The dolastatins 25. Conformational isomerism of *N*-benzyloxycarbonyl-*N*-methylisoleucinol and related substances[1]

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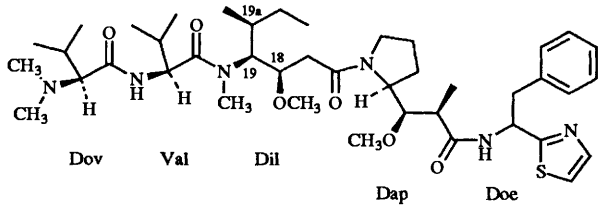
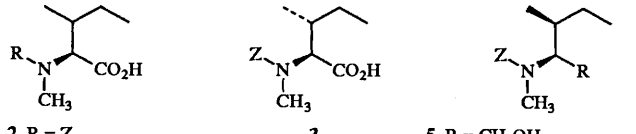
Conformational isomerism in a selection of *N*-Z-*N*-methylisoleucine derivatives was detected and investigated using variable temperature NMR spectroscopy. Thermodynamic values for the restricted rotation of conformational isomers were derived.

Dolastatin 10 is one of the most potent antineoplastic substances presently known.[2,3] Clinical trials will soon begin using this unique anticancer drug isolated (1984) from *Dolabella auricularia* (an opisthobranch mollusc found in the Western Indian Ocean) and structurally defined by us in 1987.[4] In addition, our discovery of an efficient synthetic route to dolastatin 10 (**1**) confirmed the structure and absolute configuration.[5] The first total synthesis[5] of dolastatin 10 was followed by several partial[6] and complete[7] syntheses.

In conjunction with our total synthesis of dolastatin 10 we had an opportunity to investigate the proton NMR spectra of isoleucine derivatives **2**–**8**. Three of these compounds (**2**, **5** and **6**) were intermediates in the preparation of dolaisoleucine (Dil), an amino acid constituent of dolastatin 10.[4] Since substitution on amino acids, particularly in the side chain, is well known to affect peptide conformations,[8–13] an NMR investigation of the Dil intermediates appeared to offer some useful insights. In addition, there was a need to establish the diastereoisomeric integrity of these compounds.



1 Dolastatin 10



2 R = Z
4 R = CH_3CO

3

5 R = CH_2OH
6 R = CHO
7 R = $CH_2OC(O)CH_3$
8 R = $CH_2OC(O)C(CH_3)_3$

Z = $PhCH_2OCO$

In our synthesis of the dolaisoleucine unit of dolastatin 10, *N*-Z,*N*-methyl-(*S*)-isoleucine derivatives **5** and **6** were prepared from Z-Ile.[4b,14–18] The *N*-methyl signal in the room temperature spectra of these compounds was doubled. The H^{α} signal in the spectra of amino acid **2** and amino aldehyde **6** also appeared as a pair of doublets. The method we used to prepare Ile derivative **2** was reported to give rise to only 1.1% racemization.[15] Removal of the *N*-protecting group later in the synthetic sequence resulted in sharp NMR signals indicative of one isomer.[13] We suspected the signal-doubling phenomenon was due to the presence of the bulky benzyloxycarbonyl group

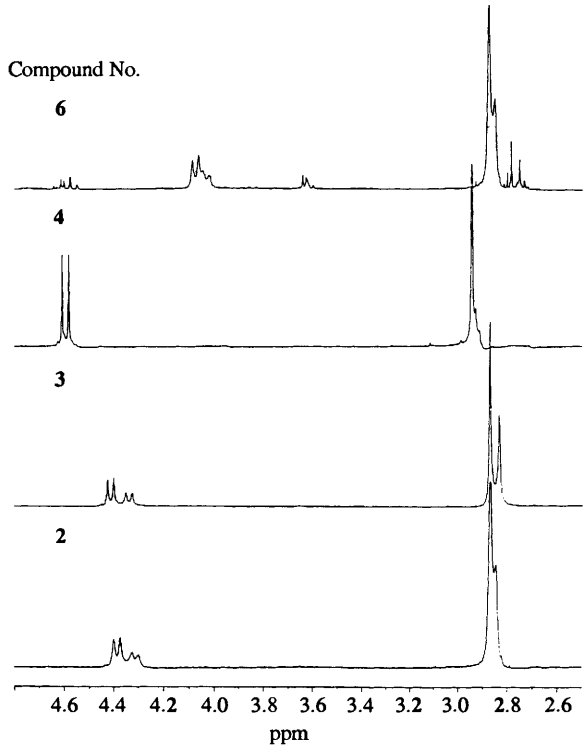


Fig. 1 ^1H NMR spectra of amides **2**, **3**, **4** and **6** at 25 °C

which hinders rotation, the result being rotational/conformational isomers detectable at room temperature. Such doubling of signals has been observed in the spectra of certain *N*-*tert*-butoxycarbonyl-*N*-methyl amides[6b] and in those of *cyclo*-(L-isoleucyl-D-alloisoleucine).[19] Furthermore, the presence of rotational isomerism about the C^{α}–C^{β} bond in both leucine[20] and isoleucine[21] has been demonstrated.

In order to study the effects of rotational or conformational isomerism in our Dil intermediates and to confirm the cause of the ^1H NMR signal doubling, we subjected isoleucine derivatives **2**–**8** to spectroscopic analysis. Compounds **2**, **3** and **6** each showed a pair of doublets for H^{α} and a pair of singlets representing the *N*-methyl group at room temperature (Fig. 1). Examination of H^{α}–H^{α} NMR coupling values (**2**, J = 10.59, 10.66 Hz; **3**, J = 10.17, 10.26 Hz; **6**, J = 9.76, 10.21 Hz) suggested the dominance of rotamer **A** (Fig. 2) and these values were in good agreement with Abraham's[9] coupling constant of 10.41 Hz for *trans* H^{α}, H^{β} protons in L-leucine as well as with X-ray data for L-isoleucine.[22] Carbamates **7** and **8** exhibited

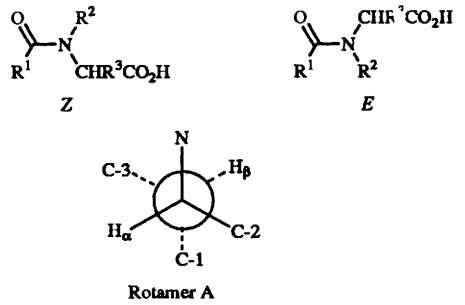


Fig. 2 Rotational isomerism in L-isoleucine carbamates (projection along the C_α–C_β axis)

analogous ^1H NMR results, but the H^α signal was more complex due to interactions with the neighbouring methylene group.

With *N*-benzoyl-*N*-alkylamino acids,[23] separate signals exist for the *Z* and *E* amide linkages. Nearly equal proportions of these isomers normally exist in solution, but in *N*-methylamino acids with a branch at C^β steric interactions between the *N*-methyl group and side chain make the *Z*-conformation more generally favoured. Interestingly, while compounds **2**, **3** and **6** with a bulky benzyloxy substituent on the carbonyl showed the doubling of signals, the non-urethane acetamide **4**, with the less bulky methyl substituent, did not demonstrate this trend. We ascribe signal doubling in the spectra of amides **2**, **3** and **6** to restricted rotation about the carbamate amide bond. In this case, the major conformer in solution was confirmed as being *E* by the utilization of the 1D cross-sectional spectra of the 2D NOESY spectrum of compound **3**. Only the *N*-methyl signal at δ 2.87 showed an NOE effect on the methylene signal at δ 5.12, demonstrating the *trans* nature of the amide bond. The ability of the *E* conformer of amides **2** and **3** to hydrogen bond intramolecularly may have some bearing on this effect.

To evaluate the energy involved in such conformational interconversions, we studied the effect of temperature on the proton NMR spectra of amides **2**–**4** and **6**–**8**. The result with amide **3** is shown in Fig. 3 along with a plot of temperature *vs.* chemical shift. The free enthalpy of activation at the coalescence temperature was calculated from the Eyring equation (1)[24]

$$k_r = (kT/h) \exp(-\Delta G^\ddagger/RT) \quad (1)$$

where h is Planck's constant and k is the Boltzman constant. The rate constant (k_r) of isomerization is given by[25] $k_r = \pi\Delta\nu_{\frac{1}{2}}/\sqrt{2}$. The $\Delta\nu_{\frac{1}{2}}$ values at the coalescence temperature were obtained by considering the *N*-methyl signals in the temperature-dependent NMR spectra of these compounds.[26] These values, along with the specific coalescence temperature and $\Delta\nu_{\frac{1}{2}}$, are shown in Table 1. The values for ΔG_{Tc} are in close agreement with the estimated lower energy barrier of 66.5 kJ mol^{-1} (15.9 kcal mol^{-1}) for similar urethane systems,[12] further supporting the existence of conformational isomerism in these compounds. The difference in the ground-state free energy of the two conformers was readily calculated from eqn. (2),[9,20] where P_{II}

$$\Delta G^\circ = -RT \ln (P_{II}/P_I) \quad (2)$$

and P_I denote the populations of the two conformers. These values are shown in Table 2.

Clearly *N*-Z,*N*-Me-Ile type amino acid derivatives exhibit conformational isomerism at ambient temperatures due to steric hindrance. In contrast Dil-OBu^t was found in a separate study[4b] to exist as one conformer at ambient temperatures. Finally, the present study confirms the lack of racemization[19] during *N*-methylation of Z-Ile using the very useful Benoiton procedure.[14–16]

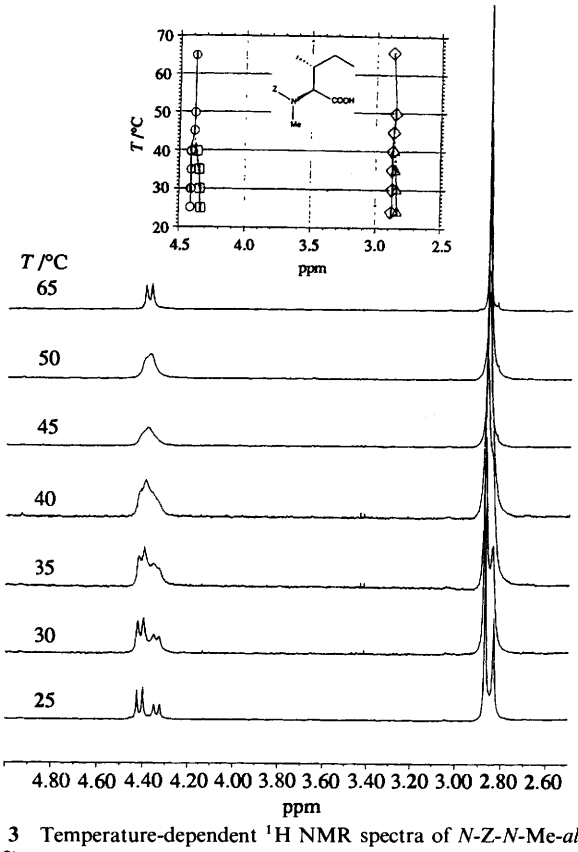


Fig. 3 Temperature-dependent ^1H NMR spectra of *N*-Z-*N*-Me-*allo*-Ile (**3**)

Experimental †

General procedures

All solvents were redistilled prior to use and stored over drying agents. The amino acids and other reagents were obtained from Sigma–Aldrich and were used as received. Flash chromatography was performed on Kieselgel 60 (0.040–0.063 mm) supplied by Merck, Darmstadt, Germany. Analytical TLC was performed on silica gel GHLF Uniplates (Analtech).

Except for the NOESY experiments using a Varian Unity 500 MHz, the high-field NMR spectra were recorded with a Bruker AM-400 narrow bore spectrometer. Processing and acquisition of data was assisted by use of an Aspect 3000 computer and pulse programmer. All spectra were obtained in 100% deuteriated acetonitrile. The 90° pulse length for ^1H was 10 ms with no applied line broadening. Infrared spectra were recorded using a Mattson 2020 Galaxy series FT-IR. Low resolution mass spectra were determined with a Finnigan-MAT 312 instrument.

N-Z,*N*-Me-Isoleucinol acetate (7)

A solution of alcohol **5** (124 mg, 0.47 mmol) was dissolved in anhydrous pyridine (0.5 cm^3) and acetic anhydride (0.1 cm^3) was added. The solution was stirred at room temperature for 1.5 h and diluted with ice–water (15 cm^3). The product was extracted into diethyl ether (2 × 15 cm^3) and the solvent extract was washed with water, 10% aqueous citric acid solution, saturated aqueous sodium bicarbonate and water, and dried over anhydrous magnesium sulfate. Removal of the solvent gave 132 mg (92%) of acetate **7** as a clear oil. No further purification was required: bp 158–160 °C (0.12 mmHg); $[\alpha]_D^{25}$ = −21 (c = 0.49, $CHCl_3$); R_f (TLC) 0.31 (4:1 hexane–acetone); ν_{max}(neat)/cm^{-1} 2964, 2935, 2879, 1743, 1701, 1454, 1406, 1340,

† For preparation and purification of compounds **2**, **3** and **4** see reference 27 and citations therein.

Table 1 $\Delta G^{\ddagger}_{(T_c)}$ values calculated from the NMe signals

N-Z,*N*-Me amide	$\Delta\nu_{\frac{1}{2}(T_c)}$/Hz	T_c/K	$\Delta G^{\ddagger}_{(T_c)}$/kcal mol^{-1}
2	11.4	316	16.52
3	15.9	318	16.40
6	8.3	303	15.99
7	13.4	328	17.04
8	8.9	323	17.04

Table 2 $\Delta G°$ values calculated from the NMe signals

	Population at 25 °C		
N-Z,*N*-Me amide	$P_{I(E)}$	$P_{II(Z)}$	$\Delta G°$/kcal mol^{-1}
2	0.690	0.310	0.47
3	0.636	0.364	0.33
6	0.691	0.309	0.48
7	0.592	0.408	0.22
8	0.526	0.474	0.18

1230, 1151 and 1031; δ_H 7.40–7.26 (m, 5 H), 5.08 (m, 2 H), 4.30–4.21 (m, 1 H), 4.13–3.95 (m, 2 H), 2.76, 2.73 (s, 3 H), 1.86, 1.83 (s, 3 H), 1.60–1.50 (m, 1 H), 1.40–1.30 (m, 1 H), 1.10–0.95 (m, 1 H) and 0.90–0.70 (m, 6 H); *m/z* (EIMS) 307 (M^+), 269, 250, 234, 206, 190 and 91 (100%) (Found: C, 66.39; H, 8.23; N, 4.59. Calc. for $C_{17}H_{25}NO_4$: C, 66.42; H, 8.20; N, 4.56%).

***N*-Z,*N*-Me-Isoleucinol pivaloyl ester (8)**

A solution of alcohol **5** (210 mg, 0.79 mmol) in anhydrous dichloromethane (6 cm^3) was treated with trimethylacetyl chloride (110 mg, 0.91 mmol) in dichloromethane (2 cm^3). The solution was cooled to 0 °C and pyridine (1 cm^3) was added (dropwise). The mixture was stirred for 1 h and then diluted with water (20 cm^3). The aqueous mixture was extracted with dichloromethane (2 × 50 cm^3). The organic phase was washed successively with water, 10% aqueous citric acid, water and aq. sodium bicarbonate (satd.), and dried over anhydrous magnesium sulfate. Removal of the solvent under reduced pressure followed by chromatography over a column of silica gel (9:1 hexane–ethyl acetate) afforded 154 mg (98%, based on recovered starting material) of the ester **(8)** as a clear oil: bp 145 °C (0.12 mmHg); $[\alpha]_D^{25} = -23$ (c = 15.1, $CHCl_3$); R_f 0.50 (5:1 hexane–acetone); ν_{max}(neat)/cm^{-1} 2966, 2877, 1716, 1701, 1456, 1404, 1323, 1284, 1161 and 1030; δ_H 7.40–7.25 (m, 5 H), 5.07 (m, 2 H), 4.25–4.19 (m, 2 H), 4.18–4.02 (m, 1 H), 2.75, 2.72 (s, 3 H), 1.68 (m, 1 H), 1.38 (m, 1 H), 1.16 (s, 9 H), 1.23–1.04 (m, 1 H), 0.93–0.30 (m, 6 H); EIMS (*m/z*): 349 (M^+), 292, 248, 234, 190, 91 (100%), 57 (Found: C, 69.06; H, 9.11; N, 4.35. Calc. for $C_{20}H_{31}NO_4$: C, 68.74; H, 8.94; N, 4.01%).

Acknowledgements

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