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Telomerase Inhibitors from Cyanobacteria: Isolation and Synthesis of Sulfoquinovosyl Diacylglycerols from *Microcystis aeruginosa* PCC 7806

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Dedicated to Professor Dr. Dieter Seebach on the occasion of his 75th birthday

Abstract: By using the Telospot assay, 27 different extracts of cyanobacteria were evaluated for telomerase inhibition. All extracts showed varying, but significant activity. We selected *Microcystis aeruginosa* PCC 7806 to identify the active compound and a bioassay guided fractionation led us to isolate mixtures of sulfoquinovosyl diacylglycerols (SQDGs), which were identified by 2D NMR and MS/MS experiments. Pure SQDG derivatives were then synthesized. The IC_{50} values of pure synthetic sulfoquinovosyl dipalmitoylglycerol and the monopalmitoylated derivative against telomerase were determined to be 17 and 40 μM, respectively. A structure–activity relationship study allowed the identification of compounds with modified lipophilic acyl groups that display improved activity.

Keywords: biological activity · enzymes · inhibitors · structure–activity relationships · structure elucidation

Introduction

Cancer remains a leading cause of death worldwide, accounting for 13% of all fatal cases according to the World Health Organization,[1] which provides a strong stimulus for the development of novel drugs. Telomerase is the ribonucleoprotein enzyme complex that adds hexameric DNA repeats to the 3′ ends of telomeres at the ends of eukaryotic chromosomes.[2] Telomerase thus counteracts the telomere shortening that stems from incomplete DNA end replication and nucleolytic processing. However, telomerase is repressed in most human tissues and in telomerase-negative cells, telomeres shorten with continuous cell division cycles and act as molecular clocks counting the number of cell divisions, and, when the telomeres are too short, cellular senescence and crisis occurs.[3] The upregulation of telomerase is the main mechanism that confers immortality on cancer cells.[4] Telomerase activity is found in 90% of malignant cells, but rarely in normal cells.[5] Therefore the inhibition of telomerase may be considered an attractive target for new chemotherapeutic agents.[6] Different small-molecule telomerase inhibitors displaying some antitumor activity have been identified,[7] however, potent compounds showing activity in the nano- or sub-nano-molar range have not yet been identified.[8]

Some of us have recently developed an assay for low- and high-throughput analysis of telomerase modulators called Telospot.[8] Telospot uses a highly efficient human telomerase expression system known as super-telomerase[9] and the detection of telomerase activity is realized without PCR amplification in macroarray format. In comparison with other telomerase detection assays, Telospot is cheaper, faster, and can be modified for medium- and high-throughput screening.

Natural products have historically been a prime source for new drugs, which still holds true in many therapeutic areas. For example, around half of all new chemical entities introduced onto the market for the treatment of cancer since the 1940s have a natural product origin.[10] Cyanobacteria (blue-green algae) are known to be an interesting and relatively unexplored source of bioactive molecules.[11] Sev-

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eral compounds displaying potent antiproliferative activity have been identified from these organisms. Cryptophycin-1 was initially discovered as a result of its antifungal activity[12] and later re-isolated[13] showing antimicrotubular activity. The synthetic derivative cryptophycin-52 was negatively evaluated in phase II clinical trials and new derivatives are currently under investigation.[14] To the best of our knowledge, no compounds with telomerase inhibitory activity isolated from cyanobacteria have yet been described in the literature.

In this paper, we report in full detail the screening, isolation, characterization, and structure–activity relationship (SAR) studies on telomerase inhibitors derived from cyanobacteria. In particular, we present the screening results of 27 strains of cyanobacteria for telomerase inhibition obtained by using the Telospot method, and the subsequent isolation, identification, and synthesis of sulfoquinovosyl diacylglycerols from *Microcystis aeruginosa* PCC 7806 as telomerase inhibitors. In addition, a SAR study was performed by synthesizing natural product analogues to investigate the role of the lipid chains in the inhibition of telomerase, which led to synthetic derivatives with higher activity.

Results and Discussion

In the context of our research program on new anticancer compounds[15] and bioactive compounds from cyanobacteria,[16] we screened a part of our cyanobacteria culture collection for telomerase inhibition by using the Telospot method. We selected 27 different strains of cyanobacteria (Table 1), cultured them for 4 months, harvested the biomass by centrifugation, and extracted the products with 70% MeOH in an ultrasonic bath. The extracts were separated from the biomass by centrifugation and evaporated with a centrifugal evaporator. The resulting dry extracts were dissolved in 70% MeOH, filtered, and distributed in a 96-well plate, with 1 mg of dry extract added to each well. The plate was dried and stored at −20 °C. These crude extracts were redissolved in 60 μL of 50% DMSO/water (v/v) to generate stock plates. After further dilution at different concentrations for a final volume of 20 μL, assay plates were screened by using the manual Telospot method, as previously reported.[8] Surprisingly, all the extracts were active at concentrations of 0.42 and 0.2 g L^{-1} (Table 1). All extracts were tested to exclude intrinsic RNAse activity.

Two strains of cyanobacteria were selected for further study: *Microcystis aeruginosa* PCC 7806 was chosen as a result of our previous experience with this strain in the context of the isolation of aerucyclamides[16b,c] and *Synechococcus* sp. PCC 6911 was selected due to its favorable growth properties and its role as a well-characterized model strain. Extracts from these two strains were fractionated by C_{18} solid-phase extraction (SPE) with MeOH concentrations ranging from 10 to 100%. Moreover, because all the cyanobacterial extracts were active, we were initially concerned that this activity was due to a common pigment of photosynthetic organisms or an artifact generated from the cyanobacterial culture medium. Therefore chlorophyll a and b, xanthophyll, and β-carotene, as well as BG11[17] and Zehnder[18] media were also included in the second screening. The IC_{50} values of all these fractions and compounds are presented in Table 2.

Neither the pigments nor the cyanobacterial media showed any significant activity. Lower IC_{50} values were obtained with the 80 and 100% MeOH fractions of the C_{18} SPE separation of *Synechococcus* sp. PCC 6911 as well as the 100% MeOH fraction of *Microcystis aeruginosa* PCC 7806. The active compound was therefore a component of the lipophilic part of the extracts. To identify the active compound we then focused on *M. aeruginosa* PCC 7806: 1.3 mg of the 100% MeOH fraction of this strain was fractionated by HPLC with a C_{18} column and a CH_3CN/water gradient directly in a 96-well plate. The solvent was evaporated and all the fractions were tested by the Telospot method after a five-fold dilution in the assay buffer contain-

Table 1. Strains selected for extract screening and results of the Telospot assay at different extract concentrations.

Strain	Origin[a]	Score		
		0.42 g L^{-1}	0.2 g L^{-1}	0.042 g L^{-1}
Anabaena	A 1403-4	0.75	0.26	0.04
Anabaena flos-aquae	EAWAG 116	0.71	0.34	0.12
Chlorogleopsis fritschii	EAWAG 002c	0.80	0.61	0.23
Fischerella cf. *major*	EAWAG 108a	0.91	0.39	0.01
Fischerella muscicola	SAG 2027	0.47	0.04	−0.18
Lyngbya	PCC 8937	0.70	0.27	0.01
Lyngbya sp.	EAWAG 140	0.17	−0.09	−0.13
Microcystis aeruginosa	PCC 7806	0.77	0.50	0.12
Microcystis aeruginosa	EAWAG 251	0.61	0.27	0.04
Microcystis aeruginosa	EAWAG 127a	0.59	0.17	−0.01
Microcystis aeruginosa	EAWAG 171	0.51	0.16	−0.07
Microcystis aeruginosa	PCC 7813	0.37	0.16	0.17
Nodularia	PCC 7804	0.80	0.17	0.07
Nostoc	78-12A	0.83	0.53	0.12
Nostoc cf. *entophytum*	EAWAG 105d	0.67	0.22	−0.02
Nostoc commune	EAWAG 122a	0.91	0.73	−0.12
Nostoc ellipsosporum	CCALA 120	0.91	0.67	0.14
Nostoc sp.	EAWAG 076	0.72	0.48	−0.03
Oscillatoria limosa	EAWAG 234	0.88	0.79	0.15
Phormidium autumnale	EAWAG 099a	0.94	0.75	−0.02
Phormidium foveolarum	EAWAG 132	0.51	0.14	0.03
Phormidium sp.	EAWAG 134	0.45	0.20	0.06
Scytonema cf. *crustaceum*	EAWAG 179a	0.69	0.36	0.08
Scytonema sp.	CCALA 177	0.79	0.41	0.16
Synechococcus sp.	PCC 6911	0.87	0.69	0.21
Tolypothrix distorata var. symplocoides	EAWAG 224a	0.55	0.25	0.08
Tolypothrix tenius	CCALA 197	0.43	0.16	0.04

[a] PCC: The Pasteur Culture Collection of Cyanobacteria, Paris, France; EAWAG: Eidgenössische Anstalt für Wasserversorgung (Swiss Federal Institute of Aquatic Science and Technology), EPFL Lausanne, Switzerland; CCALA: Culture Collection of Autotrophic Organisms, Trebon, Czech Republic; SAG: Culture Collection of Algae at the University of Göttingen, Germany.

Table 2. IC_{50} values determined for the inhibition of telomerase for SPE fractions of PCC 6911 and PCC 7806 extracts, common pigments from photosynthetic organisms, and fractions of PCC cyanobacterial media.

Fraction/pure compound	IC_{50} [mg L^{-1}]
PCC 6911 fraction 80% MeOH	8
PCC 6911 fraction 100% MeOH	17
PCC 7806 fraction 100% MeOH	20
PCC 6911 fraction 50% MeOH	96
PCC 7806 fraction 80% MeOH	98
xanthophyll	110
chlorophyll b	537
medium BG11	692
medium Z	851
PCC 6911 fraction 10% MeOH	1072
PCC7 806 fraction 10% MeOH	1097
chlorophyll a	1259
β-carotene	1549

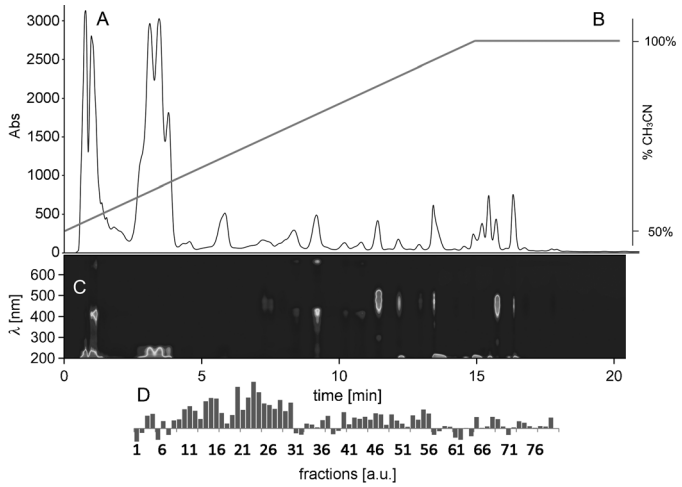


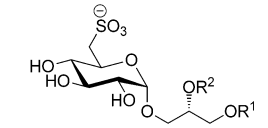
Figure 1. HPLC fractionation of the 100% MeOH C_{18} SPE fraction from *M. aeruginosa* PCC 7806. A) UV trace of the HPLC run at 225 nm, B) gradient CH_3CN/water, C) diode array trace of the HPLC run, and D) relative activities of the 80 fractions.

ing a final concentration of 2% DMSO (v/v). The results are displayed in Figure 1.

A group of fractions that eluted between 5 and 8 min (67 and 77% CH_3CN) displayed significant inhibition of telomerase. These fractions eluted just after the aerucyclamides A–D,[16b,c] which eluted between 2.5 and 4.5 min. The UV/Vis spectra of these fractions indicate that no chromophores were present and no reasonable molecular peaks were detected in the ESI-MS in positive mode.

To identify the active compounds, we decided to isolate these fractions from the *M. aeruguinosa* biomass obtained from 20 L of culture. The biomass was extracted with 80% MeOH with a sonic bath. The extract was dried first by evaporation of the MeOH under reduced pressure followed by freeze-drying to remove the water. The dry extract was redissolved in 80% MeOH and fractionated by C_{18} SPE. The 100% MeOH fraction was concentrated and then separated into four fractions by semi-preparative HPLC with a CH_3CN/water gradient. Again, these fractions were measured in the Telospot assay and they all displayed IC_{50} values between 0.01 and 0.07 g L^{-1}. The ^{1}H NMR spectrum of the four fractions revealed that the compounds present in these fractions share a common structural motif.

The general structure was investigated by 2D NMR spectroscopy, including HSQC, HMBC, COSY, and ROESY experiments, in [D_6]DMSO (for NMR data, see the Supporting Information). In the NMR spectra, signals typical of an anomeric carbon C-1′ (δ_C = 98.2 ppm) and its proton (δ_H = 4.56 ppm) reveal the presence of a carbohydrate moiety, which was corroborated by ^{1}H–^{1}H COSY interactions. The protons H-2′ (δ_H = 3.18 ppm) and H-3′ (δ_H = 3.35 ppm) are too close to the water peak to display a correlation between 2′ and 3′ in the COSY spectrum. Therefore this link was established through an HMBC correlation between H-1′ (δ_H = 4.56 ppm) and C-3′ (δ_C = 72.7 ppm). Interestingly, the chemical shifts of the C-6′ methylene (δ_C = 54.8 ppm) and its corresponding protons H_a-6′ (δ_H = 2.88 ppm) and H_b-6′ (δ_H = 2.54 ppm) are unusual for a standard carbohydrate, for which chemical shifts at around 62 ppm for the carbon atom and 3.75–3.60 ppm for the protons are expected. Moreover, the two diastereotopic protons H_a-6′ and H_b-6′ do not exhibit a correlation with a hydroxy group. A literature search indicates that the C-6′ chemical shift at 54.8 ppm is characteristic of the presence of a sulfonic acid group, which indicates that the carbohydrate is a sulfoquinovosyl moiety,[19] as confirmed by MS/MS experiments (see below). An HMBC correlation between H-1′ and C-3 (δ_C = 64.4 ppm) established the link between the anomeric position and a glycerol moiety, which was revealed by ^{1}H–^{1}H COSY interactions. From the chemical shifts, it can be concluded that the two hydroxy groups of the glycerol moiety are substituted by fatty acids, thus establishing the general structure of a sulfoquinovosyl diacylglycerol (SQDG). However, it was not possible to determine the exact structure of the fatty acid by NMR spectroscopy. Thus, the fatty acid was characterized by means of ESI-MS and ESI-MS^2 experiments in negative ion mode (Table 3).



1 R^1 = hexadecenoyl R^2 = palmitoyl
2 R^1 = palmitoyl R^2 = palmitoyl
3 R^1 = linoleoy R^2 = palmitoyl
4 R^1 = oleoyl R^2 = palmitoyl
5 R^1 = palmitoyl R^2 = H

Table 3. HRMS and MS/MS experiments performed in negative ion mode on the SQDGs present in a fresh extract of *M. aeruguinosa* PCC 7806.

	sn-1	sn-2	*m/z* $[M-H]^-$	Calcd *m/z* $[M-H]^-$	Δ [ppm]	*m/z* F1	*m/z* F2	*m/z* F3	*m/z* F4	*m/z* F5
1	C16:1	C16:0	791.4985	791.5026	5.2	535.33	537.33	255.28	253.26	225.04
2	C16:0	C16:0	793.5141	793.5145	0.5	537.19	537.16	255.21	255.21	224.99
3	C18:2	C16:0	817.5141	817.5151	1.2	561.35	537.34	255.30	279.30	225.07
4	C18:1	C16:0	819.5298	819.5248	−6.1	537.19	563.20	255.22	281.21	224.99

In these MS experiments, we first observed that all the fractions are composed of a mixture of different SQDGs. We analyzed the data of the fragmentation pattern proposed by Naumann et al. for SQDGs.[20] The presence of the sulfoquinovose was confirmed by its typical fragment at $m/z =$ 225.0. The fatty acid chains were liberated in the fragmentation process and these fragments along with the resulting sulfoquinovose moiety were observed. The fragment at $m/z = 255.2$ for the palmitic acid (C16:0) is present in all the SQDGs. Biosynthesis studies of prokaryotic anabolism suggest that hexadecanoic acid is always present at the sn-2 position.[20,21] C16:0, C16:1, C18:1, and C18:2 fatty acids were identified at the sn-1 position.

Hydroxylated fatty acids were also found in these fractions, for example, the C18:1-OH or C18:3-$(OH)_2$ moieties. We postulated that these hydroxylated fatty acids were artifacts generated during the isolation, which was confirmed by MS analysis (negative ionization mode) of a fresh 80% MeOH extract of a growing culture of *M. aeruginosa*. No hydroxylated SQDGs could be detected in the fresh extract, which indicates that these hydroxylated fatty acids were indeed degradation artifacts. Four SQDGs were identified in the *M. aeruginosa* extract and are listed in Table 3.

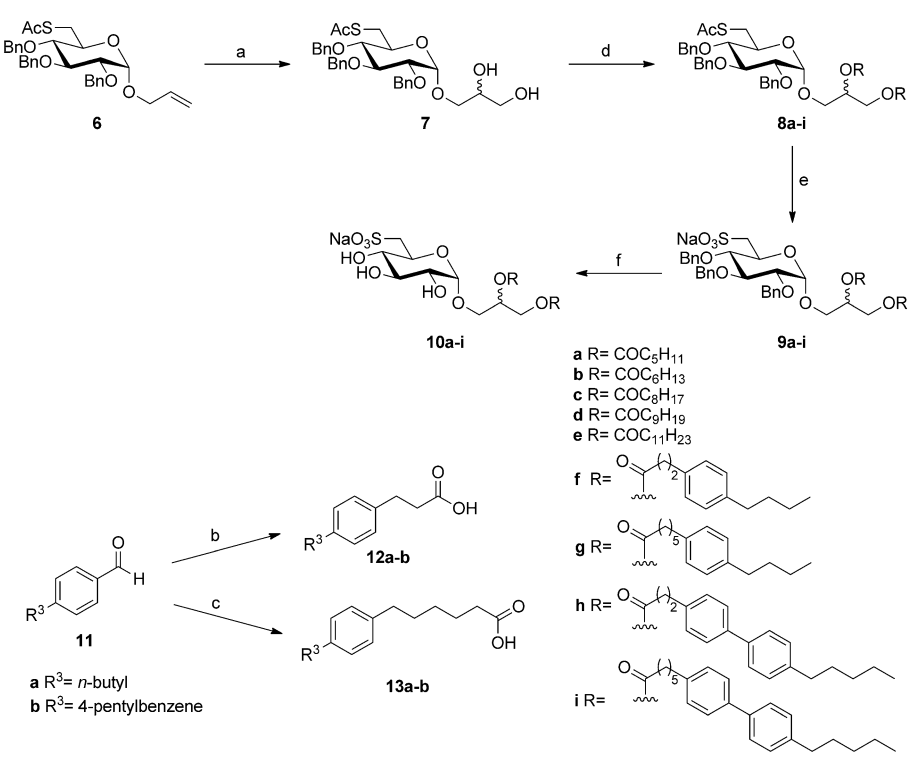
The relative configuration of the sulfoquinovose was confirmed by ROESY experiments to be identical to those of previously reported compounds.[19a,b,22] The configuration at C-2 of the glycerol unit was determined by comparison of the ^{1}H NMR chemical shifts of the isolated SQDGs with the literature data of synthetic (2*R*)- and (2*S*)-SQDG reported by Sugawara and co-workers[23] (see Table S2 in the Supporting Information). The isolated SQDG matches perfectly the data of (2*S*)-SQDG, but differences of up to 0.09 ppm compared with those reported for the 2*R* stereoisomer were observed, thus establishing the SQDG to have the 2*S* configuration.

NMR and MS analyses in negative ion mode of the second isolated fraction revealed that it was composed of the SQDGs **1**–**4**; no degradation products were identified. Based on its composition, the average molecular mass was estimated to be 805 g mol^{-1}. This fraction displayed an activity of 0.013 g L^{-1}, and therefore the average IC_{50} value for the mixture of different SQDGs was estimated to be around 16 μM.

As the SQDGs were always obtained as inseparable mixtures, we synthesized SQDG **2** following a literature procedure (see below).[24] The monoacylated derivative **5** with a palmitoyl group at the C-3 position of the glycerol was also obtained, and these two analytically pure samples were evaluated for telomerase inhibition. SQDG **2** displayed an IC_{50} value of 17 μM, which confirms the activity shown by the mixtures. The monoacylated derivative **5** was less active, displaying an activity of 40 μM.

The IC_{50} values obtained in this study are in good agreement with a previous study that estimated an IC_{50} value of 22 μM for telomerase inhibition by using the stretch PCR assay for mixtures of SQDGs isolated from edible purple laver.[25] However, compared with other telomerase inhibitors, this activity can be considered only as moderate.[7,26] Other lipidated compounds, like ceramides,[27] polyunsaturated fatty acids,[28] or the highly sulfated lipopolysaccharide axinelloside A, have also shown telomerase inhibition.[29]

Based on these structures, we conducted SAR studies with a view to improve telomerase inhibition. A previously reported SAR study of similar compounds for DNA polymerase inhibition revealed that the sulfoquinovose framework is important for biological activity,[30] which is supported by studies of the sulfonate interaction with telomerase.[25] Thus, we decided to modify only the fatty acid fragment. Several compounds with side-chains of different lengths were synthesized. As summarized in Scheme 1, the synthesis started from chiral compound **6**, prepared from D-glucose in several steps according to a literature procedure.[31] Dihy-



Scheme 1. Synthesis of sulfoquinovosyl diacylglycerols **10a–i**: Reagents and conditions: a) OsO_4, trimethylamine *N*-oxide, *t*BuOH, H_2O, RT, 75%; b) i. malonic acid, piperidine, pyridine, 120°C; ii. 10% Pd/C, EtOH, H_2, RT, 63–90% (2 steps); c) i. (4-carboxybutyl)triphenylphosphonium bromide, 1M LHMDS in THF, then **11**, THF, RT, 24 h; ii. 10% Pd/C, EtOH, H_2, RT, 44–64% (2 steps); d) fatty acid, EDCI, DMAP, CH_2Cl_2, RT, 26–90%; e) Oxone, AcOH, AcOK, RT; f) 10% Pd/C or 20% $Pd(OH)_2$/C, EtOH, H_2, RT, 24–66% (2 steps).

droxylation of the allyl group in **6** afforded the desired diol **7** in a yield of 75% as a mixture of two diastereoisomers (d.r. = 1.2:1). As the biological activity of the mixture was almost identical to that of the single diastereoisomers (see below), we proceeded with the synthesis. The acylation of diol **7** with different fatty acids in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) and 4-dimethylaminopyridine (DMAP) provided diesters **8a–i**. All of the fatty acids used were commercially available except for **12a**,**b** and **13a**,**b**, which were prepared from the corresponding aldehydes **11a** and **11b** in two steps. Thus, carboxylic acids **12a** and **12b** were obtained by a Knoevenagel condensation reaction between the aldehyde and malonic acid followed by hydrogenation of the resulting α,β-unsaturated carboxylic acid with Pd/C in ethanol. Fatty acids **13a** and **13b** were synthesized by a Wittig reaction followed by catalytic hydrogenation of the resulting alkene intermediates. Following the acylation of **7** with the fatty acids, oxidation of the thioacetate group in **8a–i** with Oxone in acetic acid gave the sodium sulfonate derivatives **9a–i**. Finally, deprotection of the benzyl groups in **9a–i** by hydrogenation with palladium in ethanol yielded the target derivatives **10a–i**.

These analogues were tested in the telomerase assay (see Figure S2 in the Supporting Information). Compounds **10a** and **10b** bearing C_6 and C_7 acyl chains, respectively, did not show any inhibition up to a concentration of 200 μM. In fact, a fatty acid with at least nine carbon atoms is necessary for the inhibition of telomerase, as displayed by compound **10c** (IC_{50} = 50 μM). Increasing the number of carbon atoms in the side-chain led to greater inhibition (**10d**: IC_{50} = 29 μM; **10e**: IC_{50} = 36 μM). The influence of a terminal alkylphenyl group strongly depended on the chain length: A shorter alkyl chain yielded a less potent inhibitor (**10f**: IC_{50} = 31 μM), whereas a significant increase in potency was observed for **10g** (IC_{50} = 20 μM). The absolute configuration at C-2 of the glycerol unit does not appear to influence the inhibition values significantly. Compound (2*S*)-**10f** was obtained by the stereoselective dihydroxylation of olefin **6** with AD-mix-α (d.r. > 8:1) and subsequent transformations as detailed in Scheme 1. Similar inhibition values were obtained for (2*S*)-**10f** as for the mixture. Finally, IC_{50} values of 14 and 11 μM were measured for **10h** and **10i**, respectively, which shows that a two-fold improvement in the inhibition was obtained by the introduction of a biphenyl motif.

The structure of the putative *Tribolium castaneum* telomerase catalytic subunit TERT was recently solved by X-ray crystallographic analysis,[32] which showed that this TERT candidate is similar to HIV reverse transcriptases, viral RNA polymerases, and β-family DNA polymerases. It is therefore not surprising that SQDGs were also found to inhibit HIV reverse transcriptases.[19a,b,33] On the other hand, free fatty acids, particularly polyunsaturated ones, have shown telomerase inhibition.[25,28] SQDGs, first isolated from *Chlorella* by Benson et al. in 1959,[34] are associated with the thylakoid membrane and are found in most photosynthetic organisms, such as cyanobacteria, higher plants, mosses, ferns, or algae.[21] Recently it was suggested that they interfere with DNA synthesis in the producing cyanobacterium.[35] It is reasonable to assume that the telomerase activity found in the lipophilic fractions of *Synechococus* sp. PCC 6911 and in the cyanobacterial crude extracts might generally be caused by SQDGs. However, the presence of other metabolites with telomerase activity cannot be ruled out at this point. This study should therefore encourage further screening of extracts of photosynthetic organisms for more potent telomerase inhibitors.

Conclusion

This broad study has encompassed different aspects, such as screening, fermentation of cyanobacteria, isolation of metabolites, structural characterization, and an SAR study carried out by the synthesis of natural product analogues to identify inhibitors of telomerase. In particular, we have demonstrated that the Telospot assay is well suited to the screening of natural product libraries. Among the 27 different strains of cyanobacteria evaluated, we have identified and characterized sulfoquinovosyl diacylglycerols (SQDG) obtained from *Microcystis aeruginosa* PCC 7806 by 2D NMR and MS/MS analyses. We also prepared pure SQDG **2** by chemical synthesis, which showed an activity against telomerase of 17 μM. In addition, to enable SAR studies on this class of natural products, we synthesized a series of compounds of which **10i** proved to be the most active. Interestingly, the introduction of more bulky and more lipophilic substituents led to higher activity. Although SQDGs are known to inhibit various DNA polymerases, such as HIV reverse transcriptase, their mode of action is poorly understood. In addition, the presence of SQDGs in *Spirulina*, a cyanobacterium used as a food supplement, as well as in many other plant-based food ingredients, and their role in telomerase inhibition and the chemoprevention of cancer should be investigated.

Experimental Section

General procedure for the acylation of glycol 7: EDCI (2.38 mmol, 2.5 equiv), DMAP (1.52 mmol, 1.6 equiv), and the corresponding fatty acid (2 equiv) were added to a solution of diol **7** (0.95 mmol, 1 equiv) in dry dichloromethane (40 mL). The reaction mixture was stirred at room temperature for 20 h and then diluted with water (20 mL). The aqueous phase was extracted with dichloromethane (3 × 20 mL) and the combined organic layers were washed with brine (1 × 20 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by flash column chromatography (SiO_2, pentane/AcOEt) to afford a colorless oil.

General procedure for oxidation and deprotection: Potassium acetate (14.9 mmol, 20 equiv) and Oxone (1.86 mmol, 2.5 equiv) were added to a solution of acylated glycol **8** (0.75 mmol, 1 equiv) in glacial acetic acid (10 mL). After 16 h at room temperature, the resulting mixture was diluted with water (40 mL). The aqueous phase was extracted with ethyl acetate (3 × 80 mL) and the combined organic layers were washed with satu-

rated $NaHCO_3$ solution (3×60 mL) and brine (1×40 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The product **9** was obtained as a sticky oil and used without further purification. The residue was dissolved in ethanol (30 mL) and treated with 10% Pd/C or 20% $Pd(OH)_2$/C. The suspension was stirred at room temperature for 16 h under an atmosphere of hydrogen. The mixture was filtered through Celite to remove the catalyst and the filtrate was concentrated. The residue was purified by flash column chromatography (SiO_2, CH_2Cl_2/EtOH) to give the target compound as a sticky oil.

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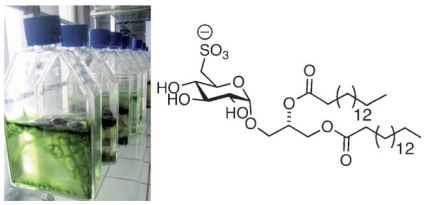
Out in the green! Screening cyanobacteria for inhibitors of telomerase led to the identification of sulfoquinovosyl diacylglycerols as the active species (see figure). A structure–activity relationship study highlighted the importance of the lipid chains for the inhibition of telomerase and a certain length was found to be essential for biological activity.



Enzyme Inhibition

M. Makhlouf Brahmi, C. Portmann, D. D'Ambrosio, T. M. Woods, D. Banfi, P. Reichenbach, L. Da Silva, E. Baudat, G. Turcatti, J. Lingner,* K. Gademann** ..... ■■■–■■■

Telomerase Inhibitors from Cyanobacteria: Isolation and Synthesis of Sulfoquinovosyl Diacylglycerols from *Microcystis aeruginosa* PCC 7806