Supplementary Material

Regioselective and enantiospecific synthesis of the HSP co-inducer arimoclomol from chiral glycidyl derivatives

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General Information

Unless preparative details are provided, all reagents were purchased from commercial suppliers and used without further purification. All solvents were of ACS reagent grade or higher and purchased from commercial suppliers without further purification. Any anhydrous solvents were purchased as such from Acros Organics or Sigma Aldrich. Thin layer chromatography (TLC) was carried out on aluminium backed silica plates. The plates were visualized under UV (254 nm) light, followed by staining with phosphomolybdic acid dip or potassium permanganate and gentle heating. During compound separations, column chromatography was carried out using a Biotage Isolera using prepacked Biotage SNAP KP-Sil silica cartridges or Biotage SNAP Ultra C18 reverse phase cartridges. Organic layers were routinely dried with anhydrous $MgSO_4$ and concentrated using a Büchi rotary evaporator. ^{1}H NMR / ^{13}C NMR spectra were run in deuterated (≥99.5%) solvents, on either a Bruker Avance 400 (400 MHz) or a Bruker Avance 600 (600 MHz). Any chemical shifts (δ) are reported as parts per million (ppm). The coupling constants (*J*) are reported in Hz and signal multiplicities are reported as singlet (s), doublet (d), triplet (t), quartet (q), pentet (p), quintet (qu), sextet (sext), doublet of doublets (dd), doublet of triplets (dt), triplet of triplets (tt), multiplet (m), or broad singlet (br. s) etc.
For mass spectrometry data acquisition was on a Waters Micromass LCT Premier electrospray time-of-flight (ESI-TOF) mass spectrometer. The observed mass and isotope pattern matched the corresponding theoretical values as calculated from the expected elemental formula. Infra-red spectra were recorded on a Shimadzu IRTracer-100 FT-IR spectrometer, using a Universal ATR accessory for sampling, with relevant absorbances quoted as ν in cm^{-1}. Optical rotations were measured on an AA-10 Automatic Polarimeter. Enantiomeric ratios were determined by Reach Separations using Supercritical Fluid Chromatography using conditions quoted below and containing a suitable modifier. Melting points were determined using Stuart SMP20 melting point equipment using closed end glass capillary tubes and are uncorrected.

Animal Testing Declaration

In vivo mouse pharmacokinetic data was generated at Pharmidex Pharmaceutical Services Ltd, UK and all animal experimental protocols carried out by this company carried out by this company have been approved by the Government Home Office and carried out in accordance with the guidelines of the Animals (Scientific Procedures) Act (1986).

Arimoclmol (2) off target pharmacology gathered using Eurofins CEREP ExpresS Study[a]

Target	% inhibition at 10 µM
A2A (h) (ag)	-8
A3 (h) (ag)	-2
alpha 1 (non-selective) (ant)	6
alpha 2 (non-selective) (ant)	-15
AT1 (h) (ant)	5
BZD (central) (ag)	-9
B2 (h) (ag)	0
CB1 (h) (ag)	-15
CCK1 (CCKA) (h) (ag)	10
D1 (h) (ant)	6
D2S (h) (ant)	-8
ETA (h) (ag)	2
GABA (non-selective) (ag)	8
GAL2 (h) (ag)	-10
CXCR2 (IL-8B) (h) (ag)	-2
CCR1 (h) (ag)	-3
H1 (h) (ant)	3
H2 (h) (ant)	**34**
MC4 (h) (ag)	-5
MT1 (ML1A) (h) (ag)	14
M1 (h) (ant)	8
M2 (h) (ant)	8
M3 (h) (ant)	13
NK2 (h) (ag)	-11
NK3 (h) (ant)	4
Y1 (h) (ag)	-13
Y2 (h) (ag)	-14
NTS1 (NT1) (h) (ag)	-5
delta 2 (DOP) (h) (ag)	-2
kappa (KOP) (ag)	-22
NOP (ORL1) (h) (ag)	2
EP4 (h) (ag)	-12
5-HT1A (h) (ag)	11
5-HT1B (ant)	4
5-HT2A (h) (ant)	8
5-HT2B (h) (ag)	**30**
5-HT3 (h) (ant)	7
5-HT5a (h) (ag)	-1
5-HT6 (h) (ag)	7
5-HT7 (h) (ag)	11
sst (non-selective) (ag)	-2
VPAC1 (VIP1) (h) (ag)	-16
V1a (h) (ag)	8

Ca2+ channel (L, verapamil site) (phenylalkylamine) (ant)	8
KV channel (ant)	-5
SKCa channel (ant)	1
Na+ channel (site 2) (ant)	11
Cl- channel (GABA-gated) (ant)	-8
norepinephrine transporter (h) (ant)	6
dopamine transporter (h) (ant)	18
5-HT transporter (h) (ant)	2

[a] Eurofins Cerep ExpresS Profile. Single point concentration at 10 μM; binding assays; n = 2 mean

Arimoclomol (2) kinase activity gathered using Invitrogen SelectScreen profiling[b]

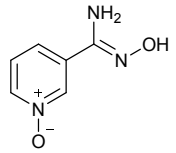
Kinase Tested	% Inhibition at 1 μM
ABL1	19
AKT1 (PKB alpha)	6
AURKA (Aurora A)	8
CAMK2A (CaMKII alpha)	3
CDK1/cyclin A2	13
CDK2/cyclin A	-8
CHEK1 (CHK1)	0
CHEK2 (CHK2)	-14
CHUK (IKK alpha)	13
CSNK1A1 (CK1 alpha 1)	19
EGFR (ErbB1)	27
EPHA2	17
EPHA3	10
EPHB4	7
FGFR1	47
FGFR2	50
FGFR3	24
FLT3	-2
GSK3A (GSK3 alpha)	21
GSK3B (GSK3 beta)	37
IRAK4	-14
JAK3	-2
KDR (VEGFR2)	-4
LCK	11
MAP2K6 (MKK6)	8
MAP4K4 (HGK)	7
MAPK1 (ERK2)	9
MAPK14 (p38 alpha) Direct	4
MAPK8 (JNK1)	4
MAPKAPK2	43
MARK1 (MARK)	0
MET (cMet)	-13
MKNK2 (MNK2)	11

MST4	-6
NEK2	-21
NTRK1 (TRKA)	-87
PAK2 (PAK65)	23
PAK4	29
PDK1 Direct	2
PIM2	-1
PLK1	1
PRKACA (PKA)	38
PRKCB2 (PKC beta II)	6
RAF1 (cRAF) Y340D Y341D	18
ROCK1	-11
SGK (SGK1)	15
SIK1	2
SRC	1
TAOK2 (TAO1)	-18

[b] Life Technologies SelectScreen Kinase Profiling. Single point concentration at 1 μM; n = 2 mean

Experimental and General Procedures

(Z)-Pyridine-3-amidoxime-1-oxide[1] **– 4**

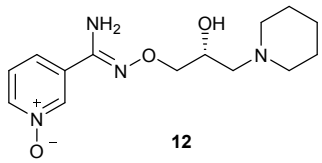


4

To a solution of 3-cyanopyridine 1-oxide (7.50 g, 62.4 mmol) in water (100 mL) was added hydroxylamine hydrochloride (5.21 g, 74.9 mmol), $NaHCO_3$ (6.94 g, 74.9 mmol) was then added portionwise over 2 minutes. The reaction mixture was stirred at room temperature for 18 hours. The precipitate was filtered, washed with water and dried under vacuum to afford the *title compound* (8.66 g, 91% yield) as a beige solid. Analytical data was consistent with literature values.

^{1}H NMR (600 MHz, d6-DMSO) δ: 10.44 (s, 1H), 8.64 (s, 1H), 8.28 (d, *J* = 6.3 Hz, 1H), 7.67 (d, *J* = 8.1 Hz, 1H), 7.47 (dd, *J* = 7.8, 6.6 Hz, 1H), 6.14 (s, 2H); ^{13}C NMR (151 MHz, d6-DMSO) δ: 147.1, 138.7, 135.7, 132.6, 126.3, 123.0

(R,Z)-3-(N'-(2-hydroxy-3-(piperidin-1-yl)propoxy)carbamimidoyl)pyridine-1-oxide[1] **– 12**



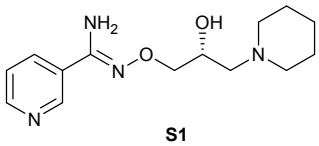
12

(*Z*)-Pyridine-3-amidoxime-1-oxide **4** (100 mg, 0.65 mmol) was partially dissolved in anhydrous DMF (6 mL) with a large stirrer bar (slurry forms after sodium hydride addition) and the solution was cooled to 0 °C under an N_2 atmosphere for 15 minutes. Some precipitate was still seen. Sodium hydride (60% wt, 34 mg, 0.85 mmol) was added with gas evolution seen. The flask was sealed and vented to a balloon. A yellow slurry was seen after 15 minutes and after further 15 minutes no further evolution of gas. Vigorous stirring was required to keep the slurry moving. After the 30 minutes (*R*)-oxiran-2-ylmethyl 3-nitrobenzenesulfonate (*ee* >97.5%) (178 mg, 0.69 mmol) was dissolved in anhydrous DMF (0.5 mL) and added dropwise to the stirring cooled solution. The flask was allowed to warm to room temperature and after 2 hours the dark brown solution showed no starting material by LCMS or TLC (20% MeOH in EtOAc). To the reaction piperidine (0.07 mL, 0.85 mmol) was added and the reaction was heated to 80 °C for 4 hours followed by stirring at room temperature for 18 hours. The reaction solvent was removed *in vacuo* and the residue was triturated with chloroform (30 mL) using sonication and then filtered. The solid, by-product sodium 3-nitrobenzenesulfonic acid, was washed with chloroform (2 x 25 mL) and the combined organic solvent was removed *in vacuo* to give a brown oil. ^{1}H NMR of the crude showed minimal impurities of starting material or intermediates with some DMF still seen as well.

The product was purified by reverse phase FCC on Biotage Isolera using Biotage Ultra SNAP 30 g C18 Si cartridge eluting with gradient elution 2-100% H_2O:MeOH both containing 0.1% NH_4OH, to afford the *title compound* (137 mg, 71% yield) as a pale brown gel. Analytical data was consistent with literature values. Optical rotation was not determined as absolute configuration and enantiomeric ratio were determined in the ultimate product of this building block **2** or **2**·citrate synthesised under the various methodologies quoted in the text. (See ESI section SFC traces)

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.50 (t, *J* = 1.4 Hz, 1H), 8.13 (ddd, *J* = 6.4, 1.7, 1.0 Hz, 1H), 7.52 – 7.42 (m, 1H), 7.23 (s, 1H), 5.04 (s, 2H), 4.02 (ddd, *J* = 10.0, 9.2, 5.6 Hz, 3H), 2.54 (s, 2H), 2.41 – 2.15 (m, 5H), 1.51 (dt, *J* = 12.0, 6.0 Hz, 4H), 1.44 – 1.32 (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 147.5, 139.7, 137.0, 132.4, 125.8, 123.5, 77.0, 65.5, 60.9, 54.7, 50.9, 26.1, 24.3.

(R,Z)-3-(N'-(2-hydroxy-3-(piperidin-1-yl)propoxy)carbamimidoyl)pyridine[2] – **S1**

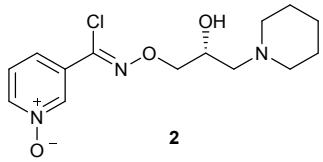


S1

Reaction was repeated as with **12** however (*Z*)-pyridine-3-amidoxime was used as the amidoxime starting material yielding the *title compound* (172 mg, 85% yield) as pale brown gel. Analytical data was consistent with literature values. Optical rotation was not determined as absolute configuration and enantiomeric ratio were determined in the ultimate product of this building block **2** or **2**·citrate synthesised under the various methodologies quoted in the text. (See ESI section SFC traces)

^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.89 – 8.83 (m, 1H), 8.64 (dd, *J* = 4.8, 1.6 Hz, 1H), 7.97 – 7.89 (m, 1H), 7.31 (ddd, *J* = 7.9, 4.8, 0.7 Hz, 1H), 4.94 (s, 2H), 4.16 – 4.06 (m, 3H), 2.60 (s, 2H), 2.43 – 2.30 (m, 4H), 1.64 – 1.53 (m, 4H), 1.44 (s, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ: 151.0, 149.9, 147.2, 133.6, 128.5, 123.5, 76.7, 65.8, 61.0, 54.8, 26.1, 24.3.

(R,Z)-3-(N'-(2-hydroxy-3-(piperidin-1-yl)propoxy)carboximidoyl chloride)pyridine-1-oxide[1] *– (R)-(+)-Arimoclomol* **– 2**

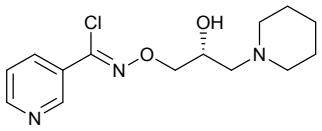


2

A solution of (*R*,*Z*)-3-(*N*'-(2-hydroxy-3-(piperidin-1-yl)propoxy)carbamimidoyl)pyridine-1-oxide **12** (205 mg, 0.70 mmol) in conc. hydrochloric acid (1.1 mL, 13.9 mmol) and water (3 mL) was cooled to -5 °C for 15 minutes. Sodium nitrite (63 mg, 0.91 mmol) in water (0.5 mL) was then added dropwise to the reaction mixture and the reaction was stirred at -5 °C for 2.5 hours. The reaction mixture was made alkaline with NaOH (7 M, 3 mL). An additional 10 mL of water was added followed by DCM (30 mL) containing EtOAc (5 mL) and the organics were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by FCC on Biotage Isolera using Biotage SNAP 10 g Si cartridge eluting with gradient elution 0-30% MeOH:DCM both containing 0.1% Et_3N to afford the *title compound* (160 mg, 73% yield) as a colourless semi-solid. Analytical data was consistent with literature values. See ESI section SFC traces for specific enantiomeric ratios of **2** synthesised under the various methodologies quoted in the text. Optical rotation was not determined as it was determined in the ultimate product of this **2**·citrate and comparative run times on SFC.

^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.63 (t, *J* = 1.4 Hz, 1H), 8.16 (ddd, *J* = 6.4, 1.6, 0.9 Hz, 1H), 7.66 – 7.62 (m, 1H), 7.25 (dd, *J* = 8.0, 6.6 Hz, 1H), 4.26 (qd, *J* = 11.3, 5.2 Hz, 2H), 4.07 (dd, *J* = 9.2, 4.7 Hz, 1H), 2.62 (s, 2H), 2.47 – 2.31 (m, 4H), 1.65 – 1.51 (m, 4H), 1.42 (s, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ: 140.3, 137.7, 133.1, 132.5, 125.7, 123.9, 78.7, 64.9, 60.9, 54.8, 25.8, 24.0.

(R,Z)-3-(N'-(2-hydroxy-3-(piperidin-1-yl)propoxy)carboximidoyl chloride)pyridine[3] *– (R)-(+)-Bimoclomol –* **1**

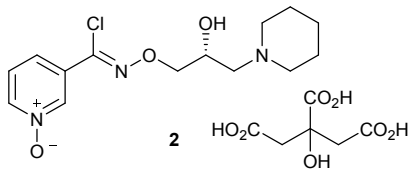


1

Reaction was repeated as with **2** however (*R,Z*)-3-(*N*'-(2-hydroxy-3-(piperidin-1-yl)propoxy)carbamimidoyl) pyridine **S1** was used as the starting material yielding the *title compound* (172 mg, 85% yield) as pale brown gel. Analytical data was consistent with literature values. See ESI section SFC traces for specific enantiomeric ratios of **2** synthesised under the various methodologies quoted in the text. Optical rotation was not determined as absolute configuration it was determined in the ultimate product of this **1**·maleate and comparative run times on SFC.

^{1}H NMR (600 MHz, $CDCl_3$) δ: 9.00 (d, *J* = 1.7 Hz, 1H), 8.59 (dd, *J* = 4.8, 1.6 Hz, 1H), 8.11 – 7.95 (m, 1H), 7.28 (ddd, *J* = 8.0, 4.8, 0.7 Hz, 1H), 4.29 – 4.22 (m, 2H), 4.04 (td, *J* = 9.9, 4.5 Hz, 1H), 2.56 (s, 2H), 2.46 – 2.26 (m, 4H), 1.58 – 1.48 (m, 4H), 1.39 (s, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ: 151.2, 148.2, 135.5, 134.5, 128.8, 123.2, 78.4, 65.1, 60.9, 54.7, 26.1, 24.2.

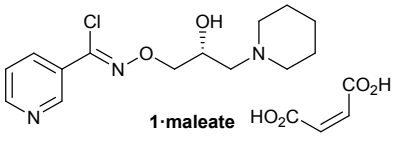
(R,Z)-3-(N'-(2-hydroxy-3-(piperidin-1-yl)propoxy)carboximidoyl chloride)pyridine-1-oxide citrate[1] *- (R)-(+)-Arimoclomol citrate* **– 2·**citrate



2

(*R,Z*)-3-(*N*'-(2-hydroxy-3-(piperidin-1-yl)propoxy)carboximidoyl chloride)pyridine-1-oxide (159 mg, 0.51 mmol) was dissolved in acetone (3 mL) and citric acid (97 mg, 0.51 mmol) was added. The reaction mixture was left to stir at room temperature for 18 hours. After this time the mixture was sonicated and the precipitate was filtered, rinsed with cold acetone (1 mL) and dried under vacuum to afford the *title compound* (165 mg, 64% yield) as a white amorphous solid. Analytical data was consistent with literature values. m.p. 161-162 °C, Acetone (lit. 163-165 °C, EtOH); $[\alpha]_D^{20}$ +8.0 (c=1, H_2O); IR vmax (neat): 3423, 3228, 2949, 2868, 1722, 1589, 1483, 1433, 1307, 1128, 972, 829 cm^{-1}; ^{1}H NMR (600 MHz, d6-DMSO) δ: 8.54 (t, *J* = 1.5 Hz, 1H), 8.39 – 8.35 (m, 1H), 7.72 – 7.68 (m, 1H), 7.55 (dd, *J* = 8.0, 6.5 Hz, 1H), 4.28 (ddd, *J* = 17.6, 13.3, 7.4 Hz, 3H), 3.35 (br. s, 2H), 3.13 – 2.74 (m, 6H), 2.59 (d, *J* = 15.2 Hz, 2H), 2.56 – 2.51 (m, 2H), 1.77 – 1.61 (m, 4H), 1.48 (s, 2H); ^{13}C NMR (151 MHz, d6-DMSO) δ: 176.6, 171.3, 140.5, 136.4, 132.7, 131.5, 126.8, 123.3, 77.8, 71.4, 63.8, 58.7, 53.1, 44.0, 30.7, 23.0, 21.9; HRMS (m/z TOF MS ES+) for $C_{14}H_{20}ClN_3O_3$ [M+H]+ calc. 314.1271, observed 314.1263; SFC *er* purity R:S >99:1.

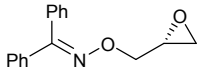
(R,Z)-3-(N'-(2-hydroxy-3-(piperidin-1-yl)propoxy)carboximidoyl chloride)pyridine maleate[3] *- (R)-(+)-Bimoclomol maleate* **– 1·**maleate



1·maleate

Reaction was repeated as with **2·citrate** however (*R,Z*)-3-(*N*'-(2-hydroxy-3-(piperidin-1-yl)propoxy)carbamimidoyl)pyridine **S1** was used as the starting material and maleic acid was used as the organic acid to afford the *title compound* (70 mg, 63% yield) as an amorphous white solid. Analytical data was consistent with literature values. m.p. 137-138 °C, Acetone (lit. 136-137 °C , iPrOH); $[\alpha]_D^{20}$ +6.0° (c=1, MeOH) (lit. $[\alpha]_D^{27}$ +5.6° (c=1, MeOH)); IR vmax (neat): 3269, 2937, 1577, 1477, 1440, 1348, 1205, 1070, 981, 864 cm^{-1}; ^{1}H NMR (600 MHz, d6-DMSO) δ: 9.09 (s, 1H), 9.01 – 8.98 (m, 1H), 8.73 (dd, *J* = 4.8, 1.5 Hz, 1H), 8.24 – 8.06 (m, 1H), 7.57 (ddd, *J* = 8.1, 4.8, 0.6 Hz, 1H), 6.02 (d, *J* = 4.0 Hz, 2H), 5.93 (s, 1H), 4.40 – 4.21 (m, 3H), 3.60 – 3.28 (m, 3H), 3.20 (d, *J* = 11.8 Hz, 1H), 3.12 – 3.05 (m, 1H), 3.03 – 2.83 (m, 2H), 1.84 – 1.55 (m, 5H), 1.38 (s, 1H); ^{13}C NMR (151 MHz, d6-DMSO) δ: 167.1, 151.7, 147.4, 136.0, 135.1, 134.6, 127.9, 123.9, 77.2, 63.1, 58.0, 54.1, 51.1, 22.2, 21.3; HRMS (m/z TOF MS ES+) for $C_{14}H_{20}ClN_3O_2$ [M+H]+ calc. 298.1322, observed 298.1319; SFC *er* purity *R*:*S* 98:2.

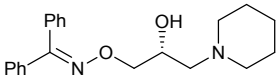
(R)-Diphenylmethanone O-oxiran-2-ylmethyl oxime[4] **– 14**



14

Benzophenoxime (1.20 g, 6.08 mmol) (previously recrystallized from MeOH, 4 mL/g, and used immediately after drying *in vacuo*) was dissolved in DMF (18 mL) and the solution was cooled to 0 °C with an ice bath and under an N_2 atmosphere. Sodium hydride (60% wt., 290 mg, 7.30 mmol) was added portionwise over 2 minutes with significant gas evolution seen. The flask was sealed and vented to a balloon. The solution was stirred for 30 minutes still on ice where it was noticed that it turned from yellow to a dark reddish orange. After 30 minutes (*R*)-oxiran-2-ylmethyl 3-nitrobenzenesulfonate (*ee* >97.5%) (1.66 g, 6.39 mmol) was dissolved in DMF (2 mL) and added dropwise to the stirring cooled solution. The flask was allowed to warm to room temperature and after 2 hours the pale brown solution showed no SM by LCMS or TLC (9:1 CyHex:EtOAc). The reaction was quenched by slow addition of water until gas evolution ceased. The reaction was then partitioned between water (50 mL) and ethyl acetate (120 mL). The organics were separated and washed with 5% LiCl solution (40 mL), brine (2 x 50 mL), dried over $MgSO_4$, filtered and silica added to the crude organics for FCC and then the solvent was removed *in vacuo*. The product was purified by FCC on Biotage Isolera using Biotage SNAP 50 g Si cartridge eluting with gradient elution 1-15% CyHex-EtOAc affording the *title compound* (1.39 g, 90% yield) as a colourless oil. Analytical data was consistent with literature values. Enantiomeric ratio and optical rotation were not determined as absolute configuration and enantiomeric ratio were determined in the ultimate product of this building block **2**·citrate or **1**·maleate.
^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.48 – 7.22 (m, 10H), 4.29 (dd, *J* = 12.4, 3.6 Hz, 1H), 4.08 (dd, *J* = 12.4, 5.7 Hz, 1H), 3.26 – 3.20 (m, 1H), 2.79 – 2.74 (m, 1H), 2.57 (dd, *J* = 5.0, 2.7 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ: 157.65, 136.41, 133.18, 129.59, 129.4, 129.0, 128.3, 128.2, 128.1, 75.1, 50.4, 45.1.

(R)-Diphenylmethanone O-(2-hydroxy-3-(piperidin-1-yl)propyl) oxime[5] **– 15**

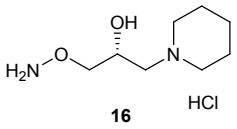


15

(*R*)-Diphenylmethanone *O*-oxiran-2-ylmethyl oxime **14** (1.38 g, 5.50 mmol) was dissolved in IPA (22 mL) and piperidine (0.56 mL, 5.70 mmol) was added. The reaction was heated to 50 °C and left stirring for 18 hours. After which LCMS indicated no remaining starting material. The solvent was removed *in vacuo* and the crude

oil subjected to multiple additions of methanol followed by removal *in vacuo* to give a thick pale brown oil. Over 24 hours, under high vacuum, the thick pale brown oil crystallised to give the *title compound* (1.85 g, quantitative yield) as a cream solid and was used without further purification as analytical data was consistent with literature values. Enantiomeric ratio and optical rotation were not determined as absolute configuration and enantiomeric ratio were determined in the ultimate product of this building block **2**·citrate or **1**·maleate.
^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.49 – 7.31 (m, 10H), 4.22 – 4.16 (m, 2H), 4.06 (td, *J* = 9.7, 4.6 Hz, 1H), 3.69 (s, 1H), 2.56 (s, 2H), 2.43 – 2.24 (m, 4H), 1.62 – 1.51 (m, 4H), 1.44 (s, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ: 157.3, 136.5, 133.2, 129.4, 129.4, 129.0, 128.3, 128.1, 128.1, 65.9, 61.3, 54.8, 26.2, 24.3.

(R)-1-(3-(aminooxy)-2-hydroxypropyl)piperidine hydrochloride – **16**



16

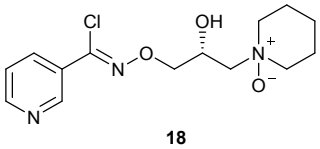
(*R*)-Diphenylmethanone *O*-(2-hydroxy-3-(piperidin-1-yl)propyl) oxime **15** (1.84 g, 5.45 mmol) was suspended in hydrochloric acid (6 M, 20 mL, 120 mmol) and heated to 95 °C for 18 hours. After 10 minutes of heating the suspension dissolved and droplets of pale yellow oil were noticed. After 18 hours LCMS indicated no remaining starting. The reaction mixture was diluted with water (2 mL) and cooled to room temperature and Et_2O was added (50 mL) and the organics were separated. The aqueous layer was washed with Et_2O (2 x 30 mL) and then separated. The aqueous layer was dried *in vacuo* until a white foam solid was seen. Repeated addition of methanol followed by toluene was carried out to dry the sample. After 6 hours under vacuum on a Büchi at 50 °C a white sticky solid (~1.3 g) was produced. This solid was seen to be highly hygroscopic, becoming a white gel within one hour. The gel was dissolved in MeOH (30 mL) and to this Amberlyst A21 was added (5 mmol/g N loading) (12 g, 60 mmol) The beads were pre-soaked with 50 mL MeOH for 20 minutes and then filtered, washed with a further 2 x 50 mL MeOH followed by 20 mL Et_2O and dried at room temperature for 15 minutes on a sintered funnel. The reaction was gently mixed using a rotary motion so to not break up the resin for 3 hours. The beads were removed by filtration and then the flask washed with 2 x 10 mL of MeOH. The beads were then washed with 3 x 30 mL fresh MeOH and the solvent of the filtrate was removed *in vacuo*. A small amount of diethyl ether was added and the product began to crystallise. The solvent was removed *in vacuo* and the solid dried *in vacuo* at 45 °C for 4 hours. The product was broken up and further dried under high vacuum for 72 hours to give the *title compound* (1.10 g, 98% yield) as a crystalline white solid.
^{1}H NMR (600 MHz, d4-MeOD) δ: 4.19 (dtd, *J* = 8.3, 5.3, 3.1 Hz, 1H), 3.60 (qd, *J* = 11.0, 5.3 Hz, 2H), 3.27 (dt, *J* = 3.2, 1.6 Hz, 1H), 3.25 – 3.01 (m, 5H), 2.97 (dd, *J* = 13.2, 10.2 Hz, 1H), 1.86 – 1.74 (m, 4H), 1.61 (s, 2H); ^{13}C NMR (151 MHz, d4-MeOD) δ: 78.2, 65.3, 60.8, 54.9, 24.2, 23.0; m.p. 101-103 °C; $[\alpha]_D^{20}$ +24.0 (c=1, MeOH)

General procedure for the thiol promoted addition of hydroxylamines into nitriles

To a suspension of the appropriate nitrile (1.0 equiv.) and (*R*)-1-(3-(aminooxy)-2-hydroxypropyl)piperidine hydrochloride **16** (1.2 equiv.) in EtOH (0.33M) in a microwave vial. Triethylamine (3.5 equiv.) was then added followed by thioglycolic acid (1.5 equiv.). The flask was sealed and the reaction mixture was stirred at 85 °C for 24 hours. TLC (20% MeOH in DCM) showed no SM so the reaction was allowed to cool to room temperature. The solvent was removed *in vacuo* and the crude reaction was purified by FCC on a Biotage Isolera using Biotage Ultra SNAP 30 g C18 Si eluting with gradient elution 2-100% H_2O:MeOH with 0.1% NH_4OH modifier. Analytical data of the products was consistent with literature and/or previous samples synthesised above.

*m*CPBA oxidation with methanesulfonic acid leading to oxidation on piperidine ring –

(R,Z)-1-(3-(((chloro(pyridin-3-yl)methylene)amino)oxy)-2-hydroxypropyl)piperidine 1-oxide - **18**



18

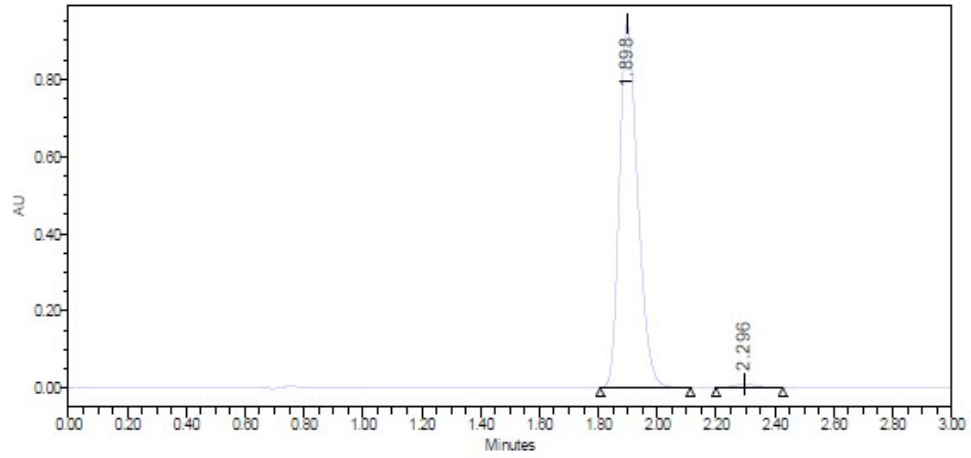
To a solution of (3*Z*)-*N*-[(2*R*)-2-hydroxy-3-(1-piperidyl)propoxy]pyridine-3-carboximidoyl chloride (75 mg, 0.25 mmol) in DCM (2 mL) at 0 °C was added methanesulfonic acid (0.05 mL, 0.76 mmol). After 5 minutes, 3-chloroperbenzoic acid (77%, 62 mg, 0.28 mmol) was added and the reaction mixture stirred for 18 hours, whilst slowly warming to room temperature. LCMS revealed some SM remaining. The reaction mixture was diluted with DCM and washed with aq. sat. $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by FCC on a Biotage Isolera using Biotage SNAP 10g Si cartridge eluting with gradient elution 0-30% MeOH in DCM to afford the *title compound* (25 mg, 31% yield) as a colourless gum.
^{1}H NMR (600 MHz, d4-MeOD) δ: 8.96 (dd, *J* = 2.2, 0.6 Hz, 1H), 8.58 (dd, *J* = 4.9, 1.5 Hz, 1H), 8.23 (ddd, *J* = 8.1, 2.2, 1.7 Hz, 1H), 7.48 (ddd, *J* = 8.1, 4.9, 0.7 Hz, 1H), 4.76 – 4.71 (m, 1H), 4.34 – 4.25 (m, 2H), 3.44 – 3.31 (m, 5H), 3.30 – 3.23 (m, 2H), 2.16 – 2.02 (m, 2H), 1.68 – 1.59 (m, 3H), 1.49 – 1.38 (m, 1H); ^{13}C NMR (151 MHz, d4-MeOD) δ: 151.9, 148.3, 136.6, 136.4, 130.5, 125.1, 78.9, 71.9, 67.1, 66.9, 65.7, 22.7, 22.0, 21.8.

Procedure for the conversion of (*R*)-(+)-Bimoclomol **1** into (*R*)-(+)-Arimoclomol **2**

To a solution of (*R*)-(+)-bimoclomol (61 mg, 0.21 mmol) in acetone (2 mL) was added benzenesulfonic acid (33 mg, 0.21 mmol). The reaction mixture was stirred at room temperature for 1.5 hours. The reaction mixture was concentrated *in vacuo*. Separately to a suspension of hydrogen peroxide-urea adduct (39 mg, 0.41 mmol) in acetonitrile (6 mL) at -5°C (ice-salt bath) was added trifluoroacetic anhydride (58 µL, 0.41 mmol) dropwise. A suspension of (*R*)-(+)-bimoclomol, **1**, benzenesulfonic acid salt, as made above, in acetonitrile (3 mL) was then added dropwise to this solution. The reaction mixture was stirred for 18 hours, whilst slowly warming to room temperature. Aqueous $Na_2S_2O_5$ solution (0.5 M, 1 mL) was added and the reaction mixture stirred for 1 hour. The reaction mixture was made alkaline with NaOH (7 M) and extracted with DCM (2 x 30 mL). The combined organics were washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by FCC on a Biotage Isolera using Biotage SNAP 10g Si cartridge eluting with gradient elution 0-35% MeOH in DCM to afford the *title compound* (35 mg, 55% yield) as a colourless semi-solid. Analytical data of the products was consistent with literature and/or previous samples synthesised above.

Chiral SFC Traces, methods and conditions

(*R*)-(+)-arimoclomol (**2**) trace of authentic sample

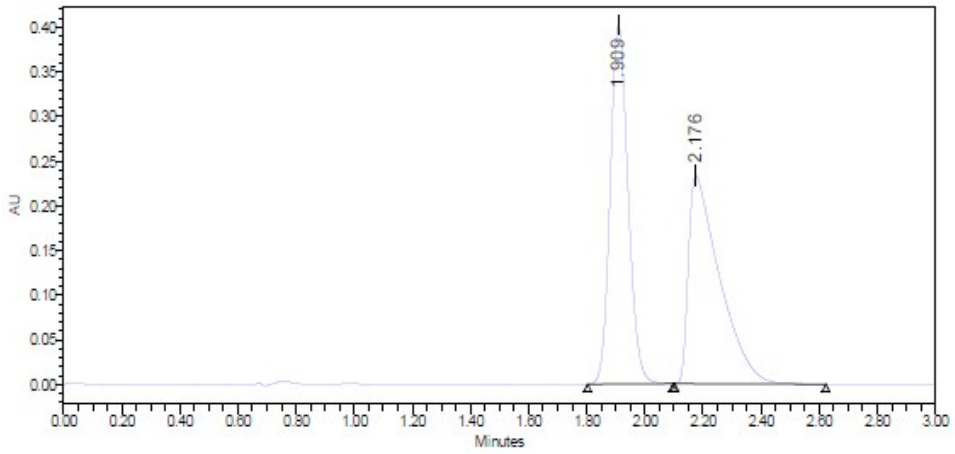


Peak Results

	Retention Time (min)	Area (µV*sec)	% Area
1	1.90	4098839	98.9
2	2.30	47370	1.1

Column Details	AMS (4.6mm x 250mm, 5um)
Column Temperature	40 C
Flow Rate	4 mL/min
Detector Wavelength	210-400nm
Injection Volume	1.0 uL
BPR	125 BarG
Isocratic Conditions	25:75 MeOH:CO2 (0.2% v/v NH3)

Racemic arimoclomol (**2**) trace

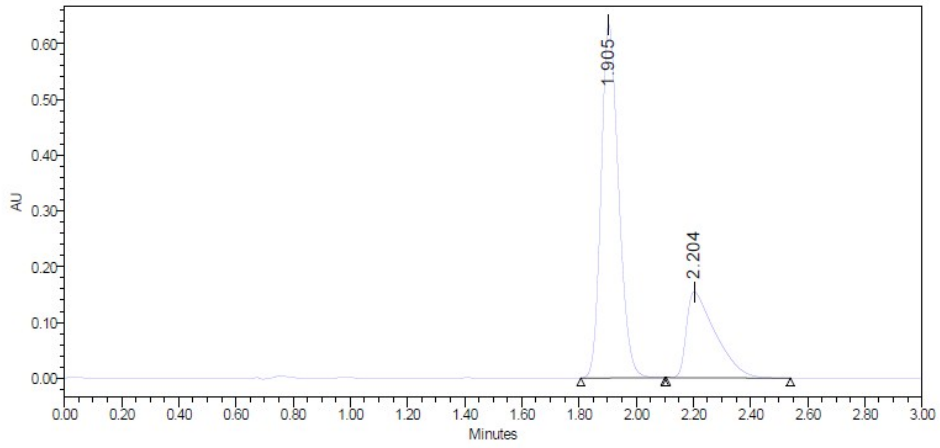


Peak Results

	Retention Time (min)	Area (µV*sec)	% Area
1	1.91	1721298	49.8
2	2.18	1732310	50.2

Column Details	AMS (4.6mm x 250mm, 5um)
Column Temperature	40 C
Flow Rate	4 mL/min
Detector Wavelength	210-400nm
Injection Volume	1.0 uL
BPR	125 BarG
Isocratic Conditions	25:75 MeOH:CO2 (0.2% v/v NH3)

(*R*)-(+)-arimoclomol (**2**) trace synthesised by resolution (Scheme 1)

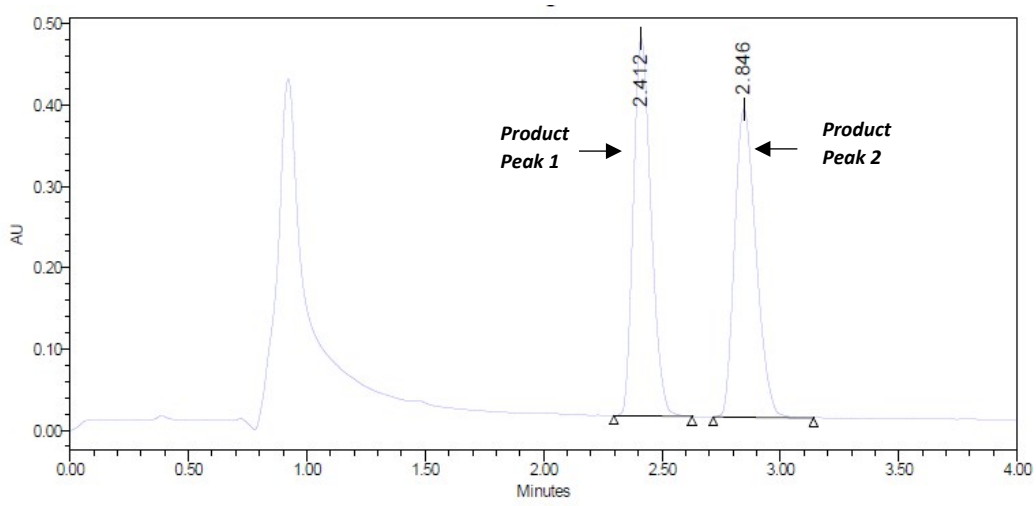


Peak Results

	Retention Time (min)	Area (µV*sec)	% Area
1	1.90	2706407	71.4
2	2.20	1082825	28.6

Column Details	Lux A1 (21.2mm x 250mm, 5um)
Column Temperature	40 C
Flow Rate	50 mL/min
BPR	100 BarG
Detector Wavelength	270 nm
Injection Volume	400 uL (12 mg)
Isocratic Conditions	40:60 MeOH:CO2 (0.2% v/v DEA)

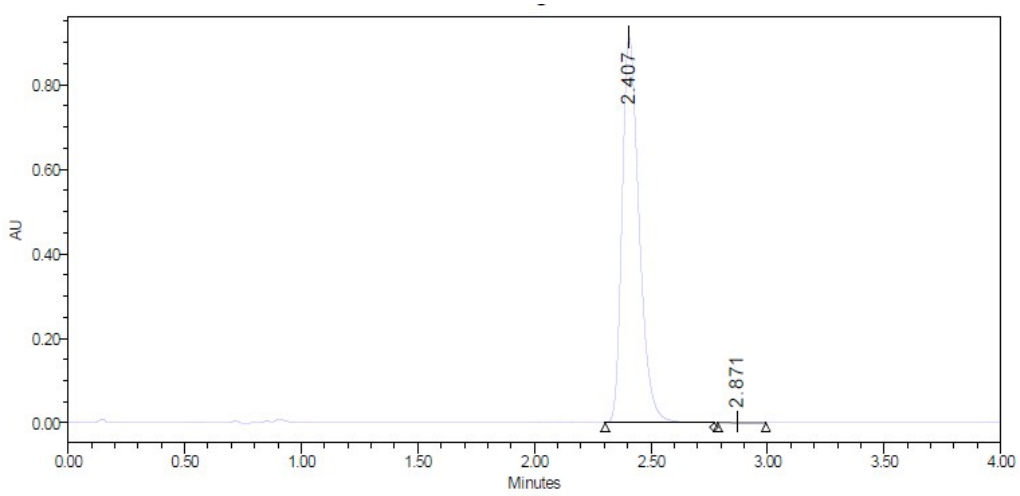
<u>Arimoclomol Preparative Chiral Purity racemate trace</u>



Peak Results

	Retention Time (min)	Area (µV*sec)	% Area
1	2.41	2412269	50.3
2	2.85	2382357	49.7

Chiral Purity Product Peak 1

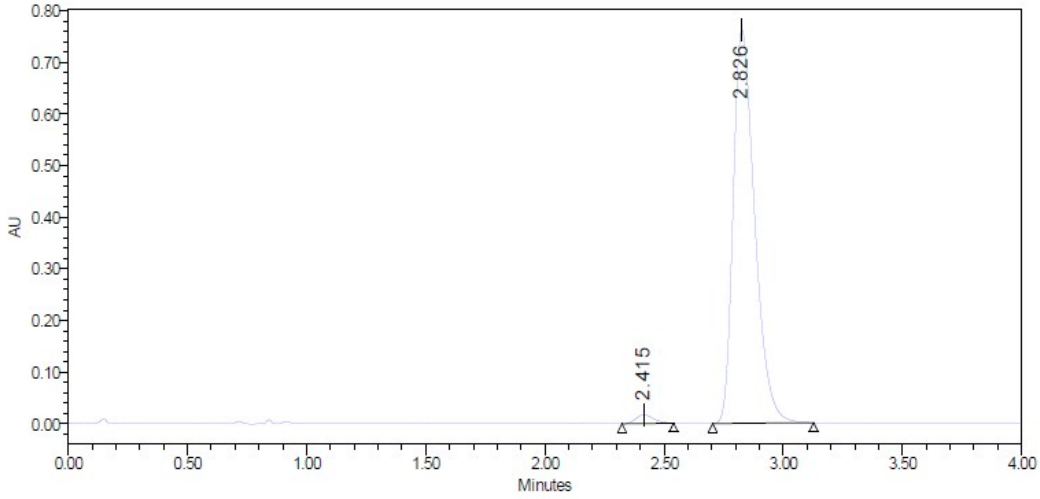


Peak Results

	Retention Time (min)	Area (µV*sec)	% Area
1	2.41	4649087	99.7
2	2.87	14118	0.3

Column Details	Amy-C (4.6mm x 250mm, 5um)
Column Temperature	40 C
Flow Rate	4 mL/min
Detector Wavelength	210-400nm
Injection Volume	1.0 uL
BPR	125 BarG
Isocratic Conditions	35:65 MeOH:CO2 (0.2% v/v DEA)

Chiral Purity Product Peak 2



Peak Results

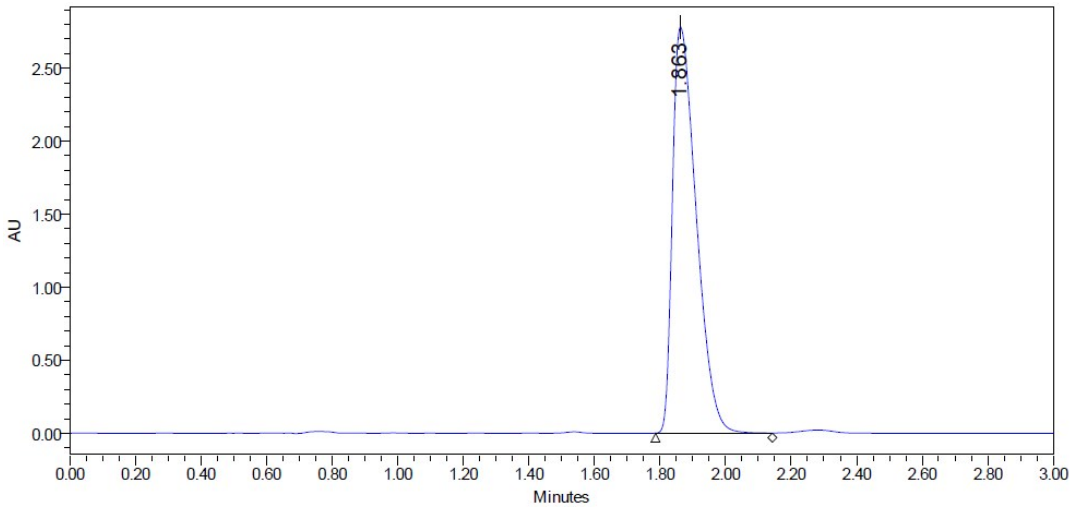
	Retention Time (min)	Area (µV*sec)	% Area
1	2.41	88803	1.8
2	2.83	4781834	98.2

Column Details	Amy-C (4.6mm x 250mm, 5um)
Column Temperature	40 C
Flow Rate	4 mL/min
Detector Wavelength	210-400nm
Injection Volume	1.0 uL
BPR	125 BarG
Isocratic Conditions	35:65 MeOH:CO2 (0.2% v/v DEA)

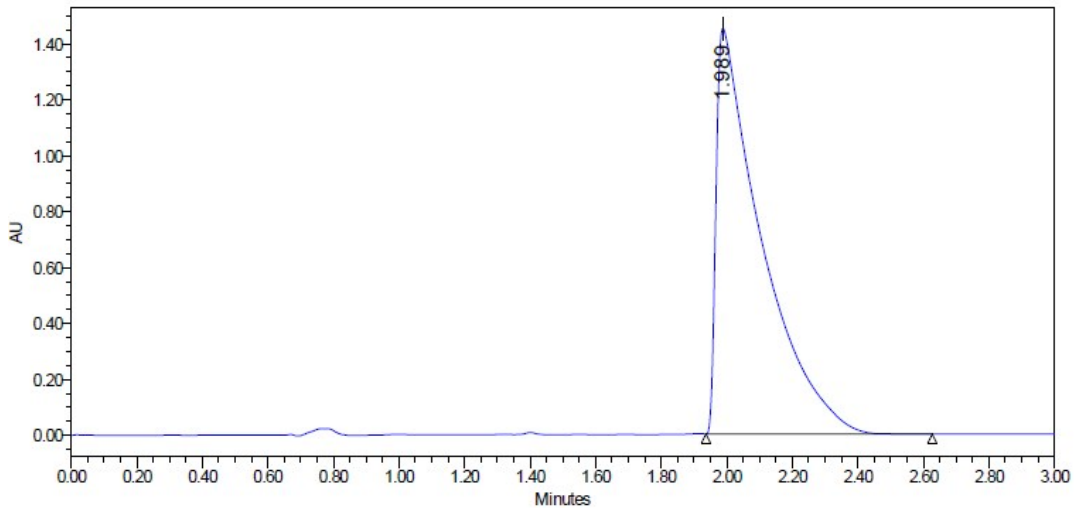
Stereochemical determination of the peaks from preparative chiral SFC

Upon analysis of the purified peaks under the same analytical condition as the authentic sample of *R*-(+)-arimoclomol (**2**) it was noted that, there was a switch in the elution order when changing from Amy-C column to AMS. Confirming the second eluting peak from the preparative SFC as *R*-(+)-arimoclomol (**2**), in comparison to authentic sample, and the first eluting peak as *S*-(-)-arimoclomol (**2**). Optical rotation of *R*-(+)-arimoclomol confirmed by $[\alpha]_D^{25}$ of the citrate salt.

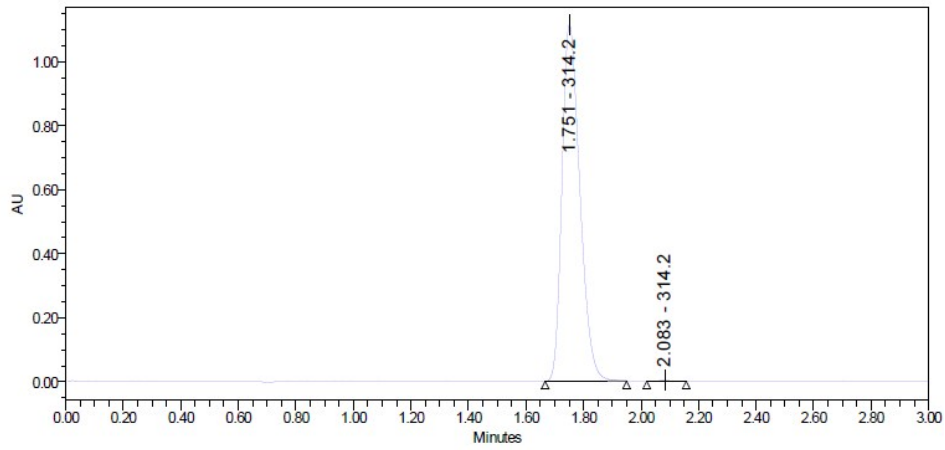
Chiral analysis of preparative Product Peak 2 on AMS column



Chiral analysis of preparative Product Peak 1 on AMS column



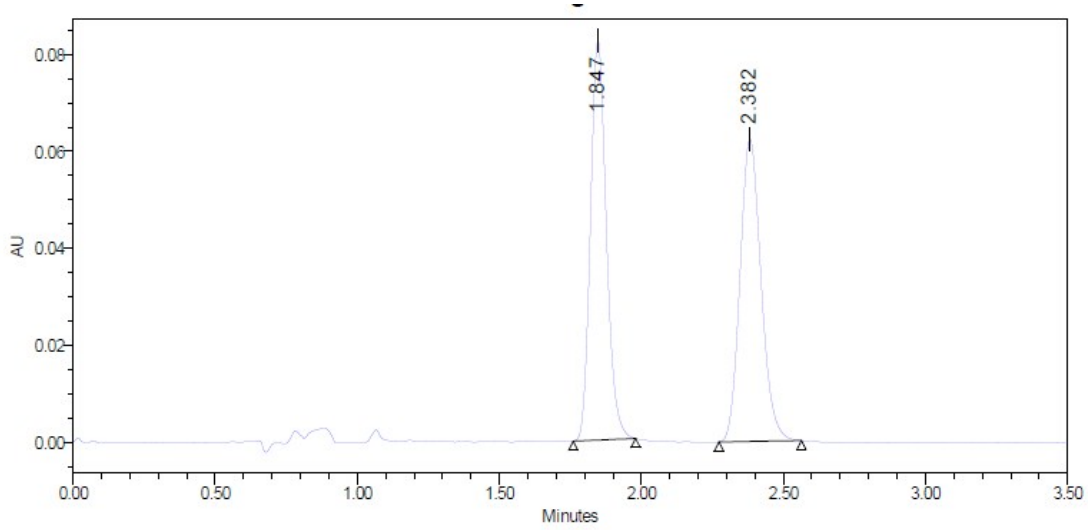
Chiral purity of R-(+)-arimoclomol (**2**) synthesised via one pot method in Scheme 2



Peak Results

	Retention Time (min)	Area (µV*sec)	% Area	Width @ 50%
1	1.75	4810967	99.8	
2	2.08	8495	0.2	

Racemic bimoclomol (**1**) trace

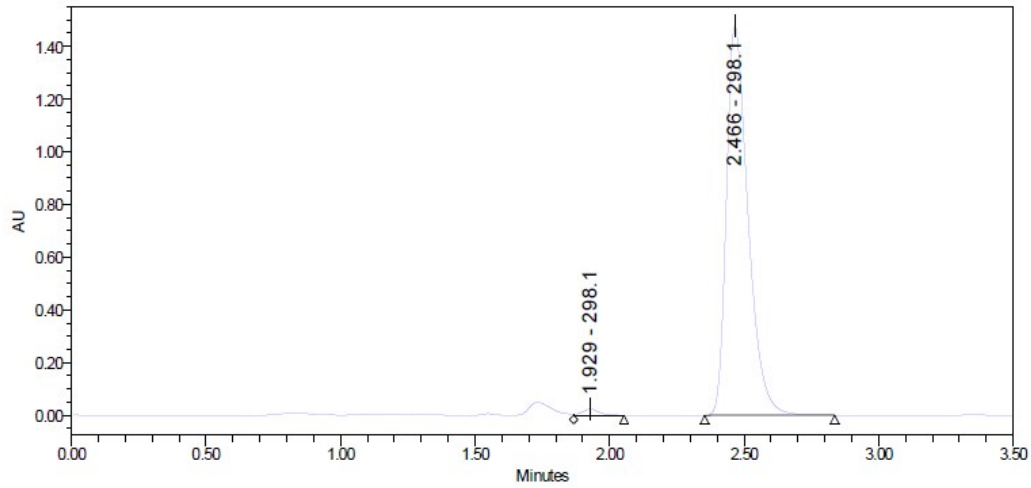


Peak Results

	Retention Time (min)	Area (µV*sec)	% Area
1	1.85	320273	49.9
2	2.38	321464	50.1

Column Details	Lux C4 (4.6mm x 250mm, 5um)
Column Temperature	40 C
Flow Rate	4 mL/min
Detector Wavelength	210-400nm
Injection Volume	1.0 uL
BPR	125 BarG
Isocratic Conditions	40:60 EtOH:CO2 (0.2% v/v NH3)

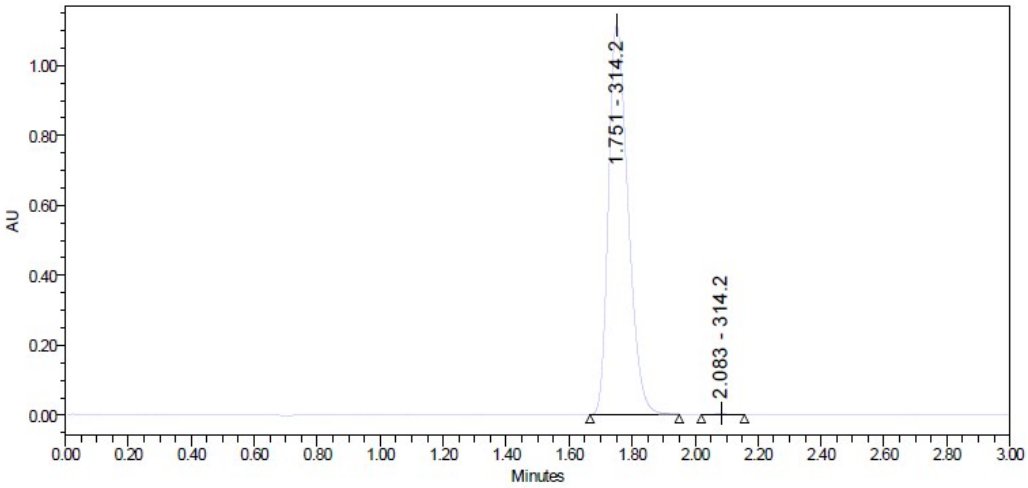
Chiral purity of *R*-(+)-bimoclomol (**1**) synthesised as in Scheme 2



Peak Results

	Retention Time (min)	Area (µV*sec)	% Area	Width @ 50%
1	1.93	97912	1.2	
2	2.47	8234833	98.8	

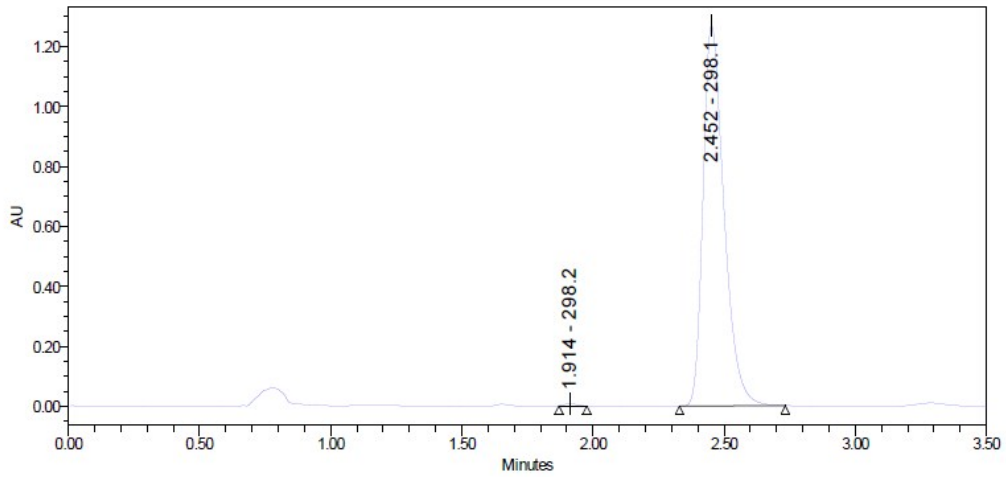
Chiral purity of *R*-(+)-arimoclomol (**2**) synthesised as in Scheme 3



Peak Results

	Retention Time (min)	Area (µV*sec)	% Area	Width @ 50%
1	1.75	4810967	99.8	
2	2.08	8495	0.2	

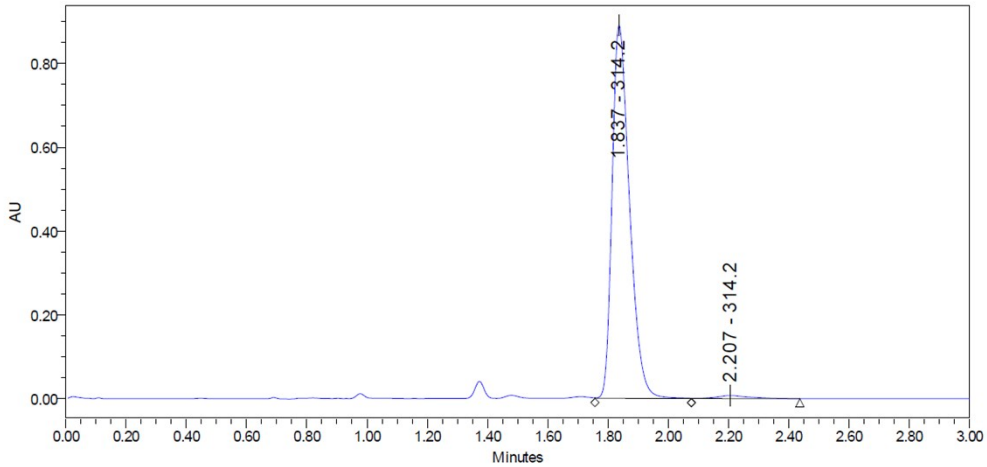
Chiral purity of *R*-(+)-bimoclomol (**1**) synthesised as in Scheme 3



Peak Results

	Retention Time (min)	Area (µV*sec)	% Area	Width @ 50%
1	1.91	22159	0.3	
2	2.45	7047904	99.7	

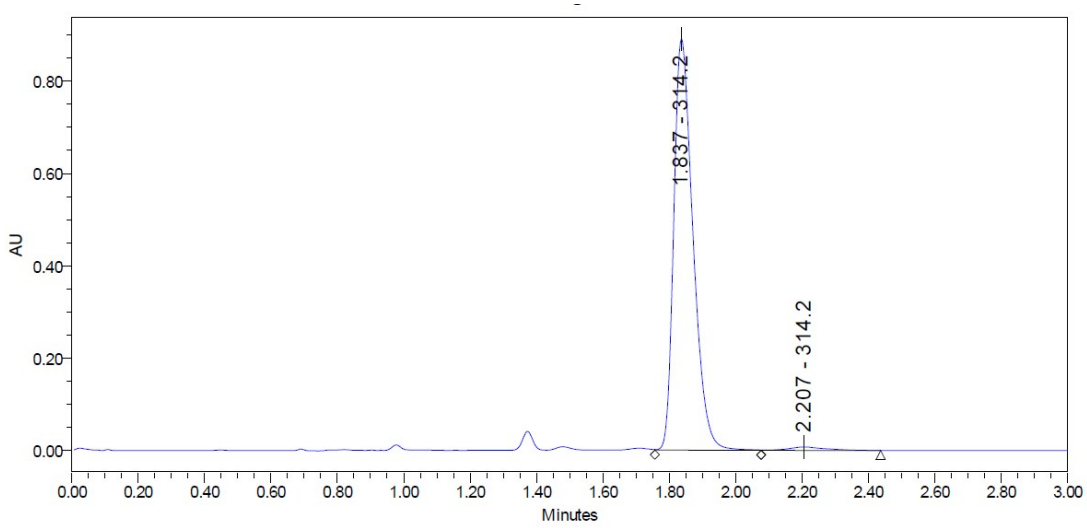
Chiral purity of *R*-(+)-arimoclomol (**2**) synthesised as in Scheme 4



Peak Results

	Retention Time (min)	Area (µV*sec)	% Area	Width @ 50%
1	1.84	3574552	98.4	0.06122
2	2.21	56655	1.6	0.10547

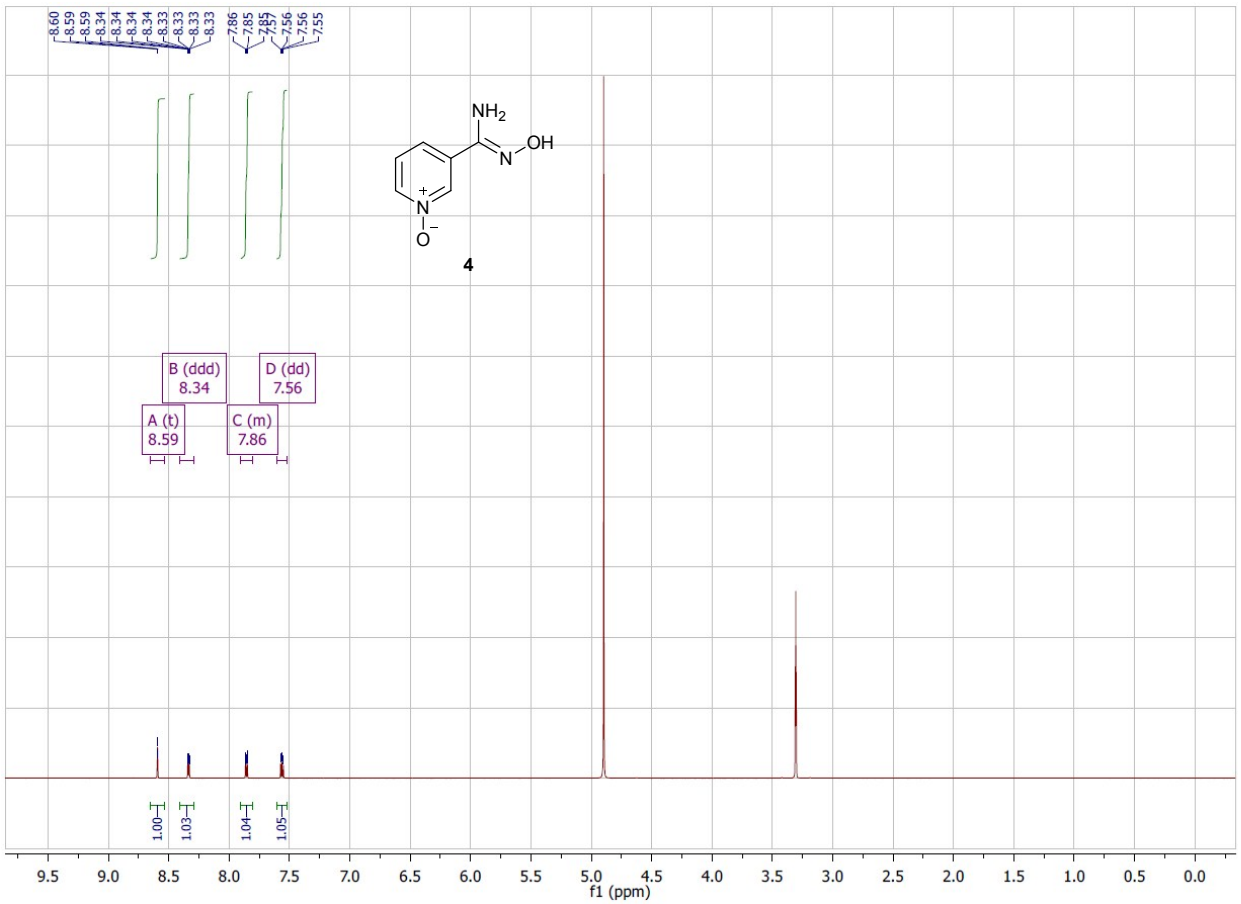
Chiral purity of *R*-(+)-arimoclomol (**2**) synthesised as in Scheme 5, method (b)

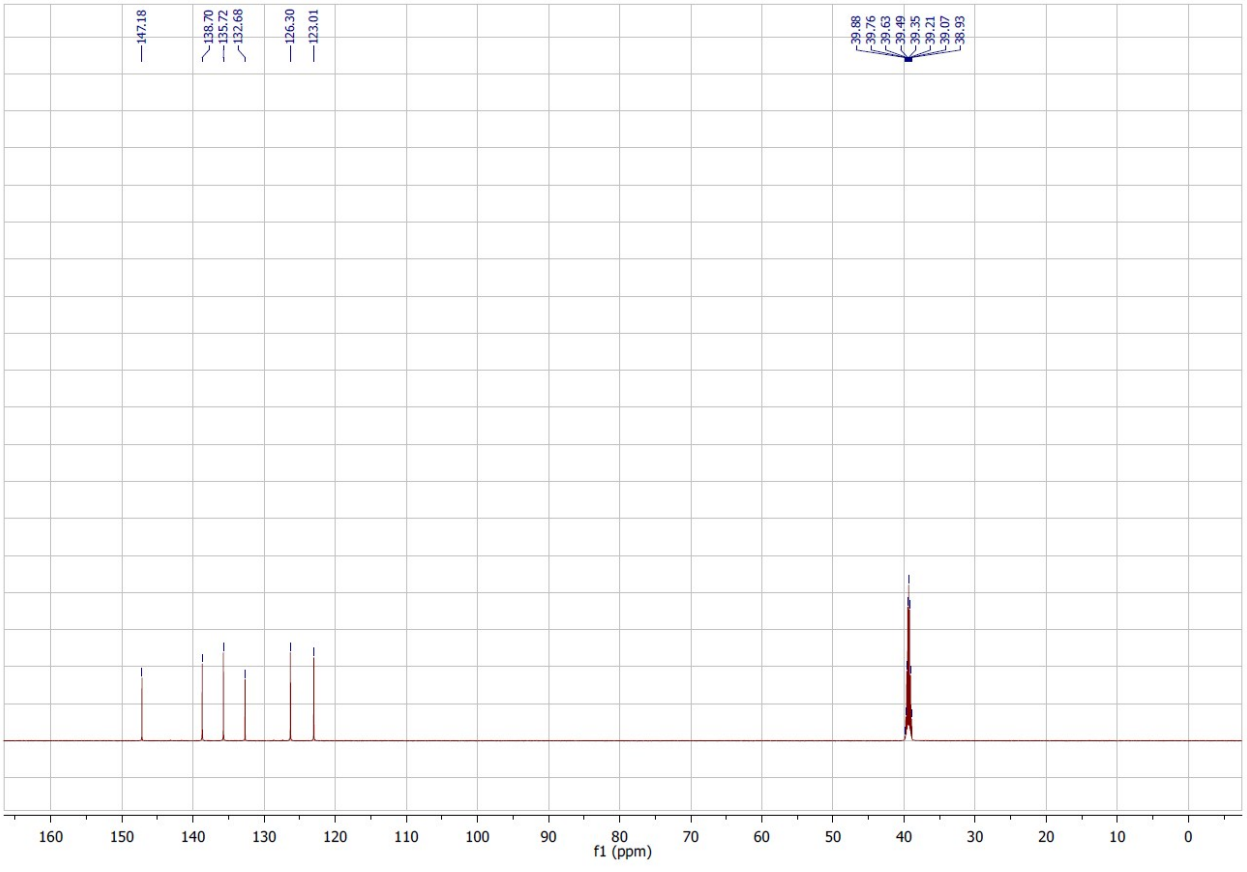


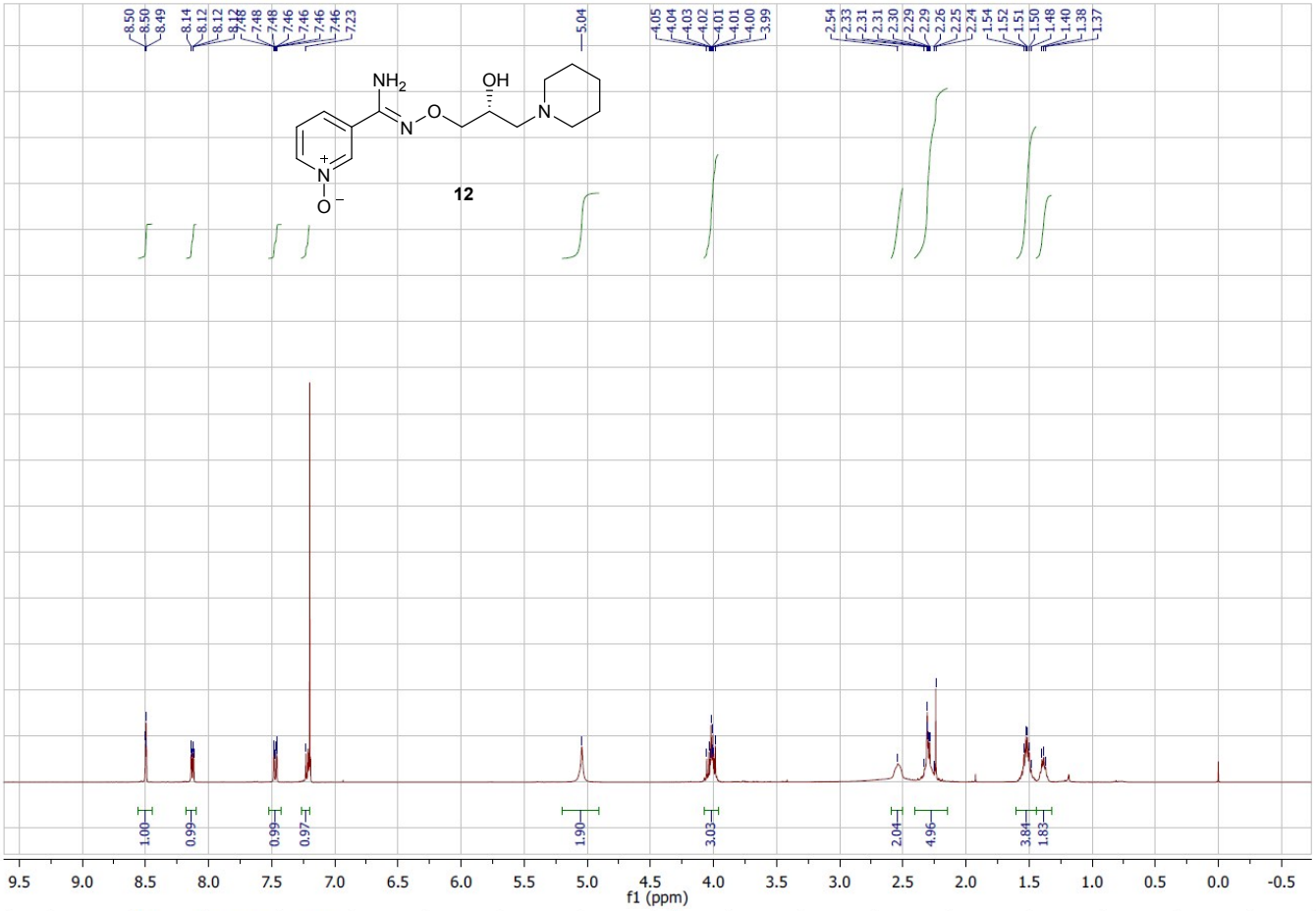
Peak Results

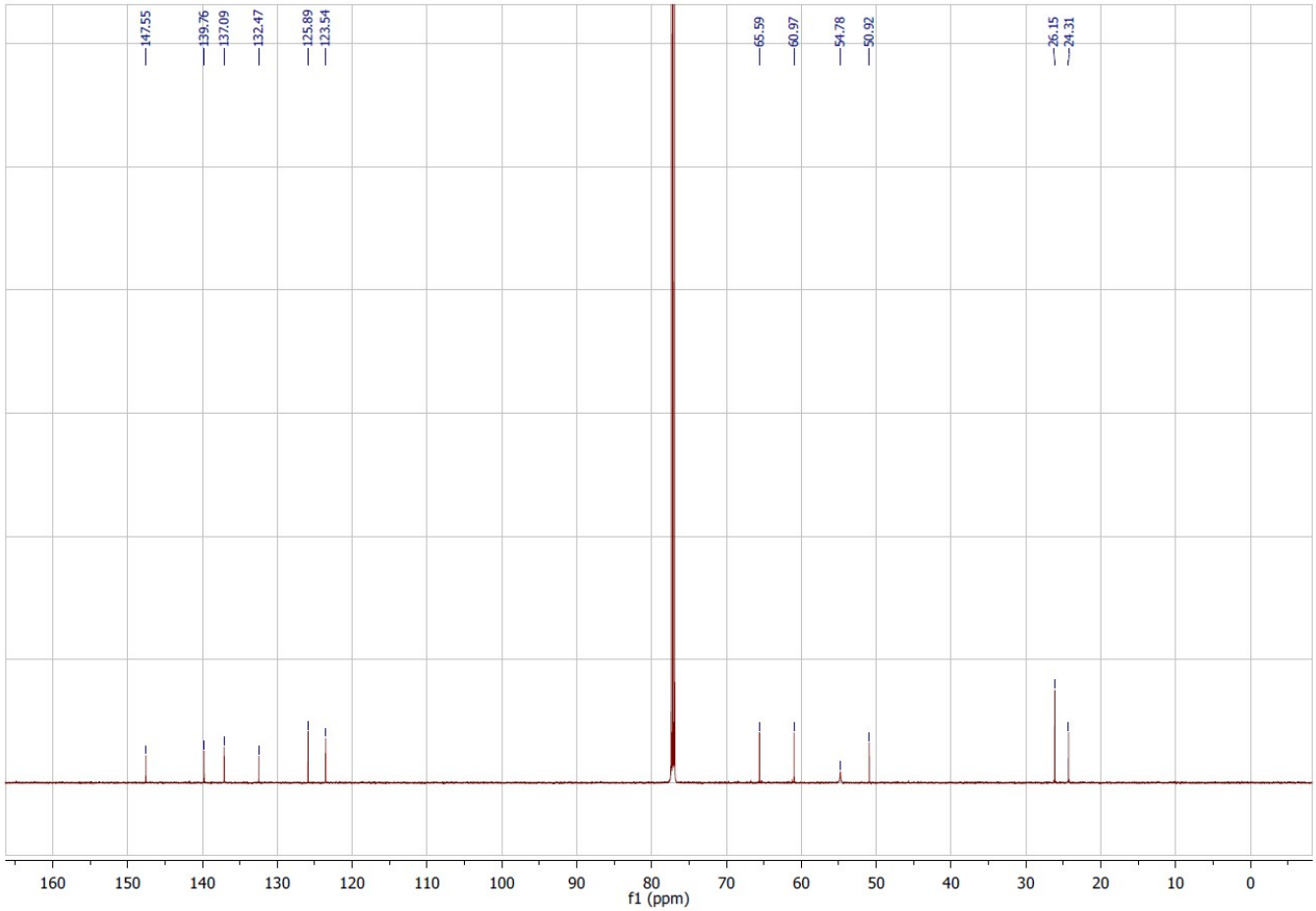
	Retention Time (min)	Area (µV*sec)	% Area	Width @ 50%
1	1.84	3574552	98.4	0.06122
2	2.21	56655	1.6	0.10547

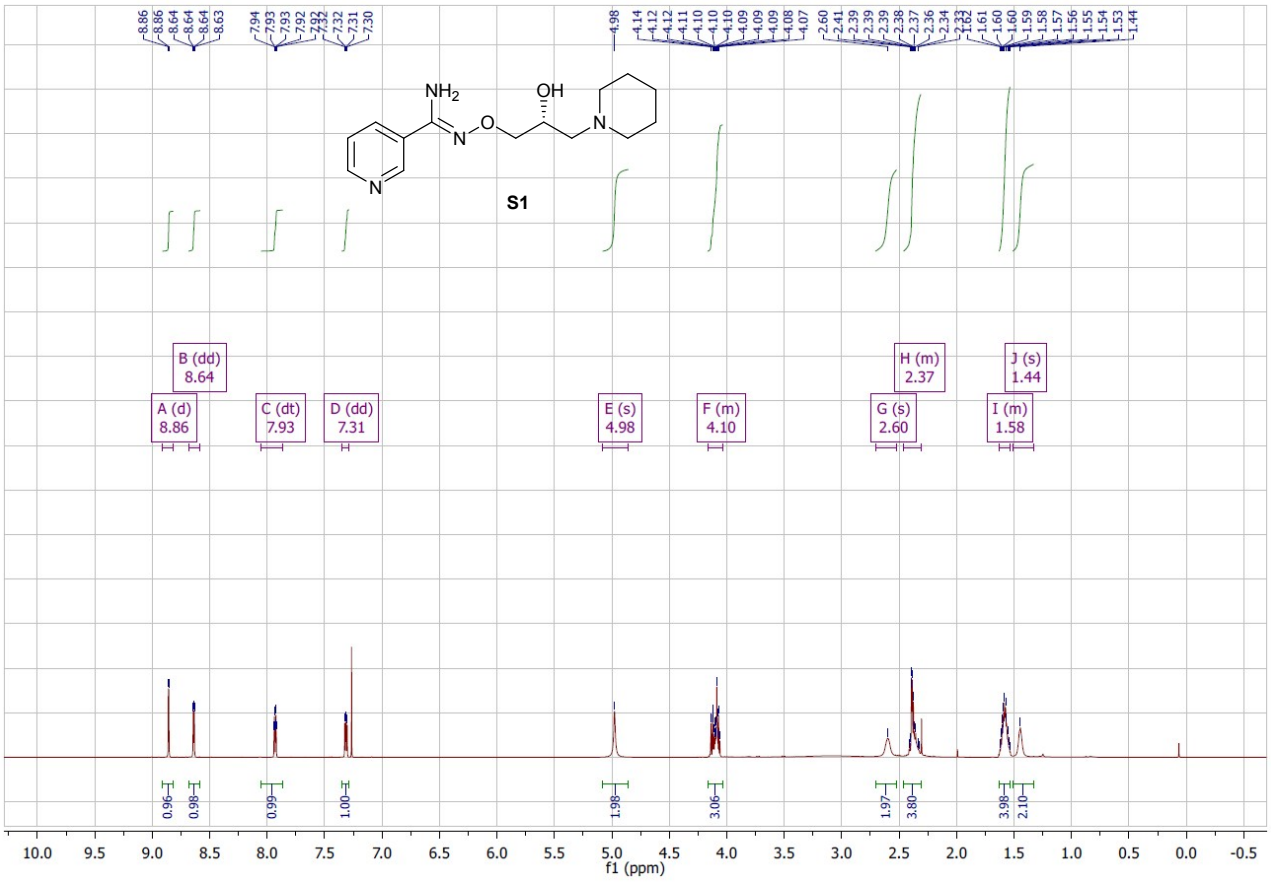
^{1}H and ^{13}C NMR Traces

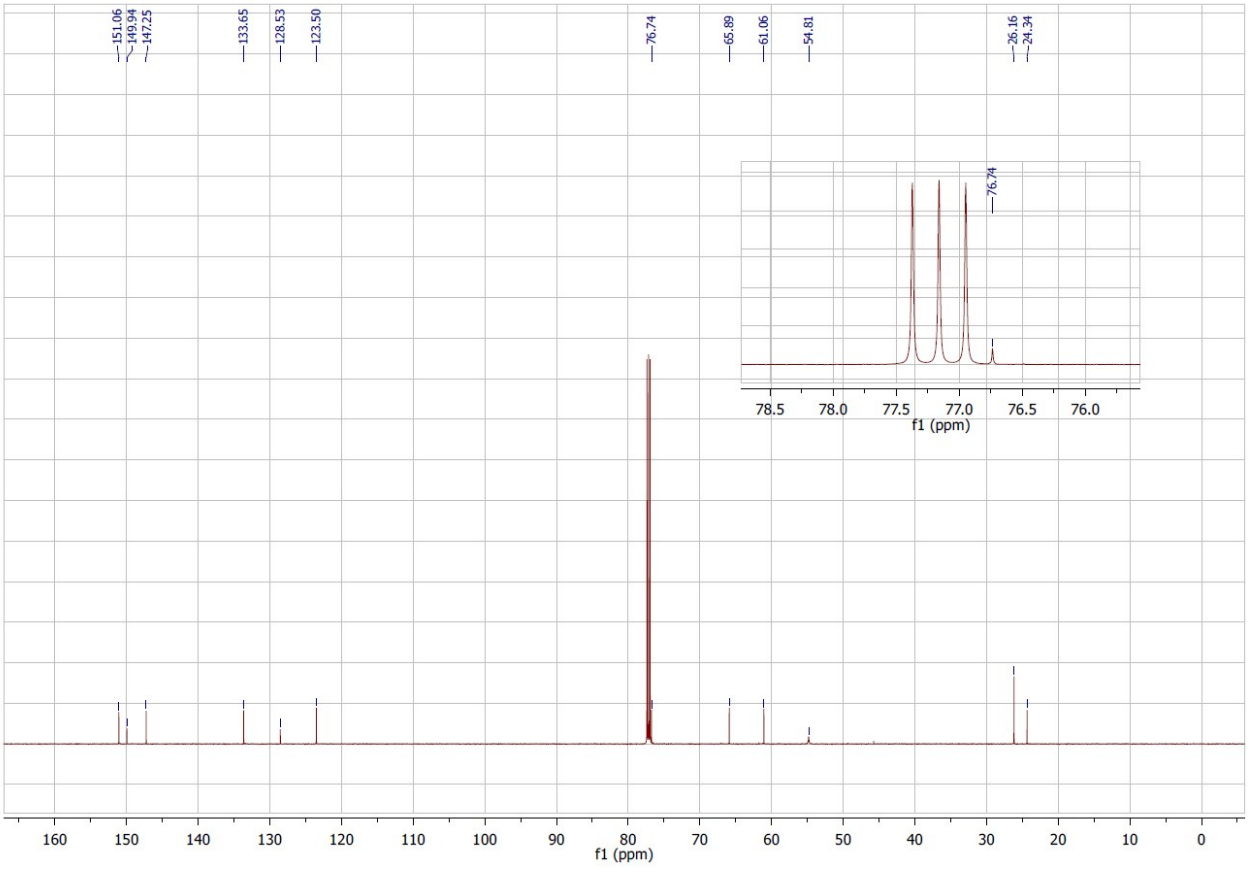





8.50
8.50
8.49
8.14
8.12
8.12
7.48
7.48
7.46
7.46
7.46
7.46
7.23
5.04
4.05
4.04
4.03
4.02
4.01
4.01
4.00
3.99
2.54
2.33
2.31
2.31
2.30
2.29
2.29
2.26
2.25
2.24
1.54
1.52
1.51
1.50
1.48
1.40
1.38
1.37
NH2
OH
N
O
N
O
12
1.00
0.99
0.99
0.97
1.90
3.03
2.04
4.96
3.84
1.83
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)


147.55
139.76
137.09
132.47
125.89
123.54
65.59
60.97
54.78
50.92
26.15
24.31
160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


NH2
OH
O
N
N
N
S1
B (dd) 8.64
A (d) 8.86
C (dt) 7.93
D (dd) 7.31
E (s) 4.98
F (m) 4.10
H (m) 2.37
G (s) 2.60
J (s) 1.44
I (m) 1.58
f1 (ppm)


151.06
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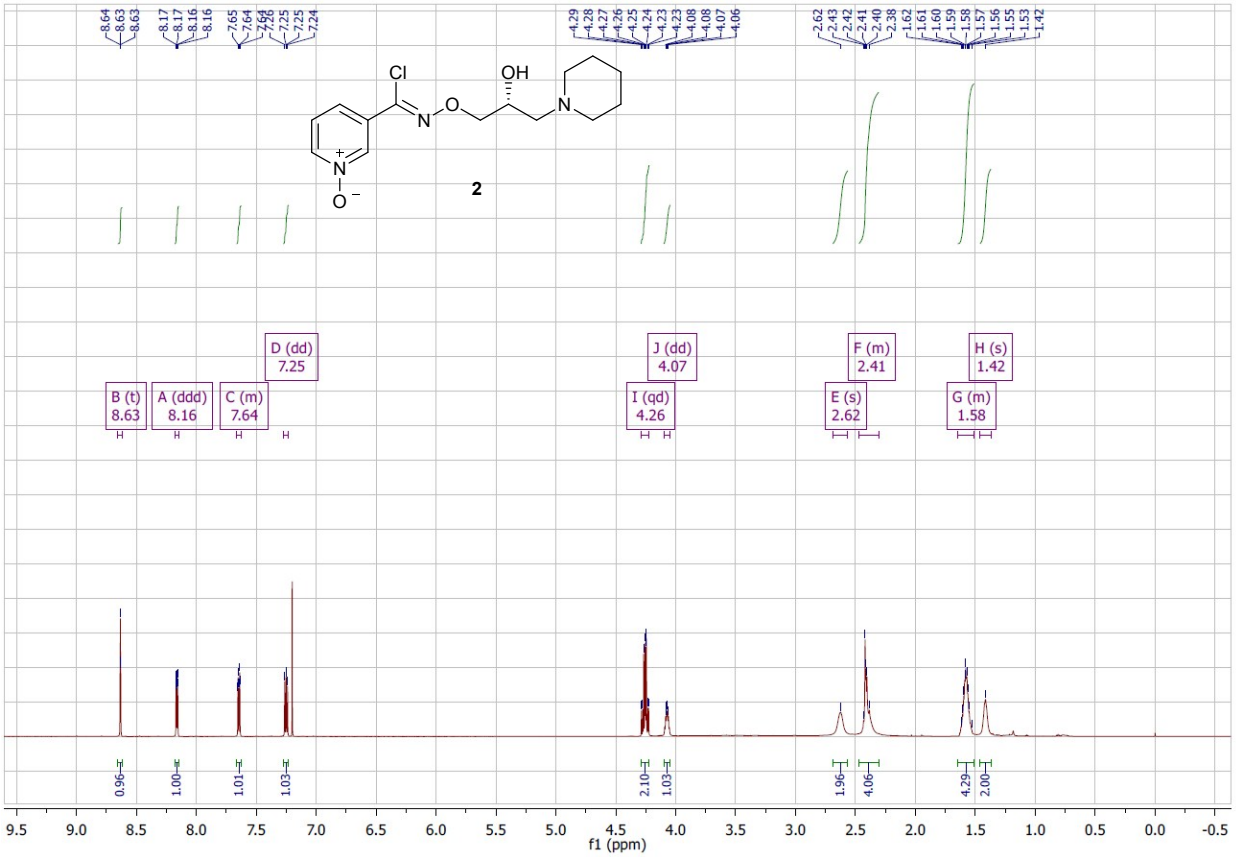
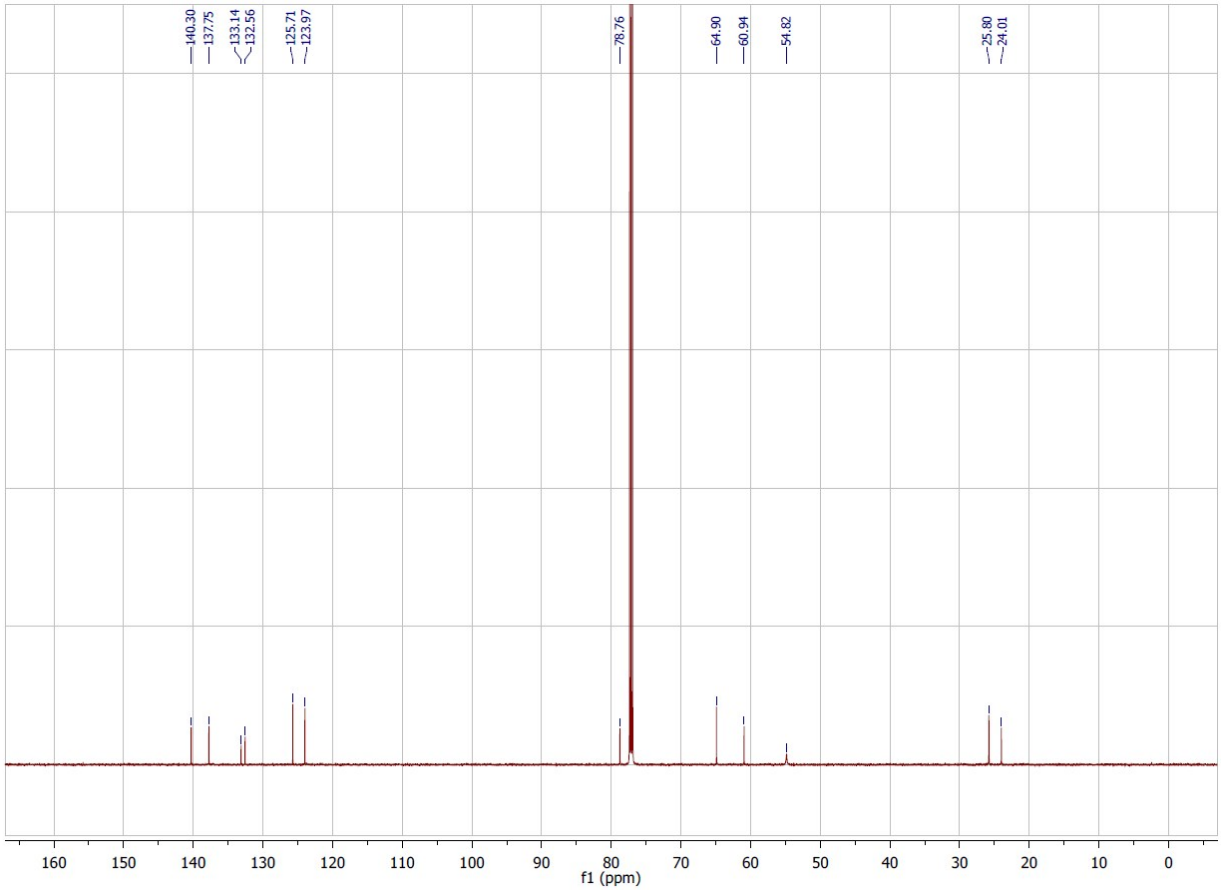
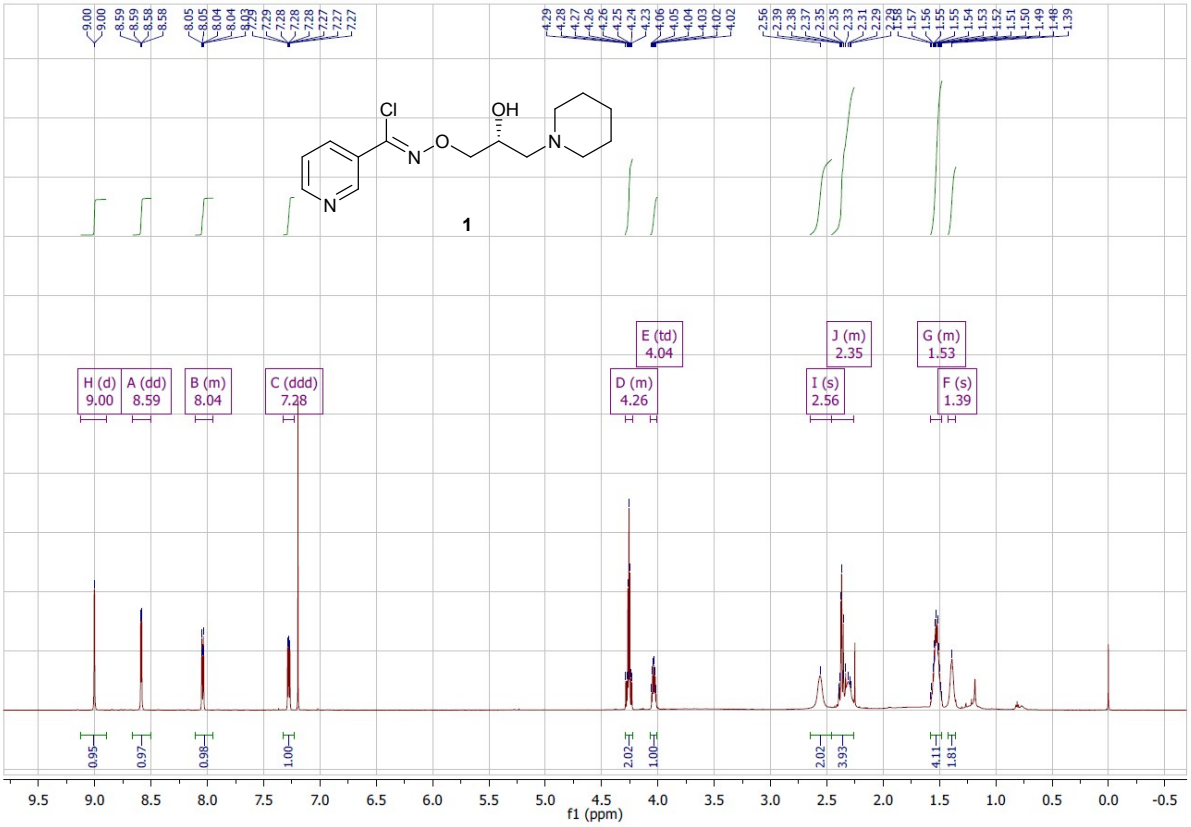
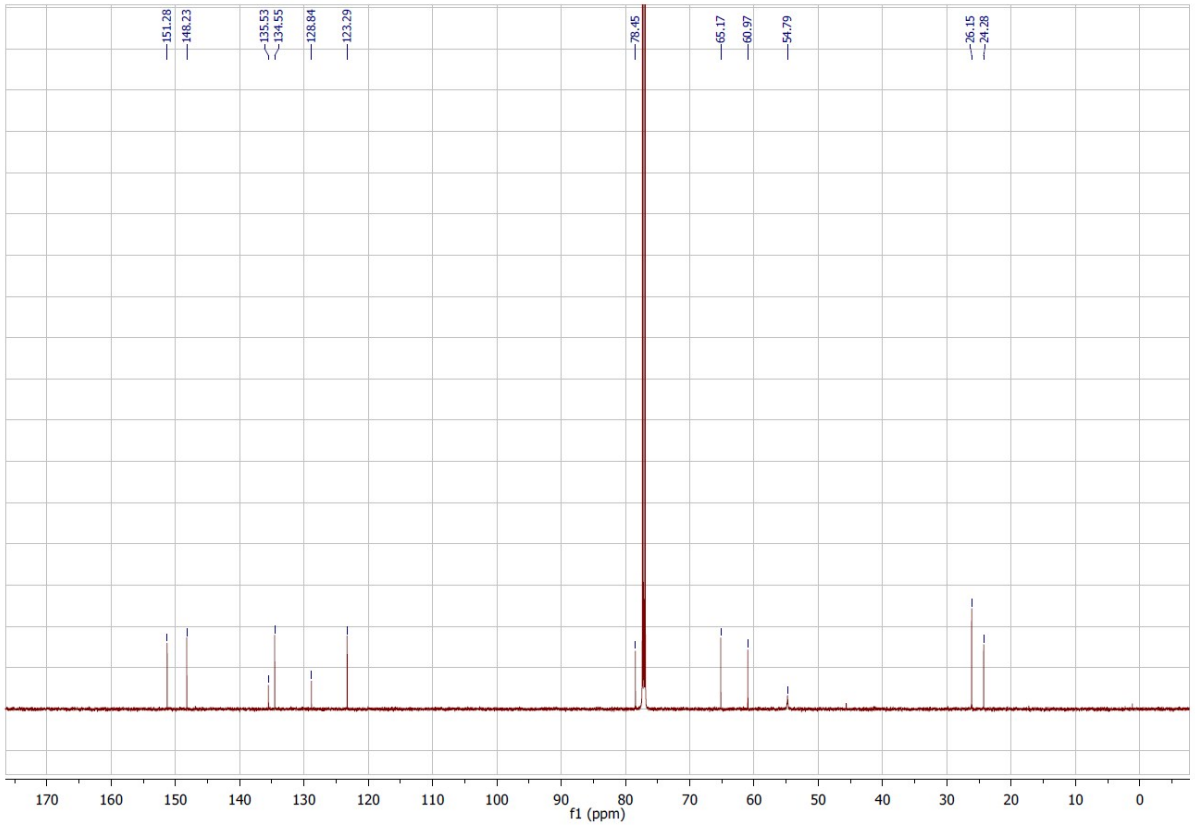
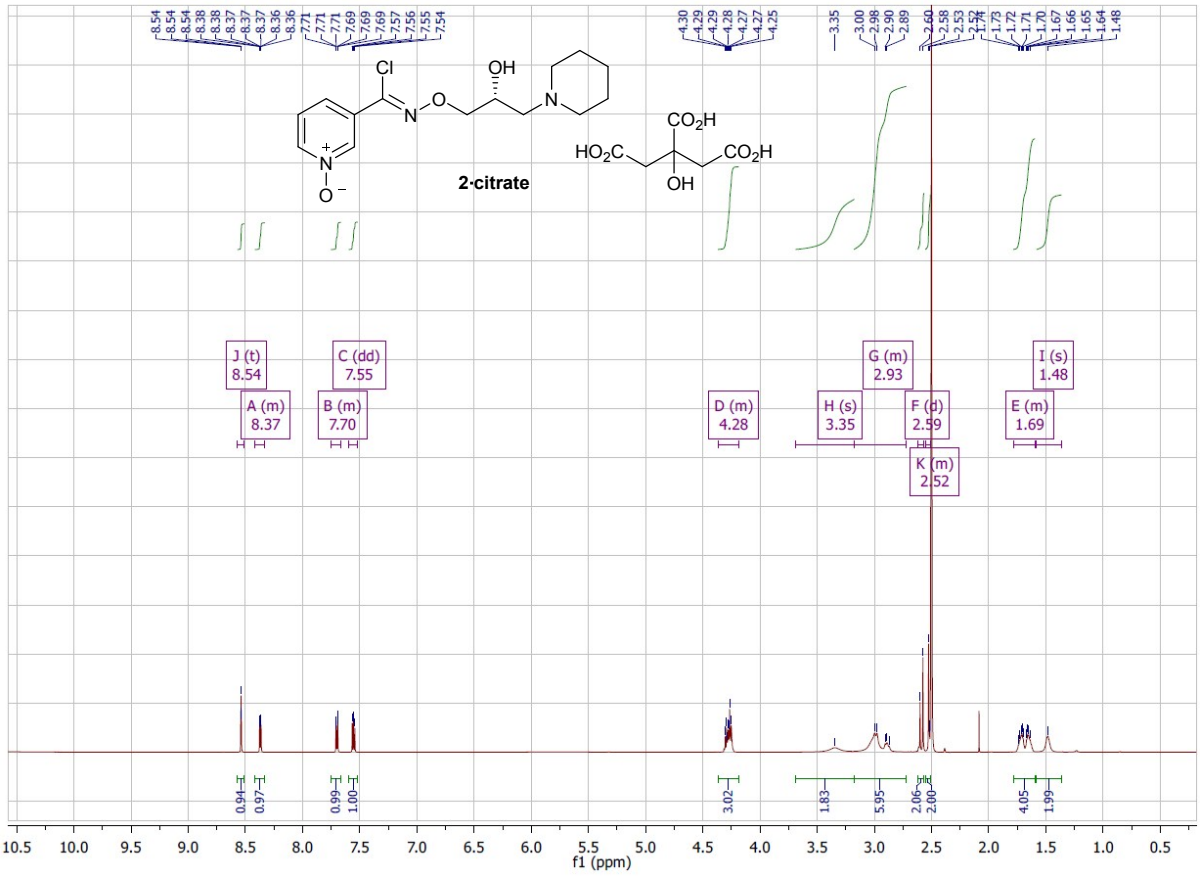
133.65
128.53
123.50
76.74
65.89
61.06
54.81
26.16
24.34
f1 (ppm)

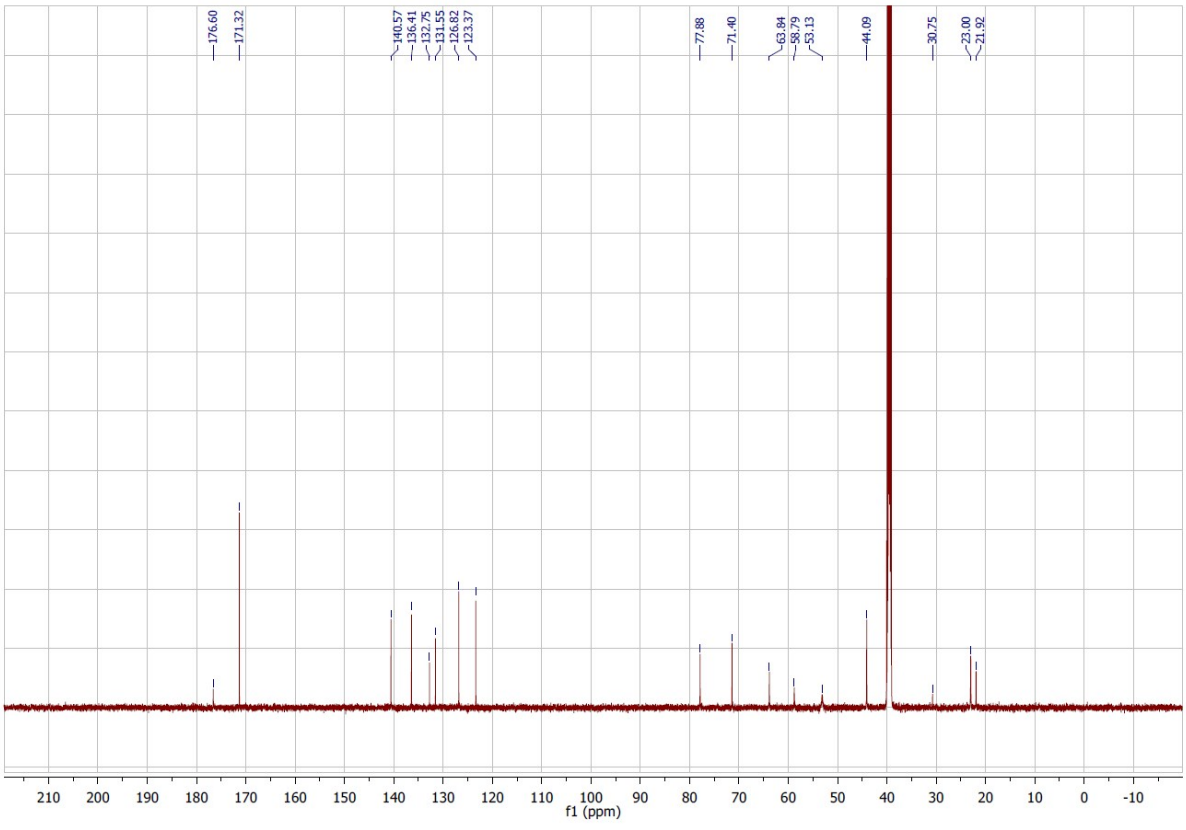

Cl
OH
N
O
N
N
O
2
B (t) 8.63
A (ddd) 8.16
C (m) 7.64
D (dd) 7.25
I (qd) 4.26
J (dd) 4.07
E (s) 2.62
F (m) 2.41
G (m) 1.58
H (s) 1.42
f1 (ppm)

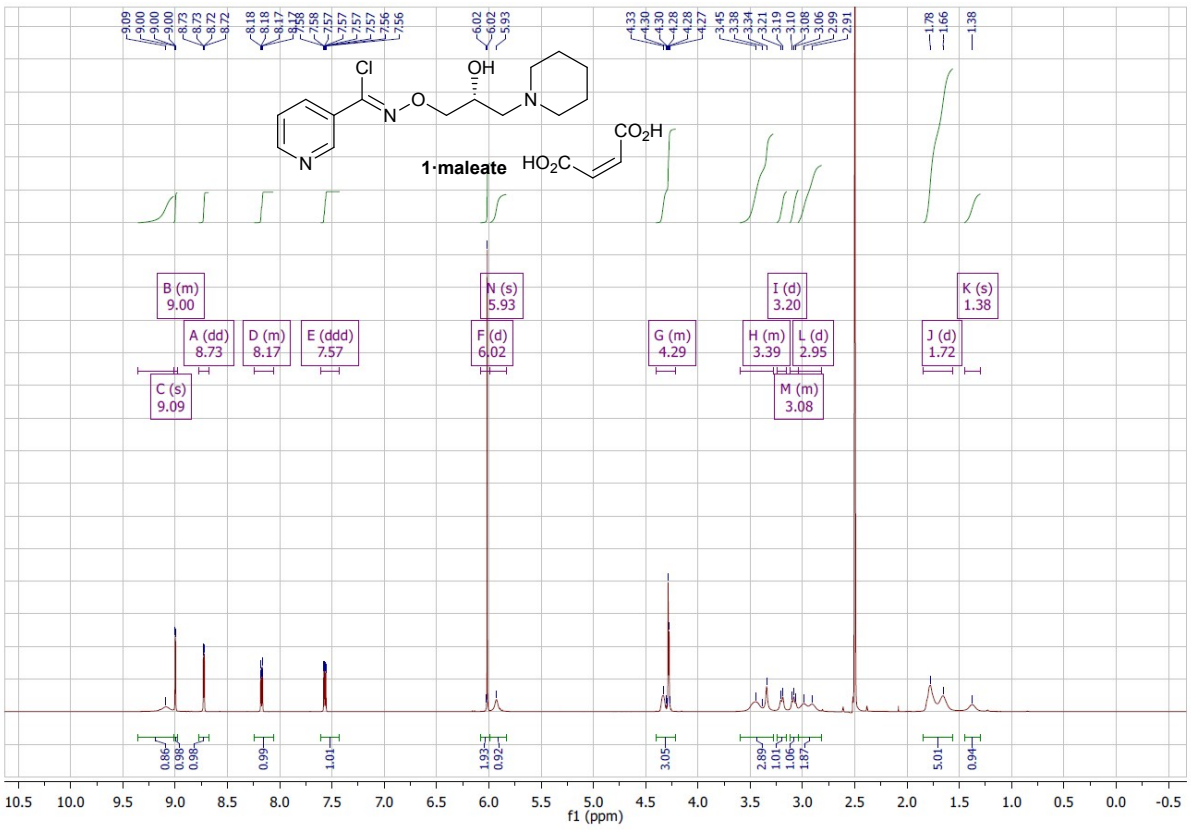

140.30
137.75
133.14
132.56
125.71
123.97
78.76
64.90
60.94
54.82
25.80
24.01
f1 (ppm)

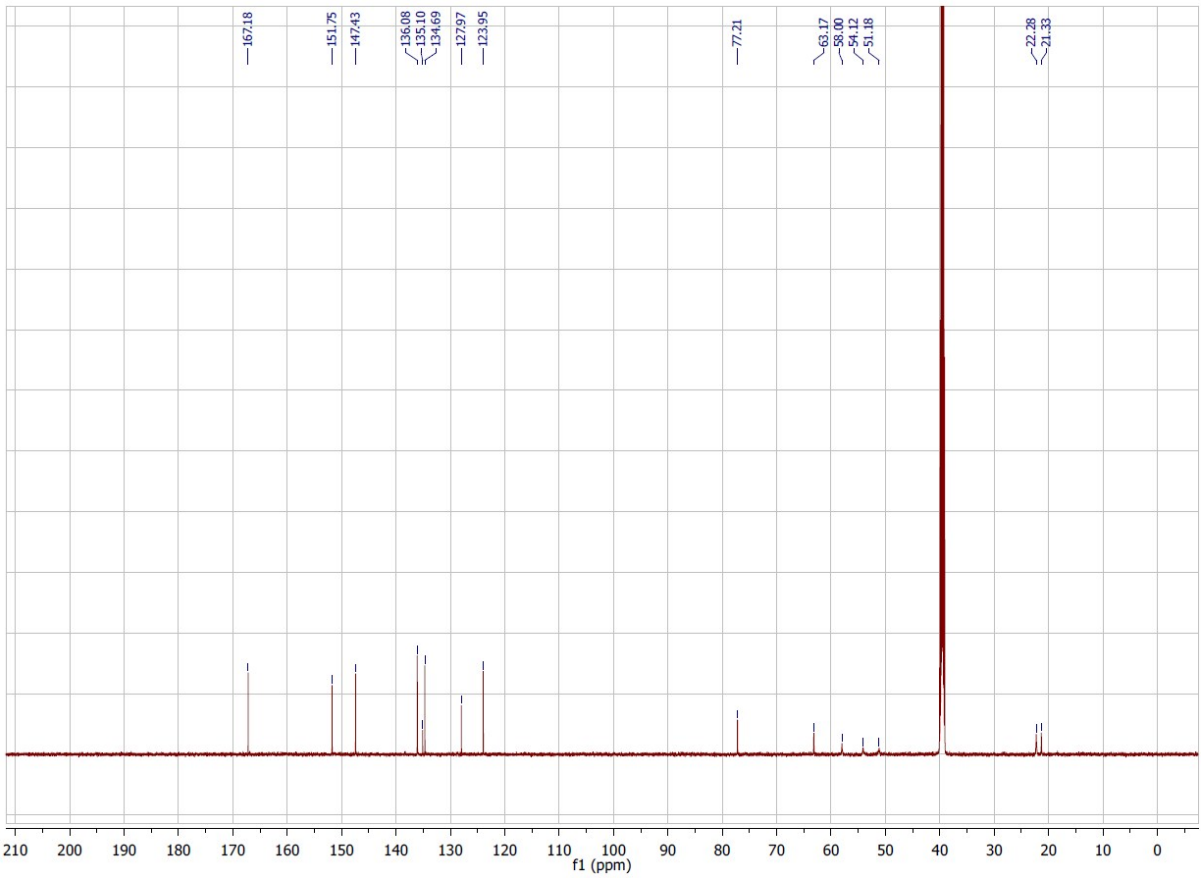

Cl
OH
N
O
N
N
1
H (d) 9.00
A (dd) 8.59
B (m) 8.04
C (ddd) 7.28
E (td) 4.04
D (m) 4.26
J (m) 2.35
I (s) 2.56
G (m) 1.53
F (s) 1.39
f1 (ppm)

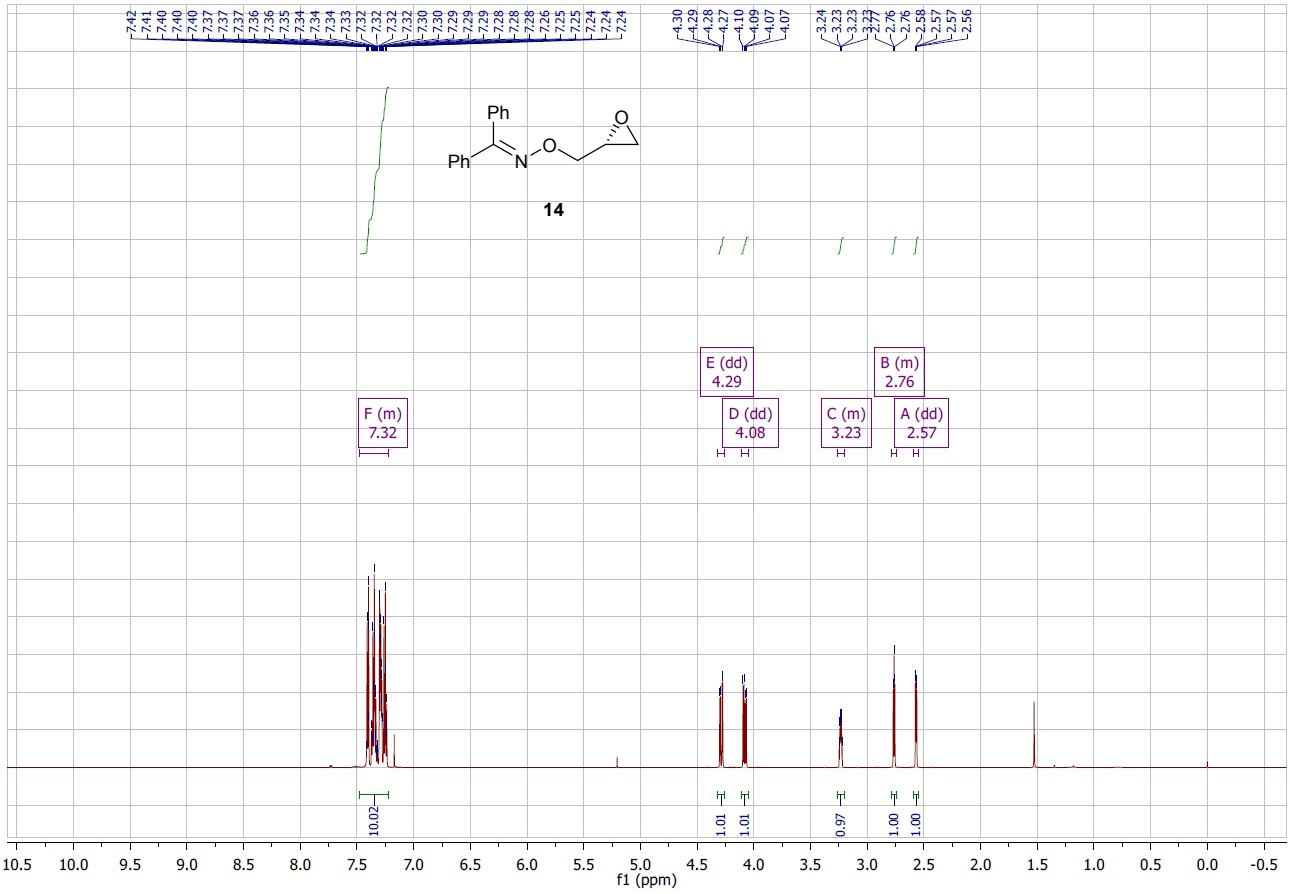

151.28
148.23
135.53
134.55
128.84
123.29
78.45
65.17
60.97
54.79
26.15
24.28
f1 (ppm)

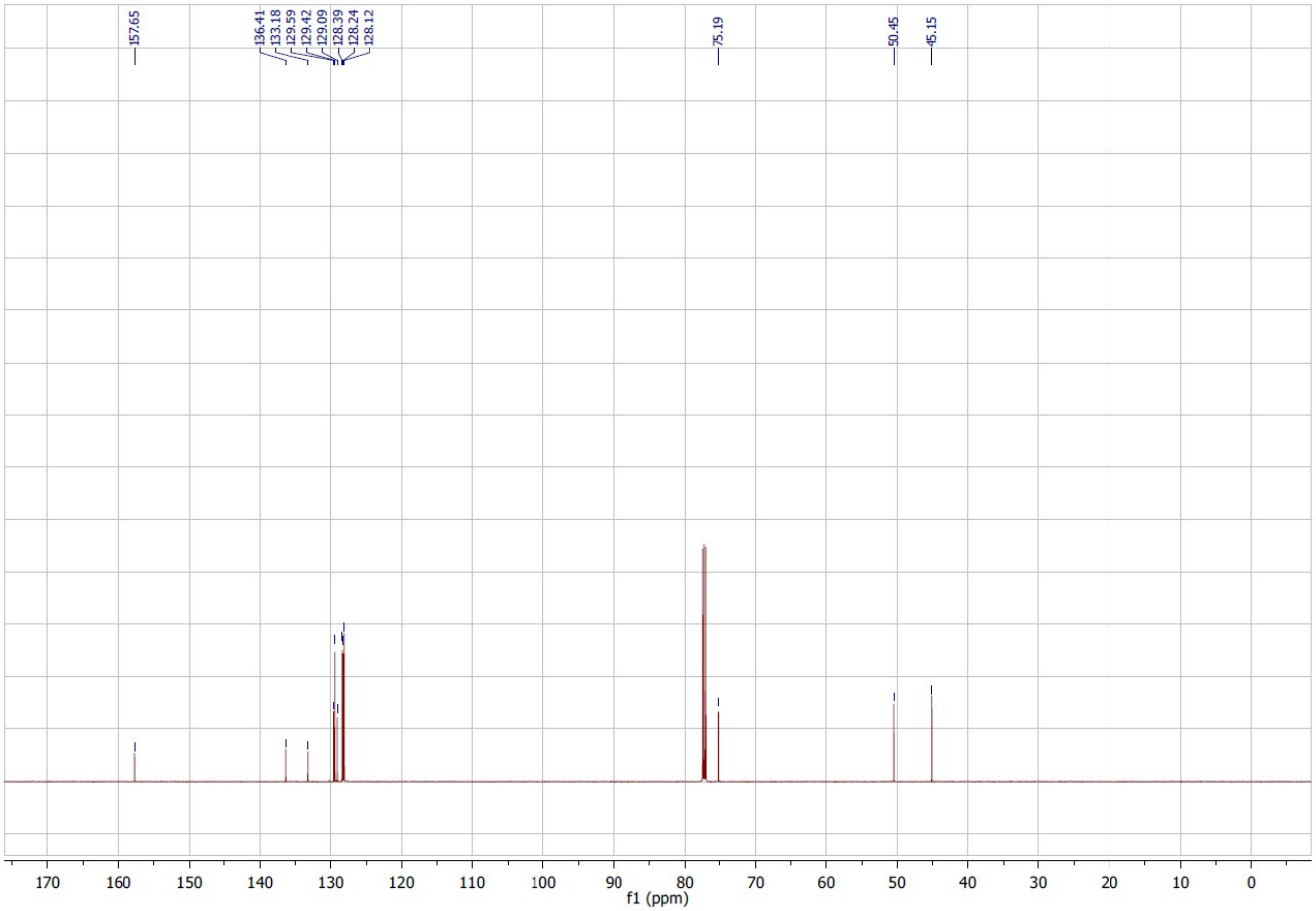

2·citrate
J (t) 8.54
A (m) 8.37
C (dd) 7.55
B (m) 7.70
D (m) 4.28
G (m) 2.93
H (s) 3.35
F (d) 2.59
K (m) 2.52
I (s) 1.48
E (m) 1.69
f1 (ppm)

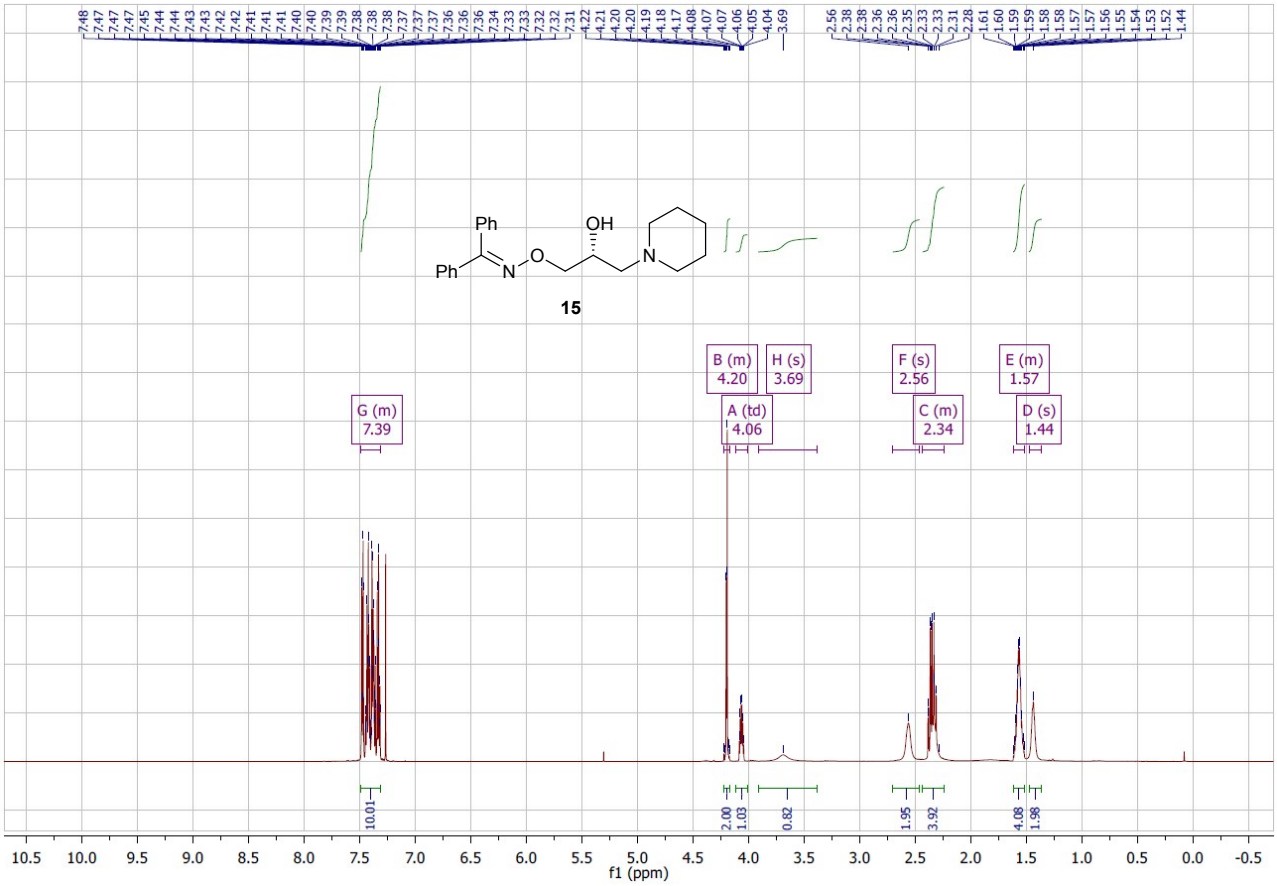

f1 (ppm)

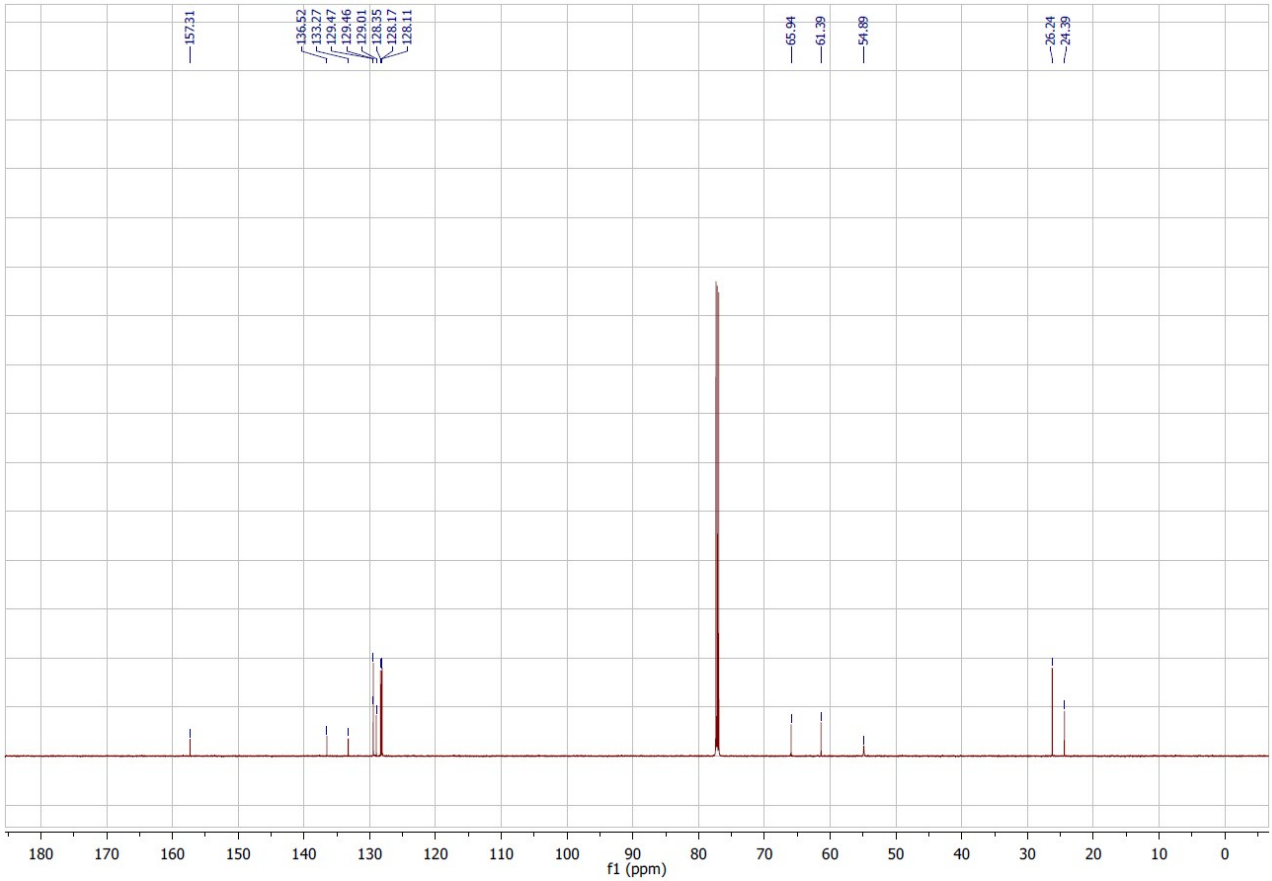

Cl
OH
N
O
N
N
1·maleate
HO2C
CO2H
B (m) 9.00
A (dd) 8.73
D (m) 8.17
E (ddd) 7.57
C (s) 9.09
N (s) 5.93
F (d) 6.02
G (m) 4.29
I (d) 3.20
H (m) 3.39
L (d) 2.95
M (m) 3.08
K (s) 1.38
J (d) 1.72
f1 (ppm)

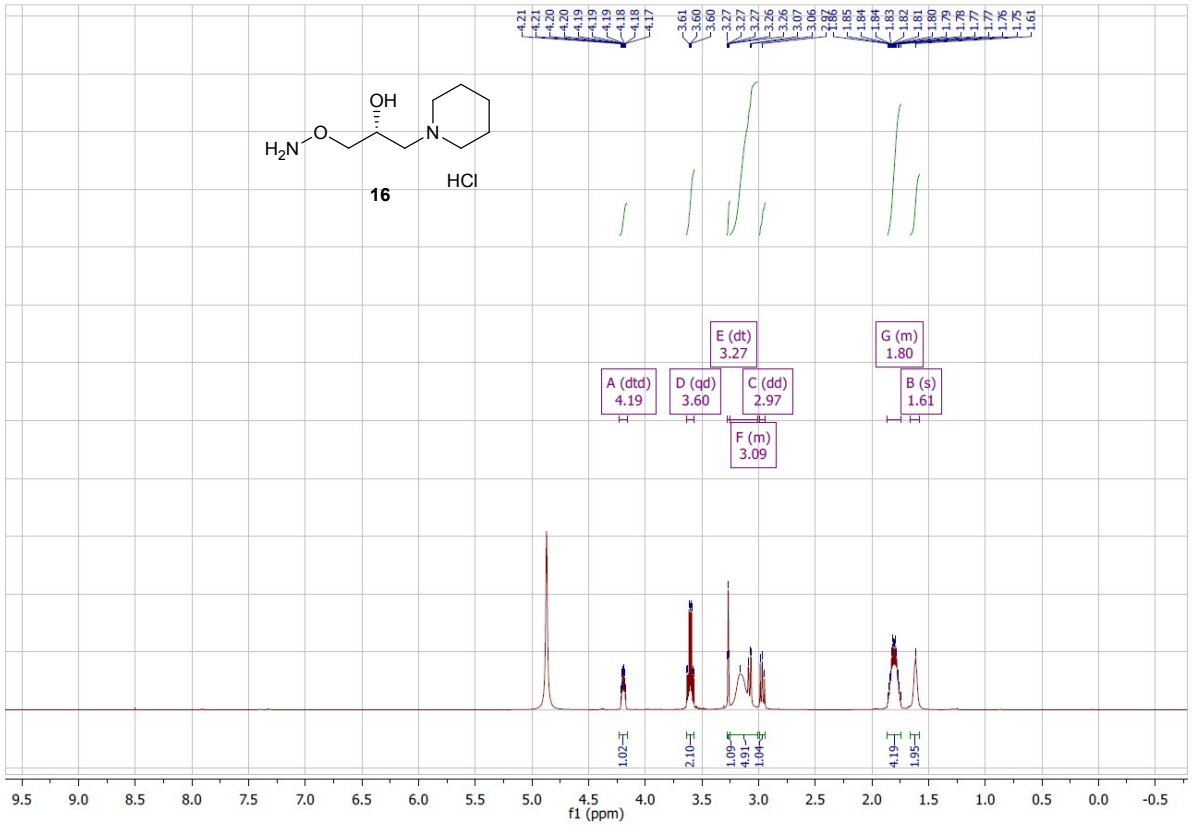

167.18
151.75
147.43
136.08
135.10
134.69
127.97
123.95
77.21
63.17
58.00
54.12
51.18
22.28
21.33
f1 (ppm)

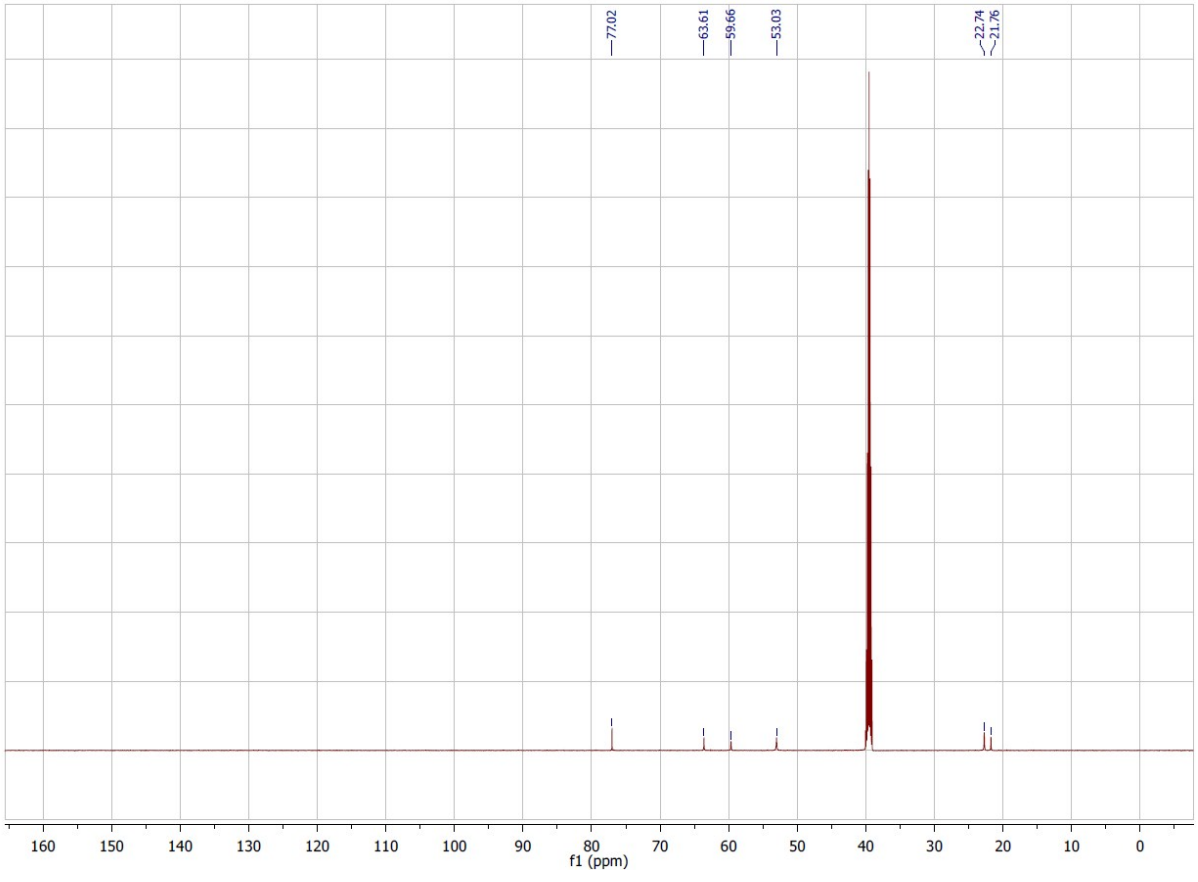

Ph
Ph
N
O
O
14
F (m)
7.32
E (dd)
4.29
D (dd)
4.08
C (m)
3.23
B (m)
2.76
A (dd)
2.57
f1 (ppm)

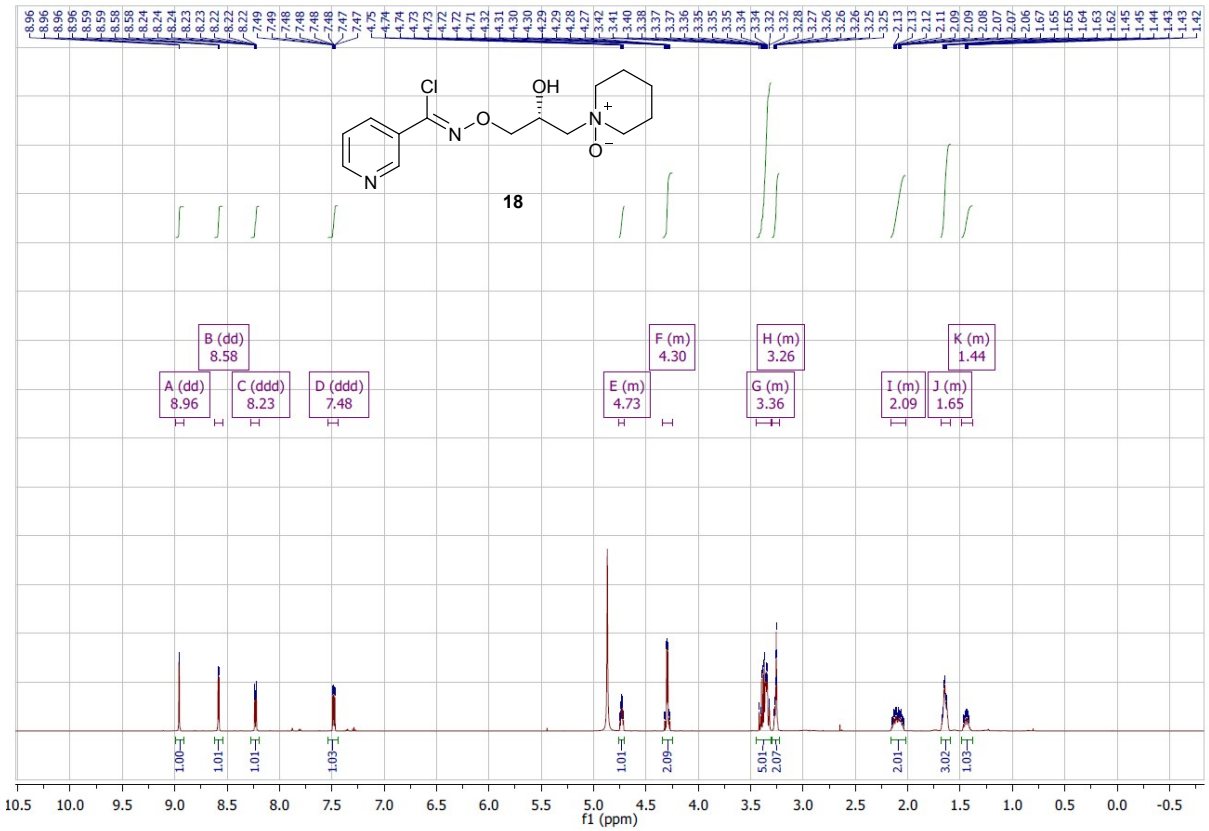

157.65
136.41
133.18
129.59
129.42
129.09
128.39
128.24
128.12
75.19
50.45
45.15
f1 (ppm)

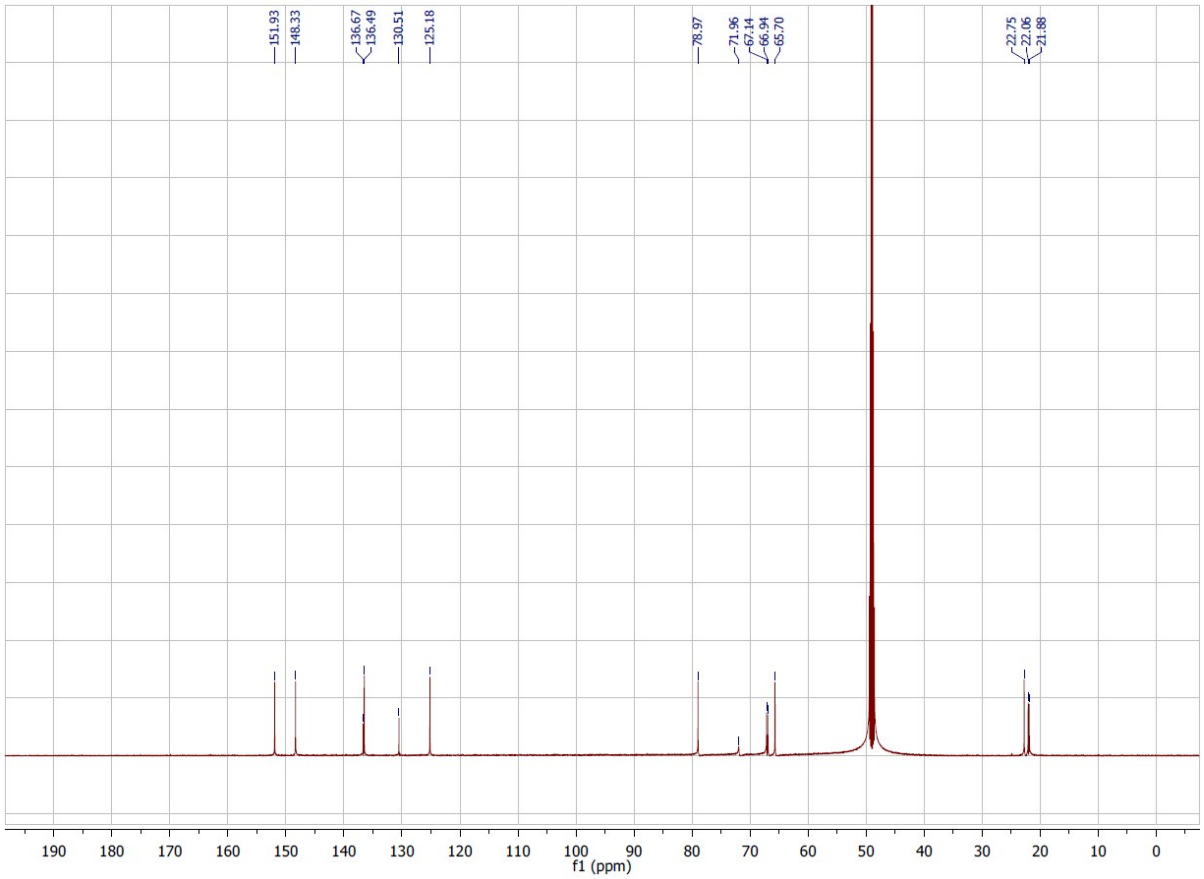

Ph
OH
Ph
N
O
N
15
G (m) 7.39
B (m) 4.20
H (s) 3.69
A (td) 4.06
F (s) 2.56
C (m) 2.34
E (m) 1.57
D (s) 1.44
10.01
2.00
1.03
0.82
1.95
3.92
4.08
1.98
f1 (ppm)


157.31
136.52
133.27
129.47
129.46
129.01
128.35
128.17
128.11
65.94
61.39
54.89
26.24
24.39
f1 (ppm)


OH
H_2N
O
N
HCl
16
A (dtd) 4.19
D (qd) 3.60
E (dt) 3.27
C (dd) 2.97
F (m) 3.09
G (m) 1.80
B (s) 1.61
f1 (ppm)


77.02
63.61
59.66
53.03
22.74
21.76
f1 (ppm)


Cl
OH
N
O
N+
O−
N
18
B (dd) 8.58
A (dd) 8.96
C (ddd) 8.23
D (ddd) 7.48
F (m) 4.30
E (m) 4.73
H (m) 3.26
G (m) 3.36
K (m) 1.44
I (m) 2.09
J (m) 1.65
f1 (ppm)


151.93
148.33
136.67
136.49
130.51
125.18
78.97
71.96
67.14
66.94
65.70
22.75
22.06
21.88
f1 (ppm)

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[3] L. Vígh, P. L. Nagy, J. Szilbereky, L. Ürögdi, A. Jednákovits, L. Jaszlits, K. Bíró, E. Màrvànyos, M. Barabás, E. Hegedüs, L. Korányi, M. Kürthy, G. Balogh, I. Horváth, Z. Török, É. Udvardy, G. Dormán, D. Medzihradszky, B. Mézes, E. Kovács, E. Duda, B. Farkas and A. Glatz, US Pat. Appl. US6653326B1, 2003.

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