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Flow Photochemical Syntheses of *trans*-Cyclooctenes and *trans*-Cycloheptenes Driven by Metal Complexation

Jessica E. Pigga[a], **Joseph M. Fox**[a]

[a]Department of Chemistry and Biochemistry University of Delaware, Newark DE 19716

Abstract

trans-Cyclooctenes and *trans*-cycloheptenes have long been the subject of physical organic study, but the broader application had been limited by synthetic accessibility. This account describes the development of a general, flow photochemical method for the preparative synthesis of *trans*-cycloalkene derivatives. Here, photoisom erization takes place in a closed-loop flow reactor where the reaction mixture is continuously cycled through Ag(I) on silica gel. Selective complexation of the *trans*-isomer by Ag(I) during flow drives an otherwise unfavorable isomeric ratio toward the *trans*-isomer. Analogous photoreactions under batch-conditions are low yielding, and flow chemistry is necessary in order to obtain *trans*-cycloalkenes in preparatively useful yields. The applications of the method to bioorthogonal chemistry and stereospecific transannulation chemistry are described.

Keywords

trans-cyclooctene; trans-cycloheptene; flow chemistry; photochemistry; bioorthogonal chemistry

The unusual reactivity and well-defined chiral structure of *trans*-cycloalkenes has made them attractive targets for synthesis for nearly 70 years.[1–3] For example, *trans*-cyclooctene possesses planar chirality and displays a high barrier to racemization (E_a = 35.6 kcal/mol),[4] and the most stable “crown” conformer has an alternating sequence of equatorial and axial hydrogens that is akin to chair cyclohexane.[5–6] The double bond of *trans*-cyclooctene is twisted severely in the crown conformation,[7] and as a consequence the HOMO of *trans*-cyclooctene is relatively high in energy.[8] *trans*-Cycloheptene also has a rigid structure with a distorted alkene.[7] The double bonds of medium-ring *trans*-alkenes are twisted.[7] *trans*-Cycloalkenes display unusual reactivity in HOMO-alkene controlled cycloaddition reactions with dienes,[9] 1,3-dipoles[8] and ketenes.[10] Strained *trans*-cycloalkenes also serve as ligands for transition metals.[11–16] This reactivity profile has made *trans*-cycloalkanes interesting targets for applications in synthesis and biology.

The broadest applications of *trans*-cycloalkenes are in the field of bioorthogonal chemistry. The inverse-electron demand Diels–Alder reaction between tetrazines and strained alkenes has a rich history in physical organic and synthetic chemistry (Figure 1).[17–19] In 2008, three groups described the bioorthogonal reactions of tetrazines with strained alkenes.[20–22]

jmfox@udel.edu.

The variant introduced by our group that used *trans*-cyclooctene is marked by exceptionally rapid kinetics, with rate constants that can exceed 10^6 $M^{-1}s^{-1}$ in the fastest cases.[23–24] With the advances including the development of fluorogenic tetrazines[25] and reactions in live cells[26] and animals[27], the tetrazine ligation has become a widely used tool for applications that span chemical biology, biomedical imaging, and materials science.[28–37]

This account describes the development of general, flow photochemical methods for the preparative synthesis of *trans*-cycloalkene derivatives and enabled applications in synthesis and bioorthogonal chemistry. Selective complexation of the *trans*-isomer by Ag(I) during flow drives an otherwise unfavorable isomeric ratio toward the *trans*-isomer. For the synthesis of *trans*-cycloalkenes in preparatively useful yields, flow chemistry is necessary as analogous photoreactions under batch-conditions are low yielding.

Synthesis of *trans*-Cyclooctenes and *trans*-cycloheptenes

While there are established routes to the parent *trans*-cycloalkenes, there were relatively few methods for preparing functionalized derivatives.[8, 16, 38–41] *trans*-Cyclooctene, which is the most studied, was first prepared in 1950 as a mixture with *cis*-cyclooctene via Hoffman elimination of trimethylcyclooctyl ammonium iodide.[1] In 1953, *trans*-cyclooctene was separated from *cis*-cyclooctene through formation of the water soluble *trans*-cyclooctene•$AgNO_3$ complex, which was subsequently decomplexed by aq. NH_4OH to provide the pure *trans*-alkene.[2] Several stereospecific methods for preparing *trans*-cyclooctene from *cis*-cyclooctene have also been described,[42–45] but a consideration for these protocols is that multistep synthesis is required to invert the alkene stereochemistry. First demonstrated by Hsung, *trans*-cycloalkenes can also be prepared by 4-**π** electrocyclic ring opening.[46–48] Recent progress has resulted in a number of new routes to heteroatom containing *trans*-cycloalkenes. Woerpel[49–57] and Tomooka[58–63] have described a number of methods for preparing oxasila-*trans*-cycloalkenes as well as the synthesis and chemistry of oxa, aza and sulfa (*E,Z*)-nonadienes.

Photochemical Syntheses of *trans*-Cycloalkenes

The singlet sensitized photoisomerization of *cis*-cyclooctene, pioneered by Inoue over 40 years ago,[64–76] is a direct method for the synthesis of *trans*-cycloalkenes from their *cis*-isomers. In 1978,[76] Inoue published the first in an series of papers on the enantioselective photoisomerization of cyclooctene, cycloheptene, and 1,3-cyclooctadiene to their *trans*-isomers.[64–75] Chiral aromatic esters function most efficiently for this process, the proposed mechanism of which is summarized in Fig 2.[74] Upon irradiation at 254 nm the aromatic ester forms a singlet excited state, which combines with *cis*-cyclooctene **1a** to form diastereomeric exciplexes **p*S*-1b** and **p*R*-1b**. These “twisted singlet”[74] exciplexes subsequently partition into the corresponding *trans*-cyclooctene (**p*S*-1c** or **p*R*-1c**) and *cis*-cyclooctene (**1a**). With chiral sensitizers, the relative rates for the formation of the “twisted singlet” exciplexes are not equal, providing the basis for enantioselectivity.

Flow Photochemical Syntheses of *trans*-Cyclooctenes

Photochemical syntheses of functionalized *trans*-cyclooctenes had been limited by low yields and by the photodegradation of the *trans*-cyclooctene. With time course monitoring, *cis*-cyclooct-4-enol irradiation produces diastereomers of *trans*-cyclooct-4-enol with a maximum of 23% conversion.[77] On prolonged irradiation (18 h), the yield drops to <5% *trans*-cyclooct-4-enol.[78]

To improve the practicality of photoisomerization, we designed a closed-loop flow reactor that drives the transformation through selective metal complexation of the *trans*-isomer.[78] Our experiments were based on the well-known observation that *trans*-cyclooctene, but not *cis*-cyclooctene, forms a complex with $AgNO_3$.[79] Cyclic olefins such as *trans*-cyclooctene interact strongly with metals due to strain relief with relatively minimal energetic cost associated with the reorganization of the hydrocarbon framework.[80] Our strategy was also influenced by classic studies on photoprotonation of cyclic alkenes by Marshall, Kropp and Beauchenmin.[81–83] A schematic of the apparatus for preparing *trans*-cyclooctenes is shown in Fig 3. A quartz reaction flask containing methyl benzoate, a singlet sensitizer, and a solution of a *cis*-cyclooctene derivative is irradiated at 254 nm. During irradiation, the reaction mixture is continuously flowed through column containing $AgNO_3$ adsorbed on silica gel.[84] The *trans*-cyclooctene derivative is selectively retained by the $AgNO_3$•silica, but the *cis*-isomer elutes back to the reaction flask, where it is photoisomerized and recirculated through the column. After consumption of the *cis*-cyclooctene, the silica is removed and stirred with NH_4OH, which liberated the *trans*-cyclooctene from the $AgNO_3$. For base sensitive substrates, NaCl can be used can be used to decomplex $AgNO_3$. The *trans*-cyclooctene derivative is then recovered by extraction. As an alternative to $AgNO_3$, Ag(I) immobilized on tosic silica gel can be used to capture *trans*-cyclooctene products at higher silver loadings without leaching, which can be especially beneficial for polar substrates or large scale photoisomerizations.[85] However, the low cost of $AgNO_3$•silica still makes it beneficial for routine (gram scale) isomerizations. Examples of *trans*-cyclooctenes that have been prepared by flow photoisomerization are given in Figure 4.

Since our original description of the flow photoisomerization protocol, it has been employed by a number of groups for *trans*-cyclooctene synthesis with applications that include radiochemistry, cellular imaging, drug delivery and materials science.[55, 86–120] There have also been a number of modifications to the flow procedure that have been introduced. To avoid the capital cost of the flow equipment, several groups have described protocols where the flow photoisomerization is mimicked by periodically stopping irradiation, capturing the *trans*-cyclooctene by filtering through $AgNO_3$ on silica, and resubjecting the filtrate to photoisomerization (254 nm). [26, 121] These procedures are more labor intensive and lower yielding than the flow chemistry protocols.

A modification of the flow system utilized a quartz tube in conjunction with a UV light.[122] In this setup, the bulk of the reaction solution resided in a reservoir flask and was continually pumped through the quartz tube where irradiation (254 nm) occurred. This setup rendered the reaction scalable without the expense of purchasing multiple quartz flasks for different reaction scales. A microflow system for cyclooctene photoisomerization has been described

which utilizes two microreactors coiled around a UV lamp and several beds of $AgNO_3$-impregnated silica that are exchangeable during irradiation.[123] This system was employed for small scale synthesis of 5-hydroxy-*trans*-cyclooctene and other *trans*-cyclooctene derivatives.

Tomooka has prepared (*E*)-4-[7]orthocyclophene without flow chemistry by directly adding $AgNO_3/SiO_2$ to a photoisomerization reaction in pentane.[63, 124] The heterogeneous solution was irradiated for 42 hours. After workup in NH_4OH, the product was isolated in 76% yield (Figure 5). The enantiomers of (*E*)-4-[7]orthocyclophene could be separated by chiral HPLC and subjected to epoxidation and Lewis-acid catalyzed, stereoselective cyclization as shown in Figure 5.

In a recent development, a liquid-liquid extraction method was developed by Rutjes. The apparatus is comprised of UV lamp and a continuously flowing heptane solution containing *cis*-cyclooctene overtop an aqueous AgNO3 solution.[77] The organic phase flows through UV-permeable FEP tubing (fluorinated ethylene propylene) wrapped around a UV lamp (254 nm) and into an $AgNO_3$ aqueous solution where *trans*-cyclooctene is trapped and *cis*-cyclooctene returns to the organic phase. This system is scalable due to the ability to maintain a consistent concentration of *cis*-cyclooctene in heptane via external addition of substrate. This method allotted up to 2.2 g/h of TCO to be produced and was employed on several of the commonly utilized *trans*-cyclooctenes.

Stereospecific Transannulation of *trans*-Cyclooctenes

Flow photoisomerization has provided access to functionalized *trans*-cyclooctenes capable of stereospecific, transannular cyclization reactions. While stereospecific, transannular cyclizations of (*E*)-cycloalkenes have been studied with larger ring systems,[125–128] few studies had been carried out on *trans*-cyclooctene derivatives. (*E*)-Thiacyclooct-4-ene has been shown to undergo acid catalyzed transannular cyclization,[129] as does the anion of (*E*)-4,5-epoxy-1-thiacyclooctane-1,1–dioxide.[130–131] As shown in Figure 6, transannular hydrobromination of 4-aza-*trans*-cyclooctene provides the pyrrolizidine framework that is common to a range of natural products.[78] Thus, treatment of **2** with bromine provides pyrrolizidine **4** in >90% isomeric purity (crude ^{1}H NMR analysis).[78] 4-Aza-*cis*-cyclooctene gives the opposite diastereomer of **2**, demonstrating that the stereochemistry of the alkene and putative bromonium ion intermediate **3** controls the diasteoselectivity of the cyclization.

Access to the pyrrolizidine alkaloids can also be realized through transannular hydroamination of a 5-aza-cyclooctene.[132] While intermolecular hydroamination of *trans*-cycloalkenes had been described by Beauchemin,[83] the transannular hydroamination of an 5-aza-*trans*-cyclooctene was previously unknown.

An initial retrosynthetic analysis for the total synthesis of hyacinthacine A2 (**7**) is displayed in Figure 7A. It was considered that **7** could arise from the hydroamination of a 5-aza-*trans*-cyclooctene **pS-6**, which would in turn arise from 5-aza-*cis*-cyclooctene **5**. A key consideration was stereocontrol in the photoisomerization step, as *trans*-cyclooctenes

possess planar chirality and are configurationally stable. Only the **p*S*** isomer of **6** would lead to the natural product, whereas the **p*R*** isomer would lead to diastereomer **8**. Generally, the photoisomerization reactions of *cis*-cyclooctenes bearing a stereogenic center are poorly diastereoselective.[78] To address the issue, it was predicted that a *trans*-ring fusion could influence diastereocontrol in the synthesis of hyacinthacine A2. Thus, acetonide **9** was expected to photoisomerize to **p*S*-10** (Figure 7B). The 8-membered ring of **p*S*-10** can adopt a crown conformation, which is the lowest energy conformer of *trans*-cyclooctene. The eight-membered ring of diastereomer **p*R*-10** is unable to adopt a crown conformation, and instead be forced to adopt the much higher energy chair conformation. The considerable difference in conformational energy between **p*S*-10** and **p*R*-10** was predicted to provide a basis for stereocontrol in the photochemical step.

The total synthesis of hyacinthacine A2 was completed as shown in Figure 8. Diene **11** was prepared in 5 steps from sucrose by a modification of a method developed by Lauritsen and Madsen.[133] Ring closing metathesis using the 2nd Generation Grubbs catalyst (Grubbs II) gave **9** in 91% yield. Flow photoisomerization (254 nm) provided **10** with 8:1 dr, favoring the p*S*-isomer. X-ray crystallography confirmed that the eight-membered ring of **p*S*-10** adopts a crown structure in the solid state (Figure 2a). The total synthesis of hyacinthacine A2 (**7**) was completed by trifluoroacetyl removal with MeLi followed by acidic treatment to give triol **12** as the ammonium salt. 5-Aza-*trans*-cyclooctene **12** smoothly underwent transannular hydroamination to give hyacinthacine A2 (**7**) upon treatment with aq. NH_4OH and adjustment to pH 7. In the hydroamination, the absolute planar chirality of **12** was transferred with excellent fidelity providing a single diastereomer of **7** in the transannular reaction.

trans-Annulation for Sulfenic Acid Detection in Live Cells

Sulfenylation (RSH –>RSOH) is a post-translational protein modification associated with cellular mechanisms for signal transduction and regulating reactive oxygen species. Flow photochemistry was used to prepare specialized sulfenic acid modifying *trans*-cycloocten-5-ol (SAM-TCO) probes for labeling sulfenic acid functionality in live cells.[134] It was reasoned that the olefinic strain of SAM-TCO's would make them particularly capable of forming thiiranium ions, and that the ring system would position a hydroxyl nucleophile for subsequent transannular attack (Fig 9). The probes enabled a new method of capturing sulfenic acids via transannular thioetherification, whereas 'ordinary' *trans*-cyclooctenes react only slowly with sulfenic acids. Bioorthogonal quenching of excess unreacted SAM-TCOs with tetrazines in live cells provided temporal control and prevented artifacts caused by cellular-lysis. A cell-based proteomic study showed that SAM-TCO probes could be used to identify and quantify known sulfenic acid redox proteins as well as targets not captured by previously established probes.

trans-Cyclooctenes in Bioorthogonal Chemistry

Due to their exceptionally fast reaction rates with tetrazines, *trans*-cyclooctenes have become broadly utilized for applications in bioorthogonal chemistry. Flow photochemistry has been integral to the discovery and synthesis of a range of functionalized *trans*-cyclooctenes that

have been used throughout this field. Most commonly, the diastereomers of 5-hydroxy-*trans*-cyclooctene are used for bioorthogonal chemistry applications (Figure 10). The equatorial diastereomer is produced as the major product in photoisomerization reactions, and can be produced on relatively large scale using flow chemistry.[78] With a second order rate constant of 8.0×10^4 $M^{-1}s^{-1}$ at 25 °C with an amido-substituted dipyridyltetrazine in water, the axial diastereomer is ~4 times faster than the equatorial diastereomer. This rate difference is even more pronounced for carbamate derivatives of 5-hydroxy-*trans*-cyclooctene, and derivatives of **ax-5-OH-TCO** have been advanced for applications in pretargeted nuclear medicine.[120] Both diastereomers of 5-hydroxy-*trans*-cyclooctene display good stability toward isomerization and have been used for applications as bioorthogonal reporters where long-term cellular or *in vivo* stability is required. The stability properties of *trans*-cyclooctenes has been summarized in a recent report.[135]

Even more rapid bioorthogonal reactions can be realized with the conformationally strained dienophiles (Figure 11).[23– 24] These bicyclic compounds adopt a half-chair conformation that in the ground state is 5.6–5.9 kcal/mol higher in energy than the crown conformer of unconstrained *trans*-cyclooctenes. As a result, cycloadditions with tetrazines are more than 2 orders of magnitude faster with these compounds. With an amido-substituted dipyridyltetrazine in water, s-TCO reacts with a second order rate constant of 3.3×10^6 $M^{-1}s^{-1}$, and has the advantage that the photochemistry precursors can be made simply on large scale.[136] With the same tetrazine, d-TCO reacts with a rate of 3.7×10^5 $M^{-1}s^{-1}$, and displays better long-term stability toward isomerization.[24] The more recently described aza-TCO also displays rapid reactivity that is intermediate between s-TCO and d-TCO.[93] aza-TCO has been utilized in the formation of fluorescent products in tetrazine ligations that do not require attachment of an extra fluorophore moiety.

The hydrophobicity of TCO and s-TCO can negatively impact the physiochemical properties of bioconjugates for some biological experiments. This has recently been linked to high levels of non-specific background fluorescence during imaging experiments, necessitating lengthy washout protocols (>2 h) to dissociate the excess reagent from the cell.[103, 137–138] While d-TCO displays reduced lipophilicity, the compound is relatively bulky compared to the parent *trans*-cyclooctene system. Accordingly, the more hydrophilic *trans*-cyclooctene dienophiles were sought.

In a seminal contribution, Jendrella synthesized 4,6-dioxo-TCO **13** and showed it to be more reactive than *trans*-cyclooctene in cycloadditions with cyclopentadiene, 2,3-dimethylbutadiene, mesitonitriloxide and diphenylketene.[139] Woerpel[49–57] and Tomooka[62] have synthesized *trans*-oxasilacycloalkenes, and have studied their reactivity in Diels-Alder and azide cycloadditions. Kele, Lemke and coworkers reported the genetic incorporation of dioxo-TCO **14** and demonstrated that the lower lipophilicity of this molecule resulted in improved washout times during imaging experiments.[103] In Diels-Alder reactions with tetrazines, the reaction rate with **2** is similar to that with the parent TCO.[103] Separately, *trans*-5-oxocene (oxo-TCO) was shown to display enhanced reactivity and hydrophilicity compared to *trans*-cyclooctene (TCO) in the tetrazine ligation reaction.[140] oxo-TCO has an improved logP 0.51 relative to 5-hydroxy-*trans*-cyclooctene (logP 1.11), d-TCO (logP 0.91). The reaction of oxo-TCO (2.2 : 1 d.r.) with an amido-substituted

3,6-dipyridyltetrazine in water occurs with a second order rate constant of 9.5×10^4 $M^{-1}s^{-1}$ in PBS at 25 °C, which is faster than either diastereomer of 5-hydroxy-*trans*-cyclooctene, and approaching the rate of bicyclic d-TCO.

As shown in Figure 12B, an ^{18}F-labeled *trans*-5-oxocene (^{18}F-oxoTCO) was utilized to develop probes for positron emission tomography imaging in mice.[137] A probe was constructed from a tetrazine conjugate of a peptide that targets the neurotensin (NT) receptor, which is upregulated in prostate, pancreatic, lung, and colorectal cancer. The tracer showed comparable tumor uptake with analogous probes constructed from ^{18}F-labeled s-TCO and d-TCO. However, the increased hydrophilicity from the oxo-TCO enabled a faster clearance rate of the tracer from non-targeting organs, which lead to significantly higher tumor to background ratio compared with s-TCO and d-TCO counterparts.

In the bioorthogonal probe-reporter strategy,[141] there are different stability requirements for the bioorthogonal reaction partners. Stability requirements are higher for the reporter molecule, which resides in the biological environment for entire duration of a biological experiment, whereas the probe need only be stable for the labeling portion of the experiment. The stability of more reactive *trans*-cyclooctenes such as s-TCO, d-TCO and oxo-TCO have been described in detail,[135] and have been used by a number of groups for cellular and *in vivo* experiments. Unfortunately, the stabilities of these compounds have sometimes been misquoted as too unstable for cellular experiments. For live cell applications, these compounds have high utility as probes, where short incubation times in the cellular enviroment are required due to the very fast kinetics of reactions with tetrazines. The primary mechanism for the deactivation of TCO reagents is isomerization to the *cis*-isomer, which decreases labeling efficiency but has the merit of not causing non-selective off-target labeling in cellular experiments. For the use of *trans*-cyclooctenes as chemical reporters, it is advisable to use the more resilient parent *trans*-cyclooctenes based on 5-hydroxy-*trans*-cyclooctene.

An additional, practical consideration for strained TCO derivatives is that non-crystalline derivatives of s-TCO and d-TCO can isomerize upon prolonged storage. The shelf-life of the most reactive *trans*-cyclooctenes can be greatly extended by ‘protecting’ them as stable Ag(I) metal complexes. [135, 142] NMR studies showed that Ag-complexation is thermodynamically favorable for *trans*-cyclooctenes with dissociation kinetics that are very rapid. Additionally, TCO•$AgNO_3$ complexes are immediately dissociated upon addition of NaCl. Thus, the most highly strained *trans*-cyclooctenes can be stabilized for long term storage through Ag(I) complexation, and then liberated on demand by addition of NaCl which is present in high concentration in cell media. The utility of Ag-TCO complexes was demonstrated in several live cell labeling experiments.[135, 142] For example, the silver nitrate complex of a highly reactive s-TCO-TAMRA conjugate was prepared, and was shown to label a protein-tetrazine conjugate in live cells with faster kinetics and similar labeling yield relative to an ‘ordinary’ TCO-TAMRA conjugate (Figure 13).

Flow Photochemical Synthesis of *trans*-Cycloheptenes and Sila-*trans*-Cycloheptenes

Like *trans*-cyclooctene, *trans*-cycloheptene has long captured the imagination of chemists. *trans*-Cycloheptene was first trapped from *trans*-1,2-cycloheptenethionocarbonate through treatment with $P(OMe)_3$.[143] In photoprotonation reactions of cyclic alkenes, including cycloheptene, photoisomerization reactions are driven by selective addition reactions of *trans*-cycloalkenes.[82–83, 144] Cycloheptene was first directly observed by Inoue who studied the singlet sensitized photoisomerization of *cis*-cycloheptene at –35 °C.[145–146] Unlike *trans*-cyclooctene, *trans*-cycloheptene is very labile and undergoes rapid isomerization under ambient conditions.[147] *trans*-Cycloheptene has also been prepared via ligand exchange from a *trans*-cycloheptene•CuOTF complex.[148] Because C-Si bonds are long, the inclusion of silicon into the cyclic backbone can alleviate olefinic strain and impart stability to *trans*-cycloalkenes. [52–57, 62] Woerpel has developed a general method for the preparation of *trans*-oxasilacycloheptenes—seven membered rings that contain *trans*-alkenes and siloxy bonds in the backbone (Figure 14), and has described their selective addition, difunctionalization and cycloaddition reactions.[52–57]

Several studies had shown that metal complexes of *trans*-cycloheptene can be isolated. [16, 149–151] Jendralla described the preparation of AgOTf and $AgClO_4$ complexes of 3-methoxy-*trans*-cycloheptene and 6-Methoxy-(*Z*),4(*E*)-cycloheptadiene through Ag-mediated ring opening of a nitrosourea derivative of bicyclo[4.1.0]heptane.[16, 150] In unspecified yields, the $AgClO_4$•3-methoxy-*trans*-cycloheptene complex was combined with a number of dienes to give the products of metal decomplexation and [4+2] cycloaddition.

Inital attempts to directly prepare *trans*-cycloheptenes and sila-analogs by flow photochemical isomerization were unsuccessful, most likely due to the susceptibility of carbocyclic TCHs to isomerization. However, flow photochemistry can be used to prepare *trans*-cycloheptenes and sila-*trans*-cycloheptenes as their isolable Ag-complexes.[152] Photoisomerizations to form sila-*trans*-cycloheptene silver nitrate (Si-TCH•$AgNO_3$) complexes were carried out at r.t. using the standard flow-photoisomerization apparatus (254 nm), with the modification that Si-TCH•$AgNO_3$ complexes were directly isolated from SiO_2 without Ag-decomplexation. The Si-TCH•$AgNO_3$ complexes are stable in neat form for >1 month in the freezer. For carbocyclic *trans*-cycloheptene•silver nitrate (TCH•$AgNO_3$) synthesis, the reactor design was modified to allow for in-line cooling and shortened residence time in the photoreactor (Figure 15). With this modified reactor, TCH•$AgNO_3$ complexes were isolated as semisolids that are moderately stable at r.t. but stable for weeks in the freezer. The scope of TCH and Si-TCH synthesis is shown in Figure 16. Aryl, Cyano, carbamate, and hydroxyl groups were tolerated in the reaction as were NHS ester and chloroalkane groups that were then used to enable conjugation to fluorophores and HaloTag fusion proteins, respectively.

TCH and Si-TCH complexes were shown to engage in a range of reactions as shown in Figures 17 and 18. The $AgNO_3$ complex of *trans*-cycloheptene underwent several transformations with *in situ* metal decomplexation. Combination with 3,6-diphenyl-1,2,4,5-tetrazine gave a pyridazine product in 98% yield. Cyclopenta-1,3-diene was also used to trap

trans-cycloheptene, delivering the [4+2] cycloaddition adduct in 81% yield as a single diastereomer. Vicinal dihydroxylation with OsO_4 and NMO gave the *trans*-diol in 82% yield as a single diastereomer. [152]

Si-TCH complex **15** was obtained treating the silver complex with NH_4OH and directly subjected to reactions cyclopentadiene, diazomethane, dichloroketene, and benzyl azide to give cycloadducts as single diastereomers in 76%–96% yields (Figure 18). With cycloadduct **16**, it was shown that the diphenylsila- group could be oxidized to diol product **17** in 76% yield. [152]

In bioorthogonal reactions, Si-TCH compounds are 1.4–2.8x more reactive than s-TCO depending upon the context of tetrazine reaction partner and reaction conditions. As shown in Figure 19, the reaction of a fluorescent dipyridyltetrazine conjugate with a Si-TCH in 9:1 water:MeOH at 25 °C proceeds with a second order rate constant k2 1.14 × 107 (+/– 5 × 10^5) $M^{-1}s^{-1}$. Remarkably, the reaction is complete within 10 *milli*seconds when the concentration of the excess reagent is only 60 μM. This is the fastest rate constant reported to date for a bioorthogonal reaction. Utility in bioorthogonal protein labeling in live cells was also demonstrated, including labeling of GFP with an unnatrual tetrazine-containing amino acid. An *in vitro* rate constant of 250,000± 15,000$M^{-1}s^{-1}$ was measured in PBS at rt. The kinetics of the in vivo tetrazine ligation were monitored in a suspension (PBS) of *E. coli* overexpressing GFP with the unnatrual tetrazine amino acid. The reaction rate of Si-TCH with **GFP-Tet** was obtained by measuring the increase in whole-cell fluorescence upon addition of **7c**. At room temperature, a second-order rate constant of 155,000 ± 20,000 $M^{-1}S^{-1}$ was measured. The reactivity and specificity of the Si-TCH reagents with tetrazines in live mammalian cells was also evaluated using the HaloTag platform. The cell labeling experiments show that Si-TCH derivatives are suitably stable to serve as highly reactive probe molecules in the cellular environment.[152]

Summary

Over the past decade, *trans*-cyclooctenes and *trans*-cycloheptenes have emerged from the physical organic literature as building blocks for synthesis and essential tool molecules for chemical biology. Enabling this transition has been the development of flow-chemistry for the photoisomerization of cycloalkenes that is driven by in-line complexation of the *trans*-isomer by Ag(I). Flow photochemical synthesis of *trans*-cyclooctenes has been broadly adopted across the chemical biology community, and recent advances have extended the method to isolable silver-complexes of *trans*-cycloheptenes and sila-*trans*-cycloheptenes.

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Biography

Jessica Pigga is a native of Scranton PA. She received the B.S. degree from Elizabethtown College where she carried out undergraduate research with Jeffrey Rood. She is currently a 3rd year Ph.D. student in the research group of Joseph Fox at the University of Delaware.

Joseph Fox is Professor of Chemistry and Biochemistry at the University of Delaware, where he also directs an NIH-funded Center of Biomedical Research Excellence. Prior to his appointment at UD in 2001, Fox received the A.B. degree from Princeton University with Maitland Jones Jr., carried out Ph.D. research at Columbia University with Thomas Katz, and was an NIH postdoctoral fellow with Stephen Buchwald at MIT.

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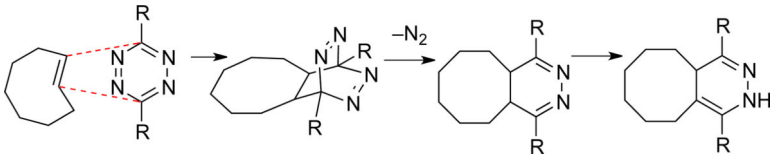


Figure 1.

Tetrazine ligation with *trans*-cycloalkenes has become a broadly used tool for chemical biology, medicine and materials science.

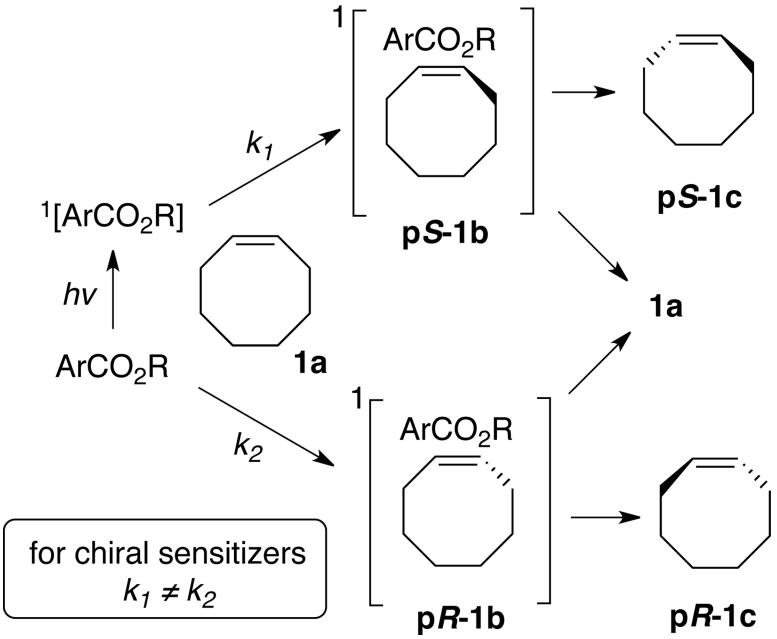


Figure 2.
Enantioselective photoisomerization based on chiral exciplex formation.

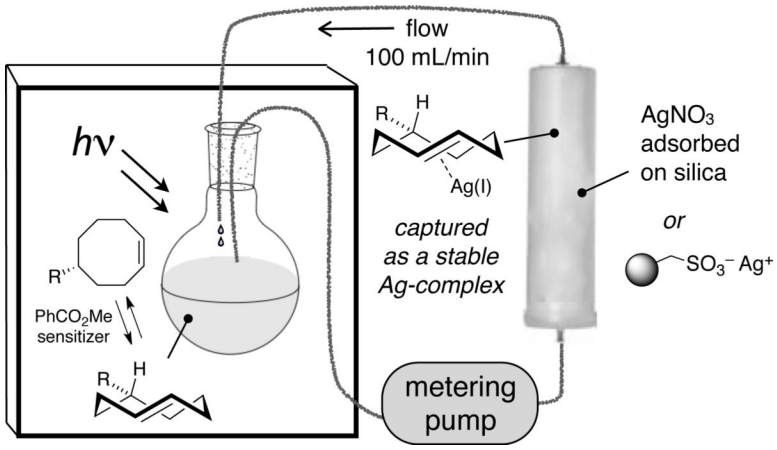


Figure 3.
Schematic of Apparatus for *trans*-Cyclooctene Synthesis

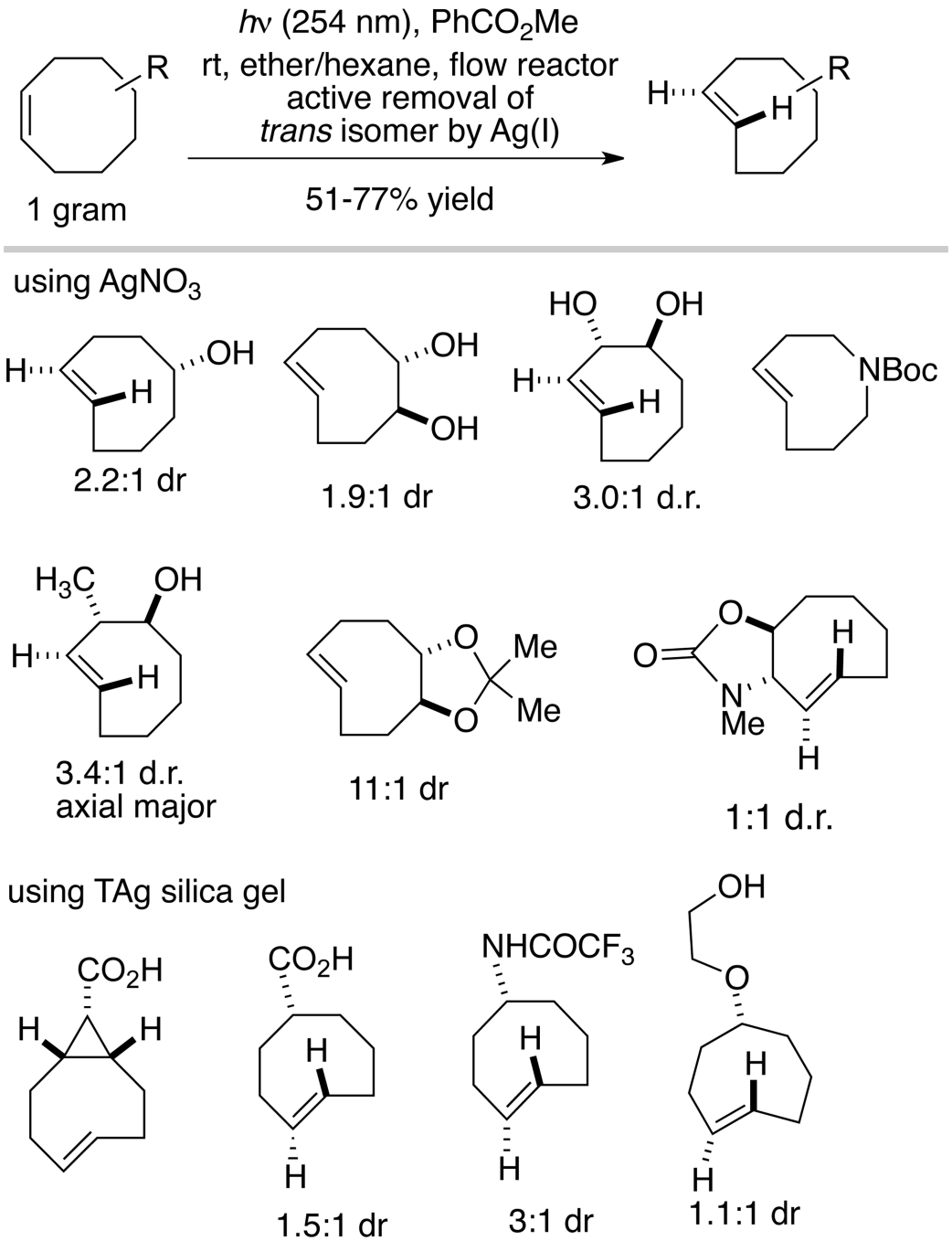


Figure 4.

Examples of *trans*-cyclooctene derivatives that have been prepared using flow photoisomerization

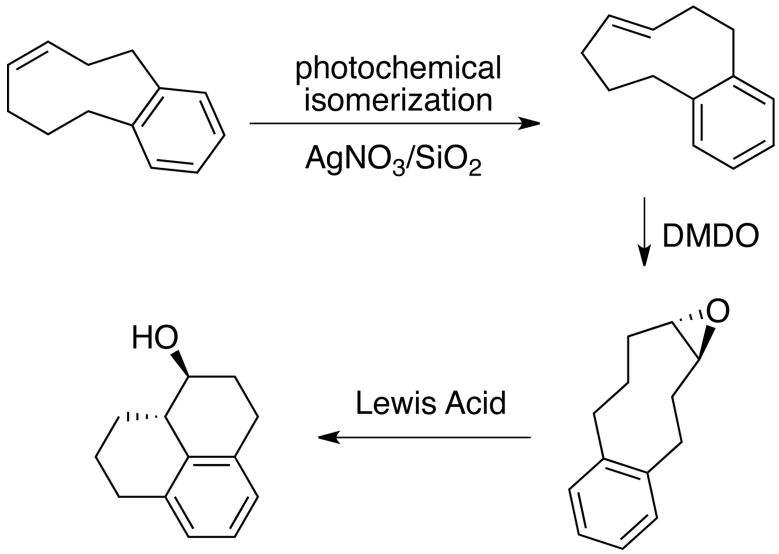


Figure 5.
Synthesis of (*E*)-4-[7]orthocyclophene without flow chemistry by directly adding $AgNO_3$ /SiO_2

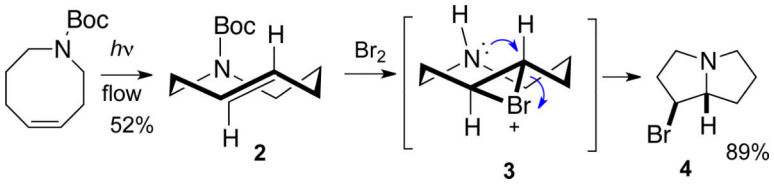


Figure 6.
Stereospecific, transannular bromoamination

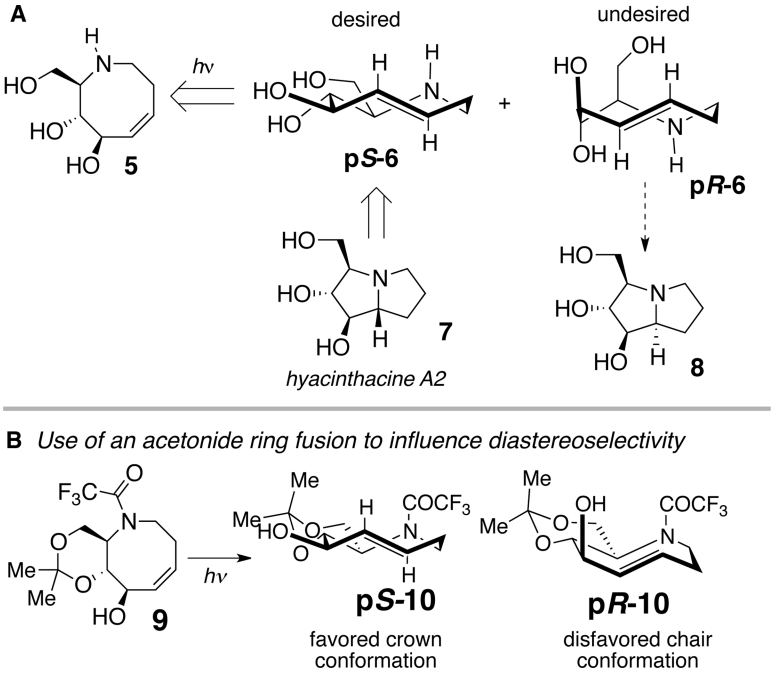


Figure 7.

(A) Retrosynthetic analysis of hyacinthacine A2 (**7**) using transannular hydroamination and diastereoselective photoisomerization as key steps. Poor diastereoselectivity in the photoisomerization step would lead to undesired isomers **p*R*-6** and **8**. (B) An acetonide ring fusion would force the minor diastereomer **p*R*-10** to adopt a high energy chair conformation, favoring the formation of **p*S*-10** in the photoisomerization 5-aza-cyclooctene **9**.

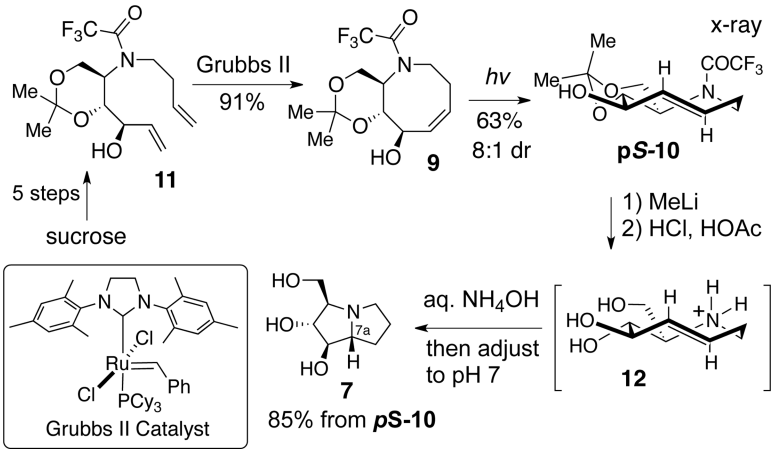


Figure 8.

Total synthesis of hyacinthacine A2 via photoisomerization and stereospecific transannular hydroamination.

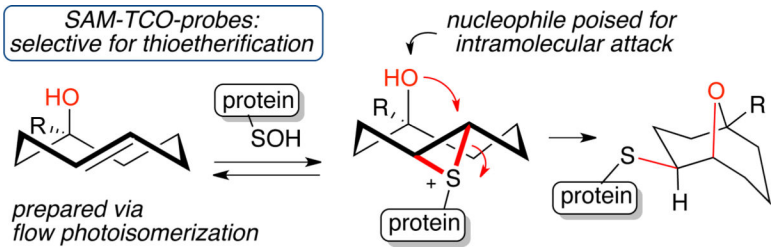


Figure 9.

SAM-TCO probes are specialized *trans*-cyclooctenes that react with sulfenic acids in cellular context via transannulation. The probes are small, cell permeant, selective, irreversible, and can be quenched *in vivo* to enable cellular reporting temporal precision.

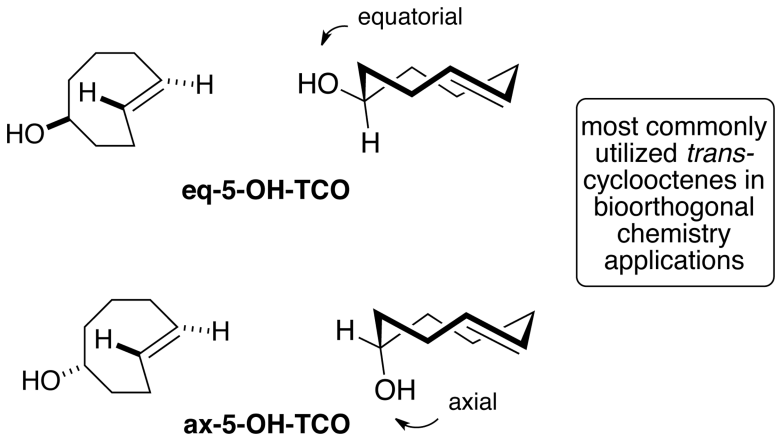


Figure 10.

Equatorial and axial diastereomers are produced as a separable mixture upon photoisomerization of 5-hydroxy-*cis*-cyclooctene. These dienophiles have been widely used for applications in bioorthogonal chemistry.

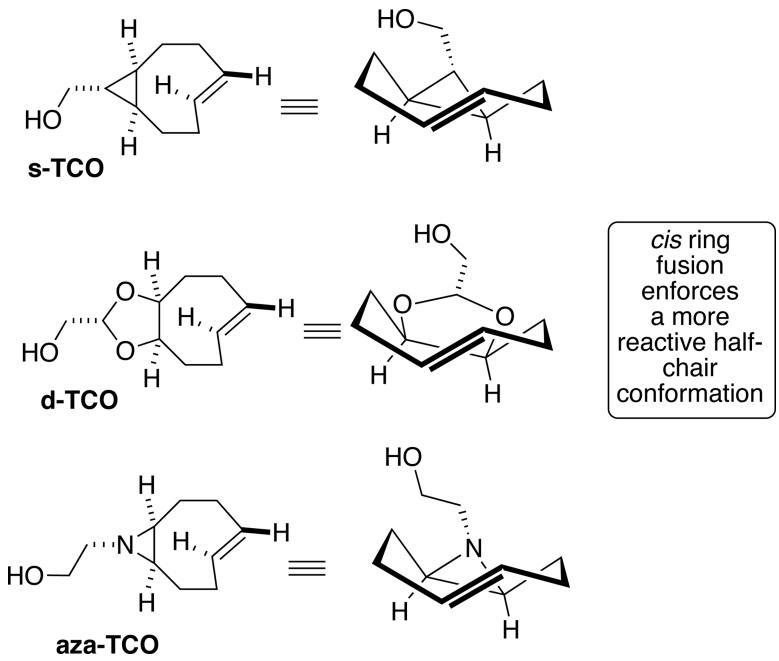
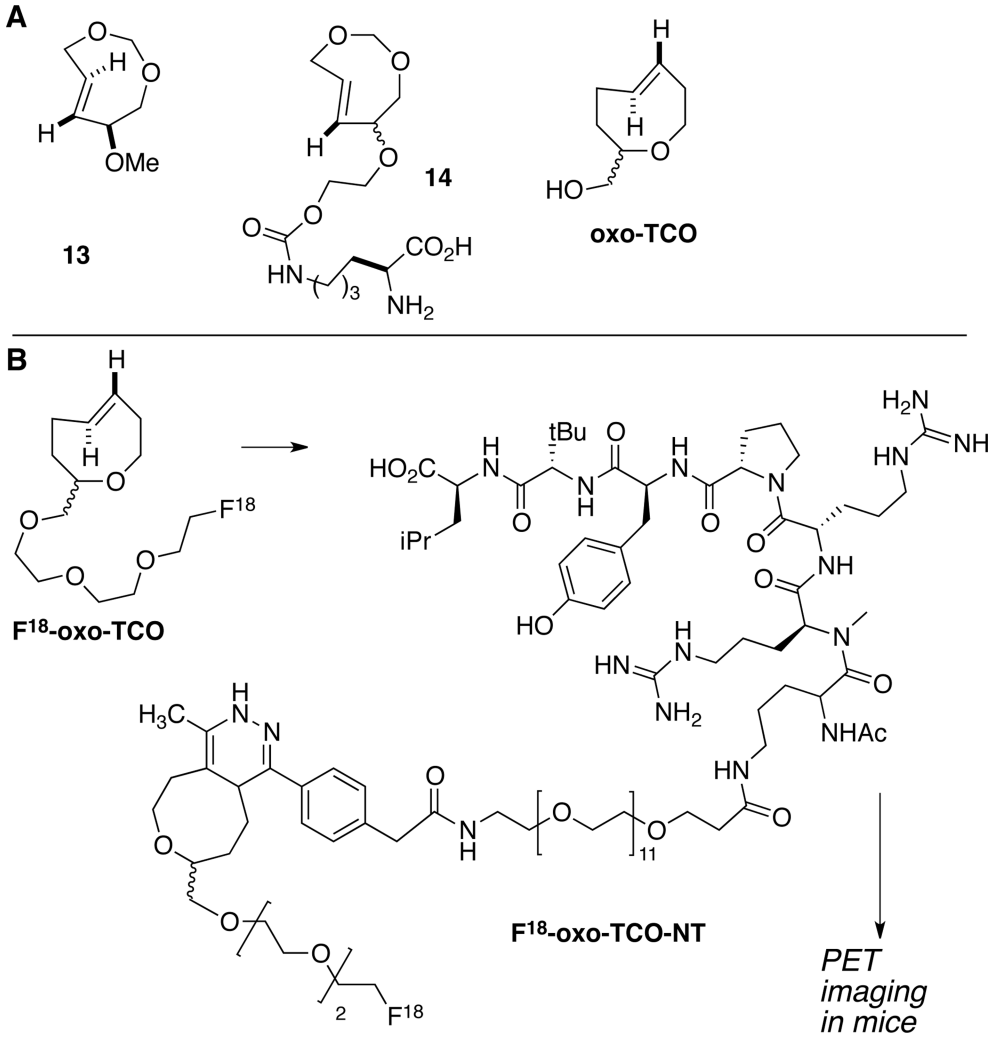


Figure 11.

Conformationally strained *trans*-cyclooctenes have faster rates of reaction than the crown conformer *trans*-cyclooctenes and can be employed in a number of bioothogonal applications.



Tumor/Muscle	0.5 h	3.5 h
^{18}F-s-TCO-NT	6.5 ± 1.5	6.4 ± 0.5
^{18}F-d-TCO-NT	3.8 ± 0.9	11.9 ± 4.3
^{18}F-oxo-TCO-NT	15.8 ± 2.2	16.2 ± 2.3

Figure 12.

(A) Hydrophilic *trans*-cyclooctene analogs. (B) ^{18}F-labeled probe based on oxo-TCO displays improved hydrophilicity for in vivo PET imaging in mice.

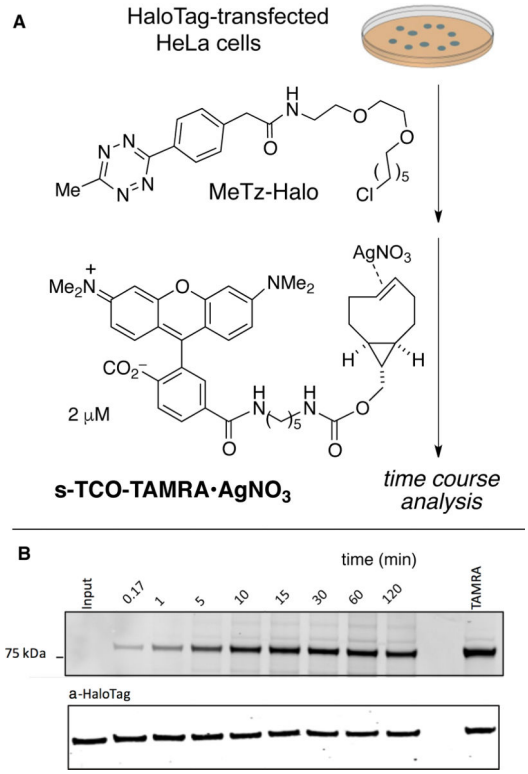


Figure 13.

(A) Incorporation of MeTz-Halo into HaloTag-transfected HeLa cells was followed by labeling with s-TCO-TAMRA•$AgNO_3$. (B) Kinetics were studied by timecourse quenching with a non-fluorescent TCO and following analysis by in-gel fluorescence.

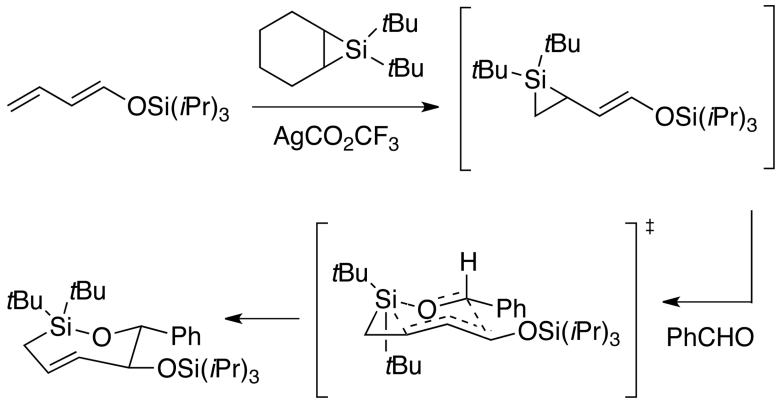


Figure 14.
Woerpel's synthesis of *trans*-oxasilacycloheptenes

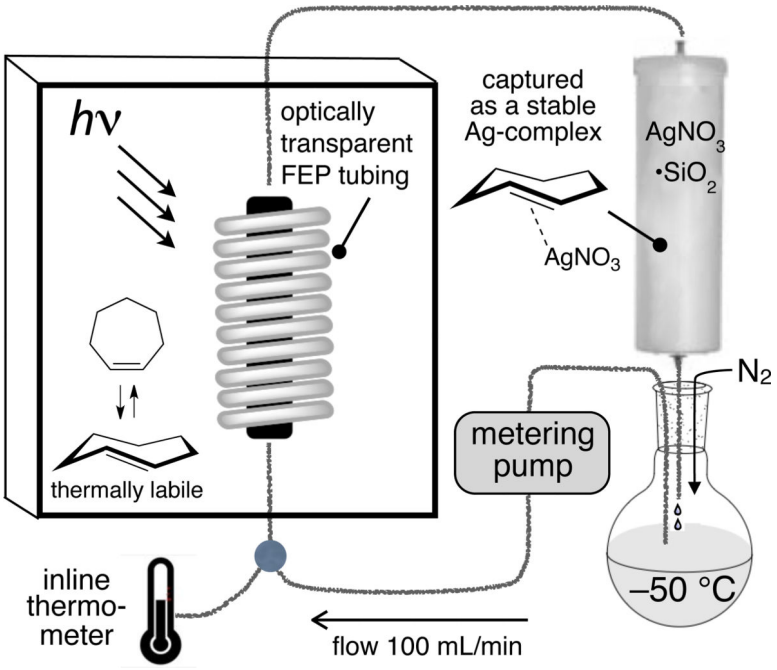


Figure 15.

Flow photoisomerization apparatus for carbocyclic *trans*-cycloheptene derivatives uses FEP tubing and inline cooling. For the synthesis of *trans*-1-sila-4-cycloheptenes, photoisomerizations could be carried out at r.t. using a conventional flow photoisomerization setup.

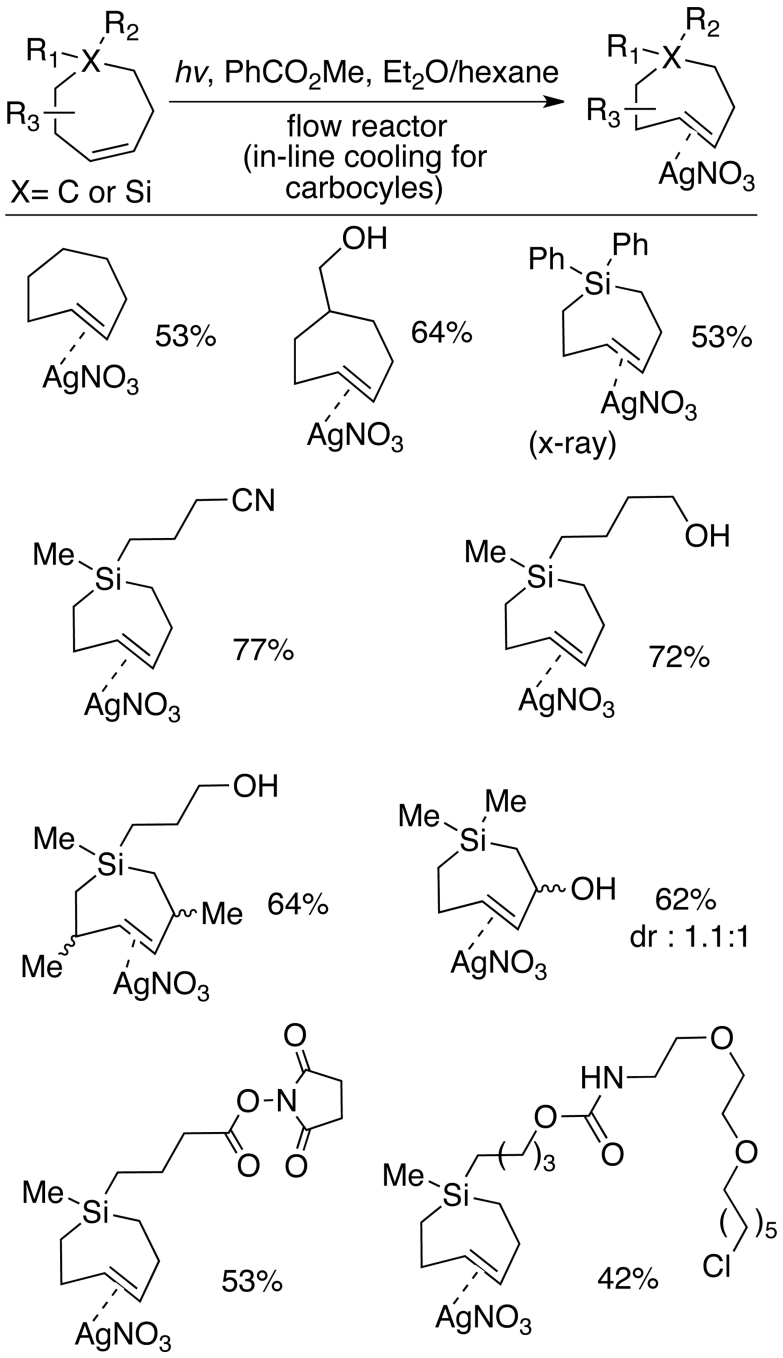


Figure 16.

Flow-photochemical synthesis of $AgNO_3$ complexes of trans-cycloheptenes and *trans*-1-sila-4-cycloheptenes.

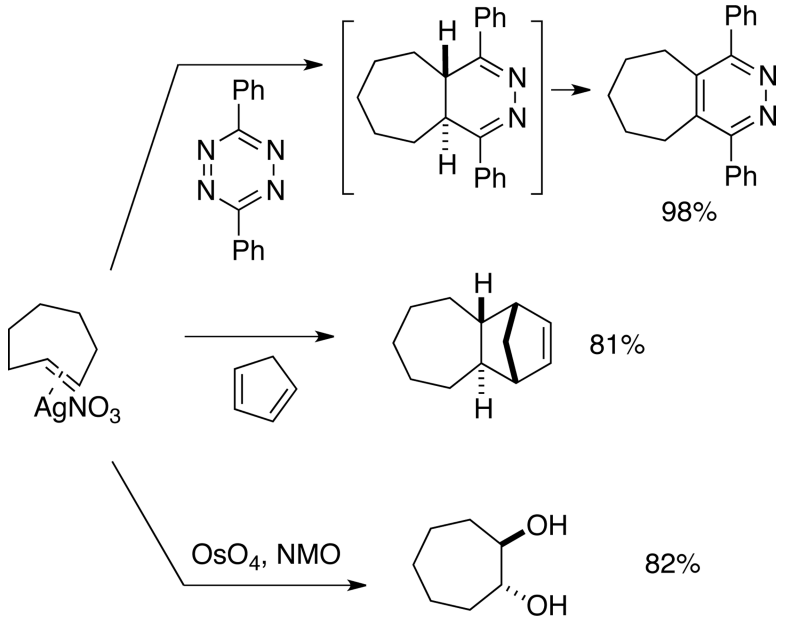


Figure 17.
Reactions of TCH•$AgNO_3$

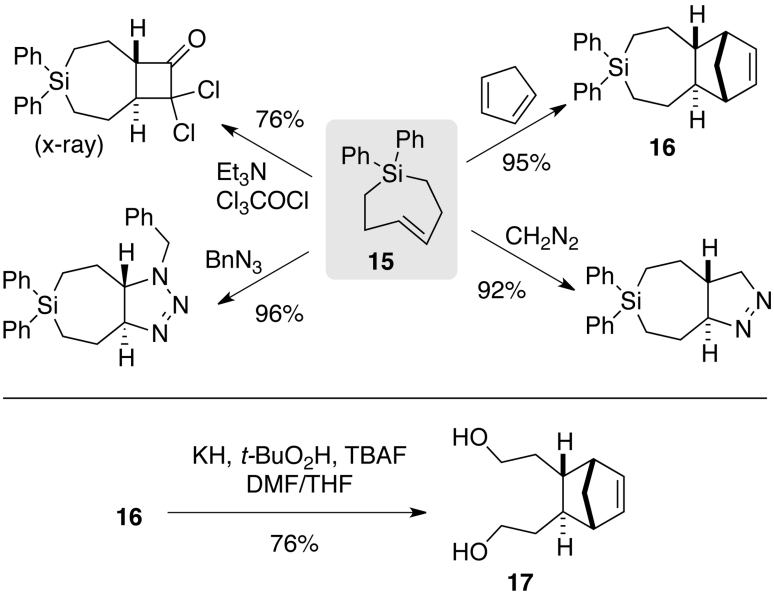


Figure 18.
Reactions of Si-TCH **15** and cycloadduct **16**.

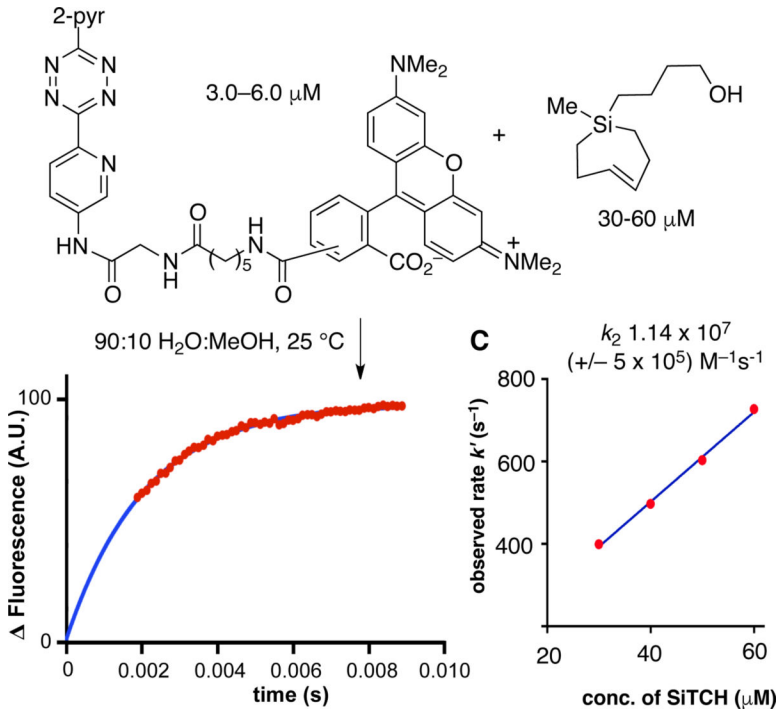


Figure 19.

Stopped flow kinetics of a Si-TCH with a TAMRA-3,6-dipyridyl-*s*-tetrazine conjugate were monitored in 9:1 H_2O:MeOH with monitoring by fluorescence. Data points are shown in red, and the fit is shown in blue. Second order rate constants (k_2) were determined by plotting k_{obs} vs. the concentration of Si-TCH.