Isocyanates in marine sponges: Axisocyanate-3, a new sesquiterpene from *Acanthella cavernosa*

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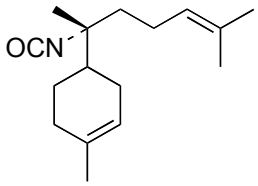
Dedicated to Professor Atta-ur-Rahman FRS on the occasion of his 65th birthday

Abstract
The isolation of axisocyanate-3 as a minor component of *Acanthella cavernosa* has been confirmed by spectroscopic characterisation of the metabolite, and by conversion of a mixture of axisothiocyanate-3 and axisocyanate-3 to their diethyl(thio)urea derivatives. The role of isocyanates in the formation of urea metabolites in sponges has been investigated through a model study with a menthyl-derived isocyanate.

Keywords: Sesquiterpenoid, isocyanate, urea, NMR, sponges

Introduction

Bioactive terpenes functionalized by the presence of isocyano, isothiocyanato and formamide groups are a common structural motif in marine sponge chemistry. Much less commonly encountered are terpenes functionalized by thiocyanato, dichloroimine or isocyanato functional groups.[1,2] Examples of the isocyanate functionality in the marine terpene literature include the bisabolene (**1**)[3] and aromadendrane (**2**)[4] sesquiterpenes, the amphilectane diterpenes **3**-**5**,[5,6] and the kalihinol A derivative **6**.[7] Novel biological activites have been described for these metabolites; compounds **3-5** are cytotoxic with **5** also showing potent and selective antiplasmodial activity,[8] while **6** inhibits the metamorphosis of barnacle larvae.[7] In contrast, isocyanate **2** is non toxic to fish and has been proposed to play a role in the detoxification of isocyano metabolites in *Acanthella cavernosa*.[4] The recent reports of symmetrical sesquiterpenes containing urea functionality in marine sponges[9-11] hint at a possible role of isocyanates in their biosynthesis, and suggest that isocyanate metabolites might occur more widely in marine sponges than has been reported.



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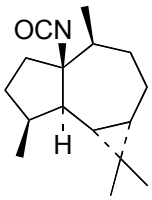

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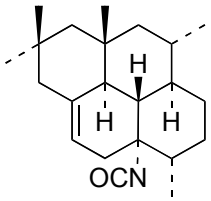

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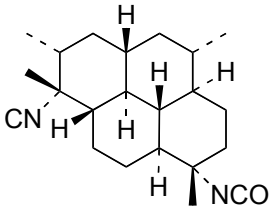

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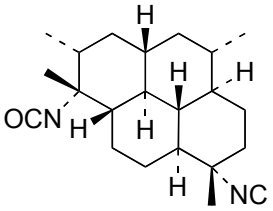

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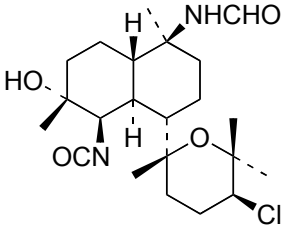

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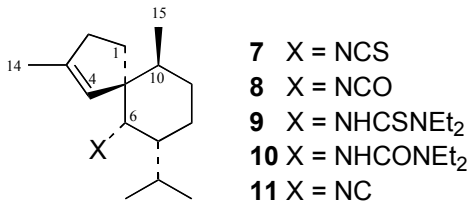
In this paper, we report the isolation of a new isocyanate with a spiroaxane skeleton from the well-known Indo-Pacific sponge *Acanthella cavernosa*, and describe a biomimetic reaction with menthyl isocyanate that may illustrate the role of terpene isocyanates in the formation of sponge natural products.

Result and Discussion

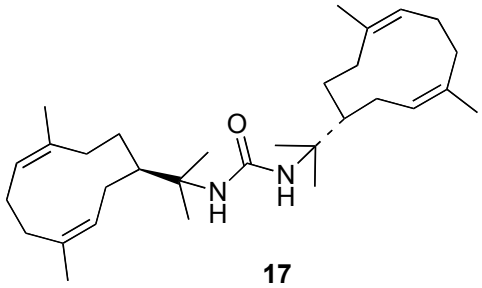
Four specimens of *Acanthella cavernosa* were collected at Tani's Reef, Mooloolaba, Australia in October 2004. Organic extracts from each sponge were examined by ^{1}H NMR, and by GC-MS which showed two clusters of peaks. A group of peaks with retention time between 10.0 – 12.0 mins and *m/z* of 231 were characteristic of sesquiterpen isocyanides, and the other cluster with a retention time of 13.5 – 15.0 mins and *m/z* 263 were characteristic of sesquiterpene isothiocyanates. In each extract, a peak with a retention time of 12.3 mins and an *m/z* of 247 was of interest since this corresponded to a sesquiterpene isocyanate metabolite. The extracts were then combined on the basis of the similar chemistry and fractionated by silica flash column chromatography followed by normal phase HPLC using ethyl acetate-hexane mixtures.

A fraction eluting from HPLC (0.25% ethyl acetate/hexane) using two columns in series was a mixture of two compounds by GC-MS with molecular ions at *m/z* 247 and 263. Examination of ^{1}H spectra showed a major component containing three methyl doublets plus a methyl singlet on a double bond, while all fifteen carbons of the terpene skeleton could be identified from 2D spectra, as was an isothiocyanate signal at δ_C 129.2. This component was identified as the spiroaxane metabolite axisothiocyanate-3 **7** by comparison with literature data.[12,13] The minor component corresponded to the isocyanate metabolite with *m/z* 247. The upfield regions of both ^{1}H and ^{13}C NMR spectra did not reveal the complexity expected for a mixture of terpenes, suggesting that the new metabolite had the same carbon skeleton as **7**. Thus

the component was likely to be the isocyanate analogue **8** named as axisocyanate-3. The ^{1}H-^{13}C connectivities for the minor component were then assigned using gHSQC data. Proton signals at δ_H 3.51 and 5.14 correlated to signals at δ_C 64.6 and 124.7 that could be assigned as C-6 and C-4 respectively. **Table 1** compares the NMR data for **7** and **8**. Generally the ^{13}C signal for an –NCO group occurs between δ_C 120-135, but no signal corresponding to this functionality could be identified in the ^{13}C spectrum of the mixture because of the small sample size. Fast relaxation of the ^{14}N nucleus also limits the detection of the linear –NCO carbon signal.



7 X = NCS
8 X = NCO
9 X = NHCSNEt$_2$
10 X = NHCONEt$_2$
11 X = NC



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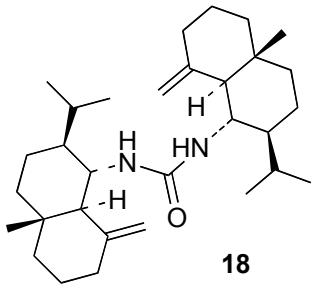

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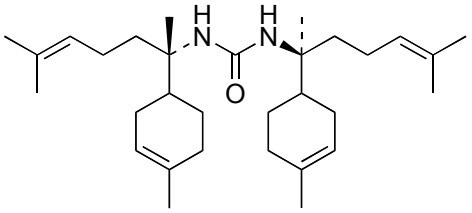

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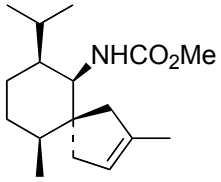

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In order to confirm the presence of the isocyanate compound, the mixture of **7** and **8** was treated with diethylamine in DCM overnight at RT to give the thiourea **9** and urea **10**. The ^{13}C NMR of the product mixture showed signals at δ_C 180.6 (thiourea) and δ_C 156.9 (urea) that had the anticipated correlations from the respective H-6 protons and from the diethylamine methylene protons in an HMBC spectrum. The two products were separated by NP HPLC (20% ethyl acetate/hexane) and their individual ^{1}H spectra recorded. The data for compound **10** were fully in accordance with the proposed spiroaxane skeleton. The $[\alpha]_D$ values for **9** and **10** were both positive, as are the values recorded for the parent axisonitrile-3 **11**[12,14] and axisothiocyanate-3 **7**,[12] hence the series of compounds are likely to share the same absolute stereochemistry shown.[14]

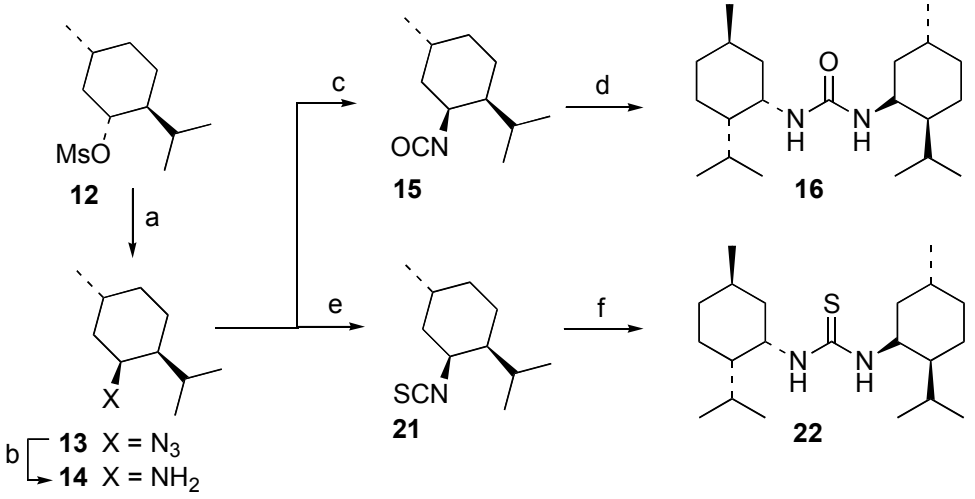
Table 1. NMR data ($CDCl_3$, 500 MHz) for axisothiocyanate-3 (**7**) and axisocyanate-3 (**8**) and their diethyl(thio)urea derivatives (**9**) and (**10**)

No.	δ ^{1}H **7**	δ ^{1}H **8**	δ ^{13}C **7**	δ ^{13}C **8**	δ ^{13}C **9**	δ ^{13}C **10**
1a 1b	1.92 1.89	1.92 1.89	35.0	35.2	32.4	32.4
2a 2b	2.24 2.24	2.24 2.24	35.9	36.0	36.1	36.0
3	-	-	144.8	144.1	143.5	142.9
4	5.12	5.14	123.9	124.7	125.3	125.6
5	-	-	58.9	58.8	60.6	60.6
6	3.67	3.51	67.4	64.6	59.2	58.7
7	1.24	1.24	45.4	45.2	45.0	45.0
8ax 8eq	1.22 1.78	1.22 1.78	25.4	25.1	25.8	25.7
9ax 9eq	1.08 1.50	1.08 1.50	31.3	31.3	31.9	31.1
10	1.68	1.68	35.0	34.7	36.4	34.9
11	1.50	1.50	30.1	30.0	29.5	29.5
12	0.91	0.90	20.8	20.9	22.8	21.3
13	0.89	0.87	20.4	20.3	20.6	20.6
14	1.74	1.72	17.0	17.0	17.1	17.0
15	0.75	0.73	16.5	16.1	16.5	16.4
NCS	-	-	129.2	-		
NCO	-	-	-	#		
C=S					180.6	
C=O						156.9

Signal not detected in 1D or HMBC spectra

Previous synthetic conversions of (+)-axisonitrile-3 **11** to (+)-axisothiocyanate-3 **7** using elemental sulphur had given rise to traces of a component with *m/z* 247 in the crude reaction product which could now be identified as axisocyanate-3, but attempts to isolate this product were unsuccesful. We conducted biomimetic model studies on a menthyl-based skeleton in order to probe the chemistry of terpene isocyanates further. The menthyl-derived mesylate **12** was converted to axial azide **13** using NaN_3 in DMF.[15] Reduction of the azide using $LiAlH_4$ in Et_2O afforded amine **14** as the sole product. Following the procedure of Isobe *et al*.,[16] amine **14** was subjected to a two-phase mixture of phosgene equivalent, triphosgene, and aqueous sodium carbonate. Isolation of menthyl isocyanate **15** was attempted, however the product solidified on standing into the stable dimeric urea **16** (**Scheme 1**). Use of triethylamine[17] instead of aqueous base led to the isolation of isocyanate **15** in 98%. Coupling of isocyanate **15** with amine **14** at RT

for 3 h also led to the formation of dimer **16**. The formation of the bis-menthyl urea is a model reaction for the formation of symmetrical terpene metabolites such as the bis-germacrane urea **17**,[9] halichonadin A **18**,[10] and bis-bisabolene urea **19**.[11] The ready formation of ureas in sponges may reflect facile hydration and decarboxylation of an isocyanate metabolite followed by attack of the amine product on unreacted isocyanate, rather than an enzymatic reaction. Sponge metabolites such as exicarbamate **20** may also be diagnostic of isocyanate functionality.[18]



Scheme 1. a. NaN_3, DMF, 80 ºC, 42 h, 59%; **b**. $LiAlH_4$, Et_2O, RT, 21 h, 51%; **c**. Et_3N, triphosgene, DCM, reflux, 2h, 98%; **d**. cmpd **14**, DCM, RT, 3h, 54%; **e**. aq. K_2CO_3, thiophosgene, DCM, reflux, ~30 mins, 68%; **f**. cmpd **14**, DCM, RT, 18h, 55%.

Samples of synthetic compounds **14** – **16**, and of a menthyl-based isothiocyanate **21** and dimeric thiourea **22** prepared according to **Scheme 1**, were subjected to antimicrobial and antiparasitic screens, but were without biological activity. The ^{13}C NMR spectrum of thiourea **22** contained two sets of signals consistent with the presence of rotamers.

Conclusion

Isocyanate metabolites are labile metabolites in marine sponges whose presence may frequently have gone undetected in isolation work owing to their sensitivity to hydrolytic conditions. By careful examination of a marine sponge extract, and by chemical derivatisation, evidence supporting the presence of axisocyanate-3 has been obtained. A model study using menthyl-based intermediates reveals that terpene isocyanates readily convert into amine and urea analogues.

Experimental Section

General experimental procedures. Optical rotations were obtained using a JASCO-P1010 polarimeter. One and two dimensional NMR spectra were acquired using Bruker DRX-500 or Bruker AMX-400 instruments. NMR spectra were obtained in deuterochloroform at room temperature. Samples were internally referenced to $CHCl_3$ at either δ_H 7.25 and δ_C 77.0. High and low resolution mass measurements were obtained from a Finnigan MAT 900 XL-Trap electrospray (ESI) mass spectrometer with a Finnigan API III electrospray source. Gas chromatography/mass spectrometry (GC-MS) spectra were recorded on a Hewlett Packard 5890A gas chromatograph, carrying a DB5 capillary column in tandem with a Hewlett Packard 5970 mass selective detector or a Shimadzu GCMS-QP5050A gas chromatograph mass spectrometer, carrying a Zebron ZB-5 capillary column (30 mL x 0.32 mm ID x 0.25 μm df, 5% phenyl polysiloxane) with a Shimadzu AOC-20i auto injector. Retention times were obtained using the following temperature ramping program: initial oven temperature 50 ºC or 100 ºC; isothermal time 3.0 minutes, ramp 16 ºC min^{-1}; final oven temperature 270 ºC.

Biological material. Specimens of *Acanthella cavernosa* Dendy 1922 (Order Halichondrida family Dictyonellidae) were collected from Tani's Reef dive site at the Inner Gneerings, a group of shoals off Mooloolaba (Australia), using SCUBA at a depth of 10-15 m on 24 October 2004. Samples were taken back to the laboratory where they were stored at -20 ºC until extraction. The sponge was dark orange in colour and globular in shape. A voucher specimen (QM G322184) is lodged at the Queensland Museum. Photographs of the sponge material are available from the authors.

Extraction and isolation of metabolites. Four frozen sponges (8.3 g each) were chopped finely and extracted with CH_2Cl_2/MeOH 1:1 (3 x 50 mL each). The extracts were filtered through a cotton wool plug, evaporated to aqueous suspension and partitioned between H_2O (20 mL) and ethyl acetate (3 x 70 mL). The organic layers were combined, dried with anhydrous $MgSO_4$ and evaporated to give dark orange crude extracts. Based on GC-MS analysis and comparison of ^1H NMR spectra, the crude extracts were combined into a single sample (281 mg) and then flash chromatographed on silica gel using a solvent gradient (100% hexane – hexane/CH_2Cl_2 5:1, 1:1, 1:5 – 100% DCM – EtOAc/CH_2Cl_2 5:1, 1:1, 1:5 – 100% EtOAc – 100% MeOH). The 100% hexane fraction contained sesquiterpene hydrocarbons including 9-aristolene[7] (4.31 mg). The combined fractions eluting with 100% hexane and hexane/CH_2Cl_2 (5:1) (17.4 mg) were further purified by NP HPLC (two columns in series, 0.25% EtOAc/hexane, 2mL/min) to give a mixture of axisothiocyanate-3 (**7**)[12] and axisocyanate-3 (**8**) (1.84 mg), an epimer of 10-isothiocyanatoguai-6-ene[19] (1.10 mg), 10-isothiocyanato-4-cadinene,[6] in addition to 1-isothiocyanatoaromadendrane[20] (6.58 mg) and 10α-isothiocyanatoalloaromadendrane[21] (0.44 mg). The next fraction eluting with hexane/CH_2Cl_2 (5:1) contained acanthene B[22] (1.08 mg). The fraction eluting with hexane/CH_2Cl_2 (1:1) was further purified by NP HPLC (two columns in series, 0.5% EtOAc/hexane, 2 mL/min) to give axisonitrile-3 (**11**)[12,14] (4.18 mg) and 1-isocyanoaromadendrane[20] (0.95 mg).

Axisothiocyanate-3 (**7**)[12] colourless oil; $[\alpha]_D^{20}$ + 152.3 (*c* = 0.3, $CHCl_3$). GC-MS *m/z* $[M]^+$ 263 (65), 248 (15), 230 (15), 205 (2), 161 (6), 121 (100); ^1H and ^{13}C NMR see **Table 1**.

Axisocyanate-3 (**8**): colourless oil; GC-MS *m/z* $[M]^+$ 247 (39), 232 (8), 205 (2), 161 (4), 121 (100); ^1H and ^{13}C NMR see **Table 1**.

Synthesis of thiourea (9) and urea (10) from a mixture of axisothiocyanate-3 (7) and axisocyanate-3 (8). A mixture of compounds **7** and **8** (1.3 mg, 4.94 μmol) was treated with diethylamine (2.05 mg, 0.028 mmol) in dry CH_2Cl_2 (9.4 μL) overnight at room temperature under N_2. The CH_2Cl_2 was removed by a steady stream of N_2 (g) to give a mixture of thiourea (**9**) and urea (**10**) (0.95 mg, 57 %). Separation by NP HPLC using 20% EtOAc/hexanes (1 mL/min) gave thiourea **9** (0.17 mg) followed by urea **10** (0.10 mg).

Thiourea (**9**): white solid: $[\alpha]_D^{20}$ + 26.2 (*c* = 0.013, $CHCl_3$); ^1H NMR ($CDCl_3$, 500MHz) δ 5.24 (1H, br s, H-4), 4.75 (1H, d, *J* = 10.0, H-6), 3.66 (4H, q, *J* = 7.0, 14.5, H-17 and H-18), 2.38 (1H, m, H-2), 2.16 (1H, m, H-1), 2.08 (1H, m, H-2), 1.84 (1H, m, H-7), 1.72 (3H, s, Me-14), 1.57 (1H, m, H-8), 1.50 (1H, m, H-1), 1.35 (1H, m, H-11), 1.30 (1H, m, H-10), 1.22 (6H, s, Me-19 and Me-20), 1.18 (1H, m, H-9), 1.03 (3H, d, *J* = 6.5, Me-12), 0.90 (1H, m, H-8), 0.83 (3H, d, *J* = 6.5, Me-13) and 0.77 (3H, d, *J* = 6.5, Me-15); ^{13}C NMR ($CDCl_3$) see **Table 1**; HREIMS *m/z* 336.2596 $[M]^+$ (calcd. for $C_{20}H_{36}N_2S$ 336.2599 (-1.0 ppm)).

Urea (**10**): white solid; $[\alpha]_D^{20}$ + 14.3 (*c* = 0.03, $CHCl_3$); ^1H NMR ($CDCl_3$, 500MHz) δ 5.24 (1H, br s, H-4), 4.00 (1H, d, *J* = 10.0, H-6), 3.25 (4H, q, *J* = 7.5, 14.5, H-17 and H-18), 2.38 (1H, m, H-2), 2.16 (1H, m, H-1), 2.08 (1H, m, H-2), 1.78 (1H, m, H-7), 1.70 (3H, s, Me-14), 1.57 (1H, m, H-8), 1.50 (1H, m, H-1), 1.35 (1H, m, H-11), 1.27 (1H, m, H-10), 1.13 (6H, t, Me-19 and Me-20), 1.18 (1H, m, H-9), 0.92 (3H, d, *J* = 6.0, Me-12), 0.87 (1H, m, H-8), 0.84 (3H, d, *J* = 6.5, Me-13) and 0.75 (3H, d, *J* = 6.5, Me-15); ^{13}C NMR ($CDCl_3$) see **Table 1**; HREIMS *m/z* 320.2823 $[M]^+$ (calcd. for $C_{20}H_{36}N_2O$ 320.2828 (-1.5 ppm)).

Synthesis of monoterpene analogues

(-)-Menthyl mesylate 12. To a solution of (-)-menthol (3 g, 19.2 mmol) in dry CH_2Cl_2 (55 mL) was added pyridine (31.05 mL, 384 mmol), a catalytic amount of DMAP (0.1 mol%), and methanesulfonic anhydride (8.69 g, 50 mmol). After stirring at room temperature for 19 h the mixture was concentrated under reduced pressure and the residue diluted with Et_2O (100 mL). The resulting solution was washed with 2M HCl made up in sat. aq. $NaHCO_3$ (250 mL) and the aqueous washings extracted with Et_2O (100 mL x 3). The combined organic phases were washed sequentially with H_2O and sat. aq. NaCl and dried (Na_2SO_4). Removal of the solvent *in vacuo* afforded crude mesylate as a yellow oil. The crude oil was subjected to column chromatography (80% CH_2Cl_2/hexane) to give mesylate **12** (4.49 g, 19.2 mmol, 100%) as a clear oil; ^1H NMR ($CDCl_3$, 400 MHz): δ 4.52 (1H, td, *J* = 4, 12 Hz), 2.97 (3H, s), 2.26 – 2.20 ($1H_{eq}$, m,), 2.08 – 2.00 (1H, dqn, *J* = 4, 8 Hz), 1.72 – 1.62 (2H, m,), 1.51 – 1.35 (2H, m), 1.24 ($1H_{ax}$, q, J = 12 Hz), 1.03 (1H, qd, *J* = 4, 12 Hz,), 0.92 (3H, d, J = 4 Hz), 0.90 (3H, d, *J* = 4 Hz), 0.87 – 0.83 (1H, m), 0.80 (3H, d, *J* = 8 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 83.3 (CH), 47.4 (CH), 42.2 (CH_2), 39.0 (CH_3), 33.7 (CH_2), 31.6 (CH), 25.7 (CH), 23.0 (CH_2), 21.8 (CH_3), 20.7 (CH_3), 15.6 (CH_3); ESI-MS *m/z* 257 $[M + Na]^+$. HRESI-MS *m/z* 257.1179 $[M+ Na]^+$ (calcd. for $C_{11}H_{22}NaSO_3$ 257.1187).

Azide 13. To a solution of (-)-menthyl mesylate **12** (2 g, 8.5 mmol) in anhydrous DMF (53 mL) was added sodium azide (1.66 g, 25.6 mmol) and the mixture was stirred for 42 h at 80 ºC. The solution was cooled to room temperature, followed by addition of Et_2O (50 mL) and washed with H_2O (3 x 100 mL), brine (100 mL), dried (Na_2SO_4) and concentrated under reduced pressure to afford crude azide as a clear oil. The crude oil was subjected to column chromatography (10% EtOAc/hexane) to give **13** (0.907 g, 5.0 mmol, 59%) as a clear oil; IR (thin film) 2105 cm^{-1} (s); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.96 (1H, q, *J* = 4 Hz), 2.00 (1H, qd, *J* = 4, 16 Hz), 1.74 – 1.62 (4H, m), 1.24 – 1.10 (3H, m), 0.92 (3H, d, J = 8 Hz), 0.88 (6H, d, J = 4Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 60.5, 47.3, 38.9, 34.8, 29.5, 26.5, 24.9, 22.2, 20.9, 20.6.

Amine 14. Lithium aluminium hydride (251 mg, 6.6 mmol) in dry Et_2O (11.42 mL) was stirred at room temperature. To the stirred suspension was added dropwise the azide **13** (600 mg, 3.3 mmol) dissolved in dry Et_2O (16.08 mL) and the mixture was brought to reflux for 2 h and then stirred at room temperature for 21 h. Ice-cold H_2O (20 mL) was added carefully followed by 10% NaOH (1 mL). The solid was removed by filtration and the ethereal layer dried (Na_2SO_4). Slow and careful removal of solvent gave amine **14** (263 mg, 1.69 mmol, 51%) as a white solid that was used without further purification; mp 91 – 92 ºC; $[\alpha]_D^{20}$ + 6.5 (*c* = 0.54, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.2 (1H, q, *J* = 3 Hz), 1.69 – 1.56 (3H, m), 1.44 (2H, br s *NH*), 1.42 – 1.35 (1H, m), 1.22 – 1.06 (2H, m), 0.89 (3H, d, *J* = 10 Hz), 0.88 (3H, d, *J* = 5 Hz), 0.86 – 0.84 (3H, m), 0.83 (3H, d, *J* = 5 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 47.9 (CH), 47.3 (CH), 43.1 (CH_2), 35.3 (CH_2), 29.2 (CH), 25.6 (CH), 23.8 (CH_2), 22.5 (CH_3), 21.3 (CH_3), 20.6 (CH_3); EI-MS (70 eV) *m*/*z* (%): 155 ($[M]^+$, 50), 140 ($[M\text{-}CH_3]^+$, 30), 98 ($[M\text{-}C_4H_9]^+$, 50), 70 ($[M\text{-}C_6H_{13}]^+$, 100). HRMS *m*/*z* 155.1675 $[M]^+$ (calcd. for $C_{10}H_{21}N$ 155.1674).

Isocyanate 15. The amine **14** (50 mg, 0.32 mmol) and triethylamine (65 mg, 0.64 mmol) were dissolved in dry CH_2Cl_2 (4 mL) and cooled to 0 ºC. Triphosgene (191 mg, 0.64 mmol) was added and the reaction was gradually brought to reflux for 2 h. The reaction mixture was allowed to cool to room temperature and then filtered through a short pad of silica. The filtrate was concentrated *in vacuo* to afford isocyanate **15** (57 mg, 0.31 mmol, 98%) as a clear oil that was used without further purification; $[\alpha]_D^{20}$ + 28.6 (*c* = 0.25, $CHCl_3$); IR (thin film): 2271 cm^{-1} (s); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.92 (1H, q, *J* = 4 Hz), 1.88 (1H, qd, *J* = 4, 12 Hz), 1.77 – 1.61 (4H, m), 1.45 (1H, td, J = 4, 16), 1.25 – 1.08 (3H, m), 0.91 (3H, d, *J* = 4 Hz), 0.89 (3H, d, *J* = 8 Hz), 0.87 (3H, d, *J* = 4 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 121.6 (NCO), 53.7 (CH), 47.8 (CH), 41.7 (CH_2), 34.6 (CH_2), 29.9 (CH), 26.4 (CH), 2.8 (CH_2), 21.9 (CH_3), 20.8 (CH_3), 20.3 (CH_3); EI-MS (70 eV) *m*/*z* (%): 181 ($[M]^+$, 30), 124 ($[M\text{-}C_4H_9]^+$, 100), 95 ($[M\text{-}C_5H_{10}O]^+$, 90). HRMS *m*/*z* 181.1461 $[M]^+$ (calcd. for $C_{11}H_{19}NO$ 181.1467).

Urea 16. Isocyanate **15** (10 mg, 0.055 mmol) was treated with amine **14** (17 mg, 0.110 mmol) in dry CH_2Cl_2 (1 mL) at room temperature for 3 h. The precipitated product was collected by filtration, washed with CH_2Cl_2 and dried *in vacuo* to afford pure **16** (10 mg, 0.030 mmol, 54%) as a white solid; mp 282-285 ºC; $[\alpha]_D^{20}$ + 32.5 (*c* = 0.092, $CHCl_3$); ^{1}H NMR (CD_3OD, 500 MHz): δ 4.07 (2H, q, *J* = 5 Hz), 1.80 – 1.71 (6H, m), 1.53 – 1.45 (2H, m), 1.43 – 1.35 (2H, m), 1.13 (2H, dq, *J* = 5, 15 Hz), 1.04 – 0.97 (4H, m), 0.94 – 0.91 (2H, m), 0.90 (12H, d, *J* = 5 Hz),

0.86 (6H, d, J = 5 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 157.5, 47.7, 46.5, 40.3, 34.7, 29.5, 26.7, 25.3, 22.3, 21.0, 20.6; ESI-MS m/z 359 $[M + Na]^+$; HRMS m/z 336.3144 $[M]^+$ (calcd. for $C_{21}H_{40}N_2O$ 336.3141).

Isothiocyanate 21. A mixture of thiophosgene (18 mg, 0.156 mmol) and CH_2Cl_2 (0.104 mL) was cooled to -5 ºC after which a solution of cooled 1M aq. K_2CO_3 (0.142 mL) was added. A mixture of amine **14** (22 mg, 0.142 mmol) and H_2O (0.03 mL) was added dropwise over 15 min with vigorous stirring and maintaining of the temperature between 0 – 5 ºC. After stirring for an additional 10 min a cold 2M aq. KOH solution (0.142 mL) was added in one portion with cooling below 0 ºC. The organic layer and three extracts (Et_2O) were dried over $MgSO_4$ and subsequently concentrated under reduced pressure to afford crude isothiocyanate as a yellow oil. The crude was subjected immediately to column chromatography (100% hexane) to give **21** (19 mg, 0.096 mmol, 68%) as a clear oil; $[\alpha]_D^{20}$ + 39.1 (c = 0.14, $CHCl_3$); IR (thin film): 2120 cm^{-1} (s); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 4.08 (1H, q, J = 3 Hz), 1.88 (1H, qd, J = 3, 15 Hz), 1.81 – 1.65 (4H, m), 1.55 – 1.48 (1H, m), 1.25 (1H, dq J = 5, 15 Hz), 1.13 (1H, ddd, J = 5, 15, 15 Hz), 1.00 – 0.94 (1H, m), 0.93 (3H, d, J = 5 Hz), 0.91 (3H, d, J = 5 Hz) 0.89 (3H, d, J = 5 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 129.7 (NCS), 56.5, 47.9, 40.4, 34.3, 29.9, 26.6, 25.1, 21.7, 20.7, 20.9. EI-MS (70 eV) m/z (%): 197 ($[M]^+$, 70), 139 ($[M\text{-}NCS]^+$, 60), 83 ($[M\text{-}C_5H_8NS]^+$, 100); HRMS m/z 197.1237 $[M]^+$ (calcd. for $C_{11}H_{19}NO$ 197.1238).

Thiourea 22. Isothiocyanate **21** (5 mg, 0.025 mmol) was treated with menthyl amine **14** (7.8 mg, 0.051 mmol) in dry CH_2Cl_2 (0.5 mL). The solution was stirred at room temperature for 18 h. The thiourea was precipitated with the aid of pentane, filtered, washed with pentane and dried *in vacuo* to afford thiourea **21** (5 mg, 0.014 mmol, 55%) as a white solid, mixture of rotational isomers 3:1 by ^{1}H NMR; mp 230 – 232 ºC; $[\alpha]_D^{20}$ + 61.1 (c = 0.018, $CHCl_3$); ^{1}H NMR (CD_3OD, 500 MHz): δ 4.07 (2H, q, J = 3 Hz), 1.99 (2H, dd, J = 3, 10 Hz), 1.84 – 1.71 (4H, m), 1.50 – 1.28 (2H, m), 1.23 – 1.11 (2H, m), 1.09 – 0.94 (8H, m), 0.91 – 0.89 (12H, m), 0.86 ($3H_{major}$, d, J = 10 Hz,), 0.85 ($3H_{minor}$, d, J = 5 Hz) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz) *major*: δ 55.8, 46.4, 39.2, 34.4, 29.3, 26.3, 24.9, 22.2, 21.1, 20.6; *minor*: δ 55.8, 46.5, 40.8, 34.4, 29.7, 27.1, 24.9, 22.2, 20.9, 20.5; EI-MS (70 eV) m/z (%): 352 ($[M]^+$, 70), 215 ($[M\text{-}C_{10}H_{17}]^+$, 100); HRMS m/z 352.2921 $[M]^+$ (calcd. for $C_{21}H_{40}N_2S$ 352.2912).

Supplementary Information Available

Figures S1-S16. ^{1}H and ^{13}C NMR data for compounds **7** – **10**, **12**, **14** – **16**, and **21** – **22**.

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References

1. Garson, M.J.; Simpson, J.S.; Flowers, A.E.; Dumdei, E.J. In *Studies in Natural Products Chemistry*, Atta-ur-Rahman Ed.; Amsterdam, 2001; vol 21, p. 329.
2. Garson, M.J.; Simpson, J.S. *Nat. Prod. Rep.* **2004**, *21*, 164.
3. Gulavita, N.K.; De Silva, E.D.; Hagadone, M.R.; Karuso, P.; Scheuer, P.J. *J. Org. Chem.* **1986**, *51*, 5136.
4. Braekman, J.C.; Daloze, D.; Moussiaux, C.; Stoller, D.; Denebourg, F. *Pure Appl. Chem.* **1989**, *61*, 509.
5. König, G.M.; Wright, A.D.; Angerhofer, C.K. *J. Org. Chem.* **1996**, *61*, 3259.
6. Mitome, H.; Shirato, N.; Miyaoka, H.; Yamada, Y.; van Soest, R.W.M. *J. Nat. Prod.* **2004**, *67*, 833.
7. Hirota, H.; Tomono, Y.; Fusetani, N. *Tetrahedron* **1996**, *52*, 2359.
8. Wright, A.D.; König, G.M.; Angerhofer, C.K.; Greenidge, P.; Linden, A.; Desqueyroux-Faúndez, R. *J. Nat. Prod.* **1996**, *59*, 710.
9. Satitpatipan, V.; Suwanborirux, K. *J. Nat. Prod.* **2004,** 67, 503.
10. Ishiyama, H.; Hashimoto, A.; Fromont, J.; Hoshino, Y.; Mikami, Y.; Kobayashi, J. *Tetrahedron* **2005**, *61*, 1101.
11. Sullivan, B.W.; Faulkner, D.J.; Okamoto, K.T.; Chen, M.H.M.; Clardy, J. *J. Org. Chem.* **1986**, *51*, 5134.
12. Di Blasio, B.; Fattorusso, E.; Magno, S.; Mayol, L.; Pedone, C.; Santacroce, C.; Sica, D. *Tetrahedron* **1976**, *32*, 473.
13. Dumdei, E.J.; Flowers, A.E.; Garson, M.J.; Moore, C.J. *Comp. Biochem. Physiol*. **1997**, *118A*, 1385.
14. Caine, D.; Deutsch, H. *J. Am. Chem. Soc.* **1978**, *100*, 8030.
15. White, J. D.; Hansen, J. D. *J. Am. Chem. Soc.* **2002,** *124*, 4950.
16. Ichikawa, Y.; Nishiyama, T.; Isobe, M. *J. Org. Chem.* **2001,** *66*, 4200.
17. Osborn, H. M. I.; Williams, N. A. O. *Org. Lett.* **2004,** 6, 3111.
18. Uy, M.M.; Ohta, S.; Yanai, M.; Ohta, E.; Hirata, T.; Ikegami, S. *Tetrahedron* **2003**, *59*, 731.
19. Jumaryatno, P.; Blanchfield, J.T.; Garson, M.J., unpublished data.
20. Mayol, L.; Piccialli, V.; Sica, D. *Tetrahedron* **1987**, *43*, 5381.
21. Ciminiello, P.; Fattorusso, E.; Magno, S.; Mayol, L. *Can. J. Chem.* **1987**, *65*, 518.
22. Burgoyne, D.L.; Dumdei, E.J.; Andersen, R.J. *Tetrahedron* **1993**, *49*, 4503.