Fully Automated Click Cyclization of a Cancer-Targeting Peptide on the Prelude®

Application Note 21 D0029155/B

Introduction

The copper-catalyzed azide-alkyne cyclization (CuAAC), the most commonly recognized variant of "click chemistry," has emerged as a powerful technique for ligation, conjugation, and cyclization reactions of peptides. It is known that cyclization can increase the metabolic stability of peptides, as well as enhancing potency or selectivity by stabilizing an active conformation. One application of the CuAAC that has generated interest is the use of this reaction to replace a disulfide bridge with the product triazole, which among other complementary properties may prevent in vivo redox chemistry. In a recent example, a novel cyclic NGR peptide analogue was synthesized on resin via click chemistry.[1]

Small peptides containing the NGR motif have generated interest in drug delivery research, as ligands containing this sequence seem to bind preferentially to a CD13 isoform expressed in tumor vasculature rather than normal tissue.[2]

In this application, we synthesize a new analogue of the cyclic cancer-targeting peptide CNGRC where we replace the disulfide bond with a triazole linkage using click chemistry (**Scheme 1**) and a fully automated, on-resin method using the **Prelude®** peptide synthesizer. We modified a convenient method that has been described for performing the cyclization, using 20% piperidine in DMF as the only base in the reaction mixture.[3]

The **Prelude**'s **Single-Shot™** delivery feature and extra amino acid bottle positions make it perfect for adding expensive special monomers to a peptide sequence or performing post-translational modifications such as cyclization. The **Prelude**'s **Single-Shot** delivery feature can deliver the entire contents of the special 10 mL **Single-Shot** amino acid vial to the reaction vessel of your choice without priming or wasting a drop.

Method

Linear Peptide Synthesis: Pentynoic acid-Asn-Gly-Arg-azidoalanine was assembled on Gly-ClTrt resin (substitution: 1.30 mmol/g) at the 40 µmol scale on a **Prelude** peptide synthesizer. (Protein Technologies, Inc., Tucson, AZ). **Deprotection:** 20% piperidine/DMF for 2 x 5 min. Washes: DMF 6 x 30 sec. **Coupling:** 5-fold excess, 1.0 mL:0.5 mL:0.5 mL 0.2M AA/0.4M HCTU/0.8M NMM in DMF for 2 x 20 min, except for Fmoc-ß-Azidoalanine, which was added in 2-fold excess using a **Single-Shot** vial and coupled once for 30 min. After the sequence was assembled, the resin was washed 6x with DCM and dried for 20 min. A small portion of the peptide-resin was removed for cleavage and analysis.

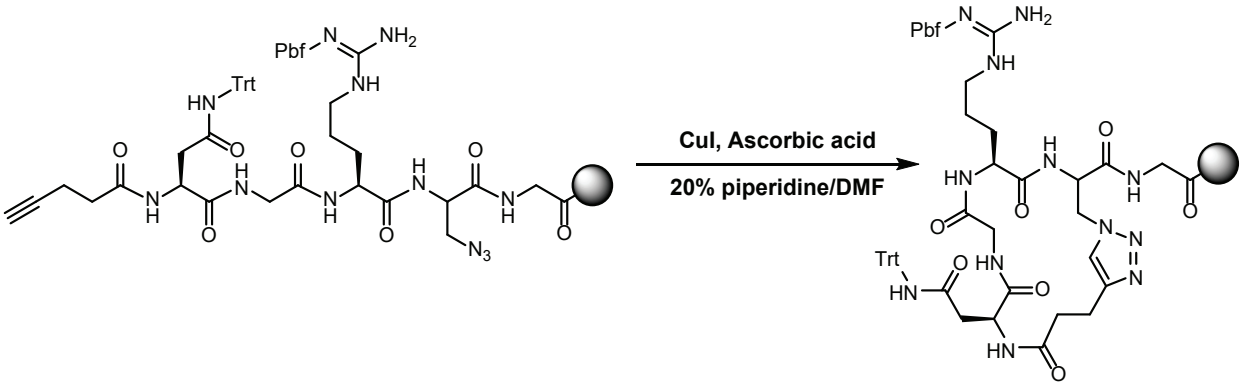
CuAAC "Click" Reaction: 5 eq. of CuI and 10 eq. of ascorbic acid in 20% piperidine/DMF were added to the RV using **Single-Shot** deliveries. Mixing was achieved by intermittent nitrogen bubbling. The reactants were drained after 18 h, and the resin washed with DMF, DIEA and sodium diethyldithiocarbamate solution in DMF (4x), DMF (3x), DCM (6x), and dried for 10 min.

Cleavage: 95/2.5/2.5 TFA/TIS/water was added to the resin and mixed with intermittent nitrogen bubbling for 2 hours. The cleavage cocktail was precipitated in cold ethyl ether and washed with ethyl ether three times, with centrifuging and decanting. The resulting solid residues were allowed to dry overnight and then analyzed by HPLC and LC/MS.

Analysis: Dry crude precipitated peptides were dissolved in water and analyzed on a Varian ProStar HPLC using a C18, 180 Å, 5 µm, 250 x 4.6 mm column (Agilent Polaris), over 60 minutes with a flow rate of 1 mL/min, and using a gradient of 5-95% B, where Buffer A is 0.1% TFA in water, and Buffer B is 0.1% TFA in acetonitrile. Detection was at 214 nm. Mass analysis was performed on a Shimadzu LCMS-2020 Single-Quad mass spectrometer, equipped with a C18, 100 Å, 2.6 µm, 50 x 2.1 mm column (Phenomenex Kinetex), over 7 minutes with a flow rate of 1 mL/min and using a gradient of 5-50% B where Buffer A is 0.1% formic acid in water and Buffer B is 0.1% formic acid in acetonitrile.

Results/Discussion

The linear NGR peptide, flanked by pentynoic acid at the N-terminus and azidoalanine-glycine at the C-terminus, was synthesized using an unoptimized protocol in 78% purity (**Figure 1a**). After treating



Scheme 1. Click reaction.

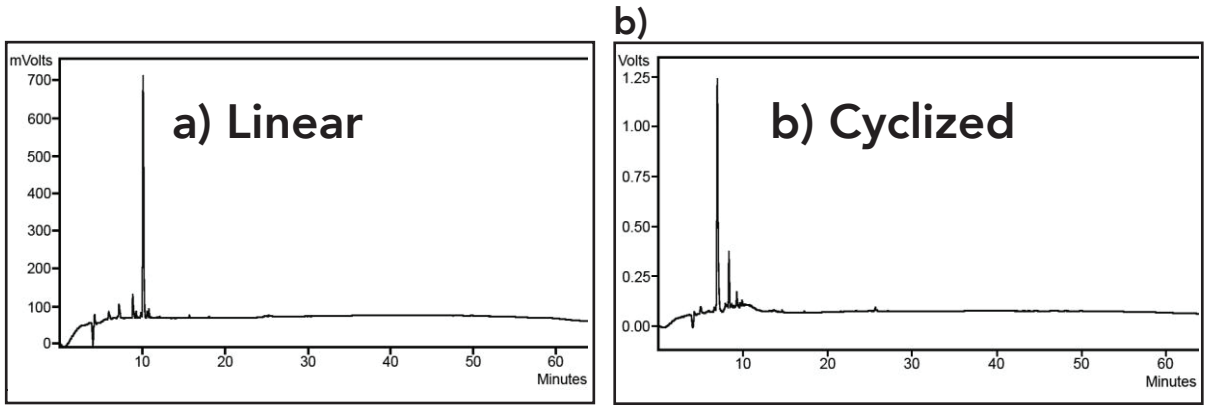


Figure 1. HPLC traces of the a) linear and b) cyclized peptide..

the peptidyl-resin with CuI and ascorbic acid in 20% piperidine/DMF solution, a clear shift in the retention time of the major peak from 10.1 min (**Figure 1a**) to 6.9 min was observed in the HPLC trace (**Figure 1b**), indicating that the cyclization reaction was successful. The product was found with 76% purity.

Conclusion

We have demonstrated that the CuAAC click reaction can be fully automated by synthesizing a novel cyclic cancer-targeting NGR peptide containing a triazole linkage on the **Prelude** peptide synthesizer. The **Prelude**'s **Single-Shot** delivery feature was used to deliver expensive special monomers and reagents without priming or wasting a drop.

References

1) Metaferia et al. Bioorg. Med. Chem. Lett. 2010, 20, 7337-7340.
2) Corti A, Cumis F, Arap W, Pasqualini R. Blood 2008, 112, 2628-2635.
3) Zhang Z, Fan E Tetrahedron Lett. 2006, 47, 665-669.