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Synthetic Remodeling of the Chartreusin Pathway to Tune Antiproliferative and Antibacterial Activities

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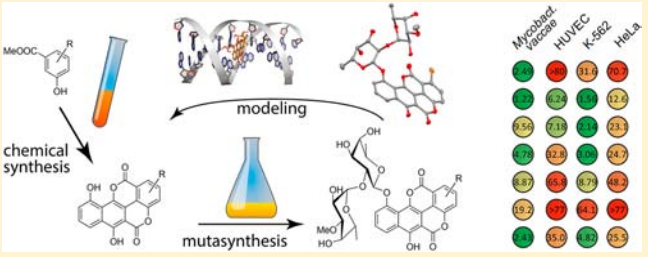
Supporting Information

ABSTRACT: Natural products of the benzonaphthopyranone class, such as chartreusin, elsamicin A, gilvocarcin, and polycarcin, represent potent leads for urgently needed anticancer therapeutics and antibiotics. Since synthetic protocols for altering their architectures are limited, we harnessed enzymatic promiscuity to generate a focused library of chartreusin derivatives. Pathway engineering of the chartreusin polyketide synthase, mutational synthesis, and molecular modeling were employed to successfully tailor the structure of chartreusin. For the synthesis of the aglycones, improved synthetic avenues to substituted coumarin building blocks were established. Using an engineered mutant, in total 11 new chartreusin analogs (desmethyl, methyl, ethyl, vinyl, ethynyl, bromo, hydroxy, methoxy, and corresponding (1→2) *abeo*-chartreusins) were generated and fully characterized. Their biological evaluation revealed an unexpected impact of the ring substituents on antiproliferative and antibacterial activities. Irradiation of vinyl- and ethynyl-substituted derivatives with blue light resulted in an improved antiproliferative potency against a colorectal cancer cell line. In contrast, the replacement of a methyl group by hydrogen caused a drastically decreased cytotoxicity but markedly enhanced antimycobacterial activity. Furthermore, mutasynthesis of bromochartreusin led to the first crystal structure of a chartreusin derivative that is not modified in the glycoside residue. Beyond showcasing the possibility of converting diverse, fully synthetic polyphenolic aglycones into the corresponding glycosides in a whole-cell approach, this work identified new chartreusins with fine-tuned properties as promising candidates for further development as therapeutics.



INTRODUCTION

Natural products and derivatives thereof represent the prime source of antitumoral agents.[1] Most potent chemotherapeutics either interfere with the cell cycle or interact with DNA, thus triggering apoptotic processes.[2] A large number of antiproliferative bacterial metabolites share an architecture consisting of a DNA-intercalating unit and sugar residues that serve to increase solubility and binding to DNA.[3−5] The aromatic polyketide glycoside chartreusin (**1**, Figure 1) from the soil-dwelling bacterium *Streptomyces chartreusis*[6] is a prominent example of this natural design. Chartreusin consists of a disaccharide (fucose and digitalose) and an unusual benzonaphthopyranone aglycone, named chartarin. While chartreusin was first investigated because of its antibacterial activity,[7,8] further studies revealed its potent antiproliferative activity against various tumor cell lines, such as murine P388 and L1210 leukemia, and B16 melanoma cells.[9] Given the remarkably high antitumor activity *in vitro*, there has been considerable interest in natural and semisynthetic chartreusin derivatives.[10] A natural analog, elsamicin A or elsamitrucin (**2**, Figure 1) is produced by an unidentified actinomycete.[11] Both elsamicin A and a semisynthetic prodrug of chartreusin, IST-622 (**3**), have reached phase II clinical trials.[12,13] Pharmacological studies revealed that chartreusin and its derivatives exert their antitumor activities through binding to DNA, radical-mediated single strand breaks, and inhibition of topoisomerase II.[14] Chartreusin and elsamicin A recognize almost the same G+C-rich DNA sequences.[14] Furthermore, analogs modified in the disaccharide portion of chartreusin retained the antileukemic effect *in vivo*.[15] Consequently, there is strong evidence that the unusual polycyclic aromatic aglycone chartarin represents a key element for bioactivity.[9,15] Interestingly, the benzonaphthopyranone chromophore is also found in potent antitumoral agents that belong to the gilvocarcin family of polyketide glycosides (**4**−**9**, Figure 1).[16−18] Yet, there is a complete lack of

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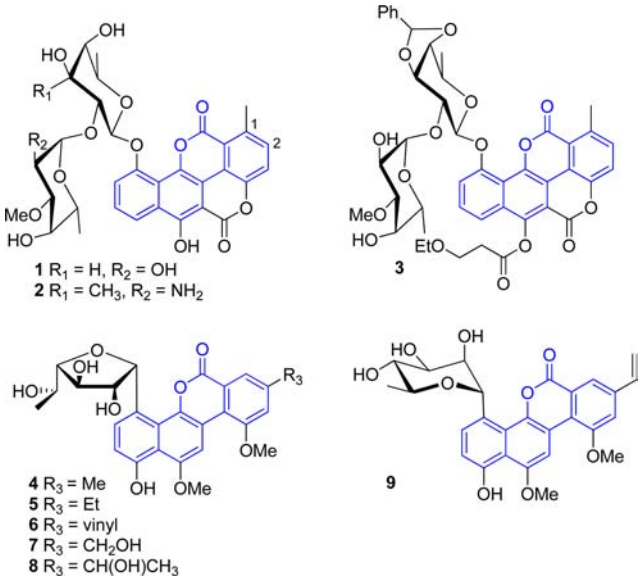

Figure 1. Chemical structures of chartreusin-type and gilvocarcin-type glycosides. Chartreusin (**1**), elsamicin A (**2**) and the semisynthetic derivative IST-622 (**3**), gilvocarcin M(**4**), E (**5**), V (**6**), H (**7**), HE (**8**), and polycarcin (**9**).

information on the impact of the chartarin ring substitution on antitumoral and antibacterial activities. Notably, there are no reported efficient synthetic methods available for altering the alkyl substituent, and to date the total synthesis of chartreusin or elsamicin A has not been achieved. Thus, with the intention of harnessing the biosynthetic potential of the chartreusin producer, we have previously elucidated the genetic basis for chartreusin biosynthesis.[19] Knowledge of the biosynthetic genes has set the basis for a targeted manipulation of this promising family of antitumoral agents. This potential has recently been highlighted by the design of a vinyl-substituted chartreusin analog, which is photoactivatable with visible light.[20]

Here we report the successful combination of chemical synthesis and biosynthesis to generate a focused library of chartreusin derivatives that are not readily accessible by total synthesis. Evaluation of these new compounds reveals the impact of ring substitution on antiproliferative and antibacterial activities of chartreusin analogs and the prospective for photoactivation.

RESULTS AND DISCUSSION

Combinatorial Biosynthesis of Homochartreusin Employing Anthracycline Synthase Genes. Variables in the biosynthetic pathway of chartreusin were considered as a way to alter the ring substitution of chartreusin (Figure 2A). Earlier stable isotope labeling studies[21] and our pathway dissection[19] revealed that the methyl substituent at C-1 represents the methyl group of the acetyl starter unit utilized by the *cha* polyketide synthase (PKS).[19,21] The product of the *cha* PKS is a decaketide that is enzymatically shaped by cyclases

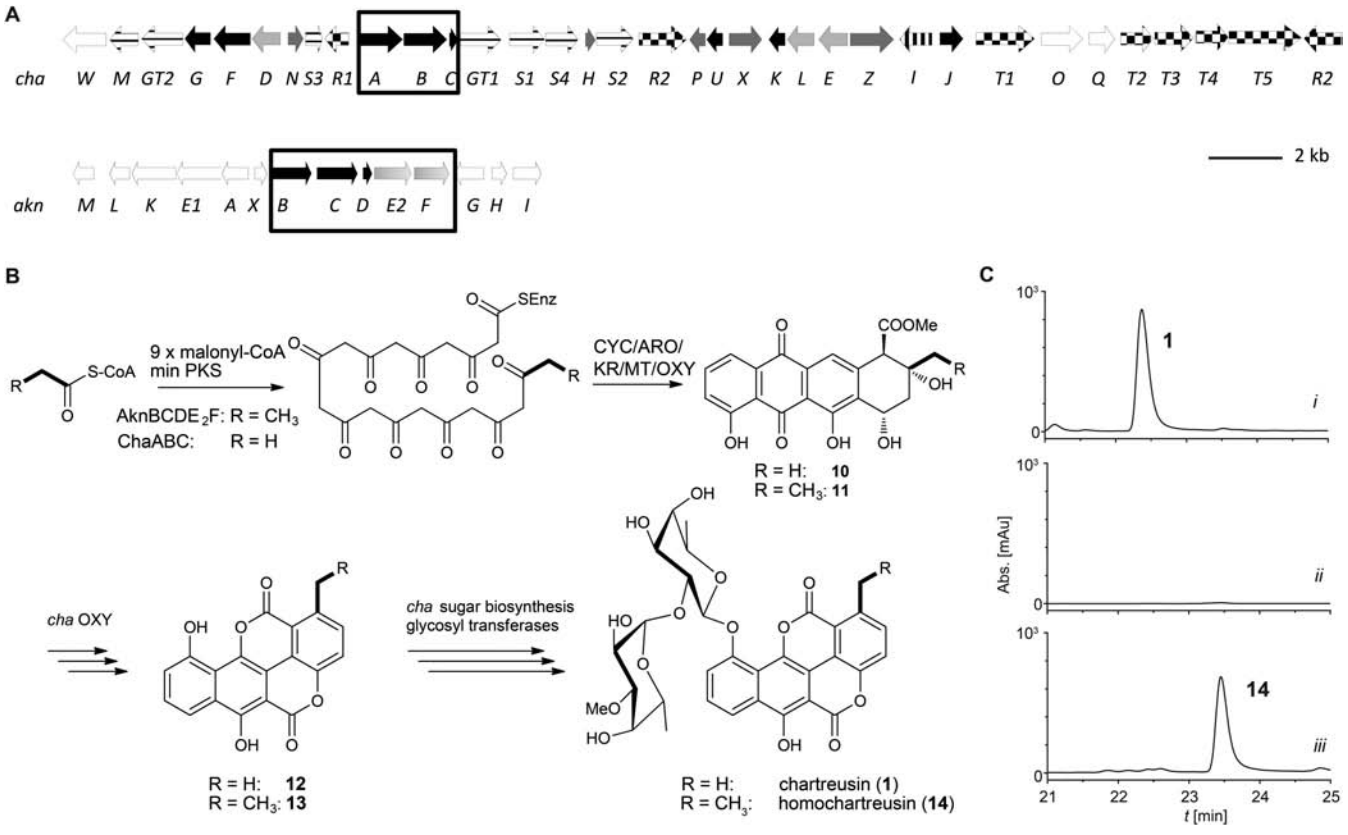

Figure 2. Combinatorial biosynthesis of ethyl-substituted chartreusin. (A) Organization of the chartreusin (*cha*) and aclacinomycin (*akn*) biosynthetic gene cluster. Gene cassettes used for complementation are highlighted. (B) Model of chartreusin biosynthesis and engineered hybrid pathway leading to homochartreusin (**14**). (C) HPLC-MS profiles (λ = 422 nm) of extracts from cultures of (*i*) heterologous host expressing the complete *cha* biosynthesis gene cluster and regulator gene *chaR1* (*S. albus*::pSC5P21/pXU343), (*ii*) Δ*chaABC* mutant (*S. albus*::pXU41/pXU343), (*iii*) Δ*chaABC* mutant supplemented with anthracycline biosynthesis gene cassette *aknBCDE2F* (*S. albus*::pXU41/pMMK1).

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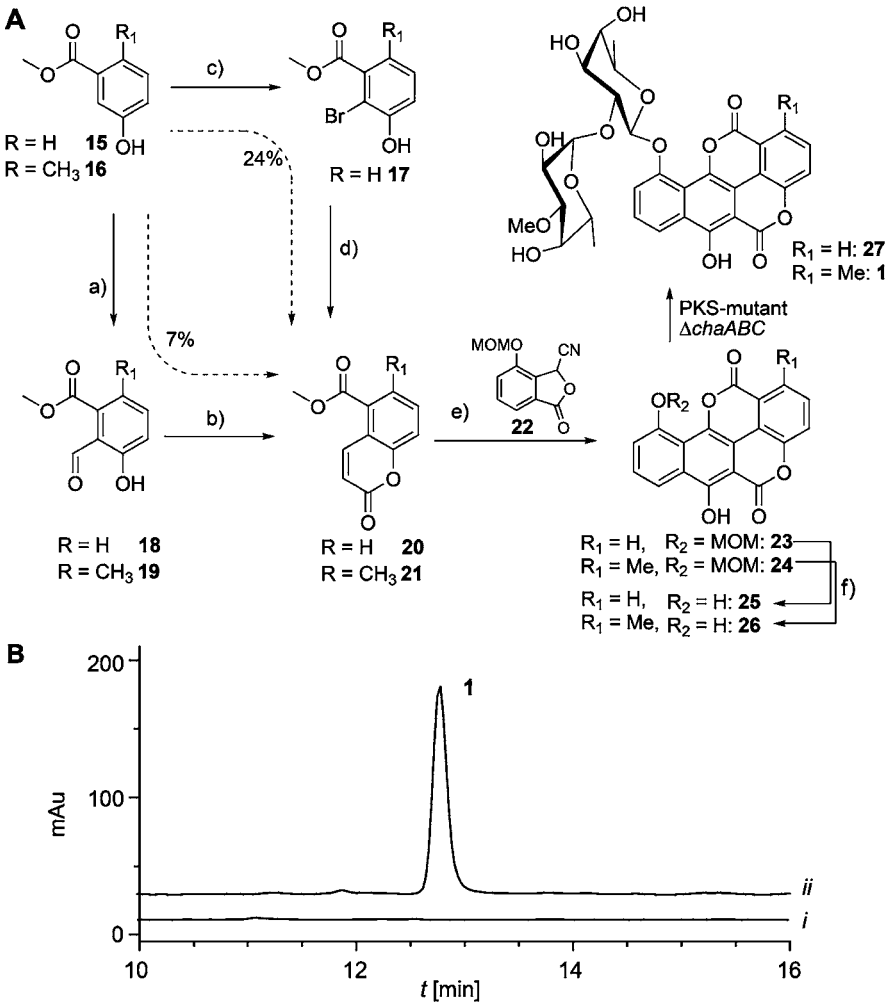

Figure 3. Convergent assembly of chartreusin and norchartreusin. (A) Synthesis of the coumarins **15** and **19** via a formylation-Wittig cascade or a halogenation-Heck cascade: Reagents and conditions: (a) polyphosphoric acid (PPA), hexamethylenetetramine, 45 min, 140 °C; (e) DEtA, Ph_3P=CH−COOEt, 20 min, reflux, 11% for R_1 = Me, 7% for R_1 = H over 2 steps; (c) 2,4,4,6-tetrabromocyclohexa-2,5-dienone, AcOH, 5 h, 50 °C, 31%; (d) methyl acrylate, $Pd(OAc)_2$, P(*o*-Tol)$_3$, DEtA, 30 min, reflux, 78%; (e) phthalide (**22**), coumarin (**20** or **21**), (KO*t*-Bu, 18 h, −60 °C to rt, 66% for R = Me, 92% for R = H; (g) BBr_3, CH_2Cl_2, 5 min, rt, 93−99%. (B) HPLC profiles (λ = 400 nm) of extracts from cultures of *S. albus*::pXU41/pXU343 (Δ*chaABC* mutant and regulator gene) supplemented with the following substrates: (*i*) DMSO (negative control), (*ii*) synthetic chartarin (**12**) in DMSO.

and aromatases to form an anthracyclic ring system (**5**) reminiscent of the doxorubicin and aclacinomycin aglycones (Figure 2B).[22] However, in contrast to the chartreusin intermediate, these canonical anthracyclines are, in part, assembled from propionate starter units.[23] It was reasoned that it would be possible to exchange the chartreusin methyl substituent for an ethyl group by swapping the PKS priming units.[24] First, in order to permit stable chartreusin production in the heterologous host, *chaR1*, a specific *cha* pathway activator gene was cloned into an expression vector (yielding pXU343), and the activator was coexpressed using a constitutive promoter. Next the genes coding for the aclacinomycin (**11**) (*akn*) PKS (*aknBCD*) and the genes required for propionate starter unit selection and incorporation (*aknE2* and *aknF*)[25] were cloned to give expression plasmid pMMK1. To construct a *cha* PKS null mutant, the genes coding for the minimal PKS (*chaABC*) were excised from the *cha* biosynthetic gene cluster (Figure 2A) using PCR targeting.[26] The resulting *cha* PKS null mutant (*S. albus*::pXU41) lost the ability to produce chartreusin or any related metabolites (Figure 2C, trace ii). However, the chartreusin chemotype could be restored by coexpressing the *chaABC* gene cassette on a replicative plasmid (pKJ01).[27] In stark contrast, the strain harboring the incomplete *cha* gene cluster and the *aknBCDE2F* cassette produced a new metabolite with the expected molecular mass of 654 amu (640 amu for chartreusin) (Figure 2C, trace iii). From MS/MS fragmentation analyses, which revealed the known glycoside cleavage pattern for chartreusin, it was inferred that the aglycone structure was altered. To elucidate its structure, the compound was isolated from a large-scale fermentation (20 L) by a combination of different chromatographic techniques. The molecular composition of compound **14**, $C_{33}H_{34}O_{14}$, was deduced from HRESI-MS measurements and ^1H and ^{13}C NMR data. The NMR spectra showed high similarity to those of **1**, suggesting a similar core structure. However, the DEPT135 spectrum displayed a signal for a methylene carbon, which is not present in chartreusin, and $^1H,^1H$-COSY measurements showed the vicinity of the methylene protons to a methyl function. The connectivity of this ethyl substituent as well as the full assignment of the

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structure of **14** was confirmed through the analyses of the HSQC and HMBC data (Supporting Information).

Mutasynthesis of a Desmethyl Analog of Chartreusin (Norchartreusin). Whereas manipulation of the starter unit selection allowed for the successful exchange of the C-1 methyl for an ethyl group, biosynthetic engineering could not be used to produce a chartreusin analog lacking the C-1 methyl group, as the PKS cannot utilize a one-carbon starter unit.[28] To overcome this limitation a mutasynthesis approach was employed by complementing the Δ*chaABC* mutant with synthetic chartarin analogs. First, mutasynthesis was explored using the natural substrate.

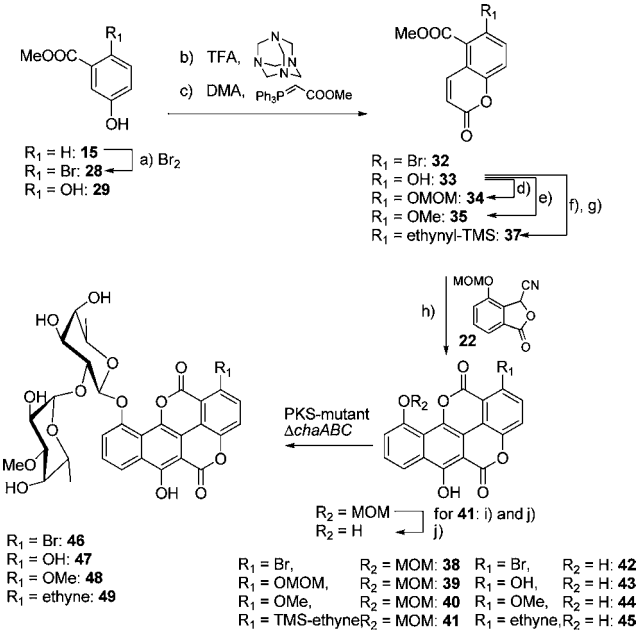
The Hauser annulation of coumarin **21** and phthalide **22** building blocks has proven a viable method to construct the pentacyclic ring system of the chartreusin aglycone.[20,29] Fusion of coumarin **21** and phthalide **22** under Hauser conditions gave 10-methoxymethyl-protected chartarin (**24**) (yield 66%), which was rapidly (<5 min) and nearly quantitatively deprotected by boron tribromide. Pure synthetic chartarin (**12**) was dissolved in dimethylsulfoxide (DMSO) and added to cultures of the Δ*chaABC* mutant (*S. albus*::pXU41/pXU343). HPLC-MS monitoring of the fermentation broth extract showed that the exogenously supplied aglycone was incorporated by the mutant and processed by glycosylation to restore chartreusin biosynthesis (Figure 3B, trace *ii*).

For the synthesis of the desmethyl analog, norchartarin (**25**), we sought to apply the same protocol. We realized, however, that the typically employed route to the coumarin **20** involved a reaction step with only unsatisfactory yields (8.3%). Because of the limitations of reported formylation reactions for non-activated aromatic systems[30] an alternative route was undertaken using a halogenation-Heck reaction sequence.[31] In order to direct the halogen into position C-2, several reagents and conditions were tested. Best results were achieved using 2,4,4,6-tetrabromocyclohexa-2,5-dienone in acetic acid at 50 °C, which yielded the desired product in good quantities and with pronounced regioselectivity. The subsequent Heck reaction provided the coumarin in high yield (78%). Thus, this new protocol is over three times more effective than the previously reported one.

Coumarin **20** was then subjected to the Hauser reaction. After deprotection of **23** the expected structure **25** was verified by NMR and MS analyses, and the surrogate was added to the mutant broth for glycosylation. HPLC-MS analysis indicated the formation of a new compound (**27**) with a molecular mass of 626 amu, which pointed to the successful production of the desmethyl variant (**27**). To verify the identity of **27**, the new compound was isolated from a 7 L fermentation culture supplemented with 70 mg of **25** (100% turnover, 13% isolated yield). The HRESI-MS, ESI-MS,[2] 1D and 2D NMR data as well as the characteristic UV−Vis spectrum fully supported the structure of norchartreusin (**27**).

Mutasynthesis of 1-Ethyne, 1-Bromo, 1-Hydroxy, and 1-Methoxy Derivatives. To introduce an ethynyl residue at position C-1 the corresponding 6-bromo coumarin (**32**) was prepared (Scheme 1). Bromination of methyl 3-hydroxybenzoate yielded **28**, which was formylated under Duff conditions.[29,32] As the reaction in polyphosphoric acid proved to be unsatisfactory trifluoroacetic acid and microwave irradiation were used, which proved to be more efficient. Unexpectedly arduous, however, was the synthesis of the bromo coumarin **32**. The Wittig reaction was hampered by

Scheme 1. Convergent Assembly of 1-Substituted Chartarins[a]



[a]Reagents and conditions: (a)CCl_4, Br_2, 5 h, rt, 57%; (b) trifluoroacetic acid, hexamethylenetetramine, 5−15 min, 120 °C, 29−48%; (c) DMA, Ph_3P=CH−COOMe, 20 min, reflux, 72−83%; (d) MeI, K_2CO_3, DMF, 2 h, rt, 84%; (e) MOMCl, *N*,*N*-diisopropylethylamine (DIPEA), CH_2Cl_2, 2 h, rt, 96%; (f) Tf_2O, DIPEA, CH_2Cl_2, 2 h, rt, 78%; (g) trimethylsilylacetylene, $Pd(PPh_3)_4$, CuI, *N*,*N*-diisopropylamine (DIPA), THF, 18 h, reflux, 63%; (h) phthalide (**22**), coumarin (**32**, **34**, **35** or **37**), (KO*t*-Bu, 18 h, −60 °C to rt, 59−71%; (i) Cs_2CO_3, MeOH, 2 h, rt; (j) BBr_3, CH_2Cl_2, 5 min, 98%.

unwanted transesterification, formation of the coumaric acid ester, and dehalogenation.

By utilizing a series of experiments to optimize the reaction conditions, we found that the regularly used solvent, diethyl aniline, induces the unwanted trans-esterification. Its substitution with dimethyl aniline resulted in only in a minor increase in yield (42% dimethylaniline (DMA) vs 36% diethylaniline (DEtA)). The main challenge associated with this step appeared to be the lactonization of the *o*-coumaric ester and that the electron-withdrawing bromine may be exchanged for a hydrogen (with formation of compound **20**) under basic and thermal conditions.[33] It became obvious that the Wittig reagent needed to be exchanged for an active ester or a related olefination reagent. While there are a large number of active esters, only a few are available in combination with a Wittig reagent.[34,35] Use of *N*-hydroxysuccinimidyl ester and the recently described polymer-bound reagent[34] raised the yield to 61% (Table 1). Particularly advantageous is the workup of this setup, which only requires filtration though a silica gel pad. We found that the use of Bestmann's ylide is even more efficient, providing the bromo coumarin **32** in 71% yield.

With the bromocoumarin at hand the next step was to introduce the ethynyl residue by means of a Sonogashira coupling with trimethylsilylacetylene.[36] Despite numerous attempts using diverse conditions the target compound could not be accessed in this way. Since the synthesis of the more reactive iodinated analog would have been intricate, the corresponding triflate was prepared (see Scheme 1). Starting

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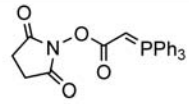
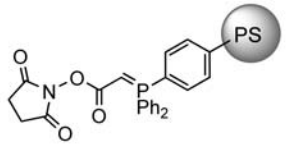
Table 1. Survey of Coumarin Synthesis Starting from the Salicylaldehyde 30a; PS = Polystyrene

Reagent	Conditions	Yield of 32
	DEtA, 20 min 220 °C	36%
	DMA, 20 min 220 °C	42%
	dioxane 2 h, 180 °C	59%
	dioxane 2 h, 160 °C	61%
Ac_2O	1 h, 160 °C	traces
$O{=}C{=}C{=}PPh_3$	THF, 2 h, 80 °C	71%

from methyl 2,5-dihydroxybenzoate (**29**) the established reaction sequence involving formylation and Wittig reaction was employed to produce hydroxycoumarin **33**, which was then converted into triflate **36**. Transformation of **36** into the trimethylsilyl (TMS)-protected ethyne (**37**), annulation, and deprotection provided the desired product. The ethyne was finally processed into the chartreusin derivative by mutasynthesis, and the new compound was isolated and fully characterized.

The hydroxycoumarin **33** proved to be useful for the mutasynthesis of two more chartreusin derivatives. The corresponding methoxymethyl (MOM)- and methoxy-substituted coumarins (**34** and **35**) were prepared and yielded hydroxychartarin (**43**) and methoxychartarin (**44**) after annulation and deprotection. Finally, the bromo-, hydroxy-, and methoxy-substituted chartarins (**42**, **43**, and **44**) were successfully glycosylated using the Δ*chaABC* mutant. All new compounds were characterized using NMR, MS, and UV−Vis techniques.

The structure of 1-bromochartreusin (**46**) was confirmed by X-ray analysis. Initially, we noted that tiny yellow crystals formed in fractions from open column chromatography. By regulating the diffusion process the crystallization conditions were optimized, and it was possible to isolate crystals suitable for X-ray analysis. Given the presence of the bromine substituent it was possible to infer the absolute configuration of **46** (Figure 4) and thus authenticate the glycoside residues of the mutasynthesis products. Notably, this is the first crystal structure of a chartreusin derivative that is not modified in the glycoside residue. As a consequence, this structure proved to be most useful for modeling experiments.

Potential Structural Role of Chartarin Substituents. To unravel a potential structural role of substituents on the pharmacophore the bromo-substituted chartreusin and other analogs were docked into a canonical B-DNA between the T and G nucleotides of the sequence GCGTA**TG**ATGCG. Biding of the glycosyl residue supports intercalation of chartarin between the DNA bases. We noted a cavity at the distal end of the aglycone, which would, in theory, allow binding of 2-substituted chartreusins with the DNA (highlighted in Figure 5).

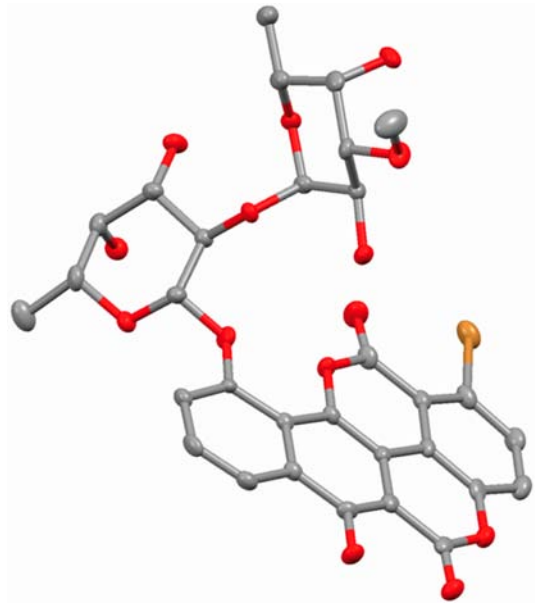


Figure 4. Crystal structure of bromochartreusin (**46**). Each unit cell includes two identical molecules, one molecule acetone and one molecule 3-pentanone.

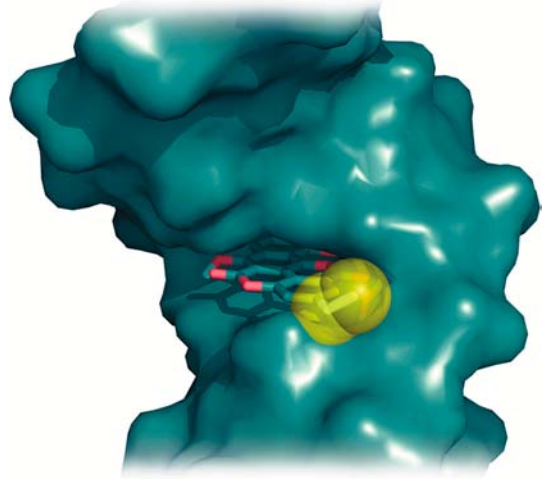


Figure 5. DNA modeling of (1→2) *abeo*-homochartreusin (**71**) into double-stranded DNA. View of the major groove, highlighting free space around the substituent at position 2 (marked in yellow).

Design of 2-Substituted Chartreusin Analogs. Encouraged by the substrate tolerance of the chartreusin glycosylating machinery and the results of the modeling experiments, we next decided to introduce a series of residues at position C-2. This substitution pattern is found naturally in gilvocarcin-type antitumor agents (**4**−**9**, Figure 1). In analogy to these natural archetypes we aimed to prepare chartreusin derivatives with methyl-, ethyl-, or vinyl groups at position C-2 of the aglycone.[18] It was envisaged that using 7-bromo-substituted coumarin as a start point would allow for the introduction of various substituents. Since it is not viable to directly brominate methyl 3-hydroxybenzoate (**15**) at this position, methyl 3-nitrobenzoate (**50**) was halogenated using dibromoisocyanuric acid (Scheme 2). The aniline **52** was generated by Béchamp reduction of the nitro group,[37,38] followed by formation and hydrolysis of the diazonium salt. The well-established Duff formylation was used to prepare the substituted salicylic

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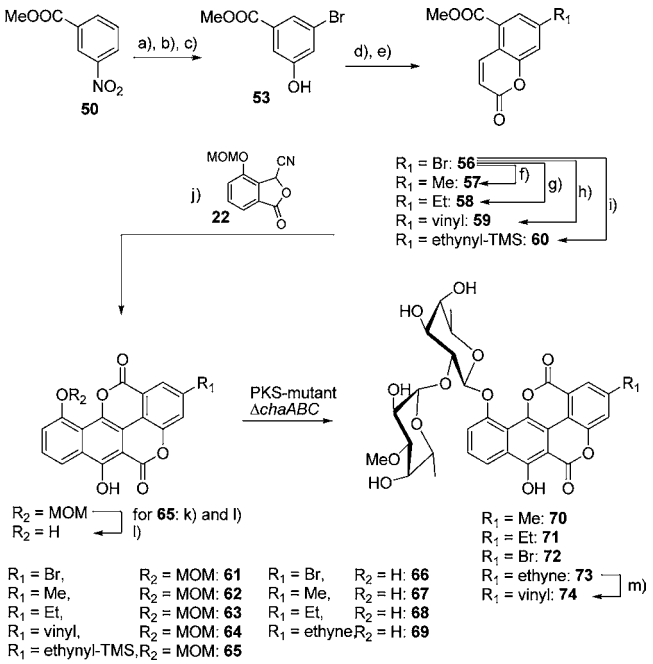
Scheme 2. Convergent Assembly of 2-Substituted Chartarins[a]



[a]Reagents and conditions: (a) H_2SO_4, dibromoisocyanuric acid, 2 h, rt, 86%; (b) Fe, MeOH, HCl, 1 h, reflux, 92%; (c) H_2SO_4, $NaNO_2$, 0 °C, then reflux, 44%; (d) PPA, hexamethylenetetramine, 45 min, 140 °C, 36%; (e) DEtA, $Ph_3P{=}CH{-}COOEt$, 20 min, reflux, 89%; (f) Me_2Zn, $Me_2N(CH_2)_2OH$, $Pd(dppf)Cl_2{\cdot}CH_2Cl_2$, THF, 1h, 60 °C, 94%, dppf = 1,1′-*bis*(diphenylphosphino)ferrocene; (g) Et_2Zn, $(CH_3)_2N$-$(CH_2)_2OH$, $Pd(dppf)Cl_2{\cdot}CH_2Cl_2$, THF, 1 h, 60 °C, 61%; (h) vinylboronic acid pinacol ester, $Pd(PPh_3)_4$, $NaHCO_3$, H_2O, dioxane, 2.5 h, 95 °C, 72%;(i) trimethylsilylacetylene, $Pd(PPh_3)_4$, CuI, DIPA, THF, 18 h, reflux, 83%; (j) phthalide (**22**), coumarin(**56**−**60**), (KO*t*-Bu, 18 h, −60 °C to rt, 60−92%; (k) Cs_2CO_3, MeOH, 2 h, rt; (l) BBr_3, CH_2Cl_2, 5 min 86−96%; (m) Lindlar catalyst, MeOH, H_2, ultrasonic, 2 h, rt, 57%.

aldehyde **54**, which was then converted into the coumarin **56** by Wittig olefination. Using a modified Negishi coupling procedure (via dimethylzinc or diethylzinc) bromocoumarin **56** was converted into methyl- and ethyl-substituted derivatives. A Suzuki reaction using vinylboronic acid pinacol ester was used to introduce the vinyl group. All four coumarins **56**−**60** were subjected to the Hauser annulation protocol. Whereas the alkyl-substituted and brominated coumarins were readily converted, the vinyl derivative **64** could not be produced in this way due to the instability of the reactant and/or the product under these conditions.

Next, we sought to convert 2-bromochartarin **66** into its corresponding vinyl derivative. However, despite testing over 80 different conditions, such as Stille- (n-Bu_3Sn-vinyl) or Suzuki reactions (O'Sheas reagent, vinylboronic acid pinacol ester)[20,39−41] in different solvents, with additives and different catalysts, the vinyl group could not be installed. To bypass this hurdle, a TMS-protected ethynyl group was introduced into the coumarin via a Sonogashira reaction, which granted access to the vinyl residue by alkyne reduction.

DMSO solutions of (1→2) *abeo*-chartarins **66**−**69** were individually added to the mutant broths, and the biotransformations yielded all four expected derivatives (**70**-**73**). To prepare the missing vinyl-substituted candidate the ethynyl-substituted derivative **73** was hydrogenated using Lindlar's catalyst. The identity of all new compounds was verified by their physicochemical data (HRESI-MS, ESI-MS^2, 1D-, 2D-NMR, UV−Vis), which were in full agreement with the proposed structures of (1→2) *abeo*-chartreusins (**70**-**74**).

Evaluation of the Antiproliferative and Cytotoxic Activities of the Novel Chartreusin Variants. The biological activities of all new chartreusin analogs were analyzed in a panel of assays employing different cancer cell lines, including human umbilical vein endothelial cells (HUVEC), human chronic myeloid leukemia in blast crisis (K-562), human cervix carcinoma (HeLa), human colon adenocarcinoma (HT-29), and human melanoma (Mel-HO) cell lines. We found that most of the chartreusin derivatives exhibit strong activity against leukemia and melanoma cell lines, whereas HUVEC and HeLa cells were substantially less sensitive toward this compound class.

Comparative activity profiling revealed that the various derivatives differ in their cytostatic potency and thus allowed for first insights into structure−activity relationships. We found that the substituent at position C-1 of the aglycone plays a crucial role for pharmacological activity. Whereas homochartreusin (**14**) and chartreusin (**1**) were found to be similarly potent, norchartreusin (**27**) proved to be almost inactive. 1-Vinylchartreusin (**75**) and hydroxychartreusin (**43**), in contrast, exhibit strong antiproliferative effects against K-562 cells (GI_{50}: 3.1 and 4.8 μM, respectively). Interestingly, the 1-ethynyl analogue and 1-methoxychartreusin do not display any cytostatic properties. Furthermore, the brominated derivatives are less potent than chartreusin and homochartreusin. Analogs with substituents at position C-2 of the aglycone were also found to possess cytostatic properties. (1→2) *Abeo*-chartreusin (**70**) is as potent as the natural congener. A surprising result was that (1→2) *abeo*-ethynylchartreusin (**73**) shows significantly increased antiproliferative and cytotoxic activities compared to its 1-ethynyl counterpart (**45**).

In addition to the antitumoral activity, the chartreusin derivatives also possess strong antibiotic properties including against methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE). 1-Vinyl- and 2-ethynyl-chartreusin are as active as the natural product and are even more potent than the reference antibiotic ciprofloxacin. A similar scenario becomes apparent in case of the VRE assay. Compounds **1**, **75** (1-vinyl), and the 1-hydroxy derivative **43** are highly active against this strain (MIC 9.59−9.76 μM). An unexpected result is the antibacterial activity of norchartreusin (**27**) against *Mycobacterium vaccae* (2.49 μM). As this compound possesses low cytotoxicity, it may be a promising lead compound for developing an antibiotic.

Impact of Aglycone Substitution and Light on Antitumoral Activity. The vinyl-substituted chartreusin derivative **75** exhibits a substantially increased antiproliferative activity upon photo activation.[20] To evaluate the effect of the substitution site, we analyzed the potential photoreactive properties of the 2-vinyl derivative (**74**) as well as the corresponding alkyne **73**. In the assay a blue gallium(III) nitride (GaN) LED was used since the emission wavelength (420 nm) of the LED matches with the absorption maximum of the chartreusins. Whereas the antiproliferative effect of **1**, **73**−**75** against colon adenocarcinoma cell line HT-29 is rather low in the absence of light (7.0−9.4 μM), incubation in the presence of the blue LED substantially increased the activity of (1→2) *abeo*-vinylchartreusin (**74**) (GI_{50} 3.1 vs 9.4 μM) (Figure

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Table 2. Survey of on Antimicrobial (MIC value [μM]), Cytotoxic (CC_{50} [μM] for HeLa), and Antiproliferative (GI_{50} [μM] for HUVEC, K-562, HT-29, and human melanoma cell line (MEL-HO) Activities of Chartreusin and Its Derivatives[a]

	MRSA	VRE	*M. vaccae*	HUVEC	K-562	HeLa	HT-29		MEl-HO	
light	w/o	w/o	w/o	w/o	w/o	w/o	w/o	w	w/o	w
1	19.5	9.76	1.22	6.24	1.56	12.6	7.96	7.65	1.56	2.34
14	38.2	19.1	9.56	7.18	2.14	23.1				
27	>100	79.8	2.49	>80	31.6	70.7				
42	70.9	35.4	8.87	65.8	8.79	48.2				
43	38.9	9.73	2.43	35.0	4.82	25.5				
44				>78	>78	>78				
45	>100	76.9	19.2	>77	64.1	>77			33.2	26.9
70	78.1	39.0	4.87	5.78	2.03	14.2				
71	>100	>100	19.1	26.6	11.6	58.1				
72	70.9	35.4	17.7	25.1	8.65	26.1				
73	19.2	4.80	19.2	7.84	3.23	13.4	8.61	1.69	2.00	1.38
74	76.6	38.3	19.2	16.9	4.90	33.7	9.35	3.06	1.03	0.48
75	19.2	9.59	4.78	32.8	3.06	24.7	7.05	0.61	0.11	0.08
cip	37.7	2.35	0.60							
van	1.08	>100								
tet	52.0	>100								
dox				0.12	0.12	0.45				
epi				0.29	0.09	0.64				
im				22.1	0.2	78.6				

[a]Test strains: MRSA 134/93; VRE 1528; *M. vaccae* 10670. Cip: ciprofloxacin; van: vancomycin; tet: tetracyclin; dox: doxorubicin; epi: epirubicin; im: imatinib.

6). Similarly, the activity of (1→2) ethynylchartreusin (**73**) was 5-fold higher in the presence of light (GI_{50} 1.7 vs 8.6 μM).

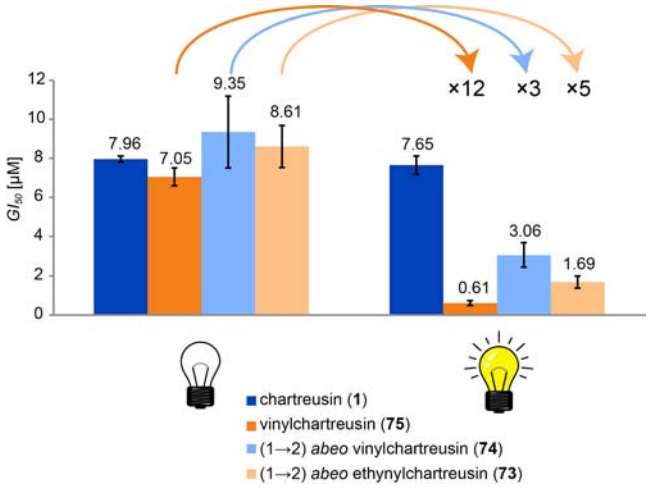

Figure 6. Evaluation of antiproliferative effect and growth inhibition values on colorectal cancer cell line HT-29 with and without light.

Interestingly, an electrophoretic mobility assay (EMSA) using a defined DNA fragment and a HPLC-HRMS-guided photoproduct assay did not give any evidence for a [2 + 2] photo adduct with thymine or other nucleobases.[20,42] Apparently photo activation of the ethynyl derivative opens an alternative reaction channel. It should be highlighted that the ethynyl functionality has been unprecedented in this compound class.

Finally, the interaction between the chartreusin variants and DNA, which is a key mechanism for native **1**, was investigated. Buffered solutions of chartreusin and its derivatives were titrated with a DNA solution, while the UV−Vis spectrum was monitored.[43] This experiment revealed a clear band shift for all chartreusins, thus corroborating their interaction with DNA (see Supporting Information).

CONCLUSION

In this study we have designed 11 new chartreusin analogs by merging the strengths of chemical synthesis with biosynthesis and biological target modeling. Since the ring substitution, namely the methyl residue, cannot be exchanged by current synthetic protocols, we explored, as an alternative, biosynthetic engineering and mutasynthesis. Initially, by deletion of the PKS genes in the *cha* gene locus and cross-complementation with anthracycline biosynthesis genes a polyketide primed with propionate in lieu of acetate was created, and thus an ethyl substituent was installed in the chartarin structure. The strategy of exchanging PKS starter unit cassettes is a powerful approach that has been employed in several impressive biosynthetic engineering studies.[23,44−47] Beyond granting access to a chartreusin analog the successful cross-complementation of the *cha* PKS mutant with an anthracycline PKS has an added value in the context of biosynthesis. Our findings reinforce the biosynthetic model involving the rearrangement of an anthracyclic polyketide scaffold into the rare benzonaphthopyranone chromophore.[19] While it would be, in principle, feasible to expand the size of the priming acyl chain, it is not viable to shorten the chain in order to yield the corresponding chartreusin analog lacking an alkyl residue (desmethyl or norchartreusin (**27**)). Thus, a chemo-biosynthetic route was explored, where the *cha* PKS null mutant, which is not capable of producing the natural chartarin aglycone, was complemented with a synthetic surrogate. For preparation of the chartarin cores, synthetic protocols toward substituted coumarins were optimized, which led to more efficient routes, higher yields, and improved regioselectivity. In all cases the mutant accepted the exogenously supplied aglycone and completed the pathway yielding chartreusin derivatives. Conceptionally related mutasynthesis approaches have been successfully applied for the

exchange of starter and extender units in polyketide and nonribosomal peptide pathways.[24,28,48−59] In this work we report an unparalleled systematic conversion of fully synthetic polyphenolic aglycones into the corresponding glycosides on a preparative scale. This efficient chemo-biosynthetic route not only led to the first crystal structure of a chartreusin derivative with a native glycosyl residue but also generated a library of aglycone-modified chartreusins. Their biological evaluation grants fresh insights into the role of ring substitution of the DNA intercalating unit. For the first time it was possible to deduce structural prerequisites for antitumoral activities of the benzonaphthopyranones. Three new chartreusin derivatives (**14**, **70**, and **73**) show potent antitumoral activity. This finding that norchartreusin lacks cytostatic properties highlights the crucial role of the aglycone substitution pattern for the pharmacological activity. Moreover, we have demonstrated that altering the substituent at position C-1 of the aglycone causes a substantial enhancement in antiproliferative cell selectivity compared to the analogs modified at position C-2. Notably, all chartreusin derivatives interfere with DNA, and two new chartreusin derivatives show an increased antiproliferative activity when irradiated with blue light. One of these compounds harbors an ethynyl group in lieu of an aliphatic group, which has not yet been reported for any photoreactive agents employed in *in vitro* cell assays. The most surprising finding was that the simple lack of the methyl group causes a dramatic decrease in cytotoxicity, but strongly enhances antibacterial activities. Specifically, the desmethyl variant **27** is active against mycobacteria, which are in the focus of antibiotic research due to the rise of tuberculosis and other life-threatening infectious diseases. Thus, the blend of biological and synthetic methods not only granted access to a library of chartreusins that are otherwise not accessible but also yielded promising candidates for further development as antitumoral or antibacterial agents (Figure 7).

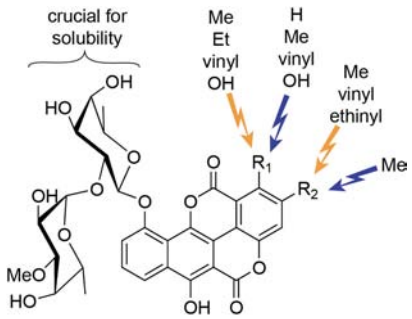

Figure 7. Overview on the impact of ring substituents on biological activity. Orange arrows highlight groups that support antiproliferative activity against K-562 leukemia cells, dark-blue arrows highlight groups that support antibacterial activity toward *M. vaccae*.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details, physicochemical data, biological assays, MS- and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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