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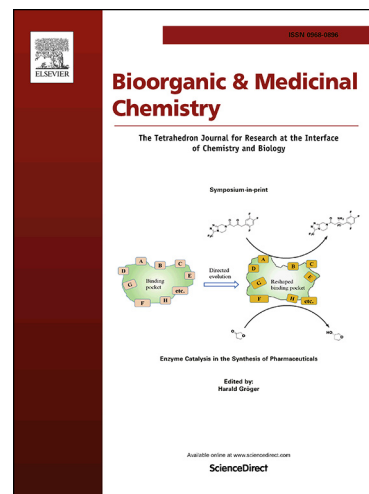
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Prosthetic groups for radioiodination and astatination of peptides and proteins: a comparative study of five potential bioorthogonal labeling strategies

Laurent Navarro, Marion Berdal, Michel Chérel, Frédéric Pecorari, Jean-François Gestin*, François Guérard*

CRCINA, Inserm, CNRS, Université d'Angers, Université de Nantes, Nantes, France

Abstract. ^{125}I - and ^{211}At -labeled azide and tetrazine (Tz) based prosthetic groups for bioorthogonal conjugation were designed and tested in a comparative study of five bioorthogonal systems. All five bioconjugation reactions conducted on a model clickable peptide led to quantitative yields within less than a minute to several hours depending on the system used. Transferability to the labeling of an IgG was demonstrated with one of the bioorthogonal system. This study provides several new alternatives to the conventional and suboptimal approach currently in use for radioiodination and astatination of biomolecules and should accelerate the development of new probes with these radionuclides for applications in nuclear imaging and targeted alpha-therapy.

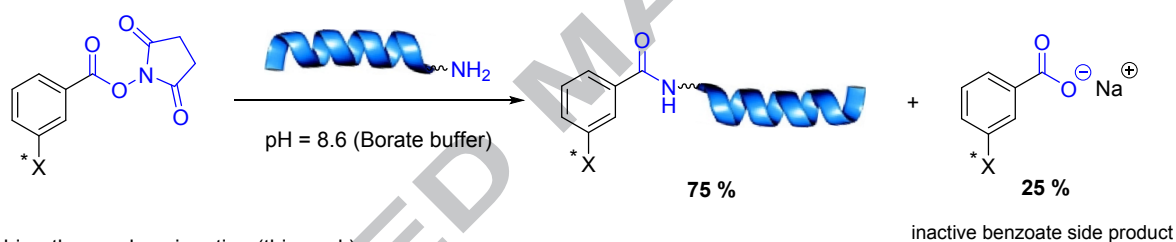
1. Introduction

Heavy radiohalogens astatine and iodine have been increasingly studied over the past decades for therapeutic or diagnostic purpose in nuclear medicine.¹ The most relevant iodine radioisotopes - ^{123}I (γ , $t_{1/2} = 13.2$ hours), ^{124}I (β^+ , $t_{1/2} = 4.18$ days), ^{125}I (γ , Auger e^- , $t_{1/2} = 59.4$ days) and ^{131}I (β^- and γ , $t_{1/2} = 8$ days) - can be used for imaging and/or therapy depending on the radiation they emit upon decay, whereas ^{211}At ($t_{1/2} = 7.2$ h, α -emitter) is a promising isotope for the treatment of small cell clusters or isolated tumor cells.^{2,3} The radioiodination strategy of relevant peptides and proteins has long been the direct electrophilic substitution on tyrosine. Despite the advantage of being a fast and simple procedure, this method exhibits limits for in vivo applications due to rapid deiodination that leads to radioiodine activity uptake in non-targeted organs (especially in thyroid and stomach). Consequently, more stable labeling strategies based on the use of a radioiodinated agent for acylation of lysine residues have been developed since then to overcome this issue.^{4,5} The lack of sufficient stability of direct electrophilic labeling with astatine is even more marked,⁶ and in this case, the use of an astatinated prosthetic group is essential to carry out any in vitro or in vivo experimentation. Thus, several astatinated agents have also been developed for conjugation to amino groups of lysine residues^{7,8} or more recently to cysteine⁹ in order to obtain sufficient label stability for biological experimentations.

The most used prosthetic groups to date are *N*-succinimidyl-3-[*I]iodobenzoate ([*I]SIB) or *N*-succinimidyl-3-[^{211}At]astatobenzoate ([^{211}At]SAB) which are comprised of an activated ester for conjugation to the lysine residues of proteins. The conjugation step requires a mildly basic aqueous solution ($\text{pH} \approx 8.5$) to make the amino group sufficiently reactive with the activated ester. However, competitive hydrolysis of the ester also occurs at this pH, leading to the

production of the inactive benzoate side product and to suboptimal conjugation yields (Figure 1a). In the most favorable cases, relatively good conjugation yields can be obtained by this approach (up to 75-80% on an intact IgG as reported by us recently¹⁰) but a minimum protein concentration of 4-5 mg/mL is necessary to favor lysine conjugation over competitive hydrolysis, a concentration that is not always compatible with antibodies at this pH due to aggregation and precipitation issues and which also limits the achievable specific activity. Recent developments in bioorthogonal click chemistry offer a growing choice of strategies to conjugate tags with high yields and specificity with biomolecules pre-modified with a complementary bioorthogonal handle. The strength of this chemistry is that it relies on the inertness or low reactivity of bioorthogonal functionalities with aqueous media and chemical groups naturally encountered in biological material.¹¹ It allows theoretically the bioconjugation of clickable tags within a short time with quantitative yields (Figure 1b). Click ligation of radioactive tags on various biomolecules are also the object of an important research effort to provide new strategies for labeling with radioisotopes that require fast, simple and efficient procedures that are difficult to reach by conventional chemistry.¹²

a) conventional lysine conjugation (ref 10)



b) bioorthogonal conjugation (this work)

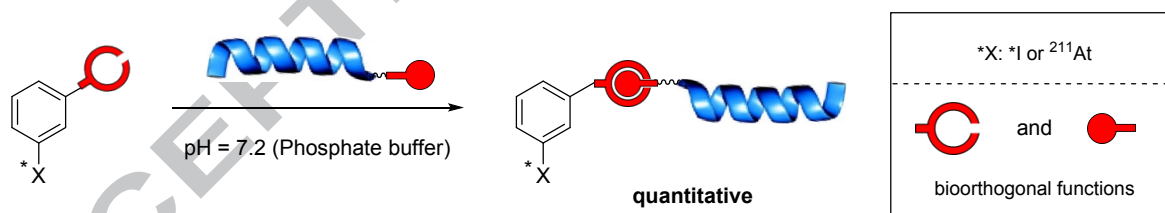


Figure 1. Bioconjugation strategies to form radiohalogenated proteins.

Recent reports have proposed the use of bioorthogonal chemistry for radioiodination of peptides or proteins with clickable prosthetic groups including the strain-promoted alkyne-azide cycloaddition (SPAAC)^{13,14} and the inverse electron demand Diels Alder cycloaddition (IEDDA) reactions,^{15,16} but to our knowledge, such strategy has not yet been reported for astatination. Yet, the necessity of fast, simple and quantitative yielding labeling procedure is even more essential for astatination with ²¹¹At because of its availability in low amounts, its shorter physical half-life compared to relevant iodine radioisotopes, and the marked radiolysis issues due to the astatine decay limiting the starting activity that can be engaged in a radiolabeling procedure,¹⁷ for the preparation of radiopharmaceuticals for clinical use, or even for animal studies.

In this context, we report herein the comparison of three or the most popular bioorthogonal reactions that we have adapted to the context of peptide or protein radiolabeling with iodine and astatine: i) the copper catalyzed alkyne azide cycloaddition (CuAAC), ii) the SPAAC, and iii) the IEDDA between tetrazines and strained alkynes or strained alkenes. On one hand, clickable radioiodinated and astatinated prosthetic groups bearing a tetrazine or an azide bioorthogonal handle were designed and synthesized from corresponding aryl iodonium salts. As reported recently, aromatic nucleophilic substitution (S_NAr) with aryl iodonium salts is an efficient strategy for the radiohalogenation of an aryl position, especially with astatine, since it is more robust than electrophilic pathways, and that purifications of labeled compounds are simplified.^{10,18} On the other hand, a simple tripeptide that was comprised of a lysine between two protected glycines was synthesized and conjugated via the amino group of the lysine to four bioorthogonal handles: i) a terminal alkyne for CuAAC, ii) a dibenzozazacyclooctyne (DIBAC) for SPAAC with azides (DIBAC/ N_3 -SPAAC), iii) a bicyclononyne (BCN) for either SPAAC with azide (BCN/ N_3 -SPAAC) or IEDDA with Tz (BCN/Tz-IEDDA) or iv) a *trans*-cyclooctene (TCO) for IEDDA with Tz (TCO/Tz-IEDDA) (Figure 2). The purpose was to identify the most adapted systems based on the following criteria: ease of access to the labeled clickable prosthetic groups, simplicity of execution, kinetics and yield of the bioorthogonal ligation. Results obtained herein on a model peptide are discussed in the perspective of protein radioiodination and astatination.

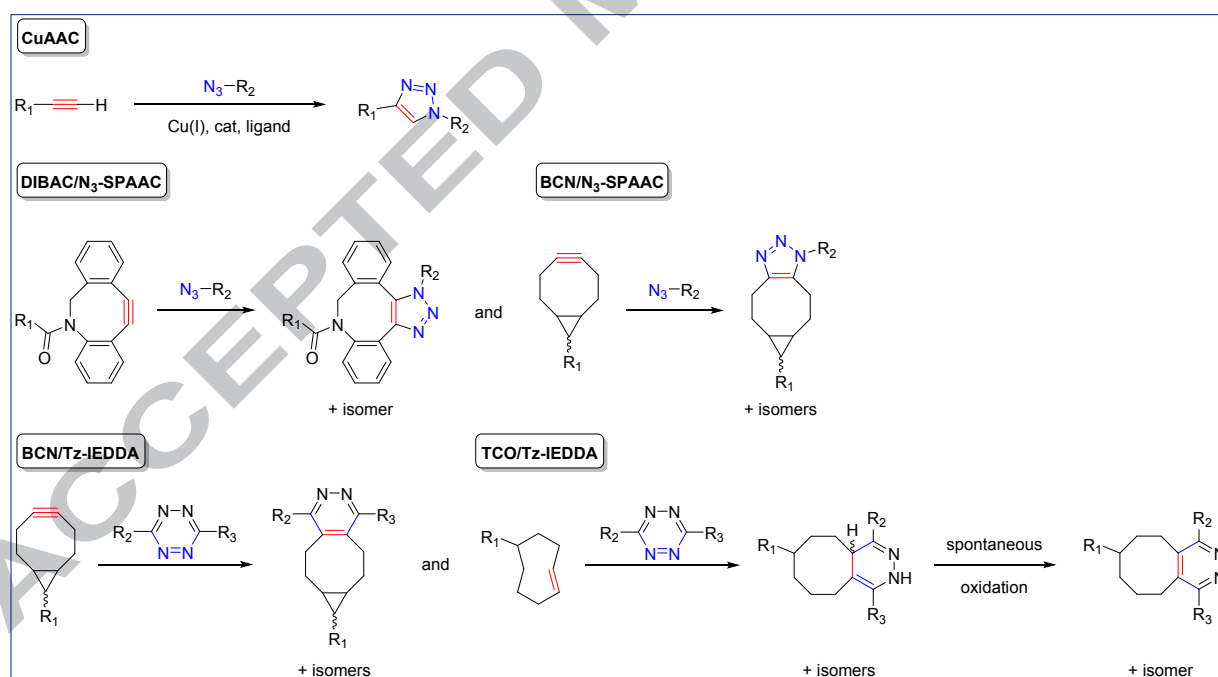
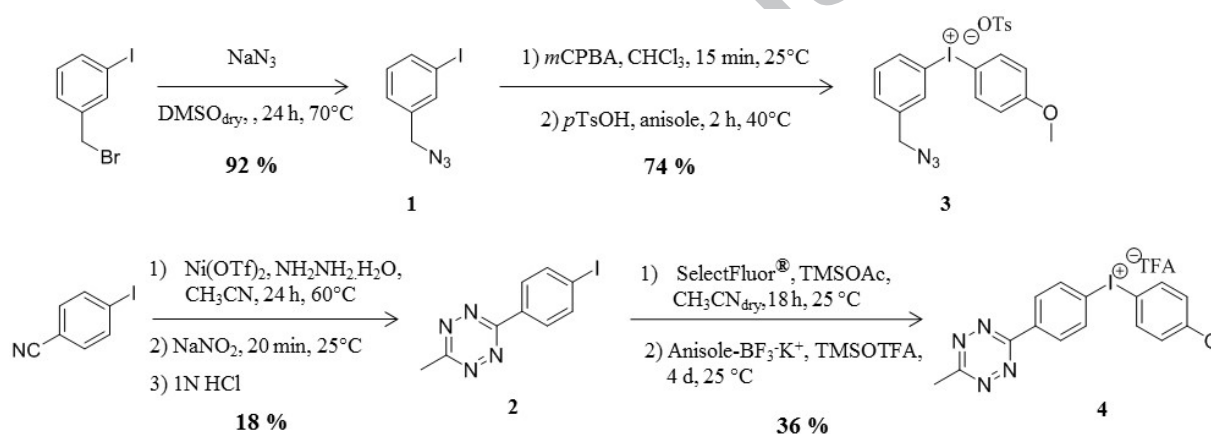


Figure 2. Bioorthogonal reactions evaluated in this report.

2. Results and discussion

2.1. Synthesis

Bifunctional precursors. In order to investigate CuAAC, SPAAC and IEDDA strategies, bifunctional precursors were designed as small aromatic compounds with functionalities allowing on one hand the substitution by the radiohalogen by S_NAr , and on the other hand the bioorthogonal ligation by one of the five reactions investigated. Aryliodonium tosylates were generated in the last synthesis step by oxidation of the corresponding iodoarenes with *m*CPBA followed by electrophilic substitution with anisole in the presence of tosic acid. The benzylazide iodonium precursor **3** was obtained in good yield whereas in the case of the tetrazine, no iodonium could be formed in sufficient yield for isolation by this approach, probably because of the strong deactivation of the aromatic ring. An alternative approach by oxidative diacetoxylation using Selectfluor[®] reported recently by Qin et al¹⁹ provided the expected compound **4** in moderate (36%) but sufficient yield (Scheme 1).

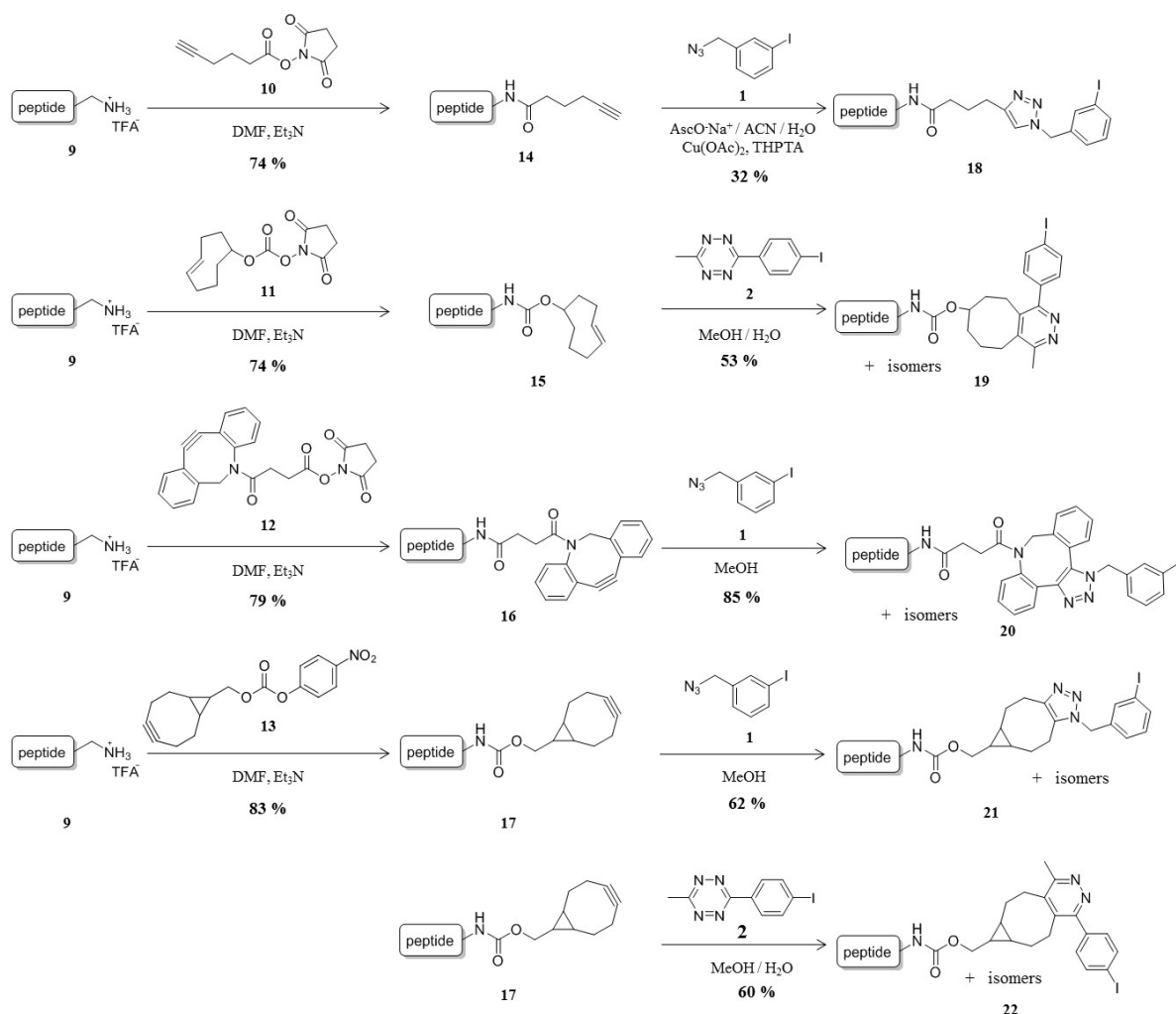


Scheme 1. Synthesis of bifunctional aryliodonium precursors.

Clickable model peptides and cold iodinated references. For the comparative study of the different bioorthogonal strategies investigated, we chose to synthesize a simple tripeptide consisting in a lysine between two protected glycines (peptide **9**). It was prepared by conventional peptide synthesis methods as a triflate salt of the free amino group (synthetic details in ESI). This peptide was designed as the most simple way to mimic a lysine on which bioconjugation by acylation is usually performed on proteins.

Four bioorthogonal groups complementary to the azide or to the tetrazine groups were then conjugated, resulting in clickable peptides **14**, **15**, **16**, **17** conjugated respectively to a terminal alkyne for CuAAC with the azide, TCO for TCO/Tz-IEDDA, DIBAC for DIBAC/ N_3 -SPAAC, or BCN for BCN/ N_3 -SPAAC or BCN/Tz-IEDDA, with yields ranging from 74 to 83%.

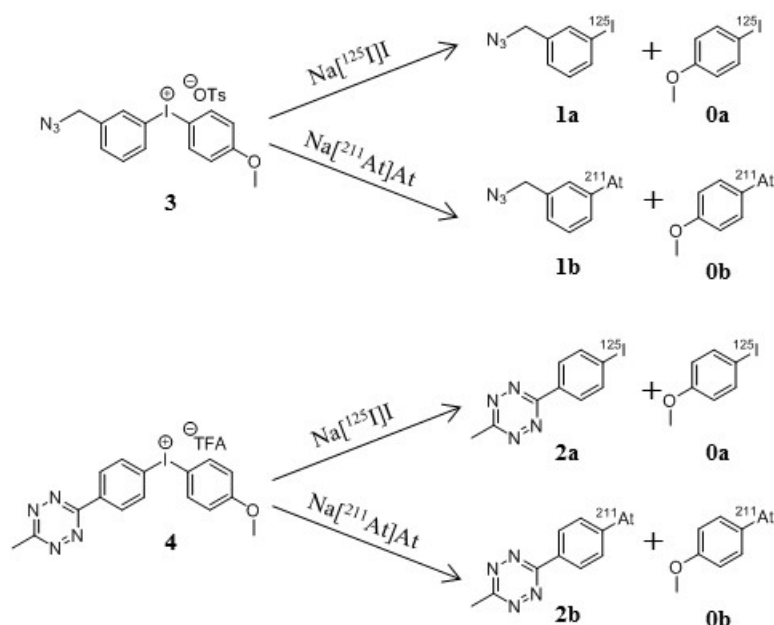
All four clickable peptides were then subjected to cycloaddition with the cold iodinated benzylazide **1** and/or methylphenyltetrazine **2**. Conversion was quantitative, resulting in five cold clicked peptides **18**, **19**, **20**, **21**, **22** with yields ranging from 32 to 85% after chromatographic purification and that were used as references for chromatographic analysis with their radioiodinated and astatinated analogues (Scheme 2, synthetic details given in ESI).



Scheme 2. Synthesis of clickable model peptides and corresponding cold iodinated clicked references. AscONa = sodium ascorbate; THPTA = Tris(3-hydroxypropyltriazolylmethyl)amine.

2.2. Radiochemistry

Labeling study of both iodonium precursors **3** and **4** with ^{125}I and ^{211}At was performed starting from conditions determined previously with other arylodonium salts, i.e., in acetonitrile with heating for 30 min,¹⁸ leading to the formation to the expected clickable radiohalogenated products (**1a**, **1b**, **2a**, **2b**) in association with a small ratio of the radiohalogenoanisole side product **0a/0b** (Scheme 3).



Scheme 3. Radioiodination and astatination of aryliodonium precursors **3** and **4**.

Similar to our previous reports,^{18,10} astatide was significantly more reactive than iodide, with lower temperatures required to reach a quantitative conversion, and a regioselectivity of substitution somewhat lower than for radioiodination (Table 1). For the synthesis of the astatinated azide derivative **1b**, the RCY was improved by changing the solvent to methanol (86% at 80°C instead of 73% in CH₃CN). Again, improved RCY in methanol is typical with astatide whereas this solvent inhibits the reaction with iodide.¹⁰ Due to the higher activation of the aryl substituted with the methyltetrazine, lower temperatures were required to form **2a** and **2b** and better regioselectivities were achieved.

Table 1. Influence of the temperature on the radiolabeling yield (RCY) of aryliodonium salts **3** and **4** reacted for 30 min with Na[¹²⁵I]I or Na[²¹¹At]At in CH₃CN. RCYs based on HPLC analyses of the crude products.

Precursor	T(°C)	¹²⁵ I (RCY %)		²¹¹ At (RCY %)	
		1a or 2a	0a	1b or 2b	0b
3	60	-	-	58	9
	80	20	2	73/86 ^a	14/13 ^a
	100	63	4	-	-
	120	82	7	-	-
4	40	-	-	86	10
	60	20	1	85	11
	80	60	2	-	-
	100	96	3	-	-

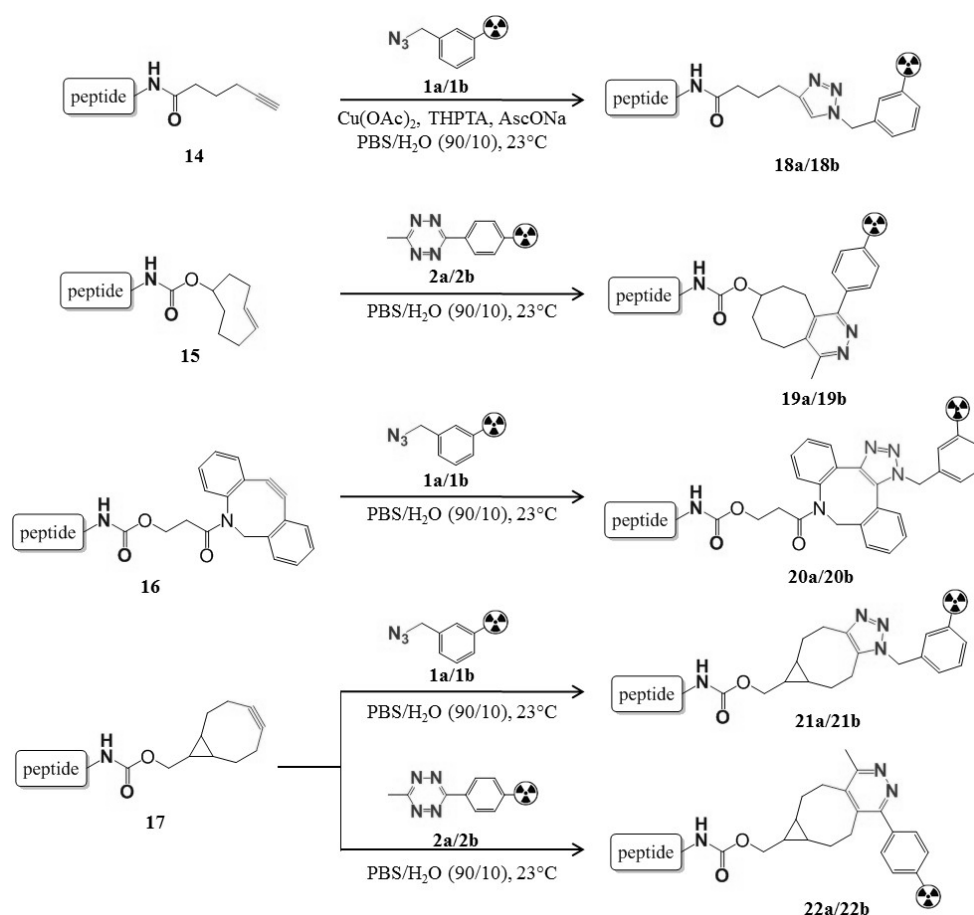
^a Solvent = methanol

Purification of the azide derivatives **1a** and **1b** was simple and fast and consisted in an evaporation of the labeling mixture to dryness followed by an extraction with diethyl ether.

Precursor **3** being insoluble in this solvent, only the product and the side products **0a/0b** were extracted. After a new evaporation to dryness, chromatographic analysis indicated the presence of **1a** or **1b** only, the anisole derivative being evaporated during the drying step (See radiochromatograms in ESI). Overall, **1a** and **1b** were obtained with a RCY of $65 \pm 5\%$ and $64 \pm 6\%$ respectively. Due to the low but non-negligible solubility of iodonium salt **4** in Et_2O , the same purification method could not be used. In this case, **2a** and **2b** were recovered by filtration on a short silica cartridge on which the iodonium salt was retained when using AcOEt as eluent. **2a** and **2b** were obtained as mixtures with anisole derivatives **0a** and **0b**, respectively, which were again eliminated during the subsequent evaporation step, providing **2a** and **2b** with RCY of $71 \pm 5\%$ and $76 \pm 4\%$.

2.3. Bioorthogonal conjugation

The four clickable peptides (**14**, **15**, **16**, **17**) and the two radioiodinated or astatinated clickable compounds **1a/1b** and **2a/2b** resulted in five bioorthogonal combinations (Scheme 4) whose reaction kinetics and yields were compared. In order to get an estimate of yields and kinetics that would be obtained when labeling relevant biomolecules such as a monoclonal antibody (mAb), we chose to fix the peptide concentration at $200 \mu\text{M}$ in phosphate-buffered saline (PBS). Such a concentration would be, in terms of bioorthogonal handles concentration, the equivalent of a 5 mg/mL mAb grafted with 6 bioorthogonal handles per mAb. 5 mg/mL is in the range of concentration usually required to perform conventional bioconjugation of radiohalogenated prosthetic groups on mAb lysine residues.^{20,21} We have thus hypothesized that if high bioorthogonal ligation RCYs (i.e. $> 75\%$) could be obtained with $200 \mu\text{M}$ peptide as initial condition, similar results could be reachable with at least 5 mg/mL mAb. This would then mean that this approach is potentially at least as good as the conventional one in terms of resulting specific activity and amount of starting mAb needed, or even better if lower mAb concentrations can be also used.



Scheme 4. Conjugation of radiohalogenated clickable prosthetic groups with model clickable peptides. AscONa = sodium ascorbate; THPTA = Tris(3-hydroxypropyltriazolylmethyl)amine.

Interestingly, all of the 5 bioorthogonal systems lead to RCYs > 99% with the radioiodinated and the astatinated compounds (Figure 3). As expected the TCO/Tz-IEDDA system was the fastest, providing quantitative RCYs within less than a minute. After optimization of the conditions (Cu^+ and THPTA ligand concentrations) the CuAAC between peptide **14** and azides **1a** or **1b** was the second fastest system with quantitative yields within 5-10 min followed by the BCN/Tz-IEDDA reaction between peptide **17** and the Tz-labeled compounds **2a** and **2b** (RCY > 99% after 10-15 min). The DIBAC/ N_3 -SPAAC and BCN/ N_3 -SPAAC reactions between azides (**1a/1b**) derivatives and peptides **16** and **17** provided the slowest kinetics with RCY > 99% reached after respectively 45-60 min and several hours.

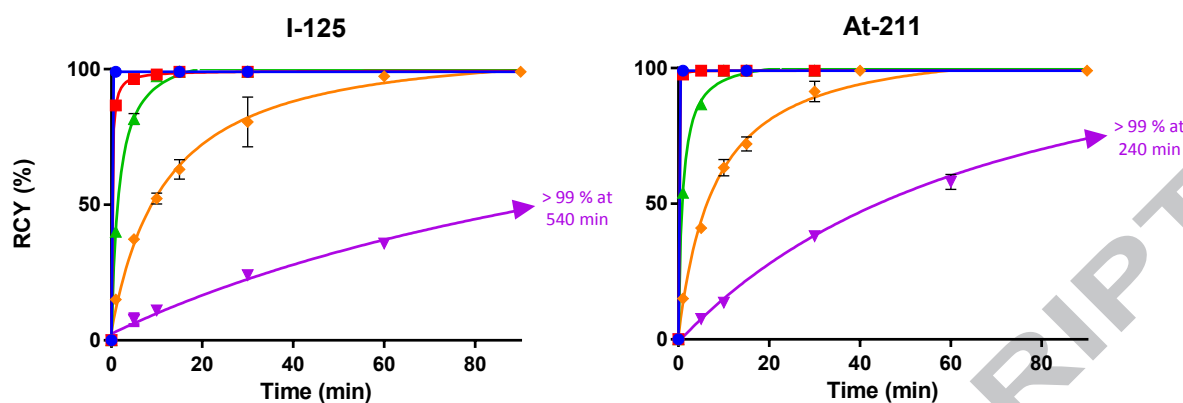


Figure 3. Kinetics of bioorthogonal conjugation between clickable peptides and radiohalogenated prosthetic groups ($n = 3$): CuAAC (■); DIBAC/ N_3 -SPAAC (◆); BCN/ N_3 -SPAAC (▼); BCN/Tz-IEDDA (▲); TCO/Tz-IEDDA (●). RCYs based on the HPLC analysis of the crude product.

Surprisingly, the cycloaddition reactions performed faster with astatinated compounds than with radioiodinated ones. Although not clearly visible in the fastest reactions, it was particularly marked in the BCN/ N_3 -SPAAC system with a reaction $t_{1/2} \approx 110$ min with radioiodine and $t_{1/2} \approx 45$ min with astatine. We investigated if the difference of lipophilicity between the iodinated and the astatinated compounds could be the origin of the kinetics differences since it has been reported previously that lipophilicity of reagents can have an impact on cycloaddition reaction rates performed in aqueous medium, with higher kinetics for the most lipophilic compounds that are forced into close proximity by hydrophobic interactions between reagents.²² Partition coefficients in water/octanol were thus determined for compounds **1a** and **1b** (procedure details in ESI). Although the lipophilicity of the astatinated compound was as expected the highest ($\log P = 2.47 \pm 0.03$), the difference with the radioiodinated one ($\log P = 2.38 \pm 0.05$) was not significant enough to comfort this hypothesis. Given the very high dilution of astatinated species used in this kinetic study (about 1 MBq/100 μ L \approx 0.01 ppb), catalysis by trace impurities associated to the ^{211}At production or generated by radiolysis of the solvent by the highly ionizing α -decay may have contributed to the accelerated kinetics with compound **1b**.

Overall, four out of the five tested bioconjugation systems exhibited reaction kinetics and RCYs compatible with the use of radioiodine isotopes and especially with the relatively short half-life of ^{211}At (BCN/ N_3 -SPAAC being too slow). However, the fastest may not necessarily be the best. Indeed, while TCO/Tz-IEDDA provided quantitative cycloaddition yields within few seconds, it resulted in two dihydropyrazine tautomers that converted spontaneously within several hours to the corresponding aromatic pyrazine (see representative radiochromatograms in ESI), a phenomenon that has been reported previously with similar compounds.^{16,23} This may raise concern regarding the lack of homogeneity of the resulting radiopharmaceutical and the consequence on pharmacokinetics, in vivo degradation of the conjugation bonds and on the carbon-radiohalogen bond stability. On the other hand, CuAAC, the second fastest system, provides the advantage of requiring fewer atoms than other systems (2 carbon and 3 nitrogen atoms), and resulting in a more hydrophilic product, a triazole core

found in many naturally occurring biological compounds. It is thus expected that the risk of altering the *in vivo* behavior of the resulting radioimmunoconjugate should be lower than with other tested systems. It must however be kept in mind that the presence of copper, and especially Cu(I) species, in the reaction media potentially generates reactive oxygen species able to alter peptides and proteins by oxidative side reactions even when a stabilizing ligand is used to lower the Cu(I) concentration required.²⁴ Extensive optimization may thus in some case be necessary to achieve high RCYs with preservation of the biomolecule activity. While these bioorthogonal systems were tested on a model peptide, we wanted to probe if the results obtained could be straightforwardly transferred to the labeling of relevant macromolecules such as antibodies. For this, the 9E7.4 IgG, a mAb developed by our group and directed against murine CD138 for targeting multiple myeloma cells²⁵ was modified by bioconjugation with a DIBAC derivative (DBCO-NHS ester) via its lysine residues. The resulting DIBAC-9E7.4 was then subjected to incubation with the astatinated azido derivative **1b**. With only 0.21 ± 0.05 DIBAC grafted per mAb, DIBAC concentration in solution was significantly lower than in peptide study ($3 \mu\text{M}$ with 2.3 mg/mL mAb vs $200 \mu\text{M}$ with the model peptide). Yet, the reaction proceeded similarly to our model with a high RCY of 90% within less than 40 min at room temperature. The immunoreactive fraction ($82 \pm 5 \%$) was similar to results obtained when astatination of this mAb is performed by the classical lysine acylation approach ($86 \pm 2\%$).¹⁰ This preliminary test on a biologically relevant biomolecule is a good indication that the different systems described in this study on a model peptide should be easily transferable for further biological investigation.

3. Conclusion

Bioorthogonal chemistry has been applied to an increasing number of biomedical applications and can provide new solutions to old problems. Meanwhile, it appears necessary to investigate alternative approaches to the conventional bioconjugation reactions developed three decades ago for labeling proteins and peptides with radioiodine and astatine. However, given the plethora of bioorthogonal system developed over the past decade, a selection and a comparison of such systems appeared necessary to us before developing specific tracers labeled by such a strategy. We showed that of the five systems tested, all provided quantitative RCYs on a model peptide, with four of them in a reasonably short time in the perspective of peptides or proteins labeling with ^{211}At ($t_{1/2} = 7.2 \text{ h}$). The choice of the optimal system will depend on the biomolecule to be labeled. Despite its impressive reaction kinetics, the TCO/Tz-IEDDA may lead to inhomogeneous product, the CuAAC has the advantage of leading to a more biocompatible ligation product (a triazole) with very fast kinetics, but the presence of copper may also be a concern regarding degradation of biomolecules to be labeled. Finally the BCN/Tz-IEDDA and the DIBAC/N₃-SPAAC might be the best compromise in several situations since, despite the slower but still largely acceptable reaction kinetics for labeling with radioiodine or ^{211}At , the limits cited above are not observed. Further developments with relevant targeting biomolecules are warranted with a specific care on their *in vivo* fate (impact on biodistribution and radiolabeling stability). Such results will be reported in due course.

4. Experimental section

4.1. Synthesis

4.1.1. General.

All reagents and solvents were obtained commercially and used without further purification unless otherwise noted. 3-iodobenzylazide (**1**) and 3-(4-iodophenyl)-6-methyl-1,2,4,5-tetrazine (**2**) were obtained by the method described respectively by Chun et al.²⁶ and Yang et al.²⁷ respectively. ¹H, ¹⁹F and ¹³C NMR spectra were recorded with a Bruker AC spectrometer at 400 (¹H), 376 (¹⁹F) and 100 (¹³C) MHz. Chemical shifts (δ) are reported in part per million (ppm) relative to tetramethylsilane (TMS) and residual solvent (CDCl₃: 7.26 ppm, DMSO-*d*₆: 2.50 ppm, CD₃CN: 1.94 ppm). The multiplicity is described by the symbols s (singlet), d (doublet), t (triplet), q (quartet), dt (doublet of triplet) and m (multiplet). Reactions were monitored by thin-layer chromatography (TLC) using 60 F₂₅₄ silica gel plates on a plastic support (Merck) and revealed either by UV lamp (254 nm), iodine, ninhydrin (10 mg/mL in EtOH) or with vanillin (10 mg/mL in H₂SO₄:EtOH (4:1)). Purifications were carried out using a Puriflash 600 (Interchim) with 30 μ m silica pre-packed columns. Mass spectrometry analyses were carried out on a CMS Expression apparatus (Advion) with electrospray ionization in positive and/or negative mode and equipped with a quadrupole analyzer. High-resolution mass spectrometry (HRMS) analyses were performed on a Synapt G2 HRMS Q-TOF mass spectrometer (Waters) equipped with an electrospray ionization (ESI) interface operating in the positive mode. HPLC analyses were carried out on a Waters Alliance e2695 HPLC system equipped with a C-18 column (Spherisorb ODS2 5 μ 4.6 mm x 25 cm, Waters) with the flow rate set at 1.50 mL/min with the following gradient: t = 0: 60% A, 40% B; t = 15 min: 100% B; with A = H₂O with 0.05% TFA and B = CH₃CN with 0.05% TFA.

The use of molecules with azide functions requires precautions: organic azides present a risk of explosion and must therefore be handled with care, in particular by avoiding high temperatures, shocks and frictions and synthesized in small quantities. Especially, if the C/N ratio is less than 1, they should never be isolated or concentrated.

4.1.2. (3-(azidomethyl)phenyl)(4-methoxyphenyl)iodonium tosylate (**3**)

To a solution of 3-iodobenzylazide (**1**) (200 mg, 0.77 mmol) in CHCl₃ (10 mL) was added *m*-chloroperbenzoic acid (193 mg, 0.86 mmol, 1.1 eq). The solution was stirred at room temperature for 15 minutes. Then, *p*-toluenesulfonic acid monohydrate (170 mg, 0.88 mmol, 1.1 eq) and anisole (424 mg, 3.88 mmol, 5 eq) were added and the solution was stirred for 2 hours at 40°C. The reaction mixture was concentrated in vacuo and the obtained residue was triturated in cold Et₂O (20 mL) until obtaining a white solid (306 mg, 74 %) / ¹H NMR (400 MHz, CD₃CN): δ 7.40-7.34 (m, 4H), 6.93 (d, 1H, 7.6 Hz), 6.82-6.78 (m, 3H), 6.47 (d, 2H, 8 Hz), 6.32 (d, 2H, 8.8 Hz), 3.77 (s, 2H, CH₂), 3.17 (s, 3H, CH₃), 1.69 (s, 3H, CH₃) / ¹³C NMR (100 MHz, CD₃CN): δ 163.8, 145.4, 141.1, 140.1, 138.5, 135.3, 135.2, 132.9, 132.6, 129.4, 126.6, 118.5, 116.8, 104.7, 56.6, 54.0, 21.3 / t_R: 7.47 min / HRMS: calculated for C₁₄H₁₃N₃OI M(+) and C₃₅H₃₃N₆O₅SI₂⁺ 2M+TsO⁻(+): 366.0103 and 903.0315, found : 366.0115 and 903.0327.

4.1.3. (4-methoxyphenyl)(4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenyl)iodonium trifluoroacetate (**4**)

To a degassed solution of 3-(4-iodophenyl)-6-methyl-1,2,4,5-tetrazine (**2**) (0.167 g, 0.53 mmol) in dry acetonitrile (15 mL) was added dropwise a solution of trimethylsilyl acetate (216 mg, 1.59 mmol, 3 eq) and SelectFluor® (277 mg, 0.745 mmol, 1.4 eq) in dry acetonitrile (10 mL). After 24h stirring at room temperature under argon, potassium (4-methoxyphenyl) trifluoroborate (130 mg, 0.583 mmol, 1.1 eq) and trimethylsilyl trifluoroacetate (105 μ L, 0.583 mmol, 1.1 eq) were added and the resulting mixture was stirred under argon for 96 hours at room temperature. The reaction mixture was concentrated in vacuo and the obtained residue was triturated in cold Et₂O (20 mL) until obtaining a purple solid (99,3 mg, 36 %). ¹H NMR (400 MHz, CD₃CN): δ 8.59 (d, 2H, meta, 8.8 Hz), 8.23 (d, 2H, ortho, 8.8 Hz), 8.06 (d, 2H, ortho, 9.2 Hz), 7.08 (d, 2H, meta, 9.2 Hz), 3.84 (s, 3H, CH₃), 3.05 (s, 3H, CH₃). ¹³C NMR (100 MHz, CD₃CN): δ 169.2, 164.5, 163.9, 138.9, 137.4, 136.5, 119.3, 118.3, 66.3, 56.8, 21.53, 15.6. ¹⁹F NMR (376 MHz, CD₃CN): δ -80.0 (CF₃COO⁻, 80%), -154.7 (BF₄⁻, 20%) / t_R: 7.82 min / HRMS: calculated for C₁₆H₁₄N₄OI M(+): 405.0212, found : 405.0219.

4.2. Radiochemistry

4.2.1. General

Na[¹²⁵I]I was obtained commercially from Perkin Elmer in 10⁻⁵ M NaOH solution with a volumic activity of 50 μ Ci/ μ L (1.85 MBq/ μ L). ²¹¹At was produced at the Arronax cyclotron facility using the ²⁰⁹Bi(α ,2n)²¹¹At reaction and recovered from the irradiated target in chloroform using a dry-distillation protocol adapted from the procedure previously reported by Lindegren et al.²⁸ Radio-HPLC analyses were performed using the system described in §4.1.1 equipped with a Flow Star LB 513 radioactivity detector (BERTHOLD Technologies). Non-radioactive iodinated compounds were analyzed using this HPLC system and their retention times (given in Table S1) were used as references for identification of their radioiodinated and astatinated analogues.

4.2.2. Radioiodination of arylidonium salts **3** and **4**

A stock solution of Na[¹²⁵I]I was prepared by diluting the commercial solution twelve times in de-ionized water. To a solution of diluted Na[¹²⁵I]I (5 μ L, 770 kBq) was added the idonium salt solution (95 μ L, 5 mM) in CH₃CN. The solution was heated to the selected temperature for 30 minutes. After return to room temperature, an aliquot was withdrawn (\approx 500 kBq) and diluted in a 3:2 H₂O/CH₃CN solution (100 μ L) and analyzed by reverse-phase HPLC. Aromatic ¹²⁵I-species were identified by comparison with the retention indexes of the non-radioactive iodinated compound.

4.2.3. Astatination of arylidonium salts **3** and **4**

A stock solution of Na[²¹¹At]At was prepared as follow: the chloroform ²¹¹At solution was evaporated to dryness under a gentle stream of nitrogen and redissolved in an appropriate volume of a 10 mg/mL sodium sulfite aqueous solution to obtain a volumic activity of about 400 kBq/ μ L. To a solution of sodium astatide (5 μ L, \approx 2 MBq) was added a solution of idonium salt (95 μ L, 5 mM) in the selected solvent (CH₃CN or MeOH). The solution was heated to the selected temperature for 30 minutes. After return to room temperature, an aliquot was withdrawn (\approx 500 kBq) and diluted in a 3:2 H₂O/CH₃CN solution (100 μ L) and analyzed by reverse-phase HPLC. Aromatic ²¹¹At-species were identified by comparison of the retention indexes of the non-radioactive iodinated compounds with an offset of \approx 0.1 min.

4.2.4. Purification of radioiodinated and astatinated benzylazide compounds **1a/1b**

After labeling, the solution was evaporated to dryness and the radioiodinated (**1a**) or astatinated (**1b**) compound was extracted with 2 x 50 μL of Et_2O (extraction efficiency: 80-90%). Et_2O was then evaporated at 40°C under a gentle stream of nitrogen. After all solvent was removed, additional drying for about five minutes allowed the complete removal of anisole derivatives **0a/0b**. The radiolabeled compound was then taken up in MeOH and the purity was measured with an aliquot diluted in a 3:2 $\text{H}_2\text{O}/\text{CH}_3\text{CN}$ solution (100 μL , \approx 600 kBq) and analyzed by reverse-phase HPLC.

4.2.5. Purification of radioiodinated and astatinated aryltetrazine compounds **2a/2b**

After labeling, the solution was evaporated to dryness and the radioiodinated (**2a**) or astatinated (**2b**) compound was recovered by filtration on a disposable Sep-Pak Vac 3cc (500 mg) silica cartridges (Waters) on which the iodonium salt was retained when using AcOEt (600 μL) as eluent. **2a** and **2b** were obtained as mixtures with anisole derivatives **0a** and **0b**, respectively. AcOEt was then evaporated at 40°C under a gentle stream of nitrogen. After all solvent was removed, additional drying for about five minutes allowed the complete removal of anisole derivatives **0a/0b**. The radiolabeled compound was taken up in MeOH and the purity was measured with an aliquot diluted in a 3:2 $\text{H}_2\text{O}/\text{CH}_3\text{CN}$ mixture (100 μL , \approx 600kBq) and analyzed by reverse-phase HPLC.

4.3. Clickable peptide radiolabeling

4.3.1. Radiolabeling of clickable peptides **15**, **16**, **17** with **1a/1b** or **2a/2b** (SPAAC and IEDDA)

To a solution of clickable peptide **15**, **16** or **17** (90 μL , 200 μM) in PBS/MeOH (99:1) was added a solution of iodinated (**1a** or **2a**) or astatinated (**1b** or **2b**) compound (1.5 MBq, 10 μL) in MeOH and the solution was stirred at room temperature. An aliquot diluted in a 3:2 $\text{H}_2\text{O}/\text{CH}_3\text{CN}$ solution (100 μL , 600kBq) was analyzed at the times chosen by reverse-phase HPLC. Products were identified by comparison of the retention indexes of the non-radioactive iodinated clickable peptides.

4.3.2. Radiolabeling of clickable peptide **14** with **1a/1b** (CuAAC)

To a solution of clickable peptide **14** (9 μL , 2.3 mM) in PBS/MeOH (99:1) was added 58.5 μL of de-ionized water, aqueous sodium ascorbate (7.5 μL , 15 mM), aqueous tris(3-hydroxypropyl)triazolylmethylamine ligand (THPTA) (5 μL , 25 mM), aqueous $\text{Cu}(\text{OAc})_2$ (10 μL , 20 mM) and a solution of iodinated (**1a**) or astatinated (**1b**) compound (1.5 MBq, 10 μL) in MeOH. The solution was stirred at room temperature and an aliquot diluted in a 3:2 $\text{H}_2\text{O}/\text{CH}_3\text{CN}$ solution (100 μL , 600 kBq) was analyzed at the times chosen by reverse-phase HPLC. Products were identified by comparison of the retention indexes of the non-radioactive iodinated clickable peptides.

4.4. 9E7.4- IgG modification with DIBAC

To a solution of 9E7.4-IgG (320 μL , 4.5 mg/mL, produced as reported previously²⁵) in borate buffer (0.3 M, pH 8.6) was added a solution of DBCO-NHS Ester (click Chemistry Tools) (15 μL , 10 mM, 15 eq) in DMSO. After 60 min of incubation at 23°C , excess of DBCO-NHS Ester was removed by filtration on centrifugal filters (Amicon[®] Ultra-2mL-30K) with PBS and the obtained 9E7.4-DIBAC was stored (2.3 mg/mL) at 4°C .

To determine the average number of reactive DIBAC groups per antibody, a solution of Cy5-azide (0.1 to 2 eq.) in methanol (2 μL) was added to a solution of 9E7.4-DIBAC (16.4 pmol) in PBS (98 μL). The reaction mixture was stirred at 23°C overnight and an aliquot was deposited on a ITLC-SG strip which was eluted with MeOH. The fractions of Cy5-Azide

bound to the IgG (bottom of ITLC-SG) and unbound (top of ITLC-SG) were determined by integrating the area of each spot using a BioRad ChemidocTM Imaging system in fluorescence detection mode. A ratio of 0.21 ± 0.05 DIBAC/IgG was measured.

4.5. 9E7.4-DIBAC radiolabeling with 1b

To **1b** in MeOH (10 μ L), prepared as described above, was added 9E7.4-DIBAC (60 μ L of a 2.3 mg/mL solution in PBS). After up to 40 min of incubation at 23°C, the conjugation yield was assessed by elution of an aliquot deposited on an ITLC-SG strip (methanol as eluent), and integration of the plate using a Cyclone phosphorimager scanner (Perkin Elmer). Purification was performed by gel filtration on a Sephadex G-25 resin loaded column (PD-10, GE healthcare) using PBS as eluent, affording the purified radiolabeled antibody with a >99% radiochemical purity as assessed by ITLC-SG.

4.6. Immunoreactivity assay

The immunoreactive fraction of **1b**-DIBAC-9E7.4 was determined using magnetic beads (Pierce, Thermo Scientific) labeled with a 40 amino acids peptide recognized by the 9E7.4 antibody according to the supplier's protocol. One picomole of radiolabeled 9E7.4 was incubated for 15 min at room temperature with 20 μ L of coated magnetic beads (10 mg/mL). Using a magnetic rack, supernatants containing non-reactive antibodies and magnetic beads were collected separately and the radioactivity in each fraction was measured in a gamma counter. Nonspecific binding was measured with the same protocol except that magnetic beads were coated with bovine serum albumin (BSA), and was below 5%.

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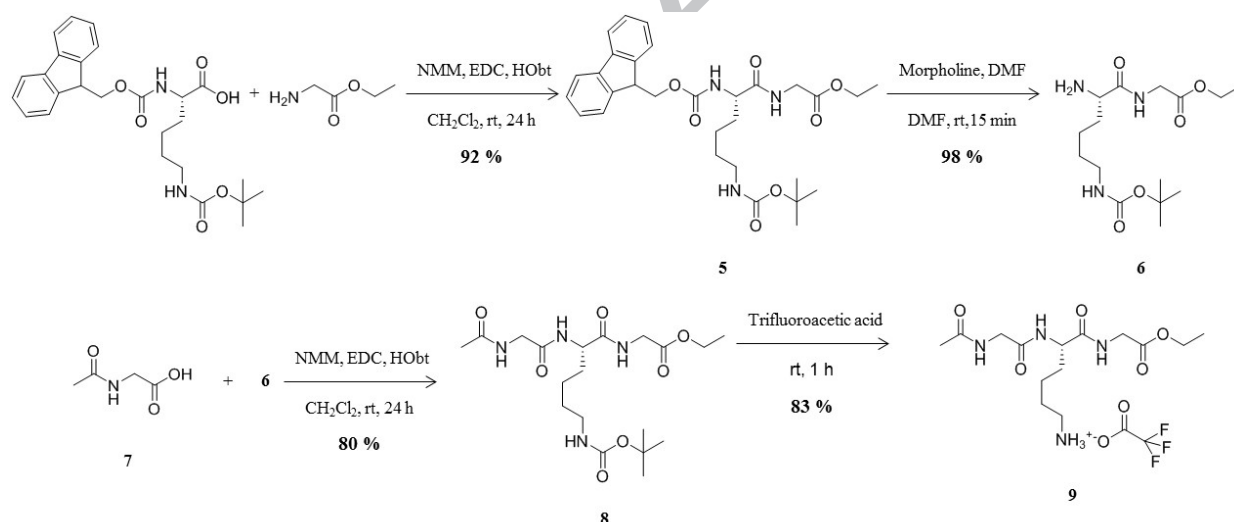
Electronic Supplementary Information

Prosthetic groups for radioiodination and astatination of peptides and proteins: a comparative study of five potential bioorthogonal labeling strategies

Laurent Navarro, Marion Berdal, Michel Chérel, Frédéric Pecorari, Jean-François Gestin*, François Guérard*

1) SYNTHESIS

1.1) Model peptide



***N*_α-Fmoc-*N*_ε-Boc-lysylglycine ethyl ester (5).** To a solution *N*_α-Fmoc-*N*_ε-Boc-lysine (9.99 g, 20.9 mmol) in anhydrous CH₂Cl₂ (150 mL) was added in the following order: *N*-methylmorpholine (5.80 mL, 52.3 mmol, 2.5 eq), *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDC) (6.13 g, 31.4 mmol, 1.5 eq), HOBT (4.37 g, 31.4 mmol, 1.5 eq) and ethylglycinate hydrochloride (3.54 g, 25.1 mmol, 1.2 eq). The solution was stirred for 24 hours under argon at room temperature. The reaction mixture was then washed with a 0.2 M aqueous HCl solution (75 mL), a 1 M aqueous Na₂CO₃ solution (50 mL) and H₂O (2 x 75mL). The organic layer was dried over MgSO₄ and concentrated under reduced pressure. The crude product was purified on silica gel (CHCl₃/MeOH as eluent) to give the desired compound as white solid (10.6 g, 92 %). ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.32 (s, 1H, NH), 7.89 (d, 2H, 7.6 Hz), 7.75-7.72 (m, 2H), 7.51 (s, 1H, NH), 7.42 (t, 2H, 7.2 Hz), 7.33

(t, 2H, 7.2 Hz), 6.78 (s, 1H, NH), 4.31-4.19 (m, 3H), 4.07 (q, 2H, 7.2 Hz), 4.02-3.98 (m, 1H, CH), 3.90-3.74 (m, 2H, CH₂), 2.89 (m, 2H), 1.68-1.51 (m, 2H, CH₂), 1.46-1.22 (m, 13H), 1.18 (t, 3H, CH₃) / ¹³C NMR (100 MHz, DMSO-*d*₆): δ 172.6, 169.7, 156.0, 155.6, 143.8, 140.8, 139.5, 137.4, 128.9, 127.6, 127.1, 125.4, 121.4, 120.1, 77.4, 65.7, 60.4, 54.4, 46.7, 40.6, 31.7, 29.4, 28.3, 22.8, 14.0 / ESI (+): M+H (554.4) / M+Na (576.4) / 2M+Na (1129.8) / Rf: 0.33 (CHCl₃/MeOH : 95/5).

***N*_ε-Boc-lysylglycine ethyl ester (6).** To a solution of *N*α-Fmoc-*N*ε-Boc-lysylglycine ethyl ester (5) (3 g, 5.4 mmol) in dry DMF (15 mL) was added morpholine (15 mL). The mixture was stirred for 5 minutes and rapidly concentrated under reduced pressure. The crude product was purified on silica gel (CHCl₃/MeOH) to give the desired compound as a colorless oil (1.75 g, 98 %). ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.24 (s, 1H, NH), 6.75 (s, 1H, NH), 4.08 (q, 2H, 7.2 Hz), 3.83-3.81 (m, 2H, CH₂), 3.15-3.12 (m, 1H, CH), 2.90-2.85 (m, 2H, CH₂), 1.90-1.68 (m, 1H), 1.59-1.51 (m, 1H), 1.42-1.24 (m, 13H), 1.18 (m, 3H, CH₃, 6.8 Hz) / ¹³C NMR (100 MHz, DMSO-*d*₆): δ 175.8, 169.9, 155.5, 77.3, 60.3, 54.9, 54.5, 40.5, 34.8, 29.4, 28.2, 22.4, 14.0 / ESI (+): M+H (332.3) / M+Na (354.3) / 2M+H (663.6) / 2M+Na (685.5) / Rf: 0.36 (CHCl₃/MeOH : 90/10).

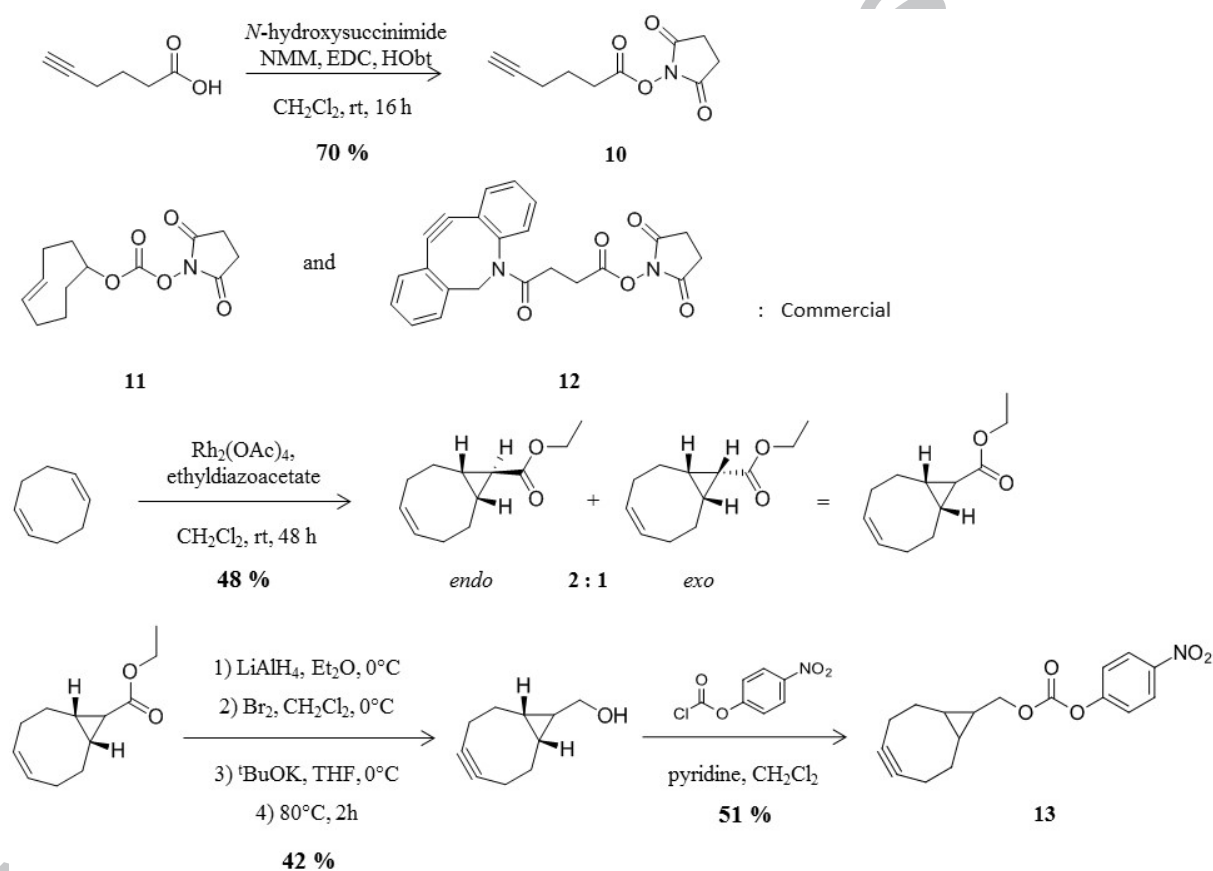
Acetylglycine (7). To a solution of glycine (5 g, 66 mmol) in H₂O (20 mL) was added in one portion and under stirring acetic anhydride (16.6 mL, 132 mmol, 2 eq). The solution was stirred for 30 minutes (exothermic reaction). When the solution was returned to room temperature (the first crystals appear) the reaction mixture was placed in the refrigerator overnight to promote crystallization. The crystals were collected by filtration, washed with ice-cold water and dried in an oven at 100 °C overnight (5.31g, 70 %). ¹H NMR (400 MHz, DMSO-*d*₆): δ 12.50 (s, 1H, OH), 8.17 (s, 1H, NH), 3.70 (d, 2H, CH₂, 5.6 Hz), 1.84 (s, 3H, CH₃) / ¹³C NMR (100 MHz, DMSO-*d*₆): δ 171.4, 169.6, 40.6, 22.3 / ESI (-): M-H (116.1).

***N*_α-(acetylglycyl)-*N*_ε-Boc-lysylglycine ethyl ester (8).** To a solution of acetylglycine (7) (0.528 g, 4.5 mmol, 1 eq) in CH₂Cl₂ (75 mL) was added in the following order: *N*ε-Boc-lysylglycine ethyl ester (6) (1.79 g, 5.4 mmol, 1.2 eq), *N*-methylmorpholine (1.30 ml, 11.7 mmol, 2.5 eq), EDC (1.33 g, 6.8 mmol, 1.5 eq) and finally HOBT (0.943 g, 6.8 mmol, 1.5 eq). The mixture was stirred for 24 hours under argon upon which the product precipitated. The obtained precipitate was collected by filtration, washed with cold CH₂Cl₂ and dried in vacuo. (1.52 g, 80 %). ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.36 (s, 1H, NH), 8.07 (s, 1H, NH), 7.99 (d, 1H, NH), 6.76 (s, 1H, NH), 4.27-4.22 (m, 1H), 4.07 (q, 2H, 7.2 Hz), 3.81-3.78 (m, 2H, CH₂), 3.71 (d, 2H, 5.6 Hz), 2.89-2.84 (m, 2H, CH₂), 1.84 (s, 3H, CH₃), 1.69-1.60 (m, 1H), 1.54-1.45 (m, 1H), 1.43-1.23 (m, 13H), 1.18 (t, 3H, CH₃, 6.8 Hz) / ¹³C NMR (100 MHz, DMSO-*d*₆): δ 172.1, 169.6, 169.5, 168.8, 155.5, 77.4, 60.4, 52.2, 42.0, 40.7, 31.8, 29.3, 28.3, 22.6, 22.5, 14.0 / ESI (+): M+H (431.4) / M+Na (459.4) / 2M+H (861.9) / Rf: 0.48 (CHCl₃/MeOH: 9:1)

***N*_α-(acetylglycyl)-lysylglycine ethyl ester trifluoroacetate salt (9).** A solution of ethyl *N*α-(acetylglycyl)-*N*ε-Boc-lysylglycine ethyl ester (8) (1.47 g, 3.42 mmol) in trifluoroacetic acid (30 mL)

was stirred for 1 hour. The reaction mixture was then concentrated under reduced pressure and the yellow oil obtained was triturated in cold Et₂O until formation of a white solid. Et₂O was removed by filtration and the white solid was dried under vacuum overnight (1.26 g, 83 %). ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.38 (s, 1H, NH), 8.11 (s, 1H, NH), 8.03 (s, 1H, NH), 7.67 (s, 3H, NH₃), 4.31-4.25 (m, 1H), 4.08 (q, 2H, 7.2 Hz), 3.82-3.80 (m, 2H, CH₂), 3.72-3.69 (m, 2H, CH₂), 2.80-2.71 (m, 2H, CH₂), 1.85 (s, 3H, CH₃), 1.75-1.63 (m, 1H, CH₂), 1.57-1.46 (m, 3H), 1.38-1.26 (m, 2H, CH₂), 1.18 (t, 3H, CH₃, 6.8 Hz) / ¹³C NMR (100 MHz, DMSO-*d*₆): δ 172.0, 169.7, 169.6, 167.0, 60.5, 52.0, 42.3, 42.0, 40.7, 31.3, 26.5, 22.4, 22.0, 14.1 / ESI (+): M (331.2) / R_f: 0.19 (CHCl₃/MeOH: 4:1).

1.2) Clickable linkers



***N*-succinimidyl 5-hexynoate (10).** To a solution of 5-hexynoic acid (500 μL, 4.3 mmol) in anhydrous CH₂Cl₂ (15 mL) were added in the following order: NMM (720 μL, 6.5 mmol, 1.5 eq), EDC (1.27 g, 6.5 mmol, 1.5 eq) and *N*-hydroxysuccinimide (0.77 g, 6.5 mmol, 1.5 eq). The solution was stirred overnight at room temperature under argon. The reaction mixture was washed with 0.2 N aqueous HCl (10 mL), H₂O (10 mL) and brine (10 mL). The organic layer was dried over MgSO₄ and concentrated under reduced pressure. The crude product was purified on silica gel (CHCl₃/EtOAc) to give the desired compound as white solid (640 mg, 70 %). ¹H NMR (400 MHz, CDCl₃): δ 2.84 (s, 4H, 2 CH₂), 2.78 (t, 2H, CH₂, 7.6 Hz), 2.35 (dt, 2H, CH₂, 6.8 Hz), 2.01 (t, 1H, CH, 2.8 Hz), 1.97 (q, 2H, CH₂, 7.2

Hz) / ^{13}C NMR (100 MHz, CDCl_3): δ 169.2, 168.1, 82.7, 70.0, 29.8, 25.7, 23.5, 17.7 / ESI: not detected / Rf: 0.58 ($\text{CHCl}_3/\text{EtOAc}$: 7/3)

TCO-NHS Ester (11). Reagent available commercially from Click Chemistry Tools.

DIBAC (DBCO-NHS Ester) (12). Reagent available commercially from Click Chemistry Tools.

Bicyclo[6.1.0]non-4-yn-9-ylmethyl (4-nitrophenyl) carbonate (13). Compound (13) was obtained according to the method described previously.¹ To a solution of 1,5-cyclooctadiene (45 mL, 0.362 mol, 8 eq) and $\text{Rh}_2(\text{OAc})_4$ (1 g, 2.26 mmol, 0.05 eq) in anhydrous CH_2Cl_2 (50 mL) was added dropwise under inert gas in 3 hours a solution of ethyl diazoacetate (5.5 mL, 45 mmol, 1 eq) in anhydrous CH_2Cl_2 (10 mL). The solution was stirred for 2 days, the residue was concentrated in vacuo and the excess of cyclooctadiene was removed by filtration over a glass filter filled silica and eluted with a mixture 200:1 (Heptane:EtOAc). While endo and exo compounds can be separated, they were kept as a mixture given the low impact on cycloaddition reaction kinetics reported. The mixture of isomers (*Endo/Exo* = 2:1) was obtained as a colorless oil (4.13g, 48%). *Endo*: Rf_{endo}: 0,31 (Heptane:EtOAc 95:5) / *Exo*: Rf_{exo}: 0,22 (Heptane:EtOAc 95:5) / ^1H NMR (400 MHz, CDCl_3): 5.64-5.59 (m, 2H, $\text{CH}_{\text{endo+exo}}$), 4.10 (q, 2H, 7.2Hz, $\text{CH}_{2\text{endo+exo}}$), 2.55-2.45 (m, 1.31H, $2\times\text{CH}_{\text{endo}}$), 2.34-2.26 (m, 0.69H, $2\times\text{CH}_{\text{exo}}$), 2.24-2.15 (m, 2H, m, $2\times\text{CH}_{\text{endo+exo}}$), 2.12-2.01 (m, 2H, m, $2\times\text{CH}_{\text{endo+exo}}$), 1.87-1.78 (m, 1.30H, $2\times\text{CH}_{\text{endo}}$), 1.70 (t, 0.65H, 8.8 Hz, CH_{endo}), 1.59-1.54 (m, 0.71H, $2\times\text{CH}_{\text{exo}}$), 1.52-1.43 (m, 0.74H, $2\times\text{CH}_{\text{exo}}$), 1.42-1.36 (m, 1.33H, $2\times\text{CH}_{\text{endo}}$), 1.26 (t, 2H, 7.2 Hz, $\text{CH}_{3\text{endo}}$), 1.25 (t, 1.05H, 7.2 Hz, $\text{CH}_{3\text{exo}}$), 1.18 (t, 4.4 Hz, 0.36H, CH_{exo}) / ^{13}C NMR (100 MHz, CDCl_3): δ 174.6 (exo), 172.4 (endo), 130.1 (exo), 129.6 (endo), 60.4 (exo), 59.9 (endo), 28.4 (exo), 28.0 (exo), 27.9 (exo), 27.2 (endo), 26.8 (exo), 24.3 (endo), 22.8 (endo), 21.4 (endo), 14.5 (endo), 14.4 (exo).

To a suspension of LiAlH_4 (277 mg, 6.95 mmol, 0.9 eq) in anhydrous Et_2O (25 mL) was added dropwise at 0°C under stirring a solution of esters obtained previously (1.5 g, 7.72 mmol) in anhydrous Et_2O (10 mL). After addition, the solution was stirred for 15 minutes at room temperature. The solution was then cooled down to 0°C and H_2O was added carefully until the grey solid had turned white (release of dihydrogen, elimination of excess LiAlH_4). MgSO_4 (5g) was added, and the solid was filtered and washed with cold Et_2O (2×50 mL). The filtrate was concentrated under reduced pressure. The desired alcohols (endo/exo) were obtained as colorless oil (1.05 g, 90 %) and were used as such in the following step.

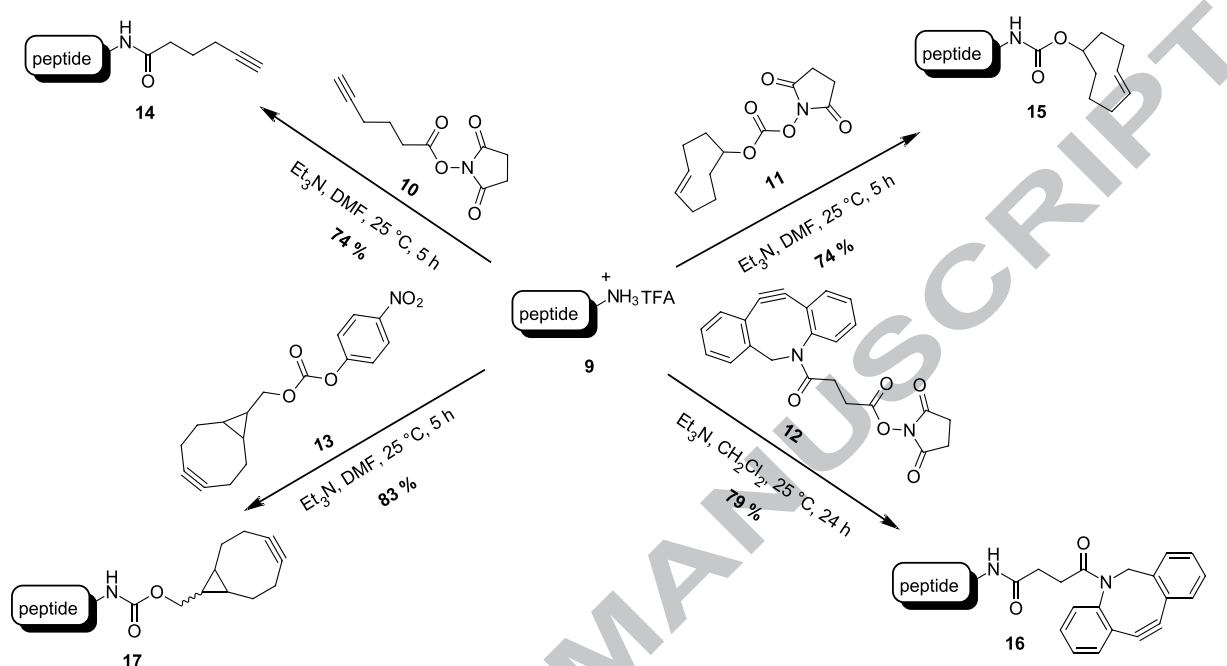
To a solution of the alcohols obtained previously (1.05 g, 6.9 mmol) in anhydrous CH_2Cl_2 (30 mL), was added Br_2 dropwise at 0°C under stirring (393 μL , 7.6 mmol, 1.1 eq) in anhydrous CH_2Cl_2 (10 mL) until the yellow colour persisted (equivalence point). The solution was then brought to room temperature and the reaction was quenched with a few drops of a 10% $\text{Na}_2\text{S}_2\text{O}_3$ solution (elimination of excess Br_2). The reaction mixture was extracted with CH_2Cl_2 (2×30 mL). The organics layers were

combined, dried over MgSO_4 and concentrated in vacuo. The desired dibromides (endo/exo) were obtained as white solid (2.13 g, 99 %) and were used as such in the following step.

To a solution of dibromides under argon obtained previously (2.05 g, 6.57 mmol) in anhydrous THF (30 mL) was added dropwise at 0°C and under stirring a solution of 1M $t\text{BuOK}$ in anhydrous THF (21.7 mL, 21.7 mmol, 3.3 eq). The solution was then refluxed for 2 hours. After cooling down to room temperature the mixture was quenched with a saturated NH_4Cl solution (2x30 mL), extracted with CH_2Cl_2 (3x20 mL). The organic layers were combined, dried over MgSO_4 and concentrated in vacuo. The crude product was purified on silica gel (Hept/EtOAc) to give the desired compounds (endo/exo, 2:1) as white solid (462 mg, 47 %). $R_{f_{\text{endo}}}$: 0.22 (Hept/EtOAc: 1:1) / $R_{f_{\text{exo}}}$: 0.25 (Hept/EtOAc: 1:1) / ^1H NMR (400 MHz, CDCl_3): δ 3.73 (d, 1.32H, 8 Hz, $\text{CH}_{2\text{endo}}$), 3.54 (d, 0.67H, 6 Hz, $\text{CH}_{2\text{exo}}$), 2.43-2.14 (m, 6H, $3\text{CH}_{2\text{endo}}+3\text{CH}_{2\text{exo}}$), 1.65-1.59 (m, 1.72H, $2\text{H}_{\text{endo}}+1\text{H}_{\text{exo}}$), 1.55-1.34 (m, 2.03H, $2\text{H}_{\text{endo}} + 2\text{H}_{\text{exo}}$), 0.96-0.91 (m, 1.32H, 2xH_{endo}), 0.69-0.66 (m, 1.05H, 3H_{exo}) / ^{13}C NMR (100 MHz, CDCl_3): δ 98.22 (exo), 98.0 (endo), 66.3 (exo), 59.2 (endo), 32.6 (exo), 28.2 (endo), 26.5 (exo), 21.8 (exo), 20.7 (endo), 20.6 (exo), 20.6 (endo), 19.20 (endo).

To a solution of bicyclononynes obtained previously (80 mg, 0.53 mmol) under argon in anhydrous CH_2Cl_2 (10 mL) was added pyridine (108 μL , 1.33 mmol, 2.5 eq). After stirring for 5 minutes, 4-nitrophenyl carbonochloridate (0.145 g, 0.692 mmol, 1.3 eq) was added in one portion in the solution and the mixture was stirred for 15 minutes. The solution was treated with saturated NH_4Cl solution (15 mL), extracted with CH_2Cl_2 (3x10 mL). The organics layers were combined, dried over MgSO_4 and concentrated in vacuo. The crude product was purified on silica gel (Hept/EtOAc) to give the desired compounds (**13**) as white solid (85 mg, 51 %). ^1H NMR (400 MHz, CDCl_3): δ 8.26 (d, 2H, $2\text{CH}_{\text{endo-exo}}$, 7.2 Hz), 7.39 (d, 2H, $2\text{CH}_{\text{arOendo-exo}}$, 9.2 Hz), 4.40 (d, 1.32H, $\text{CH}_{2\text{endo}}$, 8.4 Hz), 4.22 (d, 0.65H, $\text{CH}_{2\text{exo}}$, 6.8 Hz), 2.47-2.13 (m, 6H, $3\text{CH}_{2\text{endo}}+3\text{CH}_{2\text{exo}}$), 1.67-1.56 (m, 1.68H, $2\text{H}_{\text{endo}}+1\text{H}_{\text{exo}}$), 1.55-1.34 (m, 21.97H, $2\text{H}_{\text{endo}} + 2\text{H}_{\text{exo}}$), 1.09-1.04 (m, 1.32H, 2xH_{endo}), 0.90-0.79 (m, 1.07H, 3H_{exo}) / ^{13}C NMR (100 MHz, CDCl_3): δ 155.8 (endo), 152.8 (endo), 152.7 (exo), 145.6 (endo), 125.4 (endo), 121.9 (endo), 121.9 (exo), 98.9 (endo), 98.8 (exo), 68.1 (endo), 33.3 (exo), 29.2 (endo), 23.5 (exo), 23.1 (exo), 21.5 (endo), 21.4 (exo), 20.7 (endo), 17.4 (endo) / R_f : 0.65 (Hept/EtOAc: 4:1) / ESI (+): not detected.

1.3) Clickable peptides



***N*_α-(acetylglycyl)-*N*_ε-((hex-5-ynoyl)-lysylglycine ethyl ester (14).** To a solution of *N*_α-(acetylglycyl)-lysylglycine ethyl ester trifluoroacetate salt (**9**) (100 mg, 0.23 mmol) in dry DMF (10 mL) was added Et₃N (99 μL, 0.68 mmol, 3 eq) and *N*-succinimidyl 5-hexynoate (**10**) (70.6 mg, 0.34 mmol, 1.5 eq). The solution was stirred for 5 hours under argon. Then the crude product was concentrated under reduced pressure and directly purified on silica gel (CHCl₃/MeOH) to give the desired compound as white solid (71 mg, 74%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.32 (s, NH), 8.05 (s, NH), 7.94 (s, NH), 7.73 (s, NH), 4.28-4.23 (m, 1H), 4.08 (q, 2H, 4 Hz), 3.81 (t, 2H, 8 Hz), 3.71 (d, 2H, CH₂, 4 Hz), 3.01 (q, 2H, 8 Hz), 2.74 (s, 1H), 2.16-2.12 (m, 4H), 1.85 (s, 3H, CH₃), 1.69-1.63 (m, 3H), 1.56-1.46 (m, 1H), 1.41-1.31 (m, 2H), 1.31-1.23 (m, 2H), 1.18 (t, 3H, CH₃, 7.2 Hz) / ¹³C NMR (100 MHz, DMSO-*d*₆): δ 172.0, 171.2, 171.1, 169.5, 168.8, 84.1, 71.4, 60.3, 52.1, 41.9, 40.6, 38.3, 34.1, 31.7, 28.8, 24.2, 22.5, 22.4, 17.4, 14.0 / *t*_R: 2.11 min / HRMS: calculated for C₂₀H₃₂N₄O₆Na M(+): 447.2220, found: 447.2202.

***N*_α-(acetylglycyl)-*N*_ε-(((E)cyclooct-4-en-1-yl)oxy)carbonyl)-lysylglycine ethyl ester (15).** To a solution of *N*_α-(acetylglycyl)-lysylglycine ethyl ester trifluoroacetate salt (**9**) (83 mg, 0.18 mmol, 2 eq) in dry DMF (10 mL) was added Et₃N (78 μL, 0.56 mmol, 6 eq) and (E)-cyclooct-4-en-1-yl (2,5-dioxopyrrolidin-1-yl) carbonate (**11**) (25 mg, 0.093 mmol, 1 eq). The solution was stirred for 5 hours under argon. Then the crude product was concentrated under reduced pressure and directly purified on silica gel (CHCl₃/MeOH) to give the desired compound as white solid (33 mg, 74%). ¹H NMR (400

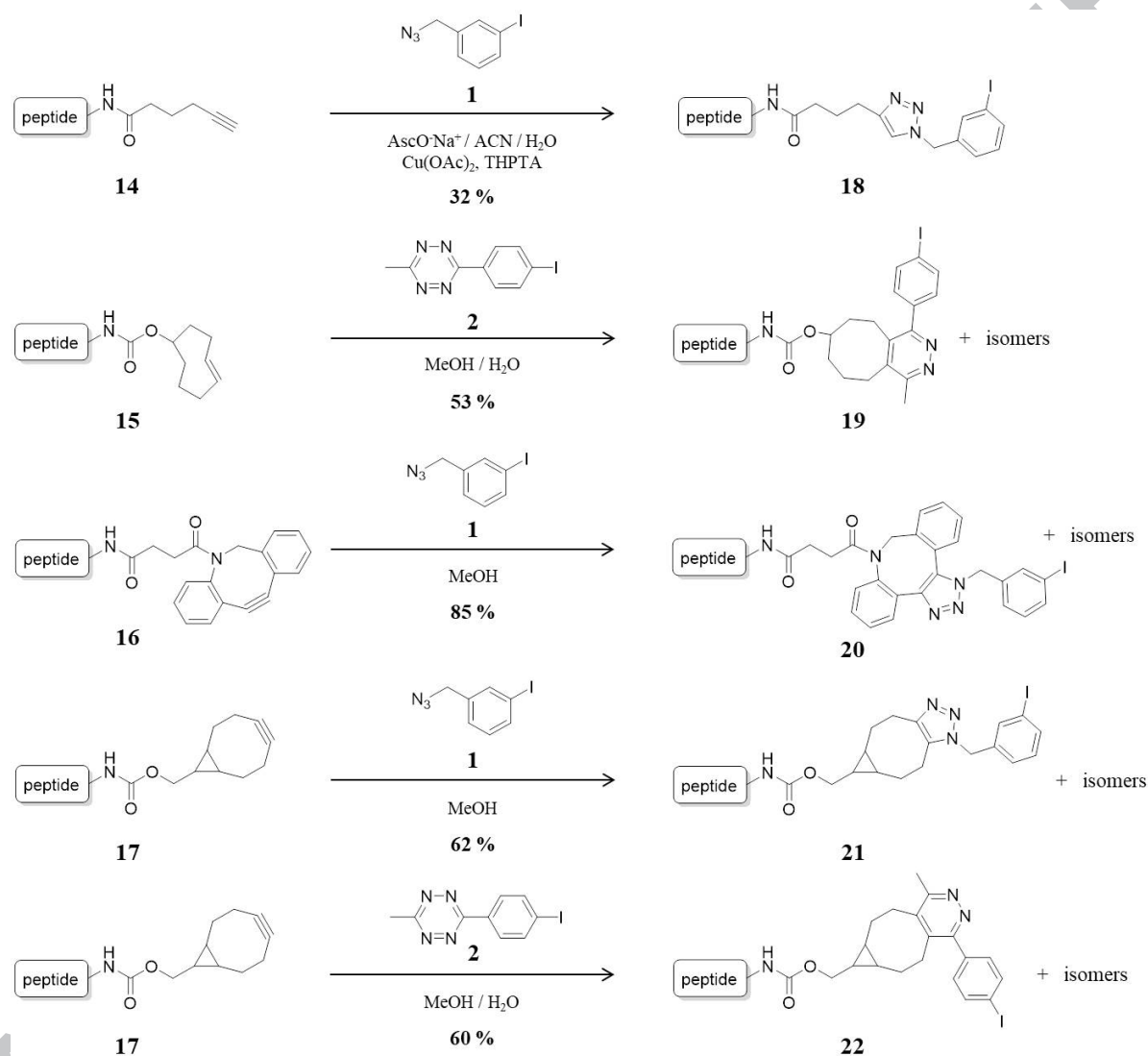
MHz, DMSO-*d*₆): δ 8.32 (s, NH), 8.04 (s, NH), 7.94 (s, NH), 6.89 (s, NH), 5.69-5.53 (m, 1.41H, =CH), 5.47-5.39 (m, 0.66H, =CH), 4.61-4.52 (m, 0.41H), 4.27-4.19 (m, 1.64H), 4.08 (q, 2H, 8 Hz), 3.80 (t, 2H, 4 Hz), 3.71 (d, 2H, 8 Hz), 2.93-2.89 (m, 2H), 2.34-1.97 (m, 4H), 1.92-1.71 (m, 6H), 1.69-1.43 (m, 5H), 1.40-1.31 (m, 2H), 1.28-1.22 (m, 2H), 1.18 (t, 3H, 8 Hz) / ¹³C NMR (100 MHz, DMSO-*d*₆): δ 172.0, 169.6, 169.5, 168.8, 155.7, 134.9, 132.5, 74.9, 74.2, 60.3, 52.2, 42.0, 40.6, 38.2, 33.7, 32.1, 31.7, 30.6, 29.1, 25.1, 24.5, 22.4, 21.9, 14.0 / HRMS: calculated for C₂₃H₃₈N₄O₇Na M(+) : 505.2638, found : 505.2656 / t_R: 4.04 min.

***N*_α-(acetylglycyl)-*N*_ε-(4-(2-Azatricyclo(10.4.0.04,9)hexadeca-1(16),4(9),5,7,12,14-hexaen-10-yn-2-yl)-4-oxobutyryl)-lysylglycine ethyl ester (16).** To a solution of *N*_α-(acetylglycyl)-lysylglycine ethyl ester trifluoroacetate salt (**9**) (38mg, 84 μmol, 1.2 eq) in anhydrous CH₂Cl₂ (5 mL), was added Et₃N (35 μL, 251 μmol, 3.6 eq) and DBCO-NHS ester (**12**) (28 mg, 69 μmol, 1.0 eq). The solution was stirred for 24 hours under argon at room temperature. The solution was washed with H₂O, dried over MgSO₄ and concentrated in vacuo. The crude product was purified on silica gel (CHCl₃/MeOH, 9/1) to give the desired compound as a white solid (34 mg, 79 %). ¹H NMR (400 MHz, CHCl₃): δ 7.70-7.57 (m, 2H), 7.55-7.52 (m, NH), 7.48-7.46 (m, 1H), 7.40-7.35 (m, 3H), 7.24-7.17 (m, 2H), 7.11-7.07 (m, NH), 6.68-6.62 (NH), 6.52-6.48 (NH), 5.15-5.07 (m, 1H, CH), 4.56-4.43 (m, 1H), 4.13 (q, 2H, 7.2 Hz), 4.02-3.90 (m, 2H), 3.87-3.81 (m, 1H, CH), 3.76-3.59 (m, 2H), 3.35-3.21 (m, 0.5H), 3.10-2.98 (m, 1H), 2.96-2.81 (m, 1H), 2.78-2.66 (m, 0.5H), 2.37-2.26 (m, 1H), 2.19-2.05 (m, 1H), 1.94 (s, 3H, CH₃), 1.91-1.71 (m, 2H), 1.69-1.56 (m, 1H), 1.50-1.27 (m, 4H), 1.22 (t, 3H, 7.2 Hz) / ¹³C NMR (100 MHz, CHCl₃): 172.8, 172.7, 172.5, 172.5, 172.4, 172.3, 171.5, 171.3, 170.0, 169.9, 169.7, 151.3, 151.3, 148.3, 148.2, 132.4, 132.3, 129.6, 128.9, 128.8, 128.4, 128.4, 128.3, 128.0, 127.8, 127.3, 127.1, 125.6, 125.6, 123.3, 123.2, 122.6, 122.5, 114.8, 114.7, 108.0, 114.7, 108.0, 107.9, 77.3, 61.5, 61.5, 53.1, 53.0, 43.4, 43.2, 41.2, 38.8, 38.8, 31.6, 31.3, 30.9, 30.5, 30.2, 28.6, 28.4, 22.9, 22.8, 22.2, 14.2, 14.2 / HRMS: calculated for C₃₃H₄₀N₅O₇ M(+) : 618.2928, found : 618.2755 / t_R: 5.33 min

***N*_α-(acetylglycyl)-*N*_ε-((bicyclo[6.1.0]non-4-yn-9-ylmethoxy)carbonyl)-lysylglycine ethyl ester (17).** To a solution of *N*_α-(acetylglycyl)-lysylglycine ethyl ester trifluoroacetate salt (**9**) (117 mg, 0.27 mmol, 1.2 eq) in anhydrous DMF (10 mL) was added Et₃N (92 μL, 0.66 mmol, 3 eq) and bicyclo[6.1.0]non-4-yn-9-ylmethyl (4-nitrobenzyl) carbonate (**13**) (69 mg, 0.22 mmol, 1 eq). The solution was stirred for 5 hours under inert gas. Then the crude product was concentrated under reduced pressure and directly purified on silica gel (CHCl₃/MeOH) to give the desired compound as white solid (92 mg, 83 %). ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.33 (s, NH), 8.05 (s, NH), 7.96 (s, NH), 7.05 (s, NH), 4.28-4.22 (m, 1H), 4.11-4.07 (q, 2H, 7.2 Hz), 4.05-4.01 (m, 1.31H), 3.86-3.82 (m, 0.62H), 3.81-3.79 (t, 2H, 5.6 Hz), 3.71 (d, 2H, 6 Hz), 2.95-2.91 (m, 2H), 2.36-1.89 (m, 6H), 1.84 (s, 3H, CH₃), 1.70-1.61 (m, 1H), 1.55-1.46 (m, 2H), 1.38-1.24 (m, 5H), 1.18 (t, 3H, 7.2 Hz), 1.04 (d, 0.65H, 6.4 Hz), 0.90-0.71 (m, 1.32H), 0.71-0.59 (m, 1H) / ¹³C NMR (100 MHz, DMSO-*d*₆): δ 172.0, 169.6, 169.5, 168.9, 156.3, 99.0, 98.9, 67.6, 61.2, 60.3, 54.9, 52.2, 42.0, 40.7, 32.9, 31.8, 29.1, 28.6,

25.5, 23.4, 22.4, 22.2, 20.8, 19.5, 17.6, 14.0 / HRMS: calculated for $C_{25}H_{38}N_4O_7Na$ M(+): 529.2638, found: 529.2644 / t_R : 3.50 min.

1.4) Clickable peptides and cold iodinated references conjugation



N_α -(acetylglycyl)- N_ϵ -(4-(1-(3-iodobenzyl)-1H-1,2,3-triazol-4-yl)butanoyl)-lysylglycine ethyl ester (**18**). To a solution of **14** (32.2 mg, 75.8 μ mol) in methanol (1.9 mL) was added 3-iodobenzylazide (**1**) (17.7 mg, 68.3 μ mol, 0.9 eq) dissolved in MeOH (500 μ L). Then, sodium ascorbate (1.5 mg, 7.6 μ mol, 0.1 eq) in water (2 mL), copper sulphate (0.19 mg, 758 nmol, 0.01 eq) in water (500 μ L) and THPTA (0.33 mg, 758 nmol, 0.01 eq) in MeOH (500 μ L) were added. The milky solution was stirred overnight at room temperature and turned clear. The crude product was concentrated under reduced pressure and

directly purified on silica gel (CHCl₃/MeOH 9:1) to give the desired compound as colorless oil (15 mg, 32 %). ¹H NMR (400 MHz, CD₃OD): δ 7.79 (s, 1H), 7.71 (s, 1H), 7.69 (s, 1H), 7.31 (d, 1H, 8 Hz), 7.14 (t, 1H, 8 Hz), 5.52 (s, 2H, CH₂), 4.42-4.35 (m, 1H), 4.16 (q, 2H, CH₂, 7.2 Hz), 3.91 (d, 2H, 6 Hz), 3.86 (s, 2H), 3.16 (t, 2H, CH₂, 6.4 Hz), 2.71 (t, 2H, CH₂, 7.6 Hz), 2.23 (t, 2H, CH₂, 7.6 Hz), 2.00 (s, 3H, CH₃), 1.95 (t, 2H, CH₂, 7.6 Hz), 1.91-1.83 (m, 1H), 1.74-1.64 (m, 1H), 1.57-1.48 (m, 2H), 1.46-1.36 (m, 2H), 1.25 (t, 3H, CH₃, 7.2 Hz) / ¹³C NMR (100 MHz, CD₃OD): δ 175.5, 174.7, 174.0, 171.7, 171.2, 149.0, 139.4, 138.7, 138.1, 131.8, 128.4, 123.6, 95.2, 62.3, 54.5, 53.9, 43.7, 42.1, 40.1, 36.4, 32.6, 29.9, 26.7, 25.8, 24.0, 22.5, 14.5 / HRMS: calculated for C₂₇H₃₉IN₇O₆ M(+) : 684.2007, found : 684.2005 / t_R: 4.12 min.

***N*_α-(acetylglycyl)-*N*_ε-(((1-(4-iodophenyl)-4-methyl-5,6,7,8,9,10-hexahydrocycloocta[*d*] pyridazin-7-yl)oxy)carbonyl)-lysylglycine ethyl ester (19).** To a solution of (15) (9.8 mg, 20.3 μmol, 1 eq) in a mixture H₂O/MeOH 1:1 (1 mL) was added 3-(4-iodophenyl)-6-methyl-1,2,4,5-tetrazine (2) (6.7 mg, 22.6 μmol, 1.1 eq). The solution was stirred for 5 hours under inert gas. The crude product was concentrated under reduced pressure and directly purified on silica gel (CHCl₃/MeOH) to give the desired compound as white solid (8 mg, 53 %). ¹H NMR (400 MHz, CD₃CN): δ 7.84 (d, 2H, 6.4 Hz), 7.22 (t, 2H, 6.4 Hz), 7.19-7.10 (m, NH), 7.01-6.90 (m, NH), 6.90-6.74 (m, NH), 5.50-5.35 (m, NH), 4.48-4.38 (m, 1H, CH), 4.29-4.19 (m, 1H), 4.12-4.06 (q, 2H, 7.2 Hz), 3.86-3.76 (m, 2H), 3.76-3.60 (m, 2H), 3.06-2.92 (m, 2H), 2.92-2.78 (m, 2H), 2.67 (s, 3H, CH₃), 2.63-2.50 (m, 2H), 1.92-1.77 (m + s, 6H, CH₃ + 3H), 1.77-1.70 (m, 1H), 1.65-1.50 (m, 4H), 1.45-1.32 (m, 2H), 1.32-1.22 (m, 2H), 1.19 (t, 3H, CH₃, 7.2 Hz) / ¹³C NMR (100 MHz, CD₃CN): δ 173.1, 171.9, 171.8, 170.7, 170.4, 170.3, 129.2, 139.1, 138.4, 132.1, 132.0, 130.5, 61.8, 54.0, 53.9, 44.0, 41.8, 41.1, 41.0, 36.2, 36.1, 34.5, 34.4, 32.1, 32.0, 30.1, 31.0, 24.8, 24.3, 24.2, 23.3, 22.9, 21.9, 20.5, 14.5 / HRMS: calculated for C₃₂H₄₄IN₆O₇ M(+) : 751.2316, found : 751.2319 / t_R: 6.3 - 7.6 - 8.2 min.

***N*_α-(acetylglycyl)-*N*_ε-(4-(5-(*m*-Iodophenyl)methyl)-3,4,5,13-tetraazatetracyclo(13.4.0.0^{2,6}.0^{7,12})nonadeca-1(19),2(6),3,7,9,11,15,17-octaen-13-yl)-4-oxobutyryl)-lysylglycine ethyl ester (20).** To a solution of 16 (33 mg, 53.4 μmol) in MeOH (4 mL) was added compound 1 (16.6 mg, 64.1 μmol, 1.2 eq) in MeOH (1 mL). The solution was stirred for 30 min at room temperature. The crude product was concentrated under reduced pressure and directly purified on silica gel (CHCl₃/MeOH 9:1) to give the desired compound as colorless oil (39 mg, 85 %). ¹H NMR (400 MHz, CDCl₃): δ 7.71-7.54 (m, 4H), 7.52-7.49 (m, 1H), 7.47-7.32 (m, 3H), 7.24-7.19 (m, 2H), 7.16-7.06 (m, 2H), 7.05-6.98 (m, NH), 6.93-6.91 (m, NH), 6.72-6.63 (m, NH), 6.51-6.40 (m, NH), 5.95 (t, 0.8H, 18.8 Hz), 5.79 (t, 0.4H, 15.2 Hz), 5.63-5.44 (m, 1.2H), 5.30-5.24 (m, 0.4H), 5.11-5.03 (m, 0.1H), 4.96-4.88 (m, 0.1H), 4.57-4.26 (m, 2H), 4.49-4.06 (m, 2H), 4.00-3.70 (m, 4H), 3.24-2.87 (m, 2H), 2.37-2.25 (m, 1H), 2.22-2.01 (m, 2H), 1.97 (s, 3H), 1.90-1.71 (m, 2H), 1.71-1.52 (m, 1H), 1.45-1.34 (m, 2H), 1.34-1.27 (m, 2H), 1.23 (t, 3H, 6.8 Hz) / ¹³C NMR (100 MHz, CDCl₃): δ

172.5, 172.4, 172.3, 172.2, 172.1, 172.0, 171.4, 171.4, 171.2, 170.1, 170.0, 170.0, 169.8, 145.4, 145.3, 143.3, 141.5, 140.2, 140.2, 138.0, 137.8, 137.8, 137.4, 137.3, 137.2, 136.6, 136.5, 136.3, 136.0, 135.3, 135.2, 133.6, 132.7, 132.3, 132.2, 131.6, 131.5, 131.4, 131.3, 131.0, 130.9, 130.3, 130.0, 129.9, 129.7, 129.5, 129.2, 128.8, 128.7, 128.4, 128.2, 128.1, 127.3, 127.2, 127.1, 127.0, 126.9, 126.6, 124.2, 124.1, 94.9, 94.6, 77.36, 61.5, 53.4, 53.3, 53.2, 53.0, 52.0, 51.7, 51.6, 51.3, 51.2, 50.7, 43.3, 41.4, 39.0, 38.7, 38.5, 31.5, 31.4, 31.2, 31.2, 30.9, 30.8, 30.1, 29.9, 29.7, 29.6, 28.9, 28.8, 28.6, 28.3, 23.0, 22.9, 22.4 / HRMS: calculated for $C_{40}H_{46}IN_8O_7$ M(+): 877.2534, found : 8.772554 / t_R : 6.19 + 6.51 min.

***N*_α-(acetylglycyl)-*N*_ε-((((6R)-1-(3-iodobenzyl)-1,4,5,5a,6,6a,7,8-octahydrocyclopropa[5,6]cycloocta[1,2-d][1,2,3]triazol-6-yl)methoxy)carbonyl)-lysylglycine ethyl ester (21).** To a solution of **17** (33 mg, 65 μmol) in MeOH (4 mL) was added compound **1** (16 mg, 62 μmol, 0.95 eq) in MeOH (1 mL). The solution was stirred overnight at room temperature. The crude product was concentrated under reduced pressure and directly purified on silica gel (CHCl₃/MeOH 9:1) to give the desired compound as colorless oil (29 mg, 62 %). ¹H NMR (400 MHz, CD₃OD): δ 7.67 (d, 1H, 6.8 Hz), 7.47 (s, 1H), 7.17-7.11 (m, 2H), 5.59-5.50 (m, 2H, CH₂), 4.40-4.37 (m, 1H), 4.20-4.09 (m, 3.32H), 3.92 (d, 2.65H, 5.6 Hz), 3.87 (s, 2H), 3.16-3.03 (m, 3H), 2.96-2.80 (m, 2H), 2.72-2.58 (m, 1H), 2.42-2.28 (m, 0.64H), 2.26-2.12 (m, 1.34H), 2.01 (s, 3H, CH₃), 1.94-1.83 (m, 1H), 1.73-1.61 (m, 2H), 1.55-1.37 (m, 5H), 1.25 (t, 3H, CH₃, 7.2 Hz), 1.10-0.95 (m, 0.8H), 0.91-0.81 (m, 1.4H), 0.78-0.67 (m, 0.8H) / ¹³C NMR (100 MHz, CD₃OD): δ 174.7, 174.0, 171.7, 171.1, 159.2, 146.4, 139.5, 138.4, 137.1, 135.7, 135.6, 131.8, 127.5, 127.4, 95.2, 69.3, 63.4, 62.3, 54.5, 51.7, 49.8, 43.8, 43.7, 42.2, 42.0, 41.4, 32.6, 30.8, 30.5, 28.0, 27.1, 26.4, 26.1, 25.6, 23.9, 23.6, 23.5, 23.4, 23.3, 23.3, 22.5, 22.4, 20.5, 20.2, 18.8, 14.5 / HRMS: calculated for $C_{32}H_{45}IN_7O_7$ M(+): 766.2425, found : 766.2421 / t_R : 5.75 min.

***N*_α-(acetylglycyl)-*N*_ε-(((1-(4-iodophenyl)-4-methyl-6,6a,7,7a,8,9-hexahydro-5H-cyclopropa[5,6]cycloocta[1,2-d]pyridazin-7-yl)methoxy)carbonyl)-lysylglycine ethyl ester (22).** To a solution of **(17)** (10 mg, 19.7 μmol) in a mixture H₂O/MeOH 1:1 (1 mL) was added 3-(4-iodophenyl)-6-methyl-1,2,4,5-tetrazine **(2)** (6.5 mg, 22 μmol, 1.1 eq). The solution was stirred for 5 hours under inert gas. The crude product was concentrated under reduced pressure and directly purified on silica gel (CHCl₃/MeOH) to give the desired compound as a white solid (9.2 mg, 60 %). ¹H NMR (400 MHz, CD₃CN): δ 7.84 (d, 2H, 8.4 Hz), 7.2 (d, 2H, 8.4 Hz), 7.13-7.08 (m, NH), 6.89-6.85 (m, NH), 6.85-6.76 (m, NH), 5.59-5.48 (m, NH), 4.28-4.22 (m, 1H, CH), 4.12-4.06 (q, 2H, 7.2 Hz), 4.06-3.99 (m, 1.3H), 3.81 (d, 2H, 6 Hz), 3.71 (d, 2.56H, 5.6 Hz), 3.05-2.95 (m, 2H), 2.86-2.74 (m, 2H), 2.67 (s, 3H, CH₃), 2.58-2.45 (m, 0.62H, CH₂_{exo}), 2.34-2.18 (m, 2.34H, CH₂_{endo}), 1.92-1.85 (m + s, 5H, CH₃ + 2CH), 1.81-1.73 (m, 1H), 1.63-1.52 (m, 2H), 1.45-1.26 (m, 5H), 1.18 (t, 3H, CH₃, 6.8 Hz), 1.10-0.99 (m, 0.68H, CH₂_{exo}), 0.90-0.79 (m, 1.35H, CH₂_{endo}), 0.75-0.66 (M, 1H) / ¹³C NMR (100MHz, CD₃CN): δ 173.0, 171.5, 170.7, 170.4, 158.9, 141.8, 139.5, 138.3, 94.6, 92.5, 62.48, 61.8, 53.9, 44.0, 41.9, 41.1, 32.1, 30.4, 30.1, 28.5, 28.4, 28.3, 23.3, 23.0, 20.9, 20.8, 14.5 / HRMS: calculated for $C_{34}H_{45}IN_6O_7$ M(+): 777.2473, found : 777.2479 / t_R : 6.8 min.

2) Determination of partition coefficients (LogP)

The partition coefficients of **1a** and **1b** were measured according to the method described by Ogawa K et al.² Namely, **1a** or **1b** were mixed with 3 mL each of 1-octanol and phosphate buffer (0.02 M, pH 7.4) in a test tube. The mixture was vortexed for 10 min. The mixture was then centrifuged at 1000 g for 5 min. 2.5 mL of 1-octanol was removed and added to 2.5 mL of new phosphate buffer (0.02 M, pH 7.4). After repeating the same procedure twice, 100 μ L and 1 mL of 1-octanol and phosphate buffer were withdrawn and their radioactivity and weight were measured. The partition coefficients were determined by calculating the ratio of cpm/mL in 1-octanol to that in the buffer, and expressed as a common logarithm (log P). Each log P determination was performed in triplicate.

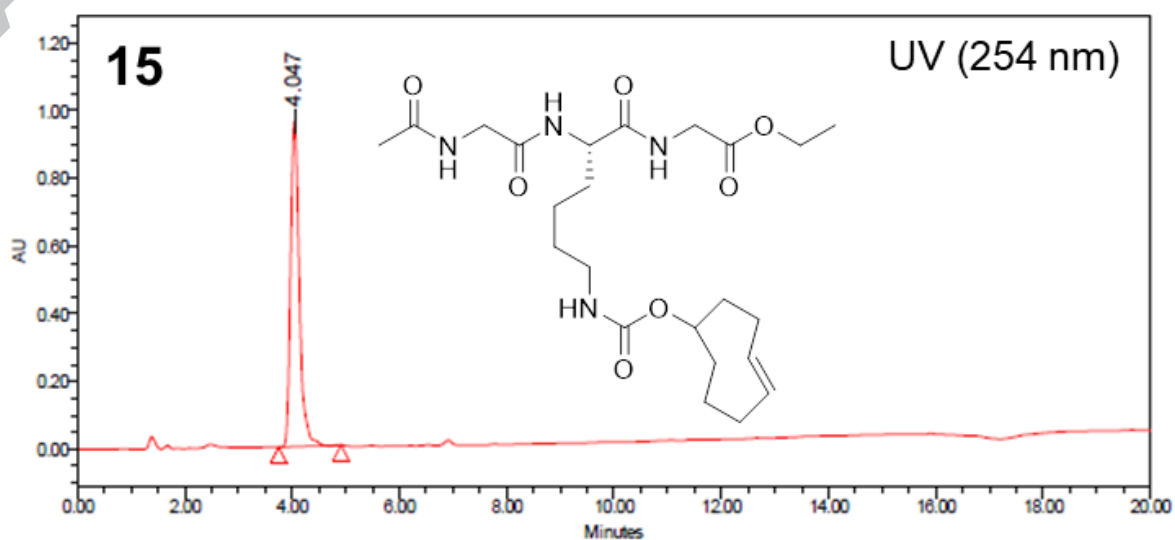
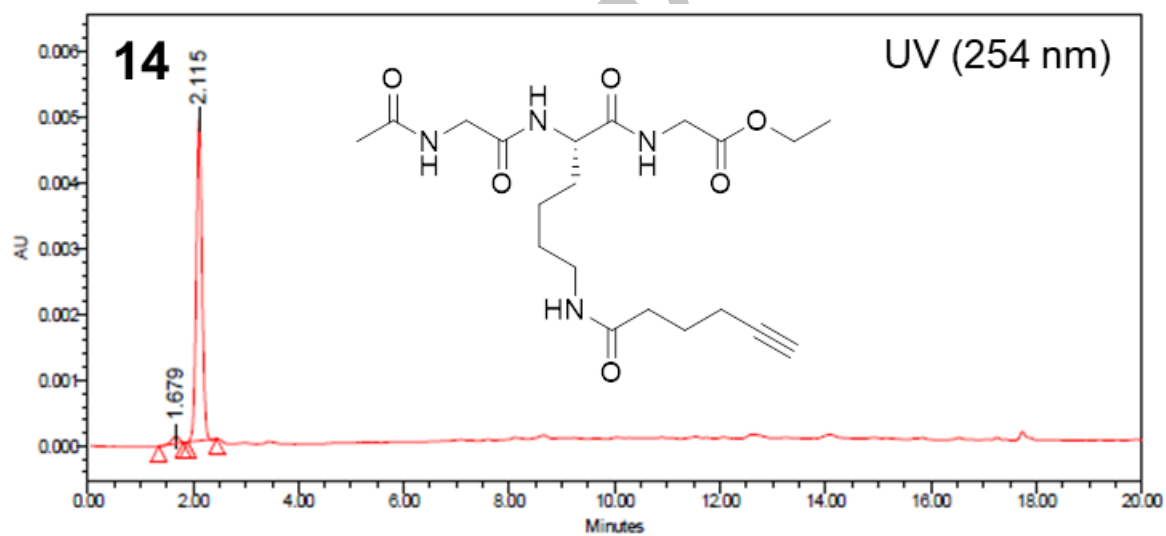
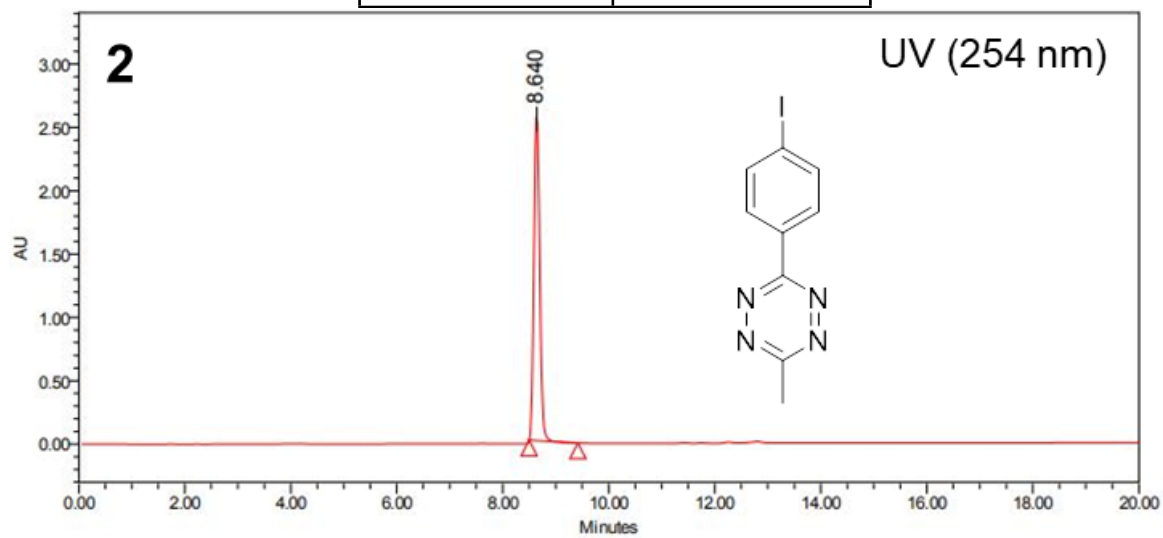
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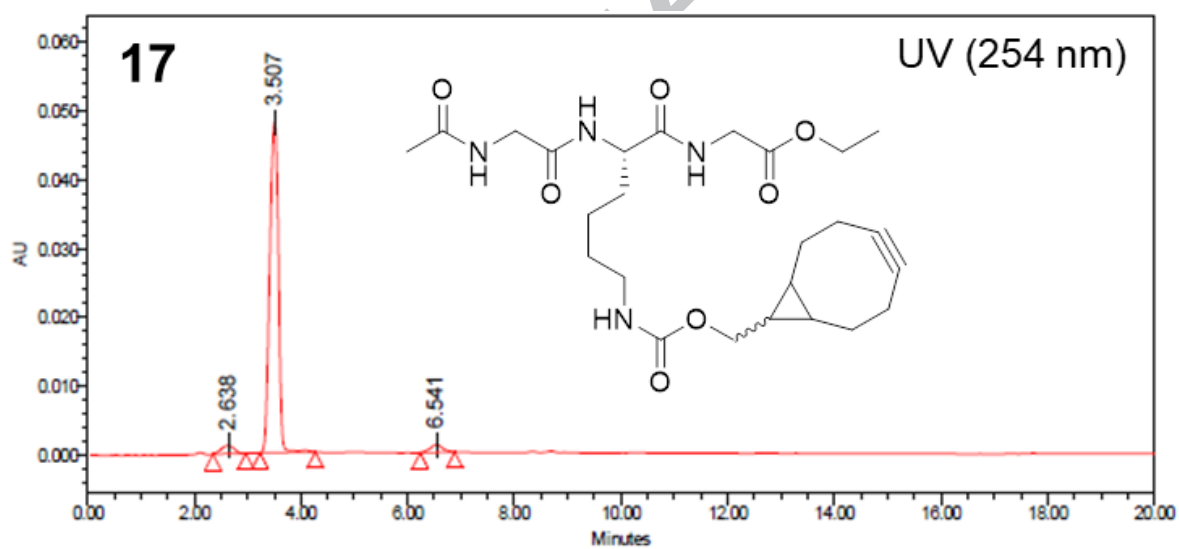
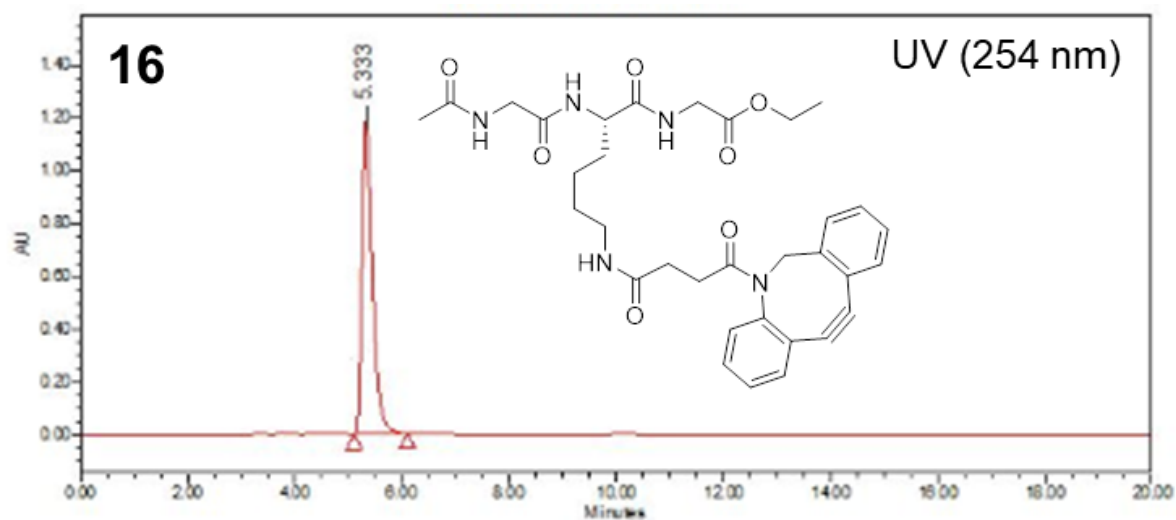
1. Dommerholt J, Schmidt S, Temming R, et al. Readily Accessible Bicyclononynes for Bioorthogonal Labeling and Three-Dimensional Imaging of Living Cells. *Angew Chem Int Ed.* 2010;49(49):9422-9425. doi:10.1002/anie.201003761
2. Ogawa K, Shiba K, Akhter N, et al. Evaluation of radioiodinated vesamicol analogs for sigma receptor imaging in tumor and radionuclide receptor therapy. *Cancer Science.* 2009;100(11):2188-2192. doi:10.1111/j.1349-7006.2009.01279.x

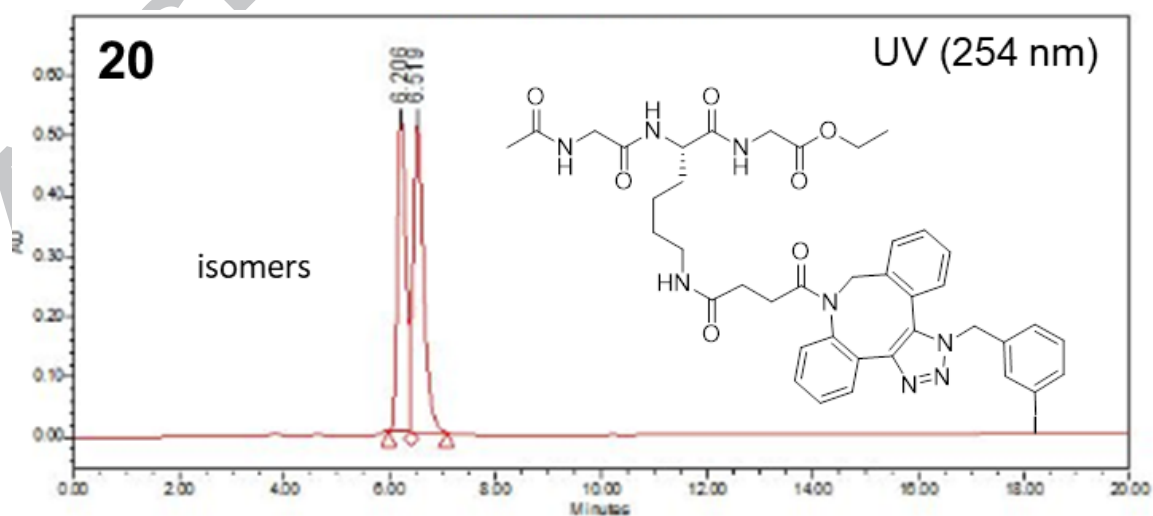
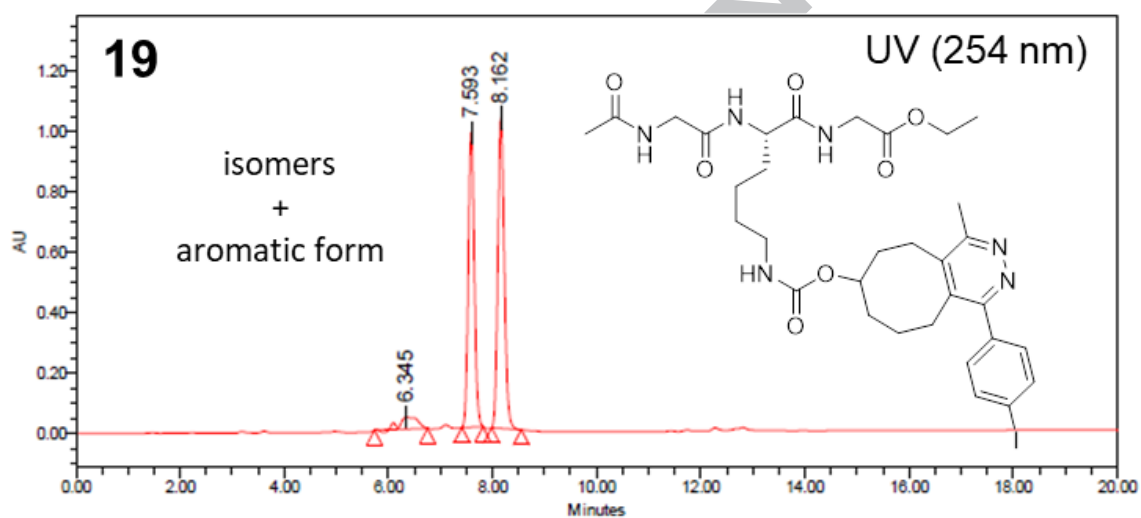
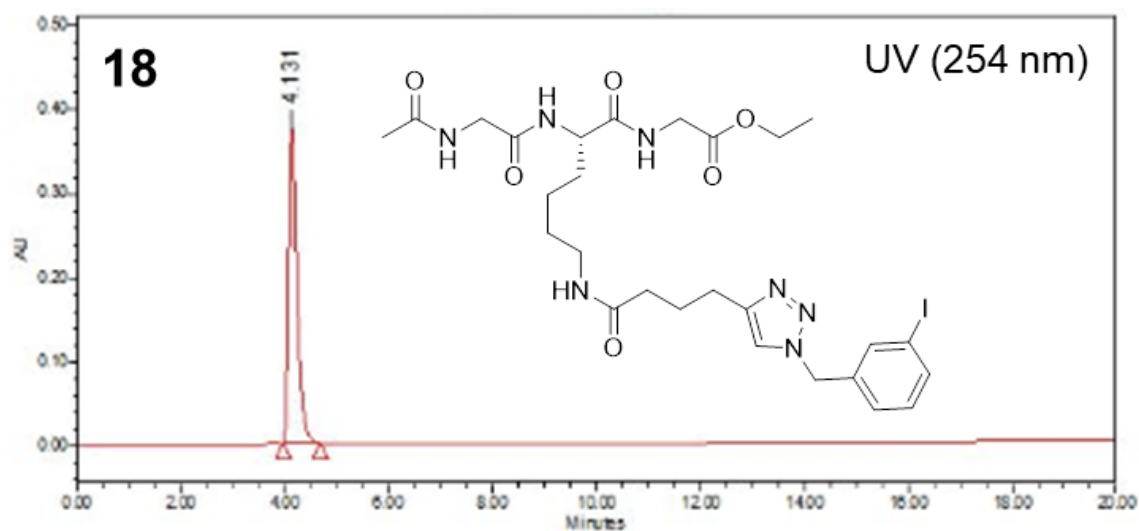
3) RETENTION TIME TABLE AND UV-HPLC CHROMATOGRAMS OF REFERENCE COMPOUNDS

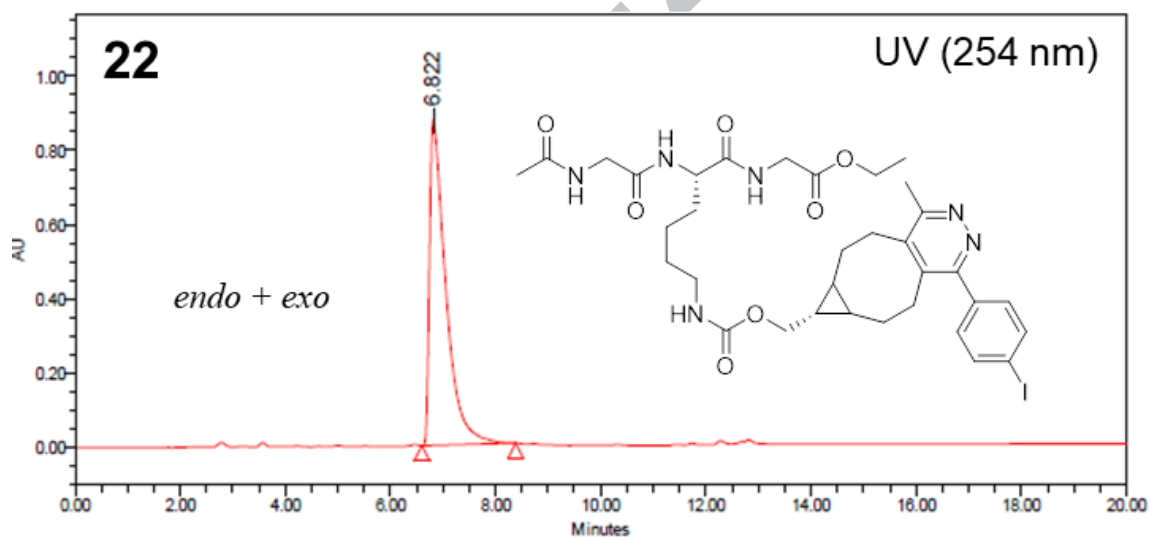
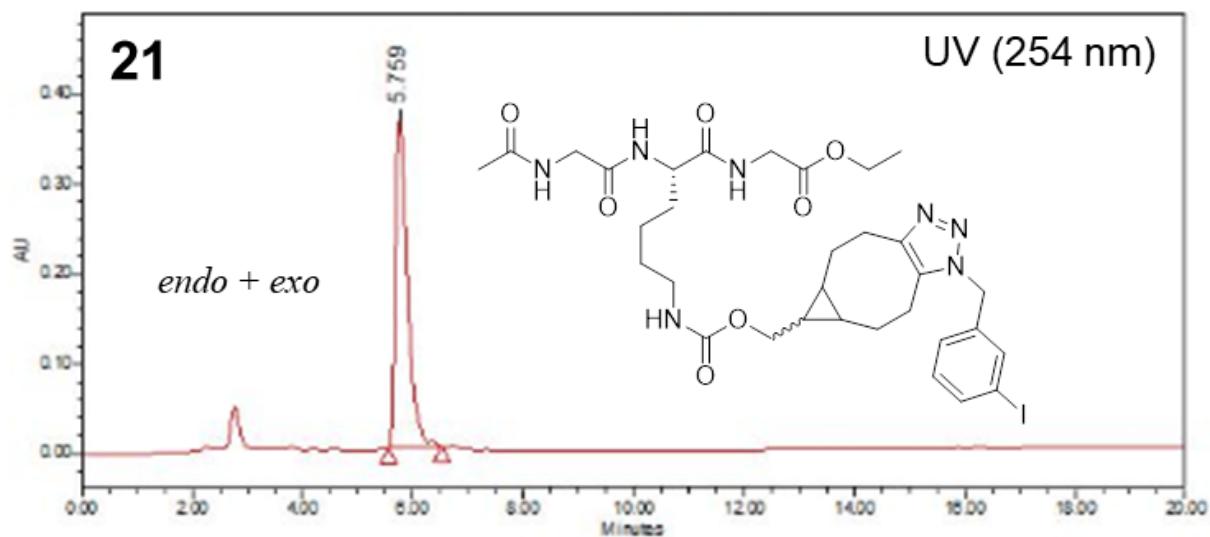
Compound	t_R (min)
1	8.3
2	8.6
14	2.1
15	4.0
16	5.3
17	3.5
18	4.1
19	6.3 + 7.6 + 8.2

20	6.2 + 6.5
21	5.8
22	6.8

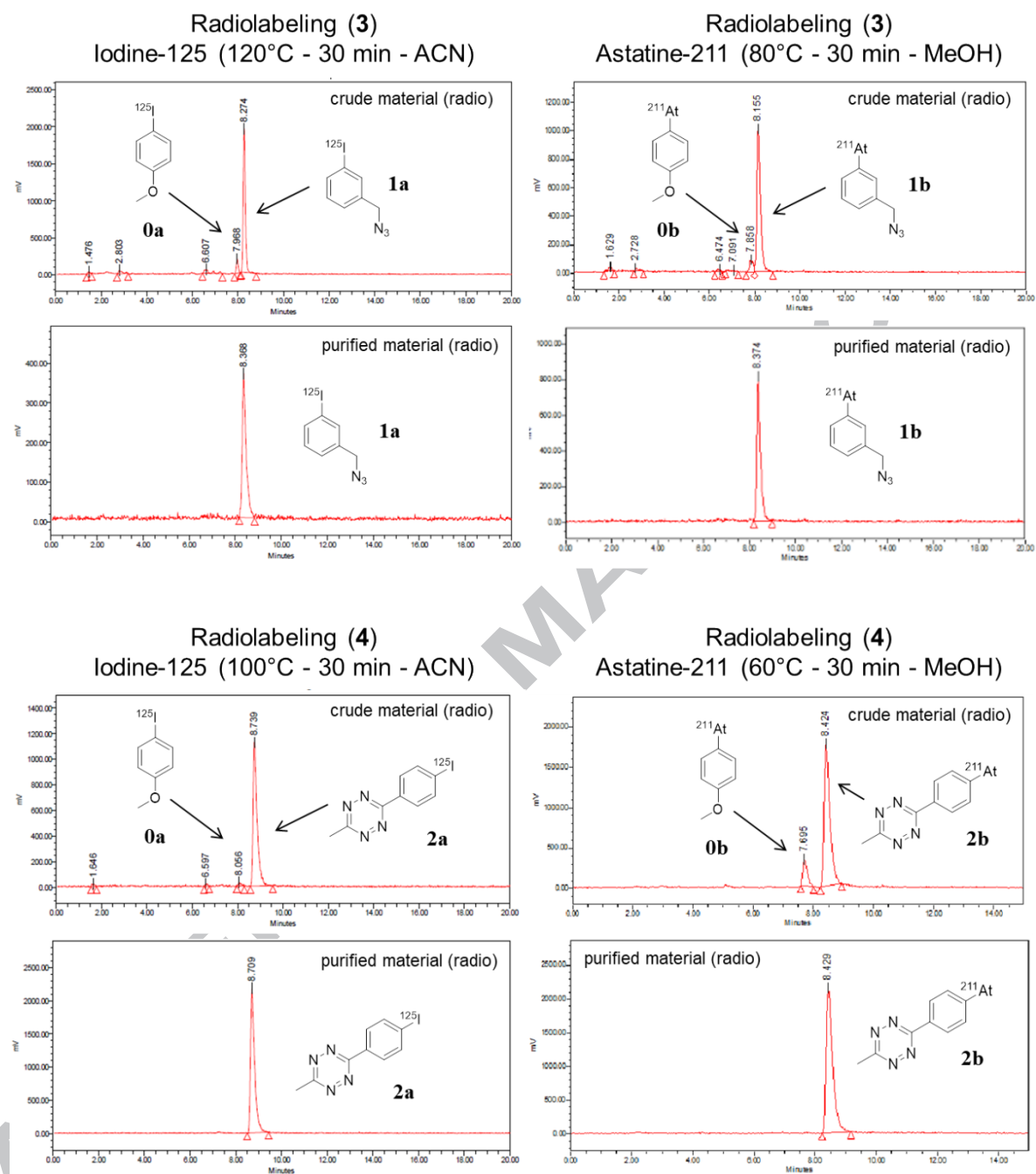


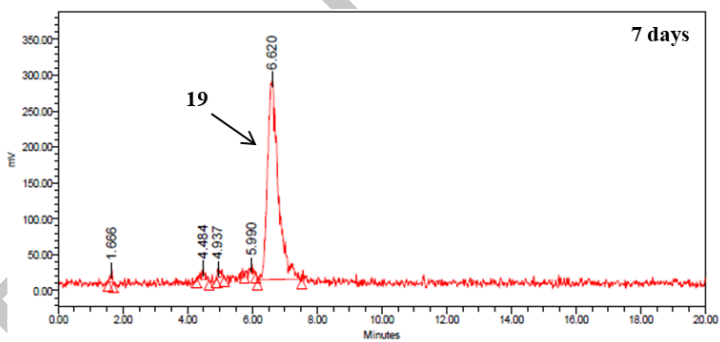
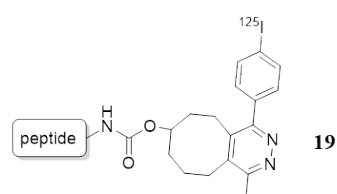
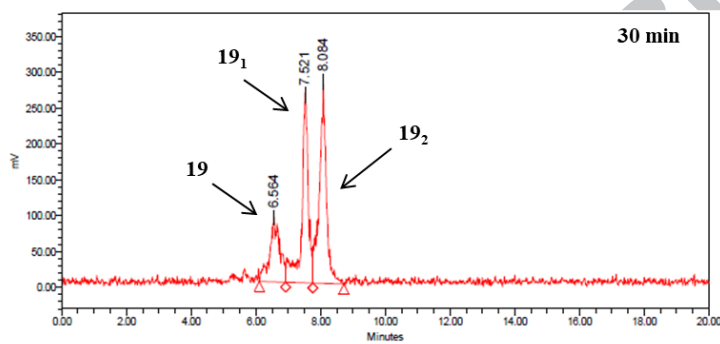
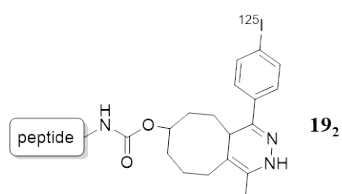
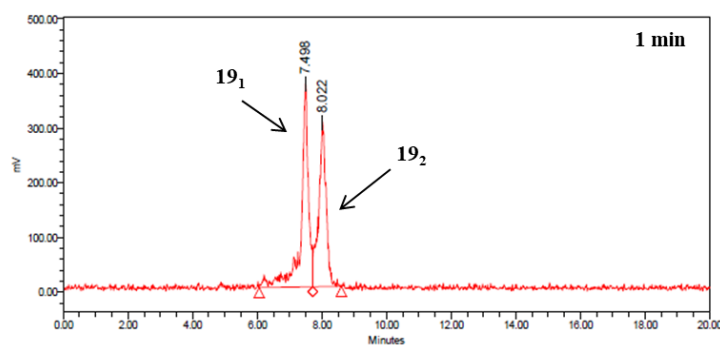
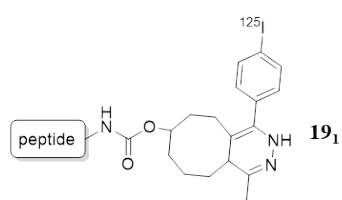


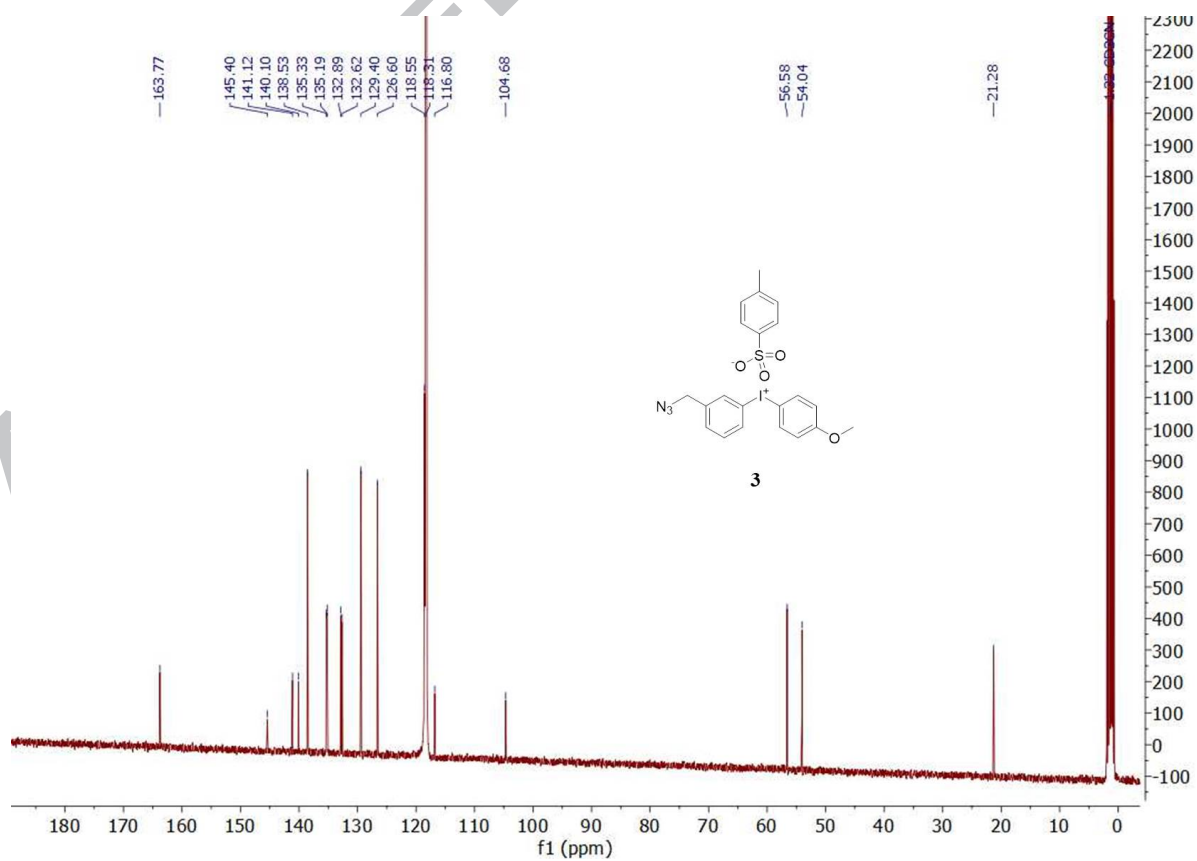
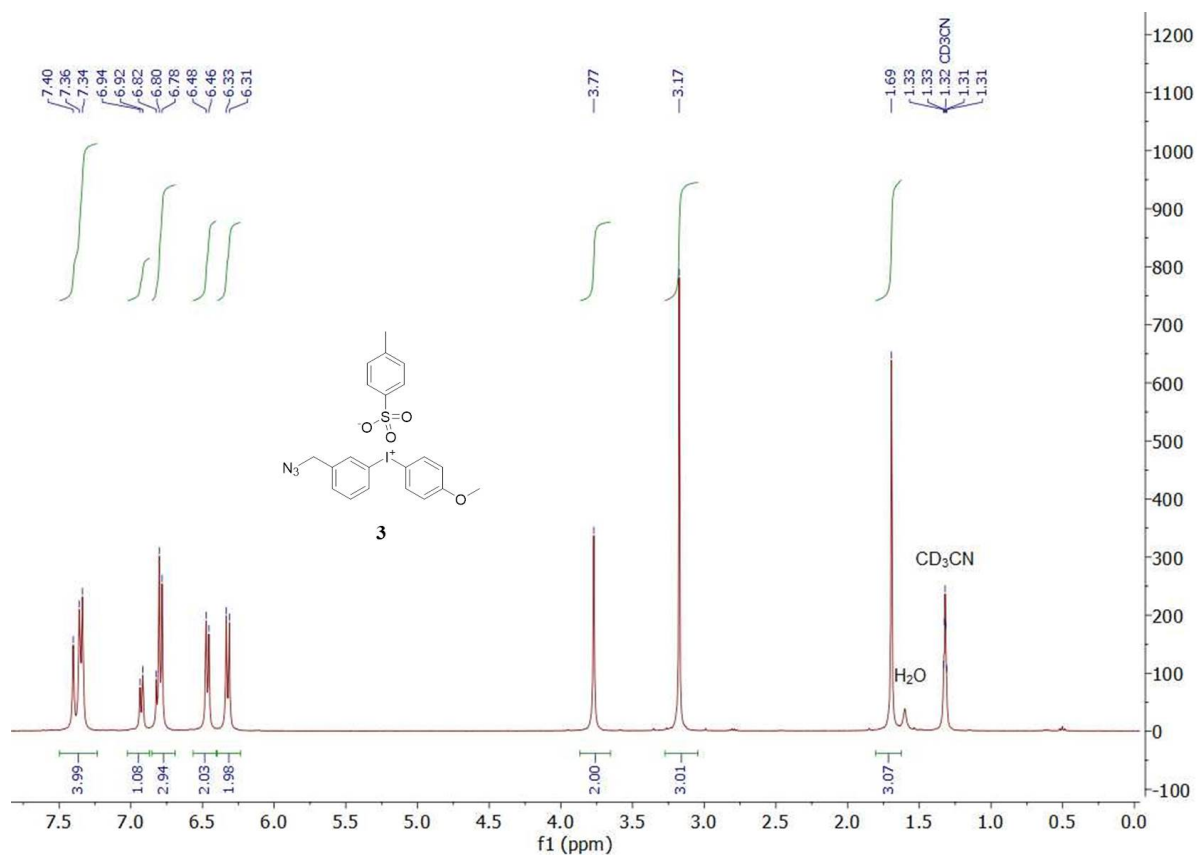




4) Radiochromatograms





5) NMR SPECTRA + HRMS

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 400.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

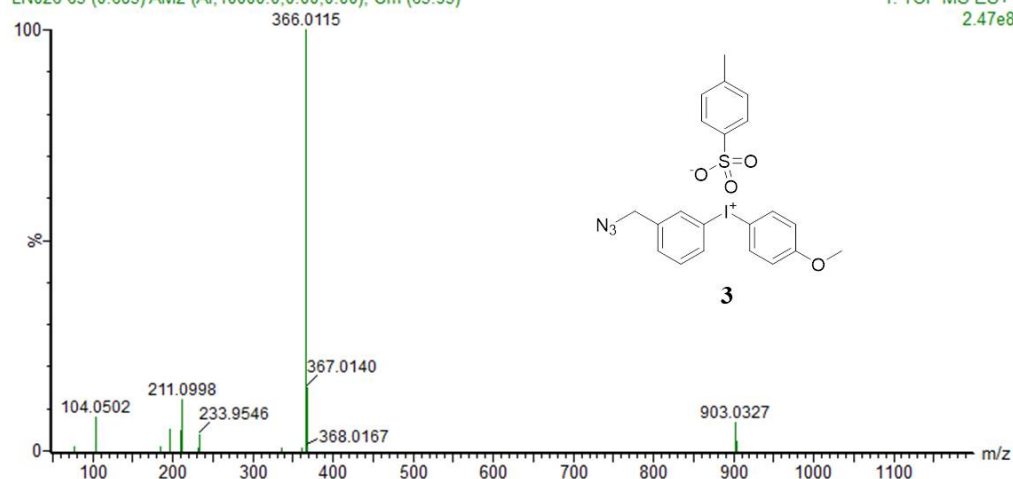
1316 formula(e) evaluated with 7 results within limits (all results (up to 1000) for each mass)

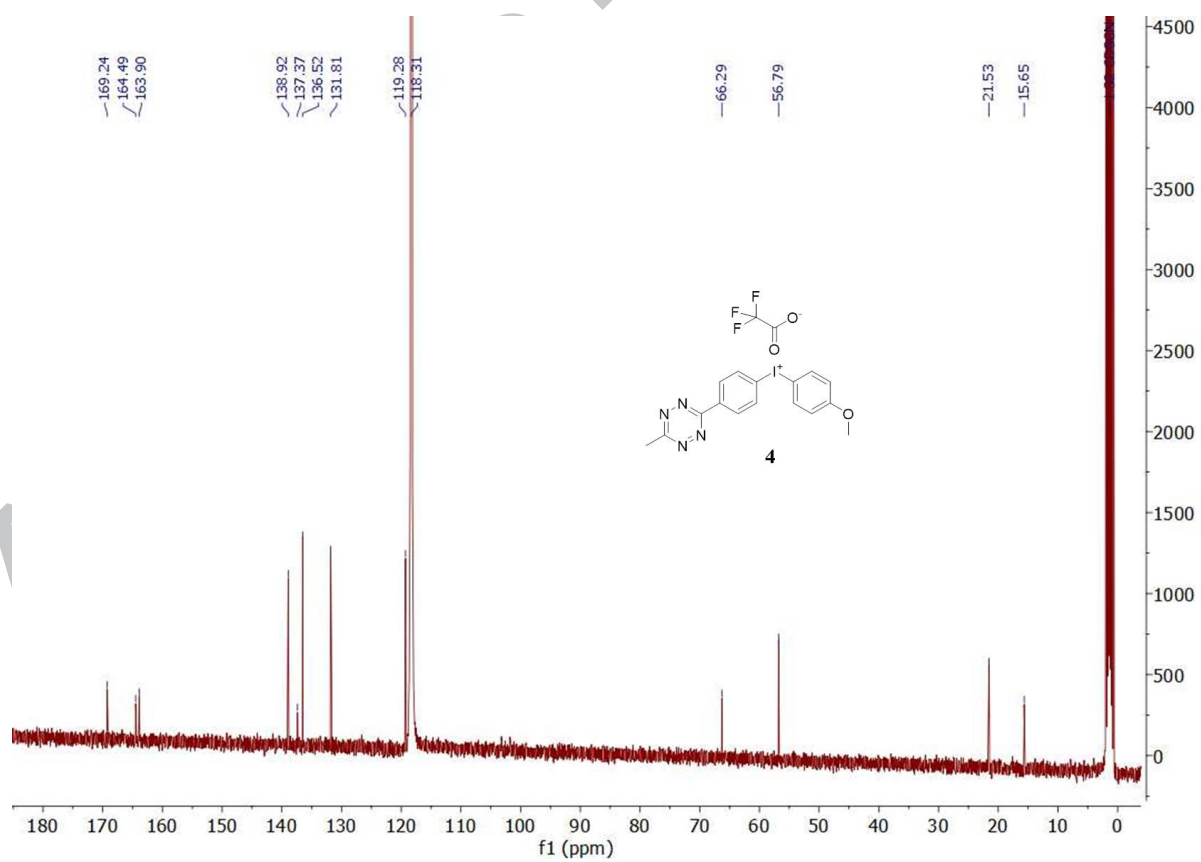
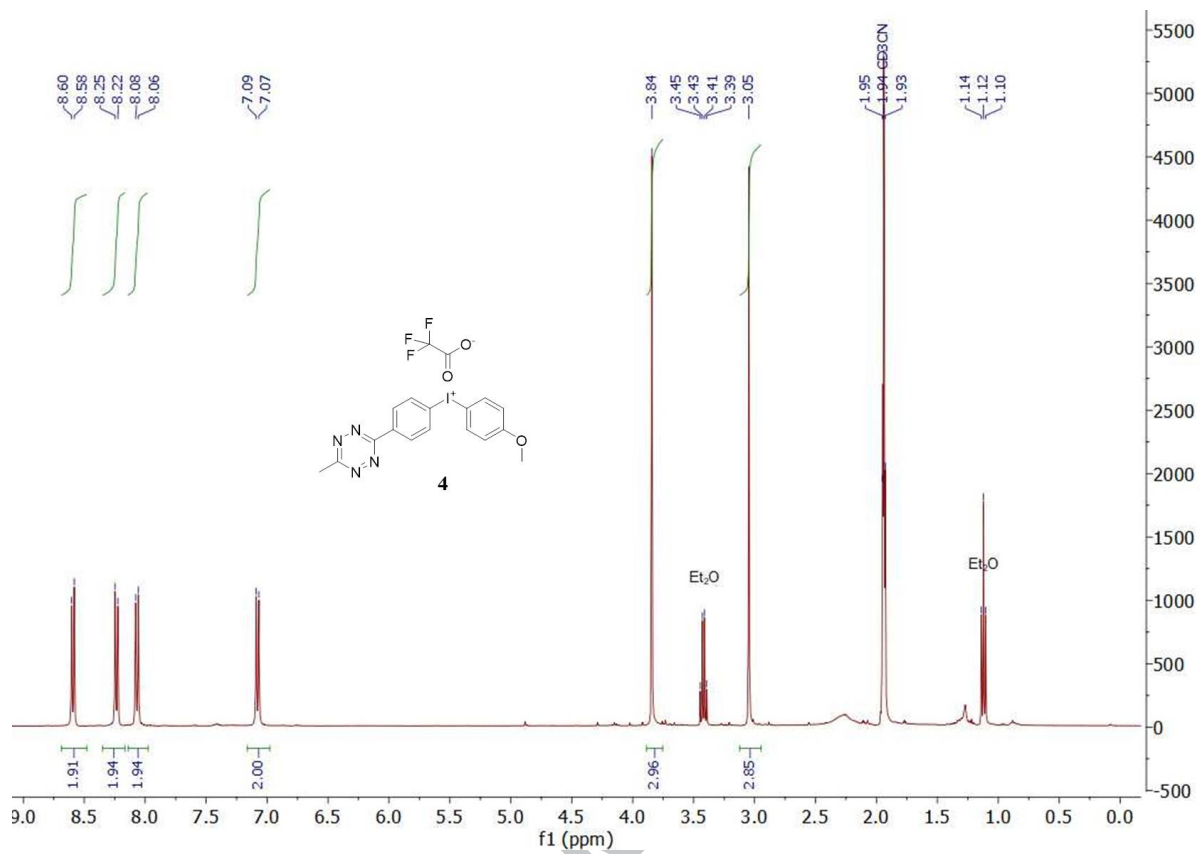
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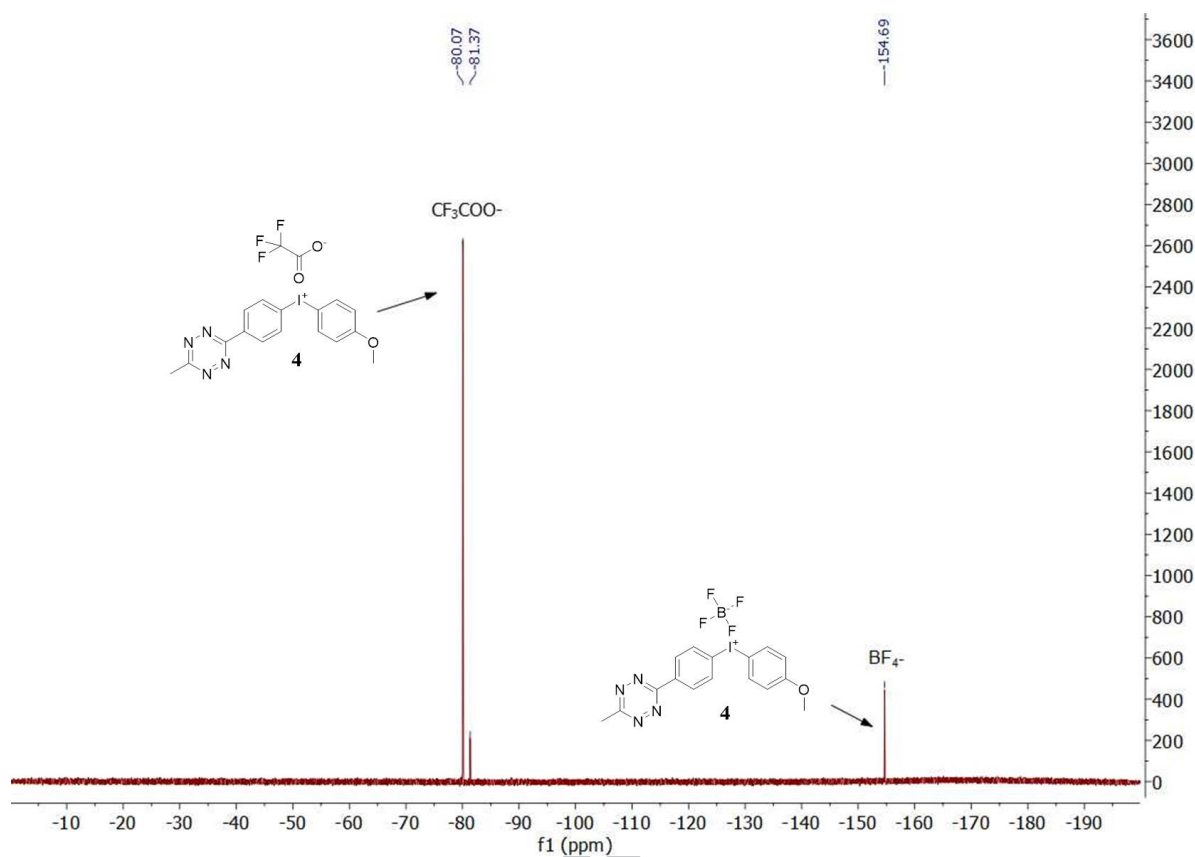
C: 0-50 H: 0-50 N: 0-20 O: 0-20 I: 0-2

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O	I
366.0115	366.0116	-0.1	-0.3	9.5	N17 O8	116.2	12.139	0.00			17	8	
	366.0116	-0.1	-0.3	-1.5	C2 H12 N3 O18	111.8	7.720	0.04	2	12	3	18	
	366.0111	0.4	1.1	16.5	C15 H4 N5 O7	108.0	3.964	1.90	15	4	5	7	
	366.0124	-0.9	-2.5	21.5	C16 N9 O3	108.4	4.300	1.36	16		9	3	
	366.0103	1.2	3.3	9.5	C14 H13 N3 O I	104.1	0.035	96.61	14	13	3	1	1
	366.0129	-1.4	-3.8	3.5	C3 H8 N7 O14	112.6	8.474	0.02	3	8	7	14	
	366.0097	1.8	4.9	11.5	C14 H8 N O11	111.3	7.246	0.07	14	8	1	11	

LN026 65 (0.685) AM2 (Ar,10000 0.0,0.0,0.00); Cm (65:95)

1: TOF MS ES+
2.47e8





Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 400.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

20315 formula(e) evaluated with 110 results within limits (all results (up to 1000) for each mass)

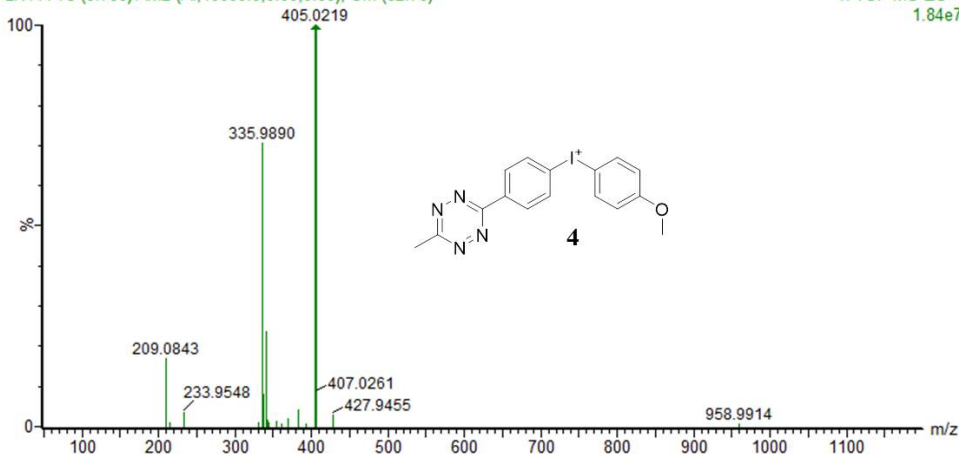
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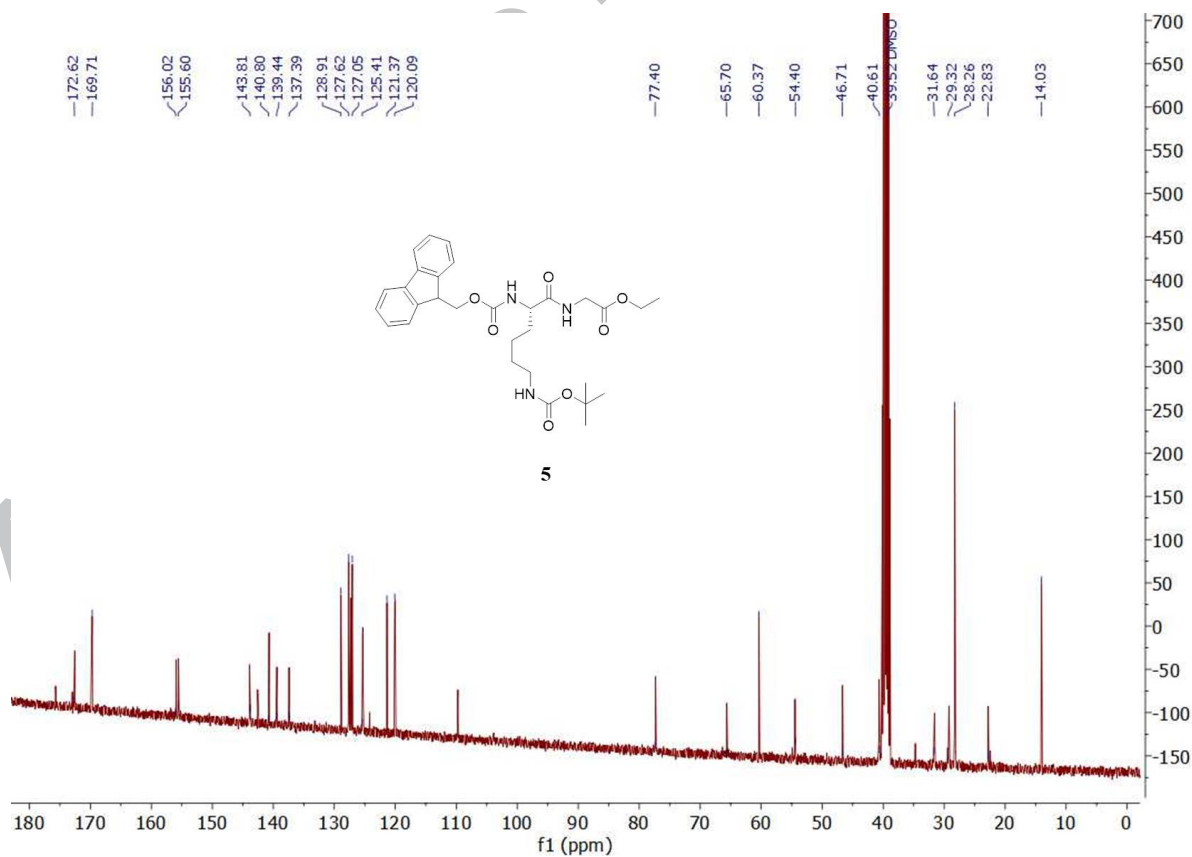
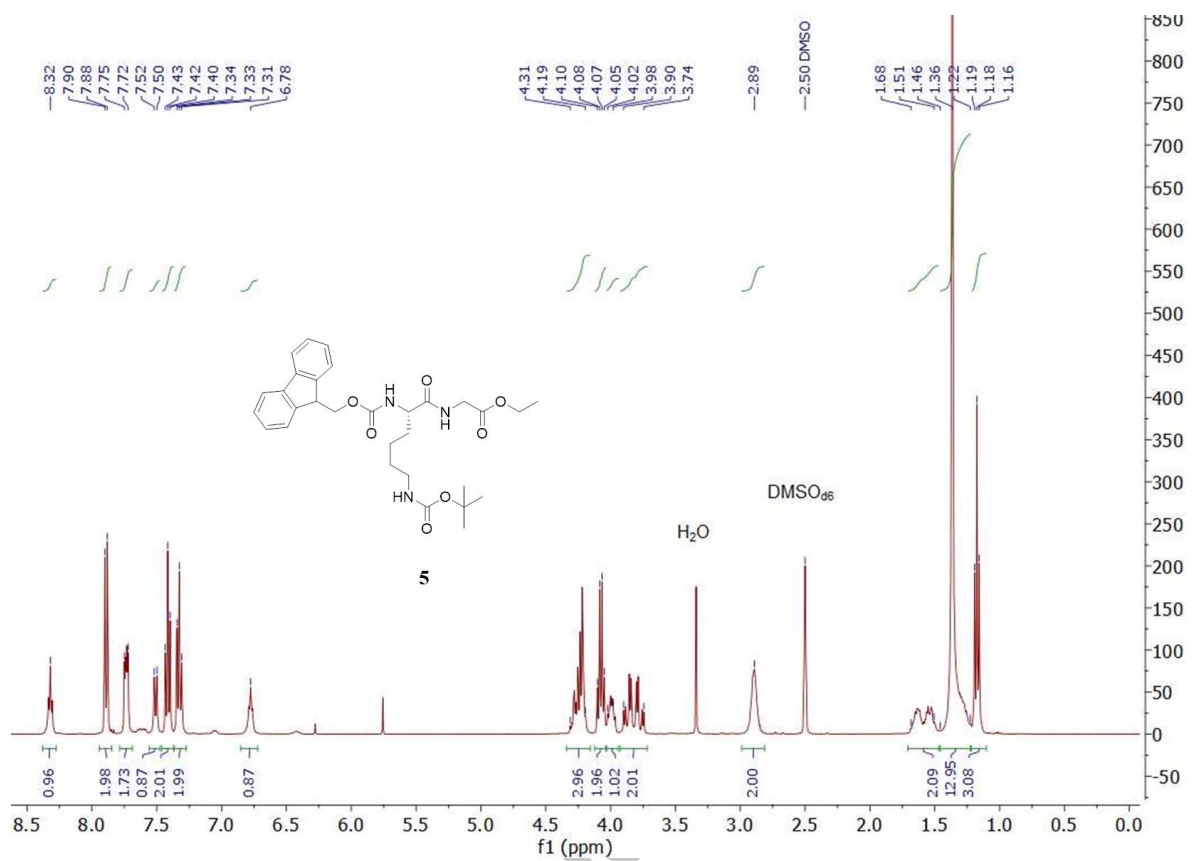
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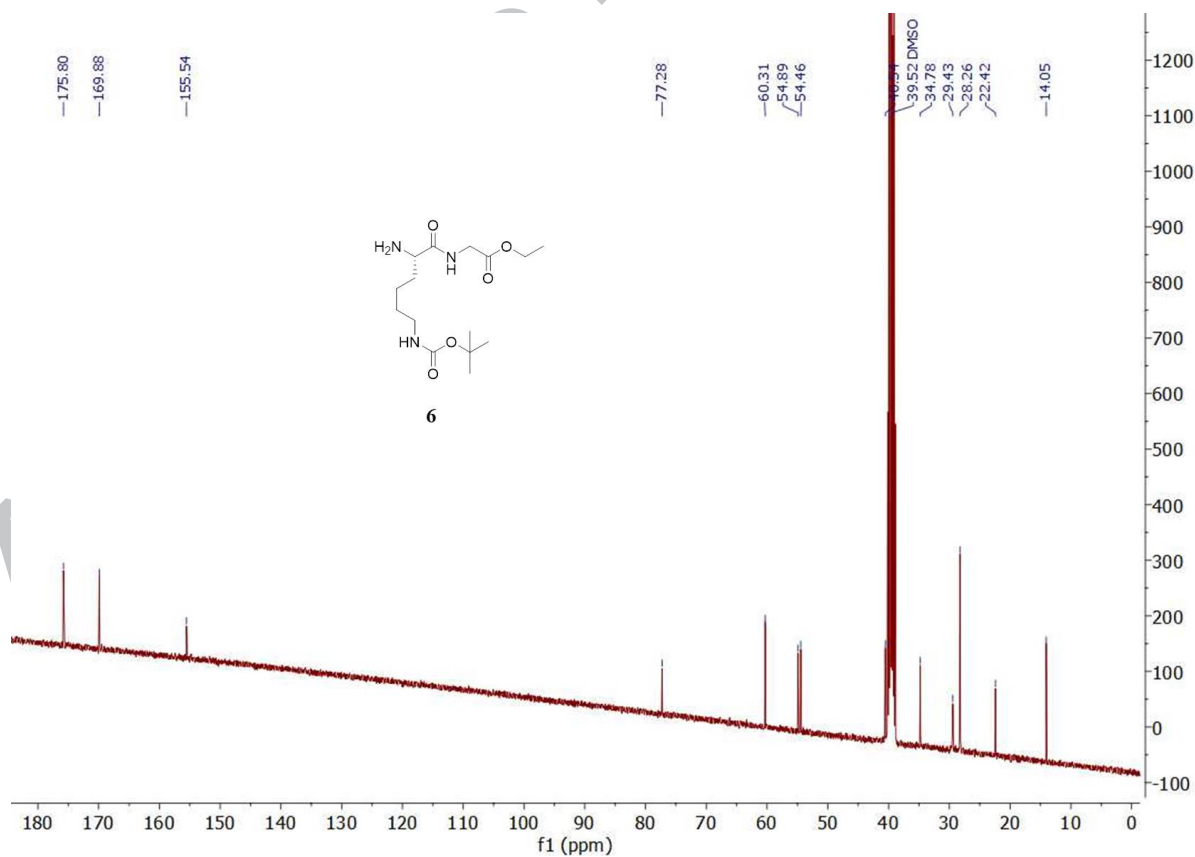
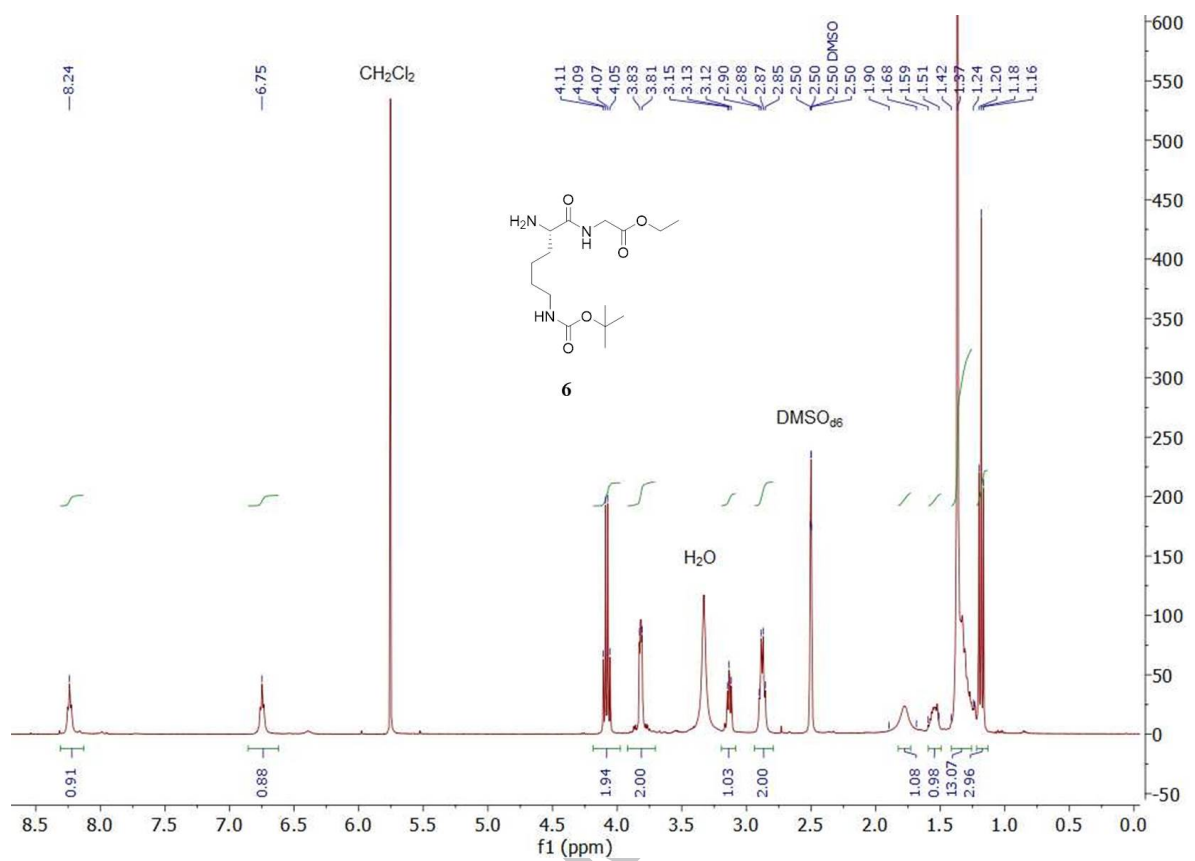
I: 0-2

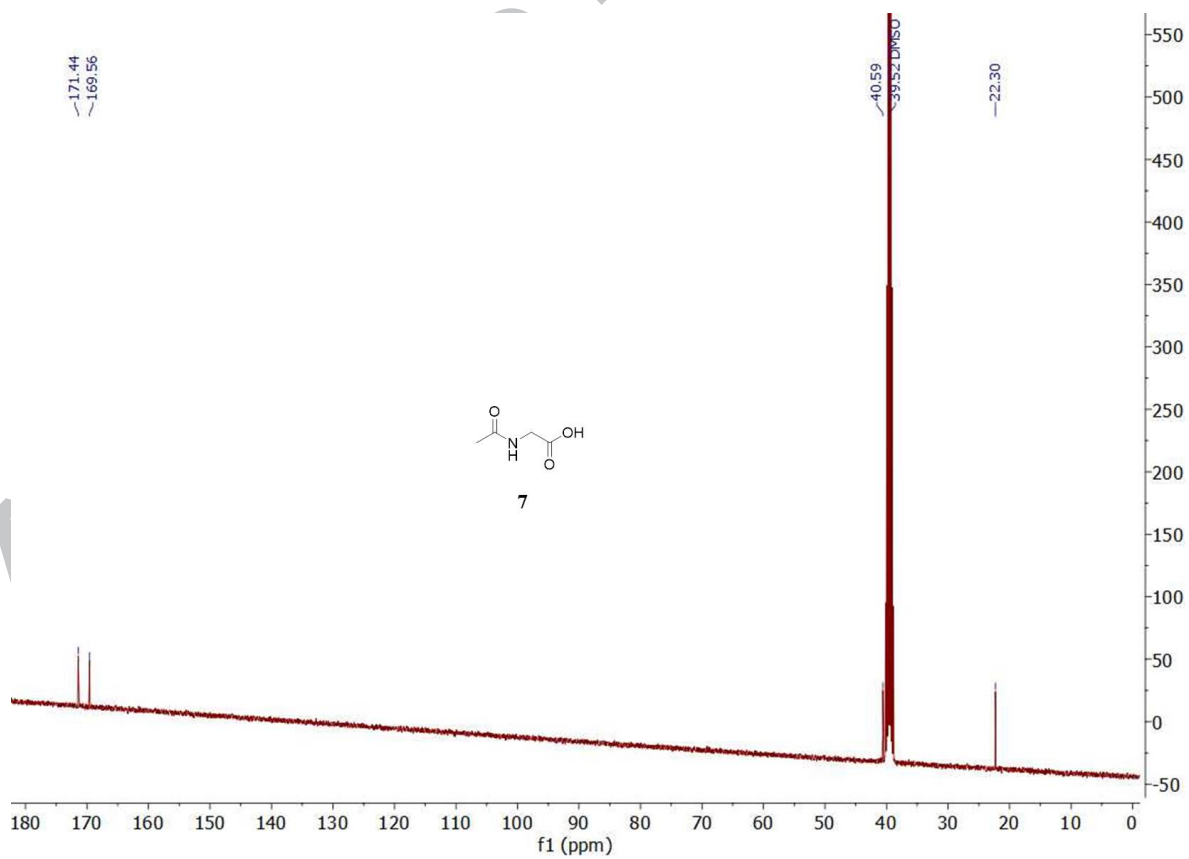
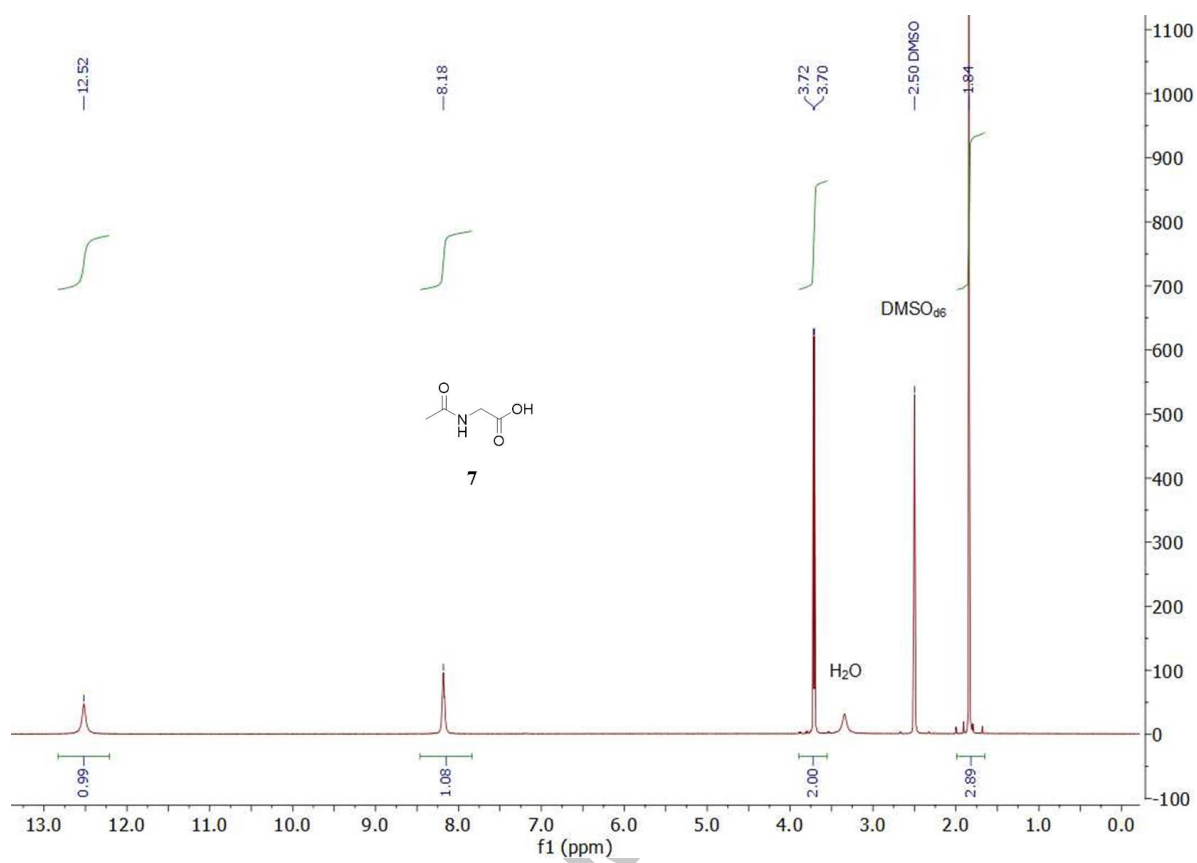
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405.0235	-1.6	-4.0	7.5	C15 H18 N2 O2 F S4	145.9	20.249	0.00	15	18	2	2	1	4		
405.0213	0.6	1.5	8.5	C15 H15 N2 O3 F2 S3	145.3	19.674	0.00	15	15	2	3	2	3		
405.0199	2.0	4.9	6.5	C15 H18 O5 I	130.2	4.545	1.06	15	18		5			1	
405.0218	0.1	0.2	14.5	C15 H7 N6 O4 F2 S	141.5	15.797	0.00	15	7	6	4	2	1		
405.0204	1.5	3.7	14.5	C16 H8 N6 F3 S2	144.2	18.507	0.00	16	8	6		3	2		
405.0206	1.3	3.2	13.5	C16 H9 N2 O11	132.0	6.344	0.18	16	9	2	11				
405.0231	-1.2	-3.0	19.5	C16 H3 N10 F2 S	141.4	15.696	0.00	16	3	10		2	1		
405.0208	1.1	2.7	5.5	C16 H22 S2 I	144.3	18.600	0.00	16	22			2	1		
405.0212	0.7	1.7	11.5	C16 H14 N4 O I	127.7	1.982	13.78	16	14	4	1			1	
405.0209	1.0	2.5	20.5	C16 N10 O F3	127.9	2.226	10.79	16		10	1	3			
405.0215	0.4	1.0	12.5	C17 H13 N2 O6 S2	144.0	18.339	0.00	17	13	2	6		2		
405.0220	-0.1	-0.2	18.5	C17 H5 N6 O7	130.5	4.790	0.83	17	5	6	7				
405.0201	1.8	4.4	12.5	C18 H14 N2 O2 F S3	145.3	19.660	0.00	18	14	2	2	1	3		
405.0228	-0.9	-2.2	17.5	C18 H9 N6 O2 S2	143.9	18.197	0.00	18	9	6	2	2	2		
405.0224	-0.5	-1.2	11.5	C18 H17 N2 O S4	145.9	20.186	0.00	18	17	2	1		4		
405.0233	-1.4	-3.5	23.5	C18 H N10 O3	131.5	5.861	0.28	18	1	10	3				

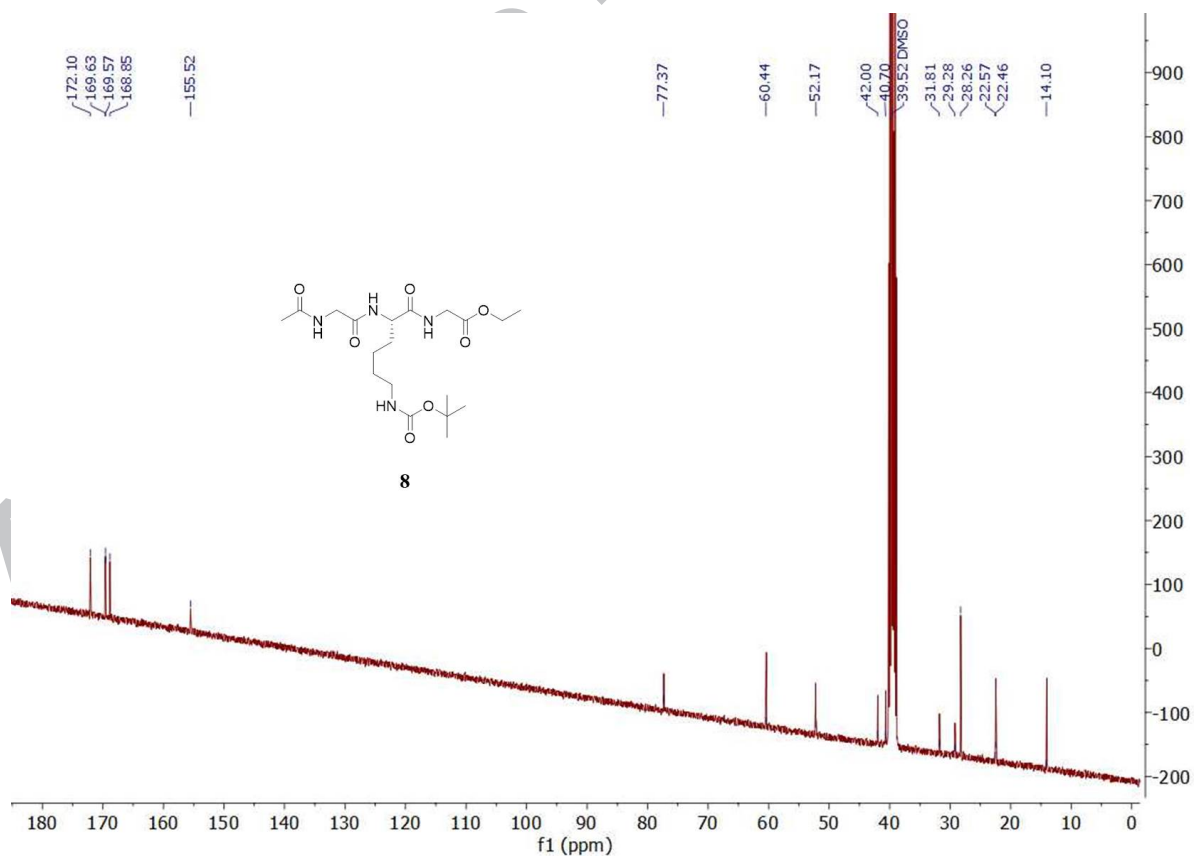
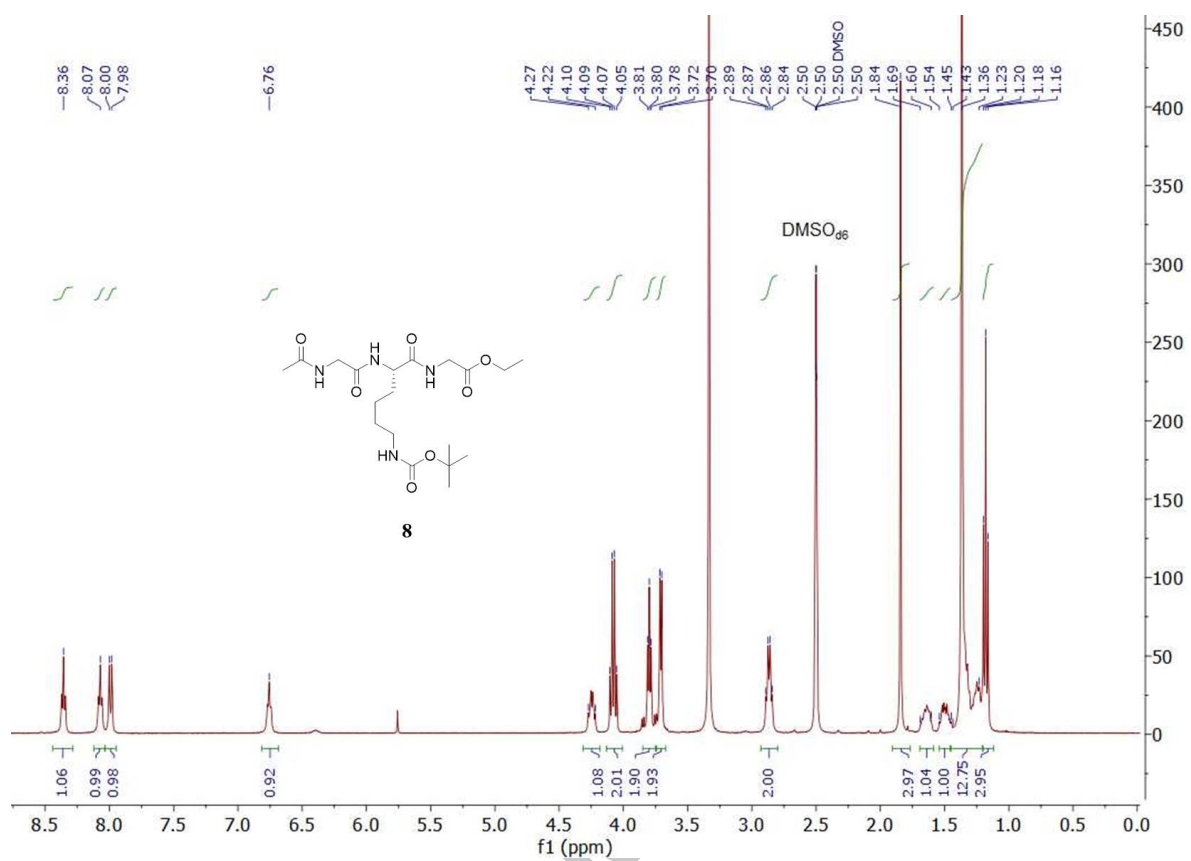
LN141 73 (0.766) AM2 (Ar,10000.0,0.00,0.00); Cm (62.76)

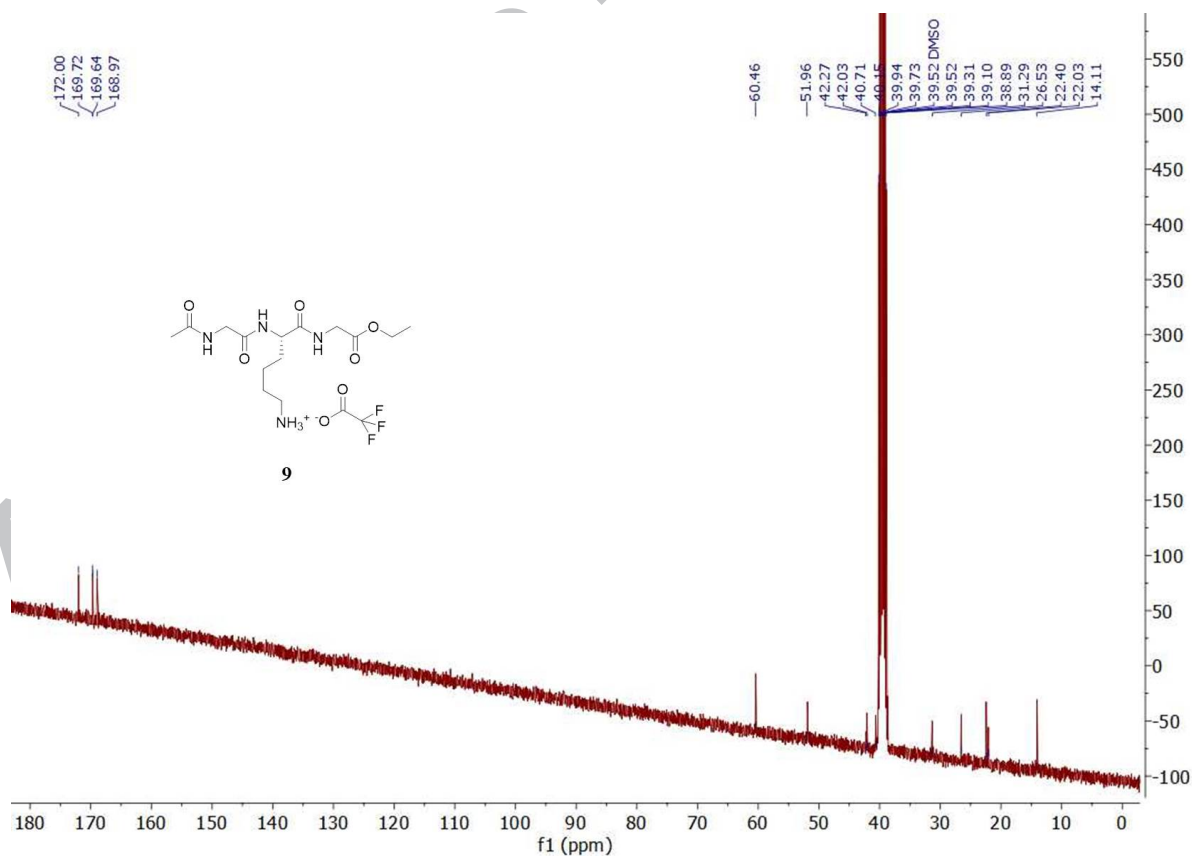
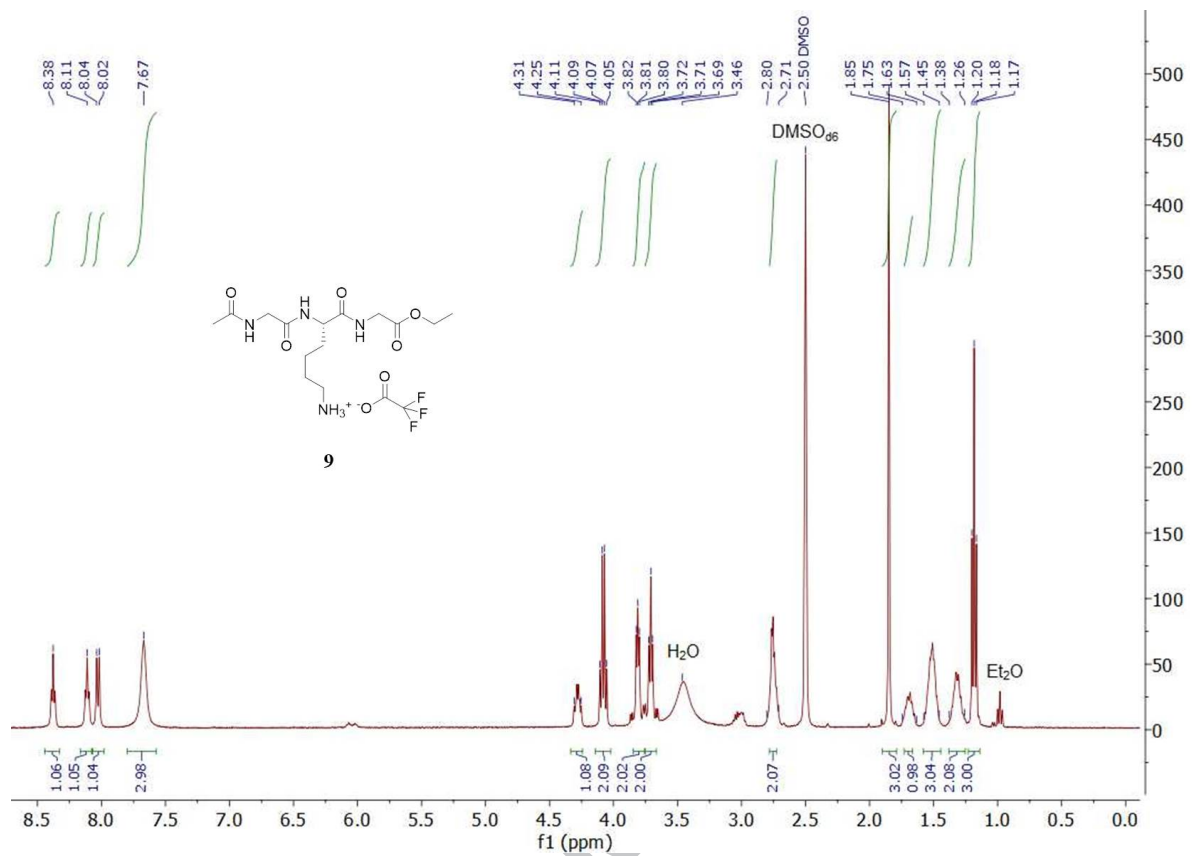
1: TOF MS ES+
1.84e7

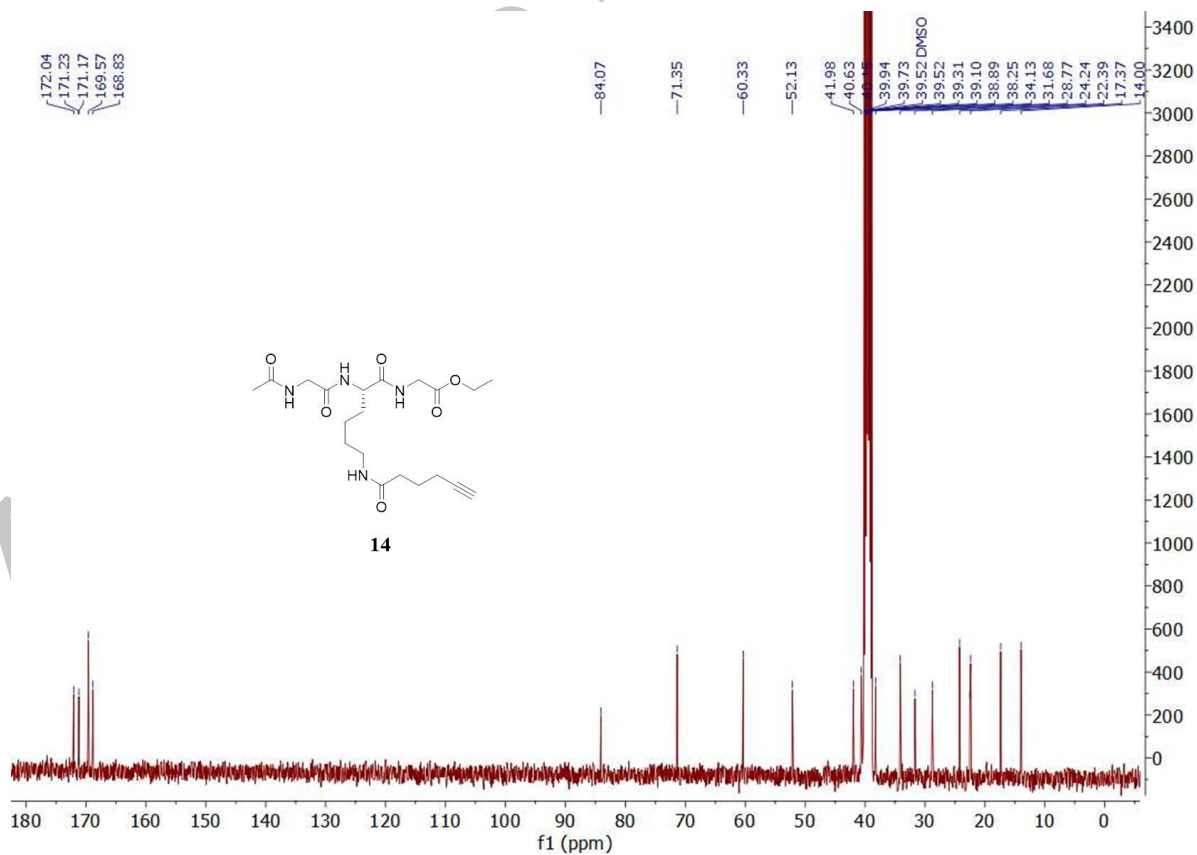
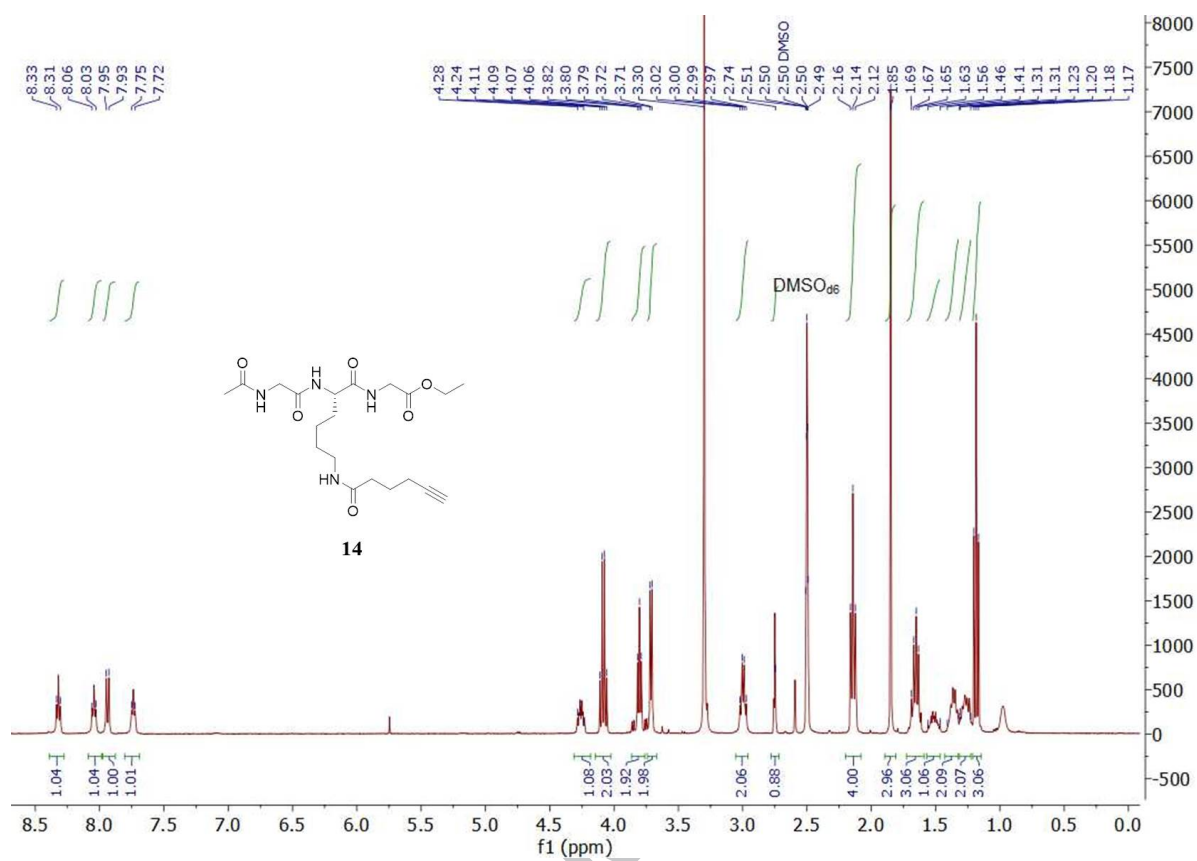












Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 400.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

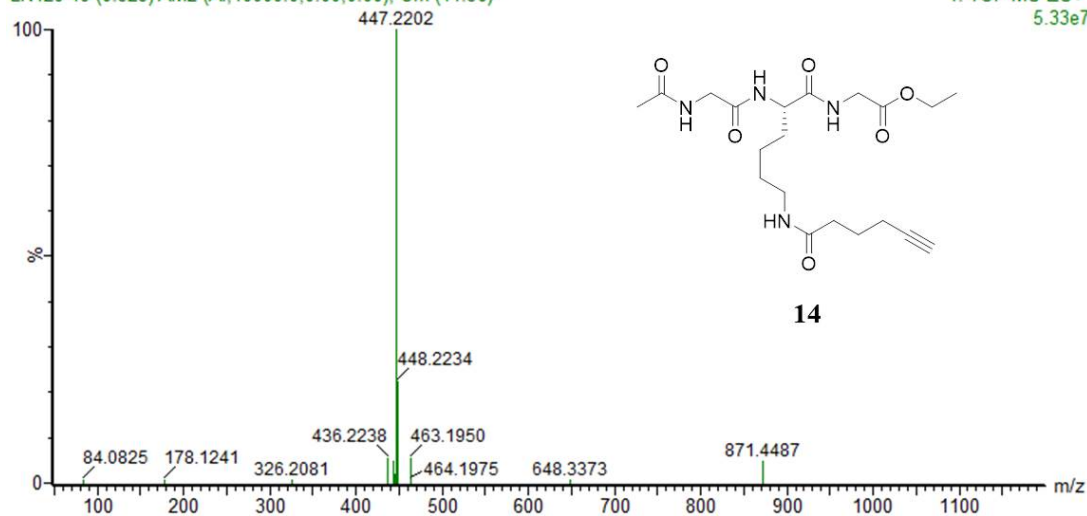
2529 formula(e) evaluated with 10 results within limits (all results (up to 1000) for each mass)

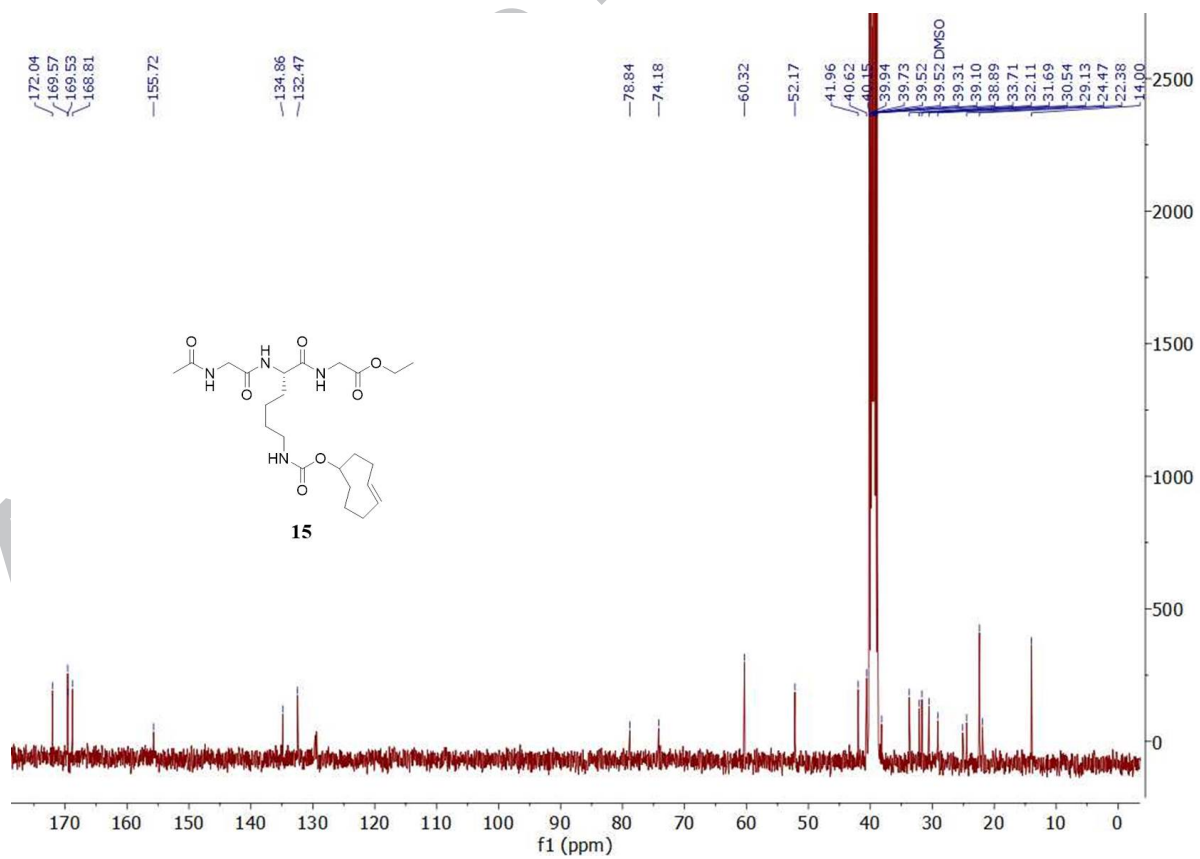
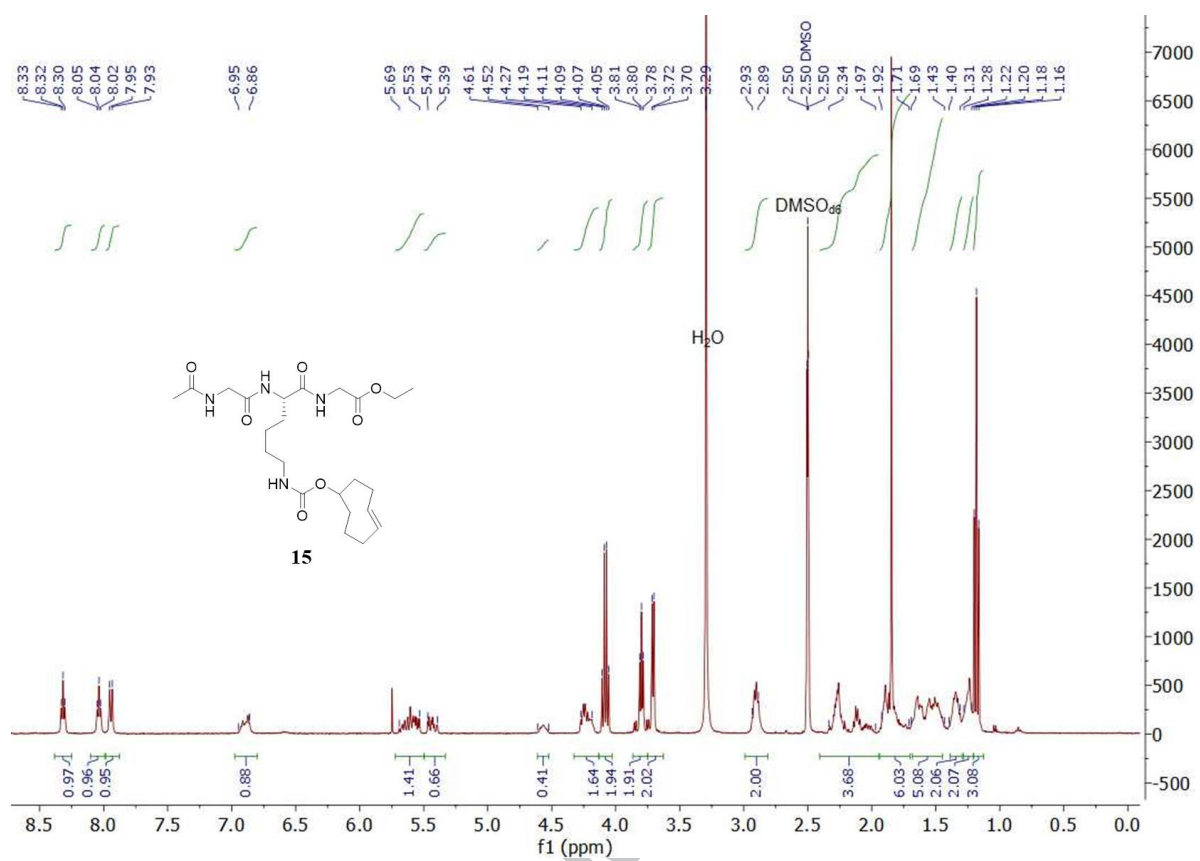
Elements Used:

C: 0-50 H: 0-50 N: 0-20 O: 0-20 Na: 0-1

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O	Na
447.2190	1.2	2.7	0.5	C16 H35 N2 O12	94.7	6.736	0.12	16	35	2	12		
447.2206	-0.4	-0.9	12.5	C17 H24 N14 Na	91.0	2.980	5.08	17	24	14		1	
447.2203	-0.1	-0.2	5.5	C17 H31 N6 O8	91.8	3.772	2.30	17	31	6	8		
447.2217	-1.5	-3.4	10.5	C18 H27 N10 O4	91.2	3.208	4.04	18	27	10	4		
447.2206	-0.4	-0.9	1.5	C19 H36 O10 Na	94.4	6.354	0.17	19	36		10	1	
447.2220	-1.8	-4.0	6.5	C20 H32 N4 O6 Na	94.1	6.131	0.22	20	32	4	6	1	
447.2185	1.7	3.8	18.5	C29 H27 N4 O	100.4	12.420	0.00	29	27	4	1		

LN129 49 (0.523) AM2 (Ar, 10000.0, 0.00, 0.00); Cm (41:56)

1: TOF MS ES+
5.33e7



Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 400.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

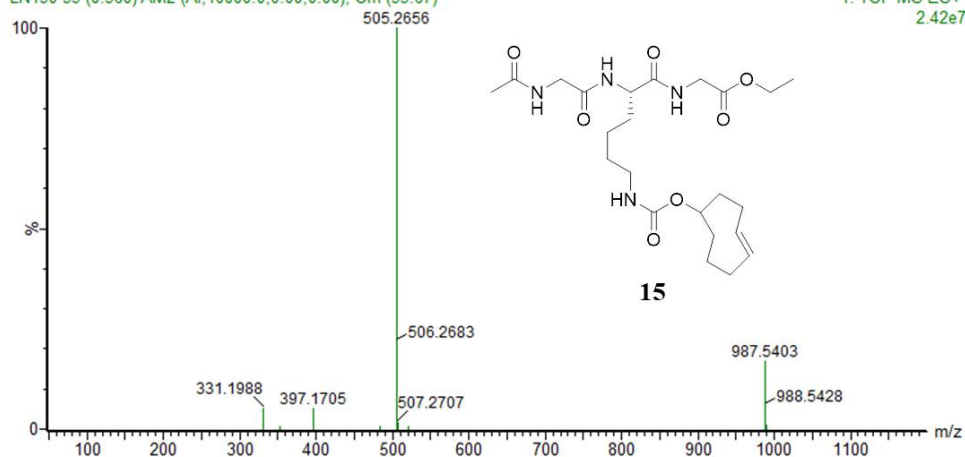
3033 formula(e) evaluated with 14 results within limits (all results (up to 1000) for each mass)

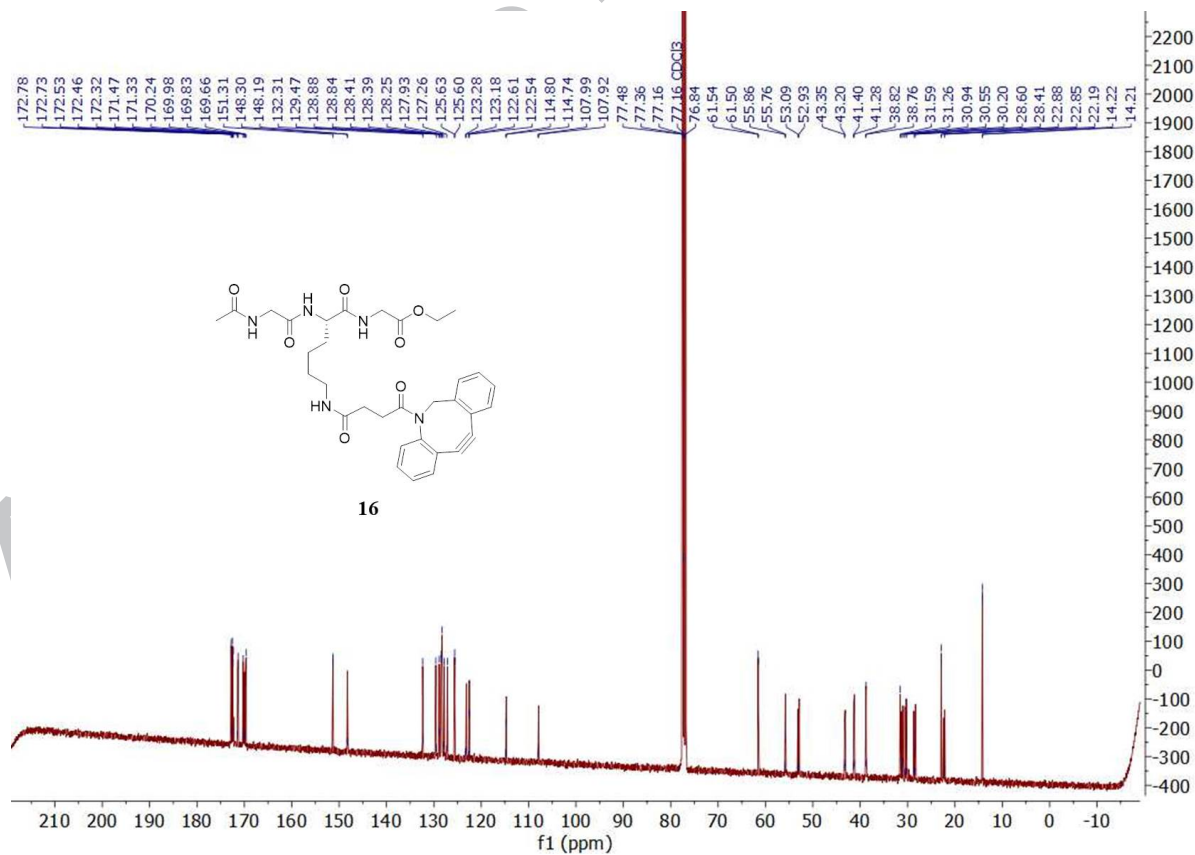
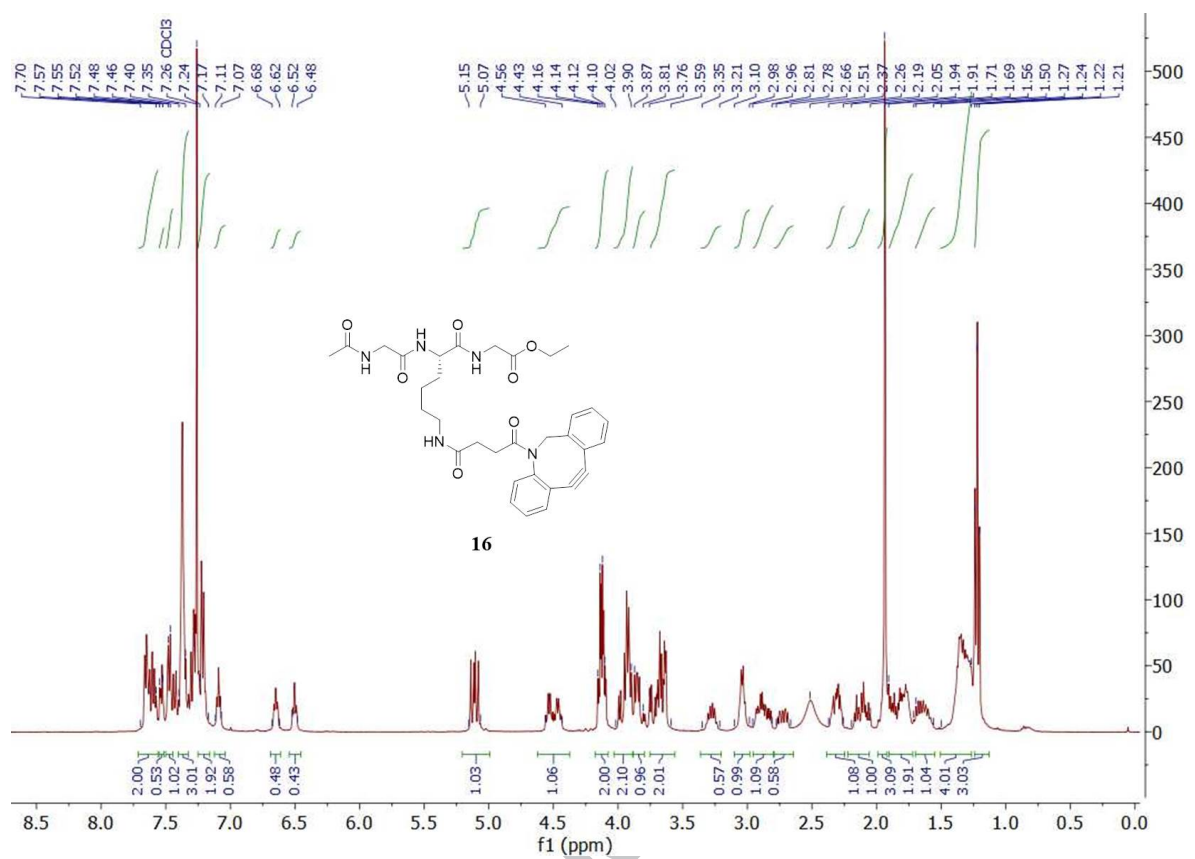
Elements Used:

C: 0-50 H: 0-50 N: 0-20 O: 0-20 Na: 0-1

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O	Na
505.2656	505.2643	1.3	2.6	-0.5	C8 H34 N16 O8 Na	41.4	4.360	1.28	8	34	16	8	1
	505.2657	-0.1	-0.2	4.5	C9 H30 N20 O4 Na	40.0	2.936	5.31	9	30	20	4	1
	505.2667	-1.1	-2.2	2.5	C10 H33 N16 O8	39.6	2.514	8.09	10	33	16	8	
	505.2681	-2.5	-4.9	7.5	C11 H29 N20 O4	39.4	2.315	9.88	11	29	20	4	
	505.2670	-1.4	-2.8	-1.5	C12 H38 N10 O10 Na	38.6	1.507	22.15	12	38	10	10	1
	505.2635	2.1	4.2	10.5	C21 H33 N10 O5	40.7	3.619	2.68	21	33	10	5	
	505.2649	0.7	1.4	15.5	C22 H29 N14 O	39.2	2.161	11.52	22	29	14	1	
	505.2638	1.8	3.6	6.5	C23 H38 N4 O7 Na	40.2	3.083	4.58	23	38	4	7	1
	505.2652	0.4	0.8	11.5	C24 H34 N8 O3 Na	38.6	1.480	22.77	24	34	8	3	1
	505.2649	0.7	1.4	4.5	C24 H41 O11	40.0	2.936	5.31	24	41		11	
	505.2662	-0.6	-1.2	9.5	C25 H37 N4 O7	40.0	2.966	5.15	25	37	4	7	
	505.2676	-2.0	-4.0	14.5	C26 H33 N8 O3	41.7	4.659	0.95	26	33	8	3	
	505.2678	-2.2	-4.4	10.5	C28 H38 N2 O5 Na	43.4	6.358	0.17	28	38	2	5	1

LN130 33 (0.360) AM2 (Ar,10000.0,0.00,0.00); Cm (33:67)

1: TOF MS ES+
2.42e7



Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 400.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

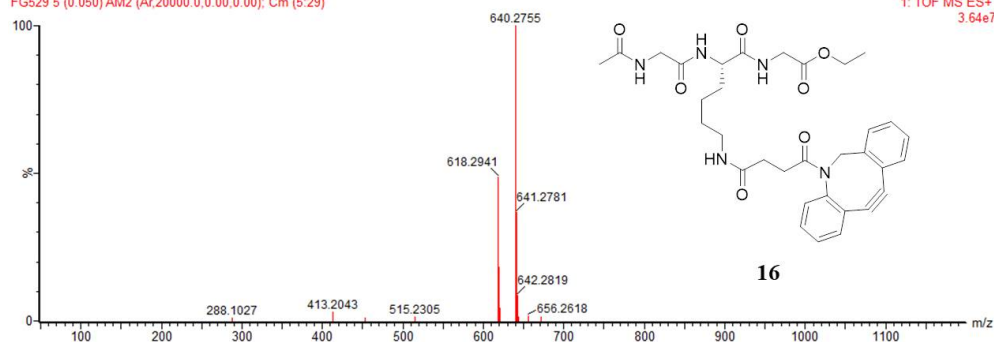
2224 formula(e) evaluated with 16 results within limits (all results (up to 1000) for each mass)

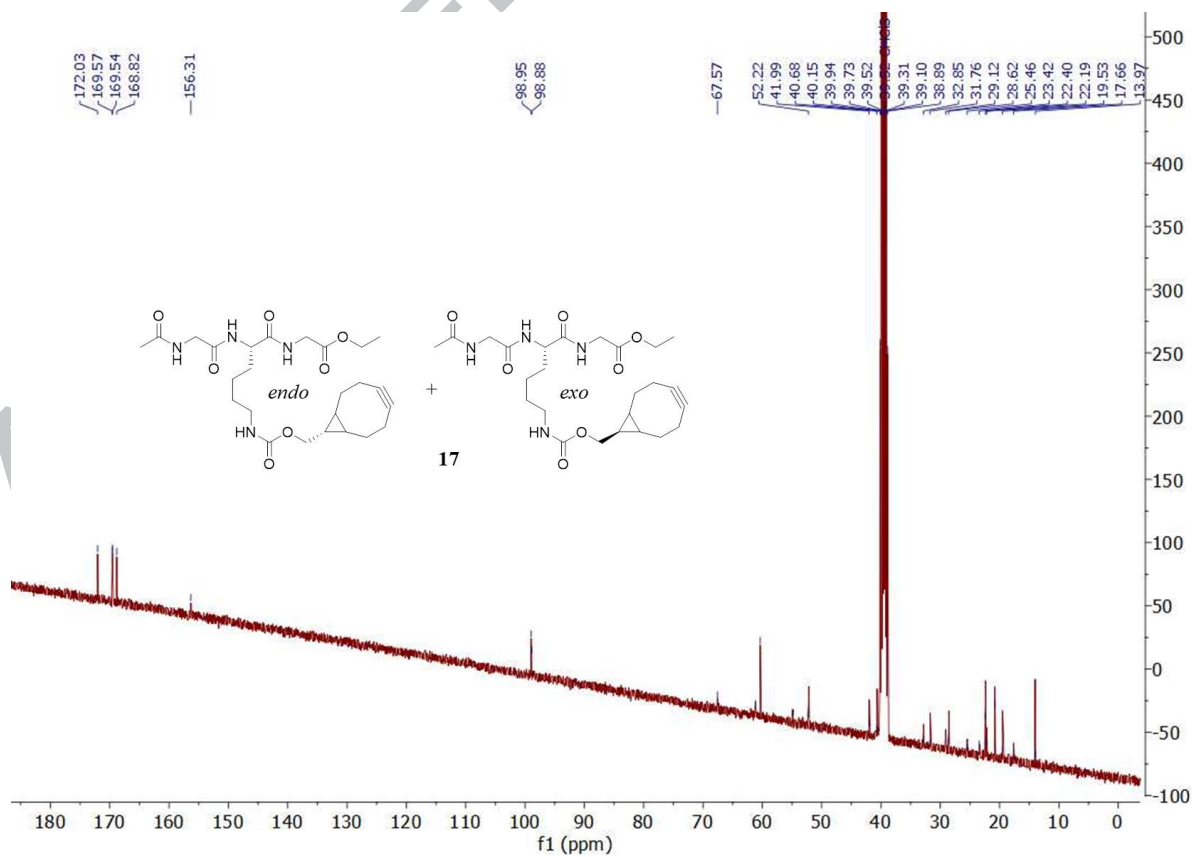
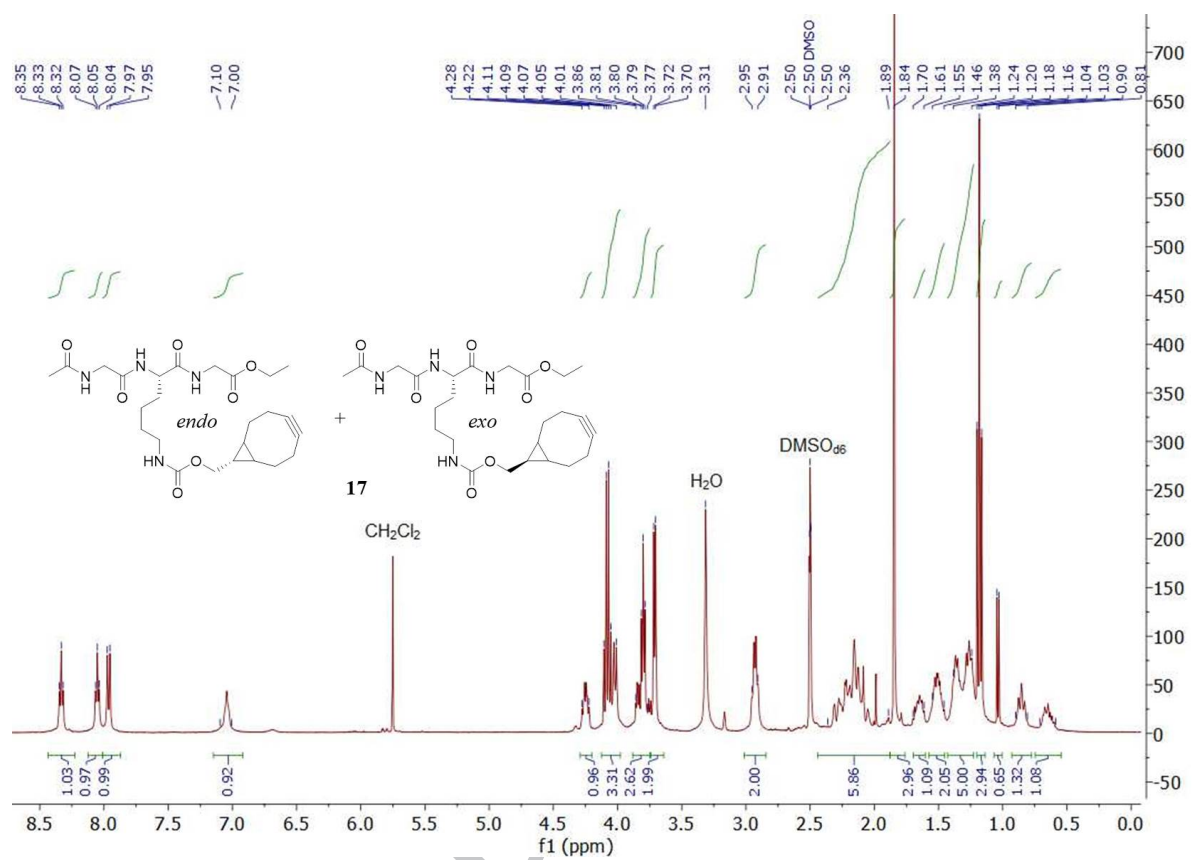
Elements Used:

C: 20-40 H: 0-100 N: 0-10 O: 0-25 I: 0-2

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O	I
618.3000	-5.9	-9.5	12.5	C27 H40 N9 O8	64.5	9.492	0.01	27	40	9	8		
618.2880	6.1	9.9	5.5	C27 H49 N5 O3 I	66.9	11.933	0.00	27	49	5	3	1	
618.2888	5.3	8.6	12.5	C28 H40 N7 O9	64.0	8.949	0.01	28	40	7	9		
618.2914	2.7	4.4	11.5	C32 H44 N O11	56.9	1.855	15.65	32	44	1	11		
618.2920	2.1	3.4	9.5	C32 H49 N3 O I	61.8	6.792	0.11	32	49	3	1	1	
618.2928	1.3	2.1	16.5	C33 H40 N5 O7	55.2	0.185	83.14	33	40	5	7		
618.2941	0.0	0.0	21.5	C34 H36 N9 O3	59.6	4.611	0.99	34	36	9	3		
618.2968	-2.7	-4.4	20.5	C38 H40 N3 O5	62.5	7.475	0.06	38	40	3	5		
618.2981	-4.0	-6.5	25.5	C39 H36 N7 O	64.6	9.564	0.01	39	36	7	1		

FG529 5 (0.050) AM2 (Ar,20000.0,0.00,0.00); Cm (5.29)





Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 400.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

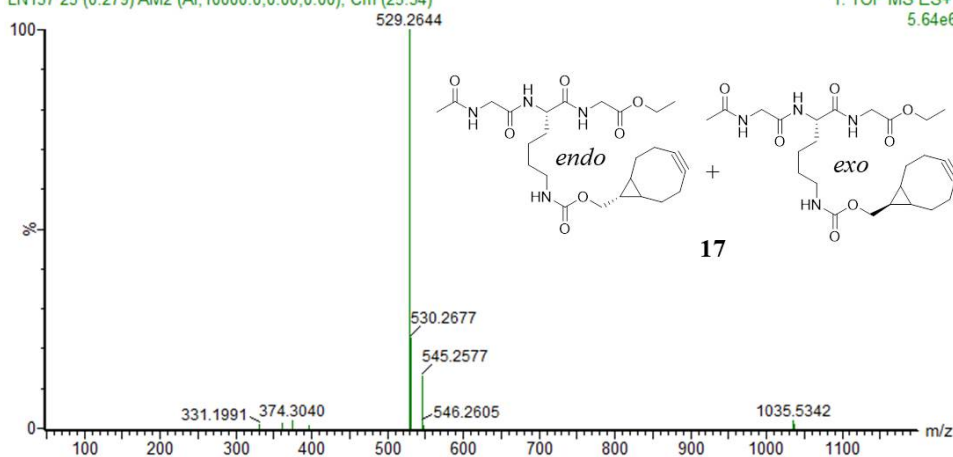
3195 formula(e) evaluated with 17 results within limits (all results (up to 1000) for each mass)

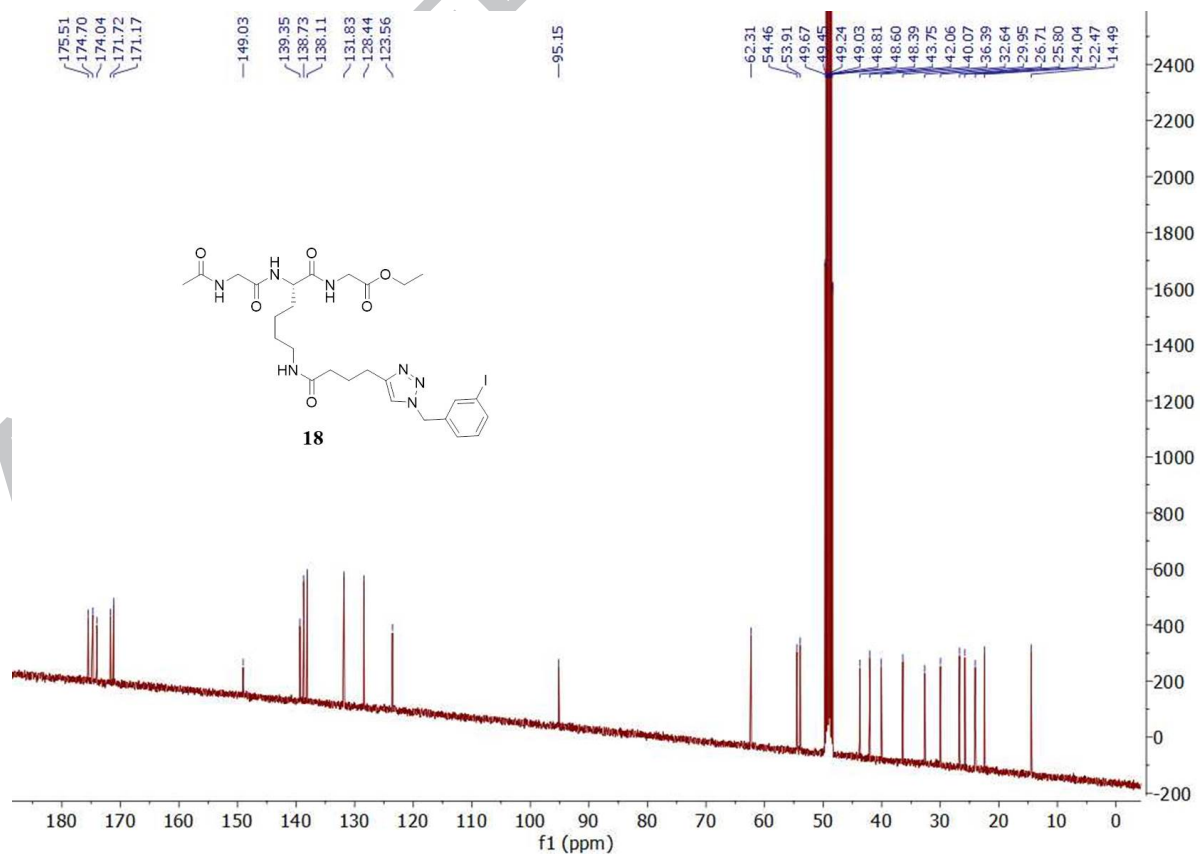
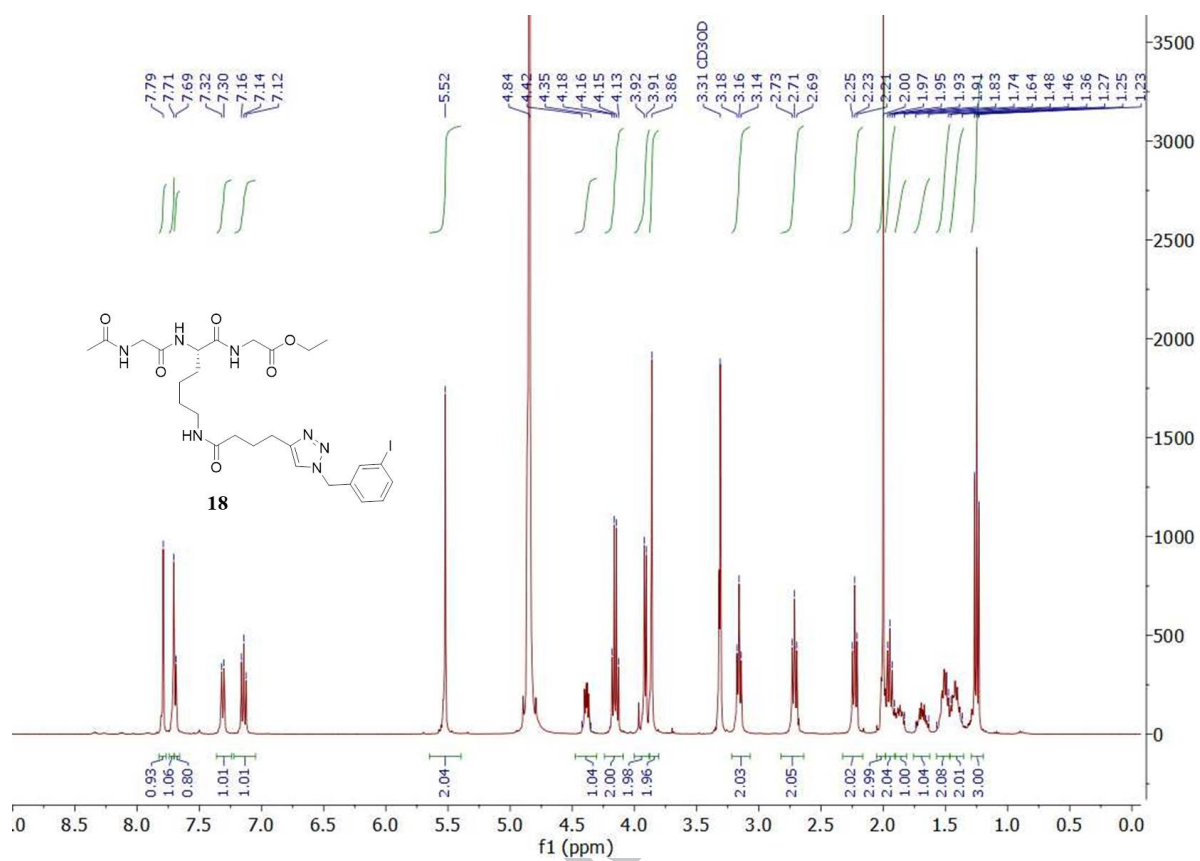
Elements Used:

C: 0-50 H: 0-50 N: 0-20 O: 0-20 Na: 0-1

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O	Na
529.2644	529.2627	1.7	3.2	0.5	C7 H33 N18 O10	39.1	5.910	0.27	7	33	18	10	
	529.2643	0.1	0.2	1.5	C10 H34 N16 O8 Na	36.8	3.601	2.73	10	34	16	8	1
	529.2654	-1.0	-1.9	-0.5	C11 H37 N12 O12	36.3	3.132	4.36	11	37	12	12	
	529.2657	-1.3	-2.5	6.5	C11 H30 N20 O4 Na	35.7	2.440	8.72	11	30	20	4	1
	529.2667	-2.3	-4.3	4.5	C12 H33 N16 O8	36.2	3.027	4.84	12	33	16	8	
	529.2670	-2.6	-4.9	0.5	C14 H38 N10 O10 Na	36.1	2.873	5.65	14	38	10	10	1
	529.2622	2.2	4.2	7.5	C22 H37 N6 O9	37.3	4.100	1.66	22	37	6	9	
	529.2625	1.9	3.6	14.5	C22 H30 N14 O Na	37.4	4.150	1.58	22	30	14	1	1
	529.2635	0.9	1.7	12.5	C23 H33 N10 O5	35.8	2.590	7.50	23	33	10	5	
	529.2625	1.9	3.6	3.5	C24 H42 O11 Na	36.5	3.263	3.83	24	42		11	1
	529.2649	-0.5	-0.9	17.5	C24 H29 N14 O	35.8	2.612	7.34	24	29	14	1	
	529.2638	0.6	1.1	8.5	C25 H38 N4 O7 Na	34.8	1.613	19.92	25	38	4	7	1
	529.2652	-0.8	-1.5	13.5	C26 H34 N8 O3 Na	35.0	1.784	16.79	26	34	8	3	1
	529.2649	-0.5	-0.9	6.5	C26 H41 O11	35.4	2.155	11.58	26	41		11	

LN137 25 (0.279) AM2 (Ar,10000.0,0.00,0.00); Cm (25:34)

1: TOF MS ES+
5.64e6



Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 400.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

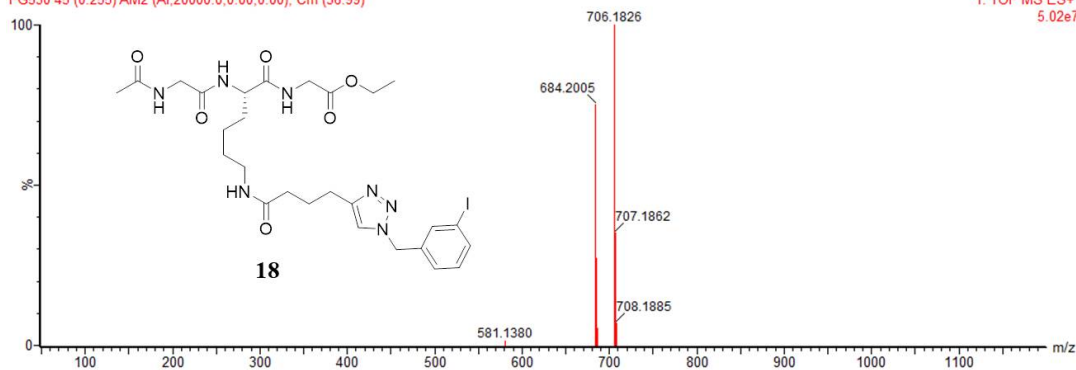
3171 formula(e) evaluated with 30 results within limits (all results (up to 1000) for each mass)

Elements Used:

C: 20-50 H: 0-100 N: 0-10 O: 0-25 I: 0-2

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O	I
684.2005	684.2007	-0.2	-0.3	11.5	C27 H39 N7 O6 I	64.1	4.489	1.12	27	39	7	6	1
	684.2001	0.4	0.6	13.5	C27 H34 N5 O16	65.3	5.750	0.32	27	34	5	16	
	684.1999	0.6	0.9	4.5	C26 H48 N5 I2	66.8	7.200	0.07	26	48	5		2
	684.2014	-0.9	-1.3	18.5	C28 H30 N9 O12	63.7	4.119	1.63	28	30	9	12	
	684.1995	1.0	1.5	31.5	C40 H26 N7 O5	68.3	8.763	0.02	40	26	7	5	
	684.1993	1.2	1.8	6.5	C26 H43 N3 O10 I	65.8	6.197	0.20	26	43	3	10	1
	684.2022	-1.7	-2.5	30.5	C44 H30 N O7	69.3	9.737	0.01	44	30	1	7	
	684.1987	1.8	2.6	8.5	C26 H38 N O20	67.0	7.117	0.06	26	38	1	20	

FG530 45 (0.255) AM2 (Ar,20000.0,0.00,0.00); Cm (36.99)

1: TOF MS ES+
5.02e7

Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 400.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

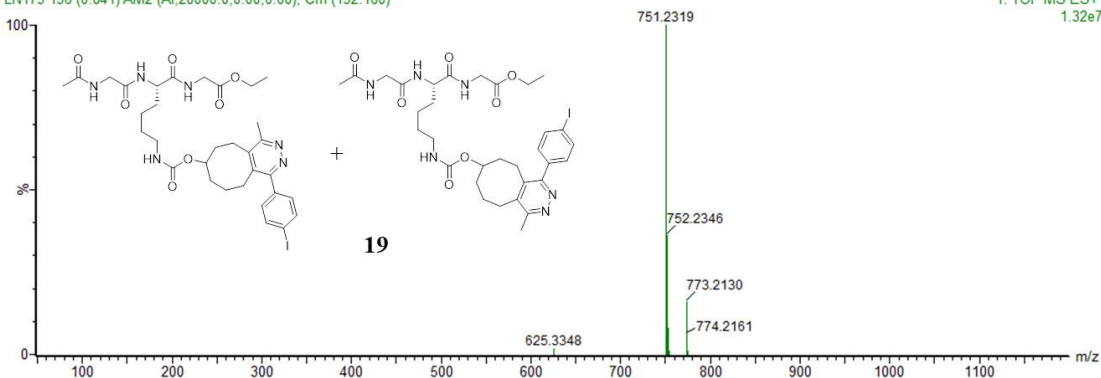
3056 formula(e) evaluated with 35 results within limits (all results (up to 1000) for each mass)

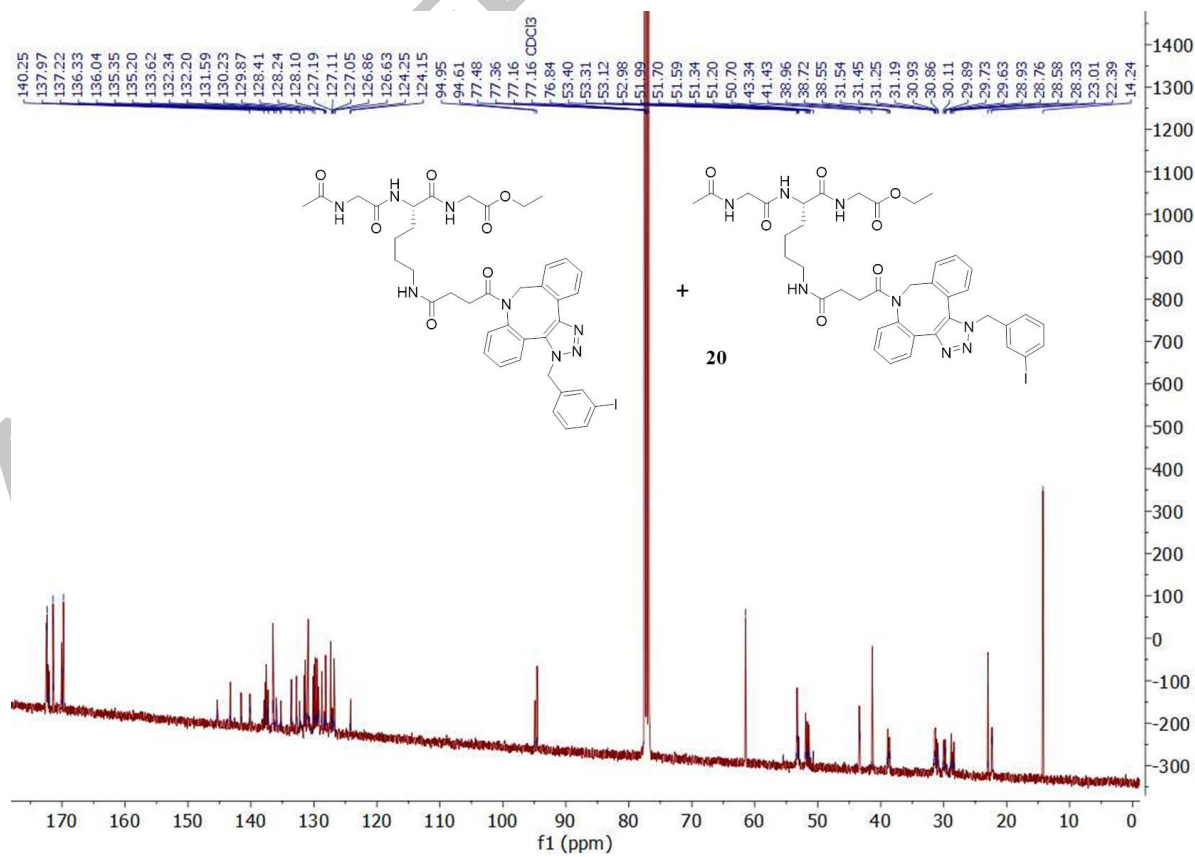
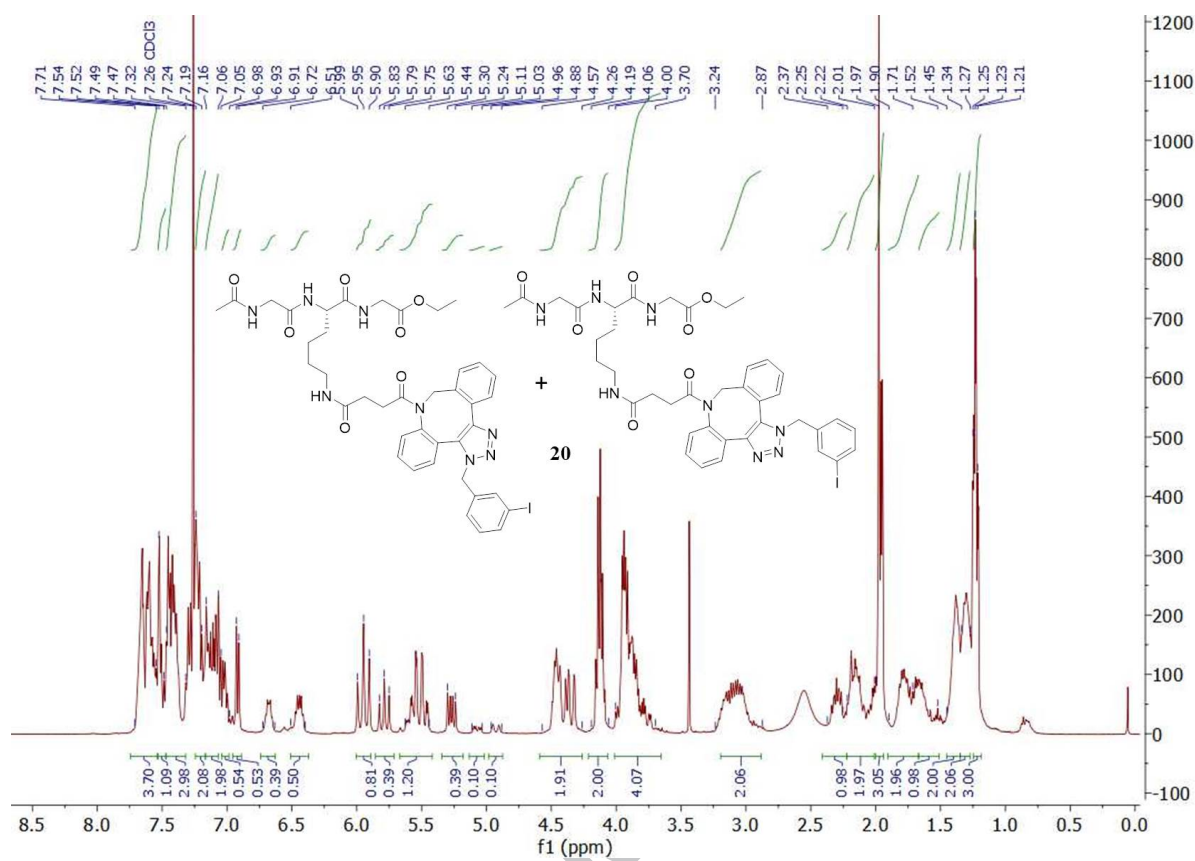
Elements Used:

C: 20-40 H: 0-100 N: 0-10 O: 0-25 I: 0-2

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O	I
751.2319	751.2316	0.3	0.4	13.5	C32 H44 N6 O7 I	56.5	1.230	29.23	32	44	6	7	1
751.2324	-0.5	-0.7	20.5	C33 H35 N8 O13	61.6	6.344	0.18	33	35	8	13		
751.2310	0.9	1.2	15.5	C32 H39 N4 O17	61.2	5.972	0.25	32	39	4	17		
751.2309	1.0	1.3	6.5	C31 H53 N4 O 12	61.0	5.759	0.32	31	53	4	1	2	
751.2329	-1.0	-1.3	2.5	C20 H43 N6 O24	64.5	9.224	0.01	20	43	6	24		
751.2330	-1.1	-1.5	18.5	C33 H40 N10 O3 I	60.0	4.767	0.85	33	40	10	3	1	
751.2335	-1.6	-2.1	0.5	C20 H48 N8 O14 I	64.6	9.323	0.01	20	48	8	14	1	
751.2303	1.6	2.1	8.5	C31 H48 N2 O11 I	55.6	4.401	66.98	31	48	2	11	1	
751.2341	-2.2	-2.9	-1.5	C20 H53 N10 O4 I2	65.4	10.186	0.00	20	53	10	4	2	
751.2297	2.2	2.9	10.5	C31 H43 O21	61.9	6.703	0.12	31	43		21		
751.2342	-2.3	-3.1	7.5	C21 H39 N10 O20	63.7	8.485	0.02	21	39	10	20		
751.2343	-2.4	-3.2	12.5	C36 H48 O9 I	62.4	7.119	0.08	36	48		9	1	
751.2295	2.4	3.2	1.5	C30 H57 O5 I2	62.0	6.778	0.11	30	57		5	2	

LN173 156 (0.841) AM2 (Ar,20000.0,0.00,0.00); Cm (152:160)

1: TOF MS ES+
1.32e7



Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 400.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

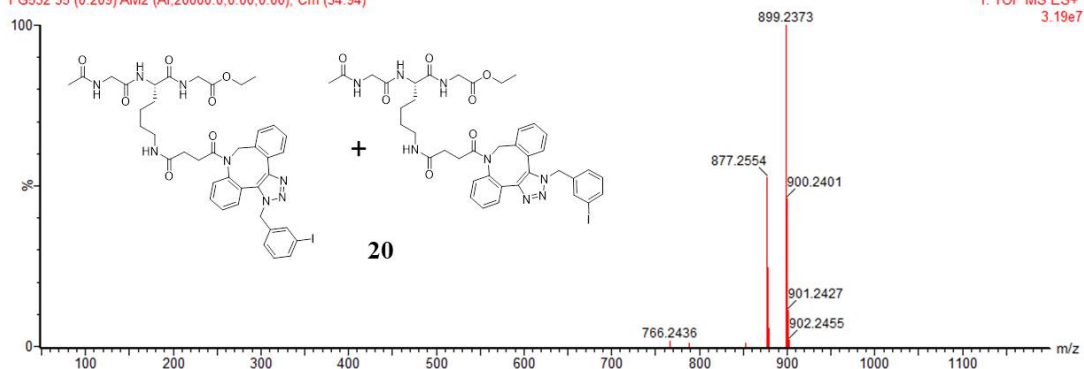
4419 formula(e) evaluated with 52 results within limits (all results (up to 1000) for each mass)

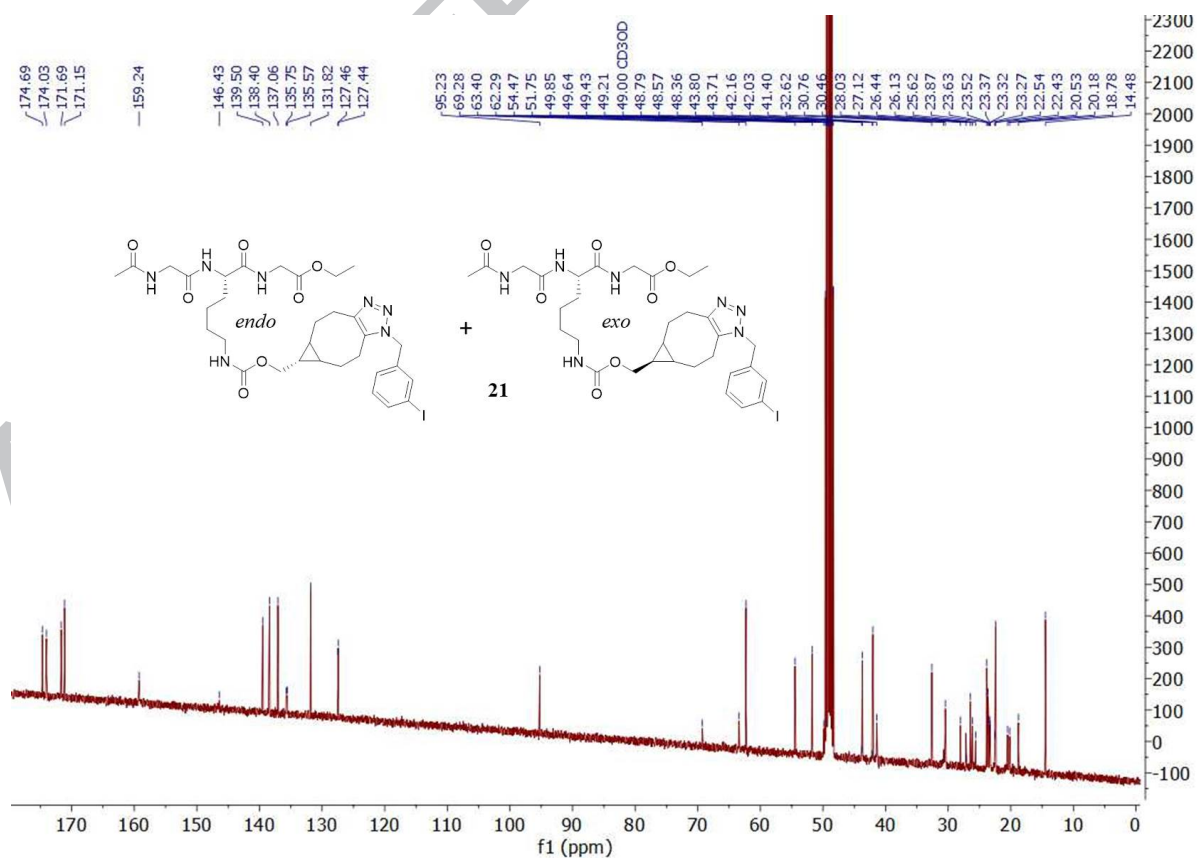
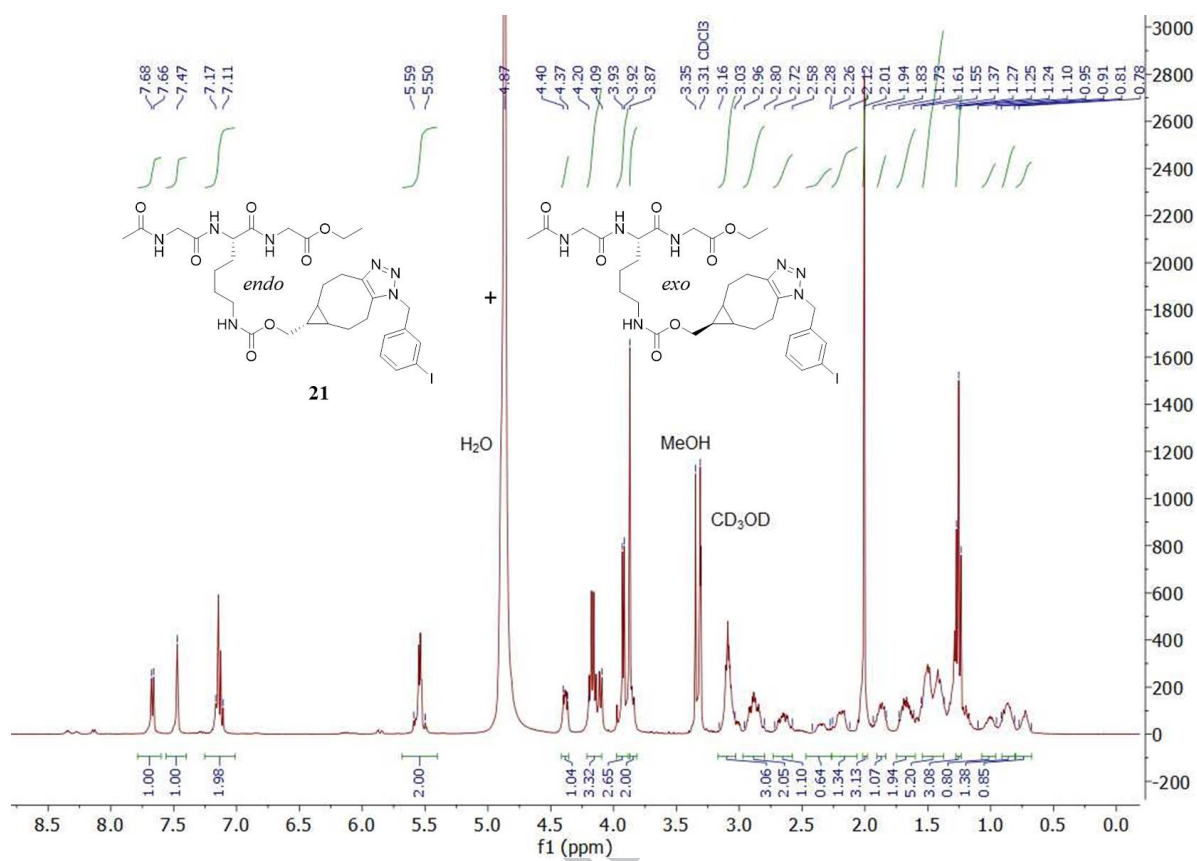
Elements Used:

C: 20-50 H: 0-100 N: 0-10 O: 0-25 I: 0-2

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O	I
877.2554	877.2554	0.0	0.0	13.5	C43 H59 O3 I2	31.2	0.960	38.30	43	59		3	2
	877.2555	-0.1	-0.1	22.5	C44 H45 O19	37.7	7.430	0.06	44	45		19	
	877.2553	0.1	0.1	8.5	C28 H50 N10 O14 I	37.9	7.645	0.05	28	50	10	14	1
	877.2547	0.7	0.8	10.5	C28 H45 N8 O24	38.0	7.746	0.04	28	45	8	24	
	877.2561	-0.7	-0.8	20.5	C44 H50 N2 O9 I	36.0	5.780	0.31	44	50	2	9	1
	877.2545	0.9	1.0	1.5	C27 H59 N8 O8 I2	39.0	8.787	0.02	27	59	8	8	2
	877.2566	-1.2	-1.4	2.5	C31 H58 O20 I	38.2	7.914	0.04	31	58		20	1
	877.2542	1.2	1.4	28.5	C41 H37 N10 O13	36.3	6.059	0.23	41	37	10	13	
	877.2568	-1.4	-1.6	27.5	C45 H41 N4 O15	38.0	7.786	0.04	45	41	4	15	
	877.2539	1.5	1.7	3.5	C27 H54 N6 O18 I	38.8	8.547	0.02	27	54	6	18	1
	877.2572	-1.8	-2.1	0.5	C31 H63 N2 O10 I2	38.5	8.282	0.03	31	63	2	10	2
	877.2534	2.0	2.3	21.5	C40 H46 N8 O7 I	32.9	2.622	7.27	40	46	8	7	1
	877.2574	-2.0	-2.3	25.5	C45 H46 N6 O5 I	36.9	6.661	0.13	45	46	6	5	1
	877.2579	-2.5	-2.8	7.5	C32 H54 N4 O16 I	37.4	7.110	0.08	32	54	4	16	1
	877.2528	2.6	3.0	23.5	C40 H41 N6 O17	36.4	6.113	0.22	40	41	6	17	

FG532 35 (0.209) AM2 (Ar.20000.0,0.00,0.00); Cm (34:94)





Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 400.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

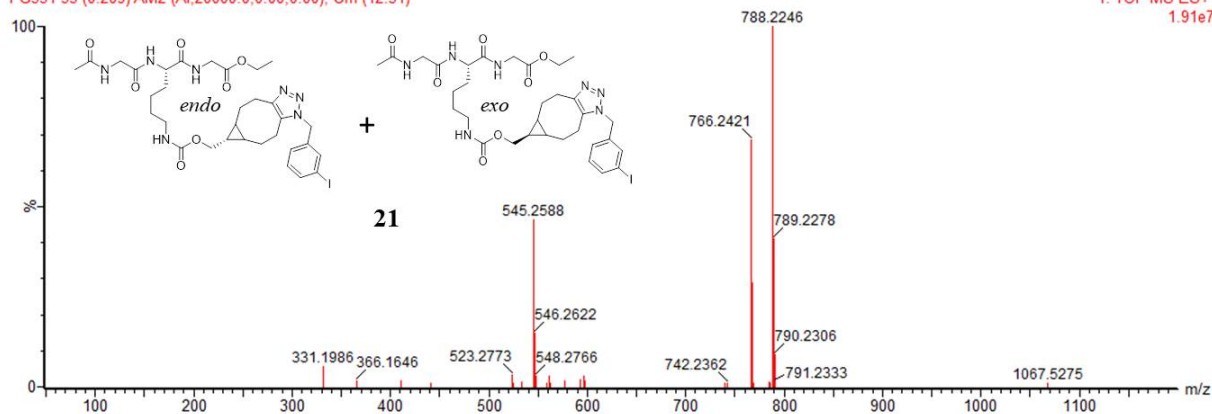
3951 formula(e) evaluated with 37 results within limits (all results (up to 1000) for each mass)

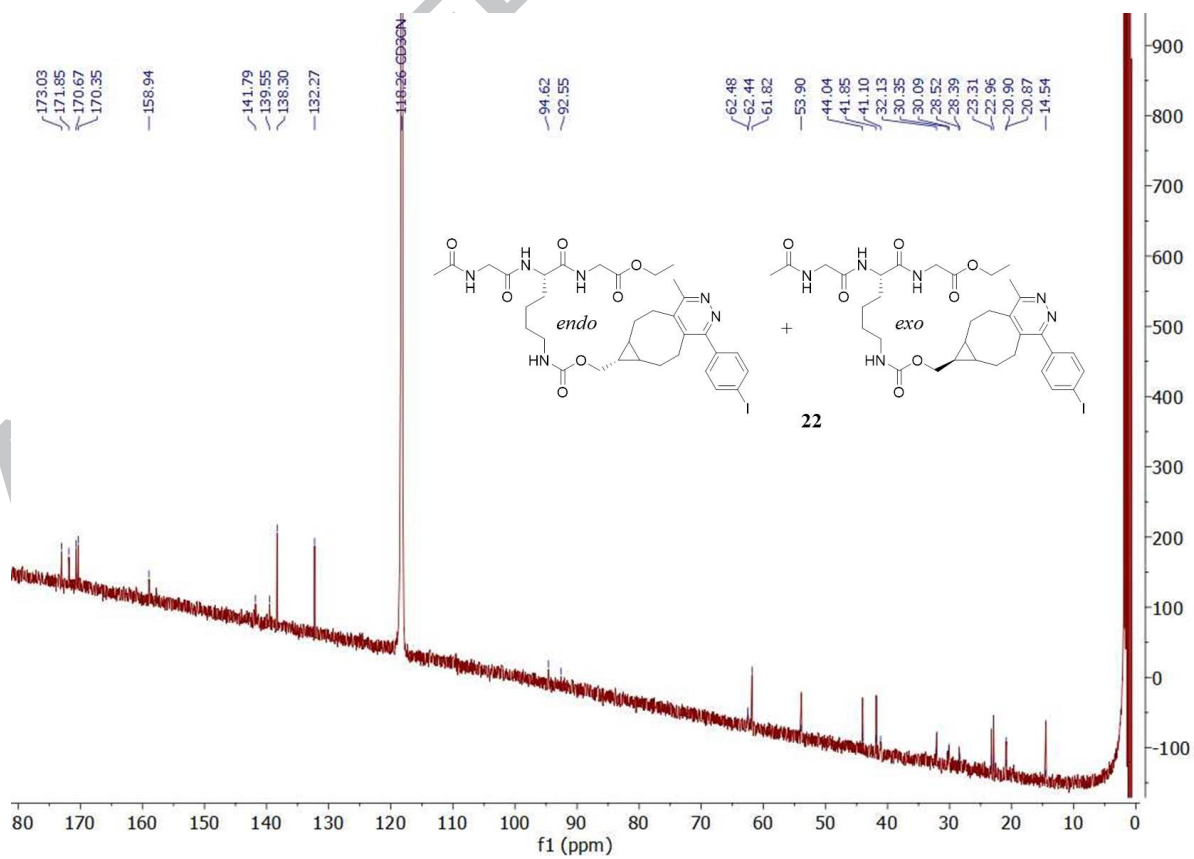
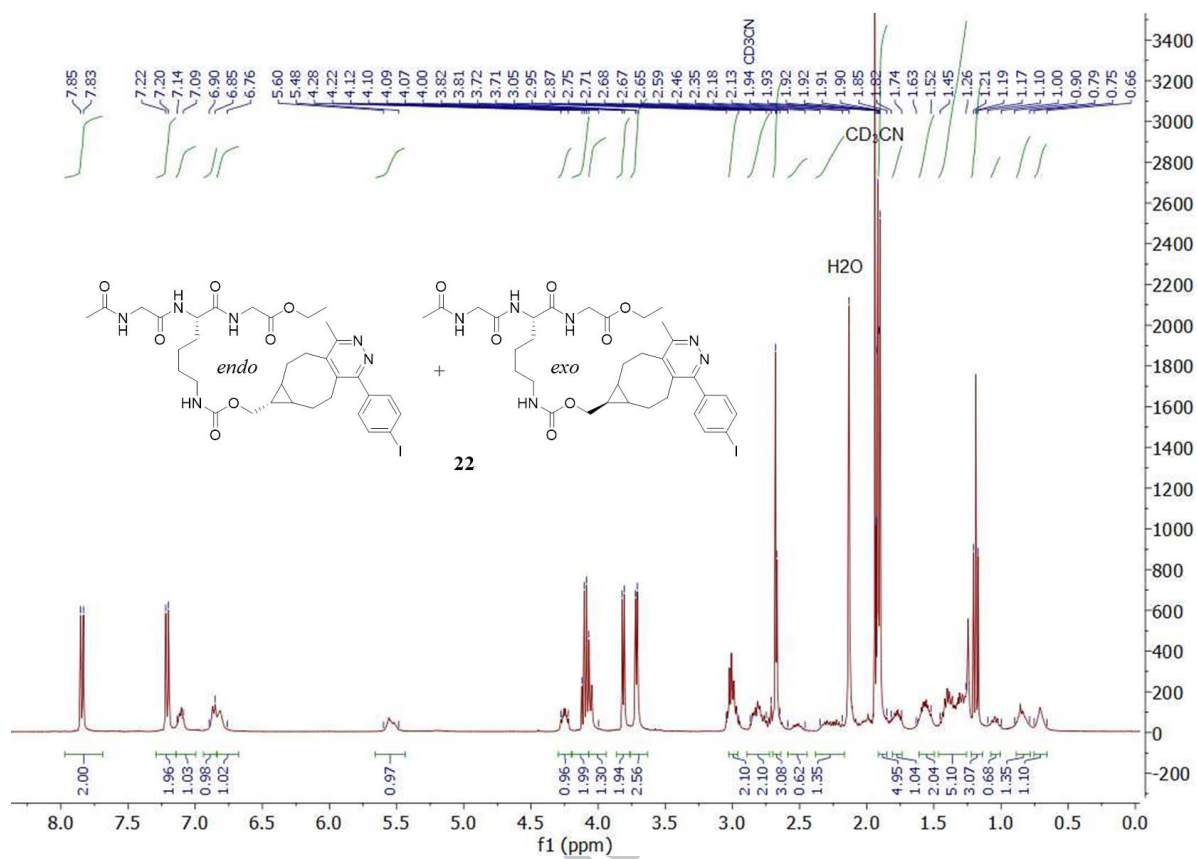
Elements Used:

C: 20-50 H: 0-100 N: 0-10 O: 0-25 I: 0-2

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O	I
766.2421	766.2419	0.2	0.3	15.5	C32 H40 N5 O17	34.0	5.277	0.51	32	40	5	17	
766.2418	766.2418	0.3	0.4	6.5	C31 H54 N5 O 12	35.7	6.923	0.10	31	54	5	1	2
766.2425	-0.4	-0.5	13.5	C32 H45 N7 O7 I	33.0	4.304	1.35	32	45	7	7	1	
766.2414	0.7	0.9	33.5	C45 H32 N7 O6	37.2	8.462	0.02	45	32	7	6		
766.2412	0.9	1.2	8.5	C31 H49 N3 O11 I	34.7	5.959	0.26	31	49	3	11	1	

FG531 35 (0.209) AM2 (Ar.20000.0,0.00,0.00); Cm (12:51)

1: TOF MS ES+
1.91e7



Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 400.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

3137 formula(e) evaluated with 32 results within limits (all results (up to 1000) for each mass)

Elements Used:

C: 20-40 H: 0-100 N: 0-10 O: 0-25 I: 0-2

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	N	O	I
777.2479	777.2480	-0.1	-0.1	21.5	C35 H37 N8 O13	103.1	6.314	0.18	35	37	8	13	
777.2473	777.2473	0.6	0.8	14.5	C34 H46 N6 O7 I	98.0	1.258	28.43	34	46	6	7	1
777.2485	777.2485	-0.6	-0.8	3.5	C22 H45 N6 O24	106.3	9.572	0.01	22	45	6	24	
777.2486	777.2486	-0.7	-0.9	19.5	C35 H42 N10 O3 I	101.0	4.252	1.42	35	42	10	3	1
777.2467	777.2467	1.2	1.5	16.5	C34 H41 N4 O17	102.7	5.984	0.25	34	41	4	17	
777.2491	777.2491	-1.2	-1.5	1.5	C22 H50 N8 O14 I	106.1	9.310	0.01	22	50	8	14	1

LN174 35 (0.209) AM2 (Ar:20000.0,0.00,0.00); Cm (27:113)

1: TOF MS ES+
1.39e8