Note

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Synthesis of ^{18}F-labelled biotin analogues

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A one-step ^{18}F-labelling strategy was used to prepare three labelled analogues of the vitamin biotin, which can be useful as tracers because of biotin's high affinity for avidin. The labelled compounds were obtained in decay-corrected yields of up to 35% and specific radioactivity of 320 GBq/μmol. When evaluated *in situ*, the analogues showed good affinity for avidin: 60–75% of the radiolabelled compounds were bound to avidin within 5 minutes. The binding was site-specific, as shown by blocking experiments with native biotin.

Keywords: ^{18}F; biotin analogues; radiolabelling; avidin; one-step labelling; nucleophilic fluorination

Introduction

Biotin (4-[(3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl]butanoic acid) is a naturally occurring vitamin that is generally found in its protein bound form. The affinity of biotin for the protein avidin, a cationic glycoprotein obtained from egg white, is the highest known ($K_d \approx 10^{-15}$ M) for a ligand and protein in nature.[1] The quaternary structure of avidin ($M_w \approx 66$ kDa) consists of a noncovalent tetramer. The monomer units combine into the active form,[2,3] so each avidin unit is capable of binding four biotin molecules.[3,4] The avidin-biotin system has been used as a tool for various purposes, such as the diagnosis[5] of, for example, tumours[6] and for isolation (affinity chromatography).[7]

The intention of the present work was to use the avidin-biotin system in combination with positron emission tomography (PET) imaging to follow the fate of the transplanted islets of Langerhans. The transplantation of islets isolated from donor pancreas into a patient with type I diabetes has been studied for decades, and patients can become independent of insulin after the transplantation. The efficiency of the procedure is, however, low; and islets from more than one pancreas are often needed to achieve independence from external insulin.[8] Initial identification of graft rejection has been hard because of the lack of suitable tools for diagnosis.[9]

To better understand the fate of the transplanted islets, a method for imaging *in vivo* is needed. PET has shown the potential to monitor islet number, mass and function.[10] A useful PET tracer for determining the fate of islets during transplantation would have high specific binding and high intracellular stability. 2-[^{18}F]Fluoro-2-deoxy-D-glucose ([^{18}F]FDG) is one tracer that has been used for this purpose.[11,12] We wanted to explore the well-known high affinity between biotin and avidin to find another suitable tracer for quantifying the fate of the transplanted islets.

Biotin has already been labelled with ^{18}F. The ^{18}F-labelled biotin analogue *N*-(3-[^{18}F]fluoropropyl)-5-[(3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl]pentanamide (**5**) (Scheme 1) binds to avidin and has been used for PET imaging of infection.[13] Another ^{18}F-labelled analogue, in which the radiolabel sits on an aromatic moiety attached to biotin, has also been prepared and evaluated *in vitro*.[14] ^{18}F-Labelled biotin has also been prepared by conjugation of pegylated biotin to [^{18}F]FDG, and this conjugate also showed binding to avidin.[15]

In the current work, three analogues of biotin that have ^{18}F labels on alkyl and ethylene glycol side-chains were prepared, and their binding abilities were investigated as a continuation of our previous work with ^{68}Ga-labelled analogues of biotin.[16]

Experimental

Experimental procedures are described in supplementary material.

Results and discussion

^{18}F-labelling

The syntheses of the ^{18}F-fluorinated target compounds **4–6** from the corresponding mesyl compounds **1–3** are outlined in Scheme 1. The ^{18}F label was placed on different side chains, alkyl and di(ethylene glycol) to obtain a set of compounds with different properties, such as size and lipopilicity.[17]

The labelled compounds **4–6** were synthesised using no-carrier-added [^{18}F]fluoride in a one-step nucleophilic substitution (Scheme 1). In short, heating a precursor with [K/K 2.2.2]$^{+}$^{18}F^{-} in 400 μL acetonitrile at 110°C for 15 minutes gave the corresponding labelled product. The crude product was purified by semipreparative HPLC, which gave the isolated product in 10–35% decay-corrected radiochemical yield (Table 1), within 60 minutes after the end of radionuclide production. Specific radioactivity was 320 GBq/μmol ($n=2$) for compound **5**[13] at the end-of-synthesis. The radiochemical purity of product always exceeded 98% after purification. Preliminary identification in the analysis of the ^{18}F-labelled compounds in

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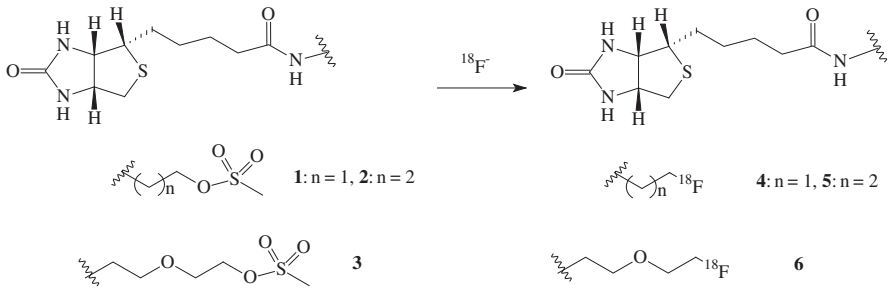


Scheme 1. ^{18}F-labelling reaction to yield compounds **4–6**.

Table 1. Isolated radiochemical yields of labelling reactions

Compound	RCY (%)*
4	13±2
5	10±3
6	35±3

RCY, radiochemical yield
*Isolated decay-corrected radiochemical yield, calculated from the amount of radioactivity at the start of synthesis and radioactivity of LC-purified product. All experiments were performed at least twice.

the liquid chromatography runs was based on the retention times of the unlabelled references.

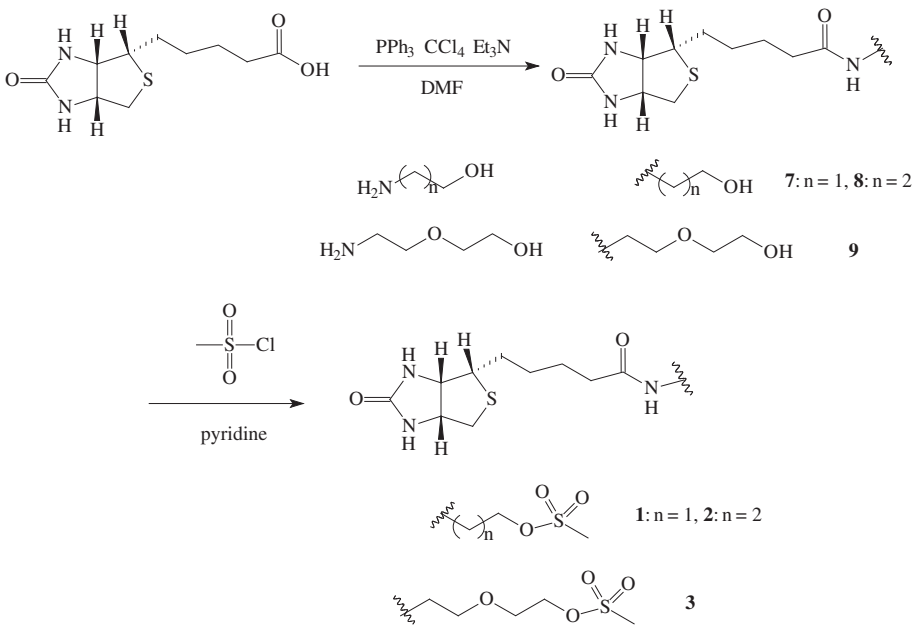
Synthesis of precursors and reference compounds

The precursors **1–3** were synthesised from biotin and the corresponding amino alcohols. The coupling was accomplished using triphenylphosphine, tetrachloromethane and triethylamine in dimethylformamide (DMF) (Scheme 2). The obtained alcohols **7–9** were mesylated using methanesulfonyl chloride in pyridine to give the desired precursors **1–3** in 11–71% yield (Scheme 2). Attempts were made to prepare the tosylated alcohols, in pyridine (with or without DMF as co-solvent) or sodium hydroxide in water. However, none of these attempts led to product, one possible reason being the lower reactivity of *para*-toluenesulfonyl chloride compared with methanesulfonyl chloride,[18] although a four carbon chain biotin amide tosylate has been published.[19] However, tosylated biotin amide compounds with shorter chains than four carbons have not been reported. A plausible reason may be the steric hindrance from internal binding of the alcohol starting compound.

The unlabelled reference substances corresponding to compounds **4–6** were synthesised by refluxing the corresponding precursors with tetrabutylammonium fluoride in THF.

Binding to avidin

The influence of fluoro-alkyl or -di(ethylene glycol) substituents on the binding of biotin to avidin was investigated *in situ*, using the same procedure as for the ^{68}Ga-labelled compounds[16]. Varying amounts of the biotin or its analogues (2–128 nmol) were incubated together with a constant amount of avidin (2 nmol). Samples were withdrawn after 5 minutes and analysed by HPLC to determine the percent consumption of avidin, and



Scheme 2. Synthesis of alcohols **7–9** and the mesylated compounds **1–3**.

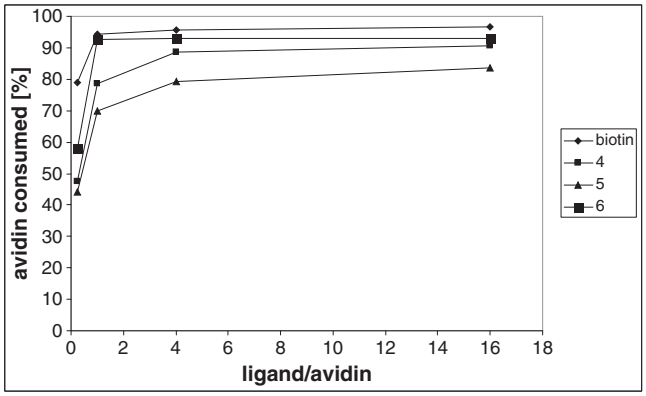


Figure 1. Saturation of the binding of biotin and nonradioactive compounds **4–6** to avidin in solution, as determined by HPLC analysis. The incubation was carried out for 5 minutes at room temperature. Data are presented as mean values ($n=2$).

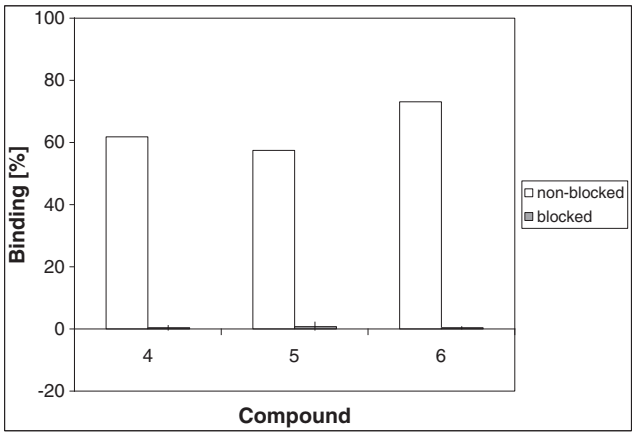


Figure 2. Percent binding of compounds **4–6** to avidin, blocked and nonblocked. Data are presented as mean values ($n=2$).

therefore indirectly determine the amount of biotin-avidin complex formed. The results of avidin-saturation experiments with nonradioactive fluoro compounds **4–6** and native biotin are shown in Figure 1. All analogues could bind to avidin. Native biotin showed the highest binding and the pegylated fluoro analogue (**6**) reached saturation close to that of biotin. The fluorinated compounds **4** and **5** reached saturation approximately 5% and 15%, respectively, below that of biotin. The ^{18}F-labelled analogues bound as well or slightly better as compared with the ^{68}Ga-labelled analogues (saturation reached 15–40% below native biotin).[16] Increasing the incubation time to 40 minutes did not increase the binding of any of the analogues.

The binding between equimolar amounts of the radiolabelled biotin analogues and avidin (67 mM each) at room temperature was studied using HPLC. Regardless of the structure, 60–75% of the labelled compounds **4–6** were bound to avidin within 5 minutes, as shown in Figure 2. Compound **6**, containing a di (ethylene glycol) chain, showed higher binding than compounds **4** and **5**, which contain alkyl chains. In contrast to our previous work with ^{68}Ga-labelled analogues, where PEG-linked analogues showed lower binding to avidin than alkyl-linked[16] in the case of ^{18}F analogues, the order was reversed. The binding was site-specific, as the binding was blocked by treating the avidin with a 50-fold excess of native biotin before adding any of the labelled compounds **4–6** (Figure 2). The limited sensitivity of the ultraviolet and radiodetectors precluded binding studies at concentration levels corresponding to the K_D of the native biotin-avidin affinity (10^{-15} M).

The binding of the biotin analogues might be high enough to make them useful in labelling avidin-treated islets of Langerhans.

Conclusion

Three ^{18}F-labelled biotin analogues have been synthesised using a one-step nucleophilic ^{18}F-fluorination strategy. The labelled compounds were obtained with a decay-corrected yield up to 35%, and the specific radioactivity for one of the ^{18}F-labelled compounds was 320 GBq/μmol. All analogues could bind to avidin *in situ*, as good as or to a slightly higher degree compared with our previously prepared ^{68}Ga-labelled biotin analogues, and 5–15% lower compared with native biotin. The binding of the tracers will be further evaluated *in vitro* and in an *in vivo* evaluation of the islet of Langerhans transplantation model.

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References

[1] E. A. Bayer, M. Wilchek, *Methods Biochem. Anal.* **1980**, *26*, 1–45.
[2] R. J. DeLange, T.-S. Huang, *J. Biol. Chem.* **1971**, *246*, 698–709.
[3] N. M. Green, *Adv. Protein Chem.* **1975**, *29*, 85–133.
[4] O. Livnah, E. A. Bayer, M. Wilchek, *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 5076–5080.
[5] M. Wilcheck, E. A. Bayer, *Anal. Biochem.* **1988**, *171*, 1–32.
[6] G. Sabatino, M. Chinol, G. Paganelli, S. Papi, M. Chelli, G. Leone, A. M. Papini, A. De Luca, M. Ginanneschi, *J. Med. Chem.* **2003**, *46*, 3170–3173.
[7] S.- C. Wu, S.- L. Wong, *Protein Exp. Purif.* **2006**, *46*, 268–273.
[8] A. M. J. Shapiro, J. R. T. Lakey, E. A. Ryan, G. S. Korbutt, E. Toth, G. L. Warnock, N. M. Kneteman, R. V. Rajotte, *N. Engl. J. Med.* **2000**, *343*, 230–238.
[9] A. M. J. Shapiro, E. Hao, J. R. T. Lakey, J. F. Elliot, R. V. Rajotte, N. M. Kneteman, *Tranplant. Proc.* **1998**, *30*, 647.
[10] B. W. Paty, S. Bonner-Weir, M. R. Laughlin, A. J. McEwan, A. M. J. Shapiro, *Transplantation* **2004**, *77*, 1133–1137.
[11] C. Toso, H. Zaidi, P. Morel, M. Armanet, A. Andres, N. Pernin, R. Baertschiger, D. Slosman, L. H. Buhler, D. Bosco, T. Berney, *Transplantation* **2005**, *79*, 353–355.
[12] T. Eich, O. Eriksson, A. Sundin, S. Estrada, D. Brandhorst, H. Brandhorst, B. Langstrom, B. Nilsson, O. Korsgren, T. Lundgren, *Transplantation* **2007**, *84*, 893–898.
[13] T. M. Shoup, A. J. Fischman, S. Jaywook, J. W. Babich, H. W. Strauss, D. R. Elmaleh, *J. Nucl. Med.* **1994**, *35*, 1685–1690.
[14] A. Najafi, A. Peterson, *Nucl. Med. Biol.* **1993**, *20*, 401–405.
[15] M. Simpson, L. Trembleau, R. W. Cheyne, T. A. D. Smith, *Appl. Radiat. Isotop.* **2011**, *69*, 418–422.
[16] E. Blom, B. Långström, I. Velikyan, *Bioconjug. Chem.* **2009**, *20*, 1146–1151.
[17] W. Zhang, S. Oya, M.- P. Kung, C. Hou, D. L. Maier, H. F. Kung, *Nucl. Med. Biol.* **2005**, *32*, 799–809.
[18] J.- i. Morita, H. Nakatsuji, T. Misaki, Y. Tanabe, *Green Chem.* **2005**, *7*, 711–715.
[19] S. J. Krivickas, E. Tamanini, M. H. Todd, M. Watkinson, *J. Org. Chem.* **2007**, *72*, 8280–8289.