'Click for PET'

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Chapter 6
Strain-Promoted 'Click' Chemistry for [^{18}F]-Radiolabelling of Bombesin

In order to develop a convenient method for rapid introduction of radiolabels via 'click' chemistry, a new route to a strained aza-dibenzocyclooctyne has been developed. The strained cycloalkyne proved to react with [^{18}F]-containing azides to give the corresponding triazoles in minutes. [Lys3]-bombesin was modified with the cycloalkyne and subsequently labeled with three [^{18}F]-containing azides, via strain-promoted 'click' chemistry. The three resulting tracers retained their high affinity for gastrin-releasing peptide receptors in vitro.

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6.1 Introduction

To better understand the complexity of living systems, scientists have sought to track biomolecules as they exist in their native environment, hoping to study biological processes as they occur at the cellular level. There are few biomolecules that naturally possess the properties allowing for their detection in a complex cellular environment, therefore methods have been developed to endow biomolecules with a reporter tag.[1] Popular techniques include fusion of fluorescent probes (such as the green fluorescent protein) to target proteins,[2] antibody conjugates,[3] and more recently, bioorthogonal chemical reporters. This last approach involves the incorporation of a unique chemical functionality into a target molecule (often using the cell's own machinery), with subsequent chemical ligation to a small-molecule probe, bestowing on the biomolecule a means by which to track it (eg. fluorescence).[1]

Azides are ideal for bioorthogonal chemical reporter strategies. They are virtually absent from living organisms and are resistant to oxidation or reaction with water (see Chapter 1, Section 1.5). As such, the Staudinger ligation of azides with functionalized phosphines and the cycloaddition of azides with alkynes have both been explored as methods for bioorthogonal labeling. These transformations proceed in water, and have been shown to work *in vivo*. The Staudinger ligation, a modification on the original Staudinger reaction,[4] involves the reaction of an azide with a triarylphosphine (**1**) to form an aza-ylide intermediate (**2**) (Scheme 1). This is followed by intramolecular trapping of the nucleophilic nitrogen atom, followed by hydrolysis of the cyclized intermediate to form an amide-bond linkage (**4**).[5]
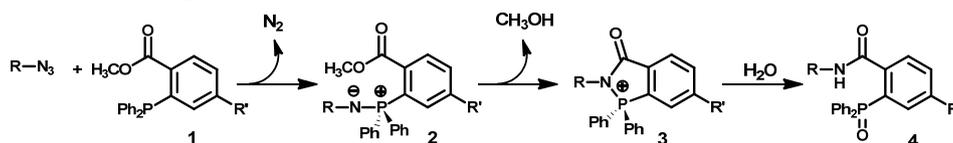
Scheme 1 The Staudinger ligation

The drawbacks of the Staudinger ligation are two-fold. Firstly, phosphines are prone to oxidation in cellular environments, thereby reducing the amount of phosphine in the system available for targeted labeling. A second disadvantage stems from the relatively slow reaction kinetics of the Staudinger ligation which limit its use in the exploration of fast biological processes.[6]

Given the ideal properties of the azide for use in chemical reporter labeling, the cycloaddition with alkynes was also explored for labeling. The copper-catalyzed azide-alkyne cycloaddition has the advantage that it is faster than the Staudinger ligation (~25

times).[1] The obvious limitation of this methodology for biological systems is the cytotoxicity of copper.[7] Other methods of activating the alkyne substrate have thus been explored, to conserve the advantages of the bioorthogonal alkyne-azide system, while avoiding the toxicity of the metal catalyst. Two main approaches have been investigated: the strategic placement of electron-withdrawing substituents (Scheme 2, A),[8] and the introduction of ring strain in alkenes (Scheme 2, B)[9] and in alkynes (Scheme 2, C).[10, 12]
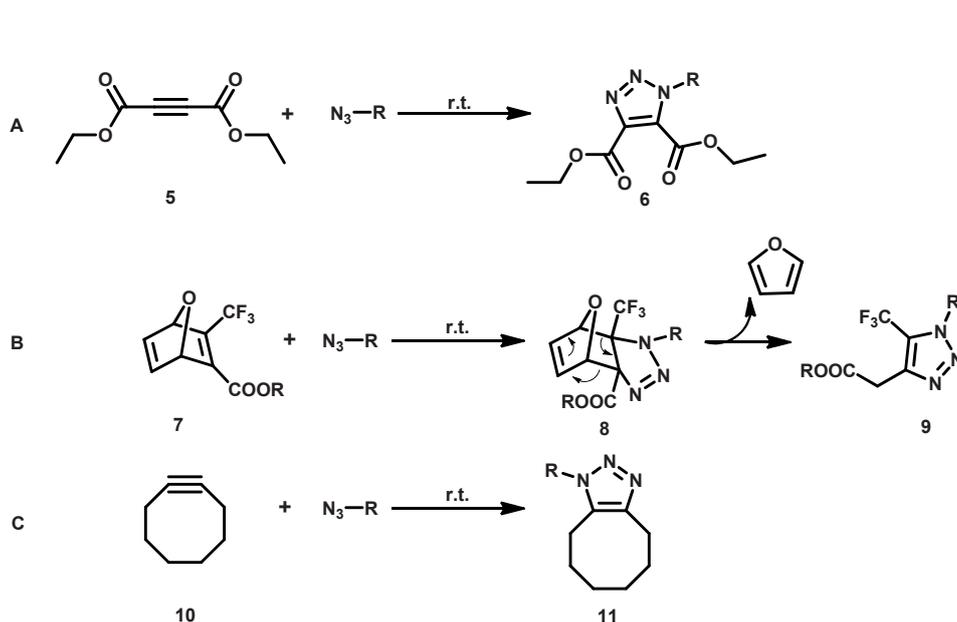
Scheme 2 Alternative approaches for activating alkynes to react with azides

Electron withdrawing groups directly adjacent to the triple bond have the effect of rendering the alkyne sufficiently electron deficient to undergo cycloaddition with azides in the absence of the copper catalyst at room temperature (Scheme 2, A). However, such a system also renders the substrate (**5**) susceptible to attack by various nucleophiles, thus effectively canceling the bioorthogonality of the system. As an alternative, strain-promoted cycloadditions between alkenes and azides were developed (Scheme 2, B). One such example is the [3+2] cycloaddition of electron-deficient oxanorbornadiene **7** with an azide at room temperature to form an intermediate triazoline (**8**). Cycloadduct **8** then undergoes a spontaneous retro-Diels-Alder reaction releasing furan, to yield triazole **9**. Yet another alternative involves cycloaddition of the smallest isolable cycloalkyne, cyclooctyne, with azides at room temperature (Scheme 2, C). The sp-hybridized carbons in cyclooctyne form a bond angle of ~160°. The triple bond is distorted towards the transition state of the

cycloaddition reaction, resulting in an accelerated reaction with azides.[11] This has proven to be a very effective means of eliminating the need for a copper catalyst in the azide-alkyne cycloaddition, and many variations of the cylooctyne motif have since been designed (Scheme 3).
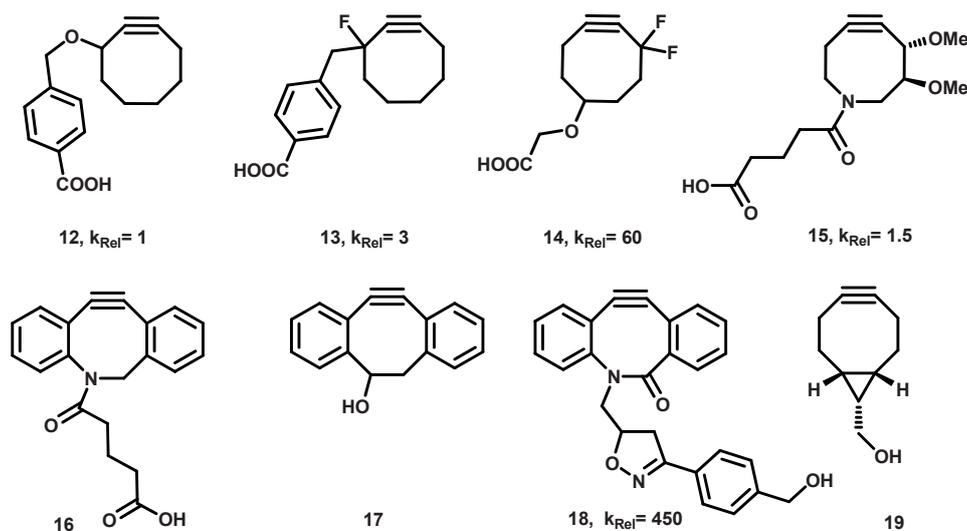

Scheme 3 Range of functionalized cyclooctynes for copper-free 'click' chemistry (k_{Rel}=relative rate)

The development of functionalized cyclooctynes was pioneered by the group of Bertozzi.[12] Functionalized cyclooctyne **12** was the first of a series of strained alkynes developed in their group.[13] Although **12** reacts with azides at room temperature, the reaction rate constants do not surpass those of the Staudinger ligation. Vast improvement in the reaction rate was achieved by the addition of first one (**13**),[14] and later two fluorine atoms (**14**)[15] adjacent to the strained triple bond. Cyclooctyne **13** had a reaction rate constant 3 times greater than **12**, and **14** had a reaction rate constant 60 times greater than **12** (Scheme 3). The next challenge became evident as **14** proved to have limited solubility in water, which can be problematic for applications in cellular environments. Thus the methoxy-substituted carboxylic acid **15** was developed.[16] While these modifications improved the solubility of the compound, the reaction rate is relatively low.[12]

Although the addition of fluorine atoms has the desired effect of improving the reactivity of the cyclooctynes towards cycloaddition with azides, the synthetic procedures to reach these compounds are quite long and low-yielding, hindering their general applicability.[12] As an alternative to the introduction of electron-withdrawing fluorine atoms,

it has been shown that the reactivity of the strained alkynes can be increased by the introduction of aryl rings (**16-18**) which induces additional ring strain,[17] or by fusion with cyclopropane (**19**).[18] The order of reactivity of the dibenzofused cyclooctynes increases from **16** to **17** to **18** (0.17, 0.36 and 0.96 $M^{-1}s^{-1}$), with **18** being to date the fastest reacting strained cyclooctyne.[11] A photo-triggered formation of a dibenzocyclooctyne has also been developed, allowing for generation of the strained alkyne from a substituted cyclopropenone via photochemical decarbonylation.[19] The cyclooctyne class of compounds has shown to have the appropriate balance of stability and reactivity and has been applied to labeling *in vitro* and even *in vivo*.[20]

Other alternatives have since emerged which focus on strain-promoted cycloadditions to reagents other than azides. These include a cycloaddition of norbornene to nitrile oxides to form isoxazolines,[21] inverse-electron demand Diels-Alder cycloadditions of tetrazines to norbornenes,[22] and tetrazines to fused cyclobutene-norbornene.[23] Cycloaddition of *trans*-cyclooctene **20** to tetrazines **21** (Scheme 4, A) proves to be the most rapid of the techniques involving strained alkenes[24] and has been explored in the fluorescent labeling of cell surface antigens and for single photon emission computed tomography imaging of antibodies (Scheme 4).[25] It has been shown that cyclooctynes (**17**) can also react very rapidly with nitrones (**24**), to form isoxazoles **25** (Scheme 4, B).[26] This is a field that is rapidly developing, which will hopefully result in a collection of reactions and reagents available for a wide variety of applications. It will then be a simple matter of choosing the appropriate one for the specific application in mind.
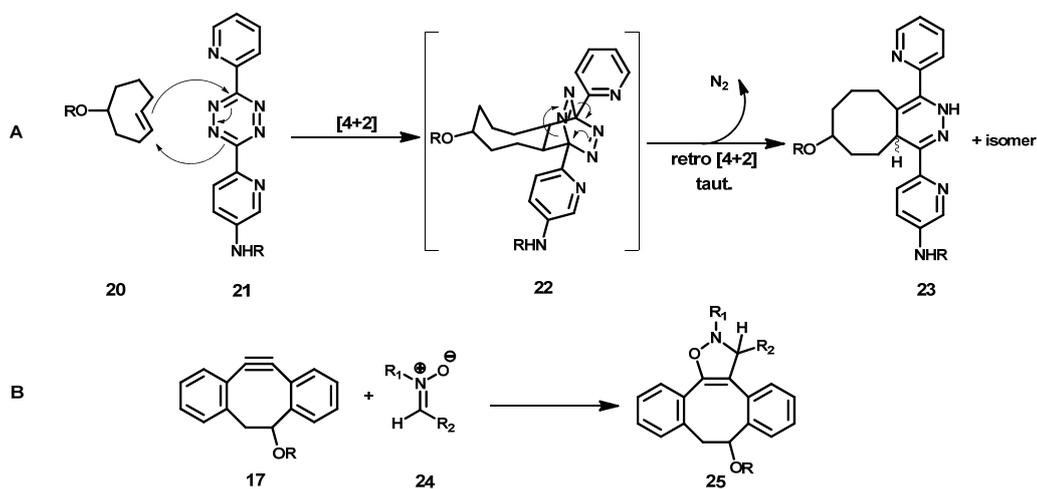
**Scheme 4** Alternative cycloadditions for rapid labeling

Copper-free 'click' chemistry could lend some advantages to the field of radiolabeling. Potential contamination of labeled compounds with traces of copper is a concern when the classic CuAAC is used to label biomolecules. Furthermore, as mentioned above, methodology for labeling by CuAAC is not amenable to extension to *in vivo* pretargeting methodology. To date, there has been one reported instance of radiochemical labeling of a peptide with [^{111}In] for SPECT using a strained cyclooctyne.[27] Given the high reaction rate of the strain-promoted azide-alkyne cycloaddition, it could be a useful means by which to label peptides with the short lived [^{18}F].

6.2 Goal

We envisioned the use of [lys3]-bombesin (see Chapter 5, Section 5.1), modified with a strained alkyne, to allow for rapid and facile labeling with [^{18}F] in the absence of possible copper contamination. A further advantage of this methodology would be the possibility to fine tune the properties of the resulting labeled peptide. The azide group can be designed to provide greater or lesser hydrophilicity, bulk or charge to the peptide in question. The stability and *in vitro* binding affinity of the resulting tracers were to be investigated.

6.3 Results and Discussion

Our starting point was to find a suitable strained alkyne with the optimal balance of reactivity and stability. Although, as aforementioned, many options are available, some initial synthetic endeavors demonstrated that the synthesis of cyclooctynes is not necessarily trivial, nor are all of the reported cyclooctynes of appropriate stability. Van Hest and van Delft reported an aza-dibenzocyclooctyne **16** (Scheme 3), which proved to be simultaneously reactive and stable.[28] For our purposes, which involve rapid 'clicking' of a short lived radioisotope as well as eventual *in vitro* and *in vivo* studies requiring a certain degree of stability, it appeared an ideal choice. The reported synthesis was followed the results of which are outlined in Scheme 5.
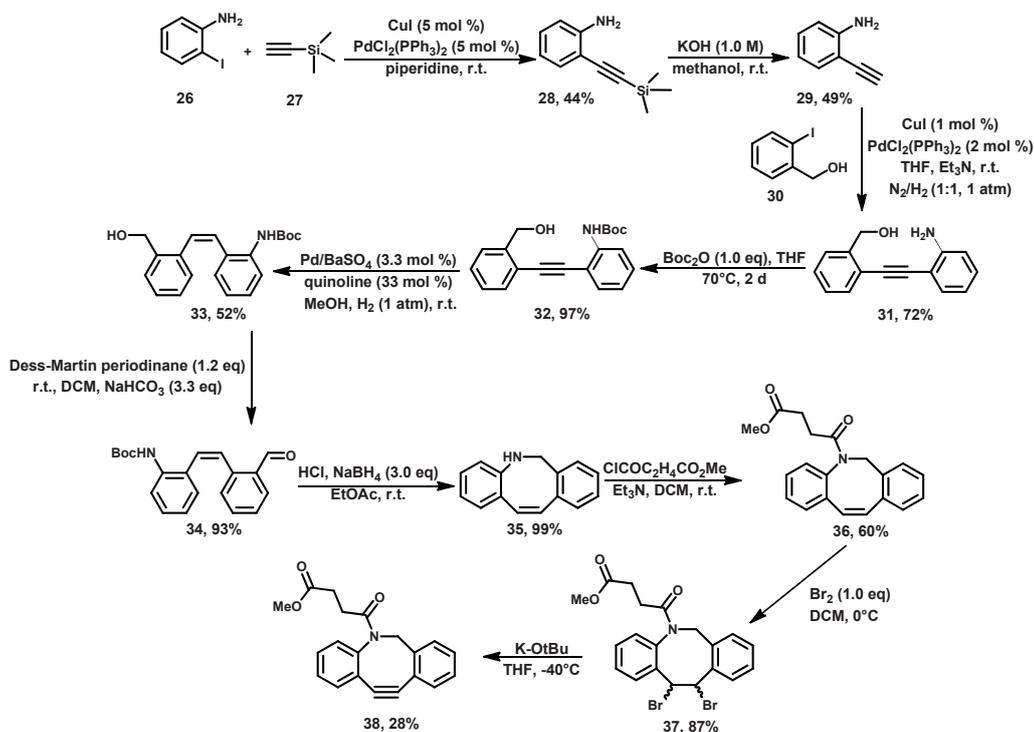
Scheme 5 Reported synthetic route to aza-dibenzocyclooctyne **38**[28]

The synthesis as shown in Scheme 5 differs from the published report in that compound **29** was prepared in two steps (Sonogoshira coupling and deprotection) rather than acquired commercially (1 g~55 €)[29] as was the case in the literature precedent.[28] A further difference is that rather than reacting **35** with glutaric acid monomethyl ester chloride as reported, we reacted it with methyl succinyl chloride, introducing a two carbon linker rather than a three carbon linker. Although in our hands, the yields proved to be somewhat lower than reported, the synthesis proceeded smoothly. Nonetheless, we envisioned an alternate, and shorter route. The retrosynthetic plan is outlined in Scheme 6.

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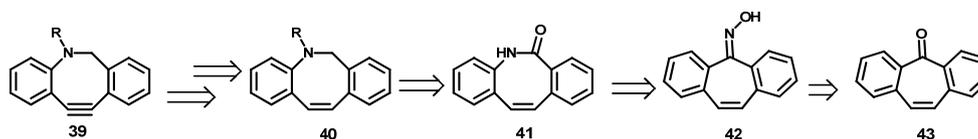
Scheme 6 Retrosynthetic route to molecule aza-dibenzocyclooctyne **39**

The aza-cyclooctyne **39** can be reached in two steps (bromination followed by elimination) from the corresponding alkene **40**. The key step of the synthesis is the transformation of oxime **42** into amide **41** via a Beckmann rearrangement (Scheme 7). Oxime **42** can be synthesized from dibenzosuberenone **43**.
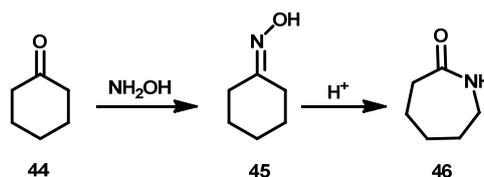
Scheme 7 Beckmann rearrangement

We started the synthesis from commercially available dibenzosuberenone (**43**), and oxime **42** was formed in 95% yield by refluxing **43** in the presence of hydroxylamine hydrochloride (Scheme 8). We initially intended to form the tosylate of **42** to provide a better leaving group for the subsequent Beckmann rearrangement, however, we were unable to form tosylate **47**. Treatment of **42** with TsCl in the presence of pyridine at reflux yielded none of **41**.[30] We also attempted the reaction in pyridine but with the addition of 1.0 eq of Et$_3$N but with no success.
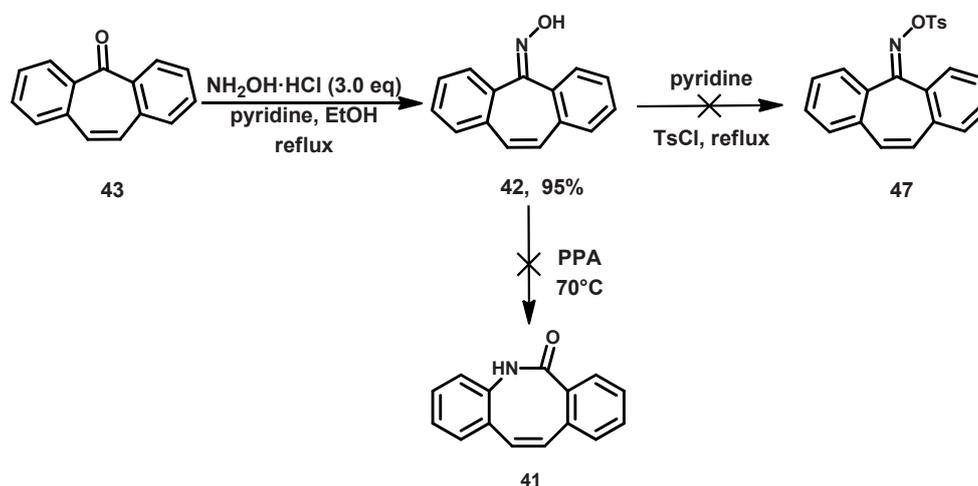
Scheme 8 Attempted reactions with oxime **42**

Treating **42** with polyphosphoric acid (PPA) at 70°C to instigate the Beckmann rearrangement also failed to give any of the expected product **41**.[31] Fortunately, treating **42** with trichlorotriazine (TCT) in dimethylformamide (DMF) at room temperature afforded amide **41** in 67% yield (Scheme 9).[32]
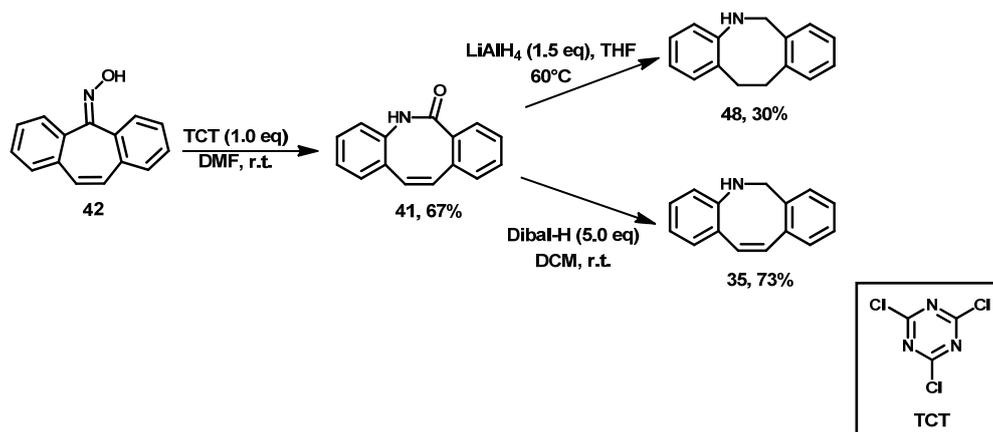

Scheme 9 Synthesis of amine **35**

The reduction of amide **41** was initially attempted using lithium aluminum hydride (LiAlH$_4$). However, at room temperature no reduction occurred, and heating the reaction mixture at 60°C reduced not only the amide, but also the alkene, affording dibenzoazocine **48** (Scheme 9). Reduction with diisobutylaluminum hydride (Dibal-H) however, gave the desired amine **35** in 73% isolated yield. Amine **35** is thus reached in three steps, rather than five, starting with the inexpensive precursor **43** (1g~1 €)[33] rather than 2-ethynylaniline **29** (1g~55 €)[29] as published (Scheme 5).[28]

A short linker can be introduced by reaction of **35** with methyl succinyl chloride (**49**) in the presence of triethylamine (Scheme 10). Subsequent bromination proceeds smoothly in 88% yield.
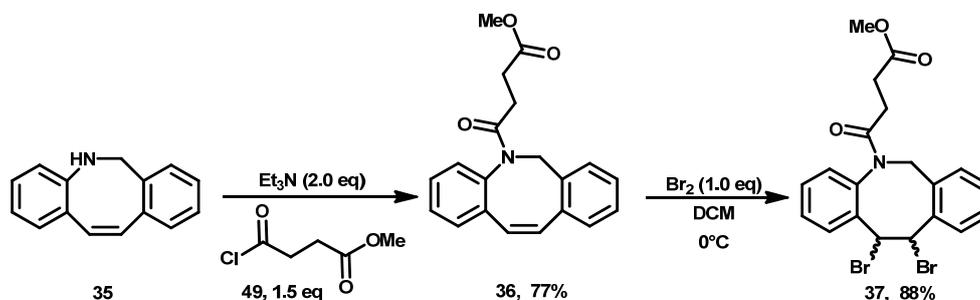
Scheme 10 Synthesis of **37**

The formation of the strained alkyne bond is the most sensitive step of the synthesis (Scheme 11). After significant optimization, it was possible to attain 65% of the desired aza-cyclooctyne **38** by treatment of **37** with a solution of potassium *tert*-butoxide in THF. Along with **38**, we also isolated the *tert*-butyl ester **50** in 22% yield. The two esters could be separated by column chromatography. Crucially, the reaction must be performed under an atmosphere of argon in freshly distilled THF, otherwise only trace amounts of **38** are produced, with the large majority of **37** converted in the mono-brominated alkene **51**.
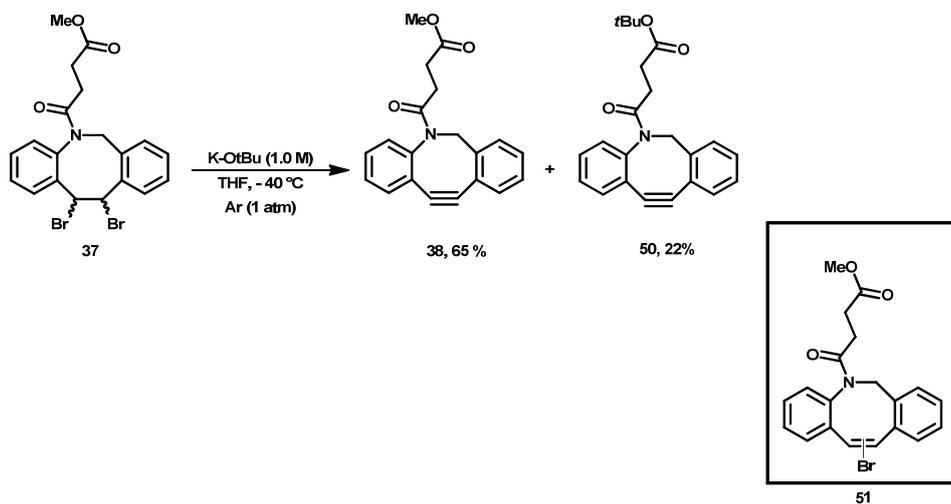
Scheme 11 Formation of Strained Alkyne **38**

To be able to attach the aza-cyclooctyne **38** to a target molecule, the ester needed to be hydrolyzed. Basic hydrolysis of **38** afforded carboxylic acid **52** in 90% yield (Scheme 12). To allow us to couple the strained alkyne to our peptide, we form the *N*-hydroxysuccinimide ester **53**.
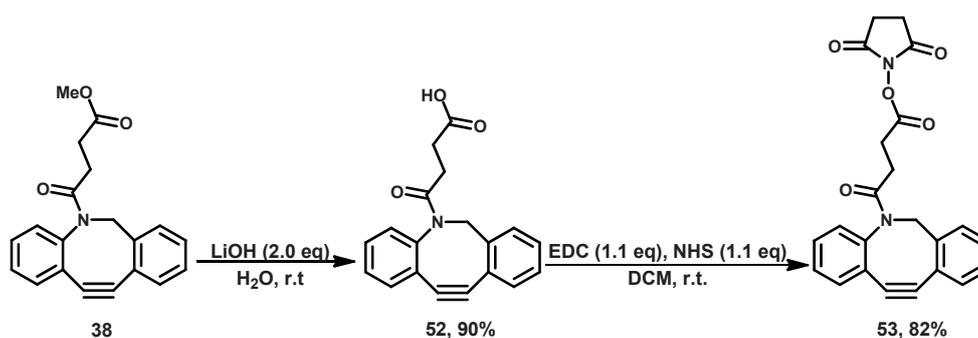
Scheme 12 Synthesis of succinimidyl ester **53**

We also attempted to hydrolyze *tert*-butyl ester **50** under various acidic and basic conditions (Table 1). Treating **50** with trifluoroacetic acid (TFA) led to a complex mixture

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of products with no remaining starting material (entry 1). Attempting hydrolysis with potassium hydroxide in THF gave no conversion (entry 2). Repeating the same experiment but with the addition of 18-crown-6 led to a complex mixture of products (entry 3). A final attempt was made using $CeCl_3 \cdot 7H_2O$ and NaI in refluxing acetonitrile, a reported procedure used for selective deprotection of *tert*-butyl esters in the presence of other acid labile protecting groups (entry 4).[34] However, this also failed to give product **52**.

Table 1 Attempted hydrolysis of *tert*-butyl ester **50**
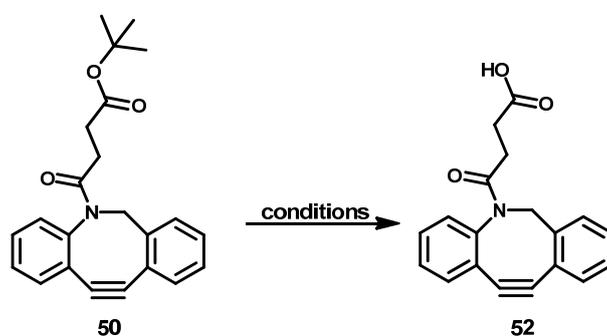
	Conditions	Result
1	TFA, DCM, r.t.	Complex mixture of products
2	KOH, THF, r.t.	Starting material only
3	KOH, 18-crown-6, THF, r.t.	Complex mixture of products
4	$CeCl_3 \cdot 7H_2O$ (1.5 eq), NaI, MeCN, reflux	Complex mixture of products

Of key importance to the field of radiochemistry is rapid conversion, allowing for the introduction of the short-lived radiolabel under biocompatible conditions. To test the reactivity of **38** in the desired time frame, it was reacted with the model compound *para*-fluorobenzyl azide to form triazole **54** (as a mixture of isomers 1:1) (Scheme 13).
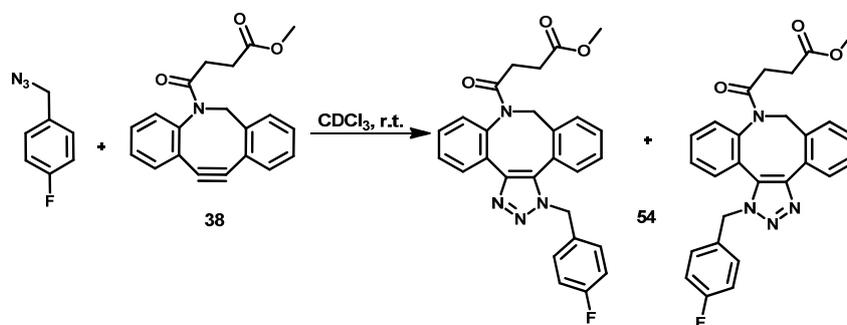
Scheme 13 Copper-free 'click' reaction of **53** with aza-dibenzocyclooctyne **38**

The reaction was performed in an NMR tube and followed in time by ^{1}H NMR. As shown in Figure 1, **38** reacted very rapidly to form triazole **54**.
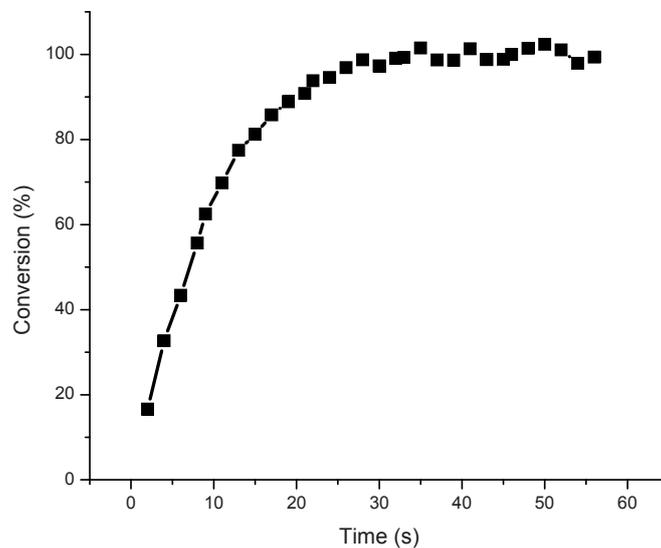
Figure 1 Conversion of **38** to **54** as a function of time (CDCl$_3$, r.t.)

 Before proceeding with the modification of bombesin, we tested the reactivity of the aza-benzocylooctyne **38** with several [^{18}F]-containing azides (Scheme 14).
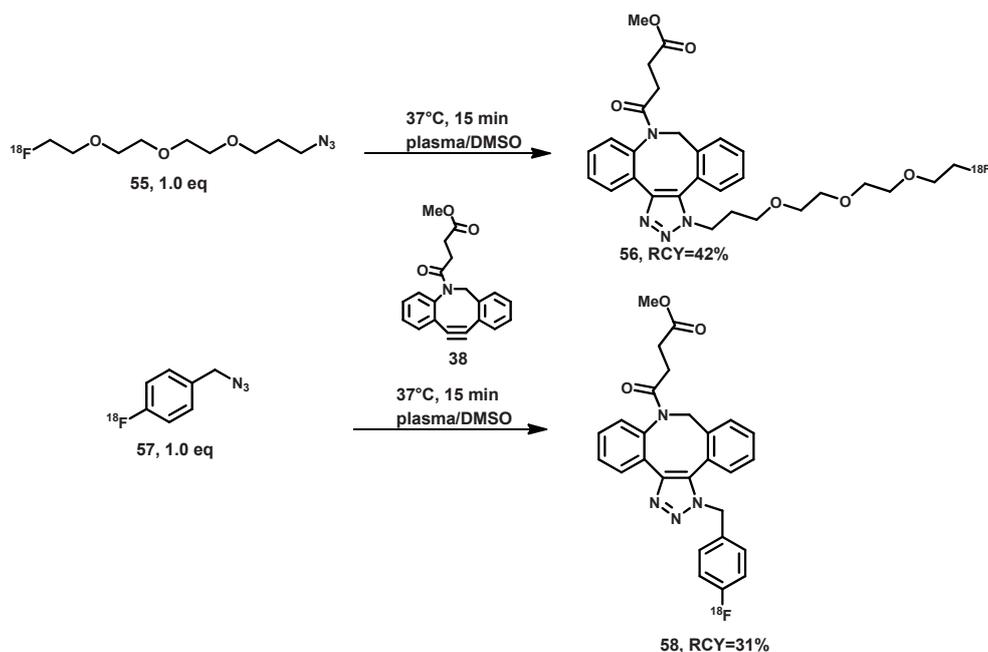

Scheme 14 Strain-promoted click chemistry for labelling with [^{18}F]

Strained alkyne **38** could be reacted with two [^{18}F]-labeled azides by simply stirring the two reactants together in a mixture of human plasma and DMSO. We found that alkyne **38** could be fully converted to the corresponding triazoles within 15 min (Scheme 14).[35] With the hydrophilic [^{18}F]-PEGylated azide **55**, triazole **56** was isolated with a radiochemical yield (RCY) of 42%. With the more lipophilic [^{18}F]-fluorobenzyl azide **57**, triazole **58** was isolated with an RCY of 31%. Furthermore, it is key to note that the reaction is performed in human plasma. Considering the eventual application of radiolabelling *in vivo*, it was important to confirm that the selected strained alkyne was not too fragile to withstand any exposure to a biological environment. Given that alkyne **38** could reach full conversion to triazoles in less than 15 min, we concluded that the reaction was fast enough for the desired time scale for labeling with [^{18}F].

Thus, succinimidyl ester **53** was conjugated to [lys3]-bombesin under basic conditions (Table 2). Full conversion to the product **Aza-DBCO-BN** (**59**) was achieved after 24 h when 5.0 eq of **53** were used with respect to [lys3]-BN in DMF along with 10.0 eq of DIPEA (entry 1). Using 2.0 eq of **53** resulted in 75% conversion after 24 h in DMF (entry

2) and performing the reaction in phosphate buffer (pH=8.1) resulted in no product formation after 24 h (entry 3) and apparent degradation of the starting material **53**. **Aza-DBCO-BN** was purified by RP-HPLC and characterized by mass spectrometry (ESI-MS).

Table 2 Modification of bombesin with **53**

[Lys3]-BN

conditions

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Aza-DBCO-BN (59)
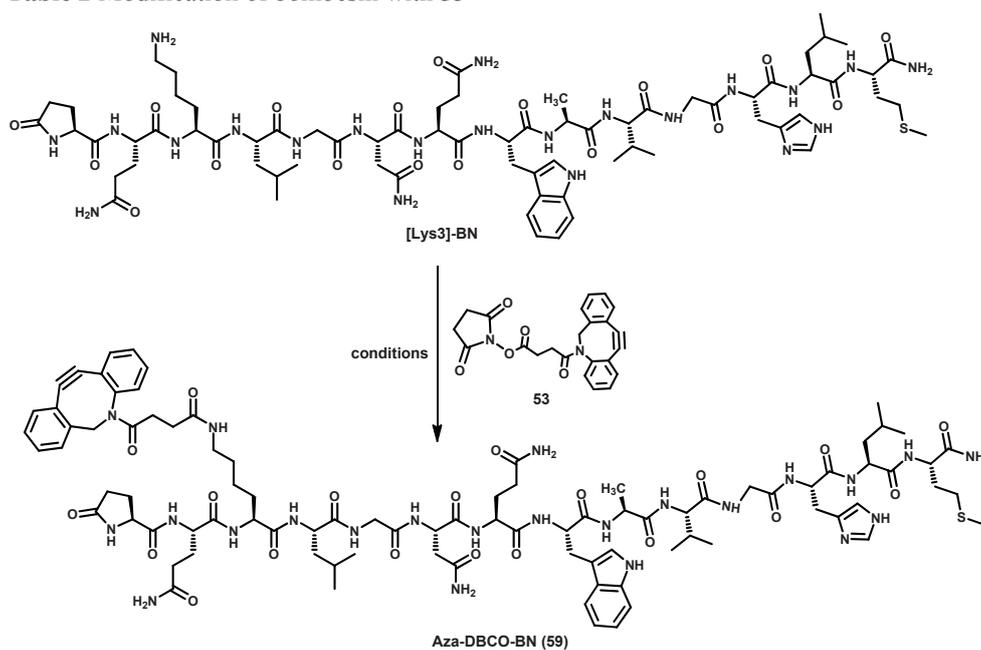
	Conditions	Conversion (Isolated Yield %)
1	5.0 eq **53**, DMF, 10.0 eq DIPEA, r.t., 24 h	100% (25)
2	2.0 eq **53**, DMF, 10.0 eq DIPEA, r.t., 24 h	75% (n.d.)
3	5.0 eq **53**, DMF, Pi buffer pH=8.1, r.t., 24 h	0% (n.d.)

With the target bombesin analogue modified with a strained alkyne, we tested the efficiency of the copper-free [3+2] cycloaddition with several [18F]-containing azides. Three [18F]-containing azides were selected to react with **Aza-DBCO-BN 59** (Scheme 15). As indicated, one advantageous aspect of this methodology is the ease with which the properties of the resulting peptidic tracer can be modified by simply changing the azides. [18F]-PEGylated azide **55**, [18F]-fluorobenzyl azide **57** and [18F]-fluoroazidobutane **65** were reacted with **Aza-DBCO-BN 59** at room temperature in DMF for 15 min, during which time complete conversion of the starting material in all cases could be detected by radio-

TLC to give triazoles [18F]-PEGTOxBN (**61**), [18F]-BnTOxBN (**63**) and [18F]-BuTOxBN (**66**), respectively.
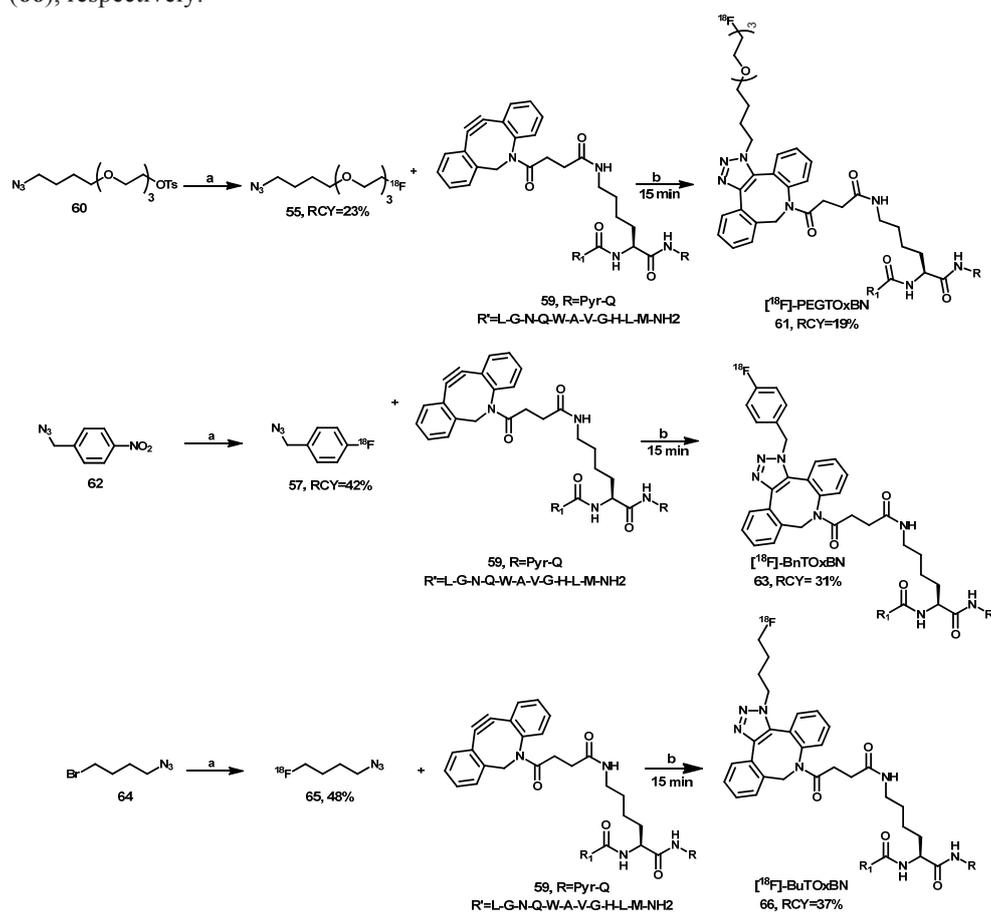
Scheme 15 Reagents and conditions: a) K[18F], MeCN, 110ºC; b) DMF, r.t.

The logarithmic partition coefficients (log P) is used to evaluate a compounds lipophilicity. It is the log of the partition coefficient, which is the ratio of a compound between two phases, typically one aqueous solvent (often water) and one hydrophobic solvent (often octanol).[36] The log P values were determined for all three tracers and were found to be -0.43, 1.27 and 0.26, respectively. This provides us with tracers ranging from the quite hydrophilic [18F]-PEGTOxBN (-0.43) to the more hydrophobic [18F]-BnTOxBN (1.27).

The binding affinity of all three tracers for gastrin-releasing peptide receptors was tested using PC3 human prostate cancer cells which overexpress GRPRs (Table 3).

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Table 3 *In vitro* binding affinity values

Tracer	IC$_{50}$
[^{18}F]-PEGTOxBN (61)	40 nM
[^{18}F]-BnTOxBN (63)	29 nM
[^{18}F]-BuTOxBN (66)	30 nM

The *in vitro* binding was determined by performing a competitive receptor binding assay with the receptor specific radioligand [^{125}I]-tyr[4]-bombesin (a displacement assay). The 50% inhibitory concentrations (IC$_{50}$) were determined to be 40 nM, 29 nM and 30 nM for **61**, **63**, and **66**, respectively. Comparing with the gold standard [^{125}I]-tyr[4]-bombesin, IC$_{50}$ of 56 nM, we conclude that all three tracers maintain high affinity for the GRPRs even post-modification and labeling. For a more detailed discussion see PhD thesis Leila Mirfeizi.

6.4 Alternate attempts at copper-free cycloadditions

We explored some alternative means by which to perform copper-free 'click' reactions. We considered the possibility of activating the alkyne by some means other than copper, ring strain, or electron withdrawing groups. Our first attempt was to use an iodine-mediated cycloaddition. The synthesis of isoquinolines has been described as proceeding via the iodine-mediated electrophilic cyclization of 2-alkynyl-1-methylene azide aromatics **67** (Scheme 16).[37] The proposed mechanism of this transformation involves coordination of I$^+$ to the triple bond (**68**), thereby activating it towards nucleophilic ring closure by the azide (**69**) (Scheme 14). Following ring closure, aromatization with the elimination of N$_2$ is undergone to form isoquinoline **70**.
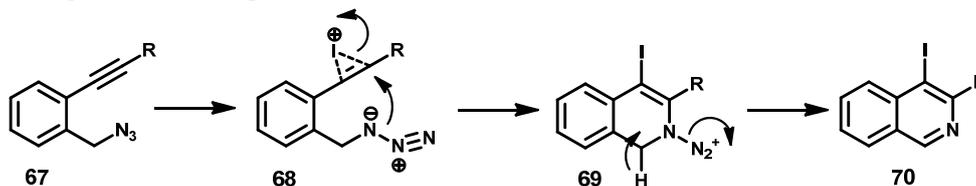
Scheme 16 Proposed mechanism for iodine-mediated electrophilic cyclization

We envisioned that a similar approach could be taken with an intermolecular system. We hoped that the iodonium would sufficiently activate the alkyne to allow for the cycloaddition with azide to occur. Although this system is no more biocompatible than is the copper-catalyzed system, we hoped in this way to be able to perform the 1,3-dipolar cycloaddition of azides and alkynes with internal alkynes. We attempted to synthesize triazoles **73** by treating a reaction mixture of benzyl azide **71** and various alkynes, both terminal and internal, with iodine (Table 3). Entry 1 shows the conditions optimized for the internal cyclization in the synthesis of isoquinolines as indicated in the literature.[34] Treating benzyl azide and phenylacetylene with an excess of iodine (5.0 eq) in DCM in the presence of sodium bicarbonate gave only bis-iodine **74**.[38] Replacing sodium bicarbonate with KI in a mixture of water/DCM also gave exclusively product **74** after 24 h (entry 2). Reducing the amount of iodine to 0.5 eq. in the absence of base gave no conversion (entry 3). Using an internal alkyne as a substrate, in the presence of iodine (5.0 eq) and the absence of base gave only bis-iodine **74** (entry 4). The addition of CuCl to the reaction mixture yielded the same result. With diphenylacetylene, no conversion was detected when it was treated with 5.0 eq of iodine in DCM, nor with the addition of KI in water (entries 6 and 7). Only starting material was recovered.

Table 3 Attempted iodine-mediated 1,3-dipolar cycloaddition of azides and alkynes
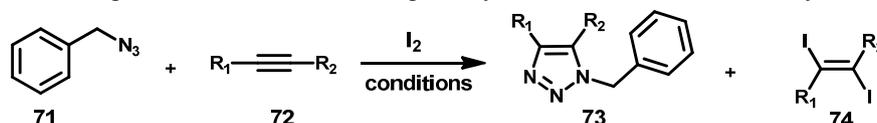
	R_1	R_2	Eq. of I_2	Base/Additives	Solvent	Product
1	Ph	H	5	NaHCO$_3$	DCM	74
2	Ph	H	5	KI	DCM/H$_2$O	74
3	Ph	H	0.5	-	DCM	-
4	Me	Me	5	-	DCM	74
5	Me	Me	5	CuCl	DCM	74
6	Ph	Ph	5	-	DCM	-
7	Ph	Ph	5	KI	H$_2$O	-

Since this approach proved unsuccessful, we also explored the use of water stable Lewis acids for the activation of alkynes as an alternative approach (Table 4).[39] Stirring benzyl azide **71** and phenylacetylene **75** in water in the presence of InCl$_3$ (1.0 eq), we were able to isolate 5% of triazole **76** after 45 h at room temperature (entry 2). Using Zn(NO$_3$)$_2$

as the Lewis acid gave similar results, yielding 5% of triazole **76** after 72 h at room temperature (entry 4). Sc(OTf)$_3$ showed no conversion to the product after 72 h (entry 5). A final example using CeCl$_3$·7H$_2$O (1.0 eq) as the Lewis acid gave 10% of triazole **76** after 72 h (entry 6).

Table 4 Lewis acid mediated 1,3-dipolar cycloaddition of azides and alkynes
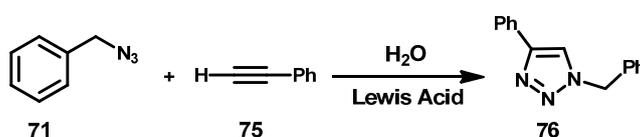
Entry	Lewis Acid	Solvent	Time	Yield
1	InCl$_3$ (1.0 eq)	H$_2$O	18 h, r.t.	-
2	InCl$_3$ (1.0 eq)	H$_2$O	45 h, r.t.	5%
3	Zn(NO$_3$)$_2$ (1.0 eq)	H$_2$O	18 h, r.t.	-
4	Zn(NO$_3$)$_2$ (1.0 eq)	H$_2$O	72 h, r.t.	5%
5	Sc(OTf)$_3$ (1.0 eq)	H$_2$O	72 h, r.t.	-
6	CeCl$_3$·7H$_2$O (1.0 eq)	H$_2$O	72 h, r.t.	10%

We also attempted the reaction using the internal alkyne, diphenylacetylene **77** (Scheme 17). Diphenylacetylene **77** was stirred with benzyl azide **71** in water in the presence of Zn(NO$_3$)$_2$ (1.0 eq). After 48 h, 10% of triazole **78** was isolated from the reaction mixture.
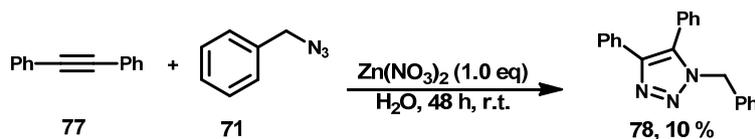
Scheme 17 Lewis acid catalyzed 1,3-dipolar cycloaddition of azides and internal alkynes

Thus it is possible to induce a [3+2] cycloaddition of an azide and an internal alkyne using a Lewis acid in water. Although the yield is low, optimization could potentially yield a useful system for accessing 1,4,5-substituted triazoles.

6.5 Conclusions

We were able to achieve rapid radiolabelling of bombesin with [^{18}F] using a very straightforward protocol. Simple stirring of the radionuclide-containing azide with the azo-dibenzocyclooctyne modified bombesin analogue for 10-15 min at room temperature suffices to reach the target peptides in modest to good yields. Furthermore, the azide can be readily varied, as we have shown, from a more lipophilic aromatic azide to a hydrophilic PEGylated azide. As a result, tracers with different properties are readily accessible from the same substrate allowing for rapid modification and fine tuning. In this way, the optimal lipophilicity for cellular uptake and metabolic clearance can be achieved. We have developed a simplified and relatively inexpensive route to the target aza-dibenzocyclooctyne **38**, hopefully rendering it accessible for future use in a clinical setting.

6.6 Future Perspectives

Although we describe herein the modification and labeling of [lys3]-bombesin and demonstrate that it maintains high affinity for the targeted receptors, ideally, this methodology would be extended to imaging making use of the technique of pretargeting.

For molecules with longer pharmacokinetics such as antibodies, and thus not amenable to the use of the short-lived [^{18}F], it would be highly advantageous to be able to administer the [^{18}F] radionuclide to the target *in vivo*. Antibodies have often been used to target diseases as they have the ability to bind selectively to tumor or other disease-associated antigens.[40] However, they are quite large molecules, which, in their natural role, remain for weeks in the bloodstream. This is a disadvantage for radionuclide imaging, as specific uptake of the labelled antibody is often masked by a large amount of radioactivity in the blood stream. Pretargeting is a method which involves the introduction of the unlabelled antibody, followed by an appropriate time interval allowing the antibody to reach its target antigen.[40] This is followed by injection with the second (radiolabeled) component, which ideally, will recognize, or attach to the antibody. In this way, superior image contrast can be achieved by increasing the target/non-target ratio. If the strain-promoted azide-alkyne cycloaddition methodology could be extended to antibodies, the use of radionuclides for imaging such targets will not be limited to the longer-lived metallic radioisotopes, and higher resolution images using [^{18}F], and better target/non-target ratios can be achieved.

Pretargeting is a technique that holds a lot of promise for improved imaging protocols.[40] The use of chemical reporters for pretargeting is an area very much limited by the scarcity of reactions that are bioorthogonal and sufficiently robust and selective to perform *in vivo* labelling. Strain promoted cycloadditions of azides and alkynes is one reaction that has proven to be amenable to *in vivo* labelling, and as such, is a very promising lead for pretargeting methodological development.

6.7 Experimental Section

General

The solution of KO*t*Bu in THF (1.0 M) should be purchased rather than prepared in the lab, otherwise full conversion is not reached (Sigma-Aldrich product 328650). Reversed phase-HPLC analyses were performed on a Shimadzu LC-20AD VP, Waters Xterra MS C18 column (3.0 x 150 mm, particle size 3.5 μm) using a gradient of MeCN/H_2O (0.1% formic acid). Gradient: from 95:5 at 0 min to 85:15 at 10 min, to 75:25 at 12 min, to 20:80 at 65 min, to 95:5 at 70 min. Flow 0.5 mL/min.

Safety

Working with azides should always be done carefully. Organic azides, particularly those of low molecular weight, or with high nitrogen content, are potentially explosive. Heat, light and pressure can cause decomposition of the azides. Furthermore, the azide ion is toxic, and sodium azide should always be handled while protected with gloves. Heavy metal azides are particularly unstable, and may explode if heated or shaken.

### Characterization of substrates and reference compounds

5*H*-Dibenzo[7]annulen-5-one oxime (42)
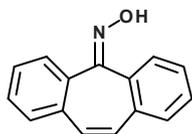
A solution of hydroxylamine was prepared by dissolving 15.6 g (0.22 mol, 3.1 eq) of $NH_2OH \cdot HCl$ in a hot mixture of absolute alcohol (100 mL) and pyridine (75 mL). To this solution was added 15.0 g (0.073 mmol, 1.0 eq) of dibenzosuberenone 43 and 20.0 mL of pyridine. The reaction mixture was heated at reflux for 3 h, and the disappearance of starting material was monitored by thin layer chromatography. After completion of the reaction, the solvent was evaporated under reduced pressure, and the product was precipitated with water. The solid was filtered, washed with water (3 x 50 mL), dissolved in chloroform, and the organic layer was washed one further time with water (10 mL). The organic layer was dried over $MgSO_4$ and the solvent evaporated to yield a pale yellow solid (15.3 g). Yield=95%. mp 187ºC. ^{1}H NMR (400 MHz, CDCl$_3$): δ 10.1 (s, 1H), 7.64-7.68 (m, 1H), 7.55-7.59 (m, 1H), 7.28-7.37 (m, 6H), 6.86 (dd, *J*=28.0, 4.0 Hz, 2H); ^{13}C NMR (100.59 MHz, CDCl$_3$): 156.3, 135.4, 134.5, 133.8, 130.8, 130.6, 130.5, 129.4, 129.2, 129.1, 128.9 (2C), 128.8, 127.8, 127.6. HRMS (ESI+) (m/z) calculated for $C_{15}H_{12}NO$ [M + H]$^+$ 222.0913, measured 222.0903.

Dibenzo[b,f]azocin-6(5H)-one (41)
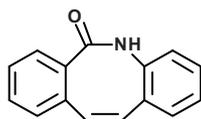
Trichlorotriazine (834 mg, 4.52 mmol) was added to DMF (1.0 mL) in a sample vial. The solution was stirred, and white precipitate formed. The formation of the catalyst was monitored by thin layer chromatography until all of the TCT had been consumed. To this solution was added oxime 42 along with DMF (10.0 mL). The reaction mixture was stirred at room temperature for 24-72 h (depending on the amount of oxime in a given reaction). More DMF was added as needed (depending on the scale of the reaction to maintain solubility). The reaction was quenched with water and DCM was added to the solution. The organic phase was washed with saturated aqueous Na_2CO_3 (2 x 10 mL), 1 N aqueous HCl (2 x 10 mL) and brine (2 x 10 mL). The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude reaction mixture was purified by column chromatography (3:1 pentane:ethyl acetate, R_f: 0.65) to give a pale yellow solid (650 mg). Yield=65%. mp 141-142°C. ^{1}H NMR (400 MHz, CDCl$_3$): δ 8.76 (s, 1 H), 7.70-7.72 (m, 1H), 7.57-7.60 (m, 1H), 7.42-7.51 (m, 6H), 6.96 (s, 2H); ^{13}C NMR (100.59 MHz, CDCl$_3$):163.5, 134.3, 133.0, 130.4, 130.2, 129.7, 129.5, 129.2, 129.0, 128.7, 128.5, 128.0, 127.7. HRMS (ESI+) (m/z) calculated for $C_{15}H_{12}NO$ [M + H]$^+$ 222.0913, measured 222.0901.

5,6-Dihydrodibenzo[b,f]azocine (35)
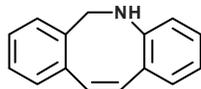
Amide 41 (2.00 g, 9.0 mmol) was dissolved in dry CH_2Cl_2 (25 mL) and 45 mL of a 1.0 M solution of Dibal-H in CH_2Cl_2 was added dropwise to the solution at room temperature with stirring. The reaction mixture was stirred under N_2 at room temperature and the conversion followed by thin layer chromatography until all of the starting material was consumed. The reaction was then carefully quenched with an aqueous solution of ammonium chloride. An aqueous solution of Rochelle salts (25 mL) was subsequently added and the mixture was stirred vigorously for 45 min. A further 50 mL of DCM was added and the organic layer collected and washed with brine. After drying over $MgSO_4$ the solvent was removed under reduced pressure to yield a yellow oil which was purified by column chromatography (2:1 pentane:ethyl acetate, R_f: 0.8). The resulting compound was a yellow solid (1.40 g). Yield=75%. ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.24-7.62 (m, 1 H), 7.16-7.24 (m, 3H), 6.97 (d, J=8.0 Hz, 1H), 6.88 (t, J=8.0 Hz, 1H), 6.60 (t, J=7.2 Hz, 1H), 6.54 (d, J=12.8 Hz, 1H), 6.47 (d, J=8.4 Hz, 1H), 6.36 (d, J=13.2 Hz, 1H), 4.59 (s, 2H), 4.29 (br s, 2H); ^{13}C NMR (100.59 MHz, CDCl$_3$): 147.1, 138.1, 136.2, 134.7, 132.7, 130.1, 128.8, 127.9, 127.6, 127.4, 127.3, 121.7, 117.9, 117.7, 49.6.

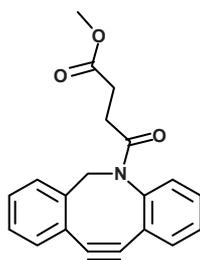**Methyl 4-(dibenzo[b,f]azocin-5(6H)-yl)-4-oxobutanoate (36)**
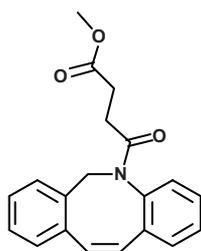
Amine 35 (2.00 g, 9.65 mmol) was dissolved in dry DCM (25.0 mL) under a N_2 atmosphere. To the stirred solution was added triethylamine (2.67 mL, 19.3 mmol) and the mixture was cooled in an ice bath, whereupon methyl succinyl chloride (1.78 mL, 14.4 mmol) was added dropwise. The reaction mixture was allowed to warm to room temperature, and stirred overnight. The reaction mixture was quenched with water and the mixture diluted with a further 20 mL of DCM. The organic layer was washed with 2 M aqueous NaOH (2 x 15 mL), 2 M aqueous HCl (2 x 15 mL), water (2 x 15 mL) and brine (1 x 10 mL). The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The resulting crude product was purified by column chromatography (4:1 pentane:ethyl acetate, R_f: 0.4) to yield a yellow-white solid (2.70 g). Yield=87%. mp 108ºC. ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.24-7.26 (m, 5H), 7.12-7.16 (m, 3H), 6.79 (d, *J*=17.2 Hz, 1H), 6.61 (d, *J*=17.2 Hz, 1H), 5.51 (d, *J*=20.0 Hz, 1H), 4.25 (d, *J*=20.8 Hz, 1H), 3.61 (s, 3H), 2.55-2.60 (m, 1H), 2.39-2.48 (m, 2H), 1.99-2.10 (m, 1H); ^{13}C NMR (100.59 MHz, CDCl$_3$): 177.4, 170.8, 140.5, 136.4, 135.8, 134.5, 132.6, 131.8, 130.8, 130.1, 128.5, 128.2, 128.0, 127.2, 126.9, 54.4, 51.6, 29.5, 29.0. HRMS (ESI+) (m/z) calculated for $C_{20}H_{19}NO_3$ $[M + Na]^+$ 344.1257, measured 344.1250.

Methyl 4-(11,12-dibromo-11,12-dihydrodibenzo[b,f]azocin-5(6H)-yl)-4-oxobutanoate (37)
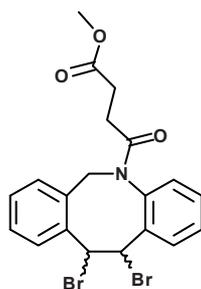
Compound 36 (1.87 g, 5.82 mmol) was dissolved in dry CH_2Cl_2 (100 mL) under a N_2 atmosphere and the reaction vessel was cooled in an ice bath. Br_2 (0.93 g, 5.82 mmol) dissolved in 5.0 mL of dry CH_2Cl_2 was added dropwise to the cooled solution and the reaction mixture was allowed to stir for 1 h. The reaction was quenched with aqueous saturated Na_2SO_3 (10 mL) and the mixture diluted with a further 50 mL of CH_2Cl_2. The organic layer was washed with saturated aqueous Na_2SO_3 (3 x 15 mL), water (2 x 15 mL) and brine (1 x 15 mL). The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The compound was purified by column chromatography (3:1 pentane:ethyl acetate, R_f: 0.3) to yield a dark solid (2.46 g). Yield=88%. mp 108ºC. ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.70 (d, *J*=7.6 Hz, 1H), 7.00-7.25 (m, 6H), 6.86 (d, *J*=7.6 Hz, 1H), 5.90 (d, *J*=9.6 Hz, 1H), 5.80 (d, *J*=14.8 Hz, 1H), 5.15 (d, *J*=10.0 Hz, 1H), 4.17 (d, *J*=14.8 Hz, 1H), 3.66 (s, 3H), 2.80-2.86 (m, 1H), 2.57-2.64 (m, 2H), 2.43-2.55 (m, 1H); ^{13}C NMR (100.59 MHz, CDCl$_3$): 173.5, 172.0, 138.3, 137.0, 136.9, 132.8, 130.8, 130.7, 130.6,
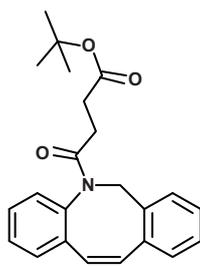
129.6, 129.5, 128.9, 128.8, 128.6, 60.0, 55.5, 52.5, 51.7, 30.6, 29.2. HRMS (ESI+) (m/z) calculated for $C_{20}H_{19}Br_2NO_3$ [M + Na]$^+$ 503.9603, measured 503.9606.

Methyl 4-(11,12-didehydrodibenzo[b,f]azocin-5(6H)-yl)-4-oxobutanoate (38)

To a cold solution (-40 ºC) of compound 37 (245.0 mg, 0.512 mmol) dissolved in 17.0 mL freshly distilled THF under Ar atmosphere was added dropwise 1.02 mL of a commercial solution of *t*BuOK (1.0 M in THF). The progress of the reaction was monitored by GC-MS. After 3 h, a further 0.4 mL of *t*BuOK solution was added and the mixture left to react for a further hour. The mixture was poured onto water (15 mL) and extracted with CH_2Cl_2 (3 x 30 mL). The combined organic layers were washed with brine (2 x 25 mL) and water (1 x 10 mL). A mixture of methyl ester, and *tert*-butyl ester products were detected in the crude ^{1}H NMR. The desired methyl ester product was isolated by column chromatography (3:1 pentane:ethyl acetate, R_f: 0.2) to give a clear yellow oil (110 mg). Yield=67%. ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.68 (d, *J*=7.2 Hz, 1H), 7.48-7.50 (m, 1H), 7.27-7.49 (m, 6H), 5.16 (d, *J*=14.0 Hz, 1H), 3.67 (d, *J*=13.6 Hz, 1H), 3.56 (s, 3H), 2.68-2.74 (m, 1H), 2.58-2.63 (m, 1H), 2.35-2.38 (m, 1H), 1.93-1.97 (m, 1H); ^{13}C NMR (100.59 MHz, CDCl$_3$): δ 173.3, 171.7, 151.4, 148.0, 132.3, 129.3, 128.8, 128.5, 128.1, 127.7, 127.1, 125.5, 123.1, 122.6, 114.9, 107.7, 55.4, 51.6, 29.6, 29.0. HRMS (ESI+) (m/z) calculated for $C_{20}H_{17}NO_3$ [M + Na]$^+$ 342.1101, measured 342.1102.

tert-Butyl 4-(11,12-didehydrodibenzo[b,f]azocin-5(6H)-yl)-4-oxobutanoate (50)

To a cold solution (-40 ºC) of compound 37 (245.0 mg, 0.512 mmol) dissolved in 17.0 mL freshly distilled THF under Ar atmosphere was added dropwise 1.02 mL of a commercial solution of *t*BuOK (1.0 M in THF). The progress of the reaction was monitored by GC-MS. After 3 h, a further 0.4 mL of *t*BuOK solution was added and the mixture left to react for a further hour. The mixture was poured onto water (15 mL) and extracted with CH_2Cl_2 (3 x 30 mL). The combined organic layers were washed with brine (2 x 25 mL) and water (1 x 10 mL). A mixture of methyl ester, and *tert*-butyl ester products were detected in the crude ^{1}H NMR. The desired methyl ester product was isolated by column chromatography (3:1 pentane:ethyl acetate, R_f: 0.5) to give a clear yellow oil (109.5 mg). Yield=22%. ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.67 (d, *J*=7.2 Hz, 1H), 7.10-7.35 (m, 7H), 5.15 (d, *J*=14.0 Hz, 1H), 3.64 (d, *J*=14.0 Hz, 1H), 2.62-2.66 (m, 1H), 2.44-2.50 (m, 1H), 2.25-2.31 (m, 1H), 1.93-1.97 (m, 1H), 1.31 (s, 9H); ^{13}C NMR (100.59 MHz, CDCl$_3$): δ 171.9, 171.7, 151.5, 148.1, 132.3, 129.2, 128.4, 128.0, 127.6, 127.0, 125.4, 123.2, 122.6, 114.9, 107.7, 80.2, 55.4, 44.4,

30.4, 22.9, 27.9. HRMS (ESI+) (m/z) calculated for $C_{23}H_{23}NO_3Na$ [M + Na]$^+$ 384.1570, measured 384.1576.

4-(11,12-Didehydrodibenzo[b,f]azocin-5(6H)-yl)-4-oxobutanoic acid (52)
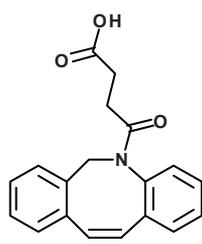
Compound 38 (42.5 mg, 0.13 mmol) was dissolved in 1.7 mL dry THF. A solution of LiOH (6.40 mg, 0.26 mmol) in 0.6 mL H_2O was added dropwise to the stirred reaction mixture. The progress of the reaction was monitored by thin layer chromatography and upon full conversion, a further 6.0 mL of H_2O was added. The reaction mixture was then made basic to a pH of 14 using 2 M aqueous NaOH. The aqueous layer was washed with CH_2Cl_2 (3 x 10 mL) and then acidified to a pH of 2 using 2 M aqueous HCl. The aqueous layer was then extracted with CH_2Cl_2 (3 x 15 mL) and the resulting organic layers of this extraction procedure were combined, dried over $MgSO_4$ and the solvent was removed under reduced pressure. Pure product was obtained as a white solid (30.6 mg). Yield= 77%. mp 163-164°C. ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.67 (d, J=7.2 Hz, 1H), 7.25-7.43 (m, 7H), 5.16 (d, J=13.6 Hz, 1H), 3.67 (d, J=13.6 Hz, 1H), 2.68-2.74 (m, 1H), 2.56-2.63 (m, 1H), 2.32-2.40 (m, 1H), 1.94-2.00 (m, 1H); ^{13}C NMR (100.59 MHz, CDCl$_3$): δ 177.8, 172.6, 151.2, 147.9, 132.5, 129.3, 128.7, 128.5, 128.1, 127.6, 127.4, 125.8, 123.2, 122.9, 115.3, 107.7, 55.9, 29.7, 29.6. HRMS (ESI+) (m/z) calculated for $C_{19}H_{15}NO_3$ [M + Na]$^+$ 328.0944, measured 328.0949.

2,5-Dioxopyrrolidin-1-yl 4-(didehydrodibenzo[b,f]azocin-5(6H)-yl)-4-oxobutanoate (53)
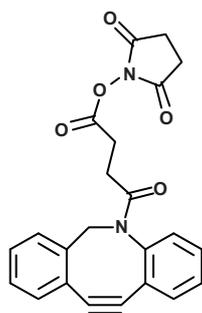
Carboxylic acid 52 (26.0 mg, 0.085 mmol) was dissolved in 5.0 mL dry CH_2Cl_2. To this solution was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (0.02 mL, 0.094 mmol) and *N*-hydroxysuccinimide (10.8 mg, 0.094 mmol). The reaction mixture was allowed to stir overnight at room temperature after which time it was diluted with a further 10 mL of CH_2Cl_2. The reaction mixture was washed with citric acid (5 %, 2 x 5 mL) and with saturated aqueous NaHCO$_3$ (2 x 5 mL) and brine (1 x 10 mL). The compound was then purified by column chromatography (1:1 pentane:ethyl acetate, R$_f$: 0.5) to yield the pure compound as a yellow oil (28.0 mg). Yield=82%. ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.68 (d, J=7.6 Hz, 1H), 7.24-7.41 (m, 7H), 5.17 (d, J=14.0 Hz, 1H), 3.69 (d, J=14.0 Hz, 1H), 2.92-2.99 (m, 1H), 2.72-2.77 (m, 1H), 2.78 (s, 4H), 2.61-2.68 (m, 1H), 2.05-2.10 (m, 1H). ^{13}C NMR (100.59 MHz, CDCl$_3$): δ 170.2, 168.9, 168.3, 151.0, 147.8, 132.3, 129.1, 128.6, 128.3, 127.8, 127.2, 125.5, 123.0,

122.7, 115.0, 107.5, 55.6, 29.2, 26.4, 25.5. HRMS (ESI+) (m/z) calculated for $C_{23}H_{18}N_2O_5$ [M + Na]$^+$ 425.1108, measured 425.1121.

1-(Azidomethyl)-4-fluorobenzene
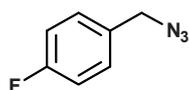
To a stirred solution of 1-(bromomethyl)-4-fluorobenzene (472.6 mg, 2.5 mmol) in a water/acetone mixture (1:4) was added NaN$_3$ (1.5 eq). The resulting suspension was stirred at room temperature for 24 h. DCM was added to the mixture and the organic layer was separated. The aqueous layer was extracted with DCM (3 x 10 mL) and the combined organic layers were dried over MgSO$_4$. Solvent was removed under reduced pressure to give the product as a pale yellow oil, sufficiently pure to use without further purification (374.0 mg). Yield= 99%. Spectroscopic data is in accordance with literature values.[41] ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.27-7.39 (m, 2H), 7.00-7.11 (m, 2H), 4.30 (s, 2H); ^{13}C (100.59 MHz, CDCl$_3$): δ 162.5 (d, J=130.7 Hz), 131.4, 129.9 (d, J= 45.2 Hz), 115.7 (d, J=110.0 Hz), 54.0; ^{19}F NMR (200 MHz, CDCl$_3$): δ -112.3.

Methyl 4-(1-(4-fluorobenzyl)-1H-dibenzo[b,f][1,2,3]triazolo[4,5-d]azocin-8(9H-yl)-4-oxobutanoate (54)
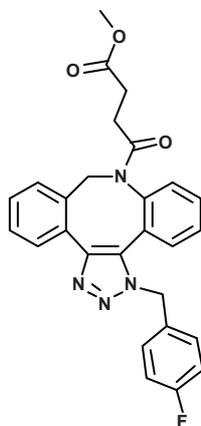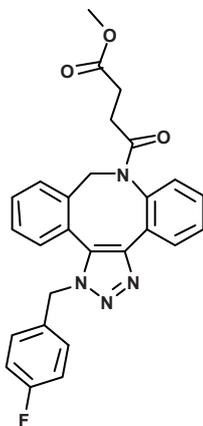
To a solution of aza-dibenzocyclooctyne 38 (80.0 mg, 0.25 mmol) dissolved in 5.0 mL CH$_2$Cl$_2$ was added 1-(azidomethyl)-4-fluorobenzene (57.0 mg, 0.38 mmol). The reaction mixture was allowed to stir for 1 h at room temperature, after which the solvent was evaporated and the crude product was purified by column chromatography (1:1 pentane:ethyl acetate) to yield the product as a white solid (94.1 mg). Yield=80%. Two isomers are formed in a 1:1 ratio as determined by ^{1}H NMR. ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.67-7.73 (m, 1H), 7.44-7.49 (m, 2H), 7.38-7.42 (m, 1H), 7.24-7.31 (m, 1H), 7.17-7.24 (m, 2H), 6.93-7.10 (m, 5H), 5.99 (d, J=16.9 Hz, 1H), 5.58 (s, 2H), 4.33 (d, J=16.9 Hz, 1H), 3.60 (s, 3H), 2.44 (m, 1H), 2.23 (m, 1H), 2.09 (m, 1H), 1.80 (m, 1H). ^{13}C NMR (100.59 MHz, CDCl$_3$): δ173.2, 171.3, 163.7, 161.3, 143.1, 140.0, 135.9, 134.9, 131.8, 131.2, 130.7, 128.9, 129.6, 129.3, 129.1, 129.0, 127.9, 127.1, 124.3, 116.0, 115.8, 52.0, 51.6, 51.4, 29.2, 28.9. HRMS (ESI+) (m/z) calculated for $C_{27}H_{23}N_4O_3F$ [M + H]$^+$ 471.1827, measured 471.1789; (ESI+) (m/z) calculated for $C_{27}H_{23}N_4O_3F$ [M + Na]$^+$ 493.1646, measured 493.1606

Peptide Chemistry
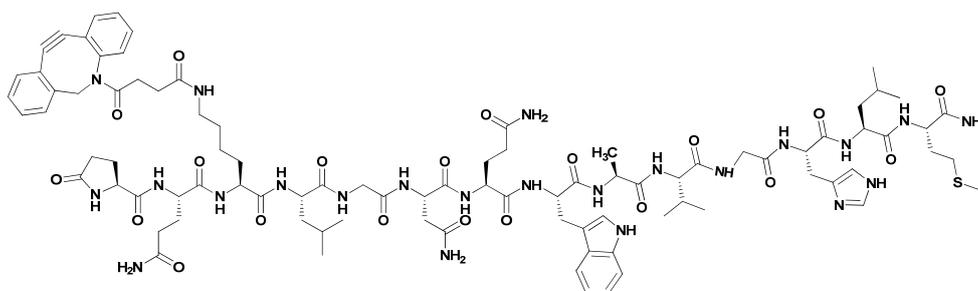
Aza-DBCO-BN (59)

[Lys3]-bombesin (0.18 mg, 1.0 eq) was weighed into a 2.0 mL Eppendorf tube along with 53 (0.5 mg, 5.0 eq). 200 μL of dry DMF and 10.0 eq of diisopropylethyl amine were added and the resulting solution was stirred at room temperature for 24 h. The solvent was removed by lyophilization. Full conversion of [lys3]-bombesin could be observed by RP-HPLC. The product was purified by preparative RP-HPLC yielding Aza-DBCO-BBN in 25% yield. HRMS (ESI+) (m/z) calculated for $C_{90}H_{123}N_{23}O_{20}S$ [M + H]$^+$ 1878.9108, measured 1878.9078. Retention time=32.0 min.

Radiochemistry General

[^{18}F] fluoride was obtained by proton bombardment of an [^{18}O] enriched water target via the ^{18}O(p,n)[^{18}F] reaction. The radioactivity was trapped by passing the target water through a preactivated Sep-Pak light QMA cartridge (Waters). A 1 mL H_2O solution of K_2CO_3 (4.5 mg) and Kryptofix 222 (20 mg) was used to elute the [^{18}F]-fluoride from the cartridge into a conical glass vial. This eluate was evaporated to dryness by three consecutive azeotropic distillations after with acetonitrile (3 × 500 μL) under a gentle stream of nitrogen gas (130°C). Analytical as well as semipreparative RP-HPLC was performed for monitoring and purification. Isolation of radiolabeled peptides was performed using a reversed-phase RP-C18 column (4.6 mm × 250 mm, 10 μm). The flow was set at 2.5 mL/min using a gradient system starting from 90% solvent A (0.01 M phosphate buffer, pH=6.0) and 10% solvent B (acetonitrile) (0-2 min) and ramped to 45% solvent A and 55% solvent B at 35 min. The analytic HPLC was performed using the same gradient system but with a reversed-phase Grace Smart RP-C18 column (4.6 mm × 250 mm, 5 μm) and a flow of 1 mL/min.

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Synthesis and radiolabelling

The reaction with cyclooctyne modified bombesin was performed in DMF at room temperature and proceeded to completion in 15 min. The resulting tracer was also purified by RP-HPLC yielding the desired triazole tracers: [^{18}F]-BnTOxBN 55 (retention time=16 min), [^{18}F]-BuTOxBN 58 (retention time=19 min) and [^{18}F]-PEGTOxBN 53 (retention time=22 min) with radiochemical yields of 31%, 37% and 19%, respectively. The specific activities were 62 GBq/μmol, 57 GBq/μmol, 60 GBq/μmol.

Cell culture: The GRPR-positive PC-3 human prostate cancer cell line (ATCC, Manassas, Virginia, USA) was cultured at 37°C in a humidified 5% CO_2 atmosphere. The cells were cultured in RPMI 1640 (Lonza, Verviers, France) supplemented with 10% fetal calf serum (Thermo Fisher Scientific Inc., Logan, Utah, USA) and subcultured twice a week after detaching with trypsin-EDTA.

In Vitro Competitive Receptor-Binding Assay: *In vitro* GRPR binding affinities and specificities of the tracers were assessed via a competitive displacement assay Experiments were performed with PC-3 human prostate cancer cells according to a method previously described.[42] The 50% inhibitory concentration (IC$_{50}$) values were calculated by fitting the data with nonlinear regression using GraphPad Prism 5.0 (GraphPad Software, San Diego, California, USA). Experiments were performed with triplicate samples. Results were plotted in sigmoidal curves for the displacement of [^{18}F]-BnTOxBN, [^{18}F]-BuTOxBN and [^{18}F]-PEGTOxBN as a function of increasing concentration of BN(1-14). The tracers displayed high affinity for binding to GRPRs within PC-3 cell with IC$_{50}$ values of 29 nM , 30 nM and 40 nM for [^{18}F]-BnTOxBN, [^{18}F]-BuTOxBN and [^{18}F]-PEGTOxBN, respectively.

Octanol/Water Partition Coefficient Study. Water partition coefficients were determined at pH =7.4. 5 μL containing 500 kBq of the radiolabeled compound in PBS was added to a vial containing 1.2 mL 1-octanol and PBS (1:1). After vortexing for 1 min, the vial was centrifuged for 5 min at 10 000 rpm to ensure complete separation of layers. Then, 40 μL of each layer was taken in a pre-weighed vial and measured in the γ-counter. Counts per unit weight of sample were calculated. The lop P values were found to be 1.27, 0.26 and -0.43 for [^{18}F]-BnTOxBN, [^{18}F]-BuTOxBN and [^{18}F]-PEGTOxBN, respectively.
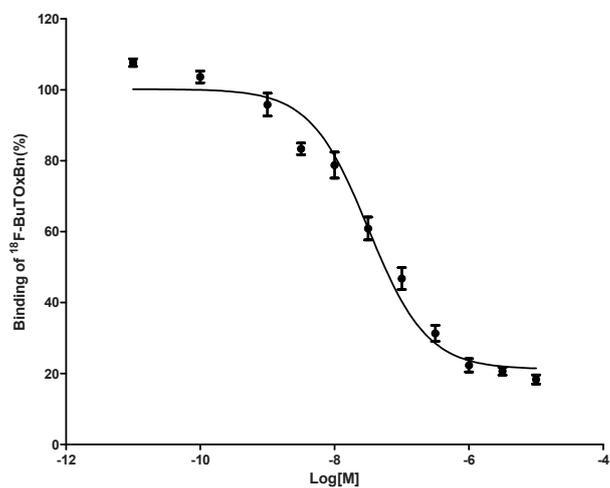
Figure 2 Competitive binding assay on PC-3 cells with [^{18}F]-BuTOxBN
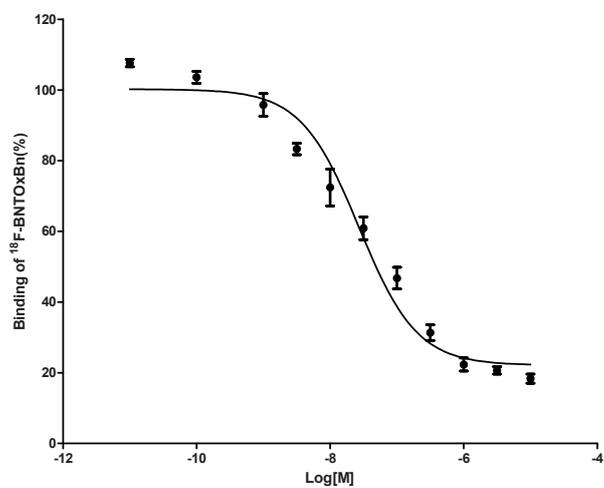
Figure 3 Competitive binding assay on PC-3 cells with [^{18}F]-BnTOxBN
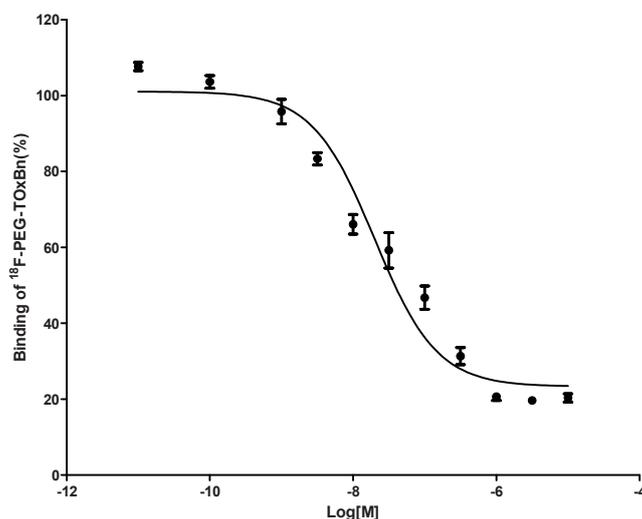
Figure 4 Competitive binding assay on PC-3 cells with [^{18}F]-PEGTOxBN

6.8   References and Notes

(1)   J. A. Prescher, C. R. Bertozzi. *Nat. Chem. Biol.* **2005**, *1*, 13-21.

(2)   J. Lippincott-Schwartz, G. H. Patterson. *Science* **2003**, *300*, 87-91; J. Zhang, R. E. Campbell, A. Y. Ting, R. Y. Tsien. *Nat. Rev. Mol. Cell Biol.* **2002**, *3*, 906-918.

(3)   T. F. Massoun, S. S. Gambhir. *Genes Dev.* **2003**, *17*, 545-580.

(4)   H. Staudinger, J. Meyer. *Helv. Chim. Acta* **1919**, *2*, 635-646.

(5)   E. Saxon, C. R. Bertozzi. *Science* **2000**, *287*, 2007-2010.

(6)   J. C. Jewett, C. R. Bertozzi. *Chem. Soc. Rev.* **2010**, *39*, 1272-1279.

(7)   A. J. Link, D. A. Tirrell. *J. Am. Chem. Soc.* **2003**, *125*, 11164-11165.

(8)   Z. Li, T. Seo, J. Ju. *Tetrahedron Lett.* **2004**, *45*, 3143-3146.

(9)   G. Wittig, A. Krebs. *Chem. Ber.* **1961**, *94*, 3260-3275.

(10) S. S. van Berkel, A. J. Dirks, M. F. Debets, F. L. van Delft, J. J. L. M. Cornelissen, R. J. M. Nolte, F. P. J. T. Rutjes. *ChemBioChem* **2007**, *8*, 1504-1508.

(11) D. H. Ess, G. O. Jones, K. N. Houk. *Org. Lett.* **2008**, *10*, 1633-1636; F. Schoenebeck, D. H. Ess, G. O. Jones, K. N. Houk. *J. Am. Chem. Soc.* **2009**, *131*, 8121-8133.

(12) M. F. Debets, S. S. van Berkel, J. Dommerholt, A. J. Dirks, F. P. J. T. Rutjes, F. L. van Delft. *Acc. Chem. Res.* **2011**, *44*, 805-815.

(13) N. J. Agard, J. A. Preschner, C. R. Bertozzi. *J. Am. Chem. Soc.* **2004**, *126*, 15046-15047.

(14) N. J. Agard, J. M. Baskin, J. A. Prescher, A. Lo, C. R. Bertozzi. *ACS Chem. Biol.* **2006**, *1*, 644-648.

(15) J. M. Baskin, J. A. Prescher, S. T. Laughlin, N. J. Agard, P. V. Chang, I. A. Miller, A. Lo, J. A. Codelli, C. R. Bertozzi. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 16793-16797.

(16) E. M. Sletten, C. R. Bertozzi. *Org. Lett.* **2008**, *10*, 3097-3099.

(17) X. Ning, J. Guo, M. A. Wolfert, G. J. Boons. *Angew. Chem. Int. Ed.* **2008**, *47*, 2253-2255; A. Kuzmin, A. Poloukhtine, M. A. Wolfert, V. V. Popik. *Bioconjugate Chem.* **2010**, *21*, 2076-2085; J. C. Jewett, E. M. Sletten, C. R. Bertozzi. *J. Am. Chem. Soc.* **2010**, *132*, 3688-3690.

(18) J. Dommerholt, S. Schmidt, R. Temming, L. J. A. Hendriks, F. P. J. T. Rutjes. *Angew. Chem. Int. Ed.* **2010**, I49*, 9422-9425*.

(19) A. A. Poloukhtine, N. E. Mbua, M. A. Wolfert, G. –J. Boons, V. V. Popik. *J. Am. Chem. Soc.* **2009**, *131*, 15769-15776.

(20) S. T. Laughlin, J. M. Baskin, S. L. Amacher, C. R. Bertozzi. *Science* **2008**, *320*, 664-667; A. B. Neef, C. Schultz. *Angew. Chem. Int. Ed.* **2009**, *48*, 1498-1500; Y. Zou, J. Yin. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5664-5667; M. Fernandez-Suarez, H. Baruah, L. Martinez-Hernandez, K. T. Zie, J. M. Baskins, C. R. Bertozzi, A. Y. Ting. *Nat. Biotechnol.* **2007**, *25*, 1483-1487; P. V. Chang, J. A. Prescher, E. M. Sletten, J. M. Baskin, I. A. Miller, N. J. Agard, A. Lo, C. R. Bertozzi. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 1821-1826; E. M. Sletten, C. R. Bertozzi. *Angew. Chem. Int. Ed.* **2009**, *39*, 1272-1279.

(21) K. Gutsmiedl, C. T. Wirges, V. Ehmke, T. Carell. *Org. Lett.* **2009**, *11*, 2405-2408.

(22) N. K. Devaraj, R. Weissleder, S. A. Hilderbrand. *Bioconjugate Chem.* **2008**, *19*, 2297-2299.

(23) R. Pipkorn, W. Waldeck, B. Didinger, M. Koch, G. Mueller, M. Wiessler, K. Braun. *J. Pept. Sci.* **2009**, *15*, 235-241.

(24) M. L. Blackman, M. Royzen, J. M. Fox. *J. Am. Chem. Soc.* **2008**, *130*, 13518-13519.

(25) R. Rossin, P. R. Verkerk, S. M. van den Bosch, R. C. M. Vulders, I. Verel, J. Lub, M. S. Robillard. *Angew. Chem. Int. Ed.* **2010**, *49*, 3375-3378.

(26) X. Ning, R. P. Temming, J. Dommerholt, J. Guo, D. B. Ania, M. F. Debets, M. A. Wolfert, G.-J. P. H. Boons, F. L. van Delft. *Angew. Chem. Int. Ed.* **2010**, *49*, 3065-3068; C. S. McKay, J. Moran, J. P. Pezacki. *Chem. Commun.* **2010**, *46*, 931-933.

(27) M. E. Martin, S. G. Parameswarappa, M. S. O'Dorisio, F. C. Pigge, M. K. Schultz. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4805-4807. .

(28) M. J. Debets, S. S. van Berkel, S. Schoffelen, F. P. J. T. Rutjes, J. C. M. van Hest, F. L. van Delft. *Chem. Commun.* **2009**, 97-99.

(29) Sigma-Aldrich product 597651

(30) S. R. Wilson, R. A. Sawicki, J. C. Huffman. *J. Org. Chem.* **1981**, *46*, 3887-3891; K. Maruoka, T. Miyazaki, M. Ando, Y. Matsumura, S. Sakane, K. Hattori, H. Yamamoto. *J. Am. Chem. Soc.* **1983**, *105*, 2831-2843.

(31) D. G. Powell, L. J. Mathias. *J. Am. Chem. Soc.* **1990**, *112*, 669-675.

(32) L. de Luca, G. Giocomelli, A. Porcheddu. *J. Org. Chem.* **2002**, *67*, 6272-6274.

(33) Sigma-Aldrich product D31737

(34) E. Marcantoni, M. Massaccesi, E. Torregiani. *J. Org. Chem.* **2001**, *66*, 4430-4432.

(35) Both isomers of the triazole were detected, but were collected and treated as one compound.

(36) A. Leo, C. Hansch, D. Elkins. *Chem. Rev.* **1971**, *6*, 525-616.

(37) D. Fischer, H. Tomeba, N. K. Pahadi, N. T. Patil, Z. Huo, Y. Yamamoto. *J. Am. Chem. Soc.* **2008**, *130*, 15720-15725; D. Fischer, H. Tomeba, N. K. Pahadi, N. T. Patil, Z. Huo, Y. Yamamoto. *Angew. Chem. Int. Ed.* **2007**, *46*, 4764-4766.

(38) V. L. Heasley, D. F. Shellhamer, L. E. Heasley, D. B. Yaeger. *J. Org. Chem.* **1980**, *45*, 4649-4652.

(39) S. Kobayashi, C. Ogawa. *Chem. Eur. J.* **2006**, *12*, 5954-5960.

(40) D. M. Goldenberg, R. M. Sharkey, G. Paganelli, J. Barbet, J. F. Chatal. *J. Clin. Oncol.* **2006**, *24*, 832-834.

(41) H. Ankait, E. Biehl. *Tetrahedron Lett.* **2009**, *50*, 4677-4682.

(42) X. Chen, R. Park, Y. Hou, M. Tohme, A. H. Shahinian, J. R. Bading, P. S. Conti. *J. Nucl. Med.* **2004**, *45*, 1390-1397.