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Synthesis and in vitro evaluation of [^{18}F]fluoroethyl triazole labelled [Tyr^3]octreotate analogues using click chemistry

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ABSTRACT

A novel class of alkyne linked [Tyr^3]octreotate analogues have been labelled by a copper catalysed azide-alkyne cycloaddition reaction (CuAAC) to form a 1,4-substituted triazole using the reagent [^{18}F]2-fluoroethyl azide. An unexpected variability in reactivity during the CuAAC reaction was observed for each alkyne analogue which has been investigated. Two lead alkyne linked [Tyr^3]octreotate analogues, G-TOCA (**3a**) and βAG-TOCA (**5a**) have been identified to be highly reactive in the click reaction showing complete conversion to the [^{18}F]2-fluoroethyl triazole linked [Tyr^3]octreotate analogues FET-G-TOCA (**3b**) and FET-βAG-TOCA (**5b**) under mild conditions and with short synthesis times (5 min at 20 °C). As well as ease of synthesis, in vitro binding to the pancreatic tumour AR42J cells showed that both FET-G-TOCA and FET-βAG-TOCA have high affinity for the somatostatin receptor with IC_{50} of 4.0 ± 1.4, and 1.6 ± 0.2 nM, respectively.

PET is becoming increasingly important for the early detection of disease in oncology and neurology. Radio-labelled peptides in particular are being investigated more frequently for the detection of disease, the monitoring of treatment, and in peptide receptor radiotherapy (PRRT).[1,2] The peptide [Tyr^3]octreotate (TOCA) (Fig. 1) has previously been labelled with various radioisotopes for the purpose of imaging[3–5] and PRRT[6] of neuroendocrine tumours. [Tyr^3]octreotate is a somatostatin analogue that has a longer biological halflife (1.5–2 h) than somatostatin and retains receptor specificity.[7] It has been found that somatostatin receptors, of which there are five subtypes (sstr 1–5), are over expressed on the surface of neuroendocrine tumours.[8] This over expression enables selective targeting of tumours with a radiolabelled octreotate analogue. The first discovered eight amino acid sequenced peptide to mimic somatostatin was octreotide. It was found that the cyclic octapeptide contained the important sites for binding to the somatostatin receptor and was initially used as an opiate anatagonist[9] for the treatment of painful conditions such as acute and chronic pancreatitis.[10] Comparing octreotide to [Tyr^3]octreotate, the latter has been shown to have a higher affinity for the somatostatin receptors,[5,11] it appears that substituting phenylalanine for tyrosine and threoninol for threonine at the C-terminus increases affinity. Nonetheless, [^{111}In]-DTPA-octreotide (Octreoscan™) is still the peptide of choice in the clinic and was approved by the FDA as an imaging agent for somatostatin receptor positive neuroendocrine tumours in 1994.[8]

Using prosthetic groups is the strategy generally employed when labelling peptides or other macromolecules to overcome the limitations of $^{18}F^-$ such as basicity and poor reactivity.[12] The approaches used to date all vary in the number of steps involved, the overall reaction time, isolated yield and method of isolation. Octreotide has previously been labelled with ^{18}F-modified organic prosthetic groups. The initial strategy employed by Hostetler et al.[13] was to directly label the N-terminus of octreotide with the activated ester of [^{18}F]fluorobenzoic acid ([^{18}F]FBA). Subsequent biodistribution studies showed that the [^{18}F]fluorobenzoyl-octreotide analogue was too lipophilic and showed significant uptake in the liver. Octreotide has also been directly labelled at the N-terminus with 2-[^{18}F]fluoropropionate 4-nitrophenylester, which itself involves three chemical modification steps to synthesise.[14] The main drawback to this chemistry is the need to Boc-protect the lysine side chain of octreotide during conjugation which requires removal in the final step.[15]

Schottelius et al.[16] wanted to develop an ^{18}F-labelled octreotate analogue with improved tumour uptake and better pharmacokinetics compared to previous analogues. The authors chose to

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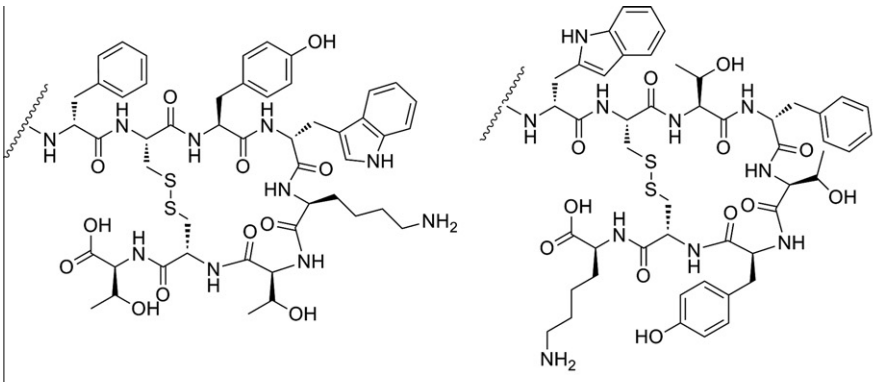


R² = [Tyr³]Octreotate (TOCA)

R³ = Scrambled octreotate peptide (NH-[W-c-(CTFTYC)K])

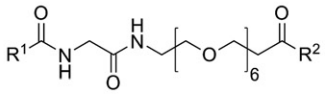
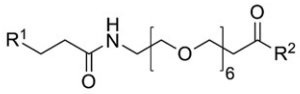
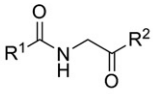
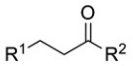
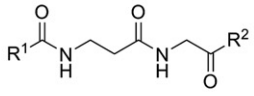
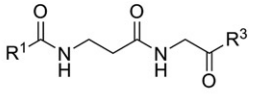
	R¹ = H—	**R¹ =** ^{18}F-triazole
	G-PEG-TOCA **(1a)**	FET-G-PEG-TOCA **(1b)**
	E-PEG-TOCA **(2a)**	FETE-PEG-TOCA **(2b)**
	G-TOCA **(3a)**	FET-G-TOCA **(3b)**
	E-TOCA **(4a)**	FETE-TOCA **(4b)**
	βAG-TOCA **(5a)**	FET-βAG-TOCA **(5b)**
	βAG-[W-c-(CTFTYC)K] **(6a)**	FET–βAG-[W-c-(CTFTYC)K] **(6b)**

Figure 1. The five alkyne (**1a**–**5a**) and triazole (**1b**–**5b**) analogues and the scrambled negative control alkyne (**6a**) and triazole (**6b**).

modify octreotate with carbohydrate groups in order to reduce lipophilicity, to consequently reduce hepatic elimination and conversely aid renal elimination. A glucose modified octreotate (Gluc-Lys-TOCA) was developed, and the lysine of the peptide labelled with 2-[^{18}F]fluoropropionate 4-nitrophenylester. The final product Gluc-Lys([^{18}F]FP)-TOCA was evaluated in patients by Meisetschläger et al.[17] and was found to be superior to [^{111}In]-DTPA-octreotide at detection of neuroendocrine tumours. The authors of this study found that while the radiotracer showed improvements in tumour uptake, the lengthy synthesis time (3 h) and low yields (20–30%) made it a non-viable option for clinical use.[17] Schottelius et al.[16] also labelled two other carbohydrate analogues, Cel-*S*-Dpr-[Tyr³]octreotate and Gluc-S-Dpr[Tyr³]octreotate with [^{18}F]fluorobenzaldehyde to give the oxime-derivatised radiotracers. The Cel-*S*-Dpr([^{18}F]FBOA)-[Tyr³]octreotate showed improved tumour uptake compared to Gluc-Lys([^{18}F]FP)-TOCA and had a shorter synthesis time (50 min) with improved yields (65–85%).

The most recently published ^{18}F-labelled octreotide analogue was the [^{18}F]AlF-NOTA-octreotide.[18] The advantage of using the [^{18}F]aluminium fluoride labelling strategy is that the fluorine-18 azeotropic drying step is not required, meaning shorter overall reaction times. By HPLC, the product was observed as two isomers, equating to approximately 50% incorporation of [^{18}F]AlF into the

NOTA chelate, the remainder was stated as non-chelated [^{18}F]AlF. The authors commented that the two isomers could be separated by HPLC. When re-analysed they saw re-equilibration to the two isomers. The conformation of these two isomers has not been established to date.

Click chemistry has been utilised previously in fluorine-18 labelling of peptides.[19–22] Since the reaction is efficient it can be applied to the synthesis of radiolabelled tracers and ligands with short lived isotopes (halflife ^{18}F, 109.7 min) for positron emission tomography (PET).[23,24]

Marik and Sutcliffe[25] took the approach of labelling terminal alkynes with fluorine-18 and adding the azide moiety to various peptides. They initially employed $CuSO_4$/Na-ascorbate as a catalytic system but only isolated the labelled peptide in 10% yield. Improvements were observed when $CuSO_4$ was replaced by CuI with addition of *N,N*-diisopropylethylamine (DIEA). Sirion et al.[26] found in their investigations that using CuI gave traces of the 1,5-substituted triazole by-product. The authors synthesized four mesylate precursors, two acetylene and two azides all of which were labelled using [^{18}F]TBAF, with *t*BuOH as solvent.[27]

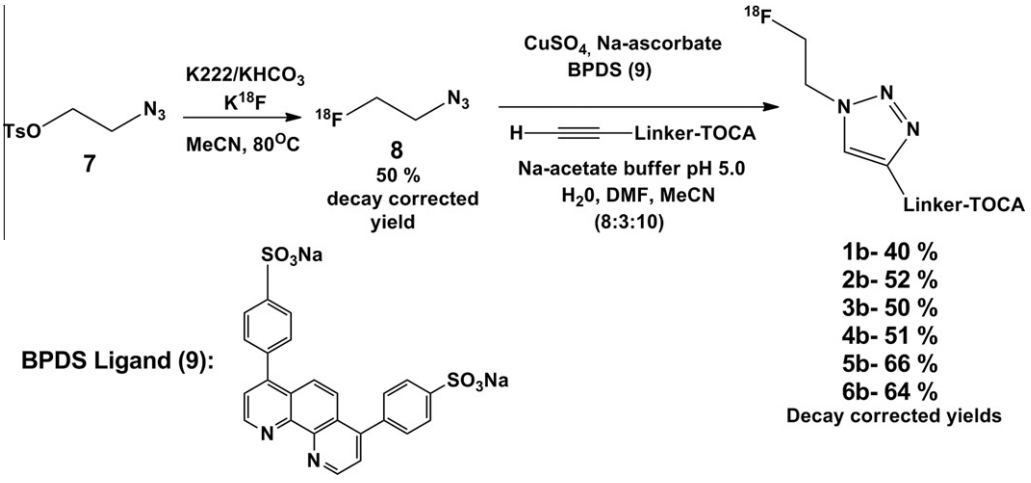
Herein we report fluorine-18 labelling of [Tyr^3]octreotate analogues with [^{18}F]2-fluoroethyl azide. Our aim in labelling [Tyr^3]octreotate via CuAAC was for a two-step method, reduced synthesis times, and an ^{18}F-labelled peptide with improved tumour uptake and pharmacokinetic parameters compared to previously labelled analogues. The triazole ring is known to be a stable mimic of the amide group,[28] therefore we anticipated the addition of our labelled precursor would not impact biological efficacy significantly.

To find a suitable candidate, five alkyne functionalised octreotate analogues were screened (Fig. 1). We also designed a scrambled peptide (**6b**) (Fig. 1) as a negative control to show no specificity to the somatostatin receptor. Due to the selective nature of the CuAAC reaction we anticipated that the purification of a final ^{18}F-labelled peptide would be greatly simplified. We modified octreotate at the N-terminus, since modification here has not been shown to reduce binding affinity in previous examples. The linkers between the octreotate and the alkyne functionality were chosen to complement the peptide and for ease of synthesis. Two analogues were designed containing polyethylene glycol groups, which we hypothesised would improve pharmacokinetics as previously reported for other PEGylated molecules.[29]

The standards, [^{19}F]-**1b–6b**, were synthesised using copper powder, and initial reactions carried out using DMF as solvent. Solvent is thought to play an important role in the success of the click reaction. It is well known that water can accelerate the rate of reaction[30] and this occurred to varying degrees when synthesising [^{19}F]-**1b–6b**. The synthesis of [^{19}F]-**1b** proceeded slowly (>3 h) when using solely DMF. Addition of water (DMF/H_2O (3:2)) enhanced the rate of reaction and had reached completion within 15 min.

The octreotate alkynes (**1a–5a**) and scrambled analogue (**6a**) were labelled using [^{18}F]fluoroethyl azide (**8**) (Scheme 1). The method used to synthesise **8** was slightly modified to that employed by Glaser et al.[23] We found that the use of $KHCO_3$ instead of K_2CO_3 during the [^{18}F]fluoride drying step gave more consistent isolated yields after purification by distillation, due to the enhanced stability of the precursor, **7** using the milder base.

The variability in click reaction rates observed for the alkyne-peptides **1a–5a**, can be attributed to the variation in linker, since the peptide moiety remains unchanged. The electronic property of the group adjacent to the terminal alkyne centre is believed to neither enhance nor reduce the rate of reaction.[31] Contrary to this, we found that terminal alkynes directly substituted with an amide moiety (**1a**, **3a**, **5a**, **6a** (Fig. 1)) had enhanced reactivity compared to those directly substituted with an ethyl-linked amide (**2a** and **4a**, (Fig. 1)). Similar results were found by Li et al.[32] during their investigations of the 1,3-Huisgen cycloaddition reaction and separately by Golas et al.[33] who investigated the effect of electron withdrawing groups and steric hindrance around the azide moiety on the rate of the CuAAC. It can be seen that **1a** reacts more slowly that **3a** and **5a** (Table 1, conditions B). Electronically they are similar, all being directly linked amide substituted terminal alkynes, but **1a** contains six sequential ethylene glycol groups. It is possible that the PEG chain is surrounded by a bulky water cloud, which could sterically hinder the alkyne functionality.[34] Alkynes **2a** and **4a** are comparable in the same respect (PEGylated vs non-PEGylated); we observed that **2a** shows a slower rate of reaction during the CuAAC. When using alkynes **1a** and **2a**, increasing the reagent concentrations during the click reaction, we were able to achieve complete incorporation of **8** with more reproducible results (conditions D, Table 1). It is known that Cu(II) is a lewis acid which is able to weakly bind to oxygen donors such as ethers. It is therefore possible that the Cu(II) binds to the PEG groups and is therefore unable to participate in the click reaction,[35] increasing the concentration of Cu(II) overcomes this. Analogues **1b–6b** were isolated as formulated products (10% EtOH/PBS buffer) to give decay corrected yields of 40–64%, based on starting [^{18}F]fluoroethyl azide activity. The specific activity measured for the analogues ranged from 4.8 to 12.3 GBq/μmol with respect to the alkyne starting material (pseudo-specific activity). Reaction rate was increased using higher

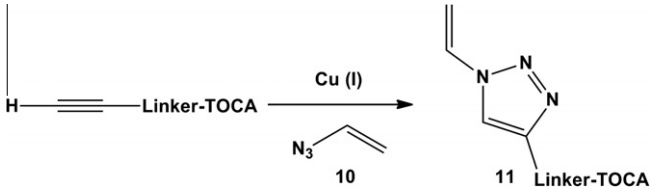


Scheme 1. The reaction pathway to the ^{18}F-labelled triazoles.

Table 1
Radiochemical analytical yields observed using HPLC analysis

Alkyne	Analytical yields (reaction time, min)			
	Conditions A[a]	Conditions B[b]	Conditions C[c]	Conditions D[d]
1a	—	19% (30)	>98% (30)	>98% (30)
2a	—	0% (30)	0%[e] (30)	>98% (30)
3a	—	>98% (5)	>98% (5)	—
4a	14% (30)	47% (30)	97% (5)	>98% (30)
5a	—	>98% (5)	—	—
6a	—	77% (30)	—	>98% (15)

All reactions carried out using 100 μl of [^{18}F]fluoroethyl azide (**8**).
[a] Conditions A: Alkyne (2 mg), $CuSO_4$ (2 equiv) , Na-ascorbate (2.2 equiv), pH 5.0, rt.
[b] Conditions B: Alkyne (2 mg), $CuSO_4$ (2 equiv), Na-ascorbate (2.2 equiv), **9** (4 equiv), pH 5.0, rt.
[c] Conditions C: Alkyne (2 mg), $CuSO_4$ (2 equiv), Na-ascorbate (2.2 equiv), **9** (4 equiv), pH 5.0, 80 °C.
[d] Conditions D: Alkyne (2 mg), $CuSO_4$ (4 equiv), Na-ascorbate (4.4 equiv), **9** (5 equiv), pH 5.0, rt.
[e] No alkyne remains in the reaction only the by-products are found in the UV trace.



Scheme 2. Suspected pathway to form by-product **11**.

temperatures with reaction times being reduced, but this led to significant by-product formation and made purification more difficult (Table 1, conditions C).

A general trend found during the click reaction with analogues **1a–6a** were two stable by-products seen during HPLC monitoring. The two by-products **11** and **12** (see Supplementary data, Fig. S1) were isolated from the reaction media using **5a** (Fig. 1). They were separated, collected and analysed by collision induced dissociation mass spectrometry (CID-MS). The more polar compound **12** was elucidated to be the alkyne precursor (**5a**) during CID-MS analysis. Although this was found to be the case, when the reaction mixture was admixed with **5a** and assessed chromatographically, by-product **12** did not co-elute on the HPLC trace. We found that reactions showing incomplete incorporation of [^{18}F]2-fluoroethyl azide (**8**) did not significantly proceed any further, even in the presence of **12**, suggesting insufficient reactivity of this species in the CuAAC reaction. The by-product **11** was analysed and found to correspond to the 1-vinyl triazole (Scheme 2). Presumably **11** was formed via the click reaction with azidoethene (**10**), which we suspect to be a by-product from an elimination mechanism in the initial labelling step. Kim et al.[36] reported a similar occurrence using 4-tosyloxy-1-butyne; they observed elimination to form vinyl acetylene, which was then able to react with the azide in the click reaction. The main issue with by-product **11** is the similar retention time to the radiolabelled product during purification, making it difficult to obtain the highest yield possible as well as high specific radioactivity. Another by-product, which could be predicted, is the 1-hydroxyethyl triazole, this was not significantly observed.

The experiments carried out using the $CuSO_4$/Na-ascorbate method were generally done using sodium acetate buffer (AB) at pH 5.0. When an experiment using **3a** was carried out using distiled water the conversion to radiolabelled product was slower, showing 78% conversion at 5 min compared to >98% conversion using the buffered system.

We carried out a one-pot reaction in which distillation of **8** was avoided. In this reaction we reduced the amount of **7** (1.3 μmol) and observed an analytical yield >95% of **8** under the same conditions as used previously. The reaction was attempted with **2a** and **3a**, both of which were used in excess of **7**, (3.4 μmol, 2.7 μmol, respectively). Both showed slower reaction rates, presumably due to competing side reactions. Another variable that can affect the rate of reaction is the volume of [^{18}F]2-fluoroethyl azide. A reaction carried out using conditions B (Table 1) with **2a** and 50 μl of [^{18}F]2-fluoroethyl azide (**8**) gave 84% analytical conversion to **2b** after 30 min. When using 100 μl of **8** under the same conditions no reaction was observed (Table 1). The solvent system plays a critical role in the CuAAC reaction.[26] The additional acetonitrile leads to a change in concentration and solvent ratios, which could affect the rate of reaction.

We have found that using BPDS (**9**) (Scheme 1), a Cu(I) stabilizing ligand,[37] greatly enhances the rate of the click reaction. We evaluated the reaction without any ligand in the early stages of our investigation. Reactions carried out with **4a** without **9** (Table 1, conditions A) showed 14% conversion to the desired product but on addition of **9** (conditions B), gave 47% conversion. Using the same reagent concentrations but heating the reaction to 80 °C gave >98% conversion to **4b** after 5 min (Table 1, conditions C); although the conversion to product at this temperature was excellent, an increase in by-products was observed, some of which co-eluted with **4b**.

The use of copper wire as an alternative source of catalytic Cu(I) was investigated and showed promising results. The experiments were carried out using **3a** due to its enhanced reactivity compared to other analogues using the $CuSO_4$/Na-ascorbate method. We found that pre-mixing the copper wire and alkyne then heating before addition of **8** showed the reaction to be complete within 5 min (Table 2, entry 2). Without pre-mixing the two components, the click reaction took longer to reach completion (Table 2, entry 1). Other pH buffer systems (Table 2, entries 4–6) were also applied, but all proved inferior to the sodium acetate buffer solution (pH 5.0, 250 mM) (Table 2, entry 1). $CuSO_4$ and BPDS (**9**) were added to the reaction using copper wire, the rationale behind this approach being that the added $CuSO_4$ would improve the comproportionation reaction by increasing the concentration of available Cu(II).[38,39] The ligand (**9**) as previously mentioned, was added to stabilise the Cu(I) species. The strategy worked well and showed improvement in yields at room temperature (Table 2, entry 7). To establish whether this was a tandem effect reactions were carried out to investigate both additional reagents separately (Table 2, entries 7 and 8). It appears that that both reagents affect the rate to some extent but used together they have a greater impact on the rate of the reaction. MonoPhos™[40] was investigated as an alternative ligand, but proved less efficient in the reaction (Table 2, entry 10).

Log *D* values were measured using the ^{18}F-labelled triazole. As expected the log *D* values for the analogues **1b** and **2b** were the lowest due to the PEGylation, and the analogue with the highest log *D* value was **4b** (Table 3). The receptor affinities for [^{19}F]**1b–6b** were determined using a competitive binding assay in AR42J tumours cells with [^{111}In]-OctreoScan as the labelled radiotracer. The half-maximal inhibitory concentration (IC_{50}) values were calculated and the results of the displacement curves are summarised (Table 3). As a reference peptide, the IC_{50} value was measured for octreotide and was found to be 14.7 ± 7.7 nM. The IC_{50} values for the octreotate analogues were found to be comparable or lower than octreotide, indicating high binding affinity for the somatostatin receptor; all showed affinity in the nanomolar range (Table 3). The introduction of the fluoroethyl triazole moiety decreased the affinity of [^{19}F]-**1b–5b**, but not significantly, the values were still below or comparable to octreotide. In this study the compound

Table 2
Copper wire catalysed experiments using alkyne **3a** (analytical radiochemical yield by HPLC)

	Reaction temperature	pH	Solvents (v/v)	Pre-activation time (min)	Other additives	Analytical yield (%) at:		
						5 min	15 min	30 min
1	80 °C	5.0	DMF/AB (1:3)	0	–	52	95	–
2	80 °C	5.0	DMF/AB (1:3)	30	–	>98	–	–
3	rt	5.0	DMF/AB (1:3)	150	–	0	7	30
4	80 °C	6.0	DMF/PB (1:3)	0	–	4	–	–
5	80 °C	4.0	DMF/FB (1:3)	0	–	3	–	–
6	80 °C	8.0	DMF/TB (1:3)	0	–	2	–	–
7	rt	5.0	DMF/AB (1:3)	0	40 mol % $CuSO_4$, BPDS (**9**) (2 equiv)	67	>98	–
8	rt	5.0	DMF/AB (1:3)	0	40 mol % $CuSO_4$,	0	0	48
9	rt	5.0	DMF/AB (1:3)	0	BPDS (**9**) (2 equiv)	35	54	>98
10	rt	5.0	DMSO/AB (7:3)	0	MonoPhos ligand (2 equiv)	0	0	0

All buffers are 250 mM concentrations. Sodium acetate buffer (AB) pH 5.0; sodium phosphate buffer (PB) pH 6.0; ammonium formate buffer (FB) pH 4.0; Tris buffer (TB) pH 8.0. Each experiment contained 100–120 mg of a copper wire coil, diameter 0.1 mm, and was used with out any treatment (rt = room temperature). All reactions carried out using 100 μl of [^{18}F]fluoroethyl azide (**8**).

Table 3
Competitive binding assay IC_{50} values using **1a–5a** and [^{19}F]-**1b–6b** displacing [^{111}In]-DTPA-octreotide on AR42J tumour cells and Log D values of [^{18}F]-**1b–6b**

Peptides	Alkyne analogue (a) IC_{50} (nM)	Triazole analogue (b) IC_{50} (nM)	Control	Log *D* of ^{18}F-triazole
1	5.3 ± 0.75	10.8 ± 5.9	–	−2.68
2	5.1 ± 2.1	13.2 ± 7.8	–	−2.77
3	1.4 ± 0.1	4.0 ± 1.4	–	−1.83
4	1.8 ± 0.8	2.9 ± 1.3	–	−1.5
5	1.0 ± 0.3	1.6 ± 0.2	–	−2.26
6	–	>10.0 (mM)	–	−1.14
Octreotide	–	–	14.7 ± 7.7	–

N.B. (n = 4 for each concentration and assay repeated three times). Log *D* partition co-efficient measurements **1b–5b** (n = 3), **6b** (n = 6). IC_{50} values of AlF-NOTA-octreotide, Ga-NOTA-octreotide found by Laverman et al.[18] were 3.6 ± 0.6, 13.0 ± 3.0 nM, respectively. Literature IC_{50} value of octreotide = 0.5 nM used in a competitive binding assay with ^{125}I-somatostatin-14.[41]

[^{19}F]-**5b** showed the highest affinity with an IC_{50} value of 1.6 ± 0.2 nM. PEGylation of the peptides, through the addition of six sequential ethylene glycol groups, appeared not to significantly affect the overall affinity of [^{19}F]-**1b** and [^{19}F]-**2b** giving IC_{50} values of 10.8 ± 5.9 nM and 13.2 ± 7.8 nM, respectively. In comparison [^{19}F]-**6b**, which contained a scrambled amino acid sequence, showed low affinity giving an IC_{50} value >10 mM. This result showed that our analogues are specifically binding to the somatostatin receptor.

In conclusion, we have screened five novel alkyne functionalised octreotate analogues in the click reaction with [^{18}F]2-fluoroethyl azide. Our studies found the most reactive alkynes were G-TOCA (**3a**) and βAG-TOCA (**5a**), showing complete conversion to the labelled triazole FET-G-TOCA (**3b**) and FET-βAG-TOCA (**5b**) in 5 min at room temperature using optimised conditions. As well as efficiency in the click reaction both analogues have shown high binding affinities to the somatostatin receptor. A full in vivo study of all five analogues will be carried out to evaluate tumour uptake and pharmacokinetics. We will use the scrambled analogue (**6b**), which shows no affinity for somatostatin receptors, as a control in vivo.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.03.016.

References and notes

1. Chen, X. Y.; Park, R.; Hou, Y. P.; Khankaldyyan, V.; Gonzales-Gomez, I.; Tohme, M.; Bading, J. R.; Laug, W. E.; Conti, P. S. *Eur. J. Nucl. Med. Mol. Imaging* **2004**, *31*, 1081.
2. Lei, M.; Tian, M.; Zhang, H. *Curr. Med. Imaging Rev.* **2009**, *6*, 33.
3. Nikolopoulou, A.; Maina, T.; Sotiriou, P.; Cordopatis, P.; Nock, B. A. *J. Pept. Sci.* **2006**, *12*, 124.
4. Li, W. P.; Lewis, J. S.; Kim, J.; Bugaj, J. E.; Johnson, M. A.; Erion, J. L.; Anderson, C. J. *Bioconjug. Chem.* **2002**, *13*, 721.
5. Reubi, J. C.; Schar, J. C.; Waser, B.; Wenger, S. *Eur. J. Nucl. Med. Mol. Imaging* **2000**, *27*, 273.
6. Teunissen, J.; Krenning, E.; deJong, F.; de Rijke, Y. *Eur. J. Nucl. Med. Mol. Imaging* **2009**, *36*, 1758.
7. Weiner, R. E.; Thakur, M. L. *Semin. Nucl. Med.* **2001**, *31*, 296.
8. Rufini, V.; Calcagni, M. L.; Baum, R. P. *Semin. Nucl. Med.* **2006**, *36*, 228.
9. Maurer, R.; Gaehwiler, B. H.; Buescher, H. H.; Hill, R. C.; Roemer, D. *PNAS* **1982**, *79*, 4815.
10. Uhl, W.; Anghelacopoulos, S. E.; Friess, H.; Buchler, M. W. *Dig. Int. J. Gastroenterol.* **1999**, *60*, 23.
11. Wild, R.; Schmitt, J. S.; Ginj, M.; Macke, H. R.; Bernard, B. F.; Krenning, E.; Jong, M.; Wenger, S.; Reubi, J. C. *Eur. J. Nucl. Med. Mol. Imaging* **2003**, *30*, 1338.
12. Okarvi, S. M. *Eur. J. Nucl. Med.* **2001**, *28*, 929.
13. Hostetler, E. D.; Edwards, W. B.; Anderson, C. J.; Welch, M. J. *J. Labelled Comp. Radiopharm.* **1999**, *42*, S720.
14. Guhlke, S.; Coenen, H. H.; Stocklin, G. *Appl. Radiat. Isot.* **1994**, *45*, 715.
15. Guhlke, S.; Wester, H. J.; Bruns, C.; Stocklin, G. *Nucl. Med. Biol.* **1994**, *21*, 819.
16. Schottelius, M.; Poethko, T.; Herz, M.; Reubi, J. C.; Kessler, H.; Schwaiger, M.; Wester, H. J. *Clin. Cancer Res.* **2004**, *10*, 3593.
17. Meisetschläger, G.; Poethko, T.; Stahl, A.; Wolf, I.; Scheidhauer, K.; Schottelius, M.; Herz, M.; Wester, H. J.; Schwaiger, M. *J. Nucl. Med.* **2006**, *47*, 566.
18. Laverman, P.; McBride, W. J.; Sharkey, R. M.; Eek, A.; Joosten, L.; Oyen, W. J. G.; Goldenberg, D. M.; Boerman, O. C. *J. Nucl. Med.* **2010**, *51*, 454.
19. Li, Z. B.; Wu, K.; Chen, K.; Chin, F. T.; Chen, X. *Bioconjug. Chem.* **2007**, *18*, 1987.
20. Ramenda, T.; Kniess, T.; Bergmann, R.; Steinbacha, J.; Wuest, F. *Chem. Commun.* **2009**, *48*, 7521.
21. Hausner, S. H.; Marik, J.; Gagnon, M. K. J.; Sutcliffe, J. L. *J. Med. Chem.* **2008**, *51*, 5901.
22. Mamat, C.; Ramenda, T.; Wuest, F. R. *Mini-Rev. Org. Chem.* **2009**, *6*, 21.
23. Glaser, M.; Arstad, E. *Bioconjug. Chem.* **2007**, *18*, 989.
24. Glaser, M.; Robins, E. G. *J. Labelled Comp. Radiopharm.* **2009**, *52*, 407.
25. Marik, J.; Sutcliffe, J. L. *Tetrahedron Lett.* **2006**, *47*, 6681.

26. Sirion, U.; Kim, H. J.; Lee, J. H.; Seo, J. W.; Lee, B. S.; Lee, S. J.; Seung, J. O.; Chi, D. Y. *Tetrahedron Lett.* **2007**, *48*, 3953.
27. Kim, D. W.; Ahn, D. S.; Oh, Y. H.; Lee, S.; Kil, H. S.; Jun, Oh. S.; Lee, S. J.; Kim, J. S.; Ryu, J. S.; Moon, D. H.; Chi, D. Y. *J. Am. Chem. Soc.* **2006**, *128*, 16394.
28. Bock, V. D.; Speijer, D.; Hiemstra, H.; Maarseveen, J. H. *Org. Biomol. Chem.* **2007**, *5*, 971.
29. Veronese, F. M. *Drug Discovery Today* **2005**, *10*, 1451.
30. Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004.
31. Hein, J. E.; Fokin, V. V. *Chem. Soc. Rev.* **2010**, *39*, 1302.
32. Li, Z.; Tae Seok, S.; Jingyue, J. *Tetrahedron Lett.* **2004**, *45*, 3143.
33. Golas, P. L.; Tsarevsky, N. V.; Matyjaszewski, K. *Macromol. Rapid Commun.* **2008**, *29*, 1167.
34. Shiffman, M. L. *Curr. Hepat. Rep.* **2003**, *2*, 17.
35. Kuang, G. C.; Michaels, H. A.; Simmons, J. T.; Clark, R. J.; Zhu, L. *J. Org. Chem.* **2010**, 6540.
36. Hong Kim, D.; Seong Choe, Y.; Tae Kim, B. *Appl. Radiat. Isot.* **2010**, *68*, 329.
37. Gill, H. S.; Tinianow, J. N.; Ogasawara, A.; Flores, J. E.; Vanderbilt, A. N. *J. Med. Chem.* **2009**, *52*, 5816.
38. Gopin, A.; Ebner, S.; Attali, B.; Shabat, D. *Bioconjug. Chem.* **2006**, *17*, 1432.
39. Bonnet, D.; Ilien, B.; Galzi, J.; Riche, S.; Antheaune, C.; Hibert, M. *Bioconjug. Chem.* **2006**, *17*, 1618.
40. Campbell-Verduyn, L. S.; Mirfeizi, L.; Dierckx, R. A.; Elsinga, P. H.; Feringa, B. L. *Chem. Commun.* **2009**, *16*, 2139.
41. Decristoforo, C.; Melendez-Alafort, L.; Sosabowski, J. K.; Mather, S. J. *J. Nucl. Med.* **2000**, *41*, 1114.