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Synthesis of a Tyr^3-octreotate conjugated *closo*-carborane [$HC_2B_{10}H_{10}$]: a potential compound for boron neutron capture therapy

Esther Schirrmacher,[a] Ralf Schirrmacher,[a,*] Carmen Beck,[a] Walter Mier,[b] Norbert Trautman[a] and Frank Rösch[a]

[a]*Institute of Nuclear Chemistry, University of Mainz, D-55177 Mainz, Germany*
[b]*Universitätsklinikum Heidelberg, Department of Nuclear Medicine, D-69120 Heidelberg, Germany*

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Abstract—A novel Tyr^3-octreotate conjugated *closo*-carborane as a potential compound for boron neutron capture therapy was obtained via Fmoc solid phase peptide synthesis. The boron cluster [$C_2B_{10}H_{11}$] was introduced through the reaction of 6,9-bis(acetonitrile)decaborane and 5-hexynoic acid yielding a new *closo*-carborane conjugated carboxylic acid which was coupled subsequently with solid phase conjugated Tyr^3-octreotate. The final boron-containing peptide was purified by preparative reverse phase HPLC and structural identity was proved applying MALDI-TOF mass spectrometry.

Boron neutron capture therapy (BNCT) originates from the idea of delivering a boron compound with enriched ^{10}B to tumour cells and subsequent exposure of those cells to a thermal neutron beam inducing the $^{10}B(n,\alpha)^7Li$ nuclear reaction.[1] The energetic ^4He and ^7Li products are responsible for injuring cancer cells. The application of boron compounds for cancer treatment, e.g. for high grade gliomas and anaplastic astrocytomas has received attention due to the fact that these tumour entities cannot be easily cured via surgery, chemotherapy or conventional radiation therapy. Clinical treatments of patients with brain tumours with BNCT in the early 1960s failed due to a lack of boron compounds with high selectivity for the target tissue. In addition, neutron beams which could deposit the required thermal neutron flux at depths greater than a few centimetres were not available.[2] Today an increasing number of boron compounds have been synthesised[3] but among these, only the *p*-dihydroxy-[^{10}B]boryl-phenylalanine-fructose complex (BPA-Fr) and [^{10}B]BSH have been applied in clinical trials.[4,5] BPA-Fr has the disadvantage of bearing only one boron atom, which is far from being ideal considering that the concentration of ^{10}B should be exceptionally high in the target tissue. The application of boron clusters in BNCT offers a new approach for enhancing the boron density in the target tissue. Some novel boron clusters containing polyamines for example have been reported which displayed no distinct affinity to tumour cells, but may function as building blocks for further syntheses.[6,7]

There is also a demand for novel targeting concepts for delivering ^{10}B-enriched compounds to tumours.

The use of peptides as targeting vectors is a promising approach to assess tumours expressing specific peptide-

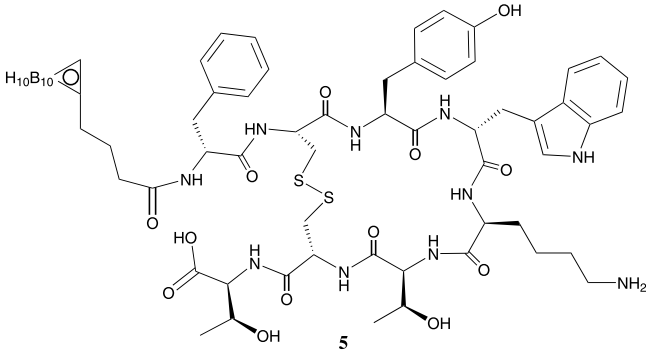

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Figure 1. The Tyr^3-octreotate conjugated *closo*-carborane [$HC_2B_{10}H_{10}$] synthesised.

Keywords: *closo*-carborane; Tyr^3-octreotate; boron neutron capture therapy (BNCT); somatostatin receptor.

* Corresponding author. Tel.: +49-6131-3925371; fax: +49-6131-3925253; e-mail: schirrmacher@mail.kernchemie.uni-mainz.de

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receptors on the cell surface.[8–10] Tyr^3-octreotate [H-D-Phe-cyclo[Cys-Tyr-D-Trp-Lys-Thr-Cys]-Thr-OH] is a somatostatin analogue with high affinity for the somatostatin receptor subtype-2. This subtype is over-expressed on the cell surface of several tumours of the neuroendocrine system, making somatostatin analogues eligible compounds for tumour targeting.[11–13]

A variety of radiolabelled octreotide and octreotate derivatives are involved in clinical routine and clinical research, both for diagnosis and radiotherapy.[14] These derivatives demonstrate selective accumulation in the corresponding tumours. Recently, the pattern of cellular uptake of the radiolabelled compounds has been investigated in detail. In particular, octreotate derivatives have been proven to internalise effectively into tumour cells.[15,16] As a prerequisite for efficient BNCT is an internalisation of the boron containing compound into the target cell, Tyr^3-octreotate-boron clusters seem to be promising compounds for achieving a high concentration of ^{10}B in the tumour cell.

Furthermore, Tyr^3-octreotate can be labelled with radionuclides such as ^{123}I enabling the evaluation of the biodistribution in vivo. Taking these considerations into account, we synthesised compound **5** (Fig. 1) as a candidate for the application of BNCT.

The synthesis of the boron cluster 5,6-dicarba-*closo*-dodecaboranyl hexynoic acid (**4**) was achieved by reacting 6,9-bis(acetonitrile) decaborane (**6**)[17] (Fig. 2) and 5-hexynoic acid methyl ester (**2**), which was synthesised via esterification of 5-hexynoic acid (**1**) (Fluka, Germany) with methanol under acidic conditions. The reaction conditions for coupling followed Valliant et al. for the synthesis of a *closo*-carborane analogue of tamoxifen.[18] Two molecules of acetonitrile were substituted by the alkynyl carbon atoms resulting in the formation of the *closo*-carborane. The resulting boron labelled ester **3** was converted into the free acid **4** by treatment with 6N HCl and finally purified via column chromatography. The Tyr^3-octreotate was synthesised applying standard Fmoc solid phase peptide synthesis via a batchwise procedure, i.e. 4 molar equiv. of the amino acid, 4 equiv. of 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium (HBTU), 8 equiv. of diisopropylethylamine (DIPEA) in DMF at 25°C. Cyclisation of the linear octapeptide D-Phe-Cys(Acm)-Tyr(*t*Bu)-D-Trp(Boc)-Lys(Boc)-Thr(*t*Bu)-Cys(Acm)-Thr(*p*-benzyloxyoxyphenylalcohol)-resin by formation of the disulphide bridge was achieved with 4 equiv. of thallium(III) trifluoroacetate in DMF at 25°C.[19] The coupling of **4** to the peptide was performed analogously yielding the final fully protected compound **5** on the solid support, which was deprotected and removed from the resin in one step applying TFA:water:triisopropylsilane (95:2.5:2.5). The crude boron cluster conjugated peptide **5** was precipitated by the addition of cold diethyl ether and purified via preparative gradient HPLC.[20] Structural identities of compounds **2**, **3** and **4** were proven with ^{1}H, ^{13}C, and ^{11}B NMR, FD mass spectrometry and elemental analysis. Compound **5** was identified by MALDI-TOF mass spectrometry (Table 1).[21]

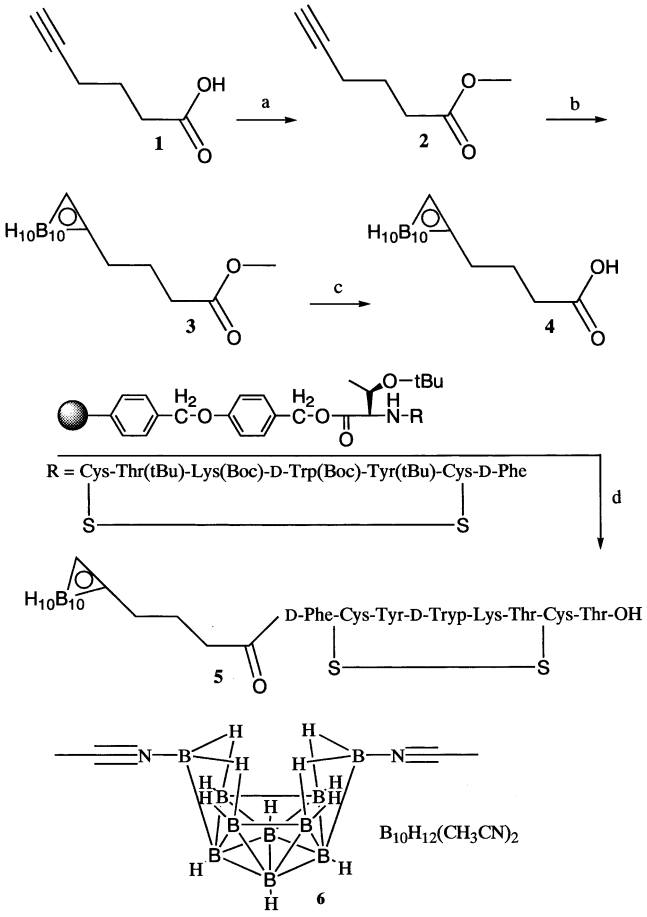

Figure 2. Synthesis of Tyr^3-octreotate conjugated [$HC_2B_{10}H_{10}$] *closo*-carborane. *Reagents and conditions*: (a) methanol, H_2SO_4, reflux, 1 day, 80%; (b) $B_{10}H_{12}(CH_3CN)_2$, toluene, argon, reflux, 95%; (c) 6N HCl, 74%; (d) 4 equiv. **4**, 4 equiv. HBTU, 8 equiv. DIPEA, 17%.

In conclusion, the synthesis of a novel Tyr^3-octreotate conjugated [$HC_2B_{10}H_{10}$] *closo*-carborane **5** was achieved in an overall yield of 17%.[22] This compound might serve as a new substance for BNCT due to (a) its possible high affinity for somatostatin receptor expressing tumours and (b) possible internalisation into tumour tissue as well as (c) the number of boron atoms, ten per targeting molecule. To assess the biodistribution data after in vivo application in humans, the labelling of this compound with radionuclides such as ^{123}I is planned. Additionally, binding experiments to prove the affinity of **5** to the human somatostatin receptor subtype 2 are under investigation.

Table 1. Analytical data for compounds **2**, **3** and **4** (^{1}H, ^{13}C, ^{11}B NMR; FD MS, elemental analysis, melting points)

Compound	^{1}H NMR (400 MHz)	^{13}C NMR (400 MHz)	FD MS	^{11}B NMR[d] (400 MHz)	Elemental analysis (%)	Melting points (°C)[c]
2	δ ($CDCl_3$) = 3.7 (s, 3H), 2.4 (t, 2H, J = 7.4 Hz), 2.2 (m, 2H), 1.95 (t, 1H, J = 2.7 Hz), 1.8 (m, 2H)	δ (DMSO-d_6) = 172.9, 83.7, 71.8, 51.4, 32.2, 23.5, 17.2	126.6 $[M]^+$	–	Calcd: C, 66.65; H, 7.99. Found: C, 66.38; H, 7.82	–[b]
3	δ ($CDCl_3$) = 3.7 (s, 3H), 3.6 (s, 1H), 3.2–2.4 (br s, 5H), 2.3 (t, 2H, J = 7 Hz), 2.25 (t, 2H, J = 8.5 Hz), 2.2–1.85 (br s, 3H), 1.8 (m, 2H), 1.75–1.4 (br s, 2H)	δ ($CDCl_3$) = 169.3, 74.4, 60.9, 51.6, 36.9, 32.5, 24.1	244.5 $[M]^+$	δ ($CDCl_3$) = −2.3, −5.7, −9.3, −11.5, −12.2, −13.1	Calcd: C, 34.41; H, 8.25. Found: C, 34.63; H, 8.41	62
4[a]	δ (DMSO-d_6) = 5.2 (s, 1H), 2.3 (t, 2H, J = 8.5 Hz), 2.2 (t, 2H, J = 7 Hz), 1.6 (m, 2H)	δ (DMSO-d_6) = 179.0, 76.4, 63.3, 36.1, 32.7, 24.5	230.5 $[M]^+$	δ (DMSO-d_6) = −3.3, −6.3, −9.9, −11.8, −13.1	Calcd: C, 31.29; H, 7.88. Found: 31.21; H, 7.97	142

[a] The 10 protons from the *closo*-carborane cluster were observed as a broad peak from 1.5 to 3.0 ppm.
[b] Compound **2** was a colourless liquid.
[c] Melting points are uncorrected.
[d] ^{11}B-peaks were broad singlets and only the centre of the peak is displayed.

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20. Column: LiChrosorb® RPselectB 250×10 (Merck Darmstadt, Germany), gradient eluent: 100% H_2O+0.1% TFA after 30 min 100% acetonitrile+0.1% TFA; retention time: R_t = 24 min. The octreotate derivative **5** was a white solid after lyophilisation and the overall yield of the purified peptide was 17%.
21. Molecular weight determinations were carried out by MALDI-TOF mass spectrometry using a Micromass TofspecE. The main observed masses were m/z = 1260.9 for [$^{10}B_2{}^{11}B_8$], m/z = 1259.9 for [$^{10}B_3{}^{11}B_7$] and m/z = 1261.9 for [$^{10}B_1{}^{11}B_9$]; calculated masses (with relative intensities): 1255.71 (0.1%); 1256.71 (0.5%); 1257.71 (2.6%); 1258.71 (8.7%); 1259.71 (20%); 1260.71 (30.2%); 1261.71 (27%); 1262.71 (10.9%); with the abundance of 19.9% ^{10}B and 80.1% ^{11}B
22. To establish the synthesis of **5** non-enriched decaborane (Alfa Aesar, Germany) was used. Highly enriched decaborane is commercially available and can be applied in the syntheses without modifications.