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Synthesis of Potential Anti-HIV GP120 Inhibitors Using a Lysine Template

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Various acylated proteins have been reported in the literature to possess anti-HIV activity. Described here is the preparation of lysine monomers, dimers and trimers acylated with various anhydrides and dioxalanones as simplified mimics of the acylated proteins. Compounds were assayed against HIV-infected C8166 cells and some showed weak anti-HIV activity.

Keywords: GP120 inhibitors; HIV; Lysine oligomers; Acylated proteins

INTRODUCTION

Despite the recent advances in combination therapy for the treatment of HIV/AIDS there is still a need for new drugs to treat this disease. In particular it would be beneficial to have drugs active against new targets in the HIV life cycle. One target of particular interest is HIV entry into the cell. The adsorption of the virus to the cell is mediated by the strong and specific binding of the virus envelope glycoprotein gp120 to the CD4 receptor in the presence of a specific chemokine receptor.[1] The formation of a complex between all 3 factors causes structural changes within the envelopes leading to fusion of virus and cell and hence delivery of the viral core. The inhibition of interaction of gp120 with the cells has already been established for many high molecular weight polyanionic compounds including aurintricarboxylic acid,[2] tannins,[3] dextran sulfate,[4] heparin,[4] cafeoylquinic acids,[5] polysulfates,[6] and polycarboxylates.[7] Whilst more recent publications dealing specifically with inhibitors directed at the gp120 chemokine co-receptor interaction, have identified certain peptides[8] and distamycin analogues[9] as viable leads. Unfortunately these large polyanionic compounds exhibit little efficacy *in vivo*. Problems including poor oral absorption, intestinal-overgrowth of certain bacilli; anti-coagulant effects and binding to plasma proteins prevent these polyanionic compounds reaching their target and gives rise to potential side effects.

We were interested by a report of the anti-HIV activity of various proteins which had been acylated at the N-position of lysine residues with succinic anhydride,[10] *cis*-aconitic anhydride[10] or 3-hydroxyphthalic anyhydride.[11] Whilst the proteins and the anhydrides have no activity of their own, the acylation of the proteins incorporates an anionic charge which gives rise to activity. Presumably the protein is acting as a scaffold which holds the charges in the correct stereochemical orientation for pharmacological activity. The mode of action of these compounds is probably binding to the V3 loop of gp120 and a so called second scavenger receptor on the target cell surface,[12,13] preventing the proper function of gp120.

Proteins in general make poor drug candidates, owing to size, stability and possible immunological problems. Therefore we decided to see if we could make lysine scaffolds to mimic the protein, which should circumvent these problems and could potentially be applied to some of the large polyanionic compounds highlighted above. We proposed to

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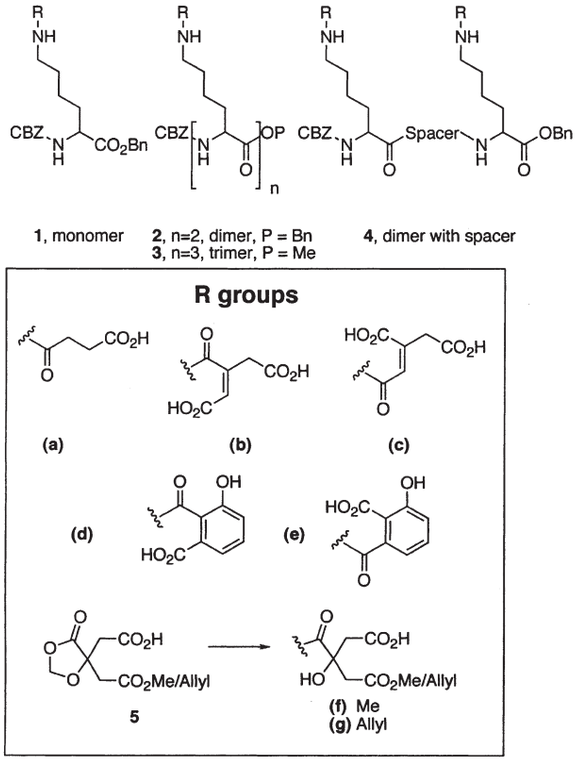

FIGURE 1 Acylated lysine oligomers.

make monomers, dimers and trimers of lysine and then acylate them with various anhydrides. The range of target molecules is shown in Figure 1. In addition to succinic anhydride, *cis*-aconitic anhydride and 3-hydroxyphthalic anyhydride, a range of dioxalanones (**5**, Figure 1) were also used.[14] Previous studies within the group had shown these dioxalanones to have modest anti-HIV activity; however this proved to be non-reproducible and it may be possible that this activity was due to a small amount of polymeric material. Should the lysine oligomers act as a good scaffold, then a further design process could be carried out to improve the characteristics of the molecules further.

EXPERIMENTAL

General

Where applicable all glassware has been oven-dried overnight and all reactions were carried out under an inert atmosphere of N_2. T.l.c. was carried out on precoated silica plates (Merck Kieselgel 60 F_{254}) with visualisation *via* U.V. light and/or $KMnO_4$ solution. ^1H NMR, ^{13}C and ^{13}C DEPT spectra were recorded on a Bruker Avance DPX300 at 300 MHz and 75 Mz respectively and coupling constants (J values) are in hertz. I.R. spectra were obtained on a Perkin Elmer 1600 FTIR spectrometer as thin films or as solids *via* a diffuse reflectance accessory using a potassium bromide matrix. Low resolution mass spectra were recorded on a Fisons VG platform electrospray mass spectrometer. High resolution mass spectra (HRMS) were determined by the EPSRC Mass Spectrometry Centre, Swansea, UK.

General Synthetic Procedures

Procedure 1: Benzyl Esterification of Acids

Acid (1 equivalent), benzyl alcohol (10 equivalent) and thionyl chloride (1 equivalent) were heated at 50°C under an atmosphere of N_2 for 3 h. The mixture was reduced *in vacuo*, the crude material was dissolved in MeOH, triturated with EtOAc and filtered to yield the title compounds.

Ammendments: (**1a**) 2 equivalents of thionyl chloride.

Procedure 2: Acylation of Amine Salts

A solution of anhydride or dioxalanone (1 equivalent), amine salt (1 equivalent) and triethylamine (3 drops) in dioxane (10 mL per mmol) was heated at 80°C under an atmosphere of N_2 for 4–5 h. The mixture was reduced *in vacuo* and the crude material was purified by flash column chromatography on silica to yield the title compounds.

Ammendments: (**2a**) 2 equivalents of anhydride. (**2b**) 2 equivalents of anhydride, 15 h, room temperature. (**2c**) 2 equivalents of dioxalanone, 15 h. (**2d**) 2 equivalents of dioxalanone, 72 h then add DMAP (10 mg) to mixture. (**2e**) 2 equivalents of dioxalanone, add DMAP (10 mg) at start, 48 h. (**2f**) 2 equivalents anhydride, 6 days. (**2g**) 3 equivalents of anhydride, 6 days. (**2h**) 2 equivalents of anhydride, DMF, 28 h, room temperature, trituration with cold ether. (**2i**) 2 equivalents of anhydride, DMF, 54 h, room temperature, trituration with cold diethyl ether.

Procedure 3: TPTU Methodology

DIPEA (2.7 equivalents) was added to a stirred solution of acid (1 equivalent) and TPTU (1.1 equivalents) in dioxane (10 mL per mmol of acid). The resultant mixture was stirred for 1 h at room temperature under an atmosphere of N_2. Amine (1 equivalent) in dioxane (5 mL per mmol) was added and the mixture was stirred for a further 15 h. The reaction mixture was reduced *in vacuo* and the crude product was purified by flash column chromatography to yield the title products.

Procedure 4: Fmoc Cleavage from Rink Amide Resin or Resin with Attached Amino Acid

The resin (pre-swollen in DMF) was shaken in a 20% solution of piperidine in DMF (5 mL) over 30 min. The presence of free amine groups was confirmed, after washing with DMF, by using the ninhydrin test.

Procedure 5: Attachment of N-α-Fmoc N-ε-Boc-L-lysine to Resin

The resin (pre-swollen in DMF) was shaken in a solution of N-α-Fmoc N-ε-Boc-L-lysine (2 equivalents), TBTU (2 equivalents), HOBT (2 equivalents) and DIPEA (4 equivalents) in DMF (5 mL) over 1 h. The absence of free amine groups was confirmed, after washing with DMF, by using the ninhydrin test.

Procedure 6: Cleavage of Lysine Oligomers from the Resin

The resin (pre-swollen in DCM) was shaken in a 10% solution of TFA in DCM (5 mL) over 30 min and the resultant solution was isolated. The resin was washed with 5% TFA in DCM (3 × 10 mL) and was then shaken in a 90% solution of TFA in DCM (5 mL) for a further 30 min. The resultant solution was isolated and reduced *in vacuo* with the previously collected solution and washings to yield yellow/brown oils. Trituration with cold diethyl ether yielded the title products as white/cream precipitates.

6-Amino-2-benzyloxycarbonylamino-hexanoic Acid Benzyl Ester Hydrochloride Salt **7**

Procedure 1. White foam; (R_f 0.5, MeOH/EtOAc, 1:1); (0.750 g, 67%). δ_H (d_6-acetone) 8.4 (2H, br s, NH_2), 7.4 (10H, m, 10 × aromatic CH), 7.0 (1H, d, $J = 8.0$, NH), 5.2 (2H, d, $J = 1.7$, CH_2), 5.1 (2H, d, CH_2), 4.3 (1H, dd, $J = 5.9, 7.8$, CH), 2.9 (2H, m, CH_2), 2.1 (2H, m, CH_2), 1.9 (4H, m, 2 × CH_2). δ_C (d_6-acetone) 170.2, 154.4, (C = O), 135.5, 134.3 (C), 126.4, 126.3, 126.0, 125.7, 123.9 (aromatic CH), 66.6, 66.1 (CH_2), 54.8 (CH), 46.9, 30.9, 27.2, 23.2 (CH_2). ν_{max} (film) cm^{-1} ~3400 (CONH), 3032 (NH_3^+), 2953 (CH_2), 1706 (C = O ester + amide), 1654 (NH_3^+), 1522 (CONH), 1498 (Ar), 1455 (CH_2), 739, 697 (5H Ph). M/z 371 (M + H). HRMS $C_{20}H_{27}O_4N_2$ (M + H) = 371.1971; found 371.1964.

2-Benzyloxycarbonylamino-6-(3-carboxy-propionylamino)-hexanoic Acid Benzyl Ester **1a**

Procedure 2. White solid; (R_f 0.38, petrol/ethyl acetate, 1:1); (0.147 g, 47%). δ_H (d_6-acetone) 11.0 (1H, br s, CO_2H), 7.4 (10H, m, 10 × aromatic CH), 7.2 (1H, d, $J = 2.5$, CH_2), 5.2 (2H, d, $PhCH_2$), 5.1 (2H, d, $J = 0.79$, $PhCH_2$), 4.3 (1H, m, CH), 3.2 (2H, m, CH_2), 3.0 (2H, d, $J = 2.9$, CH_2), 2.6 (2H, m, CH_2), 2.5 (2H, m, CH_2), 1.9 (2H, m, CH_2), 1.5 (2H, m, CH_2). δ_C (d_6-acetone) 173.5, 172.6, 171.9, 156.7, (C = O), 137.6, 136.7 (C), 128.8, 128.7, 128.4, 128.3, 128.2 (aromatic CH), 66.5, 66.3 (CH_2), 54.6 (CH), 38.9, 31.4, 30.6, 29.6, 29.5, 23.2 (CH_2). ν_{max} (KBr) cm^{-1} ~3500–2700 (CO_2H), 3320, 3064 (CONH), 2950 (CH_2), 1737 (C = O ester), 1718 (C = O acid), 1693, 1538 (C = O amide), 1455 (CH_2), 733, 696 (5H Ph). M/z 469 (M − H). HRMS $C_{25}H_{39}O_7N_2$ (M − H) = 469.1975; found 469.1967.

3-(5-Benzyloxycarbonyl-5-benzyloxycarbonylamino-pentylcarbamoyl)pent-2-enedioic Acid **1b**

Procedure 2. Yellow oil; (R_f 0.7, EtOAc/MeOH with 1% AcOH, 1:1); (0.093 g, 18%). δ_H (d_6-acetone) 7.4 (10H, m, 10 × aromatic CH), 6.5 (1H, s, CH), 5.1 (4H, m, 2 × CH_2), 4.2 (1H, m, CH_2), 1.4 (4H, m, 2 × CH_2). ν_{max} (film) cm^{-1} 2970, 2870 (CH_2), 1734, (C = O ester), 1718 (C = O acid), 1687 (C = O α, β unsaturated acid), 812 (R_2C = CRH), 750, 687 (5H Ph). M/z 525, (M − H). HRMS $C_{27}H_{29}O_9N_2$ (M − H) = 525.1873; found 525.1877.

N-(5-Benzyloxycarbonyl-5-benzyloxycarbonylamino-pentyl)-3-hydroxy-phthalamic Acid **1d** and *N*-(5-benzyloxycarbonyl-5-benzyloxycarbonylamino-pentyl)-6-hydroxy-phthalamic Acid **1e**

Procedure 2. **1d** yellow solid; (R_f 0.5, EtOAc); (0.03 g, 13%). δ_H (d_6-acetone) 7.4 (10H, m, 10 × aromatic CH), 6.7 (1H, m, aromatic CH), 6.5 (2H, m, 2 × aromatic CH) 5.0 (4H, m, 2 × CH_2), 4.2 (1H, m, CH), 3.6 (4H, m, 2 × CH_2), 1.8 (2H, m, CH_2), 1.4 (2H, m, CH_2). ν_{max} (film) cm^{-1} ~3500–3200 (OH), 2943, 2832, (CH_2), 1710 (C = O ester), 1698 (C = O acid), 1453 (CH_2), 750 (3H, Ph). M/z 533, (M − H). HRMS $C_{29}H_{29}O_8N_2$ (M − H) = 533.1924; found 533.1925.

1e yellow solid; (R_f 0.3, EtOAc); (0.02 g, 9%). δ_H (d_6-acetone) 7.2 (11H, m, 11 × aromatic CH), 6.6 (2H, m, 2 × aromatic CH), 5.1 (4H, m, 2 × CH_2), 4.1 (1H, m, CH), 2.8 (4H, m, 2 × CH_2), 1.7 (2H, m, CH_2), 1.4 (2H, m, CH_2). ν_{max} (film) cm^{-1} ~3500–3200 (OH), 2833, (CH_2), 1720 (C = O ester), 1690 (C = O acid), 1443 (CH_2), 770 (3H, Ph). M/z 533, (M − H). HRMS $C_{29}H_{29}O_8N_2$ (M − H) = 533.1924; found 533.1923.

3-(5-Benzyloxycarbonyl-5-benzyloxycarbonylamino-pentylcarbamoyl)-3-hydroxy-pentanedioic Acid Monomethyl Ester **1f**

Procedure 2. White precipitate; (R_f 0.5, methanol/ethyl acetate 1:1; (0.039 g, 7%). δ_H (d_6-acetone) 7.3 (10H, m, 10 × aromatic CH), 5.4 (2H, d, $J = 6.6$, $PhCH_2$), 5.0 (2H, d, Bn CH_2), 4.2 (1H, m, CH), 3.6 (3H, s, OCH_3), 3.2 (2H, m, CH_2), 2.9 (4H, m, 2 × CH_2), 2.0 (2H, m, CH_2), 1.8 (4H, m, 2 × CH_2). δ_C (d_6-acetone) 171.3, 170.7, 167.6, 161.5, 154.7 (C = O), 135.6, 134.7 (C), 126.8, 126.7, 126.4, 126.2 (CH), 93.1 (CH_2), 73.9 (C), 64.6, 64.3 (CH_2), 52.6 (CH), 49.7 (CH_3), 39.1, 29.4, 27.4, 21.2 (CH_2). ν_{max} (film) cm^{-1} 3700–3300 (CO_2H), 3385 (CONH), 2951 (CH_2), 1799 (C = O ester), 1734 (C = O acid), 1654 (CONH), 1498 (Ar), 1438 (CH_2/CH_3), 1057 (R_3COH), 739, 698 (5H Ph). M/z 557

(M − H). HRMS $C_{28}H_{33}O_{10}N_2$ (M − H) = 557.2135; found 557.2128.

2-Benzyloxycarbonylamino-6-*tert*-butoxycarbonylamino-hexanoic Acid **8**

Sodium bicarbonate (0.599 g, 71 mmol) was added to a solution of Nα-Cbz-L-lysine **6** (1.0 g, 3.57 mmol) stirred in THF (10 mL) and H_2O (10 mL). Di *t*-butyldicarbonate (1.8 g, 8.2 mmol) in THF (10 mL) was added and the resultant solution was stirred at room temperature for 1.5 h. Sodium bicarbonate (0.1 g, 1.1 mmol) and di *t*-butyldicarbonate (0.6 g, 2.7 mmol) was added, the solution was stirred for a further 30 min and reduced *in vacuo* to yield the crude product as a yellow oil. The crude material was purified by flash column chromatography on silica using a gradient elution of 100% petrol to 10:1 petrol/ethyl acetate to yield the title compound **8** (R_f 0.1, 1:1 methanol/ethyl acetate), as a cream precipitate (1.165 g, 86%). δ_H (d_6-acetone) 7.4 (5H, m, 5 × aromatic CH), 6.6 (1H, m, NH), 6.1 (1H, m, NH), 5.14 (1H, J_{AB} = 12.5, $PhCH_2$), 4.96 (1H, J_{AB} = 12.5, $PhCH_2$), 4.1 (1H, m, CH), 3.3 (2H, m, CH_2), 3.0 (2H, m, CH_2), 1.9 (2H, br m, CH_2), 1.4 (11H, s + m, *t*-Bu + CH_2). δ_C (d_6-acetone) 180.9, 159.0, 158.4 (C = O), 139.9 (C), 130.7, 130.3, 129.9 (aromatic CH), 80.0 (C), 68.2 (CH_2), 58.6 (CH), 42.6, 34.8, 33.1 (CH_2), 28.3 (CH_3), 25.4 (CH_2). ν_{max} (KBr) cm^{-1} 3600–2700 (CO_2H), 3353, 3339 (CONH), 2930 (CH_2), 1722 (C = O ester), 1697 (CONH), 1593, 1504 (Ar), 1455 (CH_2/CH_3), 1366 (*t*-Bu), 772, 700 (5H Ph). M/z 379 (M − H). HRMS $C_{19}H_{27}O_6N_2$ (M − H) = 379.1869; found 379.1869.

2-Benzyloxycarbonylamino-6-*tert*-butoxycarbonylamino-hexanoic Acid Pentafluorophenyl Ester **9**

Dicyclohexylcarbodiimide (0.124 g, 0.6 mmol) was added to a solution of **8** (0.153 g, 0.4 mmol) and pentafluorophenol (0.096 g, 0.52 mmol) in ethyl acetate (10 mL) cooled at 0°C under an atmosphere of N_2. The mixture was allowed to warm to room temperature, stirred for 40 min, filtered and reduced *in vacuo* to yield the crude product as a yellow oil. The crude material was purified by flash column chromatography on silica using a gradient elution of 100% petrol ether to 5:1 petrol ether/ethyl acetate to yield the title compound **9**, as a white crystaline solid (0.139 g, 61%). δ_H (d_6-acetone) 7.4 (5H, m, 5 × Ph CH), 7.2 (1H, d, *J* = 7.3, NH), 6.0 (1H, m, NH), 5.1 (2H, s, $BnCH_2$), 4.6 (1H, dd, *J* = 4.9, 8.2, CH), 3.1 (2H, d, *J* = 6.0, CH_2), 2.8 (2H, m, CH_2), 1.6 (4H, br m, 2 × CH_2), 1.4 (9H, s, *t*-Bu). δ_C (d_6-acetone) 169.5, 156.6, 156.4 (C = O), 137.4 (C), 128.7, 128.3 (CH), 78.0 (C), 66.6 (CH_2), 54.6 (CH), 39.9, 31.0, 29.8 (CH_2), 28.1 (CH_3), 23.0 (CH_2). δ_F (d_6-acetone) 155.0 (m), 164.5 (m), 105.1 (m). ν_{max} (film) cm^{-1} 3342 (CONH), 2982, 2936 (CH_2), 1737 (C = O ester), 1521 (C = O amide), 1456 (CH_2/CH_3), 1392, 1373 (*t*-Bu), 1245, 756, 698 (C–F). M/z 569 (M + H). HRMS $C_{25}H_{28}O_6N_2F_5$ (M + H) = 547.1868; found 547.1866.

2-(2-Benzyloxycarbonylamino-6-*tert*-butoxycarbonylamino-hexanoylamino)-6-*tert*-butoxycarbonylamino-hexanoic Acid **11**

Et_3N (1 mL) was added to a stirred solution of **9** (0.5 g, 2 mmol) and **10** (1.15 g, 2.02 mmol) in EtOAc (20 mL) under an atmosphere of N_2. The resultant solution was stirred at room temperature for 15 h, filtered and reduced *in vacuo* to yield a yellow oil. The crude material was purified by flash column chromatography on silica using a gradient elution of 10:1 petrol ether/EtOAc to 100% EtOAc to 1:1 EtOAc/MeOH to yield the title compound **11** as a cream foam (0.97 g, 79%). δ_H (d_6-acetone) 7.4 (5H, m, 5 × aromatic CH), 5.2 (2H, m, $PhCH_2$), 4.4 (1H, m, CH), 4.2 (1H, m, CH), 3.0 (4H, m, 2 × CH_2), 1.9 (4H, m, 2 × CH_2), 1.7 (4H, m, 2 × CH_2), 1.4 (8H, m, 4 × CH_2), 1.3 (18H, s, 2 × *t*-Bu). δ_C (d_6-acetone) 172.4, 156.5, 156.3, 156.2, 151.8 (C = O), 137.6 (C), 128.7, 128.2, 128.1 (aromatic CH), 77.9 (C), 66.3, 60.0, (CH_2), 55.1 (CH), 40.3, 40.2 (CH_2), 28.2 (*t*-Bu), 23.0 (CH_2). ν_{max} (film in CH_2Cl_2) cm^{-1} 3700–3300 (CO_2H), 3421 (CONH), 2976 (CH_2), ~1700 (C = O), 1652 (C = O amide), 1455 (CH_2/CH_2) 1366 (*t*-Bu). m/z 607, (M − H).

6-Amino-2-(6-amino-2-benzyloxycarbonylamino-hexanoylamino)-hexanoic Acid Benzyl Ester Dihydrochloride Salt **12**

Procedure 1a. The compound was isolated as a white precipitate; (0.567 g, 67%). δ_H (d_6-acetone) 7.4 (10H, m, 10 × aromatic CH), 5.1 (~4H, m, 2 × CH_2), 2.9 (4H, m, 2 × CH_2), 1.8–1.6 (8H, m, 4 × CH_2), 1.3 (4H, m, 2 × CH_2). ν_{max} (film) cm^{-1} 3036 (amino salt), 2952, (CH_2), 1706 (C = O ester), 1455 (CH_2), 739, 698 (5H, Ph). M/z 499, (M + H). HRMS $C_{27}H_{37}O_5N_4$ (M + H) = 499.2920; found 499.2924.

2-[2-Benzyloxycarbonylamino-6-(3-carboxy-propionylamino)-hexanoylamino]-6-(3-carboxy-propionylamino-hexanoic Acid Benzyl Ester **2a**

Procedure 2a. White precipitate; (R_f 0.7, EtOAc/MeOH, 1:1); (0.026 g, 26%). δ_H (d_6-acetone) 7.7 (1H, d, NH), 7.4 (10H, m, 10 × aromatic CH), 6.6 (1H, d, NH), 5.1 (4H, m, 2 × $PhCH_2$), 4.4 (1H, m, CH), 4.1 (1H, m, CH), 3.1 (4H, m, 2 × CH_2), 2.6 (4H, m, 2 × CH_2), 2.5 (4H, t, *J* = 6.6, 2 × CH_2), 1.8 (4H, m, 2 × CH_2), 1.5 (8H, m, 4 × CH_2). ν_{max} (film) cm^{-1} 3376 (amide), 2936 (CH_2), 1725 (C = O ester, acid), 1429 (CH_2), 750, 687 (5H, Ph). M/z 697, (M − H). HRMS $C_{35}H_{45}O_{10}N_4$ (M − H) = 697.3085; found 697.3076.

2-({5-Benzyloxycarbonylamino-5-[1-benzyloxycarbonyl-5-(3,4-dicarboxy-but-2-enoylamino)-pentylcarbamoyl]-pentylcarbamoyl}-methylene)-succinic Acid **2c**

Procedure 2b. Yellow oil; (R_f 0.05, EtOAc/MeOH, 1:1); (0.32 g, 20%). δ_H (d_4-MeOH) 7.3

(10H, m, 10 × aromatic CH), 6.9 (1H, s, CH), 5.9 (1H, s, CH), 5.1 (4H, m, 2 × $PhCH_2$), 4.6 (1H, m, CH), 4.2 (1H, m, CH), 3.6 (4H, m, 2 × CH_2), 3.3 (8H, m, 4 × CH_2), 1.9 (4H, m, 2 × CH_2), 1.5 (4H, m, 2 × CH_2). ν_{max} (film) cm^{-1} 2947, 2834, (CH_2), 1721 (C = O ester, acid), 1680 (C = O α, β unsatd.), 1620 (C = C), 1444 (CH_2), 750, 687 (5H, Ph). M/z 809, (M − H). HRMS $C_{39}H_{45}O_{15}N_4$ (M − H) = 809.2881; found 809.2885.

3-{5-[5-(2-Allyloxycarbonylmethyl-3-carboxy-2-hydroxy-propionylamino)-1-benzyloxycarbonyl-pentycarbamoyl]-5-benzyloxycarbonylamino-pentycarbamoyl}-3-hrdroxy-pentanedioc Acid Monomethyl Ester **2f**

Procedure 2e. White precipitate; (R_f 0.1, 1:1 EtOAc/MeOH); (0.06 g, 45%). δ_H (d_4-MeOH) 7.3 (10H, m, 10 × aromatic CH), 5.1 (4H, m, 2 × CH_2), 4.3 (1H, m, CH), 4.1 (1H, m, CH), 3.6 (6H, m, 2 × CH_3), 3.0 (4H, m, 2 × CH_2), 2.8–2.7 (4H, m, 2 × CH_2), 2.7–2.5 (4H, m, 2 × CH_2), 1.7 (2H, m, CH_2), 1.6 (2H, m, CH_2), 1.5–1.3 (8H, m, 4 × CH_2). ν_{max} (film) cm^{-1} 3600–3200 (OH), 2900 (CH_2, CH_3), ∼1700 (C = O ester, acid), 1643 (C = O amide), 780 (5H Ph). M/z 873, (M − H). HRMS $C_{41}H_{53}O_{17}N_4$ (M − H) = 873.3406; found 873.3400.

3-{5-[5-(2-Allyloxycarbonylmethyl-3-carboxy-2-hydroxy-propionylamino)-1-benzyloxycarbonyl-pentycarbamoyl]-5-benzyloxycarbonylamino-pentycarbamoyl}-3-hrdroxy-pentanedioc Acid Monoallyl Ester **2g**

Procedure 2d. Yellow oil; (R_f 0.1, EtOAc/MeOH, 1:1); (0.03 g, 23%). δ_H (d_6-acetone) 7.7 (2H, br m, 2 × NH), 7.4 (10H, m, 10 × aromatic CH), 5.9 (2H, m, 2 × allyl CH), 5.5–5.0 (8H, m, 2 × allyl CH_2 + 2 × $PhCH_2$), 4.5 (4H, m, 2 × allyl CH_2), 4.4 (1H, m, CH), 4.1 (1H, m, CH), 3.2 (4H, m, 2 × CH_2), 3.0–2.7 (8H, m, 4 × CH_2), 1.8 (4H, m, 2 × CH_2), 1.5 (8H, m, 4 × CH_2). ν_{max} (film) cm^{-1} ∼3600–3200 (OH), 3416 (amide), 2844 (CH_2), 1750 (C = O ester), 1700 (C = O acid), 1660 (C = O amide), 1650 (C = C), 1470, 1440 (CH_2), 739, 698 (5H, Ph). M/z 925, (M − H). HRMS $C_{45}H_{57}O_{17}N_4$ (M − H) = 925.3719; found 925.3722.

6-(2-Benzyloxycarbonylamino-6-*TERT*-butoxycarbonylamino-hexanoylamino) Hexanoic Acid **13**

Procedure 3. Yellow oil (R_f 0.1, 0.05 in 1:1 petroleum ether/EtOAc) (0.37 g, 79%). δ_H (d_4-MeOH) 7.8 (1H, d, J = 6.7, NH), 7.3 (5H, m, 5 × aromatic CH), 6.6 (1H, d, J = 8.9, NH), 6.4 (1H, t, J = 6.0, NH), 5.0 (2H, d, $PhCH_2$), 4.1 (1H, m, CH), 3.2 (2H, m, CH_2), 3.1 (2H, m, CH_2), 2.3 (2H, t, J = 7.2, CH_2), 1.6 (2H, m, CH_2), 1.4 (4H, m, 2 × CH_2), 1.3 (15H, m + s, 3 × CH_2 + *t*-Bu). ν_{max} (film) cm^{-1} 2939, 2868, (CH_2, CH_3), 1701 (C = O ester, acid), 1652 (C = O amide), 1452 (CH_2, CH_3), 1391, 1365 (*t*-Bu), 750, 687 (5H, Ph). M/z 492, (M − H). HRMS $C_{25}H_{37}O_7N_3$ (M − H) = 492.2710; found 492.2715.

2-[6-(2-Benzyloxycarbonylamino-6-*TERT*-butoxycarbonylamino-hexanoylamino)-hexanoylamino]-6-*TERT*-butoxycarbonylamino Hexanoic Acid **14**

Procedure 3. Yellow oil; (R_f = 0.4, hexane/EtOAc, 1:1); (0.338 g, 90%). δ_H ($CDCl_3$) 7.3 (5H, m, 5 × aromatic CH), 5.0 (2H, br s, $PhCH_2$), 4.3 (1H, br s, CH), 4.0 (1H, m, CH), 3.2 (2H, m, CH_2), 2.9 (4H, m, 2 × CH_2), 2.2 (2H, m, CH_2), 1.7–1.4 (14H, m, 7 × CH_2), 1.4 (22H, m + s, 2 × CH_2 + 2 × *t*-Bu). M/z 720 (M − H), 722 (M + H).

6-Amino-2-[6-(6-amino-2-benzyloxycarbonylamino-hexanoylamino)-hexanoylamino]-hexanoic Acid Benzyl Ester Dihydrochloride Salt **15**

Procedure 1. Brown precipitate; (0.069 g, 81%). δ_H (d_4-MeOH) 7.4 (10H, m, 10 × aromatic CH), 5.2 (4H, m, 2 × $PhCH_2$), 4.3 (1H, m, CH), 4.0 (1H, m, CH), 3.4 (2H, m, CH_2), 2.9 (4H, m, 2 × CH_2), 2.3 (∼2H, m, CH_2), 1.7–1.3 (18H, br m, 9 × CH_2). δ_C (d_4-MeOH) 175.0, 172.1 (C = O), 141.7 (Ph C), 128.6, 128.5, 128.3, 128.0, 127.9, 127.3, 126.9, 126.3 (aromatic CH), 67.0, 66.7, 64.2 (CH_2), 52.5, 48.9 (CH), 39.5, 39.2, 31.7, 30.2, 28.9, 27.0, 26.3, 25.5, 22.9 (CH_2). ν_{max} (film) cm^{-1} 3395 (CONH), 2948, 2840 (CH_2), 1710 (C = O ester), 1641 (C = O amide), 1447 (CH_2), 734, 699 (5H, Ph). M/z 612 (M + H). HRMS $C_{33}H_{50}O_6N_5$ (M − H) = 612.3761; found 612.3760.

2-{6-[2-Benzyloxycarbonylamino-6-(3-carboxy-propionylamino)-hexanoylamino]-hexanoylamino}-6-(3-carboxy-propionylamino)-hexanoic Acid Benzyl Ester **16a**

Procedure 2f. Cream precipitate; (R_f = 0.5, EtOAc/MeOH, 1:1); (0.069 g, 43%). δ_H (d_4-MeOH) 7.3 (10H, m, 10 × aromatic CH), 5.3 (4H, m, 2 × $PhCH_2$), 4.3 (1H, m, CH), 4.0 (1H, m, CH), 3.3 (2H, m, CH_2), 3.0 (4H, m, 2 × CH_2), 2.4 (8H, m, 4 × CH_2), 2.3 (2H, m, CH_2), 1.7–1.3 (18H, br m, 9 × CH_2). δ_C (d_4-MeOH) 178.2, 176.2, 174.9, 173.5, (C = O), 138.0, 137.2 (Ph C), 129.5, 129.4, 129.3, 129.2, 128.9, 128.8 (aromatic CH), 67.7 (CH_2), 53.8, 49.8 (CH), 40.1, 39.9, 36.4, 32.9, 32.3, 31.8, 31.6, 30.6, 29.9, 27.4, 24.1 (CH_2). ν_{max} (film) cm^{-1} 3479 (CONH), 2926, 2859 (CH_2), 1720 (C = O ester, acid), 1639 (C = O amide), 1411 (CH_2), 720, 693 (Ph). M/z 810 (M − H). HRMS $C_{41}H_{56}O_{12}N_5$ (M − H) = 810.3925; found 810.3927.

2-{6-[2-Benzyloxycarbonylamino-6-(2-carboxy-3-hydroxy-benzoylamino)-hexanoylamino]-hexanoylamino}-6-(2-carboxy-3-hydroxy-benzoylamino)-hexanoic Acid Benzyl Ester **16e**

Procedure 2f. Clear oil; (R_f = 0.7, EtOAc/MeOH, 1:1); (0.025 g, 11%). δ_H (d_6-acetone) 7.9–6.9 (16H, m, 16 × Ph CH), 5.0 (4H, m, 2 × Bn CH_2), 4.4 (1H, m, CH), 4.1 (1H, m, CH), 3.6 (2H, m, CH_2), 3.3 (4H, m, 2 × CH_2), 2.3 (2H, m, CH_2), 2.3 (2H, m, CH_2), 1.8–1.2 (18H, br m, 9 × CH_2). ν_{max} (film) cm^{-1} 3600–3200

(OH), 2945, 2865 (CH_2), 1700 (C = O ester, acid), 1631 (C = O amide), 1444 (CH_2), 756, 693 (Ph). M/z 939 (M − H), 469 (M − 2H). HRMS $C_{49}H_{56}O_{14}N_5$ (M − H) = 938.3824; found 938.3826.

N-{5-amino-1-[5-amino-1-(5-amino-1-carbamoyl-pentyl Carbamoyl)-pentyl Carbamoyl] Pentyl}-benzamide **17**

Procedure 4, 5, 4, 5, 4, 5, 4, 3(using benzoic acid), 6, clear oil; 38%. δ_H (d_4-MeOH) 7.9 (2H, d, $J = 6.7$, 2 × aromatic CH), 7.5 (3H, m, 3 × aromatic CH), 4.3 (1H, m, CH), 4.2 (2H, m, 2 × CH), 2.9 (6H, m, 3 × CH_2), 1.9–1.4 (18H, br m, 9 × CH_2). δ_C (d_4-MeOH) 177.1, 175.4, 174.6, 171.1 (C = O), 135.4 (Ph C), 133.5, 130.0, 129.1 (Ph CH), 56.4, 55.3, 54.7, (CH), 41.0, 40.9, 32.9, 32.4, 28.5, 28.4, 24.5, 24.2, (CH_2). ν_{max} (film) cm^{-1} 3306 ($CONH/NH_2$), 2946 (CH_2), 1670 (C = O amide), 1576 (NH_2), 1539 (C = O amide), 1455 (CH_2), 756, 722 (Ph). M/z 506 (M + H), 254 (M + 2H). HRMS $C_{25}H_{44}O_4N_7$ (M + H) = 506.3455; found 506.3455.

N-{5-[2-benzoylamino-6-(3-carboxy-propionyl-amino)-hexanoylamino]-5-[1-carbamoyl-5-(3-carboxy-propionylamino)-pentylcarbamoyl]-pentyl}-succinamic Acid **17a**

Procedure 2h. White precipitate, 41%. δ_H (d_4-MeOH) 7.9 (2H, d, 2 × aromatic CH), 7.6 (3H, m, 3 × aromatic CH), 4.5 (1H, m, CH), 4.4 (2H, m, 2 × CH), 3.1 (6H, m, 3 × CH_2), 2.6 (6H, m, 3 × CH_2), 2.4 (6H, m, 3 × CH_2), 1.9–1.4 (18H, m, 9 × CH_2). ν_{max} (film) cm^{-1} 3600–3300 (OH), 3305 (CONH), 2944 (CH_2), 1718 (C = O, acid), 1648 (C = O amide), 721 (Ph). M/z 805 (M − H), 402 (M − 2H). HRMS $C_{37}H_{54}O_{13}N_7$ (M − H) = 804.3780; found 804.3781.

2-{[5-({1-Carbamoyl-5-{(2-carboxyhydroxyphe-nyl)carboxamido]pentyl}carbamoyl)-5-{(2-car-boxyhydroxyphenyl)carboxamido]pentyl}carba-moyl)-5-(phenylcarboxamido)pentyl]carba-moyl}hydroxybenzoic Acid **17d,e**

Procedure 2i. White precipitate, 57%. δ_H (d_4-MeOH) 7.9 (2H, m, 2 × aromatic CH), 7.6–7.3 (9H, m, 9 × aromatic CH), 7.1 (1H, m, aromatic CH), 6.9 (1H, m, aromatic CH) 6.7 (1H, m, aromatic CH), 4.5 (1H, m, CH), 4.3 (2H, m, 2 × CH), 3.1 (6H, m, 3 × CH_2), 2.0–1.4 (18H, br m, 9 × CH_2). ν_{max} (film) cm^{-1} 3450–3350 (OH), 3334 (CONH), 2944, 2831 (CH_2), 1653 (C = O amide, C = O aryl acid), 1449 (CH_2), 757 (5H, Ph and 3H, Ph). M/z 997 (M − H), 498 (M − 2H). HRMS $C_{49}H_{54}O_{16}N_7$ (M − H) = 996.3627; found 996.3621.

Biological Assays

The anti-HIV activity and cytotoxicity of compounds was assessed in C8166 cells infected with HIV-1 111B. Microtiter well plates were used to mix 4,00,000 cells with five fold dilutions of compounds in growth medium before adding virus at the multiplicity of infection of 10 CCID50 (50% cell culture infectious dose). The inhibition of infection was monitored by examining syncytia, by measuring cell viability using the XTT-formazan method,[16] and by estimating viral antigen p24 by ELISA (enzyme linked immunosorbent assay) using commercial kits (from Coultar) and methods provided by them. The antigen assay is most sensitive and was used for calculating the values for EC_{50}. The TC_{50} was calculated by the XTT method (Table I).[17]

TABLE I Assay results for lysine oligomers

	C8166 cells	
Compound	EC_{50} (μM)	TC_{50} (μM)
Monomer		
7	>200	200
1a	>100	250
1b	40	100
1d	>500	>500
1e	50	125
1f	>100	250
Dimer		
12	500	1000
2a	>250	250
2c	10	250
2f	25	>250
2g	10	500
Spacer		
15	80	200
16a	100	400
16e	10	40
Trimer		
17	200	300
17a	100	200
17d,e	62	250

EC_{50} is the effective concentration to reduce viral load by 50%. TC_{50} is the concentration that kills 50% of uninfected cells.

RESULTS

Synthesis

Preparation of the Lysine Monomers (Figure 2)

Commercially available N-α-Cbz-L-lysine **6** was protected as the benzyl ester **7**, with concomitant formation of the hydrochloride salt using thionyl

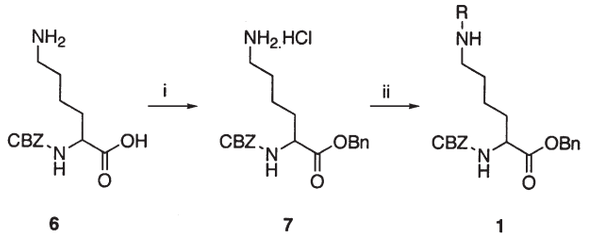

FIGURE 2 (i) BnOH, $SOCl_2$, 67%, (ii) anhydride or dioxalanone, Et_3N. **1a**, 47%; **1b**, 18%; **1d**, 13%; **1e**, 9%; **1f**, 7%.

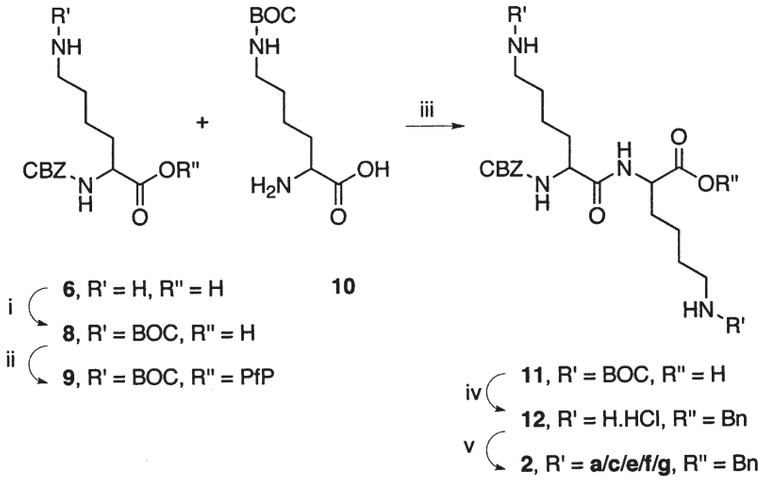

FIGURE 3 (i) $NaHCO_3$, $(BOC)_2O$, THF/H_2O, 86%, (ii) PfP, DCC, EtOAc, 61%, (iii) Et_3N, EtOAc, 79%, (iv) $SOCl_2$, BnOH, 50C, 67%, (v) anhydride or dioxalanone, Et_3N, DMAP. **2a**, 26%; **2c**, 20%; **2f**, 45%; **2g**, 23%.

chloride in good yields. Treatment of the protected lysine monomer **7** with succinic anhydride, aconitic anhydride, 3-hydroxyphthalic anhydride and the dioxalanone **5f** at 60°C gave the required products. It was necessary to carry out the acylation using *cis*-aconitic anhydride at room temperature in order to minimise the *in situ* decarboxylation of the product. Acylation with *cis*-aconitic anhydride and 3-hydroxyphthalic anhydride could give rise to regioisomers. *cis*-Aconitic gave rise to only one regioisomer **1b**, whilst two regioisomers could be isolated from the reaction with 3-hydroxyphthalic anhydride. The regiochemistry of **1b**, **1d**, and **1e** was tentatively determined by comparison of NMR spectra with that predicted by ACD labs NMR prediction software (ACD labs 2.03, Toronto, Canada, Nov 96).

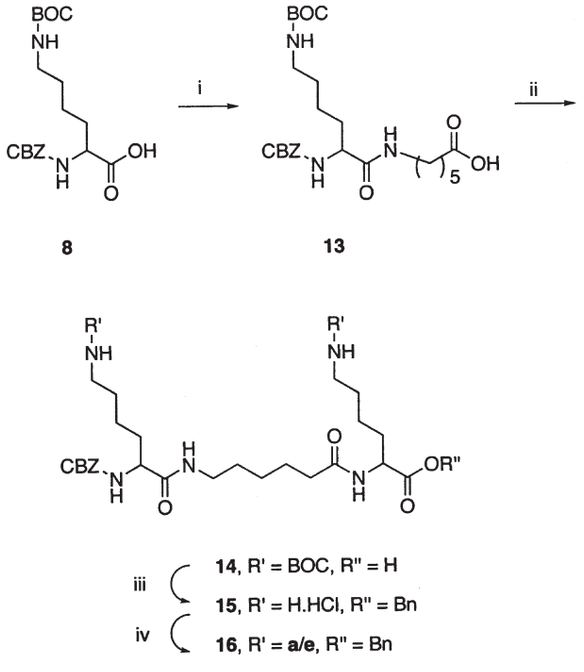

FIGURE 4 (i) TPTU, DIPEA, dioxane, 6-aminohexanoic acid, 90%, (ii) TPTU, DIPEA, **10**, 90%, (iii) $SOCl_2$, BnOH, 81%, (iv) anhydride, Et_3N, dioxane, **16a**, 47%; **16e**, 9%.

Preparation of Lysine Dimers (Figure 3)

The lysine dimers were prepared by coupling using pentafluorophenol activation of the lysine derivative **8** followed by coupling with N-ε-BOC-L-lysine **10**. The carboxylic acid group of the resultant dimer (**11**) was esterified as the benzyl ester together with concomitant BOC deprotection (**12**). The resultant diamines were then acylated. In several cases the products were contaminated with triethylamine; this was removed by treatment with Dowex ion exchange acidic cationic resin.

Preparation of Lysine Dimers Separated by Spacers (Figure 4)

A series of compounds were also made in which the lysines were separated by spacers. The pentafluorophenol methodology outlined above did not prove successful for preparation of these molecules; however use of TPTU[15] gave successful formation of the required scaffold which was then acylated. Thus N-α-CBZ-N-ε-BOC-L-lysine (**8**) was activated with TPTU and then coupled with 6-aminohexanoic acid. The resultant product was then activated again with TPTU and coupled with N-ε-BOC-L-lysine (**10**) to give the required scaffold. C-terminal protection with concomitant BOC deprotection (**15**) followed by acylation with succinic anhydride and 3-hydroxyphthalic anhydride gave the required products (**16a** and **16e** respectively). Attempts to introduce

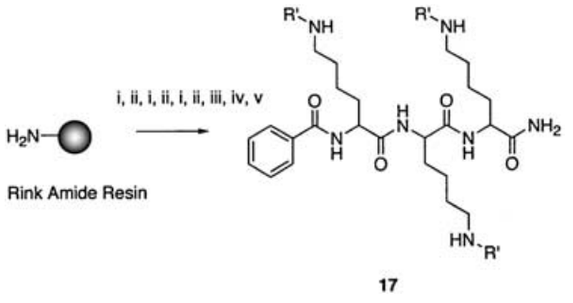

FIGURE 5 (i) N-α-Fmoc-N-ε-BOC-L-lysine, TBTU, HOBT, DIPEA, DMF, (ii) 20% piperidine, DMF. (iii) benzoic acid, TBTU, HOBT, DIPEA, DMF. (iv) 5% TFA in CH_2Cl_2 then 95% TFA in CH_2Cl_2, (v) anhydride, Et_3N, DMF. **17a**, 41%; **17d,e**, 57%.

glycine as a spacer by this methodology were unsuccessful, as were attempts to introduce 4-aminobenzoic acid as a spacer using BOP reagent.

Preparation of Lysine Trimers (Figure 5)

The lysine trimer scaffold was successfully prepared using the TPTU methodology. Acylation was successfully carried out with succinic anhydride. However the methodology was greatly improved when the trimers were prepared on solid phase using standard peptide synthesis technology. It was noted that the 3-hydroxyphthalate derived amides were slightly unstable, decomposing to the cyclic imide. Therefore compounds prepared by this route were treated with care, and column chromatography of the final products was avoided. The products were prepared as the C-terminal amides.

Antiviral Activity

Compounds were assayed against acute HIV-1 infection using C8166 cell lines infected by HIV-1 IIIB (Table I).

The acylated monomers showed little anti-HIV activity, with only the *cis*-aconitic (**1b**) and 3-hydroxyphthalic (**1e**) derivatives showing weak activity against HIV-infected C8166 cells. For the dimers the *cis*-aconitic (**2c**) and allyl dioxalanone (**2g**) derivatives showed activity at 10 μM and the methyl dioxalanone (**2f**) at 25 μM. With a spacer, the hydroxylphthalic derivative **16e** showed some activity in C8166 cells; however this was accompanied by an increase in toxicity. The hydroxyphthalic trimers (**17d**,**e**) showed activity at 62 μM.

Comparing results between classes of compounds, the following trends can be observed: the succinic derivatives showed little activity as monomer, dimer or trimer (**1a**, **2a**, **16a**, **17a**); the methyl dioxalanones showed an increase in activity on going from a monomer to a dimer (**1f**, **2f**,); the trends with hydroxyphthalic and aconitic derivatives is less clear partially due to different isomeric forms predominating during the synthetic procedures.

CONCLUSION

A series of lysine monomers, dimers and trimers have been prepared and acylated with various anhydrides and dioxalanones as simplified mimics of acylated proteins. Some of the resultant compounds showed weak anti-HIV activity against HIV-infected C8166 cells. These mimics produce insufficient activity for further study. Probably larger acylated lysine oligomers are required for good activity and these compounds may be subject to delivery problems.

Acknowledgements

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