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Bioorganic & Medicinal Chemistry 16 (2008) 5232–5246
Bioorganic & Medicinal Chemistry

Total syntheses of (±)-ovalicin, C4(S^*)-isomer, and its C5-analogs and anti-trypanosomal activities

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Received 28 January 2008; revised 25 February 2008; accepted 3 March 2008
Available online 6 March 2008

This publication is dedicated to Professor E.J. Corey on the occasion of his 80th birthday.

Abstract—Total syntheses of (±)-ovalicin, its C4(S^*)-isomer **44**, and C5-side chain intermediate **46** were accomplished via an intramolecular Heck reaction of (*Z*)-3-(*tert*-butyldimethylsilyloxy)-1-iodo-1,6-heptadiene and a catalytic amount of palladium acetate. Subsequent epoxidation, dihydroxylation, methylation, and oxidation led to (3S^*,5R^*,6R^*)-5-methoxy-6-(*tert*-butyldimethylsilyloxy)-1-oxaspiro[2.5]octan-4-one (**2**), a reported intermediate. The addition of a side chain with *cis*-1-lithio-1,5-dimethyl-1,4-hexadiene (**27**) followed by oxidation afforded (±)-ovalicin. The functional group manipulation afforded a number of regio- and stereoisomers, which allow the synthesis of analogs for bioevaluation. The structure of **44** was firmly established via a single-crystal X-ray analysis. The stereochemistry at C4 generated from the addition reactions of alkenyllithium with ketones **2**, **40**, and **45** is dictated by C6-alkoxy functionality. Anti-trypanosomal activities of various ovalicin analogs and synthetic intermediates were evaluated, and C5-side chain analog, **46**, shows the strongest activity. Compound **44** shows antiproliferative effect against HL-60 tumor cells in vitro. Compounds **46** and a precursor, (3S^*,4R^*,5R^*,6R^*)-5-methoxy-4-[(*E*)-(1′,5′-dimethylhexa-1′,4′-dienyl)]-6-(*tert*-butyldimethylsilyloxy)-1-oxaspiro[2.5]octan-4-ol (**28**), may be explored for the development of anti-parasitic drugs.

1. Introduction

In search of small molecules that block the parasitic growth of protozoan parasite *Trypanosoma brucei* (*T. brucei*)[1,2] and possess anti-angiogenic activity, we studied the total synthesis of (±)-ovalicin (**1**)[3] and its analogs. The parasitic hemoflagellate *T. brucei* causes human African Trypanosomiasis or sleeping sickness.[1,2] The disease affects over 55 million people in Africa, and the transmission of parasite between mammalian hosts is largely through *Glossina* genus tsetse fly bites. The parasite replicates in the blood, passes the blood–brain barrier, and invades the central nervous system resulting in loss of consciousness, coma, and eventually death if left untreated.[1] Our interest in finding anti-trypanosomal agents[4,5] led us to study the synthesis and bioevaluation of angiogenesis inhibitors, ovalicin and its analogs. Fumagillin, structurally similar to ovalicin, has been shown to inhibit methionine aminopeptidase 2 (MetAP2) leading to anti-angiogenesis.[4] Since MetAP2 presents in parasites,[4] syntheses and studies of anti-trypanosomal activities of ovalicin and its analogs were undertaken. Several total syntheses of ovalicin have appeared starting from functionalized six-membered ring compounds,[6–11] but an acyclic starting material is rare.[12] Moreover, anti-trypanosomal activity of ovalicin or its analogs has not been reported previously. Herein, we report the total syntheses of (±)-ovalicin, its C4(S^*) isomer, and C5-side chain isomer utilizing an intramolecular Heck reaction to generate a functionalized cyclohexene intermediate from an acyclic alkenyl iodide, and their anti-trypanosomal activities.

Keywords: Total synthesis of ovalicin; Ovalicin analogs; Anti-trypanosomal activities; Anti-parasitic; Methionine aminopeptidase 2.
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doi:10.1016/j.bmc.2008.03.009

2. Results and discussion

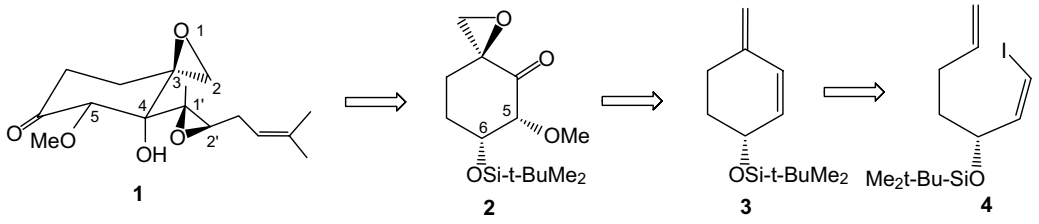
2.1. Synthesis of (±)-ovalicin

Our retrosynthetic analysis of ovalicin (**1**) is depicted in Scheme 1 utilizing compound **2**, a key intermediate reported by Barton[8] and others,[10,12] because the stereochemistry at C5 and 6 of compound **2** controls the stereochemistry at C4 of **1** in the subsequent addition reaction. Compound **2** is synthesized via an epoxidation of the exo-methylene function of diene **3** followed by dihydoxylation and functional group transformation. The functionalization of diene **3** provides various stereoisomers at C4–C6 for bioevaluation and studies of the stereochemical addition reactions at C4 of **2** and its stereoisomers leading to **1** and its analogs, respectively. Diene **3** is derived from an intramolecular Heck reaction of alkenyl iodide **4**, which is readily synthesized from 3-butenylmagnesium bromide and *cis*-3-iodopropenal (**7**).

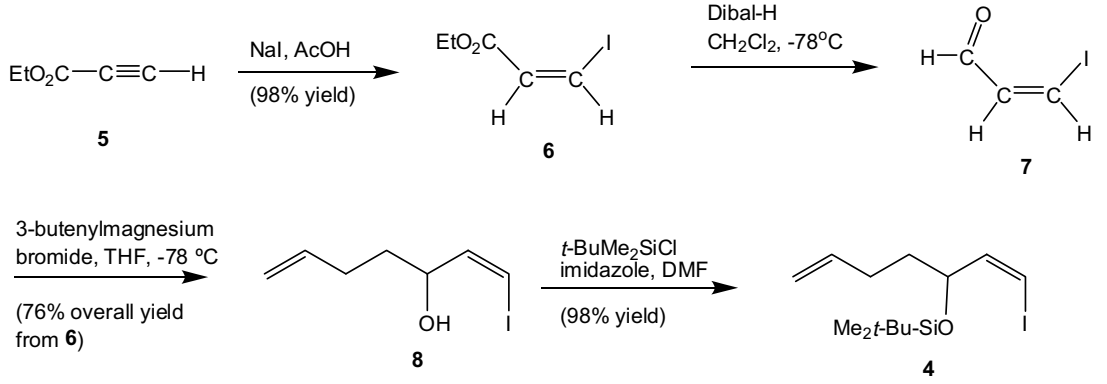
Alkenyl iodide **4** was readily synthesized from ethyl propiolate (**5**) by a sequence of reactions depicted in Scheme 2. Addition reaction of **5** with sodium iodide in acetic acid[13] followed by reduction of the ester function of resulting adduct **6** with Dibal-H in dichloromethane[13,14] and addition with 3-butenylmagnesium bromide afforded alcohol **8**. The aldehyde intermediate, **7**, from the reduction of **6**, is unstable and was used in the Grignard reaction without purification. Silylation of alcohol **8** with *tert*-butyldimethylsilyl chloride gave silyl ether **4**.

Both alcohol **8** and silyl ether **4** were studied in the ring-closing reactions. It has been reported that compounds containing hydroxyl moiety underwent successful intramolecular Heck reaction with palladium acetate, triphenylphosphine, and potassium carbonate in acetonitrile at 80 °C,[15] however under similar reaction conditions, 4-methylene-2-cyclohexenol (**9**) was not detected from the cyclization reaction of alcohol **8**. By using additives silver phosphate and proton sponge in DMF, alcohol **8** was converted to **9** in a 61% yield (Scheme 3). Under similar reaction conditions, silyl ether **4** gave a 95% yield of cyclized product **3**. Since subsequent steps involved oxidant and reactive reagents, silyl ether protected **3**[16] was used in our synthesis. In the epoxidation of **3**, an oxidizing reagent such as MCPBA does not provide satisfactory results, but methyl(trifluoromethyl)dioxirane (generated in situ)[16,17] gave an 81% yield of an inseparable mixture (1:1) of compounds **10** and **11** (determined from ^{1}H NMR spectrum). Subsequent dihydroxylation of **10** and **11** with OsO_4 and *N*-methylmorpholine *N*-oxide (NMO) afforded diols **12** and **13** (85% yield), which can be partially separated by silica gel column chromatography. A convenient separation method was found by selective mono-benzoylation of **12** and **13** (1:1) with benzoyl chloride and triethylamine providing benzoate **14** (43% yield) and unreacted diol **13** (49% recovery), which are readily separated. It appears that C4–axial–OH function of **12** is more accessible than other hydroxyl functions of **12** and **13**. Stereochemistry at C3-6 of **12** and **13** was determined from the subsequent conversion into the known compound, **2**, and 2D NOESY NMR spectroscopic study of a subsequent intermediate (vide infra).

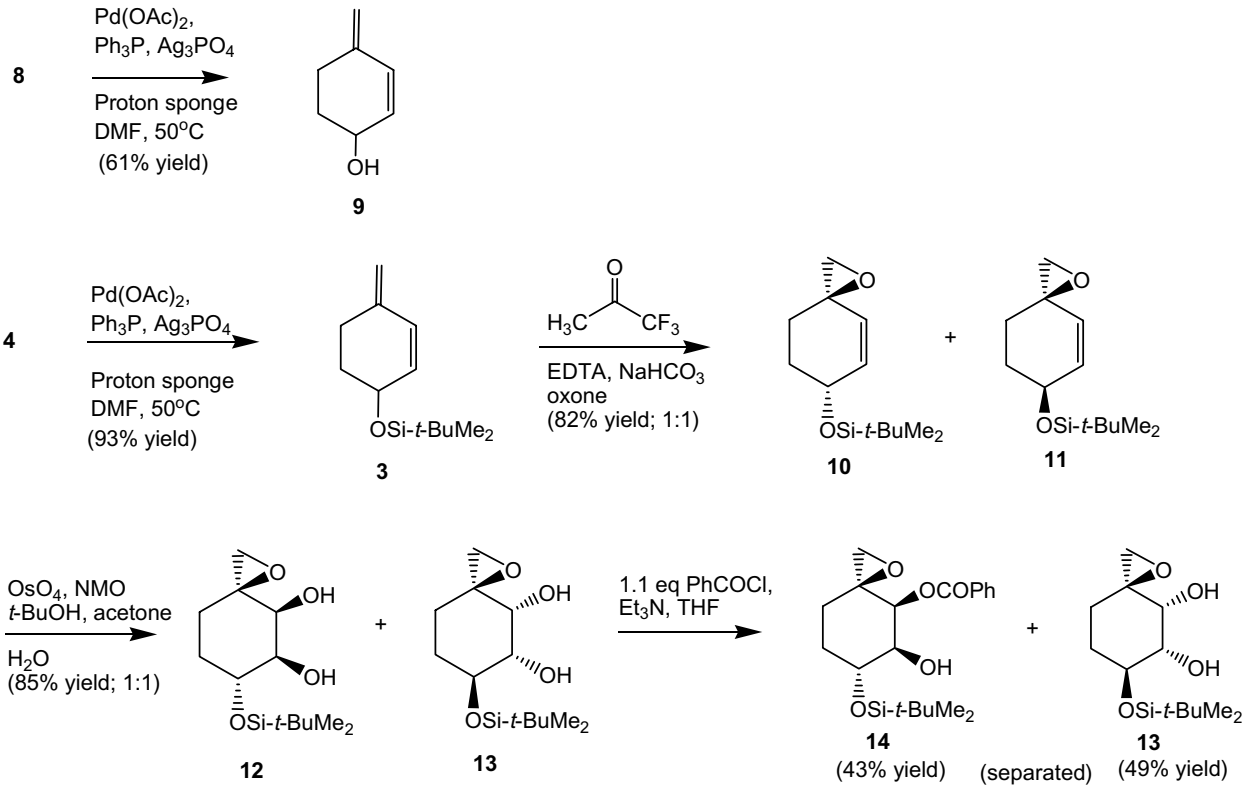
Compounds **13** and **14** possess the same stereochemistry at C6 and C5, respectively, as that of **2**, and they were converted into **2** via two separated routes (Schemes 4 and 5). Oxidation of the hydroxyl function of **14** with *o*-iodoxybenzoic acid (IBX) in DMSO[18] followed by



Scheme 1. Retrosynthesis of ovalicin (**1**).



Scheme 2. Synthesis of **4**.



Scheme 3. Synthesis of **14**.

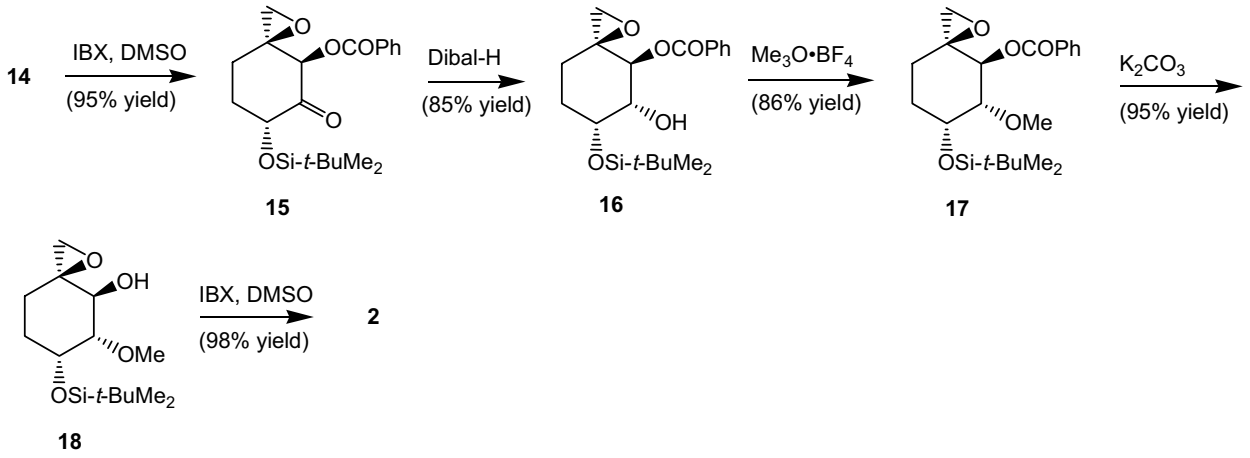
stereoselective reduction with diisobutylaluminum hydride (Dibal-H) in THF at −78 °C provided alcohol **16** as a single stereoisomer at C5. NMR spectra and TLC appearances of stereoisomers **14** and **16** are different. Methylation of alcohol **16** with trimethyloxonium tetrafluoroborate and proton sponge followed by basic hydrolysis and oxidation with IBX afforded ketone **2**. NMR spectral data of **2** are identical to those reported.[12]

The conversion of diol **13** to ketone **2** required a lengthier route because monomethylation of the *cis*-dihydroxyl functions and inversion and re-protection of C6 silyl ether moiety are needed (Scheme 5). However, a regioisomer such as compound **20** is available for analog synthesis (vide infra). As shown in the analog synthesis, the stereochemistry at C6 (R^*-configuration) of **2** is crucial for the generation of the correct stereochemistry at C4 of ovalicin in the addition reaction with the alkenyllithium (vide infra). Monomethylation of diol **13** with $Me_3O{\cdot}BF_4$ and proton sponge led to an 81% yield of methyl ether **19** and **20** in a ratio of 1.2:1 along with a small amount (6% yield) of the dimethoxylated product. Other methylation reactions were attempted such as the uses of methyl iodide or methyl triflate and sodium hydride or amine bases resulted in no methylation product. Fortuitously, **19** and **20** are separable by silica gel column chromatography. Compound **20** was used in the synthesis of analogs for bioevaluation (*vide infra*), and its regiochemistry was verified by its 2D NOESY spectrum in which C4–OMe (δ 3.42 ppm) shows NOE correlation with C2–H (δ 2.93 ppm) of the oxiran moiety. Benzoylation of **19** with benzoyl chloride and pyridine followed by removal of the silyl ether-protecting group with tetra-*n*-butylammonium fluoride (TBAF) and oxidation with IBX generated ketone **23** in excellent yield (83% yield in three steps). Ketone **23** was stereoselectively reduced with K-Selectride in THF to furnish alcohol **24** as a single stereoisomer. C6-S^*-isomer of **24** was not detected. Silylation of alcohol **24** with *tert*-butyldimethylsilyl chloride followed by basic hydrolysis of C4 benzoyl function and oxidation with IBX gave ketone **2** in a 93% overall yield (three steps).

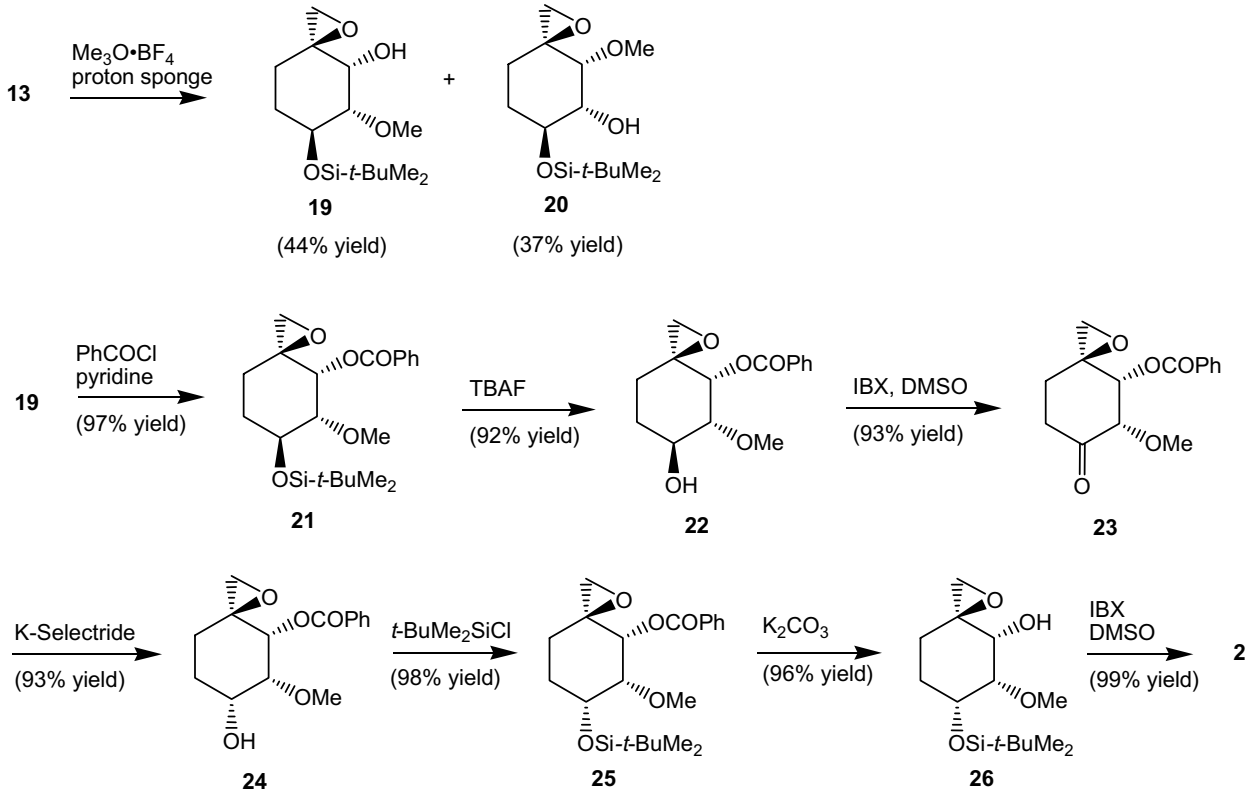
The synthesis of ovalicin **1** from **2** is similar to that reported.[8,10,12] Hence, the addition reaction of ketone **2** with *cis*-1-lithio-1,5-dimethyl-1,4-hexadiene (**27**)[6] in DME-toluene gave **28** as a single stereoisomer (Scheme 6). Compound **27** was generated in situ as described[6] utilizing a Shapiro reaction from acetone 2,4,6-triisopropylbenzenesulfonylhydrazone and 1-bromo-3-methyl-2-butene. The C6-silyloxy and C5-methoxy of **2** shielded the *si*-face of the carbonyl function resulting in the exclusive addition of alkenyllithium **27** from the *re*-face. Removal of the silyl ether-protecting group with TBAF followed by oxidation with IBX and epoxidation with vanadyl acetylacetone and *tert*-butylhydroperoxide afforded (±)-ovalicin (**1**) whose NMR data are identical to those reported.[8,12]

2.2. Syntheses of C4(S^*)-isomer 44 and C5-adduct 46

Although a number of total syntheses of ovalicin have been reported,[6–12] the synthesis of ovalicin analogs is limited.[11] Since a number of analogs are needed for our bioevaluation and examination of the effect of C6-



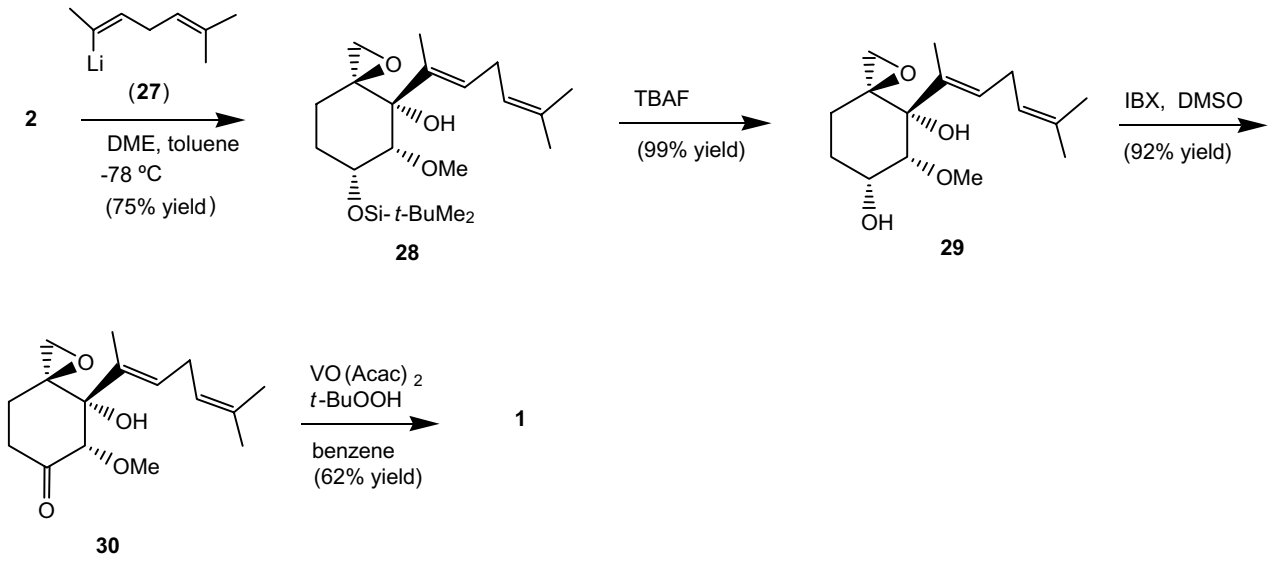
Scheme 4. Synthesis of **2**.



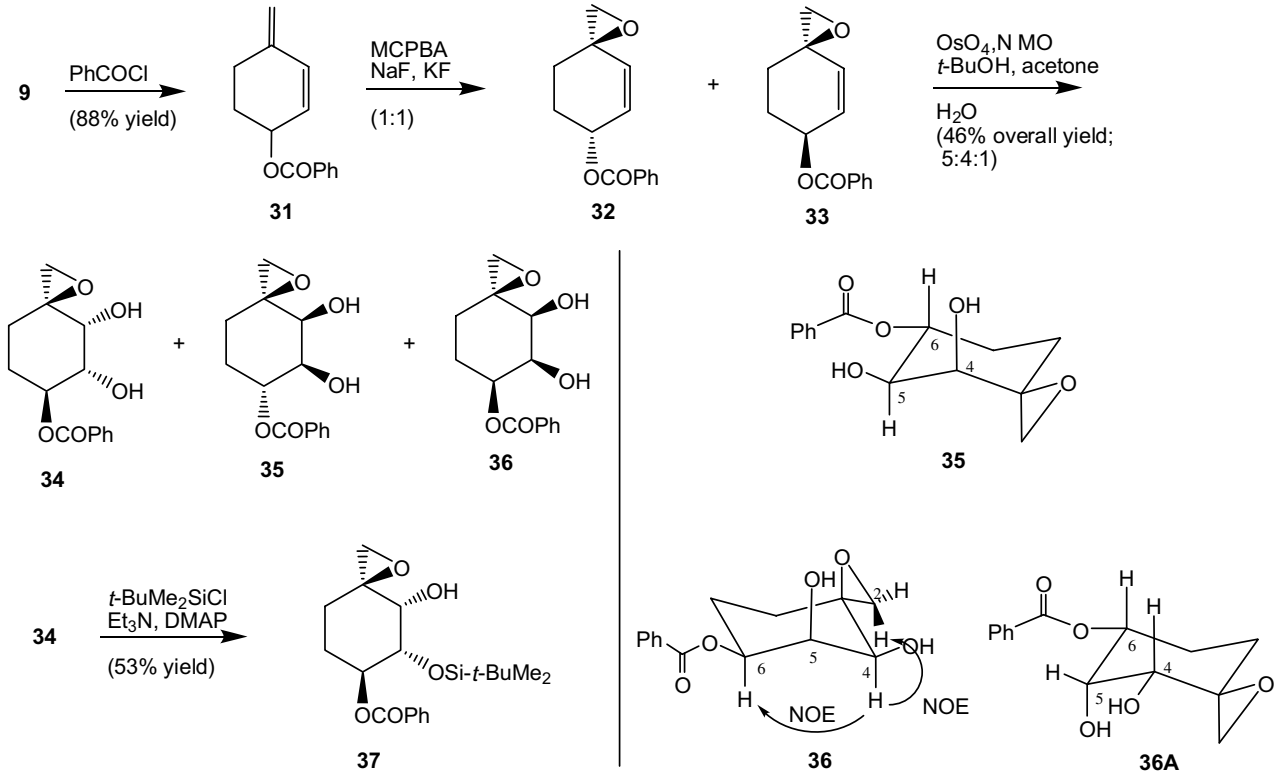
Scheme 5. Synthesis of **2** from **13**.

stereochemistry onto the alkenyllithium addition reaction, C4(S^*)-isomer of ovalicin, **44**, was synthesized and characterized. C5-adduct, compound **46** was synthesized and evaluated as well.

Instead of the silyl ether-protecting group at C6 of **2**, a benzoyl-protecting group was used in this synthesis, which provides benzoate analogs for bioevaluation (vide infra). Benzoylation of alcohol **9** with benzoyl chloride and triethylamine gave benzoate **31** (Scheme 7). Epoxidation of **31** with MCPBA–NaF–KF in dichloromethane[19] gave epoxides **32** and **33** in a 1:1 ratio, which decomposed slowly on silica gel column. The crude mixture of **32** and **33** was subjected to the dihydroxylation conditions, OsO_4 and NMO, to give a 46% overall yield of diols **34**, **35**, and **36** in a ratio of 5:4:1, which were separated by silica gel column chromatography. The relative stereochemistry of a major product, diol **34**, was established from a single-crystal X-ray analysis of its C5-monosilyl ether derivative, **37**, from the silylation reaction with *tert*-butyldimethylsilyl chloride and triethylamine (Fig. 1). Stereochemistry of the other major product, **35**, was assigned based on NMR coupling constant J values and the approach of osmium reagent predominantly from the opposite face of C6-benzoyloxy group, similar to that of the formation of compound **34**. Hence, C5–H of **35** shows a doublet of doublet at δ 4.01 ppm with J values of 7.6 Hz (axial–axial coupling)



Scheme 6. Synthesis of (±)-ovalicin (**1**).



Scheme 7. Synthesis of **37**.

and 3.2 Hz (axial–equatorial coupling). Stereochemistry of the minor product, **36**, was determined from a NMR 2D NOESY spectrum. The C4–H, δ 3.82 ppm, of **36** exhibits NOE correlations with C2–H_a, δ 3.15 ppm, and C6–H, δ 5.24 ppm. The stereoisomer of **36** or **36A** is not present. Compound **35** was used in bioevaluation (vide infra) and not investigated further since it possesses the same stereochemistry at C6 as that of **2**.

C4(S^*)-isomer of ovalicin, **44**, was synthesized from diol **34**. Monomethylation of diol **34** with trimethyloxonium tetrafluoroborate afforded a 1.3:1 ratio of methyl ether **38** and **39**, which were separated by silica gel column chromatography (Scheme 8). Compound **39** was not investigated further. Swern oxidation of **38** with trifluoroacetic anhydride and DMSO provided ketone **40**, which was subjected to the addition reaction with alkenyllithium **27**. Adducts **41** and **42** were isolated in a ratio of 1.5:1. Oxidation of alcohol **38** with IBX in DMSO at 25 °C resulted in a poor yield of **40** (42% yield). It is likely at 25 °C, product **40** undergoes E_1cb reaction under IBX-DMSO reaction conditions, while the Swern

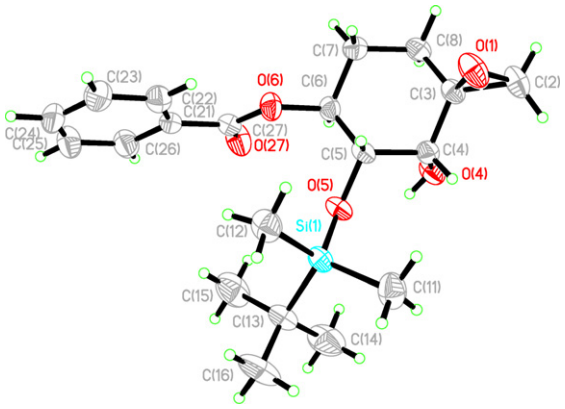


Figure 1. An ORTEP drawing of the single-crystal X-ray analysis of compound **37**.

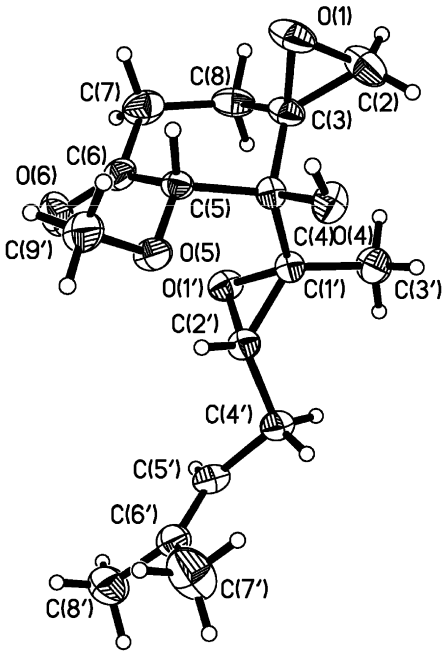
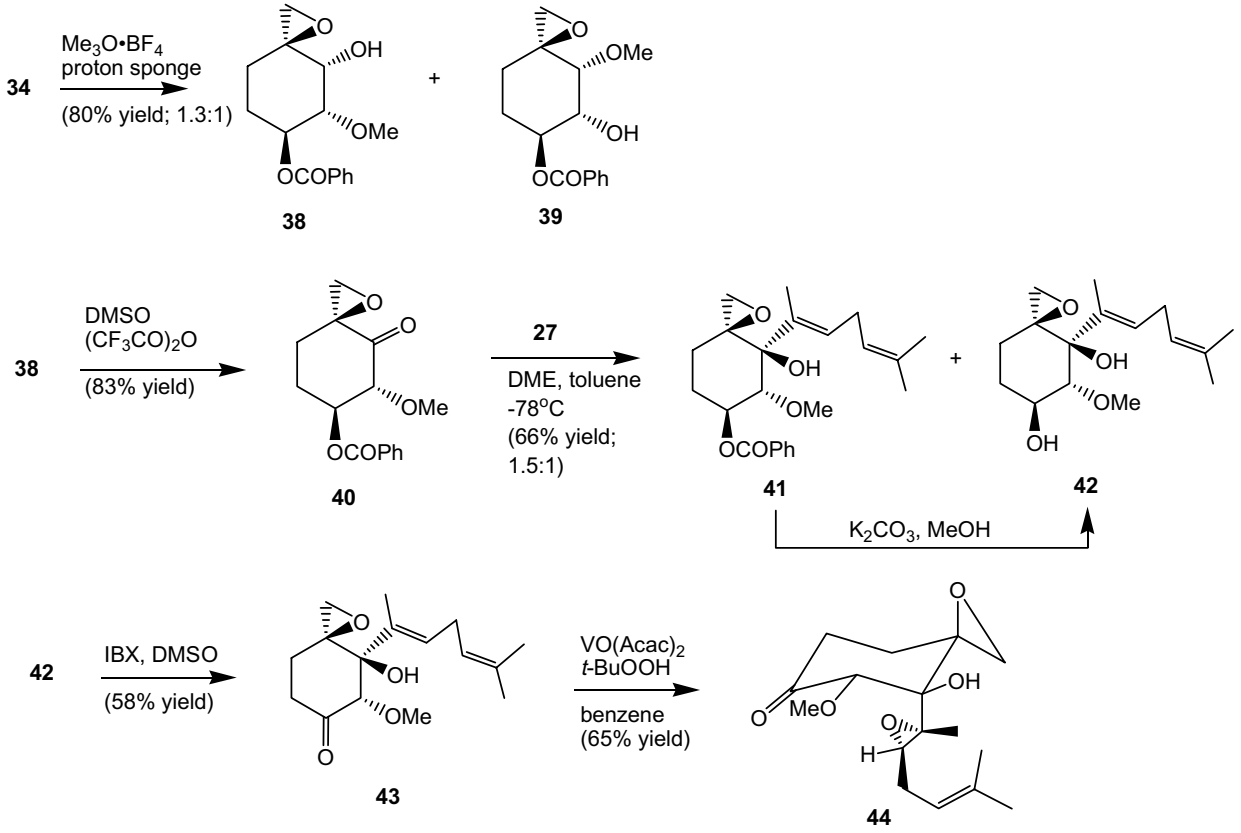


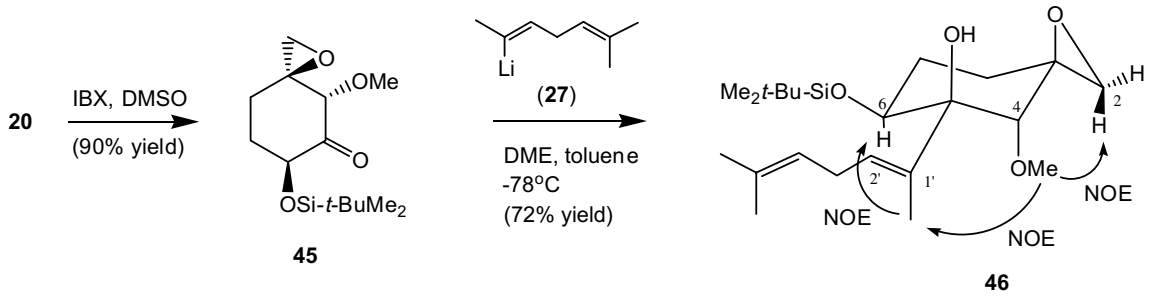
Figure 2. An ORTEP drawing of the single-crystal X-ray analysis of compound **44**.

oxidation was carried out at low temperature (−78 to 0 °C) in which elimination reaction does not occur. Compound **42** likely derived from the cleavage of the benzoyl ester function of **41** with **27** in situ. Basic hydrolysis of **41** with K_2CO_3 in methanol produced **42** (95% yield). Oxidation of **42** with IBX-DMSO followed by epoxidation with VO(Acac)$_2$-*t*-BuOOH furnished C4(*S**)-isomer **44**, of which the stereochemistry was affirmed from a single-crystal X-ray analysis (Fig. 2). Hence, the carbonyl addition reaction of compound **40** with alkenyllithium **27** took place from the opposite face of C6 benzoyloxy moiety and the same face of C5 methoxy group. The X-ray crystal structure reveals the axially oriented epoxy-alkenyl side chain, which is unusual.

C5-side chain analogs, such as compound **46**, have not been reported previously. For our bioevaluation, **46**



Scheme 8. Synthesis of C4(*S**)-isomer of ovalicin, **44**.



Scheme 9. Synthesis of C5-analog **46**.

was synthesized from alcohol **20** (Scheme 9) and evaluated (vide infra). Hence, oxidation of **20** with IBX-DMSO afforded ketone **45**, which upon addition reaction with alkenyllithium **27** gave a 72% yield of **46** as a single stereoisomer. The stereochemistry was assigned based on NMR 2D NOESY spectroscopy in which C4-methoxy, δ 3.29 ppm, shows NOE correlations with the side chain C1′-methyl, δ 1.75 ppm, and C2–H, δ 2.74 ppm. And, C6–H, δ 4.27 ppm, of **46** exhibits NOE correlations with C1′-methyl and C2′–H, δ 5.75 ppm. Hence, the carbonyl addition reaction of compound **45** with alkenyllithium **27** took place from the opposite face of C6 silyloxyl moiety and the same face of C4 methoxy group.

2.3. Bioevaluation

Various synthetic intermediates, compounds **28**, **34**, **35**, and **40**, and ovalicin analogs, compounds **41**, **43**, and **46**, were tested against *T. brucei* in vitro by following our reported methods.[20,21] C5-side chain compound, **46**, possesses the most potent inhibitory activity in vitro with an IC_{50} value of 0.28 ± 0.07 μM (Table 1). In comparison, ovalicin precursor **28**, a C4-side chain compound, is slightly less active with an IC_{50} of 0.40 ± 0.05. It is surprising to find that a structurally simple diol analog, **35**, has an IC_{50} of 0.72 ± 0.15 and is 2.5-fold less active than **46**. Compounds **34** and **40**, **41** and **43** are less active. The anti-trypanosomal activities of compounds **28** and **46** are greater than those previously reported for 5′-modified adenosine derivatives.[21] Biological targets of this class of compounds and efficacy in animal model will be studied. The utilization of ovalicin analogs, such as **46**, should be explored as anti-parasitic agents, as fumagillin and TNP-470,[4] possessing a similar skeleton as that of ovalicin, have been shown to inhibit methionine aminopeptidase 2 resulting in anti-malaria and anti-leishmaniasis.[4]

Table 1. In vivo antitrypanosmal activity of ovalicin analogs and synthetic intermediates

Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
28	0.40 ± 0.05	**41**	4.20 ± 0.40
34	325 ± 30	**43**	41.5 ± 4.0
35	0.72 ± 0.15	**46**	0.28 ± 0.07
40	47.3 ± 4.0		

3. Conclusion

(±)-Ovalicin, its C4(S^*)-isomer, and C5-side chain analog were synthesized via an intramolecular Heck reaction utilizing a catalytic amount of palladium acetate. Subsequent epoxidation, dihydroxylation, methylation, and oxidation led to ketone **2**, a reported key intermediate. The aforementioned functional group manipulation afforded a number of regio- and stereoisomers, which allow the synthesis of analogs for bioevaluation. The stereochemistry at C4 generated from the addition reactions of alkenyllithium with ketones **2**, **40**, and **45** is dictated by C6-alkoxy functionality. Anti-trypanosomal activities of various ovalicin analogs and synthetic intermediates were evaluated, and C5-side chain analog, **46**, shows the strongest activity. Compounds **46** and **28** may be used for the development of anti-parasitic drugs.

4. Experimental

4.1. General methods

Unless otherwise indicated, NMR spectra were obtained at 400 MHz for ^1H and 100 MHz for ^{13}C in $CDCl_3$, and reported in ppm. High-resolution Mass spectra were obtained from Maldi and ESI spectrometers. Maldi spectra were taken using 2,5-dihydroxybenzoic acid as a matrix. ESI spectra were acquired on a LCT Premier (Waters Corp., Milford MA) time of flight mass spectrometer. The instrument was operated at 10,000 resolution (W mode) with dynamic range enhancement that attenuates large intensity signals. The cone voltage was 60 eV. Spectra were acquired at 16,666 Hz pusher frequency covering the mass range 100–1200 μ and accumulating data for 2 s per cycle. Mass correction for exact mass determinations was made automatically with the lock mass feature in the MassLynx data system. A reference compound in an auxiliary sprayer is sampled every third cycle by toggling a 'shutter' between the analysis and reference needles. The reference mass is used for a linear mass correction of the analytical cycles. Samples are presented in Methanol Plus 0.1% formic acid as a 20 μL loop injection using an auto injector (LC PAL, CTC Analytics AG, Zwingen, Switzerland). Tetrahydrofuran and diethyl ether were distilled over sodium and benzophenone before use. Methylene chloride was distilled over CaH_2 and toluene and benzene were distilled over $LiAlH_4$.

4.1.1. (*Z*)-1-Iodo-1,6-heptadien-3-ol (8). To a three-neck flask equipped with a dropping funnel and a reflux condenser under argon were added 1.6 g (67 mmol) of magnesium turnings and 30 mL THF, and the mixture was stirred vigorously at 70 °C. To it, 3.0 g (22 mmol) of 4-bromo-1-butene was added dropwise through a dropping funnel and the resulting mixture was stirred at 75 °C for 2 h, cooled to 25 °C to give the Grignard reagent, which was maintained under argon. To a solution of (*Z*)-3-iodopropenal (**7**, generated from 16.5 mmol of **6**)[14] in 40 mL of dichloromethane at −78 °C under argon, was added the above 3-butenylmagnesium bromide via a cannula. The solution was stirred at −78 °C for 1 h, warmed to 25 °C, diluted with 100 mL of water, and extracted three times with diethyl ether (50 mL each). The combined organic layer was washed with aqueous $NaHCO_3$ and brine, dried (anhydrous Na_2SO_4), concentrated, and column-chromatographed on silica gel using a gradient mixture of hexane and diethyl ether as eluant to give 2.98 g (76% overall yield; based on **6**) of **8** a colorless liquid. ^{1}H NMR δ 6.35 (d, J = 8 Hz, 1H), 6.27 (t, J = 8 Hz, 1H), 5.90–5.70 (m, 1H), 5.07 (dd, J = 18, 4 Hz, 1H), 4.98 (dd, J = 12, 4 Hz, 1H), 4.43 (q, J = 8 Hz, 1H), 2.25–2.05 (m, 2H), 1.80–1.50 (m, 31H); ^{13}C NMR δ 143.4, 138.2, 115.4, 82.7, 74.2, 35.1, 29.5; HRMS calcd for $C_7H_{12}IO$ (M+H$^+$) 238.9927, found 238.9931.

4.1.2. (*Z*)-1-Iodo-3-(*tert*-butyldimethylsilyloxy)-1,6-heptadiene (4). To a cold (0 °C) solution of 5.6 g (23 mmol) of alcohol **8** in 40 mL of DMF under argon, were added 4.8 g (71 mmol) of imidazole, 2.0 g (16.4 mmol) of 4-dimethylaminopyridine (DMAP), and 4.6 g (31 mmol) of *tert*-butyldimethylsilyl chloride. The reaction solution was stirred at 25 °C for 12 h, diluted with 100 mL of water, and extracted twice with diethyl ether (50 mL each). The organic layer was washed with 1 N HCl (30 mL), aqueous $NaHCO_3$, and brine, dried (anhydrous Na_2SO_4), concentrated, and column-chromatographed on silica gel using a gradient mixture of hexane and diethyl ether as eluant to give 8.1 g (98% yield) of **4**. ^{1}H NMR δ 6.19 (s, 2H), 5.90–5.75 (m, 1H), 5.03 (d, J = 18 Hz, 1H), 4.92 (d, J = 12 Hz, 1H), 4.31 (q, J = 6 Hz, 1H), 2.25–2.05 (m, 2H), 1.70–1.50 (m, 2H), 0.88 (s, 9H), 0.09 (s, 3H), 0.05 (s, 3H); ^{13}C NMR δ 144.8, 138.6, 114.9, 80.07, 75.2, 36.21, 29.4, 26.06, 18.3, −3.98. HRMS calcd for $C_{13}H_{26}IOSi$ (M+H$^+$) 353.0792, found 353.0799.

4.1.3. 3-Methylene-6-(*tert*-butyldimethylsilyloxy)-1-cyclohexene (3). A mixture of 0.42 g (1.18 mmol) of **4**, 27 mg (0.12 mmol) of $Pd(OAc)_2$, 63 mg (0.24 mmol) of PPh_3, 0.49 g (1.18 mmol) of Ag_3PO_4, and 0.51 g (2.36 mmol) of proton sponge was dried under vacuum and maintained under argon. To it was added 5 mL of DMF, and the solution was stirred at 50 °C for 8 h, diluted with water, and extracted with diethyl ether. The combined organic layer was washed with water, and brine, dried ($MgSO_4$), concentrated, and column-chromatographed on silica gel using hexane as an eluant to give 0.25 g (93% yield) of **3**:[16] ^{1}H NMR δ 6.12 (d, J = 8 Hz, 1H), 5.71 (d, J = 8 Hz, 1H), 4.80 (d, J = 8 Hz, 2H), 4.40–4.30 (m, 1H), 2.55–2.45 (m, 1H), 2.35–2.25 (m, 1H), 1.95–1.85 (m, 1H), 1.65–1.55 (m, 1H), 0.82 (s, 9 H), 0.01 (s, 3H), 0.01 (s, 3H); ^{13}C NMR δ 142.6, 133.9, 130.2, 111.9, 67.1, 32.9, 27.9, 26.1, 18.4, −4.3.

4.1.4. 4-Methylene-2-cyclohexen-1-ol (9). A solution of 1.0 g (4.2 mmol) of **8**, 94 mg (0.42 mmol) of $Pd(OAc)_2$, 0.22 g (0.84 mmol) of Ph_3P, 1.8 g (4.2 mmol) of Ag_3PO_4, and 1.98 g (0.24 mmol) of proton sponge in 60 mL of DMF was stirred at 50 °C under argon for 10 h. The solution was cooled to 25 °C, diluted with water (200 mL), and extracted three times with dichloromethane (50 mL each). The combined organic layer was washed with water, and brine, dried ($MgSO_4$), concentrated, and column-chromatographed on silica gel using a gradient mixture of hexane and diethyl ether as eluant to give 0.28 g (61% yield) of **9**: ^{1}H NMR ($CDCl_3$)δ 6.21 (d, J = 9 Hz, 1H), 5.81 (d, J = 9 Hz, 1H), 4.88 (s, 2H), 4.40–4.30 (m, 1H), 2.60–2.20 (m, 2H), 2.10–1.60 (m, 2H), 1.56 (bs, 1H); ^{13}C NMR ($CDCl_3$) δ 142.1, 132.2, 131.4, 112.9, 66.1, 32.4, 27.3; HRMS calcd for $C_7H_{11}O$ (M+H$^+$) 111.0804, found 111.0811.

Silylation of **9** with *tert*-butyldimethylsilyl chloride, imidazole, and DMAP in dichloromethane gave a 97% yield of **3**.

4.1.5. (3*R,6*R**) and (3*R**,6*S**)-6-(*tert*-Butyldimethylsilyloxy)-1-oxaspiro[2.5]oct-4-ene (10 and 11).** A three-necked round-bottom flask was equipped with a dry-ice reflux condenser and a stir bar and maintained under argon. To it, were added 10.5 g (46.8 mmol) of **3** and 125 mL of EDTA (0.4 mM)/CH_3CN/THF (9:8.8:2.2), and the solution was cooled to 0 °C. Dry ice and acetone were added to the condenser to maintain at −78 °C. To the above solution, were added 19.7 g (0.23 mol) of sodium bicarbonate and 2.53 mL (26.9 mmol) of 1,1,1-trifluoroacetone. Oxone (14.4 g, 23.4 mmol) was added to the solution over 20 min in portions. The resulting mixture was stirred at 0 °C for 1 h, added 2.53 mL (26.9 mmol) of 1,1,1-trifluoroacetone, and stirred 0 °C for 6 h. The reaction was monitored by TLC, and a small amount of **3** remained. Prolonged reaction time led to the decomposition of the products. The mixture was diluted with CH_2Cl_2 and aqueous Na_2SO_4, and the organic layer was separated, washed with brine, dried (anhydrous Na_2SO_4), concentrated, and column-chromatographed on silica gel using a 1:1 mixture of hexane and diethyl ether as eluant. The silica gel was pretreated with hexane containing 1% of triethylamine by sonication for 1 h. The chromatography provided 9.10 g (82% yield) of a mixture of epoxides **10** and **11** (1:1) and 0.95 g (9% recovery) of **3**. The ratio of the two isomeric products was determined from their ^{1}H NMR spectrum. ^{1}H NMR (of one isomer) δ 6.0 (d, J = 10 Hz, 1H), 5.22 (t, J = 10 Hz, 1H), 4.35–4.25 (m, 1H), 2.84 (d, J = 5 Hz, 1H), 2.76 (d, J = 5 Hz, 1H), 1.95–1.85 (m, 2H), 1.80–1.60 (m, 2H), 0.89 (s, 9H), 0.03 (s, 3H), 0.029 (s, 3H); ^{1}H NMR (of another isomer) δ 6.00 (d, J = 10 Hz, 1H), 5.22 (t, J = 10 Hz, 1H), 4.35–4.25 (m, 1H), 2.84 (d, J = 5 Hz, 1H), 2.76 (d, J = 5 Hz, 1H), 1.95–1.85 (m, 2H), 1.80–1.60 (m, 2H), 0.89 (s, 9H), 0.03 (s, 3H),

0.029 (s, 3H); ^{13}C NMR (two isomers) δ 139.4, 138.2, 130.0, 128.8, 66.8, 65.9, 55.3, 54.9, 31.7, 30.9, 27.8, 26.0, 18.3, −4.4, −4.48. HRMS calcd for $C_{13}H_{25}O_2Si$ (M+H$^+$) 241.1624, found 241.1631.

4.1.6. (3*S,4*S**,5*R**,6*R**)-6-(*tert*-Butyldimethylsilyloxy)-1-oxaspiro[2.5]octane-4,5-diol (12) and (3*S**,4*R**,5*S**,6*S**)-6-(*tert*-butyldimethylsilyloxy)-1-oxaspiro[2.5]octane-4,5-diol (13).** To a solution of 9.0 g (37.4 mmol) of a mixture of epoxides **10** and **11** in 665 mL of acetone–water (3:1), was added 90 mL of *tert*-butanol. To it, were added 13.1 g (0.112 mol) of *N*-methylmorpholine-*N*-oxide (NMO) and 0.50 g (1.97 mmol) of osmium tetroxide, and the solution was stirred at 25 °C for 36 h. The solution was distilled under vacuum to remove acetone, diluted with water, and extracted twice with CH_2Cl_2. The organic layer was washed with brine, dried (anhydrous Na_2SO_4), concentrated, and column-chromatographed on silica gel using a mixture of hexane and diethyl ether (1:1) as eluant to give 8.71 g (85% yield) of diols **12** and **13** in 1:1 ratio. The ratio of the two isomeric products was determined from their ^{1}H NMR spectrum. These two isomers can be separated partially by column chromatography at this stage. Based on NMR spectra of pure diol **13** obtained in the following reaction (vide infra), NMR spectral data of diol **12** are deduced (by subtracting signals of **13**). Compound **12**: ^{1}H NMR δ 3.42–3.92 (m, 1H), 3.86 (dd, J = 7.6, 3.2 Hz, 1H), 3.88–3.66 (m, 1H), 2.84 (d, J = 5 Hz, 1H), 2.45 (d, J = 5 Hz, 1H), 1.90–1.80 (m, 2H), 1.60–1.40 (m, 2H), 0.91 (s, 9H), 0.11 (s, 3H), 0.10 (s, 3H); ^{13}C NMR δ 75.8, 71.4, 71.2, 59.0, 49.7, 28.8, 26.9, 26.0, 14.4, −4.3, −4.5; HRMS calcd for $C_{13}H_{27}O_4Si$ (M+H$^+$) 275.1679, found 275.1676.

4.1.7. (3*S,4*S**,5*R**,6*R**)-4-Benzoyloxy-6-(*tert*-butyldimethylsilyloxy)-1-oxaspiro[2.5]-5-octanol (14) and (3*S**,4*R**,5*S**,6*S**)-6-(*tert*-butyldimethylsilyloxy)-1-oxaspiro-[2.5]octane-4,5-diol (13).** To a solution of 6.7 g (24.5 mmol) of mixture of **12** and **13** (1:1) in 150 mL THF under argon at 0 °C, were added 10.2 mL (73 mmol) of triethylamine and 3.1 mL (27 mmol) of benzoyl chloride. The solution was stirred at 0 °C for 1 h and 25 °C for 12 h, diluted with dichloromethane, and washed with 1 N HCl. The organic layer was washed with aqueous $NaHCO_3$, and brine, dried (anhydrous Na_2SO_4), concentrated, and column-chromatographed on silica gel using a mixture of hexane and ethyl acetate (1:1) as eluant to give 3.97 g (43% yield) of **14** and 3.3 g (49% recovery) of **13**.

Compound **14**: ^{1}H NMR δ 8.07 (d, J = 7 Hz, 2H), 7.58 (t, J = 7 Hz, 1H), 7.49 (t, J = 7 Hz, 2H), 5.49 (d, J = 3 Hz, 1H), 4.20–4.10 (m, 1H), 4.00–3.95 (m, 1H), 2.82 (d, J = 5 Hz, 1H), 2.61 (d, J = 5 Hz, 1H), 2.25–2.05 (m, 2H), 2.05 (s, 1H), 1.75–1.55 (m, 2H), 0.94 (s, 9 H), 0.13 (s, 3H), 0.12 (s, 3H); ^{13}C NMR δ 152.5, 141.5, 133.5, 130.0, 128.7, 74.5, 71.2, 70.6, 58.8, 49.6, 27.1, 26.8, 25.9, 18.2, −4.5, −4.7. HRMS calcd for $C_{20}H_{30}O_5SiNa$ (M+Na$^+$) 401.1760, found 401.1754.

Diol **13**: ^{1}H NMR δ 4.02–3.95 (m, 1H), 3.84–3.80 (m, 2H), 2.94 (d, J = 5 Hz, 1H), 2.69 (d, J = 5 Hz), 2.50 (br s, 2H), 1.95–1.87 (m, 1H), 1.75–1.60 (m, 3H), 0.91 (s, 9H), 0.11 (s, 3H), 0.10 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 74.8, 70.36, 70.01, 59.6, 51.1, 28.3, 26.3, 25.8, 17.9, −4.7, −4.8; HRMS calcd for $C_{13}H_{27}O_4Si$ (M+H$^+$) 275.1679, found 275.1676.

4.1.8. (3*S,4*R**,6*R**)-4-Benzoyloxy-6-(*tert*-butyldimethylsilyloxy)-1-oxaspiro[2.5]-5-octanone (15).** To 33 mg (0.087 mmol) of alcohol **14** under argon, was added a solution of 97 mg (0.35 mmol) of IBX in 2 mL of DMSO, and the solution was stirred at 25 °C for 12 h. The solution was diluted with diethyl ether, washed with water, and brine, dried ($MgSO_4$), concentrated, and column-chromatographed on silica gel using a gradient mixture of hexane and diethyl ether as eluant to give 31 mg (95% yield) of **15**. ^{1}H NMR δ 8.12–8.07 (m, 2H), 7.64–7.60 (m, 1H), 7.50–7.46 (m, 2H), 6.24 (s, 1H), 4.37 (t, J = 3.4 Hz, 1H), 3.04 (d, J = 4.4 Hz, 1H), 2.76 (d, J = 4.4 Hz, 1H), 2.15 ~ 2.03 (m, 2H), 1.55–1.51 (m, 1H), 1.47–1.43 (m, 1H); ^{13}C NMR δ 201.0, 165.2, 133.7, 130.2, 129.3, 128.7, 75.5, 72.7, 61.9, 50.1, 31.2, 26.7, 25.9, 18.3, −4.7, −5.0; HRMS calcd for $C_{20}H_{28}O_5SiNa$ (M+Na$^+$) 399.1598, found 399.1605.

4.1.9. (3*S,4*S**,5*S**,6*R**)-4-Benzoyloxy-6-(*tert*-butyldimethylsilyloxy)-1-oxaspiro[2.5]-5-octanol (16).** To a cold (−78 °C) solution of 11 mg (0.03 mmol) of **15** in 0.5 mL of THF under argon, was added 46 μL (0.046 mmol) of diisobutylaluminum hydride (1 M in toluene). The solution was stirred at −78 °C for 8 h, added 30 μL of acetic acid, and diluted with diethyl ether. The organic layer was washed with water, and brine, dried ($MgSO_4$), concentrated, and column-chromatographed on silica gel using a gradient mixture of hexane and diethyl ether to give 9.6 mg (85% yield) of **16**. ^{1}H NMR δ 8.09–8.05 (m, 2H), 7.58–7.54 (m, 1H), 7.47–7.43 (m, 2H), 5.54 (d, J = 10 Hz, 1H), 4.29–4.25 (m, 1H), 3.90 (td, J = 10, 3 Hz, 1H), 2.87 (d, J = 4.8 Hz, 1H), 2.67 (d, J = 4.4 Hz), 2.22–2.18 (m, 2H), 1.90–1.86 (m, 2H), 0.97 (s, 9H), 0.15 (s, 3H), 0.14 (s, 3H); ^{13}C NMR δ 165.6, 133.5, 130.1, 130.0, 128.7, 77.4, 73.7, 71.7, 71.5, 50.6, 28.5, 26.8, 26.0, 18.3, −4.8, −4.9; HRMS calcd for $C_{20}H_{30}O_5SiNa$ (M+Na$^+$) 401.1760, found 401.1752.

4.1.10. (3*S,4*S**,5*S**,6*R**)-4-Benzoyloxy-5-methoxy-6-(*tert*-butyldimethylsilyloxy)-1-oxaspiro[2.5]-octane (17).** A solution of 27 mg (68 μmol) of **16**, 35 mg (0.16 mmol) of proton sponge, and 12 mg (82 μmol) of Me_3OBF_4 in 2 mL of dichloromethane under argon was stirred at 0 °C for 10 h, diluted with diethyl ether, washed with water, and brine, dried (anhydrous Na_2SO_4), concentrated, and column-chromatographed on silica gel using a gradient mixture of hexane and diethyl ether as eluant to give 23 mg (86% yield) of **17**: ^{1}H NMR δ 8.10–8.06 (m, 2H), 7.59–7.55 (m, 1H) 7.49–7.45 (m, 2H), 5.59 (d, J = 9 Hz, 1H), 4.34–4.30 (m, 1H), 3.50 (dd, J = 9, 3 Hz, 1H), 3.41 (s, 3H), 2.76 (d, J = 4.4 Hz, 1H), 2.65 (d, J = 4.4 Hz, 1H), 2.32–2.28 (m, 1H), 1.88–1.84 (m, 2H), 1.44–1.40 (m, 1H), 0.92 (s, 9H), 0.11 (s, 3H), 0.08 (s, 3H); ^{13}C NMR δ 166.1, 133.3, 130.1, 130.0, 128.6, 82.7, 70.9, 68.6, 59.1, 58.9, 51.1, 28.9, 27.0, 26.0, 18.4,

−4.4, −4.9; HRMS calcd for $C_{21}H_{32}O_5SiNa$ (M+Na$^+$) 415.1917, found 415.1906.

4.1.11. (3*S,4*S**,5*S**,6*R**)-5-Methoxy-6-(*tert*-butyldimethylsilyloxy)-1-oxaspiro[2.5]octan-4-ol (18).** A solution of 23 mg (59 μmol) of **17** and 81 mg (0.59 mmol) of potassium carbonate in 2 mL of methanol was stirred at 0 °C for 8 h, diluted with diethyl ether, washed with water, and brine, dried ($MgSO_4$), concentrated, and column-chromatographed on silica gel using a mixture of hexane and diethyl ether (2:1) as eluant to give 16 mg (95% yield) of **18**: ^{1}H NMR δ 4.34–4.30 (m, 1H), 4.10 (dd, J = 9, 6 Hz, 1H), 3.44 (s, 3H), 3.13 (d, J = 4.7 Hz, 1H), 3.07 (dd, J = 9, 2.2 Hz, 1H), 2.62 (d, J = 4.7, 1H), 2.37–2.33 (m, 1H), 2.29–2.25 (m, 1H), 1.99 (d, J = 6.4, 1H), 1.80 ∼ 1.68 (m, 1H), 1.25–1.21 (m, 1H), 0.91 (s, 9H), 0.11 (s, 3H), 0.08 (s, 3H); ^{13}C NMR δ 85.5, 67.6, 66.9, 60.2, 57.8, 50.1, 29.0, 26.5, 26.0, 18.3, −4.5, −4.8; HRMS calcd for $C_{14}H_{29}O_4Si$ (M+H$^+$) 289.1830, found 289.1837.

4.1.12. (3*S,5*R**,6*R**)-5-Methoxy-6-(*tert*-butyldimethylsilyloxy)-1-oxaspiro[2.5]octan-4-one (2).** A solution of 41 mg (0.14 mmol) of **18** and 0.16 g (0.56 mmol) of IBX in 5 mL of DMSO under argon was stirred at 25 °C for 12 h, diluted with diethyl ether, washed with water, and brine, dried ($MgSO_4$), and concentrated to give 39.9 mg (98% yield) of 2.[12] This material was used in the next step without purification. Its proton and carbon-13 NMR spectral data are similar to those reported.[12] ^{1}H NMR δ 4.46–4.42 (m, 1H), 3.98 (d, J = 2.6 Hz, 1H), 3.44 (s, 3H), 3.29 (d, J = 4.7 Hz, 1H), 2.77 (d, J = 4.7 Hz, 1H), 2.52–2.48 (m, 1H), 2.10–2.0 (m, 2H), 1.58–1.54 (m, 1 H), 0.92 (s, 9H), 0.08 (s, 3H), 0.07 (s, 3H); ^{13}C NMR δ 202.48, 87.5, 72.3, 60.7, 58.5, 51.4, 28.9, 27.1, 25.9, 18.3, −4.4, −5.0; HRMS calcd for $C_{14}H_{26}O_4SiNa$ (M+Na$^+$) 309.1498, found 309.1499.

4.1.13. (3*S,4*R**,5*S**,6*S**)-5-Methoxy-6-(*tert*-butyldimethylsilyloxy)-1-oxaspiro[2.5]octan-4-o1 (19), (3*S**,4*R**,5*S**,6*S**)-4-methoxy-6-(*tert*-butyldimethylsilyloxy)-1-oxaspiro[2.5]octan-5-o1 (20), and (3*S**,4*R**,5*S**,6*S**)-4,5-dimethoxy-6-(*tert*-butyldimethylsilyloxy)-1-oxaspiro[2.5]-octane.** A solution of 0.27 g (0.97 mmol) of diol **13**, 0.50 g (2.3 mmol) of proton sponge, and 0.17 g (1.2 mmol) of $Me_3O{\cdot}BF_4$ in 5 mL of dichloromethane was stirred at 0 °C for 10 h under argon, diluted with diethyl ether, washed with water, and brine, dried (anhydrous Na_2SO_4), concentrated, and column-chromatographed using a mixture of hexane and ethyl acetate (3:1) as eluant to give 0.123 g (44% yield) of **19**, 0.103 g (37% yield) of **20** and 18 mg (6% yield) of the dimethylated by-product.

Compound **19** (more polar isomer): ^{1}H NMR δ 4.03–3.99 (m, 1H), 3.84–3.80 (m, 1H), 3.45 (s, 3H, OMe), 3.38 (dd, J = 6.2, 3.0 Hz, 1H), 2.91 (d, J = 4.8 Hz, 1H), 2.67 (d, J = 4.8 Hz, 1H), 2.32 (br s, 1H), 1.92–1.88 (m, 1H), 1.70 ∼ 1.60 (m, 3H), 0.91 (s, 9H), 0.10 (s, 3H), 0.09 (s, 3H); ^{13}C NMR δ 84.5, 70.3, 68.6, 59.2, 58.8, 51.5, 29.1, 26.2, 25.9, 18.3, −4.6; HRMS calcd for $C_{14}H_{29}O_4Si$ (M+H$^+$) 289.1830, found 289.1841.

Compound **20** (less polar isomer): ^{1}H NMR δ 4.00–3.90 (m, 1H), 3.85–3.75 (m, 1H), 3.42 (s, 3H), 3.35 (d, J = 4 Hz, 1H), 2.93 (d, J = 5 Hz, 1H), 2.68 (d, J = 5 Hz, 1H), 2.69 (d, J = 5 Hz, 1H), 1.95–1.85 (m, 1H), 1.70–1.60 (m, 3H), 0.91 (s, 9H), 0.11 (s, 3H), 0.089 (s, 3H); ^{13}C NMR δ 80.4, 74.3, 70.6, 58.6, 58.0, 51.9, 28.7, 26.6, 26.0, 18.2, −4.4, −4.6; HRMS calcd for $C_{14}H_{29}O_4Si$ (M+H$^+$) 289.1830, found 289.1826.

4.1.14. (3*S,4*R**,5*S**,6*S**)-4,5-Dimethoxy-6-(*tert*-butyldimethylsilyloxy)-1-oxaspiro[2.5]octane.** ^{1}H NMR δ 4.08–3.98 (m, 1H), 3.45 (s, 3H), 3.41 (s, 3H), 3.39–3.35 (m, 1H), 3.35–3.30 (m, 1H), 2.95 (d, J = 5 Hz, 1H), 2.71 (d, J = 5 Hz, 1H), 1.90–1.80 (m, 1H), 1.70–1.50 (m, 3H), 0.91 (s, 9H), 0.11 (s, 3H), 0.09 (s, 3H); ^{13}C NMR δ 83.9, 79.7, 69.1, 59.1, 58.6, 58.3, 52.3, 29.3, 26.5, 26.0, 18.3, −4.54, −4.59; HRMS calcd for $C_{15}H_{31}O_4Si$ (M+H$^+$) 303.1992, found 303.1997.

4.1.15. (3*S,4*R**,5*S**,6*S**)-4-Benzoyloxy-5-methoxy-6-(*tert*-butyldimethylsilyloxy)-1-oxaspiro[2.5]-octane (21).** To a solution of 0.123 g (0.43 mmol) of **19** in 2 mL of pyridine under argon, was added 0.11 mL (0.86 mmol) of benzoyl chloride. After stirring at 40 °C for 15 min, the solution was diluted with diethyl ether, washed with aqueous NH_4Cl, aqueous $NaHCO_3$, water, and brine, dried ($MgSO_4$), concentrated, and column-chromatographed on silica gel using a mixture of hexane and diethyl ether (4:1) as eluant to give 0.164 g (97% yield) of **21**: ^{1}H NMR δ 8.02 (d, J = 7.0 Hz, 2H), 7.55 (t, J = 7.0 Hz, 1H), 7.45 (t, J = 7 Hz, 2H), 5.34 (d, J = 2.9 Hz, 1H), 4.09 (td, J = 6.6, 3.3 Hz, 1H), 3.50 (dd, J = 6.6, 2.9 Hz, 1H), 3.42 (s, 3H), 3.04 (d, J = 5.1 Hz, 1H), 2.68 (d, J = 5.1 Hz, 1H), 2.00–1.90 (m, 1H), 1.82–1.60 (m, 3H), 0.93 (s, 9H), 0.12 (s, 3H), 0.11 (s, 3H); ^{13}C NMR δ 165.7, 133.3, 130.3, 129.9, 128.6, 83.4, 72.1, 69.5, 59.0, 57.7, 52.1, 29.3, 27.0, 26.0, 18.3, −4.5, −4.7; HRMS calcd for $C_{21}H_{32}O_5SiNa$ (M+Na$^+$) 415.1917, found 415.1920.

4.1.16. (3*S,4*R**,5*S**,6*S**)-4-Benzoyloxy-5-methoxy-1-oxaspiro[2.5]-6-octanol (22).** To a cold (0 °C) solution of 0.15 g (0.39 mmol) of **21** in 3 mL of THF under argon, was added 0.78 mL (0.78 mmol) of tetra-*n*-butylammonium fluoride in THF (1 M solution). The solution was stirred at 0 °C for 4 h, diluted with diethyl ether, washed with water, and brine, dried ($MgSO_4$), concentrated, and column-chromatographed on silica gel using a gradient mixture of hexane and diethyl ether as eluant to give 99 mg (92% yield) of **22**: ^{1}H NMR δ 8.02 (d, J = 7 Hz, 2H), 7.55 (t, J = 7 Hz, 1H), 7.45 (t, J = 7 Hz, 2H), 5.09 (d, J = 3 Hz, 1H), 4.18–4.08 (m, 1H), 3.43 (s, 3H), 3.39 (d, J = 3 Hz, 1H), 2.96 (d, J = 4.4 Hz, 1H), 2.73 (d, J = 4.4 Hz, 1H), 2.35–2.25 (m, 1H), 2.22–2.12 (m, 1H), 1.90–1.70 (m, 1H), 1.40–1.20 (m, 1H); ^{13}C NMR δ165.6, 133.6, 130.0, 129.8, 128.7, 83.9, 72.1, 68.7, 57.6, 57.5, 53.2, 28.2, 26.7; HRMS calcd for $C_{15}H_{19}O_5$ (M+H$^+$) 279.1227, found 279.1233.

4.1.17. (3*S,4*R**,5*S**)-4-Benzoyloxy-5-methoxy-1-oxaspiro[2.5]-6-octanone (23).** To a solution of 71 mg (0.25 mmol) of **22** in 2 mL of DMSO under argon, was added 0.29 g (1.0 mmol) of IBX, and the solution

was stirred at 25 °C for 12 h, diluted with diethyl ether, washed with water, and brine, dried ($MgSO_4$), concentrated, and column-chromatographed on silica gel using a gradient mixture of hexane and diethyl ether as eluant to give 66 mg (93% yield) of **23**: ^{1}H NMR δ 7.80–7.96 (m, 2H), 7.56–7.52 (m, 1H), 7.45–7.41 (m, 2H), 5.25 (dd, J = 3.7, 1.8 Hz, 1H), 4.33 (dd, J = 3.7, 1.1 Hz, 1H), 3.48 (s, 3H), 3.21 (d, J = 4.4 Hz, 1H), 2.93 (dd, J = 4.4 Hz, 1H), 2.80–2.76 (m, 1H), 2.66–2.62 (m, 2H), 1.65–1.50 (m, 1H); ^{13}C NMR δ 205.1, 165.4, 133.8, 130.1, 129.3, 128.7, 83.1, 58.5, 57.2, 54.5, 37.1, 29.9, 28.7; HRMS calcd for $C_{15}H_{17}O_5$ ($M+H^+$) 277.1071, found 277.1079.

4.1.18. (3*S,4*R**,5*S**,6*R**)-4-Benzoyloxy-5-methoxy-1-oxaspiro[2.5]-6-octanol (24).** To a cold (−78 °C) solution of 33 mg (0.12 mmol) of **23** in 0.5 mL of THF under argon, was added 0.14 mL (0.14 mmol) of K-Selectride (1 M solution in THF), and the solution was stirred at −78 °C for 2 h, diluted with diethyl ether, washed with water, and brine, dried ($MgSO_4$), concentrated, and column-chromatographed on silica gel using a mixture of hexane and ethyl acetate (3:1) as eluant to give 31 mg (93% yield) of **24**: ^{1}H NMR δ 8.10–8.06 (m, 2H), 7.65–7.61 (m, 1H), 7.48–7.44 (m, 2H), 5.18 (d, J = 3.3 Hz, 1H), 4.20–4.10 (m, 1H), 3.71 (t, J = 3.3 Hz, 1H), 3.50 (s, 3H), 3.02 (d, J = 4.7 Hz, 1H), 2.71 (d, J = 4.7 Hz, 1H), 2.48 (d, J = 5 Hz, 1H), 2.24–2.16 (m, 1H), 2.05–1.90 (m, 2H), 1.50–1.30 (m, 1H); ^{13}C NMR δ 165.7, 133.5, 130.0, 129.8, 128.8, 79.9, 72.5, 68.1, 58.8, 57.7, 52.4, 27.8, 24.8; HRMS calcd for $C_{15}H_{19}O_5$ ($M+H^+$) 279.1227, found 279.1235.

4.1.19. (3*S,4*R**,5*S**,6*R**)-4-Benzoyloxy-5-methoxy-6-(*tert*–butyldimethylsilyloxy)-1-oxaspiro[2.5]-octane (25).** To a cold (0 °C) solution of 40 mg (0.14 mmol) of **24** in 2 mL of DMF under argon, were added 29 mg (0.42 mmol) of imidazole, 2 mg of DMAP, and 33 mg (0.22 mmol) of *tert*-butyldimethylsilyl chloride. The solution was stirred at 0 °C for 8 h, diluted with diethyl ether, washed with water, and brine, dried ($MgSO_4$), concentrated, and column-chromatographed on silica gel using a mixture of hexane and diethyl ether (4:1) as eluant to give 55 mg (98% yield) of **25**: ^{1}H NMR δ 8.06–8.02 (m, 2H), 7.59–7.55 (m, 1H), 7.46–7.42 (m, 2H), 5.24 (d, J = 2.6 Hz, 1H), 4.02–3.92 (m, 1H), 3.74 (t, J = 2.6 Hz, 1H), 3.54 (s, 3H), 3.10 (d, J = 5.1 Hz, 1H), 2.65 (d, J = 5.1 Hz, 1H), 2.10–1.90 (m, 1H), 1.82–1.60 (m, 3H), 0.92 (s, 9H), 0.11 (s, 3H), 0.09 (s, 3H); ^{13}C NMR δ 152.6, 133.4, 130.1, 130.0, 128.6, 82.6, 71.6, 71.2, 61.0, 57.6, 51.3, 28.4, 27.9, 26.0, 18.4, −4.48, −4.53; HRMS calcd for $C_{21}H_{32}O_5SiNa$ ($M+Na^+$) 415.1917, found 415.1916.

4.1.20. (3*S,4*R**,5*S**,6*R**)-5-Methoxy-6-(*tert*-butyldimethylsilyloxy)-1-oxaspiro[2.5]octan-4-o1 (26).** A cold (0 °C) solution of 18 mg (45 μmol) of **25** and 63 mg of potassium carbonate in 2 mL of methanol was stirred for 8 h, diluted with diethyl ether, washed with water, and brine, dried ($MgSO_4$), concentrated, and column-chromatographed on silica gel using a mixture of hexane and diethyl ether (2:1) as eluant to give 13 mg (96% yield) of **26**: ^{1}H NMR δ 4.32–4.26 (m, 1H), 4.20 (d, J = 8 Hz, 1H), 3.44 (s, 3H), 3.42–3.38 (m, 1H), 3.31–3.27 (m, 1H), 2.83 (d, J = 4.4 Hz, 1H), 2.74 (d, J = 4.4 Hz, 1H), 2.59–2.40 (m, 1H), 1.90–1.70 (m, 2H), 1.15–1.02 (m, 1H), 0.92 (s, 9H), 0.15 (s, 3H), 0.12 (s, 3H); ^{13}C NMR δ 79.0, 74.0, 70.5, 59.9, 56.7, 53.2, 29.0, 25.9, 22.3, 18.2, −4.8, −4.9; HRMS calcd for $C_{14}H_{29}O_4Si$ ($M+H^+$) 289.1830, found 289.1824.

Oxidation of **26** with IBX in DMSO gave a 99% yield of **2**, whose NMR spectra are identical to those derived from **18** (*vide supra*).

4.1.21. (3*S,4*R**,5*R**,6*R**)-5-Methoxy-4-[(*E*)-(1′,5′-dimethylhexa-1′,4′-dienyl)]-6-(*tert*-butyldimethylsilyloxy)-1-oxaspiro[2.5]octan-4-ol (28).** Alkenyllithium **27** was prepared by following the reported procedure.[6,8,10,12] To a cold (−78 °C) solution of 21 mg (73 μmol) of **2** in 1 mL of toluene under argon, was added 1.22 mL (0.146 mmol) of **27**, and the solution was stirred at −78 °C for 2 h, warmed to 0 °C, diluted with diethyl ether, washed with water, and brine, dried ($MgSO_4$), concentrated, and column-chromatographed on silica gel using a mixture of hexane and diethyl ether (2:1) as eluant to give 22.1 mg (75% yield) of **28**[8,12]: ^{1}H NMR δ 5.71 (t, J = 7 Hz, 1H), 5.20–5.13 (m, 1H), 4.86 (br s, 1H), 4.50–4.40 (m, 1H), 3.51 (d, J = 2.6 Hz, 1H), 3.45 (s, 3H), 2.81 (d, J = 5.1 Hz, 1H), 2.80–2.70 (m, 2H), 2.60–2.45 (m, 1H), 2.42 (d, J = 5.1 Hz, 1H), 1.9 ∼ 1.75 (m, 2H), 1.67 (s, 6H), 1.61 (s, 3 H), 1.22–1.18 (m, 1H), 0.92 (s, 9H), 0.14 (s, 3H), 0.13 (s, 3H); ^{13}C NMR δ 132.7, 131.7, 127.8, 123.0, 80.6, 79.3, 68.6, 62.2, 57.8, 50.7, 28.7, 27.3, 25.9, 25.8, 25.5, 18.1, 18.0, 14.2, −4.7, −4.8; HRMS calcd for $C_{22}H_{44}O_4SiN$ ($M + NH_4^+$) 414.3040, found 414.3036.

4.1.22. (3*S,4*R**,5*R**,6*R**)-5-Methoxy-4-[(*E*)-(1′,5′-dimethylhexa-1′,4′-dienyl)]-1-oxaspiro[2.5]-octane-4,6-diol (29).**[12] To a cold (0 °C) solution of 12 mg (0.03 mmol) of **28** in 1 mL of THF under argon, was added 60 μL (0.06 mmol) of tetra-*n*-butylammonium fluoride (1 M solution in THF), and the solution was stirred at 0 °C for 6 h, diluted with diethyl ether, washed with water, and brine, dried ($MgSO_4$), concentrated, and column-chromatographed on silica gel using a gradient mixture of hexane and diethyl ether to give 8.4 mg (99% yield) of **29**[12]: ^{1}H NMR δ 5.65 (t, J = 7.0 Hz, 1H), 5.10 (th, J = 7.0, 1.0 Hz, 1H), 4.40–4.30 (m, 1H), 3.61 (d, J = 3.3 Hz, 1H), 3.49 (s, 3H), 3.26 (bs, 1H), 3.15 (br d, J = 7 Hz, 1H), 2.79 (d, J = 5.1 Hz, 1H), 2.75 (t, J = 7 Hz, 2H), 2.45 (d, J = 5.1 Hz, 1H), 2.50–2.35 (m, 1H), 2.10–2.00 (m, 1H), 1.90–1.80 (m, 1H), 1.68 (s, 6H), 1.62 (s, 3H), 1.30–1.20 (m, 1H); ^{13}C NMR δ 133.9, 132.3, 127.5, 122.6, 80.0, 79.9, 67.0, 61.5, 57.8, 50.4, 27.8, 27.3, 25.9, 25.2, 18.0, 14.3; HRMS calcd for $C_{16}H_{30}NO_4$ ($M + NH_4^+$) 300.2169, found 300.2171.

4.1.23. (3*S,4*R**,5*S**)-4-Hydroxy-5-methoxy-4-[(*E*)-(1′,5′-dimethylhexa-1′,4′-dienyl)]-1-oxaspiro[2.5]octan-6-one (30).** A solution of 4.7 mg (17 μmol) of **29** and 19 mg (67 μmol) of IBX in 0.5 mL of DMSO under argon was stirred at 25 °C for 12 h, diluted with diethyl ether, washed with water, and brine, dried ($MgSO_4$), concentrated, and column-chromatographed using a mixture

of hexane and diethyl ether (2:1) to give 4.3 mg (92% yield) of **30**:[12] ^{1}H NMR δ 5.64 (t, J = 7 Hz, 1H), 5.10 (bt, J = 7 Hz, 1H), 4.26 (s, 1H), 3.50 (s, 3H), 2.85 (d, J = 4.9 Hz, 1H), 2.80–2.62 (m, 4H), 2.61 (d, J = 4.9 Hz, 1H), 2.53–2.42 (m, 2H), 1.70 (s, 6H), 1.62 (s, 3H), 1.60–1.48 (m, 1H); ^{13}C NMR δ 207.7, 133.8, 132.6, 127.9, 122.3, 85.9, 83.1, 61.1, 59.7, 51.2, 37.1, 30.5, 27.2, 25.9, 18.0, 14.6; HRMS calcd for $C_{16}H_{28}NO_4$ ($M + NH_4^+$) 298.2018, found 298.2020.

4.1.24. (±)-Ovalicin (1).[8,10,12] To a cold (5 °C) solution of 4.0 mg (14 μmol) of **30** and 1.1 mg (4.3 μmol) of $VO(Acac)_2$ in 0.2 mL of benzene, was added 4.0 μL (28 μmol) of *t*-BuOOH. The resulting brown solution was stirred at 25 °C for 1.5 h and subjected to column-chromatographic separation using a mixture of hexane and diethyl ether (2:1) as eluant to give 2.6 mg (62% yield) of (±)-ovalicin:[12] ^{1}H NMR δ 5.18 (t, J = 6.6 Hz, 1H), 4.23 (s, 1H), 3.57 (s, 3 H), 3.14 (s, 1H), 3.10 (d, J = 4.2 Hz, 1H), 2.90 (t, J = 6.3 Hz, 1H), 2.73 (d, J = 4.2 Hz, 1H), 2.72–2.62 (m, 2H), 2.51–2.47 (m, 1H), 2.45–2.39 (m, 1H), 2.18–2.12 (m, 1H), 1.75 (s, 3H), 1.66 (s, 3H), 1.45–1.41 (m, 1H), 1.37 (s, 3H); ^{13}C NMR δ 206.8, 135.7, 118.2, 86.3, 78.8, 60.7, 60.5, 59.5, 57.0, 51.5, 36.9, 30.5, 27.2, 25.9, 18.2, 14.6; HRMS calcd for $C_{16}H_{24}O_5Na$ ($M+Na^+$) 319.1522, found 319.1515.

4.1.25. 3-Methylene-6-(benzoyloxy)cyclohexene (31). To a cold (0 °C) solution of 0.64 g (5.8 mmol) of alcohol **9** and 4.8 mL (35 mmol) of triethylamine under argon, was added 2.6 mL (15 mmol) of benzoyl chloride, and the solution was slowly allowed to warm to 25 °C and stirred for 6 h, diluted with diethyl ether, washed with 1 N HCl, aqueous sodium bicarbonate, and brine, dried (anhydrous Na_2SO_4), concentrated, and column-chromatographed on silica gel using a mixture of hexane and diethyl ether (3:1) as eluant to give 1.09 g (88% yield) of **31**: ^{1}H NMR δ 8.14–8.10 (m, 2H), 7.57–7.53 (m, 1H), 7.45–7.41 (m, 2H), 6.32 (d, J = 9.6 Hz, 1H), 5.90 (dd, J = 9.6, 3.6 Hz, 1H), 5.61 (q, J = 3.6 Hz, 1H), 4.95 (s, 2H), 2.70–2.50 (m, 1H), 2.47–2.43 (m, 1H), 2.21–2.11 (m, 1H), 1.02–1.92 (m, 1H); ^{13}C NMR δ 166.3, 141.5, 133.3, 133.0, 130.2, 129.7, 128.4, 127.5, 113.9, 68.7, 28.5, 26.9; HRMS calcd for $C_{14}H_{15}O_2$ ($M+H^+$) 215.1067, found 215.1070.

4.1.26. (3*R,8*R**) and (3*R**,8*S**)-6-(Benzoyloxy)-1-oxaspiro[2.5]oct-4-ene (32 and 33).** To a cold (0 °C) solution of 1.0 g (4.7 mmol) of diene **31** in 25 mL of dichloromethane under argon, were added 0.33 g (7.9 mmol) of sodium fluoride, 0.19 g (3.3 mmol) of potassium fluoride, and 1.2 g (7.0 mmol) of *meta*-chloroperbenzoic acid (MCPBA). The mixture was stirred at 25 °C for 4 h, diluted with diethyl ether, washed with aqueous sodium thiosulfate, aqueous sodium bicarbonate, and brine, dried (anhydrous Na_2SO_4), and concentrated to give 1.0 g (93% yield) of a mixture of **32** and **33** (1:1; based on integration in ^{1}H NMR spectrum), which was used in the following step without further purification. ^{1}H NMR δ (for one isomer) 8.08–8.04 (m, 2H), 7.59–7.55 (m, 1H), 7.47–7.43 (m, 2H), 6.18 (d, J = 11 Hz, 1H), 5.66–5.60 (m, 1H), 5.50 (t, J = 11 Hz, 1H), 2.90 (d, J = 5 Hz, 1H), 2.88 (d, J = 5 Hz, 1H), 2.86–2.96 (m, 2H), 1.83–2.34 (m, 2H); ^{1}H NMR δ (for another isomer) 8.08–8.04 (m, 2H), 7.59–7.55 (m, 1H), 7.47–7.43 (m, 2H), 6.16 (d, J = 11 Hz, 1H), 5.64–5.56 (m, 1H), 5.50 (t, J = 11 Hz, 1H), 2.95 (d, J = 5 Hz, 1H), 2.86 (d, J = 5 Hz, 1H), 2.86–2.96 (m, 2H), 1.83–2.34 (m, 2H); ^{13}C NMR (two isomers) δ 166.3, 166.2, 133.6, 133.3, 133.29, 133.2, 130.5, 133.0, 132.8, 130.4, 129.9, 129.8, 128.6, 128.59, 68.3, 67.7, 55.2, 55.19, 55.1, 55.09, 27.4, 27.39, 27.3, 27.29; HRMS calcd for $C_{14}H_{15}O_3$ ($M+H^+$) 231.1016, found 231.1022.

4.1.27. (3*S,4*R**,5*S**,6*S**)-6-(Benzoyloxy)-1-oxaspiro[2.5]-octane-4,5-diol (34), (3*S**,4*S**,5*R**,6*R**)-6-(benzoyloxy)-1-oxaspiro[2.5]octane-4,5-diol (35), and (3*S**,4*S**,5*R**,6*S**)-6-(benzoyloxy)-1-oxaspiro[2.5]octane-4,5-diol (36).** To a solution of 1.00 g (4.3 mmol) of epoxides **32** and **33** (1:1) in 80 mL of acetone and water (3:1) and 3.5 mL of *tert*-butanol, were added 1.64 g (14.0 mmol) of NMO and 0.24 mg (0.93 mmol) of OsO_4. The solution was stirred at 25 °C for 12 h, and acetone was removed by vacuum distillation. The remaining solution was extracted with diethyl ether, and the organic layer was washed with water, and brine, dried (anhydrous Na_2SO_4), concentrated, and column-chromatographed on silica gel using a gradient mixture of hexane and diethyl ether as eluant to give 0.28 g (25% yield) of **34**, 0.23g (20% yield) of **35**, and 57 mg (5% yield) of **36**. Stereochemistry of **36** was determined from a NMR 2D NOESY experiment.

Compound **34**: ^{1}H NMR δ 8.08–8.04 (m, 2H), 7.61–7.57 (m, 1H), 7.48–7.44 (m, 2H), 5.35 (td, J = 7.6, 3.6 Hz, 1H), 4.11 (dd, J = 7.6, 3.6 Hz, 1H), 3.73 (d, J = 3.6 Hz, 1H), 3.21 (s, 1H), 2.94 (d, J = 4.8 Hz, 1H), 2.76 (d, J = 4.8 Hz, 1H), 2.2–2.0 (m, 2H), 1.9–1.6 (m, 2H); ^{13}C NMR δ 166.6, 133.3, 129.8, 129.7, 128.4, 73.0, 72.8, 72.6, 58.7, 51.7, 26.0, 25.7; HRMS calcd for $C_{14}H_{17}O_5$ ($M+H^+$) 265.1076, found 265.1081.

Compound **35**: ^{1}H NMR δ 8.07–8.03 (m, 2H), 7.61–7.57 (m, 1H), 7.48–7.44 (m, 2H), 5.41 (td, J = 7.6, 4 Hz, 1H), 4.00 (dd, J = 7.6, 3.2 Hz, 1H), 3.86 (d, J = 3.2 Hz, 1H), 2.88 (d, J = 5 Hz, 1H), 2.65 (d, J = 5 Hz, 1H), 2.3–1.7 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 166.5, 133.5, 130.0, 129.9, 128.7, 73.6, 73.1, 72.8, 51.9, 50.6, 26.0, 24.6; HRMS calcd for $C_{14}H_{17}O_5$ ($M+H^+$) 265.1076, found 265.1069.

Compound **36**: ^{1}H NMR ($CDCl_3$) δ 8.07 (d, J = 7 Hz, 2H), 7.58 (t, J = 7 Hz, 1H), 7.46 (t, J = 7 Hz, 2H), 5.23 (dt, J = 9.2, 4 Hz, 1H, C6H), 4.38 (br s, 1H), 3.83 (d, J = 2.5 Hz, 1H), 3.07 (d, J = 5 Hz, 1H, C2H), 2.71 (d, J = 5 Hz, 1H, C2H), 2.27–2.17 (m, 1H), 2.25–1.75 (m, 1H), 1.60–1.56 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 166.3, 133.5, 130.3, 130.0, 128.7, 72.9, 71.3, 71.1, 58.9, 51.1, 26.7, 24.7; HRMS calcd for $C_{14}H_{17}O_5$ ($M+H^+$) 265.1076, found 265.1080.

4.1.28. (3*S,4*R**,5*S**,6*S**)-6-Benzoyloxy-4-hydroxy-5-(*tert*-butyldimethylsilyloxy)-1-oxaspiro[2.5]-octane (37).** To a cold (0 °C) solution of 0.26 g (0.98 mmol) of diol **34** and 25 mg (0.20 mmol) of DMAP in 10 mL dichloromethane under argon, were added 0.28 mL (2.0 mmol)

of Et_3N and 0.23 g (1.5 mmol) of *t*-butyldimethylsilyl chloride. The solution was stirred at 25 °C for 6 h, diluted with aqueous NH_4Cl, and extracted with ethyl acetate. The organic layer was washed with 1 N HCl, aqueous $NaHCO_3$, and brine, dried (anhydrous Na_2SO_4), concentrated, and column-chromatographed on silica gel using a mixture of hexane and diethyl ether (2:1) as eluant to give 0.196 g (53% yield) of **37**: ^{1}H NMR δ 8.05 (d, J = 7 Hz, 2H), 7.58 (t, J = 7 Hz, 1H), 7.45 (t, J = 7 Hz, 2H), 5.36 (td, J = 8, 4.4 Hz, 1H), 3.93 (td, J = 8, 3.2 Hz, 1H), 3.69 (d, J = 3.2 Hz, 1H), 2.91 (d, J = 4.8 Hz, 1H), 2.75 (d, J = 4.8 Hz, 1H), 2.34 (s, 1H), 2.18–2.12 (m, 1H), 2.12–2.08 (m, 1H), 1.95–1.85 (m, 1H), 1.55–1.49 (m, 1H), 0.83 (s, 9H), 0.10 (s, 3H), 0.02 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 165.9, 133.2, 130.0, 129.8, 128.5, 73.6, 73.4, 72.9, 58.7, 51.8, 26.3, 25.8, 25.7, 18.0, −4.6, −4.7; HRMS calcd for $C_{20}H_{30}O_5SiNa$ ($M+Na^+$) 401.1760, found 401.1758.

Crystallization of **37** in diethyl ether gave white crystals, mp 121–123 °C, whose structure was determined by a single-crystal X-ray analysis (Fig. 1).[22]

4.1.29. (3*S,4*R**,5*S**,6*S**)-6-Benzoyloxy-4-hydroxy-5-methoxy-1-oxaspiro[2.5]octane (38) and (3*S**,4*R**,5*S**,6*S**)-6-benzoyloxy-5-hydroxy-4-methoxy-1-oxaspiro[2.5]octane (39).** To a cold (0 °C) solution of 0.19 g (0.74 mmol) of diol **34** in 3 mL of dichloromethane under argon, were added 0.31 g (1.5 mmol) of proton sponge and 0.11 g (0.73 mmol) of Me_3OBF_4, and the solution was stirred at 0 °C for 7 h. To it, 20 mg (0.14 mmol) of Me_3OBF_4 was added, and the solution was stirred at 0 °C for 1.5 h, diluted with diethyl ether, washed with 1 N HCl, aqueous sodium dicarbonate, and brine, dried ($MgSO_4$), concentrated, and column-chromatographed on silica gel using a mixture of hexane and ethyl acetate (3:2) as eluant to give 93 mg (46% yield) of compound **38** and 69 mg (34% yield) of **39**.

Compound **38**: ^{1}H NMR δ 8.08–8.04 (m, 2H), 7.61–7.57 (m, 1H), 7.49–7.45 (m, 2H), 5.43 (td, J = 8, 4 Hz, 1H), 3.77 (dd, J = 8, 4 Hz, 1H), 3.68 (dd, J = 7, 4 Hz, 1H), 3.49 (s, 3H), 2.93 (d, J = 4.8 Hz, 1H), 2.76 (d, J = 4.8 Hz, 1H), 2.51 (d, J = 7 Hz, 1H), 2.15–2.09 (m, 2H), 1.85–1.79 (m, 1H), 1.60–1.52 (m, 1H); ^{13}C NMR δ 166.0, 133.2, 129.9, 129.8, 128.6, 81.8, 71.3, 71.3, 58.8, 58.7, 51.9, 26.1, 26.09; HRMS calcd for $C_{15}H_{19}O_5$ ($M+H^+$) 279.1227, found 279.1223.

Compound **39**: ^{1}H NMR δ 8.09–8.05 (m, 2H), 7.58–7.54 (m, 1H), 7.47–7.43 (m, 2H), 5.32 (td, J = 9, 4 Hz, 1H), 3.99 (td, J = 9, 4 Hz, 1H), 3.46 (s, 3H), 3.18 (d, J = 4 Hz, 1H), 2.92 (d, J = 4.8 Hz, 1H), 2.81 (d, J = 4.8 Hz, 1H), 2.51 (br s, 1H), 2.20–2.14 (m, 2H), 1.90–1.80 (m, 1H), 1.43–1.37 (m, 1H); ^{13}C NMR δ 166.6, 133.2, 130.0, 129.4, 128.6, 83.0, 73.3, 72.8, 58.4, 57.2, 52.7, 26.3, 26.2; HRMS calcd for $C_{15}H_{19}O_5$ ($M+H^+$) 279.1227, found 279.1236.

4.1.30. (3*S,5*R**,6*S**)-6-(Benzoyloxy)-5-methoxy-1-oxaspiro[2.5]-4-octanone (40).** To a cold (−78 °C) solution of 14 mg (0.17 mmol) of DMSO in 1.5 mL of dichloromethane under argon was added 30 mg (0.15 mmol) of trifluoroacetic anhydride, and the solution was stirred for 0.5 h. To it, a solution of 16 mg (58 μmol) of **38** in 1 mL of dichloromethane was added, and the solution was stirred at −78 °C for 1 h, warmed to 0 °C, diluted with diethyl ether, washed with aqueous NH_4Cl, and brine, dried (anhydrous Na_2SO_4), concentrated, and column-chromatographed on silica gel using a mixture of hexane and ethyl acetate (1:1) as eluant to give 13 mg (83% yield) of compound **40**: ^{1}H NMR δ 7.99 (d, J = 8 Hz, 2H), 7.58 (t, J = 8 Hz 1H), 7.45 (t, J = 8 Hz, 2H), 5.5 (td, J = 6, 3 Hz, 1H), 3.88 (d, J = 6 Hz, 1H), 3.41 (s, 3H), 3.01 (d, J = 6 Hz, 1H), 2.9 (d, J = 6 Hz, 1H), 2.50–2.38 (m, 2H), 2.28–2.18 (m, 1H), 1.82–1.74 (m, 1H); ^{13}C NMR δ 203, 165, 137, 133.5, 129.7, 128.6, 83.9, 72.7, 59.0, 54.04, 29.9, 27.3, 24.4; HRMS calcd for $C_{15}H_{17}O_5$ ($M+H^+$) 277.1071, found 277.1077.

4.1.31. (3*S,4*S**,5*R**,6*S**)-6-Benzoyloxy-5-methoxy-4-[(*E*)-(1′,5′-dimethylhexa-1′,4′-dienyl)]-1-oxaspiro[2.5]octan-4-ol (41) and (3*S**,4*S**,5*R**,6*S**)-5-methoxy-4-[(*E*)-(1′,5′-dimethylhexa-1′,4′-dienyl)]-1-oxaspiro[2.5]-4,6-octanediol (42).** To a cold (−78 °C) solution of 49 mg (0.18 mmol) of ketone **40** in 0.5 mL of diethyl ether and 0.5 mL of toluene under argon, was added 2.7 mL (0.36 mmol) of alkenyllithium **27**, and the solution was stirred for 1 h, and slowly warmed to 25 °C. The reaction solution was diluted with aqueous NH_4Cl and extracted with CH_2Cl_2. The organic layer was washed with brine, dried (anhydrous Na_2SO_4), concentrated, and column-chromatographed on silica gel using a gradient mixture of hexane and ethyl acetate as eluant to give 28 mg (40% yield) of **41** and 18 mg (26% yield) of **42**.

Compound **41**: ^{1}H NMR δ 8.08 (d, J = 9 Hz, 2H), 7.59 (t, J = 9 Hz, 1H), 7.56 (t, J = 9 Hz, 2H), 6.13 (t, J = 7 Hz, 1H), 5.58 (td, J = 8, 4 Hz, 1H), 5.14 (t, J = 7 Hz, 1H), 3.60 (s, 3H), 3.56 (d, J = 8 Hz, 1H), 3.3 (d, J = 5 Hz, 1H), 2.82 (d, J = 5 Hz, 1H), 2.82–2.74 (m, 2H), 2.25–2.15 (m, 1H), 1.714 (s, 3H), 1.712 (s, 3H), 1.65 (s, 3H); HRMS calcd for $C_{23}H_{34}NO_5$ ($M + NH_4^+$) 404.2431, found 404.2435.

Compound **42**: ^{1}H NMR δ 5.90 (t, J = 7 Hz, 1H, C2′H), 5.11 (t, J = 7 Hz, 1H, C4′H), 3.89 (td, J = 10, 4 Hz, 1H), 3.68 (s, 3H), 3.16 (d, J = 10 Hz, 1H), 3.14 (d, J = 5 Hz, 1H), 2.74 (d, J = 5 Hz, 1H), 2.68 (bs, 1H), 2.27 (s, 1H), 2.07–1.97 (m, 2H), 1.72 (s, 3H), 1.69 (s, 3H), 1.63 (s, 3H), 1.38–1.25 (m, 1H), 1.25–1.15 (m, 1H); ^{13}C NMR δ 132.8, 132.5, 131.1, 122.5, 93.5, 70.8, 62.7, 62.4, 55.8, 51.3, 28.9, 28.0, 27.5, 25.9, 18.1, 13.7; HRMS calcd for $C_{16}H_{30}NO_4$ ($M + NH_4^+$) 300.2169, found 300.2173.

Treatment of **41** with 5 equiv of K_2CO_3 in methanol afforded a 95% yield of compound **42**.

4.1.32. (3*S,4*S**,5*S**)-4-Hydroxy-5-methoxy-4-[(*E*)-(1′,5′-dimethylhexa-1′,4′-dienyl)]-1-oxaspiro[2.5]-6-octanone (43).** To a solution of 7 mg (25 μmol) of **42** in 1 mL DMSO under argon was added 14 mg (50 μmol) of IBX, and the solution was stirred at 25 °C for 5 h, diluted with aqueous NH_4Cl, and extracted with diethyl ether. The organic layer was washed with brine, dried

(anhydrous Na_2SO_4), concentrated, and column-chromatographed on silica gel using a mixture of hexane and diethyl ether (1:1) as eluant to give 4 mg (58% yield) of **43**: ^{1}H NMR δ 5.76 (t, J = 7 Hz, 1H), 5.02 (t, J = 7 Hz, 1H), 4.01 (s, 1H), 3.58 (s, 3H), 3.43 (d, J = 5 Hz, 1H), 2.91 (d, J = 5 Hz, 1H), 2.75–2.67 (m, 2H), 2.54–2.46 (m, 1H), 2.42 (s, 1H), 2.25–2.15 (m, 2H), 1.67 (s, 3H, CH_3), 1.63 (s, 3H), 1.59 (s, 3H), 1.45 (ddd, J = 14, 7, 2 Hz, 1H); ^{13}C NMR δ 209.2, 132.5, 132.3, 131.4, 121.7, 91.0, 79.2, 61.5, 61.1, 52.2, 36.8, 27.5, 27.2, 25.8, 18.1, 12.5; HRMS calcd for $C_{16}H_{28}NO_4$ ($M + NH_4^+$) 298.2018, found 298.2015.

4.1.33. (3*S,4*S**,5*S**,1′*S**,2′R)-4-Hydroxy-5-methoxy-4-[2′-methyl-3′-(3″-methylbut-2″-enyl)oxiran-2′-yl-1-oxaspiro[2.5]-6-octanone (44).** To a cold (0 °C) solution of 3.5 mg (13 μmol) of **43** in 0.5 mL of toluene under argon, were added 0.3 mL (2.5 μmol) of vanadyl acetoacetonate (19 mM solution in toluene) and 0.15 mL (20 μmol) of *tert*-butylhydroperoxide in toluene (0.1 mL of *t*-BuOOH in 10 mL of toluene). The solution was stirred at 0 °C for 3 h, subjected to a silica gel column, and eluted with a mixture of hexane and ethyl acetate (1:1) to give 2.4 mg (65% yield) of **44**: ^{1}H NMR δ 5.10 (t, J = 8 Hz, 1H), 3.85 (s, 1H), 3.62 (t, J = 6 Hz, 1H), 3.51 (s, 3H), 3.38 (d, J = 5 Hz, 1H), 2.95 (d, J = 5 Hz, 1H), 2.74–2.66 (m, 1H), 2.62 (s, 1H, OH), 2.58–2.48 (m, 2H), 2.34–2.26 (m, 1H), 2.18–2.08 (m, 1H), 1.714 (s, 3H), 1.711 (s, 3H), 1.32 (s, 3H), 1.57–1.50 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 212.5, 130.9, 128.0, 88.4, 86.5, 61.0, 60.4, 59.5, 53.0, 51.8, 36.5, 29.9, 27.7, 25.6, 22.2, 13.4; HRMS calcd for $C_{16}H_{24}O_5Na$ ($M+Na^+$) 319.1522, found 319.1518.

Crystallization of the material from diethyl ether gave white crystals, mp 116–118 °C, which was used in a single-crystal X-ray analysis (Fig. 2).[22]

4.1.34. (3*S,4*S**,6*S**)-4-Methoxy-6-(*tert*-butyldimethylsilyloxy)-1-oxaspiro[2.5]octan-5-one (45).** To a solution of 31 mg (0.11 mmol) of **20** in 1 mL of DMSO under argon, was added 0.18 g (0.65 mmol) of IBX, and the solution was stirred at 25 °C for 12 h, diluted with diethyl ether, washed with water, and brine, dried ($MgSO_4$), concentrated, and column-chromatographed on silica gel using a mixture of hexane and diethyl ether (2:1) as eluant to give 28 mg (90% yield) of **45**: ^{1}H NMR δ 4.34 (dd, J = 5.4, 3.4 Hz, 1H), 4.25 (s, 1H), 3.43 (s, 3H), 3.02 (d, J = 5.2 Hz, 1H), 2.57 (d, J = 5.2 Hz, 1H), 2.40–2.32 (m, 1H), 2.02–1.94 (m, 1H), 1.82–1.74 (m, 1H), 1.70–1.62 (m,1H), 0.93 (s, 9H), 0.10 (s, 6H); ^{13}C NMR δ 206.0, 83.2, 75.1, 61.6, 59.5, 49.9, 30.4, 26.7, 25.9, 18.3, −4.67, −4.90; HRMS calcd for $C_{14}H_{26}O_4SiNa$ ($M+Na^+$) 309.1498, found 309.1506.

4.1.35. (3*S,4*S**,5*R**,6*S**)-4-Methoxy-5-[(*E*)-(1′, 5′-dimethylhexa-1′,4′-dienyl)–6-(*tert*-butyldimethylsilyloxy)-1-oxaspiro[2.5]octan-5-ol (46).** To a cold (−78 °C) solution of 25 mg (87 μmol) of **45** in 1 mL of toluene under argon, was added 1.5 mL (0.17 mmol) of alkenyllithium **27**. The solution was stirred at −78 °C for 2 h, warmed to 0 °C, diluted with diethyl ether, washed with water, and brine, dried ($MgSO_4$), concentrated, and column-chromatographed on silica gel using a mixture of hexane and diethyl ether (2:1) as eluant to give 25 mg (72% yield) of **46**: ^{1}HNMR δ 5.75 (td, J = 7.0, 1.1 Hz, 1H, C2′H), 5.18–5.08 (m, 1H, C4′H), 4.28 (dd, J = 10, 5 Hz, 1H, C6H), 3.29 (s, 3H, OMe), 2.75 (d,J = 4.8 Hz, 1H, C2H), 2.78–2.70 (m, 2H, C3′H), 2.73 (s, 1H, C4H), 2.62 (d, J = 4.8 Hz, 1H, C2H), 2.28 (s, 1H, OH), 2.26 ∼ 1.84 (m, 3H), 1.76 (s, 3H, CH_3C=), 1.67 (s, 3H, CH_3C=), 1.62 (s, 3H, CH_3C=), 1.24–1.16 (m, 1H), 0.84 (s, 9H), 0.08 (s, 3H), 0.07 (s, 3H); ^{13}C NMR δ 138.1, 131.5, 126.1, 123.2, 88.5, 78.4, 70.1, 59.2, 56.4, 50.5, 28.0, 27.4, 26.6, 25.9, 25.87, 18.2, 17.9, 14.4, −3.11, −4.76; HRMS calcd for $C_{22}H_{44}O_4SiN$ ($M + NH_4^+$) 414.3040, found 414.3031.

The stereochemistry was determined from a 2D NOESY NMR spectrum of **46**.

4.2. Culturing of parasites

The bloodstream form of *Trypanosoma brucei* 427 strain was maintained under the standard cell culture conditions (37 °C, 5% CO_2). The parasites were grown in complete HMI-9 medium containing 10% FBS, 10% Serum Plus and 1 × Penicillin/Streptomycin.[20,21]

4.3. Luciferase assay[20,21]

Luciferase assay was used to measure ATP-bioluminescence in *T. brucei* cultured in 96-well plates at 37 °C for 48 h. Parasites were diluted to 1.0×10^5 cells/mL in complete HMI-9 medium. One hundred microliters (100 μL) of the diluted parasites were aliquoted into sterile 96-well flat white opaque culture plates (Greiner). Each compound was serially diluted from 10 μM to 0.1 μM in DMSO and then mixed in the appropriate wells containing parasites. The treated parasites were then incubated for 48 h at 37 °C with 5% CO_2 before monitoring viability. To measure the viability of the parasites after treatment with each compound, the parasites were lysed in the wells by adding 100 μL of CellTiter-Glo™ (Promega). After lysis, the ATP-bioluminescence of the 96-well plates was measured with a SpectraFluor Plus multidetection plate reader (Tecan).

Acknowledgments

D.H.H. gratefully acknowledges financial support from the National Science Foundation (CHE-0555341 and NSF43529), National Institutes of Health (National Institute of Aging, R01AG025500), American Heart Association, Heartland Affiliate (0750115Z), and American Chemical Society PRF (40345-AC). We thank Dr. James H. McKerrow at University of California in San Francisco for advice and support.

References and notes

1. Horn, D. *Curr. Mol. Med.* **2004**, *4*, 563.
2. Welburn, S. C.; Maudlin, I. *Parasitol. Today* **1999**, *15*, 399.

3. Bollinger, P.; Sigg, H.-P.; Weber, H.-P. *Helv. Chim. Acta* **1973**, *56*, 819.
4. Zhang, P.; Nicholson, D. E.; Bujnicki, J. M.; Su, X.; Brendle, J. J.; Ferdig, M.; Kyle, D. E.; Milhous, W. K.; Chiang, P. K. *J. Biomed. Sci.* **2002**, *9*, 34.
5. Hua, D. H.; Tamura, M.; Egi, M.; Werbovetz, K.; Delfin, D.; Salem, M.; Chiang, P. K. *Bioorg. Med. Chem.* **2003**, *11*, 4357.
6. Corey, E. J.; Dittami, J. P. *J. Am. Chem. Soc.* **1985**, *107*, 256.
7. Corey, E. J.; Guzman-Perez, A.; Noe, M. C. *J. Am. Chem. Soc.* **1994**, *116*, 12109.
8. Barton, D. H. R.; Bath, S.; Billington, D. C.; Gero, S. D.; Quiclet-Sire, B.; Samadi, M. *J. Chem. Soc., Perkin Trans. 1* **1995**, 1551.
9. Barco, A.; Benetti, S.; De Risi, C.; Marchetti, P.; Pollini, G. P.; Zanirato, V. *Tetrahedron: Asymmetry* **1998**, *9*, 2857.
10. Takahashi, S.; Hishinuma, N.; Koshino, H.; Nakata, T. *J. Org. Chem.* **2005**, *70*, 10162.
11. Yamaguchi, J.; Toyoshima, M.; Shoji, M.; Kakeya, H.; Osada, H.; Hayashi, Y. *Angew. Chem. Int. Ed.* **2006**, *45*, 789.
12. Tiefenbacher, K.; Arion, V. B.; Mulzer, J. *Angew. Chem. Int. Ed.* **2007**, *46*, 2690.
13. Marek, I.; Alexakis, A.; Normant, J. F. *Tetrahedron Lett.* **1991**, *32*, 5329.
14. Marek, I.; Meyer, C.; Normant, J.-F. *Org. Syn.* **1997**, *74*, 194.
15. Korbe, S.; de Meijere, A.; Labahn, T. *Helv. Chim. Acta* **2002**, *85*, 3161.
16. Compound **3** was synthesized previously via a different route from (+)-limonene oxide. Evarts, J. B.; Fuchs, P. L. *Tetrahedron Lett.* **2001**, *42*, 3673.
17. Yang, D.; Wong, M.-K.; Yip, Y.-C. *J. Org. Chem.* **1995**, *60*, 3887.
18. Nicolaou, K. C.; Montagnon, T.; Baran, P. S.; Zhong, Y. L. *J. Am. Chem. Soc.* **2002**, *124*, 2245.
19. Urones, J. G.; Marcos, I. S.; Perez, B. G.; Lithgow, A. M.; Gomez, D. D. P. M.; Basabe, P.; Garrido, N. M. *Tetrahedron* **1995**, *51*, 1845.
20. Rapp, M.; Haubrich, T. A.; Perrault, J.; Mackey, Z. B.; McKerrow, J. H.; Chiang, P. K.; Wnuk, S. F. *J. Med. Chem.* **2006**, *49*, 2096.
21. Chiang, P. K.; Bujnicki, J. M.; Su, X. Z.; Lanar, D. E. *Curr. Mol. Med.* **2006**, *6*, 309.
22. The authors have deposited atomic coordinates for compounds **37** and **44** with the Cambridge Crystallographic Data Centre. The coordinates can be obtained on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.