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Bioorganic & Medicinal Chemistry 13 (2005) 4071–4077
Bioorganic & Medicinal Chemistry

Comparison of the anti-influenza virus activity of cyclopentane derivatives with oseltamivir and zanamivir in vivo

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Received 14 February 2005; revised 24 March 2005; accepted 25 March 2005
Available online 25 April 2005

Abstract—Cyclopentane derivatives, designated as BCX-1812, BCX-1827, BCX-1898, and BCX-1923, were tested in parallel with oseltamivir carboxylate and zanamivir for the in vivo activity in mice infected with A/Turkey/Mas/76 X A/Beijing/32/92 (H6N2) influenza virus. The compounds were tested orally and intranasally at different dose levels. BCX-1812, BCX-1827, and BCX-1923 showed more than 50% protection at 1 mg/kg/day dose level on oral treatment. The intranasal treatment was 100% effective even at 0.01 mg/kg/day for all four compounds. On comparison with oseltamivir carboxylate and zanamivir, these four cyclopentane derivatives have shown equal or better efficacies. The synthesis of two new compounds, BCX-1898 and BCX-1923, is also described.

1. Introduction

Infection by influenza viruses is a major concern of health particularly among young children, the elderly, immuno-compromised individuals, and persons with chronic cardiovascular or respiratory diseases.[1–3] In the United States alone, 20,000–40,000 people die annually of serious complications from influenza, which include pneumonia and worsening of underlying medical conditions. The development of vaccines to prevent or treat influenza infection has been significantly hampered by the high mutability of the virus.[4] The obvious choice remains the development of effective drugs, which are not susceptible to mutation. Although the replication cycle of the influenza virus indicates several potential molecular targets (hemaglutinin,[5,6] neuraminidase,[7] M2 proteins,[8] and endonuclease[9]), neuraminidase (NA) has been extensively targeted for drug design. In general, NA is thought to permit the spread of the virus from cell to cell and from the site of infection.[10] Therefore, compounds that inhibit NA can protect the host from viral infection and retard its propagation.

Analogs of neuraminic acid, such as 2,3-didehydro-2-deoxy-*N*-acetylneuraminic acid (**1**, DANA), are known to inhibit NA in vitro with a K_i value of approximately 4 μM.[11] The crystal structure of NA complexed with sialic acid and DANA was reported previously.[12] Based upon the structure based drug design (SBDD), von Itzstein discovered the 4-guanidino analog of DANA, zanamivir (**2**), which is a potent NA inhibitor.[13–15] Zanamivir received FDA approval in 1999 and is effective against both influenza A and B. Due to its highly polar nature, **2** requires administration by oral inhalation. Another compound, oseltamivir carboxylate [**3**, ethyl (3*R*,4*R*,5*S*)-4-acetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexen-1-carboxylate], reported by Kim et al. was also approved by the FDA in 1999.[16] Oseltamivir carboxylate is effective against both A and B neuraminidases and is orally active, but it has been reported to cause vomiting and nausea. NA inhibitors can also be used as prophylactic agents and oseltamivir carboxylate was recently approved by the FDA for this use. A number of aromatic compounds were also designed to inhibit neuraminidase but none of those compounds was potent enough to go further for clinical trials.[17–20] Based upon SBDD, Abbott Laboratories designed five-membered pyrrolidine derivatives[21–23] (**4**) and we designed cyclopentane derivatives[24–28] (**5**) as potent neuraminidase inhibitors. None of the pyrrolidine derivatives has gone into clinical trials yet, but one of the cyclopentane derivatives, BCX-1812 (RWJ-270201), went as far as clinical trial III, but failed to show statistical difference in efficacy probably due to lower bioavailability. BCX-1812 is highly potent against a wide range of strains of

Keywords: Cyclopentane derivatives; Neuraminidase inhibitors; In vivo activity.
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doi:10.1016/j.bmc.2005.03.048

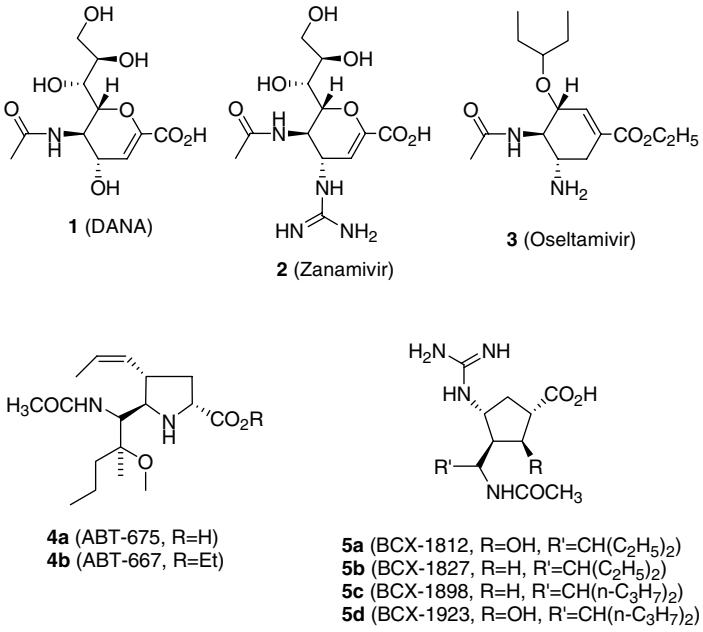


Figure 1.

influenza virus. The detailed structure–activity relationship of the cyclopentane series revealed a number of other compounds, which were equally potent in vitro. A recent report has compared the in vitro activity of BCX-1812 and other analogs (BCX-1827, BCX-1898, and BCX-1923) with zanamivir and oseltamivir.[29] The effect of oral administration of BCX-1812, BCX-1827, and BCX-1923 was compared with zanamivir and oseltamivir carboxylate in ferrets to protect against influenza virus infection.[26]

Our continued efforts to discover a better neuraminidase inhibitor that is effective against both types A and B viruses does not rapidly select for resistance, is safe without side effects, and led us to compare the in vivo activity of these cyclopentane analogs with FDA-approved drugs zanamivir and oseltamivir in mice. Therefore, in this paper, we describe the synthesis of some of these previously unreported cyclopentane derivatives (BCX-1898 and BCX-1923) and present the comparative data from in vivo studies of these four cyclopentane derivatives (BCX-1812, BCX-1827, BCX-1898, and BCX-1923), and both FDA-approved drugs, zanamivir and oseltamivir. Since in vitro activity of these compounds was found to be almost similar to one another, we were interested to see the effect of the presence and absence of the hydroxyl group (BCX-1812 and BCX-1827) and also the effect of incorporation of two carbon atoms in the side chain (BCX-1923 and BCX-1898) on the anti-influenza activity of in vivo studies in mice (Fig. 1).

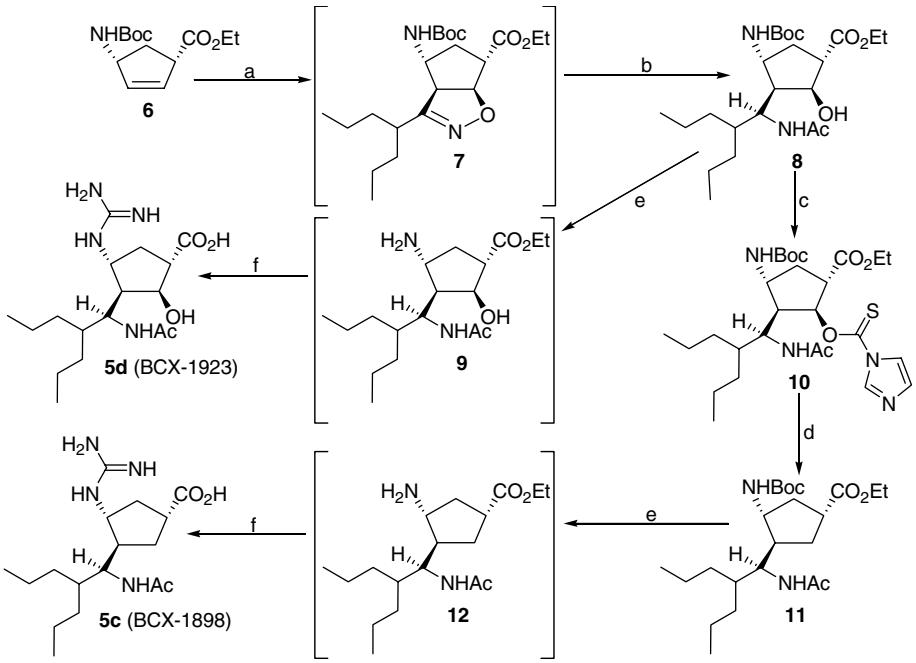
2. Chemistry

The synthesis of compounds **5a** and **5b** has been reported earlier.[25] The synthesis of **5c** and **5d** is illustrated in Scheme 1. Cyclopentene derivative **6**[25] underwent 3+2 cycloaddition reaction with the nitrile oxide derived from 2-propyl-1-nitropentane prepared from 2-propyl-1-bromopentane[30] to give cycloadduct **7** the same way as reported by us earlier on the synthesis of BCX-1812.[25] Other isomers of **7** generated during the reaction were removed by chromatography. After chromatography, compound **7** was still contaminated with some by-products generated from phenyl isocyanate; therefore, it was used for the next reaction without further purification.

The isoxazoline ring of **7** was then opened by hydrogenolysis in methanol in the presence of PtO_2 and an equivalent amount of HCl at 100 psi followed by acetylation to give **8**. The opening of the isoxazoline ring favored the formation of the desired isomer as discussed in our previous publication.[25] Compound **8** was treated with hydrochloric acid in ether for removal of the *tert*-butoxycarbonyl group and the resultant amine **9** was isolated as hydrochloride. Amine **9** was taken for guanylation with pyrazole carboxamidine hydrochloride in the presence of diisopropylethylamine to give the desired guanidine compound, which upon basic hydrolysis of ester with NaOH yielded the target **5d**. The next target was deoxygenated product **5c**, which was obtained from **8**. Compound **8** on reaction with thiocarbonyl diimidazole yielded **10**, which on free radical reaction with tributyltin hydride in the presence of AIBN toluene at 100 °C gave deoxygenated product **11**. Compound **11** was treated with hydrochloric acid in ether for removal of the *tert*-butoxycarbonyl group, and the resultant amine **12** on guanylation with pyrazole carboxamidine hydrochloride and basic hydrolysis gave the desired target **5c**.

3. Results and discussion

The ability of BCX-1812 (RWJ-270201), BCX-1827, BCX-1898, and BCX-1923 to inhibit the NA activity



Scheme 1. Reagents: (a) $O_2N–CH_2–CH$ (n-C_3H_7)$_2$, PhNCO, Et_3N; (b) i. PtO_2, H_2, HCl; ii. Ac_2O, Et_3N; (c) thiocarbonyldiimidazole; (d) $(Bu)_3SnH$, AIBN; (e) HCl, ether; (f) i. Pyrazole-carboxamidine·HCl; ii. NaOH.

of several influenza A (19 different strains of H1N1, H3N2, and H5N1) and influenza B (five different strains) viruses was tested and compared to those of zanamavir and oseltamivir in a recent publication.[29] The IC_{50} values of BCX-1812, BCX-1827, BCX-1898, and BCX-1923 ranged from <0.01 to 21.0 μM for influenza A strains and from 0.02 to 8.0 μM for influenza B strains, which is comparable to oseltamivir and zanamivir.

In the mouse influenza model, viral infection leads to loss of body weight and high mortality, and this decrease in body weight correlates with the pulmonary viral titer and the pulmonary lesion score. Therefore, the efficacy of all of these compounds was evaluated on the basis of weight loss and survival rate measured for 21 days post-infection, for treated infected animals relative to untreated infected (control) animals.

A comparison of efficacy of BCX-1827 and BCX-1898 with the oseltamivir in vivo mouse influenza model (oral treatment) shows that at a dose of 10 mg/kg/day, all of these compounds showed complete protection against the influenza virus (Table 1). When the dose was lowered to 1 mg/kg/day, BCX-1827 and oseltamivir showed 40–50% protection (measured by mean day to death), whereas BCX-1898 at this dose level (1 mg/kg/day) showed only a 10% protection against the influenza virus. In the vehicle treated group, there were no survivors.

In another experiment (Table 2), the oral efficacy of BCX-1827 and BCX-1923 was compared with BCX-1812. In all of the three drug groups, the 10 mg/kg/day group demonstrated complete protection against lethality. At 1 mg/kg/day, all of the drug groups showed comparable efficacy: BCX-1827 and BCX-1923 demonstrated 80% protection and BCX-1812 showed 70% protection; 0% survival in the vehicle-treated group. All of the drugs also showed a dose response relationship when

Table 1. Comparison of efficacy of BCX-1827 and BCX-1898 with oseltamivir in the in vivo mouse influenza model (oral treatment)

Drug	Dose, mg/kg/day (q.d.)	Survivors/total	Mean day to death[a]
BCX-1827	10	10/10**	
	1	5/10*	8.6 + 1.36*
BCX-1898			
	10	9/9**	
	1	1/10	10.0 + 0.99*
Oseltamivir	1	4/10*	11.0 + 0.83**
Vehicle	—	0/10	6.3 + 0.26
Vehicle uninfected	—	5/5	

*$p < 0.05$, **$p < 0.001$ versus saline, infected.
[a] Mean day to death of mice dying prior to day 22.

Table 2. Comparison of efficacy of BCX-1827 and BCX-1923 with RWJ-270201 in the in vivo mouse influenza model (oral treatment)

Drug	Dose, mg/kg/day (q.d.)	Survivors/total	Mean day to death[a]
BCX-1812	10	9/9**	
	1	7/10*	7.0 + 0.58
BCX-1923			
	10	10/10**	
	1	8/10**	7.0 + 1.0
BCX-1827	10	8/8**	
	1	8/10**	7.5 + 0.50
Vehicle infected	—	0/10	6.8 + 0.29
Saline uninfected	—	4/4	

*$p < 0.05$, **$p < 0.001$ versus saline, infected.
[a] Mean day to death of mice dying prior to day 22.

the weight loss of infected mice was followed over time. In general, a lower dose resulted in greater weight loss while a higher dose resulted in lower weight loss. These oral treatment experiments suggest that all four cyclopentane derivatives have comparable efficacy at 10 mg/kg/day with the FDA-approved drug, oseltamivir carboxylate, while at the lower dose level of 1 mg/kg/day, BCX-1898 is less efficacious but the other three (BCX-1812, BCX-1827, and BCX-1923) are comparable.

For intranasal experiments, BCX-1898 and BCX-1923 were compared first to oseltamivir and zanamivir (Table 3). At the 0.1 mg/kg/day dose, complete protection against lethality was observed for all of the drugs. At 0.01 mg/kg/day, both BCX-1898 and BCX-1923 demonstrated complete protection (10 out of 10 survived) and in the oseltamivir carboxylate group, nine out of 10 survived. However, in the zanamivir group only two out of nine mice survived. In the vehicle-treated group, none of the 10 mice survived and the mean day to death was 8.4 days. In the second experiment, BCX-1827 and BCX-1923 were compared to BCX-1812 and oseltamivir carboxylate (Table 4). At 0.01 mg/kg/day, complete protection against lethality was observed in all drug groups except for the oseltamivir carboxylate group where only four out of 10 survived. At 0.001 mg/kg/day, both BCX-1827 and BCX-1923-treated groups demonstrated efficacy (four out of nine and five out of 10 survived), whereas BCX-1812 and oseltamivir carboxylate treated groups demonstrated no significant efficacy (two out of 10 survived). In the vehicle-treated group, none of the 10 mice survived and the mean day to death was 7.4 days. These data suggest that BCX-1827, BCX-1923, and BCX-1812 have superior activity to zanamivir at the 0.01 mg/kg/day dose level, and probably with oseltamivir carboxylate also when given intranasally in the mouse influenza model.

These studies suggest that all four cyclopentane derivatives are 100% effective intranasally even at the 0.01 mg/kg/day dose level and show better activity than zanamivir. When given orally, BCX-1812, BCX-1827, and BCX-1923 demonstrate complete protection at 10 mg/kg/day and about 50% protection at 1 mg/kg/day, which is comparable to zanamivir and oseltamivir. It seems that the effect of removing hydroxyl from BCX-1812 and increasing two carbon atoms in the side chain does not make any difference in in vivo activity. However, unsatisfactory Phase III clinical results with BCX-1812 compel us to believe that the other two compounds, BCX-1827 and BCX-1923, may also not be orally bioavailable because of their similar structures and the activity of these compounds in mice. On the other hand, these compounds might have very good chances of becoming a drug if other routes of administration, such as intranasal as investigated in this study, are used. Similarly, different administration routes, such as intravenous, intramuscular, and inhalation, may also be investigated. Another possibility is the investigation of prodrugs, since these molecules have carboxylic acid, guanidino, and a hydroxyl group (BCX-1812 and BCX-1923) present in the molecule required for prodrug preparation.

Table 3. Comparison of efficacy of BCX-1898 and BCX-1923 with oseltamivir carboxylate and zanamivir in the in vivo mouse influenza model (intranasal treatment)

Drug	Dose mg/kg/day (q.d.)	Survivors/total	Mean day to death[a]
BCX-1898	0.1	10/10**	
	0.01	10/10**	
BCX-1923	0.1	10/10**	
	0.01	10/10**	
Oseltamivir carboxylate	0.1	10/10**	
	0.01	9/10**	19.0 + 0.0*
Zanamivir	0.1	10/10**	
	0.01	2/9	11.3 + 1.4*
Saline	—	0/10	8.4 + 0.27
Saline uninfected	—	5/5	

*$p < 0.05$, **$p < 0.001$ versus saline, infected.
[a] Mean day to death of mice dying prior to day 20.

Table 4. Comparison of efficacy of BCX-1827 and BCX1923 with BCX-1812 and oseltamivir carboxylate in the in vivo mouse influenza model (intranasal treatment)

Drug	Dose, mg/kg/day (q.d.)	Survivors/total	Mean day to death[a]
BCX-1812	0.01	10/10**	
	0.001	2/10	10.9 + 0.93**
BCX-1827	0.01	10/10**	
	0.001	4/9*	10.2 + 0.73**
BCX-1923	0.01	10/10**	
	0.001	5/10*	11.6 + 1.96*
Oseltamivir carboxylate	0.01	4/10*	10.7 + 0.80**
	0.001	2/10	9.25 + 0.65*
Vehicle	—	0/10	7.4 + 0.22
Vehicle uninfected	—	5/5	

*$p < 0.05$, **$p < 0.001$ versus saline, infected.
[a] Mean day to death of mice dying prior to Day 21.

4. Conclusion

In conclusion, we have discovered four cyclopentane derivatives, which have similar or better efficacy in vivo in comparison with zanamivir and oseltamivir when given orally and intranasally. The compounds have great promise to become drugs, if administration routes other than oral are chosen or prodrugs are investigated.

5. Experimental

5.1. Mouse influenza model

Mice (13–18 g) were anesthetized and then infected intranasally with an LD_{90} dose of the A/Turkey/Mas/76 X A/Beijing/32/92 (H6N2) influenza virus.[28] Drugs were prepared in 0.5% CMC and administered orally 4 h prior to virus exposure and continued daily for 5 days. About 10 mice were used in each of the drug- and vehicle-treated groups, while five uninfected mice served as normal controls. Parameters for evaluation

of anti-viral activity included reduction in weight loss, prevention of death, and/or increase in mean day to death determined through 21 days. Since it was technically difficult to evaluate all of the drugs in the same experiment, two separate experiments were performed to compare the efficacy of these compounds to BCX-1812 (RWJ-270201) and oseltamivir. Since zanamivir was not found effective orally based upon the literature reports, it was not included in oral studies.

5.2. Intranasal efficacy in mouse influenza model

Mice (15–20 g) were anesthetized and infected with A/Turkey/Mas/76 X A/Beijing/32/92 (H6N2) influenza virus as described before.[28] Drugs were prepared in saline and administered intranasally 4 h prior to virus exposure and continued daily for 5 days. The number of mice and the parameters evaluated to demonstrate efficacy were the same as described earlier. Again, because of technical difficulties, the drugs were compared in two separate experiments.

5.3. General methods

Commercially available solvents and reagents were used as received. All reactions were conducted under a dry nitrogen atmosphere. Melting points were obtained in open capillary tubes in a Mel-Temp II melting point apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlab, Inc. (Norcross, GA). Mass spectra were obtained on a Fison Trio 2000 quadrupole mass spectrometer. ^{1}H NMR spectra were recorded on a Bruker AM400 or Bruker AM360 spectrometer using tetramethylsilane as the internal standard. IR spectra were run on a BioRad FTS-7 FTIR spectrometer. Flash column chromatography was carried out using 230–400 mesh silica gel. Thin layer chromatography was used as an indicator for the completion of the reactions and was performed on K 6 F silica gel 60 Å plates. The spots on TLC were visualized by UV and/or spraying the plate with 1 M ammonium sulfate in 1 N sulfuric acid and heating the plate on a hot plate. Organic solvent extracts in the isolation procedures were dried over anhydrous magnesium sulfate.

5.4. (−)-Ethyl (1*S*,2*S*,3*R*,4*R*)-3-[(1*S*)-1-(acetylamino)-2-(propylpentyl)]-4-[(*tert*-butoxycarbonyl)amino]-2-hydroxycyclopentanecarboxylate (8)

1-Bromo-2-propylpentane (43.2 g, 224.0 mmol) was added to a mixture of sodium nitrite (27.9 g, 392.0 mmol) in DMSO (600 mL) at 10–15 °C over a period of 1 h. The mixture was stirred at room temperature for 16 h, diluted with water (600 mL) and extracted with ether (3 × 200 mL). The combined organic extract was washed with water (2 × 400 mL), dried ($MgSO_4$), filtered, and the filtrate concentrated to give 16.2 g (45%, 78% pure by ^{1}H NMR) of 1-nitro-2-propylpentane, which was used without purification in the next step.

A mixture of 1-nitro-2-propylpentane (16 g, 75.4 mmol) and Et_3N (1.0 mL, 7.2 mmol) in benzene (75 mL) was added dropwise to a refluxing solution of (−) ethyl 4-*tert*-butoxycarbonylaminocyclopentene-1-carboxylate (16.1 g, 62.9 mmol) and phenyl isocyanate (14.65 mL, 132.1 mmol) in benzene (125 mL) over a period of 1 h. The mixture was further heated at reflux for 16 h, the solids removed by filtration and the filtrate concentrated to give an oil, which was purified by flash chromatography on silica gel using ethyl acetate (5–20%) in hexanes to give 15.75 g (62%) of the desired product, (+)-ethyl (3a*R*,4*R*,6*S*,6a*S*)-4-[(*tert*-butoxycarbonyl)-amino]-3-(1-propylbutyl)-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]-isoxazole-6-carboxylate (**7**). The product was contaminated with by-products generated from phenyl isocyanate, which was used in the next step without further purification.

A mixture of **7** (15 g, 39.8 mmol), concd HCl (3.3 mL, 39.8 mmol), and PtO_2 (1.5 g) in methanol (250 mL) was hydrogenated at 100 psi for 48 h. The catalyst was removed by filtration, the filtrate concentrated, and the residue taken in CH_2Cl_2 (200 mL) and treated with Ac_2O (4.5 g, 43.8 mmol) and triethylamine (4.0 g, 39.8 mol). The reaction mixture was stirred at room temperature for 2 h and quenched with ice water (50 mL). After neutralization with NH_4OH, the organic layer was separated and washed with brine (50 mL), dried over $MgSO_4$, and concentrated under vacuum to furnish 17.6 g of crude product as oil. Purification by flash column chromatography on silica gel using 50%, 75%, and 100% EtOAc in hexanes gave 10.59 g (61%) of the desired product as a colorless oil. An analytical sample was obtained by the addition of ether/hexanes (1:5) to a portion of oil and storing in the freezer overnight. The crystals obtained were collected by filtration to furnish the product as a white solid: mp 128–129 °C; ^{1}H NMR ($CDCl_3$): δ 0.88 (m, 6H), 1.18 (m, 2H), 1.28 (m, 9H), 1.40 (m, 2H), 1.44 (s, 9H), 1.69 (m, 1H), 1.97 (dd, J = 10 and 4.2 Hz, 1H), 2.08 (s, 3H), 2.49 (m, 1H), 2.81 (m, 1H), 3.99 (t, J = 9.8 Hz, 1H), 4.15 (m, 1H), 4.20 (d, J = 3.8 Hz, 1H), 4.72 (d, J = 9.3 Hz, 1H), 7.44 (d, J = 10.0 Hz, 1H); IR (KBr): 3395, 3292, 2959, 1733, 1685, 1630, 1520, 1169 cm^{-1}; MS (ES^+), *m*/*z*: 444.0. Anal. ($C_{23}H_{42}N_2O_6$).

5.5. (−)-Ethyl (1*S*,2*S*,3*R*,4*R*)-3-[(1*S*)-1-(acetylamino)-2-(propylpentyl)]-4-[(*tert*-butoxycarbonyl)amino]-2-[(1*H*-imidazol-1-ylcarbonothioyl)oxy]cyclopentanecarboxylate (10)

To a mixture of **8** (34.3 g, 77.6 mmol) in CH_2Cl_2 (500 mL) was added 1,1′-thiocarbonyl diimidazole (34.5 g, 194.1 mmol) and the mixture heated at reflux for 16 h. The reaction mixture was cooled and washed with 0.25 N HCl (2 × 500 mL), water (500 mL), and brine (500 mL). The organic layer was dried and concentrated under vacuum to furnish 49.0 g of crude product. Purification by flash column chromatography on silica gel using 40–90% EtOAc in hexanes gave the desired product (32.1 g) as a white foam: mp 58–60 °C; ^{1}H NMR ($CDCl_3$): δ 0.72 (t, J = 7.0 Hz, 3H), 0.89 (t, J = 7.0 Hz, 3H), 1.06 (m, 1H), 1.16 (m, 1H), 1.30 (m, 10H), 1.46 (s, 9H), 1.89 (m, 1H), 1.99 (s, 3H), 2.47 (m, 2H), 3.10 (m, 1H), 4.23 (m, 3H), 4.46 (m, 1H), 4.91 (d, J = 8.7 Hz, 1H), 6.00 (d, J = 4.7 Hz, 1H), 6.28 (br s,

1H), 7.08 (s, 1H), 7.71 (s, 1H), 8.43 (s, 1H); IR (KBr): 3296, 2959, 1714, 1391, 1286, 1224, 1168 cm^{-1}; MS (ES^+), *m/z*: 553.9. Anal. ($C_{27}H_{44}N_4O_6S$).

5.6. (−)-Ethyl (1*R*,3*R*,4*R*)-3-[(1*S*)-1-(acetylamino)-2-(propylpentyl)]-4-[(*tert*-butoxycarbonyl)amino]cyclopentanecarboxylate (11)

To a solution of **10** (22.2 g, 40.0 mmol) in toluene (550 mL) at 100 °C was added tributyltin hydride (13.8 mL, 51.4 mmol) followed by AIBN (0.4 g, 0.25 mmol). The reaction mixture was further heated at the same temperature for 10 min and concentrated under vacuum. The residue obtained was dissolved in acetonitrile (400 mL) and washed with hexanes (3 × 400 mL). The acetonitrile layer was concentrated to give the crude product as oil. Purification by flash column chromatography on silica gel using first hexanes to remove excess tributyltin hydride and then 40–50% EtOAc in hexanes gave 15.5 g (91%) of **11** as a white foam: mp 81–83 °C; ^1H NMR ($CDCl_3$): δ 0.88 (m, 6H), 1.06 (m, 1H), 1.13–1.40 (m, 10H) 1.43 (s, 9H), 1.75 (m, 3H), 2.00 (s, 3H) 2.08 (m, 2H) 2.24 (m, 1H), 2.84 (m, 1H), 3.82 (m, 2H), 4.13 (q, J = 7.0 Hz, 2H), 4.99 (d, J = 6.5 Hz, 1H), 6.35 (br s, 1H); IR (KBr): 3333, 3054, 2932, 1710, 1511, 1265, 1170 cm^{-1}; MS (ES^+), *m/z*: 427.8. Anal. ($C_{23}H_{42}N_2O_5$).

5.7. (−)-(1*R*,3*R*,4*R*)-3-[(1*S*)-1-(Acetylamino)-2-(propylpentyl)]-4-{[amino(imino)methyl]amino}cyclopentanecarboxylic acid (5c)

To a solution of **11** (10.0 g, 23.5 mmol) in ether (125 mL) was added an ethereal solution of HCl (1 N, 125 mL, 125.0 mmol) and the reaction mixture stirred at room temperature for 24 h. After concentration, the residue was washed thoroughly with ether and dried to give 8.52 g (100%) of (−)-ethyl (1*R*,3*R*,4*R*)-3-[(1*S*)-1-(acetylamino)-2-(propylpentyl)]-4-aminocyclopentanecarboxylate hydrochloride (**11**), which was used as such for the next reaction.

A mixture of **11** (8.5 g, 23.5 mmol) in DMF (65 mL) was treated with diisopropylethylamine (10.6 g, 82.2 mmol) and 1*H*-pyrazole-1-carboxamidine hydrochloride (6.9 g, 47.0 mmol) and the mixture heated at 60 °C for 24 h. After removal of DMF under reduced pressure, the brown residue was taken in NaOH (1 N, 80 mL) and stirred at room temperature for 6 h. The reaction mixture was extracted with ethyl acetate (3 × 100 mL) and the aqueous layer concentrated. The residue was dissolved in boiling methanol (100 mL) and filtered to remove insoluble material. The filtrate on concentration and purification on silica gel column using chloroform/methanol/ammonium hydroxide (80:18:2 to 60:22:8) mixture as eluent gave 5.13 g of the compound, which was again recrystallized from ethanol (200 mL) to give 2.05 g (25.6%) of **5c** as an off-white solid: mp 250–253 °C; ^1H NMR (D_2O): 3.83 (d, J = 10.4 Hz, 1H) 3.51 (q, J = 8.0 Hz, 1H), 2.71–2.58 (m, 1H), 2.31–2.09 (m, 2H), 2.07–1.95 (m, 1H), 1.90 (s, 3H), 1.69–1.53 (m, 2H), 1.49–1.10 (m, 7H), 0.85–0.73 (m, 6H); IR (KBr): 3395, 3196, 2960, 2933, 2870, 1661, and 1550 cm^{-1}; MS (ES^+), *m/z*: 341.48. Anal. ($C_{17}H_{32}N_4O_3 \cdot 2.75\ H_2O$).

5.8. (−)-(1*S*,2*S*,3*R*,4*R*)-3-[(1*S*)-1-(Acetylamino)-2-(propylpentyl)]-4-{[amino(imino)methyl]amino}-2-hydroxycyclopentanecarboxylic acid (5d)

To a solution of **8** (10.4 g, 23.5 mmol) in ether (125 mL) was added an ethereal solution of HCl (1 N, 125 mL, 125.0 mmol) and the reaction mixture stirred at room temperature for 24 h. After concentration, the residue was washed thoroughly with ether and dried to give 8.7 g (98%) of (−)-ethyl (1*R*,3*R*,4*R*)-3-[(1*S*)-1-(acetylamino)-2-(propylpentyl)]-4-amino-2-hydroxycyclopentanecarboxylate hydrochloride (**9**), which was used as such for the next reaction.

A mixture of **11** (8.7 g, 22.9 mmol) in DMF (65 mL) was treated with diisopropylethylamine (10.6 g, 82.2 mmol) and 1*H*-pyrazole-1-carboxamidine hydrochloride (6.9 g, 47.0 mmol) and the mixture heated at 60 °C for 24 h. After removal of DMF under reduced pressure, the brown residue was taken in NaOH (1 N, 80 mL) and stirred at room temperature for 6 h. The reaction mixture was extracted with ethyl acetate (3 × 100 mL) and the aqueous layer concentrated to 30 mL in volume and left at room temperature for 1 h. The solid material was separated out, collected by filtration, and dried under vacuum to give 2.35 g (28%) of **5d** as a white powder: mp 248–249 °C; ^1H NMR (D_2O): 4.38 (d, J = 4.5 Hz, 1H) 4.33 (dd, J = 1.5 and 9.6 Hz, 1H), 3.86–3.82 (m, 1H), 2.71 (dd, J = 3.58 and 8.52, 1H), 2.57–2.51 (m, 1H), 2.18–2.23 (m, 1H), 1.96 (s, 3H), 1.80 (dt, J = 4.5 and 14.7 Hz, 1H), 1.67 (t, J = 8.6 Hz, 1H), 1.25–1.48 (m, 6H), 1.02–1.05 (m, 2H), 0.89 (t, J = 7.2 Hz, 3H), 0.84 (t, J = 7.2 Hz, 3H); IR (KBr): 3344, 2958, 2932, 2872, 1656, 1554, 1393, 1300 cm^{-1}; MS (ES^+), *m/z*: 357.60 (M^{+1}). Anal. ($C_{17}H_{32}N_4O_4$)·H_2O.

Acknowledgements

The authors would like to thank Drs. Charlie Bugg and Claude Bennett for their encouragement throughout this work. The authors would also like to express appreciation to Linda Kay First for preparation of this manuscript.

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