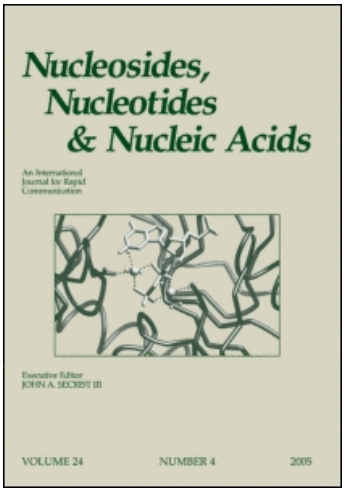

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Tc-99m-Labeling of Modified RNA

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Tc-99m-LABELING OF MODIFIED RNA

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ABSTRACT: The synthesis of Tc-99m-labeled, modified RNA is reported. This new class of radiopharmaceuticals is of potential interest as target specific imaging agents. The preparation of N_3S-RNA was achieved by coupling protected MAG_2-units to amino modified ON's. The N_3S-RNA was Tc-99m-labeled with 90-95% radiochemical yield and specific activities of 37MBq/nmol leading to 1:1-Tc-99m-N_3S-aptamers.

Oligonucleotides (ON's) with rigid secondary structures and sub-nM affinities for extracellular targets can be identified by the SELEX-process[1]. Radioactive labeled aptamers, showing in vivo accumulation in pathologic tissues by recognizing disease-specific targets, could be useful for SPECT-diagnosis in nuclear medicine. Because of its low cost, widespread availability and ideal physical properties, Tc-99m is the isotope of choice for SPECT-imaging. Therefore, methods for high yield synthesis of conjugates between aptamers and Tc-99m binding cores and protocols for efficient Tc-99m-labelings of prepared conjugates have to be established. In the last years several conjugates between HYNIC-, MAG_3- and N_4-chelators with antisense-ON's or DNA's have been synthesized and labeled successfully with Tc-99m[2, 3]. For in vivo applications unmodified RNA and DNA molecules are too unstable against digestion by endo- and exonucleases. Partial replacement of 2'-H atoms in DNA's and of 2'-OH groups in RNA's by e.g. 2'-amino-, 2'-methoxy- or 2'-fluoro-substituents in combination with 3'-caps generates molecules showing high stabilities against nuclease degradation. For preparation of N_3S-conjugated aptamers during solid phase synthesis or for postsynthetic

Figures 1–3

couplings to amino modified ON's in solution or on solid support an NHS-ester of ^{t}Bu-S-protected mercaptoacetyl-$(Gly)_2$-OH **1** was synthesized and coupled to e.g. a L-Selectin binding RNA 5'-{NH_2-$(CH_2)_6$-O-P}-GGAGUCUUAGGCAGCGCGUUU-UCGAGCUACUCC-3'-3'-dT (**2**). The RNA **2** is stabilized against enzymatic degradation by introduction of 2'-F atoms in each C- and U-unit and by capping the 3'-end with a 3'-3'-linked dT. The resulting RNA 5'-{^{t}BuS-S-CH_2-CO-$(Gly)_2$-NH-$(CH_2)_6$-O-P}-GGAGUCUUAGGCAGCGCGUUUUCGAGCUACUCC-3'-3'-dT (**3**) bears the protected N_3S-chelator fixed by an alkylphosphato linker to the 5'-end. The S-protecting group was cleaved by treatment of **3** with an excess of DTT. As analyzed by HPLC (Fig. 1) and TLC (Fig. 2), the Tc-99m-labeling of purified N_3S-RNA 5'-{HS-CH_2-CO-$(Gly)_2$-NH-$(CH_2)_6$-O-P}-GGAGUCUUAGGCAGCGCGUUUUCGAGCUAC-UCC-3'-3'-dT (**4**) was achieved with 95% radiochemical yield. PAGE-analysis (Fig. 3) of Tc-99m-labeled **4** showed one major band confirming the formation of the 1:1-Tc-99m complex **5**. Alternatively, the protected N_3S-RNA **3** was directly radiolabeled to the Tc-99m complex **5** (90% yield). In conclusion, S-protected MAG_2 building blocks were developed and coupled with good yields to amino-modified RNA's and DNA's in solution or on solid support. The prepared MAG_2-amide-oligonucleotides were Tc-99m-labeled with good yields and specific activities of 37MBq/nmol leading to 1:1-Tc-99m N_3S-aptamers.

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