A synthesis-enabled relative stereochemical assignment of the C1–C28 region of hemicalide†

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Through synthesising both candidate diastereomers of a model C1–C28 fragment of the potent cytotoxic marine polyketide hemicalide, an assignment of the relative configuration between the C1–C15 and C16–C26 regions has been achieved. By detailed NMR comparisons with the natural product, the relative stereochemistry between these two 1,6-related stereoclusters is elucidated as 13,18-syn rather than the previously proposed 13,18-anti relationship. A flexible and modular strategy using an advanced C1–C28 ketone fragment 22 is outlined to elucidate the remaining stereochemical features and achieve a total synthesis.

Extracted from the marine sponge *Hemimycale* sp., hemicalide (1, Fig. 1) was reported to display impressive picomolar IC₅₀ values against a panel of human cancer cell lines. Initial studies pointed towards a novel antimitotic mechanism of action via microtubule destabilisation, but its low isolation yield (0.5 mg) precluded further biological evaluation.¹ While extensive 1D and 2D-NMR experiments were able to ascertain the planar structure of hemicalide, the paucity of material rendered its full 3D structural elucidation elusive. Indeed, all 21 stereocentres were left unassigned in the patent application, leading to over 2 million possible permutations.

Through a combination of computational NMR shift predictions and spectroscopic corroboration from synthesised model fragments, we, together with the Ardisson and Cossy/Meyer groups, have assigned the relative configuration of hemicalide for the C8–C13 stereohexad 2,²,³ C16–C26 dihydroxylactone ³⁴,⁵ and C35–C42 hydroxylactone ⁴.⁶ To date, the relative and absolute configuration of the C26–C34 polyacetate region, and the isolated C45 stereocentre remained unassigned.⁶ While efforts have been reported by the Ardisson and Cossy/Meyer groups towards joining the fragments together, previously reported synthetic studies on the full C1–C27 fragment have targeted the 13,18-anti diastereomer ⁵ᵃ;⁵,⁷ only one of the two diastereomeric possibilities between the C1–C15 and the C16–C26 regions. Through the synthesis and detailed NMR spectroscopic comparisons of
both candidate diastereomers, we herein report the likely relative configuration of the full C1–C28 region of hemicalide as in the 13,18-syn diastereomer 5b.

Our approach needed to be modular and highly stereoselective to allow for the facile synthesis of both enantiomers of each fragment. Given that the majority of the missing stereochemical information lies in the C27–C34 region, a flexible strategy was devised involving a late stage aldol/reduction sequence to forge the C27 and C29 stereocentres (Scheme 1). A cross-coupling was envisaged to connect the C34–C46 hydroxylactone 6 with each enantiomer of the C29–C33 aldehyde, as well as the C1–C15 and C16–C28 fragments 8 and 9 together.

Our first quest towards determining the complete stereochemistry of hemicalide began with the C1–C28 region, consisting of the C1–C15 and the C16–C28 stereocenters. With computational methods proving useful in elucidating the relative configuration within the isolated fragments,2,4 we initially sought to employ our DPff methodology2 to elucidate their relative stereochemical relationship. However, due to the size and flexibility of the virtual fragments involved, it proved to be too computationally demanding to accurately model the C1–C28 truncate. As such, we turned towards synthesising both candidate diastereomers of the full C1–C28 region to elucidate the relative configuration between these two stereocenters. We envisioned that meaningful NMR differences could be observed for each diastereomer despite the relatively remote 1,6-related chiral environments between C13 and C18 adjacent to the connecting E,E-diene.8

The stereohedra in the C1–C15 region 8 was installed by asymmetric boron-mediated aldol reactions. Starting from aldehyde 10, an Evans aldol reaction (Bu3BOTf, DIPEA)9 with oxazolidinone 11 gave adduct 12 as a single diastereomer, setting up the 1,2-syn relationship at C12 and C13 (Scheme 2). TES protection and auxiliary cleavage (LiBH4) followed by a Swern oxidation gave aldehyde 13. Using our standard conditions (c-HexCl, BF3, Et2O, 78 °C), 14 was engaged with aldehyde 13 to yield the 1,2-anti,1,4-syn adduct 15 (20:1 dr). The C11 hydroxyl group was TES protected before submitting to a controlled reduction (DIBAL) to afford alcohol 16 (20:1 dr, see the ES† for confirmation of stereochemistry). Methylation (Me3OBF4, Proton Sponge™), 4 Å MS, r.t., 72%; (i) DDQ, CH2Cl2, pH 7 buffer, 0 °C to r.t., 99%.

The phosphonate 18 (Scheme 3) was prepared in three steps from sorbic acid. We previously reported in the enantiomeric series the synthesis of the C16–C28 ketone 9 in 16 steps from aldehyde 19. A Dess–Martin oxidation of alcohol 17 provided aldehyde 20, which was subjected to an HWE olefination with phosphonate 18 to afford the C1–C15 vinyl iodide as a single geometric isomer. At this juncture, the planned cross-coupling step required the appendage of a stannane handle onto either the C1–C15 or the C16–C28 vinyl iodide (21 and 9 respectively). As the C16 vinyl stannane proved to be highly prone to decomposition, we instead converted the vinyl iodide 21 to the corresponding stannane 8 under Wulff–Stille conditions (Pd(PPh3)4Cl2, (Me3Sn)2)11 With the two key fragments in hand, a modified Stille coupling12 afforded the advanced C1–C28 ketone 22. By repeating the Stille coupling with ent-8, the 13,18-anti diastereomer 23 was also obtained in an analogous manner. Encouragingly, NMR comparisons in CDCl3 indicated clear differences between the
protected 13,18-syn and 13,18-anti diastereomers despite the distal nature of the two stereoclusters.

Initial exploratory studies showed that deprotecting the C16–C28 ketone resulted in the concomitant hemiacetal engagement with the C27 carbonyl. Furthermore, the natural product contains a methyl ether at C27 and its presence is expected to aid a more selective reduction (NaBH4) and methylation (Me3O). Attempts at converting the C27 carbonyl to the corresponding methyl ether. As such, we looked to transform the C27 carbonyl to the corresponding methyl ether. At this stage, we were able to compare both the 1H and 13C NMR chemical shifts of our model truncates 5b and 28 with the 13,18-anti truncate 5a of Lecourt et al. and hemicalide itself. Notably the 13,18-anti acid 28 correlated well with the spectroscopic data reported for 5a (see the ESI†). The differences did not appear to be particularly diagnostic in the 1H NMR spectra for both diastereomers (Table 1, entries 4 and 5) and to previously published values for 1H and 13C NMR shifts respectively. Overall, the absolute and maximum errors recorded for the 13,18-syn diastereomers (Table 1, entries 1 and 2) were noticeably smaller than the corresponding 13,18-anti diastereomers (Table 1, entries 4 and 5) and to previously published values for 5a (entry 3). Interestingly, while the natural product was proposed to be isolated as the carboxylate salt,3 in situ treatment of the free acid with Na2CO3 effected complete acid deprotonation, which sharpened the carbon signals at C1–C3 in the sodium salts 29 and 30. At this stage, we were able to compare both the 1H and 13C NMR chemical shifts of our model truncates 5b and 28 with the 13,18-anti truncate 5a and hemicalide itself. Notably the 13,18-anti acid 28 correlated well with the spectroscopic data reported for 5a (see the ESI†). The differences did not appear to be particularly diagnostic in the 1H NMR spectra for both diastereomers (Table 1, entries 4 and 5) and to previously published values for 1H and 13C NMR shifts respectively. Overall, the absolute and maximum errors recorded for the 13,18-syn diastereomers (Table 1, entries 1 and 2) were noticeably smaller than the corresponding 13,18-anti diastereomers (Table 1, entries 4 and 5) and to previously published values for 5a (entry 3). Interestingly, while the natural product was proposed to be isolated as the carboxylate salt,3 the correlation for both diastereomers of salts 29 and 30 (entries 2 and 5) was poorer than for the corresponding acids 5b and 28 (entries 1 and 4), particularly in the C1–C7 triene region (see the ESI†). This suggests that hemicalide was likely isolated as the acid rather than the carboxylate salt.

carboxylic acid (see the ESI†), though this did not appear to significantly affect the signals for the remainder of the molecule. The effect of the protonation state on the 13C NMR was most noticeable for C1–C3, where presumed proton exchange kinetics resulted in peak broadening in acids 5b and 28. To verify this hypothesis, and noting that the natural product was proposed to be isolated as the carboxylate salt,3 in situ treatment of the free acid with Na2CO3 effected complete acid deprotonation, which sharpened the carbon signals at C1–C3 in the sodium salts 29 and 30. At this stage, we were able to compare both the 1H and 13C NMR chemical shifts of our model truncates 5b and 28 with the 13,18-anti truncate 5a of Lecourt et al. and hemicalide itself. Notably the 13,18-anti acid 28 correlated well with the spectroscopic data reported for 5a (see the ESI†). The differences did not appear to be particularly diagnostic in the 1H NMR spectra for both diastereomers (Table 1, entries 4 and 5) and to previously published values for 1H and 13C NMR shifts respectively. Overall, the absolute and maximum errors recorded for the 13,18-syn diastereomers (Table 1, entries 1 and 2) were noticeably smaller than the corresponding 13,18-anti diastereomers (Table 1, entries 4 and 5) and to previously published values for 5a (entry 3). Interestingly, while the natural product was proposed to be isolated as the carboxylate salt,3 the correlation for both diastereomers of salts 29 and 30 (entries 2 and 5) was poorer than for the corresponding acids 5b and 28 (entries 1 and 4), particularly in the C1–C7 triene region (see the ESI†). This suggests that hemicalide was likely isolated as the acid rather than the carboxylate salt.
In conclusion, we have firmly established the relative configuration between the C8–C13 and C16–C24 stereoclusters in hemicalide, where NMR correlations of advanced fragments decisively supported the reassigned 13,18-syn relationship. Additionally, NMR comparisons in the C1–C7 triene region indicated that hemicalide was likely isolated as the acid rather than the carboxylate salt. Our highly flexible construction of the advanced C1–C28 ketone 22 also enables the synthesis of the enantiomer ent-22. We hope to then achieve a bioassay-guided determination of hemicalide’s absolute configuration, as well as ascertaining preliminary structure–activity relationships in a drug development context.14

We thank the Woolf Fisher Trust (NYSL), the EPSRC (CIM) for financial support and the National Mass Spectrometry Centre (Swansea) for mass spectra.

Conflicts of interest
There are no conflicts to declare.

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