“Clickable” albumin binders to modulate pharmacokinetic properties of theranostic radioligands

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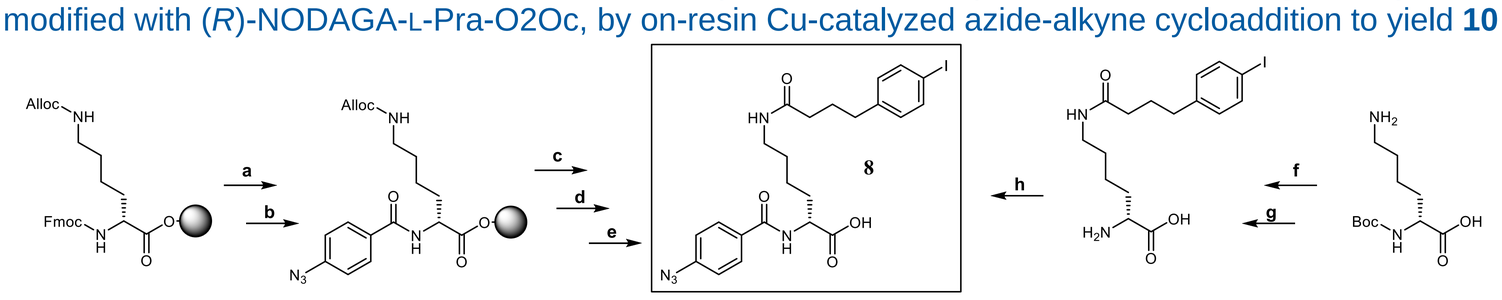
Introduction and Objective

Reversible binding of theranostic radioligands for tumour targeting to human serum albumin (HSA) increases their blood circulation time and can lead to higher accumulation in the target tissue. *N*$^{\alpha}$Acetyl-*N*$^{\varepsilon}$-IPB-lysine (IPB…4-(4-iodophenyl)butanoyl) was recently described as potent binder to HSA.[1] For late-stage modification of different classes of molecules (proteins, peptides, small molecules) with this albumin binder, we developed “clickable” *N*$^{\varepsilon}$-IPB-lysines bearing azide/alkyne functionalities. Application to dual targeting of somatostatin receptor subtype 2 (SSTR2) and HSA is highlighted.

Synthetic Approaches

For the selective acylation of lysine, a solid-phase synthesis concept (2-ClTrtCl-resin) was established starting from Fmoc-Lys(Alloc)-OH. For upscaling (4 mmol), a synthesis in solution starting from Boc-Lys-OH was elaborated, which provides the desired building blocks in three steps (Scheme 1). To demonstrate the suitability of the “clickable” albumin binders, compound **6** was coupled to the SSTR2 agonist Tyr^3-octreotate (TATE, D-Phe-c(Cys-Tyr-D-Trp-Lys-Thr-Cys)-Thr), modified with (*R*)-NODAGA-L-Pra-O2Oc, by on-resin Cu-catalyzed azide-alkyne cycloaddition to yield **10 (**Figure 1**)**.



Scheme 1. Synthetic approaches for selective acylation of lysine exemparily shown for compound (**8**)
a) 20% piperidine/DMF, 2×10 min; **b)** 4-azidobenzoic acid, HATU, DIPEA, 2 h; **c)** 5mol% $Pd(PPh_3)_4$, phenylsilane, CH_2Cl_2, 2×10min; **d)** 4-(4-iodophenyl)butanoic acid, HATU, DIPEA, 2 h; **e)** HFIP/CH_2Cl_2 1:4 (v/v, 3×10 min each 3 mL); **f)** 4-(4-iodophenyl)butanoic acid NHS ester, THF/H_2O 1:1 (v/v), $NaHCO_3$, 2 h; **g)** CH_2Cl_2/TFA 1:1 (v/v), 2 h; **h)** 4-azidobenzoic acid NHS ester, CH_3OH/THF 1:1 (v/v), Et_3N, 2 h

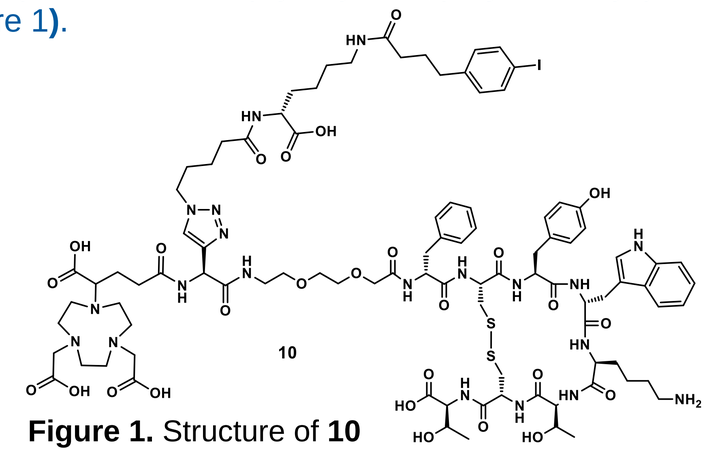


Figure 1. Structure of **10**

Characterisation of compounds

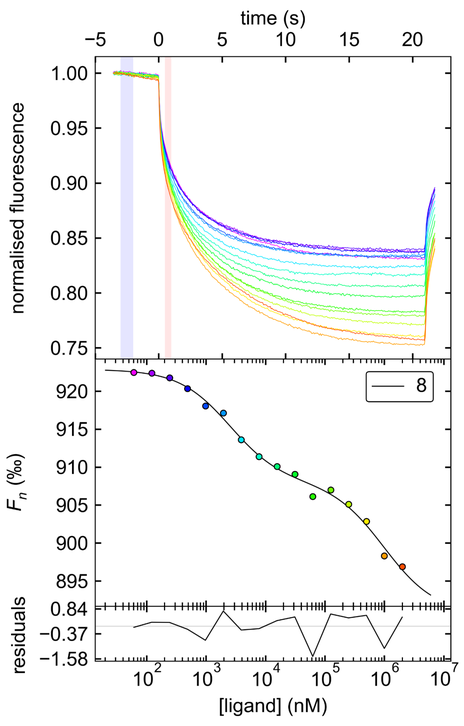
Characterisation of N$^{\varepsilon}$-IPB-lysines towards HSA binding

HSA binding was characterised by a microscale thermophoresis-based assay (MST, Monolith, NanoTemper Technologies) and a fluorimetric competitions assay (Figure 2). For MST, HSA (fatty acid free) was labeled using the Protein Labeling Kit RED-Maleimide according to the manufacture’s instructions. For the competition assay, the change in fluorescence intensity of *N*$^{\alpha}$-6-FAM-*N*$^{\varepsilon}$-IPB-D-lysine (**9**) by displacement from HSA was recorded. All measurements were performed in PBS (pH 7.4, 2% DMSO) at 37°C. Samples were loaded into Monolith NT.115 capillaries. Data of three independently pipetted measurements were analysed by the MST analysis software PALMIST[2] to provide K_d values for the different *N*$^{\varepsilon}$-IPB-lysines (see Table below).

- All novel *N*$^{\varepsilon}$-IPB-lysines showed similar affinity to HSA as *N*$^{\alpha}$-Acetyl-*N*$^{\varepsilon}$-IPB-lysine (**1** or **2**)
- Configuration of lysine (*S* or *R*) seems to be of minor importance for binding to HSA
- Esterification and amidation of the α-carboxylic group lead to significant loss of binding affinity to HSA
- Data from MST assay indicate binding of *N*$^{\varepsilon}$-IPB-lysines to a second binding site at HSA with low affinity ($K_{d,2}$ = >500 µM for **8**, Figure 2)

No.	R =	Config.	X	K_d (µM)
1	Acetyl	*R*	OH	7.0 8.0*
2		*S*	OH	3.1
3		*S*	OCH_3	110.0
4		*S*	NH_2	70.0
5	5-Pentynoyl	*R*	OH	7.0
6	5-Azidopentanoyl	*R*	OH	8.0
7		*S*	OH	6.0
8	4-Azidobenzoyl	*R*	OH	1.2 2.6*
9	6-FAM	*R*	OH	0.23

* Determined by MST assay, all other K_d values were determined by the fluorimetric competition assay



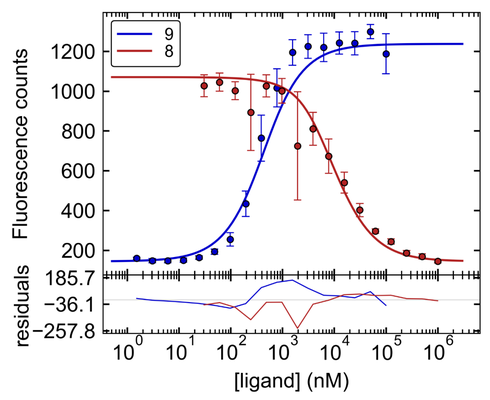
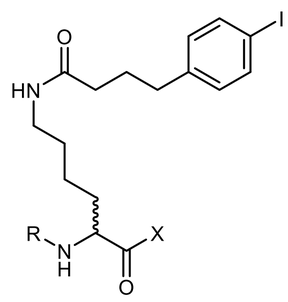


Figure 2. MST (left) and fluorescence (right) data for the interaction of **8** with HSA
Left: The top panel shows the thermophoretic time-traces from one experiment (color-coded, purple and red representing the lowest and highest concentration of **8**, respectively, shaded blue and pink areas were used for calculation of F_n) . The lower panel shows the resulting binding curve (color-coding of data points according to their respective time-trace). **Right:** Binding curves for **9** (ligand is HSA) and **8** (in the presence of **9**) are shown in blue and red, respectively.

*Characterisation of ^{64}Cu-**10** towards HSA and SSTR2 binding*

To determine binding of ^{64}Cu-**10** to plasma proteins in comparison to its alkyne analog ^{64}Cu-**10A**, a ultrafiltration assay was applied using Centrifee Ultrafiltration devices (30 kDa nominal molecular weight limit, 4104, Millipore, Figure 3).[3] Mouse pheochromocytoma cell (MPC) membranes were used for saturation binding experiments to quantify affinities of ^{64}Cu-**10** and ^{64}Cu-**10A** towards SSTR2.[4]



- ^{64}Cu-**10** binds almost completely to plasma proteins in contrast to its alkyne analogue ^{64}Cu-**10A** demonstrating successful targeting of HSA
- Both compounds maintained excellent binding affinity to SSTR2 with K_d values of 1.2 and 2.6 nM for ^{64}Cu-**10** and ^{64}Cu-**10A**, respectively (K_d = 1.8 nM for ^{64}Cu-DOTA-TATE)

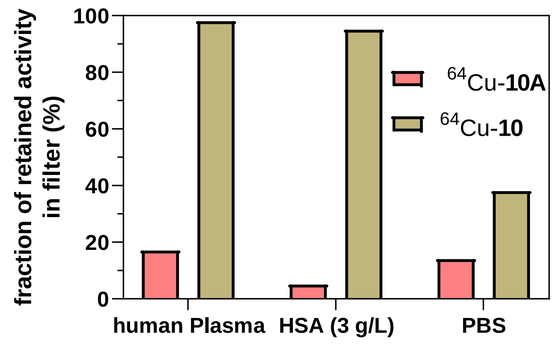


Figure 3. Results of ultrafiltration assay for ^{64}Cu-**10** and ^{64}-**10A**

Conclusion and Outlook

The synthesis of “clickable” *N*$^{\varepsilon}$-IPB-lysines has been established. The compounds maintained their binding potency to HSA. Application for the late-stage modification of sstr2 agonist TATE was successful and the dual targeting behavior of ^{64}Cu-**10** towards HSA and sstr2 was demonstrated. In current studies, biodistribution and tumour uptake of ^{64}Cu-**10** (*vs*. ^{64}Cu-**10A**) will be studied preclinically in MPC tumour bearing mice by small animal PET imaging.

References

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