Synthesis of derivatives of potent antitumor bistramides D and A leading to the first crystal structure of natural bistramide D†

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We report a crystalline derivative of bistramide D synthesized from natural bistramide A, and its structure was determined by X-ray analysis.

Since the discovery of bistramide A, in New Caledonia[1] (near Nouméa or Ua and N'Do Islands, on the south coast) and in Australia[2] (at Heron Island Reef on the Great Barrier Reef), new members of the family (bistramides B, C, D and K) have been isolated.[3] All of these compounds (Scheme 1) are macrolide metabolites from a common source, namely *Lissoclinum bistratum* Sluiter, an ascidian which is a marine organism. Bistramides show numerous biological activities, such as antiproliferative,[4a–d] neurotoxic[1,5] and immunomodulating[6] properties. They exhibit cytotoxic properties[3] against a variety of human cancer cells and induce terminal differentiation *in vitro* towards various tumor cell lines including the human non-small cell lung carcinoma (NSCLC-N6). These cells are completely blocked in the G1 phase with bistramide K.[4c,7] More interestingly, bistramide D, and in particular bistramide K, have been presented as less toxic than bistramides A, B and C.[3,6,8] For this reason, we focused, during the last years,[9] our research essentially on these two attractive bistramides, D and K, for which neither total syntheses nor a firm assignment of their configuration have been mentioned in the literature.

In recent times, the total syntheses of bistramide C[10,11a] and bistramide A,[12] or its corresponding fragments,[13] have been described. In addition, many innovative efforts have been developed, over the past few years, for the determination of the structure of these bistramides.

Originally, the structure of the carbon backbone of bistramide A (BST A) was elucidated in 1992 by a 2D Inadequate experiment.[14] We have reported recently[9] the 4(*R*) absolute configuration of bistramide D (12 stereogenic centers leading to 4096 possible stereoisomers) as well as the relative stereochemistry of the pyran and spiro moieties by an extensive NMR study on derivatives of bistramides A and D (Scheme 1). Then, other groups[10–12] described the assignment of the absolute configuration of either bistramide C[10,11] or bistramide A,[12] both structurally different from bistramide D (Scheme 1). On the one hand, the structure

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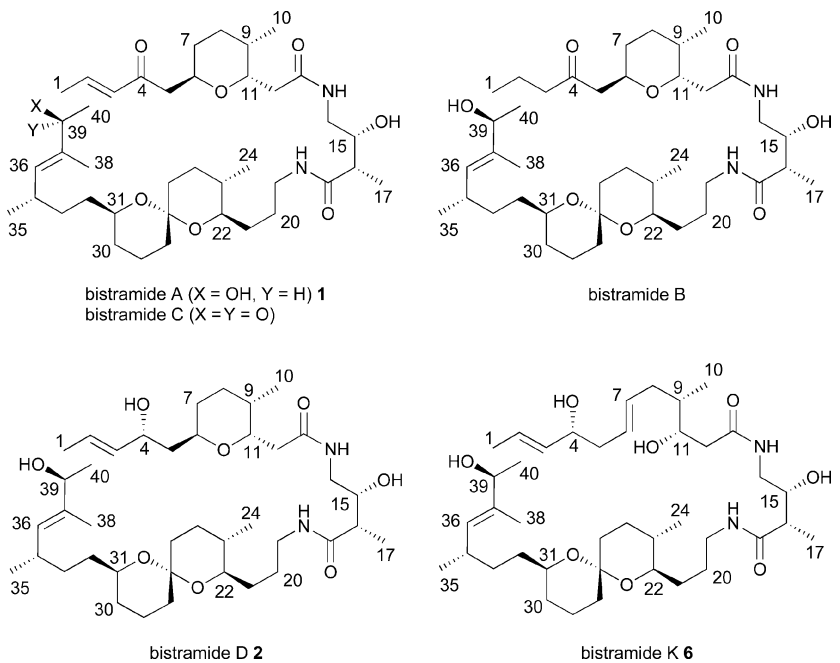


Scheme 1

assignment of bistramide C, reported by Wipf *et al.*,[10,11] was based on an elegant computational method, in addition to the synthesis of selected fragments suitable for chiroptical analysis according to van't Hoff's principle of optical superposition. This led to the total synthesis of bistramide C which was then correlated with the natural product.[11a] On the other hand, the method used by Kozmin and co-workers[12] for the determination of the structure of bistramide A (Scheme 1) involved the diastereocontrolled total synthesis and comparison with the natural product.

However, no firm assignment has been made of the stereostructure of bistramide D. Nevertheless, information relating to each compound of the bistramide class would be useful for the comprehension of their specific structure–activity relationship. In this paper, we are now able to report the first crystalline structure of a bistramide D derivative.‡

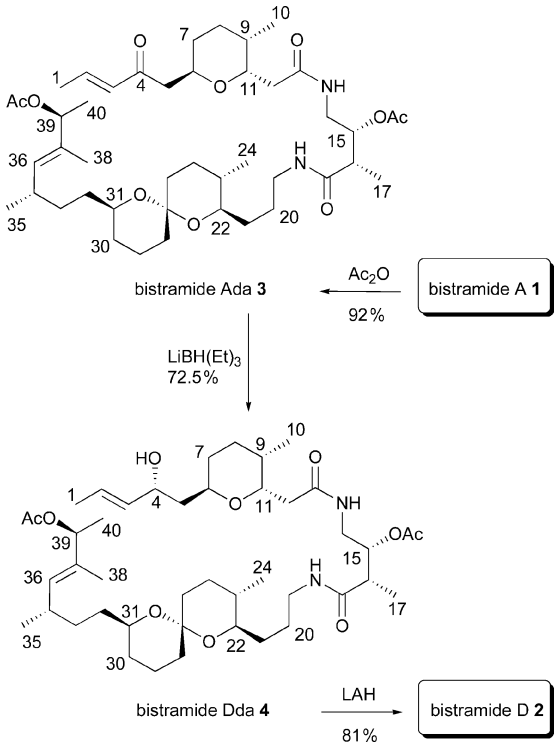
As a part of our programme directed towards the study of bistramides D and K, both including a hydroxy group at C-4 (Scheme 1), it was necessary to know their stereostructures in order to plan a synthetic approach, avoiding the 4096 or 2048 possible stereoisomers, respectively. Indeed, the unusually complex structure of these two promising marine macrolides leads to some difficulties in the assignment of the configuration of the multiple stereogenic centers. Furthermore, it is not really well established today that all additional bistramides have any correlation between them, or if the same absolute configuration is produced in their biosynthesis.

Until now, the results reported independently and successively by the groups of Wipf and Kozmin reveal an identical absolute configuration with regard to the common stereogenic centers of bistramides A and C (Scheme 1). Following an alternative strategic scheme, based on studies of derivatives of natural bistramide, we have resolved the structural assignment of bistramide D.

In our point of view, it was of interest to determine the absolute configuration of bistramide D, a third member of this group with one more stereogenic center at C-4, to know if this related compound has the same stereostructure as bistramides A and C. This complementary example would give further support to the fact that the known bistramides are likely to have the same stereostructure in the entire family, as postulated in the literature by Wipf *et al*.[11b]

Because of the structural complexity of bistramide D and its great number of possible stereoisomers, we have made the choice to direct our research on derivatives of the natural bistramides. To this end we have concentrated our efforts into an extensive multidimensional NMR study on several derivatives.

We have recently[9] reported the successful synthesis of bistramide D (BST D **2**) by stereoselective reduction [$LiBH(Et)_3$] at C-4 of BST A **1** (Scheme 2). ^{13}C NMR analysis of this synthetic BST D **2** revealed that the C-4 carbonyl signal in BST A **1** (198.9 ppm) was replaced by a CH–OH signal (72.0 ppm) in agreement with what was reported for an authentic sample of bistramide D. In spite of severe overlap in the proton NMR spectrum, unambiguous assignment of the structure was possible. It has been established by extensive NMR studies (500 MHz ^1H and 125 MHz ^{13}C, combined with 2D correlations: COSY, ROESY, HSQC, HMBC) and HPLC analysis that this hemisynthetic BST D isomer **2** matched in every respect with natural bistramide D.† This stereoselective reduction of the carbonyl function at C-4 into the corresponding natural configuration allows us to consider the determination of the



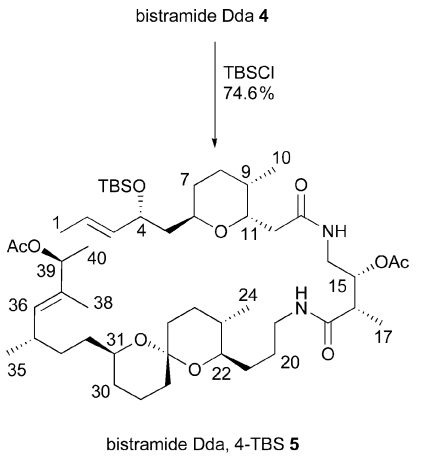
Scheme 2

absolute configuration of the three hydroxy groups at C-4, C-15 and C-39 of BST D **2** by the well known Mosher's method. To make a distinction between them, it seems obvious to start from BST A **1**, whose the two hydroxy groups, at C-15 and C-39, can be easily protected followed by the stereoselective reduction of the carbonyl group.

During the course of our examination, identical BST D **2** was also obtained under a similar stereoselective reduction [$LiBH(Et)_3$] of the C-4 carbonyl group of the diacetyl-protected bistramide A (BST Ada **3**), followed by reduction ($LiAlH_4$) of the two acetoxy groups on the resulting BST Dda **4** (Scheme 2). This approach was necessary in order to determine first on the BST Dda **4** the (*R*) absolute configuration[9] of the newly created C-4 center by Mosher's method. The absolute configuration of the other hydroxy group at C-15 was later determined by the same protocol.

This strategy was of importance since the silylation of the C-4 hydroxy position of BST Dda **4** resulted unexpectedly in the formation of a crystalline derivative **5** (BST Dda, 4-TBS)§ of bistramide D (Scheme 3) suitable for X-ray analysis. The absolute configurations at C-4(*R*) and C-15(*R*), which we had elucidated earlier, allowed us to assign the absolute configuration of **5** ¶ depicted in Fig. 1. This is the first crystallographic characterization of bistramide D.

According to this X-ray structure of bistramide D, and consequently of bistramide A, and according to the reported structural analysis concerning bistramides A and C,[10–12] the absolute configuration of the stereogenic centers seems to be extended to all members of the bistramide family. Thereby, the biologically attractive bistramide K **6**, which also includes a hydroxy group at position C-4, and which differs from the other members of the family only by a pyran ring-opened structure and a supplementary unsaturation, must probably contain the same



Scheme 3

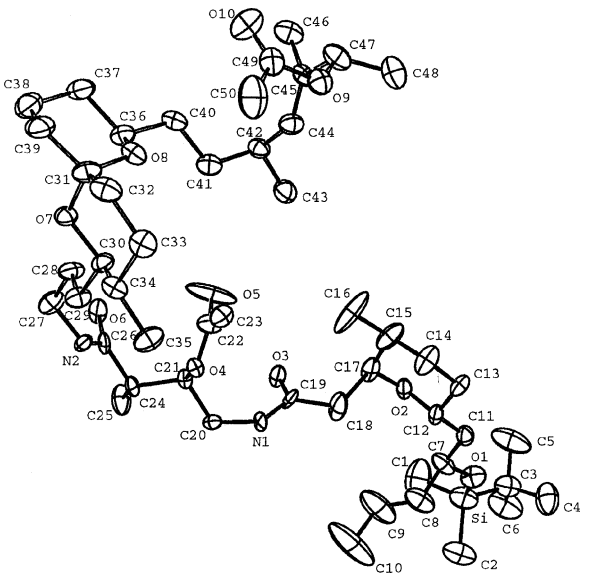


Fig. 1 ORTEP drawing of compound **5** (with 50% probability ellipsoids).

stereogenic centers. Furthermore, comparison of the structure of the elucidated bistramides A, C and D underlines that the observed biological activity is probably due to the fragment C1–C11. Indeed, the C-4 hydroxy group present in bistramides D and K (without the pyran moiety) is replaced by a carbonyl function in bistramides A, C and B, which have been shown to be more toxic. Also, in spite of the presence of a carbonyl function at C-39 for bistramide C – all other bistramides include a hydroxy group at this position – we can therefore imagine that this position has no effect on the biological activity.

In summary, we have determined the absolute configuration of a chemical derivative of bistramide D by radiocrystallographic analysis starting from the natural material. We assume that the same absolute configuration can be extended to the related bistramides K and B.

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Notes and references

‡ While this manuscript was submitted, Kozmin and co-workers reported simultaneously a communication concerning the structure of a bistramide A–actin complex.[15]

§ Experimental procedure for **5**: bistramide Dda **4** (270.8 mg; 0.342 mmol) and imidazole (76 mg; 3.26 equiv.) were dissolved under argon in DMF (5 mL) and then TBDMSCl (177 mg; 3.43 equiv.) was added in one portion. The mixture was stirred for16 h at room temperature before hydrolysis by water (30 mL), and diluted with diethyl ether (50 mL). Vigorous stirring was continued for 1.5 h and then the aqueous layer was extracted with diethyl ether (2 × 15 mL). The combined organic layers were washed with water (6 × 50 mL), brine (15 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure to obtain a crude product. The residue was purified by column chromatography on silica gel (AcOEt–CH_2Cl_2 1 : 1) to afford **5** (231 mg; 74.6%) as a white solid. In order to have some crystals of this compound for X-ray analysis, we recrystallized it in hot pentane. Mp = 127–128 °C (pentane). $[a]_D^{20}$ +84 (*c* 1.19 in CH_2Cl_2). Elemental analysis for $C_{50}H_{88}N_2O_{10}Si$ (905.327): calc. C, 66.33; H, 9.80; N, 3.09. Found: C, 66.20; H, 9.92; N, 2.85%. ^1H and ^{13}C NMR data can be seen in the ESI.

¶ Crystal data for **5**. $C_{50}H_{88}N_2O_{10}Si$, M = 905.31, monoclinic, a = 14.371(2), b = 9.5023(10), c = 21.501(3) Å, β = 103.55(5)°, V = 2854.4(6) Å^3, T = 173 K, space group $P12_11$, Z = 2, μ = 0.091 mm^{-1}, R = 0.0812, wR_2 = 0.2129, 11 544 reflections measured, 3551 unique (R_{int} = 0.07), the final R was 0.0812. CCDC reference number 255731. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b603767d.

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