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"Clicktophycin-52": A Bioactive Cryptophycin-52 Triazole Analogue

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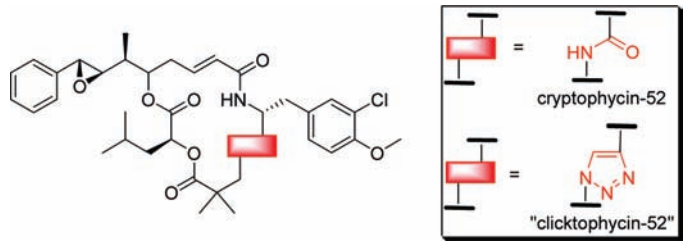
ABSTRACT



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-galactosylceramide,[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 cells[14] 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).

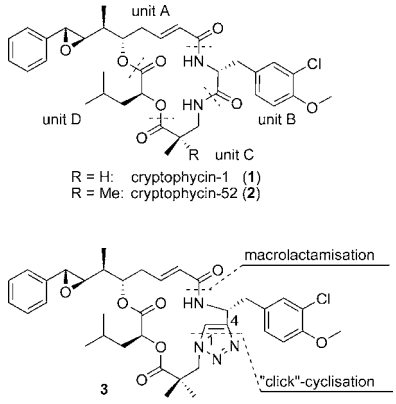
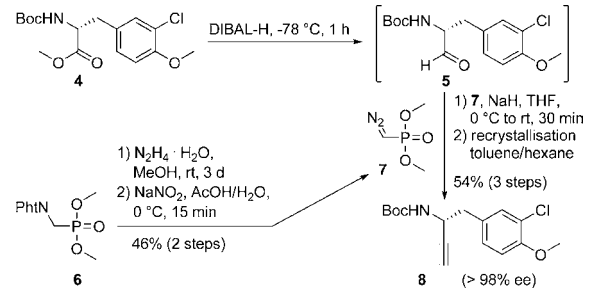


Figure 1. Structures of cryptophycins and their triazole analogue **3**.

Several elegant approaches as well as ample structure−activity relationship studies have been reviewed.[15]

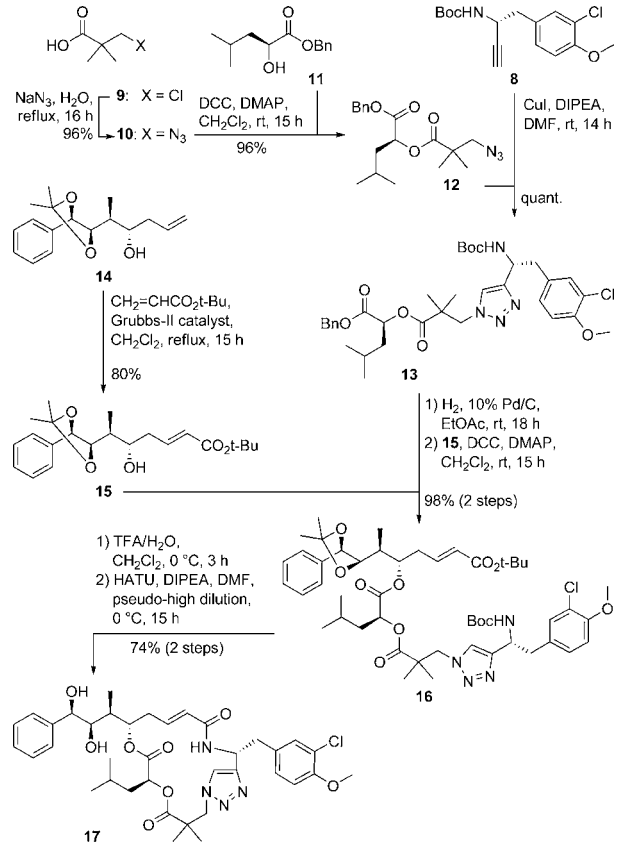
The unit B alkyne building block **8** represents a key intermediate in the synthesis of the cryptophycin-52 triazole analogue **3** and was obtained by Seyferth-Gilbert homologation[16] of aldehyde **5**. Starting with reduction of **4**[17] with DIBAL-H, aldehyde **5** was not purified because of its 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 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).

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



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



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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**.

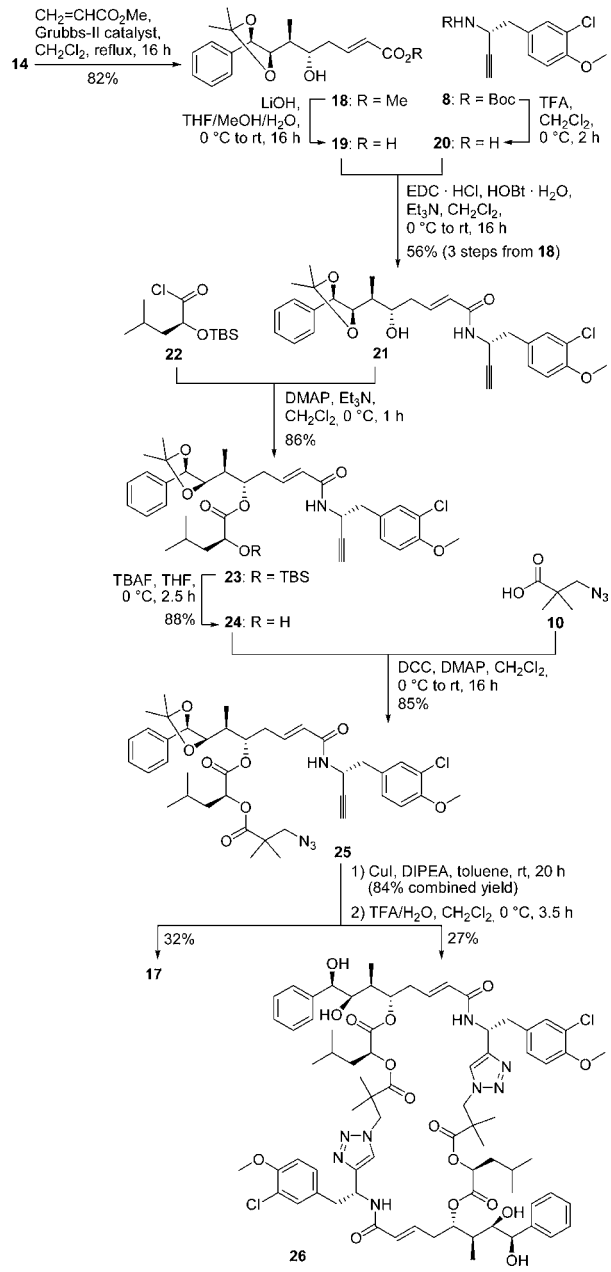
"Click" [3 + 2]-cyclizations have been introduced by Meldal et al. in 2004.[22] They typically afford a mixture of corresponding cyclomonomers, cyclodimers, and cyclotrimers, which is explained by competing complexation of one Cu^{I} ion by two acetylide moieties.[3] The cyclo-oligomers sometimes even 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



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 H_2O/*t*-BuOH/$CuSO_4$/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.

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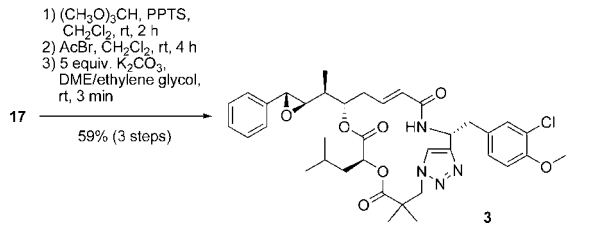
Finally, *syn*-diol **17** was converted into epoxide **3** in 59% yield over three steps (Scheme 4).[19,25] The final epoxide

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 IC_{50} of 3.2 nM. Hence, in this one-point comparison, it is only about five times less potent than the parent cryptophycin-52 (**2**) (IC_{50} = 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, ^{1}H and ^{13}C 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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