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Stereoselective Total Synthesis of Racemic BCX-1812 (RWJ-270201) for the Development of Neuraminidase Inhibitors as Anti-influenza Agents

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A convergent and versatile racemic total synthesis of the anti-influenza agent BCX-1812 (RWJ-270201) was accomplished on the basis of a sequence of stereoselective reactions. Despite intensive research to develop neuraminidase inhibitors to treat infections due to influenza, currently available agents are still in the need of optimization with respect to selectivity and potency, as well as to minimize adverse effects. Our synthetic approach, introduced in this report, is highly exploitable for further derivatization due to flexibility that will eventually accommodate diversified substituents. In addition, the size of the core ring can be varied depending on the size of the diene used for the preparation of the key cycloadduct **10** using an acylnitroso-based hetero-Diels−Alder reaction. Elaboration of **10** to methyl ester **14** followed by a precedented [3+2] dipolar cycloaddition gave bicyclic isoxazoline **17** in a regio- and stereoselective fashion. Incorporation of the peripheral guanidino group and subsequent deprotection provided the target molecule. The details of the synthesis are described herein.

Introduction

Influenza, commonly called flu, is an acute viral infection of the upper respiratory tract, accompanied by fever, headache, myalgia, prostration, coryza, sore throat, and cough. It is typically caused by influenza A and B viruses, the RNA viruses of *orthmyxoviridae*. Influenza A accounts for most of these viral infections. In the U.S. alone, the flu is estimated to affect 10−20% of the population every year, leading to approximately 20000 deaths from influenza itself and its associated complications. Thus, despite recent advances in the molecular and cellular biology of influenza virus, influenza continues to cause significant pandemic mortality and morbidity.[1]

The tricyclic amines amantadine (Symmetrel, **1**) and rimantadine (Flumadine, **2**) (Figure 1) have been indicated for both prophylaxis and treatment of influenza for decades.[2] The mechanism of action of these agents includes interference of the ion channel of the viral M2 protein, the channel for the transportation of protons into the interior of the virion at the early replication stage. Thus, inhibition of the M2 ion channel results in arresting acid-activated release of the M1 protein from the ribonucleoprotein (RNP) during uncoating of viruses after their entry into the host cell. Therefore, subsequent transport of RNP into the nucleus is terminated. These agents are effective only against influenza A since the M2 protein exists only in the type A virus, not in type B.

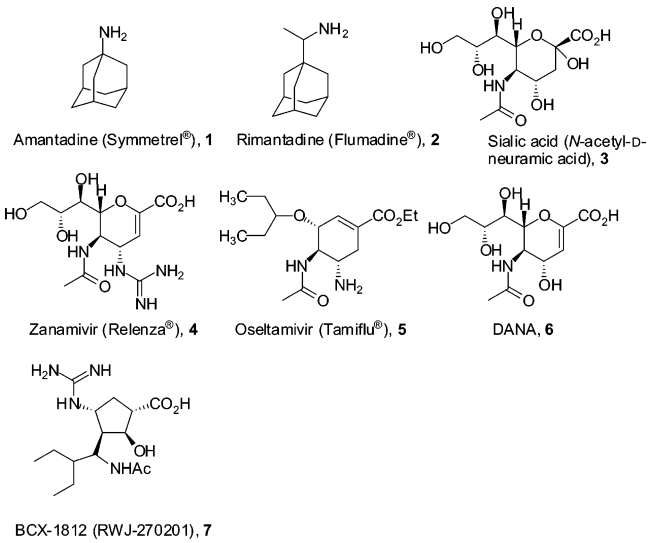

FIGURE 1. Structures of neuraminidase inhibitors.

In addition, M2 inhibitors can be associated with severe adverse effects, especially on the gastrointestinal tract and central nervous system, and the development of drug resistance has consequently led to decreased use of these agents.

Neuraminidase (NA), the enzyme projecting outward over the viral surface, has emerged as a new therapeutic target for anti-influenza treatment.[3−5] When NA cleaves the terminal sialic acid (*N*-acetyl-D-neuranic acid, **3**) from

(1) Bridges, C. B.; Fukuda, K.; Uyeki, T. M.; Cox, N. J.; Singleton, J. A. *MMWR Recomm. Rep.* **2002**, *51* (RR-3), 1−31.
(2) Hayden, F. G.; Hay, A. J. *Curr. Top. Microbiol. Immunol.* **1992**, *176* 119−130.
(3) Roberts, N. A. *Prog. Drug Res.* **2001**, *56*, 195−237.

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hemagglutinin (HA), new virus particles can be released from the infected cell and spread the infection (Figure 1). Therefore, inhibition of NA ceases the spread of the infection, trapping viruses inside epithelial cells.

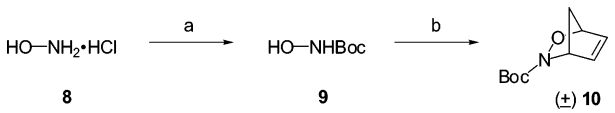
Zanamivir (Relenza, **4**) and oseltamivir (Tamiflu, **5**) are the first antiviral drugs of this new class of NA inhibitors (Figure 1). Zanamivir and oseltamivir are effective against both influenza A and B viruses. Zanamivir was approved by the FDA in 1999 for the treatment of the influenza infection. Due to the poor oral bioavailability of this compound, it must be administrated by oral inhalation. Oseltamivir, an ester prodrug to be converted to the active free carboxyl form upon administration, was also approved by the FDA in 1999 as an orally active inhibitor for both treatment and prophylaxis of influenza. The most common side effects reported with zanamivir included headache and diarrhea, whereas oseltamivir caused nausea and vomiting.

NA is a mushroom-shaped tetrameric glycoprotein with an axis that functions as a membrane anchor. Each monomer unit contains six antiparallel four-stranded β-sheets, aligned in a topologically identical format. The catalytic active site of NA, located in a concave cleft of the surface of the protein, is highly conserved in all known subtypes of influenza A and B viruses, and engages the active site of NA as an ideal target for broad-spectrum inhibitors. Nine subtypes, N1−N9, have been isolated for type A NA, while no subtypes have been identified for type B. Determination of the crystal structure of NA accelerated the design of specific NA inhibitors that fit in the active site and displayed selective, broad-spectrum anti-influenza activity.[6,7] For example, several reports about the rational drug design and synthesis of zanamivir,[8] a guanidino analogue derived from a non-selective inhibitor, 2-deoxy-2,3-didehydro-D-*N*-acetylneuraminic acid (DANA, **6**) (Figure 1) and oesltamivir[9−13] have been found as precedents.

Another promising NA inhibitor known as BCX-1812 (RWJ-270201, **7**) is currently under evaluation in clinical trials (Figure 1).[14−16] This compound demonstrated notable selectivity and potency against NA for a wide range of influenza A and B viruses. It has been emphasized that the relative positions of the interacting functional groups are essential for the NA inhibitory potency, rather than the absolute positions of the central rings.

Despite extensive research related to the design and syntheses of NA inhibitors, the above agents may not confer ideal therapeutic characteristics due to several drawbacks including the previously mentioned unwanted side effects. The establishment of a practical, efficient, and versatile synthetic route would contribute to further structure−activity relationship (SAR) studies needed for the development of safe and potent NA inhibitors. Thus, we sought to design and demonstrate an efficient and stereocontrolled synthetic sequence to provide appropriate scaffolds for the development of the novel NA inhibitors. Our synthetic approach was designed with versatility in mind to subsequently enable structural modification of both the core and substituents for more elaborate SAR studies. To illustrate our progress, in this paper, a convergent, stereoselective synthesis of tetrasubstituted cyclopentane **7** is described that emphasizes the utility of acylnitroso cycloaddition chemistry.

SCHEME 1[a]



[a] Reagents: (a) Boc_2O, $NaHCO_3$, THF/H_2O (4:1), 89%; (b) $NaIO_4$, Cp, $MeOH/H_2O$ (4:1), 81%.

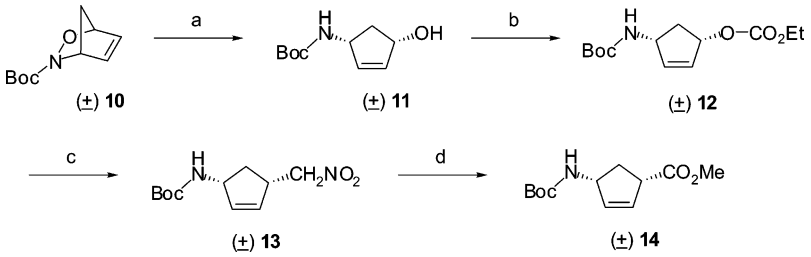
Results and Discussion

Synthesis. As the starting material to synthesize NA inhibitor **7**, hydroxylamine hydrochloride (**8**) was protected by a Boc group (Scheme 1). The resulting Boc-protected hydroxylamine **9** was subjected to our previously developed method of hetero-Diels−Alder cycloaddition employing freshly distilled cyclopentadiene and $NaIO_4$ as an oxidant to yield cycloadduct **10**.[17,18]

The synthesis of the key intermediate methyl ester **14** began with the racemic cycloadduct **10** (Scheme 2). Reductive N−O bond cleavage of **10** using $Mo(CO)_6$ and $NaBH_4$ gave allylic alcohol **11**,[17,18] which was converted to its ethyl carbonate **12** by treatment with ethyl chloroformate. The ethyl carbonate moiety was replaced by the nitromethyl group by reaction of **12** with $MeNO_2$ and a catalytic amount of Pd(0), separately prepared in situ from $Pd(OAc)_2$ and PPh_3, to furnish **13**.[19,20] Primary nitro compound **13** was transformed to the carboxylic acid by employing a mixture of $NaNO_2$ and AcOH in DMF,[21] followed by treatment with a catalytic amount of (TMS)-

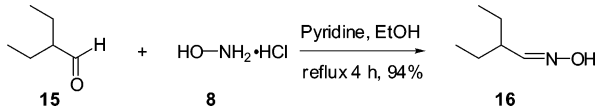
(4) Abdel-Magid, A. F.; Maryanoff, C. A.; Mehrman, S. J. *Curr. Opin. Drug Discovery Dev.* **2001**, *4*, 776−791.
(5) McKimm-Breschkin, J. L. *Expert Opin. Pharmacother.* **2002**, *3*, 103−112.
(6) Colman, P. M.; Varghese, J. N.; Laver, W. G. *Nature* **1983**, *303*, 41−44.
(7) Varghese, J. N.; Laver, W. G.; Colman, P. M. *Nature* **1983**, *303*, 35−40.
(8) von Itzstein, M.; Wu, W.-Y.; Kok, G. B.; Pegg, M. S.; Dyason, J. C.; Jin, B.; Phan, T. V.; Smythe, M. L.; White, H. F.; Oliver, S. W.; Colman, P. M.; Varghese, J. N.; Ryan, D. M.; Woods, J. M.; Bethell, R. C.; Hotham, V. J.; Cameron, J. M.; Penn, C. R. *Nature* **1993**, *363*, 418−423.
(9) Williams, M.; Bischofberger, N.; Swaminathan, S.; Kim, C. U. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2251−2254.
(10) Zhang, L.; Williams, M. A.; Mendel, D. B.; Escarpe, P. A.; Chen, X.; Wang, K.-Y.; Graves, B. J.; Lawton, G.; Kim, C. U. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1751−1756.
(11) Kim, C. U.; Lew, W.; Williams, M. A.; Liu, H.; Zhang, L.; Swaminathan, S.; Bischofberger, N.; Chen, M. S.; Mendel, D. B.; Tai, C. Y.; Laver, W. G.; Stevens, R. C. *J. Am. Chem. Soc.* **1997**, *119*, 681−690.
(12) Kim, C. U.; Lew, W.; Williams, M. A.; Wu, H.; Zhang, L.; Chen, X.; Escarpe, P. A.; Mendel, D. B.; Laver, W. G.; Stevens, R. C. *J. Med. Chem.* **1998**, *41*, 2451−2460.
(13) Karpf, M.; Trussardi, R. *J. Org. Chem.* **2001**, *66*, 2044−2051.
(14) Sorbera, L. A.; Graul, A.; Castaner, J. *Drugs Future* **2000**, *25*, 249−251.
(15) Babu, Y. S.; Chand, P.; Bantia, S.; Kotian, P.; Dehghani, A.; El-Kattan, Y.; Lin, T.-H.; Hutchison, T. L.; Elliott, A. J.; Parker, C. D.; Ananth, S. L.; Horn, L. L.; Laver, G. W.; Montgomery, J. A. *J. Med. Chem.* **2000**, *43*, 3482−3486.
(16) Chand, P.; Kotian, P. L.; Dehghani, A.; El-Kattan, Y.; Lin, T.-H.; Hutchison, T. L.; Babu, Y. S.; Bantia, S.; Elliott, A. J.; Montgomery, J. A. *J. Med. Chem.* **2001**, *44*, 4379−4392.
(17) Zhang, D.; Süling, C.; Miller, M. J. *J. Org. Chem.* **1998**, *63*, 885−888.
(18) Mulvihill, M. J.; Gage, J. L.; Miller, M. J. *J. Org. Chem.* **1998**, *63*, 3357−3363.
(19) Deardorff, D. R.; Savin, K. A.; Justman, C. J.; Karanjawala, Z. E.; Sheppeck, J. E., II.; Hager, D. C.; Aydin, N. *J. Org. Chem.* **1996**, *61*, 3616−3622.
(20) Zhang, D.; Ghosh, A.; Süling, C.; Miller, M. J. *Tetrahedron Lett.* **1996**, *37*, 3799−3802.

SCHEME 2[a]



[a] Reagents: (a) $Mo(CO)_6$, $NaBH_4$, MeCN/H_2O (20:1), 66%; (b) ethyl chloroformate, pyridine/CH_2Cl_2 (1:1), 92%; (c) $MeNO_2$, Et_3N, $Pd(OAc)_2$, PPh_3, THF, 70%; (d) (i) $NaNO_2$, AcOH, DMF, (ii) (TMS)Cl, MeOH, 58% in two steps.

SCHEME 3



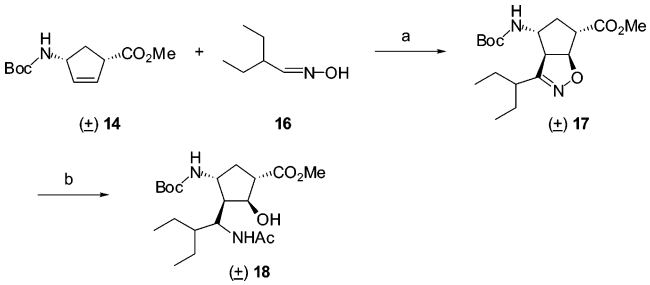
Cl in MeOH to give the corresponding methyl ester **14**. DMF rather than DMSO proved to be the preferred solvent for this transformation. When DMF was employed as the solvent, only the desired *syn* isomer **14** was formed in 58% yield in two steps, whereas employment of DMSO as the solvent generated a mixture of stereoisomers (*syn*:*anti* = 3:2) of **14**. While subsequent elaboration described below was carried out on racemic **14**, either enantiomer could be prepared starting from the corresponding antipodes of **11**, available from enzymatic resolutions.[18]

With ready access to **14**, we utilized a precedented nitrile oxide [3+2] dipolar cycloaddition for incorporation of key additional framework components and functionality. The 1,4-*cis* relationship of the rather bulky Boc-protected amine and methyl ester groups of **14** was anticipated to induce selective reaction of the nitrile oxide from the opposite face. However, while the regiochemistry of the reaction was less predictable, precedent indicated[16] that it would proceed as desired. Nitrile oxides can be derived from the corresponding oximes in situ, and it has been generally known that nitrile oxides bearing a wide range of substituents are capable of participating in [3+2] dipolar cycloaddition reactions. As the precursor for [3+2] dipolar cycloaddition with compound **14**, oxime **16** containing diethyl units was prepared (Scheme 3). The commercially available aldehyde **15** was stirred with hydroxylamine **8** at reflux for 4 h to produce oxime **16** as a colorless oil in 94% yield.[16]

As depicted in Scheme 4, reaction of dipolarophile **14** and oxime **16**, which was transformed to the corresponding nitrile oxide dipole in situ in the presence of NaOCl and Et_3N, induced a [3+2] dipolar cycloaddition to provide bicyclic isoxazoline **17**. As confirmed by X-ray crystallographic analysis, the desired isomer **17** was the only isolated product despite the possibility for generation of other unwanted stereo- and regioisomers. The isoxazoline ring of **17** was then subjected to hydrogenolysis in MeOH containing PtO_2 and an equivalent amount of HCl at 40 psi.[16] Subsequent acetylation furnished compound **18** as the precursor to the final product.

Compound **14** can also be prepared by the previously reported method starting with Vince's lactam **19**[22] (Scheme 5). After the protection by the Boc group, basic hydrolysis using 2 N NaOH aqueous solution gave compound **20** in 86% yield.[23] Compound **20** was then converted to methyl ester **14** by the same method described in Scheme 2 using a catalytic amount of (TMS)Cl in MeOH. The products from both routes were confirmed to be identical by comparison of their spectroscopic data. While this is a very practical route to **14**, use of Vince's lactam is not as versatile as the acylnitroso cycloaddition approach that can provide access to a number of related scaffolds.

SCHEME 4[a]



[a] Reagents: (a) NaOCl (bleach), Et_3N, CH_2Cl_2, 61%; (b) (i) H_2, PtO_2, HCl, MeOH, (ii) Ac_2O, Et_3N, CH_2Cl_2, 82% in two steps.

Upon the removal of the Boc protecting group, compound **18** was then converted to the corresponding protected guanidino derivative **21** by treatment with 1,3-bis(*t*-Boc)-2-methyl-2-thiopseudourea in the presence of $HgCl_2$ (Scheme 6). Subsequent basic hydrolysis with 1 N NaOH afforded the corresponding acid **22** in 95% yield. Cleavage of the Boc protecting groups using TFA in CH_2Cl_2 in the presence of Et_3SiH as the *tert*-butyl cation scavenger furnished the desired final product **7** in 78% yield. The overall yield of the entire synthesis from **8** in Scheme 1 was calculated to be 5.4%.

Conclusion

In conclusion, we have established an efficient and stereoselective synthetic sequence for compound **7** which can provide a foundation for the search of novel NA inhibitors. Promising leads can be further investigated stepwise, as well as in parallel fashion using a variety of dienes in the initial acylnitroso cycloaddition,[24,25] to

(21) Matt, C.; Wagner, A.; Mioskowski, C. *J. Org. Chem.* **1997**, *62*, 234−235.

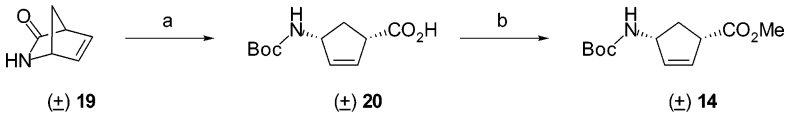
(22) Daluge, S.; Vince, R. *J. Org. Chem.* **1978**, *43*, 2311−2320.

(23) Daluge, S. M.; Martin, M. T.; Sickles, B. R.; Livingston, D. A. *Nucleosides, Nucleotides Nucleic Acids* **2000**, *19*, 297−327.

(24) Shireman, B. T.; Miller, M. J.; Jonas, M.; Wiest, O. *J. Org. Chem.* **2001**, *66*, 6046−6056.

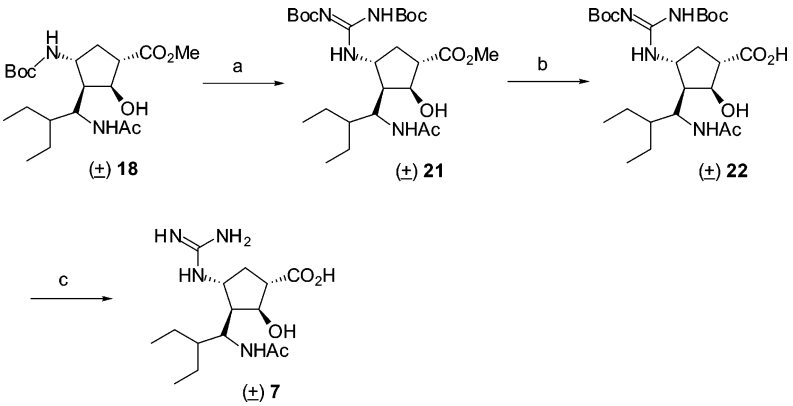
(25) Shireman, B. T.; Miller, M. J. *J. Org. Chem.* **2001**, *66*, 4809−4813.

SCHEME 5[a]



[a] Reagents: (a) (i) Boc_2O, DMAP, THF, (ii) 2 N NaOH, 86% in two steps; (b) (TMS)Cl, MeOH, 95%.

SCHEME 6[a]



[a] Reagents: (a) (i) HCl gas, ether, (ii) 1,3-bis(*t*-Boc)-2-methyl-2-thiopseudourea, $HgCl_2$, Et_3N, DMF, 82% in two steps; (b) 1 N NaOH, EtOH/THF (1:1), 95%; (c) TFA, Et_3SiH, CH_2Cl_2, 78%.

provide structurally and biologically optimized NA inhibitors. Enzymatic preparation of compound **11**,[18] generating the intermediates in optically pure forms, can lead to asymmetric syntheses of NA inhibitors. Further research focusing on synthetic modifications to examine structure−activity relationships is in progress.

Experimental Section

(±)-*cis*-4-*N*-*tert*-Butoxycarbonylamino-2-cyclopenten-1-ol (11). Cycloadduct **10**[17,18] (3.0 g, 15.2 mmol) was dissolved in MeCN/H_2O (20:1, v/v, 150 mL) in a flask equipped with a reflux condenser, and $Mo(CO)_6$ (792 mg, 3.0 mmol) was added at room temperature. To a slightly warm solution, around 30 °C, was added $NaBH_4$ (1.15 g, 30.4 mmol) in one portion, and the reaction mixture was heated at reflux for 12 h. After the reaction was monitored for completion by TLC, the mixture was cooled to 0 °C and stirred for 1 h. The solid inorganic byproducts were separated by filtration through a thin pad of Celite. The solvent was concentrated by rotary evaporation. Silica gel flash chromatography using hexanes/EtOAc (8:2, v/v) yielded **11** as a white solid. Recrystallization was carried out using hexanes/EtOAc (9:1, v/v): 2.0 g, 66% yield; mp[26] 72−73 °C (lit.[17] mp 64−65.5 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.90 (dt, 1H, J = 5.5, 1.8 Hz), 5.78 (ddd, 1H, J = 5.5, 2.1, 1.0 Hz), 5.12 (br s, 1H), 4.63−4.61 (m, 1H), 4.46−4.37 (m, 1H), 3.83 (br s, 1H), 2.66 (ddd, 1H,, J = 14.3, 7.8, 7.5 Hz) 1.50−1.44 (m, 1H), 1.40 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.5, 136.1, 134.3, 79.6, 75.1, 54.8, 41.4, 28.5; IR (neat) 3332, 2977, 1686, 1524, 1170 cm^{-1}; HRMS (FAB) (*m*/*z*) [M + H]$^+$ calcd for $C_{10}H_{18}NO_3$ 200.1287, found 200.1275.

(±)-*cis*-Carbonic Acid 4-*tert*-Butoxycarbonylaminocyclopent-2-enyl Ethyl Ester (12). Compound **11** (1.6 g, 8.2 mmol) was dissolved in pyridine/CH_2Cl_2 (1:1, v/v, 160 mL), and ethyl chloroformate (0.97 mL, 9.8 mmol) was added at room temperature. The reaction mixture was stirred at the same temperature for 3 h, being monitored for completion by TLC. The mixture was quenched with aqueous saturated NH_4Cl solution, and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were washed with water and brine and dried over anhydrous Na_2SO_4. The mixture was filtered and concentrated by rotary evaporation. The resulting residue was separated by silica gel flash column chromatography using hexanes/EtOAc (9:1, v/v) to afford the desired compound **12** as a white solid. Recrystallization was carried out using hexanes/CH_2Cl_2 (9:1, v/v): 2.0 g, 92% yield; mp 49−50 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.01−5.96 (m, 2H), 5.45−5.42 (m, 1H), 4.68 (br s, 1H), 4.18 (q, 2H, J = 7.1 Hz), 2.81 (ddd, 1H, J = 14.6, 7.5, 7.3 Hz), 1.61 (m, 1H), 1.42 (s, 9H), 1.29 (t, 3H, J = 7.1 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.1, 154.8, 137.8, 131.7, 81.0, 79.7, 64.1, 54.4, 38.6, 28.5, 14.4; IR (neat) 3369, 2980, 1743, 1712, 1258 cm^{-1}; HRMS (FAB) (*m*/*z*) [M + H]$^+$ calcd for $C_{13}H_{22}NO_5$ 272.1498, found 272.1494.

(±)-*cis*-4-*N*-*tert*-Butoxycarbonylamino-1-nitromethylcyclopent-2-ene (13). Ethyl carbonate **12** (1.4 g, 5.0 mmol) was dissolved in $MeNO_2$ (15 mL), and a catalytic amount of Et_3N (20 μL) was added. A mixture of $Pd(OAc)_2$ (11 mg, 0.05 mmol) and PPh_3 (52 mg, 0.20 mmol), which had been stirred separately in THF (1 mL) for 5 min, was transferred to the above solution via cannula under argon. The reaction mixture was stirred at 50 °C for 7 h. After the reaction was complete as monitored by TLC, the mixture was concentrated by rotary evaporation. Silica gel flash chromatography using hexanes/EtOAc (85:15, v/v) yielded **13** as a white solid. Recrystallization was carried out using hexanes/CH_2Cl_2 (9:1, v/v): 848 mg, 70% yield; mp 74.5−75.5 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.02 (d, 1H, J = 8.3 Hz), 5.75−5.70 (m, 2H), 4.64 (dd, 1H, J = 13.2, 6.4 Hz), 4.52 (dd, 2H, J = 13.2, 8.3 Hz), 3.27−3.22 (m, 1H), 2.44 (ddd, 1H, J = 13.8, 8.7, 8.4 Hz) 1.38 (s, 9H), 1.29 (ddd, 1H, J = 13.7, 5.6, 5.4 Hz); ^{13}C NMR (150 MHz, DMSO-d_6) δ 154.9, 134.8, 131.6, 79.2, 77.7, 55.5, 42.5, 34.4, 28.2; IR (neat) 3349, 1686, 1574, 1529 cm^{-1}; HRMS (FAB) (*m*/*z*) [M + H]$^+$ calcd for $C_{11}H_{19}N_2O_4$ 243.1345, found 243.1323.

(±)-Methyl *cis*-4-*tert*-Butoxycarbonylaminocyclopent-2-ene-1-carboxylate (14). Nitro compound **13** (606 mg, 2.5 mmol) was dissolved in DMF (35 mL). $NaNO_2$ (1.0 g, 15.0 mmol) and AcOH (2.9 mL, 50.0 mmol) were added to the above solution at room temperature. The reaction mixture was stirred at 40 °C for 10 h, being monitored for completion by TLC. The mixture was diluted with a 5% $NaHCO_3$ solution and washed with ether (3 × 15 mL). The aqueous layer was

(26) The melting point of **11** was measured after intensive purification since it was revealed to be highly sensitive to the purity.

then acidified with a 10% HCl solution and extracted with ether (3 × 15 mL). The combined organic layers were washed with water and brine and dried over anhydrous Na_2SO_4. The mixture was filtered and concentrated by rotary evaporation. The resulting residue was separated by silica gel flash column chromatography using hexanes/EtOAc (7:3 to 5:5, v/v) to give the acid intermediate as a white solid, which was directly used for methylation upon drying in vacuo. This acid intermediate (352 mg, 1.55 mmol) was dissolved in MeOH (150 mL) containing a catalytic amount of (TMS)Cl. The reaction mixture was stirred at room temperature for 2 h, being monitored for completion by TLC. The mixture was concentrated by rotary evaporation. The resulting residue was purified by silica gel flash column chromatography using hexanes/EtOAc (9:1, v/v) to afford the desired methyl ester **14** as a white solid. Recrystallization was carried out using hexanes/EtOAc (95:5, v/v): 350 mg, 58% yield over two steps; mp 36−37 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.80−5.75 (m, 2H), 4.95 (d, 1H, J = 8.2 Hz), 4.75−4.64 (m, 1H), 3.63 (s, 3H) 3.42−3.38 (m, 1H), 2.43 (ddd, 1H, J = 13.9, 8.4, 8.4 Hz), 1.78 (ddd, 1H, J = 13.9, 4.4, 4.3 Hz), 1.36 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.9, 155.2, 134.9, 131.1, 79.2, 55.8, 52.2, 49.2, 34.5, 28.4; IR (neat) 3368, 2978, 1736, 1712, 1508, 1171 cm^{-1}; HRMS (FAB) (m/z) [M + H]$^+$ calcd for $C_{12}H_{20}NO_4$ 242.1392, found 242.1416.

(±)-Methyl 4-*tert*-Butoxycarbonylamino-3-(1-ethylpropyl)-4,5,6,6a-tetrahydro-3aH-cyclopenta[d]isoxazole-6-carboxylate (17). To a mixture of olefin **14** (193 mg, 0.8 mmol), oxime **16** (415 mg, 3.6 mmol), and Et_3N (33.5 μL, 0.24 mmol) in CH_2Cl_2 (12 mL) was added bleach (8.9 mL, 7.2 mmol from a 6% NaOCl solution). The reaction mixture was stirred at reflux for 20 h. After the reaction was monitored for completion by TLC, the reaction mixture was extracted with CH_2Cl_2 (3 × 15 mL). The combined organic layers were washed with water and brine and dried over anhydrous Na_2SO_4. The mixture was filtered and concentrated by rotary evaporation. The resulting residue was purified by silica gel flash column chromatography using hexanes/EtOAc (7:3, v/v) to give compound **17** as a white solid. Recrystallization was carried out using hexanes/CH_2Cl_2 (9:1, v/v): 173 mg, 61% yield; mp[27] 107−109 °C (lit.[16] mp 66 °C, for the nonracemic form); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.60 (d, 1H, J = 7.4 Hz), 5.20 (dd, 1H, J = 9.2, 1.5 Hz), 4.26−4.16 (m, 1H), 3.75 (s, 3H), 3.57 (d, 1H, J = 9.2 Hz), 3.19 (d, 1H, J = 7.3 Hz), 2.54−2.45 (m, 1H), 2.16−1.98 (m, 2H), 1.77−1.55 (m, 4H), 1.43(s, 9H), 0.91 (t, 3H, J = 7.4 Hz) 0.87 (t, 3H, J = 7.5 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.3, 161.3, 155.1, 87.2, 79.6, 63.6, 55.7, 52.7, 52.3, 40.6, 33.5, 28.5, 25.8, 24.1, 12.2, 10.9; IR (neat) 3370, 2966, 1713, 1170 cm^{-1}; HRMS (FAB) (m/z) [M + H]$^+$ calcd for $C_{18}H_{30}N_2O_5$ 355.2233, found 355.2231.

(±)-Methyl 3-(1-Acetylamino-2-ethylbutyl)-4-*tert*-butoxycarbonylamino-2-hydroxycyclopentanecarboxylate (18). To a solution of **17** (106 mg, 0.3 mmol) in MeOH (10 mL) were added concd HCl (29.7 μL, 0.3 mmol) and PtO_2 (10.2 mg, 0.045 mmol). Using a Parr apparatus, the mixture was stirred vigorously at 40 psi of hydrogen pressure for 10 h. After the solution was purged with nitrogen for 30 min, the catalyst was filtered off through a thin pad of Celite. The filtrate was concentrated by rotary evaporation and dried in vacuo to give an amine hydrochloride salt as the intermediate. To this amine hydrochloride salt (118 mg, 0.3 mmol) in CH_2Cl_2 (6 mL) were added Et_3N (42 μL, 0.3 mmol) and Ac_2O (31 μL, 0.33 mmol), and the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was monitored for completion by TLC. The reaction mixture was then diluted with water and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were washed with water and brine and dried over anhydrous Na_2SO_4. The mixture was filtered and concentrated by rotary evaporation. The resulting residue was purified by silica gel flash column chromatography using hexanes/EtOAc (7:3 to 5:5, v/v) to furnish the desired compound **18** as a white solid. Recrystallization was carried out using hexanes/CH_2Cl_2 (9:1, v/v): 99 mg, 82% yield for two steps; mp 120−122 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56 (d, 1H, J = 9.9 Hz), 4.75 (d, 1H, J = 9.4 Hz), 4.24 (d, 1H, J = 4.5 Hz), 4.17−4.11 (m, 1H), 4.05−3.99 (m, 1H), 3.71 (s, 3H), 2.85−2.80 (m, 1H), 2.49 (ddd, 1H, J = 13.5, 9.0, 9.0 Hz), 2.09 (s, 3H), 2.02−1.96 (m, 1H), 1.69 (ddd, 1H, J = 13.7, 8.3, 5.8 Hz), 1.44−1.23 (m, 15H), 0.86 (t, 3H, J = 6.9 Hz), 0.79 (t, 3H, J = 7.1 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.7, 171.7, 156.2, 80.1, 77.8, 52.3, 52.0, 50.5, 49.1, 48.2, 43.6, 33.4, 28.5, 23.4, 22.0, 21.4, 10.7, 10.2; IR (neat) 3351, 2964, 1688, 1170 cm^{-1}; HRMS (FAB) (m/z) [M + H]$^+$ calcd for $C_{20}H_{37}N_2O_6$ 401.2652, found 401.2636.

(±)-Methyl 3-(1-Acetylamino-2-ethylbutyl)-4-[{(*tert*-butoxycarbonylamino)(*tert*-butoxycarbonylimino)methyl}amino)]-2-hydroxycyclopentanecarboxylate (21). Compound **18** (385 mg, 0.96 mmol) was dissolved in anhydrous ether (90 mL), and HCl gas was passed through the solution for 30 min. The white precipitate of the amine hydrochloride salt intermediate was collected and dried in vacuo. To this amine salt (303 mg, 0.9 mmol) in DMF (6 mL) were added Et_3N (319 mg, 3.15 mmol), 1,3-bis(*tert*-butoxycarbony)-2-methyl-2-thiopseudourea[28] (261 mg, 0.9 mmol), and $HgCl_2$ (243 mg, 0.9 mmol). The reaction mixture was stirred at room temperature for 10 h. Analytical TLC was periodically carried out. After complete consumption of the starting materials was observed, the mixture was diluted with EtOAc (6 mL) and filtered through Celite. The filtrate was diluted with water (30 mL) and extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with water and brine and dried over anhydrous Na_2SO_4. The solution was filtered and concentrated by rotary evaporation. The residue was purified by flash chromatography using hexanes/EtOAc (5:5 to 3:7, v/v) to yield **21** as a white foam. Recrystallization was carried out using hexanes/EtOAc (7:3, v/v): 427 mg, 82% yield over two steps; mp 167−169 °C dec; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.37 (s, 1H), 8.68 (d, 1H, J = 10.0 Hz), 8.57 (d, 1H, J = 8.4 Hz), 4.50−4.37 (m, 1H), 4.27−4.23 (m 1H), 4.21 (s, 1H), 3.94 (t, 1H, J = 10.0 Hz), 3.68 (s, 3H), 2.82−2.77 (m, 1H), 2.50 (ddd, 1H, J = 13.6, 8.9, 8.9 Hz), 2.07 (s, 3H), 2.01−1.99 (m, 1H), 1.81 (ddd, 1H, J = 13.6, 6.8, 5.8 Hz), 1.47 (s, 9H), 1.44 (s, 9H), 1.53−1.15 (m, 5H), 0.77 (t, 3H, J = 7.0 Hz), 0.72 (t, 3H, J = 7.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.1, 171.7, 162.8, 155.8, 153.1, 83.9, 79.9, 78.1, 52.3, 52.0, 50.9, 49.3, 48.6, 43.3, 33.9, 28.4, 28.1, 23.4, 22.5, 21.6, 10.7, 10.1; IR (neat) 3278, 2974, 1725, 1648, 1615, 1157, 1136 cm^{-1}; HRMS (FAB) (m/z) [M + H]$^+$ calcd for $C_{26}H_{47}N_4O_8$ 543.3394, found 543.3363.

(±)-3-(1-Acetylamino-2-ethylbutyl)-4-[{(*tert*-butoxycarbonylamino)(*tert*-butoxycarbonylimino)methyl}amino)]-2-hydroxycyclopentanecarboxylic Acid (22). Compound **21** (271 mg, 0.5 mmol) was dissolved in EtOH/THF (1:1, v/v, 6 mL) and further treated with 1 N NaOH (3 mL, 3 mmol) at room temperature for 1 h. The resulting solution was acidified using ice-cold 1 N HCl solution and quickly extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with water and brine and dried over anhydrous Na_2SO_4. The solution was filtered and concentrated by rotary evaporation. The resulting white foam was recrystallized with hexanes/EtOAc (8:2, v/v): 264 mg, 95% yield; mp 219−220 °C dec; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.40 (s, 1H), 8.93 (d, 1H, J = 10.0 Hz), 8.61 (d, 1H, J = 8.3 Hz), 4.49−4.36 (m, 2H), 4.01 (t, 1H, J = 9.5 Hz), 2.86 (t, 1H, J = 7.0 Hz), 2.53 (ddd, 1H, J = 13.4, 8.9, 8.8 Hz), 2.13 (s, 3H), 2.08−2.03 (m, 1H), 1.92 (ddd, 1H, J = 13.9, 8.1, 7.0 Hz), 1.49 (s, 9H), 1.47 (s, 9H), 1.55−1.16 (m, 6H), 0.80 (t, 3H, J = 6.8 Hz), 0.75 (t, 3H, J = 7.1 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.8, 172.3, 162.8, 156.0, 153.1, 84.0, 80.1, 78.1, 51.7, 51.0, 49.6, 48.9, 43.5, 33.7, 28.5, 28.2, 23.3, 22.6, 21.7, 10.8, 10.3; IR (neat) 3274, 2969, 1723, 1651, 1614,

(27) Compound **17** was observed to sinter at 66 °C, which corresponded to the literature melting point.

(28) Aldrich Chemical Co., Inc., Catalog No. 43,990-8.

1138 cm^{-1}; HRMS (FAB) (*m*/*z*) $[M + H]^+$ calcd for $C_{25}H_{45}N_4O_8$ 529.3237, found 529.3250.

(±)-3-(1-Acetylamino-2-ethylbutyl)-4-[{aminoimino-methyl}amino)]-2-hydroxycyclopentanecarboxylic Acid (7). A solution of **22** (127 mg, 0.24 mmol) in CH_2Cl_2 (12 mL) containing TFA (0.72 mL) and Et_3SiH (0.19 mL, 1.2 mmol) was stirred at room temperature for 24 h. The solvent was removed by rotary evaporation, and water (20 mL) was added to the residue. The aqueous solution was extracted with ether (3 × 10 mL) to remove organic impurities. The aqueous layer was concentrated, and the residue was subjected to silica gel flash column chromatography using $CHCl_3$/MeOH/aq NH_4OH (75:20:5 to 50:40:10, v/v) to furnish the final product **7** as a white solid. Recrystallization was carried out using water/MeOH (5:5, v/v): 61 mg, 78% yield; mp >250 °C; ^1H NMR (600 MHz, D_2O) δ 7.52 (d, 1H, J = 10.4 Hz), 4.41−4.37 (m, 2H), 3.89−3.86 (m, 1H), 2.84−2.82 (m, 1H), 2.59 (ddd, 1H, J = 7.1 Hz), 2.24−2.20 (m, 1H), 1.96 (s, 3H), 1.84−1.80 (m, 1H), 1.48−1.40 (m, 4H), 1.05−0.97 (m, 2H) 0.93 (t, 3H, J = 7.1 Hz) 0.88 (t, 3H, J = 6.9 Hz); ^{13}C NMR (150 MHz, DMSO-d_6 + D_2O) δ 182.4, 175.3, 157.2, 76.7, 56.7, 55.1, 51.8, 51.5, 45.0, 35.6, 24.4, 23.6, 22.6, 13.8, 13.0; IR (KBr) 3189, 2968, 1668, 1203, 1137 cm^{-1}; HRMS (FAB) (*m*/*z*) $[M + H]^+$ calcd for $C_{15}H_{29}N_4O_4$ 329.2189, found 329.2170.

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Supporting Information Available: ^1H and ^{13}C NMR spectra for compounds **7**, **12**−**14**, **17**, **18**, **21**, and **22** and the X-ray crystal structure of compound **17**. This material is available free of charge via the Internet at http://pubs.acs.org.

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