DEVELOPING CYCLIC OPIOID ANALOGUES: FLUORESCENTLY LABELED BIOCONJUGATES OF BIPHALIN

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Synthesis of unknown key intermediates and final compounds characterization

N-(2-(3,4-dibromo-2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)ethyl)-5-(dimethylamino)naphthalene-1-sulfonamide (**1**): Commercially available dansylchloride was functionalized with *tert*-Butyl *N*-(2-aminoethyl)carbamate following the procedure described by Youziel *et al.*[1] The *N*-Boc protected compound has been already reported in literature and characterized. This intermediate was deprotected with TFA:DCM = 1:1 at r.t. for 1h, then the solvent was removed in rotary evaporator washing with DCM repetitively and dried in high vacuum. The crude peptide was used as such for the following reaction without further purification. 3,4-dibromofuran-2,5-dione was dissolved in AcOH at r.t. then the intermediate TFA salt was added and the system was allowed to reflux at around 110°C for 9h. At r.t. a green-yellow solid precipitate was formed; the liquid was decanted and the solid was dried in high vacuum to yield the desired product **1** in 98% yield after precipitation, pure on TLC (AcOEt:*n*-hexane = 1:1; R*f* = 0.6). ESI-LRMS calc. for $C_{18}H_{17}Br_2N_3O_4S$: 528.9 [M]; found: 529.2 $[M+H]^+$; ^{1}H NMR (DMSO-d_6) δ: 8.42 (d, 1H, *J* = 8.7 Hz, H-4 aromatic dansyl mojety), 8.16-8.13 (m, 2H, NH and H-6 aromatic dansyl mojety), 8.02 (d, 1H, *J* = 7.5 Hz, H-3 aromatic dansyl mojety), 7.61-7.51 (m, 2H, H-5 and H-2 aromatic dansyl mojety), 7.24 (d, 1H, *J* = 7.5 Hz, H-1 aromatic dansyl mojety), 3.43 (t, 2H, CH_2 aliphatic chain), 3.01 (q, 2H, *J* = 6 Hz, NH-C$\underline{H_2}$ aliphatic chain), 2.82 (s, 6H, 2*CH_3). ^{13}C NMR (300 MHz) δ: 164.3, 150.6, 142.8, 133.7, 128.4, 127.3, 125.8, 124.9, 123.4, 119.2, 117.3, 47.3, 46.8, 37.1.

3-(3,4-dibromo-2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-N-(4-methyl-2-oxo-2H-chromen-7-yl)propanamide (**2**): Commercially available 7-Amino-4-methylcoumarin was reacted with Boc-β-Ala-OH following the procedure reported by Heltweg *et al*.[2] to obtain an intermediate compound pure on silica gel TLC plate (AcOEt 100%, R*f* = 0.8), in 63% yield after reaction work-up. This intermediate was deprotected with TFA:DCM = 1:1 at r.t. for 1h, then the solvent was removed in rotary evaporator washing with DCM repetitively and dried in high vacuum. The intermediate was used as such for the following reaction without further purification. 3,4-dibromofuran-2,5-dione was dissolved in AcOH at r.t. then the intermediate TFA salt was added and the system was allowed to reflux at around 110°C for 9h. At r.t. a yellow solid precipitate was formed; the liquid was decanted and the solid was dried in high vacuum to yield a crude intermediate product, which was purified on silica gel column chromatography (eluent: 100% AcOEt) to give compound **2** in 40% yield after isolation. TLC: AcOEt 100%; R*f* = 0.6. ESI-LRMS calc. for $C_{17}H_{12}Br_2N_2O_5$: 481.9 [M]; found: 482.5 $[M+H]^+$; ^{1}H NMR (DMSO-d_6) δ: 7.89 (d, 1H, *J* = 8.7 Hz, H-4 aromatic coumarin mojety), 7.72 (d, 1H, H-2 aromatic coumarin mojety), 7.44-7.40 (m, 2H, H-3 + NH), 6.45 (s, 1H, H-1 aromatic coumarin mojety), 3.40,3.26 (t, 4H, 2*CH_2 aliphatic chain under DMSO), 2.44 (s, 3H, CH_3). ^{13}C NMR (300 MHz) δ: 173.3, 164.1, 160.3, 154.6, 152.5, 138.9, 126.5, 124.7, 118.1, 115.4, 112.9, 111.1, 40.8, 32.1, 19.6.

3,4-dibromo-1-(4-methyl-2-oxo-2H-chromen-7-yl)-1H-pyrrole-2,5-dione (**3**): 3,4-dibromofuran-2,5-dione was dissolved in AcOH at r.t. then the 7-Amino-4-methylcoumarin was added and the system was allowed to reflux at around 110°C for 9h. At r.t. a yellow solid precipitate was formed; the liquid was decanted and the solid was dried in high vacuum to give a yellow powder product **3**, pure on TLC plate (100% AcOEt, R*f* = 0.8), with 71% yield after reaction work-up. ESI-LRMS calc. for $C_{14}H_7Br_2NO_4$: 410.8 [M]; found: 411.5 $[M+H]^+$;

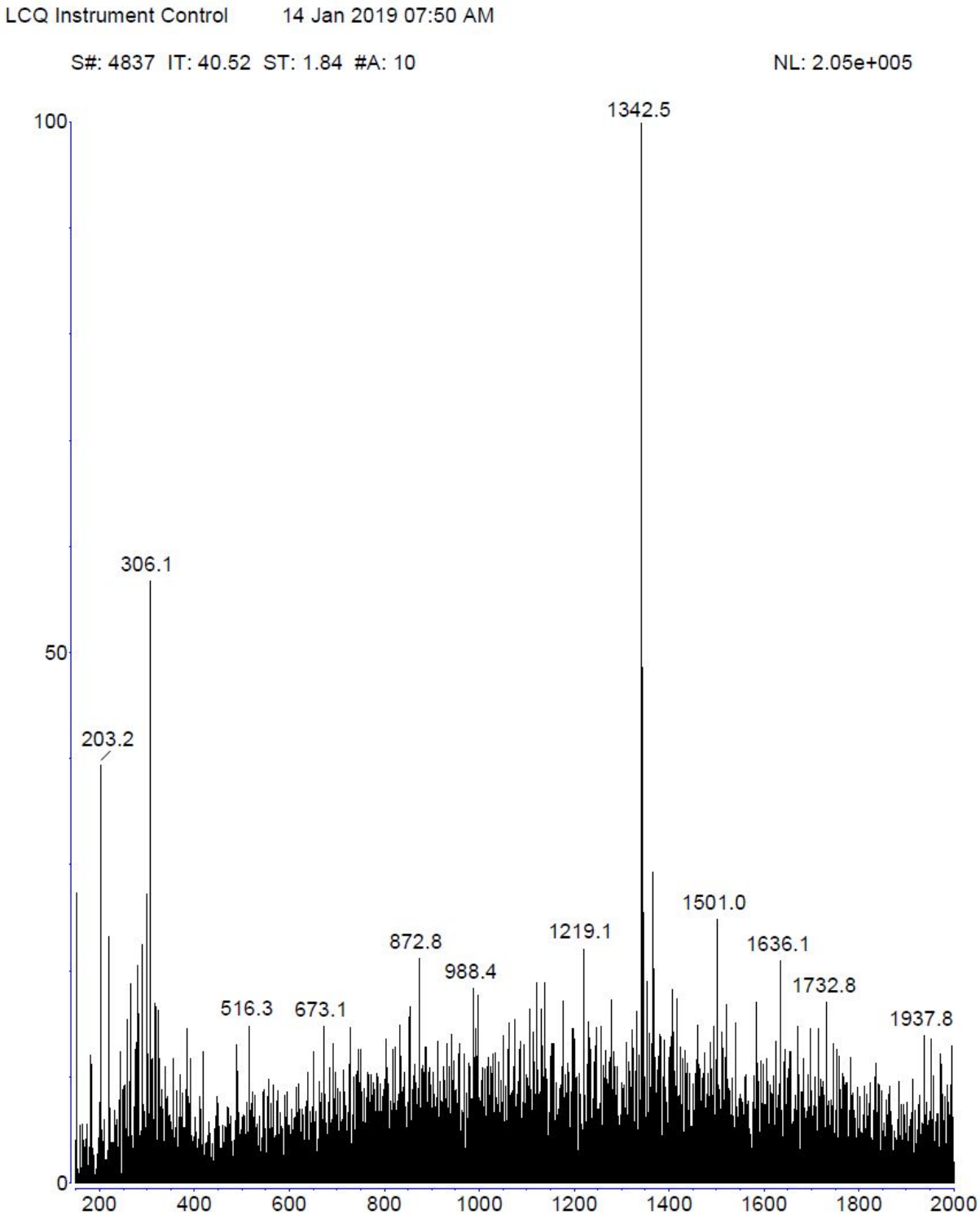
^{1}H NMR ($CDCl_3$) δ: 7.71 (d, 1H, *J* = 9 Hz, H-2 aromatic coumarin mojety), 7.44-7.35 (m, 2H, H-3 + H-4 aromatic coumarin mojety), 6.34 (s, 1H, H-1 aromatic coumarin mojety), 2.46 (s, 3H, CH_3). ^{13}C NMR (300 MHz) δ: 163.4, 159.8, 153.2, 153.1, 134.5, 130.3, 126.4, 122.9, 119.7, 115.3, 114.8, 110.1, 18.5.

Compound **1D**: The desired product has been obtained in good overall yield (71%) following the procedure previously reported by us;[3] Rt (HPLC) = 15.60 min (284 nm). ^{1}H NMR (DMSO-d_6) δ: 1.88 (s, 6H, 2*CH_3 aromatic dansyl mojety), 2.34-2.65 (4H, m, Tyr $^{\beta}CH_2$; 4H, m, Phe $^{\beta}CH_2$), 2.91 (4H, d, D-Cys $^{\beta}CH_2$), 3.03 (4H, m, Gly $^{\alpha}CH_2$), 3.08 (q, 2H, *J* = 6 Hz, NH-C$\underline{H}_2$ aliphatic chain), 3.48 (t, 2H, CH_2 aliphatic chain), 3.91-4.11 (2H, t, Tyr $^{\alpha}$CH; 2H, t, Phe $^{\alpha}$CH), 4.71-4.81 (2H, m, D-Cys $^{\alpha}$CH), 6.61 (4H, dd, Tyr Ar), 7.01 (4H, dd, Tyr Ar), 7.09-7.26 (10H, m, Phe Ar; 3H, m, H-5, H-2, H-1 aromatic dansyl mojety), 8.04-8.21 (6H, d, Tyr NH_3^+; 2H, d, D-Cys; 3H, m, NH and H-6, H-3 aromatic dansyl mojety), 8.68-8.82 (2H, d, Phe NH; 2H, t, Gly NH; d, 1H, H-1 aromatic dansyl mojety), 9.33 (2H, s, OH), 10.31 (2H, s, NH-NH). ESI-LRMS: Calcd exact mass without TFA for $C_{64}H_{71}N_{13}O_{14}S_3$ m/z: 1341.4 [M]; found 1342.5 $[M+H]^+$.

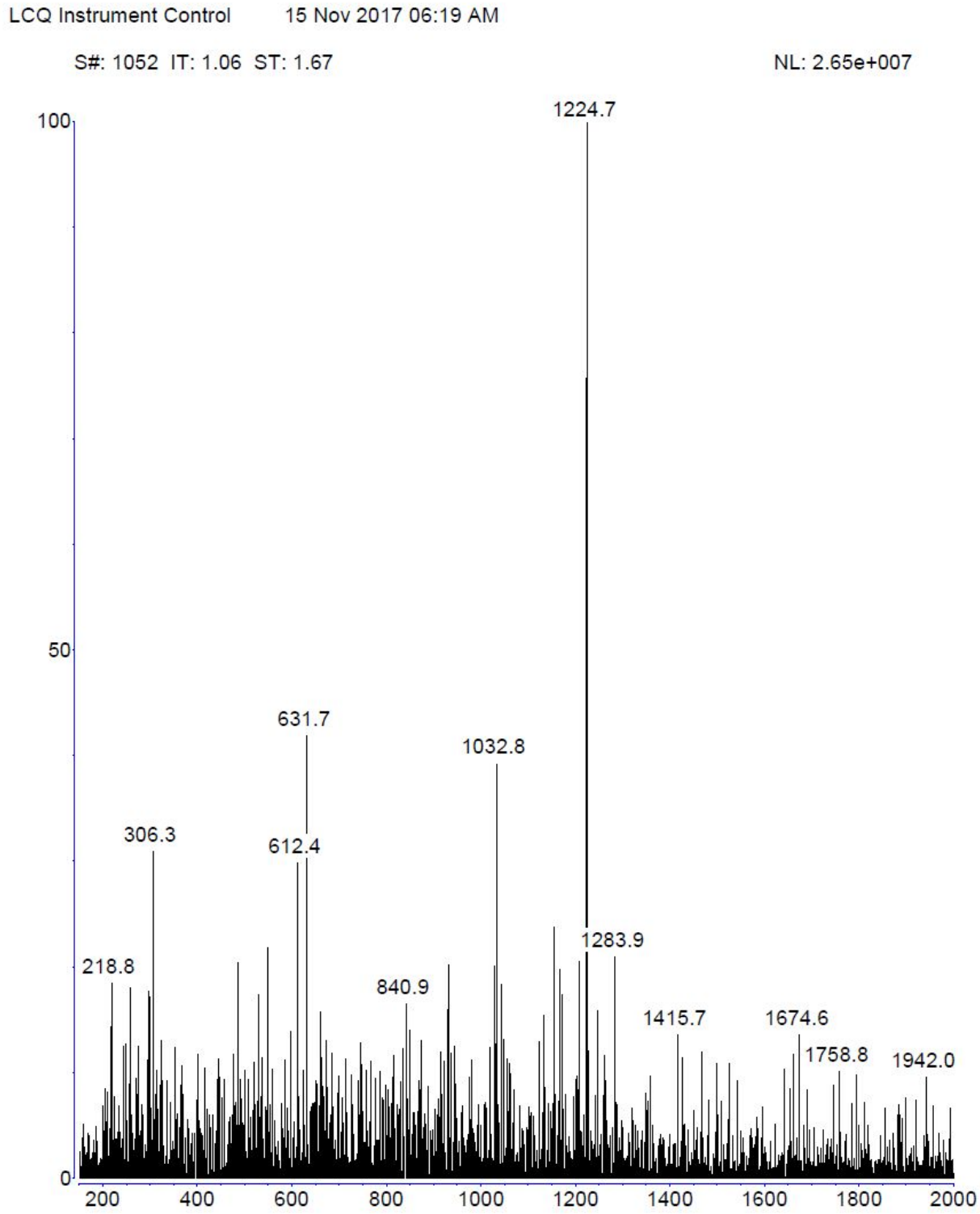
Compound **1C**: The desired product has been obtained in good overall yield (61%) following the procedure previously reported by us;[3] Rt (HPLC) = 15.85 min (284 nm). ^{1}H NMR (DMSO-d_6) δ: 1.21 (s, 3H, CH_3 coumarin mojety), 2.34-2.65 (4H, m, Tyr $^{\beta}CH_2$; 4H, m, Phe $^{\beta}CH_2$), 2.97 (4H, d, D-Cys $^{\beta}CH_2$), 3.01 (4H, m, Gly $^{\alpha}CH_2$), 3.91-4.11 (2H, t, Tyr $^{\alpha}$CH; 2H, t, Phe $^{\alpha}$CH), 4.71-4.81 (2H, m, D-Cys $^{\alpha}$CH), 6.44 (s, 1H, H-1 aromatic coumarin mojety), 6.61 (4H, dd, Tyr Ar), 7.01 (4H, dd, Tyr Ar), 7.09-7.26 (10H, m, Phe Ar), 7.38-7.40 (m, 2H, H-3 + H-4 aromatic coumarin mojety), 7.82 (d, 1H, *J* = 8.9 Hz, H-2 aromatic coumarin mojety), 8.01-8.17 (6H, d, Tyr NH_3^+; 2H, d, D-Cys NH), 8.68-8.82 (2H, d, Phe NH; 2H, t, Gly NH), 9.29 (2H, s, OH), 10.16 (2H, s, NH-NH). ESI-LRMS: Calcd exact mass without TFA for $C_{60}H_{61}N_{11}O_{14}S_2$ m/z: 1223.3 [M]; found 1224.7 $[M+H]^+$.

Compound **2C**: The desired product has been obtained in good overall yield (58%) following the procedure previously reported by us;[3] Rt (HPLC) = 15.90 min (284 nm). ^{1}H NMR (DMSO-d_6) δ: 1.21 (s, 3H, CH_3 coumarin mojety), 2.34-2.65 (4H, m, Tyr $^{\beta}CH_2$; 4H, m, Phe $^{\beta}CH_2$), 2.42-2.75 (4H, m, $^{\beta}$Ala 2*$^{\beta}CH_2$), 2.97 (4H, d, D-Cys $^{\beta}CH_2$), 3.01 (4H, m, Gly $^{\alpha}CH_2$), 3.91-4.11 (2H, t, Tyr $^{\alpha}$CH; 2H, t, Phe $^{\alpha}$CH), 4.71-4.81 (2H, m, D-Cys $^{\alpha}$CH), 6.44 (s, 1H, H-1 aromatic coumarin mojety), 6.61 (4H, dd, Tyr Ar), 7.01 (4H, dd, Tyr Ar), 7.09-7.26 (10H, m, Phe Ar), 7.38-7.40 (m, 2H, H-3 + H-4 aromatic coumarin mojety), 7.82 (d, 1H, *J* = 8.9 Hz, H-2 aromatic coumarin mojety), 8.01-8.17 (6H, d, Tyr NH_3^+; 2H, d, D-Cys NH; 1H, s, NH aromatic coumarin mojety), 8.66-8.82 (2H, d, Phe NH; 2H, t, Gly NH), 9.35 (2H, s, OH), 10.21 (2H, s, NH-NH). ESI-LRMS: Calcd exact mass without TFA for $C_{63}H_{66}N_{12}O_{15}S_2$ m/z: 1294.4 [M]; found 1228.4, 1250.5 (fragmentation occurs).[4]

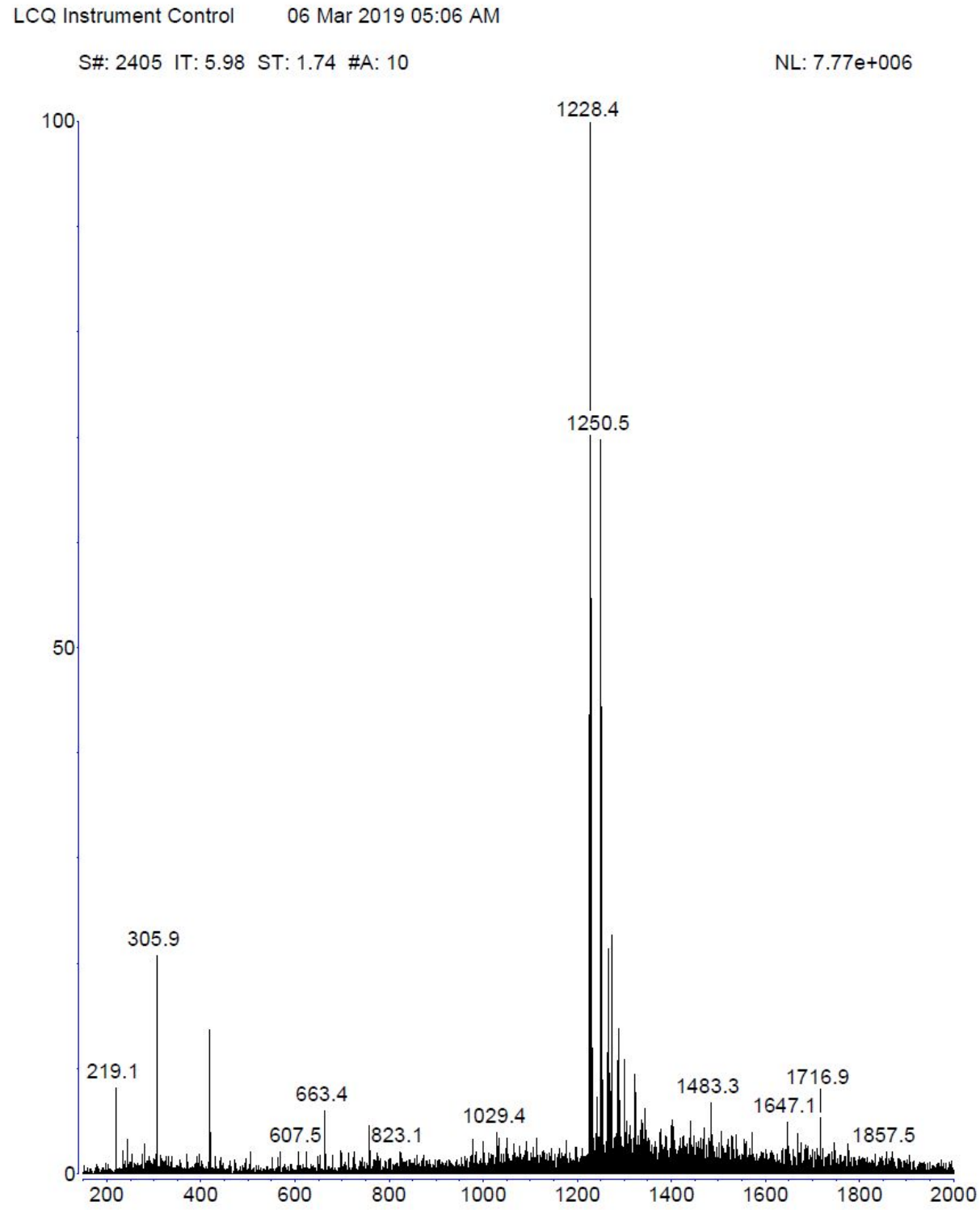
ESI-LRMS of compound **1D**



ESI-LRMS of compound **1C**

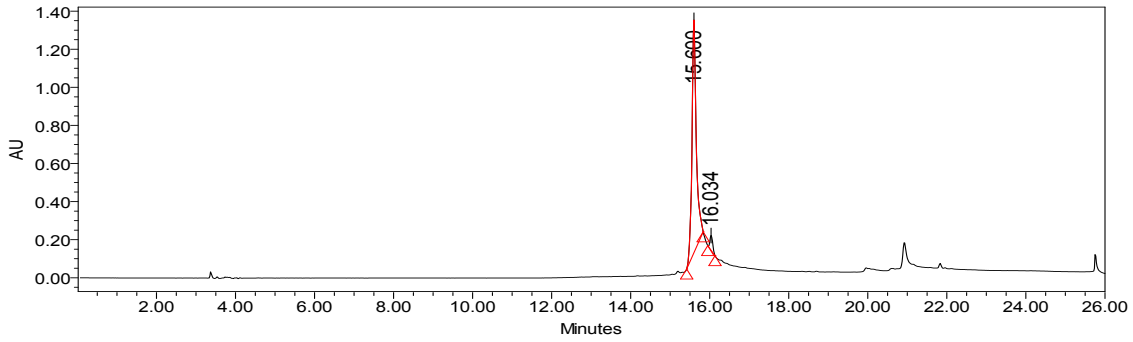


ESI-LRMS of compound **2C**



RP-HPLC analytical traces for compounds **1D**, **1C**, **2C** at 284 nm

1D



	Retention Time	Area	% Area	Height
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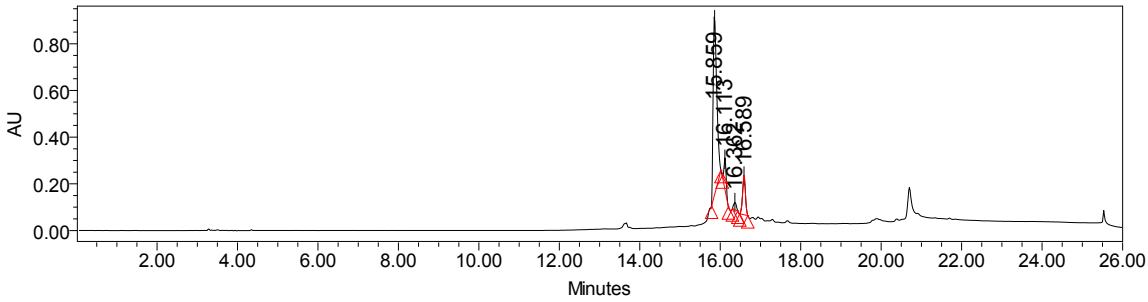
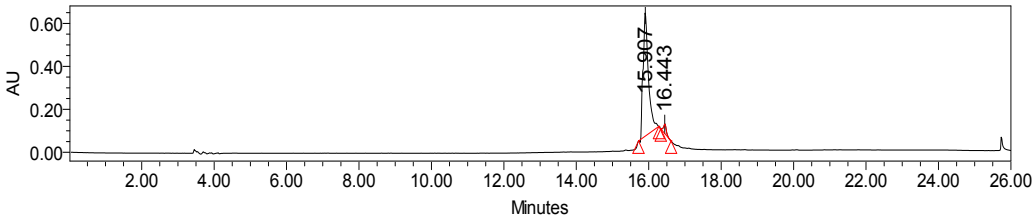
2	16.034	383241	3.96	77032

1C



	Retention Time	Area	% Area	Height
3	16.587	53057	3.97	11794
2	16.364	20039	1.50	4326
1	15.859	1263392	94.53	151699

2C



	Name	Retention Time	Area	% Area	Height
2		16.443	242800	3.95	44093
1		15.907	5908058	96.05	573870

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