Pergamon

Tetrahedron Letters 39 (1998) 9403–9406

TETRAHEDRON LETTERS

SYNTHESIS OF Tc-99m-LABELED, MODIFIED RNA [1]

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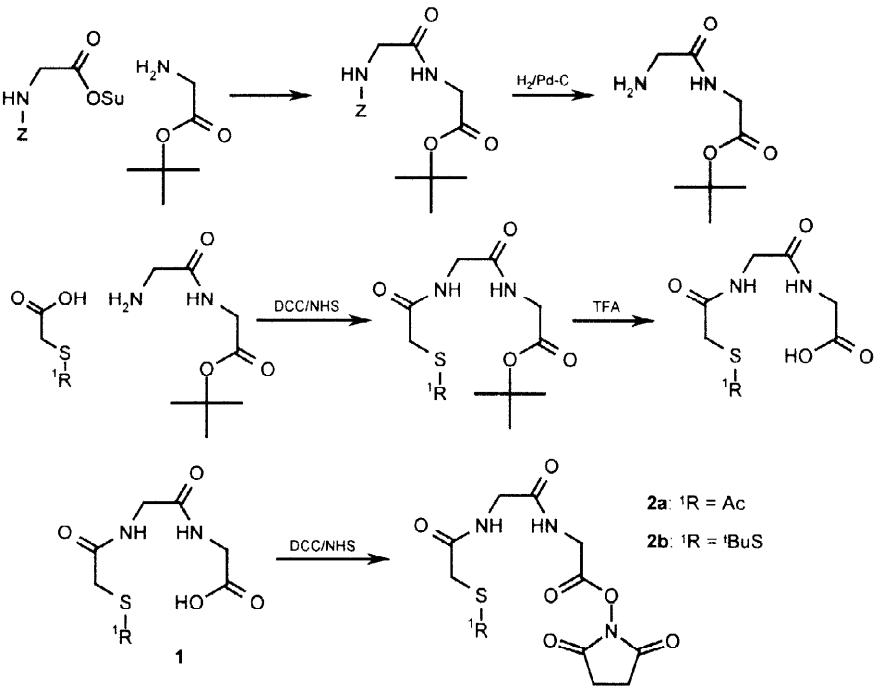
Received 17 September 1998; accepted 9 October 1998

Summary: 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-conjugated RNA was achieved by coupling S-protected MAG_2-units to amino modified RNA in solution or on solid support. The starting S-protected MAG_2 building blocks (^{1}R-S-CH_2-CO-Gly-Gly-^{2}R: ^{1}R = Ac, ^{t}Bu-S; ^{2}R = OH, OSu) were obtained by a simple 4- or 5-step synthesis. The MAG_2-amide-RNA-conjugates were successfully Tc-99m-labeled with high yield and specific activities of 37MBq/nmol leading to 1:1-Tc-99m-N_3S-aptamers.

Oligonucleotide-aptamers with rigid secondary structures, sub-nM affinities and high selectivities for extracellular targets can be identified by the SELEX-process[2]. 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- or N_4-chelators with antisense-oligonucleotides or DNA's have been synthesized and labeled successfully with Tc-99m[3, 4]. For in vivo applications unmodified RNA and DNA molecules are too unstable against cleavage 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 with high stabilities against nuclease degradation. The aim of this work was to develop regioselective and robust methods for the preparation of Tc-99m-N_3S-aptamers stabilized for in vivo applications.

0040-4039/98/$ - see front matter
PII: S0040-4039(98)02193-5

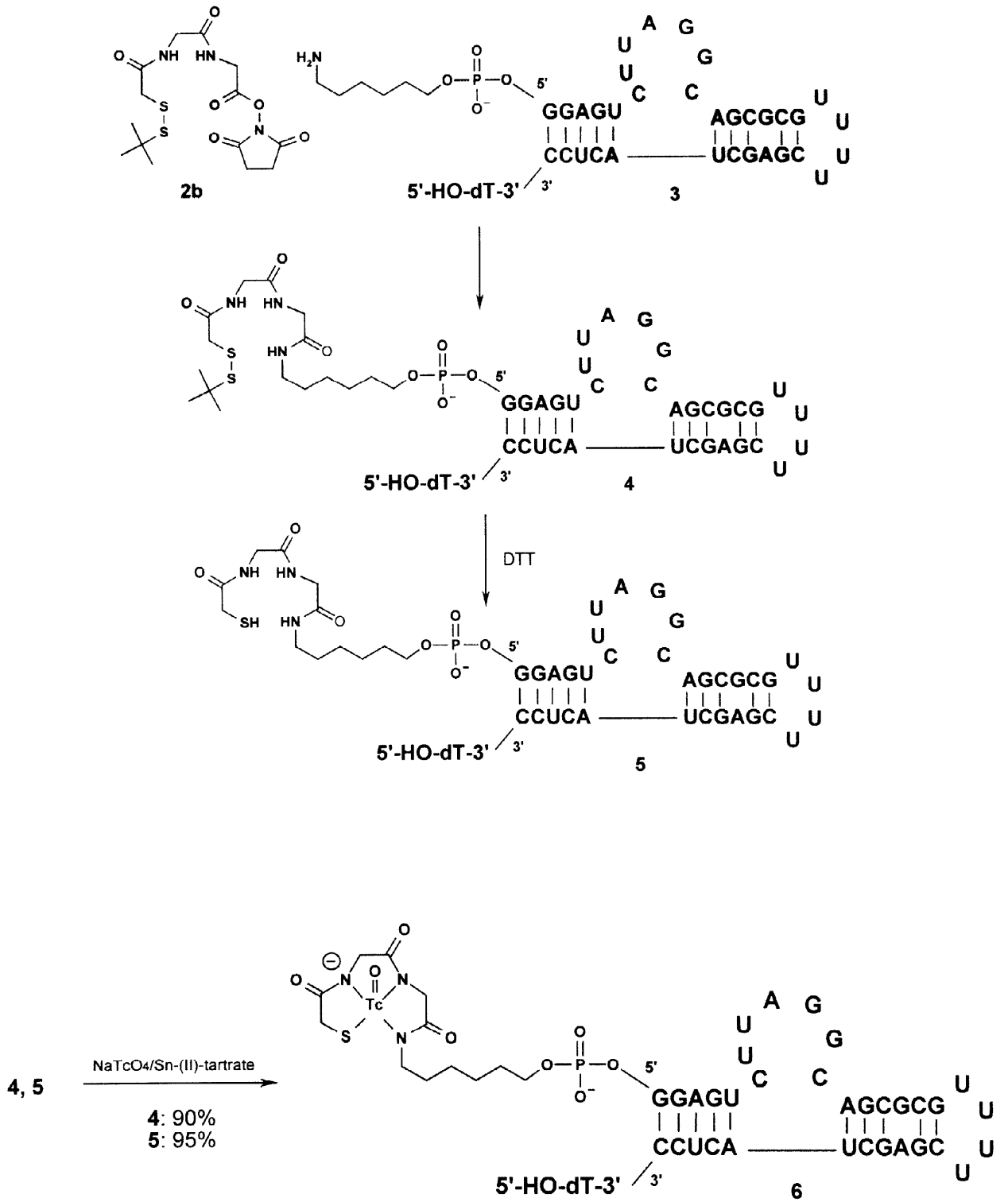
For preparations of N_3S-conjugated aptamers during solid phase synthesis or for postsynthetic couplings to unprotected amino modified oligonucleotides (ON) in solution ^{t}BuS- and S-acetyl-protected mercaptoacetyl-$(Gly)_2$-OH building blocks were synthesized as outlined in Scheme 1.



Scheme 1: Synthesis of MAG_2 **1** and **2**.

Reaction of Z-Gly-OSu with NH_2-Gly-O^{t}Bu followed by hydrogenolytic removal of the Z-protecting group yielded the dipeptide NH_2-Gly-Gly-O^{t}Bu. The amino group of the peptide was acetylated by S-acetyl- or S-^{t}BuS-protected mercaptoacetic acid using DCC/NHS as condensing reagents. After chromatographic purification of the fully protected MAG_2 units, the deprotection of C-termini was achieved in TFA with 80-90% yield. The reaction sequence allows the synthesis of ^{1}R-S-CH_2-CO-Gly-Gly-OH (**1**) in 10-15g amounts. ^{t}BuS-protected MAG_2 was transformed by DCC/NHS to the corresponding N-hydroxy succinimide ester **2b**.

Coupling reaction of **2b** with the L-Selectin binding RNA **3** has been investigated (Scheme 2). The aptamer **3** 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 starting RNA **3** was presynthesized on solid support followed by the introduction of an 6-aminohexyl linker at the 5'-end of the sequence[5]. Conjugation of the NHS-ester **2b** with the 34-mer RNA **3** in solution led to a high yield of compound **4**. RNA **4** bears the protected N_3S-chelator fixed by an 5'-alkylphosphato linker to the modified ON. The S-protecting group was cleaved by treatment of **4** with an excess of DTT yielding the N_3S-RNA **5**. For Tc-99m-labeling studies the MAG_2-amide-RNA **5** was purified by ion exchange and RP-chromatography (Scheme 2).



Scheme 2: Synthesis of N_3S-RNA **5** and Tc-99m-N_3S-RNA **6**.

The N_3S-aptamer **5** was Tc-99m labeled by direct reduction of pertechnetate in the presence of disodium tartrate in phosphate buffer solution (pH = 8.5, Scheme 2) yielding the RNA **6**.
As shown by HPLC-analysis (Figure 1), the incorporation of Tc-99m in the N_3S-RNA **5** was achieved with 95% radiochemical yield (sum of Tc-99m tartrate and pertechnetate-99m < 0.5%). In the preparation of Tc-99m-N_3S-RNA **6** less than 5% colloids/Tc-dioxide could be detected by TLC. PAGE-analysis of Tc-99m labeled **5** showed one major band confirming the formation of the 1:1-Tc-99m complex **6** (Figure 2).

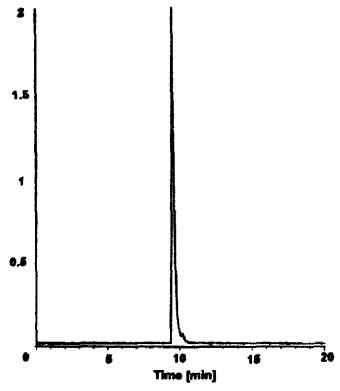


Figure 1: HPLC of **6**

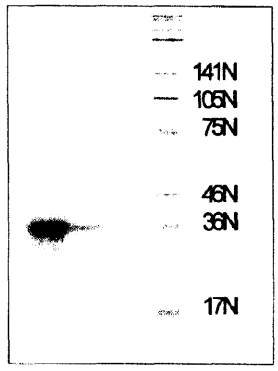


Figure 2: PAGE of **6**

Alternatively, the protected N_3S-RNA **4** was directly radio-labeled to the Tc-99m aptamer **6** using tartrate as co-ligand (90% yield, Scheme 2).

S-protected MAG_2 building blocks were coupled with good yields to amino-modified RNA's in solution or on solid support. The prepared MAG_2-amide-aptamers were Tc-99m-labeled with good yield and specific activities of 37MBq/nmol leading to 1:1-Tc-99m-N_3S-RNA's.

References and Notes

1. Dedicated to Professor Wolfgang Steglich on the occasion of his 65th birthday.

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