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Design and synthesis of macrocyclic peptidyl hydroxamates as peptide deformylase inhibitors

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Abstract—Macrocyclic peptidyl hydroxamates were designed, synthesized, and evaluated as peptide deformylase (PDF) inhibitors. The most potent compound exhibited tight, slow-binding inhibition of *Escherichia coli* PDF ($K_I^* = 4.4$ nM) and had potent antibacterial activity against Gram-positive bacterium *Bacillus subtilis* (MIC = 2–4 μg/mL).

Peptide deformylase (PDF), an essential enzyme for bacterial protein biosynthesis and maturation, catalyzes the removal of *N*-formyl group from newly synthesized polypeptides.[1] PDF is being actively pursued as a target for designing novel antibiotics to treat antibiotic resistant pathogens.[2,3] Over the past decade, numerous PDF inhibitors have been reported; two of the inhibitors have already advanced to phase I clinical trials for treatment of upper respiratory tract infections.[4] We have previously reported that macrocyclic *N*-formylhydroxylamine **1** (Fig. 1) and its congeners are highly potent, selective inhibitors against PDF (e.g., $K_I^* = 0.33$ nM for **1** against *Escherichia coli* PDF) and have broad-spectrum antibacterial activity against clinically significant pathogens.[5] However, synthesis of compound **1** was somewhat lengthy (11 steps), primarily due to an inefficient preparation of the *N*-formylhydroxylamine moiety (eight steps). In this work, we attempt to replace the *N*-formylhydroxylamine moiety with another metal chelating group, the synthetically more accessible hydroxamate. Thus, macrocyclic hydroxamates **2a**–**c** (Fig. 1) were designed, synthesized, and evaluated as potential PDF inhibitors. Compound **2a** is isosteric to inhibitor **1** except that the *N*-formylhydroxylamine moiety in the latter is replaced by a hydroxamate. Compound **2b** contains a proline as the P_2' residue, because previous studies have shown that the proline confers excellent potency and pharmacokinetic properties.[6] Compound **2c** should be synthetically very accessible because its chiral hydroxamate group can be readily prepared from D-aspartic acid.

Retrosynthetic analysis shows that the macrocycles can be conveniently prepared via olefin metathesis from diene **3**, which in turn can be prepared from acid **4** and amine **5** (Scheme 1). This synthetic strategy allows for the convergent synthesis of macrocycles of different ring sizes and specificity determinants by using the common acid **4** and varying the structure of the alkenyl amine **5**.

Synthesis of acid **4** started from the commercially available 5-hexenoic acid, which was stereoselectively alkylated at the C-2 position with a *t*-butoxycarbonylmethyl group using 4*S*-benzyloxazolidin-2-one as the chi-

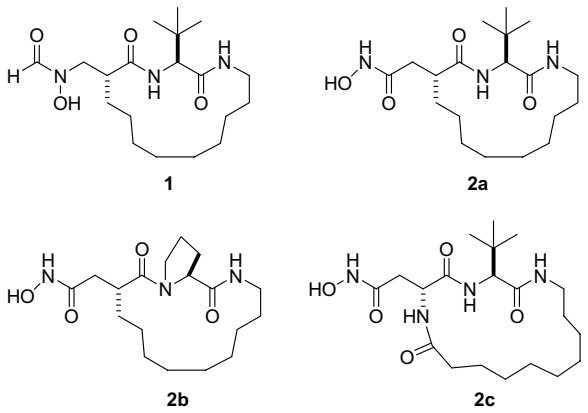

Figure 1. Structures of PDF inhibitors.

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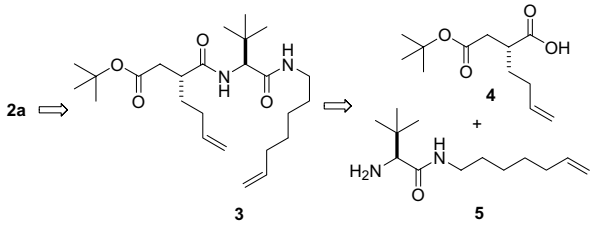
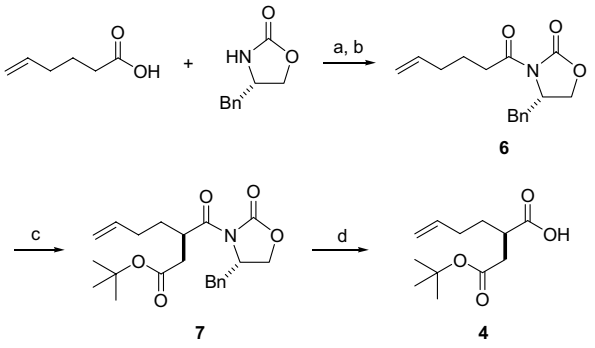
ral auxiliary group[7] (Scheme 2). Removal of the auxiliary group by hydrolysis produced the desired acid **4**, which was used as the common building block for the preparation of various cyclic hydroxamates.

The 6-heptenylamine needed for the preparation of amine **5** was obtained by reducing 5-hexenyl nitrile with $LiAlH_4$ (Scheme 3). The resulting amine was protected with a Boc group to facilitate its isolation. Removal of the Boc group by TFA, condensation with *N*-Boc-*tert*-leucine, and deprotection with TFA afforded amine **5**. Amine **5** was coupled to the common acid **4** to give diene **9**. The terminal alkenes were crosslinked using Grubbs's ruthenium catalyst[8] followed by catalytic hydrogenation (Pd/C and H_2) to produce the 15-membered macrocycle **10**. The *t*-butyl ester was cleaved with TFA treatment and the resulting acid was coupled with *O*-*t*-butylhydroxylamine to give the *t*-butyl protected hydroxamate group. Finally, the *t*-butyl group on the hydroxamate was removed by treatment with TFA and aqueous HCl to give hydroxamate **2a**.[9] Compound **2b** was synthesized in a similar manner except that *N*-Boc-proline was used instead of *N*-Boc-*tert*-leucine.

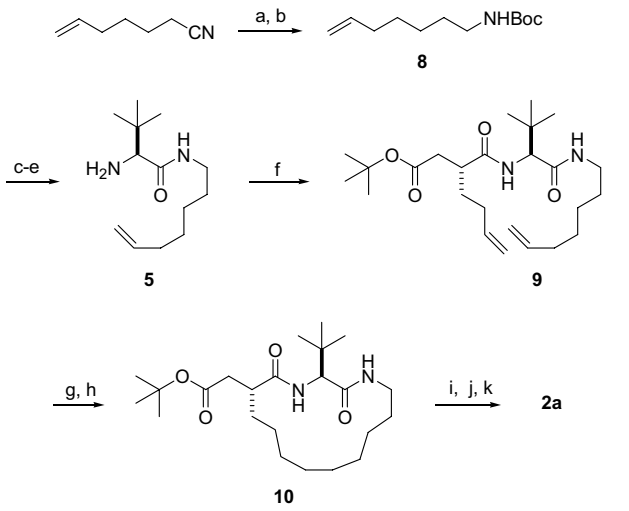
Compound **2c** was synthesized from *N*-Fmoc-Asp(*t*-Bu)-OH, which was condensed with amine **5** (Scheme 4). After removal of the N-terminal Fmoc group, the resulting amine **11** was acylated with 4-pentenoic acid and EDC. Cyclization of the diene **12** was achieved with the second-generation Grubbs's catalyst, which afforded better yields than the first-generation catalyst. The configuration of the ring C=C bond in intermediate **13** was not determined. To install the hydroxamate group, alkene **13** was treated with TFA to cleave the *t*-butyl ester



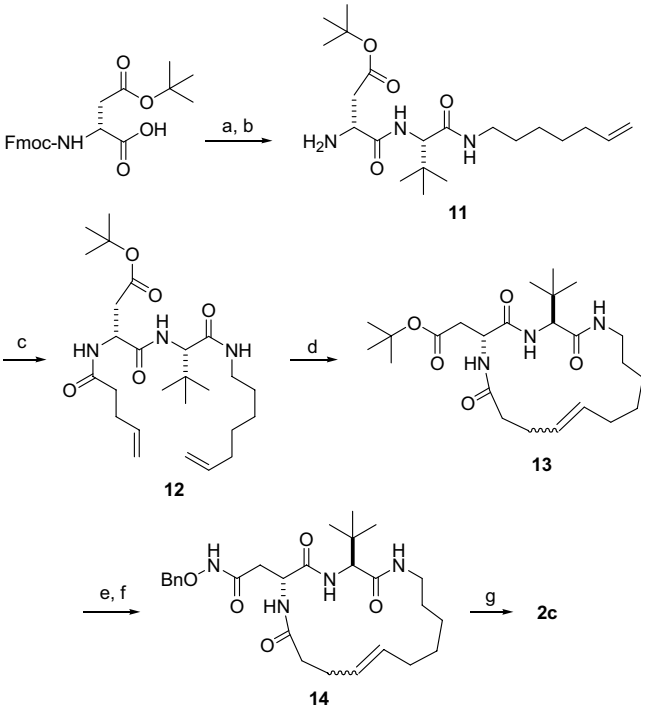
Scheme 1. Retrosynthetic analysis of **2a**.



Scheme 2. (a) Pivaloyl chloride, TEA, THF; (b) LiCl, 86% (two steps); (c) LDA, THF, *t*-butylbromoacetate, 50%; (d) H_2O_2, LiOH, H_2O, THF, 96%.



Scheme 3. (a) $LiAlH_4$, Et_2O; (b) $(Boc)_2O$, NaOH aq, 85% (two steps); (c) TFA, CH_2Cl_2; (d) *N*-Boc-L-*tert*-Leu-OH, EDC, HOAt, Et_3N, CH_2Cl_2, 78%; (e) TFA, CH_2Cl_2; (f) **4**, EDC, Et_3N, CH_2Cl_2, 42%; (g) 2^{nd}-generation Grubbs's catalyst, CH_2Cl_2, reflux, 55%; (h) H_2, 10% Pd/C, MeOH, EtOAc; (i) TFA; (j) *t*-$BuONH_2$ ·HCl, EDC, Et_3N, HOAt, CH_2Cl_2, 40%; (k) TFA, 37% HCl, 48%.



Scheme 4. (a) *tert*-Leu-NH$(CH_2)_5CH{=}CH_2$, EDC, DIPEA, CH_2Cl_2, 64%; (b) 20% piperidine; (c) $CH_2{=}CHCH_2CH_2CO_2H$, EDC, Et_3N, CH_2Cl_2, 71%; (d) second-generation Grubbs's catalyst, 76%; (e) TFA; (f) $BnONH_2$, EDC, Et_3N, CH_2Cl_2, 54%; (g) H_2, 10% Pd/C, MeOH, EtOAc, 24%.

and the resulting acid was coupled with *O*-benzylhydroxylamine to give cycloalkene **14**. Catalytic hydrogenation of **14** reduced the ring C=C bond and simultaneously removed the benzyl group from the hydroxamate moiety. Unfortunately, the hydroxamate was unstable under the hydrogenolysis condition and

Table 1. Inhibition constants of Co-EcPDF by compounds **1** and **2a–c**

Compound	K_I, nM	K_I^*, nM	k_5, min^{-1}	k_6, min^{-1}
1	109 ± 5[a]	0.33 ± 0.15[a]	1.2[a]	0.0038 ± 0.0010[a]
2a	210 ± 30	4.4 ± 0.7	12	0.26 ± 0.02
2b	16,000 ± 4000	1400 ± 200	3.1	0.30 ± 0.01
2c	>20,000	ND[b]	ND	ND

[a] Data from Ref. 5.
[b] ND, no slow binding detected.

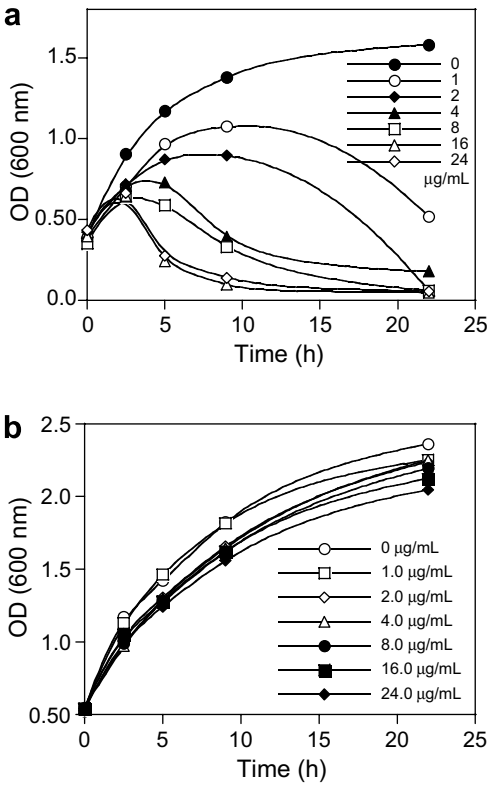


Figure 2. Antimicrobial activity of compound **2a** against *B. subtilis* (a) and *E. coli* (b).

even under carefully controlled conditions, substantial reduction of the hydroxamate to the corresponding amide was observed. As a result, the hydrogenation step had rather poor yields (24%).

Compounds **2a–c** were tested for inhibition of cobalt(II)-substituted *E. coli* PDF (Co-EcPDF)[10] using a continuous assay and peptide *N*-formyl-Met-Leu-*p*-nitroanilide (f-ML-*p*NA) as substrate.[11] Compounds **2a** and **2b** exhibited slow-binding inhibition[12] that can be described by equation

$$E + I \overset{K_I}{\rightleftharpoons} E \cdot I \underset{k_6}{\overset{k_5}{\rightleftharpoons}} E \cdot I^*$$

where K_I is the equilibrium constant for the formation of the initial E·I complex, whereas k_5 and k_6 are the forward and reverse rate constants for the slow interconversion of E·I and E·I*, respectively. The overall potency of the inhibitor is described by the equilibrium constant $K_I^* = K_I \cdot k_6/(k_5 + k_6)$. The equilibrium and rate constants for **2a** and **2b** are listed in Table 1. Compound **2a** is a highly potent inhibitor, having a K_I^* value of 4.4 nM against EcPDF. The corresponding *N*-formylhydroxylamine (compound **1**) is also a slow-binding inhibitor and has a K_I^* value of 0.33 nM.[5] Compound **2b**, which has a proline at the P_2' position, is also a slow-binding but significantly weaker inhibitor ($K_I^* = 1400$ nM). Compound **2c**, which was synthetically most accessible, was a very poor inhibitor ($K_I > 20{,}000$ nM). This is not unexpected, because previous studies have shown that PDF has stringent requirement for a hydrophobic residue such as methionine and norleucine at the P_1' site.[13] Thus, both *N*-formylhydroxylamine and hydroxamate are effective metal-chelating groups when attached to proper macrocycles.

Next, compounds **2a** and **2b** were tested for antibacterial activities in vitro. *Bacillus subtilis* and *E. coli* overnight cultures were diluted sixfold into fresh LB medium containing 0–24 μg/mL inhibitor and cell growth was monitored at 600 nm. Compound **2a** had potent antibacterial activity against *B. subtilis*, with a minimal inhibitory concentration (MIC) of 2–4 μg/mL. Interestingly, in contrast to most of the reported PDF inhibitors, which are usually bacteriostatic, compound **2a** exhibited bactericidal activity. When added to cell cultures in the exponential phase, the cell density continued to increase for several hours and then gradually decreased to near zero as a result of cell lysis (Fig. 2a). It had only weak activity against the Gram-negative *E. coli* cells (MIC > 24 μg/mL) (Fig. 2b). Compound **2b** showed weak activity against *B. subtilis* (MIC > 24 μg/mL) and was inactive against *E. coli* cells (not shown).

In summary, a novel class of macrocyclic peptidyl hydroxamates has been prepared from commercially available 5-hexenoic acid (nine steps). One of the inhibitors showed potent inhibition of EcPDF and bactericidal activity against Gram-positive bacteria. Further optimization of the ring size and P_2' side chain may lead to highly potent, selective PDF inhibitors.

Acknowledgment

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9. **2a**: ^{1}H NMR (400 MHz, CD_3OD): δ 8.04 (br s, 0.8H), 4.32–4.27 (m, 1H), 3.69–3.64 (m, 1H), 2.89–2.81 (m, 2H), 2.38–2.32 (m, 1H), 2.17–2.11 (m, 1H), 1.59–1.22 (m, 16H), 1.02 (s, 4.5H), 0.99 (s, 4.5H). ^{13}C NMR (100 MHz, CD_3OD): δ 176.6, 172.6, 170.7, 62.1, 43.7, 38.9, 35.5, 29.0, 28.7 (d), 28.6 (d), 28.2, 27.8, 27.1, 26.8, 26.5. ESI-HRMS: Calcd for $C_{19}H_{35}N_3O_4Na^+$ ($[M+Na]^+$), 392.2520; found, 392.2537. **2b**: ^{1}H NMR (400 MHz, CD3OD): δ 8.37 (br s, 0.4H), 4.25 (br s, 1H), 3.40–3.24 (m, 3H), 2.85–2.81 (m, 2H), 2.45–2.37 (m, 2H), 2.06–1.83 (m, 4H), 1.54–1.33 (m, 14H). ^{13}C NMR (100 MHz, CD_3OD): δ 177.4, 174.2, 169.4, 61.3, 47.5, 40.8, 38.2, 32.5, 32.0, 31.3, 30.4, 30.3 (d), 30.2, 27.8, 27.3. ESI-HRMS: Calcd for $C_{18}H_{31}N_3O_4H^+$ ($[M+H]^+$), 354.2387; found, 354.2399.
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