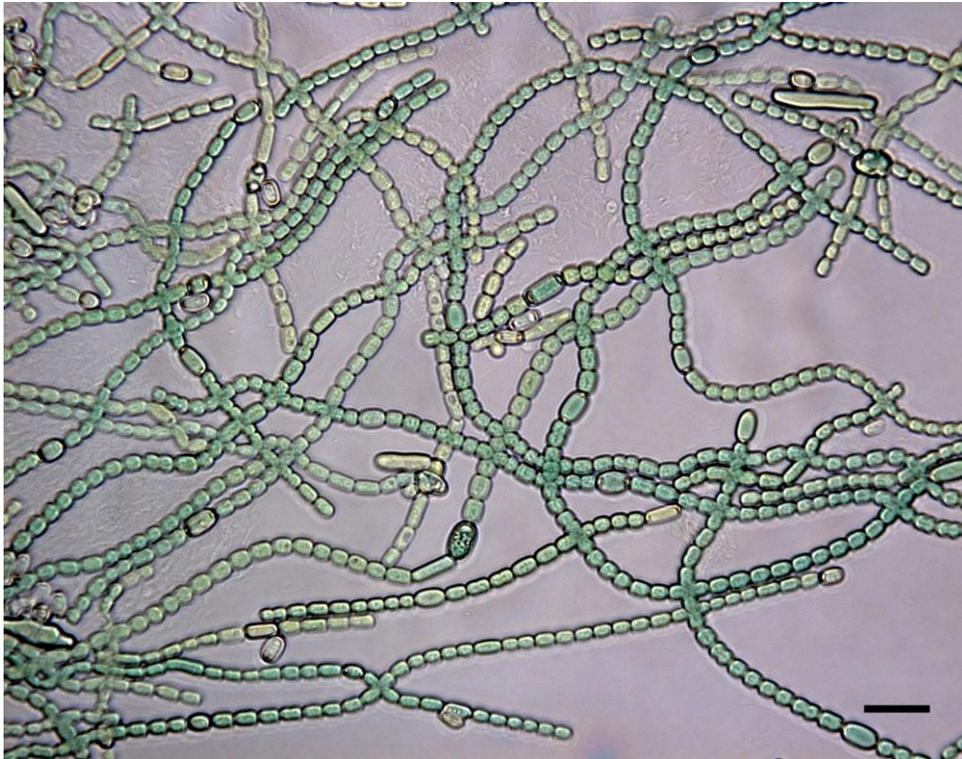


Supplementary material

Table S1. Code numbers and genera of the cyanobacteria included in this study.

Code number	Genus
1	<i>Nostoc sp.</i>
2	<i>Anabaena sp.</i>
3	<i>Anabaena sp.</i>
4	<i>Anabaena sp.</i>
5	<i>Calothrix sp.</i>
6	<i>Nostoc sp.</i>
7	<i>Nostoc sp.</i>
8	<i>Anabaena sp.</i>
9	<i>Anabaena sp.</i>
11	<i>Anabaena sp.</i>
12	<i>Anabaena sp.</i>
13	<i>Anabaena sp.</i>
14	<i>Anabaena sp.</i>
15	<i>Calothrix sp.</i>
16	<i>Calothrix sp.</i>
18	<i>Calothrix sp.</i>
19	<i>Calothrix sp.</i>
20	<i>Anabaena sp.</i>
21	<i>Calothrix sp.</i>
22	<i>Nostoc sp.</i>
23	<i>Anabaena sp.</i>
25	<i>Anabaena sp.</i>
26	<i>Anabaena sp.</i>
27	<i>Anabaena sp.</i>
28	<i>Nostoc sp.</i>
29	<i>Anabaena sp.</i>
30	<i>Anabaena sp.</i>
31	<i>Anabaena sp.</i>
33	<i>Anabaena sp.</i>
34	<i>Cyanothece sp.</i>
35	Unidentified
36	<i>Nostoc sp.</i>
37	<i>Anabaena sp.</i>
38	<i>Cyanothece sp.</i>
39	<i>Anabaena sp.</i>
41	<i>Nostoc sp.</i>
44	<i>Anabaena sp.</i>
45	<i>Anabaena sp.</i>
46	<i>Anabaena sp.</i>
47	<i>Calothrix sp.</i>
48	<i>Anabaena sp.</i>

Figure S1. The microscopic appearance of the *Anabaena* strain M30 in culture. This appearance is representative of all the benthic *Anabaena* strains studied. The bar represents 20 μm .



Bioguided Partial Purification of the Apoptogenic Constituent in the Aqueous M44-Extract

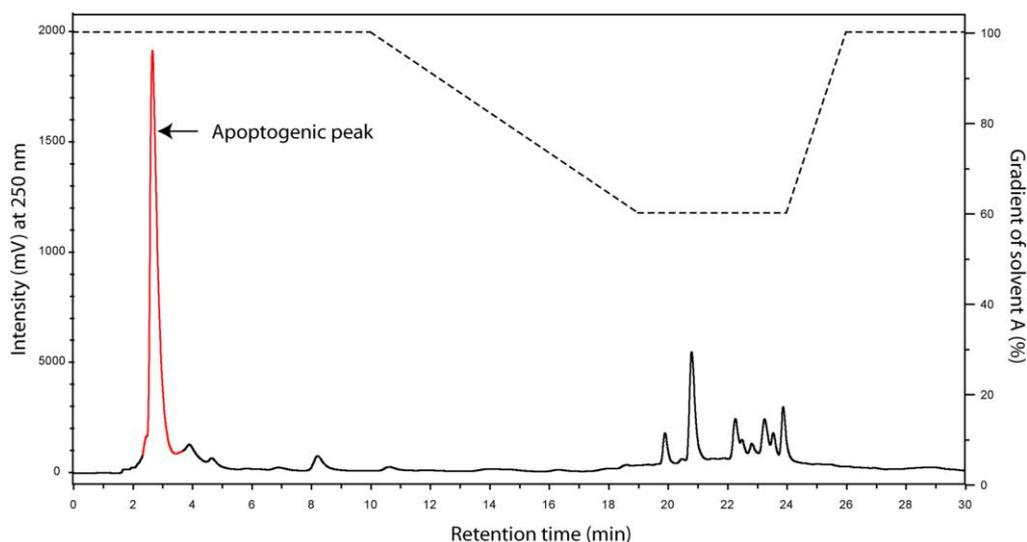
Solid phase extraction

The residue of the dried water extract from 3 mg original dry weight was dissolved in 0.15 mL water, added 0.6 mL methanol, and next 0.15 mL chloroform. The mixture was vortexed, added 0.45 mL water, vortexed again, and centrifuged at $6000 \times g$ for 2 min at room temperature. The upper aqueous/methanol phase contained the bioactivity, and was collected, evaporated, and dissolved in 1 mL of 10% aqueous methanol. This sample was applied to a mixed mode reversed phase and strong anion exchanger (Oasis®MAX 186000367, Waters Corporation, Milford, USA) solid phase extraction (SPE) cartridge, conditioned with 3 mL methanol and equilibrated with 3 mL of 10% aqueous methanol. After sample application the column was washed with another 3 mL of 10% aqueous methanol. The bioactivity appeared in the combined flow through and wash fractions, which was evaporated and redissolved in 60 μL of aqueous 10mM Triethylamine formic acid (TEAF) buffer, pH 3 just prior to reversed phase HPLC chromatography, as shown in Figure S2.

Reversed phase chromatography

In order to allow loading of the sample in aqueous solution we had to use a C-18 reversed phase matrix with polar end-capping (Aquasil). As shown below (Figure S2) the activity was very weakly retained by the column.

Figure S2. RP-HPLC separation of the analytes in the apoptogenic aqueous extract of cyanobacterial strain M44. The sample (60 μ L) prepurified by solid phase extraction (see above) was further purified by chromatography on a column (Aquasil C18; 3 \times 150 mm, Thermo Hypersil-Keystone) coupled to a Merck-Hitachi LaChrom HPLC system (VWR, West Chester, USA). The mobile phases were 10mM TEAF pH 3 (A), and 100% methanol (B). The flow rate was 0.5 mL/min and monitoring wavelength was 250nm. The bioactivity eluted in fraction 2. -3.8 min (red).



The recovery of the activity was reasonable (55–60 %) after the Aquasil HPLC step (Table S2).

Table S2. Recovery of the M44 cell death inducing activity in the different steps of the bio-guided isolation procedure. The results are given as mean \pm SEM, n = 3.

Bioactive fraction	Recovery (%)
Water extract supernatant	100
Water-methanol phase	96 \pm 4.2
SPE flow through fraction	88 \pm 2.5
RP-HPLC fraction (2.2-3.8min)	58 \pm 2.2

The HPLC peak containing the apoptosis-inducing substance (Figure S2) was not homogeneous, as it eluted only slightly after the flow through fraction. The inhomogeneity was evidenced by further HPLC chromatography on various column systems, including hydrophilic interaction chromatography (HILIC), which resulted in several resolved peaks. Unfortunately, most of the bioactivity was lost during these procedures, which blocked efforts for chemical characterisation of the anti-AML activity in the M44 extract.