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## NMR-based assignment of isoleucine vs. *allo*-isoleucine stereochemistry†

Zoe J. Anderson, Christian Hobson, Rebecca Needley, Lijiang Song,  
Michael S. Perryman, Paul Kerby and David J. Fox \*

A simple  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectrometric analysis is demonstrated that permits differentiation of isoleucine and *allo*-isoleucine residues by inspection of the chemical shift and coupling constants of the signals associated with the proton and carbon at the  $\alpha$ -stereocentre. This is applied to the estimation of epimerisation during metal-free *N*-arylation and peptide coupling reactions.

### Introduction

The assignment of the relative stereochemistry of amino-acid residues within complex natural products is normally achieved by degradation of the product to its constituent fragments, followed by reaction with a chiral derivatising agent, commonly Marfey's reagent.<sup>1</sup> During peptide coupling, epimerisation is usually measured by HPLC separation of the diastereoisomeric products.<sup>2</sup>  $^1\text{H}$ ,  $^{13}\text{C}$  and  $^{15}\text{N}$  NMR spectrometry can also be used to calculate the level of epimerisation, either *via* chiral derivatisation or with a chiral shift reagent.<sup>3</sup> Recently, during the structural assignment<sup>4</sup> and synthesis<sup>5</sup> of the azolemycins, we noted that the  $^1\text{H}$  NMR spectrum of the isoleucine portion of the natural product was significantly different from its *allo*-isoleucine diastereoisomer.

L-Isoleucine is one of the common amino-acids found in eukaryotic and prokaryotic proteins,<sup>6</sup> but is unusual in that it possesses two stereogenic centres. It can therefore exist as four distinct stereoisomers (Fig. 1).<sup>7</sup> Epimerisation at the  $\alpha$ -centre can occur due to resonance stabilisation and electron-withdrawing effects from the amino- and carboxy-groups to give *D-allo*-isoleucine. In contrast, the  $\beta$ -centre is distanced from

these effects and so is much more difficult to epimerise to give the less common *L-allo*-isoleucine stereoisomer.<sup>7a,8</sup> This lack of reactivity allows the  $\beta$ -configuration at the carbon to act as a marker for epimerisation at the  $\alpha$ -carbon. Isoleucine as a marker for the study of racemisation during peptide synthesis has been known for a number of years,<sup>9</sup> but the final assessment of chiral purity is usually performed by hydrolysis followed by analytical chromatography.<sup>10</sup>

To our knowledge, *only two reports* exist describing the specific differentiation of *L*-isoleucine and *D-allo*-isoleucine by  $^1\text{H}$  NMR spectrometry. In 1983, Khatskevich *et al.* reported that both the chemical shift and the coupling constants for the hydrogen at the  $\alpha$ -carbon differed for *N*-benzoyl-*L*-isoleucine methyl ester and *N*-benzoyl-*D-allo*-isoleucine methyl ester ((*2S,3S*) diastereoisomer,  $\delta_{\text{H}}$  ppm 4.70, dd,  $^3J_{\text{CH-CH}}$  5.15 Hz),  $^3J_{\text{CH-NH}}$  8.62 Hz; ((*2R,3S*) diastereoisomer,  $\delta_{\text{H}}$  ppm 4.90, dd,  $^3J_{\text{CH-CH}}$  4.30 Hz,  $^3J_{\text{CH-NH}}$  9.0 Hz).<sup>11</sup> Similarly, in 2003 Biron and Kessler noted that, when hydrolysing *N* $^{\alpha}$ -methyl-*N* $^{\alpha}$ -*o*-NBS-*L*-isoleucine methyl ester, epimerisation at the  $\alpha$ -stereocentre could be observed by the appearance of a new peak (corresponding to the *D-allo*-isoleucine stereoisomer  $\alpha$ -CH) downfield from the usual peak observed for the *L*-isoleucine-derived stereoisomers.<sup>12</sup> During the course of our studies into peptide bond synthesis, we decided to use this difference between *L*-isoleucine and its epimer, *D-allo*-isoleucine by  $^1\text{H}$  NMR spectrometry to easily assess the comparative levels of epimerisation during a range of reactions of molecules with a C-terminal *L*-isoleucine residue.

A range of C- and N-substituted isoleucine-containing molecules were synthesised using pure *L*-isoleucine and then repeated for comparison with a mixture of *L*-isoleucine and *D-allo*-isoleucine by standard methods (Fig. 2, see ESI† for details). *N*-Undec-10-enoyl **5a** and *N*-benzoyl-*L*-isoleucine **6a** were epimerised to a mixture of *L*-isoleucine and *D-allo*-isoleucine derivatives using the method of du Vigneaud *et al.*<sup>13</sup> Compounds lacking an N-terminal acyl group were synthesised

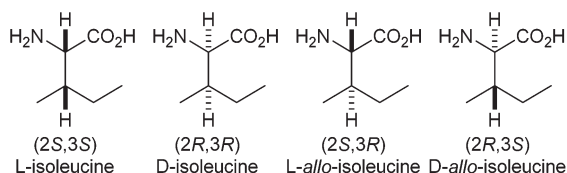
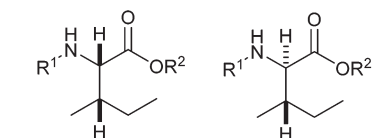


Fig. 1 Isoleucine and *allo*-isoleucine stereochemistry.

Department of Chemistry, University of Warwick, Gibbet Hill Road, Coventry, CV4 7AL, UK. E-mail: d.j.fox@warwick.ac.uk

† Electronic supplementary information (ESI) available: Synthesis details, NMR spectra and chiral HPLC chromatograms. See DOI: 10.1039/c7ob01995e



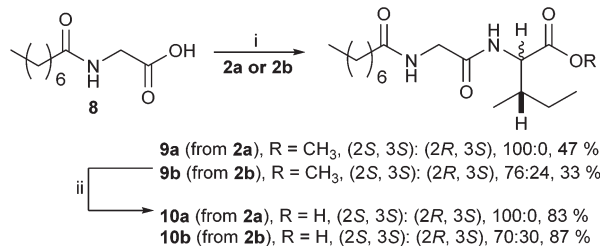


- 1,  $R^1 = H$ ,  $R^2 = H$ , (2S, 3S): (2R, 3S), 76:24  
 2a,  $R^1 = H$ ,  $R^2 = CH_3$ , (2S, 3S): (2R, 3S), 100:0  
 2b,  $R^1 = H$ ,  $R^2 = CH_3$ , (2S, 3S): (2R, 3S), 67:33  
 3a,  $R^1 = Ts$ ,  $R^2 = CH_3$ , (2S, 3S): (2R, 3S), 100:0  
 3b,  $R^1 = Ts$ ,  $R^2 = CH_3$ , (2S, 3S): (2R, 3S), 84:16  
 4a,  $R^1 = Ts$ ,  $R^2 = H$ , (2S, 3S): (2R, 3S), 100:0  
 4b,  $R^1 = Ts$ ,  $R^2 = H$ , (2S, 3S): (2R, 3S), 69:31  
 5a,  $R^1 = 10$ -Undecenoyl,  $R^2 = H$ , (2S, 3S): (2R, 3S), 100:0  
 5b,  $R^1 = 10$ -Undecenoyl,  $R^2 = H$ , (2S, 3S): (2R, 3S), 80:20  
 6a,  $R^1 = Bz$ ,  $R^2 = H$ , (2S, 3S): (2R, 3S), 100:0  
 6b,  $R^1 = Bz$ ,  $R^2 = H$ , (2S, 3S): (2R, 3S), 78:22  
 7a,  $R^1 = Bz$ ,  $R^2 = CH_3$ , (2S, 3S): (2R, 3S), 100:0  
 7b,  $R^1 = Bz$ ,  $R^2 = CH_3$ , (2S, 3S): (2R, 3S), 50:50

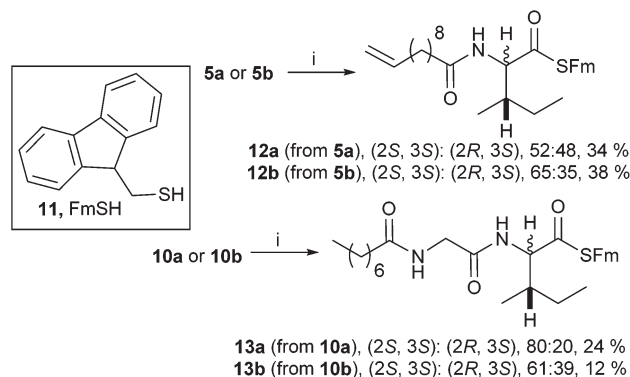
**Fig. 2** Isoleucine derivatives prepared with L-isoleucine and a mixture of L-isoleucine and D-allo-isoleucine stereochemistry.

from an epimeric mixture of D-allo-isoleucine and L-isoleucine, prepared from L-isoleucine according to the method of Yamada *et al.*<sup>14</sup> In addition, a model dipeptide octanoyl-glycyl-isoleucine was prepared as both the methyl ester **9** and the acid **10**, again diastereoisomerically pure and as a mixture (Scheme 1).

For the isoleucine derivatives **2** to **7**, differences of L-isoleucine and D-allo-isoleucine stereochemistry due to epimerisation were readily observed by the appearance of a new peak, assignable as the proton at the  $\alpha$ -stereocentre of D-allo-isoleucine by comparison with the equivalent compound prepared from a diastereoisomeric mixture of amino-acids. Compounds **5** and **10** were coupled to fluorenylmethane thiol **11** according to the method of Crich *et al.*,<sup>15</sup> to give the corresponding thioesters **12** and **13** respectively (Scheme 2). In each case, considerable epimerisation at the L-isoleucine stereocentre of the products **12a** and **13a** was readily identified by the appearance of a new peak in the  $^1H$  NMR spectrum, assignable as the proton at the  $\alpha$ -stereocentre of D-allo-isoleucine, by comparison with the equivalent products **12b** and **13b** prepared from the diastereoisomeric mixtures **5b** and **10b**. N-Propyl amides **15** and **16** were prepared by deprotection of the thioesters and reaction with the sulphonamide of propylamine **14**.



**Scheme 1** Reagents and conditions: (i) EDCl, HOBt, NMM, EtOH, 0 °C to rt, 17 h; (ii) LiOH, THF, H<sub>2</sub>O, 0 °C, 4 h.

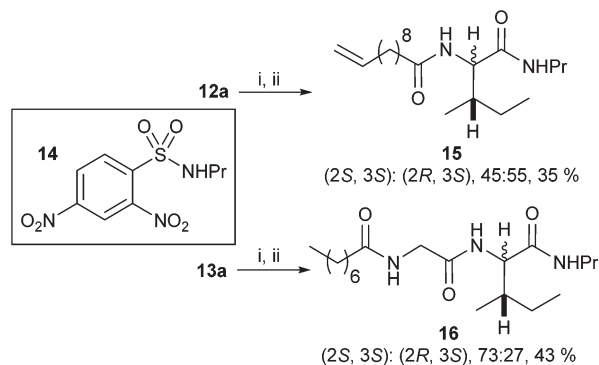


**Scheme 2** Reagents and conditions: (i) **11**, PyBOP,  $iPr_2NEt$  CH<sub>2</sub>Cl<sub>2</sub>, -20 °C, 4 h.

In each case, the products were obtained as a mixture of diastereoisomers in roughly the same proportions as the starting materials. Again, it was possible to distinguish between the L-isoleucine and D-allo-isoleucine derivatives by  $^1H$  NMR spectrometry (Scheme 3). For comparison, both **15** and **16** were also prepared using a direct PyBOP mediated peptide coupling reaction between propylamine and the equivalent acids **5a** and **10a** (not shown, see ESI†).

The  $^1H$  NMR spectra of the isoleucine derivatives were recorded in CDCl<sub>3</sub>, CD<sub>3</sub>OD and (CD<sub>3</sub>)<sub>2</sub>SO, both as a mixture of diastereoisomers and, where possible, as the single diastereoisomer (Tables 1–3). 700 MHz  $^1H$  NMR spectra were obtained and, after calibration against the internal solvent peak, the chemical shifts and coupling constants for the  $\alpha$ -CH hydrogen were measured.  $^{13}C$  NMR spectra were also obtained for most of these compounds at 100 MHz in CDCl<sub>3</sub> only. The  $^{13}C$  chemical shifts corresponding to the  $\alpha$ -CH were measured (Table 1).

The general trends noted earlier were further supported by these results. In all cases we found that the  $\alpha$ -CH chemical shift for the D-allo-isoleucine diastereoisomer occurs at a higher chemical shift than that for the L-isoleucine diastereoisomer. In addition, the  $^3J_{CH-CH}$  coupling constants for the L-isoleucine diastereoisomer  $\alpha$ -CH are always larger, and the



**Scheme 3** Reagents and conditions: (i) Piperidine, DMF, rt, 1.5 h; (ii) Cs<sub>2</sub>CO<sub>3</sub>, DMF, **14**, rt, 1 h.

**Table 1** Chemical shifts and coupling constants of L-isoleucine and D-allo-isoleucine diastereoisomers **3** to **16**

Entry	$\alpha\text{-CH}$ (ppm)		$^3J_{\text{CH-CH}}$ <sup>a</sup> (Hz)		$^3J_{\text{CH-NH}}$ <sup>a</sup> (Hz)		$\alpha\text{-CH}$ (ppm)	
	L	D-allo	L	D-allo	L	D-allo	L	D-allo
3	3.80	3.90	5.5	4.0	10.0	10.0	60.2	59.0
4	3.87	3.99	4.9	3.3	9.5	9.9	59.9	58.6
5	4.62	4.75	5.1	4.0	8.0	8.6	56.5	55.2
6	4.86	4.98	4.9	3.9	8.1	8.5	57.0	55.7
7	4.84	4.95	5.2	4.0	8.4	8.8	56.8	55.8
9	4.54	4.65	5.3	4.1	8.4	8.8	56.7	55.6
10	4.53	4.69	5.3	4.5	7.5	8.5	56.9	n/a
12	4.63	4.76	5.1	4.0	8.2	9.1	63.2	61.8
13	4.53	4.66	4.8	4.0	8.8	8.8	63.8	62.3
15	4.21	4.32	7.9	6.6	7.9	8.4	57.5	57.0
16	4.34	4.45	7.5	5.7	7.5	8.8	56.7	55.6

<sup>a</sup> To the nearest 0.1 Hz.**Table 2** Chemical shifts and coupling constants of L-isoleucine and D-allo-isoleucine diastereoisomers **2** to **16** in CD<sub>3</sub>OD

Entry	$\alpha\text{-CH}$ (ppm)		$^3J_{\text{CH-CH}}$ <sup>a</sup> (Hz)	
	L	D-allo	L	D-allo
2	4.00	4.03	4.0	3.9
3	3.70	3.82	7.0	5.0
4	3.67	3.82	5.5	4.5
5	4.37	4.54	5.8	4.7
6	4.57	4.74	6.4	5.0
7	4.56	4.74	6.9	5.6
9	4.42	4.56	6.0	4.4
10	4.40	4.55	5.3	4.0
12	4.31	4.48	6.1	5.1
13	4.33	4.48	5.7	4.8
15	4.15	4.31	8.3	6.7
16	4.20	4.35	7.5	5.7

<sup>a</sup> To the nearest 0.1 Hz.**Table 3** Chemical shifts and coupling constants of L-isoleucine diastereoisomers and D-allo-isoleucine diastereoisomers **1–16** in (CD<sub>3</sub>)<sub>2</sub>SO

Entry	$\alpha\text{-CH}$ (ppm)		$^3J_{\text{CH-CH}}$ <sup>a</sup> (Hz)		$^3J_{\text{CH-NH}}$ <sup>a</sup> (Hz)	
	L	D-allo	L	D-allo	L	D-allo
1	3.05	3.08	3.2	2.9	<i>b</i>	<i>b</i>
2	3.91	3.92	4.2	3.8	<i>b</i>	<i>b</i>
3	3.54	3.69	7.5	6.0	9.0	10.0
4	3.52	3.67	6.0	4.5	9.0	9.5
5	4.17	4.35	6.5	5.0	8.1	8.5
6	4.33	4.53	7.6	5.8	7.6	8.5
7	4.36	4.55	7.7	6.4	7.7	8.0
9	4.23	4.39	6.6	5.1	8.4	8.6
10	4.19	4.36	5.9	4.4	8.6	8.8
12	4.15	4.33	7.4	5.1	7.4	8.7
13	4.15	4.33	6.2	5.1	8.4	8.6
15	4.10	4.25	8.5	6.3	8.5	9.0
16	4.12	4.25	7.5	5.7	8.8	9.2

<sup>a</sup> To the nearest 0.1 Hz. <sup>b</sup> Not measurable.

$^3J_{\text{CH-NH}}$  coupling constants are usually smaller, than those for the D-allo-isoleucine diastereoisomer.

Generally, the difference in chemical shifts corresponding to the  $\alpha\text{-CH}$  of L-isoleucine and D-allo-isoleucine was greatest in DMSO-d<sub>6</sub> and smallest in CDCl<sub>3</sub>, but the  $\alpha\text{-CH}$  <sup>1</sup>H NMR peaks were always sufficiently distinct not to overlap at both 700 MHz and 400 MHz. This suggests they would be suitable for the approximate assignment of relative stereochemistry of isoleucine residues in compounds with unknown relative stereochemistry. The <sup>13</sup>C NMR data also suggest a useful trend with the chemical shift corresponding to the  $\alpha\text{-CH}$  of the D-allo-isoleucine derivative always occurring at a lower chemical shift than that for the L-isoleucine derivative, in most cases by more than 1 ppm. Taken in conjunction with the <sup>1</sup>H NMR data, the <sup>13</sup>C NMR data are also likely to prove extremely useful for the approximate assignment of relative stereochemistry of isoleucine residues in novel natural products.

## N-Arylation and peptide coupling reactions

N-Heteroaryl-amine derivatives, including N-aryl- $\alpha$ -amino-acid derivatives, provide important building blocks for a number of biologically active products.<sup>16</sup> We considered whether the NMR-based trends described above for distinguishing L-iso- and D-allo-isoleucine derivatives would apply to N-aryl compounds. A range of methods for N-arylation have been described, including the use of copper, palladium and nickel catalysis.<sup>16e,17</sup> N-Arylations of chiral amino-acids have been reported,<sup>17c,18</sup> and while some methods involve harsh conditions that risk racemisation, other methods are much milder and can occur without loss of stereochemical integrity as part of amino-acid analysis.<sup>19</sup>

To test these methods L-isoleucine methyl ester hydrochloride **2a** was heteroarylated with four imidoylchloride-like aryl chlorides in metal free conditions (Table 4). As expected in the mildly basic reaction conditions, <sup>1</sup>H NMR analysis indicated that arylations proceeded without significant epimerisation (**17a–20a**) when compared to reactions of a diastereoisomeric mixture of L-iso- and D-alloisoleucine methyl esters **17b–20b** (not drawn, see ESI†). The product ratios measured by <sup>1</sup>H NMR were confirmed by HPLC. Peptide couplings of N-arylated amino-acids have been used in the synthesis of a variety of peptidomimetic molecules, including DNA topoisomerases,<sup>20</sup> cathepsin S. inhibitors,<sup>21</sup> and benzoxaboroles.<sup>22</sup> We decided to extend the current study to include the synthesis of two N-aryl isoleucylglycine methyl esters, as well as the related N-benzoyl and N-tosyldipeptides for comparison. N-Aryl methyl esters **18a** and **20a** were hydrolysed and coupled to glycine methyl ester without isolation of the acids. Acids **4a** and **6a** were coupled directly (Table 5). Dipeptides **21a–23a** were produced as essentially single diastereoisomers, by comparison by both NMR spectrometry and HPLC with authentic stereoisomeric mixture **21b–24b** (not drawn, see ESI†). The N-benzoyl product **24a** was produced as a near equal mixture



Table 4 *N*-Arylation reactions of isoleucine methyl ester

Product	Heterocycle	Yield %	(2 <i>S</i> ,3 <i>S</i> ):(2 <i>R</i> ,3 <i>S</i> ) by NMR	(2 <i>S</i> ,3 <i>S</i> ):(2 <i>R</i> ,3 <i>S</i> ) by HPLC
17a		55 <sup>a</sup>	>95 : 5	>99 : 1
18a		30 <sup>b</sup>	>95 : 5	99 : 1
19a		33 <sup>b</sup>	>95 : 5	>99 : 1
20a		36 <sup>b</sup>	>95 : 5	>99 : 1

<sup>a</sup> Microwave, 80 °C, sealed tube, 2 h. <sup>b</sup> 20 °C, 1 atm, 12 h.

Table 5 Peptide coupling reactions of *N*-arylated isoleucine derivatives

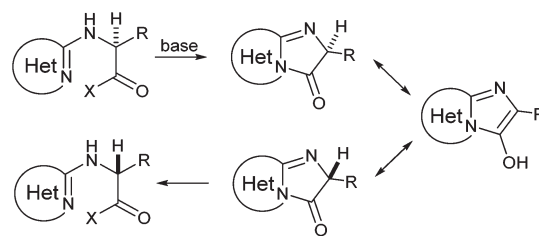
Entry	R	Yield	(2 <i>S</i> ,3 <i>S</i> ):(2 <i>R</i> ,3 <i>S</i> ) by NMR	(2 <i>S</i> ,3 <i>S</i> ):(2 <i>R</i> ,3 <i>S</i> ) by HPLC
21a		50%	>95 : 5	>99 : 1
22a		76%	97 : 3	>97 : 3
23a		84%	>95 : 5	>99 : 1
24a		54%	44 : 56	Not measured

Reagents (i) LiOH H<sub>2</sub>O, THF then EDCI, HOBt, NMM, EtOH, 0 °C to rt, 17 h.

of diastereoisomers. While it is well known that *N*-benzoyl amino acids tend to racemise during standard peptide coupling reactions,<sup>23</sup> and that *N*-sulfonyl amino acids do not,<sup>24</sup> to the best of our knowledge, there is little quantitative information regarding the levels of epimerisation of the C-terminal *N*-arylated amino-acids during these peptide coupling reactions. There are two main mechanisms of epimerisation during peptide coupling reactions, deprotonation of the

α-carbon and 5(4*H*)-oxazolone formation.<sup>9c</sup> In practise, these are normally minimised by careful control of reaction temperature, pH and *N*-substituent on the C-terminal amino-acid. Generally, *N*-amido amino-acids are more prone to racemisation than *N*-carbamate amino-acids (e.g. *N*-Cbz, *N*-Boc or *N*-Fmoc amino acids) having a greater tendency to oxazolone formation.<sup>9c,25</sup> Despite this, the benzoxazole and triazine active esters do not epimerise during peptide coupling *via* the related imidazolinone even though the heterocyclic nitrogens are in an analogous position to that of the amide oxygen in the benzamide (Scheme 4). Such a difference may be due to reduced nitrogen nucleophilicity because of increased steric crowding compared to the amide. Relative basicity does not correlate with nucleophilicity in this case as, even though 2-amino-4,6-dimethoxy-*s*-triazine is a very weak base in water,<sup>26</sup> 2-amino-benzoxazole has a p*K*<sub>aH</sub>(H<sub>2</sub>O) of 3.73,<sup>27</sup> and is therefore significantly more basic than benzamide (p*K*<sub>aH</sub>(H<sub>2</sub>O) = −1.53).<sup>28</sup>

Analysis of the <sup>1</sup>H NMR and <sup>13</sup>C NMR data for the *N*-aryl esters and the dipeptides show that the trends in relative chemical shift and coupling constants for the (2*S*,3*S*)- and



Scheme 4 Potential racemization mechanism of *N*-imidoyl amino-acids during peptide coupling.



**Table 6** Chemical shifts and coupling constants L-isoleucine and D-*allo*-isoleucine diastereoisomers of *N*-aryl and dipeptide compounds in CDCl<sub>3</sub>

Entry	$\alpha\text{-CH}$ (ppm)		$^3J_{\text{CH-CH}}$ <sup>a</sup> (Hz)		$^3J_{\text{CH-NH}}$ <sup>a</sup> (Hz)		$\alpha\text{-CH}$ (ppm)	
	L	D- <i>allo</i>	L	D- <i>allo</i>	L	D- <i>allo</i>	L	D- <i>allo</i>
17	4.76 <sup>b</sup>	4.93 <sup>b</sup>	<sup>c</sup>	<sup>c</sup>	<sup>c</sup>	<sup>c</sup>	58.5	57.4
18	4.58	4.70	5.5	4.5	8.5	9.0	60.4	59.4
19	4.63 <sup>b</sup>	4.79 <sup>b</sup>	<sup>c</sup>	<sup>c</sup>	8.5	9.0	62.1	60.5
20	4.59	4.73	5.0	4.5	8.5	9.5	57.9	56.8
21	4.30 <sup>b</sup>	4.48 <sup>b</sup>	<sup>c</sup>	<sup>c</sup>	<sup>c</sup>	<sup>c</sup>	61.7	60.8
22	4.47	4.64	7.5	5.0	7.5	8.5	59.3	58.1
23	3.55	3.67	4.0	5.5	8.5	8.5	61.5	60.2
24	4.57	4.69	7.0	5.5	8.5	8.5	57.9	57.1

<sup>a</sup> To the nearest 0.5 Hz. <sup>b</sup> Value is the centre of an undefined multiplet. <sup>c</sup> Not measurable.

(2*R*,3*S*)-diastereoisomers described above apply to these compounds as well (Table 6).

While L-isoleucine is the most common diastereoisomer in natural products, D-*allo*-isoleucine is also fairly common in microbial peptides, including the sporidomolides, stendomyacin, angolide, actinomycin C, calpinactam and peptidolipin N. A.<sup>29</sup> In addition, D-*allo*-isoleucine has been identified in peptides isolated from the skin of the yellow bellied toad, *Bombina variegata*.<sup>30</sup> Unsurprisingly, considering the comparative stability of the  $\beta\text{-CH}$  of isoleucine peptides containing L-*allo*-isoleucine and D-isoleucine are far less common, though a few examples do exist.<sup>31</sup> Alteration of relative stereochemistry of isoleucine within a peptide can have significant effects of its structure and folding, and potentially its biological activity.<sup>31d,32</sup> The ability to distinguish between, and potentially predict, the stereochemistry isoleucine diastereoisomers by a non-destructive method such as NMR, instead of peptide hydrolysis, is valuable, particularly for complex natural proteins, which are often only obtained in minute quantities. While in this study we cannot distinguish between L-*allo*-isoleucine and D-*allo*-isoleucine, and between the L- and D-isoleucine, the comparative rarity of the D- and L-*allo* forms means that this method will prove extremely useful. This method can be applied to the structure of the antibiotic peptide teixobactin.<sup>33</sup> By hydrolysis, and derivatisation using Marfey's method, teixobactin was found to contain three L-isoleucines and one D-*allo*-isoleucine. It is worth noting that the full assignments of the NMR spectra of the peptide in deuteriated DMSO show that in the <sup>1</sup>H NMR spectrum the peak corresponding to the  $\alpha\text{-CH}$  hydrogen of the D-*allo*-isoleucine residue has a higher chemical shift (4.36 ppm) than the peaks due to the  $\alpha\text{-CH}$  hydrogens of the three L-isoleucine residues (4.29, 4.12 and 4.03 ppm), and that in the <sup>13</sup>C NMR spectrum the peak corresponding to the  $\alpha\text{-CH}$  carbon of the D-*allo*-isoleucine has a lower chemical shift (56.8 ppm) than those corresponding to  $\alpha\text{-CH}$  carbons of the three L-isoleucine residues (57.3, 57.8 and 57.9 ppm), in agreement with the trends observed in Tables 1–3 and 6.

It is unclear at this point as to whether the differences observed are due to purely stereochemical differences in amino-acid derivatives with otherwise similar backbone con-

formations, or if the stereochemical differences cause a significant change in backbone conformation which is itself the reason for the observed differences in the NMR spectra.<sup>34</sup> Differentiation between these two possibilities may be effected by studying pairs of stereoisomers with defined conformations e.g. involving cyclic peptides of various sizes. Conformational analysis would be supported with molecular modelling. While the analysis of the spectra from teixobactin,<sup>33</sup> which contains both linear and cyclic isoleucine-containing regions, indicates that the trends presented in this paper may be applicable to more complex peptides, the issues of conformation and additional stereochemistry are the subjects of future work in our laboratory and results will be reported in due course.

## Conflicts of interest

There are no conflicts to declare.

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