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A simple method for the oxidation of α-amino acid esters to α-oximino esters

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ABSTRACT

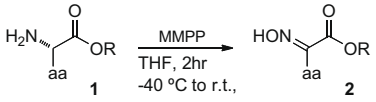
Magnesium bis(monoperoxyphthalate) hexahydrate (MMPP) was found to be an effective reagent for the oxidation of various α-amino acid esters to the corresponding α-oximino acid esters. This transformation could be completed under mild conditions within 2.5 h using 1.1 equiv of MMPP in THF. Clean oximino esters were obtained after quenching and extracting the reaction from sodium thiosulfate solution. The O-phosphorylated derivative of 2-oximinoglutarate exhibited slow binding inhibitory potency for the metallopeptidase prostate-specific membrane antigen (PSMA) with an IC_{50} value of 58 nM.

Our recent research efforts have been aimed at developing potent inhibitors for glutamate carboxypeptidases, specifically prostate-specific membrane antigen (PSMA). The most potent inhibitor for this enzyme that we have identified to date is a relatively simple molecule; *N*-phosphoryl glutamic acid which exhibited an IC_{50} value of 70 pM against PSMA.[1,2] While direct tetrahedral intermediate analogs of peptide hydrolysis serve as potent inhibitors of metalloproteaseas and metallopeptidases, we have begun to explore late transition state analogs as putative slow-tight binding inhibitors of the metallopeptidase PSMA. Toward that end, we have initiated the development of phosphorylated derivatives of α-oximino acids. In order to prepare this class of compounds, it requires the ready access of oxime analogs of various amino acids, that is, α-oximino acids. However, there is little precedent for the preparation of this class of compounds from amino acids. In this Letter, we describe the simple transformation of amino acid esters into the corresponding α-oximino acid esters in high yield and the subsequent synthesis and evaluation of a phosphorylated derivative of 2-oximinoglutarate as an inhibitor of PSMA.

Our initial studies were aimed at the preparation of the 2-oximinoglutarate diester (**2a**–**c**). There are few reports on the transformation of amino acids to α-oximino acids. These include the use of oxidants such as dimethyldioxirane, Na_2WO_4, and $MeReO_3/H_2O_2$, in addition to multi-step transformations in which tyrosine was the amino acid substrate.[3,4] A similar example demonstrated that tyrosine methyl ester could be oxidized to the corresponding α-oximino acid ester with Na_2WO_4 and 30% H_2O_2, which required column purification.[5] It has also been reported recently that primary amines could be effectively oxidized to oximes with DPPH and WO_3/Al_2O_3 but required long reaction times.[6] In general, these methods provide variable yields as a result of side reactions, in particular, the overoxidation and nitroso dimer formation.[5] In our initial attempts to oxidize **1a** with MCPBA, overoxidation to the nitro analog was indeed observed. Consequently, we turned our attention to MMPP, which can be used to carry out a wide variety of oxidation reactions.[7]

After optimizing the reaction conditions (solvent, temperature, reaction time, and concentration) for the transformation of **1a** to the corresponding α-oximino acid ester **2a**, we applied these conditions to various amino acid esters (Table 1).[8,9] In all cases, clean oximes were obtained after quenching the reaction with saturated $Na_2S_2O_3$ and extracting from $NaHCO_3$, without the need for chromatographic purification. It is noteworthy to mention that the oxime moiety was extremely sensitive to acidic conditions, leading to rapid hydrolysis.[10] While most amino acid esters listed

Table 1
Synthesis of α-oximino acid esters[8,9]



aa = amino acid side chain
R = Me, tBu, Bn

Amino acid esters **1**	α-Oximino acid esters **2** (% yield)
1a Glu(OBn)-OBn	89
1b Glu(OMe)-OMe	69
1c Glu(O*t*Bu)-O*t*Bu	77
1d Gly-OBn	71
1e Leu-OBn	78
1f Phe-OBn	79
1g Asn-O*t*Bu	27
1h Lys(Fmoc)-OMe	54
1i Ser-OBn	68
1j Trp-OMe	30

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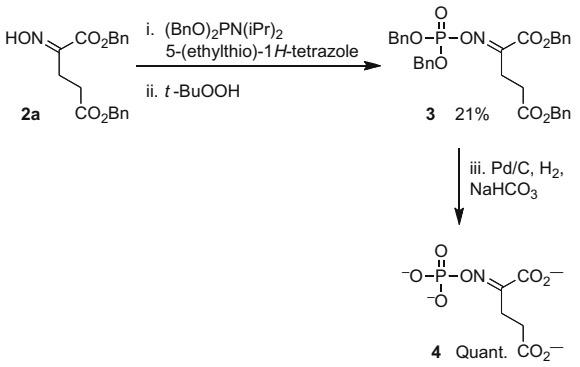

Scheme 1. Synthesis of phosphoryl oxime gluatamate.[12,13]

in Table 1 were neutralized by basic extraction prior to reaction with MMPP, we were unable to obtain **1g** in this form and consequently we observed considerable overoxidation to the corresponding α-nitro analog. The low yield of **2j** is presumably due to the competing oxidation of the indole nitrogen. Overall, the oxidation of most protected amino acids to their respective α-oximino acid ester analogs with MMPP gave good yields.

There are limited examples for the preparation of protected phosphoryl oximes.[11] We prepared the phosphorylated α-oximino acid as shown in Scheme 1.[12,13] As a late stage transition state analog peptitometic inhibitor of PSMA, phosphorylated α-oximino acid **4** was found to have an IC_{50} of 276 nM but when preincubated with the enzyme for 10 min, the IC_{50} was enhanced to 58 nM. These results suggest that the phosphorylated α-oximino acid **4** may exhibit the characteristics of slow-tight binding.[14,15] Furthermore, the *O*-phosphoryloxime motif may serve as an alternative zinc-binding group in the design of metalloprotease inhibitors.

Acknowledgments

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Supplementary data

Supplementary data (NMR data for compounds **2a**–**j**, **3**, and **4**) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.11.045.

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8. All solvents used in the reactions were both anhydrous and obtained as such from commercial sources. All other reagents were used as supplied unless otherwise stated. ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded on either a Varian Vx 300 or Inova 500 MHz instrument. ^{1}H NMR chemical shifts are relative to TMS (d = 0.00 ppm), $CDCl_3$ (d = 7.26 ppm), CD_3OD (d = 4.87 and 3.31 ppm), or D_2O (d = 4.87 ppm). ^{13}C NMR chemical shifts are relative to CD_3OD (d = 49.15 ppm) or $CDCl_3$ (d = 77.00 ppm). ^{31}P NMR chemical shifts in $CDCl_3$, CD_3OD, or D_2O were externally referenced to 85% H_3PO_4 (d = 0.00 ppm) in $CDCl_3$, CD_3OD, and D_2O, respectively. Maldi-High resolution mass spectrometry (Maldi-HRMS) was performed by the Laboratory for Bioanalysis and Biotechnology Center (LBB2) at Washington State University.
9. *General procedure for -oximino acid esters* **2**: Amino acid HCl salts were extracted in CH_2Cl_2 from saturated $NaHCO_3$, washed with brine, and the resulting organic layer was dried over $MgSO_4$, then concentrated in vacuo. MMPP (1.1 mmol, 1.1 equiv) was suspended in THF (3 mL), purged with Ar (g), and stirred at −40 °C. A neutralized amino acid ester (1 mmol) was dissolved in THF (2 mL) and was added via a syringe to the MMPP solution. The reaction was stirred for 20 min at −40 °C, the cooling bath was removed, and the reaction was stirred for an additional 2 h at room temperature. The reaction mixture was dissolved in ethyl acetate (15 mL), washed thrice with saturated sodium thiosulfate (15 mL each), washed with saturated sodium bicarbonate (15 mL), washed with brine (15 mL), and the organic layer was dried over Na_2SO_4 and concentrated in vacuo to either a white solid or colorless oil.
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12. *Synthesis of intermediate* **3**: In a 25 mL round-bottomed flask, acetonitrile (12 mL) was added to a mixture of 5-(ethylthio)-1*H*-tetrazole (216 mg, 1.658 mmol) and (*Z*)-dibenzyl 2-(hydroxyimino) pentanedioate (**2a**, 283 mg, 0.829 mmol) under an $Ar_{(g)}$ atmosphere. The solution was cooled to 0 °C and dibenzyl *N*,*N*-diisopropylphosphoramidite (409 μL, 1.243 mmol) was added slowly. The reaction was stirred for 1.5 h at room temperature, then filtered. To the resulting filtrate was added 5-(ethylthio)-1*H*-tetrazole (110 mg, 0.845 mmol) and *tert*-butyl hydroperoxide solution (70 wt % in H_2O, 1 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 10 min, the ice bath was removed, and the reaction was stirred for an additional 1 h at room temperature. The reaction mixture was concentrated in vacuo, extracted with ethyl acetate from saturated $NaHCO_3$, and washed with brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo to give a pale yellow oil. The product was purified by flash chromatography (2:8, acetone/hexane, v:v; R_f = 0.18) to give a white solid (21% yield, mp 48–49 °C).
13. *Synthesis of phosphoryl oxime* **4**: THF (1 mL) was added to a mixture of precursor of **3** (46 mg, 0.083 mmol), 10% Pd/C (5.5 mg), and $NaHCO_3$ (4.1 equiv, 28.4 mg, 0.338 mmol). The mixture was stirred vigorously, purged with argon and hydrogen. Then, distilled H_2O (0.4 mL) was added. The hydrogenolysis reaction was run for 2.5 h at rt. The reaction solvent was filtered through a 0.2 μm PTFE micropore filtration disk (Whatman), removed in vacuo and pumped overnight to yield white salts. Yield. Quantitative.
14. *PSMA inhibition assay and IC_{50} determination*: Inhibition studies were performed as described previously with only minor modifications. Working solutions of the substrate (*N*-[4-(phenylazo)benzoyl]-glutamyl-γ-glutamic acid, PAB-Glu-γ-Glu) and all inhibitors were prepared in Tris buffer (50 mM, pH 7.4). Working solutions of purified PSMA were appropriately diluted in Tris buffer (50 mM, pH 7.4) with 1% TritonX detergent to provide from 15% to 20% conversion of substrate to product in the absence of inhibitor. A typical incubation mixture (final volume 250 μL) was prepared by the addition of either 25 μL of an inhibitor solution or 25 μL Tris buffer (50 mM, pH 7.4) to 175 μL Tris buffer (50 mM, pH 7.4) in a test tube. A volume of the 25 μL PAB-Glu-γ-Glu (10 μM) was added to the above-mentioned solution. The enzymatic reaction was initiated by the addition of 25 μL of the PSMA working solution. In all cases, the final concentration of PABGlu-γ-Glu was 1 μM while the enzyme was incubated with five serially diluted inhibitor concentrations to provide a range of inhibition from 10% to 90%. The reaction was allowed to proceed for 15 min with constant shaking at 37 °C and was terminated by the addition of 25 μL methanolic TFA (2% trifluoroacetic acid by volume in methanol) followed by vortexing. The quenched incubation mixture was quickly buffered by the addition of 25 μL K_2HPO_4 (0.1 M), vortexed, and centrifuged (10 min at 7000g). An 85 μL aliquot of the resulting supernatant was subsequently quantified by HPLC as previously described. IC_{50} values were calculated using KaleidaGraph 3.6 (Synergy Software).
15. *PSMA preincubation inhibition studies*: This assay was conducted as described above for IC_{50} determination except that PSMA was preincubated with inhibitor **4** (60 nM) for 10 min with constant shaking at 37 °C prior to the addition of PABGlu-γ-Glu.