

¹⁸F-labeled analogues of anti-tumor agent calixarene 0118

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Objectives: Calix[4]arene compound 0118 (Figure 1A), a topomimetic of the antiangiogenic amphipathic peptide anginex targeting galectin-1, is currently in Phase I clinical trials with terminal cancer patients [1]. Radiolabelled analogues of compound 0118 may serve as a development tool in PK/PD studies of this class of calix[4]arene compounds, and may prove highly valuable for patient stratification and therapy monitoring. So far, such radiotracers have not been described. In this work, we have designed compound 0118 analogues containing a terminal alkyne functional group for introduction of an F-18 label via Cu(I)-catalyzed 1,3-dipolar cycloaddition of 2-[¹⁸F]fluoroethylazide.

Methods: The alkyne-functionalized calix[4]arene precursors and the cold 2-[¹⁹F]fluoroethyltriazole reference compounds (Figure 1B) were synthesized starting from tetrahydroxycalix[4]arene and purified to >99%. In the optimized, semi-automated procedure for the Click-reaction, 2-[¹⁸F]fluoroethylazide [2] was directly distilled onto the pre-cooled vial (-40°C) containing the alkyne-functionalized calix[4]arene (3.0 μmol in 500 μL of DMSO). After addition of a freshly prepared catalyst solution (9 μmol of CuSO₄ in 150 μL of H₂O and 90 μmol of Na-ascorbate in 100 μL of 0.5 M sodium phosphate buffer pH 6.0), the mixture was reacted for 15 min at 80°C, purified by semi-preparative HPLC, and formulated in ethanol.

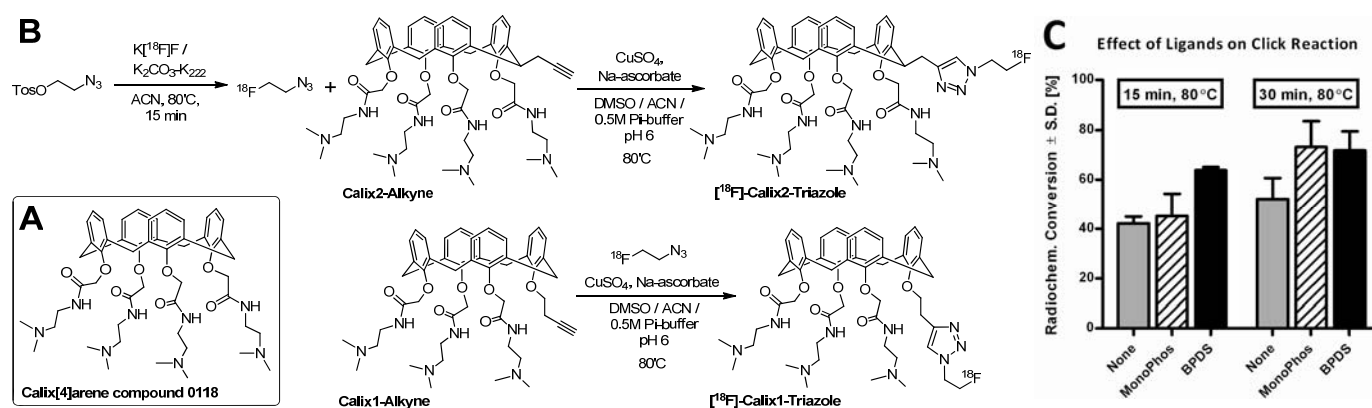


Figure 1. A) Structure of calix[4]arene compound 0118; B) Radiosynthesis of ¹⁸F-labeled analogues of calixarene 0118; C) Effect of ligands on Click-reaction with Calix2-Alkyne (n = 3).

Results: 2-[¹⁸F]fluoroethylazide was obtained in 53 ± 5 % (n = 8) decay-corrected isolated radiochemical yield starting from [¹⁸F]F⁻ in a total synthesis time of 60 min including purification by co-distillation with acetonitrile. Conditions for the Click-reaction with the Calix2-Alkyne precursor (Figure 1B) were optimized in terms of reaction time, excess of CuSO₄/Na-ascorbate, and addition of the Cu(I)-stabilizing ligands BPDS and Monophos. Even with 0.5eq/5eq CuSO₄/Na-ascorbate, however, maximum radiochemical conversions in the presence of BPDS or Monophos (0.55eq) were generally just about 75% (Figure 1C). Complete conversion was achieved only when using a large excess of 3eq/30eq CuSO₄/Na-ascorbate (15 min at 80°C), yielding [¹⁸F]Calix2-Triazole in >99% radiochemical purity in a total synthesis time of less than 2 h, including preparative HPLC and formulation. The same conditions for the Click-reaction also proved effective for the lower-rim modified precursor, Calix1-Alkyne, giving access to the corresponding [¹⁸F]Calix1-Triazole, which was obtained in an overall decay-corrected isolated radiochemical yield of 19.5 ± 2.4 % (n = 3) and a radiochemical purity of >98%.

Conclusions: We have designed structurally closely related chemical analogues of anti-tumor agent calix[4]arene 0118 containing a terminal alkyne functional group, successfully developed a multi-step synthetic strategy for their preparation, and optimized radiolabeling conditions for Click-labeling with 2-[¹⁸F]fluoroethylazide. Biological evaluation of both radiotracers is currently in progress.

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References: [1] Dings, RPM et al. (2012), J. Med. Chem., 55, 5121-5129. [2] Kobus, D et al. (2009), Appl. Rad. Isot, 67, 1977-1984.