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Pyrrolidinobenzoic Acid Inhibitors of Influenza Virus Neuraminidase: Modifications of Essential Pyrrolidinone Ring Substituents

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Abstract—We recently reported the first benzoic acid, 1-[4-carboxy-2-(3-pentylamino)phenyl]-5,5-bis(hydroxymethyl)pyrrolidin-2-one (**8**), that is a potent inhibitor of avian influenza A neuraminidase (N9) and, unlike other reported potent neuraminidase inhibitors, does not contain a basic aliphatic amine or guanidine nor a simple *N*-acetyl grouping. However, **8** was a poor inhibitor of influenza B neuraminidase. In the present study we further evaluated **8** as an inhibitor of human influenza A NA isolates, and it was effective against N2 NA but found to be 160-fold less active against N1 NA. We also synthesized analogues of **8** involving moderate modifications of essential substituents on the pyrrolidinone ring. Specifically, the aminomethyl (**9**), hydroxyethyl (**10**), and aminoethyl (**11**) analogues were prepared. Only the most conservative change (compound **9**) resulted in continued effective inhibition of influenza A, in addition to a noteworthy increase in the activity of **9** for N1 NA. The effectiveness of **9** against influenza B neuraminidase was furthermore improved 10-fold relative to **8**, but this activity remained 50-fold poorer than for type A NA.

Introduction

Influenza virus causes major respiratory tract disease in humans resulting every year in excess morbidity and mortality. Epidemics of influenza occur every winter and are responsible for approximately 20,000 deaths per year in the United States.[1] It is particularly dangerous to the very young, elderly, and to those who have suppressed immune systems. Vaccination has been the main preventive measure and has provided limited control, but vaccines must be reformulated each year in response to antigenic drift and may be ineffective against new variants of influenza viruses. While influenza drugs are an attractive alternative to vaccines, until recently the only effective drugs were amantidine and rimantidine, which have limited use since they are selective for influenza A viruses and susceptible strains rapidly become resistant.

A new viral target has recently been exploited to develop clinically useful anti-influenza drugs. This target, the neuraminidase (NA, also called sialidase), is one of two major surface glycoproteins of influenza viruses; the second is the hemagglutinin (HA). Both play important roles in the infectious and antigenic properties of the virus. HA is required for attachment of the virus to host cells through interaction with sialic acid residues. NA catalyses the cleavage of α-glycosidic bonds to the terminal sialic acid residues to facilitate spread of newly-budded virus.[2]

Neuraminidase is a tetrameric 240 kDA protein and appears as mushroom-shaped heads on thin stalks projecting out of the virion membrane. Based on their antigenic properties, nine subtypes (N1–N9) of neuraminidase have been found in influenza A viruses, while no distinct subtypes of neuraminidase have been described in influenza B viruses. Despite the fact that the protein sequence identity among different strains is only about 30%, the catalytic site for all influenza A and B virus neuraminidases is completely conserved, which is

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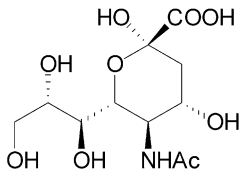
one reason that NA has been considered an attractive target for antiviral drug development.

Influenza neuraminidase contains 18 conserved amino acid residues in the active site. Mutations of these conserved residues generally result in enzyme inactivation,[2d] suggesting that the virus may not easily escape sialidase-targeted drug therapy. Sialic acid (**1**, Neu5Ac, NANA), the product of the enzyme reaction, is a weak inhibitor with a K_i of about 1 mM. The first moderately effective inhibitor to be described (over 25 years ago!) is the dehydrated analogue of sialic acid, 2-deoxy-2,3-didehydro-*N*-acetylneuraminic acid (**2**, Neu5Ac2en, DANA, K_i 10 μM).[3] In recent years, several very potent inhibitors of both influenza A and B NA have been developed that are either in human clinical trials or have been marketed as anti-influenza drugs. Zanamivir[4] (GG167, 4-Guan-Neu5Ac2en) (**3**) is a potent inhibitor (IC_{50} 5 nm) of both influenza A and B virus neuraminidases. Zanamivir, although orally inactive and rapidly excreted from the body, is effective in both the prevention and the treatment of influenza when administered directly into the lungs or nose.[5] The first orally active neuraminidase inhibitor to be marketed was based upon GS 4071 (**4**), a potent inhibitor of both influenza A and B NA.[6a] Its ethyl ester serves as the orally active prodrug form, GS 4104 (**5**).[6b] A second orally active influenza neuraminidase inhibitor, BCX-1812 (**6**),[7] also a potent inhibitor of both influenza A and B NA, recently entered Phase III human clinical trials. Other pharmaceutical companies continue to develop new influenza neuraminidase inhibitors.[8]

In addition to the carboxylate and *N*-acetyl groupings, two common structural features of the clinically useful neuraminidase inhibitors are (1) considerable stereochemical complexity and (2) a basic functionality (aliphatic amine or guanidine). We have been interested in developing neuraminidase inhibitors that are chemically simpler and, if possible, avoid the basic functionality. Toward this end, we have pursued benzoic acids as neuraminidase inhibitors. The benzene ring was selected as a template because it provides planarity near the carboxylate group that mimics the transition state-like structure of Neu5Ac2en and its derivatives. Further, the benzene ring has no stereogenic centers at substitution sites, and such inhibitors potentially offered a more economical synthesis of anti-influenza drugs. Our lead benzoic acid inhibitor[9] was BANA 113 (**7**, K_i 2.5 μM). Optimization of BANA 113 resulted in the potent benzoic acid inhibitor **8**, which contained a substituted pyrrolidinone ring in place of the *N*-acetyl functionality and exhibited an IC_{50} of 48 nm against influenza A NA (N9, an avian strain).[10] Thus compound **8** satisfied both of the above goals, since it contains no chiral carbons and also lacks a strongly basic nitrogen. However, compound **8** is nevertheless a chiral molecule resulting from atropisomerism due to restricted rotation about the aromatic C to pyrrolidinone N bond, and this compound was prepared and evaluated as the racemic mixture. This compound class is unique among known NA inhibitors.

Although the structures of the catalytic sites in type A and B neuraminidases appear to be identical, compound **8** is highly selective for influenza virus A NA and was much less effective against influenza virus B NA. This observation is consistent with earlier reports for NA inhibitors that induce a conformational change in Glu278 upon ligand binding,[11,12] since this conformational change in type B NA is energetically unfavorable.[11] This alteration in binding site structure is required to accomodate many ligands that present a hydrophobic moiety in the Glu278 binding pocket. However, some of the most potent inhibitors with broad spectrum activity against both types A and B NA also contain this hydrophobic moiety (as in compounds **5** and **6**), creating uncertainties regarding the optimization of type A-selective inhibitors to include type B activity.

Here, we continued the evaluation of **8** as an inhibitor of influenza A NA, targeting the human isolates N1 and N2. Additionally, in order to begin exploring structure–activity relationships for the unique pyrrolidinone ring in **8**, we proposed two types of modifications to the



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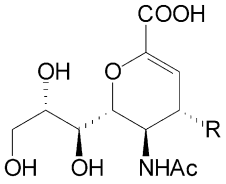
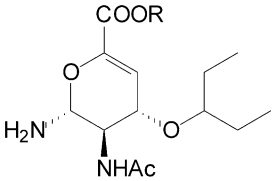
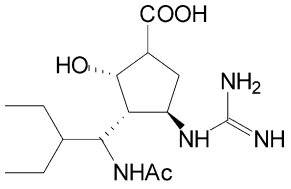


2 R = OH
3 R = guanidine



4 R = H
5 R = Et



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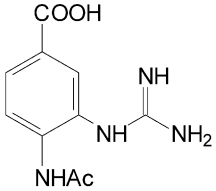

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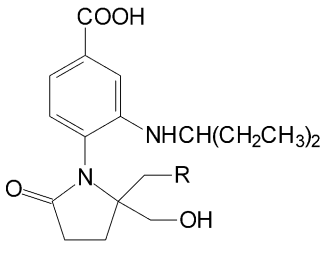


8 R = OH
9 R = NH_2
10 R = CH_2OH
11 R = CH_2NH_2

essential pyrrolidinone side chains: (1) the introduction of one basic aliphatic amino functionality in place of a hydroxy group (target **9**), and (2) conversion of the hydroxymethyl and aminomethyl groups on **8** and **9**, respectively, to their one carbon larger homologues (targets **10** and **11**).

Chemistry

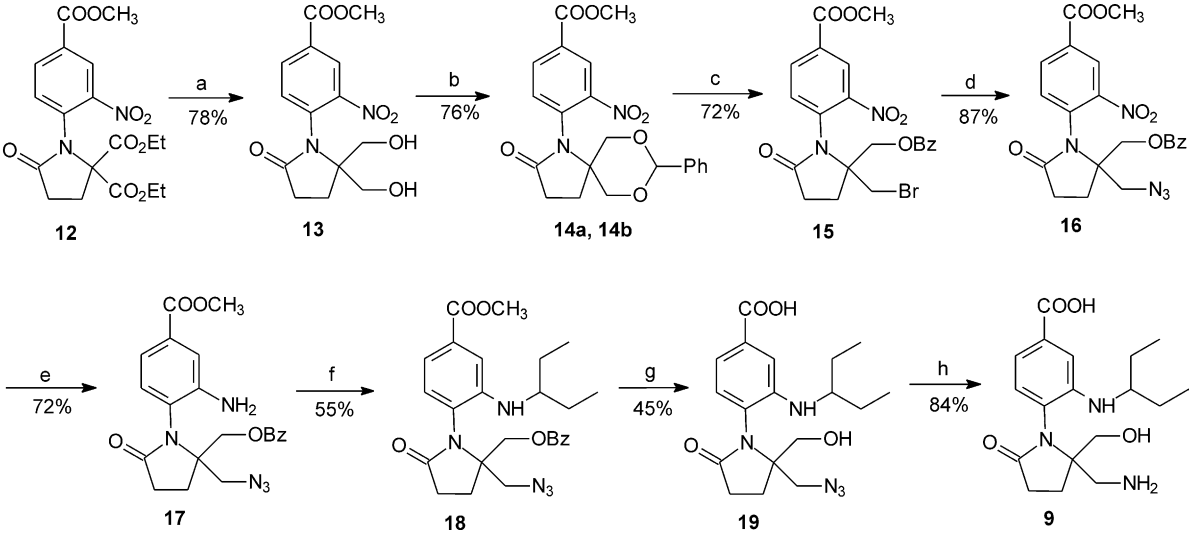
We first pursued the synthesis of analogue **9**, which is essentially isosteric with **8** but contains a basic aliphatic amino functionality. As shown in Scheme 1, diester **12**[10a] was reduced with sodium borohydride to provide the bis-(hydroxymethyl) derivative **13**. This was reacted with benzaldehyde in the presence of TsOH at reflux to give a mixture of separable diastereomeric products **14a** and **14b**. Ring opening of the acetal mixture **14a**, **14b** was done with NBS in benzene to give bromo compound **15** as a diastereomeric mixture (the same mixture was produced from reacting either **14a** or **14b** separately). Displacement of the bromine with azide was accomplished using sodium azide in DMF to give **16**. The nitro group in compound **16** was reduced to the amine **17** using sodium dithionite, and **17** was subjected to reductive amination with 3-pentanone and sodium cyanoborohydride to give product **18**. Removal of the benzoate group and ester hydrolysis was done in one step using 1 N sodium hydroxide. Acidification and recrystallization of the precipitate gave the pure azide **19**. Reduction of the azide to give amine **9** was accomplished using catalytic hydrogenation in methanol.

We also proposed analogues of **8** and **9** containing chain-extended substituents. X-ray structural studies[10a] of **8** in complex with NA revealed that a hydroxy group in **8** replaces an ordered water in the C4 subsite. However, the alcohol of **8** in the enzyme complex does not quite reach the original location of the ordered water. Modeling suggested that lengthening the side chain by one carbon atom might further enhance this interaction, assuming that minor repositioning of the benzoic acid template is permitted. This modification was achieved by preparing the hydroxyethyl (**10**) and aminoethyl (**11**) analogues, while maintaining one hydroxymethyl group in each case.

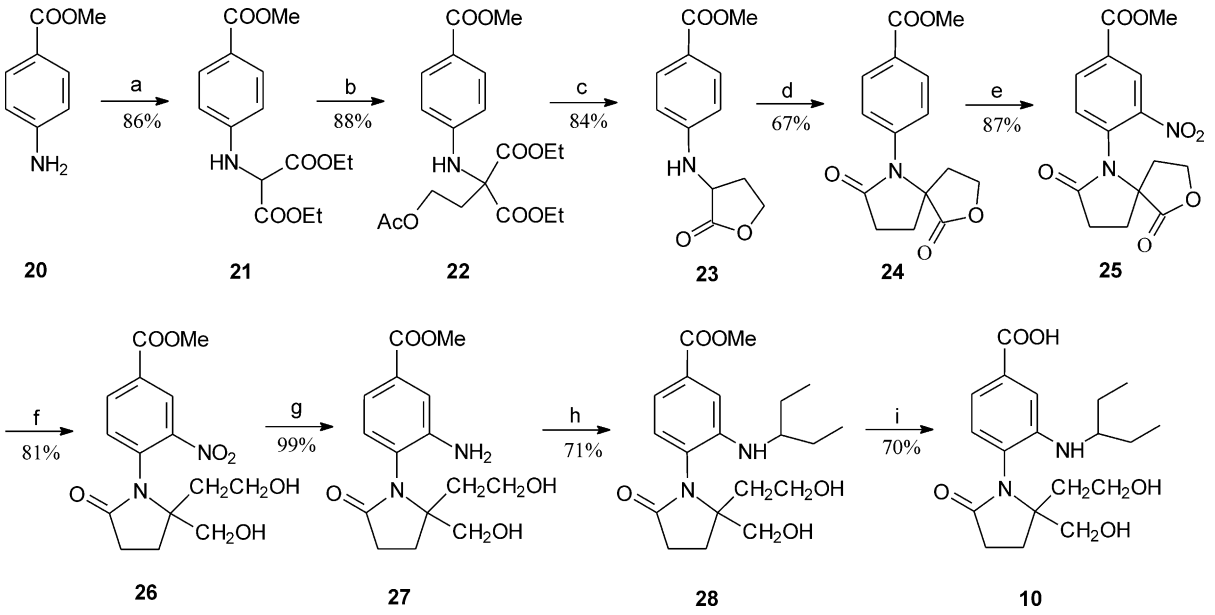
The synthesis of the 5-(hydroxyethyl)-5-(hydroxymethyl)lactam **10** was accomplished as summarized in Scheme 2. Aniline **20** was reacted with diethyl bromomalonate to give **21**. Malonate **21** was alkylated to provide **22**, which upon treatment with NaOMe underwent cyclization and decarboxylation to yield lactone **23**. The Michael reaction of **23** with ethyl acrylate gave the spiro compound **24,** and subsequent nitration with nitronium tetrafluoroborate proceeded in greater than 90% yield to provide **25**. Reduction of the lactone with $NaBH_4$ gave the diol **26**. Catalytic hydrogenation of the nitro functionality gave the corresponding amine (**27**). Reductive coupling of **27** with 3-pentanone in the presence of $NaCNBH_3$ and acetic acid gave **28**, which on saponification yielded the target compound **10**.

As summarized in Scheme 3, compound **11** was prepared in an analogous but regioselective manner starting with intermediate diol **26**. In this procedure, the seven-membered benzylidene acetal **29** was obtained in high yields from **26** using benzaldehyde as solvent (neat). Under standard reaction conditions in organic solvent we obtained poor yields. Ring opening of the acetal with NBS occurred regioselectively on the least hindered carbon to give the desired bromoester **30** in good yield (85%). Substitution with azide to provide the azidobenzoate **31** was followed by selective reduction of the nitro functionality using sodium dithionate to give **32**. The reductive amination of 3-pentanone gave **33**, which underwent saponification to give **34**. The catalytic reduction of azide **34** using H_2/Pd/C gave the final target **11**.

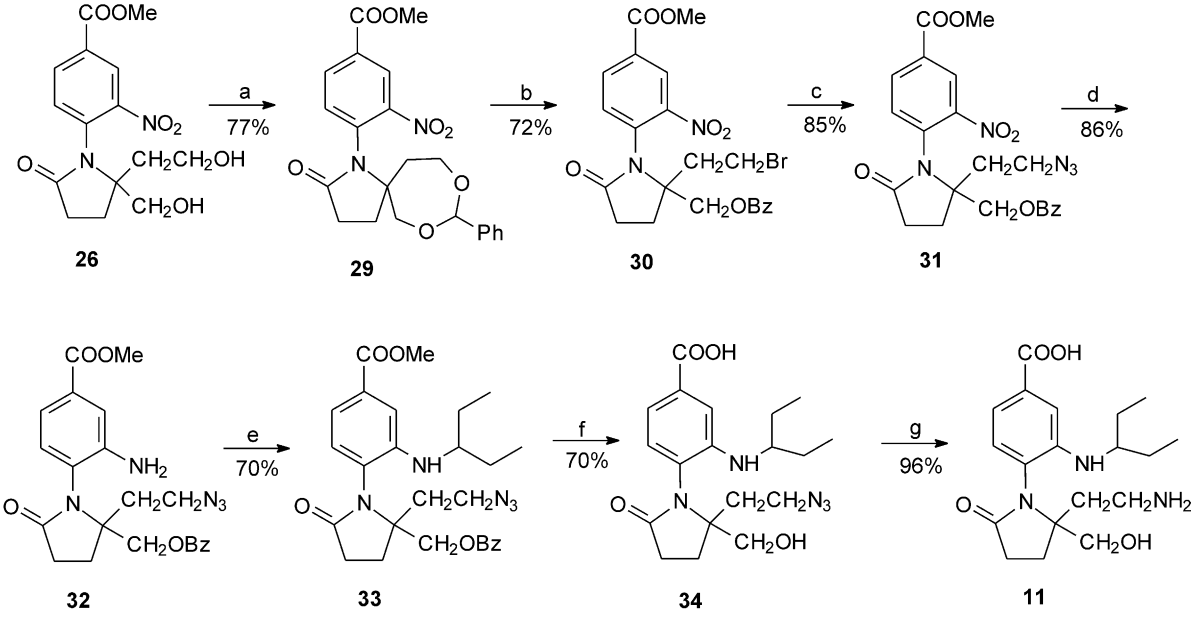
As similarly discussed earlier for **8**, it should be noted that compounds **9**–**11** in Schemes 1–3, while containing



Scheme 1. Reagents: (a) $NaBH_4$, THF–MeOH; (b) PhCHO, TsOH, $PhCH_3$; (c) NBS, benzene; (d) NaN_3, DMF, heat; (e) $Na_2S_2O_4$, THF–H_2O; (f) 3-pentanone, $NaCNBH_3$, HOAc; (g) NaOH; (h) H_2/Pd/C, MeOH.



Scheme 2. Reagents: (a) diethyl bromomalonate; (b) NaH, DMF, 2-bromoethyl acetate; (c) NaOMe, MeOH; (d) LDA, THF, −78 °C, ethyl acrylate; (e) $NO_2^+BF_4^-$, CH_2Cl_2; (f) $NaBH_4$, THF–MeOH; (g) H_2/Pd/C, MeOH; (h) 3-pentanone, $NaCNBH_3$, AcOH, $C_2H_4Cl_2$; (i) 1 N NaOH.



Scheme 3. Reagents: (a) benzaldehyde, TsOH; (b) NBS, benzene, 38 °C; (c) NaN_3, DMF, 75 °C; (d) $Na_2S_2O_4$, THF–H_2O; (e) 3-pentanone, $NaCNBH_3$, HOAc; (f) NaOH, MeOH; (g) H_2/Pd/C, MeOH.

only one chiral carbon, exist as diastereomers due to atropisomerism resulting from restricted rotation about the aromatic C to pyrrolidinone N single bond. In these cases, the diastereomeric mixtures for intermediates and products were not separable by silica chromatography, and targets **9**, **10**, and **11** were all isolated and evaluated as the approximately 1:1 diastereomeric mixture.

Biological Assays

All compounds were evaluated for in vitro inhibitory activity of NA using intact virions of influenza virus A (N1 and N2 subtypes), and influenza virus B. Colorimetric detection of the enzyme product was employed via a thiobarbiturate assay,[13] and IC_{50} values were determined from a plot of inhibitor concentration versus fractional enzyme activity. The results are contained in Table 1.

The most effective new compound, **9**, was further evaluated for its ability to inhibit viral replication in Madine Darby canine kidney (MDCK) cells using A/Udorn/72 (see Table 1).

Results and Discussion

X-ray crystallographic studies of the early NA inhibitor, Neu5Ac2en (DANA, **2**), in complex with NA clearly

Table 1. In vitro inhibitory activities of benzoic acid analogues for influenza virus A and B neuraminidases

Compd	IC_{50} values (μM) Type of influenza virus neuraminidase		
	N1	N2	B/Lee
3 (GG167)	0.006	0.015	
7[a]			8
8[b,c]	79[d]	0.49	271
9[b,c]	0.88	0.52 [e] ($EC_{50} = 4 \pm 1$ μM)	26
10	>1000[f]	59	>1000[f]
11	180	41	>1000[f]
19	268		>670[f]

[a]Ref 11a.
[b]Compound **8** was evaluated as its racemic mixture, and compounds **9**–**11** and **19** as an approximately 1:1 mixture of diastereomers.
[c]Compounds **8** and **9** were measured side by side against all virus strains.
[d]In order to estimate the accuracy of these results, this IC_{50} determination was repeated in four separate experiments. The mean (±SEM) $IC_{50} = 73 \pm 13$ μM.
[e]The concentration that reduced viral yield by 50% in a viral replication assay using A/Udorn/72 virus and MDCK cells.
[f]No significant inhibition was observed at the highest tested concentration, which is given in the table.

defined a compact catalytic site containing four binding subsites with which all reported NA inhibitors interact (see Fig. 1; N9 numbering). The carboxylate makes tight salt-bridge interactions with an arginine triad consisting of Arg 119, 294, and 372 (C2-subsite). The 4-hydroxyl group occupies the C4-subsite near Asp 152 and Glu 120. The acetamido group fits into the hydrophobic pocket (C5-subsite) formed by Trp 180, Ile 224, and Arg 226. The C8 and C9 hydroxyl groups of the glycerol side chain undergo interactions with the C6-subsite containing Glu 278.

The structure of the bis-(hydroxymethyl) compound **8** in complex with the N9 neuraminidase[10a] (Fig. 1) revealed that one of the hydroxymethyl groups hydrogen bonds to an ordered water located in the C5-subsite, whereas the other hydroxymethyl group replaces an ordered water located in the C4-subsite. The carboxylate makes salt bridges with Arg 119, 294, and 372, and the pyrrolidinone ring sits in the hydrophobic pocket created by Trp 180 and Ile 224. The 3-pentylamino moiety occupies the glycerol subsite of Neu5Ac2en, which is in this case a hydrophobic pocket created by the rotation of Glu 278 (Glu 275 using type B numbering) to form an intramolecular salt-bridge interaction with Arg 226. This movement in the catalytic site is required to create the hydrophobic pocket, and it has been suggested[11] that rotation of Glu 275 in type B NA is energetically more costly, resulting in a poorer inhibition constant ($IC_{50} = 271$ μM for **8**) for many compounds that contain such hydrophobic groups. However, GS 4071, which is a potent inhibitor of both type A and type B NA, contains a similar lipophilic grouping in the glycerol pocket but undergoes tight binding to type B NA, possibly due to differences in the extent of rotation induced for Glu 275. Apparently,

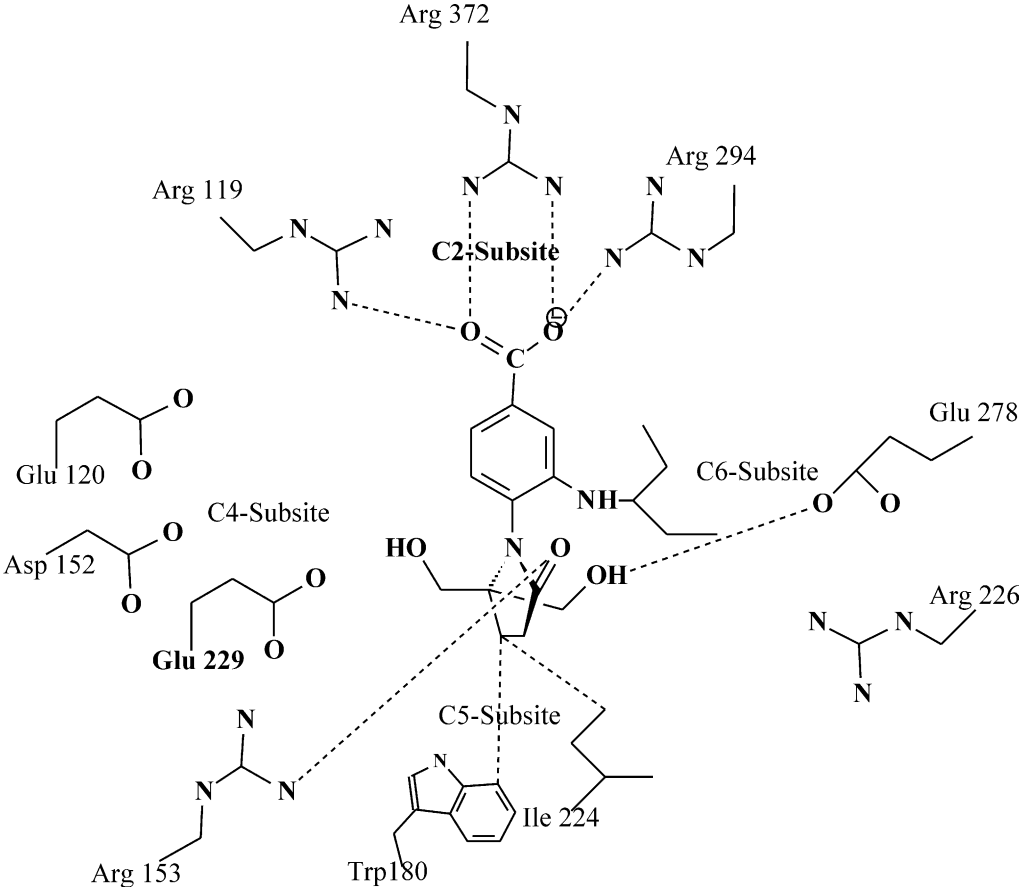


Figure 1. Depiction of the interactions for compound **8** in the active site of influenza neuraminidase.

subtle structural changes not yet clearly defined contribute to the potency of inhibition and type A selectivity seen for hydrophobic inhibitors. We therefore proposed to evaluate the effects of modest changes in the essential pyrrolidinone side chains for compound **8**.

As shown in Table 1, we first evaluated **8** as an inhibitor of NA from two human influenza isolates, N1 and N2. Interestingly, compound **8** was highly selective for N2 NA over N1 NA; in fact, the activity against N1 NA was only slightly poorer than that for B/Lee NA.

We also evaluated the newly synthesized analogues of **8** against the same viral strains of NA. The most conservative modification to **8** resulted in the aminomethyl analogue **9**. As shown in Table 1, compound **9** exhibited potent inhibitory activity, comparable to **8**, against N2 NA. However compound **9** was also approximately 100-fold more active than **8** against N1 NA, revealing a broader spectrum activity against the two most important human strains of influenza A NA. Thus the spectrum of activity for **9** makes it more attractive than compound **8** for further development into inhibitors of influenza virus A infections in humans. Its synthetic precursor, the azide **19,** exhibits an IC_{50} of only 268 μM against N1 NA, revealing the importance of the amino grouping. While compound **9** was 10-fold more effective than **8** against influenza B NA, it remains 50-fold selective for the inhibition of type A over type B NA.

Interestingly, the homologues of **8** and **9**, compounds **10** and **11**, each exhibited relatively poor activity against NA from both influenza viruses A and B. This result suggests that these side chains are too large for the C4 and C5 subsites, or that the rigidity of the catalytic site prevents small adjustments in the position of the benzoic acid core. This further emphasizes the sensitivity of the NA catalytic site to relatively modest changes in inhibitor structure.

To confirm that the inhibition of NA by compound **9** would result in antiviral actions, the EC_{50} for reduction of viral yield in MDCK cells was determined using influenza virus A/Udorn/72. As shown in Table 1, compound **9** was highly effective in this assay with an EC_{50} value of 4 μM.

In summary, properly substituted pyrrolidinobenzoic acids can serve as novel structural templates for potent inhibitors of influenza A NA. We were able to improve the cross-reactivity against type B influenza, but, as for many other reported NA inhibitors containing hydrophobic substituents, the activity of examples prepared thus far shows at least 50-fold selectivity for influenza A over type B NA.

Experimental

Neuraminidase inhibition assays

Influenza virus strains A/PR/8/34 (N1), A/Udorn/72 (N2), and B/Lee/40 were grown in embryonated chicken eggs as described.[14] IC_{50} determinations were carried out using the thiobarbiturate colorimetric assay described by Aymard-Henry et al.[13] but adapted to 96-well plates. The enzymatic reaction was carried out in a 200-μL solution containing 0.125 mM $CaCl_2$, 0.4 mM $MgCl_2$, 75 mM NaCl, 0.25% NaN_3, 50 mM phosphate buffer (pH 5.9), and 0.5% DMSO. The reactions included neuraminidase in the form of intact virions in allantoic fluid, 10 mg/mL of the substrate fetuin (Sigma), and inhibitor at various dilutions. The reaction mixtures were incubated for 1 h at 37 °C, after which formation and extraction of the chromophore were carried out as described.[13] The data was analyzed by plotting the enzyme fractional activity against the inhibitor concentration, and the IC_{50} was determined from the linear region of the dose–response curve.

Inhibition of viral replication assay

MDCK cell monolayers in 24-well plates were washed free of serum and then infected with 100 TCID (tissue culture infectious dose) of A/Udorn/72 virus. After virus was adsorbed to the cells for 30 min, 0.5-mL solution of serum-free infection medium[15] containing trypsin (1 μg/mL) and 10-fold dilutions of NA inhibitor **9** was added to each well, and the plates were incubated at 37 °C in 5% CO_2 for 48 h. The yield of virus in each well was measured by hemagglutination titration and compared to wells with no inhibitor.[16] The EC_{50} was calculated from a narrow-range experiment in which doubling dilutions of inhibitor were used. The EC_{50} is defined as the inhibitor concentration at which virus output is reduced to 50%, averaged from duplicate experiments.

Chemistry

NMR data was recorded on a Bruker ARX 300 MHz spectrometer. IR spectra were obtained on a Bruker FT/OPUS spectrophotometer. Mass spectra were obtained using electrospray ionization on a MicroMass Platform LCZ LC/MS with HP1100HPLC/autoinjector and diode array detector. Flash column chromatography was carried out using silica gel (40 μm, Mallinckrodt, Inc.). TLC was performed using silica gel (250 μM layer, Whatman), and spots were visualized by irradiation with UV light (UV-254 nm). Melting points were determined on a Mel-Temp electrothermal melting point apparatus (Laboratory Devices) and are uncorrected. Anhydrous reactions were carried out in oven-dried glassware under a nitrogen atmosphere. Compound **12** (Scheme 1) was prepared as previously described.[10a] We also previously described[10a] a synthesis for **13**, but here we describe an improved procedure. GG167 was a gift from BioCryst Pharmaceuticals.

Note that pyrrolidino-benzenes containing one or more chiral carbons were synthesized as diastereomeric mixtures due to atropisomerism via restricted rotation about the pyrrolidinone N to benzene C bond. In all cases the diastereomeric atropisomers were not separable by silica chromatography, and spectral and biological data are reported for the mixture.

1-(4-Methoxycarbonyl-2-nitrophenyl)-5,5-bis-(hydroxymethyl)pyrrolidin-2-one (13). A solution of nitrodiester **12**[10a] (10.0 g, 2.45 mmol) was dissolved in a THF–MeOH mixture (1:1, 135 mL) and cooled to zero degrees under a nitrogen atmosphere. Sodium borohydride (5.14 g, 15.2 mmol) was added in small amounts over a period of 1.5 h at 0 °C, and the mixture was stirred for another 2.5 h at 7–10 °C. The reaction mixture was quenched with ice cold 6 N HCl (31.5 mL) while stirring and then concentrated to dryness under vacuum. The yellow solid residue was dissolved in chloroform (75 mL) and washed with water (60 mL) and brine (40 mL). The combined aqueous layers were further extracted with chloroform (5 × 60 mL). The combined organic layers were dried (Na_2SO_4) and concentrated to give a pale yellow fluffy solid, which was triturated with ether and filtered to give **13** (6.2 g, 78%) as a pale yellow solid: mp 143–144 °C (ethanol–ether). MS (ES) *m/z* 325 (M + H); IR (KBr): 3419, 1729, 1664 cm^{-1}; ^1H NMR (CD_3OD) δ 2.00–2.30 (m, 2H), 2.47–2.68 (m, 2H), 3.54–3.92 (m, 4H), 3.97 (s, 3H), 7.75 (d, 1H), 8.31 (dd, 1H), 8.60 (d, 1H). Compound **13** was identical to that previously described.[10a]

(1-(4-Methoxycarbonyl-2-nitrophenyl)-5,5-*spiro*(3,5-dioxa-4-phenylcyclohexyl)-pyrrolidin-2-one (14a and 14b). The diol **13** (2.00 g, 6.17 mmol) was dissolved in dry toluene (80 mL), benzaldehyde (2.00 mL, 19.6 mmol) and pTsOH (25 mg) were added, and the mixture was heated at reflux using a Dean–Stark trap for 24 h. The solution was concentrated to dryness, and the residue was dissolved in ethyl acetate (150 mL) and washed with 5% sodium bicarbonate (2 × 10 mL), water (10 mL), and brine (10 mL), and dried (Na_2SO_4). The organic layer was concentrated to dryness and the residue was purified on a flash silica gel column (9:1 ether–ethanol). The two diastereomeric products **14a** and **14b** (total yield 1.93 g, 76.2%) were isolated. For isomer **14a** (1.1 g): mp 155–156 °C; R_f 0.76 (9:1 ether–ethanol); MS (ES) *m/z* 413 (M + H); IR (KBr) 1734, 1698 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.64 (br m, 4H), 3.69 (d, 1H), 4.00 (s, 3H), 4.13 (d, 1H), 4.28 (m, 2H), 5.33 (s, 1H), 7.25–7.44 (m, 6H), 8.34 (d, 1H), 8.70 (br s, 1H). For isomer **14b** (836 mg): mp 203–204 °C; R_f 0.31 (9:1 ether–ethanol); MS (ES) *m/z* 413 (M + 1); IR (KBr) 1728, 1696 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.88–2.05 (m, 2H), 2.59–2.68 (m, 2H), 3.70–3.82 (m, 2H), 3.95 (s, 3H), 4.56 (m, 2H), 5.45 (s, 1H), 7.20–7.30 (m, 5H), 7.76 (d, 1H), 8.15 (dd, 1H), 8.65 (d, 1H). Anal. calcd for $C_{21}H_{20}N_2O_7$: C, 61.16; H, 4.85; N, 6.80. Found: C, 61.12; H, 4.90; N, 6.81.

1-(4-Methoxycarbonyl-2-nitrophenyl)-5-bromomethyl-5-(benzoyloxymethyl)-pyrrolidin-2-one (15). The benzylidene acetal **14** (1.80 g, 4.36 mmol) (either **14a**, **14b**, or a mixture) was dried under vacuum and dissolved in anhydrous benzene (150 mL). Dry NBS (1.20 g, 6.70 mmol) was added, and the mixture was stirred under nitrogen at room temperature for 30 h. The solution was concentrated to dryness, the residue was dissolved in ethyl acetate (150 mL), and this was washed with water (5 × 60 mL) followed by brine (50 mL). The organic layer was dried (Na_2SO_4) and evaporated to dryness. The residue was purified on a flash silica gel column (5% methanol–chloroform) to give **15** (1.52 g, 72.3%) as a solid: mp 187–188 °C (ethyl acetate–hexane). MS (ES) *m/z* 491 (M + H); IR (KBr) 1723, 1704 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.27–2.53 (m, 2H), 2.66–2.87 (m, 2H), 3.63–3.89 (m, 2H), 3.99 (s, 3H), 4.59–4.92 (m, 2H), 7.24–8.71 (m, 8H). Anal. calcd for $C_{21}H_{19}N_2O_7Br$: C, 51.43; H, 3.88; N, 5.71. Found: C, 51.49; H, 3.83; N, 5.57.

1-(4-Methoxycarbonyl-2-nitrophenyl)-5-azidomethyl-5-(benzoyloxymethyl)-pyrrolidin-2-one (16). The bromo compound **15** (1.10 g, 2.24 mmol) was dissolved in DMF (5 mL), sodium azide (650 mg, 10.0 mmol) was added, and the mixture was heated at 90 °C for 20 h. The reaction mixture was diluted with ethyl acetate (120 mL), washed with water (2 × 25 mL) and brine (10 mL), dried (Na_2SO_4), and evaporated to dryness to give **16** (880 mg, 87.1%) as a white solid: mp 214–215 °C (ethyl acetate–hexane). MS (ES) *m/z* 454 (M + H); IR (KBr) 2108, 1726, 1699 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.21–2.40 (m, 2H), 2.63–2.80 (m, 2H), 3.70–3.90 (m, 2H), 3.97 (s, 3H), 4.45–4.71 (m, 2H), 7.26–7.50 (m, 5H), 7.97 (d, 1H), 8.30 (d, 1H), 8.58 (d, 1H). Anal. calcd for $C_{21}H_{19}N_5O_7 \cdot 0.5H_2O$: C, 54.54; H, 4.11; N, 15.15. Found: C, 54.85; H, 4.29; N, 14.84.

1-((2-Amino-4-methoxycarbonyl)phenyl)-5-azidomethyl-5-(benzoyloxymethyl)-pyrrolidin-2-one (17). The nitro derivative **16** (800 mg, 1.76 mmol) was dissolved in THF (20 mL) and water (8 mL) was added. To this stirring mixture was added sodium dithionite (650 mg, 3.73 mmol), and this was stirred at room temperature for 2 h. The solution was warmed to 40 °C for 10 min and then allowed to cool to room temperature. Another portion of sodium dithionite (150 mg) was added and stirring was continued for another 3 h. The reaction mixture was concentrated to dryness under vacuum, the solid residue was triturated with ethyl acetate (3 × 10 mL), and the suspension was filtered. The filtrate was concentrated to dryness and the oily residue was purified by flash chromatography (5% methanol in chloroform) to yield **17** (540 mg, 72.3%) as a solid: mp 61–62 °C (ether–methanol). MS (ES) *m/z* 424 (M + H); IR (KBr) 3361, 2109, 1723 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.21–2.43 (m, 2H), 2.67–2.88 (m, 2H), 3.44–3.81 (m, 2H), 3.88 (s, 3H), 4.26–4.52 (m, 2H), 7.03–7.66 (m, 7H), 7.92 (d, 1H). Anal. calcd for $C_{21}H_{21}N_5O_5 \cdot 0.5\ CH_3OH$: C, 58.76; H, 5.23; N, 15.94. Found: C, 58.37; H, 4.97; N, 15.51.

1-(4-Methoxycarbonyl-2-(3-pentylamino)phenyl)-5-azidomethyl-5-(benzoyloxymethyl)-pyrrolidin-2-one (18). In a flame-dried flask, aniline **17** (1.40 g, 3.31 mmol), 3-pentanone (32 mL, 31.7 mmol) and AcOH (9.30 mL) were stirred under nitrogen for 36 h. Sodium cyanoborohydride (1.91 g, 30.4 mmol) was added and the mixture stirred for 6 h. An additional portion of sodium cyanoborohydride (1.91 g, 30.4 mmol) was added and the mixture stirred for 6 more hours. The reaction was quenched with saturated $NaHCO_3$ solution (10 mL), extracted with EtOAc (3×25 mL), and the organic extracts were washed with brine (20 mL) and dried (Na_2SO_4). The organic layer was concentrated under vacuum, and the crude oily residue (2.50 g) was dissolved

in hexane (100 mL) and concentrated to help remove the side product, 3-pentanol, as an azeotrope. The crude mixture was purified by flash chromatography (silica) using stepwise elution with hexane (500 mL), then 20% ether in hexane (500 mL), and finally 40% ether in hexane (250 mL) to afford **18** (0.90 g, 55%) as an oil. Starting material (0.23 g) and a cyclized byproduct (0.2 g) were also recovered. MS (ES) *m/z* 494 (M + H); IR ($CHCl_3$) 2110, 1720, 1630 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.92 (t, 3H), 0.99 (t, 3H), 1.27 (m, 2H), 1.52 (m, 2H); 2.29 (m, 2H); 2.65 (m, 2H), 3.16–3.41 (m, 1H), 3.51–3.74 (m, 3H), 3.87, 3.90 (s, 3H), 4.25–4.52 (m, 2H), 6.98–7.67 (m, 7H), 7.89 (d, 1H). Anal. calcd for $C_{26}H_{31}N_5O_5 \cdot 0.5H_2O$: C, 62.14; H, 6.42; N, 13.93. Found: C, 62.59; H, 6.54; N, 13.03.

1-(4-Carboxy-2-(3-pentylamino)phenyl)-5-azidomethyl-5-hydroxymethyl-pyrrolidin-2-one (19). The ester **18** (370 mg, 0.750 mmol) was dissolved in methanol (1 mL), 1 N NaOH (2.5 mL, 2.5 mmol) was added, and the mixture was stirred overnight at room temperature. The solution was concentrated to dryness, and the residue was dissolved in water (1.5 mL) and acidified to pH 1 using 1 N HCl at zero degrees. After further cooling in an ice bath the mixture was filtered. The solid material was triturated with ether (2 × 10 mL) and chloroform (5 mL), and then filtered. The collected solid was purified on a flash silica gel column (9:1 ether–ethanol) to give **19** (126 mg, 44.8%) as a solid: mp 157–158 °C (methanol). MS (ES) *m/z* 376 (M + H); IR (KBr) 3387, 2109, 1710, 1632 cm^{-1}; ^1H NMR (CD_3OD) δ 0.92 (t, 6H), 1.57 (m, 4H), 2.50 (m, 4H), 2.93–3.72 (m, 5H), 6.90–7.51 (m, 3H). Anal. calcd for $C_{18}H_{25}N_5O_4$: C, 57.59; H, 6.71; N, 18.65. Found: C, 57.55; H, 6.71; N, 18.37.

1-(4-Carboxy-2-(3-pentylamino)phenyl)-5-aminomethyl-5-hydroxymethyl-pyrrolidin-2-one (9). The azide **19** (80 mg, 0.21 mmol) was dissolved in methanol (15 mL), 10% Pd/C (40 mg) was added, and the mixture was hydrogenated at 40 psi for 2.5 h. The solution was filtered and the filter washed with methanol. The filtrate and the washings were combined and concentrated under vacuum to give a solid residue, which was triturated three times with ether and filtered to give **9** (62 mg, 84%) as a solid: mp > 162 °C (dec) (methanol). MS (ES) *m/z* 350 (M + H), IR (KBr) 3375, 1694, 1587 cm^{-1}; ^1H NMR (CD_3OD) δ 0.94 (m, 6H), 1.57 (m, 4H), 2.50 (m, 4H), 2.93–3.72 (m, 5H), 6.90–7.51 (m, 3H). Anal. calcd for $C_{18}H_{27}N_3O_4 \cdot 0.75H_2O$: C, 59.57; H, 7.49; N, 11.57. Found: C, 59.32; H, 7.52; N, 11.27.

Diethyl 2-[4-(Methoxycarbonyl)phenylamino]malonate (21). A mixture of methyl *p*-aminobenzoate (**20**) (3.10 g, 20.5 mmol) and diethyl bromomalonate (2.45 g, 10.2 mmol) was heated at 112 °C for 15 h in an oven. The reaction mixture was cooled, suspended in ether (70 mL), and then washed with 2 N HCl (3×15 mL), water (15 mL) and brine (10 mL). The organic layer was dried (Na_2SO_4) and concentrated to give a solid, which was crystallized from ether/hexane to give **21** (2.8 g, 86%): mp. 67–69 °C. MS (ES) *m/z* 310 (M + H); ^1H NMR ($CDCl_3$) δ 7.90 (d, 2H), 6.60 (d, 2H), 5.21 (br s, 1H), 4.80 (d, 1H), 4.31 (q, 4H), 3.82 (s, 3H), 1.31 (t, 6H). Anal. calcd for $C_{15}H_{19}NO_6$: C, 56.52; H, 4.35; N, 10.15. Found: C, 56.55; H, 4.40; N, 10.13.

Diethyl 2-(2-Acetyloxyethyl)-2-[(4-methoxycarbonyl)phenylamino]malonate (22). To a suspension of 60% NaH in mineral oil (0.52 g, 13 mmol) in 12 mL of anydrous DMF was added amine **21** (4.0 g, 13 mmol). The resulting mixture bubbled, was stirred at room temperature for 15 min, and 2-bromoethyl acetate (2.4 g, 14 mmol) was added. The mixture was stirred for 36 h at room temperature. The reaction was quenched with 1 N HCl (10 mL) and the product was extracted into ethyl acetate (3×50 mL). The combined organic extracts were washed with H_2O (25 mL), dried (Na_2SO_4) and evaporated to give an oil (5.1 g) that was purified by flash chromatography (silica gel, 5 × 40 cm, 2:1 ether/hexane) to give an oil (4.5 g, 88%) (R_f 0.60). The oil was crystallized from ether to give **22** (4.1 g, 80%) as white crystals: mp 88–89 °C. MS (ES) *m/z* 396 (M + H); ^1H NMR ($CDCl_3$) δ 7.83 (d, 2H), 6.58 (d, 2H), 5.70 (s, 1H), 4.33–4.17 (m, 4H), 4.05 (t, 2H), 3.85 (s, 3H), 2.77 (t, 2H), 1.93 (s, 3H), 1.22 (t, 6H). Anal. calcd for $C_{19}H_{26}NO_8$: C, 57.71; H, 6.37; N, 3.54 Found: C, 57.77; H, 6.30; N, 3.51.

2-[(4-Methoxycarbonylphenyl)amino]-γ-butyrolactone (23). To an ice cold suspension of **22** (1.00 g, 2.53 mmol) in dry methanol (12 mL) was added freshly cut Na (0.250 g, 10.9 mmol). The mixture was stirred for 1 h at 0 °C and then quenched with 1 N HCl (10 mL). The methanol was removed under vacuum, the aqueous residue was extracted with ethyl acetate (3×25 mL), and the organic layers were dried (Na_2SO_4) and concentrated to give an oil (0.550 g). This was purified by flash chromatography (silica gel, 2 × 40 cm, ether) to give **23** (0.500 g, 84.0%) as a white solid: mp 136–138 °C. MS (ES) *m/z* 236 (M + H); ^1H NMR ($CDCl_3$) δ 7.90 (d, 2H), 6.64 (d, 2H), 4.70 (br s, 1H), 4.53 (t, 1H), 4.40–4.32 (m, 1H), 4.23 (dd, 1H), 3.87 (s, 3H), 2.96–2.90 (m, 1H), 2.23–2.15 (m, 1H). Anal. calcd for $C_{12}H_{13}NO_4$: C, 61.27; H, 5.57; N, 5.95. Found: C, 61.19; H, 5.65; N, 5.89.

Spiro 2-(γ-butyrolactone)-5-[*N*-(4-methoxycarbonylphenyl)pyrrolidin-2-one] (24). A solution of **23** (0.050 g, 0.21 mmol) in dry THF (1 mL) was cooled to −78 °C and lithium diisopropylamide (0.30 g, 0.28 mmol) was added. After 20 min of stirring, ethyl acrylate (0.030 g, 0.30 mmol) was added and the stirring continued for 4 h at −70 to −60 °C. The reaction mixture was quenched with 1 N HCl (1 mL), the product was extracted into ethyl acetate (3×15 mL), and the organic extracts were dried (Na_2SO_4) and concentrated to give an oil (0.50 g, 82%). This was purified by flash chromatography (silica gel, 1.5 × 36 cm, 6.4% ethanol in ether, R_f = 0.50) to give **24** (0.040 g, 67%) as a white solid: mp 102–104 °C. MS (ES) *m/z* 290 (M + H); ^1H NMR ($CDCl_3$) δ 8.11–8.07 (m, 2H), 7.33–7.27 (m, 2H), 4.37–4.20 (m, 2H), 3.92 (s, 3H), 2.96–2.84 (m, 1H), 2.68–2.55 (m, 3H), 2.29–2.22 (m, 1H). Anal. calcd for $C_{15}H_{15}NO_5$: C, 62.28; H, 5.23; N, 4.84. Found: C, 62.31; H, 5.18; N, 4.82.

Spiro 2-(γ-butyrolactone)-5-[*N*-(4-methoxycarbonyl-2-nitrophenyl)pyrrolidin-2-one] (25). An ice-cold solution of

24 (0.080 g, 0.28 mmol) in methylene chloride (2 mL) was treated with nitronium tetrafluoroborate (0.19 g, 1.4 mmol). The reaction mixture was stirred at 0 °C for 6 h and at room temperature for 10 h. The reaction mixture was quenched with H_2O (2 mL) and the CH_2Cl_2 was removed under vacuum. The aqueous residue was extracted with ethyl acetate (5×15 mL), and the combined extracts were washed with brine (5 mL), dried (Na_2SO_4) and concentrated to give a crude yellow solid (0.090 g). This was purified by flash chromatography (silica gel, 1.5×40 cm, 5% ethanol in ether, R_f = 0.60) to give **25** (0.080 g, 87%) as a white solid: mp 147–150 °C. MS (ES) *m*/*z* 335 (M + H); ^{1}H NMR ($CDCl_3$) δ 8.65 (d, 1H), 8.35 (dd, 1H), 7.53 (d, 1H), 4.33–4.38 (m, 2H), 3.99 (s, 3H), 2.91–2.79 (m, 1H), 2.70–2.60 (m, 2H), 2.52–2.43 (m, 3H). Anal. calcd for $C_{15}H_{14}N_2O_7$: C, 53.89; H, 4.22; N, 8.37. Found: C, 53.49; H, 4.26; N, 8.23.

5-(2-Hydroxyethyl)-5-hydroxymethyl-*N*-(4-methoxycarbonyl-2-nitrophenyl)pyrrolidin-2-one (26). To an ice cold solution of **25** (0.040 g, 0.12 mmol) in a mixture of dry methanol (0.50 mL) and dry THF (0.50 mL) was added $NaBH_4$ (0.20 g, 0.50 mmol) in small portions over 25–30 min. The reaction mixture was stirred at 0–5 °C for 4 h and quenched with 1 N HCl (0.75 mL). The solvent was removed under vacuum and the aqueous residue was extracted with ethyl acetate (4×15 mL). The combined extracts were dried (Na_2SO_4) and concentrated to give a crude semisolid (0.040 g). This was purified by flash chromatography (silica gel, 1.5 × 25 cm, 6.4% ethanol in ether, R_f = 0.40) to give **26** (0.030 g, 81%) as yellow crystals: mp 151–153 °C. MS (ES) *m*/*z* 339 (M + H); ^{1}H NMR (CD_3OD) δ 8.59 (s, 1H), 8.33 (d, 1H), 7.77 (d, 1H), 3.98 (s, 3H), 3.87–3.73 (dd, 2H), 3.62 (t, 2H), 2.76–2.60 (m, 1H), 2.47–2.31 (m, 3H), 1.94–1.91 (m, 1H), 1.74–1.71 (m, 1H). Anal. calcd for $C_{15}H_{18}N_2O_7$: C, 53.25; H, 5.36; N, 8.28. Found: C, 53.14; H, 5.44; N, 8.22.

***N*-(2-Amino-4-methoxycarbonylphenyl)-5-(2-hydroxyethyl)-5-(hydroxymethyl)pyrrolidin-2-one (27).** A mixture of **26** (0.050 g, 0.15 mmol) and 10% Pd/C (0.05 g) in methanol (10 mL) was hydrogenated on a Parr shaker at 38 psi for 1 h. The reaction mixture was diluted with methanol (10 mL), filtered, the filter was washed with additional methanol (10 mL), and the filtrate was concentrated to dryness to give **27** (0.045 g, 99%) as an oil. MS (ES) *m*/*z* 309 (M + H); ^{1}H NMR (CD_3OD) δ 7.65–6.98 (m, 3H), 3.87 (s, 3H), 3.78–3.51 (m, 3H), 3.28–3.39 (m, 1H), 2.74–2.65 (m, 1H), 2.52–2.19 (m, 3H), 1.95–1.62 (m, 2H). Anal. calcd for $C_{15}H_{20}N_2O_5$: C, 58.43; H, 6.53; N, 9.09. Found: C, 58.65; H, 6.55; N, 9.31.

5-(2-Hydroxyethyl)-5-hydroxymethyl-*N*-[4-methoxycarbonyl-2-(3-pentylamino)phenyl]pyrrolidin-2-one (28). A solution of **27** (0.090 g, 0.29 mmol) in 1,2-dichloroethane (1 mL) and acetic acid (0.5 mL) was treated with 3-pentanone (0.18 g, 2.0 mmol) and $NaCNBH_3$ (68 mg, 1.1 mmol). The resulting mixture was stirred at room temperature for 18 h. Additional 3-pentanone (0.18 g, 2.0 mmol) and $NaCNBH_3$ (68 mg, 1.1 mmol) were added and the stirring continued for 17 h. To the reaction mixture was added satd $NaHCO_3$, and this was extracted with ethyl acetate (5×20 mL). The combined organic layers were dried (Na_2SO_4) and concentrated to give an oil (90 mg, 82%). This was purified by flash chromatography (silica gel, 1.5×40 cm, 8% ethanol in ether, R_f = 0.60) to give **28** (78 mg, 71%) as a white solid: mp 141–143 °C. MS (ES) *m*/*z* 379 (M + H); ^{1}H NMR ($CDCl_3$) δ 7.55 (d, 1H), 7.50 (d, 1H), 7.48 (d, 1H), 4.25 (br s, 2H), 3.92 (s, 3H), 3.89–3.82 (m, 2H), 3.35–3.29 (m, 2H), 3.17 (d, 1H), 2.70–2.45 (m, 3H), 2.18–2.09 (m, 1H), 1.95–1.84 (m, 2H), 1.81–1.47 (m, 4H), 0.98–0.92 (m, 6H). Anal. calcd for $C_{20}H_{30}N_2O_5$: C, 63.47; H, 7.98; N, 7.40. Found: C, 62.57; H, 7.86; N, 7.32.

***N*-[4-Carboxy-2-(3-pentylamino)phenyl]-5-(2-hydroxyethyl)-5-(hydroxymethyl)pyrrolidin-2-one (10).** A suspension of **28** (0.060 g, 0.16 mmol) in 1 N NaOH (1.0 mL, 1.0 mmol) was stirred at room temperature for 17 h. The reaction mixture was neutralized with acetic acid and evaporated to dryness under high vacuum. The resulting solid was dissolved in H_2O (3 mL) and extracted with ethyl acetate (7×15 mL). The combined organic layers were dried (Na_2SO_4) and concentrated to give a semisolid, which was triturated with ether and filtered to give **10** (40 mg, 70%) as white crystals: mp 188–190 °C. MS (ES) *m*/*z* 365 (M + H); ^{1}H NMR ($CDCl_3$/CD_3OD) δ 7.50 (br s, 1H), 7.48–7.40 (m, 1H), 7.08–7.00 (d, 1H), 3.82–3.69 (m, 2H), 3.44 (d, 1H), 3.35–3.27 (m, 1H), 3.25 (d, 1H), 2.79–2.68 (m, 1H), 2.60–2.39 (m, 2H), 2.22–2.10 (m, 1H), 1.93–1.42 (m, 6H), 1.00–0.88 (m, 6H). Anal. calcd for $C_{19}H_{28}N_2O_5$: C, 62.61; H, 7.74; N, 7.68. Found: C, 62.33; H, 7.76; N, 7.63.

Spiro 5-[*N*-(4-Methoxycarbonyl-2-nitrophenyl)pyrrolidin-2-one]-5-(1,3-dioxa-2-phenylcycloheptane) (29). A suspension of **26** (3.00 g, 9.73 mmol), benzaldehyde (120 mL, 1.18 mol), and 5–10 mg of *p*-toluene sulphonic acid was stirred at room temperature for 24 h. To the reaction mixture was added saturated $NaHCO_3$ (1 mL) followed by water (25 mL), and this was extracted with EtOAc (4 × 25 mL). The combined organic layers were washed with water (50 mL), brine (50 mL), and dried ($MgSO_4$). The organic layer was concentrated under vacuum to give an oil (130 mg). The crude oil was purified by silica column chromatography, using stepwise elution, first with 20% EtOAc in hexane (300 mL), and subsequently with 80% EtOAc in hexane to give the desired compound **29** (3.2 g, 77%; combined weight of isomers A and B).

Isomer A. MS (ES) *m*/*z* 427 (M + H); ^{1}H NMR ($CDCl_3$) δ 8.70 (d, 1H), 8.30–8.31 (d, 1H), 7.46–7.36 (m, 6H), 5.78 (s, 0.6H), 5.47 (s, 0.4H), 4.08–4.01 (m, 1H), 3.99 (s, 3H), 3.63–3.45 (m, 4H), 2.73–2.51 (m, 3H), 2.11–2.07 (m, 1H). Anal. calcd for $C_{22}H_{22}N_2O_7$: C, 61.97; H, 5.20; N, 6.57. Found: C, 61.62; H, 5.38; N, 6.36.

Isomer B. MS (ES) *m*/*z* 427 (M + H);^{1}H NMR ($CDCl_3$) δ 8.61 (d, 1H,), 8.18–8.15 (dd, 1H), 7.37–7.22 (m, 6H), 5.72 (s, 1H), 4.08–3.99 (m, 1H), 3.95 (s, 3H),

3.81–3.70 (m, 4H), 2.66–2.52 (m, 3H), 2.20–2.13 (m, 1H) and 1.90–1.70 (m, 1H).

5-Benzoyloxymethyl-5-(2-bromoethyl)-*N*-(4-methoxycarbonyl-2-nitrophenyl)pyrrolidin-2-one (30). A solution of **29** (isomers A and B combined, 2.75 g, 6.45 mmol) in anhydrous benzene (117 mL) was treated with *N*-bromosuccinimide (1.60 g, 8.99 mmol) at ambient temperature and stirred under nitrogen for 23 h. Benzene (63 mL) was added, and stirring was continued for an additional 3 h. The reaction mixture was concentrated under vacuum, the residue was suspended in water (50 mL) and the product was extracted into chloroform (3 × 50 mL). The combined organic layers were dried ($MgSO_4$) and concentrated to give an oily residue. This was placed on a flash chromatography column (ether) to give **30** (2.35 g, 72.3%) as a white powder: mp 69–71 °C. MS (ES) *m/z* 505 (M + H); ^{1}H NMR ($CDCl_3$) δ 8.68 (s, 0.6H), 8.58 (s, 0.4H), 8.32–8.26 (m, 1H), 7.98–7.96 (d, 1H), 7.66–7.26 (m, 5H), 4.73–4.41 (m, 2H), 3.98 (s, 3H), 3.51–3.46 (m, 1H), 3.36–3.33 (m, 1H), 2.71–2.24 (m, 6H). Anal. calcd for $C_{22}H_{21}N_2O_7Br$: C, 52.33; H, 4.19; N, 5.55. Found: C, 52.24; H, 4.31; N, 5.39.

5-(2-Azidoethyl)-5-benzoyloxymethyl-*N*-(4-methoxycarbonyl-2-nitrophenyl)pyrrolidin-2-one (31). Compound **30** (1.90 g, 3.76 mmol) was dissolved in DMF (5.7 mL), NaN_3 (0.300 g, 4.61 mmol) was added, and the mixture was stirred at 70–75 °C for 16 h. The DMF was removed under vacuum at 40 °C, the residue was diluted with EtOAc (25 mL), and the mixture was washed with water (4×15 mL) followed by brine (15 mL). The organic layer was dried ($MgSO_4$) and concentrated. The residue was purified by flash chromatography (ether) to give **31** (1.48 g, 85.0%) as pale yellow needles: mp 46–48 °C. MS (ES) *m/z* 468 (M + H); ^{1}H NMR ($CDCl_3$) δ 8.66 (s, 1H), 8.28–8.25 (d, 1H), 7.98–7.95 (d, 1H), 7.64–7.22 (m, 5H), 4.72–4.40 (m, 2H), 3.97 (s, 3H), 3.61–3.57 (t, 1H), 3.45–3.40 (t, 1H), 2.77–2.08 (m, 5H) and 1.94–1.84 (m, 1H). Anal. calcd for $C_{22}H_{21}N_5O_7$: C, 56.53; H, 4.53; N, 14.98. Found: C, 56.32; H, 4.53; N, 14.75.

***N*-(2-Amino-4-methoxycarbonylphenyl)-5-(2-azidoethyl)-5-(benzoyloxymethyl)pyrrolidin-2-one (32).** Compound **31** (1.50 g, 3.21 mmol) was dissolved in THF (22 mL) and water (9.6 mL), and to this was added $Na_2S_2O_7$ (2.14 g, 12.2 mmol). After stirring under nitrogen for 20 h, more $Na_2S_2O_7$ (0.197 mg, 1.13 mmol) was added, and the mixture was stirred for an additional 5 h at room temperature. The THF was removed under vacuum and the aqueous residue was extracted with EtOAc (5 × 25 mL). The aqueous layer was concentrated to dryness and the residue was triturated with EtOAc (25 mL) and filtered. The combined organic layers were dried ($MgSO_4$) and concentrated to give an oil (1.31 g). This was purified by flash chromatography (8% ethanol in ether) to give **32** (1.20 g, 86.0%) as a white solid: mp 65–68 °C; MS (ES) *m/z* 438 (M + H); ^{1}H NMR (CD_3OD) δ 8.01–7.98 (m, 1H), 7.68–7.63 (m, 0.5H), 7.54–7.45 (m, 3H), 7.39–7.36 (m, 0.5H), 7.31–7.24 (m, 2H), 7.13–7.10 (d, 0.5H), 7.02–7.00 (d, 0.5H), 4.72–4.56 (dd, 1H), 4.36–4.35 (dd, 1H), 3.90 (s, 3H), 3.88–3.87 (d, 1H), 3.66–3.48 (m, 1H), 3.46–3.39 (q, 1H), 2.72–2.63 (m, 2H), 2.44–2.00 (m, 4H), 2.04–2.00. Anal. calcd for $C_{22}H_{23}N_5O_5$: C, 60.44; H, 5.30; N, 16.00. Found: C, 60.56; H, 5.18; N, 15.91.

5-(2-Azidoethyl)-5-benzoyloxymethyl-*N*-[4-methoxycarbonyl-2-(3-pentylamino)phenyl]pyrrolidin-2-one (33). A solution of **32** (0.19 g, 0.43 mmol) in 2 mL of 1,2-dichloroethane was treated with 1 mL of acetic acid and stirred under nitrogen. After 10 min, 3-pentanone (0.39 g, 4.5 mmol) and $NaCNBH_3$ (0.14 g, 2.2 mmol) were added, and stirring was continued for 5 h. Another portion of 3-pentanone (0.39 g, 4.5 mmol) and $NaBH_3CN$ (0.14 g, 2.2 mmol) was added, followed by an additional five portions, each added every 5 h. No further progress in reaction was observed. To the reaction mixture was added saturated $NaHCO_3$ (8 mL), and this was concentrated under high vacuum. The white residue was triturated with ether (5 × 25 mL). The combined ether washes were shaken with H_2O (10 × 20 mL), brine (20 mL) and dried ($MgSO_4$). This was concentrated to give a green oil (180 mg), which was placed on a flash chromatography column (ether) to give recovered **32** (100 mg) and the desired product **33** (70 mg, 70% conversion) as an oil. This material was carried forward without additional purification. MS (ES) *m/z* 508 (M + H); ^{1}H NMR ($CDCl_3$) δ 8.02–7.99 (d, 1H), 7.78–7.76 (d, 1H), 7.63–7.56 (m, 1H), 7.50–7.27 (m, 4H), 7.03–6.99 (m, 1H), 4.66–4.62 (d, 0.5H), 4.53–4.49 (d, 0.5H), 4.38 (s, 1H), 4.28–4.18 (m, 1H), 4.01–3.98 (d, 0.5H), 3.90 (s, 3H), 3.85–3.82 (d, 0.5H), 3.52–3.29 (m, 2H), 2.86–2.57 (m, 2H), 2.34–2.27 (m, 2H), 2.10–2.07 (t, 1H), 1.98–1.90 (t, 1H), 1.66–1.40 (m, 4H), 1.01–0.95 (m, 3H), 0.83–0.77 (m, 3H).

5-(2-Azidoethyl)-*N*-[4-carboxy-2-(3-pentylamino)phenyl]-5-(hydroxymethyl)pyrrolidin-2-one (34). A solution of **33** (0.047 g, 0.093 mmol) in 1 N NaOH (1 mL) and methanol (1 mL) was stirred at ambient temperature for 12 h. The mixture was acidified to pH 3 with acetic acid and concentrated to dryness under high vacuum. The white solid residue was triturated with EtOAc (2×10 mL), and the combined organic washes were extracted with water (10 mL) and brine (10 mL). The organic layer was concentrated to give a white solid, which was triturated with ether (5 mL) followed by hexane (5 mL). The insoluble residue was purified by flash chromatography (4% ethanol in ether) to give **34** (25 mg, 70%) as a solid: mp 164–166 °C; MS (ES) *m/z* 390 (M + H); ^{1}H NMR (CH_3OD) δ 7.39 (d, 0.4H), 7.33–7.32 (d, 0.6H), 7.30–7.27 (m, 0.4H), 7.23–7.19 (dd, 1H), 6.99–6.96 (d, 0.6H), 3.64–3.60 (t, 1H), 3.52–3.35 (m, 3H), 3.27–3.16 (m, 1H), 2.81–2.64 (m, 1H), 2.57–2.29 (m, 2H), 2.27–2.12 (m, 1H), 1.99–1.88 (m, 1H), 1.82–1.73 (m, 1H), 1.68–1.41 (m, 4H), 0.99–0.88 (m, 6H). Anal. calcd for $C_{19}H_{27}N_5O_5$: C, 57.27; H, 7.08; N, 17.57. Found: C, 57.60; H, 6.96; N, 17.22.

5-(2-Aminoethyl)-*N*-[4-carboxy-2-(3-pentylamino)phenyl]-5-(hydroxymethyl)pyrrolidin-2-one (11). A suspension of **34** (0.065 g, 0.167 mmol), 10% Pd/C (0 073 g), and methanol (15 mL) was reacted on a Parr shaker for 3 h at 40 psi H_2. The reaction mixture was diluted with MeOH (50 mL), and the mixture was filtered and concentrated to

give an off-white solid residue. The solid was suspended in ether and filtered to give **11** (0.060 g, 96%) as a powder: mp 203–205 °C. MS (ES) m/z 364 (M + H); ^{1}H NMR (35 °C, DMSO-d_6) δ 7.23–7.01 (m, 1.8H), 6.98–6.95 (d, 0.6H), 6.83–6.81 (d, 0.6H), 5.18 (bs, 2H), 4.1 (bs, 1H), 3.49–3.47 (d, 2H), 3.23–3.19 (d, 2H), 2.77–2.62 (m, 1H), 2.29–2.21 (m, 2H), 2.12–1.97 (m, 1H), 1.88–1.62 (m, 2H), 1.58–1.43 (m, 3.4H), 1.42–1.29 (m, 1.6H), 0.93–0.78 (m, 6H). Anal. calcd for $C_{19}H_{29}N_3O_4$: C, 61.34; H, 8.12; N, 11.29. Found: C, 61.19; H, 8.01; N, 11.02.

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