BIO- AND AIR-TOLERANT CARBON–CARBON BOND FORMATIONS VIA ORGANOMETALLIC RUTHENIUM CATALYSIS

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Dedicated to Dr. Alfred Bader on the occasion of his 85th birthday in recognition of his outstanding contributions as a chemist, enterpreneur, and benefactor.

Selected [2+2+2] cycloadditions, alkene–alkyne coupling and fusion of enyne with diazo compound, all triggered by an artificial organometallic ruthenium catalyst are demonstrated to proceed under ambient aerobic aqueous conditions in presence of bodily fluids or cell lysate. To the best of our knowledge, these are the first examples of bio- and air-tolerant C–C bond formation catalyzed by an artificial organometallic compound.

Keywords: Ruthenium; Organometallic catalysis; C–C coupling; [2+2+2] Cycloadditions; Alkene–alkyne coupling; Enyne-diazocompound fusion; Alkynes; Arenes; Heterocycles; Nitrogen-based organic cations; N-heteroaromatic cations; Helical structures; Helquat; Bio-, air-, and water-tolerant transformations; *E. coli* cell lysate; Human serum; Bioorthogonal reactions; Non-coded biocompatible chemistry.

Construction of carbon–carbon (C–C) bonds is the essence of organic chemistry[1]. In this respect, catalysis by organometallics (compounds with carbon–metal bonds) has become a powerful tool[2]. However, catalytic manifolds involving C–M bonds are widely viewed as incompatible with water, air, thiols, and organic electrophiles[3–5]. Consequently, the general lack of robust catalytic processes supported under aerobic aqueous physiological conditions represents a fundamental obstacle in the application of organometallic catalysis towards *in situ* synthesis in chemical biology, diagnostics, and therapeutics. Referring to this seldom discussed synthetic aspect of bioorganometallic chemistry[6], notable examples of C–H (ref.[7]), C–N (ref.[8]), and C–S (ref.[6]) bond formations catalyzed by organometallics under aerobic

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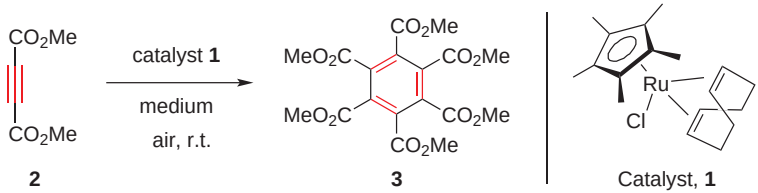
conditions in complex cellular media have been reported. In this vein, the identification of organometallic processes that form C–C bonds under physiological conditions represents a particularly important challenge[9–11].

RESULTS AND DISCUSSION

Here, we show that C–C bond formation can be performed using an artificial ruthenium organometallic catalyst in presence of aqueous aerobic media such as bodily fluids or cell lysate under mild conditions. Recently, Meggers and Streu effected the uncaging of amines from the corresponding allylcarbamates in living cells using commercially available catalyst **1** (ref.[6]). In other work directed towards organic synthesis in bio-media, the groups of Bertozzi and Sharpless have shown alkyne functionality to be significantly biorthogonal[12,13]. Inspired by these findings, we speculated that bio-tolerant C–C bond forming processes could be found among the rich set of known alkyne-based transformations catalyzed by **1** (ref.[14]). We observed that [2+2+2] cycloaddition of dimethyl acetylenedicarboxylate (**2**) using catalyst **1** (1 mole %)[15] was not only air-tolerant[16] but also proceeded in presence of water without any added organic co-solvent (Scheme 1,



SCHEME 1

TABLE I

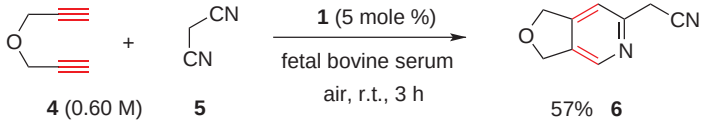
Bio- and air-tolerant [2+2+2] cycloaddition of alkyne **2**[a]

Entry	Catalyst **1**, %	Time, h	Medium	Yield, %[b]
1	1	5.5	H_2O	76[c]
2	5	25	*Rattus norveg.* urine[d]	72
3	5	4	*E. coli* cell lysate	76
4	0	20	*E. coli* cell lysate	0[c,e]
5	5	22	fetal bovine serum	59
6	5	24	human serum[f]	52

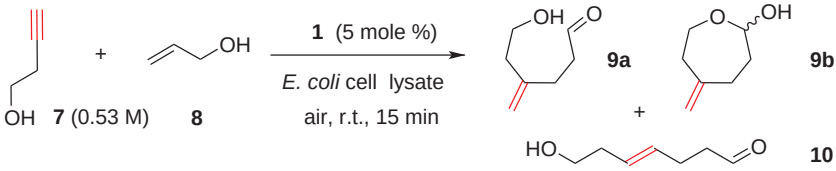
[a] Medium (1 ml), **2** (100 µl, 0.81 mmol). [b] Isolated yields of **3**. [c] 2 ml of medium used. [d] From *Rattus norvegicus* adult males. [e] Starting material **2** was recovered in 56% yield. [f] From male, age 31.

Table I, entry 1)[17]. Under these mild aerobic conditions, the starting material was consumed within 5.5 h and hexamethyl mellitate **3** was isolated in 76% yield. Notably, the reaction progressed to give similarly good isolated yields when performed with urine of *Rattus norvegicus*, cell lysate from *Escherichia coli*, fetal bovine serum, and human serum (entries 2–6). To the best of our knowledge, this is the first example of C–C bond formation catalyzed by an artificial organometallic compound in the presence of air and bodily fluids or cell lysate.

The study was extended to include [2+2+2] cycloaddition affording pyridine derivative **6** (Scheme 2)[16a,18,19], and Alder-ene-type coupling of alkyne **7** with allyl alcohol (**8**)[16a,20,21], to give a mixture of aldehydes **9** and **10** (Scheme 3). Of note is that aldehyde functionality is generated in this process. Such a feature might be further explored in the context of the follow-up reactivity profile of aldehydes in living systems[22]. Product **9** is present as a mixture of the open chain aldehyde **9a** and its cyclic seven-membered hemiacetal isomer **9b** (**9a**:**9b** 75:25). Interestingly, synthesis of **9** thus represents organometallic assembly of a carbon framework reminiscent of aldoheptose in a bio-relevant environment *in situ*.



SCHEME 2

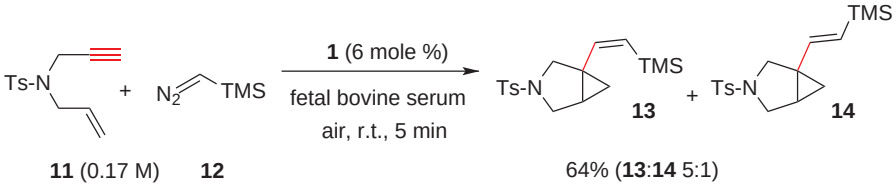


SCHEME 3

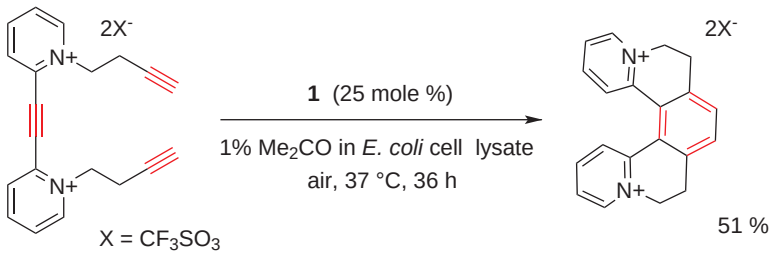
An example of enyne-diazo compound fusion to give bicyclic cyclopropanes **13** and **14** further expands the scope of bio-tolerant transformations catalyzed by **1** (Scheme 4)[16a,23,24]. This transformation proceeds remarkably fast with all the media tested (*E. coli* cell lysate, combined yield of **13** and **14** is 60%; *Rattus norvegicus* urine, 57%; human serum, 45%).

Heterogeneous catalysis and transformations taking place 'on water'[5c] cannot be excluded as substrates, products and also the catalyst **1** in examples in Table I and Schemes 2–4 were partly insoluble under the reaction

conditions presented. Therefore, we next turned to a transformation where better homogeneity of the reacting mixture is ensured. Specifically, we examined the feasibility of synthesis of our novel water-soluble helical extended diquat (helquat)[25] in *E. coli* cell lysate with an added 1% acetone to aid solubilization of the catalyst (Scheme 5).



SCHEME 4



SCHEME 5

As judged by NMR analysis of the evaporated reaction mixture, progressive disappearance of the peaks corresponding to triyne **15** and emergence of peaks diagnostic of helquat **16** demonstrates the feasibility of this process under aerobic bio-relevant conditions (Fig. 1). Increase in yield from 33% after 2.5 h to 51% after 36 h, demonstrates that the (C_5Me_5)Ru-based organometallic catalyst stays active for extended periods in this medium[26].

Notably, the reaction proceeds effectively even in the presence of considerable amounts of bovine serum albumin, a protein known for its promiscuous binding profile (Fig. 2)[27].

Potentially, a reaction proceeding via organometallic intermediates might be viewed as an entry to a collection of species with potential bioactivity (Fig. 3). The possibility of searching through such species generated *in situ* in presence of biological targets contained in the reaction medium, might allow for investigation of binding or reactive events otherwise difficult or even impossible to realize. Such an approach seems particularly attractive in light of the fact that (C_5H_5)Ru and other Ru-arene organometallics have been shown to be potent and selective bioactive entities[28].

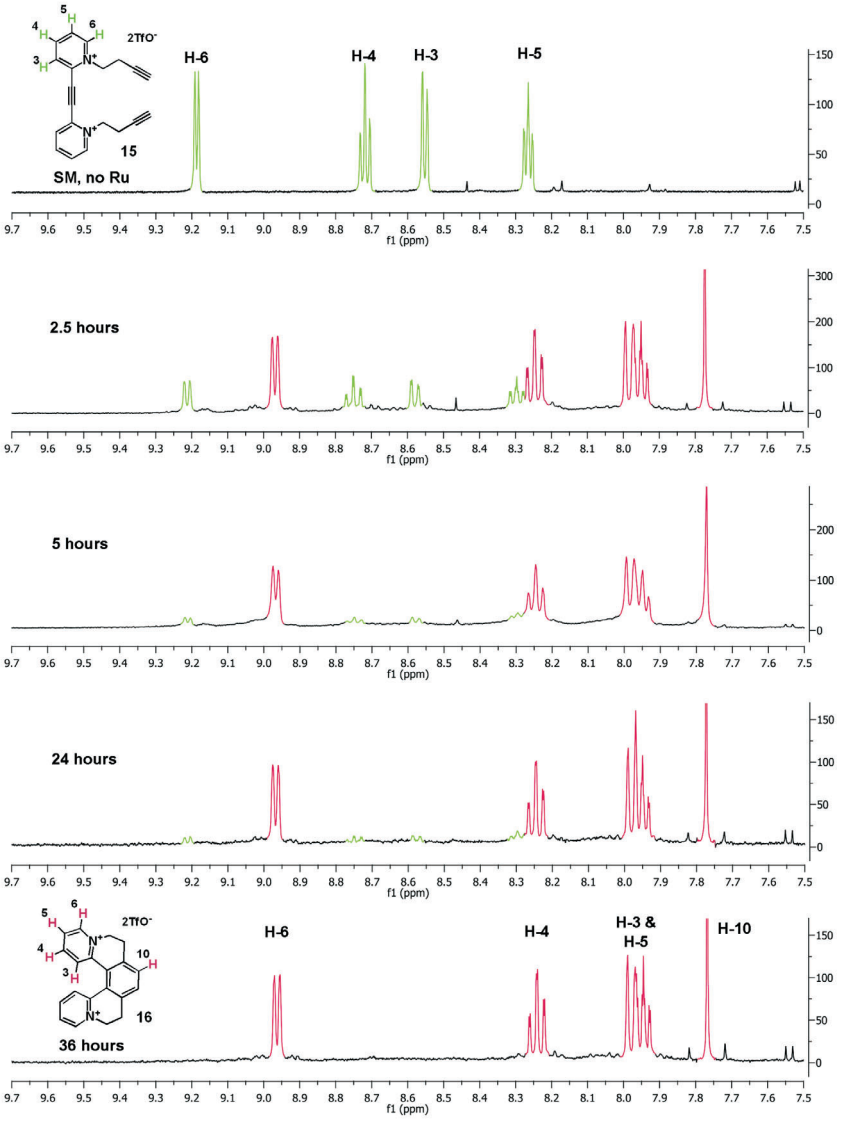


FIG. 1

^{1}H NMR (400 MHz, D_2O) monitored progression of Ru-catalyzed cycloaddition of **15** in *E. coli* cell lysate (Scheme 5). From solvation of starting material **15** in *E. coli* cell lysate before addition of catalyst **1** (top panel) to end of reaction after 36 h as judged by disappearance of **15** (bottom panel)

In summary, we have presented evidence that formation of C–C bonds via ruthenium organometallic catalysis can be achieved in the presence of bio-relevant media such as fetal bovine serum or *E. coli* cell lysate under aerobic ambient conditions. This will be of conceptual and practical interest for organometallic carbon framework construction targeted towards bio-applications including chemical biology, diagnostics, and therapeutics.

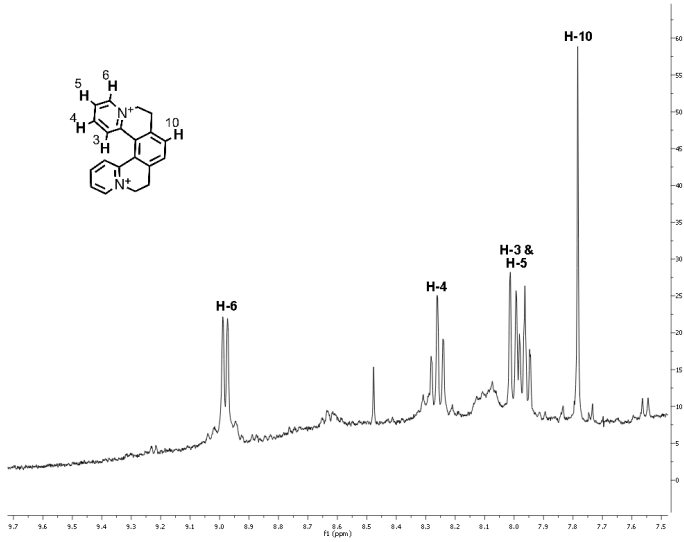


FIG. 2
^{1}H NMR (400 MHz, D_2O) of reaction mixture from transformation **15** → **16** in the presence of bovine serum albumin and *E. coli* cell lysate

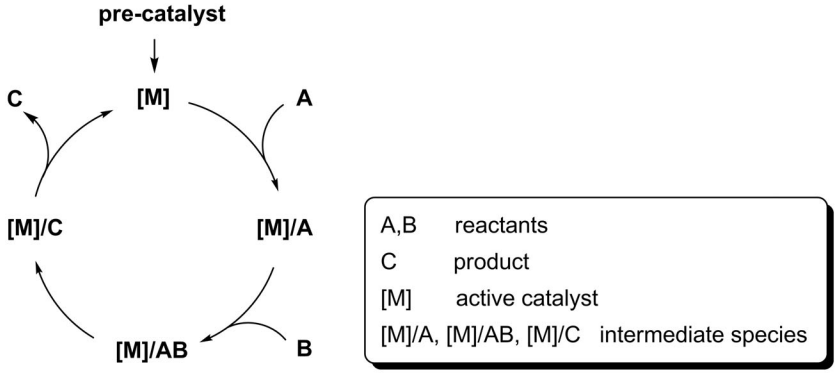


FIG. 3
An un-natural organometallic reaction in bio-relevant media might be viewed as an entry to a collection of species with potential bioactivity

EXPERIMENTAL

Unless otherwise noted, all reactions were carried out under aerobic conditions with no efforts taken to exclude air and moisture. Liquids and solutions were added via needle and syringe unless otherwise stated. Thin-layer chromatography (TLC) analysis was performed on silica gel plates (Silica gel 60 F_{254}-coated aluminium sheets, Merck, cat. No. 1.05554.0001) and visualized by UV (UV lamp 254/365 nm, Spectroline® Model ENF – 240C/FE) and, either chemical staining with $KMnO_4$ [$KMnO_4$ (1% aq.), Na_2CO_3 (2% aq.)], or $Ce(SO_4)_2$ [$Ce(SO_4)_2{\cdot}4H_2O$ (1%), $H_3P(Mo_3O_{10})_4$ (2%) in sulfuric acid (10% aq.)]. TLC analysis of dications was achieved using Stoddart's magic mixture[29] ($MeOH:NH_4Cl_{aq}$(2 M):$MeNO_2$ 70:20:10) as eluent on silica gel plates. Flash chromatography was performed on silica gel 60 (Fluka, cat. No. 60741) with the indicated eluent. All dilutions performed to determine the yield of **16** were performed with volumetric glassware and/or Hamilton Microliter™ syringes.

Materials

Unless otherwise stated, all starting materials and reagents were obtained from commercial suppliers and used without further purification. Demineralized water obtained from the Water Purification Facility at the Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic was used unless otherwise stated. Demineralization was accomplished via filtration through ion exchange columns (Lewatit S100 for catex column, Lewatit MP500 for anex column) in a demineralization ion exchange station type ID-PP and ID-KP (Kavalier, Votice, Czech Republic).

Rattus norvegicus urine. The urine from several male rats (*Rattus norvegicus* species, Wistar, supplier: Bio-Test s.r.o. Konárovice, Czech Republic) was pooled and kept frozen (–20 °C) until further use. Before an experiment, it was thawed in a 30 °C water bath.

Escherichia coli cell lysate. *Escherichia coli* DH5α transformed with plasmid pET24a (Novagen, cat. No. 69749-3) were grown in LB broth (Sigma, L3022) containing kanamycin (40 μg/ml, Fluka 60615). 40 ml of LB media were inoculated with 4 μl of saturated culture and grown overnight at 37 °C and 220 rpm. The culture was spun down at 3500*g* for 15 min and the pellet was resuspended in 10 ml of phosphate buffered saline (PBS) and sonicated (6 × 1 min at 3-min intervals). The crude lysate was then spun down at 10000*g* for 30 min at 4 °C and the supernatant was decanted. The solution was stored at –20 °C. Before an experiment, it was thawed in a 30 °C water bath. The residue content of the material was 35 mg/ml.

Fetal bovine serum (FBS). FBS was purchased from Invitrogen-Gibco (cat. No. 10270-106). Before use, FBS was heated at 65 °C for 30 min to inactivate the complement system. The residue content of the material was 30 mg/ml.

Human serum. One of the authors (male, 31) donated the blood (B negative blood type). All blood analysis data were within normal range and HIV 1 + 2 negative. The serum was obtained by centrifugation of the whole blood. Serum was stored in the freezer (–20 °C) and thawed before use in a 30 °C bath. The residue content of the material was 100 mg/ml.

Hexamethyl Benzene-1,2,3,4,5,6-hexacarboxylate (**3**). General Procedure

Dimethyl acetylenedicarboxylate **2** was added dropwise via Hamilton syringe under open flask conditions to a suspension of [Cp*Ru(cod)Cl] **1** in the described medium. The reaction flask was closed with a stopper to prevent evaporation and the reaction mixture stirred for

the described time at room temperature (r.t.). Formation of the product and consumption of the starting material was detected by TLC (petroleum ether:ethyl acetate 50:50, product R_F 0.29, starting material R_F 0.85). The reaction mixture was diluted with water and extracted with ethyl acetate (5 ×). The combined organic phases were dried over anhydrous Na_2SO_4. The mixture was filtered and the solvent was removed in vacuo to give crude product which was purified by flash chromatography on silica gel (petroleum ether:ethyl acetate 60:40). Hexamethyl benzene-1,2,3,4,5,6-hexacarboxylate **3** was obtained as colorless crystals. The spectroscopic characterization data are in agreement with the literature[17].

Table I, entry 1. Using dimethyl acetylenedicarboxylate **2** (100 µl, 115.6 mg, 0.813 mmol, 1 equiv.), [Cp*Ru(cod)Cl] **1** (3.5 mg, 9.2 µmol, 1 mole %), water (2 ml) as the medium, and a reaction time of 5.5 h, hexamethyl benzene-1,2,3,4,5,6-hexacarboxylate **3** was obtained as colorless crystals in 76% yield (88.3 mg, 0.207 mmol).

Table I, entry 2. Using dimethyl acetylenedicarboxylate **2** (100 µl, 115.6 mg, 0.813 mmol, 1 equiv.), [Cp*Ru(cod)Cl] **1** (15.5 mg, 40.8 µmol, 5 mole %), urine of *Rattus norvegicus* (1 ml) as the medium, and a reaction time of 25 h, hexamethyl benzene-1,2,3,4,5,6-hexacarboxylate **3** was obtained as colorless crystals in 72% yield (83.3 mg, 0.195 mmol).

Table I, entry 3. Using dimethyl acetylenedicarboxylate **2** (100 µl, 115.6 mg, 0.813 mmol, 1 equiv.), [Cp*Ru(cod)Cl] **1** (15.5 mg, 40.8 µmol, 5 mole %), *E. coli* DH5α cell lysate (1 ml) as the medium, and a reaction time of 4 h, hexamethyl benzene-1,2,3,4,5,6-hexacarboxylate **3** was obtained as colorless crystals in 76% yield (87.3 mg, 0.205 mmol).

Table I, entry 4. Using dimethyl acetylenedicarboxylate **2** (100 µl, 115.6 mg, 0.813 mmol, 1 equiv.), *E. coli* DH5α cell lysate (2 ml) as the medium, and a reaction time of 20 h, but omitting [Cp*Ru(cod)Cl] **1** (0 mg, 0 µmol, 0 mole %), the starting material dimethyl acetylenedicarboxylate **2** was obtained as an oil in 72% yield (83.3 mg, 0.195 mmol). The spectroscopic characterization data are in agreement with that of the commercial sample.

Table I, entry 5. Using dimethyl acetylenedicarboxylate **2** (100 µl, 115.6 mg, 0.813 mmol, 1 equiv.), [Cp*Ru(cod)Cl] **1** (15.5 mg, 40.8 µmol, 5 mole %), fetal bovine serum (1 ml) as the medium, and a reaction time of 22 h, hexamethyl benzene-1,2,3,4,5,6-hexacarboxylate **3** was obtained as colorless crystals in 59% yield (68.6 mg, 0.161 mmol).

Table I, entry 6. Using dimethyl acetylenedicarboxylate **2** (100 µl, 115.6 mg, 0.813 mmol, 1 equiv.), [Cp*Ru(cod)Cl] **1** (15.6 mg, 41.1 µmol, 5 mole %), human serum (1 ml) as the medium, and a reaction time of 24 h, hexamethyl benzene-1,2,3,4,5,6-hexacarboxylate **3** was obtained as colorless crystals in 52% yield (60.2 mg, 0.141 mmol).

2-(1,3-Dihydrofuro[3,4-*c*]pyridin-6-yl)acetonitrile (**6**)

Propargyl ether **4** (62 µl, 56.7 mg, 0.602 mmol, 1 equiv.) was added dropwise over 15 min via Hamilton syringe to a stirring suspension of malononitrile **5** (61.6 mg, 0.932 mmol, 1.5 equiv.) and [Cp*Ru(cod)Cl] **1** (11.4 mg, 30 µmol, 5 mole %) in fetal bovine serum (1 ml). The reaction mixture was then stirred at r.t. for 3 h. Formation of the product was detected by TLC (hexanes:ethyl acetate 50:50, R_F 0.18). The reaction mixture was diluted with water and extracted with DCM (1 ×) and ethyl acetate (3 ×). The organic fractions were combined and dried over anhydrous Na_2SO_4. The mixture was filtered and concentrated in vacuo to yield the crude product which was purified by flash chromatography on silica gel (hexanes: ethyl acetate 40:60). 2-(1,3-Dihydrofuro[3,4-*c*]pyridin-6-yl)acetonitrile **6** was obtained as a colorless amorphous solid in 57% yield (55.0 mg, 0.343 mmol). The spectroscopic characterization data are in agreement with the literature[18].

6-Hydroxy-4-methylenehexanal (**9**) and (*E*)-7-Hydroxyhept-4-enal (**10**)

Alkyne **7** (40 μl, 37.1 mg, 0.529 mmol, 1 equiv.) was added dropwise via Hamilton syringe under open flask conditions to a stirring suspension of allyl alcohol **8** (110 μl, 93.8 mg, 1.614 mmol, 3.1 equiv.) and [Cp*Ru(cod)Cl] **1** (10.3 mg, 0.0271 mmol, 5 mole %) in *E. coli* DH5α cell lysate (1 ml). The reaction mixture was then stirred at r.t. for 15 min. Formation of two products was detected by TLC (*n*-pentane:diethyl ether 50:50, product **9**, R_F 0.23; product **10**, R_F 0.10). The reaction mixture was diluted with water and extracted with diethyl ether (3 ×). The organic fractions were combined and dried over anhydrous Na_2SO_4. The mixture was filtered and concentrated in vacuo to give the crude product which was purified by flash chromatography on silica gel (*n*-pentane:diethyl ether 50:50). A 68:32 ratio of isomeric products **9** and **10** was obtained as a yellowish oil in 58% yield (39.3 mg, 0.307 mmol). Repeated chromatography allowed samples of each isomer to be isolated (**9** as a 76:24 ratio of the open chain and cyclic hemiacetal isomers **9a** and **9b**). The spectroscopic characterization data are in agreement with the literature[20].

(*Z*)-3-Tosyl-1-(2-(trimethylsilyl)vinyl)-3-azabicyclo[3.1.0]hexane (**13**) and
(*E*)-3-Tosyl-1-(2-(trimethylsilyl)vinyl)-3-azabicyclo[3.1.0]hexane (**14**)

(Trimethylsilyl)diazomethane **12** (110 μl, 2 M solution in diethyl ether, 0.220 mmol, 1.2 equiv.) and [Cp*Ru(cod)Cl] **1** (4 mg, 10.5 μmol, 6 mole %) were successively added within 1 min under open flask conditions to a stirring suspension of enyne **7** (45.8 mg, 0.1836 mmol, 1 equiv.)[30] in fetal bovine serum (1 ml). The reaction mixture was then stirred vigorously at r.t. for 5 min. Formation of the products was detected by TLC (hexanes:ethyl acetate 80:20, product R_F 0.57). The reaction mixture was diluted with water and extracted with diethyl ether (3 ×). The organic fractions were combined and dried over anhydrous Na_2SO_4. The mixture was filtered and concentrated in vacuo to yield the crude product which was purified by flash chromatography on silica gel (hexanes:ethyl acetate 15:1, R_F 0.20). An inseparable 5:1 mixture of isomers **13** and **14** was obtained as a brownish oil in 64% yield (39.2 mg, 0.117 mmol) and a small amount of desilylated by-product was also isolated in 6% yield (2.7 mg, 0.010 mmol). The spectroscopic characterization data are in agreement with the literature[23,31].

6,7,12,13-Tetrahydro-5,14-diaza[5]helicinium Trifluoromethanesulfonate (**16**)

Procedure to demonstrate reaction development in transformation **15** → **16**. One drop of acetone (0.01 ml) was added directly to the bottom of a RBF containing [Cp*Ru(cod)Cl] **1** (1.7 mg, 4.48 μmol, 25 mole %). A solution of 2,2′-(ethyne-1,2-diyl)bis(1-(but-3-ynyl)-pyridinium)bis(trifluoromethanesulfonate) **15** (10.6 mg, 18.1 μmol, 1 equiv.)[25] in *E. coli* DH5α cell lysate (1.5 ml) was added to the mixture of catalyst and acetone. The resulting solution was stirred at 37 °C. Aliquots (0.3 ml) of the reaction mixture were removed at reaction times of 2.5, 5, 24, and 36 h. These aliquots were concentrated in vacuo and analyzed by ^{1}H NMR (Fig. 1).

Procedure for yield determination in transformation **15** → **16**. One drop of acetone (0.01 ml) was added directly to the bottom of a RBF containing [Cp*Ru(cod)Cl] **1** (1.6 mg, 4.28 μmol, 25 mole %). A solution of 2,2′-(ethyne-1,2-diyl)bis(1-(but-3-ynyl)pyridinium)bis(trifluoromethanesulfonate) **15** (10.0 mg, 17.1 μmol, 1 equiv.)[25] in *E. coli* DH5α cell lysate (1.5 ml) was added to the mixture of catalyst and acetone. The resulting solution was stirred at 37 °C

for 36 h. The yield of this reaction was determined by ^{1}H NMR with comparison to DMSO as an internal standard.

Procedure for transformation **15** → **16** *with added bovine serum albumin*. One drop of acetone (0.01 ml) was added directly to the bottom of a RBF containing [Cp*Ru(cod)Cl] **1** (0.8 mg, 2.14 μmol, 25 mole %). A solution of 2,2′-(ethyne-1,2-diyl)bis(1-(but-3-ynyl)pyridinium)-bis(trifluoromethanesulfonate) **15** (5.0 mg, 8.6 μmol, 1 equiv.)[25] and bovine serum albumin (26.4 mg) in *E. coli* DH5α cell lysate (1 ml) was added to the mixture of catalyst and acetone. The resulting solution was stirred at 37 °C for 36 h. The reaction mixture was concentrated in vacuo. ^{1}H NMR analysis indicates formation of 6,7,12,13-tetrahydro-5,14-diaza-[5]helicinium trifluoromethanesulfonate **16** and consumption of the starting material **15** (Fig. 2).

Supporting Information Available

Further analytical data, scanned NMR spectra, and yield determination for transformation in Scheme 5 are available free of charge at http://dx.doi.org/10.1135/cccc2009053.

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