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Review

Inverse Electron-demand Diels-Alder 반응을 이용한 핵의학 영상 프로브의 합성 및 활용

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Synthesis of PET and SPECT Radiotracers Using Inverse Electron-demand Diels-Alder Reaction

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초 록

1,2,4,5-테트라진 유도체를 이용한 inverse electron-demand Diels-Alder (IEDDA) 반응은 다양한 생체물질, 고분자, 나노물질 복합체의 효율적인 합성에 폭넓게 활용되고 있다. IEDDA는 유기용매에서뿐만 아니라 생리학적 조건 하에서도 매우 특이적이며 빠른 반응속도를 가지고 있는 것으로 알려져 있다. 이러한 특성으로 인해 본 반응은 다양한 생물학적 활성을 가지는 물질의 방사성동위원소 표지와 분자영상 및 질병 치료를 위한 방사성의약품 개발에도 활발히 응용되고 있다. 본 리뷰 논문은 IEDDA 반응을 방사화학 및 핵의학 분야에서 이용한 최근 연구 동향 및 연구 결과 그리고 향후 전망에 대해 소개하고자 한다.

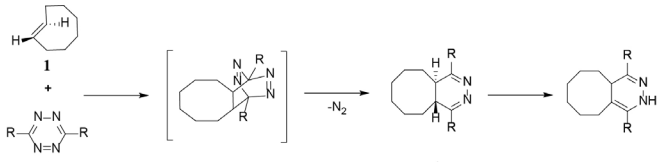
Abstract

Inverse electron-demand Diels-Alder reactions (IEDDA) between tetrazine derivatives and strained dienophiles have attracted a lot of attention for the efficient conjugation of biomolecules, polymers, and nanomaterials. Excellent specificity, exceptionally fast reaction rate, and biocompatibility are key features of IEDDA. Therefore, it has also been applied to the development of new labeling methods using several radioisotopes and development of radiotracers to carry out various nuclear imaging as well as therapeutic studies. The purpose of this review is to introduce the reader to the recent advances and applications of IEDDA in the fields of radiochemistry and nuclear medicine.

Keywords: *Inverse electron-demand Diels-Alder reaction, Radiolabeling, Nuclear imaging, Diagnosis, Therapy*

1. Introduction

In recent years, inverse electron-demand Diels-Alder (IEDDA) reaction between a 1,2,4,5-tetrazine and a strained alkene (or strained alkyne) is extensively investigated and utilized for the bioorthogonal and biocompatible labeling of a variety of small molecules, biomolecules, and living cells[1,2]. Due to the excellent specificity and rapid reaction rate of IEDDA, it has been employed to the radiolabeling of various biologically active molecules for nuclear imaging such as positron emission tomography (PET) and single-photon emission computerized tomography (SPECT) scan. Moreover, their results have successfully been applied to the syntheses of new radiotracers and molecular imaging agents. In addition to a few examples of in vitro radiolabeling of biomolecules, IEDDA has also been studied for *in vivo* pretargeted imaging of tumors in animal xenograft models[3]. In this review, we first aim to introduce the basics of IEDDA including its reaction mechanism and reaction rate. Second, the synthesis of radiolabeled products



Scheme 1. Reaction mechanism of IEDDA.

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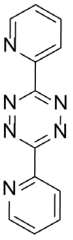
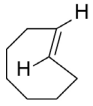
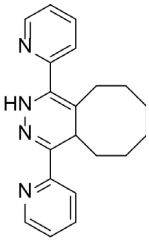
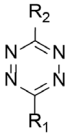
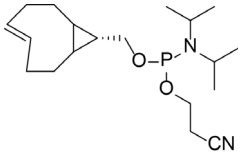
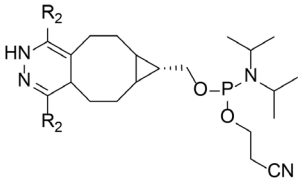
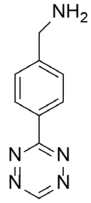
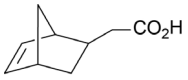
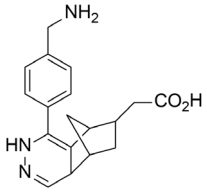
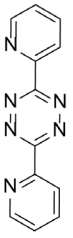
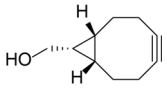
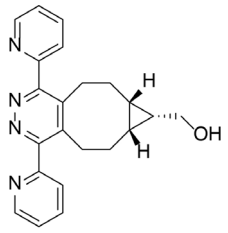
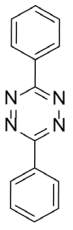
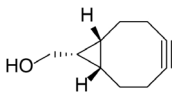
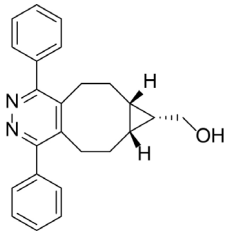
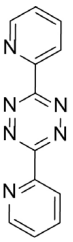
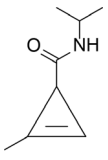
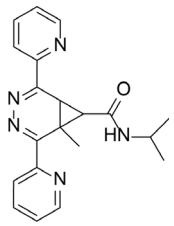
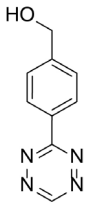
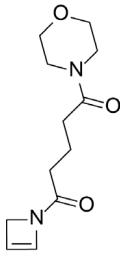
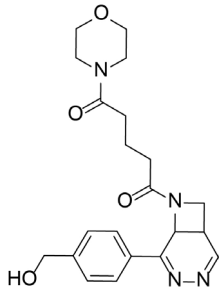
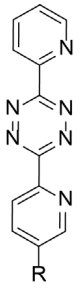
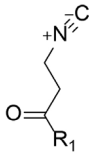
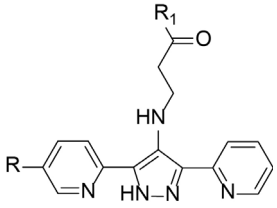
Table 1. Reaction Rate of IEDDA Between Various Tetrazines and Dienophiles

Entry	Tetrazine	Dienophile	Product	Reaction Rate ($M^{-1}s^{-1}$)	Ref
1	 **6**	 **1**	 **7**	2000	[4]
2	 **8**	 **9**	 **10**	380,000	[5]
3	 **11**	 **12**	 **13**	1.6-2.0	[6,11,14]
4	 **6**	 **14**	 **15**	44.8	[7]
5	 **16**	 **14**	 **17**	3.3	[7]
6	 **6**	 **18**	 **19**	0.15	[8]

To be continued

Table 1. Continued

Entry	Tetrazine	Dienophile	Product	Reaction Rate ($M^{-1}s^{-1}$)	Ref
7	 **20**	 **21**	 **22**	0.39	[9]
8	 **23** R = Biotin	 **24**	 **25** R = Biotin	0.014	[10]

using IEDDA and their applications for imaging, therapy of diseases, and biodistribution studies were discussed.

1.1. Reaction mechanism and general features of IEDDA

The mechanism of IEDDA between 1,2,4,5-tetrazine and strained alkene is shown in the Scheme 1. A dienophile **1** and tetrazine **2** reacts to form a bicyclic intermediate **3**. Next, the highly strained adduct **3** is rapidly converted to the corresponding 4,5-dihydropyridazine **4** via a retro-Diels-Alder reaction upon release of N_2. A subsequent 1,3-prototropic isomerization gives the corresponding 1,2-dihydro product **5**[4].

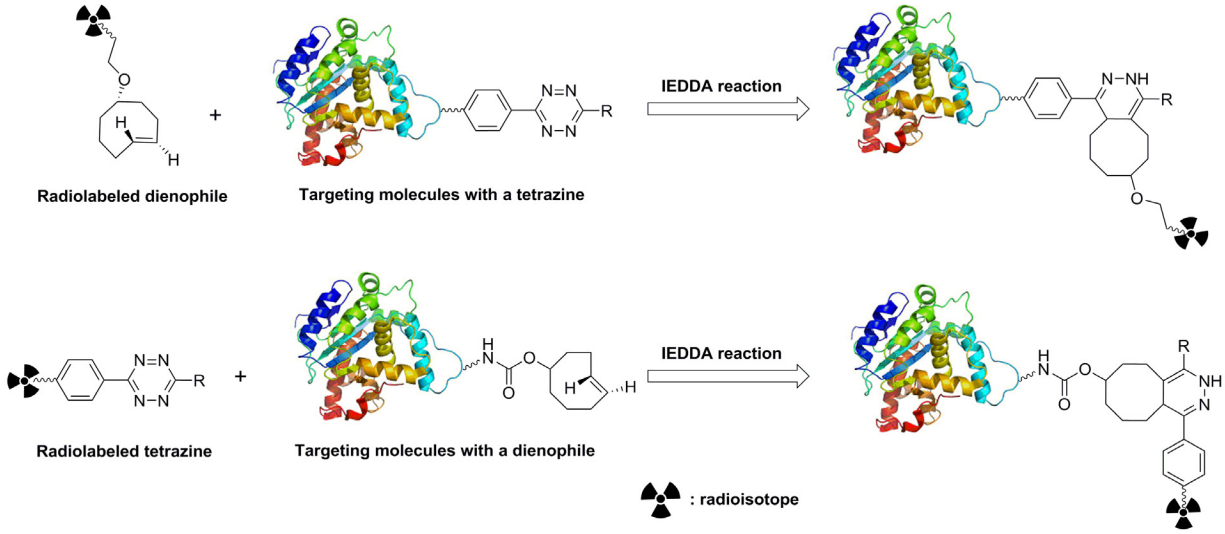
Since the first IEDDA using 1,2,4,5-tetrazine and trans-cyclooctyne (TCO) was reported in 2008, various tetrazine derivatives and dienophiles have been investigated to develop a new bioorthogonal ligation. The second order reaction rate (k_2) of between 1,2,4,5-tetrazine and trans-cyclooctyne (TCO) was normally as high as 10^3 $M^{-1}s^{-1}$ which is much faster than any other well-established bioconjugation methods including strain-promoted azide-alkyne click chemistry (also known as Copper-free click reaction, k_2 = 1-2 $M^{-1}s^{-1}$). Table 1 showed several representative examples on IEDDA. To date, the reaction rate (k_2) of the fastest IEDDA, using *trans*-bicyclo[6.1.0]nonene as a dienophile, was 380,000 $M^{-1}s^{-1}$ (entry 2)[5]. Cyclooctyne analogs, which are strained alkyne, were also highly reactive toward the tetrazine structure (entry 4 and 5). The reaction rate of tetrazine ligation using these substrates was slower than that of TCO, however a cyclooctyne substrate is easier to prepare and moreover it is known to more stable structure than TCO analogs which are prone to isomerization to its (Z)-isomer under physiological conditions. Therefore a few conjugation studies have also been reported by using tetrazine and cyclooctyne derivatives. Norborene, cyclobutene, and cyclopropane substrates have been investigated in the same reaction, however these derivatives showed much lower efficiencies (entry 3, 6-8).

2. Applications of IEDDA for the Synthesis of Radiolabeled Products

Several radioisotopes, which frequently used in the nuclear medicine, and their physical properties are shown in Table 2. These radioisotopes are mainly produced by either a medical cyclotron or a nuclear reactor. Positron emitting (β^+) radioisotopes are used in the PET imaging and the energy of β^- decay can be utilized in the therapy of disease. Most of ^{18}F-labeling is carried out by a nucleophilic substitution reaction between the fluoride anion ($^{18}F^-$) and a precursor bearing a good leaving group, while ^{11}C-labeled methyl ($^{11}CH_3I$ or $^{11}CH_3OTf$) is normally used as an electrophile for the synthesis of radiolabeled product. Metal radioisotopes can be labeled with a target molecule which contains a suitable chelating agent. As increasing demand of efficient radiochemical reactions as well as development of new radiopharmaceuticals for diagnosis and therapeutic purposes, various radiolabeled tetrazines and dienophiles have been reported. Scheme 2 illustrates a strategy about the synthesis of radiotracers using IEDDA. A radiolabeled dienophile (e.g. TCO) or tetrazine derivative was reacted with targeting biomolecules (or small molecules) which were modified with a suitable functional group to provide the desired products. The following sections will introduce the recently published results on the synthesis of

Table 2. Properties of Commonly Used Radioisotopes in the Field of Nuclear Medicine

Radioisotope	Decay half-life	Decay mode	Decay product	Applications
^{18}F	109.8 min	Positron emission (β^+) Electron capture	^{18}O	PET imaging
^{11}C	20.3 min	Positron emission (β^+) Electron capture	^{11}B	PET imaging
^{68}Ga	68 min	Positron emission (β^+) Electron capture	^{68}Zn	PET imaging
^{64}Cu	12.7 h	Positron emission (β^+) Beta decay (β^-) Electron capture	^{64}Ni ^{64}Zn ^{64}Ni	PET imaging, Therapy
^{89}Zr	78.4 h	Positron emission (β^+) Electron capture	^{89}Y	PET imaging
^{99m}Tc	6.01 h	Isomeric transition	^{99}Tc	SPECT imaging
^{111}In	2.80 days	Electron capture	^{111}Cd	SPECT imaging
^{177}Lu	6.72 days	Beta decay (β^-)	^{177}Hf	Therapy
^{124}I	4.18 days	Positron emission (β^+) Electron capture	^{124}Te	PET imaging
^{125}I	59.4 days	Electron capture	^{125}Te	SPECT imaging



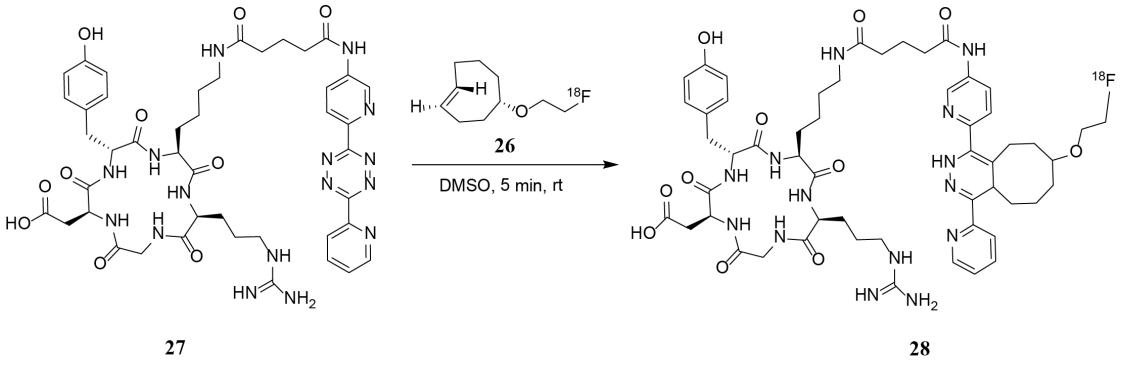
Scheme 2. An overview of IEDDA reaction for synthesis of radiotracers.

nuclear imaging tracers using IEDDA.

2.1. Synthesis of PET imaging tracers

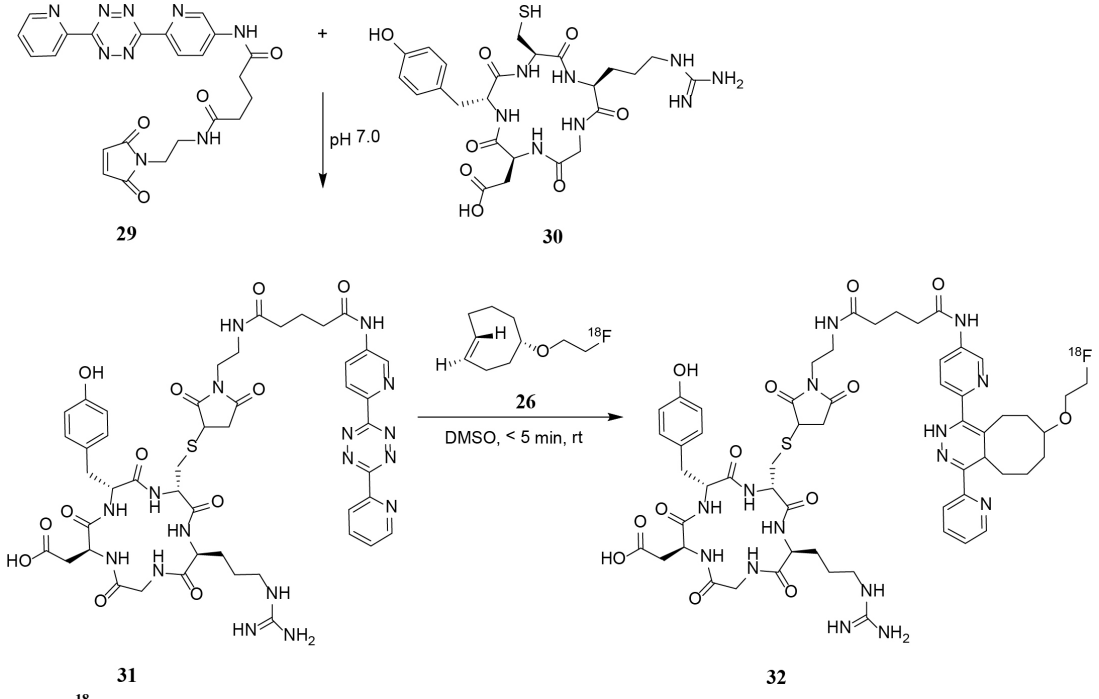
^{18}F is one of the most important radioisotopes for PET imaging because of its favorable physical characteristics[12]. For last few decades, a lot of small molecule precursors and automated synthesis systems have been developed for the production of ^{18}F-labeled radiopharmaceuticals[13]. However, incorporation of ^{18}F in a complex bioactive molecule has been limited because of relatively short decay half-life (109.8 min) and low nucleophilicity of ^{18}F in an aqueous solvent system. Under these circumstances, IEDDA can be applied as a highly efficient method to produce ^{18}F-labeled macromolecules. As the tetrazine ligation can proceed under mild conditions, including ambient temperature, neutral pH and aqueous media, it is far superior as compare to the classical nucleophilic ^{18}F-labeling method which involves an anhydrous solvent system and harsh conditions such as high reaction temperature and basic pH environment.

In 2010, Fox et al. applied IEDDA to the ^{18}F-labeling of small molecules. The radiolabeled TCO **26** (Table 3, entry 1) could be synthesized by a nucleophilic substitution reaction of the tosylated precursor in 71% radiochemical yield. IEDDA reaction between tetrazine **6** and TCO **26** yield **49** in more than 98% radiochemical yield in 10 sec[15]. Using the similar protocol, a radiolabeling reaction between **26** and the tetrazine conjugated cRGD peptide was performed to give the desired product **28** in more than 98% radiochemical yield within 10 sec (Scheme 3). It was the first report applying IEEDA to the synthesis of ^{18}F-labeled cancer targeting peptide. The product **28** was successfully used for PET imaging of female nude mice bearing U87MG tu-



27 28

Scheme 3. Synthesis ^{18}F-labeled cRGD peptide using IEDDA (i).



29 30 31 32

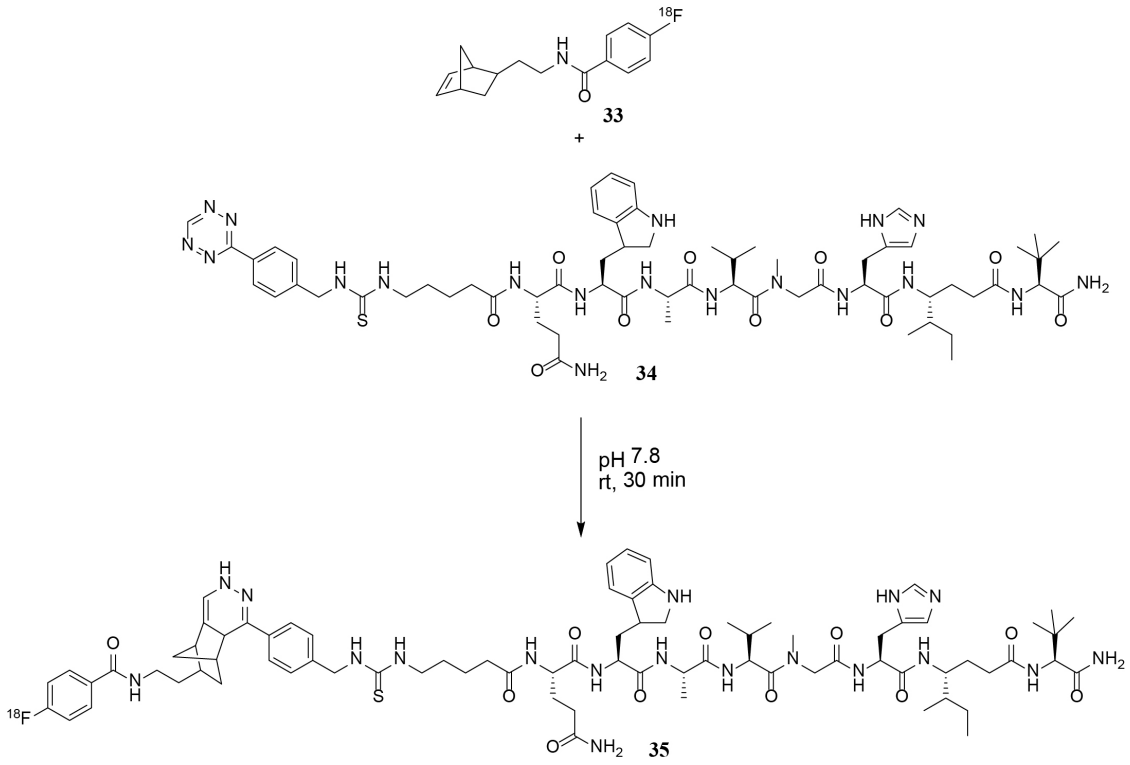
Scheme 4. Synthesis ^{18}F-labeled cRGD peptide using IEDDA (ii).

mor[16]. In another report, a maleimide group conjugated tetrazine was used to give the cRGD peptide substrate **31** (Scheme 4). The ^{18}F-labeled RGD peptide was obtained in 95% radiochemical yield by using ^{18}F-labeled TCO **26**[17].

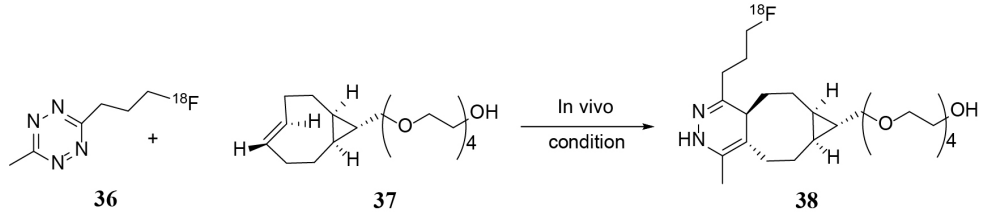
Weissleder and coworkers synthesized ^{18}F-AZD2281 **51**, a poly-ADP-ribose-polymerase1, as a PET imaging tracer (Table 3, entry 2). In this report, ^{18}F-labeled TCO **26** and a tetrazine group containing AZD2281 **50** were incubated for 3 minutes. To avoid HPLC purification of the crude product, a magnetic TCO-scavenger resin were used to remove unreacted tetrazine containing AZD2281. ^{18}F-labeled AZD2281 **51** was obtained in 92% radiochemical yield[18]. Wu and coworkers used IEDDA for ^{18}F-labeling of exendin-4, a peptide hormone, to target the glucagon- like peptide-1 receptor (GLP-1R). A ^{18}F-labeled TCO and tetrazine-conjugated exendin-4 **52** were incubated at room temperature for 5 min to prepare the final product **53** in 80% radiochemical yield and > 99% radiochemical purity after HPLC purification (Table 3, entry 3)[19]. PET imaging study in small animals indicated that the ^{18}F-labeled product (**53**) could specifically bind to GLP-1R.

In order to demonstrate another functional group pair for IEDDA, Knight et al. used ^{18}F-labeled norbornene derivative for the labeling of bombesin peptide **34** (Scheme 5). An amino norbornene was reacted with [^{18}F]fluorobenzoate to give the corresponding ^{18}F-labeled norbornene **33**. A tetrazine functionalized bombesin peptide was reacted with **33** to get final product with in 30 min at 46% radiochemical yield. *In vivo* stability of ^{18}F-labeled bombesin peptide **35** was evaluated in normal mice and approximately 90% of the product was remained intact up to 30 min after administration[20].

In 2014, Mikula and coworkers developed a new ^{18}F-labeled tetrazine **36**, which has ability to across the blood brain barrier. This tracer showed high *in vivo* and *in vitro* stability and fast distribution in the brain. To verify IEDDA in a living subject, **36** and TCO **37** was sequentially administered to the same mice at 20 min interval (Scheme 6). IEDDA reaction in the blood was completed in 30 min and **36** was



Scheme 5. Synthesis of ^{18}F-labeled bombesin peptide using IEDDA.



Scheme 6. *In vivo* reaction between [^{18}F]-labeled tetrazine 36 and TCO analog 37.

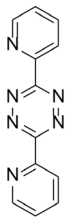
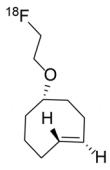
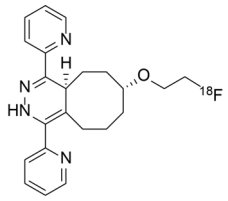
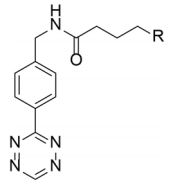
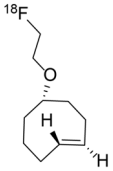
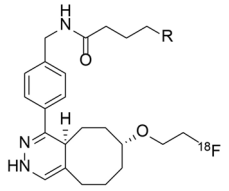
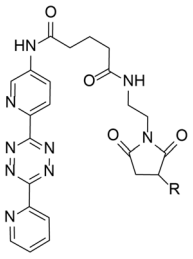
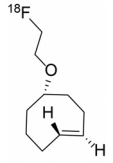
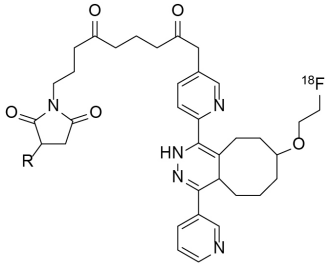
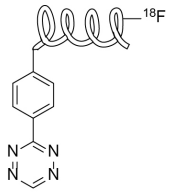
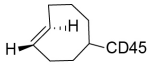
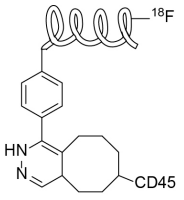
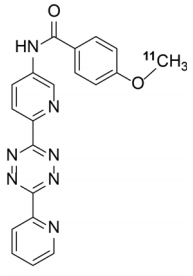
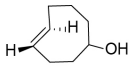
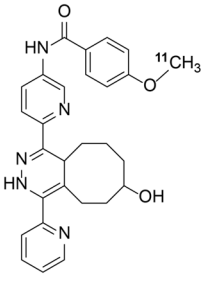
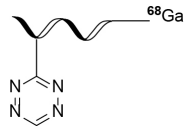
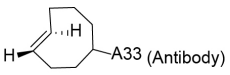
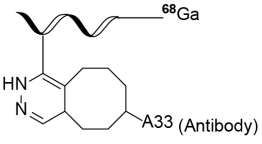
fully converted to the desired product **38** as determined by radio-TLC analysis Therefore, this method will be quite useful for further biomedical imaging studies[21].

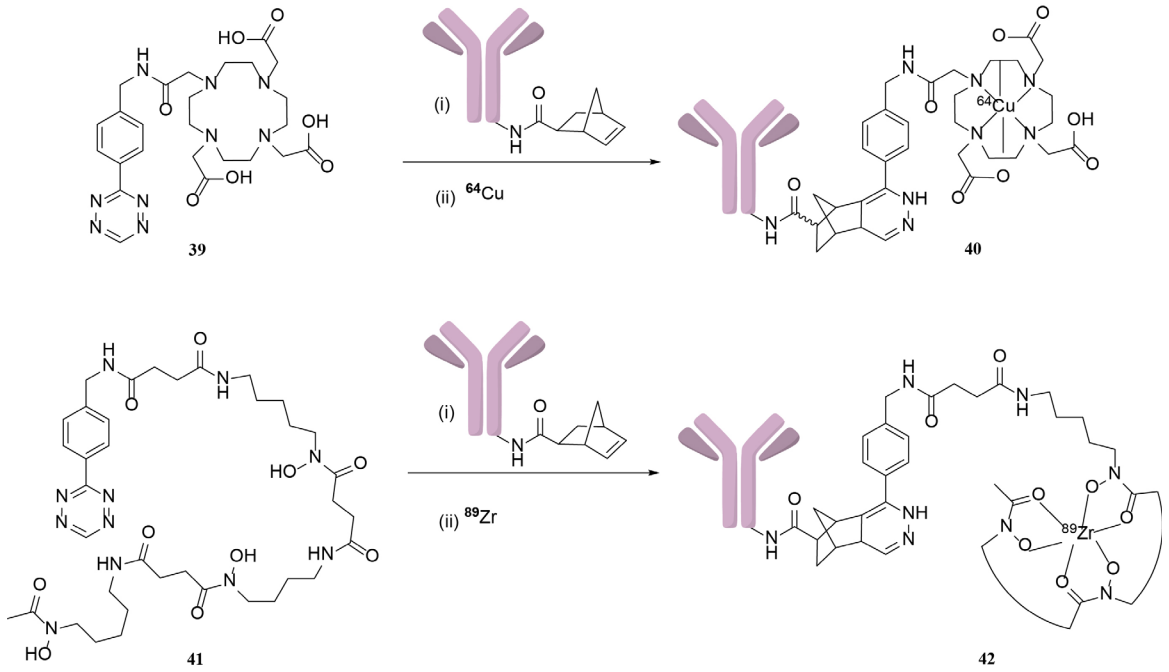
^{11}C is also a highly useful radioisotope for the synthesis of PET imaging agent. Especially ^{11}C-labeled methyl triflate and methyl iodide are the most prominent synthons for nucleophilic methylation of alcohols, amines, and thiols, commonly used for the production of various radiotracers and radiopharmaceuticals. In 2013, Herth and coworkers reported IEDDA between ^{11}C-labeled tetrazine **57** and a simple TCO derivative (Table 3, entry 5)[23]. The product **57** was synthesized at 80 ℃ within two minutes from the corresponding 4-hydroxybenzoate precursor in relatively low radiochemical yield (33%). The radiolabeling reaction between **57** and TCO **58** was completed within 20 sec to afford the desired structure **59** in 98% radiochemical yield. Although some successful ligation were reported, ^{11}C-labeled tetrazine or TCO have not been studied as much as ^{18}F-labeled analogs due to its shorter decay half-life (20 min).

As previously shown in the Table 1, several radioactive metals such as ^{64}Cu, ^{89}Zr and ^{68}Ga have been applied to preclinical PET imaging and clinical trials. In general, a target molecules to be used in the imaging study was conjugated with a chelating agent such as diethylenetriaminepentaacetic acid (DTPA), 1,4,7-triazacyclononane-triacetic acid (NOTA), and 1,4,7,10-tetraazacyclododecane-tetraacetic acid (DOTA). The labeling procedures of metal radioisotopes are normally simpler than those of ^{18}F and ^{11}C and therefore incorporation of radioactive metals in the target molecules provided high radiochemical yields. Lewis group successfully applied IEDDA to synthesize radioactive metal labeled PET tracers (Scheme 7)[24]. In this study, DOTA conjugated tetrazine **39** and deferoxamine (DFO) conjugated tetrazine **41** were prepared for the chelation of radioactive metals. A norbornene bearing trastuzumab was reacted with tetrazine chelators **39** (DOTA) and **41** (DFO). The chelator bearing antibodies were then labeled with ^{64}Cu and ^{89}Zr respectively. The radiolabeled products could be obtained with high radiochemical yield (> 80%) and specific activity (> 2.9 mCi/mg). PET imaging studies demonstrated that radiolabeled antibodies were quite stable *in vivo* conditions and showed a specific uptake in HER2-positive BT-474 tumor cells.

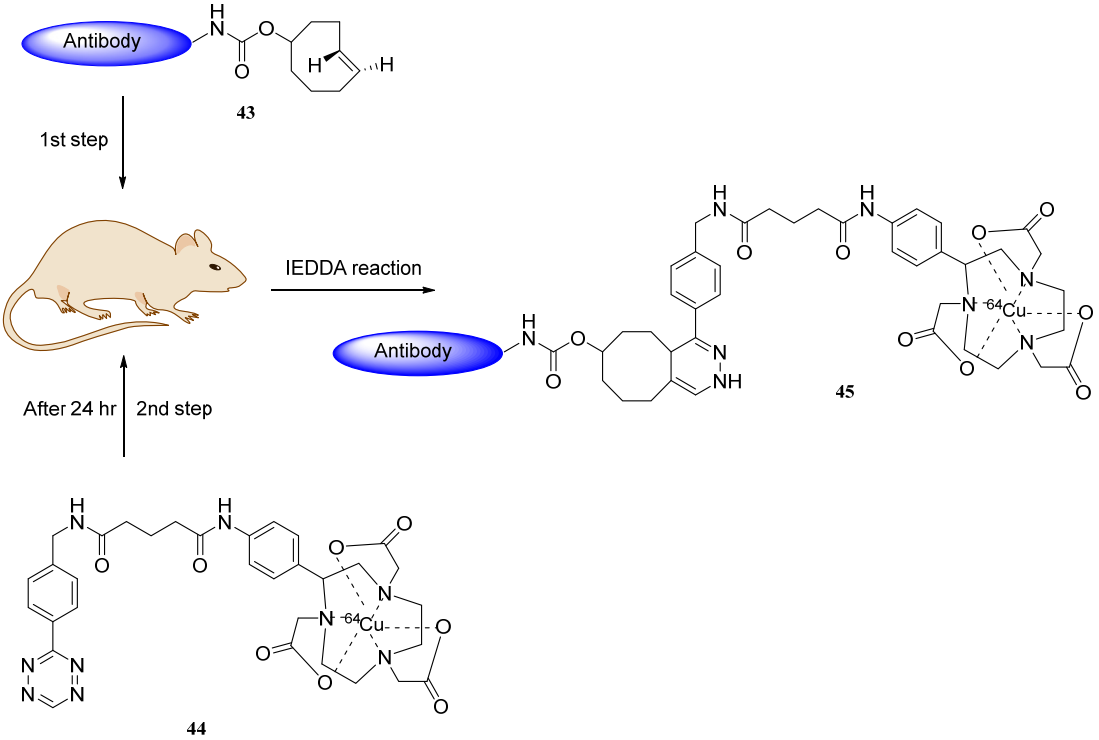
Recently, the pretargeted imaging strategy has gained increasing

Table 3. Examples on the Synthesis of PET Radiotracer Using IEDDA

Entry	Tetrazine	Dienophile	Product	Ref
1	 **6**	 **26**	 **49**	[15]
2	 **50** R = AZD2281	 **26**	 **51** R = AZD2281	[18]
3	 **52** R = Cys^{40}-exendin-4	 **26**	 **53** R = Cys^{40}-exendin-4	[19]
4	 **54**	 **55**	 **56**	[22]
5	 **57**	 **58**	 **59**	[23]
6	 **60**	 **61**	 **62**	[27]



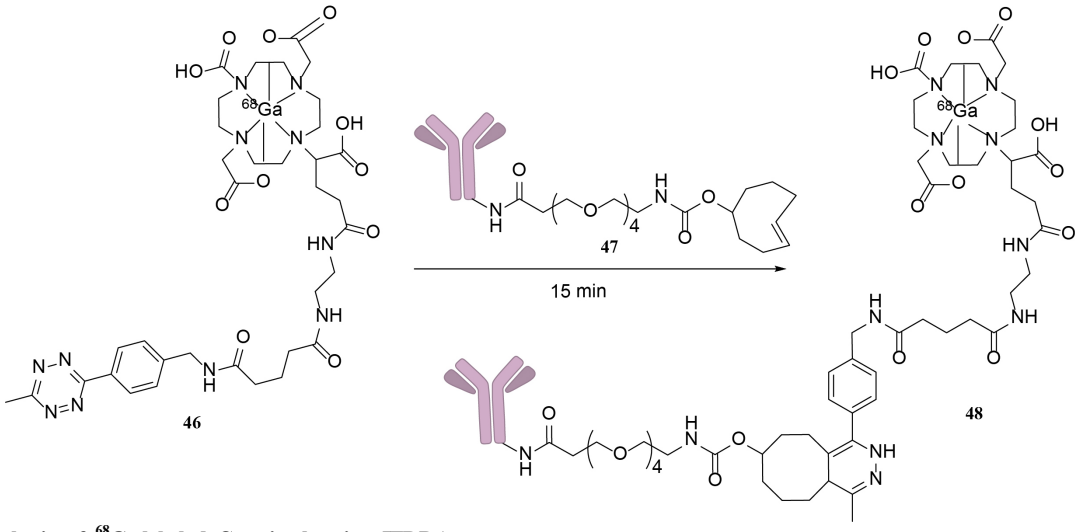
Scheme 7. Synthesis of ^{89}Zr and ^{64}Cu-labeled trastuzumab using IEDDA.



Scheme 8. Pretargeted strategy using ^{64}Cu-labeled tetrazine.

attention. Selective delivery of radioisotopes to the region of interest (e.g. tumor) can be achieved by using a two-step procedure. In the first step, the tumor is pretargeted with an antibody and after some time a radiolabeled small molecules which can be rapidly conjugated with the antibody was administrated. The region of interest was imaged by a radiolabeled small molecule. This method provided a more rapid clearance of the radioactivity from normal tissues due to the fast pharmacokinetics of the radiolabeled small molecules compared to antibodies. Due to the excellent reaction rate and specificity, radiolabeled tetrazine derivatives have been applied to the pretargeted imaging studies.

Lewis group demonstrated *in vivo* IEDDA using a 64Cu-labeled tetrazine derivative and TCO bearing A33 antibody **43** (Scheme 8). For



Scheme 9. Synthesis of ^{68}Ga-labeled Cetuximab using IEDDA.

this experiment, TCO bearing A33 **43** was first injected to SW1222 xenograft models. After 24 h post injection, ^{64}Cu-labeled tetrazine **44** was intravenously administrated to the same mice. The results revealed that the pretargeted strategy provided better a better tumor to background ratio as compared to the control experiment using *in vitro* radiolabeled A33 antibody[25]. Recently, Aboagye et al. reported ^{68}Ga-labeled tetrazine **46** and TCO modified Cetuximab **47** for pretargeted PET imaging of EGFR-expressing A431 tumors (Scheme 9). After 23 h administration of the antibody **47**, **46** was injected intravenously to the same tumor bearing mice. PET imaging showed the significantly advanced results from the pretargeted approach over the traditional direct labeling procedure[26].

In the pretargeted imaging study, radiolabeled small molecule tetrazine and TCO often underwent rapid renal or hepatobiliary clearance. To increase the blood circulation time of the functional group, tetrazine group containing polymers consisted of dextran scaffolds were designed by Weissleder group[22]. An 18F-labeled polymer modified tetrazine **54** (Table 3, entry 4) and TCO bearing CD45 monoclonal antibodies **55** were investigated in a living mice. PET imaging study showed excellent conversion of reactants and high tumor uptake in the xenograft bearing mice. These results demonstrated that the radiolabeled polymer **54** will be a promising imaging agent that can be applied to *in vivo* bioorthogonal chemistry. In another example, Devaraj and coworkers synthesized a dextran polymer **60** which contains both tetrazine group for IEDDA and DTPA for ^{68}Ga-labeling (Table 3, entry 6). A TCO bearing A33 antibody **61** was applied to the *in vivo* pretargeted imaging. PET imaging results showed that a polymer probe **60** has sufficient capability to target A33 biomarkers in LS147T xenografts bearing mice[27].

2.2. Synthesis of SPECT imaging tracers

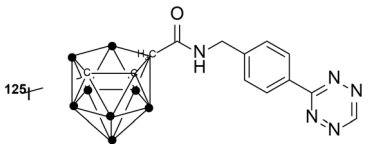
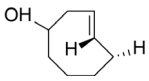
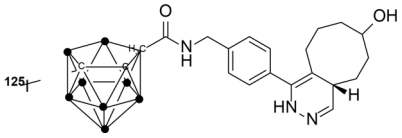
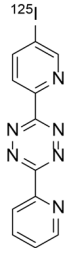
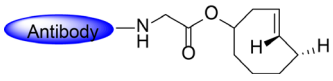
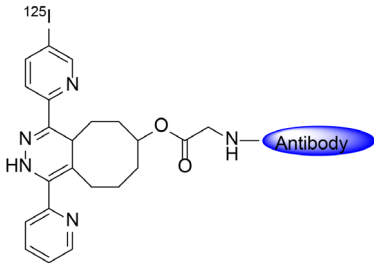
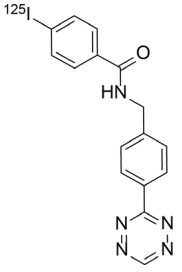
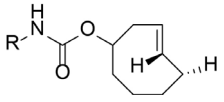
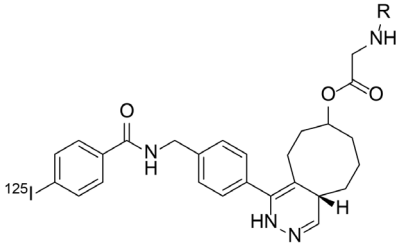
Radioactive iodines (^{125}I, ^{123}I and ^{131}I) have been used to prepare various radiotracers for *in vivo* SPECT imaging. In general, the traditional radioiodination method via an electrophilic substitution reaction gave high radiochemical yields in a short time. But the radiolabeled tracer synthesized by the above reaction normally showed a considerable deiodination in a living subjects and liberated radioactive iodines were rapidly accumulated in some specific organs such as thyroid and stomach, which resulted in the high background signals in the biomedical images. Moreover a strong oxidant which requires radioiodination reaction often caused decreased biological activity of the molecules. To address these problems, radioactive iodine labeled tetrazine or TCO group can be used as an alternative method for the efficient and site-specific radioiodination of biomolecules.

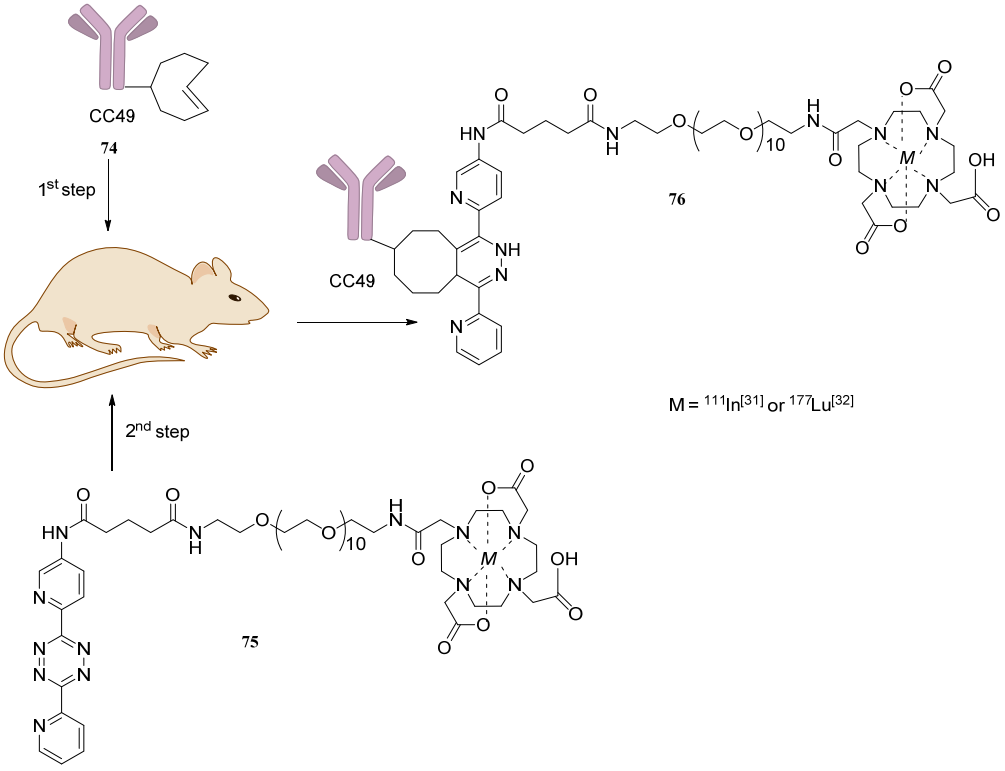
In 2015, Valliant group reported IEDDA ligation between ^{123}I-labeled carborane-tetrazine **63** and (*E*)-cyclooct-4-enol TCO **64** (Table 4, entry 1). The second order rate constant for this ligation was found to be 199 ± 26 $M^{-1}s^{-1}$. The ^{123}I-labeled carborane-tetrazine **63** was further applied to label TCO-modified H520 cells[28]. Valliant group also investigated IEDDA to synthesize radiolabeled VEGFR2 antibody by using the ^{125}I-labeled tetrazine derivative (Table 4, entry 2)[29]. The radiolabeled product **66** was prepared from a stannylated precursor in more than 80% radiochemical yield. As a next step, TCO-modified anti-VEGFR2 was incubated with **67** for 5 minutes to give the desired product **68** in 67% radiochemical yield. Biodistribution study of the radiolabeled antibody **68** was carried out to examine *in vivo* stability of the product.

Recently Jeon group reported ^{125}I-labeled tetrazine **69** for efficient radiolabeling of biomolecules (Table 4, entry 3)[30]. For the radiolabeling application, TCO conjugated cRGD peptide **70** and human serum albumin (HSA) **71** were prepared. These substrates were reacted with **69** under mild condition to provide the radiolabeled products **72** and **73** respectively with excellent radiochemical yields (> 99%). The biodistribution study on the ^{125}I-labeled HSA **73** was performed in normal ICR mice and demonstrated minimal loss of radioactive iodine *in vivo*. These results indicated that **69** can be used as a valuable prosthetic group for radioiodination of biomolecules in the future study.

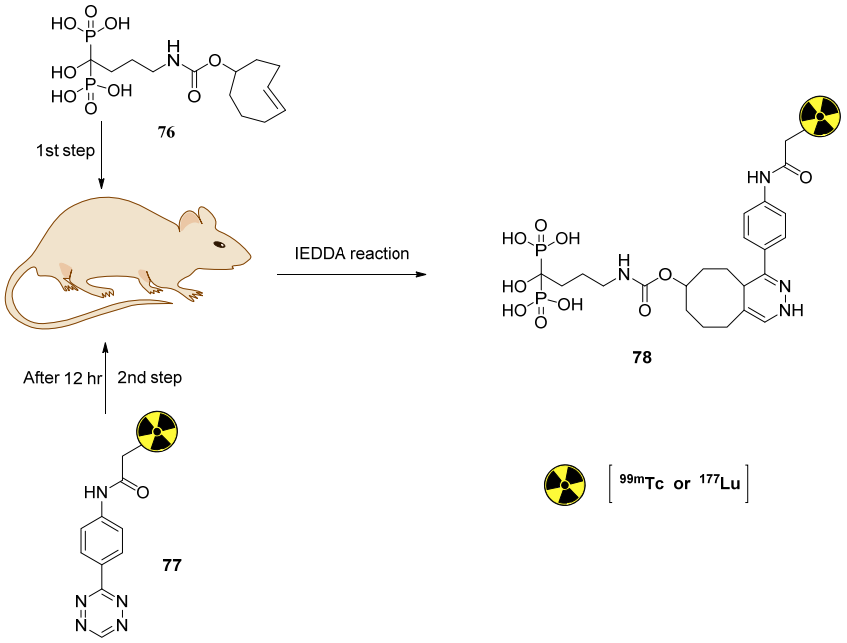
Radioisotopes for SPECT scan have also been applied to the pretar-

Table 4. Synthesis of Radioactive Iodine Labeled Radiotracers Using IEDDA

Entry	Tetrazine	Dienophile	Product	Ref
1	 **63**	 **64**	 **65**	[28]
2	 **66**	 anti-VEGFR2 (antibody) **67**	 anti-VEGFR2 (antibody) **68**	[29]
3	 **69**	 R= c(RGD) peptide **70**, HSA **71**	 R= c(RGD) peptide **72**, HSA **73**	[30]



Scheme 10. ***In vivo*** **pretargeted strategy using the radiolabeled tetrazine 75.**



Scheme 11. Tetrazine ligation between TCO-bisphosphonate 76 and radiolabeled tetrazine 77.

geted strategy. In 2010, Robillard group reported a pretargeted tumor imaging based on the IEDDA between ^{111}In-labeled tetrazine and a cancer targeting monoclonal antibody. In this work, a TCO bearing CC49 antibody **74** was first injected to colon cancer bearing xenograft model (Scheme 10). After 24 hours, ^{111}In or ^{177}Lu labeled tetrazine **75** was then injected to the same mice. SPECT images showed the specific delivery of radioisotope and enhanced tumor uptake values. Quantitative imaging analysis revealed that the tumor to normal tissue ratio was quite high[31]. Later, they reported a pretargeted radioimmunotherapy using the similar strategy. To achieve a high tumor to background ratio of the radioisotope, ^{177}Lu-labeled tetrazine tracers and TCO conjugated antibody CC49 **74** was tested in animal experiments. SPECT/CT scan in carcinoma xenografts indicated that significantly high tumor to background ratio and specific tumor images were obtained[32].

Recently, Valliant et al. demonstrated a pretargeted bone imaging and radiotherapy based on IEDDA between TCO conjugated bisphosphonate and radiolabeled tetrazine (Scheme 11). In the experiment, normal male mice were first injected with TCO-bisphosphonate **76** for accumulation of the dienophile in the skeleton. After 12 hours post injection, ^{177}Lu-labeled tetrazine for therapy or ^{99m}Tc-labeled tetrazine **77** for imaging were administrated intravenously. SPECT/CT study showed high radioactivity concentrations were imaged in the knees and shoulder. These results indicated that TCO-bisphosphonate can be utilized to target the functionalized tetrazine to bone tissues[33].

3. Conclusions

Decay half-life of radioisotope is normally considered as one of the most important factor for the optimization of radiochemical procedure. In addition, the site-specific labeling at a target molecule is highly desirable because randomly modified bioactive molecules often resulted in decreased their biological activity. To meet these requirements, IEDDA has been extensively investigated to develop advanced radiolabeling methods and moreover it can be a powerful alternative to existing radiolabeling strategies. Especially, IEDDA gave several promising results from pretargeted *in vivo* studies and therefore it is expected that this chemistry will be utilized for the specific molecular imaging and therapy of disease. Consequently, IEDDA will be continuously used as a keystone for the development of various radiopharmaceuticals which offer benefits across preclinical investigations and clinical applications.

Acknowledgment

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