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Design and synthesis of a siderophore conjugate as a potent PSMA inhibitor and potential diagnostic agent for prostate cancer

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Abstract—A siderophore conjugate was designed as a potential PSMA inhibitor and diagnostic agent for prostate cancer. A semi-rigid spacer was incorporated to avoid competitive participation of iron binding by the enzyme inhibitor relative to the siderophore component. Biological test results showed that, even with the extended scaffold, this compound is a potent PSMA inhibitor with an IC_{50} of 4 nM. This siderophore conjugate may be useful for detection of prostate-derived cancer cells by magnetic resonance imaging (MRI).

1. Introduction

In the brain, NAALADase (*N*-acetylated α-linked acidic dipeptidase) releases *N*-acetyl aspartate and glutamate from both the neuronal peptide *N*-acetylaspartylglutamate (NAAG) and folate polyglutamate.[1] NAAG is the most abundant and widely distributed peptide transmitter in the mammalian nervous system, and NAALADase is believed to play a key role in modulating the release of glutamate. As a therapeutic target, NAALADase inhibition has been suggested to have potential benefits over the existing receptor-based strategies because it represents an upstream mechanism for regulation of synaptic glutamate that could reduce transmission at a number of glutamatergic receptors rather than inhibiting a single receptor subtype.[2] Therefore, NAALADase represents an intriguing target for drug discovery aimed at unmet medical needs.[3]

2-PMPA (**1**) is a potent and selective NAALADase inhibitor and has been shown to provide significant protection against injury in rats after transient middle cerebral artery occlusion.[4] Using 2-PMPA as a template, extensive structure–activity relationship (SAR) studies have been carried out and some potent inhibitors of NAALADase, such as urea-based compounds[5] and GPI5232,[6] were identified. Recent studies on the two enantiomers of 2-PMPA and GPI5232 have clearly demonstrated that potent inhibition of NAALADase is specific to the (*S*)-enantiomer, which has an absolute configuration corresponding to L-glutamate.[7] The high potency of these phosphinate-based inhibitors can be attributed to the strong chelation of the phosphonate group to an active site zinc ion as well as to the interaction of the glutamate portion of the inhibitor with the glutamate recognition site of NAALADase.

A protein having NAALADase activity is also expressed in the human prostate parenchyma, from where it was first cloned and named prostate-specific membrane antigen (PSMA).[8] PSMA is a 110 kDa type II transmembrane protein that is expressed by prostate tumor cells and as such is a clear cellular target for the development of methods for reliable diagnosis of prostate cancer and for selective drug delivery.[8c–g] The X-ray crystal structure of PSMA has been elucidated and its catalytic mechanism has been proposed.[9] PSMA is highly homologous to NAALADase, and inhibitors of NAALADase also strongly bind to PSMA. For example, phosphinate-based inhibitor VA-033 (**2**, IC_{50} = 12.5 nM) was found to serve as a potent inhibitor of NAALADase activity

Keywords: PSMA; NAALADase; Siderophore conjugate; Inhibitor.
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associated with PSMA that is expressed on LNCaP human prostate cancer cells and by tumor cells in vivo.[10] The structurally similar phenylalkylphosphonamidates had been reported by Berkman's group as potent PSMA inhibitors.[11] Very recently, we reported some enantiomerically pure phosphinate-based analogues **3** as potent NAALADase inhibitors.[12] In the next stage of our investigations, we wished to design a suitably labeled PSMA inhibitor that could be employed as a possible diagnostic agent for prostate cancer cells.

As a labeling strategy, we chose to employ a siderophore. Such compounds are well known to form highly stable complexes with Fe(III) and Gd(III), and the resulting metal complexes can be detected by magnetic resonance imaging (MRI).[13] We and others have reported examples of Fe and Gd binders directed toward prostate cancer detection.[12] Our initial compound was an analogue of **3** with R being a form of siderophore **4**.[12a] However, closer consideration of its structural features indicated that this type of analogue is subject to conformational effects on activity. In particular, we hypothesized that a longer, rigidified linker was needed between the PSMA binding region and the siderophore region to avoid interfering interactions between these two groups. The risk is that otherwise one of the carboxylate groups of the inhibitor may displace one of the hydroxamate groups of the siderophore to chelate physiological Fe(III) or added Gd(III). Presumably, this interaction may impair the ability of the inhibitor to bind to the active site of PSMA. Related competitive binding interactions have been documented in siderophore analogues containing catechol-salicylate moieties.[14] We therefore considered using different linker groups to further restrict the conformations of the analogues, and incorporation of linker **5** was anticipated to allow metal chelation of the siderophore portion while allowing the glutamate-derived hydroxyphosphinyl portion to remain free to bind to the PSMA active site. The proposed compound (**6**) may therefore be useful for detection of prostate-derived cells by MRI if inclusion of the extended linker would not have a detrimental effect on normal PSMA binding. Herein, we describe the synthesis of **6** and assay results to indicate that compounds of this type can be potent PSMA inhibitors (Figs. 1 and 2).

2. Results and discussion

The retrosynthetic analysis of conjugate **6** is shown in Scheme 1. Compound **6** could be assembled from the

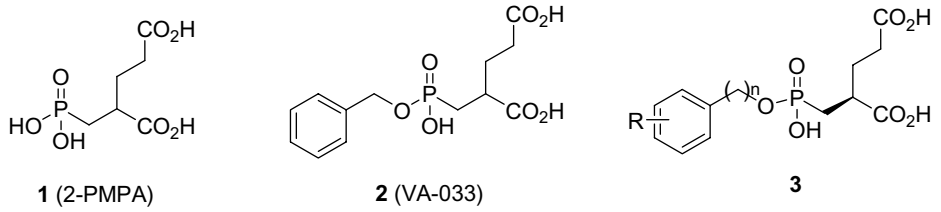


Figure 1. Inhibitors of NAALADase and PSMA.

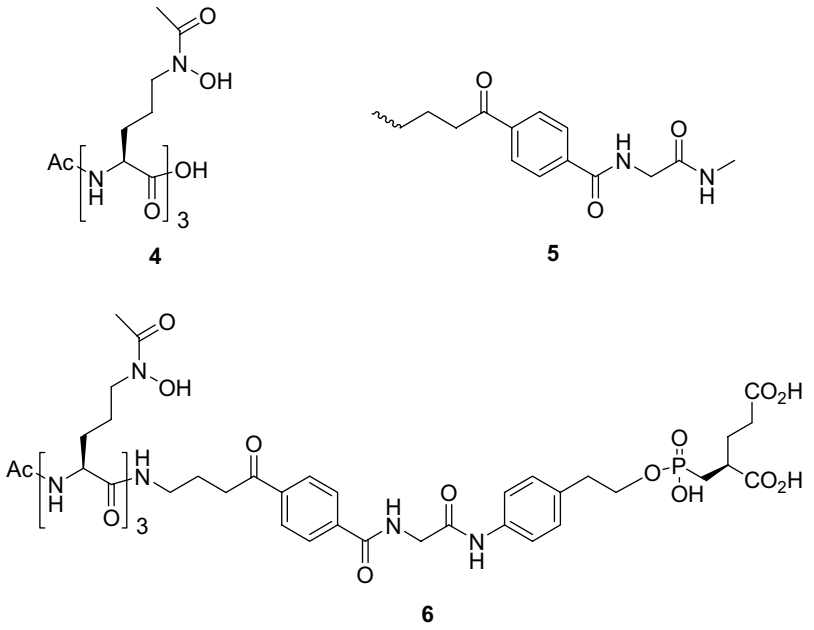
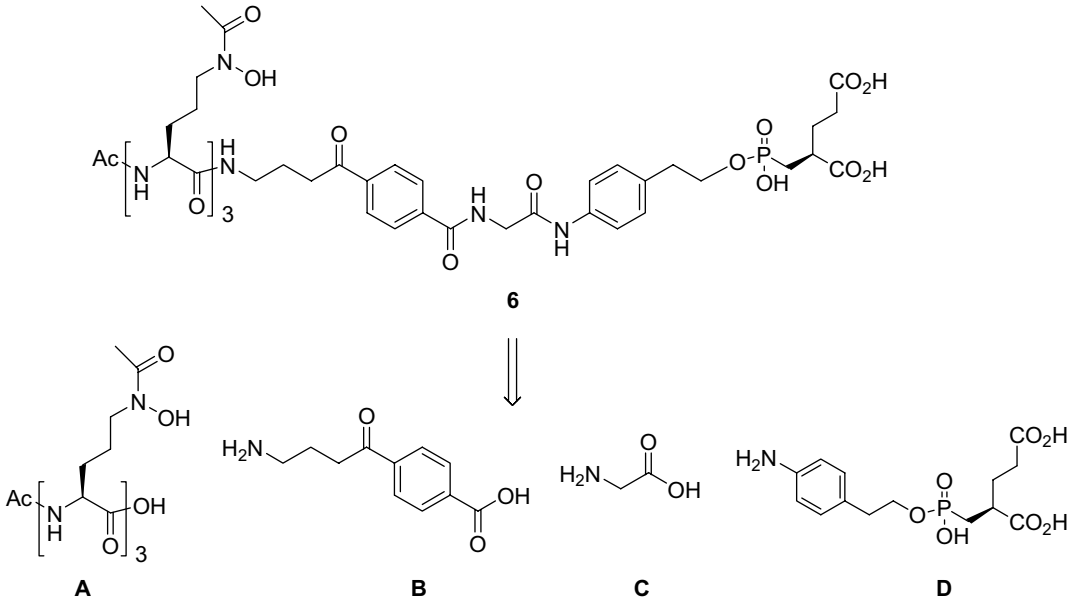


Figure 2. Proposed siderophore-including inhibitor **6**, its precursor siderophore component **4**, and linker **5**.

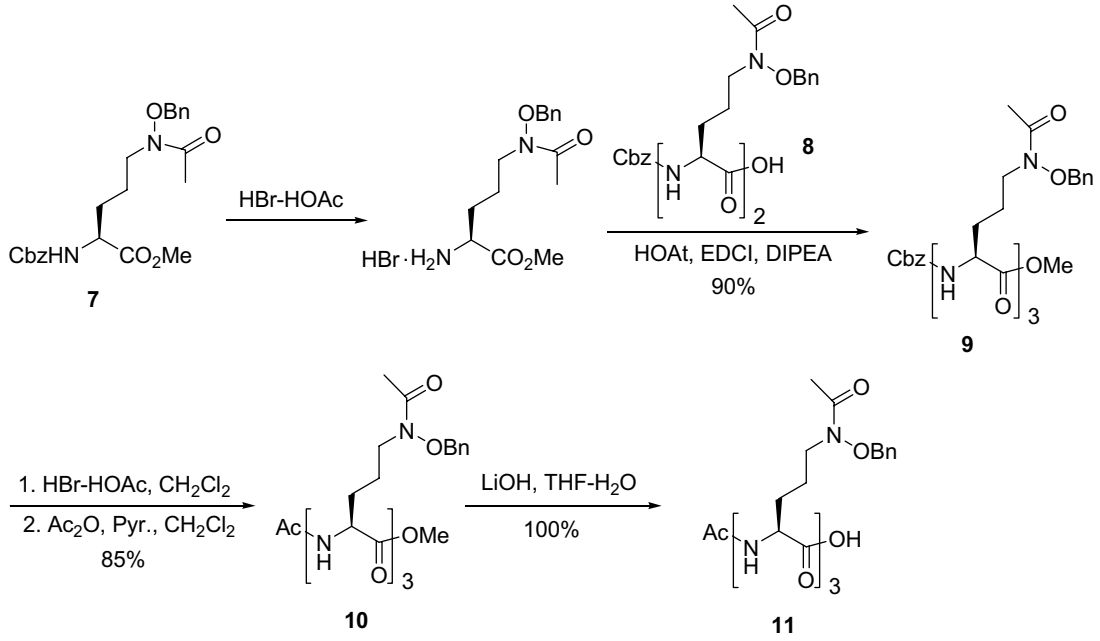


Scheme 1. Retrosynthetic analysis of proposed conjugate **6**.

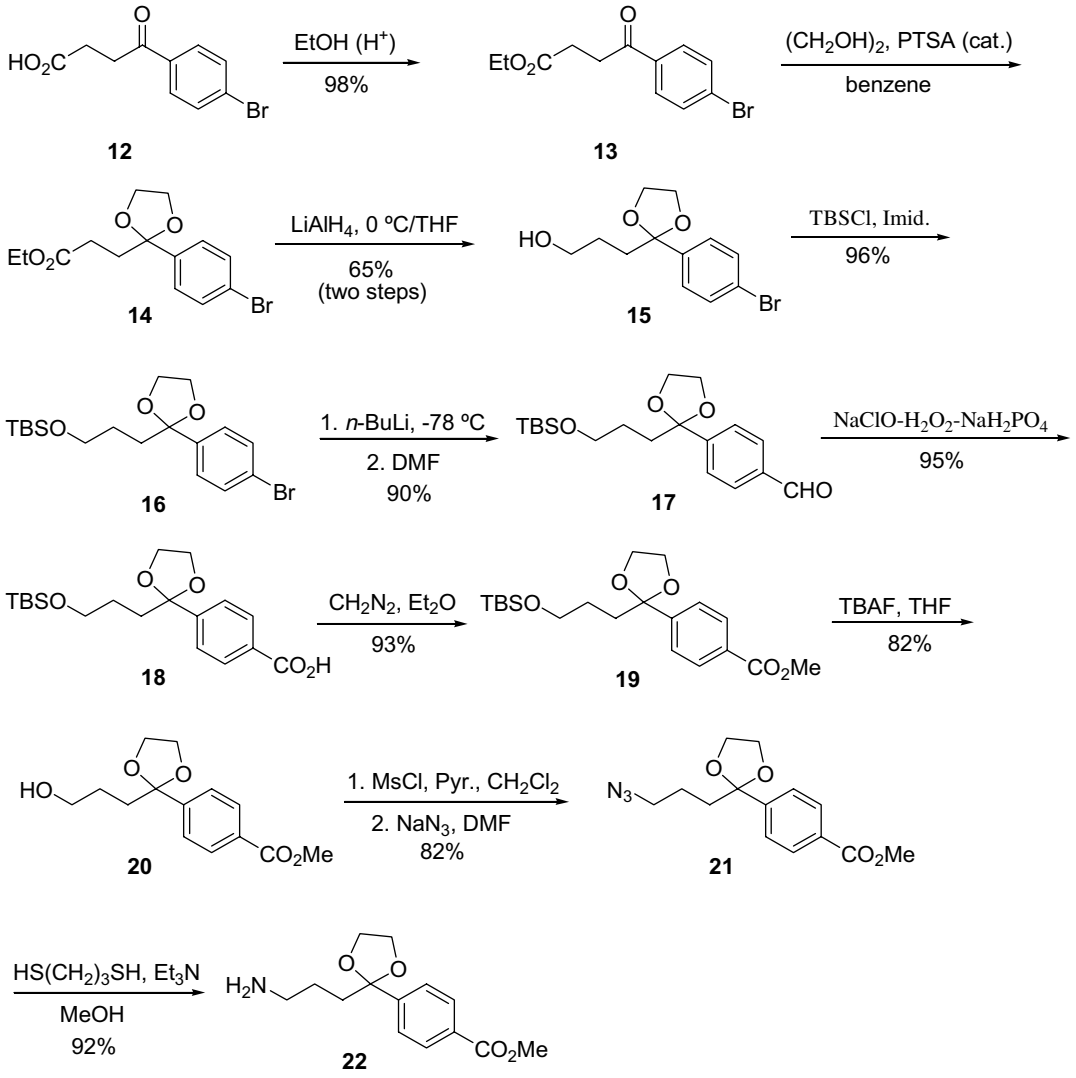
following four fragments: tripeptide component **A**, amino acid component **B**, glycine **C**, and phosphonate **D**.

The synthesis began with the removal of the Cbz group of fully protected amino acid **7**[15] by treatment with 33% HBr/acetic acid. The free amine was released from the resulting HBr salt and coupled to dipeptide acid **8**[16] to produce tripeptide methyl ester **9** in 90% yield. ^{13}C NMR analysis of tripeptide **9** indicated three sharp signals at 53.36 ppm, 52.38 ppm, and 51.43 ppm for the stereogenic methine carbons, supporting the formation of a single diastereomer. Removal of the Cbz group with 33% HBr/acetic acid and subsequent acetylation gave tripeptide methyl ester **10** in 85% yield. Saponification of the methyl ester gave tripeptide acid **11** in quantitative yield. Again, ^{13}C NMR revealed three sharp singlets at 53.56 ppm, 52.51 ppm, and 52.04 ppm assigned to the three stereogenic methine carbons, indicating that, within the limits of detection, only one diastereomer was produced (Scheme 2).

Several approaches were tried to prepare the linker. Since the linker includes carbonyl, carboxylic acid, and free amino groups, caution had to be taken to choose suitable protecting groups for these functional groups. The successful route is summarized in Scheme 3. Ethyl



Scheme 2. Synthesis of tripeptide **11**.

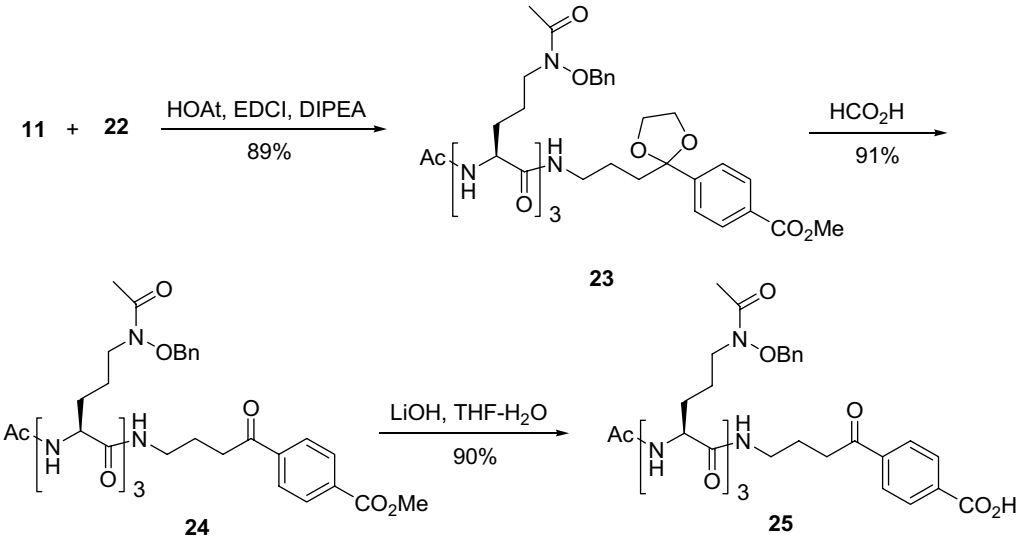


Scheme 3. Synthesis of linker **22**.

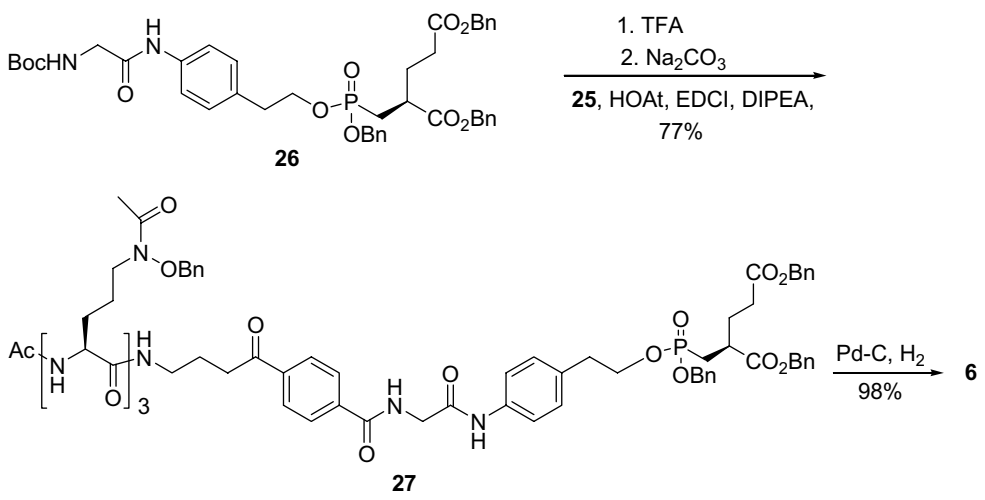
ester **13** was obtained in 97% yield by heating a solution of **12** at reflux in ethanol in the presence of a catalytic amount of H_2SO_4.[17] Treatment of **13** with ethylene glycol in the presence of a catalytic amount of *p*-toluenesulfonic acid (PTSA) gave ketal **14** as an inseparable mixture with some unconsumed starting material **13**. The mixture was then subjected to reduction by 2 equiv of $LiAlH_4$ at 0 °C in THF. The resulting alcohol **15** was isolated in 65% overall yield for the two steps. Alcohol **15** was then protected with a *tert*-butyldimethylsilyl (TBS) group to afford **16** in 96% yield. Upon treatment of **16** with 1.1 equiv of *n*-BuLi in THF at −78 °C for 30 min, the resulting aryllithium was quenched with DMF to give aldehyde **17** in 90% isolated yield. Oxidation with sodium chlorite—hydrogen peroxide afforded acid **18** in 90% yield.[18] Next, methylation with an ethereal solution of diazomethane provided methyl ester **19** in 93% yield.[19] After removal of the TBDMS protecting group by TBAF in THF, alcohol **20** was obtained in 82% yield. Azide **21** was prepared from alcohol **20** via its mesylate in 82% yield. Reduction of azide **21** proved to be difficult. We first tried to use Ph_3P/H_2O or Pd–C/H_2,[20] but both methods led to the formation of complicated product mixtures. In the end, $HS(CH_2)_3SH$ was used as a reducing reagent in the presence of Et_3N to produce free amine **22** in 92% yield.[21]

Free amine **22** was coupled to tripeptide acid **11** to give **23** in 89% yield (Scheme 4). In the next deprotection step, we first used *p*-toluenesulfonic acid (PTSA) in wet acetone,[22] but the reaction was incomplete after heating at reflux overnight, and isolation of the product from the unconsumed starting material proved to be difficult. Alternatively, when HOAc–THF–H_2O (v/v/v, 3:1:1) was used,[23] the reaction was incomplete even when the reaction mixture was heated at 70 °C for 24 h, and the increased reaction time led to some decomposition. We finally succeeded with the deprotection by using neat formic acid to obtain ketone **24** in 91% yield.[24] Subsequent hydrolysis readily provided acid **25** (Scheme 5).

Removal of the *N*-Boc group of **26**[12] provided the free amine, which was used immediately in a coupling reaction with acid **25** to provide the final precursor **27**.



Scheme 4.



Scheme 5.

The last global deprotection provided the target molecule **6** in 98% yield.

The in vitro NAALADase inhibitory potency of the synthesized inhibitor was measured at Guilford Pharmaceuticals Inc. using *N*-acetyl-L-aspartyl-[^{3}H]-L-glutamate as a substrate and human recombinant NAALADase[25] as previously reported.[26] 2-PMPA (**1**) with an IC_{50} of 0.3 nM was used as an assay reference. Results of the biological assays showed that designed conjugate **6** retains potent activity with an IC_{50} of 4 nM. The iron and gadolinium complexes of this compound are the subject of separate studies that will be reported in due course.

3. Conclusion

In summary, a siderophore-containing, enantiomerically pure phosphonic acid **6** conjugate has been designed and synthesized as a potential diagnostic agent for prostate cancer. Despite incorporation of an extended linker to promote iron binding only by the trihydroxamate siderophore component, this compound revealed outstanding activity in a biological assay. This observation has demonstrated that a large, added component such as tripeptide **4** is well tolerated upon conjugation with the active phosphonic acid derivative **3**. MRI experimental studies of iron and gadolinium complexes of **6** and related analogues will test the concept of selective detection of prostate cancer cells through use of magnetic resonance imaging of suitably functionalized PSMA binding agents. The results will facilitate future design of additional analogues as diagnostic agents for prostate cancer and, by logical extension, as therapeutic agents for selective drug delivery.

4. Experimental

4.1. General

All reactions were carried out under argon by using standard techniques. Solvents were dried under nitrogen by standard procedures, distilled before use, and stored

under argon. NMR spectra were recorded on a Varian Unityplus 300 MHz spectrometer or Varian Inova 500 MHz spectrometer. Optical rotations were recorded on a Perkin-Elmer model 343 polarimeter. Mass spectra were recorded on a JEOL JMS-AX505 HA Double Sector mass spectrometer. The chemical shifts of ^{31}P NMR spectra are reported in relation to 85% H_3PO_4.

4.1.1. Methyl N^5-acetyl-N^5-(benzyloxy)-N^2-(benzyloxycarbonyl)-L-ornithyl-N^5-acetyl-N^5-(benzyloxy)-L-ornithyl-N^5-acetyl-N^5-(benzyloxy)-L-ornithinate (9). To a solution of **7**[14] (835 mg, 1.95 mmol) in CH_2Cl_2 (20 mL) was added 33% HBr glacial acetic acid solution (8 mL). The reaction mixture was stirred at 25 °C for 30 min under argon, then diluted with $CHCl_3$ (40 mL), and concentrated in vacuo. The resulting oil was redissolved in and concentrated twice each using $CHCl_3$ (30 mL) and toluene (30 mL). The resulting residue was dissolved in CH_3CN (60 mL), and **8** (1.1 g, 1.62 mmol) and diisopropylethylamine (0.65 mL, 3.73 mmol) were added, followed by the addition of HOAt (242 mg, 1.78 mmol) and EDCI (342 mg, 1.78 mmol). The reaction mixture was stirred at 25 °C for 48 h, diluted with ethyl acetate (300 mL), and then washed with 1 N HCl (15 mL), saturated aq $NaHCO_3$ (15 mL), and brine. After being dried, filtered, and concentrated, the residue was purified by flash column chromatography, eluting with EtOAc–CH_3OH (100:3 → 100:5) to provide 1.4 g (90%) of the product as a colorless oil. $[\alpha]_D^{20}$ −2.8° (c = 1.6, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$) δ: 7.42–7.30 (m, 20H), 7.22 (d, J = 8 Hz, 1H), 7.12 (d, J = 8 Hz, 1H), 5.63 (d, J = 8 Hz, 1H), 5.09 (s, 2H), 4.90–4.74 (m, 6H), 4.62–4.58 (m, 1H), 4.50–4.38 (m, 2H), 4.22–4.02 (m, 2H), 3.78–3.60 (m, 7H), 3.60–3.42 (m, 2H), 2.13 (s, 3H), 2.11 (s, 3H), 2.08 (s, 3H), 1.88–1.48 (m, 12); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 173.4, 172.6, 172.4, 171.9, 156.5, 136.5, 134.4, 134.3, 129.45, 129.41, 129.25, 129.20, 128.96, 128.94, 128.92, 128.6, 128.2, 128.1, 76.5, 67.0, 53.3, 52.6, 52.4, 52.1, 51.4, 44.7, 43.8, 43.4, 30.8, 29.7, 29.3, 29.0, 23.4, 23.3, 23.2, 20.6; HR-FABMS Calcd for $C_{51}H_{65}N_6O_{12}$ $(M+H)^+$ 953.4660. Found: 953.4698.

4.1.2. Methyl N^5-acetyl-N^5-(benzyloxy)-N^2-acetyl-L-ornithyl-N^5-acetyl-N^5-(benzyloxy)-L-ornithyl-N^5-acetyl-N^5-(benzyloxy)-L-ornithinate (10). To a solution of **9** (175 mg, 0.183 mmol) in CH_2Cl_2 (10 mL) was added a 33% HBr glacial acetic acid solution (3 mL). The reaction mixture was stirred at 25 °C for 30 min under argon, diluted with $CHCl_3$ (20 mL), concentrated in vacuo, and redissolved in and concentrated twice each using $CHCl_3$ (15 mL) and toluene (15 mL). The resulting residue was dissolved in CH_2Cl_2 (15 mL). Pyridine (0.2 mL) and acetic anhydride (0.2 mL) were added at 0 °C, and the resulting mixture was stirred at 25 °C overnight, then diluted with CH_2Cl_2, and washed with 1 N HCl, 10% aq Na_2CO_3, and brine. After the resulting solution was dried, filtered, and concentrated, the crude product was purified by flash chromatography, eluting with EtOAc–CH_3OH (10:1 → 8:1) to afford 135 mg (85%) of the product as a white solid: mp 91–92 °C, $[\alpha]_D^{20}$ −7.9° (c = 0.9, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$) δ: 7.37 (br s, 10H), 7.31 (d, J = 8 Hz, 1H), 7.24 (d, J = 8 Hz, 1H), 6.76 (br s, 1H), 4.84–4.78 (m, 4H), 4.70–4.58 (m, 2H), 4.50–4.44 (m, 1H), 4.02 (br s, 2H), 3.72–3.50 (m, 4H), 3.63 (s, 3H), 2.10 (s, 3H), 2.09 (s, 3H), 2.06 (s, 3H), 1.97 (s, 3H), 1.82–1.50 (m, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 173.1, 172.4, 172.3, 171.8, 170.4, 134.3, 129.33, 129.31, 129.1, 129.0, 128.8, 76.3, 52.2, 52.0, 51.8, 51.5, 44.7, 43.9, 43.5, 30.1, 29.5, 28.9, 23.3, 23.2, 23.19, 23.11, 20.5; HR-FABMS Calcd for $C_{45}H_{61}N_6O_{11}$ $(M+H)^+$ 861.4398. Found: 861.4379.

4.1.3. N^5-Acetyl-N^5-(benzyloxy)-N^2-acetyl-L-ornithyl-N^5-acetyl-N^5-(benzyloxy)-L-ornithyl-N^5-acetyl-N^5-(benzyloxy)-L-ornithine (11). To a solution of **10** (150 mg, 0.174 mmol) in THF–H_2O (v/v = 1:1, 6 mL) was added $LiOH{\cdot}H_2O$ (22 mg, 0.522 mmol). The reaction mixture was stirred at 25 °C for 1 h and then diluted with EtOAc (50 mL) and 1 N HCl (5 mL). The aq layer was extracted with EtOAc twice. The combined organic layers were washed with brine. After the resulting solution was dried, filtered, and concentrated, the product was obtained in 100% yield (148 mg) as a colorless syrup: $[\alpha]_D^{20}$ +7.4° (c = 0.50, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$) δ: 7.68 (br s, 1H), 7.50 (br s, 1H), 7.36 (br s, 15H), 7.12 (br s, 1H), 4.82–4.76 (m, 6H), 4.70–4.60 (m, 2H), 4.40–4.36 (m, 1H), 4.00 (br s, 1H), 3.84 (br s, 1H), 3.64 (br s, 2H), 3.52 (br s, 2H), 2.06 (s, 3H), 2.055 (s, 3H), 2.050 (s, 3H), 1.95 (s, 3H), 1.88–1.56 (m, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 173.5, 173.1, 172.9, 172.6, 172.3, 170.9, 134.2, 129.37, 129.35, 129.0, 128.8, 76.37, 76.31, 52.56, 52.51, 51.8, 44.8, 44.4, 43.7, 30.3, 30.0, 29.8, 28.5, 23.4, 23.16, 23.11, 20.5; HR-FABMS Calcd for $C_{44}H_{59}N_6O_{11}$ $(M+H)^+$ 847.4242. Found: 847.4268.

4.1.4. Ethyl 4-(4-bromophenyl)-4-oxobutanoate (13). To a solution of 3-(4-bromobenzoyl)propionic acid (25 g, 93.9 mmol) in ethanol (180 mL) was added concd H_2SO_4 (2.5 mL), and the reaction mixture was heated at reflux for 3 h. After the mixture was cooled to 25 °C, the excess ethanol was evaporated, the residue was dissolved in ethyl acetate (200 mL), and the organic solution was washed with 10% aqueous $NaHCO_3$ and brine. After the resulting solution was dried, filtered, and evaporated, the product was obtained as a white solid in 97% yield (28.01 g): mp 57–58 °C (lit. 58–59 °C)[13]; ^1H NMR (300 MHz, $CDCl_3$) δ: 7.82 (2H, d, J = 8.7 Hz), 7.58 (2H, d, J = 8.7 Hz), 4.14 (2H, q, J = 7.2 Hz), 3.24 (2H, t, J = 6.6 Hz), 2.72 (2H, t, J = 6.6 Hz), 1.24 (2H, t, J = 7.2 Hz).

4.1.5. 1,3-Dioxolane-2-(4-bromophenyl)-2-propanol (15). To a solution of **13** (8 g, 28.05 mmol) in benzene (100 mL) were added ethylene glycol (3.85 mL, 70.1 mmol) and *p*-toluenesulfonic acid (80 mg, 0.42 mmol), and the reaction mixture was heated at reflux for 16 h. After cooling to 25 °C, the reaction mixture was washed with 10% aq $NaHCO_3$ and brine. After the resulting solution was dried, filtered, and evaporated, the crude product **14** was obtained as a mixture with some unconsumed **13**.

The above crude product was dissolved in dry THF (100 mL). $LiAlH_4$ (1.84 g, 48.6 mmol) was then added

portionwise at 0 °C. The resulting mixture was stirred at 0 °C for 2 h. Then H_2O (2.4 mL) was added slowly, followed by 15% NaOH solution (2.4 mL) and H_2O (8 mL). After stirring for another 30 min at 0 °C, the mixture was filtered through a Celite pad to remove the resulting white solid. The solid was washed with THF. The combined organic layers were concentrated to provide the crude product which was purified by flash column chromatography on silica gel (1:1 hexanes/EtOAc) to afford 4.47 g (65%) of the product as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.46 (2H, d, J = 8.7 Hz), 7.31 (2H, d, J = 8.7 Hz), 4.04–3.99 (2H, m), 3.76–3.72 (2H, m), 3.60 (2H, t, J = 6.6 Hz), 2.07 (1H, m, OH), 2.00–1.92 (2H, m), 1.63–1.54 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 131.51, 128.31, 127.83, 125.93, 122.24, 110.20, 64.80, 64.73, 62.96, 37.17, 27.00; HR-FABMS Calcd for $C_{12}H_{14}BrO_3$ $(M-H)^+$ 285.0126. Found: 285.0112.

4.1.6. 2-(4-Bromo-phenyl)-2-(3-*tert*-butyl-dimethylsilyloxy)propyl-1,3-dioxolane (16). To a solution of **15** (3.3 g, 11.49 mmol) in DMF (45 mL) were added TBDMSCl (2.77 g, 18.38 mmol) and imidazole (1.96 g, 28.73 mmol), and the reaction mixture was stirred at 25 °C overnight, then diluted with CH_2Cl_2, and washed with cold H_2O and brine. After the resulting solution was dried, filtered, and evaporated, the residual DMF was evaporated under high vacuum. The remaining residue was purified by flash column chromatography on silica gel (hexanes/EtOAc 12:1 → 8:1) to afford 4.42 g (96%) of the product as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.44 (2H, d, J = 8.7 Hz), 7.31 (2H, d, J = 8.7 Hz), 4.02–3.97 (2H, m), 3.76–3.72 (2H, m), 3.56 (2H, t, J = 6.6 Hz), 1.96–1.87 (2H, m), 1.59–1.49 (2H, m), 0.86 (9H, s), 0.01 (6H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 131.35, 128.15, 127.80, 125.90, 122.00, 110.32, 64.72, 63.30, 36.93, 27.22, 26.10, 18.43, −5.15; HR-FABMS Calcd for $C_{18}H_{28}BrO_3Si$ $(M-H)^+$ 399.0991. Found: 399.0974.

4.1.7. 2-(4-Carbaldehyde-phenyl)-2-(3-*tert*-butyl-dimethylsilyloxy)propyl-1,3-dioxolane (17). To a solution of **16** (4.1 g, 10.21 mmol) in THF (50 mL) at −78 °C was added dropwise a 1.6 M *n*-BuLi solution in hexanes (7 mL, 11.22 mmol) by syringe. After the addition, the mixture was stirred at −78 °C for another 40 min before DMF (2.36 mL, 30.64 mmol) was added. The reaction mixture was then warmed to 25 °C slowly. The volatiles were evaporated, and the residue was dissolved in ethyl acetate and washed with cold H_2O and brine. After the resulting solution was dried, filtered, and concentrated, the residue was purified by flash column chromatography on silica gel (hexanes/EtOAc 10:1 → 7:1) to afford 3.32 g (90%) of the product as a white solid: mp 49–50 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.98 (1H, s, CHO), 7.84 (2H, d, J = 8.7 Hz), 7.61 (2H, d, J = 8.7 Hz), 4.04–3.99 (2H, m), 3.77–3.72 (2H, m), 3.56 (2H, t, J = 6.6 Hz), 1.95–1.90 (2H, m), 1.59–1.49 (2H, m), 0.84 (9H, s), 0.01 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 191.92, 149.87, 136.37, 129.79, 126.73, 110.40, 64.95, 63.14, 36.90, 27.21, 26.14, 18.49, −5.11; HR-FABMS Calcd for $C_{19}H_{31}O_4Si$ $(M+H)^+$ 351.1992. Found: 351.2014.

4.1.8. 2-(4-Carboxylic-phenyl)-2-(3-*tert*-butyl-dimethylsilyloxy)propyl-1,3-dioxolane (18). To a solution of **17** (3.1 g, 8.84 mmol) in CH_3CN (60 mL) was added a solution of NaH_2PO_4 (610 mg, 4.42 mmol) in water (12 mL) at 0 °C, followed by the addition of 30% H_2O_2 (1.64 mL, 15.92 mmol) and a solution of $NaClO_2$ (1.60 g, 17.68 mmol) in water (12 mL). The reaction mixture was stirred at 25 °C for 1 h (tlc showed the conversion to be complete). Na_2SO_3 (2.23 g, 17.68 mmol) was added to destroy the excess HOCl and H_2O_2. Five minutes later, 10% citric acid solution was added to adjust the pH to 2, followed by the addition of EtOAc (50 mL). The aq layer was extracted with EtOAc twice. The combined organic extracts were washed with cold water and brine. After being dried, filtered, and concentrated, the acid was obtained in 95% yield (3.08 g) as a white solid: mp 103–104 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.08 (2H, d, J = 8.7 Hz), 7.57 (2H, d, J = 8.7 Hz), 4.07–4.02 (2H, m), 3.79–3.75 (2H, m), 3.58 (2H, t, J = 6.6 Hz), 1.98–1.92 (2H, m), 1.61–1.51 (2H, m), 0.86 (9H, s), 0.01 (6H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 171.96, 148.95, 130.34, 129.01, 110.37, 64.86, 63.19, 36.81, 27.13, 26.14, 18.53, −5.10; HR-FABMS Calcd for $C_{19}H_{31}O_5Si$ $(M+H)^+$ 367.1941. Found: 367.1938.

4.1.9. 2-(4-Methoxycarbonyl-phenyl)-2-(3-*tert*-butyl-dimethylsilyloxy)propyl-1,3-dioxolane (19). To a solution of **18** (2.6 g, 7.1 mmol) in diethyl ether (30 mL) at −5 °C was added a freshly prepared ethereal solution of diazomethane in small portions until the gas evolution ceased, and the solution became yellow. After the mixture was stirred for another 10 min, excess diazomethane was destroyed by addition of acetic acid dropwise until the yellow color disappeared. The volatiles were evaporated, and the residue was purified by flash column chromatography on silica gel (hexanes/EtOAc 5:1) to afford 2.5 g (93%) of the product as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.01 (2H, d, J = 8.7 Hz), 7.52 (2H, d, J = 8.7 Hz), 4.07–3.99 (2H, m), 3.85 (3H, s), 3.79–3.73 (2H, m), 3.57 (2H, t, J = 6.6 Hz), 1.95–1.92 (2H, m), 1.58–1.52 (2H, m), 0.80 (9H, s), −0.05 (6H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 167.03, 147.93, 129.80, 129.62, 126.01, 110.31, 64.76, 63.11, 52.23, 36.79, 27.10, 26.08, 18.45, −5.16; HR-FABMS Calcd for $C_{20}H_{33}O_5Si$ $(M+H)^+$ 381.2097. Found: 381.2093.

4.1.10. 1,3-Dioxolane-2-(4-methoxycarbonyl-phenyl)-2-propanol (20). To a solution of **19** (700 mg, 1.84 mmol) in THF (10 mL) was added a 1.0 M TBAF solution in THF (5.5 mL, 5.5 mmol), the reaction mixture was stirred at 25 °C overnight. The volatiles were evaporated. The residue was dissolved in CH_2Cl_2 and washed with cold water and brine. After the resulting solution was dried, filtered, and evaporated, the residue was purified by flash chromatography on silica gel (hexanes/EtOAc 2:1 → 1:3) to provide 400 mg (82%) of the product as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.01 (2H, d, J = 8.4 Hz), 7.52 (2H, d, J = 8.4 Hz), 4.06–4.02 (2H, m), 3.91 (3H, s), 3.78–3.73 (2H, m), 3.62 (2H, t, J = 6.3 Hz), 2.02–1.97 (3H, m), 1.67–1.58 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 167.05, 147.82, 130.17, 129.75, 126.07, 110.32, 64.89, 62.97, 52.18, 37.07,

26.98; HR-FABMS Calcd for $C_{14}H_{17}O_5$ $(M-H)^+$ 265.1076. Found: 265.1072.

4.1.11. 1,3-Dioxolane-2-(4-methoxycarbonyl-phenyl)-2-propyl azide (21). To a solution of **20** (350 mg, 1.31 mmol) in CH_2Cl_2 (10 mL) were added pyridine (0.637 mL, 7.88 mmol) and methanesulfonyl chloride (0.4 mL, 5.25 mmol) at 0 °C. After stirring at 0 °C for 30 min, the reaction mixture was stirred at 25 °C for an additional 15 h. It was then diluted with CH_2Cl_2 (70 mL) and washed with cold water (10 mL) and brine (10 mL). After the resulting solution was dried, filtered, and concentrated, the residue was dissolved in DMF (10 mL), and NaN_3 (513 mg, 7.88 mmol) was added. The reaction mixture was stirred at 25 °C for 36 h, diluted with CH_2Cl_2, and washed with cold water twice. After the resulting solution was dried, filtered, and concentrated, the residue was purified by flash chromatography, eluting with hexanes/EtOAc (12:1 → 6:1 → 4:1) to afford 315 mg (82%) of the product as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.98 (2H, d, J = 8.0 Hz), 7.49 (2H, d, J = 8.0 Hz), 4.05–3.98 (2H, m), 3.88 (3H, s), 3.80–3.75 (2H, m), 3.25 (2H, t, J = 6.5 Hz), 1.94–1.91 (2H, m), 1.70–1.59 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 166.83, 147.46, 129.93, 129.64, 125.83, 109.74, 64.74, 52.17, 51.36, 37.22, 23.25; HR-FABMS Calcd for $C_{14}H_{18}N_3O_4$ $(M+H)^+$ 292.1297. Found: 292.1292.

4.1.12. 1,3-Dioxolane-2-(4-methoxycarbonyl-phenyl)-2-propyl amine (22). To a solution of **21** (80 mg, 0.274 mmol) in methanol (5 mL) were added 1,3-propanedithiol (138 μL, 1.373 mmol) and Et_3N (191 μL, 1.373 mmol) under an atmosphere of N_2. The reaction mixture was stirred at 25 °C for 19 h, and the volatiles were then evaporated. The residue was purified by flash chromatography, eluting with CH_2Cl_2/MeOH/Et_3N (100:8.3:4) to afford 67 mg (92%) of the product as a yellow oil. ^{1}H NMR (500 MHz, CD_3OD) δ: 7.99 (2H, d, J = 8.0 Hz), 7.56 (2H, d, J = 8.0 Hz), 4.05–3.99 (2H, m), 3.91 (3H, s), 3.81–3.75 (2H, m), 2.64 (2H, t, J = 7.5 Hz), 1.93 (2H, t, J = 8.0 Hz), 1.59–1.50 (2H, m); ^{13}C NMR (125 MHz, CD_3OD) δ: 168.31, 149.51, 131.03, 130.56, 127.23, 111.20, 65.91, 52.80, 42.51, 38.74, 27.66; HR-FABMS Calcd for $C_{14}H_{20}NO_4$ $(M+H)^+$ 266.1392. Found: 266.1375.

4.1.13. N^5-Acetyl-N^5-(benzyloxy)-N^2-acetyl-L-ornithyl-N^5-acetyl-N^5-(benzyloxy)-L-ornithyl-N^5-acetyl-N^5-(benzyloxy)-L-ornithyl-N-[1,3-dioxolane-2-(4-methoxycarbonyl-phenyl)-2-propyl]-amide (23). To a suspension of **11** (108 mg, 0.127 mmol) and **22** (37 mg, 0.141 mmol) in CH_3CN (5 mL) were added diisopropylethylamine (29 μL, 0.166 mmol), HOAt (19.2 mg, 0.141 mmol), and EDCI (27 mg, 0.141 mmol). The reaction mixture was stirred at 25 °C for 22 h. The volatiles were evaporated, and the residue was purified by flash column chromatography, eluting with EtOAc–CH_3OH (10:1) and then CH_2Cl_2/CH_3OH (100:6.5) to provide 124 mg (89%) of the product as a white amorphous solid: mp 83–84 °C, $[\alpha]_D^{20}$ −40.6° (c = 0.70, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.96 (2H, d, J = 8.0 Hz), 7.54 (1H, br s), 7.47 (2H, d, J = 8.0 Hz), 7.40–7.32 (15H, m), 7.21–7.18 (1H, m), 6.84–6.82 (1H, m), 6.74–6.72 (1H, m), 4.83–4.75 (6H, m), 4.50–4.39 (3H, br s), 4.02–3.90 (3H, m), 3.88 (3H, s), 3.87–3.42 (6H, m), 3.21–3.17 (2H, m), 2.09 (3H, s), 2.07 (3H, s), 2.02 (3H, s), 1.97 (3H, s), 1.90–1.48 (16H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 173.12, 172.93, 172.62, 172.34, 171.74, 171.46, 171.21, 166.96, 147.87, 134.35, 129.77, 129.67, 129.63, 129.57, 129.37, 129.35, 129.17, 129.15, 129.10, 128.88, 128.86, 125.93, 109.96, 76.43, 76.42, 76.37, 64.73, 53.33, 53.23, 52.41, 52.29, 52.19, 44.47, 44.21, 39.36, 37.52, 29.54, 29.44, 28.48, 23.74, 23.57, 23.23, 23.16, 20.59, 20.53; HR-FABMS Calcd for $C_{58}H_{76}N_7O_{14}$ $(M+H)^+$ 1094.5450. Found: 1094.5477.

4.1.14. N^5-Acetyl-N^5-(benzyloxy)-N^2-acetyl-L-ornithyl-N^5-acetyl-N^5-(benzyloxy)-L-ornithyl-N^5-acetyl-N^5-(benzyloxy)-L-ornithyl-N-[4-(4-methoxycarbonyl-phenyl)-4-oxobutyl]-amide (24). To a 10-mL flask containing **23** (80 mg, 0.073 mmol) was added HCO_2H (6.6 mL), and the reaction mixture was stirred at 25 °C for 3 h until the conversion was complete as indicated by tlc. The volatiles were evaporated, and the residue was purified by flash column chromatography, eluting with CH_2Cl_2/CH_3OH (100:6) to provide 70 mg (91%) of the product as a white solid: mp 59–60 °C, $[\alpha]_D^{20}$ −9.1° (c = 1.10, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.09 (2H, J = 9.0 Hz), 8.99 (2H, J = 9.0 Hz), 7.78 (1H, br s), 7.40–7.25 (15H, m), 6.97–6.90 (2H, m), 4.84–4.76 (6H, m), 4.48–4.39 (1H, m), 4.37–4.25 (2H, m), 3.94 (3H, s), 3.90–3.51 (6H, m), 3.40–3.26 (2H, m), 3.12–2.99 (2H, m), 2.60 (1H, br s), 2.11 (3H, s), 2.09 (3H, s), 2.04 (3H, s), 1.98 (3H, s), 1.97–1.53 (14H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 199.59, 173.48, 173.22, 173.07, 172.62, 171.87, 166.42, 140.28, 134.36, 134.21, 133.76, 129.87, 129.41, 129.38, 129.25, 129.15, 128.92, 128.87, 128.18, 76.45, 76.42, 54.25, 53.03, 52.55, 44.81, 44.13, 38.77, 36.20, 29.05, 28.83, 27.65, 24.08, 23.88, 23.81, 23.73, 23.13, 20.70, 20.60, 20.55; HR-FABMS Calcd for $C_{56}H_{72}N_7O_{13}$ $(M+H)^+$ 1050.5188. Found: 1050.5238.

4.1.15. N^5-Acetyl-N^5-(benzyloxy)-N^2-acetyl-L-ornithyl-N^5-acetyl-N^5-(benzyloxy)-L-ornithyl-N^5-acetyl-N^5-(benzyloxy)-L-ornithyl-N-[4-(4-carboxy-phenyl)-4-oxobutyl]-amide (25). To a solution of **24** (66 mg, 0.063 mmol) in THF–H_2O solution (1:1, 5 mL) was added LiOH·H_2O (6.6 mg, 0.157 mmol). The reaction mixture was stirred at 25 °C for 1 h and diluted with EtOAc (40 mL), followed by the addition of 1 N HCl (1 mL). The layers were separated, and the aq layer was extracted with EtOAc twice. The combined organic layers were washed with brine, dried, filtered, and concentrated to afford 58 mg (90%) of the product as a white solid: mp 77–78 °C, $[\alpha]_D^{20}$ −12.5° (c = 1.0, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.02 (2H, J = 8.0 Hz), 7.93 (2H, J = 8.0 Hz), 7.88 (1H, br s), 7.40–7.26 (15H, m), 7.23–7.05 (2H, m), 4.85–4.72 (6H, m), 4.57–4.38 (3H, m), 3.96–3.50 (6H, m), 3.40–3.31 (2H, m), 3.12–2.96 (2H, m), 2.10 (3H, s), 2.08 (3H, s), 2.06 (3H, s), 2.00 (3H, s), 1.97–1.58 (14H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 199.50, 173.54, 173.30, 172.16, 172.86, 172.32, 172.26, 171.96, 168.00, 140.17, 134.20, 134.05, 130.19, 129.42, 129.25, 129.20, 128.91, 128.11, 76.45, 53.93, 53.04, 44.68, 44.30, 44.21, 38.87, 36.09, 30.44, 29.01,

28.10, 23.90, 23.73, 23.11, 20.56; HR-FABMS Calcd for $C_{55}H_{70}N_7O_{13}$ $(M+H)^+$ 1036.5032. Found: 1036.4990.

4.1.16. Compound 27. To a solution of **26**[12] (60 mg, 0.0776 mmol) in CH_2Cl_2 (5 mL) was added TFA (0.4 mL). The resulting reaction mixture was stirred at 25 °C for 1 h, then diluted with CH_2Cl_2 (100 mL), and washed with saturated aq Na_2CO_3 (15 mL). The aq layer was extracted with CH_2Cl_2 twice. The combined extracts were washed with brine (10 mL). After the solution was dried ($MgSO_4$), filtered, and concentrated, the resulting amine was obtained as a colorless oil (30 mg).

To a suspension of the amine (30 mg, 0.0445 mmol) and acid **25** (40 mg, 0.0386 mmol) in CH_3CN (4 mL) were added diisopropylethylamine (10 μL, 0.0535 mmol), HOAt (7.5 mg, 0.0535 mmol), and EDCI (11 mg, 0.0535 mmol). The reaction mixture was stirred at 25 °C for 24 h. The volatiles were evaporated, and the residue was purified by flash column chromatography, eluting with CH_2Cl_2–MeOH (100:8) to provide 50 mg (77%) of the product as a white solid: mp 83–85 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.20 (1H, br s), 8.07 (1H, br s), 8.02 (2H, d, J = 8.5 Hz), 7.96 (2H, d, J = 8.5 Hz), 7.70 (1H, br s), 7.49 (2H, d, J = 8.0 Hz), 7.43–7.21 (30H, m), 7.08 (2H, dd, J = 8.0, 3.5 Hz), 6.91 (1H, br s), 5.07–4.78 (12H, m), 4.46–4.22 (5H, m), 4.16–4.00 (3H, m), 3.97–3.76 (3H, m), 3.64–3.40 (4H, m), 3.26–2.96 (3H, m), 2.88–2.74 (3H, m), 2.52–2.20 (5H, m), 2.11 (3H, s), 2.079 (3H, s), 2.075 (3H, s), 1.96 (3H, s), 1.95–1.22 (17H, m); ^{31}P NMR (121 MHz, $CDCl_3$) δ: 29.04; ESI-MS Calcd for $C_{92}H_{109}N_9O_{20}P$ $(M+H)^+$ 1690.75. Found: 1690.

4.1.17. Compound 6. To a solution of **27** (30 mg, 0.018 mmol) in methanol (5 mL) was added 10% Pd–C (10 mg), the flask was purged with argon, then the argon was replaced with H_2, and the reaction mixture was stirred under H_2 atmosphere for 2 h. After the mixture was filtered through filter paper, the solvent was evaporated from the filtrate to provide 20 mg (98%) of the product as a yellow solid: mp 57–59 °C, $[\alpha]_D^{20}$ −8.3° (c = −0.5, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ: 7.82 (2H, d, J = 7.0 Hz), 7.50 (2H, d, J = 6.0 Hz), 7.30 (2H, d, J = 7.0 Hz), 7.22 (2H, d, J = 6.0 Hz), 4.39–4.04 (6H, m), 4.73–3.50 (7H, m), 3.33–3.30 (2H, m), 3.25–3.17 (2H, s), 2.96–2.88 (2H, m), 2.72–2.62 (3H, m), 2.40–2.23 (2H, m), 2.092 (3H, s), 2.090 (3H, s), 2.08 (3H, s), 1.98 (3H, s), 1.96–1.25 (16H, m); ^{13}C NMR (125 MHz, CD_3OD) δ: 178.01, 176.64, 174.98, 174.20, 174.04, 173.99, 173.92, 173.83, 170.63, 169.96, 148.29, 138.19, 135.41, 132.66, 130.70, 129.86, 128.78, 121.53, 67.00, 55.22, 55.01, 54.83, 54.71, 52.27, 44.65, 40.85, 40.21, 37.63, 36.41, 35.08, 32.41, 31.03, 30.89, 30.65, 30.61, 30.37, 30.02, 29.92, 29.77, 29.64, 29.44, 28.73; ^{31}P NMR (121 MHz, $CDCl_3$) δ: 26.98; FAB-MS Calcd for $C_{50}H_{73}NaN_9O_{20}P$ $(M+H+Na)^+$ 1173.46. Found: 1174.

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References and notes

1. Carter, R. E.; Coyle, J. T. In *Handbook of Proteolytic Enzymes*; Barrett, A. J., Rawlings, N. D., Woessner, J. F., Eds.; Academic: New York, 1998; pp 1434–1437.
2. (a) Bruno, V.; Battaglia, G.; Copani, A.; Giffard, R. G.; Raciti, G.; Raffaele, R.; Shinozaki, H.; Nicoletti, F. *Eur. J. Pharmacol.* **1995**, *7*, 1906; (b) Choi, D. W.; Rothman, S. M. *Annu. Rev. Neurosci.* **1990**, *13*, 171.
3. Zhou, J.; Neale, J. H.; Pomper, M. G.; Kozikowski, A. P. *Nat. Rev. Drug Discov.* **2005**, *4*, 1015.
4. Jackson, P. F.; Cole, D. C.; Slusher, B. S.; Stetz, S. L.; Ross, L. E.; Donzanti, B. A.; Trainor, D. A. *J. Med. Chem.* **1996**, *39*, 619.
5. (a) Kozikowski, A. P.; Zhang, J.; Nan, F.; Petukhov, P. A.; Grajkowska, E.; Wroblewski, J. T.; Yamamoto, T.; Bzdega, T.; Wroblewska, B.; Neale, J. H. *J. Med. Chem.* **2004**, *47*, 1729–1738; (b) Kozikowski, A. P.; Nan, F.; Conti, P.; Zhang, J.; Ramadan, E.; Bzdega, T.; Wroblewska, B.; Neale, J. H.; Pshenichkin, S.; Wroblewski, J. T. *J. Med. Chem.* **2001**, *44*, 298.
6. Jackson, P. F.; Tays, K. L.; Maclin, K. M.; Ko, Y.-S.; Li, W.; Vitharana, D.; Tsukamoto, T.; Stoermer, D.; Lu, X.-C. M.; Wozniak, K.; Slusher, B. S. *J. Med. Chem.* **2001**, *44*, 4170.
7. (a) Vitharana, D.; France, J. E.; Scarpetti, D.; Bonneville, G. W.; Majer, P.; Tsukamoto, T. *Tetrahedron: Asymmetry* **2002**, *13*, 1609; (b) Tsukamoto, T.; Majer, P.; Vitharana, D.; Ni, C.; Hin, B.; Lu, X.-C. M.; Thomas, A. G.; Wozniak, K. M.; Calvin, D. C.; Wu, Y.; Slusher, B. S.; Scarpetti, D.; Bonneville, G. W. *J. Med. Chem.* **2005**, *48*, 2319.
8. (a) Carter, R. E.; Feldman, A. R.; Coyle, J. T. *Proc. Natl. Acad. Sci.* **1996**, *93*, 749; (b) Tiffany, C. W.; Lapidus, R. G.; Merion, A.; Calvin, D. C.; Slusher, B. S. *Prostate* **1999**, *39*, 28; (c) Asatiani, E.; Gelmann, E. P. *Expert Opin. Ther. Targets* **2005**, *9*, 283; (d) Elsasser-Beile, U.; Wolf, P.; Gierschner, D.; Buhler, P.; Schultze-Seemann, W.; Wetterauer, U. *Prostate* **2006**, *66*, 1359; (e) Farokhzad, O. C.; Cheng, J. J.; Teply, B. A.; Sherifi, I.; Jon, S.; Kantoff, P. W.; Richie, J. P.; Langer, R. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 6315; (f) Chu, T. C.; Shieh, F.; Lavery, L. A.; Levy, M.; Richards-Kortum, R.; Korgel, B. A.; Ellington, A. D. *Biosens. Bioelectron.* **2006**, *21*, 1859; (g) Misra, P.; Humblet, V.; Pannier, N.; Maison, W.; Frangioni, J. V. *J. Nucl. Med.* **2007**, *48*, 1379.
9. (a) Davis, M. I.; Bennett, M. J.; Thomas, L. M.; Bjorkman, P. J. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 5981; (b) Oliver, A. J.; Wiest, O.; Helquist, P.; Miller, M. J.; Tenniswood, M. *Bioorg. Med. Chem.* **2003**, *11*, 4455.
10. Tang, H.; Brown, M.; Ye, Y.; Huang, G.; Zhang, Y.; Wang, Y.; Zhai, H.; Chen, X.; Shen, T. Y.; Tenniswood, M. *Biochem. Biophys. Res. Commun.* **2003**, *307*, 8, Other

diagnostic methods, see:; (a) Foss, C. A.; Mease, R. C.; Fan, H.; Wang, Y.; Ravert, H. T.; Dannals, R. F.; Olszewski, R. T.; Heston, W. D.; Kozikowski, A. P.; Pomper, M. G. *Clin. Cancer Res.* **2005**, *11*, 4022; (b) Ohnishi, S.; Lomnes, S. J.; Laurence, R. G.; Gogbashian, A.; Mariani, G.; Frangioni, J. V. *Mol. Imaging* **2005**, *4*, 448.

11. (a) Wone, D. W. G.; Rowley, J. A.; Garofalo, A. W.; Berkman, C. E. *Bioorg. Med. Chem.* **2006**, *14*, 67; (b) Mallari, J. P.; Choy, C. J.; Hu, Y.; Martinez, A. R.; Hosaka, M.; Toriyabe, Y.; Maung, J.; Blecha, J. E.; Pavkovic, S. F.; Berkman, C. E. *Bioorg. Med. Chem.* **2004**, *12*, 6011; (c) Maung, J.; Mallari, J. P.; Girtsman, T. A.; Wu, L. Y.; Rowley, J. A.; Santiago, N. M.; Brunelle, A. N.; Berkman, C. E. *Bioorg. Med. Chem.* **2004**, *12*, 4969.
12. (a) Ding, P.; Helquist, P.; Miller, M. J. *Org. Biomol. Chem.* **2007**, *5*, 826; (b) Humblet, V.; Misra, P.; Frangioni, J. V. *Contrast Med. Mol. Imaging* **2006**, *1*, 196.
13. (a) Richardson, N.; Davies, J. A.; Raduchel, B. *Polyhedron* **1999**, *18*, 2457; (b) Roosenberg, J. M., II; Lin, Y. M.; Lu, Y.; Miller, M. J. *Curr. Med. Chem.* **2000**, *7*, 159; (c) Luo, M.; Fadeev, E. A.; Groves, J. T. *J. Am. Chem. Soc.* **2005**, *127*, 1726; (d) Chaubet, F.; van Duong, K. N.; Courtieu, J.; Gaudemer, A.; Gref, A.; Crumbliss, A. L.; Caudle, M. T. *Can. J. Chem.* **1994**, *72*, 2361.
14. Dhungana, S.; Heggemann, S.; Heinishc, L.; Möllmann, U.; Boukhalfa, H.; Crumbliss, A. L. *Inorg. Chem.* **2001**, *40*, 7079.
15. (a) Benz, G. *Liebigs Ann. Chem.* **1984**, 1424; (b) Benz, G.; Schmidt, D. *Liebigs Ann. Chem.* **1984**, 1434; (c) Lin, Y.-M.; Miller, M. J. *J. Org. Chem.* **1999**, *64*, 7451; (d) Lee, B. H.; Miller, M. J. *Tetrahedron Lett.* **1984**, *25*, 927.
16. (a) Dolence, E. K.; Minnick, A. A.; Miller, M. J. *J. Med. Chem.* **1990**, *33*, 461; (b) Dolence, E. K.; Lin, C.-E.; Miller, M. J. *J. Med. Chem.* **1991**, *34*, 956.
17. Sonpatki, V. M.; Herbert, M. R.; Sandvoss, L. M.; Seed, A. J. *J. Org. Chem.* **2001**, *66*, 7283.
18. Dalcanale, E.; Montanari, F. *J. Org. Chem.* **1986**, *51*, 567.
19. Holzapfel, C. W.; Pettit, G. R. *J. Org. Chem.* **1985**, *50*, 2323.
20. (a) Nagarajan, S.; Ganem, B. *J. Org. Chem.* **1987**, *52*, 5044; (b) Hendry, D.; Hough, L.; Richardson, A. C. *Tetrahedron Lett.* **1987**, *28*, 4601.
21. Lim, M.-I.; Marquez, V. E. *Tetrahedron Lett.* **1983**, *24*, 5559.
22. Sterzycki, R. *Synthesis* **1979**, 724.
23. Ding, P.; Ghosez, L. *Tetrahedron* **2002**, *58*, 1565.
24. Chen, C.; Layton, M. E.; Sheehan, S. M.; Shair, M. D. *J. Am. Chem. Soc.* **2000**, *122*, 7424.
25. Barinka, C.; Rinnova, M.; Sacha, P.; Rojas, C.; Majer, P.; Slusher, B. S.; Konvalinka, J. *J. Neurochem.* **2002**, *80*, 477.
26. Rojas, C.; Frazier, S. T.; Flanary, J.; Slusher, B. S. *Anal. Biochem.* **2002**, *310*, 50.