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COMMUNICATION

Total syntheses of subereamollines A and B†

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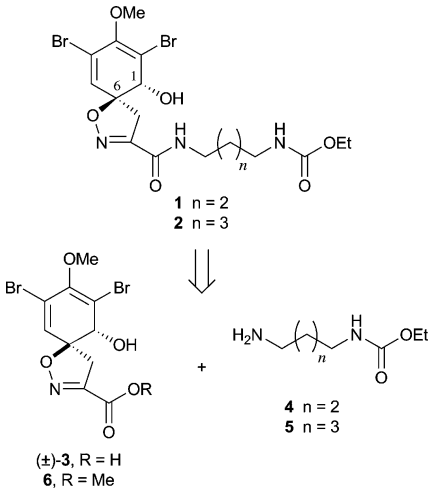
The first total syntheses of (+)- and (−)-subereamollines A and B are reported. The enantiomeric forms of the natural products were obtained by preparative chiral HPLC separation of the corresponding racemates.

Marine sponges of the order Verongida are a rich source of bromotyrosine-derived natural products. Many of these compounds are biologically active and exhibit a range of activities including antiviral,[1] antimicrobial,[2] antifungal,[3] Na^+/K^+ ATPase inhibition,[4] anti-HIV,[5] HDAC inhibition,[6] antifouling,[7] histamine H3 antagonism,[8] mycothiol *S*-conjugate amidase inhibition,[9] and isoprenylcysteine carboxy methyltransferase (Icmt) inhibition.[10] Anticancer activity has also been reported, for example, the spirocyclohexadienylisoxazolines purealidin P and Q are cytotoxic against murine lymphoma K1210 (IC_{50} 2.8 and 0.95 μg mL^{-1} respectively) and human epidermal carcinoma KB (nasopharynx) (IC_{50} 7.6 and 1.2 μg mL^{-1} respectively) cell lines.[11,12] Other structurally related compounds have also exhibited cytotoxic properties.[13]

When the extracts of the Red Sea sponge *Suberea mollis* were reinvestigated recently, two new bromotyrosine-derived secondary metabolites containing the spirocyclohexadienylisoxazoline moiety were identified; subereamollines A (**1**) and B (**2**) (Scheme 1).[14] Small quantities (2–5 mg) were isolated and **1** was found to have modest antimicrobial activity against *Staphylococcus aureus*, however, their cytotoxic activity was not reported. As part of our ongoing investigation into the anticancer properties of bromotyrosine-derived natural products,[15] the subereamollines were attractive synthetic targets.

Both **1** and **2** contain a 2,4-dibromo-1-hydroxy-3-methoxyspirocyclohexadienylisoxazole moiety attached to a carbamate containing side chain *via* an amide linkage. Although not explicitly stated in the isolation paper,[14] the absolute stereochemistries at C(1) and C(6) were determined to be (*R*) and (*S*) based on the optical rotations of **1** {$[\alpha]_D$ +156.5 (*c* 0.55, MeOH)} and **2** {$[\alpha]_D$ +22.9 (*c* 6.25, CH_2Cl_2)}. These data correlated with that reported for (+)-aerothionin {$[\alpha]_D$ +210 (*c* 1.7, MeOH)} whose absolute configuration was determined by X-ray crystallography and circular dichroism (CD) spectroscopy.[16]



Scheme 1 Retrosynthetic analysis of subereamollines A (**1**) and B (**2**).

We envisaged that **1** and **2** could be synthesised by coupling spiro acid (±)-**3** with either amine **4** or **5** (Scheme 1). Interestingly, **3** has been isolated from the marine sponge *Pseudoceratina* sp. as an optically pure isomer with its absolute configuration defined as 1*S*,6*R* by CD analysis.[17] However, the only reported synthesis of (±)-**3** is as an intermediate during the synthesis of purealin.[18] The corresponding methyl ester **6**[19–22] has been synthesised in an enantioenriched form (74% *ee*) *via* an asymmetric oxidative spirocyclisation which required a multi-step synthesis of a chiral auxiliary.[23] An alternative approach led to enantiopure **6** by resolution of (±)-**6** by derivatisation with (−)-camphanic chloride.[24]

Our approach to the naturally occurring enantiomers of **1** and **2** involved chiral HPLC separation of the corresponding racemates as this would require no additional synthetic operations and would also deliver enantiopure material required for biological screening.

Aldehyde **8** was prepared in 92% yield over two steps *via* bromination of 2-hydroxy-4-methoxybenzaldehyde (**7**) with *N*-bromosuccinimide and subsequent benzyl protection of the phenolic oxygen (Scheme 2). Conversion of **8** into the corresponding azlactone **9** was achieved by heating the aldehyde with *N*-acetylglycine and sodium acetate in the presence of acetic anhydride (Erlenmeyer conditions).[25] Saponification of the crude azlactone **9** with barium hydroxide and subsequent condensation with *O*-benzylhydroxylamine furnished the carboxylic

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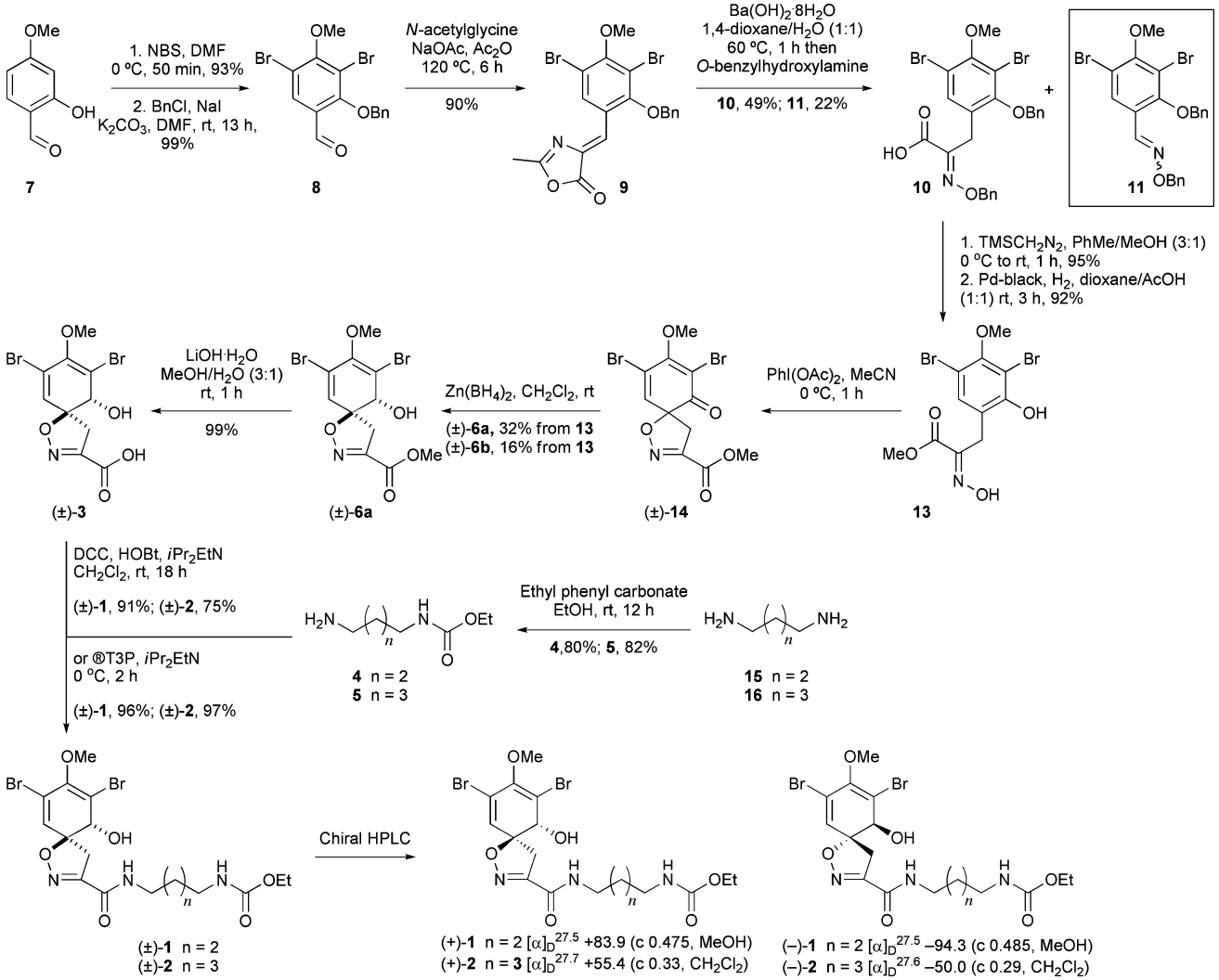
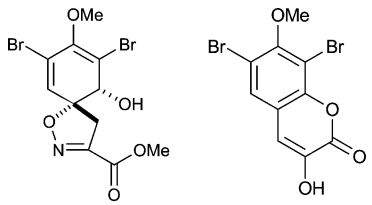
† Electronic supplementary information (ESI) available: experimental procedures for the preparation of compounds **1–6** and **8–14** and full characterisation of these compounds. ^1H and ^{13}C NMR spectra of **1** and **2**. CCDC reference numbers 787666 and 787667. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c0ob00636j



Scheme 2 Synthesis route to **1** and **2**.

acid **10** in 49% yield. This yield did not improve when **9** recrystallised from acetone was used. Furthermore, oxime **11** was discovered to be a major by-product (22% yield), which may have arisen from reversion of **9** to its parent aldehyde under the basic conditions and subsequent condensation with the *O*-benzylhydroxylamine. Azlactones are known to hydrolyse efficiently under acidic conditions (*e.g.* 3 N HCl, reflux, 24 h)[26] to give α-keto acid derivatives which can then be converted to the corresponding oxime acids.[27] However, when these conditions were applied to **9** this unexpectedly resulted in the loss of the phenolic benzyl protecting group and spontaneous lactonisation to produce coumarin **12** (in 68% yield) as the only observable product.[28]



(±)-**6b** **12**

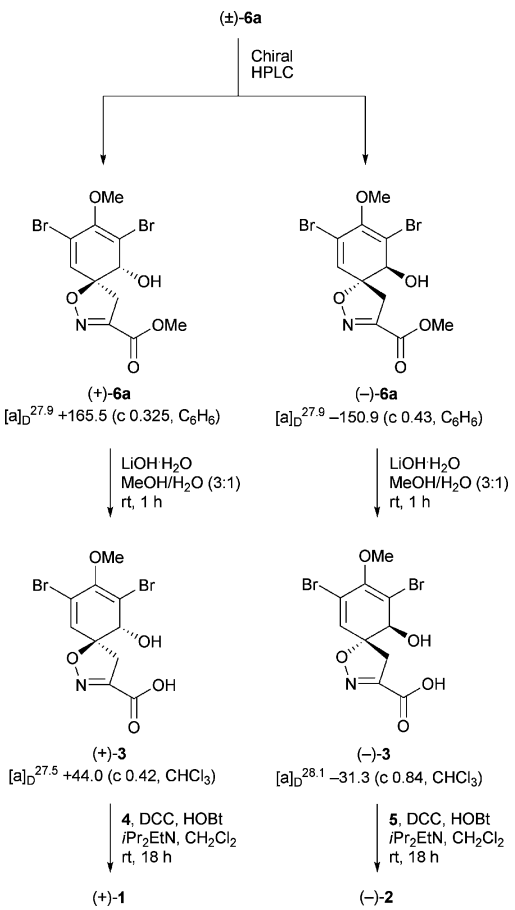
The cyclisation precursor, oxime methyl ester **13**, was obtained first by treatment of **10** with trimethylsilyldiazomethane to obtain its methyl ester then removal of the benzyl groups *via* hydrogenolysis over palladium black.[18] The oxidative cyclisation to transform **13** to spiroisoxazoline (±)-**14** in other syntheses has been achieved with thallium(III) trifluoroacetate,[19] electroorganic oxidation[20] and *N*-bromosuccinimide.[18] However, we found that iodobenzene diacetate[21,22] was the most convenient reagent on this occasion since the crude material could be taken on directly to the next step following an aqueous work up. Diastereoselective reduction of (±)-**14** with $Zn(BH_4)_2$[29] furnished the desired *trans*-isomer (±)-**6a** in a 2.03 : 1 *dr* over the undesired *cis*-isomer (±)-**6b**. X-Ray crystallographic analysis confirmed the relative stereochemistry of each isomer.[30] The methyl ester was then hydrolysed with lithium hydroxide to furnish (±)-**3** in an overall yield of 11% from aldehyde **7**.

Putrescine (**15**) and cadaverine (**16**) were converted to the amine coupling partners **4** and **5** in one step *via* dropwise addition of ethyl phenyl carbonate[31] to minimize *bis*-carbamylation. Spiro acid (±)-**3** was then coupled to amine **4** or **5** in the presence of *N*,*N*′-dicyclohexylcarbodiimide (DCC) and hydroxybenzotriazole

(HOBt) to afford the natural products subereamolline A (**1**) and B (**2**) respectively in 91% and 75% yield as a racemic mixture. Upon reinvestigation of this coupling step, it was found that the use of the coupling agent propylphosphonic anhydride (®T3P) furnished **1** and **2** in 96% and 97% yields respectively.

The ^{1}H and ^{13}C NMR spectra of synthetic **1** and **2** matched those of the natural materials.[32] We have also established that the originally reported ^{13}C chemical shift assignments for C-2 (δ_C 122.9) and C-4 (δ_C 114.3) should be switched on the basis of COLOC NMR experiments reported for related spirocyclised products.[33] This is also supported by the HMBC correlations in the natural products.[34]

The enantiomeric forms of (±)-**1** and (±)-**2** were separated using preparative chiral HPLC (CHIRALPAK AD-H column and 10% iPr$_2$OH in hexane at a flow rate of 5 mL min^{-1}) (Scheme 2). The optical rotations of (+)-**1** (t = 40.3 min) and (+)-**2** (t = 48.3 min) agreed with those observed for natural **1** and **2**. To confirm the absolute stereochemistries at C(1) and C(6), (+)-**6** (t = 30.9 min) and (–)-**6** (t = 32.9 min) were obtained from chiral HPLC separation of (±)-**6** (Scheme 3).[35] Hydrolysis of (+)-**6** and (–)-**6** under the basic conditions furnished acids (+)-**3** and (–)-**3** that were then coupled with amines **4** and **5** respectively. HPLC analysis of the products showed them to be (+)-**1** and (–)-**2** respectively (see Electronic Supplementary Information), thus confirming the original stereochemical assignments at C(1) and C(6).



Scheme 3

MTS assays[36] were performed to evaluate the cytotoxic activity of compounds (±)-**1**, (±)-**2**, (+)-**1**, (+)-**2**, **12** and **13** against an ovarian cancer (SK-OV-3) cell line. Following 48 h treatment, none of the compounds exhibited cytotoxic activities up to a concentration of 100 μM.

In conclusion, the first total syntheses of the bromotyrosine-derived natural products subereamolline A (**1**) and B (**2**) have been reported. The enantiomeric forms of **1** and **2** were obtained by chiral HPLC separation and, in addition, the stereochemistries at C(1) and C(6) in the naturally occurring enantiomers (+)-**1** and (+)-**2** were confirmed to be *R* and *S* respectively.

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35 Both (+)-**6a** (t = 30.9 min) and (−)-**6a** (t = 32.9 min) were converted to their corresponding camphanate esters and their NMR spectral data matched that reported by Nishiyama and co-workers (see Ref. 24).
36 The (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium) (MTS) assay gives a colourimetric measure of the number of viable cells present *in vitro*.