Semisynthesis and biological evaluation of amidochelocardin derivatives as broad-spectrum antibiotics

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Abstract: To address the global challenge of emerging antimicrobial resistance, the hitherto most successful strategy to new antibiotics has been the optimization of validated natural products; most of these efforts rely on semisynthesis. Herein, we report the semisynthetic modification of amidochelocardin, an atypical tetracycline obtained *via* genetic engineering of the chelocardin producer strain. We report modifications at C4, C7, C10 and C11 by the application of methylation, acylation, electrophilic substitution, and oxidative C-C coupling reactions. The antibacterial activity of the reaction products was tested against a panel of Gram-positive and Gram-negative pathogens. The emerging structure–activity relationships (SARs) revealed that positions C7 and C10 are favorable anchor points for the semisynthesis of optimized derivatives. The observed SAR was different from that known for tetracyclines, which underlines the pronounced differences between the two compound classes.

Keywords: antibiotics, chelocardin, tetracycline, semisynthesis, natural product, Gram-negative bacteria

Introduction

Efficient antibiotics constitute an indispensable pillar of modern medicine. Therefore, the loss of their efficacy by the emergence of multidrug-resistant bacterial pathogens represents one of the big challenges for global health today.[1-3] Since the current drug-development pipeline is insufficient to counteract antimicrobial resistance,[4] there is an urgent need to find new classes of antibiotics that are able to overcome current resistance mechanisms, ideally by addressing a different mode of action.

Figure 1. Structures of oxytetracycline, chelocardin and amidochelocardin. Different positions selected for chemical derivatization are marked as colored circles.

Natural products of microbial origin have been the richest source of novel antibiotics so far.[5] One of the strategies to discover novel promising natural product-based lead structures is to re-examine molecules, which were reported as potent antibiotics in the past, but not developed further for various reasons ('look back approach'). In this context chelocardin (CHD), a so-called atypical tetracycline produced by actinomycete *Amycolatopsis sulphurea*, attracted our interest: The mode of action of CHD, albeit not entirely elucidated, is most likely different from that of the structurally related tetracyclines (Figure 1), as suggested by the lack of cross-resistance and distinct cellular effects.[6-9] Moreover, CHD has demonstrated efficacy in humans in a past phase 2 study and is active against clinically relevant Gram-positive and Gram-negative pathogens.[10] In spite of the similarity of the CHD and tetracycline[11] scaffolds, important structural differences that induce different 3D pharmacophores include (i) the aromatization of ring C, (ii) the presence of a methyl group at C9 and (iii) the primary amine at C4. An important progress in chelocardin chemistry was achieved by genetic engineering of the producer strain that led to the replacement of the acetyl group at the C2 position of CHD by a carboxamido moiety, as prevalent in tetracyclines. The resulting compound, called amidochelocardin or 2-carboxamido-2-deacetyl-chelocardin (CDCHD), has on average a 2–4 fold increased antibacterial activity compared to CHD. Remarkably, whereas CHD does not show any activity against *Pseudomonas aeruginosa* (>64 $\mu\text{g mL}^{-1}$), CDCHD has a MIC₅₀ of 8 $\mu\text{g mL}^{-1}$. [12] These data have encouraged us to select the CDCHD scaffold as a lead structure for a new broad-spectrum antibiotic, and access new derivatives by a combination of biosynthetic engineering[13] and chemical derivatization of CDCHD. This article will focus on our chemical derivatization efforts. Initial plans for late-stage functionalizations of CDCHD were inspired by the synthetic procedures available for tetracyclines, which were among the first natural compounds to be used as semisynthesis platforms. [14] Since Conover's catalytic hydrogenolysis of auromycin (chlorotetracycline) to tetracycline itself at Pfizer in

1952,[15] many blockbuster antibiotics derived from tetracyclines were obtained by semisynthesis, the most recent being sarecycline[16] and omadacycline[17], both approved by the FDA in 2018. In this study, we report our efforts to functionalize CDCHD at the most tractable positions for derivatization, namely the C4 amino group, at the electron-rich C7 position, and at the C10/C11 positions.

Derivatization of the C4 amino function

Methylation: The primary amine at C4 is the most nucleophilic position in CDCHD. Given the fact that tetracyclines are *N,N*-dimethylated at this position, we hypothesized that a similar substitution would improve the activity of CDCHD. For that purpose, CDCHD was subjected to reductive amination in the presence of paraformaldehyde, triethylamine, and hydrogen gas with palladium on carbon to yield the mono and the di-substituted compounds **1** and **2** (Figure 2A).[18] Harsher agents like methyl iodide led to unselective methylations at a variety of oxygen or nitrogen atoms, even after the protection of the C4-amino group as described below.

Acylation, carbamoylation and sulfonylation: Various functional groups, such as benzyl carbamate, acetyl or methanesulfonamide were introduced under Schotten–Baumann conditions with short reaction times to obtain the respective derivatives **3**, **7** and **12** in yields between 16 and 38%.[19] The ethyl carbamate **4** and the arylamide **11** were prepared by reacting CDCHD with ethyl chloroformate or 4-methoxybenzoyl chloride in THF in the presence of DIPEA in 24 and 31% yields, respectively. The imides **5** and **6** were synthesized by heating CDCHD with maleic anhydride at 100 °C or phthalic anhydride at 120 °C in 15 and 18% yields, respectively. Coupling of CDCHD with *N*-Boc- β -Ala using propylphosphonic anhydride (T3P) in a mixture of EtOAc and pyridine at 40 °C followed by Boc-deprotection using TFA afforded β -alanine amide **8** in 5% yield over two steps. The 4-methylpiperazine-1-acetamide **9** and acrylamide **10** were obtained from the reaction between CDCHD and the corresponding acid chloride in *N*-methyl-2-pyrrolidone (NMP) in 7 and 5% yields, respectively (Figure 2A).

Derivatization at the C7 position

The C7 position has been a hotspot in tetracycline derivatization, as the chloro-substituent in the first congener has been substituted with hydrogen, dimethylamine, fluorine, or aminoxyalkyl residues. Thus, an aromatic substitution was attempted at the electron-rich position with various electrophiles, beginning with sources of halogens (Figure 2B).

Aromatic electrophilic substitution: To introduce fluorine at the C7 position, the C4 amino group was protected with Fmoc in presence of sodium bicarbonate in a mixture of acetonitrile and water with a yield of 27%. An electrophilic fluorination was performed with xenon difluoride in 6% yield.[20, 21] Deprotection of the amine function in presence of piperidine in *N,N*-dimethylformamide afforded compound **13** in 26% yield. An alternative protocol used Selectfluor[®] in acetonitrile to directly fluorinate the unprotected compound to give **13** in 15% yield.

Figure 2: Chemical derivatizations of CDCHD. a) (HCHO)_n, NEt₃, [Pd/C], H₂ 1 atm, MeOH, rt, 18 h, 4% (**1**) and 11% (**2**); b) CbzCl, NaHCO₃, THF/H₂O, rt, 30 min, 30%; c) Ethyl chloroformate, DIPEA, THF, rt, overnight, 24%; d) Maleic anhydride, acetic acid, 100 °C, 48 h, 15%; e) Phthalic anhydride, DIPEA, 120 °C, 1 h, 18%; f) AcCl, Na₂CO₃, THF/H₂O, rt, 30 min, 38%; g) 1) *N*-Boc-β-Ala, T3P, EtOAc, pyridine, 40 °C, 17 h, 22% 2) TFA, DCM, rt, 1h, 21%; h) 4-Methylpiperazine-1-acetyl chloride, NMP, rt, 16 h, 7%; i) Acryloyl chloride, NMP, 0 °C – rt, 18h, 5%; j) 4-Methoxybenzoyl chloride, DIPEA, THF, rt, overnight, 31%; k) MsCl, NaHCO₃, THF/H₂O, rt, 30 min, 16%; l) 1) Fmoc-Cl, NaHCO₃, MeCN/Water 4:1, rt, 30 min, 27% 2) XeF₂, DCE, rt, 10 min, 6% 3) 20% piperidine in DMF, rt, 1 h, 26%; m) NCS, TFA, rt 30 min, **28%**; n) NBS, TFA, rt, 30 min, 17%; o) NIS, TFA, rt, 30 min, 17%; p) Nitric acid, sulfolane, 0 °C, 15 min, 4%; q) PhI(OAc)₂, AcOH, ArH, HFIP, 3h, rt, 15% (**11**), 13% (**12**); r) 1) Boc₂O, NEt₃, THF, 1h, 40%, 2) Si(tBu)₂(OTf)₂, NEt₃, DMF, 12%, 3) TFA, DCM, rt, 1h, 39%; s) AcCl 10 equiv., HFIP, rt, 9%; t) 4-Br-phenylacetyl chloride 10 equiv., HFIP, rt, 11%.

Using trifluoroacetic acid as solvent and stoichiometric amounts of the corresponding *N*-halosuccinimide, the desired chloro- and bromo- derivatives **14** and **15** were isolated in moderate yields.[22] However, the application of *N*-iodosuccinimide under otherwise identical reaction conditions did not yield an iodinated amidochelocardin, but the quinone **17** as the major product. We hypothesized that an iodinated compound may be formed initially, that was, however, prone to further oxidation by *N*-iodosuccinimide and subsequent hydrolysis to result in the quinone scaffold.

Finally, CDCHD was also subjected to nitration using fuming nitric acid in sulfolane at 0 °C.[23] The desired molecule **16** was obtained in a poor yield of 4%. In spite of several attempts to reduce **16**, we could not isolate the respective 7-amino analog.

C-C cross-coupling attempts: The halogenated compounds **14** and **15** opened promising perspectives for further functionalization by metal-catalyzed cross-coupling reactions (Figure 3). Different conditions were tried for Suzuki–Myaura reactions with phenylboronic acid, using palladium[24] or nickel[25, 26] catalysts, but coupling products were not observed by LC-MS, neither with the free C4-amino amidochelocardin nor with the Boc-protected version. Instead, the debrominated compound was formed, indicating that an oxidative insertion was possible, but subsequent steps were unsuccessful. Other types of coupling reactions were attempted, such as Buchwald–Hartwig reactions with various nucleophiles (secondary amines, anilines,[27] thiols[28] and cyanide[29]), but turned out to be non-productive. Stille reactions[30, 31] were performed with the corresponding vinyl and phenyl tin reagents but led predominantly to a dehalogenated product. Ultimately, Ullmann-type reactions were tried in the presence of sodium azide,[32] in order to take advantage of the high nucleophilicity of this species. But even with a stoichiometric amount of copper iodide, no conversion to the desired compound was achieved. Confronted with these difficulties and the additional challenge to work with a small amount of starting material, we examined alternative strategies to form carbon-carbon bonds on the naphthalene-diol moiety.

Figure 3: Summary of attempts to perform metal-catalyzed couplings on C7-halogenated derivatives of CDCHD.

Metal-free C-H functionalization: Direct CH-functionalizations appeared as relevant transformations for our substrate,[33] especially as some do not require a protection of the C4-amino group. A metal-free method using hypervalent iodine species for coupling phenols to nucleophilic arenes was reported in 2013 by Morimoto *et al.* [34, 35] According to the proposed mechanism, the iodine (III) reagent allows the formation of a cationic phenoxenium intermediate, which can then react with various electron-rich arenes (Figure 4).

Figure 4: Proposed mechanism for metal free CH-arylation

Indeed, with the substrates mesitylene and anisole, the desired coupling products **18** and **19** were obtained in low yields of 15% and 13%, respectively (Figure 2B). However, with less nucleophilic substrates like furan, propargylic alcohol or malonates, the corresponding coupling products were not obtained.

Derivatization at the C10 and C11 hydroxy functions

The modification at the C10 and C11 position followed two objectives. First, functional group protection was sought that facilitates derivatization reactions at other positions. Second, the importance of the free OH groups for antibacterial activity was probed. We note that for tetracyclins, the respective positions are mandatory for activity.

Bis-protection: Trost *et al.* described the use of the di-*tert*-butylsilylene protecting group in the preparation of a tetracycline-derived scaffold called deoxypillaromycinone.[36] They investigated particularly the reactivity of various oxygen functions towards silylation and remarkably, naphthalene-1,8-diols were found to be more reactive than aliphatic 1,3- or 1,2-diols. To avoid any issue of selectivity, the C4-amino function of CDCHD was Boc-protected first. Next, the aromatic diol was silylated with $\text{Si}(\text{tBu})_2(\text{OTf})_2$ in the presence of trimethylamine in DMF (Figure 2C). Cleavage of the Boc protecting group in presence of TFA in methylene chloride gave, after purification by preparative HPLC, the silylene derivative **20** in 39% yield.

Selective esterification at the C10 position: CDCHD was subjected to Friedel–Crafts acylation in the presence of aluminum chloride and acylchloride under heating conditions. Whereas CDCHD was not converted into the C7-acylated product, the use of an excess of acetyl chloride in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) at room

temperature afforded the corresponding O10-acylated ester **21** in a selective manner (Figure 2C).[37] The same conditions were applied with 4-bromobenzoyl chloride to give the ester **22** in 11% yield. The regioselectivity of these reactions was proven by a shift of the C10 signal in ^{13}C -NMR ($\delta = 157$ ppm in CDCHD and $\delta = 148$ ppm in **22** using methanol- d_4 as solvent) and by a ^1H NOESY experiment that exhibited a correlation between protons of the methyl group at C9 and the *ortho*-proton of the benzoyl ester (see supporting information).

Antimicrobial activity of semisynthetic analogues

The antibiotic activity of each compound against a panel of clinically relevant Gram-positive and Gram-negative bacteria is summarized in Table 1 (MIC values in μM are given in Table S1 of the Supporting information). The minimal inhibitory concentration (MIC) data for the *N*-substituted derivatives **1–12** showed that most modifications at the C4-amino group were not tolerated and dramatically decreased the activity against Gram-negative pathogens ($>64 \mu\text{g/mL}$ against *Klebsiella pneumoniae* and *P. aeruginosa*). Only small aliphatic substituents with 3–4 atoms (**4**, **8** and **10**) displayed moderate-weak activities against *K. pneumoniae* and *Escherichia coli* (MIC 16–32 $\mu\text{g/mL}$). On the other hand, the activity against Gram-positive strains (*Enterococcus faecium* and *Staphylococcus aureus*) was improved, or at least retained, compared to the parent compounds CHD and CDHCD by introducing bulky hydrophobic substituents such as the carbamates (**3** and **4**), imides (**5** and **6**) and the amides (**10** and **11**) showing very favorable MIC values of 0.125–8 $\mu\text{g/mL}$. On the contrary, small or polar substituents severely diminished the activity against Gram-positive species (**1**, **2**, **7–9** and **12**). The reduction of susceptibility in Gram-negative compared to Gram-positive bacteria could be due to poor permeation of the compounds through the Gram-negative cell wall as indicated by regaining the activity of **5**, **6** and **10** against *E. coli* in the presence of the permeability enhancer PMBN. Moreover, higher activities of CHD and CDCHD against the efflux mutant *P. aeruginosa* PA14 ΔmexAB compared to the wild type strain *P. aeruginosa* PA14 imply that efflux plays a role for this chemical class. Remarkably, compound **2**, which possesses the same *N,N*-dimethyl feature as the typical tetracyclines, was found to be inactive, thus underlining the potential difference in mode of action between CDCHD and the tetracyclines. These data are consistent with a previous report on four acylated derivatives of CDHCD.[13] Within the C7-substituted, halogenated compound series **13–15**, potency seemed to decrease with the atom size ($\text{F} > \text{Cl} > \text{Br}$). In particular, the fluorinated compound showed comparable activities to the parent CDCHD and even had slightly higher potency against *S. aureus* or *P. aeruginosa*. However, an electron-withdrawing group at C7 is not generally favorable, because the introduction of a nitro group as in **16** led to a complete loss of activity (MIC $> 64 \mu\text{g/mL}$ against all strains). The increase of the size of the substituent at C7, as in **18** and **19**, also led to a drop in MIC values against Gram-negative bacteria. Nevertheless, **18** recovered potency against *E. coli* in the presence of the membrane permeabilizer (PMBN), highlighting a potential size limitation in the compound uptake.

Tetracyclines are known to complex Mg^{2+} cations with the hydroxy functions of the Southern half of the molecule in order to cross the outer membrane of Gram-negative pathogens *via* the porin channels OmpF and

OmpC.[38] The analogs **20–22** were tested to find out whether equivalent essential pharmacophore points exist in amidochelecardins. The quinone **17** showed poor or no activity against *S. aureus*, *K. pneumoniae* and *P. aeruginosa*. The relevance of the phenols for antibacterial activity was further underlined by the silylated **20**, where both phenolic functions are blocked. Indeed, only activity against the Gram-positive *S. aureus* could be detected (1 µg/mL), whereas the activity against Gram-negative pathogens was abolished. A MIC value of 8 µg/mL was measured against *E. coli* in the presence of PMBN, implying that the molecule could traverse a permeabilized outer membrane without metal chelation. Finally, the C10-acylated **21** showed moderate MIC values of 16 µg/mL against Gram-positive strains, no activity against *P. aeruginosa*, and promising activity of 8 µg/mL against *E. coli*. Activities against both Gram-negative strains tested were improved in the presence of PMBN, pointing out again an uptake issue by Gram-negative pathogens. Thus in principle, a modified C10 position is compatible with anti-Gram-negative activity. However, the enlargement of the acyl-substituent as in **22** is detrimental for activity against *P. aeruginosa* and *E. coli*, while MIC values of 4 µg/mL were measured against *E. faecium* and *S. aureus*. In order to exclude that the antibiotic activity of **21** and **22** is caused by CDCHD released upon cleavage of the ester function under the experimental conditions, we performed stability measurements in bacterial media. Thus, **21** and **22** incubated with medium and medium with *E. coli* were re-extracted and analyzed by LC-MS. Both chromatograms revealed the presence of intact **21** or **22**, respectively, and none of them contained a peak of the parent compound CDCHD, confirming the stability of the ester function under the assay conditions (Figure 5).

Conclusions

This study reports structure–activity relationships of the ‘atypical tetracycline’ amidochelecardin based on its semisynthetic modification. Notable differences between the CDCHD and the tetracycline SAR supports the biologically-driven hypothesis of their different modes of action. Indeed, we could establish that the replacement of the free amine at C4 by a dimethylamino group was detrimental for the activity of amidochelecardin against Gram-negative bacteria. We also highlight that small substitutions at the C7 position as well as ester functions at the O10 position were tolerated and retained in both Gram-positive and -negative pathogens. A fluorine substitution at C7 was particularly favorable for antibacterial activity. These positions are currently investigated as anchors for attaching a tag to perform target fishing experiments, which should allow elucidation of the mode of action of these atypical tetracyclines. In general, most reactions had moderate–low yields. This underlines the difficulties of modifying and purifying unprotected, highly functionalized natural products by semisynthesis on small reaction scales of typically 20 mg (or even less) CDCHD. It also implies that the establishment of a total synthesis route to (amido)chelecardin is a worthwhile endeavor to optimize this promising lead structure.

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Table 1 : MIC (Minimum Inhibitory Concentration) values of semisynthetic CDCHD derivatives against bacterial pathogens in µg/mL

X = CH₃ for chelocardin (CHD)

X= NH₂ for amidochelocardin (CDCHD) and compounds **1-22**

Cmpd	R ¹	R ²	R ³	<i>E. faecium</i> DSM-20477	<i>S. aureus</i>	<i>K. pneumoniae</i> DSM-30104	<i>P. aeruginosa</i>	<i>E. coli</i>
CHD (Na-citrate)	NH ₂	H	H	8	8 ^c	2	64 ^d ; 4 ^f	2 ^g
CDCHD (Na-citrate)	NH ₂	H	H	4	8 ^c	2	16 ^d ; 0.5 ^f	0.5 ^g
1	NHCH ₃	H	H	> 64	> 64 ^a	> 64	> 64 ^d	-
2	N(CH ₃) ₂	H	H	> 64	> 64 ^a	> 64	> 64 ^d	-
3	NHCBz	H	H	0.25	> 64 ^c	> 64	> 64 ^d	-
4	NHCO ₂ Et	H	H	-	2 ^c	16	> 64 ^d ; 64 ^f	-
5	Maleimide	H	H	-	2 ^c	64	> 64 ^{d,f}	> 64 (8) ^h
6	Phthalimide	H	H	-	0.125 ^c	> 64	> 64 ^{d,f}	> 64 (0.5) ^h
7	NHAc	H	H	32	> 64 ^c	> 64	> 64 ^d	-
8	NHCO(CH ₂) ₂ NH ₂	H	H	> 64	64 ^b	-	32 ^f	16 (8) ^g
9	NHCOCH ₂ N (C ₂ H ₄) ₂ NCH ₃	H	H	> 64	> 64 ^b	-	32 ^f	64 (32) ^g
10	NHCOCH=CH ₂	H	H	8	4 ^b	-	> 64 ^f	32 (2) ^g
11	NHCO-(4- MeOC ₆ H ₄)	H	H	-	4 ^c	> 64	> 64 ^d ; 64 ^f	-
12	NHSO ₂ CH ₃	H	H	32	> 64 ^c	> 64	> 64 ^d	-
13	NH ₂	F	H	-	4 ^c	8	8 ^d ; 4 ^f	-
14	NH ₂	Cl	H	-	4 ^b	8 (8)	16 ^c	-
15	NH ₂	Br	H	-	8 ^b	8 (4-8)	32 ^c	-
16	NH ₂	NO ₂	H	> 64	> 64 ^c	> 64	> 64 ^d	-
17	NH ₂	Quinone	-	-	32 ^b	> 64 (64)	> 64 ^c	-
18	NH ₂	Mesityl	H	-	0.5 ^c	32-64	> 64 ^d	>64 (8) ^h

19	NH ₂	4-MeO- C ₆ H ₄	H	-	1 ^c	> 64	> 64 ^d	32 (2) ^h
20	NH ₂	H	Si(<i>t</i> Bu) ₂ - O _(C11)	-	1 ^c	> 64	> 64 ^d	64 (8) ^h
21	NH ₂	H	Ac	16	16 ^b	-	> 64 (1) ^f	8 (4) ^g
22	NH ₂	H	CO(4- BrC ₆ H ₄)	4	4 ^b	-	> 64 (>64) ^f	> 64; (64) ^g

^a *S. aureus* DSM-346, ^b *S. aureus* Newman, ^c *S. aureus* DSM-11822, ^d *P. aeruginosa* PA14, ^e *P. aeruginosa* DSM-1128, ^f *P. aeruginosa* PA14ΔmexAB, ^g *E. coli* DSM-1116, ^h *E. coli* ΔTolC. MIC values between brackets were measured in the presence of the outer membrane permeabilizer PMBN (polymyxin B nonapeptide).

Figure 5: A) Superimposed LC-MS chromatograms of CDCHD in stock solution (red), **21** in stock solution (purple), re-extraction of **21** from the medium (blue) and re-extraction of **21** from the *E. coli* suspension (green); B) superimposed LC-MS chromatograms of CDCHD in stock solution (red), **22** in stock solution (purple), re-extraction of **22** from the medium (blue) and re-extraction of **22** from the *E. coli* suspension (green).

Experimental section

Microbiological assays. All bacterial indicator strains were obtained from the German Collection of Microorganisms and Cell Cultures (*Deutsche Sammlung von Mikroorganismen und Zellkulturen*, DSMZ) or were part of the internal strain collection. Minimal inhibitory concentrations (MIC) were determined by microbroth dilution assays. Overnight cultures were diluted in Mueller-Hinton broth (0.4 % beef extract, 0.15 % starch, 1.75 % casein hydrolysate, pH 7.4) to achieve a final inoculum of ca. 5×10^6 CFU/mL. Serial dilutions of each compound were prepared from H₂O stocks (Na-CHD/Na citrate and Na-CDCHD/Na citrate) or MeOH stocks (all semi-synthetic derivatives) in sterile 96-well plates. The cell suspension was added and microorganisms were grown for 16-18 h at 30 or 37 °C. Growth inhibition was assessed by visual inspection and MIC values were determined as the lowest concentration of antibiotic at which no visible growth was observed.

Chemistry. General information. Unless otherwise noted, all reagents were purchased from commercial suppliers and used without further purification.

The starting material CDCHD was produced by fermentation on a gram scale as described before in Lesnik et al., *Angew. Chem. Int. Ed.* 2015.

All solvents used for workup and purification were of HPLC grade. Moisture-sensitive reactions were performed in dried glassware under argon atmosphere, unless otherwise noted. Preparative RP high-pressure liquid chromatography (HPLC) was performed on a Phenomenex Gemini C18 RP-column 00G-4436-NO, 10 μ m, 110 A, 250×10.00 mm (5 mL/min) or on a Phenomenex Gemini C18 RP-column 00G-4435-PO-AX, 5 μ m, 110 A, 250×21.20 mm (10 mL/min) or on a Thermo Fisher Scientific BDS Hypersil C18 RP-column 28105-259370, 5 μ m, 250×30 mm, (25 mL/min) or a Macherey-Nagel Nucleosil 100-7 VP C18 RP column 715691-1116949, 250×40 mm (from 25 to 50 mL/min) using a Thermo Fisher Scientific Dionex Ultimate 3000 HPLC system. Eluents, gradients and additives are given in parentheses. The product-containing fractions were combined, diluted with milliQ H₂O (min. 1:1/solvent:H₂O), frozen and lyophilized. **In some cases, in particular concerning compounds 13-22, the products did not differ much in polarity from the starting material and co-eluted with it. Considering furthermore the limited amounts of starting material and the moderate-low yields of conversions, we could not avoid the presence of <10% impurities.** Nuclear Magnetic Resonance (NMR) spectra were recorded on a Bruker Avance III, a Bruker Avance III HD with cryoprobe system, or Bruker Avance Neo 500 MHz with prodigy cryoprobe system. ¹H NMR spectra were recorded at 500 MHz and 700 MHz. ¹³C NMR spectra were recorded at 126 MHz and 176 MHz. Chemical shifts are reported in ppm relative to the solvent signal. Multiplicity is indicated as follows: s (singlet); bs (broad singlet); d (doublet); t (triplet); q (quartet); m (multiplet); dd (doublet of doublets), etc. Liquid chromatography-coupled high resolution mass spectrometry (HRMS) data were recorded using a Dionex Ultimate 3000 HPLC system equipped with a DAD detector and a Bruker maXis HD QTOF mass detector with electrospray ionization (ESI) or a Q Exactive Focus (Thermo Scientific) mass spectrometer.

(4*R*,4*aS*,12*aS*)-3,10,11,12*a*-Tetrahydroxy-6,9-dimethyl-4-(methylamino)-1,12-dioxo-1,4,4*a*,5,12,12*a*-hexahydrotetracene-2-carboxamide [1] and **(4*R*,4*aS*,12*aS*)-4-(dimethylamino)-3,10,11,12*a*-tetrahydroxy-6,9-dimethyl-1,12-dioxo-1,4,4*a*,5,12,12*a*-hexahydrotetracene-2-carboxamide [2]** Paraformaldehyde (22 mg) was added to a solution of CDCHD (20 mg, 1 equiv) in methanol (4 mL) and triethylamine (40 μ L, 6 equiv) under nitrogen atmosphere. The resultant mixture was sonicated, followed by the addition of palladium on carbon. The flask was then bubbled with hydrogen for 10 min and then it was allowed to stir under hydrogen gas balloon pressure at room temperature for 16 h. Reaction was filtered through a pad of celite and the filtrate was evaporated under vacuum. The crude was then purified by preparative HPLC using acetonitrile / water (0.1% formic acid) with a gradient from 20 to 90% of acetonitrile to afford the mono-methylated compound as a yellow solid (1 mg, 4 %) and the dimethylated compound as a yellow solid (1.5 mg, 11 %). Mono-methylated compound [1]: $^1\text{H NMR}$ (700 MHz, Methanol- d_4) δ 7.46 (d, J = 8.6 Hz, 1H), 7.22 (d, J = 8.7 Hz, 1H), 3.32 (dd, J = 14.6, 3.5 Hz, 1H), 3.09 (dd, J = 14.6, 11.8 Hz, 1H), 3.00 (dd, J = 11.7, 3.4 Hz, 1H), 2.54 (s, 3H), 2.36 (s, 3H), 2.34 (s, 3H). The proton at C4 is not visible because it is hidden by the water peak at 4.87 ppm. $^{13}\text{C NMR}$ (176 MHz, Methanol- d_4) δ 176.5, 175.1, 151.8, 150.7, 134.4, 130.1, 130.0, 124.4, 119.2, 118.8, 117.3, 116.3, 92.6, 85.1, 64.9, 56.54, 26.8, 25.4, 16.0, 16.0, 12.8. **HRMS (ESI)** calculated for $\text{C}_{22}\text{H}_{23}\text{N}_2\text{O}_7$ (M+H) 427.1500, found 427.1493. Dimethylated compound [2]: $^1\text{H NMR}$ (700 MHz, Methanol- d_4) δ 7.47 (d, J = 8.6 Hz, 1H), 7.23 (d, J = 8.7 Hz, 1H), 3.31 (dd, J = 13.0, 2.0 Hz, 1H), 3.08 – 3.00 (m, 2H), 2.95 (s, 3H), 2.53 (s, 3H), 2.36 (s, 3H), 2.32 (s, 3H). The proton at C4 is not visible because it is hidden by the water peak at 4.87 ppm. $^{13}\text{C NMR}$ (176 MHz, Methanol- d_4) δ 174.9, 174.0, 151.6, 150.7, 134.3, 134.1, 130.1, 129.9, 124.1, 119.2, 118.5, 117.2, 116.2, 92.5, 85.2, 66.5, 54.5, 30.0, 26.6, 15.9, 15.8, 12.6. **HRMS (ESI)** calculated for $\text{C}_{23}\text{H}_{25}\text{N}_2\text{O}_7$ (M+H) 441.1656, found 441.1662.

Benzyl((1*R*,4*aS*,12*aS*)-3-carbamoyl-2,4*a*,6,7-tetrahydroxy-8,11-dimethyl-4,5-dioxo-1,4,4*a*,5,12,12*a*-hexahydrotetracen-1-yl)carbamate [3] An aqueous solution of NaHCO_3 (7.5 mg, 7 equiv) was slowly added to a vigorously stirred solution of CDCHD (5 mg, 1 equiv) and benzoyl chloride (10 μ L) in THF/water 5:1 (3 mL). The reaction mixture was stirred at RT for 30 min and then acidified to pH 2 with a 5 N HCl solution. The reaction mixture was then extracted with methylene chloride (2x), and the combined organic layers were dried over sodium sulfate, filtered and evaporated under vacuum. The crude compound was purified by preparative HPLC using acetonitrile / water (0.1% formic acid) with a gradient from 20 to 90% of acetonitrile to afford the pure product as a yellow solid (2 mg, 30%). $^1\text{H NMR}$ (500 MHz, Methanol- d_4) δ 7.53 – 7.30 (m, 7H), 5.38 (d, J = 3.2 Hz, 1H), 5.21 (s, 2H), 3.44 – 3.36 (m, 1H), 3.01 (dt, J = 12.4, 4.9 Hz, 1H), 2.78 – 2.67 (m, 1H), 2.36 (d, J = 4.8 Hz, 3H), 2.34 (s, 3H). $^{13}\text{C NMR}$ (126 MHz, Methanol- d_4) δ 175.1, 164.2, 159.2, 156.6, 139.0, 138.2, 136.3, 130.9, 129.5, 129.1, 129.0, 123.7, 120.9, 115.6, 113.0, 109.9, 67.9, 43.5, 26.7, 15.6, 14.2. **HRMS (ESI)** calculated for $\text{C}_{29}\text{H}_{27}\text{N}_2\text{O}_9$ (M+H) 547.1711, found 547.1714.

Ethyl((1*R*,4*aS*,12*aS*)-3-carbamoyl-2,4*a*,6,7-tetrahydroxy-8,11-dimethyl-4,5-dioxo-1,4,4*a*,5,12,12*a*-hexahydrotetracen-1-yl)carbamate [4] Ethyl chloroformate (5.2 μ L, 1.5 equiv) and DIPEA (32 μ L, 5 equiv) were added to a solution of CDCHD (15 mg, 1 equiv) in THF (3 mL). The resulting mixture was stirred at room

temperature overnight. After concentration under vacuum, the reaction mixture was extracted with EtOAc and the organic layer was washed with saturated aqueous NaCl solution dried over sodium sulfate, filtered and evaporated under vacuum. The crude compound was purified by preparative HPLC using Biphenyl column, MeOH / water (0.05 % formic acid) with a gradient from 15 to 95% of MeOH to afford the pure product as a yellow solid (4.3 mg, 24%). **¹H NMR (500 MHz, Acetone-*d*₆)** δ 10.11 (*br s*, 1H), 9.27 (*s*, 1H), 8.14 (*br s*, 1H), 7.51 (*d*, *J* = 8 Hz, 1H), 7.36 (*d*, *J* = 8.5 Hz, 1H), 6.86 (*br s*, 1H), 6.12 (*br s*, 1H), 5.53 (*br s*, 1H), 4.13 (*q*, *J* = 7.0 Hz, 2H), 3.55 (*br d* = 17.0 Hz, 1H), 3.06 (*br s*, 1H), 2.98-2.71 (*m*, 1H), 2.36 (*s*, 3H), 2.29 (*s*, 3H), 1.24 (*t*, *J* = 7.0 Hz, 3H). **¹³C NMR (126 MHz, Acetone-*d*₆)** δ 196.3, 190.4, 175.0, 164.2, 157.8, 156.4, 138.7, 136.2, 131.0, 123.3, 120.5, 115.3, 112.7, 109.5, 98.7, 79.4, 61.4, 53.5, 43.1, 26.5, 15.5, 15.0, 14.2. **HRMS (ESI)** calculated for C₂₄H₂₅N₂O₉ (M+H) 485.1560, found 485.1531.

(4*R*,4*aS*,12*aS*)-4-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-3,10,11,12*a*-tetrahydroxy-6,9-dimethyl-1,12-dioxo-1,4,4*a*,5,12,12*a*-hexahydrotetracene-2-carboxamide [5] Maleic anhydride (16 mg, 3 equiv) was added to a solution of CDCHD (22 mg, 1 equiv) in acetic acid (4 mL). The resulting mixture was stirred at 100 °C for 2 days. After cooling, the reaction mixture was extracted with EtOAc, and the organic layer was washed with saturated aqueous NaCl solution, dried over sodium sulfate, filtered and evaporated under vacuum. The crude compound was purified by preparative HPLC using Biphenyl column, MeOH / water (0.05 % formic acid) with a gradient from 50 to 95% of MeOH to afford the pure product (4 mg, 15%). **¹H NMR (500 MHz, Acetone-*d*₆)** δ 10.19 (*br s*, 1H), 9.63 (*s*, 1H), 8.46 (*br s*, 1H), 7.56 (*d*, *J* = 8.5 Hz, 1H), 7.42 (*d*, *J* = 8.0 Hz, 1H), 6.98 (*s*, 2H), 5.89 (*br s*, 1H), 4.88 (*br s*, 1H), 3.41 (*br s*, 1H), 3.33 (*dd*, *J* = 4.5; 17.0 Hz, 1H), 3.10 (*d*, *J* = 17.0 Hz, 1H), 2.33 (*s*, 3H), 2.31 (*s*, 3H). **¹³C NMR (126 MHz, Acetone-*d*₆)** δ 174.5, 171.1, 166.8, 156.8, 139.2, 136.4, 136.0, 135.2, 129.6, 120.8, 120.8, 115.6, 112.8, 109.1, 76.8, 52.4, 41.6, 24.7, 15.5, 14.4. **HRMS (ESI)** calculated for C₂₅H₂₁N₂O₉ (M+H) 493.1247, found 493.1218.

(4*R*,4*aS*,12*aS*)-4-(1,3-Dioxoisindolin-2-yl)-3,10,11,12*a*-tetrahydroxy-6,9-dimethyl-1,12-dioxo-1,4,4*a*,5,12,12*a*-hexahydrotetracene-2-carboxamide [6] To CDCHD (17 mg, 1 equiv), phthalic anhydride (31 mg, 5 equiv) and DIPEA (36 μL, 5 equiv) were added. The resulting mixture was heated at 120 °C for 1 h. After cooling, the reaction mixture was extracted with EtOAc, and the organic layer was washed with saturated aqueous NaCl solution, dried over sodium sulfate, filtered and evaporated under vacuum. The crude compound was dissolved in MeCN, filtered and purified by preparative HPLC using Biphenyl column, MeOH / water (0.05 % formic acid) with a gradient from 70 to 95% of MeOH to afford the pure product (4.0 mg, 18 %). **¹H NMR (500 MHz, Acetone-*d*₆)** δ 10.19 (*br s*, 1H), 9.66 (*s*, 1H), 8.49 (*br s*, 1H), 8.05-7.79 (*m*, 4H), 7.57 (*d*, *J* = 8.0 Hz, 1H), 7.43 (*d*, *J* = 7.5 Hz, 1H), 5.92 (*br s*, 1H), 5.09 (*br s*, 1H), 3.57 (*br s*, 1H), 3.44-3.16 (*m*, 2H), 2.32 (*s*, 3H), 2.31 (*s*, 3H). **¹³C NMR (126 MHz, Acetone-*d*₆)** δ 174.6, 168.2, 166.6, 156.8, 139.2, 136.4, 135.6, 135.5, 132.9, 132.5, 129.8, 124.4, 124.1, 120.8, 115.7, 112.8, 109.2, 76.9, 52.8, 41.5, 24.8, 15.5, 14.4. **HRMS (ESI)** calculated for C₂₉H₂₃N₂O₉ (M+H) 543.1404, found 543.1376.

(4R,4aS,12aS)-4-Acetamido-3,10,11,12a-tetrahydroxy-6,9-dimethyl-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide [7] To a solution of CDCHD (10 mg, 1 equiv) and acetyl chloride (3 μ L, 1.5 equiv) in THF (1 mL) was added dropwise an aqueous solution of sodium carbonate (100 μ L, 0.7 M). The reaction mixture was stirred at room temperature for 30 min. It was then acidified to pH 2 with a 5 N HCl solution and diluted with methylene chloride (5 mL). The organic layer was separated, dried over sodium sulfate and evaporated under vacuum. Obtained crude compound was purified by preparative HPLC using acetonitrile / water (0.1% formic acid) with a gradient from 20 to 90% of acetonitrile to afford the pure compound as a orange solid (5 mg, 38%). ¹H NMR (700 MHz, Methanol-d₄) δ 7.47 (d, *J* = 8.5 Hz, 1H), 7.32 (d, *J* = 8.4 Hz, 1H), 3.39 (dd, *J* = 17.3, 5.5 Hz, 1H), 3.02 – 2.95 (m, 1H), 2.75 (dd, *J* = 17.1, 12.9 Hz, 1H), 2.36 (s, 3H), 2.33 (s, 3H), 2.17 (s, 3H). ¹³C NMR (176 MHz, Methanol-d₄) δ 175.2, 173.8, 164.1, 156.5, 138.9, 136.3, 130.8, 123.6, 121.0, 115.6, 112.9, 109.9, 98.7, 43.0, 26.7, 22.6, 15.6, 14.2. HRMS (ESI) calculated for C₂₃H₂₃N₂O₈ (M+H) 455.1449, found 455.1453.

(4R,4aS,12aS)-4-(3-Aminopropanamido)-3,10,11,12a-tetrahydroxy-6,9-dimethyl-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide [8] Step 1: Amide coupling To a stirred solution of CDCHD (41 mg, 0.1 mmol, 1 equiv) and *N*-Boc- β -alanine (19 mg, 0.1 mmol, 1 equiv) in EtOAc (0.5 mL) and pyridine (0.3 mL), propylphosphonic anhydride (T3P) 50% solution in EtOAc (0.13 mL, 2 equiv) was added. The reaction was stirred at 40 °C for 17 h. The mixture was poured onto water (10 mL), acidified by addition of HCl (1 M) to pH 5–6 and extracted with EtOAc (10 mL $\times$ 2). The combined organic layers were dried over sodium sulfate, and the solvent was removed under reduced pressure. The crude material was purified by preparative HPLC using acetonitrile (0.05% formic acid) / water (0.05% formic acid) as eluent with a gradient from 10 to 90% of acetonitrile to afford the pure product (13 mg, 22%). HRMS (ESI) calculated for C₂₉H₃₄N₃O₁₀ (M+H) 584.2244, found 584.2204. Step 2: Boc-deprotection To a stirred solution of **8a** (12 mg, 0.02 mmol, 1 equiv) in DCM (6 mL), TFA (1 mL) was added. The reaction was stirred at room temperature for 1 h. Solvent was removed under reduced pressure, and the residue was purified by preparative HPLC using acetonitrile (0.05% formic acid) / water (0.05% formic acid) as eluent with a gradient from 10 to 60% of acetonitrile to afford the pure product as yellow solid (2 mg, 21%). ¹H NMR (500 MHz, DMSO-d₆) δ 9.23 (s, 1H), 8.50 (s, 1H), 8.31 (s, 1H), 7.14 (d, *J* = 8.1 Hz, 1H), 6.77 (d, *J* = 7.9 Hz, 1H), 5.98 (s, 1H), 5.31 (s, 1H), 3.41–2.99 (m, 6H), 2.65 (m, 2H), 2.10 (s, 6H). HRMS (ESI) calculated for C₂₄H₂₆N₃O₈ (M+H) 484.1712, found 484.1691.

(4R,4aS,12aS)-3,10,11,12a-Tetrahydroxy-6,9-dimethyl-4-(2-(4-methylpiperazin-1-yl)acetamido)-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide [9] To a stirred mixture of 2-(4-methylpiperazin-1-yl)acetic acid (10 mg, 0.063 mmol, 1 equiv) in THF (3 mL), thionyl chloride (12 mg, 0.1 mmol, 1.6 equiv) was added followed by DMF (1 drop) under nitrogen atmosphere. The reaction was stirred at 60 °C for 1 h. Volatiles were removed under reduced pressure, and the yellow residue was directly added to a stirred solution of CDCHD (21 mg, 0.05 mmol, 0.8 equiv) in *N*-methyl-2-pyrrolidone (NMP) (1 mL). The reaction mixture was further stirred at room temperature under nitrogen atmosphere for 16 h. The crude material was purified by preparative HPLC using acetonitrile (0.05% formic acid) / water (0.05% formic acid) as eluent with a gradient from 10 to

90% of acetonitrile to afford the pure product (2 mg, 7%). **¹H NMR (700 MHz, DMSO-*d*₆)** δ 7.25 (s, 1H), 7.12 (s, 1H), 7.02 (s, 1H), 6.83 (s, 1H), 6.69 (s, 1H), 5.44–5.32 (m, 3H), 3.28 (s, 2H), 3.03 (m, 1H), 2.88 (m, 1H), 2.60 (m, 2H), 2.52 (m, 4H), 2.40 (m, 4H), 2.18–2.14 (m, 9H). **HRMS (ESI)** calculated for C₂₈H₃₃N₄O₈ (M+H) 553.2298, found 553.2276.

(4*R*,4*aS*,12*aS*)-4-Acrylamido-3,10,11,12*a*-tetrahydroxy-6,9-dimethyl-1,12-dioxo-1,4,4*a*,5,12,12*a*-hexahydrotetracene-2-carboxamide [10] In a heat-dried Schlenk tube, CDCHD (33 mg, 0.08 mmol, 1 equiv) was dissolved in *N*-methyl-2-pyrrolidone (1.7 mL). The reaction mixture was cooled down to 0 °C, and acryloyl chloride (7.7 μL, 0.095 mmol, 1.2 equiv) was added. The resulting brown reaction mixture was kept stirring and was slowly left to warm up to room temperature. After 18 h, water (2 mL) and EtOAc (5 mL) was added. The organic layer was separated and dried over sodium sulfate, filtered, and volatiles were removed on a rotary evaporator. The crude was purified by preparative HPLC using 20–100% gradient (acetonitrile (0.1% formic acid) in water (0.1% formic acid)). After removing water and acetonitrile from the pure fractions under vacuum, the yellow product was obtained (2 mg, 5%). **¹H NMR (500 MHz, DMSO-*d*₆)** δ 9.22 (s, 2H), 8.66 (d, *J* = 8.7 Hz, 1H), 7.50 (d, *J* = 8.4 Hz, 1H), 7.30 (d, *J* = 8.2 Hz, 1H), 7.13 (s, 1H), 6.46 (dd, *J* = 17.1, 10.3 Hz, 1H), 6.21 (dd, *J* = 17.1, 1.8 Hz, 1H), 5.72 (dd, *J* = 10.3, 1.8 Hz, 1H), 5.61 (s, 1H), 3.33 – 3.02 (m, 2H), 2.88 – 2.76 (m, 1H), 2.76 – 2.65 (m, 1H), 2.32 (s, 3H), 2.26 (s, 3H). **¹³C NMR (126 MHz, DMSO-*d*₆)** δ 173.5, 165.6, 137.5, 135.5, 131.7, 130.7, 126.8, 119.3, 114.6, 112.3, 112.1, 109.1, 98.2, 42.0, 26.4, 15.8, 14.3. **HRMS (ESI)** calculated for C₂₄H₂₃N₂O₈ (M+H) 467.1454, found 467.1430.

(4*R*,4*aS*,12*aS*)-3,10,11,12*a*-Tetrahydroxy-4-(4-methoxybenzamido)-6,9-dimethyl-1,12-dioxo-1,4,4*a*,5,12,12*a*-hexahydrotetracene-2-carboxamide [11] 4-Methoxybenzoyl chloride (6 μL, 1 equiv.) and DIPEA (25 μL, 5 equiv.) were added to a solution of CDCHD (12 mg, 1 equiv.) in THF (3 mL). The resulting mixture was stirred at room temperature overnight. After concentration under vacuum, the reaction mixture was extracted with EtOAc, and the organic layer was washed with saturated aqueous NaCl solution, dried over sodium sulfate, filtered and evaporated under vacuum. The crude compound was purified by preparative HPLC using Biphenyl column, MeOH/ water with a gradient from 15 to 95% of MeOH to afford the pure product (5.0 mg, 31%). **¹H NMR (500 MHz, Methanol-*d*₄)** δ 7.92 (d, *J* = 8.5 Hz, 2H), 7.41 (d, *J* = 7.0 Hz, 1H), 7.26 (d, *J* = 6.5 Hz, 1H), 7.02 (d, *J* = 8.5 Hz, 2H), 3.87 (s, 3H), 3.41–3.25 (m, 1H), 3.16–3.04 (m, 1H), 2.92–2.76 (m, 1H), 2.29 (s, 3H), 2.28 (s, 3H). **¹³C NMR (126 MHz, Methanol-*d*₄)** δ 173.7, 168.9, 162.8, 155.3, 137.6, 134.8, 129.7, 129.2, 126.1, 122.0, 119.4, 114.1, 113.4, 111.6, 108.7, 78.2, 67.8, 54.6, 41.5, 25.6, 14.2, 12.8. **HRMS (ESI)** calculated for C₂₉H₂₇N₂O₉ (M+H) 547.1717, found 547.1687.

(4*R*,4*aS*,12*aS*)-3,10,11,12*a*-Tetrahydroxy-6,9-dimethyl-4-(methylsulfonamido)-1,12-dioxo-1,4,4*a*,5,12,12*a*-hexahydrotetracene-2-carboxamide [12] An aqueous solution of NaHCO₃ (15 mg, 7 equiv) was slowly added to a vigorously stirred solution of CDCHD (10 mg, 1 equiv) and methanesulfonyl chloride (10 μL) in THF/water 5:1 (3 mL). The reaction mixture was stirred at rt for 30 min and then acidified to pH 2 with HCl solution (5 N). The reaction mixture was then extracted with methylene chloride (2x), and the combined organic layers were

dried over sodium sulfate, filtered and evaporated under vacuum. The crude compound was purified by preparative HPLC using acetonitrile / water (0.1% formic acid) with a gradient from 20 to 90% of acetonitrile to afford the pure product as an orange solid (1.8 mg, 16 %). **¹H NMR (500 MHz, Methanol-d₄)** δ 7.47 (d, *J* = 8.7 Hz, 1H), 7.35 (d, *J* = 8.5 Hz, 1H), 5.05 (d, *J* = 4.3 Hz, 1H), 3.47 (dd, *J* = 17.4, 5.3 Hz, 1H), 3.15 (s, 3H), 2.98 – 2.89 (m, 1H), 2.66 (dd, *J* = 17.8, 12.5 Hz, 1H), 2.37 (s, 3H), 2.31 (s, 3H). **¹³C NMR (126 MHz, Methanol-d₄)** δ 175.3, 164.4, 156.8, 139.2, 136.5, 130.9, 123.9, 121.2, 115.8, 113.1, 110.1, 98.6, 45.5, 41.6, 26.6, 15.7, 14.3. **HRMS (ESI)** calculated for C₂₂H₂₃N₂O₉S (M+H) 491.1119, found 491.1123.

(4*R*,4*aS*,12*aS*)-4-Amino-7-fluoro-3,10,11,12*a*-tetrahydroxy-6,9-dimethyl-1,12-dioxo-1,4,4*a*,5,12,12*a*-

hexahydrotetracene-2-carboxamide [13] An aqueous solution of sodium bicarbonate was slowly added to the solution of CDCHD (50 mg, 1 equiv) and Fmoc-Cl (37.5 mg, 1.2 equiv). The reaction was stirred at room temperature for 30 min and then acidified to pH 6 by addition of HCl solution (6 N) and extracted with ethyl acetate (2 × 5 mL). After drying the combined organic layers over sodium sulfate, volatiles were removed under vacuum. The crude compound was then suspended in methanol (3 mL), and water was added (1 mL). The product started to appear as a dark brown precipitate appeared and was subsequently filtered. The pure compound was obtained in 27% yield (41 mg). To a solution of Fmoc-protected CDCHD (40 mg, 1 equiv) in 1,2-dichloroethane (8 mL), was added XeF₂ (43 mg, 4 equiv). The mixture was then stirred at 35 °C for 3 h. Water (5 mL) was added, and the reaction mixture was stirred for an additional 10 min. The organic layer was then separated, dried over sodium sulfate and concentrated under reduced pressure. The crude compound was purified by preparative HPLC using acetonitrile/ water (0.1% HCOOH) with a 10 to 90% gradient to afford the pure compound (3 mg, 6%). A solution of piperidine in DMF (2%) was added to the Fmoc-protected fluorinated CDCHD (7 mg), and the reaction mixture was stirred at room temperature for 1 hour. Then it was directly injected in a preparative HPLC and purified using acetonitrile/ water (0.1% TFA) from 10% to 90% of acetonitrile to afford the pure compound as an orange solid (1.5 mg, 26 %). **¹H NMR (700 MHz, Methanol-d₄)** δ 7.26 (d, *J* = 14.8 Hz, 1H), 4.87 (d, *J* = 4.5 Hz, 1H), 3.38 (dd, *J* = 17.1, 5.4 Hz, 1H), 3.16 – 3.09 (m, 1H), 2.79 (dd, *J* = 17.1, 12.7 Hz, 1H), 2.54 (d, *J* = 6.9 Hz, 3H), 2.30 (s, 3H). **¹³C NMR (176 MHz, Methanol-d₄)** δ 201.0, 174.9, 164.2, 163.3, 154.3, 153.3, 152.9, 130.8, 127.2, 127.1, 122.7, 122.6, 122.2, 114.2, 110.1, 78.6, 54.3, 41.6, 26.2, 17.2, 17.1, 15.6. **¹⁹F NMR (471 MHz, Methanol-d₄)** δ -77.0 (TFA), -155.0. **HRMS (ESI)** calculated for C₂₁H₂₀FN₂O₇ (M+H) 431.1249, found 431.1256. Other procedure for preparing [13]: Selectfluor® (20 mg, 0.056 mmol, 1 equiv) was added as a solid to a solution of HCOOH salt of CDCHD (25 mg, 0.055 mmol, 1 equiv) in MeCN (1.5 mL) and allowed to stir for 1 h at room temperature. The reaction mixture was then evaporated and purified by preparative HPLC acetonitrile / water (0.1% formic acid) with a gradient from 10 to 90% of acetonitrile to afford the pure product (3.9 mg, 15%). NMR and HRMS data are consistent with the previous description.

(4*R*,4*aS*,12*aS*)-4-Amino-7-chloro-3,10,11,12*a*-tetrahydroxy-6,9-dimethyl-1,12-dioxo-1,4,4*a*,5,12,12*a*-

hexahydrotetracene-2-carboxamide [14] *N*-chlorosuccinimide (5 mg, 1.5 equiv) was added to a solution of CDCHD (10 mg, 1 equiv) in trifluoroacetic acid (1 mL). The resultant mixture was stirred at room temperature

for 30 min. Solvent was removed under flow of nitrogen and the crude was then purified by preparative HPLC using acetonitrile / water (0.1% TFA) with a gradient from 20 to 90% of acetonitrile to afford the pure product as an orange solid (2 mg, 28%). **¹H NMR (700 MHz, DMSO-*d*₆)** δ 9.56 (s, 1H), 9.35 (s, 1H), 8.62 (s, 2H), 7.64 (s, 1H), 4.72 (d, *J* = 4.0 Hz, 1H), 3.25 (dd, *J* = 17.4, 5.2 Hz, 1H), 3.10 – 3.04 (m, 1H), 2.70 (dd, *J* = 17.2, 12.9 Hz, 1H), 2.59 (s, 3H), 2.22 (s, 3H). **¹³C NMR (176 MHz, DMSO-*d*₆)** δ 190.5, 190.4, 189.2, 172.6, 157.9, 157.7, 138.3, 133.9, 132.4, 120.7, 114.1, 108.6, 96.7, 76.9, 52.1, 48.6, 25.5, 19.0, 14.9. **HRMS (ESI)** calculated for C₂₁H₂₀ClN₂O₇ (M+H) 447.0953, found 447.0917.

(4*R*,4*aS*,12*aS*)-4-Amino-7-bromo-3,10,11,12*a*-tetrahydroxy-6,9-dimethyl-1,12-dioxo-1,4,4*a*,5,12,12*a*-hexahydrotetracene-2-carboxamide [15] *N*-bromosuccinimide (19 mg, 2 equiv) was added to a solution of CDCHD (30 mg, 1 equiv) in trifluoroacetic acid (2.4 mL). The resulting mixture was stirred at room temperature for 30 min. Solvent was removed under flow of nitrogen, and the crude was then purified by preparative HPLC using acetonitrile / water (0.1%TFA) with a gradient of acetonitrile (10 to 90%) to afford the pure product as an orange solid (3.5 mg, 17%). **¹H NMR (500 MHz, Methanol-*d*₄)** δ 7.83 (s, 1H), 3.15 (dt, *J* = 12.6, 4.9 Hz, 1H), 2.78 (dd, *J* = 17.3, 13.1 Hz, 1H), 2.69 (s, 3H), 2.27 (s, 3H). **¹³C NMR (126 MHz, Methanol-*d*₄)** δ 200.8, 174.9, 157.1, 143.9, 137.4, 133.0, 124.7, 123.2, 115.8, 110.0, 108.2, 97.7, 78.6, 54.2, 41.7, 26.7, 20.6, 15.3. **HRMS (ESI)** calculated for C₂₁H₂₀BrN₂O₇ (M+H) 491.0448, found 491.0456.

(4*R*,4*aS*,12*aS*)-4-Amino-3,10,11,12*a*-tetrahydroxy-6,9-dimethyl-7-nitro-1,12-dioxo-1,4,4*a*,5,12,12*a*-hexahydrotetracene-2-carboxamide [16] Fuming nitric acid (10.5 μL) was added to a solution of CDCHD (20 mg) in sulfolane (0.5 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 15 min, quenched with cold water (100 μL) and purified by preparative HPLC using acetonitrile / water (0.1% formic acid) with a gradient from 20 to 90% of acetonitrile to afford the pure product as an orange solid (0.8 mg, 4%). **¹H NMR (700 MHz, Methanol-*d*₄)** δ 8.00 (s, 1H), 4.92 (d, *J* = 4.4 Hz, 1H), 3.39 (dd, *J* = 17.3, 5.3 Hz, 1H), 3.28 – 3.19 (m, 1H), 2.87 (dd, *J* = 17.1, 12.8 Hz, 1H), 2.38 (s, 3H), 2.15 (s, 3H). **¹³C NMR (176 MHz, Methanol-*d*₄)** δ 174.9, 163.1, 162.9, 141.3, 136.1, 134.0, 131.6, 120.7, 119.1, 117.5, 111.1, 97.8, 78.7, 54.2, 41.6, 26.6, 17.2, 15.4. **HRMS (ESI)** calculated for C₂₁H₂₀N₃O₉ (M+H) 458.1194, found 458.1198.

(4*R*,4*aS*,12*aS*)-4-Amino-3,11,12*a*-trihydroxy-6,9-dimethyl-1,7,10,12-tetraoxo-1,4,4*a*,5,7,10,12,12*a*-octahydrotetracene-2-carboxamide [17] *N*-iodosuccinimide (45 mg, 5 equiv) was added to a solution of CDCHD (15 mg, 1 equiv) in a mixture AcOH/TFA 1:1 (3 mL) and the reaction mixture was stirred at room temperature for 30 min. After evaporation of volatiles, the obtained crude compound was purified by preparative HPLC using acetonitrile / water (0.1% TFA) with a gradient from 10 to 90% of acetonitrile to afford the pure quinone compound as a yellow solid (3.5 mg, 17%). **¹H NMR (700 MHz, Methanol-*d*₄)** δ 6.82 (d, *J* = 1.4 Hz, 1H), 3.42 (dd, *J* = 18.7, 6.6 Hz, 1H), 3.14 (ddd, *J* = 10.9, 6.6, 4.3 Hz, 1H), 2.84 (dd, *J* = 18.6, 11.0 Hz, 1H), 2.52 (s, 3H), 2.15 (d, *J* = 1.4 Hz, 3H). **¹³C NMR (176 MHz, Methanol-*d*₄)** δ 194.3, 191.9, 187.8, 174.8, 162.0, 151.2, 148.3, 139.1, 134.8, 132.5, 124.9, 116.7, 97.9, 78.7, 54.6, 41.1, 28.6, 16.2, 15.5. **HRMS (ESI)** calculated for C₂₁H₁₉N₂O₈ (M+H) 427.1136, found 427.1136.

(4R,4aS,12aS)-4-Amino-3,10,11,12a-tetrahydroxy-7-mesityl-6,9-dimethyl-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide [18] To a stirred solution of TFA salt of CDCHD (20 mg, 0.038 mmol, 1 equiv) and mesitylene (11 μ L, 0.076 mmol, 2 equiv) in HFIP (400 μ L), acetic acid (4.4 μ L, 0.076 mmol, 2 equiv) was added at room temperature. To the mixture, phenyliodine(III) diacetate (PIDA) (12.2 mg, 0.038 mmol, 1 equiv) was sequentially added, and the reaction mixture was stirred for an additional 3 h. The reaction mixture was then evaporated under vacuum and purified by preparative HPLC acetonitrile / water (0.1% formic acid) with a gradient from 10 to 90% of acetonitrile to afford the pure product as a red solid (3 mg, 15%). ¹H NMR (700 MHz, Methanol-d₄) δ 7.05 (s, 1H), 6.89 (s, 1H), 6.86 (s, 1H), 3.17 (dd, J = 17.1, 5.1 Hz, 1H), 3.02 – 2.89 (m, 1H), 2.72 (dd, J = 16.3, 13.1 Hz, 1H), 2.30 (s, 3H), 2.29 (s, 3H), 1.87 (s, 3H), 1.76 (s, 3H), 1.67 (s, 3H). ¹³C NMR (176 MHz, Methanol-d₄) δ 173.7, 157.0, 143.1, 140.2, 137.8, 137.7, 137.3, 137.1, 131.9, 129.6, 129.3, 129.1, 124.7, 121.3, 114.6, 109.9, 79.4, 54.9, 42.1, 27.0, 21.4, 21.3, 21.0, 17.1, 15.7. HRMS (ESI) calculated for C₃₀H₃₁N₂O₇ (M+H) 531.2126, found 531.2121

(4R,4aS,12aS)-4-Amino-3,10,11,12a-tetrahydroxy-7-(4-methoxyphenyl)-6,9-dimethyl-1,12-dioxo-1,4,4a,5,12,12a-hexahydrotetracene-2-carboxamide [19] To a stirred solution of TFA salt of CDCHD (20 mg, 0.038 mmol, 1 equiv) and anisole (8.25 μ L, 0.076 mmol, 2 equiv) in HFIP (400 μ L), acetic acid (4.4 μ L, 0.076 mmol, 2 equiv) was added at room temperature. To the mixture, PIDA (18 mg, 0.038 mmol, 1.5 equiv) was sequentially added, and the reaction mixture was stirred for an additional 3 h. The reaction mixture was then evaporated and purified by preparative HPLC acetonitrile / water (0.1% formic acid) with a gradient from 10 to 90% of acetonitrile to afford the pure product as an orange solid (2.6 mg, 13%). ¹H NMR (700 MHz, Methanol-d₄) δ 7.35 (s, 1H), 7.17 (dd, J = 8.3, 2.0 Hz, 1H), 7.05 (dd, J = 8.3, 1.9 Hz, 1H), 6.95 (dd, J = 8.4, 2.5 Hz, 1H), 6.91 (dd, J = 8.4, 2.6 Hz, 1H), 3.83 (s, 3H), 3.22 (dd, J = 17.0, 5.1 Hz, 1H), 3.14 (dt, J = 12.6, 4.9 Hz, 1H), 2.68 (dd, J = 16.8, 12.9 Hz, 1H), 2.32 (s, 3H), 1.77 (s, 3H). ¹³C NMR (176 MHz, Methanol-d₄) δ 200.5, 174.9, 160.3, 156.4, 141.3, 138.6, 137.7, 132.4, 131.7, 131.6, 131.3, 125.4, 121.0, 114.9, 114.8, 114.5, 109.7, 97.7, 78.5, 56.0, 55.9, 54.3, 41.9, 26.3, 20.5, 15.6. HRMS (ESI) calculated for C₂₈H₂₇N₂O₈ (M+H) 519.1762, found 519.1767.

(8aS,9R,12aS)-9-Amino-2,2-di-tert-butyl-10,12a-dihydroxy-4,7-dimethyl-12,13-dioxo-8,8a,9,12,12a,13-hexahydrotetraceno[1,12-de][1,3,2]dioxasiline-11-carboxamide [20] The Boc-protected CDCHD (20 mg, 0.39 mmol, 1 equiv) was dissolved in DMF (2 mL) and then triethylamine (30 μ L, 0.195 mmol, 5 equiv) and the silyl reagent (25.4 μ L, 0.078 mmol, 2 equiv) were subsequently added. After 1 h, the reaction mixture was quenched with a saturated aqueous NH₄Cl solution and then extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered and evaporated under vacuum. The crude was purified by preparative HPLC using 60% to 70% of acetonitrile in water. After lyophilization of the pure fractions, 3 mg of pure Boc-protected silylated compound were obtained (12%). This compound was dissolved in DCM 200 μ L, and then 2 drops of trifluoroacetic acid were added. The reaction mixture was allowed to stir until completion of the deprotection monitored by LCMS. The reaction mixture was then evaporated and purified by preparative HPLC acetonitrile / water (0.1% formic acid) with a gradient from 10 to 90% of acetonitrile to afford the pure product as a yellow solid (1 mg, 39%). ¹H NMR (500 MHz, Methanol-d₄) δ 7.54 (d, J = 8.7 Hz, 1H), 7.48 (d, J = 8.7 Hz, 1H), 4.81

(d, $J = 4.3$ Hz, 1H), 3.43 (dd, $J = 17.4, 6.9$ Hz, 1H), 3.05 (ddd, $J = 11.3, 6.8, 4.3$ Hz, 1H), 2.85 (dd, $J = 17.5, 11.6$ Hz, 1H), 2.45 (s, 3H), 2.36 (s, 3H), 1.19 (s, 9H), 1.10 (s, 9H). **^{13}C NMR (126 MHz, Methanol- d_4)** δ 174.9, 153.6, 151.5, 137.5, 134.7, 132.1, 124.9, 122.4, 117.5, 116.4, 115.8, 78.5, 41.6, 27.4, 26.8, 26.7, 22.3, 22.2, 15.6, 14.5. **HRMS (ESI)** calculated for $\text{C}_{29}\text{H}_{37}\text{N}_2\text{O}_7$ ($\text{M}+\text{H}$) 553.2365, found 553.2375.

(6a*S*,7*R*,10a*S*)-7-Amino-9-carbamoyl-8,10a,12-trihydroxy-2,5-dimethyl-10,11-dioxo-6,6a,7,10,10a,11-hexahydrotetracen-1-yl acetate [21] TFA-CDCHD (5 mg, 0.0095 mmol, 1 equiv) was dissolved in HFIP (300 μL), and acyl chloride (0.095 mmol, 10 equiv) was added dropwise. After 2 h of stirring at room temperature, the reaction mixture was evaporated and subjected to preparative HPLC using water acetonitrile 10 to 90 % (0.1% HCOOH) as a gradient. After lyophilization of the pure fractions, the desired product was obtained as an orange solid (0.4 mg, 9%). **^1H NMR (700 MHz, Methanol- d_4)** δ 7.88 (d, $J = 8.7$ Hz, 1H), 7.64 (d, $J = 8.7$ Hz, 1H), 3.43 (dd, $J = 17.1, 5.5$ Hz, 1H), 3.15 – 3.09 (m, 1H), 2.87 (dd, $J = 16.6, 13.2$ Hz, 1H), 2.47 (s, 3H), 2.42 (s, 3H), 2.31 (s, 3H). **^{13}C NMR (176 MHz, Methanol- d_4)** δ 171.9, 163.1, 148.3, 139.5, 135.2, 131.2, 129.5, 123.6, 123.7, 118.6, 111.5, 78.8, 68.3, 54.4, 41.6, 26.4, 21.2, 16.1, 14.5. **HRMS (ESI)** calculated for $\text{C}_{23}\text{H}_{23}\text{N}_2\text{O}_8$ ($\text{M}+\text{H}$) 455.1449, found 455.1452.

(6a*S*,7*R*,10a*S*)-7-Amino-9-carbamoyl-8,10a,12-trihydroxy-2,5-dimethyl-10,11-dioxo-6,6a,7,10,10a,11-hexahydrotetracen-1-yl 4-bromobenzoate [22] TFA-CDCHD (5 mg, 0.0095 mmol, 1 equiv) was dissolved in HFIP (300 μL) and 4-bromobenzoyl chloride (25 mg, 0.114 mmol, 12 equiv) was added dropwise. After 2 h of stirring at room temperature, the reaction mixture was evaporated and subjected to preparative HPLC using water/acetonitrile 10 to 90% (0.1% HCOOH) as a gradient. After lyophilization of the pure fractions, the desired product was obtained as an orange solid (0.6 mg, 11%). **^1H NMR (700 MHz, Methanol- d_4)** δ 8.17 (dd, $J = 8.5, 1.9$ Hz, 2H), 7.94 (dd, $J = 8.7, 2.4$ Hz, 1H), 7.81 (d, $J = 8.4$ Hz, 2H), 7.69 (d, $J = 8.7$ Hz, 1H), 4.81 (d, $J = 3.9$ Hz, 1H), 3.44 (dd, $J = 17.1, 5.4$ Hz, 1H), 3.14 – 3.08 (m, 1H), 2.91 – 2.84 (m, 1H), 2.50 (s, 3H), 2.34 (s, 3H). **^{13}C NMR (176 MHz, Methanol- d_4)** δ 166.3, 163.0, 148.2, 139.5, 135.2, 133.4, 133.2, 131.4, 130.5, 129.9, 129.7, 123.5, 118.7, 111.5, 78.8, 50.0, 49.7, 41.6, 26.4, 16.1, 14.5. **HRMS (ESI)** calculated for $\text{C}_{28}\text{H}_{24}\text{BrN}_2\text{O}_8$ ($\text{M}+\text{H}$) 595.0711 found 595.0717.

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