Machines *vs* Malaria: a Flow-Based Preparation of the Drug Candidate OZ439

Shing-Hing Lau,[§a] Alicia Galván,[§a] Rohan R. Merchant,[a] Claudio Battilocchio,[a] José A. Souto,[a,b] Malcolm B. Berry[c] and Steven V. Ley*[a]

[a] Innovative Technology Centre, Chemistry Department, University of Cambridge, Lensfield Road, CB2 1EW, Cambridge, UK.
[b] Departamento de Química Orgánica, Universidade de Vigo, Vigo, 36310, Spain.
[c] GlaxoSmithKline, Stevenage, SG1 2NY, UK.

Electronic Supplementary Information

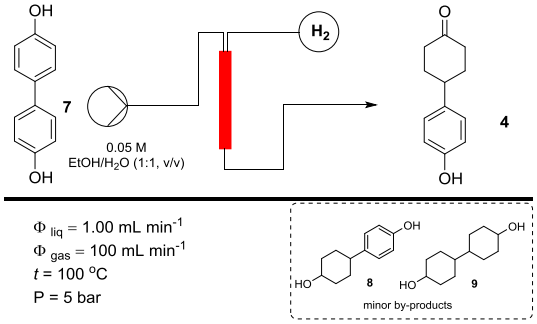
TABLE OF CONTENTS

1. General information

Unless stated otherwise, reagents were obtained from commercial sources and used without purification. New compounds have been fully characterized. NMR characterization was performed on reported ones. ^{1}H-NMR spectra were recorded on Bruker Avance DPX-400 or DPX-600 (600 MHz), with the residual solvent peak as the internal reference ($CDCl_3$ = 7.26 ppm, DMSO-d_6 = 2.52 ppm). ^{1}H resonances are reported to the nearest 0.01 ppm. ^{13}C-NMR spectra were recorded on the same spectrometer with proton decoupling, with the solvent peak as the internal reference ($CDCl_3$ = 77.00 ppm, DMSO-d_6 = 40.45 ppm). All ^{13}C resonances are reported to the nearest 0.01 ppm. DEPT 135, COSY, HMQC, and HMBC experiments were used to aid structural determination and spectral assignment. The multiplicity of ^{1}H signals are indicated as: s =singlet, d = doublet, dd = doublet of doublets, ddd = doublet of doublets of doublets, t = triplet, q = quadruplet, sext = sextet, m = multiplet, br = broad, or combinations of thereof. Coupling constants (*J*) are quoted in Hz and reported to the nearest 0.1 Hz. Where appropriate, measures of the same coupling constant are averaged. Unless stated otherwise, reagents were obtained from commercial sources and used without purification. The removal of solvent under reduced pressure was carried out on a standard rotary evaporator. High resolution mass spectrometry (HRMS) was performed using a Waters Micromass LCT Premier™ spectrometer using time of flight with positive ESI, or a Bruker BioApex 47e FTICR spectrometer using (positive or negative) ESI or EI at 70 eV to within a tolerance of 5 ppm of the theoretically calculated value. Infrared spectra were recorded on a Perkin-Elmer Spectrum RX One FT-IR ATR (Attenuated Total Reflectance) spectrometer. The samples were prepared as thin films deposited on the ATR, unless otherwise specified. Only structurally important absorptions are quoted. The flow experiments were performed on a Vapourtec R2^{+}/R4 module,[1] Uniqsis Flowsyn module[2] and HEL FlowCAT module.[3] All gas-flow reactions were performed either with a gas-liquid T-piece reactor or HEL FlowCAT[3] as described to introduce gases into a continuous flow stream. Omnifit® columns[4] were used for the containment of polymer-supported reagent or other solid reagents.

2. Preparation of OZ439 (3)

2.1 Preparation of 4-(4-hydroxyphenyl)cyclohexan-1-one (4).



A solution of 4,4'-dihydroxybiphenyl (**7**) (2.8 g, 15 mmol) in EtOH/H_2O (0.05 M, 1:1) was continuously passed through the HEL FlowCAT trickle bed reactor (flow rate 1.0 mL min^{-1}), packed with 1.8 g of Pd/C catalyst (20% mol) and heated at 100 °C. The pressure of the system was set at 5 bar and the H_2 feed was set at 0.1 L min^{-1}. The reaction output was concentrated *in vacuo* to afford the crude product. Recrystallization from acetone yielded the desired product (**4**) (1.71 g, 58%) as a white solid.

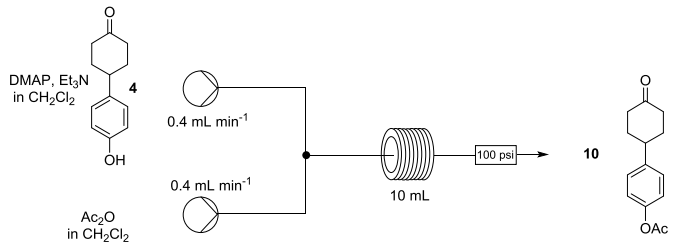
^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.14 (s, 1H), 7.05 (t, *J* = 8.5 Hz, 2H), 6.70 (d, *J* = 8.5 Hz, 2H), 2.90 (m, 1H), 2.22 (m, 2H), 2.30 (m, 2H), 2.08 (m, 2H), 1.90 (m, 2H).

^{13}C-NMR (120 MHz, DMSO-d_6) δ 210.8, 156.1, 135.9, 127.9, 115.5, 41.3, 41.3, 34.2.

2.2 **Optimisation for the selective continuous hydrogenation of 4,4'-dihydroxybiphenyl (7).**

Entry	Solvent	Conc. (M)	GF (L min^{-1})	T (°C)	Ø (mL min^{-1})	Catalyst	Ratio by ^{1}H-NMR (%)			
							7	4	8	9
1	EtOH	0.25	0.2	50	1	5% Pd/C	100	0	0	0
2	EtOH	0.05	0.2	100	1	5% Pd/C	65	16	26	8
3	EtOH	0.05	0.2	100	1	5% Pt/C	68	7	20	15
4	EtOH	0.05	0.2	100	1	10% Pd/C	31	4	33	32
5	EtOH:H_2O (1:1)	0.05	0.2	100	1	10% Pd/C	47	26	20	7
6	EtOH:H_2O (1:1)	0.05	0.2	100	1	5% Pd/Al_2O_3	99	trace	trace	0
7	EtOH:H_2O (1:1)	0.05	0.2	100	1	5% Rh/Al_2O_3	99	trace	trace	0
8	EtOH:H_2O (1:1)	0.05	0.2	100	1	20% Pd/C	8	41	14	37
9	**EtOH:H_2O (1:1)**	**0.05**	**0.1**	**100**	**1**	**20% Pd/C**	**11**	**63**	**16**	**10**
10	EtOH:H_2O (1:1)	0.05	0.1	110	1	20% Pd/C	13	59	18	10
11	EtOH:H_2O (1:1)	0.05	0.1	120	1	20% Pd/C	43	34	17	7
12	EtOH:MeOH:H_2O (5:1:4)	0.05	0.1	100	1	20% Pd/C	44	42	12	2
13	EtOH:MeOH:H_2O (2:1:2)	0.05	0.1	100	1	20% Pd/C	27	42	22	9
14	EtOH:MeOH:H_2O (1:2:2)	0.05	0.1	100	1	20% Pd/C	13	61	20	6
15	IPA	0.05	0.1	100	1	20% Pd/C	37	26	20	17
16	IPA:H_2O (1:1)	0.05	0.1	100	1	20% Pd/C	50	38	11	1
17	IPA:H_2O (4:1)	0.05	0.1	100	1	20% Pd/C	55	33	12	0

2.3 Flow acetylation of 4-(4-hydroxyphenyl)cyclohexan-1-one (4) to prepare 4-(4-oxocyclohexyl)phenyl acetate (10).

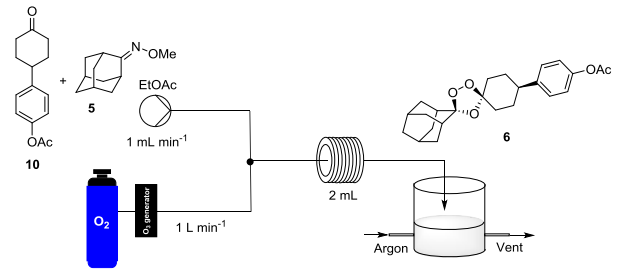


A solution of 4-(4-hydroxyphenyl)cyclohexan-1-one (**4**) (3.80 g, 20.0 mmol), 4-dimethylaminopyridine (DMAP, 124 mg, 1.0 mmol) and Et_3N (6.48 mL, 89.7 mmol), in CH_2Cl_2 (80 mL) was combined with a solution of acetic anhydride (2.44 g, 24.0 mmol, 0.3 M in CH_2Cl_2) in a T-piece (each stream was run at a flow rate of 0.4 mL min^{-1}) and reacted in a 10 mL PFA reactor coil, at room temperature. The output of the reaction was passed through an Omnifit® column containing Silica gel and a 100 psi BPR, directed into a round-bottom flask. After collection of the organic reaction solution, the solvent was removed *in vacuo* to afford product (**10**) (4.60 g, 99%) as a yellowish solid. The product was used in the next stage without need for further purification.

^{1}H-NMR (600 MHz, $CDCl_3$) δ 7.26 (d, *J* = 8.4 Hz, 2H), 7.05 (d, *J* = 8.4 Hz, 2H), 3.05 (tt, *J* = 3.4, 12.2 Hz, 1H), 2.53-2.51 (m, 4H), 2.30 (s, 3H), 2.24-2.21 (m, 2H), 1.97-1.90 (m, 2H).

^{13}C-NMR (120 MHz, $CDCl_3$) δ 210.9, 169.6, 149.2, 142.3, 127.6, 121.6, 42.2, 41.3, 34.0, 21.1.

2.4 Preparation of 4-((1*R*,3*S*,4''*S*,5*R*,5'*S*,7*R*)-dispiro[adamantane-2,3'-[1,2,4]trioxolane-5',1''-cyclohexan]-4''-yl)phenyl acetate (6).

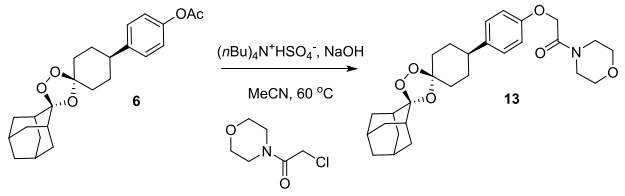


A 100 mL solution of *O*-methyl 2-adamantanone oxime (**5**) (0.2 M in EtOAc) and 4-(4-oxocyclohexyl)phenyl acetate (**10**) (0.1 M in EtOAc) was pumped at a flow rate of 1.0 mL min^{-1} and combined with the ozone stream (1.25 bar, flow rate 1.0 L min^{-1}) at a T piece connector, entering the 2 mL reactor coil. The reactor output was collected directly in a flask under a constant flow of argon to remove excess ozone. The solvent was removed to provide the crude product. Recrystallization in Et_2O afforded compound (**6**) (6.3 g, 70%) as a white solid. The isolated compound matches the spectroscopic data previously reported in literature.[5]

^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.20 (d, *J* = 8.4 Hz, 2H), 6.99 (d, *J* = 8.4 Hz, 2H), 2.60 – 2.49 (m, 1H), 2.27 (s, 3H), 2.11 – 1.63 (m, 22H).

^{13}C-NMR (100 MHz, $CDCl_3$) δ 169.5, 148.9, 143.7, 127.6, 121.3, 115.5, 111.3, 108.3, 42.3, 36.7, 36.3, 34.7, 34.6, 31.4, 26.8, 26.4.

2.5 Preparation of 2-(4-((1*R*,3*S*,4''*S*,5*R*,5'*S*,7*R*)-dispiro[adamantane-2,3'-[1,2,4]trioxolane-5',1''-cyclohexan]-4''-yl)phenoxy)-1-morpholinoethan-1-one (13).



To a solution of acetate (**6**) (200 mg, 0.5 mmol) in dry acetonitrile (5 mL) were added powdered NaOH (120 mg, 3.0 mmol) and tetrabutylammonium hydrogen sulphate (34 mg, 0.1 mmol). The mixture was stirred at 25 ^{o}C for 30 min. 2-Chloro-1-morpholinoethan-1-one (164 g, 1 mmol) was added and the reaction was stirred at 60 ^{o}C overnight. The inorganic solid was filtered off and washed with CH_2Cl_2. After removal of the solvents, the residue was diluted in EtOAc (15 mL). The combined organic layer was washed with water and brine, dried over anhydrous $MgSO_4$ and filtered. The solvent was removed *in vacuo* to afford amide (**13**) as a pale brown solid (239 mg, 99%).

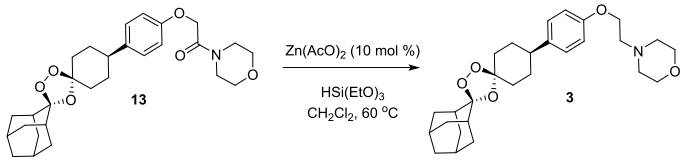
^{1}H-NMR (600 MHz, $CDCl_3$) δ 7.14 (d, *J* = 8.7 Hz, 2H), 6.88 (d, *J* = 8.7 Hz, 2H), 4.67 (s, 2H), 3.68-3.62 (m, 8H), 2.51 (m, 1H), 2.06 – 1.71 (m, 22H).

^{13}C-NMR (120 MHz, $CDCl_3$) δ 166.7, 156.0, 150.0, 127.8, 114.5, 111.4, 108.4, 67.8, 66.8, 66.8, 46.0, 42.1, 34.8, 31.6, 26.9, 26.5.

FT-IR (neat, υ_{max} cm^{-1}) 2915, 1651, 1512, 1444, 1113.

HRMS (ESI^+, m/z $[M+H]^+$) calcd for $C_{28}H_{38}NO_6$ 484.2620, found 484.2626.

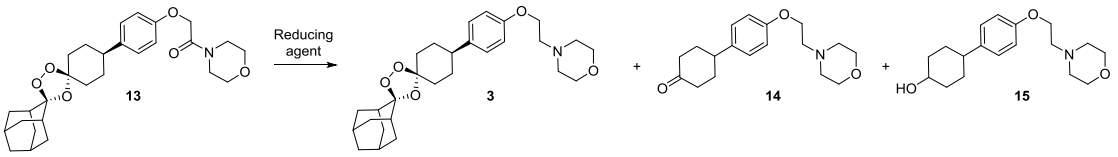
2.6 Reduction of 2-(4-((1*R*,3*S*,4''*S*,5*R*,5'*S*,7*R*)-dispiro[adamantane-2,3'-[1,2,4]trioxolane-5',1''-cyclohexan]-4''-yl)phenoxy)-1-morpholinoethan-1-one (13) to 4-(2-(4-((1*R*,3*S*,4''*S*,5*R*,5'*S*,7*R*)-dispiro[adamantane-2,3'-[1,2,4]trioxolane-5',1''-cyclohexan]-4''-yl)phenoxy)ethyl)morpholine (3, OZ439).



A 10 mL schlenk tube containing a stirrer bar was charged with zinc acetate (4 mg, 0.02 mmol). Dry THF (1 mL) and triethoxysilane (0.12 mL, 0.6 mmol) were added respectively after purging the schlenk tube with argon. The resulting mixture was stirred for 0.5 h at room temperature. A solution of amide (**13**) (100 mg, 0.2 mmol) in CH_2Cl_2 (1 mL) was added to the mixture under argon. The reaction mixture was stirred at 60 ^{o}C for 20 h and monitored by TLC. After complete disappearance of the starting material, 1M NaOH solution (5 mL) was added to the reaction mixture. The reaction mixture was vigorously stirred for 3 h and then extracted with EtOAc (3 x 5 mL); the combined organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to yield OZ439 (**3**) (81 mg, 86%) as a white solid. The isolated compound matches the spectroscopic data previously reported in literature.[6]

[1]H-NMR (400 MHz, $CDCl_3$) δ 7.05 (d, *J* = 8.4 Hz, 2H), 6.79 (d, *J* = 8.4 Hz, 2H), 4.02 (t, *J* = 5.5 Hz, 2H), 3.70 – 3.63 (m, 4H), 2.72 (t, *J* = 5.5 Hz, 2H), 2.56 – 2.39 (m, 5H), 2.07 – 1.58 (m, 22H).
[13]C-NMR (100 MHz, $CDCl_3$) δ 156.8, 138.1, 127.3, 114.1, 110.9, 108.0, 66.6, 65.5, 57.4, 53.8, 41.7, 36.5, 36.1, 34.5, 34.4, 31.4, 26.6, 26.2.

2.7 Table of reagents screened for the reduction of 2-(4-((1*R*,3*S*,4''*S*,5*R*,5'*S*,7*R*)-dispiro[adamantane-2,3'-[1,2,4]trioxolane-5',1''-cyclohexan]-4''-yl)phenoxy)-1-morpholinoethan-1-one (13)

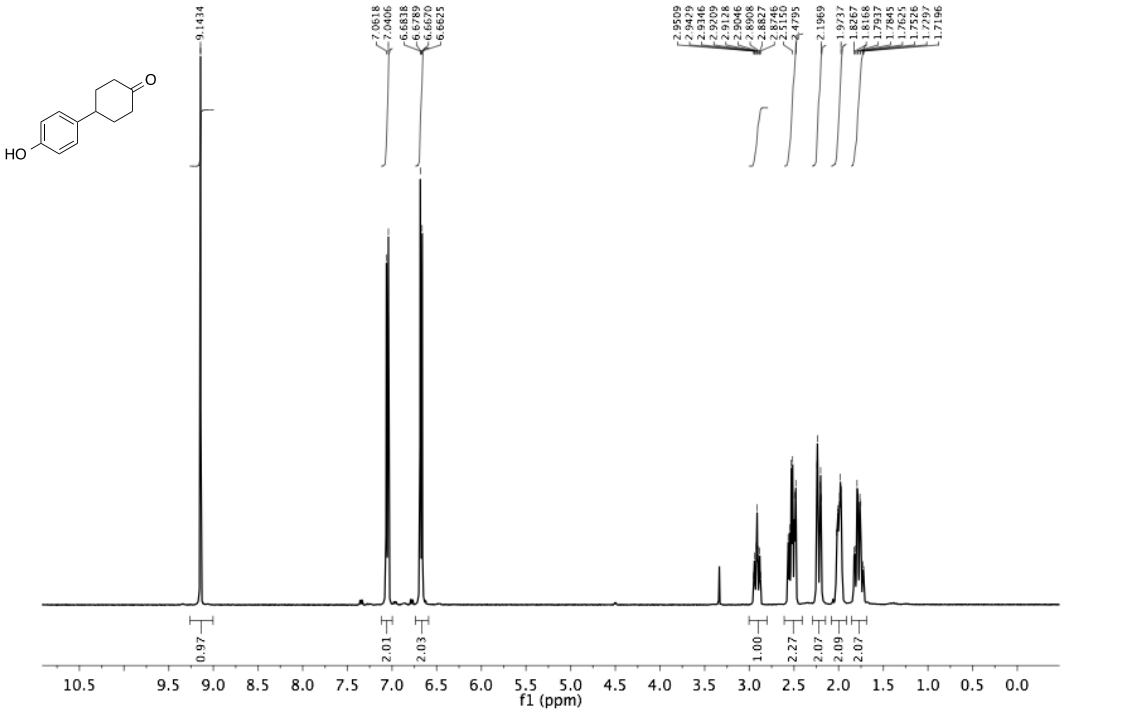


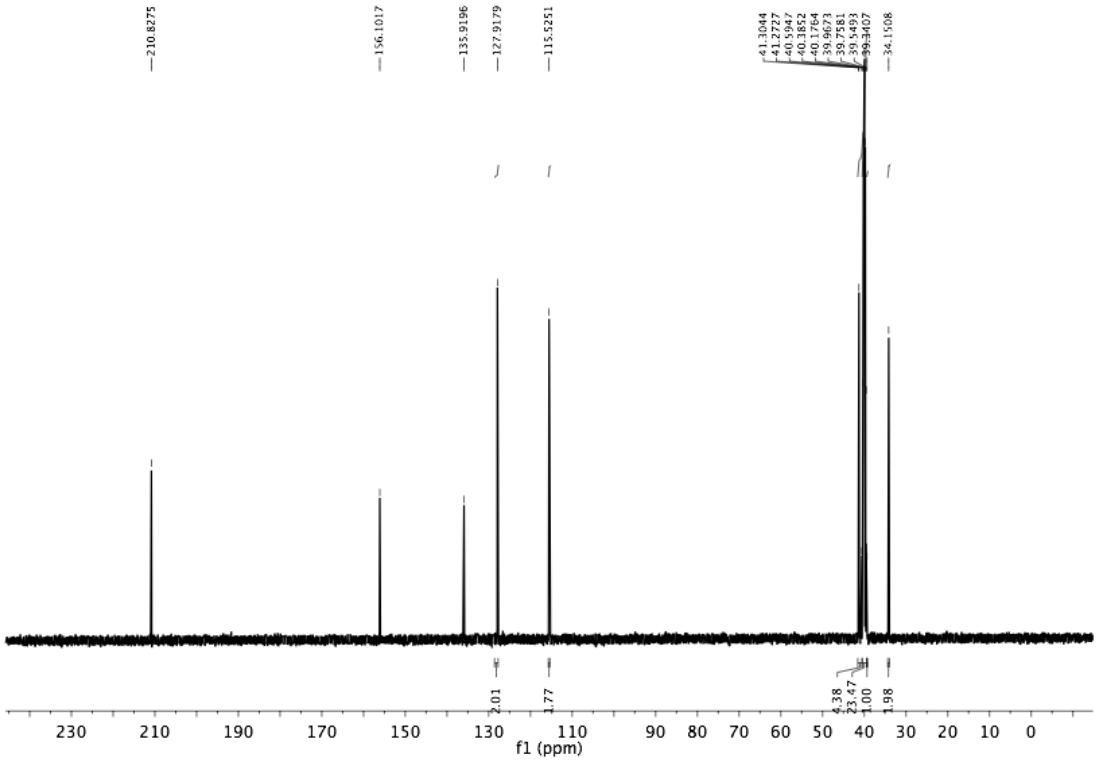
Entry	Reducing agent (equiv.)	Conc. [M][a]	T (°C)	t (h)	Product
1	BH_3·THF (1.3)	0.05	rt	3	**13**
2	BH_3·THF (1.3)	0.05	50	3	**13**
3	BH_3·THF (3.0)	0.05	50	15	**13**
4	BH_3·THF (3.0)	0.3	rt	15	**14** + **3** (traces)
5	BH_3·THF (3.0)	0.3	-78	2	**13**
6	BH_3·DMS (3.0)	0.3	rt	15	**14** + **3**
7	BH_3·DMS (1.5)	0.3	rt	15	**14** + **3**
8	BH_3·DMS (1.5)	0.3	rt	1	**14** + **3**
9	Superhydride (3.0)	0.3	rt	15	**14** + **3** (traces)
10	$NaBH_4$/TFA (3.0)	0.3	rt	15	**14**
11	$LiAlH_4$ (1.0)	0.3	rt	15	**15**
12	$NaBH_4$/Tf_2O (3.0)	0.3	0	15	**13** + complex mix
13	$Zn(OAc)_2$ (10 mol%), $(EtO)_2SiH$ (3.0)	0.3	rt	22	**13**
14	**$Zn(OAc)_2$ (10 mol%), $(EtO)_2SiH$ (3.0)**	**0.3**	**60**	**22**	**3 (86% yield)**

[a] Concentration of the starting material **13**.

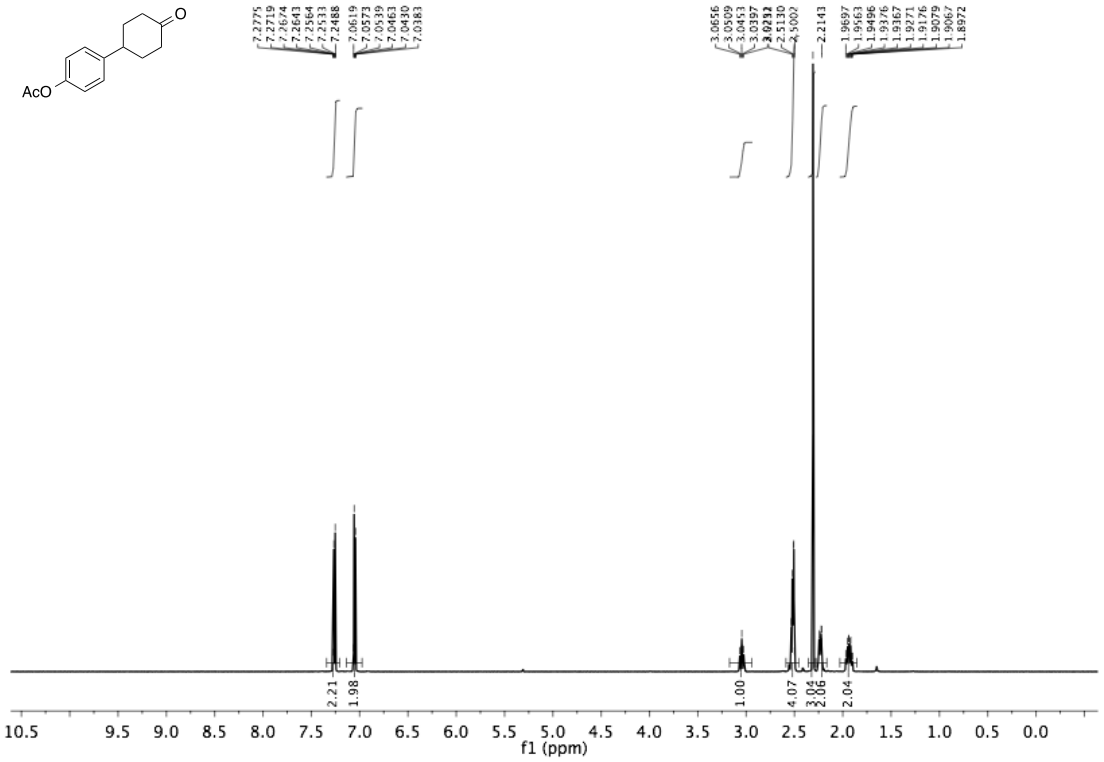
3. ^{1}H- and ^{13}C-NMR Spectra

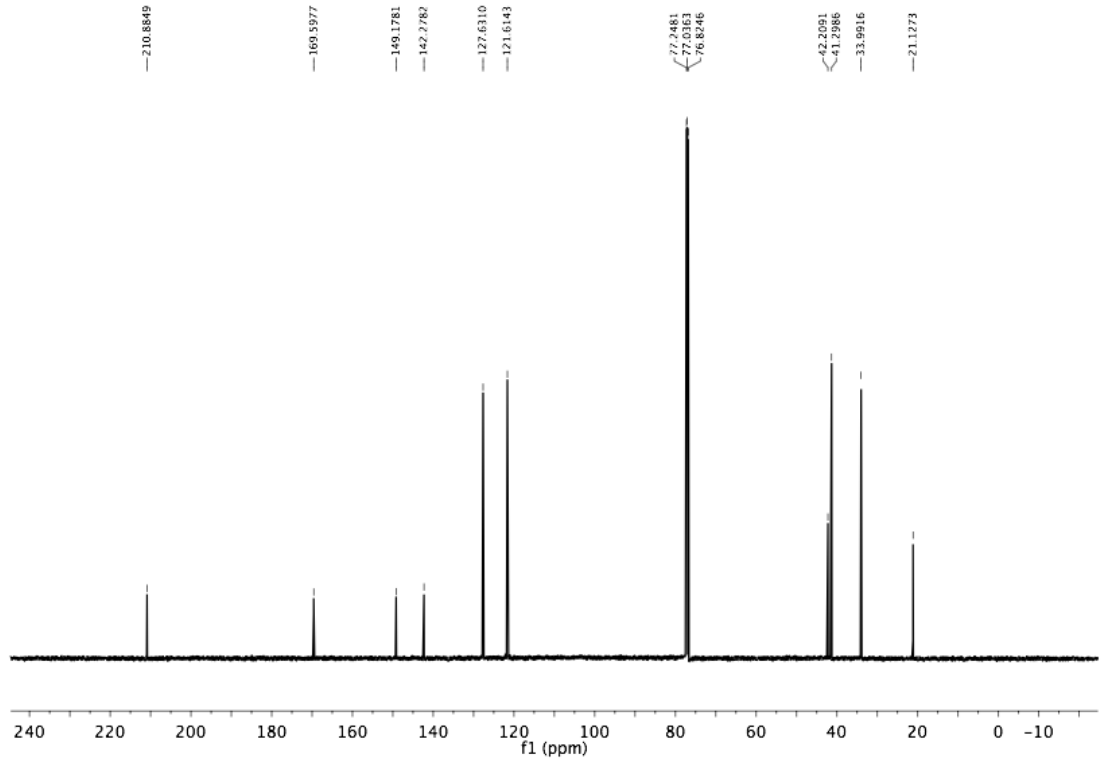
4-(4-Hydroxyphenyl)cyclohexan-1-one (4)



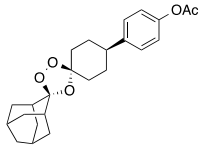


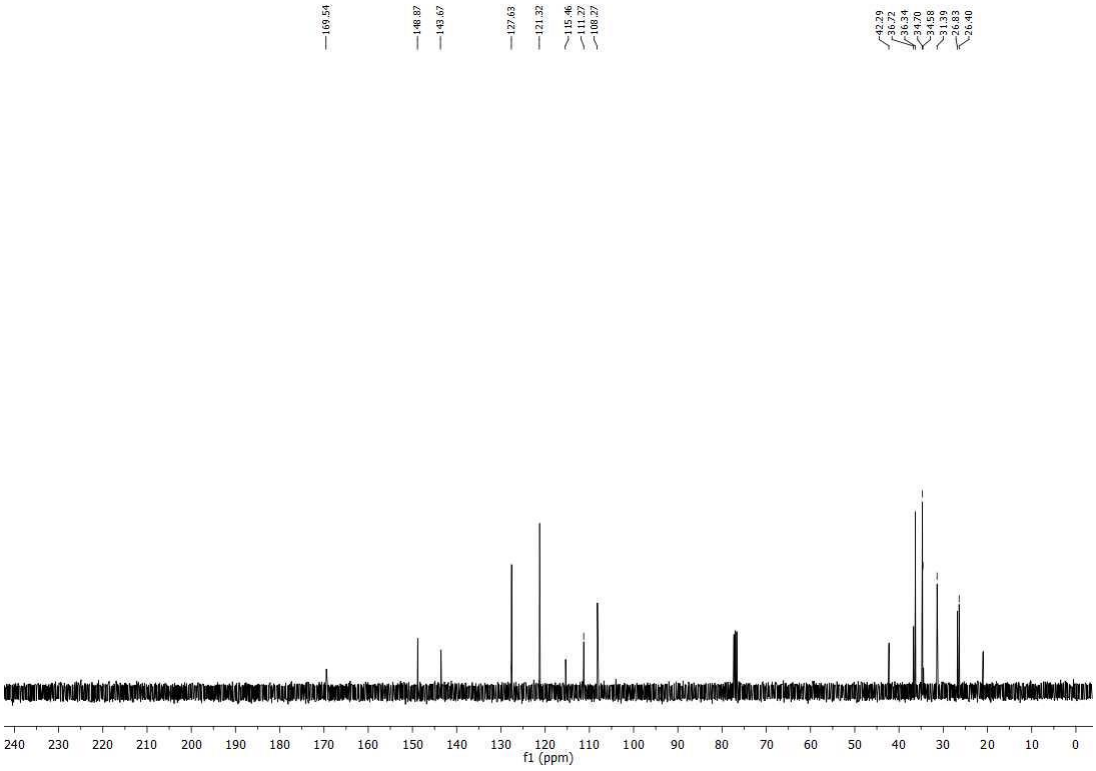
4-(4-Oxocyclohexyl)phenyl acetate (10)



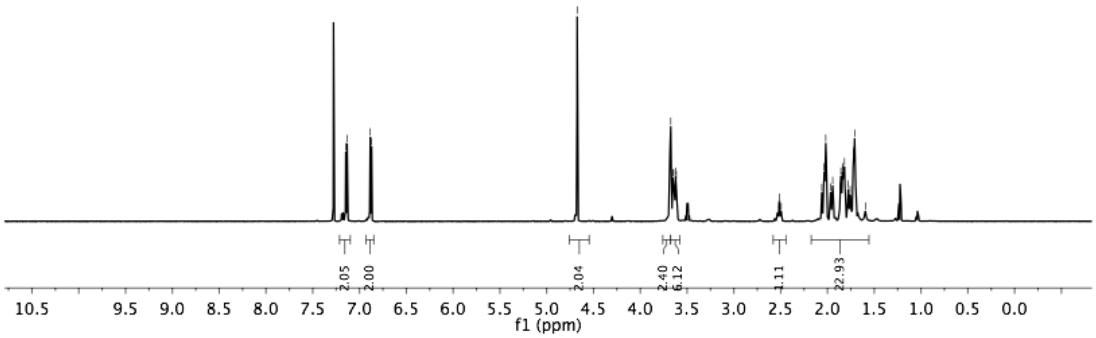


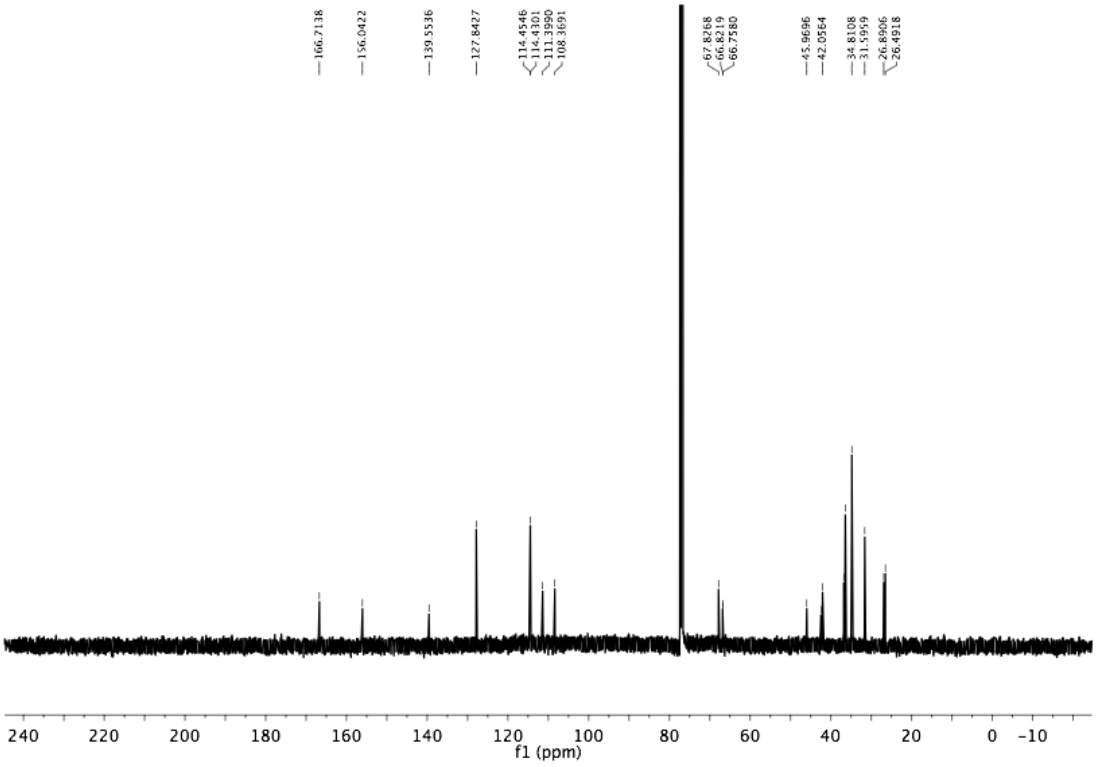
4-((1*R*,3*S*,4''*S*,5*R*,5'*S*,7*R*)-dispiro[adamantane-2,3'-[1,2,4]trioxolane-5',1''-cyclohexan]-4''-yl)phenyl acetate (6)



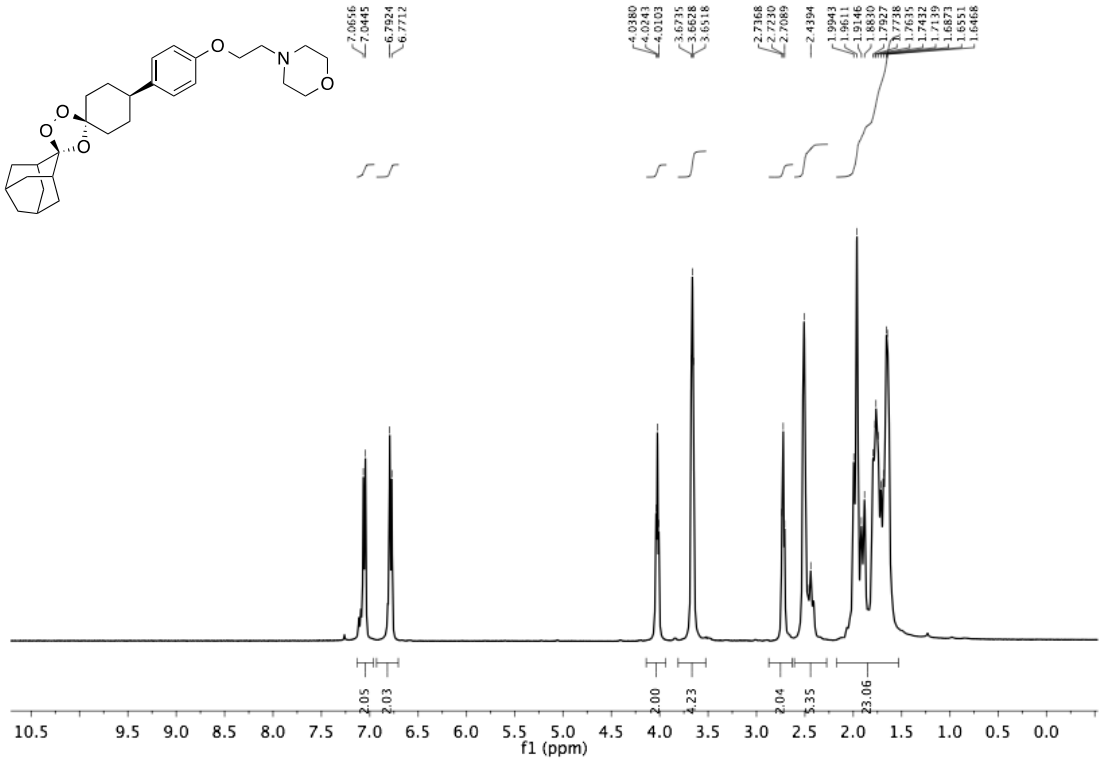


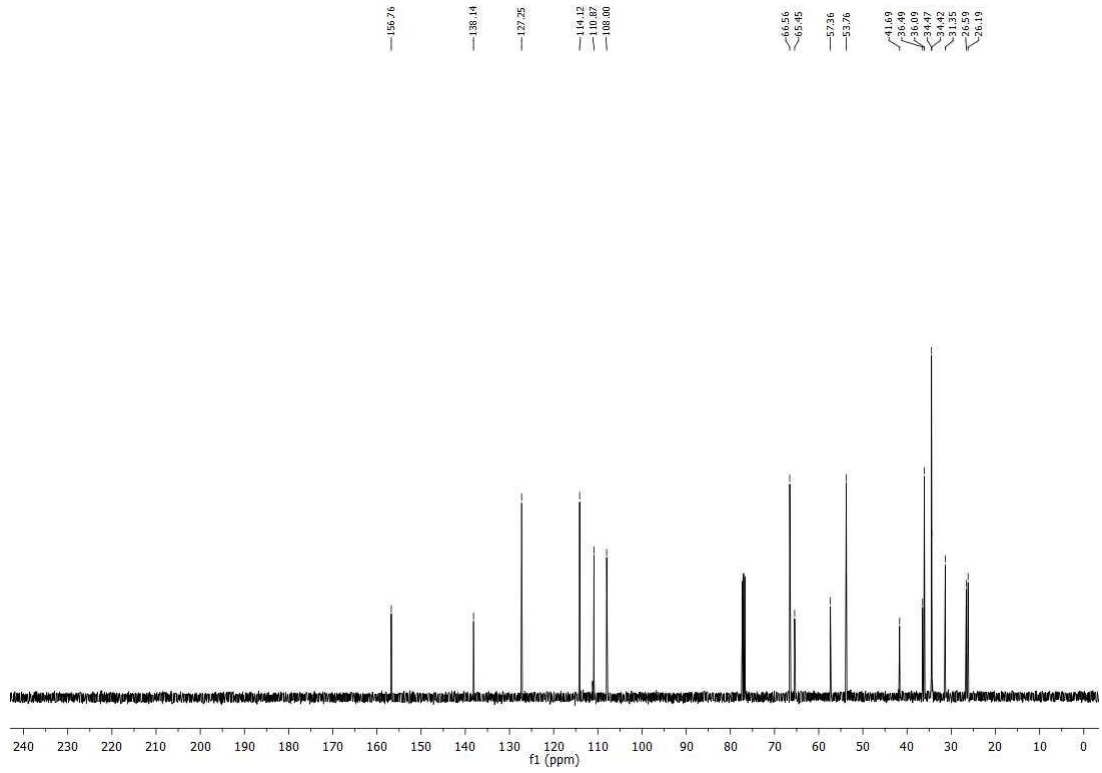
2-(4-((1*R*,3*S*,4''*S*,5*R*,5'*S*,7*R*)-dispiro[adamantane-2,3'-[1,2,4]trioxolane-5',1''-cyclohexan]-4''-yl)phenoxy)-1-morpholinoethan-1-one (13)





4-(2-(4-((1*R*,3*S*,4''*S*,5*R*,5'*S*,7*R*)-dispiro[adamantane-2,3'-[1,2,4]trioxolane-5',1''-cyclohexan]-4''-yl)phenoxy)ethyl)morpholine (3, OZ439)





4. References

1. **www.vapourtec.co.uk/**

2. **http://www.uniqsis.com/**

3. **http://www.helgroup.com/reactor-systems/hydrogenation-catalysis/flowcat/**

4. **http://kinesis.co.uk/omnifit-chromatography-columns/**

5. Tang, Y.; Dong, Y.; Karle, J. M.; DiTusa, C. A.; Vennerstrom, J. L. *J. Org. Chem.* **2004**, *69*, 6470-6473.

6. Charman, S. A.; Arbe-Barnes, S.; Bathurst, I. C.; Brun, R.; Campbell, M.; Charman, W. N.; Chiu, F. C.; Chollet, J.; Craft, J. C.; Creek, D. J.; Dong, Y.; Matile, H.; Maurer, M.; Morizzi, J.; Nguyen, T.; Papastogiannidis, P.; Scheurer, C.; Shackleford, D. M.; Sriraghavan, K.; Stingelin, L.; Tang, Y.; Urwyler, H.; Wang, X.; White, K. L.; Wittlin, S.; Zhou, L.; Vennerstrom, J. L. *Proc. Natl. Acad. Sci.* **2011**, *108*, 4400-4405.