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High-Yielding, Two-Step ^{18}F Labeling Strategy for ^{18}F-PARP1 Inhibitors

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Keywords

^{18}F-labeling; PET imaging; PARP1; cycloaddition; tetrazine

Positron emission tomography (PET) of labeled metabolites, drugs, proteins and nanomaterials[1-3] is rapidly emerging as a powerful imaging tool to detect and stage disease, to study human biology, to investigate pharmacokinetics and pharmacodynamics of new drugs, or to measure treatment efficacy in clinical trials.[4-8] ^{18}F is one of the most commonly used isotopes for clinical imaging given its half-life, ease of production, wide availability, and compatibility with microfluidics syntheses.[9] Despite extensive use and well established procedures of labeling some small molecules, facile ^{18}F platform-type universally adaptable labeling strategies are still largely missing. This is especially true for rapid labeling of small molecules that emerge from high throughput screens or for optimizing hybrid and modular imaging agents. Bioorthogonal chemistries represent one avenue to develop such generic labeling platforms.

To date, several bioorthogonal reactions have been described[10-15] but only few have been adapted for isotope labeling. The most popular ones is the 1,3-dipolar cycloaddition, “click” reaction, between azides and alkynes.[9, 16] These reactions were found to be particularly useful for in vitro ^{18}F-fluorination of biomolecules and are now commonly used.[1, 17-20] Our search for alternative more rapid, selective, and chemically accessible coupling reactions without need for a catalyst led us to investigate the [4+2] inverse electron demand Diels-Alder cycloaddition using *trans*-cyclooctenes (TCO) and tetrazines (Tz).[21, 22, 22] Advantages of the TCO/Tz labeling strategy include a) fast reaction times in excess of 6,000 $M^{-1}s^{-1}$, b) high selectivity, c) no need for elevated temperatures or catalysts, d) biocompatible reaction conditions and e) activatable tetrazines.[22]

Here we extend our previous work on TCO and Tz chemistry[21-24] and show that the Diels-Alder cycloaddition can be used for rapid ^{18}F labeling of drugs. This strategy allows one to piggy-back onto the development of the vast number of small molecule affinity ligands, peptides, and antibodies targeting different enzymes such as kinases[25-27], receptors and other proteins. Using PARP1 as a model target[28-31] and AZD2281 as a well

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developed nM affinity ligand, we show that the TCO/Tz strategy can be used to efficiently label the drug scaffold in very short times and at exceptionally high yields.

This contrasts to conventional synthesis of directly ^{18}F-fluorinated AZD2281 derivatives where yields are much lower. We show the successful fully automated synthesis of a ^{18}F-labeled TCO, its conjugation with a tetrazine-modified AZD2281, and compare affinities of the parent drugs and intermediates. The described platform-based methodology is modular and could easily be adapted to other molecularly targeted drug scaffolds of interest.

There are three generic ^{18}F labeling strategies for AZD2281: a) de novo synthesis of the native compound substituting ^{18}F for the aryl fluorine atom, b) conventional tosylation and subsequent fluorination of hydroxy-AZD2281 derivatives and c) prosthetic group labeling followed by conjugation to the parent compound. The first choice would result in a chemically identical drug but synthetic steps would be lengthy, require purification of intermediates, may not be feasible given the short half-life of ^{18}F and would be impractical for repeat for on-demand synthesis in a clinical setting. We therefore focused on the prosthetic group approach using the TCO/Tz chemistry. Advantages over the copper catalyzed 1,3-dipolar cycloaddition include: a) no need for elevated temperatures, b) no need for removal of catalysts (important for human use) and c) faster kinetics, important when working with short-lived isotopes. While ^{18}F labeling of either TCO or Tz is conceivable, preliminary experiments demonstrated the substituted 1,2,4,5-tetrazine moiety was not stable to commonly used mild nucleophilic fluorination conditions (tetrabutyl ammonium bicarbonate/potassium fluoride (pH 8.5, 40 °C). Therefore, focus was turned to the design and synthesis of an ^{18}F-labeled TCO in which labeling would take place away from the cyclooctenyl ring due to susceptibility of that system to isomerization to bicyclo[3.3.0]octenes.[32] (*Z*)-2-(Cyclooct-4-enyloxy)acetic acid, **2**, was prepared in 63% yield over two steps from commercially available 9-oxabicyclo[6.1.0]non-4-ene (Scheme 1). Carboxylic acid **2** was converted to (*E*)-2-(cyclooct-4-enyloxy)ethanol, **4**, first by $LiAlH_4$ reduction to give (*Z*)-2-(cyclooct-4-enyloxy)ethanol, **3**, in 78% yield, followed by photochemical cis/trans isomerization and isolation of the (*E*)-isomers by the previously described cycle/trap method.[15] The major (*E*)-cyclooctyl stereoisomer was isolated by column chromatography and converted to the corresponding tosylate **5** in 84% yield. (*E*)-5-(2-Fluoroethoxy)cyclooct-1-ene, $\mathbf{6^{19F}}$, was prepared in 91% yield by the treatment of **5** with tetrabutylammonium fluoride (TBAF) in THF. All previously unknown compounds were fully characterized by ^{1}H, ^{13}C and ^{19}F NMR.

As a precursor for the chemoselective reactive PARP1 inhibitor AZD2281-Tz **9**, 4-[[4-fluoro-3-(4-(5-oxopentanamide) piperazine-1-carbonyl)phenyl]methyl]-2H-phthalazin-1-one, **7**, was generated according to known literature procedures (Scheme 2).[24] This precursor was reacted with **8** [21] in the presence of polymer-supported dicyclohexylcarbodiimide (DCC)-beads to yield **9** as a pink solid. Cycloadduct $\mathbf{10^{19F}}$ was prepared by the addition of DMSO solutions of **9** and $\mathbf{6^{19F}}$ at rt and subsequent HPLC purification.

Radiofluorination of **5** was performed following a modified procedure previously described for the ^{18}F-labeling of 1-azido-2-(2-(2-^{18}F-fluoroethoxy)ethoxy)ethane.[33] In brief, ^{18}F-fluoride n.c.a. ($^{18}F^-$) in ^{18}O-enriched H_2O obtained from PETNET and tetrabutylammonium bicarbonate ($^{n}Bu_4NHCO_3$) were dried by azeotropic distillation of the acetonitrile/water mixture under reduced pressure and a stream of argon. Tosylate **5** in DMSO was added to the dried $^{18}F^-$(n.c.a.)/($^{n}Bu_4NHCO_3$) and heated to 90 °C for 10 minutes. Filtration of the reaction mixture through alumina-N removed unreacted $^{18}F^-$ prior to HPLC purification. Having a low UV absorbance, verification of the identity of desired $\mathbf{6^{18F}}$ product was confirmed in a separate experiment by HPLC injection of $\mathbf{6^{19F}}$, fraction collection at the

same elution as observed for radioactive **6**^{18F}, and NMR analysis of the non-radioactive concentrate. HPLC purified **6**^{18F} was isolated from the collected HPLC solvents by C18 solid phase extraction (SPE) and eluted with dichloromethane (DCM) to give 7.7 ± 3.4 mCi (n = 16) **6**^{18F} in 44.7 ± 7.8% decay-corrected radiochemical yield (dcRCY) in an average time of 41 min from the start of drying of [^{18}F]-F$^-$ (n.c.a.). Analytical HPLC demonstrated >93% radiochemical purity of **6**^{18F}. Tetrazine **9** in DMSO was added to the **6**^{18F}/DCM solution, stirred for 3 min and subjected to HPLC purification (Scheme 2). C18 SPE provided **10**^{18F} in 59.6 ± 5.0% isolated dcRCY (n = 3) with >96% radiochemical purity.

Compound **9** and AZD2281-Tz/*trans*-cyclooctene inverse electron demand Diels-Alder products **10**^{19F}, **10**^{16O} and **10**^{18O} were analyzed using HPLC-ESI/MS spectroscopy (Figure 2). Under the reaction conditions for this cycloaddition, it was found that the initial dihydropyridazine products underwent aromatization to the corresponding pyridazines. LC/MS data confirmed the aromatization of the formed heterocycle, giving m/z-values of 792.6, 790.5 and 792.6, respectively (calc. 792.4, 790.4 and 792.4). To verify their elemental composition, compounds **9**, **10**^{19F}, **10**^{16O} and **10**^{18O} were also subjected to high resolution mass spectrometry. All measured values reflected the calculated masses. Furthermore, high resolution mass spectrometry allowed distinction between **10**^{19F} and **10**^{18O}, whose masses differ by 0.0085 g/mol, confirming the radioactive decay of **10**^{18F} to **10**^{18O} (Figure 2).

In addition to the above described cycloadducts, we also prepared fluorinated AZD2281 analogs (Scheme 3) using nucleophilic substitution. Two candidates were designed and synthesized based on known literature procedures.[34] 4-[[4-Fluoro-3-(piperazine-1-carbonyl)phenyl]methyl]-2*H*-phthalazin-1-one was acylated with 6-hydroxyhexanoic acid or hydroxyacetic acid to give **12** and **15**. These hydroxy-AZD2281 derivatives were converted to the corresponding tosylates, **13** and **16**. Attempts to fluorinate **13** resulted in decomposition of starting material while the reaction of tosylate **16** with sodium fluoride provided **17**^{19F} in 16% yield. Standard ^{18}F-radiolabeling K222/K_2CO_3 conditions, even at low temperatures (40°C), resulted in decomposition of the starting sulfonate ester **16**. Experiments were conducted to test the use of microwave heating at short time intervals with no positive results. Attempts to use the less alkaline tetrabutyl ammonium bicarbonate ($^nBu_4NHCO_3$) as a phase transfer catalyst also resulted in decomposition of the starting materials and <1% radiochemical yields (RCY) of **17**^{18F}.

The above results show that the chemoselective approach resulted in significantly higher yields of ^{18}F labeled AZD2281 analogs and in much shorter time. The next question was therefore how the TCO/Tz ligand would affect target affinity. To assess this, a colorimetric assay was employed to measure PARP1 activity (Figure 3). The published value for AZD2281 is 5 nM,[34, 35] identical to what we observed in our assay. Conventionally fluorinated **17**^{19F} had an IC_{50} of 5.2 ± 1.1 nM, consistent with the small side group. Compound **9** showed an IC_{50} of 8.4 ± 1.3 nM, quite remarkable given the bulkier side chain. Cycloaddition fluorinated **10**^{19F} had an IC_{50} of 17.9 ± 1.1 nM (Figure 3), still in the low double digit nanomolar range and likely sufficient for imaging purposes. These findings are also in agreement with previous results showing that modification of AZD2281 at the piperazine-position only minimally perturbs the ability to bind PARP1.[24] In summary, these results show that the cycloaddition approach can achieve rapid and high yield fluorination yields under mild conditions. Introduction of the hexahydrocycloocta[*d*]pyridazine group seems to only minimally affect affinity of AZD2281 with PARP1. While this retention binding affinity may not be achieved by all targeted small molecules due to the relative size of the hyzahydrocycloocta[d]pyridazine linker, further research in modified linkers to distance binding moiety and pyrazine may be warranted. It is envisioned that this labelling strategy will also have particular utility for the

radiofluorination of peptides, antibodies and nanomaterials where size of the hexahydrocycloocta[d]pyridazine linker will of lesser importance.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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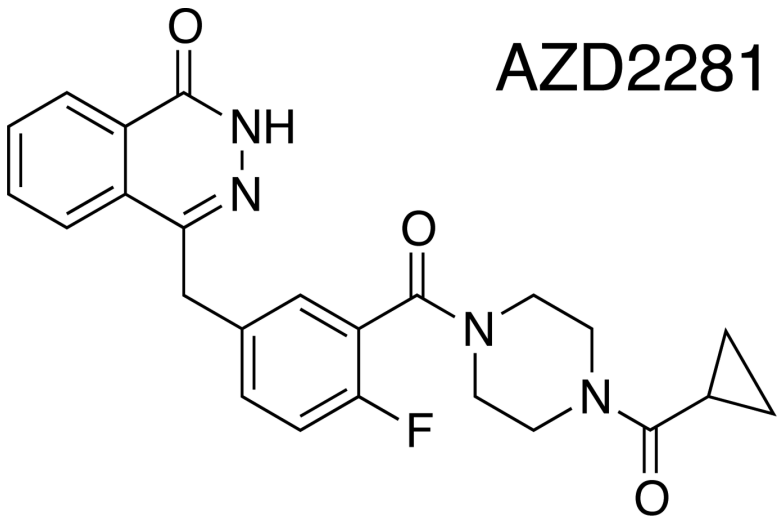
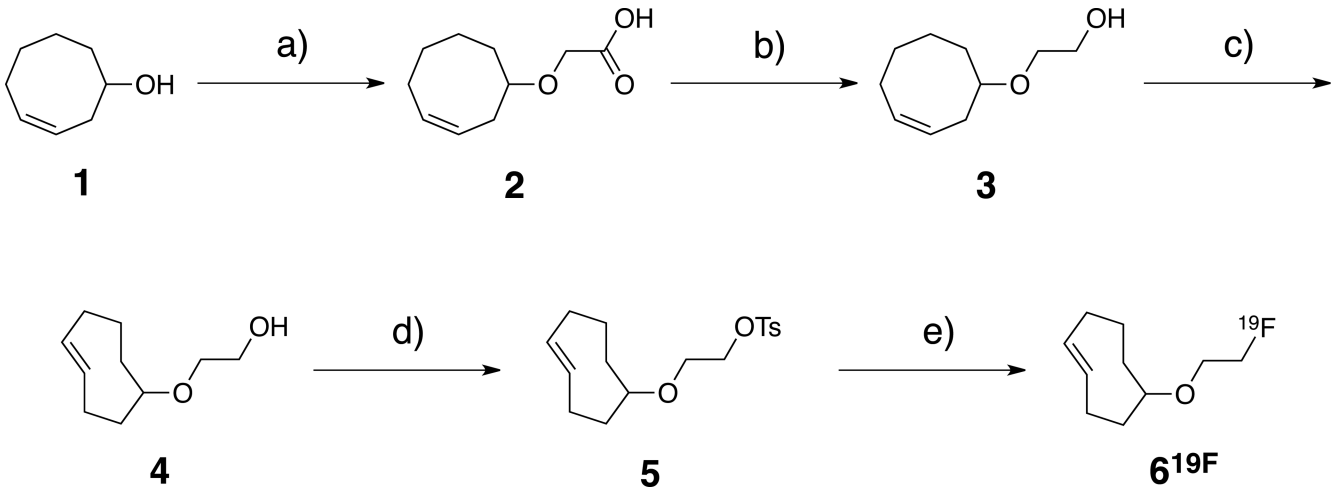
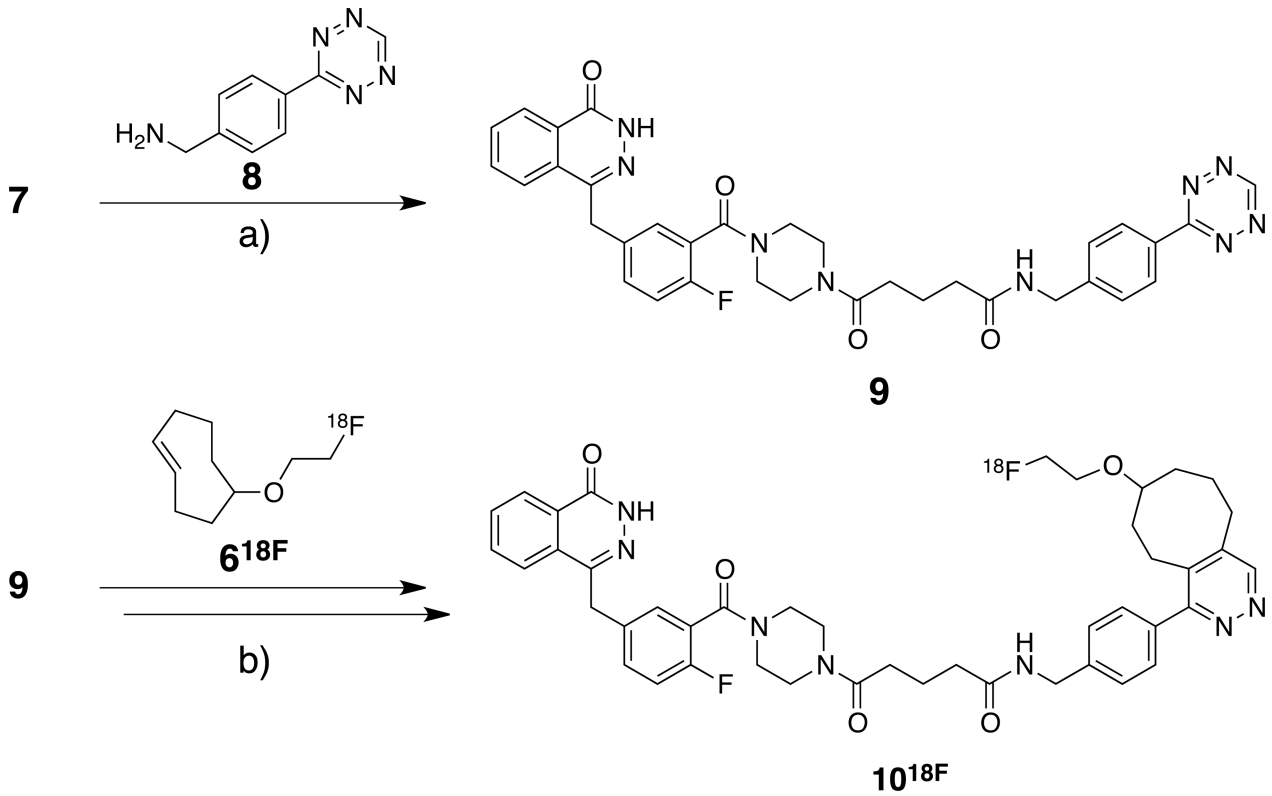


Figure 1.
Structure of AZD2281



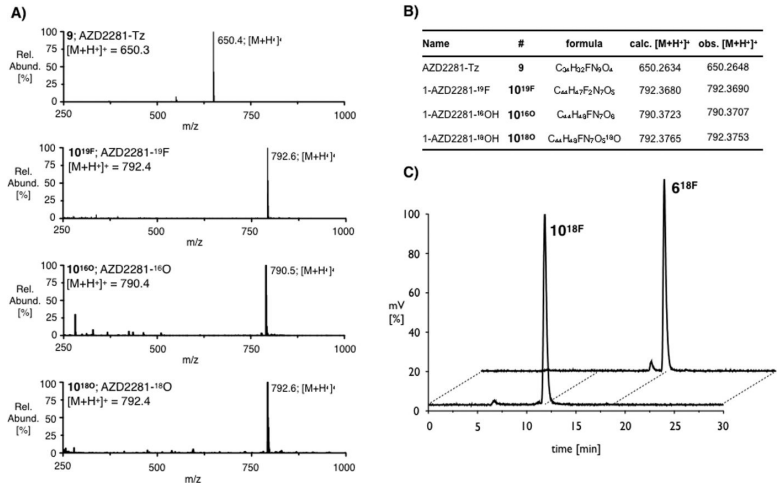
Scheme 1.

Synthetic scheme for the synthesis of radiolabeled ^{18}F-TCO, **6^{18F}**; *Reagents and conditions:* a) NaH, ICH_2CO_2H, reflux, 4h, (THF); b) $LiAlH_4$, 0 °C to rt, 24h, (Et_2O); c) hν, rt, 8h, (Et_2O/hexanes); d) TsCl, Et_3N, rt, 2 h, (MeCN); e) TBAF, rt, 2 h, (THF).



Scheme 2.

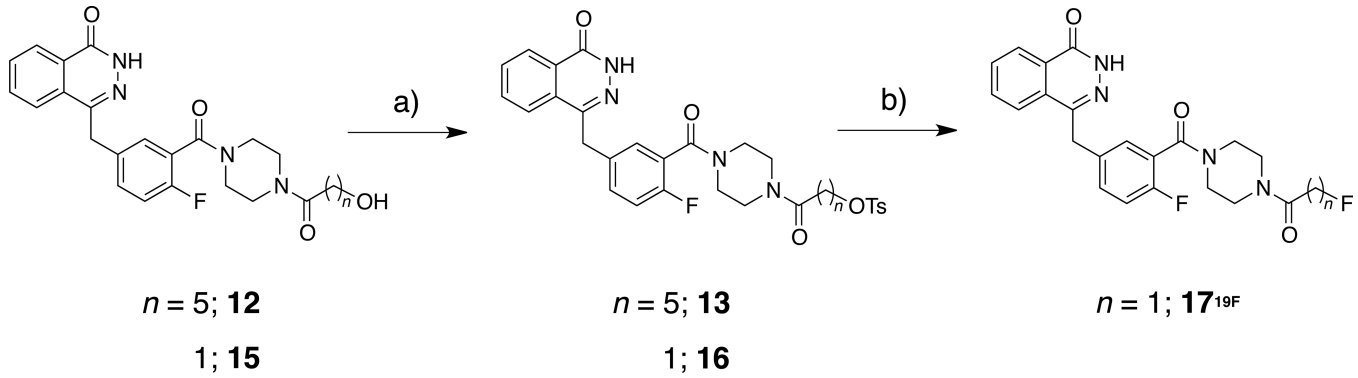
Synthetic scheme for the synthesis of radiolabeled AZD2281-^{18}F **10^{18F}**; *Reagents and conditions:* a) polystyrene-bound DCC, Et_3N, rt, 7h, (DCM); b) rt, 3 min, (DCM).



Name	#	formula	calc. $[M+H^+]^+$	obs. $[M+H^+]^+$
AZD2281-Tz	**9**	$C_{24}H_{32}FN_9O_4$	650.2634	650.2648
1-AZD2281-^{19}F	**10^{19F}**	$C_{44}H_{47}F_2N_7O_5$	792.3680	792.3690
1-AZD2281-^{16}OH	**10^{16O}**	$C_{44}H_{48}FN_7O_6$	790.3723	790.3707
1-AZD2281-^{19}OH	**10^{18O}**	$C_{44}H_{48}FN_7O_5{}^{18}O$	792.3765	792.3753

Figure 2.

a) LC-ESI/MS traces and b) High resolution mass spectra results for AZD2281-derivatives **9**, **10^{19F}**, **10^{16O}**, **10^{18O}**; c) HPLC radiotraces of ^{18}F-labeled compounds **6^{18F}** and **10^{18F}**.



Scheme 3.

Synthetic scheme for the synthesis of conventionally fluorinated AZD2281-derivatives; *Reagents and conditions:* a) HBTU, Et_3N, 6-hydroxyhexanoic acid or 2-hydroxyacetic acid, rt, 60 min, (DMF); b) TsCl, Et_3N, rt, over night, (DCM).

A)

Class	Name	#	IC_{50} [nM]	Time ^{18}F	RCY	Conjugation technique
	AZD2281-Tz	**9**	8.4 ± 1.3	NA	NA	
PARP1i	1-AZD2281-^{19}F	**10^{19F}**	17.9 ± 1.1	3 min	60%	DA Cycloaddition
	2-AZD2281-^{19}F	**17^{19F}**	5.2 ± 1.1	30 min	<1%	Nucleophilic substitution
TCO	^{19}F-TCO	**6^{19F}**	> 4000	NA		

B)

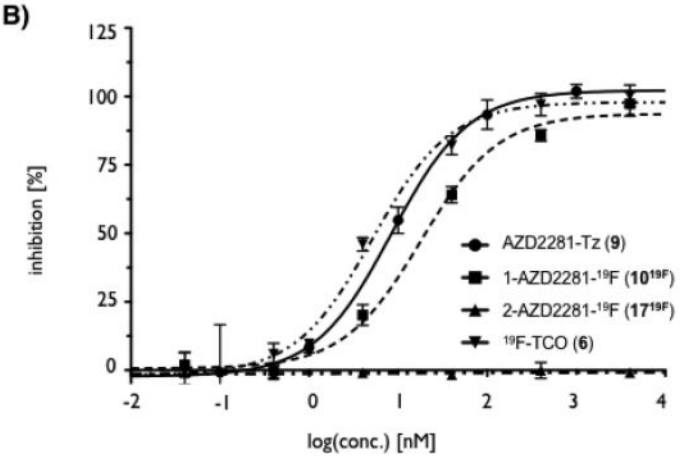


Figure 3.

a) IC_{50}s and radiochemical yields and b) IC_{50} curves for PARP1-inhibitors **9**, **10^{19F}**, **17^{19F}** and *trans*-cyclooctene **6^{19F}**.