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Microwave-assisted synthesis of hydroxyethylamine dipeptide isosteres

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Abstract—Microwave irradiation with calcium trifluoromethanesulfonate as a catalyst enabled epoxide opening with protected amino acids in high yields and with very short reaction times. Using this improved method, a series of hydroxyethyleneamine dipeptide isosteres was synthesized.

1. Introduction

Proteases are peptide-bond-cleaving enzymes that offer a promising area for therapeutic intervention, such as in the treatment of AIDS, hypertension, stroke, cancer and malaria.[1–4] A number of protease inhibitors has been developed as transition-state mimetics of the scissile peptide bond (Fig. 1). Phosphinates,[3] hydroxyethylcarbonyls (statines),[4] hydroxyethylamines,[5] hydroxymethylcarbonyls (norstatines),[6] hydroxyethylenes[7] and other types of transition-state mimetics[2,8,9] have proven to be potent inhibitors of renin,[9] HIV protease,[10] angiotensin converting enzyme,[11] matrix metalloproteases[3] and malarial proteases.[4,12]

We were interested in the synthesis of the hydroxyethylamine (HEA) isostere. An obvious and straightforward synthetic approach to HEA isosteres is via the opening of the epoxide ring with the appropriate amino acid. However, in the majority of the reported synthetic procedures, this reaction has been by-passed and other synthetic strategies have been used instead.[13–15] There are only a few reports describing the opening of epoxides with amino-acid amino groups. Prolonged reaction times, high excess of reagents and low-to-moderate yields are the most common drawbacks of these procedures.[16–26] Very low yields have been obtained especially in cases when a catalyst has not been used.[16] More recently, $LiClO_4$ has been shown to improve the outcome; however, large excesses of amino acid and catalyst were still needed.[19,20]

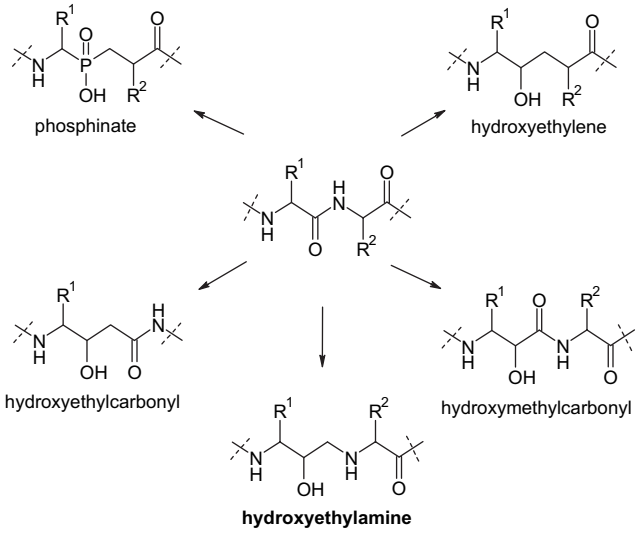

Figure 1. Common transition-state mimetics of the scissile peptide bond.

Recently, we reported that calcium trifluoromethanesulfonate, $Ca(OTf)_2$, is a better catalyst for epoxide opening with amino acids.[27] It is also well known that microwaves can be beneficial in many aspects of epoxide chemistry.[28–30] For this reason, we attempted to improve yields and accelerate the reaction in a microwave reactor, as depicted in Scheme 1. Here, we show that microwave-assisted synthesis of HEA dipeptide isostere shows superior yields and reaction times to those of all of the methods published to date.

2. Results and discussion

We synthesized a series of HEA dipeptide isosteres using different epoxides and various carboxy (*C*)-protected amino

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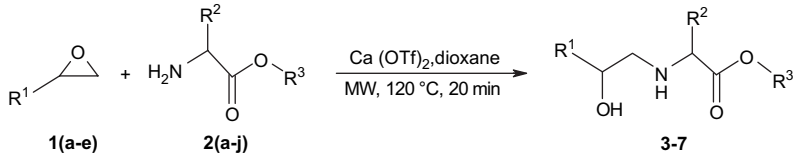


Scheme 1. Microwave-assisted opening of epoxides with *C*-protected amino acids, catalyzed by calcium trifluoromethanesulfonate.

acids. The reaction was optimized with respect to the solvent as presented in Table 1. The best microwave acceleration of the reaction was achieved in dioxane, which is known to be almost transparent for microwaves.[30] Therefore, we assume that the calcium triflate and other polar species in the reaction mixture interacted with the electric field in a way that differs fundamentally from conventional heating. The beneficial effects of the microwaves were reduced if the reaction was performed without the calcium trifluoromethanesulfonate (Table 1).

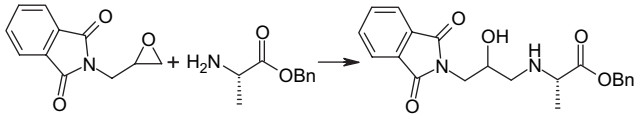
We found the yields to be highly dependent on the reaction time and temperature, so a fine balance between these factors had to be arrived at. Reaction temperatures higher than 120 °C led to significant increases in the side products detected. Increasing the reaction time did not improve the yields significantly.

When compared to previously reported methods,[16–26] significant improvements were also achieved in the amount of amino acid used. The minimal excess of 0.1 equiv was found to provide very few side products and high yields, regardless of the nature of the amino-acid carboxyl-protecting group. It is worth noting that the preparation of free amino-acid esters prior to the reaction did in fact have a profound influence on the outcome of the reaction. Any water or base present in trace amounts significantly influenced the reaction profile and lowered the yields. Anhydrous dioxane stored over 4 Å molecular sieves was used, and the amino-acid ester was extensively dried before the reaction.

Several aspects of the reaction were addressed by careful choice of the epoxides and amino acids used. First, the absence of racemization was unambiguously shown by using enantiomerically pure epoxide **1c**, since it gave only the product in the form of one diastereoisomer when it reacted with a chiral amino acid. Whenever racemic epoxide and enantiopure amino acid were used, the reaction gave two diastereoisomers in a 1:1 ratio, which was determined from the ratio of integrals from ^{1}H NMR spectra. This also demonstrates the second important aspect studied: a chiral amino acid shows no preference for any of the epoxide enantiomers, as shown by ^{1}H and H–H COSY NMR spectra. Furthermore, microwave-assisted conditions allow only a minimal excess of amino acid to be used, which in turn facilitates purification. Last but not least, total regioselectivity was seen from NMR spectra, giving only the desired HEA products.

The synthesized HEA dipeptide isosteres are presented in Table 2. All of the yields obtained in the microwave reactor are higher than 70% and significantly better than previously reported procedures, which means that this type of reaction can be used in the complex, multiple-step synthesis of HEA isosteres.

Table 1. Optimization of the reaction with respect to the solvent[a]



1b 2a 4b

Solvent	Yield[b] (%)
Acetonitrile	17
Tetrahydrofuran	51
Dimethylformamide	<10
Dichloromethane	42
Methanol	<10
Dioxane	75
Dioxane[c]	No product detected

[a] Reaction conditions: 1 equiv of epoxide **1b**, 1.1 equiv of protected amino acid **2a**, 1 equiv of $Ca(OTf)_2$, 6 mL of solvent, microwave heating, 120 °C, 20 min.
[b] Yields of isolated product **4b** after circular chromatography.
[c] No catalyst ($Ca(OTf)_2$) added.

3. Conclusion

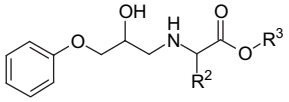
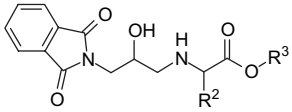
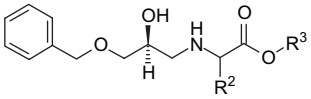
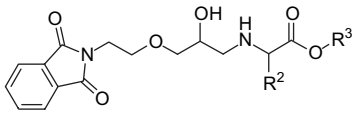
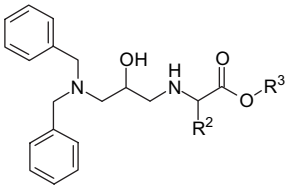
To summarize, we present a very efficient method for epoxide opening with *C*-protected amino acids that yields a series of HEA dipeptide isosteres, important building blocks for the synthesis of transition-state analogue enzyme inhibitors. Calcium trifluoromethanesulfonate as a catalyst combined with microwave acceleration made it possible to perform this reaction with high yields and very short reaction times.

4. Experimental

4.1. General

Chemicals from Aldrich Chemical Co. and Fluka were used without further purification. Analytical TLC was performed on Merck silica gel (60 F 254) plates (0.25 mm) and the components were visualized with ultraviolet light and charred with 20% sulfuric acid in ethanol and ninhydrine. The microwave reactions were performed using a Discover® (CEM Corporation) microwave synthesis system. Circular chromatography was carried out on a Chromatotron® centrifugal thin-layer chromatograph (Harrison Research) using silica gel 60 GP_{254}-containing gypsum. Melting points were determined on a Reichert hot-stage microscope and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AVANCE DPX_{300} spectrometer in $CDCl_3$ solution with TMS as the internal standard. IR spectra were obtained on a Perkin–Elmer 1600 FT-IR spectrometer. Microanalyses were performed on a 240 C Perkin–Elmer CHN analyzer. Mass spectra were obtained using a VG-Analytical Autospec Q mass spectrometer.

Table 2. Hydroxyethylamine dipeptide isosteres obtained via Scheme 1

Entry	Epoxide (R^1)	Protected amino acid (R^2, R^3)	Product	Yield[a] (%)	
				Normal[b]	MW
					
1	$PhOCH_2$ (**1a**)	L-Ala (CH_3, Bn) (**2a**)	**3a**	61	74
2		L-Ala (CH_3, Et) (**2b**)	**3b**	68	79
3		L-Ala (CH_3, *t*-Bu) (**2c**)	**3c**	54	70
4		L-Phe (CH_2Ph, Et) (**2d**)	**3d**	65	79
5		L-Val ($CH(CH_3)_2$, Bn) (**2e**)	**3e**	76	89
					
6	$PhtCH_2$ (**1b**)[b,c]	Gly (H, Bn) (**2f**)	**4a**	52	71
7		L-Ala (CH_3, Bn) (**2a**)	**4b**	74	75
8		L-Val ($CH(CH_3)_2$, Bn) (**2e**)	**4c**	63	84
9		D,L-Met ($(CH_2)_2SCH_3$, Et) (**2g**)	**4d**	72	83
10		D,L-Met ($(CH_2)_2SCH_3$, Bn) (**2h**)	**4e**	67	73
11		L-Phe (CH_2Ph, Et) (**2d**)	**4f**	62	80
12		L-Ala (CH_3, *t*-Bu) (**2c**)	**4g**	61	78
					
13	$PhCH_2OCH_2$ (**1c**)	Gly (H, Bn) (**2f**)	**5a**	—	81
14		L-Phe (CH_2Ph, Et) (**2d**)	**5b**	—	80
					
15	$PhtCH_2CH_2O$ (**1d**)[c,d]	D-Ala (CH_3, Bn) (**2i**)	**6a**	51	70
16		D,L-Met ($(CH_2)_2SCH_3$, Et) (**2g**)	**6b**	71	78
17		L-Lys ($(CH_2)_4NHCbz$, Me) (**2j**)	**6c**	—	80
					
18	$Bn_2NCH(CH_3)$ (**1e**)[e]	L-Ala (CH_3, Bn) (**2a**)	**7**	77	81

[a] Yields of isolated products after circular chromatography.
[b] Conventional heating in acetonitrile for 4 h using calcium triflate as catalyst.[27]
[c] Pht=Phthalimido group.
[d] Compound **4** was synthesized according to previously reported procedures.[31,32]
[e] Compound **5** was synthesized according to previously reported procedures.[33,34]

4.2. General procedures and characterization of products 3–7

The *C*-protected amino acid salt (2.0 mmol) was suspended in 70 mL dichloromethane and washed with 0.5 M sodium hydroxide solution (3×10 mL), water (5 mL) and brine (2×10 mL). The organic phase was dried with sodium sulfate and evaporated under reduced pressure, without exceeding 40 °C. The free amino acid (1.1 mmol) was immediately dissolved in 6 mL anhydrous dioxane containing epoxide (1.0 mmol). The $Ca(OTf)_2$ (0.5 mmol) was suspended and the reaction mixture was stirred for 20 min at 120 °C and 2–3 bar pressure in a Discover® microwave reactor. After cooling to ambient temperature with compressed

air, the catalyst was removed by filtration and the solvent evaporated under reduced pressure, giving a crude product that was purified by circular chromatography using ethylacetate/hexane (2/1) as an eluent.

4.2.1. Benzyl 2-(2-hydroxy-3-phenoxypropylamino)propanoate (3a). White solid (0.244 g, 74%); mp 71–75 °C; ^{1}H NMR (300 MHz, $CDCl_3$): (2 diastereoisomers)* δ (ppm)=1.37 (d, 3H, J=7.0 Hz, CH_3), 1.70 (br s, 1H, OH), 2.65 (2.85)* (dd+dd, 1H, J=12.2, 7.3 Hz, CH_aCH), 2.76 (2.98)* (dd+dd, 1H, J=12.2, 3.6 Hz, CH_bCH), 3.45 (3.46)* (q+q, 1H, J=7.0 Hz, CHCOO), 3.93–4.08 (m, 3H, OCH_2CH), 5.19 (5.20)* (s+s, 2H, $COOCH_2$), 6.88–7.02 (m, 3H, Ph–H), 7.24–7.42 (m, 7H, Ph–H); MS (EI) m/z: 330 $(MH)^+$; MS (FAB) m/z: 330 $(MH)^+$; HRMS Calcd for $C_{19}H_{24}NO_4$ m/z: 330.170534 $(MH)^+$, found 330.171350; IR (KBr, cm^{-1}): 3436, 1647, 1496, 1246, 755, 695. Anal. Calcd for $C_{19}H_{24}NO_4$ (%): C, 69.28; H, 7.04; N, 4.25. Found: C, 69.01; H, 7.28; N, 4.26.

4.2.2. Ethyl 2-(2-hydroxy-3-phenoxypropylamino)propanoate (3b). White solid (0.211 g, 79%); mp 42–47 °C; ^{1}H NMR (300 MHz, $CDCl_3$): (2 diastereoisomers)* δ (ppm)= 1.22 (t, 3H, J=7.1 Hz, CH_3), 2.57 (2.86)* (dd+dd, 1H, J=12.2, 7.3 Hz, CH_aCH), 2.72 (3.00)* (dd+dd, 1H, J=12.2, 3.7 Hz, CH_bCH), 2.92 (dd, 1H, J=13.5, 7.6 Hz, CH_aPh), 3.03 (dd, 1H, J=13.6, 6.1 Hz, CH_bPh), 3.08 (br s, 1H, OH), 3.48–3.56 (m, 1H, CHCOO), 3.87–3.98 (m, 3H, OCH_2CH), 4.17 (q, 2H, J=7.1 Hz, $COOCH_2$), 6.86–7.00 (m, 3H, Ph–H), 7.18–7.34 (m, 7H, Ph–H); MS (EI) m/z: 268 $(MH)^+$; MS (FAB) m/z: 268 $(MH)^+$; HRMS Calcd for $C_{14}H_{22}NO_4$ m/z: 268.154883 $(MH)^+$, found 268.155570; IR (KBr, cm^{-1}): 3480, 1719, 1638, 1497, 1458, 1247, 1018, 752, 691. Anal. Calcd for $C_{14}H_{21}NO_4\times 2/3H_2O$ (%): C, 60.20; H, 8.06; N, 5.01. Found: C, 59.97; H, 7.63; N, 4.74.

4.2.3. *tert*-Butyl 2-(2-hydroxy-3-phenoxypropylamino)-propanoate (3c). White solid (0.207 g, 70%); mp 60–65 °C; ^{1}H NMR (300 MHz, $CDCl_3$): (2 diastereoisomers)* δ (ppm)=1.29 (1.30)* (d+d, 3H, J=7.0 Hz, CH_3), 1.49 (s, 9H, $3\times CH_3$), 1.98 (br s, 1H, OH), 2.64 (2.85)* (dd+dd, 1H, J=12.2, 7.2 Hz, CH_aCH), 2.76 (2.98)* (dd+dd, 1H, J=12.2, 3.7 Hz, CH_bCH), 3.25 (3.26)* (q+q, 1H, J=7.0 Hz, CHCOO), 3.93–4.09 (m, 3H, OCH_2CH), 6.88–7.02 (m, 3H, Ph–H), 7.25–7.34 (m, 2H, Ph–H); MS (EI) m/z: 296 $(MH)^+$; MS (FAB) m/z: 296 $(MH)^+$; HRMS Calcd for $C_{16}H_{26}NO_4$ m/z: 296.186184 $(MH)^+$, found 296.187020; IR (KBr, cm^{-1}): 3286, 2980, 1726, 1600, 1499, 1369, 1253, 1160, 1081, 851, 759, 694. Anal. Calcd for $C_{16}H_{25}NO_4$ (%): C, 65.06; H, 8.53; N, 4.74. Found: C, 65.02; H, 8.56; N, 5.09.

4.2.4. Ethyl 2-(2-hydroxy-3-phenoxypropylamino)-3-phenylpropanoate (3d). White solid (0.271 g, 79%); mp 46–47 °C; ^{1}H NMR (300 MHz, $CDCl_3$): (2 diastereoisomers)* δ (ppm)=1.22 (t, 3H, J=7.1 Hz, CH_3), 2.57 (2.86)* (dd+dd, 1H, J=12.3, 7.4 Hz, CH_aCH), 2.72 (3.00)* (dd+dd, 1H, J=12.3, 3.7 Hz, CH_bCH), 2.88–3.06 (m, 2H, CH_2Ph), 3.48–3.56 (m, 1H, CHCOO), 3.87–3.98 (m, 3H, OCH_2CH), 4.17 (q, 2H, J=7.1 Hz, $COOCH_2$), 6.86–7.00 (m, 3H, Ph–H), 7.18–7.34 (m, 7H, Ph–H); MS (EI) m/z: 344 $(MH)^+$; MS (FAB) m/z: 344 $(MH)^+$; HRMS Calcd for $C_{19}H_{24}NO_4$ m/z: 344.186184 $(MH)^+$, found 344.187050; IR (KBr, cm^{-1}): 3480, 1719, 1638, 1497, 1458, 1247, 1018, 752, 691. Anal. Calcd for $C_{20}H_{25}NO_4$ (%): C, 69.95; H, 7.34; N, 4.08. Found: C, 70.27; H, 7.50; N, 4.07.

4.2.5. Benzyl 2-(2-hydroxy-3-phenoxypropylamino)-3-methylbutanoate (3e). White solid (0.318 g, 89%); mp 71–75 °C; ^{1}H NMR (300 MHz, $CDCl_3$): (2 diastereoisomers)* δ (ppm)=0.87–1.01 (m, 6H, $2\times CH_3$), 1.58 (br s, 1H, OH), 1.92–2.09 (m, 1H, CH), 2.54 (2.89)* (dd+dd, 1H, J=12.2, 7.2 Hz, CH_aCH), 2.67 (3.01)* (dd+dd, 1H, J=12.2, 3.8 Hz, CH_bCH), 3.08 (3.09)* (d+d, 1H, J=5.9 Hz, CHCOO), 3.26 (s, 1H, NH), 3.90–4.05 (m, 3H, OCH_2CH), 5.19 (5.20)* (s+s, 2H, $COOCH_2$), 6.88–7.04 (m, 3H, Ph–H), 7.26–7.46 (m, 7H, Ph–H); MS (EI) m/z: 358 $(MH)^+$; MS (FAB) m/z: 358 $(MH)^+$; HRMS Calcd for $C_{21}H_{28}NO_4$ m/z: 358.201834 $(MH)^+$, found 358.202752; IR (KBr, cm^{-1}): 3440, 2960, 1730, 1598, 1495, 1459, 1244, 1177, 1041, 752, 693. Anal. Calcd for $C_{21}H_{28}NO_4\times 2/3H_2O$ (%): C, 68.34; H, 7.98; N, 3.50. Found: C, 68.27; H, 7.73; N, 3.79.

4.2.6. Benzyl 2-(3-(1,3-dioxoisoindolin-2-yl)-2-hydroxypropylamino)acetate (4a). Colourless oil (0.261 g, 71%); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm)=1.60 (br s, 1H, OH), 2.69 (dd, 1H, J=12.5, 7.2 Hz, CH_bCH), 2.82 (dd, 1H, J=12.5, 3.8 Hz, CH_aCH), 3.26 (br s, 1H, NH), 3.49 (s, 2H, CH_2), 3.72–3.86 (m, 2H, CH_2NPht), 3.90–3.98 (m, 1H, CHOH), 5.16 (s, 2H, CH_2Ph), 7.31–7.41 (m, 5H, Ph–H), 7.71–7.74 (m, 2H, Pht–H), 7.84–7.87 (m, 2H, Pht–H); MS (EI) m/z: 369 $(MH)^+$; MS (FAB) m/z: 369 $(MH)^+$; HRMS Calcd for $C_{20}H_{21}N_2O_5$ m/z: 369.145047 $(MH)^+$, found 369.146250. IR (NaCl, cm^{-1}): 3470, 2917, 1710, 1398, 1190, 1029, 722. Anal. Calcd for $C_{20}H_{21}N_2O_5$ (%): C, 65.21; H, 5.47; N, 7.60. Found: C, 64.99; H, 5.63; N, 7.51.

4.2.7. Benzyl 2-(3-(1,3-dioxoisoindolin-2-yl)-2-hydroxypropylamino)propanoate (4b). Colourless oil (0.287 g, 75%); ^{1}H NMR (300 MHz, $CDCl_3$): (2 diastereoisomers)* δ (ppm)=1.33 (1.35)* (s+s, 3H, CH_3), 1.60 (br s, 1H, OH), 2.50 (2.72)* (dd+dd, 1H, J=12.4, 6.5 Hz, CH_aCH), 2.69 (2.90)* (dd+dd, J=12.4, 3.8 Hz, 1H, CH_bCH), 3.21 (br s, 1H, NH), 3.42 (q, 1H, J=7.0 Hz, CHCH$_3$), 3.72–3.86 (m, 2H, CH_2NPht), 3.90–3.98 (m, 1H, CHOH), 5.17 (5.18)* (s+s, 2H, CH_2–Ph), 7.31–7.41 (m, 5H, Ph–H), 7.73–7.76 (m, 2H, Pht–H), 7.86–7.90 (m, 2H, Pht–H); MS (EI) m/z: 383 $(MH)^+$; MS (FAB) m/z: 383 $(MH)^+$; HRMS Calcd for $C_{21}H_{23}N_2O_5$ m/z: 383.160697 $(MH)^+$, found 383.161550; IR (NaCl, cm^{-1}): 3470, 2917, 1773, 1709, 1395, 1154, 1029, 725. Anal. Calcd for $C_{21}H_{23}N_2O_5$ (%): C, 65.96; H, 5.80; N, 7.33. Found: C, 65.51; H, 5.53; N, 7.67.

4.2.8. Benzyl 2-(3-(1,3-dioxoisoindolin-2-yl)-2-hydroxypropylamino)-3-methylbutanoate (4c). Colourless oil (0.344 g, 84%); ^{1}H NMR (300 MHz, $CDCl_3$): (2 diastereoisomers)* δ (ppm)=0.89–0.95 (m, 6H, CH(CH_3)$_2$), 1.60 (br s, 1H, OH), 1.97 (m, 1H, CH), 2.37 (2.71)* (dd+dd, 1H, J=12.4, 7.3 Hz, CH_aCH), 2.53 (2.89)* (dd+dd, 1H, J=12.4, 3.8 Hz, CH_bCH), 3.04 (dd, 1H, J=5.9, 3.0 Hz, CH), 3.21 (br s, 1H, NH), 3.67–3.81 (m, 2H, CH_2NPht), 3.85–3.93 (m, 1H, CHOH), 5.15 (s, 2H, CH_2–Ph), 7.30–7.36 (m, 5H, Ph–H), 7.70–7.73 (m, 2H, Pht–H), 7.84–7.87

(m, 2H, Pht–H); MS (EI) *m/z*: 411 $(MH)^+$; MS (FAB) *m/z*: 411 $(MH)^+$; HRMS Calcd for $C_{23}H_{27}N_2O_5$ *m/z*: 411.191997 $(MH)^+$, found 411.193150; IR (NaCl, cm^{-1}): 3470, 2961, 1773, 1714, 1467, 1395, 1154, 1031, 725. Anal. Calcd for $C_{23}H_{27}N_2O_5\times H_2O$ (%): C, 64.48; H, 6.78; N, 6.54. Found: C, 64.44; H, 6.85; N, 6.67.

4.2.9. Ethyl 2-(3-(1,3-dioxoisoindolin-2-yl)-2-hydroxypropylamino)-4-(methylthio)butanoate (4d). Colourless oil (0.315 g, 83%); ^1H NMR (300 MHz, $CDCl_3$): (4 diastereoisomers)* δ (ppm)=1.24–1.31 (m, 3H, CH_3), 1.59 (br s, 1H, OH), 1.73–1.87 (m, 1H, CH_aCHCOO), 1.92–2.05 (m, 1H, CH_bCHCOO), 2.09 (2.10)* (s+s, 3H, CH_3S), 2.44 (2.76)* (dd+dd, 1H, J=12.4, 7.3 Hz, CH_aCH), 2.55 (2.90)* (dd+dd, 1H, J=12.4, 3.8 Hz, CH_bCH), 2.61 (2.62)* (t+t, 2H, J=7.0, 2.5 Hz, CH_2S), 3.21 (br s, 1H, NH), 3.37 (dd, 1H, J=8.4, 5.1 Hz, CHCOO), 3.67–3.81 (m, 2H, CH_2NPht), 3.85–3.93 (m, 1H, C*H*OH), 4.19 (4.20)* (q+q, 2H, CH_2CH_3), 7.70–7.73 (m, 2H, Pht–H), 7.84–7.87 (m, 2H, Pht–H); MS (EI) *m/z*: 381 $(MH)^+$; MS (FAB) *m/z*: 381 $(MH)^+$; HRMS Calcd for $C_{18}H_{25}N_2O_5S_1$ *m/z*: 381.148419 $(MH)^+$, found 381.149050; IR (NaCl, cm^{-1}): 3470, 2917, 1773, 1708, 1395, 1190, 1028, 725. Anal. Calcd for $C_{18}H_{25}N_2O_5S_1\times H_2O$ (%): C, 54.26; H, 6.58; N, 7.03. Found: C, 54.59; H, 6.27; N, 7.19.

4.2.10. Benzyl 2-(3-(1,3-dioxoisoindolin-2-yl)-2-hydroxypropylamino)-4-(methylthio)butanoate (4e). Colourless oil (0.323 g, 73%); ^1H NMR (300 MHz, $CDCl_3$): (4 diastereoisomers)* δ (ppm)=1.60 (br s, 1H, OH), 1.73–1.87 (m, 1H, $CHCH_a$), 1.92–2.05 (m, 1H, $CHCH_b$), 2.04 (2.05)* (s+s, 3H, CH_3S), 2.44 (2.76)* (dd+dd, 1H, J=12.4, 7.3 Hz, CH_aCH), 2.58 (2.61)* (m, 2H, CH_2S), 2.55 (2.90)* (dd+dd, 1H, J=12.4, 3.8 Hz, CH_bCH), 3.11 (3.24)* (br s+s, 1H, NH), 3.40 (dd, 1H, J=8.2, 5.1 Hz, CH), 3.67–3.81 (m, 2H, CH_2NPht), 3.85–3.93 (m, 1H, CHOH), 5.16 (5.17)* (s+s, 2H, CH_2Ph), 7.31–7.41 (m, 5H, Ph–H), 7.70–7.73 (m, 2H, Pht–H), 7.84–7.87 (m, 2H, Pht–H); MS (EI) *m/z*: 443 $(MH)^+$; MS (FAB) *m/z*: 443 $(MH)^+$; HRMS Calcd for $C_{23}H_{27}N_2O_5S_1$ *m/z*: 443.164069 $(MH)^+$, found 443.165120; IR (NaCl, cm^{-1}): 3466, 2916, 1772, 1712, 1395, 1170, 1026, 725.

4.2.11. Ethyl 2-(3-(1,3-dioxoisoindolin-2-yl)-2-hydroxypropylamino)-3-phenylpropanoate (4f). Colourless oil (0.317 g, 80%); ^1H NMR (300 MHz, $CDCl_3$): (2 diastereoisomers)* δ (ppm)=1.19 (1.18)* (t+t, 3H, J=7.1 Hz, CH_3), 1.59 (br s, 1H, OH), 2.38 (2.68)* (dd+dd, J=12.3, 6.8 Hz, 1H, CH_aCH), 2.69 (2.90)* (dd+dd, J=12.7, 4.0 Hz, 1H, CH_bCH), 2.87–3.02 (m, 2H, CH_2Ph), 3.21 (br s, 1H, NH), 3.46 (m, 1H, $CHCH_2$), 3.63–3.77 (m, 2H, CH_2NPht), 3.80–3.86 (m, 1H, CHOH), 4.12 (4.13)* (q+q, 2H, J=7.1 Hz, CH_2CH_3), 7.17–7.28 (m, 5H, Ph–H), 7.70–7.73 (m, 2H, Pht–H), 7.83–7.86 (m, 2H, Pht–H); MS (EI) *m/z*: 397 $(MH)^+$; MS (FAB) *m/z*: 397 $(MH)^+$; HRMS Calcd for $C_{22}H_{25}N_2O_5$ *m/z*: $(MH)^+$ 397.176347, found 397.177000; IR (NaCl, cm^{-1}): 3470, 2937, 1772, 1713, 1395, 1191, 1029, 725. Anal. Calcd for $C_{22}H_{25}N_2O_5$ (%): C, 66.65; H, 6.10; N, 7.07. Found: C, 66.37; H, 6.39; N, 7.25.

4.2.12. *tert*-Butyl 2-(3-(1,3-dioxoisoindolin-2-yl)-2-hydroxypropylamino)propanoate (4g). Colourless oil (0.272 g, 78%); ^1H NMR (300 MHz, $CDCl_3$): (2 diastereoisomers)* δ (ppm)=1.26 (d, 3H, J=7.0 Hz, CH_3), 1.44 (1.45)* (s+s, 9H, $(CH_3)_3C$), 2.19 (br s, 1H, NH), 2.50 (2.72)* (dd+dd, 1H, J=12.4, 7.0 Hz, CH_aCH), 2.62 (2.85)* (dd+dd, 1H, J=12.4, 3.8 Hz, CH_bCH), 3.21 (q, 1H, J=7.0 Hz, C*H*CH_3), 3.70–3.84 (m, 2H, CH_2NPht), 3.87–3.98 (m, 1H, CHOH), 7.69–7.76 (m, 2H, Pht–H), 7.83–7.88 (m, 2H, Pht–H); MS (EI) *m/z*: 349 $(MH)^+$; MS (FAB) *m/z*: 349 $(MH)^+$; HRMS Calcd for $C_{18}H_{25}N_2O_5$ *m/z*: 349.176347 $(MH)^+$, found 349.177750; IR (NaCl, cm^{-1}): 3326, 2983, 1722, 1399, 1276, 1165, 1025, 845, 719. Anal. Calcd for $C_{18}H_{25}N_2O_5$ (%): C, 62.05; H, 6.94; N, 8.04. Found: C, 61.55; H, 6.88; N, 8.37.

4.2.13. Benzyl 2-((*R*)-3-(benzyloxy)-2-hydroxypropylamino)acetate (5a). Colourless oil (0.267 g, 81%); ^1H NMR (300 MHz, $CDCl_3$): δ (ppm)=2.69 (dd, 1H, J=12.1, 7.1 Hz, CH_aCH), 2.78 (dd, 1H, J=12.1, 4.0 Hz, CH_bCH), 3.41–3.61 (m, 3H, CH_2O+NH), 3.47 (s, 2H, $NHCH_2COO$), 3.86 (m, 1H, *CH*OH), 4.55 (s, 1H, $PhCH_2O$), 5.17 (s, 2H, CH_2Ph), 7.26–7.38 (m, 10H, Ph–H); MS (FAB) *m/z*: 330 $(MH)^+$; IR (KBr, cm^{-1}): 3432, 2860, 1730, 1453, 1193, 1098, 743. Anal. Calcd for $C_{19}H_{23}N_1O_4\times 1.7H_2O$ (%): C, 63.33; H, 7.32; N, 3.89. Found: C, 62.94; H, 6.82; N, 4.03.

4.2.14. Ethyl 2-((*R*)-3-(benzyloxy)-2-hydroxypropylamino)-3-phenylpropanoate (5b). Colourless oil (0.286 g, 80%); ^1H NMR (300 MHz, $CDCl_3$): δ (ppm)=1.21 (t, 3H, J=7.2 Hz, CH_3), 1.72 (br s, 1H, OH), 2.48 (dd, 1H, J=12.1, 8.0 Hz, CH_aCH), 2.86 (dd, 1H, J=12.1, 3.8 Hz, CH_bCH), 2.97–3.04 (m, 2H, CH_2Ph), 3.42–3.51 (m, 3H, O–CH_2+CHCOO), 3.71–3.81 (m, 1H, C*H*OH), 4.15 (q, 2H, J=7.1 Hz, $COOCH_2$), 4.54 (s, 2H, CH_2O), 7.15–7.40 (m, 10H, Ph–H); MS (EI) *m/z*: 358 $(MH)^+$; MS (FAB) *m/z*: 358 $(MH)^+$; HRMS Calcd for $C_{21}H_{28}NO_4$ *m/z*: 358.201834 $(MH)^+$, found 358.202500; IR (KBr, cm^{-1}): 3432, 2860, 1730, 1453, 1193, 1098, 743. Anal. Calcd for $C_{21}H_{28}NO_4\times 1/4H_2O$ (%): C, 69.69; H, 7.66; N, 3.87. Found: C, 69.98; H, 7.66; N, 3.87.

4.2.15. Benzyl 2-{3-[2-(1,3-dioxoisoindolin-2-yl)-ethoxy]-2-hydroxypropylamino}propanoate (6a). Oil (0.298 g, 70%); ^1H NMR (300 MHz, $CDCl_3$): (2 diastereoisomers)* δ (ppm)=1.31 (1.32)* (d+d, 3H, J=7.0 Hz, CH_3), 2.16 (br s, 1H, OH), 2.49 (2.66)* (dd+dd, 1H, J=12.0, 7.3 Hz, CH_aCH), 2.56 (2.75)* (dd+dd, 1H, J=12.0, 4.2 Hz, CH_bCH), 3.31–3.56 (m, 3H, OCH_2+CHCOO), 3.66–3.80 (m, 3H, $PhtNCH_2$+C*H*OH), 3.90 (t, 2H, J=5.5 Hz, CH_2–O), 5.08–5.20 (m, 2H, $COOCH_2$), 7.28–7.44 (m, 5H, Ph–H), 7.67–7.77 (m, 2H, Ph–H), 7.80–7.88 (m, 2H, Ph–H); MS (EI) *m/z*: 427 $(MH)^+$; MS (FAB) *m/z*: 427 $(MH)^+$; HRMS Calcd for $C_{23}H_{27}N_2O_6$ *m/z*: 427.186912 $(MH)^+$, found 427.187500; IR (KBr, cm^{-1}): 3378, 2932, 1773, 1710, 1395, 1130, 1025, 722. Anal. Calcd for $C_{23}H_{27}N_2O_6\times 3/4H_2O$ (%): C, 62.79; H, 6.30; N, 6.37. Found: C, 62.66; H, 6.51; N, 6.62.

4.2.16. Ethyl 2-{3-[2-(1,3-dioxoisoindolin-2-yl)-ethoxy]-2-hydroxypropylamino}-4-(methylthio)butanoate (6b). Oil (0.331 g, 78%); ^1H NMR (300 MHz, $CDCl_3$): (2 diastereoisomers)* δ (ppm)=1.27 (1.28)* (t+t, 3H, J=7.1 Hz, CH_3), 1.72–2.06 (m, 3H, $CHCH_2$+OH), 2.09 (2.10)* (s+s, 3H, CH_3), 2.44 (2.72)* (dd+dd, 1H, J=12.1, 7.2 Hz,

CH_aCH), 2.51 (2.80)* (dd+dd, 1H, J=12.1, 4.3 Hz, CH_bCH), 2.55–2.64 (m, 2H, CH_2S), 3.31 (3.34)* (t+t, 1H, J=5.3 Hz, CHCOO), 3.42–3.58 (m, 2H, OCH_2), 3.68–3.80 (m, 3H, PhtNCH_2+CHOH), 3.91 (t, 2H, J=5.5 Hz, CH_2O), 4.11–4.23 (m, 2H, $COOCH_2$), 7.68–7.76 (m, 2H, Ph–H), 7.82–7.89 (m, 2H, Ph–H); MS (EI) m/z: 425 $(MH)^+$; MS (FAB) m/z: 425 $(MH)^+$; HRMS Calcd for $C_{20}H_{29}N_2O_6S$ m/z: 425.174634 $(MH)^+$, found 425.175520; IR (KBr, cm^{-1}): 3468, 2917, 1773, 1714, 1467, 1395, 1190, 1026, 722. Anal. Calcd for $C_{20}H_{29}N_2O_6S$ (%): C, 56.59; H, 6.65; N, 6.60. Found: C, 56.42; H, 6.82; N, 6.88.

4.2.17. Methyl 16-(1,3-dioxoisoindolin-2-yl)-12-hydroxy-3-oxo-1-phenyl-2,14-dioxa-4,10-diazahexadecane-9-carboxylate (6c). Colourless oil (0.433 g, 80%); ^{1}H NMR (300 MHz, $CDCl_3$): (2 diastereoisomers)* δ (ppm)=1.36–1.74 (m, 6H, 3×CH_2), 2.43 (2.68)* (dd+dd, 1H, J=12.1, 7.3 Hz, CH_aCH), 2.50 (2.76)* (dd+dd, 1H, J=12.1, 4.2 Hz, CH_bCH), 3.16–3.25 (m, 3H, CH_2NHCOO+CH), 3.45 (3.48)* (dd+dd, 1H, J=7.9, 5.9 Hz, OCH_a), 3.53 (3.57)* (dd+dd, 1H, J=3.8, 0.9 Hz, OCH_b), 3.70 (3.71)* (s+s, 3H, CH_3), 3.71–3.78 (m, 3H, PhtNCH_2+CHOH), 3.90 (t, 2H, J=5.5 Hz, CH_2O), 4.88 (br s, 1H, NH), 5.11 (s, 2H, $COOCH_2$), 7.30–7.38 (m, 5H, Ph–H), 7.68–7.76 (m, 2H, Ph–H), 7.82–7.89 (m, 2H, Ph–H); MS (EI) m/z: 542 $(MH)^+$; MS (FAB) m/z: 542 $(MH)^+$; HRMS Calcd for $C_{28}H_{36}N_3O_8$ m/z: 542.250241 $(MH)^+$, found 542.251560; IR (KBr, cm^{-1}): 3366, 2943, 1709, 1395, 1249, 1026. Anal. Calcd for $C_{28}H_{36}N_3O_8$ (%): C, 62.10; H, 6.51; N, 7.76. Found: C, 62.09; H, 6.78; N, 7.70.

4.2.18. Benzyl 2-(3-(dibenzylamino)-2-hydroxybutylamino)propanoate (7). Colourless oil (0.361 g, 81%); ^{1}H NMR (300 MHz, $CDCl_3$): (2 diastereoisomers)* δ (ppm)=1.10 (1.16*) (d+d, 3H, J=6.6 Hz, CH_3CHCOO), 1.27 (d, 3H, J=6.9 Hz, CH_3CHN), 1.54 (br s, 1H, OH), 2.40 (2.85)* (m+m, 1H, CH_aCH), 2.62 (3.17)* (m+m, 1H, CH_bCH), 3.32–3.51 (m, 3H, PhCH_2N+CH_3CHCOO), 3.64–3.90 (m, 3H, PhCH_2N+CH_3CHN), 4.00 (4.76)* (dd+dd, 1H, J=10.9, 3.2 Hz, CHOH), 5.12 (s, 2H, CH_2–Ph), 7.20–7.35 (m, 15H, Ph–H); MS (EI) m/z: 447 $(MH)^+$; MS (FAB) m/z: 447 $(MH)^+$; HRMS Calcd for $C_{28}H_{35}N_2O_3$ m/z: 447.264768 $(MH)^+$, found 447.265950; IR (NaCl, cm^{-1}): 3326, 2933, 1737, 1454, 1376, 1135, 1027, 699.

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