The synthesis of WAY-derivatives to find linker location and a new linker to attach a fluorescence tag for homo- or hetero-dimer of 5-HT receptor ligands

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A Thesis Presented to

the Faculty of the Department of Chemistry

University of Houston

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

By

Soo Jeong Kim

May 2014

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ABSTRACT

Serotonin action or inaction is known to correlate with depression, obesity and drug addiction. The serotonin receptor has been studied as a possible target for cocaine addiction therapy. Previously, 5-HT_{2A} receptor antagonist, M100907 and 5-HT_{2C} receptor agonist, WAY 163909 have been studied as molecules to control behaviors related to addiction. Additionally, serotonin receptor families such as 5-HT_{2A} and 5-HT_{2C} receptors have been found to exist as dimers and oligomers. The focus of this thesis has been on the synthesis and bioactivity of multivalent ligands of M100907 and WAY 163909 for homo- and hetero dimeric ligands.

As an extension of previous research, this study includes development of WAY derivatives, which can be developed for homo- or hetero dimeric ligands.

In addition, a new linker for homodimers of M100907 derivatives has been developed. Through this new linker, a fluorescence tag can be attached to the dimeric ligands. This will be used to both probe the activity of receptor dimers as well as visualize them.

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List of Schemes

A. Introduction

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List of abbreviations

AcOH: Acetic acid

Boc: *tert*-Butoxycarbonyl

Cbz: Carboxybenzyl

CSA: (1S)-(+)-10-Camphorsulfonic acid

DMF: Dimethylformaide

DML: Designed multiple ligands

EDCI: 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide

EtOAc: Ethyl Acetate

HOBT: Hydroxybenzotriazole

LC-MS: Liquid chromatography-mass spectroscopy

MeOH: Methanol

Mesyl: Methanesulfonyl

n-BuLi: n-Butyllithium

NMR: Nuclear magnetic resonance

PCC: Pyridinium chlorochromate

PDC: Pyridinium dichromate

p-TSA: *p*-Toluenesulfonic acid

TBAF: Tetrabutylammonium fluoride

TBDPS: *tert*-Butyldiphenylsilyl

TBS: *tert*-Butyldimethylsilyl

Tf (Triflyl): Trifluoromethanesulfonyl

THF: Tetrahydrofuran

TLC: Thin layer chromatography

TMEDA: Tetramethylethylenediamine

TFA: Trifluoroacetic acid

TPAP: Tetrapropylammonium perruthenate

Ts: *p*-Toluenesulfonyl

NMO: N-methylmorpholine N-oxide

PPA: Polyphosphoric acid

Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is one of the important neurotransmitters in central nervous system (CNS). Serotonin works through its receptors.[1, 3] Based on their structural and operational characteristics, serotonin receptors are classified into seven families (5-HT_1 to 5 HT_7).[2] Among those seven families, only the 5-HT_3 receptor is a ligand-gated ion channel receptor, all others are G-protein-coupled receptors (GPCRs). Serotonin and its receptors are well known to be involved in various diseases such as major depression, anxiety, social phobia, obesity, schizophrenia and drug addiction.[1,2]

Based on those important roles, research on serotonin and its receptors, especially their critical role in behaviors related to addiction, have been conducted. Dopamine neurotransmission and indirect activation of dopamine receptors have been known as a central mediator of the cocaine responses.[3,4] However, dopamine receptor-targeted pharmacological treatment for cocaine addiction has not been successful because controlling dopamine receptors has little efficacy and causes various side effects resulting from dopamine's many physiological functions.[6] Instead of controlling the dopamine receptor, serotonin

receptors have been the focus as an alternative target for cocaine addiction treatment.

Cocaine-withdrawal symptoms include depression, ahedonia, insomnia, fatigue, hyperphagia and drug craving.[3] Depression is aggravated indicating a drop or disruption of 5-HT neurotransmission induced by cocaine withdrawal.[3] Preclinical studies have shown that the duration of cocaine self-administration is directly proportional to the drop in 5-HT level in the nucleus accumbens region of the brain during cocaine withdrawal.[3, 7] Interestingly, dopamine in the nucleus accumbens also decreased in cocaine withdrawal, but the deficit was smaller than the drop of the 5-HT level.[3]

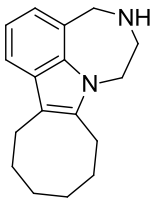
Based on preclinical studies, there are two possible approaches to alleviate drug craving.[3] One way is to apply indirect 5-HT agonists to counter the decreases of 5-HT level during 5-HT deficit. This suppresses conditioned stimulus-induced seeking behavior. Additionally, 5-HT_{1B} and 5-HT_{2C} receptor agonists diminish effects of cocaine itself. Another way is to reduce motivational effects of cocaine by 5-HT_{1A} and 5HT_{2A} receptor antagonists and cocaine-associated conditioned stimuli by 5-HT_{2A} receptor antagonists.

Our research group has focused on the 5-HT_{2A} and 5-HT_{2C} receptors because studies have shown that the 5-HT_{2A} and 5-HT_{2C} receptors play important roles in the effects of cocaine. For example, in discriminative stimulus effects of cocaine in rats, a 5-HT_{2A} receptor antagonist and 5-HT_{2C} receptor agonist showed the ability to inhibit the physiological responses resulting from cocaine administration.[8] Additionally, a 5-HT_{2A} receptor antagonist been shown to have the ability to prevent cue-evoked reinstatement of cocaine-seeking behavior in a rats.[9]

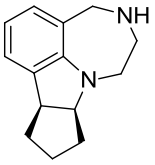
However, to date many studies have demonstrated the existence of homodimer and heterodimer of G-protein-coupled receptors (GPCRs) including 5-HT receptors.[10,11] One of the first examples is expression of 5-HT_{1B} receptor in Sf9 cells which indicates that 5-HT_{1B} receptor may form homodimer.[12] Additionally, the formation of homodimers of both 5-HT_{1B} and 5-HT_{1D} receptors has been observed. Direct evidence of heterodimerization resulting from a physical association of the 5-HT_{1B} receptor with the 5-HT_{1D} receptor has also been reported.[13] The 5-HT_{2C} receptor exists as homodimers in the plasma membrane of living cells as determined by fluorescence correlation spectroscopy (FCS) photon counting histogram (PCH) data.[14] and by combination of immunoprecipitation,

Westernblot, bioluminescence resonance energy transfer (BRET) spectroscopy and fluorescence resonance energy transfer (FRET) spectroscopy.[10]

Recently, there has been growing interest in the design of multiple ligands (DMLs) or multivalent ligands which modulate simultaneously multiple targets of relevance to a disease.[15, 16] 5-HT receptors are also targeted because enhanced efficacy and improved safety from DMLs may be expected[15, 16] as well as the role of receptor dimerization can be probed.[17a] In addition, divalent ligands for 5-HT serotonin receptors might have better pharmacological profiles than their respective monomeric ligands.[17a, 18, 19, 20] Affinity and selectivity of the multivalent ligands are affected by a combination of three main variables involved in multivalent ligands design: linker distance, point of attachment, structure of ligand fragment.[18]



WAY-470 (**1**)



WAY-163909 (**2**)

Figure 1. WAY-470 and WAY-163909

Our research group has been interested in the synthesis of WAY-470 (**1**) and WAY-163909 (**2**) derivatives to use in the development of multivalent ligands. Both WAY-470 and WAY-163909 have high affinity and good selectivity for the 5-HT_{2C} receptor as full agonists.[21] To find a proper ligand fragment and linker position, WAY-470 derivatives such as compounds **3**, **4**, **5** and **6** were prepared and tested as 5-HT_{2C} receptor agonists. However, the Ca^{+2} stimulation assay of those compounds showed they are not 5 HT_{2C} receptor agonists.

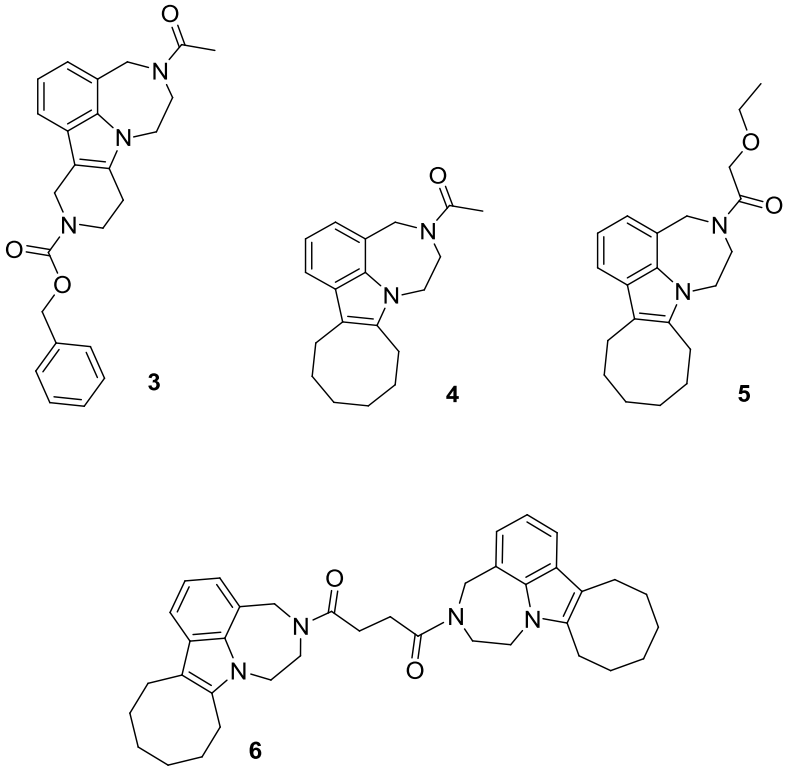


Figure 2. Previous WAY-derivatives

Additionally, WAY-163909 derivatives such as **7**, **8**, **9** and **10** were synthesized and tested. However, EC_{50} values of those compounds were not as good as the value of WAY-163909 (**2**) (Table 1).[21] Therefore, to synthesize designed multiple ligands with the desired activity, it is necessary to develop different derivatives.

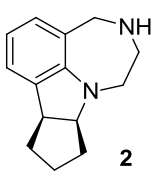
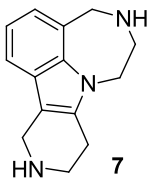
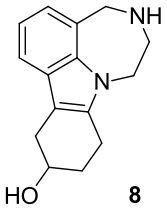
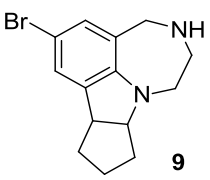
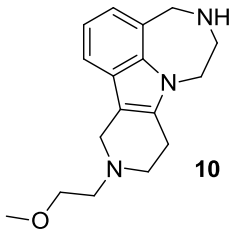
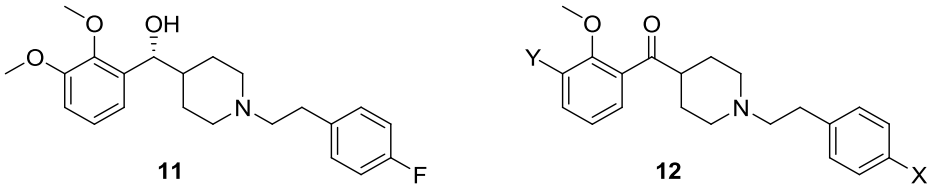
 **2**	 **7**	 **8**	 **9**	 **10**
EC_{50} 71 ± 15nM	EC_{50} 30 ± 14 μM	EC_{50} > 10 μM	EC_{50} > 10 μM	EC_{50} > 10 μM

Table 1. EC_{50} values of WAY-163909 derivatives



11 **12**

Figure 3. M100907 (11) and its derivatives (12) to find a proper linker site

Prior to this work, the synthesis of dimeric 5-HT_{2A} receptor antagonists and their optimization has been reported.[17a] Despite selectivity and high affinity of M-100907 (**11**) (IC_{50} = 3.3 nM), the structure-activity relationship

studies with derivatives of this molecule to develop multiple ligands or attach fluorescent tags and bioaffinity labels were rarely conducted. In that work, our group determined the proper site (Y on **12**) to attach a linker without significantly decreasing functional activity using the M100907 derivatives (**12**) which have ketone instead of the secondary alcohol.

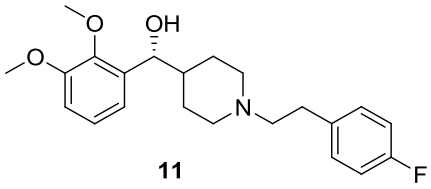
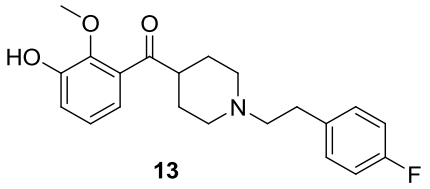
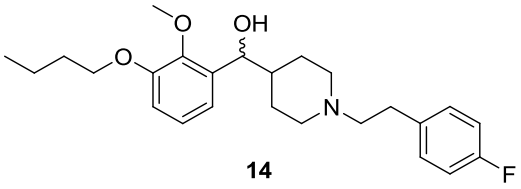
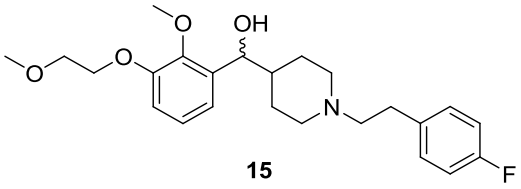
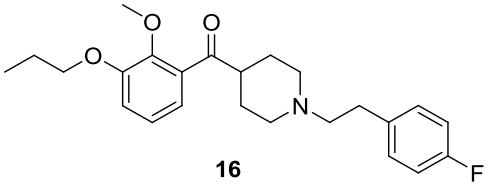
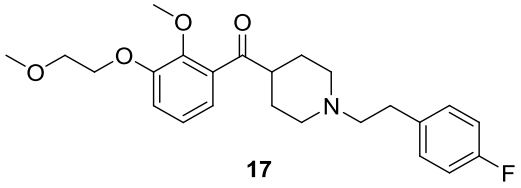
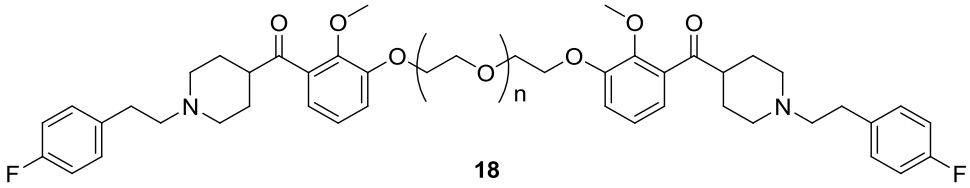
 **11**	 **13**
IC_{50} 3.3 nM	IC_{50} 2.7 ± 1.6 nM
 **14**	 **15**
IC_{50} 134 ± 30 nM	IC_{50} 120 nM
 **16**	 **17**
IC_{50} 873 ± 399 nM	IC_{50} 34 ± 16 nM

Table 2. IC_{50} values of M100907 derivatives

The M100907 derivatives showed reasonable potency as inhibitors of 5-HT $_{2A}$ receptor in Ca^{2+} assay in CHO cells (Table 2, **13** and **17**). Proper linker type

like ethylene glycol group was also found to show less deleterious inhibition of 5-HT-induced intracellular Ca^{2+} release (Table 2, **14** vs **15**, **16** vs **17**). To synthesize homodimer of M100907 derivative (**18**), proper linker length was tested resulting in increase of antagonist potency when linker length increased. Linker lengths with 12-18 atoms show comparable inhibition (Table 3). A portion of this thesis presents work to develop a linker that possesses a site at which a fluorescent tag can be attached.



18

Figure 4. Homodimer of M100907 derivative

n	atoms	IC_{50}
1	6	181 ± 71 nM
2	8	56 ± 14 nM
3	12	28 ± 16 nM
4	14	32 ± 6 nM
5	18	34 ± 10 nM
6	21	154 ± 25 nM
7	24	373 ± 153 nM

Table 3. Potencies of Homodimer as a Function of Length

Therefore, in this report, first of all, development of WAY-163909 derivatives will be discussed. Then, the synthesis of homodimer of M100907 derivatives with a new linker to which a fluorescence tag can be attached will be discussed.

Chapter 1. Synthesis of WAY Derivatives

Our group has worked to develop WAY derivatives that can be linked to other molecules in order to design multiple ligands with the desired activity. Based on the structure of WAY-163909 (**2**), structure-activity relationship (SAR) study progressed. A proper linker attachment site and ligand fragments with high activity have been studied (Figure 1.1). Different spots on benzene ring were tested to probe how each location affects bioactivity. Also, free amine was proved to be important to show agonistic activity.

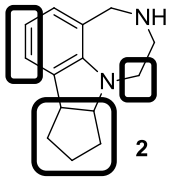


Figure 1.1. Structural Modification of WAY-163909

From the previous research, compound **8,** which has cyclohexane ring with alcohol substituent, did not possess good EC_{50} value (> 10 μM). As one of many SAR studies, we decided to change the substitution sites on the cyclohexane ring. The type of substituent is also changed from alcohol to ethylene

glycol group to prepare for synthesis of bivalent ligands. Therefore, three desired derivatives would be compounds **1.1**, **1.2** and **1.3** (Figure 1.2).

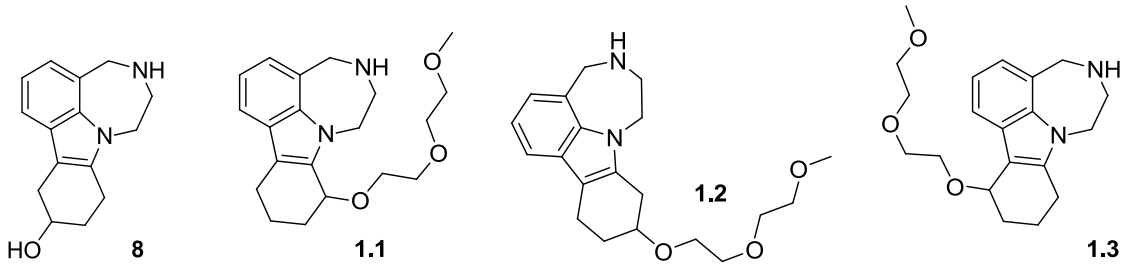
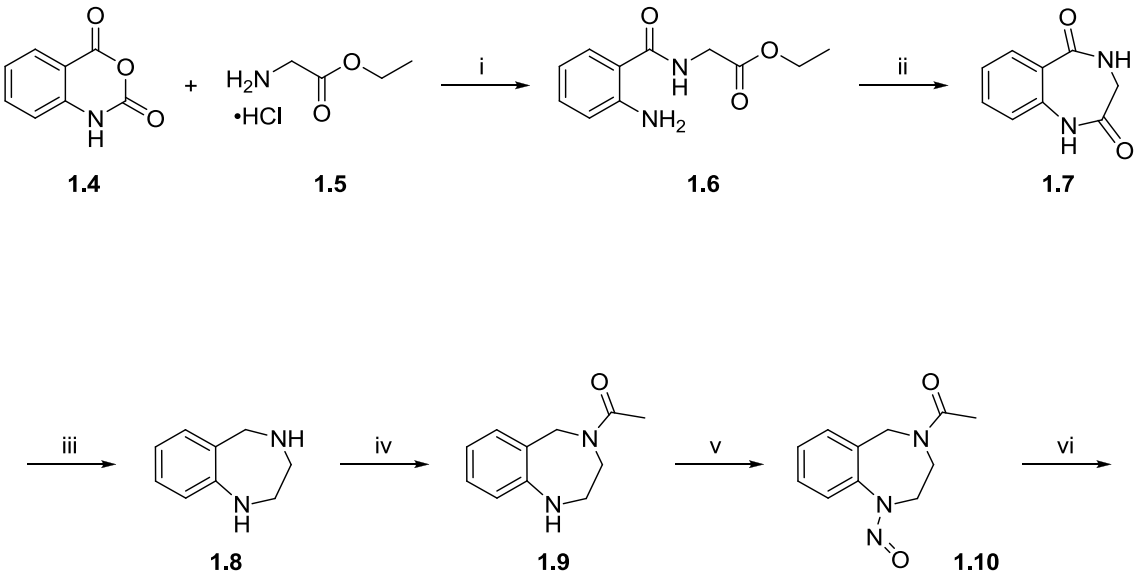
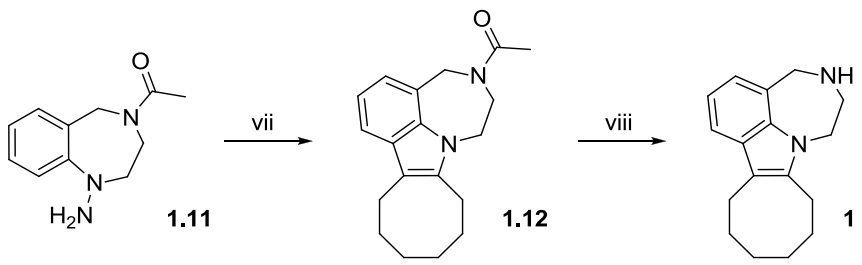


Figure 1.2. Desired WAY derivatives

To synthesize **1.1**, **1.2** and **1.3**, the modified synthetic route of WAY-470 from the previous research of Sabb was used (Scheme 1.1).[22]

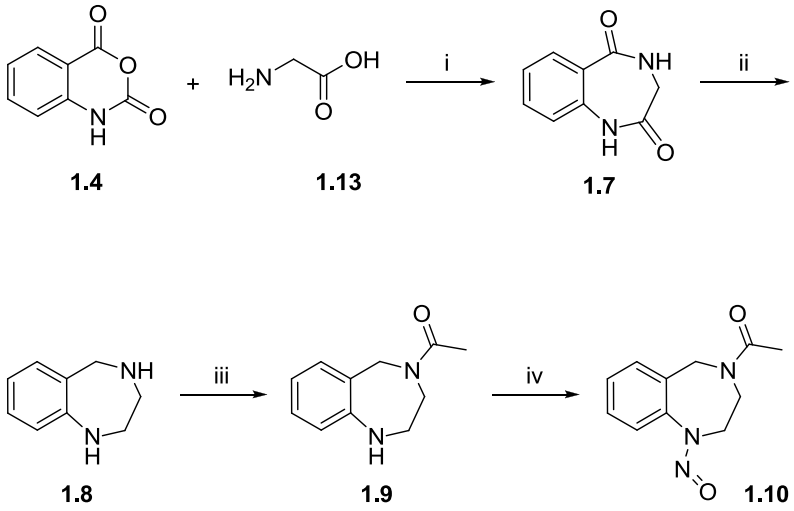


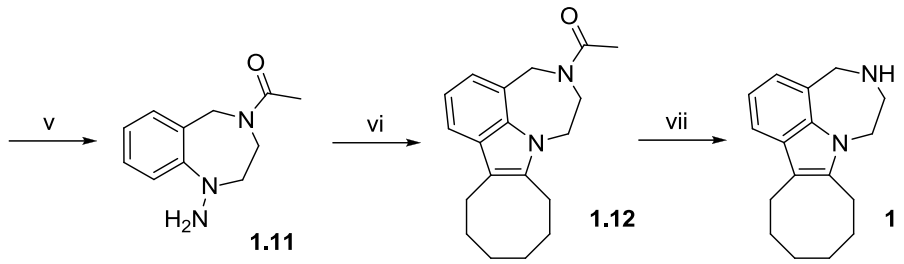


Reagents and conditions : (i) pyridine, reflux, 24 h, 40-50%; (ii) acetic acid, r.t., 24 h, 40-70%; (iii) lithium aluminum hydride, THF, r.t., 65-75%; (iv) acetic anhydride, triethylamine, ether, r.t., 24 h, 90%; (v) sodium nitrite, aq. HCl; (vi) zinc, acetic acid; (vii) cyclooctanone, acetic acid, 75-90 ˚C, 1.5 h, 20%; (viii) aq. NaOH, MeOH, or conc. HCl

Scheme 1.1. Synthetic Route to WAY-470 by Sabb

In the modified route[21] (Scheme 1.2), using glycine (**1.13**) instead of using glycine ester hydrochloride (**1.5**), increased the yield from 20% to 86%. Additionally, compound **1.7** could be formed only in a single reaction. Purification is also easy without column chromatography.

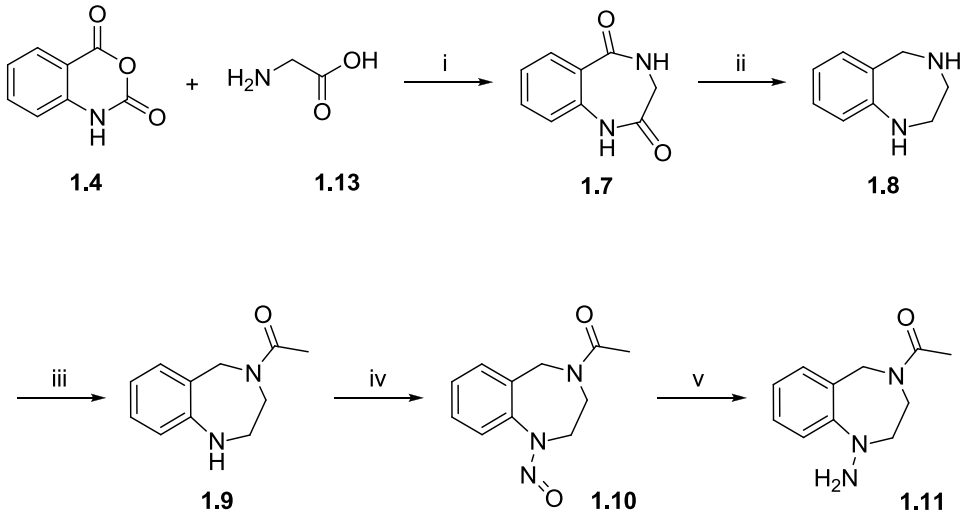




Reagents and conditions : (i) 6M NaOH, 100 ˚C, 4 h, L-tartaric acid, 100 ˚C, 1 h, 86%; (ii) lithium aluminum hydride, THF, reflux, 24 h, 93%; (iii) K_2CO_3, acetic anhydride, CH_3CN, r.t., 21 h, 89%; (iv) sodium nitrite, aq. HCl, r.t., 24 h, 86%; (v) $TiCl_4$, Mg, CH_2Cl_2, ethyl ether, 0.75 h, 91%; (vi) cyclooctanone, acetic acid, reflux, 15 h, 90%; (vii) 6M NaOH, reflux, 5 d, 83%

Scheme 1.2 Modified Synthetic Route to WAY-470

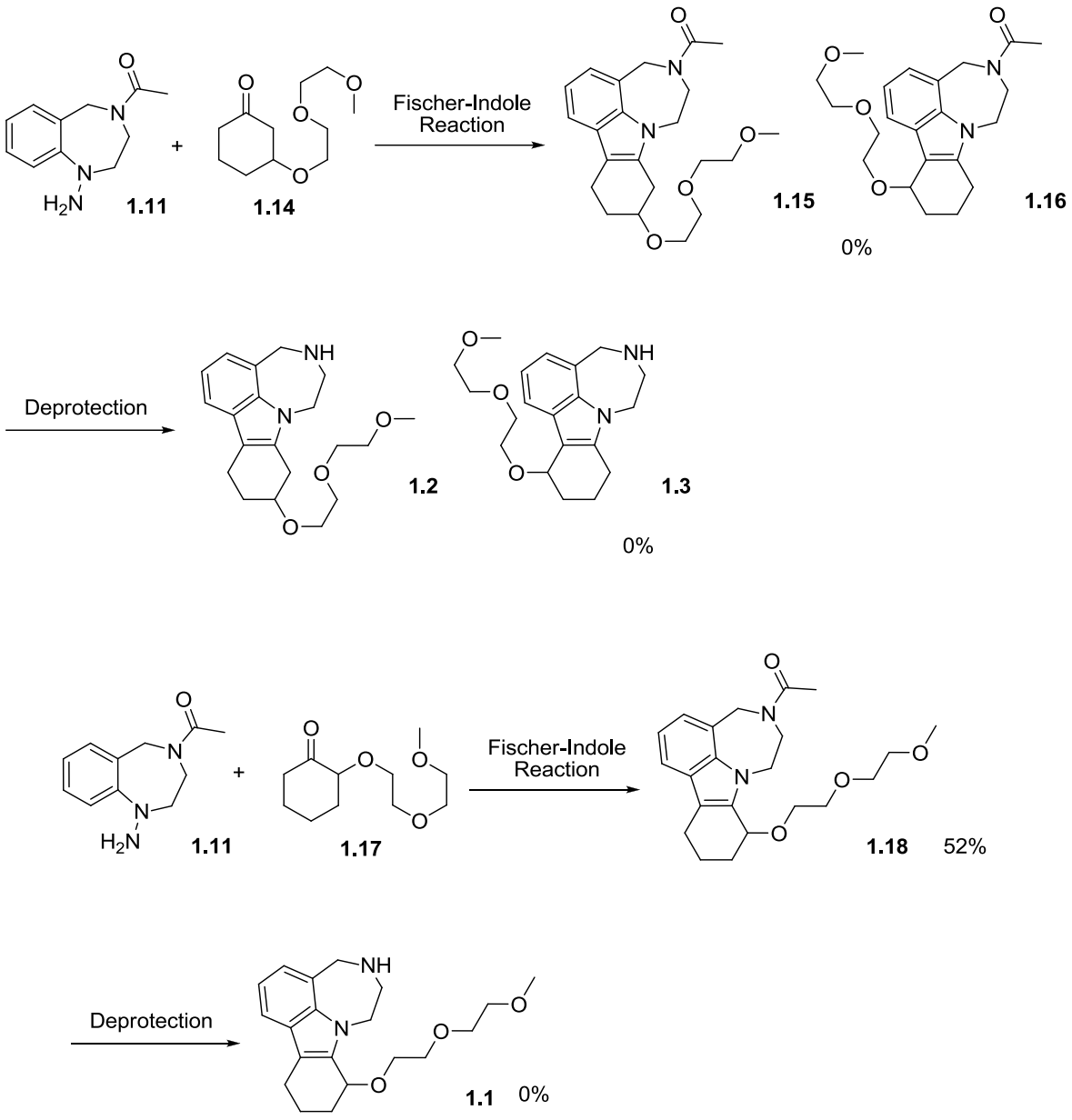
The modified route is used to obtain compound **1.11**. Reaction yields were similar to previous research (Scheme 1.3).



Reagents and conditions : (i) 6M NaOH, 100 ˚C, 4 h, L-tartaric acid, 100 ˚C, 1.5 h, 71%; (ii) lithium aluminum hydride, THF, 65 ˚C, 21 h, 99%; (iii) K_2CO_3, acetic anhydride, CH_3CN, r.t., 21 h, 90%; (iv) sodium nitrite, aq. HCl, r.t., 41 h, 98%; (v) $TiCl_4$, Mg, CH_2Cl_2, ethyl ether, 0.75 h, 93%

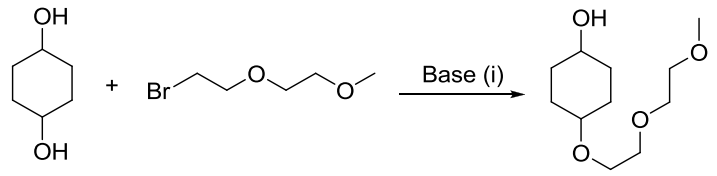
Scheme 1.3 Modified Synthetic Route to 1.11

Ethylene glycol substituted cyclohexanones (**1.14**, **1.17**) react with **1.11** under acidic conditions to synthesize **1.15**, **1.16** and **1.18** (Scheme 1.4).



Scheme 1.4 Synthetic Route to New WAY-derivatives

For preparation of the compounds **1.14** and **1.17**, 1,4-cyclohexanediol (**1.19**) which has less steric hindrance than 1,3- or 1,2-cyclohexanediol is tested first in S_N2 reaction (Scheme 1.5, Table 1.1). However, the reaction didn't provide the desired product even though a number of conditions were used. The starting materials didn't react (Entry 1, 3) or they decomposed during the reaction (Entry 2, 4) as determined by thin layer chromatography (TLC).



1.19 **1.20** **1.21**

Scheme 1.5 Test Study of S_N2 Reaction using 1,4-cyclohexanediol

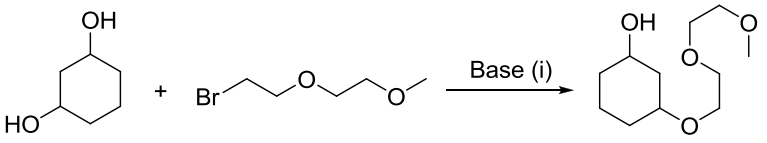
Entry	Base	Solvent	Time	Temp.	Yield
1	NaH	DMF	1 d → 1 d	r.t. → 50 °C	0%
2	NaH	DMF	26 h	50 °C	0%
3	K_2CO_3	CH_3CN	7 h	r.t.	0%
4	K_2CO_3	CH_3CN	44 h → 1 d	r.t. → 50 °C	0%

Table 1.1. Reaction conditions (i) for Scheme 1.5

However, interestingly, 1,3-cyclohexanediol reacted (**1.22**) with 1-bromo-2-(2-methoxyethoxy)ethane (**1.20**) using NaH as a base (Scheme 1.6, Table 1.2). The reaction on the first attempt (Entry 1) because NaH was directly added

to 1,3-cyclohexanediol (**1.22**) without ice-bath to cool down the reaction mixture. However modified reacton condictions provided the desired product. This procedure was also applied to the above reactions for 1,4-cyclohexanediol (**1.19**).

1,3-cyclohexanediol (**1.22**) in DMF was cooled to 0 ˚C. Then, NaH in dimethylformamide was slowly added to reaction mixture of 1,3-cyclohexanediol (**1.22**) via cannula. After that, the temperature was increased to room temperature over 1 hour, then the reaction mixture was heated to 50 ˚C for 2 hours. 1-bromo-2-(2-methoxyethoxy) ethane (**1.20**) was added later after cooling. When DMF was used as a solvent, the yield was only 14% even though the reaction time was 3 days and the temperature was upto 150 ˚C (Entry 2). However, when tetrahydrofuran (THF) was used, the reaction time was 20 hours at 80 ℃ providing the better yield, 48% (Entry 3).



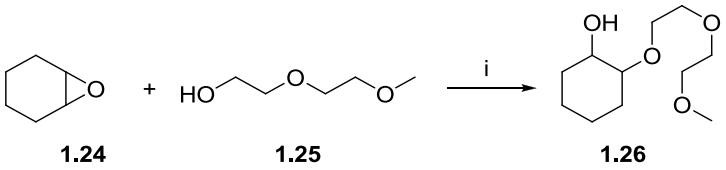
1.22 **1.20** **1.23**

Scheme 1.6 Preparation of 1.23 using 1,3-cyclohexanediol

Entry	Base	Solvent	Time	Temp.	Yield
1	NaH	DMF	21 h → 22 h	80 °C → 95 °C	0%
2	NaH	DMF	3 d	110 °C → 150 °C	14%
3	NaH	THF	20 h	80 °C	48%

Table 1.2. Reaction conditions (i) for Scheme 1.6

For the preparation of 2-(2-(2-methoxyethoxy) ethoxy) cyclohexanol (**1.26**), a different strategy was applied (Scheme 1.7).[23] Cyclohexene oxide (**1.24**) reacted with 2-(2-methoxyethoxy) ethanol (**1.25**) using catalytic amount of NaH for 4 hours at 110 ℃. The maximum reaction yield was 84% on 5 g scale.



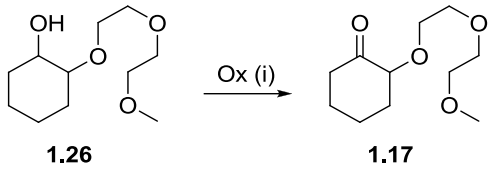
1.24 **1.25** **1.26**

Reagents and conditions : (i) NaH, 110 °C, 4 h, 84%

Scheme 1.7 Preparation of 1.26 using Cyclohexene Oxide

To oxidize the alcohol on the cyclohexane ring (Scheme 1.8), pyridinium chlorochromate (PCC) and pyridinium dichromate (PDC) were applied (Table 1.3). However, even though the starting material (**1.26**) almost disappeared under PCC, the desired product was not indentified in the crude reaction mixture by NMR (Entry 1). The reaction using PDC proved the desired product but in low yield. A new spot was seen by TLC, but purification was

difficult because the amount of the desired product was small (Entry 2). When the reaction time was increased, the yield was improved, but still only to 10%, with many side products visible on a TLC plate (Entry 3). It seems that pyridinium salts are inefficient or too strong to oxidize the alcohol compound which has an ethylene glycol chain in the same molecule.



1.26 1.17

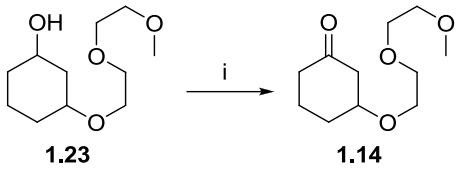
Scheme 1.8 Oxidation of 1.26

Entry	Oxidant	Solvent	Time	Temp.	Yield
1	PCC	CH_2Cl_2	5 h	r.t.	0%
2	PDC	CH_2Cl_2	4 h → 20 h	0 °C → r.t.	0%
3	PDC	CH_2Cl_2	43 h	r.t.	10%
4	TPAP, NMO	CH_3CN	21.5 h	r.t.	62%

Table 1.3. Reaction conditions (i) for Scheme 1.8

To increase the reaction yield, tetrapropylammonium perruthenate (TPAP) and co-oxidant N-methylmorpholine N-oxide (NMO) were chosen.[24] The reaction using activated 4 Å molecular sieves (500 mg/mmol) gave a 62% yield (Table 1.3, Entry 4).

Using these reaction conditions, compound **1.23** was also oxidized in 57% (Scheme 1.9). 4 Å molecular sieves should be freshly activated for every reaction, otherwise, reaction yields decreased to 24% with **1.26** and 27% with **1.23**.

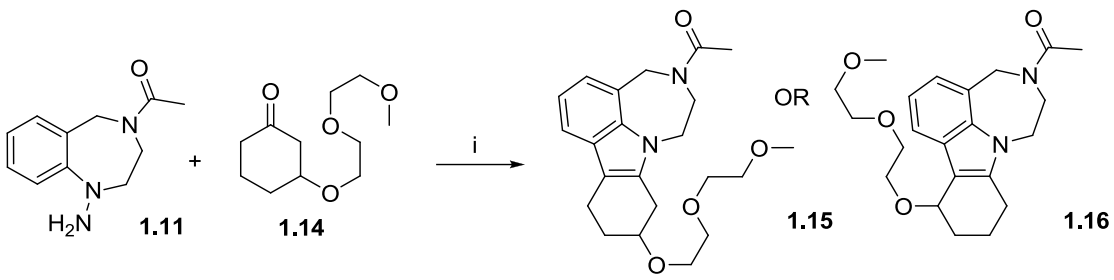


1.23 **1.14**

Reagents and conditions : (i) TPAP, NMO, CH_3CN, 4 Å molecular sieves, 17 h, r.t. 57%

Scheme 1.9 Oxidation of 1.23

For synthesis of the desired WAY-derivatives, the key step is the Fischer-Indole reaction. **1.14** was tried first using *p*-toluenesulfonic acid (*p*-TSA) (Scheme 1.10, Table 1.4). However, reaction did not proceed, with only decomposition of the starting material (Entry 1-3). After purification of the side products, they were analyzed by NMR spectroscopy. From the analysis, **1.27** was identified (Figure 1.3). The main issue was, because it is easily decomposed, **1.11** needs to be freshly prepared for each Fischer-Indole reaction. However, for those three reactions (Entry 1-3), **1.11** was prepared in a large scale and stored in a refrigerator.



Scheme 1.10 Fischer-Indole Reaction with 1.11 and 1.14

Entry	**1.11**	**1.14**	Acid	Solvent	Time	Temp.	Yield
1	1 eq	1.2 eq	*p*-TSA 1.1 eq	Toluene	20.5 h	75 ˚C	0%
2	1 eq	2.4 eq	*p*-TSA 1.2 eq	Toluene	19 h	75 ˚C	0%
3	1.2 eq	1 eq	*p*-TSA 1.2 eq	Toluene	2.5 h	105 ˚C	0%
4	1.2 eq	1 eq	*p*-TSA 1.2 eq	Toluene	2.5 h	105 ˚C	0%
5	1.2 eq	1 eq	*p*-TSA 1.2 eq	Toluene	2.5 h	105 ˚C	0%

Table 1.4. Reaction conditions (i) for Scheme 1.10

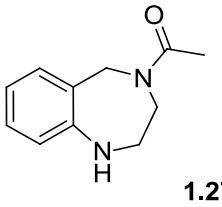
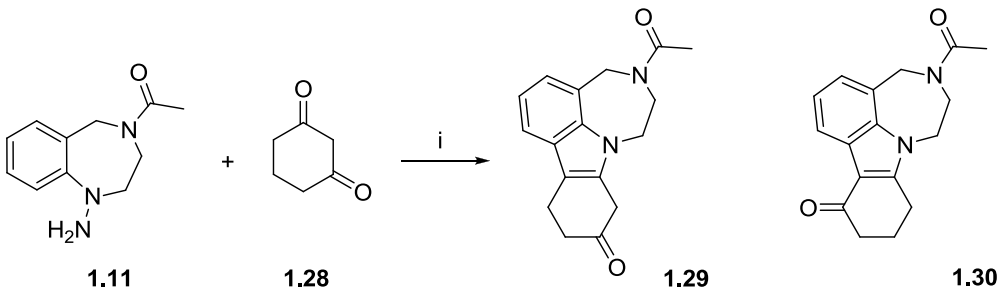


Figure 1.3. Side Product from the Fischer-Indole Reaction with 1.11 and 1.14

However, despite using freshly prepared **1.11** (Entry 4, 5), both **1.15** and **1.16** where not formed. Since **1.14** has diethylene glycol group at the 3-position, it seemed to undergo β-elimination to give cyclohexenone.

To find a new strategy, a model study was conducted with 1,3-cyclohexanedione (**1.28**) in the same conditions (Scheme. 1.11). As expected, 1,3-cyclohexanedione (**1.28**) provided the desired products.



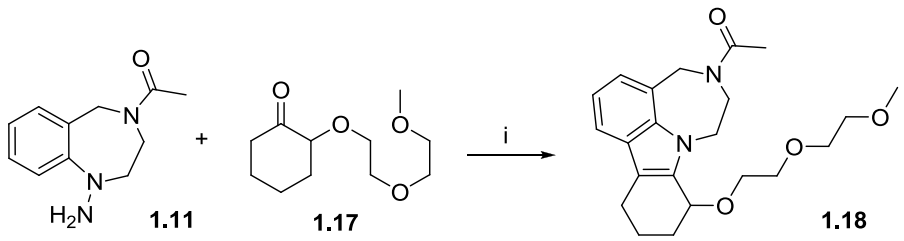
1.11 **1.28** **1.29** **1.30**

Reagents and conditions : (i) **1.11** (1.1 eq), **1.28** (1 eq), *p*-TSA (1.1 eq), toluene, 110 ˚C, 3 h, 0%

Scheme 1.11 Fischer-Indole Reaction with 1.11 and 1.28

Fischer-Indole reaction was also attempted with **1.17** (Scheme 1.12, Table 1.5). As with **1.14**, the desired product was not formed. After purification and analysis of all side products from the reaction (Entry 1), 2-(2-methoxyethoxy) ethanol (**1.25**) was the only product identified. It seems the ethylene glycol group was cleaved from the cyclohexanone when it was exposed to the acidic reaction conditions. Therefore, it was decided to reduce the reaction time even though starting materials were remained. After changing the ratio between reagents and reaction time, reaction yields increased to a maximum of 52% (Entry 2-4). Because it seemed that ethylene glycol group is liable to a Brønsted acid, Lewis acids, like

$ZnCl_2$ were tried (Entry 7). However, under those conditions starting materials did not react. In addition, other acids such as trifluoroacetic acid (TFA) and polyphosphoric acid (PPA) were applied to this reaction, resulting in much lower yields (Entry 5, 6).



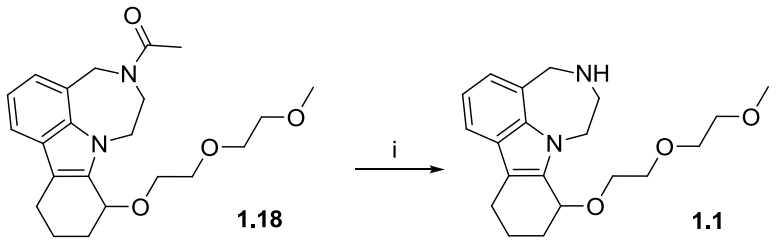
Scheme 1.12 Fischer-Indole Reaction with 1.11 and 1.17

Entry	**1.11**	**1.17**	Acid	Solvent	Time	Temp.	Yield
1	1 eq	1.5 eq	*p*-TSA 1.2 eq	Toluene	13 h	75 ˚C	0%
2	1.2 eq	1 eq	*p*-TSA 1.2 eq	Toluene	2 h	105 ˚C	1.4%
3	1 eq	1.05 eq	*p*-TSA 1.2 eq	Toluene	3 h	105 ˚C	8%
4	1.1 eq	1 eq	*p*-TSA 1.2 eq	Toluene	5 h	105 ˚C	52%
5	1.1 eq	1 eq	TFA	-	14 h	70 ˚C	4%
6	1.1 eq	1 eq	PPA excess	EtOH	3.5 h	reflux	4%
7	1.1 eq	1 eq	$ZnCl_2$ 1.2 eq	Toluene	4.5 h	105 ˚C	0%

Table 1.5. Reaction conditions (i) for Scheme 1.12

To deprotect the acetyl group, basic conditions were used following the previous research (Scheme 1.13, Table 1.6, Entry 1, 2). When 6M NaOH was used in methanol (MeOH), ethylene glycol group was (Entry 1). The concentration was considered to be too high so 1M NaOH was also applied to the deprotection

(Entry 2), but, the result was same. Acidic condition using hydrochloric acid (HCl) were tried (Entry 3). First, 1% of HCl was added to **1.18** in MeOH. After stirring the reaction mixture for 17.5 hours at room temperature, new spot did not appear on TLC plate. The temperature was increased to 70 ℃, stirring for 6 hours, but, still no reaction was observed. Because the concentration of HCl seemed too low, 1M HCl was added to the reaction stirring for 24 hours at 70 ℃. One tiny new spot on TLC plate appeared, but after aqueous work-up, it disappeared.



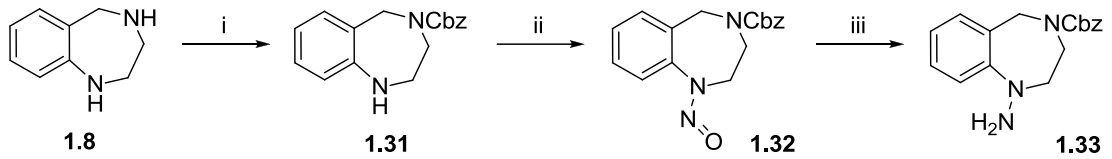
Scheme 1.13 Deprotection of Acetyl group

Entry	Reagent	Solvent	Time	Temp.	Yield
1	6M NaOH	MeOH	22 h	95 ˚C	0%
2	1M NaOH	MeOH	21.5 h	105 ˚C	0%
3	1% HCl	MeOH	17.5 h → 6 h	r.t. → 70 ˚C	0%
	→ 1M HCl		→ 24 h	→ 70 ˚C	

Table 1.6. Reaction conditions (i) for Scheme 1.13

Since the ethylene glycol group seemed to be sensitive to both acidic and basic conditions, we decided to change the amine protecting group from

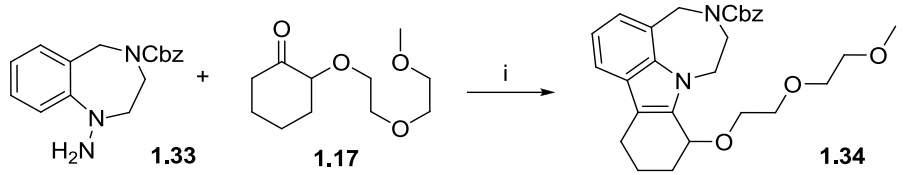
acetyl to carboxybenzyl (Cbz). To prepare **1.33**, the amine next to benzylic carbon of **1.8** was protected with carboxybenzyl group in 95% followed by nitration of the amine in 93%. Reduction of N-nitroso group resulted in the hydrazine compound **1.33** in 89% yield (Scheme 1.14).



Reagents and conditions : (i) **1.8** (1 eq), Benzyl chloroformate (1.1 eq), $NaHCO_3$ (6 eq), CH_2Cl_2/H_2O (1/1), 24 h, r.t., 95%. (ii) **1.31** (1 eq), $NaNO_2$ (1.2 eq), 0.8M HCl (1.5 eq), 5 h, r.t., 93%. (iii) **1.32** (1 eq), $TiCl_4$ (4 eq), Mg (4 eq), CH_2Cl_2/Et_2O (4/1), 0.75 h, r.t. 89%.

Scheme 1.14 Preparation of 1.33

Because **1.17** showed the better result in Fischer-Indole reaction than **1.14**, **1.17** was reacted with **1.33** under acidic conditions (Scheme 1.15, Table 1.7). As before, *p*-TSA was used in toluene refluxing the reaction mixture for 1.5 hours (Entry 1). However, for **1.33**, the desired product (**1.34**) was not formed. Even though the reaction time was increased to 24.5 h (Entry 2), the result did not change. NMR spectroscopy of both entry 1 and 2 did not include any aromatic protons indicating the starting **1.33** was decomposed. Lewis acid was not that different with *p*-TSA showing with decomposition of starting materials as the only result (Entry 3).

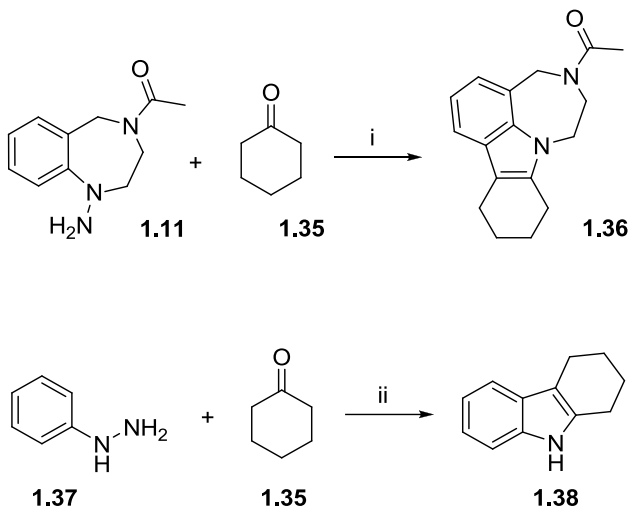


Scheme 1.15. Fischer-Indole Reaction with 1.33 and 1.17

Entry	**1.31**	**1.17**	Acid	Solvent	Time	Temp.	Yield
1	1.1 eq	1 eq	*p*-TSA 1.2 eq	Toluene	1.5h	110 ˚C	0%
2	1.1 eq	1 eq	*p*-TSA 1.2 eq	Toluene	24.5h	110 ˚C	0%
3	1.1 eq	1 eq	$ZnCl_2$ 1.2 eq	Toluene	4.5h	110 ˚C	0%

Table 1.7. Reaction conditions (i) for Scheme 1.15

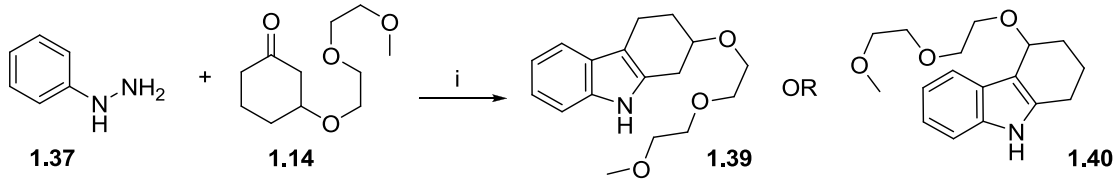
Alternative route was considered, Fischer-Indole reaction would be the first step in the alternative route because it is the most important and difficult step. Additionally, the new hydrazine **1.37** is commercially available. For model-study, cyclohexanone was reacted with **1.37** using *p*-TSA in H_2O.[25] As a result, **1.37** showed better reaction yield in 97% than **1.11** in 37% (Scheme 1.16).



Reagents and conditions : (i) **1.35** (1 eq), **1.11** (1.2 eq), *p*-TSA (1.2 eq), toluene, 2 h, 105 ˚C, 35%; (ii) **1.35** (1 eq), **1.37** (1 eq), *p*-TSA (1 eq), H_2O, 2.5 h, 80 ˚C, 97%

Scheme 1.16 Comparisons of Fischer-Indole Reactions with Two Hydrazines

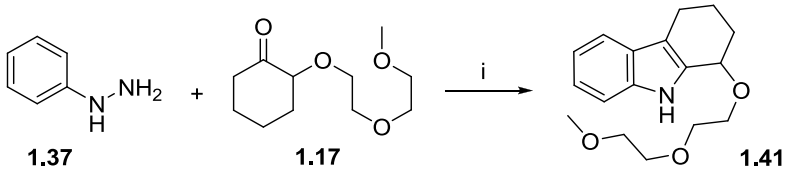
Unlike the model-study, the desired ketones **1.14** and **1.17** did not react with **1.37**. In the case of **1.14** using **1.37**, the result was not different from the previous one using **1.11,** showing decomposition of compounds (Scheme 1.17).



Reagents and conditions : (i) **1.14** (1 eq), **1.37** (1. eq), *p*-TSA (1 eq), H_2O, 2 h, 80 ˚C, 0%

Scheme 1.17 an Alternative Fischer-Indole Reaction with 1.14

1.17 was expected to show better result because it provided 52% yield in the previous reaction (Scheme 1.18, Table 1.8). In the case of *p*-TSA, several solvents were tested. However, during the reaction in toluene (Entry 1), cyclohexane ring of **1.17** decomposed. Since in the model study with **1.38**, H_2O seemed to be good solvent, H_2O used for the synthesis of **1.41** (Entry 2). After the reaction, some crystals were filtered. Since the crystals were not pure, they were purified by column chromatography. However, the NMR of the purified product did not include any peaks for diethylene glycol. The reaction residue after filtration was extracted with CH_2Cl_2, and then, monitored by NMR showing diethylene glycol peaks. Those results indicate during the reaction, diethylene glycol was cleaved from the cyclohexane ring. Entry 3 using mixed solvents with H_2O and CH_3CN and Entry 4 didn't work either. Strong Brønsted acids such as H_2SO_4 and HCl were not effective for **1.17** with diethylene glycol group showing decomposition of the substituents (Entry 6, 7). $ZnCl_2$ was not useful either just forming inorganic salts (Entry 8).



Scheme 1.18 an Alternative Fischer-Indole Reaction with 1.17

Entry	**1.37**	**1.17**	Acid	Solvent	Time	Temp.	Yield
1	1.1 eq	1 eq	*p*-TSA 1.2 eq	Toluene	4.5h	110 ˚C	0%
2	1 eq	1 eq	*p*-TSA 1 eq	H_2O	2h	80 ˚C	0%
3	1 eq	1 eq	*p*-TSA 1.2 eq	H_2O/CH_3CN	3d	80 ˚C	0%
4	1 eq	1 eq	*p*-TSA 1.2 eq	CH_3CN	5h	80 ˚C	0%
5	1 eq	2 eq	TFA	-	4h	70 ˚C	0%
6	1 eq	1 eq	H_2SO_4 1 eq	Toluene	6h	107 ˚C	0%
7	1 eq	1 eq	HCl 1 eq	Toluene	6h	110 ˚C	0%
8	1 eq	1 eq	$ZnCl_2$ 1.2 eq	Toluene	4h	110 ˚C	0%

Table 1.8. Reaction conditions (i) for Scheme 1.18

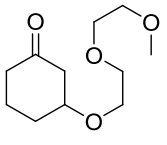
Even though Fischer-Indole reaction was not that effective for **1.14**, from **1.17**, **1.18** was formed in 52% yield. Deprotection of acetyl group should be studied for further research. WAY-derivatives studied here could be used for homo- or heterodimer of 5-HT receptor ligands.

Experimental

General Procedures:

Unless otherwise noted, all reagents were purchased from Sigma-Aldrich or Acros and used as received. Thin layer chromatography (TLC) was performed on silica gel 60 F_{254} pre-coated plates (0.25 mm) from Silicycle and components were visualized by UV light (254 nm) and/or 10% phosphomolybdic acid in ethanol stain, anisaldehyde or $KMnO_4$ solution. Chromatography was performed using Silicycle silica gel 230-400 (particle size 40-63 μm) mesh. ^{1}H and ^{13}C NMR were recorded on JEOL ECX-400 NMR spectrometer (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR) in chloroform-*d* at ambient temperature and/or 318 K (45 °C). Chemical shifts were referenced to the residual chloroform-H peak at 7.26 ppm for ^{1}H NMR and 77.16 ppm for ^{13}C NMR. Chemical shifts are reported in parts per million (ppm, δ). Multiplicity is indicated as s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublet, dt = doublet of triplet. Coupling constants (*J*) are reported in Hz. Liquid chromatography-mass spectrometry (LC-MS) was performed on a Thermo Finningan Surveyor instrument equipped with MSQ Plus single quadrupole detector.

3-(2-(2-methoxyethoxy)ethoxy)cyclohexanone (1.14)



1.14

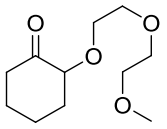
N-Methylmorpholine N-oxide (0.69 g, 5.89 mmol) was added to 3-(2-(2-methoxyethoxy)ethoxy)cyclohexanol (**1.23**) (0.85 g, 3.93 mmol) in 10 mL CH_3CN with 4Å molecular sieves (2.45 g). Tetrapropylammonium perruthenate (0.14 g, 0.39 mmol) was added to the mixture and stirred at room temperature for 17 hours. The reaction mixture was filtered through Celite® and silica gel. The filtrate was concentrated under reduced pressure, taken up in 200 mL CH_2Cl_2 and washed with 80 mL of saturated Na_2SO_4 once, 80 mL of saturated NaCl once and 80 mL saturated $CuSO_4$ once. The organic layer was dried over $MgSO_4$, filtered and concentrated. The crude products were purified by column chromatography (EtOAc : Hexane = 3 : 1) to afford 3-(2-(2-methoxyethoxy) ethoxy) cyclohexanone (**1.14**) in 57% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm); 3.81–3.72 (m, 1H), 3.64–3.56 (m, 6H), 3.54–3.48 (m, 2H), 3.35 (s, 3H), 2.60 (dd, *J* = 14.1, 3.4 Hz, 1H), 2.44 (dd, *J* = 14.1, 7.3 Hz, 1H), 2.28 (dd, *J* = 6.4 Hz, 2H), 2.06–1.91 (m, 2H), 1.86–1.74 (m, 1H), 1.69–1.55 (m, 1H);

^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) 209.75, 72.00, 70.80, 70.69, 67.84, 59.14, 47.70, 41.18, 29.93, 29.78, 20.67;

TLC R_f 0.3 (EtOAc : Hexane = 3 :1)

2-(2-(2-methoxyethoxy)ethoxy)cyclohexanone (1.17)



1.17

N-Methylmorpholine N-oxide (1.61 g, 13.7 mmol) was added to 2-(2-(2-methoxyethoxy)ethoxy)cyclohexanol (**1.26**) (2 g, 9.16 mmol) in 10 mL of CH_3CN with 4 Å molecular sieves (4.7 g). Tetrapropylammonium perruthenate (0.16 g, 0.46 mmol) was added to the mixture which was stirred at room temperature. After 21.5 hours, the reaction mixture was filtered through Celite® and silica gel. The filtrate was concentrated under reduced pressure, taken up in 400 mL CH_2Cl_2 and washed with 100 mL saturated Na_2SO_4 once, 100 mL saturated NaCl once and 100 mL saturated $CuSO_4$ once. The organic layer was dried over $MgSO_4$, filtered and concentrated. The crude products was purified by column chromatography

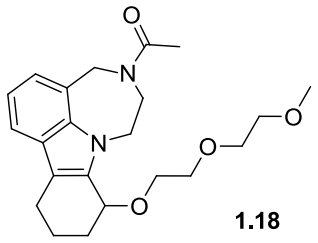
(EtOAc : Hexane = 1 : 1) to provide 2-(2-(2-methoxyethoxy)ethoxy)cyclohexanone (**1.17**) in 62% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm); δ 3.95–3.74 (m, 2H), 3.71–3.48 (m, 7H), 3.35 (s, 3H), 2.54–2.13 (m, 3H), 2.02–1.56 (m, 5H);

^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) δ 210.40, 83.44, 71.97, 70.93, 70.54, 69.45, 59.10, 40.72, 34.70, 27.77, 23.37;

TLC R_f 0.19 (EtOAc : Hexane = 1 :1)

1-(11-(2-(2-methoxyethoxy)ethoxy)-1,2,8,9,10,11-hexahydro-[1,4]diazepino[6,7,1-*j k*]carbazol-3(4*H*)-yl)ethanone (1.18)



1.18

2-(2-(2-methoxyethoxy)ethoxy)cyclohexanone (**1.17**) (0.2 g, 0.94 mmol) and 1-(1-amino-2,3-dihydro-1H-benzo[e][1,4]diazepin-4(5H)-yl)ethanone (**1.11**) (0.22 g, 1.04 mmol) dissolved in 2 mL toluene stirring for 0.5 hours. *p*-Toluenesulfonic acid

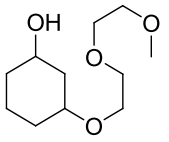
(0.22 g, 1.13 mmol) was added. The reaction mixture was refluxed at 105 ˚C for 5 hours. The mixture was cooled to room temperature, taken up in 50mL H_2O and extracted with 30 mL CH_2Cl_2 three times. The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography (EtOAc : Hexane = 3 :1) to provide 1-(11-(2-(2-methoxyethoxy)ethoxy)-1,2,8,9,10,11-hexahydro-[1,4]diazepino[6,7,1-jk]carbazol-3(4H)-yl)ethanone (**1.18**) in 52% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) δ 7.55 (dd, *J* = 21.5, 7.7 Hz, 1H), 7.19–7.03 (m, 2H), 5.12–4.95 (m, 2H), 4.84 (s, 1H), 4.17–4.01 (m, 2H), 3.92–3.77 (m, 2H), 3.74–3.60 (m, 4H), 3.60–3.51 (m, 2H), 3.54–3.42 (m, 1H), 3.38 (d, *J* = 2.7 Hz, 3H), 3.00 (t, *J* = 6.1 Hz, 2H), 2.72–2.60 (m, 2H), 2.26–2.17 (m, 2H), 2.14 (s, 3H);

^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) 192.45, 131.44, 131.06, 126.53, 124.83, 123.77, 120.93, 120.35, 77.45, 77.13, 76.81, 60.52, 51.60, 50.57, 47.60, 46.05, 45.59, 40.27, 40.16, 31.07, 29.81, 24.61, 22.24, 21.99, 14.31;

TLC R_f 0.2 (EtOAc : Hexane = 3 :1)

3-(2-(2-methoxyethoxy)ethoxy)cyclohexanol (1.23)



1.23

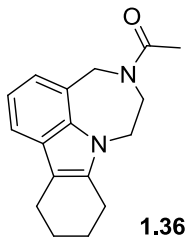
1,3-cyclohexanediol (1.65 g, 14.2 mmol) in THF 15 mL was slowly added to NaH (0.39 g, 16.4 mmol) at -78 ˚C. Temperature was increased slowly to room temperature for 2 hours. The reaction mixture was stirred for 1 hour at room temperature, and then, heated at 50 ˚C for 4 hours. 1-bromo-2-(2-methoxyethoxy)ethane (1.47 mL, 10.9 mmol) was added to the mixture. For 19.5 hours, the mixture was refluxed at 78 to 80 ˚C. After that, it was cooled to room temperature. 2N HCl was added until the pH is 4. The mixture was concentrated under reduced pressure. The residue was taken up in 500 mL water and then, extracted 3 times with 300 mL CH_2Cl_2. The combined organic layers are dried over MgSO4 and filtered. It was concentrated under reduced pressure to afford 3-(2-(2-methoxyethoxy)ethoxy)cyclohexanol (**1.23**) in 48% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm); δ 3.84–3.42 (m, 10H), 3.36 (s, 3H), 1.98 (d, *J* = 12.3 Hz, 1H), 1.90–1.33 (m, 6H), 1.33–1.05 (m, 1H);

^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) 76.33, 72.02, 70.88, 70.66, 68.28, 67.69, 59.15, 38.97, 34.16, 30.35, 18.02;

TLC R_f 0.2 (EtOAc : Hexane = 3 :1)

1-(1,2,8,9,10,11-hexahydro-[1,4]diazepino[6,7,1-*jk*]carbazol-3(4*H*)-yl)ethanone (1.36)



1.36

Cyclohexanone (**1.35**) (0.1 g, 1.02 mmol) and 1-(1-amino-2,3-dihydro-1H-benzo[e][1,4]diazepin-4(5H)-yl)ethanone (**1.11**) (0.26 g, 1.22 mmol) were dissolved in 2 mL toluene. After 0.3 hours, *p*-toluenesulfonic acid (0.23 g, 1.22 mmol) was added. The reaction mixture was refluxed at 105 °C for 2 hours. After cooling to room temperature, the mixture was taken up in 50 mL H_2O and extracted with 30 mL CH_2Cl_2 5 times. The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by

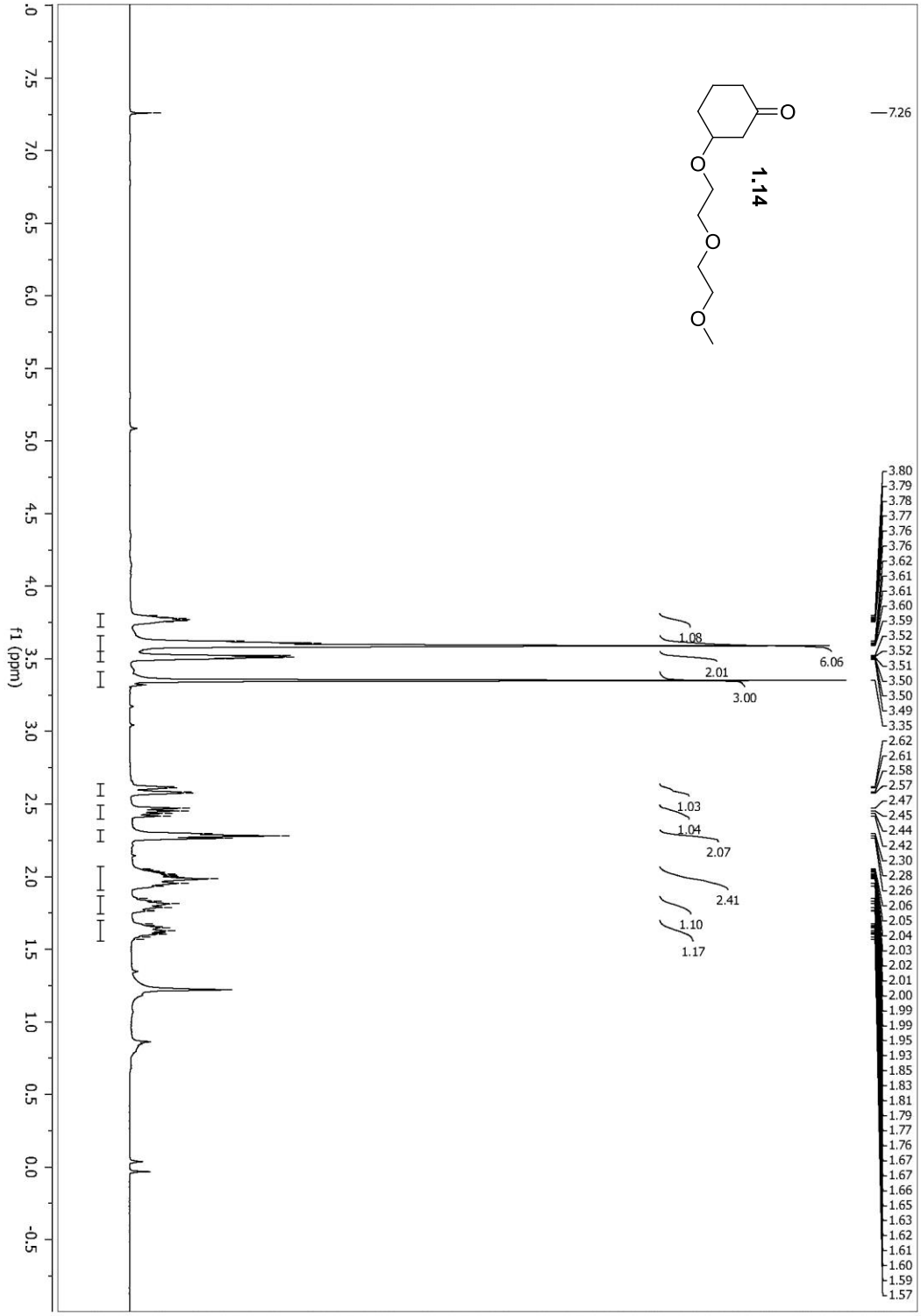
column chromatography (EtOAc : Hexane = 1 : 3 to 95 : 1) to provide 1-(1,2,8,9,10,11-hexahydro-[1,4]diazepino[6,7,1-*jk*]carbazol-3(4H)-yl)ethanone (**1.36**) in 37% yield.

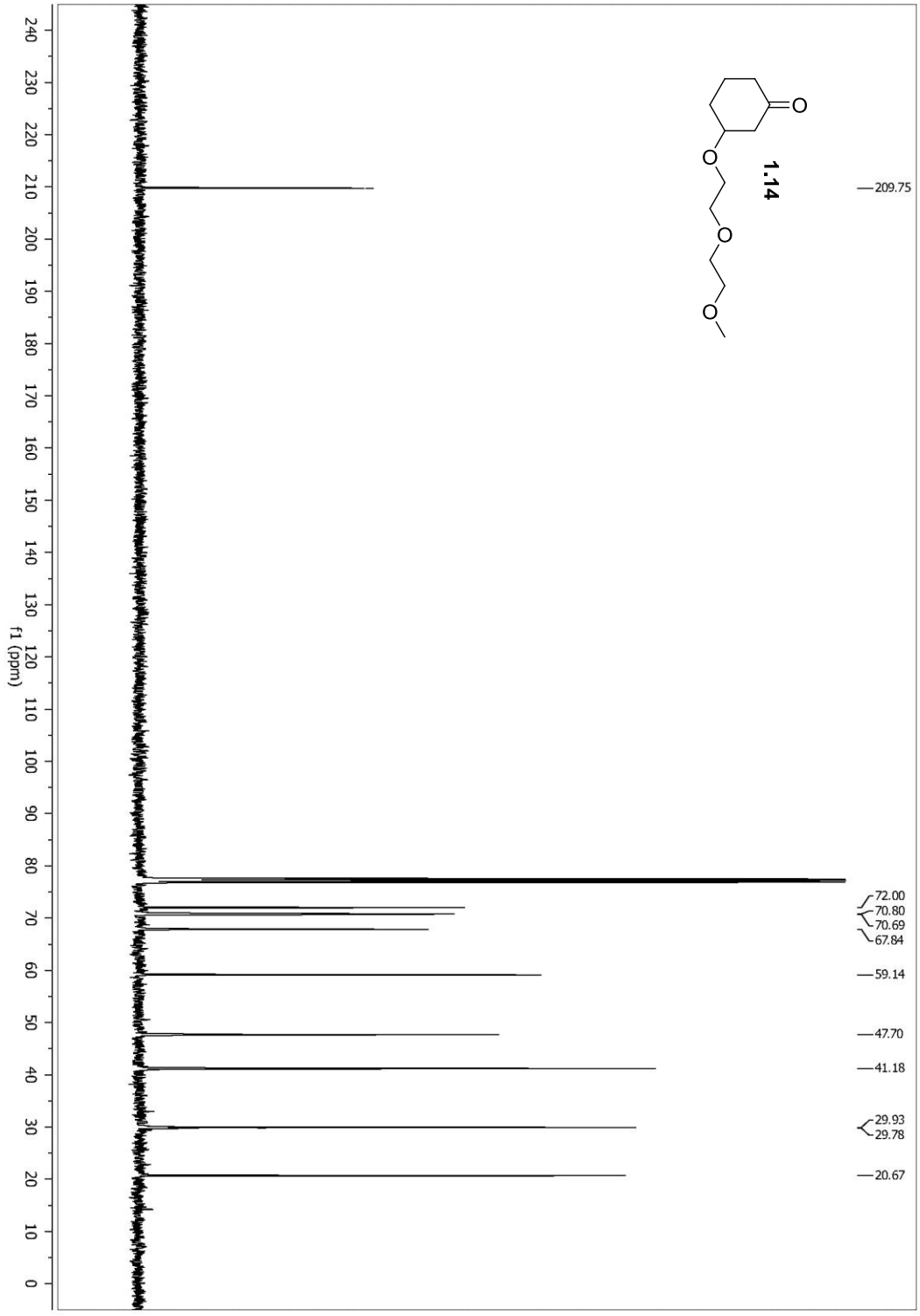
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) δ 7.42 (d, *J* = 7.8 Hz, 1H), 7.04 (dd, *J* = 7.6 Hz, 1H), 6.93 (d, *J* = 7.2 Hz, 1H), 4.82 (s, 2H), 2.77–2.61 (m, 4H), 2.17 (s, 3H), 2.00–1.80 (m, 4H);

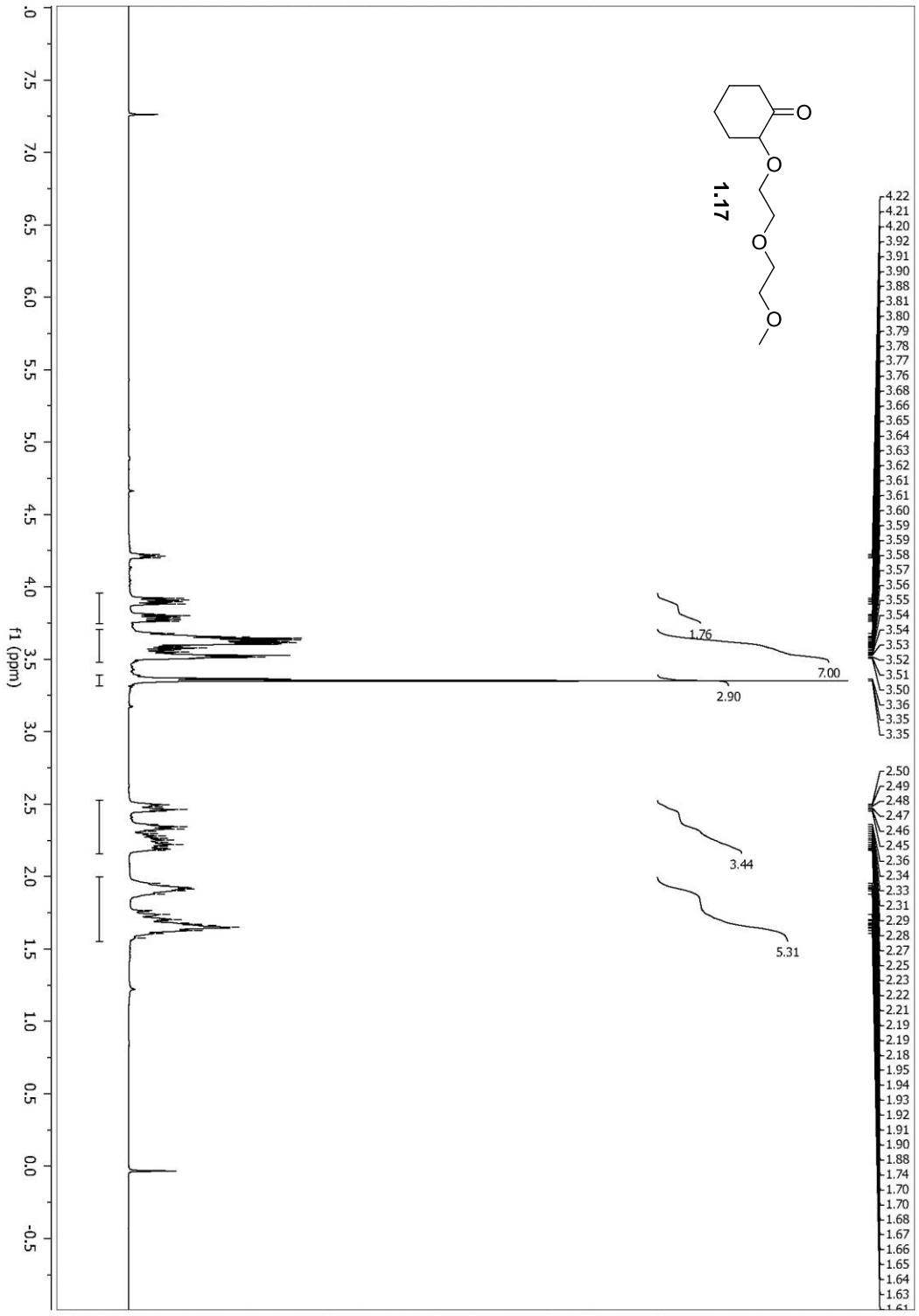
^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) δ 170.16, 137.28, 135.43, 129.43, 121.72, 119.06, 118.97, 117.23, 111.29, 77.47, 77.15, 76.83, 54.52, 47.56, 45.43, 23.40, 23.00, 22.77, 22.10, 21.11;

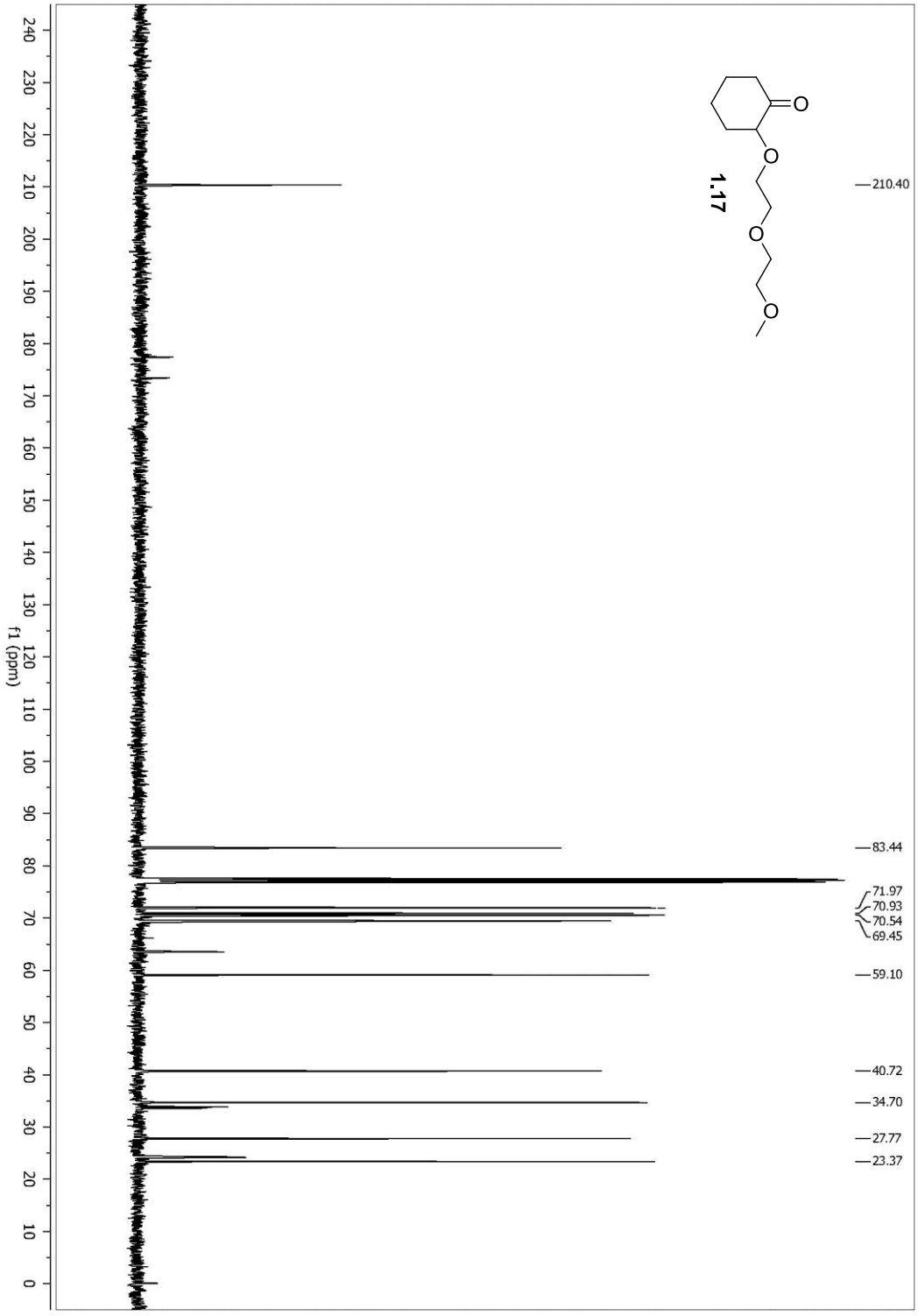
TLC R_f 0.4 (EtOAc : Hexane = 3 :1)

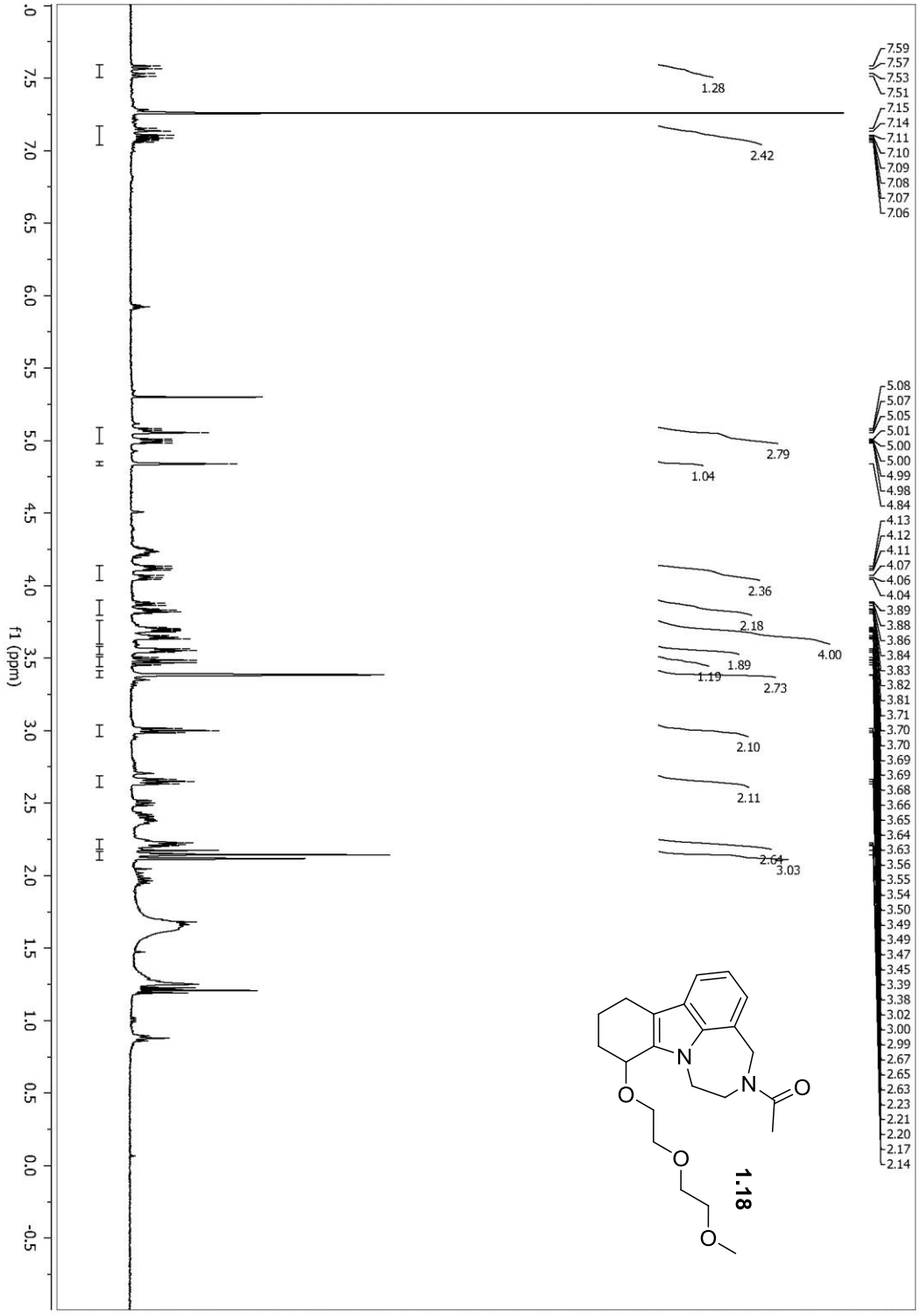
^{1}H NMR and ^{13}C NMR Spectra

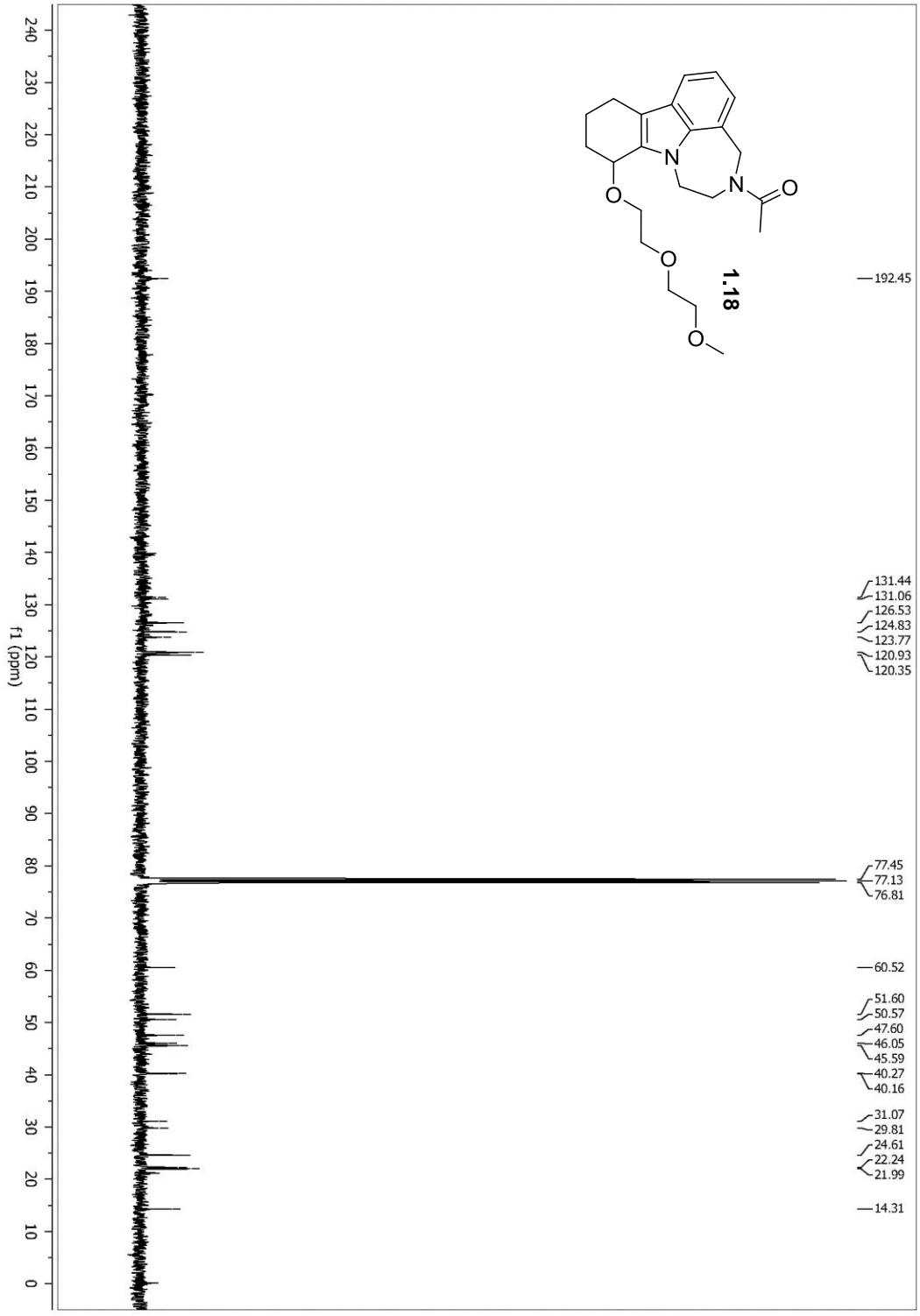



1.14
209.75
72.00
70.80
70.69
67.84
59.14
47.70
41.18
29.93
29.78
20.67
f1 (ppm)


1.17
1.76
7.00
2.90
3.44
5.31
f1 (ppm)


1.17
210.40
83.44
71.97
70.93
70.54
69.45
59.10
40.72
34.70
27.77
23.37
f1 (ppm)


f1 (ppm)
1.28
2.42
2.79
1.04
2.36
2.18
4.00
1.89
1.19
2.73
2.10
2.11
2.64
3.03
1.18


f1 (ppm)
1.18
192.45
131.44
131.06
126.53
124.83
123.77
120.93
120.35
77.45
77.13
76.81
60.52
51.60
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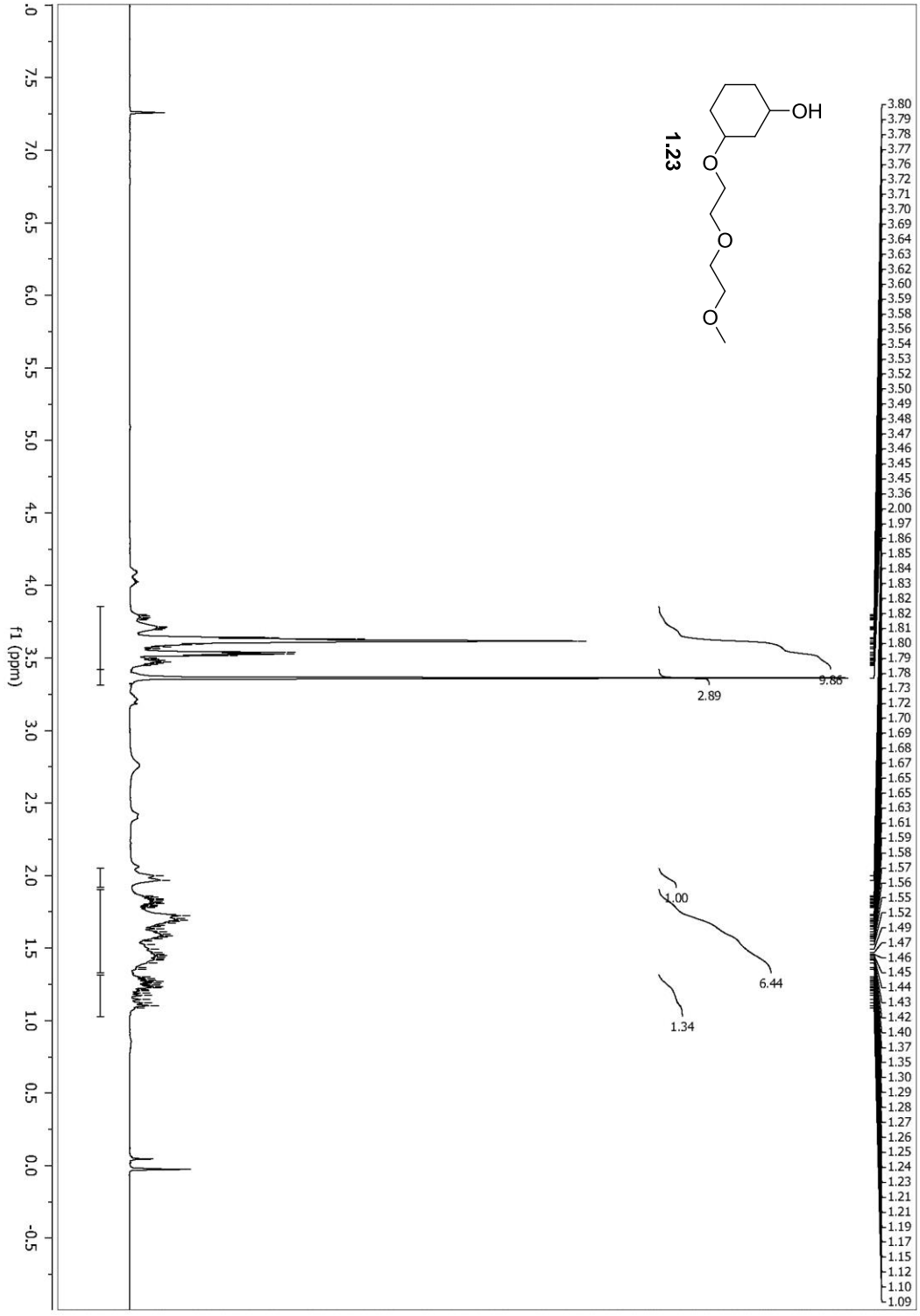
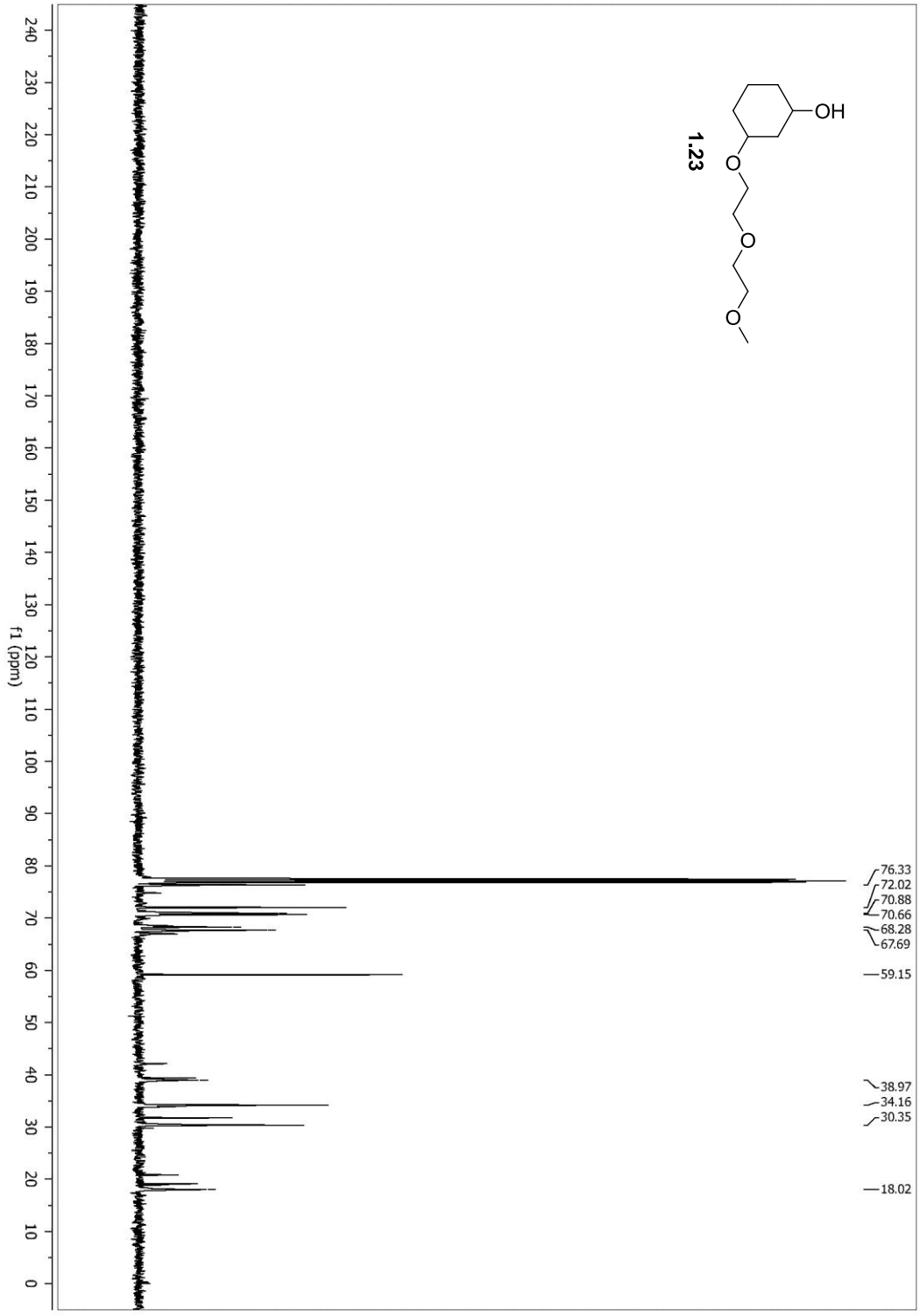
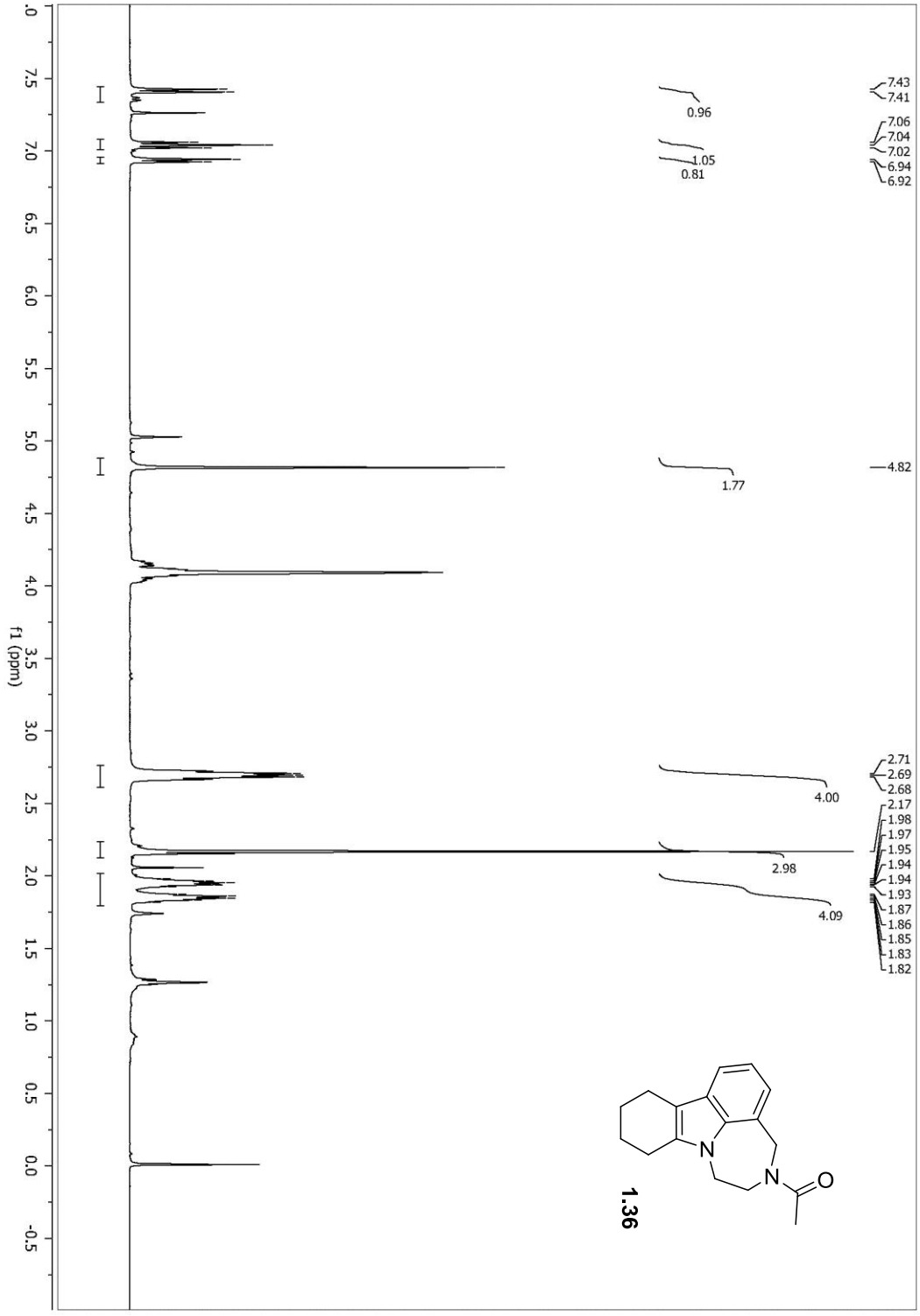
31.07
29.81
24.61
22.24
21.99
14.31


1.23
OH
O
O
O
f1 (ppm)
9.86
2.89
1.00
6.44
1.34


1.23
OH
O
O
O
f1 (ppm)
240
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
76.33
72.02
70.88
70.66
68.28
67.69
59.15
38.97
34.16
30.35
18.02


f1 (ppm)
0.96
1.05
0.81
1.77
4.00
2.98
4.09
7.43
7.41
7.06
7.04
7.02
6.94
6.92
4.82
2.71
2.69
2.68
2.17
1.98
1.97
1.95
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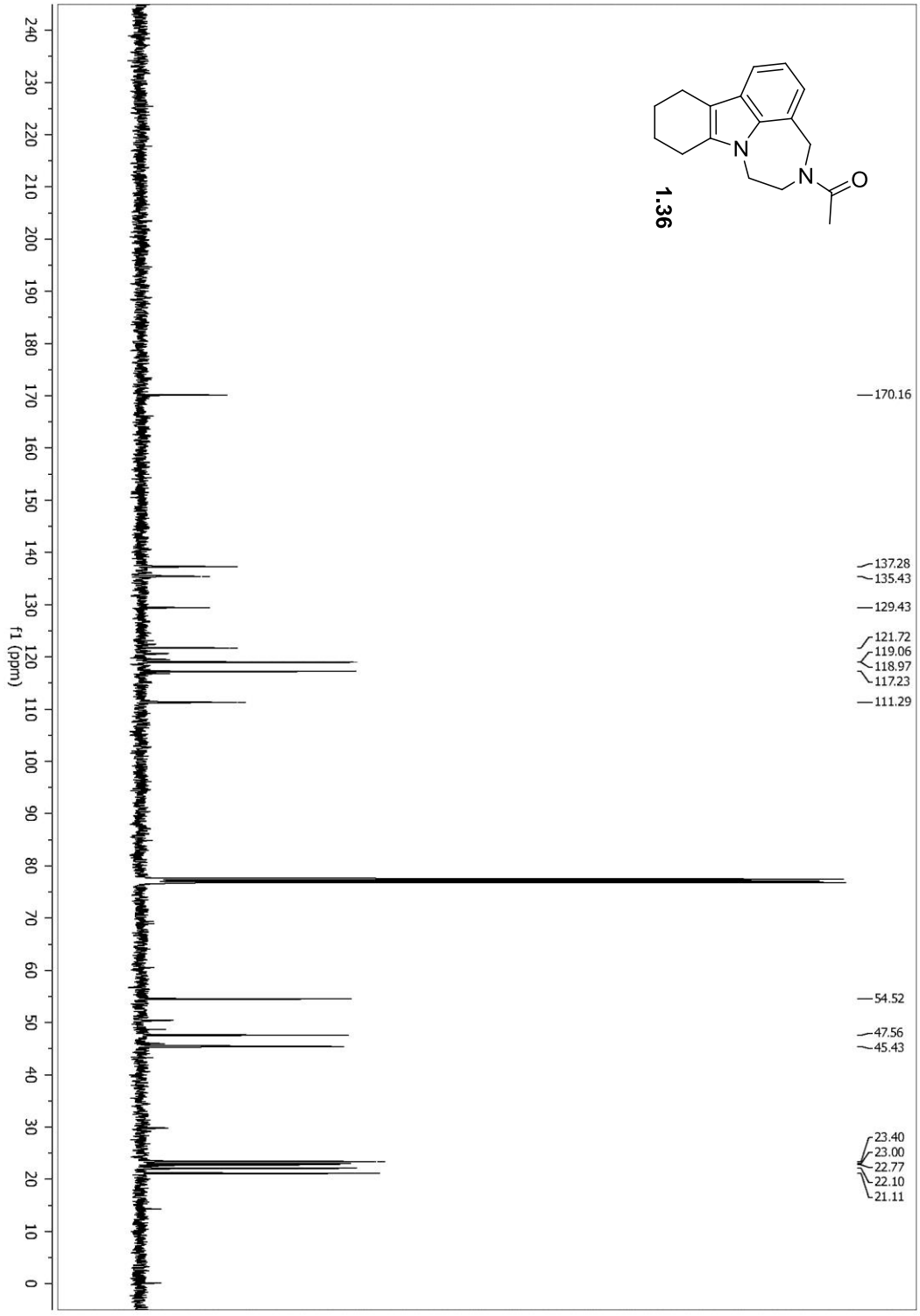
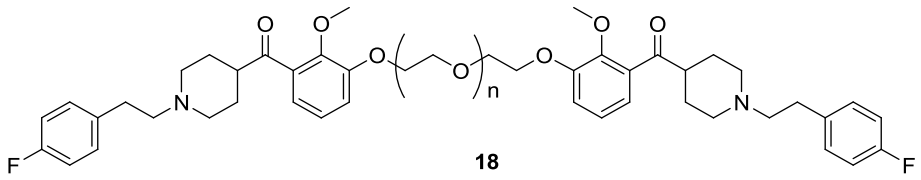
1.93
1.87
1.86
1.85
1.83
1.82
N
N
O
1.36


1.36
170.16
137.28
135.43
129.43
121.72
119.06
118.97
117.23
111.29
54.52
47.56
45.43
23.40
23.00
22.77
22.10
21.11
f1 (ppm)

Chapter 2. Preparation of a New Linker for Homodimer of M100907 derivative

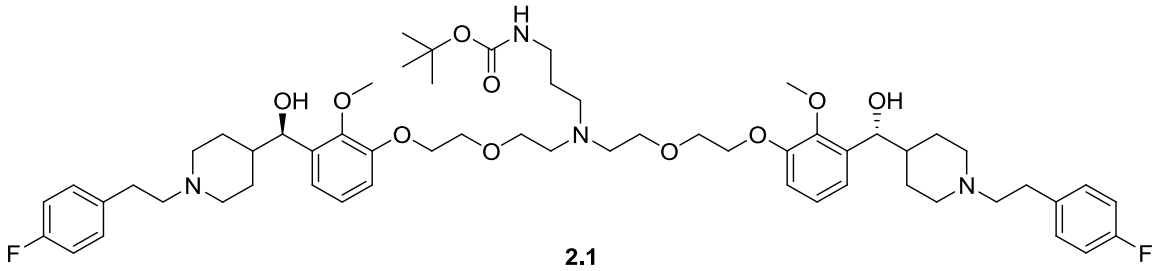
Previously, the homodimer of M100907 derivatives (**18**) was synthesized by our group (Figure 2.1). The proper linker type, linker length and linker position were determined.[17a] Its potency was also proved as an antagonist at the 5-HT_{2A} receptor. However, because the homodimer cannot be visualized on cells it is not possible to observe binding phenomenon to 5-HT_{2A} receptor.



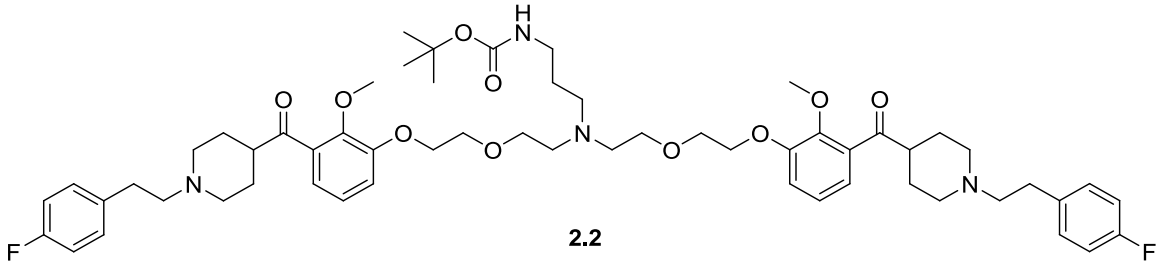
18

Figure 2.1. The Homodimer of M100907 derivatives

Therefore, a new linker that can attach a fluorescence tag was designed (Figure 2.2). The new linker contains an amine which can link to another carbon chain. At the end of the carbon chain, there is another amine protected by Boc group which can be removed and used to attach a fluorescence tag. The ligands will be used to both probe the activity of receptor dimers as well as visualize them. Additionally, heterodimer of WAY-derivative and M100907 derivative will be synthesized using this new linker.

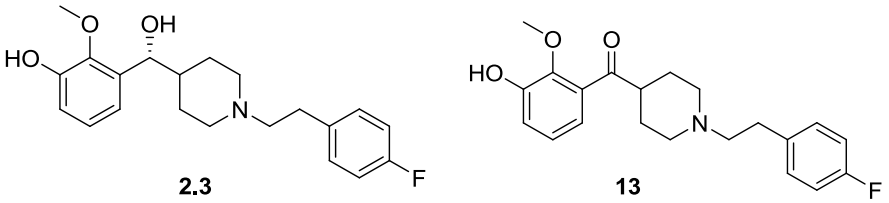


2.1



2.2

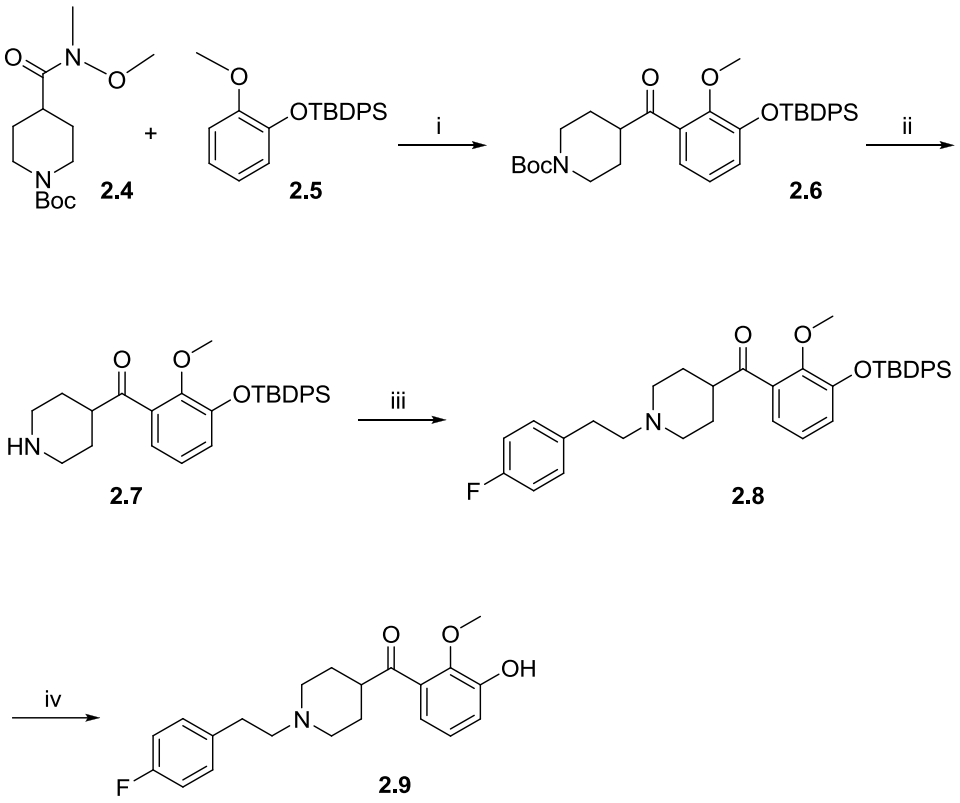
Figure 2.2. The Homodimer of M100907 derivatives with a New Linker



2.3 13

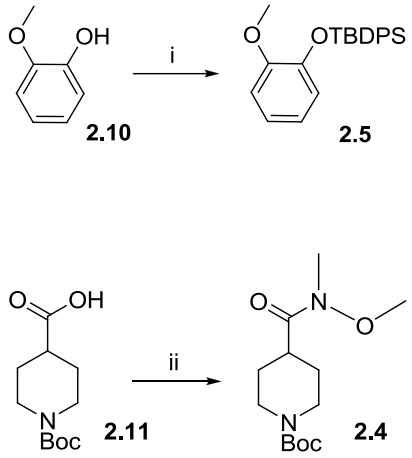
Figure 2.3. The Desired M100907 derivatives

The M100907 derivatives (**2.3**, **13**) (Figure 2.3) were synthesized using previous report (Scheme 2.1).[17] Two starting materials (**2.4**, **2.5**) were prepared following Scheme 2.2. An alcohol of guaiacol (**2.10**) was protected as the *tert*-butyldiphenylsilyl ether in 72% yield. Weinreb amide was prepared using the amino acid coupling reagents 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) and hydroxybenzotriazole (HOBT) in 50% yield.



Reagents and conditions : (i) n-BuLi, TMEDA, THF, 20 h, r.t., 67%; (ii) TFA, 21 h, r.t., 81%; (iii) 4-fluorophenethyl bromide, N,N-diisopropylethylamine, CH_3CN, 22 h, 85 ˚C, 70%; (iv) NH_4F, MeOH, 70 ˚C, 0.4 h, 91%

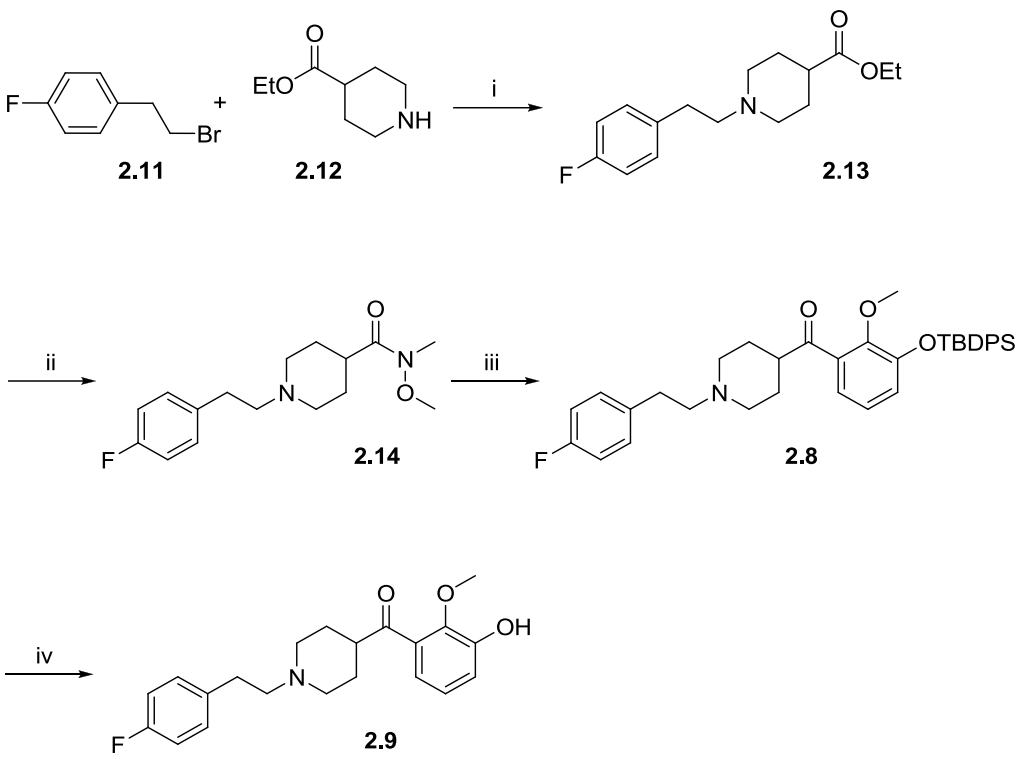
Scheme 2.1. Previous Synthetic Route 1 to 2.9



Reagents and conditions : (i) TBDPSCl, imidazole, 4-dimethylaminopyridine, CH_2Cl_2, 24 h, r.t., 72%; (ii) 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, hydroxybenzotriazole, N,N-diisopropylethylamine, N,O-dimethylhydroxylamine hydrochloride, DMF, 50 °C, 26 h, 50%

Scheme 2.2. Preparation of 2.5 and 2.4

Metalation of **2.5** using n-butyl lithium (n-BuLi) and coupling with **2.4** performed with some effort. Given that n-butyl lithium is very moisture sensitive and was not fresh, the reaction did not proceed on the first attempt. Because n-butyl lithium was old, 2.3 equivalent of n-butyl lithium was added resulting in 24% yield. For metalation of **2.5**, the reaction mixture was refluxed. However, it did not improve the reaction. Instead of refluxing, tetramethylethylenediamine (TMEDA) was added resulting in **2.6** in 67% yield. The Boc-protecting group of **2.6** was removed by reaction with TFA in 81% yield. 4-Fluorophenethyl bromide was attached to the amine of **2.7** in 70% yield. TBDPS protecting group from **2.7** was removed by ammonium fluoride (NH_4F) in 91% yield.

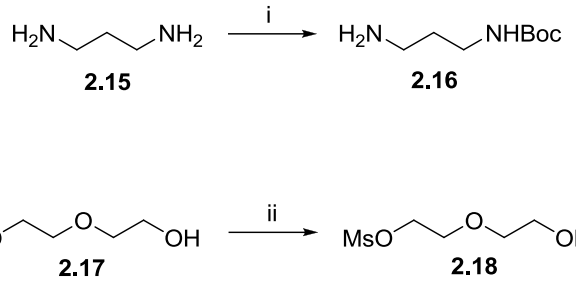


Reagents and conditions : (i) K_2CO_3, DMF, 20 h, 90 ˚C, 96%; (ii) N,O-dimethylhydroxylamine hydrochloride, ethylmagnesium bromide, THF, 21 h, r.t., 35%; (iii) **2.5**, n-BuLi, TMEDA, THF, 24 h, r.t., 53%; (iv) NH_4F, MeOH, 70 ˚C, 0.2 h, 90%

Scheme 2.3. Previous Synthetic Route 2 to 2.9

Because starting materials are commercially available, another published synthetic route to **2.9** was also tried (Scheme 2.3).[26] Once it was determined that the 4-fluorophenyl section of the molecule could not be modified d this route proved to be more efficient. For synthesis of **2.3**, ketone would be reduced from **2.7** in scheme **2.1**. After optical resolution, when 4-fluorophenethyl bromide is attached, the reaction should be refluxed.

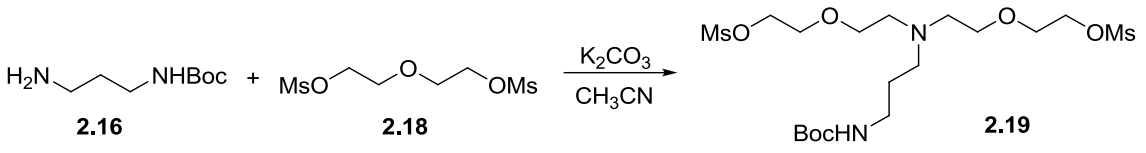
Because of the reasons above, another published synthetic route to **2.9** was also followed (Scheme 2.3). 4-Fluorophenethyl bromide was attached to ethyl isonipecotate in 96% yield. In the original literature, **2.8** was synthesized directly from **2.13** without purification of the amide **2.14**. However, this procedure did not proceed exactly as expected. It seems that Weinreb amide **2.14** was not formed or many side products from the synthesis of the Weinreb amide influenced the next coupling reaction, resulting in failure of the next step. Therefore, it was necessary to purify Weinreb amide **2.14** to modify this step. **2.14** was purified in 35% yield. As concerned above, formation of Weinreb amide **2.14** was not that efficient. However, this amide is white-yellowish solid, it is easy to purify in large scale. Then, metalations of **2.5** using n-BuLi and TMEDA and coupling with **2.14** were conducted showing 53% yield. Deprotection of TBDPS group resulted in 90% yield. In future, another M100907 derivative **2.3** (Figure 2.3) will be prepared for further research.



Reagents and conditions : (i) di-*tert*-butyl dicarbonate, THF, 18 h, r.t., 79%; (ii) triethylamine, methanesulfonyl chloride, CH_2Cl_2, 18 h, r.t., 91%

Scheme 2.4. Preparation of 2.16 and 2.18

To synthesize the linker the first strategy was scheme 2.5. Starting **2.15** was prepared by mono-protection of 1,3-diaminopropane (Scheme 2.4).[27] In this reaction, di-*tert*-butyl dicarbonate was added slowly at 0 ˚C otherwise, to prevent both amines from being protected. For 7 g scale reaction, the crude product was not purified showing 79% crude yield, but for 5g scale reaction, 60% yield was shown with purification. Diol from diethylene glycol (**2.17**) was activated by methanesulfonyl group (Ms) in 91%.

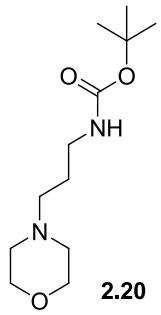


Scheme 2.5. Synthesis of 2.19 using 2.18

Entry	**2.16**	**2.18**	K_2CO_3	Time	Temp.	Yield
1	1 eq	2 eq	10 eq	19.5 h	85 ˚C	0%
2	1 eq	4 eq	10 eq	48 h	85 ˚C	0%
3	1 eq	2 eq	10 eq	96 h	r.t.	0%
4	1 eq	1 eq	1 eq	24h → 24h	r.t. → 85 ˚C	0%

Table 2.1. Reaction conditions for Scheme 2.5

Connection of two ethylene glycol groups to the amine was conducted using potassium carbonate (K_2CO_3) (Scheme 2.5, Table 2.1). From the first two trials (Entry 1, 2), the only product obtained was compound **2.20** in figure 2.4. This result indicates that mesyl group was to reactivity. After one mesyl group of a **2.18** is attacked by the amine of **2.16**, before another **2.18** can react the other mesyl group of the first **2.18** reacts forming stable 6-membered ring.



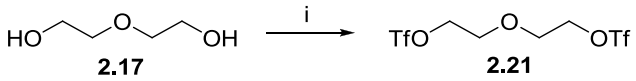
2.20

Figure 2.4. Side Product from the Reaction in Scheme 2.5

To reduce reaction rate, the reaction was conducted at room temperature without heating (Table 2.1, Entry 3, 4). However, at room

temperature, no reaction occurred, even after 24 hours. After temperature was increased, two starting materials reacted, but only **2.20** was formed (Entry 4). The result from running the reaction at room temperature for 4 days was same as Entry 4.

Trifluoromethanesulfonyl group (Triflyl, Tf) was also too reactive (Scheme 2.6, Table 2.2). The purified compound from entry 1 did not contain peaks for alkyl group of diethylene. In the case of entry 2, the first purification using column chromatography, seemed to provide the desired product with an impurity showing two spots on TLC. A second column provided a pure product, as determined by TLC. However, the following day, the pure product had decomposed to two spots like the TLC from first purification. This observation indicates that **2.21** decomposed easily.



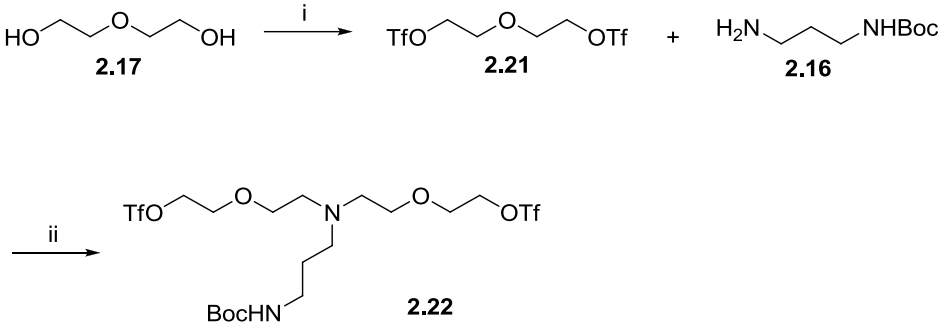
2.17 **2.21**

Scheme 2.6. Preparation of 2.21

Entry	**2.17**	Tf_2O	Base	Time	Temp.	Yield
1	1 eq	2.2 eq	Pyridine 2.2 eq	3 h	-10 ˚C	0%
2	1 eq	2 eq	Et_3N 2 eq	1.3 h	r.t.	0%

Table 2.2. Reaction conditions (i) for Scheme 2.6

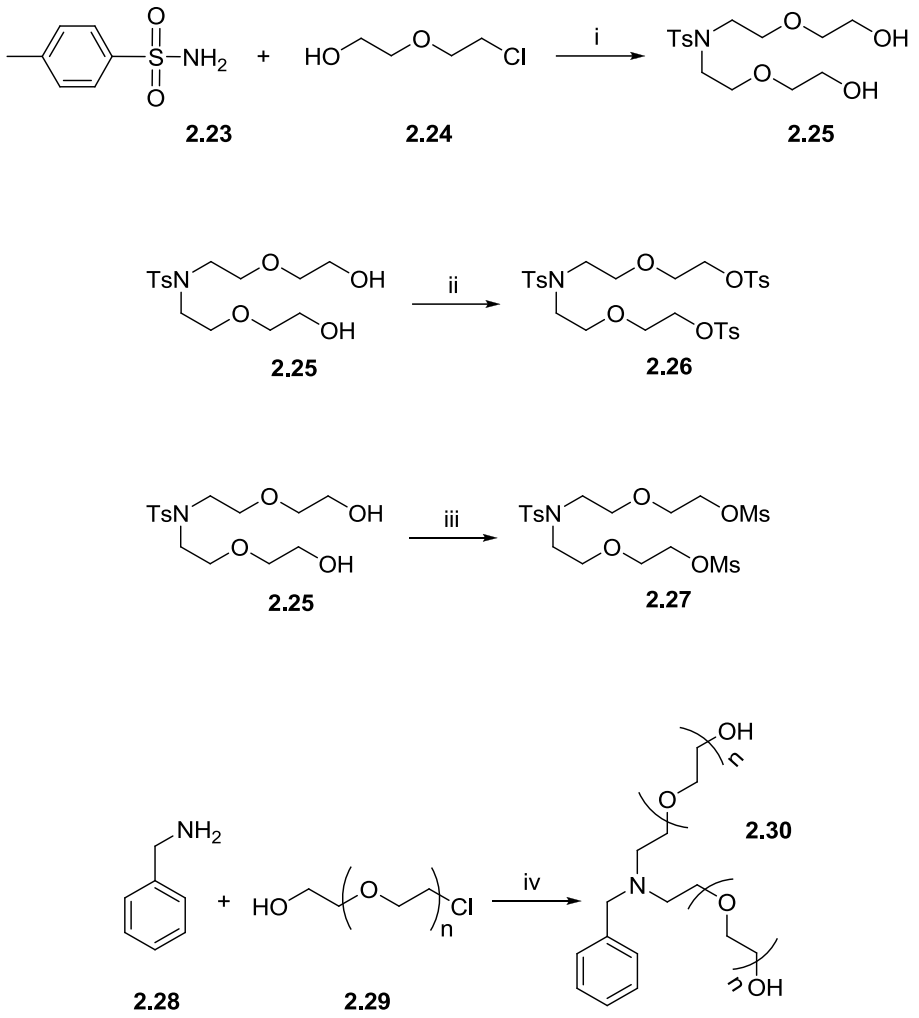
Therefore, without any aqueous work-up and purification, **2.21** was used directly for the next reaction (Scheme 2.7). Unfortunately, after the reaction, **2.20** in Figure 2.4 was isolated indicating triflyl group maybe too reactive. Since both mesyl and triflyl groups show the intramolecular reaction an alternative route was planned.



Reagents and conditions : (i) Et_3N, Tf_2O, CH_2Cl_2, 1 h, r.t.; (ii) K_2CO_3, CH_3CN, 24 h, 90 ˚C, 0%

Scheme 2.7. Synthesis of 2.22 using 2.21

Few similar synthetic methods could be found in literature (Scheme 2.8). First, *p*-toluenesulfonamide (**2.23**) reacted with 2-(2-chloroethoxy) ethanol (**2.24**) in dimethylformamide (DMF) using K_2CO_3 for 4 days.[28] Then, two alcohol groups are also activated by *p*-toluenesulfonyl (Ts) groups[29] or mesyl groups.[28] Another way is to use benzylamine (**2.28**) and K_2CO_3 in DMF to produce **2.30**.[30]

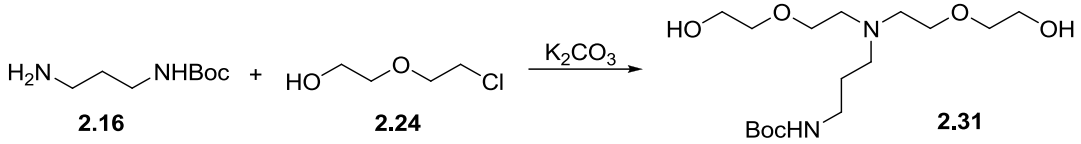


Reagents and conditions : (i) K_2CO_3, DMF, 96 h, reflux, 77%; (ii) NaOH, tosyl chloride, H_2O, THF, 4 h, 0 ˚C, 87%; (iii) methanesulfonyl chloride, pyridine, overnight, 0 ˚C, 96%; (iv) K_2CO_3, KI, DMF, overnight, 110 ˚C, 77%

Scheme 2.8. Similar Reactions from Literatures

Based on the results from literature, scheme 2.9 was planned (Table 2.3). However, from the first trial, a significant amount of unreacted **2.24** and unidentified side product were obtained (Entry 1). Reaction solvent and temperature were changed to acetonitrile at 85 ˚C, but the reagents didn't dissolve

well even at 85 ˚C (Entry 2). Thus, H_2O was added, refluxing at 90 ˚C. However, only **2.24** was isolated (Entry 2,3) even though only H_2O was used at 110 ˚C (Entry 3). Longer reaction time provided many side products were formed, without the desired product (Entry 4).

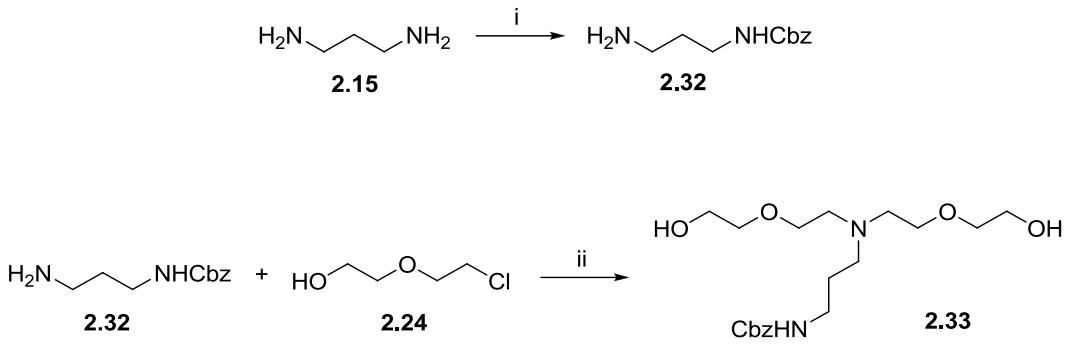


Scheme 2.9. Synthesis of 2.31 using 2.24

Entry	**2.16**	**2.22**	K_2CO_3	KI	Solvent	Time	Temp.	Yield
1	1 eq	2 eq	3.6 eq	2.3 eq	DMF	24 h	110 ˚C	0%
2	1 eq	2 eq	3.6 eq	2.3 eq	CH_3CN → H_2O	23 h → 24 h	85 ˚C → 90 ˚C	0%
3	1 eq	2 eq	3.6 eq	2.3 eq	H_2O	24 h	110 ˚C	0%
4	1 eq	2 eq	3.6 eq	2.3 eq	H_2O	96 h	110 ˚C	0%

Table 2.3. Reaction conditions for Scheme 2.9

The amine-protecting group was changed to carboxybenzyl (Cbz) group (Scheme 2.10). **2.32** was prepared in 13% yield.[31] Cbz-protected diaminopropane **2.32** reacted with **2.24** in toluene using K_2CO_3 for 24 hours at 165 ˚C. As a result, only a single **2.24** was attached to the amine of **2.32** in 8% yield indicating **2.34** in figure 2.5.



Reagents and conditions : (i) Benzyl chloroformate, CH_2Cl_2, 48 h, r.t., 13%; (ii) K_2CO_3, toluene, 24 h, 165 °C, 0%

Scheme 2.10. Synthesis of 2.33 using 2.32 and 2.24

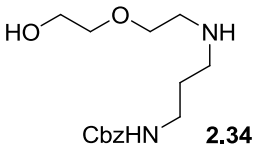
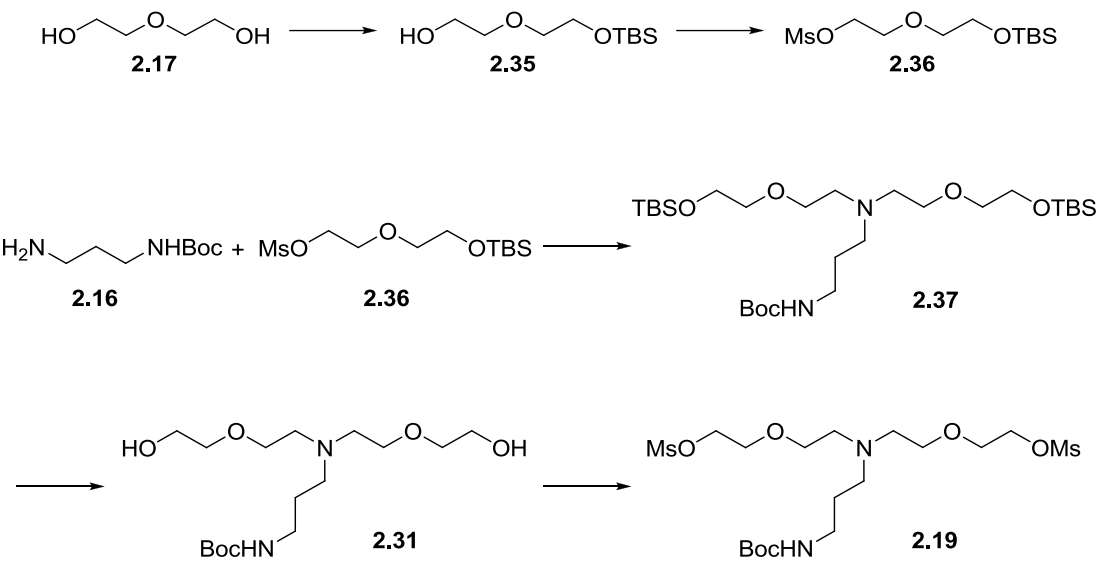


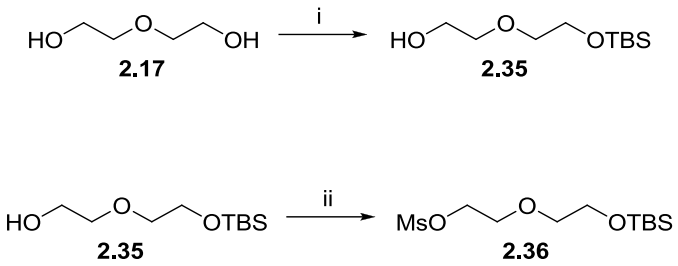
Figure 2.5. Side Product from the Reaction in Scheme 2.10

Because of the difficulties of attaching two diethylene glycols, we decided to go step by step. Instead of using same activating groups such as mesyl and triflyl to diol on diethylene glycol, *tert*-butyldimethylsilyl (TBS) group is used to protect one alcohol (Scheme 2.11). Then another alcohol is activated by a mesyl group. These 2 equilivents of ether **2.36** were attached to amine **2.16**. After removal of the TBS protecting group provided diol will be activated again by mesyl group.



Scheme 2.11. Synthetic plan for 2.19

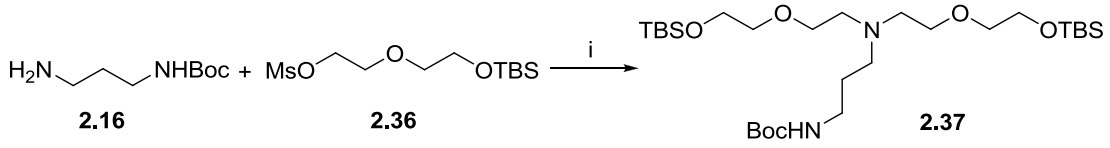
By this alternative strategy, **2.35** was prepared in 99% yield (Scheme 2.12).[32] Because the reaction proceeds clean especially in large scale like 6-10g and during aqueous work-up imidazole, diethylene glycol and all impurities are removed, it is not necessary to purify the crude product. The next step also works well without purification showing pure product in 98% yield.[33]



Reagents and conditions : (i) imidazole, TBSCl, CH_2Cl_2, 20 h, r.t., 99%; (ii) Et_3N, MsCl, CH_2Cl_2, 24 h, r.t., 98%

Scheme 2.12. Preparation of 2.36

The reaction for attaching **2.36** to **2.16** was conducted (Scheme 2.13, Table 2.4). Using K_2CO_3 in 1 : 1 ratio of acetonitrile and water **2.37** was formed in maximum 76% yield (Entry 3). When Na_2CO_3 was used, the reaction yields were a little bit lower in 48% (Entry 2). The interesting result is when only 2 equivalents of **2.36** were used, yields decreased almost in half (Entry 4).



Scheme 2.13. Synthesis of 2.37

Entry	**2.16**	**2.36**	Base	Solvent	Time	Temp.	Yield
1	1 eq (0.2g)	4 eq	K_2CO_3 10 eq	CH_3CN/H_2O (1/1)	24 h	90 ˚C	68%
2	1 eq (0.2g)	4 eq	Na_2CO_3 2.2 eq	CH_3CN/H_2O (1/1)	36 h	90 ˚C	48%
3	1 eq (0.2g)	4 eq	K_2CO_3 10 eq	CH_3CN/H_2O (1/1)	24 h	90 ˚C	76%
4	1 eq (0.2g)	2 eq	K_2CO_3 10 eq	CH_3CN/H_2O (1/1)	30 h	90 ˚C	32%
5	1 eq (0.5g)	4 eq	K_2CO_3 10 eq	CH_3CN/H_2O (1/1)	23 h	90 ˚C	0%
6	1 eq (0.5g)	4 eq	K_2CO_3 10 eq	CH_3CN	48 h	90 ˚C	54%
7	1 eq (1.6g)	4 eq	K_2CO_3 10 eq	CH_3CN	18 h	90 ˚C	0%
8	1 eq (2.3g)	5 eq	K_2CO_3 10 eq	CH_3CN	18 h	90 ˚C	0%
9	1 eq (1.1g)	4 eq	K_2CO_3 10 eq	CH_3CN	19 h	90 ˚C	40%

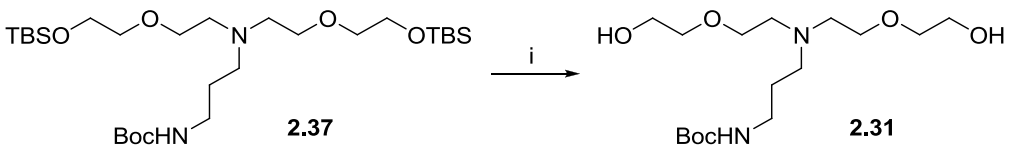
Table 2.4. Reaction conditions (i) for Scheme 2.13

When reaction scale goes up, the solubility of **2.36** in water decreased resulting in no reaction (Entry 5). Therefore, for large scale, only acetonitrile should be used. In addition, the rate and sequence of adding reagents affect the reaction yields. **2.36** in CH_3CN was usually added to **2.16** in CH_3CN. In large scale, **2.36** should be added slowly in ice-bath otherwise, it warms affecting the reaction yields in 0% (Entry 7). After **2.36** in CH_3CN was added to **2.16** in CH_3CN cooled with an ice-bath, it was stirred at room temperature for 30 minutes, then K_2CO_3

was added. However, if K_2CO_3 is added to **2.16** in CH_3CN before **2.36**, **2.37** was not formed (Entry 8).

To remove TBS protecting groups, triethylamine trihydrofluoride ($Et_3N{\cdot}3HF$) was first used. After 72 hours, **2.37** had been consumed but the desired product was not formed (Entry 1).

Based on those considerations above, the amount of $Et_3N{\cdot}3HF$ and reaction time were reduced. As a result, 12% of **2.31** could be obtained using 5 equivalents of $Et_3N{\cdot}3HF$ for 1 hour (Entry 3). Using 2 equivalents of $Et_3N{\cdot}3HF$ for 1 hour provided the best result (41% yield) (Entry 5). 2 equilivalents and longer reaction times resulted in no product being isolated.



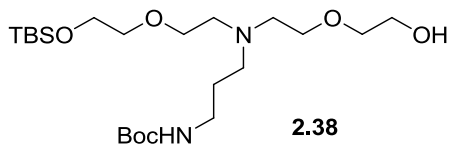
Scheme 2.14. Deprotection of TBS groups

Entry	**2.37**	reagent	Solvent	Time	Temp.	Yield
1	1 eq	$Et_3N{\cdot}3HF$ 10 eq	CH_2Cl_2	72 h	r.t.	0%
2	1 eq	$Et_3N{\cdot}3HF$ 5 eq	CH_2Cl_2	20 h	r.t.	0%
3	1 eq	$Et_3N{\cdot}3HF$ 5 eq	CH_2Cl_2	1 h	r.t.	12%
4	1 eq	$Et_3N{\cdot}3HF$ 2 eq	CH_2Cl_2	24 h	r.t.	0%
5	1 eq	$Et_3N{\cdot}3HF$ 2 eq	CH_2Cl_2	1 h	r.t.	41%
6	1 eq	TBAF 4 eq	THF	29 h	r.t.	0%
7	1 eq	TBAF 3 eq	THF	1.5 h	r.t.	6%
8	1 eq	CSA 2 eq	CH_2Cl_2/MeOH (1/1)	2.5 h	0 ˚C	41%
9	1 eq (0.1g)	2M HCl	AcOH/H_2O/MeOH (8/1/1)	48 h	r.t.	71%
10	1 eq (0.5g)	1M HCl	AcOH/H_2O/MeOH (8/1/1)	48 h	r.t.	57%
11	1 eq (1.5g)	1M HCl (0.1 eq)	AcOH/H_2O/MeOH (8/1/1)	18 h	r.t.	19% (32%-mono)

Table 2.5. Reaction conditions (i) for Scheme 2.14

Tetrabutylammonium fluoride (TBAF) shows similar results. When reaction time was shortened to 1.5 hours, a 6% yield was obtained (Entry 6, 7). Removing TBAF from the reaction mixture was proved difficult. (1S)-(+)-10-camphorsulfonic acid was used in mixed solvents (CH_2Cl_2/MeOH = 1/1) for 2.5 hours at 0 ˚C (Entry 8) resulting in 41% yield.

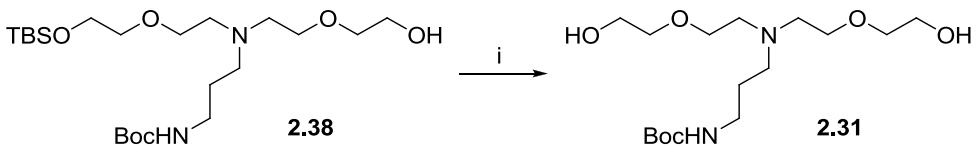
The best yield of 71% was with 2N HCl in 8 : 1 : 1 ratios of AcOH : H_2O : MeOH (Entry 9). From the original reference[34], the amount of HCl was not measured quantitatively with just one drop of 2N HCl being added. However, for large scale reactions, we assumed it is approximately 0.1 equivalents of 1N HCl (Entry 10). When scaled up, the reaction efficiency decreased (Entry 9, 10 and 11). However, interestingly, from entry 11, 32% of **2.38** in figure 2.6 was obtained.



2.38

Figure 2.6. Side Product from Entry 11 in Table 2.5

For further investigation, **2.38** was reacted in 8 : 1 : 1 ratios of AcOH : H_2O : MeOH with 0.1 equivalents of 1N HCl for 48 hours at room temperature (Scheme 2.15). As a result, **2.31** was formed in 85% yield. These results will be very useful in the future. Double M100907 derivative can be attached to **2.31** at the same time. In addition, single M100907 derivative can be attached to **2.38** first, and then, TBS group will be removed. After deprotection, another M100907 derivative will be combined or WAY-derivatives can be attached for synthesis of heterodimer.



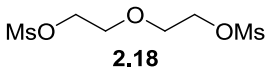
Reagents and conditions : (i) 1N HCl, AcOH/H_2O/MeOH (8/1/1), 48 h, r.t., 85%

Scheme 2.15. Deprotection of TBS group

For further research, reactivating diol on **2.31** and attaching M100907 derivatives will be conducted. In addition, as mentioned above, preparation of **2.3** also would be performed. With the linker studied in this research, homo- or heterodimer of 5-HT receptor ligands which a fluorescence tag can be attached will be synthesized.

Experimental

2,2'-oxybis(ethane-2,1-diyl) dimethanesulfonate (2.18)



2.18

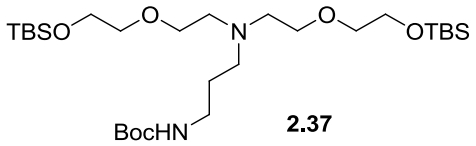
Diethylene glycol (**2.17**) (5 g, 47.11 mmol) in 50 mL CH_2Cl_2 was cooled to 0 ˚C. Triethylamine (13.14 mL, 94.22 mmol) was added slowly. After 10 minutes, methanesulfonyl chloride (7.29 mL, 94.22 mmol) was added over 10 minutes. After 10 minutes, the reaction mixture was warmed to room temperature and stirred for 18 hours. 500 mL H_2O was added to the mixture and the mixture was extracted with 300 mL CH_2Cl_2 3 times. The combined organic layer was washed with saturated 100 mL NaCl once and 100 mL H_2O twice. Then, the organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure to afford 2,2'-oxybis(ethane-2,1-diyl) dimethanesulfonate (**2.18**) in 91% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 4.39 – 4.32 (m, 4H), 3.81 – 3.73 (m, 4H), 3.05 (d, *J* = 1.0 Hz, 6H);

^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) δ 69.14, 69.10, 69.05, 68.98, 37.74, 37.71;

TLC R_f 0.49 (EtOAc : Hexane = 3 :1)

***tert*-butyl-10-(2-(2-(tert-butyldimethylsilyloxy)ethoxy)ethyl)-2,2,3,3-tetramethyl-4,7-dioxa-10-aza-3-silatridecan-13-ylcarbamate (2.37)**



2.37

2-(2-(tert-butyldimethylsilyloxy)ethoxy)ethyl methanesulfonate (**2.36**) (2.81 g, 9.41 mmol) in 3 mL CH_3CN was added to *tert*-butyl-3-aminopropylcarbamate (**2.16**) (0.41 g, 2.35 mmol) in 3 mL H_2O (for over 0.5 g of **2.16**, only CH_3CN used). The mixture was stirred for 0.5 hours at room temperature. K_2CO_3 (3.25 g, 23.53 mmol) was added. The reaction mixture was refluxed for 24 hours at 90 °C. (When only CH_3CN was used, the mixture was concentrated under reduced pressure.) 200 mL CH_2Cl_2 was added for extraction. (When only CH_3CN was used, after concentratioin 200 mL H_2O was also added.) The mixture was extracted with 200 mL CH_2Cl_2 three times.. The combined organic layer was washed with 100 mL saturated NaCl (x1). The organic layer was dried over Na_2SO_4, filtered and concentrated. The crude material was purified with column chromatography (3%

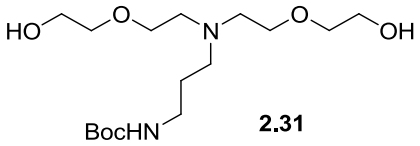
EtOAc in hexane to 20% EtOAc in hexane for small scale under 0.5 g, EtOAc : hexane = 1 : 9 to 2 : 3 for large scale over 0.5 g). *tert*-butyl-10-(2-(2-(tert-butyldimethylsilyloxy)ethoxy)ethyl)-2,2,3,3-tetramethyl-4,7-dioxa-10-aza-3-silatridecan-13-ylcarbamate (**2.37**) was afforded in 76% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.61 (s, 1H), 3.74 (dd, *J* = 5.4 Hz, 4H), 3.52 (dt, *J* = 10.7, 5.7 Hz, 8H), 3.17 (d, *J* = 5.8 Hz, 2H), 2.67 (dd, *J* = 6.0 Hz, 4H), 2.58 (dd, *J* = 6.4 Hz, 2H), 1.65 – 1.50 (m, 2H), 1.42 (s, 10H), 0.88 (s, 19H), 0.05 (s, 12H);

^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) δ 156.22, 78.63, 72.80, 72.58, 69.87, 62.78, 54.09, 53.40, 39.55, 28.60, 26.75, 26.01, 18.45;

TLC R_f 0.08 (EtOAc : Hexane = 2 : 3)

***tert*-butyl 3-(bis(2-(2-hydroxyethoxy)ethyl)amino)propylcarbamate (2.31)**



2.31

tert-butyl10-(2-(2-(tert-butyldimethylsilyloxy)ethoxy)ethyl)-2,2,3,3-tetramethyl-4,7-dioxa-10-aza-3-silatridecan-13-ylcarbamate (**2.37**) (0.1 g, 0.17 mmol) was

dissolved in 3 mL 8:1:1 ratio of acetic acid : H_2O : methanol. After the reaction mixture was cooled to 0 ˚C, two drops of 1N HCl were added. The mixture was stirred at room temperature for 2 days. Excess amount of toluene was added to the reaction mixture. Then, the reaction mixture was concentrated under reduced pressure. Without aqueous work-up, the crude product was purified by column chromatography (CH_2Cl_2 : methanol = 2 : 1) to provide *tert*-butyl 3-(bis(2-(2-hydroxyethoxy)ethyl)amino)propylcarbamate (**2.31**) in 71% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.26 (s, 1H), 3.85 – 3.55 (m, 14H), 3.19 (dd, *J* = 12.4, 6.1 Hz, 2H), 2.86 (d, *J* = 35.3 Hz, 6H), 1.88 – 1.76 (m, 2H), 1.44 (s, 9H);

^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) 79.25, 72.73, 68.10, 61.58, 54.66, 52.19, 38.90, 29.79, 28.54, 26.10;

TLC R_f 0.1 (CH_2Cl_2 : MeOH = 2 : 1)

tert-butyl-10-(2-(2-hydroxyethoxy)ethyl)-2,2,3,3-tetramethyl-4,7-dioxa-10-aza-3-silatridecan-13-ylcarbamate (**2.38**) (0.46 g, 1.00 mmol) was dissolved in 20 mL 8:1:1 ratios of acetic acid : H_2O : methanol. After the reaction mixture was cooled to 0 ˚C, 1N HCl (0.11 mL, 0.11 mmol) was added. The mixture was stirred at 0 ˚C for 0.5 hours and then, warmed to room temperature and stirred for 2 days. Excess

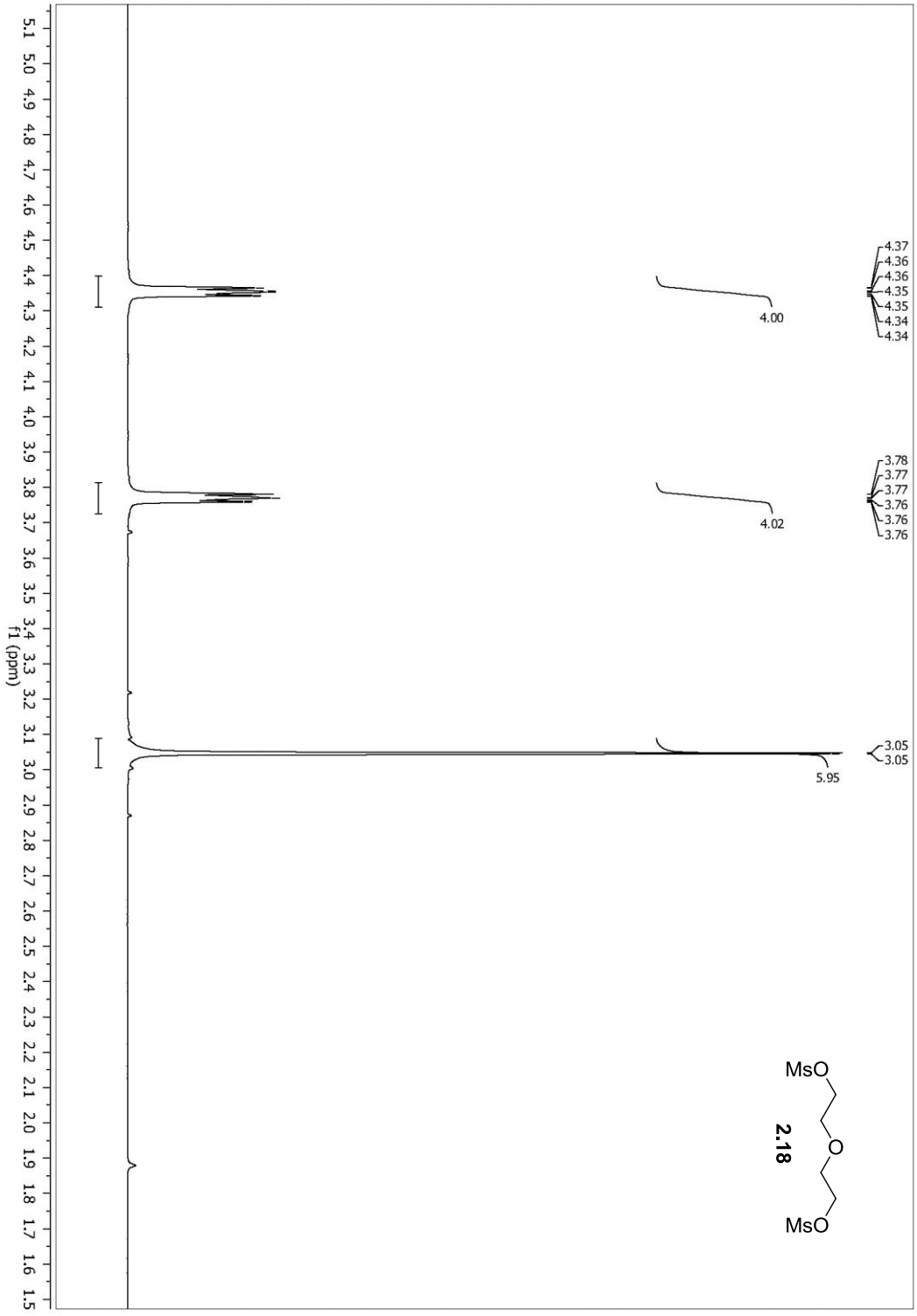
amount of toluene was added to remove solvents. Without aqueous work-up, the crude product was purified by column chromatography (CH_2Cl_2 : methanol = 4 : 1) to provide *tert*-butyl 3-(bis(2-(2-hydroxyethoxy)ethyl)amino)propylcarbamate (**2.31**) in 85% yield.

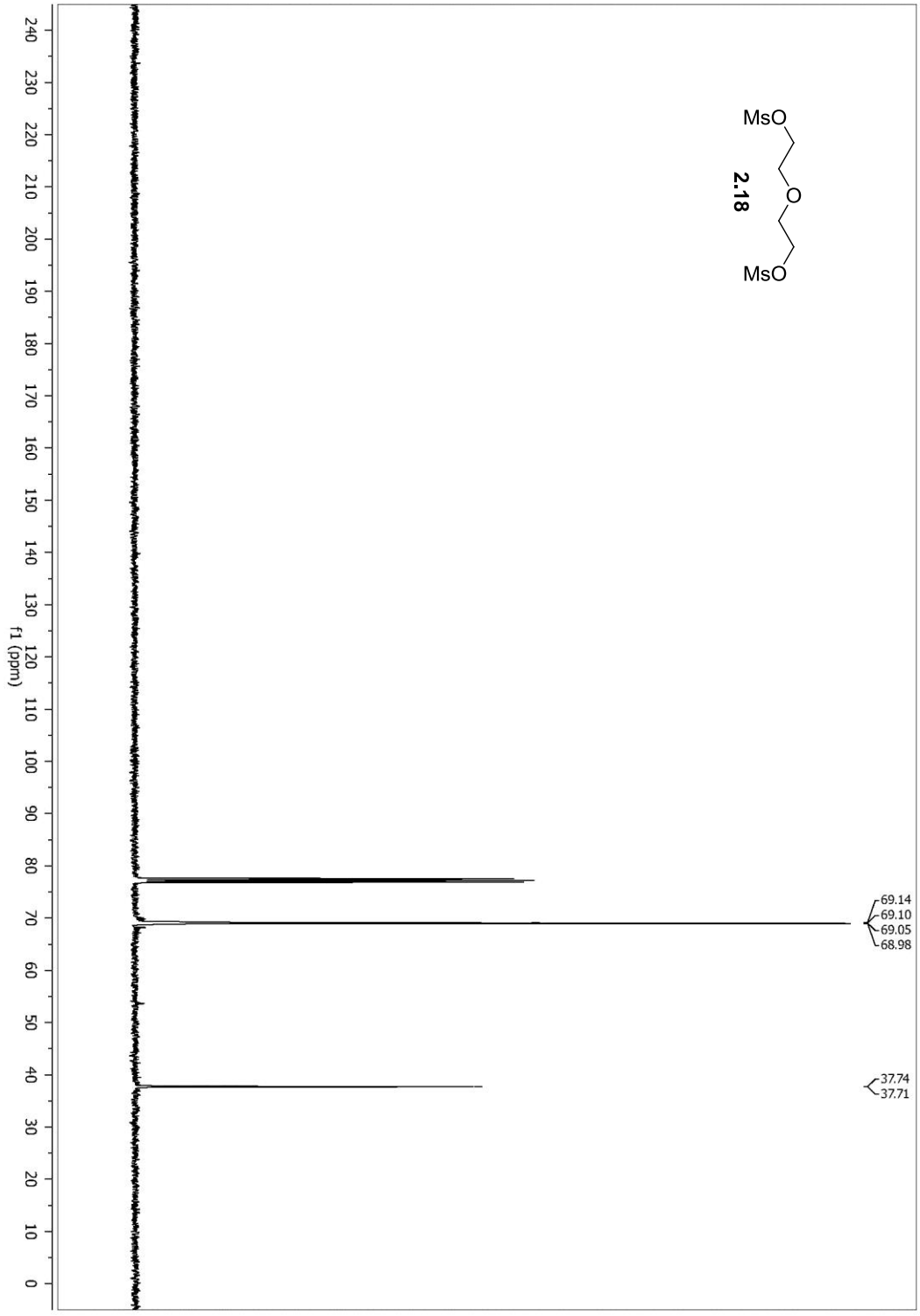
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.26 (s, 1H), 3.85 – 3.55 (m, 14H), 3.19 (dd, *J* = 12.4, 6.1 Hz, 2H), 2.86 (d, *J* = 35.3 Hz, 6H), 1.88 – 1.76 (m, 2H), 1.44 (s, 9H);

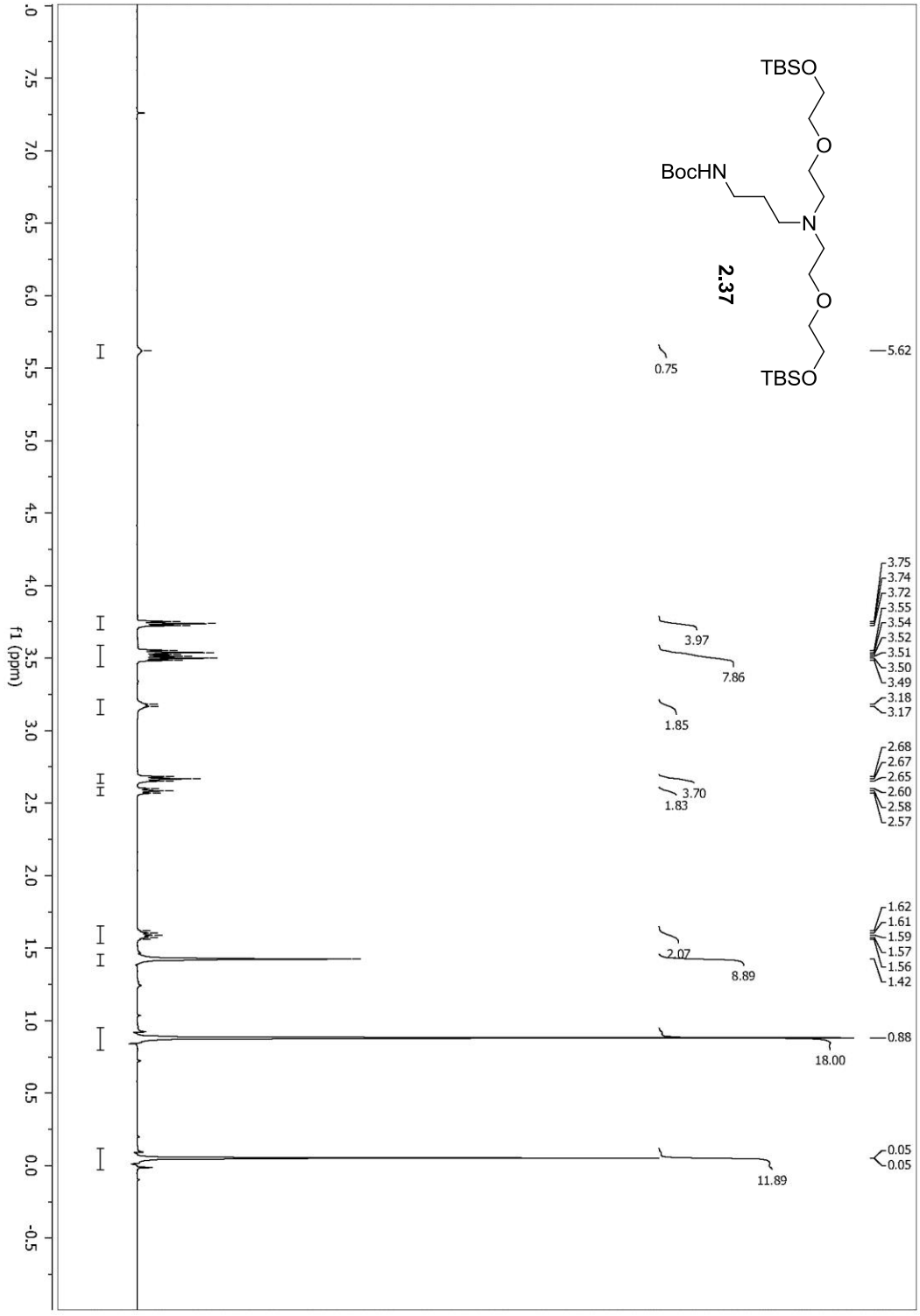
^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) 79.25, 72.73, 68.10, 61.58, 54.66, 52.19, 38.90, 29.79, 28.54, 26.10;

TLC R_f 0.1 (CH_2Cl_2 : MeOH = 2 : 1)

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




MsO
O
MsO
2.18
69.14
69.10
69.05
68.98
37.74
37.71
f1 (ppm)


TBSO
O
BocHN
N
2.37
O
TBSO
f1 (ppm)
0.75
5.62
3.97
7.86
1.85
3.70
1.83
2.07
8.89
18.00
11.89
3.75
3.74
3.72
3.55
3.54
3.52
3.51
3.50
3.49
3.18
3.17
2.68
2.67
2.65
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1.61
1.59
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1.56
1.42
0.88
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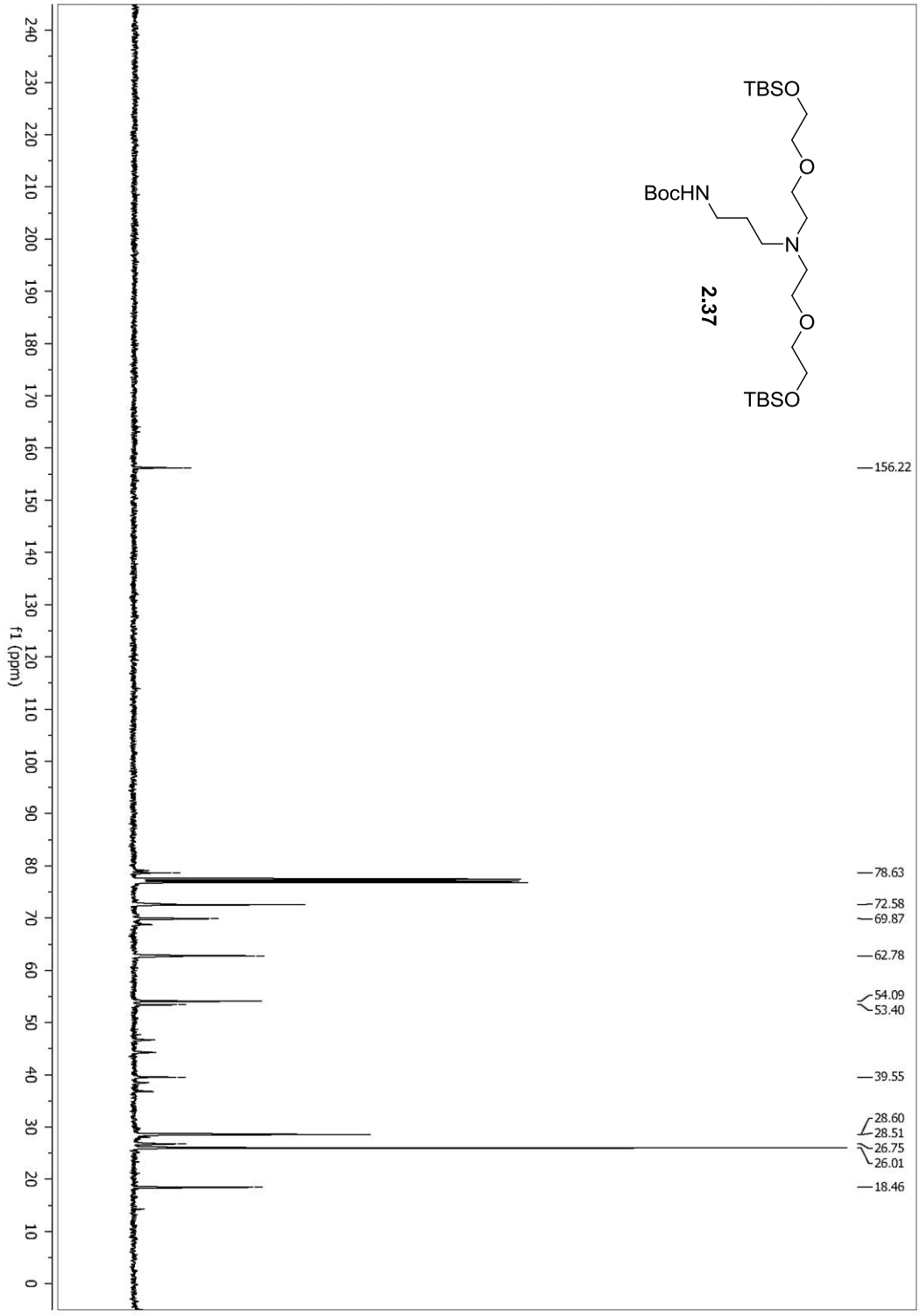
0.05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BocHN
N
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O
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78.63
72.58
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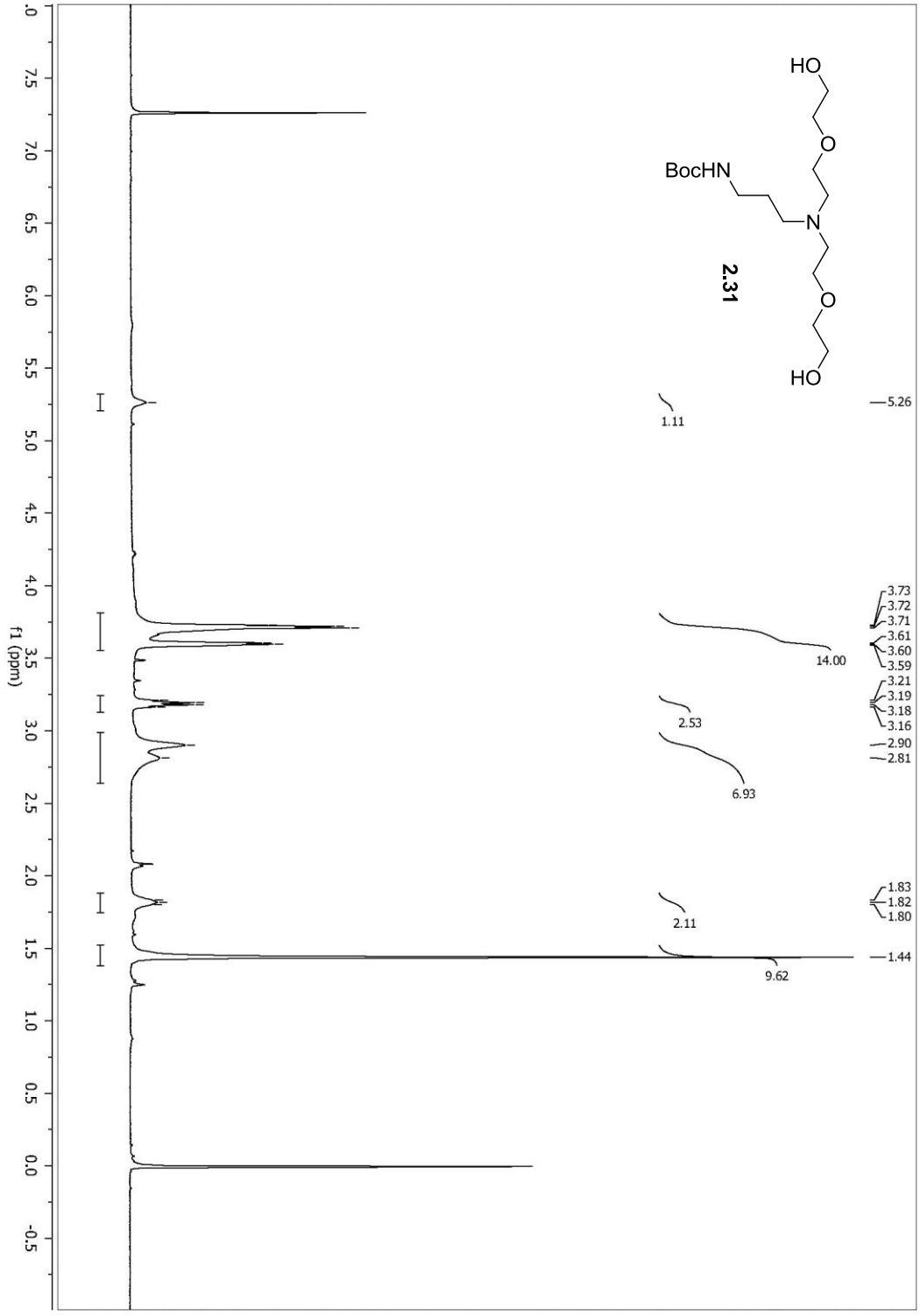
28.60
28.51
26.75
26.01
18.46
f1 (ppm)


HO
O
BocHN
N
O
HO
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5.26
14.00
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3.71
3.61
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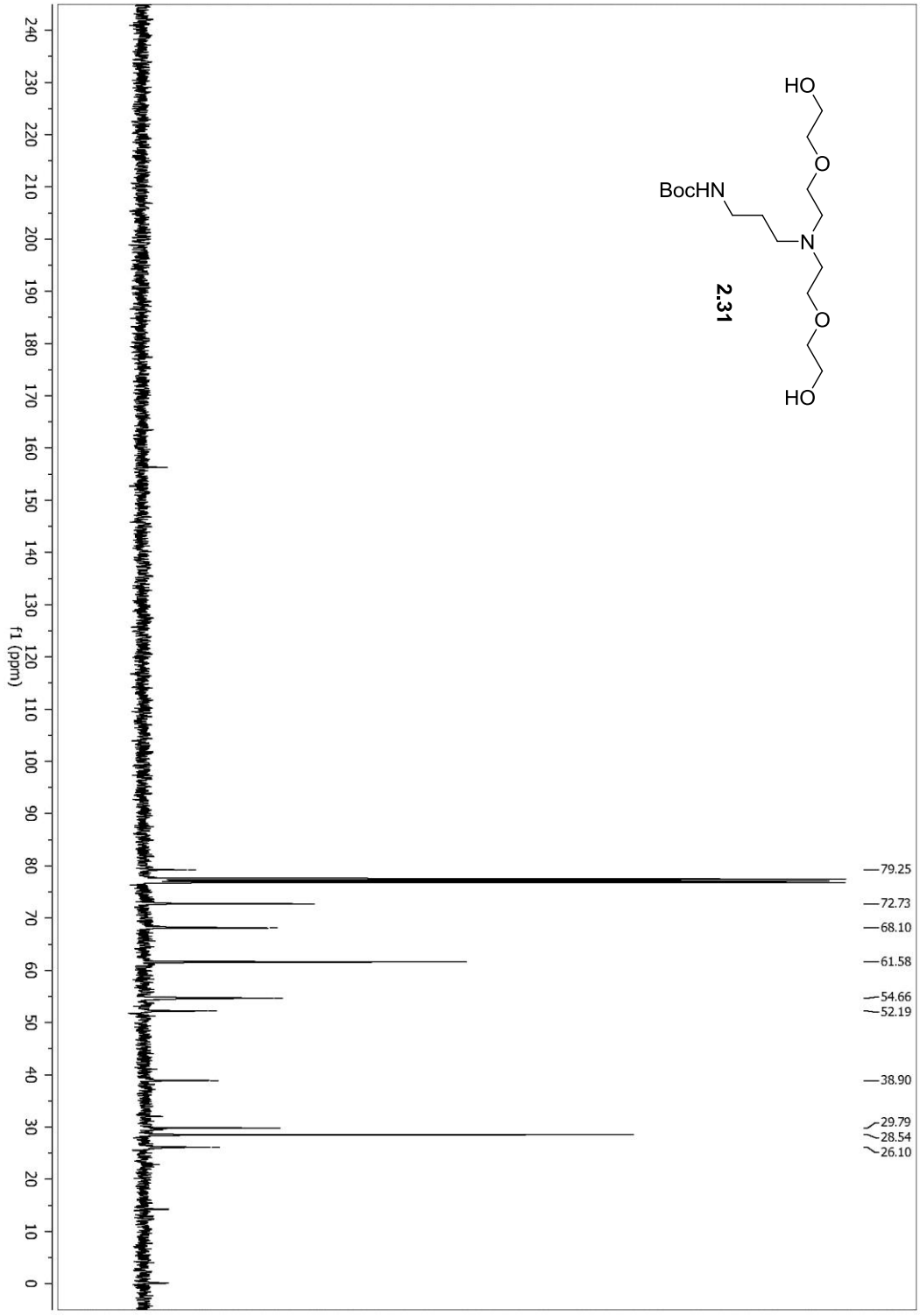
3.59
2.53
3.21
3.19
3.18
3.16
2.90
2.81
6.93
2.11
1.83
1.82
1.80
9.62
1.44
f1 (ppm)


HO
O
BocHN
N
2.31
O
HO
f1 (ppm)
240
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
79.25
72.73
68.10
61.58
54.66
52.19
38.90
29.79
28.54
26.10

Conclusion

Development of WAY derivatives such as **1.1, 1.2** or **1.3** was studied. Fischer-Indole reaction with **1.11** and **1.17** using *p*-toluenesulfonic acid in toluene at 105 ˚C afforded **1.18** in 52% yield. Further study for deprotection of acetyl group from **1.18** to synthesize **1.1** is necessary. In case of synthesis of **1.2** or **1.3**, Fischer-Indole reaction using **1.14** was not successful. It might be because of electronic effects of *meta*-substituents. Instead of **1.11**, different amide **1.37** was also tried for both **1.17** and **1.14**, but that strategy was not effective. Alternative strategy for making **1.2** or **1.3** would be studied in the future. WAY-derivatives studied here would be used for homo- or heterodimer of 5-HT receptor ligands.

A new linker which can attach fluorescence tag to the homo- or hetero- dimeric ligands was developed. For making homodimers of M100907 derivatives with the new linker such as **2.1** and **2.2**, M100907 derivative **13** was prepared using previous methods. Another derivative **2.3** is also working now, but not included in this paper. To develop a new linker, many synthetic methods were tried. Fortunately, **2.37** was formed successfully in 76% yield from **2.16** and **2.36** using K_2CO_3 in CH_3CN. Many reaction conditions for deprotection of TBS

groups from **2.37** were tested. Among them, Using 1N HCl in 8 : 1 : 1 ratios of AcOH/H_2O/MeOH at room temperature showed the best result in 71% yield. In the future, reactivation of diol of **2.31** and attachment of M100907 derivatives would be conducted. With the new linker studied here, homo- or heterodimer of 5-HT receptor ligands which a fluorescence tag can be attached will be synthesized.

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