Electronic Supplementary Information for

Hydrophilic azaspiroalkenes as robust bioorthogonal reporters

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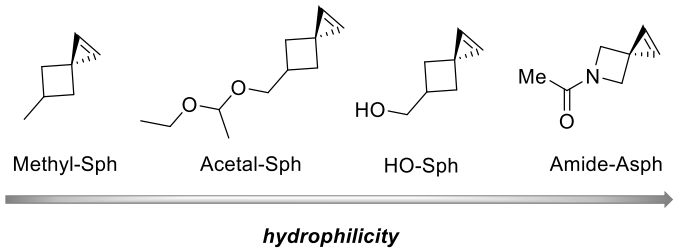
Table of Contents

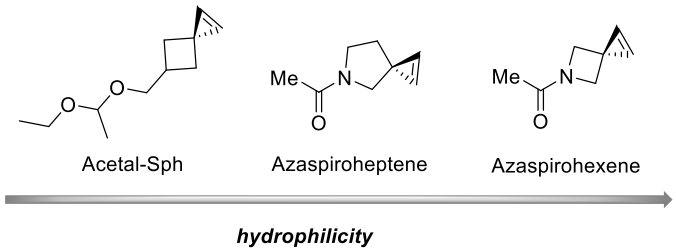
Table S1. Determination of R_f values for azaspirohexene and spirohexene derivatives in TLC. [a]



Structure	**Acetal- Sph**	**HO-Sph**	**Amide-Asph**
R_f value	>0.9	0.3	<0.1

[a] An eluent of 30% ethyl acetate/hexanes was used in TLC analysis.

Table S2. Determination of Log *P* values for spiroalkenes. [a]



Spiroalkene	**Acetal- Sph**	**Azaspiroheptene**	**Azaspirohexene**
Log *P*	0.13	-0.12	-0.95

[a] The partition coefficients in 1-octanol/H_2O (1:1) were determined using LC-MS for quantification. See experimental procedure for details.

Table S3. Crystal data and structure refinement for compound **1f**.

	Single crystal of **1f**
CCDC	1864586
Empirical formula	$C_9H_9N_3O$
Formula weight	175.19
Temperature/K	90
Crystal system	orthorhombic
Space group	Pnma
a/Å	6.1464(11)
b/Å	6.8359(12)
c/Å	19.339(3)
α/°	90
β/°	90
γ/°	90
Volume/Å^3	812.6(2)
Z	4
ρ_{calc}g/cm^3	1.432
μ/mm^{-1}	0.099
F(000)	368.0
Crystal size/mm^3	0.16 × 0.1 × 0.01
Radiation	MoKα (λ = 0.71073)
2Θ range for data collection/°	4.212 to 61.146
Index ranges	$-8 \le h \le 8$, $-9 \le k \le 9$, $-26 \le l \le 27$
Reflections collected	12301
Independent reflections	1339 [R_{int} = 0.0609, R_{sigma} = 0.0305]
Data/restraints/parameters	1339/0/76
Goodness-of-fit on F^2	1.124
Final R indexes [I>=2σ (I)]	R_1 = 0.0501, wR_2 = 0.1100
Final R indexes [all data]	R_1 = 0.0672, wR_2 = 0.1155
Largest diff. peak/hole / e Å^{-3}	0.44/-0.31

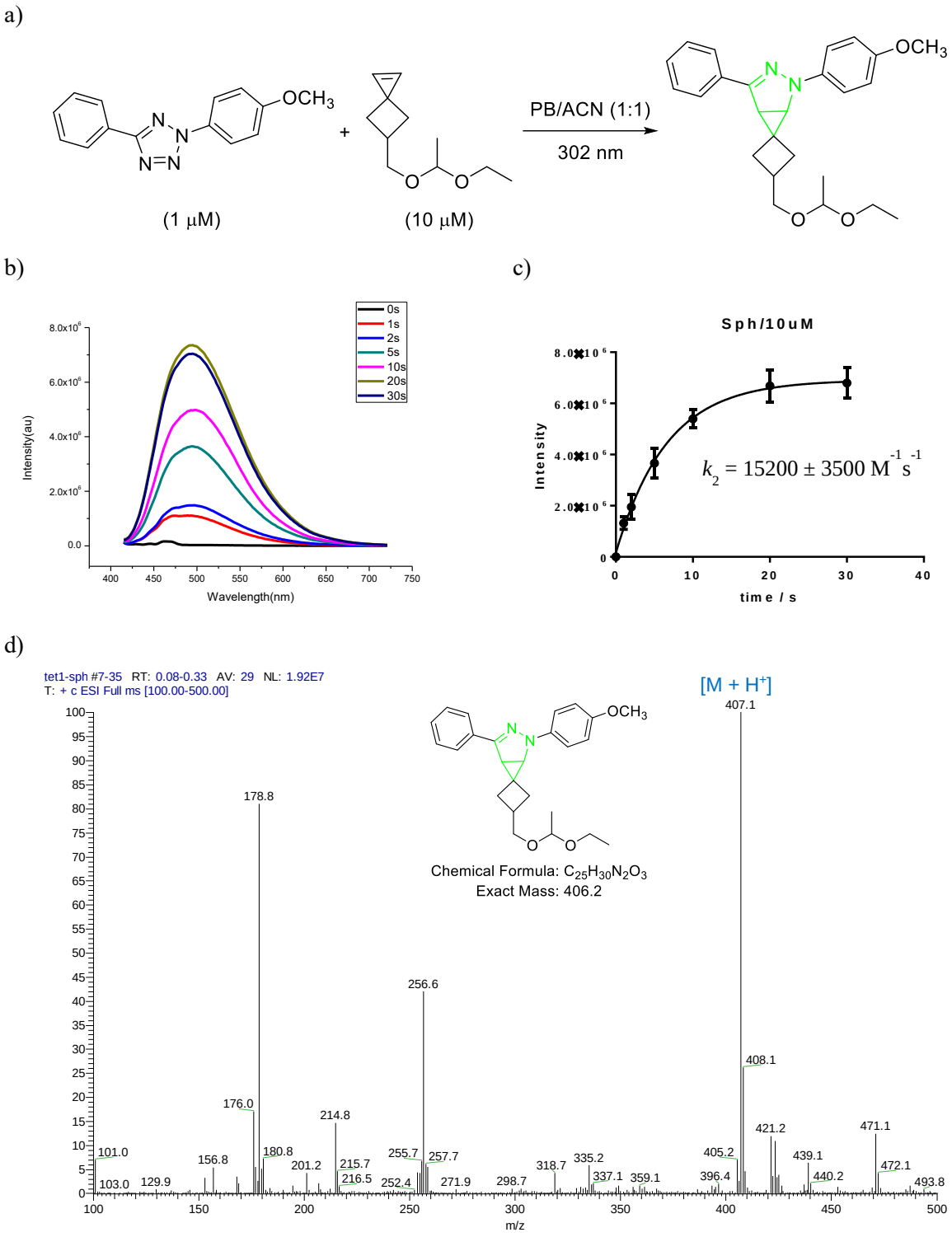


Fig. S1. Fluorescence-based kinetics measurement of the cycloaddition between **Tet-1** (1 μM) and spirohexene (10 μM) in phosphate buffer/acetonitrile (1:1) under 302 nm photoirradiation. (**a**) Reaction scheme. (**b**) Time course of the cycloaddition reaction monitored by a spectrofluorometer (λ_{ex} = 405 nm). (**c**) Plots of relative fluorescence intensity vs. reaction time. The amounts of pyrazoline adducts were fitted to an exponential rise to maximum equation, $y = (y_0\text{-}a)\ e^{-kt} + a$, to give k. The second-order rate constant, k_2, was calculated using the following equation: k_2 = k/[dipolarophile]. Measurements were repeated three times at each time point to derive the mean and standard deviation. (**d**) LC-MS analysis of the reaction mixture to verify the formation of the desired pyrazoline adduct.

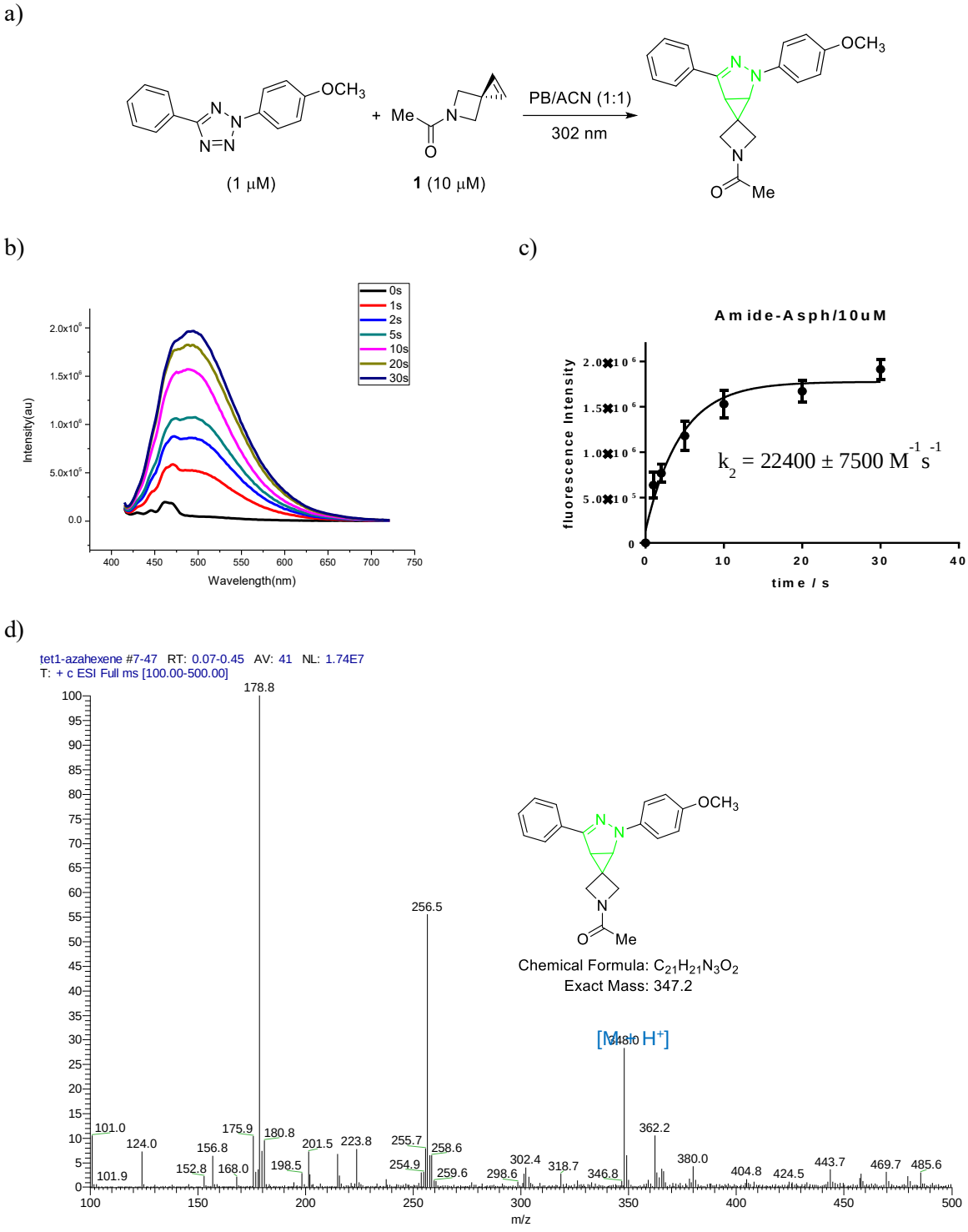


Fig. S2. Fluorescence-based kinetics measurement of the cycloaddition between **Tet-1** (1 μM) and azaspirohexene (10 μM) in phosphate buffer/acetonitrile (1:1) under 302 nm photoirradiation. (**a**) Reaction scheme. (**b**) Time course of the cycloaddition reaction between **Tet-1** and azaspirohexene monitored by a spectrofluorometer (λ_{ex} = 405 nm). (**c**) Plots of relative fluorescence intensity vs. reaction time. The amounts of pyrazoline adducts were fitted to an exponential rise to maximum equation, $y = (y_0\text{-}a)\ e^{-kt} + a$, to give k. The second-order rate constant, k_2, was calculated using the following equation: k_2 = k/[dipolarophile]. Measurements were repeated three times at each time point to derive the mean and standard deviation. (**d**) LC-MS analysis of the reaction mixture to verify the formation of the desired pyrazoline adduct.

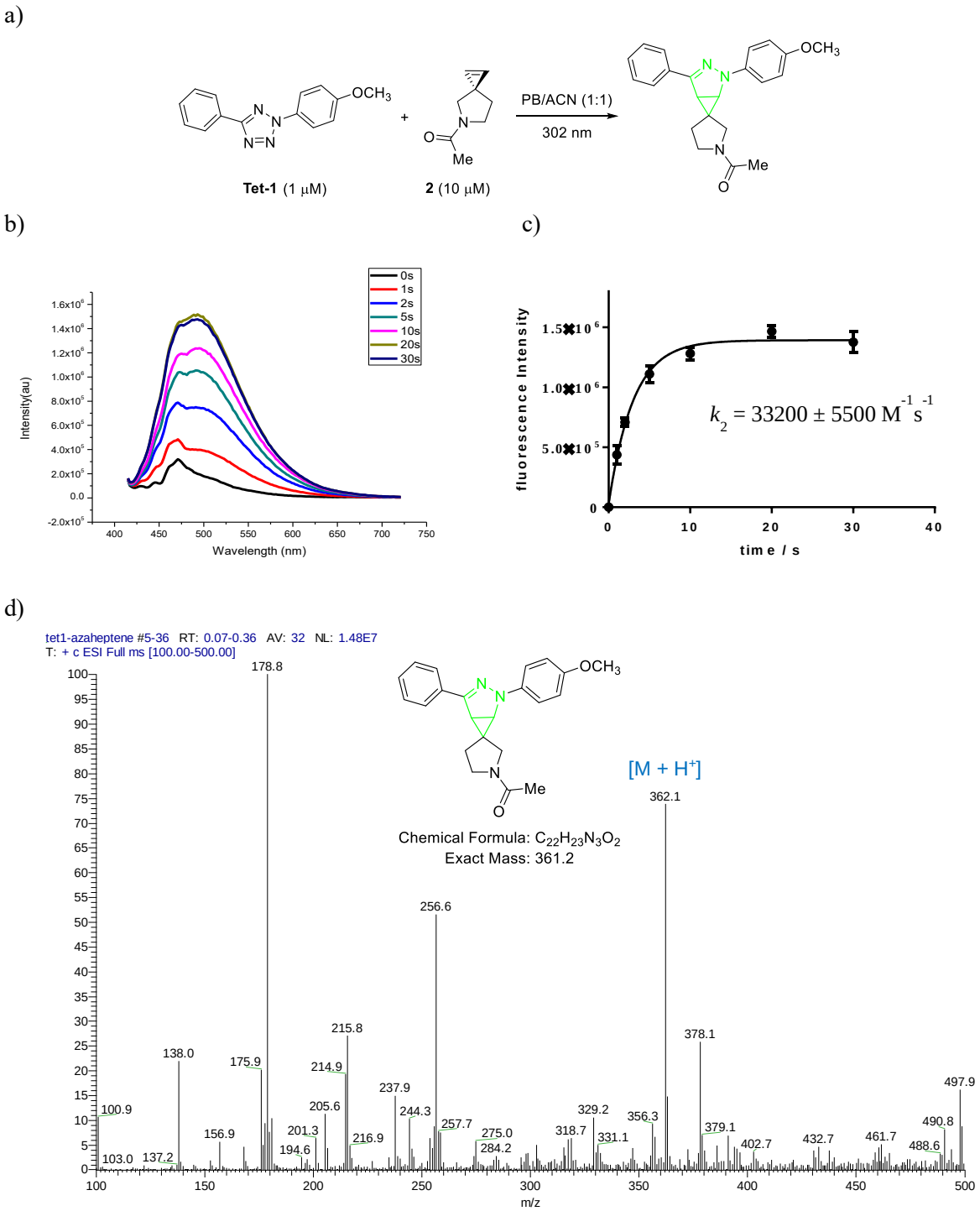
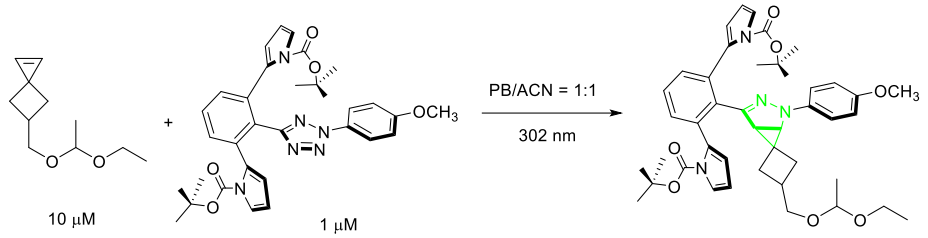


Fig. S3. Fluorescence-based kinetics measurement of the cycloaddition between **Tet-1** (1 μM) and azaspiroheptene (10 μM) in phosphate buffer/acetonitrile (1:1) under 302 nm photoirradiation. (**a**) Reaction scheme. (**b**) Time course of the cycloaddition reaction between **Tet-1** and azaspiroheptene monitored by a spectrofluorometer (λ_{ex} = 405 nm). (**c**) Plots of relative fluorescence intensity vs. reaction time. The amounts of pyrazoline adducts were fitted to an exponential rise to maximum equation, $y = (y_0\text{-}a)\ e^{-kt} + a$, to give k. The second-order rate constant, k_2, was calculated using the following equation: k_2 = k/[dipolarophile]. Measurements were repeated three times at each time point to derive the mean and standard deviation. (**d**) LC-MS analysis of the reaction mixture to verify the formation of the desired pyrazoline adduct.

a)



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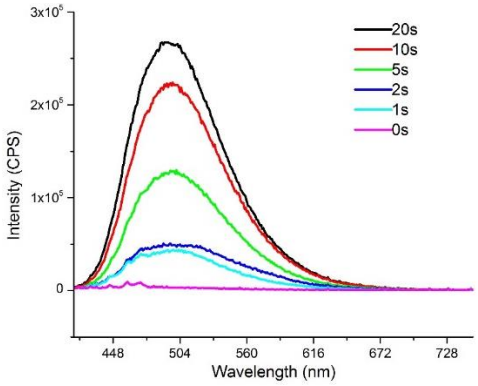

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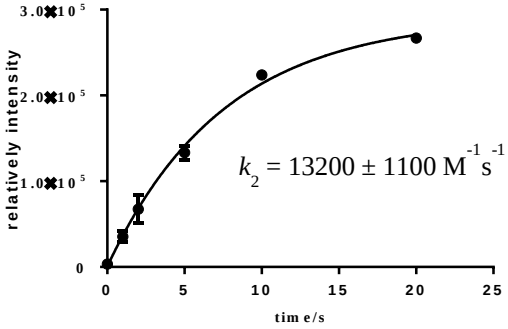

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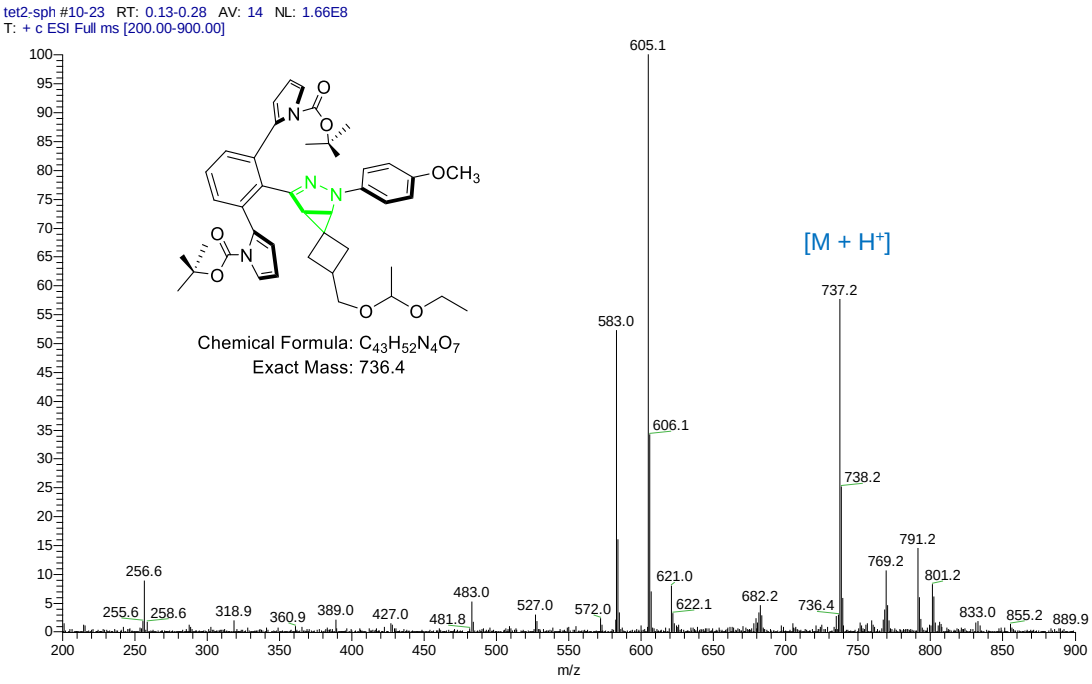


Fig. S4. Fluorescence-based kinetics measurement of the cycloaddition reaction between **Tet-2** (1 μM) and spirohexene (10 μM) in phosphate buffer/acetonitrile (1:1) under 302 nm photoirradiation. (**a**) Reaction scheme. (**b**) Time course of the cycloaddition reaction monitored by a spectrofluorometer (λ_{ex} = 405 nm). (**c**) Plots of relative fluorescence intensity vs. reaction time. The amounts of pyrazoline adducts were fitted to an exponential rise to maximum equation, $y = (y_0\text{-}a)\, e^{-kt} + a$, to give k. The second-order rate constant, k_2, was calculated using the following equation: k_2 = k/[dipolarophile]. Measurements were repeated three times at each time point to derive the mean and standard deviation. (**d**) LC-MS analysis of the reaction mixture to verify the formation of the desired pyrazoline adduct.

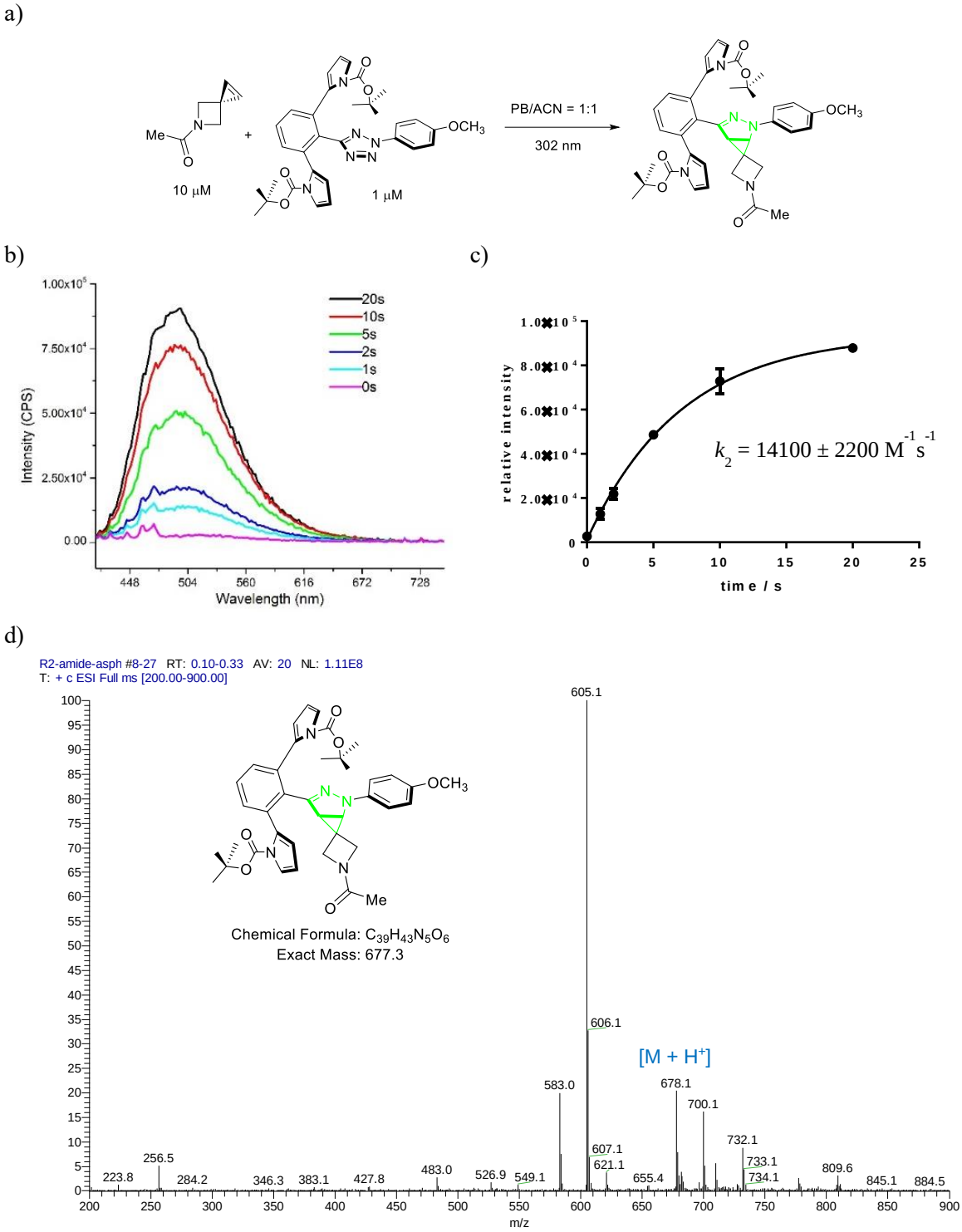
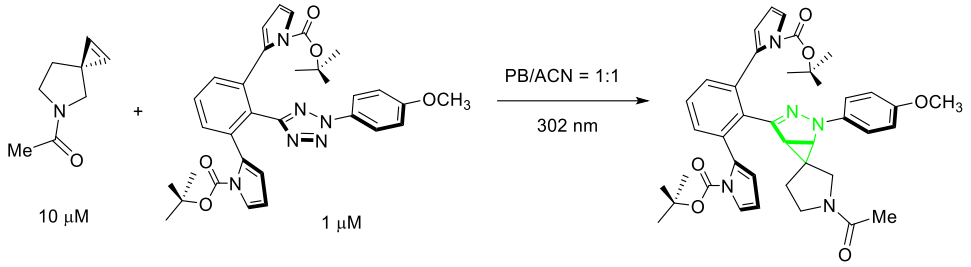


Fig. S5. Fluorescence-based kinetics measurement of the cycloaddition between **Tet-2** (1 μM) and azaspirohexene (10 μM) in phosphate buffer/acetonitrile (1:1) under 302 nm photoirradiation. (**a**) Reaction scheme. (**b**) Time course of the cycloaddition reaction monitored by a spectrofluorometer (λ_{ex} = 405 nm). (**c**) Plots of relative fluorescence intensity vs. reaction time. The amounts of pyrazoline adducts were fitted to an exponential rise to maximum equation, $y = (y_0\text{-}a)\, e^{-kt} + a$, to give k. The second-order rate constant, k_2, was calculated using the following equation: k_2 = k/[dipolarophile]. Measurements were repeated three times at each time point to derive the mean and standard deviation. (**d**) LC-MS analysis of the reaction mixture to verify the formation of the desired pyrazoline adduct.

a)



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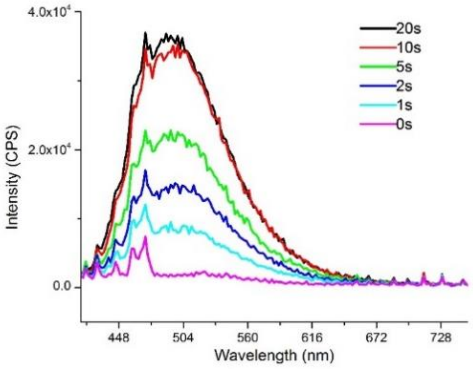

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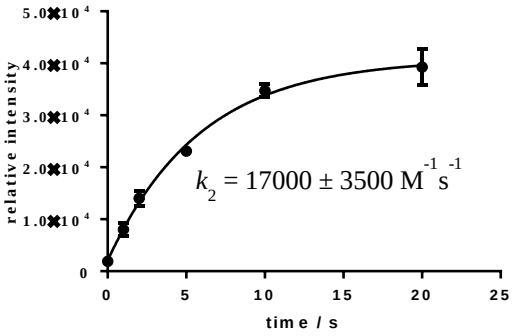

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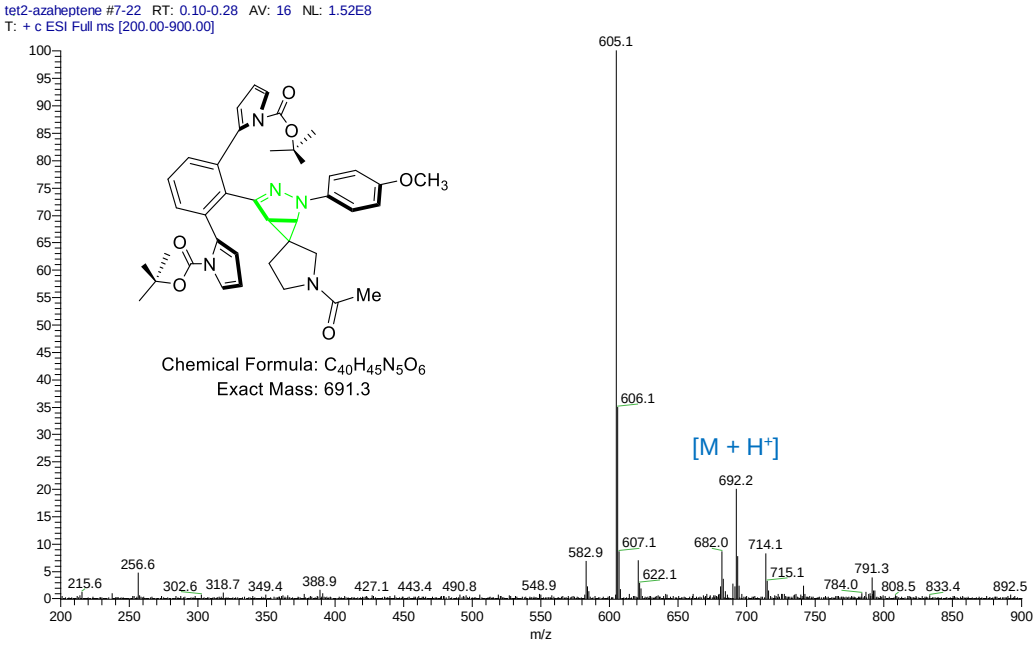


Fig. S6. Fluorescence-based kinetics measurement of the cycloaddition between **Tet-2** (1 μM) and azaspiroheptene (10 μM) in phosphate buffer/acetonitrile (1:1) under 302 nm photoirradiation. (**a**) Reaction scheme. (**b**) Time course of the cycloaddition reaction monitored by a spectrofluorometer (λ_{ex} = 405 nm). (**c**) Plots of relative fluorescence intensity vs. reaction time. The amounts of pyrazoline adducts were fitted to an exponential rise to maximum equation, $y = (y_0\text{-}a)\, e^{-kt} + a$, to give k. The second-order rate constant, k_2, was calculated using the following equation: k_2 = k/[dipolarophile]. Measurements were repeated three times at each time point to derive the mean and standard deviation. (**d**) LC-MS analysis of the reaction mixture to verify the formation of the desired pyrazoline adduct.

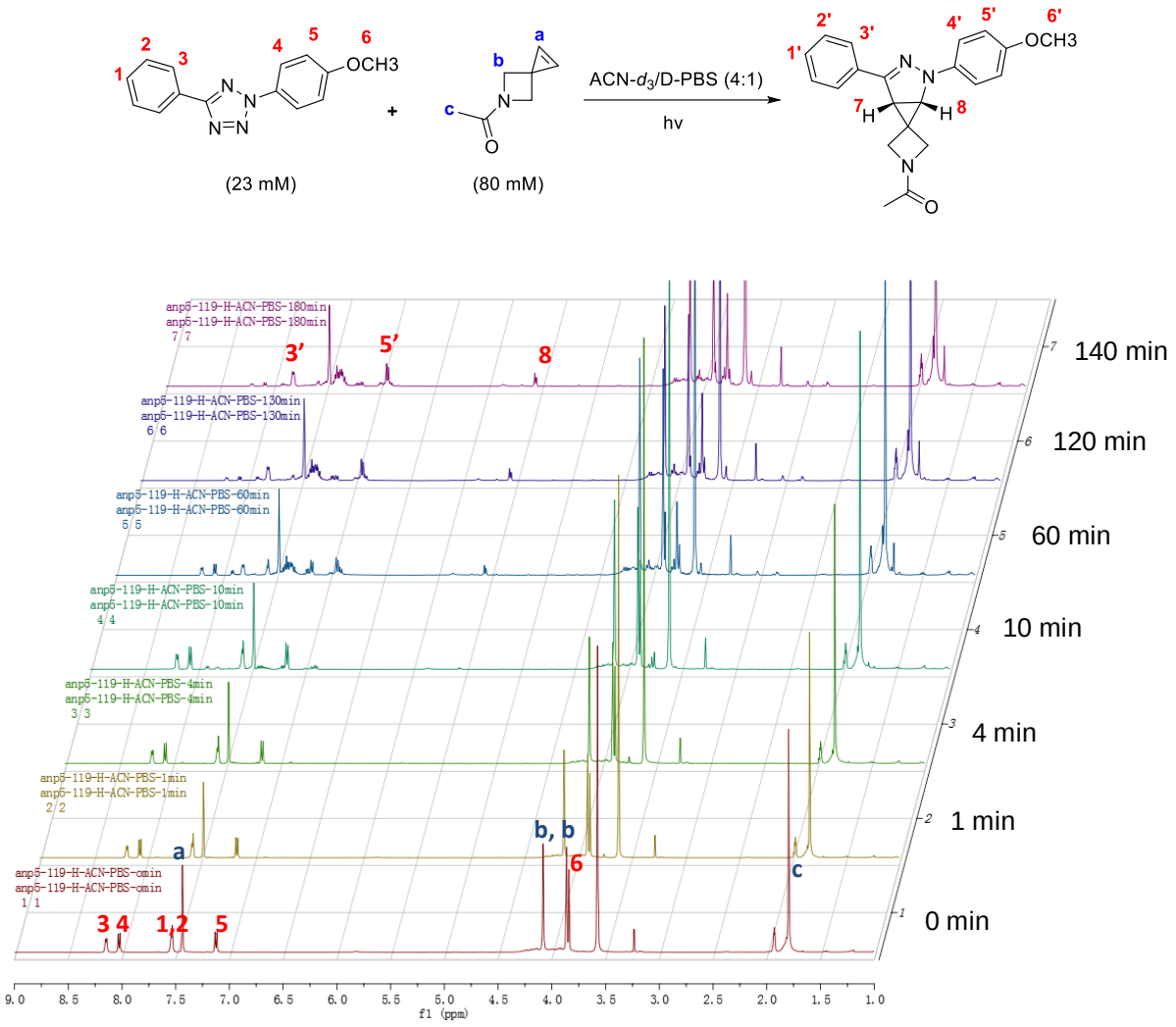


Fig. S7. The cycloaddition reaction between **Tet-1** and azaspirohexene in mixed CD_3CN/deuterated phosphate-buffered saline (4:1) monitored by ^1H NMR. The samples were irradiated with a handheld 302 nm UV lamp and the spectra were recorded immediately after the indicated time of photoirradiation. The pyrazoline product was confirmed by LC-MS.

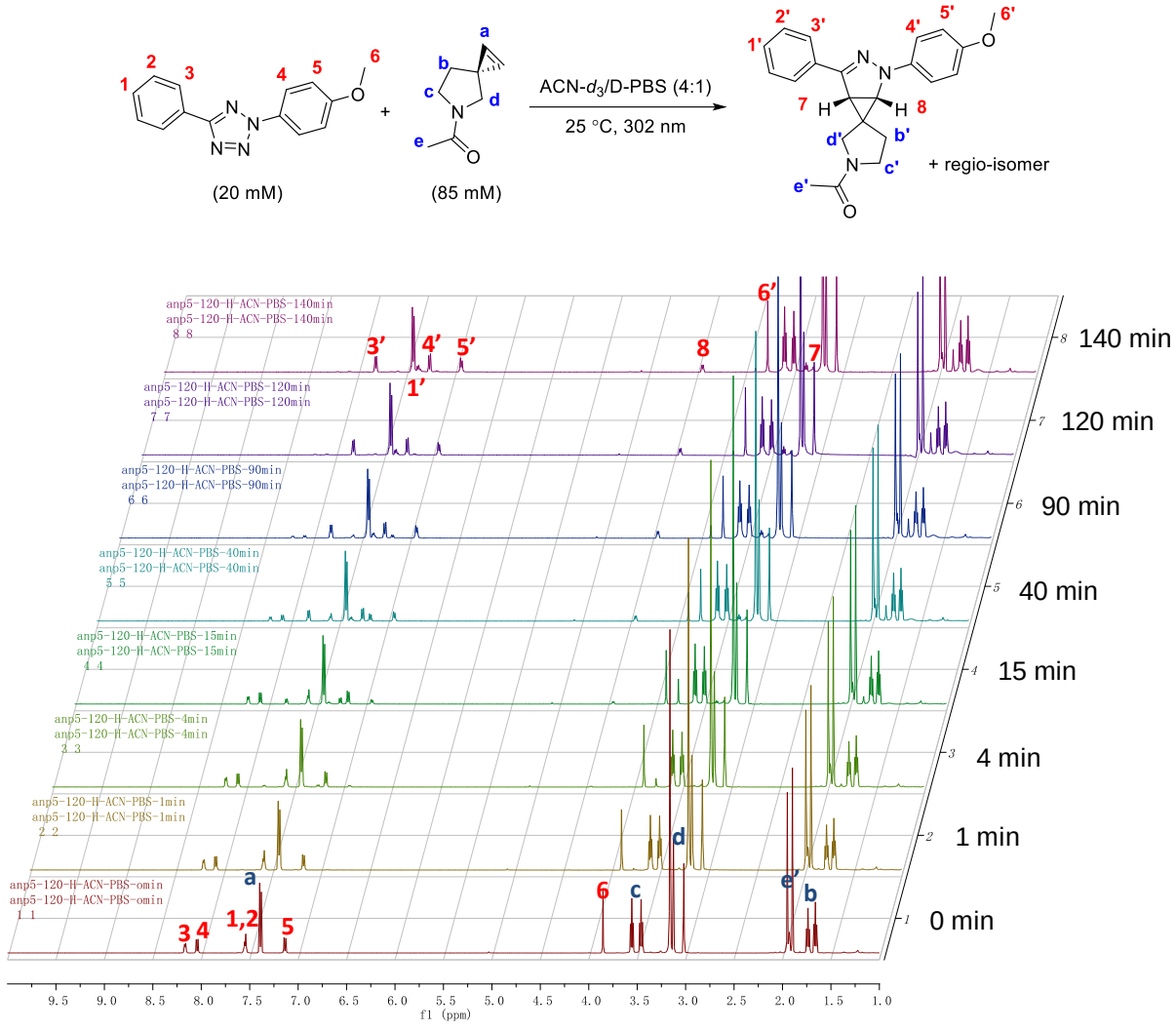


Fig. S8. The cycloaddition reaction between **Tet-1** and azaspiroheptene in mixed CD_3CN/deuterated phosphate-buffered saline (4:1) monitored by ^1H NMR. The samples were irradiated with a handheld 302 nm UV lamp and the spectra were recorded immediately after the indicated time of photoirradiation. The pyrazoline product was confirmed by LC-MS.

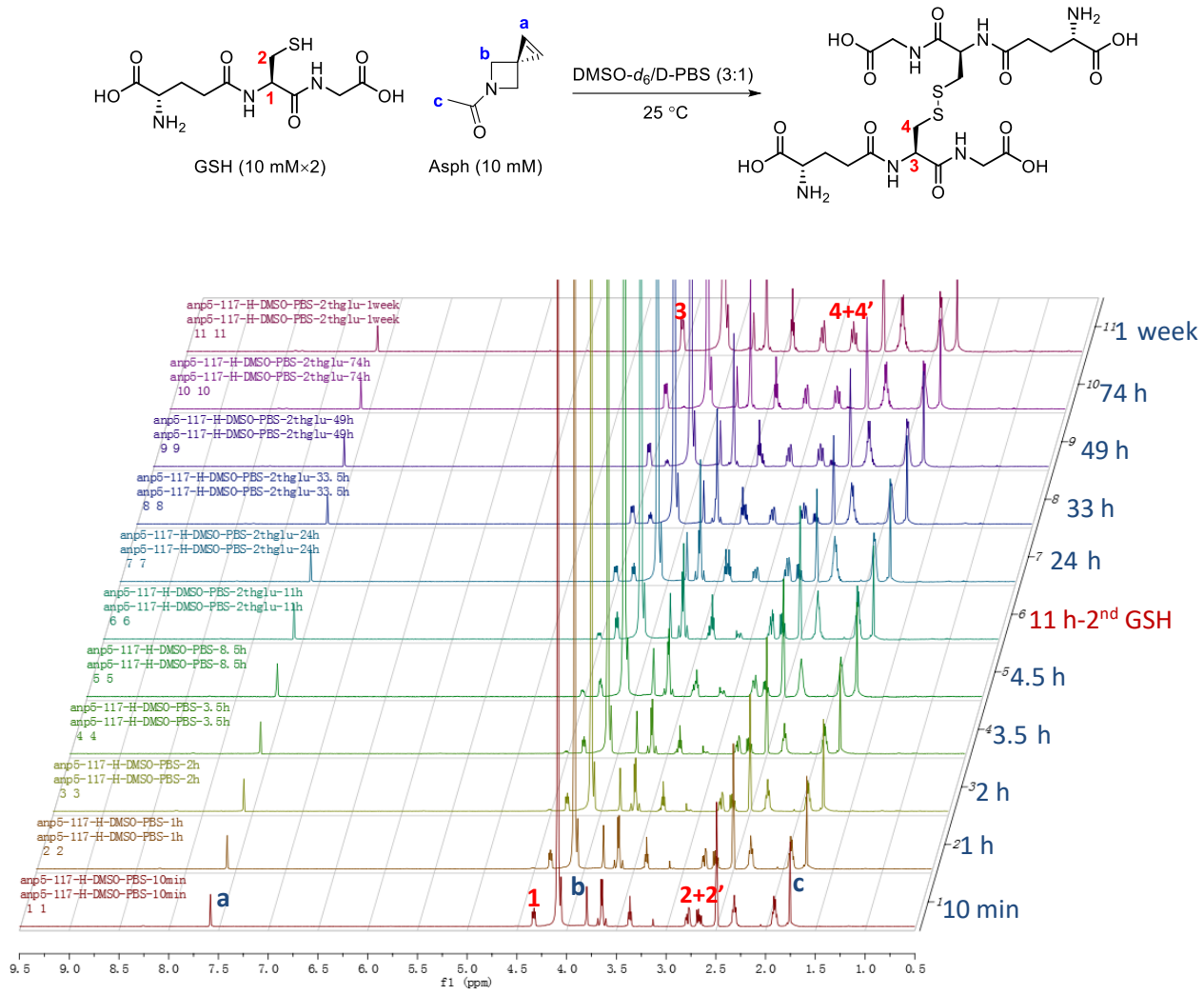


Fig. S9. Stability of azaspirohexene towards glutathione at room temperature in mixed DMSO-d_6/deuterated PBS buffer (3:1) as monitored by ^{1}H NMR. Conditions: 10 mM azaspirohexene was incubated with 10 mM GSH (reduced form) in deuterated solvent. The formation of GSH dimer was observed in ^{1}H NMR and confirmed by LC-MS. The second batch of GSH was added after 11 hours to ensure enough GSH in solution. Azaspirohexene remained intact after the prolonged incubation.

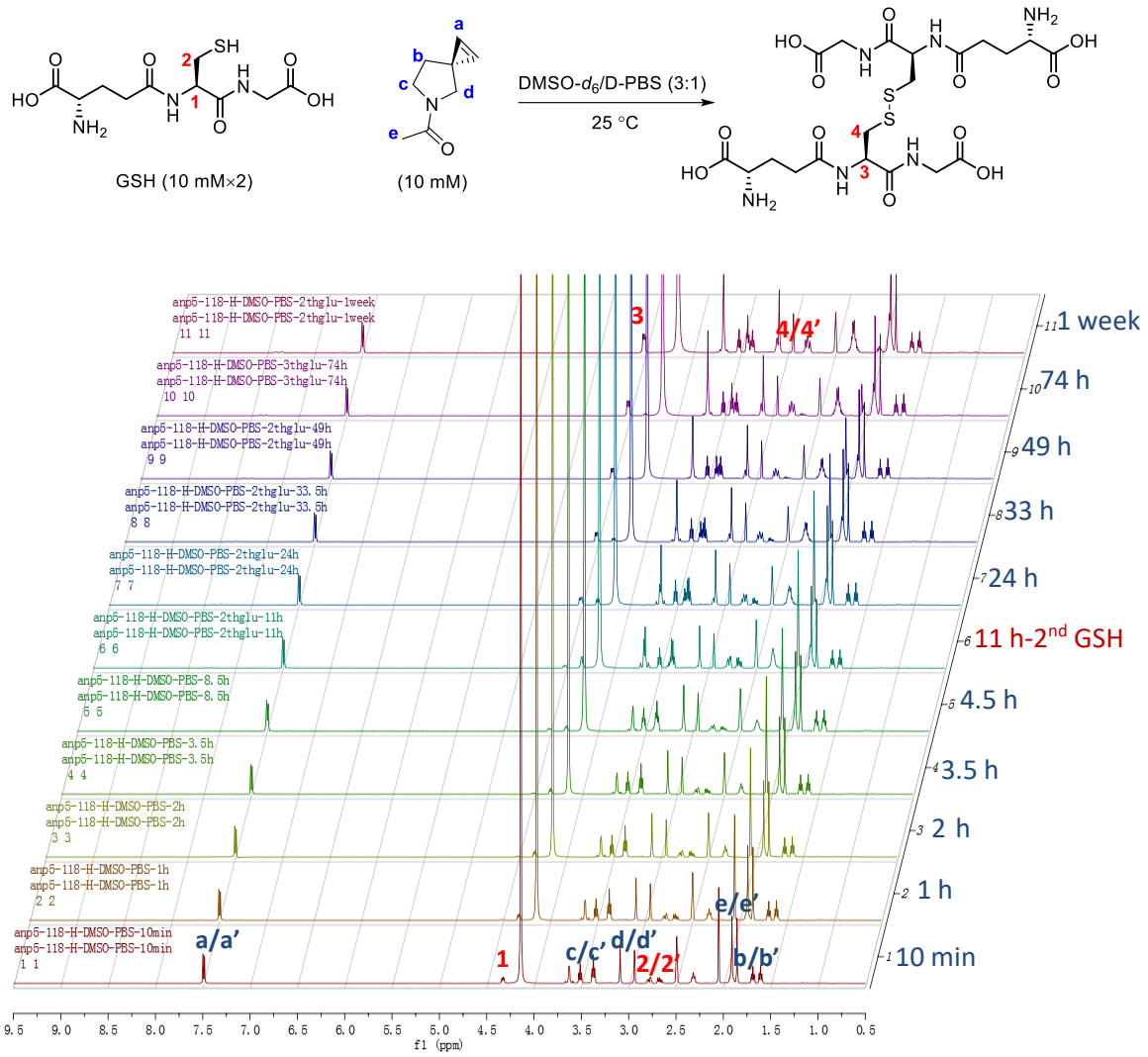
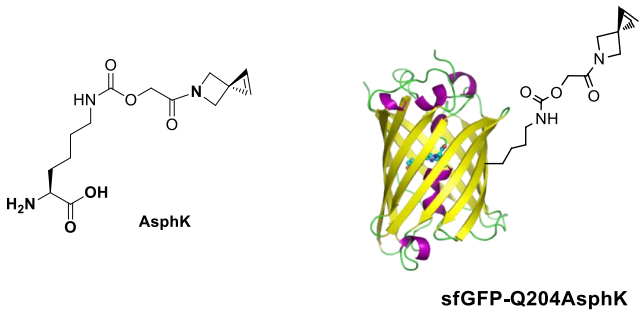


Fig. S10. Stability of azaspiroheptene towards glutathione at room temperature in mixed DMSO-d_6/deuterated PBS buffer (3:1) as monitored by ^{1}H NMR. Conditions: A solution of 10 mM azaspiroheptene was incubated with 10 mM GSH (reduced form) in deuterated solvent. The formation of GSH dimer was observed in ^{1}H NMR and confirmed by LC-MS. The second batch of GSH was added after 11 hours to ensure enough GSH in solution. Azaspiroheptene remained intact after the prolonged incubation.

a)



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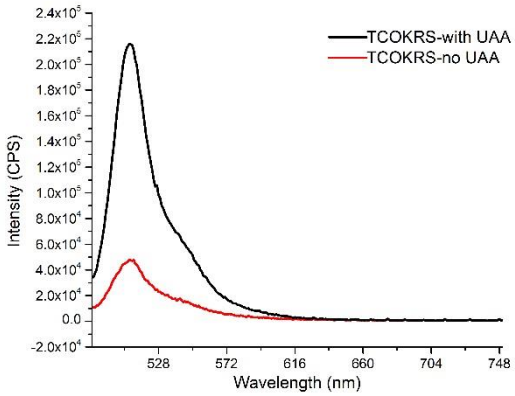

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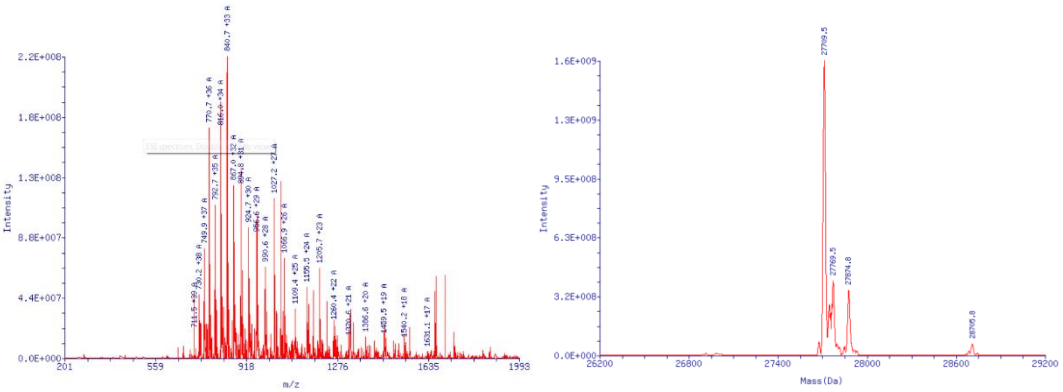


Fig. S11. Site-specific incorporation of AsphK into sfGFP-Q204TAG in *E. coli*. (**a**) Structures of AsphK and AsphK-encoded sfGFP. (**b**) Assessment of AsphK incorporation into sfGFP-Q204TAG by fluorescence spectra; λ_{ex} = 480 nm. The fluorescence lysates from bacterial cells expressing sfGFP-Q204TAG in the presence or absence of 1 mM AsphK was measured directly. (**c**) The charge ladder and the de-convoluted mass of sfGFP-Q204AsphK: calcd 27875.1, found 27874.8. The glutamine-incorporation product sfGFP-Q204 was also observed: calcd 27709.1 found 27709.3.

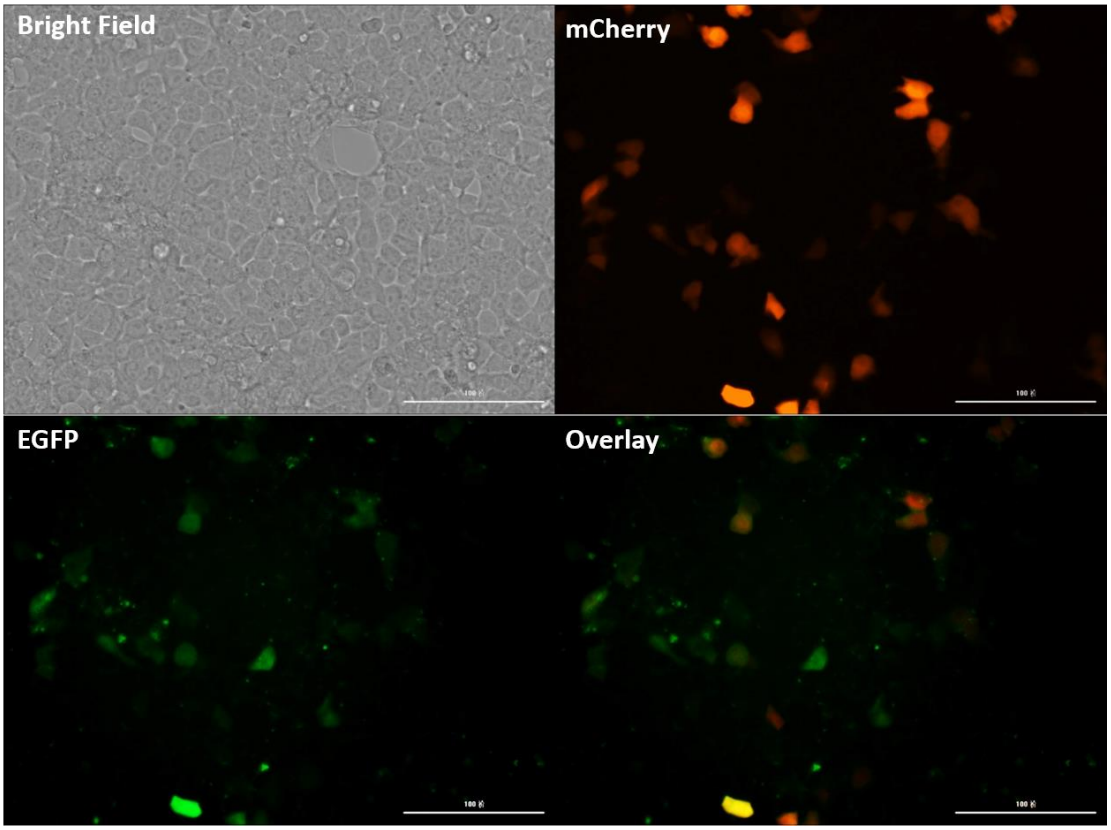


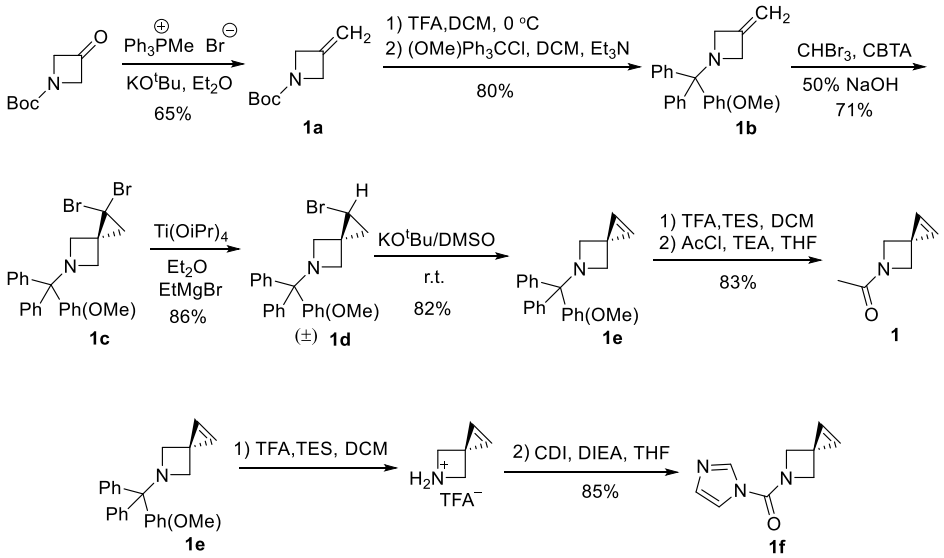
Fig. S12. Bright-field and fluorescence micrographs of HEK293T cells transfected with the plasmids encoding mCherry-TAG-EGFP and TCOKRS/tRNA and cultured in DMEM medium supplemented with 10% FBS and 1 mM AsphK. Scale bar = 100 μm.

General Information

Solvents and chemicals were purchased from commercial sources and used directly without further purification. Flash chromatography was performed with SiliCycle P60 silica gel (40-63 μm, 60 Å). ^{1}H NMR spectra were recorded with Inova-300, -400 or -500 MHz spectrometers and chemical shifts were reported in ppm using either TMS or deuterated solvents as internal standards (TMS, 0.00; $CDCl_3$, 7.26; CD_3OD, 3.31; DMSO-d_6, 2.50, D_2O, 4.63). Multiplicity was reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. ^{13}C NMR spectra were recorded at 75.4 MHz, and chemical shifts were reported in ppm using deuterated solvents as internal standards ($CDCl_3$, 77.0; DMSO-d_6, 39.5; CD_3OD, 49.05). Fluorescence spectra were recorded using 1-cm plastic cuvettes on a Horiba FluoroMax-4 spectrofluorometer at 25 ºC. IR spectra were taken neat using a Perkin Elmer 1760 Fourier Transform Infrared (FTIR) spectrometer, and wave numbers were reported in cm^{-1} for characteristic peaks. Low-resolution mass spectrometry was performed on a Thermo LCQ mass spectrometer. All fluorescence images were acquired with a Lionheart^{TM} FX automated live cell imager. The cLogP values were calculated with Molinspiration. The molecular optimization, orbitals, and energy levels of spiroalkenes were obtained from DFT calculations using Gaussian09 program[S1] at B3LYP/6-31++G** level.

Experimental Procedures and Characterization Data

Scheme S1



***tert*-Butyl 3-methyleneazetidine-1-carboxylate (1a)**[S2]**:** Exocyclic alkene **1a** was prepared from the commercially available *tert*-butyl 3-oxoazetidine-1-carboxylate as a colorless liquid (5.43 g, 65% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.99 (m, J = 2.5 Hz, 2H), 4.48 (t, J = 2.5 Hz, 4H), 1.45 (s, 9H).

1-((4-Methoxyphenyl)diphenylmethyl)-3-methyleneazetidine (1b): To a solution of alkene **1a** (0.5 g, 2.96 mmol) in 3 mL DCM cooled in an ice-water bath was added 3 mL trifluoroacetic acid (TFA) dropwise, and the mixture was stirred for 30 minutes. Then, DCM and TFA were removed under reduced pressure to afford a colorless liquid, which was used directly in subsequent steps without further

purification. The above liquid was re-dissolved in 10 mL anhydrous DCM. To the resulting solution were added Et_3N (2.1 mL, 14.8 mmol) and (chloro(4-methoxyphenyl)methylene)dibenzene (0.91 g, 2.96 mmol), and the mixture was stirred at room temperature for 6 hours. The reaction was quenched by pouring the mixture into 20 mL water and extracted with DCM (3×15 mL). The organic layers were separated, washed with brine (3×15 mL), and concentrated to dryness under reduced pressure. The residue was purified by silica gel chromatography using hexanes/ethyl acetate as eluent to give the title product as a colorless solid (0.81 g, 80% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.49 (d, J = 10.0 Hz, 4H), 7.39 (d, J = 5.0 Hz, 2H), 7.26 (t, J = 5.0 Hz, 4H), 7.18 (t, J = 5.0 Hz, 2H), 6.81 (d, J = 10.0 Hz, 2H), 4.66 (s, 2H), 3.75 (s, 3H), 3.72 (s, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.93, 143.19, 141.74, 134.56, 130.50, 129.05, 127.55, 126.26, 112.83, 103.18, 74.11, 56.27, 55.07; ESI-MS calcd for $C_{24}H_{24}NO$ 342.5 [M+H$^+$], found 342.5.

1,1-Dibromo-5-((4-methoxyphenyl)diphenylmethyl)-5-azaspiro[2.3]hexane (1c): To a mixture of alkene **1b** (0.49 g, 1.44 mmol), cetrimonium bromide (52 mg, 0.14 mmol) in DCM (0.5 mL) was added bromoform (0.25 mL, 2.87 mmol) and stirred at room temperature. Then, 50% NaOH (0.58 g dissolved in 0.58 mL water) was added dropwise and the mixture turned dark during vigorously stirring. The reaction was quenched by pouring the mixture into 15 mL water after more than 20 hours and extracted with DCM (3×15 mL). The organic layers were separated, washed with brine (3×15 mL), and concentrated to dryness under reduced pressure. The residue was purified by silica gel chromatography using hexanes/ether acetate (50:1) as eluent to give the title product as a colorless solid (0.52 g, 71% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.51 (d, J = 10.0 Hz, 4H), 7.41 (d, J = 10.0 Hz, 2H), 7.29 (t, J = 5.0 Hz, 4H), 7.20 (t, J = 5.0 Hz, 2H), 6.84 (d, J = 10.0 Hz, 2H), 3.77 (s, 3H), 3.42 (d, J = 10.0 Hz, 2H), 3.23 (d, J = 10.0 Hz, 2H), 1.47 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.06, 143.02, 134.57, 130.39, 129.00, 127.68, 126.44, 113.00, 74.28, 55.15, 53.55, 32.58, 31.45, 30.60; ESI-MS calcd for $C_{25}H_{23}Br_2NNaO$ 535.0 [M+Na$^+$], found 534.9.

(±)-1-Bromo-5-((4-methoxyphenyl)diphenylmethyl)-5-azaspiro[2.3]hexane (1d): To a solution of dibromo-azaspiro[2.3]hexane **1c** (315 mg, 0.61 mmol) in 10 mL anhydrous Et_2O containing 10 mol% Ti(O^{i}Pr)$_4$ (18.0 μL, 0.06 mmol) under argon was slowly added 3 M ethyl magnesium bromide solution in Et_2O (230 μL, 0.80 mmol) at room temperature through syringe. The mixture was stirred at room temperature for more than 24 hours. The reaction was then quenched and filtered through a thin layer of Celite, and the filtrate was extracted with Et_2O. The organic layers were separated, dried over anhydrous Na_2SO_4, and evaporated to dryness in vacuum. The residue was purified by silica gel chromatography using hexanes/ethyl acetate (50:1) as eluent to give the title product as a colorless solid (229 mg, 86% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50 (d, J = 9.0 Hz, 4H), 7.39 (d, J = 9.0 Hz, 2H), 7.24 (t, J = 6.0 Hz, 4H), 7.18 (t, J = 6.0 Hz, 2H), 6.83 (d, J = 9.0 Hz, 2H), 3.78 (s, 3H), 3.45 (d, J = 9.0 Hz, 1H), 3.24 (t, J = 6.0 Hz, 1H), 3.19 (d, J = 12.0 Hz, 1H), 3.78 (dd, J = 6.0, 6.0 Hz, 1H), 1.06 (t, J = 9.0 Hz, 1H), 0.70 (dd, J = 6.0, 6.0 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.89, 143.23, 143.20, 134.72, 130.41, 129.01, 127.66, 127.54, 126.24, 112.84, 74.09, 55.04, 53.59, 53.40, 24.78, 21.67, 18.81; ESI-MS calcd for C_5H_9BrN 162.0 [M−trityl$^+$+H$^+$+H$^+$], found 162.0.

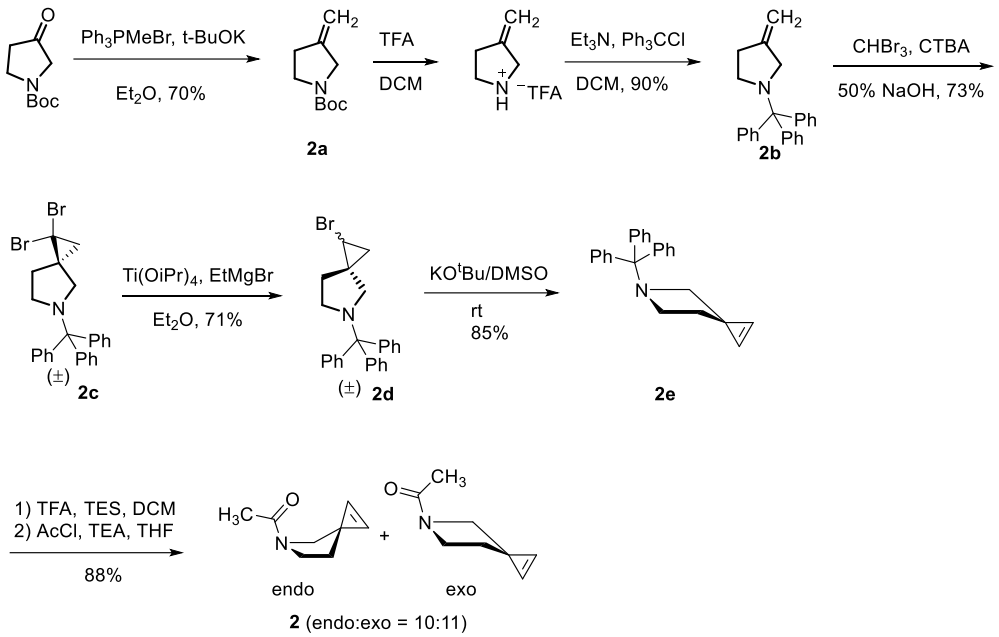
5-((4-Methoxyphenyl)diphenylmethyl)-5-azaspiro[2.3]hex-1-ene (1e): To a solution of monobromo-azaspiro[2.3]hexane **1d** (600 mg, 1.38 mmol) in 1 mL anhydrous DMSO under argon was added dropwise a solution of KO^{t}Bu (248 mg, 2.21 mmol) in 1 mL anhydrous DMSO, and the mixture was

stirred at room temperature for 2 hours until all the starting material was consumed. The reaction was monitored by withdrawing small aliquots of the reaction mixture quenching with water, extracting with Et_2O, drying and analyzing by ^1H NMR. The entire reaction was then quenched by pouring the mixture into icy water and extracted with Et_2O (3×15 mL). The organic layers were separated, dried over anhydrous $MgSO_4$, and evaporated to dryness in vacuum. The residue was purified by chromatography using hexane/ethyl acetate (15:1) as the eluent to give the title product as a colorless foamy oil (400 mg, 82% yield): ^1H NMR (300 MHz, $CDCl_3$) δ 7.53 (d, J = 9.0 Hz, 4H), 7.42 (d, J = 9.0 Hz, 2H), 7.26 (t, J = 6.0 Hz, 4H), 7.18 (d, J = 6.0 Hz, 2H), 7.12 (s, 2H), 6.83 (d, J = 9.0 Hz, 2H), 3.76 (s, 3H), 3.24 (s, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.74, 143.91, 135.51, 130.57, 129.22, 127.36, 126.00, 118.17, 112.65, 74.16, 59.67, 55.08, 20.28; ESI-MS calcd for $C_{25}H_{24}NO$ 354.2 [$M+H^+$], found 353.9.

1-(5-Azaspiro[2.3]hex-1-en-5-yl)ethanone (1): To a solution of azaspirohexene **1e** (240 mg, 0.68 mmol) and triethylsilane (217 μL, 1.36 mmol) in DCM (1mL) at 0 °C was added dropwise TFA (1 mL). Then the reaction solution was stirred at room temperature for 1 hour before evaporating the solvent. To the above mixture was added 2 mL DCM and extracted with water (3×2 mL), the water was collected and water was evaporated to dryness in vacuum to get the crude azaspirohexene-TFA salt, which can be used directly in subsequent steps without further purification. To the above salt in THF was added triethylamine (280 μL, 2.04 mmol) and stirred at room temperature for 5 minutes, then acetyl chloride (145 μL, 2.04 mmol) was slowly added to the reaction mixture and stirred for 1 hour. The solvent was evaporated in vacuum, and the residue was purified by chromatography using ethyl acetate/methanol (15:1) as the eluent to give the title product as colorless oil (69 mg, 83% yield): ^1H NMR (300 MHz, $CDCl_3$) δ 7.36 (s, 2H), 4.12 (s, 2H), 4.02 (s, 2H), 1.91 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.87, 116.37, 62.16, 59.38, 19.38, 19.06; IR (neat) 3326, 2944, 2832, 1647, 1449, 1271, 1115, 1023, 737, 703, 628; ESI-MS calcd for $C_7H_{10}NO$ 124.1 [$M+H^+$], found 124.1.

(1*H*-Imidazol-1-yl)(5-azaspiro[2.3]hex-1-en-5-yl)methanone (1f): To a solution of azaspirohexene **1e** (240 mg, 0.68 mmol) and triethylsilane (217 μL, 1.36 mmol) in DCM (1mL) at 0 °C was added dropwise TFA (1 mL). Then, the mixture was stirred at room temperature for 1 hour before evaporating the solvent. To the above mixture was added 2 mL DCM and extracted with water (3×2 mL), the water phase was collected and the water was evaporated to dryness in vacuum to get the crude azaspirohexene-TFA salt, which can be used directly in subsequent steps without further purification. To the above salt was added THF (2 mL) followed by *N*,*N*-diisopropylethylamine (355 μL, 2.04 mmol) and stirred at room temperature for 5min, then 1,1'-Carbonyldiimidazole (121 mg, 0.75 mmol) was added to the reaction mixture and stirred for 1h. The solvent was evaporated in vacuum and the residue was purified by chromatography using ethyl acetate/methanol (15:1) as the eluent to give the title product as colorless solid (101 mg, 85% yield): ^1H NMR (500 MHz, $CDCl_3$) δ 8.02 (s, 1H), 7.41 (s, 2H), 7.33 (s, 1H), 7.07 (s, 1H), 4.33 (s, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.87, 116.37, 62.16, 59.38, 19.38, 19.06; ^{13}C NMR (125 MHz, $CDCl_3$) δ 149.90, 136.32, 129.71, 116.77, 116.00, 19.84; ESI-MS calcd for $C_9H_9N_3O$ 176.1 [$M+H^+$], found 176.0.

Scheme S2



***tert*-Butyl 3-methylenepyrrolidine-1-carboxylate (2a)**: The alkene compound **2a** was synthesized by following the reported procedure with 70% yield[S3].

3-Methylene-1-tritylpyrrolidine (2b): The alkene compound **2b** was obtained as colorless solid using the same synthetic procedure as compound **1b** with 90% yield: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.51 (d, J = 10.0 Hz, 6H), 7.23 (t, J = 5.0 Hz, 6H), 7.12 (t, J = 5.0 Hz, 3H), 4.78 (d, J = 5.0 Hz, 2H), 3.01 (s, 2H), 2.43 (t, J = 5.0 Hz, 2H), 2.37 (d, J = 5.0 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 148.09, 142.27, 129.30, 127.30, 126.00, 104.21, 74.41, 52.04, 46.75, 30.60; ESI-MS calcd for $C_{24}H_{24}N$ 326.2 [M+H$^+$], found 325.9.

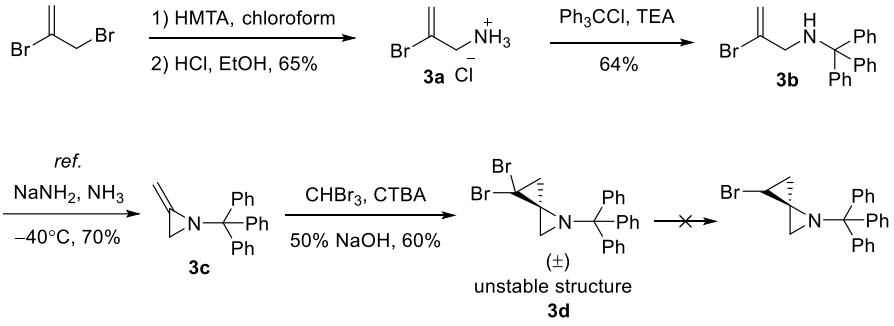
(±)-1,1-Dibromo-5-trityl-5-azaspiro[2.4]heptane (2c): The dibromo compound **2c** was obtained as colorless solid using the same synthetic procedure as compound **1c** with 73% yield: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.51 (d, J = 10.0 Hz, 6H), 7.26 (t, J = 10.0 Hz, 6H), 7.17 (t, J = 10.0 Hz, 3H), 2.95 (d, J = 10.0 Hz, 1H), 2.79 (dd, J = 5.0, 5.0 Hz, 1H), 2.40 (m, 1H), 2.28 (m, 1H), 2.24 (d, J = 10.0 Hz, 1H), 1.67 (m, 1H), 1.54 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 142.14, 129.26, 127.43, 126.19, 74.32, 55.89, 47.82, 37.59, 35.34, 34.56, 34.09; ESI-MS calcd for $C_{25}H_{24}Br_2N$ 496.0 [M+H$^+$], found 496.0.

(±)-1-Bromo-5-trityl-5-azaspiro[2.4]heptane (2d): The diastereoisomeric mixture of **2d** was obtained as a colorless solid using the same procedure as compound **1d** with 71% yield: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.55 (d, J = 5.0 Hz, 6H), 7.50 (d, J = 5.0 Hz, 6H), 7.28–7.24 (br, 12H), 7.24–7.14 (br, 6H), 3.00 (m, 2H), 2.83 (d, J = 10.0 Hz, 1H), 2.70 (m, 2H, 2.46 (d, J = 10.0 Hz, 1H), 2.36 (m, 2H), 2.26 (d, J = 10.0 Hz, 1H), 2.21 (d, J = 10.0 Hz, 1H), 2.14 (m, 1H), 1.81 (m, 1H), 1.65 (m, 2H), 1.20–1.17 (br, 2H), 0.82–0.81 (br, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 142.42, 142.37, 129.37, 129.24, 127.37, 127.31, 126.08, 126.03, 74.35, 74.32, 55.11, 53.23, 47.34, 47.23, 33.48, 31.56, 31.19, 29.67, 29.13, 26.38, 26.21, 22.63, 21.77; ESI-MS calcd for $C_{25}H_{25}BrN$ 418.1 [M+H$^+$], found 418.2.

5-Trityl-5-azaspiro[2.4]hept-1-ene (2e): The azaspiroheptene **2e** was obtained as a colorless foamy oil using the same procedure as compound **1e** with 85% yield: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.52 (d, J = 10.0 Hz, 6H), 7.24 (t, J = 10.0 Hz, 6H), 7.14 (t, J = 10.0 Hz, 3H), 2.45 (t, J = 5.0 Hz, 2H), 2.16 (s, 2H), 1.52 (t, J = 5.0 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 142.70, 129.44, 127.18, 125.82, 116.69, 74.57, 55.78, 47.31, 34.97, 23.89; ESI-MS calcd for $C_{25}H_{24}N$ 338.2 [M+H$^+$], found 337.9.

1-(5-Azaspiro[2.4]hept-1-en-5-yl)ethanone (2): The compound **2** was obtained as a colorless solid using the same procedure as compound **1** with 88% yield: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32 (s, 2.4H), 7.30 (s, 2H), 3.60 (m, 4.4H), 3.20 (s, 2H), 3.17 (s, 2.4H), 2.06 (s, 3H), 2.00 (s, 3.6H), 1.83 (t, J = 5.0 Hz, 2H), 1.74 (t, J = 5.0 Hz, 2.4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.03, 116.24, 116.06, 55.75, 53.46, 47.13, 44.96, 36.07, 34.72, 25.42, 24.19, 22.41, 21.78; IR (neat) 2927, 2860, 1737, 1625, 1458, 1438; ESI-MS calcd for $C_8H_{12}NO$ 138.1 [M+H$^+$], found 138.1.

Scheme S3



2-Bromoprop-2-en-1-aminium chloride (3a): The alkene salt was synthesized according to the literature procedure[S4]: To a solution of hexamethylene tetramine (4.29 g, 30.7 mmol) in chloroform (40 mL) in two-necked flask under reflux was dropwise added 2,3-dibromopropene (6.1 mL, 30.7 mmol). After the addition is complete, the reaction mixture was stirred under reflux for 3 hours and allowed to stand overnight at room temperature. Then, the mixture was cooled in an ice bath and the salt was collected by filtration to give the crude solid around 10 g. Then the solid was dissolved in a warm solution prepared from 20 mL water, 100 mL ethanol and 24 mL concentrated HCl. A white precipitate of ammonium chloride formed and the reaction mixture was allowed to stand at room temperature overnight and the white solid was removed by filtration. When the reaction mixture was concentrated to 30 mL, the solid was removed again by filtration, then the mother liquid was concentrated and dried to give the product as a white solid (6.5 g, 65% yield): ^{1}H NMR (500 MHz, CD_3OD) δ 6.17 (s, 1H), 5.83 (s, 1H), 3.92 (s, 2H); ESI-MS calcd for C_3H_7NBr 136.0 [M-Cl$^+$], found 135.9.

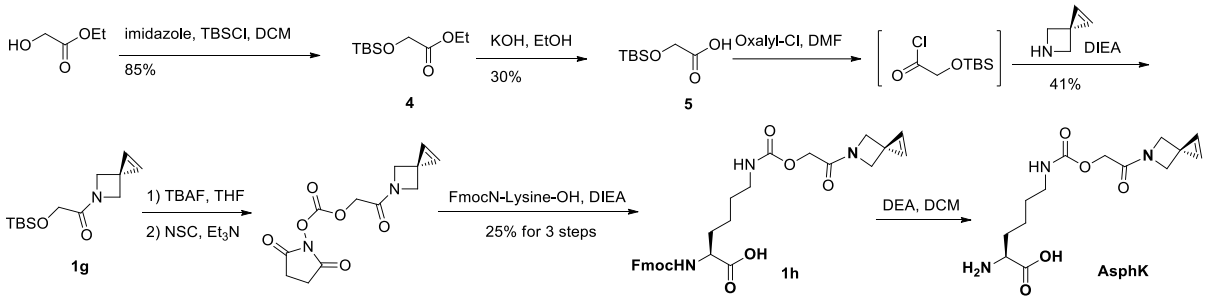
2-Bromo-*N*-tritylprop-2-en-1-amine (3b): To a suspension of alkene salt **3a** (1.50 g, 8.72 mmol) in DCM was added triethylamine (7.30 mL, 52.3 mmol), the mixture was stirred for 5 minutes at room temperature before triphenylchloromethane (2.20 g, 7.89 mmol) was added. Then the mixture was stirred at room temperature for 12h, the reaction was quenched by pouring the mixture into 20 mL water and extracted with DCM (3×15 mL). The organic layers were separated, washed with brine (3×15 mL), and concentrated to dryness under reduced pressure. The residue was purified by silica gel chromatography using hexanes/ethyl acetate (30:1, then 15:1) as eluent to give the title product as a

colorless solid (1.90 g, 64% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.48 (d, J = 10.0 Hz, 6H), 7.25 (t, J = 10.0 Hz, 6H), 7.15 (t, J = 10.0 Hz, 3H), 6.02 (s, 1H), 5.52 (s, 1H), 2.98 (s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 145.54, 132.79, 128.36, 127.93, 126.43, 116.38, 70.61, 52.28; ESI-MS calcd for $C_{22}H_{21}NBr$ 378.1 [M+H$^+$], found 378.1.

2-Methylene-1-tritylaziridine (3c): 2-Methyleneaziridine (**3c**) was synthesized according to the literature procedure[S5]: To a two-necked flask fitted with dry ice condenser was added sodium amide and the system flushed with ammonia. A dry ice/acetone mixture was added to the condenser and ammonia condensed into the flask. Alkene **3b** (1.2 g, 3.2 mmol) was added slowly and the resulting solution stirred for 6 hours. The reaction was allowed to warm up to room temperature, and then diluted with diethyl ether and quenched by dropwise addition of water. The mixture was stirred in diethyl ether for 2 minutes before extraction with diethyl ether, washed with 10% sodium hydroxide and water. The crude product was recrystallized in ethyl acetate and hexane mixture to give the product as a white solid (700 mg, 74% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45 (d, J = 10.0 Hz, 6H), 7.26 (m, 9H), 4.44 (s, 1H), 4.33 (s, 1H), 1.81 (s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 143.53, 130.76, 129.07, 127.40, 126.89, 82.89, 25.40; ESI-MS calcd for $C_{22}H_{20}N$ 298.2 [M+H$^+$], found 298.1.

(±)-4,4-Dibromo-1-trityl-1-azaspiro[2.2]pentane (3d): To a mixture of mthyleneaziridine **3c** (300 mg, 1.01 mmol), cetrimonium bromide (36 mg, 0.1 mmol) in DCM (0.3 mL) under argon was added bromoform (0.18 mL, 2.02 mmol) and stirred at room temperature. Then 50% NaOH (400 mg dissolved in 0.4 mL water) was added dropwise with vigorously stirring. After 12 hours, the reaction was quenched by pouring the mixture into 15 mL water and extracted with DCM (3×15 mL). The organic layers were separated, washed with brine (3×15 mL), and concentrated to dryness under reduced pressure. The residue was purified by silica gel chromatography using hexanes/ether acetate (50:1) as eluent to give the title compound as a colorless solid (350 mg, 74% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.54 (d, J = 10.0 Hz, 6H), 7.23 (m, 9H), 2.48 (s, 1H), 1.90 (s, 1H), 1.82 (d, J = 10.0 Hz, 1H), 1.71 (d, J = 10.0 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 143.81, 129.73, 127.42, 126.88, 75.00, 47.07, 32.64, 28.27, 26.85; ESI-MS calcd for $C_{23}H_{20}NBr_2$ 468.0 [M+H$^+$], found 467.9.

Scheme S4



2-((*tert*-butyldimethylsilyl)oxy)acetate (5)[S6]: To a solution of ethyl glycolate (1.9 mL, 20 mmol) and imidazole (2.04 g, 30 mmol) in Dichloromethane was added a solution of TBSCl (3.32 g, 22 mmol) at ice bath. After stirring for 3 hours, the mixture was diluted with dichloromethane and the organic layer was washed with water, followed by brine, dried over Na_2SO_4 and purified by silica gel chromatography using hexanes/ether acetate to give **4** as a clear liquid (3.7 g, 85% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.23 (s, 2H), 4.19 (m, 2H), 1.27 (t, J = 6.8 Hz, 3H), 0.92 (s, 9H), 0.10 (s, 6H). Then, to a solution of

ester **4** (2.8 g, 12.8 mmol) in EtOH (100 mL) was added a solution of KOH in ethanol (10 mL), the mixture was stirred at room temperature overnight. The reaction mixture was concentrated, and water (10 mL) was added and the mixture was carefully added concentrated HCl to reach pH 4. Then the mixture was extracted with ethyl acetate, washed with water, dried over Na_2SO_4, and purified by silica gel chromatography using DCM/hexanes followed by hexanes/ether acetate as eluents to give compound **5** as a clear liquid (0.73 g, 30% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.22 (s, 2H), 0.94 (s, 9H), 0.15 (s, 6H).

2-((*tert*-Butyldimethylsilyl)oxy)-1-(5-azaspiro[2.3]hex-1-en-5-yl)ethanone (1g): To a solution of acid **5** (209 mg, 1.1 mmol) in dichloromethane (10 mL) was added oxalyl chloride (94 µL, 1.1 mmol) and a drop of DMF at ice bath. The mixture was stirred at room temperature until no bubble released (around 10 min), then azaspirohexene amine with DIEA (522 µL, 3.0 mmol) in dichloromethane (5 mL) obtained from **1e** (360 mg, 1.0 mmol) was added and stirred for 30 min before evaporating the solvents. After purification by silica gel chromatography using ethyl acetate/hexanes, the titled product was obtained as a colorless oil (105 mg, 41% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34 (s, 2H), 4.25 (s, 2H), 4.20 (s, 2H), 4.04 (s, 2H), 0.87 (s, 9H), 0.06 (s, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.85, 116.71, 63.96, 62.89, 60.49, 25.79, 25.71, 20.43, 18.29, -5.53, -5.66; ESI-MS calcd for $C_{13}H_{24}NO_2Si$ 254.2 [M+H$^+$], found 254.1.

(*S*)-2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-6-(((2-oxo-2-(5-azaspiro[2.3]hex-1-en-5-yl)ethoxy)carbonyl)amino)hexanoic acid (1h): To a solution of TBS ether **1g** (105 mg, 0.41 mmol) in THF (10 mL) was added 1 M TBAF (0.54 mL, 0.54 mmol), and the mixture was stirred at room temperature for 30 min. Then the mixture went through a silica filter to remove the TBAF salt and washed with ethyl acetate, the solution was concentrated to give the hydroxyl product. Then to the hydroxyl product in acetonitrile (10 mL) *N*,*N*-disuccinimidyl carbonate (100 mg, 0.39 mmol) was added and the mixture was stirred at room temperature for 12 hours. The solution was concentrated to give the crude active ester. Then to the active ester in DMF was added Fmoc-lysine-OH hydrochloric acid salt (93 mg, 0.23 mmol) and DIEA (125 µL, 0.714 mmol). The mixture was stirred at room temperature for 10 hours and the solvents were evaporated. The residue was purified by silica gel chromatography to five the titled compound as a colorless oil (55 mg, 25% yield): ^{1}H NMR (500 MHz, CD_3OD) δ 7.80 (d, J = 7.5 Hz, 2H), 7.71–7.64 (m, 2H), 7.52 (s, 2H) , 7.39 (t, J = 7.4 Hz, 2H), 7.31 (t, J = 7.4 Hz, 2H), 7.07 (s, 1H), 4.47 (s, 2H), 4.41 – 4.27 (m, 2H), 4.22 (t, J = 6.7 Hz, 1H), 4.16 (s, 2H), 4.05 (s, 1H), 3.99 (s, 2H), 3.12 (m, 2H), 1.84 (s, 1H), 1.73–1.62 (m, 1H), 1.58 – 1.34 (m, 4H); ^{13}C NMR (75 MHz, CD_3OD) δ 168.00, 157.16, 156.67, 143.94, 143.81, 141.16, 127.39, 126.76, 124.84, 119.53, 115.82, 66.37, 61.31, 60.60, 60.05, 54.42, 53.40, 48.43, 48.15, 48.10, 48.09, 48.07, 48.04, 47.99, 47.98, 47.96, 47.95, 47.93, 47.92, 47.87, 47.82, 47.80, 47.79, 47.77, 47.76, 47.74, 47.73, 47.71, 47.70, 47.68, 47.66, 47.65, 47.58, 47.30, 47.02, 46.73, 40.23, 31.51, 29.04, 22.63, 19.39, 17.32; ESI-MS calcd for $C_{29}H_{32}N_3O_7$ 534.2 [M+H$^+$], found 534.1.

(*S*)-2-Amino-6-(((2-oxo-2-(5-azaspiro[2.3]hex-1-en-5-yl)ethoxy)carbonyl)amino)hexanoic acid (AsphK): To a solution of amino acid **1h** (55 mg, 0.10 mmol) in dichloromethane (10 mL) was added diethyl amine (10 mL) and the mixture was stirred at room temperature for 10 hours. The solvents were evaporated and diethyl ether (10 mL) was added. The mixture was extracted with water. The water phase was dried to give the titled amino acid in quantitative yield: ^{1}H NMR (400 MHz, D_2O) δ 7.32 (s,

2H), 4.37 (s, 2H), 4.10 (s, 2H), 3.90 (s, 2H), 3.73 (m, 1H), 2.97 (m, 2H), 1.56–1.18 (m, 6H); ^{13}C NMR (75 MHz, CD_3OD) δ 173.87, 168.51, 157.20, 115.58, 115.48, 61.47, 60.81, 60.25, 58.55, 54.94, 40.11, 28.50, 26.41, 23.22, 21.70, 19.112; ESI-MS calcd for $C_{11}H_{22}N_3O_5$ 312.2 [M+H$^+$], found 312.1.

Determination of partition coefficient (Log *P*) using LC-MS

Appropriate amounts of 20 mM spiroalkene stock solution in DMSO were diluted into DCM to obtain 100 μL standard solutions with final concentrations of 1, 2.5, 5, and 10 mM for Sph-acetal and 0.1, 0.25, 0.5, 1, and 2.5 mM for azaspirohexene/azaspiroheptene. Five μL of the above standard samples were injected into the mass spectrometer and the intensities of molecular ions were recorded and plotted to give the standard curves. Afterwards, a twenty-five or fifty μL of the spiroalkene stock solution were mixed thoroughly with 1.0 mL 1-octanol/H_2O (1:1) in microcentrifuge tubes before the mixture was allowed to stand at room temperature for phase separation. Then, 50 μL solutions were carefully withdrawn from the octanol and water phases using pipettes (without disturbing the other phase) and injected directly into the mass spectrometer. The intensities of molecular ions were extracted from the LC-MS data, and the spiroalkene concentrations in each phase were determined by comparing ion intensities to those of the standard curves. The partition coefficients were calculated using the following equation: Log P = log ([spiroalkene]$_{oct}$/[spiroalkene]$_{water}$).

Site-specific incorporation of SphK or AsphK into sfGFP

Two hundred μL of overnight culture of BL21(DE3) cells co-transformed with pET-sfGFP-Q204TAG and pEvol-TCOKRS plasmids were diluted with fresh 50 mL LB broth containing 100 μg/mL ampicillin and 34 μg/mL chloramphenicol. The cells were allowed to grow at 37 °C to an OD_{600} ~0.6 before supplemented with 1 mM AsphK and the protein expression was induced with 0.2% arabinose and 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG). After incubation for 6 hours (37 °C, 280 rpm), the cells were pelleted in 50 mL conical tubes and resuspended with 4 mL binding buffer (10 mM imidazole, 300 mM NaCl in 50 mM Na_2HPO_4 pH 8.0), lysed by sonication on ice, then centrifuged. The lysates were transferred to 15 mL conical tubes containing 100 μL of Ni-NTA resin slurry (Thermo HisPur™) and incubated for 2 hours with gentle shaking. After removing the supernatant by centrifuge, the resin was washed three times with wash buffer (50 mM imidazole, 300 mM NaCl in 50 mM Na_2HPO_4, pH 8.0) and the proteins were eluted with 4 mL of elution buffer (250 mM imidazole, 300 mM NaCl in 50 mM Na_2HPO_4 pH 8.0). Protein yields were calculated based on the concentration of the protein quantified by Pierce™ BCA protein assay kit (Thermo). The purified protein was incubated with 100 μM tetrazole-Cy5 in PBS and irradiated with a 302-nm UV lamp for 1 min. The mixture was then added equal volume of 2× SDS loading buffer, heated at 95 °C for 10 min before being loaded onto 4-12% SDS-PAGE gel (GenScript). The in-gel fluorescence was detected by illuminating the gel with a 365-nm UV lamp. The proteins on the gel were stained with Coomassie blue. Three μg of the protein was analyzed by LC/ESI-MS and molecular mass of the protein was obtained by deconvoluting its multiply charged ladders using ProMass software (Thermo Scientific).

Site-specific incorporation of AsphK into mCherry-TAG-EGFP-HA in mammalian cells

Human Embryonic Kidney 293T (HEK293T) cells were seeded in 6-well plate and grown in DMEM supplemented with 10% FBS (HyClone™. GE Healthcare Life Sciences) and 10 μg/mL gentamycin (Gibco) and 2.5 μg/mL plasmocin at 37 °C, 5% CO2 until ~80% confluency. The medium was replaced

with DMEM supplemented with 1.0 mM AsphK and cells were transfected using polyethylenimine (Sigma-Aldrich) in Opti-MEM® (Gibco) with two plasmids: one encoding TCOKRS/$tRNA_{CUA}$ pair and the other encoding mCherry-TAG-EGFP-HA. Control experiments were performed using DMEM without unnatural amino acids. After 48 hours, live cell images were recorded. The cells were lysed by modified RIPA buffer (25 mM Tris· HCl, pH 7.4, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS, 1 mM EDTA, 1 mM PSMF). Twenty μL lysates were loaded to an SDS-PAGE gel, separated at 140 V for 50 minutes, and then transferred to a PVDF membrane (Thermo Fisher Scientific). The membrane was blocked in 1% casein in TBST (50 mM Tris, 150 mM NaCl, 0.05% Tween-20, pH 7.6) at 4 °C overnight, and then incubated with mouse anti-HA tag antibody (1:5000, Thermo Fisher Scientific) in TBST at room temperature for 1 h. The membrane was washed with TBST (5 min x 6) before addition of the secondary goat anti-mouse horseradish peroxidase conjugate (1:4000, Santa Cruz Biotech). After 30 minutes, the membrane was washed with TBST (5 min x 6), and incubated in 100 mM Tris buffer, pH 9.5, before addition of Pierce™ ECL Western Blotting Substrate (Thermo Fisher Scientific) and incubation for 5 min. The blot was exposed to an X-ray film (Phenix).

Reference:

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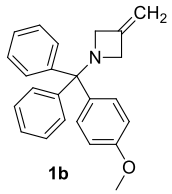
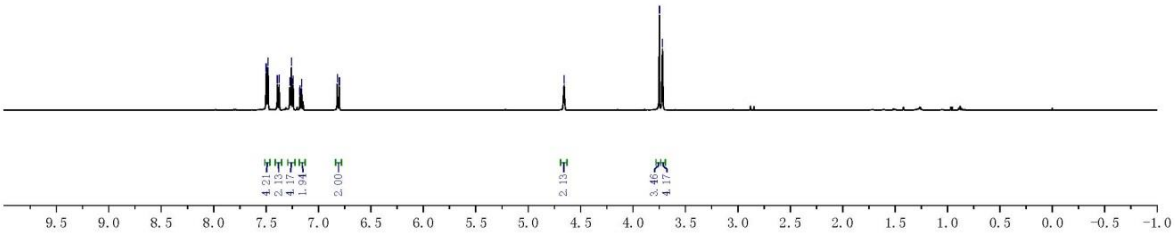
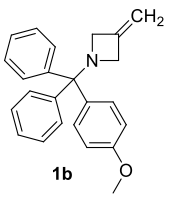
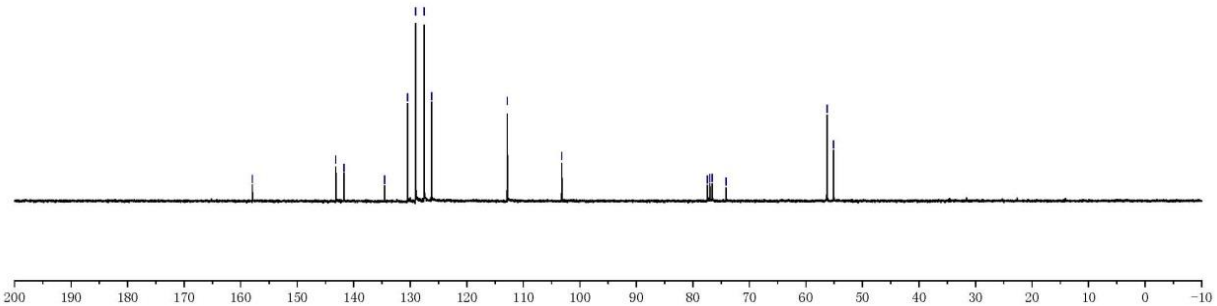
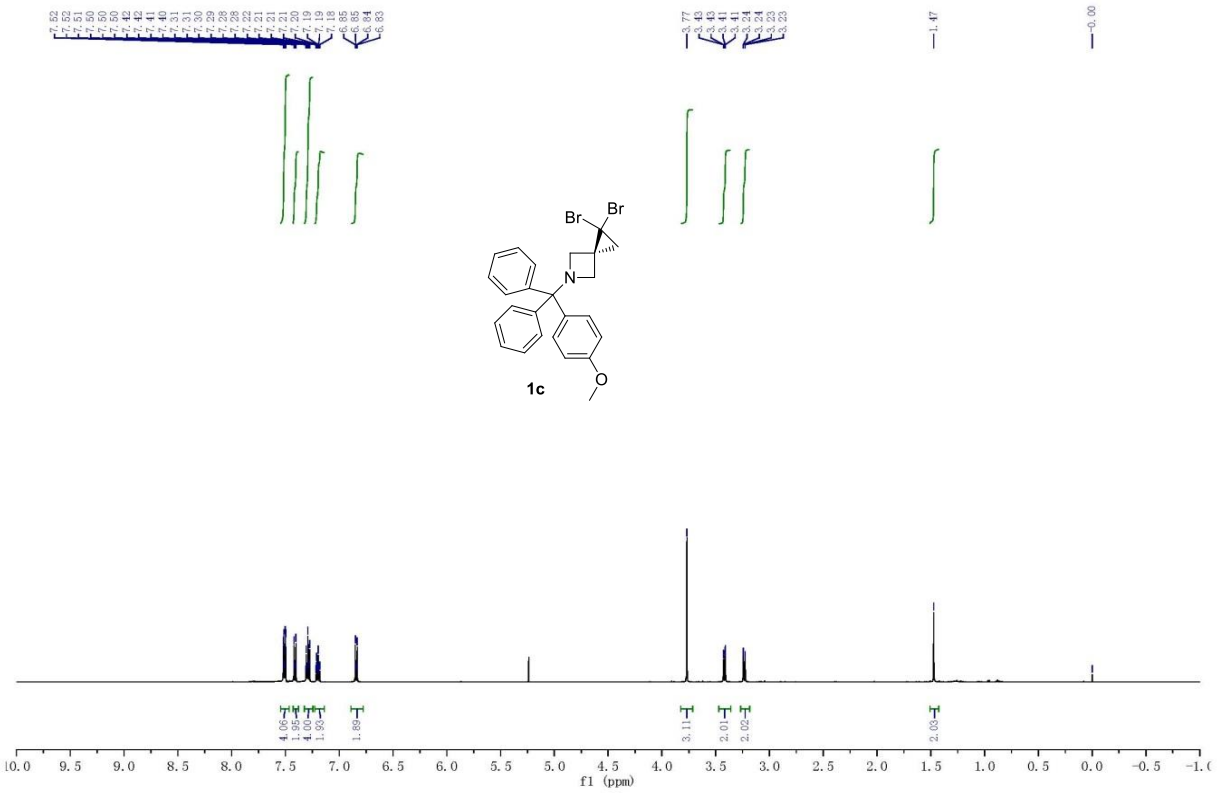
^{1}H and ^{13}C NMR Spectra







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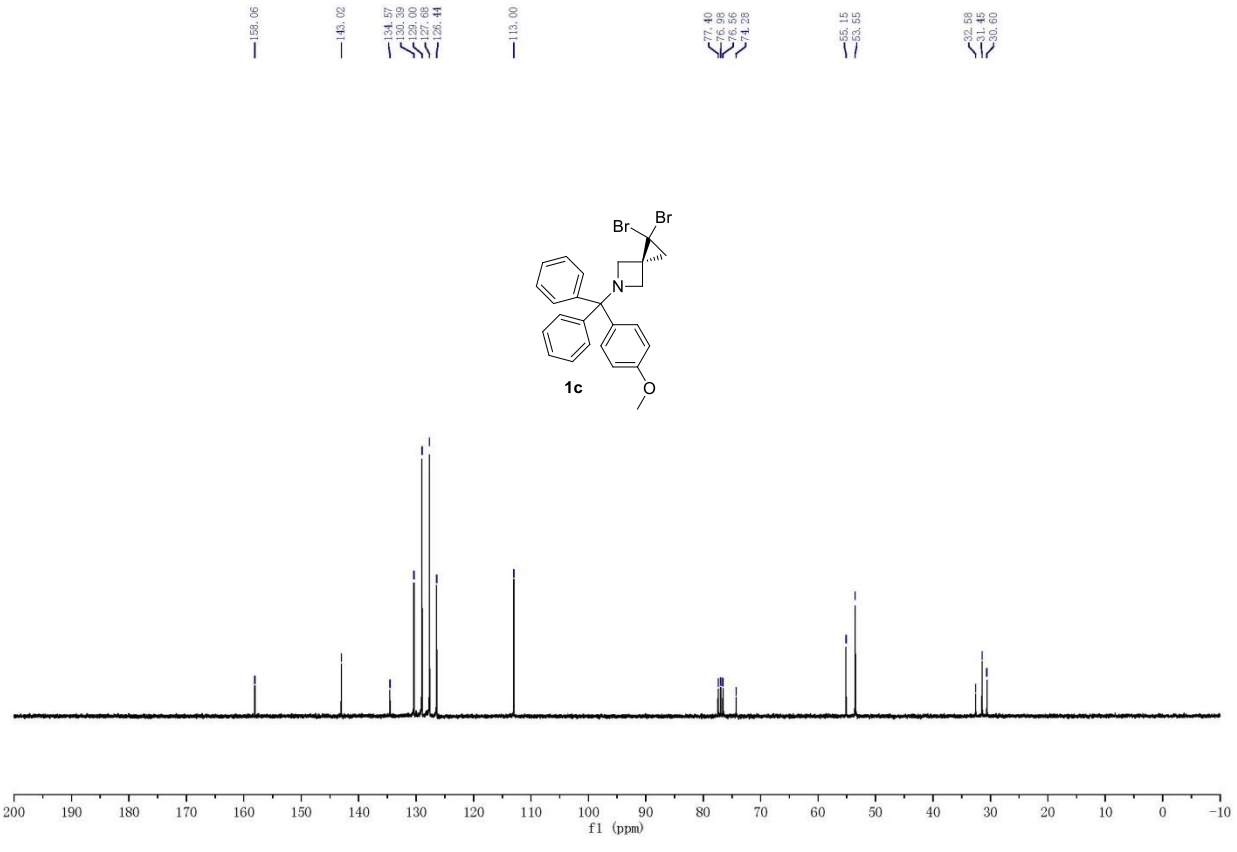
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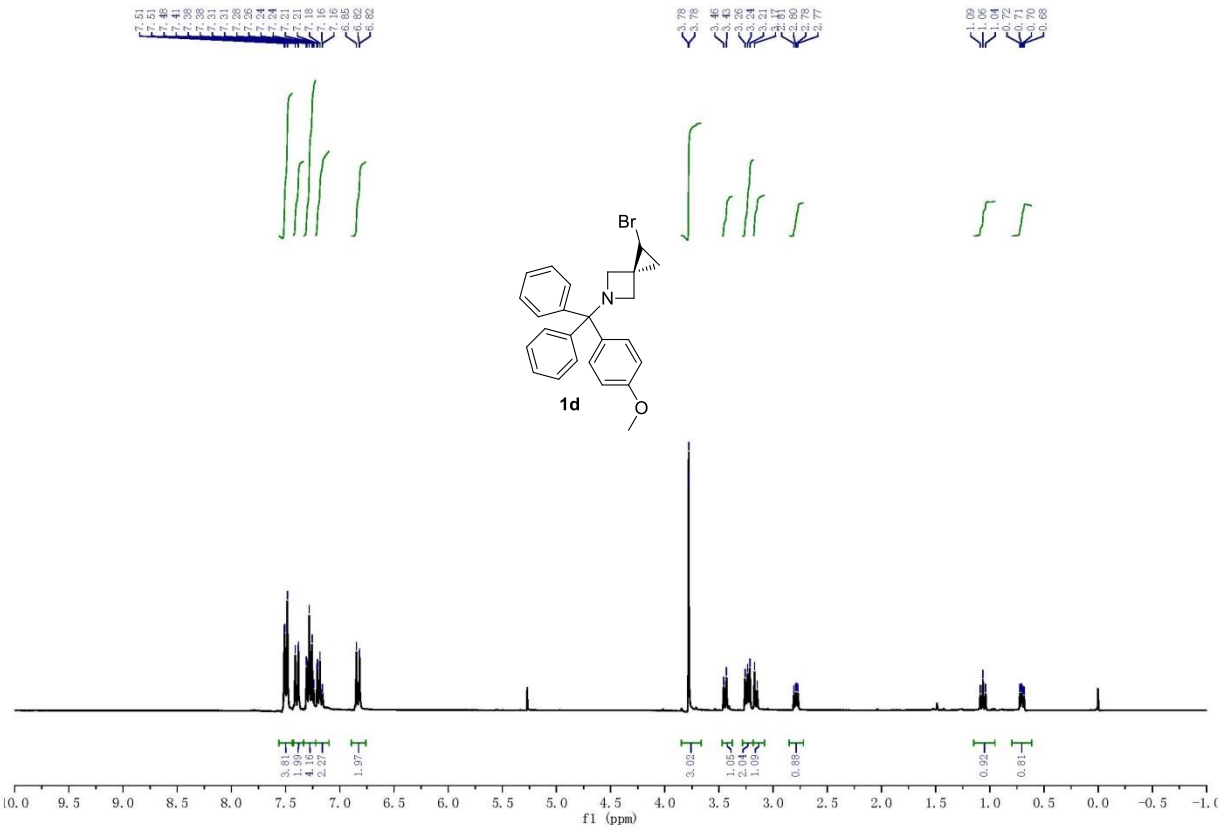
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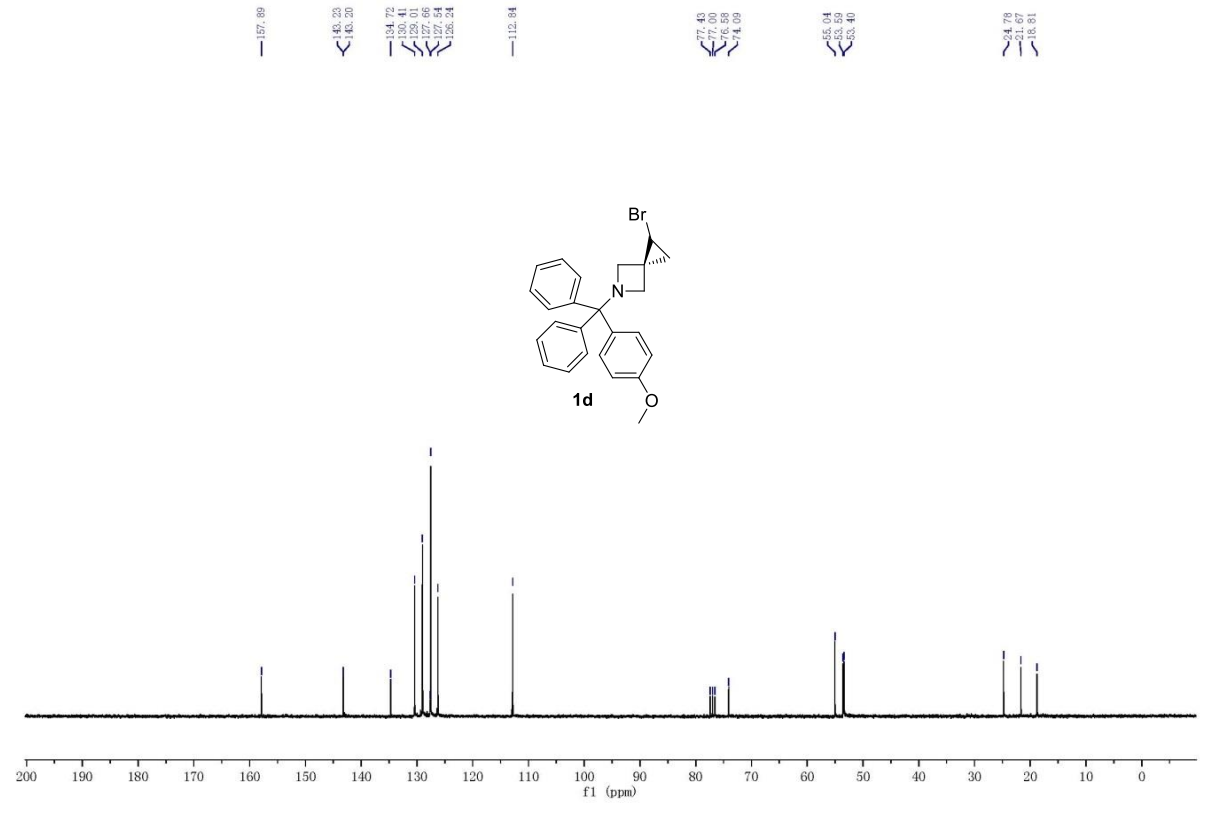
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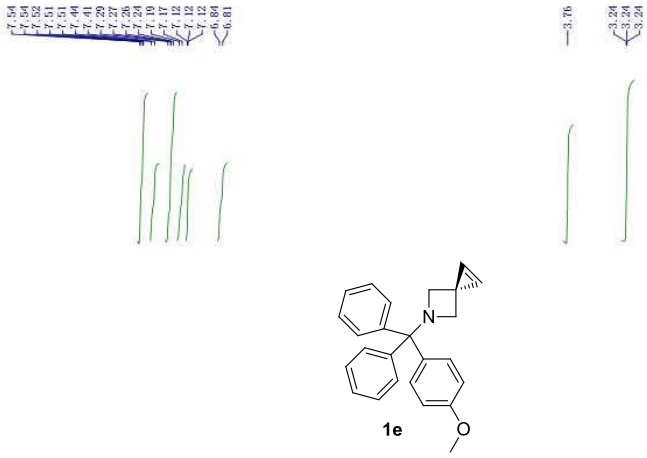
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7.12
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6.81
3.76
3.24
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1e
O
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2.63
3.90
2.00
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3.04
4.21
f1 (ppm)


157.74
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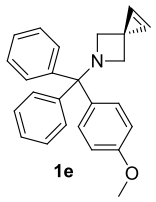
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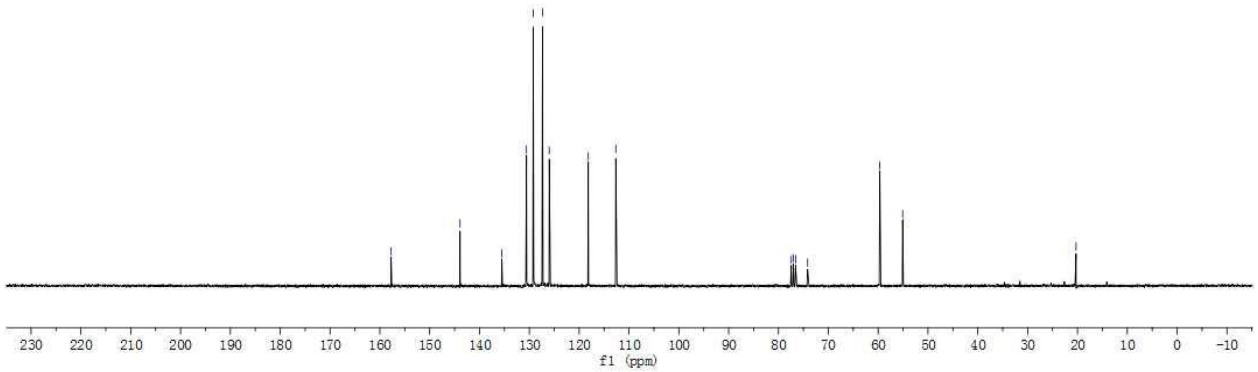
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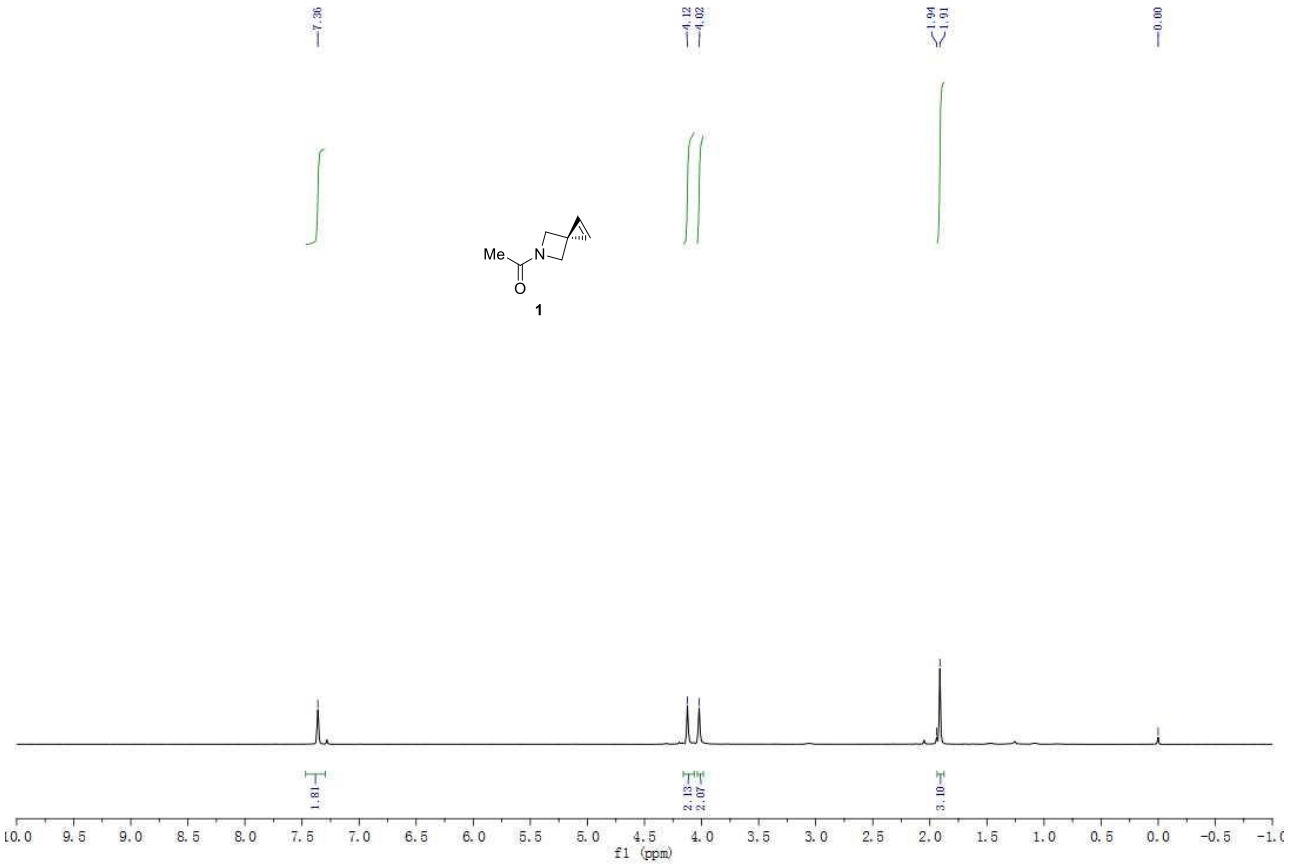
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O
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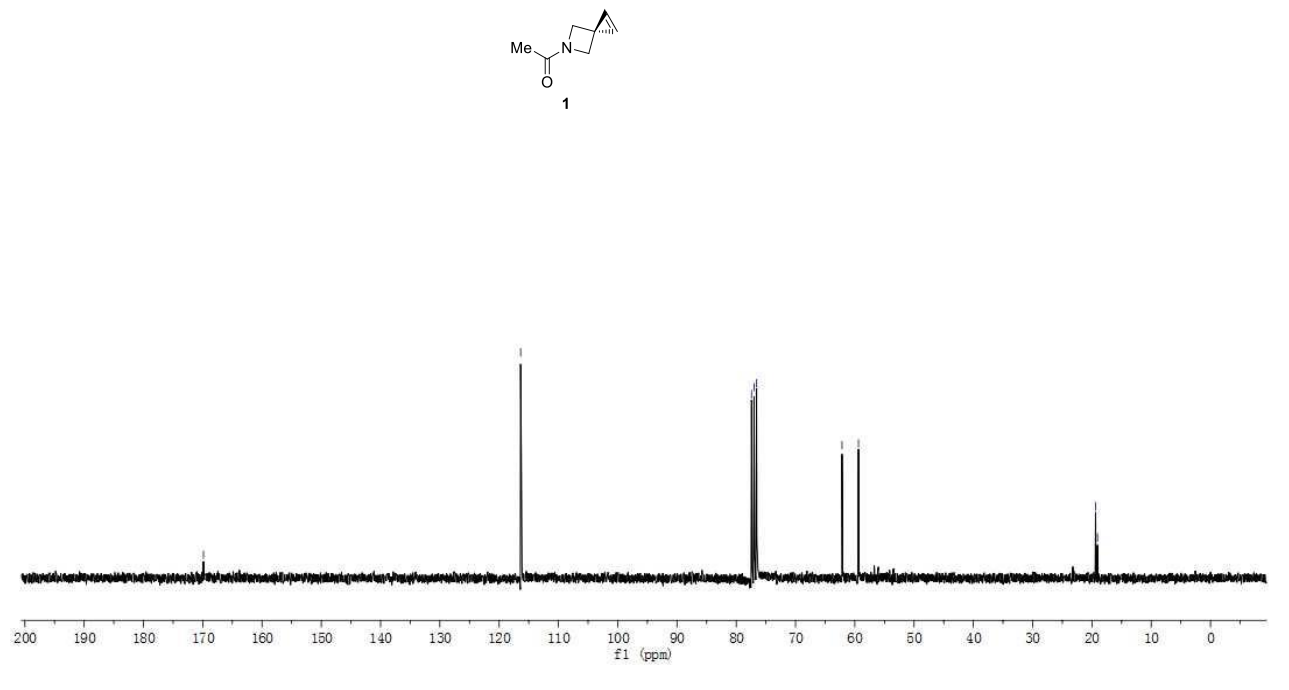
3.10
f1 (ppm)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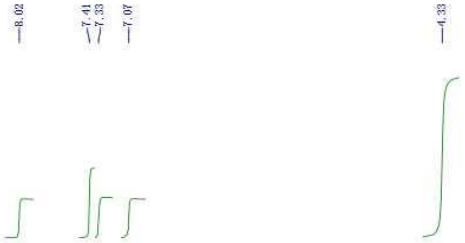
19.06
Me
N
O
1
f1 (ppm)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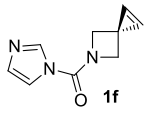
7.33
7.07
4.33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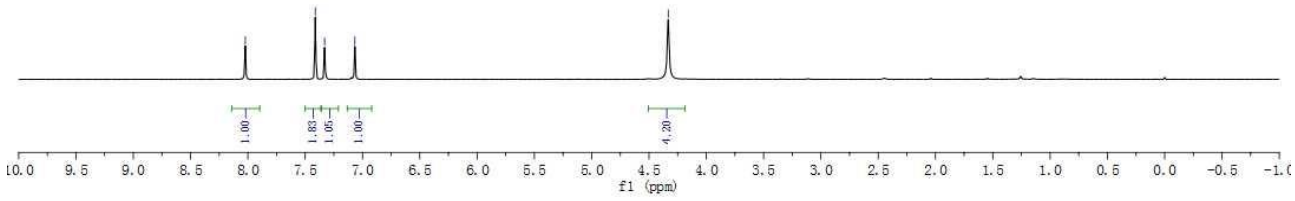
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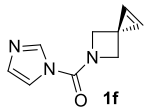
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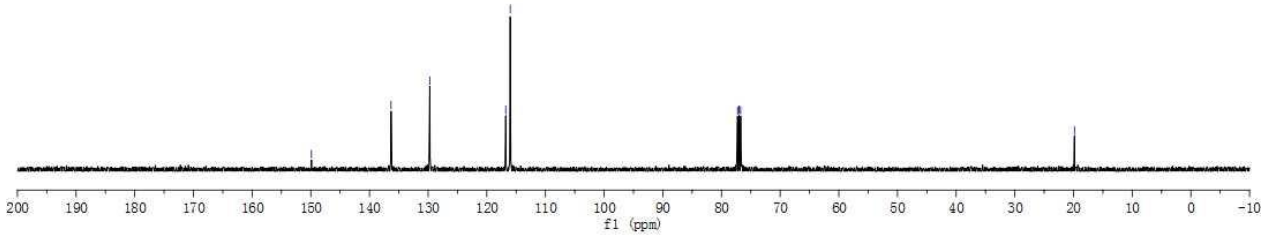
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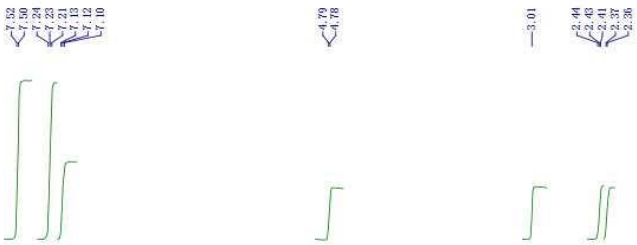
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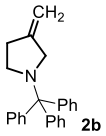
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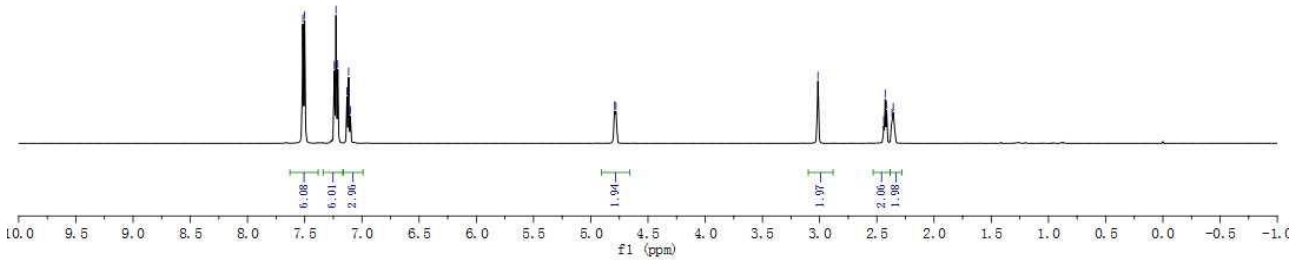
Ph
Ph
Ph

2b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f1 (ppm)


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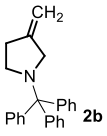
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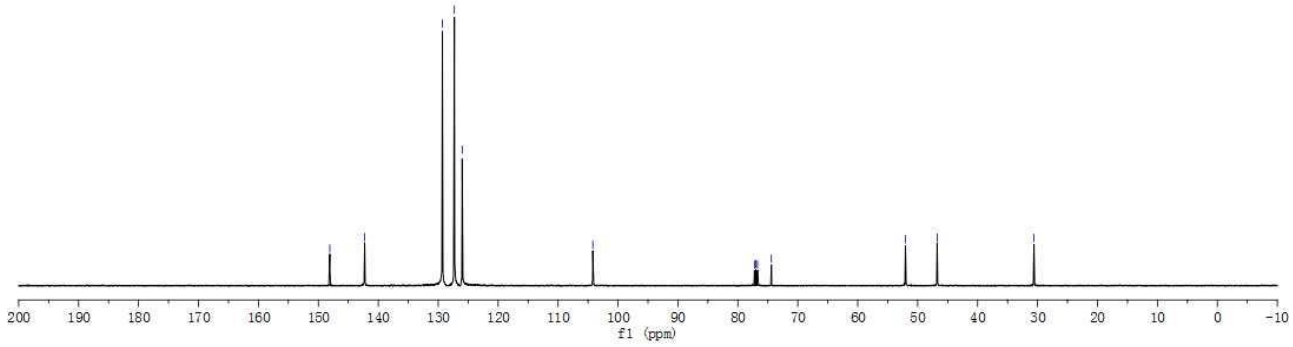
Ph
Ph

2b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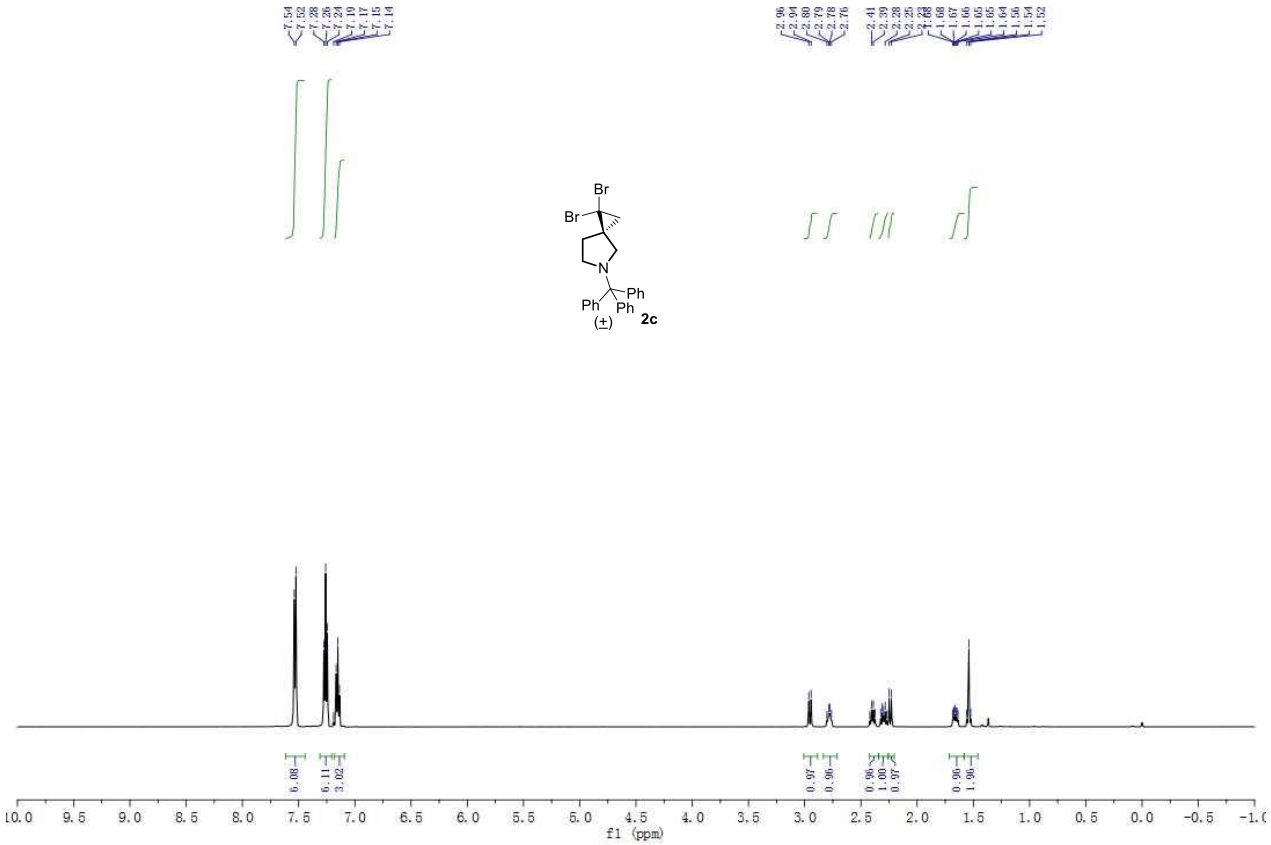
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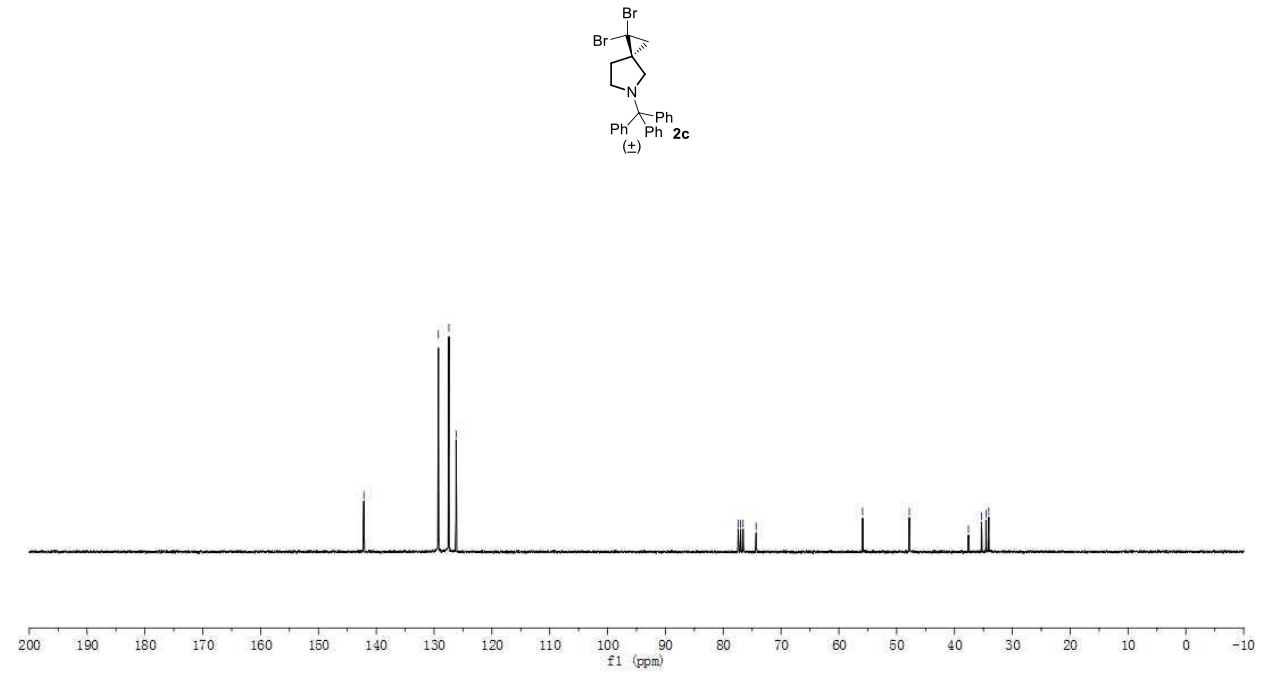
Ph
Ph
Ph
(±)
2c
f1 (ppm)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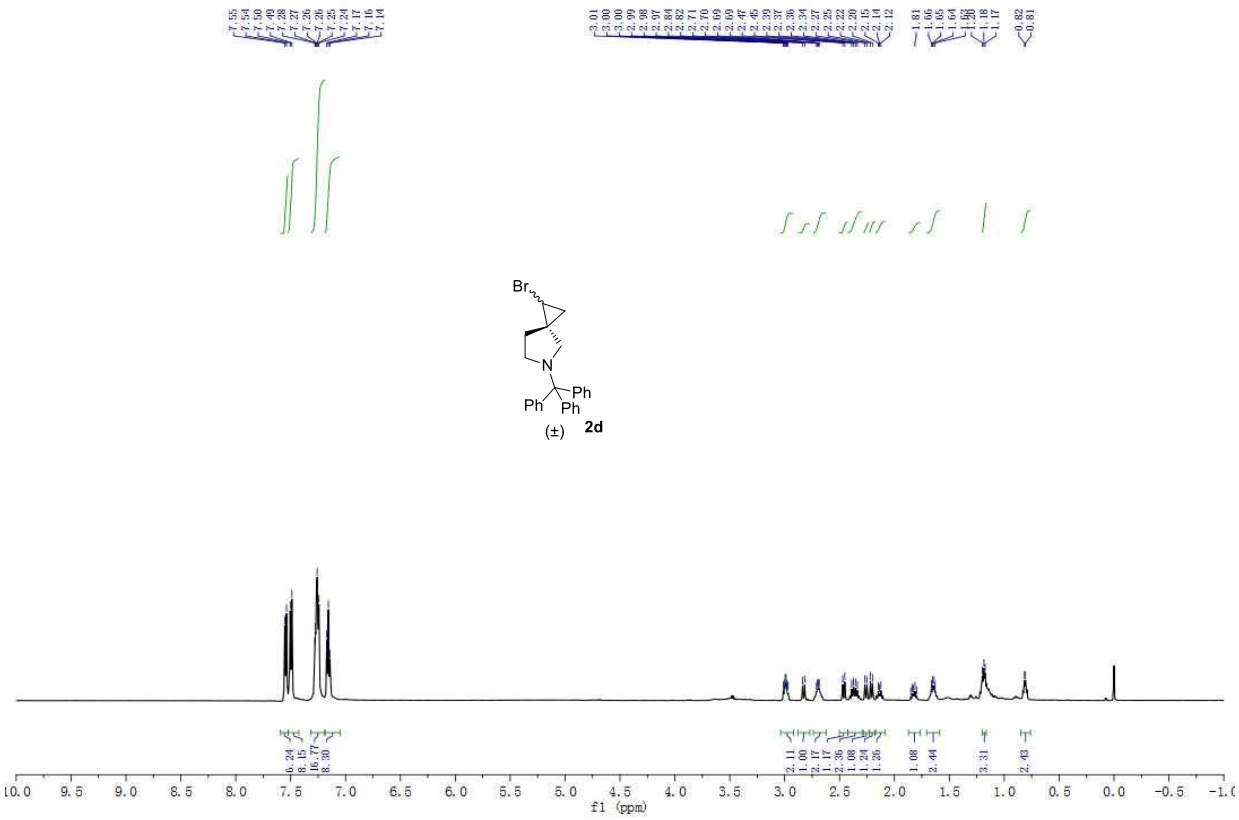
Ph
Ph
Ph
(±)
2c
f1 (ppm)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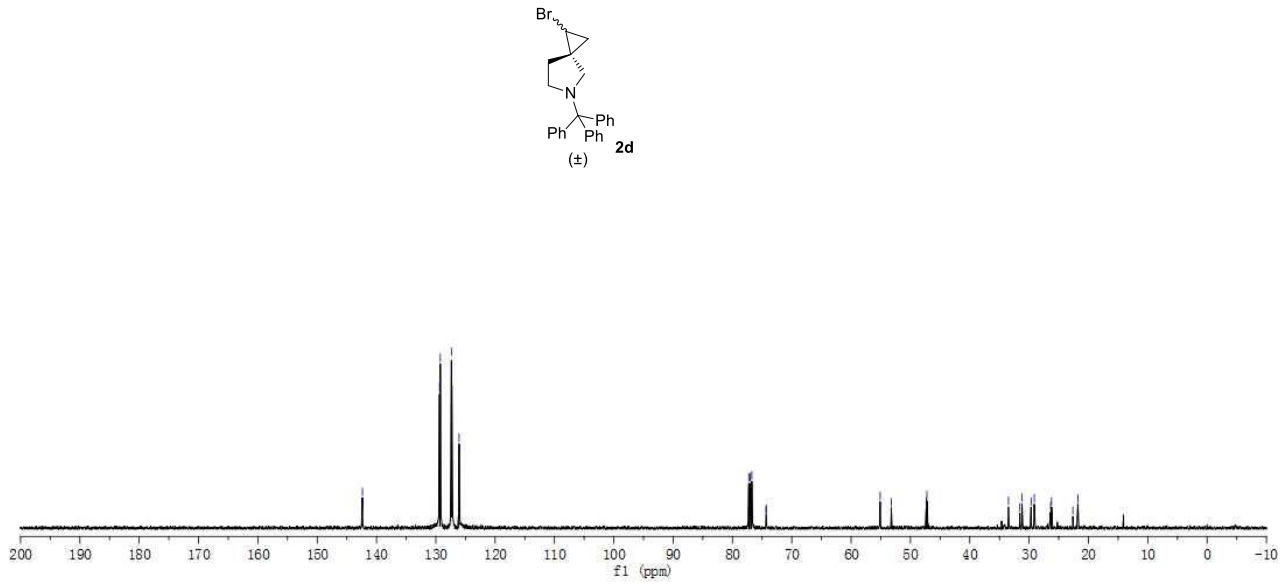
Ph
Ph
Ph
(±) 2d
f1 (ppm)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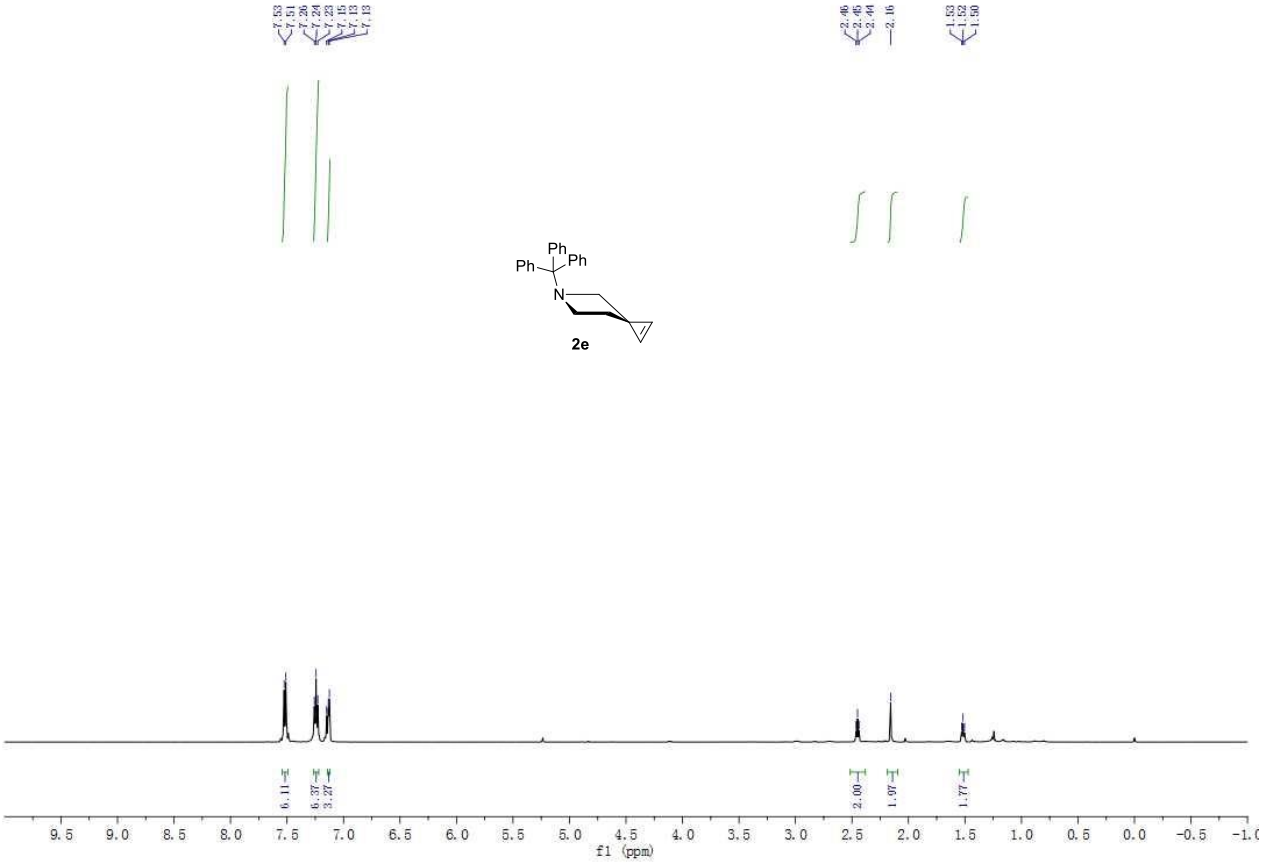
Ph
Ph
Ph
(±) 2d
f1 (ppm)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1.52
1.50
Ph
Ph
Ph
N
2e
6.11
6.37
3.27
2.00
1.07
1.77
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)
142.70
129.04
127.18
125.82
116.69
77.24
76.99
76.73
74.57
55.78
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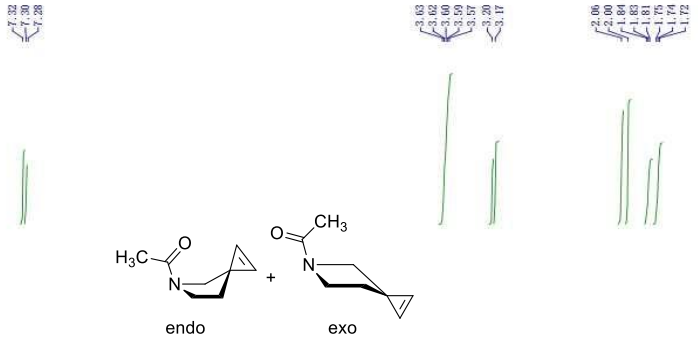
f1 (ppm)



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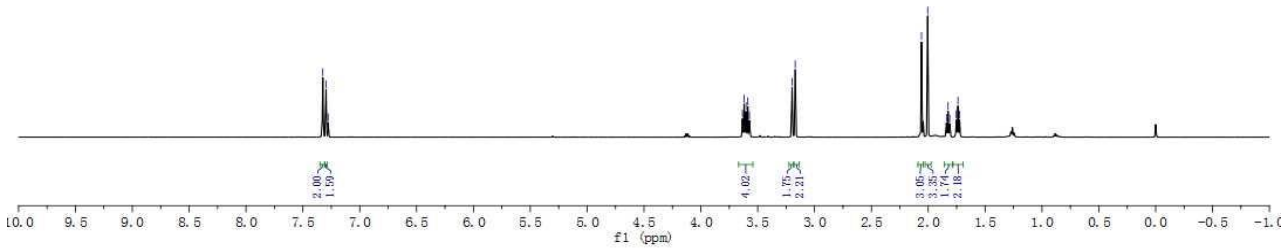

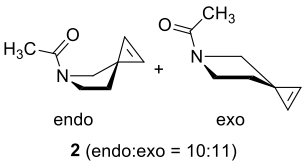

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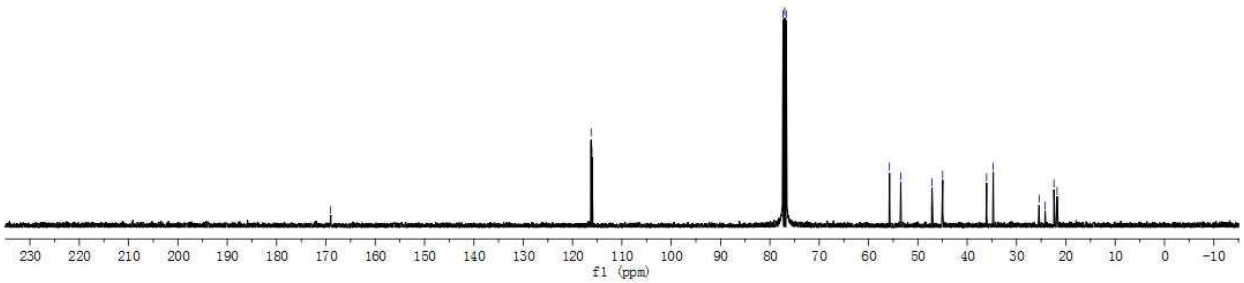
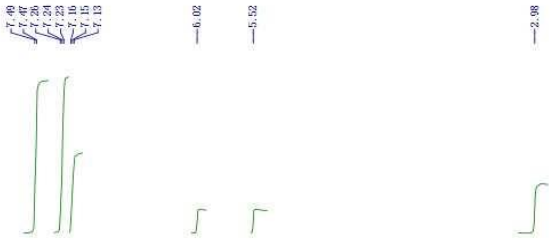
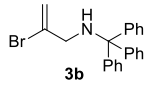
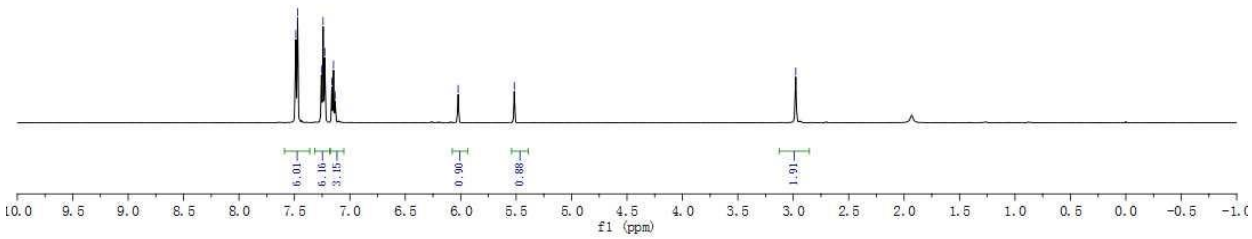
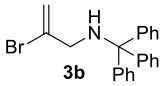






3b





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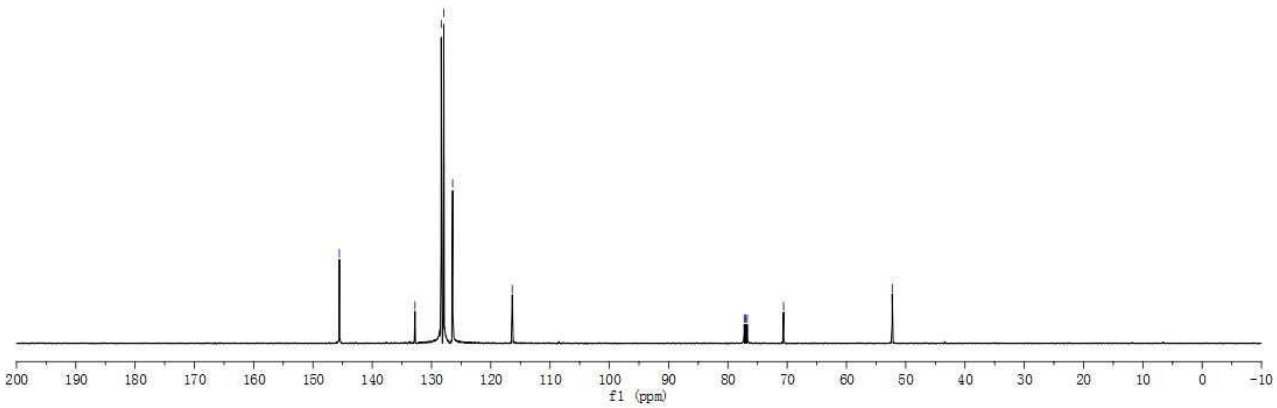

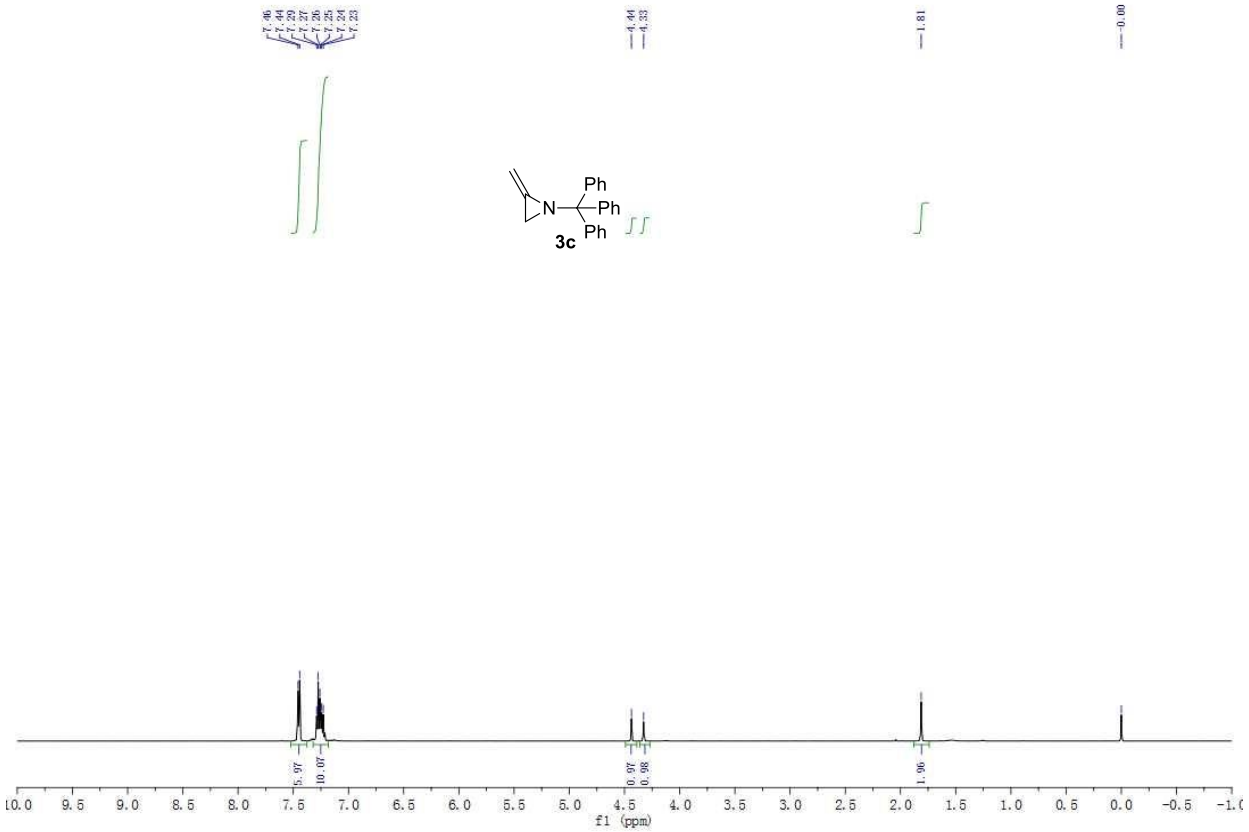
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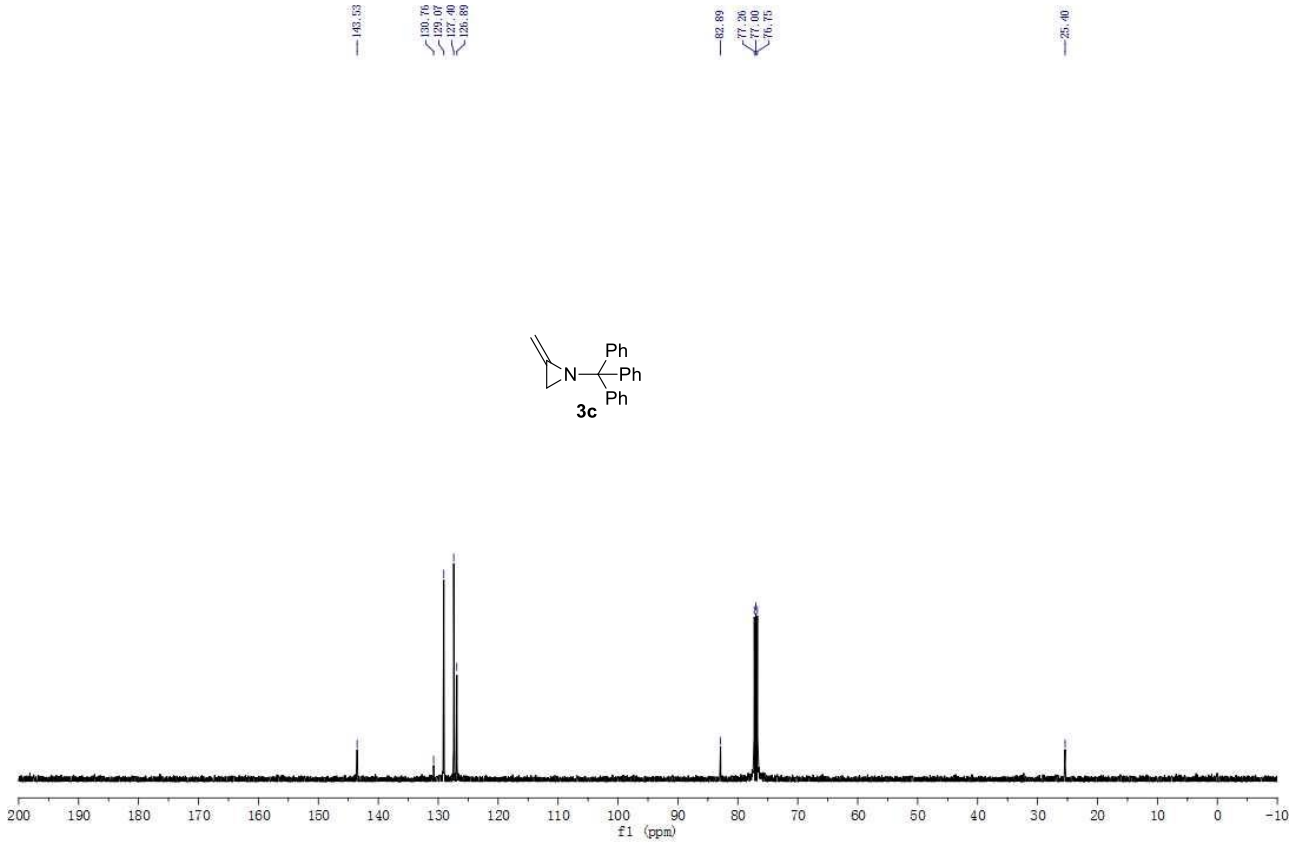
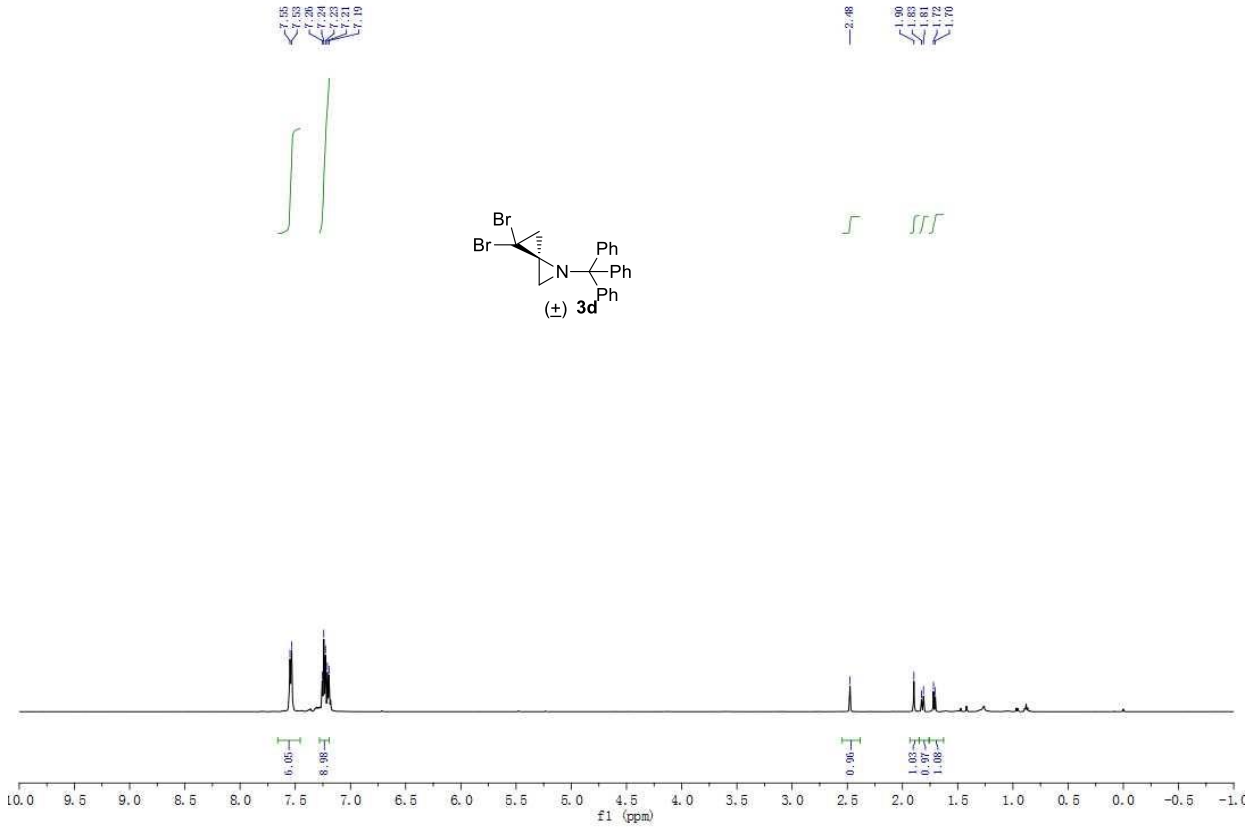
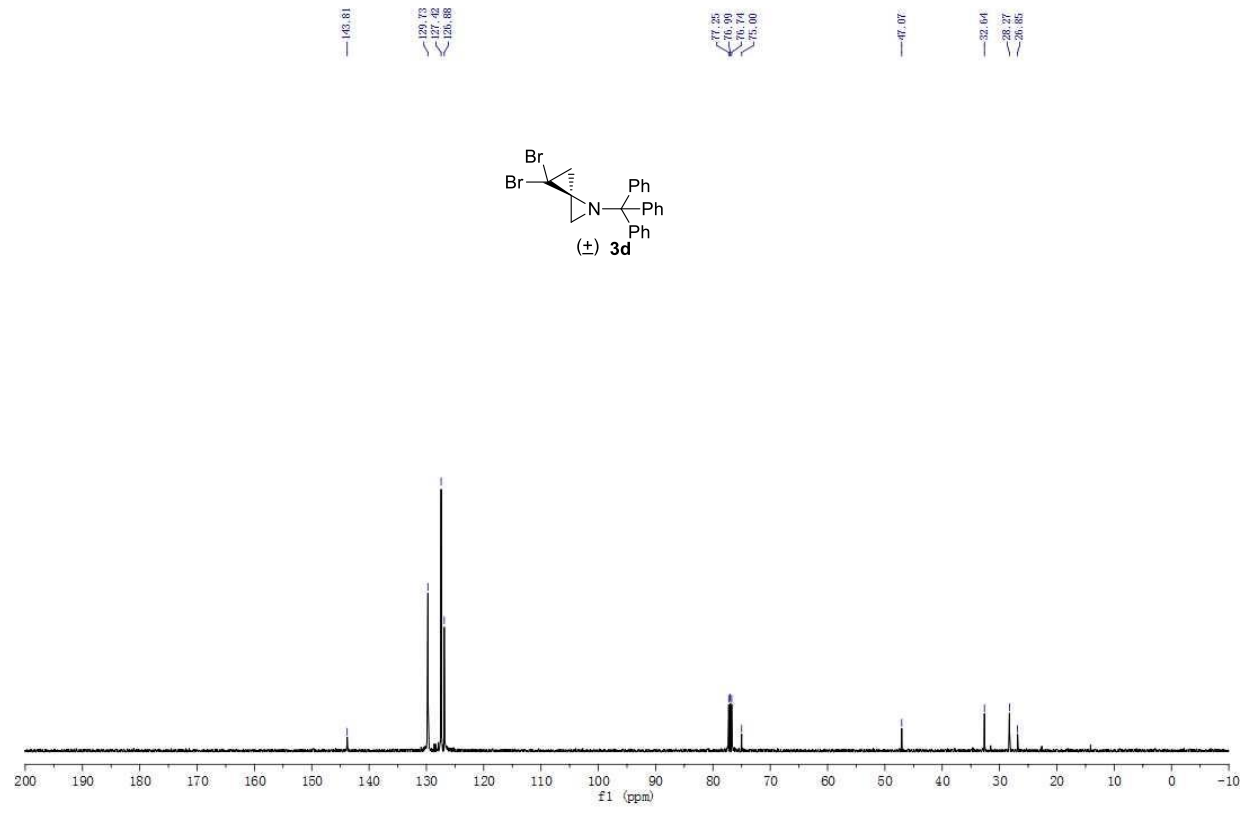
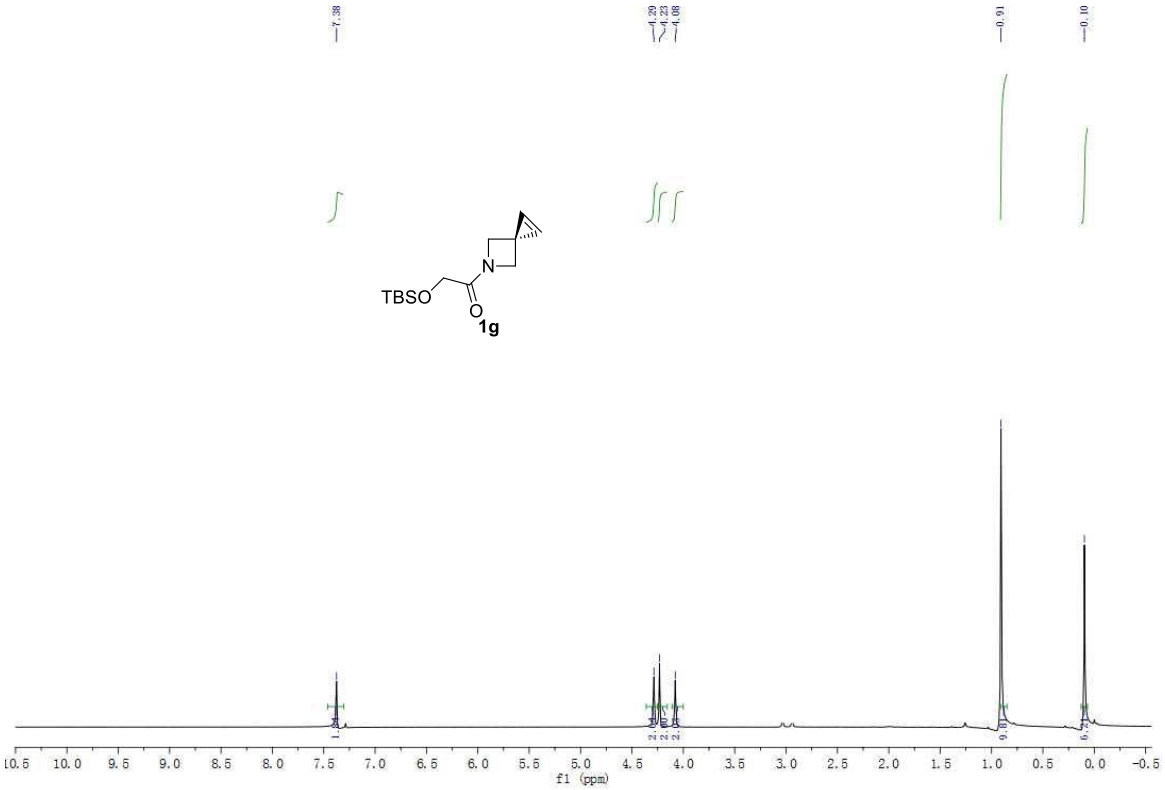
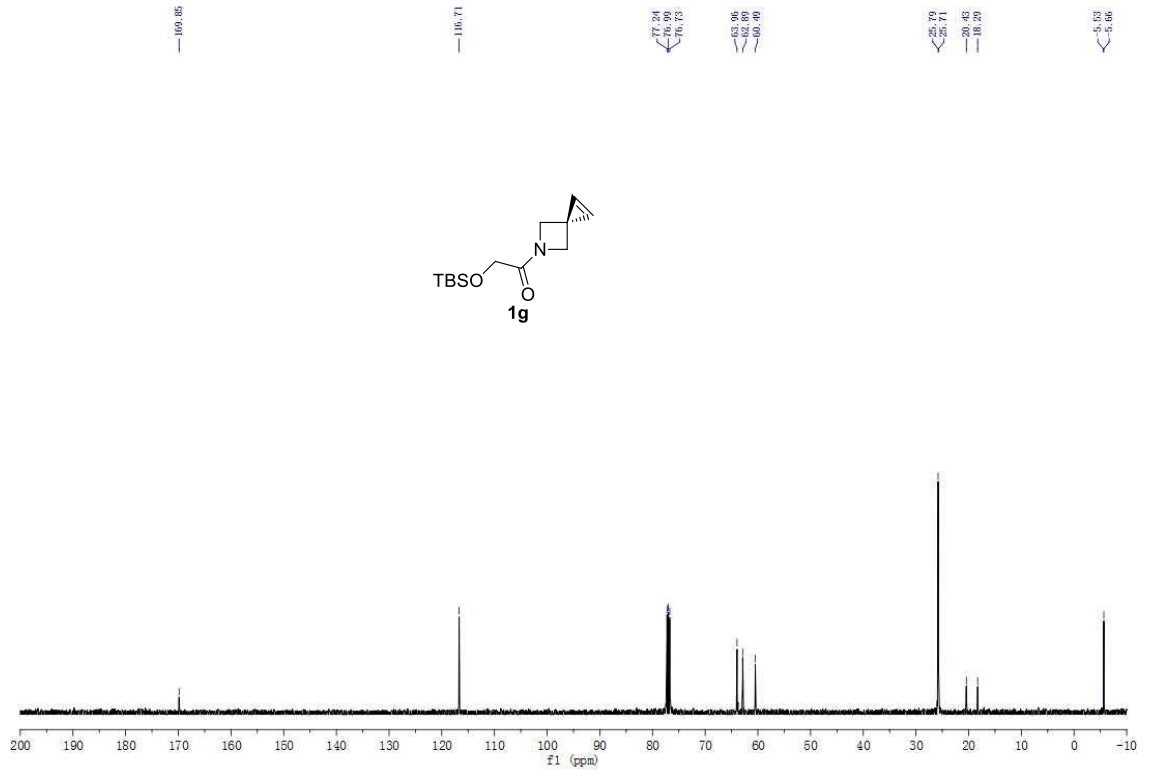
7.23
4.44
4.33
1.81
0.00
Ph
Ph
Ph
N
3c
5.97
10.07
0.97
0.98
1.96
f1 (ppm)

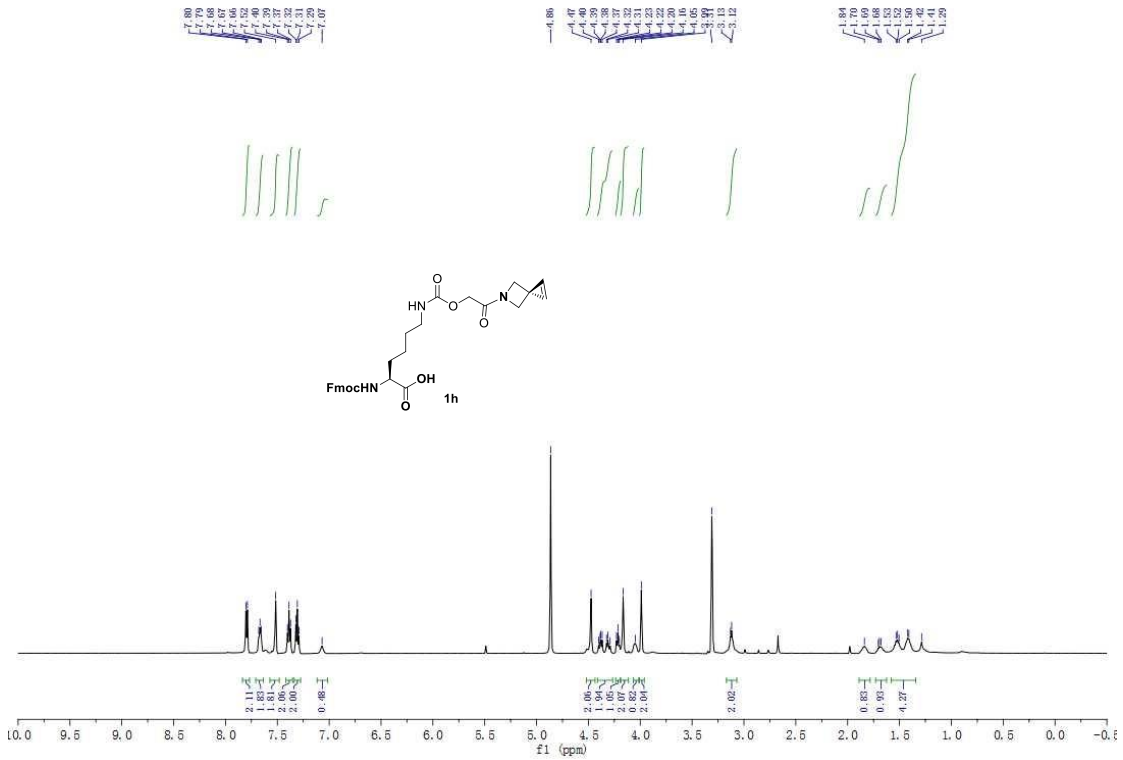

143.53
130.76
129.07
127.40
126.89
82.89
77.26
77.00
76.75
25.40
Ph
Ph
Ph
N
3c
f1 (ppm)

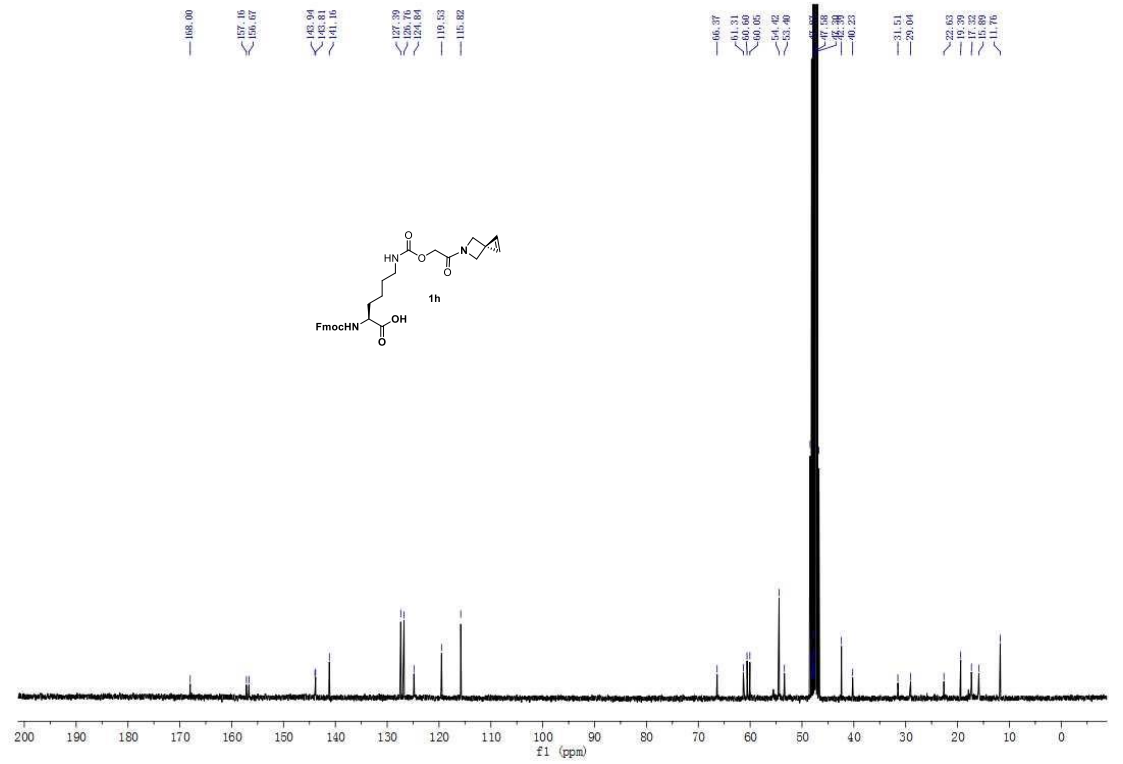

7.55
7.53
7.36
7.24
7.23
7.21
7.19
2.48
1.90
1.83
1.81
1.72
1.70
Br
Br
Ph
Ph
Ph
N
(±) 3d
6.05
8.98
0.96
1.03
0.97
1.08
f1 (ppm)

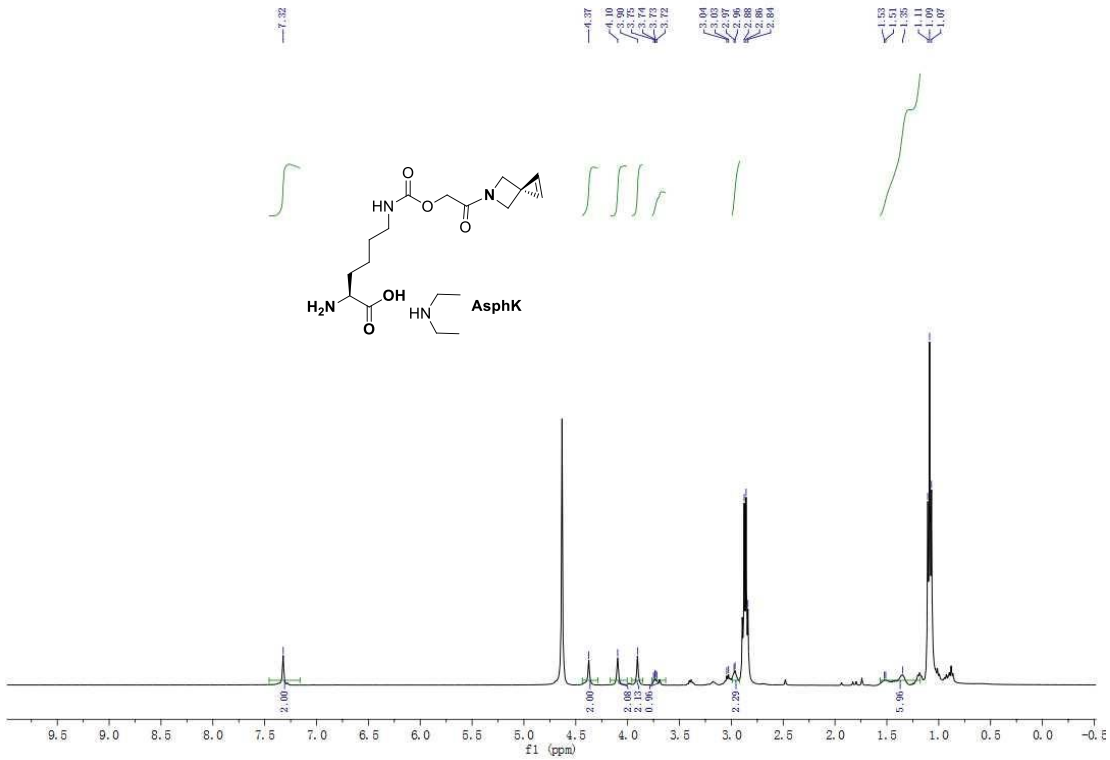

143.81
129.73
127.62
126.88
77.25
76.99
76.74
75.00
47.07
32.64
28.27
26.85
Br
Br
Ph
Ph
Ph
N
(±) 3d
f1 (ppm)

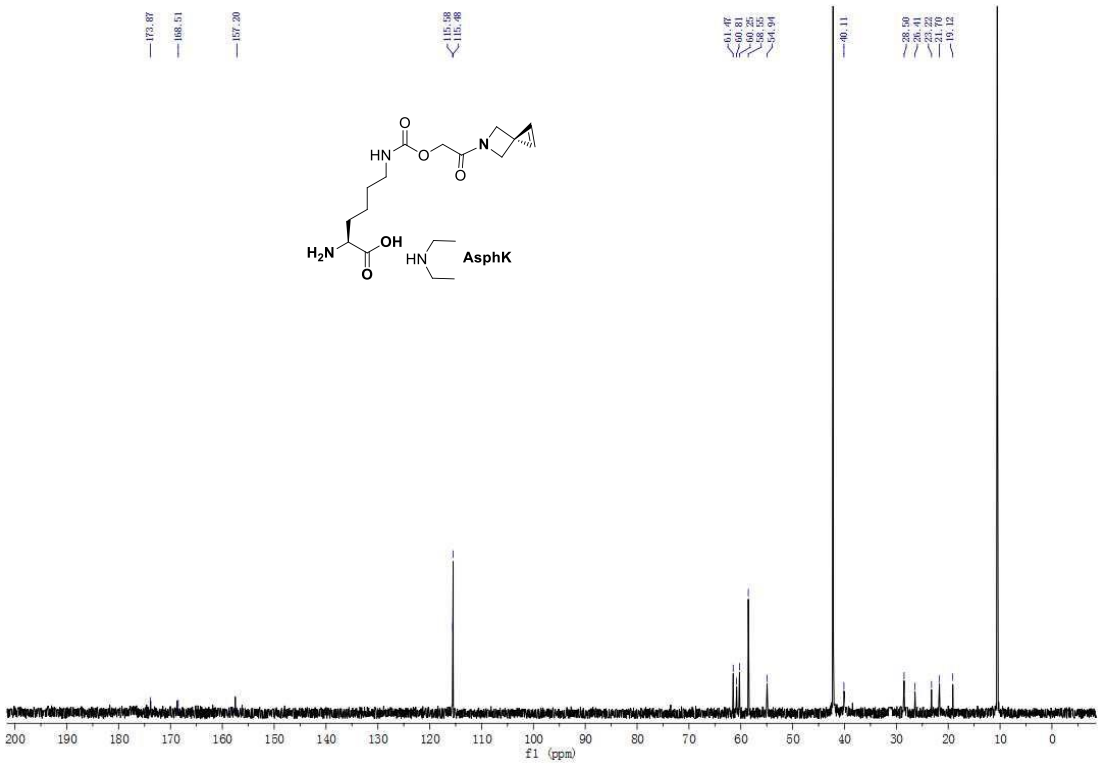

7.38
4.29
4.23
4.08
0.91
0.10
TBSO
O
N
1g
f1 (ppm)


169.85
116.71
77.24
76.99
76.73
63.96
62.89
60.49
25.79
25.71
20.43
18.29
-5.53
-5.66
TBSO
O
N
1g
f1 (ppm)


FmocHN
OH
1h
f1 (ppm)


1h
FmocHN
OH
f1 (ppm)


7.32
4.37
4.10
3.90
3.75
3.74
3.73
3.72
3.04
3.03
2.97
2.96
2.88
2.86
2.84
1.53
1.51
1.35
1.11
1.09
1.07
HN
O
O
N
O
H2N
OH
HN
AsphK
2.00
2.00
2.08
2.13
0.96
2.29
5.96
f1 (ppm)


173.87
168.51
157.20
115.58
115.48
61.47
60.91
60.25
58.55
54.94
40.11
28.50
26.41
23.22
21.79
19.12
HN
O
O
N
O
H2N
OH
HN
AsphK
f1 (ppm)