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Synthesis of methyl sulfomycinate, sulfomycinic amide and sulfomycinine, degradation products of the sulfomycin thiopeptide antibiotics

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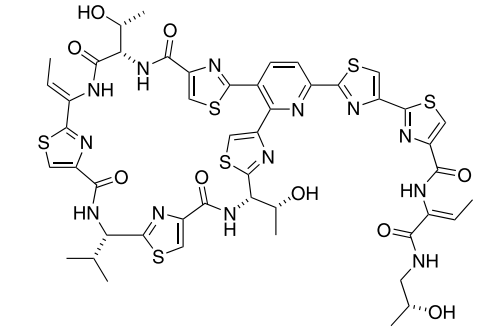
Abstract—The convergent synthesis of methyl sulfomycinate and sulfomycinic amide, two acidic methanolysis products of the sulfomycin thiopeptide antibiotics, is achieved starting from diethoxyacetonitrile. Further confirmation of structure is obtained by heating methyl sulfomycinate at 110 °C in hydrochloric acid to give (±)-sulfomycinine hydrochloride, the acid hydrolysate of sulfomycin I.

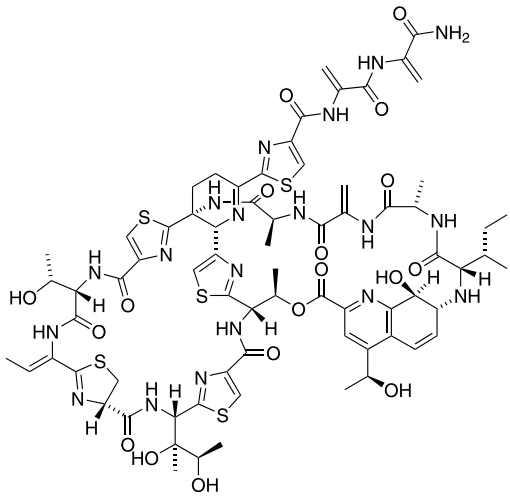
1. Introduction

The thiopeptide antibiotics are a class of biologically active natural products comprising 29 different families and at least 76 structurally distinct sulfur-containing cyclic peptide secondary metabolites isolated from the mycelial cake of actinomycetes. Their biological properties have attracted attention from a broad consortia of scientists and include features as diverse as the inhibition of bacterial protein synthesis, *tipA* promotion, renin inhibition and anti-malarial activity.[1] Since the first isolation of these natural products in 1948,[2] structure elucidation of metabolites such as micrococcin P_1 (**1**) by NMR[3] and the stunningly complex thiostrepton (**2**) by X-ray crystallography (Fig. 1)[4] has been supported by synthetic studies. The laboratory preparation of promothiocin A (**3**)[5,6] and amythiamicin D (**4**)[7] (Fig. 2) served to verify the constitution and stereochemistry of these thiopeptide families and recently the groundbreaking synthesis of thiostrepton (**2**) was reported,[8,9] demonstrating that the synthesis of any members of this antibiotic class is potentially within reach.

The sulfomycins (**5**) (Scheme 1) are one class of thiopeptide antibiotics isolated from *Streptomyces viridochromogenes* subsp. *sulfomycini* ATCC 29776 and MCRL-0368 with strong inhibitory activity against Gram-positive bacteria.[10] Chemical degradation studies,[11,12] in combination with ^1H and ^{13}C NMR spectroscopic and FAB mass spectrometric



micrococcin P_1 (**1**)



thiostrepton (**2**)

Figure 1. Micrococcin and thiostrepton thiopeptide antibiotics.

Keywords: Sulfomycin; Thiopeptide antibiotics; Chemical degradation; Thiazoles.
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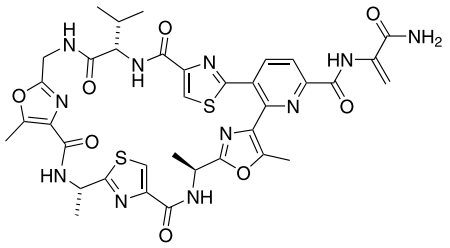


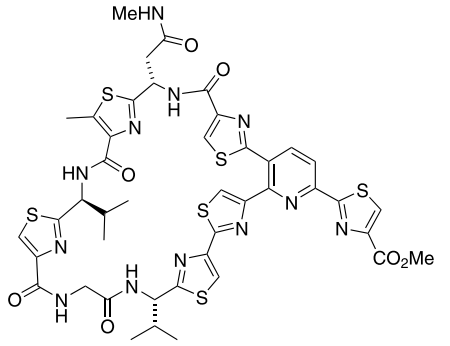
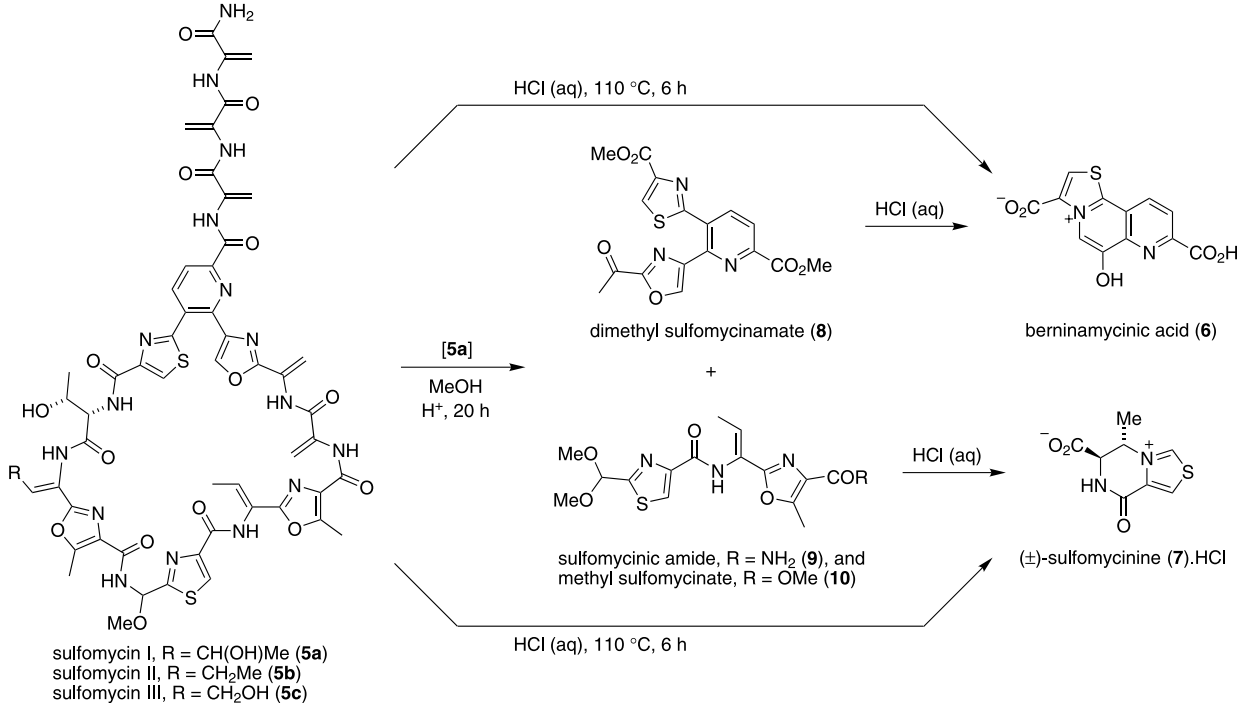


Figure 2. Promothiocin and amythiamicin thiopeptide antibiotics.

data,[13,14] elucidated their structure which contains an oxazole–thiazole–pyridine series *d* domain but differs between factors in the identity of one alkenyl substituent (*R*) located on the cyclic peptide backbone. In these degradation experiments, the acid hydrolysis of sulfomycin I in concentrated hydrochloric acid at 110 °C gave berninamycinic acid (**6**) and sulfomycinine (**7**) hydrochloride, both of which have been identified by X-ray crystallographic methods and chemical synthesis.[15,16] Acidic methanolysis of sulfomycin using Amberlyst 15 ion-exchange resin gave dimethyl sulfomycinamate (**8**), sulfomycinic amide (**9**), and methyl sulfomycinate (**10**), the latter of which was transformed to **7** in order to provide corroborating data on structure (Scheme 1). Our synthesis of dimethyl sulfomycinamate (**8**),[17,18] by Bohlmann-Rahtz reaction of an oxazolylenamine, further verified the identity of this acidic methanolysis product to complement the findings of Kelly,[19] but the structures of degradation products **9** and **10**, which were proposed on the basis of IR, UV, ^{1}H NMR spectroscopic and MS data for **10**,[11] are unconfirmed. In order to validate a route toward the sulfomycins, and as part of our interest in the total synthesis[20,21] and stereochemistry of thiopeptide antibiotics,[22] we set out to address this shortfall and establish a convergent synthesis of methyl sulfomycinate (**10**).

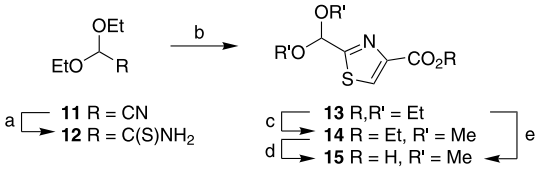
2. Results and discussion

Methyl sulfomycinate (**10**) is a modified tripeptide consisting of two fragments: an oxazole-containing dipeptide **20** related to a known (−)-muscoride A building block,[23] and a 2-formylthiazole-4-carboxylate residue **15** that is also a component of the antibiotic althiomycin,[24] present as its dimethyl acetal. Our convergent strategy assembled each heterocyclic component in turn and then coupled them together with subsequent dehydration to elaborate the aminopropenyl unit of **10**. Starting with diethoxyacetonitrile (**11**), treatment with ammonium sulfide in methanol at room temperature according to our recently reported procedure gave thioamide **12** in excellent yield.[25] Thiazole **13** was prepared by reaction with ethyl bromopyruvate in ethanol under Hantzsch conditions. Transacetalization under acidic conditions with *p*-toluenesulfonic acid (TsOH) in methanol gave dimethyl acetal **14**, which

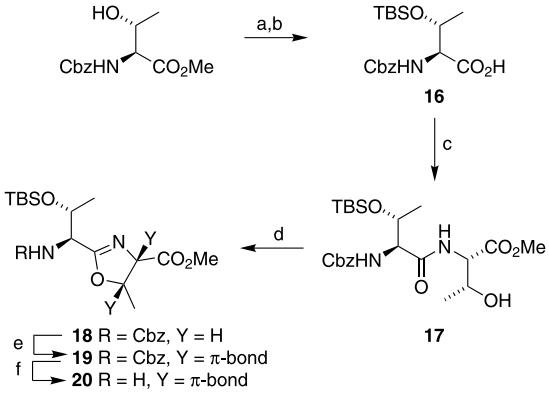


Scheme 1. Structure and chemical degradation of the sulfomycins.

was saponified using lithium hydroxide in methanol–water either directly or after isolation to give thiazole-4-carboxylic acid **15** in reasonable overall yield (Scheme 2).



Scheme 2. Synthesis of thiazole **15**. Reagents and conditions: (a) $(NH_4)_2S$, MeOH, RT, 18 h (100%); (b) ethyl bromopyruvate, EtOH, reflux, 1 h (100%); (c) TsOH, MeOH, reflux, 6 h (66%); (d) LiOH, MeOH–H_2O, RT, 18 h (64%); (e) TsOH, MeOH, reflux, 6 h; LiOH, MeOH–H_2O, 48 h (54%).



Scheme 3. Synthesis of oxazole **20**. Reagents and conditions: (a) TBDMSCl, imidazole, DMAP, DMF, RT, 36 h (100%); (b) LiOH, MeOH–H_2O, RT, 18 h (96%); (c) HCl·H-Thr-OMe, pyBOP, Et_3N, CH_2Cl_2, 0 °C–RT, 18 h (83%); (d) Deoxo-Fluor, CH_2Cl_2, −20 °C, 18 h (65%); (e) $CBrCl_3$, DBU, CH_2Cl_2, −20 °C, 18 h (52%); (d+e) without isolation of pure **18** (52%); (f) H_2, Pd–C, MeOH, RT, 3 h (93%).

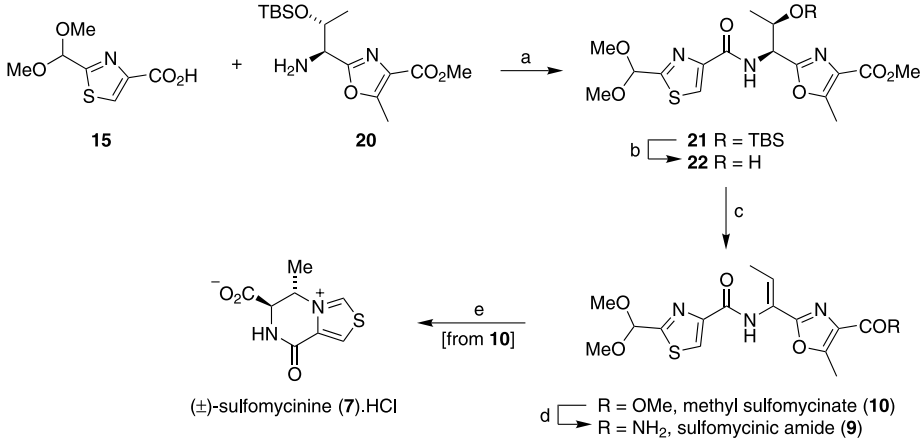
The other component, oxazole **20**, was prepared according to a modified method of Pattenden[23] from an *O*-silyl ether derivative of threonine **16** (Scheme 3).[26,27] Peptide coupling using benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (pyBOP) in dichloromethane gave dipeptide **17** which was cyclized to oxazoline **18** with [bis(2-methoxyethyl)amino]sulfur trifluoride (Deoxo-Fluor)[28] and oxidized to the oxazole **19** with $CBrCl_3$–DBU.[29] Hydrogenolysis of the benzyloxycarbonyl (Cbz) protecting group over Pd–C in methanol gave amine **20** which was coupled with thiazole-acid **15** using pyBOP to give tripeptide **21** (Scheme 4). Finally protodesilylation by treatment with TBAF in THF gave alcohol **22**, which was dehydrated via the corresponding methanesulfonate derivative to give methyl sulfomycinate (**10**). The physical and spectroscopic properties of the synthetic material [mp 122–123 °C; UV (MeOH)/nm λ_{max} 246 (log ε 4.40)] were in very good agreement with literature data on the degradation product [lit.[11] mp 124–124.5 °C; lit.[11] UV (MeOH)/nm λ_{max} 247 (log ε 4.38)] confirming the outcome of Abe's methanolysis studies[11] and providing a viable route to this region of the sulfomycin cyclic peptides.

Further confirmation of structure was obtained by carrying out both the known ammonolysis and hydrolysis of **10**, in methanolic ammonia at room temperature for the former or in hydrochloric acid at 110 °C for the latter, to obtain sulfomycinic amide (**9**) and sulfomycinine (**7**) hydrochloride, respectively, with physical properties that corroborated known data.[16] This convergent synthesis thus confirms both the structure of methyl sulfomycinate (**10**) and the outcome of its chemical degradation, as well as providing a method for the preparation of this fragment applicable to the total synthesis of the sulfomycin thiopeptide antibiotics.

3. Experimental

3.1. General

Commercially available reagents were used without further purification; solvents were dried by standard procedures. Light petroleum refers to the fraction with bp 40–60 °C. Flash chromatography was carried out using Merck Kieselgel 60 H silica or Matrex silica 60. Analytical thin layer chromatography was carried out using aluminium-backed plates coated with Merck Kieselgel 60 GF_{254} that were visualised under UV light (at 254 and/or 360 nm). Melting points (mp) were determined on a Kofler hot stage apparatus and are uncorrected. Infra-red (IR) spectra were



Scheme 4. Synthesis of methyl sulfomycinate (**10**). Reagents and conditions (a) pyBOP, CH_2Cl_2, 0 °C–RT, 18 h (88%); (b) TBAF, THF, 0 °C–RT, 5 h (77%); (c) $MeSO_2Cl$, CH_2Cl_2, RT, 1 h; Et_3N, CH_2Cl_2, RT, 18 h (60%); (d) NH_3, MeOH, RT, 2 d (24%); (e) HCl (aq), 110 °C, 2 h (27%).

recorded in the range 4000–600 cm^{-1} on a Perkin-Elmer 1600 series FTIR spectrometer using KBr disks for solid samples and thin films between NaCl plates for liquid samples and are reported in cm^{-1}. Nuclear magnetic resonance (NMR) spectra were recorded in $CDCl_3$ at 25 °C unless stated otherwise, using either a Bruker DPX 400 or 500 Avance instrument and were reported in ppm; *J* values were recorded in Hz and multiplicities were expressed by the usual conventions (s=singlet, d=doublet, t=triplet, app=apparent, b=broad, m=multiplet). Low-resolution mass spectra (MS) were determined using a Fisons VG Platform II Quadrupole instrument using atmospheric pressure chemical ionization (APcI) unless stated otherwise. ES refers to electrospray ionization, CI refers to chemical ionization (ammonia) and EI refers to electron ionization. High-resolution mass spectra denote the mass of the ion (mass of electron is 0.00055 Da) unless stated otherwise. Specific rotations were measured at the indicated temperature using an AA-1000 (Optical Activity Ltd) polarimeter at the sodium D line and are given in deg $cm^3\ g^{-1}\ dm^{-1}$ with concentration *c* in $10^{-2}\ gcm^{-3}$. Microanalyses were recorded using a Perkin-Elmer 240C Elemental Analyzer. In vacuo refers to evaporation at reduced pressure using a rotary evaporator and diaphragm pump, followed by the removal of trace volatiles using a vacuum (oil) pump.

3.1.1. 2,2-Diethoxythioacetamide (12). A mixture of diethoxyacetonitrile (1.12 ml, 8.06 mmol) and ammonium sulfide (50 wt% in H_2O; 1.5 ml, 11.0 mmol) in MeOH (100 ml) was stirred overnight. The mixture was evaporated in vacuo to give the title compound as a pale yellow solid (1.5 g, 100%), mp 92–93 °C (lit.[30] mp 81–82 °C) (Found: MH^+, 164.0743. $C_6H_{13}NO_2S$ requires MH^+, 164.0740) (Found: C, 44.1; H, 8.0; N, 8.4; S, 19.5. Calcd for $C_6H_{13}NO_2S$: C, 44.2; H, 8.0; N, 8.6; S, 19.6%); IR (KBr)/cm^{-1} ν_{max} 3371, 3256, 2971, 2916, 1604, 1424, 1368, 1246, 1121, 1057, 960, 918, 824, 724; ^1H NMR (400 MHz; $CDCl_3$) δ 7.87 (1H, bs, NH), 7.62 (1H, bs, NH), 5.05 (1H, s, CH), 3.76 (2H, dq, *J*=9.5, 7 Hz, 2OC*H*H), 3.67 (2H, dq, *J*=9.5, 7 Hz, 2OCH*H*), 1.18 (6H, t, *J*=7 Hz, 2Me); ^{13}C NMR (125 MHz; $CDCl_3$) δ 202.2 (C), 103.0 (CH), 62.9 (CH_2), 15.1 (Me); *m/z* (EI) 163 (M^+, 2%), 118 (15), 103 (87).

3.1.2. Ethyl 2-(diethoxymethyl)thiazole-4-carboxylate (13). Ethyl bromopyruvate (1.71 ml, 13.6 mmol) was added to a stirred solution of 2,2-diethoxythioacetamide (**12**) (1.22 g, 7.47 mmol) in EtOH (16 ml) over 4 Å molecular sieves (14 g) and the mixture was heated at reflux for 1 h. The solution was allowed to cool, filtered through Celite® and evaporated in vacuo. Purification twice by column chromatography on SiO_2, eluting with Et_2O–light petroleum (4:1) and Et_2O–light petroleum (1:1) (R_f 0.28), gave the title compound as a pale yellow oil (1.94 g, 100%) (Found: MH^+, 260.0956. $C_{11}H_{17}NO_4S$ requires MH^+, 260.0951); IR (film)/cm^{-1} ν_{max} 3441, 3113, 2983, 2359, 1694, 1556, 1454, 1391, 1368, 1332, 1216, 1101, 1020, 953, 882, 860, 767, 704; ^1H NMR (400 MHz; $CDCl_3$) 8.10 (1H, s, 5-H), 5.63 (1H, s, CH), 4.32 (2H, q, *J*=7.1 Hz, CH_2), 3.69 (2H, dq, *J*=9.5, 7 Hz, 2OC*H*H), 3.60 (2H, dq, *J*=9.5, 7 Hz, 2OCH*H*), 1.35 (3H, t, *J*=7.1 Hz, Me), 1.18 (6H, app t, *J*=7.1 Hz, Me); ^{13}C NMR (100 MHz; $CDCl_3$) δ 170.2 (C), 161.4 (C), 147.1 (C), 128.5 (CH), 98.7 (CH), 62.8 (CH_2), 61.5 (CH_2), 15.1 (Me), 14.4 (Me); *m/z* (APcI) 260 (MH^+, 37%).

3.1.3. Ethyl 2-(dimethoxymethyl)thiazole-4-carboxylate (14). *p*-TsOH monohydrate (30 mg, 20 mol%) was added to a stirred solution of ethyl 2-(diethoxymethyl)thiazole-4-carboxylate (**13**) (200 mg, 0.77 mmol) in dry MeOH (5 ml) and the mixture heated at reflux for 6 h. After evaporating in vacuo, the mixture was partition between saturated aqueous $NaHCO_3$ solution (15 ml) and CH_2Cl_2 (30 ml). The aqueous layer was further extracted with CH_2Cl_2 (2×30 ml) and the organic extracts were combined, dried (Na_2SO_4) and evaporated in vacuo. Purification by column chromatography on SiO_2, eluting with EtOAc–light petroleum (1:1) (R_f 0.41), gave the title compound as a brown oil (118 mg, 66%) (Found: MH^+, 232.0638. $C_9H_{13}NO_4S$ requires MH^+, 232.0637); IR (film)/cm^{-1} ν_{max} 3439, 3106, 2981, 1730, 1454, 1369, 1332, 1248, 1216, 1095, 1021, 950.1, 860, 766; ^1H NMR (400 MHz; $CDCl_3$) δ 8.14 (1H, s, 5-H), 5.52 (1H, s, CH), 4.35 (2H, q, *J*=7.1 Hz, CH_2), 3.39 (6H, s, MeO), 1.34 (3H, t, *J*=7.1 Hz, Me); ^{13}C NMR (100 MHz; $CDCl_3$) δ 168.9 (C), 161.4 (C), 147.3 (C), 128.6 (CH), 100.3 (CH), 61.6 (CH_2), 54.0 (Me), 14.4 (Me); *m/z* (APcI) 232 (MH^+, 73%).

3.1.4. 2-(Dimethoxymethyl)thiazole-4-carboxylic acid (15). LiOH monohydrate (108 mg, 2.6 mmol) was added to a stirred solution of ethyl ester **14** (100 mg, 0.43 mmol) in MeOH–$_2$O (5:1) (4 ml) and the solution was stirred overnight. After evaporating in vacuo, the mixture was partitioned between citric acid (1 M; 8 ml) and CH_2Cl_2 (35 ml). The aqueous layer was further extracted with CH_2Cl_2 (2×20 ml) and the organic extracts were combined, washed with brine (30 ml), dried (Na_2SO_4) and evaporated in vacuo to give the title compound as a brown solid (56 mg, 64%), mp 112–113 °C (EtOAc) (Found: MH^+ 204.0325. $C_7H_9NO_4S$ requires MH^+ 204.0325); IR (KBr)/cm^{-1} ν_{max} 3391, 2885, 2698, 2586, 2511, 1726, 1503, 1484, 1459, 1399, 1320, 1205, 1092, 1062, 984, 953, 905, 872, 820, 770, 732, 670; ^1H NMR (400 MHz; $CDCl_3$) δ 9.40 (1H, bs, OH), 8.25 (1H, s, 5-H), 5.52 (1H, s, CH), 3.36 (6H, s, Me); ^{13}C NMR (100 MHz; $CDCl_3$) δ 168.5 (C), 161.3 (C), 147.4 (C), 129.1 (CH), 100.2 (CH), 53.1 (Me); *m/z* (APcI) 204 (MH^+, 100%).

3.1.5. 2-(Dimethoxymethyl)thiazole-4-carboxylic acid (15) from 13. *p*-TsOH monohydrate (300 mg, 156 mmol) was added to a stirred solution of ethyl 2-(diethoxymethyl)thiazole-4-carboxylate (**13**) (2 g, 7.7 mmol) in MeOH (60 ml) and the mixture heated at reflux for 6.5 h. After cooling to room temperature, LiOH monohydrate (1.9 g, 46 mmol) and water (12 ml) were added and the solution was stirred for 48 h at room temperature. After evaporating in vacuo, the mixture was partitioned between citric acid (1 M; 40 ml) and CH_2Cl_2 (80 ml). The aqueous layer was further extracted with CH_2Cl_2 (2×60 ml) and the organic extracts were combined, dried (Na_2SO_4) and evaporated in vacuo to give the title compound as an off-white solid (0.85 g, 54%), mp 115–116 °C, with identical spectroscopic properties.

3.1.6. Z-L-Thr(TBS)-OMe. Imidazole (1.5 g, 22 mmol), DMAP (0.92 g, 7.5 mmol) and TBDMSCl (2.3 g, 15 mmol) were added successively to a solution of Z-L-Thr-OMe (3.0 g, 11 mmol) in DMF (11 ml). The mixture was stirred at room temperature for 36 h, acidified with hydrochloric acid (1 M; 15 ml) and extracted with ether (3×40 ml). The combined organic extracts were washed with H_2O (3×60 ml), dried (Na_2SO_4) and evaporated in vacuo to give the title compound as a pale yellow oil (4.3 g, 100%) (Found: MH^+, 382.2048. $C_{19}H_{31}NO_5Si$ requires MH^+, 382.2044); $[\alpha]_D^{30}$ −6.6 (*c*2.05, $CHCl_3$) {lit.[31] $[\alpha]_D^{24}$ −7.31 (*c*3.55, $CHCl_3$)}; IR (film)/cm^{-1} ν_{max} 3449, 2954, 2856, 1730, 1507, 1472, 1436, 1378, 1344, 1315, 1255, 1209, 1174, 1129, 1101, 1071, 1030, 1004, 963, 838, 810, 777; ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.35 (5H, PhH), 5.42 (1H, bd, J=9.8 Hz, NH), 5.13 (2H, s, CH_2O), 4.42 (1H, dq, J=6.3, 1.7 Hz, β-CH), 4.25 (1H, dd, J=9.8, 1.7 Hz, α-CH), 3.67 (3H, s, MeO), 1.17 (3H, d, J=6.3 Hz, Me) 0.8 (9H, s, CMe_3), −0.01 (3H, s, *Me*SiMe), −0.05 (3H, s, MeSi*Me*); ^{13}C NMR (100 MHz; $CDCl_3$) δ 171.4 (C), 156.8 (C), 136.3 (C), 128.6 (CH), 128.5 (CH), 122.3 (CH), 69.8 (CH), 67.2 (CH_2), 59.9 (CH), 52.3 (Me), 25.6 (Me), 20.8 (Me), 17.8 (C), −4.39 (Me), −5.34 (Me); *m*/*z* (APcI) 382 (MH^+, 100%).

3.1.7. Z-L-Thr(TBS)-OH 16. LiOH monohydrate (1.05 g, 25.0 mmol) was added to a stirred solution of Z-L-Thr(TBS)-OMe (1.5 g, 3.93 mmol) in MeOH–H_2O (5:1) (40 ml). After stirring for 18 h, the mixture was partitioned between hydrochloric acid (1 M; 40 ml) and CH_2Cl_2 (60 ml). The aqueous layer was further extracted with CH_2Cl_2 (2×60 ml) and the organic extracts were combined, dried (Na_2SO_4) and evaporated in vacuo to give the title compound as a colourless solid (1.38 g, 96%), mp 149–150 °C (triturated with light petroleum–EtOAc) (lit.[27] mp 154–157 °C) (Found: MH^+, 368.1883. $C_{18}H_{29}NO_5Si$ requires MH^+, 368.1888); $[\alpha]_D^{31}$ +10.3 (*c*2.82, $CHCl_3$) {lit.[27] $[\alpha]_D^{22}$ +10.5 (*c*1.69, $CHCl_3$)}; IR (KBr)/cm^{-1} ν_{max} 3440, 3035, 2956, 2856, 1702, 1600, 1514, 1455, 1413, 1344, 1308, 1257, 1102, 1042, 1005, 975, 836, 813, 778, 733, 696; ^{1}H NMR (500 MHz; $CDCl_3$) δ 9.6 (1H, bs, CO_2H), 7.3 (5H, PhH), 5.43 (1H, bd, J=8.6 Hz, NH), 5.04 (2H, s, CH_2), 4.40 (1H, qd, J=6.3, 2.4 Hz, β-H), 4.24 (1H, dd, J=8.6, 2.4 Hz, α-H), 1.11 (3H, d, J=6.3 Hz, Me), 0.75 (9H, s, CMe_3), −0.02 (3H, s, *Me*SiMe), −0.03 (3H, s, MeSi*Me*); ^{13}C NMR (125 MHz; $CDCl_3$) δ 175.7 (C), 156.7 (C), 136.1 (C), 128.6 (CH), 128.3 (CH), 128.2 (CH), 68.5 (CH), 67.3 (CH_2), 59.4 (CH), 25.7 (Me), 20.3 (Me), 17.9 (C), −4.6 (Me) and −5.1 (Me); *m*/*z* (CI) 368 (MH^+, 28%), 277 (100).

3.1.8. Z-L-Thr(TBS)-L-Thr-OMe 17. Et_3N (0.95 ml, 6.8 mmol) was added dropwise over 30 min to a stirred solution of acid **16** (1.0 g, 2.7 mmol), HCl.H-L-Thr-OMe (0.509 g, 3.0 mmol) and pyBOP (1.56 g, 3.0 mmol) in dry CH_2Cl_2 (13 ml) at 0 °C. The solution was allowed to warm to room temperature and stirred for a further 18 h. After concentrating in vacuo, purification by column chromatography on silica, eluting with light petroleum–EtOAc (1:1) (R_f 0.49), gave the title compound as a colourless solid (1.1 g, 83%), mp 133–134 °C (light petroleum–EtOAc) (Found: MH^+, 483.2521. $C_{23}H_{38}N_2O_7Si$ requires MH^+, 483.2521); $[\alpha]_D^{32}$ +13.1 (*c*1.05, $CHCl_3$); IR (KBr)/cm^{-1} ν_{max} 3446, 3357, 2955, 1752, 1723, 1670, 1507, 1253, 1207, 1132, 1102, 1078, 967, 839, 780; ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.43 (1H, bd, J=8.8 Hz, NH), 7.20 (5H, PhH), 5.74 (1H, bd, J=5.7 Hz, NH), 5.01 (1H, d, J=12 Hz, C*H*H), 4.92 (1H, d, J=12 Hz, CH*H*), 4.42 (1H, dd, J=8.8, 1.7 Hz, C*H*CO_2Me), 4.21 (2H, 2β-H), 4.14 (1H, m, α-H), 3.60 (3H, s, Me), 2.05 (1H, bs, OH), 1.05 (3H, d, J=6.4 Hz, Me), 1.01 (3H, d, J=6.2 Hz, Me), 0.78 (9H, s, CMe_3), 0.04 (3H, s, *Me*SiMe), 0.00 (3H, s, MeSi*Me*); ^{13}C NMR (100 MHz; $CDCl_3$) δ 171.1 (C), 170.1 (C), 156.2 (C), 136.1 (C), 128.6 (CH), 128.2 (CH), 128.1 (CH), 68.4 (CH), 67.6 (CH), 67.0 (CH_2), 59.1 (CH), 57.3 (CH), 52.5 (Me), 25.7 (C), 19.9 (Me), 17.9 (Me), 17.4 (Me), −4.8 (Me), −5.0 (Me); *m*/*z* (APcI) 483 (MH^+, 100%).

3.1.9. (4*S*, 5*S*, 1′*S*, 2′*R*)-Methyl *N*-(benzyloxy)carbonyl-2-[1-amino-2-(*tert*-butyldimethylsilyloxy)prop-1-yl]-5-methyloxazoline-4-carboxylate (18). [Bis(2-methoxyethyl)amino]sulfur trifluoride (Deoxo-Fluor) (0.37 ml, 2.0 mmol) was added dropwise to a stirred solution of Z-L-Thr(TBS)-L-Thr-OMe **17** (0.95 g, 1.97 mmol) in dry CH_2Cl_2 (30 ml) at −20 °C. The solution was stirred for 18 h and then quenched by the addition of saturated aqueous $NaHCO_3$ solution (30 ml). After warming to room temperature, the mixture was extracted with CH_2Cl_2 (3×40 ml). The organic extracts were combined, dried (Na_2SO_4) and evaporated in vacuo. Purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (R_f 0.39), gave the title compound as a colourless oil (595 mg, 65%); $[\alpha]_D^{32}$ +7.4 (*c*1.05, $CHCl_3$); IR (film)/cm^{-1} ν_{max} 3330, 3034, 2954, 2856, 2358, 1731, 1674, 1504, 1383, 1258, 1212, 1101, 836, 778, 698; ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.33 (5H, PhH), 5.45 (1H, bd, J=9.5 Hz, NH), 5.11 (1H, d, J=13.7 Hz, C*H*H), 5.08 (1H, d, J=13.7 Hz, CH*H*), 4.90 (1H, dq, J=10.4, 6.3 Hz, 5-H), 4.78 (1H, d, J=10.4 Hz, 4-H), 4.42 (1H, d, J=9.5 Hz, NHC*H*), 4.35 (1H, q, J=6.3 Hz, CH), 3.75 (3H, s, OMe), 1.25 (3H, d, J=6.3 Hz, 5-Me), 1.17 (3H, d, J=6.3 Hz, Me), 0.80 (9H, s, CMe_3), 0.00 (3H, s, *Me*SiMe), −0.06 (3H, s, MeSi*Me*); ^{13}C NMR (100 MHz; $CDCl_3$) δ 170.0 (C), 168.8 (C), 156.4 (C), 136.4 (C), 128.6 (CH), 128.5 (CH), 128.1 (CH), 78.1 (CH), 71.3 (CH), 69.3 (CH), 67.0 (CH_2), 55.5 (CH), 52.1 (Me), 25.7 (Me), 20.6 (Me), 17.9 (C), 16.3 (Me), −4.4 (Me), −5.0 (Me); *m*/*z* (APcI) 483 (100%), 465 (MH^+, 50).

3.1.10. (1′*S*, 2′*R*)-Methyl *N*-(benzyloxy)carbonyl-2-[1-amino-2-(*tert*-butyldimethylsilyloxy)prop-1-yl]-5-methyloxazole-4-carboxylate (19). $BrCCl_3$ (0.88 ml, 8.9 mmol) and DBU (0.88 ml, 5.9 mmol) were added successively to a stirred solution of the oxazoline **18** (0.60 g, 1.28 mmol) in dry CH_2Cl_2 (24 ml) at −20 °C. After stirring for 18 h, the mixture was poured into saturated aqueous $NaHCO_3$ solution (40 ml) and extracted with ethyl acetate (3×60 ml). The organic extracts were combined, dried (Na_2SO_4) and evaporated in vacuo. Purification by column chromatography on SiO_2, eluting with light petroleum–EtOAc (R_f 0.63), gave the title compound as a colourless oil (0.31 g, 52%) (Found: MH^+ 463.2258. $C_{23}H_{34}N_2O_6Si$ requires MH^+, 463.2259); $[\alpha]_D^{33}$ −5.7 (*c*2.75, $CHCl_3$); IR (film)/cm^{-1} ν_{max} 3436, 3352, 2954, 2925, 2857, 2361, 1732, 1622, 1587, 1504, 1442, 1352, 1253, 1211, 1100, 965, 915, 838, 810, 778, 739, 698; ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.35 (5H, PhH), 5.72 (1H, bd, J=9.4 Hz, NH), 5.15 (2H, s, CH_2), 4.93 (1H, dd, J=9.4, 2.1 Hz, 1′-H),

4.42 (1H, qd, J=6.2, 2.1 Hz, 2′-H), 3.90 (3H, s, OMe), 2.60 (3H, s, 5-Me), 1.26 (3H, d, J=6.2 Hz, 2′-Me) 0.78 (9H, s, CMe_3), 0.03 (3H, s, *Me*SiMe), −0.17 (3H, s, MeSi*Me*); ^{13}C NMR (100 MHz; $CDCl_3$) δ 162.6 (C), 160.9 (C), 156.4 (C), 156.4 (C), 136.2 (C), 128.6 (CH), 128.2, (CH), 127.8 (CH), 127.5 (C), 70.0 (CH), 67.3 (CH_2), 55.5 (CH), 52.0 (Me), 25.5 (C), 20.4 (Me), 17.8 (C), 11.9 (Me), −4.7 (Me), −5.5 (Me); *m/z* (APcI) 463 (MH^+, 100%).

3.1.11. Oxazole 19 from Z-L-Thr(TBS)-L-Thr-OMe 17. Deoxo-Fluor (0.72 ml, 3.9 mmol) was added dropwise to a stirred solution of Z-L-Thr(TBS)-L-Thr-OMe **17** (1.87 g, 3.87 mmol) in dry CH_2Cl_2 (60 ml) at −20 °C. The solution was stirred for 18 h and then quenched by the addition of saturated aqueous $NaHCO_3$ solution (30 ml). After warming to room temperature, the mixture was extracted with CH_2Cl_2 (3×100 ml). The organic extracts were combined, dried (Na_2SO_4) and evaporated in vacuo to give the crude oxazoline **18** as a brown oil (2.1 g). The residue was dissolved in dry CH_2Cl_2 (70 ml) and cooled to −20 °C. $BrCCl_3$ (1.7 ml, 17.4 mmol) and DBU (2.6 ml, 17.4 mmol) were added and the solution was stirred at −20 °C for 18 h. The mixture was poured into saturated aqueous $NaHCO_3$ solution (70 ml) and extracted with ethyl acetate (3× 60 ml). The organic extracts were combined, dried (Na_2SO_4) and evaporated in vacuo. Purification by column chromatography on SiO_2, eluting with light petroleum–Et_2O (R_f 0.15), gave the title compound as a colourless oil (0.94 g, 52%) with identical physical and spectroscopic properties.

3.1.12. (1′*S*, 2′*R*)-Methyl 2-[1-amino-2-(*tert*-butyldimethylsilyloxy)prop-1-yl]-5-methyloxazole-4-carboxylate (20). A solution of oxazole **19** (0.94 g, 2.0 mmol) in MeOH (34 ml) was stirred over Pd–C (10 wt%; 817 mg) under an atmosphere of H_2 for 3 h. The mixture was filtered through Celite® and evaporated in vacuo to give the title compound as a colourless solid, mp 75–76 °C (623 mg, 93%) (Found: MH^+329.1890, $C_{15}H_{28}N_2O_4Si$ requires MH^+329.1891); $[\alpha]_D^{31}$ −29.3 (*c*2.78, $CHCl_3$); IR (film)/cm^{-1} ν_{max} 3387, 2955, 2857, 1721, 1621, 1441, 1351, 1256, 1205, 1098, 970, 837.0, 776, 666; ^{1}H NMR (500 MHz; $CDCl_3$) δ 9–6 (2H, bs, NH), 4.45 (2H, 1′,2′-H), 3.85 (3H, s, OMe), 2.53 (3H, s, 5-Me) 1.35 (3H, bs, Me) 0.75 (9H, s, CMe_3), 0.05 (3H, s, *Me*SiMe), −0.1 (3H, s, MeSi*Me*); ^{13}C (100 MHz; $CDCl_3$) δ 164.0 (C), 162.8 (C), 156.3 (C), 127.2 (C), 70.7 (CH), 56.5 (CH), 52.0 Me), 25.7 (Me), 20.5 (Me), 17.8 (C), 11.9 (Me), −4.5 (Me), −5.3 (Me); *m/z* (APcI) 329 (MH^+, 100%).

3.1.13. Amide 21. Et_3N (0.50 ml, 3.6 mmol) was added dropwise over 30 min to a stirred solution of acid **15** (325 mg, 1.6 mmol), amine **20** (420 mg, 1.3 mmol) and pyBOP (830 mg, 1.6 mmol) in dry CH_2Cl_2 (13 ml) at 0 °C. The solution was allowed to warm to room temperature and stirred for a further 18 h. After concentrating in vacuo, purification by column chromatography on silica, eluting with light petroleum–EtOAc (1:1) (R_f 0.31), gave the title compound as a colourless oil (580 mg, 88%) (Found: MH^+514.2044, $C_{22}H_{35}N_3O_7SSi$ requires MH^+, 514.2038); $[\alpha]_D^{25}$ +16 (*c*2.3, $CHCl_3$); IR (film)/cm^{-1} ν_{max} 3409, 3116, 2956, 2857, 2400, 1731, 1682, 1621, 1538, 1499, 1471, 1352, 1258, 1186, 1098, 973, 910, 838, 756, 666; ^{1}H NMR (400 MHz; $CDCl_3$) δ 8.3 (1H, s, CH), 8.2 (1H, d, J=8.9 Hz, NH) 5.72 (1H, s, CH), 5.41 (1H, dd, J=8.9, 2.8 Hz, CH), 4.65 (1H, qd, J=6.1, 2.8 Hz, C*H*Me), 4.07 (3H, s, OMe), 3.68 (3H, s, OMe) 3.4 (3H, s, OMe), 2.75 (3H, s, Me), 1.41 (3H, d, J=6.1 Hz, CH*Me*), 1.05 (9H, s, CMe_3), 0.22 (3H, s, *Me*SiMe), 0.03 (3H, s, MeSi*Me*); ^{13}C NMR (100 MHz; $CDCl_3$) δ 167.9 (C), 162.7 (C), 161.1 (C), 160.7 (C), 156.4 (C), 149.5 (C), 127.5 (C), 125.0 (CH), 100.3 (CH), 70.0 (CH), 54.1 (Me), 53.8 (Me), 53.4 (CH), 52.0 (Me), 25.5 (Me), 20.7 (Me), 17.8 (C), 12.0 (Me), −4.6 (Me), −5.4 (Me); *m/z* (CI^+) 514 (MH^+, 95%), 159 (100).

3.1.14. Alcohol 22. A solution of TBAF (1.0 M; 1.8 mmol) in THF (1.8 ml) was added to a stirred solution of silyl ether **21** (580 mg, 1.1 mmol) in dry THF (21 ml) at 0 °C. After warming to room temperature, the mixture was stirred for 4.5 h and then partitioned between H_2O and EtOAc. The aqueous layer was further extracted with EtOAc (twice) and the combined organic extracts were dried (Na_2SO_4) and evaporated in vacuo. Purification by column chromatography on SiO_2, eluting with EtOAc (R_f 0.31), gave the title compound as a colourless solid (344 mg, 77%), mp 159–160 °C (25% EtOAc–light petroleum) (Found: MH^+400.1171. $C_{16}H_{21}N_3O_7S$ requires MH^+, 400.1173); $[\alpha]_D^{27}$ −14.7 (*c*2.3, $CHCl_3$); IR (KBr)/cm^{-1} ν_{max} 3458, 3277, 3123, 2956, 2825, 2362, 1715, 1668, 1540, 1497, 1453, 1391, 1376, 1334, 1247, 1217, 1192, 1148, 1098, 1063, 982, 828, 797; ^{1}H NMR (400 MHz; $CDCl_3$) δ 8.11 (1H, s, CH), 8.03 (1H, d, J=9.3 Hz, NH) 5.45 (1H, s, CH), 5.24 (1H, dd, J=9.3, 2.9 Hz, CH), 4.51 (1H, qd, J=6.3, 2.9 Hz, C*H*Me), 3.82 (3H, s, OMe), 3.61 (1H, s, OH), 3.37 (6H, s, OMe), 2.53 (3H, s, Me), 1.22 (3H, d, J=6.3 Hz, CH*Me*); ^{13}C NMR (100 MHz; $CDCl_3$) δ 168.2 (C), 162.4 (C), 161.3 (C), 160.6 (C), 156.9 (C), 149.3 (C), 127.3 (C), 125.5 (CH), 99.9 (CH), 67.7 (CH), 53.7 (Me), 52.0 (Me), 51.9 (CH), 19.2 (Me), 12.1 (Me); *m/z* (CI^+) 400 (MH^+, 100%).

3.1.15. Methyl sulfomycinate (10). A solution of alcohol **22** (344 mg, 0.86 mmol), Et_3N (1.2 ml, 8.6 mmol) and MsCl (0.53 ml, 6.9 mmol) in dry CH_2Cl_2 (20 ml) was stirred for 1 h at room temperature. The solution was partitioned between H_2O (45 ml) and CH_2Cl_2 (30 ml) and the aqueous layer was further extracted with $CHCl_3$ (2×40 ml). The combined organic extracts were dried (Na_2SO_4) and evaporated in vacuo. The residue was dissolved in dry CH_2Cl_2 (20 ml), Et_3N (2.0 ml, 14.4 mmol) was added and the mixture was stirred for 18 h. After evaporating in vacuo, purification by column chromatography on SiO_2, eluting with EtOAc (R_f 0.44), gave the title compound as a colourless solid (198 mg, 60%), mp 122–123 °C (EtOAc) (lit.[11] mp 124–124.5 °C) (Found: MH^+382.1066. $C_{16}H_{19}N_3O_6S$ requires MH^+, 382.1067); IR (KBr)/cm^{-1} ν_{max} 3372, 3116, 2924, 2845, 2363, 2344, 1719, 1684, 1618, 1527, 1469, 1439, 1351, 1233, 1189, 1101, 1062; UV (MeOH)/nm λ_{max} 246 (log ε 4.40) [lit.[11] 247 (log ε 4.38)]; ^{1}H NMR (400 MHz; $CDCl_3$) δ 8.65 (1H, bs, NH), 8.14 (1H, s, CH) 6.68 (1H, q, J=7.2 Hz, CH), 5.55 (1H, s, CH), 3.82 (3H, s, OMe), 3.41 (6H, s, OMe), 2.57 (3H, s, Me), 1.84 (3H, d, J=7.2 Hz, CH*Me*); ^{13}C NMR (100 MHz; $CDCl_3$) δ 168.1 (C), 162.7 (C), 159.0 (C), 157.5 (C), 156.5 (C), 149.7 (C), 128.9 (CH), 128.1 (C), 125.7 (CH), 122.1 (C), 99.9 (CH), 53.6 (Me), 52.0 (Me), 14.5 (Me), 12.2 (Me); *m/z* (CI^+) 382 (MH^+, 100%).

3.1.16. Sulfomycinic amide (9). A saturated solution of methanolic NH_3 (20 ml) was added to methyl sulfomycinate (**10**) (70 mg, 0.18 mmol) at room temperature. The mixture was stirred at this temperature for 2 days and then evaporated in vacuo. Purification by column chromatography on SiO_2, eluting with EtOAc, gave the title compound as a colourless solid (16 mg, 24%), mp 188–189 °C (EtOAc) (lit.[11] mp 194.5–195 °C); (Found: MH^+ 367.1070. $C_{15}H_{18}N_4O_5S$ requires MH^+, 367.1071); IR (KBr)/cm^{-1} ν_{max} 3474, 3360, 3284, 3153, 3090, 2940, 2842, 1687, 1636, 1604, 1555, 1531, 1497, 1482, 1449, 1419, 1369, 1333, 1305, 1229, 1211, 1195, 1168, 1104, 1082, 1042, 1016, 990, 968, 956, 836, 789, 762, 721, 707, 684; ^{1}H NMR (400 MHz; $CDCl_3$) δ 8.71 (1H, bs, NH), 8.13 (1H, s, CH) 6.84 (1H, bs, N*H*H), 6.61 (1H, q, J=7.2 Hz, CH), 5.58 (1H, bs, NH*H*), 5.53 (1H, s, CH), 3.37 (6H, s, OMe), 2.55 (3H, s, Me), 1.84 (3H, d, J=7.2 Hz, CH*Me*); ^{13}C NMR (100 MHz; $CDCl_3$) δ 168.2 (C), 164.0 (C), 159.0 (C), 156.7 (C), 153.9 (C), 149.7 (C), 129.4 (C), 128.7 (CH), 125.7 (CH), 122.2 (C), 99.8 (CH), 53.6 (Me), 14.5 (Me), 11.9 (Me); *m/z* (APcI) 367 (MH^+, 18%).

3.1.17. (±)-Sulfomycinine (7)·HCl (6-carboxy-5-methyl-8-oxo-5,6,7,8-tetrahydrothiazolo[3,4-*a*]pyrazinium chloride). Methyl sulfomycinate (**10**) (67 mg, 0.76 mmol) was stirred in hydrochloric acid (6 M; 9 ml) in a Carius tube at 110 °C for 2 h. After cooling, the mixture was evaporated in vacuo, by forming an azeotrope with MeOH. The residue was triturated with MeOH to give the title compound as a colourless solid (12 mg, 27%), mp 203–204 °C (dec.) (EtOAc) (lit.[12] mp 205–207 °C) (Found: $[M-Cl]^+$ 213.0325. $C_8H_8N_2O_3S$.HCl requires $[M-Cl]^+$, 213.0328); IR (KBr)/cm^{-1} ν_{max} 3455, 3197, 3096, 3055, 2370, 2288, 1740, 1686, 1575, 1436, 1192, 887, 761; UV (MeOH)/nm λ_{max} 230 (log ε 3.76) [lit.[12] 230 (log ε 3.85)]; ^{1}H NMR (500 MHz; D_2O) δ 10.15 (1H, d, J=2.4 Hz, exch D_2O, 3-H), 8.77 (1H, d, J=2.4 Hz, 1-H), 5.51 (1H, dq, J=1.5, 6.9 Hz, 5-H), 4.46 (1H, d, J=1.5 Hz, 6-H), 1.61 (3H, d, J=6.9 Hz, Me); ^{13}C NMR (125 MHz; D_2O) δ 172.3 (C), 159.7 (CH), 156.7 (C), 136.1 (C), 131.5 (CH), 59.8 (CH), 57.5 (CH), 19.6 (Me); *m/z* (ES) 215 ($[M-Cl]^+$, 100%).

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