Discovery of EST73502, a dual μ-opioid receptor agonist and σ_1 receptor antagonist clinical candidate for the treatment of pain.

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Analytical data of final compounds: purity, NMR, HRMS, enantiomeric purity by chiral HPLC and optical rotations for enantiopure compounds.

Chemical characterization of intermediates: NMR.

Experimental methods for determination of activity in selectivity panel: guinea-pig sigma-2 receptor (σ2R) and the following human receptors: 5-HT1A, 5-HT2B, α1A adrenoreceptor, α2A adrenoreceptor, dopamine transporter (DAT), δ-opioid receptor (DOR), k-opioid receptor (KOR), histamine H1, norepinephrine transporter (NET), serotonin transporter (SERT).

Analytical data of final compounds

comp	Form	Purity (%)	NMR	HRMS $[M+H]^+$ (diff ppm)
14a	HCl	98	^{1}H NMR (400 MHz, CD_3OD) δ 7.41 – 7.24 (m, 10H), 4.76 (d, *J* = 14.5 Hz, 1H), 4.46 (d, *J* = 14.5 Hz, 1H), 4.34 (q, *J* = 6.7 Hz, 1H), 3.50 (br s, *J* = 17.6 Hz, 2H), 3.44 (d, *J* = 12.8 Hz, 1H), 3.40 – 3.26 (m, 3H), 3.23 – 3.11 (m, 2H), 3.11 – 3.03 (m, 2H), 2.44 – 2.28 (m, 1H), 1.98 – 1.77 (m, 2H), 1.74 – 1.54 (m, 1H), 1.47 (d, *J* = 6.7 Hz, 3H).	379.2391 (-3.05)
14b	Free base	95	^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 – 7.27 (m, 4H), 7.25 – 7.17 (m, 6H), 4.15 (q, *J* = 6.8 Hz, 1H), 3.68 – 3.51 (m, 2H), 3.26 (d, *J* = 12.1 Hz, 1H), 2.97 – 2.78 (m, 5H), 2.62 (m, 4H), 2.35 (m, 1H), 2.04 (d, *J* = 14.1 Hz, 1H), 1.77 – 1.49 (m, 4H), 1.41 (d, *J* = 6.8 Hz, 3H).	393.2553 (-4.1)
14c	HCl	96	^{1}H NMR (400 MHz, CD_3OD) δ 7.43 – 7.23 (m, 5H), 4.21 (q, *J* = 6.7 Hz, 1H), 3.56 (d, *J* = 13.8 Hz, 2H), 3.46 (d, *J* = 12.9 Hz, 1H), 3.43 – 3.35 (m, 2H), 3.28 – 3.15 (m, 3H), 3.13 – 3.03 (m, 2H), 2.81 – 2.71 (m, 1H), 2.33 (d, *J* = 14.0 Hz, 1H), 2.08 – 1.73 (m, 3H), 1.39 (d, *J* = 6.7 Hz, 3H), 0.92 – 0.79 (m, 2H), 0.77 – 0.57 (m, 2H).	329.2224 (0.08)
14d	Free base	99	^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 – 7.26 (m, 2H), 7.23 – 7.17 (m, 3H), 4.86 (sept, *J* = 6.8 Hz, 1H), 4.17 (q, *J* = 6.8 Hz, 1H), 3.15 (d, *J* = 12.3 Hz, 1H), 2.96 (d, *J* = 12.3 Hz, 1H), 2.87 – 2.79 (m, 2H), 2.75 – 2.60 (m, 4H), 2.60 – 2.43 (m, 1H), 2.36 (t, *J* = 10.1 Hz, 1H), 2.03 (d, *J* = 14.5 Hz, 1H), 1.75 – 1.67 (m, 2H), 1.63 – 1.49 (m, 1H), 1.44 (d, *J* = 6.8 Hz, 3H), 1.11 (d, *J* = 6.9 Hz, 3H), 1.07 (d, *J* = 6.7 Hz, 3H).	331.2379 (0.34)
14e	HCl	95	^{1}H NMR (400 MHz, CD_3OD) δ 7.39 – 7.24 (m, 5H), 4.22 (q, *J* = 6.7 Hz, 1H), 3.61 – 3.51 (m, 3H), 3.51 – 3.32 (m, 5H), 3.28 – 3.15 (m, 2H), 3.13 – 3.05 (m, 2H), 2.40 (d, *J* = 14.5 Hz, 1H), 2.09 – 1.97 (m, 1H), 1.96 – 1.80 (m, 2H), 1.41 (d, *J* = 6.7 Hz, 3H), 1.14 (t, *J* = 7.2 Hz, 3H).	317.2226 (-0.73)
14f	HCl	97	^{1}H NMR (400 MHz, CD_3OD) δ 7.40 – 7.24 (m, 5H), 4.24 (q, *J* = 6.7 Hz, 1H), 3.62 – 3.51 (m, 3H), 3.44 – 3.34 (m, 3H), 3.28 – 3.15 (m, 2H), 3.14 – 3.05 (m, 2H), 2.99 (s, 3H), 2.45 (d, *J* = 14.1 Hz, 1H),	303.2072 (-1.6)

			2.02 (t, J = 13.9 Hz, 1H), 1.96 – 1.80 (m, 2H), 1.42 (d, J = 6.7 Hz, 3H).	
14g	HCl	100	^{1}H NMR (400 MHz, CD_3OD) δ 7.42 – 7.24 (m, 5H), 4.24 (q, J = 6.7 Hz, 1H), 3.61 – 3.52 (m, 3H), 3.42 – 3.33 (m, 3H), 3.27 – 3.15 (m, 2H), 3.14 – 3.08 (m, 2H), 2.98 (s, 3H), 2.45 (ddd, J = 15.5, 5.3, 2.7 Hz, 1H), 2.10 – 1.99 (m, 1H), 1.95 – 1.84 (m, 2H), 1.42 (d, J = 6.7 Hz, 3H).	303.2078 (-3.65)
14h	HCl	100	^{1}H NMR (400 MHz, CD_3OD) δ 7.40 – 7.25 (m, 5H), 4.25 (q, J = 6.7 Hz, 1H), 3.61 – 3.49 (m, 3H), 3.44 – 3.34 (m, 3H), 3.28 – 3.17 (m, 2H), 3.14 – 3.06 (m, 2H), 2.99 (s, 3H), 2.44 (br d, J = 14.2 Hz, 1H), 2.06 – 1.96 (m, 1H), 1.96 – 1.82 (m, 2H), 1.42 (d, J = 6.7 Hz, 3H).	303.2071 (-1.17)
14i	HCl	100	^{1}H NMR (400 MHz, CD_3OD) δ 7.40 – 7.22 (m, 5H), 4.17 (s, 2H), 3.61 – 3.52 (m, 2H), 3.45 – 3.34 (m, 4H), 3.29 – 3.20 (m, 2H), 3.15 – 3.05 (m, 2H), 3.00 (s, 3H), 2.22 (d, J = 13.7 Hz, 2H), 1.99 (t, J = 13.6 Hz, 2H).	289.1921 (-3.79)
14j	HCl	100	^{1}H NMR (400 MHz, CD_3OD) δ 7.40 – 7.24 (m, 5H), 4.13 (dd, J = 5.9, 4.1 Hz, 1H), 3.63 – 3.49 (m, 3H), 3.44 – 3.35 (m, 3H), 3.28 – 3.16 (m, 2H), 3.13 – 3.05 (m, 2H), 2.99 (s, 3H), 2.41 (br s, 1H), 2.09 – 1.98 (m, 1H), 1.97 – 1.78 (m, 4H), 0.97 (t, J = 7.4 Hz, 3H).	317.2224 (-0.1)
14k	Free base	99	^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 – 7.26 (m, 2H), 7.22 – 7.17 (m, 3H), 3.93 (d, J = 2.5 Hz, 1H), 3.39 (d, J = 12.0 Hz, 1H), 2.97 (s, 3H), 2.90 (d, J = 12.0 Hz, 1H), 2.85 – 2.78 (m, 2H), 2.77 – 2.69 (m, 2H), 2.65 – 2.59 (m, 2H), 2.50 (td, J = 11.2, 3.5 Hz, 1H), 2.44 – 2.36 (m, 1H), 2.24 (td, J = 11.6, 2.4 Hz, 1H), 2.18 – 2.10 (m, 1H), 1.77 – 1.62 (m, 2H), 1.61 – 1.52 (m, 1H), 1.03 (d, J = 7.0 Hz, 3H), 0.86 (d, J = 6.8 Hz, 3H).	331.2379 (-0.7)
14l	HCl	100	^{1}H NMR (400 MHz, CD_3OD) δ 7.46 – 7.40 (m, 2H), 7.39 – 7.23 (m, 8H), 5.18 (s, 1H), 3.83 (d, J = 12.9 Hz, 1H), 3.68 – 3.61 (m, 1H), 3.59 – 3.53 (m, 1H), 3.42 – 3.22 (m, 5H), 3.14 – 3.07 (m, 2H), 3.03 (s, 3H), 2.59 (d, J = 15.7 Hz, 1H), 2.18 – 1.94 (m, 3H).	365.2221 (0.32)
14m	HCl	99	^{1}H NMR (400 MHz, CD_3OD) δ 7.39 – 7.22 (m, 5H), 3.64 – 3.55 (m, 2H), 3.49 (s, 2H), 3.44 – 3.36 (m, 2H), 3.34 – 3.24 (m, 2H), 3.14 – 3.06 (m, 2H), 3.03 (s, 3H), 2.10 – 2.00 (m, 2H), 1.98 – 1.86 (m, 2H), 1.43 (s, 6H).	317.2234 (-3.4)

14n	HCl	94	^{1}H NMR (400 MHz, CD_3OD) δ 7.38 – 7.24 (m, 5H), 3.58 (d, *J* = 11.6 Hz, 2H), 3.50 (s, 2H), 3.42 – 3.35 (m, 2H), 3.17 (t, *J* = 12.9 Hz, 2H), 3.11 – 3.05 (m, 2H), 3.01 (s, 3H), 2.30 (d, *J* = 14.9 Hz, 2H), 1.97 (t, *J* = 14.3 Hz, 2H), 1.29 (q, *J* = 4.6 Hz, 2H), 1.14 – 1.05 (m, 2H).	315.208 (-3.92)
14o	HCl	99	^{1}H NMR (400 MHz, CD_3OD) δ 7.39 – 7.25 (m, 5H), 4.23 (q, *J* = 6.7 Hz, 1H), 3.62 – 3.52 (m, 3H), 3.51 – 3.45 (m, 1H), 3.43 – 3.33 (m, 4H), 3.27 (d, *J* = 12.8 Hz, 1H), 3.24 – 3.16 (m, 1H), 3.15 – 3.08 (m, 2H), 2.39 (dd, *J* = 15.4, 2.4 Hz, 1H), 2.12 – 2.02 (m, 1H), 1.97 – 1.85 (m, 2H), 1.41 (d, *J* = 6.7 Hz, 3H), 1.15 (t, *J* = 7.2 Hz, 3H).	317.2227 (-1.06)
14p	HCl	98	^{1}H NMR (400 MHz, CD_3OD) δ 7.41 – 7.30 (m, 2H), 7.22 – 7.10 (m, 2H), 4.23 (q, *J* = 6.6 Hz, 1H), 3.65 – 3.53 (m, 3H), 3.52 – 3.34 (m, 5H), 3.28 – 3.21 (m, 2H), 3.20 – 3.12 (m, 2H), 2.41 (d, *J* = 14.3 Hz, 1H), 2.10 – 1.80 (m, 3H), 1.41 (d, *J* = 6.7 Hz, 3H), 1.15 (t, *J* = 7.2 Hz, 3H).	335.2144 (-4.41)
14q	HCl	100	^{1}H NMR (400 MHz, CD_3OD) δ 7.37 (td, *J* = 8.0, 6.1 Hz, 1H), 7.17 – 7.08 (m, 2H), 7.02 (td, *J* = 8.6, 2.6 Hz, 1H), 4.23 (q, *J* = 6.7 Hz, 1H), 3.62 – 3.52 (m, 3H), 3.52 – 3.45 (m, 1H), 3.45 – 3.34 (m, 4H), 3.30 – 3.16 (m, 2H), 3.16 – 3.08 (m, 2H), 2.40 (d, *J* = 14.9 Hz, 1H), 2.10 – 1.98 (m, 1H), 1.98 – 1.81 (m, 2H), 1.41 (d, *J* = 6.7 Hz, 3H), 1.15 (t, *J* = 7.2 Hz, 3H).	335.2128 (0.37)
14r	HCl	99	^{1}H NMR (400 MHz, CD_3OD) δ 7.38 – 7.30 (m, 2H), 7.12 – 7.06 (m, 2H), 4.23 (q, *J* = 6.7 Hz, 1H), 3.61 – 3.45 (m, 4H), 3.43 – 3.33 (m, 4H), 3.26 (d, *J* = 12.8 Hz, 1H), 3.20 (td, *J* = 13.2, 2.3 Hz, 1H), 3.14 – 3.06 (m, 2H), 2.44 – 2.35 (m, 1H), 2.10 – 1.99 (m, 1H), 1.97 – 1.81 (m, 2H), 1.41 (d, *J* = 6.7 Hz, 3H), 1.15 (d, *J* = 14.4 Hz, 3H).	335.2144 (-4.21)
14s	HCl	98	^{1}H NMR (400 MHz, CD_3OD) δ 7.27 – 7.15 (m, 3H), 4.23 (q, *J* = 6.7 Hz, 1H), 3.64 – 3.57 (m, 2H), 3.55 (d, *J* = 12.9 Hz, 1H), 3.52 – 3.46 (m, 1H), 3.45 – 3.35 (m, 4H), 3.28 – 3.17 (m, 4H), 2.42 (d, *J* = 14.3 Hz, 1H), 2.07 – 1.91 (m, 2H), 1.90 – 1.79 (m, 1H), 1.42 (d, *J* = 6.7 Hz, 3H), 1.15 (t, *J* = 7.2 Hz, 3H).	353.2034 (0.23)
14t	HCl	97	^{1}H NMR (400 MHz, CD_3OD) δ 7.46 – 7.36 (m, 1H), 7.05 – 6.95 (m, 2H), 4.23 (q, *J* = 6.7 Hz, 1H), 3.60 (d, *J* = 12.3 Hz, 2H), 3.54 (d, *J* = 12.9 Hz, 1H), 3.52 – 3.45 (m, 1H), 3.43 – 3.33 (m, 4H), 3.27 (d,	353.2033 (0.63)

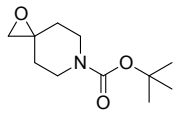
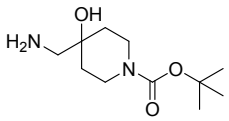
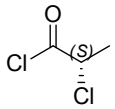
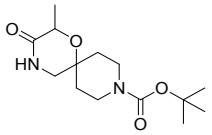
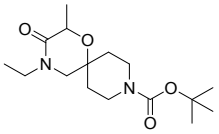
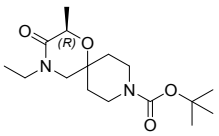
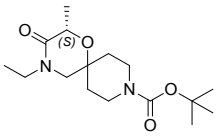
			J = 12.8 Hz, 1H), 3.24 – 3.12 (m, 3H), 2.45 – 2.36 (m, 1H), 2.11 – 2.01 (m, 1H), 1.98 – 1.83 (m, 2H), 1.41 (d, *J* = 6.7 Hz, 3H), 1.15 (t, *J* = 7.2 Hz, 3H).	
14u	HCl	99	^{1}H NMR (400 MHz, CD_3OD) δ 7.20 – 7.12 (m, 2H), 7.11 – 7.03 (m, 1H), 4.22 (q, *J* = 6.7 Hz, 1H), 3.59 (d, *J* = 12.2 Hz, 2H), 3.54 (d, *J* = 12.9 Hz, 1H), 3.52 – 3.45 (m, 1H), 3.44 – 3.33 (m, 4H), 3.28 – 3.20 (m, 2H), 3.19 – 3.12 (m, 2H), 2.40 (dd, *J* = 15.5, 2.3 Hz, 1H), 2.09 – 1.99 (m, 1H), 1.98 – 1.80 (m, 2H), 1.41 (d, *J* = 6.7 Hz, 3H), 1.14 (t, *J* = 7.2 Hz, 3H).	353.2028 (1.61)
14v	HCl	99	^{1}H NMR (400 MHz, CD_3OD) δ 7.42 – 7.33 (m, 1H), 7.08 – 7.00 (m, 2H), 4.23 (q, *J* = 6.8 Hz, 1H), 3.66 – 3.57 (m, 2H), 3.54 (d, *J* = 12.8 Hz, 1H), 3.51 – 3.45 (m, 1H), 3.45 – 3.33 (m, 4H), 3.29 – 3.16 (m, 4H), 2.42 (d, *J* = 15.4 Hz, 1H), 2.06 – 1.91 (m, 2H), 1.90 – 1.79 (m, 1H), 1.41 (d, *J* = 6.7 Hz, 3H), 1.14 (t, *J* = 7.2 Hz, 3H).	353.2045 (-3.07)
14w	HCl	98	^{1}H NMR (400 MHz, CD_3OD) δ 8.65 (d, *J* = 5.3 Hz, 1H), 8.09 (t, *J* = 7.7 Hz, 1H), 7.64 (d, *J* = 8.0 Hz, 1H), 7.57 (t, *J* = 6.4 Hz, 1H), 3.65 – 3.56 (m, 4H), 3.53 (s, 2H), 3.47 (q, *J* = 7.2 Hz, 2H), 3.42 (t, *J* = 7.6 Hz, 2H), 3.29 – 3.22 (m, 2H), 2.32 – 2.23 (m, 2H), 2.15 – 2.03 (m, 2H), 1.30 (dd, *J* = 7.9, 4.6 Hz, 2H), 1.17 (t, *J* = 7.2 Hz, 3H), 1.09 (dd, *J* = 7.9, 4.6 Hz, 2H).	330.2174 (0.78)
14x	HCl	100	^{1}H NMR (400 MHz, CD_3OD) δ 7.12 (dd, *J* = 14.1, 4.9 Hz, 2H), 7.08 – 7.00 (m, 1H), 4.21 (q, *J* = 6.6 Hz, 1H), 3.62 – 3.44 (m, 4H), 3.43 – 3.33 (m, 4H), 3.25 (d, *J* = 13.0 Hz, 1H), 3.17 (dd, *J* = 10.6, 5.9 Hz, 3H), 2.38 (d, *J* = 15.0 Hz, 1H), 2.17 – 2.04 (m, 1H), 2.00 – 1.89 (m, 2H), 1.41 (d, *J* = 6.7 Hz, 3H), 1.15 (t, *J* = 7.2 Hz, 3H).	353.2048 (-3.48)
17	HCl	98	^{1}H NMR (400 MHz, CD_3OD) δ 7.39 – 7.28 (m, 2H), 7.20 – 7.08 (m, 2H), 4.24 (q, *J* = 6.8 Hz, 1H), 3.50 – 3.36 (m, 2H), 3.38 (d, *J* = 12.7 Hz, 1H), 3.29 – 3.21 (m, 3H), 3.21 (d, *J* = 12.7 Hz, 1H), 3.17 – 3.06 (m, 3H), 2.41 (d, *J* = 13.1 Hz, 1H), 2.01 – 1.93 (m, 1H), 1.92 – 1.80 (m, 2H), 1.42 (d, *J* = 6.8 Hz, 3H).	307.1813 (-0.97)

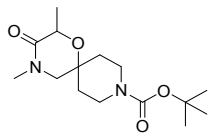
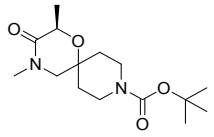
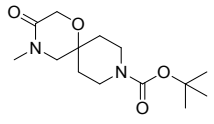
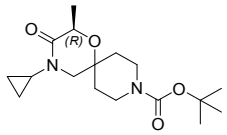
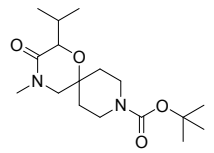
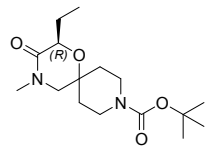
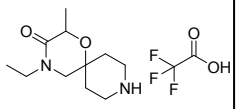
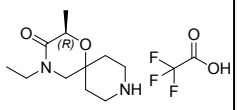
Conditions for enantiomeric excess by chiral HPLC and optical rotation of enantiopure compounds.

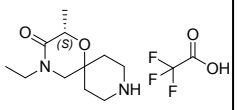
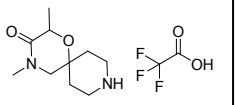
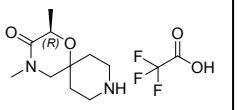
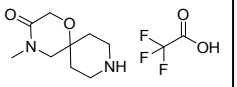
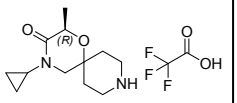
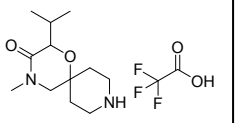
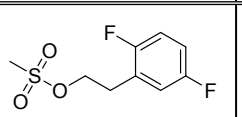
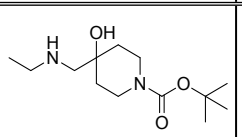
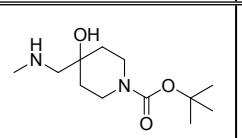
Compd	Chiral HPLC	Ee%	[α]20D
14c	Chiralcel OJ 250x 4.6 mm, 10um Heptane/EtOH-0.2%DEA 90:10 Flow 0.8 mL/min	97.2%	11.0 (c 0.26, MeOH)
14g	Chiralcel OJ 250x 4.6 mm, 10um Heptane/ EtOH-0.2%DEA 90:10 Flow 0.8 mL/min	98.1%	12.33 (c 0.31, MeOH)
14h	Chiralcel OJ 250x 4.6 mm, 10um Heptane/ IPA-0.2%DEA 90:10 Flow 0.8 mL/min	96.1% (Determined as free base)	-9.92 (c 0.22, MeOH)
14j	Chiralcel OJ 250x 4.6 mm, 10um Heptane/EtOH-0.2%DEA 90:10 Flow 0.5 mL/min	98.6%	19.73 (c 0.06, MeOH)
14o	Chiralpak ADH 250x 4.6 mm, 5um Heptane/ EtOH-0.2%DEA 90:10 Flow 0.5 mL/min	98.5%	12.84 (c 0.04, MeOH)
14p	Chiralpak ADH 250x 4.6 mm, 5um Heptane/EtOH-0.2%DEA 90:10 Flow 0.5 mL/min	98.8%	4.68 (c 0.26, MeOH)
14q	Chiralcel OJ 250x 4.6 mm, 10um Heptane/EtOH-0.2%DEA 90:10 Flow 0.8 mL/min	97.6%	15.91 (c 0.29, MeOH)
14r	Chiralpak ADH 250x 4.6 mm, 5um Heptane/ EtOH 70:30 Flow 0.5 mL/min	98.6% (Determined at Boc stage, **11-1R**)	3.60 (c 0.29, MeOH)
14s	Chiralcel OJ 250x 4.6 mm, 10um Heptane/EtOH-0.2%DEA 90:10Flow 0.5 mL/min	98.8%	14.60 (c 0.26, MeOH)

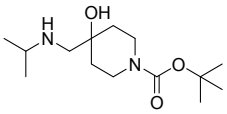
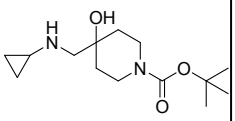
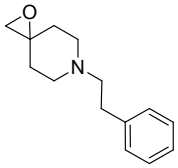
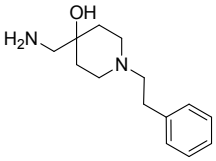
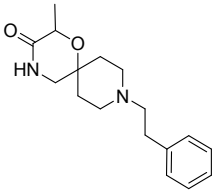
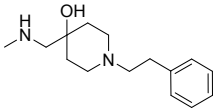
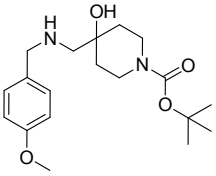
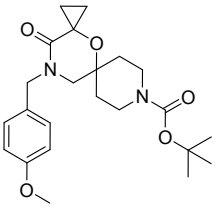
14t	Chiralpak ADH 250x 4.6 mm, 5um Heptane/ EtOH 70:30 Flow 0.5 mL/min	98.6% (Determined at Boc stage, **11-1R**)	16.26 (c 0.26, MeOH)
14u	Chiralcel OJ 250x 4.6 mm, 10um Heptane/EtOH-0.2%DEA 95:5 Flow 0.5 mL/min	99.0	15.93 (c 0.25, MeOH)
14v	Chiralpak ADH 250x 4.6 mm, 5um Heptane/ EtOH 70:30 Flow 0.5 mL/min	98.6% (Determined at Boc stage, **11-1R**)	15.95 (c 0.26, MeOH)
14x	Chiralcel OJ 250x 4.6 mm, 10um Heptane/EtOH-0.2%DEA 95:5 Flow 0.5 mL/min	95.4%	-15.75 (c 0.29, MeOH)

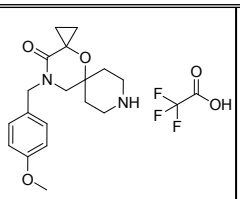
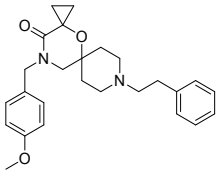
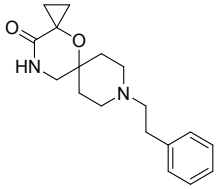
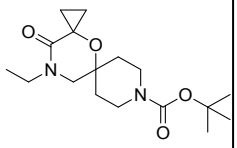
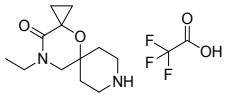
Chemical characterization of intermediates

comp	Structure	Chemical name	NMR
6		*tert*-butyl 1-oxa-6-azaspiro[2.5]octane-6-carboxylate	^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 3.77 – 3.63 (m, 2H), 3.42 (ddd, *J* = 13.3, 9.5, 3.7 Hz, 2H), 2.68 (s, 2H), 1.79 (ddd, *J* = 13.7, 9.5, 4.4 Hz, 2H), 1.46 (s, 9H), 1.44 – 1.40 (m, 2H).
7		*tert*-butyl 4-(aminomethyl)-4-hydroxypiperidine-1-carboxylate	^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm: 3.86 (br s, 2H), 3.17 (t, *J* = 12.0 Hz, 2H), 2.60 (s, 2H), 1.51 (d, *J* = 12.4 Hz, 2H), 1.46 (s, 9H), 1.43 – 1.32 (m, 2H).
8-1S		(*S*)-2-chloropropanoyl chloride	^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm: 4.66 (q, *J*=7.0 Hz, 1H), 1.82 (d, *J*=6.8 Hz, 3H).
9-1		*tert*-butyl 2-methyl-3-oxo-1-oxa-4,9-diazaspiro[5.5]undecane-9-carboxylate	^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm: 5.75 (br s, 1H, NH), 4.20 (q, *J* = 6.8 Hz, 1H), 3.82 (br s, 2H), 3.38 (d, *J* = 12.0 Hz, 1H), 3.26 (t, *J* = 11.9 Hz, 1H), 3.10 (dd, *J* = 11.9, 4.8 Hz, 1H), 3.05 (t, *J* = 11.0 Hz, 1H), 2.16 (d, *J* = 15.2 Hz, 1H), 1.67 – 1.49 (m, 3H), 1.46 (s, 9H), 1.46 (d, *J* = 6.8 Hz, 3H).
11-1		*tert*-butyl 4-ethyl-2-methyl-3-oxo-1-oxa-4,9-diazaspiro[5.5] undecane-9-carboxylate	^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm: 4.16 (q, *J* = 6.8 Hz, 1H), 3.81 (br s, 2H), 3.55 – 3.44 (m, 1H), 3.38 (d, *J* = 12.2 Hz, 1H), 3.35 – 3.15 (m, 2H), 3.04 (t, *J* = 11.6 Hz, 1H), 2.98 (d, *J* = 12.2 Hz, 1H), 2.10 – 2.01 (m, 1H), 1.65 – 1.48 (m, 2H), 1.46 (s, 9H), 1.43 (d, *J* = 6.8 Hz, 3H), 1.43 – 1.35 (m, 1H), 1.12 (t, *J* = 7.2 Hz, 3H).
11-1R		(*R*)-*tert*-butyl 4-ethyl-2-methyl-3-oxo-1-oxa-4,9-diazaspiro[5.5] undecane-9-carboxylate	^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm: 4.15 (q, *J* = 6.8 Hz, 1H), 3.81 (br s, 2H), 3.56 – 3.45 (m, 1H), 3.38 (d, *J* = 12.3 Hz, 1H), 3.36 – 3.19 (m, 2H), 3.04 (t, *J* = 11.8 Hz, 1H), 2.97 (d, *J* = 12.2 Hz, 1H), 2.10 – 2.01 (m, 1H), 1.66 – 1.49 (m, 2H), 1.46 (s, 9H), 1.43 (d, *J* = 6.8 Hz, 3H), 1.44 – 1.37 (m, 1H), 1.12 (t, *J* = 7.2 Hz, 3H).
11-1S		(*S*)-*tert*-butyl 4-ethyl-2-methyl-3-oxo-1-oxa-4,9-diazaspiro[5.5] undecane-9-carboxylate	^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm: 4.15 (q, *J* = 6.8 Hz, 1H), 3.82 (br s, 2H), 3.56 – 3.45 (m, 1H), 3.38 (d, *J* = 12.3 Hz, 1H), 3.35 – 3.28 (m, 1H), 3.28 – 3.19 (m, 1H), 3.04 (t, *J* = 12.1 Hz, 1H), 2.97 (d, *J* = 12.2 Hz, 1H), 2.06 (ddd, *J* = 14.0, 5.4, 2.8 Hz, 1H), 1.65 – 1.49 (m, 2H), 1.46 (s, 9H), 1.43 (d, *J* = 6.8 Hz, 3H), 1.44 – 1.38 (m, 1H), 1.12 (t, *J* = 7.2 Hz, 3H).

11-2		*tert*-butyl 2,4-dimethyl-3-oxo-1-oxa-4,9-diazaspiro[5.5]undecane-9-carboxylate	^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm: 4.17 (q, *J* = 6.7 Hz, 1H), 3.82 (br s, 2H), 3.42 (d, *J* = 12.2 Hz, 1H), 3.24 (t, *J* = 11.2 Hz, 1H), 3.03 (t, *J* = 11.4 Hz, 1H), 2.97 (d, *J* = 12.1 Hz, 1H), 2.97 (s, 3H), 2.15 – 2.06 (m, 1H), 1.63 – 1.50 (m, 2H), 1.46 (s, 9H), 1.44 (d, *J* = 6.8 Hz, 3H), 1.44 – 1.39 (m, 1H).
11-2R		(*R*)-*tert*-butyl 2,4-dimethyl-3-oxo-1-oxa-4,9-diazaspiro[5.5]undecane-9-carboxylate	^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm: 4.17 (q, *J* = 6.7 Hz, 1H), 3.82 (br s, 2H), 3.42 (d, *J* = 12.2 Hz, 1H), 3.24 (t, *J* = 11.2 Hz, 1H), 3.03 (t, *J* = 11.4 Hz, 1H), 2.97 (d, *J* = 12.1 Hz, 1H), 2.97 (s, 3H), 2.15 – 2.06 (m, 1H), 1.63 – 1.50 (m, 2H), 1.46 (s, 9H), 1.44 (d, *J* = 6.8 Hz, 3H), 1.44 – 1.39 (m, 1H).
11-3		*tert*-butyl 4-methyl-3-oxo-1-oxa-4,9-diazaspiro[5.5]undecane-9-carboxylate	^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm: 4.14 (s, 2H), 3.84 (s, 2H), 3.19 (s, 2H), 3.10 (t, *J* = 12.4 Hz, 2H), 2.99 (s, 3H), 1.88 (d, *J* = 12.1 Hz, 2H), 1.56 – 1.47 (m, 2H), 1.46 (s, 9H).
11-4R		(*R*)-*tert*-butyl 4-cyclopropyl-2-methyl-3-oxo-1-oxa-4,9-diazaspiro[5.5]undecane-9-carboxylate	^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm: 4.13 (q, *J* = 6.8 Hz, 1H), 3.81 (br s, 2H), 3.28 (d, *J* = 12.3 Hz, 1H), 3.26 – 3.17 (m, 1H), 3.08 – 3.00 (m, 1H), 3.01 (d, *J* = 12.4 Hz, 1H), 2.73 (ddd, *J* = 11.2, 7.4, 4.1 Hz, 1H), 1.99 (d, *J* = 12.3 Hz, 1H), 1.59 – 1.47 (m, 3H), 1.46 (s, 9H), 1.41 (d, *J* = 6.7 Hz, 3H), 0.90 – 0.76 (m, 2H), 0.72 – 0.63 (m, 1H), 0.58 – 0.50 (m, 1H).
11-5		*tert*-butyl 2-isopropyl-4-methyl-3-oxo-1-oxa-4,9-diazaspiro[5.5]undecane-9-carboxylate	^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm: 3.97 – 3.78 (m, 3H), 3.40 (d, *J* = 12.1 Hz, 1H), 3.27 – 3.12 (m, 1H), 2.97 (s, 3H), 2.99 – 2.86 (m, 1H), 2.89 (d, *J* = 12.1 Hz, 1H), 2.46 – 2.36 (m, 1H), 2.11 (d, *J* = 14.1 Hz, 1H), 1.64 – 1.34 (m, 3H), 1.46 (s, 9H), 1.03 (d, *J* = 7.0 Hz, 3H), 0.86 (d, *J* = 6.8 Hz, 3H).
11-6R		(*R*)-*tert*-butyl 2-ethyl-4-methyl-3-oxo-1-oxa-4,9-diazaspiro[5.5]undecane-9-carboxylate	^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm: 4.00 (dd, *J* = 7.3, 3.4 Hz, 1H), 3.85 (br s, 2H), 3.41 (d, *J* = 12.1 Hz, 1H), 3.22 (t, *J* = 12.5 Hz, 1H), 2.97 (s, 3H), 2.99 – 2.88 (m, 1H), 2.94 (d, *J* = 12.1 Hz, 1H), 2.12 (d, *J* = 16.7 Hz, 1H), 2.03 – 1.89 (m, 1H), 1.85 – 1.72 (m, 1H), 1.46 (s, 9H), 1.45 – 1.37 (m, 3H), 0.97 (t, J = 7.4 Hz, 3H).
12-1 TFA salt		4-ethyl-2-methyl-1-oxa-4,9-diazaspiro[5.5]undecan-3-one trifluoroacetate	^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm: 8.90 (br s, 2H), 4.18 (q, *J* = 6.7 Hz, 1H), 3.59 – 3.30 (m, 6H), 3.19 – 3.07 (m, 2H), 2.30 (dd, *J* = 15.4, 2.5 Hz, 1H), 2.07 – 1.95 (m, 1H), 1.94 – 1.78 (m, 2H), 1.46 (d, *J* = 6.8 Hz, 3H), 1.15 (t, *J* = 7.2 Hz, 3H).
12-1R TFA salt		(*R*)-4-ethyl-2-methyl-1-oxa-4,9-diazaspiro[5.5]undecan-3-one trifluoroacetate	^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm: 8.90 (br s, 2H), 4.18 (q, *J* = 6.7 Hz, 1H), 3.59 – 3.30 (m, 6H), 3.19 – 3.07 (m, 2H), 2.30 (dd, *J* = 15.4, 2.5 Hz, 1H), 2.07 – 1.95 (m, 1H), 1.94 – 1.78 (m, 2H), 1.46 (d, *J* = 6.8 Hz, 3H), 1.15 (t, *J* = 7.2 Hz, 3H).

12-1S TFA salt		(*S*)-4-ethyl-2-methyl-1-oxa-4,9-diazaspiro[5.5]undecan-3-one trifluoroacetate	^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm: 7.95 (br s, 2H), 4.27 (q, *J* = 6.8 Hz, 1H), 3.64 – 3.53 (m, 2H), 3.51 – 3.34 (m, 4H), 3.26 – 3.12 (m, 2H), 2.34 (dd, *J* = 15.3, 2.2 Hz, 1H), 2.08 – 1.98 (m, 1H), 1.94 – 1.79 (m, 2H), 1.47 (d, *J* = 6.8 Hz, 3H), 1.17 (t, *J* = 7.2 Hz, 3H).
12-2 TFA salt		2,4-dimethyl-1-oxa-4,9-diazaspiro[5.5]undecan-3-one trifluoroacetate	^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm: 8.59 (br s, 2H), 4.22 (q, *J* = 6.7 Hz, 1H), 3.53 (d, *J* = 12.7 Hz, 1H), 3.45 – 3.31 (m, 3H), 3.20 – 3.08 (m, 1H), 3.13 (d, *J* = 12.7 Hz, 1H), 3.03 (s, 3H), 2.36 (d, *J* = 15.2 Hz, 1H), 2.08 – 1.96 (m, 1H), 1.93 – 1.82 (m, 2H), 1.47 (d, *J* = 6.8 Hz, 3H).
12-2R TFA salt		(*R*)-2,4-dimethyl-1-oxa-4,9-diazaspiro[5.5]undecan-3-one trifluoroacetate	^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm: 8.59 (br s, 2H), 4.22 (q, *J* = 6.7 Hz, 1H), 3.53 (d, *J* = 12.7 Hz, 1H), 3.45 – 3.31 (m, 3H), 3.20 – 3.08 (m, 1H), 3.13 (d, *J* = 12.7 Hz, 1H), 3.03 (s, 3H), 2.36 (d, *J* = 15.2 Hz, 1H), 2.08 – 1.96 (m, 1H), 1.93 – 1.82 (m, 2H), 1.47 (d, *J* = 6.8 Hz, 3H).
12-3 TFA salt		4-methyl-1-oxa-4,9-diazaspiro[5.5]undecan-3-one trifluoroacetate	^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm: 8.73 (br s, 1H), 8.61 (br s, 1H), 4.23 (s, 2H), 3.40 (d, *J* = 12.5 Hz, 2H), 3.32 (s, 2H), 3.23 (dd, *J* = 22.8, 11.4 Hz, 2H), 3.05 (s, 3H), 2.14 (d, *J* = 14.3 Hz, 2H), 1.99 (td, *J* = 14.6, 3.7 Hz, 2H).
12-4R TFA salt		(*R*)-4-cyclopropyl-2-methyl-1-oxa-4,9-diazaspiro[5.5]undecan-3-one trifluoroacetate	^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm: 9.09 (br s, 1H), 8.92 (br s, 1H), 4.13 (q, *J* = 6.8 Hz, 1H), 3.36 (d, *J* = 12.8 Hz, 1H), 3.39 – 3.27 (m, 3H), 3.15 (d, *J* = 12.8 Hz, 1H), 3.15 – 3.06 (m, 1H), 2.80 – 2.70 (m, 1H), 2.21 (dd, *J* = 15.0, 2.0 Hz, 1H), 2.03 – 1.93 (m, 1H), 1.89 – 1.77 (m, 2H), 1.43 (d, *J* = 6.7 Hz, 3H), 0.94 – 0.79 (m, 2H), 0.72 – 0.54 (m, 2H).
12-5 TFA salt		2-isopropyl-4-methyl-1-oxa-4,9-diazaspiro[5.5]undecan-3-one trifluoroacetate	^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm: 9.19 (br s, 2H), 3.92 (d, *J* = 2.5 Hz, 1H), 3.48 (d, *J* = 12.4 Hz, 1H), 3.39 – 3.30 (m, 3H), 3.04 (d, *J* = 12.5 Hz, 1H), 3.07 – 2.96 (m, 1H), 3.01 (s, 3H), 2.42 (dtd, *J* = 13.7, 6.7, 2.4 Hz, 1H), 2.37 – 2.30 (m, 1H), 2.09 – 2.01 (m, 1H), 1.94 – 1.78 (m, 2H), 1.04 (d, *J* = 7.0 Hz, 3H), 0.88 (d, *J* = 6.8 Hz, 3H).
13-1		2,5-difluorophenethyl methanesulfonate	^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.10 – 6.90 (m, 3H), 4.43 (t, *J* = 6.7 Hz, 2H), 3.08 (t, *J* = 6.7 Hz, 2H), 2.94 (s, 3H).
16-1		*tert*-butyl 4-hydroxy-4-((ethylamino)methyl)piperidine-1-carboxylate	^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 3.84 (br s, 2H), 3.16 (t, *J* = 11.7 Hz, 2H), 2.69 (q, *J* = 7.1 Hz, 2H), 2.51 (s, 2H), 1.52 – 1.35 (m, 4H), 1.45 (s, 9H), 1.09 (t, *J* = 7.1 Hz, 3H).
16-2		*tert*-butyl 4-hydroxy-4-((methylamino)methyl) piperidine-1-	^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 3.82 (br s, 2H), 3.15 (t, *J* = 11.5 Hz, 2H), 2.49 (s, 2H), 2.47 (s, 3H), 1.44 (s, 9H), 1.54 – 1.34 (m, 4H).

		carboxylate	
16-3		*tert*-butyl 4-hydroxy-4-((isopropylamino)methyl)piperidine-1-carboxylate	^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 3.84 (br s, 2H), 3.16 (t, *J* = 11.5 Hz, 2H), 2.76 (d, *J* = 6.4 Hz, 1H), 2.48 (s, 2H), 1.45 (s, 9H), 1.43 – 1.31 (m, 4H), 1.05 (d, *J* = 6.3 Hz, 6H).
16-4		*tert*-butyl 4-((cyclopropylamino)methyl)-4-hydroxypiperidine-1-carboxylate	^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 3.82 (s, 2H), 3.14 (t, *J* = 11.4 Hz, 2H), 2.63 (s, 2H), 2.22 – 2.15 (m, 1H), 1.44 (s, 9H), 1.50 – 1.33 (m, 4H), 0.48 – 0.42 (m, 2H), 0.33 – 0.28 (m, 2H).
19		6-phenethyl-1-oxa-6-azaspiro[2.5]octane	^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.31 – 7.25 (m, 2H), 7.23 – 7.17 (m, 3H), 2.87 – 2.79 (m, 2H), 2.74 – 2.58 (m, 8H), 1.88 (ddd, *J* = 13.2, 9.0, 4.3 Hz, 2H), 1.63 – 1.53 (m, 2H).
20		4-(aminomethyl)-1-phenethylpiperidin-4-ol	^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.30 – 7.25 (m, 2H), 7.22 – 7.15 (m, 3H), 2.85 – 2.79 (m, 2H), 2.76 (dt, *J* = 7.0, 3.2 Hz, 2H), 2.66 – 2.59 (m, 4H), 2.44 (td, *J* = 10.8, 4.7 Hz, 2H), 1.66 – 1.54 (m, 4H).
21		2-methyl-9-phenethyl-1-oxa-4,9-diazaspiro[5.5]undecan-3-one	^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.32 – 7.26 (m, 2H), 7.23 – 7.17 (m, 3H), 6.10 (br s, 1H, NH), 4.21 (q, *J* = 6.8 Hz, 1H), 3.37 (d, *J* = 11.9 Hz, 1H), 3.12 (dd, *J* = 11.9, 4.8 Hz, 1H), 2.85 – 2.78 (m, 2H), 2.74 – 2.67 (m, 1H), 2.67 – 2.60 (m, 2H), 2.59 – 2.48 (m, 1H), 2.35 (td, *J* = 11.2, 2.1 Hz, 1H), 2.20 (d, *J* = 14.0 Hz, 1H), 1.77 – 1.61 (m, 4H), 1.46 (d, *J* = 6.8 Hz, 3H).
22		4-((methylamino)methyl)-1-phenethylpiperidin-4-ol	^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.31 – 7.26 (m, 2H), 7.23 – 7.17 (m, 3H), 2.88 – 2.76 (m, 4H), 2.69 – 2.60 (m, 2H), 2.53 (s, 2H), 2.49 (s, 3H), 2.53 – 2.44 (m, 2H), 1.70 – 1.57 (m, 4H).
23		*tert*-butyl 4-hydroxy-4-(((4-methoxybenzyl)amino)methyl) piperidine-1-carboxylate	^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.25 – 7.20 (m, 2H), 6.89 – 6.84 (m, 2H), 3.80 (s, 3H), 3.89 – 3.70 (br s, 2H), 3.78 (s, 2H), 3.15 (t, *J* = 11.7 Hz, 2H), 2.55 (s, 2H), 1.54 – 1.47 (m, 2H), 1.45 (s, 9H), 1.43 – 1.35 (m, 2H).
24		*tert*-butyl 12-(4-methoxybenzyl)-13-oxo-4-oxa-8,12-diazadispiro[2.1.5.3]tridecane-8-carboxylate	^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.18 (d, *J* = 8.6 Hz, 2H), 6.87 (d, *J* = 8.7 Hz, 2H), 4.54 (s, 2H), 3.81 (s, 3H), 3.63 (br s, 2H), 3.18 (s, 2H), 3.06 (t, *J* = 11.4 Hz, 2H), 1.86 (d, *J* = 13.5 Hz, 2H), 1.43 (s, 9H), 1.48 – 1.34 (m, 4H), 0.98 (q, *J* = 4.5 Hz, 2H).

25 TFA salt		12-(4-methoxybenzyl)-4-oxa-8,12-diazadispiro[2.1.5.3]tridecan-13-one trifluoroacetate	^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 9.31 (br s, 1H), 8.94 (br s, 1H), 7.20 – 7.16 (m, 2H), 6.92 – 6.84 (m, 2H), 4.54 (s, 2H), 3.80 (s, 3H), 3.24 (s, 2H), 3.20 (d, *J* = 12.1 Hz, 2H), 3.11 – 2.97 (m, 2H), 2.09 (d, *J* = 14.4 Hz, 2H), 1.75 (td, *J* = 14.5, 4.3 Hz, 2H), 1.46 (q, *J* = 4.7 Hz, 2H), 0.97 (q, *J* = 4.7 Hz, 2H).
26		12-(4-methoxybenzyl)-8-phenethyl-4-oxa-8,12-diazadispiro[2.1.5.3]tridecan-13-one	^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.30 – 7.25 (m, 2H), 7.23 – 7.17 (m, 5H), 6.88 – 6.84 (m, 2H), 4.55 (s, 2H), 3.80 (s, 3H), 3.19 (s, 2H), 2.80 – 2.68 (m, 2H), 2.58 – 2.47 (m, 4H), 2.31 (td, *J* = 11.4, 2.7 Hz, 2H), 1.97 – 1.87 (m, 2H), 1.66 – 1.48 (m, 2H), 1.42 (dd, *J* = 7.9, 4.5 Hz, 2H), 0.99 (dd, *J* = 7.9, 4.5 Hz, 2H).
27		8-phenethyl-4-oxa-8,12-diazadispiro[2.1.5.3]tridecan-13-one	^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 7.32 – 7.27 (m, 2H), 7.23 – 7.17 (m, 3H), 5.83 (br s, 1H, NH), 3.37 (d, *J* = 2.7 Hz, 2H), 2.84 – 2.77 (m, 2H), 2.71 – 2.57 (m, 4H), 2.39 (t, *J* = 10.2 Hz, 2H), 2.08 – 2.00 (m, 2H), 1.79 – 1.70 (m, 2H), 1.37 (dd, *J* = 8.0, 4.6 Hz, 2H), 1.02 (q, *J* = 4.6 Hz, 2H).
28		*tert*-butyl 12-ethyl-13-oxo-4-oxa-8,12-diazadispiro[2.1.5.3]tridecane-8-carboxylate	^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 3.79 (s, 2H), 3.45 (q, *J* = 7.2 Hz, 2H), 3.30 (s, 2H), 3.10 (t, *J* = 11.2 Hz, 2H), 2.00 – 1.90 (m, 2H), 1.60 – 1.49 (m, 2H), 1.46 (s, 9H), 1.36 (dd, *J* = 7.8, 4.5 Hz, 2H), 1.13 (t, *J* = 7.2 Hz, 3H), 0.94 (dd, *J* = 7.8, 4.5 Hz, 2H).
29 TFA salt		12-ethyl-13-oxo-4-oxa-8,12-diazadispiro[2.1.5.3]tridecane trifluoroacetate	^{1}H NMR (400 MHz, CD_3OD) δ ppm: 3.49 (s, 2H), 3.46 (q, *J* = 7.2 Hz, 2H), 3.33 – 3.27 (m, 1H), 3.20 – 3.07 (m, 3H), 2.23 – 2.15 (m, 2H), 1.93 – 1.83 (m, 2H), 1.29 (dd, *J* = 8.0, 4.6 Hz, 2H), 1.15 (t, *J* = 7.2 Hz, 3H), 1.04 (dd, *J* = 8.0, 4.6 Hz, 2H).

Selectivity panel experimental methods.

Guinea pig σ_2R radioligand assay. The binding of test compounds to guinea pig σ_2R was studied in guinea pig brain membranes. [^{3}H]-Di-*o*-tolylguanidine (DTG) (Perkin Elmer, Code NET-986) was used as the radioligand. The assay was carried out with 200 µg of membrane suspension, [^{3}H]-DTG (10 nM) in either absence or presence of either buffer or 10 µM haloperidol for total and non-specific binding, respectively. Binding buffer contained Tris-HCl (50 mM, pH 8) and σ_1R was blocked with (+)-SKF10047 at 400 nM. Plates were incubated at 25 °C for 120 min. After the incubation period, the reaction mixture was transferred to MultiScreen HTS, FC plates (Millipore), filtered and plates were washed 3 times with ice-cold 50 mM Tris–HCl (pH 7.4). Filters were dried and counted at approximately 40% efficiency in a MicroBeta scintillation counter (Perkin-Elmer) using EcoScint liquid scintillation cocktail.

Human 5-HT$_{1A}$ receptor radioligand assay. Human 5-HT$_{1A}$ enriched membranes (10 µg) were incubated with 2 nM radiolabeled [3H]-8-hydroxy-DPAT in 250 µL of assay buffer containing 50 mM Tris-HCl, 5 mM $MgCl_2$, pH 7.4. NSB (non-specific binding) was measured by adding 10 µM 5-HT in 25 µL volume. Final DMSO concentration was 0.1% (v/v). After 2 h incubation at 37 ºC, binding reaction was terminated by filtering 200 µL through Multiscreen GF/C (Millipore) presoaked in 150 µL of 0.5 % polyethyleneimine in Vacuum Manifold Station, followed by 3 washes with 200 µL/well of ice-cold filtration buffer containing 50 mM Tris-HCl, pH 7.4

Human 5-HT$_{2B}$ receptor radioligand assay. Human 5-HT$_{2B}$ enriched membranes (5 µg) were incubated with 1nM radiolabeled [3H]-LSD in 250 µL of assay buffer containing 50 mM Tris-HCl, 4 mM $CaCl_2$, 01% ascorbic acid, pH 7.4. NSB (non-specific binding) was measured by adding 50 µM 5-HT in 2.5 µL volume. Final DMSO concentration was 0.1% (v/v). After 30 min incubation at 37 ºC, binding reaction was terminated by filtering 200 µL through Multiscreen GF/C (Millipore) presoaked in 150 µL of 0.5 % polyethyleneimine in Vacuum Manifold Station, followed by 3 washes with 200 µL/well of ice-cold filtration buffer containing 50 mM Tris-HCl, pH 7.4 .

Human α_2A adrenoreceptor radioligand assay. Human α_2A adrenoreceptor enriched membranes (5 µg) were incubated with 0.5 nM of radiolabeled [3H]-MK912 in 250 µL of assay buffer

containing 25 mM sodium phosphate, pH 7.4. NSB (non-specific binding) was measured by adding 10 μM rauwolascine in 25 μL volume. Final DMSO concentration was 0.1% (v/v). After 30 min incubation at 25 ºC, binding reaction was terminated by filtering 200 μL through Multiscreen GF/C (Millipore) presoaked in 150 μL of 0.5 % polyethyleneimine in Vacuum Manifold Station, followed by 3 washes with 200 μL/well of ice-cold filtration buffer containing 25 mM sodium phosphate, pH 7.4.

Human dopamine transporter (DAT) radioligand assay. Human Dopamine transporter (DAT) enriched membranes (5 μg) were incubated with 2 nM of radiolabeled [3H]-GBR12935 in 250 μL of assay buffer containing 50 mM Tris-HCl, 100 mM NaCl, pH 7.4. NSB (non-specific binding) was measured by adding 10 μM GBR12909 in 25 μL volume. Final DMSO concentration was 0.1% (v/v). After 120 min incubation at 4 ºC, binding reaction was terminated by filtering 200 μL through Multiscreen GF/C (Millipore) presoaked in 150 μL of 0.5 % polyethyleneimine in Vacuum Manifold Station, followed by 3 washes with 200 μL/well of ice-cold filtration buffer containing 50 mM Tris-HCl, 100 mM NaCl, pH 7.4.

Human δ-opioid receptor (DOR) radioligand assay. Human δ-opioid receptor (DOR) enriched membranes (3.5 μg) were incubated with 1.5 nM of radiolabeled [3H]-DADLE in 250 μL of assay buffer containing 50 mM Tris-HCl, pH 7.4. NSB (non-specific binding) was measured by adding 10 μM DPDPE in 25 μLvolume. Final DMSO concentration was 0.1% (v/v). After 90 min incubation at 25 ºC, binding reaction was terminated by filtering 200 μL through Multiscreen GF/C (Millipore) presoaked in 150 μL of 0.5 % polyethyleneimine in Vacuum Manifold Station, followed by 3 washes with 200 μL/well of ice-cold filtration buffer containing 50 mM Tris-HCl, pH 7.4.

Human κ-opioid receptor (KOR) radioligand assay. Human human opioid κ receptor (KOR) enriched membranes (10 μg) were incubated with 0.25 nM of radiolabeled [3H]-diprenorphine in 250 μL of assay buffer containing 50 mM Tris-HCl, pH 7.4. NSB (non-specific binding) was measured by adding 200 μM U69.593 in 25 μL volume. Final DMSO concentration was 0.1% (v/v). After 90 min incubation at 25 ºC, binding reaction was terminated by filtering 200 μL through

Multiscreen GF/C (Millipore) presoaked in 150 µL of 0.5 % polyethyleneimine in Vacuum Manifold Station, followed by 3 washes with 200 µL/well of ice-cold filtration buffer containing 50 mM Tris-HCl, pH 7.4.

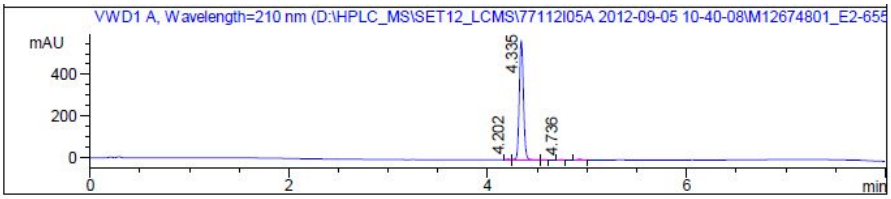
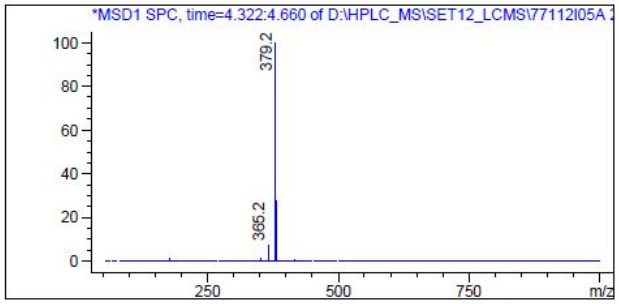
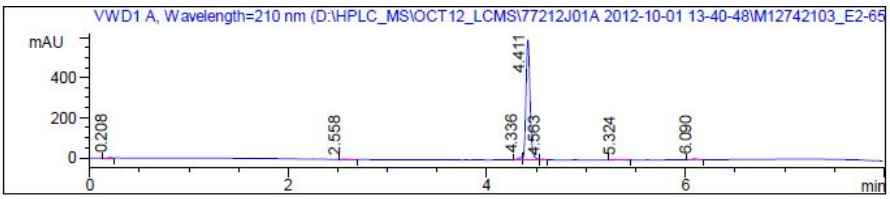
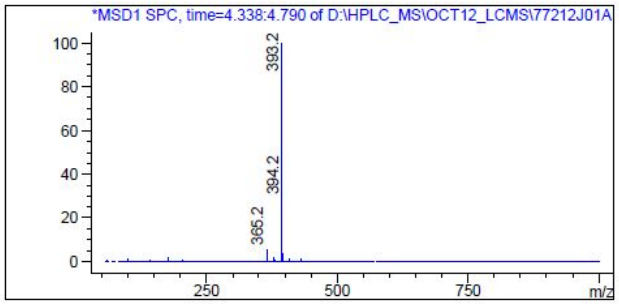
Human histamine H_1 receptor radioligand assay. Human histamine H_1 receptor enriched membranes (25 µg) were incubated with 2.4 nM of radiolabeled [3H]-pyrilamine in 250 µL of 8.6 mM KH_2PO_4, 41.4 mM Na_2HPO_4 Na+/K+ phosphate buffer, pH 7.4. NSB (non-specific binding) was measured by adding triprolidine 10 µM in 25 µL volume. Final DMSO concentration was 0.1% (v/v). After 90 min incubation at 25 ºC, binding reaction was terminated by filtering 200 µL through Multiscreen GF/C (Millipore) presoaked in 150 µL of 0.5 % polyethyleneimine in Vacuum Manifold Station, followed by 3 washes with 200 µL/well of ice-cold 8.6 mM KH_2PO_4, 41.4 mM Na_2HPO_4 Na+/K+ phosphate buffer, pH 7.4

Human norepinephrine transporter (NET) radioligand assay. Human norepinephrine transporter (NET) enriched membranes (1 µg) were incubated with 5 nM of radiolabeled [3H]-nisoxetine in 250 µL of assay buffer containing 50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, pH 7.4. NSB (non-specific binding) was measured by adding 10 µM desipramine in 2.5 µL volume. Final DMSO concentration was 1% (v/v). After 60 min incubation at 4 ºC, binding reaction was terminated by filtering 200 µL through Multiscreen GF/C (Millipore) presoaked in 150 µL of 0.5 % polyethyleneimine in Vacuum Manifold Station, followed by 3 washes with 200 µL/well of ice-cold filtration buffer containing 50 mM Tris-HCl, 0.9% NaCl, pH 7.4.

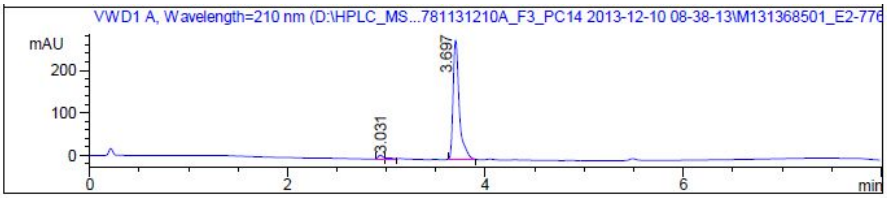
Human serotonin transporter (SERT) radioligand assay. Human serotonin transporter (SERT) enriched membranes (3.5 µg) were incubated with 3 nM of radiolabeled [3H]-imipramine in 250 µL of assay buffer containing 50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, pH 7.4. NSB (non-specific binding) was measured by adding 200 µM imipramine in 25 µL volume. Final DMSO concentration was 0.1% (v/v). After 30 min incubation at 27 ºC, binding reaction was terminated by filtering 200 µL through Multiscreen GF/C (Millipore) presoaked in 150 µL of 0.5 % polyethyleneimine in

Vacuum Manifold Station, followed by 3 washes with 200 μL/well of ice-cold filtration buffer containing 50 mM Tris-HCl, 0.9% NaCl, pH 7.4.

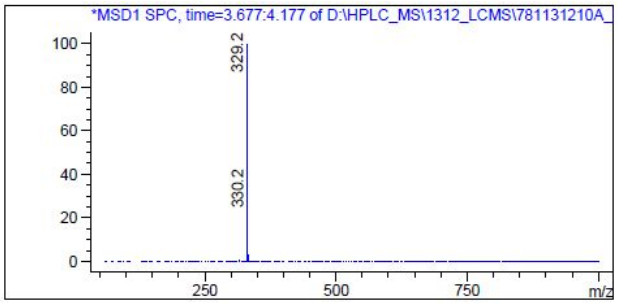
HPLC purity

Compound	HPLC chromatogram	Purity (%)
14a	 **# Meas. Ret Peak Width Area Response** 1 4.202 MF 0.045 10.263 0.602 2 4.335 FM 0.048 1.674e3 98.215 3 4.567 MM 0.040 2.228 0.131 4 4.736 MM 0.047 2.792 0.164 5 4.921 MM 0.051 15.142 0.889 	98
14b	 **# Meas. Ret Peak Width Area Response** 1 0.208 BV 0.050 9.518 0.530 2 2.558 VB 0.041 5.086 0.283 3 4.336 VV 0.042 35.813 1.994 4 4.411 VV 0.043 1.708e3 95.092 5 4.563 VV 0.045 12.259 0.682 6 5.324 BB 0.061 9.421 0.524 7 6.090 BB 0.047 16.060 0.894 	95

14c

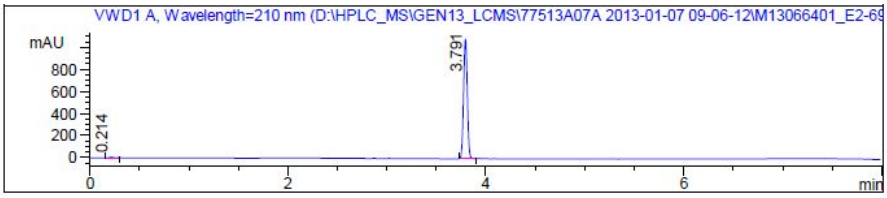


#	Meas. Ret	Peak	Width	Area	Response
1	2.938	MF	0.057	31.676	2.487
2	3.031	FM	0.078	13.217	1.038
3	3.697	BB	0.066	1.229e3	96.475

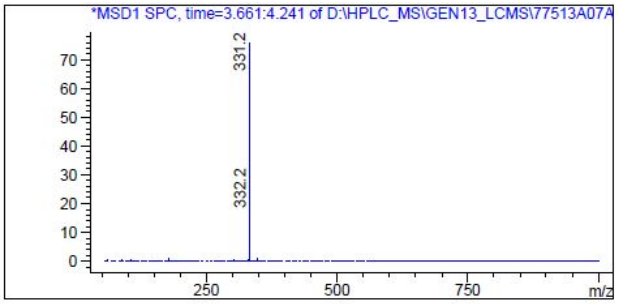


96

14d

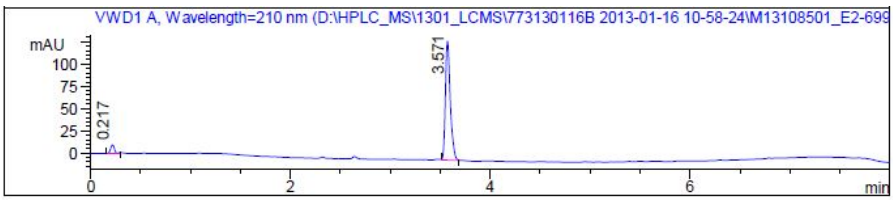


#	Meas. Ret	Peak	Width	Area	Response
1	0.214	BB	0.050	24.506	0.850
2	3.791	BB	0.042	2.858e3	99.150



99

14e



#	Meas. Ret	Peak	Width	Area	Response
1	0.217	BB	0.042	25.542	5.301
2	3.571	BB	0.051	456.259	94.699

95

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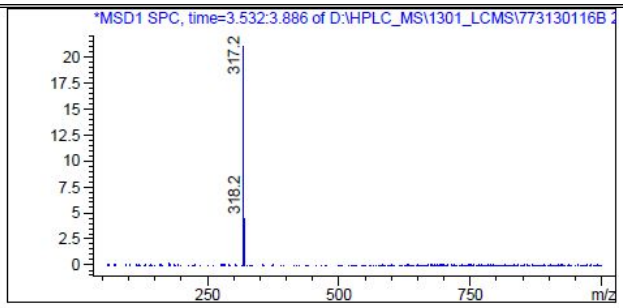
<tr><td></td><td> 
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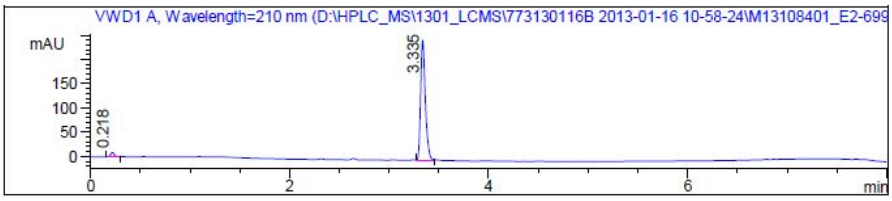
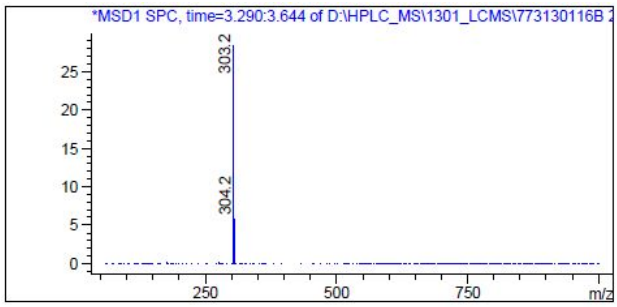
<tr><td>14f</td><td> 

Meas. Ret Peak Width Area Response

#	Meas. Ret	Peak	Width	Area	Response
1	0.218	BB	0.042	23.025	2.739
2	3.335	BB	0.052	817.554	97.261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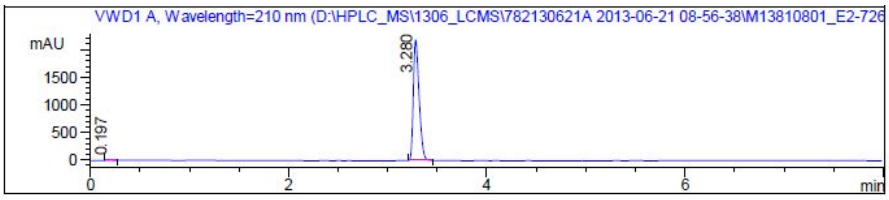
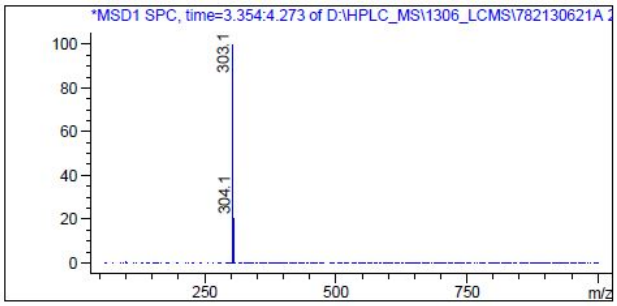
<tr><td>14g</td><td> 

Meas. Ret Peak Width Area Response

#	Meas. Ret	Peak	Width	Area	Response
1	0.197	BB	0.037	10.251	0.115
2	3.280	BB	0.064	8.916e3	99.885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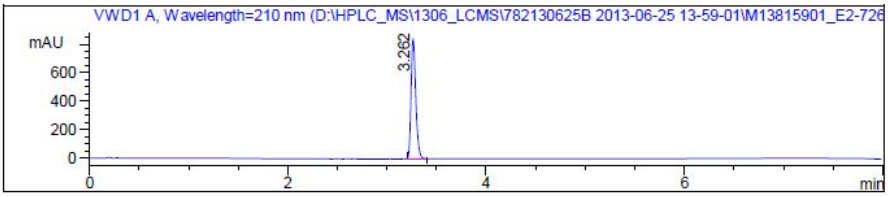
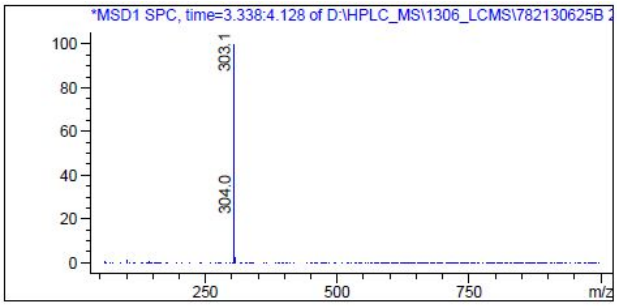
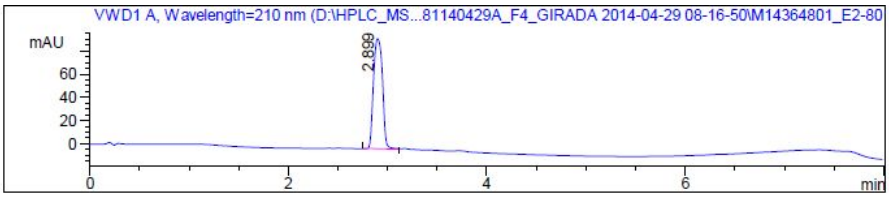
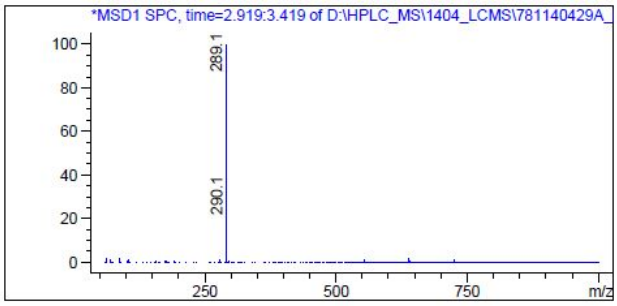
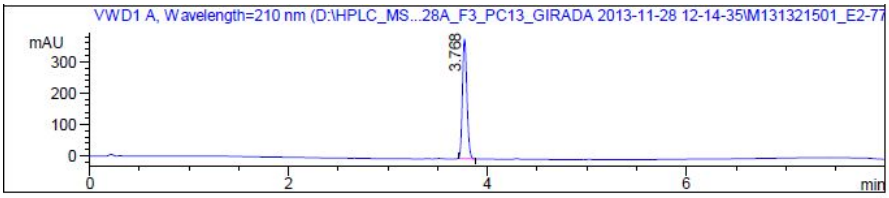
14h	 \# Meas. Ret Peak Width Area Response 1 3.262 BB 0.051 2.791e3 100.000 	100
14i	 \# Meas. Ret Peak Width Area Response 1 2.899 BB 0.106 601.394 100.000 	100
14j	 \# Meas. Ret Peak Width Area Response 1 3.768 BB 0.050 1.208e3 100.000	100

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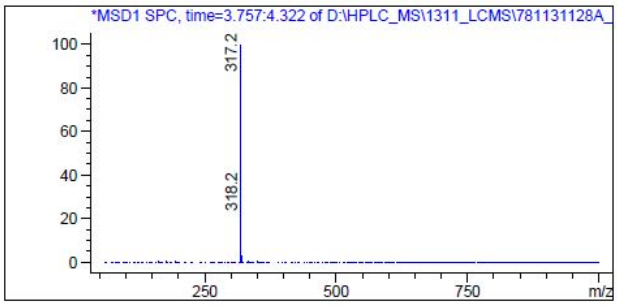
<tr><td></td><td> 
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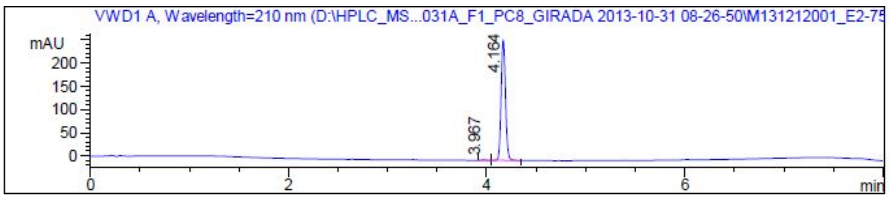
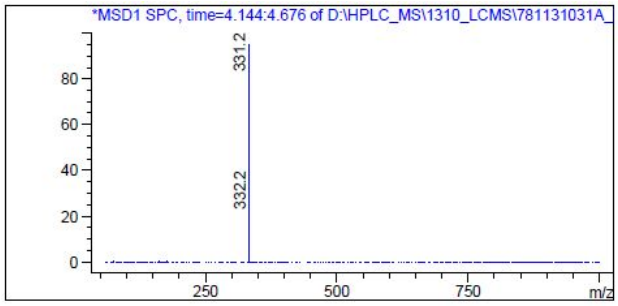
<tr><td>14k</td><td> 

#	Meas. Ret	Peak	Width	Area	Response
1	3.967	MM	0.060	6.816	0.844
2	4.164	MM	0.051	800.674	99.156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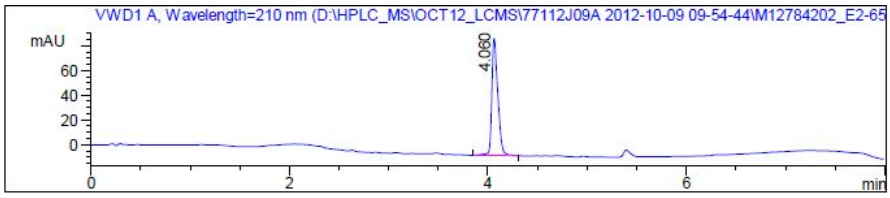
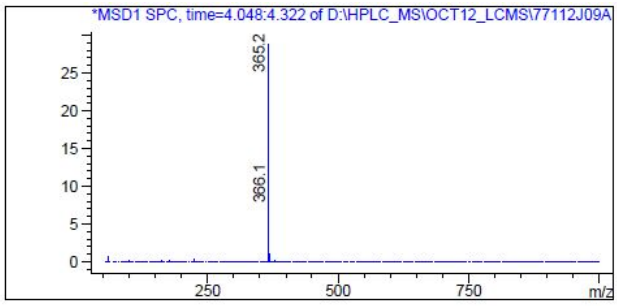
<tr><td>14l</td><td> 

#	Meas. Ret	Peak	Width	Area	Response
1	4.060	BB	0.061	402.643	100.000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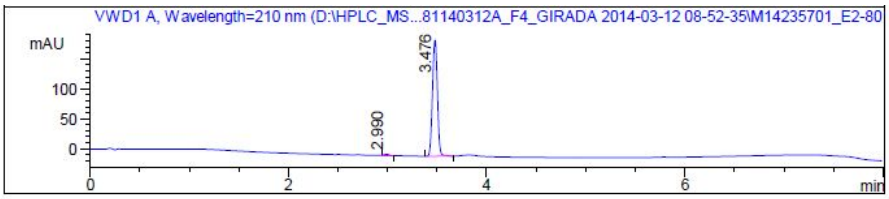
<tr><td>14m</td><td> 
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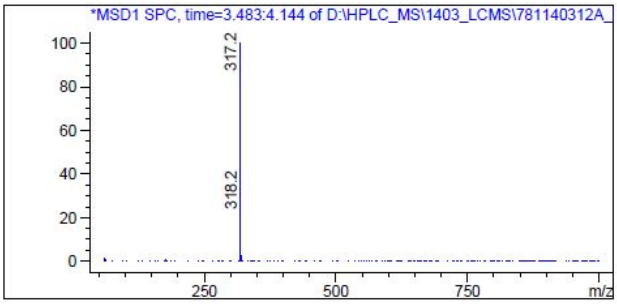
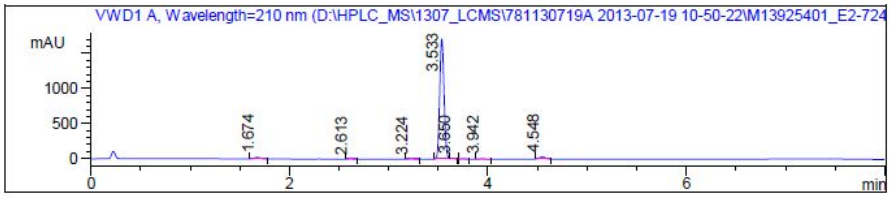
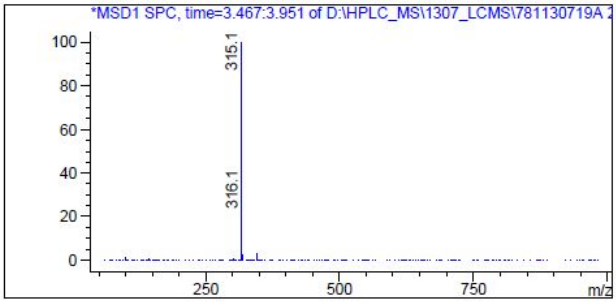
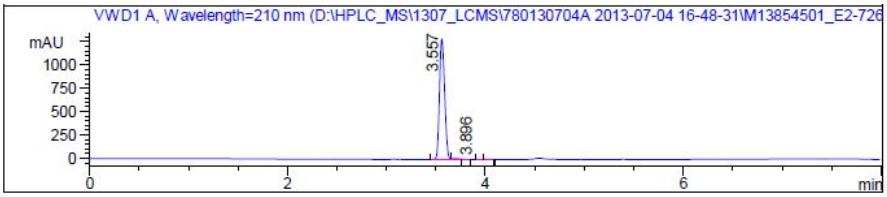
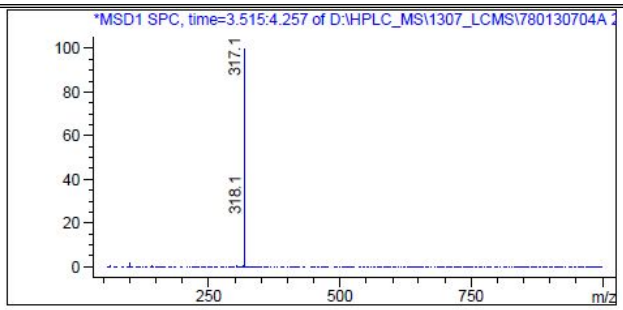
	# Meas. Ret Peak Width Area Response 1 2.990 MM 0.054 6.863 1.008 2 3.476 MM 0.058 673.985 98.992  	
14n	 # Meas. Ret Peak Width Area Response 1 1.674 BB 0.058 93.042 1.657 2 2.613 BB 0.041 13.902 0.248 3 3.224 BB 0.048 19.087 0.340 4 3.533 MM R 0.051 5.259e3 93.635 5 3.650 MM T 0.045 22.520 0.401 6 3.756 MM 0.052 32.896 0.586 7 3.942 BB 0.057 33.868 0.603 8 4.548 MM 0.063 142.190 2.532  	94
14o	 # Meas. Ret Peak Width Area Response 1 3.557 MM R 0.060 4.684e3 99.408 2 3.698 MM T 0.054 16.206 0.344 3 3.896 MM R 0.067 7.377 0.157 4 4.028 MM T 0.057 4.291 0.091	99



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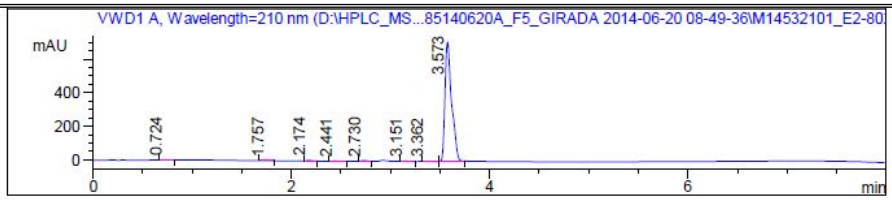
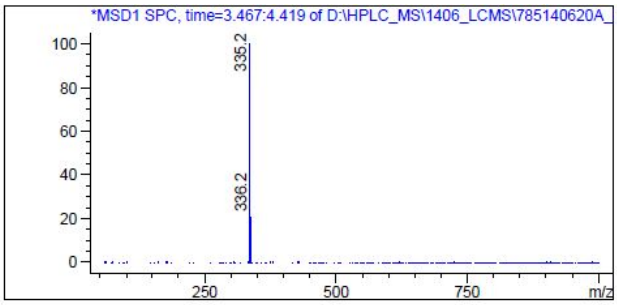


#	Meas. Ret	Peak	Width	Area	Response
1	0.724	FM	0.081	4.054	0.119
2	1.757	MM	0.092	5.658	0.165
3	2.174	MM	0.070	17.407	0.509
4	2.441	MM	0.109	6.975	0.204
5	2.730	MM	0.061	19.490	0.570
6	3.151	MM	0.073	12.119	0.354
7	3.362	FM	0.077	11.488	0.336
8	3.573	MF	0.078	3.343e3	97.743



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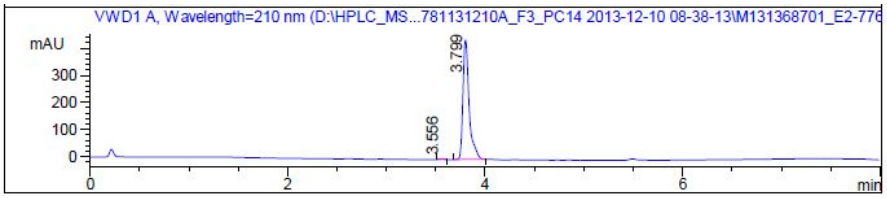
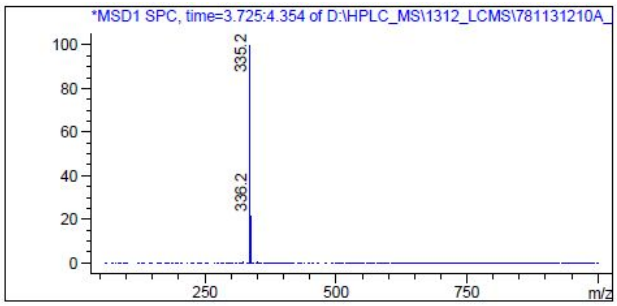


#	Meas. Ret	Peak	Width	Area	Response
1	3.556	MM	0.050	3.905	0.203
2	3.799	MM	0.072	1.919e3	99.797



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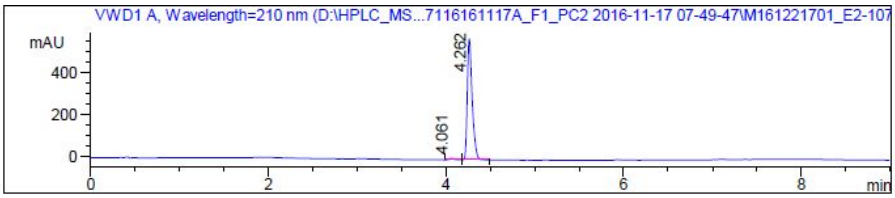
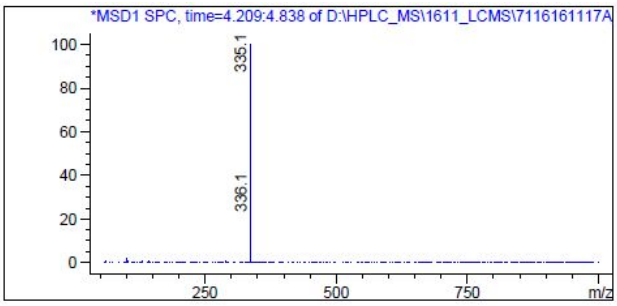


#	Meas. Ret	Peak	Width	Area	Response
1	4.061	BV	0.055	26.758	1.208
2	4.262	VB	0.058	2.188e3	98.792



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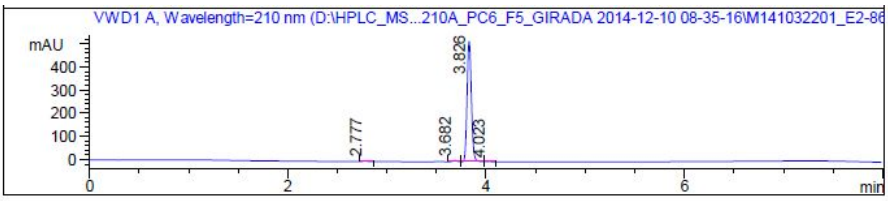
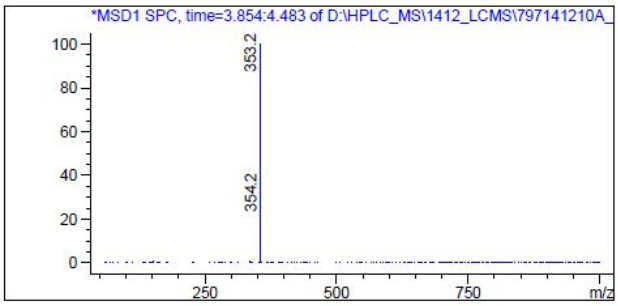


#	Meas. Ret	Peak	Width	Area	Response
1	2.777	MM	0.057	10.161	0.633
2	3.682	BV	0.049	15.970	0.995
3	3.826	MM	0.050	1.575e3	98.151
4	4.023	MM	0.074	3.539	0.221



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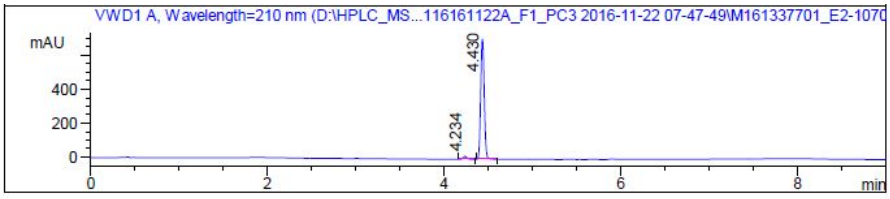
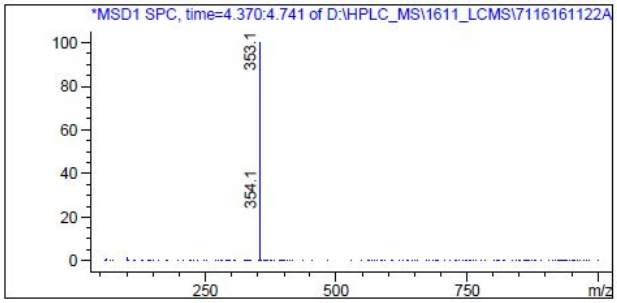


#	Meas. Ret	Peak	Width	Area	Response
1	4.234	BB	0.045	52.223	2.570
2	4.430	BB	0.044	1.980e3	97.430

97



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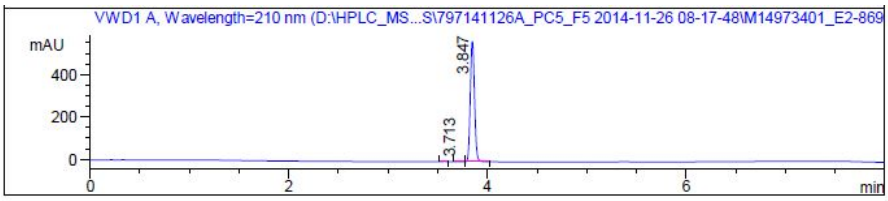
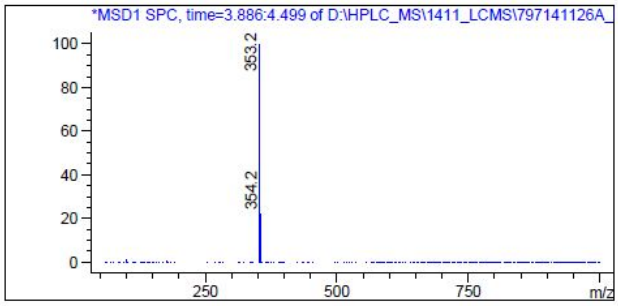


#	Meas. Ret	Peak	Width	Area	Response
1	3.567	MM	0.046	4.413	0.263
2	3.713	MM	0.050	4.130	0.246
3	3.847	MM	0.049	1.668e3	99.490



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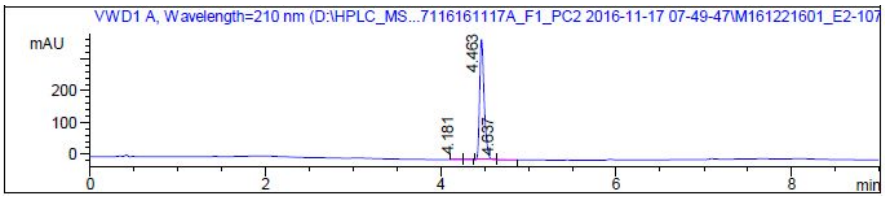
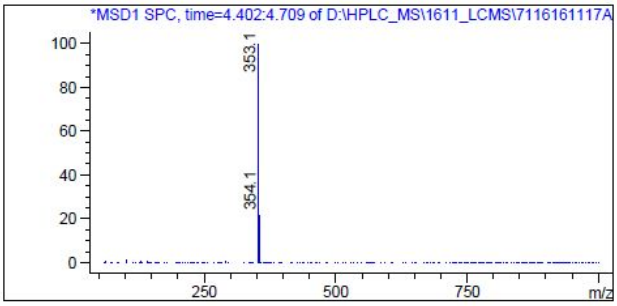


#	Meas. Ret	Peak	Width	Area	Response
1	4.181	MM	0.080	2.575	0.187
2	4.295	MM	0.066	3.614	0.262
3	4.463	MF	0.060	1.371e3	99.336
4	4.637	FM	0.114	2.967	0.215



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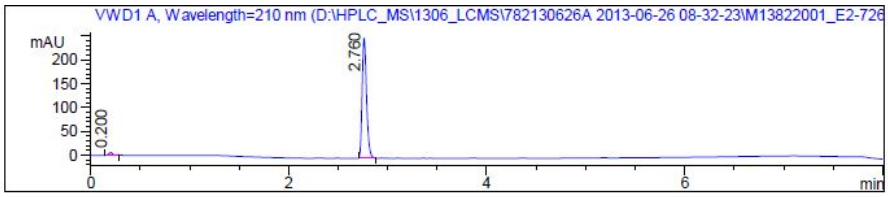
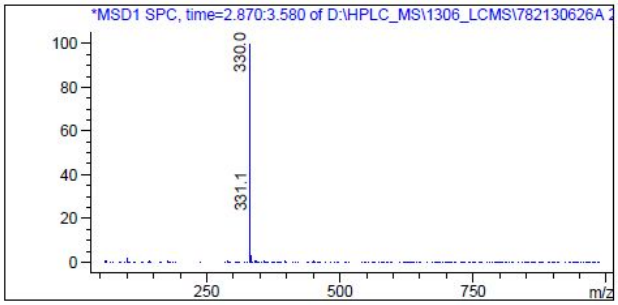


#	Meas. Ret	Peak	Width	Area	Response
1	0.200	BB	0.043	15.935	1.994
2	2.760	BB	0.048	783.125	98.006



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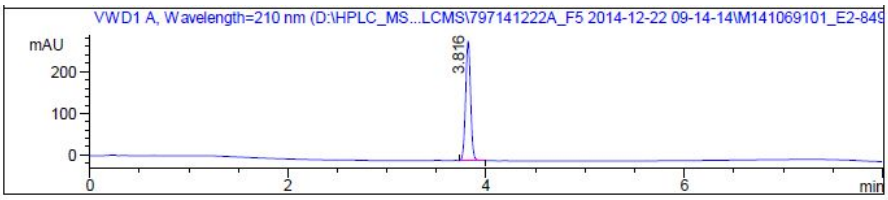
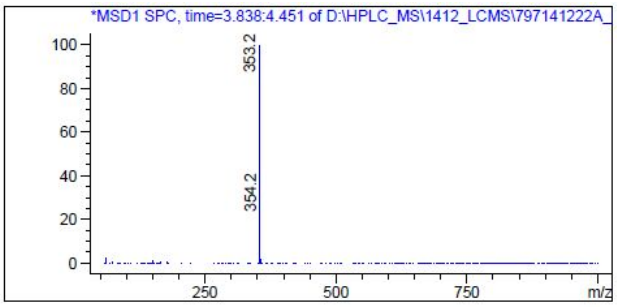


#	Meas. Ret	Peak	Width	Area	Response
1	3.816	MM	0.058	1.003e3	100.000



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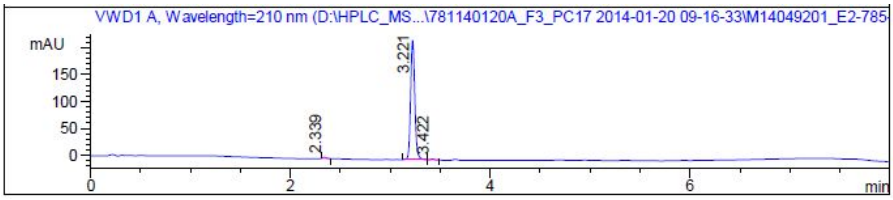
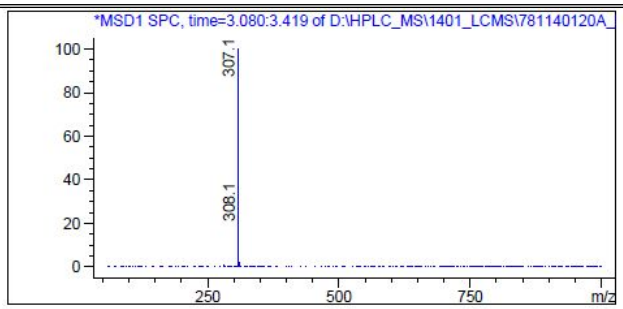


#	Meas. Ret	Peak	Width	Area	Response
1	2.339	MM	0.045	6.594	0.997
2	3.221	MM	0.048	648.891	98.113
3	3.422	MM	0.053	5.887	0.890

98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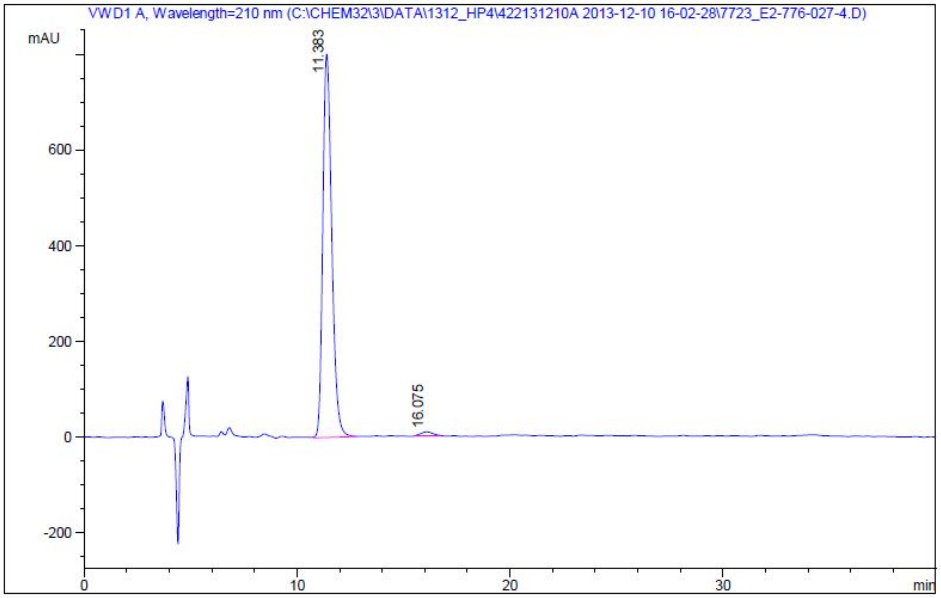
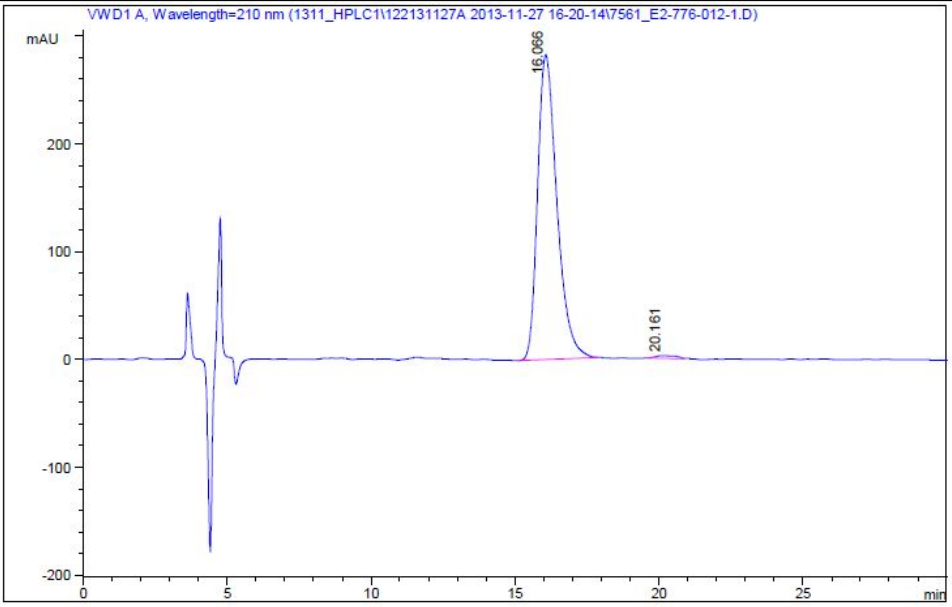
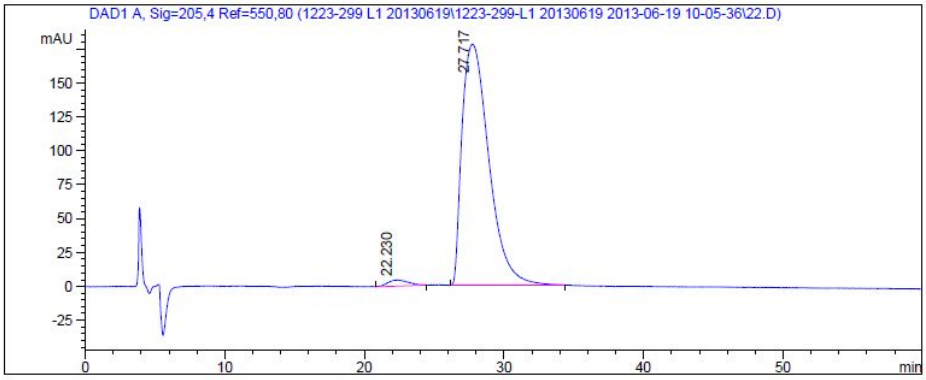
60
40
20
0
307.1
308.1
250
500
750
m/z

Optical purity by chiral HPLC

Compound	HPLC chromatogram	ee (%)
14c	 |# |Meas. R |Pea | Width | Area |Height |Area % | --- | 1 | 11.383 |MM | 0.490 | 23539.252 |801.280 | 98.599| | 2 | 16.075 |MM | 0.675 | 334.565 | 8.255 | 1.401| ---	97.2
14g	 |# |Meas. R |Pea | Width | Area |Height |Area % | --- | 1 | 16.066 |MM | 0.778 | 13199.116 |282.610 | 99.057| | 2 | 20.161 |MM | 0.823 | 125.594 | 2.543 | 0.943| ---	98.1

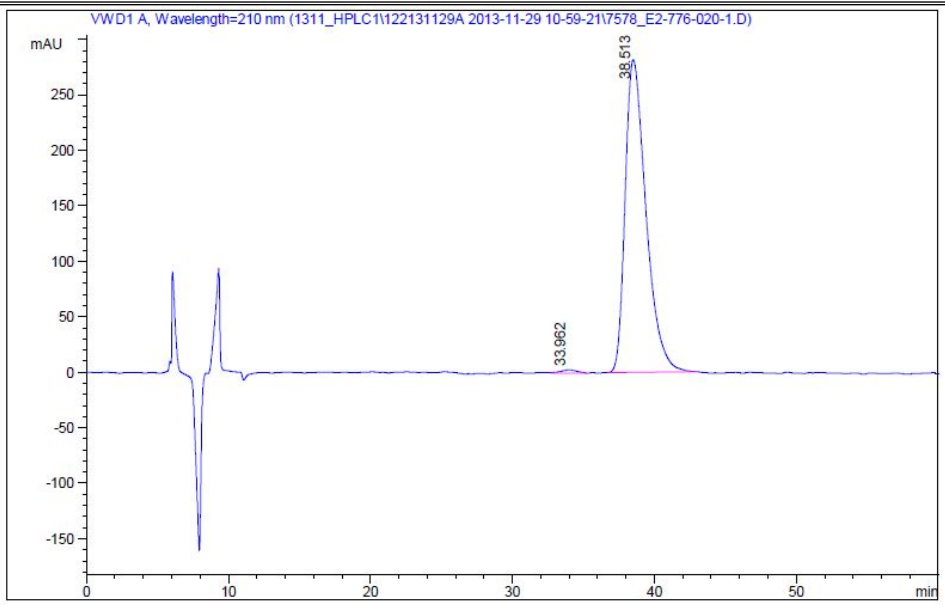
14h



Peak #	RT [min]	Type	Width [min]	Area	Area %
1	22.230	MM	1.755	475.941	1.955
2	27.717	MM	2.236	23873.652	98.045

96.1

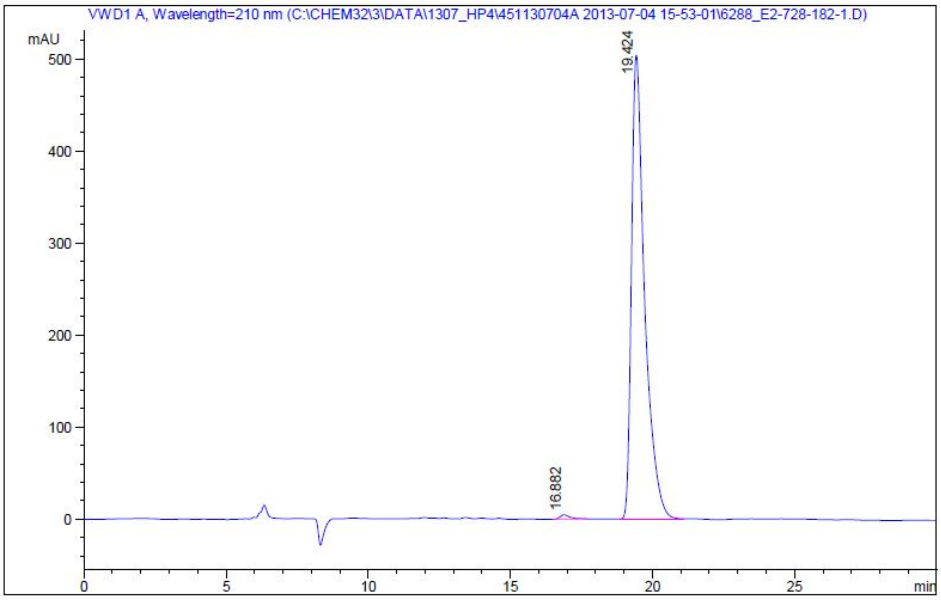
14j



#	Meas. R	Pea	Width	Area	Height	Area %
1	33.962	VV	0.932	209.329	2.665	0.724
2	38.513	MM	1.699	28694.928	281.568	99.276

98.6

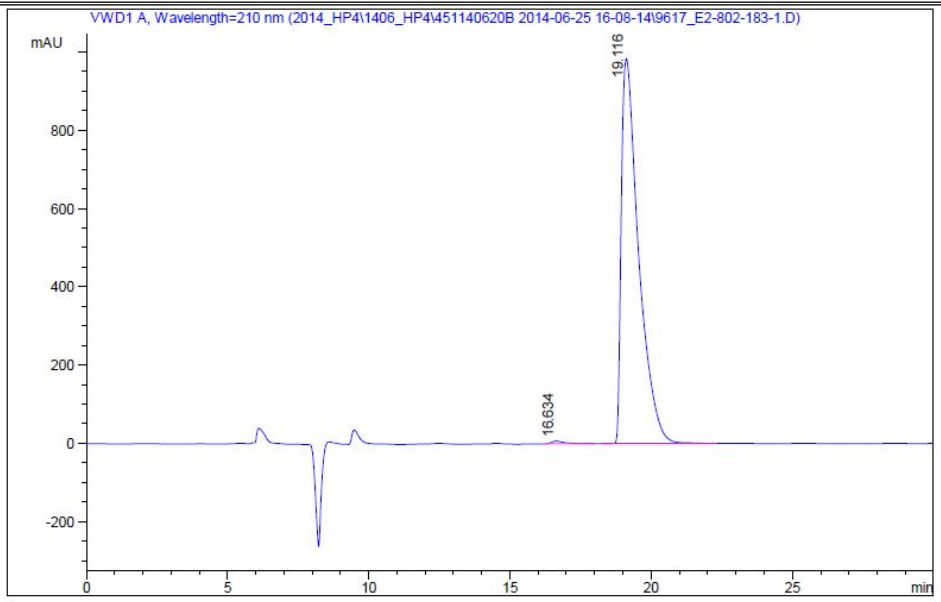
14o



#	Meas. R	Pea	Width	Area	Height	Area %
1	16.882	BB	0.376	122.337	4.822	0.748
2	19.424	BB	0.475	16240.536	503.992	99.252

98.5

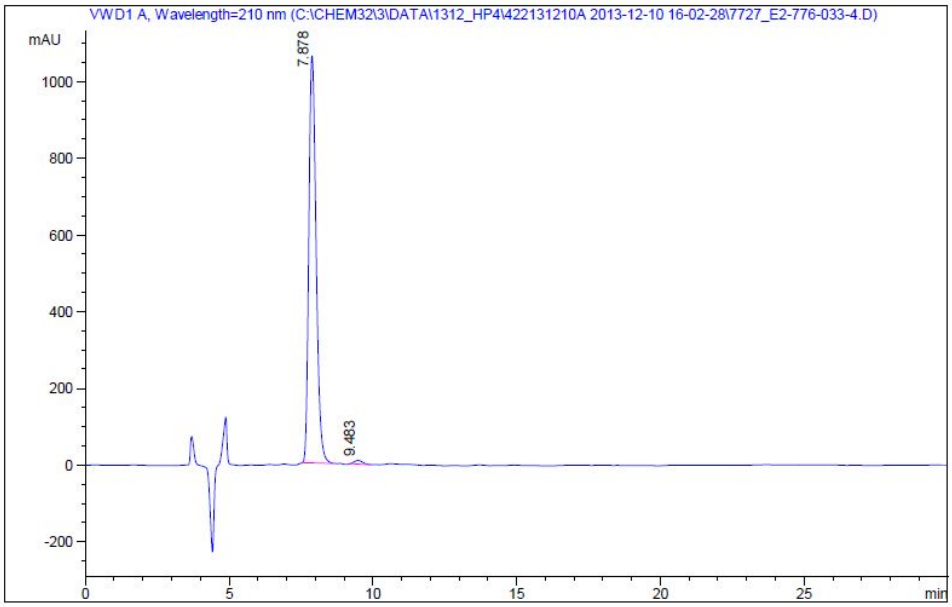
14p



#	Meas. R	Pea	Width	Area	Height	Area %
1	16.634	MM	0.571	249.112	7.276	0.590
2	19.116	MM	0.711	41940.266	983.517	99.410

98.8

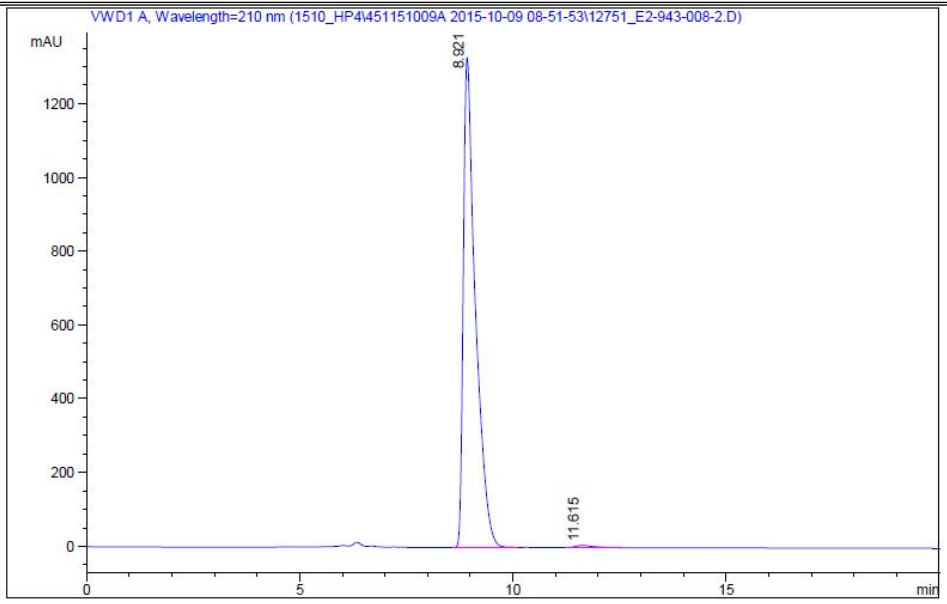
14q



#	Meas. R	Pea	Width	Area	Height	Area %
1	7.878	MM	0.294	18749.268	1.061e3	98.822
2	9.483	MM	0.366	223.503	10.191	1.178

97.6

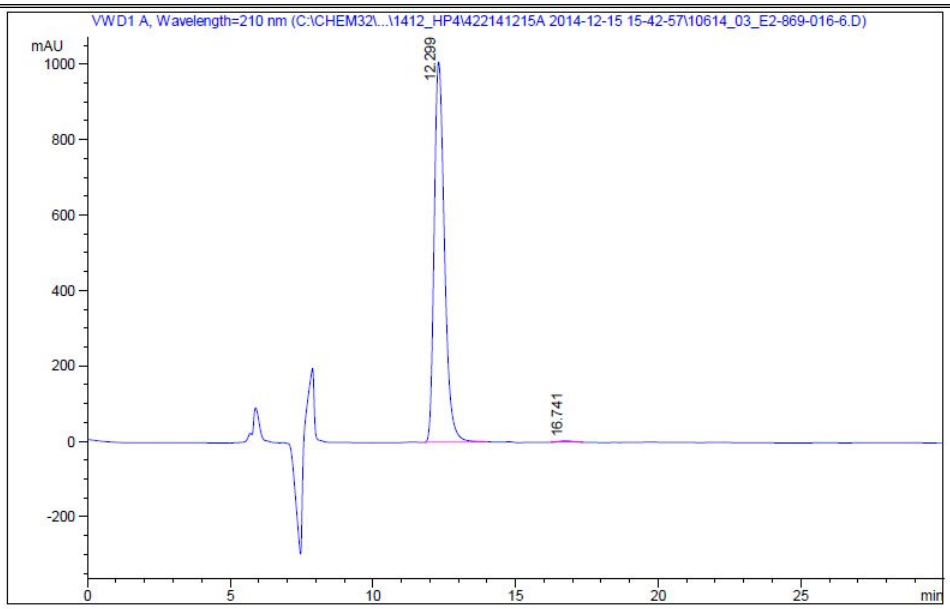
14r



#	Meas. R	Pea	Width	Area	Height	Area %
1	8.921	BB	0.282	26183.822	1.328e3	99.308
2	11.615	BB	0.424	182.577	6.254	0.692

98.6[a]

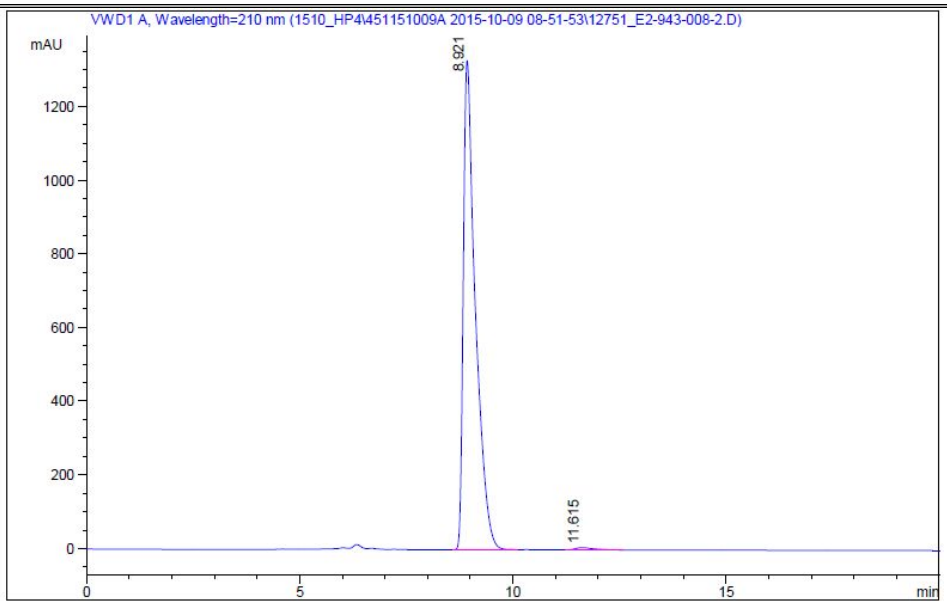
14s



#	Meas. R	Pea	Width	Area	Height	Area %
1	12.299	MM	0.417	25252.156	1.009e3	99.428
2	16.741	MM	0.547	145.390	4.430	0.572

98.8

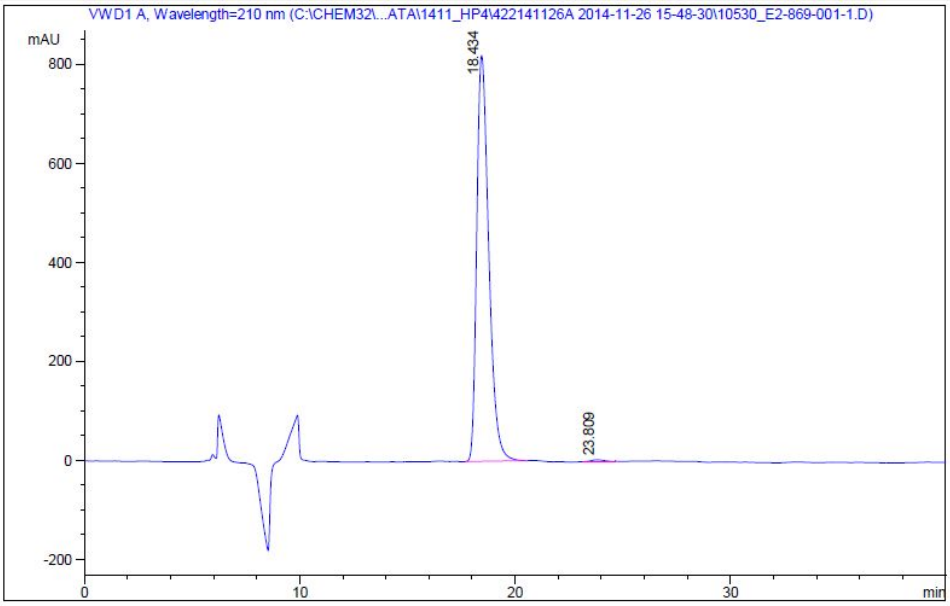
14t



#	Meas. R	Pea	Width	Area	Height	Area %
1	8.921	BB	0.282	26183.822	1.328e3	99.308
2	11.615	BB	0.424	182.577	6.254	0.692

98.6[a]

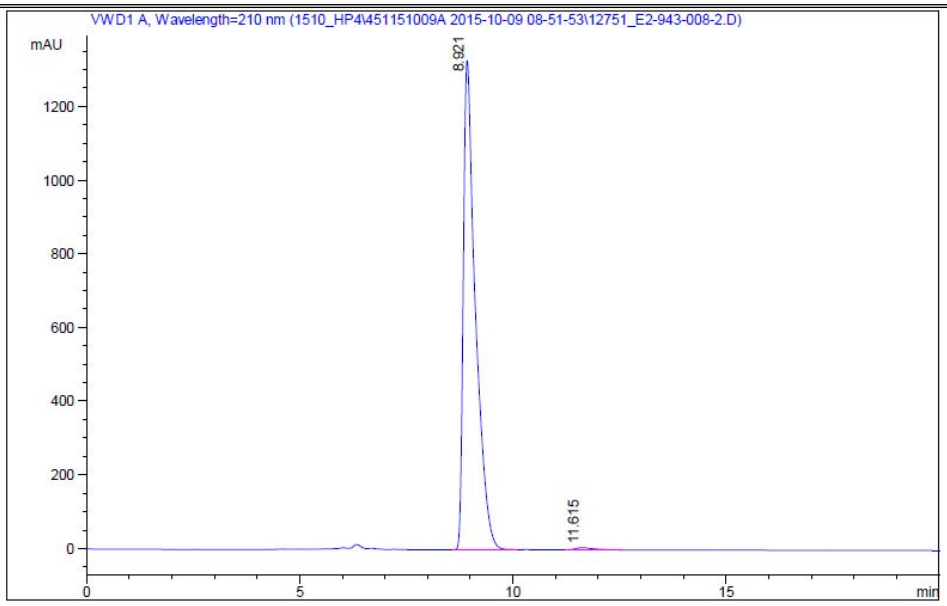
14u



#	Meas. R	Pea	Width	Area	Height	Area %
1	18.434	MM	0.647	31838.379	819.853	99.519
2	23.809	MM	0.682	153.808	3.760	0.481

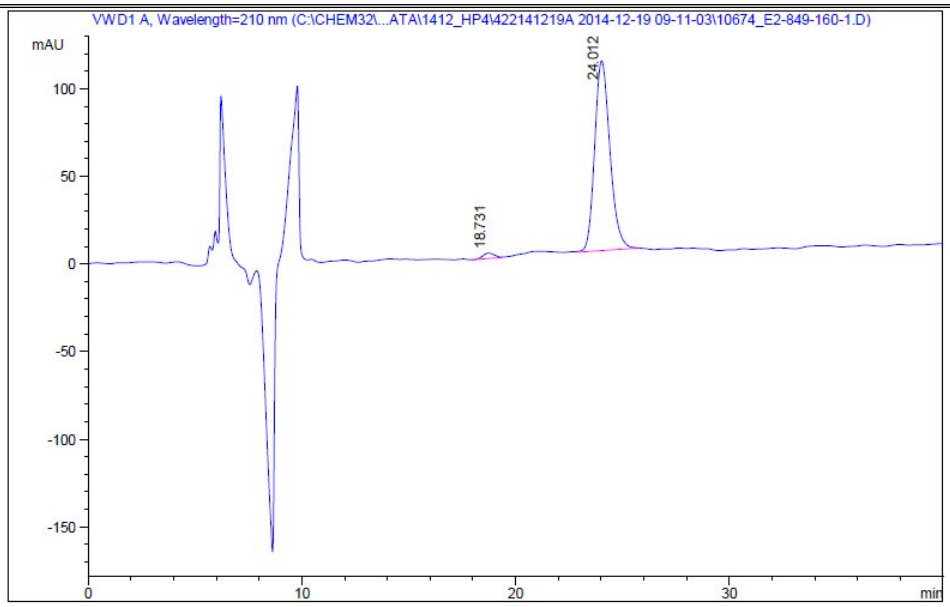
99.0

14v



#	Meas. R	Pea	Width	Area	Height	Area %
1	8.921	BB	0.282	26183.822	1.328e3	99.308
2	11.615	BB	0.424	182.577	6.254	0.692

98.6[a]

14x	 | # | Meas. R | Pea | Width | Area | Height | Area % | | 1 | 18.731 | BV | 0.576 | 125.527 | 3.225 | 2.271 | | 2 | 24.012 | MM | 0.830 | 5402.676 | 108.499 | 97.729 |	95.4

[a] ee% determined at Boc-morpholinone precursor stage (compound **11-1R**)