

“Clicktophycin-52”: A Bioactive Cryptophycin-52 Triazole Analogue

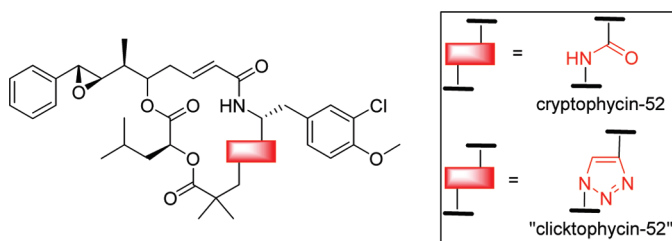
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ABSTRACT



An endocyclic *trans*-amide linkage within the macrocyclic antitumor agent cryptophycin-52 was replaced by a 1,4-disubstituted 1*H*-1,2,3-triazole ring. Macrocyclisation of the triazole analogue was accomplished by macrolactamization as well as by Cu(I)-mediated “click”-cyclization. Compared to cryptophycin-52, *in vitro* cytotoxicity of “clicktophycin-52” against the multidrug resistant human cancer cell line KB-V1 is only slightly reduced.

Since the Cu^I catalyzed azide–alkyne coupling was discovered by the workgroups of Meldal and Sharpless,¹ this so-called “click”-reaction found numerous applications.^{2–4} Although size and dipole moment of the metabolically inert 1,4-disubstituted triazole ring are larger compared to a *trans*-amide bond,² the overall physicochemical properties are similar enough to enable these triazoles to act as *trans*-amide mimetics.^{3,5–12} The bioisosterism has been exemplified by triazole analogues of a matrix metalloprotease inhibitor,⁶ of the immuno stimulating natural compound α -galactosylcer-

amide,⁷ and of capsaicin in its role as agonist of the vanilloid-receptor TRPV1.⁹ Further examples are triazole analogues of the tyrosinase inhibitor *cyclo*-[Pro-Tyr-Pro-Val],¹⁰ of the histone deacetylase inhibitor apicidin,⁸ and of peptides containing the pharmacophoric residues of somatostatin.¹¹ Moreover, X-ray analysis revealed the triazole ring within an analogue of the HIV-1-protease inhibitor amprenavir to interact with the enzyme in the same way as an amide bond in the parent compound.¹²

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Cryptophycins are macrocyclic depsipeptides produced, for example, by cyanobacteria of genus *Nostoc*.¹³ Especially, cryptophycin-1 (**1**) displays high cytotoxicity against multidrug resistant cancer cells¹⁴ and against solid tumors implanted in mouse xenografts.^{13a} However, the synthetic drug candidate cryptophycin-52 (**2**) failed in phase II clinical trials because of neurotoxicity.¹⁵ Cryptophycin derivatives have been shown to possess a *trans*-amide bond between units B and C and a *cis*-amide bond between units A and B.^{13b} We envisioned to replace the peptide linkage between cryptophycin units B and C by a 1,4-disubstituted 1*H*-1,2,3-triazole ring to probe the bioequivalence. Cryptophycins can be retrosynthetically subdivided into four amino and hydroxy carboxylic acid building blocks (units A–D, Figure 1).

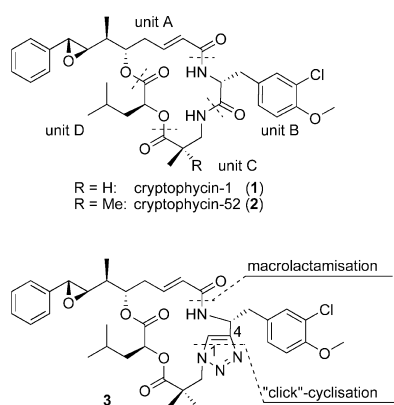


Figure 1. Structures of cryptophycins and their triazole analogue **3**.

Several elegant approaches as well as ample structure–activity relationship studies have been reviewed.¹⁵

The unit B alkyne building block **8** represents a key intermediate in the synthesis of the cryptophycin-52 triazole analogue **3** and was obtained by Seyferth–Gilbert homologation¹⁶ of aldehyde **5**. Starting with reduction of **4**¹⁷ with DIBAL–H, aldehyde **5** was not purified because of its intrinsic stereochemical lability.¹⁸ The required Seyferth reagent dimethyldiazomethyl phosphonate (**7**) was obtained in two steps from phthalimide **6** according to the slightly modified original

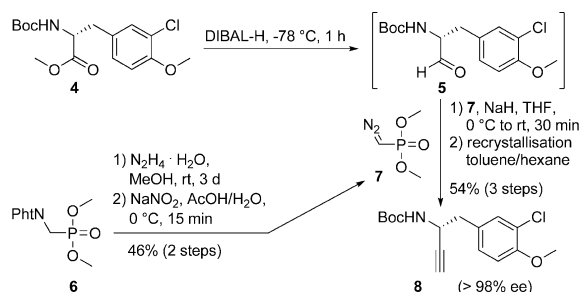
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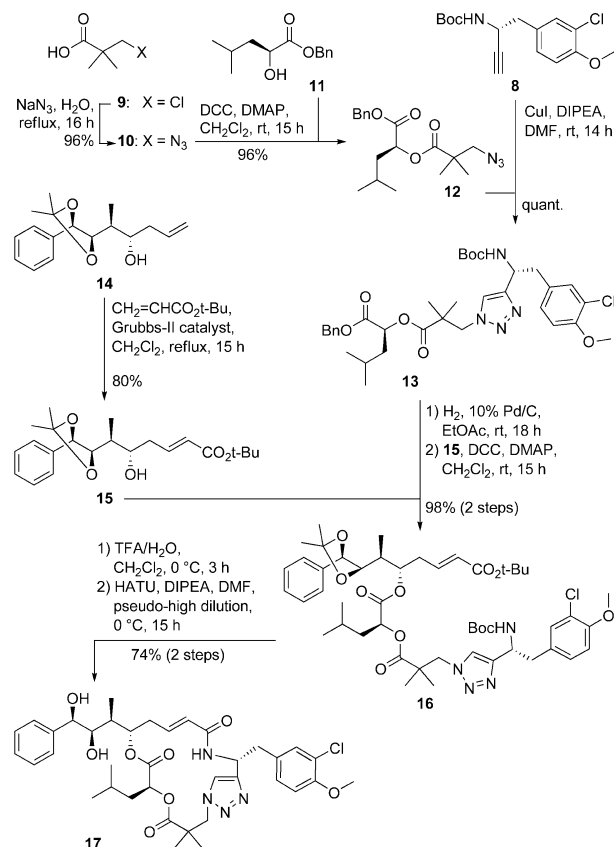
Scheme 1. Synthesis of Unit B Alkyne **8**



procedure of Seyferth et al.^{16a} Phosphonate **7** was deprotonated with sodium hydride and the resulting ylide reacted in situ with **5** yielding alkyne **8**. Analysis of the enantiomeric purity of **8** revealed an enantiomeric excess of 68%. A 3-fold recrystallization improved its optical purity (>98% ee, chiral HPLC, Chiralpak AD, eluent: *i*-PrOH/hexane 1: 10 v/v).

We first assembled the corresponding *seco*-compound **16** as starting material of a macrolactamization reaction to investigate whether cyclization of the cryptophycin-52 triazole analogue is generally feasible. In this previously developed cyclization strategy,¹⁹ ring closure occurs by amide formation between units A and B (Scheme 2).

Scheme 2. Coupling Strategy 1: Ring Closure by Macrolactamization



The unit C building block azidopivalic acid (**10**) was obtained from commercially available chloropivalic acid (**9**). DCC/DMAP-mediated condensation of **10** with the unit D precursor **11**²⁰ afforded CD segment **12**. The subsequent [3 + 2]-cycloaddition between **12** and unit B alkyne **8** was performed in the presence of CuI, affording BCD segment **13**. The corresponding unit A precursor **15** was obtained by completely *E*-selective cross-metathesis reaction of **14** and *tert*-butyl acrylate, mediated by Grubbs' second generation catalyst.²¹ Hydrogenolytic deprotection of BCD segment **13** was followed by esterification with unit A precursor **15** to afford the *seco*-compound **16**. All three acid-labile protective groups of **16** were cleaved simultaneously and the product was directly subjected to macrolactamisation under pseudohigh dilution conditions to afford **17** in 74% yield starting from **16**.

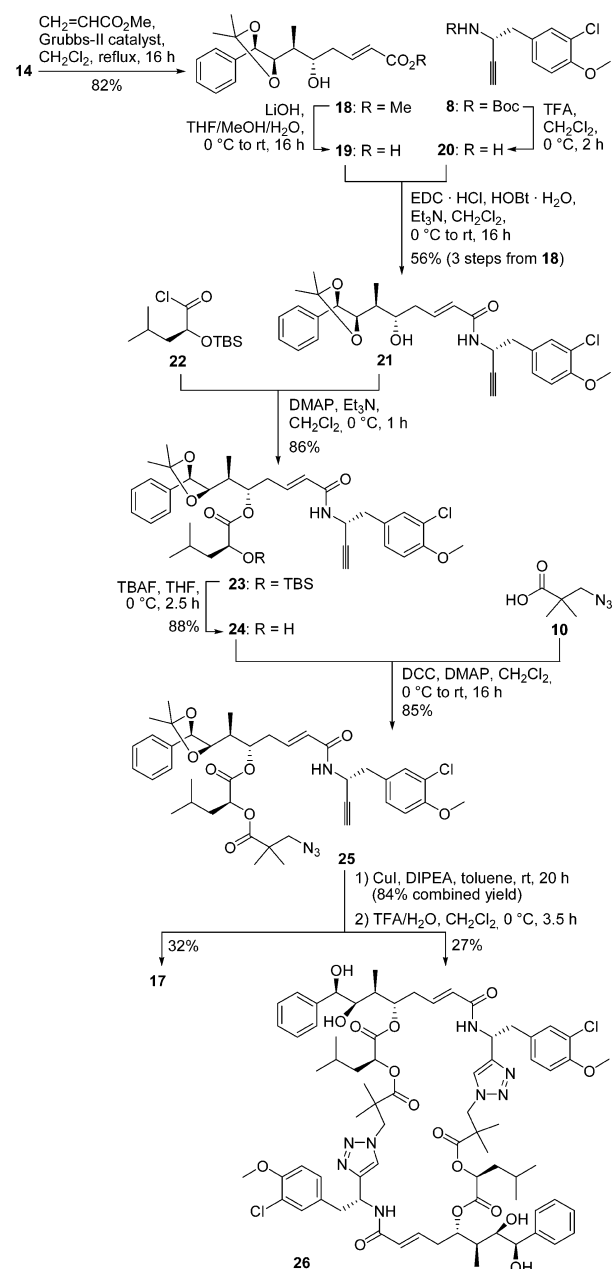
"Click" [3 + 2]-cyclizations have been introduced by Meldal et al. in 2004.²² They typically afford a mixture of corresponding cyclomonomers, cyclodimers, and cyclotrimers, which is explained by competing complexation of one Cu^I ion by two acetylide moieties.³ The cyclo-oligomers sometimes even are formed as the main products,^{23a} while acyclic oligomers are not observed.³

The corresponding linear precursor **25** was synthesized to study the macrocyclisation of "clicktophycin-52", a cryptophycin-52 triazole analogue, by Cu^I catalyzed azide-alkyne coupling (Scheme 3).

The previously described unit A methyl ester **18** was carefully saponified and crude **19** was used directly because of its limited stability. Selective coupling of **19** and amine **20** was mediated by EDC/HOAt. The resulting AB segment **21** was condensed with freshly prepared unit D precursor **22**²⁴ to yield TBS-protected DAB-segment **23**. Cleavage of the silyl ether affording secondary alcohol **24** was followed by Steglich esterification with azidopivalic acid (**10**) to give the *seco*-precursor **25**.

Schreiber and co-workers optimized reaction conditions for Cu^I catalyzed solution phase ring closure of 17-membered peptidomimetics. By using CuI/DIPEA in toluene, cyclomonomers were obtained as main products in yields ranging from 53 to 83%, while unwanted cyclo-oligomers were only obtained as minor byproduct.^{23b} Under similar conditions, van Maarseveen et al. achieved a high yielding cyclization

Scheme 3. Coupling Strategy 2: Macrocyclization by "Click" Reaction



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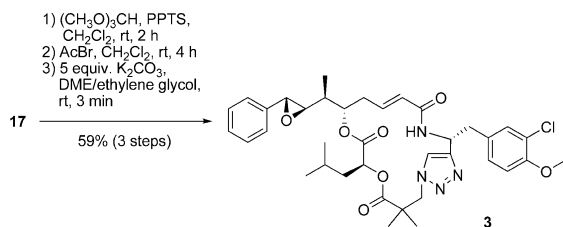
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of otherwise inaccessible cyclotetrapeptide mimetics.⁷ Following the first procedure,^{23b} we cyclized the cryptophycin precursor **25** at room temperature in 4 mM toluene solution. An inseparable mixture of cyclomonomer and cyclodimer was obtained in a combined yield of 84%. Alternative solvent/Cu^I source combinations such as DMF/CuI and H₂O/*t*-BuOH/CuSO₄/ascorbate lead to incomplete conversions. After acidic cleavage of the acetonides, free diols **17** and **26** were cleanly separated by column chromatography. Cyclomonomer **17** was isolated as main product in 32% yield over two steps, whereas cyclodimer **26** was obtained in 27% yield.

Finally, *syn*-diol **17** was converted into epoxide **3** in 59% yield over three steps (Scheme 4).^{19,25} The final epoxide

Scheme 4. Diol-Epoxide Conversion to “Clicktophycin-52” (**3**)



formation was performed in a potassium carbonate/ethylene glycol/DME emulsion as reaction medium.²⁶

In cytotoxicity assays against the multidrug resistant human cervix carcinoma cell line KB-V1, triazole analogue **3** exhibited an IC₅₀ of 3.2 nM. Hence, in this one-point comparison, it is only about five times less potent than the parent cryptophycin-52 (**2**) (IC₅₀ = 0.7 nM). The triazole analogue possibly may show improved activity over cryptophycin-52 when assayed against a broad panel of tumor cells.

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Since NMR signals of most carbon and hydrogen atoms within units A and D of **3** are markedly shifted compared to those of cryptophycin-52 (**2**), a distinct influence of the triazole ring on the preferred conformation of the macrocycle seems likely (see Supporting Information).

After all, in comparison to the 16-membered ring of **2**, the macrocyclic structure of its triazole-analogue **3** is widened to a 17-membered ring.

In conclusion, the largely maintained bioactivity of “clicktophycin-52” (**3**) compared to cryptophycin-52 (**2**) underlines the considerable bioequivalence of *trans*-amides and 1,4-disubstituted 1*H*-1,2,3-triazoles as linkages within peptidic structures.

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Supporting Information Available: Experimental procedures, full spectroscopic data, ¹H and ¹³C NMR spectra, and details on the cytotoxicity assays. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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