

Click Chemistry as a Powerful and Chemoselective Tool for the Attachment of Targeting Ligands to Polymer Drug Carriers

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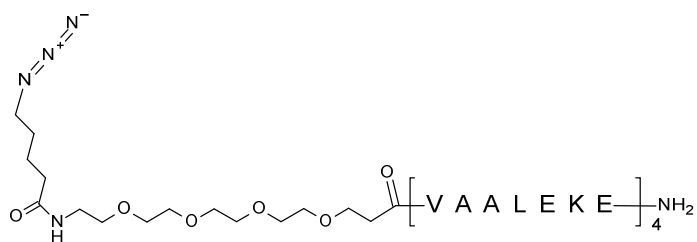
Supplementary Information

Synthesis of peptide azides E and K

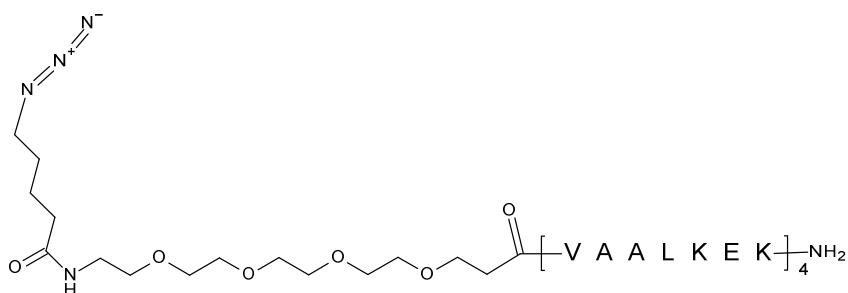
The loading of the 2-chlorotrityl chloride resin with Fmoc-amino acid in DCM in the presence of DIPEA (4 equiv) was assessed by the spectrophotometric determination of the Fmoc group (0.37 mmol/g) released with 25% piperidine in DMF. The linear fully protected heptapeptide was assembled using an AVSP-2 multiple automatic peptide synthesizer (Development Workshops of the Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic), starting from the C-terminus using standard Fmoc procedures, by consecutive addition of the N-Fmoc-protected amino acid (2.5 equiv), PyBOP (2.5 equiv), HOBt (2.5 equiv), and DIPEA (5.0 equiv) in DMF. The Fmoc groups were removed using piperidine–DMF (1:4). The Fmoc-(Fmoc-Hmb)Ala-OH derivative was used for the incorporation of the Ala residue following Leu in peptides. The following Fmoc-Ala-OH (10 equiv) was coupled as its symmetric anhydride preformed with DIC (10 equiv) in 50% DMF–DCM. Cleavage of the protected peptides from the resin was performed with a 30% solution of HFIP in DCM, 25 mL/g of resin, for 2 h. The resin was filtered off and rinsed with DCM, the filtrate was concentrated under vacuum, and the oily residue was precipitated with Et₂O. The precipitate was isolated by filtration and dried in vacuum and characterized. Fully protected heptapeptides VAALKEKE and VAALKEK (0.05 mmol) were attached to the amino group of the TentaGel Rink amide resin in DMSO using DIC (0.1 mmol) as a coupling agent. The condensation was carried out for 6 h at 25 °C. The resin was incubated with acetic anhydride (10 equiv) and DIPEA (20 equiv) in DMF for 30 min to end-cap possible unreacted amino groups. The peptide resin was washed with DMF, deprotected with 25% piperidine and 2% DBU in DMF, and washed again with DMF and DMSO. The condensation (with 0.15 mmol of the protected heptapeptide), acetylation, deprotection, and washing steps were repeated until four repeating heptads were assembled. Then the peptide resin was condensed with Fmoc-Peg₄ (2.5 equiv) using PyBOP (2.5 equiv), HOBt (2.5 equiv), and DIPEA (5.0 equiv) in DMF. After removal of Fmoc, the N-terminus was modified with 5-azidopentanoic acid under the same conditions as described above. The peptides were cleaved from the resin with a mixture of TFA–H₂O–TIPS (95:2.5:2.5). The crude peptides were purified on a semipreparative Chromolith C18 column using a gradient elution of acetonitrile–water with 0.1% TFA¹.

Abbreviations

1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU), dichloromethane (DCM), *N,N'*-diisopropylcarbodiimide (DIC), *N,N*-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), 1,1,1,3,3,3-hexafluoropropan-2-ol (HFIP), glycylglycine, ethyldiisopropylamine (DIPEA), 1-hydroxybenzotriazole (HOBt), trifluoroacetic acid (TFA), triisopropylsilane (TIPS), (benzotriazol-1-yloxy)-trispyrrolidinophosphonium hexafluorophosphate (PyBOP), 9-fluorenylmethoxycarbonyl (Fmoc), α -*O*-(2-carboxyethyl)- ω -*O*-(2-Fmoc-aminoethyl)tetraethylene glycol (Fmoc-Peg₄), 2-hydroxy-4-methoxybenzyl (Hmb).

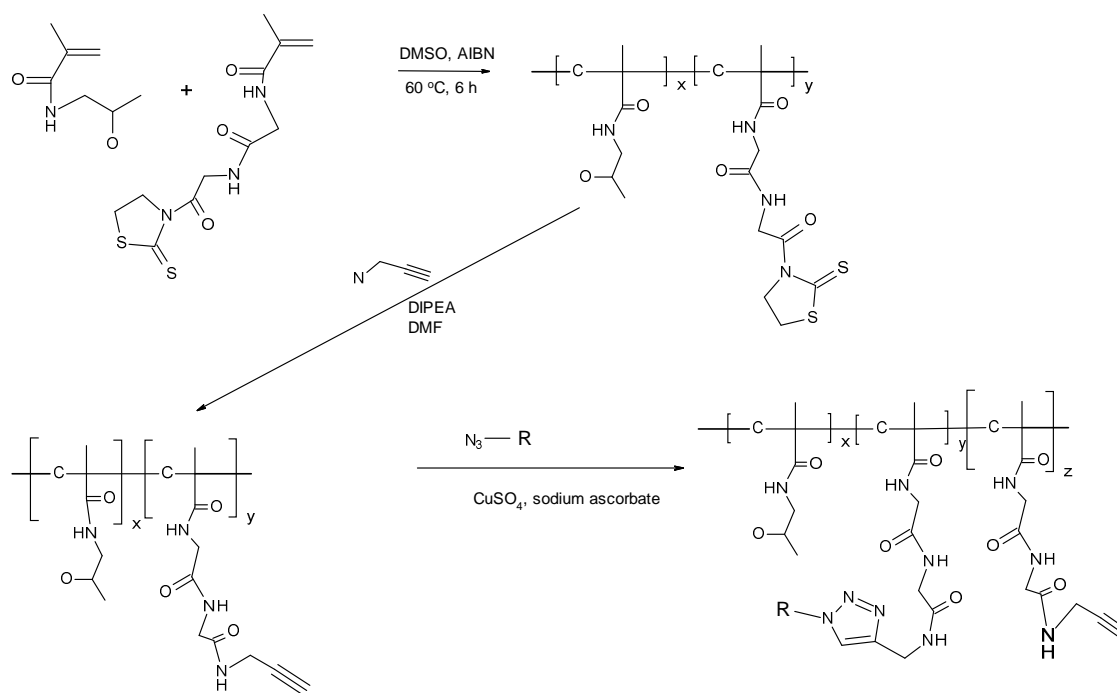


Peptide E

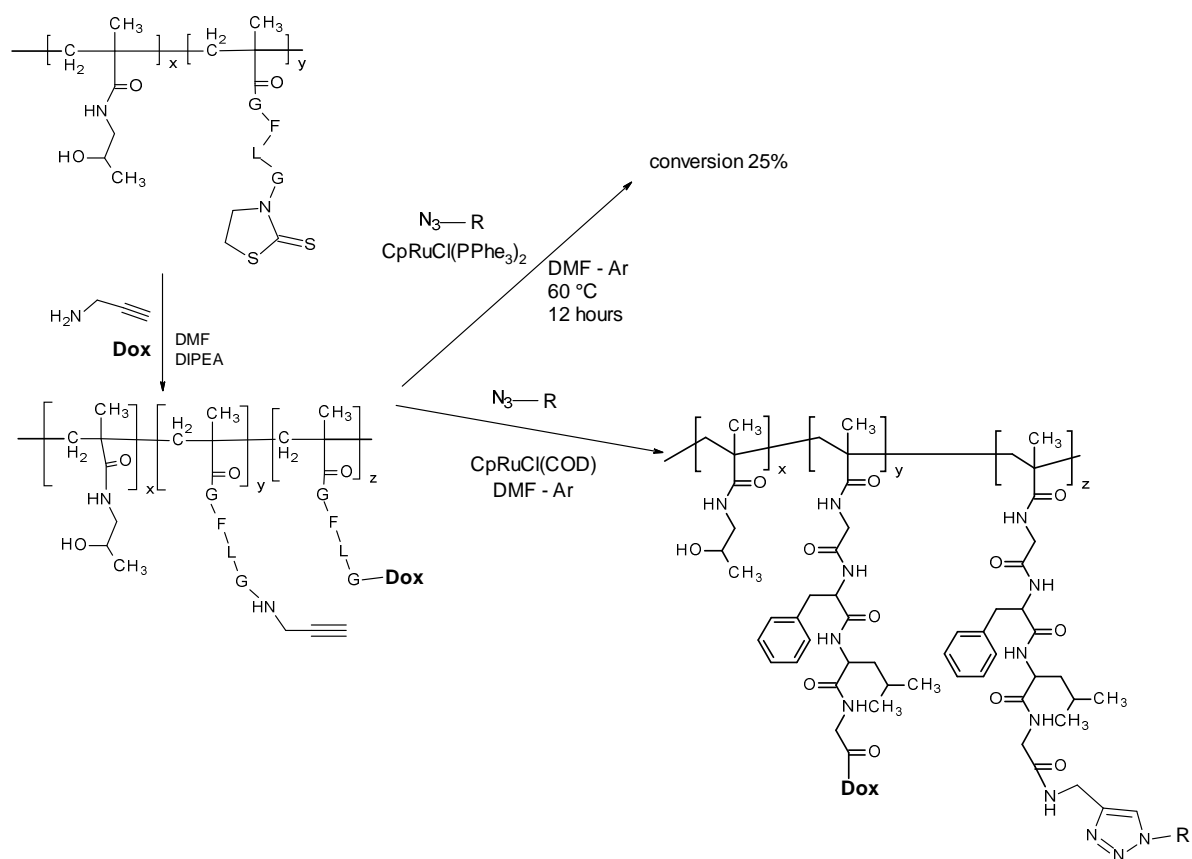


Peptide K

Scheme S1. Structures of peptide azides E and K.



Scheme S2. Click chemistry using copper (II) sulfate in the presence of sodium ascorbate.



Scheme S3. Click chemistry catalyzed by ruthenium complexes.

Reference

Pechar, M.; Pola, R.; Laga, R.; Ulbrich, K.; Bednarova, L.; Malon, P.; Sieglova, I.; Kral, V.; Fabry, M.; Vanek, O. Coiled Coil Peptides as Universal Linkers for the Attachment of Recombinant Proteins to Polymer Therapeutics. *Biomacromolecules* **2011**, *12* (10), 3645-3655.