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Establishment of the absolute configuration of the bioactive marine alkaloid eudistomin X by stereospecific synthesis

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ABSTRACT

A stereospecific synthesis of both enantiomers of the marine alkaloid eudistomin X using the amino acid chiral pool is achieved. Comparison of ^{1}H and ^{13}C NMR chemical shifts of the synthetic product as either the free base, mono-salt or disalt with those reported for the natural product established that the ascidian metabolite was originally characterised as a mono-salt and that the absolute configuration was (10*R*).

Marine organisms of the class Ascidiacea (ascidians) are noted for their abilities to biosynthesise secondary metabolites derived from amino acids.[1,2] After cyclic peptides, one of largest subclasses of metabolites reported are the β-carboline alkaloids, exemplified by eudistomins C (**1**),[3] G (**2**), H (**3**) and I (**4**),[4] woodinine (**5**),[5] eudistalbin A (**6**)[6] and eudistomidin B (**7**)[7] (Fig. 1). Biosynthetically, these alkaloids are derived from tryptophan/tryptamine condensation with a second amino acid, as evidenced by ^{3}H-labelled L-proline incorporation into eudistomins G and H.[8] The eudistomin alkaloids appear to serve ecological roles related to inhibition of larval settlement[9] though much of the discovery and synthetic chemistry attention paid to this class of alkaloid derives from their potent pharmacological activities.[10] Of the 65 β-carboline-containing alkaloids reported from ascidians to date,[11] only 13 contain an asymmetric centre at C-10 with defined configuration, potentially suggestive of the configuration of the second amino acid utilised in the biosynthetic pathway. The oxathiazepine ring containing examples, eudistomins C (**1**), E, F, K, K-sulfoxide, debromo K and L have (10*S*) configuration consistent with biosynthetic incorporation of D-cysteine,[3] as do the structurally-simpler analogues, eudistomidin C[7a] and an *N*-methyl analogue.[12]

The (10*S*) absolute configuration of woodinine (**5**),[5] eudistalbin A (**6**)[6] and eudistomidins B (**7**)[7] and G[7c] is consistent with the

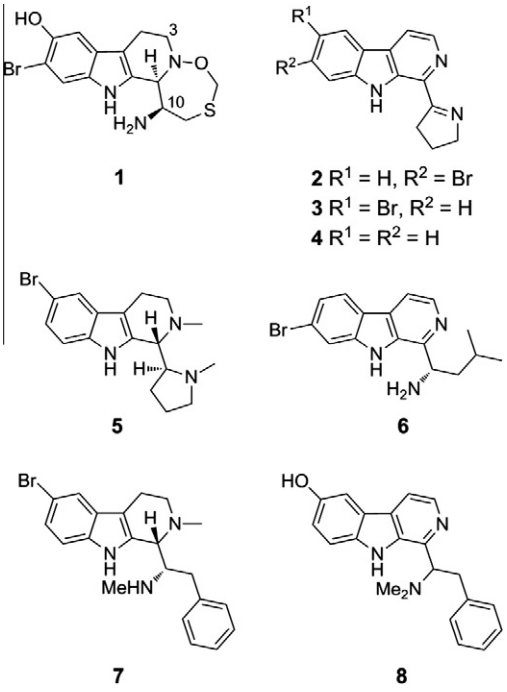

Figure 1. Structures of eudistomins C (**1**), G (**2**), H (**3**) and I (**4**), woodinine (**5**), eudistalbin A (**6**), eudistomidin B (**7**) and eudistomin X (**8**).

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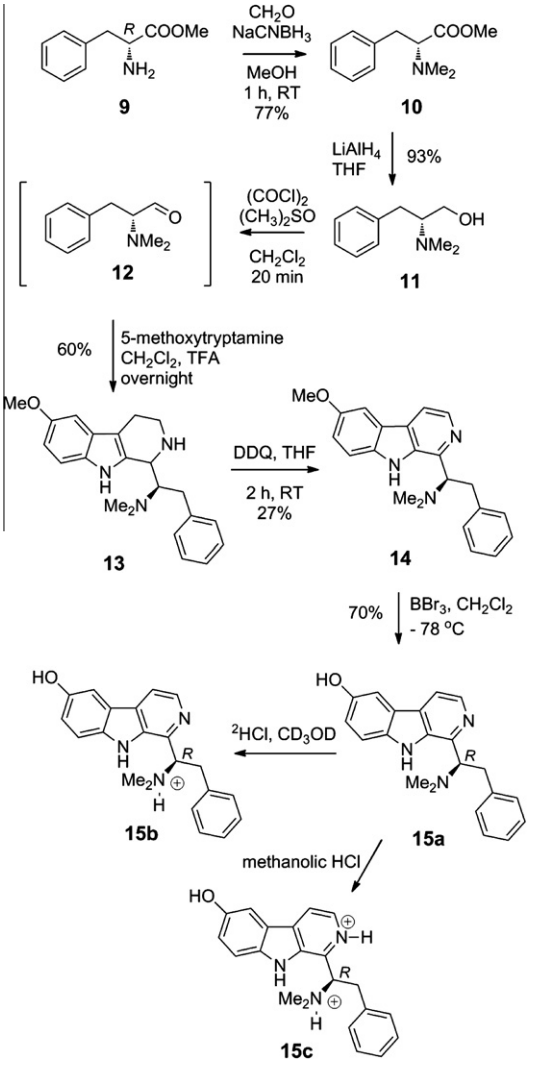
incorporation of L-proline, L-leucine and L-phenylalanine, respectively, during the biosynthesis of these natural products.

The 2003 publication of eudistomin X (**8**) from a Micronesian collection of *Eudistoma* sp. was of interest to us due to it being chiral, but with undefined configuration and that it exhibited relatively potent in vitro antimicrobial properties.[13] We undertook the stereospecific synthesis of both enantiomers of **8**, using the amino acid phenylalanine as the chiral pool source, to address two questions: what is the C-10 configuration of eudistomin X, and is there any influence of the C-10 configuration on the biological activity of the alkaloid? Our synthesis of eudistomin X made use of the classical Pictet-Spengler β-carboline ring-forming reaction between an appropriately substituted (1*H*-indol-3-yl)ethanamine and a chiral aldehyde, derived from phenylalanine.[14] (*R*)-(−)-2-(*N*,*N*-dimethylamino)-3-phenyl-propionic acid methyl ester (**10**) ($[\alpha]_D$ −5.0 (*c* 2.84, ethyl acetate), lit.[15] data for the (*S*) enantiomer $[\alpha]_D$ +4.7 (*c* 2.84, ethyl acetate)) was prepared in 77% yield by reaction of the amino acid methyl ester (**9**) with formaldehyde and sodium cyanoborohydride in methanol (Scheme 1).[16] Reduction of the ester functionality present in **10** using $LiAlH_4$ in THF afforded chiral aminoalcohol **11** ($[\alpha]_D$ +19.3 (*c* 1.00, CH_2Cl_2), lit.[17] data for the (*S*) enantiomer, also as the free base $[\alpha]_D$ −18.4 (*c* 1.00, CH_2Cl_2)) in 93% yield. Oxidation under Swern conditions yielded unstable (*R*)-2-(dimethylamino)-3-phenylpropanal (**12**) that was used in the next step without purification. Pictet-Spengler reaction of chiral aldehyde **12** with 5-methoxytryptamine yielded the expected tetrahydro-β-carboline (**13**) as a yellow foam in 60% yield. The subsequent sequence of oxidation with DDQ in THF (27% yield), de-*O*-methylation using BBr_3 in dichloromethane (70% yield), and finally silica gel column chromatography using 5% MeOH/CH_2Cl_2 with 2% NH_3 as eluent afforded (10*R*)-eudistomin X as the free base (**15a**). The optical rotation observed for **15a** ($[\alpha]_D$ −106.1 (*c* 0.49, MeOH)) was substantially different from that reported for the natural product ($[\alpha]_D$ −7.0 (*c* 0.49, MeOH)).[13] Comparison of the ^1H and ^{13}C NMR data observed for our synthetic **15a**[18] with



Scheme 1. Total synthesis of (10*R*)-eudistomin X (**15a**).

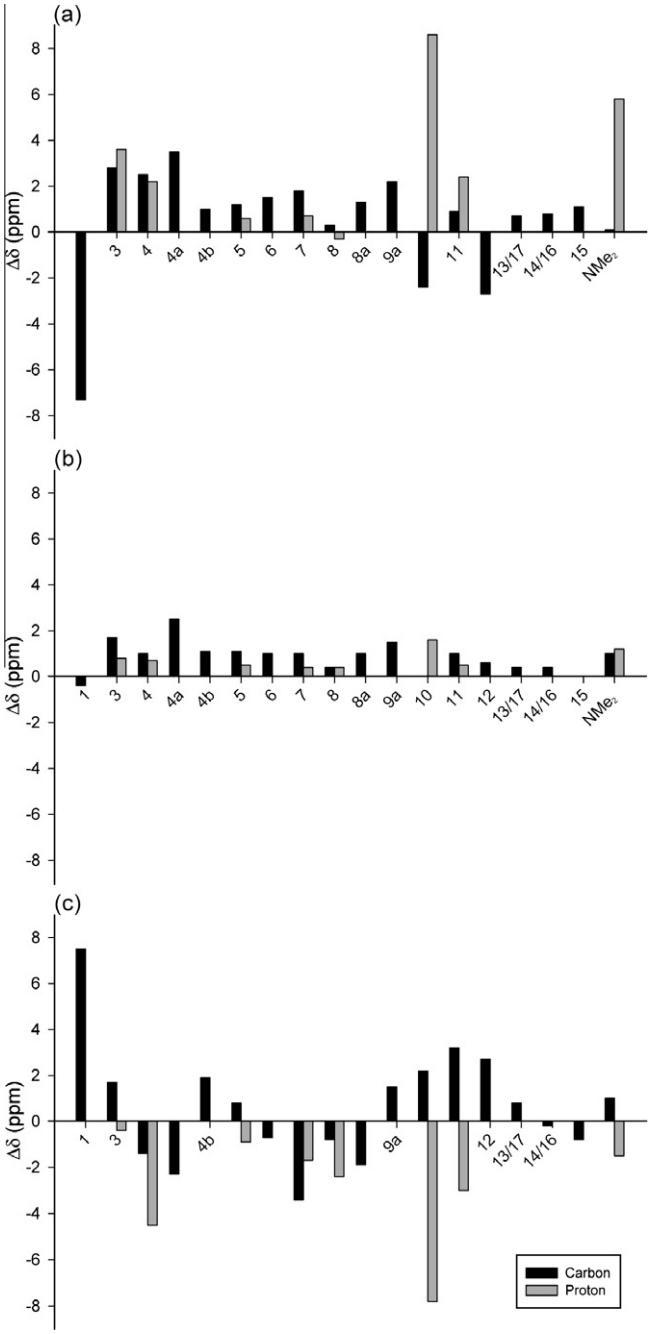


Figure 2. Plots of difference observed for ^1H and ^{13}C NMR data ($\Delta\delta = \delta_{literature} - \delta_{synthetic}$) varying by alkaloid numbered position for (a) free base **15a**, (b) mono-salt **15b**, and (c) dihydrochloride **15c** compared to published data for eudistomin X.[13] All data acquired in the same solvent (CD_3OD) at 6 mg/mL concentration. Note that $\Delta\delta$ ^1H values have been arbitrarily scaled by a factor of 10 for clarity.

the literature data for the natural product[13] indicated major chemical shift differences centred upon C-1, H-10 and the dimethylamino group (Fig. 2a). We speculated that the differences between these two data sets were the result of differing protonation states, at the more basic tertiary alkyl amine functionality, between our synthetic sample and the characterised natural product. Sub-samples of synthetic (10*R*)-eudistomin X free base (**15a**) were converted either into a mono-deuterochloride salt **15b** by careful titration with 1 mol equiv of ^{2}HCl (monitored by ^{1}H NMR spectroscopy) or to the dihydrochloride salt (**15c**), by dissolution in excess methanolic HCl.

Confirmation of the free base, dimethylammonium salt and di-salt natures of **15a**, **15b** and **15c**, respectively, were made by examination of the ^{15}N NMR chemical shifts indirectly acquired with the aid of ^{1}H–^{15}N HMBC NMR experiments (Table 1).

As expected, only minor deshielding of the ^{15}N NMR resonance observed for the dimethylamino group was detected for the mono-salt **15b**,[19] but the pyridine ^{15}N resonance N-2 shifted significantly upon protonation (**15c**).[20] Re-acquisition of the ^{1}H and ^{13}C NMR data for **15b** and **15c**[18] and comparison with published data established that the natural product had in fact been characterised as a mono-salt (of unknown counter-ion) (Fig. 2). This conclusion was further supported by comparison of the optical rotation data observed for **15a** ($[\alpha]_D$ −106.1), **15b** ($[\alpha]_D$ −7.2 (*c* 0.49, MeOH)) and **15c** ($[\alpha]_D$ +10.5 (*c* 0.49, MeOH)), for which excellent agreement with the reported rotation of the natural product ($[\alpha]_D$ −7.0)[13] was observed again for the mono-salt **15b**. This confirmed the structure of eudistomin X and established the absolute configuration of the natural product as (10*R*).

In order to realise our second goal of investigating the influence of the C-10 configuration on the antibacterial activity of eudistomin X, we repeated the synthesis utilising *S*-phenylalanine as the chiral pool starting material. As expected, unnatural (10*S*)-eudistomin X exhibited identical ^{1}H and ^{13}C NMR data, in all protonation states, to those observed for (10*R*)-eudistomin X, and showed similar magnitude but opposite sign optical rotation data to those observed earlier for the natural stereoisomer series.[21]

We evaluated penultimate methyl ether **14** and natural product **15a** in both the natural (10*R*) and unnatural (10*S*) configurations for in vitro antibacterial activity towards three species of Gram-negative marine bacteria (*Aeromonas salmonicida*, *Listonella anguillarum* and *Shewanella baltica*), Gram-negative (*Escherichia coli* DH5a) and Gram-positive bacteria (*Mycobacterium smegmatis* mc^{2}155 and *Staphylococcus aureus* Newman). Of the four compounds tested, natural (10*R*)-configuration of methyl ether **14** inhibited all three marine bacteria with modest potency (25–30 μM), while the remaining compounds were inactive (MIC ⩾250 μM). While all four compounds exhibited weak growth inhibition of *M. smegmatis* (MIC 250 μM; highest test concentration was 1000 μM), methyl ether **14** was the more active growth inhibitor against *S. aureus* (MIC 250 μM)—the other test compounds proved inactive. None of the test compounds were active towards *E. coli* (MIC ⩾1000 μM) and they were also inactive towards a panel of human tumour cell lines in testing by the Developmental Therapeutics Program, NCI. These results fail to provide any clear evidence of a C-10 stereochemical bias for biological activity.

Table 1
^{15}N NMR (40.6 MHz) chemical shifts observed for **15a**, **15b** and **15c**[a]

	N-2	N-9	NMe_2
15a	292.9	111.4	37.9
15b	289.3	111.1	47.5
15c	189.3	120.4	51.6

[a] Determined indirectly from ^{1}H–^{15}N HMBC NMR experiments, optimised for $^{x}J_{HN}$ 6 Hz, acquired in CD_3OD solvent and referenced to liquid NH_3 using urea as an external standard.

In summary, we have completed the first synthesis of eudistomin X in a stereoselective manner that defines the natural product as bearing (10*R*) configuration. Prior to this study, ascidian-derived β-carboline alkaloids bearing a stereogenic centre at C-10, the configuration of which had been rigorously determined by synthesis, were limited to those putatively derived from the biosynthetic incorporation of D-cysteine,[3] L-proline,[5] L-leucine[6] and L-phenylalanine.[7] We can now expand this series to include D-phenylalanine. The wide variation in $[\alpha]_D$ data observed for various protonation states of the same alkaloid (**15a**, **15b** and **15c**) should serve as an extra caution to natural product chemists who attempt to assign configuration solely by analogy.[22] We are currently expanding our library of natural (10*R*) and unnatural (10*S*) β-carboline alkaloids related to eudistomin X and are evaluating them for a wider range of biological properties to determine more categorically the influence of C-10 configuration on ecological and potential pharmaceutical activities.

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18. Compound characterisation data for **15a**, **15b** and **15c**. *(10R)-Eudistomin X free base (**15a**)*: $[\alpha]_D$ −106.1 (*c* 0.49, MeOH); IR ν_{max} (neat) 3221, 1824, 1567, 1493, 1434, 1196, 1025 cm^{-1}; R_f (5% MeOH/CH_2Cl_2) 0.29; ^{1}H NMR (400 MHz, CD_3OD) δ 8.07 (1H, d, *J* = 5.4 Hz, H-3), 7.77 (1H, d, *J* = 5.4 Hz, H-4), 7.45 (1H, d, *J* = 2.4 Hz, H-5), 7.38 (1H, d, *J* = 8.8 Hz, H-8), 7.07 (1H, dd, *J* = 8.8, 2.4 Hz, H-7), 6.92 (3H, m, H-14/H-16, H-15), 6.82 (2H, m, H-13/H-17), 4.16 (1H, dd, *J* = 10.4, 4.4 Hz, H-10), 3.47 (1H, dd, *J* = 13.1, 10.4 Hz, H-11a), 3.33 (1H, dd, *J* = 13.1, 4.4 Hz, H-11b), 2.37 (6H, s, NMe_2); ^{13}C NMR (100 MHz, CD_3OD) δ 152.2 (C-6), 144.7 (C-1), 139.9 (C-12), 137.3 (C-9a), 136.9 (C-3), 136.8 (C-8a), 130.2 (C-13/C-17), 130.1 (C-4a), 129.0 (C-14/C-16), 126.9 (C-15), 122.9 (C-4b), 119.5 (C-7), 114.9 (C-4), 113.5 (C-8), 106.6 (C-5), 71.4 (C-10), 43.5 (NMe_2), 38.0 (C-11); ^{15}N NMR (40.6 MHz, CD_3OD) δ 292.9 (N-2), 111.4 (N-9), 37.9 (NMe_2); (+)-ESIMS *m/z* 332 $[M+H]^+$; (+)-HRESIMS $[M+H]^+$ 332.1751 (calcd for $C_{21}H_{22}N_3O$, 332.1757). *(10R)-Eudistomin X mono-salt (**15b**)*: $[\alpha]_D$ −7.2 (*c* 0.49, MeOH) [lit.[13] −7.0 (*c* 0.49, MeOH)]; ^{1}H NMR (400 MHz, CD_3OD) δ 8.42 (1H, d, *J* = 5.4 Hz, H-3), 7.97

(1H, d, *J* = 5.4 Hz, H-4), 7.45 (1H, d, *J* = 2.4 Hz, H-5), 7.31 (1H, d, *J* = 8.8 Hz, H-8), 7.08 (1H, dd, *J* = 8.8, 2.4 Hz, H-7), 6.94 (5H, m, H-13/H-17, H-14/H-16, H-15), 5.20 (1H, dd, *J* = 11.2, 4.3 Hz, H-10), 3.70 (1H, dd, *J* = 12.1, 4.3 Hz, H-11a), 3.59 (1H, dd, *J* = 12.1, 11.2 Hz, H-11b), 2.98 (6H, s, NMe_2); ^{13}C NMR (100 MHz, CD_3OD) δ 152.8 (C-6), 138.0 (C-9a, C-3), 137.1 (C-8a), 136.6 (C-1), 136.1 (C-12), 131.4 (C-4a), 130.4 (C-13/C-17), 129.4 (C-14/C-16), 128.1 (C-15), 122.7 (C-4b), 120.5 (C-7), 116.7 (C-4), 113.5 (C-8), 106.7 (C-5), 68.4 (C-10), 42.5 (NMe_2), 37.6 (C-11); ^{15}N NMR (40.6 MHz, CD_3OD) δ 289.3 (N-2), 111.1 (N-9), 47.5 (NMe_2).

*(10R)-Eudistomin X dihydrochloride salt (**15c**):* $[\alpha]_D$ +10.5 (*c* 0.49, MeOH); ^1H NMR (400 MHz, CD_3OD) δ 8.59 (1H, d, *J* = 6.2 Hz, H-4), 8.52 (1H, d, *J* = 6.2 Hz, H-3), 7.62 (2H, m H-5, H-8), 7.33 (1H, dd, *J* = 8.8, 2.5 Hz, H-7), 7.25 (2H, d, *J* = 7.5 Hz, H-13/H-17), 7.04 (2H, m, H-14/H-16), 6.96 (1H, m, H-15), 6.13 (1H, br m, H-10), 4.09 (1H, dd, *J* = 13.8, 12.4 Hz, H-11a), 4.02 (1H, dd, *J* = 13.8, 5.5 Hz, H-11b), 3.20 (6H, br s, NMe_2); ^{13}C NMR (100 MHz, CD_3OD) δ 154.7 (C-6), 140.6 (C-8a), 136.9 (C-4a), 134.3 (C-12), 131.0 (C-3), 130.0 (C-13/C-17 and C-14/C-16), 128.9 (C-15), 128.7 (C-1), 125.5 (C-7), 121.7 (C-4b), 119.3 (C-4), 114.8 (C-8), 107.1 (C-5), 66.4 (C-10), 42.8 (NMe_2), 35.2 (C-11), C-9a not observed; ^{15}N NMR (40.6 MHz, CD_3OD) δ 189.3 (N-2), 120.4 (N-9), 51.6 (NMe_2).

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21. (10*S*)-Eudistomin X free base $[\alpha]_D$ +91.0 (*c* 0.49, MeOH); (10*S*)-eudistomin X mono hydrochloride salt $[\alpha]_D$ +7.3 (*c* 0.49, MeOH); (10*S*)-eudistomin X dihydrochloride salt $[\alpha]_D$ −9.8 (*c* 0.49, MeOH).
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