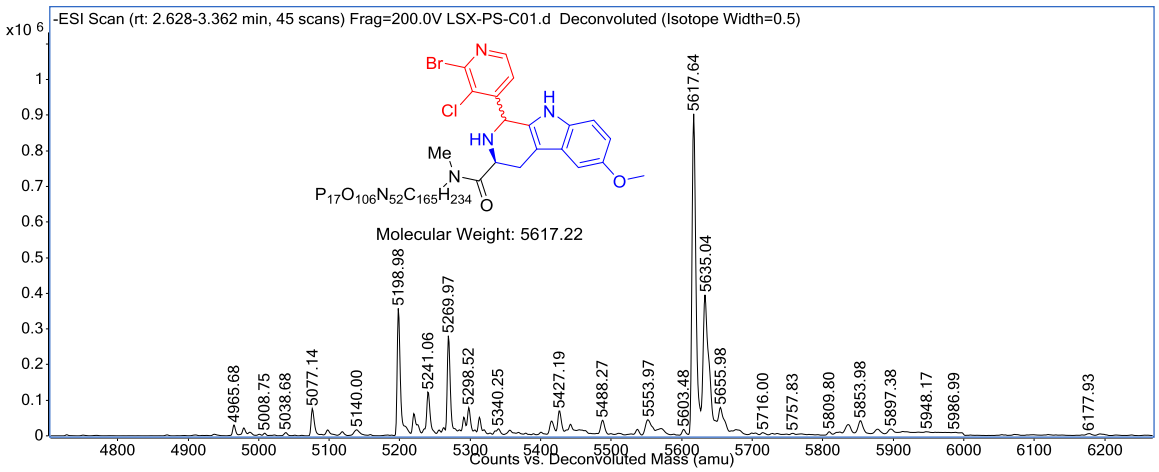


Fig. S81. Deconvoluted mass of **4ew**

Figure S82, Mass Spectrum of 4ex, related to Figure 4.

Percent conversion: 98%
Exact mass: 5600.03
Observed: 5600.33

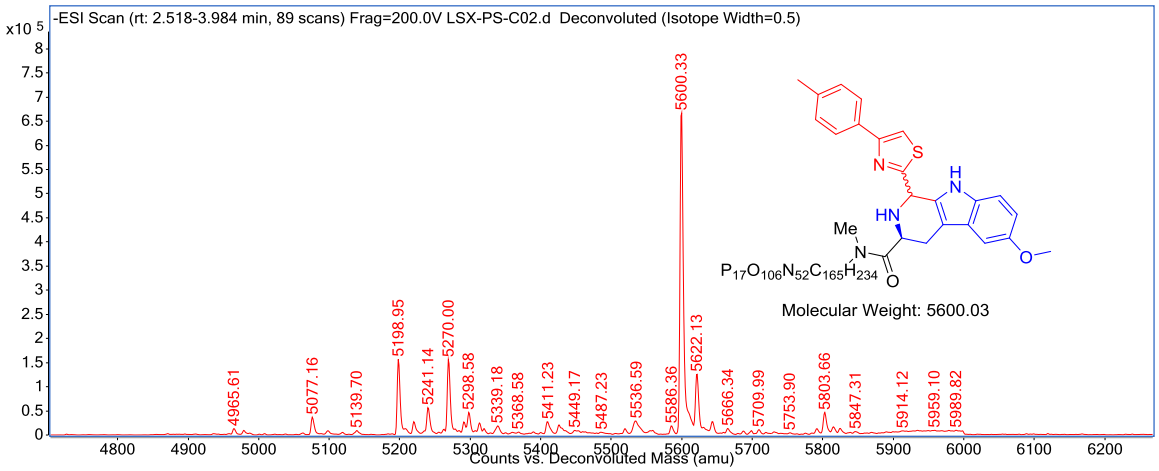


Fig. S82. Deconvoluted mass of **4ex**

Figure S83, Mass Spectrum of 4ey, related to Figure 4.

Percent conversion: 72.60%
Exact mass: 5565.39
Observed: 5565.71

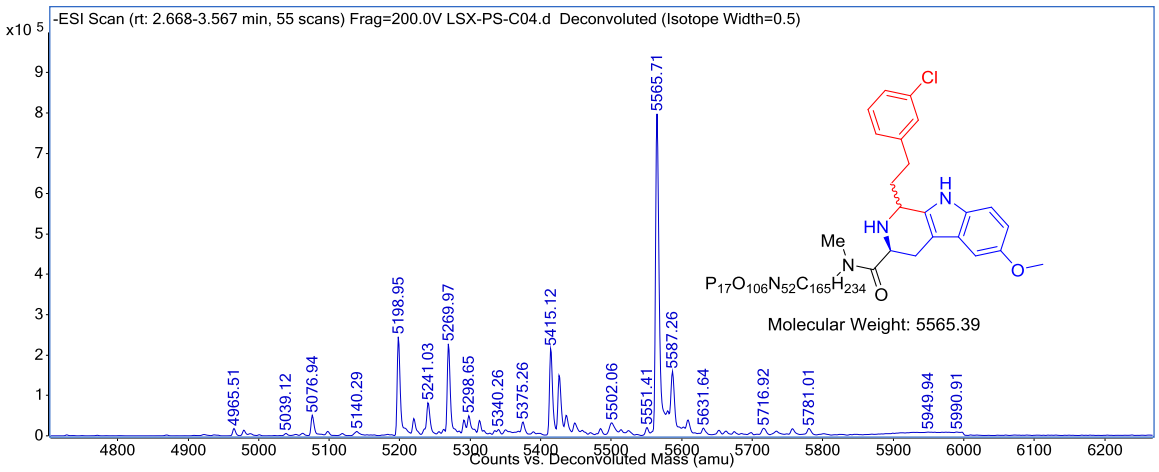


Fig. S83. Deconvoluted mass of **4ey**

Figure S84, Mass Spectrum of 4ez, related to Figure 4.

Percent conversion: 98%
Exact mass: 5556.32
Observed: 5556.65

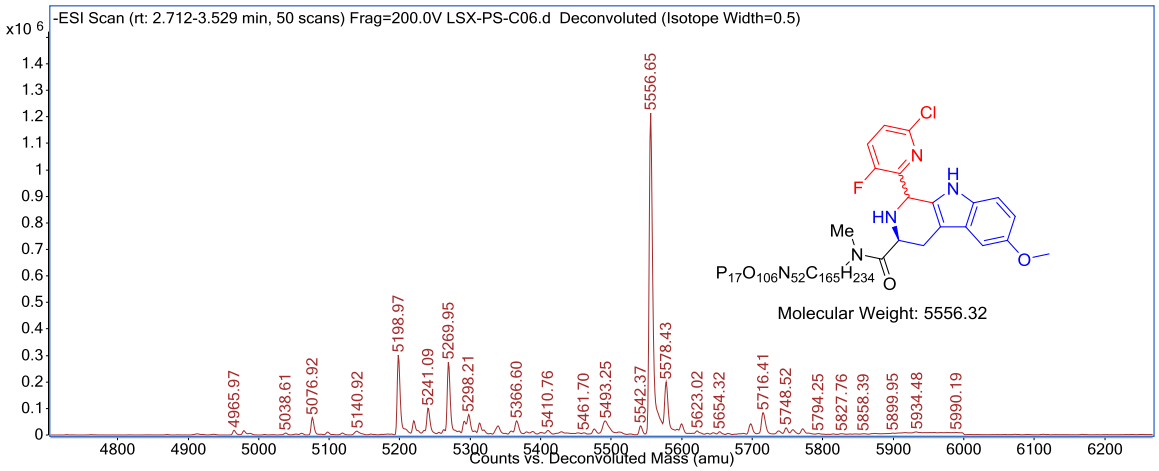


Fig. S84. Deconvoluted mass of **4ez**

Figure S85, Mass Spectrum of 4fa, related to Figure 4.

Percent conversion: 55.85%
Exact mass: 5626.79
Observed: 5626.67

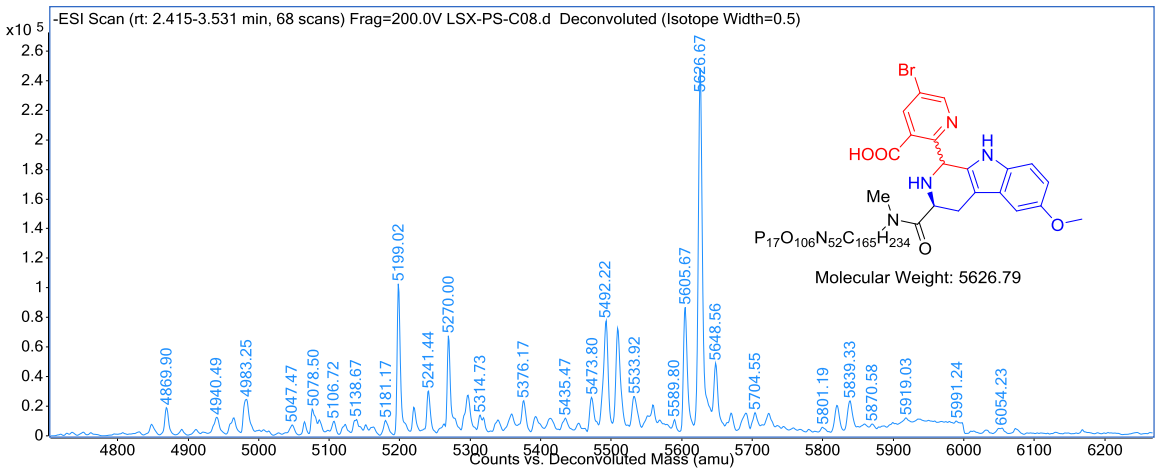


Fig. S85. Deconvoluted mass of **4fa**

Figure S86, Mass Spectrum of 4fb, related to Figure 4.

Percent conversion: 0
Exact mass: 5739.04
Observed: NO

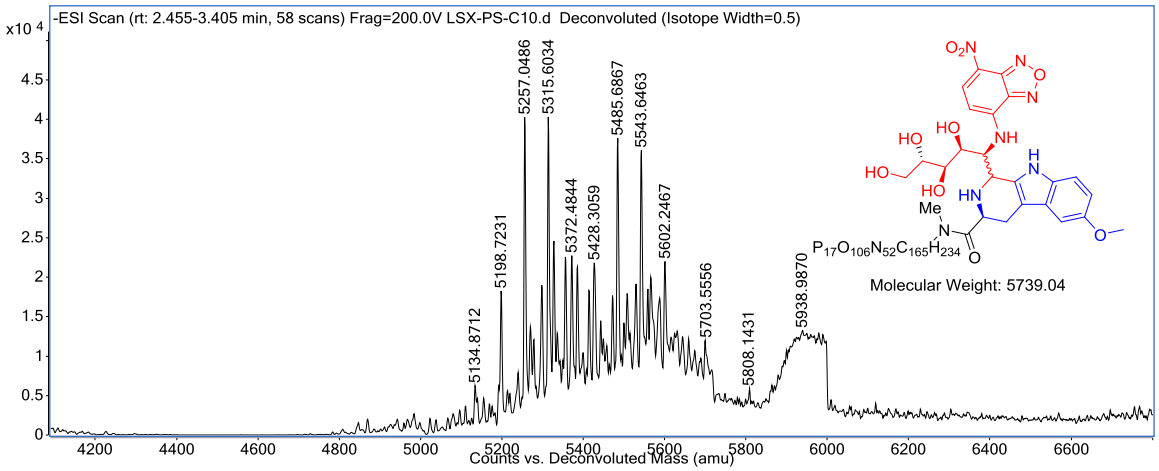


Fig. S86. Deconvoluted mass of **4fb**

Figure S87, Mass Spectrum of 4fc, related to Figure 4.

Percent conversion: 66.67%
Exact mass: 5562.35
Observed: 5562.5587

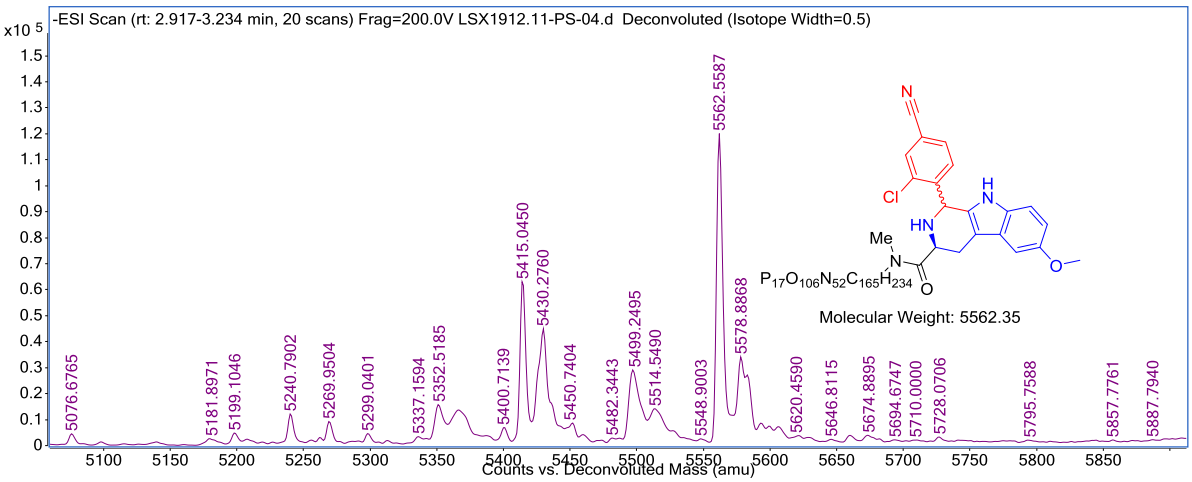


Fig. S87. Deconvoluted mass of **4fc**

Figure S88, Mass Spectrum of 4fd, related to Figure 4.

Percent conversion: 81.43%
Exact mass: 5530.95
Observed: 5531.2255

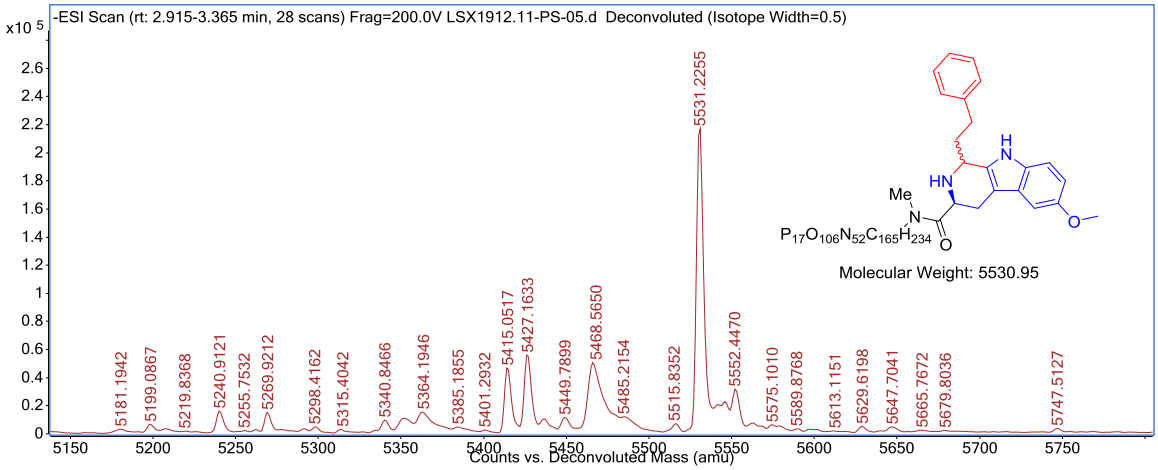


Fig. S88. Deconvoluted mass of **4fd**

Figure S89, Mass Spectrum of 4fe, related to Figure 4.

Percent conversion: 51.43%
Exact mass: 5626.79
Observed: 5627.0993

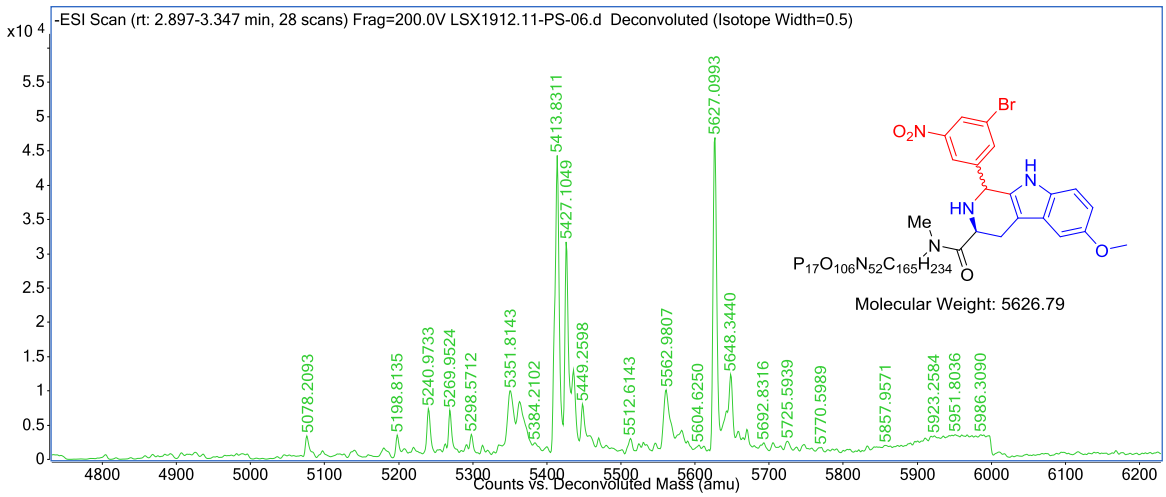


Fig. S89. Deconvoluted mass of **4fe**

Figure S90, Mass Spectrum of 4ff, related to Figure 4.

Percent conversion: 45.74%
Exact mass: 5547.89
Observed: 5546.4868

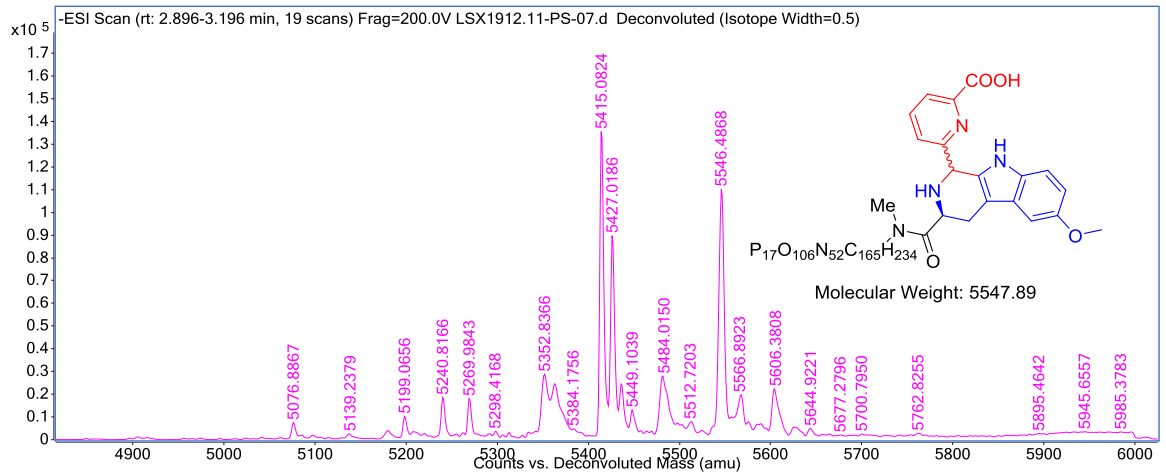


Fig. S90. Deconvoluted mass of **4ff**

Figure S91, Mass Spectrum of 4fg, related to Figure 4.

Percent conversion: 58.70%
Exact mass: 5615.89
Observed: 5615.2219

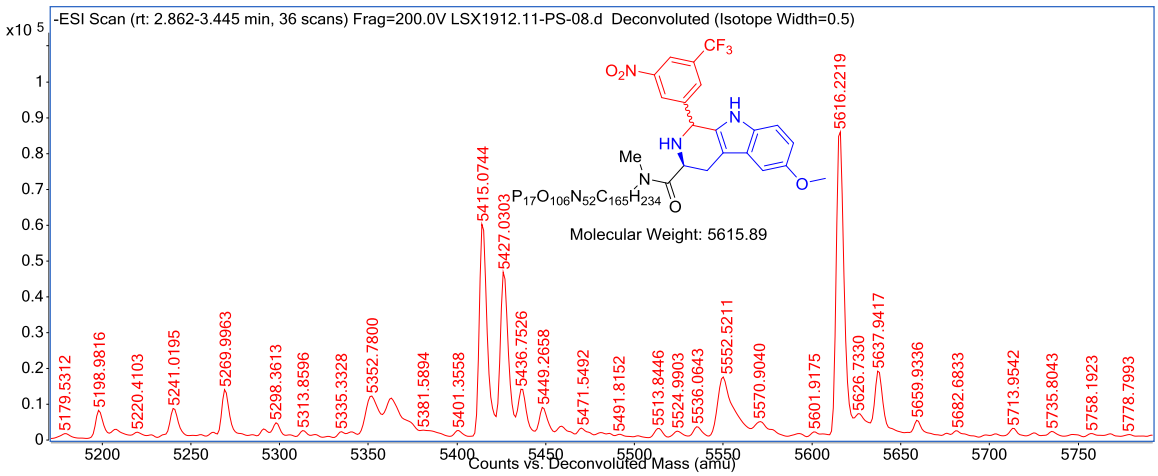


Fig. S91. Deconvoluted mass of **4fg**

Figure S92, Mass Spectrum of 4fh, related to Figure 4.

Percent conversion: 37.04%
Exact mass: 5531.70
Observed: 5530.1939

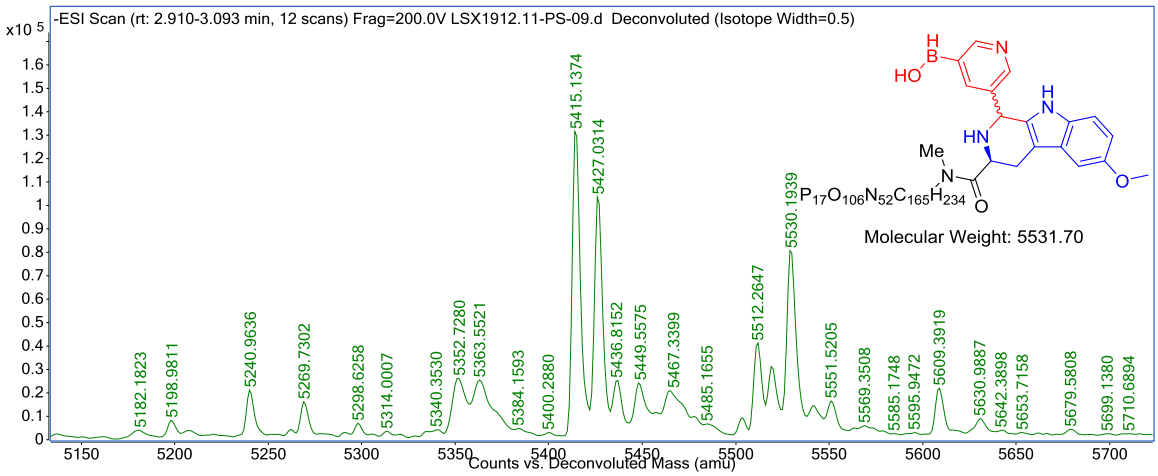
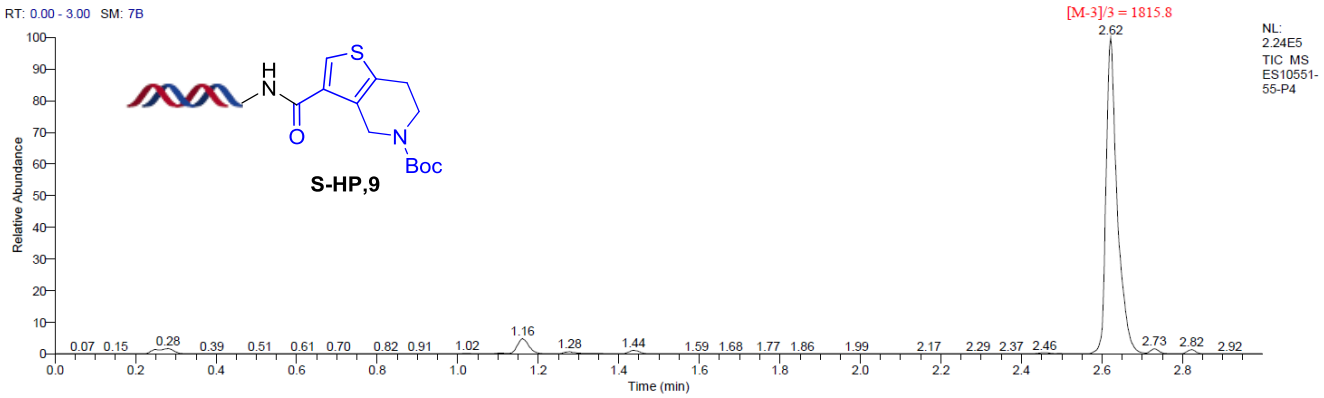


Fig. S92. Deconvoluted mass of **4fh**

Figure S93, LC Trace and Mass of 9, related to Figure 7.

Following General Procedure 5
Percent conversion: 95.29%
Exact mass: 5449.83
Triply charged mass [M-3]/3, calculated: 1815.61; observed:1815.8



RT	Peak Height	Peak Width	Peak Area	Area %
1.16	10889.55	0.16	21645.79	4.71
2.62	245583.85	0.21	437934.39	95.29

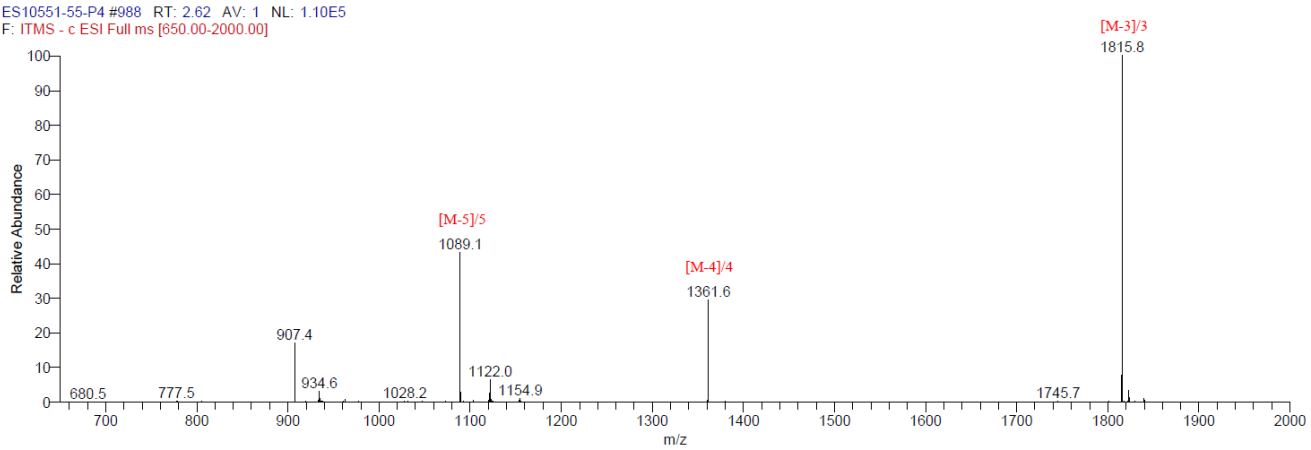
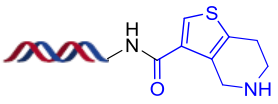
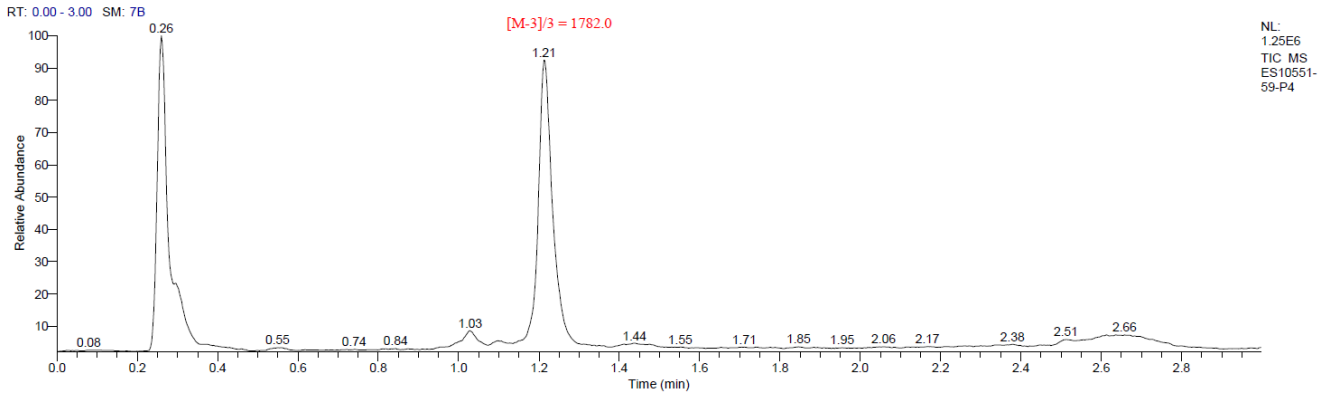


Fig. S93. LC trace and mass of **9**

Figure S94, LC Trace and Mass of 10, related to Figure 7.

Following **General Procedure 5**
Percent conversion: 92.45%
Exact mass: 5349.83
Triply charged mass [M-3]/3, calculated: 1782.28; observed:1782.0





RT	Peak Height	Peak Width	Peak Area	Area %
1.03	75201.39	0.18	243060.60	7.55
1.21	1172769.32	0.28	2975952.47	92.45

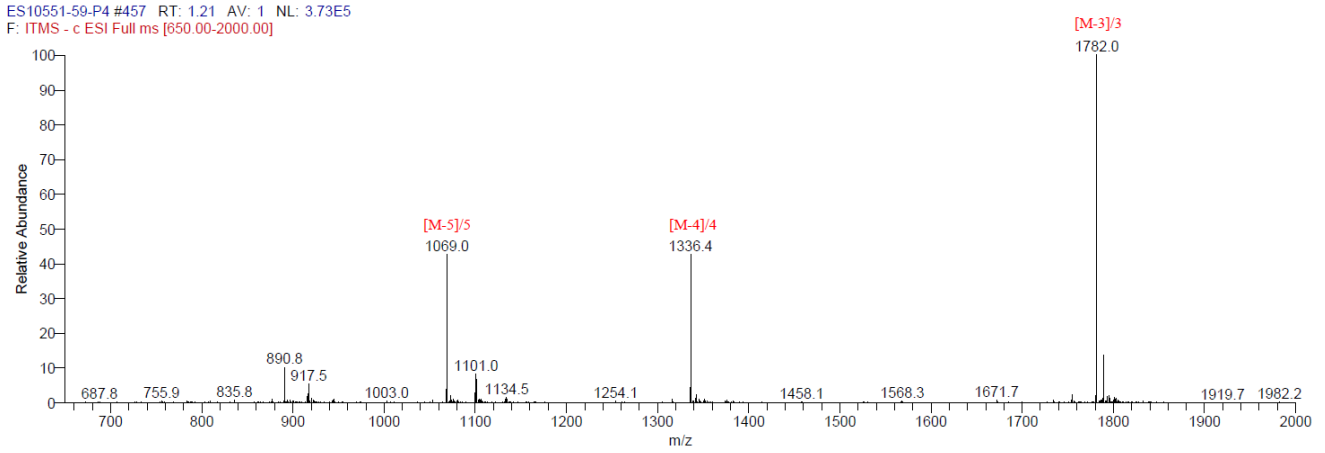
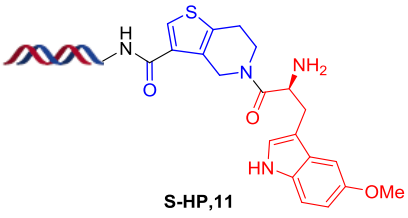


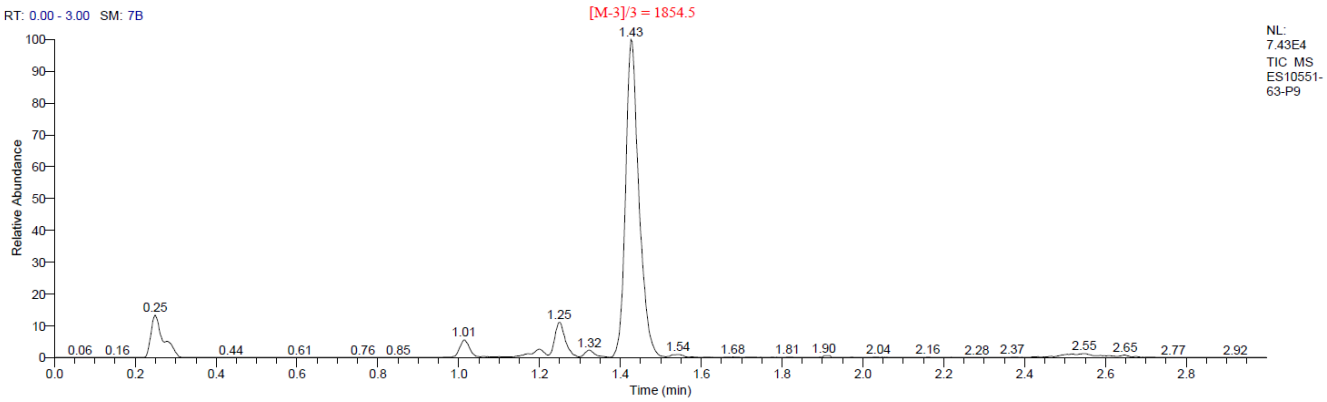
Fig. S94. LC trace and mass of **10**

Figure S95, LC Trace and Mass of 11, related to Figure 7.

Following **General Procedure 1**
Percent conversion: 88.95%
Exact mass: 5566.32
Triply charged mass [M-3]/3, calculated: 1854.44; observed:1854.5



S-HP,11



RT	Peak Height	Peak Width	Peak Area	Area %
1.25	8969.07	0.22	21303.06	11.05
1.43	80179.27	0.14	171406.83	88.95

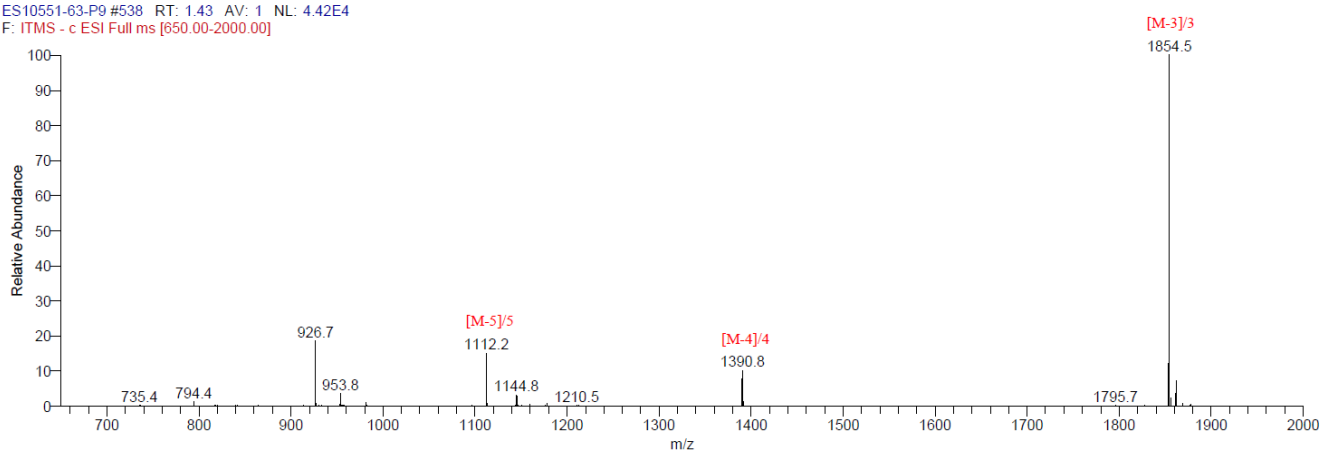
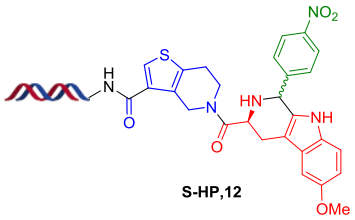


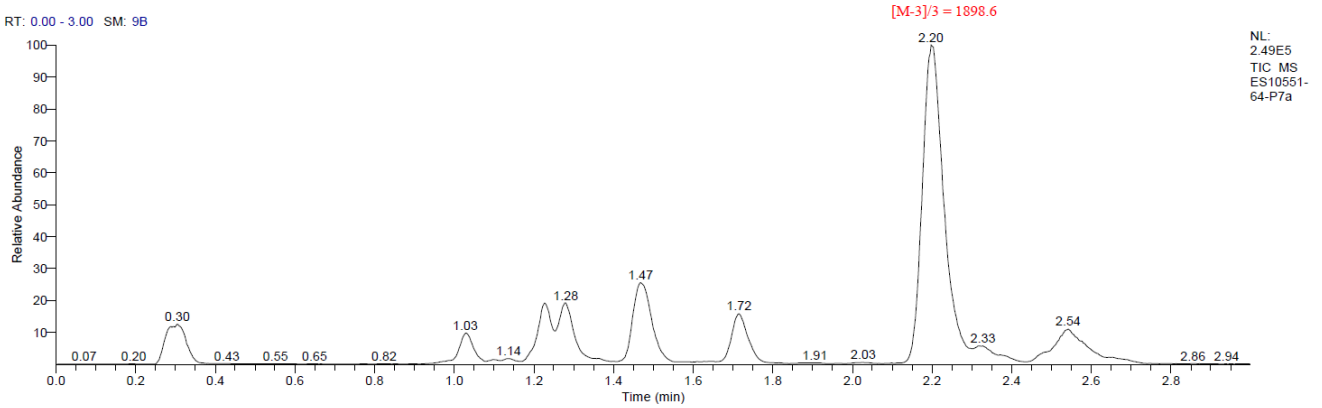
Fig. S95. LC trace and mass of **11**

Figure S96, LC Trace and Mass of 12, related to Figure 7.

Following **General Procedure 2**
Percent conversion: 55.32%
Exact mass: 5699.44
Triply charged mass [M-3]/3, calculated: 1898.81 observed:1898.6



S-HP,12



RT	Peak Height	Peak Width	Peak Area	Area %
1.23	53660.90	0.33	284509.43	15.68
1.47	65770.35	0.22	223109.96	12.29
1.72	42742.23	0.32	124866.96	6.88
2.20	264922.45	0.37	1004065.09	55.32
2.54	28023.39	0.48	178383.38	9.83

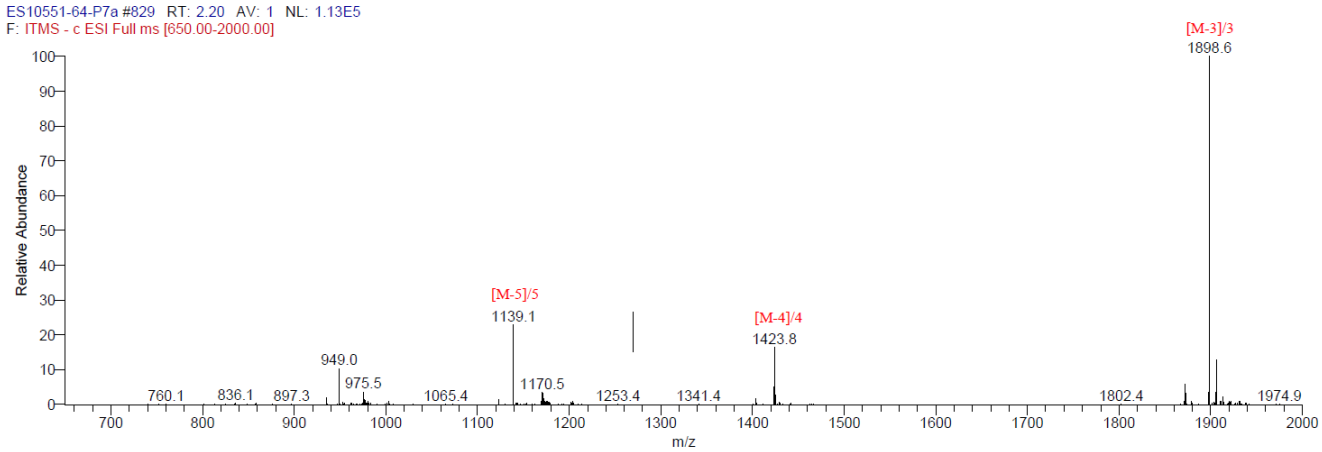


Fig. S96. LC trace and mass of **12**

Figure S97, Mass Spectrum of 13, related to Figure 7.

Percent conversion: 95%
Exact mass: 5112.37
Observed: 5112.8975

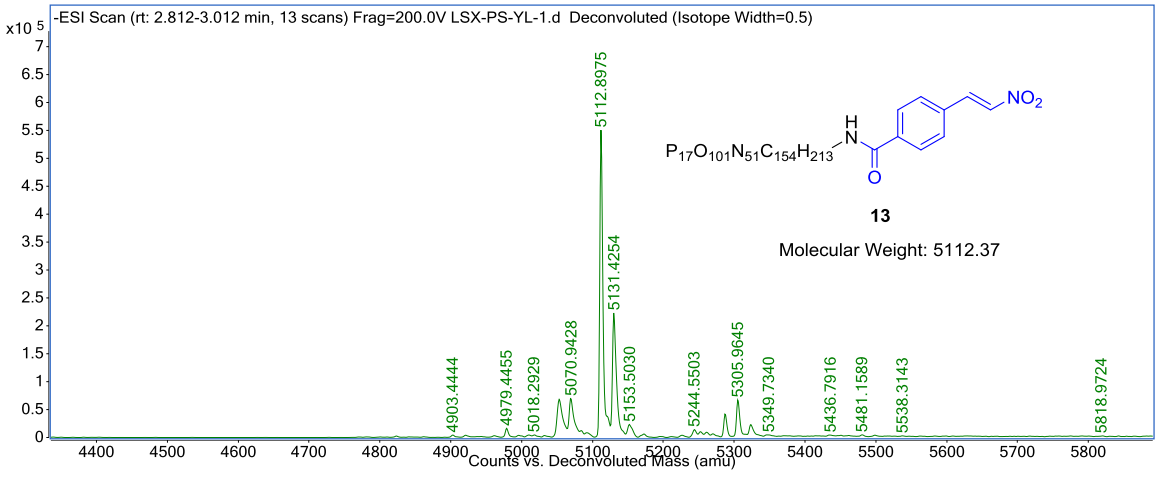
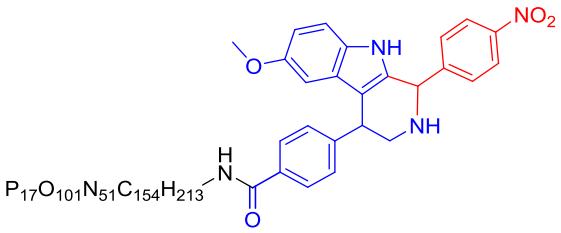


Fig. S97. Deconvoluted mass of **13**

Figure S98, Mass Spectrum of 8a, related to Figure 7.

Percent conversion: 50%
Exact mass: 5362.67
Observed: 5362.87



Molecular Weight: 5362.67

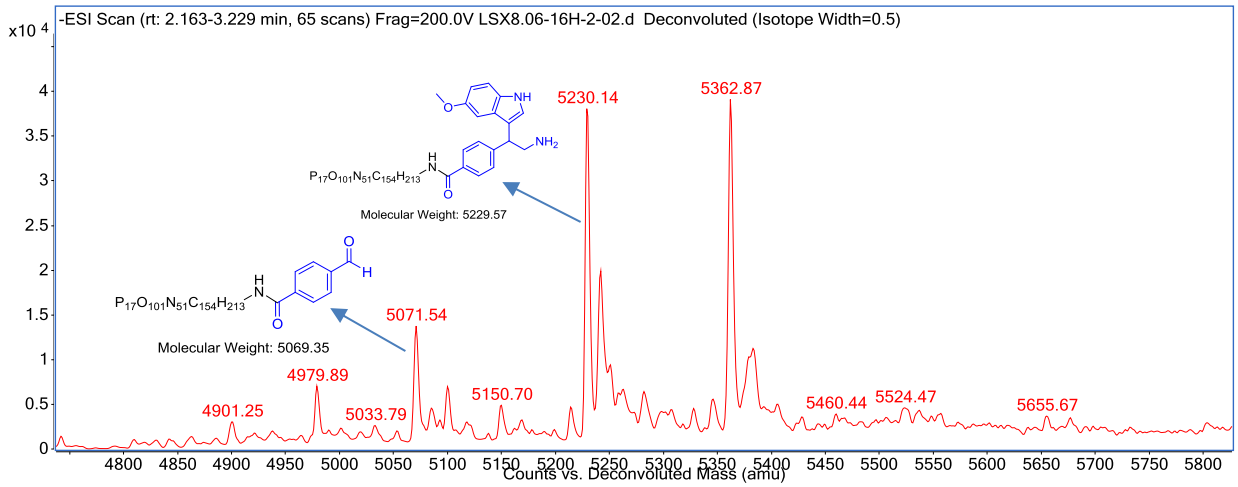
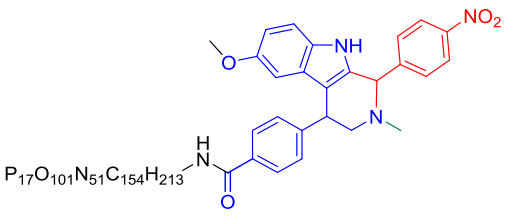


Fig. S98. Deconvoluted mass of **8a**

Figure S99, Mass Spectrum of 14a, related to Figure 7.

Percent conversion: 95%
Exact mass: 5376.70
Observed: 5377.3636



Molecular Weight: 5376.70

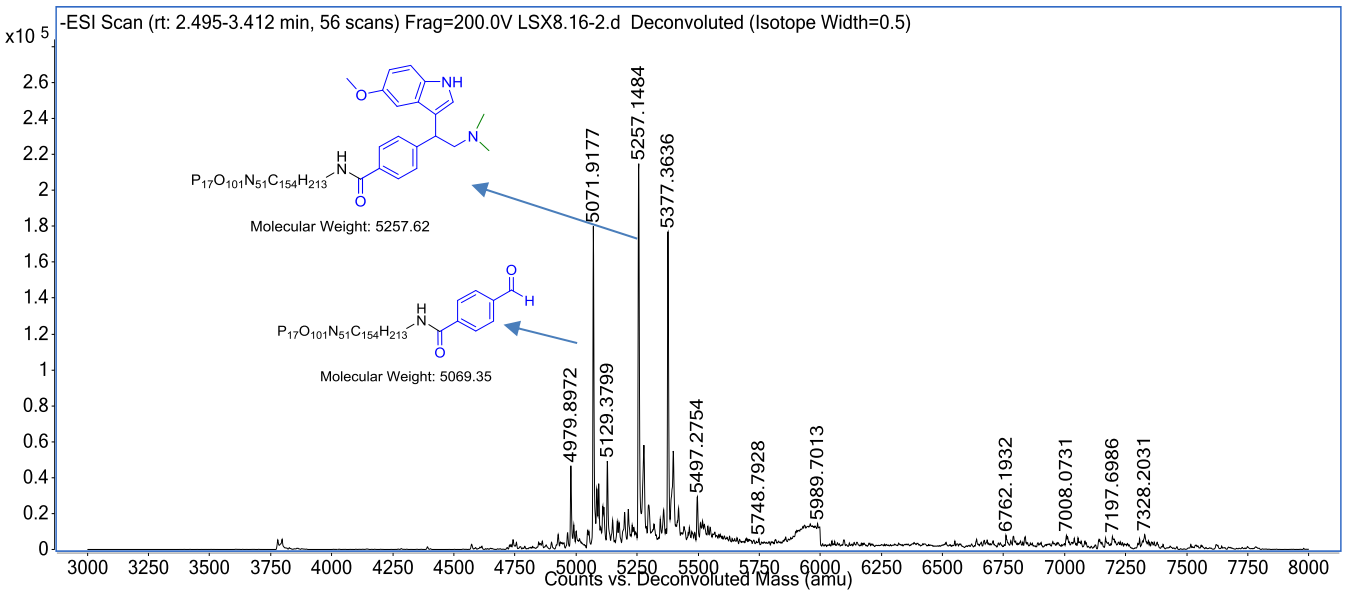
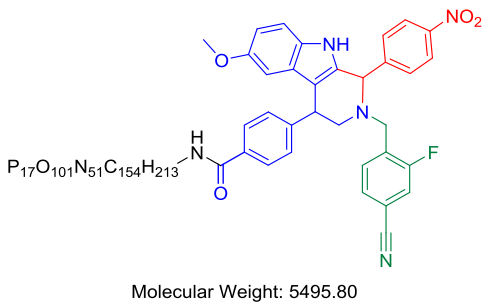


Fig. S99. Deconvoluted mass of **14a**

Figure S100, Mass Spectrum of 14b, related to Figure 7.

Percent conversion: 95%
Exact mass: 5495.80
Observed: 5496.4340



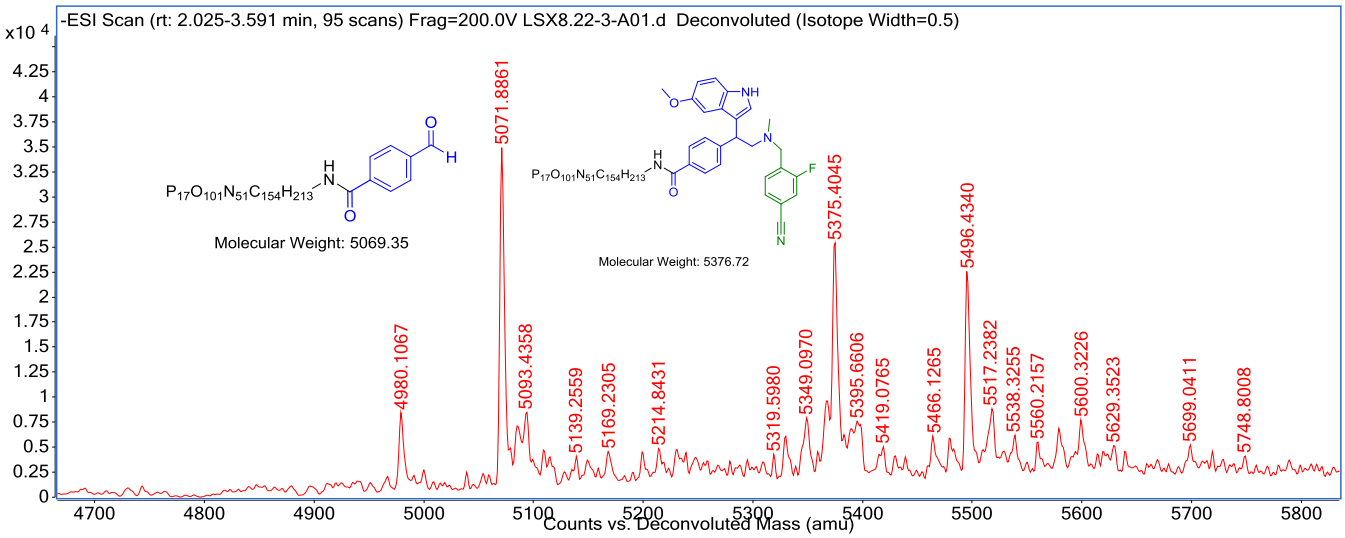
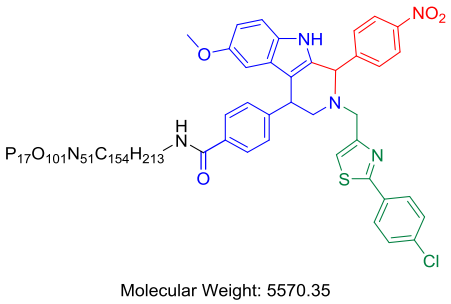


Fig. S100. Deconvoluted mass of **14b**

Figure S101, Mass Spectrum of 14c, related to Figure 7.

Percent conversion: 68.97%
Exact mass: 5570.35
Observed: 5570.8703



Molecular Weight: 5570.35

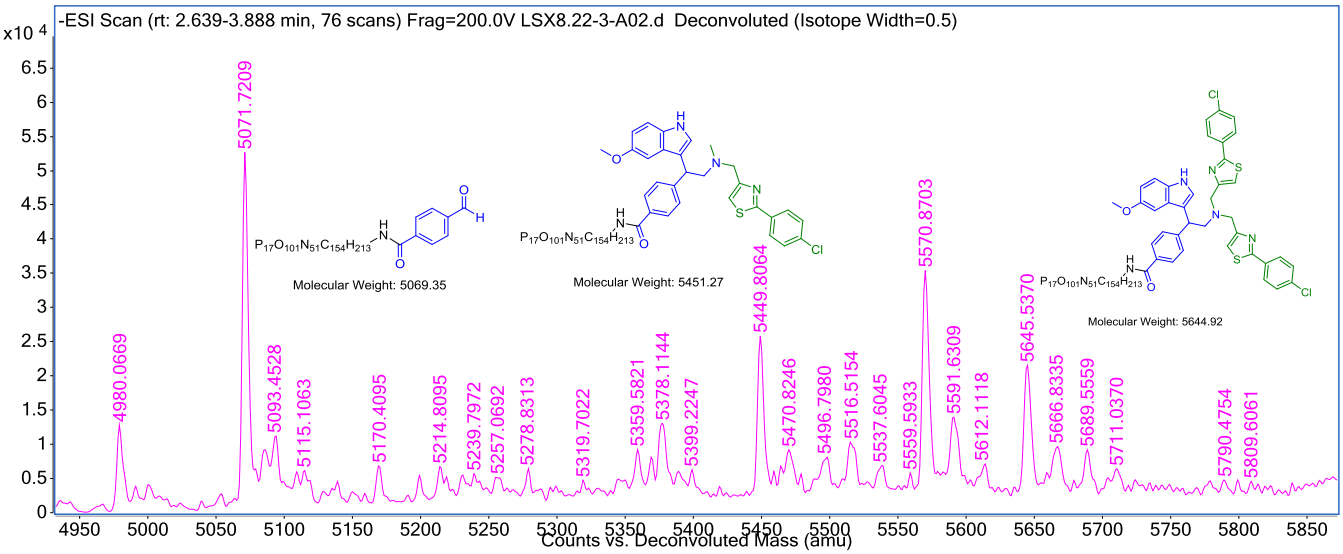
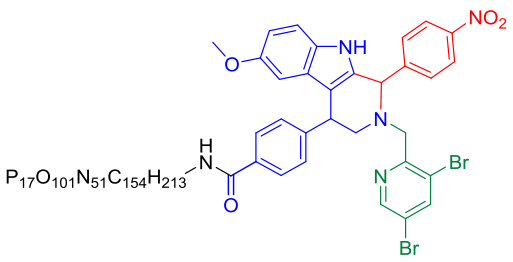


Fig. S101. Deconvoluted mass of **14c**

Figure S102, Mass Spectrum of 14d, related to Figure 7.

Percent conversion: 60.98%
Exact mass: 5611.58
Observed: 5612.3937



Molecular Weight: 5611.58

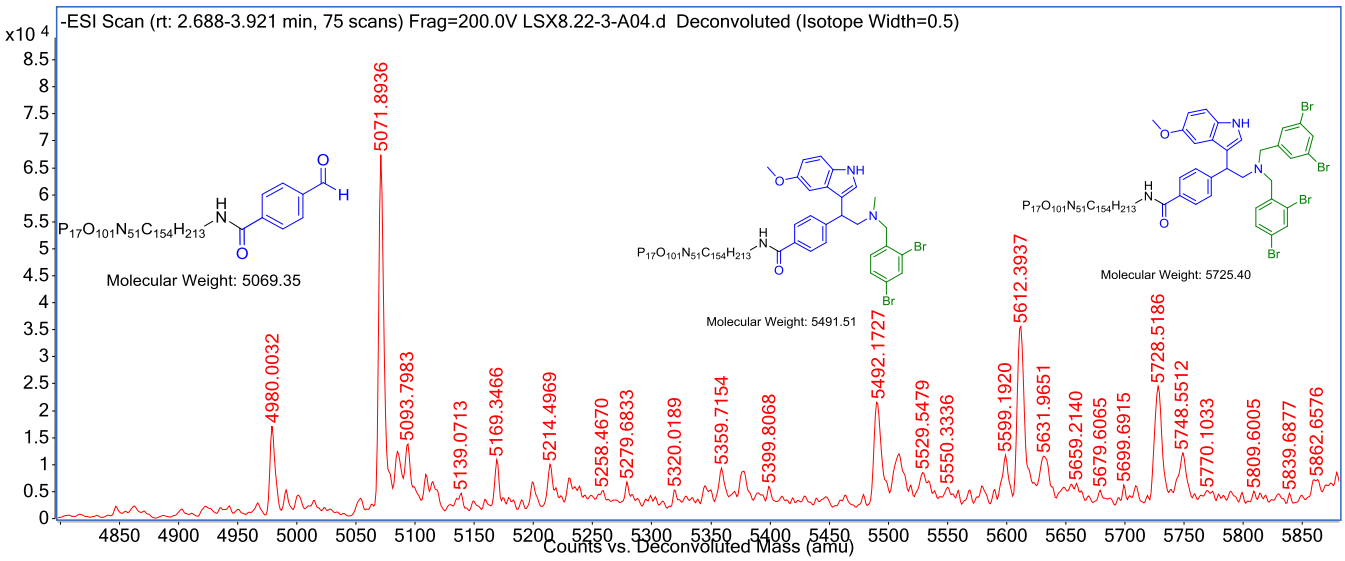
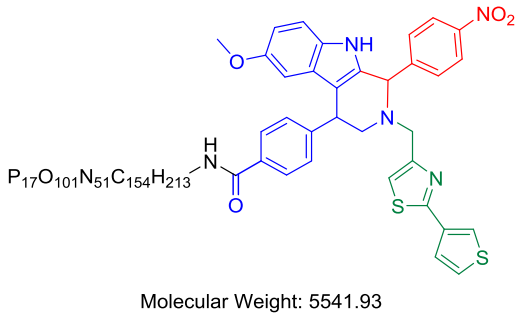


Fig. S102. Deconvoluted mass of **14d**

Figure S103, Mass Spectrum of 14e, related to Figure 7.

Percent conversion: 56.67%
Exact mass: 5541.93
Observed: 5542.3905



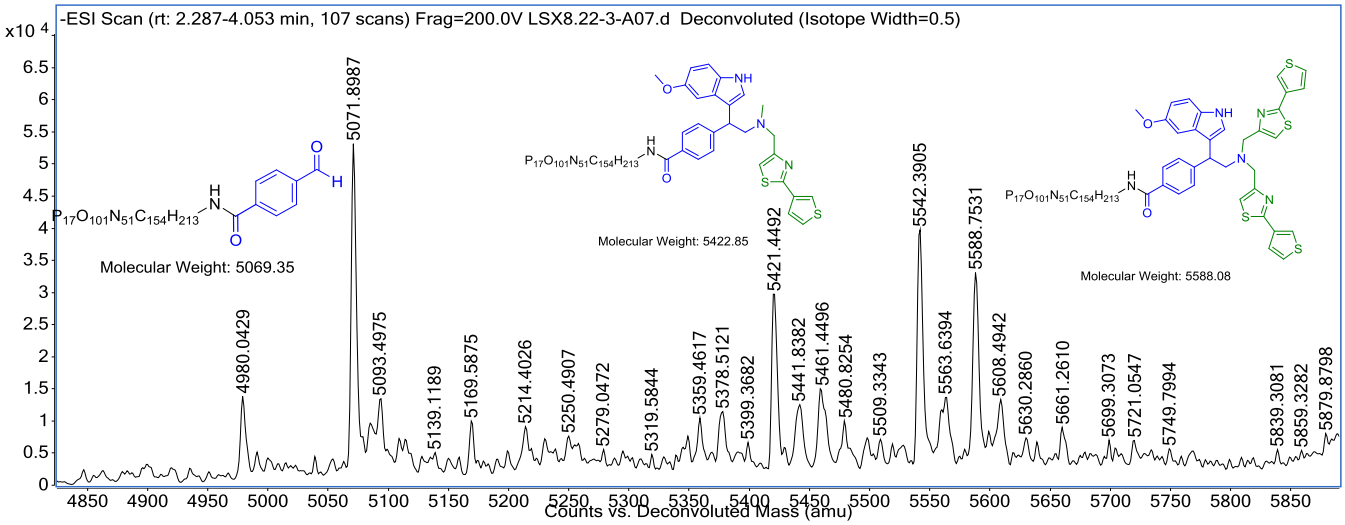
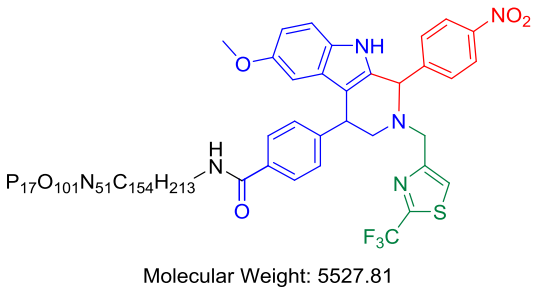


Fig. S103. Deconvoluted mass of **14e**

Figure S104, Mass Spectrum of 14f, related to Figure 7.

Percent conversion: 58.33%
Exact mass: 5527.81
Observed: 5528.3546



Molecular Weight: 5527.81

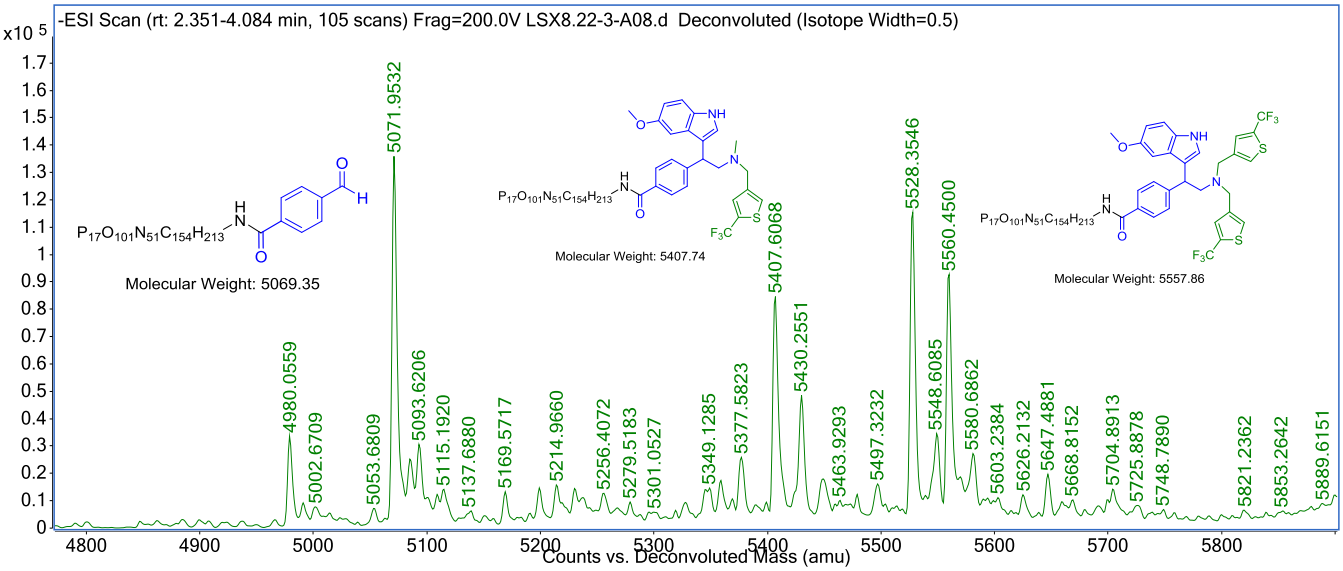
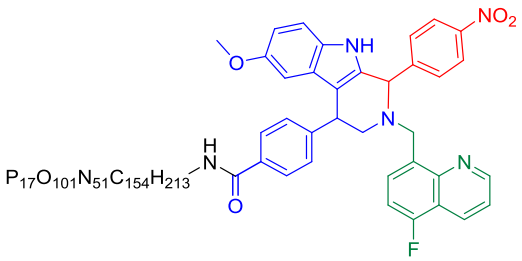


Fig. S104. Deconvoluted mass of **14f**

Figure S105, Mass Spectrum of 14g, related to Figure 7.

Percent conversion: 54.55%
Exact mass: 5521.84
Observed: 5522.4649



Molecular Weight: 5521.84

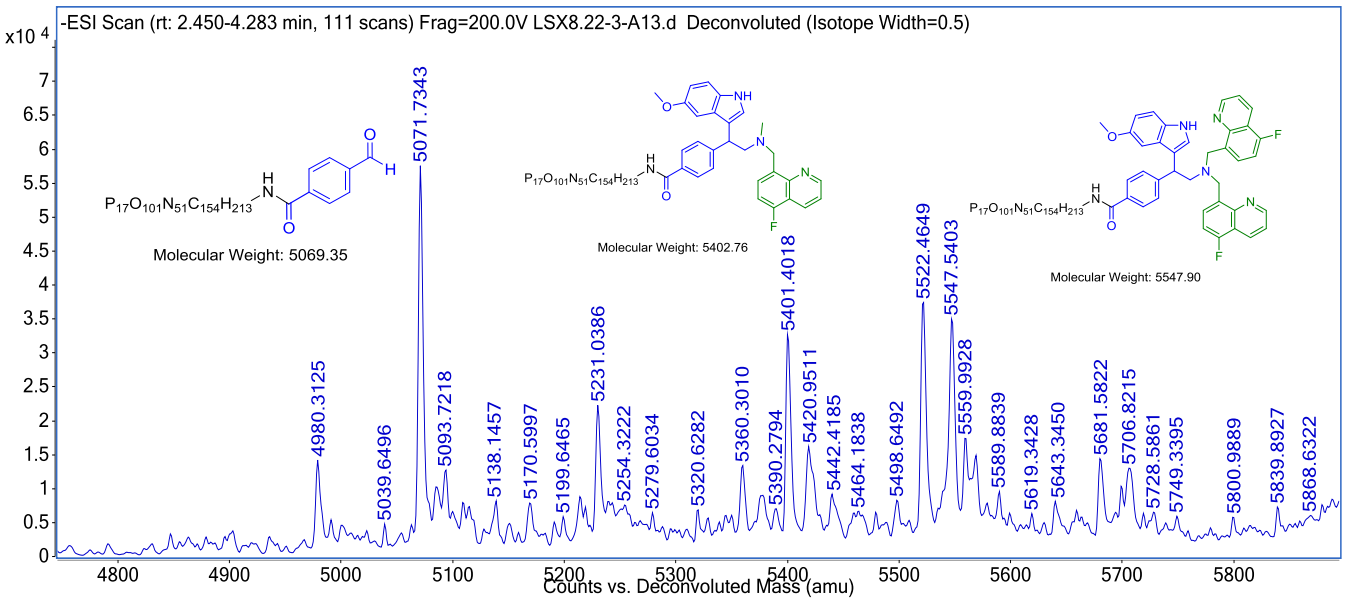
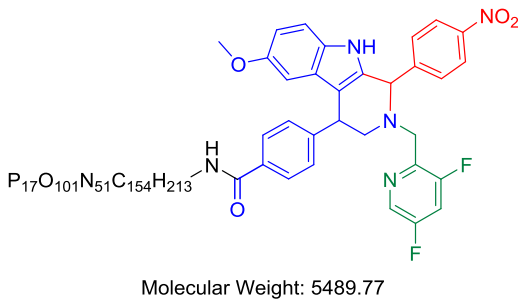


Fig. S105. Deconvoluted mass of **14g**

Figure S106, Mass Spectrum of 14h, related to Figure 7.

Percent conversion: 59.32%
Exact mass: 5489.77
Observed: 5489.4860



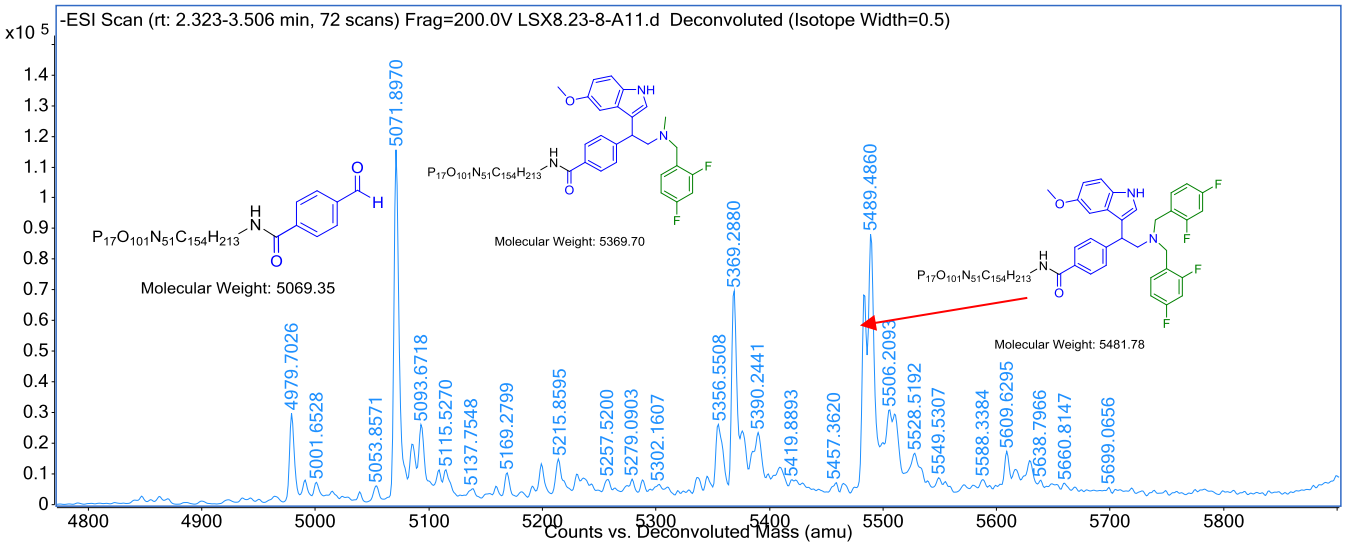
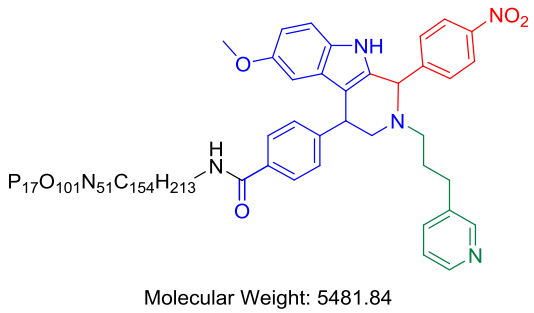


Fig. S106. Deconvoluted mass of **14h**

Figure S107, Mass Spectrum of 14i, related to Figure 7.

Percent conversion: 36.97%
Exact mass: 5481.84
Observed: 5482.4366



Molecular Weight: 5481.84

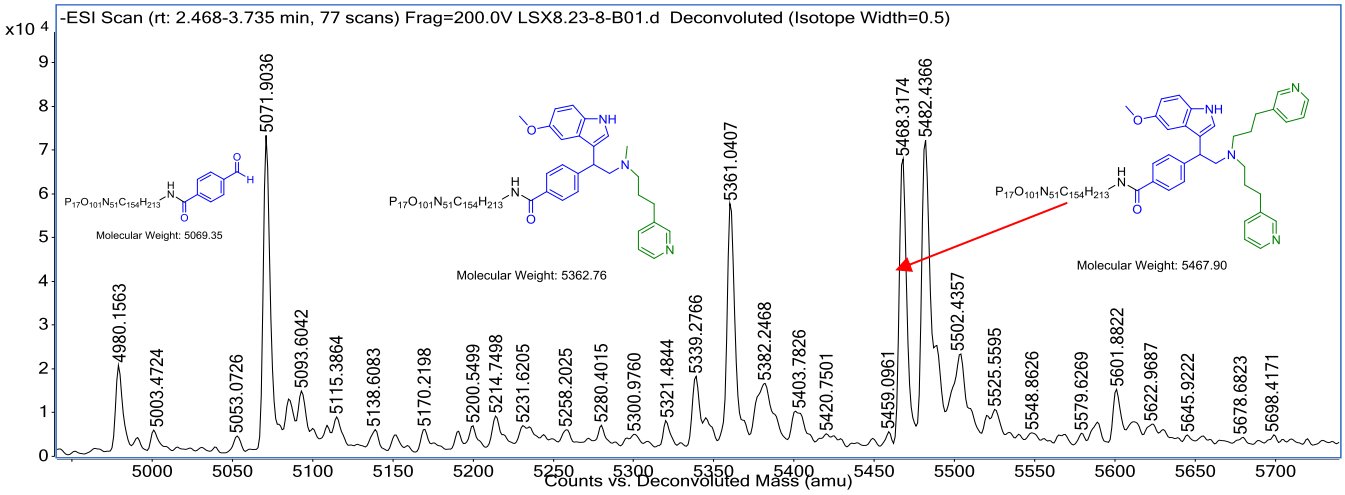
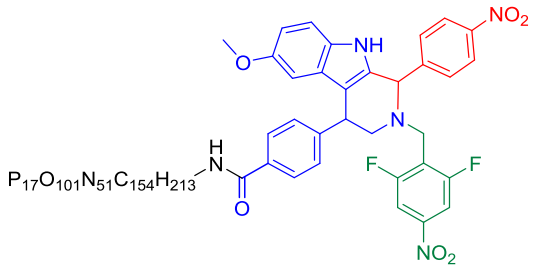


Fig. S107. Deconvoluted mass of **14i**

Figure S108, Mass Spectrum of 14j, related to Figure 7.

Percent conversion: 44.16%
Exact mass: 5533.78
Observed: 5534.1750



Molecular Weight: 5533.78

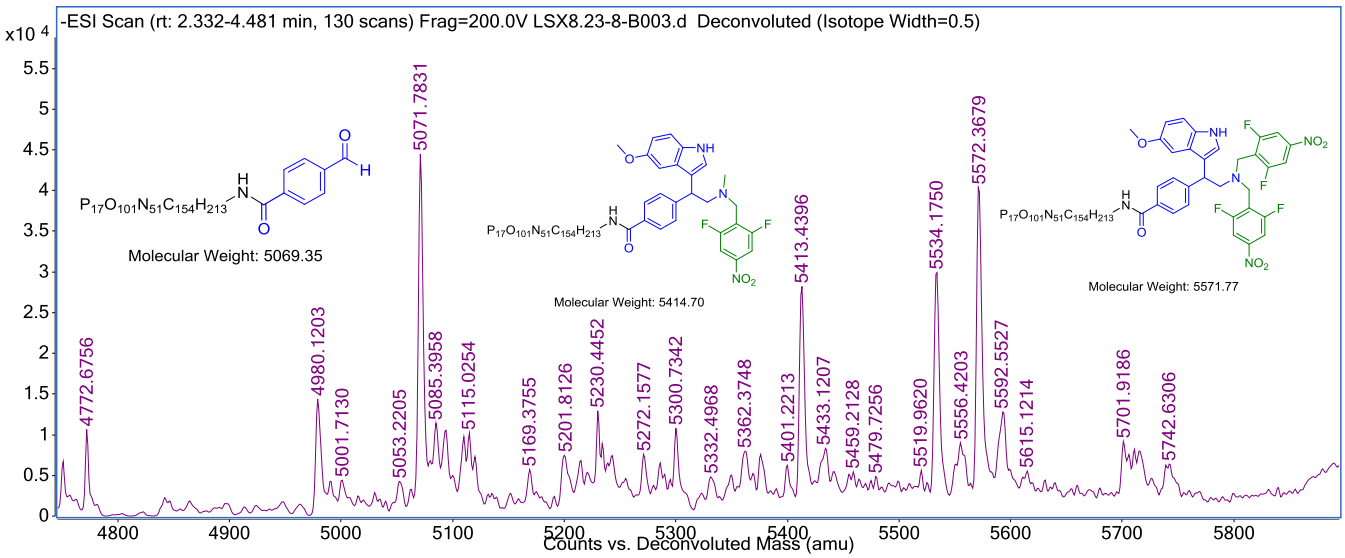
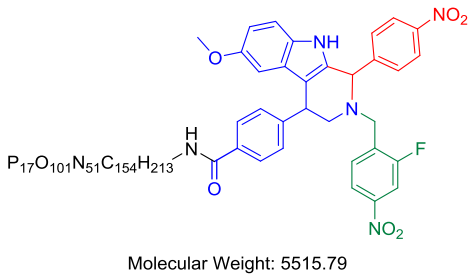


Fig. S108. Deconvoluted mass of **14j**

Figure S109, Mass Spectrum of 14k, related to Figure 7.

Percent conversion: 20.17%
Exact mass: 5515.79
Observed: 5516.4274



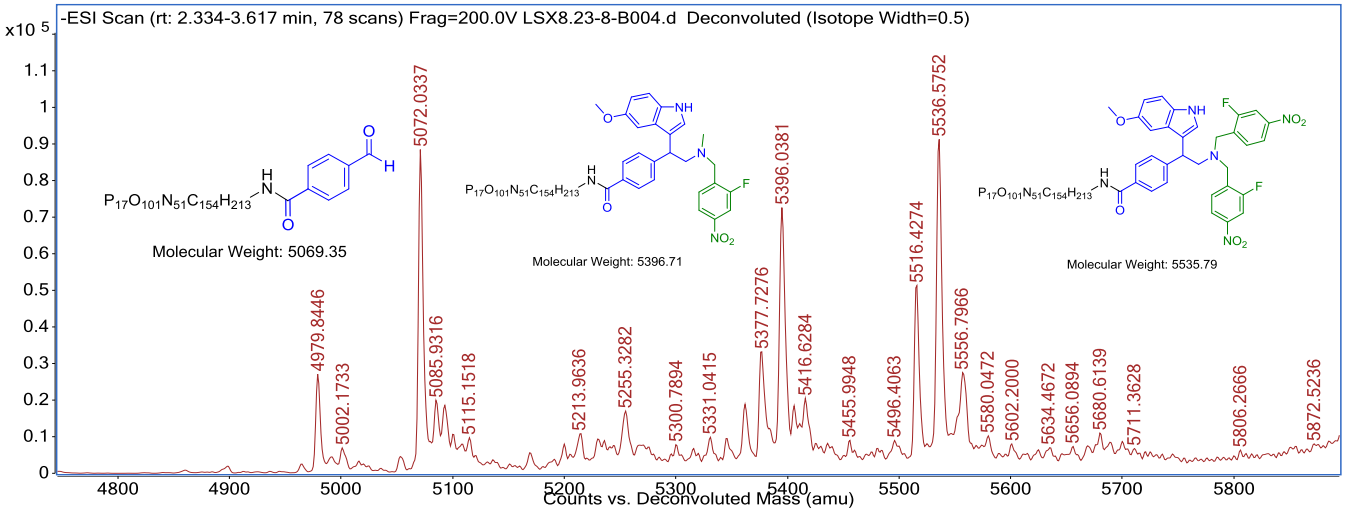
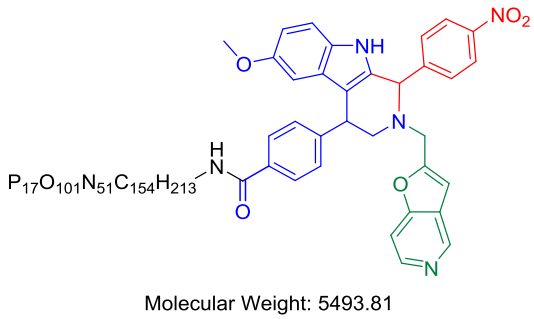


Fig. S109. Deconvoluted mass of **14k**

Figure S110, Mass Spectrum of 14l, related to Figure 7.

Percent conversion: 57.38%
Exact mass: 5493.81
Observed: 5492.5954



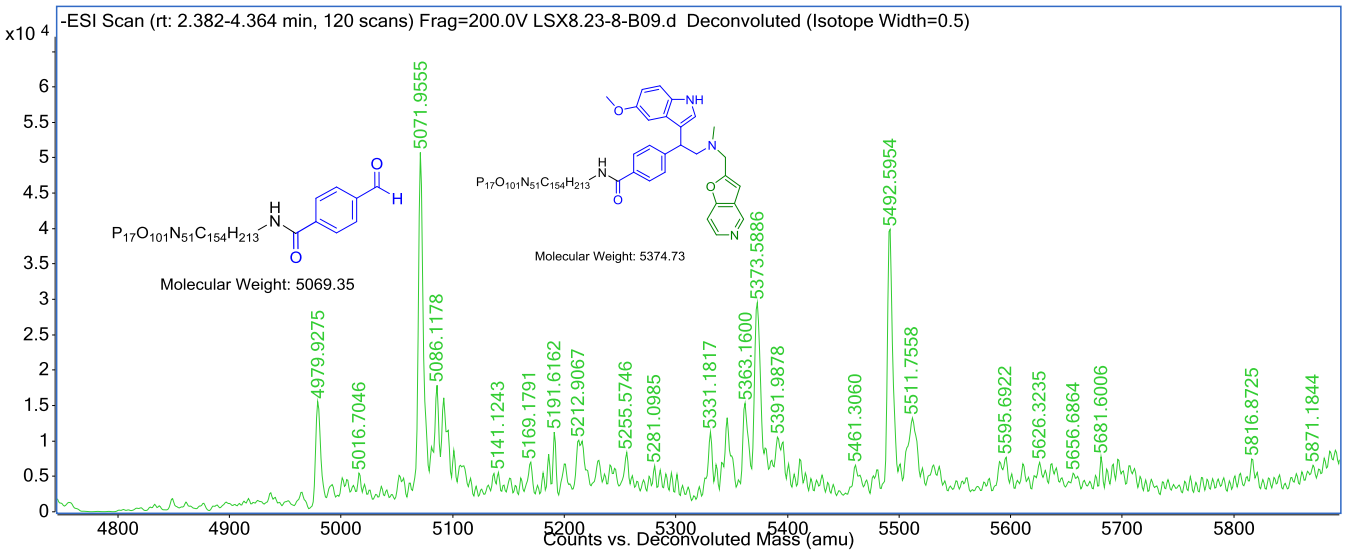


Fig. S110. Deconvoluted mass of **14l**

Tables

Table S5. DNN and KNN comparison, related to Figure 4.

In order to compare the performance of DNN and conventional similarity-based ML methods, a KNN model was implemented based on the same training dataset, and its optimal parameter K=9 (searching range 1-50) was determined by 5-fold cross validation as the same way as DNN. As summarized in the following table, both precision and recall of DNN are higher than the values of KNN on internal test dataset. (ECFP4 as fingerprint)

model	DNN		KNN	
metrics	precision	recall	precision	recall

Internal test dataset	0.81	0.37	0.6	0.2

Table S6. ECFP4 and MACCS comparison, related to Figure 4.

ECFP4 and MACCS keys were separately taken as input of DNN and trained with the same procedures. The performance of two fingerprints on internal test dataset is summarized as following, where both precision and recall of model trained with MACCS are lower than that with ECFP4 on internal test dataset.

fingerprint	ECFP4		MACCS	
metrics	precision	recall	precision	recall
Internal test dataset	0.81	0.37	0.78	0.23

Table S7. The number of clusters: the number of structures with different threshold of the train dataset, internal test dataset, top300 candidates and external test dataset, related to Figure 4.

Table S7. The number of clusters: the number of structures with different threshold

Threshold	0.4	0.5	0.6	0.7
Train	283:1325	520:1324	900:1324	1178:1324
	(1:4.68)	(1:2.55)	(1:1.47)	(1:1.12)
Internal test	135:331	220:331	300:331	324:331
	(1:2.45)	(1:1.50)	(1:1.10)	(1:1.02)
Top300	58:300	94:300	159:300	259:300
	(1:5.17)	(1:3.19)	(1:1.89)	(1:1.16)
External test	13:34	18:34	25:34	33:34
	(1:2.62)	(1:1.89)	(1:1.36)	(1:1.03)

Table S8 Quantitation Result of the concentration check group, related to Figure 6.

Check Group	Dilution Fold	Average(Ct)	Concentration(Copies/μL)	Original Concentration(Copies/μL)	Original Amount(Copies)	Average Amount(Copies)
No Reaction	1.65E+03	7.29	1.39E+08	2.29E+11	2.29E+13	1.64E+13
	1.65E+04	11.17	1.05E+07	1.73E+11	1.73E+13	
	1.65E+05	14.99	8.17E+05	1.35E+11	1.35E+13	
	1.65E+06	18.65	7.10E+04	1.17E+11	1.17E+13	
Pictet-Spengler	1.65E+03	9.4	3.42E+07	5.64E+10	5.64E+12	4.04E+12
	1.65E+04	13.09	2.90E+06	4.79E+10	4.79E+12	
	1.65E+05	17.08	2.02E+05	3.33E+10	3.33E+12	
	1.65E+06	21.03	1.45E+04	2.39E+10	2.39E+12	

Transparent Methods

SI-1 Machine learning model

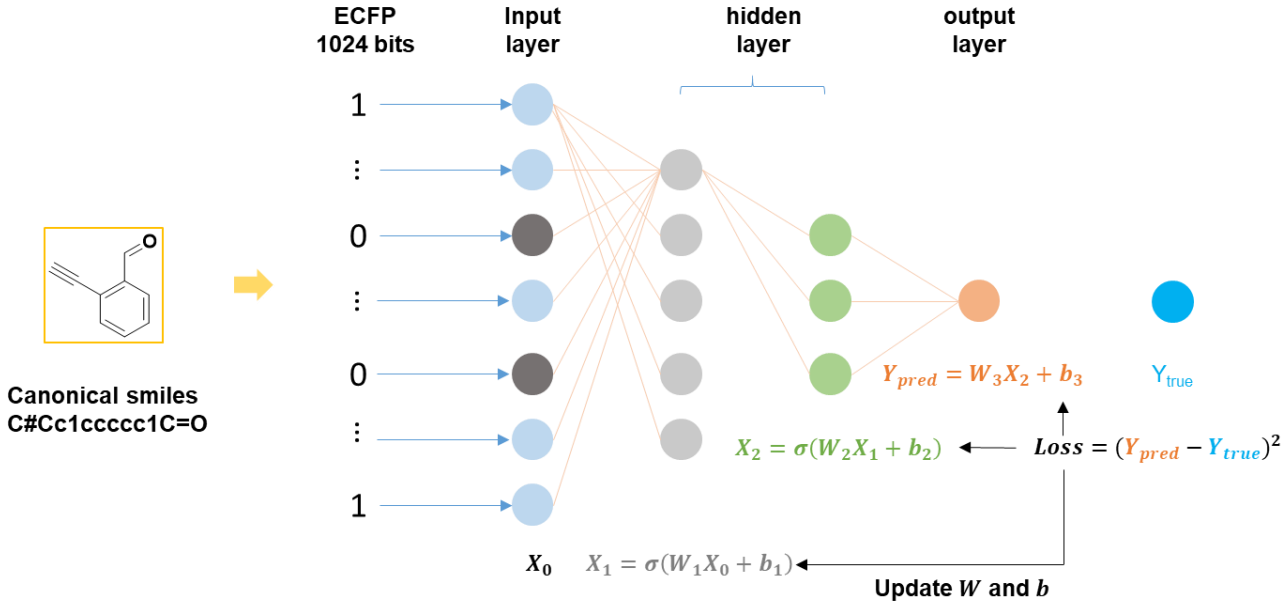
When training the model, molecules initially represented by ECFP4 fingerprints were fed into multiple hidden layers defined below:

$$X_L = \sigma(W_L X_{L-1} + b_L)\ (L \geq 1)$$

where L represents the L-th layer of the model, X_L represents the L-th representation of the molecule. When L=1, X_0 is the input feature ECFP. W_L and b_L represent the weight matrix and bias for the L-th layer. σ is a function for nonlinear transformation. The cost function J(Θ) of the model is as following:

$$J(\Theta) = \left(Y_{pred} - Y_{true}\right)^2$$

Cost function (here is the mean squared error, MSE) applied to a batch of all training data is minimized with respect to the model parameters Θ. Given predicted Y_{pred} and the true Y_{true}, Θ is updated according to the gradient of the prediction.

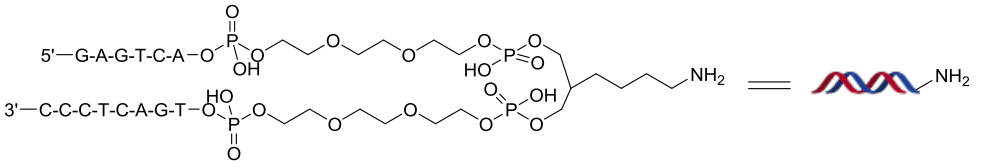


SI-2 General Experimental

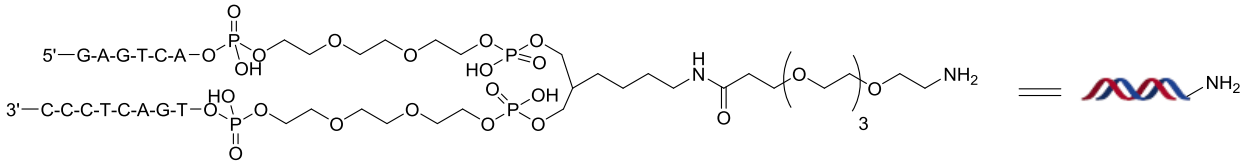
Dimethylsulfoxide (DMSO), 1-methyl-2-pyrrolidinone (NMP), and 2-Propanol (*i*-PrOH) and *N*,*N*-dimethylacetamide (DMAc), EtOH were purchased from Sigma-Aldrich. HATU (CAS: 148893-10-1), *N*,*N*-Diisopropylethylamine (DIPEA), NaCl, NaOAc were purchased from TCI. The $MgCl_2$ was purchased from *J&K*. The ddH_2O was obtained by passing the Milli-Q Direct. The buffer was purchased from

Vazyme. On-DNA reaction yields were determined by UV traces of LC/MSanalysis. The centrifugeinstruments including Allegra X-15R, eppendorf-5424R.

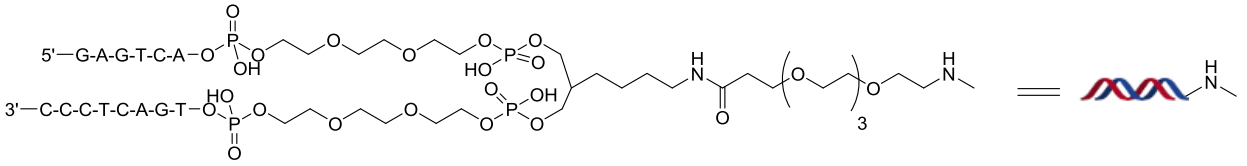
SI-3 HP, S-HP and Me-S-HP Material



HP (Exact Mass: 4937.23)



S-HP (Exact Mass: 5184.48)



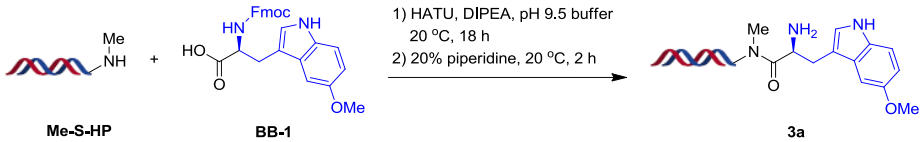
Me-S-HP (Exact Mass: 5198.48)

SI-4 General Procedure

SI-4-1 EtOH Precipitation for DNA substrate

To a DNA reaction mixture was added 10% (V/V) 5 M NaCl solution and 2.5−3 folds the volume of absolute ethanol. The colloidal solution was then allowed to stand at −80 °C for 2 h. The solutions were centrifuged at 4°C for 30 min at 4000 g; the supernatants were discarded. And the DNA pellet was dried at 30°C for 1 h in vacuo. General, ethanol precipitation was performed after each chemical reaction.

SI-4-2 General Procedure 1 for DNA-conjugated tryptamine



Me-S-HP **BB-1** **3a**

1) Acylation of Me-S-HP

To a 15 mL tube was added HATU (200 mM in DMSO, 500 μL, 100 eq.), DIPEA (200 mM in DMSO, 500μL, 100 eq.) and amino acid (200 mM in DMAc, 300 μL, 60 eq.). This solution was eddied, then centrifuged and stood at 20 °C for 15 min to make the activated ester.

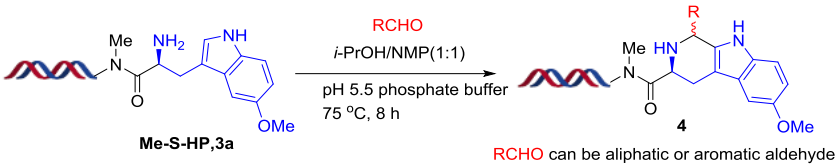
Next, the freshly prepared active ester solution was transferred to the Me-S-HP solution (1 mM in pH 9.5 sodium borate buffer, 1.00 mL, 1 eq.). After addition, the solution was eddied, centrifuged and stood at

20 °C for 2 h. Then the reaction mixture were treated with the second addition of the activated ester solution. The tube was centrifuged, eddied, re-centrifuged and stood at 20 °C for 16 h. After reaction, ethanol precipitation was done.

2) De-Fmoc

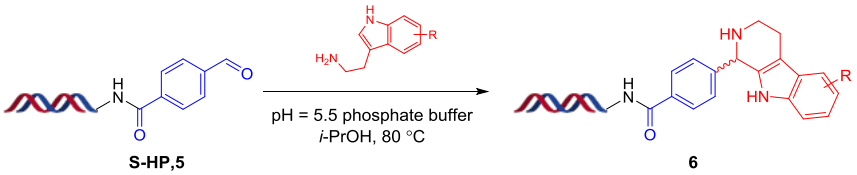
The solid of DNA substrate that from acylation was dissolved in 500 μL ddH_2O to make the 1 mM solutions in 15 mL tube. Then to the DNA solutionwas added 20% piperidine (500 μL). The tube was eddied, centrifuged and stood at 20 °C for 2 hr. After reaction, ethanol precipitation was done.

SI-4-3 General Procedure 2 for DNA-compatible Pictet-Spengler reaction



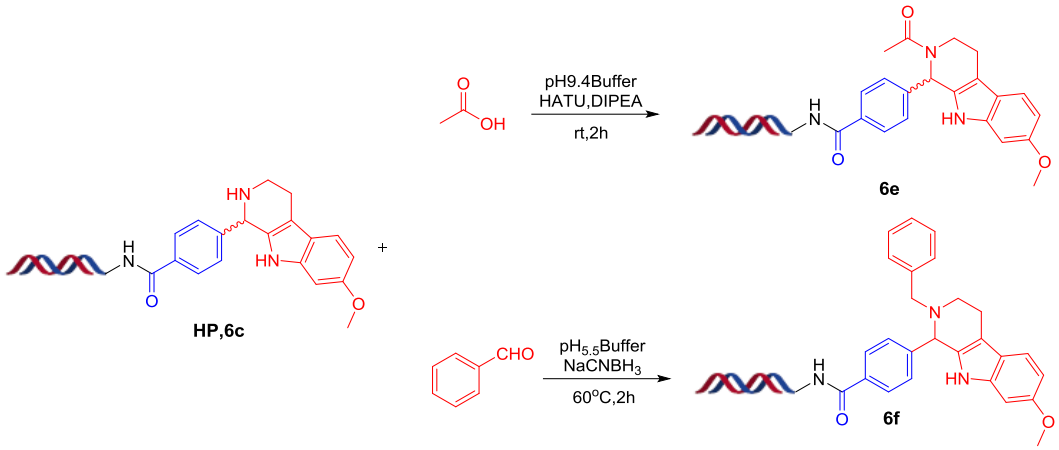
To the solution of DNA-conjugated tryptamine substrate **3**(1 mM in pH 5.5 sodium phosphate buffer, 9.00 μL, 1 *eq.*) was added aldehyde solution (400 mM in NMP, 4.0 μL, 180 *eq.*) in a 96-well plate. The plate was centrifuged, eddied and re-centrifuged. Then the pure *i*-PrOH (4.0 μL) was added to the mixed solution. The mixture was heated in PCR at 75 °C for 8 hr. After then, ethanol precipitation was done.

SI-4-4 General Procedure 3 for DNA-compatible Pictet-Spengler reaction



To the solution of DNA-conjugated aldehyde substrate **5**(1 mM in pH 5.5 sodium phosphate buffer, 10.0 μL, 1 *eq.*) was added tryptamines (400 mM in *i*-PrOH, 5.0 μL, 200 *eq.*) in a 96-well plate. The plate was centrifuged, eddied and re-centrifuged. Then the pure *i*-PrOH (5.0 μL) was added to the mixed solution. The mixture was heated in PCR at 80 °C for 16 hr. After then, ethanol precipitation was done.

SI-4-5 General Procedure 4 for amine capping



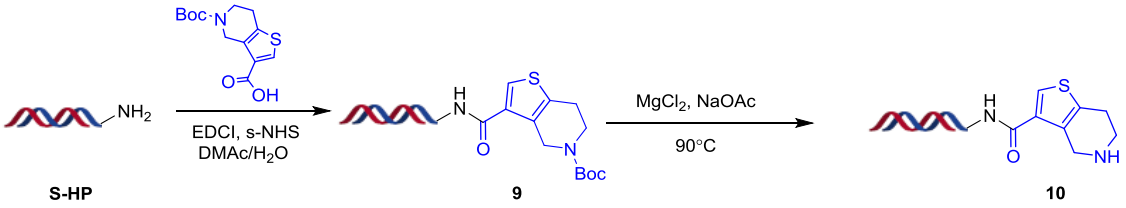
1) Acylation of substrate **6c**

To a 600 μL tube was added HATU (200 mM in DMA, 5 μL, 200 eq.), DIPEA (200 mM in DMA, 5 μL, 200 eq.) and acetic acid (200 mM in DMA, 5 μL, 200 eq.). This solution was mixed by vortex, then centrifuged and stand at 20 °C for 10 min to make the activated ester. Next, the freshly prepared active ester solution was transferred to the HP solution (2.5uL, 2 mM in water, 1 eq.), which was added 2.5uL pH 9.4 buffer solution. After addition, the solution was vortex, centrifuged and stood at 20°C for 2 h. After reaction, ethanol precipitation was done.

2) Reductive amination of **6c**

To the solution of DNA-conjugated amine substrate **6c** (1 mM in pH 5.5 sodium phosphate buffer, 5.00 μL, 1 eq.) was added aldehyde solution (200 mM in DMA, 5 μL, 200 eq.) and $NaCNBH_3$ solution (400 mM in water, 2.5 μL, 200 eq.) in a 250 uL tube. Then the mixture was heated in PCR at 60 °C for 2 hr. After then, ethanol precipitation was done.

SI-4-6 General Procedure 5 for DNA-conjugated amino acids synthesis



1) Acylation of S-HP

To a 15 mL tube was added the solution of EDCI (200 mM in DMSO, 125 μL, 50 *eq.*), *s*-NHS (200 mM in DMSO/ddH_2O=1/1, 75 μL, 30 *eq.*) and BocN-amino acid. This solution was eddied, then centrifuged and stood at 20 °C for 15 min to make the activated ester.

Next, the freshly prepared active ester solution was transferred to the S-HP solution (1 mM in pH 9.5 sodium borate buffer, 500 μL, 1 *eq.*). After addition, the solution was eddied, centrifuged and stood at 20 °C for 2 h. Then the reactions were treated with the second addition of the activated ester solutions. The tube was centrifuged, eddied, re-centrifuged and stood at 20 °C for 16 h. After then, ethanol precipitation was done.

2) De-Boc

The solid of DNA substrate **9** was dissolved in 500 μL ddH_2O to make the 1 mM solution in 15 mL tube. Then to the DNA solution was added 500 μL NaOAc aq. (75 mM in ddH_2O, 75 eq.) and 250 μL $MgCl_2$ aq. (1 mM in ddH_2O, 0.5 *eq.*). The solution was mixed and stood at 90 °C for 16 hr. After then, ethanol precipitation was done.

SI-5 DNA Damage Evaluation

Pictet-Spengler reaction was performed with a DNA conjugated compound with a double stranded DNA coding region to mimic the library component. The product was then ligated to an oligonucleotide to generate a full-length DNA fragment and examined by bioanalyzer (Figure 3). The concentration of DNA was affected by Pictet-Spengler reaction somehow, because there was some liquid phase change and an EtOH precipitation process in the Pictet-Spengler reaction, which could result some loss of the DNA during these steps. The length of the product has no change compared to the control group on the other hand, indicating that the Pictet-Spengler reaction did not affect the maneuverability of the DNA ligation.

SI-5-1 QPCR Test

The concentration and the amplification efficiency of the ligation products were assessed by qPCR after ethanol precipitation. Two parallel experimental groups were set up to determine 1) if the Pictet-Spengler reaction affects the ligation efficiency or the remaining DNA quantity 2) if the Pictet-Spengler reaction affects the amplification efficiency by PCR. qPCR was performed with the SYBR Green Master Mix kit (Thermo) on a Real-Time PCR System (QuantStudio 7 Flex). All samples were run in triplicates and subjected to PCR cycles as follows: 95 °C heat activation for 5 min followed by 40 cycles of 95 °C denaturation (10 seconds each), 55 °C annealing (15 seconds each), and 72 °C extension (30 seconds each). The result showed a slight difference in the starting concentration of the template, suggesting possible degradation or loss of DNA during the process of reaction, consistent with the observation by Bioanalyzer (**Fig. 4**). To further assess the amplification efficiency, the quantity of the full length DNA templates was first normalized based on the Bioanalyzer result and qPCR with serial dilution was performed. Linear fitting was then calculated respectively based on the CT values. The slope, which dictates

the amplification efficacy, was compared between the experimental groups. No significant difference was observed between the Pictet-Spengler reaction group and the negative control group, indicated no obvious impact on PCR efficiency by the reaction. Moreover, melting curves of the qPCR products were examined and no peak shift or multiple peaks were observed, suggesting no significant alteration of DNA species after the reaction. Thus, in summary, the DNA remained in good integrity after the Pictet-Spengler reaction.

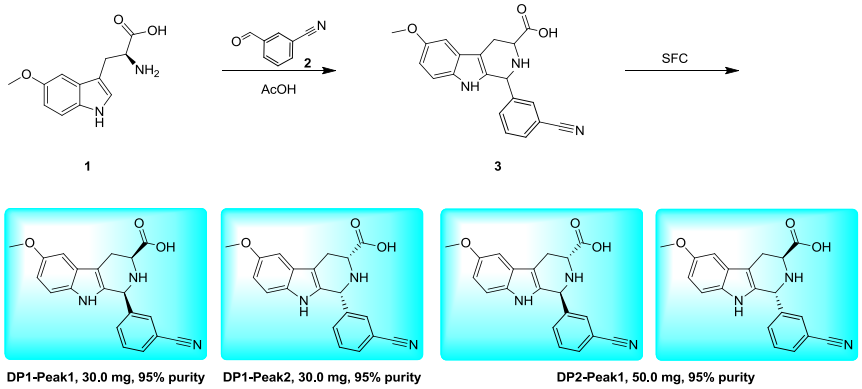
SI-5-2 Next-generation sequencing.

2 μL of the 1.65e+5 folds dilution sample was used as a template for PCR amplification. To a PCR tube was added diluted sample (2 μL), 10x high fidelity PCR buffer (5 μL), 50 mM $MgSO_4$ (2 μL), 10 mM dNTP mix (1 μL), Platinum Taq DNA Polymerase (0.2 μL), 10 μM forward primer (2 μL), 10 μM reverse primer (2 μL), and nuclease-free water (35.8 μL). The PCR products were purified by the Agencourt AMPure XP Beads method. The purified samples were sent for next-generation sequencing (Illumina NovaSeq). Bowtie2 was used to map the sequenced reads by local alignment. The detailed mapping identity were extracted from CIGAR string and XM flag in the SAM format. The results of NGS showed that all samples retained the right sequence as expected (**Figure 6**), indicating that the chemical reactions did not affect the encodability of DNA tags.

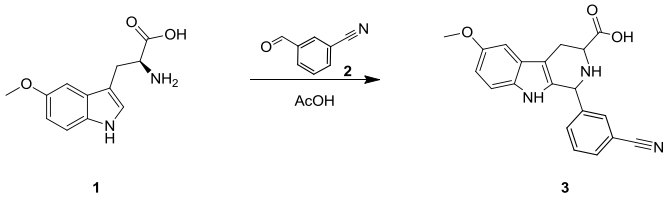
In conclusion, our data revealed that the Pictet-Spengler reactions used in this paper caused no damage to DNA, and thus could potentially be used for the encoded library construction.

SI-6 Off-DNA Validation of PS Reaction

SI-6-1 Synthetic Scheme



SI-6-2 General procedure for preparation of intermediate 3

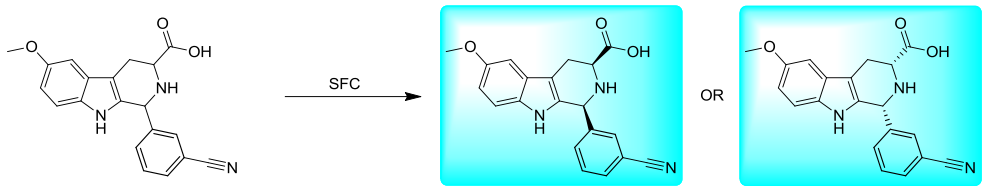


To a solution of **Compound 1** (500 mg, 2.13 mmol, 1.00 eq) in AcOH (5.00 mL) was added **Compound 2** (307.88 mg, 2.35 mmol, 1.10 eq). The mixture was stirred at 110 °C for 5 hrs. LCMS (EW22081-2-P1A1, RT1=0.700, RT2=0.735) showed **Compound 1** was consumed completely and two main peaks with desired was detected. The mixture was concentrated under reduced pressure to give a residue. **Compound 3** contained two parts (triturated product and prep-HPLC product). The crude product was triturated with CH_3CN at 25 °C for 30 min and obtained **Compound 3** (300 mg, 100% purity) as a yellow solid, which was confirmed by LCMS (EW22081-2-P1A3), HPLC (EW22081-2-P1H1), H NMR (EW22081-2-P1R3), SFC (EW22081-2-P1S2_c2). And the mother liquor was purified by prep-HPLC (column: Phenomenex luna C18 15*40mm*15um; mobile phase: (water (0.1%TFA)-CAN); B%: 10%-40%, 10min) and got another **Compound 3** (320 mg, 69.8% purity) as a yellow solid, which was confirmed by LCMS (EW22081-2-P1A7), SFC (EW22081-2-P1S4_d1), SFC (EW22081-2-P1S4_d2).

^{1}H NMR: EW22081-2-P1R3, (400 MHz, MeOD)

δ 7.89 (t, J = 15.6 Hz, 2 H), 7.79 (d, J = 8.0 Hz, 1 H), 7.68 (t, J =15.6 Hz, 1H), 7.15 (d, J =8.8 Hz, 1H), 7.06 (d, J = 2.0 Hz, 1H), 6.80 (dd, J_1 = 8.8 Hz, J_2 = 2.4 Hz, 1H), 5.86 (s, 1H), 4.15 (dd, J_1 = 12 Hz, J_2 = 5.2 Hz, 1H), 3.83 (s, 3H), 3.50 (dd, J_1 = 15.6 Hz, J_2 = 4.4 Hz, 1H), 3.20 - 3.10 (m, 1H).

SI-6-3 General procedure for preparation of DP1-Peak1 and DP1-Peak2



Compound 3 was purified by prep-SFC (column: DAICEL CHIRALCEL OJ (250mm*30mm, 10um); mobile phase: (0.1%NH3H2O ETOH); B%: 40%-40%, 3.6 min; 180 min) to go two product. DP1-Peak1 (0.08 g, 24.5% yield, 98.0% purity) was obtained as a off-white solid, which was confirmed by H NMR (EW22081-3-P1R3), LCMS (EW22081-3-P1A1), HPLC (EW22081-3-P1H1), SFC (EW22081-3-P1S1_c1), NOE (EW22081-3-P1N1), C NMR (EW22081-3-P1C3). DP1-Peak2 (0.10 g, 30.3% yield, 97.0% purity) was obtained as a yellow solid, which was confirmed by H NMR (EW22081-3-P1R4), LCMS (EW22081-3-P1A2), HPLC (EW22081-3-P1H2), SFC (EW22081-3-P1S2_c1), NOE (EW22081-3-P1N2), C NMR (EW22081-3-P1C4).

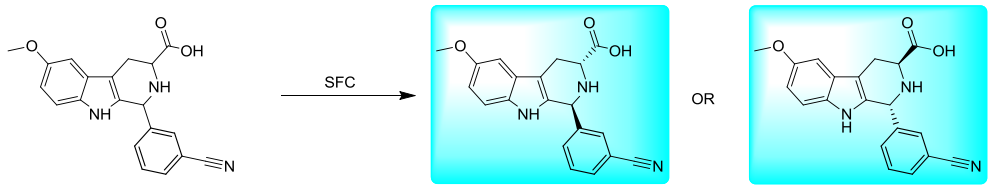
^{1}H NMR: EW22081-3-P1R3, (400 MHz, DMSO-d_6)

δ 10.23 (s, 1H), 7.82 (d, *J* = 6.8 Hz, 2H), 7.72 (d, *J* = 8.0 Hz, 1H), 7.58 (t, *J* = 15.6 Hz, 1H), 7.08 (d, *J* = 8.8 Hz, 1H), 6.97 (d, *J* = 2.2 Hz, 1H), 6.66 (dd, J_1 = 8.8 Hz, J_2 = 2.0 Hz, 1H), 5.35 (s, 1H), 3.75 (s, 3H), 3.72 (s, 1H), 3.06 (d, *J* = 14.8 Hz, 1H), 2.81 (t, *J* = 26.0 Hz, 1H).

^{1}H NMR: EW22081-3-P1R4, (400 MHz, DMSO-d_6)

δ 10.24 (s, 1H), 7.82 (d, *J* = 6.4 Hz, 2H), 7.72 (d, *J* = 8.0 Hz, 1H), 7.58 (t, *J* = 16.0 Hz, 1H), 7.08 (d, *J* = 8.8 Hz, 1H), 6.97 (d, *J* = 2.4 Hz, 1H), 6.66 (dd, J_1 = 8.4 Hz, J_2 = 2.0 Hz, 1H), 5.36 (s, 1H), 3.75 (s, 3H), 3.72 (s, 1H), 3.07 (d, *J* = 15.2 Hz, 1H), 2.81 (t, *J* = 24.8 Hz, 1H).

SI-6-4 General procedure for preparation of DP2-Peak1

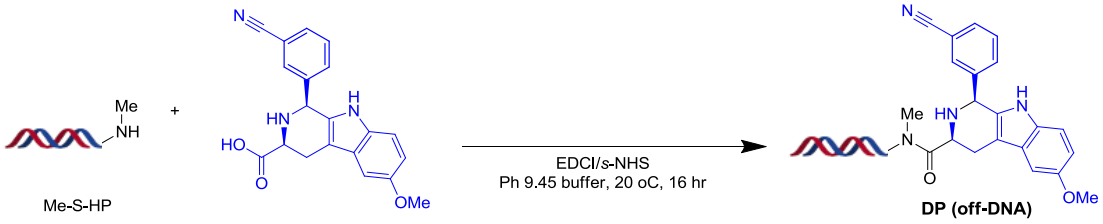


Compound 3 was purified by prep-SFC (column: DAICEL CHIRALPAK IG (250mm*30mm, 10um); mobile phase: (0.1%NH3H2O ETOH); B%: 45%-45%, 4.6min; 50min) to go two product. DP2-Peak1 (0.15 g, 394.68 umol, 45.70% yield, 91.4% purity) was obtained as a yellow solid, which was confirmed by H NMR (EW22081-4-P1R3), LCMS (EW22081-4-P1A1), HPLC (EW22081-4-P1H1), NOE (EW22081-4-P1E1), C NMR (EW22081-4-P1C1), SFC (EW22081-4-P1S2_d11).

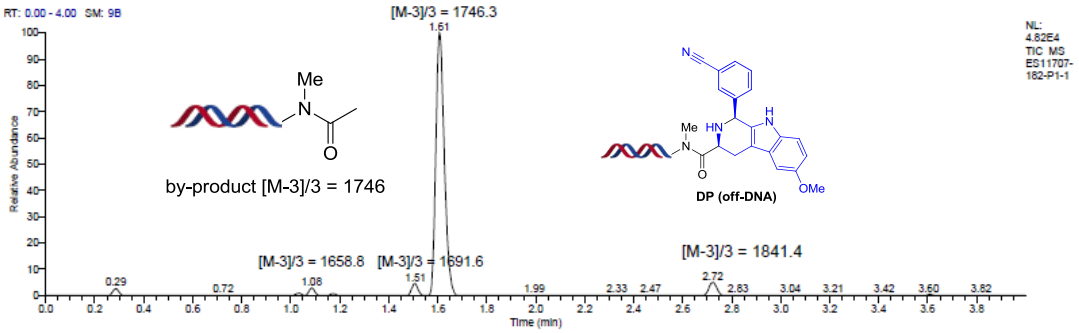
^{1}H NMR: EW22081-4-P1R3, EW22081-4-P1R2, (400MHz, DMSO-d_6)

δ 10.57 (s, 1H), 7.76 (t, *J* = 20.8 Hz, 2H), 7.61 (d, *J* = 7.6 Hz, 1H), 7.55 (t, *J* = 15.2 Hz, 1H), 7.14 (d, *J* = 8.8 Hz, 1H), 6.99 (d, *J* = 2.4 Hz, 1H), 6.70 (dd, J_1 = 8.8 Hz, J_2 = 2.4 Hz, 1H), 5.53 (s, 1H), 3.76 (s, 3H), 3.73 (d, *J* = 6.4 Hz, 1H), 3.12 (dd, J_1 = 15.2 Hz, J_2 = 5.2 Hz, 1H), 2.94 (dd, *J* 1= 15.2 Hz, J2 = 7.6, 1H).

SI-6-5 LC Trace and Mass of DP

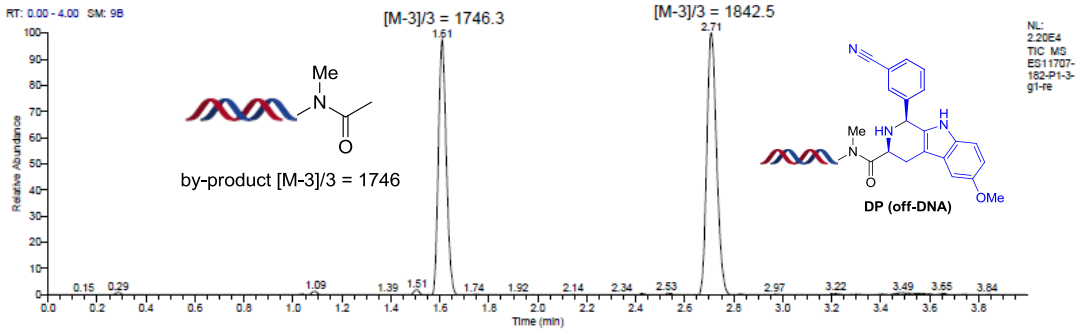


Following **General Procedure 1**
Yield: 4.14%
Exact mass: 5528.10
Triply charged mass [M-3]/3, calculated: 1841.7; observed: 1841.4



RT	Peak Height	Peak Width	Peak Area	Area %
1.08	1792.03	0.04	2141.68	1.69
1.50	2568.62	0.05	3770.00	2.97
1.61	53830.47	0.10	115768.49	91.20
2.72	2614.04	0.06	5257.59	4.14

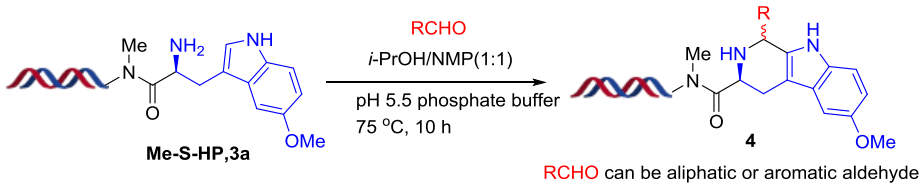
DP (off-DNA)+4af (retain time = 2.71)



RT	Peak Height	Peak Width	Peak Area	Area %
1.61	23531.58	0.09	48002.13	44.13
2.71	23648.51	0.11	60762.34	55.87

Fig.40. LC trace and mass of **DP**

SI-7 Mass Spectrum of 34 Aldehyde Building Blocks



Materials

Product **3a**: 1 mM in sodium phosphate buffer (250 mM, pH = 5.5)

Aldehyde: 400 mM in NMP

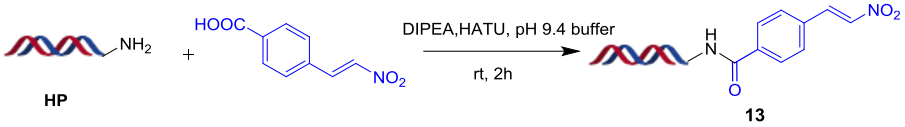
Sodium phosphate buffer, pH = 5.5, 250 mM

Procedure

To **3a** solution (5 nmol, 5 μL), was added to a solution of aldehyde (400 mM in NMP, 2.25 μL, 180 eq). Then the *i*-PrOH (2.25 μL) was added to the mixed solution. The mixture was vortexed. Heat the reaction

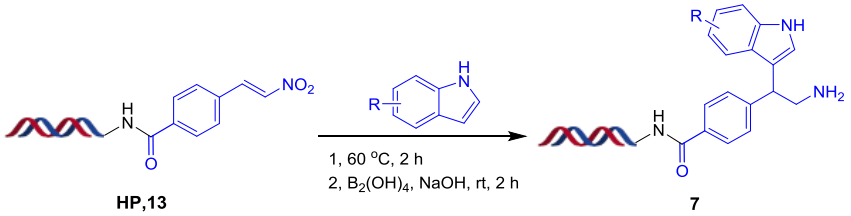
mixture in PCR at 75 °C for 10 h. After the reaction, add 40.5 μL water, then add 5 M NaCl solution (10 % by volume) and cold ethanol (2.5 times by volume, ethanol stored at -20 °C). The mixture was stored at a -80 °C freezer for more than 30 minutes. Centrifuge the sample for around 30 minutes at 4 °C in a micro-centrifuge at 10000 rpm. The above supernatant was removed and the pellet (precipitate) was cooled in liquid nitrogen and then placed on a lyophilizer. After lyophilization, the dry pellet was recovered.

SI-8 General Procedure for DNA-conjugated nitroalkene 13



To a 600 μL tube was added HATU (200 mM in DMA, 50 μL, 100 eq.), DIPEA (200 mM in DMA, 50 μL, 100 eq.) and 4-(2-nitrovinyl) benzoic acid (200 mM in DMA, 100 μL, 200 eq.). This solution was mixed by vortex, then centrifuged and stand at 20 °C for 10 min to make the activated ester. Next, the freshly prepared active ester solution was transferred to the HP solution (50 μL, 2 mM in water, 1 eq.), which was added 50 μL pH 9.4 buffer solution. After addition, the solution was vortex, centrifuged and stood at 20 °C for 2 h. After reaction, ethanol precipitation was done.

SI-9 General Procedure for DNA conjugated indole substituted amine 7



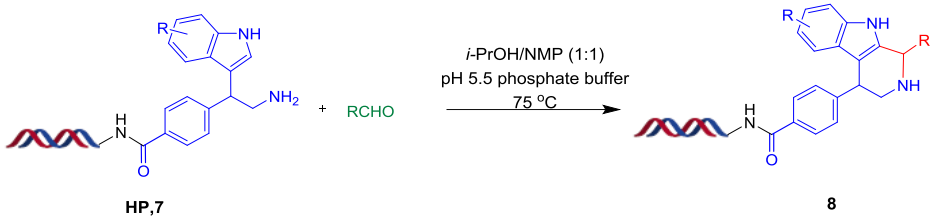
1) Addition of 6-methoxy-1*H*-indole

To the solution of DNA-conjugated nitroalkene **13** (1 mM in water, 50.00 μL, 1 eq.) was added 6-methoxy-1*H*-indole solution (200 mM in DMA, 50.0 μL, 200 eq.) in a 250 μL tube. The mixture was vortex. Then the mixture was heated in PCR at 60 °C for 2 hr. After then, ethanol precipitation was done.

2) Nitro reduction

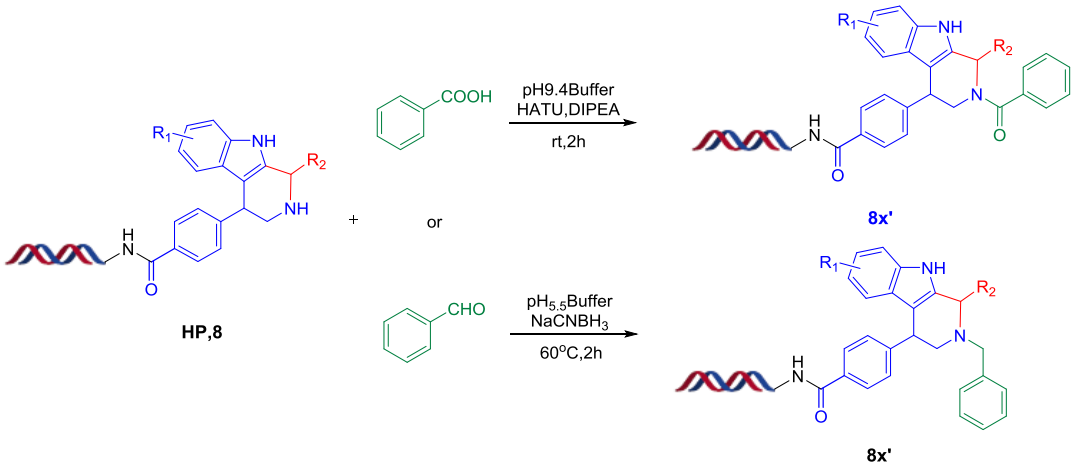
To the solution of DNA-conjugated substrate (1 mM in water, 50.00 μL, 1 eq.) was added $B_2(OH)_4$ solution (100 mM in water, 50.0 μL, 100 eq.) in a 250 μL tube. The mixture was vortex. After addition, the solution was vortex, centrifuged and stood at 20 °C for 2 h. After reaction, ethanol precipitation was done.

SI-10 General Procedure for DNA-compatible Pictet-Spengler reaction



To the solution of DNA-conjugated indole substituted amine **7** (1 mM in pH 5.5 sodium phosphate buffer, 5.00 μL, 1 eq.) was added 4-nitrobenzaldehyde solution (400 mM in NMP, 2.25 μL, 180 eq.) in a 250 μL tube. Then the pure *i*-PrOH (4.0 μL) was added to the mixed solution. The mixture was heated in PCR at 75 °C for 16 hr. After then, ethanol precipitation was done.

SI-10-1 General Procedure for amine capping



1) Acylation of substrate **8**

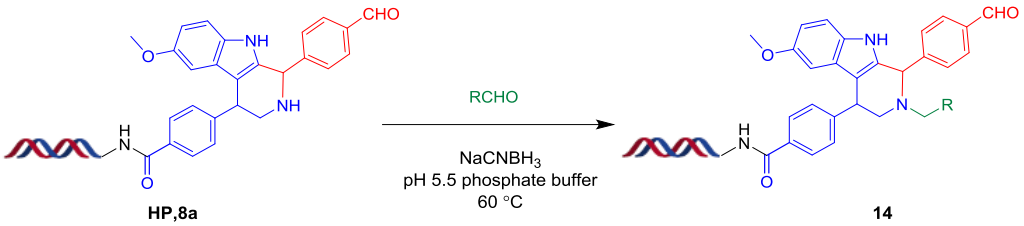
To a 600 µL tube was added HATU (200 mM in DMA, 5 µL, 200 eq.), DIPEA (200 mM in DMA, 5 µL, 200 eq.) and benzoic acid (200 mM in DMA, 5 µL, 200 eq.). This solution was mixed by vortex, then centrifuged and stand at 20 °C for 10 min to make the activated ester. Next, the freshly prepared active ester solution was transferred to the HP solution (2.5uL, 2 mM in water, 1 eq.), which was added 2.5uL pH 9.4 buffer solution. After addition, the solution was vortex, centrifuged and stood at 20°C for 2 h. After reaction, ethanol precipitation was done.

2) Reductive amination of **8**

To the solution of DNA-conjugated amine substrate **8** (1 mM in pH 5.5 sodium phosphate buffer, 5.00 µL, 1 eq.) was added aldehyde solution (200 mM in DMA, 5 μL, 200 eq.) and $NaCNBH_3$ solution (400

mM in water, 2.5 μL, 200 eq.) in a 250 uL tube. Then the mixture was heated in PCR at 60 °C for 2 hr. After then, ethanol precipitation was done.

SI-11 General Procedure for amine capping



To the solution of DNA-conjugated amine substrate **8a** (1 mM in pH 5.5 sodium phosphate buffer, 5.00 µL, 1 eq.) was added aldehyde solution (400 mM in NMP, 2.5 μL, 200 eq.) and $NaCNBH_3$ solution (400 mM in water, 2.5 μL, 200 eq.) in a 250 uL tube. Then the mixture was heated in PCR at 60 °C for 2 hr. After then, ethanol precipitation was done.