Labeling of HPMA based polymers via click chemistry methodology

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Introduction: During the last decades the development of polymer based drug targeting systems for medical diagnosis and treatment has been of increasing interest in pharmaceutical research.[1] For medical applications sufficient knowledge about the *in vivo* behavior is required. Here, positron emission tomography (PET) can be used to visualize the *in vivo* fate of potential drug targeting systems in living organisms. With the development of versatile labeling strategies e.g. via small ^{18}F-labeling synthons, various polymeric systems can be visualized without interfering structure and properties of the macromolecular systems. The "click" reaction between terminal alkynes and azides ideally complies with the requirements of fluorine-18 labeling[2] and was used for the labeling of an alkyne functionalized HPMA-based model polymer under mild conditions.

Experimental: For the labeling of alkyne functionalized polymers the ^{18}F-labeling precursor 2-(2-(2-azido-ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (**3**) was synthesized in 2 steps beginning with 2,2'-ethylenedi-oxydiethanol (**1**) (cf. Figure 1).

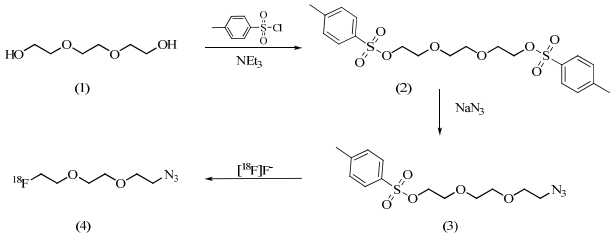

Figure 1. Synthesis and ^{18}F-labeling of the azide functionalized labeling precursor **3** towards the new ^{18}F-synthon **4**.

Fluorine-18 labeling of **3** was performed using a solution of 3,3 mg (10 µmol) precursor, 15 mg Kryptofix® 222, 15 µL 1M K_2CO_3-solution in 1 mL of MeCN at 100 °C. Purification of the synthon **4** was accomplished using HPLC (Luna RP18, MeCN:H_2O 50:50, flow: 4 mL/min, t_R: 6,5 min). After diluting the HPLC product fraction with water, **4** was loaded on a C18-SepPak cartridge and eluted with 0.8 mL of DMSO.
For labeling of the alkyne functionalized polymer via click chemistry (cf. Figure 2), the eluted solution of **4** was added to a mixture of 100µL 0.6M sodium ascorbate solution, 50 µL 0.4 M $CuSO_4$-solution, 50 µL H_2O and 2 mg of polymer **5** and the reaction mixture was stirred at RT.

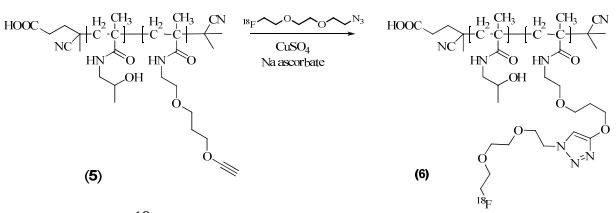

Figure 2. ^{18}F-labeling of HPMA-based polymer via Cu(I)catalysed 1,2,3-triazole formation with the ^{18}F-synthon **4**.

The ^{18}F-labeled polymer (**6**) was separated using size exclusion chromatography (HiTrap™ Desalting Column, Sephadex G-25 Superfine, flow: 0.5 mL/min, pure water).

Results: Nucleophilic fluorination of synthon (**3**) was achieved in up to 80% RCY within 10 min (cf fig. 3).

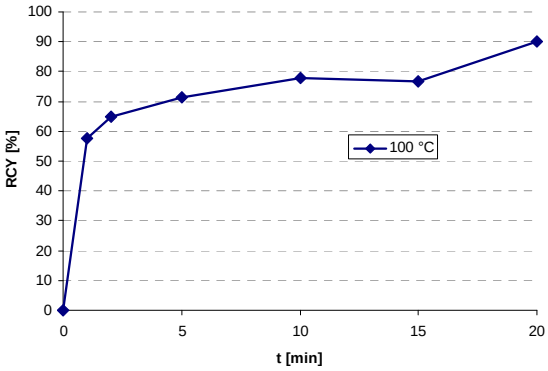

Figure 3. RCY of **4** from ^{18}F-labeling using Kryptofix® 222/K_2CO_3 in MeCN at 100 °C.

Purification of the azide labeling synthon **4** was accomplished successfully via RP-HPLC and SPE and labeling of an alkyne functionalized HPMA-based model polymer could be performed using click chemistry methodology in 60% RCY within 40 min (cf Figure 4).

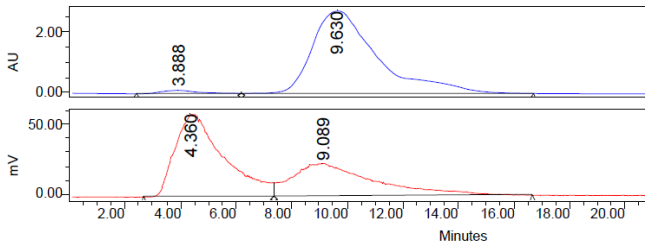

Figure 4. Size exclusion chromatogram of the ^{18}F-labeled polymer (**6**). top: UV-chromatogram. bottom: radioactive chromatogram with labeled polymer at t_R:4.3 min and free 4 at t_R:9.1 min.

References

[1] R. Duncan, Nat. Rev. Cancer 2006, 6 (9), 688-701.
[2] T. L. Ross, Curr. Radiopharmaceuticals 2010, 3 (3), 202-223.

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