Natural Products Synthesis

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Synthesis and Biological Activity of Largazole and Derivatives**

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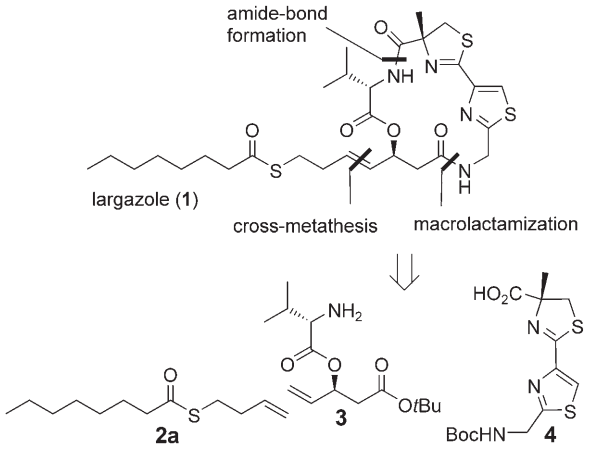
The search for new pharmaceutically relevant lead structures still has a focus on natural products.[1] In particular, cytotoxic compounds isolated from marine sources display a rich structural diversity.[2] However, the often highly potent compounds frequently lack selectivity for cancer cells over nontransformed wild-type cells. Largazole (**1**), which was recently isolated in scarce amounts by Luesch and co-workers from cyanobacteria of the genus *Symploca*, appears to be an exception to this pattern.[3] The growth-inhibitory activity of **1** is considerably higher for cancer cell lines ($GI_{50} = 7.7$ nM) than for the corresponding nontransformed cells ($GI_{50} = 122$ nM).[3] This excellent property makes **1** an important synthetic target. A synthesis should provide enough material for further biological studies to establish the biological profile of **1** in more detail and to determine the mode of action of **1** and the origin of its observed growth-inhibition selectivity.

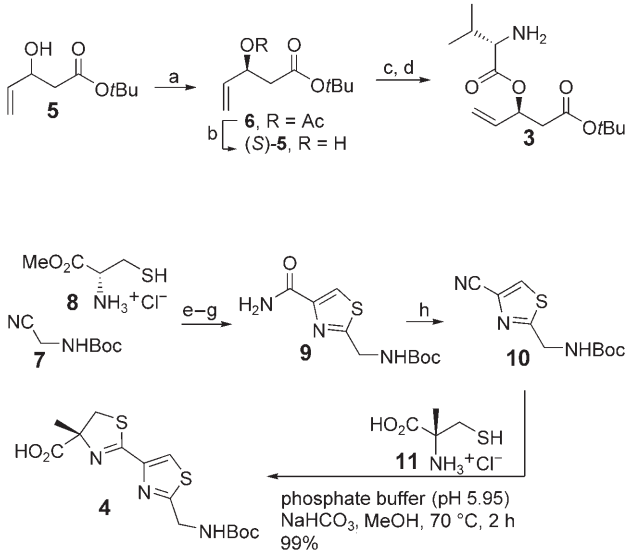
The structure of largazole (**1**) consists of several uncommon structural motifs, such as a 4-methylthiazoline unit, which, in analogy to didehydromirabazole,[4] is fused linearly to a thiazole ring, and a sensitive thioester moiety. We chose a synthetic approach that would enable the late-stage preparation of different analogues from a common precursor. This aim is reflected in our retrosynthetic strategy (Scheme 1): We planned to assemble **1** by a cross-metathesis reaction between the alkene **2a**[5] and the cyclic core in the form of the cyclic terminal alkene **13** (see Scheme 3), which in turn should be accessible from the fragments **3** and **4** through the formation of two amide bonds.

The synthesis of fragment **3** started with an enzymatic resolution of alcohol **5** with Amano lipase PS to provide acetate **6** with excellent enantioselectivity (Scheme 2).[6] The subsequent hydrolysis of **6** required very mild conditions, as elimination to the conjugated diene occurs as a competing reaction. This side reaction was mitigated by the use of potassium carbonate in methanol. The optically pure allylic alcohol (*S*)-**5** obtained in this way in 82% yield was esterified with Fmoc-L-valine to give the amine fragment **3** after cleavage of the Fmoc group with piperidine.

The cyano-substituted thiazole **10**[7] was prepared in four steps in an overall yield of 39% from *N*-Boc-aminoacetonitrile (**7**) and cysteine methyl ester hydrochloride (**8**). Base-catalyzed condensation gave the thiazoline, which was oxidized directly to the thiazole with bromotrichloromethane.



Scheme 1. Retrosynthesis of largazole (**1**). Boc = *tert*-butoxycarbonyl.



Scheme 2. Reagents and conditions: a) Amano lipase PS, vinyl acetate, 30°C, 16 h, 45%, >95% *ee*; b) K_2CO_3, MeOH, 10°C, 15 min, 82%; c) Fmoc-L-valine, *N*,*N*′-diisopropylcarbodiimide, DMAP, CH_2Cl_2, room temperature, 97%; d) piperidine, DMF, 20 min, room temperature, 96%; e) NEt_3, MeOH, 60°C, 3 h; f) $CBrCl_3$, DBU, CH_2Cl_2, room temperature, 10 h; g) NH_3, MeOH, 2 days, room temperature, 44% over 3 steps; h) trifluoroacetic anhydride, NEt_3, CH_2Cl_2, 0°C, 1 h, 89%. DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, DMAP = 4-dimethylaminopyridine, DMF = *N*,*N*-dimethylformamide, Fmoc = 9-fluorenylmethoxycarbonyl.

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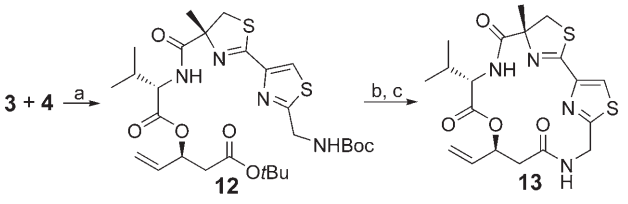
Ammonolysis led to amide **9**, which was converted into nitrile **10** by dehydration with trifluoroacetic anhydride and triethylamine. The subsequent transformation of **10** with α-methylcysteine required some optimization, as the methods established by Pattenden and co-workers[8] gave the desired condensation product in only modest yield and low purity. Unexpectedly mild conditions (phosphate buffer (pH 5.95)/methanol, 70 °C, 2 h)[9] resulted in a fast and clean reaction of **10** with (*R*)-α-methylcysteine hydrochloride (**11**)[10] to provide the pure thiazoline–thiazole **4** in virtually quantitative yield and high purity after simple acid–base extraction.

The carboxylic acid **4** was coupled with the amine fragment **3** under standard conditions in the presence of HATU (Scheme 3). The *tert*-butyl ester and the Boc group



Scheme 3. Reagents and conditions: a) HATU, *N*,*N*-diisopropylethylamine, DMF, room temperature, 20 min, 96%; b) TFA, Et_3SiH, CH_2Cl_2, 0 °C→RT, 1.5 h, 88%; c) HATU, *N*,*N*-diisopropylethylamine, THF, 4 mM, 0 °C, 16 h, 77–89%. HATU = *O*-(7-azabenzotriazol-1-yl)tetramethyluronium hexafluorophosphate, TFA = trifluoroacetic acid.

were cleaved in the following step with a mixture of trifluoroacetic acid and triethylsilane. The slow addition of the resulting ammonium salt to a dilute solution of HATU and Hünig base in THF at 0 °C led to the desired ring closure. Lactam **13** was isolated in a remarkably high yield of 89% on a 40 μmol scale. However, the yield dropped to 77% when the reaction was scaled up by a factor of 25.

Some interesting properties of lactam **13** can be identified from its X-ray crystallographic data (Figure 1).[11] The planar alignment of the thiazoline–thiazole moiety forces nitrogen atoms N25 and N26 to point into the center of the macrocycle. This results, with the amide nitrogen atom (N24) and the ester oxygen atom O20, in a potential chelating pocket for metal

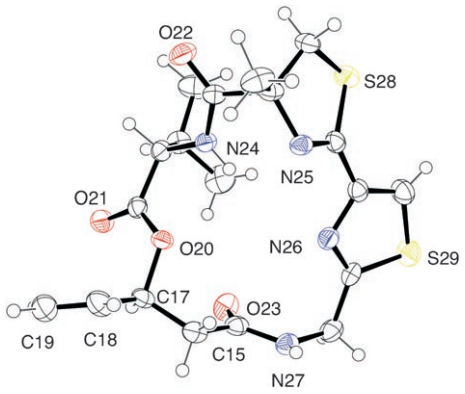
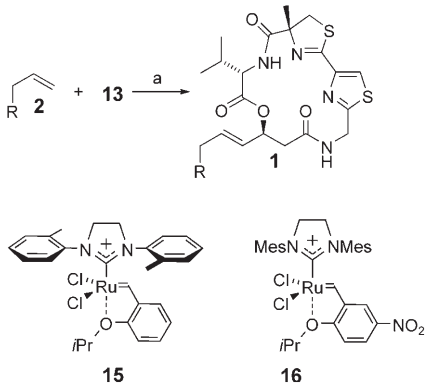


Figure 1. ORTEP representation of **13** (probability ellipsoids at 50%).

ions.[12] Furthermore, the virtually perfect orthogonality of O20 to the carbonyl group at C15 and the C18–C19 double bond predisposes the ester group to an *anti* elimination and thus accounts for the base sensitivity of compound **13**.

Next, we optimized the crucial cross-metathesis step. As expected, the sulfur substituent in the δ position of compound **2a** proved to be problematic. The screening of ruthenium-based metathesis catalysts showed that only a catalyst loading of 10–15% in 1,2-dichloroethane at 80–100 °C resulted in significant conversion (Table 1). The Hoveyda–Grubbs II catalyst caused significantly less decomposition than the Grubbs I and Grubbs II catalysts and provided **1** in 44% yield (Table 1, entries 1–3). The sterically less demanding variant **15**[13] gave the product in considerably lower yield than the parent Hoveyda–Grubbs II catalyst (Table 1, entry 4). The

Table 1: Optimization of the cross-metathesis reaction.[a]



Entry	Catalyst	2	R	Product	Yield [%][b]
1	Grubbs I	**2a**	$CH_2SC(O)(CH_2)_6CH_3$	**1**	5[c]
2	Grubbs II	**2a**	$CH_2SC(O)(CH_2)_6CH_3$	**1**	11[c]
3	Hoveyda–Grubbs II	**2a**	$CH_2SC(O)(CH_2)_6CH_3$	**1**	44[d]
4	**15**	**2a**	$CH_2SC(O)(CH_2)_6CH_3$	**1**	13[e]
5	**16**	**2a**	$CH_2SC(O)(CH_2)_6CH_3$	**1**	75
6	Hoveyda–Grubbs II	**2b**	$(CH_2)_9CH_3$	**1b**	73
7	**16**	**2c**	CH_2Br	**1c**	92
8	**16**	**2d**	$CH_2OC(O)(CH_2)_6CH_3$	**1d**	37
9	**16**	**2e**	$(CH_2)_2SC(O)(CH_2)_6CH_3$	**1e**	69
10	**16**	**2f**	$(CH_2)_3SC(O)(CH_2)_6CH_3$	**1f**	58

[a] Reaction conditions: **2** (4 equiv), catalyst (10%, +5% after 2 h), 0.1 M in 1,2-dichloroethane, 90 °C, 3–12 h. Mes = 2,4,6-trimethylphenyl. [b] Combined yield of the isolated product (*cis* and *trans*). [c] Compound **13** was recovered in 68% yield. [d] Compound **13** was recovered in 44% yield. [e] Compound **13** was recovered in 77% yield.

p-nitro-substituted catalyst **16** developed by Grela and co-workers[14] showed significantly higher activity and gave largazole[15] in 75% yield with a *trans*/*cis* ratio of 6:1 (Table 1, entry 5). The derivatives **1b**–**1f** were prepared in comparable yields (37–92%) and *trans*/*cis* selectivities (4:1–10:1) under the optimized conditions (Table 1, entries 6–10).

The antiproliferative activity of synthetic largazole (**1**) and derivatives **1b**–**f** was investigated in MTT assays (MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) against the human epithelial carcinoma cell line A432

and the preadipocyte cell line 3T3L1 (Table 2). Synthetic **1** displayed only weak selectivity for the cancer cell line (GI_{50} = 49 nM) over the 3T3 cells (GI_{50} = 127 nM). The advanced intermediate **13**, which contains a terminal double bond, and the analogue **1b**, in which the side chain has been replaced

Table 2: Antiproliferative activity of **1** and analogues **1b–g** in the MTT assay.[a]

Cell line	**1**	**1b**	**1d**	**1e**	**1f**	**1g**[15]
A431	49	n.d.[b]	n.d.[b]	n.d.[b]	n.d.[b]	126
3T3L1	127	n.d.[b]	n.d.[b]	n.d.[b]	n.d.[b]	1200
SI	2.6	–	–	–	–	9.5

[a] GI_{50} values are given in nM. [b] n.d. = not determined; no activity was observed up to a concentration of 5 μM.

with a C_{13} alkyl chain, showed no growth-inhibitory activity, even at a concentration of 5 μM. Thus, the possibility that the biological activity originates solely from the cyclic core structure can be ruled out. The replacement of the thioester functionality with an ester group in **1d** also led to a complete loss of activity.

It stood to reason that the octanoic thioester of **1** plays the role of a protecting group, rapidly hydrolyzed under physiological conditions, for the free thiol. Indeed, the free thiol **1g** ($R = CH_2SH$)[16] displayed a strong antiproliferative activity. Remarkably, the activity profile of **1g** is not identical to that of compound **1**. The free thiol **1g** showed slightly lower potency (GI_{50} = 126 nM; GI_{50}(**1**) = 49 nM) but a significantly higher specificity (SI = 9.5; SI(**1**) = 2.6) against the wild-type cells with respect to the thioester **1**. This finding might be explained by a reduced uptake of the free thiol by the wild-type cells. Surprisingly, thioester derivatives **1e** and **1f**, in which the side chain is one or two carbon atoms longer than that in **1**, showed no activity at all. This result underlines how important it is for the thio functionality to be positioned at the right distance from the cyclic core.

In summary, we have described a short synthesis of largazole (19% overall yield, nine steps in the longest linear sequence), the modular nature of which enabled us to prepare several analogues of **1**. We determined the biological activities of the synthetic compounds in MTT assays and demonstrated the necessity of the thiobutenyl group for an antiproliferative effect. The free thiol derivative **1g** displayed improved selectivity relative to that of **1**. Studies to modify the activity of these compounds and elucidate their mode of action on a molecular level are ongoing.[17]

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[11] Crystallographic data for **13**: $C_{19}H_{24}N_4O_4S_2$, M = 436.55, orthorhombic, space group $P2_12_12_1$, a = 9.6075(2), b = 10.9467(2), c = 20.3175(3) Å, V = 2136.80(7) Å^3, Z = 4, ρ_{calcd} = 1.357 Mg m^{-3}, T = 223 K, reflections collected: 3647, independent reflections: 3628 (R_{int} = 0.023), R(all) = 0.0394. wR(gt) = 0.0997, Flack parameter −0.06(7). CCDC 686672 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
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[15] All spectroscopic data of synthetic largazole (**1**) are in good agreement with those of natural largazole; see the Supporting Information.
[16] Compound **1g** was synthesized by monoalkylation of bis(trimethylsilyl)thiol with bromide **1c**.
[17] Note added in proof: For another synthesis of largazole see: Y. Ying, K. Taori, H. Kim, J. Hong, H. Leusch, *J. Am. Chem. Soc.*, **2008**, *130*, 8455–8459.

