Biosynthesis of Diisocyanoadociane, a Novel Diterpene from the Marine Sponge *Amphimedon* sp. Crystal Structure of a Monoamide Derivative

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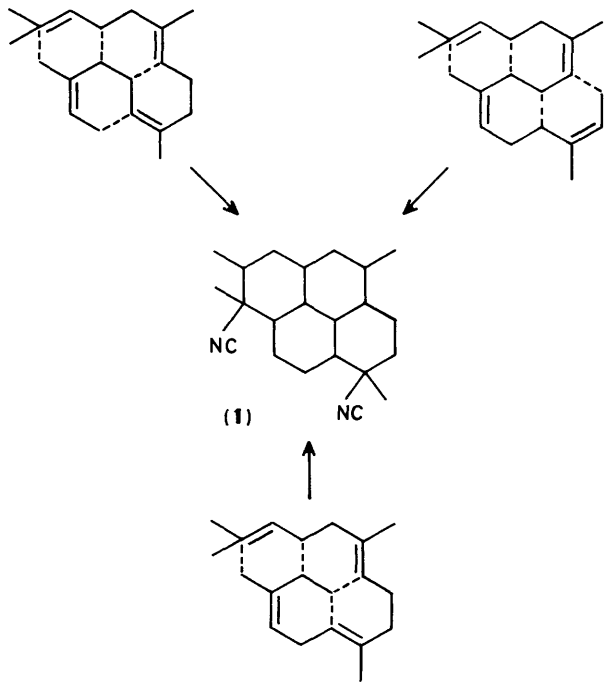
Sodium [^{14}C]cyanide is efficiently incorporated into diisocyanoadociane [1,8-di-isocyano-1,2,5,8-tetramethylperhydropyrene, (**1**)], the major metabolite of the haplosclerid sponge *Amphimedon* sp. It is established by stepwise chemical degradation that the radiolabel is associated with the two isocyanide carbons. Zinc [^{14}C]cyanide and isobutyraldehyde [^{14}C]cyanohydrin are effective precursor alternatives to sodium [^{14}C]cyanide and more suitable for use in stable isotope study. Attempts to demonstrate an amino acid origin of the cyanide utilised by *Amphimedon* sp. include tracer studies with [U-^{14}C]alanine, [2-^{14}C]glycine, [U-^{14}C]leucine and [guanidino-^{14}C]arginine; none of these precursors were used for isocyanide synthesis. Single *X*-ray crystallographic structure analysis of a *p*-bromobenzamide degradation product (**17**) established that the absolute configuration for diisocyanoadociane is (1*R*,2*R*,3a*S*,5*S*,6*R*,8*S*,8a*S*,10a*S*,10b*S*,10c*S*).

Diisocyanoadociane (**1**)[1,2] (1,8-di-isocyano-1,2,5,8-tetramethylperhydropyrene),† is the major antimicrobial metabolite from an encrusting sponge, *Amphimedon* sp. (ex. *Adocia* sp.), common to the midshelf reefs of the Great Barrier Reef. The biosynthesis of diisocyanoadociane is of interest for three reasons: (i) the ring skeleton is unique and may result from novel cyclisation of a C_{20} skeleton during which methyl migration occurs (Scheme 1). At least three different foldings of the C_{20} precursor lead to the desired skeleton; (ii) the biochemical origin of isocyanide groups is unclear; (iii) the metabolite may be of bacterial rather than sponge origin. Over twenty different terpenes containing an isocyanide substituent have been isolated from marine sponges,[1–7] although this functional group is rarely observed in terrestrial metabolism.[8] Most of the isocyanides isolated from marine sources co-occur with their corresponding isothiocyanates, formamide, and amine derivatives.[3–7,9] Many of these compounds possess antimicrobial or cytotoxic activity. Bergquist[10] proposes that their presence in the organism may confer an advantage by helping to preserve the specificity of association of the sponge and its preferred microfloral symbionts.

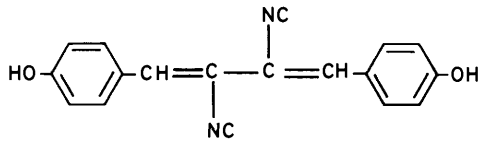
The biosynthetic origin of isocyanides is obscure. Xanthocillin (**2**), one of the first isocyanides isolated, has been the subject of extensive biosynthetic study.[11–15] The source of the 1,4-diarylbutadiene system has been identified as tyrosine by precursor study with ^{14}C, ^{15}N, and ^{2}H isotopes. Acetate, formate, and methionine were tested as possible precursors for the isocyanide carbons[11] as were glycine and serine, all without success. More recently, methionine has been reported to act as precursor to the isocyanide groups in the antibiotics hazimycin factors 5 and 6 [structures (**3**) and (**4**)].[16]

It was initially considered that marine isocyanide biosynthesis proceeds *via* capture of a carbonium ion or equivalent by ammonia to give an amine which would then be alkylated by the C_1 pool in the same fashion as proposed for xanthocillin. Subsequent dehydration generates the isocyanide group. This proposal was supported by the co-occurrence of formamide derivatives alongside the isocyanides for most of the terpene skeletons isolated to date. However, in pioneering biosynthetic work on sponge terpenes, Sodano *et al.*[17] supplied ^{14}C-labelled axamide-I (**5**) to the mediterranean sponge *Axinella cannabina*

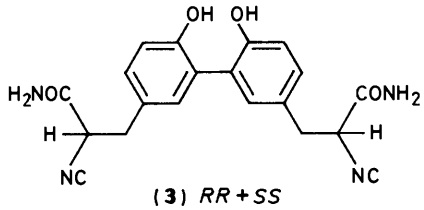


Scheme 1.

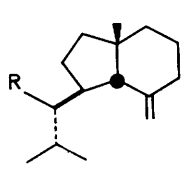
† I.U.P.A.C. nomenclature for diisocyanoadociane is presented in this paper. Previously, a numbering scheme based on the hypothetical hydrocarbon epiamphilectane has been used.[2] The key isocyanide-substituted carbons, C(1) and C(8), were numbered C(20) and C(7) respectively in a preliminary communication.[32]



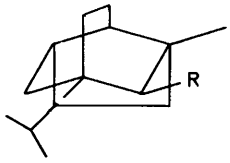
(2)



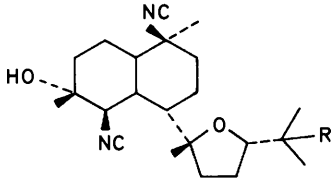
(3) *RR*+*SS*
(4) *RS*



(5) R=NHCHO
(6) R=NC



(7) a; R = NC
b; R = NHCHO
c; R = NCS



(8) R=Cl
(9) R=NC

	R′	R″
(1)	NC	NC
(10)	NHCHO	NHCHO
(11)	NHCHO	NH_2
(12)	NH_2	NH_2
(13)	$NHCONHC_6H_4Cl$-*m*	$NHCONHC_6H_4Cl$-*m*
(14)	NH_2	NHCHO
(15)	NHCHO	$NHCONHC_6H_4Cl$-*m*
(16)	NHCHO	$NHCSNHC_6H_4Br$-*p*
(17)	NC	$NHCOC_6H_4Br$-*p*
(18)	NHCHO	$NHCOC_6H_4Br$-*p*

but failed to obtain incorporation into axisonitrile-I (**6**). The recovery of ^{14}C-labelled fatty acids indicated precursor utilisation although over 85% radioactivity was recovered unchanged.

Scheuer's group provided evidence that the reverse transformation, *i.e.*, RNC→RNHCHO (where R represents a typical terpene skeleton), may be the major pathway to formamides.[18] Samples of ^{13}C-labelled isopupukeanane (**7a**) were converted into the corresponding formamide (**7b**) and isothiocyanate (**7c**) derivatives in low yield, as assessed by mass spectrometry. [^{13}C]Formate was not utilised by the sponge. An attractive mechanism for the direct formation of an isocyanide terpene is the capture of a carbocation or equivalent by the ambident nucleophile cyanide. Circumstantial evidence in favour of this mechanism is provided by the isolation of the highly oxygenated kalihinol series of metabolites by Scheuer's group.[7] The co-occurrence of kalihinols B and F [(**8**) and (**9**)] is supportive of a mode of formation in which the terminal double bond of a C_{20} precursor is attacked by either chloride ion or cyanide ion, possibly on an epoxide intermediate.

In this paper, we present results[19] of tracer experiments with sodium [^{14}C]cyanide and other precursors which establish the origin of the two isocyanide groups. Details of chemical studies which allowed the specificity of incorporation of precursors to be checked are presented. A single crystal *X*-ray structure determination of a derivative of diisocyanoadociane confirms the absolute configuration of the group of metabolites produced by *Amphimedon* sp. and removes the current ambiguity in the literature as to the correct enantiomeric form of diterpene isocyanide metabolites.

Biosynthetic Studies.—All experiments were carried out at John Brewer Reef, 75 km north-east of Townsville. Sponge transplants were prepared according to the method of Wilkinson;[20] the survival rate of transplants was 70—80% over a six month period in 1984—1985, dropping to less than 50% during the second summer period 1985—1986.

In situ incorporations were carried out using the method of Thompson *et al.*;[21] during the incubations, uptake of precursors was monitored by analysis of water samples taken at 2—3 h intervals. Results for the utilisation of sodium [^{14}C]cyanide (Figure 1) indicate uptake of approximately 80% radioactivity. The sponges were then returned to an underwater grid at −14 m for incubation periods of up to one month prior to work-up.

First, the incorporation of two C_1 precursors, cyanide and formate, was assessed (Table 1). The data establish clearly that formate was not incorporated (although uptake did occur, Figure 1); in contrast there was efficient utilisation of cyanide for diisocyanoadociane production. The highest incorporation levels were obtained in experiments of approximately one month duration. It was essential to confirm that the label was associated with the two isocyanide groups, and the degradative sequence shown in Scheme 2 was therefore developed (see Chemical Studies Section for details). The results of degradation of ^{14}C-labelled (**1**) are shown in Table 2. The bisformamide (**10**) retained over 96% activity of the parent di-isocyanide. On removal of one formyl group to give (**11**), 49% total activity was

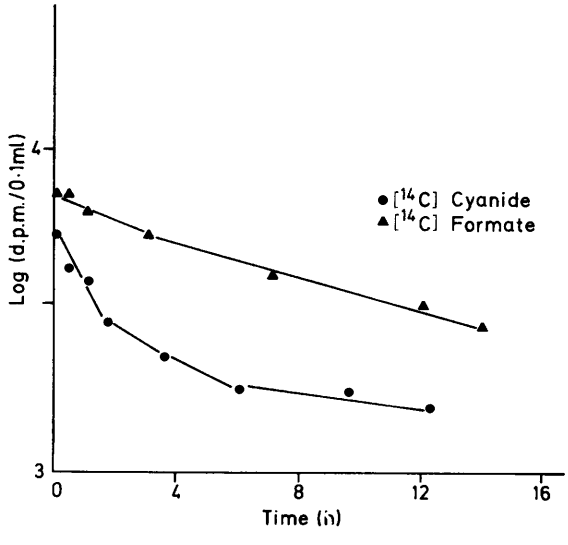


Figure 1. Uptake of sodium [^{14}C]cyanide or sodium [^{14}C]formate by *Amphimedon* sp.

Table 1. Incorporation of labelled precursors into diisocyanoadociane (**1**)

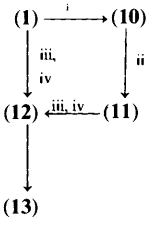
Expt.	Incubation (days)	Precursor fed (μCi)	Amount	Weight (1) (mg)	Specific activity[b] (μCi mmol^{-1})	% Incorporation
1	18	[^{14}C]Cyanide	40	34	0.76(3)	0.20
2	18	[^{14}C]Cyanide	23	26	1.32(3)	0.46
3	34	[^{14}C]Cyanide	63	86	4.35(1)	1.83
4	30	[^{14}C]Formate	125	165		

[a] Experiments 1 and 4 used 20 day old transplants, experiments 2 and 3 used freshly prepared transplants. [b] Number of recrystallisations required to give constant specific activity in brackets.

Table 2. Degradation of [^{14}C]cyanide-labelled diisocyanoadociane

Expt.	Compound	Weight (mg)	Specific activity μCi mmol^{-1}	% Radio-activity
6	(**1**)	26	1.32	100.0
	(**12**)	5	0.02	1.5
	(**12**)[b]	3	<0.01	<0.8
7	(**1**)[a]	67	0.82	100.0
	(**10**)	65	0.79	96.3
	(**12**)	12	0.03	3.7
	(**11**)	32	0.40	48.8
	(**13**)	9	<0.01	<1.2

[a] Compound (**1**) (16.5 mg) of specific activity 4.35 μCi mmol^{-1} was diluted with non-radioactive (**1**) (68 mg) and recrystallised twice from analytical grade hexane to give 67 mg of specific activity 0.82 μCi mmol^{-1}. [b] Following a second purification *via* the hydrochloride.



Scheme 2. *Reagents and conditions:* i, AcOH, 25 °C, 12 h; ii, 2.5M NaOH, 100 °C, 3 h, then 25 °C for 20 h; iii, 6M HCl, 100 °C, 4 h, then 25 °C for 20 h; iv, dil. OH^-; v, *m*-chlorophenyl isocyanate, 25 °C, 18 h

retained. In contrast, the diamine (**12**) retained low level activity (1.5—3.7%) which almost certainly derived from amide impurities since a second purification of the diamine *via* its hydrochloride salt gave radioactive-free material. Conversion of the diamine product into the bis(*m*-chlorophenylurea) derivative (**13**) also resulted in loss of ^{14}C activity. Thus the isocyanide substituents of (**1**) are selectively labelled by cyanide and the label is equally distributed between the two positions.

In the reaction of alkyl halides with metal cyanides,[22] nitrile formation is generally favoured except in the presence of silver ions; however isocyanide formation may be reversible. The possibility that the [^{14}C]cyanide precursor was chemically exchanging with the isocyanide groups of diisocyanoadociane was investigated by allowing a sample of diisocyanoadociane to stand in water containing [^{14}C]cyanide (100 μCi l^{-1}). After 35 days, the isocyanide was reisolated and recrystallised to constant specific activity. The specific activity (1.18 μCi mmol^{-1}) was about one quarter of that obtained in the biosynthetic experiment. However, when this chemically pure sample was subjected to silica gel chromatography followed by further recrystallisation, the ^{14}C content was found to be negligible. Our interpretation of these data is the conversion of cyanide into formate followed by reaction with isocyanide substituents generating formamide impurities at tracer level. These could not be satisfactorily removed by recrystallisation alone. This result illustrates a common difficulty in biosynthetic study using radioisotopes.

With chemical exchange excluded as a mechanism for the incorporation of [^{14}C]cyanide, the biosynthetic experiment supports the utilisation of the carbon atom of cyanide in isocyanide biosynthesis. A biomimetic synthesis of diisocyanoadociane from trimethylsilyl cyanide has recently been reported.[23] In order to provide evidence for the utilisation of cyanide-derived nitrogen or the intact incorporation of a cyanide unit, stable isotope study with ^{15}N (mass spectrometric detection) or ^{13}C–^{15}N (n.m.r. detection) is required. There are a number of experimental considerations to bear in mind when progressing from a ^{14}C tracer study to stable isotope study which are particularly relevant in a marine biosynthetic study of this type. (i) Concentration of precursor. The successful [^{14}C]cyanide study discussed above used 110 μg cyanide in a total incubation volume of *ca.* 4 l, a concentration of 5 × 10^{-7}M. Typical concentrations of common nutrients (amino acids, sugars) in sea water are 0—25 μg l^{-1},[24] *i.e.* similar to those used in the cyanide experiment. As filter feeders, sponges may not be able to take up precursors effectively at non-physiological concentrations. The concentration levels required for effective precursor incorporation with ^{13}C or ^{15}N are minimally 50 mg l^{-1}, *i.e.* three orders of magnitude higher than those used in the successful ^{14}C study.

(ii) Sensitivity of detection. Dilution values for detectable incorporation of singly labelled ^{13}C precursors (by n.m.r.) are 100 or less; the dilution values for the incorporation of double label (^{13}C–^{15}N) precursors will be up to an order of magnitude higher. The best dilution value obtained with sodium cyanide in our tracer study (Table 3) was 2.66 × 10^4.

(iii) Toxicity of precursor. The proven role of cyanide as an enzyme inhibitor[25] precludes incorporation study with high concentration levels of this precursor. These fears were confirmed in preliminary studies with [^{14}C]cyanide at concentration levels of 50—250 mg l^{-1}. Although all experimental animals survived, loss of symbionts was observed during incubation; subsequently cell disintegration occurred and fragile transplants were collected. The quantity of organic extract isolated in each experiment was inversely proportional to the cyanide concentration used. Only the lowest dosage sample contained any diisocyanoadociane (by t.l.c., n.m.r.) and the presence of minute traces of (**1**) in the other two experiments was only detectable by h.p.l.c. The organic components were presumably used as source of carbon and nitrogen by the decaying sponge.

In order to avoid these problems, we considered organic and inorganic forms of cyanide which might generate cyanide at non-toxic and non-saturating concentrations. Cyanohydrins have been tested as alternative precursors of cyanide in biosynthetic studies on xanthocillin.[26] Inorganic cyanide can also be stabilised by co-ordination to a transition metal. Of the

Table 3. Incorporation of organic and inorganic cyanides by *Amphimedon* sp.

Expt.	Precursor	Quantity supplied: Wt (mg)	Quantity supplied: Radioactivity (μCi)	Specific activity (**1**) μCi mmol^{-1}	Dilution factor
1	Sodium cyanide	0.05	63	4.350	26 666
2	Isobutyraldehyde cyanohydrin	23.80	100	0.066	12 606
3	$Ni(CN)_2$	30.90	100	0.055	15 127
4	$K_2Ni(CN)_4$	62.15	100	0.017	48 941
5	$Zn(CN)_2$	29.35	100	0.070	11 428
6	$K_2Zn(CN)_4$	53.9	100	0.005	161 616

metal ions known to be present in biological tissues we considered Fe, Ni, Zn, and Co as candidates. Metal cyanide complexes[27] of Fe and Co are very stable and would not be expected to release CN^- except in the presence of a good nucleophile (such as RS^- in biological systems). Cyanide complexes of Fe^{2+}, Fe^{3+}, and Co^{3+} are kinetically inert to cyanide exchange[28] whereas those of Ni^{2+} and Zn^{2+} are very labile.[27-29] Table 3 shows the results of incorporation of charged and neutral complexes of Ni^{2+} and Zn^{2+}. As anticipated, neutral complexes such as $Zn(CN)_2$ are effective precursors. The charged complexes $K_2Ni(CN)_4$ and $K_2Zn(CN)_4$ were also incorporated, surprisingly since we had anticipated that they might fail to pass across cell membranes. In two of the successful incorporations (zinc cyanide and isobutyraldehyde cyanohydrin), degradation to the diamine (**12**) confirmed that the ^{14}C label was associated with the isocyanide carbons. Dosage experiments to determine the effectiveness of these two precursors at concentrations suitable for ^{13}C–^{15}N study are currently in progress.

The origin of the cyanide accumulated in (**1**) by *Amphimedon* sp. is of interest. The concentration of cyanide in sea-water is not documented and is presumably negligible, except perhaps in industrially polluted waters. By analogy with terrestrial metabolic processes such as the biosynthesis of cyanogenic glycosides,[25,30] or ethylene,[31] the ultimate source of the cyanide may be an amino acid. A number of amino acids have been tested as potential precursors of diisocyanoadociane, including [U-^{14}C]alanine, [U-^{14}C]leucine, [2-^{14}C]glycine, and [guanidino-^{14}C]arginine. In every case, precursor uptake could be demonstrated both by loss of ^{14}C from the incubation medium and by subsequent detection of ^{14}C in the sponge tissue (by whole tissue digest), but none of these precursors generated labelled diisocyanoadociane. Sponges contain many unusual amino acids along with the common protein amino acids, and it is highly possible that one of these specific amino acids may act as a cyanide precursor. Confirmation of intact utilisation of cyanide by ^{13}C–^{15}N precursor study would add weight to the idea that an amino acid is the ultimate isocyanide precursor.

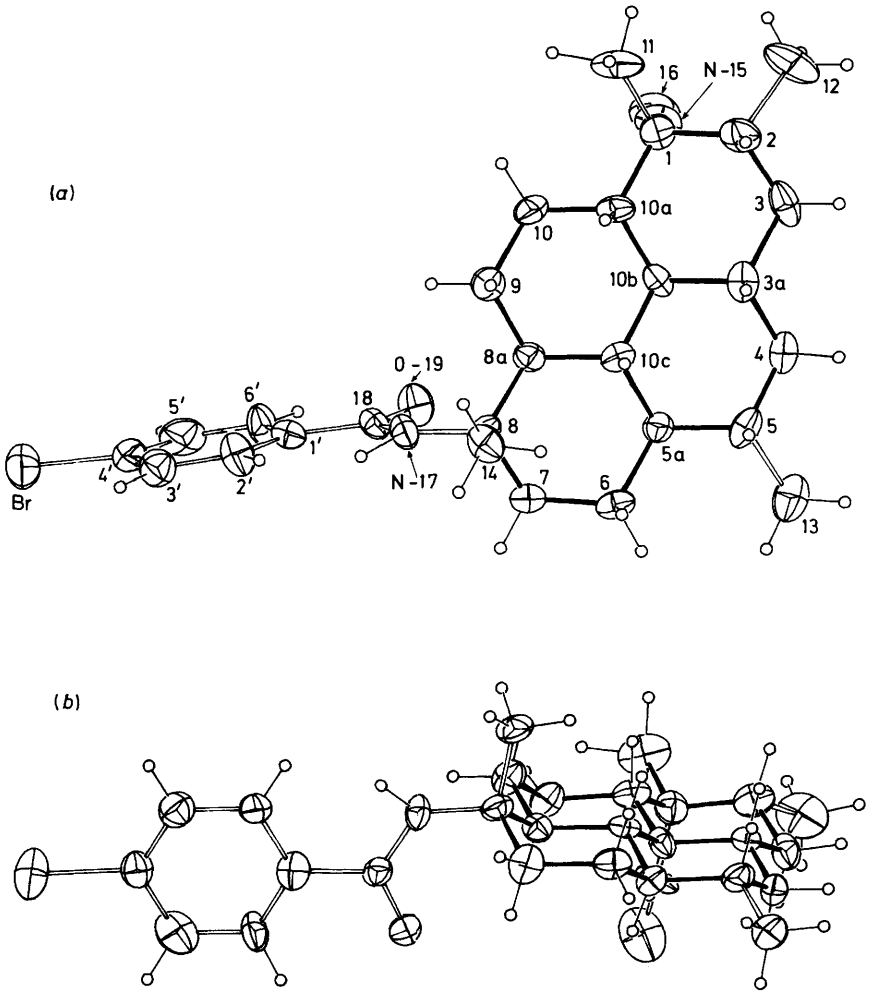
Although we have demonstrated utilisation of [^{14}C]acetate,[19] [2-^{14}C]mevalonolactone, [2-^{14}C]mevalonate (*N*,*N*-dibenzyl ethylene diamine salt) and of other terpene precursors such as [U-^{14}C]leucine by this sponge, none of these precursors generated labelled terpenes. Instead, we have detected[32] rapid and efficient synthesis of labelled carotenoids such as β,β[1]-carotene or zeaxanthin which are typical products of algal or bacterial metabolism. These results suggest competition between sponge and symbiont cells for the basic terpene precursors. Thus it may not be feasible to test for terpene synthesis in sponge-symbiont associations.

Chemical Studies.—To provide evidence for a specific labelling pattern, it was necessary to separate the two C_1 units from the main C_{20} skeleton of diisocyanoadociane. The degradative sequence followed is shown in Scheme 2. Mild hydrolysis of diisocyanoadociane yielded the bisformamide (**10**) in which the formyl group at C-8 exists as a 70:30 mixture of *trans* and *cis* rotamers. The geometry at C-1 appeared to be exclusively *trans*; no *cis* isomer could be detected by n.m.r. Two sets of signals each for the C-1 methyl, C-1 formyl, and NH protons could be assigned by n.O.e. difference spectroscopy to molecules possessing *trans* or *cis* geometry at C-8 respectively. Full details of the spectroscopic assignments for (**10**) are given in the Experimental section. Conversion of compound (**1**) into a diamine (**12**) required vigorous treatment with 4M HCl. In the total hydrolysis, it proved difficult to isolate clean samples of formic acid by ion exchange chromatography of the aqueous residues. Conversion of the formic acid obtained into a *p*-bromophenacyl derivative was very pH dependent and gave low yields; attempted derivatisation with phenylenediamine did not work in dilute aqueous solution.[33]

These difficulties were avoided by stepwise degradation of bisformamide (**10**) using 2M NaOH to remove selectively one of the two formyl groups. The monoamide product (**11**) was shown by ^1H and ^{13}C n.m.r. to be a single positional isomer, presumably (**11**) rather than the isomeric (**14**) because the C(8) substituent of (**1**) is equatorial and would be expected to hydrolyse faster than the axial C(1) group under basic conditions.

Support for structure (**11**) was provided by ^1H n.O.e. difference spectroscopy. The formyl proton of (**11**) gives rise to two doublets at δ 8.08 (12.2 Hz coupling) and 8.22 (*J* 2.2 Hz) assigned to the *trans* and the *cis* rotamers respectively. The ratio of *trans*:*cis* was 93:7. Irradition of the doublet at δ 8.08 resulted in n.O.e. enhancements to the methyl signals at δ 1.36 (s, 1-Me) and δ 0.87 (d, *J* 6.7 Hz, 2-Me). Irradiation of 1-Me at δ 1.36 gave n.O.e. enhancements to the 2-Me and formyl proton. If the monoamide had possessed the alternative structure (**14**), each irradiation experiment would have resulted in n.O.e. enhancement of a single signal alone (excluding the small n.O.e.s onto ring protons).

Before these n.m.r. studies were undertaken, we had planned a single crystal *X*-ray study to resolve the ambiguity over the structure of (**11**). Monoamide (**11**) itself was unsuitable for structure determination, therefore a number of derivatives were prepared. Monoamide (**11**) could be converted into urea (**15**) or thiourea (**16**) by treatment with *m*-chlorophenyl isocyanate or *p*-bromophenyl isothiocyanate respectively; both these compounds were relatively insoluble and the combination of polar groups around a lipophilic core did not facilitate recrystallisation. Attempts to prepare a toluene-*p*-sulphonate (TsCl, pyridine) or acetate (Ac_2O, NaOAc) gave product mixtures. Treatment with an excess of *p*-bromobenzoyl chloride in triethylamine gave a reasonable yield of a *p*-bromobenzamide product (**17**) for which ^1H and ^{13}C n.m.r. data indicated the loss of a formyl group but which still analysed for the presence of two nitrogens. The e.i. mass spectrum showed a weak peak at



Figures 2(*a*) and (*b*). Projections of the molecule of (**17**). 20% Thermal ellipsoids and crystallographic numbering are shown for the non-hydrogen atoms; hydrogen atoms have an arbitrary radius of 0.1 Å

m/*z* 498 (addition of *p*-bromobenzoyl group plus loss of water) and a strong peak at *m*/*z* 469 (M^+ − HCN). I.r. spectroscopy revealed the presence of an isocyanide (2 120 cm^{-1}), confirming dehydration of the formamido substituent during derivatisation. Treatment of the monoamide (**11**) with one equivalent of *p*-bromobenzoyl chloride in pyridine gave a mixture of products from which a formamido-(*p*-bromobenzamide) derivative (**18**) plus the above isocyano-(*p*-bromobenzamide) derivative (**17**) could be isolated by chromatography. The compound (**17**) proved suitable for single crystal *X*-ray structure analysis (see Experimental section). The structure and connectivity determined were consistent with that proposed on the basis of spectroscopic evidence and the relative stereochemistry confirmed that no epimerisation had taken place during any of the hydrolytic or derivatisation reactions. Owing to the presence of bromine, it was possible to propose an absolute configuration. As shown in Figure 2(*a*), compound (**17**) has absolute configuration 1*R*,2*R*,3a*S*,5*S*,6*R*,8*S*,8a*S*,10a*S*,10b*S*,10c*S* and by extension, diisocyanoadociane (**1**) also possesses this absolute stereochemistry. This result is in accordance with that recently proposed for diisocyanoadociane by enantioselective total synthesis.[2,3] The ambiguity[2] in the literature as to the correct enantiomeric representation of diterpene isocyanides in *Amphimedon* sp. and *Hymeniacidon amphilecta* is thus resolved.

Experimental

Solutions were dried over Na_2SO_4, m.p.s were determined with a Kofler hot-stage apparatus. I.r. spectra were recorded with a Perkin-Elmer 297 spectrophotometer for solutions in chloroform. Low resolution electron impact mass spectra were obtained on a V.G. Micromass 7070 F or an AEI MS-902 instrument at 70 eV. High resolution accurate mass measurements were determined under electron impact conditions on an A.E.I. MS 902 mass spectrometer. Chemical ionization mass spectra using ammonia as reagent gas were run on a V.G. Micro mass 7070F spectrometer. ^1H N.m.r. spectra were recorded on a Bruker AM300 or Jeol JNM GX400 spectrometer for solutions in deuteriochloroform with $SiMe_4$ as internal standard and ^{13}C n.m.r. spectra were recorded on a Bruker AM300 for solutions in deuteriochloroform with deuteriochloroform as internal standard. Silica chromatography was carried out under reduced pressure using silica gel (Merck 7736). T.l.c. plates were visualised by spraying with a solution of vanillin (2%) on conc. sulphuric acid. H.p.l.c. was performed using a Waters Associates HPLC system (590 pump, R403 differential refractometer) using a Waters μ-porasil semi-preparative column (5 mm × 30 cm). Radioactive samples were counted as solutions in aqueous or organic scintillation fluid (Amersham; 4 ml) on a LKB Rack Beta Scintillation counter and standardised externally. Radioactive precursors were

Table 4. Non-hydrogen atom coordinates for (**17**) (Br defines the origin)

Atom	*x*	*y*	*z*
Br	−0.491 9(2)	0.273 2(2)	0*
C(1)	0.249(1)	−0.411(1)	0.266 2(7)
C(11)	0.201(1)	−0.520(1)	0.233 9(7)
N(15)	0.181(1)	−0.385(1)	0.317 7(7)
C(16)	0.122(1)	−0.363(1)	0.356 3(9)
C(2)	0.374(1)	−0.434(1)	0.288 2(7)
C(12)	0.388(1)	−0.539(1)	0.335 6(7)
C(3)	0.430(1)	−0.325(2)	0.319 9(6)
C(3a)	0.430(1)	−0.212(1)	0.276 4(5)
C(4)	0.484(1)	−0.101(1)	0.305 4(6)
C(5)	0.487(1)	0.007(1)	0.264 1(7)
C(13)	0.543(1)	0.119(1)	0.297 5(7)
C(5a)	0.362(1)	0.034(1)	0.236 3(5)
C(6)	0.364(1)	0.143(1)	0.189 6(6)
C(7)	0.238(1)	0.161(1)	0.161 3(7)
C(8)	0.189(1)	0.051(1)	0.126 4(6)
C(14)	0.268(1)	0.024(1)	0.069 2(5)
C(8a)	0.187(1)	−0.053(1)	0.175 1(5)
C(9)	0.132(1)	−0.168(1)	0.146 0(6)
C(10)	0.127(1)	−0.270(1)	0.196 0(6)
C(10a)	0.250(1)	−0.299(1)	0.221 3(6)
C(10b)	0.308(1)	−0.188(1)	0.250 1(6)
C(10c)	0.312(1)	−0.079(1)	0.205 0(5)
N(17)	0.072(1)	0.078(1)	0.101 5(5)
C(18)	−0.028(1)	0.109(1)	0.133 4(7)
O(19)	−0.033 0(8)	0.102 5(7)	0.192 4(5)
C(1′)	−0.139(1)	0.148(1)	0.099 8(8)
C(2′)	−0.143(1)	0.157(1)	0.034 0(8)
C(3′)	−0.247(2)	0.199(1)	0.003 2(8)
C(4′)	−0.347(1)	0.224(1)	0.037 3(8)
C(5′)	−0.341(2)	0.217(1)	0.103 9(10)
C(6′)	−0.237(1)	0.177(1)	0.134 4(7)

Table 5. Non-hydrogen interatomic distances (Å)

Atoms	Distance	Atoms	Distance
C(1)–C(2)	1.48(2)	C(8)–C(14)	1.52(2)
C(1)–C(10a)	1.55(2)	C(8)–N(17)	1.43(2)
C(1)–C(11)	1.48(2)	C(8a)–C(9)	1.53(2)
C(1)–N(15)	1.35(2)	C(8c)–C(10c)	1.54(2)
C(16)–N(15)	1.07(2)	C(9)–C(10)	1.55(2)
C(2)–C(3)	1.51(2)	C(10)–C(10a)	1.50(2)
C(2)–C(12)	1.54(2)	C(10a)–C(10b)	1.52(2)
C(3)–C(3a)	1.55(2)	C(10b)–C(10c)	1.53(2)
C(3a)–C(4)	1.49(2)	N(17)–C(18)	1.33(2)
C(3a)–C(10b)	1.49(2)	C(18)–O(19)	1.25(2)
C(4)–C(5)	1.48(2)	C(18)–C(1′)	1.49(2)
C(5)–C(5a)	1.53(2)	C(1′)–C(2′)	1.39(2)
C(5)–C(13)	1.56(2)	C(2′)–C(3′)	1.40(2)
C(5a)–C(6)	1.56(2)	C(3′)–C(4′)	1.35(2)
C(5a)–C(10c)	1.52(2)	C(4′)–C(5′)	1.41(3)
C(6)–C(7)	1.53(2)	C(5′)–C(6′)	1.39(2)
C(7)–C(8)	1.52(2)	C(6′)–C(1′)	1.34(2)
C(8)–C(8a)	1.54(2)	C(4′)–Br	1.86(1)

purchased from Amersham Australia Pty. Ltd. or the Australian Atomic Energy Commission. Ether refers to diethyl ether.

Incorporation of Precursors.—Collected sponges were cut into 18 cm^3 pieces (approximately 5—10 g after freeze-drying) and attached underwater to numbered PVC plastic plaques using nylon cable ties. The plaques were attached to a fixed grid at −14 m. Where possible, the sponge transplants were established in advance to allow repair of damaged tissue. The ^{14}C-labelled precursors were incorporated into the sponge transplants using methods developed by Thompson *et al.*[21] The sponges were transferred to small plastic buckets containing 2—4 l of unfiltered, well-aerated sea-water and the precursors were added as solutions in aqueous solution. All experiments were maintained under dim natural light and ambient temperature (approximately 26 °C) for 12—14 h. The sponges were returned to the −14 m fixed grid for up to 35 days, collected, and frozen until work-up. All radioactive products were carefully purified by h.p.l.c. and recrystallisation to constant specific activity.

Table 6. Non-hydrogen interbond angles (°)

Atoms	Angles	Atoms	Angles
C(2)–C(1)–C(10a)	109(1)	C(8)–N(17)–C(18)	128(1)
C(2)–C(1)–C(11)	109(1)	N(17)–C(18)–C(1′)	121(1)
C(2)–C(1)–C(16)	108(1)	N(17)–C(18)–O(19)	122(1)
C(10a)–C(1)–C(11)	111(1)	C(1′)–C(18)–O(19)	117(1)
C(10a)–C(1)–N(15)	109(1)	C(8)–C(8a)–C(9)	111(1)
C(11)–C(1)–N(15)	110(1)	C(8)–C(8a)–C(10c)	114(1)
C(1)–N(15)–C(16)	176(2)	C(9)–C(8a)–C(10c)	111(1)
C(1)–C(2)–C(3)	113(1)	C(8a)–C(9)–C(10)	110(1)
C(1)–C(2)–C(12)	116(1)	C(10)–C(10a)–C(1)	112(1)
C(3)–C(2)–C(12)	106(1)	C(10)–C(10a)–C(10b)	110(1)
C(2)–C(3)–C(3a)	112(1)	C(1)–C(10a)–C(10b)	114(1)
C(3)–C(3a)–C(4)	115(1)	C(9)–C(10)–C(10a)	111(1)
C(3)–C(3a)–C(10b)	112(1)	C(3a)–C(10b)–C(10a)	112(1)
C(4)–C(3a)–C(10b)	111(1)	C(3a)–C(10b)–C(10c)	110(1)
C(3a)–C(4)–C(5)	115(1)	C(10a)–C(10b)–C(10c)	113(1)
C(4)–C(5)–C(5a)	111(1)	C(5a)–C(10c)–C(8a)	110(1)
C(4)–C(5)–C(13)	113(1)	C(5a)–C(10c)–C(10b)	112(1)
C(5a)–C(5)–C(13)	112(1)	C(8a)–C(10c)–C(10b)	112(1)
C(5)–C(5a)–C(6)	112(1)	C(18)–C(1′)–C(2′)	121(1)
C(5)–C(5a)–C(10c)	110(1)	C(18)–C(1′)–C(6′)	118(1)
C(6)–C(5a)–C(10c)	111(1)	C(2′)–C(1′)–C(6′)	120(1)
C(5a)–C(6)–C(7)	110(1)	C(1′)–C(2′)–C(3′)	121(1)
C(6)–C(7)–C(8)	114(1)	C(2′)–C(3′)–C(4′)	119(1)
C(7)–C(8)–C(8a)	106(1)	C(3′)–C(4′)–Br	122(1)
C(7)–C(8)–C(14)	110(1)	C(5′)–C(4′)–Br	119(1)
C(8a)–C(8)–C(14)	113(1)	C(3′)–C(4′)–C(5′)	119(1)
C(7)–C(8)–N(17)	110(1)	C(4′)–C(5′)–C(6′)	121(2)
N(17)–C(8)–C(14)	106(1)	C(1′)–C(6′)–C(5′)	119(2)
N(17)–C(8)–C(8a)	113(1)		

Isolation of Diisocyanoadociane (**1**).—Freeze-dried sponge (2.89 g) was extracted with light petroleum (40—60 °C; 5 × 50 ml). The combined organic phases were concentrated to a brown oil (95 mg) which was further purified by rapid (vacuum) silica gel chromatography with hexane–dichloromethane as eluant to give a pale orange solid (51 mg). Further purification either by semi-preparative h.p.l.c. using 7% ethyl acetate in hexane at 4 ml min^{-1} (R_t 6.5 min) followed by recrystallisation, or by recrystallisation alone gave diisocyanoadociane (28 mg, 0.96%), as white needles from hexane, m.p. 109—110 °C* (Found: M^+, 324.2565. $C_{22}H_{32}N_2$ requires 324.2569); $\nu_{max.}$(KBr) 2 130 and 2 140 cm^{-1}; δ_H 0.88 (3 H, d, *J* 6 Hz), 1.06 (3 H, d, *J* 6 Hz), 1.29 (3 H, m), 1.37 (3 H, m), and 0.5—2.2 (20 H, m); δ_C 16.03 (q), 19.6 (q), 20.4 (q), 23.9 (q), 25.1 (t), 25.7 (2 C, t), 36.5 (d), 37.7 (t), 40.0 (t), 40.5 (d), 40.7 (d), 42.2 (t), 45.3 (d), 45.9 (d), 47.7 (d), 48.3 (d), 48.8 (d), 50.4 (t, *J* 4.5 Hz), 65.1 (t, *J* 4.5 Hz), 155.8 (t, *J* 4 Hz), and 153.0 (t, *J* 4 Hz) p.p.m.; *m/z* 324, 309, 297 (M^+ − HCN), 282, 271, 270 (100%, M^+ − 2HCN), 256, 255, 241, 228, 215, 213, 201, 199, 185, 159, 145, 143, 131, 117, 107, 105, and 95.

Bisformamide (**10**).—Diisocyanoadociane (176 mg, 0.54 mmol) was allowed to stand in glacial acetic acid (10 ml) overnight, then diluted with sodium carbonate solution (10%; 10 ml) and extracted into ether (4 × 10 ml). The combined organic extracts were washed with sodium carbonate solution

* No melting point range quoted in reference 1.

(10%) to remove residual acid, dried, then concentrated to give the bisamide (**10**) (190 mg, 97%), as a white powder from CH_2Cl_2–ether (1:1), m.p. 133—137 °C* (Found: 360.2763. $C_{22}H_{36}N_2O_2$ requires 360.2777); $\nu_{max.}$ 2 950, 2 860, and 1 680 cm^{-1}; δ_H 0.88 (3 H, d, *J* 6.6 Hz, 5-Me), 0.89 (3 H, d, *J* 6.4 Hz, 2-Me), 1.20 (s, 8-Me, *cis* rotamer), 1.24 (s, 8-Me, *trans* rotamer), 1.36 (s, 1-Me, *cis* at C-8), 1.37 (s, 1-Me, *trans* at C-8), 5.08 (br s, 8-NH, *cis* at C-8), 5.65 (d, *J* 12.2 Hz, 8-NH, *trans* at C-8), 5.65 (d, *J* 11.9 Hz, 1-NH, *cis* at C-8), 5.65 (d, *J* 12.7 Hz, 1-NH, *trans* at C-8), 8.05 (d, *J* 2.2 Hz, 8-NHC*H*O, *cis* at C-8), 8.08 (d, *J* 11.9 Hz, 1-NHC*H*O, *cis* at C-8), 8.08 (d, *J* 12.7 Hz, 1-NHC*H*O, *trans* at C-8), and 8.25 (d, *J* 12.2 Hz, 8-NHC*H*O, *trans* at C-8); δ_C 15.49 (q), 19.33 (q), 19.71 (q), 22.25 (q), 24.87 (t), 25.14 (t), 25.59 (t), 26.40 (t), 36.66 (d), 37.51 (t), 41.08 (d), 41.62 (t), 42.52 (t), 46.10 (d), 46.69 (d), 48.20 (d), 49.79 (d), 50.37 (d), 55.57 (s), 57.16 (s), 162.6 (s), and 163.7 (s) p.p.m.; *m/z* 360, 345, 329, 315, 270 (100%), 255 (42%), 241, 228, 215, 112, 84, and 70.

Monoamide (**11**).—The bisamide (**10**) (45 mg, 0.125 mmol) was stirred under reflux with sodium hydroxide (2.5M, 4 ml) and ethanol (4 ml) for 3 h, then allowed to stand at room temperature overnight. The solution was extracted with dichloromethane (4 × 5 ml) and the combined organic phases washed with water (2 × 2 ml), dried, and concentrated to give the monoamide (**11**) (32 mg, 77%), as white needles from dichloromethane–ether, m.p. 157.5—158 °C (Found: C, 75.5; H, 11.6; N, 8.30%; M^+, 332.2829. $C_{21}H_{36}N_2O$ requires C, 75.85; H, 10.91, N, 8.42%; M^+ 332.2827); $\nu_{max.}$(Nujol) 3 340, 3 260, 3 220, and 1 670 cm^{-1}; δ_H 0.86 (3 H, d, *J* 6.6 Hz, 5-Me), 0.87 (3 H, d, *J* 6.7 Hz, 2-Me), 0.93 (3 H, s, 8-Me), 1.36 (3 H, s, 1-Me), 4.88 (br, 1-NH, *cis* isomer), 5.64 (d, *J* 12.2 Hz, 1-NH, *trans* isomer), 8.08 (d, J, 12.2 Hz, NHCHO, *trans* isomer), and 8.22 (d, *J* 2 Hz, NHCHO, *cis* isomer, ratio of *trans*:*cis* is 93:7); δ_C 15.6, 19.8, 21.6, 22.4, 25.2, 27.2, 36.9, 37.6, 41.1, 41.2, 42.8, 42.9, 46.4, 47.4, 48.8, 51.2, 52.0, 57.2, and 163.7 p.p.m.; *m/z* 332, 317, 289, 270, 255, and 70 (100%).

Diamine (**12**).—Diisocyanoadociane (85 mg, 0.26 mmol) was refluxed with conc. HCl (2 ml) and water (2 ml) for 4 h, the solution was cooled to room temperature overnight, then extracted with dichloromethane (2 × 2 ml), made alkaline with dilute sodium hydroxide solution, and extracted again with dichloromethane (4 × 4 ml). The combined organic extracts were washed with water (2 × 2 ml), dried, and concentrated to give the diamine (**12**) as a buff powder (67 mg, 84%) from ether, m.p. 105—108 °C (Found: 304.2875. $C_{20}H_{36}N_2$ requires 304.2878); $\nu_{max.}$($CHCl_3$) 3 650, 3 050, 2 960, 2 920, 2 860, 1 630, and 1 580 cm^{-1}; δ_H 0.80 (3 H, d, *J* 6 Hz), 0.85 (3 H, d, *J* 6 Hz), and 0.95 (6 H, s); δ_C 15.51, 19.65, 21.73, 25.18, 25.58, 26.19, 27.23, 36.99, 38.24, 41.07, 41.71, 42.61, 43.12, 46.64, 47.49, 48.84, 50.70, 51.21, 51.81, and 53.06 p.p.m.; *m/z* 304, 389, 270, 255, 221, 204, 162, 84, and 70 (100%).

Bis-(3-chlorophenylurea) (**13**).—The diamine (**12**) (30 mg, 0.1 mmol) was stirred at room temperature with 3-chlorophenyl isocyanate (100 µl, 127 mg, 0.4 mmol) and dry dichloromethane (2 ml). The mixture was filtered and the filtrate purified by chromatography using 20% methanol in dichloromethane to give a solid (38 mg) which was triturated with ether. The ether-soluble portion, on recrystallisation from dichloromethane–ether, gave a white powder (15 mg, 24.6%), m.p. 194—196 °C (Found: C, 65.9; H, 7.55; N, 8.8. $C_{34}H_{44}Cl_2N_4O_2$ requires C, 66.77; H, 7.25; N, 9.16%); $\nu_{max.}$($CHCl_3$) 3 320, 2 920, 2 860, and 1 650 cm^{-1}; δ_H 0.5–2.0 (32 H), 4.50 (1 H, NH), 5.30 (1 H, NH), 6.90 (2 H, m, 4′-,4-H), 7.15 (2 H, dt, *J* 8, 2 Hz, 5′-,5-H), 7.40 (2 H, dd, *J* 8, 2 Hz, 6′-, 6-H), 7.55 (2 H, b, 2′-, 2-H), 7.90 (1 H, NH), and 8.00 (1 H, NH); *m/z* (c.i.m.s.) 484 (MH^+ − $NH_2C_6H_4Cl$), 441, 407, 331, 314, 288, and 271.

1-*Formamido*-8-(3-*chlorophenyl*) (**15**).—The monoamide (**11**) (25 mg, 0.075 mmol) was stirred with 3-chlorophenyl isocyanate (200 µl) in dry dichloromethane (1 ml) for 48 h. The solution was filtered and the filtrate purified by silica chromatography using 10% methanol in dichloromethane to give the urea (**13**), as a white powder (21 mg, 57%) from dichloromethane–ether (1:3), m.p. 191—193 °C (Found: C, 68.65; H, 8.8; N, 8.35. $C_{28}H_{40}ClN_3O_2$ requires C, 69.19; H, 8.29; N, 8.64%); $\nu_{max.}$($CHCl_3$) 3 350 (br), 2 920, 2 860, 1 655 (br, s), 1 580, and 740 cm^{-1}; δ_H 0.3—2.0 (32 H), 5.83 (1 H, NH), 6.33 (1 H, d, *J* 12.7 Hz, N*H*CHO, *trans* isomer), 6.90 (1 H, d, *J* 6.9 Hz, 4′-H), 7.16 (1 H, t, *J* 8 Hz, 5′-H), 7.38 (1 H, d, *J* 8 Hz, 6′-H), 7.48 (1 H, b, 2′-H), 7.93 (1 H, NH), and 8.11 (1 H, d, *J* 12.7 Hz, NHC*H*O, *trans* isomer); *m/z* (c.i.m.s.) 484—486 (M − H^+), 359 (MH^+ − $NH_2C_6H_4Cl$), 333, 316, and 271.

1-*Formamido*-8-(p-*bromophenyl*)*thiourea* (**16**).—A mixture of the monoamide (48 mg, 0.14 mmol) and *p*-bromophenyl isothiocyanate (39 mg, 0.18 mmol) was kept at room temperature over 3 days in dichloromethane (2 ml). The mixture was purified by silica chromatography using 20% methanol in dichloromethane to give the thiourea (**16**) as a white powder (68 mg, 89%) with limited solubility in methanol; $\nu_{max.}$ 3 430, 3 335, 3 302, 2 720, 1 675, 1 660, 1 635, 1 620, 1 565, 1 545, 1 485, and 820 cm^{-1}; δ_H 0.88 (6 H, d, *J* 5.5 Hz), 1.26 (3 H, s), 1.36 (3 H, s), 5.85 (1 H, br s, NH), 5.95 (1 H, d, *J* 12 Hz, N*H*CHO), 7.07 (2 H, d, *J* 9 Hz, ArH), 7.53 (2 H, d, *J* 9 Hz, ArH), and 8.07 (1 H, d, *J* 12 Hz, NHC*H*O); *m/z* 374, 316, 271, 255, 185, 183, 173, and 171; (c.i.m.s.) 375 (MH^+ − $NH_2C_6H_4Br$), 333, and 316.

1-*Isocyano*-8-(p-*bromobenzamide*) (**17**).—The monoamide (33 mg, 0.1 mmol) was stirred in dichloromethane (2 ml) with freshly recrystallised *p*-bromobenzoyl chloride (Fluka; 60 mg, 0.2 mmol, 2 equiv.) and triethylamine (20 mg, 0.2 mmol) for 4 h. The mixture was diluted with water (2 ml) and extracted into dichloromethane (3 × 2 ml). The combined organic extracts were washed with dilute sodium carbonate solution and water, dried, and concentrated to give a white solid (70 mg) which was purified by chromatography on silica gel using 2% methanol in chloroform to give compound (**17**) as a white powder (23 mg, 46.4%) from dichloromethane–ether (1:1), m.p. 211—213 °C (Found: C, 66.6; H, 7.75; N, 5.40%; M^+ 469.1977. $C_{28}H_{37}BrN_2O$ requires C, 67.70; H, 7.50; N, 5.63%; M^+ 469,1980); $\nu_{max.}$(Nujol) 2 720, 2 660, 2 120, 1 640, 1 570, and 840 cm^{-1}; δ_H 0.88 (3 H, d, *J* 6.5 Hz), 1.03 (3 H, d, *J* 6.5 Hz), 1.35 (3 H, s, Me), 1.56 (3 H, s, Me), 5.75 (1 H, br s, NH), and 7.56 (4 H, br s, ArH); *m/z* 496, 469 (M^+ − HCN), 454, 497, 270, 255, 200, 185, and 183.

1-*Formamido*-8-(p-*bromobenzamide*) (**18**).—The monoamide (78 mg, 0.23 mmol), *p*-bromobenzoyl chloride (58 mg, 0.27 mmol), and pyridine (19 mg, 0.02 ml, 0.24 mmol) were stirred in dry dichloromethane (2 ml) for 2 h. The mixture was then diluted with water (5 ml) and extracted with dichloromethane (3 × 3 ml). The combined organic extracts were washed with 0.5M HCl (2 ml) to remove pyridine and unchanged monoamide (**11**), water (2 ml), dried, and concentrated to a semi-solid (104 mg) which was further purified by silica gel chromatography using 2% methanol in dichloromethane to give (**18**) as a white solid (33 mg, 30%) which was not purified further [Found: 499.1966. $C_{27}H_{36}{}^{79}BrN_2O_2$ (M^+ − Me) requires 499.1963]; $\nu_{max.}$ 3 300 (br), 2 720, 2 120, 2 120, 1 675, 1 640, 1 580, 1 530, and 840 cm^{-1}; δ_H 0.89 (6 H, d, *J* 6.5 Hz), 1.35 (3 H, s), 1.37 (3 H, s), 5.65 (1 H, d, *J* 12 Hz, NHCHO), 5.89 (1 H, s, NH), 7.55 (4 H, s,

* No melting point range quoted in reference 1.

ArH), and 8.08 (1 H, d, *J* 12 Hz, NHCHO); *m/z* 514, 499, 315, 297, and 270.

Isobutyraldehyde Bisulphite Addition Compound.[34]—Isobutyraldehyde (5.0 g, 69 mmol) was added to a vigorously stirred, freshly prepared solution of sodium metabisulphite (16.6 g, 87.2 mmol) in water (20 ml) at 0 °C and stirring continued for 30 min. The white solid formed was removed by filtration, washed with ethanol, and dried *in vacuo* (9.5 g, 78.2%), m.p. >250 °C; $\nu_{max.}$(Nujol) 3 600—3 200, 1 225, 1 160, 1 040, and 635 cm^{-1}.

2-*Hydroxy*-3-*methylbutanonitrile.*—Sodium cyanide (0.51 g, 0.01 mol) in water (5 ml) was added dropwise to a chilled solution of the isobutyraldehyde bisulphite addition compound (2.0 g, 0.011 mol) in water (10 ml) over 5 min. The mixture was stirred for 1 h, filtered, then extracted into ether (3 × 10 ml). The combined organic extracts were dried (Na_2SO_4) and concentrated to give 2-hydroxy-3-methylbutanonitrile[35] as a pale oil (0.89 g, 90%) which was not purified further; $\nu_{max.}$($CHCl_3$) 3 700—3 100 (OH stretch), 3 000—2 850, and 2 240 cm^{-1}; δ_H 1.06 (3 H, d, *J* 7 Hz), 1.09 (3 H, d, *J* 7 Hz), 2.02 (1 H, m), 3.51 (1 H, br, OH), and 4.27 (1 H, d, *J* 5 Hz). The cyanohydrin was stable to hydrolysis in dilute sodium hydrogen carbonate, pH 8.5—9.0, over a 24 h period.

2-*Hydroxy*-3-*methylbutano*[^{14}C]*nitrile.*—Sodium [^{14}C]cyanide (100 μCi, 58 mCi $mmol^{-1}$) was added to a solution of isobutyraldehyde bisulphite addition compound (45.8 mg, 0.25 mmol) in sea-water (5 ml). The mixture was stirred for 2 min, a solution of unlabelled sodium cyanide (11.8 mg, 0.24 mmol) in water (10 ml) was added, and the mixture swirled for several further min, then added directly to the incubation bucket as isobutyraldehyde cyanohydrin (23.8 mg, 0.24 mmol, 416 μCi $mmol^{-1}$).

[^{14}C]*Nickel Cyanide Complexes.*—(*a*) Sodium [^{14}C]cyanide (100 μCi, 58 mCi $mmol^{-1}$) was added to a solution of nickel chloride hexahydrate (59.4 mg, 0.25 mmol) in sea-water (5 ml), followed by potassium cyanide (31.3 mg, 0.48 mmol) in sea-water (10 ml) to generate [^{14}C]nickel cyanide[36] (30.9 mg, 0.24 mmol, 416 μCi $mmol^{-1}$). (*b*) Sodium [^{14}C]cyanide (100 μCi, 58 mCi $mmol^{-1}$) was added to a solution of nickel chloride hexahydrate (59.4 mg, 0.25 mmol) in sea-water (5 ml) followed by potassium cyanide (62.6 mg, 0.96 mmol) in sea-water (10 ml). The solution was swirled until the orange colour of potassium tetracyanonickelate[36] (62 mg, 0.24 mmol, 416 μCi $mmol^{-1}$) was produced, and then added to the incubation bucket.

[^{14}C]*Zinc Cyanide Complexes.*—(*a*) Sodium [^{14}C]cyanide (100 μCi, 58 mCi $mmol^{-1}$) was added to zinc sulphate heptahydrate (71.9 mg, 0.25 mmol) in sea-water (1 ml) followed by sodium cyanide (24.5 mg, 0.50 mmol) to give [^{14}C]zinc cyanide[26] (29.35 mg, 0.25 ml, 400 μCi $mmol^{-1}$). (*b*) Sodium [^{14}C]cyanide (100 μCi, 58 mCi, $mmol^{-1}$) was added to zinc sulphate heptahydrate (71.9 mg, 0.25 mmol) in sea-water (1 ml) followed by unlabelled sodium cyanide (49 mg, 1.00 mmol) in sea-water (5 ml) to give [^{14}C]sodium tetracyanozincate[28] (53.85 mg, 0.25 mmol, 400 μCi $mmol^{-1}$).

Sponge Tissue Digest.—Freeze-dried tissue was finely ground. Samples (about 1 mg) were accurately weighed into glass scintillation vials and wetted with distilled water (200 μl), N.C.S. reagent (Amersham: 1 ml) was added carefully and the solution was left standing in the dark for 3—4 h. Glacial acetic acid (40 μl) was added, followed by scintillation fluid (O.C.S. Amersham, 16 ml) and the samples were counted in the usual way. All sponge samples were counted in triplicate.

Crystal Structure Determination.—A unique data set (*h*, *k*, *l*) was measured, together with its counterpart in 'Friedel pairs' ($\bar{h}$, $\bar{k}$, $\bar{l}$) within the limit $2\theta_{max.} = 50°$ using an ENRAF-Nonius CAD-4 four-circle diffractometer in conventional $2\theta/\theta$ scan mode at 295 K. *N* Independent reflections were obtained, N_o with $I > 1.5\sigma(I)$* being considered 'observed' and used in the full-matrix refinement after Gaussian absorption correction and solution of the structure by the heavy atom method. Anisotropic thermal parameters were refined for the non-hydrogen atoms; $(x, y, z, U_{iso})_H$ were included, constrained at estimated values. The two data sets were refined independently; at convergence, conventional *R*,*R'* on |*F*| were 0.065, 0.037 (*h*, *k*, *l*); 0.065, 0.038 ($\bar{h}$, $\bar{k}$, $\bar{l}$).For the alternative chirality 0.074, 0.047 (*h*, *k*, *l*); 0.074, 0.048 ($\bar{h}$, $\bar{k}$, $\bar{l}$) were obtained (N,N_o = 1 645, 1 211 (*h*, *k*, *l*); 1 645, 1 165 ($\bar{h}$, $\bar{k}$, $\bar{l}$). Statistical weights derived from $\sigma^2(I) = \sigma^2(I)_{diff} + 0.000\,1\sigma^4\ (I)_{diff}$ were employed. Neutral atom complex scattering factors[37] and the XTAL program system[38] implemented by S. R. Hall on a Perkin-Elmer 3240 computer were used. Pertinent results are given in the Figures and Tables 4—6. Thermal and hydrogen atom parameters are available on request from the Cambridge Crystallographic Data Centre.†

Crystal Data.—$C_{28}H_{37}BrN_2O$, *M* = 497.5. Tetragonal, space group $P4_1$, (C_4^2, No. 76), *a* = 11.045(3), *c* = 21.14(1) Å, *U* = 2 579 Å^3, D_c = 1.28 g cm^{-3}, *Z* = 4, *F*(000) = 1 048. Monochromatic Mo-K_α radiation, λ = 0.710 69 Å, μ_{Mo} = 16.5 cm^{-1}, specimen: 0.20 × 0.13 × 0.50 mm, $A^*_{min.,max.}$ = 1.23, 1.38.

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† For details, see para. 5.6.3, Instructions for Authors, *J. Chem. Soc., Perkin Trans. 1*, 1988, Issue 1.

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