Synthesis, Radiolabelling and *In Vitro* and *In Vivo* Evaluation of a Novel Fluorinated ABP688 Derivative for the PET Imaging of Metabotropic Glutamate Receptor Subtype 5

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Supporting Information

Chemistry

3-((*Tert*-butyldimethylsilyl)oxy)propan-1-ol (2): A flame dried flask was charged with anhydrous *N*,*N*`-dimethylformamide (25 mL) and at ambient temperature under N_2 atmosphere 1,3-propanediol (1.00 mL, 1.05 g, 13.8 mmol, d=1.05) was added and the resulting colourless solution was treated with diisopropylethylamine (22.5 mL, 17.0 g, 131 mmol, d=0.755) in one portion and pale yellow biphasic mixture was vigorously stirred and further treated with a solution of *tert*-butyldimethylchlorosilane (2.08 g, 13.8 mmol) in DMF (15 mL) dropwise over 16 min during which time the mixture turned cloudy and it was allowed to stir for 6 h. After this time the mixture was partitioned between H_2O (30 mL) and Et_2O (50 mL) and the two layers were well shaken and separated. The aqueous phase was extracted with Et_2O (2x50 mL). The combined organic extracts were washed with 2M aq. HCl (2x50 mL; CAUTION: vigorous bubbling), saturated aq. $NaHCO_3$ (1x50 mL), brine (1x50 mL), dried (Na_2SO_4) and concentrated *in vacuo* to give crude mixture as pale yellow oil (3.78 g). The crude product was purified by chromatography on a silica gel column (eluting with EtOAc:pentane 3:7) to afford title compound (1.29 g, 6.77 mmol, 49%) as a colourless oil: IR (neat) 3358, 2954, 2930, 1741, 1472, 1373, 1251, 1094, 962, 835, 776 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.84 (t, *J* = 5.6 Hz, 2H), 3.80 (dd, *J* = 10.9, 5.4 Hz, 2H), 2.54 (t, *J* = 5.3 Hz, 1H), 1.78 (quint, *J* = 5.6 Hz, 1H), 0.90 (s, 9H), 0.08 (s, 6H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 63.0 (2), 62.6 (2), 34.3 (2), 26.1 (3C, 3), 18.4 (0), −5.3 (2C, 3) ppm; MS (ES+) *m/z* 191 $(M + H)^+$; HRMS (EI+) *m/z* 191.1462 (calcd. for $C_9H_{23}O_2Si$: 191.1467).

3-((*Tert*-butyldimethylsilyl)oxy)propyl 2-bromoacetate (3): A stirred solution of 3-((tert-butyldimethylsilyl)oxy)propan-1-ol (818 mg, 4.30 mmol) in anhydrous dichloromethane (9 mL) was allowed to cool to 0 °C (the ice bath) and under N_2 atmosphere it was then treated with triethylamine (0.66 mL, 478 mg, 4.73 mmol, d=0.726) in one portion followed by 4-dimethylaminopyridine (26.0 mg, 0.22 mmol) and finally bromoacetylbromide (0.38 mL, 867 mg, 4.30 mmol, d=2.31) was added dropwise over 10 min during which time the mixture turned orange and then yellow and cloudy and it was allowed to stir and slowly warm to ambient temperature over 16 h. After this time the mixture was partitioned between H_2O (20 mL) and CH_2Cl_2 (10 mL) and the two layers were well shaken and separated. The aqueous phase was extracted with CH_2Cl_2 (2x20 mL). The combined organic extracts were washed with H_2O (3x20 mL), brine (1x20 mL), dried (Na_2SO_4) and concentrated *in vacuo* to give crude mixture as brown oily residue (2.49 g). The crude mixture was purified by

chromatography on a silica gel column (eluting with gradient EtOAc:pentane 1:18 to EtOAc:pentane 1:9) to afford title compound (489 mg, 1.57 mmol, 36%) as a colourless oil: IR (neat) 2954, 2931, 2854, 1742, 1473, 1277, 1257, 1103, 1008, 969, 834 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 4.28 (t, *J* = 6.4 Hz, 2H), 3.83 (s, 2H), 3.71 (t, *J* = 6.0 Hz, 2H), 1.87 (quint, *J* = 6.2 Hz, 1H), 0.89 (s, 9H), 0.05 (s, 6H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.4 (0), 63.5 (2), 59.3 (2), 31.7 (2), 26.1 (3C, 3), 26.0 (2), 18.5 (0), −5.2 (2C, 3) ppm; HRMS (EI+) *m/z* 252.9890 ($(M - C_4H_9)^+$ calcd. for $C_7H_{14}BrO_3Si$; 252.9896).

(3-(2-Bromoethoxy)propoxy)(*tert*-butyl)dimethylsilane (4a): A stirred solution of 3-((tert-butyldimethylsilyl)oxy)propyl 2-bromoacetate (709 mg, 2.28 mmol) in chloroform (2.2 mL) under N_2 atmosphere was treated with indium(III) bromide (40.4 mg, 0.11 mmol) in one portion and triethylsilane (1.46 mL, 1.06 g, 9.12 mmol, d=0.728) was then added dropwise (addition time < 1 min) and the resulting heterogeneous mixture was allowed to heat at 60 °C (oil bath temperature) over 5 h in a flask equipped with condenser. Immediately upon heating mixture turned creamy and yellow. After this time the mixture was allowed to cool to ambient temperature and it was then diluted with H_2O (12 mL) and CH_2Cl_2 (20 mL) and the two layers were well shaken and separated. The aqueous phase was further extracted with CH_2Cl_2 (2x20 mL). The combined organic extracts were washed with brine (1x30 mL), dried (Na_2SO_4) and concentrated *in vacuo* to give crude mixture as brown oily residue (2.49 g). The crude product was purified by chromatography on a silica gel column (eluting with EtOAc:pentane 1:18) to afford the title compound (287 mg, 0.97 mmol, 42%) as a mixture with **(3-(2-bromoethoxy)propoxy)triethylsilane (4b)** in a 5:1 NMR ratio, respectively: IR (neat) 2954, 2928, 2857, 1471, 1255, 1098, 1006, 836, 776, 745, 666 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 3.74 (t, *J* = 6.3 Hz, 4H, overlapped), 3.71 (t, *J* = 6.2 Hz, 4H, overlapped), 3.59 (t, *J* = 6.2 Hz, 2H, **4b**), 3.58 (t, *J* = 6.3 Hz, 2H, **4a**), 3.46 (t, *J* = 6.3 Hz, 4H, overlapped), 1.81 (quint, *J* = 6.2 Hz, 4H, **4b**), 1.79 (quint, *J* = 6.2 Hz, 4H, **4a**), 0.96 (t, *J* = 8.0 Hz, 9H, **4b**), 0.89 (s, 9H, **4a**), 0.60 (q, *J* = 8.0 Hz, 6H, **4b**), 0.05 (s, 6H, **4a**) ppm; MS (EI+) *m/z* 239 $(M - C_4H_9)^+$ for **4a** and 267 $(M - C_2H_5)^+$ for **4b**. This mixture was used for the next step.

(*E*)-3-(Pyridin-2-ylethynyl)cyclohex-2-enone *O*-(2-(3-((*tert*-butyldimethylsilyl)oxy)propoxy)ethyl) oxime (6a) and **(*E*)-3-(pyridin-2-ylethynyl)cyclohex-2-enone *O*-(2-(3-(triethylsilyl)oxy)propoxy)ethyl) oxime (6b)**: A flame dried flask was charged with (*E*)-3-(pyridin-2-ylethynyl)cyclohex-2-enone oxime (185 mg, 0.87 mmol) and anhydrous *N*,*N*`-dimethylformamide (10 mL) was added and clear pale yellow mixture was treated with sodiumhydride (50 mg of 60% suspension in oil, 1.25 mmol) and the resulting bright yellow heterogeneous mixture was stirred at ambient temperature under N_2 atmosphere for 36 min during which time the mixture turned orange. After this time a mixture of (3-(2-bromoethoxy)propoxy)(*tert*-butyl)dimethylsilane and (3-(2-bromoethoxy)propoxy)triethylsilane (5:1 ratio, respectively, 287 mg, 0.96 mmol) in DMF (7.5 mL) was added dropwise over 8 min during which time the mixture turned brown and it was allowed to stir further for 70 min. After this time the crude mixture was quenched with saturated aq. $NaHCO_3$ (10 mL) and it was diluted with H_2O (40 mL) and Et_2O (80 mL) and the two layers were well shaken and separated. The aqueous phase was extracted with Et_2O (2x80 mL). The combined organic extracts were washed with H_2O (3x40 mL), brine (1x40 mL),

dried (Na_2SO_4) and concentrated *in vacuo* to give the crude mixture as a brown oily residue (293 mg). The crude product was used for the next step without purification.

(*E*)-3-(Pyridin-2-ylethynyl)cyclohex-2-enone *O*-(2-(3-hydroxypropoxy)ethyl) oxime (7): At ambient temperature under N_2 atmosphere round bottom flask was charged with the crude mixture of (*E*)-3-(pyridin-2-ylethynyl)cyclohex-2-enone O-(2-(3-((tert-butyldimethylsilyl)oxy)propoxy)ethyl) oxime and (*E*)-3-(pyridin-2-ylethynyl)cyclohex-2-enone O-(2-(3-((triethylsilyl)oxy)propoxy)ethyl) oxime (293 mg, 0.68 mmol) and anhydrous tetrahydrofuran (12.3 mL) was added and the resulting clear orange mixture was further treated with tetrabutylammoniumfluoride solution in THF (1.4 mL, 1.36 mmol, c=1M) dropwise over 5 min and the resulting brown mixture was allowed to stir at ambient temperature under N_2 for 70 min. After this time the mixture was partitioned between H_2O (30 mL) and EtOAc (40 mL) and the two layers were well shaken and separated. The aqueous phase was extracted with EtOAc (2x40 mL, slow separation of phases). The combined organic extracts were washed with H_2O (3x30 mL), brine (1x40 mL), dried (Na_2SO_4) and concentrated *in vacuo* to give crude mixture as brown oil. The crude product was purified by chromatography on a silica gel column (eluting with gradient EtOAc:pentane 9:1 to 100% EtOAc) to give the title compound (131 mg, 0.42 mmol) as a pale yellow oil: IR (neat) 3412, 2936, 2869, 2196, 1581, 1463, 1428, 1358, 1248, 1122, 1059, 978, 958, 861, 779 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.60 (ddd, *J* = 4.9, 1.7, 0.9 Hz, 1H), 7.66 (td, *J* = 7.8, 1.8 Hz, 1H), 7.45 (dt, *J* = 7.8, 1.0 Hz, 1H), 7.23 (ddd, *J* = 7.6, 4.9, 1.2 Hz, 1H), 6.58 (t, *J* = 1.6 Hz, 1H), 4.26 (bdm, *J* = 4.6 Hz, 2H), 3.78 (t, *J* = 5.5 Hz, 2H), 3.71 (bdm, *J* = 4.8 Hz, 2H), 3.72 (bdm, *J* = 3.5 Hz, 2H), 3.69 (dd, *J* = 5.7 Hz, 2H), 2.57 (dd, *J* = 6.4 Hz, 2H), 2.40 (td, *J* = 6.1, 1.6 Hz, 2H), 1.84 (quint, *J* = 5.6 Hz, 2H), 1.80 (quint, *J* = 6.3 Hz, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.0 (0), 150.2 (1), 143.4 (0), 136.4 (1), 131.2 (1), 127.6 (0), 127.5 (1), 123.0 (1), 92.0 (0), 90.2 (0), 73.5 (2), 70.9 (2), 69.8 (2), 62.4 (2), 32.0 (2), 29.6 (2), 22.4 (2), 21.0 (2) ppm; MS (ES+) *m/z* 315 $(M + H)^+$; HRMS (ESI) *m/z* 315.1710 (calcd. for $C_{18}H_{23}N_2O_3$: 315.1703).

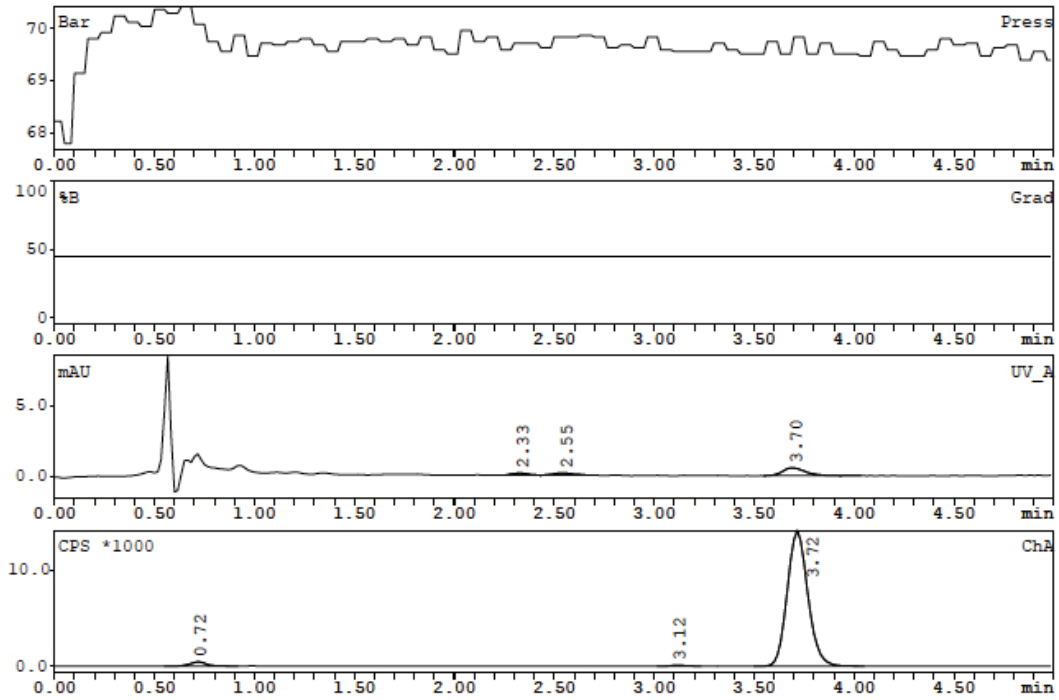
(*E*)-3-(2-(((3-(Pyridin-2-ylethynyl)cyclohex-2-en-1-ylidene)amino)oxy)ethoxy)propyl methanesulfonate (8): A stirred solution of (*E*)-3-(pyridin-2-ylethynyl)cyclohex-2-enone O-(2-(3-hydroxypropoxy)ethyl) oxime (131 mg, 0.42 mmol) in anhydrous tetrahydrofuran (4.2 mL) was treated with triethylamine (39 µL, 57.0 mg, 0.50 mmol, d=1.477) in one portion and methanesulfonylchloride (117 µL, 85.0 mg, 0.84 mmol, d=0.726) was then added dropwise (addition time < 1 min) and the resulting pale yellow mixture was allowed to stir at ambient temperature under N_2 atmosphere for 22 min during which time white precipitate formed. After this time the mixture was partitioned between H_2O (16 mL) and EtOAc (20 mL) and the two layers were well shaken and separated. The aqueous phase was extracted with EtOAc (2x20 mL). The combined organic extracts were washed with H_2O (3x16 mL), brine (1x16 mL), dried (Na_2SO_4) and concentrated *in vacuo* to give crude mixture as pale yellow oil. The crude product was purified by chromatography on a silica gel column (eluting with gradient EtOAc:pentane 4:1 to 100% EtOAc) to afford the title compound (138 mg, 0.35 mmol, 85%) as a pale yellow oil: IR (neat) 2933, 2870, 2197, 2097, 1580, 1462, 1428, 1352, 1173, 1124, 1061, 887, 863, 842, 779, 629 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.59 (bdm, *J* = 4.4 Hz, 1H), 7.65 (td, *J* = 7.8, 1.8 Hz, 1H), 7.44 (bdm, *J* = 7.8 Hz, 1H), 7.22 (ddd, *J* = 7.6, 4.9, 1.1 Hz, 1H), 6.56 (t, *J* = 1.5 Hz, 1H),

4.34 (t, *J* = 6.2 Hz, 2H), 4.24 (bdm, *J* = 4.7 Hz, 2H), 3.70 (bdm, *J* = 4.8 Hz, 2H), 3.59 (t, *J* = 5.9 Hz, 2H), 3.00 (s, 3H), 2.57 (dm, *J* = 6.5 Hz, 2H), 2.40 (td, *J* = 6.2, 1.4 Hz, 2H), 2.01 (quint, *J* = 6.0 Hz, 2H), 1.80 (quint, *J* = 6.4 Hz, 2H) ppm; ^{13}C NMR (100MHz, $CDCl_3$) δ 155.9 (0), 150.3 (1), 143.4 (0), 136.3 (1), 131.1 (1), 127.6 (0), 127.4 (1), 123.0 (1), 92.1 (0), 90.0 (0), 73.7 (2), 69.6 (2), 67.5 (2), 66.6 (2), 37.3 (3), 29.6 (2), 29.5 (2), 22.5 (2), 20.9 (2) ppm; MS (ES+) *m/z* 393 $(M + H)^+$; HRMS (ESI) *m/z* 393.1489 (calcd. for $C_{19}H_{25}N_2O_5S$: 393.1479).

(*E*)-3-(Pyridin-2-ylethynyl)cyclohex-2-enone *O*-(2-(3-fluoropropoxy)ethyl) oxime (PSS223): A flame dried round bottom flask was charged with Kryptofix-222® (150 mg, 0.40 mmol) and potassium fluoride (23.0 mg, 0.40 mmol) and at ambient temperature under N_2 atmosphere anhydrous acetonitrile (3.2 mL) was added. Resulting colourless solution was further treated with a solution of (*E*)-3-(2-(((3-(pyridin-2-ylethynyl)cyclohex-2-en-1-ylidene)amino)oxy)ethoxy)propyl methanesulfonate (78.0 mg, 0.20 mmol) in anhydrous acetonitrile (3.2 mL) dropwise over 2 min during which time the mixture turned pale orange and the mixture was allowed to heat at 80 °C (oil bath temperature) for 40 min. The mixture was then allowed to cool to ambient temperature and then diluted with H_2O (15 mL) and EtOAc (25 mL) and the two layers were well shaken and separated. The aqueous phase was extracted with EtOAc (2x25 mL, slow separation of phases). The combined organic extracts were washed with H_2O (3x15 mL), brine (1x15 mL), dried (Na_2SO_4) and concentrated *in vacuo* to give the title compound (44.1 mg, 0.14 mmol, 70%) as a colourless oil: IR (neat): 3050, 2925, 2869, 2205, 1580, 1462, 1358, 1124, 1060, 978, 864, 778, 741 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (ddd, *J* = 4.9, 1.8, 0.9 Hz, 1H), 7.65 (td, *J* = 7.8, 1.8 Hz, 1H), 7.44 (dt, *J* = 7.8, 1.0 Hz, 1H), 7.22 (ddd, *J* = 7.6, 5.0, 1.2 Hz, 1H), 6.57 (t, *J* = 1.6 Hz, 1H), 4.55 (dt, *J* = 47, 5.9 Hz, 2H), 4.25 (dm, *J* = 4.8 Hz, 2H), 3.71 (dm, *J* = 4.9 Hz, 2H), 3.61 (t, *J* = 6.2 Hz, 2H), 2.58 (ddm, *J* = 6.4 Hz, 2H), 2.40 (td, *J* = 6.0, 1.5 Hz, 2H), 1.96 (dquint, *J* = 26, 6.0 Hz, 2H), 1.80 (quint, *J* = 6.4 Hz, 2H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.0 (0), 150.3 (1), 143.6 (0), 136.3 (1), 131.2 (1), 127.45 (0), 127.42 (1), 123.0 (1), 92.4 (0), 90.1 (0), 81.5 (d, *J* = 141 Hz, 2), 73.8 (2), 69.6 (0), 67.0 (d, *J* = 5.5 Hz, 2), 31.0 (d, *J* = 20 Hz, 2), 29.6 (2), 22.5 (2), 21.0 (2) ppm; ^{19}F NMR (376 MHz, $CDCl_3$) δ −221.8 (ddt, *J* = 46, 25 Hz) ppm; MS (ES+) *m/z* 317 $(M + H)^+$; HRMS (ESI) *m/z* 317.1663 (calcd. for $C_{18}H_{22}FN_2O_2$: 317.1660).

HPLC chromatograph of [^{18}F]-PSS223: Quality control

Measurement SDM-RS-40A ETH Zuerich Page 1/1
D:\GINA_NT\MGLUR5\ISO45_ACN IN 5MIN\SDM-RS-40A Print date: 05.09.2011



Sample description

Study:	MGLUR5		
Measurement:	SDM-RS-40A, injection :	20.06.2011 10:38	
Method:	ISO45_ACN IN 5MIN from:	01.04.2011 05:30: Position:	1
Station number:	2		
Inj. volume (µl):	5.0		
Radio detector: raytest Gabi Star	Serial Nr.: #30168 raytest GINA star 20.04.09 V4.8		

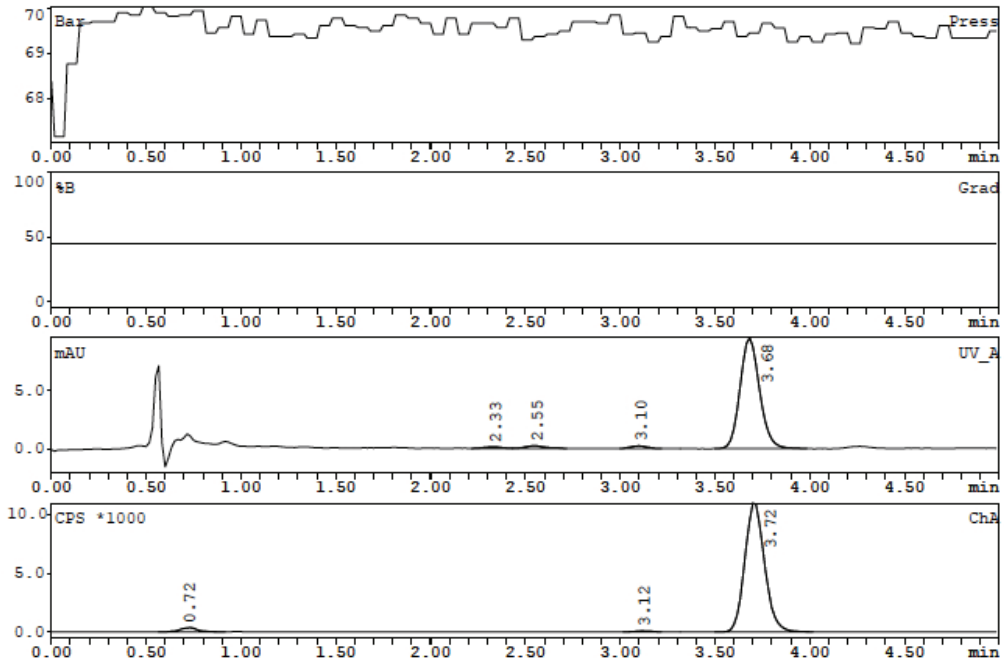
Integration ChA

Substance	R/T min	Type	Area Counts	%Area %
Reg #1	0.72	BB(	2661.8	2.55
Reg #2	3.12	BB(	496.8	0.48
Reg #3	3.72	BB(	101096.1	96.97
Sum in ROI			104254.7	
Area			106831.4	
Ext. BKG			0.00 CPS	

Integration UV_A

Substance	R/T min	Type	Area mAU*min	%Area %
Reg #1	2.33	BD(	0.785731	12.57
Reg #2	2.55	DB(	1.003935	16.06
Reg #3	3.70	BB(	4.462353	71.37
Sum in ROI			6.252019	

HPLC chromatograph of [^{18}F]-PSS223: Coinjection with cold reference



Sample description

Study:	MGLUR5				
Measurement:	SDM-RS-40CONIJECTION, injection :	20.06.2011	10:49		
Method:	ISO45_ACN IN 5MIN from:	01.04.2011	05:30:	Position:	1
Station number:	2				
Inj. volume (µl):	5.0				
COINJECTION WITH COLD REFERENCE					
Radio detector: raytest Gabi Star	Serial Nr.: #30168 raytest GINA star 20.04.09 V4.8				

Integration ChA

Substance	R/T min	Type	Area Counts	%Area %
Reg #1	0.72	BB(	2254.93	2.73
Reg #2	3.12	BB(	410.08	0.50
Reg #3	3.72	BB(	79818.78	96.77
Sum in ROI			82483.79	
Area			85725.75	
Ext. BKG			0.00 CPS	

Integration UV_A

Substance	R/T min	Type	Area mAU*min	%Area %
Reg #1	2.33	BD(	0.82671	1.15
Reg #2	2.55	DB(	1.66486	2.33
Reg #3	3.10	BB(	1.28342	1.79
Reg #4	3.68	BB(	67.80675	94.73
Sum in ROI			71.58175	