Pyrrolidinobenzoic Acid Inhibitors of Influenza Virus Neuraminidase: the Hydrophobic Side Chain Influences Type A Subtype Selectivity

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Abstract

Neuraminidase (NA) plays a critical role in the life cycle of influenza virus and is a target for new therapeutic agents. A series of influenza neuraminidase inhibitors with the pyrrolidinobenzoic acid scaffold containing lipophilic side chains at the C3 position have been synthesized and evaluated for influenza neuraminidase inhibitory activity. The size and geometry of the C3 side chains have been modified in order to investigate structure-activity relationships. The results indicated that size and geometry of the C3-side chain are important for selectivity of inhibition against N1 vs N2 NA, important type A influenza variants that infect man, including the highly lethal avian influenza.

Keywords

Neuraminidase; avian influenza; pyrrolidinobenzoic acid

1. Introduction

Annual epidemics of influenza virus are responsible for considerable morbidity and mortality[1], and pandemics are particularly devastating[2–4]. Infection is especially dangerous to the very young, elderly, and to those who have suppressed immune systems[5–7]. 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[2, 8]. The replicative cycle of influenza virus offers several virus-specific events which could be considered as targets for chemotherapeutic intervention.[9] One of these is influenza neuraminidase (NA), which catalyzes the cleavage of the terminal sialic acid attached to host cell glycoproteins and glycolipids.[10, 11] This process is believed to be necessary for the release of newly formed virus from infected cells and for efficient spread of virus in the respiratory tract.

In recent years several very potent inhibitors of both influenza A and B NA have been developed.[12–18] Early studies on NA inhibition revealed that 2,3-didehydro-2-deoxy-*N*-

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acetylneuraminic acid (**1a**) (Neu5Ac2en), an analogue of sialic acid, is a modest inhibitor with a K_i of 4 μM, and is believed to be a transition state analog due to planarity near the carboxylate. Solving the crystal structure of **1a** in complex with NA was crucial in that it provided for the structure-based drug design[19] of Relenza® (**1b**), which was followed by Tamiflu® (the prodrug **1d; 1c** is the active form), and Rapiacta® (**1e**, peramivir), and all have been marketed as anti-influenza drugs. These drugs have in common a nonaromatic central ring containing a carboxylate and several side chains with defined stereochemistry, including the *N*-acetyl moiety, a strongly basic nitrogen, and (except for Relenza®) a hydrophobic group. The requirement for multiple chiral centers in some cases has resulted in high manufacturing costs.

We have developed a number of NA inhibitors containing the benzoic acid ring scaffold.[20–24]. The benzene ring was selected as a template because it provides planarity near the carboxylate as found in structures **1a–1d** and, further, the benzene ring has no stereogenic centers at substitution sites. Such inhibitors thus potentially offered a more economical synthesis of anti-influenza drugs. Our lead benzoic acid inhibitor[20] was 4-(*N*-acetylamino)-3-guanidinobenzoic acid (**2**) (BANA 113, K_i 2.5 μM). Further optimization of **2** resulted in the potent benzoic acid inhibitor **3** (1-[4-carboxy-2-(3-pentylamino)phenyl]-5,5-bis(hydroxymethyl)pyrrolidin-2-one), which is unique among potent inhibitors in that it contains a substituted pyrrolidinone ring in place of the *N*-acetyl functionality. Note that compound **3** is also unique in that it contains no chiral carbons and lacks a strongly basic nitrogen. Compound **3** exhibited an IC_{50} of 48 nM against influenza A NA (N9, an avian strain),[22] but was less effective against other type A NAs (see Table 1).

Here we describe the synthesis and evaluation of additional inhibitors related to **3**, with special emphasis on determining effects on the N1 and N2 NAs of human viruses. Given the importance of the hydrophobic side chain in potent inhibitors **1c**, **1e**, and **3**, we proposed for this study modifications to the hydrophobic side chain at the C_3 position of **3** by incorporating alkyl or ether moieties.

2. Chemistry

We first pursued the synthesis of analogue **10a–e**, which introduced lipophilic ether side chains (similar to **1c**) at C_3. These were prepared as shown in Scheme 1. 3-Methyl-4-nitrobenzoic acid was esterified with MeOH to give **4**, and reduced with Zn/HCl to afford methyl 3-methyl-4-aminobenzoate (**5**). Intermediate **5** was reacted with diethyl bromomalonate to give **6**, which was *N*-acylated with 3-bromopropionic acid and PCl_3 to provide **7**. This underwent cyclization in the presence of NaH to yield lactam **8**, which was treated with NBS to produce the bromo compound **9** in 18% overall yield. There were three steps involved in the conversion of compound **9** to the final acids **10a–e** and intermediates were not purified. The first step was the alkylation with the appropriate alkoxide to form the corresponding ether, In this step, the methyl benzoate of **9** was partially transesterified by the alkoxide to provide a mixture of methyl benzoate and/or alkyl benzoate (for example, 2-propyl, etc.). The ethers containing mixed benzoate esters were used for the next steps without further purification. The second step was selective reduction of the ethyl malonate esters on the pyrrolidinone ring to form the diols. The third step was hydrolysis of the mixed benzoate esters using aqueous NaOH to afford the final acids **10a–e.**

Next, compounds **13a**, **13c**, and **14b** were designed as analogues of **3** that contain an alkyl side chain in place of the aliphatic amino side chain at the C_3 position. The preparations of **13a**, **14b** and **13c** are described in Scheme 2. Compounds **11a–c** were synthesized through a palladium-catalyzed cross-coupling reaction between methyl 4-amino-3-iodobenzoate and the corresponding vinyl bromide (**a–c**). While the syntheses of intermediates **12a–c** from the

intermediate **11a–c** each involved three major steps (in one "pot"), only the purified intermediates are shown in Scheme 2: The first step was the introduction of the pyrrolidinone ring, which was accomplished by reductive amination of the aniline with diethyl ketomalonate, *N*-alkylation with 3-bromopionic aid in the presence of PCl_3, and cyclization in the presence of NaH. We found that overall yields were highest when these reactions were carried out without purifying the intermediates. The alkenes **12a and 12b** were purified at this stage, while alkane **12c** was obtained after further hydrogenation in the presence of Pd/C. Final compounds **13a** and **13c** were prepared by catalytic hydrogenation of the alkene, selective reduction of the malonate esters to diols using $NaBH_4$, and saponification of the benzoates to the final acids. Compounds **13a** and **13c** were synthesized following the same sequence except that the intermediate were purified at different stage. Compound **13b** was prepared in a similar fashion, but without the catalytic hydrogenation step, to allow the alkene to be preserved in the larger side chain of final product **13b**, and the catalytic hydrogenation of **13b** then afforded **14b**.

3. Biological Assays

All compounds were evaluated for in vitro inhibitory activity against NA using intact purified virions of influenza virus A (current vaccine strains of H1N1 and H3N2 subtypes) and influenza virus B using the fluorogenic substrate $2'$-(4-methylumbelliferyl)-α-D-N-acetylneuraminic acid [32], and IC_{50} values were determined using Prism software. The results are contained in Table 1.

4. Results and Discussion

The numerous crystal structures that have been reported for a variety of type A and B neuraminidases (NAs) demonstrate that this site is highly conserved and, essentially, structurally identical irregardless of the source. Thus it is not surprising to find that inhibitors often show similar effects for all subtypes. For example, with oseltamivir carboxylate (**1c;** the active form of Tamiflu®), inhibition (IC_{50}) of N1 NAs ranged from 0.30–0.80 nM, N2 NAs ranged from 0.68–1.78 nM, and type B NAs ranged from 5.00–24.3 nM[28]. One recent exception to this structural conservation among NA binding sites is the observation that type A NAs can be grouped into two structural classes. The Group 1 NAs include N1, which in some crystal structures (but not all) shows an additional cavity next to the active site that is not present in N2 (a member of Group 2 NAs). The cavity is created by movement of the "150 loop" (residues 147-152) and has been suggested to provide a new target for drug design.[29] While, as mentioned previously, selectivity of inhibition for N1 vs N2 NAs is not observed for the potent clinically used inhibitors, any observed selectivity might be rationalized based on this observation.

As shown in Table 1, the parent compound for the present study, pyrrolidinone **3**, indeed exhibited significant selectivity for N2 vs N1 NA. The much poorer inhibition seen for type B NA is commonly observed for certain inhibitors containing a hydrophobic side chain, and has been rationalized based on an energetically unfavorable conformational change in Glu276 upon ligand binding.[30,31] Type A influenza causes more serious disease in humans and is the major target of clinically useful NA inhibitors. Since pyrrolidinone **3** exhibited good activity against N2 NA (relatively rigid binding site), but poor activity against N1 NA (may contain extra binding cavity), here we proposed to determine if smaller or larger side chains of different presentation might improve N1 activity without decreasing N2 activity. Particular choices for these side chains were limited by synthetic accessibility and resources but attempted to vary side chain length, presentation, and branching size. For this study we chose to measure inhibition against NAs of recently circulating H3N2 and H1N1 viruses, using the current vaccine strains in most cases.

In evaluating the analogs of **3** described in Table 1, we were therefore interested in two trends- changes in potency of inhibition and changes in selectivity for N1 vs N2 NA. Analogs of **3** included in this study were those containing aliphatic side chains with the same branching position(**13a–c**, and **14b**), relative to the benzene ring, as found in **3,** and those containing ether side chains branched one atom further from the benzene ring (**10a–e**).

For the latter series, the most effective inhibitors against N1 and N2 NA are **10b** and **10c**. If the hydrophobic chain is too small (**10a**) or too large (**10d–e**) potency is lost. Further, **10c** is less selective than **10b** for N2 vs N1 NA. While **10b** and **10c** exhibit nearly the same IC_{50} against N2, **10c** is much more effective against N1, and is the most potent N1 NA inhibitor of this series, with dramatically improved (80–100 fold) N1 activity compared to the parent **3**. The activities of **10b** and **10c** for N2 NA are similar to that for the parent **3**.

For the former series (**13a–c**, **14b**), all but **13c** are nM inhibitors against N2 NA that exhibit similar potency to each other and to the parent **3**. Within this series, compounds **13b** and **14b** are also the least selective for N2 vs N1 NA and exhibit a 30–40 fold increase in N1 NA inhibition compared to the parent **3**.

As discussed above, for the series of compounds presented, N2 activity is always better than N1 activity, suggesting that any involvement of the 150-loop for binding of these inhibitors results in a penalty in all but the most potent N1 inhibitors such as **10c**.

5. Conclusions

In summary, none of these analogs resulted in significantly enhanced potency, relative to **3**, against N2 NA. However, relatively subtle changes in the nature of the hydrophobic group did yield up to 100-fold improvements against N1 NA. In this regard the best compounds (**10c**, **13b**, and **14b**) all contain the same 3-pentyl grouping, but this was contained in different structures with either a one or two atom spacer to the benzene ring. It is interesting that this spacer length made little difference in activity.

It is important to note that **3,** and the analogs in Table 1, can only undergo three subsite interactions with NA, unlike the more potent inhibitors **1b**, **1c,** and **1e**, which all contain four interactions (for example **1c** contains substituents that occupy subsites for the carboxylate, *N*-acetyl, amino, and hydrophobic groups). Thus we anticipate that introduction of the missing basic group to provide a fourth interaction on pyrrolidinobenoic acids should also provide highly potent inhibitors. Current studies are pursuing such targets.

6. Experimental Section

6.1. Neuraminidase inhibition assays

Influenza virus strains A/Uruguay/716/07 (H3N2), A/Brisbane/59/07 (H1N1), A/Solomon Islands/3/06 (H1N1), A/Wisconsin/67/05 (H3N2), A/OK/3052/09 (H1N1pdm09), and B/Malaysia/5206/04 were grown in embryonated chicken eggs. IC_{50} determinations were carried out using the fluorogenic substrate 2′-(4-methylumbelliferyl)-α-D-N-acetylneuraminic acid[32] adapted to a 96-well plate. The reactions included neuraminidase in the form of purified intact virions, preincubated for 30 minutes at room temperature with two-fold dilutions of inhibitor in 50 mM sodium acetate, pH 6.0, 100 μM $CaCl_2$, 320 μM $MgCl_2$ and 60 mM NaCl, before adding 100 μM of the substrate (Sigma). The reaction mixtures were incubated for 15 minutes at 37 °C, after which the reaction was stopped with 5 volumes of 0.2 M glycine-NaOH, pH 11. The IC_{50}s were determined by Prism software using the log (inhibitor) versus normalized response option and cross-checked 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.

6.2. General experimental procedures

NMR data were recorded on Bruker ARX 300 or 400 MHz spectrometer. 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, Silicycle, Inc.). TLC was performed on silica gel (25 μm platesWhatman), and spots were visualized with UV light (UV-254 nm). Melting points were determined on a Mel-Temp electrothermal melting point apparatus (Laboratory Devices) and are uncorrected.

Methyl 3-Methyl-4-nitro-benzoate (4)[27]—To a solution of 3-methyl-4-nitrobenzoic acid (25.0 g, 138 mmol) in anhydrous methanol (400 mL) was added conc. H_2SO_4 (2 mL), at room temperature. The resulting solution was refluxed for 15h. The reaction mixture was concentrated. The solid was filtered and washed with cold methanol and dried to obtain the ester **4** (24 g, 89%) as a colorless solid. mp 78–80 °C.

Methyl 4-Amino-3-methylbenzoate (5)[27]—Methyl 3-methyl-4-nitrobenzoate (23 g, 117.84 mmol) was dissolved in ethyl acetate (400 mL) and conc.HCl (58 mL). Then the solution was cooled to 0 °C and Zn powder (46.2 g, 707 mmol) was added in portions over 20 minutes with stirring. The mixture was allowed to warm up to room temperature and stirring was continued for 15h. The reaction mixture was passed through a bed of Celite and washed with ethyl acetate (100 mL). The filtrate was washed with sat.NaHCO$_3$ solution (4 × 100 mL), water (3 × 100 mL) and brine (2 × 100 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated to dryness to obtain the amino compound **5** (17.4 g, 90.0%) as a colorless solid. mp 111–113 °C; ^1H NMR (300 MHz, CDCl$_3$) δ 2.18 (s, 3H), 3.85 (s, 3H), 4.02 (s, br, 2H), 6.65 (d, J = 8.1Hz, 1H), 7.76-7.72 (m, 2H); ^{13}C NMR (75 MHz, CDCl$_3$) δ 17.2, 51.7, 113.8, 119.5, 121.1, 129.4, 132.3, 149.5, 167.6; MS (ES) m/z 166 (M+1).

Diethyl 2-(4-Methoxycarbonyl-2-methyl-phenylamino)malonate (6)—A mixture of methyl 4-amino-3-methylbenzoate **5** (10.0 g, 60.5 mmol) and diethylbromomalonate (7.90 g, 33.3 mmol) was heated at 120 °C for 22h. The mixture was then cooled to room temperature and triturated with ethyl acetate (100 mL). The separated solid was filtered and washed with ethyl acetate (50 mL). The filtrate was concentrated and the crude product was purified by flash silica chromatography (75% hexane in EtOAc) to give a pale yellow solid (5.53 g, 56.0%). mp 40–42 °C; ^1H NMR (300 MHz, CDCl$_3$) δ 1.30 (t, J = 7.1 Hz, 6H), 2.27 (s, 3H), 3.85 (s, 3H), 4.30 (q, J = 7.1 Hz, 4H), 4.82 (d, J = 6.8 Hz, 1H), 5.17 (d, J = 6.8 Hz, 1H), 6.50 (d, J = 8.3 Hz, 1H), 7.77–7.81 (m, 2H); ^{13}C NMR (100 MHz, Acetone-d6) δ 14.3, 17.2, 51.6, 60.6, 63.0, 110.3, 120.3, 122.8, 130.1, 132.3, 148.7, 167.1, 168.0; MS (ES) m/z 324 (M+1).

Diethyl 2-[(3-Bromo-propionyl)-(4-methoxycarbonyl-2-methyl-phenyl)-amino]malonate (7)—To a solution of 3-bromopropionic acid (2.44 g, 16.0 mmol) in toluene (25 mL), PCl$_3$ (3.49 g, 25.4 mmol) was added and the mixture was heated at 110 °C for 2h. The mixture was cooled to room temperature, and a solution of **6** (2.35 g, 7.26 mmol) in toluene (10 mL) was added dropwise. Heating was continued at 100 °C for an additional 20h. The reaction mixture was concentrated and diluted with EtOAc (100 mL), washed with sat.NaHCO$_3$ solution (2 × 75 mL), water (2 × 75 mL) and brine (2 × 75 mL). The organic layer was dried over anhydrous Na_2SO_4, evaporated and the crude product was purified by flash silica chromatography (25% EtOAc in hexanes; Rf 0.5) to afford **7** as a colorless solid

(2.86 g, 85.0%). mp 104–106°C; ^{1}H NMR (300 MHz, CDCl$_3$) δ; 1.12 (t, J = 6 Hz, 3H), 1.31 (t, J = 7.2 Hz, 3H), 2.43 (s, 3H), 2.49–2.70 (m, 2H), 3.49–3.63 (m, 2H), 3.93 (s, 3H), 4.00–4.18 (m, 2H), 4.30 (q, J = 7.2 Hz, 2H), 5.05 (s, 1H), 7.58 (d, J = 8.4 Hz, 1H), 7.90 (dd, J = 8.4 Hz, 1.5 Hz, 1H), 8.00 (s, 1H); ^{13}C NMR (75 MHz, CDCl$_3$) δ 13.9, 14.1, 18.1, 26.1, 37.3, 52.5, 62.4, 65.2, 128.7, 129.7, 131.2, 132.9, 137.7, 142.9, 165.2, 165.6, 166.3, 170.1; MS (ES) m/z 458, 460 (M+1).

5,5-Bis(ethoxycarbonyl)-1-(4-methoxycarbonyl-2-methylphenyl)pyrrolidin-2-one (8)—To a suspension of NaH (0.300 g, 12.6 mmol) in anhydrous DMF (35 mL) was added a solution of **7** (5.26 g, 11.5 mmol) in anhydrous DMF (20 mL) dropwise for a period of 15 min at 0 °C. The mixture was stirred at room temperature for 4h. The reaction mixture was diluted with EtOAc (200 mL) and washed with water (4 × 150 mL) and brine (2 × 150 mL). The organic layer was dried over anhydrous Na$_2$SO$_4$, evaporated and the crude product was purified by flash silica chromatography (25% EtOAc in hexanes, Rf 0.4) to afford pure **8** as a colorless solid (4.0 g, 92%); mp 68–70 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.93 (s, 1H), 7.85 (d, J = 8.3 Hz, 1H), 7.52 (d, J = 8.3 Hz, 1H), 4.34 (m, 2H), 3.93 (m, 2H), 3.90 (s, 3H), 2.98 (m, 1H), 2.65 (m, 2H), 2.52 (m, 1H), 2.17 (s, 3H), 1.30 (t, J = 7.1 Hz, 6H), 0.88 (t, J = 7.1 Hz, 6H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 13.8, 14.4, 18.5, 29.3, 29.7, 52.6, 62.7, 63.1, 74.6, 128.2, 128.6, 130.3, 132.2, 138.4, 140.6, 166.8, 167.0, 1170.2, 174.6; MS (ES) m/z 378(M+1).

Diethyl 1-(2-(Bromomethyl)-4-(methoxycarbonyl)phenyl)-5-oxopyrrolidine-2,2-dicarboxylate (9)—To a solution of **8** (1.6 g, 4.2 mmol) and AIBN (70 mg, 0.40 mmol) in CCl$_4$ (36 mL), NBS (0.80 g, 4.6 mmol) was added. The mixture was refluxed for 3 h. The solid was filtered and the filtrate was concentrated to dryness. The obtained residue was chromatographed on a flash silica gel column (hexane/EtOAc, 2:1 v/v) to yield **9** (1.0 g, 52%) as a white solid. mp 58–60 °C; TLC R$_f$ 0.4, (hexane/EtOAc, 1:1 v/v). ^{1}H NMR (400 MHz, CDCl$_3$) δ 0.86 (t, J=7.1 Hz, 3H), 0.95 (t, J=7.1 Hz, 3H), 2.53 (m, 1H), 2.67 (m, 2H), 2.96 (m, 1H), 3.93 (s, 3H), 4.01 (m, 2H), 4.34 (m, 2H), 4.35 (d, J = 11.4 Hz, 1H), 4.40 (d, J = 11.4 Hz, 1H), 7.48 (d, J = 8.4 Hz, 1H), 7.97 (dd, J = 1.9, 8.4 Hz, 1H), 8.21 (d, J = 1.9 Hz, 1H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 13.7, 14.4, 29.3, 29.4, 29.8, 52.8, 62.8, 63.4, 74.6, 128.4, 130.5, 130.7, 132.9, 137.1, 140.5, 166.3, 166.4, 170.1, 175.2; MS (ES) m/z 456, 458(M+1).

Note: In the following conversion of **9** to **10a–e**, overall yields were highest when intermediates were not isolated and purified.

4-(2,2-Bis(hydroxymethyl)-5-oxopyrrolidin-1-yl)-3-(alkoxymethyl)benzoic Acid (10a–e)

General procedure: After the alcohol was treated with 4 mL of butyllithium (1.6 M) at −10 °C**,** compound **9** (1 mmol) was added at room temperature. The resulting mixture was stirred for 15 h at room temperature. The reaction mixture was diluted with ethyl acetate (50 mL), and washed with saturated NaHCO$_3$ (3 × 20 mL), water (2 × 20 mL) and brine (2 × 20 mL). The organic layer was dried (Na$_2$SO$_4$) and concentrated. The residue was chromatographed on a flash silica gel column to afford an oil. The oil was dissolved in dry MeOH (3 mL) and THF (3 mL) and cooled to 0°C under an argon atmosphere. Then NaBH$_4$ (189 mg, 5.00 mmol) was added in portions over 20 min. The mixture was allowed to warm to room temperature, stirred for 1–2 h, and quenched by adding cold water (5 mL), This mixture was then extracted with EtOAc (3 × 10 mL), and the combined organic extracts were washed with water (1 × 5 mL) and brine (1 × 5 mL), dried (Na$_2$SO$_4$) and evaporated to dryness. The residue was chromatographed on a flash silica gel column to afford a semisolid. The semisolid in MeOH (5 mL) was treated with a 1M solution of NaOH (5 mL) and the solution

was stirred at room temperature for 1–2h. The mixture was neutralized by using 1N HCl. The solvent was evaporated and the residue was purified by flash chromatography to obtain the benzoic acids **10a–e**. The three steps overall yields were calculated from bromo compounds **9** to the benzoic acids **10a–e.**

4-(2,2-Bis(hydroxymethyl)-5-oxopyrrolidin-1-yl)-3-(isopropoxymethyl)benzoic Acid (10a)—Colorless solid: mp 146–148 °C; yield 20%; TLC R_f 0.3, (EtOAc/MeOH, 4:1, v/v). ^{1}H NMR(400 MHz, CD_3COCD_3) δ 1.01(d, J=6.1 Hz, 6H), 2.12(m, 2H), 2.39(m, 2H), 3.09(d, J=11.8 Hz, 1H), 3.14(d, J=11.8 Hz, 1H), 3.40(d, J=11.7 Hz, 1H), 3.46(d, J=11.7 Hz, 1H), 3.55(m, 1H), 4.18(d, J=12.9 Hz, 1H), 4.54(d, J=12.9 Hz, 1H), 7.25(d, J=8.3 Hz, 1H), 7.78(dd, J=8.3, 2 Hz, 1H), 8.04(d, J=2 Hz, 1H); ^{13}C NMR (100 MHz, CD_3COCD_3) δ 21.3, 21.5, 25.1, 30.4, 63.0, 63.8, 65.8, 72.2, 72.4, 129.1, 129.4, 130.6, 131.0, 138.5, 139.9, 168.3, 178.1; Purity by HPLC 100%; MS (ES) m/z 338 (M+1).

4-(2,2-Bis(hydroxymethyl)-5-oxopyrrolidin-1-yl)-3-(2-butoxymethyl)benzoic Acid (10b)—Colorless solid: mp 156–158 °C; yield 29%; TLC R_f 0.3, (AcOEt/MeOH, 4:1, v/v). ^{1}H NMR(400 MHz, CD_3COCD_3) δ 0.76–0.81(m, 3H), 1.01–1.08(m, 3H), 1.35–1.52(m, 2H), 2.18(m, 2H), 2.38(m, 2H), 3.19–3.28(m, 2H), 3.41(m, 1H), 3.53–3.61(m, 2H), 4.23(d, J=12.7 Hz, 0.5H), 4.33(d, J=12.7 Hz, 0.5H), 4.64(d, J=12.6 Hz, 0.5H), 4.70(d, J=12.6 Hz, 0.5H), 7.38(d, J=8.2 Hz, 1H), 7.81(m, 1H), 8.14(m, 1H); ^{13}C NMR (100 MHz, CD_3COCD_3) δ 10.4, 10.6, 19.8, 20.0, 31.3, 64.3, 64.4, 65.1, 65.2, 67.1, 67.3, 72.2, 72.3, 77.9, 78.2, 130.0, 130.1, 131.1, 131.2, 131.4, 131.8, 140.6, 140.8, 140.9, 141.2, 167.8, 167.9, 176.8, 176.9; Purity by HPLC 96%; MS (ES) *m/z* 352 (M+1).

4-(2,2-Bis(hydroxymethyl)-3-((3-pentyloxy)methyl)-5-oxopyrrolidin-1-yl)benzoic Acid (10c)—Colorless solid: mp 177–179 °C; yield 20%; TLC R_f 0.3, (EtOAc/MeOH, 4:1, v/v). ^{1}H NMR(400 MHz, CD_3COCD_3) δ 0.67(d, J=7.5 Hz, 6H), 1.33(m, 2H), 2.08(m, 2H), 2.29(m, 2H), 3.09–3.17(m, 3H), 3.44(d, J=11.5 Hz, 1H), 3.48(d, J=11.5 Hz, 1H), 4.18(d, J=12.9 Hz, 1H), 4.54(d, J=12.9 Hz, 1H), 7.28(d, J=8.2 Hz, 1H), 7.71(dd, J=8.2, 1.9 Hz, 1H), 8.07(d, J=1.9 Hz, 1H); ^{13}C NMR (100 MHz, CD_3COCD_3) δ 10.2, 10.4, 14.8, 23.7, 26.1, 26.7, 26.9, 31.3, 64.3, 65.1, 67.6, 72.2, 83.4, 130.0, 130.4, 131.1, 1231.5, 140.5, 141.2, 167.8, 176.8; Purity by HPLC 98%; MS (ES) m/z 366 (M+1).

4-(2,2-Bis(hydroxymethyl)-5-oxopyrrolidin-1-yl)-3-(phenoxymethyl)benzoic Acid (10d)—Colorless solid: mp 198–200 °C; yield 20%; TLC R_f 0.5, (EtOAc/MeOH, 4:1, v/v). ^{1}H NMR(400 MHz, CD_3OD) δ 2.33(m, 2H), 2.63(m, 2H), 3.36(d, J=11.5 Hz, 1H), 3.42(d, J=11.5 Hz, 1H), 3.64(d, J=11.7 Hz, 1H), 3.70(d, J=11.7 Hz, 1H), 5.04(d, J=13.6 Hz, 1H), 5.40(d, J=13.6 Hz, 1H), 6.95(m, 1H), 7.04(d, J=7.8 Hz, 2H), 7.28(m, 2H), 7.55(d, J=8.2 Hz, 1H), 8.04(dd, J=8.2, 1.9 Hz, 1H), 8.31(d, J=1.9 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 26.6, 31.2, 64.5, 65.1, 67.2, 73.6, 116.3, 122.5, 130.8, 131.0, 132.4, 139.6, 139.9, 160.5, 169.6, 179.6; Purity by HPLC 97%; MS (ES) m/z 372 (M+1).

3-((Benzyloxy)methyl)-4-(2,2-bis (hydroxymethyl)-5-oxopyrrolidin-1-yl)benzoic Acid (10e)—Colorless solid: mp 198–200 °C; yield 20%; TLC R_f 0.5, (AcOEt/MeOH, 4:1, v/v). ^{1}H NMR(400 MHz, CD_3COCD_3) δ 2.05(m, 1H), 2.15(m, 1H), 2.27(m, 1H), 2.38(m, 1H), 3.33–3.44(m, 2H), 4.13(w, 1H), 4.44–4.58(m, 5H), 7.14–7.31(m, 6H), 7.86(dd, J=8.2, 1.9 Hz, 1H), 8.14(d, J=1.9 Hz, 1H); ^{13}C NMR (100 MHz, CD_3COCD_3) δ 22.6, 31.8, 62.7, 63.7, 69.7, 73.7, 128.8, 129.1, 129.6, 130.5, 130.6, 131.5, 138.7, 139.7, 142.0, 167.6, 175.5; Purity by HPLC 98%; MS (ES) m/z 386 (M+1).

General Procedure for the Palladium-Catalyzed Cross-coupling Reaction (11a–c)—To a solution of vinyl bromide **a–c** (6.7 mmol) in dioxane (30 mL) at 25 °C

under argon were successively added Et$_3$N (3.70 mL, 26.9 mmol), Pd (PPh$_3$)$_4$ (779 mg, 0.670 mmol), and pinacolborane (2.90 mL, 20.2 mmol) dropwise. The solution was heated to 80 °C for 1 h. After cooling, water (5 mL) was added dropwise followed by methyl 4-amino-3-iodobenzoate (1.5 g, 5.4 mmol) dissolved in dioxane (15 mL) and Ba(OH)$_2$·8H$_2$O (6.40 g, 20.2 mmol). The solution was heated to 90 °C for 8 h. After cooling, the mixture was filtered through Celite, brine was added (40 mL), and the solution was extracted with ethyl acetate (3 × 50 mL). After drying over magnesium sulfate and evaporation of the solvents under vacuum, the residue was purified by flash chromatography to obtain the benzoates

Methyl 4-Amino-3-(2-propylpent-1-ynyl)benzoate (11a)—Colorless oil, yield 76 %, TLC R$_f$ 0.4 (hexane/EtOAc 1/1 v/v). ^{1}H NMR(400 MHz, CDCl$_3$) δ 0.81(t, J=7.4 Hz, 3H), 0.97(t, J=7.4 Hz, 3H), 1.40(m, 2H), 1.54(m 2H), 2.02(m, 2H), 2.17(m, 2H), 3.85(s, 3H), 4.10(w, 2H), 6.00(s, 1H), 6.64(d, J=8.4Hz, 1H),7.68(d, J=2.0 Hz, 1H), 7.74(dd, J=8.4, 2.0 Hz, 1H); ^{13}C NMR(100 MHz, CDCl$_3$) δ 14.4, 14.4, 21.6, 21.6, 33.1, 38.7, 52.0, 114.0, 119.5, 120.2, 123.6, 130.0, 132.5, 147.3, 149.1, 167.9; MS (ES) *m/z* 262 (M+1).

Methyl 4-Amino-3-(2-ethylbut-1-ynyl)benzoate (11b)—Colorless oil, yield 73 %, TLC R$_f$ 0.4 (hexane/EtOAc 1/1 v/v). ^{1}H NMR(400 MHz, CDCl$_3$) δ 0.97(t, J=7.6, 3H), 1.14(t, J=7.4 Hz, 3H), 2.08(q, J=7.4 Hz, 2H), 2.23(q, J=7.6 Hz, 2H), 3.85(s, 3H),4.07(w, 2H), 5.96(s, 1H), 6.64(d, J=8.4 Hz, 1H), 7.69(d, J=2.0 Hz, 1H), 7.75(dd, J=8.4, 2.0 Hz, 1H); ^{13}C NMR(100 MHz, CDCl$_3$) δ 12.9, 13.0, 24.1, 28.6, 51.5, 113.6, 117.8, 119.2, 123.2, 129.7, 132.0, 148.6, 150.2, 167.4; MS (ES) *m/z* 234 (M+1).

Methyl 4-Amino-3-(2-methylprop-1-ynyl) benzoate (11c)—Colorless oil, yield 70 %, TLC R$_f$ 0.4 (hexane/EtOAc 1/1 v/v). ^{1}H NMR(400 MHz, CDCl$_3$) δ 1.70(d, J=0.4 Hz, 3H), 1.91(d, J=0.8 Hz, 3H), 3.84(s, 3H), 4.12(w, 2H), 6.00(s, 1H), 6.64(d, J=8.4 Hz, 1H), 7.69(d, J=1.7 Hz, 1H), 7.75(dd, J=8.4, 1.7 Hz, 1H); ^{13}C NMR(100 MHz, CDCl$_3$) δ 19.8, 19.8, 26.3, 52.0, 114.1, 119.5, 120.2, 123.5, 132.5, 139.3, 149.1, 167.8; MS (ES) *m/z* 206 (M+1).

Note: In the following conversion of **11a–b** to **12a–b**, and for **11c** to **12c**, overall yields were highest when intermediates were not isolated and purified.

Diethyl 1-(-2-Alkenyl-4-methoxycarbonylphenyl)-5-oxopyrrolidine-2,2-dicarboxylate (12a–b)

General procedure: To a solution of methyl 3-alkenyl-4-aminobenzoate (**11a–b**) (3.83 mmol) and diethylketomalonate (1.33 g, 7.66 mmol) in toluene (40 mL), p-toluene sulfonic acid (73 mg, 0.38 mmol) and molecular sieves (4 g) were added. The reaction mixture was refluxed 15 h, then transferred to a separatory funnel and washed with saturated NaHCO$_3$ (3×40mL) and brine (2×40mL). The organic layer was dried (Na$_2$SO$_4$), filtered, and evaporated to dryness. The residue was chromatographed on a flash silica gel column to afford an oil. To a solution of the oil in 1, 2-dichloroethane (8 mL), NaBH$_4$ (144 mg, 3.80 mmol) was added followed by slow addition of glacial acetic acid (0.3 mL). After the mixture was stirred 15 h at r.t., the reaction was quenched by adding cold water (1 mL). The mixture was washed with saturated NaHCO$_3$ (2×10mL) and brine (2×10mL), and the organic layer was dried (Na$_2$SO$_4$), filtered, and evaporated to dryness. The residue was chromatographed on a flash silica gel column to afford an oil. To a solution of the oil in toluene (5 mL), phosphorus trichloride (0.40 mL, 4.6 mmol) was added. The mixture was refluxed 15 h. The cooled reaction mixture was washed with water (2×5mL). The combined aqueous layers were extracted with ethyl acetate (2×10mL). The organic layers ware combined, and washed with saturated NaHCO$_3$ (2×10mL) and brine (2×10mL), dried

(Na$_2$SO$_4$), filtered, and evaporated to dryness. The residue was chromatographed on a flash silica gel column to afford an oil. A solution of the oil in dry DMF (4 mL) was added dropwise to a stirred suspension of NaH (16 mg, 0.68 mmol) in dry DMF (4 mL) over 10 minutes under Ar. The mixture was stirred at room temperature for 1h. The NaH was quenched with water (0.1 mL), and the mixture was diluted with ethyl acetate (20 mL). This was washed with NaHCO$_3$ (2×10mL) and brine (4×10mL), dried (Na$_2$SO$_4$), filtered, and concentrated. The obtained residue was chromatographed on a flash silica gel column to obtain the benzoic acids **12a–b**. The four step overall yields are given below.

Diethyl 1-(4-(Methoxycarbonyl)-2-(2-propylpent-1-enyl) phenyl)-5-oxopyrrolidine-2,2-dicarboxylate (12a)—Colorless solid: mp 88–90 °C, yield 17 %, TLC R$_f$ 0.59 (hexane/EtOAc 1/1 v/v). ^{1}H NMR(400 MHz, CDCl$_3$) δ 0.86(m, 3H), 0.93(t, J=7.3 Hz, 3H), 0.94(t, J=7.3 Hz, 3H), 1.30(m, 3H), 1.53(m 4H), 2.09(m, 4H), 2.42(m, 1H), 2.58(m, 2H), 2.98(m, 1H), 3.90(m, 1H), 3.91(s, 3H), 3.93(m, 1H), 5.90(s, 1H), 4.32(m, 2H), 7.58(d, J=8.3 Hz, 1H), 7.90(dd, J=8.3, 2.0 Hz, 1H), 7.94(d, J=2.0 Hz, 1H); ^{13}C NMR(100 MHz, CDCl$_3$) δ 14.3, 14.7, 21.4, 22.0, 29.3, 29.4, 33.5, 39.0, 52.6, 62.9, 74.5, 120.3, 128.6, 129.1, 130.0, 131.8, 139.1, 140.3, 147.2, 167.1, 174.6, MS (ES) *m/z* 474 (M+1).

Diethyl 1-(2-(2-Ethylbut-1-enyl)-4-(methoxycarbonyl) phenyl)-5-oxopyrrolidine-2,2-dicarboxylate (12b)—Colorless solid: mp 85–87°C, yield 20 %, TLC R$_f$ 0.53 (hexane/EtOAc 1/1 v/v). ^{1}H NMR(300 MHz, CDCl$_3$) δ 0.91(m, 3H), 1.04(m, 6H), 1.26(m, 3H), 2.14(m, 4H), 2.58(m, 2H), 3.89(s, 3H), 4.44-3.8(m, 4H), 5.90(s, 1H), 7.59(d, J=8.3 Hz, 1H), 7.88(dd, J=8.3, 2.0 Hz, 1H), 7.92(d, J=2.0 Hz, 1H); ^{13}C NMR(75 MHz, CDCl$_3$) δ 12.6, 12.8, 14.2, 24.2, 28.7, 28.8, 29.0, 52.2, 62.4, 74.1, 118.3, 128.2, 128.9, 129.6, 131.2, 138.7, 139.9, 149.6, 166.7, 174.3; MS (ES) *m/z* 446 (M+1).

Diethyl 1-(4-(Methoxycarbonyl)-2-(2-methylprop-1-enyl) phenyl)-5-oxopyrrolidine-2,2-dicarboxylate (12c)—To a solution of **11c** (2.60 g, 12.6 mmol) and diethyl ketomalonate (6.6 g, 38 mmol) in toluene (130 mL), p-toluene sulfonic acid (242 mg, 1.30 mmol) and molecular sieves (13g) were added. The reaction mixture was refluxed overnight. The cooled reaction mixture was washed with saturated NaHCO$_3$ (3×80mL) and brine (2×80mL). The organic layer was dried (Na$_2$SO$_4$), filtered, and evaporated to dryness. The residue was chromatographed on a flash silica gel column to afford an oil. To a solution of the oil in 1, 2-dichloroethane (30 mL), NaBH$_4$ (630 mg, 33.2 mmol) was added followed by the slow addition of glacial acetic acid (2.5 mL). After the mixture was stirred overnight at r.t., the reaction was quenched by adding cold water (2 mL) and diluted with ethyl acetate (90 mL). The mixture was washed with saturated NaHCO$_3$ (2×30mL) and brine (2×30mL), and the organic layer was dried (Na$_2$SO$_4$), filtered, and evaporated to dryness. The residue was chromatographed on a flash silica gel column to afford an oil. To a solution of the oil in toluene (20 mL), phosphorus trichloride (3.60 mL, 28.7 mmol) was added. The mixture was refluxed overnight. After quenching with water (50mL), the reaction mixture was diluted with ethyl acetate (100 mL). The combined aqueous layers were extracted with ethyl acetate (2×50mL). The organic layers were combined, washed with saturated NaHCO$_3$ (2×60mL) and brine (2×60mL), dried (Na$_2$SO$_4$), filtered and evaporated to dryness. The residue was chromatographed on a flash silica gel column to afford an oil. A solution of the oil in dry DMF (20 mL) was added dropwise to a stirred suspension of NaH (128 mg, 3.2 mmol) in dry DMF (20 mL) over 10 minutes under Ar. The mixture was stirred at room temperature for 2h. The NaH was quenched with water (0.5 mL), and the mixture was diluted with ethyl acetate (100 mL). The mixture was washed with NaHCO$_3$ (2×50mL) and brine (4×50mL), dried (Na$_2$SO$_4$), filtered, and evaporated to dryness. The residue was chromatographed on a flash silica gel column to afford a colorless semisolid. The semisolid was dissolved in THF (6 mL). Palladium on carbon (30 mg, 5%) was added. The mixture was stirred under a

hydrogen atmosphere (approximately one atmosphere from an attached balloon reservoir) for 4 h. The mixture was filtered and concentrated to yield **12c** (0.6 g, 11% for 5 steps, TLC R_f 0.50 (hexane/EtOAc 1/1 v/v) as a colorless solid; m.p. 73–75 °C. ^{1}H NMR(300 MHz, CDCl$_3$) δ 0.96-0.90(m, 9H), 1.29(t, J=5.3 Hz, 3H), 1.98(m, 1H), 2.20(dd, J=5.9, 11.9 Hz, 1H), 2.36(dd, J=5.0, 11.9 Hz, 1H), 2.44(m, 1H), 2.65(m, 2H), 3.05(m, 1H), 3.84(m, 1H), 3.90(s, 3H), 4.01(m, 1H), 4.29–4.35(m, 2H), 7.55(d, J=8.2 Hz, 1H), 7.87(dd, J=8.2, 2.0 Hz, 1H), 7.94(d, J=2.0 Hz, 1H); ^{13}C NMR(75 MHz, CDCl$_3$) δ 13.2, 13.9, 22.3, 22.9, 27.6, 28.8, 29.0, 39.8, 62.2, 62.6, 74.2, 127. 6, 128.8, 129.7, 130.6, 140.3, 141.0, 165.9, 166.6, 169, 7, 174.7; MS (ES) *m/z* 420 (M+1).

4-(2,2-Bis (hydroxymethyl)-5-oxopyrrolidin-1-yl)-3-(2-propylpentyl)benzoic Acid (13a)

Compound **12a** (0.30 mmol) was dissolved in THF (3 mL). Palladium on carbon (14 mg, 5%) was added. The mixture was stirred under a hydrogen atmosphere (approximately one atmosphere from an attached balloon reservoir) for 5 h. The mixture was filtered and evaporated to dryness. The residue was dissolved in dry MeOH (1 mL) and THF (1 mL) and cooled to 0 °C under argon. NaBH$_4$ (54.0 mg, 1.46 mmol) was added. The mixture was warmed to room temperature, stirred for 5 h, and quenched by adding cold water (0.1 mL). The mixture was evaporated to dryness leaving a solid which was dissolved in dichloromethane (10 mL). The organic layer was washed with brine (2×5mL), dried (Na$_2$SO$_4$), filtered, and evaporated to dryness. The residue was dissolved in MeOH (2 mL) and 1M NaOH (2 mL) was added. After the mixture was stirred for 2 h at room temperature, the pH was adjusted to 2 with 1 M HCl, and the mixture was evaporated to dryness, leaving a solid which was dissolved in methanol (3 mL). The organic layer was concentrated. The obtained residue was chromatographed on a flash silica gel column to obtain the benzoic acids **13a**. Colorless solid: mp 203–204 °C, yield 42 % for three steps, TLC R_f 0.38 (AcOEt/MeOH/AcOH 9:1:0.5, v/v/v). ^{1}H NMR(400MHz, CD$_3$OD) δ 0.78(m, 6H), 1.33-1.11(m, 8H), 1.76(m, 1H), 2.20(m, 2H), 2.44-2.32(m, 2H), 2.54(m, 1H), 2.73(m, 1H), 3.24(d, J=11.2 Hz, 1H), 3.34(d, J=11.2 Hz, 1H), 3.50(d, J=11.6 Hz, 1H),3.54(d, J=11.6 Hz, 1H), 7.33(d, J=8.1 Hz, 1H), 7.78(d, J=8.1 Hz, 1H), 7.90(s, 1H); ^{13}C NMR(100 MHz, CD$_3$OD) δ 15.2, 21.3, 21.4, 27.2, 32.0, 37.0, 37.3, 37.7, 38.3, 65.0, 65.4, 73.3, 129.0, 131.5, 132.3, 132.6, 141.5, 143.8, 170.2, 179.8; Purity by HPLC 98%; MS (ES) *m/z* 378 (M+1).

4-(2,2-Bis (hydroxymethyl)-5-oxopyrrolidin-1-yl)-3-(2-ethylbut-1-enyl)benzoic Acid (13b)

Compound **12b** (168 mg, 0.380 mmol) was dissolved in dry MeOH (1.2 mL) and THF (1.2 mL) and cooled to 0°C under argon. NaBH$_4$ (71.0 mg, 1.89 mmol) was added. The mixture was warmed to room temperature, stirred for 5 h, and quenched by adding cold water (0.1 mL). The mixture was evaporated to dryness leaving a solid which was dissolved in dichloromethane (10 mL). The organic layer was washed with brine (2×5mL), dried (Na$_2$SO$_4$), filtered, and evaporated to dryness. The residue was dissolved in MeOH (3 mL) and 1M NaOH (3 mL) was added. After the mixture was stirred for 1 h at room temperature, the pH was adjusted to 2 with 1 M HCl, and the mixture was evaporated to dryness, leaving a solid which was dissolved in methanol (2 mL). The organic layer was concentrated. The obtained residue was chromatographed on silca gel (EtOAc/MeOH/AcOH=40:2:1, R_f=0.48) to yield acid **13b** (47 mg, 36% in two steps) as a colorless solid: m.p. 189–191 °C; ^{1}H NMR(300 MHz, CD$_3$OD) δ 1.15-1.09(m, 6H), 2.45-2.15(m, 6H), 2.66-2.50(m, 2H), 3.22(d, J=11.2 Hz, 1H), 3.43(d, J=11.2 Hz, 1H), 3.62(d, J=11.8 Hz, 1H), 3.73(d, J=11.8 Hz, 1H), 6.24(d, J=8.0 Hz, 1H), 7.46(d, J=8.0 Hz, 1H), 7.94(d, J=8.0 Hz, 1H), 7.96(s, 1H); ^{13}C NMR(75 MHz, CD$_3$OD) δ 11.6, 11.8, 23.7, 25.3, 28.2, 29.3, 29.8, 63.0, 64.0, 71.3, 78.0, 120.0, 128.3, 129.4, 131.8, 138.8, 138.9, 148.5, 177.1; MS (ES) *m/z* 348 (M+1).

4-(2,2-Bis (hydroxymethyl)-5-oxopyrrolidin-1-yl)-3-isobutylbenzoic Acid (13c)

Compound **12c** (419 mg, 1.00 mmol) was dissolved in dry MeOH (3 mL) and THF (3

mL) and cooled to 0 °C under argon. $NaBH_4$ (190 mg, 5.00 mmol) was added. The mixture was warmed to room temperature, stirred for 2 h, and quenched by adding cold water (0.5 mL). The mixture was evaporated to dryness leaving a solid which was dissolved in dichloromethane (20 mL). The organic layer was washed brine (2×10mL), dried (Na_2SO_4), filtered, and evaporated to dryness. The residue was dissolved in MeOH (2 mL) and 1M NaOH (2 mL) was added. After the mixture was stirred for 2 h at room temperature, the pH was adjusted to 2 with 1 M HCl, and the mixture was evaporated to dryness, leaving a solid which was dissolved in methanol (3 mL). The organic layer was concentrated. The obtained residue was chromatographed on silca gel (EtOAc/MeOH/AcOH=40:2:1, R_f=0.57) to yield acid **13c** (99 mg, 31% in two steps) as a colorless solid. m.p. 168–170 °C: ^{1}H NMR(300 MHz, CD_3OD) δ 1.10(d, J=2.5 Hz, 3H), 1.12(d, J=2.5 Hz, 3H), 2.31(m, 1H), 2.46–2.50(m, 2H), 2.60–2.88(m, 4H), 3.52(d, J=11.2Hz, 1H), 3.62(d, J=11.2Hz, 1H), 2.69–2.42(m, 3H), 2.85(m, 1H), 3.35(d, J=11.1 Hz, 1H), 3.46(d, J=11.1 Hz, 1H), 3.80(d, J=11.7 Hz, 1H), 3.83(d, J=11.7 Hz, 1H), 7.62(d, J=8.2 Hz, 1H), 8.06(dd, J=8.2, 2.0 Hz, 1H), 8.20(d, J=2.0 Hz, 1H); ^{13}C NMR(100 MHz, CD_3OD) δ 21.2, 21.9, 25.3, 27.4, 30.0, 39.1, 63.2, 63.6, 71.4, 127.3, 129.6, 130.2, 130.5, 139.6, 141.8, 168.2, 177.9; Purity by HPLC 97%; MS (ES) *m/z* 322 (M+1).

4-(2,2-Bis (hydroxymethyl)-5-oxopyrrolidin-1-yl)-3-(2-ethylbutyl)benzoic Acid (14b)—Compound **13b** (20.0 mg, 0.058 mmol) was dissolved in THF (0.5 mL). Palladium on carbon (2 mg, 5%) was added. The mixture was stirred under a hydrogen atmosphere (approximately one atmosphere from an attached balloon reservoir) for 5 h. The mixture was filtered and concentrated to yield **14b** (20 mg, 99%) as a colorless solid. m. p. 168–170 °C; ^{1}H NMR(300 MHz, CD_3OD) δ 0.91(t, J=7.3 Hz, 6H), 1.36(m, 4H), 1.70(m, 1H), 2.30(m, 2H), 2.69-2.42(m, 3H), 2.85(m, 1H), 3.35(d, J=11.1 Hz, 1H), 3.46(d, J=11.1 Hz, 1H), 3.61(d, J=11.7 Hz, 1H), 3.66(d, J=11.7 Hz, 1H), 7.43(d, J=8.2 Hz, 1H), 7.89(d, J=8.2 Hz, 1H), 8.01(s, 1H); ^{13}C NMR(75 MHz, CD_3OD) δ 10.0, 10.1, 25.0, 25.2, 25.4, 30.2, 34.4, 40.2, 63.3, 63.6, 71.5, 127.2, 129.7, 130.6, 130.8, 139.7, 142.0, 168.4, 178.0. MS (ES) *m/z* 350 (M+1).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

References and notes

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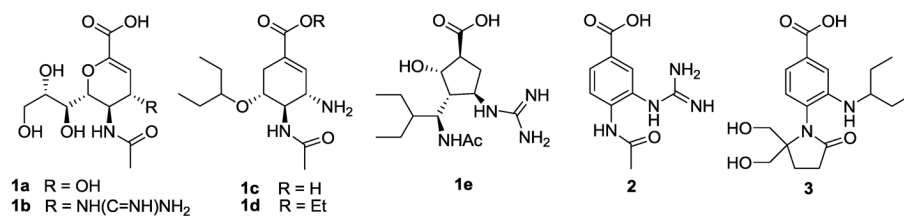
**Figure 1.**
Structures of clinically marketed neuraminidase inhibitors compared to benzoic acid inhibitors.
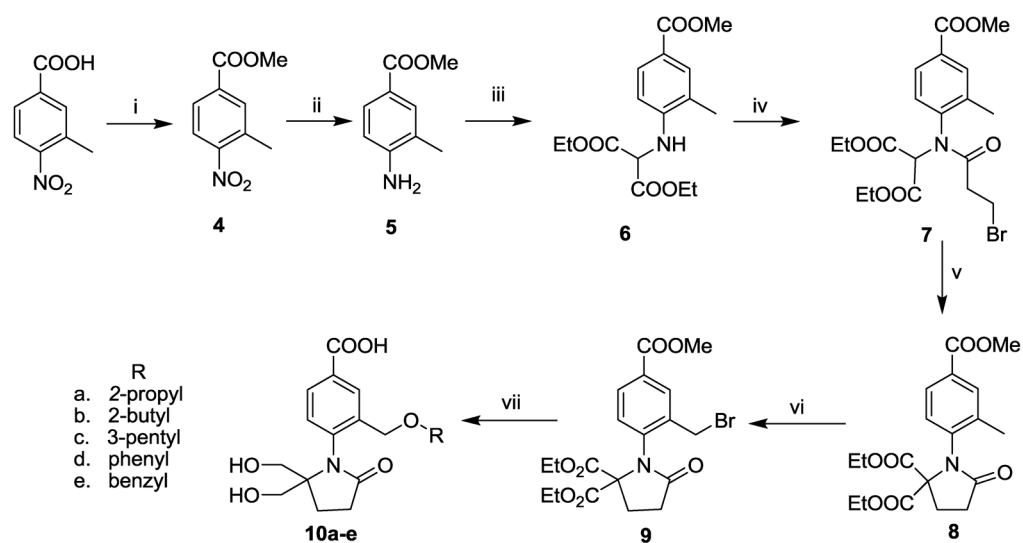
Scheme 1[a]

[a]Reagents and conditions: (i) MeOH, H_2SO_4, reflux, 15h; (ii) Zn/HCl, EtOAc, 0–20 °C 15h; (iii) diethyl bromomalonate, 120 °C, 22h; (iv) 3-bromopropionic acid, PCl_3, toluene, 100 °C, 22h; (v) NaH, 0–20 °C, 4h; (vi) CCl_4, NBS, AIBN, 80 °C, 3h; (vii) a, BuLi, ROH, −10–20 °C 15h; b, $NaBH_4$, MeOH, THF, −21h; c, NaOH, MeOH, 20 °C, 1–2h.
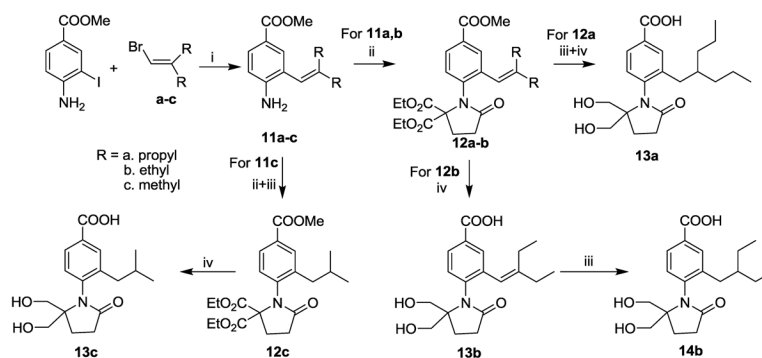
Scheme 2[a]

[a]Reagents and conditions: : (i) a, vinyl bromide, dioxane, Et$_3$N, Pd(PPh$_3$)$_4$, pinacolborane, 80 °C, 1h; b, methyl 4-amino-3-iodobenzoate, Ba(OH)$_2$·8H$_2$O, water, 90°C, 8h; (ii) a, p-TSA, toluene, diethyl ketomalonate, reflux, 19h; b, NaBH$_4$, DEC, AcOH, 15h; c, 3-bromopropionic acid, PCl$_3$, toluene, 100°C, 20h; d, NaH, 0–20 °C, 4h; (iii) Pd/C, H$_2$, MeOH, 25 °C; (iv) a, NaBH$_4$, MeOH/THF, 2–5h; b, NaOH, MeOH, 20 °C, 1–2h.

Table 1

In Vitro Inhibitory Effects of Benzoic Acid Analogues on Influenza A and B Neuraminidases

	Inhibition of Neuraminidase, IC_{50} (µM)			
	H3N2	**H1N1**		**B**
Compounds	**A/Uruguay/716/07**	**A/Brisbane/59/07**	**A/Solomon Islands/3/06**	**B/Malaysia/5206/04**
3[a]	0.49		79	271
10a	4000 ± 15	>10000	N/A[b]	N/A
10b	0.20 ± 0.19	16 ± 2.1	11 ± 4.4	80 ± 30
10c	0.50 ± 0.10	1.0 ± 0.21	0.80 ± 0.07	30 ± 1.4
10d	40 ± 8.1	420 ± 45	400 ± 25	N/A
10e	200 ± 95	N/A	1600 ± 255	N/A
	H3N2	H1N1		B
Compounds	A/Wisconsin/05	A/Brisbane/59/07	A/OK 3052/09	B/Malaysia/2506/04
13a	0.50 ± 0.15	N/A	N/A	190 ± 10
13b	0.41 ± 0.070	2.4±0.9	3.4 ± 1.2	120 ± 16
13c	3.0 ± 0.90[c]	70 ± 7.8	N/A	140 ± 17
14b	0.20 ± 0.090	1.6 ± 0.60	3.2 ± 1.3	32 ± 3.4

[a] Data from ref 24 using A/PR/8/34 (N1), A/Udorn/72 (N2), and B/Lee/40.

[b] N/A= data not available

[c] A/Uruguay/716/07.