KLS Supporting Information

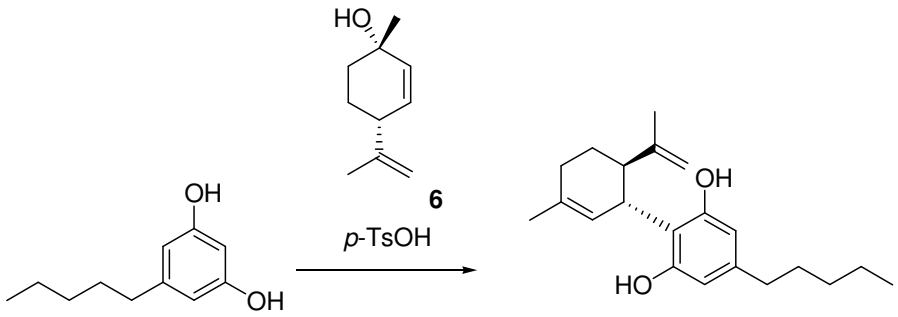
General

In the examples below, unless otherwise stated, temperatures are given in Celsius (°C); operations were carried out at room or ambient temperature, "rt," or "RT," (typically a range of from about 18-25 °C; evaporation of solvent was carried out using a rotary evaporator under reduced pressure (typically, 4.5-30 mm Hg) with a bath temperature of up to 60 °C; the course of reactions was typically followed by thin layer chromatography (TLC); melting points are uncorrected; products exhibited satisfactory ^{1}H-NMR and/or microanalytical data; and the following conventional abbreviations are also used: L (liter(s)), mL (milliliters), mmol (millimoles), g (grams), mg (milligrams), min (minutes), and h (hours).

Unless otherwise specified, all solvents and reagents were purchased from suppliers and used without further purification. Reactions were conducted under a blanket of nitrogen unless otherwise stated. Compounds were visualized under UV lamp (254 nM). ^{1}H NMR and C^{13} NMR spectra were recorded on a 300 MHz NMR instrument.

Synthesis of Cannabidiol (1)

Synthesis scheme for 1.



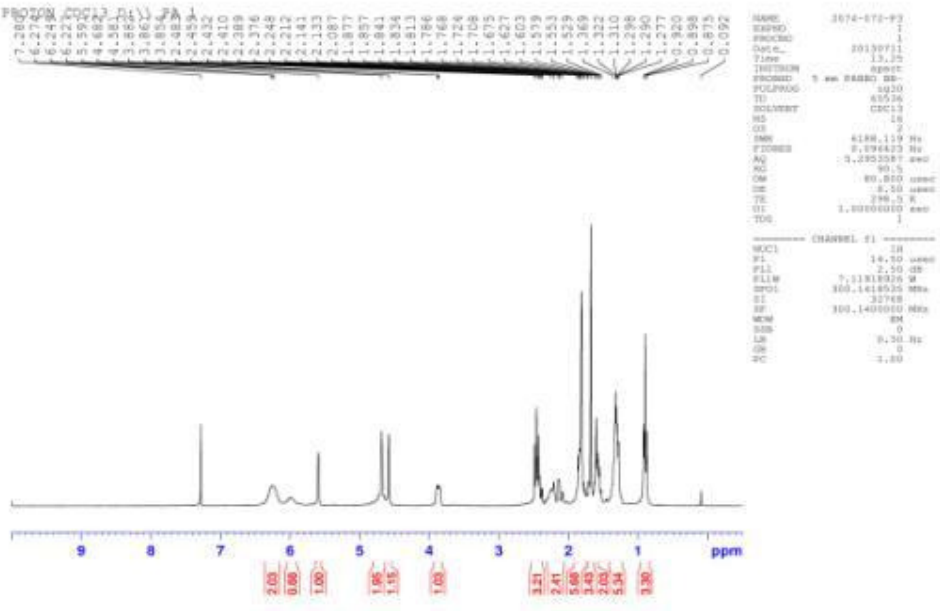
1-1 **Compound 1**

A solution of olivetol (**1-1**) (0.40 g, 2.2 mol, 1 equiv.), *p*-TsOH (40 mg, 0.21 mmol, 0.1 equiv.) and compound **6** (0.47 g, 3.1 mmol, 1.4 equiv.) in toluene (28 mL) was stirred at RT for 1.5 hours. TLC analysis indicated ~70% conversion of the starting olivetol. The reaction was stopped at this point and EtOAc (30 mL) was added to dilute the reaction mixture, which was then washed by saturated $NaHCO_3$ aqueous solution (3 x 50 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated to give crude compound **1** (0.9 g). It was purified by column chromatography to give compound **1** (140 mg, yield 20%). HPLC purity: 97%. LC/MS (ESI): m/z 315 (M+1). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 6.40-6.20 (br s, 2H), 6.10-5.90 (br s, 1H), 5.59 (s, 1H), 4.68 (s, 2H), 4.58 (s, 1H), 3.90-3.80 (m, 1H), 2.50-2.40 (m, 3H), 2.30-2.00 (m, 2H), 1.90-1.70 (m, 5H), 1.67 (s, 3H), 1.65-1.50 (m, 2H), 1.40-1.20 (m, 4H), 0.90 (t, J = 6.6 Hz, 3H). The analytical data are attached below.

Optical Rotation of **1**: $[\alpha]_D^{22}$= -121.4 (*c* 1.00, EtOH), the average of two measurements: -121.7 and -121.1

Literature: $[\alpha]_D^{22}$= -125 (Ben-Shabat, 2006).

H-NMR spectrum of compound **1**

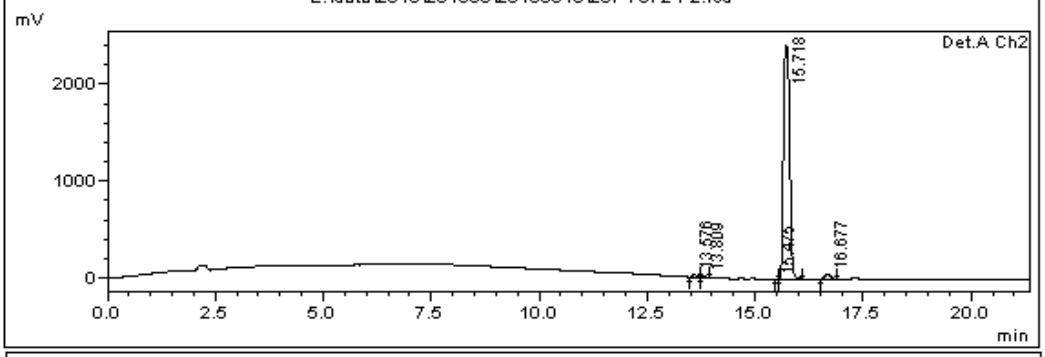


HPLC chromatogram of compound **1**

==== Shimadzu LCsolution Analysis Report ====

E:\data\2013\201306\20130618\2074-072-P2.lcd

Sample Name : Sample ID:
Tray# : 1 Vail #: 67
Injection Volume : 5 uL Data File Name: 2074-072-P2.lcd
Method File Name : method(15cm)-1.lcm Batch File Name:
Data Acquired : 2013-6-18 16:48:39 Data Processed: 2013-6-19 8:56:17

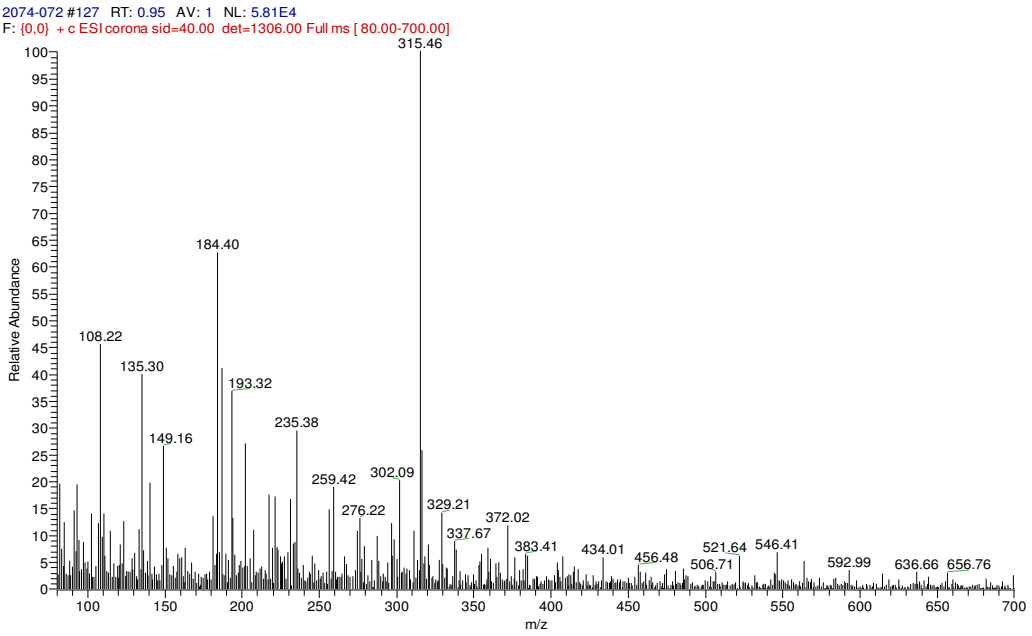


Peak Table

Detector A Ch2 220nm

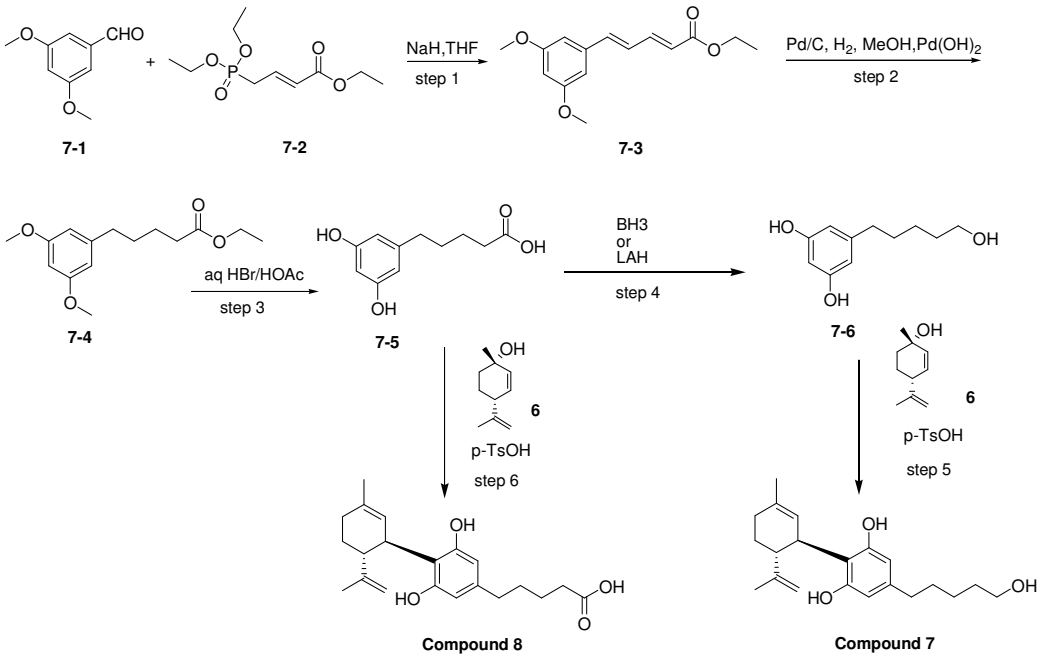
Peak#	Ret. Time	Area	Area %
1	13.576	196762	0.799
2	13.809	89950	0.365
3	15.475	-1691	-0.007
4	15.718	23894995	97.060
5	16.677	438803	1.782
Total		24618818	100.000

LC/MS of compound **1**

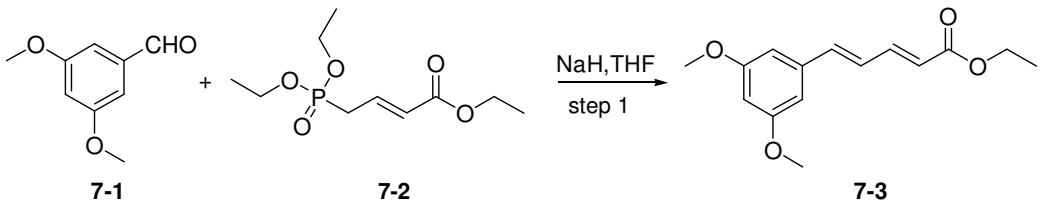


Synthesis of Compounds 7 and 8

Synthesis Scheme for 7 & 8



STEP 1:



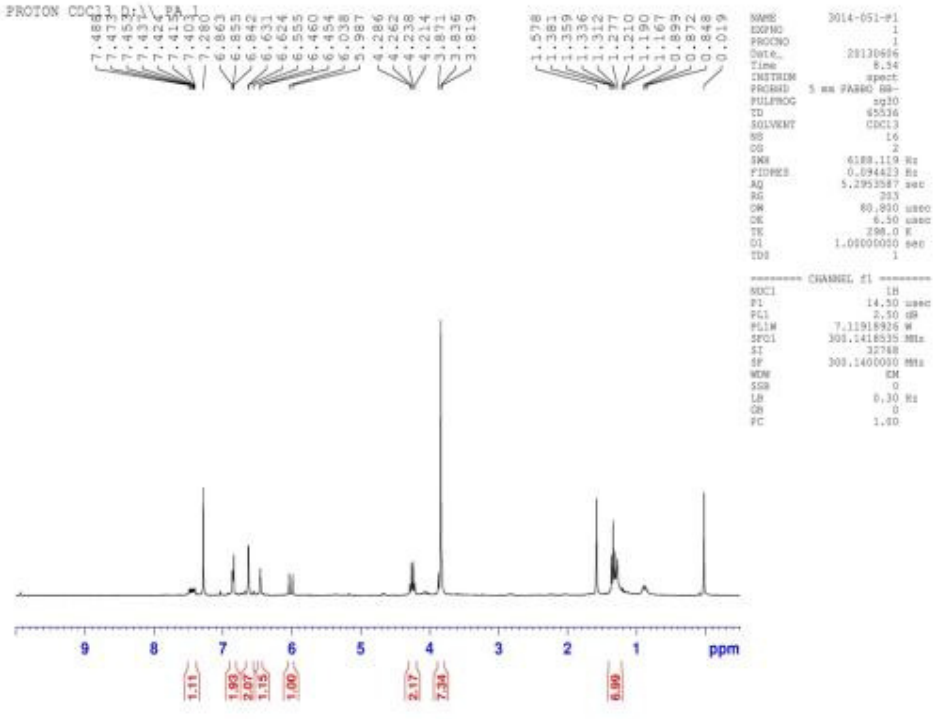
To a suspension of NaH (0.87 g, 22 mmol, 1.2 equiv.) in THF cooled by an ice-salt bath was added a solution of **7-2** (5 g, 20 mmol, 1.1 equiv.) in THF (50 mL) over 30 min and followed by the addition of a solution of **7-1** (2.77 g, 18 mmol, 1.0 equiv.) in THF over 35 min. After the addition, the ice-salt bath was removed and the reaction was stirred at RT for one h, and then heat to 60 °C for another h. TLC analysis indicated the consumption of **7-1**. The reaction mixture was cooled to RT and quenched by water (120 mL), extracted by MTBE (3 x 120 mL). The organic phase was back washed with brine (2 x 120 mL), dried over Na_2SO_4, filtered and

concentrated to give crude product **7-3** (3.7 g , yield 84%), which was directly used for the next step without further purification. HPLC purity: 88%. ^{1}H-NMR (300 MHz, CDCl3) δ 7.41-7.48 (m, 1H), 6.86 (m, 2H), 6.63 (s, 2H), 6.46 (s, 1H), 6.02 (d, J = 15.3 Hz, 1H), 4.25 (q, J = 7.2 Hz, 2H), 3.82 (s, 6H), 1.33 (t, J = 6.9 Hz, 3H). The analytical data are attached below.

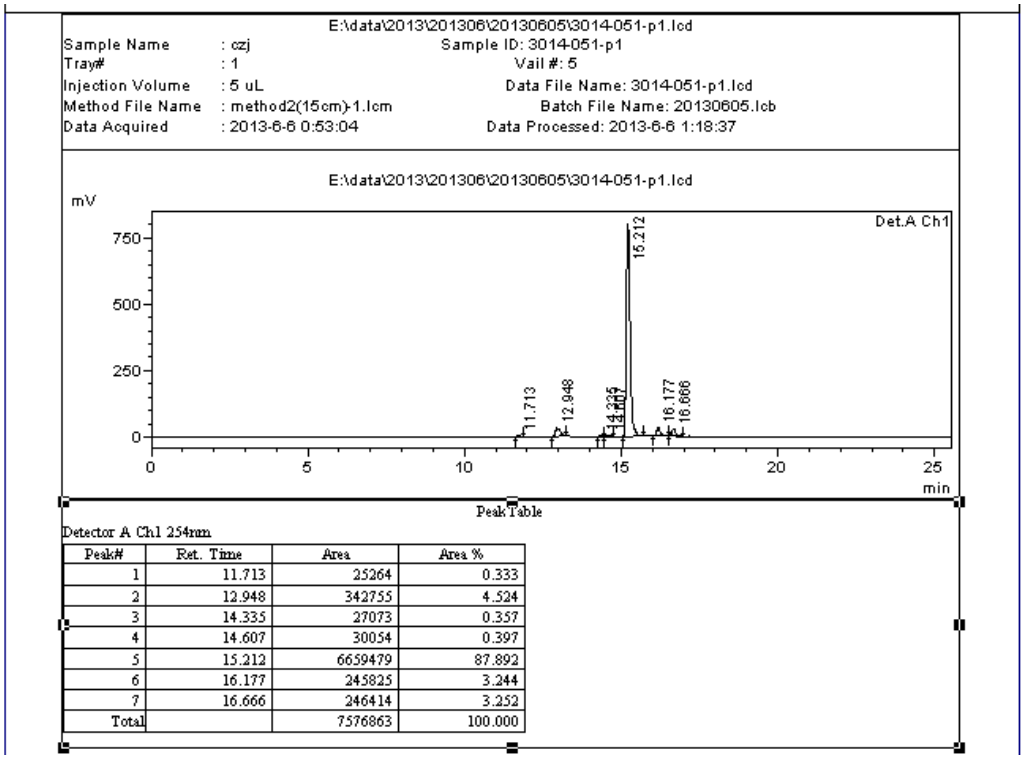
Batch Summary of Step 1

Batch #	**SM (7-1)**	**(7-3)**	**Yield (%)**	**HPLC (%area)**
3014-051	2.77 g	3.70 g	84	88
2074-063	2.4g	4.0g	105	93
3020-011	3.0g	4.62g	97	96

^{1}H-NMR of intermediate **7-3**



HPLC chromatogram of intermediate **7-3**

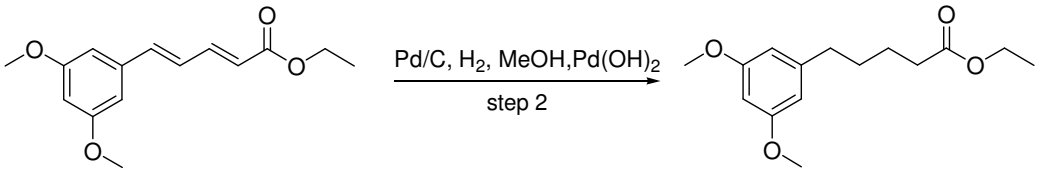


Peak Table

Detector A Ch1 254nm

Peak#	Ret. Time	Area	Area %
1	11.713	25264	0.333
2	12.948	342755	4.524
3	14.335	27073	0.357
4	14.607	30054	0.397
5	15.212	6659479	87.892
6	16.177	245825	3.244
7	16.666	246414	3.252
Total		7576863	100.000

STEP 2:



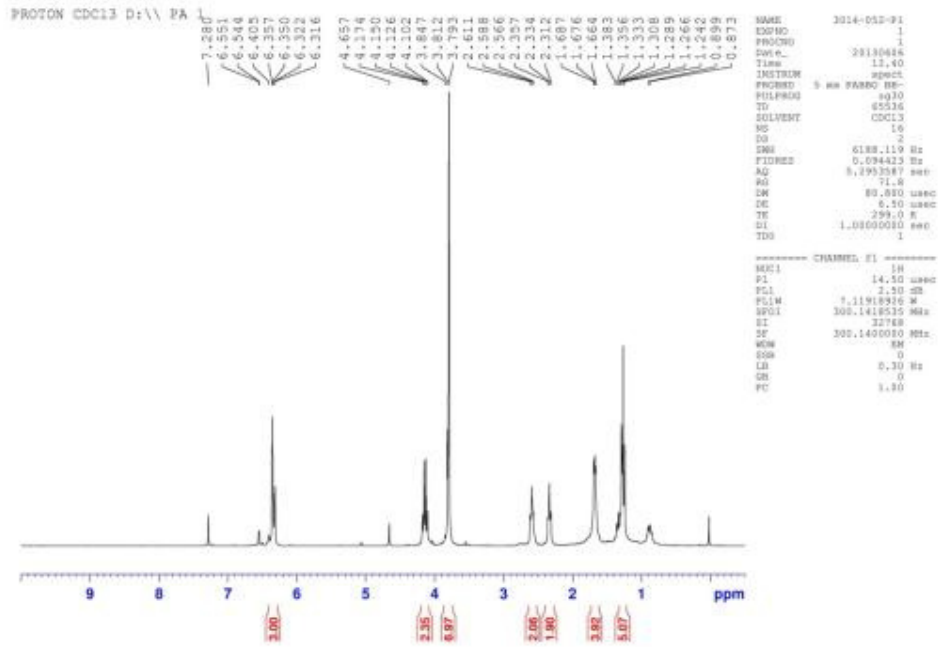
7-3 **7-4**

A suspension of crude intermediate **7-3** (3.57 g, 14 mmol, 88% purity) and 10% Pd-C (0.74 g, 20% wt over intermediate **7-3**) in methanol (74 mL) was stirred with a hydrogen balloon at RT for 2 hours. The Pd/C was filtered off through a pad of Celite. The filtrate was concentrated to give crude **7-4** (3.5 g, yield 93%) as light yellow oil. ^{1}H-NMR (300Hz, $CDCl_3$) δ 6.55-6.32 (m, 3H), 4.38 (q, J = 7.2 Hz, 2H), 3.79 (s, 6H), 2.61-2.57 (m, 2H), 2.36-2.31 (m, 2H), 1.69-1.66 (m, 4H), 1.31 (t, J = 5.7 Hz, 3H). The analytical data are attached below.

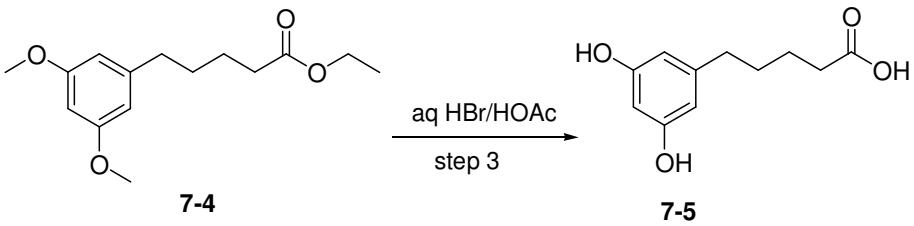
Batch Summary of Step 02

Batch #	SM (7-3)	Product (7-4)	Yield (%)	HPLC (%area)
3014-052	3.70 g	3.50 g (crude)	93%	n/a
2074-064	4.0g	3.88g（crude）	95%	62
3020-012	4.6g	4.48g（crude）	96%	82

^{1}H-NMR of intermediate **7-4**



STEP 3:



7-4 **7-5**

In order to demethylate and saponify the ethers and ester, crude **7-4** (4.4 g, 16.5 mmol, 1 equiv.) and 40% hydrobromic acid (50 mL) in AcOH (50 mL) was refluxed for 3-4 h. The reaction mixture was cooled to RT and added ice water (150 mL). The resulting mixture was extracted by EtOAc (3 x 150 mL). The organic phase was back

washed by brine (2 x 200 mL), dried and concentrated to give crude product (4.8 g) as black oil. The crude product was purified through column chromatography (eluted by EtOAc/Hexane 1:2) to give **7-5** (2.49 g, yield 71%). HPLC purity: 99.6%. ^{1}H-NMR (300 MHz, DMSO-d^{6}) δ 11.96 (s, 1H), 9.01 (s, 2H), 6.02 (s, 3H), 2.37 (m, 2H), 2.23-2.19 (m, 2H), 1.50-1.49 (m, 4H). The analytical data is attached below.

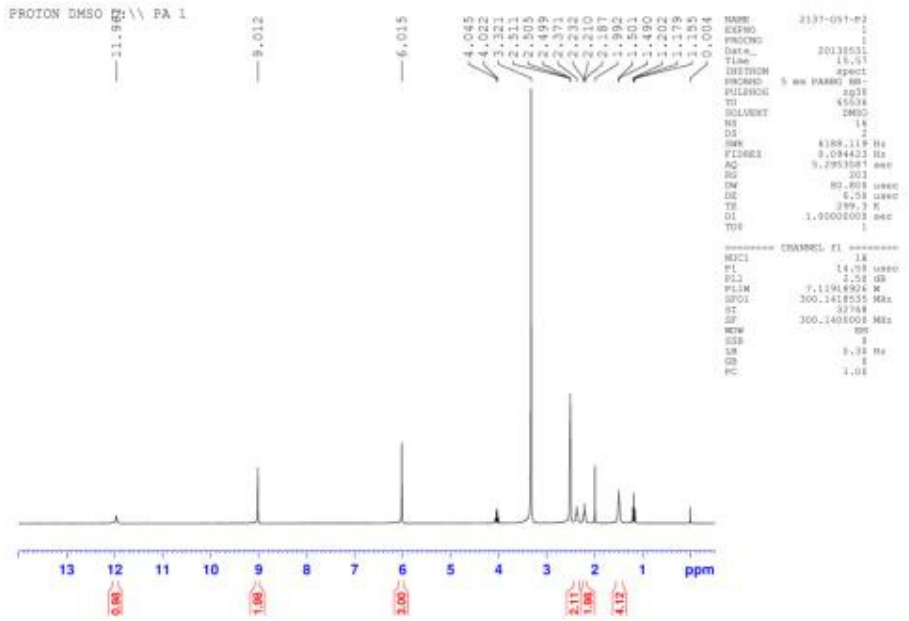
Note:

1. The crude **7-5** (lot#: 3014-053) was used directly for the next step, and found the residual AcOH and EtOAc consumed too much LiAlH4. It is better to purify the intermediate **7-5** before the reduction by LAH.

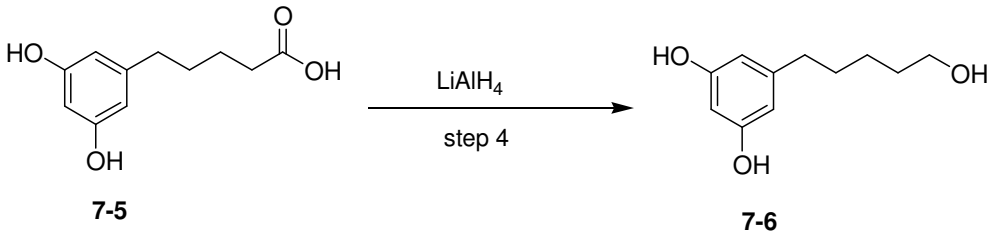
Batch Summary of Step 3

Batch #	SM (**7-4**)	Product (**7-5**)	Yield (%)	HPLC (%area)
2137-057	0.94 g	0.66 g	89%	63
3020-013	4.40 g	2.49 g	71.7%	99%
3014-053	3.50 g	3.40 g (crude)	126%	n/a (Residual HOAc & EtOAc)

^{1}H-NMR spectrum of intermediate **7-5**



STEP 4:

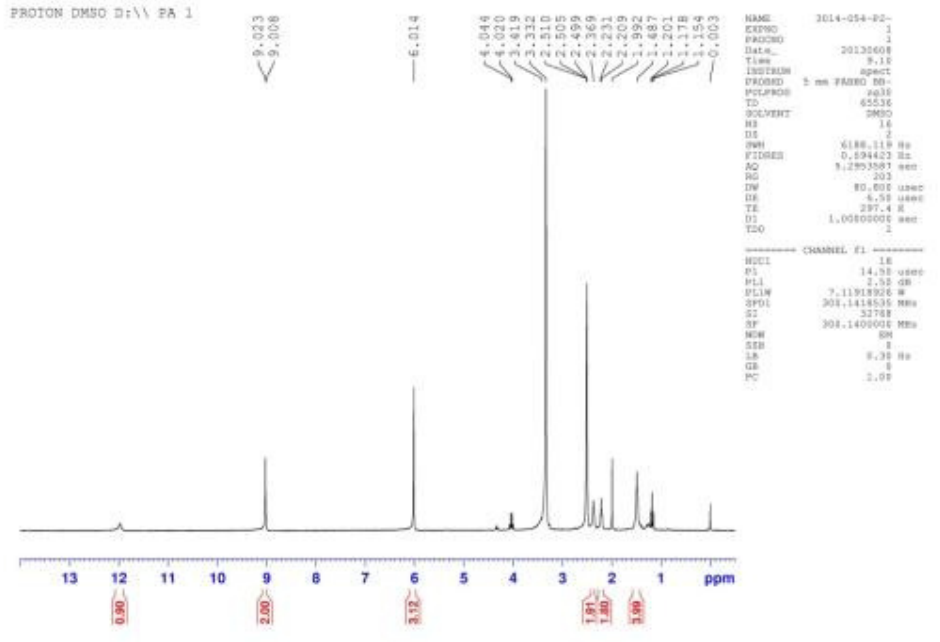


7-5 **7-6**

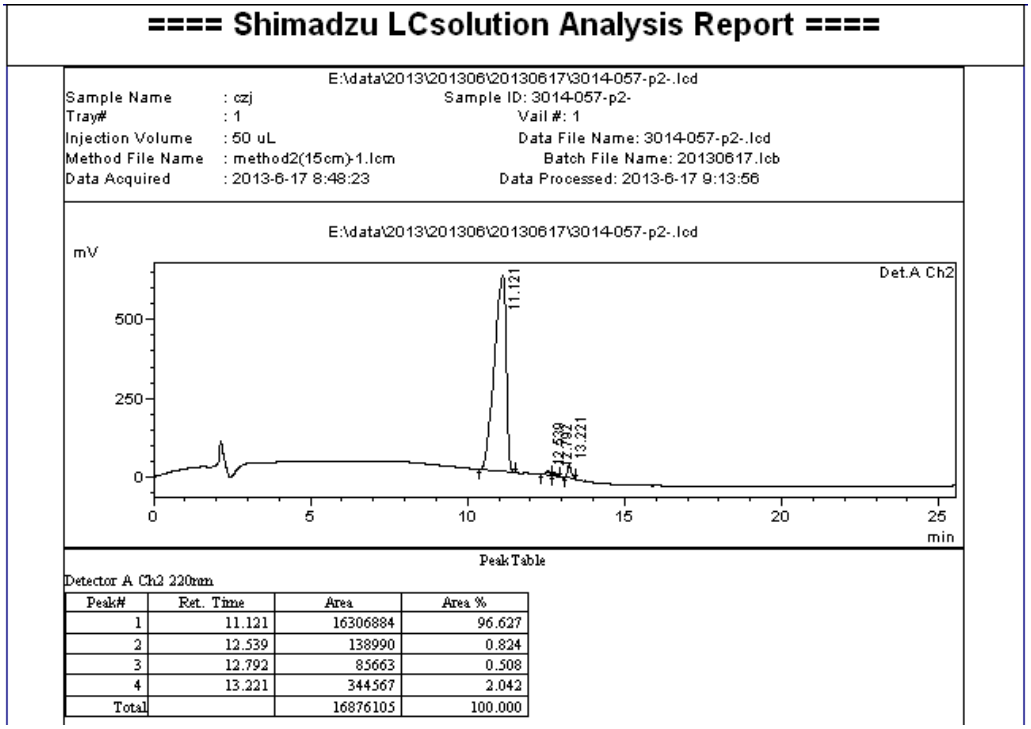
To a suspension of $LiAlH_4$ (0.34 g, 9.1 mmol, 1.1 equiv.) in THF (30 mL) was added a solution of **7-5** (1.8 g, 8.3 mmol, 1.0 equiv.) in THF (18 mL) over 50 min and the reaction mixture was continued stirring for 20 min. The reaction mixture was then heated to 60 °C with stirring for 2 hours. Another 1.0 equivalent of $LiAlH_4$ was added as solid and the reaction was continued at RT for overnight. TLC analysis showed ~5% of intermediate **7-5** remaining. The reaction was quenched by the addition of 1M HCl (150 mL). The product was extracted by EtOAc (2 x 100 mL). The organic layer was back washed by brine (2 x 100 mL), dried over Na_2SO_4 and concentrated to give crude **7-6** (2.0 g). The crude **7-6** was purified through column chromatography (eluent: DCM/EtOAc = 3/1) to provide pure intermediate **6** (0.8 g, yield 48%). HPLC purity: 97%; ^{1}H-NMR（300Hz, DMSO-d^6） δ: 9.00 (s, 2H), 6.02 (s, 3H), 4.33 (s, 1H), 3.40 (t,

J = 6.3Hz, 2H), 2.36 (t, *J* = 7.5, 2H), 1.54-1.40 (m, 4H), 1.38-1.24 (m, 2H). The analytical data is attached below.

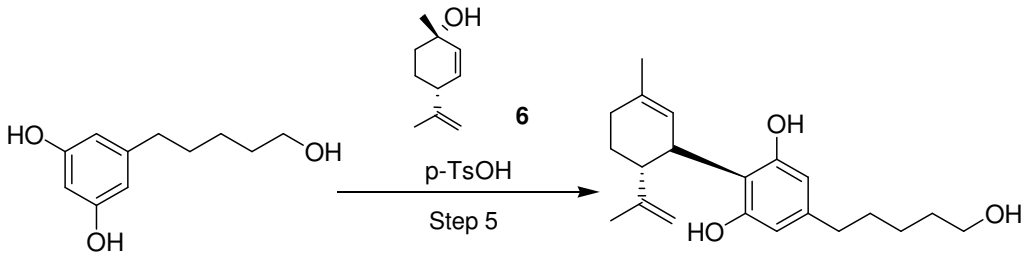
^{1}H-NMR spectrum of intermediate **7-6**



HPLC chromatogram of intermediate **7-6**

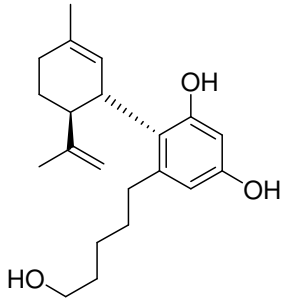


STEP 5: Synthesis of compound 7



7-6 **Compound 7**

A mixture of intermediate **7-6** (0.48 g, 2.4 mmol, 1.0 equiv.), **6** (0.56 g, 3.7 mmol, 1.5 equiv.) and *p*-TsOH (0.088 g, 0.46 mmol, 0.2 equiv.) in the mixture of DCM and THF (ratio of DCM/THF = 4:1, 24 mL) was stirred at RT for 2.5 hours. TLC analysis showed ~50% of conversion of intermediate **7-6**. The reaction was quenched by the addition of aqueous solution of $NaHCO_3$ (20 mL) to pH = 5-6 and extracted by EtOAc (2 x 20 mL) The organic layer was back washed by brine twice, dried and concentrated to give crude compound **7** (1.0 g). The crude compound **7** was purified through column chromatography to give pure product compound **7** (200 mg, 25%), and the less pure 4-regio-isomer (200 mg). The less pure 4-regio-isomer was further purified by preparative HPLC to produce 100 mg (12%) of pure 4-regio-isomer of **7**.



4-regioisomer of 7

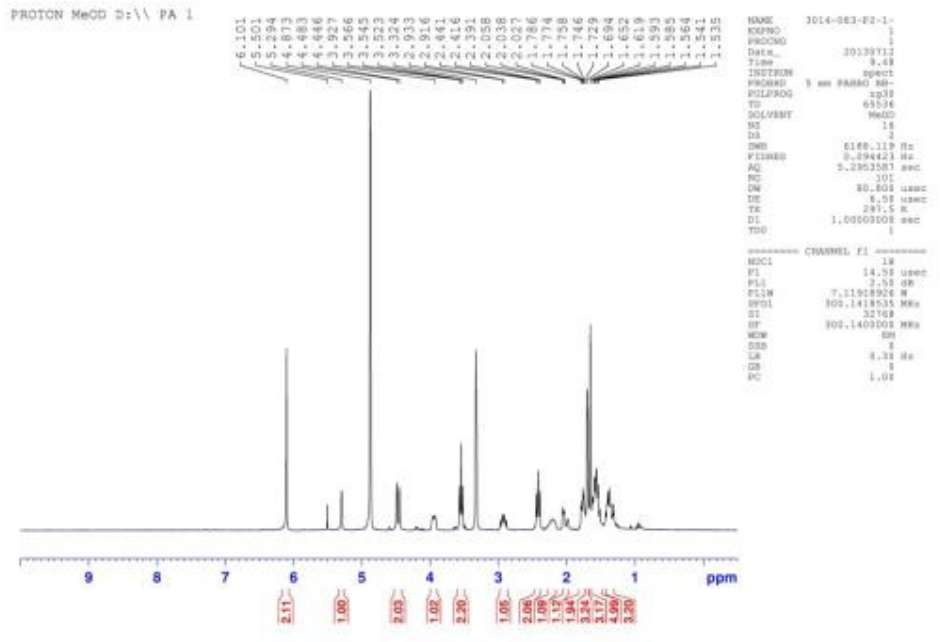
Analytical data of compound **7**

HPLC purity: 95.9%. LC/MS (ESI): m/z 331 (M+1). 1H-NMR（300 Hz, CD_3OD）δ 6.01 (s, 2H), 5.29 (s, 1H), 4.46 (d, J = 11.1Hz, 2H), 3.96-3.93 (m, 1H), 3.54 (t, J = 6.6Hz, 2H), 2.93-2.92 (m, H), 2.42 (t, J = 7.5Hz, 2H), 2.25-2.15 (m, 1H), 2.06-2.03 (m, 1H), 1.79-1.72 (m, 2H), 1.69 (s, 3H), 1.65 (s, 3H), 1.62-1.59 (m, 3H), 1.56-1.54 (m, 3H). The analytical data are attached below.

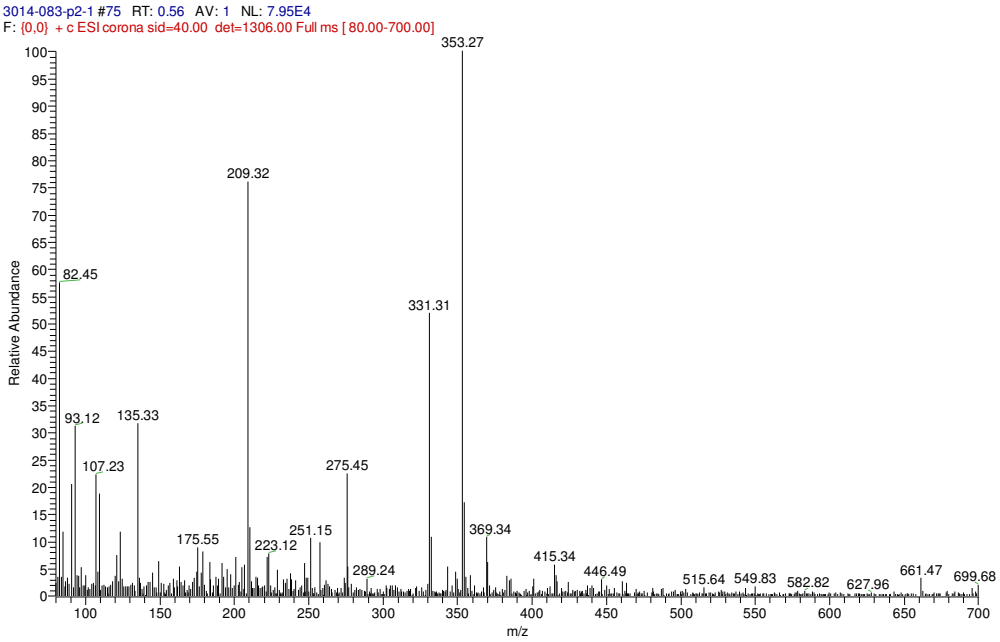
Analytical data of 4-regio-isomer of **7**

HPLC purity: 99%. LC/MS (ESI): m/z 331 (M+1). H-NMR（300Hz, $CDCl_3$） δ 6.20 (s, 2H), 5.51 (s, 1H), 4.64 (s, 1H), 4.47 (s, 1H), 3.64 (t, J = 6.3Hz, 2H), 3.53-3.50 (m, 1H), 2.62-2.59 (m, 1H), 2.48-2.47 (m, 1H), 2.30-2.20 (m, 2H), 2.12-2.06 (m, 1H), 1.83-1.74 (m, 5H), 1.64-1.42 (m, 7H), 1.40-1.35 (m, 2H). The analytical are attached below.

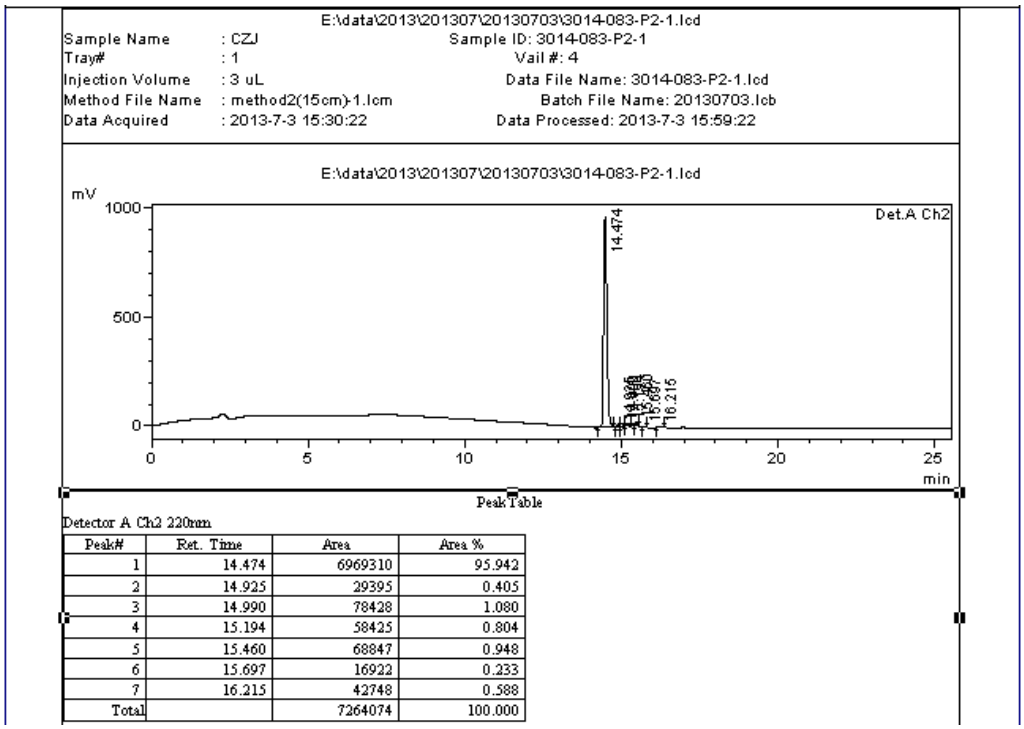
^{1}H-NMR of compound **7**



LC/MS of compound **7**

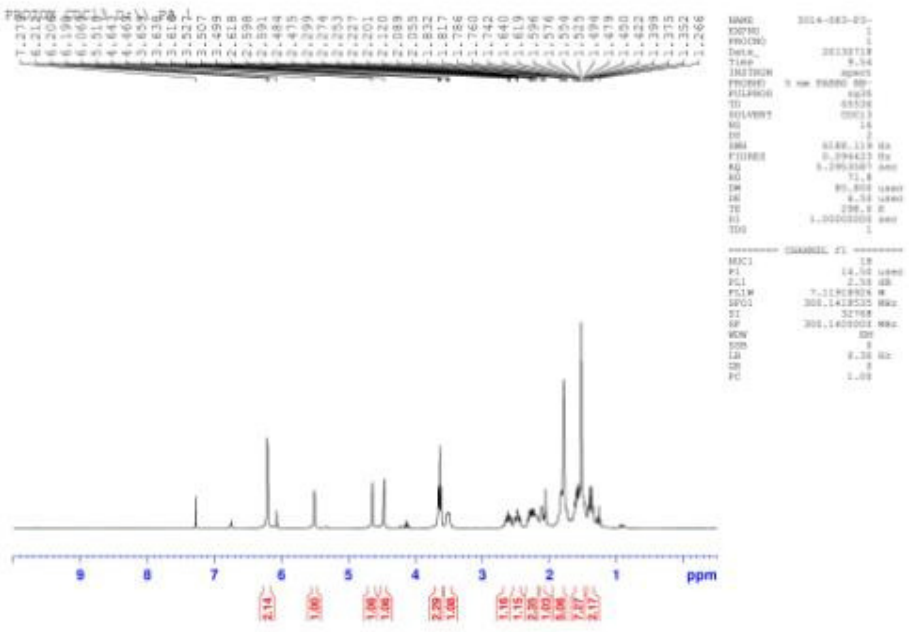


HPLC chromatogram of compound **7**

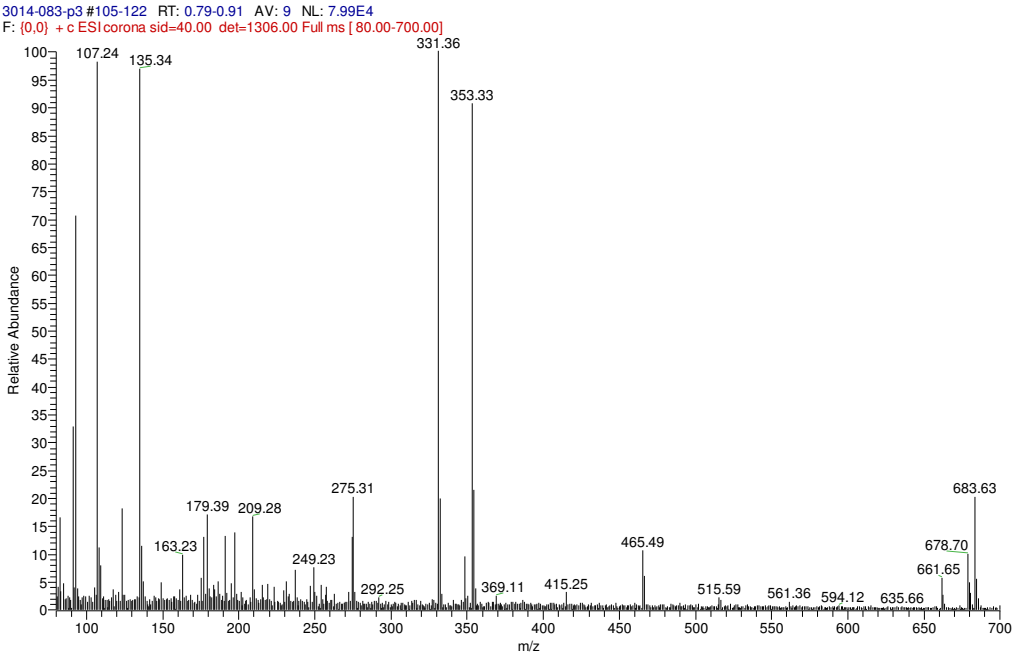


Peak#	Ret. Time	Area	Area %
1	14.474	6969310	95.942
2	14.925	29395	0.405
3	14.990	78428	1.080
4	15.194	58425	0.804
5	15.460	68847	0.948
6	15.697	16922	0.233
7	16.215	42748	0.588
Total		7264074	100.000

[1]H-NMR of the 4-regio-isomer of compound **7**



LC/MS of the 4-regio-isomer of compound **7**

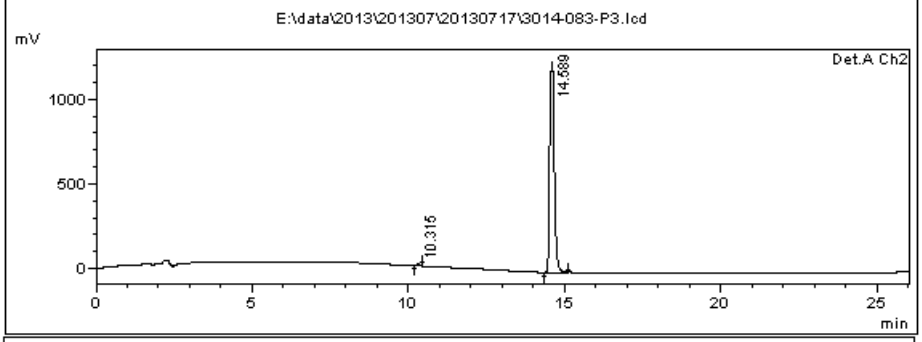


HPLC chromatogram of the 4-regio-isomer of compound **7**

==== Shimadzu LCsolution Analysis Report ====

E:\data\2013\201307\20130717\3014-083-P3.lcd

Sample Name	: CZJ	Sample ID: 3014-083-P2-2	
Tray#	: 1	Vail #: 20	
Injection Volume	: 12 uL	Data File Name: 3014-083-P3.lcd	
Method File Name	: method2(15cm)-1.lcm	Batch File Name: 20130717.lcb	
Data Acquired	: 2013-7-18 4:51:04	Data Processed: 2013-7-18 5:17:06	

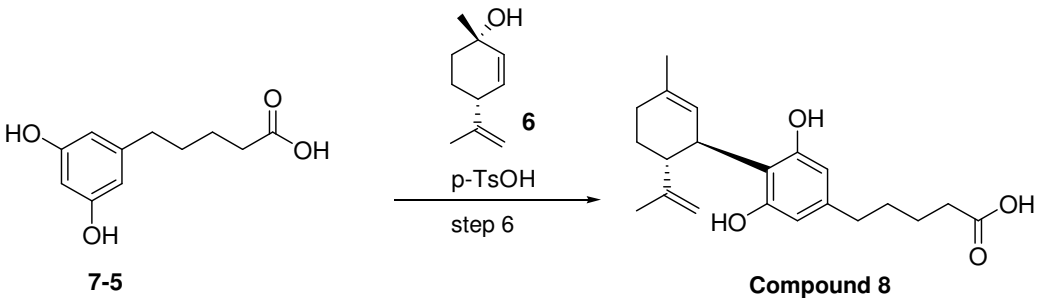


Peak Table

Detector A Ch2 220nm

Peak#	Ret. Time	Area	Area %
1	10.315	59508	0.461
2	14.589	12851890	99.539
Total		12911397	100.000

STEP 6: Synthesis of compound 8



To a suspension of intermediate **7-5** (1.0 g, 4.75 mmol, 1.0 equiv.) and *p*-TsOH (0.18 g, 0.95 mmol, 0.2 equiv.) in 20% THF in DCM (50 mL) was slowly added compound **6** (1.08 g, 7.09 mmol, 1.5 equiv.) dropwise at RT. The resulting mixture was stirred at RT for 30 min. TLC analysis showed 50% conversion of intermediate **7-5**. The reaction was then quenched by the addition of EtOAc (50 mL) and diluted with aqueous $NaHCO_3$ to pH = 3~4. The organic phase was separated, washed by brine twice, dried and concentrated to give crude product **8** (2.3 g) as yellow oil. The crude **8** was first purified through column chromatography and then further purified by preparative HPLC to give the desired compound **8** (220 mg, yield: 13.6%). Additionally 200 mg of the 4-regio-isomer of compound **8** was isolated as well.

Analytical data of compound **8**:

HPLC purity: 99.6%; LC/MS (ESI): m/z 345 (M+1), m/z 367 (M+Na); ^{1}H-NMR (300 MHz, CD_3OD) δ 6.10 (s, 2H), 5.29 (s, 1H), 4.46 (d, J = 10.5 Hz, 2H), 3.96-3.93 (m, 1H), 2.96-2.91 (m, 1H), 2.43 (t, J = 6.9 Hz, 2H), 2.36 (t, J = 6.9 Hz, 2H), 1.78-1.60 (m, 14H). The analytical data are attached below.

Analytical data of the 4-regio-isomer of compound **8:**

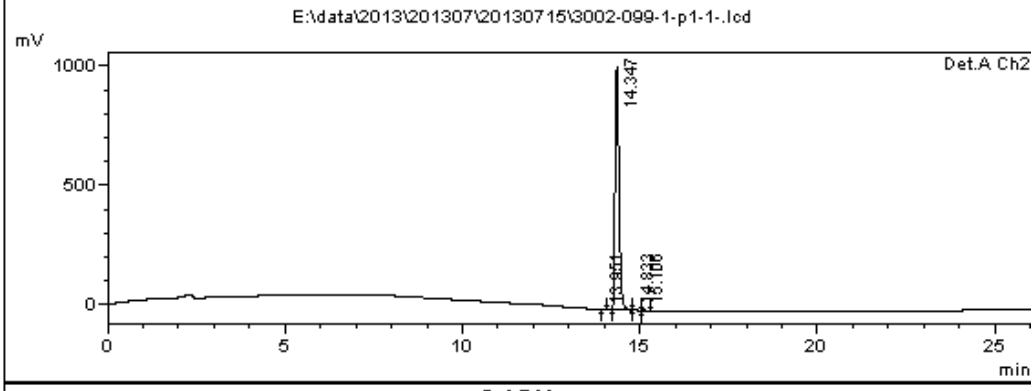
HPLC purity: 96%. LC/MS (ESI): m/z 345 (M+1), m/z 367 (M+Na). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 6.30-6.20 (m, 2H), 6.06 (s, 1H), 5.53 (s, 1H), 4.67 (s, 1H), 4.48 (s, 1H), 3.55-3.45 (m, 1H), 2.70-2.60 (m, 1H), 2.50-2.10 (m, 7H), 1.90-1.75 (m, 5H), 1.70-1.60 (m, 3H), 1.50 (s, 3H). The analytical data are attached below.

HPLC chromatogram of compound **8**

==== Shimadzu LCsolution Analysis Report ====

E:\data\2013\201307\20130715\3002-099-1-p1-1-.lcd

Sample Name	: ls	Sample ID:	
Tray#	: 1	Vail #: 31	
Injection Volume	: 3 uL	Data File Name: 3002-099-1-p1-1-.lcd	
Method File Name	: method2(15cm)-1.lcm	Batch File Name: 20130716.lcb	
Data Acquired	: 2013-7-16 10:43:13	Data Processed: 2013-7-16 11:09:17	

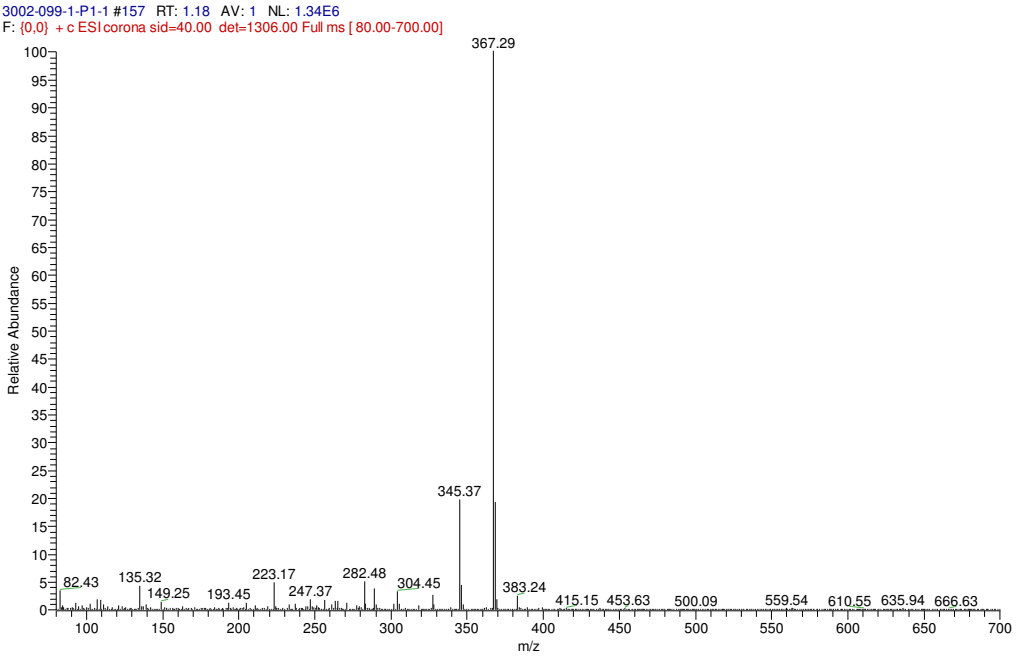


Peak Table

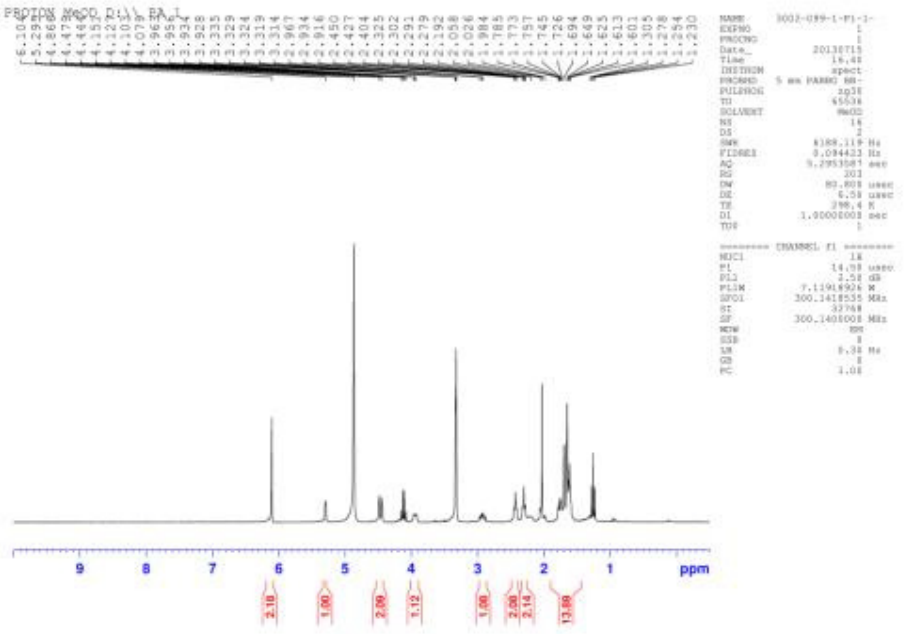
Detector A Ch2 220nm

Peak#	Ret. Time	Area	Area %
1	13.951	8205	0.106
2	14.347	7693963	99.655
3	14.833	8631	0.112
4	15.106	9783	0.127
Total		7720582	100.000

LC/MS of compound **8**



[1]H-NMR spectrum of Compound **8**

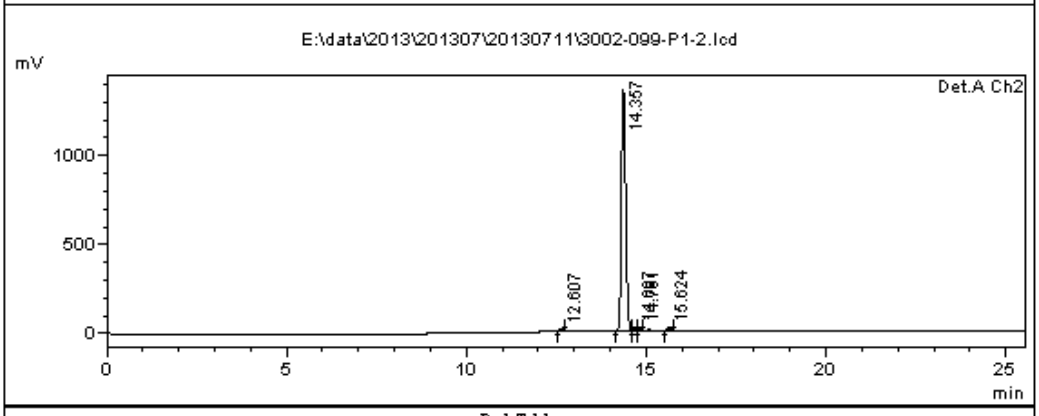


HPLC chromatogram of the 4-regio-isomer of compound **8**

==== Shimadzu LCsolution Analysis Report ====

E:\data\2013\201307\20130711\3002-099-P1-2.lcd

Sample Name	: LS	Sample ID: 3002-099-P1-2	
Tray#	: 1	Vail #: 91	
Injection Volume	: 3 uL	Data File Name: 3002-099-P1-2.lcd	
Method File Name	: method1(15cm)-MeOH+H2O.lcm	Batch File Name: 20130711.lcb	
Data Acquired	: 2013-7-11 10:24:56	Data Processed: 2013-7-11 10:50:30	

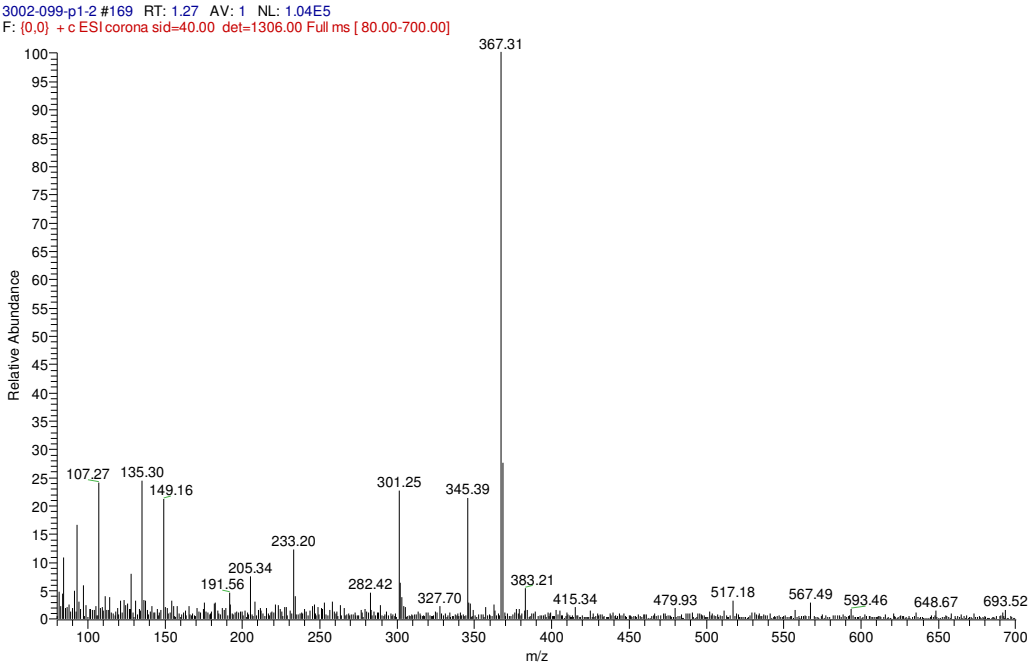


Peak Table

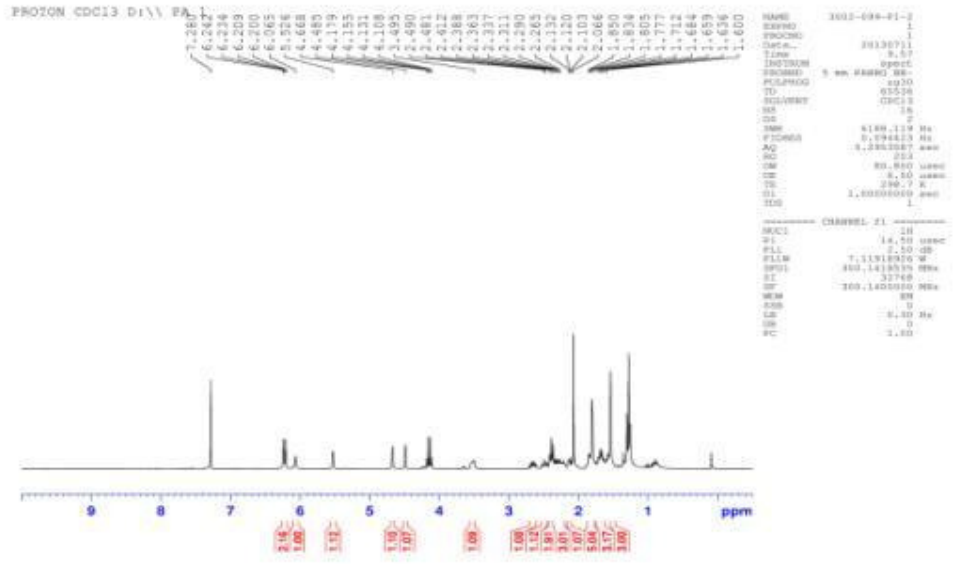
Detector A Ch2 220nm

Peak#	Ret. Time	Area	Area %
1	12.607	98925	0.848
2	14.357	11225299	96.263
3	14.697	87714	0.752
4	14.781	118724	1.018
5	15.624	130448	1.119
Total		11661110	100.000

LC/MS of the 4-regio-isomer of compound **8**

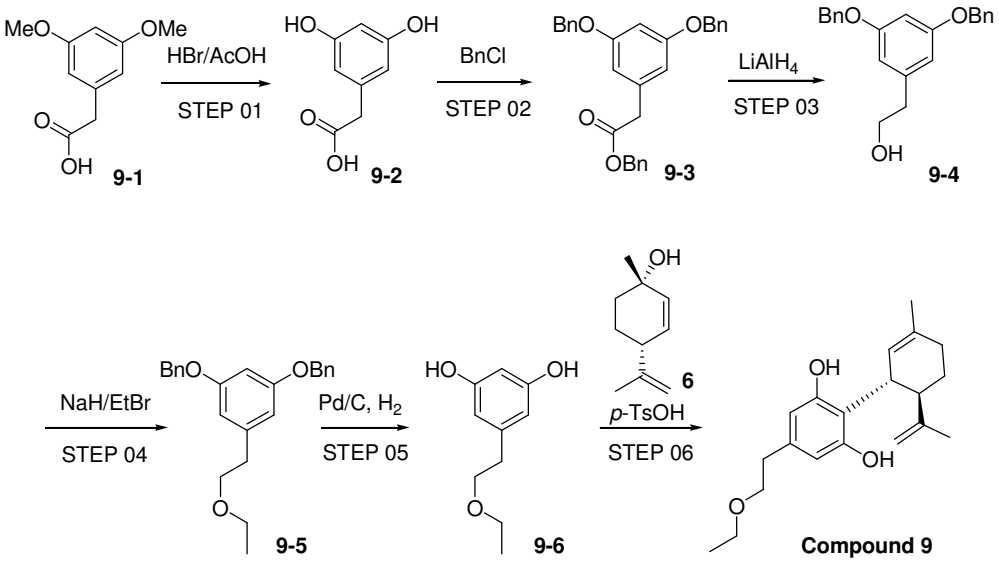


^{1}H-NMR spectrum of the 4-regio-isomer of compound **8**

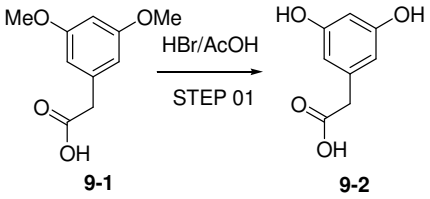


Synthesis of Compound 9

Synthesis Scheme Compound 9

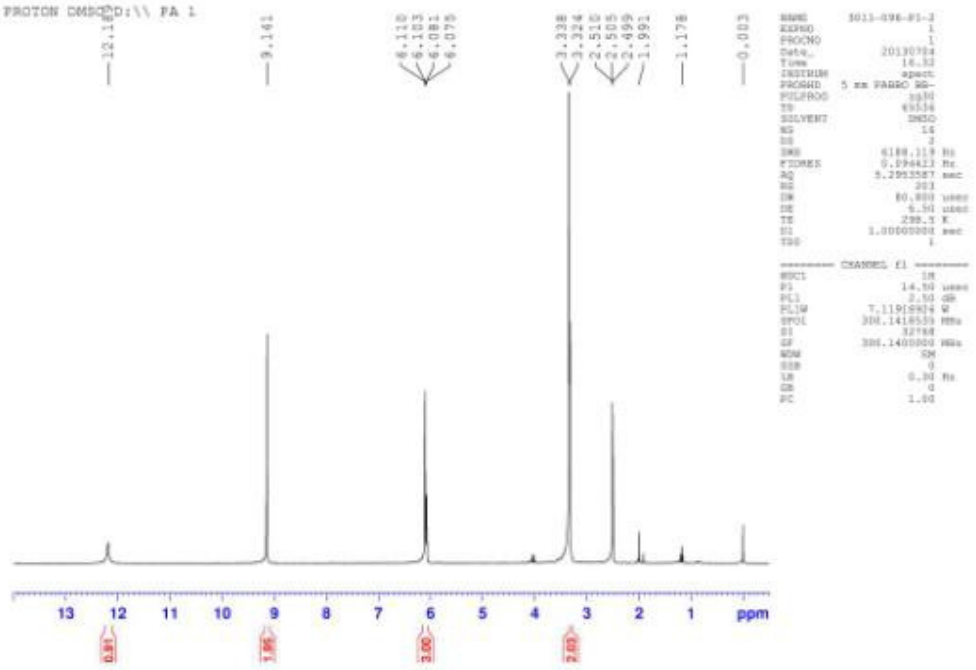


STEP 1:

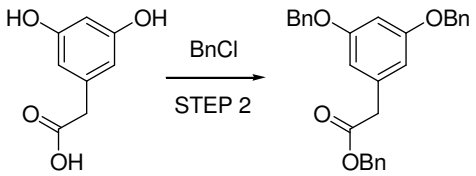


The solution of compound **9-1** (2.4 g, 12.2 mmol, 1.0 equiv.) in 40% hydrobromic acid (40 mL) and AcOH (40 mL) was refluxed overnight (~18 h). TLC analysis indicated the full consumption of the starting material **9-1**. The reaction mixture was then concentrated in vacuo. The resulting residue was dissolved in EtOAc (50 mL) and back washed with 10% brine (2 x 50 mL), dried and concentrated to give crude **9-2** (1.9 g, yield 92%). The crude **9-2** was used directly for the next step without further purification. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 12.19 (s, 1H), 9.14 (s, 1H), 6.11 (s, 1H), 6.10 (s, 1H), 6.08 (s, 1H), 3.34 (s, 2H). The analytical data is attached below.

^{1}H-NMR of intermediate **9-2**



STEP 2:

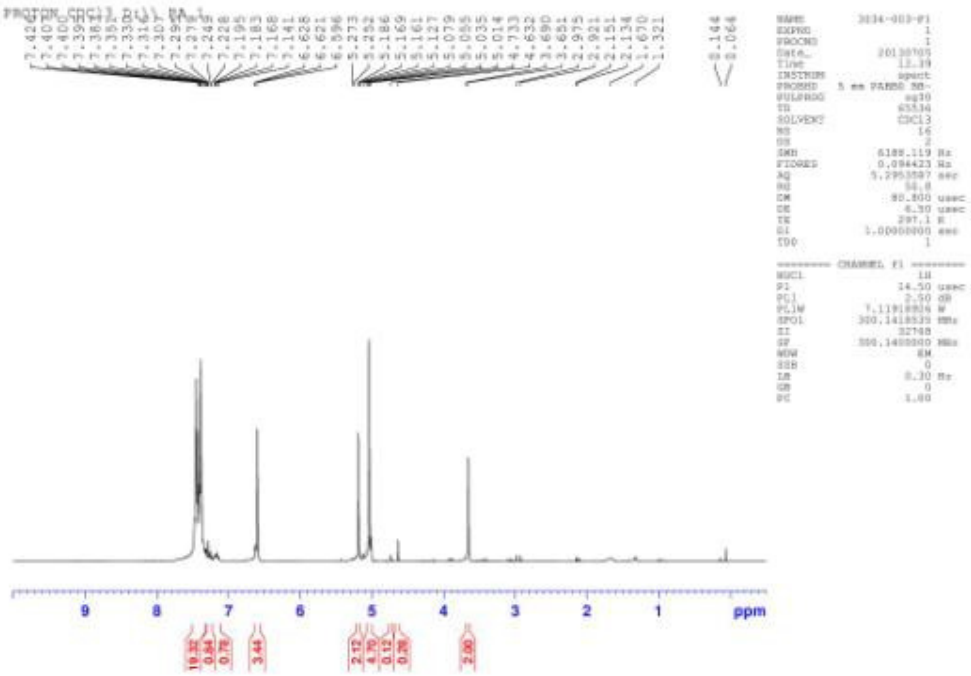


9-2 **9-3**

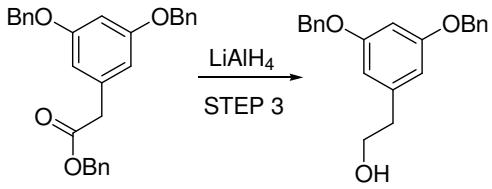
To a suspension of **9-2** (1.9 g, 11.2 mmol, 1.0 equiv.) and K_2CO_3 (6.2 g, 45.2 mmol, 4.0 equiv.) in DMF (15 mL) was stirred at RT for 30 min. Benzyl chloride (4.7 g, 37.3 mmol, 3.0 equiv.) was added and the reaction mixture was heated to 70 °C overnight. After the consumption of the starting material **9-2** by TLC, water (20 mL) was added to quench the reaction. The quenched mixture was extracted with EtOAc (3 x 30 mL). The combined organic layer was back washed with 10% brine (3 x 30 mL), dried over Na_2SO_4, filtered and concentrated to give crude **9-3** (~5 g, yield 100%). The crude **9-3** was used directly for the next step without further purification. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.43-7.28 (m, 15H), 6.62 (s, 3H), 5.27 (s, 2H), 5.13 (s,

4H), 3.65 (s, 2H). The analytical data is attached below.

^{1}H-NMR of intermediate **9-3**



STEP 3



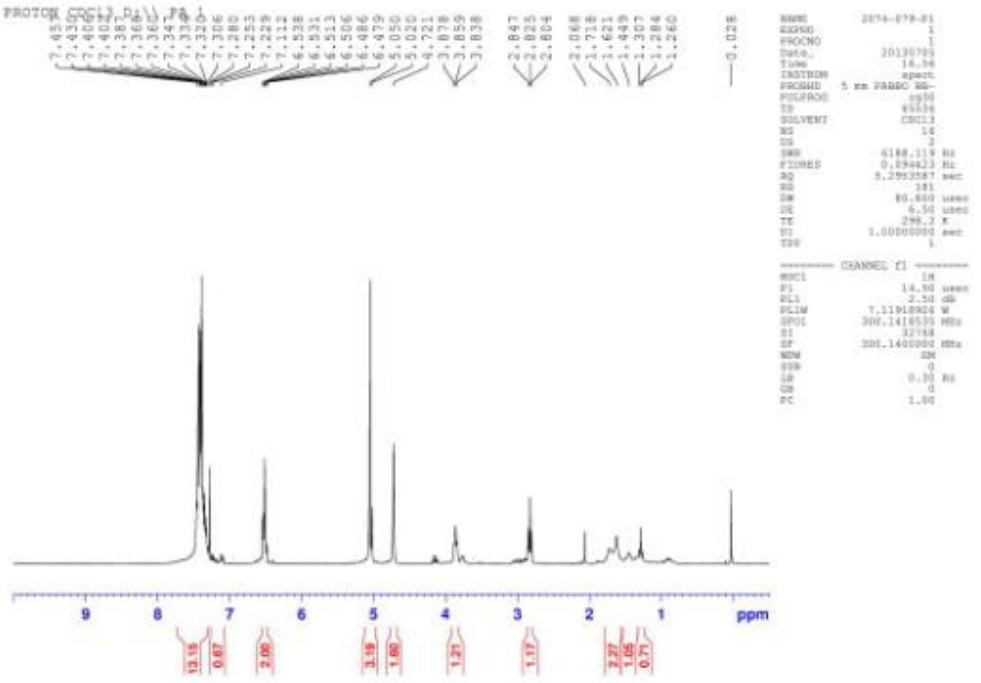
9-3 **9-4**

To a solution of **9-3** (5.0 g, 11.4 mmol, 1.0 equiv.) in dry THF (45.0 mL) was added $LiAlH_4$ (1.7 g, 45.6 mmol, 4.0 equiv.) as solid. The resulting suspension was stirred at RT. After **9-3** was completely consumed as indicated by TLC (~1.5 h), water (5.0 mL) was added, followed by the addition of 15 % KOH (5.0 mL) and water (30.0 mL) in order. The mixture was filtered and the filter cake was washed by EtOAc (2 x 10 mL). The water phase was extracted with EtOAc (30 mL). The combined organic phases were dried over Na_2SO_4, filtered and concentrated to give **9-4** (4.1 g, yield 100%) brown oil which was used directly for the next step without further

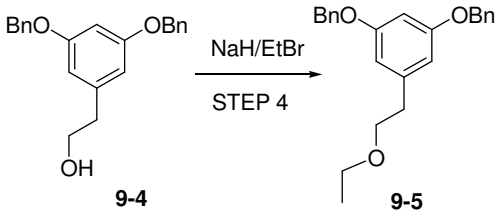
purification.

^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.80-7.30 (m, 13H), 6.54-6.48 (m, 2H), 5.05 (s, 2H), 5.02 (s, 1H), 4.72 (s, 1H), 3.86 (t, J = 6.6 Hz, 1H), 2.83 (t, J = 6.6 Hz, 1H). The analytical data is attached below.

^{1}H-NMR spectrum of intermediate **9-4**



STEP 4:



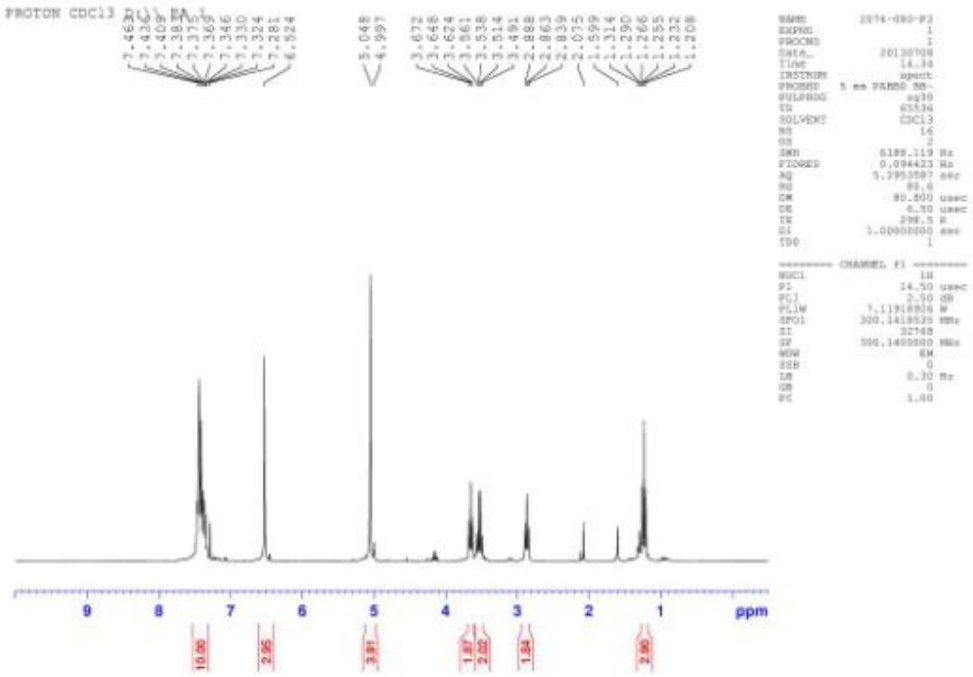
9-4 **9-5**

To a solution of crude **9-4** (4.0 g, 12 mmol, 1.0 equiv.) in DMF (35 mL) was added 60% NaH (1.44 g, 36 mmol, 3.0 equiv.) and stirred at RT for 30 min. The solution of bromoethane (1.96 g, 18 mmol, 1.5 equiv.) in DMF (5 mL) was added dropwise at RT. After **9-4** was consumed as indicated by TLC, ice-water (50 mL) was added to quench the reaction. The aqueous phase was extracted with EtOAc (3 x 40

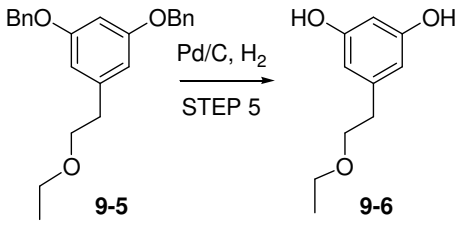
mL). The combined EtOAc phase was washed with 10% brine (3 x 50 mL), dried over Na_2SO_4, filtered, concentrated (4.6 g as brown oil), and purified by column chromatography (Hex/ EtOAc = 20/1) to provide **9-5** (1.68 g, yield 39%) as colorless oil.

^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.46-7.33 (m, 10H), 6.52 (s, 3H), 5.05 (s, 4H), 3.65 (t, *J* = 7.2Hz, 2H), 3.56-3.49 (m, 2H), 2.87(t, *J* = 7.5Hz, 2H), 1.29-1.21(m, 3H). Analytical data is attached below.

^{1}H-NMR of intermediate **9-5**



STEP 5:



9-5 **9-6**

To a solution of **9-5** (1.68 g, 4.6 mmol, 1.0 equiv.) in EtOAc (30.0 mL) was added Pd/C (0.16 g) after purged by N_2. The resulting suspension was stirred at RT with H_2 balloon. After **9-5** was consumed as indicated by TLC, the reaction mixture was filtered through a pad of Celite. The EtOAc solution were dried over Na_2SO_4, filtered and concentrated in vacuo to yield 0.88 g (100%) of **9-6** as colorless oil, which was used for the next step without further purification.

^{1}H-NMR (300 MHz, $CDCl_3$) δ 9.04 (s, 2H), 6.07 (s, 3H), 3.51-3.40 (m, 4H), 2.62-2.51(m, 2H), 1.10 (t, J = 6.9Hz, 4H). The analytical data is attached below.

Notes

1. Intermediate **9-6** was initially to be synthesized from methyl protected precursor (**Fig 9-1**). However, it was hard to demethylate using BBr_3, HBr/HOAc, or methionine, to give the desired intermediate **9-6** without affecting the ethyl ether linkage.

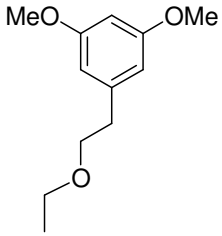
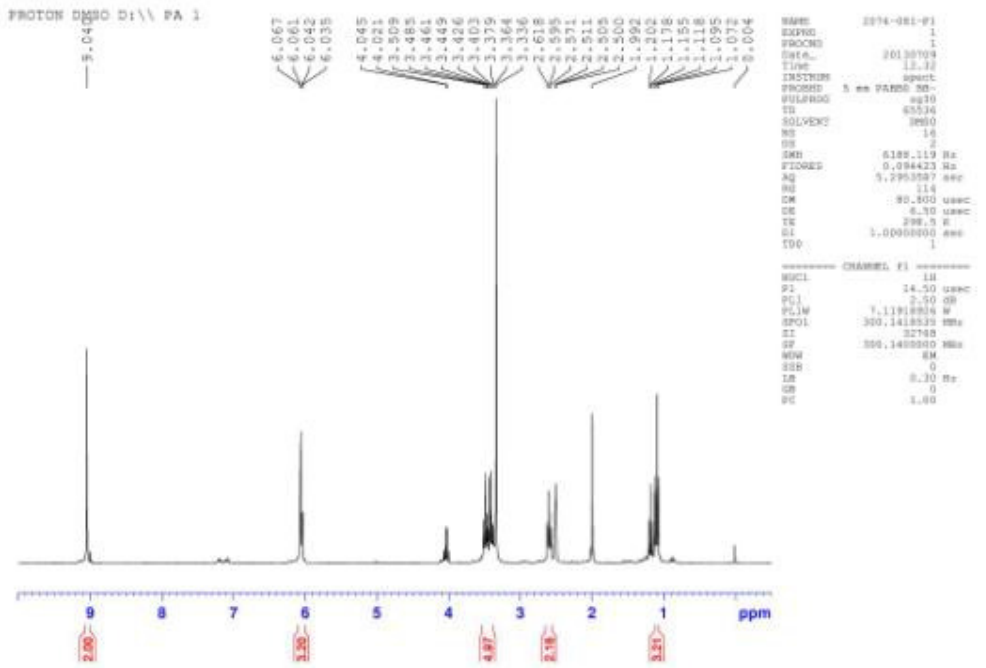
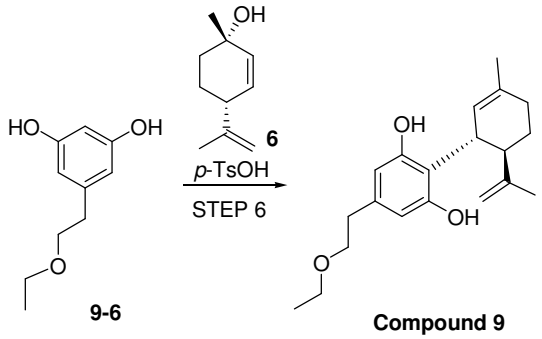


Fig 9-1

^{1}H-NMR spectrum of intermediate **9-6**



STEP 6:



9-6 **Compound 9**

To a solution of **9-6** (0.4 g, 2.2 mmol, 1.0 equiv.) in DCM (20.0 mL) was added *p*-TsOH (0.08 g, 0.44 mmol, 0.2 equiv.) and **6** (0.34 g, 2.2 mmol, 1.0 equiv.). The mixture was stirred under N_2 at RT for 10 min. and saturated $NaHCO_3$ aqueous solution (20 mL) was added to quench the reaction. The DCM phase was separated and dried over Na_2SO_4, filtered, concentrated in vacuo, and purified by column chromatography (Hex/EtOAc = 15/1) to provide 120 mg of product **9** and 110 mg of 4-regio-isomer of **9**. The product **9** and its regio-isomer were further purified by

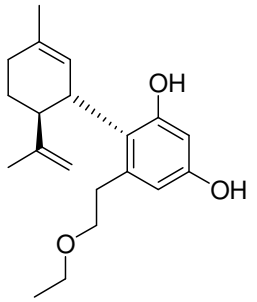
preparative HPLC to afford 80 mg of compound **9** (12%) and 80 mg (12%) of its 4-regio-isomer were obtained.

Analytical data of compound **9**

HPLC purity: 97.6%. LC/MS (ESI): m/z 339 (M+Na). 1H-NMR (300 MHz, $CDCl_3$) δ 6.50-6.20 (brs, 2H), 6.10-5.90 (brs, 1H), 5.57 (s, 1H), 4.67-4.60 (m, 2H), 4.56 (s, 1H), 3.89-3.86 (m, 1H), 3.61 (t, *J* = 7.5 Hz, 2H), 3.51 (q, *J* = 7.2 Hz, 2H), 2.76 (t, *J* = 7.5 Hz, 2H), 2.45-2.00 (m, 3H), 1.90-1.70 (m, 5H), 1.67 (s, 3H), 1.40-1.10 (m, 3H).

Analytical of 4-regio-isomer of compound **9**

HPLC purity: 97%. LC/MS (ESI): m/z 339 (M+Na). ^{1}H-NMR (300 MHz, $CDCl_3$) *δ* 6.25 (s, 2H), 6.07 (s, 1H), 5.53 (s, 1H), 4.67 (m, 2H), 4.49 (s, 1H), 3.61-3.58 (m, 1H), 3.53-3.48 (m, 4H), 2.90-2.80 (m, 1H), 2.65-2.45 (m, 2H), 2.30-2.00 (m, 2H), 1.90-1.70 (m, 5H), 1.58 (s, 3H), 1.30-1.10 (m, 3H). The analytical data are attached below.

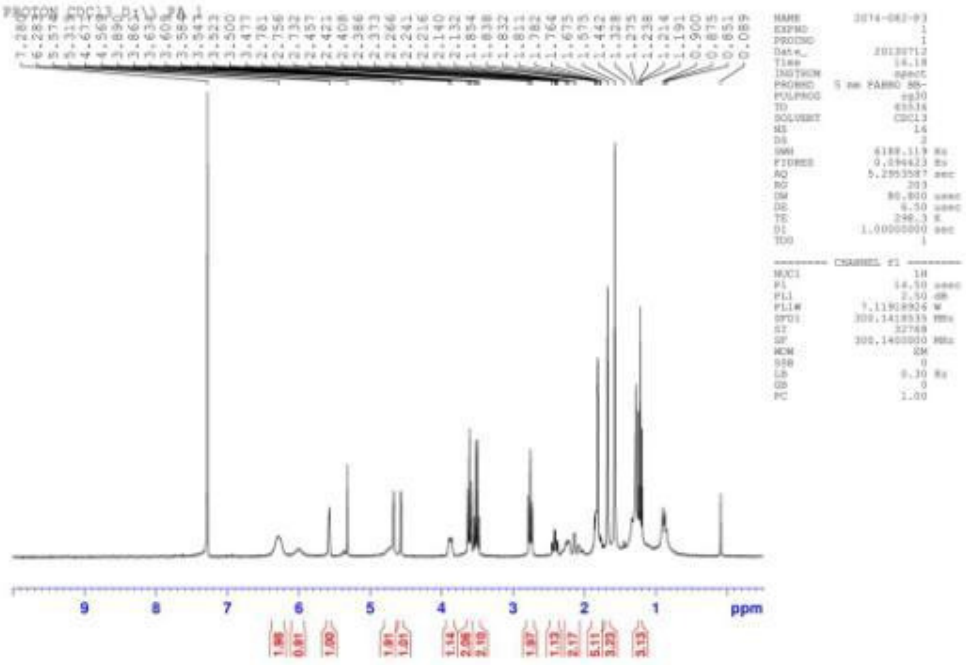


4-regio-isomer of 9

NOTES:

1. The reaction went much faster compard to the other substrates because intermediate **9-6** has good solubility in DCM.
2. Longer reaction time caused greater impurities by TLC analysis. The reaction was usually stopped before **9-6** was completely consumed.

^{1}H-NMR spectrum of compound **9**

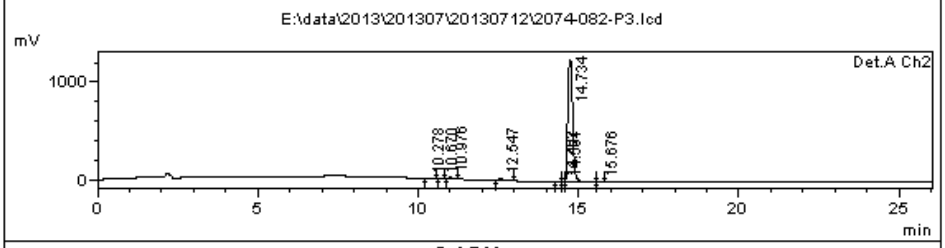


HPLC chromatogram of compound **9**

==== Shimadzu LCsolution Analysis Report ====

E:\data\2013\201307\20130712\2074-082-P3.lcd

Sample Name	: JK	Sample ID: 2074-082-P3	
Tray#	: 1	Vail #: 6	
Injection Volume	: 20 uL	Data File Name: 2074-082-P3.lcd	
Method File Name	: method2(15cm)-1.lcm	Batch File Name: 20130712.lcb	
Data Acquired	: 2013-7-12 14:47:10	Data Processed: 2013-7-12 16:53:45	

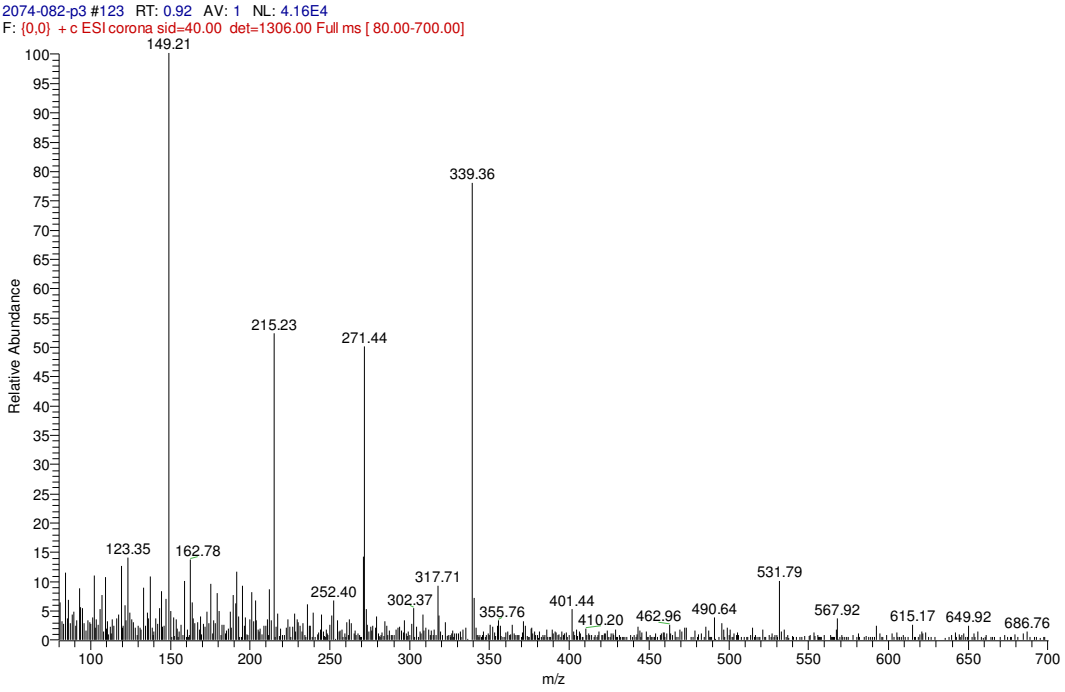


Peak Table

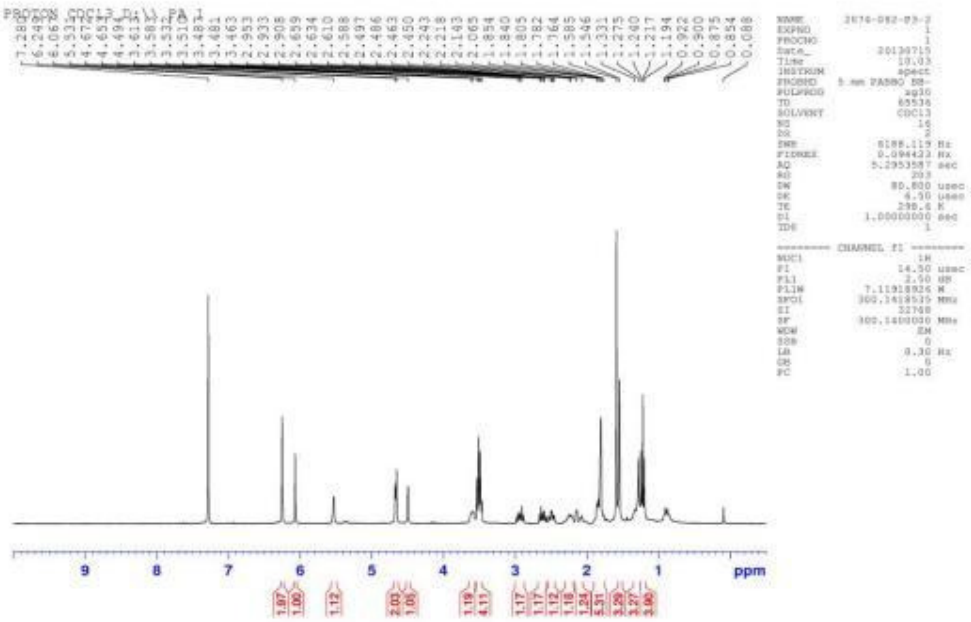
Detector A Ch2 220nm

Peak#	Ret. Time	Area	Area %
1	10.278	11847	0.088
2	10.670	3113	0.023
3	10.976	110064	0.820
4	12.547	159385	1.188
5	14.402	5384	0.040
6	14.531	11935	0.089
7	14.734	13106386	97.678
8	15.676	9826	0.073
Total		13417939	100.000

LC/MS of compound **9**



^{1}H-NMR spectrum of the 4-regio-isomer of compound **9**

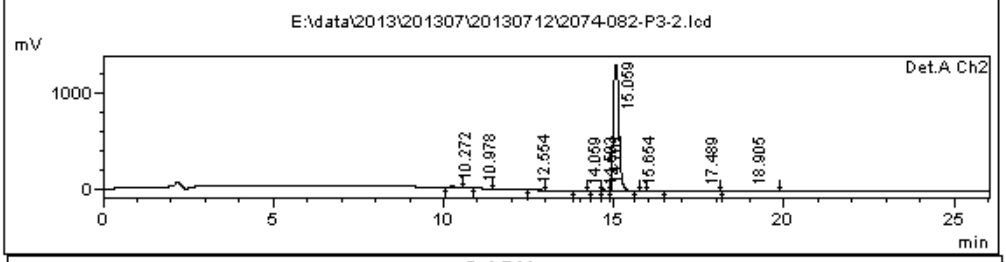


HPLC chromatogram of the 4-regio-isomer of compound **9**

==== Shimadzu LCsolution Analysis Report ====

E:\data\2013\201307\20130712\2074-082-P3-2.lcd

Sample Name	: JK	Sample ID: 2074-082-P3-2	
Tray#	: 1	Vail #: 20	
Injection Volume	: 25 uL	Data File Name: 2074-082-P3-2.lcd	
Method File Name	: method2(15cm)-1.lcm	Batch File Name: 20130712.lcb	
Data Acquired	: 2013-7-12 23:02:01	Data Processed: 2013-7-16 10:12:09	

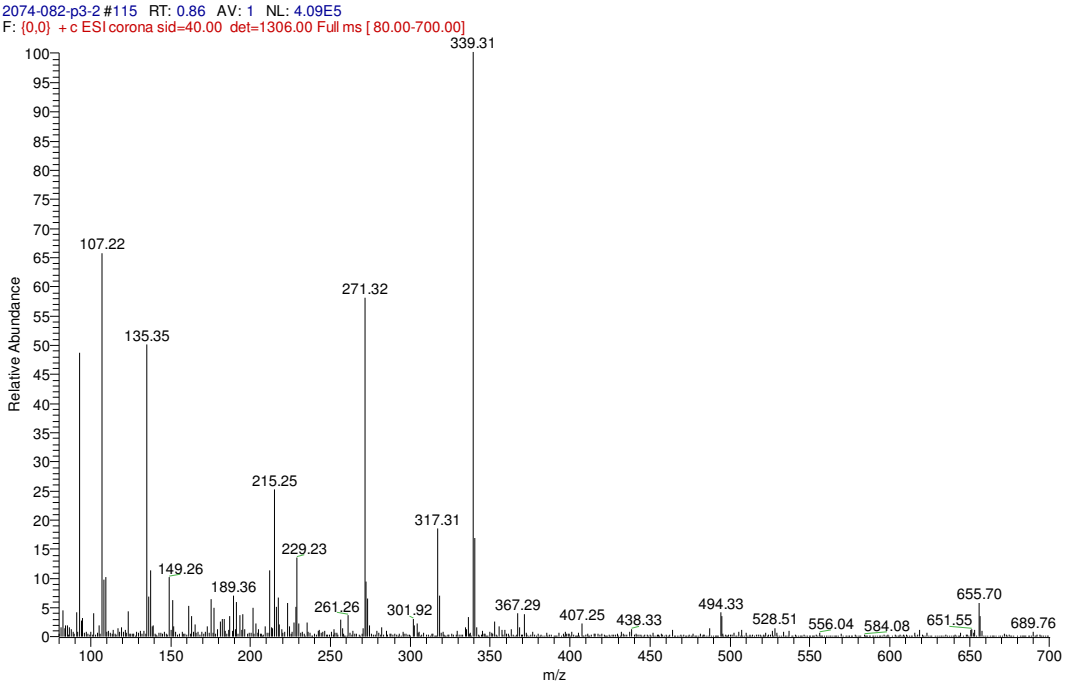


Peak Table

Detector A Ch2 220nm

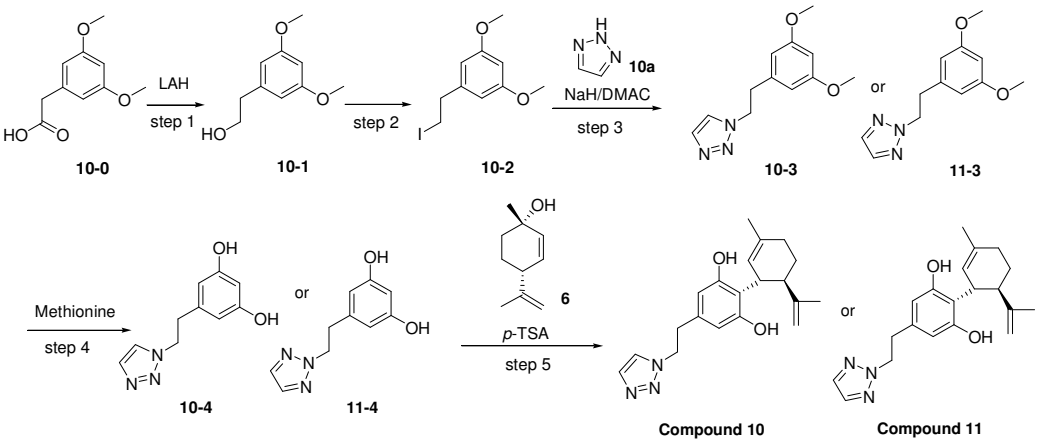
Peak#	Ret. Time	Area	Area %
1	10.272	147923	0.919
2	10.978	57217	0.355
3	12.554	55192	0.343
4	14.059	6948	0.043
5	14.503	20877	0.130
6	14.702	112873	0.701
7	15.059	15626879	97.079
8	15.654	7118	0.044
9	17.489	27520	0.171
10	18.905	34517	0.214
Total		16097063	100.000

LC/MS of the 4-regio-isomer of compound **9**

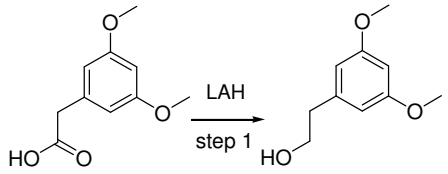


Synthesis of Compounds 10 and 11

Synthesis Scheme for Compounds 10 and 11



STEP 1:

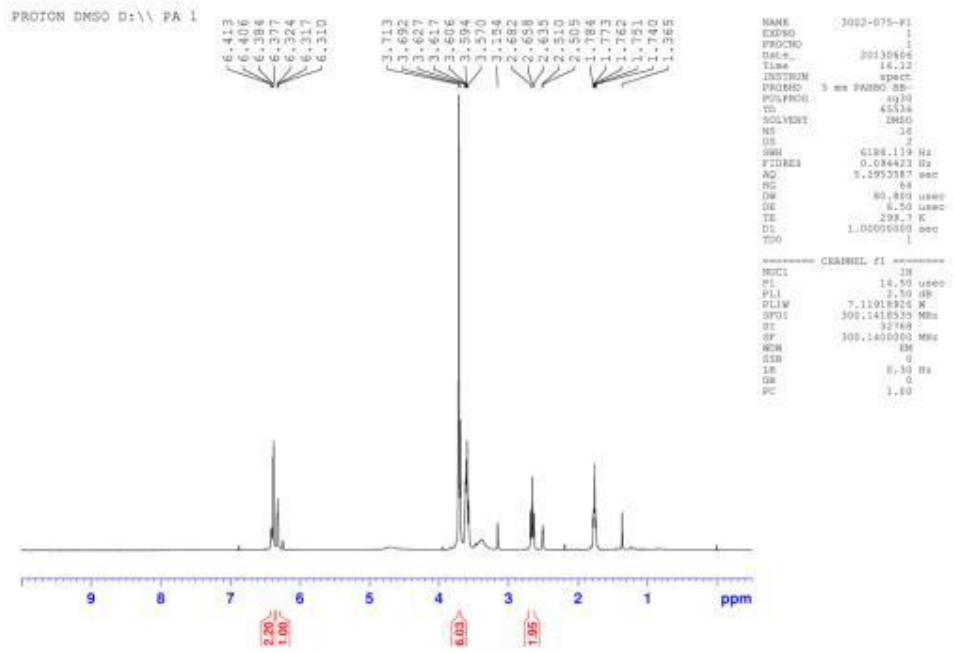


To a suspension of $LiAlH_4$ (0.43 g, 11mmol, 1.1 equiv.) in THF (25 mL) was added a solution of **10-0** (2.0 g, 10 mmol, 1.0 equiv.) in THF (20 mL) dropwise at the rate of keeping the inner temperature below 30 °C and the mixture was continued stirring for additional 30 min. TLC analysis indicated the consumption of **10-0**, so water (1 mL) was added slowly to quench the reaction, followed by 15% aqueous KOH (1 mL) and water (3 mL) in order. The solid formed was filtered off and the filtered cake was washed with THF (2 x 30 mL). The combined filtrate was dried over Na_2SO_4, concentrated to obtain the crude **10-1** as yellow oil (1.6 g, yield 86%). HPLC purity: 86%. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 6.41-6.31 (m, 3H), 3.71 (s, 6H), 3.59 (m, 2H), 2.66 (t, J = 7.2 Hz, 2H). The analytical data are attached below.

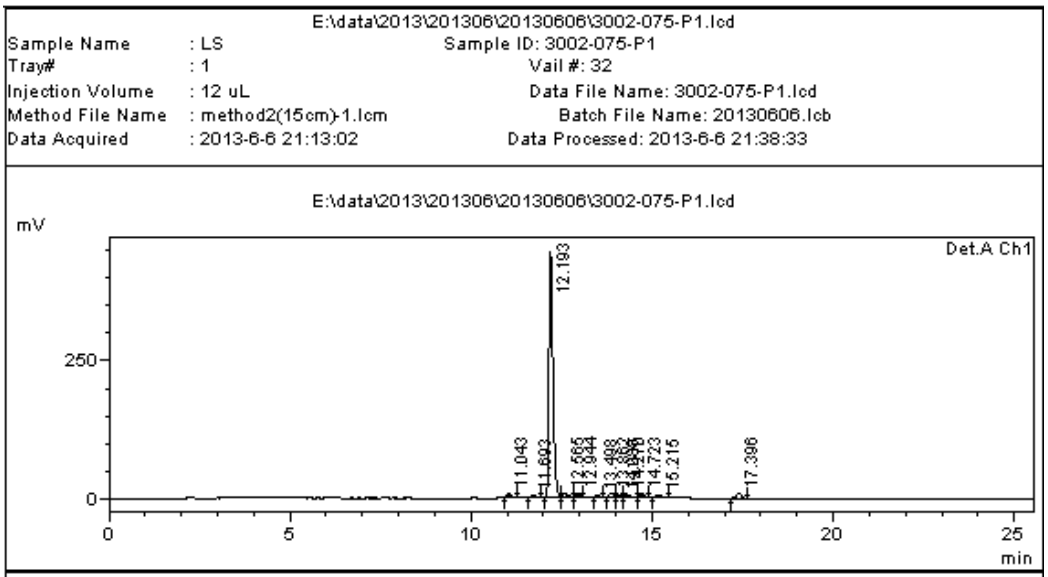
Batch Summary of Step 1

Batch #	**10-0**	Product (**10-1**)	Yield (%)	HPLC (%)
2137-047	2.0 g	1.65 g (crude)	89%	76%
3002-075	2.0 g	1.60 g (crude)	86%	86%

^{1}H-NMR of the intermediate **10-1**



HPLC chromatogram of the intermediate **10-1**

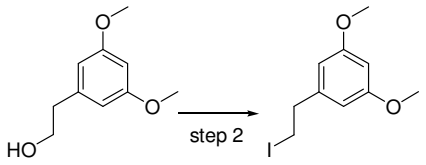


Peak Table

Detector A Ch1 254nm

Peak#	Ret. Time	Area	Area %
1	11.043	63164	1.608
2	11.693	36907	0.940
3	12.193	3377452	85.988
4	12.565	71409	1.818
5	12.944	42345	1.078

STEP 2:



10-1 **10-2**

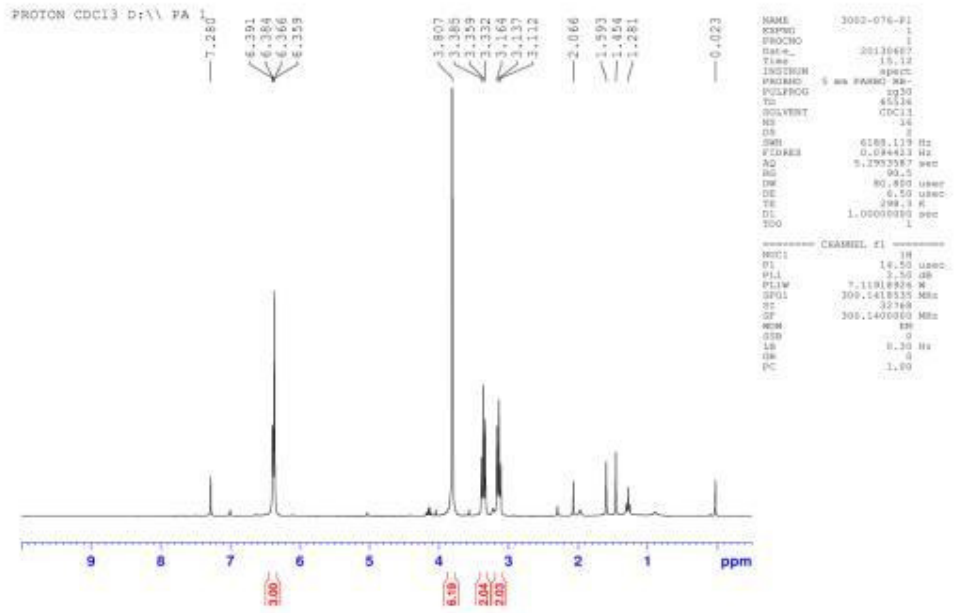
To a mixture of PPh_3 (4.56 g, 17.4 mmol, 1.5 equiv.), iodine (4.41 g, 17.4 mmol, 1.5 equiv.) and imidazole (1.97 g, 29.0 mmol, 2.5 equiv.) in DCM (80 mL) was added a solution of **10-1** (2.11 g, 11.6 mmol, 1.0 equiv.) in DCM (25 mL) and the resulting mixture was continued stirring for at RT 45 min. TLC analysis indicated the complete consumption of **10-1**. An aqueous solution of $NaHSO_3$ (100 mL) was added to quench the reaction. The water phase was extracted by Et_2O (3 x 100 mL). The combined organic phase was dried over Na2SO4, filtered and concentrated to give crude **10-2** (8.60 g) as yellow oil. The crude **10-2** was purified by column chromatography to provide pure product **10-2** (2.16 g, yield 64%) as a yellow oil. HPLC purity: 96%.

^{1}H-NMR (300 MHz, $CDCl_3$) δ 6.39-6.36 (m, 3H), 6.36 (s, 6H), 3.36 (t, J = 8.1 Hz, 2H), 3.14 (t, J = 7.8 Hz, 2H). The analytical data are attached below.

Batch Summary of Step 2

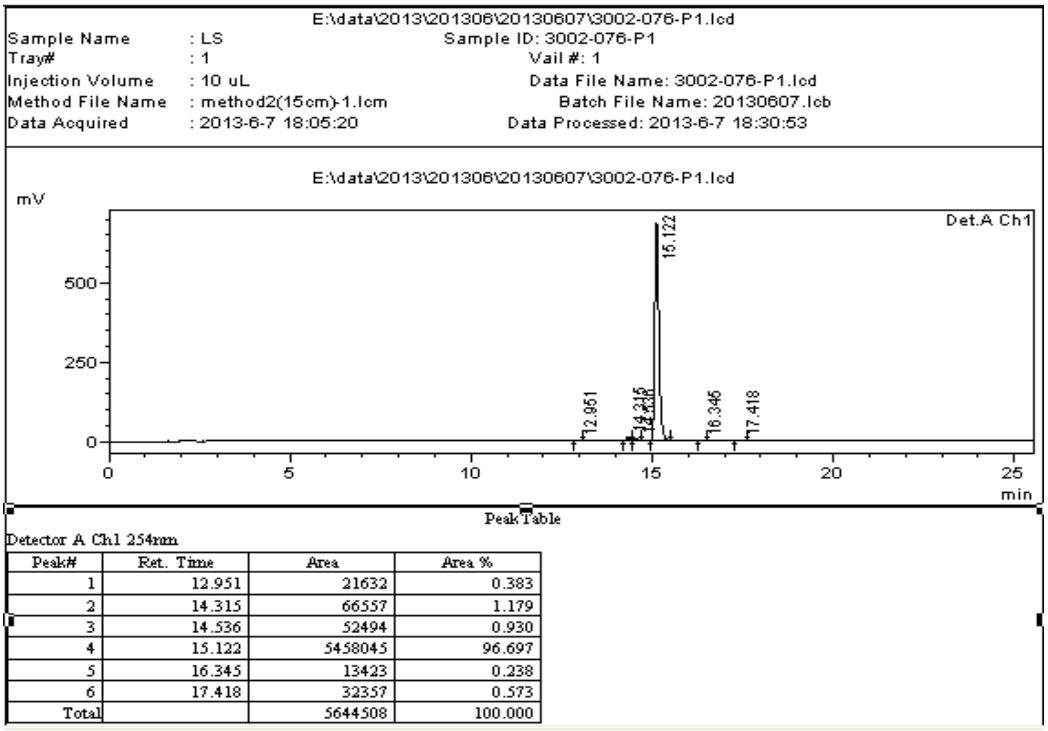
Batch #	SM (**10-1**)	Product (**10-2**)	Yield (%)	HPLC (%)
2137-048	100mg	330mg (crude)	206%	N/A
3002-063	300mg	277mg	58%	95%
3002-070	1g	1.04g	65%	95%
3002-076	2.11g	2.16g	64%	96%

^{1}H-NMR spectrum of the intermediate **10-2**



HPLC chromatogram of the intermediate **10-2**

==== Shimadzu LCsolution Analysis Report ====

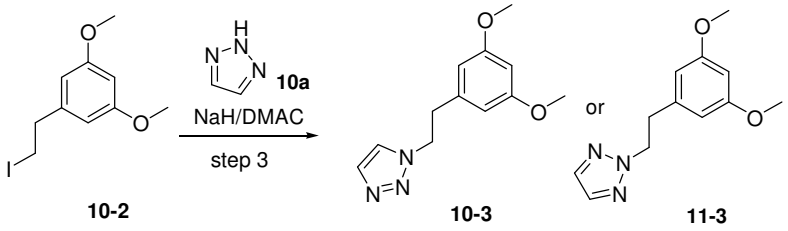


PeakTable

Detector A Ch1 254nm

Peak#	Ret. Time	Area	Area %
1	12.951	21632	0.383
2	14.315	66557	1.179
3	14.536	52494	0.930
4	15.122	5458045	96.697
5	16.345	13423	0.238
6	17.418	32357	0.573
Total		5644508	100.000

STEP 3:



10-2 **10-3** **11-3**

To a solution of 2*H*-1,2,3-triazole (**10a**, 0.28 g, 4.05 mmol, 1.0 equiv.) in dimethylacetamide (72 mL) was added NaH (60%, 0.2 g, 5.0 mmol, 1.2 equiv.) and stirred at RT for 30 min. The intermediate **10-2** (1.2 g, 4.1 mmol, 1.0 equiv.) was added and the resulting mixture was stirred at RT for 14 h. TLC analysis showed the completion of the reaction. Water (100 mL) was added to quench the reaction, which was extracted by EtOAc (3 x 50 mL). The EtOAc phase was back washed by brine (3 x 50 mL), dried, concentrated, and purified by column chromatography to give **11-3**

(0.52 g, yield 68%, less polar, eluted by EtOAc/Hexane = 1/10) and **10-3** (0.13 g, yield 17%, polar one, eluted by EtOAc/Hexane = 1/3)

Analytical data of **11-3**

^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.62 (d, J = 1.8 Hz, 2H, 2H), 6.32 (m, 3H), 4.68 (t, J = 7 Hz, 2H), 3.77 (s, 6H), 3.23 (t, J = 7 Hz, 3H). The analytical data is attached below.

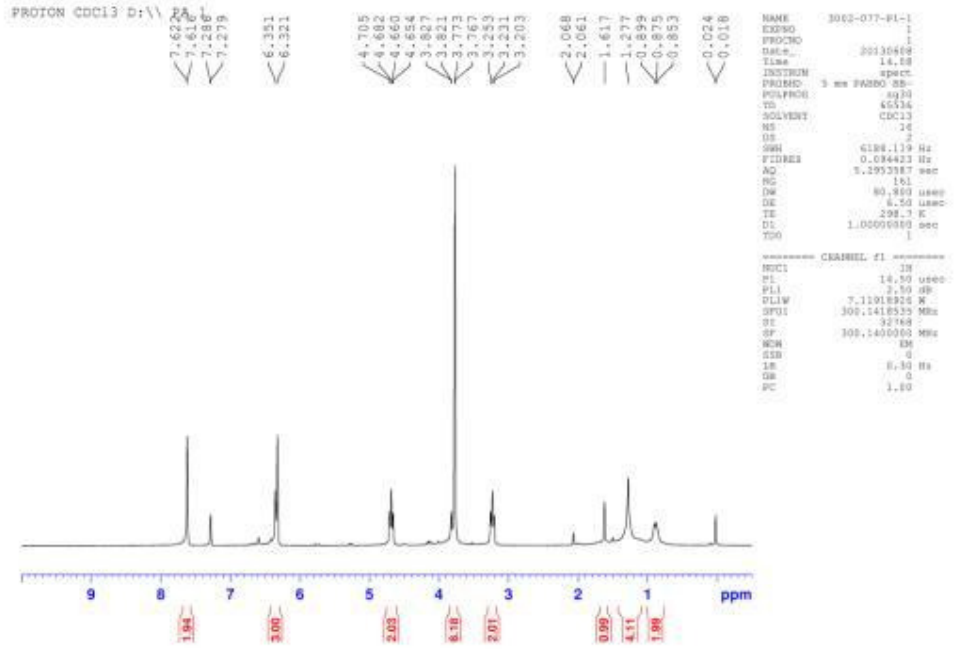
Analytical data of **10-3**

^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.64 (s, 1H), 7.31 (m, 1H), 6.36 (s, 1H), 6.23 (s, 2H), 4.63 (t, J = 7 Hz, 2H), 3.75 (s, 6H), 3.19 (t, J = 7 Hz, 2H). The analytical data is attached below.

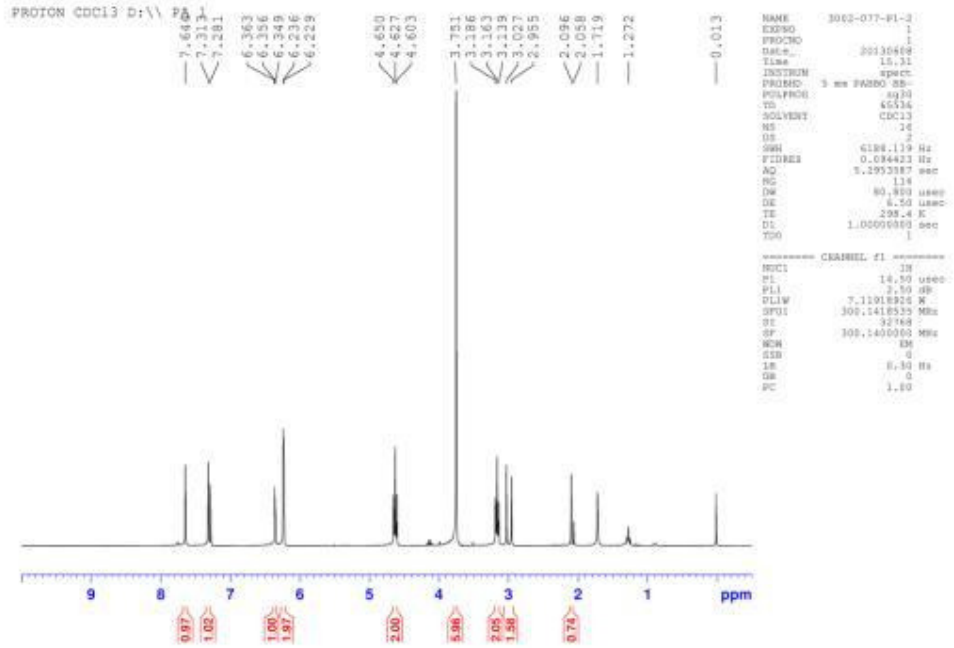
Batch Summary of Step 3

Batch #	SM	product	Yield (%)	Purity
3002-071	1.0g	**11-3**：0.4g	72%	Both H-NMR ok
		10-3：0.18g	32.4%	
3002-077	1.2g	**11-3**：0.52g	68%	Both H-NMR ok
		10-3：0.13g	17%	

H-NMR spectrum of intermediate **11-3**

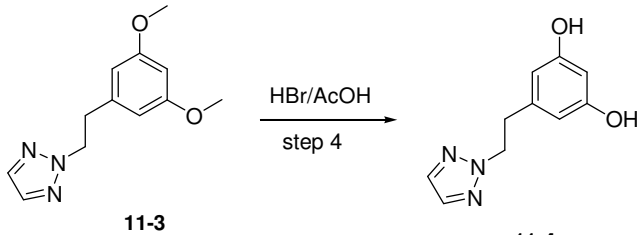


H-NMR spectrum of intermediate **10-3**



STEP 4:

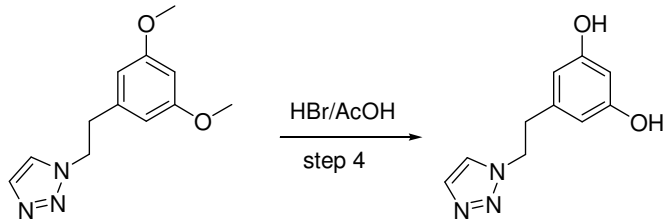
Synthesis of the intermediate **11-4**



11-3 **11-4**

A solution of **11-3** (0.52 g, 2.22 mmol, 1.0 equiv.) in 40% HBr/AcOH (1:1) (22 mL) was refluxed for 12 h under the protection by nitrogen. TLC analysis indicated the completion of the reaction. The reaction mixture was concentrated to dryness. The residue was dissolved in EtOAc (50 ml) and treated with a solution of saturated $NaHCO_3$ to adjust the pH to 5-6. The organic phase was separated and the aqueous phase was extracted by EtOAc (2 x 5 mL). The combined organic phase was dried over Na_2SO_4, filtered and concentrated to give crude **11-4** (0.44 g) as a brown solid. The crude **11-4** was used directly for the next step without further purification. ^{1}H-NMR (300 MHz, CD3OD) δ 7.66 (s, 2H), 6.10 (s, 3H), 4.62 (t, J = 7.5 Hz, 2H), 3.11 (t, J = 7.5 Hz, 2H).

Synthesis of the intermediate **10-4**



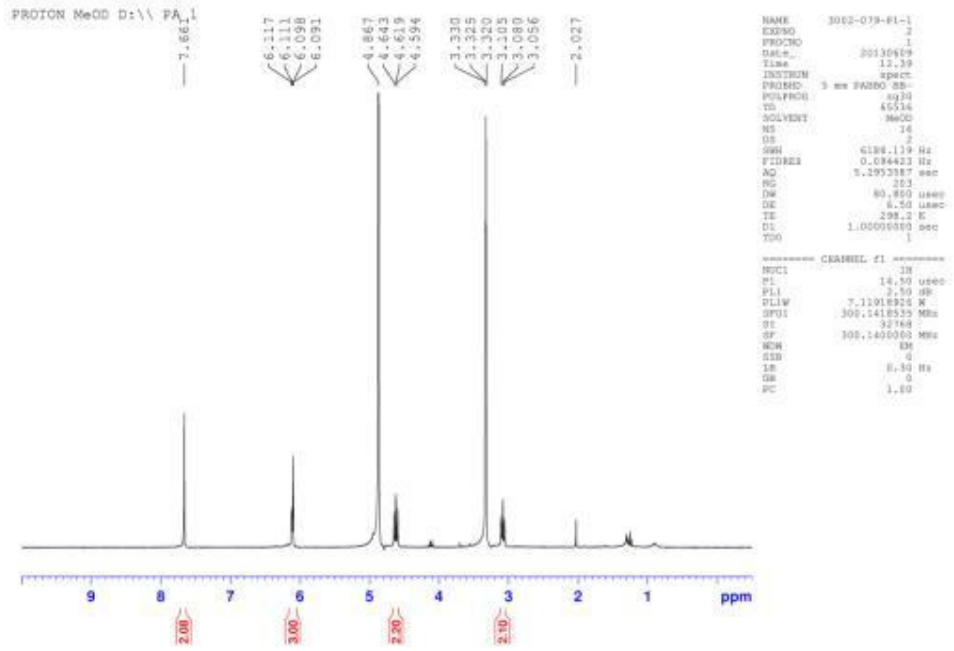
10-3 **10-4**

Using the same procedure as for **11-4**, 0.11 g of crude **10-4** was obtained from **10-3** (0.13 g). ^{1}H-NMR (300 MHz, CD_3OD) δ 7.76 (s, 1H), 7.66 (s, 1H), 6.09 (m, 3H), 4.64 (t, J = 7.0 Hz, 2H), 3.05 (t, J = 7.0 Hz, 2H).

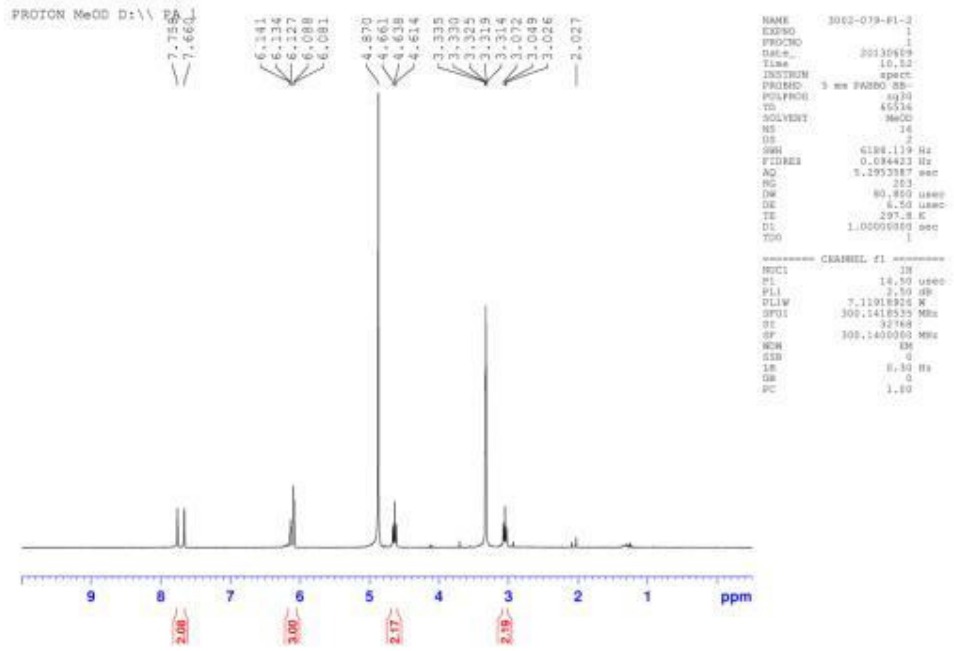
Batch Summary of Step 4

Batch #	SM	product	Yield (%)	Purity
3002-073	**11-3**：0.4g	**11-4**：0.315g	89.5%	Both ^{1}H-NMR ok
	10-3：0.18g	**10-4**：0.12g	75.8%	
3002-079	**11-3**：0.52g	**11-4**：0.44g	96%	Both ^{1}H-NMR ok
	10-3：0.13g	**10-4**：0.11g	96%	

^{1}H-NMR spectrum of the intermediate **11-4**

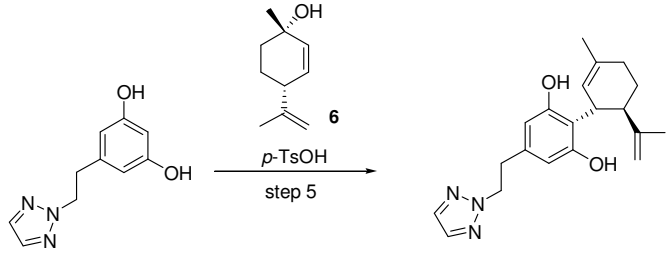


^{1}H-NMR spectrum of the intermediate **10-4**



STEP 5:

Synthesis of Compound 11



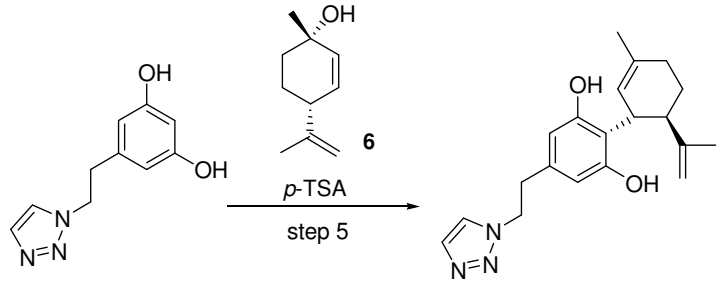
11-4 **Compound 11**

To a mixture of **11-4** (0.40 g, 1.95 mmol, 1.0 equiv.) and *p*-TsOH (74 mg, 0.43 mmol, 0.2 equiv.) in a mixed solvent of THF/DCM (1:4) (20 mL) was slowly added **6** (0.44 g, 2.89 mmol, 1.5 equiv.) dropwise. The resulting mixture was continued stirring at RT for 1-2 h. The reaction mixture was diluted by EtOAc (40 mL) and washed by saturated aqueous $NaHCO_3$ solution (2 x 30 mL). The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography (eluent: EtOAc/hexane = 1:5) to give crude compound **11** (136 mg) and the 4-regio-isomer of **11** (160 mg). The starting material **11-4** (167 mg) was

recovered. The crude compound **11** was combined with another batch (batch # 3002-096) and further purified by preparative HPLC to produce 140 mg of compound **11**.

HPLC purity: 97%. LC/MS (ESI): m/z 340 (M+1), m/z 362 (M+Na). ^{1}H-NMR (300 MHz, CD_3OD) δ 7.66 (s, 2H), 6.08 (s, 2H), 5.25 (S, 1H), 4.59 (t, J = 7.5 Hz, 2H), 4.43 (m, 2H), 3.98-3.89 (m, 1H), 3.02 (t, J = 7.8 Hz, 2H), 2.95-2.85 (m, 1H), 2.2-2.00 (m, 2H), 1.80-1.70 (m, 2H), 1.68 (s, 3H), 1.63 (s, 3H).

Synthesis of Compound 10



10-4 **Compound 10**

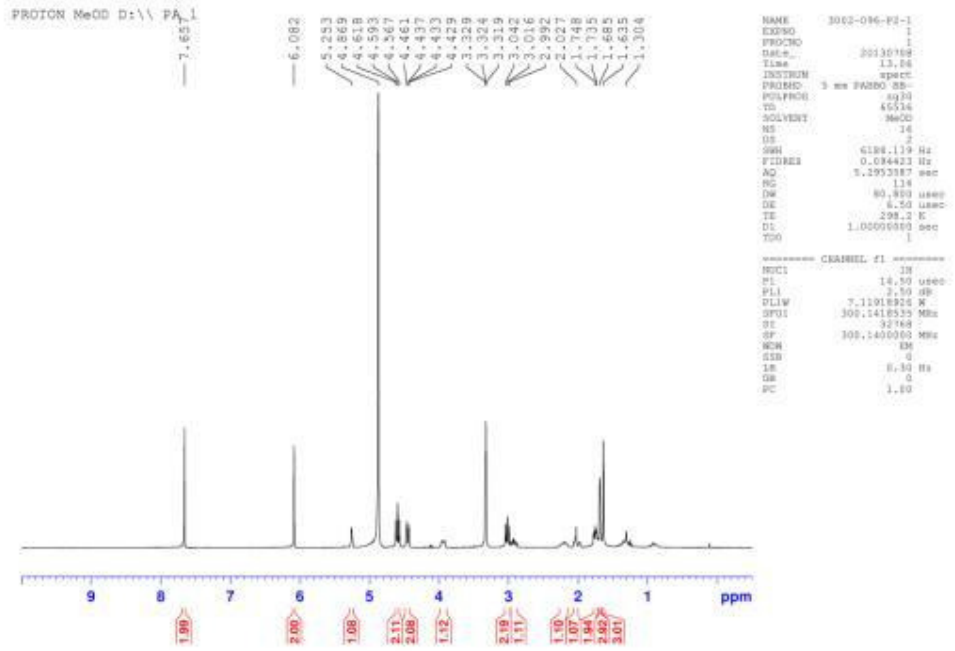
Using the same procedure as the one for compound **11**, compound **10** (9 mg) was obtained from 200 mg of **10-4**.

HPLC purity: 98%. LC/MS (ESI): m/z 340 (M+1), m/z 362 (M+Na). ^{1}H-NMR (300 MHz, CD_3OD) δ 7.63 (s, 1H), 7.61 (s, 1H), 6.04 (s, 2H), 5.26 (s, 1H), 4.61 (t, J = 6.9 Hz, 2H), 4.45 (d, J = 3 Hz, 2H), 4.00-3.92 (m, 1H), 3.00-2.90 (m, 3H), 2.30-2.00 (m, 2H), 1.80-1.70 (m, 2H), 1.68 (s, 3H), 1.64 (s, 3H).

Batch Summary of Step 5

Batch #	SM	Product compound **11**	Yield (%)	HPLC (%)
3002-095	**11-4**：400 mg	**11**：140 mg	15%	97%
3002-096	**11-4**：167 mg			
3002-097	**10-4**：200 mg	**10**：9 mg	3%	98%

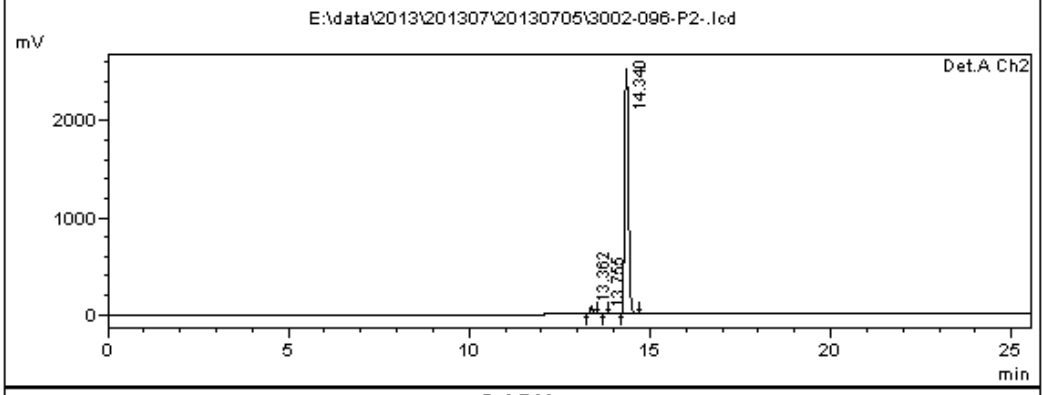
[1]H-NMR spectrum of compound **11**:



HPLC chromatogram of compound **11**

E:\data\2013\201307\20130705\3002-096-P2-.lcd

Sample Name	: LS	Sample ID: 3002-096-P2	
Tray#	: 1	Vail #: 31	
Injection Volume	: 5 uL	Data File Name: 3002-096-P2-.lcd	
Method File Name	: method1(15cm)-MeOH+H2O.lcm	Batch File Name: 20130705.lcb	
Data Acquired	: 2013-7-5 21:05:19	Data Processed: 2013-7-5 21:30:52	

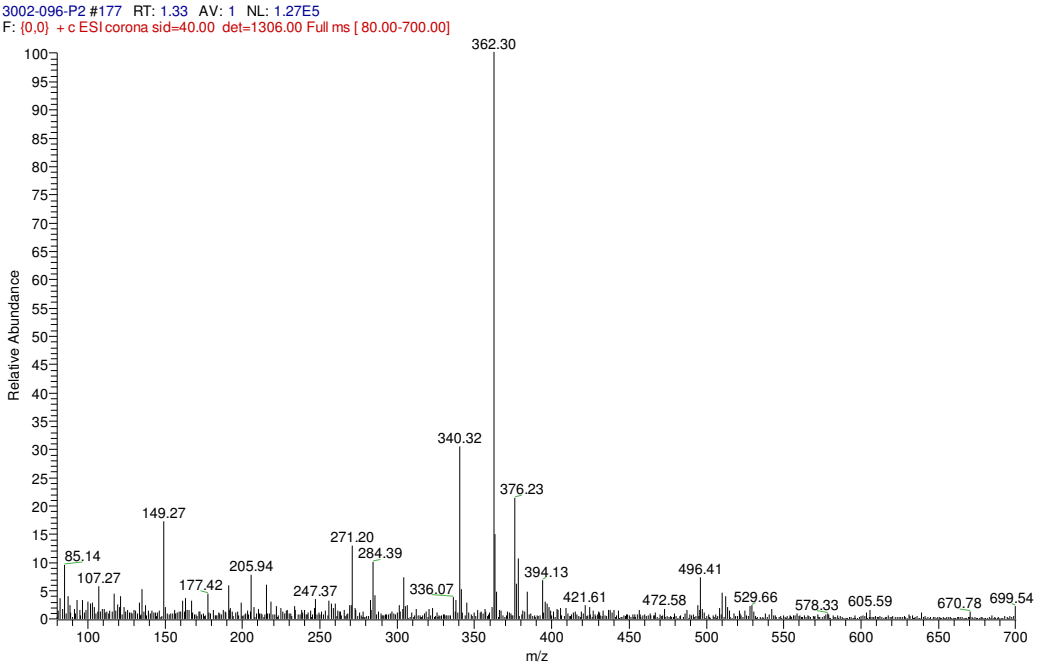


Peak Table

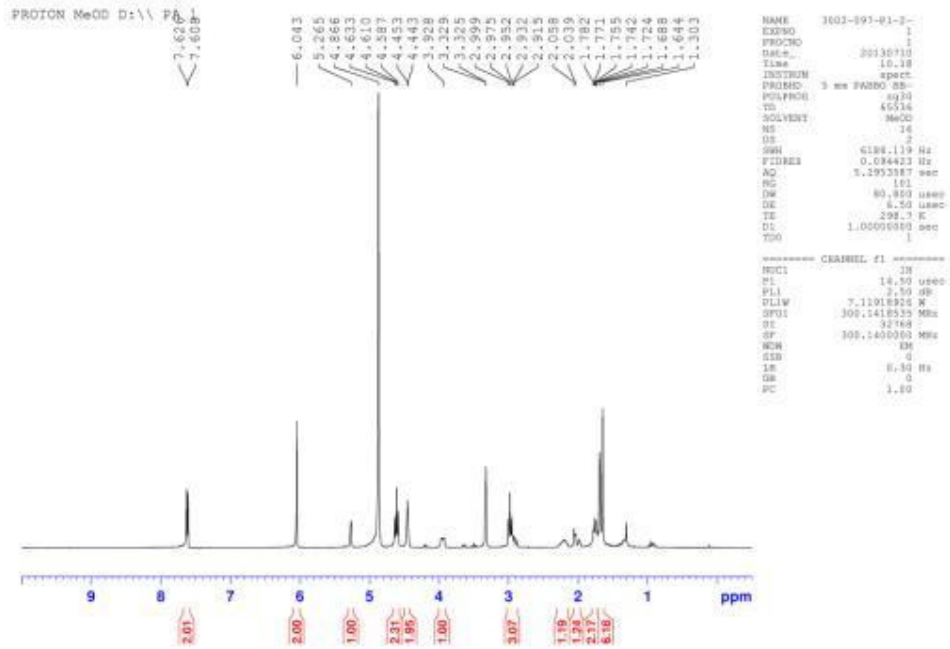
Detector A Ch2 230nm

Peak#	Ret. Time	Area	Area %
1	13.362	433306	2.260
2	13.755	70860	0.370
3	14.340	18670467	97.371
Total		19174632	100.000

LC/MS of compound **11**

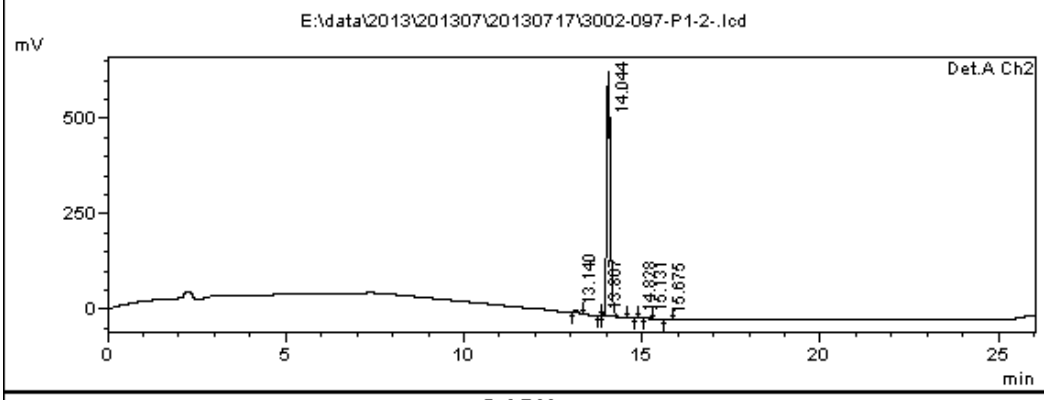


^{1}H-NMR spectrum of compound **10**:



HPLC chromatogram of compound **10**

Sample Name	: LS	Sample ID: 3002-097-P1-2-	
Tray#	: 1	Vail #: 91	
Injection Volume	: 5 uL	Data File Name: 3002-097-P1-2-.lcd	
Method File Name	: method2(15cm)-1.lcm	Batch File Name: 20130717.lcb	
Data Acquired	: 2013-7-17 17:02:15	Data Processed: 2013-7-17 17:28:18	

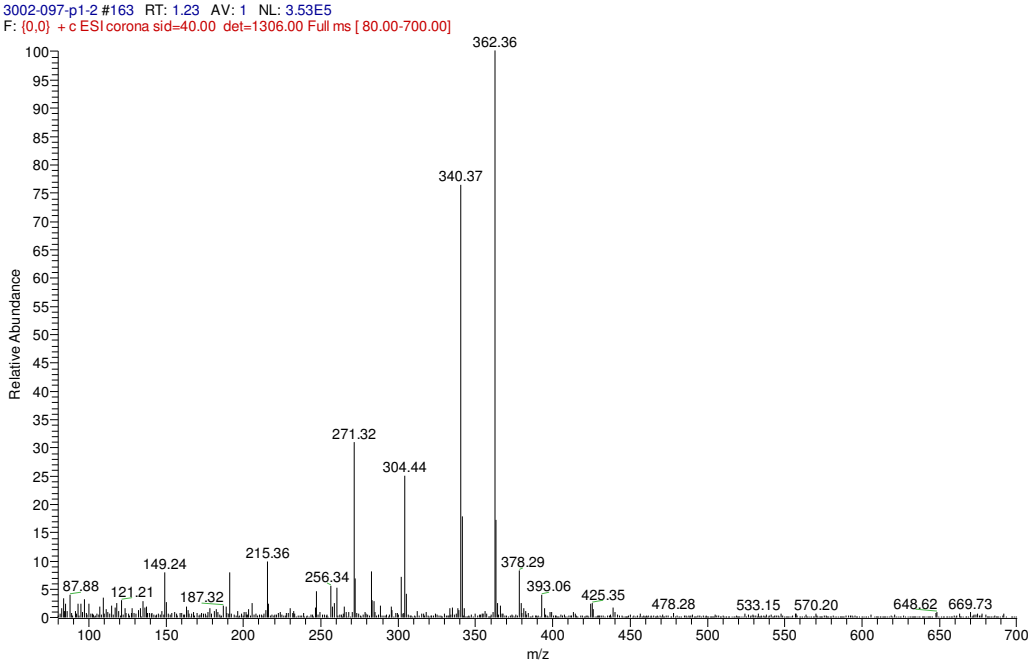


Peak Table

Detector A Ch2 220nm

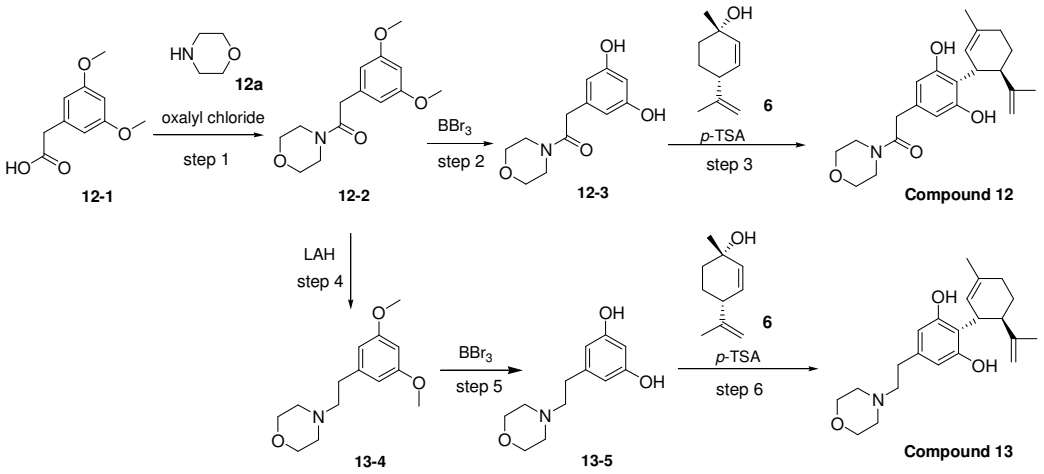
Peak#	Ret. Time	Area	Area %
1	13.140	43180	0.983
2	13.867	7035	0.160
3	14.044	4320641	98.362
4	14.828	5447	0.124
5	15.131	9599	0.219
6	15.675	6670	0.152
Total		4392573	100.000

LC/MS of compound **10**

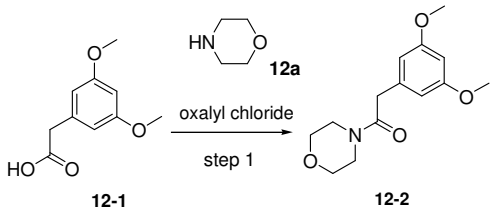


Synthesis of Compounds 12 and 13

Synthesis Scheme for Compounds 12 and 13



Step 1：



Compound **12-1** (5.0 g, 25 mmol, 1.0 equiv.) and catalytic amount of DMF (3 drops) under nitrogen was dissolved in DCM (50 mL). To the solution was added oxalyl chloride (9.7 g, 25 mmol, 1.0 equiv.) in DCM (20 mL) dropwise and stirred at RT for 1 h. The reaction mixture was then evaporated to dryness and diluted with DCM (50 mL). To the resulting DCM solution, **12a** (2.88 g, 33.0 mmol, 1.3 equiv.) was added slowly. After the addition, TEA (5.1 g, 51 mmol, 2.0 equiv.) was added to the reaction mixture, which was stirred for at RT one h. The reaction was quenched by addition of water (100 mL) and EtOAc (100 mL). The organic phase was separated and the water phase was extracted by EtOAc (50 mL). The combined organic phase was washed by water (2 x 200 mL), followed by brine (2 x 200 mL). The organic phase was dried over Na_2SO_4 and concentrated to give the crude product **12-2** (6.33 g, yield 93%) as yellow solid. The crude **12-2** was directly used for the next step without

further purification. HPLC purity: 96.2%. ^{1}H-NMR (300 MHz, CD_3OD) δ 6.43-6.39 (m, 3H), 3.77 (s, 6H), 3.73 (s, 2H), 3.65-3.62 (m, 4H), 3.52 (m, 4H). The analytical data are attached below.

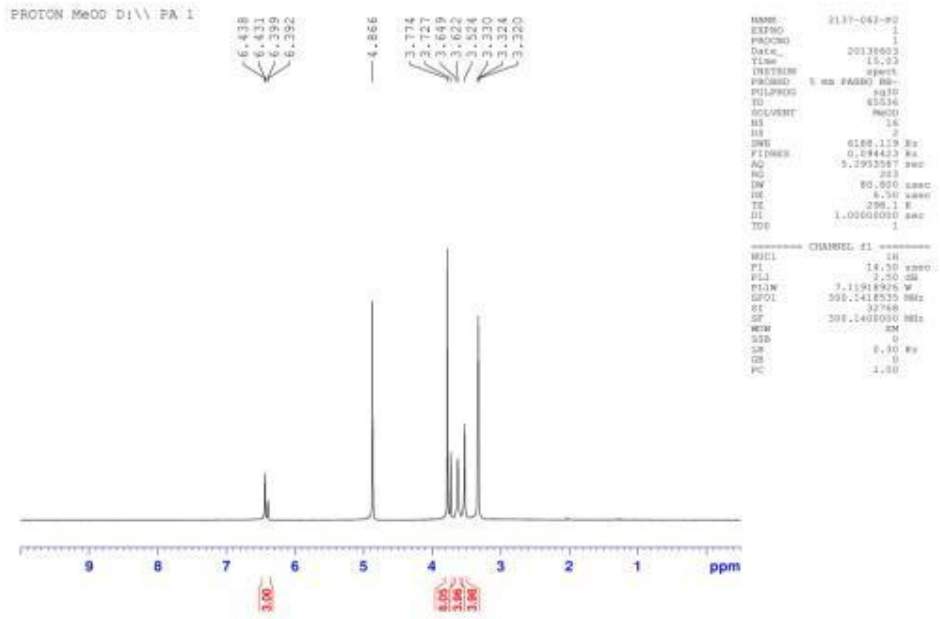
NOTES:

1. EDCI/HOBt had also been used for this step, but it gave the lower yield (49%~79%). (ref: 2137-039, 040, 053, 062, 071).

Batch Summary of Step 1

Batch #	SM (**12-1**)	Product (**12-2**)	Yield (%)	HPLC (%area)
2137-039	500 mg	420 mg（crude）	62.1%	93%
2137-040	500 mg	350 mg（crude）	51.8%	86%
2137-053	800 mg	530 mg (purified)	49%	78%
2137-062	2 g	1.48 g (purified）	55%	99%
2137-071	1.5 g	1.6 g (crude）	79%	96%
3020-025	5 g	6.33 g（crude）	94%	96%

^{1}H-NMR spectrum of intermediate 12-2

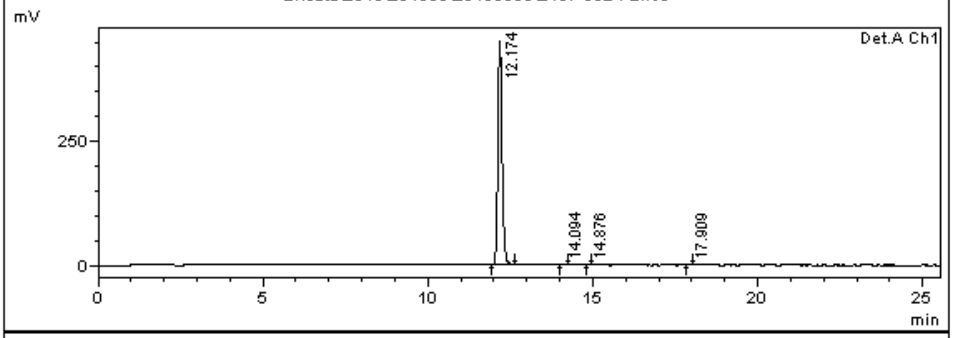


HPLC chromatogram of intermediate **12-2**

==== Shimadzu LCsolution Analysis Report ====

E:\data\2013\201306\20130603\2137-062-P2.lcd

Sample Name : QT
Sample ID: 2137-062-P2
Tray# : 1
Vail #: 46
Injection Volume : 50 uL
Data File Name: 2137-062-P2.lcd
Method File Name : method2(15cm)-1.lcm
Batch File Name: 20130603.lcb
Data Acquired : 2013-6-3 16:29:30
Data Processed: 2013-9-4 10:53:56

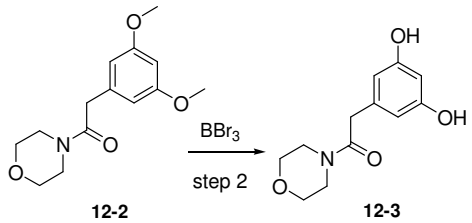


Peak Table

Detector A Ch1 254nm

Peak#	Ret. Time	Area	Area %
1	12.174	3877330	99.607
2	14.094	8701	0.224
3	14.876	2650	0.068
4	17.909	3949	0.101
Total		3892629	100.000

Step 2:



12-2 **12-3**

A 1N solution of BBr_3 (22.6 g, 90.2 mmol, 8 equiv.) in DCE (90 mL) was slowly added to a solution of intermediate **12-2** (3.0 g，11 mmol, 1.0 equiv.) in DCE (300 mL) with cooling by ice-salt bath. After the addition, the reaction mixture was continued stirring for 10 min and the ice-salt bath was removed and the reaction mixture was stirred at RT for 4 h. TLC analysis indicated that the reaction was almost complete. The pH value of the reaction mixture was adjusted to around 7 by the addition of the saturated aqueous $NaHCO_3$ (~200 mL). It was extracted with a mixture of THF/EtOAc (1:1) (4 x 200 mL). The combined organic layers were back washed with brine (2 x 400 mL), dried over Na_2SO_4, filtered and concentrated to provide crude **12-3** (2.63 g) as viscous oil. The crude **12-3** was purified by column chromatography (eluent: DCM/MeOH = 25/1) to give **12-3** as white solid (1.07 g, yield 40%). HPLC purity: 98.2%. ^{1}H-NMR (300 MHz, CD_3OD) δ 6.20 (m, 3H), 3.63-3.62 (m, 6H), 3.50 (s, 4H). The analytical data are attached below.

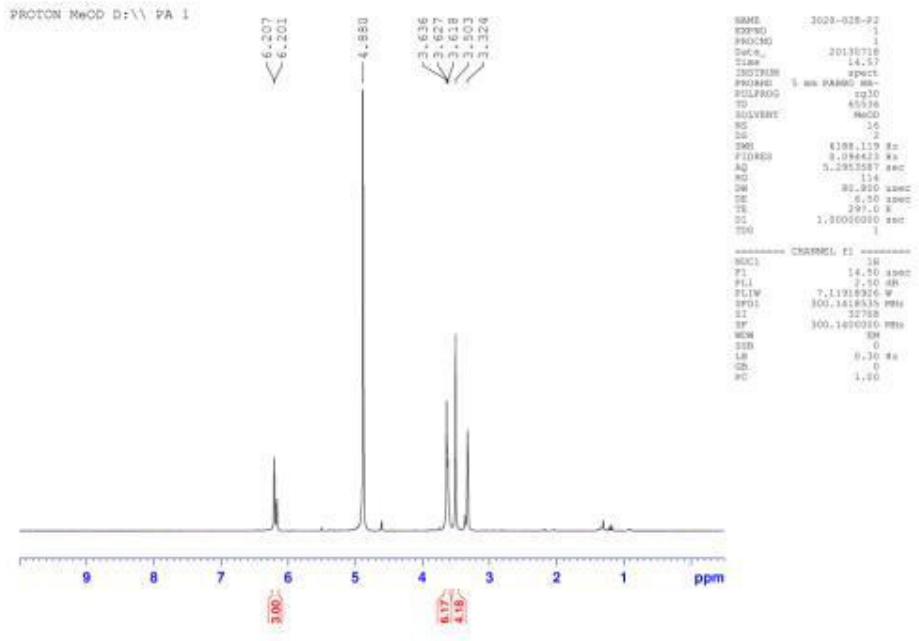
NOTES:

1. MsOH/methionine had been tested for the demethylation. It gave the same result as BBr_3.

Batch Summary of Step 2

Batch #	SM (**12-2**)	Product (**12-3**)	Yield (%)	HPLC (%)
2137-068	1.4 g	660 mg	53%	75%
2137-072	1.5 g	660 mg	49%	n/a
3020-028	3 g	1.07 g	40%	98%

[1]H-NMR of intermediate **12-3**

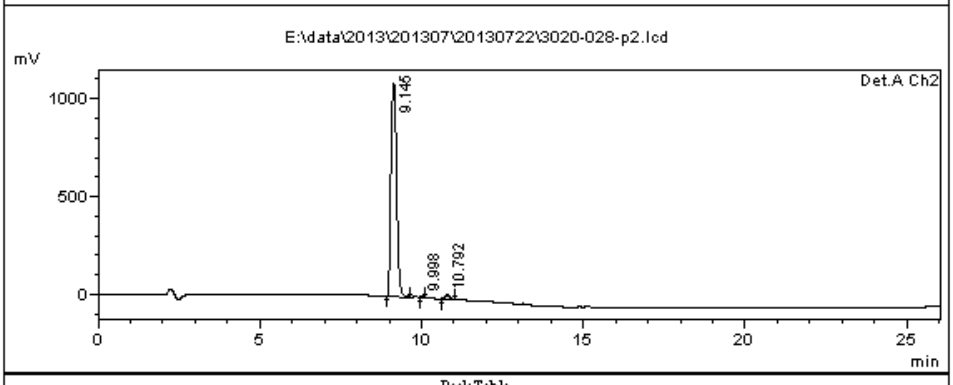


HPLC chromatogram of intermediate **12-3**

==== Shimadzu LCsolution Analysis Report ====

E:\data\2013\201307\20130722\3020-028-p2.lcd

Sample Name	: qt	Sample ID: 3020-028-p2	
Tray#	: 1	Vail #: 78	
Injection Volume	: 20 uL	Data File Name: 3020-028-p2.lcd	
Method File Name	: method2(15cm)-1.lcm	Batch File Name: 20130722.lcb	
Data Acquired	: 2013-7-22 12:52:11	Data Processed: 2013-8-21 16:08:45	

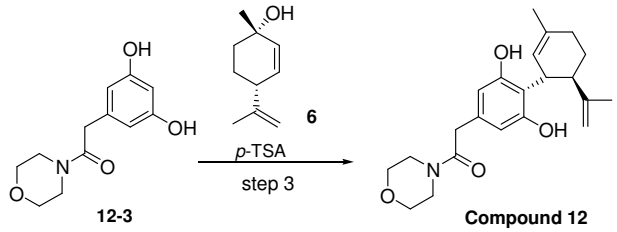


Peak Table

Detector A Ch2 220nm

Peak#	Ret. Time	Area	Area %
1	9.145	13292751	98.272
2	9.998	20129	0.149
3	10.792	213657	1.580
Total		13526537	100.000

Step 3: Synthesis of compound 12



To a suspension of compound **12-3** (0.43 g, 1.81 mmol, 1.0 equiv.) in the mixture of DCM/THF (4:1, 43 mL), was added BF_3-Et_2O (0.77 g，5.42 mmol, 3.0 equiv.) and reagent **6** (0.38 g, 1.81 mmol, 1.0 equiv.). The resulting mixture was stirred at RT for 20 min. TLC analysis indicated ~50% of conversion of compound **12-3**. The reaction was stopped at this point and quenched by saturated $NaHCO_3$. The organic phase was separated. The water phase was extracted by THF/EtOAc (1:1) (3 x 50 mL). The combined organic phase was washed by brine (2 x 100 mL), dried and concentrated to give crude product (0.6 g) as yellow oil. The crude product was combined with other three batches and purified by flash chromatography column (eluent: EtOAc/hexane = 1/2), and then further purified through preparative TLC (eluent: DCM/MeOH = 20/1) to provide the desired compound **12** (43 mg). HPLC: 95.4%. LC/MS (ESI): m/z 372 (M+1); m/z 394 (M+Na). ^{1}H-NMR (300 MHz, CD_3OD) δ 6.16 (s, 2H), 5.27 (s, 1H), 4.44 (d, J = 9.9 Hz, 2H), 3.97-3.94 (m, 1H), 3.61-3.58 (m, 6H), 3.46-3.42 (m, 4H), 2.99-2.91 (m, 1H), 2.81 (s, 1H), 2.02 (d, J = 14.4 Hz, 1H), 1.77-1.73 (m, 2H), 1.69 (s, 3H), 1.64 (s, 3H). The analytical data are attached below.

NOTES:

1. Intermediate **12-3** has poor solubility in the most of common solvents. The mixed solvent DCM/THF (4/1) showed slightly better solubility than a single solvent.
2. Other solvents, such as DCM, nitrobenzene, toluene, DMSO, DCM/dioxane, DCM/HOAc, have been tested for the reaction, but none of them showed the significant amount of the desired product based TLC analysis. They showed a low conversion of the starting material **12-3** or the messy reaction based on TLC.

Batch Summary of Step 3

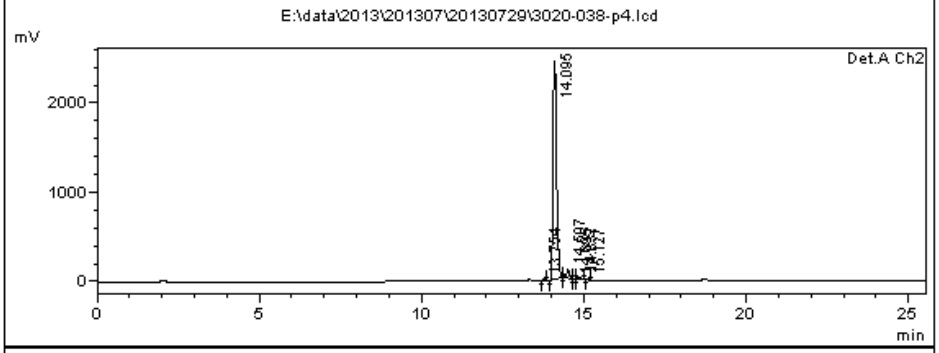
Batch #	SM (**12-3**)	Compound **12**	Yield (%)	HPLC (%)
3020-016	750 mg	23 mg	2%	97.3%
3020-032	50 mg	14 mg (crude)	18%	n/a
3020-034	200 mg	50 mg (crude)	16%	78%
3020-036	350 mg	67 mg	5%	95%
3020-037	500 mg			
3020-038	430 mg	43 mg	4.5%	95.4%
3020-031	120 mg			
2074-085	40 mg			

HPLC chromatogram of compound **12**

==== Shimadzu LCsolution Analysis Report ====

E:\data\2013\201307\20130729\3020-038-p4.lcd

Sample Name : qt Sample ID:
Tray# : 1 Vail #: 101
Injection Volume : 35 uL Data File Name: 3020-038-p4.lcd
Method File Name : method1(15cm)-MeOH+H2O.lcm Batch File Name: 20130726.lcb
Data Acquired : 2013-7-27 5:20:35 Data Processed: 2013-7-29 8:39:44

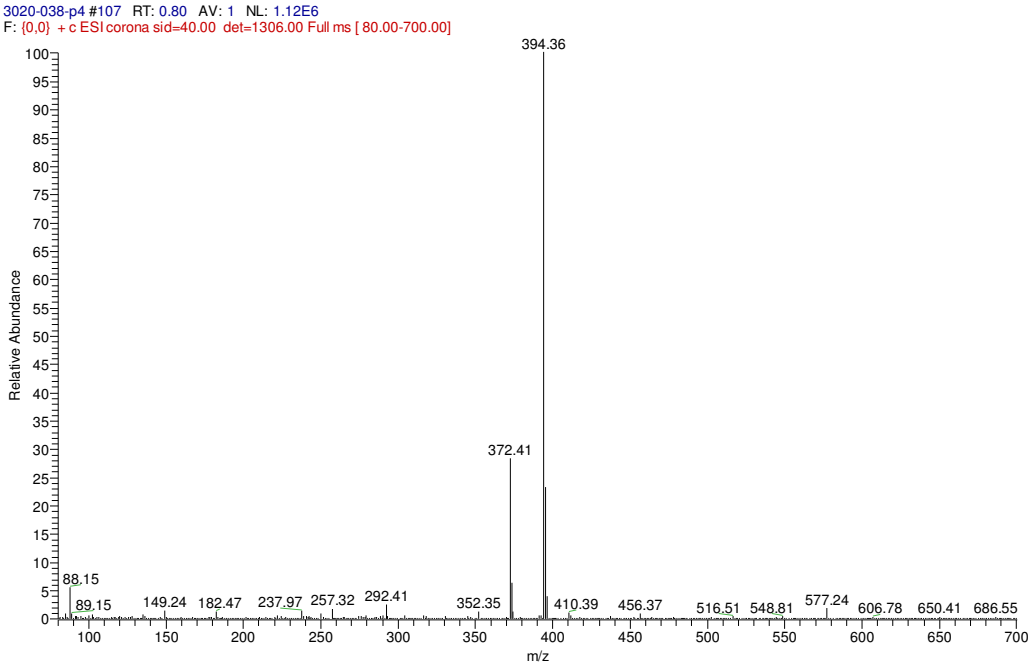


Peak Table

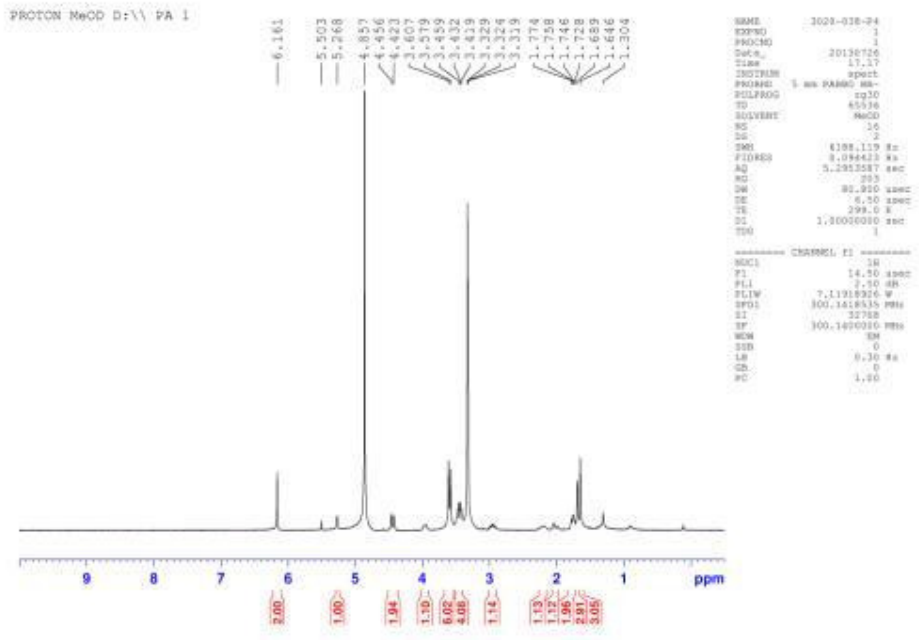
Detector A Ch2 220nm

Peak#	Ret. Time	Area	Area %
1	13.754	72874	0.375
2	14.095	18558645	95.398
3	14.507	594891	3.058
4	14.695	13398	0.069
5	14.837	168284	0.865
6	15.127	45850	0.236
Total		19453943	100.000

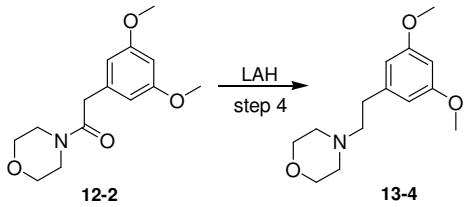
LC/MS of compound **12**



^{1}H-NMR spectrum of compound **12**

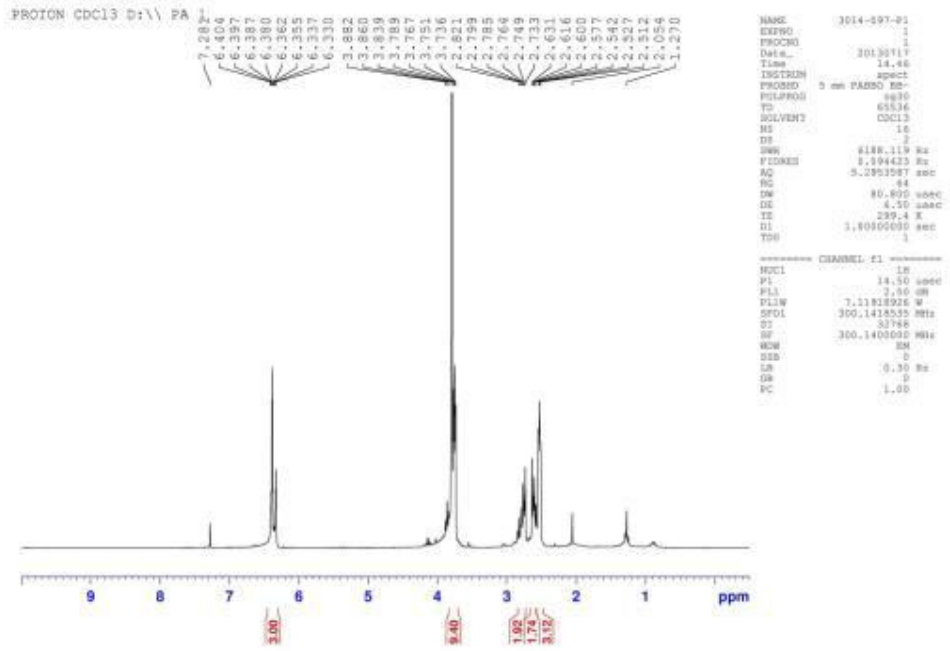


Step 4

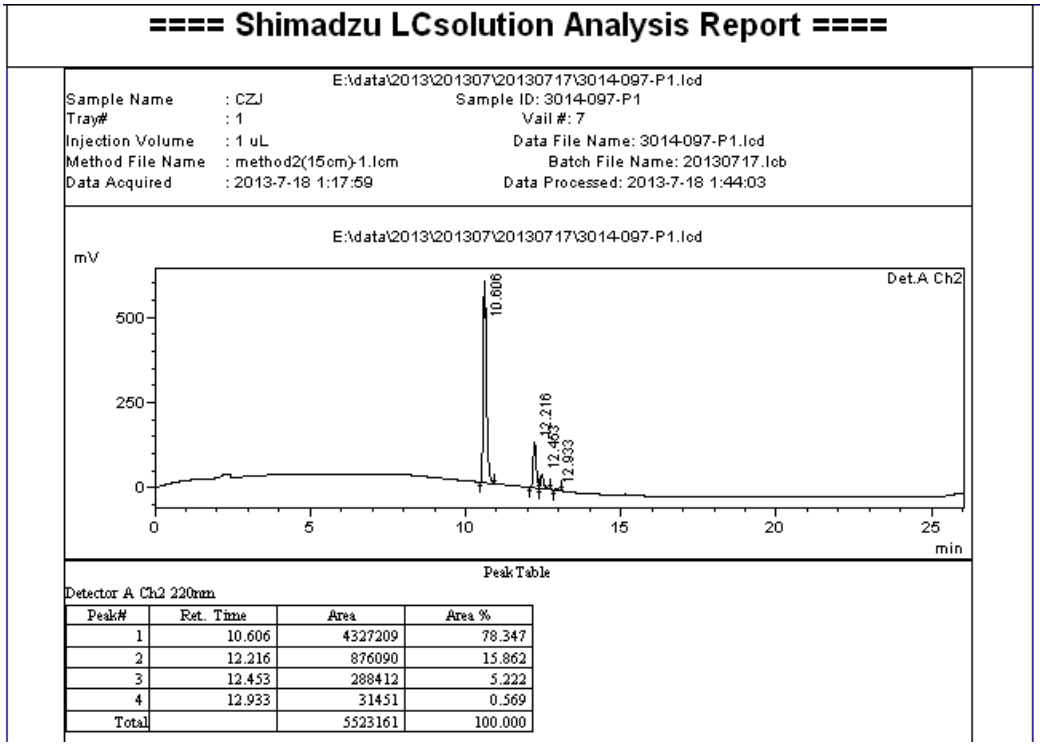


To a suspension of $LiAlH_4$ (1.72 g, 44.0 mmol, 4 equiv.) in THF was added a solution of **12-2** (3.0 g, 11.0 mmol, 1.0 equiv.) dropwise at RT over ~40 min and the resulting mixture was stirred for one h. TLC analysis indicated the consumption of intermediate **12-2**. Water (4.5 mL) was added slowly to the reaction mixture, followed by 15% KOH aqueous solution (4.5 mL) and water (13.5 mL). The resulting solid was filtered off and the filter cake was washed by THF. The filtrate was concentrated to dryness and re-dissolved by EtOAc (2 x 20 mL). The EtOAc solution was back washed by brine (2 x 20 mL), dried and concentrated to give crude product **13-4** (2.68 g) in 78% purity by HPLC. The crude **13-4** was directly used for the next step without further purification. ^{1}H-NMR (300Hz, $CDCl_3$) δ 6.37 (s, 3H), 3.79 (s, 6H), 3.77-3.74 (m, 4H), 2.82-2.73 (m, 2H), 2.63-2.58 (m, 2H), 2.54-2.53 (m, 4H). The analytical data are attached below.

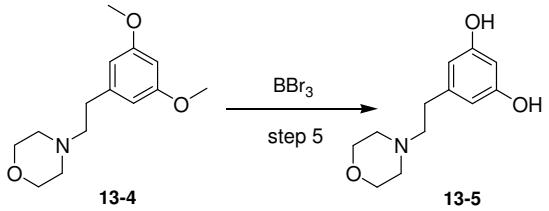
^{1}H-NMR spectrum of crude intermediate **13-4**



HPLC chromatogram of crude intermediate **13-4**

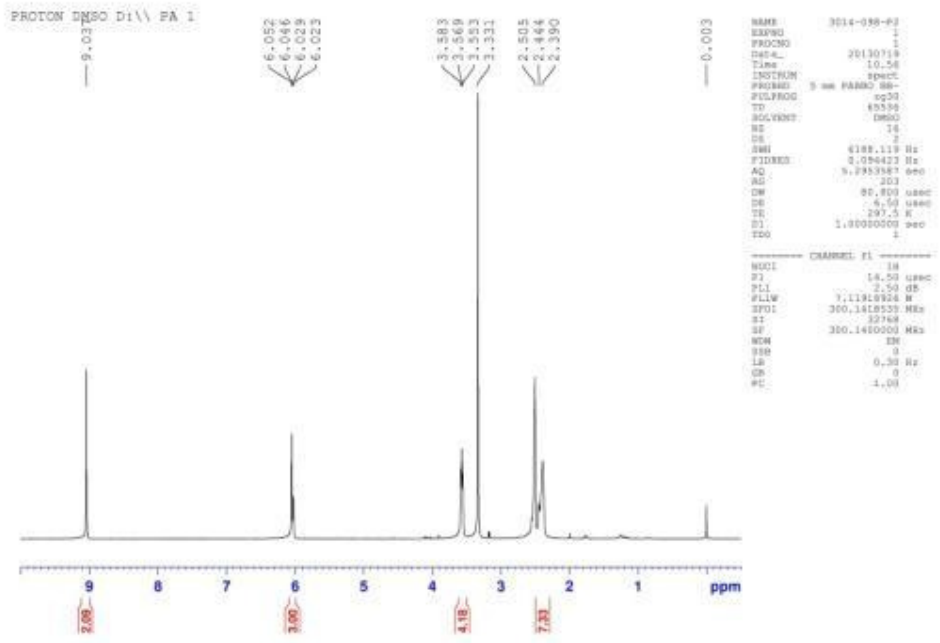


Step 5

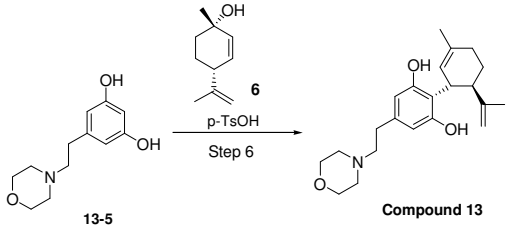


To a solution of crude intermediate **13-4** (2.68 g, 78% HPLC purity, 11.0 mmol, 1 equiv.) in DCE (322 mL) was added a solution of BBr_3 (21.35 g, 85.0 mmol, 8 equiv.) in DCE (24 mL) at 0 °C over 40 min. The resulting mixture was stirred at 0 °C for 2 hours. TLC analysis indicated the completion of the reaction. Saturated Na_2CO_3 solution was added to adjust the pH to ~7 and extracted with EtOAc/THF (1:1) mixture (4 x 200 ml). The organic phase was washed by brine (2 x 500 mL), dried over Na_2SO_4 and concentrated to give crude **13-5** (2.25 g), which was subsequently slurried in MeOH (10 mL) for one h. The suspension was filtered to give intermediate **13-5** (1.25 g, yield 50%). ^{1}H-NMR (300Hz, DMSO-*d*6) δ 9.04 (s, 2H), 6.03 (m, 3H), 3.58-2.55 (m, 4H), 2.51-2.44 (m, 8H). The analytical data is attached below.

^{1}H-NMR spectrum of the intermediat**e 13-5**



Step 6: synthesis of compound **13**



To the suspension of intermediate **13-5** (450 mg, 2.0 mmol, 1.0 equiv.) in DCM/THF mixture (1:1, 45 mL) was slowly added BF_3-Et_2O (0.57 g, 4.0 mmol, 2 equiv.) and reagent **6** (0.31 g, 2.0 mmol, 1.0 equiv.) in order. The reaction mixture was stirred for 30 min and TLC analysis showed around 60% conversion of the intermediate **13-5.** The reaction was stopped at this point by adding saturated $NaHCO_3$ solution to adjust the pH to 7. The mixture was extracted by EtOAc (3 x 50 mL). The organic layer was back washed by brine (2 x 100 mL), dried and concentrated to give crude product (750 mg), which was combined with another batch of same scale and slurried in DCM (10 mL). The solid was filtered off to give intermediate **13-5** (290 mg). The filtrate was concentrated to dryness and the residue was purified by column chromatography (eluent: EtOAc/Hexane = 1/1) to provide the

4-regio-isomer of the desired compound **13** (350 mg, HPLC purity 94%) and the desired compound **13** (245 mg) with only 58% HPLC purity. The obtained compound **13** was further purified by preparative TLC to provide pure desired compound **13** (75 mg, yield 5.2%). The analytical data are attached below.

Analytical data of compound **13**

HPLC: 95%. LC/MS (ESI): m/z 358 (M+1). ^{1}H-NMR（300 MHz, $CDCl_3$）δ 6.25 (br s, 2H), 5.57 (s, 1H), 4.68 (s, 1H), 4.54 (s, 1H), 3.97-3.93 (m, 1H), 3.77-3.74 (m, 4H), 2.69-2.58 (m, 4H), 2.55-2.52 (m, 4H), 2.43-2.41 (m, 1H), 2.41-2.21 (m, 2H), 2.14-2.09 (m, 1H), 1.90-1.88 (m, 1H), 1.81 (s, 3H), 1.71 (s, 3H).

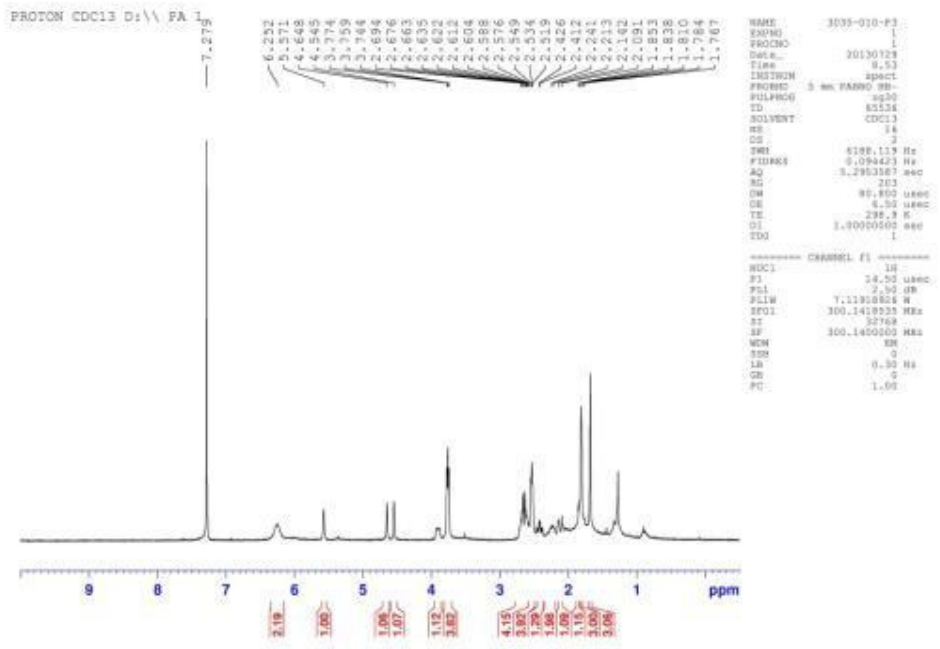
Analytical data of the 4-regio-isomer of compound **13**

HPLC: 94%. LCMS (ESI): m/z 358 (M+1). H-NMR（300MHz, $CDCl_3$）δ 6.24 (s, 2H), 6.08 (s, 1H), 5.53 (s, 1H), 4.68 (s, 1H), 4.50 (s, 1H), 3.78-3.75 (m, 4H), 3.53-3.50 (m, 1H), 2.85-2.81 (m, 1H), 2.58-2.42 (m, 8H), 2.24-2.02 (m, 1H), 1.88-1.81 (m, 1H), 1.77-1.71 (m, 1H), 1.761 (s, 6H).

NOTES:

1. Other catalysts, such TfOH, p-TsOH, HOAc and TFA, have also been evaluated. None of them gave satisfactory results. The reaction was messy or had low conversions of SM **13-5**.

[1]H-NMR of compound **13**

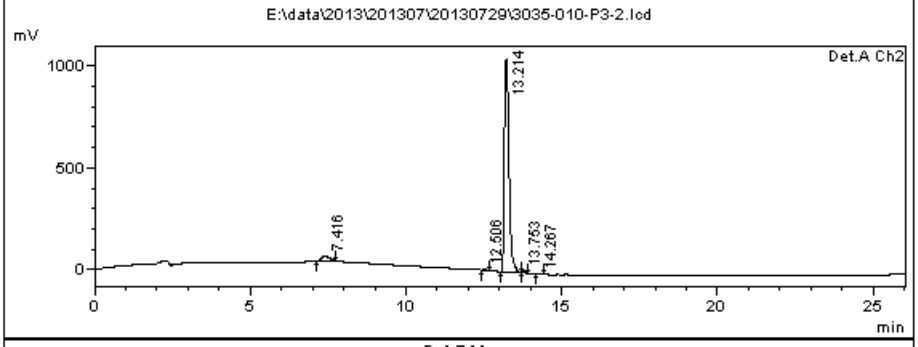


HPLC chromatogram of compound **13**

==== Shimadzu LCsolution Analysis Report ====

E:\data\2013\201307\20130729\3035-010-P3-2.lcd

Sample Name : CZJ
Sample ID: 3035-010-P3-
Tray# : 1
Vail #: 3
Injection Volume : 7 uL
Data File Name: 3035-010-P3-2.lcd
Method File Name : method2(15cm)-1.lcm
Batch File Name: 20130729.lcb
Data Acquired : 2013-7-29 9:49:26
Data Processed: 2013-7-29 13:22:44

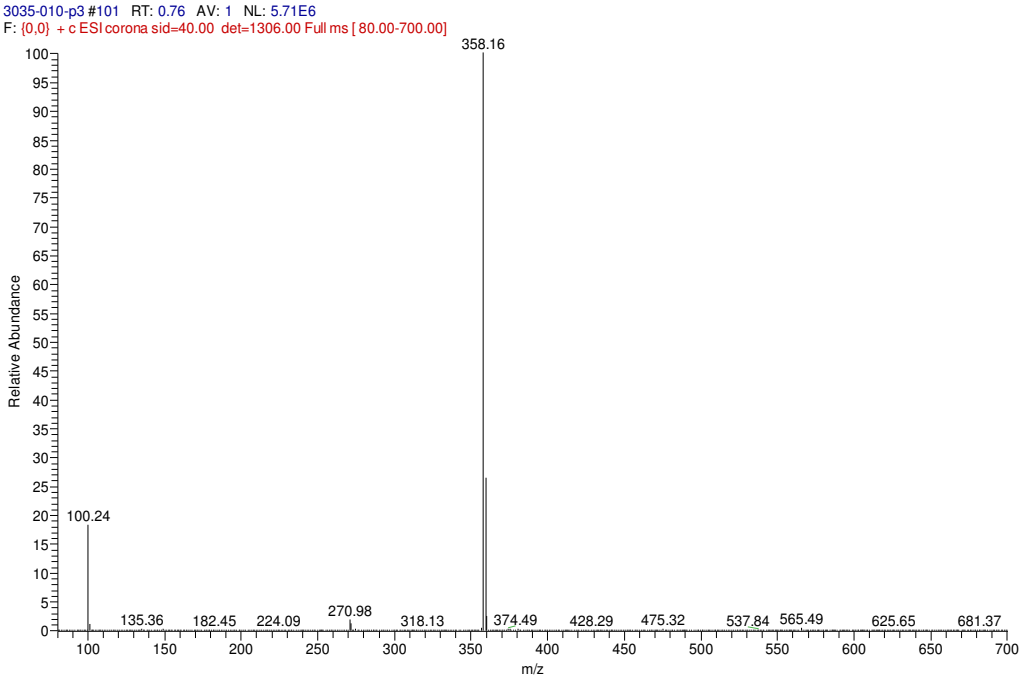


Peak Table

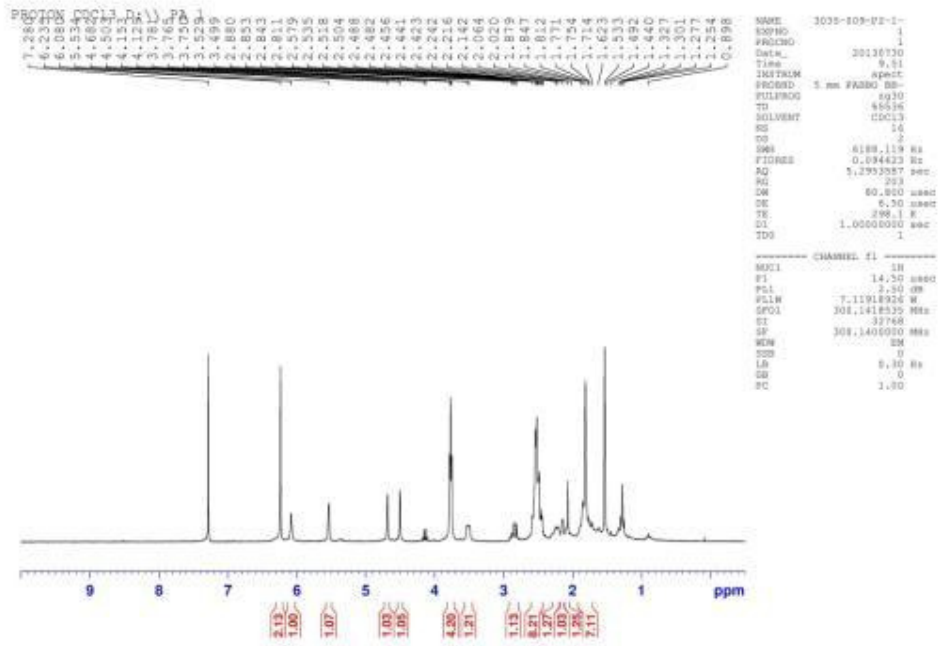
Detector A Ch2 220nm

Peak#	Ret. Time	Area	Area %
1	7.416	427662	3.736
2	12.506	37741	0.330
3	13.214	10912746	95.343
4	13.753	44796	0.391
5	14.267	22786	0.199
Total		11445731	100.000

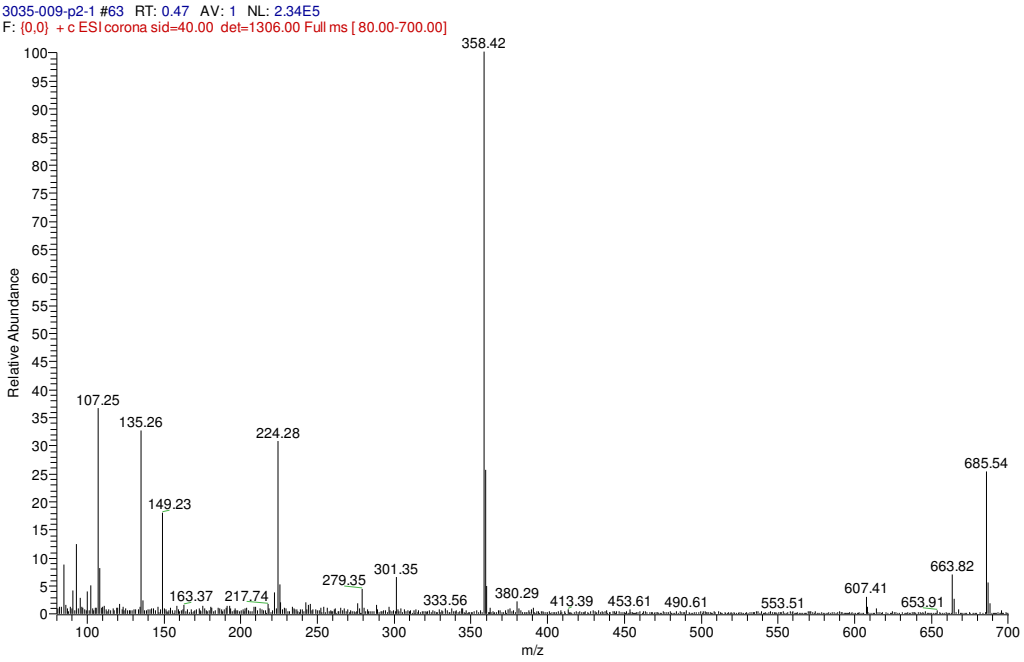
LC/MS of compound **13**



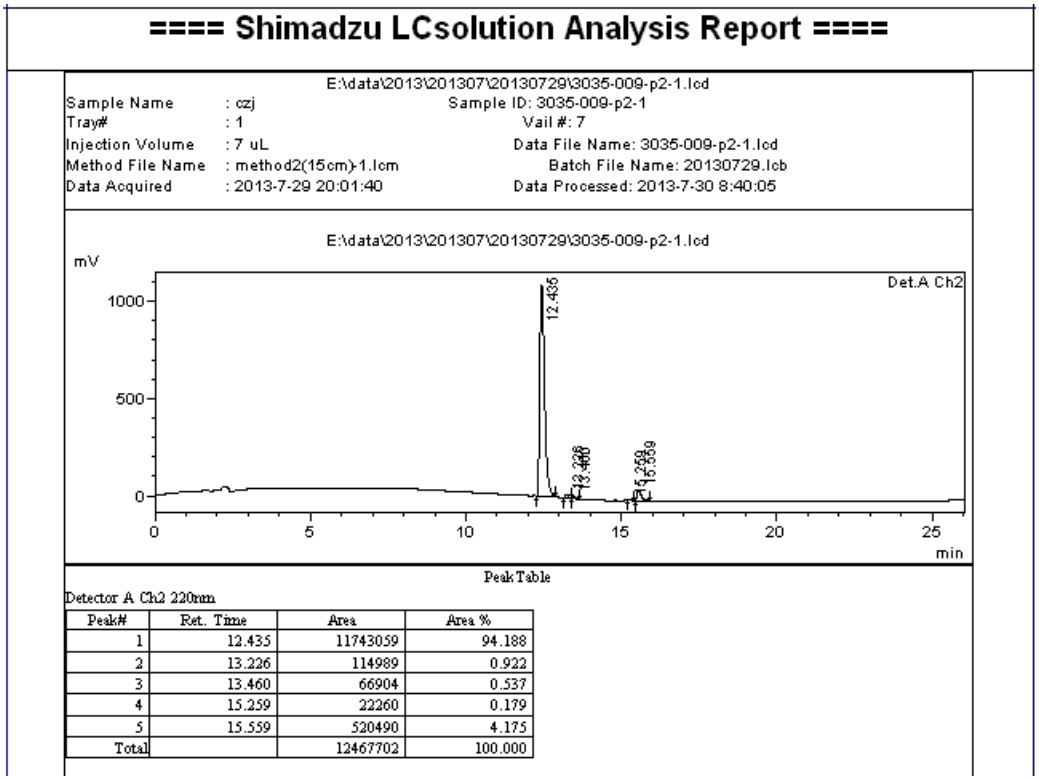
^{1}H-NMR spectrum of the 4-regio-isomer of compound **13**



LC/MS of the 4-regio-isomer of compound **13**



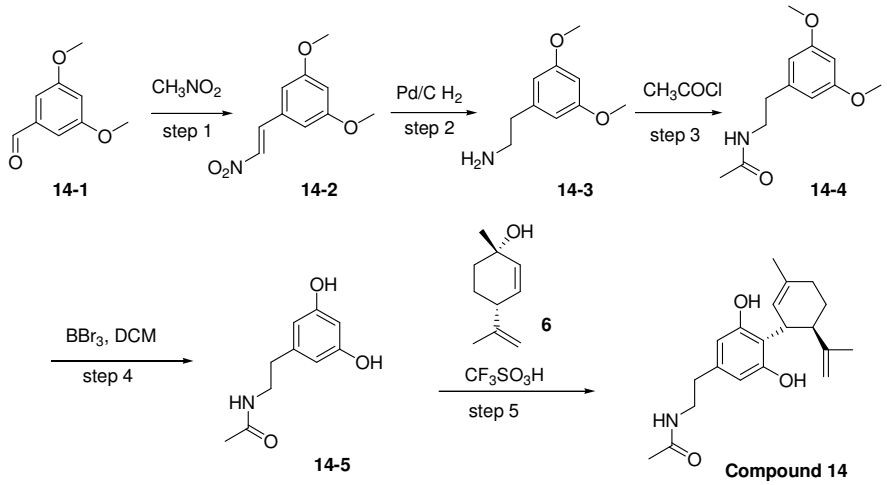
HPLC chromatogram of the 4-regio-isomer of compound **13**



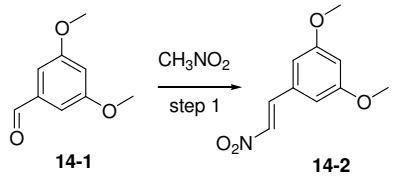
Peak#	Ret. Time	Area	Area %
1	12.435	11743059	94.188
2	13.226	114989	0.922
3	13.460	66904	0.537
4	15.259	22260	0.179
5	15.559	520490	4.175
Total		12467702	100.000

Synthesis of Compound 14

Synthesis Scheme for Compound 14:

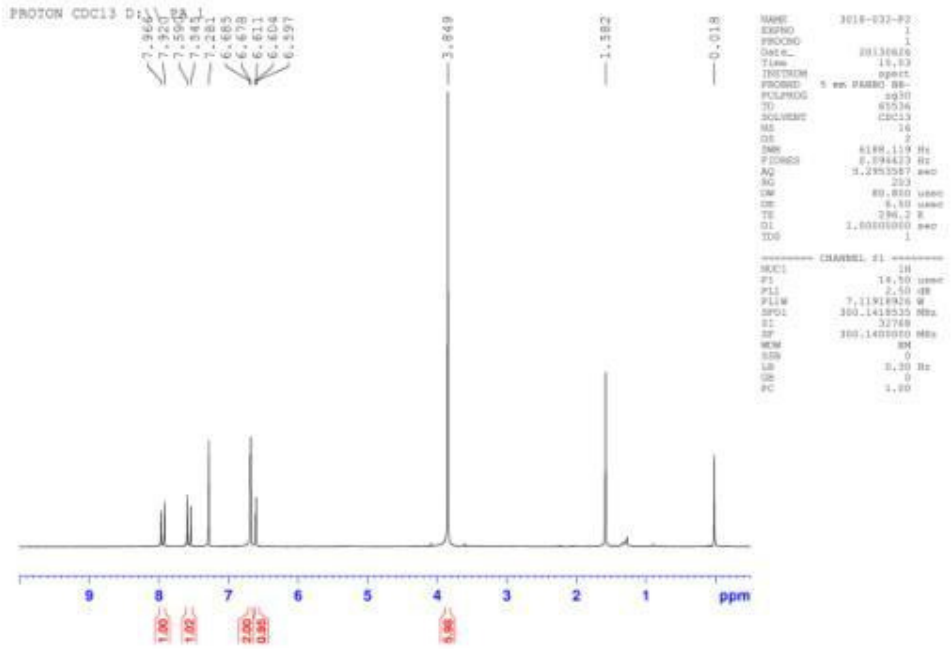


STEP 1:

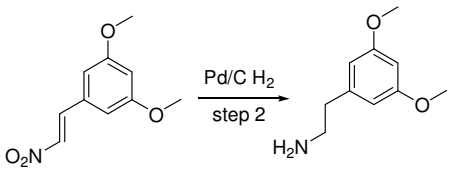


A suspension of **14-1** (6.0 g, 36 mmol, 1.0 equiv.), CH_3NO_2 (11 g, 180 mmol, 5.0 equiv.) and $CH_3CO_2NH_4$ (8.35 g, 180 mmol, 5.0 equiv.) in CH_3CO_2H (180 mL) was heated to 90 ^oC and stirred for overnight. After the completion of the reaction by ^1H-NMR analysis, the reaction was concentrated to dryness. The residue was adjusted pH to 9 by the addition of aqueous K_2CO_3 and then extracted with DCM (3 x 150 mL). The combined organic layer was dried over Na_2SO_4, filtered, and concentrated to give crude product (**14-2**), which was purified by column chromatography to give the pure product **14-2** (4.7 g). ^1H-NMR (300MHz, $CDCl_3$) δ 7.95 (d, J = 15 Hz, 1H), 7.57 (d, J = 12 Hz, 1H), 6.69 (d, J = 3 Hz, 2H), 6.61 (t, J = 3 Hz, 1H), 3.85 (s, 6H). The analytical data is attached below.

^{1}H-NMR spectrum of intermediate **14-2**



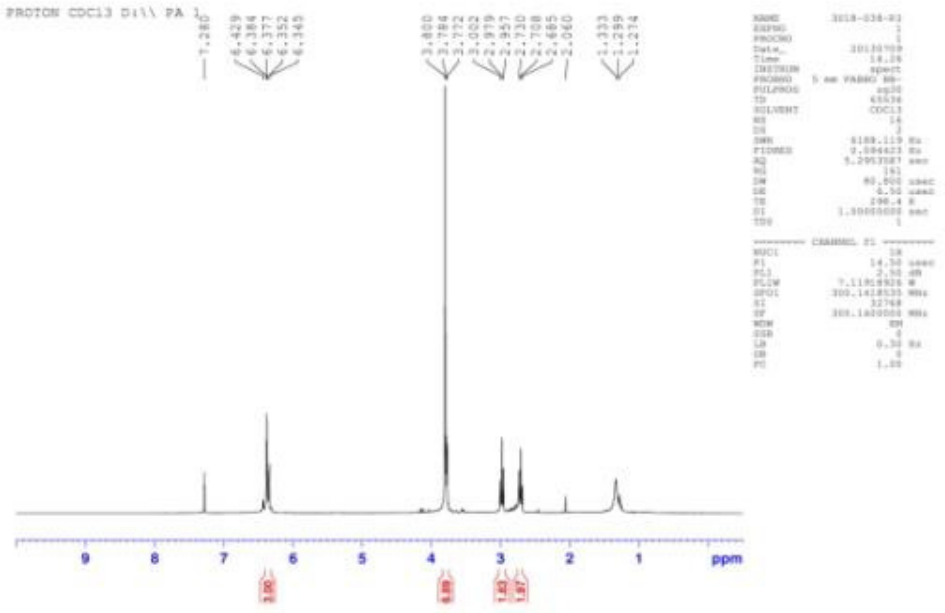
STEP 2:



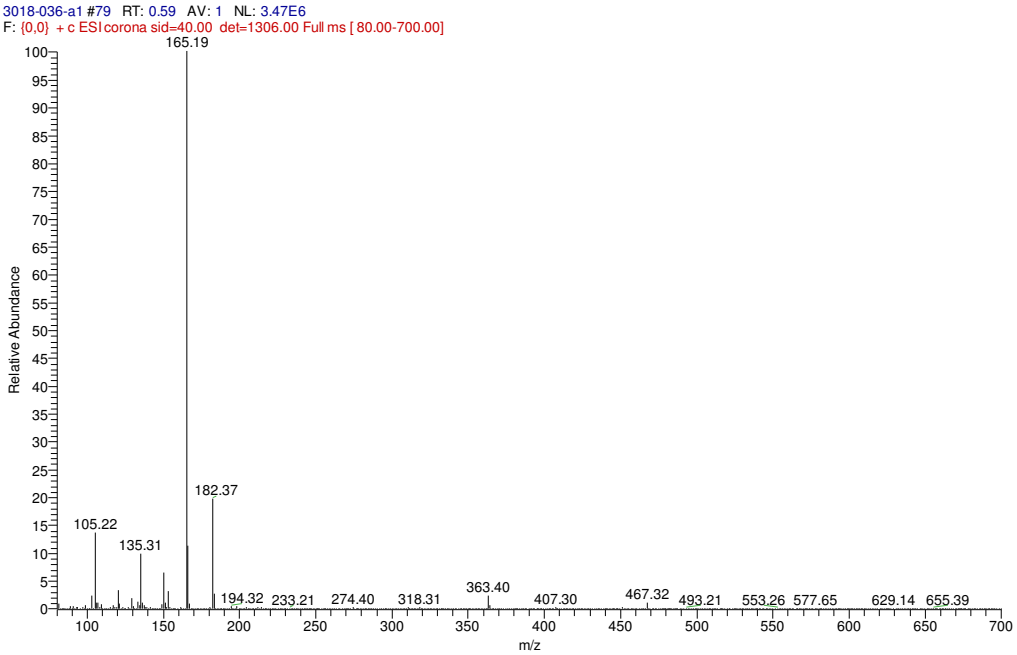
14-2 **14-3**

To a solution of **14-2** (3.4 g, 16 mmol, 1.0 equiv.) in dry THF (60 mL) was added $LiAlH_4$ (2.5 g, 65 mmol, 4.0 equiv.) in portions over 20 mins at 0 ^oC. After addition, the suspension was stirred at 50 ^oC for 6 days. After the completion of the reaction as indicated by TLC, the suspension was cooled to 0 ^oC. Water (2.5 mL) was added dropwise to the suspension under N_2 at the rate of keeping the temperature below 15 ^oC. Then 15% aq. NaOH (2.5 mL) and water (2.5 mL) were added in orders to the suspension. The suspension was filtered, and the filtrate was concentreated. The residue was purified by column chromatography to give the desired product **14-3** (1.6 g, 54% yield). LC/MS (ESI): m/z 182 (M+H). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 6.43-6.35 (m, 3H), 3.80 (s, 6H), 3.00-2.96 (t, J = 6.6 Hz, 2H), 2.73-2.69 (t, J = 6.6 Hz, 2H). The analytical data are attached below.

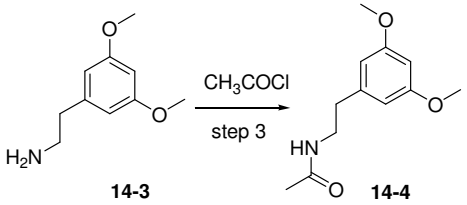
^{1}H-NMR spectrum of intermediate **14-3**



LC/MS of intermediate **14-3**



STEP 3:



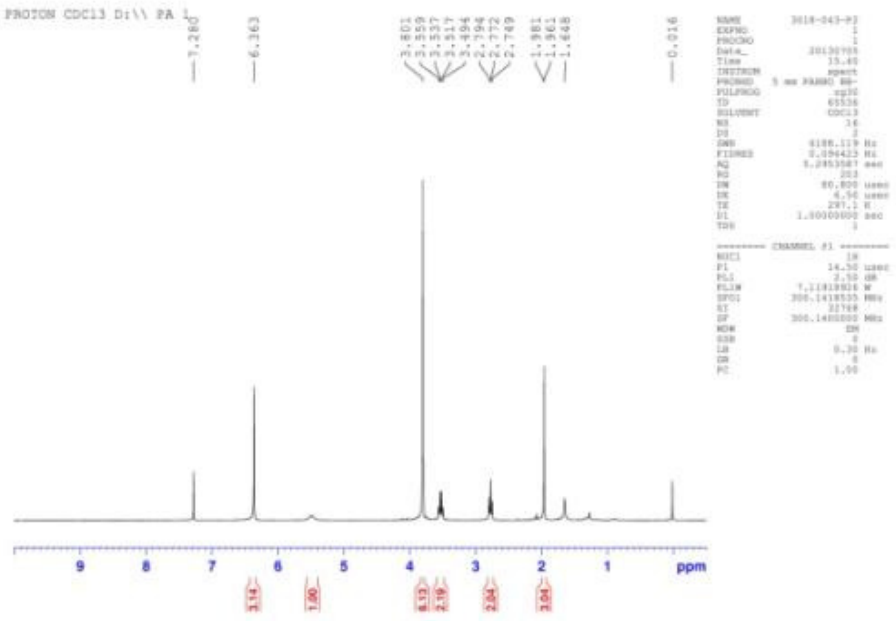
14-3 **14-4**

A solution of **14-3** (500 mg, 2.76 mmol, 1.0 equiv.) and TEA (279 mg, 2.76 mmol, 1.0 equiv.) in DCM (50 mL) was added acetyl chloride (325 mg, 4.1 mmol, 1.5 eq) over 5 mins at 0 ^{o}C under N_2 atmosphere. After completion of the reaction by TLC, the reaction mixture was concentrated to dryness and purified by column chromatography to give 600 mg of the desired product **14-4**. LC/MS (ESI): m/z 224 (M+H). ^{1}H-NMR (300MHz, $CDCl_3$) δ 6.36 (s, 3H), 3.80 (s, 6H), 3.56-3.49 (m, 2H), 2.79-2.75 (m, 2H), 1.96 (s, 3H). Analytical data is attached below.

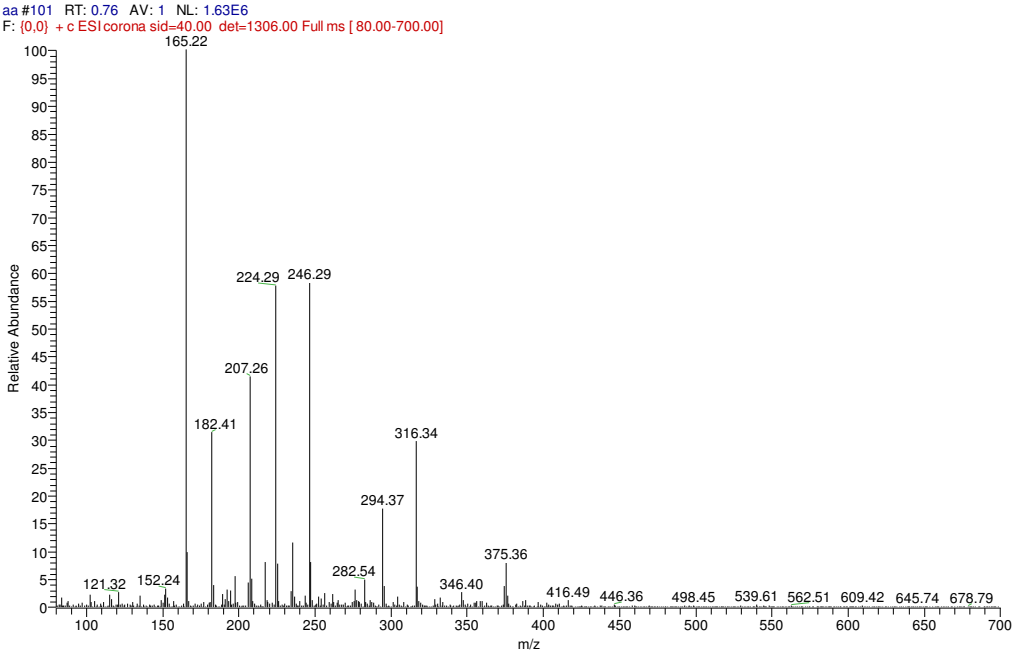
Batch summary of step 3

Batch #	SM (**14-3**)	Prodcut (**14-4**)	purity
3018-043	500 mg	600 mg	^{1}H-NMR: OK
3018-045	1.6 g	1.3 g	^{1}H-NMR: OK

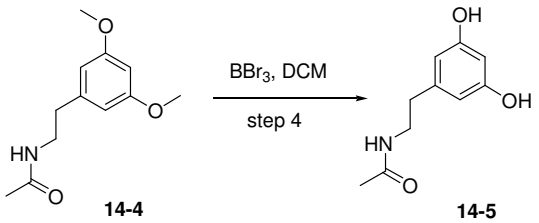
[1]H-NMR spectrum of intermediate **14-4**



LC/MS of intermediate **14-4**

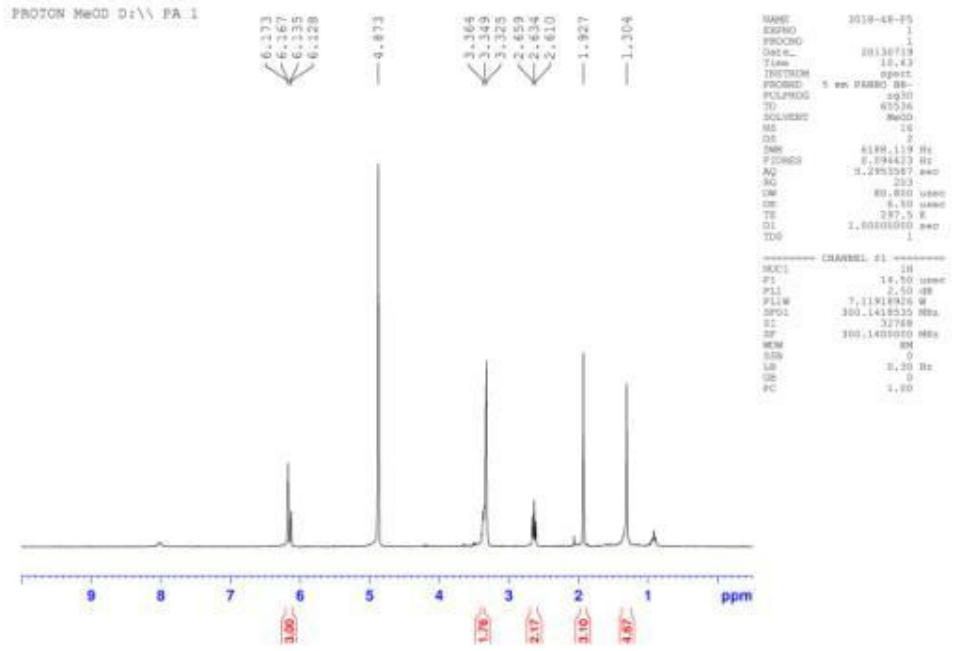


STEP 4

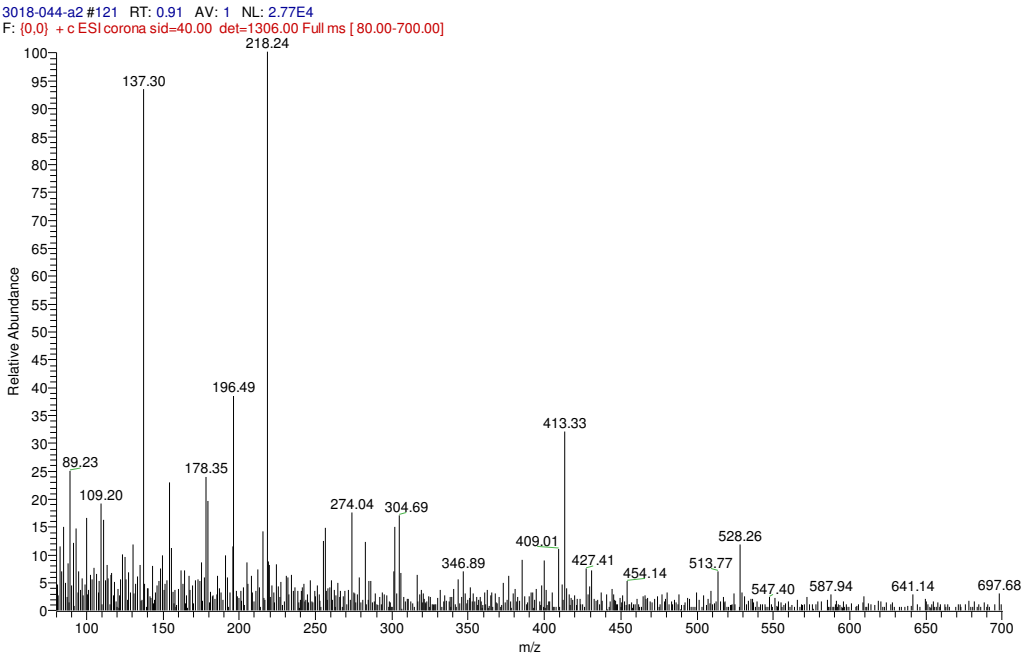


To a solution of **14-4** (1.3 g, 5.8 mmol, 1.0 equiv.) in DCM (50 mL) under N_2 atmosphere was added BBr_3 (14.6 g, 58 mmol, 10 equiv.) at 0 ^oC over 10 min. After the addition, the reaction mixture was allowed to warm to RT and stirred overnight. After the completion of the reaction by TLC, the reaction was cooled to 0 ^oC and quenched with water (50 mL) over 10 min. The organic phase was separated and the aqueous phase was extracted with DCM (15 x 100 mL). The combined organic phase was dried over Na_2SO_4, concentrated and purified by column chromatography to give the desired product **14-5** (1.1 g, 97% yield). LC/MS (ESI): m/z 196 (M+1). ^{1}H-NMR (300MHz, $CDCl_3$) δ 6.17-6.13 (m, 3H), 3.36-3.33 (t, J = 7.2 Hz, 2H), 2.64 (t, J = 7.2 Hz, 2H), 1.93 (s, 3H). The analytical data are attached below.

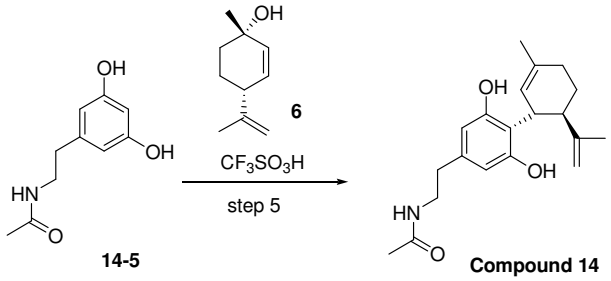
[1]H-NMR spectrum of intermediate **14-5**



LC/MS of intermediate **14-5**



STEP 5

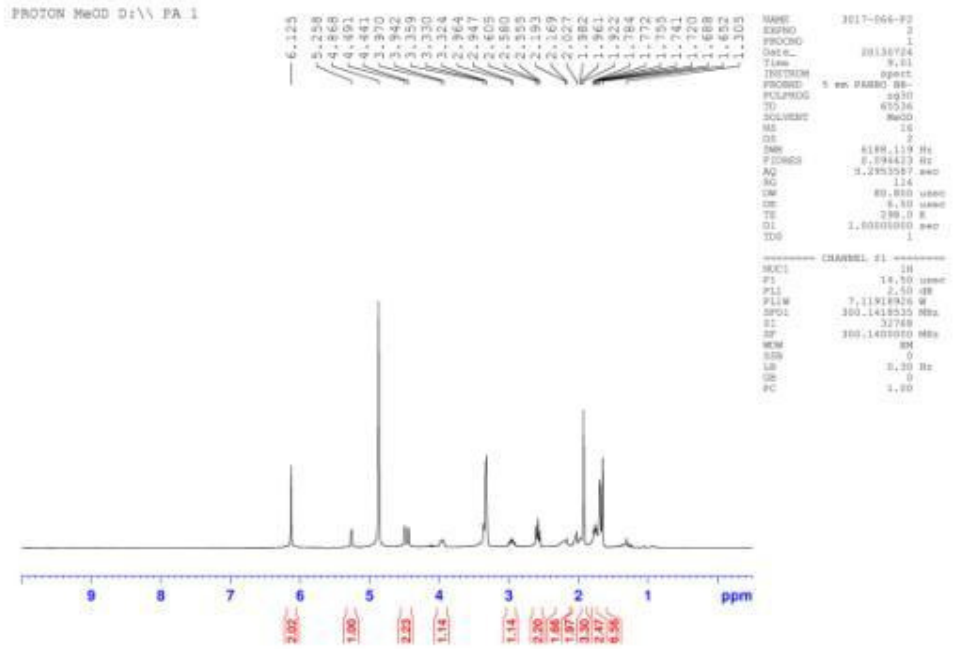


To a solution of **14-5** (500 mg, 2.56 mmol, 1.0 equiv.) and CF_3SO_3H (382 mg, 2.56 mmol, 1.0 equiv.) in a mixed solvent of THF/DCM (1:3, 40 mL) was added the solution of **6** (390 mg, 2.56 mmol, 1.0 equiv.) in DCM (5 mL) over 5 min. The mixture was stirred at RT for about 30 min and TLC analysis indicated the 30-40% conversion of the starting material **14-5**. The reaction was stopped at that point by adding aqueous $NaHCO_3$ solution to quench the reaction and adjust the pH to 8-9. The resulting mixture was extracted by DCM (2 x 100 mL) and the combined organic phase was dried and concentrated to dryness. The residue was purified by column chromatography to provide 95 mg of impure compound **14.** The impure **14** was combined with another batch (112 mg of crude **14** from 300 mg of **14-5**), and further purified by preparative HPLC to give compound **14** (105 mg, 12.5% yield). HPLC purity: 96.9%. LC/MS (ESI): m/z 352.3 (M+Na). ^{1}H-NMR (300MHz, $CDCl_3$) δ 6.13 (s, 2H), 5.26 (s, 1H), 4.47 (d, J = 15.0 Hz, 2H), 3.95 (m, 1H), 2.96-2.93 (m, 1H), 2.61-2.56 (m, 2H), 2.03 (m, 2H), 1.98-1.96 (m, 2H), 1.96 (s, 3H), 1.78-1.74 (m, 2H), 1.69-1.65 (m, 6H). Analytical data ares attached below.

NOTES:

1. This reaction was messy by TLC, as with other analogs. It was better to quench the reaction before the SM **14-5** was consumed completely. The longer reaction time will result in more impurities, which make the purification of the desired compound more challenging.
2. The unreacted SM **14-5** could be recovered.
3. *p*-TsOH was also evaluated as the catalyst for this substrate and the reaction gave lower conversion of starting material **14-5** based on TLC analysis.

[1]H-NMR spectrum of compound **14**

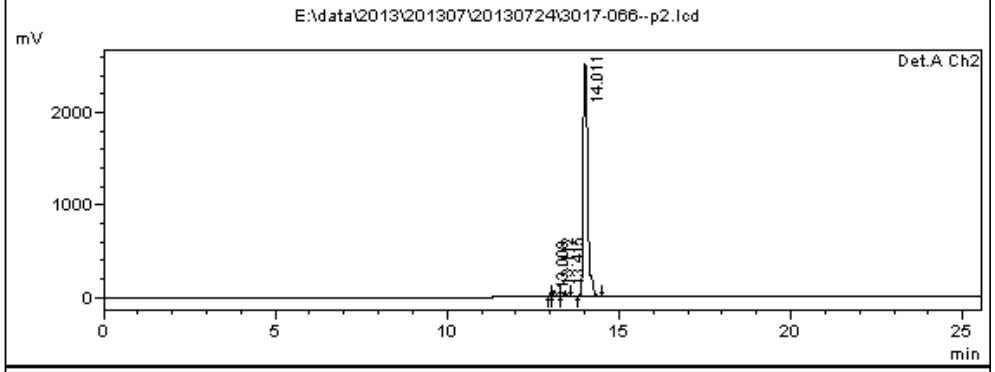


HPLC chromatogram of compound **14**

==== Shimadzu LCsolution Analysis Report ====

E:\data\2013\201307\20130724\3017-066--p2.lcd

Sample Name	: pld	Sample ID: 3017-066-p2-1	
Tray#	: 1	Vail #: 61	
Injection Volume	: 5 uL	Data File Name: 3017-066--p2.lcd	
Method File Name	: method1(15cm)-MeOH+H2O.lcm	Batch File Name: 20130724.lcb	
Data Acquired	: 2013-7-24 9:43:58	Data Processed: 2013-7-24 10:09:30	

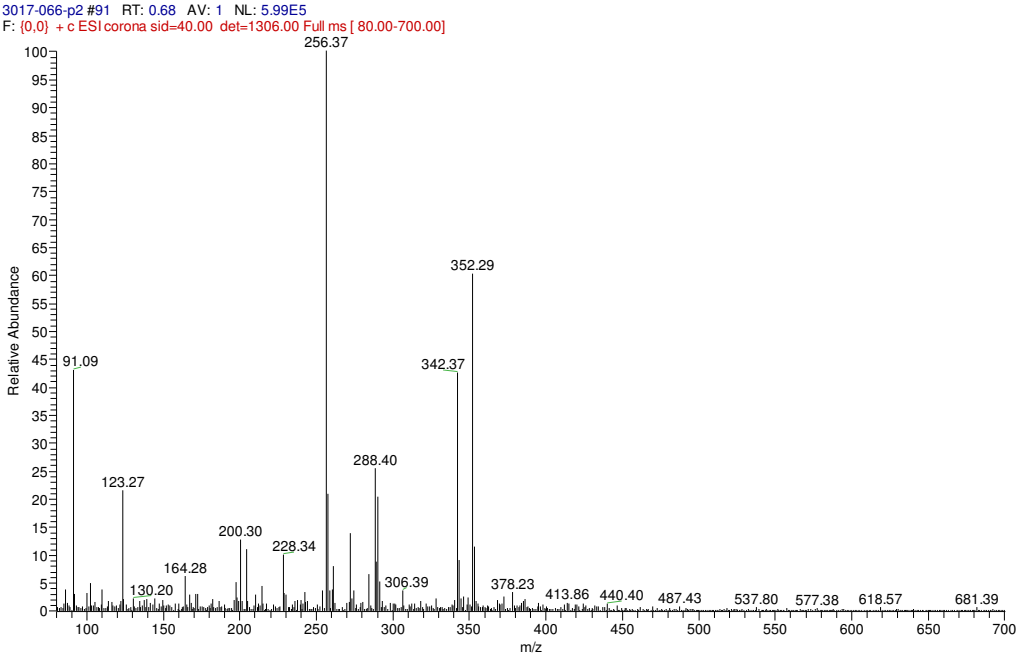


Peak Table

Detector A Ch2 220nm

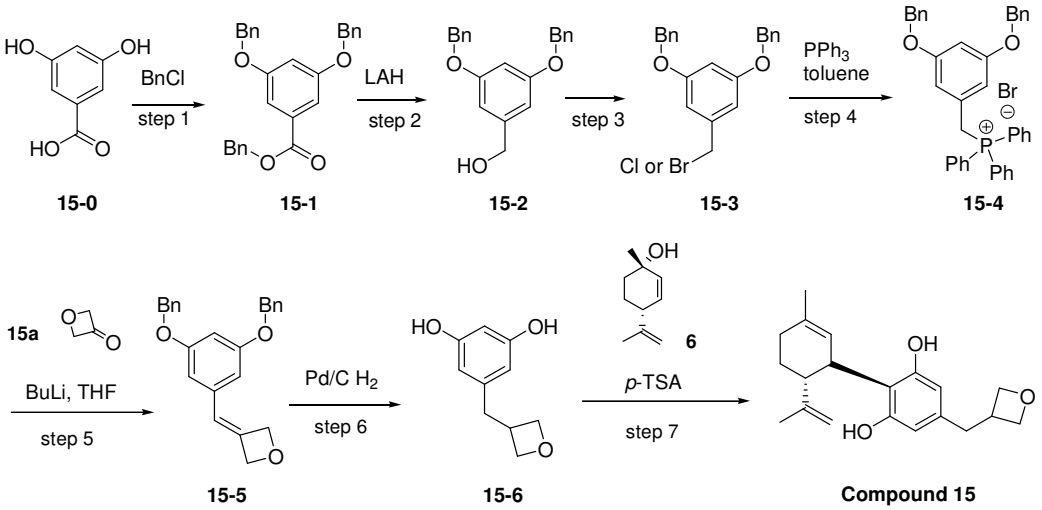
Peak#	Ret. Time	Area	Area %
1	13.008	76459	0.323
2	13.112	361583	1.528
3	13.415	287670	1.216
4	14.011	22931691	96.932
Total		23657402	100.000

LC/MS of of compound **14**

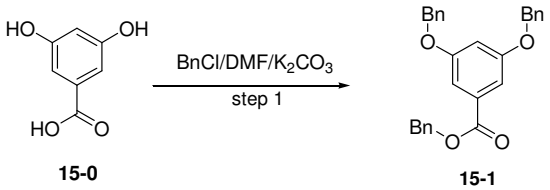


Synthesis of Compound 15

Synthesis Scheme for Compound 15



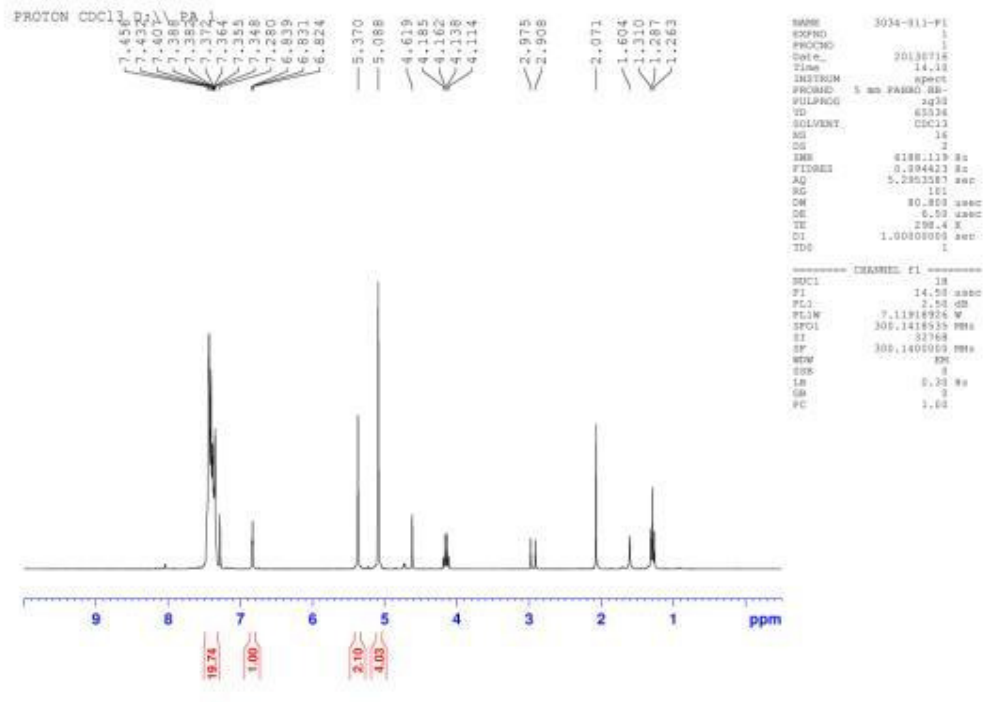
STEP 1:



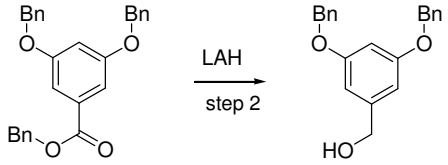
To a solution of 3,5-dihydroxybenzoic acid (**15-0**) (8.0 g, 52 mmol, 1.0 equiv.) in DMF (25 mL) was added K_2CO_3 (28.6 g, 210 mmol, 4 equiv.). The mixture was stirred at RT for 30 min. The solution of BnCl (21.6 g, 171 mmol, 3.3 equiv.) in DMF (25 mL) was added and the resulting suspension was stirred at 70 ^oC overnight. The progress of the reaction was monitored by TLC. After the SM was consumed, water (50 mL) was added to quench the reaction. The reaction mixture was extracted with EtOAc (3 x 50 mL), and the combined organic phase was washed with 10 % brine (3 x 50 mL), dried over Na_2SO_4, filtered and concentrated in vacuo to give crude product **15-1** (25 g, yield 113%) as brown solid, which was used for the next step without further purification.

^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.50-7.28 (m, 17H), 6.84 (s, 1H), 5.37 (s, 2H), 5.09 (s, 4H). The analytical data is attached below.

[1]H-NMR spectrum of intermediate **15-1**



STEP 2:



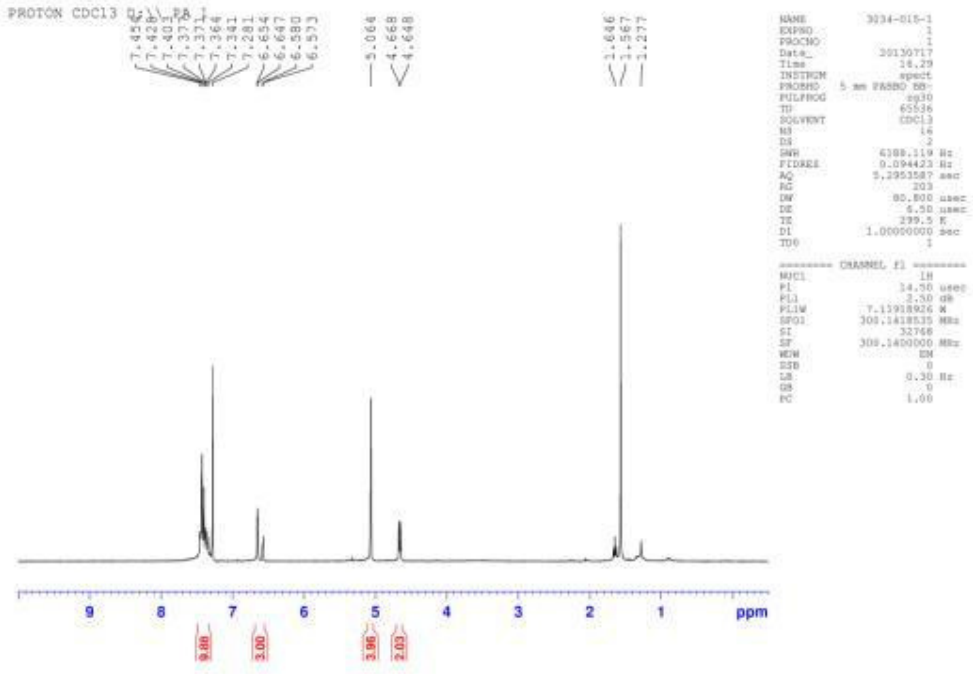
15-1 **15-2**

To a suspension of $LiAlH_4$ (14 g, 0.37 mol, 4 equiv.) in THF (100 mL), was added the solution of **15-1** (39 g, 0.092 mol, 1 equiv.) in THF (100 mL) over 20 min and the resulting mixture was stirred at RT for one hour. TLC analysis showed the completion of the reaction. To the reaction mixture was then slowly added water (40 mL), 15% KOH aqueous solution (40 mL), and water (120 mL) in order. The resulting solid was filtered off. The organic phase was separated off and the aqueous layer was extracted by EtOAc (100 mL). The combined organic layer was dried, concentrated, and purified by column chromatography (EtOAc/Hexane = 1/8) to give the desired product **15-2** (18 g, yield 61%).

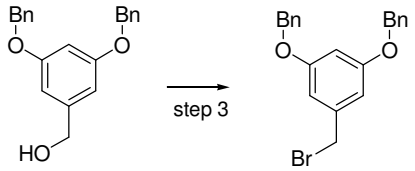
[1]H-NMR (300 MHz, $CDCl_3$) δ 7.50-7.30 (m, 10H), 6.66-6.50 (m, 3H), 5.06 (s,

4H), 4.65 (d, J = 6.0 Hz, 2H). The analytical data is attached below.

^{1}H-NMR spectrum of intermediate **15-2**



STEP 3:



15-2 **15-3**

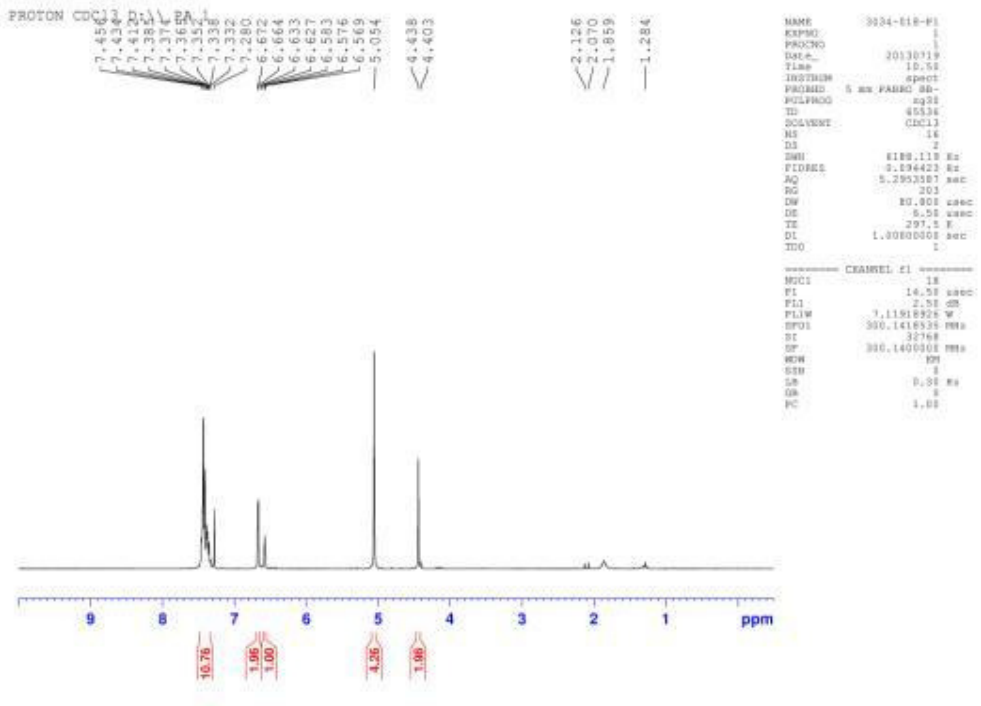
To a solution of **15-2** (18 g, 56 mmol, 1.0 equiv.) in CH_3CN (100 mL) was added the solution of PBr_3 (22.8 g, 84 mmol, 1.5 equiv.) in CH_3CN (50 mL) dropwise at 0 to 5 °C. After the addition, the reaction mixture was continued stirring at 0-5 °C for 2 h. Water (50 mL) was added over 30 min and the resulting solid was filtered, re-dissolved in EtOAc (100 mL), washed by brine (100 mL), dried and concentrated to obtain crude product **15-3** (17 g, yield 78%).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.45-7.33 (m, 10H), 6.67 (s, 2H), 6.58 (s, 1H), 5.05 (s, 4H), 4.44 (s, 2H). The analytical data is attached below.

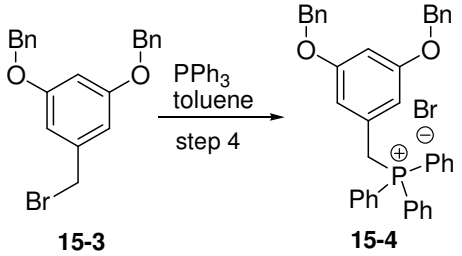
NOTES:

1. During the addition of the water to quench the reaction, a vigorous stirring could improve the quality of the solid for easy filtration.

^{1}H-NMR spectrum of intermediate **15-3**



STEP 4:

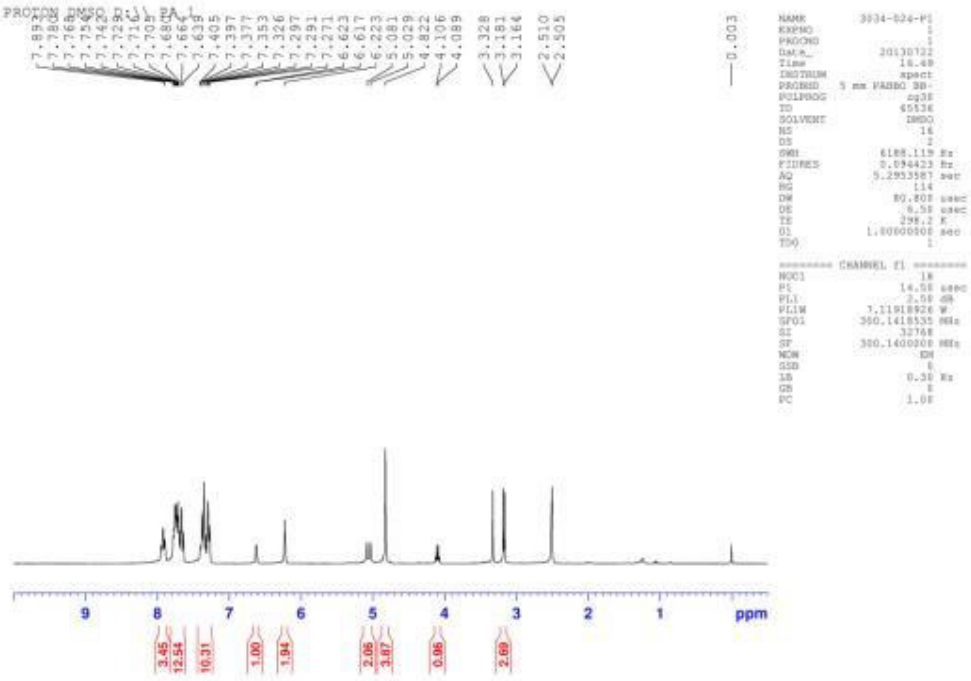


A solution of **15-3** (16 g, 42 mmol, 1.0 equiv.) and triphenylphosphine (12 g, 46 mmol, 1.1 equiv.) in toluene (100 mL) was refluxed for 3-4 h. The starting material **15-3** was completely consumed as indicated by TLC. The reaction mixture was cooled to RT and the solid was collected by filtration. The solid was sonicated in methanol/petroleum ether (1:20, 220 mL) for one hour, filtered and the filter cake was

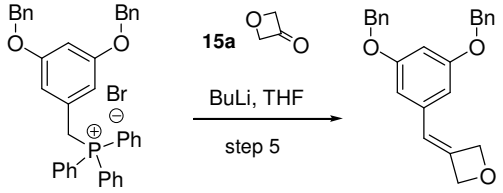
washed by petroleum ether (3 x 20 mL) to give product **15-4** (22 g, yield 81%) as s white solid.

^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.89-7.78 (m, 3H), 7.77-7.68 (m, 12H), 7.40-7.21 (m, 10H), 6.62 (s, 1H), 6.22 (s, 2H), 5.08 (s, 1H), 5.03 (s, 1H), 4.82 (s, 4H). The analytical data is attached below.

H-NMR spectrum of intermediate **15-4**



STEP 5:

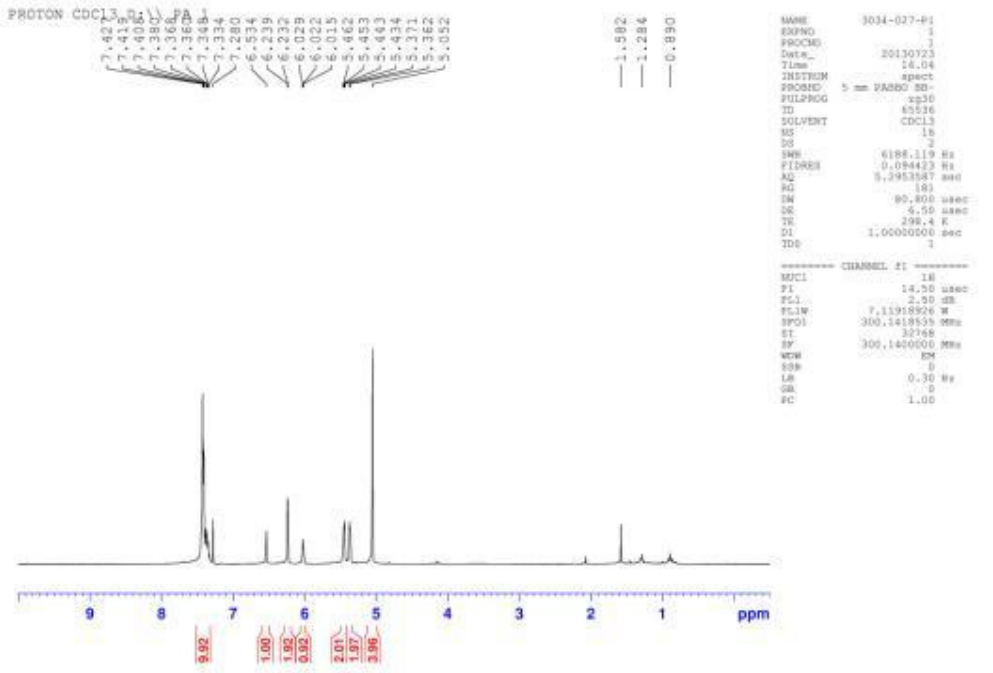


15-4 **15-5**

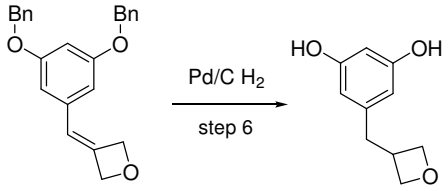
To a suspension of **15-4** (3.35 g, 5.2 mmol, 1.5 equiv.) in anhydrous THF (30 mL) was added *n*-BuLi (2.5 M in THF, 2.2 mL, 5.6 mmol, 1.6 equiv.) dropwise at 0 °C. After continued stirring for 20 min, the solution of **15a** (0.25 g, 3.5 mmol, 1.0 equiv.) in dry THF (10 mL) was slowly added dropwise. After the addition, the cold bath was removed and the reaction mixture was stirred at RT for one h. TLC analysis showed the consumption of **15a**. Water (50 mL) was added to quench the reaction, which was extracted with EtOAc (3 x 40 mL). The combined organic layers were dried, concentrated, and purified by column chromatography (EtOAc/Hexane = 1/10) to give pure product **15-5** (0.70 g, yield 56%).

^{1}H-NMR (300 MHz, CDCl3) δ 7.42-7.33 (m, 10H), 6.53 (s, 1H), 6.23 (d, J = 2.1 Hz, 2H), 6.02 (m, 1H), 5.45 (m, 2H), 5.37 (m, 2H), 5.05 (s, 4H). The analytical data is attached below.

^{1}H-NMR spectrum of intermediate **15-5**



STEP 6:

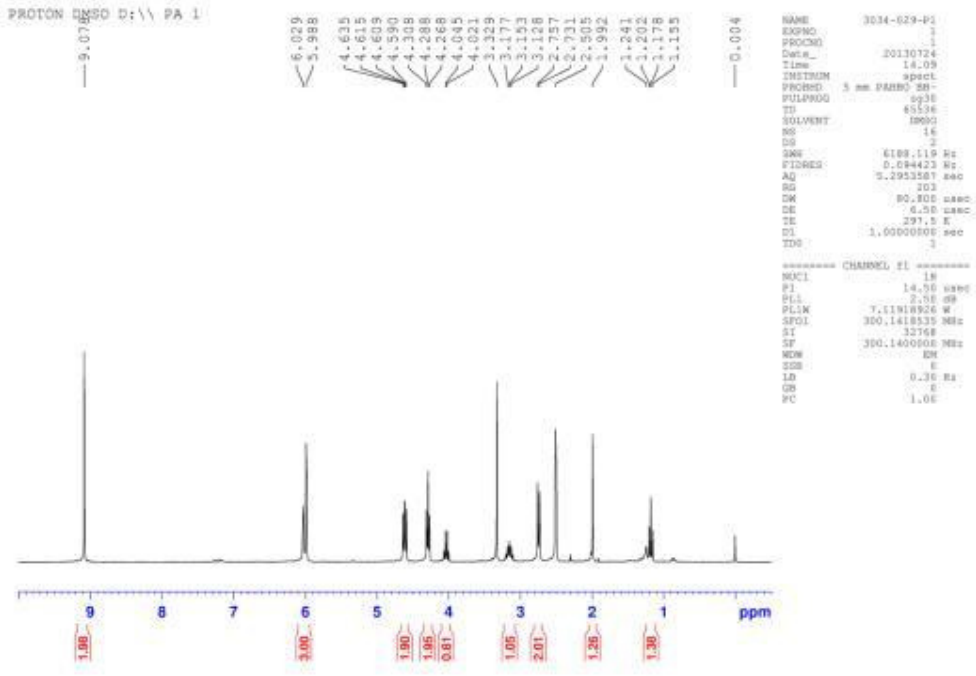


15-5 **15-6**

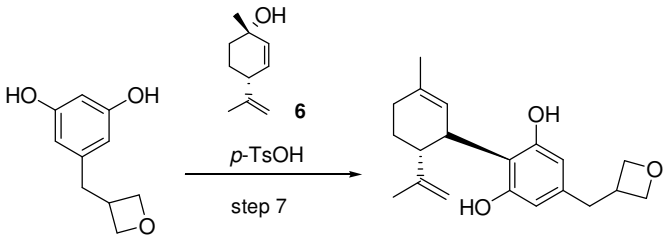
After purging with nitrogen, the suspension of **15-5** (0.7 g, 2.2 mmol) and 10% Pd/C (0.7g) in EtOAc (100 mL) was stirred under hydrogen (balloon) at RT for 4 h. TLC indicated the consumption of **15-5**. The suspension was filtered through Celite and concentrated to give crude **15-6** (0.40 g, yield 114%), which was directly used for the next step without further purification.

^{1}H-NMR (300 MHz, $CDCl_3$) δ 9.07 (s, 2H), 6.02-5.99 (m, 3H), 4.63-4.59 (m, 2H), 4.29 (t, J = 6.0 Hz, 2H), 3.18-3.13 (m, 1H), 2.75-2.73 (m, 2H). The analytical data is attached below.

^{1}H-NMR spectrum of intermediate **15-6**



STEP 7:



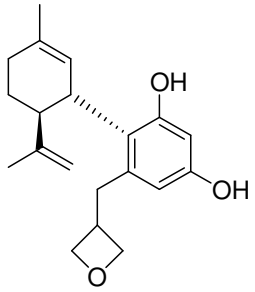
15-6 **Compound 15**

To a suspension of **15-6** (0.35 g, 2.0 mmol, 1.0 equiv.) in $CHCl_3$ (35 mL) was added *p*-TsOH (70 mg, 0.4 mmol, 0.2 equiv.) and **6** (0.29 g, 2.0 mmol, 1.0 equiv.) and the resulting mixture was stirred at RT for 10 min. Saturated aqueous $NaHCO_3$ was added to quench the reaction and to adjust the pH to 9~10. The organic phase was separated and the aqueous phase was extracted with DCM (2 x 20 mL). The combined organic phase was dried over Na_2SO_4, filtered, concentrated, and purified by column chromatography (EtOAc/Hexane = 1/5) to give the desired compound **15** (90 mg) and its 4-regio-isomer (60 mg). Both products were further purified by preparative HPLC

to give 64 mg (10%) of the desired compound **15** and 26 mg (4%) of its 4-regio-isomer.

Analytical data of the compound **15**: HPLC purity: 99%. LC/MS (ESI): m/z 315 (M+H), m/z 337 (M+Na). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 6.30-6.00 (m, 3H), 5.56 (s, 1H), 5.00-4.80 (m, 3H), 4.67 (s, 1H), 4.56 (s, 1H), 4.46 (t, *J* = 6.0 Hz, 2H), 3.87-3.84 (m, 1H), 3.29-3.24 (m, 1H), 2.88 (d, *J* = 7.8 Hz, 2H), 2.43-2.38 (m, 1H), 2.30-2.20 (m, 1H), 2.10-2.00 (m, 1H), 1.90-1.75 (m, 5H), 1.67 (s, 3H). The analytical data are attached below.

Analytical data of the 4-regio-isomer of compound **15**: HPLC purity: 98%. LC/MS (ESI): m/z 315 (M+H), m/z 337 (M+Na). ^{1}H-NMR (300 MHz, CDCl3) δ 6.25 (s, 1H), 6.05 (s, 2H), 5.50 (s, 1H), 4.90-4.70 (m, 4H), 4.50-4.40 (m, 3H), 3.53-3.48 (m, 1H), 3.30-3.20 (m, 1H), 3.05-2.90 (m, 1H), 2.80-2.70 (m, 1H), 2.50-2.40 (m, 1H), 2.30-2.10 (m, 2H), 1.90-1.70 (m, 5H), 1.57 (s, 3H). The analytical data are attached below.

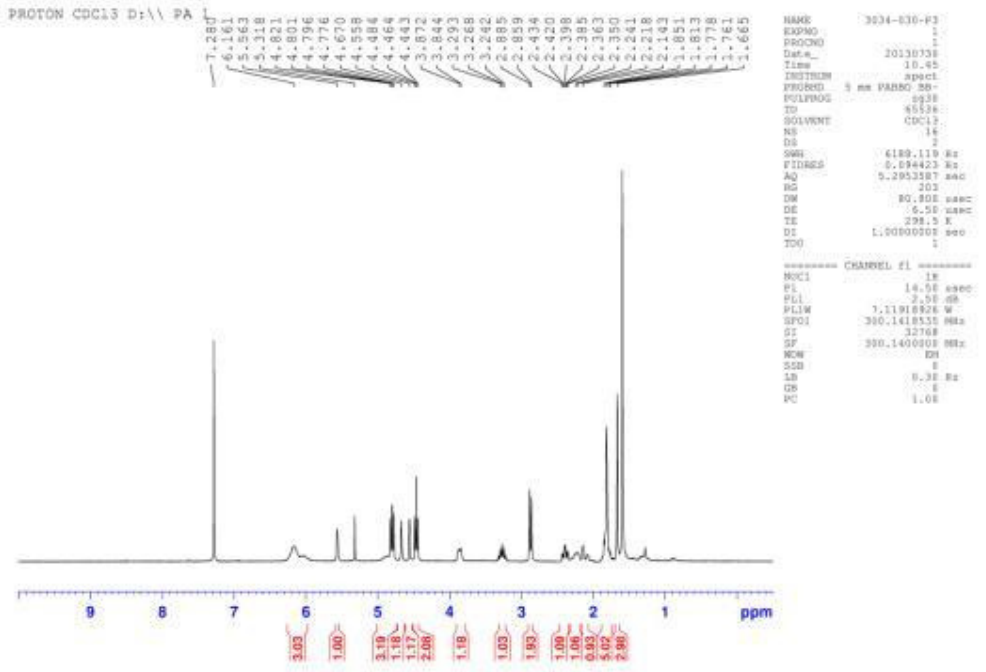


4-regio-isomer of 15

NOTES:

1. Based on TLC analysis, the reaction was quite messy. It was recommended to quench the reaction before the complete consumption of the starting material **15-6** because a longer reaction will result in more impurities and make the purification more difficult.
2. The starting material **15-6** has less solubility in $CHCl_3$. All other components have better solubility than **15-6** in $CHCl_3$. More than 1 equiv. of **6** usually caused the formation of more impurities.

^{1}H-NMR spectrum of compound **15**



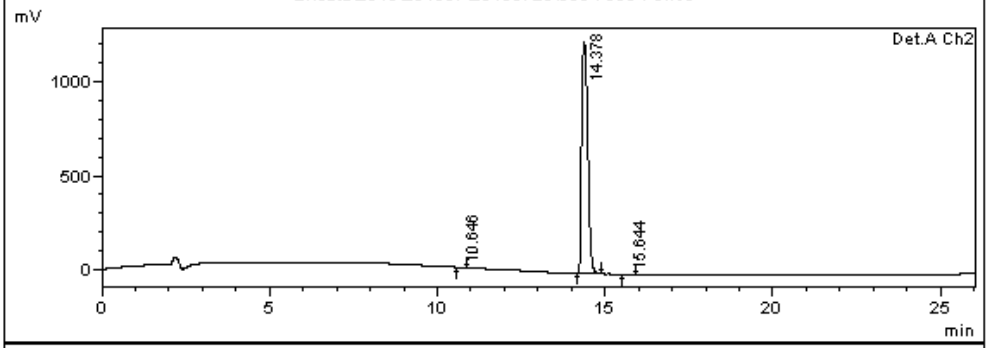
HPLC chromatogram of compound **15**

==== Shimadzu LCsolution Analysis Report ====

E:\data\2013\201307\20130729\3034-030-P3.lcd

Sample Name	: CY	Sample ID:	
Tray#	: 1	Vail #: 62	
Injection Volume	: 30 uL	Data File Name: 3034-030-P3.lcd	
Method File Name	: method2(15cm)-1.lcm	Batch File Name: 20130729.lcb	
Data Acquired	: 2013-7-29 18:15:06	Data Processed: 2013-7-30 8:29:34	

E:\data\2013\201307\20130729\3034-030-P3.lcd

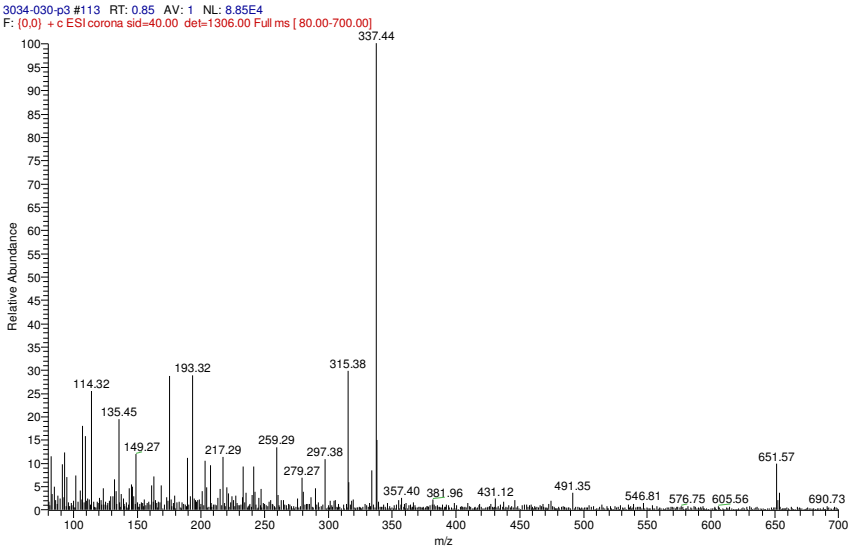


Peak Table

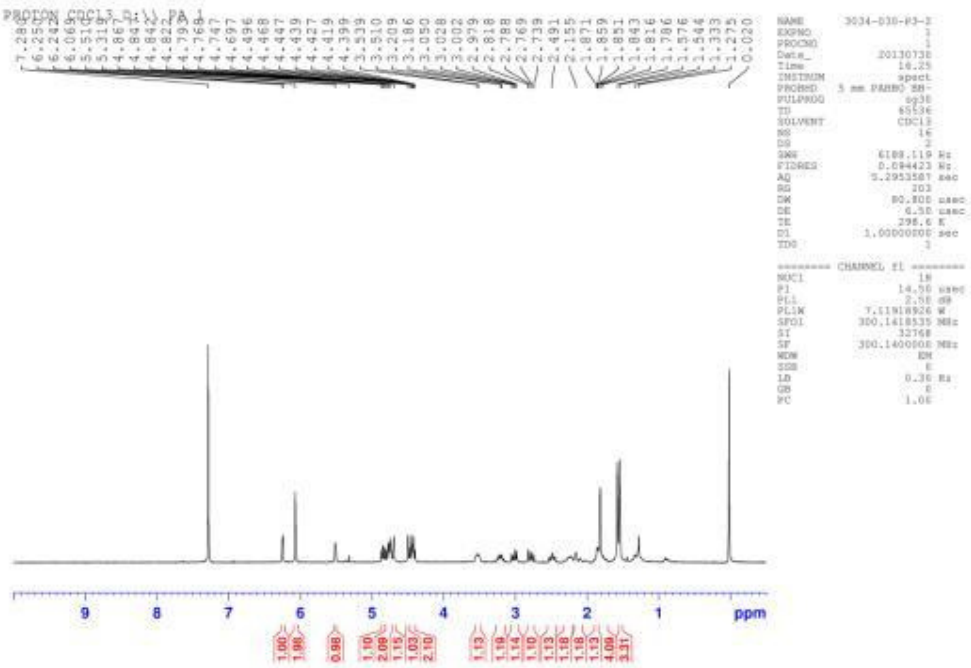
Detector A Ch2 220nm

Peak#	Ret. Time	Area	Area %
1	10.646	7470	0.048
2	14.378	15588307	99.908
3	15.644	6901	0.044
Total		15602679	100.000

LC/MS of compound **15**



[1]H-NMR spectrum of the 4-regio-isomer of compound **15**

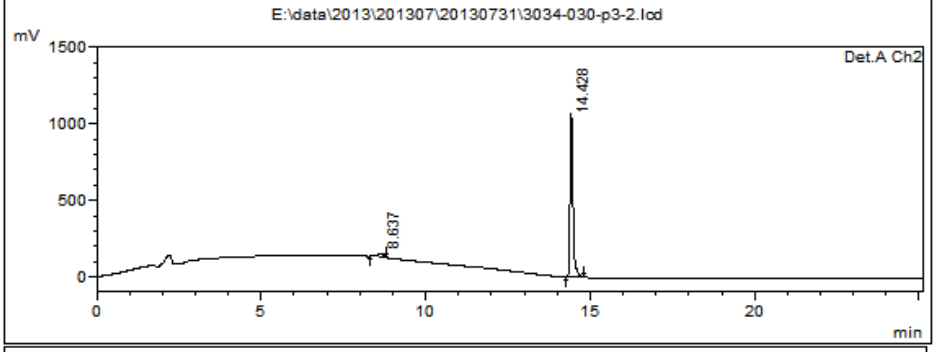


HPLC chromatogram of the 4-regio-isomer of compound **15**

==== Shimadzu LCsolution Analysis Report ====

E:\data\2013\201307\20130731\3034-030-p3-2.lcd

Sample Name	: cy	Sample ID:	
Tray#	: 1	Vail #: 3	
Injection Volume	: 4 uL	Data File Name: 3034-030-p3-2.lcd	
Method File Name	: method(15cm)-1.lcm	Batch File Name: 20130731.doc.lcb	
Data Acquired	: 2013-7-31 12:38:39	Data Processed: 2013-7-31 13:18:44	

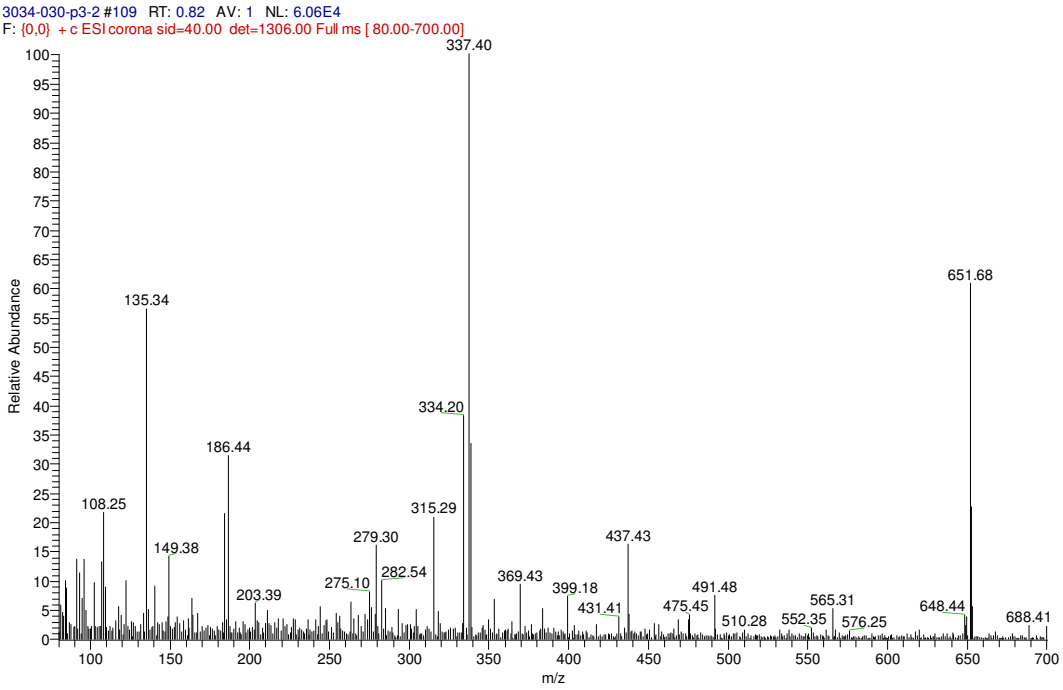


PeakTable

Detector A Ch2 220nm

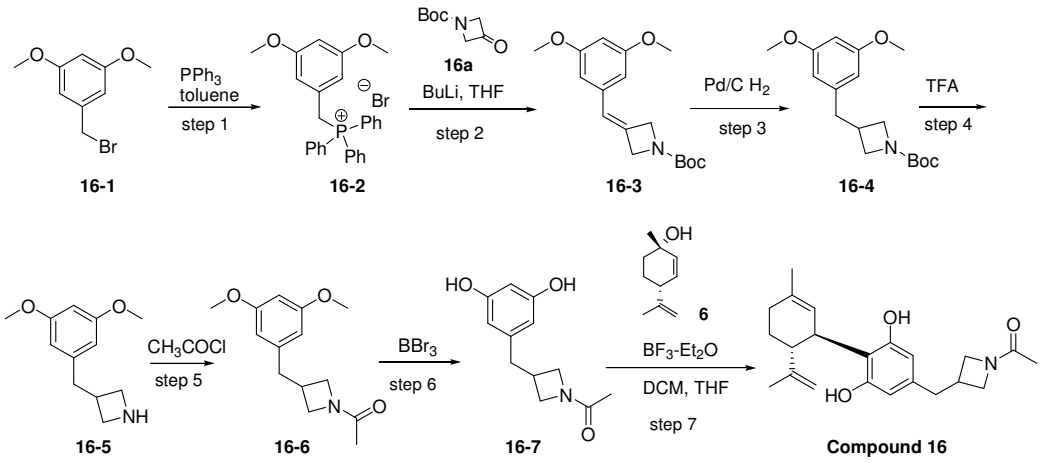
Peak#	Ret. Time	Area	Height	Area %	Height %
1	8.637	319694	20827	4.605	1.920
2	14.428	6623039	1064114	95.395	98.080
Total		6942733	1084941	100.000	100.000

LC/MS of the 4-regio-isomer of compound **15**

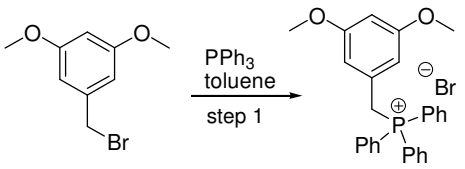


Synthesis of Compound 16

Synthesis Scheme for Compound 16



STEP 1:



16-1 **16-2**

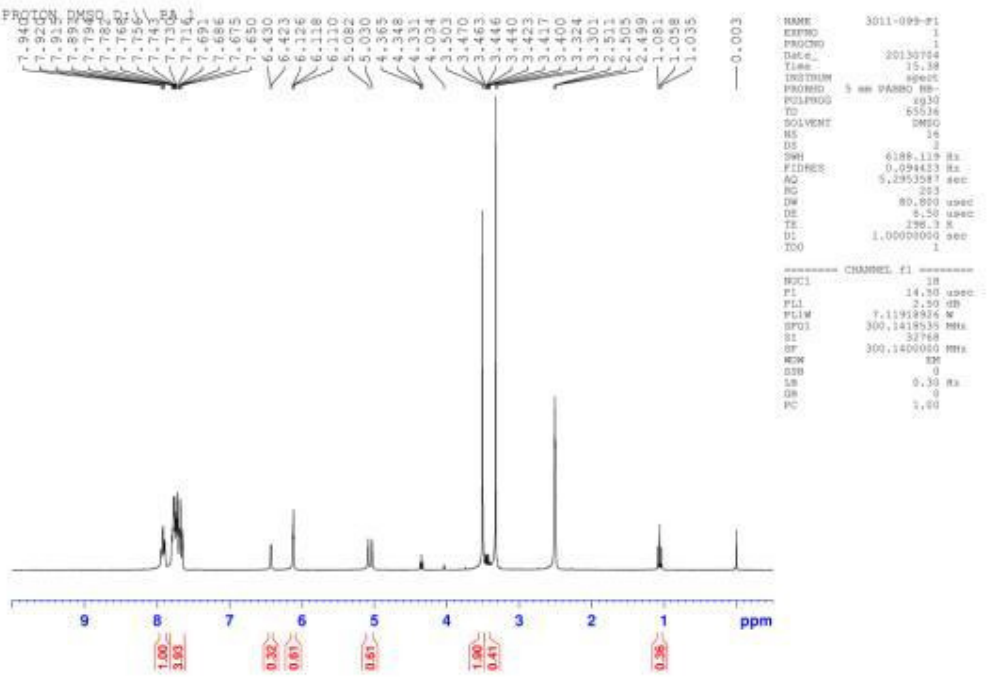
The solution of **16-1** (12 g, 52 mmol, 1.0 equiv.) and triphenylphosphine (15 g, 57 mmol, 1.1 equiv.) in toluene (100 mL) was refluxed for 3-4 h. TLC analysis indicated that the starting material was consumed completely. The reaction mixture was cooled to RT and the resulting solid was filtered and then sonicated in methanol/petroleum ether (1:20, 220 mL) for one h, filtered and washed with petroleum ether (3 x 20 mL) to give a crude product **16-2** (25 g, yield 97%) as white solid that was used directly for the next step.

^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.94-7.89 (m, 3H), 7.79-7.65 (m, 12H), 6.43 (s, 1H), 6.12 (t, J = 2.4 Hz, 2H), 5.08 (s, 1H), 5.03 (s, 1H), 3.50 (s, 6H). The analytical data is attached below.

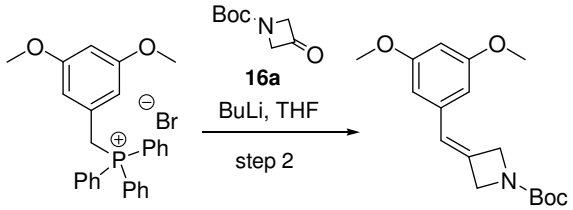
Batch Summary of Step 1

Batch #	SM (**16-1**)	Product (**16-2**)	Yield (%)	Purity
3011-081	3.0 g	6.0 g	93.6%	^{1}HNMR ok
3011-099	12 g	25 g	97.5%	^{1}HNMR ok

[1]H-NMR spectrum of intermediate **16-2** (integrations should by 3x)



STEP 2:

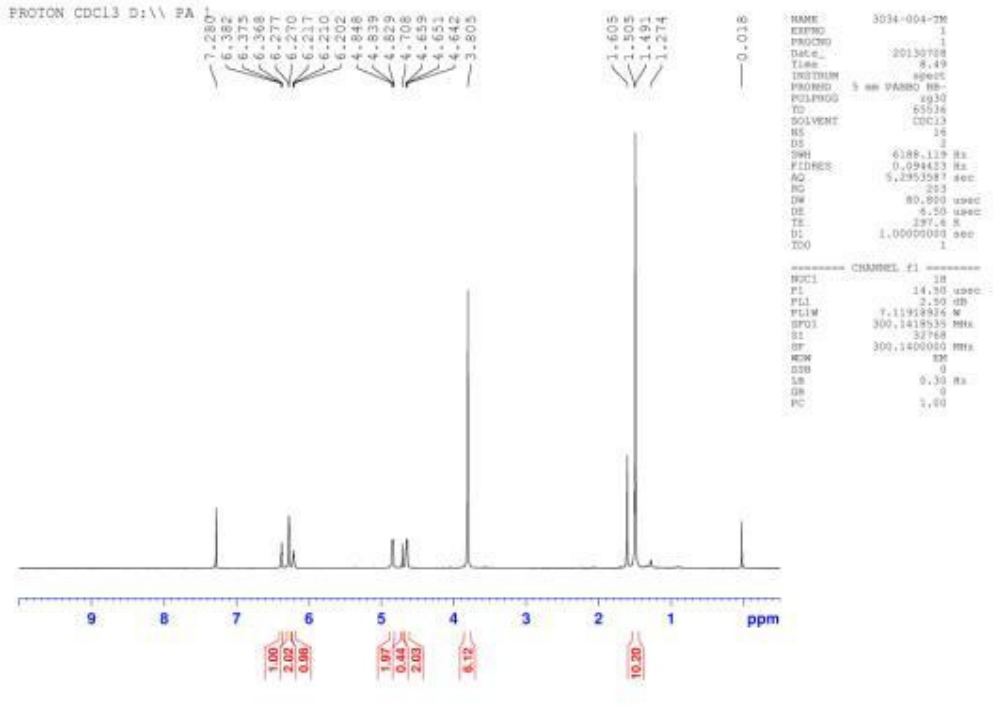


16-2 **16-3**

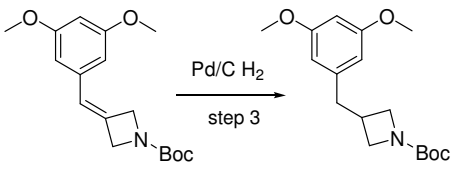
To a suspension of **16-2** (23.07 g, 46.76 mmol, 2.0 equiv.) in anhydrous THF (150 mL) was added *n*-BuLi (2.5 M in THF, 21 mL, 52.5 mmol, 2.2 equiv.) dropwise at ~ 0 °C. After stirred for 20 min, the solution of **16a** (4.00 g, 23.3 mmol, 1.0 equiv.) in dry THF (50 mL) was added dropwise. The cooling bath was removed and the reaction mixture was stirred at RT for one h, when TLC analysis showed the consumption of **16a**. Water (200 mL) was added to quench the reaction, which was extracted with EtOAc (3 x 100 mL), dried, concentrated, and purified by column chromatography (EtOAc/Hexane = 1/15) to give pure product **16-3** (4.5 g, yield 63%) as pale yellow oil that solidified on standing. [1]H-NMR (300 MHz, $CDCl_3$) δ 6.38-6.36

(m, 1H), 6.27 (s, 2H), 6.21 (s, 1H), 4.85-4.83 (m, 2H), 4.66-4.64 (m, 2H), 3.80 (s, 6H), 1.50 (s, 9H). The analytical data is attached below.

^{1}H-NMR spectrum of intermediate **16-3**



STEP 3:



16-3 **16-4**

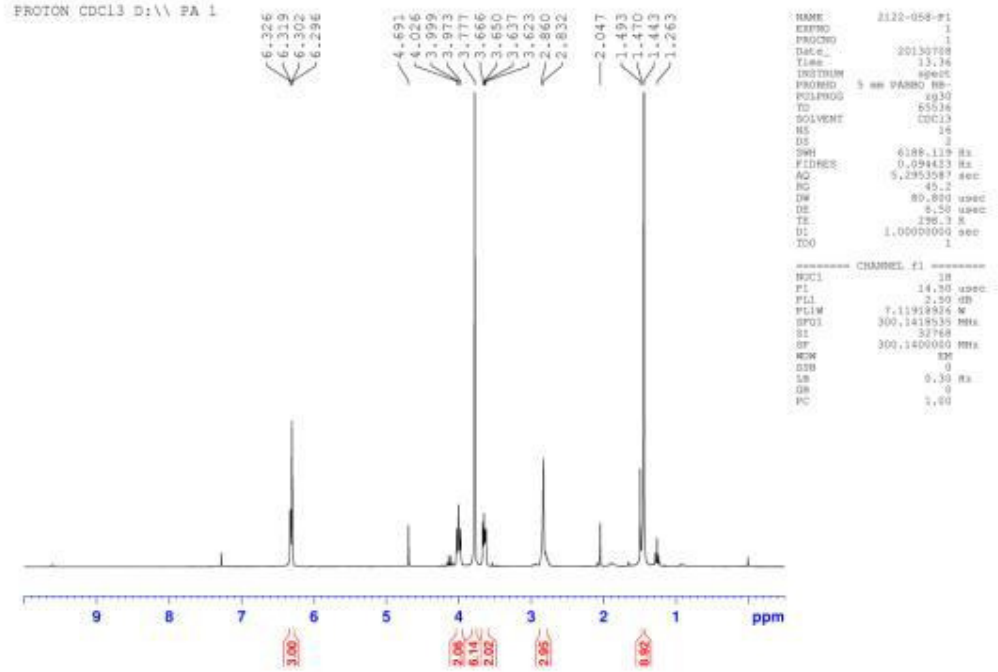
After purging with nitrogen, the suspension of **16-3** (4.50 g, 14.7 mmol) and 10% Pd/C (4.0 g) in EtOAc (800 mL) was stirred under hydrogen (balloon) at RT for 4 h, when TLC indicated the full consumption of **16-3**. The reaction mixture was filtered through Celite and concentrated to give crude **16-4** (5 g, yield 110%), which was directly used for the next step.

^{1}H-NMR (300 MHz, CDCl3) δ 6.32-6.29 (m, 3H), 4.03-3.97 (m, 2H), 3.78 (s, 6H), 3.67-3.62 (m, 2H), 2.86-2.76 (m, 3H), 1.47 (s, 9H). The analytical data is attached below.

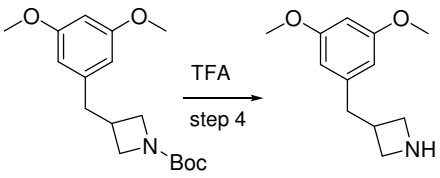
Batch Summary of Step 3

Batch #	SM (**16-3**)	Product (**16-4**)	Yield (%)	Purity
3034-001	0.8 g	0.8 g	99%	^{1}HNMR ok
2122-058	4.5 g	5.0 g	110%	^{1}HNMR ok

H-NMR spectrum of intermediate **16-4**



STEP 4:

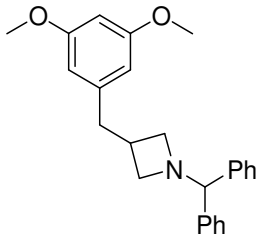


16-4 **16-5**

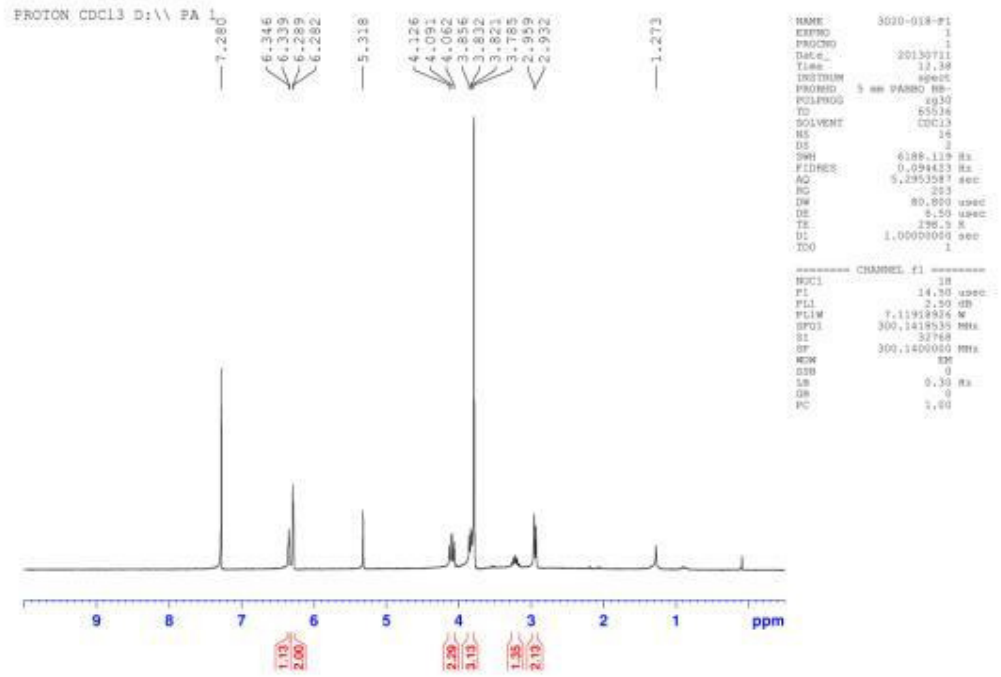
Deprotection of nitrogen was accomplished by treating compound **16-4** (1.50 g, 4.88 mmol) with TFA (10 mL) in DCM (30 mL) at 0 °C for 40 min. The reaction mixture was concentrated and the residue was dissolved in DCM (20 mL). The mixture was treated with aqueous $NaHCO_3$ to pH = 8-9. The organic layer was separated and the aqueous layer was extracted with DCM (3 x 20 mL). the combined organic phases were dried over Na_2SO_4, filtrated, and concentrated to give 1.0 g of the crude product **16-5** (1.0 g, yield 99%) as white solid, which was used for the next step without further purification. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 6.35 (s, 1H), 6.28 (s, 2H), 4.09 (t, J = 10.5 Hz, 2H), 3.83 (t, J = 7.2 Hz, 2H), 3.78 (s, 6H), 3.28-3.10 (m, 1H), 2.94 (d, J = 8.1 Hz, 2H). The analytical data is attached below.

Notes

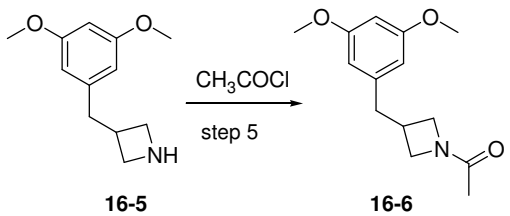
1. Diphenylene was used initially as the protecting group on nitrogen of intermediate **16-4**, however deprotection of dibenzylene group was very difficult using common hydrogenation conditions.



^{1}H-NMR spectrum of intermediate **16-5**



STEP 5:

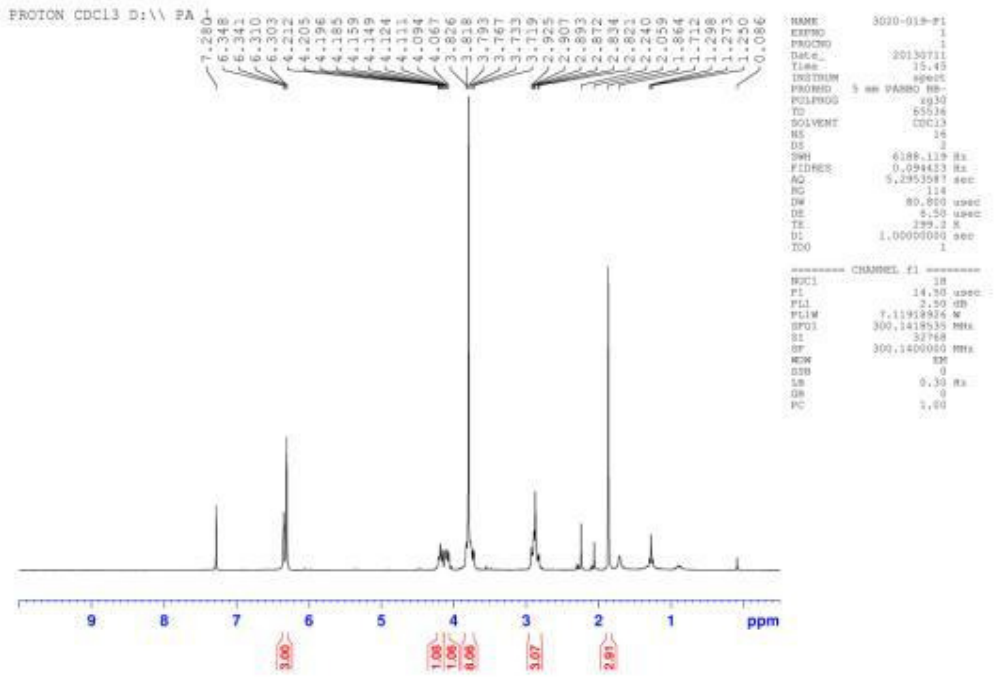


16-5 **16-6**

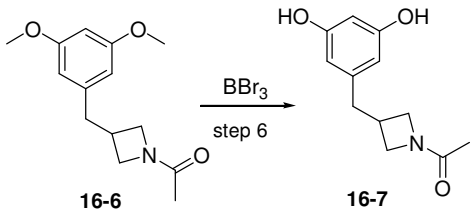
The mixture of compound **16-5** (1.0 g, 4.83 mmol, 1 equiv.), TEA (0.98 g, 9.68 mmol, 2 equiv.) and CH_3COCl (0.46 g, 5.8 mmol, 1.2 equiv.) in DCM (20 mL) was stirred at RT for one h. Water was added to quench the reaction and the organic layer was separated. The aqueous layer was extracted with ethyl acetate (2 x 20 mL) and the combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated to give a crude product **16-6** (0.97 g, yield 80%) as yellow oil.

^{1}H-NMR (300 MHz, $CDCl_3$) δ 6.35-6.30 (m, 3H), 4.30-4.00 (m, 2H), 3.90-3.70 (m, 8H), 3.00-2.80 (m, 3H), 1.86 (s, 3H). The analytical data is attached below.

[1]H-NMR spectrum of intermediate **16-6**



STEP 6:



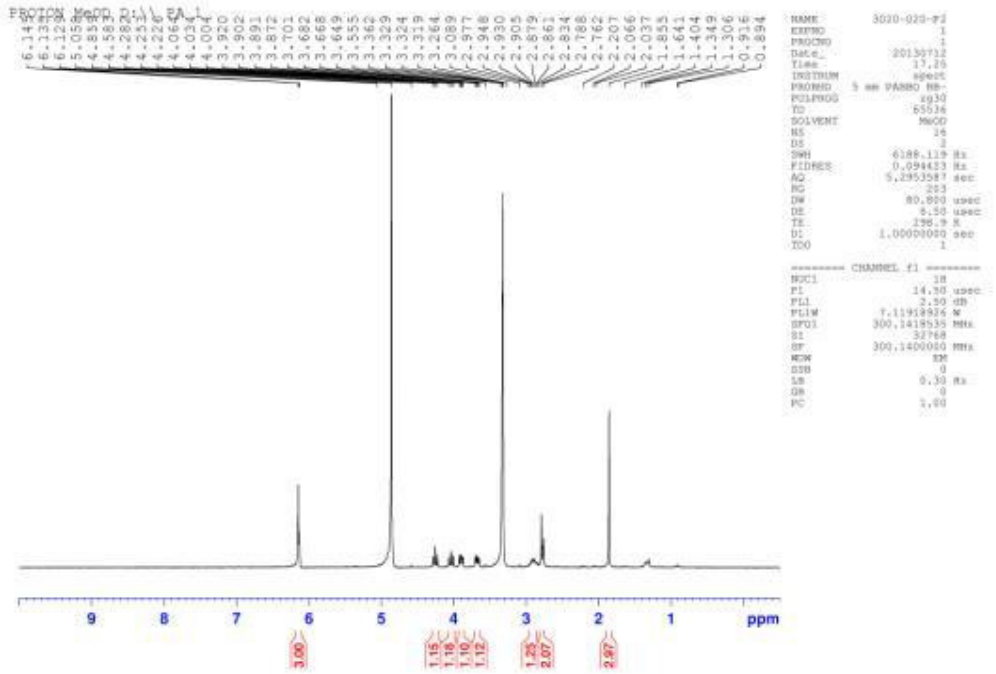
16-6 **16-7**

A solution of BBr_3 (7.70 g, 30.8 mmol, 8.0 equiv.) in DCE (30 ml) was slowly added to the solution of compound **16-6** (0.96 g, 3.86 mmol, 1.0 equiv.) in DCE (100 mL) under nitrogen over 20 min at - 5 to 0 °C. The resulting reaction mixture was stirred at RT for another 2.5 h until TLC indicated reaction completion. An aqueous solution of NH_4Cl (80 mL) was added to quench the reaction and the organic layer was separated. The aqueous layer was extracted with ethyl acetate (4 x 100 mL) and the combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated to give a crude product (0.69 g) as yellow solid, which was purified by column chromatography to give **16-7** (0.44 g, yield 51%).

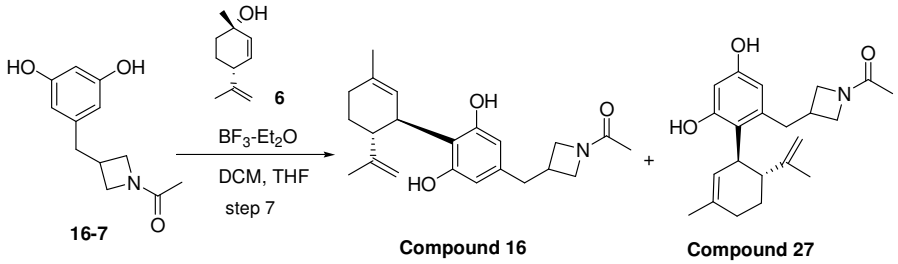
[1]H-NMR (300 MHz, CD_3OD) δ 6.14 (m, 3H), 4.28-4.20 (m, 1H), 4.00-3.95 (m,

1H), 3.95-3.85 (m, 1H), 3.60-3.50 (m, 1H), 2.95-2.80 (m, 1H), 2.78 (m, 2H), 1.86 (s, 3H). The analytical data is attached below.

^{1}H-NMR spectrum of intermediate **16-7**



STEP 7:



To a solution of **16-7** (350 mg, 1.58 mmol, 1 equiv.) and BF_3-Et_2O (673 mg, 4.74 mmol, 3 equiv.) in DCM/THF (4:1, 50 mL) was added a solution of **6** (241 mg, 1.58 mmol, 1 equiv.) in DCM/THF (4:1, 3 mL) at RT over 15 min. After the addition, the mixture was stirred at RT for additional 50 min. TLC analysis showed 20-30% conversion of **16-7**. The reaction was stopped at that point by the addition of aqueous $NaHCO_3$ (20 mL) was added to quench the reaction. The aqueous layer was extracted with ethyl acetate/THF (1:1) (3 x 20 mL). The combined organic layers were dried

over Na_2SO_4, filtrated and concentrated to dryness. The residue was purified through column chromatography to give the crude product **16** (138 mg) with 70-80% purity and 220 mg of intermediate **16-7** recovered. The crude compound **16** (138 mg) was further purified by preparative TLC to provide 50 mg of compound **16** with 90% purity at 254nm by HPLC. The recovered **16-7** (220 mg) was subsequently converted to compound **16** (40 mg, ~82% purity) using the same procedure. Two batches were combined to give compound **16** (86 mg, yield 13%) with 84% purity at 254 nm and ~95% purity at 230 nm. Additionally, ~100 mg of the 4-regio-isomer **27** (yield 17%) was obtained as well.

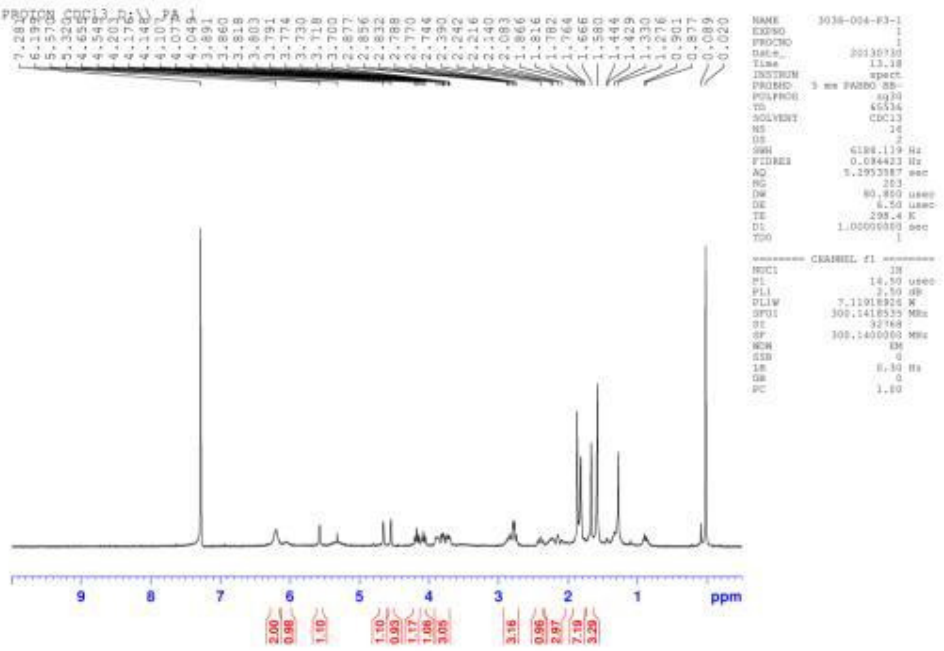
Analytical data of compound **16**

HPLC purity: 94%. LC/MS (ESI): m/z 356 (M+1), m/z 378 (M+Na). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 6.35-6.15 (br s, 2H), 6.15-5.95 (br s, 1H), 5.55 (s, 1H), 4.65 (s, 1H), 4.55 (s, 1H), 4.25-4.15 (m, 1H), 4.15-4.00 (m, 1H), 3.95-3.85 (m, 1H), 3.85-3.65 (m, 3H), 2.90-2.70 (m, 3H), 2.45-2.35 (m, 1H), 2.30-2.00 (m, 3H), 1.90-1.80 (m, 7H), 1.67 (s, 3H). The analytical data is attached below.

Analytical data of the 4-regio-isomer **27**

HPLC: 97.6%. LC/MS (ESI): m/z 356 (M+1), m/z 378 (M+Na). H-NMR (300 MHz, CDCl3) δ 6.28 (s, 1H), 6.13 (s, 1H), 6.07 (m, 1H), 5.50 (m, 1H), 5.40-5.30 (m, 1H), 4.69 (s, 1H), 4.50 (s, 1H), 4.30-4.00 (m, 3H), 3.85-3.50 (m, 2H), 3.50-3.40 (m, 1H), 3.00-2.40 (m, 4H), 2.30-2.00 (m, 3H), 1.88 (s, 3H), 1.82 (s, 3H), 1.52 (s, 3H). The analytical data is attached below.

[1]H-NMR spectrum of compound **16**:

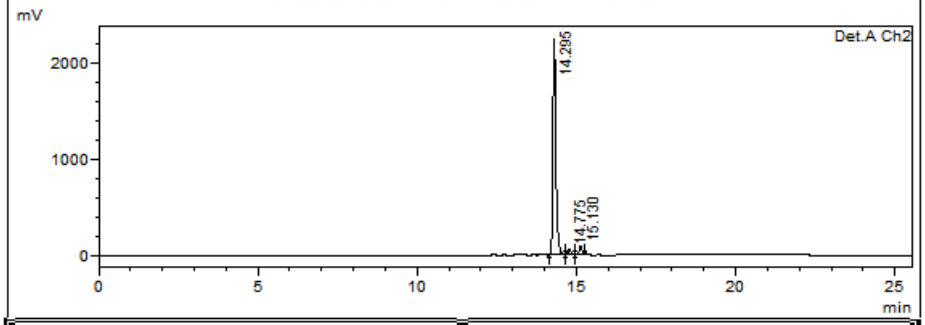


HPLC chromatogram of compound **16**

==== Shimadzu LCsolution Analysis Report ====

E:\data\2013\201307\20130730\3036-004-P3-1.lcd

Sample Name : Sample ID:
Tray# : 1 Vail #: 33
Injection Volume : 20 uL Data File Name: 3036-004-P3-1.lcd
Method File Name : method1(15cm)-MeOH+H2O.lcm Batch File Name: 20130730.doc.lcb
Data Acquired : 2013-7-30 11:12:46 Data Processed: 2013-7-30 11:38:17

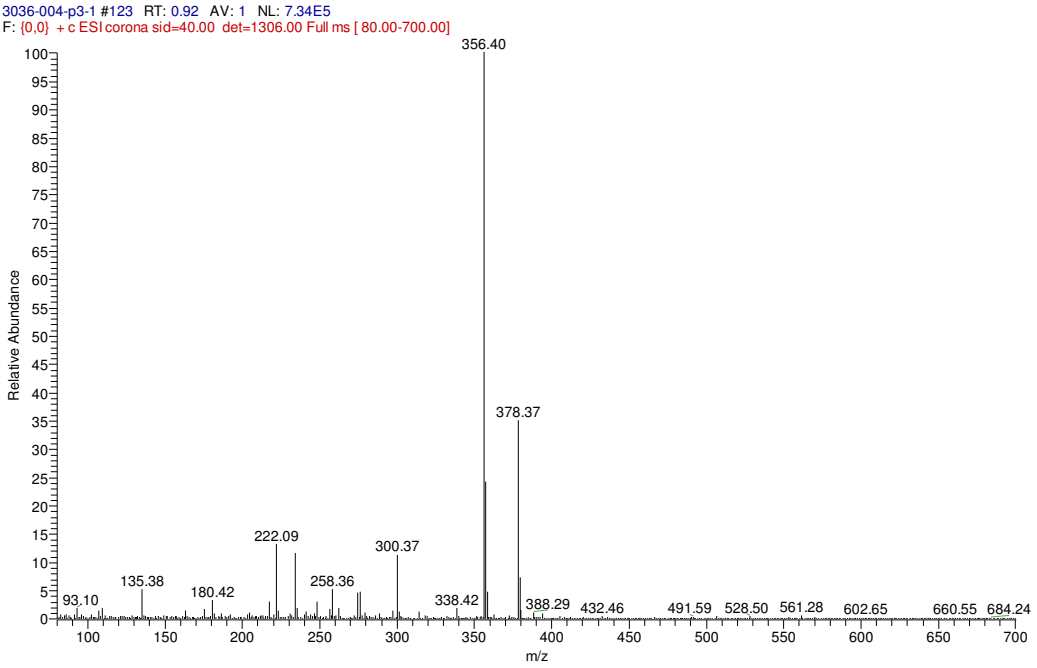


PeakTable

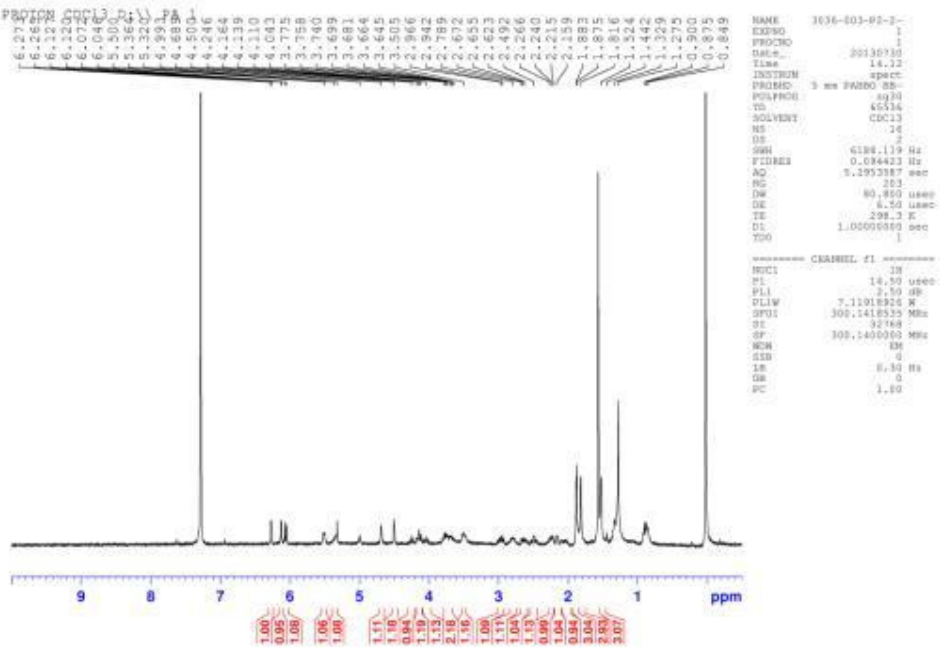
Detector A Ch2 230nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	14.295	14336534	2248586	94.597	94.298
2	14.775	374592	61280	2.472	2.570
3	15.130	444236	74691	2.931	3.132
Total		15155362	2384557	100.000	100.000

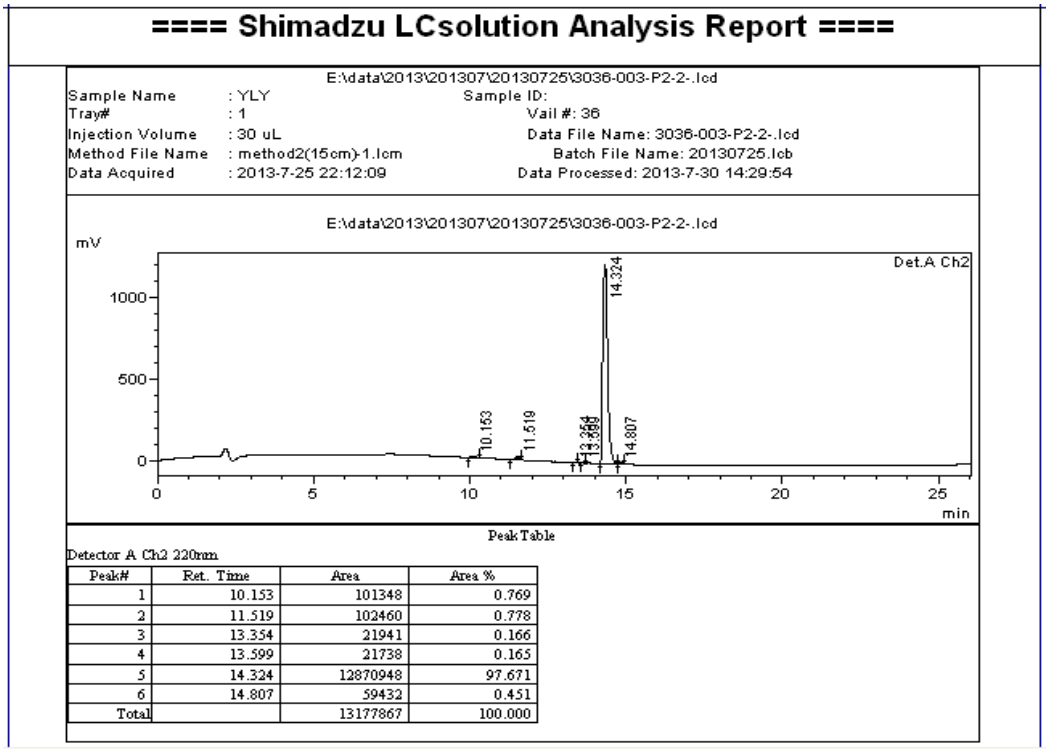
LC/MS of compound **16**



H-NMR spectrum of 4-regio-isomer **27**



HPLC chromatogram of 4-regio-isomer **27**



==== Shimadzu LCsolution Analysis Report ====

E:\data\2013\201307\20130725\3036-003-P2-2-.lcd

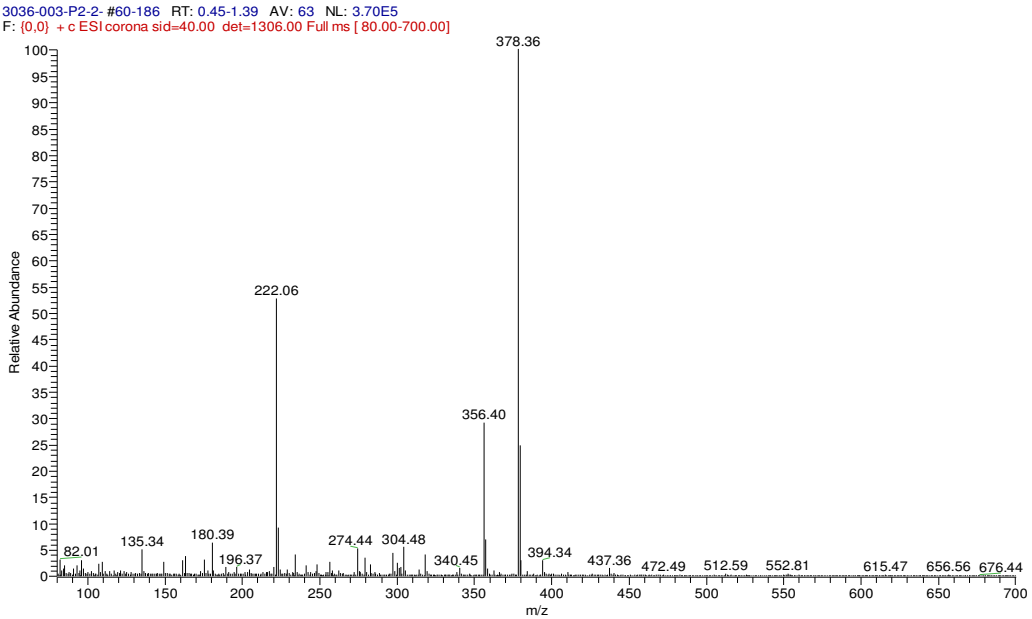
Sample Name	: YLY	Sample ID:	
Tray#	: 1	Vail #: 36	
Injection Volume	: 30 uL	Data File Name:	3036-003-P2-2-.lcd
Method File Name	: method2(15cm)-1.lcm	Batch File Name:	20130725.lcb
Data Acquired	: 2013-7-25 22:12:09	Data Processed:	2013-7-30 14:29:54

Peak Table

Detector A Ch2 220nm

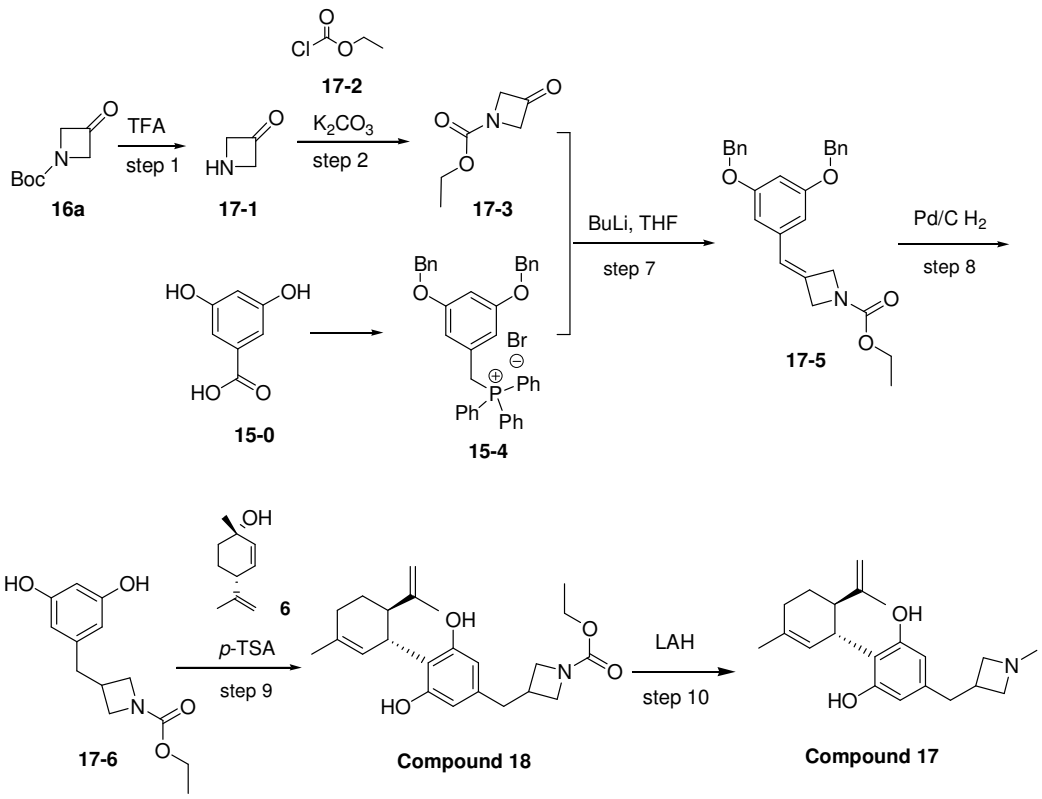
Peak#	Ret. Time	Area	Area %
1	10.153	101348	0.769
2	11.519	102460	0.778
3	13.354	21941	0.166
4	13.599	21738	0.165
5	14.324	12870948	97.671
6	14.807	59432	0.451
Total		13177867	100.000

LC/MS of 4-regio-isomer **27**

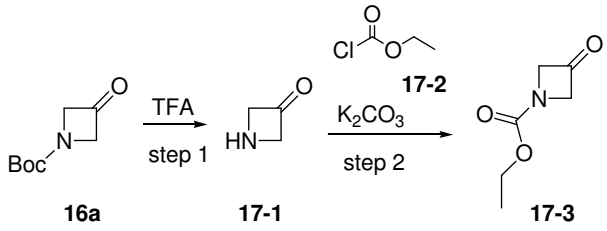


Synthesis of compound 17 and 18

Synthesis Scheme for Compounds 17 and 18



STEP 1 & 2:

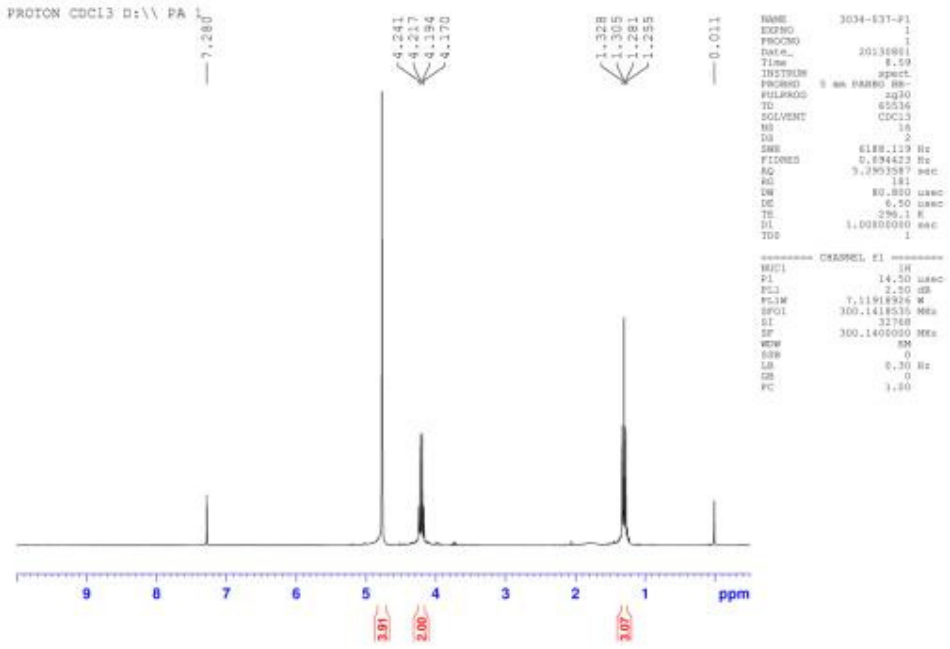


A solution of **16a** (4.3 g, 25.1 mmol, 1 equiv.) in 30% TFA in DCM was stirred at RT for 2 hours. TLC analysis indicated the disappearance of **16a**. The reaction mixture was concentrated to dryness on a rotary evaporator to give crude **17-1**. The crude **17-1** was dissolved in THF (20 mL) and treated with ethyl chloroformate (**17-2**) (4.07 g, 37.7 mmol, 1.5 equiv.). To the resulting mixture, an aqueous solution of

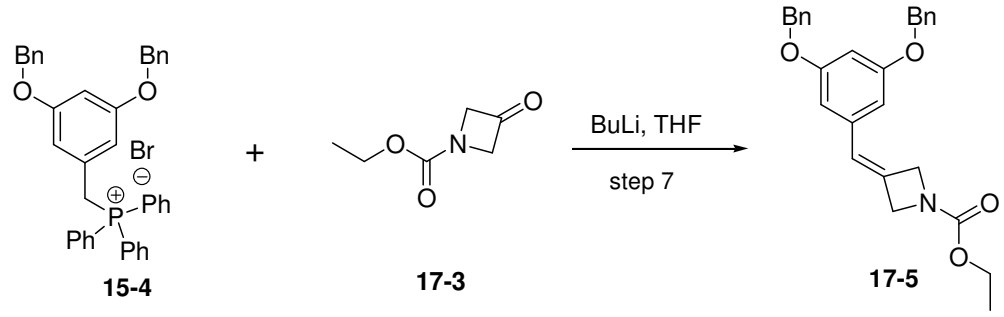
K_2CO_3 (10.4 g, 75.3 mmol, 3 equiv.) in water (20 mL) was added dropwise at 0 °C. After the addition, the reaction mixture was allowed to warm to RT and stirred for 1.5 h. Since TLC analysis indicated the completion of the reaction, it was extracted with EtOAc (3 x 30 mL), back washed by brine (30 mL), dried and concentrated to give product **17-3** (3.7 g, yield 102%) as solid.

^{1}H-NMR (300 MHz, $CDCl_3$) δ 4.77 (s, 4H), 4.20 (q, J = 7.1 Hz, 2H), 1.30 (t, J = 7.1 Hz, 3H). The analytical data is attached below.

^{1}H-NMR spectrum of intermediate **17-3**



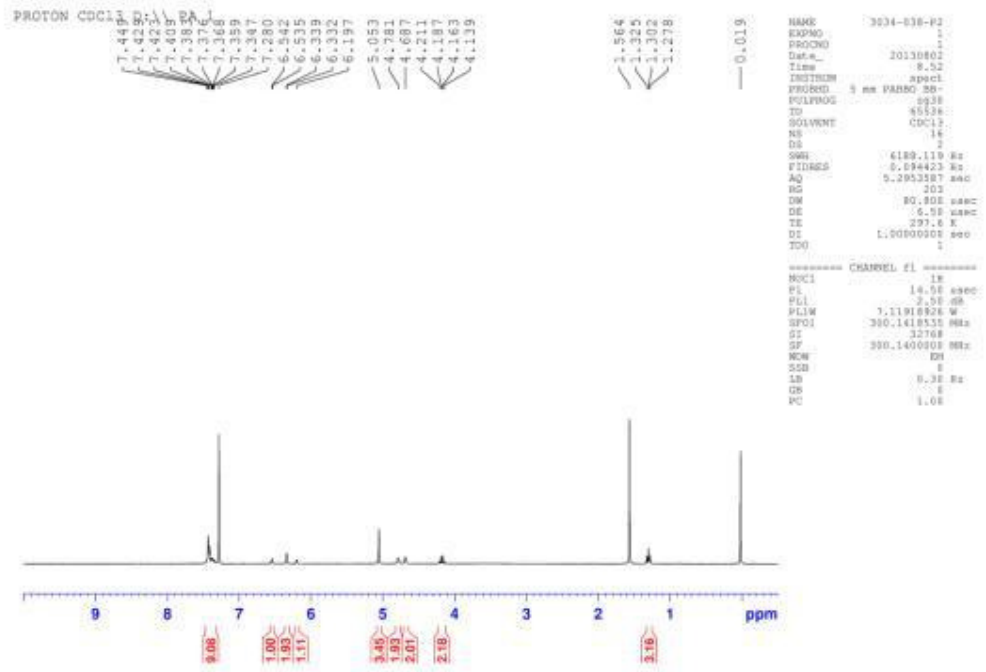
Step 7



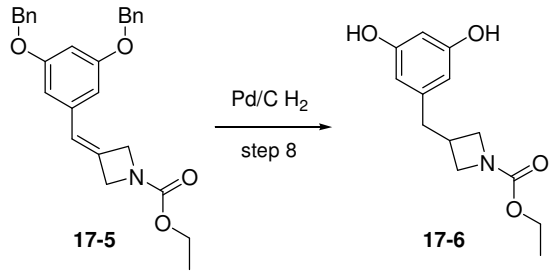
Under nitrogen, *n*-BuLi in hexane (2.5 M, 15.1 mL, 38 mmol, 2 equiv.) was added to a suspension of **15-4** (8.10 g, 12.6 mmol, 1.0 equiv.) in dry THF (150 mL) at -5 to 0 °C over 20 min. After stirring at 0 °C for 20 min, a solution of **17-3** (3.60 g, 25.2 mmol, 2 equiv.) in THF (100 mL) was added dropwise. After the addition, the ice-salt cooling bath was removed and the reaction was allowed warm to RT with continued stirring for one additional h. Water (150 mL) was added to quench the reaction, which was extracted by EtOAc (3 x 100 mL). The combined organic phase was washed with brine (100 mL), dried, concentrated, and purified by column chromatography (EtOAc/Hexane = 1/10) to provide the desired product **17-5** (4.0 g, yield 37%).

^{1}H-NMR (300 MHz, CDCl3) δ 7.77-7.37 (m, 10H), 6.54-6.53 (m, 1H), 6.33 (d, *J* = 1.9 Hz, 2H), 6.19 (s, 1H), 5.05 (s, 4H), 4.78 (s, 2H), 4.68 (s, 2H), 4.21-4.13 (q, *J* = 7.1 Hz, 2H), 1.30 (t, *J* = 7.1 Hz, 3H). The analytical data is attached below.

^{1}H-NMR spectrum of intermediate **17-5**



Step 8

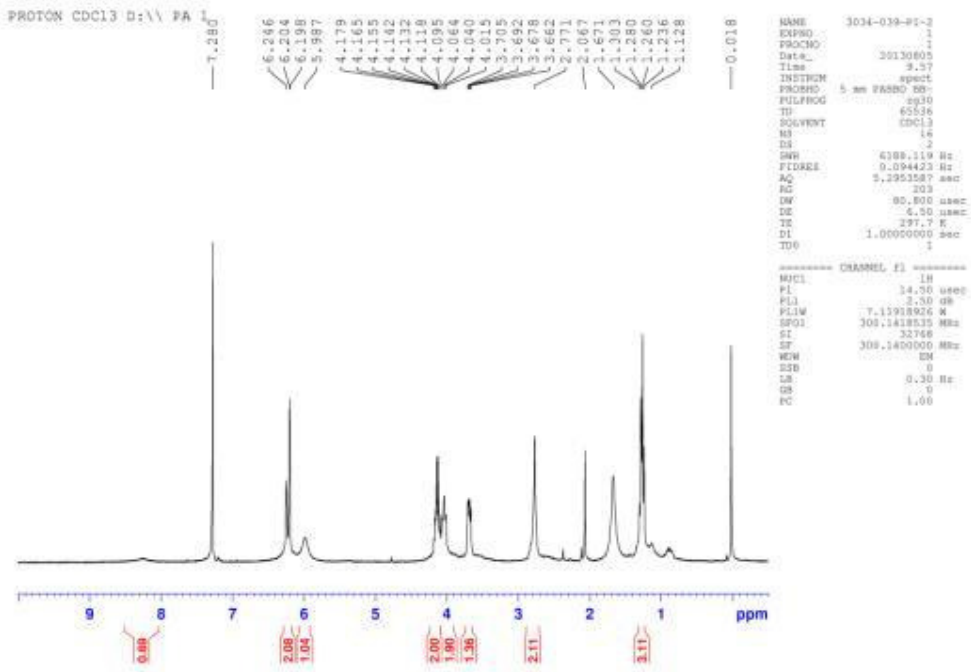


17-5 **17-6**

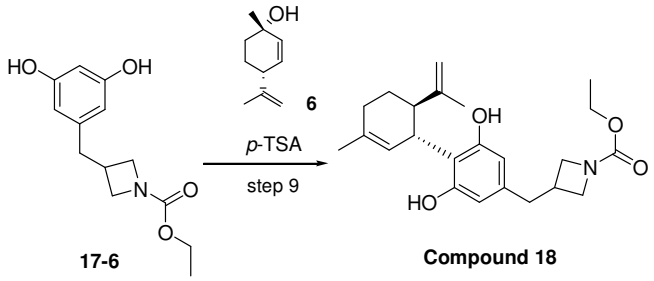
A suspension of intermediate **17-5** (4.0 g, 9.3 mmol) and 10% Pd/C (1.0 g) in EtOAc (400 mL) was stirred under a hydrogen balloon at RT for 4 hours. The reaction mixture was filtered through Celite and concentrated to give a crude product **17-6** (2.2 g, yield 94%), which was used without further purification.

LC/MS (ESI): m/z 252 (M+1). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 6.24-6.19 (m, 2H), 5.98 (brs, 1H), 4.17-4.11 (m, 2H), 4.06-4.01 (m, 2H), 3.70-3.67 (m, 2H), 2.77 (m, 3H), 1.30-1.23 (m, 3H). The analytical data is attached below.

^{1}H-NMR spectrum of intermediate **17-6**



Synthesis of Compound 18

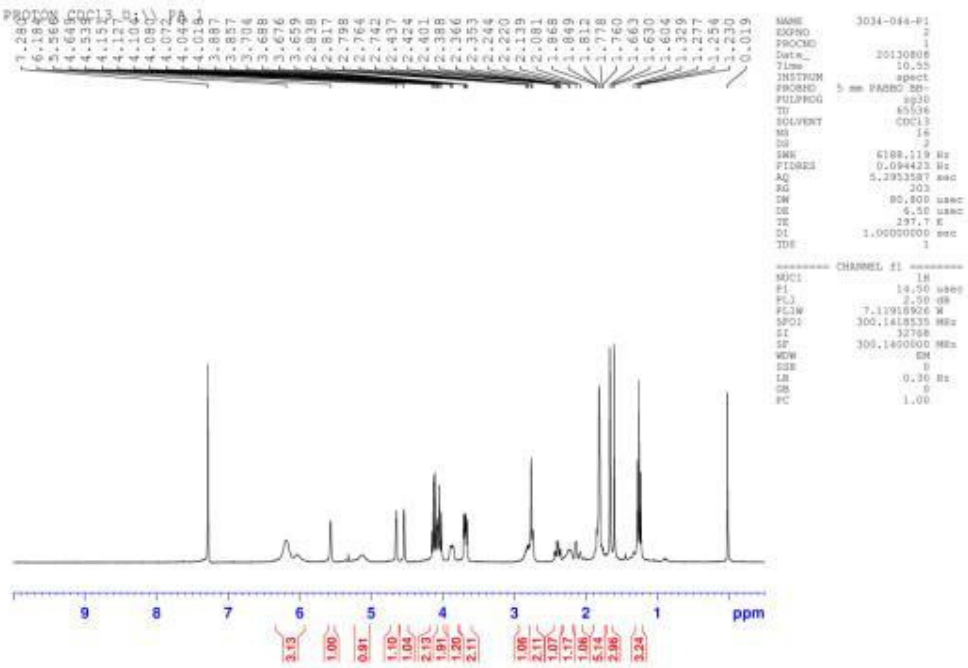


Four parallel batches were carried out at the scale of 0.45-0.7 g of **17-6**. The representative procedure is summarized below.

To a suspension of **17-6** (0.45 g, 1.79 mmol, 1.2 equiv.) in $CHCl_3$ (45 mL), was added *p*-TsOH (68 mg, 0.39 mmol, 0.26 equiv.) and **6** (0.23 g, 1.5 mmol, 1 equiv.) and the resulting mixture was stirred at RT for 10 min. TLC analysis indicated ~60-70% conversion of the starting material **17-6**. A saturated $NaHCO_3$ solution was added to the reaction mixture to adjust the pH to 9-10. The organic phase was separated and the aqueous phase was extracted by DCM (2 x 50 mL). The combined organic phase was dried over Na_2SO_4, filtered and concentrated to provide crude compound **18**, which was combined three other batches of crude compound **18** and purified by column chromatography (EtOAc/Hexane = 1/3) and preparative HPLC (0.40 g of compound **18** was obtained, 14%).

HPLC: 99%. LC/MS (ESI): m/z 408 (M+Na). ^{1}H-NMR (300 MHz, CDCl3) δ 6.18-6.03 (m, 3H), 5.56 (s, 1H), 5.12 (s, 1H), 4.64 (s, 1H), 4.53 (s, 1H), 4.15-4.04 (m, 4H), 3.90-3.86 (m, 1H), 3.70-3.65 (m, 2H), 2.86-2.74 (m, 3H), 2.43-2.35 (m, 1H), 2.24-2.21 (m, 1H), 2.13-2.08 (m, 1H), 1.86-1.76 (m, 5H), 1.66 (s, 3H), 1.25 (t, J = 7.1 Hz, 3H). The analytical data are attached below.

[1]H-NMR spectrum of compound **18**

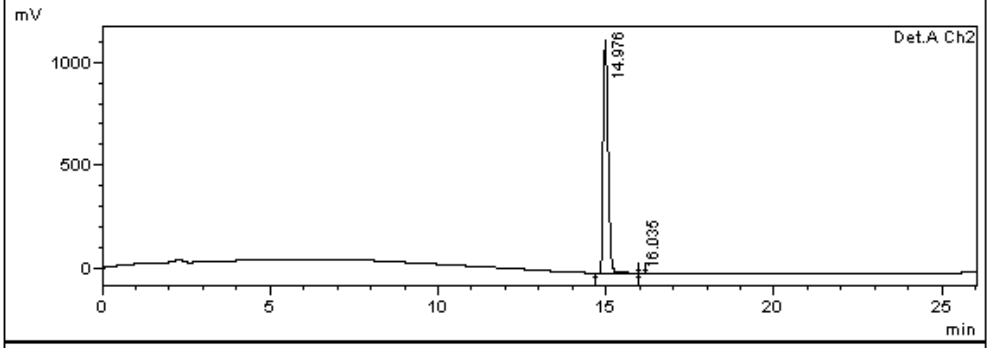


HPLC chromatogram of compound **18**

==== Shimadzu LCsolution Analysis Report ====

E:\data\2013\201308\20130808\3034-044-P1.lcd

Sample Name : CY Sample ID:
Tray# : 1 Vail #: 39
Injection Volume : 2 uL Data File Name: 3034-044-P1.lcd
Method File Name : method2(15cm)-1.lcm Batch File Name: 20130808.doc.lcb
Data Acquired : 2013-8-8 8:55:01 Data Processed: 2013-8-8 9:23:26

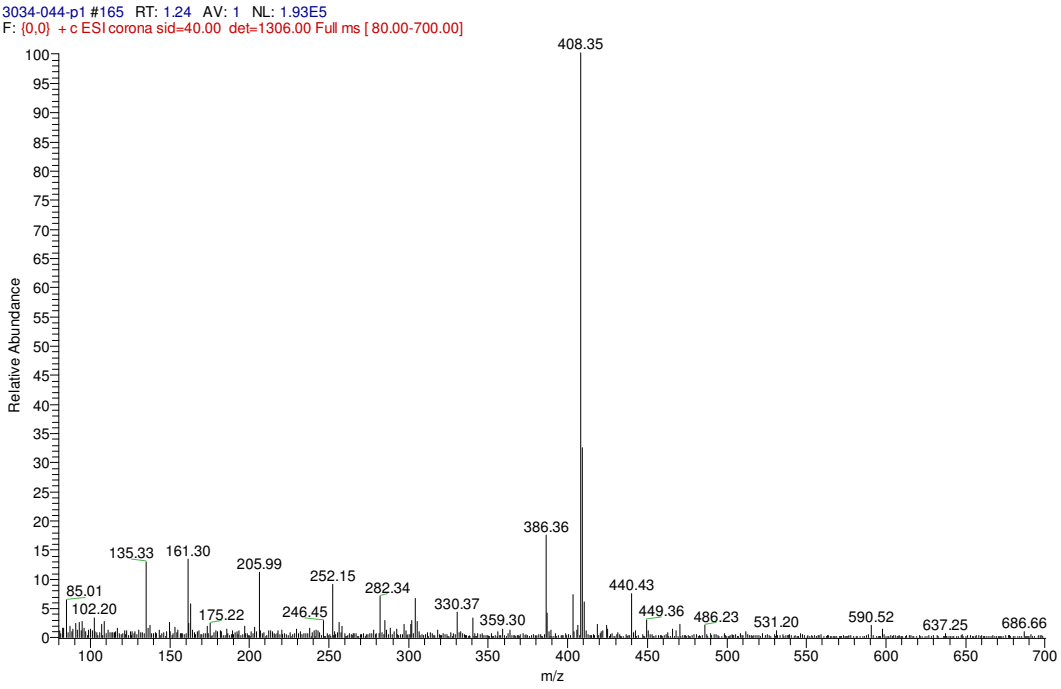


Peak Table

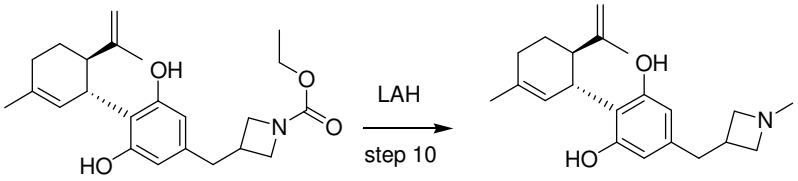
Detector A Ch2 220nm

Peak#	Ret. Time	Area	Area %
1	14.976	11387508	99.986
2	16.035	1637	0.014
Total		11389145	100.000

LC/MS of compound **18**



Synthesis of Compound 17



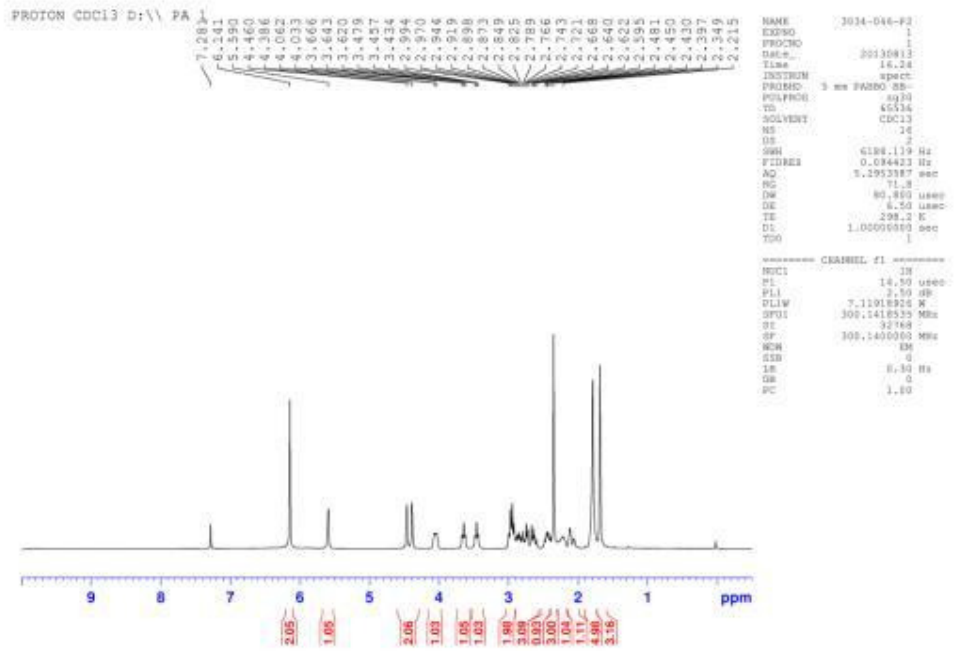
Compound 18 **Compound 17**

Under nitrogen, $LiAlH_4$ (74 mg, 3.9 mmol, 10.0 equiv.) was added to a solution of compound **18** (0.15 g, 0.39 mmol, 1.0 equiv.) in MTBE (15 mL). The suspension was heated to reflux and stirred for 4 h. After cooling with an ice-salt bath, water (0.2 mL), 15% KOH aqueous solution and water (0.6 mL) were slowly added sequentially. The solid was filtered and washed by MTBE (3 x 20 mL). The combined filtrates were dried over Na_2SO_4, filtered and concentrated to give crude compound **17** (150 mg) as brown oil, which was purified through preparative HPLC to provide compound **17** (72 mg, yield 56%).

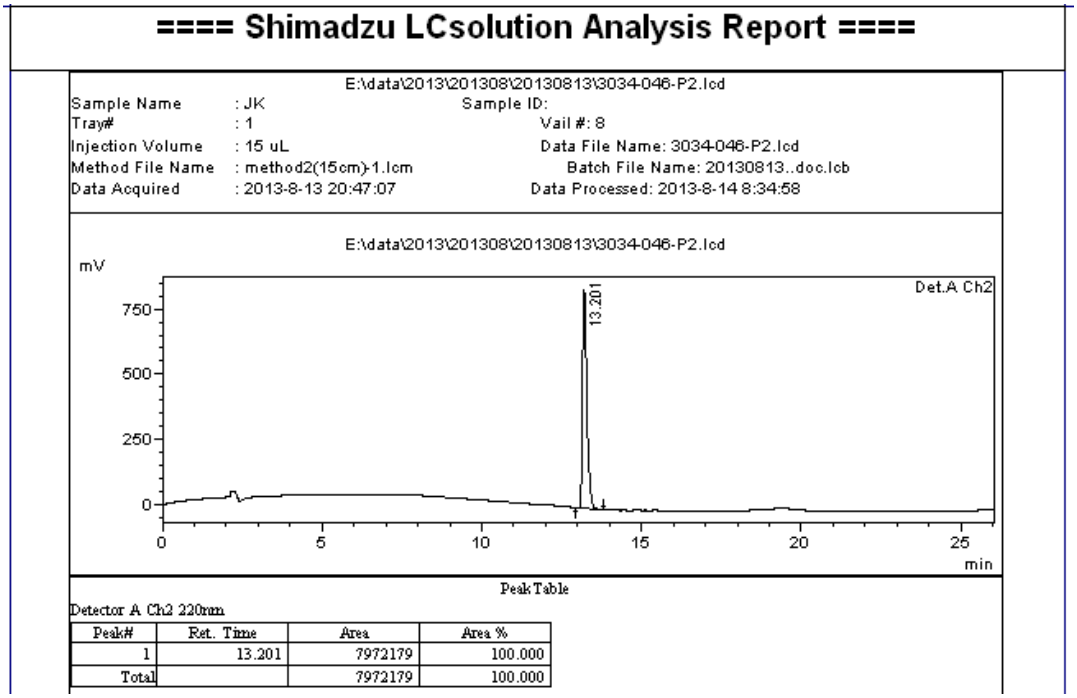
HPLC: ~100%. LC/MS (ESI): m/z 328 (M+1). ^{1}H-NMR (300 MHz, $CDCl_3$) δ

6.14 (s, 2H), 5.59 (s, 1H), 4.46 (s, 1H), 4.38 (s, 1H), 4.06-4.03 (m, 1H), 3.64 (t, *J* = 6.9 Hz, 2H), 3.46 (t, *J* = 6.6 Hz, 2H), 3.00-2.50 (m, 5H), 2.50-2.40 (m, 1H), 2.35 (s, 3H), 2.30-2.00 (m, 2H), 1.80 (br s, 5H), 1.68 (s, 3H). The analytical data are attached below.

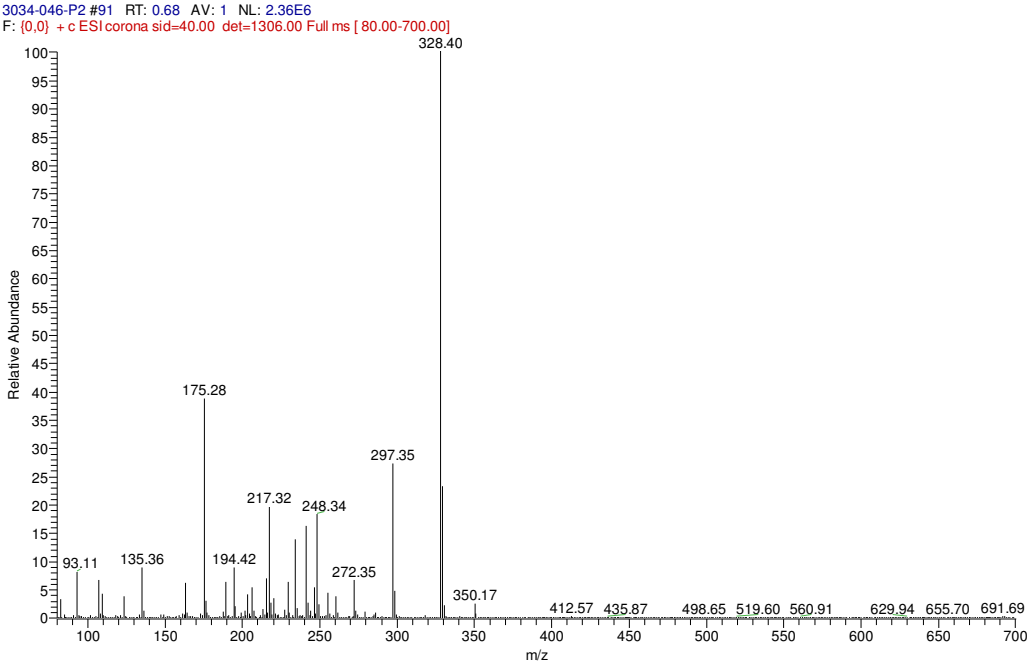
^{1}H-NMR spectrum of compound **17**



HPLC chromatogram of compound **17**



LC/MS of compound **17**



Biological Assays

Culture Model

Dissociated hippocampal cultures derived from embryonic day 18 rats were employed as the primary screening system to test for toxicity responses as well as neuroprotective actions. This cellular system was chosen because a limited number of compounds were to be tested thereby permitting the use of a low throughput screening system that is derived from a brain area that is highly susceptible to seizures. In brief, hippocampal tissue was obtained commercially through Brain Bits (Springfield, IL) and cultures prepared with slight modifications to methods previously described (Brewer et al. 1993). Tissue was dissociated with a papain-based kit from Worthington Biochemical Corporation (Lakewood, NJ) that is modeled after the method described by Huettner and Baughman (1986). The hippocampal neurons were platted at low density (10,000 cell/well) in a 96-well format and maintained in serum-free medium consisting of neurobasal medium supplemented with B27 and GlutaMAX (Gibco). Pre-coated poly-L-lysine-coated plates (BD Biosciences, Franklin Lakes, NJ) were used because of the preferential adherence and survival of neurons on this matrix support. Prior to the initiation of experiments between days 11 and 21 in vitro, a complete change of medium was performed in a working volume of 100 μL. This medium differed from the medium utilized for plating in that the B27-AO did not contain the five antioxidants present in the standard B27 (Gibco, product #10889). This change of medium was designed to decrease the background levels of antioxidants to facilitate the damage and death with glutamate or hydrogen peroxide treatment. The rationale for this change of medium was twofold: (1) because loss of antioxidant control may also be a component of epileptogenesis (Waldbaum and Patel 2010; Wu et al. 2010); and (2) because the goal was to obtain a significant and reproducible toxicity signal in hippocampal neurons. Thus, this decrease in the background antioxidant concentration was used to recapitulate a disease model for epilepsy as well as a means to produce neurotoxicity at the lowest concentration of toxin. Both the amount of glutamate and hydrogen peroxide used in the assays, as well as the time of treatment and duration of the experiment were designed to be

relevant to the disease. Further, all time parameters employed in these studies were empirically determined to be within the limits of reversible toxic events.

In vitro neuroprotection testing:

The central objective of all neuroprotective assays was their relevancy to oxidative stress related to hepatic encephalopathy (HE) and other or diseases associated with oxidative stress in general. These studies use phenotypic assays of neuroprotection because the molecular targets mediating the action of cannabidiol-like substances are unknown. Both the amount of ethanol and ammonia used in the assays, as well as the time of treatment and duration of the experiment, were designed to be relevant to HE (Ong et al, 2003). Further, all time parameters employed in these studies were empirically determined to be within the limits of reversible toxic events after treatment with cannabidiol (Hamelink et al., 2005) and cannabidiol-like substances, yet using amounts of ethanol (30 mM) and ammonia (300 μM ammonium acetate) that were relevant to the disease.

Cultures were treated within the period of culture vulnerability for toxins relevant to HE: between days 11 and 22 after cell plating. The test agents were evaluated with the two assays during a 5 hour test period. For all assays, a 96-well format was used. For the screen, log concentration-effect studies were conducted 10 nM to 100 μM with 5 replications. Cultures were given a complete change of medium prior to the initiation of the treatment period. Testing for neuroprotection from ammonium acetate and ethanol was tested separately to demonstrate the relevance to HE. At the conclusion of the test period, the cultures were evaluated with fluorescent dye-based assays for cell death (propidium iodide) and for neuronal viability (CFDA). These two standard assays were chosen because they could be measured as multiplexed determinations within a single well thereby monitoring both an increasing (cell death) parameter and a decreasing parameter (neuronal viability) after a toxic treatment of the hippocampal cultures. For the cell death assessment with the propidium iodide, slight modification to a method previously described was used (Sarafian et al. 2002). Propidium iodide (PI) stock solution of 1 mg/mL (1.5 mM) was diluted 1:30 in DPBS, for a final working concentration of 50 μM. After removal of the growth medium, 50

μL of the 50 μM PI solution was added to the cultures and allowed to incubate in the dark at room temperature for 15 min. On every plate, wells without cells were used to provide a blank reading that was used to subtract background fluorescence. The cultures were assessed for fluorescence intensity at Ex536/Em590 nm in a CytoFluor fluorimeter (Perceptive Biosystems). Results were expressed in relative fluorescent units and EC_{50}s calculated from the dose–responses of the test compound and compared to values obtained from controls and wells treated with toxin alone. All compounds were screened with an N = 5 at each test concentration and the EC50 value determined. For compounds of highest interest (CBD, **1**; KLS-13007, **10**; KSL-13019, **16**; KLS-13022, **18**), the EC50 ± SE was determined from three replicate experiments with N = 5 for each experiment.

CB1 binding assay:

Purpose: test 3 compounds in binding assay at 10 μM.

Protocol:

Cell membrane homogenates (20 μg protein) are incubated for 120 min at 37°C with 0.5 nM [^{3}H]CP 55940 in the absence or presence of the test compound in a buffer containing 50 mM Tris-HCl (pH 7.4), 5 mM $MgCl_2$, 2.5 mM EDTA and 0.3% BSA.

Nonspecific binding is determined in the presence of 10 μM WIN 55212-2.

Following incubation, the samples are filtered rapidly under vacuum through glass fiber filters (GF/B, Packard) presoaked with 0.3% PEI and rinsed several times with an ice-cold buffer containing 50 mM Tris-HCl (pH 7.4) and 0.5% BSA using a 96-sample cell harvester (Unifilter, Packard). The filters are dried then counted for radioactivity in a scintillation counter (Topcount, Packard) using a scintillation cocktail (Microscint 0, Packard).

The results are expressed as a percent inhibition of the control radioligand specific binding.

The standard reference compound is CP 55940 which is tested in each experiment at several concentrations to obtain a competition curve from which its IC_{50} is calculated.

Reference: Rinaldi-Carmona et al., 1996

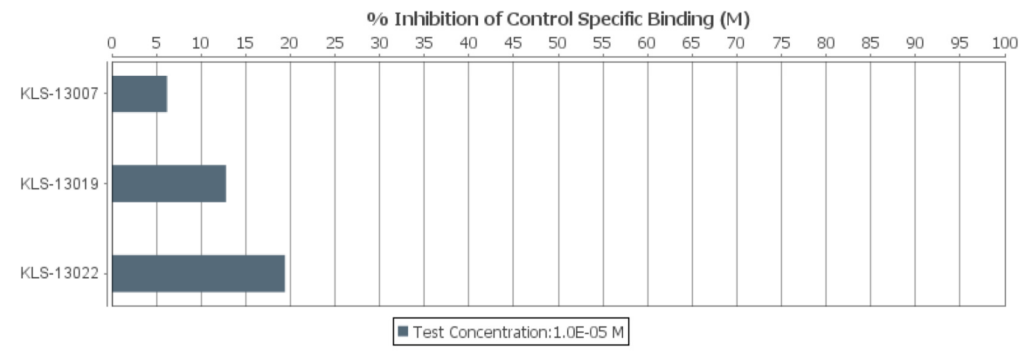


Figure 1. Histogram for CB1(h) (agonist radioligand)

KLS-13007 is **10**, KSL-13019 is **16**, KLS-13022 is **18**

CB2 binding assay:

Purpose: test 3 compounds in binding assay at 10 µM.

Protocol:

Cell membrane homogenates (12 µg protein) are incubated for 120 min at 37°C with 0.8 nM [^{3}H]WIN 55212-2 in the absence or presence of the test compound in a buffer containing 50 mM Hepes/Tris (pH 7.4), 5 mM MgCl2, 2.5 mM EGTA and 0.1% BSA. Nonspecific binding is determined in the presence of 5 µM WIN 55212-2.

Following incubation, the samples are filtered rapidly under vacuum through glass fiber filters (GF/B, Packard) presoaked with 0.3% PEI and rinsed several times with ice-cold 50 mM Tris-HCl using a 96-sample cell harvester (Unifilter, Packard). The filters are dried then counted for radioactivity in a scintillation counter (Topcount, Packard) using a scintillation cocktail (Microscint 0, Packard).

The results are expressed as a percent inhibition of the control radioligand specific binding.

The standard reference compound is WIN 55212-2 which is tested in each experiment at several concentrations to obtain a competition curve from which its IC_{50} is calculated.

Reference: Munro et al., 1993

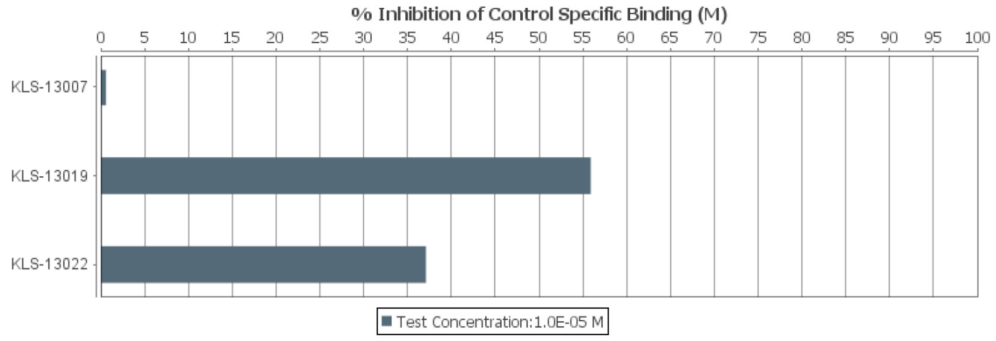


Figure 2. Histogram for CB2(h) (agonist radioligand)

KLS-13007 is **10**, KSL-13019 is **16**, KLS-13022 is **18**

Reference Compound	IC_{50} (M)	K_i (M)	nH
CB_1 *(h)* (agonist radioligand)			
CP 55940	5.2E-10 M	4.6E-10 M	0.8
CB_2 *(h)* (agonist radioligand)			
WIN 55212-2	9.5E-10 M	6.2E-10 M	1.3

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