Semi-synthesis of bioactive fluorescent analogues of the cytotoxic marine alkaloid discorhabdin C

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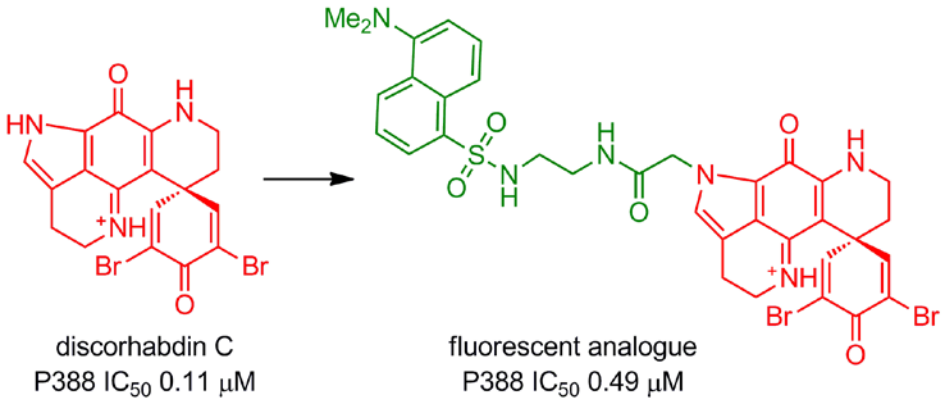
Keywords:

Marine natural product; discorhabdin; alkaloid; fluorescent probe; cytotoxicity

Graphical abstract



Abstract

Semi-synthetic *N*-13 alkylated analogues of the cytotoxic marine alkaloid discorhabdin C have been found to exhibit cytotoxicity towards tumour cell lines at comparable levels to that of the natural product. Incorporation of an ethylene diamine linker facilitated the synthesis of a variety of fluorophore-labelled probes, of which dansyl analogue **20** exhibited biological activity, providing a tool for mechanism of action and cellular localization studies. An alternative probe design was also exemplified, whereby a bioactive alkyne-terminated analogue (**24**) was found to undergo Huisgen 1,3-dipolar cycloaddition 'click' reactions with fluorescent azides, enabling studies directed towards activity-based protein profiling.

Keywords:

Discorhabdin C ; Pyrroloiminoquinone; Cytotoxic; Fluorophore; Semi-synthesis

1. Introduction

The discorhabdins, prianosins and epinardins are a series of pyrroloiminoquinone alkaloids isolated from marine sponges of the family Latrunculiidae Topsent, 1922.[1,2] Structural variation of the alkaloids typically occurs in the spiro di(enone) ring system, which can include the presence of a thia-linkage between C-5 and C-8 (e.g. discorhabdins A (**1**, Fig. 1)[3] and D (**2**)[4]), degrees of reduction or oxidation at positions C-1 through C-5 (e.g. discorhabdins X (**3**)[5] and Z (**4**)[6]), or ring closure between *N*-18 and C-2 (e.g. discorhabdins D, X and Z).

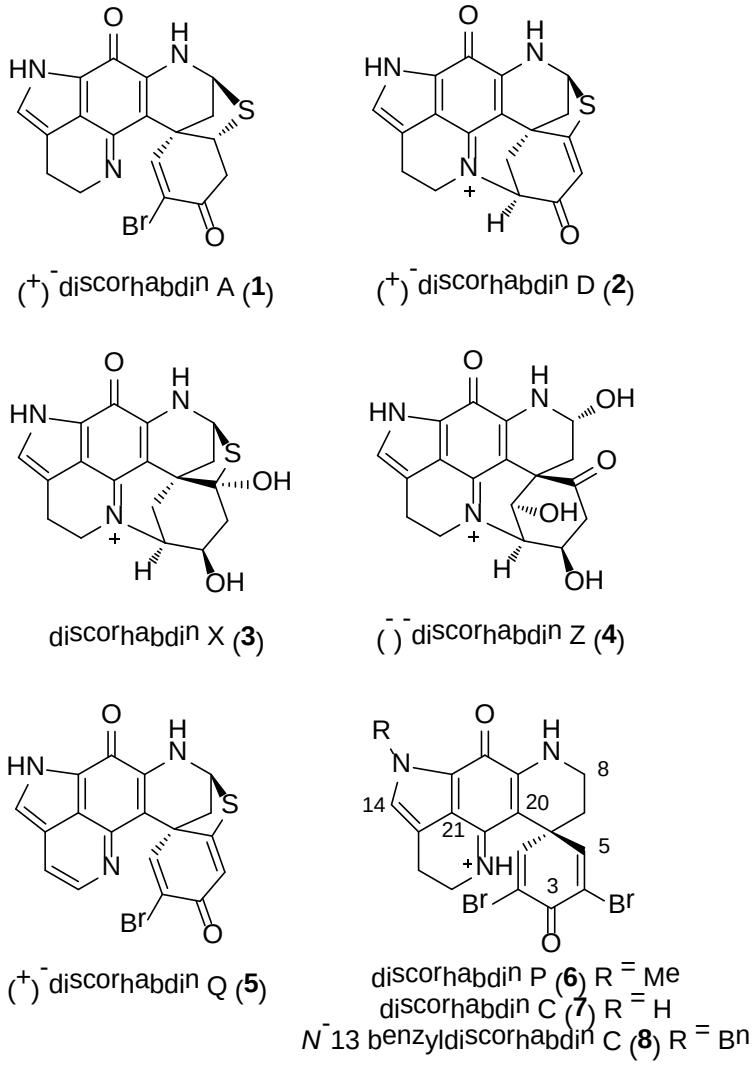


Fig. 1. Structures of natural products discorhabdins A, C, D, P, Q, X and Z and semi-synthetic benzyl analogue **8**.

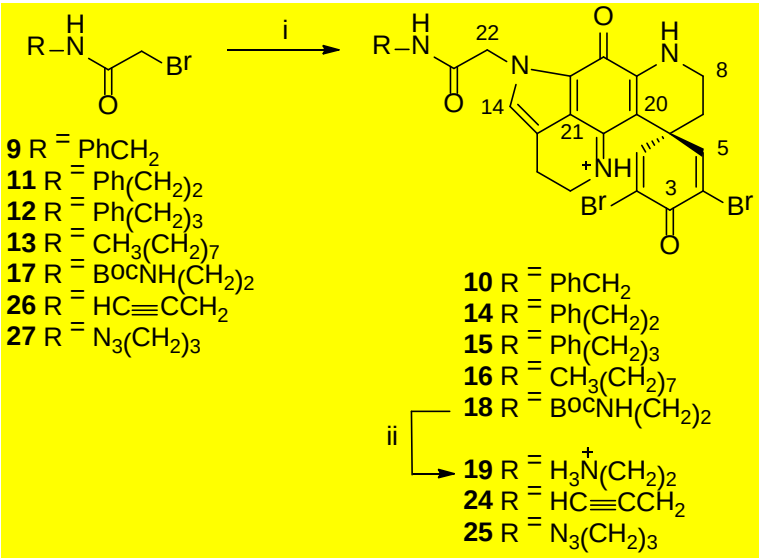
Several natural products in the series contain the $\Delta^{16(17)}$ dehydropyrroloiminoquinone moiety (e.g. discorhabdin Q (**5**)[7]) and four examples are known that contain substitution (methylation) at *N*-13 (e.g. discorhabdin P (**6**)[8]).

Due to their potent cytotoxicity, enzyme inhibitory and antimalarial properties, this class of alkaloid continues to attract attention.[9] A structure-cytotoxicity model of the discorhabdins has been proposed, with a requirement of both the iminoquinone core and the spiro (α-bromo) enone structure being essential, and that C-2–*N*-18 ring closure, substitution at C-1, and unsaturation at C-16(17) are detrimental to activity.[2,9] Notably, the presence of a methyl group at the pyrrole *N*-13 position is tolerated, with discorhabdin P (**6**) retaining much the same levels of cytotoxic potency of the demethyl analogue discorhabdin C (**7**).[10] We thus considered it of interest to explore the influence on cytotoxicity of substituents at *N*-13 of discorhabdin C that varied with lipophilicity and steric bulk. With no specific cellular target(s) of the discorhabdins having been identified,[2,9] we speculated that further modification at *N*-13 of **7** that incorporated fluorescent moieties could lead to analogues with utility in mechanism of action and sub-cellular localisation studies.[11] Herein we report the semi-synthesis and in vitro antitumour evaluation of such a library of analogues.

2. Results and discussion

We have previously reported that reaction of discorhabdin C (**7**) with excess MeI in acetone utilising K_2CO_3 as the base afforded the *N*-13 methyl analogue discorhabdin P (**6**) in good yield.[10] The reaction was found to work equally well using benzylbromide

as the alkylating agent giving *N*-13 benzyldiscorhabdin C (**8**, Fig. 1)[12] in 82% yield, suggesting that alkylation held scope for the preparation of our library of analogues. To increase the generality of substituents that could be appended to *N*-13, we next trialled the use of a 2-bromoacetamide linker subunit. Reaction of discorhabdin C with 2-bromo-*N*-benzylacetamide (**9**)[13] under our now standard reaction conditions afforded **10** in 69% yield (Scheme 1).



Scheme 1. Reagents and conditions: (i) discorhabdin C (**7**), K_2CO_3, acetone, 70 °C, 2 h., 23-85%; (ii) H_2O (0.05 %TFA), 70 °C, 1 h, 49%.

Selective attachment of the *N*-benzylacetamido moiety to the *N*-13 position of discorhabdin C was established by NMR, whereby HMBC correlations were observed between the H_2-22 methylene resonance (δ_H 5.06, 2H, s) of the linker to ^{13}C resonances at δ_C 122.7 (C-12), δ_C 132.4 (C-14) and δ_C 165.5 (C-23). Using similar methodology, reaction with 2-bromoacetamides **11**,[13] **12**[13] and **13**[14] gave discorhabdin C analogues **14** (in 47% yield), **15** (42%) and **16** (48%) respectively. Preliminary evaluation of the cytotoxic properties of *N*-13-benzyl (**8**), and amide-linked analogues **10**, **14**, **15** and **16** towards the P388 murine leukaemia cell line (Table 1) identified that the presence of such bulky and lipophilic substituents had little effect on

biological activity, with all five being essentially equi-potent to discorhabdins C (**7**) and P (**6**).

Table 1

In vitro anti-tumour activities (μM) of discorhabdin C and analogues

Compound (NSC)[a]	**P388 IC_{50}**[b]	**GI_{50}**[c]	**TGI**	**LC_{50}**
6 (740265)	0.31[d]	0.47	2.1	12.5
7 (626162)	0.11[d]	0.11	0.32	2.0
8 (740558)	0.31	0.14	0.5	2.9
10	0.21	nt[e]		
14 (742956)	0.15	0.27	1.0	4.2
15	0.23	nt		
16 (750320)	0.12	0.47	1.5	7.0
18 (743581)	0.47	1.3	4.0	9.7
19 (743580)	>7.9	inactive at single dose.[f]		
20 (743578)	0.49	0.83	2.6	9.0
21	>24	nt		
22	>21	nt		
23	>6.1	nt		
24 (747185)	0.34	0.64	1.9	6.0
25	1.30	nt		

[a] NSC number is the NCI reference number for each compound. Search for this number at http://dtp.cancer.gov to view complete information of all in vitro assay profiles.

[b] IC_{50} against the P388 D1 murine leukaemia cell line. Positive control was mitomycin C (IC_{50} 0.5 μM).

[c] GI_{50} (50% growth inhibition), TGI (total growth inhibition) and LC_{50} (50% cell kill) data are averaged calculated mean micro-molar values obtained from two experiments at the NCI.

[d] Data taken from Ref. 10.

[e] not tested.

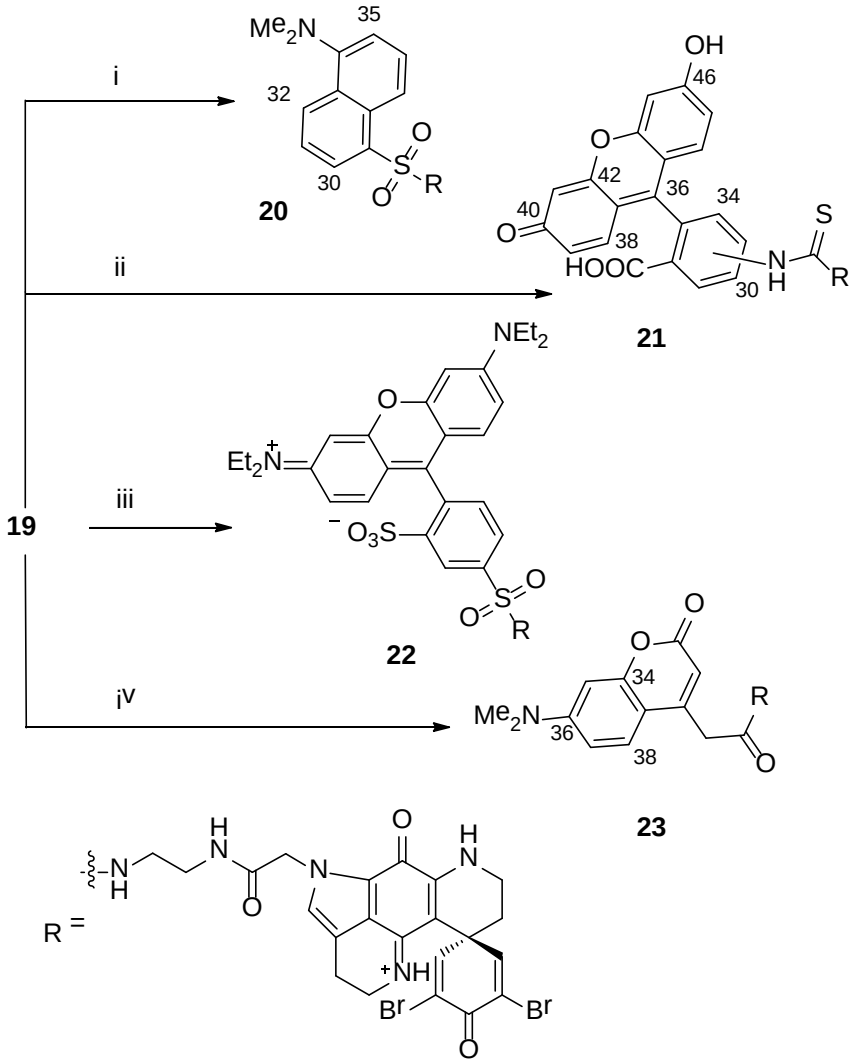
[f] Considered inactive in single dose (100 μM) testing at NCI.

Analogues **6**, **8**, **14** and **16** were also evaluated for cytotoxicity towards a panel of human tumour cell lines as part of the Developmental Therapeutics Program of the

National Cancer Institute,[15] and in all cases were found to exhibit panel average GI_{50}, TGI and LC_{50} values similar to those observed for discorhabdins C (**7**).

With this promising result, we sought to introduce a linker that would facilitate further attachment of a variety of fluorophores. To this end, we reasoned that a linker comprised of a *tert*-butoxycarbonyl protected ethylenediamine subunit would be suitable, as deprotection to the corresponding primary amine would provide an attachment point for fluorophores containing amine-reactive functionality. *tert*-Butyl 2-(2-bromoacetamido)ethylcarbamate (**17**)[16] was prepared in 91% yield by reaction of commercially available *tert*-butyl 2-aminoethylcarbamate with bromoacetylbromide. Subsequent reaction with discorhabdin C (**7**) yielded carbamate **18** (85%), which upon stirring in aqueous acid solution afforded amine **19** as the bis-TFA salt (49%). Interestingly, while carbamate **18** displayed potent cytotoxicity towards the P388 murine leukaemia cell line, the more polar amine analogue **19** was inactive (Table 1). The fluorophores selected for attachment to **19** were dansyl, fluorescein, lissamine rhodamine and 7-dimethylaminocoumarin-4-acetic acid, all of which have found application in cellular localization and mechanism of action studies.[11,17] Thus reaction of amine **19** with dansylchloride yielded the fluorescent sulfonamide **20**, with fluorescein-5(6)-isothiocyanate (5(6)-FITC) yielding analogue **21** as a mixture of regioisomers, or with lissamine rhodamine B sulfonyl chloride affording sulfonamide **22** (Scheme 2). Attachment of the 7-dimethylaminocoumarin-4-acetic acid (DACA) fluorophore proceeded by synthesis of the carboxylic acid using a literature route[18] followed by conversion into the activated *N*-hydroxysuccinimde ester[19] and finally reaction with amine **19** to yield fluorescent amide **23**. Biological evaluation of fluorescent analogues **20 - 23** (Table 1) revealed that only the dansylated discorhabdin **20** retained the same degree of cytotoxicity exhibited by the unfunctionalised natural

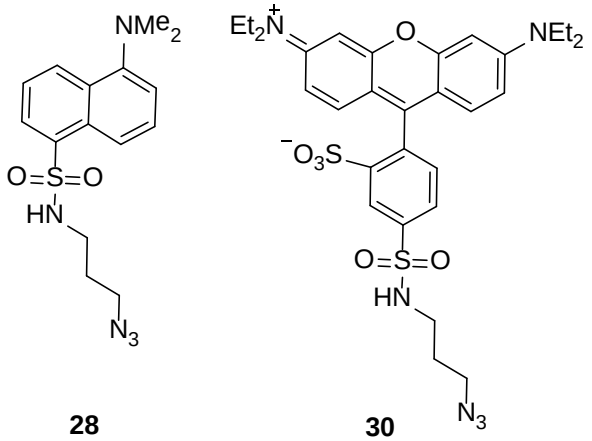
product **7**. While it could be speculated that the highly polar derivatives **21** and **22** may lack adequate cell penetration properties, leading to reduced cytotoxic potency, it is not obvious why the DACA analogue **23** lacked activity compared with dansyl labelled **20**.



Scheme 2. Reagents and conditions: (i) 5-(Dimethylamino) naphthalene-1-sulfonyl chloride, K_2CO_3, DMF, r.t., 1.5 h, 60%; ii) 5(6)-fluorescein-isothiocyanate, K_2CO_3, acetone, r.t., 1.5 h, 20%; iii) lissamine rhodamine sulfonyl chloride, K_2CO_3, DMF, 60 °C, 1.5 h, 27%; iv) NHS-activated 7-dimethylaminocoumarin-4-acetic acid, K_2CO_3, acetone, r.t., 1.5 h, 55%.

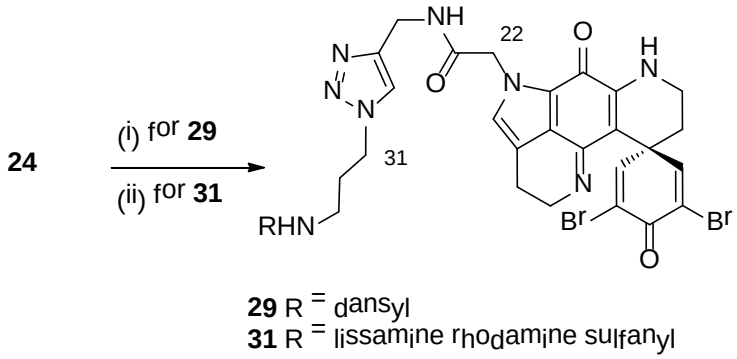
An alternative fluorescence-based method to identify cellular targets of small molecules is to introduce an alkyne or azide moiety as a 'latent secondary handle'[20]

which can be subsequently converted into a fluorescent tag via Huisgen 1,3-dipolar cycloaddition 'click' chemistry. This method is particularly suited to the investigation of cellular targets of covalently reactive small molecules, and has found widespread use in activity-based protein profiling (ABPP).[21] As the spiro (di)enone of the discorhabdin alkaloids is a necessary requirement for potent cytotoxicity,[1,2,9] we consider it likely that some examples of the alkaloids are acting as Michael acceptors and reacting irreversibly with (a) cellular target(s). Suitable alkyne or azide functionalized analogues of discorhabdin C (**24**, **25**) were prepared by reaction of discorhabdin C with either 2-bromo-*N*-propargylacetamide (**26**)[22] or with 2-bromo-*N*-(3-azidopropyl)-acetamide (**27**)[23,24] respectively (Scheme 1). Of the two products, alkyne **24** retained cytotoxic activity comparable to the natural product **7** (Table 1) making it of potential use in ABBP studies. The ability of **24** to undergo Huisgen 1,3-dipolar cycloaddition with suitable azide-linked fluorophores was confirmed by copper (I) mediated reaction with dansyl azide **28** (Fig. 2),[25] which afforded **29** in 58% yield, and with lissamine azide **30**,[26] yielding **31** (51%) (Scheme 3).



28 **30**

Fig. 2. Azide functionalised fluorophores **28** and **30**.



Scheme 3. Reagents and conditions: (i) *N*-(3-azidopropyl)-5-(dimethylamino)-1-naphthalenesulfonamide (**28**), CuI, DIPEA, CH_2Cl_2, r.t., 30 min., 58%; ii) *N*-(3-azidopropyl)-lissamine rhodamine sulphonamide (**30**), CuI, DIPEA, MeCN, r.t., 30 min., 51%.

3. Conclusion

We have developed a general synthetic route that allows for the selective derivatisation of the *N*-13 position of the cytotoxic marine alkaloid discorhabdin C. The versatility of the 2-bromo-*N*-substituted acetamide linker group used in this study was demonstrated by the successful preparation of fluorophore and alkyne tagged analogues that retain the sub-micromolar cytotoxic potency of the original natural product. The methodology should allow ready conversion of pyrroloiminoquinone natural products, whether they be discorhabdins or the structurally related makaluavmines,[1,2] into labelled probes suitable for biological investigation. Both dansyl analogue **20** and alkyne **24** should prove useful in enabling studies of the sub-cellular localisation and protein targets of the discorhabdin family of biologically active marine natural products.

4. Experimental

4.1. General Experimental Procedures. Infrared spectra were run as dry films on an ATR crystal and acquired with a Perkin Elmer Spectrum One Fourier Transform infrared spectrometer with a Universal ATR Sampling Accessory. NMR spectra were recorded on either a Bruker Avance DRX-600 spectrometer operating at 600 MHz for ^{1}H nuclei and 150 MHz for ^{13}C nuclei, a Bruker Avance DRX-400 spectrometer operating at 400 MHz for ^{1}H nuclei and 100 MHz for ^{13}C nuclei or a Bruker Avance DRX-300 spectrometer operating at 300 MHz for ^{1}H nuclei and 75 MHz for ^{13}C nuclei. Proto-deutero solvent signals were used as internal references (DMSO-d_6: δ_H 2.50, δ_C 39.43; CD_3OD: δ_H 3.30, δ_C 49.05; $CDCl_3$: δ_H 7.25, δ_C 77.0). Standard Bruker pulse sequences were utilized. MS data were acquired on either a VG-7070 or a Bruker micrOTOF Q II mass spectrometer. Flash column chromatography was performed using reversed-phase Merck Lichroprep RP-18, cyanopropyl, or phenyl solid supports, or with Davisil silica gel, or by size exclusion chromatography on Pharmacia Biotech Sephadex LH-20. Analytical reversed-phase HPLC was run on either a Waters 600 HPLC photodiode array system using either an Alltech C_8 column (3 μm Econosphere Rocket, 33 × 7 mm) (System A) or an Alltech C_{18} column (3 μm Econosphere Rocket, 33 × 7 mm) (System B) or a Dionex UltiMate 3000RS using a Grace C_8 column (3 μm Platinum, 33 × 7 mm) (System C) and eluting with a linear gradient of H_2O (0.05% TFA) to MeCN over 13.5 min at 2 mL/min and monitoring at 330 nm. Bromoacetamides **9**,[13] **11**,[13] **12**,[13] **13**,[14] **17**[16] and **26**[22] and fluorescent reagents *N*-hydroxysuccinimidyl-7-dimethylamino-4-coumarinacetate,[18,19] dansyl azide **28**,[25] and lissamine azide **30**[26] were prepared by published routes and data obtained were in agreement with those previously reported. The synthesis of bromoacetamide **27** has been previously reported[24] but without experimental details.

*4.1.1. Discorhabdin C (**7**).* Discorhabdin C was isolated from specimens of *Latrunculia (Latrunculia) trivetricillata* (MNP 6116) collected from the Three Kings Islands, New Zealand using protocols previously reported.[27] Spectroscopic data recorded for the alkaloid were identical to those reported previously[28] and sample purity used for subsequent semi-synthetic preparations was judged >98% by analytical HPLC-DAD and NMR.

*4.1.2. Discorhabdin P (**6**).* Discorhabdin C trifluoroacetate salt (**7**) (10.2 mg, 17.7 µmol) was dissolved in dry acetone (1 mL) along with excess K_2CO_3 (30.0 mg, 0.217 mmol) and CH_3I (30 µL, 92.7 µmol), turning the solution from red/purple to orange. The mixture was heated to reflux and left to stir for 1 h. The crude mixture was loaded directly onto a reversed-phase C_{18} flash chromatography column and washed with six column volumes of water (0.05% TFA). Elution with a gradient solvent mixture of 0-30% MeOH (0.05% TFA) yielded a single red/purple band. The solution was dried in vacuo to yield **6** (10.0 mg, 96%) as a red/purple non-crystalline trifluoroacetate salt. R_T 6.41 min (System A); ^{1}H and ^{13}C NMR and HRMS data matched those previously reported.[10]

*4.1.3. N-13 Benzyldiscorhabdin C (**8**).*[12] Discorhabdin C trifluoroacetate salt (**7**) (5.0 mg, 8.7 µmol) was dissolved in dry acetone (2 mL) to which were added benzylbromide (20 µL, 168 µmol) and K_2CO_3 (4.6 mg, 0.033 mmol). The mixture was kept at reflux for 4 h after which time the solvent was removed in vacuo and the crude product purified by C_{18} flash column chromatography (methanol/water/0.05% trifluoroacetic acid) to afford **8** (4.7 mg, 82%). R_T 7.3 min (System A); ν_{max} (ATR) 3343, 1676, 1584, 1535, 1438, 1329, 1188, 1133 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.15 (1H, br s, NH-9), 8.19 (1H, br s, NH-18), 7.70 (2H, s, H-1/5), 7.65 (1H, s, H-14), 7.37-7.31 (5H, m, benzyl), 5.49 (2H, s, H_2-22), 3.66 (2H, td, *J* =

7.5, 2.8 Hz, H_2-17), 3.62-3.57 (2H, br m, H_2-8), 2.82 (2H, t, *J* = 7.5 Hz, H_2-16), 1.99 (2H, t, *J* = 5.4 Hz, H_2-7); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 171.3 (C-3), 165.6 (C-11), 152.5 (C-19), 151.5 (C-10), 151.1 (C-1/C-5), 136.7 (C-23), 131.4 (C-14), 128.7 (C-25/27), 128.1 (C-26), 127.5 (C-24/28), 124.0 (C-21), 122.6 (C-2/C-4), 121.8 (C-12), 119.6 (C-15), 92.0 (C-20), 51.7 (C-22), 44.6 (C-6), 43.4 (C-17), 38.3 (C-8), 33.5 (C-7), 17.9 (C-16); HRFABMS (nitrobenzylalcohol) *m/z* $[M+H]^+$ 551.9912 (calcd for $C_{25}H_{20}{}^{79}Br_2N_3O_2$ 551.9922), 553.9888 (calcd for $C_{25}H_{20}{}^{79}Br^{81}BrN_3O_2$ 553.9902), 555.9893 (calcd for $C_{25}H_{20}{}^{81}Br_2N_3O_2$ 555.9881).

*4.1.4. N-13-Benzylacetamido discorhabdin C (**10**).* Discorhabdin C trifluoroacetate salt (**7**) (7.0 mg, 12 µmol) was dissolved in dry acetone (0.5 mL), followed by addition of dry K_2CO_3 (9.9 mg, 72.4 µmol) and 2-bromo-*N*-benzylacetamide (**9**)[13] (29.9 mg, 0.132 mmol), and the mixture was purged under N_2. The mixture was heated with stirring at 70 °C for 2 h., after which the crude product was subjected to reversed-phase C_{18} flash column chromatography (0-50% MeOH (0.05% TFA)), and reversed-phase CN flash column chromatography (0-10% MeOH (0.05% TFA)) to yield **10** as a purple non-crystalline trifluoroacetate salt (6.0 mg, 69%). R_T 6.31 min (System B); ν_{max} (ATR) 3276, 1676, 1587, 1541, 1325, 1200 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.12 (1H, br s, NH-9), 8.80 (1H, t, *J* = 5.8 Hz, NH-24), 8.23 (1H, br s, NH-18), 7.74 (2H, s, H-1/H-5), 7.40 (1H, s, H-14), 7.37-7.24 (5H, m, 2H-27/2H-28/H-29), 5.06 (2H, s, H_2-22), 4.32 (2H, d, *J* = 5.8 Hz, H_2-25), 3.69 (4H, obscured by water, H_2-8/H_2-17), 2.83 (2H, t, *J* = 7.2 Hz, H_2-16), 2.02 (2H, br t, *J* = 4.8 Hz, H_2-7); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 171.3 (C-3), 165.7 (C-11 or C-23), 165.5 (C-23 or C-11), 152.6 (C-19), 151.5 (C-10), 151.1 (C-1/C-5), 138.7 (C-26), 132.4 (C-14), 128.3 (C-28/C-30), 127.2 (C-27/C-31), 126.9 (C-29), 123.5 (C-21), 122.7 (C-12), 122.6 (C-2/C-4), 118.8 (C-15), 91.9 (C-20), 50.8 (C-22), 44.6 (C-6), 43.5 (C-17), 42.3 (C-25), 38.3 (C-8), 33.5 (C-7), 17.9 (C-16); (+)-ESIMS *m/z* 609 $[M+H]^+$; (+)-HRESIMS *m/z* $[M+H]^+$

609.0115 (calcd. for $C_{27}H_{23}{}^{79}Br_2N_4O_3$, 609.0131), 611.0106 (calcd. for $C_{27}H_{23}{}^{79}Br^{81}BrN_4O_3$, 611.0113), 613.0088 (calcd. for $C_{27}H_{23}{}^{81}Br_2N_4O_3$, 613.0092).

*4.1.5. N-13-Phenethylacetamido discorhabdin C (**14**).* Discorhabdin C trifluoroacetate salt (**7**) (10.8 mg, 23.4 µmol) was dissolved in dry acetone (2 mL), followed by addition of dry K_2CO_3 (29.0 mg, 0.21 mmol) and 2-bromo-*N*-phenethylacetamide (**11**)[13] (138 mg, 0.57 mmol), and the mixture was purged under N_2. The mixture was heated with stirring at 70 °C for 2 h., after which the crude reaction mixture was purified by reversed-phase C_{18} flash column chromatography (0-50% MeOH (0.05% TFA)), and three successive steps of Sephadex LH-20 column chromatography (MeOH (0.05% TFA)) to yield **14** as a purple non-crystalline trifluoroacetate salt (5.1 mg, 47%). R_T 7.45 min (System B); ν_{max} (ATR) 3271, 1676, 1587, 1541, 1325, 1200 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 10.09 (1H, br s, NH-9), 8.39 (1H, t, *J* = 5.2 Hz, NH-24), 8.22 (1H, br s, NH-18), 7.73 (2H, s, H-1/H-5), 7.36 (1H, s, H-14), 7.35-7.20 (5H, m, 2H-28/2H-29/H-30), 4.96 (2H, s, H_2-22), 3.67 (2H, td, *J* = 7.6, 4.7 Hz, H_2-17), 3.62 (2H, br s, H_2-8), 3.31 (2H, td, *J* = 7.2, 5.2 Hz, H_2-25), 2.83 (2H, t, *J* = 7.6 Hz, H_2-16), 2.73 (2H, t, *J* = 7.2 Hz, H_2-26), 2.02 (2H, t, *J* = 5.6 Hz, H_2-7); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 171.3 (C-3), 165.5 (C-11/C-23), 152.6 (C-19), 151.4 (C-10), 151.1 (C-1/C-5), 139.1 (C-27), 132.3 (C-14), 128.6 (C-28 or C-29), 128.3 (C-29 or C-28), 126.1 (C-30), 123.4 (C-21), 122.6 (C-2/C-4/C-12), 118.7 (C-15), 91.9 (C-20), 50.8 (C-22), 44.6 (C-6), 43.5 (C-17), 40.5 (C-26), 38.2 (C-8), 35.0 (C-25), 33.5 (C-7), 17.9 (C-16); (+)-ESIMS *m/z* 623 $[M+H]^+$; (+)-HRESIMS *m/z* $[M+H]^+$ 623.0270 (calcd. for $C_{28}H_{25}{}^{79}Br_2N_4O_3$, 623.0288), 625.0255 (calcd. for $C_{28}H_{25}{}^{79}Br^{81}BrN_4O_3$, 625.0270), 627.0248 (calcd. for $C_{28}H_{25}{}^{81}Br_2N_4O_3$, 627.0249).

*4.1.6. N-13-Phenpropylacetamido discorhabdin C (**15**).* Discorhabdin C trifluoroacetate salt (**7**) (11.0 mg, 19 μmol) was dissolved in dry acetone (2 mL), followed by addition of dry K_2CO_3 (20.0 mg, 0.145 mmol) and 2-bromo-*N*-(3-phenyl-propyl)-acetamide (**12**)[13] (39.9 mg, 0.156 mmol), and the mixture was purged under N_2. The mixture was heated with stirring at 70 °C for 2 h., after which the crude product was purified by reversed-phase C_{18} flash column chromatography (0-50% MeOH (0.05% TFA)), and reversed-phase CN flash column chromatography (0-10% MeOH (0.05% TFA)) to yield **15** as a purple non-crystalline trifluoroacetate salt (6.0 mg, 42%). R_T 6.76 min (System C); ν_{max} (ATR) 3289, 1676, 1587, 1538, 1325, 1199 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 10.08 (1H, br s, NH-9), 8.34 (1H, t, *J* = 5.4 Hz, NH-24), 8.21 (1H, br s, NH-18), 7.73 (2H, s, H-1/H-5), 7.37 (1H, s, H-14), 7.31-7.15 (5H, m, 2H-29/2H-30/H-31), 4.98 (2H, s, H_2-22), 3.71 (4H, obscured by water, H_2-8/H_2-17), 3.09 (2H, td, *J* = 7.1, 5.4 Hz, H_2-25), 2.82 (2H, t, *J* = 7.5 Hz, H_2-16), 2.60 (2H, t, *J* = 7.1 Hz, H_2-27), 2.02 (2H, m, H_2-7), 1.72 (2H, p, *J* = 7.1 Hz, H_2-26); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 171.3 (C-3), 165.5 (C-11/C-23), 152.6 (C-19), 151.4 (C-10), 151.1 (C-1/C-5), 141.5 (C-28), 132.3 (C-14), 128.2 (C-29/C-30), 125.7 (C-31), 123.4 (C-21), 122.6 (C-2/C-4/C-12), 118.7 (C-15), 91.9 (C-20), 50.9 (C-22), 44.6 (C-6), 43.5 (C-17), 38.6 (C-25), 38.3 (C-8), 33.5 (C-7), 32.3 (C-27), 30.8 (C-26), 17.9 (C-16); (+)-ESIMS *m/z* 637 $[M+H]^+$; (+)-HRESIMS *m/z* $[M+H]^+$ 637.0426 (calcd. for $C_{29}H_{27}{}^{79}Br_2N_4O_3$, 637.0444), 639.0409 (calcd. for $C_{29}H_{27}{}^{79}Br^{81}BrN_4O_3$, 639.0426), 641.0395 (calcd. for $C_{29}H_{27}{}^{81}Br_2N_4O_3$, 641.0405).

*4.1.7. N-13-Octylacetamido discorhabdin C (**16**).* Discorhabdin C trifluoroacetate salt (**7**) (8.0 mg, 14 μmol) was dissolved in dry acetone (2 mL), followed by addition of dry K_2CO_3 (20.0 mg, 0.145 mmol) and 2-bromo-*N*-octylacetamide (**13**)[14] (64.0 mg, 0.256 mmol), and the mixture was purged under N_2. The mixture was heated with stirring at 70 °C for 2 h.,

after which the crude product was purified by a combination of reversed-phase C_{18} and phenyl flash column chromatography (0-50% MeOH (0.05% TFA)) to yield **16** as a purple non-crystalline trifluoroacetate salt (5.0 mg, 48%). R_T 7.34 min (System C); ν_{max} (ATR) 3247, 2927, 1673, 1585, 1537, 1323 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 10.08 (1H, br s, NH-9), 8.24 (2H, m, NH-18/NH-24), 7.73 (2H, s, H-1/H-5), 7.36 (1H, s, H-14), 4.95 (2H, s, H_2-22), 3.68 (2H, t, *J* = 7.5 Hz, H_2-17), 3.61 (2H, br s, H_2-8), 3.07 (2H, dt, *J* = 6.9, 6.9 Hz, H_2-25), 2.82 (2H, t, *J* = 7.5 Hz, H_2-16), 2.01 (2H, t, *J* = 7.5 Hz, H_2-7), 1.41 (2H, br s, H_2-26), 1.25 (10H, br s, H_2-27/H_2-28/H_2-29/H_2-30/H_2-31), 0.88 (3H, br t, *J* = 6.9 Hz, H_3-32); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 171.3 (C-3), 165.4 (C-11/C-23), 152.6 (C-19), 151.4 (C-10), 151.1 (C-1/C-5), 132.3 (C-14), 123.4 (C-21), 122.6 (C-2/C-4/C-12), 118.7 (C-15), 91.9 (C-20), 50.8 (C-22), 44.6 (C-6), 43.5 (C-17), 38.7 (C-25), 38.2 (C-8), 33.5 (C-7), 31.2 (C-27), 28.9 (C-26), 28.6 (C-28/C-29), 26.3 (C-30), 22.0 (C-31), 17.9 (C-16), 13.9 (C-32); (+)-ESIMS *m/z* 631 $[M+H]^+$; (+)-HRESIMS *m/z* $[M+H]^+$ 631.0921 (calcd. for $C_{28}H_{33}{}^{79}Br_2N_4O_3$, 631.0914), 633.0903 (calcd. for $C_{28}H_{33}{}^{79}Br^{81}BrN_4O_3$, 633.0896), 635.0883 (calcd. for $C_{28}H_{33}{}^{81}Br_2N_4O_3$, 635.0875).

*4.1.8. N-13- (tert-Butyl 2-acetamido ethylcarbamate) discorhabdin C (**18**).* Discorhabdin C trifluoroacetate salt (**7**) (10.2 mg, 21.9 µmol) was dissolved in dry acetone (1 mL). Dry K_2CO_3 (41.0 mg, 0.297 mmol) was added, turning the mixture colour from dark purple to orange. *tert*-Butyl 2-(2-bromoacetamido)ethylcarbamate (**17**)[16] (147 mg, 0.52 mmol) was added, and heated under N_2 at 70 °C for 1 h. The crude product was purified by reversed-phase C_{18} flash column chromatography (0-40% MeOH (0.05% TFA)) yielding **18** as a non-crystalline red/purple trifluoroacetate salt (12.4 mg, 85%). R_T 6.43 min (System B); ν_{max} (ATR) 3272, 1676, 1587, 1539, 1325, 1200, 1174 cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz) δ 10.12 (1H, br s, NH-9), 8.37-8.33 (1H, br m, NH-24), 8.22 (1H, br s, NH-18), 7.73 (2H, s, H-

1/H-5), 7.36 (1H, s, H-14), 6.85 (1H, t, *J* = 5.6 Hz, NH-27), 4.95 (2H, s, H_2-22), 3.67 (2H, td, *J* = 7.5, 2.7 Hz, H_2-17), 3.63-3.59 (2H, br m, H_2-8), 3.10 (2H, dt, *J* = 6.2, 6.2 Hz, H_2-25), 2.99 (2H, dt, *J* = 6.2, 6.2 Hz, H_2-26), 2.82 (2H, t, *J* = 7.5 Hz, H_2-16), 2.01 (2H, t, *J* = 5.2 Hz, H_2-7), 1.38 (9H, s, 3 × H_3-31); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 171.4 (C-3), 165.8 (C-23), 165.5 (C-11), 155.6 (C-28), 152.6 (C-19), 151.5 (C-10), 151.1 (C-1/C-5), 132.3 (C-14), 123.5 (C-21), 122.7 (C-12/C-2/C-4), 118.8 (C-15), 91.9 (C-20), 77.8 (C-30), 50.9 (C-22), 44.7 (C-6), 43.5 (C-17), 39.4 (C-26), 38.9 (C-25), 38.3 (C-8), 33.5 (C-7), 28.2 (3 × C-31), 17.9 (C-16); HRFABMS *m/z* $[M+H]^+$ 662.0625 (calcd for $C_{27}H_{30}{}^{79}Br_2N_5O_5$, 662.0614), 664.0604 (calcd for $C_{27}H_{30}{}^{79}Br^{81}BrN_5O_5$, 664.0593), 666.0599 (calcd for $C_{27}H_{30}{}^{81}Br_2N_5O_5$, 666.0573).

*4.1.9. N-13 (2-Aminoethyl acetamido) discorhabdin C (**19**).* *N*-13-(*tert*-Butyl 2-acetamido ethylcarbamate) discorhabdin C trifluoroacetate salt (**18**) (8.9 mg, 11.4 µmol) was dissolved in aqueous HCl (2 mL, 1 M) and heated at 80 °C for 30 min. The crude product was purified by reversed-phase C_{18} flash column chromatography (0-20% MeOH (0.05% TFA)), yielding **19** as a purple non-crystalline trifluoroacetate salt (8.1 mg, 49%). R_T 6.00 min (System B); ν_{max} (ATR) 3253, 1674, 1588, 1541, 1326, 1200 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.09 (1H, br s, NH-9), 8.56 (1H, t, *J* = 5.6 Hz, NH-24), 8.24 (1H, s, NH-18), 7.90 (3H, br s, NH_3-27), 7.72 (2H, s, H-1/H-5), 7.36 (1H, s, H-14), 4.98 (2H, s, H_2-22), 3.69 (2H, td, *J* = 7.6, 2.8 Hz, H_2-17), 3.65-3.59 (2H, br m, H_2-8), 3.34 (2H, dt, *J* = 6.0, 5.6 Hz, H_2-25), 2.89 (2H, dt, *J* = 6.0, 6.0 Hz, H_2-26), 2.83 (2H, t, *J* = 7.5 Hz, H_2-16), 2.00 (2H, t, *J* = 5.2 Hz, H_2-7); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 171.3 (C-3), 166.5 (C-11/C-23), 152.6 (C-19), 151.4 (C-10), 151.0 (C-1/C-5), 132.3 (C-14), 123.5 (C-21), 122.6 (C-2/C-4/C-12), 118.8 (C-15), 91.9 (C-20), 50.8 (C-22), 44.6 (C-6), 43.5 (C-17), 38.3 (C-8/C-26), 36.5 (C-25), 33.5 (C-7), 17.8 (C-16); HRFABMS *m/z* $[M+H]^+$ 562.0087 (calcd for $C_{22}H_{22}{}^{79}Br_2N_5O_3$, 562.0089),

564.0067 (calcd for $C_{22}H_{22}{}^{79}Br^{81}BrN_5O_3$, 564.0069), 566.0068 (calcd for $C_{22}H_{22}{}^{81}Br_2N_5O_3$, 566.0048).

*4.1.10. N-13 (Dansyl 2-aminoethyl acetamido) discorhabdin C (**20**).* *N*-13 2-Aminoethyl acetamide discorhabdin C trifluoroacetate salt (**19**) (6.8 mg, 12 μmol) was dissolved in dry dimethylformamide (0.5 mL). Excess dry potassium carbonate was added, turning the alkaloid from dark purple (salt) to orange (free base). 5-(Dimethylamino)naphthalene-1-sulfonyl chloride (dansyl chloride) (11.0 mg, 41 μmol) was added and the reaction stirred under nitrogen at room temperature for 1.5 h. Analytical HPLC indicated incomplete reaction, and so additional dansyl chloride was added (2.1 mg, 7.8 μmol) and the mixture stirred for a further 30 min. The crude product was purified by C_{18} flash column chromatography (0-30% MeOH (0.05% TFA)) to afford **20** (5.7 mg, 60%). R_T 7.37 min (System B); ν_{max} (ATR) 3280, 1675, 1587, 1540, 1324, 1200, 1142 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 10.07 (1H, br s, NH-9), 8.48 (1H, br d, *J* = 8.6 Hz, H-32), 8.31 (1H, t, *J* = 5.6 Hz, NH-24), 8.27 (1H, d, *J* = 8.6 Hz, H-37), 8.21 (1H, br s, NH-18), 8.10 (1H, dd, *J* = 7.4, 1.2 Hz, H-30), 8.08 (1H, t, *J* = 6.0 Hz, H-27), 7.71 (2H, s, H-1/H-5), 7.64 (1H, dd, *J* = 8.6, 7.4 Hz, H-31), 7.61 (1H, dd, *J* = 8.6, 7.4 Hz, H-36), 7.31 (1H, s, H-14), 7.26 (1H, d, *J* = 7.4 Hz, H-35), 4.89 (2H, s, H_2-22), 3.66 (2H, td, *J* = 7.6, 2.8 Hz, H_2-17), 3.62-3.57 (2H, br m, H_2-8), 3.09 (2H, dt, *J* = 6.4, 5.6 Hz, H_2-25), 2.84 (6H, s, $2H_3$-40), 2.83-2.79 (4H, m, H_2-16/H_2-26), 2.00 (2H, t, *J* = 5.3 Hz, H_2-7); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 171.3 (C-3), 165.8 (C-23), 165.5 (C-11), 152.6 (C-19), 151.4 (C-10), 151.0 (C-1/C-5/C-34), 135.7 (C-29), 132.2 (C-14), 129.4 (C-32), 128.9 (C-33/C-38), 128.2 (C-30), 127.9 (C-36), 123.6 (C-31), 123.5 (C-21), 122.6 (C-2/C-4/C-12), 119.0 (C-37), 118.8 (C-15), 115.2 (C-35), 91.9 (C-20), 50.8 (C-22), 45.0 (C-40/C-41), 44.6 (C-6), 43.5 (C-17), 41.5 (C-26), 39.5 (C-25), 38.2 (C-8), 33.5 (C-7), 17.8 (C-16); HRFABMS *m/z* $[M+H]^+$ 795.0598 (calcd for $C_{34}H_{33}{}^{79}Br_2N_6O_5S$,

795.0600), 797.0586 (calcd for $C_{34}H_{33}{}^{79}Br^{81}BrN_6O_5S$, 797.0579), 799.0585 (calcd for $C_{34}H_{33}{}^{81}Br_2N_6O_5S$, 799.0559).

*4.1.11. N-13 (Fluorescein 2-aminoethyl acetamido) discorhabdin C (**21**).* *N*-13 (2-Aminoethyl acetamido) discorhabdin C trifluoroacetate salt (**19**) (14.0 mg, 17.7 µmol) was dissolved in dry acetone (0.5 mL). Excess dry K_2CO_3 was added, turning the mixture colour from dark purple to orange. Fluorescein 5(6)-isothiocyanate (15.0 mg, 38.5 µmol) was added, and stirred under N_2 at room temperature for 1.5 h. The product was purified by reversed-phase C_{18} flash column chromatography (0-80% MeOH (0.05% TFA)), yielding **21** as a brown/purple non-crystalline trifluoroacetate salt (3.8 mg, 20%). R_T 6.61 min (System B); ν_{max} (ATR) 3250, 1677, 1544, 1202, 1133 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.16 (1H, br s, H-29), 10.10 (1H, br s, NH-9), 8.49 (1H, t, *J* = 6.0 Hz, NH-24), 8.23-8.16 (3H, m, NH-18/H-27/H-31), 7.73-7.71 (3H, m, H-1/H-5/H-35), 7.36 (1H, s, H-14), 7.20 (1H, d, *J* = 8.4 Hz, H-34), 6.68-6.54 (6H, m, H-38/H-39/H-41/H-45/H-47/H-48), 5.00 (2H, s, H_2-22), 3.65 (2H, td, *J* = 7.6, 2.8 Hz, H_2-17), 3.64 (2H, m, H_2-26), 3.61 (2H, br m, H_2-8), 3.36 (2H, dt, *J* = 6.0, 6.0 Hz, H_2-25), 2.80 (2H, t, *J* = 7.6 Hz, H_2-16), 2.00 (2H, br s, H_2-7) (COOH not observed); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 171.3 (C-3), 166.1 (C-23), 165.5 (C-11), 152.6 (C-19), 151.4 (C-10), 151.0 (C-1/C-5), 147.0 (C-33), 141.0 (C-30 or C-32), 132.3 (C-14), 129.8 (C-35), 128.5 (C-32 or C-30), 124.0 (C-34), 123.5 (C-21), 122.6 (C-2/C-4/C-12), 118.8 (C-15), 116.8 (C-31), 91.9 (C-20), 50.9 (C-22), 44.6 (C-6), 43.5 (C-17), 43.3 (C-26), 38.2 (C-8), 37.9 (C-25), 33.5 (C-7), 17.8 (C-16); ^{13}C NMR data only assigned up to C-35 as presence of fluorescein isomers hindered full assignment of fluorescein moiety; (+)-ESIMS *m/z* 951 $[M+H]^+$; (+)-HRESIMS *m/z* $[M+H]^+$ 951.0425 (calcd. for $C_{43}H_{33}{}^{79}Br_2N_6O_8S$, 951.0442), 953.0402 (calcd. for $C_{43}H_{33}{}^{79}Br^{81}BrN_6O_8S$, 953.0426), 955.0393 (calcd. for $C_{43}H_{33}{}^{81}Br_2N_6O_8S$, 955.0417).

*4.1.12. N-13 (Lissamine rhodamine 2-aminoethyl acetamido) discorhabdin C (**22**).* *N*-13 (2-Aminoethyl acetamido) discorhabdin C trifluoroacetate salt (**19**) (5.46 mg, 12.0 µmol) was dissolved in dry DMF (0.5 mL). Excess dry K_2CO_3 (15.0 mg, 0.109 mmol) was added, turning the mixture colour from dark purple to orange. Lissamine rhodamine sulfonyl chloride (5.9 mg, 10.2 µmol) was added, the mixture was heated to 60 °C and stirred under N_2 at room temperature for 1.5 h. The product was purified by reversed-phase C_{18} flash column chromatography (0-80% MeOH (0.05% TFA)), yielding **22** as a pink/purple non-crystalline trifluoroacetate salt (2.2 mg, 27%). R_T 7.49 min (System B); ν_{max} (ATR) 3270, 1678, 1591, 1541, 1326, 1200, 1182 cm^{-1}; ^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.12 (1H, br s, NH-9), 8.43 (1H, d, *J* = 2.8 Hz, H-30), 8.41 (1H, t, *J* = 6.0 Hz, NH-24), 8.22 (1H, br s, NH-18), 8.08 (1H, t, *J* = 6.0 Hz, H-27), 7.95 (1H, dd, *J* = 8.4, 2.8 Hz, H-34), 7.71 (2H, s, H-1/H-5), 7.51 (1H, d, *J* = 8.4 Hz, H-33), 7.39 (1H, s, H-14), 7.02 (2H, dd, *J* = 9.2, 2.2 Hz, H-42/H-47), 6.98 (2H, d, *J* = 9.2 Hz, H-41/H-48), 6.94 (2H, d, *J* = 2.2 Hz, H-44/H-45), 4.98 (2H, s, H_2-22), 3.66-3.61 (2H, m, H_2-17), 3.62 (8H, obscured by water, 4 × H_2-50), 3.61 (2H, br s, H_2-8), 3.22 (2H, dt, *J* = 6.0, 6.0 Hz, H_2-25), 2.97 (2H, dt, *J* = 6.0, 6.0 Hz, H_2-26), 2.78 (2H, t, *J* = 7.8 Hz, H_2-16), 2.00 (2H, br t, *J* = 5.4 Hz, H_2-7), 1.21 (12H, t, *J* = 7.2 Hz, 4 × H_3-51); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 171.3 (C-3), 166.0 (C-23), 165.4 (C-11), 157.3 (C-35), 157.0 (C-37/C-39), 154.9 (C-43/C-46), 152.6 (C-19), 151.5 (C-10), 151.1 (C-1/C-5), 147.9 (C-29 or C-31), 141.3 (C-31 or C-29), 133.1 (C-32), 132.6 (C-41/C-48), 132.4 (C-14), 130.7 (C-33), 126.5 (C-34), 125.5 (C-30), 123.5 (C-21), 122.6 (C-2/C-4/C-12), 118.7 (C-15), 113.5 (C-42/C-47), 113.4 (C-36/C-40), 95.3 (C-44/C-45), 91.8 (C-20), 50.8 (C-22), 45.2 (4 × C-50), 44.6 (C-6), 43.4 (C-17), 41.8 (C-26), 38.8 (C-25), 38.2 (C-8), 33.5 (C-7), 17.8 (C-16), 12.4 (4 × C-51); (+)-FABMS *m/z* 1102 $[M+H]^+$; (+)-HRFABMS *m/z* $[M+H]^+$ 1102.1461

(calcd. for $C_{49}H_{50}{}^{79}Br_2N_7O_9S_2$, 1102.1478), 1104.1466 (calcd. for $C_{49}H_{50}{}^{79}Br^{81}BrN_7O_9S_2$, 1104.1458), 1106.1440 (calcd. for $C_{49}H_{50}{}^{81}Br_2N_7O_9S_2$, 1106.1437).

*4.1.13. N-13 (Coumarin 2-aminoethyl acetamido) discorhabdin C analogue (**23**).* *N*-13 (2-Aminoethyl acetamido)discorhabdin C trifluoroacetate salt (**19**) (6.1 mg, 7.7 μmol) was dissolved in dry acetone (0.5 mL). Excess dry K_2CO_3 (15.0 mg, 0.109 mmol) was added, turning the mixture colour from dark purple to orange. NHS-activated 7-dimethylaminocoumarin-4-acetic acid[18,19] (5.3 mg, 15 μmol) was added, and the reaction stirred under N_2 at room temperature for 1.5 h. The crude product was purified by reversed-phase C_{18} flash column chromatography (0-50% MeOH (0.05% TFA)), followed by Sephadex LH-20 column chromatography (MeOH (0.05% TFA)) to yield **23** as a purple non-crystalline trifluoroacetate salt (3.8 mg, 55%). R_T 6.82 min (System B); ν_{max} (ATR) 3330, 1615, 1677, 1537, 1201, 1135 cm^{-1}; ^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.08 (1H, br s, NH-9), 8.37 (1H, br s, NH-24), 8.25 (1H, br s, NH-27), 8.22 (1H, br s, NH-18), 7.72 (2H, s, H-1/H-5), 7.52 (1H, d, *J* = 9.0 Hz, H-38), 7.35 (1H, s, H-14), 6.71 (1H, br d, *J* = 9.0 Hz, H-37), 6.60 (1H, d, *J* = 1.8 Hz, H-35), 6.00 (1H, s, H-31), 4.95 (2H, s, H_2-22), 3.67 (2H, dt, *J* = 7.8, 4.2 Hz, H_2-17), 3.61 (4H, obscured by water, H_2-8/H_2-29), 3.15 (4H, m, H_2-25/H_2-26), 3.02 (6H, s, 2 × H_3-41), 2.82 (2H, t, *J* = 7.8 Hz, H_2-16), 2.00 (2H, br s, H_2-7); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 171.3 (C-3), 168.0 (C-28), 165.8 (C-23), 165.5 (C-11), 160.7 (C-32), 155.3 (C-34), 152.7 (C-36), 152.6 (C-19), 151.4 (C-30), 151.1 (C-1/C-5), 151.0 (C-10), 132.3 (C-14), 126.0 (C-38), 123.5 (C-21), 122.7 (C-12), 122.6 (C-2/C-4), 118.8 (C-15), 109.3 (C-31), 108.9 (C-37), 108.1 (C-39), 97.4 (C-35), 91.9 (C-20), 50.9 (C-22), 44.6 (C-6), 40.0 (C-17), 39.7 (2 × C-41), 38.7 (C-8/C-29), 38.2 (C-25/C-26), 33.5 (C-7), 17.9 (C-16); (+)-FABMS *m/z* 791 $[M+H]^+$; (+)-HRFABMS *m/z* $[M+H]^+$ 791.0807 (calcd. for $C_{35}H_{33}{}^{79}Br_2N_6O_6$,

791.0828), 793.0804 (calcd. for $C_{35}H_{33}{}^{79}Br^{81}BrN_6O_6$, 793.0808), 795.0806 (calcd. for $C_{35}H_{33}{}^{81}Br_2N_6O_6$, 795.0787).

*4.1.14. N-13-Propargylacetamido discorhabdin C (**24**).* Discorhabdin C trifluoroacetate salt (**7**) (6.0 mg, 10 µmol) was dissolved in dry acetone (2 mL), followed by addition of dry K_2CO_3 (20.0 mg, 0.145 mmol) and 2-bromo-*N*-propargylacetamide (**26**)[22] (12.0 mg, 68 µmol), and the mixture was purged under N_2. The mixture was heated with stirring at 70 °C for 2 h., after which the crude product was purified by reversed-phase C_{18} flash column chromatography (0-50% MeOH (0.05% TFA)), and reversed-phase phenyl-bonded column chromatography (0-50% MeOH (0.05% TFA)) to yield **24** as a purple non-crystalline trifluoroacetate salt (3.0 mg, 45%). R_T 5.94 min (System B); ν_{max} (ATR) 3288, 1675, 1541, 1326, 1200 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 10.12 (1H, br s, NH-9), 8.79 (1H, t, *J* = 5.6 Hz, NH-24), 8.23 (1H, br s, NH-18), 7.73 (2H, s, H-1/H-5), 7.38 (1H, s, H-14), 5.00 (2H, s, H_2-22), 3.90 (2H, obscured by water, H_2-25), 3.69 (2H, td, *J* = 7.6, 2.6 Hz, H_2-17), 3.63-3.58 (2H, br m, H_2-8), 3.17 (1H, d, *J* = 1.6 Hz, H-27), 2.82 (2H, t, *J* = 7.6 Hz, H_2-16), 2.01 (2H, t, *J* = 5.6 Hz, H_2-7); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 171.3 (C-3), 165.5 (C-11/C-23), 152.6 (C-19), 151.4 (C-10), 151.1 (C-1/C-5), 132.3 (C-14), 123.5 (C-21), 122.6 (C-2/C-4/C-12), 118.8 (C-15), 91.9 (C-20), 80.5 (C-26), 73.4 (C-27), 50.6 (C-22), 44.6 (C-6), 43.5 (C-17), 38.2 (C-8), 33.5 (C-7), 28.1 (C-25), 17.8 (C-16); (+)-FABMS *m/z* 557 $[M+H]^+$; (+)-HRFABMS *m/z* $[M+H]^+$ 556.9823 (calcd. for $C_{23}H_{19}{}^{79}Br_2N_4O_3$, 556.9824), 558.9793 (calcd. for $C_{23}H_{19}{}^{79}Br^{81}BrN_4O_3$, 558.9803), 560.9797 (calcd. for $C_{23}H_{19}{}^{81}Br_2N_4O_3$, 560.9783).

*4.1.15. N-13-(3-Azidopropyl)-acetamido discorhabdin C (**25**).* Discorhabdin C trifluoroacetate salt (**7**) (12 mg, 21 µmol) was dissolved in dry acetone (2 mL), followed by

addition of dry K_2CO_3 (20.0 mg, 0.145 mmol) and 2-bromo-*N*-(3-azidopropyl)-acetamide[23,24] (**27**) (25.0 mg, 0.110 mmol), and the mixture was purged under N_2. The mixture was heated with stirring at 70 °C for 2 h., after which the crude product was purified by reversed-phase C_{18} flash column chromatography (0-50% MeOH (0.05% TFA)) to yield **25** as a purple non-crystalline trifluoroacetate salt (3.5 mg, 23%). R_T 5.25 min (System C); ν_{max} (ATR) 3247, 1674, 1543, 1327, 1200 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 10.09 (1H, br s, NH-9), 8.36 (1H, t, *J* = 5.4 Hz, NH-24), 8.22 (1H, br s, NH-18), 7.73 (2H, s, H-1/H-5), 7.37 (1H, s, H-14), 4.96 (2H, s, H_2-22), 3.80 (2H, t, *J* = 6.9 Hz, H_2-27), 3.67 (2H, obscured by water, H_2-17), 3.62 (2H, br s, H_2-8), 3.15 (2H, td, *J* = 6.9, 5.4 Hz, H_2-25), 2.82 (2H, t, *J* = 7.8 Hz, H_2-16), 2.01 (2H, br s, H_2-7), 1.68 (2H, p, *J* = 6.9 Hz, H_2-26); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 171.3 (C-3), 165.6 (C-11), 165.4 (C-23), 152.6 (C-19), 151.4 (C-10), 151.1 (C-1/C-5), 132.3 (C-14), 123.5 (C-21), 122.6 (C-2/C-4/C-12), 118.7 (C-15), 91.9 (C-20), 50.8 (C-22), 48.1 (C-27), 44.6 (C-6), 43.5 (C-17), 38.2 (C-8), 36.0 (C-25), 33.5 (C-7), 28.2 (C-26), 17.8 (C-16); (+)-FABMS *m/z* 602 $[M+H]^+$; (+)-HRFABMS *m/z* $[M+H]^+$ 602.0139 (calcd. for $C_{23}H_{22}{}^{79}Br_2N_7O_3$, 602.0151), 604.0126 (calcd. for $C_{23}H_{22}{}^{79}Br^{81}Br\ N_7O_3$, 604.0126), 606.0127 (calcd. for $C_{23}H_{22}{}^{81}Br_2N_7O_3$, 606.0110).

*4.1.16. 2-Bromo-N-(3-azidopropyl)-acetamide (**27**).* 3-Bromopropylamine hydrobromide (250 mg, 1.14 mmol) was added to a solution of NaN_3 (222 mg, 3.41 mmol) in H_2O (2 mL) and stirred at 75 °C for 16 h. The reaction mixture was then left to cool to room temperature before addition of NaOH (50.0 mg, 1.25 mmol) and extraction with CH_2Cl_2 (3 × 10 mL). The organic layer was dried with anhydrous $MgSO_4$, before concentrating in vacuo to less than 10 mL. The solution was then purged under N_2 and cooled to –20 °C to –30 °C (dry ice/acetone), before addition of bromoacetyl bromide (50 μL, 0.57 mmol) while stirring. The mixture was then stirred for a further 20 min. at this temperature and left to warm to room

temperature. The crude reaction product was washed with aqueous HCl (10%, 3 × 5 mL), sat. sodium bicarbonate (2 × 5 mL), and H_2O (5 mL), before drying the organic layer with anhydrous $MgSO_4$, filtered and concentrated in vacuo to give **27** as a pale yellow oil (127 mg, quant.); ν_{max} (ATR) 3258, 2938, 2095, 1649, 1541 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.94 (1H, br s, NH-3), 3.90 (2H, s, H_2-1), 3.41-3.37 (4H, m, H_2-4/H_2-6), 1.82 (2H, p, *J* = 6.6 Hz, H_2-5); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.1 (C-2), 49.4 (C-6), 38.1 (C-4), 29.2 (C-5 or C-1), 28.5 (C-1 or C-5); (+)-ESIMS *m/z* 243 $[M+Na]^+$; (+)-HRESIMS *m/z* $[M+H]^+$ 242.9855 (calcd. for $C_5H_9{}^{79}BrN_4NaO$, 242.9852), 244.9833 (calcd. for $C_5H_9{}^{81}BrN_4NaO$, 244.9832).

*4.1.17. Dansyl "click" discorhabdin C analogue (**29**).* *N*-13-Propargylacetamido discorhabdin C trifluoroacetate salt (**24**) (4.0 mg, 6 µmol) was dissolved in dry CH_2Cl_2 (2 mL) with *N*-(3-azidopropyl)-5-(dimethylamino)-1-naphthalenesulfonamide (**28**)[25] (5.0 mg, 15 µmol), before addition of DIPEA (5 µL, 28.7 µmol) and copper(I) iodide (cat.). The reaction mixture was stirred at room temperature for 30 min, and the resulting mixture was filtered to remove the catalyst. The filtrate was diluted with MeOH (10 mL) and concentrated under reduced pressure. Crude product purification by Sephadex LH-20 flash column chromatography (MeOH (0.05% TFA)) followed by reversed-phase C_{18} flash column chromatography (0-50% MeOH (0.05% TFA)), yielded **29** as a purple non-crystalline trifluoroacetate salt (3.5 mg, 58%). R_T 6.29 min (System C); ν_{max} (ATR) 3276, 1674, 1539, 1324, 1200, 1139 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 10.08 (1H, br s, NH-9), 8.79 (1H, br t, *J* = 5.6 Hz, NH-24), 8.47 (1H, d, *J* = 8.1 Hz, H-39), 8.29 (1H, d, *J* = 8.1 Hz, H-44), 8.20 (1H, br s, NH-18), 8.07-8.03 (2H, m, H-34/H-37), 7.79 (1H, s, H-27), 7.69 (2H, s, H-1/H-5), 7.61 (2H, t, *J* = 8.1 Hz, H-38/H-43), 7.37 (1H, s, H-14), 7.27 (1H, d, *J* = 8.1 Hz, H-42), 5.00 (2H, s, H_2-22), 4.32-4.27 (4H, m, H_2-25/H_2-31), 3.68 (2H, t, *J* = 7.0 Hz, H_2-17), 3.62-3.57 (2H, m, H_2-8), 2.84 (6H, s, 2 × H_3-47), 2.82-2.75 (4H, m, H_2-16/H_2-33),

2.00 (2H, br t, *J* = 5.4 Hz, H_2-7), 1.88 (2H, p, *J* = 7.0 Hz, H_2-32); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 171.3 (C-3), 165.6 (C-23 or C-11), 165.5 (C-11 or C-23), 152.6 (C-19), 151.4 (C-41 or C-10), 151.3 (C-10 or C-41), 151.0 (C-1/C-5), 143.9 (C-26), 135.5 (C-36), 132.3 (C-14), 129.4 (C-39), 129.0 (C-40 or C-45), 128.9 (C-45 or C40), 128.3 (C-37), 127.8 (C-43), 123.5 (C-21/C-38), 122.9 (C-27), 122.6 (C-2/C-4/C-12), 118.8 (C-15 or C-44), 118.7 (C-44 or C-15), 115.1 (C-42), 91.8 (C-20), 50.8 (C-22), 46.5 (C-31), 45.0 (2 × C-47), 44.6 (C-6), 43.4 (C-17), 39.4 (C-33), 38.2 (C-8), 34.4 (C-25), 33.5 (C-7), 30.0 (C-32), 17.8 (C-16); (+)-ESIMS *m/z* 890 $[M+H]^+$; (+)-HRESIMS *m/z* $[M+H]^+$ 890.1047 (calcd. for $C_{38}H_{38}{}^{79}Br_2N_9O_5S$, 890.1079), 892.1060 (calcd. for $C_{38}H_{38}{}^{79}Br^{81}BrN_9O_5S$, 892.1061), 894.1052 (calcd. for $C_{38}H_{38}{}^{81}Br_2N_9O_5S$, 894.1039).

*4.1.18. Lissamine rhodamine "click" discorhabdin C analogue (**31**).* *N*-13-Propargylacetamido discorhabdin C trifluoroacetate salt (**24**) (4.0 mg, 6 µmol) was dissolved in MeCN (2 mL) with *N*-(3-azidopropyl)-lissamine rhodamine sulphonamide (**30**)[26] (6.0 mg, 9.4 µmol), before addition of DIPEA (5.0 µL, 28.7 µmol) and copper(I) iodide (cat.). The reaction mixture was stirred at room temperature for 30 min, and the resulting mixture was filtered to remove the catalyst. The crude product was purified by reversed-phase C_{18} flash column chromatography (0-50% MeOH (0.05% TFA)) to yield **31** as a purple non-crystalline trifluoroacetate salt (4.0 mg, 51%). R_T 7.40 min (System C); ν_{max} (ATR) 3381, 1678, 1591, 1181 cm^{-1}; ^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.08 (1H, br s, NH-9), 8.78 (1H, br s, NH-24), 8.42 (1H, s, H-37), 8.22 (1H, br s, H-18), 8.10 (1H, br s, NH-34), 7.94 (2H, br s, H-27/H-41), 7.73 (2H, s, H-1/H-5), 7.50 (1H, d, *J* = 7.8 Hz, H-40), 7.36 (1H, s, H-14), 7.05 (4H, m, H-48/H-49/H-54/H-55), 6.94 (2H, m, H-51/H-52), 5.00 (2H, s, H_2-22), 4.38 (2H, br t, H_2-31), 4.34 (2H, br s, H_2-25), 3.64 (12H, m, H_2-8/H_2-17/4 × H_2-57), 2.93 (2H, m, H_2-33), 2.82 (2H, m, H_2-16), 2.00 (2H, m, H_2-7), 1.97 (2H, m, H_2-32), 1.21 (12H, m, 4 × H_3-

58); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 171.3 (C-3), 165.6 (C-23 or C- 11), 165.5 (C-11 or C-23), 157.2 (C-42), 157.0 (C-44/C-46), 155.0 (C-50/C-53), 152.6 (C-19), 151.4 (C-10), 151.1 (C-1/C-5), 147.9 (C-36 or C-38), 144.1 (C-26), 141.2 (C-36 or C-38), 133.1 (C-39), 132.6 (C-35/C-48), 132.3 (C-14), 130.8 (C-40), 126.5 (C-41), 125.6 (C-37), 123.5 (C-21), 123.0 (C-27), 122.6 (C-2/C-4/C-12), 118.8 (C-15), 113.6 (C-49/C-54), 113.4 (C-43/C-47), 95.3 (C-51/C-52), 91.9 (C-20), 50.8 (C-22), 46.7 (C-31), 45.2 (4 × C-57), 44.6 (C-6), 43.5 (C-17), 39.9 (C-33), 38.3 (C-8), 34.4 (C-25), 33.5 (C-7), 29.9 (C-32), 17.9 (C-16), 12.4 (4 × C-58); (+)-ESIMS *m/z* 1197 $[M+H]^+$; (+)-HRESIMS *m/z* $[M+H]^+$ 1197.2030 (calcd. for $C_{53}H_{55}{}^{79}Br_2N_{10}O_9S_2$, 1197.1956), 1199.1946 (calcd. for $C_{53}H_{55}{}^{79}Br^{81}BrN_{10}O_9S_2$, 1199.1942), 1201.1931 (calcd. for $C_{53}H_{55}{}^{81}Br_2N_{10}O_9S_2$, 1201.1919).

4.2. Cytotoxicity assays.

The P388 assay used a two-fold series dilution of the sample incubated for 72 h with murine leukemia cells (ATCC CCL 46 P388D1). The concentration of the sample required to inhibit cell growth to 50% of the growth of the solvent control was determined using the absorbance obtained upon staining with MTT tetrazolium. Mitomycin C was used as a positive control, with IC_{50} 0.5 μM. Details of the NCI-60 human tumour testing protocols can be found online at http://dtp.cancer.gov.

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