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Synthesis of Bis-Glyoxylamide Peptidomimetics Derived from Bis-*N*-acetylisatins Linked at C5 by a Methylene or Oxygen Bridge

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Abstract: The bis-glyoxylamide peptidomimetics have been synthesized from bis-*N*-acetylisatins linked at C5 by ring-opening with alcohols, amines, and amino acid methyl ester hydrochlorides. X-ray images of single crystals of bis-glyoxylamide peptidomimetics have been obtained.

Keywords: antimicrobial peptides; *N*-acetylisatins; glyoxylamides; peptidomimetics; quorum sensing

1. Introduction

Many peptides show strong biological activities and are considered as a potential source of therapeutic agents. Prior work in this field has been well reviewed and describes examples of bioactive compounds [1,2]. However, their use as therapeutics has some limitations, such as poor absorption, low bioavailability, inability to cross cell membranes, and in vivo instability [3,4]. To address these issues, peptidomimetics are designed by mimicking existing peptide structures and/or function but whose backbone is not only based on α-amino acids. The modification of the peptide backbone by incorporation of a large range of non-proteinogenic amino acids enhances the proteolytic stability of the molecules, increasing their function as drugs against diseases, such as viral and bacterial infections, cancer and autoimmune diseases. Thus, synthesis of such peptidomimetics is a critical and promising approach in the development of novel pharmaceutical compounds [5–13].

Our group has reported libraries of mono-glyoxylamides derived from *N*-acylisatins [14–16]. Acyclic and cyclic glyoxamide derivatives resulting from ring-opening reactions of *N*-chloroacetylisatins expressed quorum sensing (QS) inhibition activity against *P. aeruginosa* MH602 and *E. coli* MT102 [17]. The ring-opening reactions of *N*-naphthoylisatins with amines and amino acids have been examined to give guanidine-embedded amphipathic glyoxamide-based peptidomimetics and the example of methyl {2-[2-(2-naphthylamido)phenyl]-2-oxoacetyl}arginylargininate dihydrochloride demonstrated the greatest disruption of established biofilms by 60–65% in *S. aureus*, *P. aeruginosa*, and *S. marcescens* [18]. Related *N*-sulfonylphenylglyoxamide derivatives have been synthesized as small molecular antimicrobial peptide (AMP) mimics by ring-opening reactions of *N*-sulfonylisatins with *N*,*N*-dimethylethane-1,2-diamine or *N*,*N*-dimethylpropane-1,3-diamine and were converted into their hydrochloride or iodide salts [19].

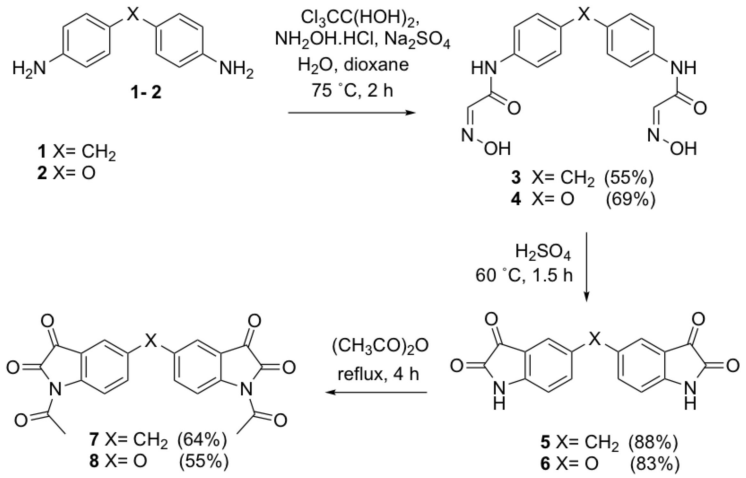
In our previous research, we reported the synthesis of bis-glyoxylamide peptidomimetics from oxalyl-bis-isatins and bis-acylisatins linked through their carbonyl groups and some analogs showed

potent QS inhibitory activity against Gram-positive bacteria [20,21]. In order to expand the library of bis-glyoxylamides, we have designed novel bis-glyoxylamide peptidomimetics from bis-*N*-acetylisatins linked at C5. In this study, we synthesized bis-*N*-acetylisatins linked by a C5 methylene or oxygen bridge and their nucleophilic ring-opening reactions were examined with alcohols, amines, and amino acid methyl ester hydrochlorides.

2. Results and Discussion

2.1. Synthesis of bis-N-acetylisatins

The bis-isatyl methane **5** and the bis-isatyl ether **6** have been reported many years ago, but without any spectroscopic information [22–24]. Therefore, details of our modified syntheses are included here. As a starting point, the intermediate bis-isonitrosoacetanilide **3** was synthesized by applying the Sandmeyer isonitrosoacetanilide isatin synthesis [25]. The starting material of methylene bridged, 4,4′-methylenedianiline **1** was initially dissolved in aqueous hydrochloric acid and heated in the presence of chloral hydrate and hydroxylamine. The product crystallized out after being left overnight at room temperature and was recrystallized from ethyl acetate to give bis-isonitrosoacetanilide **3** in 10% yield as yellow crystals. A consideration of the mechanism shows the generation of a large amount of HCl in the reaction. Therefore, in an attempt to increase the yield, the concentrated HCl solvent was replaced by dioxane. The 4,4′-methylenedianiline **1** was dissolved in the minimum amount of 1,4-dioxane instead of hydrochloric acid and this led to the successful synthesis of the target bis-isonitrosoacetanilide *3* to 55% yield (Scheme 1).



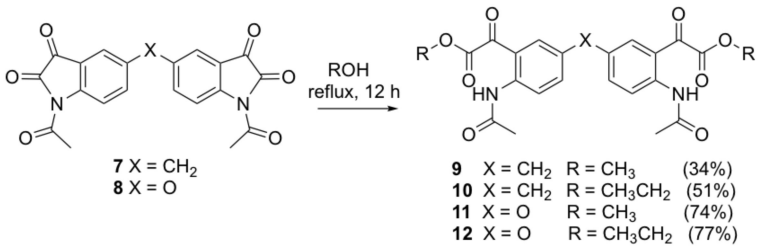
Scheme 1. Synthesis of bis-N-acetylisatins 7 and 8.

The reaction was continued by dissolving bis-isonitrosoacetanilide **3** in concentrated sulfuric acid and stirring the solution to afford the bis-isatin **5** in high yield (88%). The bis-isatin **5** was then suspended in acetic anhydride and heated at reflux to generate the target bis-*N*-acetylisatin **7** in 64% yield. In the ^{1}H NMR spectrum of compound **7**, the two CH_3 groups resonated as a singlet that integrated for 6H at δ 2.59, the CH_2 appeared as a singlet at δ 4.11 and the aromatic protons resonated as a singlet at δ 7.69, a doublet of doublet at δ 7.73, and a doublet at δ 8.85.

In the same manner, the structurally similar bis-*N*-acetylisatins **8** with oxygen as the linkage was generated from 4,4′-oxydianiline **2**. The ring closure of intermediate bis-isonitrosoacetanilide **4** gave bis-isatin **6** in 83% yield. The targeted bis-*N*-acetylisatin **8** was obtained in 55% yield by acetylating bis-isatin **6** with acetic anhydride (Scheme 1). Following the synthesis of bis-*N*-acetylisatins **7** and **8**, their nucleophilic ring-opening reactions were then carried out with alcohols, amines and amino acid methyl ester hydrochlorides, in order to generate the new class of peptide mimics.

2.2. Ring-Opening Reactions of bis-N-acetylisatins with Alcohols

The ring-opening reactions of bis-*N*-acetylisatins **7** and **8** were initially examined with the alcohols MeOH and EtOH. Bis-*N*-acetylisatin **7** was dissolved in anhydrous methanol and the solution was stirred for 24 h at room temperature. The resulting yellow crystals were recrystallized with some loss to give methyl bis-glyoxylacetate **9** in 34% yield (Scheme 2). ^{1}H NMR spectrum of bis-glyoxylacetate **9** revealed three singlets at δ 3.89, 3.90, and 10.91 corresponding to CH_3, CH_2, and two NH protons, respectively. The resonances of aromatic protons appeared as a singlet at δ 7.37 and two doublets at δ 7.35 and 8.66.

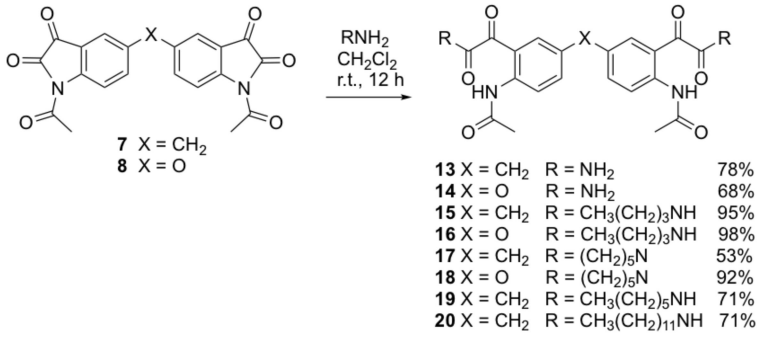


Scheme 2. Ring-opening reactions of bis-N-acetylisatins 7 and 8 with alcohols.

The analogous reaction with ethanol did not show the formation of any new products over 24 h, as confirmed by TLC analysis. Thus, the reaction mixture was heated at reflux for 3 h and during that time the solution turned dark yellow. A yellow solid precipitated upon cooling to room temperature and was recrystallized to give bis-glyoxylacetate **10** in 51% as yellow crystals. The analogous reactions of the bis-*N*-acetylisatin **8** afforded the bis-glyoxylacetates **11** and **12**.

2.3. Ring-Opening Reactions of bis-N-acetylisatins with Amines

The primary and secondary amines, butylamine and piperidine, were reacted with the bis-*N*-acetylisatins **7** and **8** by stirring the reaction mixtures for 5 h at room temperature. The bis-glyoxylamide products **15**–**18** were obtained in yields of 53–98% (Scheme 3). In the ^{1}H NMR spectrum of bis-glyoxylamide **15**, the CH_2 linkage was present as a singlet at δ 3.91, the butylamine carbon chain resonated as four multiplets at δ 0.86–0.88, 1.28–1.35, 1.47–1.52, and 3.28–3.32, the aromatic protons appeared as a doublet of doublets at δ 7.33 and two doublets at δ 8.07 and 8.50, and four NH protons resonated as two singlets at δ 6.81 and 10.79.

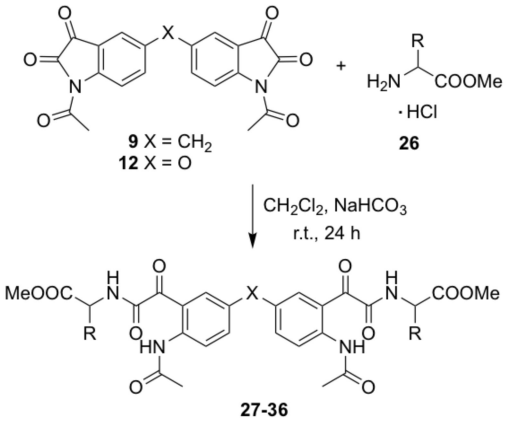


Scheme 3. Ring-opening reactions of bis-N-acetylisatins 7 and 8 with amines.

The ring-opening reactions of bis-*N*-acetylisatins **8** using primary amines with longer alkyl chains, such as hexylamine and dodecylamine were also investigated, but these reactions did not proceed at room temperature. However, by heating the reaction mixtures at reflux overnight the bis-glyoxylamide products **19** and **20** were obtained as yellow solids in moderate yields.

2.4. Ring-Opening Reactions of bis-*N*-acetylisatins with Amino Acid Methyl Esters

The reaction mixtures of bis-*N*-acetylisatins **7** and **8** with amino acid methyl ester hydrochloride salts and sodium hydrogen carbonate in dichloromethane or acetonitrile at room temperature overnight gave the corresponding bis-glyoxylamide peptide mimics **21**–**30** in yields of 26–80% as yellow solids (Scheme 4, Table 1). The ^{1}H NMR spectrum of compound **25** was typical for these peptide mimics and revealed two singlet peaks at δ 7.94 and 10.87, which integrated for 2H each and corresponded to the four NH groups. The valine subunit was identified by two multiplets and one doublet at δ 0.86–0.92, 2.16–2.21, and 4.54 which corresponded to the four methyl groups, the α-proton and the β-proton, respectively. The aromatic protons appeared as a multiplet at δ 7.31–7.41 and a doublet at δ 8.51.



Scheme 4. Ring-opening reactions of bis-N-acetylisatins 7 and 8 with amino acid methyl ester hydrochlorides.

Table 1. Bis-glyoxylamide peptidomimetics **21**–**32**.

No	Product	X	Amino Acid Methyl Esters	Yields (%)
1	**21**	CH_2	Gly OMe	55
2	**22**	CH_2	L-Leu OMe	57
3	**23**	CH_2	L-Met OMe	51
4	**24**	CH_2	L-Val OMe	80
5	**25**	CH_2	D-Phe OMe	28
6	**26**	CH_2	L-Phe OMe	48
7	**27**	O	Gly OMe	28
8	**28**	O	L-Leu OMe	26
9	**29**	O	L-Met OMe	76
10	**30**	O	L-Val OMe	46
11	**31**	O	D-Phe OMe	68
12	**32**	O	L-Phe OMe	78

The bis-glyoxylamide peptidomimetics were recrystallized from a range of solvents via slow evaporation of the solvent at room temperature. Crystals suitable for X-ray crystallography were successfully obtained for compounds **22**–**24** from methanol. Single crystal X-ray structure determination was carried out on compounds **22**–**24**.

Figure 1 shows the Oak Ridge Thermal Ellipsoid Plot (ORTEP) diagrams for the molecules **22** and for **23** and **24** (only one of the diastereoisomers similar to **22** is shown). In all the compounds, the bulky substituents, probably to avoid a steric clash, adopt *trans* positions with respect to each other. The side chains are orientationally disordered in **22** and **24**, while this is not observed in the structure of **23** that contains heavy sulfur atom. The core of the structure is well ordered. Strong intramolecular N-H···O hydrogen bonds (shown as dotted lines in Figure 1) maintain the planarity of each half of the

molecule. Weaker intramolecular C-H···O (C6-H···O1) contacts restrict the planarity of these moieties. However, the overall molecular framework is angled because of the central CH_2 link.

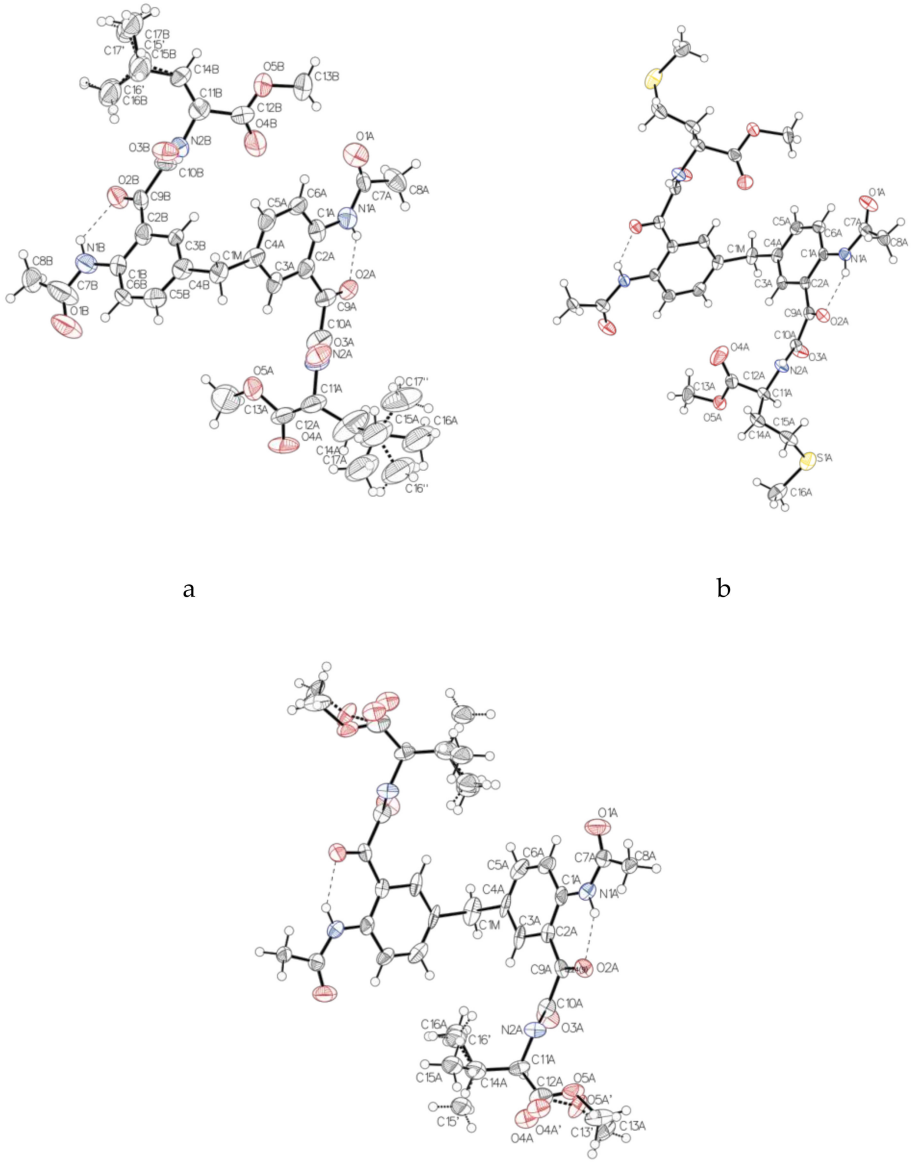


Figure 1. ORTEP diagrams of compound **22** (**a**), compound **23** (**b**), and compound **24** (**c**).

There is only one molecule in the asymmetric unit of 22 in orthorhombic space group P21212, whereas there are two molecules (diastereoisomers) in the asymmetric unit of 23 as well as in 24 in monoclinic space group C2 (Figure 2). The ring-opening reaction takes place with retention of configuration, as shown by optical rotation measurements. One point that establishes this issue unambiguously is the space group in which these compounds have crystallized. They are all chiral space groups (P2(1)2(1)2 and two in C2), which by the absence of mirror (or glide) symmetry, allow only one of the enantiomers.

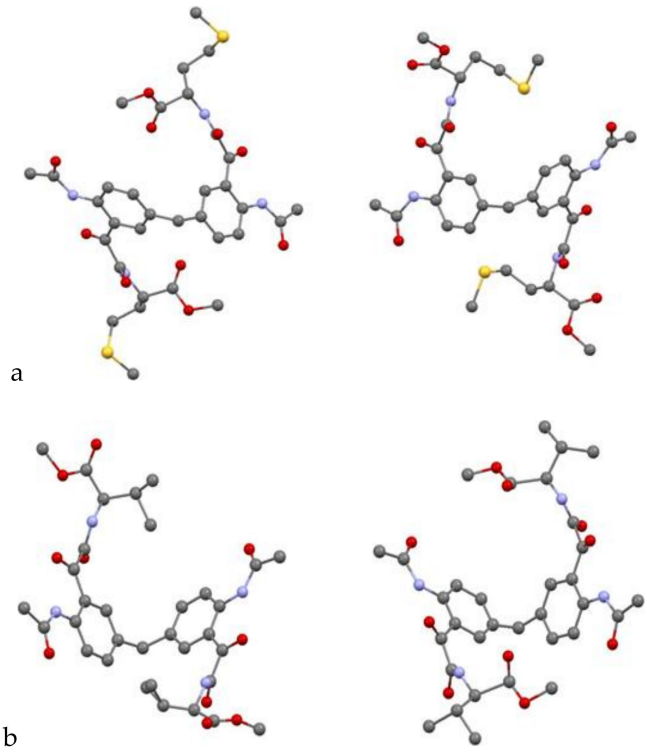


Figure 2. Two molecules in the asymmetric unit of **23** (**a**) and **24** (**b**).

3. Materials and Methods

Melting points were measured using a Reichert microscope (Gallenkamp hot stage apparatus; Mettler-Toledo Ltd, Sydney, Australia) and are uncorrected. Infrared spectra were recorded with a Thermo Nicolet 370 FTIR spectrometer with the sample prepared as a KBr pellet. NMR data were recorded using a Bruker DPX300 instrument (^{1}H 300 MHz, ^{13}C 75.6 MHz) (Bruker Pty Ltd, Preston, Victoria, Australia) at 25 °C and reported as chemical shift (δ) relative to $SiMe_4$. High resolution mass spectrometric analysis was carried out at the Biomedical Mass Spectrometry Facility, UNSW, and the spectra were recorded on Q-TOF Ultima API (Micromass; Waters, Rydalmere, NSW, Australia). Gravity column chromatography was carried out using Merck 230–400 mesh ASTM silica gel. Supplementary Materials contains NMR spectroscopic data.

N,N′-[4,4′-bis(4,1-phenylene)] bis [2-(hydroxyimino)acetamide]methylene (**3**). Concentrated sodium sulfate aqueous solution (175 mL) and a solution of 4,4′-methylenedianiline 1 (10 g, 50.4 mmol) in dioxane (15 mL) was added to a stirred solution of chloral hydrate (18.5 g, 126.1 mmol) in water (175 mL). The reaction mixture was heated slowly to 75 °C until a yellow precipitate was formed. A solution of hydroxylamine hydrochloride (8.8 g, 126.7 mmol) in water (55 mL) was then added into the resulting suspension. The temperature was increased to 75 °C and the reaction mixture was stirred for a further 2 h. The reaction mixture was cooled to room temperature and left to stand overnight. The crude product was collected by filtration and recrystallized from ethyl acetate to give the title compound as yellow crystals (9.44 g, 55%); mp 272–274 °C; IR (KBr): υ_{max} 3194, 2919, 2613, 1675, 1606, 1547, 1510, 1443, 1412, 1252, 1100, 1023, 999, 820, 765, 622, 510 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 12.13 (bs, 2H, 2 x OH) (disappears on D_2O exchange), 10.14 (bs, 2H, 2 x NH) (disappears on D_2O exchange), 7.65 (s, 2H, 2 x C<u>H</u>NOH), 7.59 (d, J = 8.5 Hz, 4H, ArH), 7.16 (d, J = 8.7 Hz, 4H, ArH), 3.85 (s, 2H, ArC<u>H</u>$_2$Ar); ^{13}C NMR (DMSO-d_6, 75.6 MHz): δ 160.4 (<u>C</u>O), 144.4 (<u>C</u>HNOH), 137.2 (ArC), 136.8 (ArC), 129.2 (ArC), 120.3 (ArC), 40.2 (Ar<u>C</u>H$_2$Ar); HRMS (+ESI) *m/z* [M + Na]$^+$ calcd for $C_{17}H_{16}N_4NaO_4$: 363.1069; found: 363.1055.

N,N′-[4,4′-bis(4,1-phenylene)]bis [2-(hydroxyimino)acetamide]oxide (**4**). This compound was prepared by the same method as compound **3** from 4,4′-oxydianiline 2 (10 g, 50.0 mmol) as orange needles (11.8 g, 69%); mp 221–223 °C; IR (KBr): υ_{max} 3185, 2931, 2622, 1669, 1596, 1521, 1510, 1433, 1410, 1232, 1096, 1021, 1000, 822, 764, 619 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 12.22 (bs, 2H, 2 x OH) (disappears on D_2O exchange), 10.22 (bs, 2H, 2 x NH) (disappears on D_2O exchange), 7.69 (d, *J* = 9.0 Hz, 4H, ArH), 7.67 (s, 2H, 2 x C<u>H</u>NOH), 6.99 (d, *J* = 9.0 Hz, 4H, ArH); ^{13}C NMR (DMSO-d_6, 75.6 MHz): δ 153.2 (ArC), 160.4 (<u>C</u>O), 144.4 (<u>C</u>HNOH), 137.2 (ArC), 121.9 (ArC), 119.1 (ArC); HRMS (+ESI) *m/z* $[M + Na]^+$ calcd for $C_{16}H_{14}N_4NaO_5$: 365.0862; found: 365.0851.

5,5′-Methylenediindoline-2,3-dione (**5**). Compound **3** (5 g, 14.7 mmol) in small portions at 60 °C was added to concentrated sulfuric acid (20 mL). The deep red reaction mixture was stirred at 60 °C for a further 30 min. Chilled water (100 mL) was added to quench the reaction. The title compound was collected by filtration as a red solid (3.96 g, 88%); mp > 300 °C; IR (KBr): υ_{max} 3450, 3109, 1622, 1472, 1271, 1200, 1141, 906, 833, 745, 731, 711, 660, 622 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 10.99 (bs, 2H, 2 x NH) (disappears on D_2O exchange), 7.51 (dd, *J* = 1.8, 8.1 Hz, 2H, ArH), 7.42 (s, 2H, ArH), 6.86 (d, *J* = 8.2 Hz, 2H, ArH), 3.89 (s, 2H, ArC<u>H</u>$_2$Ar); ^{13}C NMR (DMSO-d_6, 75.6 MHz): δ 184.8 (CO<u>C</u>ONH), 159.8 (<u>C</u>OCONH), 149.4 (ArC), 138.9 (ArC), 136.2 (ArC), 124.9 (ArC), 118.3 (ArC), 112.7 (ArC), 39.3 (Ar<u>C</u>H$_2$Ar); HRMS (+ESI) *m/z* $[M + Na]^+$ calcd for $C_{17}H_{10}N_2NaO_4$: 329.0538; found: 329.0541.

5,5′-Oxydiindoline-2,3-dione (**6**). This compound was prepared by the same method as compound 8 from compound 4 (5.0 g, 14.6 mmol) as a red solid (4.02 g, 83%); mp > 300 °C; IR (KBr): υ_{max} 3457, 3111, 1743, 1621, 1472, 1199, 1145, 907, 839, 744, 712, 660, 623 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 11.02 (bs, 2H, 2 x NH) (disappears on D_2O exchange), 7.30 (dd, *J* = 2.6, 2H, 8.5 Hz, ArH), 7.09 (s, 2H, ArH), 6.93 (d, *J* = 8.5 Hz, 2H, ArH); ^{13}C NMR (DMSO-d_6, 75.6 MHz): δ 184.4 (CO<u>C</u>ONH), 159.9 (<u>C</u>OCONH), 149.4 (ArC), 147.1 (ArC), 129.0 (ArC), 118.9 (<u>C</u>OCONH), 114.7 (ArC), 114.0 (ArC); HRMS (+ESI) *m/z* $[M + Na]^+$ calcd for $C_{16}H_8N_2NaO_4$: 331.0331; found: 331.0318.

Bis(1-acetylindoline-2,3-dione)methylene (**7**). A suspension of compound 5 (2 g, 6.5 mmol) in acetic anhydride (30 mL) was heated to reflux for 4 h. The deep red solution was cooled to room temperature. The excess acetic anhydride was removed under vacuum. The resulting brown oily residue was redissolved in ethyl acetate (20 mL) and washed with water (2 × 20 mL) and brine (2 × 20 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated under vacuum. The crude product was purified by flash column chromatography (dichloromethane) to yield the title compound as a bright yellow solid (1.63 g, 64%); mp 289–291 °C; IR (KBr): υ_{max} 1785, 1712, 1756, 1617, 1588, 1481, 1443, 1371, 1346, 1306, 1294, 1250, 1227, 1201, 1168, 1124, 1042, 996, 659, 598 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 8.22 (d, *J* = 8.5 Hz, 2H, ArH), 7.73 (dd, *J* = 1.9, 8.5 Hz, 2H, ArH), 7.69 (s, 2H, ArH), 4.11 (s, 2H, ArC<u>H</u>$_2$Ar), 2.59 (s, 6H, 2 x COC<u>H</u>$_3$); ^{13}C NMR ($CDCl_3$, 75.6 MHz): δ 180.5 (CO<u>C</u>ONH), 170.0 (<u>C</u>OCH$_3$), 158.7 (<u>C</u>OCONH), 146.8 (ArC), 138.3 (ArC), 124.5 (ArC), 124.5 (ArC), 120.5 (ArC), 117.8 (ArC), 39.2 (Ar<u>C</u>H$_2$Ar), 26.3 (CO<u>C</u>H$_3$); HRMS (+ESI) *m/z* $[M + Na]^+$ calcd for $C_{21}H_{14}N_2NaO_6$: 413.0750; found: 413.0713.

5,5′-Bis(1-acetylindoline-2,3-dione)oxide (**8**). This compound was prepared by the same method as compound **7** from compound **6** (2.0 g, 6.5 mmol) as a bright yellow solid (1.40 g, 55%); mp > 300 °C; IR (KBr): υ_{max} 3429, 1783, 1747, 1702, 1617, 1470, 1371, 1330, 1312, 1293, 1266, 1241, 1156, 850, 599, 468 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 8.31 (d, *J* = 9.2 Hz, 2H, ArH), 7.51 (dd, *J* = 2.9, 8.9 Hz, 2H, ArH), 7.31 (s, 2H, ArH), 2.57 (s, 6H, 2 x COC<u>H</u>$_3$); ^{13}C NMR ($CDCl_3$, 75.6 MHz): δ 179.9 (CO<u>C</u>ONH), 170.0 (<u>C</u>OCH$_3$), 158.6 (<u>C</u>OCONH), 154.1 (ArC), 144.4 (ArC), 128.3 (ArC), 121.8 (ArC), 119.7 (ArC), 113.9 (ArC), 26.2 (CO<u>C</u>H$_3$); HRMS (+ESI) *m/z* $[M + Na]^+$ calcd for $C_{20}H_{12}N_2NaO_7$: 415.0542; found: 415.0526.

Dimethyl 2,2′-[5,5′-bis(2-acetamido-5,1-phenylene)]bis(2-oxoacetate)methylene (**9**). A solution of compound **7** (0.15 g, 0.38 mmol) in anhydrous methanol (20 mL) was stirred at room temperature for 24 h. A massive yellow precipitate formed. The crude compound was collected by filtration and recrystallized from

n-hexane/DCM to afford the title compound as a yellow solid (0.059 g, 34%); mp 162–164 °C; IR (KBr): υ_{max} 3303, 1747, 1733, 1702, 1652, 1595, 1528, 1420, 1340, 1295, 1250, 1226, 1160, 1013 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 10.91 (bs, 2H, 2 x NH) (disappears on D_2O exchange), 8.66 (d, *J* = 8.7 Hz, 2H, ArH), 7.37 (s, 2H, ArH), 7.35 (d, *J* = 2.1 Hz, 2H, ArH), 3.90 (s, 2H, $ArC\underline{H}_2Ar$), 3.89 (s, 6H, 2 x $COOCH_3$), 2.18 (s, 6H, 2 x $COC\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 75.6 MHz): δ 190.4 ($\underline{C}OCH_3$), 169.8 ($\underline{C}OCOO$), 164.1 ($CO\underline{C}OO$), 141.7 (ArC), 138.0 (ArC), 134.5 (ArC), 133.6 (ArC), 121.6 (ArC), 117.6 (ArC), 53.4 ($COO\underline{C}H_3$), 40.2 ($Ar\underline{C}H_2Ar$), 25.9 ($CO\underline{C}H_3$); HRMS (+ESI) *m*/*z* $[M + Na]^+$ calcd for $C_{23}H_{22}N_2NaO_8$: 477.1274; found: 477.1243.

Diethyl 2,2′-[5,5′-bis(2-acetamido-5,1-phenylene)]bis(2-oxoacetate)methylene (**10**). A solution of compound **7** (0.15 g, 0.38 mmol) in absolute ethanol (20 mL) was heated to reflux for 12 h. The reaction mixture was allowed to cool to room temperature and a massive yellow precipitate formed. The crude compound was collected by filtration and recrystallized from *n*-hexane/DCM to afford the title compound as a yellow solid (0.095 g, 51%); mp 162–164 °C; IR (KBr): υ_{max} 3317, 1749, 1738, 1705, 1648, 1617, 1590, 1493, 1399, 1367, 1295, 1243, 1211, 1089, 1007 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 11.02 (bs, 2H, 2 x NH) (disappears on D_2O exchange), 8.73 (d, *J* = 9.0 Hz, 2H, ArH), 7.44 (s, 2H, ArH), 7.42 (dd, *J* = 2.2, 9.0 Hz, 2H, ArH), 4.41 (q, *J* = 7.2 Hz, 4H, 2 x $C\underline{H}_2CH_3$), 3.97 (s, 2H, $ArC\underline{H}_2Ar$), 2.24 (s, 6H, 2 x $COC\underline{H}_3$), 1.35 (t, *J* = 7.1 Hz, 6H, 2 x $CH_2C\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 75.6 MHz): δ 190.2 ($\underline{C}OCH_3$), 169.3 ($\underline{C}OCOO$), 163.3 ($CO\underline{C}OO$), 141.2 (ArC), 137.4 (ArC), 134.0 (ArC), 133.1 (ArC), 121.1 (ArC), 117.1 (ArC), 62.6 ($\underline{C}H_2CH_3$), 39.7 ($Ar\underline{C}H_2Ar$), 25.4 (OCH_3), 13.9 ($CH_2\underline{C}H_3$); HRMS (+ESI) *m*/*z* $[M + Na]^+$ calcd for $C_{25}H_{26}N_2NaO_8$: 505.1587; found: 505.1572.

Dimethyl 2,2′-[5,5′-bis(2-acetamido-5,1-phenylene)]bis(2-oxoacetate)oxide (**11**). Compound **11** was prepared by the same method as compound **9** from compound **8** (0.15 g, 0.38 mmol) as a white solid (0.129 g, 74%); mp 159–161 °C; IR (KBr): υ_{max} 3456, 1750, 1737, 1704, 1655, 1587, 1520, 1488, 1410, 1286, 1257, 1237, 1217, 1154, 1016, 970, 783 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 10.82 (bs, 2H, 2 x NH) (disappears on D_2O exchange), 8.72 (d, *J* = 9.1 Hz, 2H, ArH), 7.32 (d, *J* = 2.7 Hz, 2H, ArH), 7.22 (dd, *J* = 2.9, 9.2 Hz, 2H, ArH), 3.89 (s, 6H, 2 x $COOCH_3$), 2.19 (s, 6H, 2 x $COC\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 75.6 MHz): δ 189.6 ($\underline{C}OCH_3$), 169.7 ($\underline{C}OCOO$), 163.7 ($CO\underline{C}OO$), 151.3 (ArC), 139.2 (ArC), 127.7 (ArC), 123.2 (ArC), 122.9 (ArC), 118.6 (ArC), 53.6 ($COO\underline{C}H_3$), 25.9 ($CO\underline{C}H_3$); HRMS (+ESI) *m*/*z* $[M + H]^+$ calcd for $C_{22}H_{21}N_2O_9$: 457.1247; found: 457.1230.

Diethyl 2,2′-[5,5′-bis(2-acetamido-5,1-phenylene)]bis(2-oxoacetate)oxide (**12**). Compound **12** was prepared by the same method as compound **10** from compound **8** (0.15 g, 0.38 mmol) as a yellow solid (0.143 g, 77%); mp 104–106 °C; IR (KBr): υ_{max} 3307, 2965, 1745, 1655, 1589, 1520, 1437, 1411, 1372, 1267, 1217, 1282, 1160, 1011, 842 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 10.92 (bs, 2H, 2 x NH) (disappears on D_2O exchange), 8.79 (d, *J* = 9.3 Hz, 2H, ArH), 7.37 (d, *J* = 2.7 Hz, 2H, ArH), 7.35 (dd, *J* = 2.9, 9.3 Hz, 2H, ArH), 4.41 (q, *J* = 7.2 Hz, 4H, 2 x $C\underline{H}_2CH_3$), 2.25 (s, 6H, 2 x $COC\underline{H}_3$), 1.36 (t, *J* = 7.1 Hz, 6H, 2 x $CH_2C\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 75.6 MHz): δ 189.6 ($\underline{C}OCH_3$), 169.3 ($\underline{C}OCOO$), 162.9 ($CO\underline{C}OO$), 151.0 (ArC), 138.8 (ArC), 127.3 (ArC), 122.8 (ArC), 122.4 (ArC), 118.2 (ArC), 62.9 ($\underline{C}H_2CH_3$), 25.4 (OCH_3), 14.0 ($CH_2\underline{C}H_3$); HRMS (+ESI) *m*/*z* $[M + Na]^+$ calcd for $C_{24}H_{24}N_2NaO_9$: 507.1380; found: 507.1360.

N,N′-{4,4′-bis-[2-(2-acetamidophenyl)-2-oxoacetamide]}methylene (**13**). To a solution of compound **7** (0.15 g, 0.38 mmol) in dichloromethane (20 mL) was added concentrated ammonia solution (10 mL). The reaction mixture was stirred at room temperature for 30 min. A massive white precipitate formed. The crude product was collected by filtration and purified by recrystallization from methanol to yield the title compound **13** as a white solid (0.127 g, 78%); mp 229–232 °C; IR (KBr): υ_{max} 3310, 2933, 1692, 1641, 1588, 1522, 1466, 1411, 1359, 1310, 1279, 1201, 1177, 1132, 987, 900, 826, 735, 591 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 10.50 (bs, 2H, 2 x NH) (disappears on D_2O exchange), 7.79 (s, 4H, 2 x NH_2) (disappears on D_2O exchange), 8.01 (s, 2H, ArH), 7.50 (d, *J* = 1.9 Hz, 2H, ArH), 7.44 (dd, *J* = 2.1, 8.5 Hz, 2H, ArH), 3.99 (s, 2H, $ArC\underline{H}_2Ar$), 2.04 (s, 6H, 2 x $COC\underline{H}_3$); ^{13}C NMR (DMSO-d_6, 75.6 MHz): δ 169.2 ($\underline{C}OCONH_2$), 192.6 ($\underline{C}OCH_3$), 166.3 ($CO\underline{C}ONH_2$), 137.4 (ArC), 136.4 (ArC), 134.8 (ArC), 131.8

(ArC), 124.3 (ArC), 122.3 (ArC), 40.0 (Ar$\underline{C}H_2$Ar), 24.5 (OCH_3); HRMS (+ESI) *m/z* $[M + Na]^+$ calcd for $C_{21}H_{20}N_4NaO_6$: 447.1281; found: 447.1252.

N,N′-{4,4′-bis-[2-(2-acetamidophenyl)-2-oxoacetamide]}oxide (**14**). Compound **14** was prepared by the same method as compound **13** from compound **8** (0.15 g, 0.38 mmol) as a white solid (0.108 g, 66%); mp 183–186 °C; IR (KBr): υ_{max} 3309, 2940, 2859, 1701, 1639, 1588, 1521, 1463, 1416, 1368, 1326, 1285, 1237, 1215, 1181, 1165, 1142, 991, 942, 842, 753, 637 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 10.36 (bs, 2H, 2 x NH) (disappears on D_2O exchange), 8.00 (s, 2H, ArH), 7.74 (s, 4H, 2 x NH_2) (disappears on D_2O exchange), 7.50 (d, *J* = 1.7 Hz, 2H, ArH), 7.12 (dd, *J* = 1.7, 8.0 Hz, 2H, ArH), 2.14 (s, 6H, 2 x $COC\underline{H}_3$); ^{13}C NMR (DMSO-d_6, 75.6 MHz): δ 193.4 ($\underline{C}OCH_3$), 169.5 ($\underline{C}OCONH_2$), 166.2 ($CO\underline{C}ONH_2$), 137.1 (ArC), 136.3 (ArC), 130.9 (ArC), 128.9 (ArC), 120.5 (ArC), 113.2 (ArC), 26.1 (OCH_3); HRMS (+ESI) *m/z* $[M + Na]^+$ calcd for $C_{20}H_{18}N_4NaO_7$: 449.1073; found: 447.1059.

N,N′-{4,4′-bis-[2-(2-acetamidophenyl)-N-butyl-2-oxoacetamide]}methylene (**15**). A solution of n-butylamine (0.20 mL, 2.0 mmol) in anhydrous dichloromethane (20 mL)was added to a stirred solution of compound **7** (0.15 g, 0.38 mmol) in anhydrous dichloromethane (30 mL). The reaction mixture was stirred at room temperature for 12 h. The organic layer was diluted with dichloromethane (30 mL) and extracted in aqueous hydrochloric acid (0.5 M, 2 × 40 mL) and water (3 x 40 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated under vacuum. The crude compound was purified by gravity column chromatography (dichloromethane-ethyl acetate/3:1) to afford the title compound **15** as a yellow solid (0.196 g, 95%); mp 144–148 °C; IR (KBr): υ_{max} 3309, 2960, 1681, 1650, 1582, 1507, 1402, 1371, 1320, 1288, 1253, 1222, 1180, 1134, 1006, 833, 700 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 10.79 (bs, 2H, 2 x NH) (disappears on D_2O exchange), 8.50 (d, *J* = 8.7 Hz, 2H, ArH), 8.07 (d, *J* = 2.0 Hz, 2H, ArH), 7.33 (dd, *J* = 2.0, 8.6 Hz, 2H, ArH), 6.81 (bs, 2H, 2 x NH butylamine) (disappears on D_2O exchange), 3.91 (s, 2H, $ArCH_2Ar$), 3.31 (dd, *J* = 6.8, 13.2 Hz, 4H, 2 x $C\underline{H}_2CH_2$), 2.14 (s, 6H, 2 x $COC\underline{H}_3$), 1.47–1.52 (m, 4H, 2 x $CH_2C\underline{H}_2CH_2CH_3$), 1.28–1.35 (m, 4H, 2 x $C\underline{H}_2CH_3$), 0.87 (t, *J* = 7.6 Hz, 6H, 2 x CH_3); ^{13}C NMR ($CDCl_3$, 75.6 MHz): δ 192.4 ($\underline{C}OCH_3$), 169.5 ($\underline{C}OCONH$), 163.2 ($CO\underline{C}ONH$), 140.9 (ArC), 137.4 (ArC), 134.9 (ArC), 134.7 (ArC), 121.6 (ArC), 119.4 (ArC), 40.4 (Ar$\underline{C}H_2$Ar), 39.8 ($\underline{C}H_2CH_2CH_2$), 31.7 ($CH_2\underline{C}H_2CH_2$), 25.8 (OCH_3), 20.4 ($CH_2\underline{C}H_2CH_3$), 14.1 (CH_3); HRMS (+ESI) *m/z* $[M + Na]^+$ calcd for $C_{29}H_{36}N_4NaO_6$: 559.2533; found: 559.2513.

N,N′-{4,4′-bis-[2-(2-acetamidophenyl)-N-butyl-2-oxoacetamide]}oxide (**16**). Compound **16** was prepared by the same method as compound **15** from compound **8** (0.15 g, 0.38 mmol) and *n*-butylamine (0.20 mL, 2.0 mmol) as a yellow solid (0.202 g, 98%); mp 188–191 °C; IR (KBr): υ_{max} 3311, 2960, 1686, 1656, 1589, 1513, 1406, 1370, 1324, 1286, 1264, 1222, 1181, 1162, 1008, 847, 699 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 10.75 (bs, 2H, 2 x NH) (disappears on D_2O exchange), 7.99 (d, *J* = 3.0 Hz, 2H, ArH), 8.58 (d, *J* = 9.3 Hz, 2H, ArH), 7.25 (dd, *J* = 3.4, 9.1 Hz, 2H, ArH), 6.97 (bs, 2H, 2 x NH butylamine) (disappears on D_2O exchange), 3.34 (dd, *J* = 6.9, 13.2 Hz, 4H, 2 x $C\underline{H}_2CH_2$), 2.21 (s, 6H, 2 x $COC\underline{H}_3$), 1.51–1.56 (m, 4H, 2 x $CH_2C\underline{H}_2CH_2CH_3$), 1.32–1.39 (m, 4H, 2 x $C\underline{H}_2CH_3$), 0.91 (t, *J* = 7.2 Hz, 6H, 2 x CH_3); ^{13}C NMR ($CDCl_3$, 75.6 MHz): δ 191.3 ($\underline{C}OCH_3$), 169.0 ($\underline{C}OCONH$), 162.4 ($CO\underline{C}ONH$), 151.2 (ArC), 137.7 (ArC), 126.8 (ArC), 123.4 (ArC), 122.7 (ArC), 120.2 (ArC), 39.4 ($\underline{C}H_2CH_2CH_2$), 31.2 ($CH_2\underline{C}H_2CH_2$), 25.3 (OCH_3), 20.0 ($CH_2\underline{C}H_2CH_3$), 13.7 (CH_3); HRMS (+ESI) *m/z* $[M + Na]^+$ calcd for $C_{28}H_{34}N_4NaO_7$: 561.2325; found: 561.2300.

N,N′-[4,4′-bis-(N-{2-[2-oxo-2-(piperidin-1-yl)acetyl]phenyl}acetamide)]methylene (**17**). Compound **17** was prepared by the same method as compound **15** from compound **7** (0.15 g, 0.38 mmol) and piperidine (0.20 mL, 2.0 mmol) as a yellow solid (0.114 g, 53%); mp 181–183 °C; IR (KBr): υ_{max} 2939, 2859, 1701, 1642, 1591, 1520, 1451, 1412, 1367, 1326, 1298, 1251, 1182, 1136, 988, 851, 784, 752 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ11.15 (bs, 2H, 2 x NH) (disappears on D_2O exchange), 8.66 (d, *J* = 8.8 Hz, 2H, ArH), 7.35 (dd, *J* = 1.8, 8.7 Hz, 2H, ArH), 7.28 (d, *J* = 1.8 Hz, 2H, ArH), 3.89 (s, 2H, Ar$C\underline{H}_2$Ar), 3.55 (t, *J* = 5.5 Hz, 4H, 2 x $NC\underline{H}_2$), 3.13 (t, *J* = 5.5 Hz, 4H, 2 x $NC\underline{H}_2$), 2.18 (s, 6H, 2 x $COC\underline{H}_3$), 1.51–1.57 (m, 8H, 2 x $CH_2C\underline{H}_2CH_2C\underline{H}_2CH_2$), 1.29–1.38 (m, 4H, 2 x $CH_2C\underline{H}_2CH_2$); ^{13}C NMR ($CDCl_3$, 75.6 MHz): δ 196.4

($\underline{C}OCH_3$), 169.8 ($\underline{C}OCON$), 164.5 ($CO\underline{C}ON$), 141.3 (ArC), 137.6 (ArC), 135.0 (ArC), 133.6 (ArC), 121.4 (ArC), 118.5 (ArC), 47.5 ($N\underline{C}H_2$), 42.5 ($N\underline{C}H_2$), 40.1 ($Ar\underline{C}H_2Ar$), 25.7 ($NCH_2\underline{C}H_2$), 25.9 (OCH_3), 25.7 ($NCH_2\underline{C}H_2$), 24.6 ($CH_2\underline{C}H_2CH_2$); HRMS (+ESI) *m/z* $[M + Na]^+$ calcd for $C_{31}H_{36}N_4NaO_6$: 583.2533; found: 583.2493.

N,N′-[4,4′-bis-(N-{2-[2-oxo-2-(piperidin-1-yl)acetyl]phenyl}acetamide)]oxide (**18**). Compound **18** was prepared by the same method as compound **15** from compound **8** (0.15 g, 0.38 mmol) and piperidine (0.20 mL, 2.0 mmol) as a yellow solid (0.198 g, 92%); mp 158–160 °C; IR (KBr): υ_{max} 3444, 3288, 1682, 1557, 1489, 1415, 1371, 1335, 1303, 1289, 1253, 1126, 929, 839, 669, 617, 559 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 11.08 (bs, 2H, 2 x NH) (disappears on D_2O exchange), 8.75 (d, *J* = 8.8 Hz, 2H, ArH), 7.23 (dd, *J* = 2.8, 9.1 Hz, 2H, ArH), 7.20 (d, *J* = 1.9 Hz, 2H, ArH), 3.58 (t, *J* = 5.3 Hz, 4H, 2 x $NC\underline{H}_2$), 3.23 (t, *J* = 5.3 Hz, 4H, 2 x $NC\underline{H}_2$), 2.22 (s, 6H, 2 x $COC\underline{H}_3$), 1.63–1.65 (m, 4H, 2 x $CH_2C\underline{H}_2CH_2$), 1.51–1.57 (m, 8H, 2 x $CH_2C\underline{H}_2CH_2C\underline{H}_2CH_2$); ^{13}C NMR ($CDCl_3$, 75.6 MHz): δ 195.2 ($\underline{C}OCH_3$), 169.3 ($\underline{C}OCON$), 163.8 ($CO\underline{C}ON$), 151.3 (ArC), 138.4 (ArC), 127.0 (ArC), 122.6 (ArC), 122.4 (ArC), 119.1 (ArC), 47.1 ($N\underline{C}H_2$), 42.2 ($N\underline{C}H_2$), 26.1 ($NCH_2\underline{C}H_2$), 25.4 (OCH_3), 25.3 ($NCH_2\underline{C}H_2$), 24.2 ($CH_2\underline{C}H_2CH_2$); HRMS (+ESI) *m/z* $[M + Na]^+$ calcd for $C_{30}H_{34}N_4NaO_7$: 585.2325; found: 585.2293.

2,2′-[Methylene-bis(2-acetamido-5,1-phenylene)]bis(N-hexyl-2-oxoacetamide) (**19**). A mixture of compound **7** (0.24 g, 0.61 mmol) and hexylamine (0.12 g, 1.22 mmol) in dichloromethane (30 mL) was heated at reflux overnight. After cooling to room temperature, the solvent was evaporated in vacuo and the crude product was purified by column chromatography using silica gel and *n*-hexane/DCM as eluent. The title compound **19** was obtained as an off-white solid (0.26 g, 71%); mp 182–184 °C; IR (KBr): υ_{max} 3305, 3053, 2928, 2855, 1694, 1650, 1585, 1518, 1465, 1411, 1369, 1293, 1223, 1176, 1006, 855, 811 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 10.89 (s, 2H, 2 x NHCO), 8.56 (d, *J* = 8.6 Hz, 2H, ArH), 8.16 (d, *J* = 2.0 Hz, 2H, ArH), 7.42 (dd, *J* = 8.7, 1.9 Hz, 2H, ArH), 6.98 (t, *J* = 5.5 Hz, 2H, 2 x CONH), 4.00 (s, 2H, $ArCH_2Ar$), 3.47-3.49 (q, *J* = 6.7, 6.3 Hz, 4H, 2 x $NHC\underline{H}_2CH_2(CH_2)_3CH_3$), 2.25 (s, 6H, 2 x $COCH_3$), 1.55–1.66 (m, 4H, 2 x $NHCH_2C\underline{H}_2(CH_2)_3CH_3$), 1.30–1.44 (m, 12H, 2 x $NHCH_2CH_2(C\underline{H}_2)_3CH_3$), 0.93 (t, *J* = 6.6 Hz, 6H, 2 x $NHCH_2(CH_2)_4C\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 75.6 MHz): δ 162.8, 169.1, 192.0 (6 x C=O), 121.2, 134.5, 136.9 (6 x ArCH), 119.0, 134.3, 140.5 (6 x ArC), 40.0 (2 x $NHCH_2$), 39.7 ($Ar\underline{C}H_2Ar$), 25.4 (2 x $CO\underline{C}H_3$), 31.4 (2 x $CH_2(\underline{C}H_2)_4CH_3$), 22.5, 26.6, 29.2, 14.0 (2 x $CH_2(CH_2)_4\underline{C}H_3$); HRMS (ESI) *m/z* $[M + H]^+$ calcd for $C_{33}H_{45}N_4O_6$: 593.3333; found: 593.3326.

2,2′-[Methylene-bis(2-acetamido-5,1-phenylene)]bis(N-dodecyl-2-oxoacetamide) (**20**). Compound **20** was prepared by the same method as compound **19** from compound **7** (0.24 g, 0.61 mmol) and dodecylamine (0.23 g, 1.22 mmol) as an off-white solid (0.26 g, 69%); mp 162–164 °C; IR (KBr): υ_{max} 3344, 3293, 2919, 2850, 1694, 1650, 1586, 1519, 1467, 1412, 1370, 1294, 1224, 1177, 1014, 855, 721 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 10.89 (s, 2H, 2 x NHCO), 8.54 (d, *J* = 8.5 Hz, 2H, ArH), 8.17 (d, *J* = 2.1 Hz, 2H, ArH), 7.45 (dd, *J* = 8.6, 2.12 Hz, 2H, ArH), 6.98 (t, *J* = 5.9 Hz, 2H, 2 x CONH), 4.03 (s, 2H, $ArCH_2Ar$), 3.37–3.44 (q, *J* = 6.7, 6.8 Hz, 4H, 2 x $NHC\underline{H}_2CH_2(CH_2)_9CH_3$), 2.28 (s, 6H, 2 x $COCH_3$), 1.56-1.66 (m, 4H, 2 x $NHCH_2C\underline{H}_2(CH_2)_9CH_3$), 1.26–1.40 (m, 36H, 2 x $CH_2CH_2(C\underline{H}_2)_9CH_3$), 0.93 (t, *J* = 7.0 Hz, 6H, 2 x $CH_2(CH_2)_9C\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 75.6 MHz): δ 162.8, 169.9, 191.7 (6 x C = O), 121.6, 134.9, 136.9 (6 x ArCH), 120.6, 134.2, 139.9 (6 x ArC), 40.0 (2 x $NHCH_2$), 39.6 ($Ar\underline{C}H_2Ar$), 25.2 (2 x $CO\underline{C}H_3$), 22.7, 26.9, 29.2, 29.2, 29.4, 29.5, 29.6, 29.6, 31.3 (2 x $CH_2(\underline{C}H_2)_{10}CH_3$), 14.1 (2 x $CH_2(CH_2)_{10}\underline{C}H_3$); HRMS (ESI) *m/z* $[M + Na]^+$calcd for $C_{45}H_{68}N_4NaO_6$: 761.5211; found: 761.5207.

N,N′-(4,4′-bis-{methyl-2-[2-(2-acetamidophenyl)-2-oxoacetamido]acetate}) methane (**21**). A mixture of the glycine methyl ester hydrochloride (0.24 g, 1.9 mmol) and saturated sodium hydrogen carbonate solution (3 mL) in water (7 mL) was added to a stirred solution of compound **7** (0.15 g, 0.38 mmol)) in dichloromethane (20 mL) was. The reaction mixture was stirred at room temperature for 24 h. The organic layer was diluted with dichloromethane (20 mL) and extracted in aqueous hydrochloric acid (0.5 M, 30 mL) and water (2 × 30 mL). The combined organic extracts were dried over anhydrous sodium sulfate and concentrated under vacuum. The crude compound was purified by gravity

column chromatography (dichloromethane-ethyl acetate = 4:1) to afford the title compound as a yellow solid (0.120 g, 55%); mp 204–206 °C; IR (KBr): υ_{max} 3279, 1750, 1698, 1666, 1651, 1592, 1519, 1412, 1367, 1326, 1293, 1234, 1217, 1177, 1005, 688 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 10.49 (bs, 2H, 2 x NH) (disappears on D_2O exchange), 9.07 (d, *J* = 11.2 Hz, 2H, ArH), 7.74 (s, 2H, ArH), 7.60 (d, *J* = 1.5 Hz, 2H, 2 x α-NH Gly) (disappears on D_2O exchange), 7.49 (dd, *J* = 2.0, 8.5 Hz, 2H, ArH), 3.97 (d, *J* = 5.7 Hz, 4H, 2 x $NHCH_2COOCH_3$), 3.91 (s, 2H, $ArCH_2Ar$), 3.67 (s, 6H, 2 x $COOCH_3$), 2.05 (s, 6H, 2 x $COCH_3$); ^{13}C NMR ($CDCl_3$, 75.6 MHz): δ 189.8 ($COCH_3$), 169.5 (COCONH), 168.9 (COCONH), 163.2 ($COOCH_3$), 149.8 (ArC), 138.6 (ArC), 127.1 (ArC), 123.5 (ArC), 122.6 (ArC), 119.6 (ArC), 53.1 ($COOCH_3$), 40.9 ($NHCH_2COOCH_3$), 38.9 ($ArCH_2Ar$), 24.9 (OCH_3); HRMS (+ESI) *m*/*z* $[M + Na]^+$ calcd for $C_{27}H_{28}N_4NaO_{10}$: 591.1703; found: 591.1692.

N,N′-(4,4′-bis-{methyl-2-[2-(2-acetamidophenyl)-2-oxoacetamido]-4-methylpentanoate})methane (**22**). Compound **22** was prepared by the same method as compound **21** from compound **7** (0.15 g, 0.38 mmol) and L-leucine methyl ester hydrochloride (0.30 g, 1.9 mmol) as a yellow solid (0.149 g, 57%); mp 184–186 °C; IR (KBr): υ_{max} 3328, 2958, 1747, 1648, 1592, 1522, 1438, 1415, 1370, 1298, 1253, 1231, 1176 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 10.84 (bs, 2H, 2 x NH) (disappears on D_2O exchange), 8.53 (d, *J* = 8.8 Hz, 2H, ArH), 8.01 (d, *J* = 1.9 Hz, 2H, 2 x α-NH Leu) (disappears on D_2O exchange), 7.35 (dd, *J* = 2.1, 8.7 Hz, 2H, ArH), 7.25 (d, *J* = 8.4 Hz, 2H, ArH), 4.57–4.64 (m, 2H, 2 x $NHCHCOOCH_3$), 3.91 (s, 2H, $ArCH_2Ar$), 3.66 (s, 6H, 2 x $COOCH_3$), 2.14 (s, 6H, 2 x $COCH_3$), 1.56-1.69 (m, 4H, 2 x $CH_2CH(CH_3)_2$), 1.18–1.20 (m, 2H, 2 x $CH(CH_3)_2$), 0.89 (dd, *J* = 2.6, 5.9 Hz, 12H, 2 x $CH(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 75.6 MHz): δ 191.9 ($COCH_3$), 172.9 (COCONH), 169.5 (COCONH), 163.3 ($COOCH_3$), 141.1 (ArC), 137.7 (ArC), 134.8 (ArC), 134.6 (ArC), 121.4 (ArC), 119.0 (ArC), 52.9 ($COOCH_3$), 51.3 ($NHCHCOOCH_3$), 41.7 ($CH_2CH(CH_3)_2$), 40.3 ($ArCH_2Ar$), 25.8 (OCH_3), 25.3 ($CH(CH_3)_2$), 23.0 (CH_3), 22.2 (CH_3); HRMS (+ESI) *m*/*z* $[M + Na]^+$ calcd for $C_{35}H_{44}N_4NaO_{10}$: 703.2955; found: 703.2948.

N,N′-(4,4′-bis-{methyl-2-[2-(2-acetamidophenyl)-2-oxoacetamido]-4-(methylthio)butanoate})methane (**23**). Compound **23** was prepared by the same method as compound **21** from compound **7** (0.15 g, 0.38 mmol) and L-methionine methyl ester hydrochloride (0.38 g, 1.9 mmol) as a yellow solid (0.140 g, 51%); mp 172–174 °C; IR (KBr): υ_{max} 3292, 1746, 1695, 1663, 1642, 1589, 1523, 1433, 1412, 1294, 1250, 1219, 1173 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 10.80 (bs, 2H, 2 x NH) (disappears on D_2O exchange), 8.52 (d, *J* = 8.5 Hz, 2H, ArH), 8.01 (d, *J* = 1.9 Hz, 2H, 2 x α-NH Met) (disappears on D_2O exchange), 7.49 (d, *J* = 8.1Hz, 2H, ArH), 7.35 (dd, *J* = 2.0, 8.7 Hz, 2H, ArH), 4.68–4.77 (m, 2H, 2 x $NHCHCOOCH_3$), 3.91 (s, 2H, $ArCH_2Ar$), 3.70 (s, 6H, 2 x $COOCH_3$), 2.47 (t, *J* = 7.2 Hz, 4H, 2 x CH_2CH_2S), 2.15 (s, 6H, 2 x $COCH_3$), 2.03 (s, 6H, 2 x SCH_3), 1.05 (t, *J* = 7.2 Hz, 4H, 2 x CH_2CH_2S); ^{13}C NMR ($CDCl_3$, 75.6 MHz): δ 191.6 ($COCH_3$), 171.9 (COCONH), 169.6 (COCONH), 163.3 ($COOCH_3$), 141.1 (ArC), 137.7 (ArC), 134.8 (ArC), 134.5 (ArC), 121.5 (ArC), 118.9 (ArC), 53.2 ($COOCH_3$), 52.0 ($NHCHCOOCH_3$), 40.3 ($ArCH_2Ar$), 31.6 (CH_2CH_2S), 30.3 (CH_2CH_2S), 25.8 (OCH_3), 15.9 (SCH_3); HRMS (+ESI) *m*/*z* $[M + Na]^+$ calcd for $C_{33}H_{40}N_4O_{10}NaS_2$: 739.2084; found: 739.2071.

N,N′-(4,4′-bis-{methyl-2-[2-(2-acetamidophenyl)-2-oxoacetamido]-3-methylbutanoate})methane (**24**). Compound **24** was prepared by the same method as compound **21** from compound **7** (0.15 g, 0.38 mmol) and L-valine methyl ester hydrochloride (0.32 g, 1.9 mmol) as a yellow solid (0.201 g, 80%); mp 204–207 °C; IR (KBr): υ_{max} 3251, 2962, 1740, 1696, 1666, 1644, 1588, 1521, 1469, 1438, 1413, 1369, 1295, 1245, 1225, 1211, 1176, 1141 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 10.87 (bs, 2H, 2 x NH) (disappears on D_2O exchange), 8.51 (d, *J* = 8.6 Hz, 2H, ArH), 7.93 (d, *J* = 1.9 Hz, 2H, 2 x α-NH Val) (disappears on D_2O exchange), 7.39 (d, *J* = 8.8 Hz, 2H, ArH′), 7.32 (dd, *J* = 2.0, 8.8 Hz, 2H, ArH), 4.54 (dd, *J* = 4.9, 9.0 Hz, 2H, 2 x $NHCHCOOCH_3$), 3.89 (s, 2H, $ArCH_2Ar$), 3.67 (s, 6H, 2 x $COOCH_3$), 2.18–2.22 (m, 2H, 2 x $CH(CH_3)_2$), 2.14 (s, 6H, 2 x $COCH_3$), 0.89 (dd, *J* = 6.9, 12.7 Hz, 12H, 2 x $CH(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 75.6 MHz): δ 192.3 ($COCH_3$), 172.1 (COCONH), 169.6 (COCONH), 163.7 ($COOCH_3$), 141.1 (ArC), 137.7 (ArC), 134.7 (ArC), 134.5 (ArC), 121.4 (ArC), 118.9 (ArC), 57.6 ($NHCHCOOCH_3$), 52.8 ($COOCH_3$), 40.3 ($ArCH_2Ar$), 31.8 ($CH(CH_3)_2$), 25.8 (OCH_3), 19.4 (2 x CH_3), 18.0 (CH_3); HRMS (+ESI) *m*/*z* $[M + Na]^+$ calcd for $C_{33}H_{40}N_4NaO_{10}$: 675.2642; found: 675.2616.

N,N′-(4,4′-bis-{methyl-2-[2-(2-acetamidophenyl)-2-oxoacetamido]-3-phenylpropanoate})methane (**25**). Compound **25** was prepared by the same method as compound **21** from compound **7** (0.15 g, 0.38 mmol) and D-phenylalanine methyl ester hydrochloride (0.41 g, 1.9 mmol) as a yellow solid (0.081 g, 28%); mp 200–202 °C; $[\alpha]_D^{22}$ (c 0.1 in MeOH); IR (KBr): υ_{max} 3294, 1749, 1693, 1644, 1590, 1521, 1412, 1293, 1268, 1217, 1173, 701 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 10.79 (bs, 2H, 2 x NH) (disappears on D_2O exchange), 8.50 (d, *J* = 9.0 Hz, 2H, ArH), 7.88 (s, 2H, 2 x α-NH Phe) (disappears on D_2O exchange), 7.16–7.29 (d, 12H, ArH, ArH), 7.05 (d, *J* = 6.4 Hz, 2H, ArH), 4.86 (dd, *J* = 6.6, 14.8 Hz, 2H, 2 x $NHC\underline{H}COOCH_3$), 3.84 (s, 2H, $ArC\underline{H}_2Ar$), 3.66 (s, 6H, 2 x $COOC\underline{H}_3$), 2.99-3.17 (m, 4H, 2 x $C\underline{H}_2Ph$), 2.11 (s, 6H, 2 x $COC\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 75.6 MHz): δ 191.7 ($\underline{C}OCH_3$), 171.5 ($CO\underline{C}ONH$), 169.6 ($CO\underline{C}ONH$), 163.0 ($\underline{C}OOCH_3$), 141.1 (ArC), 137.6 (ArC), 135.7 (ArC), 134.7 (ArC), 134.5 (ArC), 129.6 (ArC), 129.1 (ArC), 127.8 (ArC), 121.4 (ArC), 118.8 (ArC), 53.7 ($NH\underline{C}HCOOCH_3$), 53.0 ($COO\underline{C}H_3$), 40.3 ($Ar\underline{C}H_2Ar$), 38.3 ($\underline{C}H_2Ph$), 25.8 (OCH_3); HRMS (+ESI) *m/z* $[M + Na]^+$ calcd for $C_{41}H_{40}N_4NaO_{10}$: 771.2642; found: 771.2610.

N,N′-(4,4′-bis-{methyl-2-[2-(2-acetamidophenyl)-2-oxoacetamido]-3-phenylpropanoate})methane (**26**). Compound **26** was prepared from compound **7** (1.00 g, 2.56 mmol) and L-phenylalanine methyl ester hydrochloride (1.21 g, 5.64 mmol) in the presence of Et_3N (1.04g, 10.2 mmol) as a yellow solid (0.78 mg, 48%); mp 201–202 °C; $[\alpha]_D^{23}$ (c 0.1 in MeOH)**;** IR υ_{max} 3291, 1742, 1690, 1642, 1594, 1523, 1418, 1289, 1272, 1212, 1171, 705 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ 10.80 (bs, 2H, 2 x NH) (disappears on D_2O exchange), 8.51 (d, *J* = 9.0 Hz, 2H, ArH), 7.89 (s, 2H, 2 x α-NH Phe) (disappears on D_2O exchange), 7.14-7.30 (d, 12H, ArH, ArH), 7.06 (d, *J* = 6.4 Hz, 2H, ArH), 4.88 (dd, *J* = 6.6, 14.8 Hz, 2H, 2 x $NHC\underline{H}COOCH_3$), 3.85 (s, 2H, $ArC\underline{H}_2Ar$), 3.67 (s, 6H, 2 x $COOC\underline{H}_3$), 2.98–3.18 (m, 4H, 2 x $C\underline{H}_2Ph$), 2.12 (s, 6H, 2 x $COC\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 101 MHz): δ 191.8 ($\underline{C}OCH_3$), 172.1 ($CO\underline{C}ONH$), 169.6 ($CO\underline{C}ONH$), 163.0 ($\underline{C}OOCH_3$), 141.1 (ArC), 137.6 (ArC), 135.7 (ArC), 134.7 (ArC), 135.6 (ArC), 129.9 (ArC), 129.2 (ArC), 128.1 (ArC), 121.2 (ArC), 119.2 (ArC), 54.1 ($NH\underline{C}HCOOCH_3$), 53.1 ($COO\underline{C}H_3$), 40.2 ($Ar\underline{C}H_2Ar$), 38.4 ($\underline{C}H_2Ph$), 25.9 (OCH_3); HRMS (+ESI) *m/z* $[M + Na]^+$ calcd for $C_{41}H_{40}N_4NaO_{10}$: 771.2642; found: 771.2612.

N,N′-(4,4′-bis-{methyl-2-[2-(2-acetamidophenyl)-2-oxoacetamido]acetate})oxide (**27**). Compound **27** was prepared by the same method as compound **21** from compound **8** (0.15 g, 0.38 mmol) and glycine methyl ester hydrochloride (0.24 g, 1.9 mmol) as a yellow solid (0.061 g, 28%); mp 180–182 °C; IR (KBr): υ_{max} 3354, 3293, 2923, 1747, 1686, 1658, 1588, 1512, 1438, 1408, 1373, 1327, 1286, 1262, 1219, 1183, 1011 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 10.69 (bs, 2H, 2 x NH) (disappears on D_2O exchange), 8.60 (d, *J* = 9.3 Hz, 2H, ArH), 7.93 (d, *J* = 3.0 Hz, 2H, 2 x α-NH Gly) (disappears on D_2O exchange), 7.36 (t, *J* = 5.3 Hz, 2H, ArH), 7.49 (dd, *J* = 3.0, 9.2 Hz, 2H, ArH), 4.05 (d, *J* = 5.5 Hz, 4H, 2 x $NHC\underline{H}_2COOCH_3$), 3.68 (s, 6H, 2 x $COOC\underline{H}_3$), 2.17 (s, 6H, 2 x $COC\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 75.6 MHz): δ 190.9 ($\underline{C}OCH_3$), 169.8 ($CO\underline{C}ONH$), 169.5 ($CO\underline{C}ONH$), 163.2 ($\underline{C}OOCH_3$), 151.6 (ArC), 138.5 (ArC), 127.8 (ArC), 123.5 (ArC), 123.1 (ArC), 119.9 (ArC), 53.1 ($COO\underline{C}H_3$), 41.5 ($NH\underline{C}H_2COOCH_3$), 25.8 ($OCH_3$). HRMS (+ESI) *m/z* $[M + Na]^+$ calcd for $C_{26}H_{26}N_4NaO_{11}$: 593.1496; found: 593.1472.

N,N′-(4,4′-bis-{methyl-2-[2-(2-acetamidophenyl)-2-oxoacetamido]-4-methylpentanoate})oxide (**28**). Compound **28** was prepared by the same method as compound **21** from compound **8** (0.15 g, 0.38 mmol) and L-leucine methyl ester hydrochloride (0.30 g, 1.9 mmol) as a yellow solid (0.068 g, 26%); mp 106–108 °C; IR (KBr): υ_{max} 3313, 2958, 1748, 1655, 1589, 1519, 1438, 1410, 1370, 1263, 1217, 1182, 1162, 1013, 830, 698 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 10.82 (bs, 2H, 2 x NH) (disappears on D_2O exchange), 8.64 (d, *J* = 9.3 Hz, 2H, ArH), 7.83 (d, *J* = 2.7 Hz, 2H, 2 x α-NH Leu) (disappears on D_2O exchange), 7.41 (d, *J* = 8.5 Hz, 2H, ArH), 7.28 (dd, *J* = 2.9, 9.2 Hz, 2H, ArH), 4.60–4.67 (m, 2H, 2 x $NHC\underline{H}COOCH_3$), 3.67 (s, 6H, 2 x $COOC\underline{H}_3$), 2.21 (s, 6H, 2 x $COC\underline{H}_3$), 1.57–1.72 (m, 6H, 2 x $C\underline{H}_2C\underline{H}(CH_3)_2$), 0.93 (d, *J* = 5.9 Hz, 12H, $CH(C\underline{H}_3)_2$); ^{13}C NMR ($CDCl_3$, 75.6 MHz): δ 191.3 ($\underline{C}OCH_3$), 172.7 ($CO\underline{C}ONH$), 169.0 ($CO\underline{C}ONH$), 162.9 ($\underline{C}OOCH_3$), 151.1 (ArC), 138.0 (ArC), 127.3 (ArC), 122.9 (ArC), 122.6 (ArC), 119.4 (ArC), 52.5 ($COO\underline{C}H_3$), 50.8 ($NH\underline{C}HCOOCH_3$), 41.3 ($\underline{C}H_2CH(CH_3)_2$), 25.3 ($OCH_3$), 24.9 ($C\underline{H}(CH_3)_2$), 22.8 ($\underline{C}H_3$), 21.8 ($\underline{C}H_3$); HRMS (+ESI) *m/z* $[M + Na]^+$ calcd for $C_{34}H_{42}N_4NaO_{11}$: 705.2748; found: 705.2720.

N,N′-(4,4′-bis-{methyl-2-[2-(2-acetamidophenyl)-2-oxoacetamido]-4-(methylthio)butanoate})oxide (**29**). Compound **29** was prepared by the same method as compound **21** from compound **8** (0.15 g, 0.38 mmol) and L-methionine methyl ester hydrochloride (0.38 g, 1.9 mmol) as a yellow solid (0.206 g, 75%); mp 102–104 °C; IR (KBr): υ_{max} 3274, 1743, 1658, 1588, 1519, 1489, 1410, 1370, 1271, 1218, 1183, 1162 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 10.77 (bs, 2H, 2 x NH) (disappears on D_2O exchange), 8.63 (d, *J* = 9.3 Hz, 2H, ArH), 7.87 (d, *J* = 2.9 Hz, 2H, 2 x α-NH Met) (disappears on D_2O exchange), 7.65 (d, *J* = 8.1 Hz, 2H, ArH), 7.29 (dd, *J* = 2.9, 9.2 Hz, 2H, ArH), 4.74 (dd, *J* = 8.8, 12.9 Hz, 2H, 2 x $NHC\underline{H}COOCH_3$), 3.72 (s, 6H, 2 x $COOC\underline{H}_3$), 2.49–2.56 (m, 4H, 2 x $C\underline{H}_2CH_2S$), 2.21 (s, 6H, 2 x $COC\underline{H}_3$), 2.07 (s, 6H, 2 x $SC\underline{H}_3$), 1.03 (t, *J* = 6.8 Hz, 4H, 2 x $CH_2C\underline{H}_2S$); ^{13}C NMR ($CDCl_3$, 75.6 MHz): δ 190.8 ($\underline{C}OCH_3$), 171.6 ($CO\underline{C}ONH$), 169.1 ($CO\underline{C}ONH$), 162.7 ($\underline{C}OOCH_3$), 151.1 (ArC), 138.0 (ArC), 127.3(ArC), 122.9 (ArC), 122.6 (ArC), 119.5 (ArC), 52.8 ($COO\underline{C}H_3$), 51.5 ($NH\underline{C}HCOOCH_3$), 31.1 ($\underline{C}H_2CH_2S$), 29.9 ($CH_2\underline{C}H_2S$), 25.3 (OCH_3), 15.5 ($S\underline{C}H_3$); HRMS (+ESI) *m/z* $[M + Na]^+$ calcd for $C_{32}H_{38}N_4O_{11}NaS_2$: 741.1876; found: 741.1854.

N,N′-(4,4′-bis-{methyl-2-[2-(2-acetamidophenyl)-2-oxoacetamido]-3-methylbutanoate})oxide (**30**). Compound **30** was prepared by the same method as compound **21** from compound **8** (0.15 g, 0.38 mmol) and L-valine methyl ester hydrochloride (0.32 g, 1.9 mmol) as a yellow solid (0.115 g, 46%); mp 99–101 °C; IR (KBr): υ_{max} 3215, 2992, 1743, 1701, 1660, 1591, 1518, 1485, 1417, 1370, 1289, 1256, 1215, 1210, 1016, 920, 836, 741 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 10.80 (bs, 2H, 2 x NH) (disappears on D_2O exchange), 8.61 (d, *J* = 9.2 Hz, 2H, ArH), 7.70 (d, *J* = 2.8 Hz, 2H, 2 x α-NH Val) (disappears on D_2O exchange), 7.47 (d, *J* = 9.1 Hz, 2H, ArH), 7.24 (dd, *J* = 2.8, 9.2 Hz, 2H, ArH), 4.54 (dd, *J* = 4.8, 9.1 Hz, 2H, 2 x $NHC\underline{H}COOCH_3$), 3.66 (s, 6H, 2 x $COOC\underline{H}_3$), 2.16 (s, 6H, 2 x $COC\underline{H}_3$), 1.18–1.20 (m, 2H, 2 x $C\underline{H}(CH_3)_2$), 0.88 (dd, *J* = 6.9, 15.3 Hz, 12H, 2 x $CH(C\underline{H}_3)_2$); ^{13}C NMR ($CDCl_3$, 75.6 MHz): δ 192.0 ($\underline{C}OCH_3$), 172.4 ($CO\underline{C}ONH$), 169.4 ($CO\underline{C}ONH$), 163.7 ($\underline{C}OOCH_3$), 151.5 (ArC), 138.5 (ArC), 127.8 (ArC), 123.1 (ArC), 123.0 (ArC), 119.7 (ArC), 57.4 ($NH\underline{C}HCOOCH_3$), 52.8 ($COO\underline{C}H_3$), 31.8 ($\underline{C}H(CH_3)_2$), 25.7 (OCH_3), 18.0 ($\underline{C}H_3$), 19.4 ($\underline{C}H_3$); HRMS (+ESI) *m/z* $[M + Na]^+$ calcd for $C_{33}H_{40}N_4NaO_{10}$: 677.2435; found: 677.2419.

N,N′-(4,4′-bis-{methyl-2-[2-(2-acetamidophenyl)-2-oxoacetamido]-3-phenylpropanoate})oxide (**31**). Compound **31** was prepared by the same method as compound **21** from compound **8** (0.15 g, 0.38 mmol) and D-phenylalanine methyl ester hydrochloride (0.41 g, 1.9 mmol) as a yellow solid (0.196 g, 68%); mp 200–202 °C; IR (KBr): υ_{max} 3298, 1745, 1669, 1587, 1518, 1496, 1439, 1410, 1370, 1267, 1218, 1180, 702 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 10.78 (bs, 2H, 2 x NH) (disappears on D_2O exchange), 8.67 (d, *J* = 9.3 Hz, 2H, ArH), 7.79 (d, *J* = 2.9 Hz, 2H, 2 x α-NH Phe) (disappears on D_2O exchange), 7.22-7.31 (m, 12H, ArH), 7.11 (dd, *J* = 1.7, 8.0 Hz, 2H, ArH), 4.86–4.93 (m, 2H, 2 x $NHC\underline{H}COOCH_3$), 3.70 (s, 6H, 2 x $COOC\underline{H}_3$), 3.00–3.19 (m, 4H, 2 x $C\underline{H}_2Ph$), 2.21 (s, 6H, 2 x $COC\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 75.6 MHz): δ 190.9 ($\underline{C}OCH_3$), 171.3 ($CO\underline{C}ONH$), 169.0 ($CO\underline{C}ONH$), 162.3 ($\underline{C}OOCH_3$), 151.1 (ArC), 138.1 (ArC), 135.2 (ArC), 129.2 (ArC), 128.8 (ArC), 127.4 (ArC), 127.3 (ArC), 123.0 (ArC), 122.6 (ArC), 119.3 (ArC), 53.2 ($NH\underline{C}HCOOCH_3$), 52.6 ($COO\underline{C}H_3$), 37.9 (2 x $\underline{C}H_2Ph$), 25.4 (OCH_3); HRMS (+ESI) *m/z* $[M + Na]^+$ calcd for $C_{40}H_{38}N_4NaO_{11}$: 773.2435; found: 773.2419.

*N,N′-(4,4′-bis-{methyl-2-[2-(2-acetamidophenyl)-2-oxoacetamido]-3-phenylpropanoate})*oxide (**32**). Compound **32** was prepared by the same method as compound **21** from compound **8** (1.00 g, 2.55 mmol), L-phenylalanine methyl ester hydrochloride (1.21 g, 5.61 mmol) and Et_3N (1.03 g, 10.20 mmol) as a yellow solid (1.50 g, 78%); mp 201–202 °C; $[\alpha]_D$ -12 (c 0.1 in MeOH); IR: υ_{max} 3292, 1743, 1671, 1582, 1520, 1494, 1440, 1411, 1371, 1265, 1211, 1178, 701 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 10.74 (bs, 2H, 2 x NH) (disappears on D_2O exchange), 8.65 (d, *J* = 9.2 Hz, 2H, ArH), 7.80 (d, *J* = 3.0 Hz, 2H, 2 x α-NH Phe) (disappears on D_2O exchange), 7.21–7.34 (m, 12H, ArH), 7.10 (dd, *J* = 1.8, 8.0 Hz, 2H, ArH), 4.87–4.91 (m, 2H, 2 x $NHC\underline{H}COOCH_3$), 3.71 (s, 6H, 2 x $COOC\underline{H}_3$), 3.00–3.20 (m, 4H, 2 x $C\underline{H}_2Ph$), 2.22 (s, 6H, 2 x $COC\underline{H}_3$); ^{13}C NMR ($CDCl_3$, 101 MHz): δ 190.8 ($\underline{C}OCH_3$), 171.2 ($CO\underline{C}ONH$), 168.5 ($CO\underline{C}ONH$), 162.0 ($\underline{C}OOCH_3$), 151.2 (ArC), 137.8 (ArC), 135.1 (ArC), 129.1 (ArC), 128.7 (ArC), 127.3 (ArC), 127.1 (ArC), 123.0 (ArC), 122.5 (ArC), 119.0 (ArC), 53.1 ($NH\underline{C}HCOOCH_3$), 52.5 ($COO\underline{C}H_3$), 37.7 (2 x $\underline{C}H_2Ph$), 25.3 (OCH_3); HRMS (+ESI) *m/z* $[M + Na]^+$ calcd for $C_{40}H_{38}N_4NaO_{11}$: 773.2435; found: 773.2428.

Single-Crystal X-Ray Diffraction

The X-ray diffraction measurements for compounds **22–24** were carried out at MX1 and MX2 beamlines at the Australian Synchrotron Facility, Melbourne. The procedure for diffraction intensity measurements on both beamlines was similar. The crystal was mounted on the goniometer using a cryo loop for diffraction measurements, and it was coated with paraffin oil and then quickly transferred to the cold stream using cryo stream attachment. Data were collected using Si<111> monochromated synchrotron X-ray radiation (λ = 0.71023 Å) at 100(2) K and were corrected for Lorentz and polarization effects using the XDS software [23]. The structure was solved by direct methods and the full-matrix least-squares refinements were carried out using SHELXL [26]. X-ray crystallographic information files (CIF) for the structures **22–24** are CCDC 1505262, 1,505, 263 and 1956306. A copy of the data can be obtained free of charge from CCDC, 12 Union Road, Cambridge CB2 1EZ, UK or email: deposit@ccdc.cam.ac.uk.

4. Conclusions

The synthesis of a library of bis-glyoxylamide peptidomimetics was achieved by nucleophilic ring-opening reactions of bis-*N*-acetylisatins linked at C5 by a methylene or oxygen bridge with alcohols, amines, and amino acid methyl ester hydrochlorides. The bis-isatins were prepared using a modified Sandmeyer isonitrosoacetanilide isatin synthesis using 1,4-dioxane instead of hydrochloric acid as the reaction solvent. Amines were the most reactive reagents for ring-opening of the bis-*N*-acetylisatins and the products were obtained in high yields. The addition of sodium bicarbonate was needed for ring-opening of bis-*N*-acetylisatins with amino acid methyl ester hydrochlorides and the reactions were complete in 24 h. The alcohols were slow to react and heating the reaction mixture at reflux was required for ethanol. The biological activity of these compounds is currently under investigation.

Supplementary Materials: The following are available online at http://www.mdpi.com/1420-3049/24/23/4343/s1, ^{1}H and ^{13}C NMR spectra for the synthesized compounds.

Author Contributions: D.S.B. and N.K. conceived and directed this project. The synthesized and spectroscopic identification of the title compounds **3–32** were conducted by V.S., R.Z. and V.A. V.S. produced the single crystal of compounds **22–24** and prepared the manuscript for publication. M.B. conducted the X-ray analysis of compounds **22–24**.

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Sample Availability: Samples of the synthesized compounds are available from the corresponding authors.