藥學碩士學位論文

Part A:

Hendrickson reagent-mediated transformation of *N*–Boc carbamates to isocyanate and its applications.

Part B:

Discovery of a novel anti-obesity agent and *in silico* approach for target identification.

Part A:

Hendrickson reagent를 사용하여 *N*-Boc carbamate로부터 형성된 isocyanate의 cyclization 및 urea formation에 대한 응용.

Part B:

항비만에 효과적인 NED-240의 발굴 및 QSAR 모델 설계와 응용.

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서울大學敎　大學院

藥學科　藥品化學專攻

李 貞 玉

Abstract

Part A: Hendrickson reagent-mediated transformation of *N*–Boc carbamates to isocyanate and its applications.

A new method for efficient and chemoselective conversion of *N*–Boc carbamates to isocyanates was developed. The reaction is carried out in a mildly acidic condition using Hendrickson's 'POP' reagent as the dehydrative reagent. The *in-situ* formed isocyanate intermediate were successfully employed to Friedel-Crafts cyclization and urea formation to provide various heterocyclic lactams and ureas, respectively.

Part B: Discovery of a novel anti-obesity agent and *in silico* approach for target identification.

The use of anti-obesity agents is highly constrained due to their frequent advent of cardiovascular-related side effects, despite the need for these agents in modern society is constantly rising. For this reason, we screened a library containing about two-hundred and fifty piper-amide-like compounds for anti-obesity activity and discovered NED-240 as a potent agent. Additional experiments in animal models revealed the possibility of NED-240 as potential anti-obesity agent with little or no toxicity. A QSAR model based on the screening results were constructed that represents positively correlative descriptors with regard to activities. This model was used to predict anti-obesity activity for libraries containing thousands of small molecules and bioactives that are indicated for distinct actions on various targets. From the calculated results, a few of the selected compounds with high predicted activities were actually effective *in vitro.* By using this method we are expecting to identify the cellular target of NED-240 that leads to the observed anti-obesity effects, as well as more of the possible lead-compounds that can be utilized for further modification of NED-240 as a potent and safe anti-obesity agent.

Contents

Part A. Hendrickson reagent–mediated transformation of *N*–Boc carbamates to isocyanate and its applications.

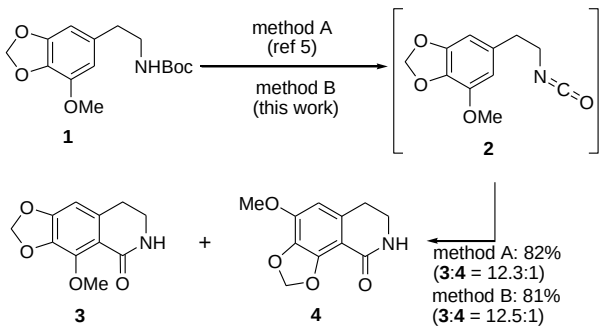
Introduction

The highly electrophilic isocyanate have been widely used as an useful intermediate to provide an array of various functional groups.[1] Treatment of isocyanates with alcohols, amines and nucleophilic aryl groups affords carbamates, urea and aryl amide,[2] respectively. Methods for preparation of isocyanates have been developed by many groups over the decade. Phosgene or its less toxic derivatives such as triphosgene have been utilized to provide a direct way to form isocyanates from amines. Alternatively, a phosgene-equivalent condition under $(Boc)_2O$/DMAP was reported by Knölker and coworkers as a safe and non-toxic method.[3] Another valuable strategy for isocyanate formation is taking advantage of carbamates, which are commonly used as amino-protecting groups in multi-step synthesis. Treatment of carbamates with halosilanes or Lewis acids would easily result in isocyanate formation.[4] Nevertheless, a chemoselective formation of isocyanates on a substrate bearing two or more different carbamate groups is rarely investigated (Scheme 1). Petillo and coworkers demonstrated that appropriate selection of chlorosilanes could differentiate two carbamates group for isocyanate formation, which is strongly affected by steric properties of alkyl substituent on oxygen of the carbamates to give the reactivity pattern: Me > *i*-Bu > *t*-Bu.[5] Although *N*-Boc carbamate group is selectively deprotected over other carbamate groups under acidic conditions, a selective transformation to isocyanate has not been studied in depth. Accordingly, a new method for a mild and chemoselective condition for isocyanates formation from *N*-Boc carbamates could assist practical synthesis of complex molecules or natural products.

During the course of total synthesis of (+)-*trans*-dihydronarciclasine, we recently found an efficient method for the synthesis of isoquinolinones (**3** and **4**) from *N*-Boc carbamate (**1**) utilizing Tf_2O (1.1 equiv) and 2-ClPy (1.5 equiv) as dehydrating agents.[6] Interestingly, intermediate study revealed that this reaction proceed through the isocyanate intermediate (**2**), which has been isolated and structurally elucidated. As part of our ongoing efforts towards the discovery of efficient conditions for dehydrative cyclization of **1**, various dehydrating reagents have been screened. To our delight, treatment of **1** with *in-situ* formed Hendrickson reagent (Tf_2O (1.5 equiv), Ph_3PO (3.0 equiv)) gave the isoquinolines (**3** and **4**) with high yield and regioselectivity (81%, **3**:**4** = 12.5:1) via the same isocyanate intermediate **2**. Hendrickson reagent has been used as a powerful dehydrating reagent for functional group transformations

and synthesis of various heterocycles.[7] Recently, Hendrickson reagent-mediated dehydrative cyclization of substrate with amide group has been reported to provide an efficient way for the synthesis of isoquinolines, β-carblines and phenanthridines.[8] Moreover, Wang and coworkers showed that *N*-CO_2Et carbamate could be used instead of amides for Hendrickson reagent mediated-dehydrative cyclization to give the corresponding ethoxy substituted product.[9] To our best knowledge, however, Hendrickson reagent has yet been utilized for isocyanate formation from *N*-Boc carbamates. This prompted us to investigate the optimal reaction condition and application in a scope of various substrate for the Hendrickson reagent-mediated isocyanate formation from *N*-Boc carbamates and subsequent dehydrative cyclization and urea formation.

Scheme 1. Preliminary Results from the Total Synthesis of (+)-*trans*-Dihydronarciclasine[a]



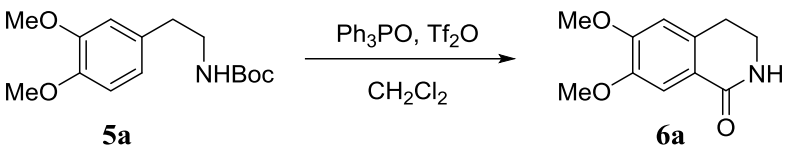
[a]Reagents and conditions: method A: Tf_2O (1.1 equiv.), 2-ClPy (1.5 equiv.), CH_2Cl_2, –78 ℃ to rt. 2 h. method B Tf_2O (1.5 equiv.), Ph_3PO (3.0 equiv.), CH_2Cl_2, 0 ℃ to 35 ℃, 2 h.

Results and Discussion

The optimal reaction condition was first investigated for the formation of isocyanate followed by Friedel-Crafts cyclization with *N*-Boc carbamate **5a** as an initial model substrate (Table 1). When **5a** was exposed to Hendrickson reagent (Tf_2O (1.5 equiv.), Ph_3PO (3.0 equiv.)) at 0 ℃, the corresponding isocyanate was rapidly generated. Subsequently, Friedel-Crafts type cyclization was facilitated by increasing temperature up to room temperature and 35 ℃ to give dihydroisoquinolin-1-one **6** in 57% and 71% yield, respectively (entries 1 and 2). Based on this result which showed a fast transformation of *N*-Boc carbamate group to isocyanate, we tested the reaction with the reduced amount of Hendrickson reagent (Tf_2O (1.2 equiv.), Ph_3PO (2.4 equiv.)). Under these conditions, isocyanate formation still occurred efficiently and the following cyclization took place in a slower rate but gave **6** with a good yield (entry 3: room

temperature, 71%, entry 4: 35 ℃, 88%). Increasing the amount of Tf_2O to 2.4 equiv. shortened the reaction time but the yield was unaffected (70%, entry 5). Addition of acids to the reaction mixture increased the product yields (94–98% vs 88%) possibly due to the facilitating effect of acids towards the intramolecular Friedel-Crafts reaction of the corresponding isocyanate intermediate (entries 6–8).

Table 1. Optimization of the Cyclization with Hendrickson Reagent[a]



5a **6a**

Entry	Ph_3PO (eq.)	Tf_2O (eq.)	Lewis acid[b](eq.)	Temp. (°C)	Time (h)	Yield (%)[c, d]
1	3	1.5	-	0 → rt	2	57
2	3	1.5	-	0 → 35	1	71
3	2.4	1.2	-	0 → rt	3.5	71
4	2.4	1.2	-	0 → 35	2	88(85)
5	2.4	2.4	-	0 → rt	1	70
6	2.4	1.2	$BF_3{\cdot}Et_2O$ (5.0)	0	1	98(97)
7	2.4	1.2	MsOH (5.0)	0	1	97
8	2.4	1.2	TfOH (5.0)	0	1	94

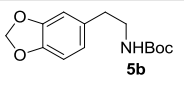
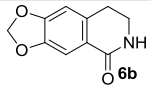
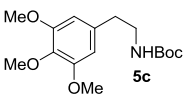
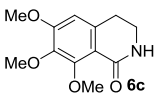
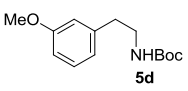
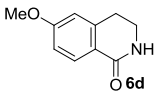
[a]Reaction conditions: **5a** (0.3 mmol), Ph_3PO, Tf_2O, CH_2Cl_2 (10 mL). [b]The values in parentheses are the equivalent of Lewis acids. [c]Determined by ^1H NMR using 1,1,2,2-tetrachloroethane as an internal standard. [d]The values in parentheses are isolated yield.

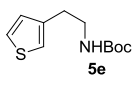
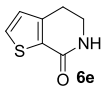
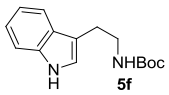
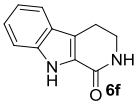
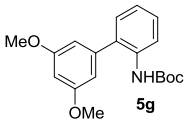
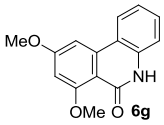
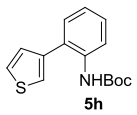
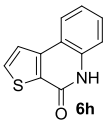
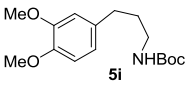
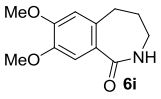
With the optimized cyclization conditions in hand, we next investigated the substrate scope of the dehydrative cyclization (Table 2). All the substrates were tested under two different

reaction conditions: with the acid additive (method A: Ph_3PO (2.4 equiv.), Tf_2O (1.2 equiv.), $BF_3{\cdot}Et_2O$ (5.0 equiv.), CH_2Cl_2, 0 ℃) or without the acid additive (method B: Ph_3PO (2.4 equiv.), Tf_2O (1.2 equiv.), CH_2Cl_2, 0 ℃ to 35 ℃). Table 2 displayed a better outcome from the two methods or result from method B if both conditions gave similar product yields. Remarkably, reactions of all the compounds with electron-rich aryl or heteroaryl groups underwent smoothly to afford the desired products in high yields.

The *N*-Boc carbamate **5b** having a methylendioxy substituent on the benzene ring was easily transformed into the 3,4-dihydroisoquinolin-1-one **6b** with the aid of acid additive (method A) in 99% yield (entry 1). Three electron-donating methoxy-substituted **5c** let cyclized efficiently without any acid additive (method B) to produce **6c** in 70% yield. One methoxy group on **5d** is good enough to undergo cyclization with the aid of an acid additive (method A) to give **6d** in 71% yield with high regioselectivity (17:1) (entry 3). Under the acid-aided cyclization condition (method A), substrates with electron-rich heteroarenes, such as thiophene and indole, underwent cyclization smoothly to provide **6e** and **6f** in 97% and 65% yield (entries 4 and 6). Substrates having Boc group on arylamine also gave the cyclized products (entries 6 and 7). The 3,5-dimethoxy substituted substrate **5g** afforded phenanthridone **6g** in 81% yield without an acid additive (method B). Also, **5h** with a thiophene ring could be transformed into **6h** in high yield (95%) under method A. Formation of a seven-membered lactam ring was explored as well by using this method. When γ-arylpropylcarbamate **5i** was exposed to acid-induced cyclization conditions (method A), benzo[*c*]azepin-1-one **6i** was produced in 90% yield (entry 8).

Table 2. Substrate Scope of Cyclization Reaction

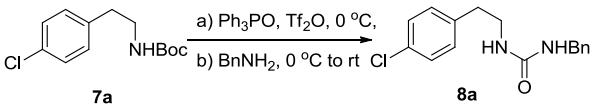
Entry	*N*-Boc carbamate	Product	Method[a]	Yield[b] (%)
1	 **5b**	 **6b**	A	99
2	 **5c**	 **6c**	B	70
3	 **5d**	 **6d**	A	71[c]

4	 **5e**	 **6e**	A	97
5	 **5f**	 **6f**	A	65
6	 **5g**	 **6g**	B	81
7	 **5h**	 **6h**	A	95
8	 **5i**	 **6i**	A[d]	90

[a]Method A: **5** (0.3 mmol), Ph_3PO (2.4 equiv), Tf_2O (1.2 equiv), $BF_3{\cdot}Et_2O$ (5.0 equiv), CH_2Cl_2, 0 ℃, Method B: **5** (0.3 mmol), Ph_3PO (2.4 equiv), Tf_2O (1.2 equiv), CH_2Cl_2, 0 ℃ to 35 ℃. [b]Isolated yield. [c]Regioisomeric mixture (17:1). [d]TfOH was used instead of $BF_3{\cdot}Et_2O$.

Next, we investigated the feasibility of employing our Hendrickson reagent-mediated isocyanate formation for the synthesis of urea (Table 3). After treatment of *N*-Boc carbamate **7a** with Hendrickson reagent at 0 ℃ for 30 min, varying amounts of benzylamine was added to seek for the optimal equivalents of amines for urea formation. Addition of 10, 5 and 3 equivalents of benzylamine to isocyanate intermediate efficiently produced urea **8a** in high yield (89–91%) (entries 1–3). On the contrary, when 2 equivalents of benzylamine was added, the yield of urea product was dramatically decreased to 27% (entry 4). The presence of TfOH derived from the triflic anhydride during the reaction might account for this low yield, presumably via reducing the amount of nucleophilic free amine by salt formation. To overcome this problem, a catalytic tertiary amine, such as NEt_3 or DIPEA, was added into the reaction mixture along with benzylamine (entries 5 and 6). Addition of NEt_3 and DIPEA increased the yield of urea formation up to 68% and 35%, respectively. Both of the additional amines, however, were unable to give better results than the 3 equivalents of benzylamine (91% vs 68% or 35%).

Table 3. Optimization of Urea Formation[a]

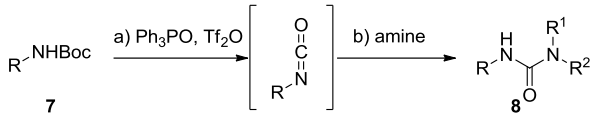


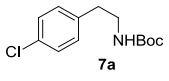
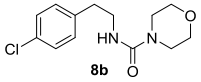
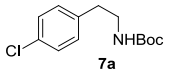
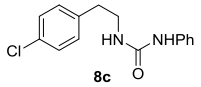
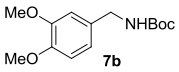
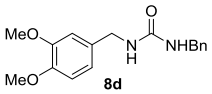
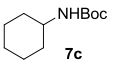
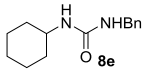
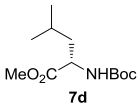
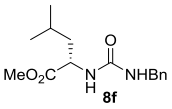
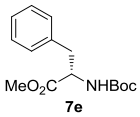
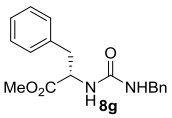
Entry	$BnNH_2$ (eq.)	Base[b] (eq.)	Time (h)	Yield[c] (%)
1	10	-	1	90
2	5	-	1	89
3	3	-	1.5	91
4	2	-	24	27
5	2	NEt_3 (2.0)	2	68
6	2	DIPEA (2.0)	24	35

[a]Reaction conditions: **7a** (0.3 mmol), Ph_3PO (1.2 equiv.), Tf_2O (2.4 equiv.), CH_2Cl_2 (10 mL), 0 ℃, 30 min; $BnNH_2$, base, 0 ℃ to rt. [b]The values in parentheses are the equivalent of bases. [c]Isolated yield.

To examine the applicability of the method for urea synthesis as described above, the reaction was performed in combination of different amines and *N*-Boc carbamate substrates (Table 4). Treatment of the isocyanate intermediate **7a** with morpholine provided urea **8b** in high yield (both 96%) (entries 1 and 2). Less nucleophilic aniline required prolonged reaction time (13 h vs 1 h) to complete the reaction, yet the product yield was unaffected (91%) (entry 3). Sterically demanding substrates, such as *N*-Boc-3,4-dimethoxybenzylamine (**7b**) and *N*-Boc-cyclohexylamine (**7b**), efficiently provided urea **8d** and **8e** in 91% and 88% yield, respectively (entries 4 and 5). *N*-Boc-Protected amino acid derivatives **7d** and **7e** could be transformed into urea **8f** and **8g** in good yield without loosing their optical purity (entries 6 and 7).

Table 4. Substrate Scope of Urea Formation[a]



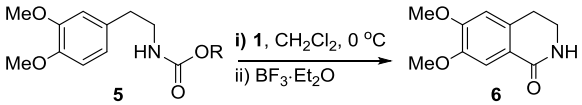
Entry	*N*-Boc Carbamate **7**	Amine	Product **8**	Yield[b] (%)
1	 **7a**	Morpholine	 **8b**	96
2	 **7a**	Aniline	 **8c**	91[c]
3	 **7b**	$BnNH_2$	 **8d**	91
4	 **7c**	$BnNH_2$	 **8e**	88
5	 **7d**	$BnNH_2$	 **8f**	98
6	 **7e**	$BnNH_2$	 **8g**	88

[a]Reaction conditions: **7** (0.3 mmol), Ph_3PO (1.2 equiv), Tf_2O (2.4 equiv), CH_2Cl_2 (10 mL), 0 ℃, 30 min; amine (3.0 equiv), 0 ℃ to rt, 1 h. [b]Isolated yield. [c]Reaction time is 13 h.

To expand the synthetic application range of these methods, we examined the chemoselectiviy of the Hendrickson reagent-mediate isocyanate formation and the subsequent cyclization reaction (Table 5). First, *N*-Boc carbamate **5a** and its *O*-alky or *O*-phenyl carbamate

derivatives were exposed to Hendrickson reagent at 0 ℃ for 5 min and formation of isocyanate was carefully monitored by the thin-layer chromatography. *N*-Boc carbamate **5a** was transformed into isocyanate almost quantitatively in 5 min (entry 1). However, neither *O*-Me or *O*-Ph carbamates were unreactive in this condition (entry 2 and 5). Instead, hydrolysis of the reaction mixture resulted in recovery of the starting material **5**. Only a trace amount of the isocyanate intermediate was observed when *O*-Et and *O*-Ph carbamates were treated with the Hendrickson reagent (entries 3 and 4). These results altogether led to the conclusion that the Hendrickson reagent-mediated isocyanate formation generally have the following pattern: *t*-Bu > Bn > Et >> Me, Ph.

Table 5. Reactivities of the carbamates in the Hendrickson reagent-mediated cyclization.[a]



Entry	Carbamate	R	Recovery of **5** [%][b]	Yield of **6** [%][b]
1	**5a**	*t*Bu	n.d. [c]	98
2	**5a-Me**	Me	88	n.d. [c]
3	**5a-Et**	Et	55	21
4	**5a-Bn**	Bn	n.d. [c]	55
5	**5a-Ph**	Ph	90	n.d. [c]

[a] 5 (0.3 mmol), Hendrickson reagent 1 (1.2 equiv.), $BF_3 \cdot Et_2O$ (5 equiv.), and CH_2Cl_2 (10 mL) were used. The reaction was carried out at 0 ℃ for 1 h. [b] Determined by ^1H NMR using 1,1,2,2-tetrachloroethane as an internal standard. [c] Not detected.

Conclusion

In conclusion, under mild Hendrickson reagent-mediated conditions, *N*-Boc carbamates are easily transformed into versatile isocyanate intermediates, which could be applied to further chemical modifications, such as Friedel-Crafts cyclization and urea synthesis. This transformation proceeds in highly chemoselective way over the other carbamates, which expands the applicability of this method for the synthesis of complex molecules or natural products.

Experimental Section

1. General methods

All chemicals were of reagent grade and used as received. All reactions were performed under an inert atmosphere of dry nitrogen using distilled dry solvents. Reactions were monitored by TLC analysis using silica gel 60 F-254 thin-layer chromatography plates. Flash column chromatography was performed on silica gel (230-400 mesh). ^{1}H NMR (300, 400 or 500 MHz) and ^{13}C NMR (75 or 100 MHz) spectra were recorded in δ units relative to the non-deuterated solvent as the internal reference. IR spectra were measured on a Fourier Transform Infrared spectrometer. High-resolution mass spectra (HRMS) were recorded using fast atom bombardment (FAB).

2. Experimental procedure and spectroscopic data analysis

2.1. Representative procedure for preparation of *N*-Boc carbamate

***tert*-Butyl (2-(7-methoxybenzo[*d*][1,3]dioxol-5-yl)ethyl)carbamate (1)**: To a solution containing 2-(7-methoxybenzo[*d*][1,3]dioxol-5-yl)ethanamine[15] CH_2Cl_2 (52 mL) were added trimethylamine, NEt_3 (2.9 mL, 20.5 mmol, 2.0 equiv), DMAP (125 mg, 1.03 mmol, 0.1 equiv), and $(Boc)_2O$ (2.5 g, 11.3 mmol, 1.1 equiv) at 0 ℃. Upon completion of the reaction as monitored by TLC, saturated solution of ammonium chloride was added and the mixture was extracted twice with CH_2Cl_2. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (hexane/EtOAc, 2:1) to give *N*-Boc carbamate **1** (2.8 g, 93%) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.42 (s, 9H), 2.68 (t, J = 6.8 Hz, 2H), 3.30−3.32 (m, 2H), 3.87 (s, 3H), 4.51 (br s, 1H), 5.92 (s, 2H), 6.33−6.36 (m, 2H); HRMS (FAB) calcd. $C_{15}H_{21}NO_5$ 295.1420 ($[M+H]^+$), found 295.1426.

The amines required for the preparation of carbamate compounds **5a-5i** and **7a-7e** are commercially available.

***tert*-butyl 3,4-dimethoxyphenethylcarbamate (5a)**:[1] White solid; 96% yield; R_f = 0.35 (hexane/EtOAc, 3:1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 1.42 (s, 9H), 2.57 (t, *J* = 7.7 Hz, 2H), 3.12–3.14 (m, 2H), 3.83 (s, 3H), 3.85 (s, 3H), 4.50 (br s, 1H), 6.68 (s, 1H), 6.71 (d, *J* = 2.0 Hz, 1H), 6.77 (d, *J* = 8.8 Hz, 1H); HRMS (FAB) calcd. $C_{15}H_{24}NO_4$ 282.1627 ($[M+H]^+$), found 282.1634.

***tert*-butyl (2-(benzo[*d*][1,3]dioxol-5-yl)ethyl)carbamate (5b)**:[1] White solid; 98% yield; R_f = 0.35 (hexane/EtOAc, 8:1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 1.42 (s, 9H), 2.69 (t, *J* = 7.0 Hz, 2H), 3.29–3.31 (m, 2H), 4.50 (br s, 1H), 5.91 (s, 2H), 6.60–6.66 (m, 2H), 6.72 (d, *J* = 7.9 Hz, 1H); HRMS (FAB) calcd. $C_{14}H_{19}NO_4$ 265.1314 ($[M+H]^+$), found 265.1311.

***tert*-butyl 3,4,5-trimethoxyphenethylcarbamate (5c)**:[1] White solid; 95% yield; R_f = 0.35 (hexane/EtOAc, 4:1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 1.42 (s, 9H), 2.72 (t, *J* = 7.1 Hz, 2H), 3.33 (td, *J* = 6.5 Hz, 2H), 3.81 (s, 3H), 3.83 (s, 6H), 4.54 (br, s, 1H), 6.38 (s, 2H); HRMS (FAB) calcd. $C_{16}H_{25}NO_5$ 311.1733 ($[M+H]^+$), found 311.1735.

***tert*-butyl 3-methoxyphenethylcarbamate (5d)**:[1] White solid; 95% yield; R_f = 0.35 (hexane/EtOAc, 4:1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 1.42 (s, 9H), 2.72 (t, *J* = 7.1 Hz, 2H), 3.33 (td, *J* = 6.5 Hz, 2H), 3.81 (s, 3H), 3.83 (s, 6H), 4.54 (br, s, 1H), 6.38 (s, 2H); HRMS (FAB) calcd. $C_{16}H_{25}NO_5$ 311.1733 ($[M+H]^+$), found 311.1735.

***tert*-butyl (2-(thiophen-3-yl)ethyl)carbamate (5e)**:[1] White solid; 75% yield; R_f = 0.30 (hexane/EtOAc, 30:1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 1.42 (s, 9H), 2.80 (t, *J* = 6.9 Hz, 2H), 3.33–3.37 (m, 2H), 4.54 (br s, 1H), 6.93 (dd, *J* = 1.1, 5.0 Hz, 2H), 6.98–6.99 (m, 1H), 7.26 (dd, *J* = 3.2, 5.0 Hz, 1H); HRMS (FAB) calcd. $C_{11}H_{18}NO_2S$ 228.1058 ($[M+H]^+$), found 228.1054.

***tert*-butyl (2-(1*H*-indol-3-yl)ethyl)carbamate (5f)**:[1] White solid; 76% yield; R_f = 0.30 (hexane/EtOAc, 5:1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 1.41 (s, 9H), 2.94 (t, *J* = 6.8 Hz, 2H), 3.44–3.46 (m, 2H), 4.58 (br s, 1H), 7.03 (s, 1H), 7.10 (td, *J* = 1.0, 7.4 Hz, 1H), 7.19 (td, *J* = 1.2, 7.5 Hz, 1H), 7.35 (d, *J* = 8.0 Hz, 1H), 7.59 (d, *J* = 7.7 Hz, 1H), 7.99 (br s, 1H); HRMS (FAB) calcd. $C_{15}H_{20}N_2O_2$ 260.1525 ($[M+H]^+$), found 260.1528.

***tert*-butyl (3',5'-dimethoxy-[1,1'-biphenyl]-2-yl)carbamate (5g)**: [1] White solid; 80% yield; R_f = 0.35 (hexane/EtOAc, 5:1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 1.45 (s, 9H), 3.80 (s, 6H), 6.48 (s, 3H), 6.60 (br s, 1H), 7.06 (td, *J* = 1.2, 7.5 Hz, 1H), 7.20 (dd, *J* = 1.7, 7.5 Hz, 1H) 7.32 (td, *J* = 1.7, 8.4 Hz, 1H), 8.10 (d, *J* = 8.3 Hz, 1H); HRMS (FAB) calcd. $C_{19}H_{23}NO_4$ 329.1627 ($[M+H]^+$), found 329.1632

***tert*-butyl (2-(thiophen-3-yl)phenyl)carbamate (5h)**: [10] White solid; 92% yield; R_f = 0.35 (hexane/EtOAc, 10:1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 1.49 (s, 9H), 6.65 (br s, 1H), 7.07 (t, *J* = 7.2 Hz, 1H), 7.16 (dd, *J* = 0.6, 4.2 Hz, 1H), 7.25 (dd, *J* = 1.7, 7.4 Hz, 1H), 7.29–7.35 (m, 2H), 7.46 (dd, *J* = 3.0, 5.1 Hz, 1H), 8.10 (d, *J* = 8.4 Hz, 1H); HRMS (FAB) calcd. $C_{15}H_{17}NO_2S$ 275.0980 ($[M+H]^+$), found 275.0982.

***tert*-butyl (3-(3,4-dimethoxyphenyl)propyl)carbamate (5i)**: [1] White solid; 88% yield; R_f = 0.35 (hexane/EtOAc, 5:1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 1.42 (s, 9H), 1.77 (q, *J* = 7.4 Hz, 2H), 2.57 (t, *J* = 7.8 Hz, 2H), 3.13–3.15 (m, 2H), 3.84 (s, 3H), 3.85 (s, 3H), 4.50 (br s, 1H), 6.68–6.78 (m, 3H); HRMS (FAB) calcd. $C_{16}H_{25}NO_4$ 295.1784 ($[M+H]^+$), found 295.1777.

***tert*-butyl 4-chlorophenethylcarbamate (7a)**: [1] White solid; 98% yield; R_f = 0.35 (hexane/EtOAc, 4:1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 1.41 (s, 9H), 2.74 (t, *J* = 6.9 Hz, 2H), 3.30−3.36 (m, 2H), 4.49 (br s, 1H), 7.10 (d, *J* = 8.3 Hz, 2H), 7.23–7.27 (m, 2H); HRMS (FAB) calcd. $C_{13}H_{19}ClNO_2$ 256.1104 ($[M+H]^+$), found 256.1100.

***tert*-butyl 3,4-dimethoxybenzylcarbamate (7b)**:[1] White solid; 92% yield; R_f = 0.35 (hexane/EtOAc, 5:1); ^{1}H NMR ($CDCl_3$, 500 MHz) δ = 1.44 (s, 9H), 3.84 (s, 6H), 4.22 (s, 2H), 4.77 (br s, 1H), 6.79–6.80 (m, 3H); HRMS (FAB) calcd. $C_{14}H_{21}NO_4$ 267.1471 ($[M+H]^+$), found 267.1467.

***tert*-butyl cyclohexylcarbamate (7c)**: [11] White solid; 85% yield; R_f = 0.35 (hexane/EtOAc, 20:1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 0.98–1.16 (m, 3H), 1.21–1.35 (m, 2H), 1.40 (s, 9H), 1.51–1.59 (m, 1H), 1.62–1.68 (m, 2H), 1.85–1.90 (m, 2H), 3.33–3.41 (m, 1H), 4.41 (br s, 1H); HRMS (FAB) calcd. $C_{11}H_{22}NO_2$ 200.1651 ($[M+H]^+$), found 200.1650.

***N*-*tert*-butoxycarbonyl-*L*-leucine methyl ester (7d)**: [3] White solid; 91% yield; R_f = 0.35 (hexane/EtOAc, 7:1); ^{1}H NMR ($CDCl_3$, 500 MHz) δ = 1.44 (s, 9H), 3.84 (s, 6H), 4.22 (s, 2H), 4.77 (br s, 1H), 6.79–6.80 (m, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ = 21.87, 22.79, 24.75, 27.39, 28.28, 28.28, 41.83, 52.01, 52.12, 79.80, 155.39, 174.00; HRMS (FAB) calcd. $C_{12}H_{24}NO_4$ 246.1705 ($[M+H]^+$), found 246.1702.

***N*-*tert*-butoxycarbonyl-*L*-phenylalanine methyl ester (7e)**:[12] White solid; 89% yield; R_f = 0.35 (hexane/EtOAc, 4:1); $[\alpha]_D$ = +47.1 (c = 1.0, $CHCl_3$) {lit. $[\alpha]_D$ = +47.0 (c = 1.0, $CHCl_3$)}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 1.39 (s, 9H), 2.99–3.13 (m, 2H), 3.69 (s, 3H), 4.57 (q, *J* = 6.7 Hz, 1H), 4.95 (d, *J* = 7.8 Hz, 1H), 7.09–

7.12 (m, 2H), 7.22–7.31 (m, 3H); HRMS (FAB) calcd. $C_{15}H_{22}NO_4$ 280.1549 ([M+H]$^+$), found 280.1551.

2.2. Optimized procedure for the Friedel-Crafts-type cyclization

Method A : Triflic anhdyride 1.0 M soln. in CH_2Cl_2 (0.24 mL, 0.24 mmol, 1.2 equiv) was added solution of Ph_3PO (130.7 mg, 0.48 mmol, 2.4 equiv) in CH_2Cl_2 (2 mL) at 0 ℃. After stirring for 30 mins until the mixture becomes opaque, *N*-Boc carbamate **5** (0.2 mmol, 1.0 equiv) in CH_2Cl_2 (2 mL) was added slowly to the mixture. The reaction mixture was warmed to 35ºC and stirring was continued. Upon completion, the reaction mixture was quenched by addition of saturated $NaHCO_3$ solution, diluted with CH_2Cl_2, washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. This residue was purified by column chromatography on silica gel (hexane/EtOAc/CH_2Cl_2/MeOH, 10:10:10:1) to yield the cyclized product **6**.

Method B : Identical to method A except for the addition of $BF_3 \cdot Et_2O$ (0.12 mL, 1.0 mmol, 5.0 equiv) 15 min after the addition of Tf_2O.

6,7-Dimethoxy-3,4-dihydroisoquinolin-1(2*H*)-one (**6a**):[5] White solid; R_f = 0.35 (hexane/EtOAc/CH_2Cl_2/MeOH,10:10:10:1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 2.87 (t, *J* = 6.6 Hz, 2H), 3.51 (td, *J* = 2.5, 6.6 Hz, 2H), 3.88 (s, 6H), 6.63 (s, 1H), 7.07 (br, s, 1H), 7.51 (s, 1H); HRMS (FAB) calcd. $C_{11}H_{14}NO_3$ 208.0974 ([M+H]$^+$), found 208.0973.

7,8-Dihydro-[1,3]dioxolo[4,5-*g*]isoquinolin-5(6*H*)-one (**6b**):[5] White solid; R_f = 0.30 (hexane/EtOAc/CH_2Cl_2/MeOH,10:10:10:1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 2.86 (t, *J* = 6.6 Hz, 2H), 3.49 (t, *J* = 6.6 Hz, 2H), 5.75 (br s, 1H), 5.98 (s, 2H), 6.63 (s, 1H), 7.50 (s, 1H); HRMS (FAB) calcd. $C_{10}H_{10}NO_3$ 192.0661 ([M+H]$^+$), found 192.0665.

6,7,8-Trimethoxy-3,4-dihydroisoquinolin-1(2*H*)-one (**6c**):[5] White solid; R_f = 0.35 (hexane/EtOAc/CH_2Cl_2/MeOH,10:10:10:1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 2.86 (t, *J* = 6.3 Hz, 2H), 3.40–3.45 (m, 2H), 3.85 (s, 3H), 3.88 (s, 3H), 3.93 (s, 3H), 5.79 (br s, 1H), 6.48 (s, 1H); HRMS (FAB) calcd. $C_{12}H_{16}NO_4$ 238.1079 ([M+H]$^+$), found 238.1086.

6-Methoxy-3,4-dihydroisoquinolin-1(2*H*)-one (**6d**):[5] White solid; R_f = 0.35 (hexane/EtOAc/CH_2Cl_2/MeOH,10:10:10:1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 2.94 (t, *J* = 6.5 Hz, 2H), 3.52 (dt, *J* = 2.7, 6.5 Hz, 2H), 3.83 (s, 3H), 5.81 (br s, 1H), 6.68 (s, 1H), 6.83 (dd, *J* = 2.1, 8.6 Hz, 1H), 8.00 (d, *J* = 8.6 Hz, 1H); HRMS (FAB) calcd. $C_{10}H_{12}NO_2$ 178.0868 ($[M+H]^+$), found 178.0861.

5,6-Dihydrothieno[2,3-*c*]pyridin-7(4*H*)-one (**6e**):[5] White solid; R_f = 0.35 (hexane/EtOAc/CH_2Cl_2/MeOH,10:10:20:1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 2.90 (t, *J* = 6.9 Hz, 2H), 3.71 (td, *J* = 2.7, 6.9 Hz, 2H), 6.31 (br s, 1H), 6.93 (d, *J* = 4.8 Hz, 1H), 7.48 (d, *J* = 4.8 Hz, 1H); HRMS (FAB) calcd. C_7H_8NOS 154.0327 ($[M+H]^+$), found 154.0326.

2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indol-1-one (**6f**):[5] White solid; R_f = 0.35 (hexane/EtOAc/CH_2Cl_2/MeOH,10:10:10:1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 3.05 (t, *J* = 7.1 Hz, 2H), 3.71 (td, *J* = 2.6, 7.1 Hz, 2H), 6.27 (br s, 1H), 7.13 (t, *J* = 7.5 Hz, 1H), 7.28 (t, *J* = 7.5 Hz, 1H), 7.47 (d, *J* = 8.1 Hz, 1H), 7.58 (d, *J* = 8.1 Hz, 1H), 9.97 (br s, 1H); HRMS (FAB) calcd. $C_{11}H_{11}N_2O$ 187.0871 ($[M+H]^+$), found 187.0875.

7,9-dimethoxyphenanthridin-6(5*H*)-one (**6g**):[5] White solid; R_f = 0.35 (hexane/EtOAc/CH_2Cl_2/MeOH,10:10:10:1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 3.98 (s, 3H), 4.00 (s, 3H), 6.60 (d, *J* = 2.4 Hz, 1H), 7.04 (d, *J* = 8.1 Hz, 1H), 7.20 (td, *J* = 7.7, 1.0 Hz, 1H), 7.27 (d, *J* = 2.7 Hz, 1H), 7.42 (td, *J* = 1.2, 7.5 Hz, 1H), 8.08 (d, *J* = 8.1 Hz, 1H), 8.48 (br s, 1H); HRMS (FAB) calcd. $C_{15}H_{14}NO_3$ 256.0974 ($[M+H]^+$), found 256.0977.

thieno[2,3-c]quinolin-4(*5H*)-one (**6h**): White solid; R_f = 0.35 (CH_2Cl_2/MeOH, 40:1); ^{1}H NMR ($CDCl_3$:acetone-d6 5:1 v/v, 300 MHz) δ = 7.10 (ddd, *J* = 1.7, 6.6, 8.2 Hz, 1H), 7.26–7.37 (m, 2H), 7.44–7.52 (m, 1H), 7.62 (d, *J* = 5.1 Hz, 1H), 7.69 (d, *J* = 5.4 Hz, 1H), 7.80 (d, *J* = 7.8 Hz, 1H); HRMS (FAB) calcd. $C_{11}H_8NOS$ 202.0327 ($[M+H]^+$), found 202.0323.

7,8-Dimethoxy-2,3,4,5-tetrahydro-1*H*-benzo[*c*]azepin-1-one (**6i**):[5] White solid; R_f = 0.30 (CH_2Cl_2/MeOH, 20:1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 2.02 (q, *J* = 6.2 Hz, 2H), 2.80 (t, *J* = 6.5 Hz, 2H), 3.14 (q, *J* = 5.3 Hz, 2H), 3.91 (s, 6H), 6.67 (s, 1H), 6.99 (br s, 1H), 7.26 (s, 1H); HRMS (FAB) calcd. $C_{12}H_{16}NO_3$ 222.1130 ($[M+H]^+$), found 222.1128.

2.3. Optimized procedure for the urea formation

Method A : Triflic anhdyride 1.0 M soln. in CH_2Cl_2 (0.36 mL, 0.36 mmol, 1.2 equiv) was added solution of Ph_3PO (200 mg, 0.72 mmol, 2.4 equiv) in CH_2Cl_2 (5 mL) at 0 ℃. After stirring for 30 mins until the mixture becomes opaque, *N*-Boc carbamate **7** (0.3 mmol, 1.0 equiv) in CH_2Cl_2 (5 mL) was added slowly to the mixture. After stirring for 15 mins corresponding amine (0.9 mmol, 3.0 equiv) was added and stirring was continued at room temperature. Upon completion, the reaction mixture was quenched by addition of saturated $NaHCO_3$ solution, diluted with CH_2Cl_2, washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. This residue was purified by column chromatography on silica gel (hexane/acetone 3:1) to yield the urea product **8**.

1-benzyl-3-(4-chlorophenethyl)urea (**8a**): White solid; R_f = 0.30 (hexane/acetone, 2:1); m.p. 162–165 ℃; ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 2.71 (t, *J* = 6.8 Hz, 2H), 3.36 (q, *J* = 6.6 Hz, 2H), 4.27 (d, *J* = 5.7 Hz, 2H), 4.50 (br s, 1H), 4.81 (br s, 1H), 7.01–7.07 (m, 2H), 7.18–7.31 (m, 7H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 35.71, 41.46, 44.53, 127.32 (3C), 128.61 (4C), 130.10 (2C), 132.13, 137.55, 139.01, 157.97; IR (solid) υ_{max} = 3314, 2932, 1616, 1569 cm^{-1}; HRMS (FAB) calcd. $C_{16}H_{18}ClN_2O$ 289.1108 ([M+H]$^+$), found 289.1103.

***N*-(4-chlorophenethyl)morpholine-4-carboxamide** (**8b**): White solid; R_f = 0.35 (hexane/acetone, 4:1); m.p. 94–98 ℃; ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 2.78 (t, *J* = 6.9 Hz, 2H), 3.26 (t, *J* = 5.0 Hz, 4H), 3.45 (q, *J* = 6.5 Hz, 2H), 3.64 (t, *J* = 5.0 Hz, 4H), 4.41 (br s, 1H), 7.10 (d, *J* = 8.7 Hz, 2H), 7.26 (d, *J* = 8.7 Hz, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 35.70, 41.91, 43.94 (2C), 66.45 (2C), 128.56, 128.69, 130.12 (2C), 132.25, 137.68, 157.56; IR ($CHCl_3$) υ_{max} = 3343, 2856, 1538, 1263 cm^{-1}; HRMS (FAB) calcd. $C_{13}H_{18}ClN_2O_2$ 269.1057 ([M+H]$^+$), found 269.1053.

1-(4-chlorophenethyl)-3-phenylurea (**8c**): White solid; R_f = 0.30 (hexane/acetone, 4:1); m.p. 158–161 ℃; ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 2.77 (t, *J* = 6.8 Hz, 2H), 3.44 (t, *J* = 6.9 Hz, 2H), 4.81 (br s, 1H), 6.41 (br s, 1H), 7.04–7.10 (m, 2H), 7.17–7.30 (m, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 35.55, 41.35, 121.54, 124.24, 128.62, 128.69 (2C), 129.33 (2C), 130.14 (2C), 132.27, 137.44, 138.18, 155.68; IR (solid) υ_{max} = 3319, 2878, 1627, 1562, 1230 cm^{-1}; HRMS (FAB) calcd. $C_{15}H_{16}ClN_2O$ 275.0951 ([M+H]$^+$), found 275.0949.

1-benzyl-3-(3,4-dimethoxybenzyl)urea (**8d**): White solid; R_f = 0.25 (hexane/acetone, 4:1); m.p. 120–122 ℃; ^{1}H NMR ($CDCl_3$, 300 MHz) δ = 3.80 (s, 3H), 3.82 (s, 3H), 4.25 (d, *J* = 5.7 Hz, 2H), 4.31 (d, *J* = 5.7 Hz, 2H), 4.79–4.82 (m, 2H), 6.75–6.76 (m, 3H), 7.21–7.27 (m, 5H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 44.48, 44.55, 55.83,

55.92, 110.76, 111.14, 119.61, 127.29, 127.37 (2C), 128.58 (2C), 131.61, 139.08, 148.29, 149.11, 157.99; IR ($CHCl_3$) υ_{max} = 3335, 2935, 1570, 1263 cm^{-1}; HRMS (FAB) calcd. $C_{17}H_{21}N_2O_3$ 301.1552 ($[M+H]^+$), found 301.1552.

1-benzyl-3-cyclohexylurea (**8e**):[14] White solid; R_f = 0.30 (hexane/acetone, 3:1); ^1H NMR ($CDCl_3$, 300 MHz) δ = 0.99–1.13 (m, 3H), 1.23–1.37 (m, 2H), 1.55–1.67 (m, 3H), 1.87–1.91 (m, 2H), 3.48–3.55 (m, 1H), 4.20 (d, *J* = 7.98 Hz, 1H), 4.34 (d, *J* = 6.0 Hz, 2H), 4.55 (br s, 1H), 7.23–7.34 (m, 5H); HRMS (FAB) calcd. $C_{14}H_{21}N_2O$ 233.1654 ($[M+H]^+$), found 233.1658.

methyl (benzylcarbamoyl)leucinate (**8f**): White solid; R_f = 0.30 (hexane/acetone, 4:1); m.p. 91–94 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ = 0.86–0.90 (m, 6H), 1.42–1.63 (m, 3H), 3.62 (s, 3H), 4.29 (t, *J* = 6.5 Hz, 2H), 4.42–4.49 (m, 1H), 5.25–5.30 (m, 2H), 7.18–7.29 (m, 5H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ = 21.91, 22.81, 24.76, 41.90, 44.38, 51.63, 52.11, 127.16, 127.38 (2C), 128.50 (2C), 139.09, 157.72, 175.19; IR ($CHCl_3$) υ_{max} = 3345, 2955, 1633, 1743 cm^{-1}; HRMS (FAB) calcd. $C_{15}H_{23}N_2O_3$ 279.1709 ($[M+H]^+$), found 279.1715.

methyl (benzylcarbamoyl)phenylalaninate (**8g**):[13] White solid; R_f = 0.30 (hexane/acetone, 4:1); $[\alpha]_D$ = +55.1 (c = 1.0, $CHCl_3$) {lit. $[\alpha]_D$ = +56.5 (c = 1.0, $CHCl_3$)}; ^1H NMR ($CDCl_3$, 300 MHz) δ = 3.02 (dd, *J* = 5.9, 11.6 Hz, 2H), 3.67 (s, 3H), 4.29 (dd, *J* = 5.9, 9.8 Hz, 2H), 4.76–4.83 (m, 1H), 5.07 (t, *J* = 5.9 Hz, 1H), 5.14 (d, *J* = 7.8 Hz, 1H), 7.07–7.09 (m, 2H), 7.20–7.34 (m, 8H); HRMS (FAB) calcd. $C_{18}H_{21}N_2O_3$ 313.1552 ($[M+H]^+$), found 313.1543.

2.4. General procedure for the preparation of carbamates associated with Table 5

Methyl (3,4-dimethoxyphenethyl)carbamate (**5a-Me**):[14] To a solution containing 3,4-dimethoxyphenethylamine (1.7 mL, 10.3 mmol, 1 equiv) in CH_2Cl_2 was added triethylamine (2.9 mL, 20.5 mmol, 2 equiv), 4-dimethylaminopyridine (125 mg, 1.03 mmol, 0.1 equiv), and methyl chloroformate (1.6 mL, 20.5 mmol, 2 equiv) at 0 °C. After stirring for 3 h at room temperature, saturated solution of ammonium chloride was added at 0 °C. The reaction mixture was extracted twice with CH_2Cl_2. The combined organic layers were washed brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (hexane/EtOAc, 3:1, R_f = 0.35) to give carbamate **5a-Me** (2.4 g, 98%) as a white solid. ^1H NMR ($CDCl_3$, 400 MHz) δ = 2.74 (t, *J* = 6.8 Hz, 2H), 3.39–3.41 (m, 2H), 3.64 (s, 3H), 3.84 (s, 3H), 3.85 (s, 3H), 4.64 (br s, 1H), 6.70 (d, *J* = 10.7 Hz, 2H), 6.79 (d, *J* = 8.0 Hz, 1H); HRMS (FAB) calcd. $C_{12}H_{17}NO_4$ 239.1158 ($[M+H]^+$), found 239.1154.

Ethyl (3,4-dimethoxyphenethyl)carbamate (**5a-Et**): [15] White solid; 90% yield; R_f = 0.35 (hexane/EtOAc, 4:1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 1.21 (t, *J* = 7.0 Hz, 3H), 2.73 (t, *J* = 6.9 Hz, 2H), 3.38–3.40 (m, 2H), 3.84 (s, 3H), 3.85 (s, 3H), 4.08 (q, *J* = 5.9 Hz, 2H), 4.62 (br s, 1H), 6.70 (d, *J* = 10.8 Hz, 2H), 6.79 (d, *J* = 8.0 Hz, 1H); HRMS (FAB) calcd. $C_{13}H_{19}NO_4$ 253.1314 ($[M+H]^+$), found 253.1310.

Benzyl (3,4-dimethoxyphenethyl)carbamate (5d): [14] White solid; 98% yield; R_f = 0.35 (hexane/EtOAc, 4:1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 2.75 (t, J = 6.7 Hz, 2H), 3.42 (q, J = 6.3 Hz, 2H), 3.82 (s, 3H), 3.84 (s, 3H), 4.71 (br s, 1H), 5.08 (s, 2H), 6.69 (d, J = 10.9 Hz, 2H), 6.78 (d, J = 8.0 Hz, 1H), 7.28–7.36 (m, 5H); HRMS (FAB) calcd. $C_{18}H_{21}NO_4$ 315.1471 ($[M+H]^+$), found 315.1464.

Phenyl (3,4-dimethoxyphenethyl)carbamate (5e): [15] White solid; 98% yield; R_f = 0.35 (hexane/EtOAc, 4:1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 2.75 (t, *J* = 6.7 Hz, 2H), 3.42 (q, *J* = 6.3 Hz, 2H), 3.82 (s, 3H), 3.84 (s, 3H), 4.71 (br s, 1H), 5.08 (s, 2H), 6.69 (d, *J* = 10.9 Hz, 2H), 6.78 (d, *J* = 8.0 Hz, 1H), 7.28–7.36 (m, 5H); HRMS (FAB) calcd. $C_{18}H_{21}NO_4$ 315.1471 ($[M+H]^+$), found 315.1464.

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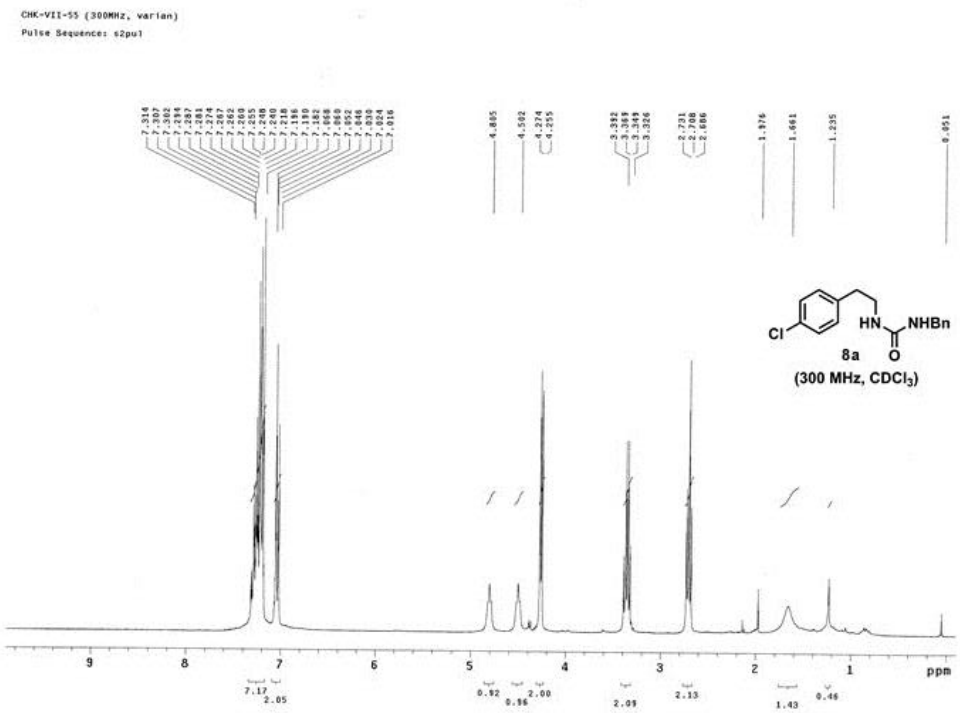
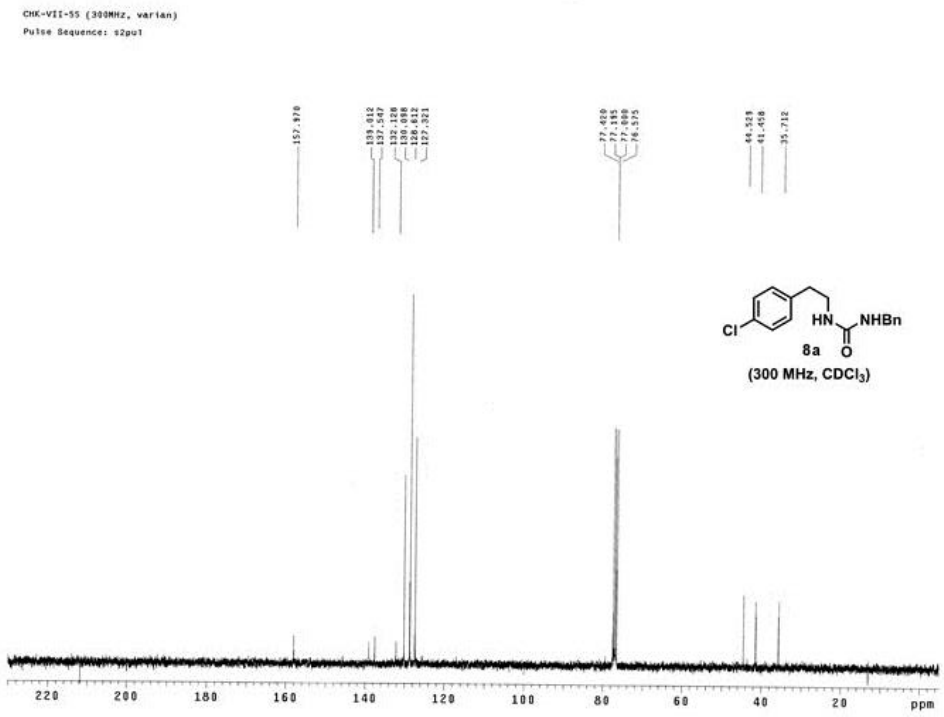
Spectral Data

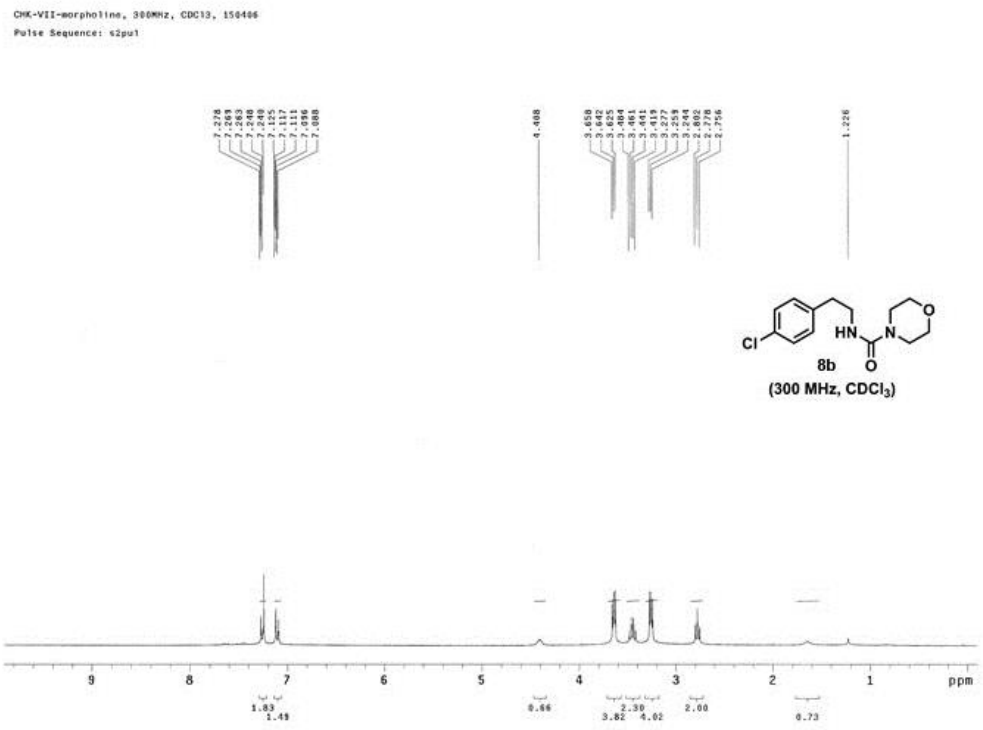
- ^{1}H NMR spectrum of **8a**



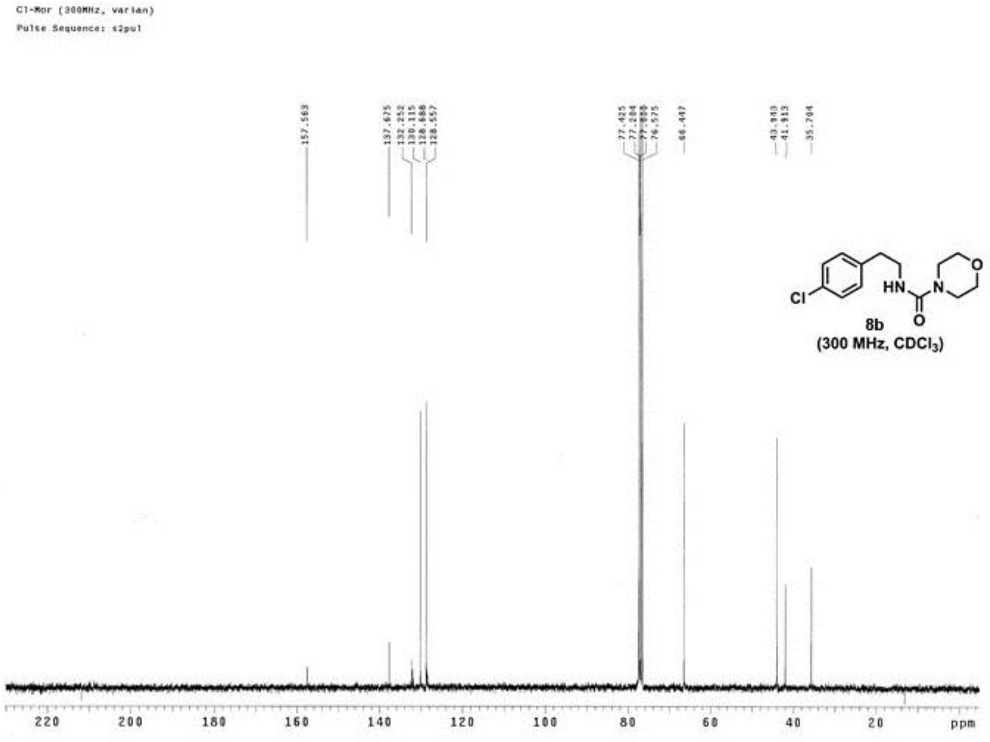
- ^{13}C NMR spectrum of **8a**



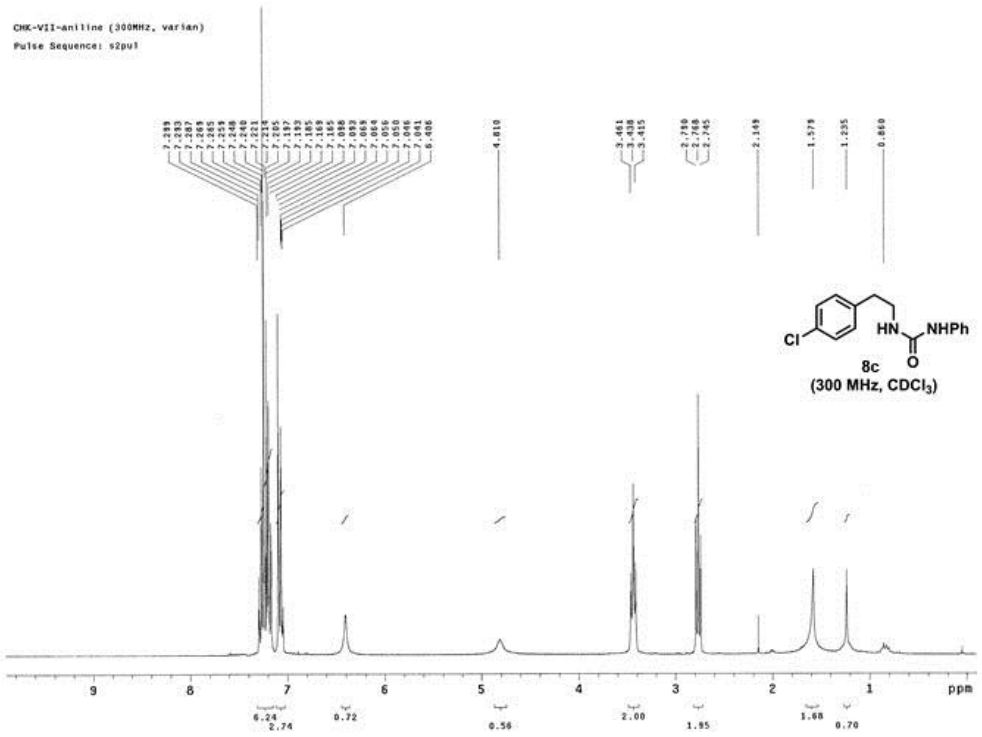
- ^{1}H NMR spectrum of **8b**



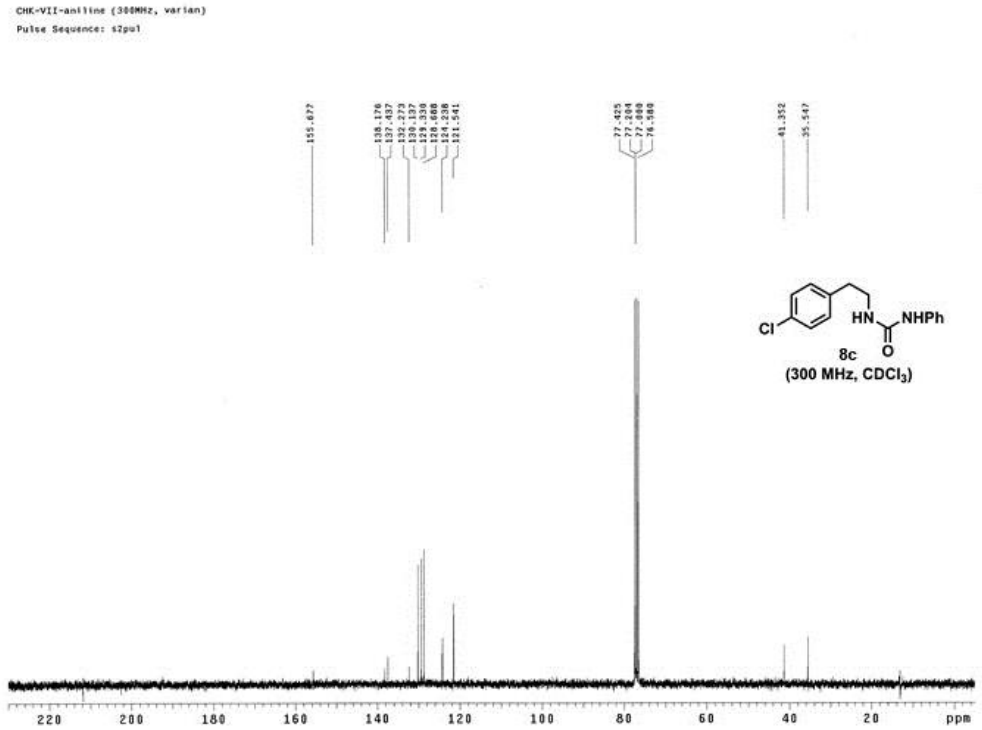
- ^{13}C NMR spectrum of **8b**



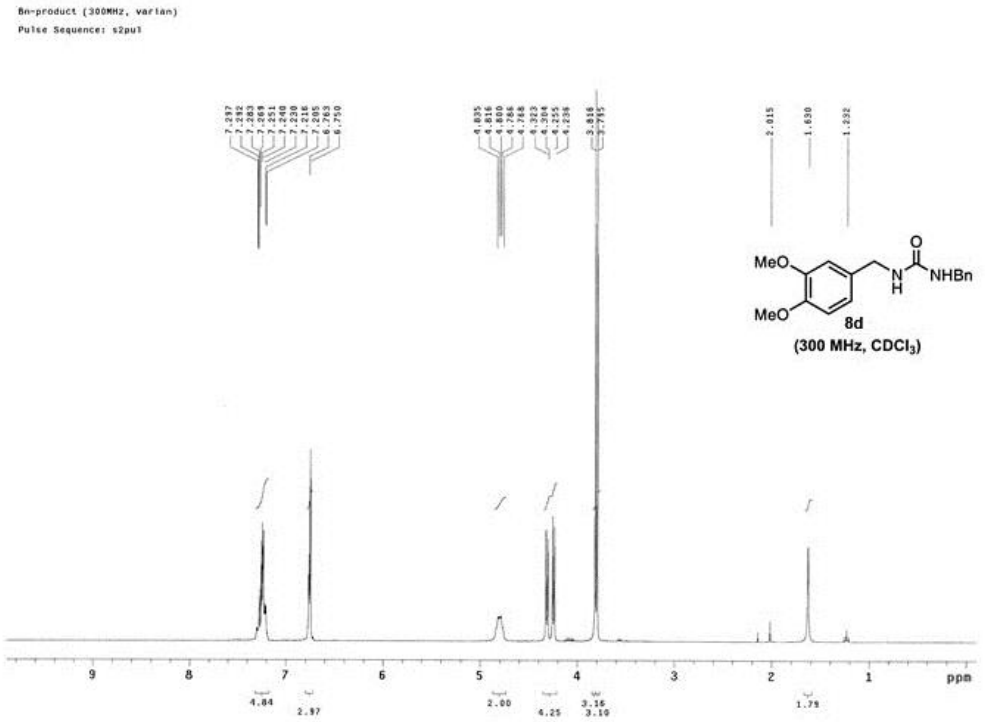
- ^{1}H NMR spectrum of **8c**



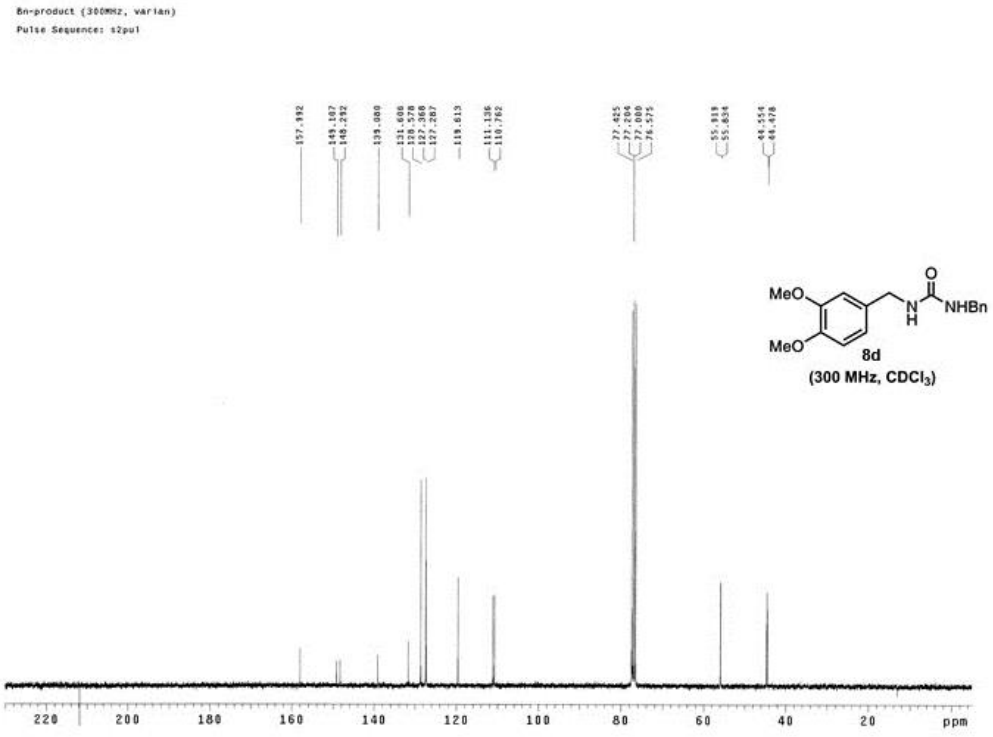
- ^{13}C NMR spectrum of **8c**



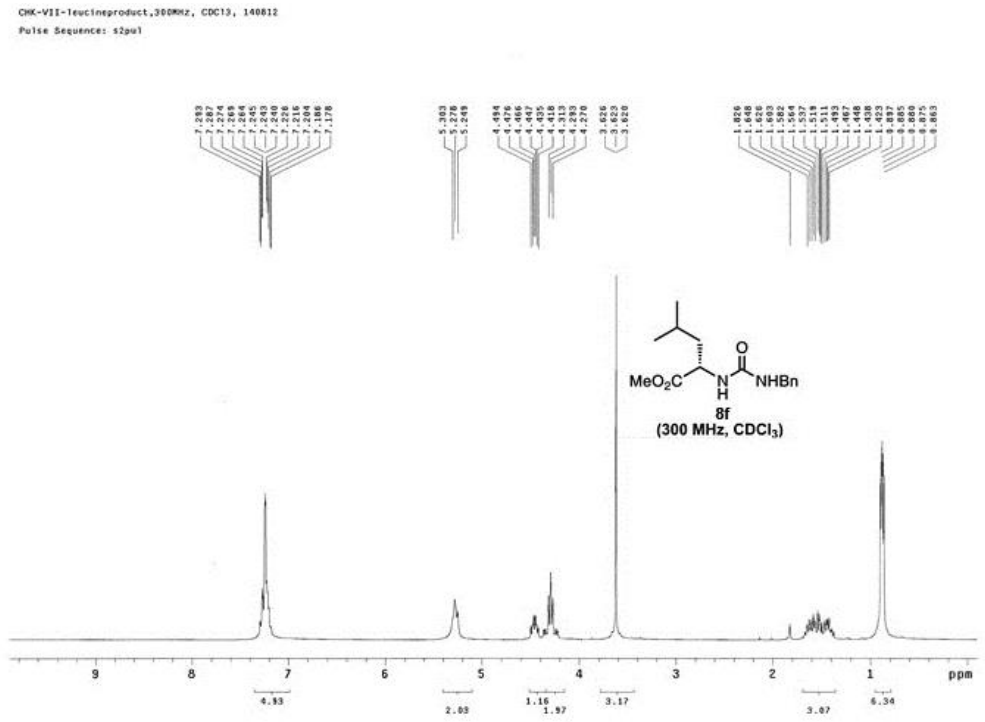
- ^{1}H NMR spectrum of **8d**



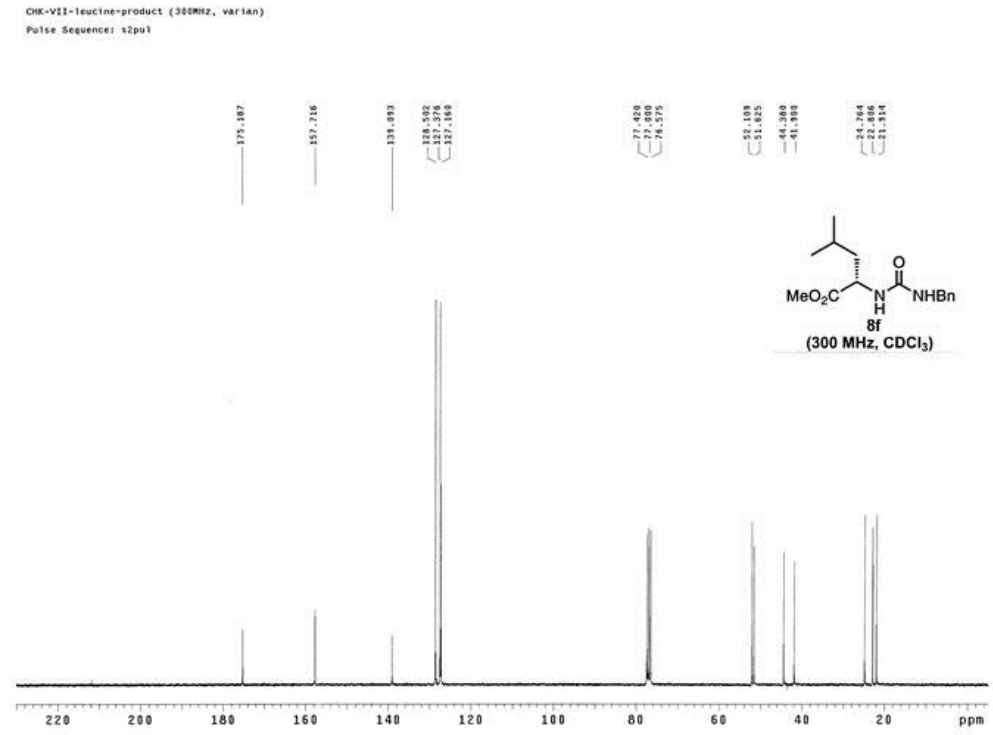
- ^{13}C NMR spectrum of **8d**



- ^{1}H NMR spectrum of **8f**



- ^{13}C NMR spectrum of **8f**



Part B: Discovery of a novel anti-obesity agent and *in silico* approach for target identification.

Introduction

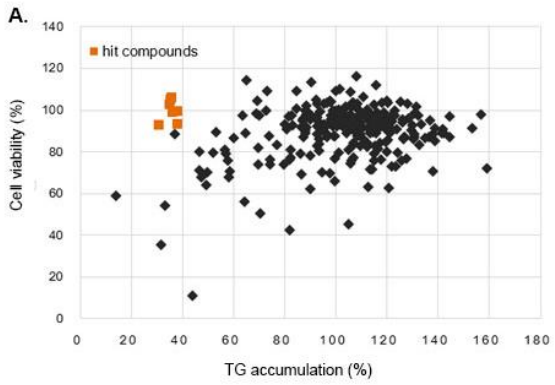
Obesity is defined as abnormal or excessive fat accumulation in adipose tissue by the World Health Organization (WHO), that became one of the most common global health problems for all age groups.[1] Worldwide increase in the prevalence of obesity threatens public health by exposing the risk of associated complications including type 2 diabetes, hyperlipidemia, hypertension, gallbladder disease, and certain types of cancers.[2] In 2010, widely used weight-loss drug, sibutramine, has been withdrawn from the market due to the advent of cardiovascular adverse events during the post-marketing study.[2,3] By now, novel anti-obesity agents approved for its use by FDA are Orlistat and Locaserin, although Locaserin has failed to gain license from the European market due to safety concerns.[2,3] Alternatively, recently approved agents such as Qsymia and Contrave are combinations of existing drugs originally indicated for other clinical conditions.[3]

Considering the complexity of the mechanisms related to the pathogenesis of obesity, recent researches show different approaches for regulating the body fat and energy balance by targeting a variety of proteins and enzymes such as β3 adrenergic receptor, GLP-1, and many other gut-derived peptide.[3] Still, drugs that are currently undergoing researches and clinical trials seem to pose undesirable side effects due to the nature of these target proteins and a demand to seek for a novel cellular target that can safely lead to weight-reduction is urgent.

Various phytochemicals such as resveratrol, quercetin, genistein, capsaicin, catechin, and dietary flavonoids are reported to inhibit adipocyte differentiation during the early stages.[4] In addition, natural piper amides, the most common constituents of the genus *Piper*, exhibited anti-obesity effect along with many other biological activities such as anti-inflammatory, antifungal, analgesic and antidepressant.[5] As a part of our efforts for the development of new anti-obesity agents, the previously reported piper-amide-like compound library was screened.[6] About 250 compounds were evaluated in terms on their activity in regulating TG accumulation in a 3T3-L1 cells using Oil Red O staining. As for the hit compounds, the effects on the intracellular expression of fatty acid synthase (FAS) and PPARγ were measured via Western blot analysis.

Results and Discussion

Our previously constructed α,β–unsaturated amide library contains 250 compounds that structurally resembles natural piper amide.[6] For screening of the library, each compound was tested on 3T3-L1 preadipocytes and adipocyte differentiation was monitored for 8 days. Upon completion of the experiment, cells were stained with Oil Red O dye and the cumulative quantity of TG was measured by fluorescence absorbance at 510 nm wavelength. Cell viability rate was also determined by performing MTT assays to avoid false positives. The influence of each test compound on TG accumulation and cell viability rate determined by MTT assay is shown in **Figure 1.** Of the 250 compounds tested, 6 compounds represented in orange box (**1A**) were identified as the hit compounds (TG accumulation < 40 % and cell viability > 90 %). The basic molecular properties and druggability based on Lipinski's rule were calculated using ADMET module in Discovery Studio 4.1 (**Table 1**.)



B.

Entry	TG accumulation (%)	Cell viability (%)
NED-109	38.0	93.2
NED-223	35.7	105.7
NED-240	38.2	99.3
NED-262	36.0	98.9
NED-275	30.7	92.5
NED-278	35.4	104.9
resveratrol	46.5	71.1

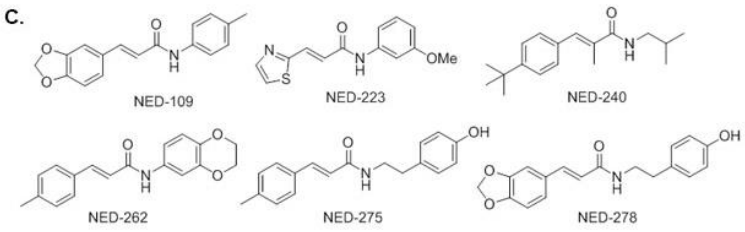


Figure 1. Screening results of the Piper-amide library. **A)** Scatter plot of cumulative quantity of triglyceride versus cell viability (%) of the 250 compounds. **B)** Identification of the hit compounds from **A). C)** Structures of the hit compounds

entry	Molecular_PSA	MW (<500)	ALog P (≤5)	H-bond donor (≤5)	H-bond acceptor (≤10)
NED-109	47.56	281.306	3.336	1	4
NED-223	79.46	260.312	1.928	1	4
NED-240	29.10	273.413	4.544	1	2
NED-262	47.56	295.332	3.354	1	4
NED-275	49.33	281.349	3.654	2	3
NED-278	67.79	311.332	2.936	2	5
resveratrol	60.69	228.243	3.090	3	3

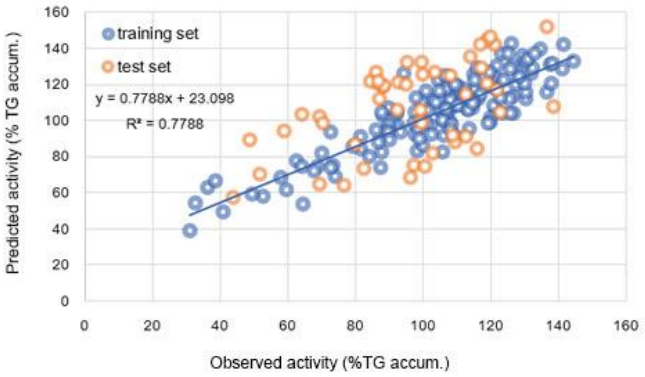
Table 1. Compliance of hit compounds to the computational parameters of oral bioavailability (Lipinski's rule of five).

A QSAR model was developed in order to explore important structural features that correlates to activity. About 160 compounds and 30 compounds with definite value for TG accumulation were distributed in training set and test set, respectively. All the compounds were minimized into their most stable conformation by using CHARMm force field prior to calculation in Discovery studio 4.1 software. Genetic function approximation algorithm was used, which automatically select for the optimal descriptors over thousands of generations created by evolutionary cross-over mechanism. The following equation **(1)** and the graph in **Figure 2.** were obtained by GFA model:

$$\begin{aligned}\text{Predicted TG accumulation (\%)} &= 186.9 - 20.241 \times \text{logD} - 3.6507 \times \text{VSA\_MR[7]} + 0.88412 \times \text{VSA\_PartialCharge[1]} \\ &+ 200.85 \times \text{BIC} - 21.363 \times \text{Kappa\_2\_AM} + 7.8376 \times \text{Kappa\_3} + 9.1805 \times \text{Dipole\_Y} \\ &+ 406.1 \times \text{Jurs\_FNSA\_2} - 1115.5 \times \text{Jurs\_RNCG} + 323.72 \times \text{Jurs\_RPCG} + 1.6445 \\ &\times \text{Jurs\_WNSA\_1} - 0.25772 \times \text{Jurs\_WNSA\_2} - 7.1175 \times \text{Jurs\_WNSA\_3} + 317.07 \\ &\times \text{Shadow\_YZfrac} - 1.0381 \times \text{Molecular\_3D\_PolarSASA} \quad (\mathbf{1})\end{aligned}$$

The predictive capability of the model was evaluated by the method developed by Roy et al. The equation **(2)** for calculating external predictability is as follow: [7]

$$r^2_{pred} = 1 - \frac{\sum\left(Y_{pred(test)} - Y_{obs(test)}\right)^2}{\sum\left(Y_{obs(test)} - \bar{Y}_{training}\right)^2} \quad (\mathbf{2})$$



r^2 = 0.7788	$r^2_{(adj)}$ = 0.7531	$r^2_{(pred)}$ = 0.7180	S.O.R p-value =4.871e-035

Figure 2. Correlation of observed activity (%TG accum.) versus predicted activity (%TG accum.) of training (blue) and test set (orange) by GFA model.

The model was verified using commercially available library from Selleck® consists of 1700 bioactive small molecules. The predicted activity (% TG accumulation) were calculated for the entire library using the equation **(1)**. Among those with highest predicted activity, SB505124, TWS119 and triclabendazole were selected based on structural similarity and commercial availability. Oil Red O dye staining test were performed on these compounds to evaluate their anti-obesity effects. The calculated results and experimentally determined inhibitory activity is shown in **Figure 3A** and **3B**, respectively. Among the three compounds tested, TWS119 exhibited remarkable TG reducing effect comparable to NED-240. TWS119 was initially identified as a small molecule that controls neuronal differentiation in embryonic stem cell.[8] Affinity-based chromatography revealed that the cellular target of TWS119 is GSK-3β, which has emerged as a key regulator in various processes involved in cellular differentiation and development. Furthermore, it has been also noted that inhibition of GSK-3β could play beneficial role in preventing differentiation of preadipocytes into adipocytes.[9] These previous researches suggest that the mechanism of how NED-240 suppresses adipogenesis might relate to its inhibitory activity against GSK-3β. Further investigation on the entire series of piper-amide library for their action on GSK-3β is needed to validate this hypothesis in future.

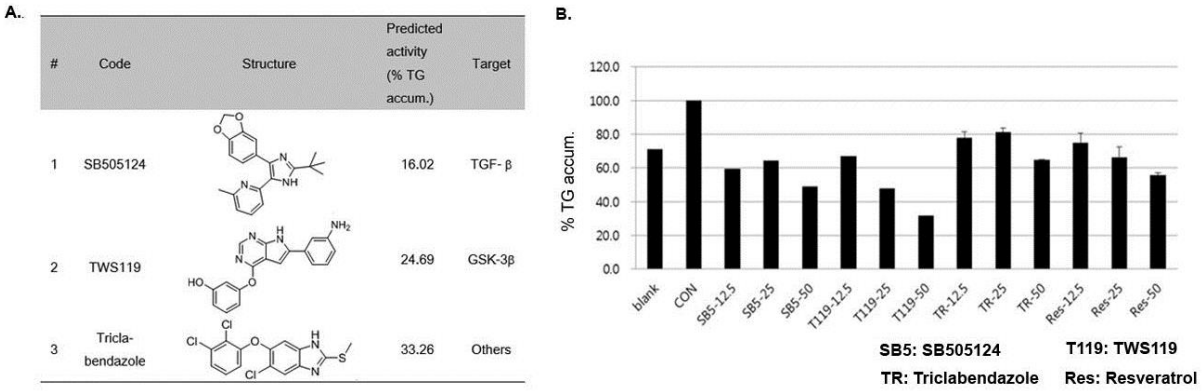


Figure 3. Predicted and observed inhibition on TG accumulation of the top 3 compounds from the Selleck® library.

Conclusion

We have tested our previously constructed piper-amide library for anti-obesity effects by Oil Red O staining on 3T3-L1 preadipocytes. A set of six compounds, NED-109, NED-223, NED-240, NED-262, NED-275, NED-278 have been identified as hit compounds that suppress triglyceride accumulation and prevent adipogenesis. NED-240 has been selected and continuing experiments are still in progress in animal models. A 2D QSAR model based on the 250 compounds from the library was constructed in order to correlate structural features with the anti-obesity effects. The equation obtained from the GFA function was further utilized in calculating external sources of commercially available bioactives for their predicted activity. From the 1700 molecules screened, we have identified TWS119 as a potent anti-obesity agent which targets GSK-3β. As a part of our attempts to determine the cellular target of NED-240, investigation on the NED-series for the activity against GSK-3β and other possible targets from the obtained data is still on-going.

Experimental Section

Analytical data of representative biaryl amide compounds

All the compounds including NED-109, NED-223, NED-240, NED-262, NED-275, NED-278 were previously reported in 2013by us.[6]

Cell culture and Oil red O staining

For screening of the library, 3T3-L1 cells were cultured in DMEM with 10% fetal bovine serum (FBS) under normoxic conditions (20% O_2, 5% CO_2, and 75% N_2) in a CO_2 incubator at 37 °C. Cells were then grown to confluence in 48-well-plates and were exposed to hormone mixture containing 10ug/ml insulin, 0.5uM dexamethasone, 0.5 mM IBMX. After 48 hours, the medium was replaced by DMEM containing insulin and adipocyte differentiation was monitored while cells were being treated with varying concentration of test sample solution every day. On day 8, cells were washed (rinsed) twice with PBS and fixed with 3.7 % formaldehyde. After fixation, cells were incubated with Oil Red O dye for 1 hour and the quantification of triglyceride was measured by fluorescence absorbance at 510 nm wavelength.

2D QSAR modelling

All calculation and analysis of data were performed using Accelrys Discovery Studio 4.1 (DS 4.1). A MOL file (sd. format) containing 190 molecules with specified values for activity were minimized and prepared using Minimize Ligands and Prepare Ligands for QSAR protocol based on CHARMm forcefield. The prepared molecules were assigned to the test and training set by Diverse Molecules method with default properties and ECFP_6 fingerprints. Genetic Function Approximation model was then built with the obtained datasets taken into account all the possible 2D and 3D descriptors. The maximum number of generation created was set to 5000 with the population size = 100. The Pareto method was used for scoring with Friedman′s lack of fit (L.O.F) and R^2.

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국 문 초 록

Part A: Hendrickson reagent를 사용하여 *N*-Boc carbamate로부터 형성된 isocyanate의 cyclization 및 urea formation에 대한 응용.

Isocyanates는 다양한 electrophilic addition 혹은 cycloaddition reactions에 응용되어 복잡한 구조의 화합물을 만들 때 쓰일 수 있는 중요한 중간체로 널리 사용된다. Hofmann, Curtius, Lossen rearrangement등 named reaction에서 isocyanate가 중간체로 형성되며 halosilane, phosphorus pentachlroride 와 chloroborane 같은 시약들을 사용하여 carbamate functional group으로부터 isocyante를 합성하는 방법들이 알려져 있다. 이러한 반응들은 주로 *N*-methoxycarbonyl (Moc) 혹은 *N*-benzyloxycarbonyl (Cbz) 를 가지고 있는 기질들을 사용하며 *N*-*tert*-butyloxycarbonyl (Boc) group 에 대한 반응성은 미미하다고 알려져 있다. 현재까지 알려진 isocyanate를 형성하는 방법들 중 다른 carbamate 기질들에 비해 *N*-Boc carbamate 기질을 선택적으로 사용하는 방법은 잘 알려져 있지 않다. 이에 본 논문에서는 Hendrickson 'POP' reagent를 사용하여 *N*-Boc carbamate를 보다 선택적으로 isocyanate로 전환하는 방법에 대하여 설명하고자 한다. 이렇게 형성된 isocyanate는 더 나아가 Friedel-Crafts-type cyclization 및 urea formation에 응용되어 더욱 효율적으로 사용 될 수 있다. 최적화 된 반응 조건에 의해 heterocyclic lactam과 urea를 높은 수득률로 얻을 수 있으며 이러한 방법은 복잡한 구조의 alkaloid나 천연물의 합성에 응용될 수 있으리라 기대한다.

주요어 : Isocyanate, Hendrickson reagent, *N*-Boc carbamate

Part B: 항비만에 효과적인 NED-240의 발굴 및 QSAR 모델 설계와 응용.

비만 억제제는 현대사회에서 각광받는 약물이지만 약물의 특성상 잦은 심장병 및 심혈관계 부작용으로 인하여 사용이 제한되고 있다. 이에 보다 안전하고 효과적인 항비만 약물을 개발하기 위하여 약 250개 정도의 piper-amide 계열의 유도체 library screening을 통해 가장 효과가 좋은 NED-240을 발굴 하였다. 이 물질은 동물 실험을 통하여 항비만 효과를 입증하였으며 독성도 거의 없어 차세대 항비만 약물로서의 선도물질로서의 가능성을 가지고 있다. 추가적으로 screening 결과를 바탕으로 QSAR모델을 구축함으로써 활성과 상관관계가 있는 구조적인 특성을 파악하였으며, 이를 다양한 scaffold와 타켓을 가지고 있는 1700개 정도의 small molecules library를 통하여 validation을 하였다. 그 결과 비만 치료에 효과적일 것 이라고 예측되는 화합물 40개를 도출해내었고 그 중 구조적 유사성 및 시중 구입 가능 여부를 고려해 선정된 세가지 물질을 Oil Red O staining을 통해 항비만 효과가 있는지 알아보았다. 그 결과로 NED-240과 유사한 정도의 활성을 띄는 TWS119를 도출할 수 있었고, 아직 NED-240의 작용 기전이 명백하지 않아 TWS119의 타겟인 GSK-3β 에 대해 추가적인 활성 여부 실험을 실시할 계획이다.

주요어: anti-obesity, piper-amide, QSAR model, TWS119, GSK-3β.

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