The Inactivation of Bacterial DD-Peptidase by β-Sultams

Supporting Information

Synthesis

N-Benzoyl-D-alanine. D-Alanine (5 g, 0.056 mol) was dissolved in water (20 ml) containing sodium hydroxide (2.25 g, 0.056 mol) and the solution cooled in an ice bath. Benzoyl chloride (8.7 g, 7.18 ml, 0.062 mol) and a solution of sodium hydroxide (2.48 g, 0.062 mol) in water (10 ml) were added to the stirred, cooled amino acid solution, alternately and in portions during 2 hours; which was then stirred for further 2 hours. The reaction mixture was boiled for 20 min, then cooled to 0 ºC and acidified carefully to Congo red with concentrated hydrochloric acid. The product was filtered, washed with 25 ml of ice-cold water and then recrystallised from about 150 ml of boiling water (yield 90%). *HPLC*: Retention Time: 4 min 12 sec; *IR* (nujol, ν, cm^{-1}): 3369, 1724, 1628; *^{1}H-NMR* (δ, ppm, d^6-DMSO): 1.43 (CH_3, d, J = 7.35 Hz), 4.48 (CH, m, J_1 = 7.35 Hz, J_2 = 7.23 Hz), 7.45-7.99 (Ph, m), 8.66 (NH, d, J = 7.23 Hz); *^{13}C-NMR* (δ, ppm, d^6-DMSO): 17.0 (CH_3), 48.3 (CH), 127.5, 128.3, 128.6, 129.3, 131.4, 134.1, 166.3 (COam), 174.3 (COac).

Mercaptoacetic acid disulfide. Sodium hydroxide (8 g, 0.2 mol) and potassium iodide (0.5 g, 3 mmol) were dissolved in water (50 ml) and cooled in ice-water. Mercaptoacetic acid (20 g, 0.217 mol) was added, then iodine (25 g, 0.1 mol) in portions for 30 min, until the red colour persisted. The solution was acidified to pH 3 with concentrated hydrochloride acid and extracted with ether. The solvent was removed to give, after cooling, a white solid product (yield 94%). *HPLC*: Retention

Time: 4 min; *IR* (nujol, ν, cm^{-1}): 1725; *^{1}H-NMR* (δ, ppm, d^6-DMSO): 3.58 (CH_2, s); *^{13}C-NMR* (δ, ppm, d^6-DMSO): 41.1 (CH_2), 170.9 (CO).

Thiolactic acid disulfide. Sodium hydroxide (11.3 g, 0.28 mol) and potassium iodide (0.7 g, 4.2 mmol) were dissolved in water (70 ml) and cooled in ice-water. (±)Thiolactic acid (30 g, 0.283 mol) was added, then iodine (40 g, 0.157 mol) in portions for 30 min, until the red colour persisted. The solution was acidified to pH 3 with concentrated hydrochloride acid and extracted with ether. The solvent was removed to give, after cooling, a diastereoisomeric mixture as a yellow solid product (yield 85%). *IR* (nujol, ν, cm^{-1}): 1718; *^{1}H-NMR* (δ, ppm, d^6-DMSO): 1.38 (CH_3, dd, J = 7.1 Hz), 3.60 (CH_2, dq, J = 7.1 Hz); *^{13}C-NMR* (δ, ppm, d^6-DMSO): 17.1 (CH_3), 47.5 (CH), 173.2 (CO).

Mercaptoacetic acid disulfide tert-butyl ester. To a solution of mercaptoacetic acid disulphide (18.5 g, 0.1 mol) in DCM (100 ml) was added concentrated H_2SO_4 (1 ml). Isobutylene was passed through the solution slowly for 6 hours, the reaction was stirred for a further 48 hours at room temperature before washing two times with $NaHCO_3$ solution and then separating the organic layer. The organic phase was dried over sodium sulphate and the solvent removed under vacuum to give a liquid (yield 80%). *HPLC*: Retention Time: 6 min 12 sec; *IR* (neat, ν, cm^{-1}): 1726; *^{1}H-NMR* (δ, ppm, d^6-DMSO): 1.48 (3xCH_3, s), 3.49 (CH_2, s); *^{13}C-NMR* (δ, ppm, d^6-DMSO): 27.9 (CH_3), 42.6 (CH_2), 81.9 (Cq), 168.4 (CO).

Thiolactic acid disulfide tert-butyl ester. To a solution of thiolactic acid disulphide (30 g, 0.14 mol) in DCM (150 ml) was added concentrated H_2SO_4 (1.5 ml). Isobutylene was passed through the solution slowly for 6 hours, the reaction was stirred for a further 48 hours at room temperature before washing two times with $NaHCO_3$ solution and then separating the organic layer. The organic phase was dried over sodium sulphate and the solvent removed under vacuum to give a liquid (yield 75%). *HPLC*: Retention Time: 8 min 50 sec; *IR* (neat, ν, cm^{-1}): 1727; *^{1}H-NMR* (δ, ppm, d^6-DMSO): 1.36 (CH_3, d, J = 7.1 Hz), 1.43 (3xCH_3, s), 3.06 (2xCH_2, dq, J = 7.1 Hz); *^{13}C-NMR* (δ, ppm, d^6-DMSO): 16.5 (CH_3), 27.5 ($CH_{3\ t\text{-}Bu}$), 47.7 (CH), 79.1 (Cq), 170.5 (CO).

Mercaptoacetic acid tert-butyl ester. To a refluxing stirred solution of mercaptoacetic acid disulfide t-butyl ester (24 g, 0.082 mol) in absolute ethanol (150 ml) was added in small portions and slowly sodium borohydride (8 g, 0.21 mol). The reaction was stirred under reflux for 2 hours. The solvent was removed under vacuum before the addition of dilute hydrochloric acid solution (0.5 mol/dm^{-3}, 100 ml). The mixture was extracted with ethyl acetate (3 x 100 ml), dried over sodium sulphate and the solvent removed under vacuum to give a dense yellow oil, which was purified by column chromatography (110 g silica) (7:3 chloroform/hexane, R_f = 0.6) to give a pale yellow oil (7.53 g, yield 60%). *HPLC*: Retention Time: 5 min; *IR* (neat, ν, cm^{-1}): 1728; *^{1}H-NMR* (δ, ppm, d^6-DMSO): 1.45 (CH_3, s), 3.13 (CH_2, s); *^{13}C-NMR* (δ, ppm, d^6-DMSO): 27.8 (CH_3), 42.6 (CH_2), 81.7 (Cq), 168.4 (CO).

Thiolactic acid tert-butyl ester. To a refluxing stirred solution of thiolactic acid disulfide t-butyl ester (32 g, 0.1 mol) in absolute ethanol (200 ml) was added in small portions and slowly sodium borohydride (10.1 g, 0.27 mol). The reaction was stirred under reflux for 2 hours. The solvent was removed under vacuum before the addition

of dilute hydrochloric acid solution (0.5 mol/dm^{-3}, 100 ml). The mixture was extracted with ethyl acetate (3 x 100 ml), dried over sodium sulphate and the solvent removed under vacuum to give a dense yellow oil, which was purified by column chromatography (110 g silica) (7:3 chloroform/hexane, Rf = 0.6) to give a pale yellow oil (6.40 g, yield 40%). *HPLC*: Retention Time: 6 min 20 sec; *IR* (neat, ν, cm^{-1}): 1731; *^{1}H-NMR* (δ, ppm, d^6-DMSO): 1.36 (CH_3, d, J = 7.2 Hz), 1.42 (3xCH_3, s), 3.03 (CH, d, J = 7.2 Hz); *^{13}C-NMR* (δ, ppm, d^6-DMSO): 20.9 (CH_3), 27.4 ($CH_{3\ t\text{-}Bu}$), 35.8 (CH), 80.4 (Cq), 172.2 (CO).

N-Benzoyl-D-Ala-thio-Glycolate tert-butyl ester. N-benzoyl-D-alanine (3.9 g, 20 mmol) was dissolved in dry THF (50 ml) and stirred under N_2 atmosphere. Mercaptoacetic acid tert-butyl ester (3.05 g, 20.6 mmol) and a solution of DCC (4.4 g, 21 mmol) in dry THF (10 ml) were added simultaneously. The mixture was stirred at room temperature for 2 hours before filtering, to separate dicyclohexylurea, then the solvent was removed under reduced pressure by rotary evaporation at 40 ºC to give a colourless oil. The product was purified by column chromatography (100 g silica) (9:1 $CHCl_3$/ethyl acetate, R_f = 0.4) and the white solid was recrystallised in ethyl acetate to give a pure product (3.53 g, yield 55%). *HPLC*: Retention Time: 4 min 25 sec; *IR* ($CHCl_3$, ν, cm^{-1}): 3333, 1728, 1695, 1652; *^{1}H-NMR* (δ, ppm, d^6-DMSO): 1.39 (3xCH_3, s), 1.42 (CH_3, d, J = 7.27 Hz), 3.62 (CH_2, s), 4.69 (CH, m, J = 7.27 Hz, J = 7.24 Hz), 7.50-7.95 (Ph, m), 9.09 (NH, d, J = 7.24 Hz); *^{13}C-NMR* (δ, ppm, d^6-DMSO): 16.9 (CH_3), 27.5 ($CH_{3\ t\text{-}Bu}$), 31.6 (CH), 55.2 (CH_2), 81.2 (Cq), 127.5, 128.3, 131.6, 133.6 (Ph), 166.7 (CO_{am}), 167.4 (CO_{est}), 201.1 (-CO-S-).

N-Benzoyl-D-Ala-thio-D-Lactate tert-butyl ester. N-benzoyl-D-alanine (2 g, 10 mmol) was dissolved in dry THF (30 ml) and stirred under N_2 atmosphere. Thiolactic acid tert-butyl ester (1.7 g, 10.5 mmol) and a solution of DCC (2.2 g, 10.6 mmol) in

dry THF (5 ml) were added simultaneously. The mixture was stirred at room temperature for 2 hours before filtering, to separate dicyclohexylurea, then the solvent was removed under reduced pressure by rotary evaporation at 40 ºC to give a colourless oil. The product was purified by column chromatography (100 g silica) (9:1 $CHCl_3$/ethyl acetate, R_f = 0.4) to give a pure product (2.00 g, yield 60%); *IR* ($CHCl_3$, ν, cm^{-1}): 3298, 1725, 1665, 1652; *^{1}H-NMR* (δ, ppm, d^6-DMSO): 1.39 (3xCH_3, s), 1.42 (CH_3, d, J = 7.27 Hz), 3.62 (CH_2, s), 4.69 (CH, m, J = 7.27 Hz, J = 7.24 Hz), 7.50-7.95 (Ph, m), 9.09 (NH, d, J = 7.24 Hz); *^{13}C-NMR* (δ, ppm, d^6-DMSO): 16.9 (CH_3), 27.5 ($CH_{3\ t\text{-}Bu}$), 31.6 (CH), 55.2 (CH_2), 81.2 (Cq), 127.5, 128.3, 131.6, 133.6 (Ph), 166.7 (CO_{am}), 167.4 (CO_{est}), 201.1 (-CO-S-).

N-Benzoyl-D-Ala-thio-Glycolate (2). To a cooled solution (0 ºC) of hydrobromic acid 45% w/v in acetic acid was added N-benzoyl-D-ala-thio-gly tert-butyl ester (1.6 g, 5 mmol). The mixture was stirred at room temperature for 1 hour and then dry ether (100 ml) was added and stirred for a further 1 hour. The mixture was evaporated under reduced pressure at 40 ºC to give a colourless oil. The thioester-peptide was purified by column chromatography (50 g silica): firstly with eluent 8:2 $CHCl_3$:ethyl acetate to remove unreacted ester and then with ethyl acetate. Pure product was obtained (0.5 g, yield 40%); *IR* ($CHCl_3$, ν, cm^{-1}): 3297, 1695, 1690, 1652; *^{1}H-NMR* (δ, ppm, d^6-DMSO): 1.42 (CH_3, d, J = 7.29 Hz), 3.65 (CH_2, s), 4.71 (CH, m, J = 7.29 Hz, J = 7.40 Hz), 7.50-7.95 (Ph, m), 9.09 (NH, d, J = 7.40 Hz); *^{13}C-NMR* (δ, ppm, d^6-DMSO): 17.0 (CH_3), 31.0 (CH), 55.2 (CH_2), 127.6, 128.4, 131.7, 133.7 (Ph), 166.8 (CO_{am}), 169.7 (CO_{ac}), 201.3 (-CO-S-).

N-Benzoyl-D-Ala-thio-D-Lactate (1). To a cooled solution (0 ºC) of hydrobromic acid 45% w/v in acetic acid was added N-benzoyl-D-ala-thio-ala tert-butyl ester (2.0 g, 6 mmol). The mixture was stirred at room temperature for 1 hour and then dry ether

(100 ml) was added and stirred for a further 1 hour. The mixture was evaporated under reduced pressure at 40 ºC to give a colourless oil. The thioester-peptide was purified by column chromatography (50 g silica): firstly with eluent 8:2 $CHCl_3$:ethyl acetate to remove unreacted ester and then with ethyl acetate. Pure product was obtained (0.4 g, yield 25%); *IR* ($CHCl_3$, ν, cm^{-1}): 3330, 1700, 1685, 1637; *^{1}H-NMR* (δ, ppm, d^6-DMSO): 1.42 (CH_3, d, J = 7.29 Hz), 3.65 (CH_2, s), 4.71 (CH, m, J = 7.29 Hz, J = 7.40 Hz), 7.50-7.95 (Ph, m), 9.09 (NH, d, J = 7.40 Hz); *^{13}C-NMR* (δ, ppm, d^6-DMSO): 17.0 (CH_3), 17.7 (CH_3), 40.0 (CH), 55.2 (CH_2), 127.6, 128.4, 131.7, 133.7 (Ph), 166.8 (CO_{am}), 172.7 (CO_{ac}), 201.3 (-CO-S-).