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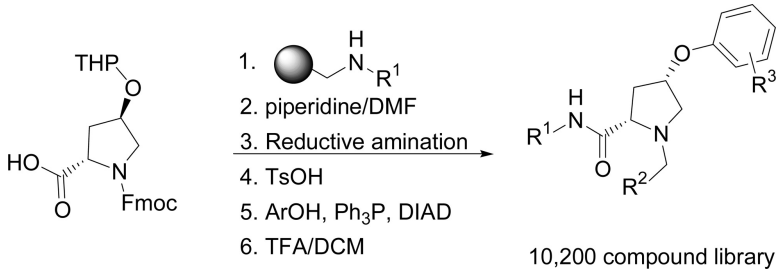

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Solid-Phase Synthesis of a Library of Hydroxyproline Derivatives

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The synthesis of a library of *N*-alkylated *O*-arylated hydroxyproline derivatives has been achieved on solid phase. The choice of *O*-protection and the optimization of the Mitsunobu reaction involving a secondary alcohol were key to the success of this synthesis. First, acylation of resin-bound amines with *N*-Fmoc-*O*-THP-hydroxyproline was accomplished readily. Subsequent deprotection of the Fmoc and reductive amination with different aldehydes resulted in the tertiary amine intermediate. The deprotection of the THP group by *p*-toluenesulfonic acid was followed by a Mitsunobu reaction with a series of phenols. Finally, the products were cleaved from the resin using trifluoroacetic acid to produce a 10 200 member library.

Introduction

The hydroxyproline motif is found in many compounds (Figure 1) with biological activity. For example, these have been shown to act as protease inhibitors: Compound **1** has an IC_{50} of 0.9 nM for thrombin,[1] while compounds **2** and **3** showed activity against the Hepatitis C virus NS3 protease ($K_i = 12$ nM and $IC_{50} = 5\ \mu$M, respectively).[2,3] Additionally, the hydroxyproline motif has been incorporated into a putative peptide β-turn mimetic, **4**.[4]

The hydroxyproline scaffold offers three points of attachment (the amine, the hydroxyl, and the carboxyl groups) making it a good candidate for library production. Several libraries[1,3,6] of hydroxyproline derivatives have been previously synthesized either in solution or on solid phase. However, these were primarily *O*-alkylated and/or *N*-acylated and the products often needed final purification. Therefore, we decided to develop the solid-phase synthesis of *N*-alkylated *O*-arylated *cis*-hydroxyproline derivatives **5**. The design and the optimization of their synthesis are described herein. Also, as an example of the efficacy of this method, we also report the synthesis and characterization of a 10 200 member library.

Results and Discussion

Strategy and Optimization of the Solid-Phase Synthesis. A solid-phase synthesis of **5** was chosen to eliminate intermediate isolation, minimize final purification, and allow the production of larger libraries using the Irori technology. We envisioned acylating a variety of resin-bound amines with hydroxyproline followed by sequential *N*-alkylation and *O*-arylation. We also considered that the order of these transformations needed to be explored to determine which sequence gave optimum results.

For the first step, FDMP resin was reacted with different amines via a reductive amination using sodium triacetoxyborohydride in 2.5% acetic acid in DCM[5] leading to the formation of the resin **7**.

Then, as reported by Boldi et al.,[6] we attempted the coupling of *N*-Fmoc-protected *trans*-4-hydroxyproline (Fmoc-Hyp) with the resin-bound amine. Several conditions were tested as follows: HOBt/DIC or HOBt/pyBOP/DIEA at different concentrations. The best results were obtained with HOBt (3 equiv), pyBOP (3 equiv), and DIEA (6 equiv) in DMF for 1 h. However, along with the product, we observed the formation of the *O*-acylated byproduct. This led us to explore hydroxyl protection to avoid this side reaction. Although an orthogonal protecting group (with respect to the resin cleavage conditions) such as TBDMS was desired, we nevertheless also considered the THP group since its cleavage requires very mild acidic conditions.

Attempts to protect Fmoc-Hyp with a TBDMS group using TBDMS chloride/imidazole or TBDMS triflate/2,6-lutidine resulted in mixtures of starting material, product, and Fmoc-deprotected product. Changing the order of protection, with TBDMS being installed prior to Fmoc, resulted in low yields of the doubly protected hydroxyproline. Therefore, we decided to explore the utility of the THP derivative **6**.

The protection of Fmoc-Hyp by a THP group was successfully achieved on a 200 g scale with quantitative yields by reacting it with dihydropyran in DCM in the presence of a catalytic amount of pyridinium *p*-toluenesulfonate.[7]

N-Fmoc-*O*-THP-Hyp **6** was coupled to the resin-bound amine **7** using DIC (0.1 M) and HOBt (0.12 M) in anhydrous DMF. The loading of resin **8** was quantified by measuring the absorbance of dibenzofulvene obtained upon cleavage of the Fmoc group. Routine loadings ranged from 70.3 to 100% (as compared to the theoretical loading calculated using the original aldehyde substitution).

Arylation of the hydroxyl group and formation of the tertiary amine could be performed by two possible strategies: (i) *O*-arylation via a Mitsunobu reaction followed by *N*-alkylation as reported by O'Connell, et al.[8] in a solution

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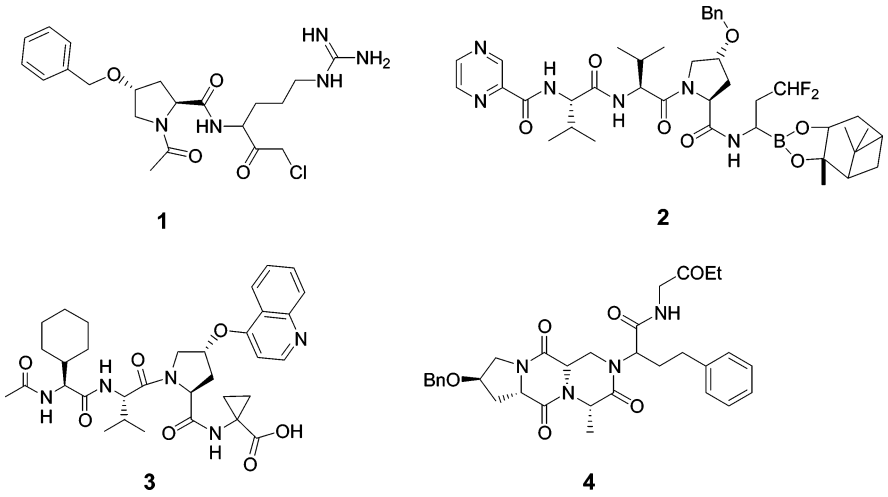
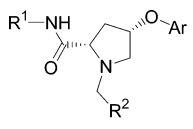


Figure 1. Biologically relevant hydroxyproline derivatives.

phase synthesis of *O*-arylated hydroxyproline derivatives or (ii) first *N*-alkylation and then *O*-arylation. Various conditions for the Mitsunobu reaction on the Fmoc-protected-Hyp (method i) were attempted, but all resulted in a mixture of the product and an unidentified impurity. As method ii seemed to yield compounds of increased purity, we decided to optimize this route. The Fmoc group of **8** was first deprotected using 20% piperidine in DMF, and this secondary amine **9** was reacted with a series of aldehydes via a reductive amination using sodium triacetoxyborohydride in 2.5% acetic acid in DCM leading to the formation of **10**. The hydroxyl group was then deprotected using a solution of *p*-toluenesulfonic acid monohydrate (5 mg/mL) in DCM/methanol (19:1).[7]

The deprotected hydroxyproline **11** was finally reacted with different phenols via a Mitsunobu reaction. Several conditions were attempted as follows: DEAD or DIAD, PPh_3 or *n*-Bu_3P, with and without base such as Et_3N or NMM, THF or DCM, and at varying concentrations.[9−11] We noticed that the use of Et_3N and the use of DCM instead of THF greatly increased the purity of the product by decreasing the formation of DEAD adducts. The best results were obtained when the reagents were added in the following order: PPh_3 (0.5 M), phenol (0.6 M), Et_3N (0.34 M), and DEAD (0.6 M) in DCM. Unfortunately, prior to production of this library, DEAD was withdrawn from the market; therefore, we had to reoptimize this step with DIAD. We found that the addition of Et_3N was not necessary for the success of the reaction and that the optimum conditions were as follows: PPh_3 (0.5 M), phenol (0.6 M), and DIAD (0.5 M) in DCM. The stereochemistry of the Mitsunobu reaction products was assumed to be cis as previously demonstrated by X-ray crystallography on a similar hydroxyproline derivative.[12]

The products **5** were recovered by cleaving the resin **12**



5

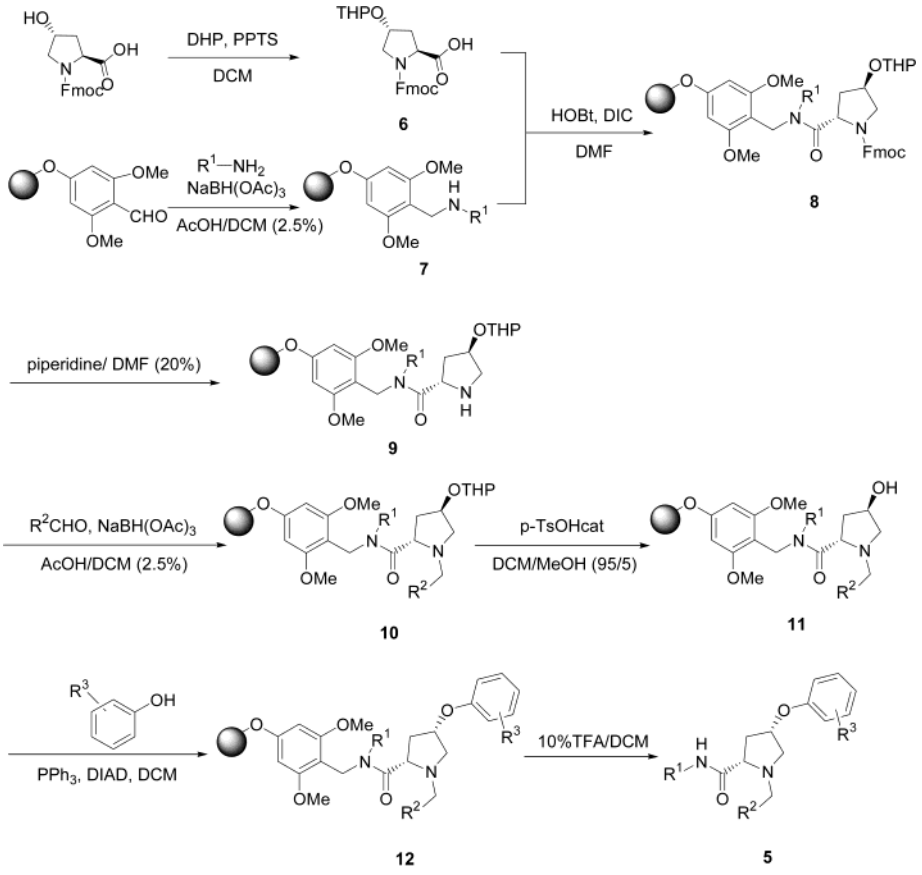
with 10% trifluoroacetic acid in DCM (higher concentrations of trifluoroacetic acid led to partial cleavage of the resin linker). About 25 compounds were prepared using the final synthetic scheme described in Scheme 1, and their analysis showed purities above 85%. Using this scheme, we decided to produce a larger library of hydroxyproline derivatives **5**.

Library Design and Synthesis. The building blocks for the library synthesis (amines, aldehydes, and phenols) were selected based on two sets of privileged structures for GPCRs as determined by the retrosynthetic combinatorial analysis procedure (RECAP).[13] Also, using the DirectedDiversity[14] platform, we studied the diversity and physicochemical properties of the "virtual" library of compounds **5** to eliminate building blocks with undesirable properties. This analysis led to the selection of 40 amines, 80 aldehydes, and 40 phenols. These building blocks were then rehearsed to determine their reactivity in our synthetic pathway. The rehearsals were accomplished by fixing two building blocks while varying the third. The analysis of this rehearsal study resulted in 15 amines, 34 aldehydes, and 20 phenols (Figures 2−4) giving satisfactory purities of final product **5**. The analysis of the data also showed that certain building blocks led to the unexpected formation of byproducts, therefore, lowering the purity of the final compounds. For instance, some amines **R1** described in Figure 5 did not couple efficiently with the *N*-Fmoc-Hyp but were instead acetylated. This most likely occurred during the acylation since the resin-bound amine is actually the acetate salt. However, this side reaction was not observed to a great extent with other resin-bound amines. We also noticed that **R1**{*6*} gave lower purity due to incomplete reaction in the subsequent Mitsunobu.

Most aldehydes (**R2**) explored in the reductive amination reacted well except for some indole aldehydes. These either did not react completely or in one case gave the acetylated product due to the acetyl group transferring from the indole N to the proline ring. All of the phenols in the Mitsunobu reaction gave very good results.

The production of this 10 200 member library (15 × 34 × 20) was accomplished by means of Scheme 1 using the Irori system. All products were fully characterized by LC-MS using ELS detection. Twenty of the products were also analyzed by NMR (Table 1), and their characterization is reported herein in the Experimental Section.

Scheme 1. Final Synthetic Scheme



The average molecular weight of the library was 503.9 g/mol, and its average cLogP was 4.65. The average purity of the library was 87.5% with 80.4% of the products having purities above 80%. **R1**{*5*} gave products with an average purity of 75% due to the formation of the acetylated amine, as observed in the rehearsal. **R2**{*7*} gave a mixture of the product and an unidentified impurity of lower mass that was not observed during rehearsal.

Conclusion

We have developed the solid-phase synthesis of a library of *cis*-hydroxyproline derivatives. It was achieved by carefully choosing the protecting groups on the hydroxyproline core (Fmoc for the *N*-protection and THP for the *O*-protection) and by choosing the appropriate synthetic sequence, *N*-alkylation and then *O*-arylation. We also explored the conditions for the Mitsunobu reaction and concluded that

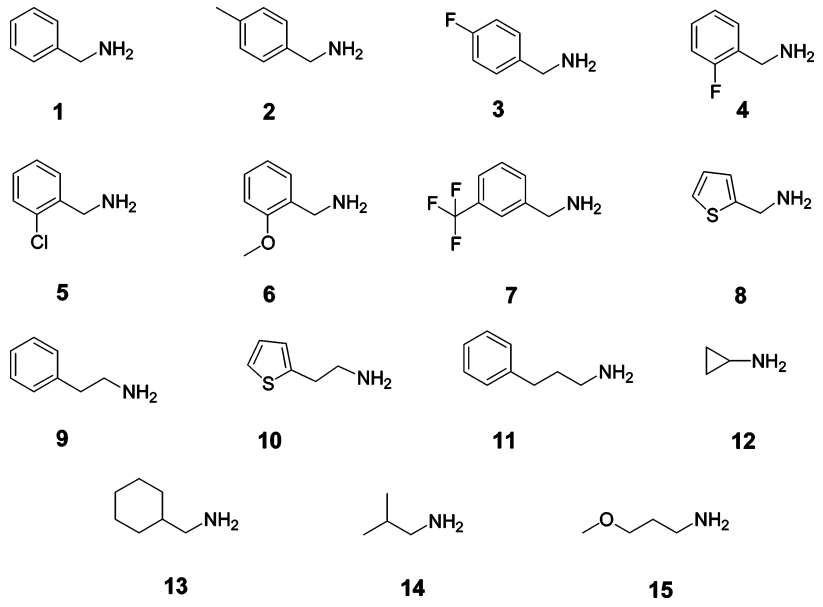


Figure 2. Set of amines **R1**{*1*−*15*} for the library.

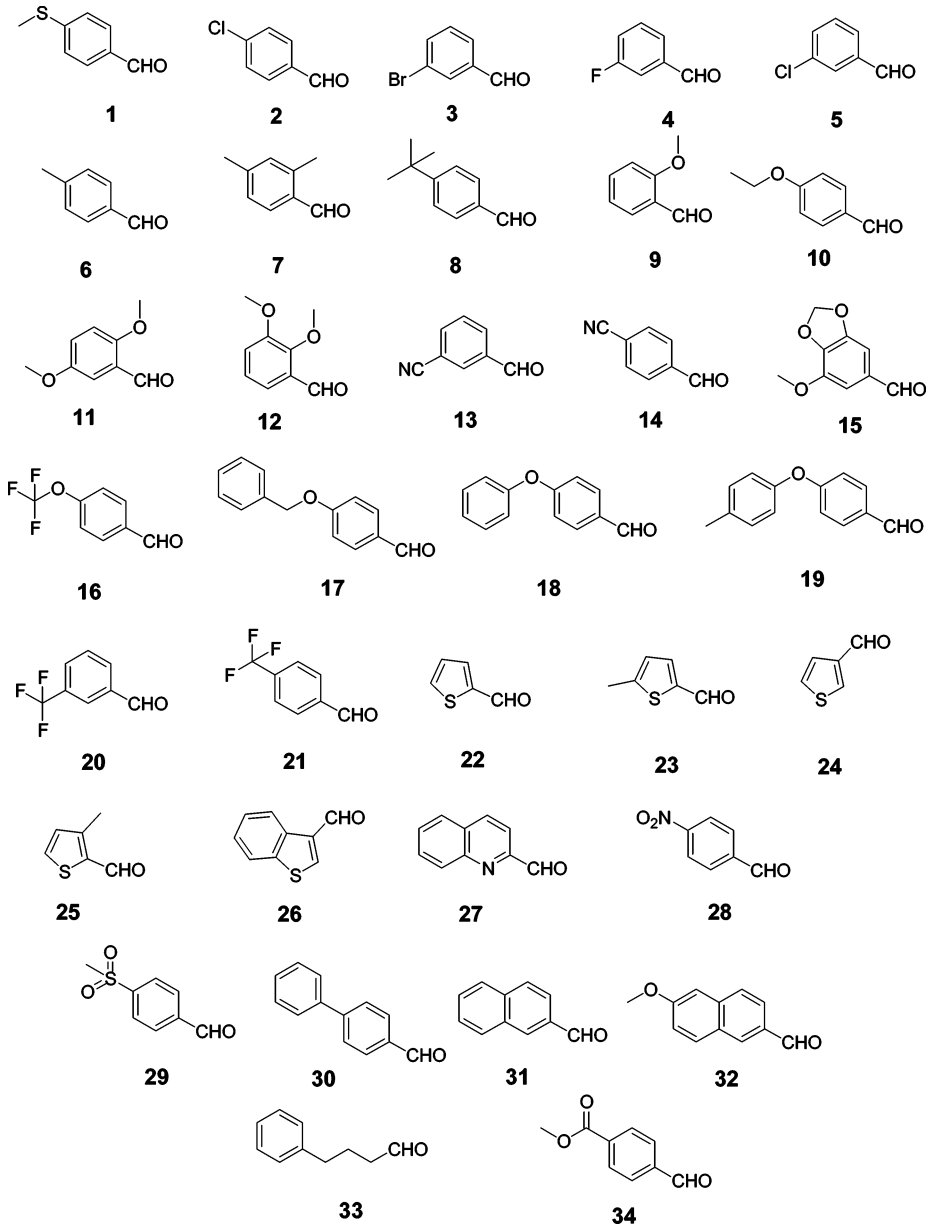


Figure 3. Set of aldehydes **R2**{*1*−*34*} for the library.

the selection of the reagents (PPh_3, DEAD/Et_3N, or DIAD) and the solvent (DCM) and the sequence of addition were crucial for the success of this step. The optimization of this synthesis led to the production of a library of 10 200 compounds.

Experimental Section

General Information. The 2-(3,5-dimethoxy-4-formylphenoxy)ethoxymethyl polystyrene (FDMP) beads of 150−300 μm (loading 1.5 mmol/g) were purchased from Polymer Laboratories (Amherst, MA). Fmoc-Hydroxyproline was purchased from Novabiochem (San Diego, CA). All other reagents were purchased from standard commercial sources and used as such without further purification. The solvents were purchased from Aldrich Chemical and Co. (Milwaukee, WI) in 18 L stainless steel containers.

The LC-MS data were recorded on a Waters ZQ electrospray mass spectrometer equipped with 4-channel MUX capabilities (Milford, MA) with ELS detection using a Princeton SPHER HTS 60 Å, 5 μm column (3 mm × 50 mm) Princeton Chromatography (Cranbury, NJ). Two mobile phases (A: 99.9% water, 0.1% TFA; B: 99.9% acetonitrile, 0.1% TFA) were employed as a gradient from 25% B to 100% B in 1.8 min and 100% B for 0.45 min with a flow rate of 6.0 mL/min. ^1H NMR spectra were recorded in 5 mm tubes on a 300 MHz Varian in $CDCl_3$ unless otherwise stated.

About 25 mg of FDMP resin was dispensed in MicroKans along with an RF tag (MicroKans and RF tags were purchased from Discovery Partners, San Diego, CA). After each step, the cans were sorted using the AutoSort-10K. At the final step, the resin was cleaved and the products were recovered in deep well plates using the Accucleave96. The quantitation and archiving of the products were done by transferring them from the deep well to tared bar-coded Matrix minitubes (Hudson, NH) using the Robbins Hydra (Sunnyvale, CA) and weighing them using the Mettler-Toledo Bohdan Balance Automator (Mt. Vernon, IL).

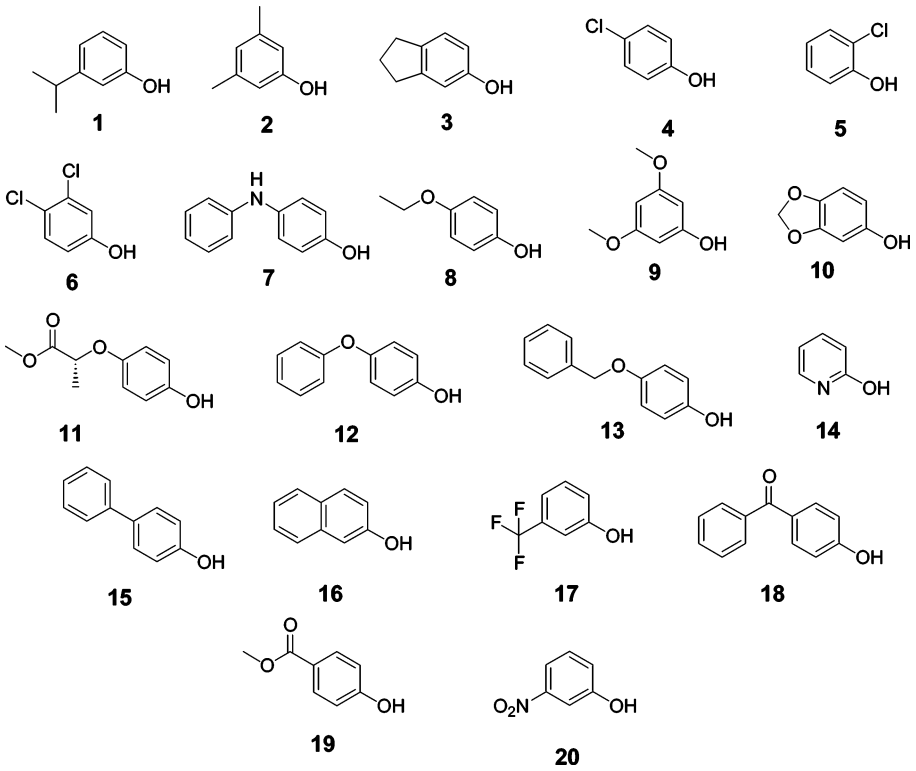


Figure 4. Set of phenols **R3**{*1*−*20*} for the library.

Table 1. Representative Set of Products **5**

entry	product **5**	R1	R2	R3	ELS purity
1	{*1*,*2*,*10*}	benzyl	4-chlorobenzyl	benzo[1,3]dioxol-5-yloxy	88.62
2	{*1*,*3*,*1*}	benzyl	3-bromobenzyl	3-isopropylphenoxy	85.74
3	{*1*,*4*,*10*}	benzyl	3-fluorobenzyl	benzo[1,3]dioxol-5-yloxy	83.66
4	{*2*,*14*,*4*}	4-methylbenzyl	4-cyanobenzyl	4-chlorophenoxy	88.66
5	{*3*,*8*,*18*}	4-fluorobenzyl	4-*tert*-butylbenzyl	4-benzoyl-phenoxy	97.25
6	{*3*,*26*,*2*}	4-fluorobenzyl	1-benzo[*b*]thiophen-3-ylmethyl	3,5-dimethylphenoxy	92.9
7	{*8*,*14*,*16*}	thiophen-2-ylmethyl	4-cyanobenzyl	2-naphthyloxy	95.49
8	{*12*,*21*,*4*}	cyclopropyl	4-trifluoromethyl)benzyl	4-chlorophenoxy	91.41
9	{*2*,*2*,*18*}	4-methylbenzyl	4-chlorobenzyl	4-benzoyl-phenoxy	96.98
10	{*2*,*14*,*2*}	4-methylbenzyl	4-cyanobenzyl	3,5-dimethylphenoxy	95.35
11	{*2*,*16*,*20*}	4-methylbenzyl	4-(trifluoromethoxy)benzyl	3-nitrophenoxy	97.4
12	{*2*,*18*,*10*}	4-methylbenzyl	4-phenoxybenzyl	benzo[1,3]dioxol-5-yloxy	90.6
13	{*2*,*21*,*8*}	4-methylbenzyl	4-trifluoromethyl)benzyl	4-ethoxyphenoxy	99.17
14	{*2*,*29*,*16*}	4-methylbenzyl	4-methanesulfonyl-benzyl	2-naphthyloxy	89.77
15	{*2*,*34*,*17*}	4-methylbenzyl	4-methyl-benzoic acid methyl ester	4-trifluoromethyl-phenoxy	92.94
16	{*8*,*27*,*18*}	thiophen-2-ylmethyl	1-quinolin-2-ylmethyl	4-benzoyl-phenoxy	94.52
17	{*8*,*27*,*20*}	thiophen-2-ylmethyl	1-quinolin-2-ylmethyl	3-nitrophenoxy	97.09
18	{*8*,*30*,*18*}	thiophen-2-ylmethyl	1-biphenyl-4-ylmethyl	4-benzoyl-phenoxy	99.37
19	{*9*,*1*,*2*}	phenethyl	4-methylsulfanyl-benzyl	3,5-dimethylphenoxy	99.69
20	{*9*,*1*,*8*}	phenethyl	4-methylsulfanyl-benzyl	4-ethoxyphenoxy	93.26

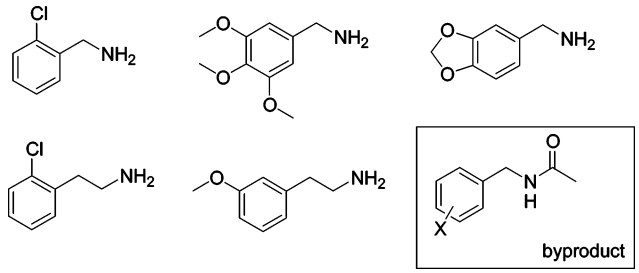


Figure 5. Selected monomers **R1** and their common byproduct.

Abbreviations are used as follows: DCM, dichloromethane; DMF, *N*,*N*-dimethylformamide; NMM, *N*-methyl morpholine; THF, tetrahydrofuran; DEAD, diethyl azodicarboxylate; DIAD, diisopropyl azodicarboxylate; HOBt, *N*-hydroxybenzotriazole; DIC, *N*,*N*-diisopropyl carbodiimide; PyBOP, benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate; DIEA, diisopropylethylamine; THP, tetrahydropyran; TBDMS, *tert*-butyldimethylsilyl; TFA, trifluoroacetic acid.

Preparation of *N*-Fmoc-*O*-THP-Hyp 6. In a 1 L round-bottom flask, *N*-Fmoc-Hyp (100 g, 283 mmol) was partially dissolved in DCM (500 mL). Then, dihydropyran (39 mL, 426 mmol) was slowly added followed by a catalytic amount of pyridinium *p*-toluenesulfonate (7.1 g, 28.3 mmol). The reaction mixture was stirred overnight at room temperature. After the completion of the reaction was checked by TLC (DCM/methanol 4:1), the mixture was washed twice with water and dried with magnesium sulfate, and the solvent was

evaporated. The final compound was obtained as a white solid in quantitative yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.8 (d, $J = 7.25$ Hz, 2H), 7.6 (d, $J = 7.25$ Hz, 2H), 7.4 (m, 2H), 7.3 (m, 2H), 4.7 (m, 1H), 4.5 (m, 1H), 4.3−4.5 (2 overlapping multiplets, 2H + 1H), 4.2 (m, 1H), 3.4−3.6 (2 overlapping multiplets, 2H + 2H), 2.1−2.6 (m, 2H), 1.4−1.8 (m, 6H). LC-MS (ELS) $m/z = 438.4$ $[M + H]^+$ (R_t = 2.02 min).

General Procedure for the Preparation of Library Compounds. Preparation of the Resin-Bound Amine 7. The resin was swollen in 2.5% acetic acid in DCM. Then, the amines (10 equiv, 0.375 M) were added. The resin was shaken for 2 h, and then, $NaBH(OAc)_3$ (13.5 equiv, 0.5 M) was added. The mixture was shaken overnight at room temperature. The solvent was then drained, and the excess of $NaBH(OAc)_3$ was eliminated by addition of DCM/methanol 1:1. The resin was finally washed three times with DCM and methanol successively and dried in vacuo.

Preparation of the Resin-Bound Hydroxyproline Amide 8. First, *N*-Fmoc-*O*-THP-Hyp (3.2 equiv, 0.12 M), HOBt (3.2 equiv, 0.12 M), and DIC (2.7 equiv, 0.1 M) were premixed for 10 min in DMF. The solution was then added to the resin-bound amine. After the solution was shaken overnight at room temperature, the solvent was drained and the resin was washed three times with DMF, DCM, and ^{i}PrOH successively. Finally, the resin was dried in vacuo. A small portion of the resin was cleaved with 10% TFA in DCM. The analysis of the cleaved product by LC-MS showed purities of **8** ranging from 97 to 100%.

Deprotection of the Fmoc Group. A mixture of 20% piperidine in DMF was added to the dried resin **8** and shaken for 30 min. After the solvent was drained, a fresh mixture of piperidine in DMF was added to the resin and it was shaken for another 30 min. The solution was drained, and the resin was washed three times with DMF, DCM, and ^{i}PrOH successively. The resin was dried in vacuo overnight.

Reductive Amination. The resin **9** obtained at the previous step was swollen in 2.5% acetic acid in DCM followed by the addition of the aldehydes (12 equiv, 0.45 M). After it was shaken for 2 h, $NaBH(OAc)_3$ (13.5 equiv, 0.5 M) was added. The resin was shaken overnight at room temperature. The solvent was then drained, and the excess of $NaBH(OAc)_3$ was eliminated by addition of DCM/methanol (1:1). The resin was finally washed three times with DCM and methanol successively and dried in vacuo.

Deprotection of the THP Group. To the resin **10**, a solution of *p*-toluenesulfonic acid monohydrate (5 mg/mL) in DCM/methanol (19:1) was added and the resin was shaken for 45 min. The solvent was drained, and this procedure was repeated. The deprotected resin **11** was finally washed three times with DCM and MeOH successively and dried in vacuo.

Mitsunobu Reaction. To the resin **11** swollen in DCM was added PPh_3 (13.3 equiv, 0.5 M), followed by the phenols (16 equiv, 0.6 M). The mixture was shaken until the reagents were completely dissolved. Then, DIAD (13.3 equiv, 0.5 M) was added slowly to prevent overheating (the reaction was very exothermic). The mixture was shaken overnight at room temperature. After the solvent was drained, the resin was washed twice with DCM and then twice with DMF, DCM, methanol, and finally with DCM. The resin was dried in vacuo.

Compound Cleavage. The compounds were cleaved from the resin by treating each MicroKan with 10% TFA in DCM for 30 min, and then, the solution was collected by filtration into deep well plates. The resin was washed with DCM for another 30 min, and the solution was also collected. The two solutions were evaporated, and the products **5** were analyzed by LC-MS as previously described. Selected products were also analyzed by NMR. In the spectra, we often noticed the presence of a common impurity (multiple peaks at δ 7.1, 4.3, 2.3, 1.27−1.25, and 0.88−0.86), which was caused by leaching from the deep well plates into which the products were cleaved.

4-(Benzo[1,3]dioxol-5-yloxy)-1-(4-chloro-benzyl)proline Benzylamide 5{*1,2,10*}**.** Yield, 1.0 mg (4%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.4−7.2 [(s, 5H) and (m, 4H) overlapping], 6.68 (d, $J = 8.57$ Hz, 1H), 6.39 (d, $J = 2.64$ Hz, 1H), 6.21 (dd, $J = 8.57$, 1.98 Hz, 1H), 5.93 (s, 2H), 4.97 (m, 1H), 4.68 (m, 1H), 4.45 (d, $J = 5.93$ Hz, 2H), 4.35 [(d, $J = 9.23$ Hz, 2H)], 4.04 (dd, $J = 49.4$, 13.18 Hz, 1H), 3.83 (m, 1H), 3.34 (d, $J = 12.52$ Hz, 1H), 2.59 and 2.35 (m, 2H). LC-MS (ELS) $m/z = 465.1$ $[M + H]^+$ (88.62%, R_t = 1.52 min).

1-(3-Bromo-benzyl)-4-(3-isopropyl-phenoxy)proline Benzylamide 5{*1,3,1*}**.** Yield, 1.0 mg (4%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.51 (s, 1H), 7.27 [(m, 3H) and (s, 5H) overlapping), 7.20 (m, 1H), 6.89 (d, $J = 7.91$ Hz, 1H), 6.69 (t, $J = 1.98$ Hz, 1H), 6.59 (dd, $J = 8.57$, 1.98 Hz, 1H), 5.08 (m, 1H), 4.95 (m, 1H), 4.45 (d, $J = 5.27$ Hz, 2H), 4.39 (d, $J = 5.27$ Hz, 2H), 4.25 (dd, $J = 31.64$, 12.52 Hz, 1H), 3.841 and 3.36 (m, 2H), 2.85 (hpt, $J = 6.59$ Hz, 1H), 2.62 and 2.33 (m, 2H), 1.22 (d, $J = 6.59$ Hz, 6H), 1.25 and 0.88. LC-MS (ELS) $m/z = 507.1$ $[M + H]^+$ (85.74%, R_t = 1.87 min).

4-(Benzo[1,3]dioxol-5-yloxy)-1-(3-fluoro-benzyl)proline Benzylamide 5{*1,4,10*}**.** Yield, 0.8 mg (4%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.28 [(s, 5H) and (m, 1H) overlapping], 7.14 (m, 3H), 6.67 (d, $J = 7.91$ Hz, 1H), 6.39 (d, $J = 2.64$ Hz, 1H), 6.21 (dd, $J = 7.91$, 2.64 Hz, 1H), 5.92 (s, 2H), 4.97 (m, 1H), 4.68 (m, 1H), 4.45 (d, $J = 5.93$ Hz, 2H), 4.39 (d, $J = 11.87$ Hz, 2H), 4.28 (dd, $J = 44.83$, 15.16 Hz, 1H), 3.81 and 3.35 (m, 2H), 2.59 and 2.37 (m, 2H). LC-MS (ELS) $m/z = 449.1$ $[M + H]^+$ (83.66%, R_t = 1.45 min).

4-(4-Chloro-phenoxy)-1-(4-cyano-benzyl)proline 4-Methyl-benzylamide 5{*2,14,4*}**.** Yield, 1.6 mg (7%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.56 (d, $J = 8.57$ Hz, 2H), 7.41 (d, $J = 8.57$ Hz, 2H), 7.23 (d, $J = 9.23$ Hz, 2H), 7.15 (m, 4H), 6.73 (d, $J = 9.23$ Hz, 2H), 4.97 [(m, 1H) and (m, 1H) overlapping], 4.40 (d, $J = 5.27$ Hz, 2H), 4.34 (d, $J = 5.93$ Hz, 2H), 4.19 (m, 1H), 3.74 and 3.17 (m, 2H), 2.52 and 2.31 (m, 2H), 2.34 (s, 3H). LC-MS (ELS) $m/z = 460.1$ $[M + H]^+$ (88.66%, R_t = 1.54 min).

4-(4-Benzoyl-phenoxy)-1-(4-*tert*-butyl-benzyl)proline 4-Fluoro-benzylamide 5{*3,8,18*}**.** Yield, 1.4 mg (7%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.76 (dd, $J = 17.8$, 7.91 Hz, 4H), 7.57 (m, 1H), 7.47 (m, 2H), 7.39 (m, 2H), 7.27 (m, 4H), 7.01 (m, 2H), 6.86 (d, $J = 8.57$ Hz, 2H), 5.23 (br s,

1H), 4.97 (m, 1H), 4.41 (2 overlapping doublets, 4H), 4.1 (m, 2H), 3.49 (d, $J = 12.52$ Hz, 1H), 2.7 and 2.37 (m, 2H), 1.30 (s, 9H). LC-MS (ELS) $m/z = 565.2$ $[M + H]^+$ (97.25%, $R_t = 1.93$ min).

1-Benzo[*b*]thiophen-3-ylmethyl-4-(3,5-dimethyl-phenoxy)-proline 4-Fluoro-benzylamide 5{*3,26,2*}**.** Yield, 1.2 mg (6%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.89 (q, $J = 2.64$ Hz, 1H), 7.76 (m, 1H), 7.42 (q, $J = 3.29$ Hz, 2H), 7.2 (2 overlapping multiplets, 2H + 1H), 6.9 (m, 2H), 6.65 (s, 1H), 6.40 (s, 2H), 5.05 (br s, 1H), 4.97 (qt, $J = 6.59$ Hz, 1H), 4.61 (m, 1H), 4.42 (d, $J = 5.93$ Hz, 2H), 4.3 (m, 2H), 3.95 and 3.48 (m and d, $J = 11.87$ Hz, 2H), 2.6 and 2.34 (2 multiplets, 2H), 2.25 (s, 6H). LC-MS (ELS) $m/z = 489.1$ $[M + H]^+$ (92.90%, $R_t = 1.87$ min).

1-(4-Cyano-benzyl)-4-(naphthalen-2-yloxy)proline (Thiophen-2-ylmethyl)amide 5{*8,14,16*}**.** Yield, 2.5 mg (10%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.76 (d, $J = 9.23$ Hz, 2H), 7.68 (d, $J = 7.91$ Hz, 1H), 7.59 (d, $J = 8.57$ Hz, 2H), 7.45 (d, $J = 7.91$ Hz, 2H), 7.36 (m, 1H), 7.23 (m, 1H), 7.07 (m, 1H), 7.0 (overlapping multiplets, 4H), 5.17 (br s, 1H), 4.95 (m, 1H), 4.61 (2 overlapping doublets, 2H + 2H), 4.22 (m, 1H), 3.83 and 3.24 (two multiplets, 2H), 2.64 and 2.37 (multiplets, 2H). LC-MS (ELS) $m/z = 468.1$ $[M + H]^+$ (95.49%, $R_t = 1.55$ min).

4-(4-Chloro-phenoxy)-1-(4-trifluoromethyl-benzyl)-proline Cyclopropylmethyl-amide 5{*12,21,4*}**.** Yield, 1.1 mg (4%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.67 (d, $J = 7.91$ Hz, 2H), 7.58 (d, $J = 8.57$ Hz, 2H), 7.23 (d, $J = 8.57$ Hz, 2H), 6.73 (d, $J = 8.57$ Hz, 2H), 6.42 (br s, 1H), 5.03 (m, 1H), 4.97 (qt, $J = 5.93$ Hz, 1H)**,** 4.55 (m, 1H), 4.35 (dd, $J = 38.89, 12.52$ Hz, 2H), 3.85 and 3.29 (m and d, $J = 11.87$ Hz, 2H), 2.5 and 2.37 (multiplets, 2H), 0.77 (m, 2H), 0.49 (m, 2H). LC-MS (ELS) $m/z = 439.1$ $[M + H]^+$ (91.41%, $R_t = 1.43$ min).

4-(4-Benzoyl-phenoxy)-1-(4-chloro-benzyl)proline 4-Methyl-benzylamide 5{*2,2,18*}**.** Yield, 2.0 mg (8%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.76 (q, $J = 9.23$ Hz, 4H), 7.58 (t, $J = 7.25$ Hz, 1H), 7.47 (t, $J = 7.91$ Hz, 2H), 7.28 (d, $J = 8.57$ Hz, 2H), 7.21 (d, $J = 8.57$ Hz, 2H), 7.16 (m, 4H), 6.85 (d, $J = 9.23$ Hz, 2H), 5.78 (br s, 1H), 5.0 (m and qt, $J = 5.93$, 1H), 4.39 (d, $J = 5.93$ Hz, 2H), 4.36 (d, $J = 5.93$ Hz, 2H), 4.06 (s, 1H), 3.73 and 3.15 [(dd, $J = 11.87$, 5.3 Hz) and (d, $J = 11.87$ Hz), 2H], 2.58 (m, 2H), 2.34 (s, 3H). LC-MS (ELS) $m/z = 539.1$ $[M + H]^+$ (96.98%, $R_t = 1.77$ min).

1-(4-Cyano-benzyl)-4-(3,5-dimethyl-phenoxy)proline 4-Methyl-benzylamide 5{*2,14,2*}**.** Yield, 1.1 mg (5%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.545 (d, $J = 8.57$ Hz, 2H), 7.34 (d, $J = 8.57$ Hz, 2H), 7.16 (m, 4H), 6.63 (s, 1H), 6.42 (s, 2H), 5.75 (br s, 1H), 4.95 (m, 1H), 4.39 (d, $J = 5.27$ Hz, 2H), 4.32 (m, 1H), 4.03 (q, $J = 13.18$ Hz, 2H), 3.56 and 2.97 [(dd, $J = 11.87$, 5.27 Hz) and (d, $J = 10.55$), 2H], 2.58 and 2.38 (two multiplets, 2H), 2.34 (s, 3H), 2.26 (s, 6H). LC-MS (ELS) $m/z = 454.2$ $[M + H]^+$ (95.35%, $R_t = 1.64$ min).

4-(3-Nitro-phenoxy)-1-(4-trifluoromethoxy-benzyl)proline 4-Methyl-benzylamide 5{*2,16,20*}**.** Yield, 0.6 mg (3%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.86 (d, $J = 7.91$ Hz, 1H), 7.63 (m, 1H), 7.44 (t, $J = 7.91$ Hz, 1H), 7.28 (buried m, 1H), 7.16 [(s, 4H) and (m, 2H) and (m, 2H) overlapping], 5.74 (br s, 1H), 5.03 (m, 1H), 4.39 [(d, $J = 5.93$ Hz, 2H) and (m, 1H)], 4.02 (m, 2H), 3.66 and 3.06 [(q, $J = 5.27$ Hz) and (d, $J = 9.89$ Hz), 2H], 2.55 (m, 2H), 2.34 (s, 3H). LC-MS (ELS) $m/z = 530.1$ $[M + H]^+$ (97.40%, $R_t = 1.69$ min).

4-(Benzo[1,3]dioxol-5-yloxy)-1-(4-phenoxy-benzyl)proline 4-Methyl-benzylamide 5{*2,18,10*}**.** Yield, 1.5 mg (5%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.37 (t, $J = 7.25$ Hz, 2H), 7.16 [(m, 4H) and (m, 3H)], 7.01 (d, $J = 7.251$ Hz, 2H), 6.93 (d, $J = 8.57$ Hz, 2H), 6.67 (d, $J = 7.91$ Hz, 1H), 6.39 (s, 1H), 6.21 (d, $J = 9.23$ Hz, 1H), 5.92 (s, 2H), 5.71 (br s, 1H), 4.9 (m, 1H), 4.39 (d, $J = 5.27$ Hz, 4H), 4.16 (m, 1H), 3.68 and 3.21 (two multiplets, 2H), 2.55 (m, 2H), 2.34 (s, 3H). LC-MS (ELS) $m/z = 537.1$ $[M + H]^+$ (90.60%, $R_t = 1.86$ min).

4-(4-Ethoxy-phenoxy)-1-(4-trifluoromethyl-benzyl)proline 4-Methyl-benzylamide 5{*2,21,8*}**.** Yield, 1.6 mg (6%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.59 (d, $J = 7.91$ Hz, 2H), 7.49 (d, $J = 7.91$ Hz, 2H), 7.16 (s, 4H), 6.8 (d, $J = 9.23$ Hz, 2H), 6.72 (d, $J = 9.23$ Hz, 2H), 5.8 (br s, 1H), 4.95 (m, 1H), 4.39 (d, $J = 5.93$ Hz, 2H), 4.29 (d, $J = 23.07$, 4.61 Hz, 2H), 4.1 (buried m, 1H), 3.96 (q, $J = 7.25$ Hz, 2H), 3.74 and 3.22 [m and (d, $J = 12.52$ Hz), 2H], 2.57 (m, 2H), 2.34 (s, 3H), 1.38 (t, $J = 7.25$ Hz, 3H). LC-MS (ELS) $m/z = 513.2$ $[M + H]^+$ (99.17%, $R_t = 1.73$ min).

1-(4-Methanesulfonyl-benzyl)-4-(naphthalen-2-yloxy)-proline 4-Methyl-benzylamide 5{*2,29,16*}**.** Yield, 1.4 mg (5%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.85 (d, $J = 8.57$ Hz, 2H), 7.75 (d, $J = 9.23$ Hz, 2H), 7.68 (d, $J = 7.91$ Hz, 1H), 7.43 [(d, $J = 8.57$ Hz, 2H] and (m, 1H) overlapping], 7.35 (t, $J = 7.25$ Hz, 1H), 7.16 (s, 4H), 7.07 (dd, $J = 9.23$, 2.64 Hz, 1H), 6.99 (m, 1H), 5.75 (br s, 1H), 5.06 and 4.93 (two multiplets, 1H), 4.39 (d, $J = 5.27$ Hz, 2H), 4.06 (m, 2H), 4.06 (buried m, 1H), 3.62 (dd, $J = 11.2$, 4.61 Hz, 2H), 3.03 (s, 3H), 2.68 and 2.45 (2 multiplets, 2H), 2.34 (s, 3H). LC-MS (ELS) $m/z = 529.1$ $[M + H]^+$ (89.77%, $R_t = 1.52$ min).

1-(4-methyl-benzoic acid methyl ester)-4-(4-trifluoromethyl-phenoxy)proline 4-Methyl-benzylamide 5{*2,34,17*}. Yield, 1.3 mg (5%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.97 (d, $J = 7.91$ Hz, 2H), 7.38 (t, $J = 7.91$ Hz, 1H), 7.32 (d, $J = 7.91$ Hz, 2H), 7.22 (s, 1H), 7.16 (s, 4H), 7.03 (s, 1H), 6.97 (d, $J = 8.57$ Hz, 1H), 5.73 (br s, 1H), 4.96 (m, 1H), 4.39 (d, $J = 5.27$ Hz, 4H), 4.04 (dd, $J = 27.69$, 12.32 Hz, 2H), 3.92 (s, 3H), 3.61 and 3.02 [(dd, $J = 11.87$, 5.27 Hz) and (d, $J = 11.87$ Hz), 1H), 2.57 and 2.45 (two multiplets, 2H), 2.34 (s, 3H). LC-MS (ELS) $m/z = 527.1$ $[M + H]^+$ (92.94%, $R_t = 1.65$ min).

4-(4-Benzoyl-phenoxy)-1-quinolin-2-ylmethyl-proline (Thiophen-2-ylmethyl)amide 5{*8,27,18*}**.** Yield, 1.4 mg (6%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.73 (d, $J = 8.57$ Hz, 1H), 8.64 (d, $J = 7.91$ Hz, 1H), 8.03 (t, $J = 7.25$ Hz, 2H), 7.85 (t, $J = 7.25$ Hz, 1H), 7.77 [(q, $J = 8.57$ Hz, 4H) and (m, 1H) overlapping], 7.57 (t, $J = 7.251$ Hz, 1H), 7.47 (t, $J = 7.91$ Hz, 2H), 6.89 [(m, 1H) and (m, 2H) overlapping], 6.79 (d, $J = 3.29$ Hz, 1H), 6.65 (dd, $J = 4.61$, 3.29 Hz, 1H), 5.1 (m, 1H), 4.6 [(m, 2H) and (s, 2H) overlapping], 4.32 (d, $J = 15.16$ Hz, 1H), 4.13 (m, 1H), 3.98 (dd, $J = 11.87$, 4.61 Hz, 1H), 3.06 (d, $J = 11.21$ Hz, 1H), 2.66 (dd,

J = 13.84, 5.93 Hz, 1H), 2.35 (m, 1H). LC-MS (ELS) m/z = 548.1 $[M + H]^+$ (94.52%, R_t = 1.76 min).

4-(3-Nitro-phenoxy)-1-quinolin-2-ylmethyl-proline (Thiophen-2-ylmethyl)amide 5{*8,27,20*}**.** Yield, 1.2 mg (6%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.79 (d, J = 9.23 Hz, 1H), 8.66 (d, J = 8.57 Hz, 1H), 8.04 (t, J = 7.25 Hz, 2H), 7.85 [(t, J = 7.25 Hz, 1H) and (m, 1H) overlapping], 7.72 (d, J = 8.57 Hz, 1H), 7.65 (m, 1H), 7.44 (t, J = 8.57 Hz, 1H), 7.2 (dd, J = 8.57, 2.64 Hz, 1H), 6.88 (d, J = 5.27 Hz, 1H), 6.8 (d, J = 3.29 Hz, 1H), 6.62 (dd, J = 5.27, 3.95 Hz, 1H), 5.08 (br s, 1H), 4.57 (m, 4H), 4.33 (dd, J = 14.5, 3.95 Hz, 1H), 4.08 (m, 1H), 3.98 (dd, J = 11.87, 5.27 Hz, 1H), 3.03 (d, J = 13.84 Hz, 1H), 2.61 and 2.46 (two multiplets, 2H). LC-MS (ELS) m/z = 489.1 $[M + H]^+$ (97.09%, R_t = 1.56 min).

4-(4-Benzoyl-phenoxy)-1-biphenyl-4-ylmethyl-proline (Thiophen-2-ylmethyl)amide 5{*8,30,18*}**.** Yield, 0.7 mg (3%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.79 (d, J = 8.57 Hz, 2H), 7.74 (d, J = 7.25 Hz, 2H), 7.57 (quadruplet and doublet overlapped, 4H + 1H), 7.47 (two multiplets overlapped, 4H + 2H), 7.39 (m, 1H), 7.18 (d, J = 5.27 Hz, 1H), 7.04 (m, 1H), 6.9 (m, 1H), 6.85 (d, J = 8.57 Hz, 2H), 5.23 (br s, 1H), 4.61 (m, 4H), 4.36 (m, 1H), 4.02 (m, 1H), 3.35 (m, 1H), 2.8 and 2.72 (two multiplets, 2H). LC-MS (ELS) m/z = 573.2 $[M + H]^+$ (99.37%, R_t = 1.91 min).

4-(3,5-Dimethyl-phenoxy)-1-(4-methylsulfanyl-benzyl)-proline Phenethyl-amide 5{*9,1,2*}**.** Yield, 1.2 mg (5%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.33−7.18 [(s, 5H) and (2 multiplets, 4H)], 6.645 (s, 1H), 6.36 (s, 2H), 5.48 (br s, 1H), 5.01 (m, 1H), 4.05 (m, 2H), 3.76 (buried multiplet, 1H), 3.54 (q, J = 7.25 Hz, 2H), 2.89 (m, 2H), 2.83 (t, J = 6.59 Hz, 2H), 2.6 (m, 2H), 2.47 (s, 3H), 2.26 (s, 6H). LC-MS (ELS) m/z = 475.2 $[M + H]^+$ (97.94%, R_t = 1.89 min).

4-(4-Ethoxy-phenoxy)-1-(4-methylsulfanyl-benzyl)proline Phenethyl-amide 5{*9,1,8*}**.** Yield, 0.5 mg (2%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.35−7.18 [(s, 5H) and (two multiplets, 4H)], 6.79 (m, 2H), 6.69 (m, 2H), 5.48 (m, 1H), 4.96 (m, 1H), 3.97 [(q, J = 6.59 Hz, 2H) and (m, 1H)] overlapping], 3.53 ([(q, J = 6.59 Hz, 2H) and (m, 2H) overlapping], 2.91 (m, 2H), 2.83 (t, J = 6.59 Hz, 2H), 2.59 (m, 2H), 2.47 (s, 3H), 1.39 (t, J = 6.59 Hz, 3H). LC-MS (ELS) m/z = 491.2 $[M + H]^+$ (93.26%, R_t = 1.77 min).

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Supporting Information Available. ^{1}H NMR spectra with internal standards and ELS LC-MS traces for each compound. NMR spectrum for the impurity leaching from the deep well plate. This material is available free of charge via the Internet at http://pubs.acs.org.

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