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Semi-synthetic preparation of the rare, cytotoxic, deep-sea sourced sponge metabolites discorhabdins P and U

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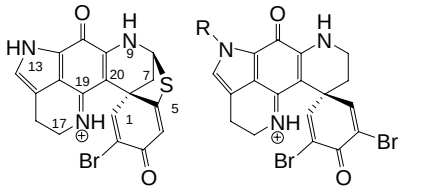
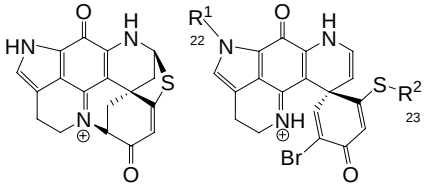
Abstract—Semi-synthetic routes to the enzyme inhibitory and potently anti-proliferative marine natural products discorhabdins P and U were developed by one-step methylation reactions of discorhabdins C and B, respectively. Two novel semi-synthetic derivatives of discorhabdin U were also prepared, one of which (**6**) exhibited significant anti-proliferative activity.

The discorhabdins are a class of cytotoxic alkaloids found in marine sponges of the families Latrunculiidae and Acarnidae.[1] Numerous naturally occurring discorhabdins typified by discorhabdin (prianosin) A,[2,3] B (**1**),[3] C (**2**),[3,4] D (**3**)[5] and more recently W,[6] along with several semi-synthetic and synthetic analogues have been reported in the literature.[7,8] The core discorhabdin structure contains a pyrido[2,3-*h*]pyrrolo[4,3,2-*de*]quinoline tetracyclic skeleton bound to various spiro-substituents at the C-6 position. *N*-Methyl pyrrole analogues are quite uncommon, with reports of only four methylated discorhabdins isolated from deepsea *Batzella* sp. sponges.[9,10] Such compounds, however, display interesting biological activity: for example, discorhabdin P (**4**), 13-*N*-methyldiscorhabdin C, was found to be an inhibitor of calcineurin and caspase CPP32 enzymes, whereas discorhabdin C (**2**) was inactive in the same assays.[9] Discorhabdin U (**5**), an 13-*N*-methyl-*S*-methyl analogue, exhibited strong in vitro cytotoxicity against PANC-1, P-388, and A-549 cell lines, comparable to those of some of the most potent of the discorhabdins.[10]

In New Zealand waters, *Latrunculia* spp. sponges are a rich source of the discorhabdin alkaloids, with organisms being divisible into producing either discorhabdins A, B or C as their major secondary metabolite, typically in isolable yields of 25–35 mg/g dry sponge weight.[11] Local sponges have also been shown to be a successful aquaculture species, thus providing a potentially sustainable and continuous supply of the discorhabdins to facilitate further biological studies.[12,13] To date, no *N*-methyl analogues have been found in any of our local *Latrunculia* spp. collections. The aim of the present study was to investigate the methylation of discorhabdin C in an effort to prepare discorhabdin P or related *N*-methyl analogues for further biological testing, and for the first time, to prepare similar analogues of discorhabdin B in an effort to expand the structure–activity relationship insight of this class of compounds.





Two possible sites for the methylation of discorhabdin C exist; N-9 and N-13. The N-13 substituent is a known

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Table 1. In vitro biological activities of compounds **1**, **2**, and **4**–**7**

Compound	P388 IC_{50}[a]	Antimicrobial[b]	
		Ec	Bs
1	44 (0.08 μM)	1	6
2	62 (0.11 μM)	1	8
4	182 (0.31 μM)	0	8
5	89 (0.16 μM)	0	3
6	81 (0.15 μM)	0	3
7	918 (1.30 μM)	0	0

[a] IC_{50} against the P388 D1 murine leukemia cell line; quantities in ng/mL.

[b] Zone of microbial inhibition against *Escherichia coli* and *Bacillus subtilis* for 30 μg of test compound on a 6 mm paper disk. Incubation for 18 h at 35 °C. Zones measured as excess radii in mm.

natural product **4**, while N-9, N-13 di-alkylated analogues were claimed but not exemplified in a patent issued for discorhabdin P.[14] Discorhabdin C was reacted with CH_3I in dry acetone to yield a single major product, the 13-*N*-methyl analogue, discorhabdin P (**4**).[15,16] Interestingly, methylation takes place exclusively at the N-13 pyrrole position, even in the presence of excess (25 equiv) CH_3I. Compound **4** was purified and fully characterized as a trifluoroacetate salt, and gave consistent data to those reported for the naturally occurring alkaloid.[9,17]

As no structure–activity relationship studies have ever been reported for discorhabdin B, the same conditions were applied to compound **1**. Discorhabdin B was reacted with CH_3I in dry acetone to yield two products; **5** and **6**. Products of this reaction were dependent upon the molar equivalents of CH_3I used, with a large excess (10–25 equiv) yielding predominantly dimethyl **5**, while two molar equivalents of CH_3I yielded mono-methyl **6**.[18] The order of discorhabdin B methylation favors the thio group preferentially and only reacts at the N-13 pyrrole position in the presence of a large excess of CH_3I. Interestingly, the mono-13-*N*-methyl derivative was not detected as a product in the latter study. Compounds **5** and **6** were purified by a combination of Sephadex LH-20, C18, and C8 flash chromatography and fully characterized as their trifluoroacetate salts.[19] A small portion of discorhabdin U (**5**) was converted to the free base (K_2CO_3) and yielded ^1H NMR spectroscopic data in full agreement with those published for the natural product.[10,20] Semi-synthetic **5** was optically active ($[\alpha]_D$ +222°), whereas the natural product is known to be non-racemic but precise optical activity data are not available for the free base species.[21]

To assess the ability to introduce other alkyl groups, discorhabdin B was reacted with an excess of benzyl bromide under dry conditions in the presence of base to yield the di-benzyl derivative **7**.[22]

Compounds **1**, **2**, and **4**–**7** were evaluated for biological activity against the murine leukemia P388 cell line as well as activity against Gram-positive and -negative bacteria. The results, summarized in Table 1, indicate that all three methylated semi-synthetic analogues **4**, **5**, and **6** exhibit potent anti-proliferative activity, comparable in magnitude with the natural products discorhabdins B (**1**) and C (**2**). Dibenzylation (**7**) led to attenuation of bioactivity.

This study has led to the development of an efficient semi-synthetic route to discorhabdins P (**4**) and U (**5**) based upon the use of discorhabdins C (**2**) and B (**1**), respectively, providing an alternative supply of these rare, cytotoxic natural products. Novel semi-synthetic discorhabdins **6** and **7** were also prepared. The finding of potent anti-proliferative activity associated with **6** clearly warrants further structure–activity investigations, results of which will be presented in due course.

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15. Samples of discorhabdins B (**1**) and C (**2**) used in this study were isolated from green and brown specimens of *Latrunculia* spp. collected from Wellington Harbor and Three Kings Islands, New Zealand, respectively.
16. Discorhabdin C trifluoroacetate salt (**2**) (10.0 mg, 17.4 μmol) was dissolved in dry acetone (2 mL) to which CH_3I (10 μL, 159 μmol) and K_2CO_3 (5 mg) were added. The reaction mixture was kept at reflux at 90 °C for 4 h under N_2 at which time analytical HPLC indicated complete consumption of starting material. The solvent

was removed in vacuo and the solid purified by C18 flash chromatography (MeOH, H_2O–TFA (0.05%)) and Sephadex LH-20 (MeOH (0.05% TFA)), yielding **4** (5.5 mg, 54% yield).

17. Naturally occurring **4** was characterized as a free base. We have found free base samples of discorhabdin alkaloids to be unstable, being susceptible to decomposition in the presence of nucleophiles, hence our characterization of the trifluoroacetate salts. NMR assignments were made by interpretation of COSY, HSQC–DEPT, and HMBC experiments. ^{1}H NMR ($(CD_3)_2SO$, 400 MHz) δ 10.10 (1H, br s, H-9), 8.16 (1H, br s, H-18), 7.70 (2H, s, H-1/5), 7.39 (1H, s, H-14), 3.91 (3H, s, H-22), 3.65 (2H, m, H-17), 3.63 (2H, m, H-8), 2.79 (2H, t, J = 7.2 Hz, H-16), 2.00 (2H, br s, H-7); ^{13}C NMR ($(CD_3)_2SO$, 100 MHz) δ 171.3 (C-3), 165.7 (C-11), 152.6 (C-19), 151.6 (C-10), 151.1 (C-1/C-5), 132.0 (C-14), 123.3 (C-21), 122.6 (C-2/C-4), 122.4 (C-12), 119.2 (C-15), 91.8 (C-20), 44.7 (C-6), 43.4 (C-17), 38.3 (C-8), 36.0 (C-22), 33.5 (C-7), 17.9 (C-16); HRFABMS (nitrobenzyl alcohol) *m/z* 475.9617 (calcd for $C_{19}H_{16}{}^{79}Br_2N_3O_2$, 475.9609), 477.9580 (calcd for $C_{19}H_{16}{}^{79}Br^{81}BrN_3O_2$, 477.9589), 479.9563 (calcd for $C_{19}H_{16}{}^{81}Br_2N_3O_2$, 479.9568).
18. Discorhabdin B trifluoroacetate salt (**1**) (15.4 mg, 29.2 μmol) was dissolved in dry acetone (2 mL) to which CH_3I (20 μL, 318 μmol) and K_2CO_3 (20 mg) were added. The reaction mixture was kept at reflux at 80 °C for 3 h under N_2 at which time analytical HPLC indicated complete consumption of starting material. The solvent was removed in vacuo and the solid purified by C18 and C8 flash (MeOH, H_2O–TFA (0.05%)) and Sephadex LH-20 (MeOH (0.05% TFA)) chromatography, yielding **5** (6.26 mg, 39% yield) and **6** (3.6 mg, 22% yield); discorhabdin B trifluoroacetate salt (**1**) (5.5 mg, 10.4 μmol) was dissolved in dry acetone (5.5 mL) to which methyl iodide (1.3 μL, 20.7 μmol) and K_2CO_3 (15 mg) were added. The reaction mixture was kept at reflux at 80 °C for 2 h under N_2 at which time the analytical HPLC indicated complete consumption of the starting material. The solvent was removed in vacuo and the solid purified by C18 and C8 flash (MeOH, H_2O–TFA (0.05%)) and Sephadex LH-20 (MeOH (0.05% TFA)) chromatography, yielding **6** (4.6 mg, 82% yield).
19. *Compound* ***5***. Dark green oil; $[\alpha]_D$ +222.2 (*c* 0.05, MeOH); ^{1}H NMR ($(CD_3)_2SO$, 600 MHz) δ 10.71 (1H, s, H-9), 8.85 (1H, s, H-18), 7.78 (1H, s, H-1), 7.42 (1H, s, H-14), 6.54 (1H, dd, J = 7.4, 4.8 Hz, H-8), 6.07 (1H, s, H-4), 4.71 (1H, d, J = 7.0 Hz, H-7), 3.88 (3H, s, H-22), 3.76 (2H, t, J = 7.9 Hz, H-17), 2.85 (2H, m, H-16), 2.42 (3H, s, H-23); ^{13}C NMR ($(CD_3)_2SO$, 150 MHz) δ 174.1 (C-3), 168.4 (C-5), 165.9 (C-11), 156.7 (C-19), 147.9 (C-1), 144.4 (C-10), 131.6 (C-14), 124.8 (C-8), 123.3 (C-2), 122.9 (C-12), 121.6 (C-21), 118.9 (C-15), 117.5 (C-4), 114.4 (C-7), 95.7 (C-20), 48.3 (C-6), 44.4 (C-17), 36.2 (C-22), 17.7 (C-16), 14.7 (C-23); HRFABMS (nitrobenzyl alcohol) *m/z* 442.02189 (calcd for $C_{20}H_{17}{}^{79}BrN_3O_2$ S, 442.02248), 444.01979 (calcd for $C_{20}H_{17}{}^{81}BrN_3O_2$ S, 444.02044).
Compound ***6***. Dark green oil; $[\alpha]_D$ +116.6 (*c* 0.05, MeOH); ^{1}H NMR (CD_3OD, 400 MHz) δ 7.78 (1H, s, H-1), 7.22 (1H, s, H-14), 6.54 (1H, d, J = 7.5 Hz, H-8), 6.15 (1H, s, H-4), 4.73 (1H, d, J = 7.5 Hz, H-7), 3.86 (2H, td, J = 8.2, 3.0 Hz, H-17), 2.94 (2H, td, J = 7.5, 3.0 Hz, H-16), 2.47 (3H, s, H-23); ^{13}C NMR (CD_3OD, 100 MHz) δ 176.2 (C-3), 171.8 (C-5), 166.8 (C-11), 160.4 (C-19), 150.0 (C-1), 146.5 (C-10), 127.5 (C-14), 125.7 (C-8), 125.7 (C-12), 124.8 (C-2), 123.3 (C-15), 121.1 (C-21), 118.4 (C-4), 115.9 (C-7), 97.3 (C-20), 50.2 (C-6), 46.0 (C-17), 19.3 (C-16), 15.2 (C-23); HRFABMS (nitrobenzyl alcohol) *m/z* 428.00735 (calcd for $C_{19}H_{15}{}^{79}BrN_3O_2$ S, 428.00683), 430.00607 (calcd for $C_{19}H_{15}{}^{81}BrN_3O_2$ S, 430.00479).
20. Compound **5** free base: dark brown oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.59 (1H, s, H-1), 6.64 (1H, s, H-14), 6.39 (1H, d, J = 7.6 Hz, H-8), 5.90 (1H, s, H-4), 4.11 (1H, d, J = 7.5 Hz, H-7), 3.95 (2H, m, H-17), 3.92 (3H, s, H-22), 2.60 (2H, t, J = 8.1 Hz, H-16), 2.31 (3H, s, H-23).
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22. Discorhabdin B trifluoroacetate salt (**1**) (8.5 mg, 16.1 μmol) was dissolved in dry acetone (2 mL) to which benzyl bromide (20 μL, 168.2 μmol) and K_2CO_3 (20 mg) were added. The reaction mixture was kept at reflux at 80° for 3 h under N_2 at which time the analytical HPLC indicated complete consumption of the starting material. The solvent was removed in vacuo and the solid purified by C18 flash (MeOH, H_2O–TFA (0.05%)) chromatography, yielding **7** (5.00 mg, 44% yield); *Compound 7*. TFA salt dark green oil; $[\alpha]_D$ −78.4° (*c* 0.05, MeOH); ^{1}H NMR (CD_3OD, 600 MHz) δ 7.72 (1H, s, H-1), 7.34 (1H, s, H-14), 7.26–7.40 (10H, m, aryl), 6.56 (1H, d, J = 7.5 Hz, H-8), 6.29 (1H, s, H-4), 5.53 (1H, d, J = 14.7 Hz, NCH_2Ph), 5.48 (1H, d, J = 14.7 Hz, NCH_2Ph), 4.72 (1H, d, J = 7.5 Hz, H-7), 4.25 (2H, s, SCH_2Ph), 3.75 (2H, m, H-17), 2.91 (2H, td, J = 7.7, 3.8 Hz H-16); ^{13}C NMR (CD_3OD, 150 MHz) δ 176.2 (C-3), 169.6 (C-5), 167.2 (C-11), 159.6 (C-19), 149.7 (C-1), 146.5 (C-10), 137.5 (aryl q), 136.4 (aryl q), 131.5 (C-14), 129.0–130.0 (aryl CH), 125.8 (C-8), 124.8 (C-2), 124.5 (C-12), 123.7 (C-21), 121.2 (C-15), 119.4 (C-4), 115.6 (C-7), 97.4 (C-20), 53.6 (NCH_2Ph), 50.1 (C-6), 45.7 (C-17), 37.1 (SCH_2Ph), 19.3 (C-16); HRFABMS (nitrobenzyl alcohol) *m/z* 594.08652 (calcd for $C_{32}H_{25}{}^{79}BrN_3O_2$ S, 594.08509), 596.08357 (calcd for $C_{32}H_{25}{}^{81}BrN_3O_2$ S, 596.08304).