Synthesis of pentenoic acid analogs as potential anti-influenza agents

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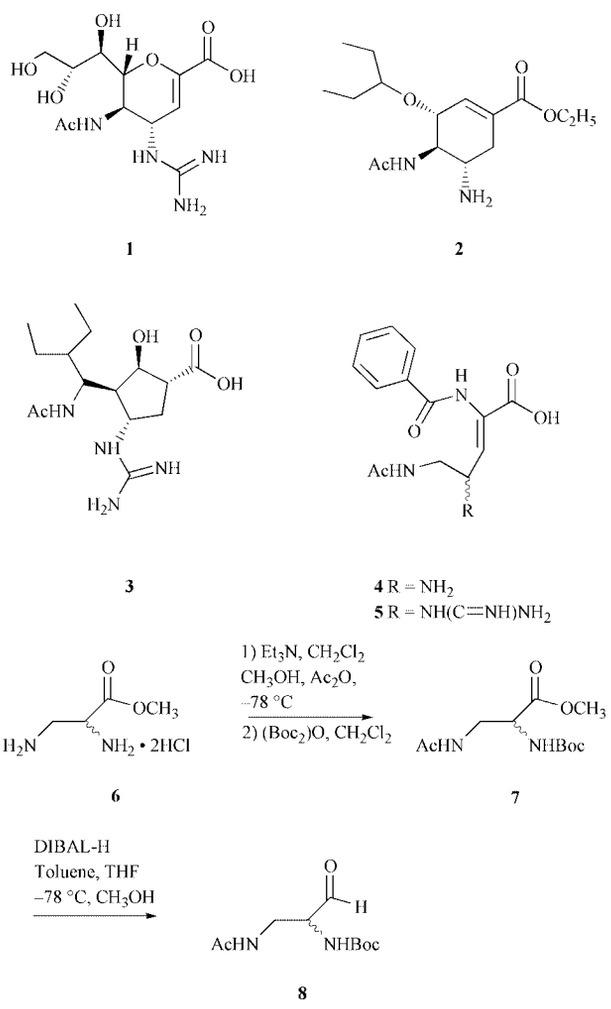
The synthesis of (2*Z*,4*RS*)-5-acetamido-4-guanidino-2-benzamidopent-2-enoic acid (sodium salt) **5** is reported. Wittig–Horner olefination of diamino protected propionaldehyde **8** with phosphonoglycine trimethyl ester **11** provided (*Z*)-olefin methyl ester **12**. The methyl ester was converted to allyl ester **15** by initial hydrolysis with lithium hydroxide followed by reaction with allyl bromide. Target compound **5** was prepared from allyl ester **15** by initial formation of bis(Boc)guanidino ester **16** followed by treatment with tetrakis(triphenylphosphine)palladium(0). The product was evaluated for activity against influenza neuraminidase and was found to be weakly active.

Introduction

Viral neuraminidase (NA) inhibitors have currently emerged as promising therapeutics for the treatment of influenza.[1–3] The approval of two such inhibitors for influenza, Relenza **1** (Zanamivir by Glaxo Wellcome/Biota) and Tamiflu **2** (Oseltamivir by Hoffman–La Roche/Gilead), further underscores the importance of NA as a valid anti-influenza drug target.[4,5] Neuraminidase is an exoglycohydrolase thought to facilitate the spread of influenza virus through the sialic acid rich mucous layer in the upper respiratory airways. Unlike amantadine and rimantadine,[6,7] inhibitors of influenza neuraminidase are effective in blocking both influenza A and B. Relenza and Tamiflu have been approved for the treatment and prevention of both influenza types. Another NA inhibitor, RWJ-270201 (BCX-1812) **3**, is under phase III trial in North America and Europe.[8–11]

Our intent became to investigate the neuraminidase inhibitory activity of modified ring-opened versions of **1**. In addition to presenting key functional groups in the proper orientation, it was hoped that these compounds would show improved absorption *via* uptake by one intestinal dipeptide transport system. In conjunction with opening the ring, the 5,6-bond of the dihydropyranyl moiety was altered to give a benzoylamido moiety in place of a 2,3,4-trihydroxybutyloxy moiety. Such compounds in our rationale would only be of interest if the olefin geometry between the carboxylic acid and guanidino or amino moieties were analogous to **1**. This would suggest that selective preparation of *Z*-olefin analogs would be of higher interest. In this regard, we chose racemic (2*Z*,4*RS*)-5-acetamido-4-amino-2-benzamidopent-2-enoic acid **4** and (2*Z*,4*RS*)-5-acetamido-4-guanidino-2-benzamidopent-2-enoic acid **5** as our target molecules. We now report on the successful preparation of **5** as the sodium salt and its evaluation for activity against influenza neuraminidase.



1 **2** **3** **4** R = NH_2, **5** R = NH(C=NH)NH_2 **6** **7** **8**

Scheme 1

Results and discussion

An efficient route to aldehyde **8** was initially required. The aldehyde was prepared in two steps from methyl (2*RS*)-2,3-diaminopropionate[12] **6** as shown in Scheme 1. Reaction of **6** with acetic anhydride initially at −78 °C followed by the addition of Boc anhydride provided a 84% yield of ester **7**. The use of methanol was found to be necessary for solubility as poorer selectivity and lower yields were observed in its absence. Aldehyde **8** was then obtained in 33% yield from **7** by reduction with DIBAL-H in THF[13] at −78 °C. Conditions to quench the reaction were carefully considered with a combination of methanol and hydrous sodium sulfate giving optimal results. Toluene azeotrope was used on the chromatographically pure material to remove traces of undesired dimethyl acetal and provide homogeneous **8**.

Commercially available (±)-*N*-(benzyloxycarbonyl)phosphonoglycine trimethyl ester **9** was reduced to amino ester **10**

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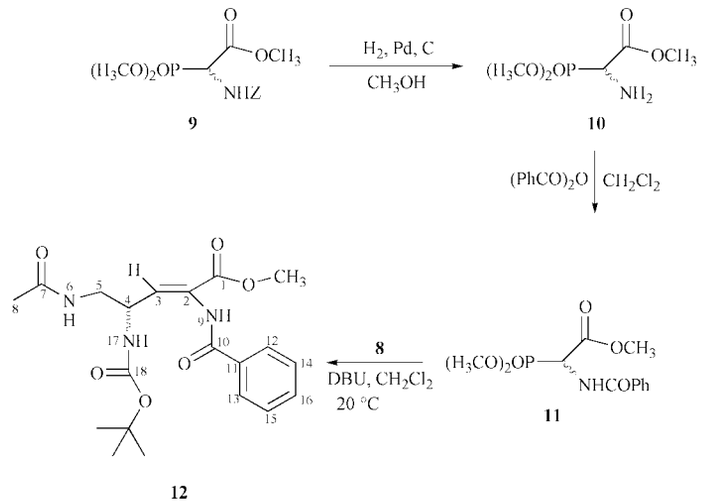
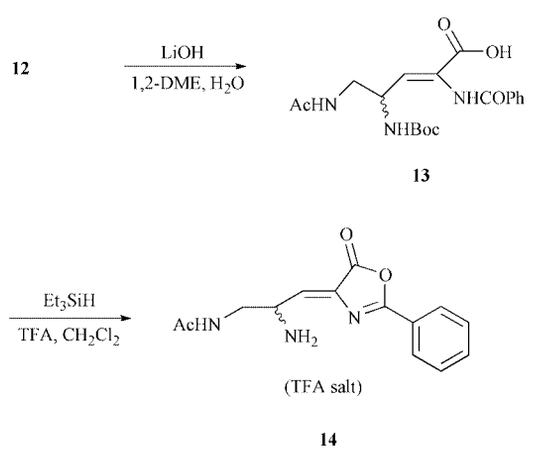


Scheme 2

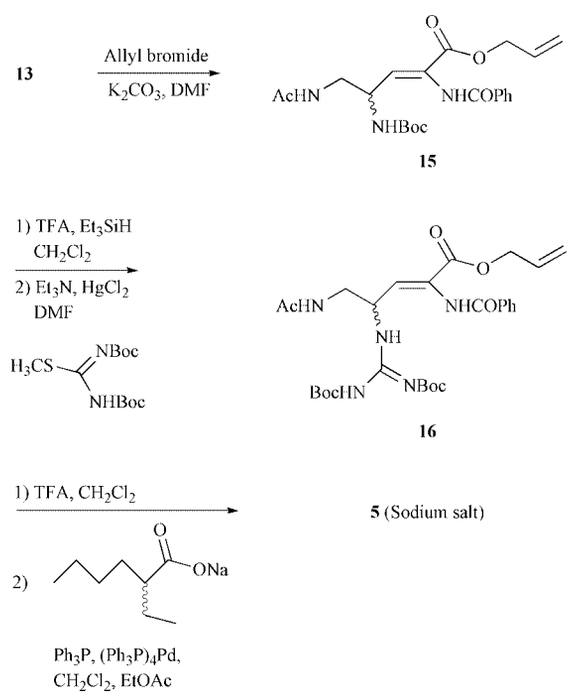
catalytically and immediately reacted with benzoic anhydride to give a 65% overall yield of (±)-*N*-benzoyl-α-phosphonoglycine trimethyl ester **11** as shown in Scheme 2. Wittig–Horner olefination[14] with aldehyde **8** at −20 °C in the presense of DBU provided the *Z* isomer **12** in 73% yield. Only a trace of the *E* isomer was obtained, which was readily removed by silica gel chromatography (1% methanol in methylene chloride). Olefin selectivity was verified by a ^{1}H NMR nuclear Overhauser enhancement (NOE) experiment. When the H-9 (10.07 ppm) proton in DMSO-d_6 was irradiated, NOE's were observed to H-4 and H-12,13 (see H numbering designation in Scheme 2), confirming that **12** was of the desired *Z* configuration.

Treatment of **12** with 2 equivalents of lithium hydroxide in 1,2-dimethoxyethane gave a 93% yield of acid **13** (Scheme 3). It



Scheme 3

was hoped that removal of the *N*-Boc protecting group with TFA would generate amino acid **4**. However, the TFA salt of azlactone **14** was isolated in 86% yield. This finding suggested that acidic removal of the Boc protecting group was not feasible in the preparation of **4** as intramolecular cyclization resulted instead. Attempts to hydrolyze **14** with base to produce **4** were unsuccessful. Since the use of base was problematic in the attempted synthesis of **4**, an alternative allyl ester was examined because of the mild palladium catalyzed[15,16] deprotection this group affords to give the target molecule. Treatment of **13** with allyl bromide[17] gave allyl ester **15** in 70% yield (Scheme 4). The ester was treated with TFA followed by *N*,*N*′-bis(Boc)-*S*-methylisothiourea [18] to give the bis(Boc)guanidino protected analog **16** in 50% yield. Removal of the guanidino *N*-Boc protecting groups with TFA followed by treatment of the resulting intermediate with tetrakis(triphenylphosphine)-palladium(0)[19] in the presence of sodium 2-ethylhexanoate [20–22]



Scheme 4

provided a 91% yield of **5**, isolated as the sodium salt. Attempts to obtain **4** using methodology analogous to that outlined in Scheme 4 were not successful.

Analogs **5** and **14** were assayed for activity against influenza neuraminidase by modification of a fluorescence assay as described by Woods *et al.*[23] Compound **5** was found to give 33% inhibition of enzyme, whereas analog **14** was devoid of inhibition. These results indicated that moderate activity was preserved by preparing a ring-opened version of **1** in conjunction with substituting oxygen for nitrogen at position 2.

Experimental

All melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. IR spectra were obtained on a Nicolet 510P FT-IR spectrometer. ^{1}H NMR spectra were obtained on a GE QE-300 spectrometer at 300.15 MHz in the solvent indicated. *J* values are given in Hz. ^{13}C NMR were obtained on a Bruker Avance 300 MHz instrument with a carbon frequency of 75 MHz. Unless otherwise indicated, mass spectra were field desorption (FD) recorded on a VG Analytical ZAB-3F instrument. High resolution mass spectra (HRMS) were obtained on a Micromass Q-tof II

instrument, positive ion electrospray. Elemental analyses were performed by the Physical Chemistry Department at Lilly Research Laboratories. Chromatography was performed using silica gel 60, partical size 0.040–0.063 mm, 230–400 mesh ATM from EM Science. Unless otherwise indicated, products were dried in high vacuum at 60 °C (<1 mmHg). TLC analyses were done on silica gel 60 precoated plates, EM Science, with a layer thickness of 250 μm.

For the neuraminidase fluorescent assay, an enzyme source of UV-inactivated Influenza B/Great Lakes crude spent culture broth was mixed with inhibitor, buffer [32.5 mM 2-morpholinoethanesulfonic acid (MES)] and 4.0 mM $CaCl_2$ monohydrate at pH 6.5, and reaction was initiated by addition of substrate (37.5 μM 2′-(4-methylumbelliferyl)-α-D-*N*-acetylneuraminic acid).[24] The reaction mixture was incubated for 1 h in Dynatech Microlite 2 plates for fluorescent measurements and terminated with 10 mM $ZnCl_2$. 4-Methylumbelliferone was quantified with an ICN Titertek Fluoroscan II Fluorometer (filters for excitation 360, emission 460) and the percent inhibition relative to no inhibitor control was determined.

Methyl (2*RS*)-3-acetamido-2-(*N*-*tert*-butoxycarbonylamino)-propionate 7

To a stirred suspension of **6** (9.0 g, 47.1 mmol) in CH_2Cl_2 (1.5 dm^3) at −78 °C under N_2 was added triethylamine (27.0 cm^3, 0.26 mol) followed by methanol (1.5 dm^3). The mixture was strirred for 30 min and then brought to −40 °C. To the clear solution was added acetic anhydride (3.3 cm^3, 35 mmol), the mixture was stirred for 30 min and then placed in an ice bath for 2 h. A solution of di-*tert*-butyl dicarbonate (20.4 g, 93.5 mmol) in CH_2Cl_2 (150 cm^3) was added, the solution was brought to room temperature, and stirred for 16 h. The solution was diluted with ethyl acetate (4 dm^3) and concentrated at 35–40 °C to a paste. The paste was partitioned between ethyl acetate (1 dm^3) and brine (1 dm^3). The organic layer was dried (Na_2SO_4) and evaporated to give an oil of the crude product. Crystallization from diethyl ether–hexanes gave **7** (7.7 g, 84% based on acetic anhydride used). Recrystallization from diethyl ether–hexanes provided an analytical sample, mp 121–123 °C; ν_{max}(KBr)/cm^{-1} 3320, 3252, 1739, 1680, 1006; δ_H($CDCl_3$) 1.45 (9 H, s), 1.98 (3 H, s), 3.63 (2 H, t, *J* 6.0), 3.80 (3 H, s), 4.36 (1 H, m), 5.46 (1 H, m), 5.95 (1 H, m); δ_C($CDCl_3$) 22.9 (q), 27.9 (q), 28.2 (q), 28.5 (q), 41.8 (t), 52.6 (q), 53.9 (d), 80.3 (s), 155.7 (s), 171.0 (s), 171.2 (s); *m*/*z* 261 (M^+) (Found: C, 51.03; H, 7.74; N, 10.57. $C_{11}H_{20}N_2O_5$ requires C, 50.76; H, 7.75; N, 10.76%).

(2*RS*)-3-Acetamido-2-(*N*-*tert*-butoxycarbonylamino)propionaldehyde 8

To a solution of **7** (4.7 g, 18.1 mmol) in anhydrous CH_2Cl_2 (400 cm^3) at −70 °C under N_2 was added a solution of 1.0 M DIBAL-H in toluene (49 cm^3, 49 mmol) over 15 min *via* syringe. The resulting solution was stirred for 1 h longer and methanol (5 cm^3) was added. The mixture was brought to room temperature as Na_2SO_4·$10H_2O$ (38 g, 0.11 mmol) was added. The mixture was stirred for 30 min at room temperature and the solvent was decanted. The solid was suspended and sonicated in ethyl acetate (400 cm^3) for 30 min. The solvent was again decanted. This procedure was repeated using 1 : 1 ethyl acetate–acetonitrile (400 cm^3). The combined solutions were evaporated to give a residue which was chromatographed over silica gel (2% acetonitrile in ethyl acetate) to give a dark yellow oil. The oil was evaporated repeatedly under reduced pressure with toluene (3 × 25 cm^3) to provide homogeneous **8** (1.37 g, 33%). The aldehyde was unstable to prolonged standing and vacuum drying at 60 °C, but could be stored in the freezer overnight; δ_H($CDCl_3$) 1.42 (9 H, s), 2.00 (3 H, s), 3.60–3.80 (2 H, m), 4.25 (1 H, m), 5.80 (1 H, m), 6.20 (1 H, m), 9.65 (1 H, s); *m*/*z* 231 (M^+).

(±)-*N*-Benzoyl-α-phosphonoglycine trimethyl ester 11

A solution of (±)-*N*-(benzyloxycarbonyl)-α-phosphonoglycine trimethyl ester (500 mg, 1.50 mmol) in methanol (50 cm^3) containing 5% palladium on carbon (50 mg, 0.02 mmol) was hydrogenated at 45 psi † for 1.5 h. The solvent was evaporated to an oil of homogeneous **10** (266 mg, 89%); δ_H($CDCl_3$) 1.80 (2 H, br s), 3.73 (6 H, s), 3.80 (3 H, s), 3.96 (1 H, d, *J* 12). A mixture of **10** (254 mg, 1.28 mmol) and benzoic anhydride (290 mg, 1.28 mmol) in CH_2Cl_2 (7 cm^3) was stirred for 4 h at room temperature. The solution was diluted to 20 cm^3 with CH_2Cl_2 and partitioned with 20 cm^3 each of 1.0 M potassium bisulfate, saturated aqueous sodium bicarbonate, brine, and dried (Na_2SO_4). The solvent was evaporated to a paste which was crystallized from ethyl ether–hexanes to give **11** (250 mg, 65%). A portion was chromatographed over silica gel (3 : 2 ethyl acetate–hexanes) to give homogeneous ester, crystallized from diethyl ether; mp 112–114 °C; ν_{max}(KBr)/cm^{-1} 3298, 1737, 1669, 1292, 1034; δ_H($CDCl_3$) 3.85 (9 H, m), 5.45 (1 H, dd, *J* 9, 22), 6.89 (1 H, br d, *J* 9), 7.50 (3 H, m), 7.84 (2 H, d, *J* 7); δ_C($CDCl_3$) 49.5 (d, *C*HP=O, $J_{^{31}P-^{13}C}$ 148), 53.4 (q), 54.0 (q, *C*H_3OP=O, $J_{^{31}P-^{13}C}$ 6), 54.2 (q, *C*H_3OP=O, $J_{^{31}P-^{13}C}$ 6), 127.3 (d), 128.6 (d), 132.2 (d), 133.1 (s), 166.8 (s, PCH*C*=O, $J_{^{31}P-^{13}C}$ 5), 167.2 (s); *m*/*z* 301 (M^+) (Found: C, 48.15; H, 5.15; N, 4.70. $C_{12}H_{16}NO_6P$ requires C, 47.85; H, 5.35; N, 4.65%).

Methyl (2*Z*,4*RS*)-5-acetamido-4-(*N*-*tert*-butoxycarbonylamino)-2-benzamidopent-2-enoate 12

To a stirred solution of **11** (350 mg, 1.16 mmol) in CH_2Cl_2 (15 cm^3) at −20 °C under N_2 was added DBU (0.19 cm^3, 1.27 mmol). The solution was stirred for 10 min and **8** (267 mg, 1.16 mmol) in CH_2Cl_2 (10 cm^3) was added over 5 min by syringe. The yellowish solution was stirred for 1 h, brought to room temperature, and stirred for 1 h longer. The solution was diluted to 125 cm^3 with CH_2Cl_2 and extracted with brine (125 cm^3). The aqueous layer was extracted with CH_2Cl_2 (125 cm^3) and the combined organics were dried (Na_2SO_4). The solvent was evaporated and the residue was chromatographed over silica gel (1% methanol in CH_2Cl_2, gradually increasing to 2% methanol in CH_2Cl_2) to give a white foam of **12** (342 mg, 73%); ν_{max}(KBr)/cm^{-1} 3315, 1733, 1661, 1517, 1160; δ_H($CDCl_3$) 1.46 (9 H, s), 2.08 (3 H, s), 3.50 (2 H, m), 3.70 (3 H, s), 4.35–4.45 (1 H, m), 6.07 (1 H, m), 6.10–6.20 (1 H, br s), 6.15 (1 H, d, *J* 12), 7.40–7.60 (3 H, m), 8.02 (2 H, d, *J* 7), 10.07 (1 H, br s); δ_C($CDCl_3$) 22.8 (q), 27.9 (q), 28.2 (q), 28.3 (q), 42.3 (t), 49.4 (d), 52.5 (q), 80.4 (s), 127.8 (d), 128.3 (d), 128.4 (d), 130.8 (s), 132.2 (d), 132.8 (s), 157.0 (s), 166.4 (s), 165.1 (s), 172.7 (s); *m*/*z* 405 (M^+) (Found: C, 59.05; H, 6.65; N, 10.37. $C_{20}H_{27}N_3O_6$ requires C, 59.25; H, 6.71; N, 10.36%).

(2*Z*,4*RS*)-5-Acetamido-4-(*N*-*tert*-butoxycarbonylamino)-2-benzamidopent-2-enoic acid 13

To a solution of **12** (50 mg, 0.12 mmol) in 1,2-dimethoxyethane (5 cm^3) was added an aqueous solution of 0.1 M lithium hydroxide (2.5 cm^3, 0.25 mmol). The solution was stirred for 3 h at room temperature and 0.2 M HCl (1.25 cm^3, 0.25 mmol) was added. The mixture was evaporated to a residue which was dissolved in the solvent mixture ethyl acetate–acetonitrile–acetic acid–water (65 : 7 : 7 : 9, 10 cm^3) and filtered through a pad of silica gel in a sintered glass funnel. The silica gel was backwashed with the above solvent mixture (2 × 10 cm^3) and the combined solutions were evaporated. The addition of ethyl ether–hexanes gave a precipitate which was filtered to provide an amorphous hygroscopic powder of **13** (44 mg, 93%); ν_{max}(KBr)/cm^{-1} 3326, 1659, 1517, 1282, 1164; δ_H($CDCl_3$) 1.40 (9 H, s), 1.98 (3 H, s), 3.30–3.60 (2 H, m), 4.30–4.50 (1 H, m), 6.50 (2 H, m), 7.10 (1 H, br s), 7.40–7.60 (3 H, m), 8.03 (2 H, d, *J* 7),

† 1 psi = 6894.76 Pa.

10.35 (1 H, br s); δ_C($CDCl_3$) 22.8 (q), 28.1 (q), 28.3 (q), 42.3 (t), 49.2 (d), 80.4 (s), 127.8 (d), 128.6 (d), 130.2 (s), 131.5 (d), 132.0 (d), 132.6 (s), 157.0 (s), 166.5 (s), 167.4 (s), 173.3 (s); *m*/*z* 392 (M^+) (Found: C, 56.73; H, 6.83; N, 9.93. $C_{19}H_{25}N_3O_6 \cdot 0.75H_2O$ requires C, 56.36; H, 6.54; N, 10.38%).

Azlactone of (2*Z*,4*RS*)-5-acetamido-4-amino-2-benzamidopent-2-enoic acid (TFA salt) 14

To a solution of **13** (30 mg, 0.08 mmol) in CH_2Cl_2 (2 cm^3) at 0 °C was added triethylsilane (0.025 cm^3, 0.16 mmol). The mixture was stirred for 5 min and TFA (1.0 cm^3, 12.98 mmol) was added. The solution was brought to room temperature and stirred for 1 h. The solvent was evaporated to give a residue which was concentrated further with acetonitrile (2 × cm^3). The resulting foam was suspended in ethyl acetate–diethyl ether (1 : 1) and filtered to give a tan amorphous resin of **14** (18 mg, 86%); ν_{max}(KBr)/cm^{-1} 3400, 3000, 1671, 1202; δ_H(DMSO-d_6) 1.81 (3 H, s), 3.20–3.40 (2 H, m), 4.10 (1 H, m), 6.28 (1 H, d, *J* 9), 7.50–7.65 (3 H, m), 7.91 (2 H, d, *J* 8), 8.13 (1 H, t, *J* 6); δ_C(DMSO-d_6) 22.5 (q), 40.3 (t), 47.9 (d), 127.7 (d), 128.1 (s), 128.4 (d), 128.7 (d), 132.0 (d), 132.7 (s), 133.2 (s), 164.8 (s), 166.1 (s), 170.5 (s); *m*/*z* 274 (M^+, free base) (Found: C, 46.85; H, 4.88; N, 9.88. $C_{14}H_{15}N_3O_3 \cdot CF_3COOH \cdot 1.25H_2O$ requires C, 46.89; H, 5.00; N, 10.25%).

Allyl (2*Z*,4*RS*)-5-acetamido-4-(*N*-*tert*-butoxycarbonylamino)-2-benzamidopent-2-enoate 15

A mixture of **13** (188 mg, 0.48 mmol), potassium carbonate (75 mg, 0.54 mmol), and allyl bromide (0.06 cm^3, 0.69 mmol) in DMF (2 cm^3) was stirred at room temperature for 1 h. The mixture was partitioned between ethyl acetate (30 cm^3) and brine (2 × 30 cm^3). The organic layer was dried (Na_2SO_4) and evaporated. The residue was chromatographed over silica gel (3 : 2 ethyl acetate–hexanes) to give a white foam of **15** (144 mg, 70%); ν_{max}($CHCl_3$)/cm^{-1} 3300, 1669, 1510, 1368, 1160; δ_H ($CDCl_3$) 1.44 (9 H, s), 2.04 (3 H, s), 3.40–3.60 (2 H, m), 4.40 (1 H, m), 4.71 (2 H, d, *J* 6), 5.28 (2 H, dd, *J* 9, 21), 5.95 (1 H, m), 6.20 (2 H, m), 6.62 (1 H, m), 7.53 (3 H, m), 8.01 (2 H, d, *J* 8), 10.10 (1 H, br s); δ_C($CDCl_3$) 22.8 (q), 28.1 (q), 28.3 (q), 42.4 (t), 49.5 (d), 66.2 (t), 80.4 (s), 118.5 (t), 127.7 (d), 128.5 (d), 128.7 (d), 130.7 (s), 131.7 (d), 132.2 (d), 132.8 (s), 155.9 (s), 164.2 (s), 166.4 (s), 172.8 (s); *m*/*z* 431 (M^+) (Found: C, 61.41; H, 6.98; N, 9.71. $C_{22}H_{29}N_3O_6$ requires C, 61.24; H, 6.77; N, 9.74%).

Allyl (2*Z*,4*RS*)-5-acetamido-4-[*N*′,*N*″-bis(*tert*-butoxycarbonyl)-guanidino]-2-benzamidopent-2-enoate 16

To a solution of **15** (130 mg, 0.30 mmol) in CH_2Cl_2 (1 cm^3) at 0 °C under N_2 was added triethylsilane (0.08 cm^3, 0.50 mmol). The solution was stirred for 2 min and TFA (1 cm^3, 13.00 mmol) was added. The mixture was stirred for 1 h as it was gradually brought to room temperature, diluted to 5 cm^3 with acetonitrile, and evaporated. The residue was evaporated further with acetonitrile (2 × 5 cm^3) to give an oil of the 4-(*RS*)-deprotected amine trifluoroacetate salt intermediate, used as such without further purification for the subsequent step; ν_{max}(KBr)/cm^{-1} 3600, 3000, 1668, 1180; δ_H(DMSO-d_6) 1.80 (3 H, s), 3.25–3.35 (2 H, m), 4.20 (1 H, m), 4.62 (2 H, m), 4.80 (1 H, m), 5.40 (2 H, dd, *J* 9, 21), 6.00 (1 H, m), 6.35 (1 H, d, *J* 5), 7.55 (3 H, m), 7.72 (2 H, d, *J* 8), 8.36 (1 H, t, *J* 6); *m*/*z* 331 (M^+, free base). The oil thus obtained (133 mg, 0.30 mmol) was dissolved in DMF (2.5 cm^3) under N_2 at 0 °C and triethylamine (0.13 cm^3, 0.93 mmol) was added. The solution was stirred for 2 min and *N*,*N*′-bis(Boc)-*S*-methylisothiourea (87 mg, 0.30 mmol) and mercuric chloride (81 mg, 0.30 mmol) were added. The mixture was brought to room temperature and stirred for 2 h, the suspension was diluted with ethyl acetate (10 cm^3), and filtered through a pad of Celite (sintered glass funnel). The filtrate was extracted with brine (2 × 30 cm^3), dried (Na_2SO_4), and concentrated to a clear oil. Chromatography over silica gel (3 : 2 ethyl acetate–hexanes) gave a clear, colorless foam of **16** (86 mg, 50%); ν_{max}($CHCl_3$)/cm^{-1} 3200, 2960, 1726, 1610, 1125; δ_H($CDCl_3$) 1.29 (9 H, s), 1.48 (9 H, s), 1.97 (3 H, s), 3.40 (2 H, m), 3.65 (1 H, m), 4.68 (2 H, d, *J* 6), 5.25 (2 H, dd, *J* 11, 25), 5.80–6.00 (1 H, m), 6.23 (1 H, d, *J* 10), 7.38–7.57 (3 H, m), 8.00 (2 H, d, *J* 7), 9.60 (1 H, br s), 11.30 (1 H, s); δ_C($CDCl_3$) 22.8 (q), 26.8 (q), 26.9 (q), 27.0 (q), 40.8 (t), 48.6 (d), 65.0 (t), 83.0 (s), 117.2 (t), 127.0 (d), 127.4 (d), 128.4 (d), 129.3 (s), 130.2 (d), 131.9 (d), 132.2 (s), 151.4 (s), 154.3 (s), 162.8 (s), 165.9 (s), 170.2 (s); *m*/*z* 574 (M^+) (Found: C, 58.74; H, 6.80; N, 12.35. $C_{28}H_{39}N_5O_8$ requires C, 58.63; H, 6.85; N, 12.21%).

(2*Z*,4*RS*)-5-Acetamido-4-guanidino-2-benzamidopent-2-enoic acid (sodium salt) 5

A mixture of **16** (75 mg, 0.13 mmol) and TFA (2 cm^3, 26 mmol) was stirred at room temperature for 3 h. The solution was diluted to 5 cm^3 with acetonitrile, evaporated, and concentrated again from acetonitrile (2 × 5 cm^3). The resulting amorphous foam was the monotrifluoroacetate salt of the deprotected guanidino allyl ester (66 mg, 100%); ν_{max}($CHCl_3$)/cm^{-1} 3350, 2975, 1674, 1280, 1230; δ_H(DMSO-d_6) 1.75 (3 H, s), 3.15–3.35 (2 H, m), 4.51 (1 H, m), 4.66 (2 H, d, *J* 6), 5.26 (2 H, dd, *J* 9, 22), 5.80–5.97 (1 H, m), 6.47 (1 H, d, *J* 6), 7.20 (3 H, br s), 7.50 (3 H, m), 7.76 (1 H, d, *J* 6), 7.95 (2 H, d, *J* 7), 8.14 (1 H, t, *J* 5), 9.87 (1 H, s). To a solution of the above monotrifluoroacetate salt (59 mg, 0.12 mmol) in CH_2Cl_2 (1 cm^3) under N_2 was added a solution of sodium 2-ethylhexanoate (42 mg, 0.25 mmol) in ethyl acetate (2 cm^3). To the solution was then added triphenylphosphine (1 mg, 0.004 mmol) and tetrakis(triphenylphosphine)palladium(0) (3.5 mg, 0.003 mmol). The clear yellow solution was stirred for 1 h at room temperature at which point a crystalline solid appeared. The mixture was stirred for 16 h and the suspension was sonicated for 30 min. The mixture was then centrifuged and decanted. The solid was treated with diethyl ether (3.5 cm^3), centrifuged, and decanted to give **5** (39 mg, 91%). A pure sample was prepared by recrystallization from acetonitrile–methanol–diethyl ether; mp 192–198 °C; δ_H(DMSO-d_6) 1.78 (3 H, s), 3.00–3.30 (2 H, m), 4.40 (1 H, m), 6.10 (1 H, d, *J* 6), 7.56 (3 H, m), 7.80–8.15 (4 H, br m), 7.92 (2 H, d, *J* 7), 8.10 (1 H, m), 9.00 (1 H, br s), 9.40 (1 H, br s); δ_C(DMSO-d_6) 22.4 (q), 42.1 (t), 56.0 (d), 127.2 (d), 128.2 (d), 128.4 (s), 128.5 (s), 130.3 (d), 131.2 (d), 159.6 (s), 166.3 (s), 169.8 (s), 172.0 (s); HRMS found MH^+ 334.1513. $C_{15}H_{20}N_5O_4$ requires 334.1515.

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