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An efficient chelator for complexation of thorium-227

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ABSTRACT

We present the synthesis and characterization of a highly efficient thorium chelator, derived from the octadentate hydroxypyridinone class of compounds. The chelator forms extremely stable complexes with fast formation rates in the presence of Th-227 (ambient temperature, 20 min). In addition, mouse biodistribution data are provided which indicate rapid hepatobiliary excretion route of the chelator which, together with low bone uptake, supports the stability of the complex in vivo. The carboxylic acid group may be readily activated for conjugation through the ε-amino groups of lysine residues in biomolecules such as antibodies. This chelator is a critical component of a new class of Targeted Thorium Conjugates (TTCs) currently under development in the field of oncology.

The clinical development of targeted radioimmunotherapies (RIT) for the treatment of cancer utilizing the high linear energy transfer (LET) of alpha-particles has, to a large extent, been limited by the availability of suitable radionuclides on a commercial scale. More recently, with the approval in May 2013 of Xofigo® for the treatment of castration resistant prostate cancer (CRPC),[1] a commercial production line for the alpha-emitting radionuclide radium-223 (Ra-223) was established. However, although the inherent bone-seeking characteristics of Ra-223 make it well suited for the targeting of bone metastases in CRPC, the paucity of efficient chelator systems limits its use in radioimmunotherapy. By utilizing the same actinium-227 generators as used in the manufacture of Ra-223,[2] highly purified thorium-227 (Th-227) may be produced. Unlike Ra-223, Th-227 exists in the 4+ oxidation state and forms stable complexes with chelators such as 1,4,7,10-tetraazacycloododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid (DOTA). However, in order to achieve sufficient labeling of DOTA-coupled antibodies, the complexation step must either be performed as a two-step process[3] or directly at elevated temperatures.[4] The harsh conditions used for direct labeling are not always compatible with the stability of complex biological macromolecules such as antibodies. In addition the slower complex formation rates affect radiolabeling yield, efficiency and specific activity.[5]

Therefore, there remains a need for alternative chelators which complex thorium-227 in near quantitative yield at ambient temperature in aqueous solutions. To this end we have developed an octadentate chelator utilizing the 3-hydroxy-*N*-methyl-2-pyridinone moiety, abbreviated henceforth as Me-3,2-HOPO, reported previously to form extremely stable thorium complexes.[6,7] The Me-3,2-HOPO groups are monoprotic acids that complex thorium-227 through the two oxygen atoms on each subunit.[8] Figure 1 shows the structural drawing of the target octadentate Me-3,2-HOPO thorium-227 complex **1**. We report herein on the synthesis, labeling and in vivo biodistribution in mice and provide evidence of superiority of this new chelator compared to DOTA for thorium-227 complexation.

The chelator comprises three key components. The Me-3,2-HOPO moiety, which complexes the thorium; a symmetrical polyamine scaffold to which the Me-3,2-HOPO moieties are coupled; and a carboxylic acid group facilitating conjugation to biomolecules such as antibodies.

Scheme 1 shows the synthesis of the polyamine scaffold component. Compound **3** was isolated in 66% yield by the addition of 4-nitrobenzyl bromide to a mixture of dimethyl malonate/NaH in tetrahydrofuran (THF). Diisobutylaluminium hydride (DIBAL-H) reduction in THF was performed to generate diol **4** in 46% yield. Care was taken to stop the reaction before completion to avoid reduction of the nitro group. Activation by mesylation of **4** with methane sulfonyl chloride and triethylamine in dichloromethane (DCM) gave compound **7**, the crude material after workup being used directly in the next step. The bis-Boc protected triamine **6** was formed in 56% yield from diethylenetriamine **5**, directly using *N*-Boc-imidazole. The fully protected precursor was prepared in

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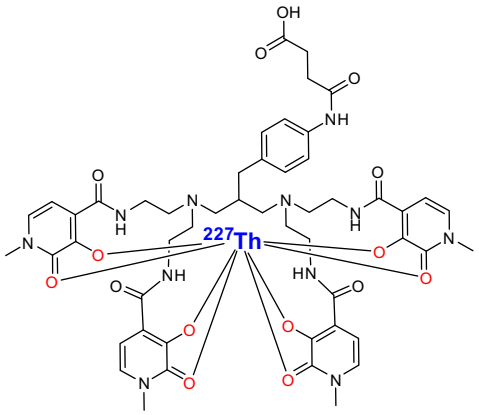
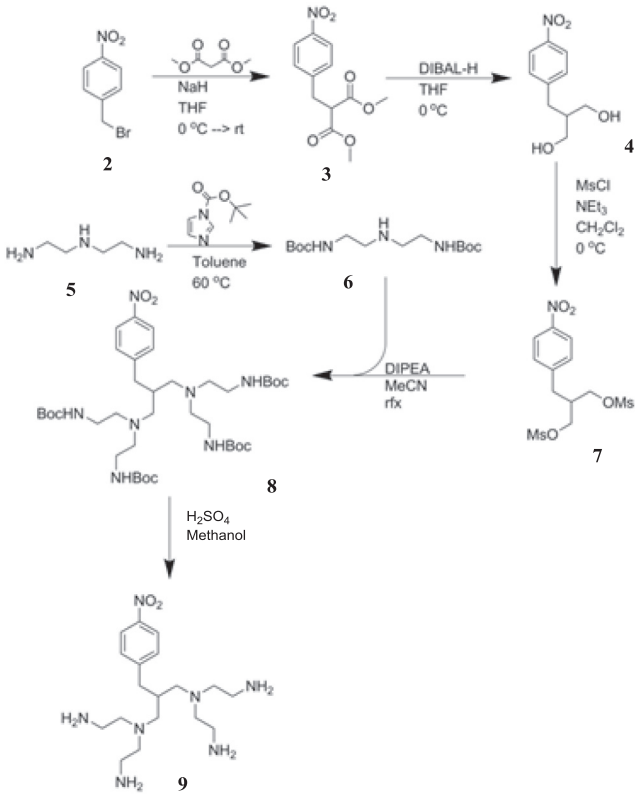


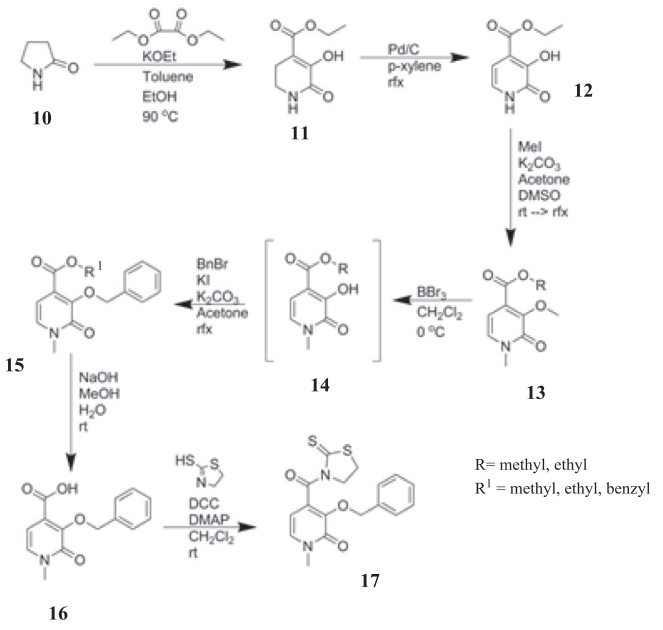
Figure 1. Drawing of the octadentate hydroxypyridinone thorium-227 complex **1**.

44% yield by combining **6** and **7** in acetonitrile using *N*,*N*-diisopropylethylamine (DIPEA) as base. Finally, the deprotected product **9** was isolated in 66% yield by addition of sulfuric acid to compound **8** in methanol.

Scheme 2 shows the synthesis of the activated Me-3,2-HOPO precursor. Construction of building block **11** poses the challenge of correctly introducing the functional group in the 4-position of the dihydropyridine ring. One approach, previously reported by Xu et al. relied on the carboxylation of 1-methyl-3-hydroxy-2-pyridinone at 5860 kPa/CO_2.[9] We have investigated an alternative method employing condensation of 2-pyrrolidinone with diethyl oxalate which also yields the correctly substituted tetrahydropyridine-4-carboxylate through a sequential ring-opening and closure under Claisen conditions.[10] In the first step, the reaction between 2-pyrrolidinone and diethyl oxalate gives compound **11**



Scheme 1. The synthesis route to the polyamine scaffold.

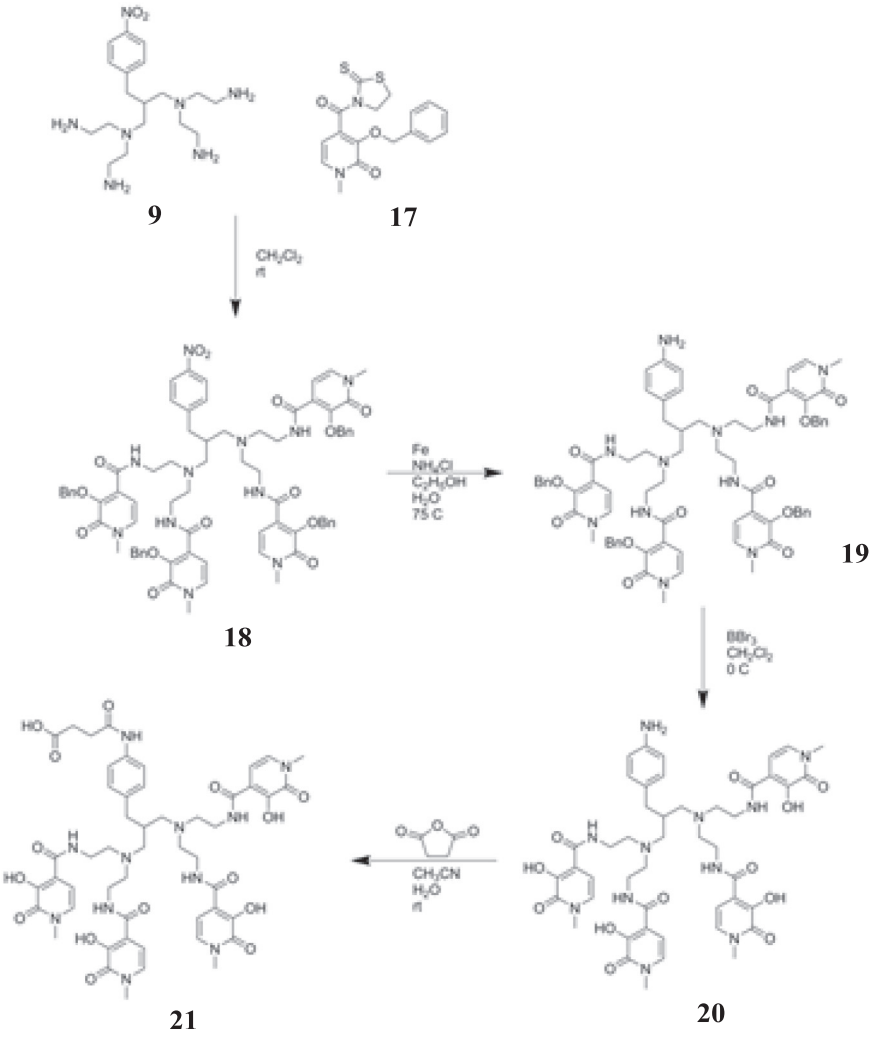


Scheme 2. The synthesis route to the activated Me-3,2-HOPO precursor.

in 73% yield. Subsequent aromatization using Pd/C has been described previously and results in a 75% yield of **12**.[11] N-methylation was then performed with an excess of methyl iodide and potassium carbonate. This reaction resulted in the formation of the methyl ether as well as partial *trans*-esterification. The *O*-methyl group was removed using BBr_3 in dichloromethane (DCM) and following quenching and isolation by filtration **14** was treated with benzyl bromide furnishing the desired benzyl ether **15**. Further transesterification occurs in this reaction; the combined yield of all three esters being 67%. Ester hydrolysis was then carried out in methanol/water with NaOH, giving carboxylic acid **16** in 89% yield. Finally conversion of the carboxylic acid with 2-thiazoline-2-thiol with *N*,*N*′-dicyclohexylcarbodiimide and 4-dimethylaminopyridine was performed in dichloromethane yielding the thiazolidino-activated and benzyl protected Me-3,2-HOPO building block **17** in 87% yield.

Scheme 3 details the synthesis of the octadentate Me-3,2-HOPO chelator compound **21**. By simply stirring compounds **9** and **17** in DCM for 16–20 h the protected chelate **18** was formed in 68% yield. Reduction of the nitro group to the amine **19** with iron/NH_4Cl, followed by removal of the benzyl protecting groups with BBr_3 gave the desired product **20** in 90% yield. In the final step, compound **21** was formed by reaction with succinic anhydride as shown in Scheme 3. On removal of the benzyl protecting groups care was taken to avoid contamination of the chelate system with unwanted metal ions. Both compounds **20** and **21**, complex a variety of metals with oxidation states of +2, +3 and +4 and in particular show high affinity for ferric ions. Complex formation and characterization was confirmed by LC–high resolution mass spectrometry using non-radioactive thorium-232 as surrogate. The full details of the synthesis and characterization steps are given in Supporting information.

Conjugation of compound **21** to monoclonal antibodies is described in detail elsewhere.[12] However, the chemistry is robust and reproducible. Briefly, the in situ pre-activation of compound **21** is performed in 4-morpholinoethanesulfonic acid (MES) buffer pH 5.5 in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and *N*-hydroxysuccinimide.[13] The activated chelator is then added to a solution of the antibody in phosphate-buffered saline (PBS) at pH 7.4 with gentle mixing for 30 min before purification of the conjugate.



Scheme 3. The synthesis route to the octatendate Me-3,2-HOPO thorium chelator, compound **21**.

In this study our aim was to investigate the labeling of compound **21** with thorium-227 (synthesis of complex **1**) as well as the pharmacokinetics of complex **1** in mice. In a typical labeling experiment[14] compound **21** is added directly to a solution of thorium and the complexation allowed to proceed for 30 min. Complex **1** as the test item for injection had a final chelator concentration of 0.0125 mg/mL (target; 0.0125 mg/mL), specific activity of 14082 kBq/mg (target 14000 kBq/mg, and thorium concentration of 188 kBq/mL (target 175 kBq/mL). An aliquot of the chelate complex was analysed by iTLC, the strips were divided into two halves and counted separately in a high performance germanium detector. Free Th-227 is known to migrate with the solvent front while chelated Th-227 remains at the application point. In this example >96% of the measured activity remained at the application point.

The blood clearance and tissue distribution of complex **1** in female C57Bl6 mice was studied (Table 1). Animals were administered complex **1** as a single slow bolus injection via the tail vein. The dose volume was 5 mL/kg (0.1 mL/20 g mouse), the target dose of complex **1** was 0.05 mg/kg, and for the radioactivity, 700 kBq/kg thorium-227. Animals were sacrificed at the following post-treatment time-points; 5 min, 1 and 4 h and on days 1, 3 and 7. Samples of blood and tissues were collected and the radioactivity measured on a high performance germanium detector. Activity was decay corrected to give the activity at time of sampling/euthanasia.

Complex **1** rapidly cleared from the blood, with $t½$ alpha approximately 0.4–1 h, and $t½$ beta in the range of 4.9–6.4 h, with almost complete elimination from the blood at 24 h. At the early time points the presence of complex **1** was observed mainly in the liver and small intestine with measured doses of 7.2% and 14.4%ID/g respectively after only 5 min. After 4 h the large intestine had a measured dose of 48%ID/g, indicating that the route of excretion was predominant biliary, consistent with the hydrophobic nature of complex **1**. Although our ultimate aim is to develop antibody-based targeted thorium conjugates with this chelator system, it is important to know the fate of any free complex **1** formed as a result of metabolic cleavage of the chelate from the antibody in vivo. We anticipate the rapid excretion of free labeled chelate through the liver will minimize the potential off-target toxicity of this potential metabolite.

It has been previously observed by ourselves and others that free thorium-227 has bone-seeking properties, with the highest uptake rates in the skeleton. Washiyama et al. demonstrated that following injection of thorium-227 citrate in mice 28.4 and 19.4% ID/g was measured in the femur after 1 and 7 days, respectively.[15] In this study we hypothesized that any instability of the complex in vivo would be reflected in high bone uptake values. The finding on days 1, 3 and 7 that femur uptake was measured at 1.8, 1.9 and 1.4%ID/g, respectively, consistent with in vivo stability of the complex **1**.

Table 1
Organ distribution of complex **1** as % injected dose per gram (%ID/g) post injection

	Th-227 (%ID/g)											
Time	5 min		1 h		4 h		Day 1		Day 3		Day 7	
Organ	Average (n = 3)	SD	Average (n = 3)	SD	Average (n = 3)	SD	Average (n = 3)	SD	Average (n = 3)	SD	Average (n = 3)	SD
Blood	3.2	0.9	1.1	0.5	0.2	0.1	0.02	0.00	0.00	0.01	0.00	0.00
Lung	3.9	2.4	0.7	0.06	0.2	0.1	0.10	0.02	0.08	0.01	0.12	0.05
Liver	7.2	4.2	1.9	0.4	1.3	0.4	1.08	0.20	0.91	0.06	0.83	0.17
Spleen	1.28	0.57	0.37	0.06	0.13	0.10	0.29	0.06	0.33	0.09	0.39	0.16
Kidney	3.89	2.11	1.99	0.3	1.73	1.04	2.26	0.38	1.66	0.08	1.37	0.29
Large Intestine + contents	0.97	0.60	12.0	19.9	48.1	19.4	0.22	0.06	0.04	0.00	0.05	0.01
Small Intestine + contents	14.4	12.2	37.8	14.4	6.83	2.11	0.10	0.02	0.04	0.00	0.04	0.01
Heart	1.61	0.72	0.32	0.03	0.14	0.12	0.08	0.01	0.07	0.01	0.09	0.02
Femur	2.17	1.47	1.40	0.35	0.33	0.15	1.81	0.21	1.94	0.61	1.43	0.53
Residual carcass	1.08	0.26	0.84	0.26	0.36	0.24	0.14	0.02	0.13	0.00	0.12	0.03
Tail	49.9	45.4	25.5	24.4	16.5	1.98	0.92	0.60	0.31	0.53	1.39	0.72

In conclusion, the discovery of new chelator systems that efficiently complex Th-227 with sufficient in vivo stability is a prerequisite for the development of antibody targeted alpha-pharmaceuticals. The optimal chelator characteristics are governed by the long half-life of Th-227 (18.7 days) coupled to the long biological half-life of the antibody in vivo. The high Relative Biological Effectiveness of the alpha particle potentially allows for therapeutic efficacy at lower radiation doses compared to beta-emitters[16,17] as the decay of Th-227 releases in total 5 alpha-particles with a total alpha energy of 32.5 MeV.[18] We provide evidence in this report that compound **21** is an efficient chelator of Th-227 and is a superior chelator to DOTA as it requires no heating to effect efficient labeling. In addition the octadentate Me-3,2-HOPO, compound **21**, demonstrates rapid complexation, which is a key requirement for the future commercial development of thorium-227 based alpha-pharmaceuticals.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.07.034.

References and notes

1. Parker, C.; Nilsson, S.; Heinrich, D.; Helle, S. I.; O'Sullivan, J. M.; Fosså, S. D.; Chodacki, A.; Wiechno, P.; Logue, J.; Seke, M.; Widmark, A.; Johannessen, D. C.; Hoskin, P.; Bottomley, D.; James, N. D.; Solberg, A.; Syndikus, I.; Kliment, J.; Wedel, S.; Boehmer, S.; Dall'Oglio, M.; Franzén, L.; Coleman, R.; Vogelzang, N. J.; O'Brien-Tear, C. G.; Staudacher, K.; Garcia-Vargas, J.; Shan, M.; Bruland, Ø. S.; Sartor, O. *N. Eng. J. Med.* **2013**, *369*, 213.
2. Morgenstern, A.; Abbas, K.; Bruchertseifer, F.; Apostolidis, C. *Curr. Radiopharm.* **2008**, *1*, 135.
3. Dahle, J.; Borrebaek, J.; Melhus, B.; Bruland, Ø. S.; Salberg, G.; Olsen, D. R.; Larsen, R. H. *Nucl. Med. Biol.* **2006**, *33*, 271.
4. Heyerdal, H.; Abbas, N.; Brevik, E. M.; Mollatt, C.; Dahle, J. *PLoS One* **2012**, *7*, e42345.
5. Brechbiel, M. W. Q. *J. Nucl. Med. Mol. Imaging* **2008**, *52*, 166.
6. Sturzbecher-Hoehne, M.; Deblonde, G. J.-P.; Abergel, R. J. *Radiochem. Acta* **2013**, *101*, 359.
7. Deblonde, G. J.-P.; Sturzbecher-Hoehne, M.; Abergel, R. J. *Inorg. Chem.* **2013**, *52*, 8805.
8. Gorden, A. E. V.; Xu, J.; Raymond, K. N. *Chem. Rev.* **2003**, *103*, 4207.
9. Xu, J.; Sonya, J.; Whisenhunt, D. W.; Durbin, P.; Raymond, K. N. *J. Am. Chem. Soc.* **1995**, *117*, 7245.
10. Synthesis described in patent application WO2013049104.
11. Fu, P. P.; Harvey, R. G. *Chem. Rev.* **1978**, *78*, 317.
12. Conjugation reactions to antibodies detailed in WO2016096843 (A1).
13. Kurzer, F.; Douraghi-Zadeh, K. *Chem. Rev.* **1967**, *67*, 107.
14. *Chelator labeling protocol:* Two stock solutions were prepared: Solution 1, comprising 0.5 mg/mL of compound **21** in 30 mM citrate buffer containing 70 mM NaCl, 2 mM EDTA, 0.5 mg/mL PABA and 5% DMSO, pH 5.5; solution 2 was identical to solution 1, without the chelate compound **21** and DMSO. A 113 μL aliquot of chelator stock solution 1 was transferred to a vial containing 792 kBq of thorium-227 (2.1 μL in 0.05 M HCl) and the volume adjusted to 250 μL by addition of stock solution 2. The labeling reaction, forming complex **1** was allowed to proceed for 30 min at ambient temperature before final dilution to a volume of 3.8 mL with stock solution 2. A 10 μL aliquot of the chelate complex was analysed by iTLC using iTLC-SG chromatography paper eluted with the mobile phase 0.1 M citric acid/citrate buffer in 0.9% NaCl, pH 5.5.
15. Washiyama, K.; Amano, R.; Sasaki, J.; Kinuya, S.; Tonami, N.; Shiokawa, Y.; Mitsugashira, T. *Nucl. Med. Biol.* **2004**, *31*, 901.
16. Supiot, S.; Faivre-Chauvet, A.; Couturier, O.; Heymann, M. F.; Robillard, N.; Kraeber-Bodéré, F.; Morandeau, L.; Mahé, M. A.; Chérel, l. M. *Cancer* **2002**, *94*, 1202.
17. Graf, F.; Fahrer, J.; Maus, S.; Morgenstern, A.; Bruchertseifer, F.; Venkatachalam, S.; Fottner, C.; Weber, M. M.; Huelsenbeck, J.; Schrekenberger, M.; Kaina, B.; Miederer, M. *PLoS One* **2014**, *9*, e88239.
18. Dahle, J.; Larsen, R. H. *Curr. Radiopharm.* **2008**, *1*, 209.