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# [6-$^{13}$C]-(2*S*,4*S*)-5-Chloroleucine: synthesis and incubation studies with cultures of the cyanobacterium, *Lyngbya majuscula*

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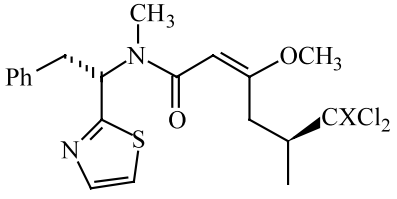
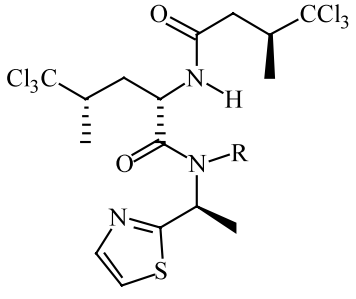
**Abstract**—[6-$^{13}$C]-(2*S*,4*S*)-5-Chloroleucine **12** was prepared in six steps and 26% overall yield from protected L-glutamic acid using $^{13}CH_3I$ as the source of isotopic label. On feeding **12** to cultures of *L. majuscula* no incorporation of isotopic label into the trichlorinated marine natural product barbamide was detected. The synthesis of a novel dichloro amino acid **16** is also described.


In recent years several natural products containing a trichloromethyl group have been isolated from sponges of the genus *Dysidea* which have symbiotic association with cyanobacteria.[1] These compounds include, for example, the dysamides,[2] dysidin,[3] dysidenin,[4] herbacic acid[5] and herbamide A.[6] It has been suggested that sponge-based dysidenins are biosynthesised from associated cyanobacteria and indeed, in 1996 a new natural product, barbamide **1** was found in extracts of the cyanobacterium *Lyngbya majuscula*.[7] Further metabolites **3**, **4** and **5** encompassing a trichloromethyl group have been identified more recently in extracts of *L. majuscula*.[8]

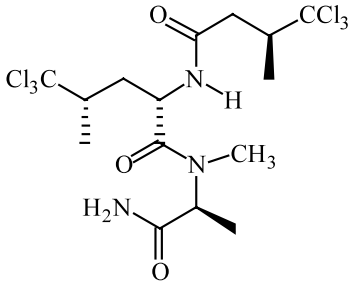
In the majority of halogenated natural products, the halogens are incorporated into positions which are suggestive of their biochemical reaction involving electrophilic species and indeed haloperoxidases which catalyse such reactions have been widely studied.[9] However, the biosynthesis of barbamide is particularly fascinating as feeding studies have revealed that the trichloromethyl group derives from the *pro-R* methyl group of L-leucine (leading to the 2*S* stereocentre of barbamide) without detectable activation to facilitate a potential nucleophilic or electrophilic chlorination process (Scheme 1).[10] In addition, a very high level of incorporation of carbon-13 into barbamide from exogenously applied [2-$^{13}$C]-5,5,5-trichloroleucine strongly suggests that trichloroleucine is also an intermediate on the pathway.[11] Hence, we have proposed that biochlorination occurs through a novel process, possibly involving radical chemistry, but the precise mechanism has



**1**, X=Cl, barbamide
**2**, X=H, dechlorobarbamide



**3**, R=$CH_3$, pseudodysidenin
**4**, R=H, nordysidenin



**5** Dysidenamide

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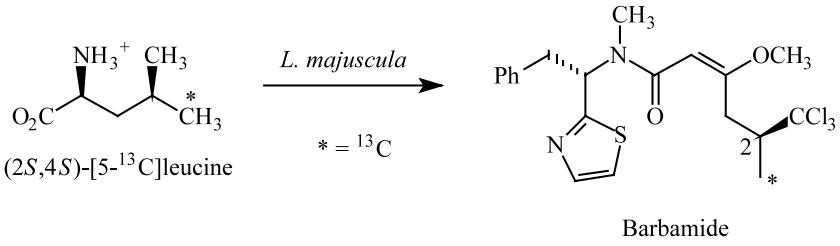


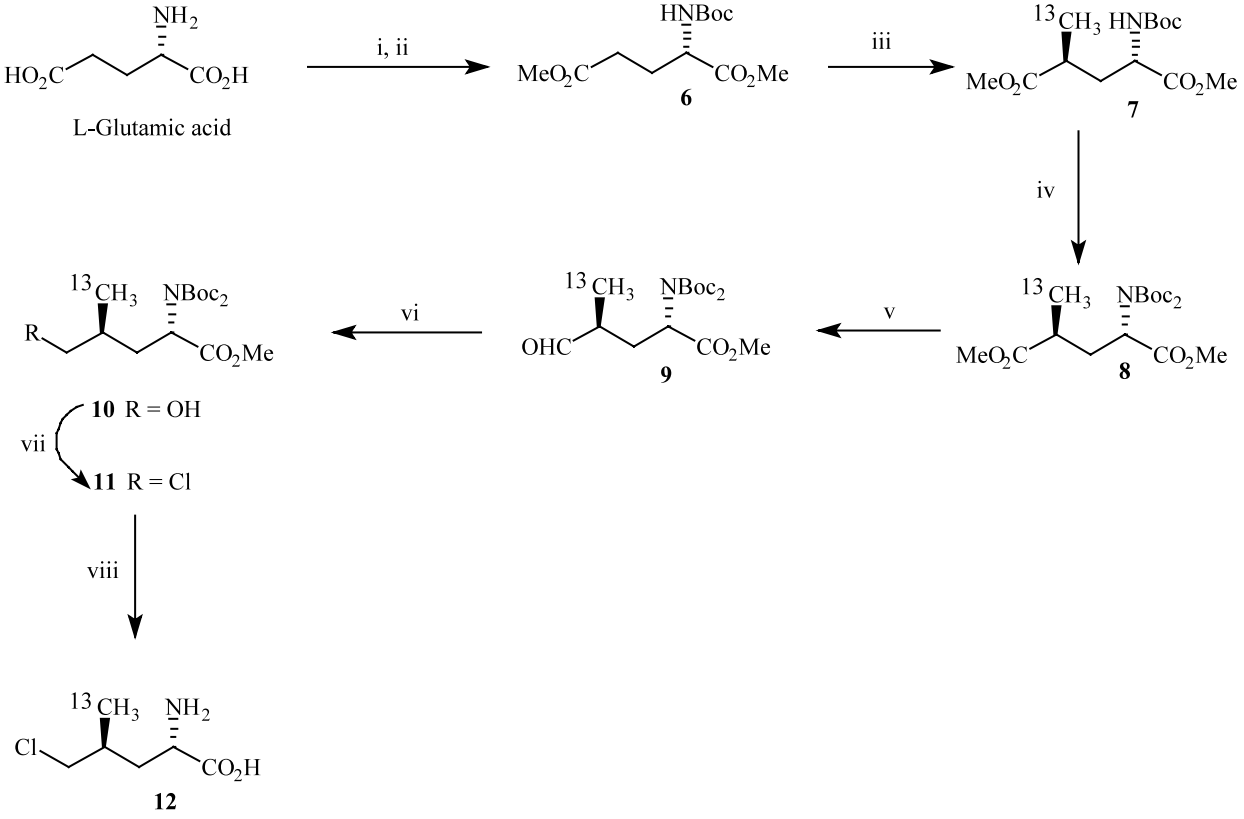

**Scheme 1.**

not yet been established.[10–12] In order to probe whether a stepwise biochlorination process is occurring via chloroleucine we required a sample of (2*S*,4*S*)-5-chloroleucine labelled with carbon-13 at any site except C-1 (as C-1 of leucine in lost in the biosynthesis of barbamide) for incubation studies.[13] We now describe the synthesis of **12** and the results of incubation studies with *L. majuscula*.

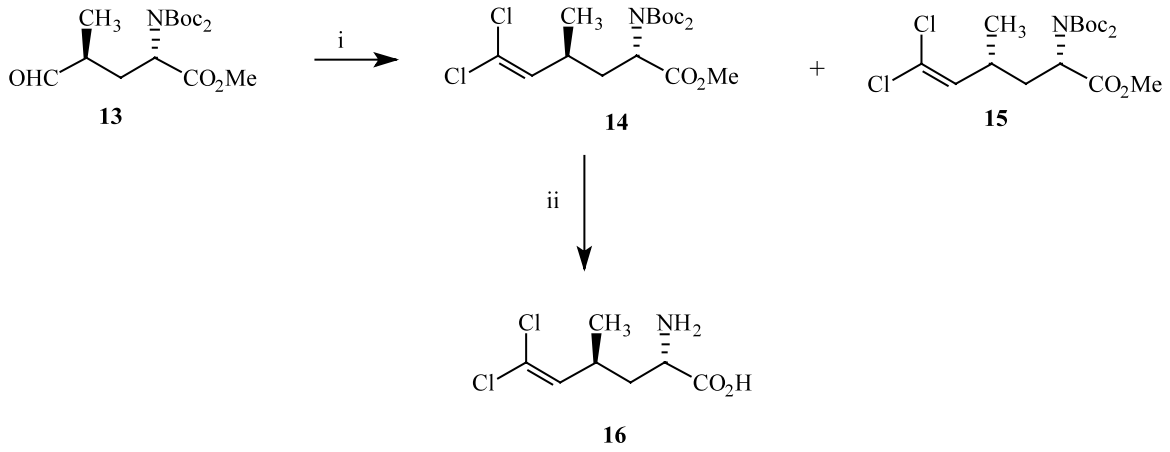
When designing a synthetic route to carbon-13 labelled compounds, ideally the expensive isotope should be introduced as late as possible in the pathway and the cost and availability of suitably labelled precursors needs to be taken into account.[14] It has been reported that reaction of the lithium enolates of *N*-Boc and *N*-CBz glutamates with a range of electrophiles gives the *anti* alkylated products with excellent diastereoselectivites (via 1,3-asymmetric induction) and in good yields.[15] Hence we proposed to use this reaction with $^{13}CH_3I$ as the electrophile to give **7**, which, following selective reduction of the γ-ester to a primary alcohol, conversion to the chloride and deprotection would give the target [6-$^{13}$C]-(2*S*,4*S*)-chloroleucine **12** (Scheme 2).

First the known *N*-Boc dimethyl ester **6** was readily prepared in two steps and 90% yield from L-glutamic acid.[16] Alkylation of **6** was optimised using unlabelled iodomethane, lithium hexamethylsilylazide at −78°C giving >60% yield of alkylated product as a single diastereomer by $^1$H and $^{13}$C NMR spectroscopy. The reaction was repeated using $^{13}CH_3I$ to give **7** in 63% yield. The $^1$H NMR spectrum of **7** showed a characteristic doublet of doublets, $J$ 128.6, 6.9 Hz at $\delta$ 1.22 assigned to the newly introduced $^{13}CH_3$.

Prior to reduction of the γ-ester, we deemed it prudent to further protect the α-amino functionality in **7** as intramolecular cyclisation of α-*N*-Cbz-amino-γ-alcohols is a facile process.[17] Treatment of **7** with $Boc_2O$,




**Scheme 2.** *Reagents and conditions*: (i) MeOH, $SOCl_2$; (ii) $Boc_2O$, $Et_3N$, MeOH; (iii) BuLi, HMDS, $^{13}CH_3I$, THF; (iv) $Boc_2O$, DMAP, $CH_3CN$; (v) DIBALH, $CH_2Cl_2$, −78°C; (vi) $NaBH_4$, MeOH; (vii) $Ph_3P$, $CCl_4$; (viii) 6 M HCl, 6 h reflux.




**Scheme 3.** *Reagents and conditions*: (i) $Ph_3P$, $Et_3N$, $CCl_4$, (ii) 6 M HCl, reflux.

DMAP in $CH_3CN$ gave the required diBoc protected diester **8** in 95% yield. Attempts to directly reduce the γ-ester to a primary alcohol **10** with DIBALH proved unsatisfactory as a mixture of products was obtained. A more efficient approach proved to be reduction of the ester **8** in $CH_2Cl_2$ to the aldehyde **9** by the dropwise addition of DIBALH (1 equiv.) at 0°C followed by further reduction of crude **9** to the required alcohol **10** using $NaBH_4$ in methanol. The primary alcohol was readily converted to the required chloride **11** using triphenylphosphine and carbon tetrachloride and finally the protecting groups were removed in refluxing 6 M HCl giving the target [6-$^{13}$C]-(2*S*,4*S*)-5-chloroleucine **12** in 40% yield over the five steps from introduction of the isotopic label in **7**.[18]

[6-$^{13}$C]-(2*S*,4*S*)-5-Chloroleucine **12** (84 mg) was fed to cultures (2 L) of *L. majuscula* and after 10 days barbamide was isolated and analysed by NMR spectroscopy but no isotope enrichment was apparent. In contrast, we have previously shown that both carbon-13 labelled L-leucine and trichloroleucine are readily incorporated into barbamide in cultures of *L. majuscula*.[10,11] Biosynthetic and precursor incorporation studies are consistent with a mixed type I PKS/NRPS origin for barbamide. One possible explanation for the fact that no incorporation of carbon-13 into barbamide was observed on feeding [6-$^{13}$C]-(2*S*,4*S*)-5-chloroleucine **12** is that only leucine, and not chloroleucine, may be activated as the proposed acyl-adenylate and thus recognised by a peptidyl-carrier protein (PCP).[19] Clearly an alternative explanation is that chloroleucine is not an intermediate in the biochlorination of leucine to trichloroleucine in *L. majuscula*.

Interestingly dechlorobarbamide **2** has been detected as a minor secondary metabolite from extracts of *L. majuscula*, hence, it is reasonable to propose that the biochlorination process does occur via dichloroleucine. Thus, an alternative biosynthetic pathway may involve oxidation of the *pro-R* methyl group of leucine to the aldehyde oxidation level followed by multiple additions of chlorine, to give dichloro- and trichloromethyl groups present in the natural products **1** and **2**. We have examined an in vitro conversion of aldehyde **13** to protected dichloroleucine by reaction with $Ph_3P/CCl_4$ under conditions reported to give dichlorides from aldehydes.[20] However, in this case unsaturated dichlorides **14** and **15** were obtained in an approximately 1:1 mixture via a process analogous to the first stage of the Corey–Fuchs reaction (Scheme 3).[21] In order to reduce the tendency for epimerisation at C-4, aldehyde **13** was treated with $Ph_3P/CCl_4/Et_3N$ giving **14** in 80% yield with <5% of the diastereomer **15** apparent by NMR spectroscopy. Hydrolysis of **14** in refluxing 6 M HCl gave the novel amino acid **16**. We have examined further methods for the preparation of *gem*-dichlorides from aldehyde **13** in which a Wittig type reaction cannot compete, e.g. with $PCl_5$[22] or $[Ph_3PCl]^+Cl^-$, but, in each case, no protected dichloroleucine was detected. Further studies are currently underway to elucidate the mechanism of biochlorination of L-leucine in *L. majuscula*.

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18. [6-$^{13}$C]-(2*S*,4*S*)-5-Chloroleucine **12**: mp 223–225°C; $[\alpha]_D$ +25.5 (*c* 1.0 in $CH_3OH$); $\delta_H$ (300 MHz, $D_2O$) 1.10 (3H, dd, *J* 126.9, 7.5, $^{13}CH_3$), 1.66 (1H, m, 3-*H*H), 2.45 (1H, m, 4-H), 2.57 (1H, m, 3-H*H*), 2.93 (1H, dd, *J* 10.4, 6.9, 5-H*H*), 3.45 (1H, dd, *J* 10.4, 6.9, 5-*H*H), 4.13 (1H, t, *J* 8.4, 2-H); $\delta_C$ (75.45 MHz, $D_2O$) 16.7 (enriched $CH_3$), 28.6 (C-3), 37.4 (C-4), 53.0 (C-5), 62.7 (C-2), 175.4 (C-1); *m*/*z* (EI) 166.0589 ($M^+$, $C_5{}^{13}CH_{12}NO_2Cl$ requires 166.0590), 131 (2), 109 (10) and 85 (100).
Dichloride **16**: mp 200°C (decomposition), $[\alpha]_D$ +21.0 (*c* 1.0 in $H_2O$), $\delta_H$ (400 MHz, $D_2O$) 1.07 (3H, d, *J* 7.5, $CH_3$), 1.89 (2H, m, 3-$H_2$), 2.76 (1H, m, 4-H), 3.66 (1H, m, 2-H), 5.87 (1H, d, *J* 9.5, 5-H); $\delta_C$ (100 MHz, $D_2O$) 18.3 ($CH_3$), 31.6 (C-3), 36.8 (C-4), 53.0 (C-2), 118.2 (C-6), 134.0 (C-5), 174.4 (C-1); *m*/*z* (CI) 212/214/216 ($MH^+$, 100/66/12) and 166/168/170 (50/35/8).
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