Supporting Information

Kinetics of Action of a Two-Stage Pro-Inhibitor of Serine β-Lactamases

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*Synthesis of **8**, **9** and **41***

N-(Benzyloxycarbonyl)-N-(benzoyl)hydroxylamine (**8**) N-(Benzyloxycarbonyl)-O-(t-butoxycarbonyl)hydroxylamine was synthesized (Scheme1) by the procedure of Guimond at el.[19] Thus, Boc_2O (0.75 mL, 3.29 mmol) was added to a suspension of benzyl N-hydroxycarbamate (500 mg, 3 mmol) in DCM (20 mL). Sodium t-butoxide (15 mg, 0.14 mmol) was added and the reaction mixture was stirred for 15 minutes at room temperature. More DCM (12 mL) was added and the reaction mixture was washed twice with sat. aqueous $NaHCO_3$, after which the organic phase was dried over Na_2SO_4, filtered and the solvent removed by evaporation under reduced pressure, yielding N-(benzyloxycarbonyl)-O-(t-butoxycarbonyl)hydroxylamine as a colorless liquid. N-(Benzyloxycarbonyl)-O-(t-butoxycarbonyl)hydroxylamine, (0.8 g, 3.0 mmol), from above**,** dissolved in DCM (15 mL) at room temperature, was treated with triethylamine (0.46 mL, 3.29 mmol) followed by benzoyl chloride (0.50 mL, 3.29 mmol). The reaction mixture was stirred overnight at room temperature. More DCM (12 mL) was added and the reaction mixture was washed twice with water, after which the organic phase was dried over Na_2SO_4, filtered and the solvent removed by evaporation under reduced

pressure. The resulting oil was purified by silica gel column chromatography using ether:hexane (20:80) as eluting solvent to afford N-(benzyloxycarbonyl-N-(benzoyl)-O-(t-butoxycarbonyl)hydroxylamine in 64% yield as a colorless solid. ^{1}H NMR (D_6-DMSO) δ 1.42 (s, 9H), 5.21 (s, 2H), 7.2-7.7 (m, 10H). FTIR (KBr, cm^{-1}) 1791, 1758, 1711. ES(+)MS 371.00 ($M + H^+$).

The product from immediately above (0.5 g, 1 mmol) was dissolved in DCM (20 mL) and the solution ice-cooled to 0 ºC and stirred under an argon atmosphere. Trifluoroacetic acid (0.2 mL, 2 mmol) was added dropwise to the solution and the reaction mixture stirred overnight at room temperature. The volatiles were removed under reduced pressure and the residue dried under an oil pump vacuum. The crude product was purified by preparative thin layer chromatography on silica gel at 4 ºC with ether:hexane (50:50) as the eluting solvent, to afford **8** in 16% yield as a colorless solid. MP 76-78 ˚C. ^{1}H NMR (D_6-DMSO) δ 5.18 (s, 2H), 7.27-7.6 (m, 10H), 10.63 (s, 1H). FTIR (KBr, cm^{-1}) 1742, 1685. HRMS (ES+) 294.0739 ($M + Na^+$), calcd for $C_{15}H_{13}NO_4Na$ 294.0742.

N-(t-Butoxycarbonyl)-O-(benzoyl)hydroxylamine (**9**) Triethylamine (0.63 mL, 4.51 mmol) in DCM (2 mL) was added to a stirred solution of t-butyl N-hydroxycarbamate (0.55 mL, 3.75 mmol) in DCM (10 mL) at room temperature and the reaction mixture stirred for 10 minutes. Benzoyl chloride (0.55 mL, 4.51 mmol) was then added dropwise and the subsequent mixture stirred for further 2 hours at room temperature. The organic phase was washed twice with 0.1M HCl, dried over Na_2SO_4, and the solvent removed by evaporation under reduced pressure. The residue was dried under an oil pump vacuum and the resulting crude product recrystallized from a cyclohexane:benzene (1:1) mixture

to afford compound **9** in 50% yield as a colorless solid. MP 82-84 ˚C. ^{1}H NMR (D_6-DMSO) δ 1.41 (s, 9H), 7.53 (t, J = 7.3, 2H), 7.68 (t, J = 6.6, 1H), 7.97 (d, J = 7.8, 2H), 10.87 (s, 1H). FTIR (KBr, cm^{-1}) 1765, 1705. ES(-)MS 235.93 ($M - H^+$)

4-Methoxyphthalic anhydride (**41**)[S1] Acetic anhydride (0.4 mL) was added to a solution of 4-methoxyphthalic acid (0.3 g, 16 mmol) in anhydrous tetrahydrofuran (1.5 mL) and the mixture was heated under reflux for 4 hours. Upon cooling to room temperature, the solvent was removed under reduced pressure to afford 4-methoxyphthalic anhydride in 99% yield as an off-white solid. MP 88-90 ˚C. ^{1}H NMR (D_6-DMSO) 3.97 (s, 3H), 7.49 (dd, J = 4.8 Hz, 1H), 7.59 (m, 1H), 8.02 (d, J = 6.0 Hz, 1H). ES(+)MS 179.00 ($M + H^+$).

Reference

S1. Gallagher, N.J., Lyons, J.F., Thomson, N.T., Yule, S.M. and Murray, C.W. (2010) Pharmaceutical combinations. *US patent* 0092474A1.

Figure S1

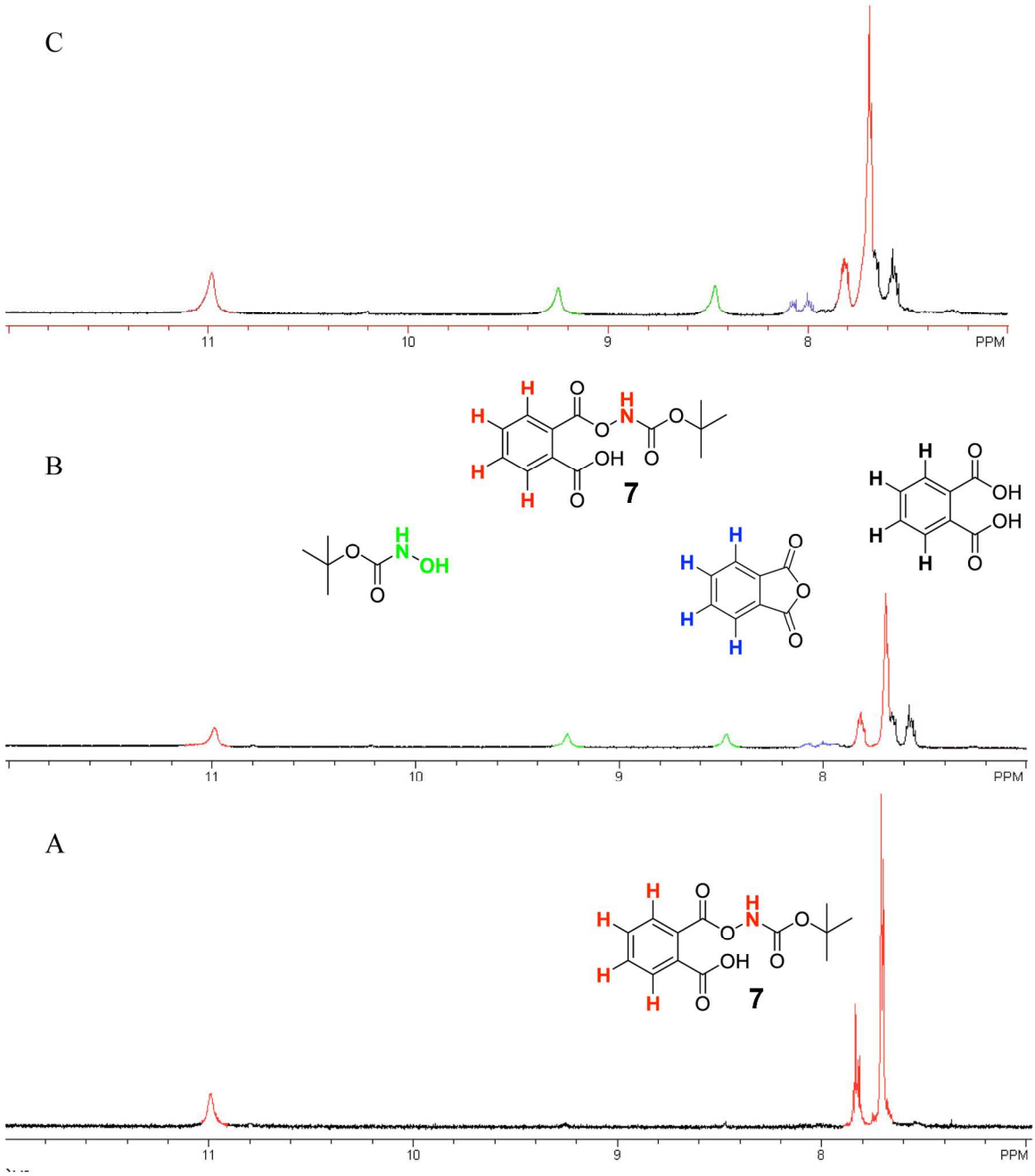

Figure S1. A. ^{1}H NMR spectrum of **7**. B. NMR spectrum showing the products of reaction of **7** in aqueous solution at pH 7.5 after 7 min. C. NMR spectrum showing the products arising from an equimolar mixture of **10** and **11** after 7 min. All spectra were taken in D_6-DMSO.

Figure S2

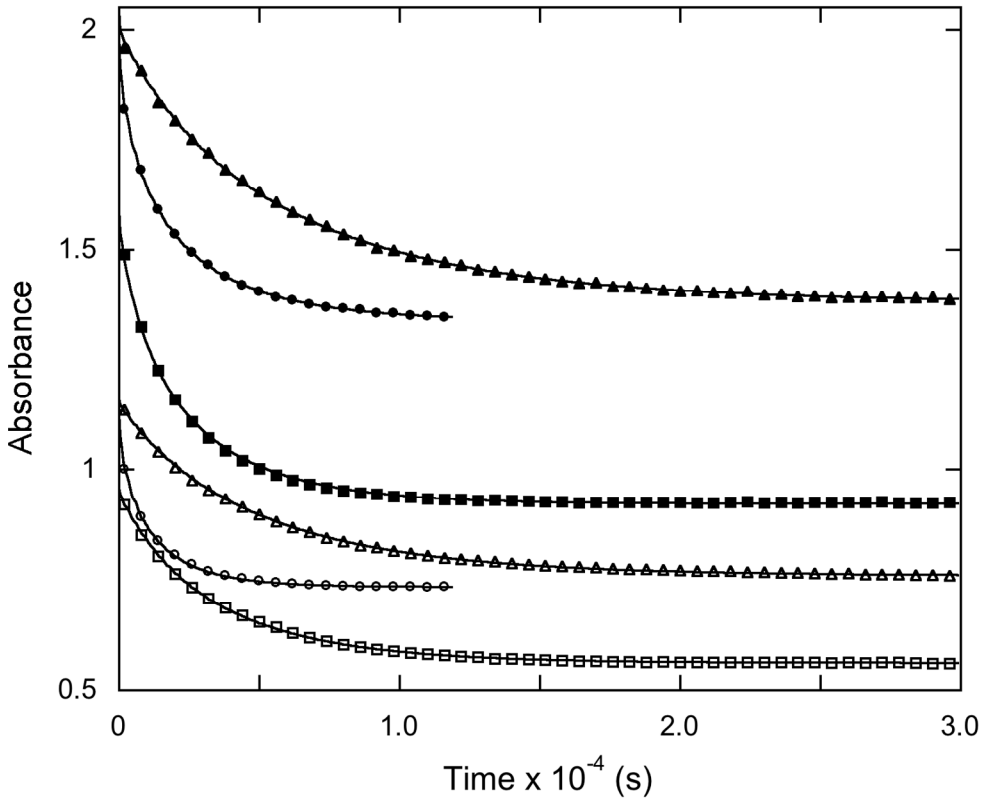

Figure S2. Absorption changes at 260 nm on spontaneous reaction of **7** (0.20 mM) (○), **7** (0.40 mM) (●), a mixture of **7** (0.20 mM) and **11** (0.50 mM)(△), a mixture of **7** (0.40 mM) and **11** (0.50 mM)(▲), a mixture of **10** (0.20 mM) and **11** (0.50 mM)(□), and a mixture of **10** (0.40 mM) and **11** (0.50 mM)(■), in aqueous solution at pH 7.5. The curves were fitted simultaneously to Scheme 2.

Figure S3

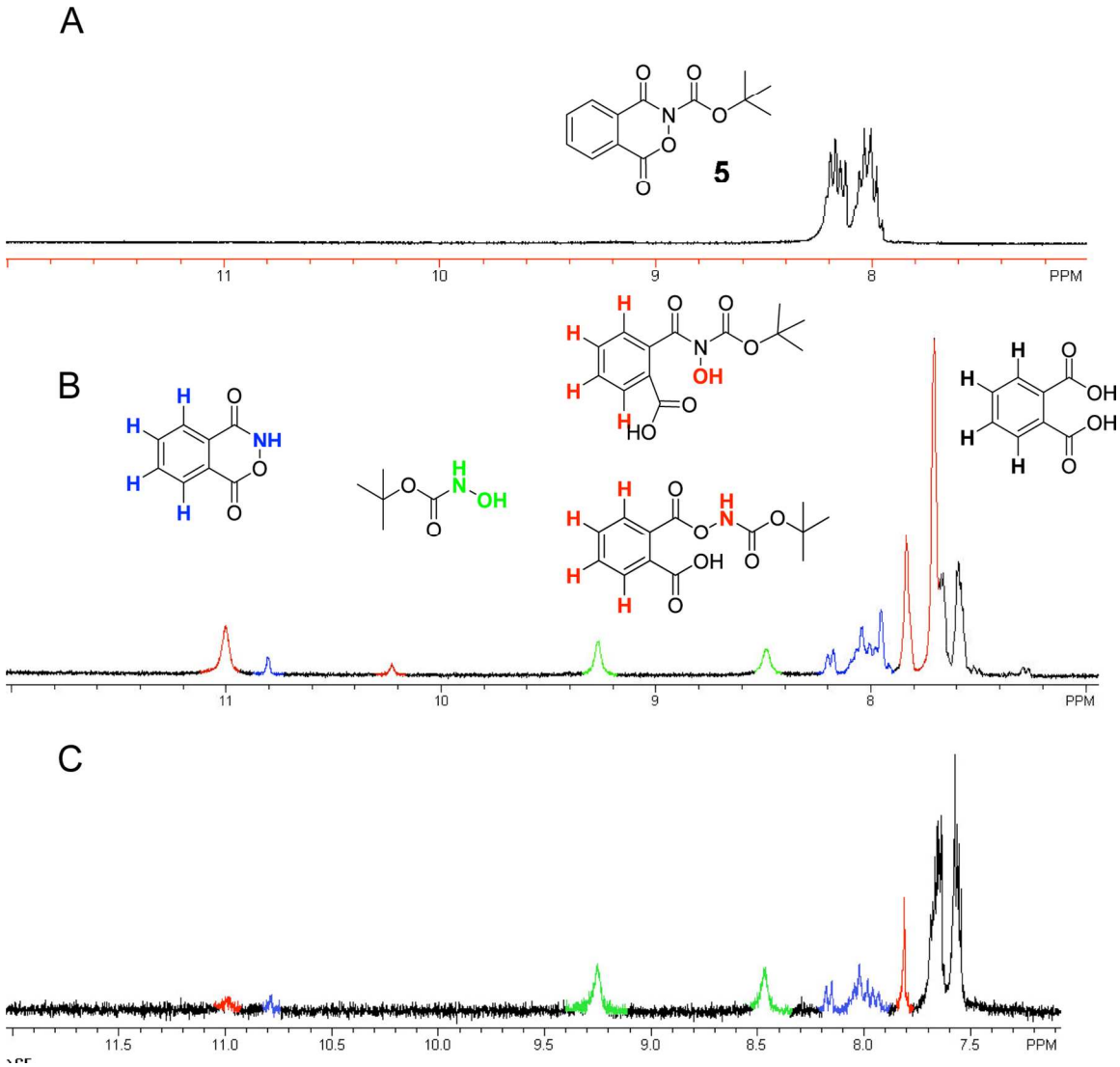

Figure S3. A. ^{1}H NMR spectrum of **5**. B. NMR spectrum showing spontaneous hydrolysis products of **5** after 3 min of reaction. C. after 90 min of reaction. All spectra were taken in D_6-DMSO.

Figure S4

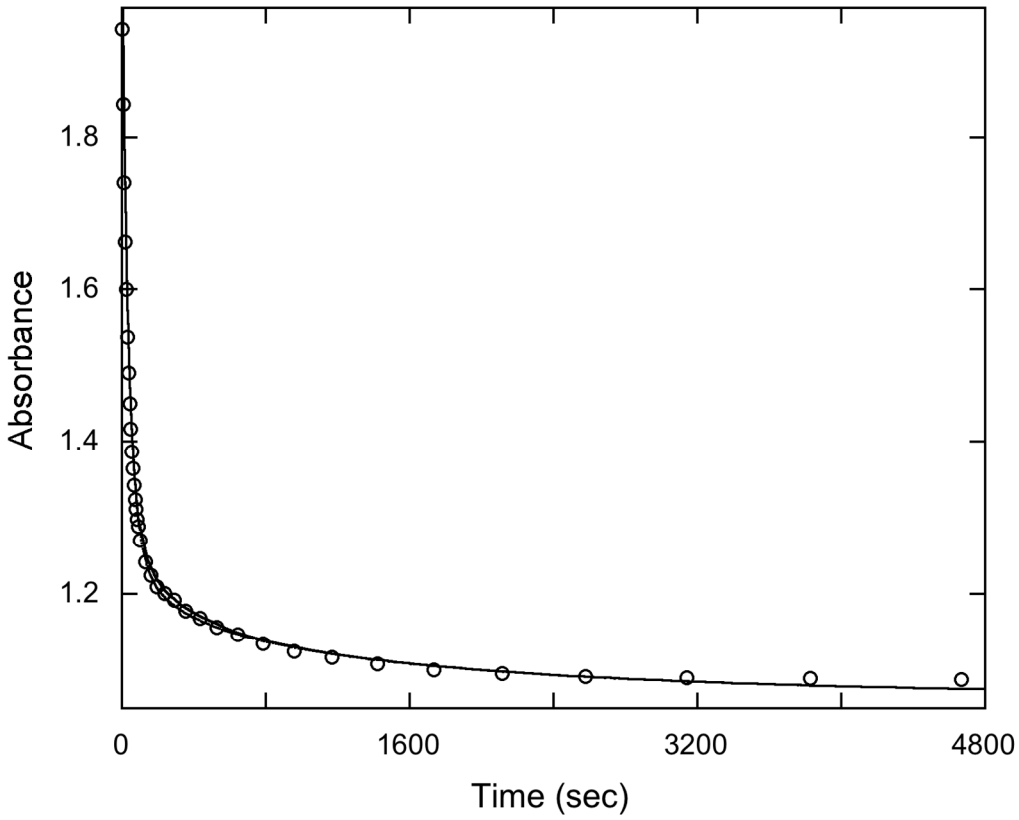

Figure S4. Spontaneous reaction of **5** (0.40 mM) in aqueous solution at pH 7.5, monitored at 260 nm. The data was fitted (solid line) to Scheme 3.

Figure S5

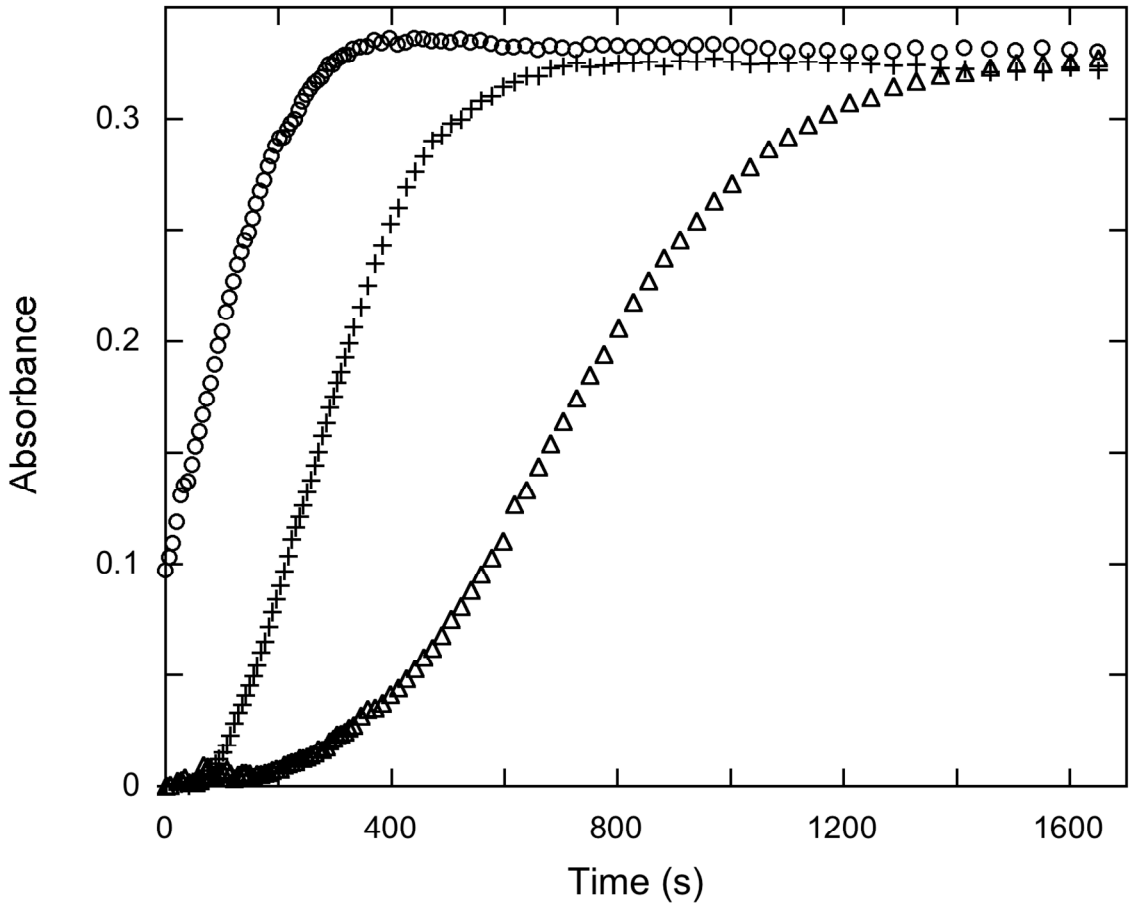

Figure S5. Relative activity (proportional to $t_{1/2}$, the time to half consumption of substrate) of **10** (+), **39** (△), and **42** (○) (10 μM each), added the substrate CENTA (50 μM) and the P99 ß-lactamase (1.0 nM).