Synthesis of a Peptidomimetic HCMV Protease Inhibitor Library

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Abstract: The human cytomegalovirus (HCMV) protease catalyzes the maturation process of the herpes virus assembly protein and plays a key role during the manufacture of viral capsid, and so is an attractive target for potential anti-herpes-virus agents with novel structures and new mechanisms. In this work, a peptidomimetic skeleton was designed and a chemical library containing 32 compounds with different substitutions on the skeleton was prepared by the oxidation of a precursor library, which was constructed from four types of building blocks: 4 carboxylic acids, 2 amines, 2 aldehydes and 2 isocyanides, based on multicomponent condensation following liquid phase strategies. The syntheses of the key building block isocyanides are presented.

Key words: combinatorial chemistry, condensation, antiviral agents, isocyanides, peptides

The human cytomegalovirus (HCMV) is a member of the herpes virus family infecting 40–80% of the general population. HCMV, like all other herpes viruses, encodes a protease that is essential for viral replication. Inhibiting this enzyme would show antiviral activity, and the enzyme has become a potential target for therapeutic agents. Peptidomimetic compounds are very promising molecules in developing new drugs, especially as protease inhibitors, and there are many successful precedents. Finding general synthetic methods for this kind of compound, especially combinatorial synthetic methods that can greatly improve the efficiency of finding new drugs, is very useful. In this paper, a peptidomimetic HCMV protease inhibitor library was designed and synthesized with a liquid-phase, multicomponent condensation reaction.

Several crystallographic reports have shown that HCMV protease represents a novel structure of serine protease and in fact possesses a unique catalytic triad. A series of peptidomimetic inhibitors of the HCMV protease has been developed, and a crystal structure of HCMV protease in complex with a peptidomimetic inhibitor (covering the P4 to P1 positions) has been determined, which revealed that the inhibitor was bound in an extended conformation, forming an anti-parallel β-sheet with the protease, and that there were five hydrogen bonds and a covalent bond between the inhibitor and the protease. On the basis of this interaction mode, a peptidomimetic library I (Figure 1) was designed for building up a combinatorial synthetic method for peptidomimetic protease inhibitors.

The library was synthesized by liquid-phase combinatorial synthesis, using a Ugi four-component condensation reaction, following a two-step strategy (Scheme 1). The first step was the Ugi condensation of four kinds of building blocks: carboxylic acids II, amines III, aldehydes IV, and isocyanides V (Figure 2), to produce the precursor library VI. This was followed by oxidation of VI to form the activated carbonyl group in compound I. Nakamura et al have also reported similar work.
In the four building blocks, IIa, IIb, III and IV were commercially available. Compounds IIc and IId can be prepared conveniently (Scheme 2).

Isocyanides V have to be produced from their precursors XIII just before use owing to their instability. The synthetic route to V is shown in Scheme 3. By following this method, two isocyanides were synthesized with R group equals benzyl (Va) and cyclohexyl (Vb), respectively.

The amidation of diethyl tartrate with corresponding amines is a good way to N,N'-disubstituted tartaric acid diamides IX. By following literature procedure, IXa was produced in good yield (95.3%), and IXb in moderate yield (76.7%). Kelly and co-workers reported an applicable convenient, high-yielding method for the preparation of esters of glyoxylic acid.8 The method consists of the cleavage of the corresponding tartrate esters by ethereal periodic acid. This method was applied here in the cleavage of tartrate amides IX to form glyoxylate amides X. Because of the poor solubility of IX in diethyl ether, several other solvents were tried, such as THF, methanol and ethanol. THF gave less improvement in solubility. Methanol and ethanol can solve all reactants well, but the reactions gave much more by-product. The mixture of diethyl ether and ethanol (95:5) was found to be the best solvent for the reaction both in solubility and good reaction. The cleavage of IX in this mixed solvent can be carried out smoothly and rapidly to produce glyoxylate amides X, and no by-product was detected. The colorless oils X were reacted immediately after preparation by addition of nitroethane under the catalyzation of potassium fluoride on alumina (KF/alumina) to generate 2-hydroxy-3-nitro-butyric amides XI. The yields of these two steps were 61.1% (XIa) and 41.3% (XIb), respectively. Alumina coated with potassium fluoride proved to be a versatile solid base for olefin- and acetylene-forming elimination, the Michael addition, aldol condensation, and the Darzens condensation.9 KF/alumina was tried as the solid base in nitroethane addition to X and the procedure was improved by packing the reaction mixture with black paper and standing at r.t. for 48 h. This decreased the formation of by-products and gave the product XI with higher purity and yield. There are two chiral centers formed in the 2-hydroxy-3-nitrobutyric amide XI molecule when nitroethane is added to glyoxylate amide X, and this theoretically produces four optical isomers. The crystalline products XIa and XIb were examined by TLC, which showed two spots with XIa and one spot with XIb. The two samples corresponding to the two spots with XIa were obtained by silica gel column chromatography. They have different melting points (98–100°C and 118–120°C, respectively).
but exactly the same $^1$H NMR spectrum. These two samples were believed to be a pair of enantiomers. The absolute configurations of the two samples have not yet been determined. The reported structure-activity relationships showed that the absolute configuration of the carbon atom did not affect the bioactivity, so the pairs of enantiomers of XI were retained unseparated.

The transformation of XI to XII was achieved by reduction of nitro group in XI to the amino group in XII. Several methods were tried, e.g. Fe/HCl, Fe/HOAc, HCOONH$_4$/10% Pd-C, NaBH$_4$/NiCl$_2$, Sn/HCl, Zn/NH$_4$Cl, and H$_2$/10% Pd-C. It was found that the HCOONH$_4$/10% Pd-C, Zn/NH$_4$Cl, H$_2$/10% Pd-C method was better than the others, but the yield and purity of the product were not satisfactory. Optimised results were obtained with P–2 Ni$^{10}$ (Scheme 4) and 4 atm H$_2$/10% Pd-C for 12 h, and then, after filtration, with Zn/NH$_4$Cl for a further 12 h. The products XII were adequate and could be used in the next reaction without purification.

\[
\text{NaBH}_4 + \text{NiCl}_2 + 2\text{H}_2\text{O} \rightarrow 95\% \text{C}_2\text{H}_5\text{OH} \rightarrow \text{B}_2\text{Ni} \rightarrow \text{P-2 Ni}
\]

Scheme 4

The formylation of XII was carried out by using HCOOEt, HCOOOCMe, DCC/HCOOH, HCOOCH$_2$CN (imidazole)$^{11}$ and DCC/HCOOH/NET$_3$/HCOOCH$_2$CH$_2$CN (imidazole), respectively. Only DCC/HCOOH/NET$_3$/HCOOCH$_2$CH$_2$CN (imidazole) gave good results. A catalytic amount of cyanoethyl formate was needed. The POCl$_3$–NET$_3$ method was used in this work, and the two reactions of isocyanides in XII were formylated at the same time to give the diformylated products XIII. The yields of the two steps (reduction and formylation) were 63.6% (XIIIa) and 52.0% (XIIIb), respectively.

The preparations of isocyanides has been reviewed.$^{12}$ The POCl$_3$–NET$_3$ method was used in this work, and the two isocyanides V were obtained from their precursors XI.

The formation of the isocyanides was detected by the characteristic $^{13}$C NMR peaks of the isocyanide groups at $\delta = 160.654$ (Va) and 158.642 (Vb), as well as the IR absorbance at 2200 cm$^{-1}$. When we tried to purify the products by silica chromatography, the isocyanides were found to be very unstable. They were therefore difficult to purify, so the procedure was improved as follows: the reaction in shorter time (treated with POCl$_3$ for 30 min instead of 1 h, then with Na$_2$CO$_3$ for 5 min instead of 30 min), then worked up and used immediately. The determinations of the Ugi-4CC products confirm the correct structures of isocyanides V.

Before the precursor library VI was synthesized (Scheme 1), a model reaction (Scheme 5) was undertaken to test the method, and the products XIV and XV were designed to be representative compounds of the precursor and target libraries, respectively. The structures of XIV and XV were confirmed by their EI–MS, elemental analysis, $^1$H NMR and IR spectra (see experimental section).

Typically equal moles of the four components reacted with each other in a Ugi reaction. But in the preparation of compound XIV, the amount of benzylamine was doubled. The extra one mole of the amine was used to transfer the formylate group in V to the hydroxy group in XIV. According to the mechanism of the Ugi reaction, on route to producing the condensation product XIV, the amino group of benzylamine first reacts with the carbonyl group of benzaldehyde to form a iminium intermediate state 1, which is attacked by the nucleophilic isocyanide carbon to form the intermediate state 2, this would produce a new chiral center C* (Scheme 6). Because the attachment of the isocyanide was equal from the two sides of the $sp^2$ plane in transition state 1, the product XIV might be formed equally as a pair of diastereomers. But in our experiment XIV was yielded as one compound, as were all the precursor library compounds VI. No structural data were obtained to show the stereostructures.

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Four carboxylic acids, 2 amines, 2 aldehydes and 2 isocyanides were chose to build up the precursor library VI, which contained 32 molecules (compounds VI01–VI32, Table 1), which were then oxidized to the target library I (compounds I01–I32, Table 2). The oxidation of a hydroxy to a carbonyl group can be carried out by many methods. In this work, Swern oxidation and Oppenauer oxidation were tried, as well as other oxidants, e.g. CrO$_3$ derivatives and DMP (Dess–Martin Periodinane).$^{13}$ DMP was found to be the best reagent to transform VI to I. The reaction was done under mild conditions, and gave neat product with high yield. DMP can be prepared from IBX (2-iodoxybenzoic acid, Scheme 7).$^{14}$
Following the general procedure, all the library molecules were synthesized, and large part of them were purified from the reaction mixtures by chromatography and determined by MS spectrometry. The yields were 12.2–45.2% for the precursor library VI preparation, 68.3–90.4% for oxidation of library VI to target library I.

In conclusion, a peptidomimetic skeleton was designed based on the interactions between HCMV protease and its peptidomimetic inhibitors. A chemical library containing 32 compounds with different substitutions on the skeleton was prepared by the oxidation of a precursor library, which was constructed from the four types of building blocks: 4 carboxylic acids, 2 amines, 2 aldehydes and 2 isocyanides based on a multicomponent condensation following liquid phase strategies. The syntheses of the key building block isocyanides are reported in detail. This liquid phase method was not satisfactory enough to be applied to build up large scale libraries because of the difficulty of purification and the low yields. It is necessary to develop solid phase procedure to improve the method.

### Table 1 The Precursor Library VI Molecules

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**Table 1** The Precursor Library VI Molecules (continued)

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<td>PhCH&lt;sub&gt;2&lt;/sub&gt;</td>
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<td>PhCH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>(CH&lt;sub&gt;3&lt;/sub&gt;)&lt;sub&gt;3&lt;/sub&gt;CHC H&lt;sub&gt;2&lt;/sub&gt;</td>
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<td>39</td>
<td>C&lt;sub&gt;8&lt;/sub&gt;H&lt;sub&gt;11&lt;/sub&gt;N&lt;sub&gt;5&lt;/sub&gt;O&lt;sub&gt;5&lt;/sub&gt; (606)</td>
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<td>PhCH&lt;sub&gt;2&lt;/sub&gt;</td>
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<sup>a</sup> EI–MS  
<sup>b</sup> TOF–MS
### Table 2  The Target Library \( I \) Molecules

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<th>No.</th>
<th>( R^1 )</th>
<th>( R^2 )</th>
<th>( R^3 )</th>
<th>( R^4 )</th>
<th>( R^5 )</th>
<th>Yield (%)</th>
<th>Formula (MW)</th>
<th>MS&lt;sup&gt;a&lt;/sup&gt; (m/z)</th>
<th>Mp (°C)</th>
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<td>PhCH(_2)</td>
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<td>PhCH(_2)</td>
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<td>PhCH(_2)</td>
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Unless otherwise mentioned, reagents were obtained commercially and used without further purification. Melting points (uncorrected) were taken with an X-4 apparatus and were uncorrected. IR (KBr), 1H NMR, 13C NMR and element data were taken with PE-983, VXR-300S, Carloerba-1106 instruments, respectively. Petroleum ether used had a boiling point range 60–90 °C.

**Benzylaminocarbonylpropylic Acid (VII)**

Anhydrous Et3N (6.5 g, 64 mmol) was added to a solution of succinic anhydride (3.1 g, 31 mmol) in anhyd CHCl3 (30 mL). After 5 min stirring, benzylamine (3.28 mL, 30 mmol) was added and the mixture was stirred at 60 °C for 12 h, and then mixed thoroughly with 12 M aq NaOH (50 mL). The aq layer was extracted and washed with CH2Cl2 (30 mL), and dried (Na2SO4). After filtration, the pH was modulated to 2 with concd HCl. A large amount of white solid was precipitated, filtered off, washed with water and dried under reduced pressure to give 5.23 g (84.2%) of VII; mp 136 °C.

**Benzyl N-(Benzylaminocarbonylpropyl)glycinate (VIIIc)**

Glycine (0.75 g, 10 mmol) and p-toluenesulfonic acid monohydrate (2.28 g, 12 mmol) were dissolved in benzyl alcohol (5 mL) and then mixed with CCl4 (120 mL). The mixture was refluxed for 8 h and the produced water was azeotropically distilled. The solvents were evaporated under vacuum and the residual solid was washed with Et2O to yield 3.33 g (97.8%) of white solid benzyl glycinate p-toluenesulfonate; mp 127–129 °C. The solid was dispersed in CHCl3 (50 mL), then extracted with 10% aq Na2CO3/sat. aq NaCl (3/18020 mL). The organic layer was dried (Na2SO4) for 30 min and filtered to give the solution of benzyl glycinate in CHCl3.

To a solution of VII (2.07 g, 10 mmol) in anhyd CHCl3 (50 mL), DCC (2.5 g, 12 mmol) was added with stirring. After 10 min, the solution of benzyl glycinate in CHCl3 prepared above was added. The mixture was stirred at r.t. for 2 h and filtered. The filtrate was washed with 10% aq Na2CO3/sat. NaCl (2/18020 mL), dried (Na2SO4) for 30 min, filtered and evaporated to obtain crude VIIIc, which was purified with silica chromatography (CHCl3–CH3OH 20:1).

Yield: 2.62 g (73.9%); white crystals; mp 136–137 °C.

IR: 3290, 3060, 1741, 1632, 1448, 1410, 891, 799 cm⁻¹.

1H NMR (DMSO-d6): δ = 8.35 (m, 2 H, NHCO), 7.36–7.19 (m, 10 H, PhH), 5.11 (s, 2 H, OCH2Ph), 4.26 (d, 2 H, J = 6.0 Hz, NCH2CO), 3.89 (d, 2 H, J = 6.0 Hz, PhCH2N), 2.41 (m, 4 H, CH2CH2).

MS (EI): m/z = 354 (M+).
Benzyl-N-(Benzylaminocarbonylpropyl)phenylalaninate (VIIId)
Following the same procedure with VIIIc, from phenylalanine (1.65 g, 10 mmol) and p-toluene sulfonic acid monohydrate (2.28 g, 12 mmol), 4.12 g (96.5%) of white solid benzyl phenylalaninate p-toluene sulfonate were obtained mp 186–188 °C.

Yield: 2.94 g (83%); white crystals; mp 144–146 °C.

Recrystallization from CH3OH–acetone–petroleum ether (5:1:1) mole, benzylamine (38.5 g, 0.36 mole), K2CO3 (0.5 g, 3.6 mmol)
A mixture of (+)-diethyl 2,3-dihydroxysuccinate (30.9 g, 0.15

**Found C, 67.83; H, 6.26; N, 7.90.**

Found C, 67.95; H, 6.58; N, 7.78.

**Benzyl-N-(Benzylaminocarbonylpropyl)phenylalaninate (VIIId)**
Following the same procedure with VIIIc, from phenylalanine (1.65 g, 10 mmol) and p-toluene sulfonic acid monohydrate (2.28 g, 12 mmol), 4.12 g (96.5%) of white solid benzyl phenylalaninate p-toluene sulfonate were obtained mp 186–188 °C.

Yield: 2.94 g (83%); white crystals; mp 144–146 °C.

Recrystallization from CH3OH–acetone–petroleum ether (5:1:1) mole, benzylamine (38.5 g, 0.36 mole), K2CO3 (0.5 g, 3.6 mmol)
A mixture of (+)-diethyl 2,3-dihydroxysuccinate (30.9 g, 0.15

**Found C, 67.83; H, 6.26; N, 7.90.**

Found C, 67.95; H, 6.58; N, 7.78.

**Benzyl-N-(Benzylaminocarbonylpropyl)phenylalaninate (VIIId)**
Following the same procedure with VIIIc, from phenylalanine (1.65 g, 10 mmol) and p-toluene sulfonic acid monohydrate (2.28 g, 12 mmol), 4.12 g (96.5%) of white solid benzyl phenylalaninate p-toluene sulfonate were obtained mp 186–188 °C.

Yield: 2.94 g (83%); white crystals; mp 144–146 °C.

Recrystallization from CH3OH–acetone–petroleum ether (5:1:1) mole, benzylamine (38.5 g, 0.36 mole), K2CO3 (0.5 g, 3.6 mmol)
A mixture of (+)-diethyl 2,3-dihydroxysuccinate (30.9 g, 0.15

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**Found C, 67.83; H, 6.26; N, 7.90.**

Found C, 67.95; H, 6.58; N, 7.78.
N-Benzyl-2-hydroxy-3-aminobutyrin Amide (XIa)

To a solution of XIa (2.38 g, 10 mmol) in CH₂OH (120 mL), a catalytic amount of 10% Pd-C, one drop of anhyd formic acid and a catalytic amount of P₂Ni (prepared as described10) were added. The mixture was stirred under 4 atm H₂ at r.t. for 12 h. After filtration, to the filtrate solution, NH₄Cl (7.0 g, 120 mmol), zinc powder (4.9 g, 75 mmol) and H₂O (5 mL) were added. After further stirring at r.t. for 12 h, the mixture was filtered and the filtrate was evaporated under vacuum to dryness to yield crude solid XIIa. This crude XIIa can be used directly in the next reaction without further purification.

IR: 3408, 3325, 3297, 3285, 1646 cm⁻¹.

Yield: 0.453 g (85.8% from XIa); white solid; mp 60–62 °C.

MS (EI): m/z = 263 (M⁺ – 1).

Anal. Calcd for C₁₂H₂₀N₂O₄ (256.30): C, 56.24; H, 7.87; N, 10.93. Found C, 67.87; H, 6.50; N, 10.61.

N-Benzyl-2-formyloxy-3-formamidobutyric Amide (XIIIa)

The crude product of XIIa (1.96 g, 9.4 mmol) was mixed with a catalytic amount of imidazole and cyanomethyl formiate (1.875 g, 25 mmol, prepared as described11) and stirred at r.t. for 48 h. After evaporation in vacuum, the mixture was separated on a silica gel column (CH₂Cl₂-MeOH, 20:1) to yield XIIIa.

Yield: 1.68 g (63.6% from XIa to XIIIa); white solid; mp 98–99 °C.

IR: 3376, 3183, 3025, 1932, 1643, 1531, 1447, 1339, 1294, 1124, 884, 690 cm⁻¹.

1H NMR (DMSO-d₆): δ = 8.27 (t, 1 H, J = 5.9 Hz, BnNHC0), 7.92 (s, 1 H, OCHO), 7.78 (m, 1 H, NHCO), 7.40–7.20 (m, 5 H, PhH), 5.90 (d, 1 H, J = 5.7 Hz, NCHO, disappeared with D₂O), 4.30 (m, 2 H, CH₂Ph), 4.22 (d, 1 H, J = 6.0 Hz, OCH), 3.92 (m, 1 H, NCH), 1.05 (d, 3 H, J = 6.6 Hz, CH₃).

MS (EI): m/z = 263 (M⁺ – 1).


N-Benzyl-2-formyloxy-3-formamidobutyric Amide (XIIIb)

Following the same procedure with XIIa, from Xib (2.30 g, 10 mmol), crude solid XIIIb was obtained.

Yield: 1.33 g (52.0% from Xib to XIIIb); white solid; mp 147–149 °C.

IR: 3500, 3412, 3330, 2936, 1526 cm⁻¹.

1H NMR (DMSO-d₆): δ = 7.99 (s, 1 H, OCHO), 7.93 (m, 1 H, NHCO), 7.51 (d, 1 H, J = 8.4 Hz, CONH), 5.78 (d, 1 H, J = 5.7 Hz, NCHO, disappeared with D₂O), 4.20 (m, 1 H, OCH), 3.92 (m, 1 H, NCH), 3.57 (m, 1 H, CH), 1.68–0.99 (m, 10 H, CH₂), 0.89 (d, 3 H, J = 6.3 Hz, CH₃).

MS (EI): m/z = 256 (M⁺).

Anal. Calcd for C₁₃H₂₁N₂O₅ (296.36): C, 61.06; H, 7.52; N, 10.93. Found C, 61.46; H, 7.48; N, 10.86.

N-Benzyl-2-hydroxy-3-[acetylglycyl-(N-benzyl-2-phenyl)glycyl]aminobutyrin Amide (XIV)

A solution of acetylglycin (0.234 g, 2 mmol), benzylamine (0.428 g, 4 mmol) and benzonitrile (0.212 g, 2 mmol) in MeOH (5 mL) was prepared in advance. The above solution of isocyanide Va in CH₂Cl₂ was added to the prepared mixture, and stirred at r.t. for 48 h. Condensed product XIV was obtained from the reaction mixture with silica gel column chromatography (eluted with CH₂Cl₂-MeOH, 20:1).

Yield: 0.357 g (33.7% from XIIIa to XIV); white solid; mp 60–62 °C.

IR: 3302, 3057, 2969, 2868, 1948, 1644, 1526, 1473, 1278, 1078, 844, 698 cm⁻¹.

1H NMR (DMSO-d₆): δ = 8.26–8.02 (m, 3 H, NH), 7.27–7.12 (m, 15 H, PhH), 5.89 (d, 1 H, J = 5.7 Hz, OH, disappeared with D₂O), 4.30–3.78 (m, 9 H, CH₂, CH₃), 1.24 (s, 3 H, CH₃CO), 1.05 (d, 3 H, J = 6.9 Hz, CH₃).

MS (EI): m/z = 531 (M⁺ + 1).


N-Benzyl-2-oxo-3-[acetylglycyl-(N-benzyl-2-phenyl)glycyl]aminobutyrin Amide (XV)

The XIV white solid (0.530 g, 1 mmol) and DMAP (0.64 g, 1.5 mmol, prepared as described13,14) were placed with a solution of CF₃COOH in CH₂Cl₂ (0.05 mol/L, 20 mL), and stirred for 30 min at r.t. Then a solution of Na₂SO₅ in sat. aq NaHCO₃ (60%, 8 mL) was added and stirred for a further10 min. The CH₂Cl₂ layer was separated out, dried (Na₂SO₄) for 30 min, and filtered. This solution of isocyanide Va in CH₂Cl₂ was used immediately in the next Ugi reaction.

A solution of acetylglycin (0.234 g, 2 mmol), benzylamine (0.428 g, 4 mmol) and benzonitrile (0.212 g, 2 mmol) in MeOH (5 mL) was prepared in advance. The above solution of isocyanide Va in CH₂Cl₂ was added to the prepared mixture, and stirred at r.t. for 48 h. Condensed product XV was obtained from the reaction mixture with silica gel column chromatography (eluted with CH₂Cl₂-MeOH, 20:1).

Yield: 0.453 g (85.8%); white solid; mp 65–66.5 °C.

1H NMR (DMSO-d₆): δ = 8.23–8.00 (m, 3 H, NH), 7.21–7.02 (m, 15 H, PhH), 4.25–3.70 (m, 8 H, CH₂, CH₃), 1.80 (s, 3 H, CH₃CO), 1.05 (d, 3 H, J = 6.7 Hz, CH₃).

MS (EI): m/z = 529 (M⁺ + 1).

Anal. Calcd for C₁₃H₂₁N₂O₅ (528.61): C, 66.18; H, 6.10; N, 10.60. Found C, 66.02; H, 6.33; N, 10.54.

Preparation of Library VI and I; General procedure

Following the same procedures with XIV and XV, the precursor library VI and the target library I were prepared. Most products were white to pale yellow solids, obtained by silica gel column chromatography, eluted with CH₂Cl₂-MeOH, 20:1.
Acknowledgement

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References