Supporting Information

Serendipitous Discovery of α-Hydroxyalkyl Esters as β-Lactamase Substrates

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Synthetic details for the preparations of the α-hydroxyalkyl esters 6 and 9 - 20.

N-(Benzyloxycarbonyl)-O-(2-D-hydroxypropanoyl)hydroxylamine (6) Anhydrous D-lactic acid was protected, activated and coupled to benzyl N-hydroxycarbamate by the procedure reported for **7** (main text). After deprotection of the hydroxyl group, the synthesis produced flat colorless crystals in 25% overall yield (mp 70-72 °C). ^{1}H NMR: ($CDCl_3$): δ 8.11 (s, 1H, NH), 7.37 (m, 5H, Ar), 5.23 (s, 2H, CH_2), 4.50 (q, 1H, J = 6.6 Hz), 1.51 (d, 3H, J = 6.6 Hz). IR (cm^{-1}) 1731, 1790. ES(-)MS m/z: 238.13.

N-(Benzyloxycarbonyl)-O-(3-phenylpropanoyl)hydroxylamine (9) Benzyl N-hydroxycarbamate (99 mg, 0.59 mmol) was dissolved in ethyl acetate (2 ml) and stirred at 0 °C. Hydrocinnamoyl chloride (88 μl, 0.59 mmol) in methylene chloride (1 ml) was added dropwise, followed by pyridine (45 μl, 0.59 mmol). The reaction mixture was stirred for thirty minutes, filtered, and the solvent removed to yield a clear oil. The crude product was purified by preparative chromatography on silica gel with hexanes/ethyl acetate (2:1) as eluent. The resulting colorless solid was recrystallized from benzene/cyclohexane to give 162 mg (31%) colorless needle-like crystals (mp 37-38 °C). ^{1}H NMR (CD$_3$CN): δ̣8̃83 (s, 1H, NH), 7̃37 (m, 5H), 7̃27 (m, 5H), 5.15 (s, 2H, CH$_2$), 2.95 (t, 2H, J = 7.5 Hz), 2.75 (t, 2H, J = 7.5 Hz). ^{13}C NMR; δ̣173.13, 157.89, 141.51, 137.32, 129.89 (m), 68.84, 34.08, 31.43. IR (KBr, cm^{-1}) 1788, 1747. ES(-)MS m/z: 298.28.

N-(Benzyloxycarbonyl)-O-(2-D-methoxypropanoyl)hydroxylamine (10) 2-D-Methoxypropionic acid was reacted with one equivalent of TBDMSCl, activated, and coupled to benzyl N-hydroxycarbamate by the procedure employed for **7**. The synthesis yielded a colorless oil in 14% overall yield which failed to crystallize. ^{1}H NMR (CDCl$_3$): δ 8.05 (s, 1H, NH), 7.37 (s, 5H), 5̃22 (s, 2H, CH$_2$), 4.07 (q, 1H, J = 6.9 Hz), 3̃41 (s, 3H, CH$_3$), 1.47 (d, 3H, J = 6.6 Hz). IR (neat, cm^{-1}) 1796, 1755. ES(-)MS m/z: 251.93 m/z, ES(+)MS m/z: 271.0 (M + Na$^+$)

D-Phenylalanine 4-nitrophenyl ester (11) Boc-D-phenylalanine 4-nitrophenyl ester (100 mg, 0.26 mmol) was dissolved in methylene chloride (3 ml) and trifluoroacetic acid (6 ml) was added. The solution was stirred for one hour, following which the solvent was evaporated to quantitatively yield the amine trifluoroacetate as a colorless solid (mp 182-185 °C). ^{1}H NMR (CD$_3$CN): δ̣8̃31 (d, 2H, J = 8.9 Hz), 7.38 (s, 5H), 7̃32̣(d, 2H, J = 8.9 Hz), 4.61 (t, 1H, J = 8.3 Hz), 3̃42 (d, 2H, J = 8.5 Hz). IR (neat, cm^{-1}) 1737. ES(+)MS m/z: 287.07.

N-(Benzyloxycarbonyl)-O-(2-D-hydroxy-2-phenylacetyl)hydroxylamine (12) D-Mandelic acid was protected, activated and coupled to benzyl N-hydroxy carbamate by the procedure reported for **7**. After deprotection of the hydroxyl group, as described, the synthesis produced colorless crystals in 14% yield (mp 68-69 °C). ^{1}H NMR (CD$_3$CN): δ

8.902 (s, 1H, NH), 7.40 (m, 10H, Ar), 537 (d, 1H, J = 6 Hz), 5.14 (s, 2H, CH_2), 4.23 (d, 1H, J = 5.4 Hz). IR (neat, cm^{-1}) 1792, 1734. ES(-)MS m/z: 300.07.

N-(Benzyloxycarbonyl)-O-(2-D-hydroxy-4-phenylbutanoyl)hydroxylamine (13) 4-Phenyl 2-D-hydroxybutanoic acid was protected, activated and coupled to benzyl N-hydroxycarbamate by the procedure reported for **7**. After deprotection of the hydroxyl, the synthesis yielded colorless crystals in 9 % overall yield (mp 42-44 °C). ^1H NMR (d_6-DMSO): δ11.14 (s, 1H, NH), 7.35 (s, 5H), 731 (m, 1H), 7.27 (d, 2H, J = 7.2 Hz), 7.19 (t, 2H, J = 7.5 Hz), 5.86 (d, 1H, J = 6.3 Hz), 5.14 (s, 2H, CH_2), 4.17 (q, 1H, CH), 2.68 (t, 2H, J = 7.8 Hz), 1.90 (m, 2H, CH_2). IR (cm^{-1}) 1787, 1732. ES(-)MS m/z: 328.20.

N-(Methyl)-N-(benzyloxycarbonyl)-O-(2-D-hydroxy-3-phenylpropanoyl)hydroxylamine (14) N-Benzyloxycarbonyl N-methyl hydroxylamine was synthesized from benzyl chloroformate and N-methyl hydroxylamine by the method of Defoin (S1). D-3-Phenyllactic acid was protected, activated and condensed with the N-methyl N-hydroxycarbamate by the procedure reported for **7**. After deprotection of the hydroxyl, the synthesis yielded a colorless oil in 34 % yield. It is noteworthy that this compound was unstable at -20 °C and required chromatographic purification immediately prior to kinetic analysis. ^1H NMR ($CDCl_3$): δ7.34 (m, 5H),7.26 (m, 5H), 519 (s, 1H, CH_2), 4.57 (s, 1H, OH),4.12 (m, 1H), 3.26 (s, 3H, CH_3), 3.14 (m, 1H),2.95 (m, 1H). IR (neat, cm^{-1}) 1790, 1715. ES(+)MS m/z: 352.13 ($M + Na^+$).

N-(Benzyloxycarbonyl)-O-[2-D-hydroxy-3-(4-biphenyl)propanoyl]hydroxylamine (15) D-3-(4-biphenyl)lactic acid was prepared from the corresponding unnatural amino acid D-4-biphenylalanine by a diazotization reaction (S2). D-4-biphenylalanine (780 mg, 3.11 mmol) was suspended in acetic acid (15 ml) and water (3 ml) with the aid of sonication. $NaNO_2$ (889 mg, 13 mmol), dissolved in water (4 ml), was added dropwise over six hours, while vigorous stirring was maintained. The acetic acid was removed by rotary evaporation, and the residue taken into ethyl acetate (100 ml) and washed with water (50 ml), 0.1 M HCl (200 ml) and brine (100 ml). The organic layer was dried over $NaSO_4$ and the solvent removed yielding 674 mg (89 %) of D-4-biphenyl lactic acid. The biphenyl lactic acid was protected, activated and coupled to benzyl N-hydroxycarbamate by the procedure reported for **7**. After deprotection of the hydroxyl group, and following recrystallization, the synthesis yielded colorless crystals, in 24% yield (mp 141-142 ^{o}C). ^{1}H NMR (DMSO): δ 11.22 (s, 1H, NH), 7.63 (d, 2H, J = 7.2 Hz), 7.56 (d, 2H, J = 7.2 Hz), 7.45 (t, 2H, J = 7.05 Hz), 7.37 (m, 8H, Ar), 5̃95 (d, 1H, J = 7.2 Hz), 5.16 (s, 1H, CH_2), 4.43 (m, 1H, CH), 2.96 (m, 2H, CH_2). IR (cm^{-1}) 1804, 1723. ES(-)MS m/z: 391.0.

N-(methoxycarbonyl)-O-(2-D-hydroxy-3-phenylpropanoyl)hydroxylamine (16)

Methyl N-hydroxycarbamate was prepared by the method of Defoin (S1). It was then condensed with TBDMS D-3-phenyllactic acid as described for compound **7**. After deprotection of the hydroxyl group the synthesis resulted in a colorless oil in 30% yield. ^{1}NMR ($CDCl_3$): δ 7.35 (s, 5H), 4.13 (dd, 1H, J = 6.6 Hz), 3.78 (s, 3H),3.60 (m, 2H). IR (cm^{-1}) 1790, 1739. ES(-)MS m/z: 238.33.

1-D-Carboxy-2-phenylethyl 2'-D-hydroxy-3'-phenylpropionate (17) *tert*-Butyl 2-D-hydroxy-3-phenylpropionate was prepared as described for compound **2** and condensed with 2-D-TBDMSO-3-phenylpropionic acid as described for compound **7**, yielding, after deprotection, a colorless oil in 14% yield. ^{1}H NMR (CD_3CN) δ̣7̃39̃7̃23̣(m, 10H), 5̃25̣(dd, 1H, J = 3.6 Hz), 4̃35̣(dd, 1H, J = 3.9 Hz), 3̃29̃2̃8̣(m, 4H). IR (neat, cm^{-1}) 1735, 1638. ES(-)MS m/z: 313.07.

Phenyl 2-D-hydroxy-3-phenylpropionate (18) D-3-Phenyllactic acid was hydroxyl protected, activated and coupled to phenol by the procedure reported for **7**. After deprotection of the hydroxyl, the synthesis yielded 28% of colorless needle-like crystals [mp 82-83 °C, lit. 81 °C (S3)]. ^{1}NMR ($CDCl_3$): δ̣7̃42̃7̃27 (m, 8H), 7̃5̣(d, 2H, J = 7.8 Hz), 4̃65̣(t, 1H), 3̃6̣̃(d, 1H). 3̃18̣(m, 2H). IR (cm^{-1}): 1748.

3-Nitrophenyl 2-D-hydroxy-3-phenylpropionate (19) D-3-Phenyllactic acid was protected, activated and coupled to *m*-nitrophenol by the procedure reported for **7**. The final recrystallization of the product yielded fine colorless crystals in 38% yield (mp 90-92 °C). ^{1}NMR (CD_3CN): δ 8.13 (d, 1H, J = 6 Hz), 7.87 (s, 1H, Ar), 7̃65 (t, 1H, J = 8.1 Hz), 7.45 (d, 1H, J = 8.7 Hz), 7.34 (m, 5H, Ar)̣,4̃7̃ (q, 1H, J = 7.2 Hz), 3.71 (d, 1H, J = 6 Hz), 3.19 (m, 2H, CH_2). IR (cm^{-1}) 1767. ES(-)MS m/z: 286.07.

3-Carboxyphenyl 2-D-hydroxy-3-phenylpropionate (20) The carboxyl group of 3-hydroxybenzoic acid was protected as the *p*-methoxybenzyl ester (S4). Thus, 3-hydroxybenzoic acid (2.5 g, 18.0 mmol) was dissolved in water (12 ml) and stirred, as $KHCO_3$ (1.8 g, 18.0 mmol) was slowly added. Stirring was maintained until the solution was homogenous, then the water was removed by rotary evaporation and the residue dried overnight with an oil pump. The resulting potassium salt of 3-hydroxybenzoic acid was then dissolved in anhydrous DMF (50 ml), followed by the addition of 4-methoxybenzyl chloride (2.8 g, 18.0 mmol) and sodium iodide (50 mg, 0.3 mmol). The mixture was stirred for 24 hours after which it was dried by rotary evaporation. The crude residue was dissolved in methylene chloride and washed three times with saturated $NaHCO_3$, followed by a final wash with water. The organic layer was dried over $MgSO_4$ and evaporated yielding a pale yellow solid (89%). D-3-Phenyllactic acid was protected, activated and coupled to the *p*-methoxybenzyl ester by the procedure reported for **7,** in 60% yield. Following removal of the TBDMS protecting group, the *p*-methoxybenzyl ester (71 mg, 0.175 mM) was dissolved in a mixture of methylene chloride (3 ml) and trifluoroacetic acid (3 ml). Anisole (19 μl) was then added and the mixture stirred for one hour. The solvent was evaporated, the residue taken into benzene and the insoluble fraction collected by filtration. The filtered solid was recrystallized from diisopropyl ether/hexanes (1:1), yielding 43 mg (86%) of fine colorless crystals (mp 130-132 °C). ^{1}H NMR: (CDCN): δ̣7.90 (d, 1H, J = 7.2 Hz), 7.63 (s, 1H), 7̃52 (t, 1H, J = 7.95 Hz), 7.34 (m, 5H), 7.28 (d, 1H, J = 7.5 Hz), 4.67 (t, 1H, J = 5.1 Hz), 3.18 (m, 2H). IR (cm^{-1}) 1760, 1698. ES(-)MS m/z: 285.20.

References

S1. Defoin, A., Pires, J., and Streith, J. (1991) From 1-(silyloxy)butadiene to 4-amino-4-deoxy-dl-erythrose and to 1-amino-1-deoxy-dl-erythritol derivatives. Hetero-Diels-Alder reactions with acylnitroso dienophiles. *Helv. Chim. Acta 74*, 1653-1670.

S2. Govardhan, C. P. (1986), Depsipeptides as β-Lactamase Substrates. *Ph.D. Thesis,* Wesleyan University, Middletown, CT.

S3. Corey, E.J. and Zhang, F.-Y. (1999) Mechanism and conditions for highly enantioselective epoxidation of α,β-enones using charge-accelerated catalysis by a rigid quaternary ammonium salt. *Chem. Lett. 1*, 1287-1290.

S4. Wyrembak, P. N. (2003) N,O-Diacyl Hydroxylamines as Novel β-Lactamase Inhibitors. *Honors Thesis,* Wesleyan University, Middletown, CT.

Table S1. Wavelengths and absorption coefficients used for spectrophotometric kinetics.

	λ (nm)	$\Delta\varepsilon$ (M^{-1} cm^{-1})
6	230	924
7, 8	233	887
9	255	*ND*
10	250	418
11	410	7830
12	245	551
13	240	700
14	233	166
15	240	*ND*
16	230	1038
17	230	*ND*
18	270	1157
19	335	1226
20	290	1834

Figure S1. Initial rates of reaction of **7** (700 μM) in the presence of the P99 β-lactamase (0.1 μM) and methanol.

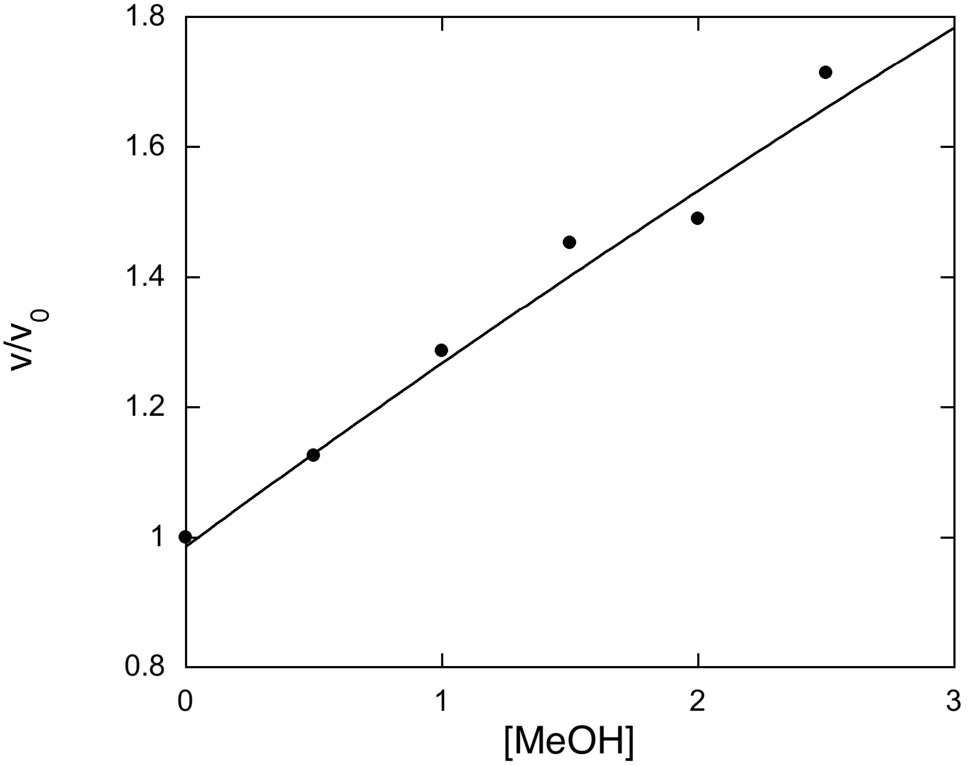

Figure S2. Stereoview of the energy-minimized tetrahedral intermediate structure formed on reaction of **7** with the P99 β-lactamase: the S-adduct (**22**). Only heavy atoms are shown.