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Synthesis and biological evaluation of vioprolide B and its dehydrobutyrine-glycine analogue†

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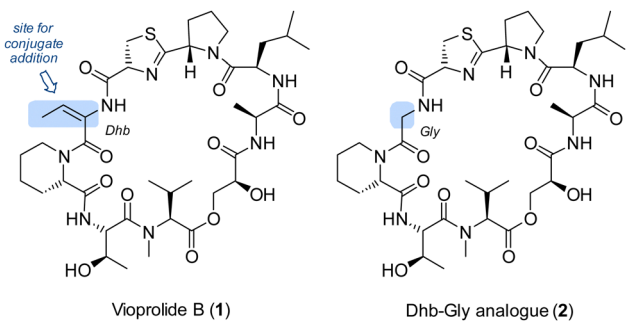
Herein, we describe the total synthesis of the depsipeptide vioprolide B and of an analogue, in which the (*E*)-dehydrobutyrine amino acid was replaced by glycine. The compounds were studied in biological assays which revealed cytotoxicity solely for vioprolide B presumably by covalent binding to cysteine residues of elongation factor eEF1A1 and of chromatin assembly factor CHAF1A.

Natural products and other biologically active compounds frequently display an α,β-unsaturated carboxylic ester or amide group as a potential Michael acceptor. The functional group invites conjugate addition reactions which can lead to irreversible binding to a target protein. If selectivity is achieved towards a specific protein and if binding leads to regulation of the protein function, this mode of action can be a useful starting point for drug discovery.[1] However, the presence of a Michael acceptor in a molecule does not necessarily imply it to be active by conjugate addition. Our groups have for some time been interested in the biological chemistry of a class of depsipeptides,[2] called vioprolides.[3] The compounds were first isolated by Reichenbach, Höfle and co-workers from the myxobacterium *Cystobacter violaceus* Cb vi35.[4] Detailed biosynthetic studies were performed in the group of R. Müller which revealed the individual steps of the nonribosomal peptide synthesis including the formation of less common structural elements.[5] A key feature of the compound class is the presence of *E*-dehydrobutyrine which evolves biosynthetically from threonine by elimination. The configuration of the double bond imposes notable strain on the molecule and prohibits peptide bond formation to the adjacent amino acid.[6] In the so far only total synthesis[7] of a vioprolide, vioprolide D,[3b] the double bond configuration was established in the final stages by $Z \rightarrow E$ isomerization. In the present study, we have interrogated the role of the double bond for the biological activity of the vioprolides. To this end, we prepared vioprolide B (**1**) which displayed in previous work a higher anticancer activity than vioprolide D.[3a] The compound was compared with a synthetic analogue **2**, in which the dehydrobutyrine (Dhb) entity was replaced by a glycine (Gly) fragment (Fig. 1). It was found that compound **2** was completely inactive in assays against HeLa and Jurkat cells. Possible biological targets of vioprolide B were identified by competitive activity-based protein profiling (ABPP) with cysteine-reactive iodoacetamide alkyne probes.[8]

The synthesis of vioprolide B commenced with the known[7c] southern fragment **3**, to the N-terminal site of which *N*-*tert*-butyloxycarbonyl(Boc)-protected pipecolic acid (*N*-Boc-Pip) was attached by peptide coupling.[9,10] After releasing the Boc-protecting group from piperidine **4**, a peptide coupling[10] of compound **5** with the northern fragment[3b] of vioprolide B was probed. Various attempts with the free C-terminal carboxylic acid failed which is why the corresponding pentafluorophenyl ester[11] **6** was prepared (see the ESI† for further details). Stirring of secondary amine **5** with the activated ester **6** at 50 °C led to a smooth bond formation to product *Z*-**7** which comprises the



Vioprolide B (**1**) Dhb-Gly analogue (**2**)

Fig. 1 Structures of vioprolide B (**1**) and an analogue **2**, in which the potential Michael acceptor (*E*)-dehydrobutyrine (Dhb) is replaced by a glycine (Gly).

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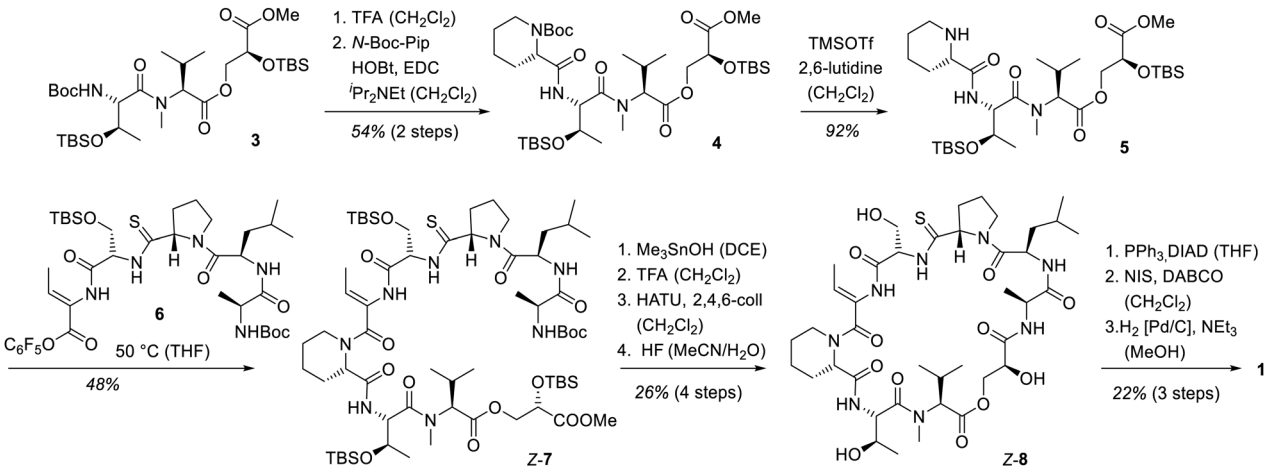
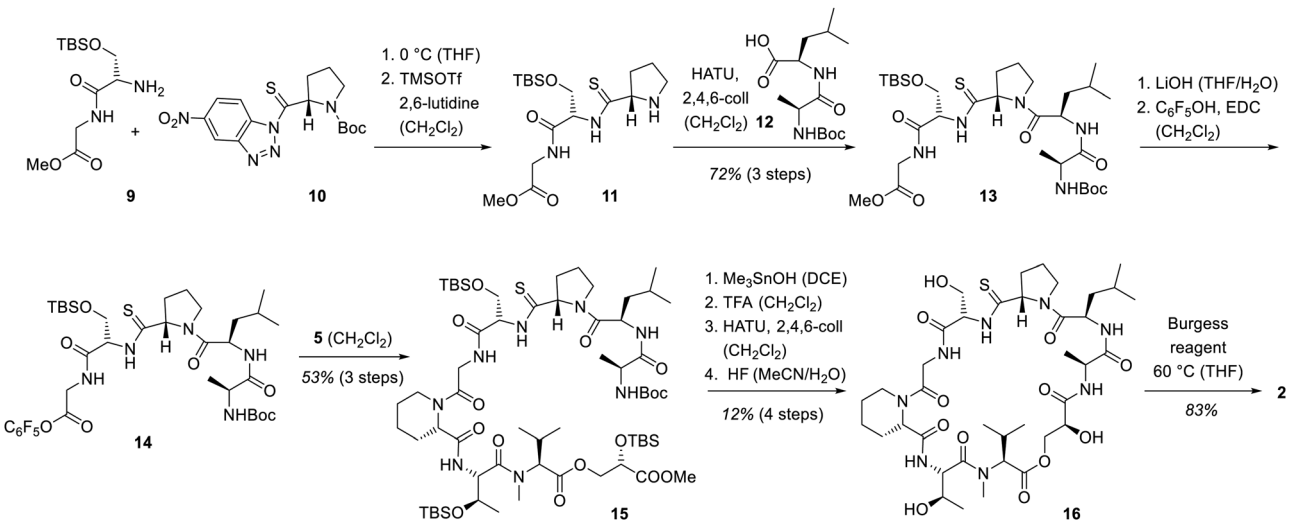


Scheme 1 Total synthesis of vioprolide B (**1**) by linkage of a northern (**6**) and southern (**5**) fragment followed by macrolacamization, thiazoline formation, and adjustment of the double configuration (*Z* → *E*) at the dihydrobutyrine fragment. Abbreviations: TFA = trifluoroacetic acid; HOBt = hydroxybenzotriazole; EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; DCE = dichloroethane; HATU = 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate; DIAD = Di-iso-propyl azodicarboxylate; NIS = *N*-iodosuccinimide; DABCO = 1,4-diazabicyclo[2.2.2]octane.

complete skeleton of the natural product. As for vioprolide D, the adjustment of the relative configuration from *Z*- to *E*-Dhb was postponed to the final step. Hence, the deprotection of the ester at the glycerate[12] was followed by removal of the *N*-Boc group at the N-terminal amino acid alanine. Macrolactamization[13] and global silyl deproctection resulted in depsipeptide *Z*-**8**. Thiazoline ring formation[14] was the final bond forming step before the adjustment of the double bond configuration was completed. Iodination and elimination[15] delivered a *Z*-configured alkenyl iodide the iodine–carbon bond of which was hydrogenolytically cleaved[16] under retention of configuration. After purification by preparative HPLC, vioprolide B (**1**) was obtained as a diastereomerically pure compound, the analytical data of which were in full agreement with the natural product (Scheme 1).

The introduction of the glycine fragment as required for analogue **9** commenced at an initial phase of the total synthesis. Glycine-serine dipeptide **9** was reacted with the known[17] activated thioproline-substituted benzotriazole **10**. Deprotection of the proline led to tripeptide **11** which was coupled with the D-leucine–alanine dipeptide **12** thus completing the assembly of the northern half of the target molecule. Since we relied again on an activated ester for combining the northern and the southern fragment, methyl ester **13** was converted into the pentafluorophenyl ester **14**. The coupling with fragment **5** was successfully performed by stirring at ambient temperature and delivered product **15**. The final steps of the synthesis followed the protocol employed for vioprolide B (Scheme 2). Macrolactamization, thiazoline formation and deprotection enabled the conversion to thioamide **16**. Thiazoline formation was



Scheme 2 Synthesis of vioprolide B analogue **2** by replacing the Dhb unit with a glycine. Burgess reagent = (methoxycarbonylsulfamoyl)triethylammonium hydroxide.

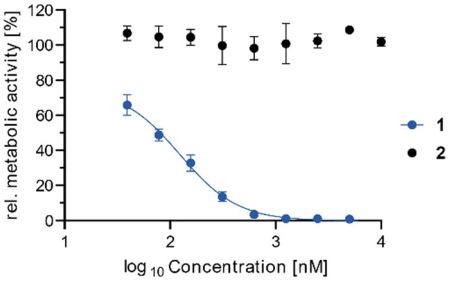


Fig. 2 Dose-dependent inhibition of Jurkat cell proliferation by compounds **1** (vioprolide B) and **2** (Dhb-Gly analogue). IC_{50} is determined by MTT assay with 48 h treatment and calculated as 123 nM (94–148 nM 95% confidence intervall) for **1** and >10 μM for **2**. Data points result from four biologically independent experiments performed in three technical replicates.

accomplished with the Burgess reagent[18] since the impurities of the Mitsunobu protocol were impossible to separate from the final product. A full comparison of the NMR data of **1** and **2** are found in the ESI.†

To evaluate the cytotoxicity of both molecules, we determined the metabolic activity of Jurkat cells upon addition of **1** and **2** *via* the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) assay (Fig. 2). Interestingly, while synthetic vioprolide B (**1**) displayed potent cytotoxicity with an IC_{50} value of 123 nM [94–148 nM 95% confidence interval (CI)], the Dhb analogue **2** lost its bioactivity (IC_{50} > 10 μM) highlighting the relevance of the Michael acceptor. The activity of the synthetic material matched well the previously studied cytotoxicity determined for vioprolide B isolated from the natural producer. The IC_{50} value for Jurkat cells had been determined as 187 ± 24 nM for the natural product.[3a]

In earlier work, we had seen that the nature of the exocyclic double bond in vioprolide D has a strong influence on its activity,[3b] and the present result further supported the key role of the *E*-Dhb entity. We, thus, hypothesized that the electrophilic Michael acceptor is crucial for the covalent interaction with nucleophilic cysteines on cellular proteins. To unravel these cellular protein targets, we performed activity-based protein profiling (ABPP) in a competitive mode utilizing the cysteine reactive iodoacetamide alkyne probe (**IAA**) (Scheme 3).[8] Jurkat cells were pre-incubated with 10 μM of **1** or **2** as control for 1 h, followed by the addition of **IAA** to label residual free cysteines in the proteome. The cells were subsequently lysed and modified with isotopically labelled light or heavy desthiobiotin azide tags (iso-DTB) *via* click chemistry.[8c] Enrichment of probe-bound proteins *via* streptavidin beads followed by tryptic digest revealed light and heavy isotopically labelled peptides which were analysed *via* LC-MS/MS. The corresponding volcano plot depicts most significantly enriched proteins which bind **IAA** treatment and disappear upon pre-incubation with **1**. Among the most significant hits, we identified the elongation factor 1-alpha 1 (eEF1A1) as well as the chromatin assembly factor 1 subunit a (CHAF1A), both with essential roles for cell viability. Moreover, using the MS-Fragger software, we identified residue Cys 31 in eEF1A1 and Cys 79 in CHAF1A as the modified sites.[19] As part of the ribosomal elongation complex, eEF1A1 catalyses the transfer of the aminoacyl-tRNA to the ribosome during protein biosynthesis.[20] CHAF1A forms the largest subunit of CAF-1, an essential chromatin assembly factor involved in the replication fork progression in DNA replication.[21]

Overall, our study provides for the first time evidence for the importance of the exocyclic double bond present in the vioprolides. We here demonstrate its essential role for cancer cell toxicity and its binding to cysteine residues within proteins regulating translation and chromatin assembly. These results highlight that the concept of targeted covalent modification is highly relevant for natural product cytotoxicity and that the structural complexity of vioprolides by itself is not sufficient for cellular target engagement.

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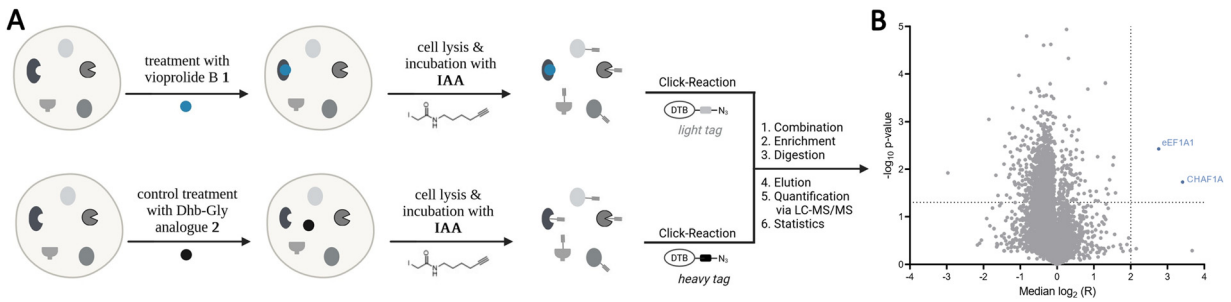

Scheme 3 (A) Schematic overview of the competitive isotopically labelled desthiobiotin azide (isoDTB)-activity-based protein profiling (ABPP) workflow. Two identical sample sets of Jurkat cells were treated *in situ* with compound **1** (upper) or **2** as control (lower), lysed, incubated with cysteine-reactive iodoacetamide alkyne (**IAA**) and clicked to isotopically labelled light respective heavy tags. Samples were combined, enriched, digested with trypsin, eluted from streptavidin beads and quantified by LC-MS/MS. The difference in MS1 signal intensity between heavy (**2**-treated, control) and light (**1**-treated) labelled peptides is represented by the competition ratio *R*. (B) The results of (A) are shown in the volcano plot and represent the median $\log_2(R)$ and the statistical $-\log_{10}(p)$ by a one-sample *t*-test of for all quantified cysteines. All data result from at least three biologically independent replicates. (A) was created with **https://BioRender.com**.

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Data availability

The data supporting this article have been included as part of the ESI.† Primary data are available at the ProteomeXchange Consortium (PRIDE partner repository with the dataset identifier PXD053104) and at Zenodo (**https://doi.org/10.5281/zenodo.11576894**).

Conflicts of interest

There are no conflicts to declare.

Notes and references

1 Reviews: (*a*) M. Gersch, J. Kreuzer and S. A. Sieber, *Nat. Prod. Rep.*, 2012, **29**, 659–682; (*b*) P. Gehrtz and N. London, *Trends Pharmacol. Sci.*, 2021, **42**, 434–447; (*c*) S.-T. Liang, C. Chen, R.-X. Chen, R. Li, W.-L. Chen, G.-H. Jiang and L.-L. Du, *Front. Pharmacol.*, 2022, **13**, 1033003.
2 Reviews: (*a*) H. I. Farah, U. Supratman, A. T. Hidayat and R. Maharani, *ChemistrySelect*, 2022, **7**, e202103470; (*b*) X. Wang, X. Gong, P. Li, D. Lai and L. Zhou, *Molecules*, 2018, **23**, 169; (*c*) S. C. Stolze and M. Kaiser, *Molecules*, 2013, **18**, 1337–1367; (*d*) S. C. Stolze and M. Kaiser, *Synthesis*, 2012, 1755–1777.
3 (*a*) V. C. Kirsch, C. Orgler, S. Braig, I. Jeremias, D. Auerbach, R. Müller, A. M. Vollmar and S. A. Sieber, *Angew. Chem., Int. Ed.*, 2020, **59**, 1595–1600; (*b*) H. A. Grab, V. C. Kirsch, S. A. Sieber and T. Bach, *Angew. Chem., Int. Ed.*, 2020, **59**, 12357–12361.
4 D. Schummer, E. Forche, V. Wray, T. Domke, H. Reichenbach and G. Höfle, *Liebigs Ann.*, 1996, 971–978.
5 (*a*) F. Yan, D. Auerbach, Y. Chai, L. Keller, Q. Tu, S. Hüttel, A. Glemser, H. A. Grab, T. Bach, Y. Zhang and R. Müller, *Angew. Chem., Int. Ed.*, 2018, **57**, 8754–8759; (*b*) F. Yan and R. Müller, *ACS Chem. Biol.*, 2019, **14**, 99–105.
6 E. Butler, L. Florentino, D. Cornut, G. Gomez-Campillos, H. Liu, A. C. Regan and E. J. Thomas, *Org. Biomol. Chem.*, 2018, **16**, 6935–6960.
7 For other synthetic efforts towards the vioprolides, see: (*a*) N. Chopin, F. Couty and G. Evano, *Lett. Org. Chem.*, 2010, **7**, 353–359; (*b*) H. Liu and E. J. Thomas, *Tetrahedron Lett.*, 2013, **54**, 3150–3153; (*c*) N. Osorio Reineke, H. A. Grab and T. Bach, *Synthesis*, DOI: **10.1055/s-0043-1763750**, in press.
8 (*a*) K. M. Backus, B. E. Correia, K. M. Lum, S. Forli, B. D. Horning, G. E. Gonzalez-Paez, S. Chatterjee, B. R. Lanning, J. R. Teijaro, A. J. Olson, D. W. Wolan and B. F. Cravatt, *Nature*, 2016, **534**, 570–574; (*b*) E. Weerapana, *et al.*, *Nature*, 2010, **468**, 790–795; (*c*) P. R. A. Zanon, L. Lewald and S. M. Hacker, *Angew. Chem., Int. Ed.*, 2020, **59**, 2829–2836.
9 (*a*) Y. Chen, M. Bilban, C. A. Foster and D. L. Boger, *J. Am. Chem. Soc.*, 2002, **124**, 5431–5440; (*b*) J. Yao, H. Liu, T. Zhou, H. Chen, Z. Miao, G. Dong, S. Wang, C. Sheng and W. Zhang, *Tetrahedron*, 2012, **68**, 3074–3085; (*c*) P. Barbie and U. Kazmaier, *Org. Lett.*, 2016, **18**, 204–207.
10 Reviews: (*a*) E. Valeur and M. Bradley, *Chem. Soc. Rev.*, 2009, **38**, 606–631; (*b*) C. A. G. N. Montalbetti and V. Falque, *Tetrahedron*, 2005, **61**, 10827–10852; (*c*) J. M. Humphrey and A. R. Chamberlin, *Chem. Rev.*, 1997, **97**, 2243–2266.
11 (*a*) L. Kisfaludy, J. E. Roberts, R. H. Johnson, G. L. Mayers and J. Kovacs, *J. Org. Chem.*, 1970, **35**, 3563–3565; (*b*) J. Yang, H. Huang and J. Zhao, *Org. Chem. Front.*, 2023, **10**, 1817–1846.
12 K. C. Nicolaou, A. A. Estrada, M. Zak, S. H. Lee and B. S. Safina, *Angew. Chem., Int. Ed.*, 2005, **44**, 1378–1382.
13 (*a*) L. A. Carpino, *J. Am. Chem. Soc.*, 1993, **115**, 4397–4398; (*b*) L. A. Carpino and A. El-Faham, *J. Org. Chem.*, 1994, **59**, 695–698; (*c*) L. A. Carpino, A. El-Faham and F. Albericio, *J. Org. Chem.*, 1995, **60**, 3561–3564.
14 (*a*) O. Mitsunobu, *Synthesis*, 1981, 1–28; (*b*) N. Galéotti, C. Montagne, J. Ponce and P. Jouin, *Tetrahedron Lett.*, 1992, **33**, 2807–2810.
15 (*a*) R. S. Hoerrner, D. Askin, R. P. Volante and P. J. Reider, *Tetrahedron Lett.*, 1998, **39**, 3455–3458; (*b*) G. J. Roff, R. C. Lloyd and N. J. Turner, *J. Am. Chem. Soc.*, 2004, **126**, 4098–4099; (*c*) P. M. T. Ferreira, L. S. Monteiro and G. Pereira, *Eur. J. Org. Chem.*, 2008, 4676–4683; (*d*) Y. Yasuno, A. Nishimura, Y. Yasukawa, Y. Karita, Y. Ohfune and T. Shinada, *Chem. Commun.*, 2016, **52**, 1478–1481.
16 (*a*) S. M. Kupchan and A. Afonso, *J. Org. Chem.*, 1960, **25**, 2217–2218; (*b*) A. P. Kozikowski and K. Sugiyama, *Tetrahedron Lett.*, 1980, **21**, 4597–4600; (*c*) D. R. Boyd, N. D. Sharma, J. F. Malone and C. C. R. Allen, *Chem. Commun.*, 2009, 3633–3635; (*d*) S. Hanessian, G. Huang, C. Chenel, R. Machaalani and O. Loiseleur, *J. Org. Chem.*, 2005, **70**, 6721–6734.
17 (*a*) M. A. Shalaby, C. W. Grote and H. Rapoport, *J. Org. Chem.*, 1996, **61**, 9045–9048; (*b*) B. McKeever and G. Pattenden, *Tetrahedron*, 2003, **59**, 2701–2712.
18 (*a*) G. M. Atkins, Jr. and E. M. Burgess, *J. Am. Chem. Soc.*, 1968, **90**, 4744–4745; (*b*) P. Wipf and P. C. Fritch, *Tetrahedron Lett.*, 1994, **35**, 5397–5400; (*c*) C. D. J. Boden and G. Pattenden, *Tetrahedron Lett.*, 1995, **36**, 6153–6156.
19 (*a*) A. T. Kong, F. da Veiga Leprevost, D. M. Avtonomov, D. Mellacheruvu and A. I. Nesvizhskii, *Nat. Methods*, 2017, **14**, 513–520; (*b*) F. Yu, *et al.*, *Nat. Commun.*, 2020, **11**, 4065.
20 T. E. Dever, J. D. Dinman and R. Green, *CSH Perspect. Biol.*, 2018, vol. 10, p. a032649.
21 M. Hoek and B. Stillman, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, **100**, 12183–12188.