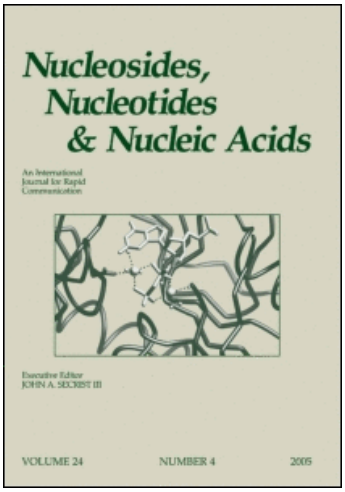


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis of Copper-64 and Technetium-99M Labeled Oligonucleotides with Macrocyclic Ligands

Sven Wagner[a]; Michael Eisenhut[b]; Ramon Eritja[c]; Franz Oberdorfer[a]
[a] Radiopharmaceutical Chemistry, German Cancer Research Center, Heidelberg [b] Department of Nuclear Medicine, Radiological University Clinic, Heidelberg [c] EMBL, Heidelberg, Germany

To cite this Article Wagner, Sven , Eisenhut, Michael , Eritja, Ramon and Oberdorfer, Franz(1997) 'Synthesis of Copper-64 and Technetium-99M Labeled Oligonucleotides with Macrocyclic Ligands', Nucleosides, Nucleotides and Nucleic Acids, 16: 7, 1789 — 1792
To link to this Article: DOI: 10.1080/07328319708006279
URL: http://dx.doi.org/10.1080/07328319708006279

NUCLEOSIDES & NUCLEOTIDES, 16(7-9), 1789-1792 (1997)

SYNTHESIS OF COPPER-64 AND TECHNETIUM-99M LABELED OLIGONUCLEOTIDES WITH MACROCYCLIC LIGANDS

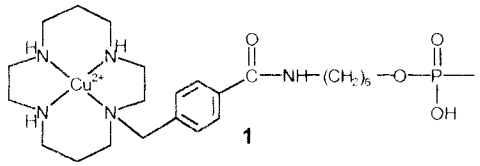
Sven Wagner[1], Michael Eisenhut[2], Ramon Eritja[3], Franz Oberdorfer[1]*

[1]German Cancer Research Center, Radiopharmaceutical Chemistry, D-69120 Heidelberg.
[2]Department of Nuclear Medicine, Radiological University Clinic, D-69120 Heidelberg.
[3]EMBL, Meyerhofstrasse 1, Postfach 10.2209, D-69012 Heidelberg, Germany.

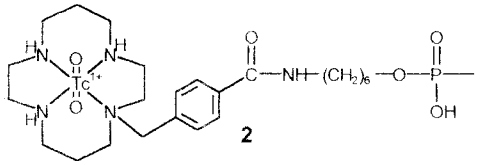
Abstract: An efficient procedure for the preparation of oligonucleotides carrying a mono-*N*-substituted azamacrocycle and its radiolabeled complex with Cu-64 and Tc-99m is reported. The new derivatives are of potential interest for Positron Emission Tomography and Single Photon Emission Tomography.

The use of radiolabeled antisense oligonucleotides markers in PET (Positron Emission Tomography) or SPECT (Single Photon Emission Tomography) is a promising technique for cancer diagnosis. There is currently a growing interest to develop methods to monitor the in vivo uptake of antisense oligonucleotides into malignant tumors[1]. Depending on the pharmacokinetic properties of the oligonucleotide, PET and SPECT may be applied to investigate the bioavailability[2,3]. To combine high signal resolution with optimal imaging times the positron-emitting radionuclide ^{64}Cu for PET studies as well as the gamma-emitting radionuclide ^{99m}Tc for SPECT experiments would fullfill these requirements. No methods have been desribed to label an oligonucleotide with ^{64}Cu. Recently, a comparison study of bifunctional chelates with copper radioisotopes conjugated to antibodies and their biodistribution was reported[4]. Due to the high stability of the Cu(II)-CPTA (Cu(II)-4-[(1,4,8,11-tetraazacyclotetradec-1-yl)methyl]benzoic acid) complex the data showed that transchelation of CPTA **3** is very unlikely. Only few examples are known to label oligonucleotides with ^{99m}Tc[5,6] and the stability of the ^{99m}Tc-label keeps still a

major problem. Cyclam structures analogous to CPTA **3** have been previously described as a very stable complex with [$^{99m}TcO_2$] . The preparation of oligonucleotides containing a ^{64}Cu(II)-CPTA complex **1** and a ^{99m}Tc(V)O_2-CPTA complex **2** at the 5'-end are described.

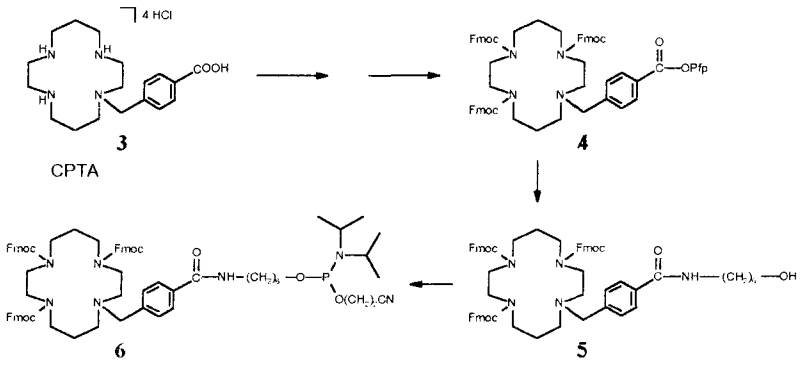


1



2

Starting from CPTA **3**[8], protection of the secondary amines was necessary to prevent further side reactions in later functionalizations at the carboxy termini[9]. The 9-fluorenylmethoxycarbonyl group (Fmoc) ideally suited for simultaneous cleavage with the conjugated oligonucleotide from the solid support was introduced in good yields following the scheme shown. A lenghty spacer arm is desirable to separate the incorporated metall complex from the nucleic acid sequence. The protected derivative **4** was first activated to a pentafluorophenol ester and in a one-pot reaction subsequent tagged to 6-amino-1-hexanol. Due to the higher degree of nucleophilicity of the primary amino function no protection of the hydroxyl group was needed to obtain almost quantitative yields of compound **5**. The phosphoramidite **6** of the Fmoc-protected CPTA was assayed using standard procedures via bis(diisopropylamino)-2-cyanoethoxyphosphine. The phosphoramidite **6** was incorporated on oligonucleotide sequences using an automated DNA synthesizer with subsequent ammonia deprotection. The following oligonucleotide sequences: CPTA-NH-C6-5' pT_8 3', CPTA-NH-C6-5' TAACTACTGAGGTCACAA 3' and CPTA-NH-C6-5' GAGATATCACCCTAGTATGAGCAG 3' were prepared. Reverse phase HPLC profiles showed a slightly longer retention time for the modified oligonucleotide than for the natural. The presence of the macrocycle was confirmed by mass spectrometry before and after Cu(II) incorporation as well as enzyme digestion analysis. High impact mass spectrometry experiments with the Cu(II)-CPTA-NH-C6-5' thymidin 3' gave indications of the high stability of the complex.



Radiolabeling of the modified oligonucleotides with $^{64}Cu(II)$ in acetate buffer at pH 5.5 followed Sephadex-G25 purification afforded the desired products as confirmed by radioactivity / UV HPLC detection. Enzyme digestion of the radiolabeled oligonucleotides and HPLC analysis results were consistent with the proposed retention times. The formation of $^{99m}TcO_2^+$ complexes was achieved using a Sn(II) salt as reducing reagent in the presence of ^{99m}Tc-pertechnetate at pH 11.5. Similar analysis revealed a slightly better radiochemical yield and a higher degree of purity for the ^{99m}Tc complex than for the ^{64}Cu complex due to the origin of the radioactive metall.

Melting temperature studies, investigations of biological properties and optimization of the radiochemical yields of these labeled oligonucleotides are currently in progress.

ACKNOWLEDGEMENTS

We are grateful to Dr. Stefan Zeisler for his kindness providing us with ^{64}Cu from our nuclear reactor TRIGA MARK 1 via Szilard-Chalmers reaction. We thank Marten Wiersma for qualified technical assistance in the DNA synthesis and Gerhard Erben for his reliable spectroscopic measurements.

REFERENCES

1. Stein, C.A., Krieg, A.M. (1994) *Antisense Res. Devel.*, **4**, 67-69.
2. Hildebrandt, M., Reske, S.N. (1996) *Nuclear Medicine*, **35**, 126-131.

3. Urbain, J.L.C., Shore, S.K., Vekemans, M.C., Cosenza, S.C., DeRiel, K., Patel, G.V., Charkes, N.D., Malmund, L.S., Reddy, E.P. (1995) *Eur. J. Nucl. Med.*, **22**, 499-504.

4. Rogers, B.E., Anderson, C.J., Connett, J.M., Guo, L.W., Edwards, W.B., Sherman, E.L.C., Zinn, K.R., Welch, M.J. (1996) *Bioconjugate Chem.*, **7**, 511-522.

5. Dewanjee, M.K., Ghalfouripour, A.K., Subramanian, M., et al. (1994) *J. Lab. Compd. Radiopharm.*, **35**, 40-42.

6. Hnatowitch, D.J., Winnard, P.Jr., Virzi, F., Fogarasi, M., Sano, T., Smith, C.L., Cantor, C.R., Rusckowski, M. (1995) *J. Nucl. Med.*, **36**, 2306-2314.

7. Riche, F., Vidal, M., Mathieu, J.P., Pasqualini, R., Fagret, D., Comet,M. (1996) *Appl. Radiat. Isot.*, **47**, 51-56.

8. Studer, M., Kaden, T.A. (1986) *Helvetica Chimica Acta*, **69**, 2081-2086.

9. Smith-Jones, P.M., (1992) *PhD Thesis*, University of Basel.