# Structure and biosynthesis of borophycin, a new Boeseken complex of boric acid from a marine strain of the blue-green alga Nostoc linckia

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# Structure and Biosynthesis of Borophycin, a New Boeseken Complex of Boric Acid from a Marine Strain of the Blue-Green Alga *Nostoc linckia*

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Borophycin (1) is the potent cytotoxin in the lipophilic extract of a marine strain of the blue-green alga (cyanobacterium) *Nostoc linckia* (Roth) Bornet ex Bornet & Flahault (UH isolate GA-5-23). The gross structure of this boron-containing compound was determined by spectral methods and its relative stereochemistry established by X-ray crystallography. Borophycin is made up of two identical halves with an overall structure reminiscent of the ionophoric antibiotics boromycin (2) and aplasmomycin (3). The biosynthesis of 1 differs from the biosynthesis of 2 and 3. All three compounds are acetate-derived polyketides that utilize a $C_3$ precursor for the starter unit and methionine for the methyl branches on the polyketide chain. Whereas phosphoglycerate or phosphoenolpyruvate has been suggested to be the $C_3$ starter unit in the biosynthesis of 2 and 3, the $C_3$ starter unit for the biosynthesis of 1 is derived from acetate and methionine, but not propionate.

In evaluating lipophilic and hydrophilic extracts of laboratory-cultured blue-green algae (cyanobacteria) for *in vitro* antitumor activity,[1] the lipophilic extract of a marine strain of *Nostoc linckia* (Roth) Bornet ex Bornet & Flahault (UH isolate GA-5-23)[2] was found to display appreciable cytotoxicity against LoVo (MIC 0.066 μg/mL) and KB (MIC 3.3 μg/mL), two human tumor cell lines used routinely in our laboratory for screening.[3] In the more meaningful Corbett and Valeriote assays,[4] however, the extract was neither solid tumor selective nor tumor selective, suggesting that further investigation of this extract might not lead to a drug that would offer any distinct advantage over drugs already in clinical use for the treatment of human cancer. Nevertheless, this algal extract was among the most LoVo-cytotoxic found in screening extracts of 665 blue-green algae,[1] and this potency prompted us to isolate, identify, and pharmacologically evaluate the active agent.

Table 1. NMR Data for 1 in DMSO-$d_6$

| position | $\delta_C$ | $\delta_H$ |
|---|---|---|
| 1 | 173.0 | |
| 2-H | 78.6 | 4.09 |
| 3 | 103.2 | |
| 4-H | 35.8 | 1.52 |
| 5-$H_2$ | 27.7 | 1.52 (*R*), 1.52 (*S*) |
| 6-$H_2$ | 24.5 | 1.20 (*R*), 1.49 (*S*) |
| 7-H | 72.3 | 4.06 |
| 8 | 49.8 | |
| 9 | 216.2 | |
| 10-$H_2$ | 42.0 | 3.33 (*R*), 2.44 (*S*) |
| 11-H | 66.2 | 3.80 |
| 12-$H_2$ | 36.5 | 1.07 (*R*), 1.26 (*S*) |
| 13-$H_2$ | 23.4 | 2.50 (*R*), 1.45 (*S*) |
| 14-H | 133.6 | 5.52 |
| 15-H | 121.4 | 5.35 |
| 16-$H_2$ | 28.5 | 3.31 (*R*), 1.84 (*S*) |
| 17-H | 75.8 | 4.35 |
| 18-$H_2$ | 24.8 | 1.70 (*R*), 1.55 (*S*) |
| 19-$H_3$ | 10.5 | 0.92 |
| 20-$H_3$ | 16.5 | 0.85 |
| 21-$H_3$ | 22.5 | 0.83 |
| 22-$H_3$ | 16.2 | 0.86 |

Successive bioassay-directed normal-phase chromatography on silica gel and reversed-phase chromatography on C-18 led to a fraction which possessed most of the cytotoxicity. Borophycin (1), the active agent,[2,5] crystallized from a methanolic solution of this fraction in 0.43% yield.

**Gross Structure Determination.** The molecular weight of 1 was concluded to be 854 on the basis of the following data: The electron-impact mass spectrum exhibited a prominent ion peak at *m/z* 854, which had to be the molecular ion peak since the normal positive-ion fast-atom bombardment (FAB) mass spectrum showed a

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(2) [7.7]Paracyclophanes (nostocyclophanes A–D) are the cytotoxins associated with terrestrial *Nostoc linckia* strain UTEX 1932 [(a) Moore, B. S.; Chen, J.-L.; Moore, R. E.; Patterson, G. M. L.; Brinen, L.; Kato, Y.; Clardy, J. *J. Am. Chem. Soc.* **1990**, *112*, 4061–3. (b) Chen, J.-L.; Moore, R. E.; Patterson, G. M. L. *J. Org. Chem.* **1991**, *56*, 4360–4].

(3) The lipophilic extract exhibited marginal antiherpes activity since it reduced plaque formation by 98% at 0.1 mg/mL, well below the MIC dose of 5 mg/mL for the uninfected mink lung host cells [Patterson, G. M. L.; Baker, K. K.; Baldwin, C. L.; Bolis, C. M.; Caplan, F. R.; Larsen, L. K.; Levine, I. A.; Moore, R. E.; Nelson, C. S.; Tschappat, K. D.; Tuang, G. D.; Boyd, M. R.; Cardellina, J. H., II; Collins, R. P.; Gustafson, K. R.; Snader, K. M.; Weislow, O. S. *J. Phycol.* **1993**, *29*, 125–30], but comparable with the KB and LoVo MICs.

(4) (a) Corbett, T. H.; Valeriote, F. A.; Polin, L.; Panchapor, C.; Pugh, S.; White, K.; Lowichik, N.; Knight, J.; Bissery, M.-C.; Wozniak, A.; LoRusso, P.; Biernat, L.; Polin, D.; Knight, L.; Biggar, S.; Looney, D.; Demchik, L.; Jones, J.; Jones, L.; Blair, S.; Palmer, K.; Essenmacher, S.; Lisow, L.; Mattes, K. C.; Cavanaugh, P. F.; Rake, J. B.; Baker, L. In *Cytotoxic Anticancer Drugs: Models and Concepts for Drug Discovery and Development*; Valeriote, F. A., Corbett, T. H., Baker, L. H., Eds.; Kluwer Academic Publishers: Norwell, 1992; pp 35–87. (b) Valeriote, F.; Moore, R. E.; Patterson, G. M. L.; Paul, V. P.; Scheuer, P. J.; Corbett, T. In *Discovery and Development of Anticancer Agents*; Valeriote, F. A.; Corbett, T. H., and Baker, L. H., Eds.; Kluwer Academic Publishers: Norwell, 1994, in press.

(5) Anti-HSV2 $IC_{90}$ is 20 ng/mL (mink lung cell MIC = 1 μg/mL), comparable with cytotoxicity $IC_{50}$ values of 7.5 ng/mL for LoVo (a human epidermoid carcinoma cell line) and 20 ng/mL for KB (a human colorectal adenocarcinoma cell line).

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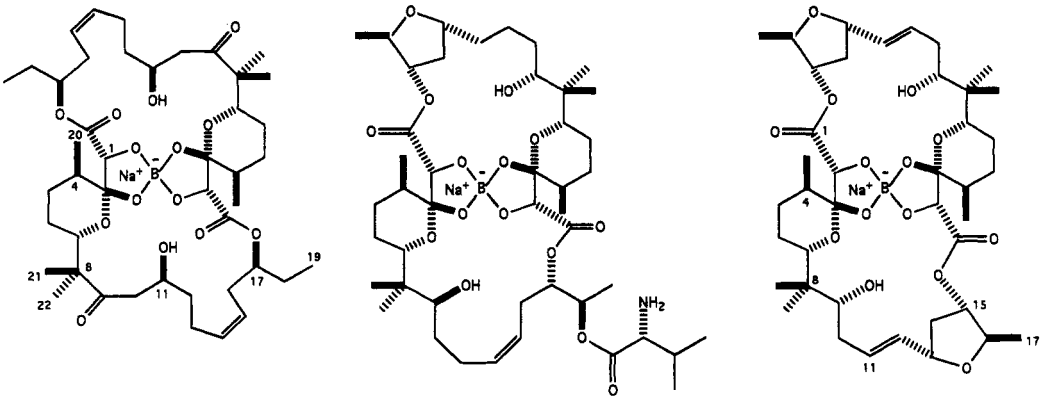
**Chart 1**



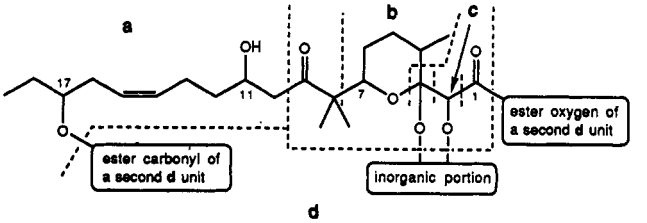
1 borophycin 2 boromycin 3 aplasmomycin

strong $MH^+$ ion peak at $m/z$ 855. To our surprise, however, only a small $MK^+$ peak could be seen at $m/z$ 893 when KCl was added to the glycerol matrix containing 1. Much larger peaks existed at $m/z$ 855, 871 (MH − Na + K) and 909 (M − Na + 2K). These observations were consistent only with the presence of sodium in borophycin. When the FAB mass spectra were reexamined, a small $m/z$ 833 peak (M − Na + 2H) could be found in most of them.

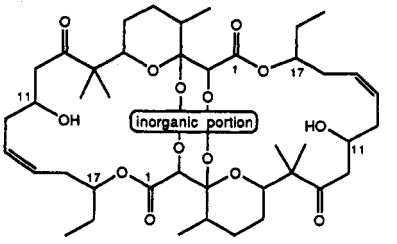
The $^{13}C$ NMR spectrum indicated that 1 was a symmetric dimer since only 22 carbon signals could be seen (Table 1). A refocused INEPT experiment established that these 22 signals were comprised of 4 methyl, 7 methylene, 7 methine, and 4 non-protonated carbon signals. This meant that 1 contained 44 carbons and 66 hydrogens attached to carbon. The number of protons attached to heteroatoms, i.e, exchangeable protons, was determined by analyzing the $^1H$ NMR spectrum of 1 in dimethyl sulfoxide-$d_6$. All but one of the $^1H$ signals could be $^1J$-correlated with the 22 carbon signals in a HMQC experiment. Although this $^1H$ signal, a doublet at 4.12 ppm ($J$ = 4 Hz), was not $^1J$-coupled to any of the carbon signals, it was vicinally coupled to a proton signal at 3.80 ppm and $^2J$-coupled to a methine carbon signal at 66.2 ppm (C-11) as shown by a HMBC experiment. The latter two signals, which did $^1J$-correlate in the HMQC spectrum, had chemical shifts indicative of a methine bearing a hydroxyl group. A comparison of the $^{13}C$ NMR spectra of 1 in MeOH-$d_4$ and MeOH-$d_3$ showed a chemical shift difference ($\Delta\delta_C$) of 0.1 ppm for the C-11 methine carbon signal; however, the chemical shifts of all of the other signals were identical in the two solvents. Therefore only two exchangeable protons, one in each moiety of this symmetrical dimer, appeared to be present in 1.

In addition to signals for a methine bearing a hydroxyl group, the $^{13}C$ and $^1H$ NMR spectra in dimethyl sulfoxide-$d_6$ revealed signals which strongly suggested that each moiety of this dimeric molecule possessed a ketone group ($\delta_C$ 216.5 ppm), an ester group ($\delta_C$ 173.0 ppm) attached via the oxygen to a methine ($\delta_C$ 75.8 ppm, $\delta_H$ 4.35 ppm), a ketal group ($\delta_C$ 103.2 ppm), and two methines bearing single ether-type oxygens ($\delta_C$ 78.6 ppm, $\delta_H$ 4.09 ppm; $\delta_C$ 72.3 ppm, $\delta_H$ 4.06 ppm). Two carbonyl absorption bands were found in the IR spectrum of 1 in $CHCl_3$ at 1690 (ketone) and 1720 (ester) $cm^{-1}$, but their positions suggested that the carbonyl oxygens were either hydrogen-bonded or chelated. Each moiety of this dimeric molecule, therefore, had to possess a minimum of six oxygens since it was certain that hydroxyl, ketone, ester, and ketal functionalities were present. This meant that 1 contained 44 carbons, 68 hydrogens, and at least 12 oxygens and 1 sodium, which accounted for 811 of the 854 molecular mass units for 1. Only 43 mass units remained unassigned. If an additional 32 mass units were attributable to two more oxygens, then the remaining 11 mass units could only be accommodated by an atom of this atomic weight, namely boron. NMR evidence provided support for boron (broad peak at −11 ppm). The molecular formula of borophycin was therefore $C_{44}H_{68}BO_{14}Na$.

Partial structures $CH_3CH_2CH(OR)CH_2(CH{=}CH)_{cis}$-$CH_2CH_2CHOHCH_2$- (**a**), where R is the ester carbonyl, and -$CH$(-O-)$CH_2CH_2CHCH_3$- (**b**) could be generated by a detailed analysis of the $^1H$ double-quantum COSY spectrum. RELAY and TOCSY experiments verified the COSY interpretations. The NMR data also indicated the presence of two methyl groups that had to be attached to the sole quaternary carbon atom (*gem*-dimethyl group) in each moiety of **5** and an oxygen-bearing methine carbon (**c**) that was not attached to any protonated carbons. The HMBC data now allowed us to assemble the various structural units, including the ketone, ester, and ketal groups, into an expanded structure **d**. First of all, the




d




e

data suggested that the methylene on the right side of unit **a** was attached to either the ketone or *gem*-dimethyl

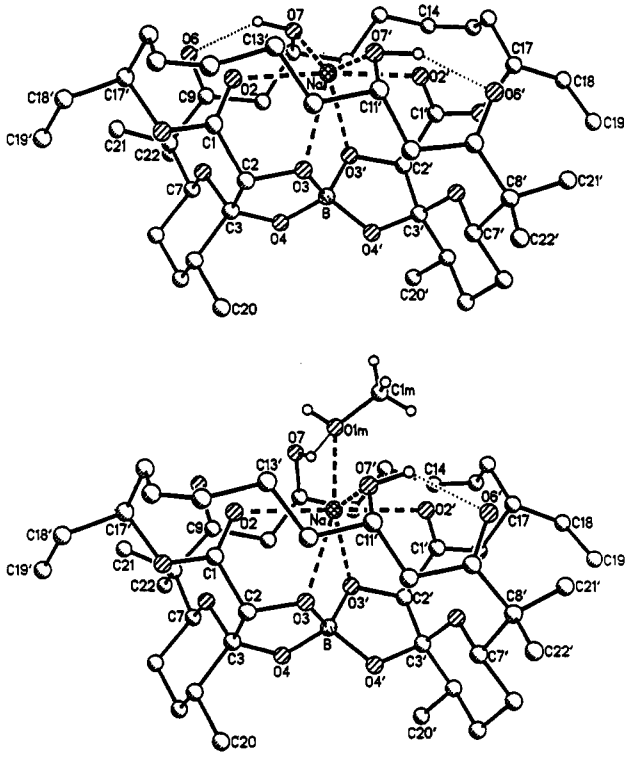



**Figure 1.** A computer-generated perspective drawing of two different crystalline conformations for borophycin (1). The top conformation is form A and the bottom one is form B.

carbon. The magnitude of the geminal coupling (18–19 Hz) between the methylene protons, however, indicated that **a** was attached to the ketone group. In this way the hydroxyl group is on a carbon which is $\beta$ to the ketone group and explains the loss of two water molecules from the molecular ion in the EIMS (only prominent fragment ions seen) and the H-bonding associated with the ketone carbonyl as shown by the IR spectrum. The proton signal for the oxygenated methine of unit **b** showed important HMBC cross peaks to the signals for the ketal carbon, the ketone carbonyl carbon, and all three carbons of the *gem*-dimethyl group, as well as the methylene carbon in **b** three bonds away. This meant that unit **b** and the ketal carbon were in a tetrahydropyran ring to which was attached successively on the oxygenated methine the *gem*-dimethyl and ketone groups. The isolated oxygen-bearing methine (**c**) carbon appeared to be attached to the ketal carbon. Although a cross peak was not observed from the unit **c** proton signal to the ketal carbon signal, a meaningful cross peak was seen to the signal for the methine carbon in unit **b** bearing the methyl group. Unit **c** was further connected to the ester carbonyl carbon since a cross peak was also seen to the signal for this carbon. Finally a cross peak between the signals for the proton of the oxygenated methine (C-17) in one unit **d** and the ester carbonyl carbon (C-1) in a second unit **d** confirmed the position of the ester group and established that two units of **d** were connected together into a macrodiolide ring as shown by **e**.

The gross structure of borophycin was completed by connecting the four oxygens on C-2 and C-3 of the two **d** units in **e** to the boron and, in turn, the sodium.

**Stereochemistry.** The complete stereostructure of **1** was given by single crystal X-ray diffraction. In Figure 1 is shown a computer-generated perspective drawing of the two different crystalline conformations. As can be most easily appreciated from the upper drawing, **1** is made up of two identical halves with an overall structure reminiscent of other naturally-occurring borate Böeseken complexes, viz., the ionophoric antibiotics boromycin[6,7] (**2**) from a terrestrial strain of *Streptomyces antibioticus*, and aplasmomycin[8,9] (**3**) from a marine strain of *Streptomyces griseus*. Their structures, including absolute stereochemistries, were elucidated by X-ray analysis[6a,8d] and confirmed by synthesis.[7,9]

The monomeric $C_{22}H_{34}O_7$ units of **1** are stitched together through diol fragments in a borate ester (O3, O4, O3′, and O4′) and through ester links (C17–O1′ and C17′–O1). As drawn, the borate ester is at the bottom and the sodium ion is at the top of a disk-like molecular assembly. The interior of the disk contains all of the oxygens and the outside of the disk possesses all of the hydrophobic alkyl groups—the overall structure expected of an ionophore. The sodium to boron distance is roughly 3.0 Å.

Borophycin represents an unusual case where the two molecules forming the asymmetric unit have distinctly different conformations and it is interesting to contrast them. In Figure 1 the top conformation is form A and the bottom one is form B. In form A all of the oxygens coordinated to the sodium ion come from **1**. The molecule has approximate but not exact two-fold symmetry, and the sodium is coordinated in a distorted trigonal prism fashion where the atom pairs O3–O3′, O2–O7′, and O2–O7 form the pairs of three-fold related atoms. The twist angles are 1°, 12°, and 53°, respectively. In form A there is an intramolecular hydrogen bond between O7–H···O6.

In form B at the bottom of Figure 1, five of the six oxygens coordinated to sodium in form A remain the same, but a methanol oxygen displaces O7. Atom O7 moves roughly 1 Å away from its former A position, and this motion is accomplished by a tiny 4° change in the torsional angle around the C10–C11 bond. This subtle change in twist leads to other displacements along the C8 to C17 chain with the largest displacements being 1.07 Å for C13 and 1.01 Å for C14. The other half of the molecule—the primed half—remains essentially unperturbed, and its mean rms deviation from form A is 0.23 Å. The coordination of the sodium ion can be described as a distorted octahedron with O2, O3, O2′, and O(methanol) forming an approximately planar, four-fold related set and O7′ and O3′ forming the perpendicular axis. O3′ is distorted 53° away from the four-fold axis. In form B the hydrogen bond between O7′ and O6′ is preserved and a hydrogen bond between O7 and O(methanol) is established.

The absolute stereochemistry of borophycin could not be determined from X-ray analysis of the sodium salt and is tentatively depicted as that shown in **1** by analogy with **2** and **3**.

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(8) Okazaki, T.; Kitahara, T.; Okami, Y. *J. Antibiot.* **1975**, *28*, 176–84. (b) Khahara, T.; Naganawa, H.; Okazaki, T.; Okami, Y.; Umezawa, H. *J. Antibiot.* **1975**, *28*, 280–85. (c) Okami, Y.; Okazaki, T.; Kitahara, T.; Umezawa, H. *J. Antibiot.* **1976**, *29*, 1019. (d) Nakamura, H.; Iitaka, Y.; Kitahara, T.; Okazaki, T.; Okami, Y. *J. Antibiot.* **1977**, *30*, 714–9. (e) Stout, T. J.; Clardy, J.; Pathirana, I. C.; Fenical, W. *Tetrahedron* **1991**, *47*, 3511–20.

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**Biosynthesis.** The oxygenation pattern of the monomeric subunit **d** of borophycin suggests a polyketide origin for the carbon skeleton from a $C_3$ starter unit. To test this hypothesis sodium [1,2-$^{13}C_2$]acetate and sodium [2,3-$^{13}C_2$]-propionate were administered to two cultures of *N. linckia* GA-5-23 in two parallel experiments. The **1** isolated from the culture to which the labeled propionate had been administered did not show any enrichment in the signals assigned to C-18 and C-19. Satellites, however, were readily discernible in the signals due to carbons C-1 to C-16 in the sample isolated from the culture to which labeled acetate had been fed. The specific incorporation was 1.35% above natural abundance. Furthermore, satellites were observed in the signals attributed to C-17 and C-18, the carbonyl and the methylene, respectively, of the $C_3$ starter unit. If therefore appears that the starter unit for the biosynthesis of **1** in GA-5-23 is derived in the same fashion as the acetate plus methionine-derived $C_3$ starter unit in asteltoxin[10] or myxovirescin $A_1$.[11] Support for this route was obtained in a feeding experiment in which [*methyl*-$^{13}$C]-L-methionine was supplied to a culture of the alga. The **1** isolated from this incubation showed four strongly and equally enhanced signals (specific incorporation 10% above natural abundance) in its $^{13}$C NMR spectrum for the methyl groups C-19, 20, 21, and 22. Thus it appears that the C3 of **1** is derived exclusively via the acetate plus methionine route, whereas propionate serves equally-well for the $C_3$ starter unit in asteltoxin. Thus, while all three boron-containing antibiotics contain a $C_3$ starter unit from a purely structural point of view, **1** differs from **2** and **3** in its origin. Phosphoglycerate or phosphoenolpyruvate has been suggested to be the $C_3$ starter unit in the biosynthesis of **2**[12] and **3**.[13]

## Experimental Section

**Spectral Analysis.** NMR spectra were determined on 11.75- and 7.05-T instruments operating at 500 and 300 MHz for $^1$H and 125 and 75 MHz for $^{13}$C. The $^{11}$B NMR spectrum of **1** was determined on the 7.05-T instrument operating at 96.5 MHz. $^1$H Chemical shifts are referenced in DMSO-$d_6$, MeOH-$d_4$, and $CDCl_3$ to residual DMSO-$d_5$, (2.49 ppm), [$CH_3$-$d_2$]MeOD (3.30 ppm), and $CHCl_3$ (7.24 ppm); $^{13}$C chemical shifts are referenced to the solvent (DMSO-$d_6$, 39.5; MeOH-$d_4$, 49.0; $CDCl_3$, 77.0 ppm); $^{11}$B chemical shifts are referenced externally to boric acid in $D_2O$. Homonuclear $^1$H connectivities were determined by using double-quantum filtered COSY, single and double relay (RCT and RCT2), and TOCSY (HOHAHA) experiments. Homonuclear $^1$H NOEs were obtained by difference NOE experiments using a 3-s irradiation period and by two-dimensional phase-sensitive NOESY and ROESY experiments. One-bond heteronuclear $^1$H–$^{13}$C connectivities were determined by proton-detected HMQC and carbon-detected HETCOR experiments; two and three bond $^1$H–$^{13}$C connectivities were determined by the HMBC experiment.

**Isolation and Cultivation of Alga.** *N. linckia* (Roth) Bornet ex Bornet & Flahault (UH isolate GA-5-23) was isolated from a marine mud sample collected at Majuro atoll in the Marshall Islands (7° 6′ 18″ N, 171° 22′ 42″ E) and purified by repeated subculture on solidified media. The cyanophyte was cultured in 20-L glass bottles containing a modified inorganic medium, designated BG-11, containing 2% NaCl. Prior to autoclaving, the pH of the medium was adjusted to 7.0 with sodium hydroxide. Cultures were illuminated continuously at an incident intensity of 200-$\mu$mol photons m$^{-2}$ s$^{-1}$ (photosynthetically active radiation) from banks of cool-white fluorescent tubes, aerated at a rate of 5 L/min with a mixture of 0.5% $CO_2$ in air and incubated at a temperature of 24 ± 1°C. After 3–4 weeks the alga was harvested by filtration. The yield of lyophilized cells was 0.3–0.4 g/L.

For the biosynthetic experiments, 225 and 500 mg amounts of a 1:1 mixture of sodium [1,2-$^{13}$C]acetate and unlabeled acetate were fed to cultures 13 and 20 days, respectively, after innoculation. Similarly 100 and 150 mg amounts of sodium [2,3-$^{13}$C]-propionate were fed on days 10 and 20, respectively, and 85-mg portions of [*methyl*-$^{13}$C]-L-methionine were fed on days 11, 16, and 21. The algal cultures were havested by filtration on day 28 and immediately freeze-dried.

**Isolation of Borophycin (1).** Freeze-dried alga (25.23 g) was extracted three times with 1-L portions of 1:1 $CH_2Cl_2$/MeOH. The extract (6.6 g) was applied to a 14 × 2 in column of silica gel (100–200 mesh). Elution was carried out with 3 bed volumes each of 3:2, 2:3, and 1:4 $CH_2Cl_2$/EtOAc followed by EtOAc. The separation was monitored by NMR analysis and most of the **1** appeared in the 1:4 $CH_2Cl_2$/EtOAc fractions. Borophycin crystallized from MeOH and mixtures of $CH_2Cl_2$/MeOH.

Borophycin had the following properties: $[\alpha]_D$ −23.7° (*c* 1.4, $CHCl_3$); FABMS *m/z* 855 ($MH^+$), 833 ($[M - Na + H]^+$); EIMS *m/z* 854 ($M^+$, base peak), 836 ($[M - H_2O]^+$), 818 ($[M - 2H_2O)^+$]; high resolution EIMS *m/z* 854.4542 (calcd for $C_{44}H_{68}BO_{14}Na$, 854.4600); high resolution FABMS *m/z* 833.4698 (calcd for $C_{44}H_{69}BO_{14}$, 833.4780); $^{13}$C NMR ($CDCl_3$/MeOH-$d_4$) δ 175.2/175.3 (C-1), 79.4/80.9 (C-2), 104.3/105.6 (C-3), 36.8/38.0 (C-4), 28.0/29.0 (C-5), 25.0/26.2 (C-6), 73.7/75.2 (C-7), 50.7/51.9 (C-8), 216.5/217.6 (C-9), 42.2/43.8 (C-10), 68.6/69.1 (C-11),[12] 36.1/37.9 (C-12), 23.9/25.1 (C-13), 134.1/135.0 (C-14), 121.7/122.7 (C-15), 29.1/29.9 (C-16), 77.1/78.3 (C-17), 25.7/26.7 (C-18), 10.9/11.5 (C-19), 16.8/16.9 (C-20), 16.7/17.3 (C-21), 23.0/23.6 (C-22); $^1$H NMR ($CDCl_3$) δ 4.27 (s, H-1), 1.62 (m, H-4 and $H_2$-5), 1.28 (m, $H_R$-6), 1.51 (m, $H_S$-6), 4.16 (dd, *J* = 11.4 and 2 Hz, H-7), 2.41 (d, *J* = −18.4 Hz, $H_S$-10), 3.47 (dd, *J* = −18.4 and 10 Hz, $H_R$-10), 3.99 (td, *J* = 10 and 4 Hz, H-11), 1.22 (m, $H_2$-12), 1.54 (m, $H_S$-13), 2.56 (br qd, $H_R$-13), 5.54 (td, *J* = 11 and 4 Hz, H-14), 5.39 (td, *J* = 11 Hz and 4 Hz, H-15), 1.82 (ddd, *J* = −13, 3 and 1 Hz, $H_S$-16), 3.49 (td, *J* = ±13 and 4 Hz, $H_R$-16), 4.48 (br dt, H-17), 1.55 (m, $H_S$-18), 1.78 (m, $H_R$-18), 0.98 (t, *J* = 7 Hz, $H_3$-19), 0.95 (d, *J* = 6 Hz, $H_3$-20), 0.91 (s, $H_3$-21), 0.97 (s, $H_3$-22). $^1$H NMR (MeOH-$d_4$) δ 4.27 (s, H-1), 1.68 (m, H-4), 1.60 (m, $H_S$-5), 1.70 (m, $H_R$-5), 1.35 (m, $H_R$-6), 1.58 (m, $H_S$-6), 4.17 (dd, *J* = 11.5 and 2 Hz, H-7), 2.53 (dd, *J* = −19 and 0.5 Hz, $H_S$-10), 3.39 (dd, *J* = −19 and 10 Hz, $H_R$-10), 3.90 (t br t, *J* = 10 and 1–2 Hz, H-11), 1.20 (m, $H_S$-12), 1.30 (m, $H_S$-12), 1.53 (m, $H_S$-13), 2.62 (qd, *J* = ±12.3 and 4 Hz, $H_R$-13), 5.56 (td, *J* = 11 and 4 Hz, H-14), 5.40 (td, *J* = 11 Hz and 4 Hz, H-15), 1.86 (ddd, *J* = −14.5, 4 and 1 Hz, $H_S$-16), 3.47 (ddd, *J* =

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(14) In MeOH-$d_3$ the C-11 signal is shifted to 69.2 ppm.

(15) The X-ray model for **1** allowed rigorous assignment of all of the signals in the proton NMR spectrum, particularly the ones for the *pro-R* and *pro-S* hydrogens of the various methylenes (see figure in supplementary material). All of the important coupling constants could be abstracted directly from the spectrum and the sizes of the couplings enabled us to distinguish between *anti* and *syn* relationships for vicinally coupled protons. Large coupling constants (10–13 Hz) were observed for *anti* protons whereas small coupling constants were found for *syn* protons. Signals for *anti* and *syn* coupled protons could be differentiated by examining the relative intensities of cross peaks in the COSY spectrum. In one case the H-11/11′ signal, a broad triplet at 3.80 ppm, exhibited intense cross peaks (large couplings) with the signals for the *anti pro-R* hydrogens on C-10/10′ (3.33 ppm) and C-12/12′ (1.07 ppm) and weak cross peaks (small couplings) with the signals for the *syn pro-S* hydrogens on C-10/10′ (2.44 ppm) and C-12/12′ (1.26 ppm). Moreover, the *pro-R* H-12/12′ signal, a 1:3:3:1 quartet of doublets, showed an intense cross peak with the *anti pro-S* H13/13′ signal and a weak cross peak with the *syn pro-R* H-13/13′ signal, whereas the *pro-S* H12/12′ signal, a triplet of broad triplets, displayed a weak cross peak with the *syn pro-R* H-13/13′ signal at 2.50 ppm and an intense cross peak with the *anti pro-S* H-13/13′ signal at 1.45 ppm. In another case the signal for axial H-7/7′, a doublet of doublets at 4.06 ppm, exhibited strong and weak cross peaks with the signals for axial *pro-R* H-6/6′ (1.20 ppm) and equatorial *pro-S* H-6/6′ (1.49 ppm), respectively.

−14.5, 12.5 and 5 Hz, $H_R$-16), 4.50 (br dt, $J$ = 9 and 5 Hz, H-17), 1.60 (m, $H_S$-18), 1.77 (m, $H_R$-18), 0.99 (t, $J$ = 7.3 Hz, $H_3$-19), 0.97 (d, $J$ = 6 Hz, $H_3$-20), 0.91 (s, $H_3$-21), 0.96 (s, $H_3$-22).

**X-Ray Crystallographic Studies.**[16] A 0.25 × 0.45 × 0.50 mm crystal grown from MeOH/$CH_2Cl_2$ was used to collect a room temperature (23 °C) data set with $2\theta \leq 114°$ and Cu K$\bar{\alpha}$ radiation (1.5418 Å). The crystal belonged to the monoclinic space group $P21$ with $a$ = 15.659(3), $b$ = 15.377(3), $c$ = 20.762(4) Å and $\beta$ = 108.44(3)° with two molecules of composition $C_{44}H_{68}BO_{14}Na$ and one $CH_3OH$ forming the asymmetric unit. A total of 6682 unique reflections were surveyed, and 5855 (88%) were judged observed [$|F_o| \geq 4\sigma(F_o)$]. The structure was solved using the SHELXTL implementation of direct methods. All non-hydrogen atoms were refined with anisotropic positional and thermal parameters using block-diagonal least-squares methods. Hydrogens were refined using a riding model. These refinements have converged to the current crystallographic residual of $R$ = 5.03%, $wR$ = 6.19% where $w = 1/(\sigma^2(F) + 0.0005(F^2))$.

(16) The author has deposited atomic coordinates for this structure with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.

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**Supplementary Material Available:** A copy of the 500-MHz $^1H$ NMR spectrum of 1 in DMSO-$d_6$ and proton assignments for 1 based on coupling constants and NOEs (2 pages). The material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.