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Double-sided α-helix mimetics

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ABSTRACT

The design and synthesis of substituted bis- and tris-benzamides is reported in which the projection of side-chain residues on both sides of an α-helix is reproduced. The scaffold is conformationally constrained by a series of intramolecular hydrogen bonds, allowing for spatial and angular mimicry of the *i*, *i*+2, *i*+4 and *i*+6 side-chains in the case of the bis-benzamide, and may be extended to higher-order oligomers.

1. Introduction

Protein–protein interactions (PPIs) are responsible for diverse biological functions ranging from signal transduction to immune response.[1] Among protein secondary structures, α-helices form the largest class and are responsible for a multitude of PPIs and the stability of higher-order structures.[2] Hence, the design of α-helix mimetics as inhibitors of aberrant interactions is a promising strategy that has attracted wide attention from the synthetic community. There are many reports in which both peptidic[3] and non-peptidic[4] oligomers project substituents in the correct spatial and angular orientation to mimic the side-chains of one face of an α-helix—frequently those of the *i*, *i*+4 and *i*+7 residues. Non-peptidic scaffolds include indanes, terphenyls, terpyridyls and polycylic ethers.[4e] In a related approach, hydrogen-bond-constrained scaffolds including enaminones,[5] benzoylureas, tris-pyridylamides and tris-benzamide have been developed as more accessible mimetics in which the syntheses are simplified and the biological properties improved (Fig. 1a,b, Ref. 4b and refs therein).

Whilst there are a growing number of synthetic scaffolds for the mimicry of side-chains on a single face of an α-helix, there have been very few examples in which the side-chain projection of two faces is reproduced.[6] Of these approaches, many do not allow extension to higher-order oligomers in which more than three or four side-chains are mimicked. The side-chains projecting from the exterior face of an α-helix have been widely implicated in the binding of multiple proteins and in bacterial cell wall sensing. Hrs-UIM (hepatocyte growth factor-regulated tyrosine kinase substrate-ubiquitin interacting motif),[7] golgi associated protein

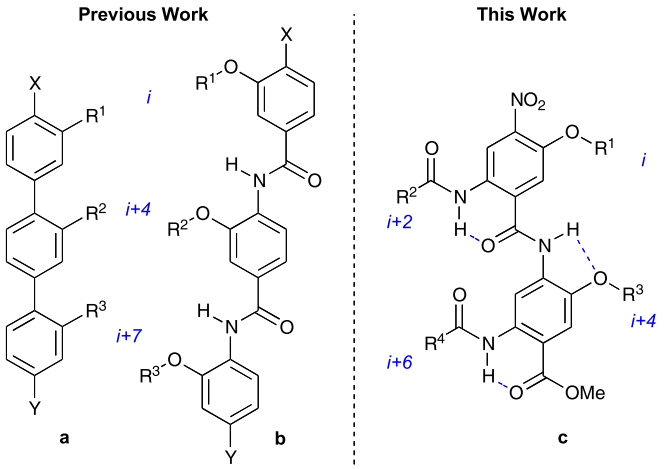

Fig. 1. Functionalized single- and double-sided α-helix mimetics: (a) terphenyl; (b) tris-benzamide; (c) bis-benzamide.

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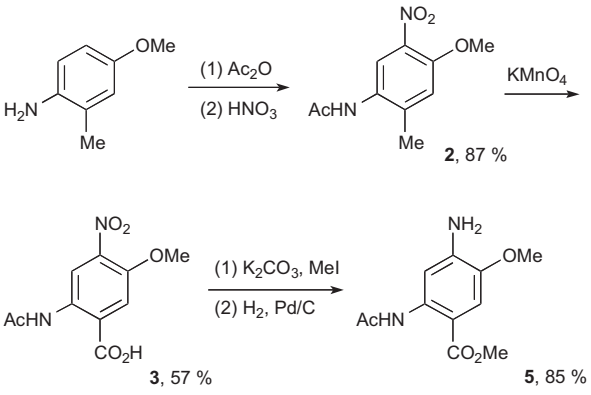
Arf-GAP1[8] and glucagon like peptide-1 (GLP-1)[9] depend of residues of both faces of the α-helix for their function. Antimicrobial peptides (magainins, defensins and protegrins) have one side of their backbone composed of cationic groups to interact with anionic phospholipids and lipopolysaccharides on the bacterial cell wall, and another side composed of hydrophobic groups to facilitate penetration of the bacterial membrane.[10]

With the goal of creating a novel scaffold for simultaneous mimicry of two faces of an α-helix, we have designed a series of double-sided mimetics based on our benzamide scaffold[11] (Fig. 1c). We anticipated that this design would adopt a constrained conformation due to intramolecular H-bonding between the amide N–H and *ortho*-alkoxy group, and mimic of the *i*, *i*+2, *i*+4 and *i*+6 residues of an α-helix. The two oligomer precursors originate from a common intermediate and may be connected via nucleophilic attack of an aromatic amine on an activated carboxyl group. A range of acyl groups, potentially including amino acids, may be incorporated in the *i*+2 and *i*+6 positions.

2. Results and discussion

Synthesis of carboxylic acid monomer **3** from 2-amino-5-hydroxybenzoic acid or 4-chloro-2-methylaniline was poor yielding; however a robust three-step procedure was established starting from *m*-cresidine in 50% overall yield (Scheme 1). Esterification of the carboxylic acid and reduction of the nitro-group gave amine **5** in 85% yield, setting the stage for coupling with **3** to form bis-benzamide **10**.



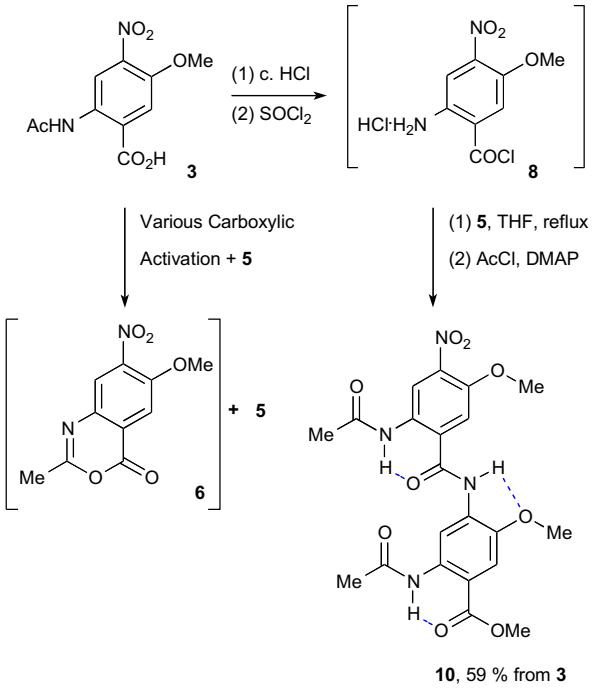
Scheme 1. Synthesis of building blocks.

Activation of **3** as an acid chloride, or the use of coupling agents such as DCC, EDCI, HATU, gave an intermediate thought to be benzoxazinone **6** and recovered amine **5**.

As an alternative we protected the amino group of **3** as the hydrochloride salt (**7**, not shown) prior to carboxyl activation coupling in refluxing tetrahydrofuran and reacylation with acetyl chloride. Overall this represents a modular and scalable six-step bis-benzamide synthesis from *m*-cresidine (Scheme 2).

The single crystal X-ray structure of bis-benzamide **10**[12a] confirms the presence of hydrogen bonds between side-chain N–Hs and the adjacent carbonyls of 1.9 and 2.0 Å, and between the main-chain N–H and *ortho*-alkoxy group of 2.1 Å in the solid state. These appear to provide a conformationally constrained scaffold in which substituent projection is in good agreement with the *i*, *i*+2, *i*+4 and *i*+6 side-chains of an α-helix; i.e., two residues on one face of an α-helix and two residues on the other face (Fig. 2).

Our synthetic strategy enabled us to synthesize heterodimers where, following amide bond formation, the free amino group may be decorated with a variety of different acyl groups to generate bis-benzamides bearing different side-chains (Scheme 3).[12b]



Scheme 2. Synthesis of functionalized bis-benzamide bearing four side-chains.

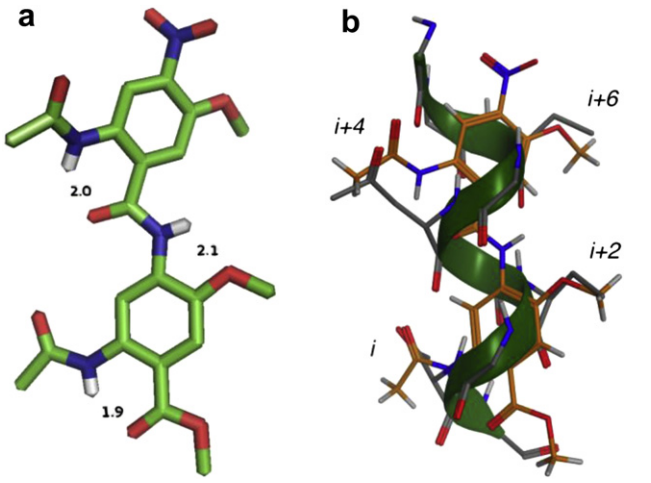
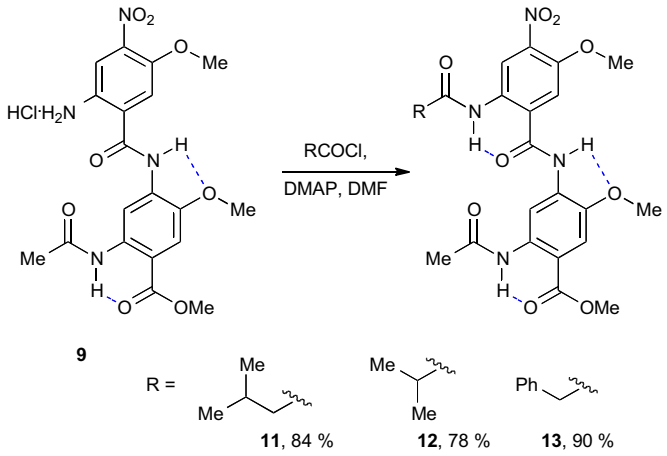


Fig. 2. (a) X-ray crystal structure of bis-benzamide **10**. (b) Substituents of bis-benzamide X-ray structure (orange) fitting the side-chains of an α-helix (grey, green ribbon) with a root-mean-square deviation of 0.434 Å.[13]

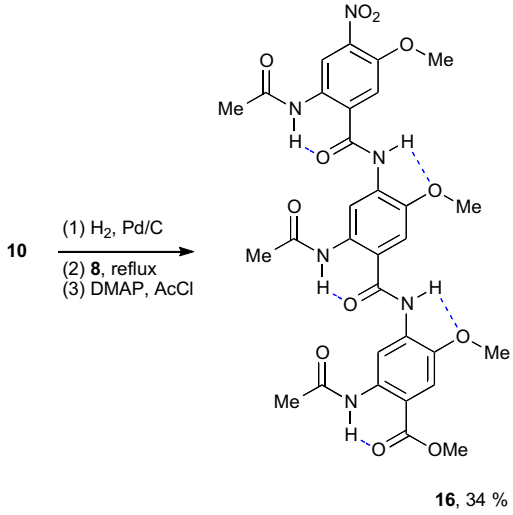
Following our standard protocol for benzamide formation, reduction of nitro-substituted bis-benzamide **10**, coupling with acid chloride **8** and acylation gave hexa-substituted tris-benzamide **16** (Scheme 4). This molecule has substituents mimicking the side-chain positions of the *i*, *i*+2, *i*+4, *i*+6, *i*+8 and *i*+10 positions.

3. Conclusions

In summary, we have designed a modular and scalable route to conformationally constrained double-sided benzamide α-helix mimetics and have efficiently synthesized a range of heterodimeric bis-benzamides and a tris-benzamide bearing six side-chains. The synthesis is amenable to extension of these mimetics to higher oligoamides and to molecules with a broad array of *N*-acyl groups.



Scheme 3. Synthesis of hetero bis-benzamides (heterodimers).



Scheme 4. Functionalized tris-benzamide.

4. Experimental section

4.1. General

Reactions were carried out under a nitrogen or argon atmosphere in oven-dried glassware unless otherwise stated. Standard inert atmosphere techniques were used in handling all air and moisture sensitive reagents. Anhydrous tetrahydrofuran and dichloromethane (from commercial sources) were obtained by filtration through activated alumina (powder ~150 mesh, pore size 58 Å, basic, Sigma–Aldrich) columns, or were dried on an MB-SPS-800 dry solvent system. Other solvents and reagents were used directly as received from commercial suppliers. Petrol refers to distilled light petroleum of fraction (30–40 °C). Flash column chromatography was carried out using VWR Kieselgel 60 silica gel (60–63 μm). Thin-layer chromatography was carried out using Merck Kieselgel 60 F_{254} (230–400 mesh) fluorescent treated silica, visualized under UV light (250 nm) and by staining with aqueous potassium permanganate solution. ^{1}H and ^{13}C NMR spectra were recorded using a Bruker 500, 400, 300 or 250 MHz spectrometer running Topspin™ software and are quoted in parts per million for measurement against a tetramethylsilane (TMS) or residual solvent peak as internal standards. Chemical shifts (δ) are given in parts per million (ppm), and coupling constants (J) are given in hertz (Hz). The ^{1}H NMR spectra are reported as follows: δ/ppm (number of protons, multiplicity, coupling constant J/Hz (where appropriate), assignment). Multiplicity is abbreviated as follows: s=singlet, br=broad, d=doublet, t=triplet, q=quartet, quint.=quintet, sept.=septet, m=multiplet, v=very. Compound names are those generated by ChemBioDraw™ (CambridgeSoft) following IUPAC nomenclature. The ^{13}C NMR spectra are reported in δ/ppm. Two-dimensional (COSY, HSQC, HMBC) NMR spectroscopy was used to assist the assignment of signals in the ^{1}H and ^{13}C NMR spectra. IR spectra were recorded on a Bruker Tensor 27 FT-IR spectrometer from a thin film deposited onto a sodium chloride plate or a diamond ATR module. Only selected maximum absorbances (ν_{max}) of the most intense peaks are reported (cm^{-1}). Low-resolution mass spectra were recorded on a Waters LCT premier XE Micromass spectrometer (ESI). High-resolution mass spectra were recorded on a Bruker MicroTof mass spectrometer (ESI) by the internal service at the Department of Organic Chemistry, University of Oxford. Melting points were recorded using a Leica Galen III hot-stage microscope apparatus and are reported uncorrected in degrees Celsius (°C).

4.2. *N*-(4-Methoxy-2-methylphenyl)acetamide (1)

Acetic anhydride (10.0 mL) was added to a stirred solution of 4-methoxy-2-methylaniline (5.77 g, 42.1 mmol) in pyridine (10.0 mL) at 0 °C. The cooling bath was removed and the mixture stirred overnight before being concentrated in vacuo. The residue was dissolved in dichloromethane (500 mL), partitioned with ammonium chloride (500 mL) and extracted with dichloromethane (3×300 mL). The organic layers were combined, dried (magnesium sulfate), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2; diethyl ether) affording the *title compound* **1** (7.40 g, 41.3 mmol, 98%) as an off-white solid: mp 130; δ_H (500 MHz, CD_3OD) 7.15 (1H, d, J 8.6, Ar–H), 6.81 (1H, d, J 2.8, Ar–H), 6.75 (1H, dd, J 8.6, 2.8, Ar–H), 3.78 (3H, s, OCH_3), 2.22 (3H, s, CH_3), 2.14 (3H, s, CH_3); δ_C (125 MHz, CD_3OD) 171.5, 158.6, 135.4, 128.7, 127.6, 115.6, 111.5, 54.8, 21.8, 17.3; IR (neat) ν_{max} 3281, 2959, 1645, 1616, 1532, 1498, 1310, 1286, 1161, 1051, 809, 705; HRMS (ESI) found 202.0833, $C_{10}H_{13}NNaO_2$ $[M+Na]^+$ requires 202.0838.

4.3. *N*-(4-Methoxy-2-methyl-5-nitrophenyl)acetamide (2)

Based on a literature procedure,[14] sodium nitrite (73 mg, 1.06 mmol) was added portion-wise to a stirred solution of **1** (189 mg, 1.06 mmol) in trifluoroacetic acid (5.0 mL) at 0 °C. The cooling bath was removed and the mixture stirred for 1 h before concentration in vacuo. The residue was dissolved in dichloromethane (10 mL), partitioned with sodium hydrogencarbonate (10 mL) and extracted with dichloromethane (3×10 mL). The organic layers were combined, dried (magnesium sulfate), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2; 19:1 dichloromethane/methanol) affording the *title compound* **2** (211 mg, 0.94 mmol, 89%) as an off-white solid: mp 185; δ_H (250 MHz, CD_3OD) 7.89 (1H, s, Ar–H), 7.15 (1H, s, Ar–H), 3.94 (3H, s, OCH_3), 2.32 (3H, s, CH_3), 2.16 (3H, s, CH_3); δ_C (125 MHz, CD_3OD) 171.5, 151.2, 141.3, 137.4, 128.6, 123.0, 115.5, 56.2, 21.9, 17.5; IR (neat) ν_{max} 1646, 1621, 1519, 1461, 1338, 1261, 1212, 1065, 1015, 760, 721; HRMS (ESI) found 247.0689, $C_{10}H_{12}N_2NaO_4$ $[M+Na]^+$ requires 247.0689.

4.4. 2-Acetamido-5-methoxy-4-nitrobenzoic acid (3)

Based on a literature procedure,[15] **2** (606 mg, 2.70 mmol) was added to a stirred solution of magnesium sulfate (406 mg, 3.38 mmol) and potassium permanganate (1.18 g, 7.44 mmol) in water (27 mL) at 80 °C. After 45 min, magnesium sulfate (204 mg, 1.70 mmol) and potassium permanganate (589 mg, 3.73 mmol)

were added. After 45 min the reaction mixture was filtered and the solids were washed with hot water (2×15 mL). The filtrate was acidified to pH 3 (3.0 N HCl), partitioned with dichloromethane (60 mL) and extracted (2×60 mL). The organic layers were combined, dried (magnesium sulfate), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2; 90:10:1 dichloromethane/methanol/acetic acid) affording the *title compound* **3** (395 mg, 1.56 mmol, 57%) as a yellow solid: mp 246 (decomp.); δ_H (500 MHz, $(CD_3)_2SO$) 10.71 (1H, s, NH), 8.75 (1H, s, Ar–H), 7.73 (1H, s, Ar–H), 3.94 (3H, s, CH_3), 2.12 (3H, s, NHCOCH_3); δ_C (125 MHz, $(CD_3)_2SO$) 169.2, 168.5, 147.1, 140.8, 134.2, 128.1, 117.2, 116.4, 57.5, 25.6; IR (neat) ν_{max} 3117, 1706, 1678, 1621, 1526, 1393, 1340, 1198, 1014, 822, 715; HRMS (ESI) found 253.0463, $C_{10}H_9N_2O_6$ $[M-H]^-$ requires 253.0466.

4.5. Methyl 2-acetamido-5-methoxy-4-nitrobenzoate (4)

Methyl iodide (47 μL, 0.74 mmol) was added to a stirred solution of **3** (170 mg, 0.67 mmol) and potassium carbonate (138 mg, 1.00 mmol) in *N*,*N*-dimethylformamide (3.0 mL) at 70 °C. After overnight heating the reaction mixture was diluted with dichloromethane (20 mL), partitioned with ammonium chloride (15 mL) and extracted with dichloromethane (3×15 mL). The organic layers were combined, dried (magnesium sulfate), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2; 20:1 chloroform/methanol) affording the *title compound* **4** (167 mg, 0.62 mmol, 93%) as a yellow solid: mp 138; δ_H (400 MHz, $CDCl_3$) 10.81 (1H, s, NH), 9.17 (1H, s, Ar–H), 7.69 (1H, s, Ar–H), 4.00 (3H, s, CH_3), 3.95 (3H, s, CH_3), 2.24 (3H, s, NHCOCH_3); δ_C (125 MHz, $CDCl_3$) 170.4, 167.0, 147.3, 142.9, 133.7, 121.2, 117.7, 115.7, 56.3, 52.8, 23.9; IR (neat) ν_{max} 1691, 1532, 1340, 1231, 1013, 785; HRMS (ESI) found 291.0582, $C_{11}H_{12}N_2NaO_6$ $[M+Na]^+$ requires 291.0588.

4.6. Methyl 2-acetamido-4-amino-5-methoxybenzoate (5)

Palladium on carbon (57 mg, wet Degussa type, 5% palladium by weight) was added to a stirred solution of **4** (764 mg, 3.35 mmol) in methanol (20 mL) and dichloromethane (20 mL). The solution was degassed three times with argon using a pump-flood procedure and placed under hydrogen. After 20 h the reaction mixture was filtered (Celite™), washed with dichloromethane (60 mL) and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2; 50:1 chloroform/methanol) affording the *title compound* **5** (725 mg, 3.05 mmol, 91%) as a pale solid: mp 149; δ_H (500 MHz, $CDCl_3$) 11.10 (1H, s, NH), 8.07 (1H, s, Ar–H), 7.28 (1H, s, Ar–H), 4.46 (2H, br, NH_2), 3.82 (3H, s, CH_3), 3.79 (3H, s, CH_3), 2.15 (3H, s, NHCOCH_3); δ_C (125 MHz, $CDCl_3$) 169.0, 168.8, 143.1, 141.4, 138.1, 111.3, 104.8, 103.4, 55.7, 51.8, 25.6; IR (neat) ν_{max} 3498, 3303, 1662, 1529, 1430, 1257, 776; HRMS (ESI) found 261.0840, $C_{11}H_{14}N_2NaO_4$ $[M+Na]^+$ requires 261.0846.

4.7. 2-Amino-5-methoxy-4-nitrobenzoic acid hydrochloride (7)

Based on a literature procedure,[16] **3** (2.05 g, 8.08 mmol) was refluxed in concentrated hydrochloric acid (36.5%, 24.0 mL) for 5 h. The mixture was allowed to attain room temperature, filtered and the solids washed with water (2×20 mL), affording the *title compound* **7** (1.72 g, 6.91 mmol, 86%) as an off-white solid: mp 230 (decomp.); δ_H (500 MHz, $(CD_3)_2SO$) 7.52 (1H, s, Ar–H), 7.26 (1H, s, Ar–H), 5.30 (2H, br, NH_2), 3.79 (3H, s, CH_3); δ_C (75 MHz, $(CD_3)_2SO$) 173.3, 150.7, 149.0, 145.4, 121.4, 118.2, 117.0, 62.1; IR (neat) ν_{max} 2788, 1699, 1622, 1501, 1403, 1351, 1237, 1204, 1124, 1041, 982, 864, 743; HRMS (ESI) found 211.0361, $C_8H_7N_2O_5$ $[M-H]^-$ requires 211.0360.

4.8. Methyl 2-acetamido-4-(2-amino-5-methoxy-4-nitrobenzamido)-5-methoxybenzoate hydrochloride (9)

Thionyl chloride (50 mL) and **7** (533 mg, 2.13 mmol) were refluxed for 1 h and then concentrated in vacuo. The residue was dissolved in tetrahydrofuran (15 mL) and a solution of **5** (637 mg, 2.66 mmol) in tetrahydrofuran (20 mL) was added. The resultant mixture was heated at reflux for 18 h, cooled to −5 °C, filtered and the solids washed with tetrahydrofuran (2×10 mL) and methanol (3×50 mL) to afford the *title compound* **9** (829 mg, 1.77 mmol, 83%) as an orange solid: mp 309 (decomp.); δ_H (500 MHz, $(CD_3)_2SO$) 10.39 (1H, s, NH), 9.91 (1H, s, NH), 8.68 (1H, s, Ar–H), 7.53 (1H, s, Ar–H), 7.47 (1H, s, Ar–H), 7.28 (1H, s, Ar–H), 6.13 (2H, br, NH_2), 3.86 (3H, s, CH_3), 3.85 (3H, s, CH_3), 3.85 (3H, s, CH_3), 2.09 (3H, s, NHCOCH_3); δ_C (125 MHz, $(CD_3)_2SO$) 169.0, 167.8, 166.4, 147.2, 143.5, 142.9, 142.6, 134.3, 132.4, 121.0, 117.8, 116.3, 115.6, 113.2, 112.7, 58.0, 57.0, 53.2, 25.3; IR (neat) ν_{max} 3376, 1691, 1672, 1607, 1523, 1420, 1357, 1250, 1079, 895, 770; HRMS (ESI) found 455.1176, $C_{19}H_{20}N_4NaO_8$ $[M+Na]^+$ requires 455.1173.

4.9. Methyl 2-acetamido-4-(2-acetamido-5-methoxy-4-nitrobenzamido)-5-methoxybenzoate (10)

Acetyl chloride (210 μL, 2.97 mmol) was added dropwise to a stirred suspension of 4-dimethylaminopyridine (362 mg, 2.97 mmol) and **9** (465 mg, 0.99 mmol) in *N*,*N*-dimethylformamide (3.0 mL) at 0 °C. The cooling bath was removed and the mixture stirred at room temperature for 1 h. The resultant pale yellow solution was diluted with dichloromethane (100 mL), partitioned with ammonium chloride (50 mL) and extracted with dichloromethane (3×50 mL). The organic layers were combined, dried (magnesium sulfate), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2; 20:1 dichloromethane/acetone) affording the *title compound* **10** (387 mg, 0.82 mmol, 82%) as an yellow solid: mp 272; δ_H (500 MHz, $(CD_3)_2SO$) 10.38 (1H, s, NH), 10.13 (1H, s, NH), 9.80 (1H, s, NH), 8.85 (1H, s, Ar–H), 8.27 (1H, s, Ar–H), 7.62 (1H, s, Ar–H), 7.46 (1H, s, Ar–H), 3.98 (3H, s, CH_3), 3.86 (3H, s, CH_3), 3.85 (3H, s, CH_3), 2.09 (3H, s, NHCOCH_3), 2.02 (3H, s, NHCOCH_3); δ_C (125 MHz, $(CD_3)_2SO$) 168.9, 168.0, 166.8, 164.1, 147.9, 145.5, 139.7, 133.5, 132.0, 131.3, 129.0, 119.9, 115.7, 115.0, 114.6, 111.7, 57.0, 56.1, 52.2, 24.2, 23.4; IR (neat) ν_{max} 1692, 1677, 1603, 1530, 1422, 1363, 1229, 1084, 788; HRMS (ESI) found 497.1287, $C_{21}H_{22}N_4NaO_9$ $[M+Na]^+$ requires 497.1279.

4.10. Methyl 2-acetamido-4-(2-isobutyramido-5-methoxy-4-nitrobenzamido)-5-methoxybenzoate (11)

Based on the procedure for the synthesis of **10**, **9** (170 mg, 0.39 mmol) and isobutyryl chloride (123 μL, 1.17 mmol) afforded the *title compound* **11** (169 mg, 0.33 mmol, 84%) as a yellow solid: mp 242; δ_H (500 MHz, $CDCl_3$) 10.92 (1H, s, NH), 10.31 (1H, s, NH), 9.69 (1H, s, NH), 9.11 (1H, s, Ar–H), 8.49 (1H, s, Ar–H), 7.53 (1H, s, Ar–H), 7.26 (1H, s, Ar–H), 3.99 (3H, s, CH_3), 3.96 (3H, s, CH_3), 3.95 (3H, s, CH_3), 2.58 (1H, sept, *J* 6.5, C*H*$(CH_3)_2$), 2.26 (3H, s, NHCOCH_3), 1.24 (6H, d, *J* 6.5, CH(CH_3)$_2$); δ_C (67 MHz, $(CD_3)_2CO$) 177.6, 168.3, 165.7, 162.4, 147.2, 142.1, 141.5, 139.2, 136.5, 132.0, 124.0, 118.3, 114.8, 114.0, 111.8, 110.0, 57.1, 56.1, 52.3, 33.6, 24.7, 19.2; IR (neat) ν_{max} 3284, 2865, 1684, 1603, 1530, 1417, 1349, 1230, 1045, 785; HRMS (ESI) found 525.4634, $C_{23}H_{26}N_4NaO_9$ $[M+Na]^+$ requires 525.4632.

4.11. Methyl 2-acetamido-5-methoxy-4-(5-methoxy-2-(3-methylbutanamido)-4-nitrobenzamido)benzoate (12)

Based on the procedure for the synthesis of **10**, **9** (180 mg, 0.41 mmol) and isovaleryl chloride (150 μL, 1.23 mmol) afforded

the *title compound* **12** (165 mg, 0.32 mmol, 78%) as a yellow solid: mp 123; δ_H (500 MHz, $(CD_3)_2SO$) 10.38 (1H, s, NH), 10.11 (1H, s, NH), 9.81 (1H, s, NH), 8.82 (1H, s, Ar–H), 8.32 (1H, s, Ar–H), 7.62 (1H, s, Ar–H), 7.46 (1H, s, Ar–H), 3.98 (3H, s, CH_3), 3.85 (6H, s, $2\times CH_3$), 2.16 (2H, d, *J* 7.0, CH_2), 2.09 (3H, s, NHCOCH_3), 1.98 (1H, sept, *J* 6.8, CH(CH_3)$_2$), 0.86 (6H, d, *J* 6.5, CH(CH_3)$_2$); δ_C (125 MHz, $CDCl_3$) 171.6, 168.9, 167.8, 165.1, 147.3, 143.1, 142.1, 136.8, 132.8, 131.7, 125.4, 119.0, 112.0, 111.9, 111.0, 110.6, 57.1, 56.2, 52.4, 47.0, 25.9, 25.4, 22.4; IR (neat) ν_{max} 3282, 2957, 1682, 1603, 1528, 1462, 1348, 1206, 999, 822, 783; HRMS (ESI) found 525.1602, $C_{23}H_{26}N_4NaO_9$ $[M+Na]^+$ requires 525.1592.

4.12. Methyl 2-acetamido-5-methoxy-4-(5-methoxy-4-nitro-2-(2-phenylacetamido)benzamido)benzoate (13)

Based on the procedure for the synthesis of **10**, **9** (184 mg, 0.42 mmol) and phenylacetyl chloride (170 μL, 1.26 mmol) afforded the *title compound* **13** (209 mg, 0.38 mmol, 90%) as a yellow solid: mp 235; δ_H (500 MHz, $(CD_3)_2SO$, $CDCl_3$) 10.39 (1H, s, NH), 10.29 (1H, s, NH), 9.82 (1H, s, NH), 8.81 (1H, s, Ar–H), 8.31 (1H, s, Ar–H), 7.61 (1H, s, Ar–H), 7.45 (1H, s, Ar–H), 7.26–7.14 (5H, m, Ar–H), 3.97 (3H, s, CH_3), 3.88 (3H, s, CH_3), 3.86 (3H, s, CH_3), 3.65 (2H, s, CH_2), 2.10 (3H, s, NHCOCH_3); δ_C (125 MHz, $(CD_3)_2SO$, $CDCl_3$) 170.5, 169.1, 167.6, 164.7, 148.0, 144.0, 141.0, 135.5, 133.7, 131.4, 130.7, 129.0, 128.6, 128.0, 127.1, 119.5, 113.0, 112.5, 111.4, 111.2, 77.3, 77.0, 76.7, 56.7, 55.8, 52.2, 24.7; IR (neat) ν_{max} 3329, 2954, 1687, 1601, 1528, 1415, 1349, 1237, 1029, 895, 786, 730; HRMS (ESI) found 573.1605, $C_{27}H_{26}N_4NaO_9$ $[M+Na]^+$ requires 573.1592.

4.13. Methyl 2-acetamido-4-(2-acetamido-4-amino-5-methoxybenzamido)-5-methoxybenzoate (14)

Based on the procedure for the synthesis of **5**, **10** (1.07 g, 2.26 mmol) afforded the *title compound* **14** (949 mg, 2.14 mmol, 95%) as a yellow solid: mp 269; δ_H (400 MHz, $(CD_3)_2SO$) 10.82 (1H, s, NH), 10.40 (1H, s, NH), 9.47 (1H, s, NH), 8.74 (1H, s, Ar–H), 7.44 (1H, s, Ar–H), 7.36 (1H, s, Ar–H), 7.29 (1H, s, Ar–H), 5.65 (2H, br, NH_2), 3.85 (3H, s, CH_3), 3.84 (3H, s, CH_3), 3.81 (3H, s, CH_3), 2.08 (3H, s, NHCOCH_3), 2.01 (3H, s, NHCOCH_3); δ_C (125 MHz, $(CD_3)_2SO$) 168.4, 168.1, 167.0, 166.4, 145.7, 142.5, 141.9, 133.9, 133.7, 132.2, 115.8, 113.7, 111.5, 110.5, 109.4, 106.6, 56.0, 55.6, 52.2, 24.4, 24.4; IR (neat) ν_{max} 3348, 2922, 1674, 1602, 1528, 1419, 1352, 1234, 1026, 878, 767; HRMS (ESI) found 467.1538, $C_{21}H_{24}N_4NaO_7$ $[M+Na]^+$ requires 467.1537.

4.14. Methyl 2-acetamido-4-(2-acetamido-4-(2-amino-5-methoxy-4-nitrobenzamido)-5-methoxybenzamido)-5-methoxybenzoate hydrochloride (15)

Based on the procedure for the synthesis of **9**, **14** (940 mg, 2.11 mmol) and **7** (481 mg, 1.92 mmol) afforded the *title compound* **15** (875 mg, 1.30 mmol, 67%); δ_H (400 MHz, $(CD_3)_2SO$) 10.41, (1H, s, NH), 10.33 (1H, s, NH), 9.91 (1H, s, Ar–H), 9.65 (1H, s, Ar–H), 8.92, (1H, s, Ar–H), 8.24 (1H, s, Ar–H), 7.55 (1H, s, Ar–H), 7.51 (1H, s, Ar–H), 7.48 (1H, s, Ar–H), 7.29 (1H, s, Ar–H), 6.16 (2H, br, NH_2), 3.92 (3H, s, CH_3), 3.89 (3H, s, CH_3), 3.88 (3H, s, CH_3), 3.86 (3H, s, CH_3), 2.11 (NHCOCH_3), 2.04 (NHCOCH_3); δ_C (100 MHz, $(CD_3)_2SO$) 168.9, 168.1, 166.9, 165.7, 165.2, 147.8, 145.3, 145.2, 142.9, 142.0, 141.5, 133.7, 131.7, 129.8, 129.7, 123.3, 120.0, 119.9, 115.4, 114.0, 112.1, 111.6, 111.5, 57.2, 56.2, 56.1, 52.3, 24.4, 23.7; HRMS (ESI) found 661.5723, $C_{29}H_{30}N_6NaO_{11}$ $[M+Na]^+$ requires 661.5713.

4.15. Methyl 2-acetamido-4-(2-acetamido-4-(2-acetamido-5-methoxy-4-nitrobenzamido)-5-methoxybenzamido)-5-methoxybenzoate (16)

Based on the procedure for the synthesis of **10**, **15** (870 mg, 1.29 mmol) and acetyl chloride (275 μL, 3.87 mmol) afforded the *title compound* **16** (470 mg, 0.69 mmol, 54%); δ_H (500 MHz, $(CD_3)_2SO$) 10.31 (1H, s, NH), 10.12 (1H, s, NH), 9.89 (1H, s, NH), 8.45 (1H, s, Ar–H), 8.21 (1H, s, Ar–H), 8.04 (1H, s, Ar–H), 7.65 (1H, s, Ar–H), 7.60 (1H, s, Ar–H), 7.53 (1H, s, Ar–H), 4.00 (3H, s, CH_3), 3.97 (3H, s, CH_3), 3.90 (3H, s, CH_3), 3.80 (3H, s, CH_3), 2.12 (3H, s, NHCOCH_3), 2.09 (3H, s, NHCOCH_3), 2.03 (3H, s, NHCOCH_3); δ_C (125 MHz, $CDCl_3$) 169.6, 168.9, 167.3, 165.3, 160.9, 152.0, 149.5, 147.9, 147.4, 142.6, 140.5, 135.6, 133.3, 131.5, 130.4, 129.3, 120.6, 118.7, 118.5, 117.0, 116.4, 113.6, 112.8, 104.9, 57.0, 55.9, 55.9, 53.2, 25.1, 24.4, 23.1; HRMS (ESI) found 703.1961, $C_{31}H_{32}N_6NaO_{12}$ $[M+Na]^+$ requires 703.1970.

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