Synthesis of a “clickable” folic acid derivative

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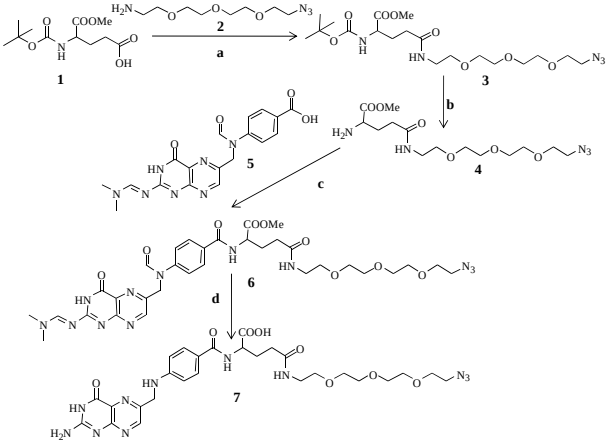
Introduction: Folic acid is a dietary vitamin, which is involved in the one-carbon metabolism in eukaryotic cells [1]. In normal cells it is taken up in its reduced form, the tetrahydrofolate, by the reduced-folate carrier (RFC). This carrier has only a low affinity for the oxidized form, folic acid. In contrast, the folate receptor (FR), a glycoprotein receptor, is able to transport the oxidized form into the cell via endocytosis. In highly proliferating cells the FR is (over)expressed because of the increased need for C1-building blocks. These are essential for *de novo* DNA-synthesis [2]. Moreover, there is a massive increased FR-expression in malignant human endothelial cancer cells. The degree of overexpression of the FR depends on the type of tumor, but can reach levels of up to 1400fold and higher. A physiological expression of the FR in the choroid plexus, thyroid and kidneys is apical and thus not directly reachable for i.v. injected compounds. Therefore, the FR represents an interesting target for tumor diagnostics and therapy.

Methods and Materials: To synthesize folic acid-PEG_4-azide (**7**), 100 mg (0.38 mmol) *N-BOC*-Glu-OMe (**1**) was coupled to 83 mg (0.38 mmol) 11-Azido-3,6,9-trioxaundecan-1-amine (**2**) using 163 mg (0.38 mmol) COMU as the activating agent of the γ-carboxylic acid of the glutamate and 2 eq. of TMP as base. The reaction occurred within 24 h at room temperature in acetonitrile. The solvent was removed under reduced pressure and resolved in dichloromethane (DCM). The crude reaction mixture was extracted 3-times with 1 M HCl-solution, 3-times with 1 M $NaHCO_3$-solution and washed with brine. The organic phase was dried with sodium sulfate and the solvent was removed *in vacuo*, obtaining 138 mg (0.31mmol) of *N-BOC*-Glu-OMe-PEG_4-N_3 (**3**). **3** was stirred in pure TFA over night at 40 °C. After removal of TFA the mixture was adjusted to a basic pH value with 1 M Na_2CO_3-solution and extracted 3-times with DCM, dried, evaporated to dryness obtaining quantitative conversion, getting 105 mg (0.31 mmol) Glu-OMe-PEG_4-N_3 (**4**). The activated ester of the protected pteroic acid (**5**) was formed by dissolving 50 mg (0.11 mmol) (**5**) in DCM adding 1 eq. DIPEA and 1 eq. ethyl chloroformate. 38 mg (0.11 mmol) of **4** were added dropwise in DCM. After 24 h the solvent was removed *in vacuo* and the crude reaction mixture was purified by column chromatography. After purification 15 mg (0.02 mmol) of protected folic acid-PEG_4-N_3 (**6**) were obtained. For deprotection, **6** was stirred for 24 h at 40 °C in 1 M NaOH-solution. The final product **7** was obtained by adjusting the pH value between 1.8 and 2.4 which led to precipitation of the deprotected folic acid derivative, getting 3 mg (0.004 mmol) of a yellow solid (*Scheme 1*).

Results and Discussion: The coupling of **1** and **2** with COMU was screened using different solvents and bases. We found that changing the solvent from DMF to acetonitrile simplifies the work up without losing efficiency. By using TMP instead of DIPEA, a higher yield during the coupling reaction could be obtained.



Scheme 1: a) COMU, MeCN, TMP, RT, 24 h. b) TFA, 12 h, 40 °C. c) Ethyl chloroformate, DCM, RT, 24 h. d) 1 M NaOH, 24 h, 40 °C.

Our overall yield by using COMU is higher than the yield using current coupling reagents. The removal of the BOC protection group was successful at 40 °C over night. The coupling of **4** with **5** revealed more problematic. In this case, it is assumed that some current peptide bond forming reagents are not suitable to form the certain amide bond. Ethyl chloroformate forms an anhydride which allowed a higher activation than COMU, HATU etc. During the purification of **6,** two spots on the TLC-plate with almost the same R_f-values were obtained. These spots are either protonation levels or a product which is partly deprotected. The final deprotection in 1 M NaOH at 40 °C followed by precipitation of **7** proceeded well, but was also accompanied by noticeable product losses.

Outlook: The “clickable” folic acid is a kind of a key compound. This compound will be coupled to a ^{18}F-labeled Alkyne-PEG-^{18}F-Synthon, to siRNA and siRNA-systems in cooperation with Prof. M. Helm (Pharmacy department) and to polymeric drug-delivery-systems in cooperation with Prof. R. Zentel (Organic Chemistry department) to characterize the *in vivo* behavior of these compounds and structures using PET imaging.

References

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Acknowledgements: We thank the Merck & Cie AG (Switzerland) for kindly providing us with protected pteroic acid. This work is further supported by the research cluster SAMT of the Johannes Gutenberg-University Mainz.