# A first attempt at a glycosilated DOTATATE-analogue

E. Schirrmacher, F. Rösch
Institut für Kernchemie, Johannes Gutenberg-Universität, Fritz-Straßmann-Weg 2, 55128 Mainz

Many tumours overexpress a variety of receptors for regulatory peptides and peptide hormones. Thus, receptor targeted diagnostic imaging and radiotherapy have become interesting concepts for the detection, localisation and therapeutic intervention of malignant neoplasms. For these purposes, a number of radiolabelled analogues of endogenous peptides have been synthesised and are presently evaluated. Prominent examples are the derivatives of octreotide, a somatostatin (sst) analogue. [$^{111}$In]DTPA-octreotide has already been approved for routine clinical scintigraphy of sst-expressing tumours. For endoradiotherapy, [$^{90}$Y]DOTATOC, a tyrosine-containing analogue of octreotide, is in ongoing clinical trials.

The clinical potential of such tracers will rely, among other factors such as high receptor affinity and selectivity, mainly on early and high tumour/background ratios combined with fast blood clearance and excretion kinetics. To reduce the whole body radiation exposure, the radiolabelled peptidic ligand should be cleared via the kidneys, but with minimal renal uptake to avoid nephrotoxicity.

Glycosylation is a strategy that has often been applied to enhance the bioavailability, resulting from absorption from the small intestine and most likely from an increased stability towards enzymatic degradation.[1] Glucosyl derivatives of Arg$^8$-vasopressin exhibited a sugar dependent renal uptake and intrarenal distribution.[2]

Our aim was thus to synthesise a glysosylated analogue of DOTATOC, namely DOTA-Tyr$^3$-ocetrotate. For this purpose, we synthesised (3,4,5-triacetoxy-6-acetoxymethyl-tetrahydro-pyran-2-ylsulfanyl)-acetic acid, a tetra-acetylated thioglucose linked to acetic acid (Fig. 1).

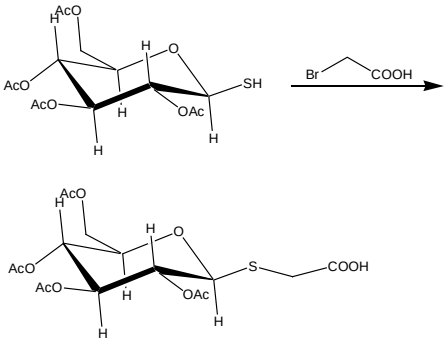


Fig. 1: synthesis of (3,4,5-triacetoxy-6-acetoxymethyl-tetrahydro-pyran-2-ylsulfanyl)-acetic acid

The peptide had to be altered to allow the binding of both the macrocyclic chelator DOTA ((4,7,10-tris-carboxymethyl-1,4,7,10tetraaza-cyclododec-1-yl)-acetic acid) and the thiosugar derivative. Therefore, the amino acid sequence of the peptide was altered to Lys-D-Phe-Cys-Tyr-D-Trp-Lys-Thr-Cys-Thr with a disulfide bond between Cys$^3$ and Cys$^8$. The synthesis followed the classic Fmoc strategy as shown in Fig.2. The tris-tBu-protected DOTA was then coupled as the 10$^{th}$ "amino acid".

Due to the drastic conditions required to deprotect especially the tBu-moieties of DOTA (24 h; TFA), it was not possible to couple the (3,4,5-triacetoxy-6-acetoxymethyl-tetrahydro-pyran-2-ylsulfanyl)-acetic acid on resin as the carbohydrate would have been cleaved under these conditions. Thus the modified DOTATATE was cleaved and fully deprotected except for the Dde protecting group of Lys$^6$ which is stable to acidic conditions. This group was introduced to allow the coupling of the carbohydrate derivative to Lys$^0$ only, because Lys$^6$ is part of the pharmacophore and should not be altered.

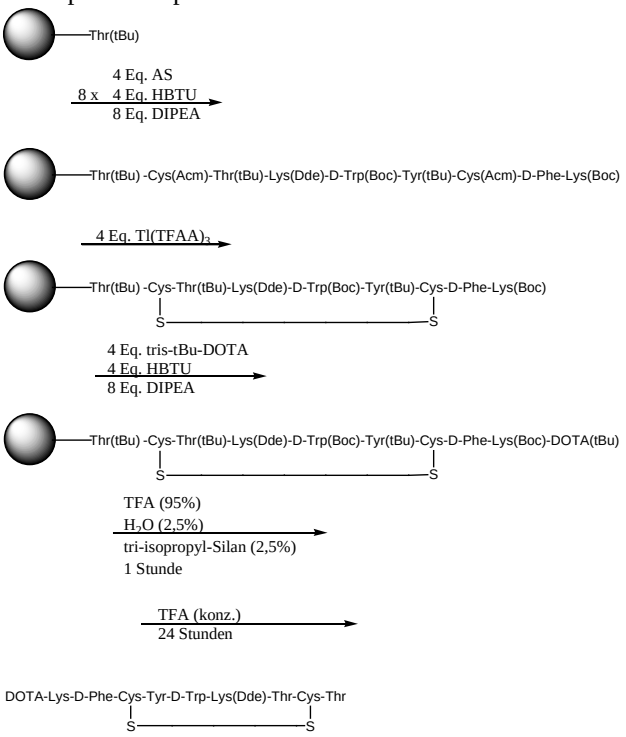


Fig. 2: synthesis of the DOTATATE derivative

After the purification of the crude peptide via semi-preparative HPLC, the (3,4,5-triacetoxy-6-acetoxymethyl-tetrahydro-pyran-2-ylsulfanyl)-acetic acid was coupled in solution to the Lys$^0$ of the peptide. Unfortunately, the complexity of the reaction mixture prevented the isolation of the pure product although MALDI-Tof analysis showed the presence of glycosylated DOTA-Lys$^0$-TATE. Subsequent treatment of the crude peptide with 2% hydrazine in DMF led to the deprotection of the Lys$^6$. The deacetylation of the sugar moiety proved not possible due to the complexity of the reaction mixture which could not be purified with the existing HPLC system.

A new strategy to obtain glycosylated DOTATATE derivatives is under investigation which will allow the on-resin coupling of all the constituents, eliminating the problems of work-up.

[1] Kihlyberg, J., Ahman, J., Walse, B., Drakenberg, T., Nilsson, A. et al. *J Med Chem*, **38**, 161-169 (1995).

[2] Suzuki, K., Susaki, H., Okuno, S., Yamada, H., Watanabe, H. K., Sugiyama, Y. *J Pharmacol Exp Ther*, **288 (2)**, 888-897 (1999).