^{18}F labelling of electron rich iodonium ylides: Application to the radiosynthesis of potential 5-HT$_{2A}$ receptor PET ligands.

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PDSP screening of 2

Target	Ki (nM)
5-HT$_{1A}$	644
5-HT$_{2A}$	1.3
5-HT$_{2B}$	3
5-HT$_{2C}$	5.4
5-HT$_{5A}$	3425
5-HT$_{6}$	36
Alpha 1A	1392.5
Alpha 2A	174
Alpha 2B	411
Alpha 2C	33
D2	2530
D3	491
D4	208
DAT	1858
H1	27

Binding affinities

Compound	K_i (nM)		
	$5\text{–}HT_{2A}$	$5\text{–}HT_{2C}$	K_i^{2C}/K_i^{2A}
1	0.21	3.1	15
2	0.95	9.9	10
3	7.2	240	33
4	1.7	83	49

Binding affinities of 1 and analogs in a [^{3}H]Cimbi-36 competition binding assay using membranes from tsA201 cells transiently expressing human 5-HT_{2A} and 5-HT_{2C} receptors. The K_i values for the compounds are given in nM.

Functional data for tested compounds

Compound	pEC_{50}		selectivity	% R_{max}	
	$5\text{–}HT_{2A}$	$5\text{–}HT_{2C}$		$5\text{–}HT_{2A}$	$5\text{–}HT_{2C}$
2	8.55±0.13	7.87±0.06	4.6	63±6	85±2
3	8.45±0.14	7.66±0.08	6	67±5	80±3
4	7.91±0.08	6.52±0.08	25	46±2	48±2
5-HT	8.35±0.02	8.81±0.05	3	100	100

Stable h5-HT_{2A}- and h5-HT_{2C}-HEK293 cell lines were used in the Ca^{2+}/Fluo-4/assay. The EC_{50} values (given in nM with $pEC_{50} \pm$ S.E.M. values) and $R_{max} \pm$ S.E.M. values (given in % of the maximal response evoked by 5-HT at the receptor) are based on 3 independent experiments.

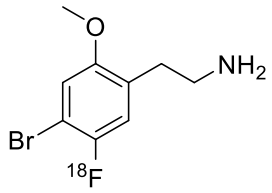
Analytical identification

Analytical method A: LUNA (Phenomenex), 5u, C18(2) 100a, 150X4.6 mm. 33 % ACN in 0,1 % H_3PO_4 1,5 ml/min

Analytical method B: Kintex (Phenomenex), 2.6, C18, 100A, 50x4.6 mm. 33 % ACN in 0,1 % H_3PO_4 1,5 ml/min

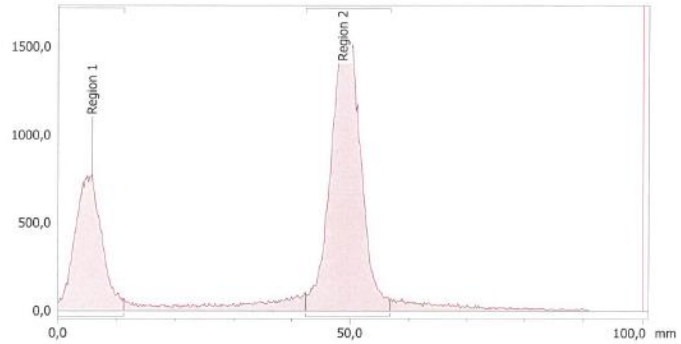
Analytical method C: LUNA (Phenomenex), 5u, C18(2) 100a, 150X4.6 mm. 20 % ACN in 25mM citric acid 1,5 ml/min

[^{18}F]8



Labeled using general procedure A
RCC TLC (EtOAc) of the Boc protected: 60 %, 79 %, 63 %, 85 %, 69 %, 87 %, 80 %, 70 %

Chromatogram: F-18

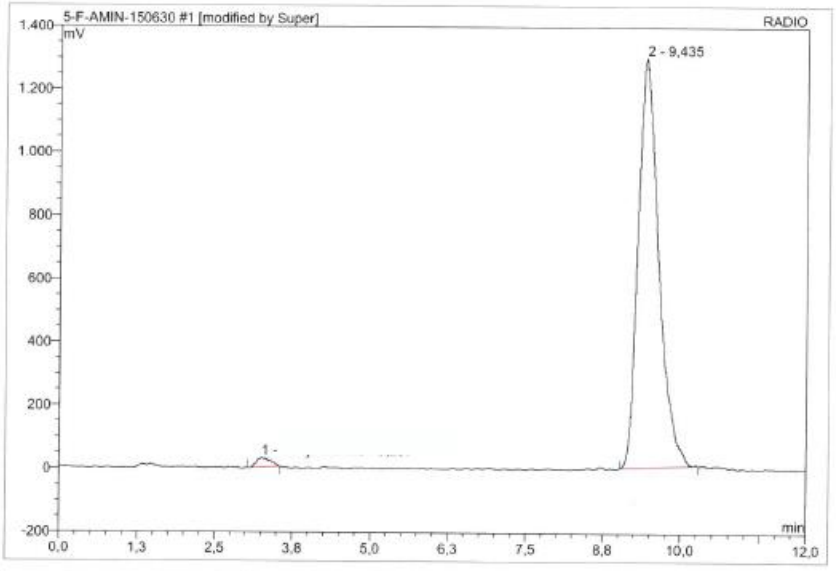


Regions: F-18

Name	Start (mm)	End (mm)	Retention (RF)	Area (Counts)	%ROI (%)	%Total (%)
Region 1	0,0	11,2	0,058	20421,0	30,00	25,96
Region 2	42,4	56,8	0,488	47658,0	70,00	60,58
2 Peaks				68079,0	100,00	86,54

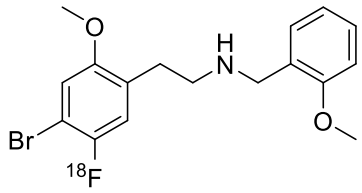
Total Area: 78669,0 Counts

Analytical method C: 9 min 24 sec



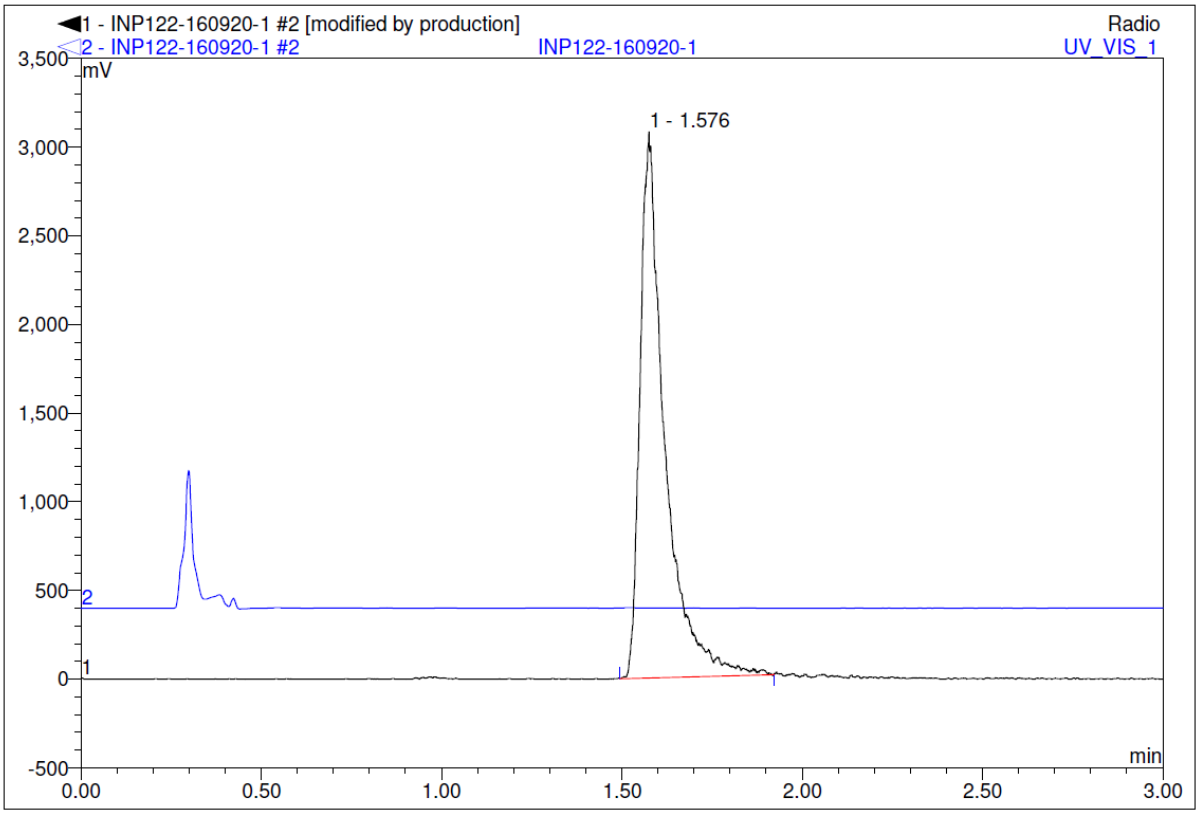
No.	Ret.Time min	Peak Name	Area mV*min	Rel.Area %	Amount	Type
1	3,26		7,610	1,51	n.a.	BMB*
2	9,44	n.a.	496,360	98,49	n.a.	BMB*
Total:			1325,077		0,000	

[^{18}F]2



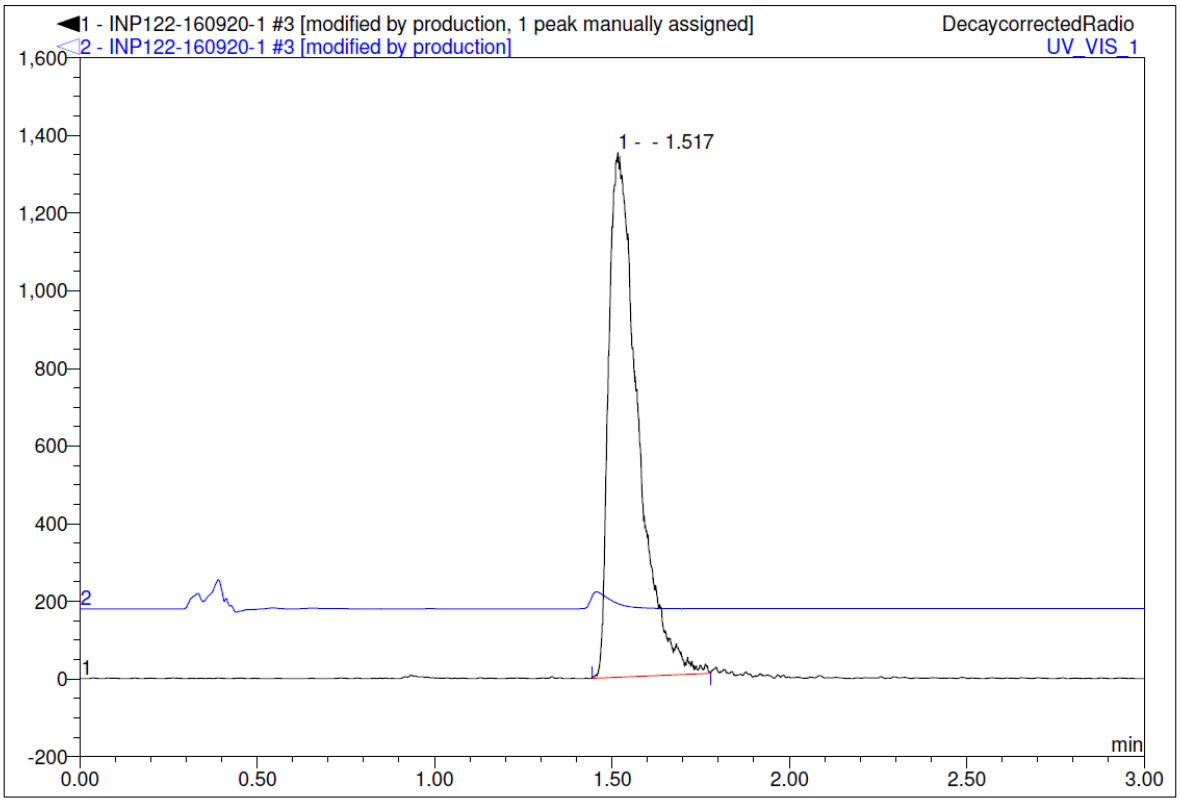
Via general procedure B via [^{18}F]4
Preparative: retention time 18 min 20 sec
Analytical A: 6 min 42 sec (not shown)
Analytical B: 2 min 33 sec
SA: 19 GBq/umol

Sample Name:	**INP122-160920-1**	*Injection Volume:*	**100.0**
Vial Number:	**BA2**	*Channel:*	**Radio**
Sample Type:	**unknown**	*Wavelength:*	**n.a.**
Control Program:	**Cimbi36**	*Bandwidth:*	**n.a.**
Quantif. Method:	**CIMBI**	*Dilution Factor:*	**1.0000**
Recording Time:	**20/9/2016 14:02**	*Sample Weight:*	**1.0000**
Run Time (min):	**3.00**	*Sample Amount:*	**1.0000**



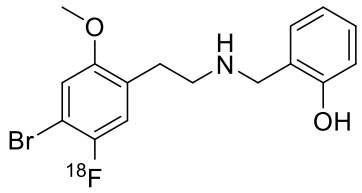
No.	Ret.Time min	Peak Name	Area mV*min	Rel.Area %	Amount mAUmin	Type
1	1.576	n.a.	243.7671	100.00	n.a.	BMB*
Total:			3079.300		0.000	

Sample Name:	**INP122-160920-1**	*Injection Volume:*	**100.0**
Vial Number:	**BA3**	*Channel:*	**DecaycorrectedRadio**
Sample Type:	**spiked**	*Wavelength:*	**n.a.**
Control Program:	**Cimbi36**	*Bandwidth:*	**n.a.**
Quantif. Method:	**CIMBI**	*Dilution Factor:*	**1.0000**
Recording Time:	**20/9/2016 14:11**	*Sample Weight:*	**1.0000**
Run Time (min):	**3.00**	*Sample Amount:*	**1.0000**



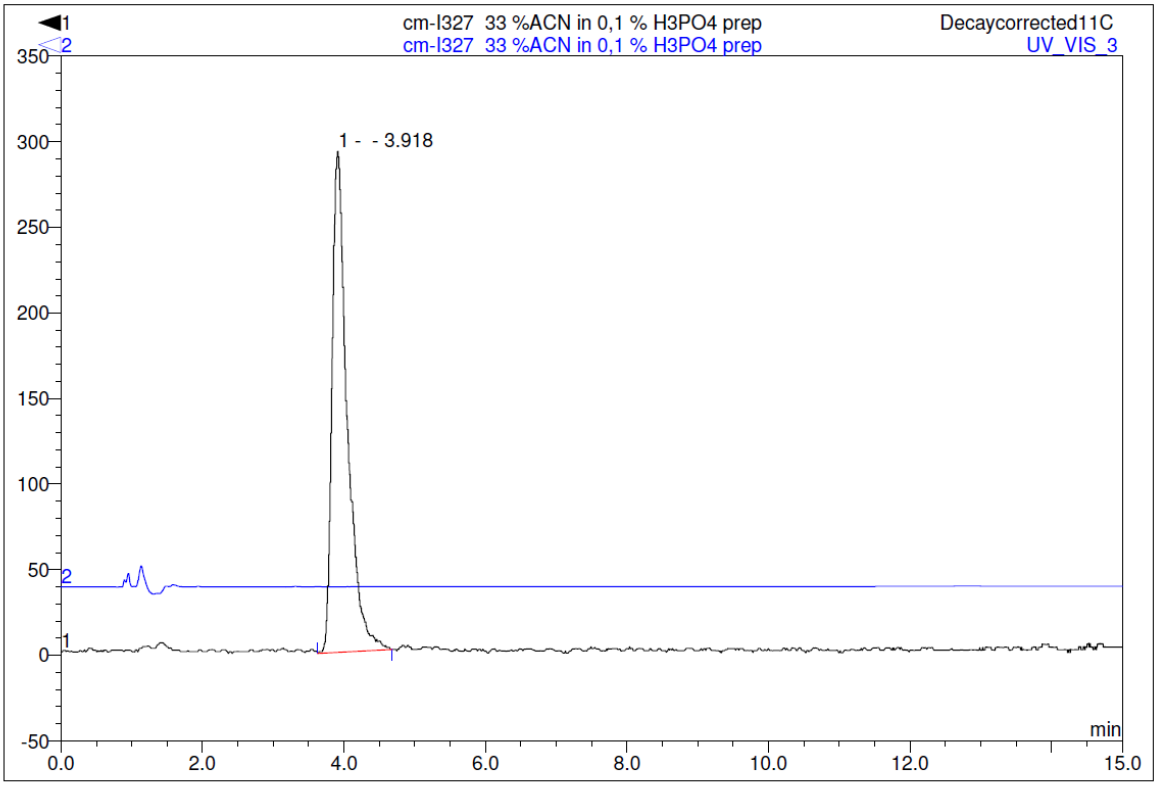
No.	Ret.Time min	Peak Name	Area *min	Rel.Area %	Amount mAUmin	Type
1	1.517		128.2688	100.00	n.a.	BMB*^
Total:			1352.213		0.000	

[^{18}F]3



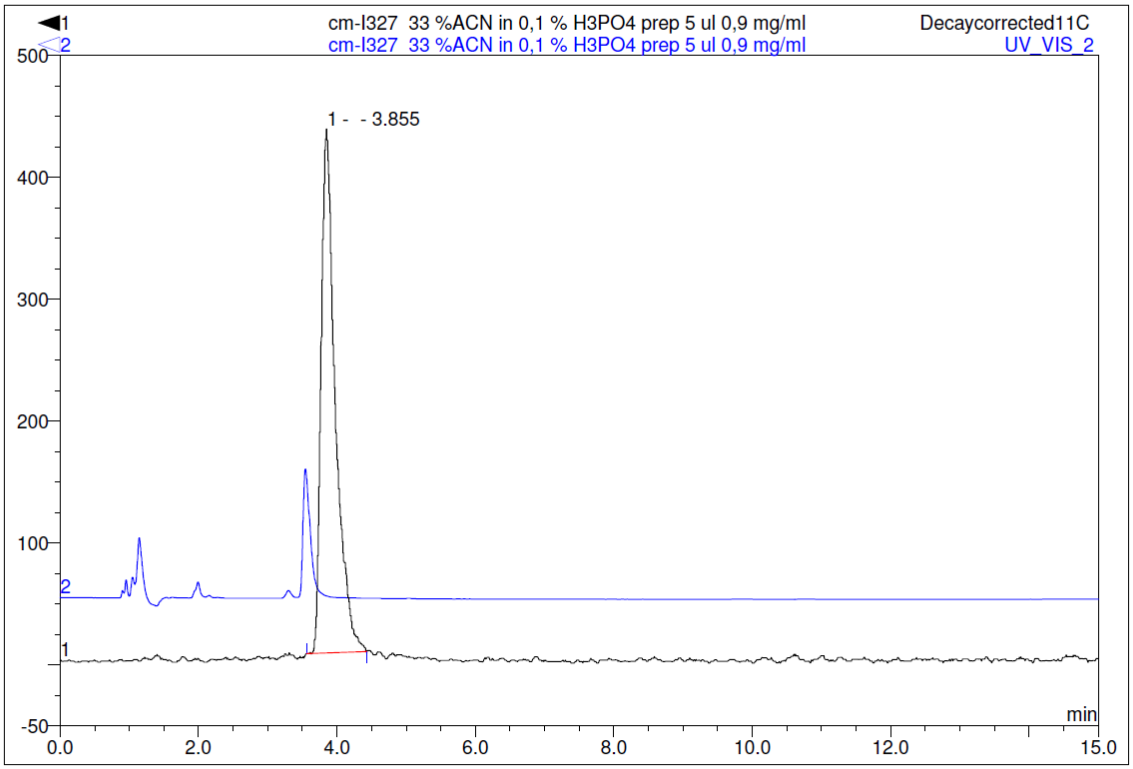
Using general procedure B via [^{18}F]4
Preparative retention time: 9 min 31 sec
Analytical: 3 min 50 sec
SA: 21 GBq/umol

Sample Name:	**cm-I327 33 %ACN in 0,1 % H3PO**	*Injection Volume:*	**50.0**
Vial Number:	**6**	*Channel:*	**Decaycorrected11C**
Sample Type:	**unknown**	*Wavelength:*	**n.a.**
Control Program:	**isocratic 100 % A, flow 1,5, 15 mi**	*Bandwidth:*	**n.a.**
Quantif. Method:	**AE105**	*Dilution Factor:*	**1.0000**
Recording Time:	**25/8/2015 13:21**	*Sample Weight:*	**1.0000**
Run Time (min):	**15.00**	*Sample Amount:*	**1.0000**



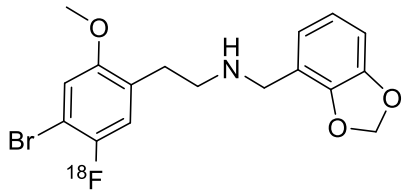
No.	Ret.Time min	Peak Name	Area *min	Rel.Area %	Amount	Type
1	3.92		72.881	100.00	n.a.	BMB*^
Total:			292.587		0.000	

Sample Name:	**cm-l327 33 %ACN in 0,1 % H3PO**	*Injection Volume:*	**50.0**
Vial Number:	**6**	*Channel:*	**Decaycorrected11C**
Sample Type:	**spiked**	*Wavelength:*	**n.a.**
Control Program:	**isocratic 100 % A, flow 1,5, 15 mi**	*Bandwidth:*	**n.a.**
Quantif. Method:	**AE105**	*Dilution Factor:*	**1.0000**
Recording Time:	**25/8/2015 14:10**	*Sample Weight:*	**1.0000**
Run Time (min):	**15.00**	*Sample Amount:*	**1.0000**



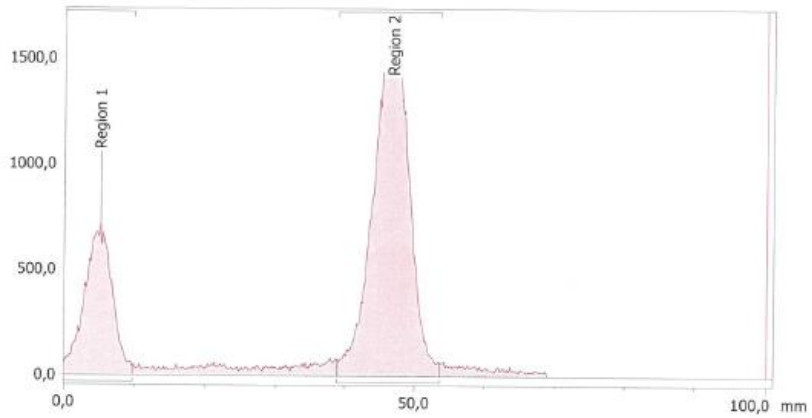
No.	Ret.Time min	Peak Name	Area *min	Rel.Area %	Amount	Type
1	3.86		102.266	100.00	n.a.	BMB*^
Total:			429.910		0.000	

[^{18}F]4



Direct labeling via general procedure A using
RCC TLC (EtOAc) of the boc protected 75 %, 52 %, 56 %, 77 %
Preparative retention time: 6 min 30 sec
Analytical: 3 min 55 sec
SA: 48 GBq/umol

Chromatogram: F-18

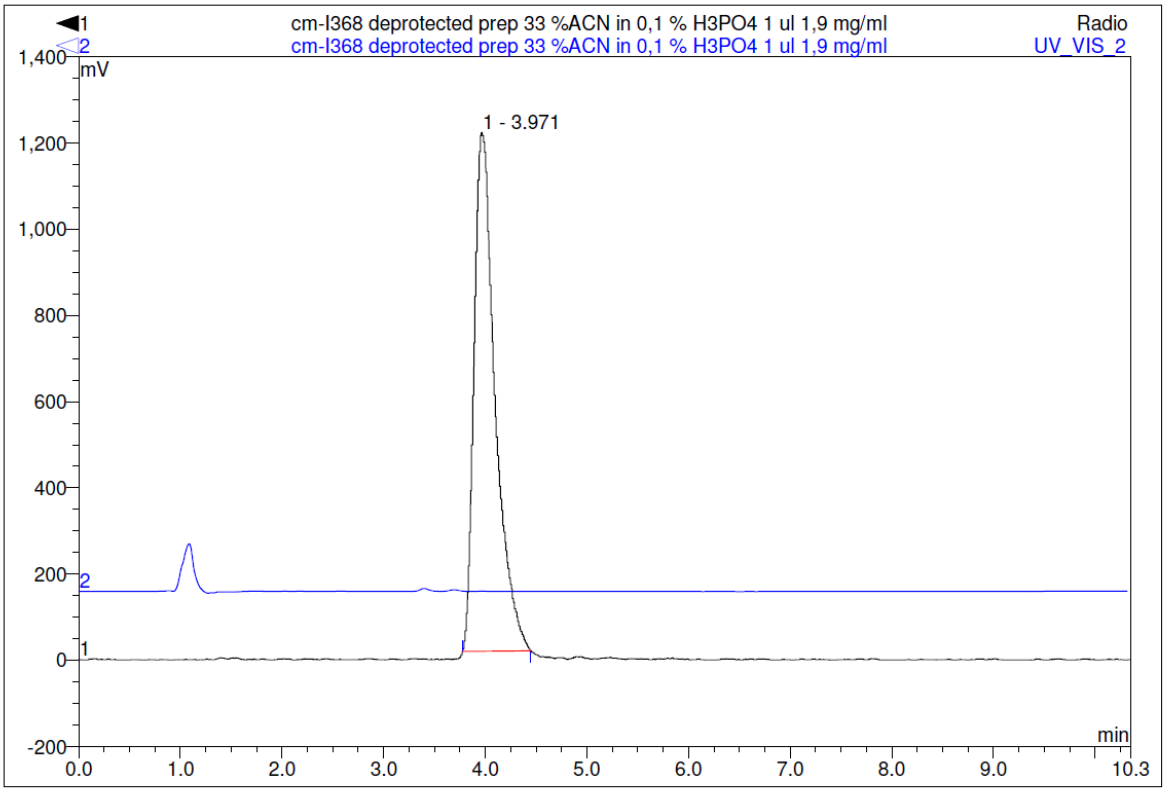


Regions: F-18

Name	Start (mm)	End (mm)	Retention (RF)	Area (Counts)	%ROI (%)	%Total (%)
Region 1	0,0	9,8	0,052	16276,0	25,01	22,09
Region 2	39,0	53,6	0,470	48792,0	74,99	66,23
2 Peaks				65068,0	100,00	88,32

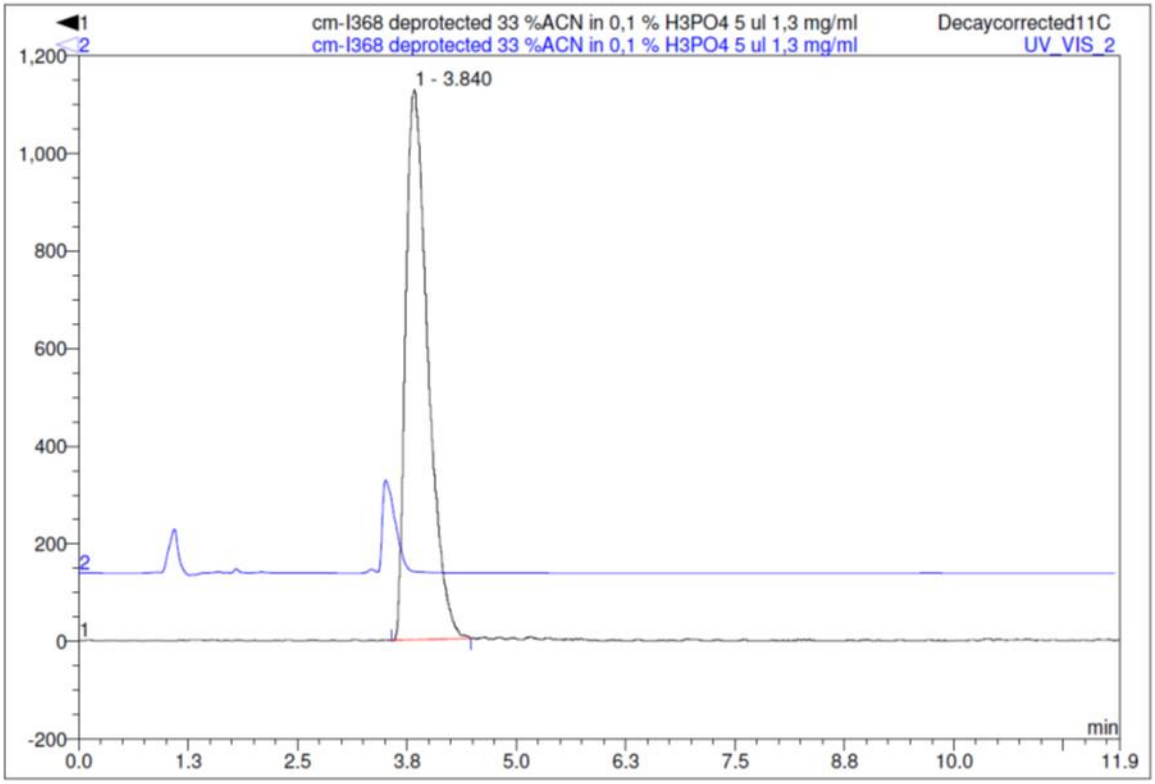
Total Area: 73673,0 Counts
Average Background: 0,0 Counts

Sample Name:	**cm-I368 deprotected prep 33 %A**	*Injection Volume:*	**50.0**
Vial Number:	**3**	*Channel:*	**Radio**
Sample Type:	**unknown**	*Wavelength:*	**n.a.**
Control Program:	**isocratic 100 % A, flow 1,5, 15 mi**	*Bandwidth:*	**n.a.**
Quantif. Method:	**AE105**	*Dilution Factor:*	**1.0000**
Recording Time:	**23/9/2015 16:08**	*Sample Weight:*	**1.0000**
Run Time (min):	**10.35**	*Sample Amount:*	**1.0000**



No.	Ret.Time min	Peak Name	Area mV*min	Rel.Area %	Amount	Type
1	3.97	n.a.	288.668	100.00	n.a.	BMB
Total:			1203.116		0.000	

Sample Name:	**cm-I368 deprotected 33 %ACN in**	*Injection Volume:*	**50.0**
Vial Number:	**3**	*Channel:*	**Decaycorrected11C**
Sample Type:	**spiked**	*Wavelength:*	**n.a.**
Control Program:	**isocratic 100 % A, flow 1,5, 15 ml**	*Bandwidth:*	**n.a.**
Quantif. Method:	**AE105**	*Dilution Factor:*	**1.0000**
Recording Time:	**23/9/2015 16:20**	*Sample Weight:*	**1.0000**
Run Time (min):	**11.89**	*Sample Amount:*	**1.0000**



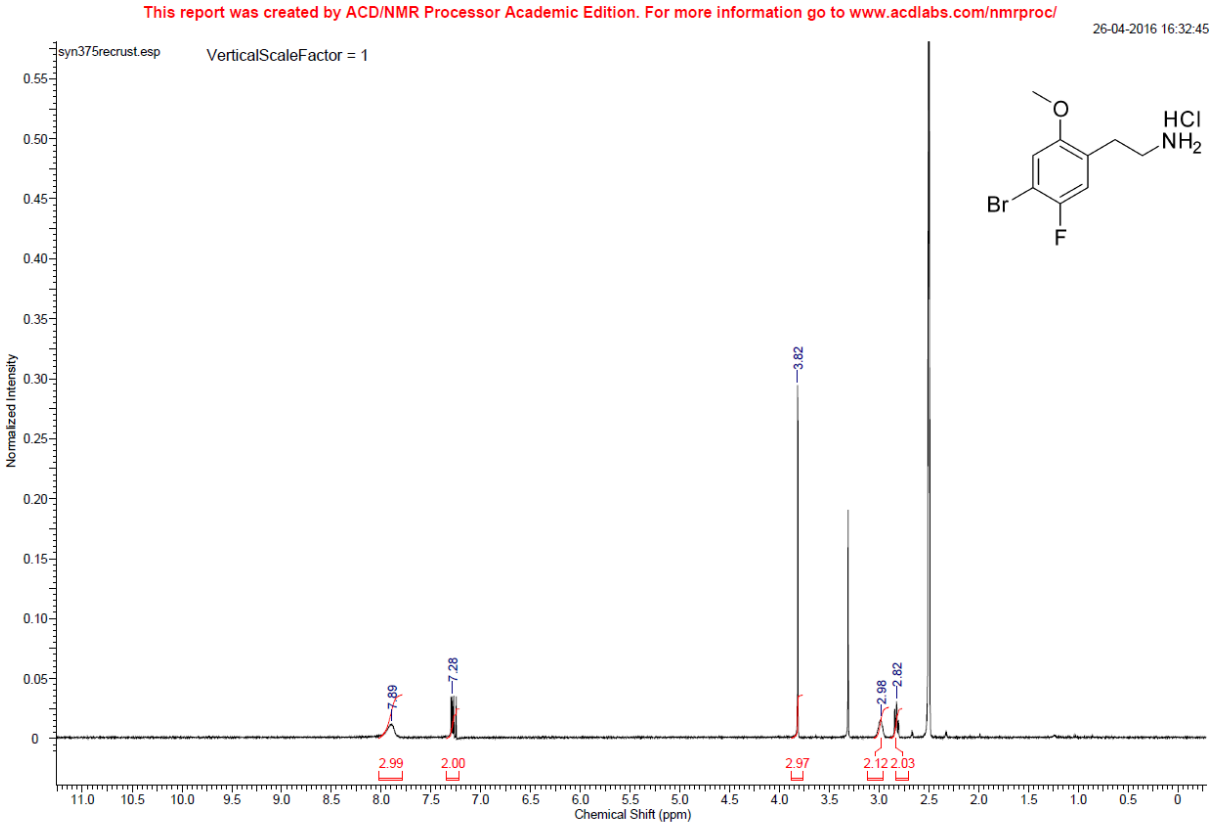
No.	Ret.Time min	Peak Name	Area *min	Rel.Area %	Amount	Type
1	3.84	n.a.	332.268	100.00	n.a.	BMB*
Total:			1127.675		0.000	

[^{11}C]2

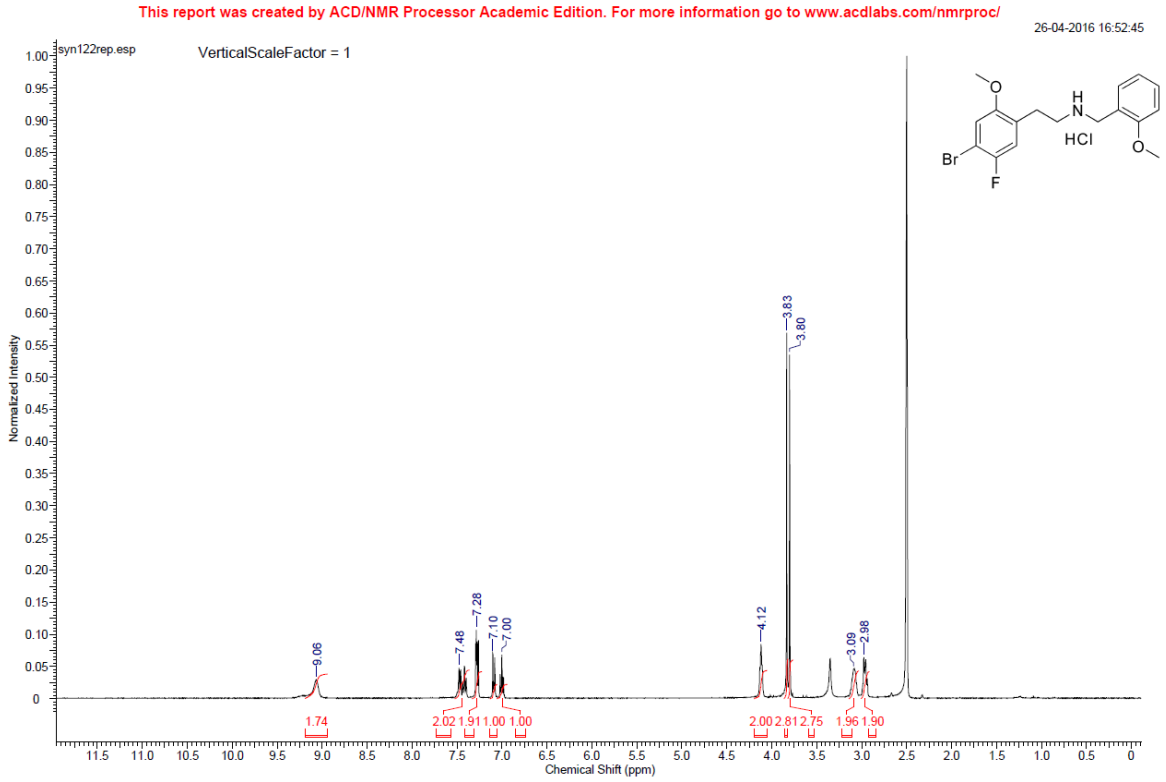
2 (0.3 mg) was dissolved in 0.3 ml acetone and added 1 M NaOH (2 ul) and heated for 50 min at 60^{0}C before reaction with [^{11}C]MeOTf for 5 min at 40^{0}C. Preparative cleaning as described. Analytical data as described for **[^{18}F]2,** typical yield for 40 min irradiation was 600 MBq, As 1100 GBq/µmol.

Copies of NMR spectra

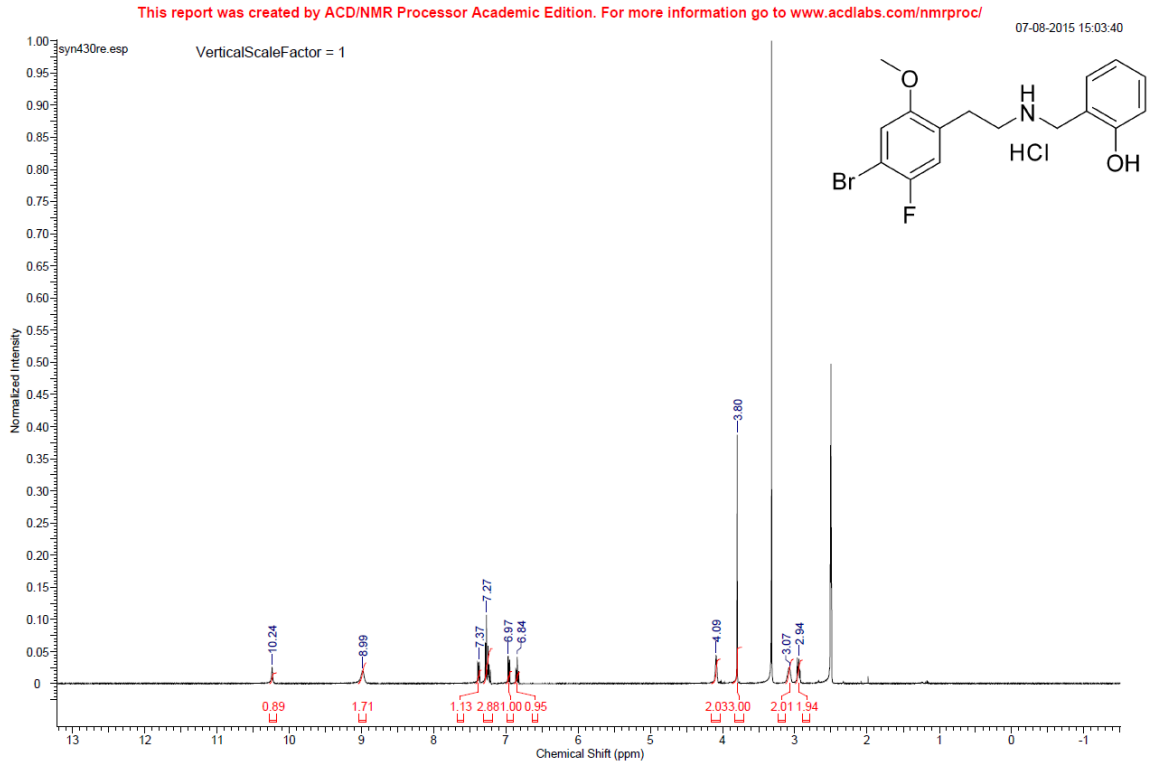
2-(4-bromo-5-(fluoro)-2-methoxyphenyl)ethan-1-amine (**9**)

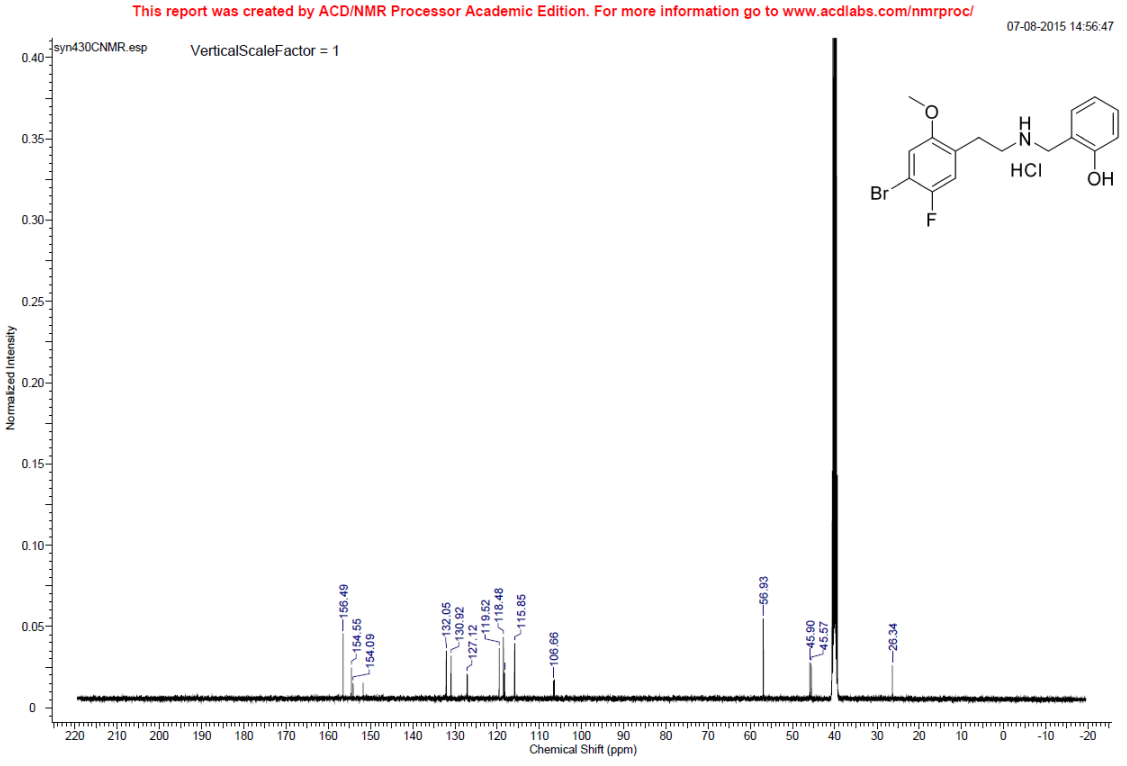


2-(4-bromo-5-(fluoro-18F)-2-methoxyphenyl)-N-(2-methoxybenzyl)ethan-1-amine (**2**)

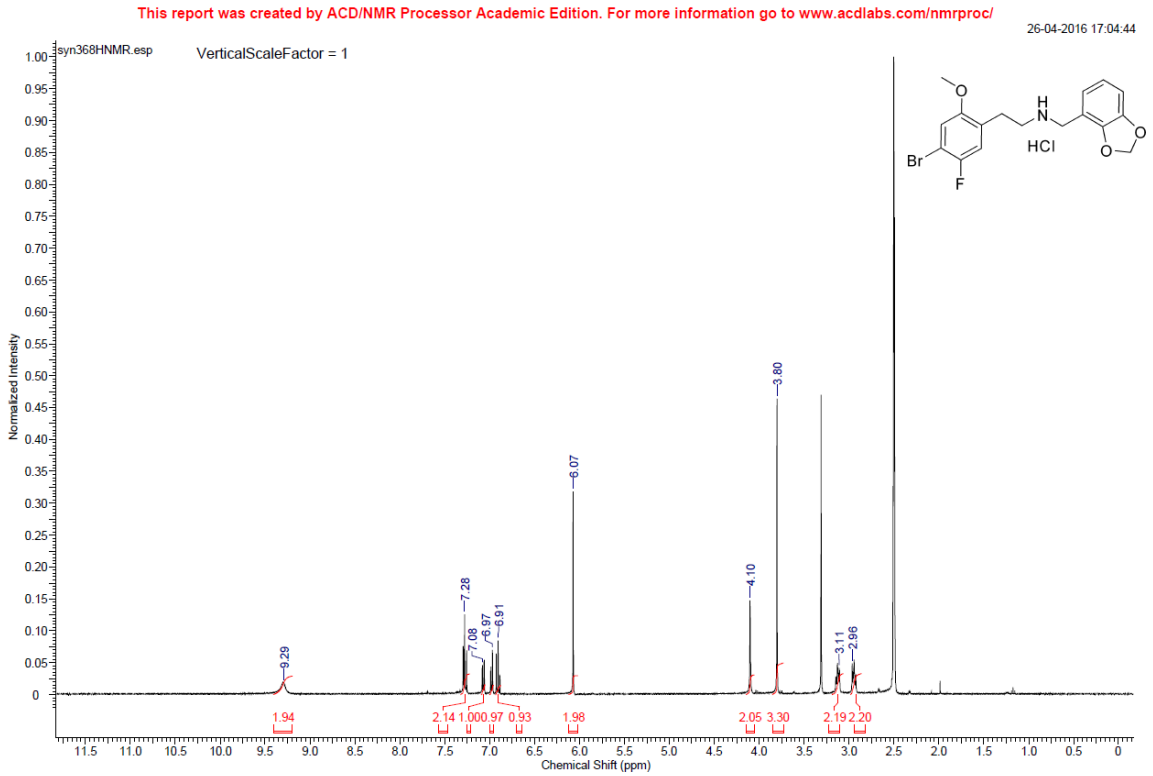


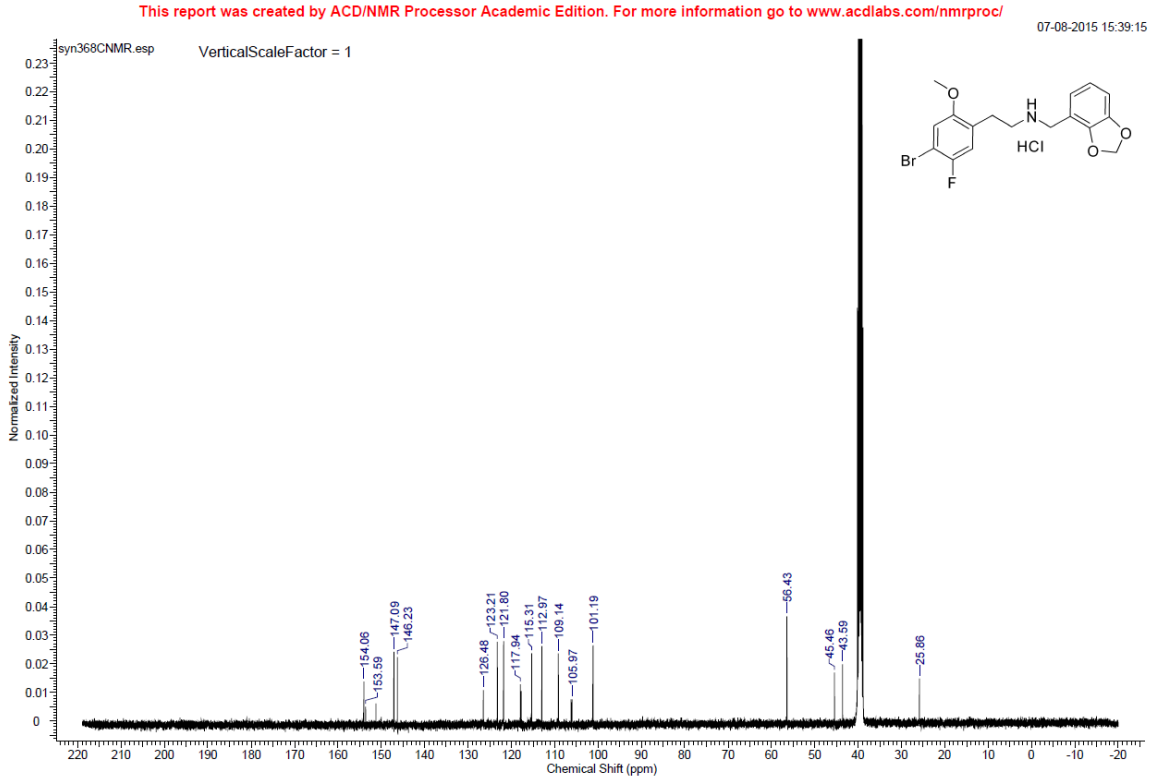
2-(((4-bromo-5-fluoro-2-methoxyphenethyl)amino)methyl)phenol hydrochloride (**3**)



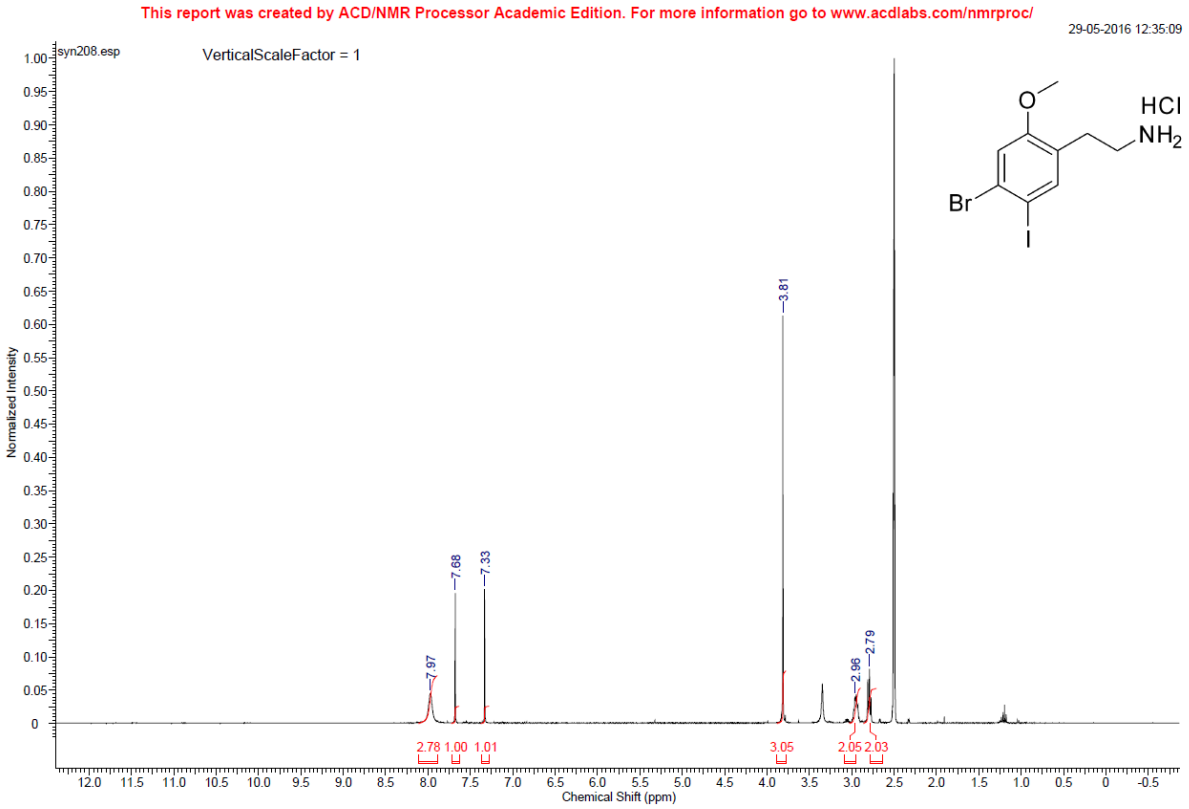


N-(benzo[d][1,3]dioxol-4-ylmethyl)-2-(4-bromo-5-fluoro-2-methoxyphenyl)ethan-1-aminehydrochloride (**4**)

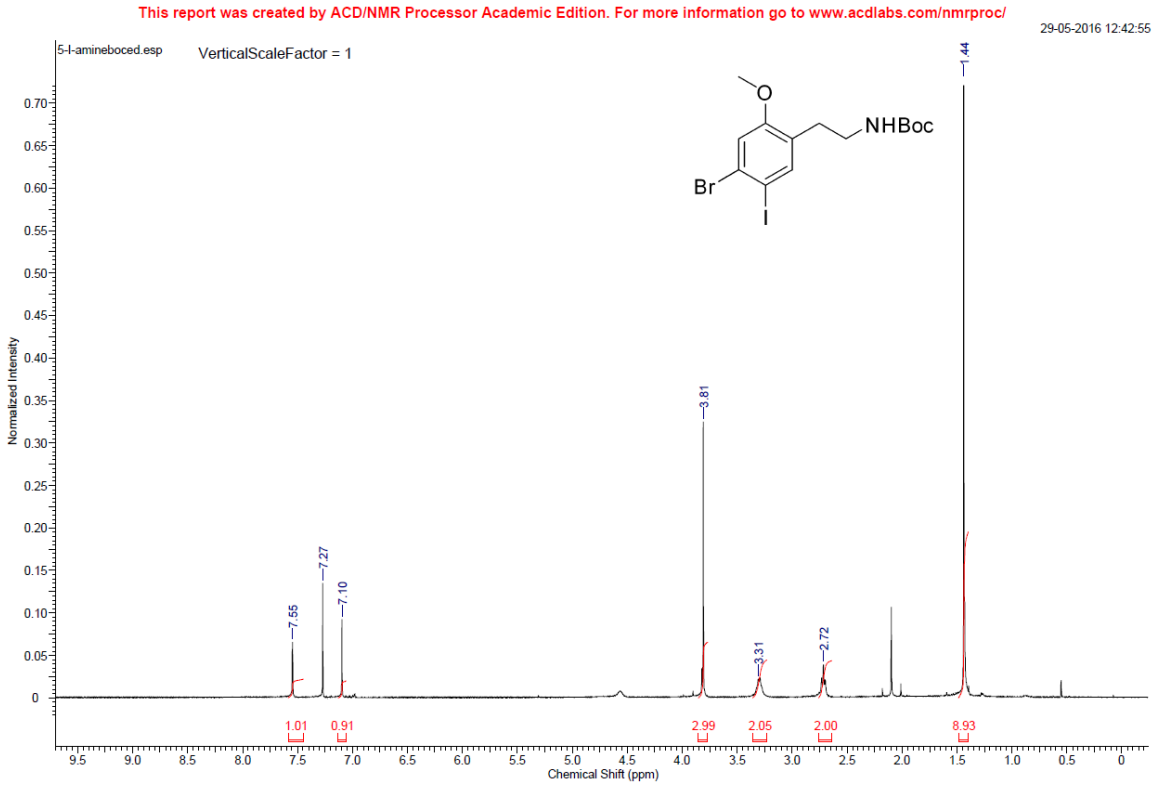


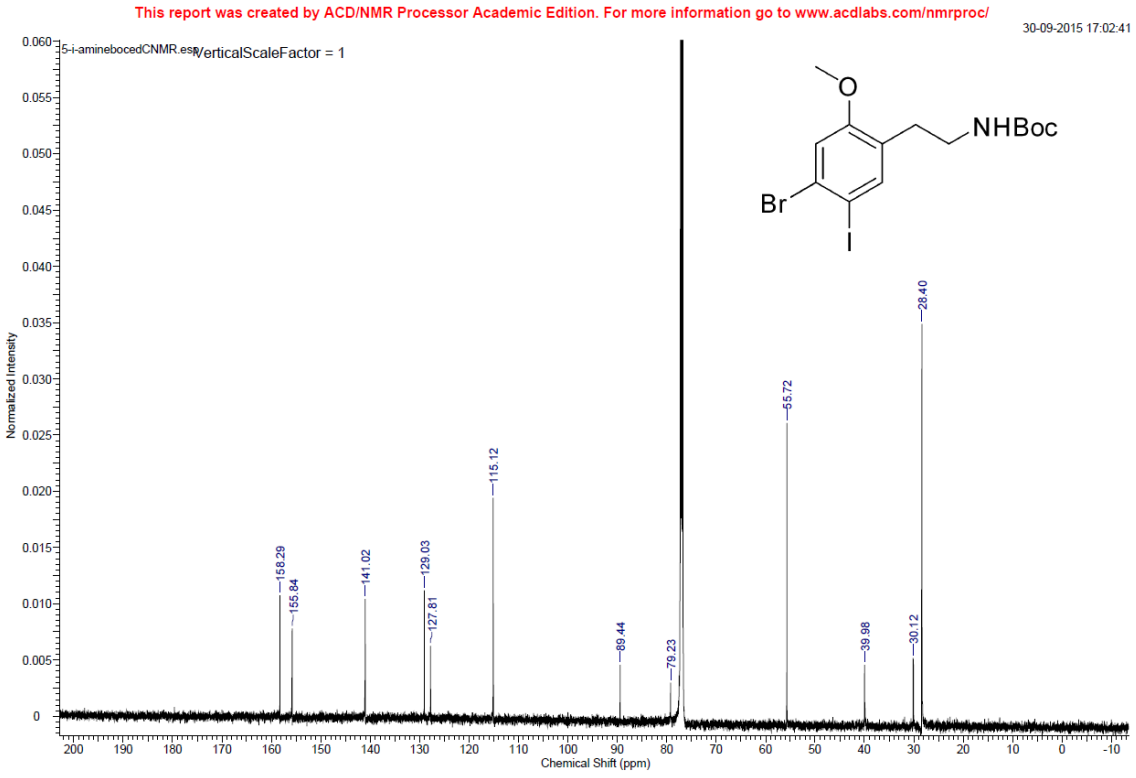


tert-butyl (4-bromo-5-iodo-2-methoxyphenethyl)carbamate (**5**)

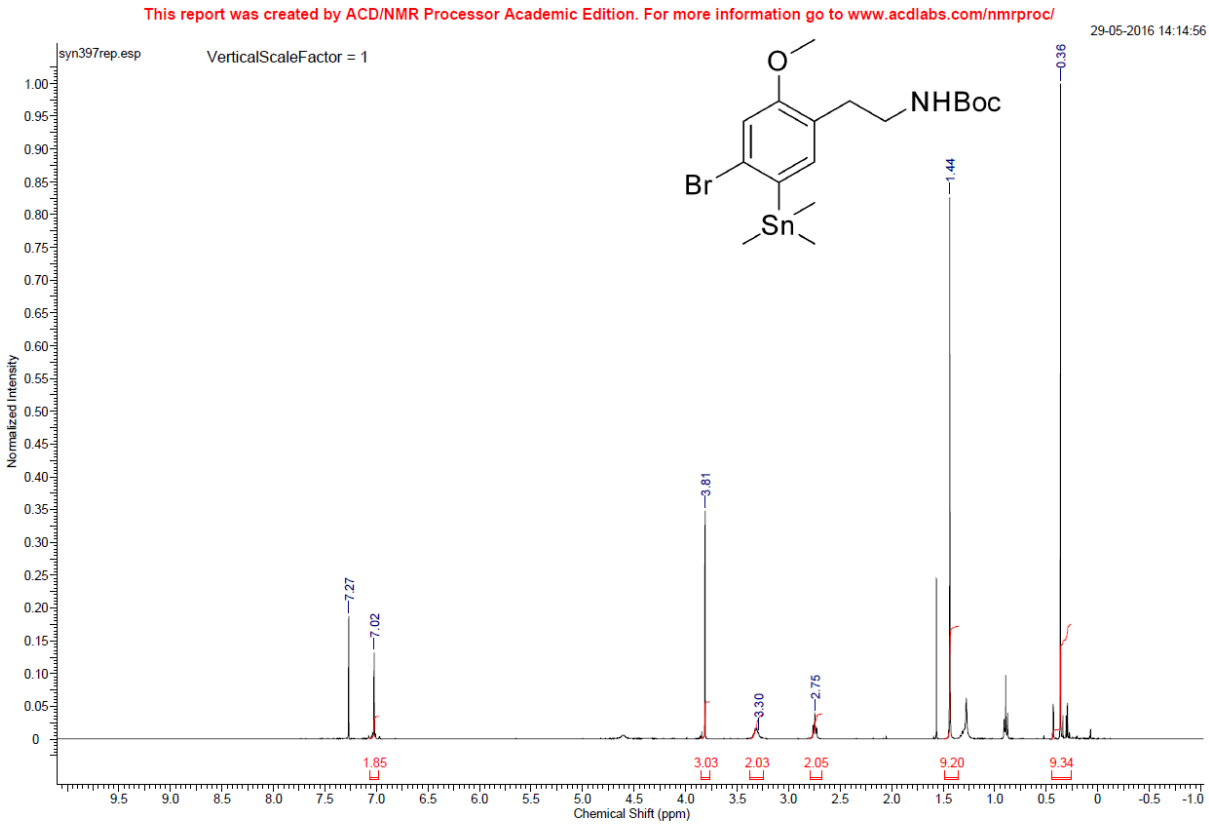


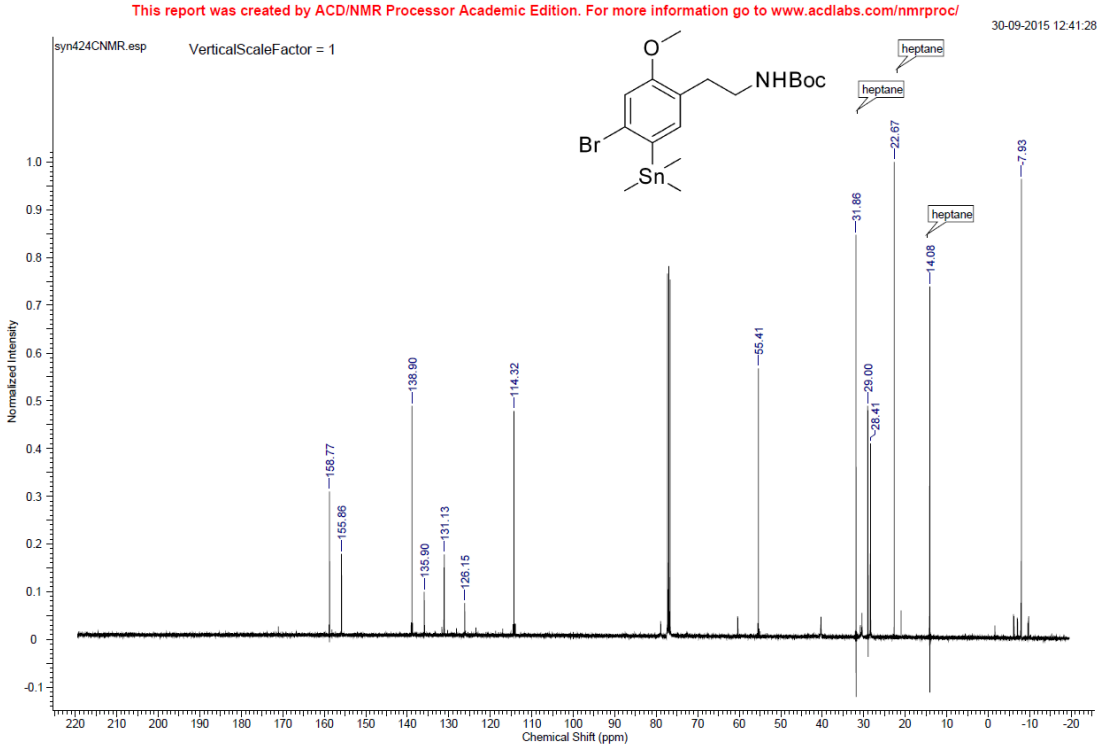
tert-butyl (4-bromo-2-methoxy-5-(trimethylstannyl)phenethyl)-carbamate (**5a**)



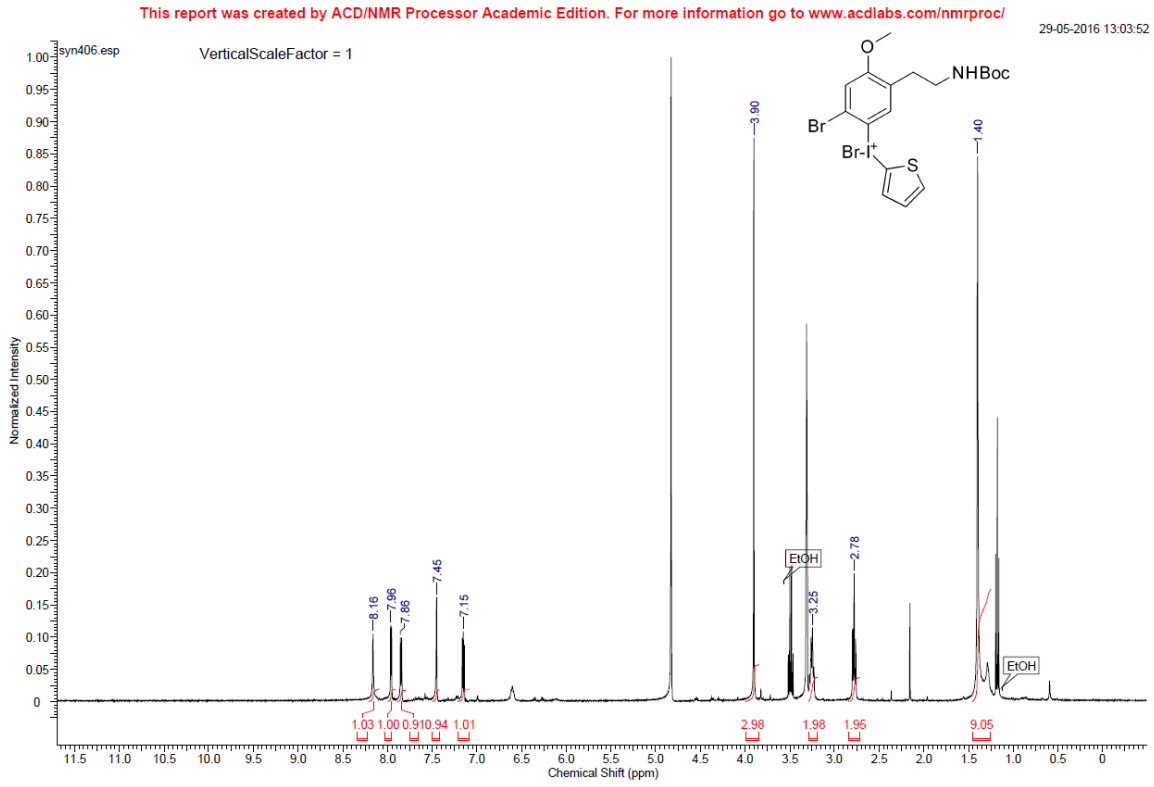


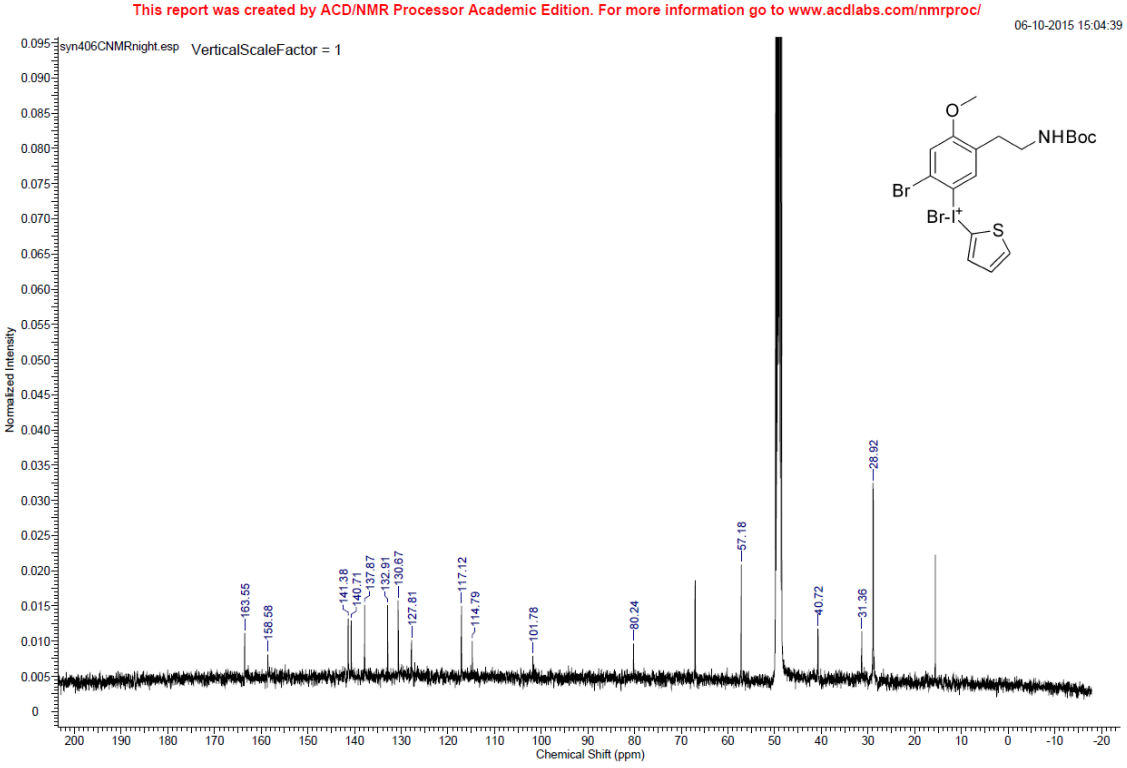
tert-butyl (4-bromo-2-methoxy-5-(trimethylstannyl)phenethyl)-carbamate (**5a**)



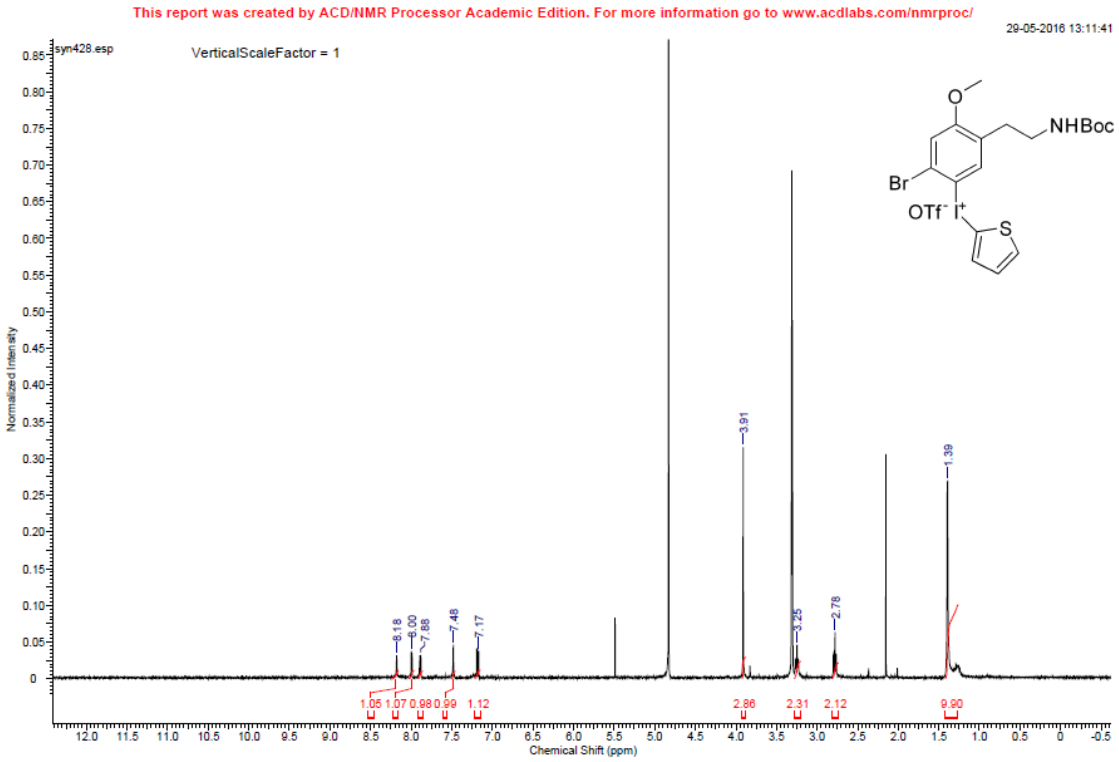


(2-bromo-5-(2-((tert-butoxycarbonyl)amino)ethyl)-4-methoxyphenyl)(thiophen-2-yl)iodonium bromide (**6a**)

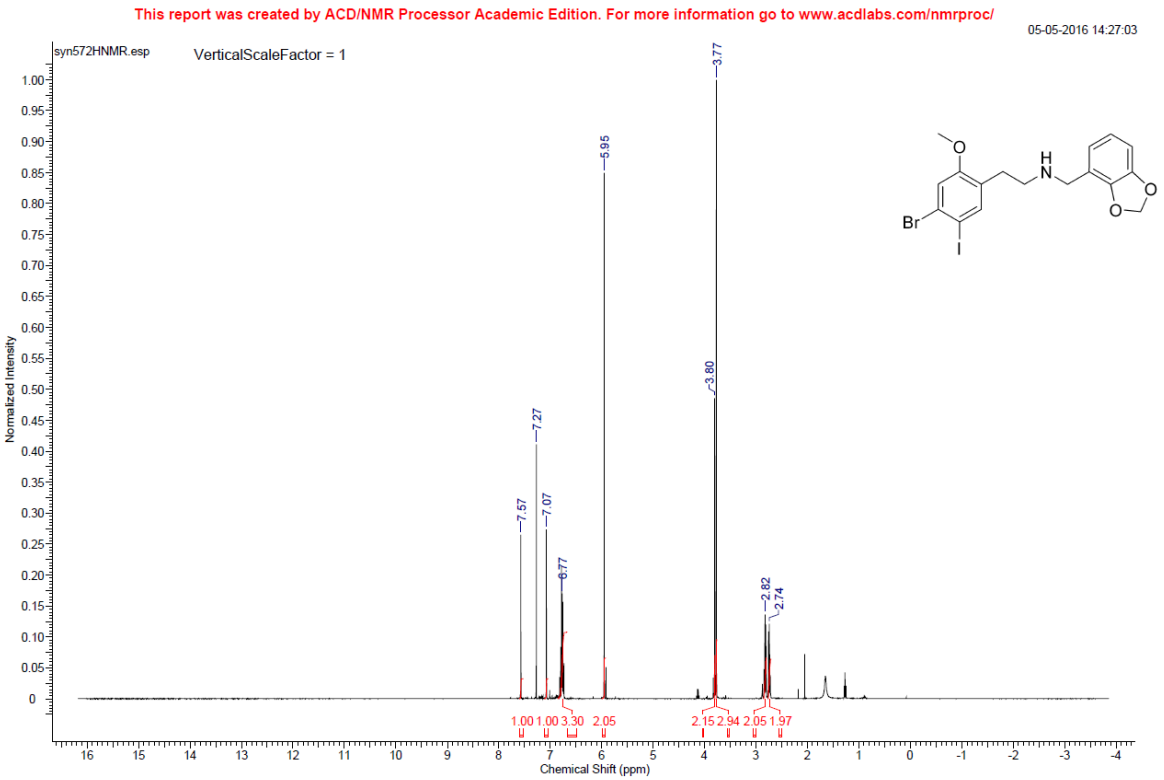


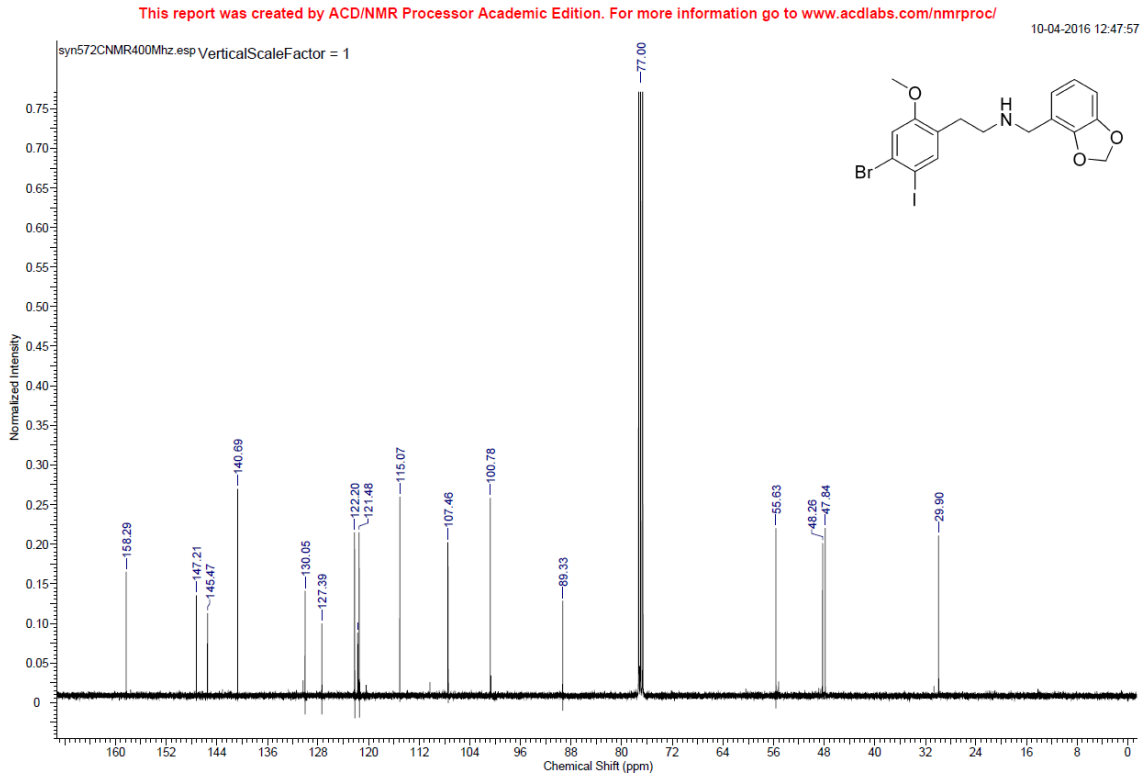


(2-bromo-5-(2-((tert-butoxycarbonyl)amino)ethyl)-4-methoxyphenyl)(thiophen-2-yl)iodonium triflate (**6b**)

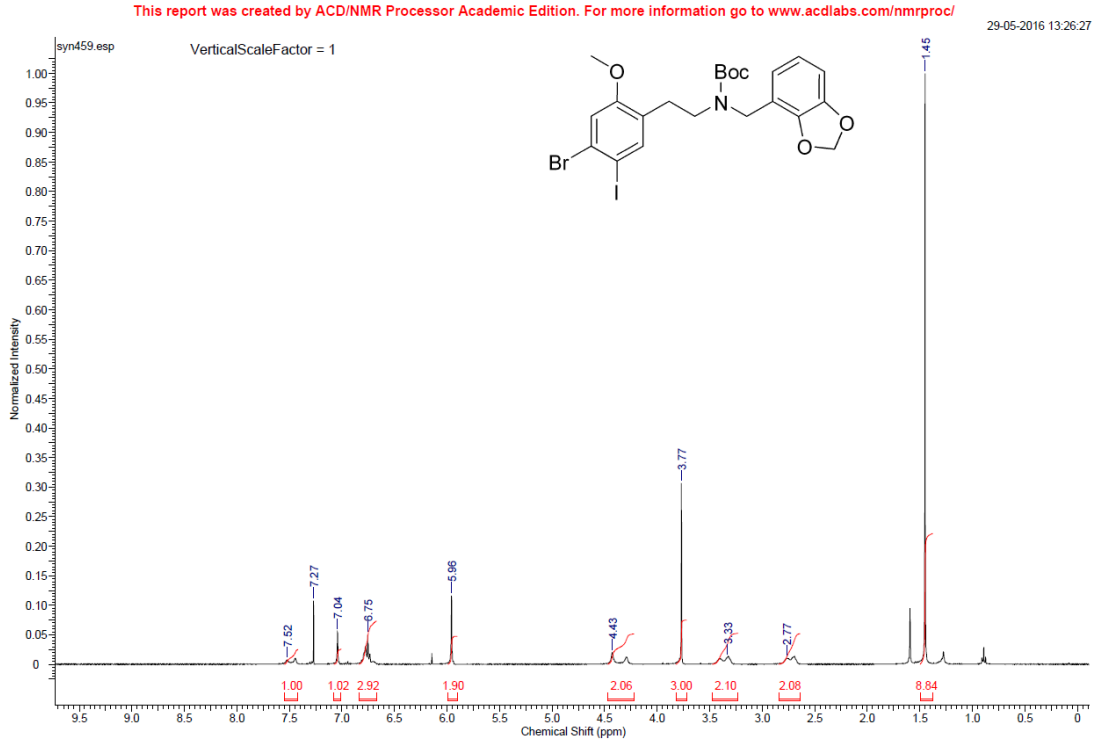


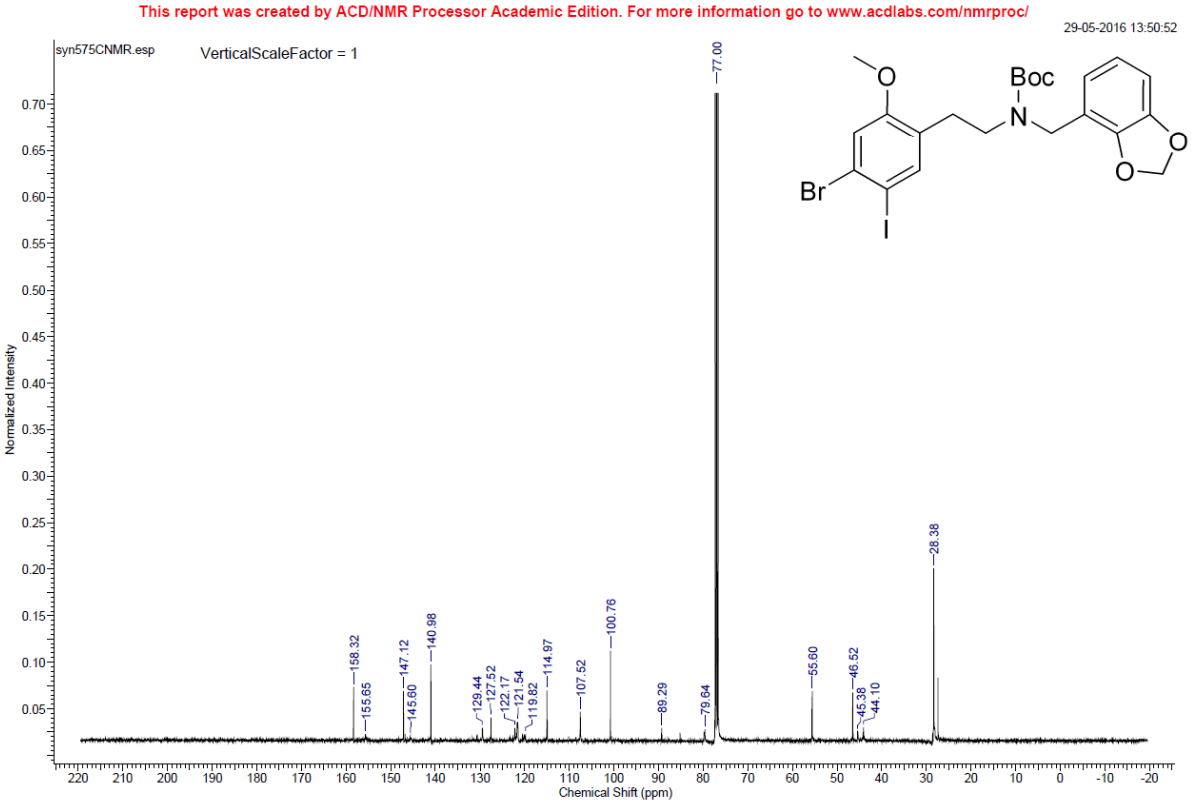
N-(benzo[d][1,3]dioxol-4-ylmethyl)-2-(4-bromo-5-iodo-2-methoxyphenyl)ethan-1-amine (**11a**)



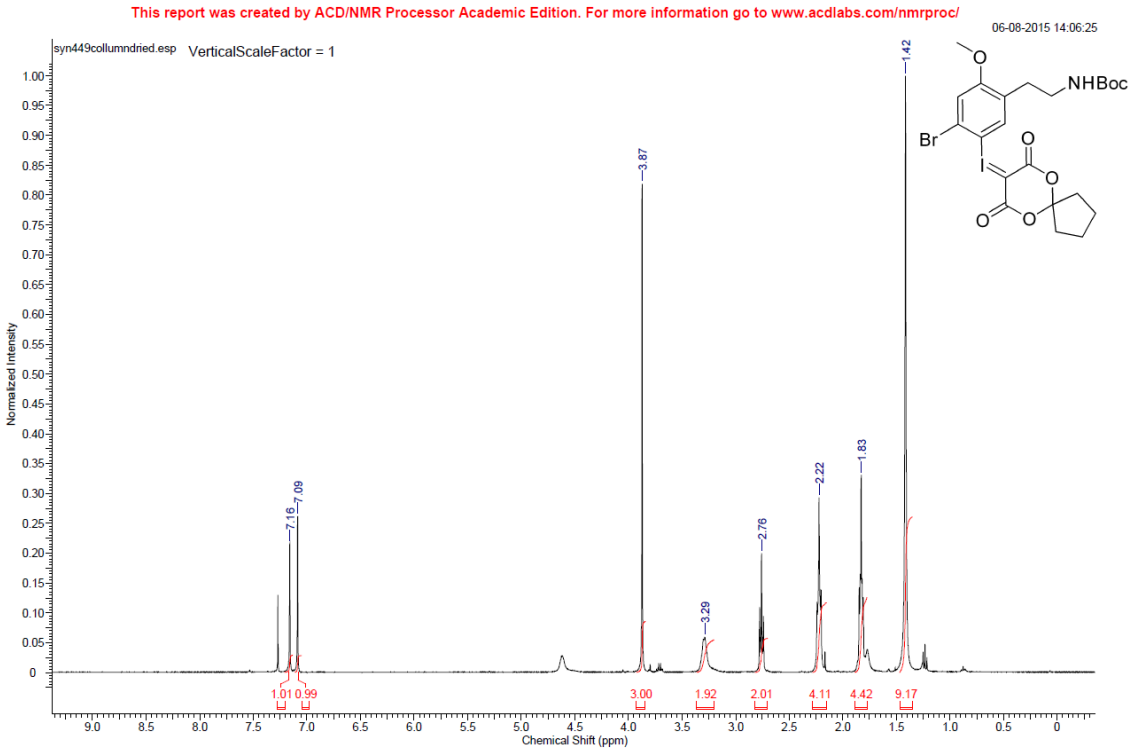


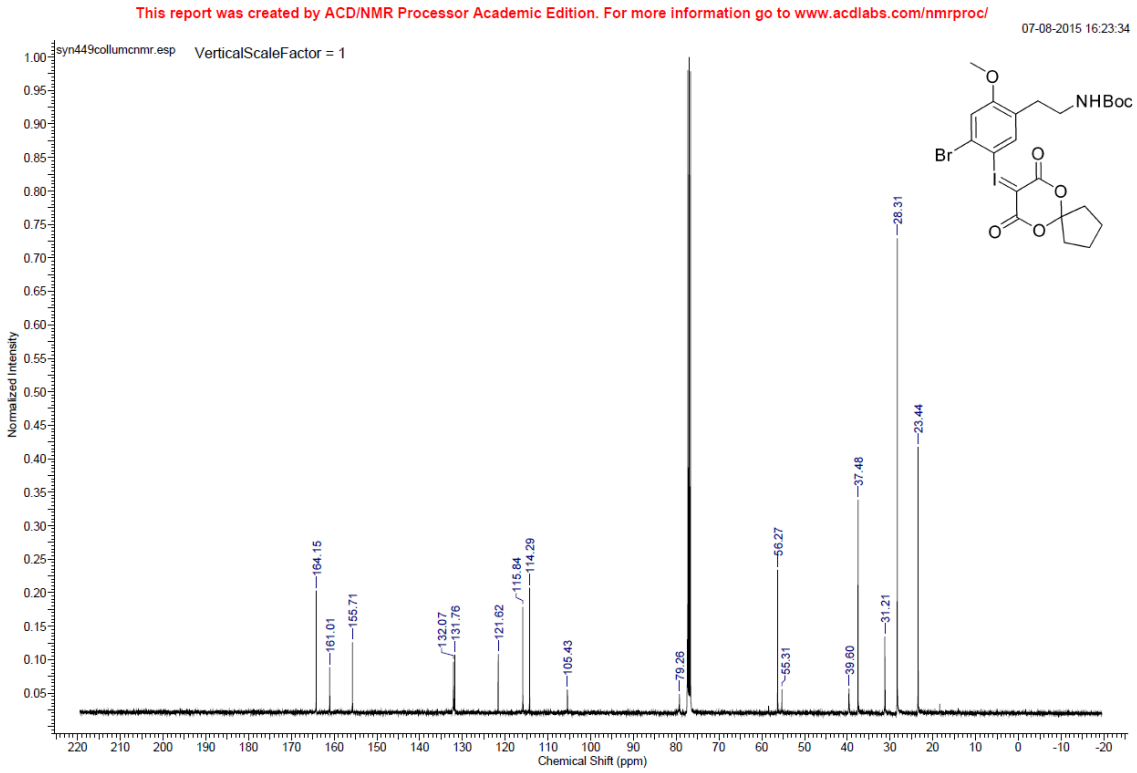
tert-butyl (benzo[d][1,3]dioxol-4-ylmethyl)(4-bromo-5-iodo-2-methoxyphenethyl)carbamate (**11**)





tert-butyl (4-bromo-5-((7,9-dioxo-6,10-dioxaspiro[4.5]decan-8-ylidene)-l3-iodanyl)-2-methoxyphenethyl)carbamate(**7**)





tert-butyl (benzo[d][1,3]dioxol-4-ylmethyl)(4-bromo-5-((7,9-dioxo-6,10-dioxaspiro[4.5]decan-8-ylidene)-l3-iodanyl)-2-methoxyphenethyl)carbamate (**10**)