Synthesis of the algicide bacillamide

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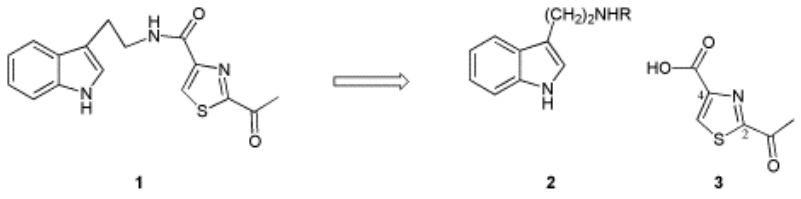
Abstract
Tryptamine and the mixed anhydride derived from 2-acetylthiazole-4-carboxylic acid and pivaloyl chloride afforded bacillamide.

Keywords: Regiospecific acetylation, bromination, chemoselective oxidation, algicide

Introduction

Bacillamide, a novel algicide active against the harmful dinoflagellate *Cochlodinium polykrikoides*, recently isolated[1] from *Bacillus* sp. SY-1, was shown by spectroscopic studies to be the amide **1** derived biosynthetically most probably from tryptamine (**2a**) and 2-acetylthiazole-4-carboxylic acid (**3**) (Scheme 1).

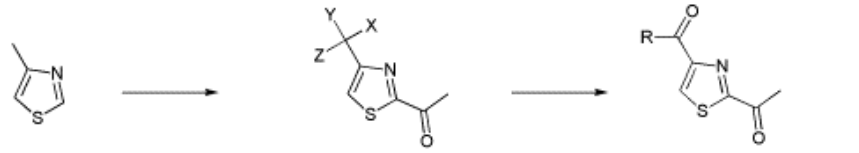


Scheme 1

As a part of a programme of studies on structure-algicidic properties, a general synthetic method for this class of compounds was deemed desirable. We report herein one such process and describe its application to the first synthesis of bacillamide starting from the commercially available 4-methylthiazole (**4**).

Results and Discussion

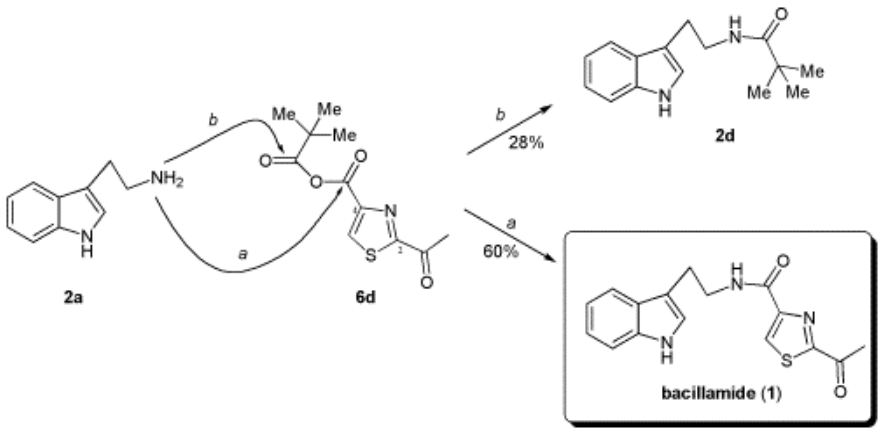
A regiospecific introduction of the acetyl group at C-2 in **4** was achieved by the procedure described by Dondoni[2] for unsubstituted thiazole, which involved metallation at C-2 followed by treatment of the resulting C-Li compound with ethyl acetate (Scheme 2). Compound **5a**,[3] thus secured in 64% yield**,** on reaction with NBS (2.5 eq) and in the presence of a catalytic quantity of AIBN in CCl_4 under reflux, furnished a chromatographically separable mixture of the monobromide **5b** (30%) (recyclable to **5c** in 50% yield), the dibromide **5c** (54%) and the tribromomethylthiazole **5d** (16%).



Scheme 2

Since attempts to convert the monobromide with $NaNO_2$/HOAc in DMSO[4] or the tribromide directly into the corresponding acid **3** by alkaline hydrolysis failed, **5c** was first solvolysed in aqueous acetone in the presence of AgOAc to the aldehyde **6a** (82%) and thence to the requisite acid **3**,[5,6] in high yield (94.5%) by oxidation with $NaClO_2$.[7]

The seemingly simple task of coupling tryptamine **2a** with acid **3**, to form bacillamide in good yield, proved to be unexpectedly difficult. For example, the following three mild methods involving activated carboxylic acid derivatives of **3** derived from *a)* benzotriazole,[8] *b)* carbonyl diimidazole,[9] or *c) via* Mitsunobu reaction,[10] all failed to give the requisite product **1** in acceptable yields. However, the mixed anhydrides **6b** and **6c** formed *in situ* from **3** and phenylchloroformate and isobutylchloroformate respectively, furnished, on reaction with tryptamine, the title compound **1** in low yields (38 and 22%, respectively). Significant amounts of carbamates of tryptamine **2b** (30%) and **2c** (26%) were also formed during the reactions indicating that the two carbonyl groups in these mixed anhydrides have similar reactivities towards tryptamine. A markedly improved yield of bacillamide (60%) was obtained with the use of the mixed anhydride **6d,** derived from the sterically more hindered pivaloyl chloride, although the formation of the by product **2d** (28%) could not be completely suppressed (Scheme 3). The former isolated as a colourless crystalline solid, mp 178-180 ºC, after preparative tlc, possessed ^{13}C and ^{1}H NMR spectra identical with those of the natural product.[11]



Scheme 3

Experimental Section

General Procedures. Melting points were determined with a microscopic hot-stage Reichert Thermovar and are uncorrected. Chromatography was performed using E. Merck silica gel 60 (70-230 mesh). Preparative thin-layer chromatography (PTLC) was performed on plates precoated with silica gel GF_{254} (0.5 mm). Infrared spectra (IR) were recorded with a Fourier Perkin-Elmer 157G and 683 infrared spectrophotometers and the frequencies reported in cm^{-1}. Nuclear magnetic resonance spectra (^1H NMR and ^{13}C NMR) were obtained with a Bruker ARX 400. Chemical shifts are reported in ppm downfield from tetramethylsilane. Mass spectra were obtained on a mass spectrometer GC-TOF Micromass GTC. Elemental analyses were carried out at the Microanalytical Laboratory of the Department. All solvents were purified by standard methods. Petroleum ether refers to fraction boiling between 60-80 ºC. Unless otherwise stated work-up is taken to mean drying the water-washed organic extract over anhydrous Na_2SO_4 or $MgSO_4$, filtering and evaporating the solvent from the filtrate under reduced pressure.

2-Acetyl-4-methylthiazole (5a). To a stirred solution of BuLi (46 mL, 73.6 mmol) in dry diethyl ether (100 mL), at -78 ºC and under an inert atmosphere, was added 4-methylthiazole (**4**) (6.1 g, 61.5 mmol) in dry Et_2O (100 mL) dropwise. The mixture after having been kept at -78 ºC (1 h) was treated with a solution of dry ethyl acetate (30 mL, 0.306 mol) in dry ether (100 mL) and then allowed to warm to room temperature. CH_2Cl_2 was added (200 mL), the solution was washed with aqueous saturated solution of $NaHCO_3$ and the product isolated in the usual manner. The residue thus obtained was purified by column chromatography (CH_2Cl_2/MeOH, 99:1) to provide the *title compound* **5a**, isolated as an orange solid, 5.71 g, (64%); mp 34 ºC (*n*-hexane) [lit.[3] mp 35 ºC (*n*-hexane)]; IR (CH_2Cl_2) ν: 2959, 2933, 2862, 2361, 1690, 1506, 1434 cm^{-1}; ^1H-NMR ($CDCl_3$) δ_H: 2.529 (s, 3H), 2.695 (s, 3H), 7.236 (s, 1H).

Bromination of 2-acetyl-4-methylthiazole. A mixture of **5a** (4.16 g, 24.2 mmol), NBS (11.0 g, 61.9 mmol) and AIBN (62 mg, 0.38 mmol) in previously distilled CCl_4 (44 mL) was, under a nitrogen atmosphere, stirred under reflux (5 h). The mixture at room temperature was filtered to remove the precipitate, and the filtrate diluted with CH_2Cl_2 (200 mL) and the organic phase washed with a 25% aqueous sodium hydrogen carbonate solution (2 x 20mL). Usual work-up followed by purification of the residue by column chromatography (CH_2Cl_2/petroleum ether, 9:1) provided in decreasing order of elution compounds **5d**, **5c** and **5b**.

2-Acetyl-4-tribromomethylthiazole (5d). Yield: 1.46 g (16%); orange oil; IR (CH_2Cl_2) ν: 3117, 1693, 1446, 1360, 1267, 1143 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ_H: 2.769 (s, 3H), 8.126 (s, 1H); ^{13}C-NMR ($CDCl_3$) δ_C: 25.13 (C-Br, by DEPT), 25.90 (CH_3), 123.61 (C*5*), 159.75 (C*4*), 167.00 (C*2*), 191.43 (C=O). Anal. Calcd for $C_6H_4NOSBr_3$: C, 19.07; H, 1.07; N, 3.71; S, 8.41. Found: C, 19.28; H, 0.76; N, 3.58; S, 8.47.

2-Acetyl-4-dibromomethylthiazole (5c). Yield: 3.9 g (54%); colourless solid; mp 68-68.5 ºC (CH_2Cl_2/*n*-hexane, 1:4); IR (CH_2Cl_2) ν: 1698, 1501, 1423, 1294, 1182 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ_H: 2.723 (s, 3H), 6.796 (s, 1H), 7.919 (s, 1H); ^{13}C-NMR ($CDCl_3$) δ_C: 25.89 (CH_3), 32.39 (CBr_2), 124.82 (C*5*), 156.68 (C*4*), 167.02 (C*2*), 191.24 (C=O); MS (CI, CH_4): *m/z* = 297.85 ($[M+H]^+$), 221.94, 219.93, 217.92, 177.92, 140.02.

Anal. Calcd for $C_6H_5NOSBr_2$: C, 24.10; H, 1.69; N, 4.68; S, 10.72. Found: C, 24.30; H, 1.56; N, 4.76; S, 10.90.

2-Acetyl-4-bromomethylthiazole (**5b).** Yield: 1.6 g (30%); colourless solid; mp 59-60 ºC (CH_2Cl_2/*n*-hexane, 1:4); IR (CH_2Cl_2) ν: 1689, 1450, 1359, 1276, 1217, 1057 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ_H: 2.711 (s, 3H), 4.616 (s, 2H), 7.637 (s, 1H); ^{13}C-NMR ($CDCl_3$) δ_C: 26.01 (CH_3), 26.22 (CH_2), 124.95 (C*5*), 154.38 (C*4*), 167.28 (C*2*), 191.39 (C=O); MS (CI, CH_4): *m/z* = 219.94 ($[M+H]^+$), 140.02. Anal. Calcd for C_6H_6NOSBr: C, 32.74; H, 2.75; N, 6.36; S, 14.57. Found: C, 32.84; H, 2.57; N, 6.57; S, 14.66.

2-Acetyl-4-formylthiazole (6a). To a stirred solution of **5c** (4.49 g, 15 mmol) in acetone-water 2:1 (30 mL), at room temperature and under N_2 atmosphere, protected from light, silver acetate (5.71 g, 34 mmol) was added. After stirring (30 h), the solution was filtered over a pad of celite and the latter washed repeatedly with acetone and methylene chloride. The residue obtained on work-up was purified by column chromatography (CH_2Cl_2/petroleum ether, 7:3) to afford the *title compound* **6a**, as a yellow solid, 1.91 g (82%); mp 57-59 ºC (CH_2Cl_2/ *n*-hexane, 3:1); IR (CH_2Cl_2) ν: 3119, 2874, 1699, 1687, 1466, 1450, 1360 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ_H: 2.772 (s, 3H), 8.449 (s, 1H), 10.110 (s, 1H); ^{13}C-NMR ($CDCl_3$) δ_C: 25.97 (*C*H_3), 132.21 (C*5*), 155.89 (C*4*), 167.96 (C*2*), 184.64 (CHO), 191.33 (C=O); MS (CI, CH_4): *m/z* = 156.01 ($[M+H]^+$). Calcd for $C_6H_5NO_2S$: C, 46.44; H, 3.25; N, 9.03; S, 20.66. Found: C, 46.34; H, 3.06; N, 9.25; S, 20.68.

2-Acetylthiazole-4-carboxylic acid (3). A mixture of **6a** (0.78 g, 5.03 mmol), aq. 50% H_2O_2 solution (0.3 mL, 5.23 mmol) and a buffer solution of NaH_2PO_4 (pH 4.3) (1.6 mL) in acetonitrile (6 mL), while being stirred at 0 ºC and under N_2 atmosphere, was treated dropwise with a solution of $NaClO_2$ (0.574 g, 7.04 mmol) in water (6 mL). The reaction was allowed to proceed for 2 hours. After left to reach room temperature, the reaction mixture was taken to dryness *in*

vacuum. The resulting residue was sublimed in high vacuum (0.2 mm Hg) providing the *title compound* **3** as a colourless solid, yield: 0.81 g (94.5%); mp 193-195 ºC (lit.[5] mp 193-195 ºC); IR (KBr) ν: 3436, 1686, 1639, 1560, 1415, 1355, 1298 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ_H: 2.638 (s, 3H, Me), 7.578 (s, 1H, D_2O exchange), 8.309 (s, 1H).

2-Acetylthiazole-4-carboxylic acid [2-(1*H*-indol-3-yl)-ethyl]-amide (Bacillamide) (1). A mixture of the acid **3** (25 mg, 0.146 mmol) and ethyl di-isopropylamine (21 µL, 0.161 mmol) in CH_2Cl_2 (2.3 mL) was stirred at 0 ºC under an inert atmosphere (15 min). A solution of pivaloyl chloride (19.8 µL, 0.161 mmol) in methylene chloride (2 mL) was added dropwise. The mixture was kept at 0 ºC for one hour to yield the mixed anhydride **6d** which was reacted immediately with tryptamine (23.5 mg, 0.161 mmol) in CH_2Cl_2 (3.5 mL). The reaction was allowed to proceed for an additional 30 min. After left to reach room temperature, the reaction mixture was washed with an aqueous saturated solution of $NaHCO_3$ (10 mL). Usual work-up afforded a residue that on purification by flash chromatography (ethyl acetate/*n*-hexane, 1:1) provided the *title compound* **1** accompanied with **2d**.

Bacillamide (1). Yield: 27.6 mg (60%); yellow solid; mp 169-170 ºC (CH_2Cl_2/ *n*-hexane, 1:1); IR (CH_2Cl_2) ν: 3401, 3311, 3106, 2922, 1687, 1660, 1544, 1481, 1456, 1359, 1267 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ_H: 2.690 (s, 3H, Me), 2.956 (t, 2H, CH_2, *J* = 7.5 Hz), 3.579 (dd, 2H, C*H*$_2$-NH, *J* = 7.0 Hz, 7.5 Hz), 6.972 (t, 1H, CH_{indole}, *J* = 7.7 Hz), 7.059 (t, 1H, CH_{indole}, *J* = 7.7 Hz), 7.191 (s, 1H, CH_{indole}-CH_2), 7.329 (d, 1H, CH_{indole}, *J* = 7.7 Hz), 7.608 (d, 1H, CH_{indole}, *J* = 7.7 Hz), 8.640 (brs, 2H, NH-C=O and CH-S), 10.820 (s, 1H, NH_{indole}); ^{13}C-NMR ($CDCl_3$) δ_C: 25.22 (*C*H_2), 25.69 (*C*H_3-C=O), 39.72 (*C*H_2-NH), 111.38 (*C*$_{indole}$), 111.64 (*C*$_{indole}$-CH_2), 118.24 (*C*$_{indole}$), 118.34 (*C*$_{indole}$), 120.96 (*C*$_{indole}$), 122.63 (*C*$_{indole}$–NH), 127.23 (*C*$_{indole}$), 130.49 (H*C*-S), 136.26 (*C*$_{indole}$), 151.51 (*C*-CONH), 159.89 (NH-*C*=O), 166.22 (*C*-$COCH_3$), 191.41 (CH_3-*C*=O). Anal. Calcd for $C_{16}H_{15}N_3O_2S$: C, 61.32; H, 4.82; N, 13.41; S, 10.23. Found: C, 61.38; H, 4.90; N, 13.26; S, 10.10.

Compound 2d. Yield: 10 mg (28%); colourless solid; mp 134-135 ºC (CH_2Cl_2/*n*-hexane, 1:3) [lit[12] mp 134-135 ºC (CH_2Cl_2/*n*-hexane, 1:3)].

Acknowledgements

We thank Fundação para a Ciência e Tecnologia (FC&T, Lisbon, Portugal) for partial financial support. One of us (V. B. C. F.) is grateful for the award of a doctoral fellowship from FC&T.

References and Footnotes

1. Jeong, S.-Y.; Isida, V.; Ito, Y.; Okada, S.; Muraami, M. *Tetrahedron Lett.* **2003**, *44*, 8005.
2. (a) Dondoni, A.; Fantin, G.; Fogagnolo, M. *Tetrahedron Lett.* **1989**, *30*, 6063. (b) Dondoni, A.; Fantin, G.; Fogagnolo, M.; Pedrini, P. *Tetrahedron* **1989**, *45*, 5141.

3. For a different procedure for the preparation of 2-acetyl-4-methylthiazole, see: Daigo, K.; Ree, L. J. *J. Am. Chem. Soc.* **1962**, 84, 1962.
4. Matt, C.; Wagner, A.; Mioskowski, C. *J. Org. Chem.* **1997**, *62*, 234.
5. Barton, M. A.; Kenner, G. W.; Sheppard, R. C. *J. Chem. Soc. (C)* **1966**, 2115. These authors obtained the acid by $KMnO_4$ oxidation of *dl*-2-(α-aminoethyl)-thiazole-4-carboxylic acid which in turn was prepared by a multi-step process.
6. The ubiquitous presence of the acid in organisms such as eubacteria and eukaryotes has led to the suggestion that it could be a co-enzyme, cf.: White, R. H. *Experientia* **1990**, *46*, 274.
7. Dalcanale, E.; Montanari, F. *J. Org. Chem.* **1986**, *51*, 567.
8. (a) Katritzky, A. R.; Shobana, N.; Pernak, J.; Afridi, A. S.; Fan, W.-Q. *Tetrahedron* **1992**, *48*, 7817. (b) Katritzky, A. R.; He, H.-Y.; Suzuki, K. *J. Org. Chem.* **2000**, *65*, 8210.
9. Okuzumi, T.; Nakanishi, E.; Tsuji, T.; Makino, S. *Tetrahedron* **2003**, *59*, 5603.
10. Brosse, N.; Pinto, M.-F.; Jamart-Grégoire, B. *J. Org. Chem.* **2000**, *65*, 4370.
11. Photocopies of spectra were kindly provided by Professor S. Okada, Graduate School of Agriculture and Life Sciences, University of Tokyo, Bunkyo-ku, Tokyo.
12. Atkinson, J. D., Eur. Pat. Appl., GB1 055 413, 1967; *Chem. Abst.* **1967**, *67*, 43678.