Supporting Information

Practical and Scalable Synthesis of $S1P_1$ Receptor Agonist ACT-209905

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1. Analytical methods

LC-MS method:

Agilent G1956B (MS, Ionisation: ESI+, APCI), Agilent G1312B Bin Pump, Agilent G1315C DAD, Agilent G1316B (thermostated column compartment), Agilent G1367C (auto sampler)

Injection volume:	2 µL	
Column:	Zorbax SB C18, 1.8 µm, 2.1 x 50 mm	
Column flow:	1 mL/min	
Eluent:	Eluent A: Water, 0.08% TFA (trifluoroacetic acid)	
	Eluent B: Acetonitrile, 0.012% TFA	
Gradient:	2.0 min	95% B
	2.8 min	95% B
	3.0 min	5% B
Pressure:	520 bar	
Temperature:	75 °C	
Detection wavelength:	210 nm	

HPLC method 1:

HPLC system Agilent 1100 with data acquisition (Chemstation Plus)

Injection volume:	5 μL	
Column:	Zorbax Eclipse XDB C18, 3.5 μm, 150 mm x 4.6 mm	
Column flow:	1.2 mL/min	
Eluent:	Eluent A: 950 mL water, pH = 2 (ca. 350-400 μl H_2SO_4 95%) + 50 mL acetonitrile	
	Eluent B: 950 ml acetonitrile + 50 mL water + 350-400 μl H_2SO_4 95%	
Gradient mode:	Time	% A
	0 min	80%
	15 min	50%
	20 min	5%
	25 min	5%
	25.1 min	80%
	30 min	80%
Pressure:	160 bar	
Temperature:	25 °C	
Detection wavelength:	255 nm	
Retention time of **1**:	7.6 min	

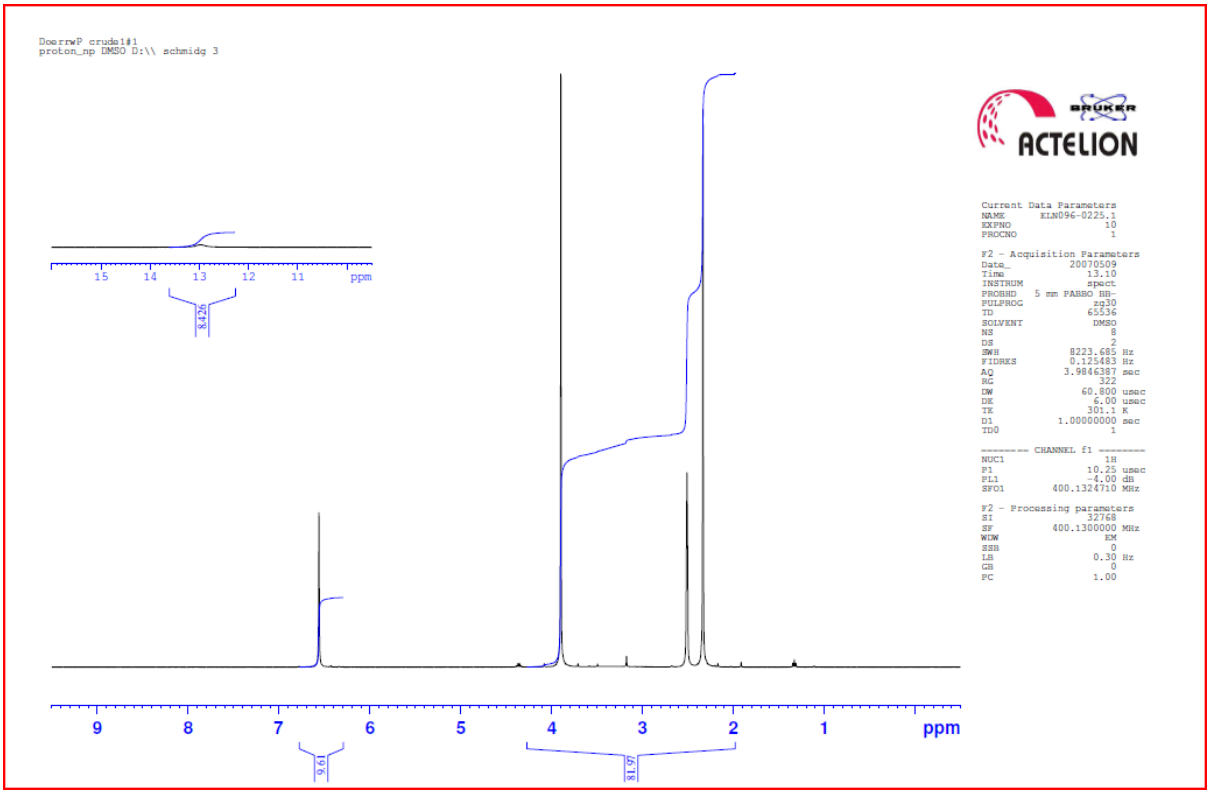
HPLC method 2:

HPLC system Agilent 1100 with data acquisition (Chemstation Plus)

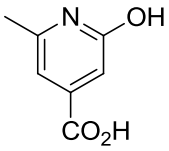
Injection volume:	5 μL
Column:	Daicel ChiralPak AD-H, 5 μm, 250 mm x 4.6 mm
Column flow:	0.8 mL/min
Eluent:	Add 1 mL diethylamine to 1 L ethanol. 150 mL of this solution + 850 mL *n*-hexan.
Gradient mode:	Isocratic
Chromatogram Time:	20 min
Pressure:	35 bar
Temperature:	25 °C
Detection wavelength:	230 nm
Retention time of **1**:	12 min
Retention time of **13**:	5.8 min

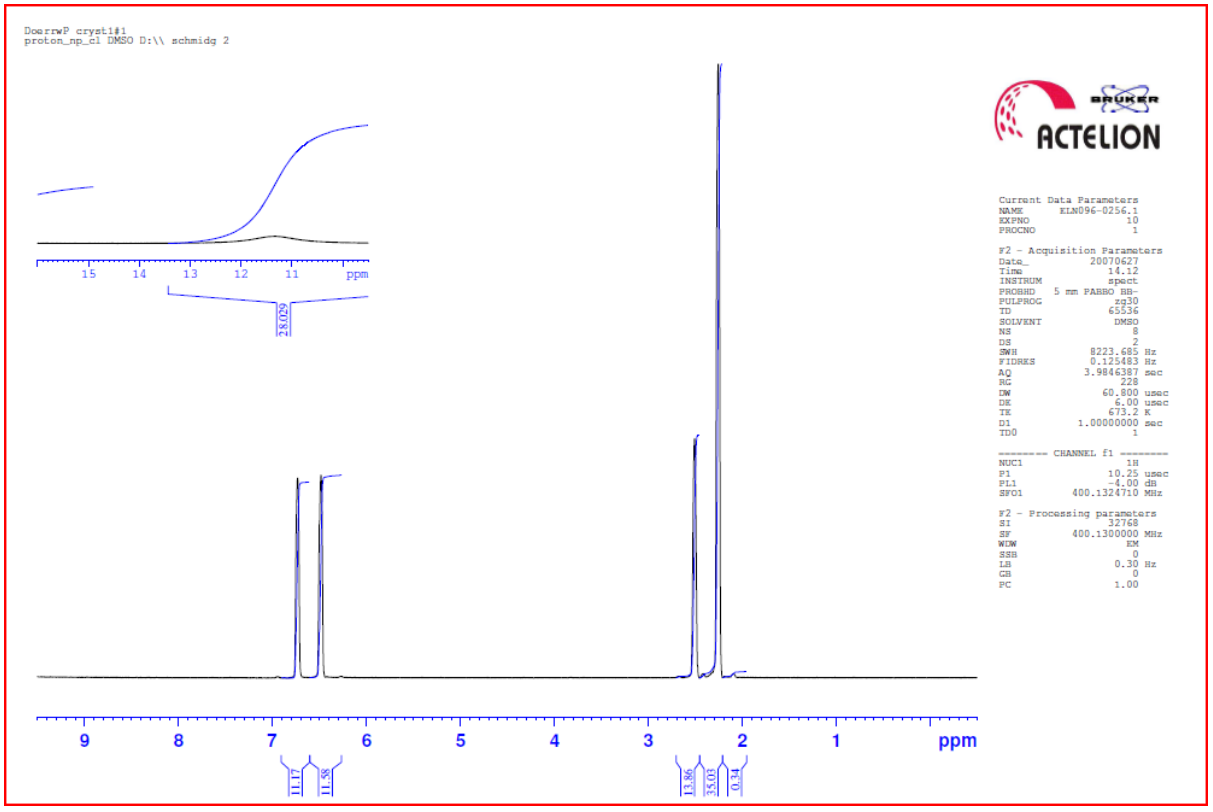
2. Representative spectra

^{1}H NMR spectrum of methyl 3-cyano-2-hydroxy-6-methyl-isonicotinate **21** (in D_6 DMSO).

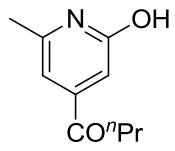


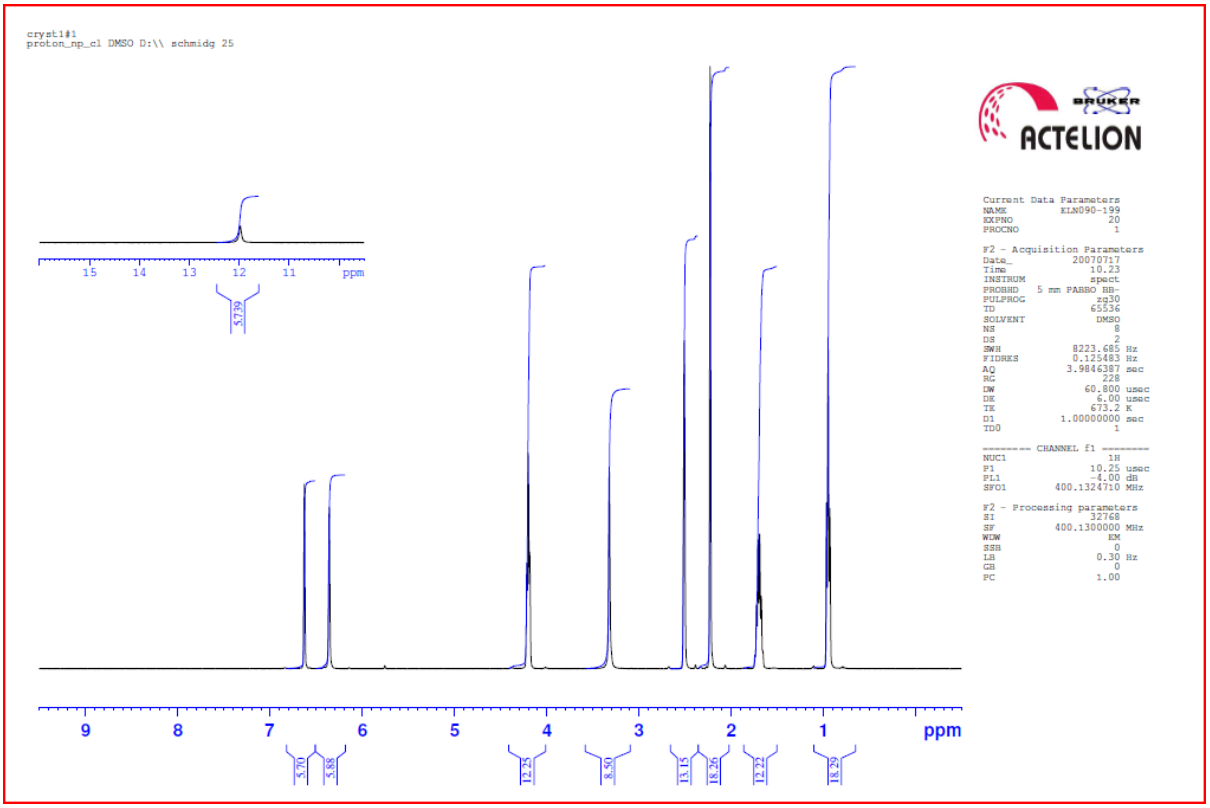
^{1}H NMR spectrum of 2-hydroxy-6-methylisonicotinic acid **25** (in D_6 DMSO).



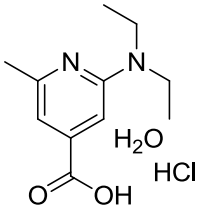


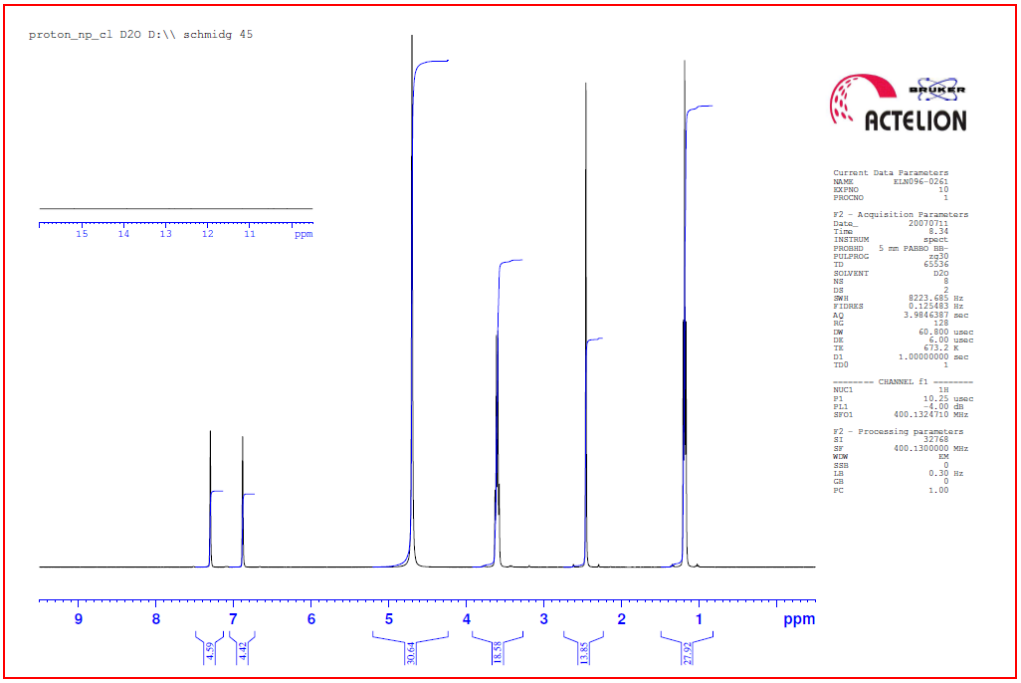
^{1}H NMR spectrum of propyl 2-hydroxy-6-methylisonicotinate **26** (in D_6 DMSO).



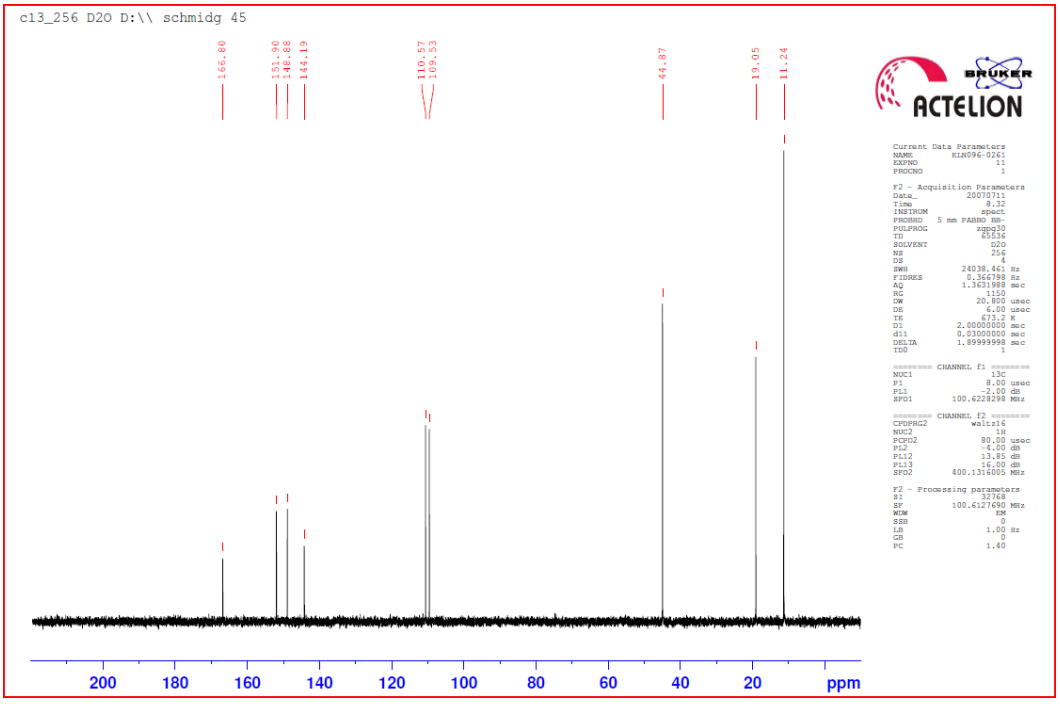


^{1}H NMR spectrum of 2-(diethylamino)-6-methylisonicotinic acid hydrochloride hydrate **6** (in D_2O).

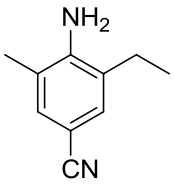


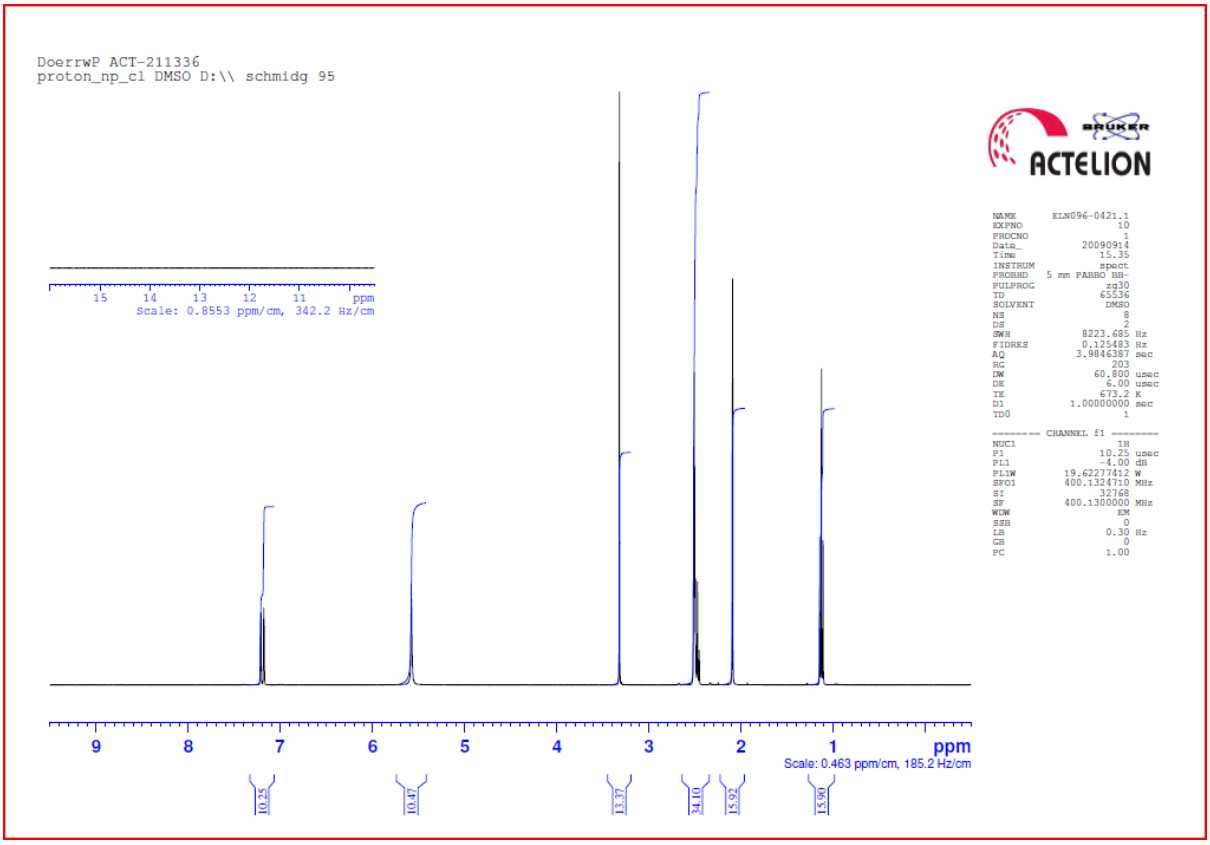


^{13}C NMR spectrum of 2-(diethylamino)-6-methylisonicotinic acid hydrochloride hydrate **6** (in D_2O).

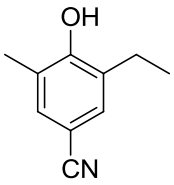


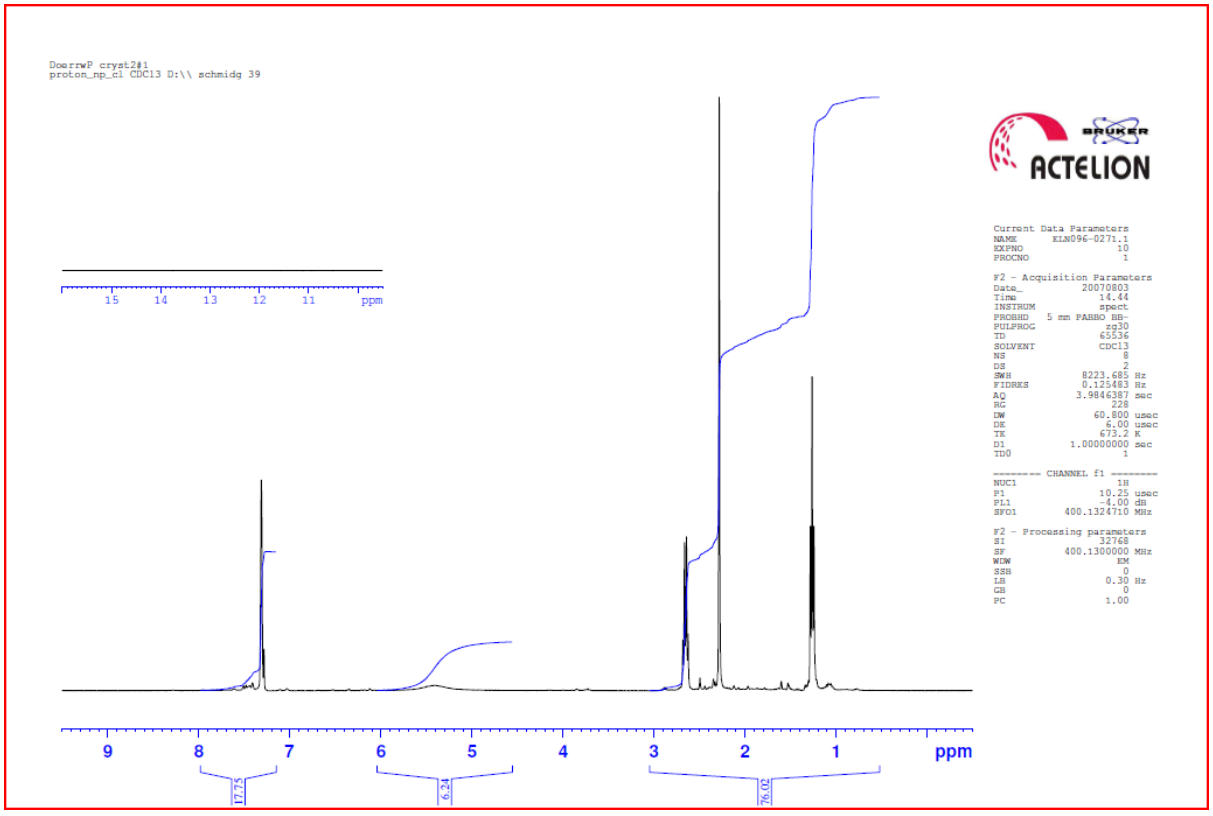
^{1}H-NMR spectrum of 4-amino-3-ethyl-5-methylbenzonitrile **31** (in D_6 DMSO)-



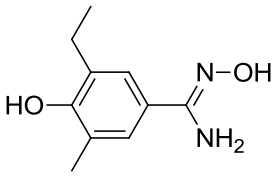


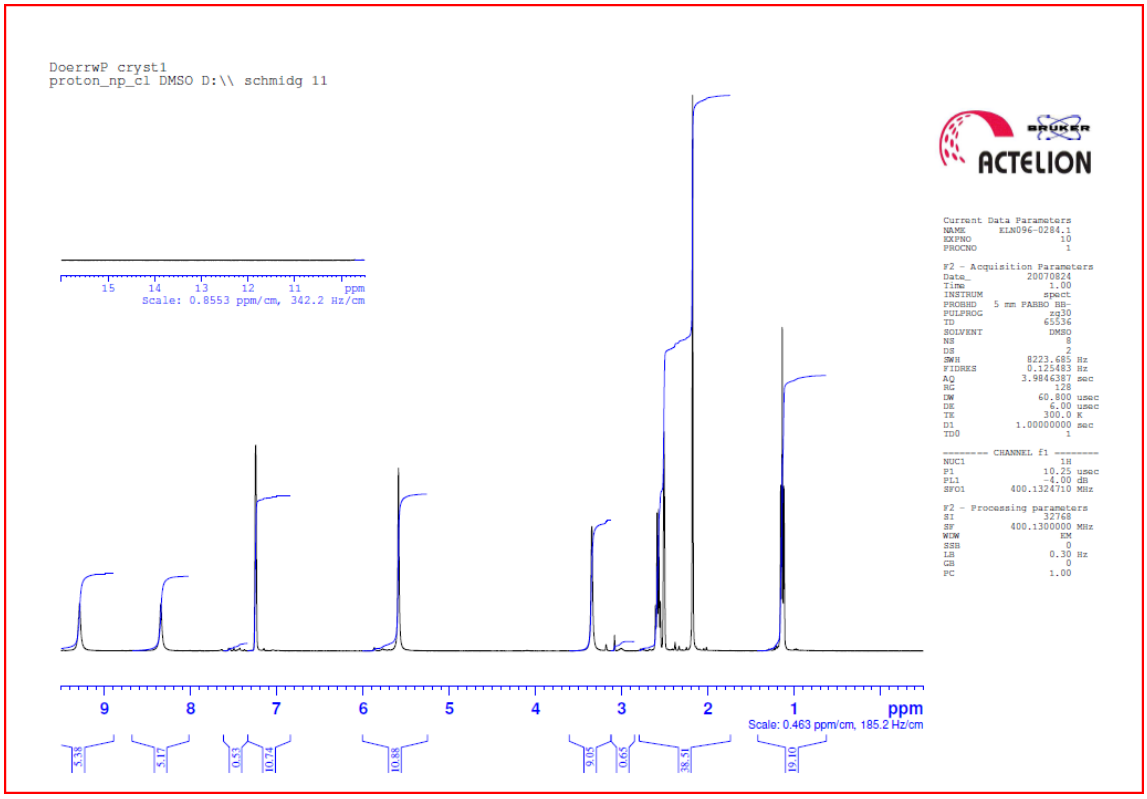
^{1}H NMR spectrum of 3-ethyl-4-hydroxy-5-methylbenzonitrile **10** (in $CDCl_3$).



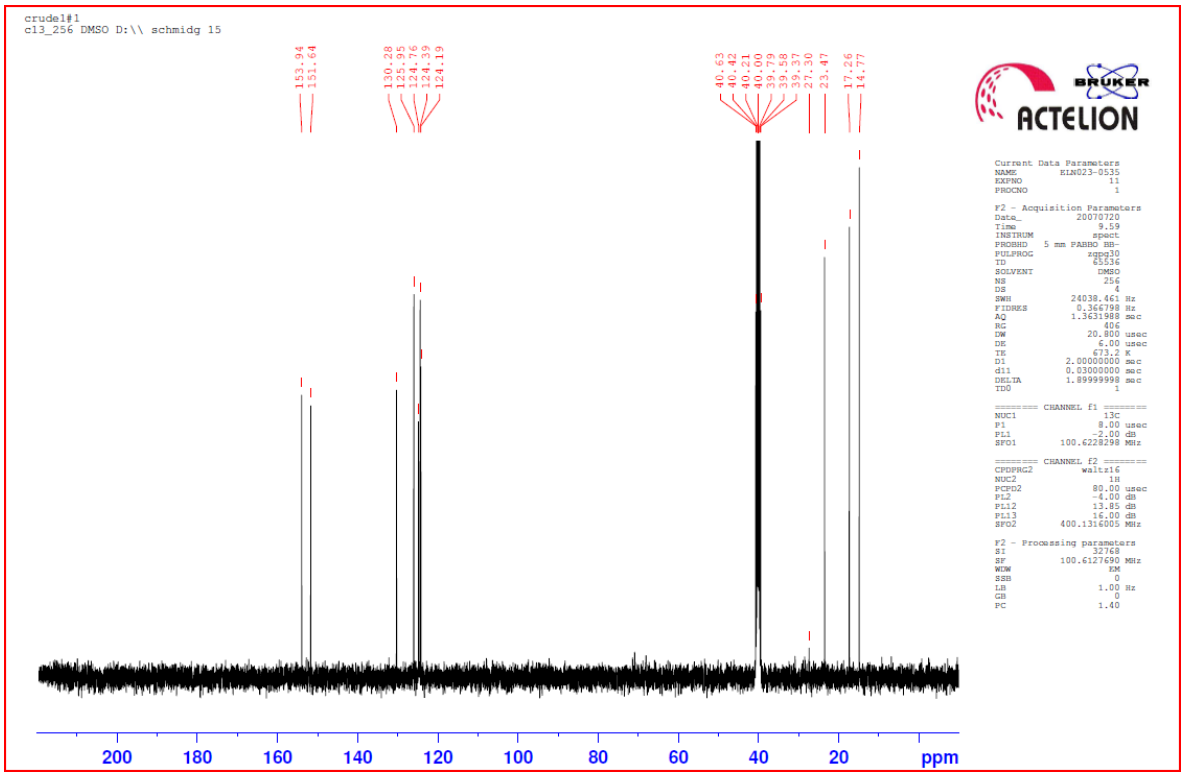


^{1}H NMR spectrum of 3-ethyl-N,4-dihydroxy-5-methylbenzimidamide **11** (in D_6 DMSO).

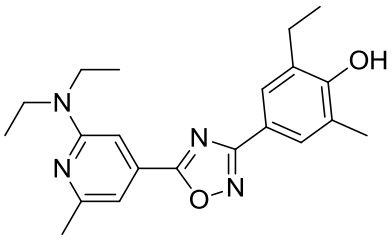


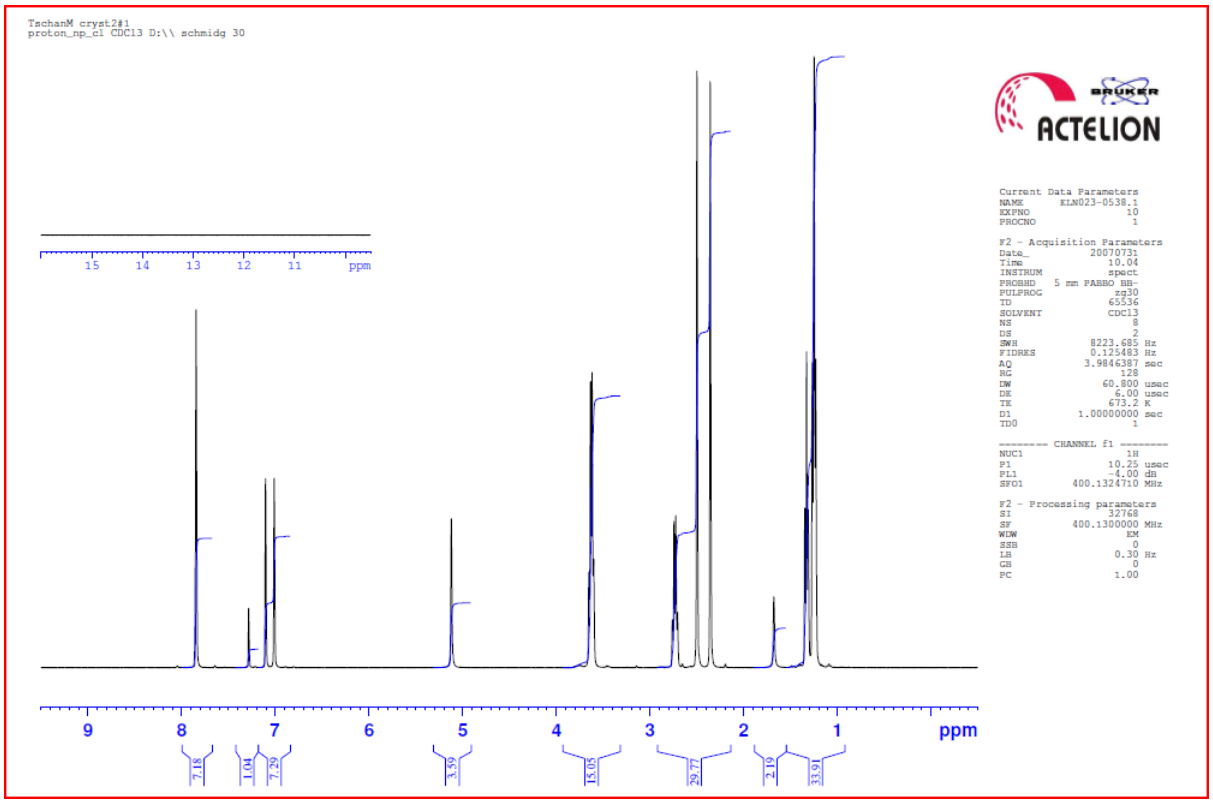


^{13}C NMR spectrum of 3-ethyl-N,4-dihydroxy-5-methylbenzimidamide **11** (in D_6 DMSO).

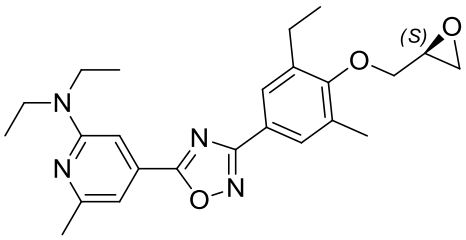


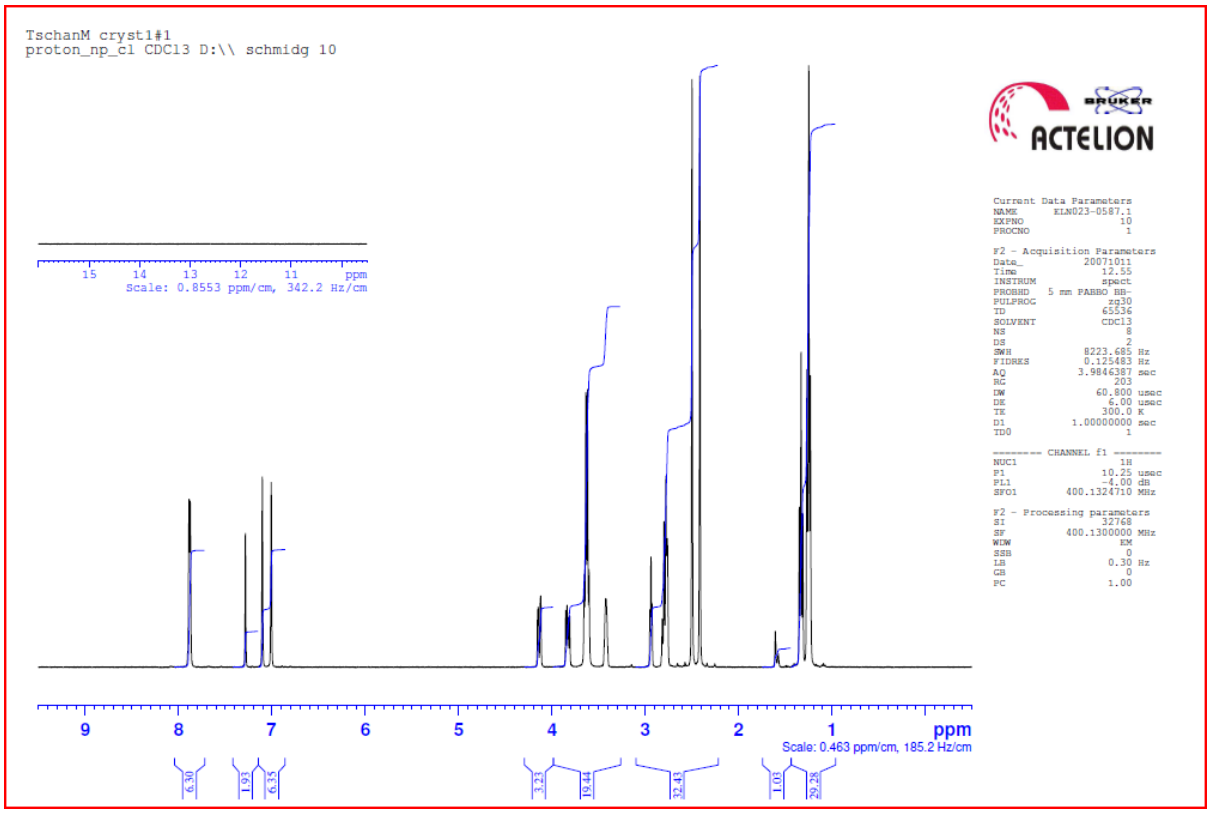
^{1}H NMR spectrum of 4-(5-(2-(diethylamino)-6-methylpyridin-4-yl)-1,2,4-oxadiazol-3-yl)-2-ethyl-6-methylphenol **12** (in $CDCl_3$).



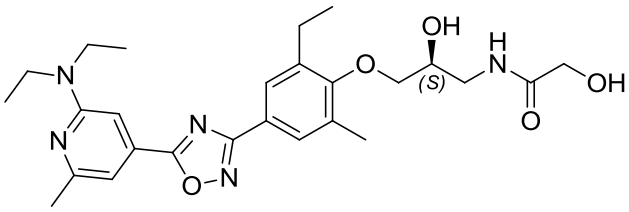


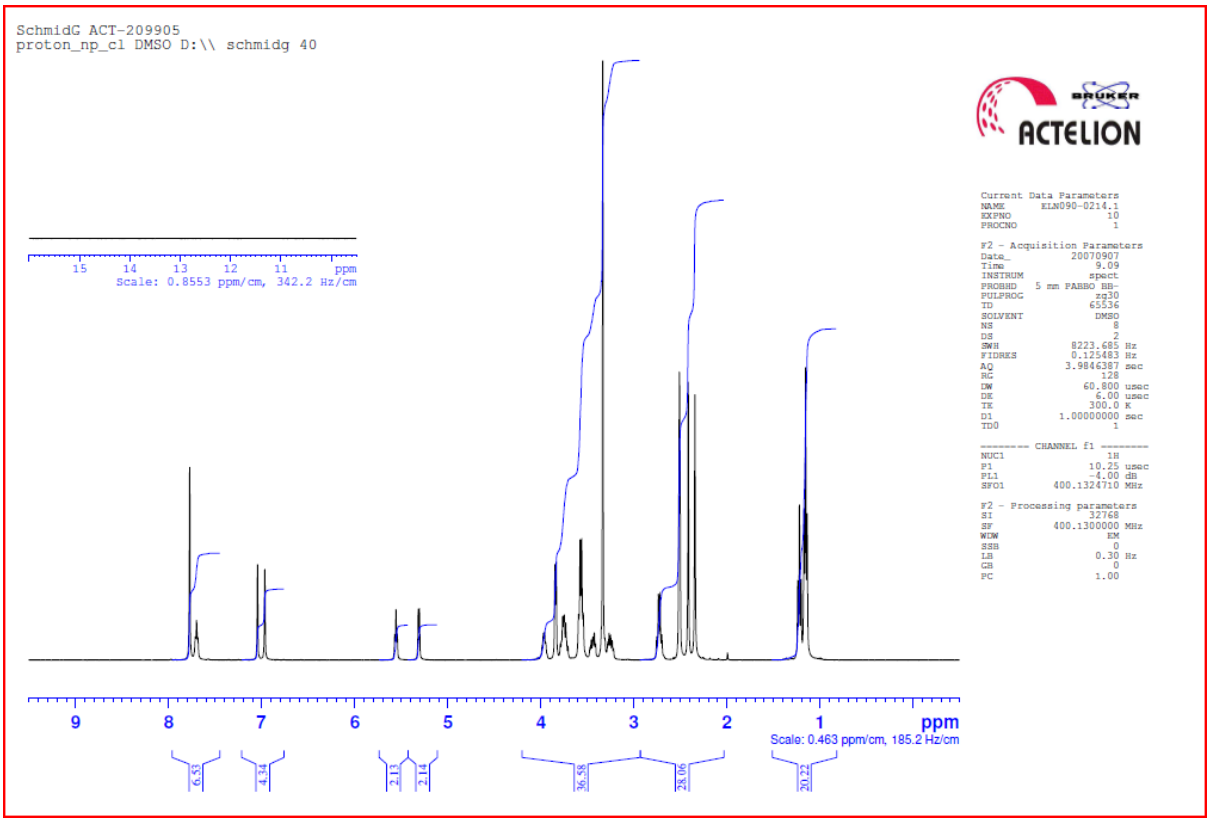
^{1}H NMR spectrum of (*S*)-N, N-diethyl-4-(3-(3-ethyl-5-methyl-4-(oxiran-2-ylmethoxy)phenyl)-1,2,4-oxadiazol-5-yl)-6-methylpyridin-2-amine **13** (in $CDCl_3$).



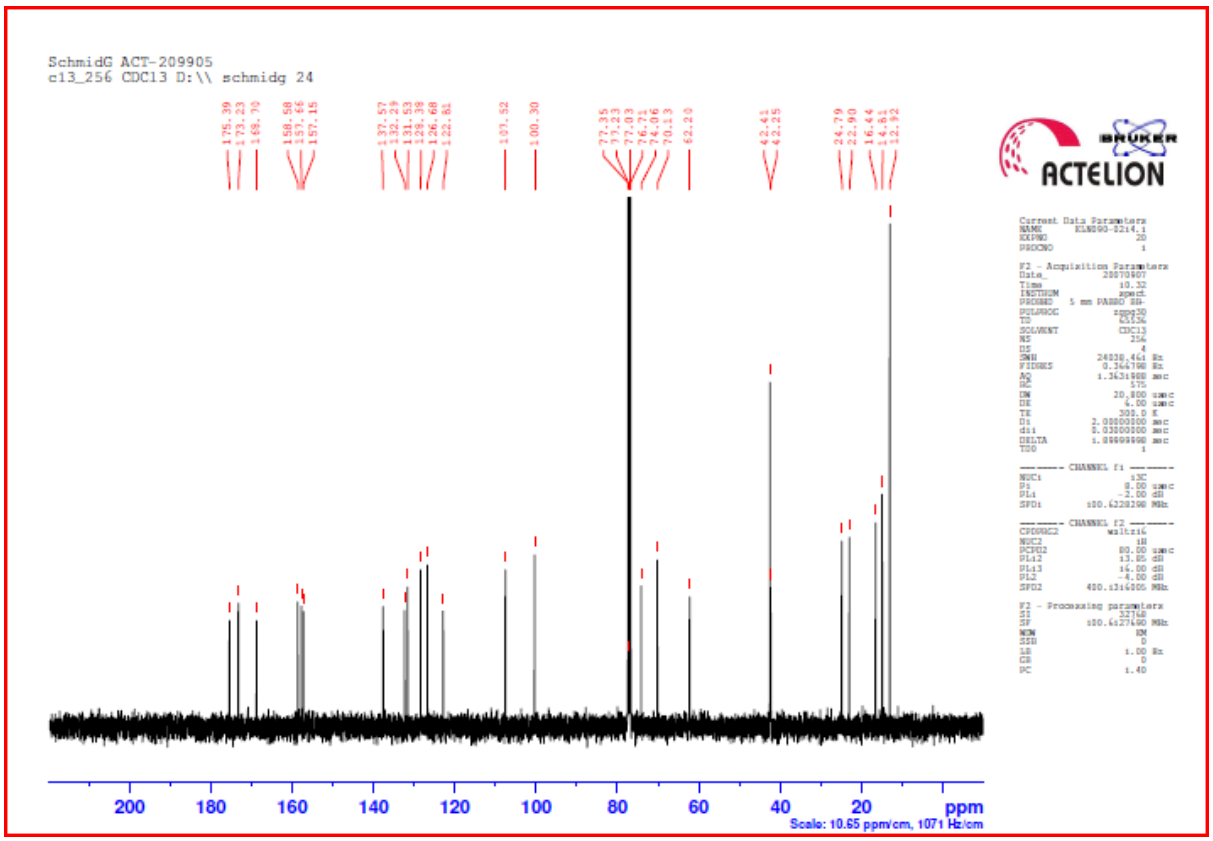


^{1}H NMR spectrum of (*S*)-N-(3-(4-(5-(2-(diethylamino)-6-methylpyridin-4-yl)-1,2,4-oxadiazol-3-yl)-2-ethyl-6-methylphenoxy)-2-hydroxypropyl)-2-hydroxyacetamide **1** (in D_6 DMSO).

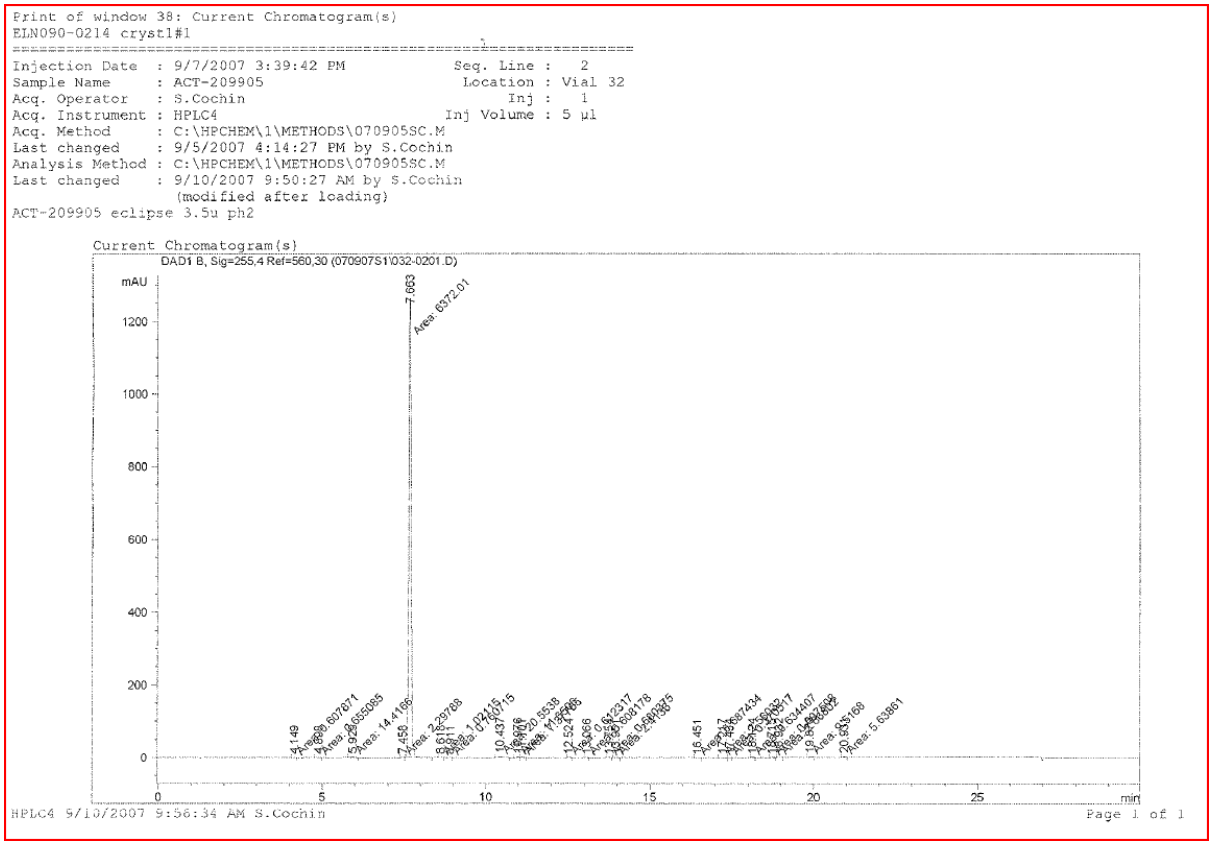




^{13}C NMR spectrum of (*S*)-N-(3-(4-(5-(2-(diethylamino)-6-methylpyridin-4-yl)-1,2,4-oxadiazol-3-yl)-2-ethyl-6-methylphenoxy)-2-hydroxypropyl)-2-hydroxyacetamide **1** (in $CDCl_3$).



HPLC trace (HPLC method 1) of (*S*)-N-(3-(4-(5-(2-(diethylamino)-6-methylpyridin-4-yl)-1,2,4-oxadiazol-3-yl)-2-ethyl-6-methylphenoxy)-2-hydroxypropyl)-2-hydroxyacetamide **1**



REVERSED PHASE
10.09.2007

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5.929	0.77	14.41662	0.22
7.458	0.97	2.29788	0.04
7.663	**1.00**	**6372.00781**	**98.66**
8.618	1.12	1.02115	0.02
8.911	1.16	0.76072	0.01
10.437	1.36	20.55378	0.32
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11.101	1.45	7.37765	0.11
12.524	1.63	0.61232	0.01
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17.434	2.28	0.37052	0.01
18.124	2.37	0.63441	0.01
18.715	2.44	0.69750	0.01
18.932	2.47	2.86802	0.04
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2

RT	RRT	Area	Area %
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4.891	0.64	0.653336	0.01
5.917	0.77	14.77023	0.23
7.445	0.97	2.35305	0.04
7.648	**1.00**	**6402.02588**	**98.64**
8.604	1.13	1.04532	0.02
8.89	1.16	0.8698	0.01
10.416	1.36	21.00886	0.32
10.957	1.43	11.79776	0.18
11.082	1.45	7.50646	0.12
12.503	1.63	0.648527	0.01
13.049	1.71	0.694111	0.01
13.744	1.80	0.657952	0.01
13.937	1.82	2.47687	0.04
16.434	2.15	0.581887	0.01
17.197	2.25	2.56998	0.04
17.416	2.28	0.485589	0.01
18.118	2.37	0.688632	0.01
18.709	2.45	0.680639	0.01
18.93	2.48	2.88383	0.04
19.876	2.60	9.51533	0.15
20.927	2.74	5.61082	0.09
		6490.173193	**100.00**

Average

RT	RRT	Area	Area %
4.279	0.56	0.628	0.01
4.895	0.64	0.654	0.01
5.923	0.77	14.593	0.23
7.452	0.97	2.325	0.04
7.656	**1.00**	**6387.017**	**98.65**
8.611	1.12	1.033	0.02
8.901	1.16	0.815	0.01
10.427	1.36	20.781	0.32
10.967	1.43	11.724	0.18
11.092	1.45	7.442	0.11
12.514	1.63	0.630	0.01
13.058	1.71	0.651	0.01
13.749	1.80	0.669	0.01
13.944	1.82	2.445	0.04
16.443	2.15	0.635	0.01
17.207	2.25	2.565	0.04
17.425	2.28	0.428	0.01
18.121	2.37	0.662	0.01
18.712	2.44	0.689	0.01
18.931	2.47	2.876	0.04
19.878	2.60	9.516	0.15
20.929	2.73	5.625	0.09
		6474.405	**100.00**

HPLC trace (HPLC method 2) of (*S*)-N-(3-(4-(5-(2-(diethylamino)-6-methylpyridin-4-yl)-1,2,4-oxadiazol-3-yl)-2-ethyl-6-methylphenoxy)-2-hydroxypropyl)-2-hydroxyacetamide **1**

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Last changed    : 06.09.2007 17:36:38  by S.Cochin
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Last changed    : 10.09.2007 10:00:12  by S.Cochin
                  (modified after loading)
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Sample Info     : ELN090-0214 cryst1#1
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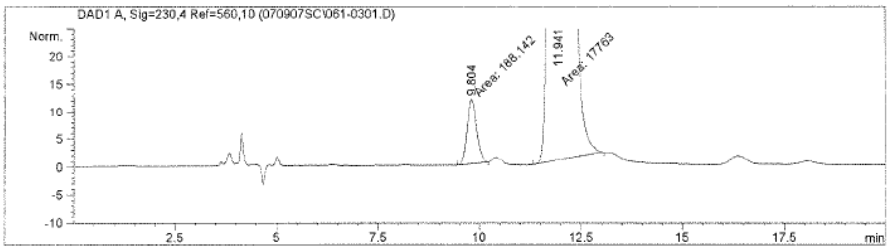


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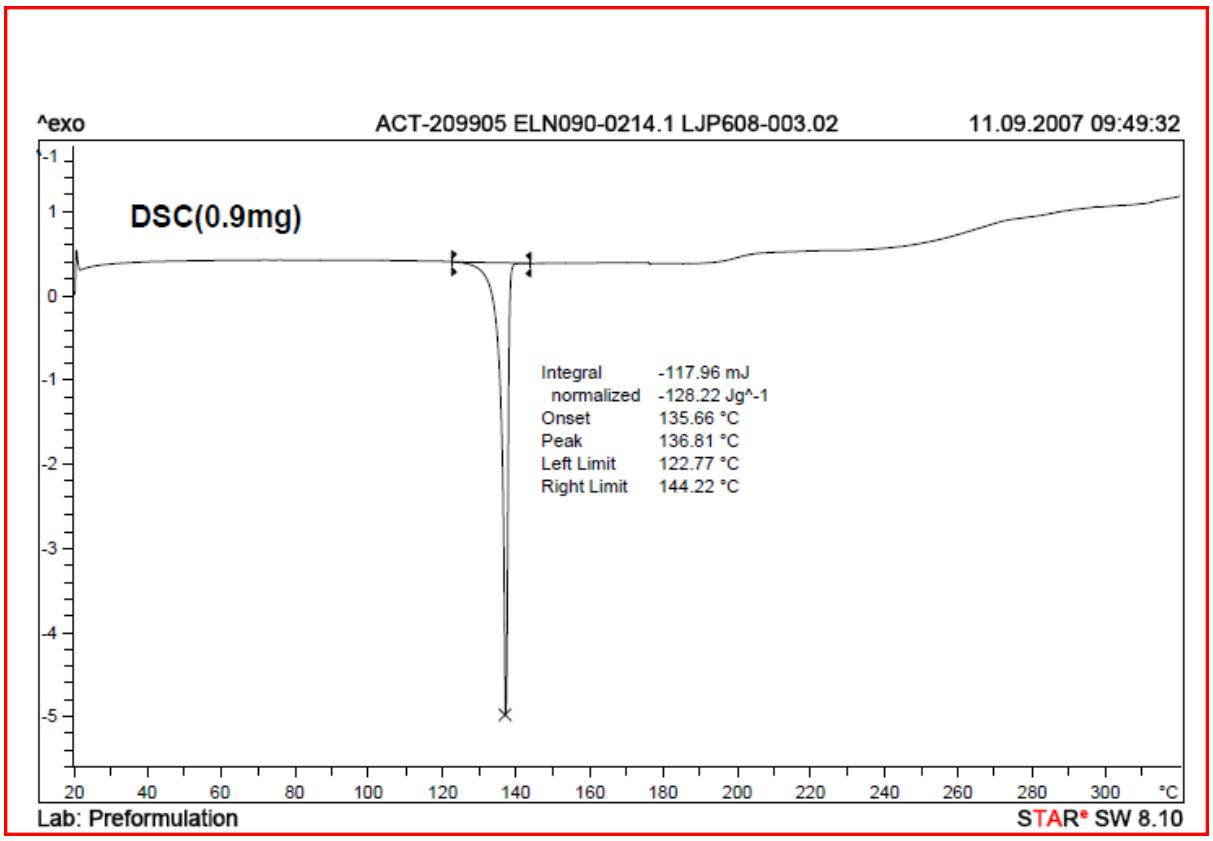
DSC of (*S*)-N-(3-(4-(5-(2-(diethylamino)-6-methylpyridin-4-yl)-1,2,4-oxadiazol-3-yl)-2-ethyl-6-methylphenoxy)-2-hydroxypropyl)-2-hydroxyacetamide **1**



3. Examples of procedures on 400-L scale (6, 11, 12)

2-(Diethylamino)-6-methylisonicotinic acid hydrochloride hydrate (6)

A 400-L enamelled reactor was charged with DCM (156 L), propyl methylisonicotinate **26** (21.277 kg, 109.2 mol, 1.0 equiv) and pyridine (13 L). The mixture was cooled to - 5 °C. Triflic anhydride (34.02 kg, 1.1 equiv) was added in 125 min at below 5 °C. The mixture was heated to 20 °C and stirred for 45 min. IPC showed 0.1 % a/a of **26**. The reaction mixture was washed with water (100 L). The organic layer (183 L) was concentrated to a minimum stirring volume at 60–70 °C (128 L solvent removed). The water content of a sample of the residue was 0.01 % w/w (Karl Fischer titration). DMSO (100 L) and Et_2NH (112.5 L) were added to the residue. The solution was heated to 50 °C for 17 h. IPC showed 0.6 % a/a of **27**. The temperature was increased to 125 °C and solvent and Et_2NH (61 L) were distilled off. The mixture was cooled to 30 °C and diluted with TBME (144 L). The solution was washed with water (151 L) and 8.3 w/w% HCl (6.5 L). The aqueous layer had a pH of 6.7 and was discarded. The organic layer was washed with water (50 L). IPC of a sample showed no diethylamine present (^{1}H NMR). Solvent (102 L) was distilled off at 90 °C jacket temperature. The mixture was treated with 25% HCl (83 L) at 20 °C and heated to 145 °C while 1-propanol and aqueous HCl were distilled off (74 L solvents were distilled off). IPC showed 0.2% a/a of **28**. The mixture was cooled to 30 °C. To the suspension was added acetone (85 L) at 30 °C. The suspension was cooled to 1 °C and aged for 9.5 h. The suspension was filtered over a 50 L glass nutsche with Teflon filter cloth 36 μm in 15 min. The solid was washed with acetone (2 x 18 L). The yellow solid **6** was dried on the nutsche with a slight stream of nitrogen for 47 h. Yield: 18.007 kg (74%). ^{1}H NMR assay: 100% w/w. The residue in the reactor was dissolved with methanol (100 L). The solution was concentrated on a rotavapor at 40 °C and reduced pressure to obtain 3.164 kg (15%) of **6** with a ^{1}H NMR assay of 94% w/w.

3-Ethyl-*N*,4-dihydroxy-5-methylbenzimidamide (11). A 400-L enamelled reactor was charged with nitrile **10** (20.925 kg, 130 mol, 1 equiv), $NH_2OH \cdot HCl$ (22.555 kg, 2.5 equiv), Et_3N (45 L, 2.5 equiv) and MeOH (176 L). The mixture was heated to 65 °C for 7.5 h. The mixture was cooled to 20 °C and 8.9 w/w% HCl (96.4 L) was added to obtain a pH of 1.79. The brown solution was concentrated at 44 °C internal temperature and reduced pressure (172 L solvent removed). The residue was washed with TBME (2 x 100 L). The organic layers were discarded. The pH of the aqueous layer was adjusted to 7.12 by addition of 30% NaOH (23 L). The resulting suspension was cooled to 2 °C and filtered. The cake was washed with water (25 L). The product was dried on the nutsche for 7.5 d with a slight stream of nitrogen. Yield (**11**): 23.16 kg (92%). ^{1}H NMR assay: 95% w/w.

4-(5-(2-(Diethylamino)-6-methylpyridin-4-yl)-1,2,4-oxadiazol-3-yl)-2-ethyl-6-methylphenol (12). A 400-L enamelled reactor was charged with isonicotinic acid **6** (21.058 kg, 80.151 mol), $HOBt \cdot H_2O$ (1.229 kg, 0.1 equiv), hydroxy-benzamidine **11** (16.3957 kg, 15.5677 kg assay corrected, 1 equiv), Et_3N (8.16 kg, 1 equiv) and THF (156 L). The mixture was cooled to 0 °C. A solution of DCC (18.2 kg, 1.1 equiv) in THF (60 L) was added to the mixture at 0 °C for 152 min. The mixture was stirred at 0 °C for 8 hours. The suspension was heated to 20 °C in 8 h and stirred at 20 °C for 3 h. The suspension was heated to reflux for 21 h and then concentrated at 75 °C whereby THF (145 L) was removed. TBME (109.5 L) was added to the residue and the mixture was cooled to 0 °C. The urea was filtered off and washed with TBME (3 x 8.5 L). The combined filtrates were washed with 3.7 w/w% aq $NaHCO_3$ solution (2 x 54 L) and water (56 L). The organic layer (170 L) was concentrated at normal pressure and 120 L solvent was removed. Acetonitrile (211 L) was added and distillation was continued (internal temperature 65-70 °C, 139 L solvent distilled off). Acetonitrile (141 L) was added and distillation was continued (internal temperature 70-80 °C, 65 L solvent distilled off). The mixture was cooled to 60 °C within 1 h and to 5 °C within 5 h. The suspension was aged for 9 h. The suspension was filtered and the cake was washed with cold acetonitrile (32 L). The cake was dried on the nutsche with a slight stream of nitrogen for 112 h. Yield (**12**): 20.32 kg (69%). ^{1}H NMR assay: 93% w/w.