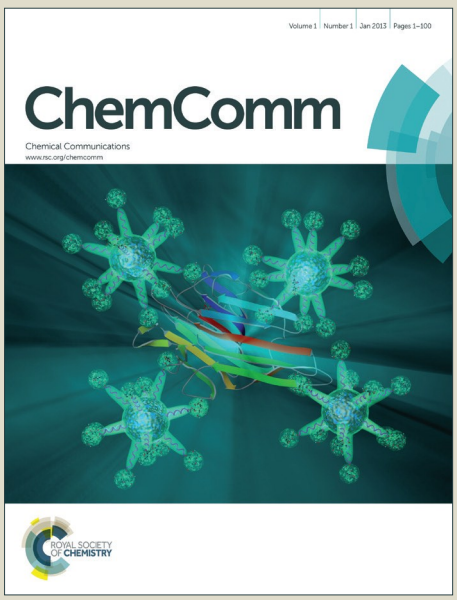
ChemComm

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: N. Ueberschaar, F. Meyer, H. Dahse and C. Hertweck, *Chem. Commun.*, 2016, DOI: 10.1039/C6CC00890A.

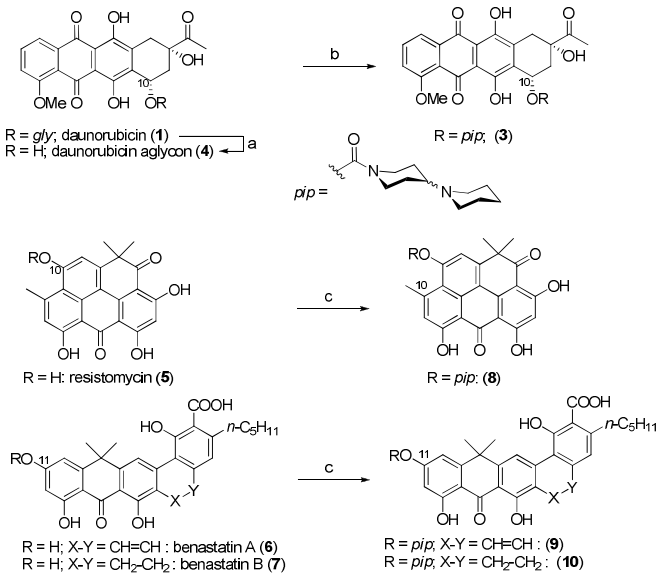


ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/chemcomm

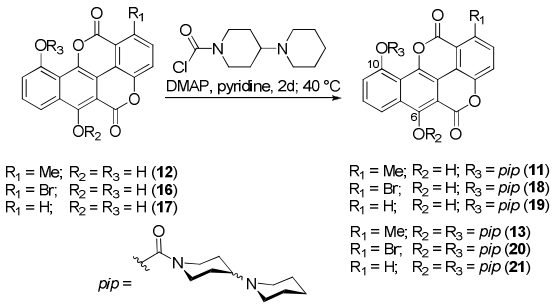
COMMUNICATION View Article Online DOI: 10.1039/C6CC00890A Journal Name

To exclude unspecific effects we prepared the 1,4'-bipiperidine-1'-carbamate adducts of polyphenols, resistomycin (**5**) and benastatin (**6, 7**) from *Streptomyces* species.[13, 14] For all derivatives antiproliferative activities were markedly decreased (see **Table 1**). Consequently, installation of the 1,4'-bipiperidine-1'-carbamate would be unfavorable for polyphenols **5**-**7**, likely because of the dramatic structural changes. A decrease in bioactivity of related carbamate derivatives has also been reported for glyfoline[15] and S16020-2.[16] Thus, we reasoned that the relatively large 1,4'-bipiperidine substituent would be more suitable for the replacement of a disaccharyl residue.



Scheme 1 Synthesis of carbamates starting from isolated natural products. a) KOH, MeOH, H_2O, 2 h, 80 °C, >99% (*gly* for daunosamine); b) DMAP, pyridine, 1,4'-bipiperidine-1'-carbonyl chloride, 72 h, 45 °C, 53%; c) DMAP, pyridine, 1,4'-bipiperidine-1'-carbonyl chloride, 24 h, r.t. 75% for **8**; 47% for **9**; 76% for **10**.

This assumption was tested using chartreusin (**2**), a potent DNA intercalator isolated from the soil-bacterium *Streptomyces chartreusis*.[4, 5] Notably, its poor solubility has hampered the development of this compound for therapy.[6, 7] Initial logD and logS calculations for a mono-substituted product (**11**) indicated a logD value comparable to **2** but an increased (10-fold higher) solubility. Aglycone **12**, which was prepared using an optimized synthetic route,[17-19] was transformed according to the established protocol. We obtained two products, the mono- and di-substituted chartreusin analogues (**11** + **13**). Both compounds were purified and fully characterized (**Scheme 2**). HMBC-NMR data and ^{13}C-NMR shift calculations revealed that **11** is mono-substituted at position C-10 as in the natural glycoside (see supplementary information **Figure S13**).



Scheme 2 Synthesis of carbamates starting from the aglycone using Steglich conditions. Yields: **12**→**11** (19%)+**13**(73%) ∑92%; **16**→**18**(14%)+**20**(84%) ∑98%; **17**→**19**(31%)+**21**(69%) ∑>99%.

We found that **11** exhibit an eight-fold lower GI_{50} value towards HUVEC cells than the natural product chartreusin (**2**). Its cytotoxicity measured using HeLa cells was only slightly lower. The di-substituted analogue **13** proved to be soluble at 10 mg/mL in aqueous buffer, which allowed the omission of the co-solvent DMSO in cell-based assays. Additionally, the antiproliferative activity of **13** is substantially lower compared to **2** (1.56 µM for **2** and 0.15 µM for **13**, for K-562 leukemia cells). Notably, the carbamic acid of the bipiperidine alone proved to be completely inactive at all cell-based assays (Table 1: *pip*-OH).

To evaluate the scope of the method we also prepared analogues of less potent chartreusin derivatives such as bromochartreusin (**14**)[18] and norchartreusin (**15**),[19] which were practically inactive in human cancer cell-line assays. By conjugation the antiproliferative potency was increased by up to two orders of magnitude. The presence of two 1,4'-bipiperidine-1'-carbamate residues further improved solubility and bioactivity, yielding compounds (**13**, **20**-**21**) that are active at nanomolar concentrations (see **Table 1**).

Because of their outstanding antiproliferative activities we sought to validate the mode of action of compounds **13**, **20**-**21** in comparison to the natural DNA intercalator **2**. We used a band-shift assay[19, 20] to evaluate DNA-intercalating properties. Buffered solutions of the test compounds (**2** and **11**-**21**) were individually titrated with a DNA solution, monitoring their UV-Vis spectra. DNA intercalation is clearly visible by a bathochromic band shift of the aglycone absorption bands due to base-pair interaction with the DNA double strand (**Figure 2**).

View Article Online
DOI: 10.1039/C6CC00890A

lead structure	daunorubicin			resistomycin		benastatin A		benastatin B		chartreusin				bromochartreusin				norchartreusin				reference compounds		
compound no.	**1**	**4**	**3**	**5**	**8**	**6**	**9**	**7**	**10**	**2**	**12**	**11**	**13**	**14**	**16**	**18**	**20**	**15**	**17**	**19**	**21**	**iri**	**cip**	***pip*-OH**
R*	*gly*[1]	H	*pip*	H	*pip*	H	*pip*	H	*pip*															
R_1**										Me	Me	Me	Me	Br	Br	Br	Br	H	H	H	H			
R_2**										H	H	H	*pip*	H	H	H	*pip*	H	H	H	*pip*			
R_3**										*gly*[2]	H	*pip*	*pip*	*gly*[2]	H	*pip*	*pip*	*gly*[2]	H	*pip*	*pip*			
M. vaccae		7.83	21.1	<0.13	21.9	0.40	18.0	0.40	17.9	1.22	>150	23.6	8.65	8.86	>125	21.1	15.9	2.5	>156	48.6	17.6	85.3	0.60	
HUVEC	0.19	98.1	13.5	0.80	30.8	11.4	62.8	19.7	17.9	6.25	>150	0.76	0.26	66.3	>125	0.59	0.83	>80	>156	0.86	0.68	4.78	>150	715
K-562	0.03	115	7.26	0.53	31.5	12.2	>72	16.1	53.8	1.56	>150	3.59	0.15	8.79	>125	1.85	0.63	31.6	>156	0.78	0.16	2.05	117	>800
HeLa	0.90	111	28.0	0.53	68.0	10.4	>72	14.5	>72	12.7	>150	17.0	11.8	48.2	117	9.94	2.79	70.7	>156	17.3	12.4	83.2	>150	>800
logD (pH 7.4)	0.91	2.20	1.90	4.84	5.29	5.11	6.85	5.39	7.12	2.46	3.75	2.66	0.81	2.72	4	2.92	1.06	1.95	3.24	2.15	0.29	0.72	-0.81	
logS (pH 7.4)	-3.53	-3.82	-4.34	-5.13	-7.37	-4.18	-10.3	-3.71	-9.62	-7.27	-6.6	-6.6	-5.12	-7.6	-7.11	-7	-5.4	-6.82	-6.11	-6.13	-4.68	-3.93	-2.28	

Table 1. Survey of antimicrobial (MIC value [µg/mL]), antiproliferative (GI_{50} [µg/mL] for HUVEC and K-562) and cytotoxic (CC_{50} [µg/mL] for HeLa) activities. Residues marked with an asterisk (*) see **Figure 1** and **Scheme 1**, with two asterisks (**) see **Scheme 2**; *pip*, 1,4'-bipiperidine-1'-carbamoyl; *gly*[1], daunosaminyl, *gly*[2], digitalosyl-fucosyl (see **Scheme 1**), pip-OH, 1,4'-bipiperidine-1'-carboxylic acid. Test strain: *Mycobacterium vaccae* 10670. Reference compounds: **iri**, irinotecan **cip**, ciprofloxacin. LogD and logS values were calculated using the ChemAxon[21] prediction software.

For chartreusin (**2**) a maximum red shift of about 8 nm was detected. When the glycoside residue is replaced by the carbamate (**11**, **18** and **19**) the band shift decreases to around 4 nm. A second *pip* substituent (as in **13**, **20**-**21**) causes a markedly reduced band shift, which correlates with decreased DNA intercalation.[20] These results are in contrast to the observed activities of the di-substituted analogues **13**, **20**-**21**.

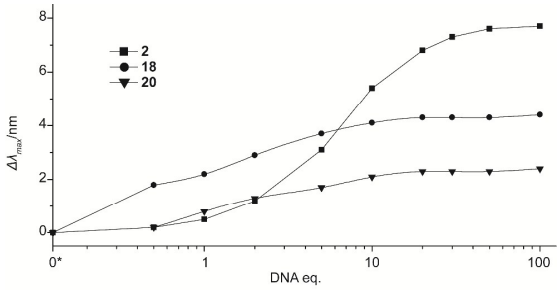


Figure 2 UV-Vis band shift titration of chartreusin (**2**) and two selected carbamates **18** and **20** as a function of added DNA equivalents. *: The zero-value within the x-axis is interpolated.

A plausible explanation of this unexpected finding would be a prodrug mechanism.[22] Thus, we monitored the fate of bromo compound **20** in cell assays, taking advantage of its unique isotopic pattern. In the presence of K-562 leukemia cells, specific (degradation) products of **20** were detected by metabolic profiling (HPLC/HRMS). We found that **20** remains intact when incubated in medium alone (negative control) (**Figure 3A**), yet two hydrolysis products are formed in the presence of K-562 cells (**Figure 3B**). High-resolution mass spectra and comparison of the retention times with the aglycone **16** and the mono-substituted product **18** (**Figure 3C**) revealed the identity of **16** (aglycone) and **18** (mono-substituted analogue). These findings strongly support the model according to which the di-carbamoylated compounds act as well-soluble prodrugs, which are converted into the mono-substituted congeners, which represent potent DNA intercalators.

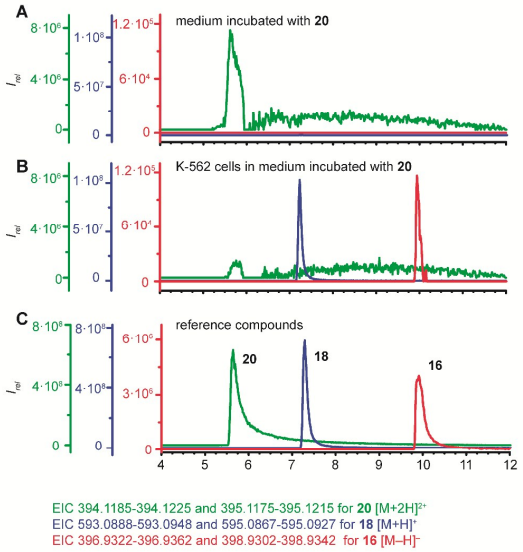


Figure 3 Metabolic profiling using HPLC/HRMS of cell culture incubated for 48 h with **20** including negative control and reference compounds. **A** Overlaid LC-EIC profiles of the medium incubated with **20**. **B** Overlaid HPLC-EIC profiles of the medium including K-562 cells incubated with **18**. **C** HPLC-EIC profiles of the corresponding reference compounds **16**, **18** and **20**.

Conclusions

In this study we have designed and evaluated a series of polyketide glycoside analogues with the aim to use bipiperidine residues as sugar surrogates. We found that it is feasible to replace the daunosamine residue in daunorubicin with the bipiperidine carbamate. This novel analogue proved to be a potent antiproliferative agent, albeit less active than the parent compound. The best results were obtained for chartreusin derivatives, where the disaccharide residue was replaced with the bicyclic heterocycle. We obtained several water-soluble chartreusin derivatives with substantially improved antiproliferative activities and decreased cytotoxicity compared to the corresponding glycosides. Water solubility was always higher for the di-substituted chartreusin derivatives, and their antiproliferative potency was tenfold higher than the natural product (**2**). Band-shift assays and in vivo monitoring revealed that the di-substituted chartreusin analogues serve as prodrugs that are cleaved in vivo. The resulting mono-substituted derivatives have pronounced DNA-intercalating

View Article Online
DOI: 10.1039/C6CC00850A
Journal Name

properties (**Figure 4**). The potential of this approach is illustrated by the fact that the bipiperidine conjugates are readily soluble in water without the need of any co-solvent. In addition, compared the parent glycosides, the antiproliferative activities of the chartreusin derivatives are up to 200 times higher. These findings may encourage related studies to replace glycoside residues in natural products. Thus not only the pharmacological properties may be improved, but also their synthesis may be greatly facilitated.

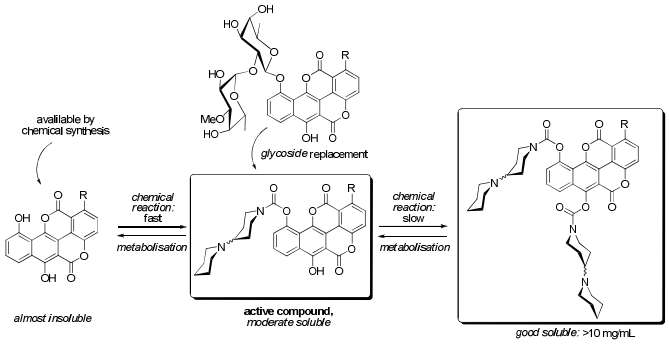


Figure 4 Overview of the synthesis and biological hydrolysis of chartreusin analogues using easy synthetic tools and metabolomics profiling. R = H, CH_3, or Br.

Acknowlegements

We thank R. Hermenau and P. Wein for assistance in synthesis, A. Perner for MS analyses, H. Heinecke for NMR measurements, C. Weigel and E.-M. Neumann for assistance in biological assays. Financial support by the BMBF (GenBioCom in the GenoMik program) to C.H. is gratefully acknowledged.

Notes and references

[#] *These authors contributed equally to the manuscript.*

[a] *Leibniz Institute for Natural Product Research and Infection Biology, Hans Knöll Institute (HKI), Beutenbergstr. 11a, D-07745, Jena, Germany. E-Mail: christian.hertweck@hki-jena.de*

[b] *Friedrich Schiller University, Jena, Germany*

† Electronic Supplementary Information (ESI) available: Experimental details, analytical data, NMR spectra and results of cytotoxicity testing. See DOI: 10.1039/c000000x/

1. A. Jemal, F. Bray, M. M. Center, J. Ferlay, E. Ward and D. Forman, *CA-Cancer J. Clin.*, 2011, **61**, 69-90.
2. D. J. Newman and G. M. Cragg, *J. Nat. Prod.*, 2012, **75**, 311-335.
3. G. M. Cragg, D. G. I. Kingston and D. J. Newman, *Anti Cancer Agents From Natural Products*, Taylor & Francis, 2005.
4. J. Portugal, *Curr. Med. Chem.*, 2003, **3**, 411-420.
5. Z. Xu, K. Jakobi, K. Welzel and C. Hertweck, *Chem. Biol.*, 2005, **12**, 579-588.
6. G. Asai, N. Yamamoto, M. Toi, E. Shin, K. Nishiyama, T. Sekine, Y. Nomura, S. Takashima, M. Kimura and T. Tominaga, *Cancer Chemother. Pharmacol.*, 2002, **49**, 468-472.
7. T. Tashiro, K. Kon, M. Yamamoto, N. Yamada, T. Tsuruo and S. Tsukagoshi, *Cancer Chemother. Pharmacol.*, 1994, **34**, 287-292.
8. F. von Nussbaum, M. Brands, B. Hinzen, S. Weigand and D. Habich, *Angew. Chem. Int. Ed.*, 2006, **45**, 5072-5129.
9. A. T. Serajuddin, *Adv. Drug. Deliv. Rev.*, 2007, **59**, 603-616.
10. S. Sawada, S. Matsuoka, K. Nokata, H. Nagata, T. Furuta, T. Yokokura and T. Miyasaka, *Chem. Pharm. Bull.*, 1991, **39**, 3183-3188.
11. Representative studies using 1,4'-bipiperidine moieties to derivatise synthetic compounds and natural products: US2007/197581 A1, 2007; WO2003/66592 A1, 2003; WO2005/68424 A1, 2005; EP1508570 A1, 2005; WO2010/127363 A1, 2010; Z. F. Tao, Z. Chen, M. H. Bui, P. Kovar, E. Johnson, J. Bouska, H. Zhang, S. Rosenberg, T. Sowin and N. H. Lin, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 6593-6601; T. Asberom, T. A. Bara, J. W. Clader, W. J. Greenlee, H. S. Guzik, H. B. Josien, W. Li, E. M. Parker, D. A. Pissarnitski, L. Song, L. Zhang and Z. Zhao, *Bioorg. Med. Chem. Lett.,* 2007, **17**, 205-207; Y. Wang, L. Li, Z. Tian, W. Jiang and J. W. Larrick, *Bioorg. Med. Chem.*, 2006, **14**, 7854-7861.
12. K. Scherlach, L. P. Partida-Martinez, H. M. Dahse and C. Hertweck, *J. Am. Chem. Soc.*, 2006, **128**, 11529-11536.
13. K. Jakobi and C. Hertweck, *J. Am. Chem. Soc.*, 2004, **126**, 2298-2299.
14. Z. Xu, A. Schenk and C. Hertweck, *J. Am. Chem. Soc.*, 2007, **129**, 6022-6030.
15. T.-L. L. Su, Chin-Tarng; Chen, Ching-Huang; Lin, Mau-Ting, *Med. Chem. Res.*, 2000, **10**, 137.
16. C. Guillonneau, A. Pierré, Y. Charton, N. Guilbaud, L. Kraus-Berthier, S. Léonce, A. Michel, E. Bisagni and G. Atassi, *J. Med. Chem.*, 1999, **42**, 2191-2203.
17. D. Mal, A. Patra and H. Roy, *Tet. Lett.*, 2004, **45**, 7895-7898.
18. N. Ueberschaar, H.-M. Dahse, T. Bretschneider and C. Hertweck, *Angew. Chem. Int. Ed.*, 2013, **52**, 6185-6189.
19. N. Ueberschaar, Z. Xu, K. Scherlach, M. Metsä-Ketelä, T. Bretschneider, H.-M. Dahse, H. Goerls and C. Hertweck, *J. Am. Chem. Soc.*, 2013, **135**, 17408-17416.
20. W. C. Krueger, L. M. Pschigoda and A. Moscowitz, *J. Antibiot.*, 1986, **39**, 1298-1303.
21. ChemAxon, *MarvinSketch*, (2015) ChemAxon Ltd.
22. P. Ettmayer, G. L. Amidon, B. Clement and B. Testa, *J. Med. Chem.*, 2004, **47**, 2393-2404.