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Sub-structure syntheses and relative stereochemistry in the bistramide (bistratene) series of marine metabolites

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Abstract—The (6*R**,9*S**,11*S**) and (22*S**,23*R**,27*R**,31*R**) stereochemistry, respectively, of the tetrahydropyranyl and spiroacetal moieties in bistramide A (**1**) have been established by stereoselective syntheses and high field NMR comparisons. Routes to the γ-amino acid moiety are outlined.

Didemnid ascidians (tunicates) are rich sources of biologically active compounds,[1] and the colonial ascidian, *L. bistratum* Sluiter, has provided five structurally related compounds, bistramides A–D and K.[2–6] Bistramide A (Fig. 1), which is identical to bistratene A,[3] exhibits potent anti-tumour activity in vitro, is cell permeable and is the only described specific activator of protein kinase Cδ, a PKC isoform. All PKCs are involved in the transduction of signals for cell proliferation, differentiation and apoptosis, processes highly relevant to cancer therapy. Bistramides B, C and D differ from A with respect to the oxidation levels in the terminal C2–C4 and C36–C39 regions.

The bistramides A–D incorporate tetrahydropyran and spiroacetal moieties linked peptidically via a γ-amino acid unit.[2–6] Bistramides A–D and K appear to be single stereoisomers, but the NMR[2–6] data had not been analysed from a stereochemical perspective. We now describe syntheses and NMR data which together establish the stereochemistry of the ring systems, and provide sub-structures for eventual linkage to form bistramide A and other isomers.

Our ^{13}C and ^{1}H NMR assignments for bistramide A (750 MHz) agree with those of Ireland,[6] and the major NOEs are summarised below for the tetrahydropyranyl and spiroacetal sub-structures **2** and **3**, respectively. Key *vic*-^{1}H–^{1}H coupling constants are consistent[7] with these structures and the portrayed stereochemistry viz (6*R**,9*S**,11*S**) for **2** and (22*S**,23*R**,27*R**,31*R**) for **3** (Fig. 2).[8]

Comparative NMR studies of synthesised, retro-synthetically derived fragments (see Fig. 1) verified these conclusions. With respect to the THP fragment, the sequences in Scheme 1, utilising either Hg(II)- or Pd(II)-mediated cyclisations of hydroxyalkenes, provided **10** and **10a** along with three other isomers. The trends in ^{13}C chemical shifts for the separable isomers are intelligible in terms of *axial* and *equatorial* sub-

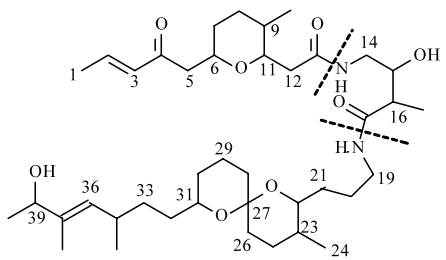

Figure 1. Bistramide A (**1**).

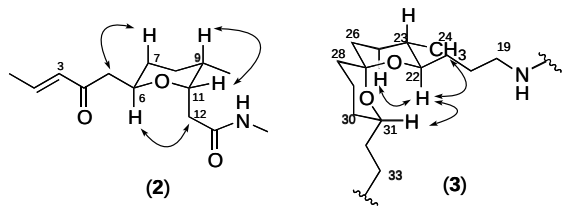

Figure 2. Observed NOEs.

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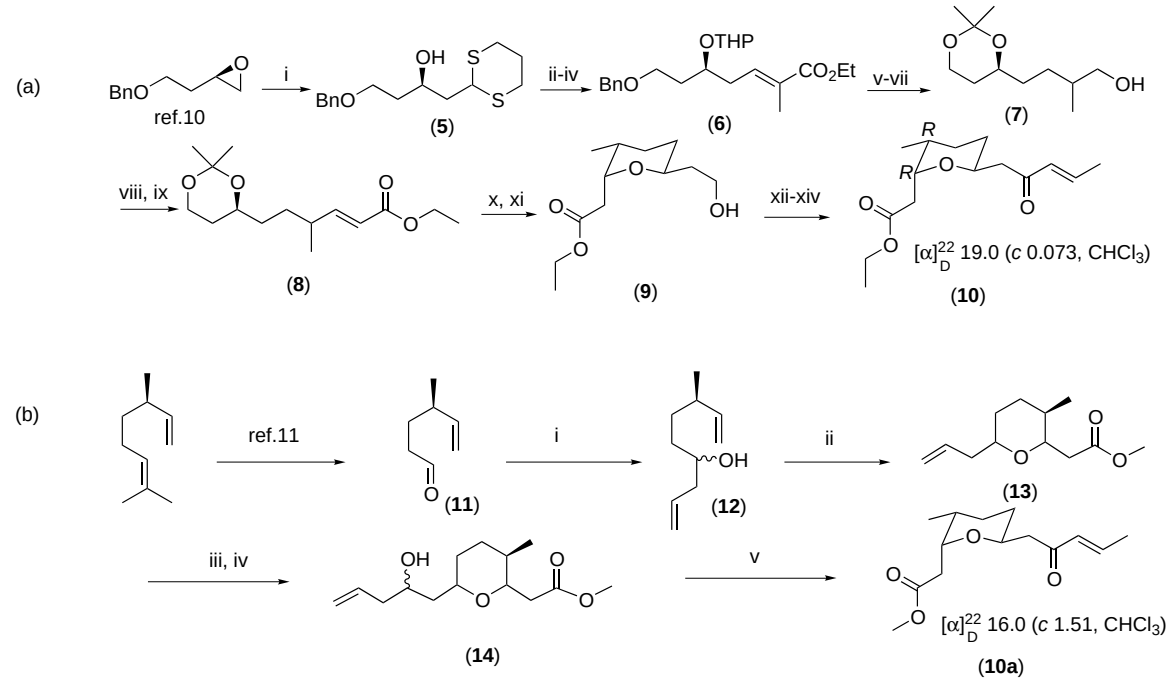


Scheme 1. (a) (i) 1,3-Dithiane, BuLi, (70%); (ii) DHP, PPTS, DCM; (iii) MeI, $CaCO_3$, (65%); (iv) $Ph_3P{=}C(CH_3)CO_2Et$; (v) H_2 Pd/C; (vi) DMP, H^+; (vii) $LiAlH_4$ (72%); (viii) TPAP, NMO; (ix) $Ph_3P{=}CHCO_2Et$, (80%); (x) H^+, MeOH; (xi) $HgOAc_2$, H^+, $NaBH_4$ (55%); (xii) TPAP, NMO; (xiii) In, allyl bromide; (xiv) TPAP, NMO, Al_2O_3 (neutral) (95%). (b) (i) Allylbromide, Zn, THF–H_2O (NH_4Cl) (85%); (ii) $PdCl_2{\cdot}2MeCN$, $CuCl_2$, MeOH, CO (1 atm) (81%); (iii) O_3, DCM, DMS, −78°C; (iv) In, allyl bromide; (v) Swern Ox-isomerisation (62% over three steps).

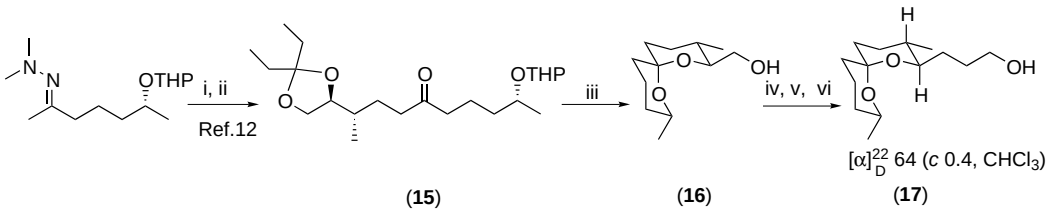
Table 1. ^{13}C and ^1H NMR data for tetrahydropyran **10**, spiroacetal **17** and corresponding positions in bistramide A

Carbon position		1	2	3	4	5	6	7	8	9	10	11	12
Bistramide A	δ_C	18.4	144.6	132.1	198.8	45.2	64.8	30.4	26.5	33.3	17.1	74.8	32.3
	δ_H	1.90	6.88	6.09	–	2.88, 2.50	4.18	1.61, 1.34	1.58, 1.27	1.89	0.84	4.04	2.73, 2.11
Isomer (**10**)	δ_C	18.2	143.1	132.4	198.8	45.8	66.9	30.5	26.5	32.6	16.5	74.1	33.0
	δ_H	1.87	6.81	6.11	–	2.76, 2.50	4.09	1.74, 1.28	1.61, 1.37	1.93	0.80	4.27	2.69, 2.33
Carbon position		20	21	22	23	24	25	26	27	28	29	30	31
Bistramide A	δ_C	25.3	30.4	74.3	34.9	18.0	27.9	36.1	95.5	35.4	19.2	31.3	69.1
	δ_H	1.80, 1.51	1.70, 1.34	3.12	1.29	0.78 (Me)	1.51, 1.42	1.57, 1.44	–	1.56, 1.35	1.80, 1.51	1.38, 1.11	3.42
Spiroacetal (**17**)	δ_C	28.5	29.5	74.4	34.4	17.8	27.8	36.1	96.0	35.0	19.1	32.7	65.3
	δ_H	1.79, 1.61	1.75, 1.46	3.21	1.36	0.82 (Me)	1.57, 1.46	1.61, 1.47	–	1.52, 1.35	1.83, 1.51	1.56, 1.15	3.66

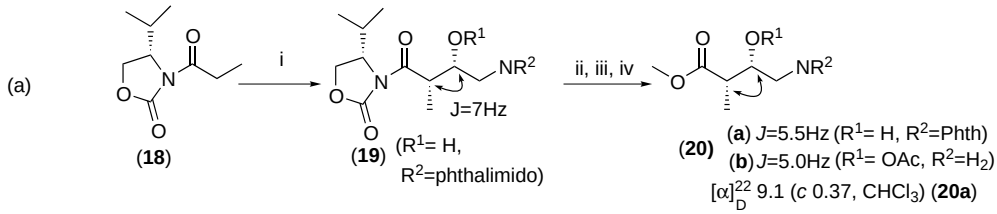
stituent induced shifts in tetrahydropyrans, and are supported by crucial *vic*-^1H–^1H coupling constants.[9] The NMR data for isomer **10** along with the data for the relevant portion of bistramide A are shown in Table 1 and confirm the stereochemistry depicted in **2**. The data for the other isomers provide inferior matches.

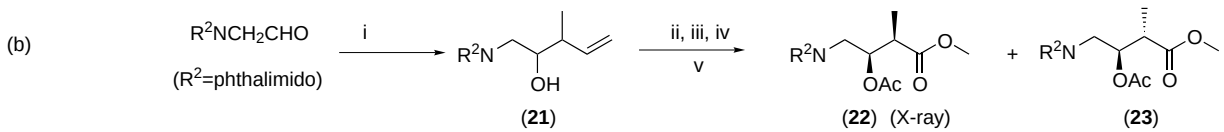
For the spiroacetal unit, the open chain keto-diol precursor was configured so that spirocyclisation would provide that diastereomer deduced from the NMR spectra to be present in bistramide A. Thus, the procedure in Scheme 2 delivers a single enantiomer of alcohol **16**,[14] which on chain extension provided **17**. This approximates the C-19–C-32 portion of bistramide A. The NMR data for **17** and bistramide A are also summarised in Table 1 and for the system from C-21 to C-30 the agreement is outstanding. Coupling constants also match very well.[15] Thus, the (22*S**,23*R**, 27*R**,31*R**) stereochemistry of the C-21–C-32 spiroacetal portion of bistramide A is confirmed.

Aldol methodology has been employed to deliver the γ-amino acid fragment.[16] For example, the boron enolate[17] ($^nBu_2BOTf$) (Scheme 3a) provides *syn*-aldol



Scheme 2. (i) BuLi, THF, HMPA, 2,2-diethyl-4-(*S*)-(2-iodo-1-(*R*)-methylethyl)-[1.3]dioxolane;[13] (ii) SiO_2 (48%, two steps); (iii) THF, H_2O conc. HCl (59%); (iv) Swern Ox., $Ph_3P{=}CHCO_2Et$ (in situ); (v) H_2 Pd/C (47%, three steps); (vi) $LiAlH_4$ (55%).





Scheme 3. (a) (i) $^iPr_2NEt$, DCM, 0°C, $^nBu_2BOTf$, 0°C, R^2NCH_2CHO (65%); (ii) NaOMe, MeOH (75%); (iii) Ac_2O, pyridine; (iv) $NH_2NH_2{\cdot}xH_2O$, EtOH (50%). (b) (i) $CH_3CH{=}CHCH_2Cl$, Zn, THF–H_2O (NH_4Cl) (87%); (ii) Ac_2O, Py, (81%); (iii) $KMnO_4/H_2O$–C_6H_6, HOAc, TBAI; (iv) CH_2N_2, ether (52% over iii and iv); (v) HPLC, hexane–EtOAc.

(**19**) (J_{vic} = 7 Hz) and thence ester **20a**, with [α]$_D^{22}$ 9.1 (*c* 0.37, $CHCl_3$). The lithium enolate (LDA) furnished an aldol mixture (2.5:1) with the *syn* isomer, alternative to **19**, predominating (J = 10.5 Hz).[18] The highly regioselective Zn-mediated α-methylallylation[19] of protected α-aminoethanal (Scheme 3b) followed by oxidation[20] of the protected homoallyl alcohol **21** and esterification provided the racemic, separable *syn* and *anti* γ-amino esters **22** and **23** (1:1). The stereochemistry of **22** (see Scheme 3b) was confirmed by X-ray analysis.[21] The $^3J_{2-3}$ values were ca. 5.0–7.0 Hz for derivatives of the *syn*-ester and 7.8 Hz for the *anti*-ester, with the corresponding value ($^3J_{15-16}$) in bistramide A being 5.5 Hz.

Overall, the data require that the (6*R**,9*S**,11*S**) stereochemistry for bistramide A applies also to bistramide C and the (22*S**,23*R**,27*R**,31*R**) stereochemistry is very likely in bistramides B, C, D and K[22] also. Further synthetic endeavours with respect to the spiroacetal appended with the C32–C40 side chain, and studies of the hydrolytically derived fragments from bistramide A are being undertaken and will be described at a later date.[23]

Acknowledgements

The authors are grateful to the Australian Research Council for support and to Dr. Ian Paterson (University of Cambridge) for helpful discussions.

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7. For example, in **2** the signal for H-11 (δ 4.04) has discernible coupling constants of 10.7 and 4.7 Hz, whereas J_{11-12} are 11.7 and 1.8 Hz. Consequently, J_{9-11}

of 4.7 Hz requires that either the CH_3 group (attached to C-9) or the CH_2CO moiety (C-12) is *axial*. The CH_3 group (attached at C-9) is confirmed as *equatorial*, because there is an NOE between CH_3 (C-10) and the H-12 proton pair. (Not shown on **2**.) Sub-structure **3** extends from C-19 to C-40 and the CH_3-doublet (C-24) (δ 0.78) provided an unambiguous starting point for the spectral assignments. In the truncated spiroacetal moiety **3**, an NOE links H-22 (δ 3.12) and H-31 (δ 3.42), requiring the (*E*,*E*) configured arrangement **3** with the alkyl appendages at C-22 and C-31 *equatorially* oriented. Furthermore, H-22 appears as a triplet of doublets (2×9.8 Hz; 2.3 Hz) which is consistent with an *axial–axial* coupling to H-23, as is the NOE correlation between H-22 (confirmed as *axially* oriented) and the (C-24) methyl group.

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9. For example, in isomer **10**, H-6 has *vic*-couplings to H-7*ax* and H-7*eq* of 10.9 and 5.4 Hz, requiring H-6 to be *axial* and the enone grouping to be *equatorial*. Similarly J_{11-9} of 4.8 Hz requires either the C-10 methyl group or the C-11 ester group to be *axial*, and the other *equatorial*. The chemical shifts of the methyl group C-10 (δ 16.5) and of C-12 (δ 33.0) require these groups to be *equatorial* and *axial*, respectively, with the latter orientation inducing the higher field shift for C-6 (δ 66.9) (γ-effect).
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14. Mesylation and reduction of alcohol **16** afforded a single 2,3,8-trimethyl-1,7-dioxaspiro[5.5]undecane with (2*R*,3*S*,6*S*,8*R*) stereochemistry and $[\alpha]_D^{22}$ 68.8 (*c* 0.08, $CHCl_3$). NMR spectra matched those from the isomer with $[\alpha]_D^{24}$ −69.4 (*c* 0.089, $CHCl_3$), previously incorrectly assigned as the (2*S*,3*R*,6*S*,8*R*) stereochemistry, because the now verified NOE between H-2 and H-8 was not detected. (Tu, Y. Q.; Hubener, A.; Zhang, H.; Moore, C. J.; Fletcher, M. T.; Hayes, P.; Dettner, K.; McErlean, C. S. P.; Kitching, W. *Synthesis* **2000**, 1956.) In this reference, the spiroacetal **40** should be **38**, with (2*S*,3*R*,6*R*,8*S*) stereochemistry. This correction establishes the stereochemical course of the reactions in Schemes 3 and 4 of that reference.
15. The data for H-22 in bistramide A (δ 3.12, td, J=9.8, 2.3 Hz) agrees well with the corresponding data for spiroacetal **17** (δ 3.21, td, 9.8 and 2.6 Hz), with one of the large couplings (9.8 Hz) requiring both H-22 and H-23 to be *axial*.
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18. There are two diastereomeric *syn* isomers (at the carbons bearing the methyl and OR^1 groups) because of the chirality of the auxiliary. After auxiliary removal, the *syn* isomers are enantiomeric. This follows from the NMR identity with **20a**, and the smaller, but opposite sign of rotation ($[\alpha]_D^{25}$ −3.8 (*c* 0.68, $CHCl_3$)) as expected for a 2.5:1 ratio of *syn* aldols, with **20a** now the minor form.
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21. We are grateful to Dr. Paul Bernhardt for this X-ray analysis.
22. Bistramides A–D and K have been described[4] as amorphous solids and dextrorotatory, with specific rotations (CH_2Cl_2 solution) of 10° for A, B and C, 8° for D and 20° for K.
23. Characterisation data for selected compounds: Compound **5**: HREIMS: calcd for $C_{15}H_{22}S_2O_2$, 298.10557; measured, 298.10526. Compound **9**: calcd for $C_{12}H_{23}O_4$ (M+H), 231.15780; measured, 231.15840. Compound **10**: calcd for $C_{15}H_{24}O_4$, 268.16557; measured, 268.16595, (NMR data for **10** is in Table 1). Compound **12**: calcd for $C_{10}H_{18}O$, 154.1357; measured, 154.1356. Compound **13**: calcd for $C_{12}H_{20}O_3$, 212.1412; measured, 212.1418. Spiroacetal **16**: for $C_{12}H_{22}O_3$ calcd C, 67.3; H, 10.3. Found C, 67.0; H, 10.6. $[\alpha]_D^{25}$ +64.0 (*c* 0.4, pentane). MS: 214 (7, M^+), 183 (57), 154 (11), 142 (29), 127 (38), 115 (78), 112 (100), 97 (45), 84 (48), 55 (90), 43 (86). ^1H NMR: ($CDCl_3$) 3.72 (1H, H_A of ABX, dd, 11.5, 2.5), 3.67 (1H, dqd, 11.5, 6.5, 2.0), 3.52 (1H, H_B of ABX, dd, 11.5, 7.5), 3.32 (1H, ddd, 10.0, 7.5, 2.5), 1.80 (1H, qt, 13.0, 4.5), 1.73–1.12 (10H, m), 1.11 (3H, d, 6.5), 0.88–0.85 (1H, m), 0.83 (3H, d, 6.5). ^{13}C NMR: ($CDCl_3$) 17.2 (CH_3), 19.1 (CH_2), 21.8 (CH_3), 27.4 (CH_2), 31.1 (CH), 32.5 (CH_2), 35.8 (CH_2), 35.9 (CH_2), 63.3 (CH), 64.0 (CH_2), 74.7 (CH), 95.8 (C); spiroacetal **17**: for $C_{14}H_{26}O_3$ calcd 242.1882; measured 242.1885 $[\alpha]_D^{25}$ +61.6 (*c* 0.17, $CHCl_3$) MS: 242 (1, M^+), 224 (2), 154 (33), 115 (19), 112 (100), 97 (13), 83 (11), 71 (7), 67 (7), 55 (21), 43 (21), 41 (20). ^1H NMR: ($CDCl_3$) 3.70–3.59 (3H, m), 3.21 (1H, td, 9.8, 2.6), 1.89–1.74 (3H, m), 1.63–1.23 (13H, m), 1.11 (3H, d, 6.3), 0.81 (3H, d, 6.5). (C_6D_6) 3.73 (1H, dqd, 11.3, 6.2, 2.2), 3.50 (2H, dt, 6.2, 2.3), 3.30 (1H, td, 9.4, 2.6), 1.98 (1H, qt, 13.6, 4.1), 1.86–1.48 (14H, m), 1.14 (3H, d, 6.0), 1.13–1.05 (1H, m), 0.71 (3H, d, 6.4). ^{13}C NMR: ($CDCl_3$) 96.0 (C), 74.4 (CH), 65.3 (CH), 63.3 (CH_2), 36.1 (CH_2), 35.6 (CH_2), 34.4 (CH_2), 32.7 (CH_2), 29.5 (CH_2), 28.5 (CH_2), 27.8 (CH_2), 21.8 (CH_3), 19.1 (CH_2), 17.8 (CH_3). Aldol **19**: for $C_{19}H_{22}O_6N_2$ calcd C, 60.96; H, 5.88; found C, 60.71; H, 6.08. $[M+1]^+$ $C_{19}H_{23}O_6N_2$ calcd 375.1558, measured 375.1564. ^1H NMR: ($CDCl_3$) 0.84–0.90 (6H, d, 7.0), 1.35 (3H, d, 7.0), 2.29 (1H, m), 3.75 (1H, m), 3.74–3.97 (2H, ABX, 14.5, 6.5, 4.0), 4.18

(1H, dd, 9.0, 3.0), 4.25 (1H, m), 4.34 (1H, t, 9.0), 4.48 (1H, m), 7.67–7.87 (4H, m). ^{13}C NMR: 12.9, 14.9, 17.9, 28.6, 40.5, 41.4, 58.3, 63.6, 70.4, 123.6, 131.9, 134.3, 169.0, 176.1 (some signal overlap). Ester **20a**: for $C_{14}H_{15}O_5N$ calcd C, 60.64; H, 5.40; found C, 60.10; H, 5.69. $[\alpha]_D^{25}$ +9.1 (*c* 0.37, $CHCl_3$). ^{1}H NMR: 1.27 (3H, d, 7.0), 2.58 (1H, m), 3.11 (1H, br s, OH), 3.66 (3H, s), 3.70–3.84 (2H, ABX, 14.0, 8.0, 4.5), 4.18 (1H, m), 7.67–7.79 (4H, m). ^{13}C NMR: 11.3, 41.4, 42.7, 51.9, 70.1, 123.3, 131.8, 134.1, 168.6 (some signal overlap).