Reiterative chiral resolution/racemization/recycle (RRR synthesis) for an effective and scalable process for the enantioselective synthesis of a dual IDO1/TDO2 inhibitor imidazoisoindole derivative.

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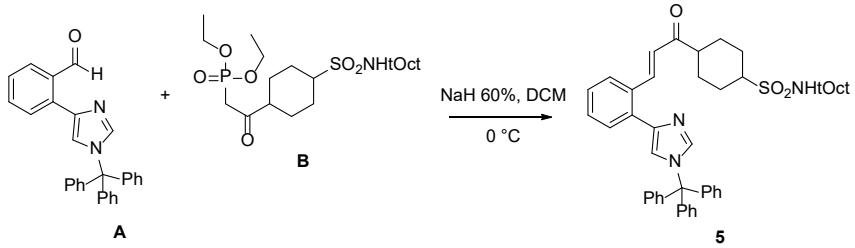
Supporting information

General remarks

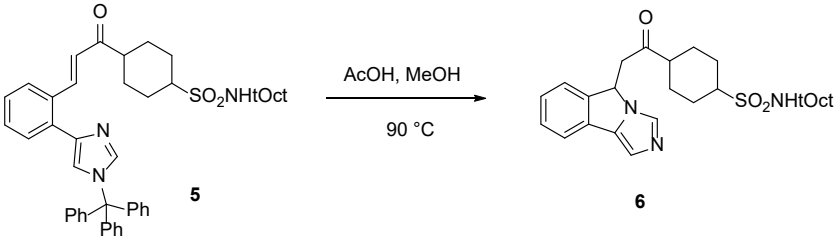
Anhydrous solvents dichloromethane (DCM) and tetrahydrofuran (THF) were obtained from Sigma-Aldrich, but the usual solvents ethyl acetate (EtOAc), dichloromethane (DCM), hexanes, methanol (MeOH) were purchased from Carlo Erba. Thin-layer chromatography (TLC) and flash-column chromatography were performed on 0.25 mm silica gel 60 F_{254} plates (E. Merck; Darmstadt, Germany) and with 230–400 mesh ASTM silica gel 60. HPLC puritiy for compound 1 was determined with a Waters HPLC (mod.e2695) with PDA (waters mod. 2998).HPLC purity for compound **6** was determined with a Waters 1525 Binary Pump with Waters 2487 Dual λ Absorbance Detector Nuclear magnetic resonance (NMR) spectra were recorded at 400 MHz and 300 MHz for ^{1}H and 100 MHz and 75 MHz for ^{13}C on a Bruker Avance 400 digital spectrometer and Bruker Avance 300 digital spectrometer (Billerica, MA). The chemical shifts (δ) were expressed in ppm and referenced to chloroform (7.26 and 77.0 ppm) , methanol (3.49 and 49.7 ppm) or dimethyl sulfoxide (2.50 and 39.5 ppm) for ^{1}H and ^{13}C NMR, respectively. High-resolution mass spectra (HRMS) were recorded on Bruker Solarix XR Fourier Transform (Bremen, Germany) equipped with a turbo ion-spray source. Melting points were determined on a Gallenkamp melting point apparatus (England). Compound **5** and compound **6** were prepared according to literature procedure.[1,2]

1. Procedure for the synthesis of (E)-*N*-(2,4,4-trimethylpentan-2-yl)-4-(3-(2-(1-trityl-1H-imidazol-4-yl)phenyl)acryloyl)cyclohexane-1-sulfonamide (compound 5)



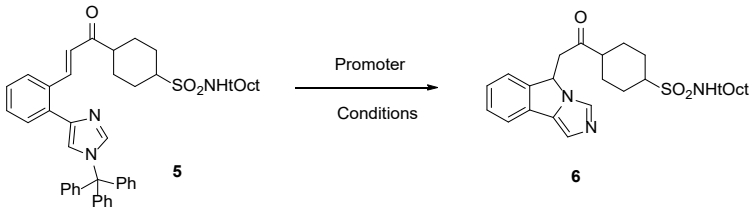
At 0 °C, to a suspension of sodium hydride (60%, 87 mg, 3.6 mmol) in dichloromethane (16 mL) was added phosphonate **B** (1.20 g, 2.65 mmol) slowly. After stirring for additional 15 min at 0 °C, the reaction mixture was treated with a solution of 2-[l-(triphenylmethyl)-lH-imidazol-4yl]benzaldehyde **A** (1.00 g, 2.41 mmol) in dichloromethane (12 mL). The resulting mixture was then stirred at room temperature for 2.5 h. The reaction was quenched by water (100 mL) and the mixture was extracted with dichloromethane (70 mL x 2). The organic phases were combined, washed with brine, and dried over Na_2SO_4. After the solvent was removed under reduced pressure, the light yellow solid was purified by flash chromatography eluting with Hexane 80/Ethyl acetate 20 to give a white solid. Yield: 89% (1.530 g). M.p. 181-182°C. HR-MS (ESI) *m/z* calculated for [$C_{45}H_{51}N_3O_3S + H^+$] 714.3723. Found: 714.3748. ^{1}HNMR ($CDCl_3$+ CD_3OD, 400 MHz) δ 7.99 (d, 1H, *J*= 15.91 Hz), 7.71 (d, 1H, *J*= 7.61 Hz), 7.63 (d, 1H, *J*= 7.61 Hz), 7.60 (s, 1H), 7.45-7.37 (m, 11H), 7.23 (d, 6H, *J* =7.61 Hz), 6.96 (s, 1H), 6.81 (d, 1H, *J*= 15.91 Hz), 2.85 (t, 1H, *J*= 9.21 Hz), 2.66 (t, 1H, *J*= 12.12 Hz), 2.31 (d, 2H, *J*= 11.41 Hz), 2.05 (d, 2H, *J*= 12.12 Hz), 1.71 (s, 2H), 1.61 (q, 2H, *J*= 10.40 Hz), 1.41 (s, 8H), 1.07 (s, 9H).^{13}CNMR ($CDCl_3$, 100 MHz) δ 201.6, 143.2, 142.1, 139.2, 139.1, 134.8, 132.5, 129. 129.8, 129.6, 128.1, 127.2, 127.0, 125.5, 121.2, 75.5, 62.9, 58.7, 55.6, 47.2, 31.6, 31.5, 29.6, 27.4, 25.8.

2. Procedure for the synthesis of 4-(2-5H-Imidazo[5,1-a]isoindol-5-yl-acetyl)-cyclohexanesulfonic acid (1,1,3,3-tetramethyl-butyl)-amide as racemate (compound 6).



To a suspension of **5** (0.150 g, 0.21 mmol) in methanol (1.2 mL) was added acetic acid (350 µL) slowly at room temperature. The resulting reaction mixture was stirred at 90 °C for 2 h. The solvent was removed under reduced pressure and the residue was dissolved in ethyl acetate (8 mL). The diluted solution was washed with sat. $NaHCO_3$ solution (5 mL x 2) and brine, and then dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by flash chromatography eluting DCM 98/MeOH 2 to yield a white-grey solid. Yield: 91% (0.90 g). M.p. 152-153°C. HR-MS (ESI) *m/z* calculated for [$C_{26}H_{37}N_3O_3S + H^+$] 472.2628. Found: 472.2633. ^{1}HNMR (CD_3OD, 300 MHz) δ 7.61 (s, 1H), 7.58 (d, 1H, *J*= 7.48 Hz), 7.45 (d, 1H, *J*= 7.53 Hz), 7.38 (t, 1H, *J*= 7.36 Hz), 7.28 (t, 1H, *J*= 7.48 Hz), 7.11 (s, 1H), 5.68 (dd, 1H, *J*= 3.36, 8.80 Hz), 3.46 (dd, 1H, *J*= 3.63, 18.68 Hz), 3.02 (dd, 1H, *J*= 9.08, 18.81), 2.85 (t, 1H, *J*=13.48), 2.49 (t, 1H, *J*= 11.81 Hz), 2.30-2.26 (m, 2H), 2.13-2.06 (m, 2H), 1.68 (s, 2H), 1.61-1.40 (m + s, 10H), 1.05 (s, 9H). ^{13}C (CD_3OD, 75 MHz) δ 210.8, 144.5, 137.6, 132.9, 129.3, 128.4, 126.7, 123.3, 119.6, 116.6, 62.5, 57.6, 56.2, 54.6, 48.9, 46.4, 31.0, 30.8, 28.9, 26.7, 26.6, 25.6, 25.5. HPLC: AD-H column, 60/40 Hexane/Isopropanol, flow: 0.8 mL/min, λ: 254 nm and 274 nm. Retention times: 15 min and 24 min.

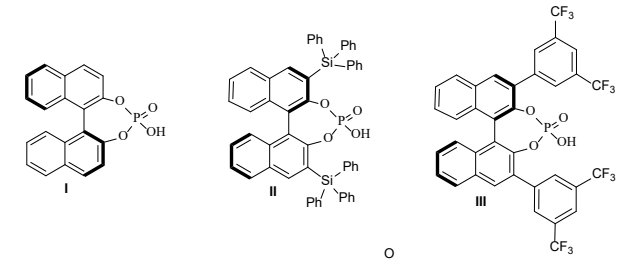
3. General procedure for deprotection/intra-molecular aza-Michael reaction using chiral promoters

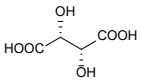


To a solution of compound **5** (0.05 mmol) in solvent (see Table 1 for details) chiral promoter was added and the reaction was stirred for the time and at temperature reported. The solvent was removed under vacuum and the crude purified by flash chromatography (from Hexane 50/Ethyl acetate 50 to DCM 98/MeOH 2). HPLC: AD-H column, 60/40 Hexane/Isopropanol, flow: 0.8 mL/min, λ: 254 nm and 274 nm. Retention times: 15 min and 24 min. $[\alpha]_D^{20:}$ -3.2 (c 0.5, MeOH).

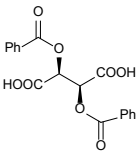
Table S1

Promoters





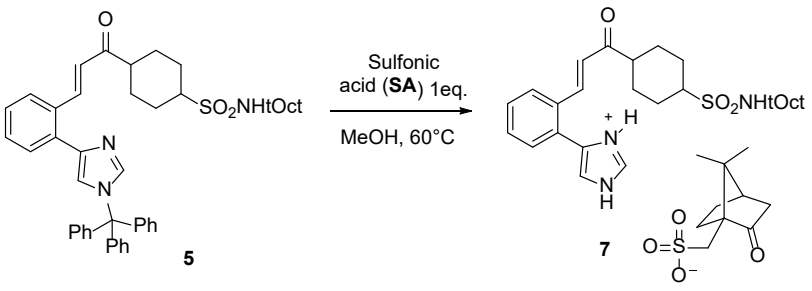
L (+) Tartaric acid



D (+) (2,3)-Dibenzoyl tartaric acid

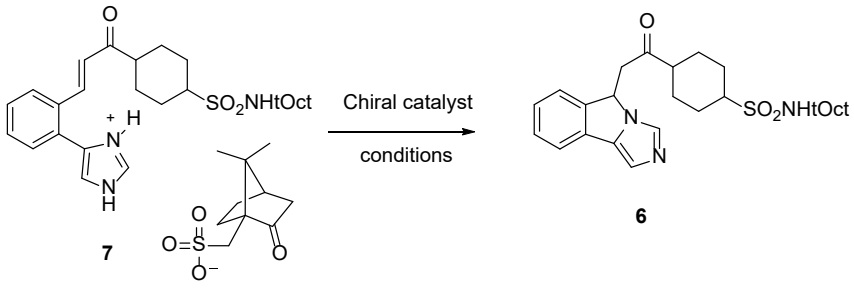
EXP	SOLVENT	PROMOTER (mol%)	TIME	T °C	YIELD 3 (%)	ee (%)
1	DCM	**I** (20%)	5 days	r.t	Traces	
2	Toluene	**I** (20%)	2 days	60°C	42	3%
3	Toluene	**I** (20%)	3 days	40°C	Traces	
4	Toluene	**I** (20%)	7 days	60°C	40	6%
5	MeOH	**I** (20%)	7 days	r.t	43	5%
6	DCM	**I** (50%)	7 days	r.t	31	**-14%**
7	DCM	**I** (50%)	7 days	r.t	30	-14%
8	DCM	**I** (100%)	7 days	r.t	66	-13%
9	Toluene	**I** (50%)	3 days	60°C	56	8%
10	THF	**I** (50%)	7 days	r.t	NO REACTION	
11	AcOEt	**I** (50%)	7 days	r.t	Traces	
12	$CHCl_3$	**I** (50%)	8 days	r.t	**74**	-12%
13	MeOH	**I** (50%)	2 days	r.t	99	8%
14	$CHCl_3$ /H_2O (1:1)	**I** (50%)	7 days	r.t	31	-13%
15	DCM	**I** /(20%) / $AgNO_3$ (20%)	4 days	r.t	14	-12%
16	DCM	**I** (50%) / AgOtf(50%)	3 days	r.t	100	-8%
17	$CHCl_3$	**I** (20%) / TFA (100mol%)	2 days	r.t	87	0
18	$CHCl_3$	**II** (100%)	7 days	r.t	Traces	
19	$CHCl_3$	**III** (100%)	7 days	r.t	Traces	
20	DCM	**III** (100%)	7 days	r.t	Traces	
21	MeOH	L-Tartaric acid (100%)	7 days	r.t	99	6%
22	MeOH	D-(2,3)-Dibenzoyl tartaric acid (100%)	4 days	r.t	88	-2%
23	$CHCl_3$	D-(2,3)-Dibenzoyl tartaric acid (100%)	7 days	r.t	99	0

4. Procedure for the synthesis of (E)-4-(2-(3-oxo-3-(4-(N-(2,4,4-trimethylpentan-2-yl)sulfamoyl)cyclohexyl)prop-1-en-1-yl)phenyl)-1H-imidazol-3-ium camphorsulfonic salt (compound 7)



To a white suspension of compound **5** (1 mmol) in MeOH (20 mL), (1*R*)-(-)-10-Camphorsulfonic acid monohydrate 98% (1 mmol, 250 mg), was added and the reaction was stirred for 1h at 60°C. The solvent was removed under vacuum and the crude was dissolved in $CHCl_3$ and precipitated by adding hexane, to yield a white solid. Yield: 70%. (500 mg). HR-MS (ESI) *m/z* calculated for $[C_{26}H_{38}N_3SO_3]^+$ 472.2633. Found: 472.2639. ^{1}HNMR ($CDCl_3$, 400 MHZ) δ 9.05 (s, 1H), 7.76-7.68 (m, 3H), 7.53-7.45 (m, 3H), 7.34-7.30 (m, 1H), 6.76 (d, 1H, *J*= 15.88 Hz), 4.51 (s, 1H), 3.36 (d, 1H, *J*= 14.64 Hz), 2.94-2.81 (d + m, 2H), 2.73 (t, *J*= 11.76 Hz, 1H), 2.54 (dt, *J*= 3.24, 14.64 Hz, 1H), 2.40-2.30 (m, 3H), 2.13-2.06 (m, 4H), 1.91-1.86 (m, 4H), 1.68 (s, 2H), 1.67-1.66 (m, 2H), 1.45 (s, 8H), 1.06 (s, 9H), 1.05 (s, 3H), 0.84 (s, 3H).

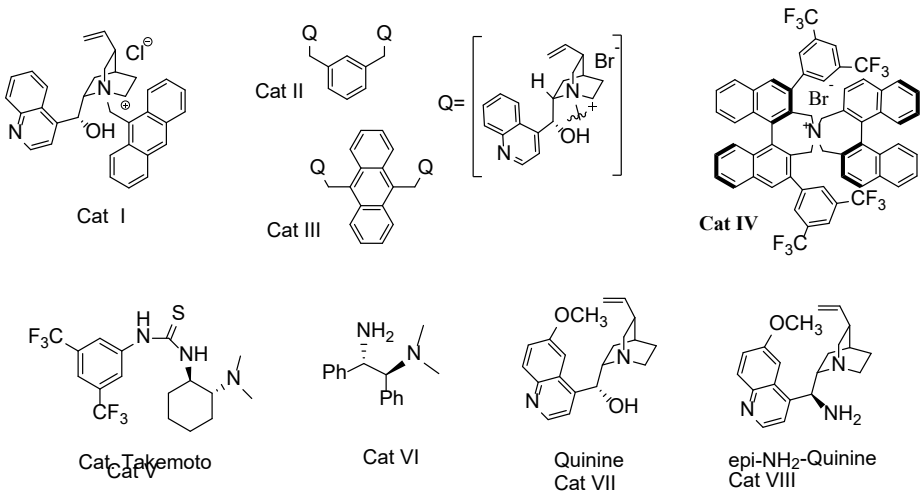
5. General procedure for intra-molecular aza-Michael reaction from sulfonic salt 7



Sulfonic salt **7** (0.05 mmol) was dissolved in solvent (see Table S2 for details) and chiral catalyst was added: the reaction was stirred for the time and at temperature reported. The solvent was removed under vacuum and the crude purified by flash chromatography (from Hexane 50/Ethyl acetate 50 to DCM 98/MeOH 2). HPLC: AD-H column, 60/40 Hexane/Isopropanol, flow: 0.8 mL/min, λ: 254 nm and 274 nm. Retention times: 15 min and 24 min.

Table S2

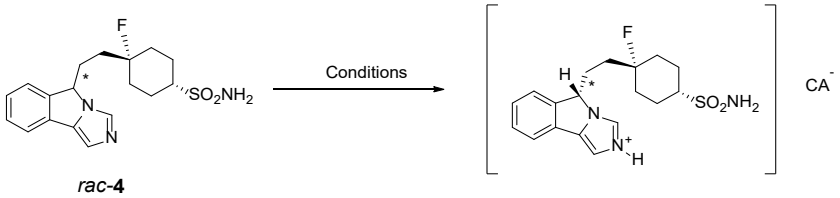
Chiral catalysts



EXP	SOLVENT	CATALYST (mol%)	TIME	T °C	YIELD 3 (%)	ee (%)
1	$CHCl_3$	K_2CO_3 (100%) (control)	24 h	r.t	99%	0
2	DCM	Cat **I** (20%) / K_2CO_3 (100%)	15h	r.t	98 %	0
3	DCM	Cat **I** (20%) / K_2CO_3 (100%)	18h	r.t	99%	0
4	DCM	Cat **I** (20%) / K_2CO_3 (100%)	4 days	0°C	75%	0
5	$CHCl_3$	Cat **II** (20%) / K_2CO_3 (100%)	4 h	r.t	99%	2%
6	$CHCl_3$	Cat **II** (20%) / K_2CO_3 (100%)	14 h	r.t	92%	0
7	$CHCl_3$	Cat **III** (20%) / K_2CO_3 (100%)	14 h	r.t	95%	0
8	DCM/ H_2O (1/1)	Cat **IV** (20%)	24 h	r.t	99%	0
9	DCM	Cat **IV** / K_2CO_3 (100%)	24 h	r.t	99%	0
10	DCM	Cat. **II** (20%)	24 h	r.t	99%	0
11	$CHCl_3/H_2O$ (1/1)	Cat. **II** (20%)	24 h	r.t	99%	0
12	$CHCl_3$	Cat. **V** (20%)	7 days	r.t	50%	0
13	$CHCl_3$	Cat. **VI** (30%)	18 h	r.t	99%	0
14	$CHCl_3$	Cat. **VI** (30%)	4 days	0°C	99%	0
15	$CHCl_3$	Cat. **VII** (20%)	14 h	r.t	99%	0
16	$CHCl_3$	Cat. **VII** (100%)	14 h	r.t	99%	0
17	DCM	Cat. **VIII** (20%)	30h	r.t	89%	0

6-

Procedures for preliminary crystallization screening on 20 mg scale.



rac-**4**

The racemic Drug Substance (batch scale: 20mg) and the chiral acid (100 mol%) were charged into the glass tube vial and the solvent (10Vol-40Vol) was added at room temperature. The obtained mixture was mixed at room temperature for a few minutes in order to observe if dissolution occurs. Then, the mixture was heated to 65°C (water bath) and stirred for 1 hour. The hot mixture was cooled down to room temperature. In case no precipitation occurred, the mixture was further cooled down to +5°C (refrigerated conditions overnight).

The solid was separated from the mother liquors and both of them were analyzed by means of the chiral HPLC method.

Table S3. Some of the results deriving from the screening of chiral acids and solvents of Table 1.

Solvent	Chiral acid	Chiral HPLC results Solid	Chiral HPLC results Mother Liquors.
2-Propanol	D-(-)-tartaric acid	(R) 49.50% -(S) 50.50%	
	R-(-)-Mandelic acid	(R) 49.86% -(S) 50.14%	
	(1R)-(−)-10-Camphorsulfonic acid	no precipitation	
	(+)-2,3-Dibenzoyl-D-tartaric acid	Nearly racemic	
	(S)-(+)-OAcetylmandelic acid	Nearly racemic	
	(+)-O,O'-Di-p-toluoyl-D-tartaric acid	Nearly racemic	
	D-(-)-quinic acid	Nearly racemic	
	(1R,3S)-(+)-Camphoric acid	Nearly racemic	
	L-(-)-Malic acid	Nearly racemic	
Acetone	D-(-)-tartaric acid	(R) 49.50% -(S) 50.50%	(R) 51.62% -(S) 48.38%
	R-(-)-Mandelic acid	(R) 49.86% -(S) 50.14%	(R) 49.73% -(S) 50.27%
	(1R)-(−)-10-Camphorsulfonic acid	no precipitation	
	(+)-2,3-Dibenzoyl-D-tartaric acid	**(R) 15.09% -(S) 84.91%**	**(R) 72.31% -(S) 27.69%**
	(S)-(+)-O-Acetylmandelic acid	no precipitation	
	(+)-O,O'-Di-p-toluoyl-D-tartaric acid	no precipitation	
	D-(-)-quinic acid	Nearly racemic	
	(1R,3S)-(+)-Camphoric acid	Nearly racemic	
	L-(-)-Malic acid	no precipitation	
Acetonitrile	D-(-)-tartaric acid	(R) 49.73% -(S) 50.27%	
	R-(-)-Mandelic acid	(R) 50.62% -(S) 49.38%	
	(1R)-(−)-10-Camphorsulfonic acid	Nearly racemic	
	(+)-2,3-Dibenzoyl-D-tartaric acid	**(R) 35.70% -(S) 64.30%**	**(R) 60.78% -(S) 39.22%**
	(S)-(+)-O-Acetylmandelic acid	Nearly racemic	
	(+)-O,O'-Di-p-toluoyl-D-tartaric Acid	Nearly racemic	
	D-(-)-quinic acid	Nearly racemic	
	(1R,3S)-(+)-Camphoric acid	Nearly racemic	
	L-(-)-Malic acid	no precipitation	

Other tested solvents gave nearly racemic or no precipitation.

- Investigation of water as crystallization co-solvent.

Racemic Drug Substance (50,000 mg; 0,138 mmol) was charged into a glass tube andAcetone-Water mixture (0.500 mL; 10 Vol) was added at room temperature. The resulting suspension was heated to reflux (external temperature, water bath 65°C) and after 10 min under stirring the mixture was a yellow solution. Then (+)-2,3-Dibenzoyl-D-tartaric acid (49,291 mg; 0,138 mmol) was added to the hot turbid solution. After 10 minutes of stirring at reflux the limpid yellow solution was left to cool naturally down to room temperature. After 15 min at room temperature the formation of a white solid was observed. The precipitate was separated from mother liquors

Table S4: Chiral HPLC results of diastereomeric salts

Entry	Co-solvent / solvent	Chiral HPLC results (solid)
1	5% H_2O/ 95% Acetone (v/v)	(R) 9.11% - (S) **90.89%**
2	10% H_2O / 90% Acetone (v/v)	(R) 5.24% - (S) **94.76%**

The trial 2 was replicated on a higher batch scale 500mg: 234mg of MSC2579448D as white solid were obtained (yield: 23.3%; chiral purity (S)%: 99.03 %;

- The effect of the dilution and the effect of the crystallization cooling temperature on the resolution efficiency and yield was investigated on 3g scale. Some representative examples are presented in the following table.

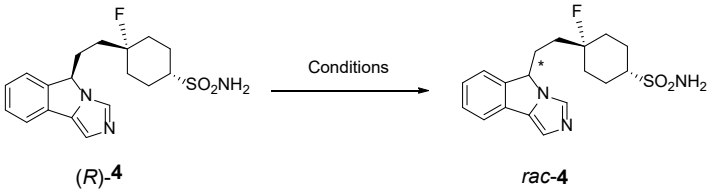
Table S5: Solvent/co-solvent effect

Entry	T (°C)	Volume (mL/g rac-1)	Yield (%)	e.r.
1	25°C	10	23.3	(R) 0.92% - (S) **99.08%**
2	25°C	5	28.30	(R) 0.86% - (S) **99.14%**
3	0/5°C	5	29.10	(R) 0.60% - (S) **99.40%**
4	-15/-20°C	5	33.70	(R) 0.20% - (S) **99.8%**

-Determination of the exact stoichiometry of the formed salt under the optimized conditions

Furthermore, it was investigated by ^{1}H-NMR analysis the molar ratio between the chiral agent and it was found that the precipitated salt if formed by two molecule of (*S*)-**4** compound and one molecule of *(+)-2,3-Dibenzoyl-D-tartaric acid.* The molar yields reported in the manuscript have been accordingly calculated.

7- Screening of conditions for racemization



(*R*)-**4** *rac*-**4**

Table S6: Preliminary screening for racemization of enantioenriched (*R*)-4 (selected examples)

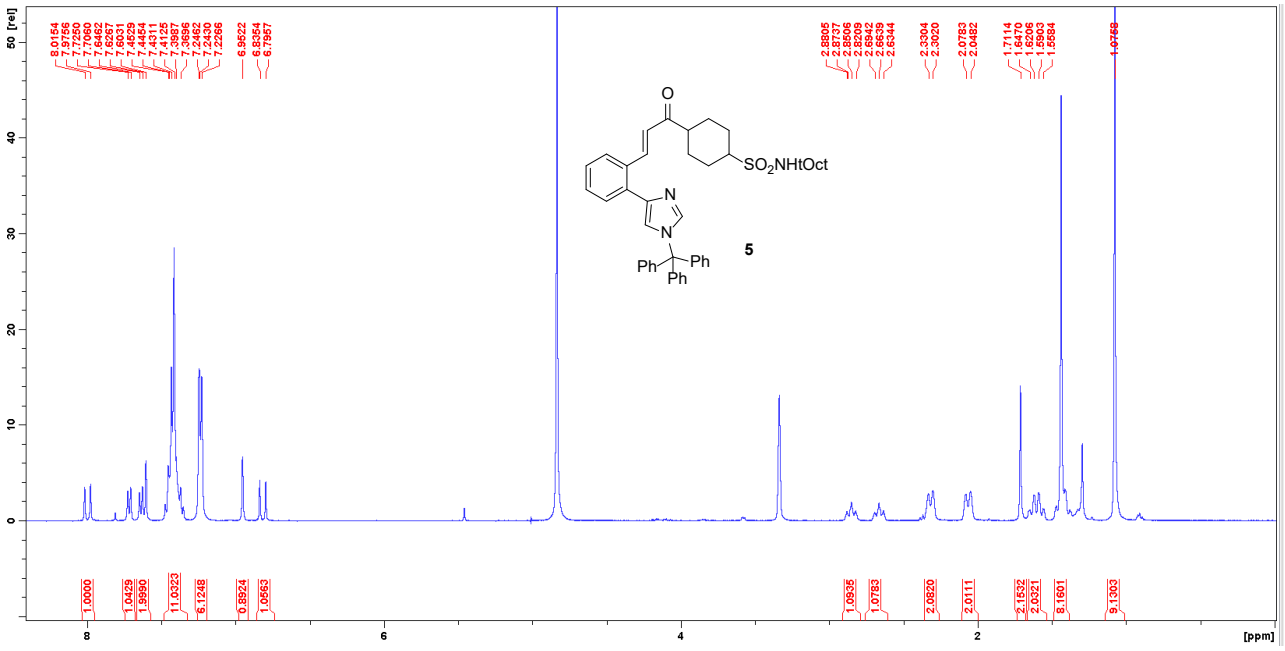
Entry	T (°C)	Solvent/base (conditions)	t (h)	e.r.
1	68°C	IPA / $NaOH_{aq}$ 0.1N (50%)	55	(R) 61.6% - (S) 38.4%
2	71°C	EtOH / $NaOH_{aq}$ 0.1N (50%)	40	(R) 50.7% - (S) 49.3%
3	78°C	IPA / Et_3N (2.2 eq)	55	(R) 98.3% - (S) 1.7%
4	72°C	EtOH / water = 75/25, NaOH = 1.8 eq	16	(R) 50.0% - (S) 50.0%

References

1. Mario Mautino, Sanjeev Kumar, Jesse Waldo, Firoz Jaipuri, Tanay Kesharwani. FUSED IMIDAZOLE DERIVATIVES USEFUL AS IDO INHIBITORS. WO2012142237A1. **2012**.

2. Sherer Brian A. CYCLOHEXYL-ETHYL SUBSTITUTED DIAZA- AND TRIAZA-TRICYCLIC COMPOUNDS AS INDOLE-AMINE-2,3-DIOXYGENASE (IDO) ANTAGONISTS FOR THE TREATMENT OF CANCER. WO 2016/037026 Al. **2016**.

^{1}HNMR AND ^{13}CNMR SPECTRA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7.3987
7.3896
7.2482
7.2460
7.2296
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6.8354
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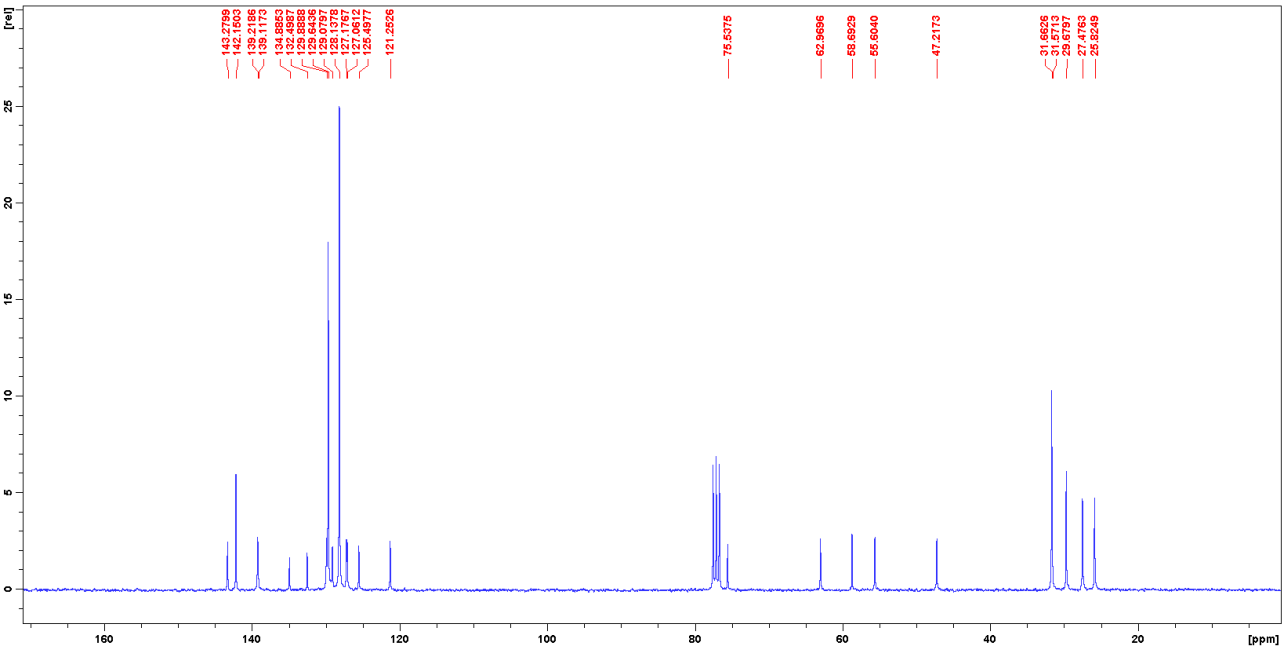
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138.1173
134.9865
132.4987
129.8888
129.6436
129.0797
128.1379
127.1767
127.0612
125.4977
121.2526
75.5375
62.9696
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47.2173
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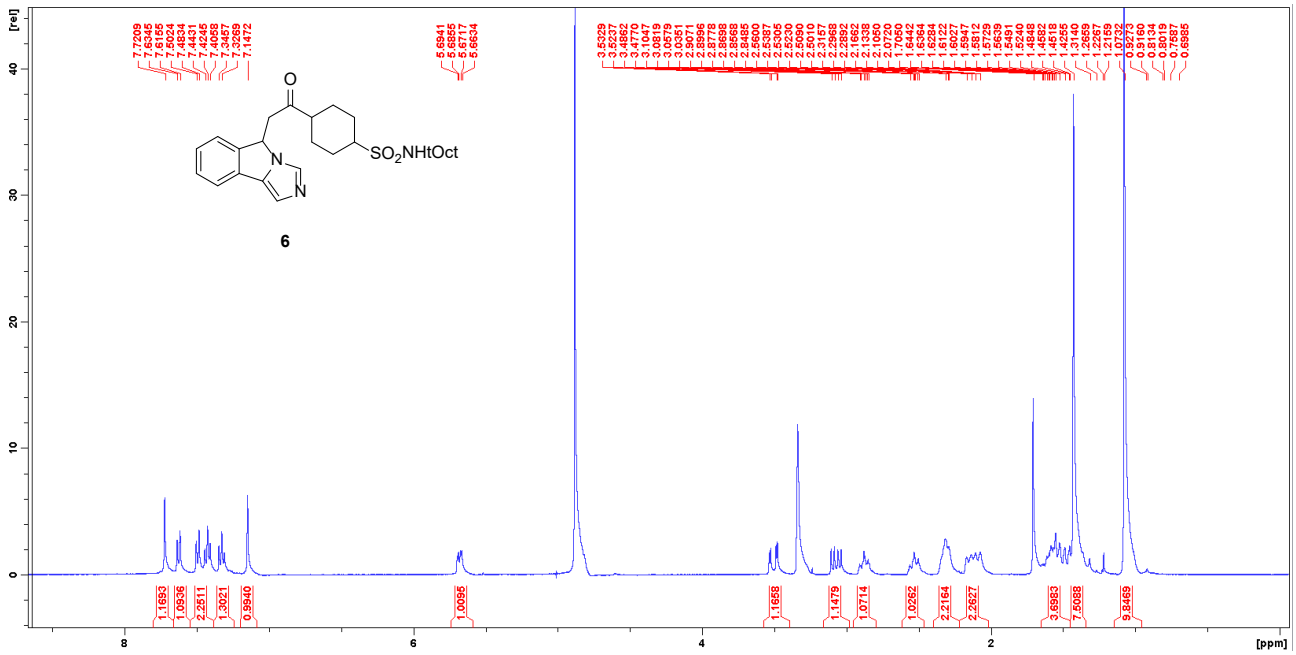
31.5713
29.6797
27.4763
25.8249
[rel]
[ppm]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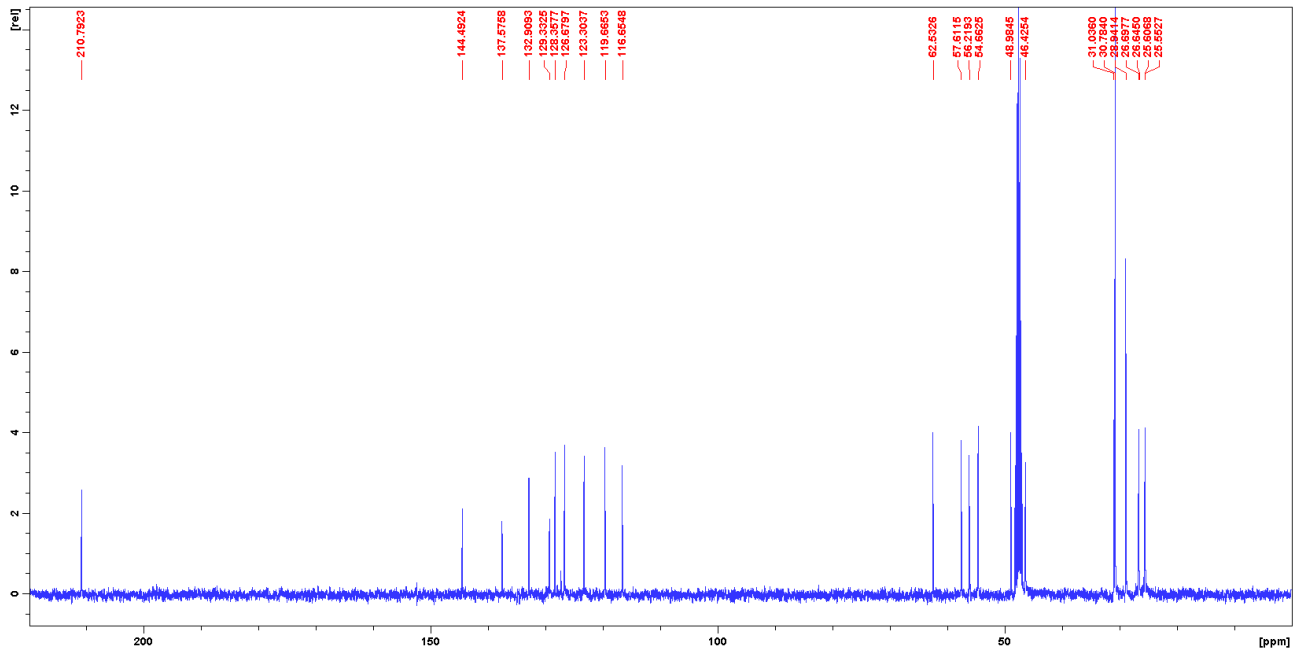
O
N
N
SO2NHtOct
6
[ppm]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132.9093
129.3325
128.8577
128.6797
123.3037
119.6653
116.6548
62.5326
57.6115
56.2193
54.6625
48.9845
46.4254
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29.8414
28.6977
26.6450
25.6068
25.5527
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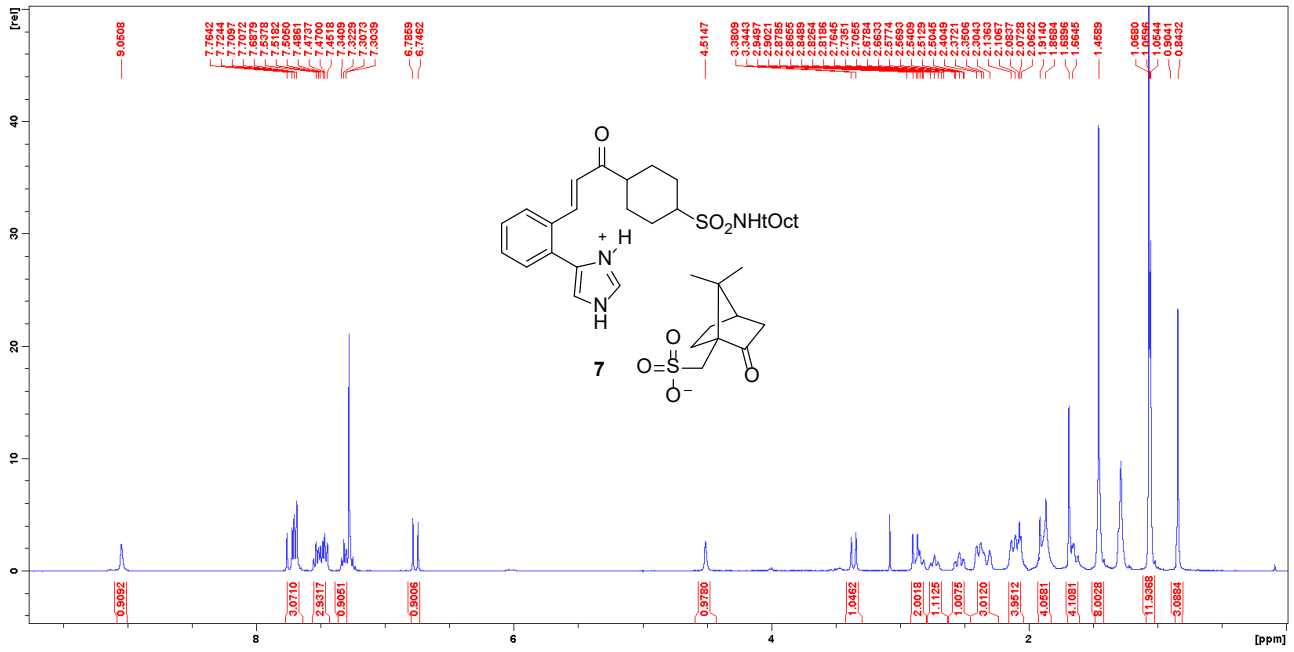
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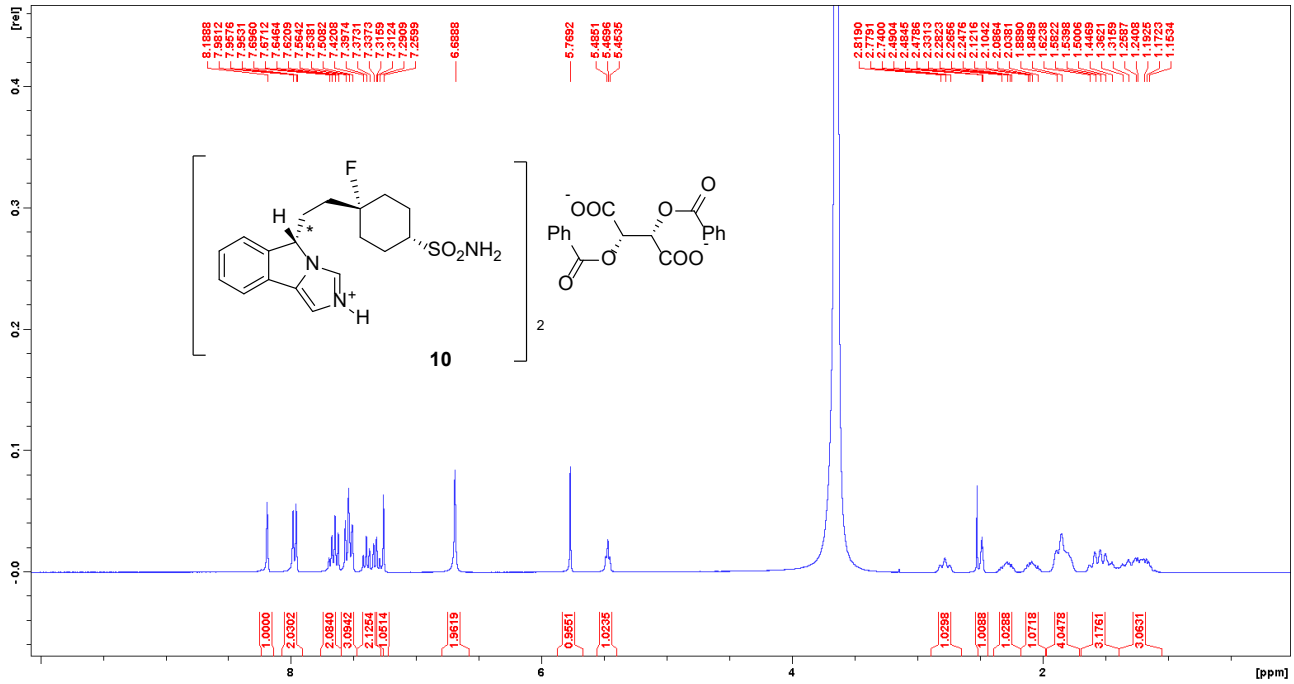
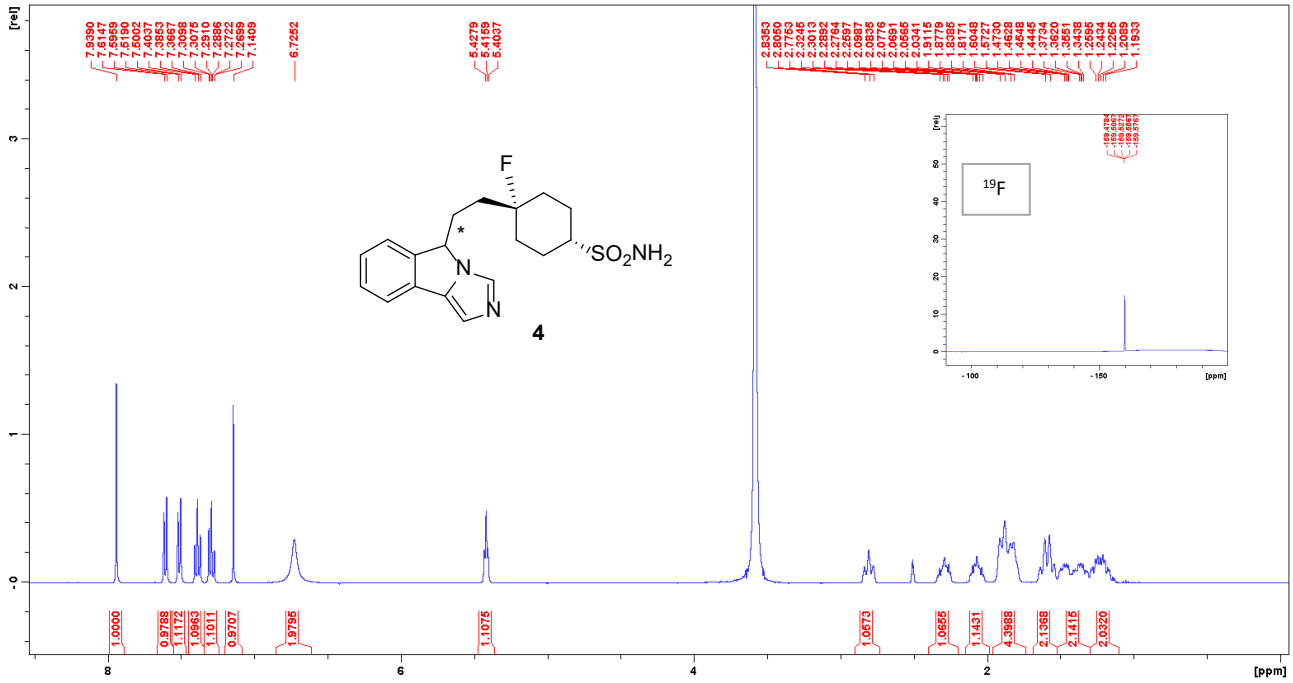
7


SO2NH2
10


F
SO2NH2
N
N
4
19F
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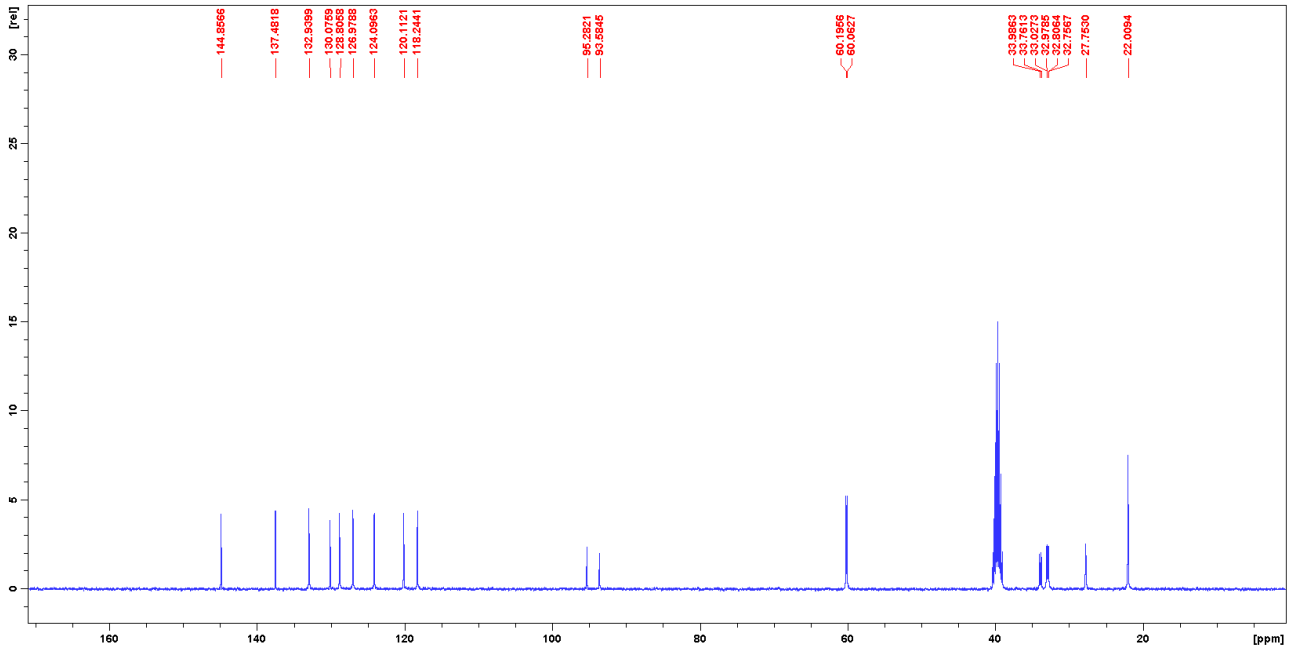
[ppm]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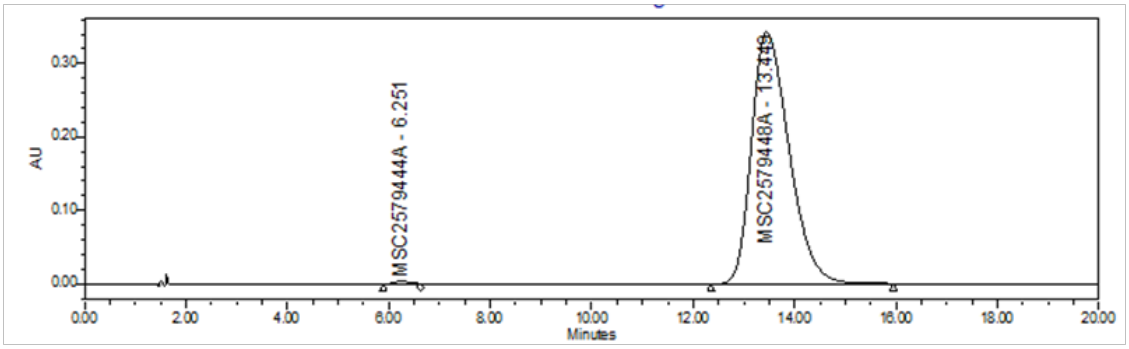
HPLC traces

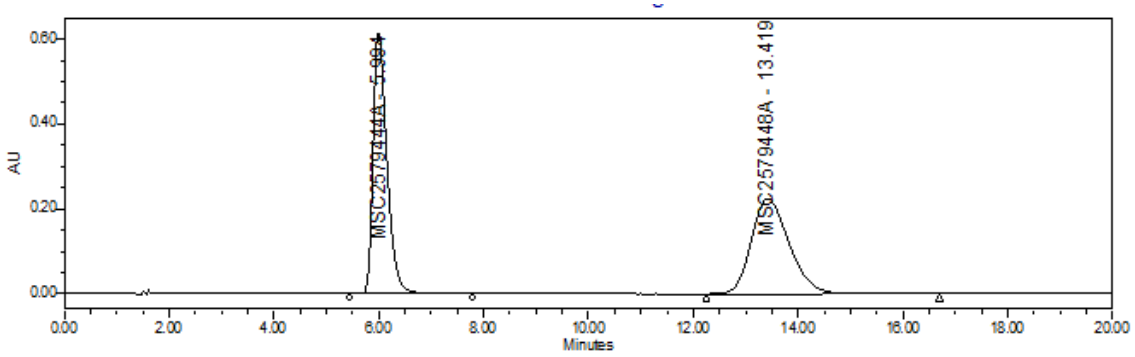


Name: MSC2579444A

	Vial	Inj	Name	RT	RT Ratio	Area	Height	% Area
1	2	2	MSC2579444A	6.25	0.465	75353.38	3750.71	0.43
Mean				6.25	0.465	75353.38	3750.71	0.43
Std. Dev.								
% RSD								

Name: MSC2579448A

	Vial	Inj	Name	RT	Area	Height	% Area	Resolution	Symmetry Factor
1	2	2	MSC2579448A	13.45	17566415.04	342750.65	99.57	7.69	1.36
Mean				13.45	17566415.04	342750.65	99.57	7.7	1.36
Std. Dev.									
% RSD									



Name: MSC2579444A

	Vial	Inj	Name	RT	RT Ratio	Area	Height	% Area	Symmetry Factor
1	2	1	MSC2579444A	5.99	0.447	11368698.19	616085.63	50.34	1.48
Mean				5.99	0.447	11368698.19	616085.63	50.34	1.48
Std. Dev.									
% RSD									

Name: MSC2579448A

	Vial	Inj	Name	RT	Area	Height	% Area	Resolution	Symmetry Factor
1	2	1	MSC2579448A	13.42	11216772.55	224698.64	49.66	8.38	1.24
Mean				13.42	11216772.55	224698.64	49.66	8.4	1.24
Std. Dev.									
% RSD									