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Ethyl 2-(*tert*-Butoxycarbonyloxyimino)-2-cyanoacetate (Boc-Oxyma) as Coupling Reagent for Racemization-Free Esterification, Thioesterification, Amidation and Peptide Synthesis

Kishore Thalluri,[a] Krishna Chaitanya Nadimpally,[a]
Maharishi Parasar Chakravarty,[a] Ashim Paul,[a] and Bhubaneswar Mandal[a,*]

[a] Department of Chemistry, Indian Institute of Technology Guwahati, Guwahati, Assam – 781039, India
Fax: (+91)-358-258-2349; phone: (+91)-361-258-2319; e-mail: bmandal@iitg.ernet.in

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Abstract: Here we report the synthesis and utility of ethyl 2-(*tert*-butoxycarbonyloxyimino)-2-cyanoacetate (Boc-Oxyma) as an efficient coupling reagent for racemization-free esterification, thioesterification, amidation reactions and peptide synthesis that uses equimolar amounts of acids and alcohols, thiols, amines or amino acids, respectively. Its application to solid phase as well as solution phase peptide synthesis is also demonstrated and a mechanistic investigation is discussed. Boc-Oxyma is similar to the well known coupling agent COMU {1-[1-cyano-2-ethoxy-2-oxoethylideneaminooxy)-dimethylaminomorpholino]uronium hexafluorophosphate} in terms of its high reactivity and mechanism of action. However, it is not only much easier to prepare, but also to recover and reuse, thereby generating far less chemical waste.

Keywords: amidation; Boc-Oxyma; coupling reagents; esterification; peptide synthesis; racemization; thioesterification

Introduction

Amidation, esterification and thioesterification reactions of carboxylic acids and amino acids are key reactions in the organic synthesis[1] of many important molecules of biological interest.[2] To date, a variety of coupling reagents have been developed for peptide synthesis[3] which are also capable of effecting these same reactions. The most effective coupling agents are those that not only afford fast reaction rates and high yields, but also the suppression of any undesired racemization.

To prevent racemization in peptide synthesis, *N*-hydroxyamine reagents, for example, HOBt (hydroxybenzotriazole) and HOAt (hydroxyazabenzotriazole), are frequently used as additives in conjunction with the coupling reagents such as carbodiimides, uronium salts, phosphonium salts, phosphates, phosphinates and immonium salts.[4] Recently, Oxyma (ethyl 2-hydroxyimino 2-cyanoacetate) has also been used as a racemization suppressant in DIC (diisopropylcarbodiimide)[5] and TFFH (tetramethylfluoroformamidinium hexafluorophosphate)[6] mediated peptide synthesis and has gained popularity as a non-explosive alternative to HOBt and HOAt. However, a major disadvantage of most coupling reagents invented to date is that they generate substantial amounts of undesired by-products and chemical waste. Moreover, the preparation of these coupling reagents is often very difficult and involves multi-step syntheses that require harsh reagents.

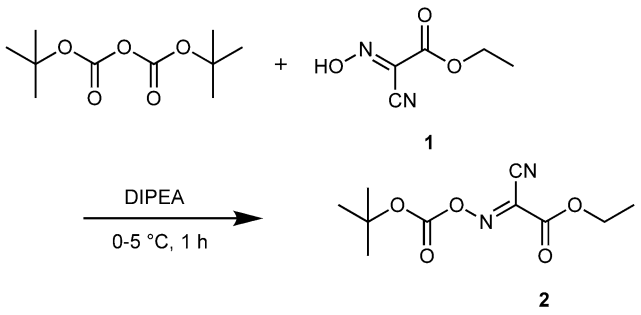
The use of Boc-protected reagents is also known to inhibit racemization and Boc_2O has been shown to be quite effective in this regard.[7] However, Boc_2O mediated coupling proceeds *via* mixed anhydride formation which inevitably generates unwanted by-products (e.g., Boc-protected amines), thus reducing the yield of the desired product, increasing waste, and complicating purification.

We wish to report herein the invention of the first member of a novel class of coupling reagent, Boc-Oxyma. Similar to the recently developed COMU (1-{[1-(cyano-2-ethoxy-2-oxoethylideneaminooxy)dimethylaminomorpholino]}uronium hexafluorophosphate), a coupling reagent leading to excellent yields with virtually no racemization,[8] Oxyma is Boc-deriv-

atized to generate a transiently stable, yet highly activated species. Boc-Oxyma readily affords the Oxyma ester of a carboxylic acid in basic milieu which in turn generates an ester, thioester, amide or peptide bond depending on the nucleophile that is added. Chiral integrity is also completely conserved.

Results and Discussion

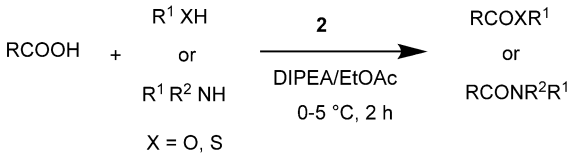
Herein, we describe the synthesis of a new coupling reagent, Boc-Oxyma, and its application as a simple, mild and racemization-free esterification, thioesterification and amidation reagent that uses nearly equimolar amounts of acids and the corresponding nucleophiles. We prepared Boc-Oxyma by the reaction of Boc_2O (1.2 equiv.) with Oxyma (1 equiv.) in the presence of DIPEA or triethylamine (1 equiv.) at 0–5 °C for 1 h (Scheme 1).



Scheme 1. Prepation of the coupling reagent, Boc-Oxyma.

Once the Oxyma was completely consumed, the solvent (chloroform) was evaporated to obtain Boc-Oxyma, which could be used for coupling reactions without further purification. The reagent thus prepared was added to the carboxylic acid or amino acid, which was predissolved in EtOAc with an equivalent amount of DIPEA, and the reaction mixture was stirred for 1 h at 0 °C for preactivation. The final ester, thioester, amide or peptide product was obtained by further stirring for about 2 h after the addition of the corresponding nucleophile (Scheme 2).

For optimization, we screened different solvents using the reaction between phenylacetic acid and benzylamine (200-mg scale) and ethyl acetate was found



Scheme 2. Esterification, amidation and thioesterification using Boc-Oxyma.

Table 1. Solvent screening.

Entry	Solvent	Yield [%][a]
1	dichloromethane	50
2	chloroform	55
3	acetonitrile	40
4	tetrahydrofuran	35
5	ethyl acetate	92
6	dimethylformamide	70

[a] Isolated yield after column chromatography (200 mg scale). Solvents were examined for the coupling of phenylacetic acid (1 equiv.) and benzylamine (1 equiv.) using **2** (1 equiv.) at 0–5 °C for 2 h.

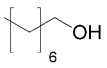
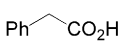
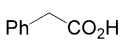
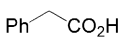
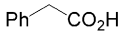
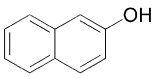
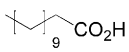
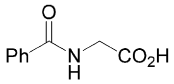
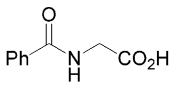
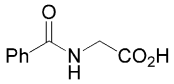
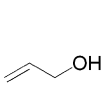
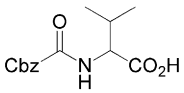
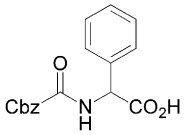
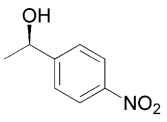
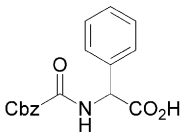
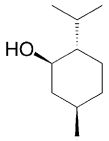
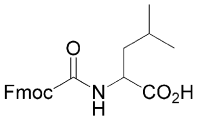
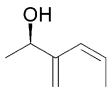
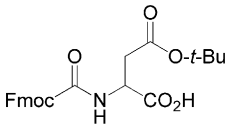
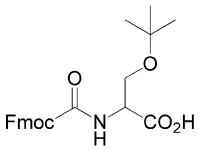
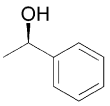
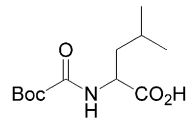
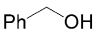
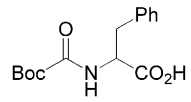
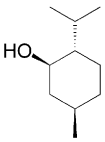
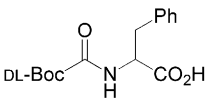
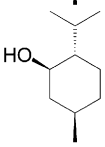
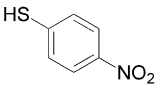
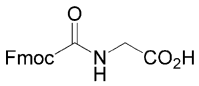
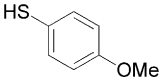
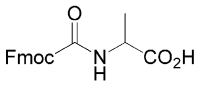
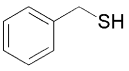
to afford higher yields for both amidations and esterifications (Table 1). Furthermore, the Oxyma that is produced as a by-product could be readily recovered by dilution of the reaction mixture with ethyl acetate, washing with sodium bicarbonate solution, acidifying the aqueous layer, and then extracting with ethyl acetate. The Oxyma thus recovered can then be used to regenerate Boc-Oxyma.

After optimization of the reaction procedure, the esterification of various acids with different alcohols was examined to explore the scope of the coupling reagent (Table 2). It was noted that the coupling of a carboxylic acid with an equimolar amount of an alcohol was completed within 2 h with good to excellent yields. The reaction works well with aromatic acids (entries 1–3), aliphatic acids (entries 4–7), including a long-chain aliphatic acid (entry 8) and amino acids (entries 9–20), and no side reactions were observed. The reaction is compatible with the common amino protecting groups (Cbz, Fmoc and Boc; entries 12–20). Various amino acids with bulky side chains also were tested and all of them resulted in excellent yields (entries 12–20).

The esterification of secondary alcohols using benzotriazole-derived coupling agents, for example, TATU [(1*H*-7-azabenzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate] and TBTU [2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate], requires a strong base, such as DBU, and the reaction does not proceed with tertiary alcohols.[9] Esterifications with *tert*-butyl alcohol have been achieved using COMU with good yield, but only when the very strong base MTBD (7-methyl-1,5,7-triazabicyclo[4.4.0]dec-5-ene) is used.[9] In contrast, Boc-Oxyma mediated esterification proceeds smoothly with a wide variety of secondary alcohols, and even with *tert*-butyl alcohol (entry 6) in good to excellent yields, all with just DIPEA as the base. Thioesterification using **2** also proceeds with excellent yields (entries 21–23, Table 2).

The potential for racemization associated with the use of Boc-Oxyma was investigated by use of the re-

Table 2. Esterification using **2** as coupling reagent.

Entry	Acids	Alcohols	Yield [%][a]
1	Ph CO_2H	MeOH	92
2	Ph CO_2H	PhOH	90
3	Ph CO_2H		94
4			93
5			90
6			87
7			95
8			93
9		MeOH	93
10		EtOH	95
11			93
12			92
13			89
14			91
15			90
16		PhOH	90
17			92
18			91
19			92
20			91[b]
21	Ph-CO_2H		87
22			91
23			92

[a] Isolated yield after column chromatography (200 mg scale).
[b] DL-Boc-Phe-OH was used as substrate.

action of L-Boc-phenylalanine with (1*R*,2*S*,5*R*)-menthol (entry 19). It was clear from various characterization data that the product obtained in this reaction contained a single diastereomer. As a control, the same reaction was carried out with DL-Boc-phenylalanine (entry 20) and the presence of the two diastereomers was demonstrated independently by HPLC (Symmetry C8, 5 μm 3.0×150 mm analytical column, 70% CH_3CN in H_2O with 0.1% formic acid, isocratic gradient, 20 min), and ^1H and ^{13}C NMR (Figure 1).

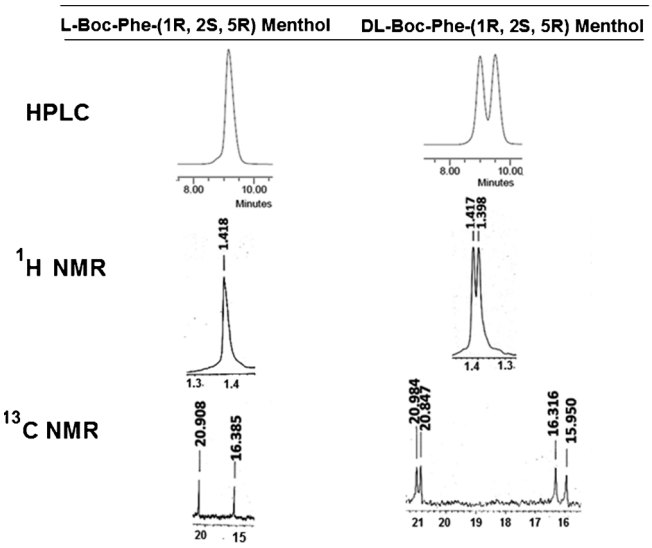


Figure 1. Examination of racemization by comparison of the HPLC profiles, ^{1}H NMR and ^{13}C NMR of the product of entries 19 and 20, respectively.

The peak with a retention time of 9 min corresponds to the pure stereoisomer (entry 19) whereas a second peak with a retention time of 9.5 min (entry 20) appeared when both diastereomers were present. The identity of the peaks in the HPLC profile was verified using ESI-MS (see the Supporting Information). In the ^{1}H and ^{13}C NMR spectra of the product of the reaction with L-Boc-phenylalanine (entry 19), the peaks at $\delta = 1.42$ ppm and $\delta = 20.9$ ppm, respectively, correspond to the tertiary butyl proton and carbon, respectively, of the Boc group of the ester product (entry 19) and confirm the presence of only one optical isomer. The appearance of twin peaks at $\delta = 1.42$ and 1.40 ppm in ^{1}H NMR and at $\delta = 21.0$ and 20.9 ppm in ^{13}C NMR of the product of the reaction with DL-Boc-Phe-OH (entry 20) indicates the presence of two diastereomers of the resulting ester. The comparison of the spectra of the single and mixed diastereomers also allowed the assignment of several other peaks. For example, the peaks at $\delta = 16.3$ and 15.9 ppm in the ^{13}C NMR were assigned to the methyl carbon of menthol ester (entry 20 and Figure 1). From the above comparative study, it can be concluded that the Boc-Oxyma mediated esterification conserves the chiral integrity of the substrate in the product. Similarly, the HPLC profiles and NMR spectra of the products of entries 13–15 and 17 also clearly indicate the absence of any racemization (see the Supporting Information).

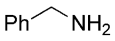
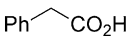
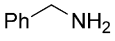
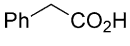
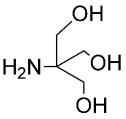
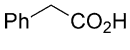
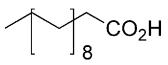
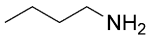
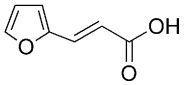
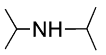
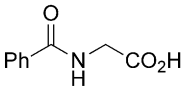
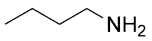
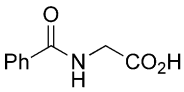
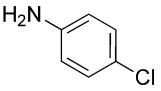
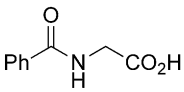
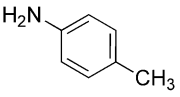
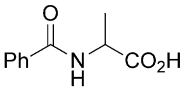
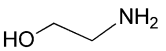
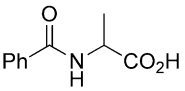
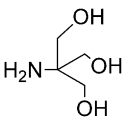
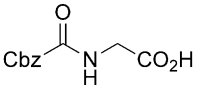
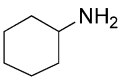
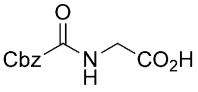
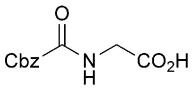
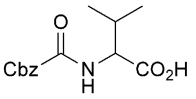
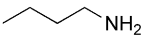
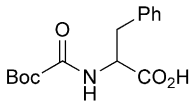
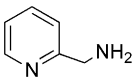
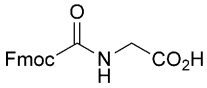
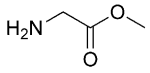
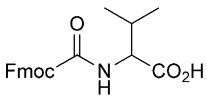
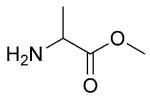
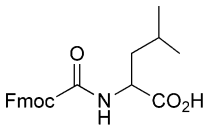
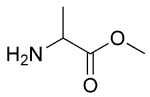
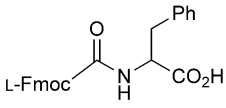
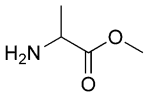
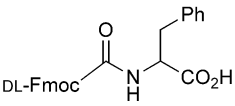
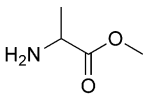
We also evaluated the scope of using Boc-Oxyma for amidation reactions under the optimized conditions by reacting a variety of amines with a selection of aromatic acids, long-chain aliphatic acids and *N*-protected amino acids containing various side chains (Table 3). All the reactions generated good to excellent yields even on a small reaction scale. The current coupling protocol is compatible with common amino protecting groups for example, Cbz (entries 35–38), Boc (entry 39) and Fmoc (entries 40–46). The reaction works well for hindered (entry 29) and non-hindered (entry 36) secondary amines. The dehydration of *N*-hydroxyalkylamides affords a facile route to a variety of heterocycles that are present in many biologically active natural products,[10] and several of these were prepared by direct coupling of the carboxylic acid and amino alcohol under mild conditions (entries 26, 33 and 34).

Also of note is the ease with which aniline derivatives undergo amidation with the use of **2**. While other reports of aniline amidation call for the use of harsh conditions,[11] the reaction proceeds smoothly with **2** at mild temperatures (0 to 5 °C) and with better yields (entries 27, 31, 32 and 37).

The use of Boc-Oxyma was also successfully applied to solution phase peptide coupling (entries 40–47). The potential for racemization during the course of amidation as well as peptide coupling was tested by comparing the HPLC profiles (Symmetry C8 5 μm 3.0×150 mm analytical column, linear gradient of 18 min, 0 to 100% CH_3CN in H_2O with 0.1% formic acid was used), as well as the ^{1}H and ^{13}C NMR spectra of Fmoc-L-Phe-L-Ala-OMe with those of the Fmoc-DL-Phe-L-Ala-OMe (Figure 2). In the HPLC profile of the former (entry 43, Table 3), a single peak with a retention time of 11.6 min was noted, which corresponds to a single stereoisomeric product, Fmoc-L-Phe-L-Ala-OMe. In the case of the latter, two peaks with retention times of 12.7 min and 14.2 min appeared in the HPLC profile, corresponding to the two diastereomers present in the product, Fmoc-DL-Phe-L-Ala-OMe (entry 44, Table 3). The identity of the peaks in the HPLC profiles was verified using ESI-MS (see the Supporting Information). The doublet centered at $\delta = 1.32$ ppm in the ^{1}H NMR and the singlet at $\delta = 17.8$ ppm in the ^{13}C NMR correspond to the protons and the carbon, respectively, of the methyl group of the alanine residue of the single optical isomer of the dipeptide product (entry 43), whereas, the doublets centered at $\delta = 1.33$ and 1.22 ppm in the ^{1}H NMR and the peaks at $\delta = 18.3$ and 18.2 ppm in the ^{13}C NMR indicate the presence of two diastereomers for the same proton and carbon, respectively (entry 44, Figure 2). From these comparative studies, it can be concluded that there was no detectable racemization in the amidation reactions mediated by **2** as the coupling reagent.

We have also synthesized a tripeptide, Z-Gly-Phe-Val-OMe (entry 47, Table 3), *via* the segment coupling of Z-Gly-Phe-OH and H-Val-OMe in solution using **2** and compared the results to those reported by

Table 3. Amidation reactions using **2** as a coupling reagent.

Entry	Acid	Amine	Yield [%][a]
24			91
25			92
26			90
27		$PhNH_2$	85
28			95
29			90
30			93
31			90
32			89
33			91
34			91
35			94
36			91
37		$PhNH_2$	87
38			93
39			91
40			91
41			90
42			88
43			89
44			91
45			90
46			91
47			90

[a] Isolated yield after column chromatography (200-mg scale). All amino acids were L-amino acids except 44 which was the DL-amino acid.

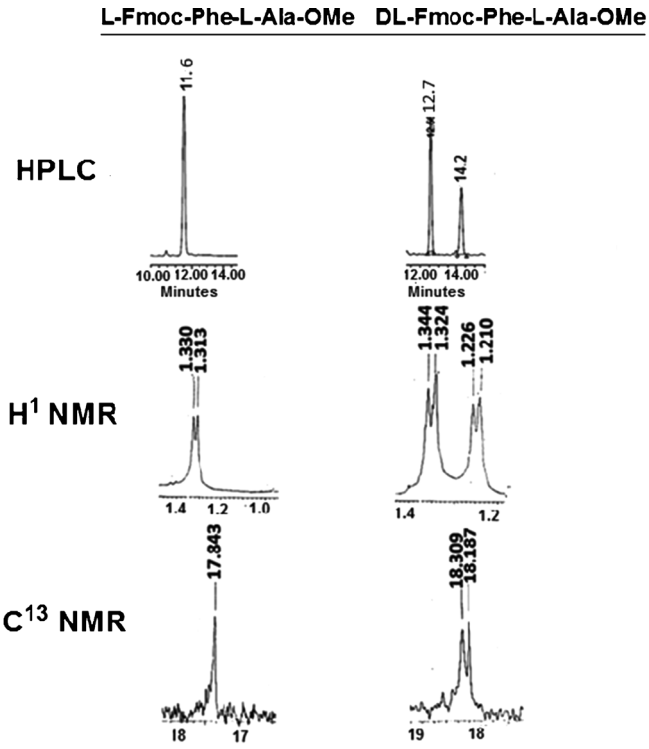


Figure 2. HPLC profiles (18 min, linear gradient, 0 to 100% CH_3CN in water), ^1H NMR and ^{13}C NMR of the products of the reactions entered as 43 and 44 in Table 3.

others with a variety of well known coupling reagents such as HATU {*N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-yl-methylene)-*N*-methylmethanaminium hexafluorophosphate *N*-oxide}, HDMA {1-[(dimethylamino)(morpholino)methylene]-1*H*-[1,2,3]triazolo[4,5-*b*]pyridinium hexafluorophosphate 3-oxide}, HBTU {*N*-[(1*H*-benzotriazol-1-yl)(dimethylamino)methylene]*N*-methylmethanaminium hexafluorophosphate *N*-oxide} and HDMB {1-[(dimethylamino)(morpholino)methylene]-1*H*-benzotriazolium hexafluorophosphate 3-oxide}.[12] Unlike the other coupling reagents, no racemization was observed while using **2** by RP-HPLC and NMR techniques (Table 4).

Table 4. Comparison of % yield and % racemization of a tripeptide synthesized using various coupling reagents.

Entry	Peptide	Coupling reagent	Yield [%]	Racemization [%]
1	Z-Gly-Phe-Val-OMe	HATU	90	1.56
2		HDMA	90	0.65
3		HBTU	89	6.90
4		HDMB	90	2.90
5		2	90	0[a]

[a] No racemization could be detected by HPLC, ^1H and ^{13}C NMR (see Supporting Information).

Table 5. HPLC retention times and observed mass (ESI-MS) of the peptides after each coupling step on solid phase.

Entry	Peptide	HPLC retention time [min]	Expected mass [*m/z*]	Observed mass [*m/z*]
1	Fmoc-L-G-NH_2	12.618	$[M+H]^+$ 410.20	410.21
2	Fmoc-I-L-G-NH_2	13.083	$[M+H]^+$ 523.29	523.31
3	Fmoc-A-I-L-G-NH_2	13.216	$[M+H]^+$ 594.32	594.35
4	Fmoc-G-A-I-L-G-NH_2	13.047	[M+Na]+ 673.33	673.36
5	Fmoc-F-G-A-I-L-G-NH_2	13.430	[M+Na]+ 820.40	820.41
6	Fmoc-N-F-G-A-I-L-G-NH_2	13.448	$[M+Na+H]^{+2}$ 467.72	467.17
7	G-N-F-G-A-I-L-G-NH_2	10.992	$[M+H]^+$ 747.41	747.35

The efficiency of the new coupling reagent was verified for solid phase peptide synthesis as well. We prepared the octapeptide H-Gly-Asn-Phe-Gly-Ala-Ile-Leu-Gly-NH_2, which contains the hexapeptide sequence NFGAIL, known to be the core sequence responsible for the initiation of aggregation of the Amylin peptide that leads to type 2 diabetes. We synthesized the said octapeptide by stepwise coupling of the constituent amino acids on rink amide MBHA resin using Fmoc chemistry, and employing **2** as the coupling reagent. Couplings proceeded smoothly at each step in less than 2 h and were confirmed by the Kaiser test. The HPLC retention times and ESI-MS data for each fragment are reported in Table 5. The yield of the peptide after cleavage from the resin followed by ether precipitaion and semi-preparative HPLC was around 40% with respect to the resin loading. The HPLC profile and ESI-MS of the purified peptide are provided in Figure 3.

Having demonstrated the applicability of **2** as a coupling reagent for esterification, thioesterification, amidation and peptide synthesis, we turned our attention to the elucidation of the mechanism by which it works. The reaction of a carboxylic acid with **2** can lead to the generation of two possible intermediates, the mixed anhydride, **[X]** (Figure 4) and the active ester, **[A]**. The mixed anhydride **[X]** would evolve *via* nucleophilic attack of the carboxylate anion at the carbonyl carbon of **2** and expulsion of the Oxyma anion (which is favored because of resonance stabilization). Subsequently, the active ester **[A]** is likely generated by further attack of the Oxyma anion at the carbonyl carbon of the mixed anhydride, followed by the release of CO_2 and *tert*-butyl alcohol.

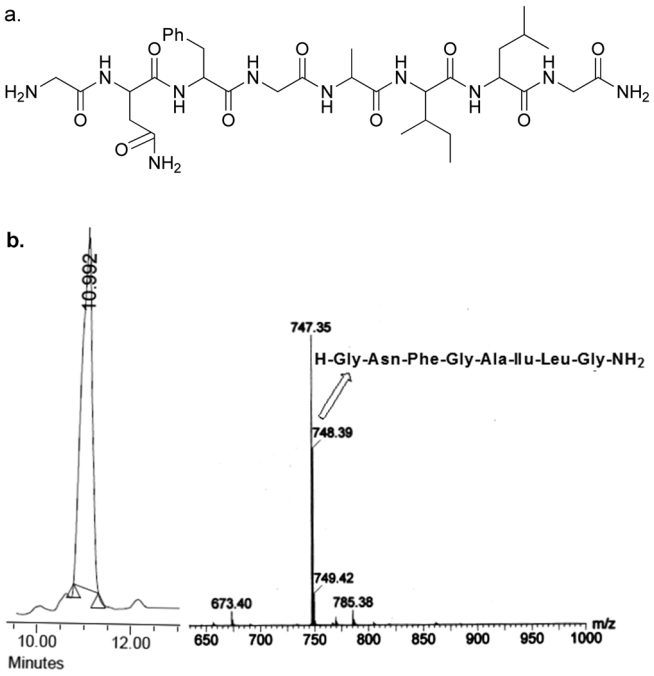


Figure 3. (a) Sequence of the peptide synthesized by SPPS using Boc-Oxyma. (b) HPLC profile and ESI-MS of the final peptide after purification.

This formation of the active ester **[A]** sets Boc-Oxyma apart from other butoxycarbonylation reagents. For instance, Takahata et al. recently reported coupling reactions using BBDI (1-*tert*-butoxy-2-*tert*-butoxycarbonyl-1,2-dihydroisoquinoline) and assumed the reactions to proceed *via* the formation of the mixed anhydride, similar to **[X]**, which then undergoes attack by a nucleophile to generate an ester or amide. They found that the reaction did not proceed with the use of *tert*-butyl alcohol as the nucleophile owing to steric hindrance.[13] In contrast, the use of 2 with *tert*-butyl alcohol readily afforded the *tert*-butyl ester product (entry 6, Table 2), further supporting the proposed conversion of the mixed anhydride to the active ester.

The lower steric congestion about the carbonyl carbon of the intermediate **[A]** allows the formation of the *tert*-butyl ester.

To identify the intermediate **[A]**, **2** was mixed with equimolar amounts of phenylacetic acid in the presence of DIPEA in $CDCl_3$ in an NMR tube and ^{13}C NMR spectra were recorded over time. Initially, at five minutes, three CO peaks were present, one corresponding to the phenyl acetic acid at 175.0 ppm (a, Figure 4) and the other two to Boc-Oxyma at 155.9 and 147.5 ppm (b and c). At 30 min, two new peaks corresponding to the expected Oxyma ester of phenylacetic acid **[A]** were clearly observed at 169.8 (d) and 163.0 ppm (e). At 60 min, only the two new peaks (d, e) could be observed, while the peaks corresponding to the starting materials (a, b and c) had disappeared, indicating complete conversion of the starting carboxylic acid and the reagent **2** to the intermediate Oxyma ester **[A]**. However, with the addition of benzylamine (the nucleophile), peaks d and e shifted to new positions, f and g, respectively, as the intermediate **[A]** was converted to the amide product and the Oxyma was regenerated. When benzyl alcohol was added as the nucleophile, instead of the peak corresponding to the amide carbonyl carbon f, the benzyl ester carbonyl carbon, h, was observed. These observations support the proposed mechanism, similar to that of TBTU and COMU,[9] in which the coupling first proceeds *via* the slow formation of the mixed anhydride **[X]**, which then quickly converts to the active Oxyma ester of the carboxylic acid **[A]**. Final attack of the added nucleophile on **[A]** generates the end product, as depicted in Figure 4.

The evolution of *tert*-butyl alcohol was confirmed by both ^{13}C NMR and ^{1}H NMR. In the ^{13}C NMR, the peak which corresponds to the *tert*-butyl group of Boc-Oxyma gradually shifted from 86.4 ppm (i) to 67.1 ppm (k, corresponding to the *tert*-butyl carbon of the *tert*-butanol that is generated, right side of panel a in Figure 4). In the ^{1}H NMR, the peak corresponding to the *tert*-butyl proton of Boc-Oxyma gradually shifted from 1.60 ppm to 1.20 ppm (corresponding to the *tert*-butyl alcohol protons) as shown in Figure 4 (panel b). Other peaks in $^{13}C/^{1}H$ NMR (Supporting Information) and characteristic bands in IR spectra (CO stretching frequencies[14] are shown in Figure 5) also support the mechanistic pathway elucidated in Figure 4. Furthermore, the evolution of CO_2 was confirmed by passing the evolved gas in a saturated solution of $Ca(OH)_2$ and observing the formation of an emulsion of $CaCO_3$ (Figure 5, panel f, a video demonstrating the course of the experiment is provided in the Supporting Information). Finally, when benzoic acid was used, the corresponding transient intermediate **[A]** could be isolated and its structure was resolved crystallographically (panel g, Figure 5), thereby proving the formation of **[A]**. All the relevant NMR and IR spectra, including that of the starting materials, are furnished in the Supporting Information.

Conclusions

The newly designed compound, ethyl 2-(*tert*-butoxycarbonyloxyimino)-2-cyanoacetate (**2**) is an efficient coupling reagent for simple, mild and racemization-free esterification, thioesterification, amidation reactions and peptide synthesis that uses equimolar amounts of acids with equimolar amounts of alcohols, thiols and amines, respectively. This method can be used for both solid phase and solution phase peptide synthesis. Boc-Oxyma is much easier to prepare than

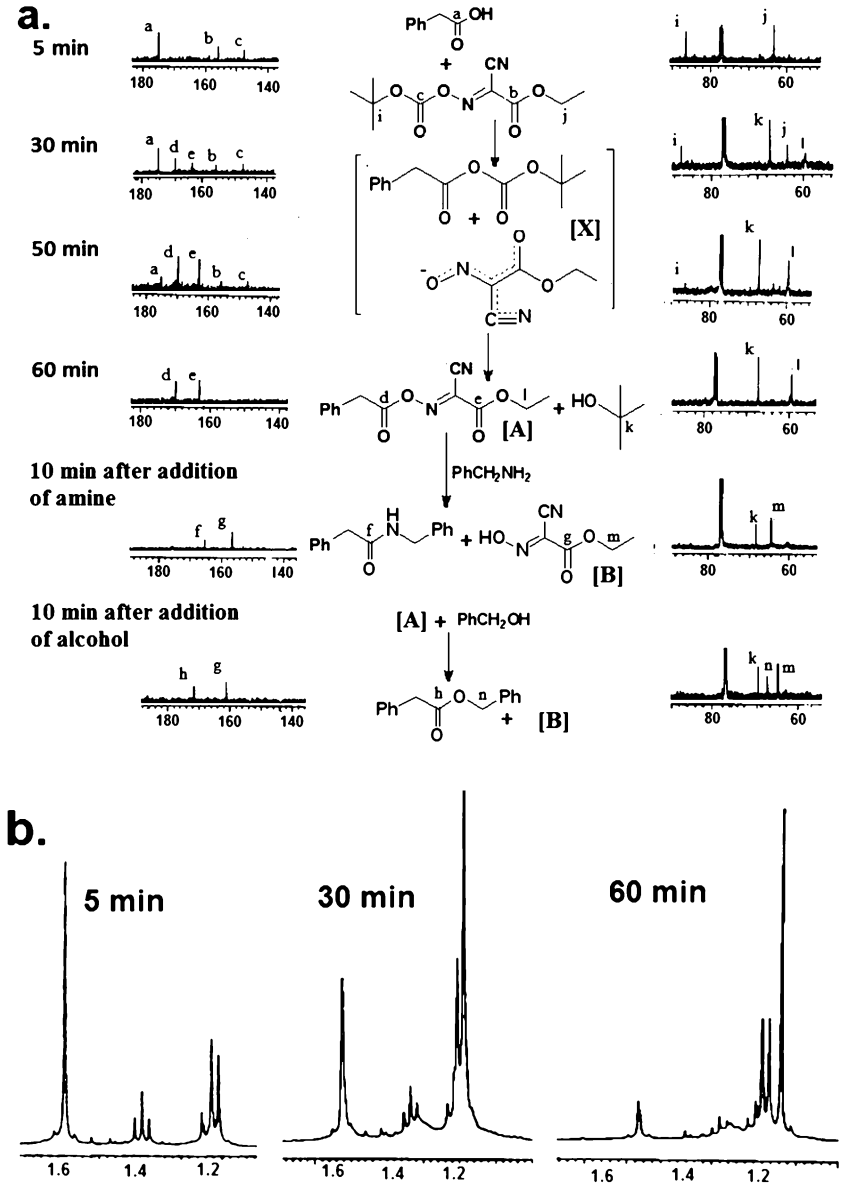
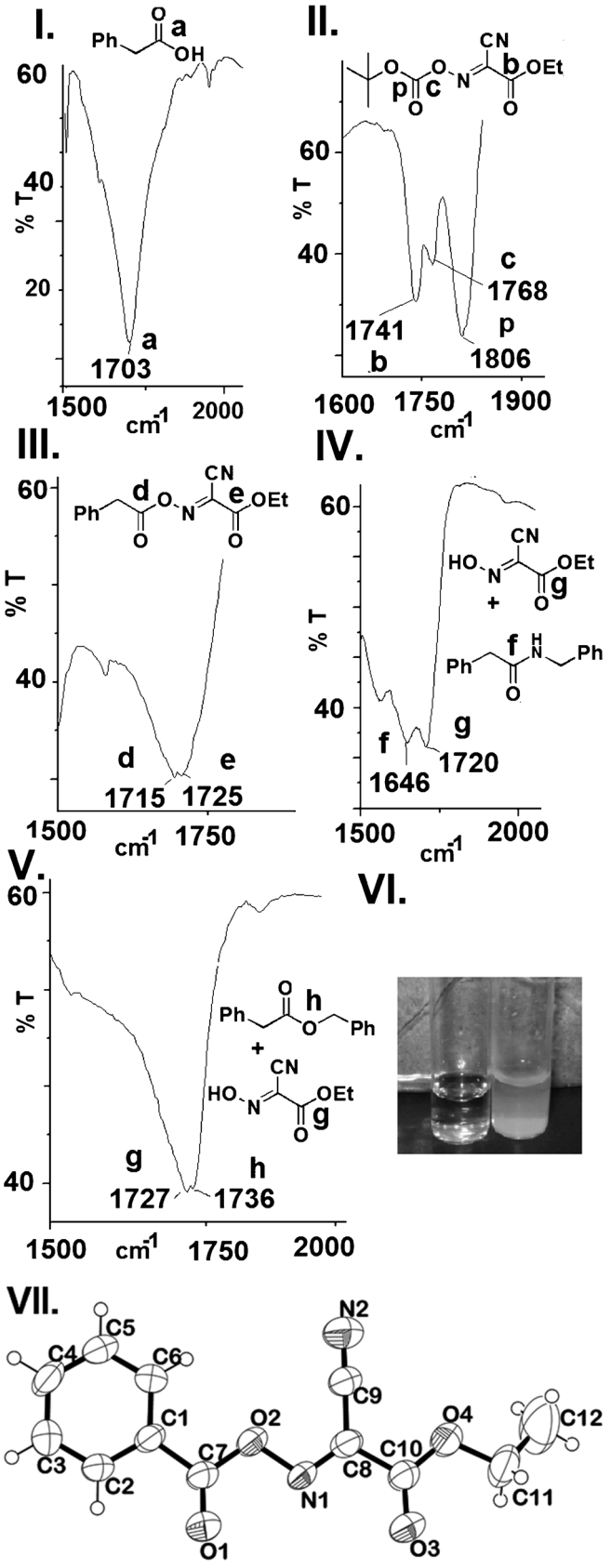


Figure 4. Plausible mechanism of Boc-Oxyma mediated coupling reaction. (a) Time dependent ^{13}C NMR experiments. δ C_{ppm} a = 175.0, b = 155.9, c = 147.5, d = 169.8, e = 163.0, h = 171.5, f = 165.9, g = 156.4, h = 171.5, i = 86.4, j = 63.4, k = 67.1, l = 59.5, m = 64.0, k = 66.6. (b) Part of the proton NMR spectra, to demonstrate the release of *tert*-butyl alcohol.

other popular coupling reagents and produces only *tert*-butyl alcohol, CO_2 and Oxyma as by-products, the latter of which can be easily recovered and reused to regenerate **2**. Thus, use of this reagent reduces chemical waste generation. Boc-Oxyma was successfully used for the esterification of secondary and tertiary alcohols with DIPEA, whereas COMU, TBTU and TATU need stronger bases. Although there are a few other Oxyma-based coupling reagents reported to date, for example, TOTU {*O*-[(ethoxycarbonyl)cyanomethylenamino]-*N,N,N′,N′*-tetramethyluronium tetrafluoroborate}, HOTU {*O*-[(ethoxycarbonyl)cyanomethylenamino]-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate}, COMU, etc.,[8] all of them are uronium salt-based coupling reagents. Boc-Oxyma is similar to them with respect to efficiency and mechanism, but completely different structurally, as it does not involve a uronium/aminium type salt. Thus, Boc-Oxyma is the first member of a new class of efficient coupling reagents. We are continuing to develop other coupling reagents in this series and will be reporting on them in the near future.



Experimental Section

Procedure for the Preparation of Ethyl 2-(*tert*-Butoxycarbonyloxyimino)-2-cyanoacetate (Boc-Oxyma)

Di-*tert*-butyl dicarbonate (1.2 equiv,) was added to a solution of ethyl cyano(hydroxyimino)acetate (Oxyma) (1 equiv.) and DIPEA (1 equiv) in 1 mL $CHCl_3$ at 0–5 °C and the reaction mixture was stirred for 1 h. (***Caution!:*** Boc_2O is a known irritant and sensitizer and should be handled with due care.) After complete disappearance of Oxyma, the solvent $CHCl_3$ and byproduct *tert*-butyl alcohol were evaporated with a rotary evaporator to obtain pure Boc-Oxyma; yield: 96%; red colored liquid. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 4.44–4.39$ (q, $J = 8$ Hz, 2H), 1.54 (s, 9H) 1.38–1.34 (t, $J = 8.4$, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 156.7$, 148.3, 129.9, 106.6, 87.4, 64.3, 27.3, 13.8; LR-MS: $m/z = 265$ $[M + Na]^+$, HR-MS (ESI): $m/z = 265.0728$, calcd. for $C_{10}H_{14}N_2NaO_5$: 265.0800; IR (KBr): $\nu = 3444$, 2925, 1764, 1742 cm^{-1}; R_f product: 0.5 (10% EtOAc/hexane).

General Procedure for the Synthesis of Esters, Amides and Thioesters

Boc-Oxyma was added to a solution of acid (1 equiv.) and DIPEA (1 equiv.) in 1 mL of EtOAc. The reaction mixture was stirred for 1 h at 0–5 °C followed by the addition of alcohol, amine or thiol (1 equiv.). The reaction mixture was stirred at the same temperature for another 2 h. After completion of the reaction, the reaction mixture was diluted with 50 mL of ethyl acetate; the organic phase was washed with 5% citric acid (2×20 mL), saturated $NaHCO_3$ (2× 20 mL) and dried over anhydrous Na_2SO_4. Finally, Na_2SO_4 was filtered off and the solvent was evaporated to obtain the product which was purified by column chromatography.

General Procedure for the Synthesis of Dipeptides

To a solution of *N*-protected amino acid (1 equiv.) and DIPEA (1 equiv.) in 1 mL of EtOAc, Boc-Oxyma (1 equiv.) was added. The reaction mixture was stirred for 1 h at 0–5 °C followed by the addition of methyl ester of the second amino acid (1 equiv.) and DIPEA (1 equiv.) in 1 mL of EtOAc. The reaction mixture was stirred at the same temperature for another 2 h. After completion of the reaction, the reaction mixture was diluted with 50 mL of ethyl acetate, the organic phase was washed with 5% citric acid (2× 20 mL), saturated $NaHCO_3$ (2×20 mL) and dried over

Figure 5. IR spectra of (I) phenyl acetic acid, (II) Boc-Oxyma, (III) the reaction mixture after 1 h, Oxyma ester of phenylacetic acid, intermediate **[A]**, (IV) 10 min after addition of benzyl amine, (V) 10 min after addition of benzyl alcohol. (VI) formation of the $CaCO_3$ emulsion when passed the evolved gas through $Ca(OH)_2$ solution, left side bottle serves as control. (VII) ORTEP molecular diagram with ellipsoid at 50% probability of **[A]**, when benzoic acid was used. CCDC 903487 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* www.ccdc.cam.ac.uk/data_request/cif.

Na_2SO_4 anhydrous. Finally, Na_2SO_4 was filtered and the solvent was evaporated. The product was purified by silica gel column chromatography.

Solid Phase Synthesis of GNFGAILG-NH_2

The octapeptide was manually assembled stepwise on Fmoc-Rink Amide MBHA resin using Fmoc/*t*-Bu protection strategy. The preactivation time of Fmoc-amino acids with Boc-Oxyma (3 equiv.) and DIPEA (5 equiv.) was 1 h. The coupling time was 2 h. Fmoc deprotection was carried out with the use of piperidine/DMF (1:4, 3×7 min). The peptide was cleaved from the resin with the use of TFA/DCM (1:1) mixture for 2.5 h. Purification of the peptide was carried out by semi-preparative HPLC and subsequent liophilization afforded the final peptide as a white powder.

Bz-Oxyma: Yield: 96%. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.18–7.51 (m, 5H), 4.52–4.46 (q, J = 8 Hz, 2H), 1.44–1.40 (t, J = 8.4, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 160.0, 157.0, 135.3, 131.7, 130.6, 129.2, 125.6, 107.0, 67.7, 13.9; LR-MS: m/z = 269 $[M+Na]^+$; HR-MS (ESI): m/z = 269.0007, calcd. for $C_{12}H_{10}N_2NaO_4$: 269.0538.

Benzoic acid methyl ester (1): Yield: 92%; white solid; mp 112 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.02–7.38 (m, 5H), 3.88 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 167.3, 133.0, 130.3, 129.7, 128.5, 52.2; LR-MS: m/z = 137 $[M+H]^+$; HR-MS (ESI): m/z = 139.0649 $[M+H]^+$, calcd. for $C_8H_9O_2$: 137.0603; IR (KBr): ν = 3451, 2961, 1730 cm^{-1}; R_f product: 0.5 (5% EtOAc/hexane).

Benzoic acid phenyl ester (2): Yield: 90%; white solid; mp 70 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.21–8.19 (m, 2H), 7.64–7.48 (m, 3H), 7.50–7.15 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 165.3, 151.2, 133.7, 130.3, 129.6, 129.5, 128.7, 126.0, 125.8; LR-MS: m/z = 221 $[M+Na]^+$; HR-MS (ESI): m/z = 221.0583 $[M+Na]^+$, calcd. for $C_{13}H_{10}NaO_2$: 221.0578; IR (KBr): ν = 3454, 2923, 1731 cm^{-1}; R_f product: 0.15 (30% EtOAc/hexane).

Benzoic acid octyl ester (3): Yield: 94%; colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.05–7.41 (m, 5H), 4.33–4.29 (t, J = 6.8 Hz, 2H), 1.80–1.73 (m, 2H), 1.46–1.41 (m, 2H), 1.37–1.28 (m, 8H), 0.89–0.87 (t, J = 4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.9, 132.9, 130.7, 129.7, 128.5, 65.3, 32.0, 29.4, 29.4, 28.9, 26.2, 22.8, 14.2; LR-MS: m/z = 235 $[M+H]^+$; HR-MS (ESI): m/z = 235.1692 $[M+H]^+$, calcd. for $C_{15}H_{23}O_2$ is 235.1698; IR (KBr): ν = 3455, 2929, 1721 cm^{-1}; R_f product: 0.3 (5% EtOAc/hexane).

Phenyl acetic acid benzyl ester (4): Yield: 93%; white solid; mp 52 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.34–7.25 (m, 10H), 5.11 (s, 2H), 3.64 (s,2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.3, 135.9, 133.9, 129.3, 128.5, 128.5, 128.2, 128.1, 127.1, 66.5, 41.3; LR-MS: m/z = 249 $[M+Na]^+$; HR-MS (ESI): m/z = 249.0893, calcd. for $C_{15}H_{14}NaO_2$: 249.0891; IR (KBr): ν = 3436, 2917, 1733 cm^{-1}; R_f product: 0.3 (5% EtOAc/hexane).

Phenyl acetic acid isopropyl ester (5): Yield: 90%; colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.33–7.23 (m, 5H), 5.04–4.97 (m, 1H), 3.57 (s, 2H), 1.22 (d, J = 0.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.2, 134.4, 129.3, 128.6, 127.0, 68.2, 41.8, 21.8; LR-MS: m/z = 179.1072 $[M+H]^+$; HR-MS (ESI): m/z = 179.0885, calcd. for $C_{11}H_{15}O_2$: 179.1072; IR (KBr): ν = 3440, 2981, 1732 cm^{-1}; R_f product: 0.3 (5% EtOAc/hexane).

Phenyl acetic acid *tert*-butyl ester (6): Yield: 87%; colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.32–7.22 (m, 5H), 3.51 (s, 2H), 1.45 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.9, 134.7, 129.2, 128.5, 126.8, 80.7, 42.6, 28.0; LR-MS: m/z = 215 $[M+Na]^+$; HR-MS (ESI): m/z = 215.0990, calcd. for $C_{12}H_{16}NaO_2$: 215.1048; IR (KBr): ν = 3230, 2989, 1754 cm^{-1}; R_f product: 0.3 (5% EtOAc/hexane).

Phenyl acetic acid naphthalen-1-yl ester (7): Yield: 95%; white solid; mp 52 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.82–7.80 (d, J = 8 Hz, 1H), 7.70–7.68 (d, J = 8.4, 1H), 7.61–7.59 (d, J = 8.4, 1H), 7.48–7.20 (m, 9H), 3.99 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.9, 146.5, 134.6, 133.5, 129.4, 128.8, 127.9, 127.4, 126.8, 126.4, 126.0, 125.3, 121.0, 118.0, 41.4; LR-MS: m/z = 285 $[M+Na]^+$; HR-MS (ESI): m/z = 285.0892, calcd. for $C_{18}H_{14}NaO_2$: 285.0891; IR (KBr): ν = 3444, 2925, 1748 cm^{-1}; R_f product: 0.3 (10% EtOAc/hexane).

Dodecanoic acid benzhydryl ester (8): Yield: 93%; white solid; mp 55 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.37–7.22 (m, 10H), 5.79 (s, 1H), 2.31–2.27 (t, J = 7.6 Hz, 2H), 1.59–1.57 (m, 2H), 1.25 (s, 16H), 0.89–0.86 (t, J = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 179.7, 158.8, 143.6, 128.4, 127.5, 126.6, 63.3, 34.1, 32.0, 29.7, 29.5, 29.4, 29.3, 29.1, 24.1, 22.7, 14.1; LR-MS: m/z = 389 $[M+Na]^+$, HR-MS (ESI): m/z = 389.2480 $[M+Na]^+$, calcd. for $C_{25}H_{34}NaO_2$: 389.2456; IR (KBr): ν3390, 2921, 1705; R_f product 0.25 (5% EtOAc/Hexane)

Benzoylamino-acetic acid methyl ester (9): Yield: 93%; white solid; mp: 82 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.83–7.81 (d, J = 7.2 Hz, 2H), 7.54–7.50 (t, J = 7.2 Hz, 1H), 7.46–7.43 (t, J = 7.2 Hz, 2H), 6.75 (br, 1H), 4.26–4.25 (d, J = 4.8 Hz, 2H), 3.80 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.6, 167.7, 133.6, 131.7, 128.4, 127.1, 52.3, 41.7; LR-MS: m/z = 216 $[M+Na]^+$; HR-MS (ESI): m/z = 216.0631 $[M+Na]^+$, calcd. for $C_{10}H_{11}NNaO_3$: 216.0637; IR (KBr): ν = 3433, 2925, 1736, 1627 cm^{-1}; R_f product: 0.15 (30% EtOAc/hexane).

Benzylamino acetic acid ethyl ester (10): Yield: 95%; white solid; mp 61 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.82–7.80 (d, J = 8 Hz, 2H), 7.53–7.49 (t, J = 6.8 Hz, 1H), 7.45–7.42 (t, J = 7.2 Hz, 2H), 6.79 (br, 1H), 4.28–4.22 (m, 4H), 1.32–1.29 (t, J = 7 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.0, 167.8, 133.4, 131.5, 128.3, 127.0, 61.2, 41.6, 13.9; LR-MS: m/z = 208 $[M+H]^+$; HRMS (ESI): m/z = 208.0982 $[M+Na]^+$, calcd. for $C_{11}H_{14}NO_3$: 208.0974; IR (KBr): ν = 3435, 3340, 1757, 1642 cm^{-1}; R_f product: 0.15 (30% EtOAc/hexane).

Benzylamino acetic acid allyl ester (11): Yield: 93%; yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.8–7.78 (d, J = 8.4 Hz, 2H), 7.52–7.48 (t, J = 8.2 Hz, 1H), 7.44–7.40 (t, J = 8 Hz, 2H), 6.68 (br, 1H), 5.96–5.86 (m, 1H), 5.36–5.31 (dd, J = 16 Hz, 1H), 5.27–5.25 (dd, J = 10.4 Hz, 1H), 4.68–4.67 (d, J = 6 Hz, 2H), 4.26–4.25 (d, J = 5.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.8, 167.8, 133.6, 131.7, 131.5, 128.5, 127.2, 118.8, 60.0, 41.8; LR-MS: m/z = 242 $[M+Na]^+$; HR-MS (ESI): m/z = 242.0791 $[M+Na]^+$, calcd. for $C_{12}H_{13}NNaO_3$: 242.0793; IR (KBr): ν = 3427, 2928, 1751, 1650 cm^{-1}; R_f product: 0.3 (30% EtOAc/hexane).

Benzyloxycarbonylamino-3-butyric acid benzyl ester (12): Yield: 92%; colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.33 (m, 10H), 5.3–5.27 (d, J = 8.8 Hz, 1H), 5.19–5.11 (m, 2H), 5.09 (s, 2H), 4.36–4.32 (m, 1H), 2.19–2.14 (m, 1H),

0.94–0.92 (d, $J=6.8$ Hz, 3H), 0.84–0.82 (d, $J=6.8$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=171.8$, 156.2, 136.3, 135.3, 128.4, 128.3, 128.3, 128.2, 127.9, 66.8, 59.0, 31.1, 18.8, 17.3; ESI-MS: $m/z=342$ $[M+H]^+$; HR-MS (ESI): $m/z=342.1714$ $[M+H]^+$, calcd. for $C_{20}H_{24}NO_4$: 342.1705; IR (KBr): $\nu=$ 3342, 2963,1727, 1538 cm^{-1}; R_f product: 0.35 (10% EtOAc/hexane).

1-(4-Nitrophenyl)ethyl 2-(benzyloxycarbonylamino)-2-phenylacetate (13): Yield: 93%; white solid; mp 155 °C. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=8.20$–8.18 (d, $J=8.4$ Hz, 2H), 7.98–7.96 (d, $J=8.4$ Hz, 1H), 7.54–7.00 (m, 12H), 5.92–5.91 (d, $J=6.4$ Hz, 1H), 5.78–5.77 (q, $J=7.6$ Hz, 1H), 5.06 (s, 2H), 1.41–1.39 (d, $J=6.8$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=170.1$, 155.5, 147.9, 147.6, 136.1, 129.0, 128.8, 128.5, 128.2, 128.1, 127.4, 127.1, 126.7, 126.2, 72.9, 67.1, 58.2, 21.7; LR-MS: $m/z=457$ $[M+Na]^+$; HR-MS (ESI): $m/z=457.1388$, calcd. for $C_{24}H_{22}N_2NaO_6$: 457.1376; IR (KBr): $\nu=3404$, 1726, 1716 cm^{-1}; R_f product: 0.2 (5% EtOAc/hexane).

2-Isopropyl-6-methylcyclohexyl 2-(benzyloxycarbonylamino)-2-phenylacetate (14): Yield: 93%; white solid; mp 60 °C. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.33$–7.12 (m, 10H), 5.98–5.96 (d, $J=6.8$ Hz, 1H), 5.36–5.34 (d, $J=7.2$ Hz, 1H), 5.06 (s, 2H), 4.75–4.64 (m, 1H), 2.03–2.00 (m, 1H),1.95–1.86 (m, 1H), 1.73–1.57 (m, 1H), 1.37–1.28 (m, 1H), 1.05–1.95 (m, 4H), 0.89–0.87 (d, $J=6.8$ Hz, 3H), 0.81–0.74 (dd, $J=6.4$ Hz, 3H), 0.58–0.56 (d, $J=6.8$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=170.2$, 155.3, 137.0, 136.2, 128.6, 128.5, 128.3, 128.2, 128.2, 128.0, 127.2, 127.0, 76.0, 66.8, 58.0, 46.7, 40.6, 39.8, 34.0, 31.3, 26.1, 23.3, 21.9, 20.6; LR-MS: $m/z=446$ $[M+Na]^+$; HR-MS (ESI): $m/z=446.2356$, calcd. for $C_{26}H_{33}NNaO_4$: 446.2307; IR (KBr): $\nu=3368$, 1735, 1708 cm^{-1}; R_f product: 0.2 (5% EtOAc/hexane).

1-Phenylethyl 2-{[(9*H*-fluoren-9-yl)methoxy]carbonylamino}-4-methylpentanoate (15): Yield: 92%; white solid; mp 150 °C. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.76$–7.74 (d, $J=$ 7.6 Hz 2H), 7.58–7.25 (m, 11H), 5.92–5.90 (q, $J=2.8$ Hz, 1H), 5.22–5.20 (d, $J=8.4$ Hz, 1H) 4.91–4.86 (q, $J=6.4$ Hz, 1H), 4.39–4.38 (d, $J=4$ Hz, 2H), 4.21–4.18 (t, $J=6.8$ Hz, 1H), 1.69- 1.52 (m, 2H), 1.49–1.48 (d, $J=6.4$ Hz, 3H), 0.97–0.95 (d, $J=6$ Hz, 3H), 0.92–0.87 (dd, $J=6$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=172.5$, 156.0, 144.1, 143.9, 141.4, 128.7, 128.2, 127.8, 127.2, 126.2, 126.1, 125.5, 125.2, 73.6, 67.0, 52.8, 47.3, 42.0, 29.8, 25.3, 23.0, 22.3; LR-MS: $m/z=480$ $[M+Na]^+$; HR-MS (ESI): $m/z=480.2764$ $[M+H]^+$, calcd. for $C_{29}H_{31}NNaO_4$: 480.2150; IR (KBr): $\nu=3429$, 1727, 1711 cm^{-1}; R_f product: 0.35 (15% EtOAc/hexane).

2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-succinic acid 4-*tert*-butyl 1-phenyl ester (16): Yield: 90%; white solid; mp 122–124 °C. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.76$–7.74 (d, $J=7.6$ Hz, 2H), 7.59–7.58 (d, $J=7.6$ Hz, 2H), 7.40–7.36 (t, $J=7.2$ Hz, 2H), 7.30–7.19 (m, 3H), 7.10–7.08 (d, $J=7.6$ Hz, 2H), 6.90–6.82 (m, 2H), 5.94–5.92 (d, $J=8.4$ Hz, 1H), 4.45–4.43 (d, $J=7.2$ Hz, 2H), 4.38–4.34 (t, $J=7.6$ Hz, 1H), 4.27–4.23 (t, $J=6.8$ Hz, 1H) 3.15–3.10 (dd, $J=4.4$ Hz, 1H), 2.92–2.87 (dd, $J=4.4$ Hz, 1H), 1.41 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=170.0$, 169.7, 156.1, 150.5, 143.7, 141.2, 129.4, 128.7, 127.6, 127.0, 126.1, 125.0, 121.2, 82.0, 67.3, 50.7, 47.0, 37.8, 27.9; LR-MS: $m/z=510$ $[M+Na]^+$; HR-MS (ESI): $m/z=510.2304$ $[M+H]^+$, calcd. for $C_{30}H_{33}NNaO_5$: 510.1893; IR (KBr): $\nu=3361$, 2935, 1750, 1720, 1690 cm^{-1}; R_f product: 0.45 (15% EtOAc/hexane).

3-*tert*-Butoxy-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-propionic acid 1-phenylethyl ester (17): Yield: 92%; white solid; mp 162 °C. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.78$–7.76 (d, $J=7.6$ Hz 2H), 7.64–7.61 (t, $J=6.4$ Hz, 2H), 7.42–7.25 (m, 9H), 5.99–5.97 (d, $J=6.4$ Hz, 1H) 4.92–4.87 (q, $J=$ 6.4 Hz, 1H), 4.54–4.53 (d, $J=2.4$ Hz, 2H), 4.42–4.36 (m, 1H), 4.27–4.26 (t, $J=5.2$ Hz, 1H), 3.91–3.89 (dd, $J=2$ Hz, 1H), 3.85–3.82 (dd, $J=2.4$ Hz, 1H), 1.51- 1.49 (d, $J=6.4$ Hz, 3H), 1.81 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=170.0$, 156.2, 144.0, 141.3, 141.0, 128.5, 128.4, 128.0, 127.7, 127.3, 127.1, 126.2, 126.0, 125.4, 73.5, 70.2, 67.2, 62.3, 54.8, 47.1, 27.3, 22.0; LR-MS: $m/z=510$ $[M+Na]^+$; HR-MS (ESI): $m/z=510.2284$ $[M+H]^+$, calcd. for $C_{30}H_{33}NNaO_5$: 510.2256; IR (KBr): $\nu=3443$, 2975, 1727, 1700 cm^{-1}; R_f product: 0.50 (15% EtOAc/hexane).

2-*tert*-Butoxycarbonylamino-4-methylpentanoic acid benzyl ester (18): Yield: 91%; white semi-solid. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.36$–7.32 (m, 5H), 5.18–5.09 (m, 2H), 4.89–4.87 (d, $J=8$ Hz, 1H), 4.33–4.32 (m, 1H), 1.40 (s, 12H), 0.90–0.88 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=$ 173.3, 155.4, 135.5, 128.5, 128.2, 128.1, 79.7, 66.8, 52.2, 41.5, 28.3, 24.7, 22.8, 21.8; LR-MS: $m/z=344$ $[M+Na]^+$; HR-MS (ESI): $m/z=344.1838$, calcd. for $C_{18}H_{27}NNaO_4$: 344.1838; IR (KBr): $\nu=3442$, 1712, 1638 cm^{-1}; R_f product: 0.3 (10% EtOAc/hexane).

L-2-*tert*-Butoxycarbonylamino-3-phenylpropionic acid 5-isopropyl-2-methylcyclohexyl ester (19): Yield: 92%; white solid; mp 162 °C. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.29$–7.14 (m, 5H), 5.01–4.99 (d, $J=7.2$ Hz, 1H), 4.73–4.68 (m,1H), 4.54–4.52 (d, $J=6.4$ Hz, 1H), 3.12–3.09 (dd, $J=6$ Hz, 1H), 3.05–3.00 (dd, $J=6$ Hz, 1H), 1.89–1.86 (m, 1H), 1.77–1.76 (m, 1H), 1.67–1.64 (m, 1H), 1.41 (s, 9H), 1.34–1.31 (m, 1H), 1.04–1.0 (m, 4H), 0.92–0.85 (dd, $J=6.4$ Hz, 6H), 0.72–0.71 (d, $J=6.8$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=171.6$, 155.2, 136.3, 129.6, 128.5, 127.0, 79.8, 75.6, 54.6, 47.0, 40.8, 38.3, 34.3, 31.5, 28.4, 26.1, 23.4, 22.1, 20.9, 16.3; LR-MS: $m/z=426$ $[M+Na]^+$; HR-MS (ESI): $m/z=426.2797$, calcd. for $C_{27}H_{37}NNaO_4$: 426.2620; Rt: RP-HPLC [isocratic gradient, 20 min (70% CH_3CN in water)]: $R_t=9.1$ min; IR (KBr): $\nu=3394$, 2945, 1721, 1687 cm^{-1}; R_f product: 0.3 (10% EtOAc/hexane).

DL-2-*tert*-Butoxycarbonylamino-3-phenylpropionic acid 5-isopropyl-2-methylcyclohexyl ester (20): Yield: 91%; white solid; mp 162 °C. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.29$–7.15 (m, 10H), 5.01–5.00 (d, $J=7.2$ Hz, 1H), 4.95–4.93 (d, $J=$ 8 Hz, 1H) 4.72–4.67 (t, $J=10.4$ Hz, 2H), 4.54–4.52 (d, $J=$ 6.4 Hz, 2H), 3.13–3.04 (dd, $J=5.2$, Hz, 2H), 3.02–2.97 (dd, $J=6$ Hz, 2H), 2.03–1.86 (m, 2H), 1.77–1.72 (m, 2H), 1.67–1.64 (m, 2H), 1.41 (s, 9H), 1.39 (s, 9H), 1.32–1.29 (m, 2H), 1.03–1.93 (m, 8H), 0.90–0.85 (dd, $J=6.4$ Hz, 12H), 0.72–0.70 (d, $J=6.8$ Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=171.7$, 171.5, 155.1, 136.2, 129.6, 129.4, 128.5, 128.4, 126.9, 79.7, 75.7, 75.5, 54.7, 54.6, 47.0, 46.8, 40.7, 38.2, 34.2, 31.4, 29.7, 28.3, 26.0, 25.8, 23.3, 23.0, 22.0, 20.9, 20.8, 16.3, 15.9. R_f product: 0.3 (10% EtOAc/hexane); RP-HPLC [isocratic gradient, 20 min (70% CH_3CN in water)]: $R_t=9$ min and 9.5 min. ESI-MS: $m/z=426.27$ and 426.28, calcd. for $C_{27}H_{37}NNaO_4$: 426.26.

Thiobenzoic acid *S*-(4-nitrophenyl) ester (21): Yield: 87%; yellow solid; mp 125 °C. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=$ 8.25–8.22 (dd, $J=2$ Hz, 2H), 7.96–7.94 (dd, $J=1.2$ Hz, 2H), 7.66–7.64 (dd, $J=2$ Hz, 2H), 7.59–7.44 (m, 3H); ^{13}C NMR

(100 MHz, $CDCl_3$): δ = 162.5, 137.4, 134.3, 133.3, 128.7, 126.3, 124.9, 124.2, 123.6; LR-MS: m/z = 280 $[M+Na]^+$; HR-MS (ESI): m/z = 280.0200 $[M+Na]^+$, calcd. for $C_{13}H_9NNaO_3S$: 280.0201; IR (KBr): =3390, 2927, 1750, 1289 cm^{-1}; R_f product: 0.5 (15% EtOAc/hexane).

(9*H*-Fluoren-9-ylmethoxycarbonylamino)thioacetic acid *S*-(4-methoxyphenyl) ester (22): Yield: 91%; white solid; mp 168 °C. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.77–7.75 (d, J = 7.6 Hz 2H), 7.61–7.60 (d, J = 6.4 Hz, 2H), 7.41–7.25 (m,7H), 6.95–6.93(d, J=6.4 Hz, 1H), 5.45 (s, 1H), 4.46–4.44 (d, J = 2.4 Hz, 2H), 4.26–4.23 (t, J = 6.8 Hz, 1H), 4.21–4.20 (d, J = 5.2 Hz, 2H), 3.82 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 197.1, 161.0, 156.3, 143.8, 141.4, 136.4, 132.8, 127.9, 127.2, 125.2, 120.1, 116.9, 115.2, 67.5, 55.5, 47.3, 29.8; LR-MS: m/z = 442 $[M+Na]^+$; HR-MS (ESI): m/z = 442.1081 $[M+H]^+$, calcd. for $C_{24}H_{21}NNaO_4S$: 442.1089; IR (KBr): ν = 3349, 1724, 1683, 1249 cm^{-1}; R_f product: 0.5 (20% EtOAc/hexane).

2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)thiopropionic acid *S*-benzyl ester (23): Yield: 92%; white solid; mp 172 °C. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.75–7.73 (d, J = 7.6 Hz, 2H), 7.60–7.25 (m, 11H), 5.28–5.27 (d, J = 2.4 Hz, 1H), 4.51–4.46 (q, J = 6.8 Hz, 1H),), 4.38–4.36 (d, J = 6.8 Hz, 2H) 4.22–4.19 (t, J = 7.2 Hz, 1H), 4.10 (s, 2H), 1.41–1.39 (d, J = 7.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 200.9, 155.7, 143.9, 143.8, 141.4, 137.0, 128.9, 128.7, 127.8, 127.4, 127.1, 125.1, 67.1, 56.7, 47.2, 33.3, 18.8; LR-MS: m/z = 440 $[M+Na]^+$. HR-MS (ESI): m/z = 440.1374 $[M+Na]^+$, calcd. for $C_{25}H_{23}NNaO_3S$: 440.1296; IR (KBr): ν3309, 1735, 1696, 1273 cm^{-1}; R_f product: 0.3 (10% EtOAc/hexane).

***N*-Benzylbenzamide (24):** Yield: 91%; white solid; mp 162 °C. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.79–7.77 (m, 2H), 7.43–7.25 (m, 8H), 4.64–4.63 (d, J = 5.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ =167.6, 138.4, 134.4, 131.6, 128.8, 128.6, 127.9, 127.5, 127.1, 44.1; LR-MS: m/z 212 $[M+H]^+$; HR-MS (ESI): m/z = 212.1073 $[M+H]^+$, calcd. for $C_{14}H_{14}NO$: 212.1075; IR (KBr): ν = 3290, 2924, 1637, 1551 cm^{-1}; R_f product: 0.45 (20% EtOAc/hexane).

***N*-Benzyl-2-phenylacetamide (25):** Yield: 92%; white solid; mp 118 °C. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.36–7.16 (m, 10H), 5.76 (br, 1H), 4.41–4.39 (d, J = 6 Hz, 2H), 3.62 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.1, 138.3, 135.0, 129.4, 129.0, 128.7, 127.5, 127.4, 127.3, 43.7, 43.6; LR-MS: m/z = 226 $[M+H]^+$; HR-MS (ESI): m/z = 226.1231, $[M+H]^+$, calcd. for $C_{15}H_{16}NO$: 226.1232; IR (KBr): ν = 3289, 2925, 1638, 1552 cm^{-1}; R_f product: 0.3 (20% EtOAc/hexane).

***N*-[2-Hydroxy-1,1-bis(hydroxymethyl)ethyl]-2-phenylacetamide (26):** Yield: 90%; white solid; mp 125 °C. ^1H NMR (400 MHz, CD_3OD): δ = 7.19–7.13 (m, 5H), 3.59 (s, 6H), 3.46 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 175.1, 136.7, 130.3, 129.7, 128.0, 63.6, 62.5, 44.3; LR-MS: m/z = 240 $[M+H]^+$; HR-MS (ESI): m/z = 240.1233 $[M+H]^+$, calcd. for $C_{12}H_{18}NO_4$: 240.1236; IR (KBr): ν = 3283, 2934, 1644, 1542 cm^{-1}; R_f product: 0.25 (80% EtOAc/hexane).

2,*N*-Diphenylacetamide (27): Yield: 85%; white solid; mp: 116 °C. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.42–7.06 (m, 10H), 3.73 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.8, 137.9, 134.7, 129.5, 129.1, 128.9, 127.5, 124.5, 120.2, 44.6; LR-MS: m/z = 212 $[M+H]^+$; HR-MS (ESI): m/z = 212.1071 $[M+H]^+$, calcd. for $C_{14}H_{14}NO$: 212.1075; IR (KBr): ν = 3285, 3256, 1657, 1601 cm^{-1}; R_f product: 0.35 (20% EtOAc/hexane).

***N*-Butyldodecanamide (28):** Yield: 95%; white solid; mp: 56 °C. ^1H NMR (400 MHz, $CDCl_3$): δ = 5.52 (br, 1H), 3.23–3.18 (q, J = 6.8 Hz, 2H), 2.14–2.10 (t, J = 7.2 Hz, 2H), 1.60–1.56 (m, 2H), 1.48–1.41 (m, 2H), 1.22 (s, 18H), 0.90–0.82 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.5, 39.2, 36.7, 31.9, 31.7, 29.6, 29.5, 29.4, 29.4, 25.9, 22.7, 20.1, 14.1, 13.7; LR-MS: m/z = 256 $[M+H]^+$; HR-MS (ESI): m/z = 256.2639 $[M+H]^+$, calcd. for $C_{16}H_{34}NO$: 256.2640; IR (KBr): ν = 3296, 2919, 1638, 1552 cm^{-1}; R_f product: 0.25 (20% EtOAc/hexane).

3-Furan-2-yl-*N*,*N*-diisopropylacrylamide (29): Yield: 90%; white solid; mp 125 °C. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.38 (d, J = 1.6 Hz, 1H),7.36–7.32 (d, J = 15.2 Hz, 1H), 6.74–6.71 (d, J = 14.8 Hz, 1H), 6.46–6.45 (d, J = 3.6 Hz, 1H), 6.40–6.39 (dd, J = 2 Hz, 1H), 4.10–4.05 (q, J = 7.4 Hz, 1H), 1.35–1.30 (d, J = 11.6 Hz, 12H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.0, 152.1, 143.6, 128.2, 118.2, 113.1, 112.2, 48.0, 46.0, 21.7, 20.8; LR-MS: m/z = 222 $[M+H]^+$; HR-MS (ESI): m/z = 222.1498 $[M+H]^+$, calcd. for $C_{13}H_{20}NO_2$: 222.1494; IR (KBr): ν = 3458, 2970, 1646 cm^{-1}; R_f product: 0.5 (20% EtOAc/hexane).

***N*-Butylcarbamoylmethylbenzamide (30):** Yield: 93%; white solid; mp 128 °C. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.82–7.38 (m, 5H), 6.78 (br, 1H), 4.12–4.10 (d, J = 4.8 Hz, 2H), 3.27–3.22 (m, 2H), 1.51–1.43 (m, 2H), 1.36–1.28 (m, 2H), 0.89–0.85 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.4, 168.2, 133.6, 132.1, 128.8, 127.4, 41.1, 39.7, 31.6, 20.2, 13.8; LR-MS: m/z = 235 $[M+H]^+$, HR-MS (ESI): m/z = 235.1458 $[M+H]^+$, calcd. for $C_{13}H_{19}N_2O_2$: 235.1447; IR (KBr): ν = 3320, 3298, 1652, 1635 cm^{-1}; R_f product: 0.2 (40% EtOAc/hexane).

***N*-[(4-Chlorophenylcarbamoyl)methyl]benzamide (31):** Yield: 90%; white solid; mp 232 °C. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.93–7.33 (m, 10H), 4.25 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$, CD_3OD for solubility): δ = 168.8, 167.8, 136.4, 133.2, 131.8, 129.1, 128.6, 128.4, 127.0, 121.1, 43.5; LR-MS: m/z = 311 $[M+Na]^+$; HR-MS (ESI): m/z = 311.0565 $[M+Na]^+$, calcd. for $C_{15}H_{13}ClN_2Na\ O_2$: 311.0563; IR (KBr): ν = 3306, 3192, 1680, 1638 cm^{-1}; R_f product: 0.25 (40% EtOAc/hexane).

***N*-(*p*-Tolylcarbamoylmethyl)benzamide (32):** Yield: 89%; white solid; mp 230 °C. ^1H NMR (400 MHz, $CDCl_3$): δ = 9.04 (br, 1H), 7.83–7.06 (m, 10H), 5.68 (br, 1H), 4.32–4.31 (d, J = 5.6 Hz, 2H), 2.27 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, CD_3OD for solubility): δ = 167.8, 167.4, 135.4, 133.4, 131.5, 130.8, 129.1, 128.3, 127.2, 119.8; LR-MS: m/z = 269 $[M+H]^+$; HR-MS (ESI): m/z = 269.1285 $[M+H]^+$, calcd. for $C_{16}H_{17}N_2O_2$: 269.1290; IR (KBr): ν = 3269, 2935, 1676, 1638 cm^{-1}; R_f product: 0.2 (40% EtOAc/hexane).

***N*-1-(2-Hydroxyethylcarbamoyl)ethylbenzamide (33**): Yield: 91%; white solid; m.p 127 °C. ^1H NMR (400 MHz, CD_3OD): δ = 7.78–7.34 (m, 5H), 4.49–4.43 (m, 1H), 3.51–3.49 (d, J = 5.2 Hz, 2H), 3.23–3.16 (m, 2H), 1.84 (s, 1H), 1.37–1.35 (d, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD): δ = 175.6, 170.1, 135.3, 133.0, 129.6, 128.7, 61.6, 51.3, 43.1, 18.4; LR-MS: m/z = 237 $[M+H]^+$; HR-MS (ESI): m/z = 237.1250 $[M+H]^+$, calcd. for $C_{12}H_{17}N_2O_3$: 237.1239; IR (KBr): ν = 3425, 3300, 1622 cm^{-1}; R_f product: 0.2 (80% EtOAc/hexane).

***N*-{1-[2-Hydroxy-1,1-bis(hydroxymethyl)ethylcarbamoyl]-ethyl}benzamide (34):** Yield: 91%; semi solid. ^{1}H NMR (400 MHz, CD_3OD): δ = 7.76–7.73 (m, 5H), 4.51–4.44 (q, J = 7.2 Hz, 1H), 3.62 (s, 6H), 1.37–1.36 (d, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD): δ = 176.0, 170.5, 135.1, 133.1, 129.7, 128.6, 63.5, 62.3, 51.8, 18.1; LR-MS: m/z = 297 $[M+H]^+$; HR-MS (ESI): m/z = 297.1455 $[M+H]^+$, calcd. for $C_{14}H_{21}N_2O_5$: 297.1450; IR (KBr): ν = 3436, 2922, 1671, 1640 cm^{-1}; R_f product: 0.2 (80% EtOAc/hexane).

Cyclohexylcarbamoylmethylcarbamic acid benzyl ester (35): Yield: 94%; brown solid; mp 110 °C. 1H NMR (400 MHz, $CDCl_3$): δ = 7.31 (s, 5H), 6.1 (br, 1H), 5.6 (br, 1H), 5.0 (s, 2H), 3.79–3.78 (d, J = 4 Hz, 2H), 3.74–3.70 (m, 1H), 1.84–1.09 (m, 10H), ^{13}C NMR (100 MHz, $CDCl_3$): δ = 168.1, 156.8, 136.3, 128.7, 128.4, 128.2, 67.3, 48.5, 44.8, 33.0, 25.6, 24.9; LR-MS: m/z = 291 $[M+H]^+$; HR-MS (ESI): m/z = 291.1710 $[M+H]^+$, calcd. for $C_{16}H_{23}N_2O_3$: 291.1709; IR (KBr): ν = 3347, 2924, 1718, 1655 cm^{-1}; R_f product: 0.1 (10% EtOAc/hexane).

2-Oxopiperidin-1-ylethylcarbamic acid benzyl ester (36): Yield: 91%; white solid; mp 114 °C ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.33–7.29 (m, 5H), 5.84 (br, 1H), 5.09 (s, 2H), 3.98–3.97 (d, J = 4 Hz, 2H), 3.54–3.52 (t, J = 5.2 Hz, 2H), 3.29–3.27 (t, J = 5.2 Hz, 2H), 1.70 (s, 2H), 1.63–1.61 (d, J = 4.8 Hz, 2H), 1.54–1.52 (d, J = 5.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.0, 156.3, 136.6, 128.5, 128.1, 128.0, 66.8, 45.4, 43.1, 42.6, 34.0, 26.2, 25.7, 25.4, 25.0, 24.3; LR-MS: m/z = 299 $[M+Na]^+$; HR-MS (ESI): m/z = 299.1366 $[M+Na]^+$, calcd. for $C_{15}H_{20}N_2NaO_3$: 299.1372; IR (KBr): ν = 3293, 2936, 1707, 1641 cm^{-1}; R_f product: 0.2 (30% EtOAc/hexane).

Phenylcarbamoylmethyl-carbamic acid-benzyl ester (37): Yield: 87%; light red solid; mp 147 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.45–7.10 (m, 10H), 5.57 (br, 1H), 5.13 (s, 2H), 3.99–3.98 (d, J = 4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.0, 156.5, 136.3, 135.3, 129.3, 128.8, 128.7, 128.5, 128.3, 128.2, 67.3, 43.0; LR-MS: m/z = 307 $[M+Na]^+$, HR-MS (ESI): m/z = 307.1062 $[M+Na]^+$, calcd. for $C_{16}H_{16}N_2NaO_3$: 307.1059; IR (KBr): ν = 3340, 2929, 1696, 1675 cm^{-1}; R_f product: 0.2 (30% EtOAc/hexane).

1-Butylcarbomyl-2-methylpropylcarbamic acid benzyl ester (38): Yield: 93%; white solid; m.p 139 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.34 (s, 5H), 5.95 (br, 1H), 5.41–5.39 (d, J = 8 Hz, 1H), 5.1 (s, 2H), 3.92–3.82 (t, J = 8.4 Hz, 1H), 3.27–3.18 (m, 2H), 2.11–2.10 (m, 1H) 1.47–1.45(m, 2H), 1.35–1.29 (m, 2H), 0.96–0.83(m, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.4, 156.7, 136.4, 128.6, 128.2, 128.0, 67.0, 60.8, 39.3, 31.6, 31.2, 20.1, 19.3, 18.2, 13.8; LR-MS: m/z = 307 $[M+H]^+$; HR-MS (ESI): m/z = 307.2032 $[M+H]^+$, calcd. for $C_{17}H_{27}N_2O_3$: 307.2022; IR (KBr): ν = 3293, 1690, 1641 cm^{-1}; R_f product: 0.1 (20% EtOAc/hexane).

2-Phenyl-1-pyridin-2-ylmethylcarbamoylethylcarbamic acid *tert*-butyl ester (39): Yield: 91%; white solid; mp 120 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.44–8.43 (d, J = 4 Hz, 1H), 7.65–7.62 (t, J = 6.4 Hz, 1H), 7.22–7.14 (m, 8H), 5.16 (br, 1H), 4.49–4.48 (d, J = 4 Hz, 2H), 4.45 (br, 1H), 3.07–3.05 (d, J = 6.4 Hz, 2H), 1.36 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.1, 156.4, 155.5, 148.9, 137.0, 136.8, 129.4, 128.7, 126.9, 122.5, 122.1, 80.2, 56.0, 44.5, 38.8, 28.4; LR-MS: m/z = 356 $[M+H]^+$; HR-MS (ESI): m/z = 356.1962 $[M+H]^+$, calcd. for $C_{20}H_{26}N_3O_3$: 356.1974; IR (KBr): ν = 3332, 1684, 1661, 1650 cm^{-1}; R_f product: 0.6 (50% EtOAc/hexane).

2-[(9*H*-Fluoren-9-ylmethoxycarbonylamino)acetylamino]-acetic acid methyl ester (40): Yield: 91%; white solid; mp 162 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.76–7.74 (d, J = 7.6 Hz, 2H), 7.58–7.56 (d, J = 7.6 Hz, 2H), 7.40–7.37 (t, J = 7.2 Hz, 2H), 7.31–7.27 (t, J = 7.2 Hz, 2H), 6.80 (br, 1H), 5.73 (br, 1H), 4.43–4.41 (d, J = 6.8 Hz, 2H), 4.22–4.19 (t, J = 7.2 Hz, 1H), 4.04–4.03 (d, J = 6.8 Hz, 2H) 3.92–3.91 (d, J = 6.8 Hz, 2H), 3.73 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.9, 169.7, 156.8, 143.7, 141.2, 128.6, 128.5, 128.3, 127.1, 67.1, 47.0, 44.2, 41.2, 33.8; LR-MS: m/z = 391 $[M+Na]^+$; HR-MS (ESI): m/z = 391.1366 $[M+H]^+$, calcd. for $C_{20}H_{20}N_2NaO_5$: 391.1270; IR (KBr): ν = 3316, 2931, 1734, 1692, 1654 cm^{-1}; R_f product: 0.2 (40% EtOAc/hexane).

2-[2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-methyl-butyrylamino]propionic acid methyl ester (41): Yield: 90%; white solid; mp: 205 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.76–7.75 (d, J = 7.6 Hz, 2H), 7.60–7.58 (d, J = 6.8 Hz, 2H), 7.41–7.37 (t, J = 7.2 Hz, 2H), 7.31–7.28 (t, J = 7.2 Hz, 2H), 6.54–6.52 (d, J = 6.8 Hz, 1H), 5.54–5.52 (d, J = 8 Hz, 1H), 4.60–4.58 (d, J = 7.2 Hz, 2H), 4.44–4.35 (m, 1H), 4.23–4.21(d, J=6.4 Hz, 1H) 4.04–4.0 (t, J = 7.4 Hz, 1H), 3.73 (s, 3H), 2.11–2.08 (m, 1H), 1.41–1.39 (d, J = 6.8 Hz, 3H) 0.98–0.96 (d, J = 9.6 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.3, 171.2, 156.6, 144.0, 141.4, 127.8, 127.2, 125.2, 120.1, 67.2, 60.4, 52.6, 48.2, 47.3, 31.9, 29.8, 19.2, 18.2; LR-MS: m/z = 447 $[M+Na]^+$; HR-MS (ESI): m/z = 447.1908 $[M+Na]^+$, calcd. for $C_{24}H_{28}N_2NaO_5$: 447.1896; IR (KBr): ν = 3296, 2953, 1745, 1692, 1650 cm^{-1}; R_f product: 0.5 (30% EtOAc/hexane).

Methyl 2-(2-{[(9*H*-fluoren-9 yl)methoxy]carbonylamino}-4-methylpentanamido)propanoate (42): Yield: 88%; white solid; mp 162 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.76–7.75 (d, J = 7.6 Hz, 2H), 7.59–7.57 (d, J = 6.8 Hz, 2H), 7.41–7.37 (t, J = 7.2 Hz, 2H), 7.32–7.28 (t, J = 7.2 Hz, 2H), 6.62–6.60 (d, J = 6.8 Hz, 1H), 5.36–5.34 (d, J = 8 Hz, 1H), 4.58–4.52 (m, 1H), 4.41–4.35 (m, 3H), 4.22–4.19 (t, J = 6.4 Hz, 1H), 3.73 (s, 3H), 1.81–1.74 (m, 1H), 1.65–1.63 (m, 2H), 1.40–1.38 (d, J = 6.8 Hz, 3H) 1.01–0.98 (d, J = 9.6 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 174.2, 163.1, 156.6, 143.8, 141.1, 129.3, 128.6, 127.5, 126.9, 124.9, 66.6, 57.4, 52.2, 46.9, 38.2, 38.3, 28.1, 18.0.; LR-MS: m/z = 439 $[M+H]^+$; HR-MS (ESI): m/z = 439.2047 $[M+H]^+$, calcd. for $C_{25}H_{31}N_2O_5$: 439.2233; IR (KBr): ν3307, 2925, 1754, 1694, 1654 cm^{-1}; R_f product: 0.5 (30% EtOAc/hexane).

2-[2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-phenyl-propionylamino]propionic acid methyl ester (43): Yield: 89%; white solid; mp 182 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.75–7.73 (d, J = 7.6 Hz, 2H), 7.53–7.50 (t, J = 6.8 Hz, 2H), 7.40–7.36 (t, J = 7.2 Hz, 2H), 7.30–7.18 (m, J = 7.2 Hz, 7H), 6.63 (br, 1H), 5.37 (br, 1H), 4.51–4.39 (m, 3H), 4.32–4.29 (m, 1H), 4.18–4.14(t, J=6.8 Hz, 1H) 3.09–3.03 (dd, J = 6 Hz, 2H), 3.68 (s, 3H), 1.33–1.31 (d, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.8, 170.9, 156.0, 143.7, 141.1, 136.5, 129.4, 128.4, 127.6, 127.0, 126.8, 125.1, 119.8, 67.0, 56.0, 52.3, 48.2, 46.9, 38.4, 17.8; LR-MS: m/z = 473 $[M+H]^+$; HR-MS (ESI): m/z = 473.2402 $[M+H]^+$, calcd. for $C_{28}H_{28}N_2NaO_5$: 473.2076; IR (KBr): ν = 3307, 2925, 1754, 1694, 1654 cm^{-1}; R_f product: 0.5 (30% EtOAc/hexane).

2-[2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-phenyl-propionylamino]propionic acid methyl ester (44): Yield:

91%; white solid; mp 182 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.76–7.74(d, J=7.6 Hz, 4H), 7.55–7.12 (m, 20H), 6.41 (br, 1H), 6.29 (br, 1H), 5.52–5.50 (d, J = 6.8 Hz, 1H), 5.45–5.43 (d, J = 6.8 Hz, 1H 4.53–4.37 (m, 6H), 4.35–4.31 (m, 2H), 4.19–4.16(t, J=6.8 Hz, 2H) 3.10–2.97 (m, 4H), 3.68 (s, 6H), 1.33–1.32 (d, J = 7.2 Hz, 3H), 1.22–1.20 (d, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.2, 173.1, 171.1, 170.8, 156.2,144.0, 141.5, 136.8, 129.6, 128.8, 128.3, 127.9, 127.6, 127.3, 127.2, 125.3, 120.2, 67.4, 56.3, 52.6, 48.3, 48.2, 47.3, 39.2, 38.9, 18.3, 18.1.

2-[2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-2-phenylacetylamino]-4-methylpentanoic acid methyl ester (45): Yield: 90%; white solid; mp 194 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.74–7.73 (d, J = 7.6 Hz, 2H), 7.56–7.22 (m, 11H), 6.21–6.20 (d, J = 4.8 Hz, 1H), 5.29(br, 1H), 4.60–4.55 (m, 1H), 4.36–4.34 (d, J = 7.2 Hz, 1H) 4.19–4.16 (t, J = 6.8 Hz, 2H), 3.61 (s, 3H), 1.63–1.60 (m, 2H), 1.54–1.47 (m, 1H), 0.92–0.91 (d, J = 4.4 Hz, 3H) 0.90–0.89 (d, J = 4.4 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.7, 170.0, 155.9, 143.7, 143.7, 141.2, 137.6, 128.8, 128.2, 127.6, 127.1, 127.0, 125.1, 119.9, 67.2, 58.1, 52.0, 51.1, 47.0, 41.0, 24.7, 22.6, 21.8; LR-MS: m/z = 501 $[M+H]^+$; HR-MS (ESI): m/z = 501.2418 $[M+H]^+$, calcd. for $C_{30}H_{33}N_2O_5$: 501.2389; IR (KBr): ν = 3433, 1742, 1684, 1655; 1654 cm^{-1}; R_f product: 0.5 (30% EtOAc/hexane).

[2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-methylbutyrylamino]phenylacetic acid methyl ester (46): Yield: 91%; white solid; mp 200 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.75–7.74 (d, J = 7.6 Hz, 2H), 7.56–7.55 (d, J = 7.6 Hz, 2H), (7.40–7.23 (m, 9H), 6.90–6.88 (d, J = 6.8 Hz, 1H), 5.54–5.52 (d, J = 6.4 Hz, 1H), 5.45–5.43 (d, J = 9.6 Hz, 1H), 4.40–4.28 (m, 1H), 4.19–4.18 (d, J = 6.8, 1H) 4.10–4.07 (t, J = 7.8 Hz, 2H), 3.7 (s, 3H), 2.16–2.13 (m, 1H), 1.01–0.99 (d, J = 6.8 Hz, 3H), 0.96–0.95 (d, J = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.7, 169.1, 156.6, 144.0, 143.9, 141.4, 135.9, 129.1, 128.7, 127.8, 127.4, 127.2, 125.2, 120.0, 67.2, 60.2, 56.7, 52.9, 47.2, 31.6, 19.2, 18.1; LR-MS: m/z = 487 $[M+H]^+$; HR-MS (ESI): m/z = 487.2373 $[M+H]^+$, calcd. for $C_{29}H_{31}N_2O_5$: 487.2233; IR (KBr): ν = 3445, 1739, 1681, 1650 cm^{-1}; R_f product: 0.5 (30% EtOAc/hexane).

2-[2-(2-Benzyloxycarbonylaminoacetylamino)-3-phenylpropionylamino]-3-methylbutyric acid methyl ester (47): Yield: 90%; white solid; mp 101 °C. 1H NMR (400 MHz, $CDCl_3$): δ = 7.32–7.13 (m, 10H), 6.99 (br, 1H), 6.2 (br, 1H), 5.8 (br, 1H), 5.08 (s, 2H), 4.58–4.54 (m, 1H), 4.43–4.38 (m, 1H), 3.85–3.84 (d, J = 4.4 Hz, 2H), 3.73 (s, 3H), 304–3.00(m, 2H), 2.14 (m, 1H), 0.94–0.93 (d, J = 7.2 Hz, 3H), 0.91–0.90 (d, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.8, 171.2, 169.4, 156.6, 136.5, 136.3, 129.3, 128.4, 128.3, 128.0, 127.9, 126.7, 66.9, 57.2, 54.3, 52.0, 44.2, 38.4, 31.1, 18.8, 18.7; LR-MS: m/z = 470 $[M+H]^+$; HR-MS (ESI): m/z = 470.2294 $[M+H]^+$, calcd. for $C_{25}H_{32}N_3O_6$:470.2291; IR (KBr): ν = 3406, 1728, 1677, 1651, 1646 cm^{-1}; R_f product: 0.1 (10% EtOAc/hexane).

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