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Communications

The Dolastatins. 21. Synthesis, X-ray Crystal Structure, and Molecular Modeling of (6*R*)-Isodolastatin 10[1a]

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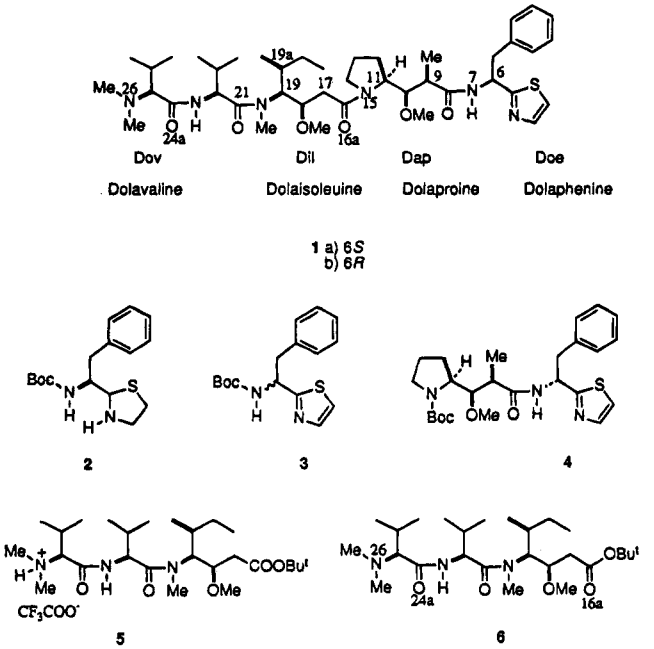
Summary: A practical synthesis of (6*R*)-isodolastatin 10 (**1b**) was devised, the X-ray crystal structure was determined, and this chiral isomer was found to have antineoplastic activity comparable to dolastatin 10 (**1a**).

The ability to mount very effective chemical defenses has allowed the shell-less mollusks, especially the sea hares and nudibranches,[2] to proceed along very successful evolutionary pathways. Certain species of sea hares such as *Dolabella auricularia* and *Aplysia kurodai* produce remarkably potent and structurally diverse antineoplastic agents. Illustrative are, respectively, dolastatin 10 (**1a**)[3] and aplyronine.[4] Of the dolastatin series,[5] the most potent has proven to be dolastatin 10.[3a] The extraordinary antitumor activity of dolastatin 10 (presently under clinical development) and the need to further explore its mechanism of action[6,7] required an X-ray crystal structure determination of the parent compound or a comparably active derivative. However, we were unable to

Chart 1



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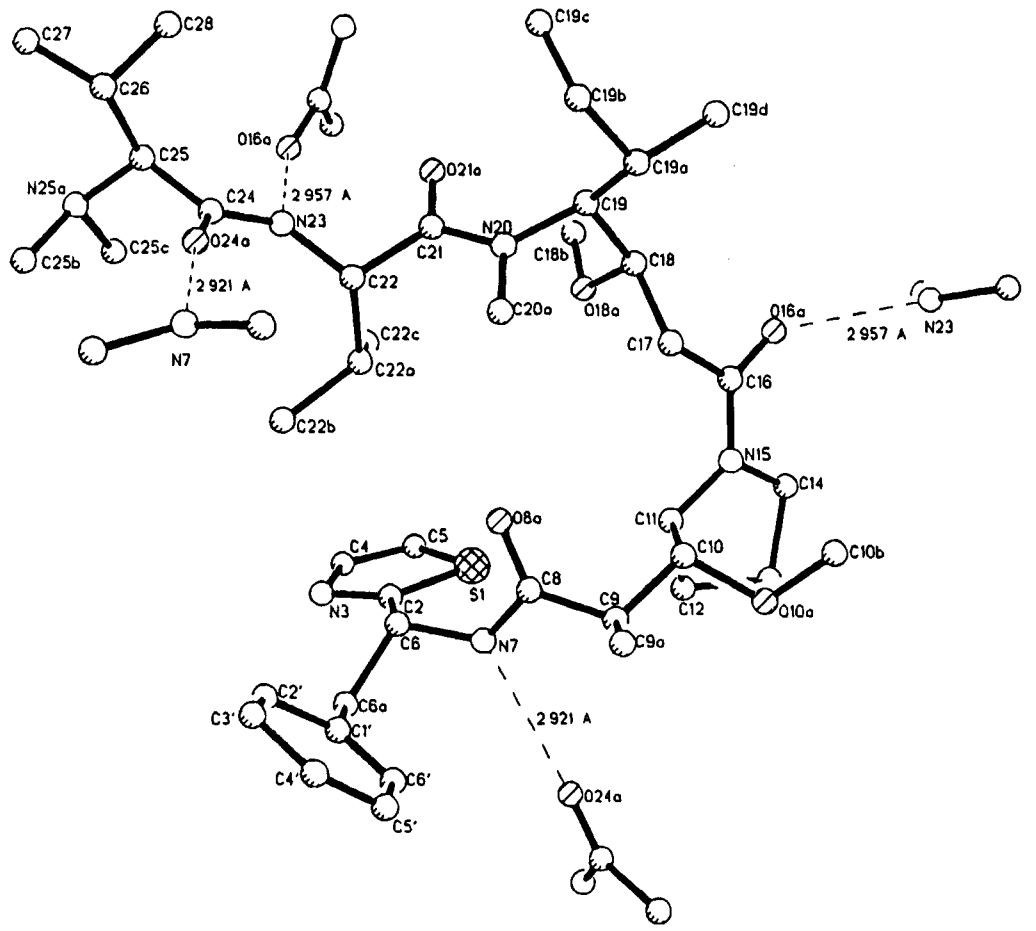

Figure 1. Computer-generated perspective drawing (less H atoms) and X-ray numbering system of (*6R*)-isodolastatin 10 (**1b**).

obtain X-ray quality crystals from dolastatin 10 or from 18 of its configurational isomers.[7a,8] Most of these isomers showed IC_{50} values in the range 30–90 nM as compared to 1 nM for dolastatin 10. We now report the synthesis of (*6R*)-isodolastatin 10 (**1b**),[9] its X-ray crystal structure determination, and the molecular modeling of this important substance (exhibits antineoplastic activity comparable to that of dolastatin 10).

For the synthesis of (*6R*)-isodolastatin 10 (**1b**), a frequent side reaction in the synthesis of dolaphenine[3c] was used to advantage (Chart 1). Manganese dioxide oxidation of the penultimate thiazolidine (**2**) often resulted in some isomerization. Hence, when the resulting optically impure dolaphenine (**3**) was coupled[3c] with dolaproine, the product was a mixture of two diastereoisomers which were easily separated by column chromatography. The more polar amide (**4**) (mp 133°, $[\alpha]_D$ −25° (c = 0.48, CH_3OH)) was found to possess the required stereochemistry for synthesis of (*6R*)-isodolastatin 10. The structure (**4**) was further confirmed by a single-crystal X-ray structural analysis.[8b] Amide **4** was deprotected with trifluoroacetic acid and coupled (DEPC)[3c] with tripeptide trifluoroacetate salt **5**[3c] to obtain (*6R*)-isodolastatin 10 (**1b**) in quantitative yield. Crystallization from diethyl ether–dichloromethane–hexane yielded tiny crystals (mp 100–103 °C, $[\alpha]_D$ −35°, (c = 0.29 in CH_3OH)) suitable (at low temperature) for X-ray crystal structure determination.[10,11]

Structure solution and refinement revealed a single peptide molecule associated with one diethyl ether solvate molecule in each asymmetric unit of the cell. Residual electron density in the unit (highest peak, 1.20 e/Å^3) suggested that additional, highly-disordered solvate molecules were also probably present. Hydrogen atoms were included at optimum calculated positions for structure factor calculations, but were not refined. The final unweighted and weighted standard agreement factors were R = 0.116 and R_w = 0.136, respectively. Calculated

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(10) Satisfactory elemental analyses and spectral data (NMR and MS) were obtained for each new compound.

(11) Crystal data for **1b**: colorless, thin plate (0.05 × 0.16 × 0.36 mm) from ether–CH_2Cl_2–hexane solution consisting of a 1:1 complex of parent molecule and ether ($C_{42}H_{68}N_6O_6S{\cdot}C_4H_{10}O$, FW = 859.22, F(000) = 1872), orthorhombic $P2_12_12_1$, a = 14.546(6) Å, b = 16.085(7) Å, c = 22.243(7) Å, V = 5204.25 Å^3, λ (Cu Kα) = 1.541 84 Å, ϱ_o (variable) = 0.954 g cm^{-3}, ϱ_c = 1.002 g cm^{-3} for Z = 4, T = 169 °K, μ = 9.1 cm^{-1}. Of the 8591 unique absorption-corrected reflections (including Friedel pairs) collected (Enraf-Nonius CAD4, 2Θ ≥ 130°), 2635 were considered observed ($I_o > 3\sigma(I_o)$). The structure was solved by direct methods (SHELXS86). All 55 non-hydrogen atoms of **1b**, as well as the five non-hydrogen atoms of the solvate ether molecule, were isotropically refined (CRYSTALS); hydrogen atoms were idealized. R = 0.116 and R_w = 0.136. The absolute stereochemistry at the eight chiral centers of **1b** as depicted in Figure 1 (SHELXTL PLUS) are as follows: 6*R*,9*R*,10*R*,-11*S*,18*R*,19*S*,19a*S*,22*S*,25*S*.[16]

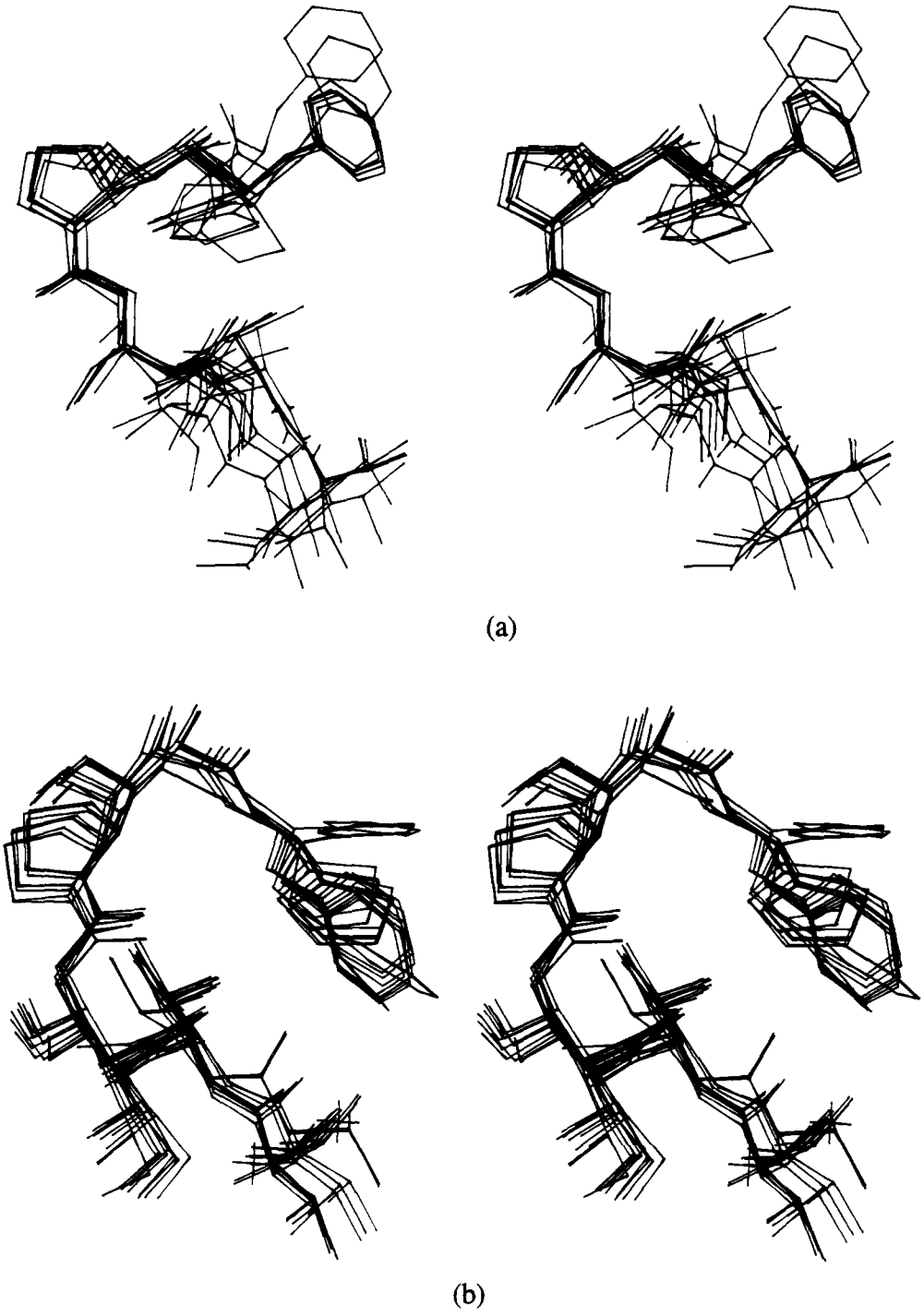

Figure 2. Stereoview of the superimposed minimized dynamic datasets of (a) *cis*- and (b) *trans*-(*6R*)-isodolastatin 10 showing good convergence.

bond distances, angles, and thermal parameters for (*6R*)-isodolastatin 10 were within accepted limits, excepting the N23—C24 and C26—C27 bonds, which were shorter than expected (1.29 Å and 1.43 Å, respectively). A computer-generated perspective drawing depicting the absolute configuration of peptide **1b** is shown in Figure 1. The absolute configuration was based upon the relative configuration established *via* X-ray correlated with the known absolute configuration of the *S*-amino acids from which (*6R*)-isodolastatin 10 was synthesized.

On the basis of the N···O bond lengths observed in an N—H···O-type hydrogen bond, none of the carbonyl groups or amide hydrogens in (*6R*)-isodolastatin 10 appear to be involved in intramolecular hydrogen bonding (N···O distance for peptides, ~2.79 ± 0.12 Å). Instead, intermolecular hydrogen bonding occurs at N7, O16a, N23, and O24a to adjacent molecules of isodolastatin 10 (see Figure 1). Importantly, the last two H-bonding sites occur in the Dov-Val amino acid unit N-terminal portion of the peptide backbone. Since peptide **1b** displays strong inhibition of tubulin assembly,[7] these positions may be involved in molecular recognition of the active binding sites in the tubulin protein groove. Also, it is likely that H-bonding at O16a plays a role in positioning of the molecule in the groove, producing a three-point attachment. Since tripeptide **6** also shows tubulin activity,[7] but lacks the N7 atom, the H-bonding at this site probably does not play a significant role in binding the molecule

to tubulin. Interestingly, the X-ray structure of (*6R*)-isodolastatin 10 proved to be very close conformationally to the biological working model we had proposed, based upon competitive tubulin binding experiments.[6,7,9a] Once the crystal coordinates for (*6R*)-isodolastatin 10 (**1b**) were secured, molecular modeling studies were undertaken.

Molecular modeling studies of isodolastatin **10** (**1b**) were performed using QUANTA/CHARMM.[12] In the crystal structure, all the amide bonds were found in the *trans* conformation except the Dil-Dap (N15—C16) peptide bond which proved to be *cis*. For the modeling studies a distance dependent dielectric constant of 4*R* was used throughout the calculations.[13] The molecule was subjected to energy minimization using the ABNR algorithm, followed by simulated annealing (at 1000 K) and cooling slowly to 50 K. During this process, the conformation of the peptide bond between Dil and Dap changed from *cis* to *trans*. The *trans*-isodolastatin structure was used for further modeling studies. The ABNR-md-ABNR protocol[14] was used to obtain the low energy conformers of the *cis*- and *tran*-isodolastatins. Both conformers were first energy minimized (ABNR) and then subjected to dyanamics at 300 K for 200 ps. Several low energy structures were selected from the dynamics trajectory and were minimized (ABNR). The potential energy of the various minimized conformers of both the *cis*- and *trans*-isodolastatins was found to be very close to that of the initial structures. A least-squares superposition of the low energy conformers of *cis*- and *trans*-isodolastatins is given in Figure 2. When the Dil-Dap peptide bond of *cis*-isodolastatin 10 was constrained at different dihedral angles and the resulting structures energy minimized a plot of the potential energy *vs* the corresponding dihedral angle showed the energy barrier between the *cis* and *trans* conformers to be ~21 kcal/mol. In the ^{1}H-NMR spectrum, in $CDCl_3$, isodolastatin 10 showed two conformers in the ratio 4:1. In the 2D NOESY spectrum the C-17 methylene protons of the Dil unit showed very strong cross peaks with only one conformer location of the dolaproine δ protons (δ 3.2 and 3.62) consistent with the *trans* conformer (a, Figure 3). Also observed were cross peaks between the Dil C-17 and

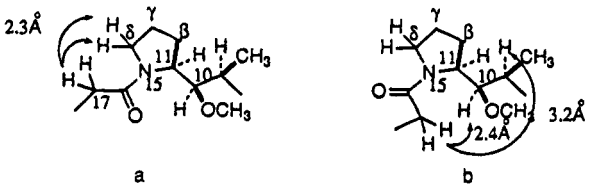

Figure 3. Characteristic NOEs observed in (a) *trans*- and (b) *cis*-(*6R*)-isodolastatin 10.

the Dap C-10 and C-11 protons corresponding to the *cis* conformer (b, Figure 3).

Isodolastatin **1b** has two well-defined backbone conformations differing in orientation of the Dil-Dap peptide bond. Both the *cis* and *trans* rotamers showed characteristic backbone conformation over the entire dynamic trajectory. Whereas the *trans* conformer has all three $\phi-\psi$[15] angles in the extended β-sheet region (second quadrant), the *cis* conformer has the $\phi-\psi$ for Dil in the right-handed α-helix region (third quadrant). Both result in a bent structure for (*6R*)-isodolastatin 10 (**1b**).

Results of the present study are expected to markedly assist in obtaining further insights into the molecular interactions between dolastatin 10 and the tubulin proteins as well as pursuing structure/activity relationships.

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(15) The backbone ϕ and ψ angles for Dil unit are defined by the atoms $C_{21}-N_{20}-C_{19}-C_{18}$ and $N_{20}-C_{19}-C_{18}-C_{17}$ and for Dap unit by the atoms $N_{16}-N_{15}-C_{11}-C_{10}$ and $N_{15}-C_{11}-C_{10}-C_9$, respectively.

(16) The author has deposited atomic coordinates for **1b** with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 IEZ, UK.