Electronic Supplementary Material (ESI) for Analyst.

Supplementary Information

Stereospecific recognition of a chiral centre over multiple flexible covalent bonds by ^{19}F-NMR

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Materials and methods

Sample preparation

Preparation of the ^{19}F-tag and analytes. The ^{19}F-tag and analytes were prepared as a 50 mM stock in various solvents (toluene, chloroform, acetonitrile, acetone, and dimethyl sulfoxide) and used in subsequent NMR experiments.

The ^{19}F-chemical shift determination of the ^{19}F-tag derivatized enantiomeric analytes. 1.0 mM each enantiomeric analyte was mixed with 0.2 mM ^{19}F-tag in chloroform except for several amines (Fig 2d, 2e, 2f, 2g) in aqueous solution with 0.5 mM analyte and 1.0 mM (Fig 2d, 2f, 2g) or 3.0 mM (Fig 2e) ^{19}F-tag. The resulting mixture was incubated for 6 h at room temperature before recording NMR spectra.

The solvent effects on ^{19}F chemical shifts of the ^{19}F-tag derivatized analytes. Three pair enantiomers, phenylglycinol, phenylpropylamine, and 2-amino-1,2-diphenylethanol were treated with the ^{19}F-tag in different organic solvents (toluene, chloroform, acetonitrile, acetone, and dimethyl sulfoxide) at room temperature for 6 h. The concentration of the ^{19}F-tag was 0.5 mM, and the concentration of the enantiomeric analytes was 1.0 mM, respectively.

Simultaneous identification of 18 enantiomeric analytes by the ^{19}F-tag. The solution of 18 analytes (each 0.1 mM) was mixed with 2.0 mM ^{19}F-tag in chloroform, and the reaction mixture was incubated at room temperature for 6 h followed by NMR measurement.

Quantitative determination of enantiomeric excess values. Several working solutions of standard two pair enantiomers, 1,2,3,4-tetrahydronaphthalen-1-amine and 1-(4-chlorophenyl)ethan-1-amine with different concentrations were prepared. The concentration of the ^{19}F-tag was 2.0 mM and the concentrations of enantiomeric analytes were 0.05-0.95 mM. The reaction mixture was incubated at 35 °C for 6 h followed by NMR measurement. The *ee* (%) value was calculated as the equation, *ee* (%) = $100 \times \frac{[S]-[R]}{[S]+[R]}$, of which [*R*] and [*S*] represent the concentrations of *R*-analytes and *S*-analytes, respectively. The *ee* value is negative when [*R*] is larger than [*S*]. The *ee* value was calculated according to the integral area of the ^{19}F peak of the amide products. The theoretical *ee* value was determined according to the gravimetrically prepared samples, and the measured errors were determined based on the signal-noise ratio of the ^{19}F signals.

Quantitative performance of the amide product by the internal standard. Commercial hexafluorobenzene was used as internal standard for quantification of chiral analytes. A mixed solution of ^{19}F-tag, hexafluorobenzene and (S)-(+)-2-amino-1-propanol with different concentrations was prepared. The concentrations of internal standard were 0.01-0.3 mM, the concentrations of ^{19}F-tag were 0.1-2.0 mM and the concentrations of (*S*)-(+)-2-amino-1-propanol were 0.2-4.0 mM. The non-proton-decoupled ^{19}F-NMR spectra were recorded with a ^{19}F frequency of 753 MHz after 6 h incubation of ^{19}F-tag with (*S*)-(+)-2-amino-1-propanol at 35 °C in $CDCl_3$. The equation used to calculate the concentration of amide product according to the ^{19}F-tag concentration was: $[\text{Amide}]_{\text{tag}} = [\text{tag}] \times \frac{a}{b}$, of which [tag] represent the concentration of the ^{19}F-tag, a represent the ^{19}F-NMR signal integral area of the amide product and b represent the sum of the integral area of all ^{19}F-NMR signals except hexafluorobenzene. The equation used to calculate the

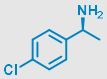
concentration of amide product according to the concentration of internal standard was: $[\text{Amide}]_{\text{internal}} = [\text{internal}] \times \frac{6a}{b'}$, of which [internal] represent the concentration of internal standard, a represent the ^{19}F-NMR signal integral area of the amide product and b' represent the ^{19}F-NMR signal integral area of the internal.

LC-MS analysis. 3.0 mM (*S*)-(+)-2-amino-1-propanol was incubated with 1.0 mM the ^{19}F-tag after 6 h at 35 °C in $CDCl_3$. The reaction mixture was then analyzed through liquid chromatography equipped with a reverse phase C18 column followed by ESI-MS analysis. The mobile phase (95% CH_3CN+5% H_2O) was used at a flow rate of 0.4 mL/min with a linear gradient from 5% H_2O to 100% H_2O within 10 min and 100% H_2O for 10-15 min. The products formed by (*S*)-(+)-2-amino-1-propanol and ^{19}F-tag were obtained with a 4.77 min retention time.

NMR experiments

The proton decoupled ^{19}F-NMR spectra of the ^{19}F-tag derivatized analytes in various solvents were recorded with a Bruker 400 MHz NMR (376 MHz for ^{19}F) spectrometer at 298K. The 2D NOESY spectrum was recorded with a standard noesygpph pulse program with a mixing time of 600 ms, and the 2D TOCSY spectrum with a standard mlevgpph pulse program with mixing time of 80 ms at 298K using a Bruker 800 MHz NMR spectrometer equipped with a TCI-cryoprobe.

Supplementary Table S1: The correlation of the actual *ee* value and the calculated *ee* value according to the ^{19}F-NMR for 1,2,3,4-tetrahydronaphthalen-1-amine and 1-(4-chlorophenyl)ethan-1-amine enantiomers.

 (*S*)-1,2,3,4-tetrahydronaphthalen-1-amine			 (*S*)-1-(4-chlorophenyl)ethan-1-amine		
actual ee (%)	calculated ee (%)	measured error (%)	actual ee (%)	calculated ee (%)	measured error (%)
90.0	90.2	0.5	90.0	91.7	0.4
60.0	60.8	0.4	60.0	59.7	0.4
30.0	29.7	0.4	30.0	28.9	0.7
0.0	0.4	0.6	0.0	-0.3	0.6
-30.0	-29.7	0.5	-30.0	-31.3	0.4
-60.0	-59.9	0.3	-60.0	-59.1	0.5
-90.0	-90.6	0.5	-90.0	-91.9	0.3

The concentration of the ^{19}F-tag was 2.0 mM and the concentrations of *R*- and *S*- chiral analytes were in the range of 0.05-0.95 mM. The proton decoupled ^{19}F NMR spectra were recorded with a ^{19}F frequency of 376 MHz after 6 h incubation for reaction mixture at 35 °C in $CDCl_3$. The errors of calculated *ee* values were determined based on the signal-noise ratio of the ^{19}F signals.

Supplementary Figures

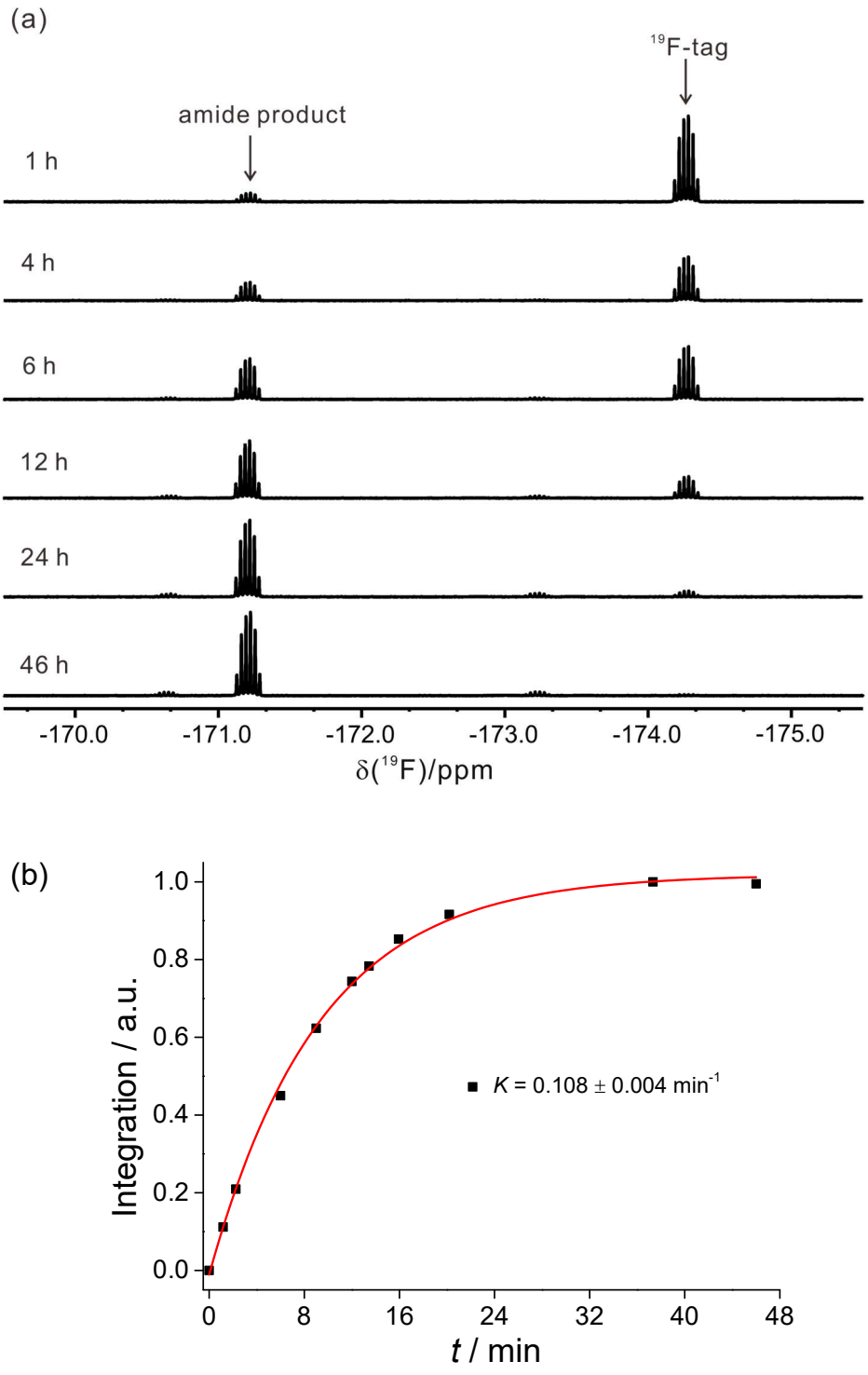


Figure S1. The kinetic formation curve of amide product monitored for the sample of 0.5 mM ^{19}F-tag after incubation with 2.5 mM (*S*)-(+)-2-amino-1-propanol in $CDCl_3$ at room temperature. The apparent formation rate was fitted by the single exponential function of ^{19}F-NMR signal integral area of the amide product with the incubation time. The non-proton-decoupled ^{19}F-NMR spectra were recorded with a ^{19}F frequency of 753 MHz at 298K.

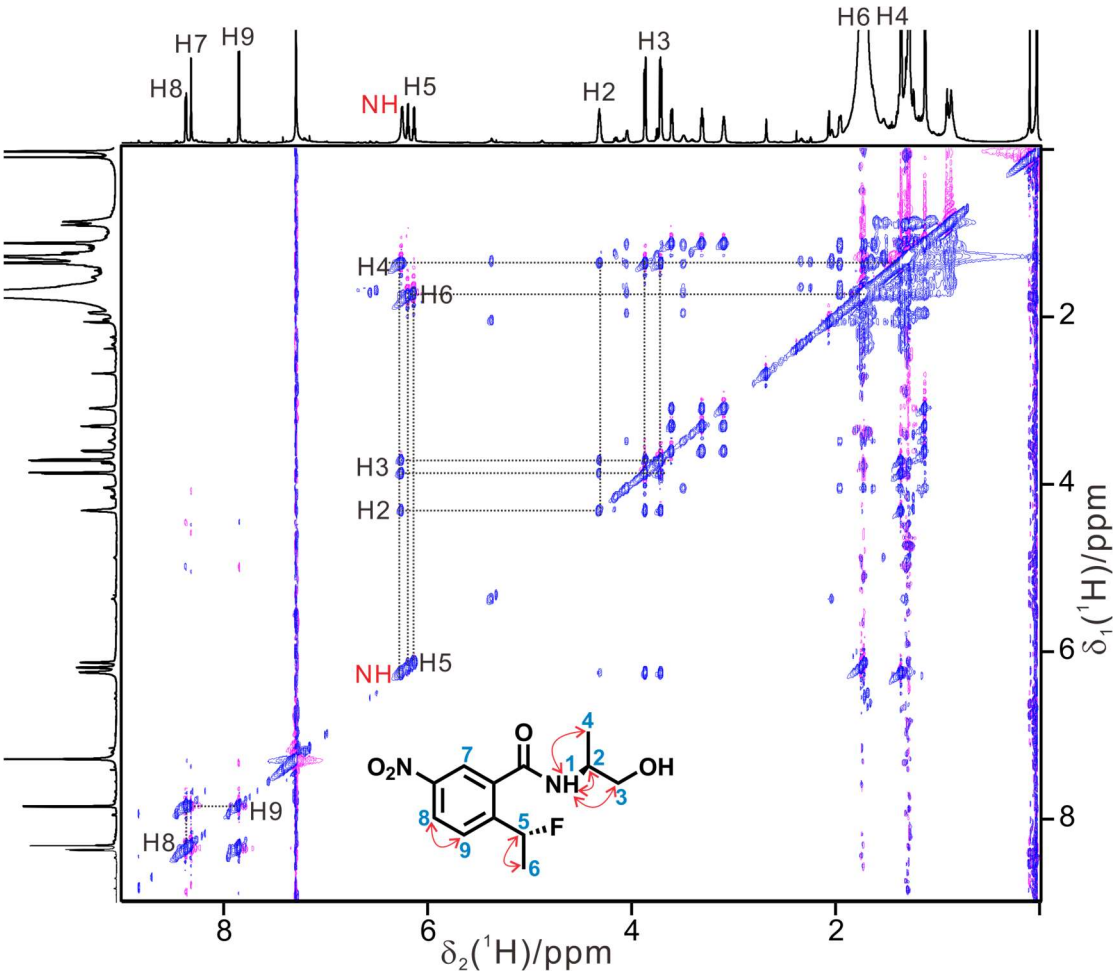


Figure S2. ^{1}H-^{1}H TOCSY spectrum was recorded for the reaction mixture of the ^{19}F-tag and the (*S*)-(+)-2-amino-1-propanol in $CDCl_3$, and the key resonances were assigned and labeled in the spectrum. The chemical structure of the amide product was shown in the inset. The correlation of resonance signals in the structure of the amide product was depicted as red arrows. The resonance signal of H6 overlapped with the peak of residual water in the solvent. The concentration of ^{19}F-tag was 1.0 mM and the concentration of (*S*)-(+)-2-amino-1-propanol was 3.0 mM. The spectrum was recorded with a proton frequency of 800 MHz after 6 h incubation for the reaction mixture at 35 °C in $CDCl_3$.

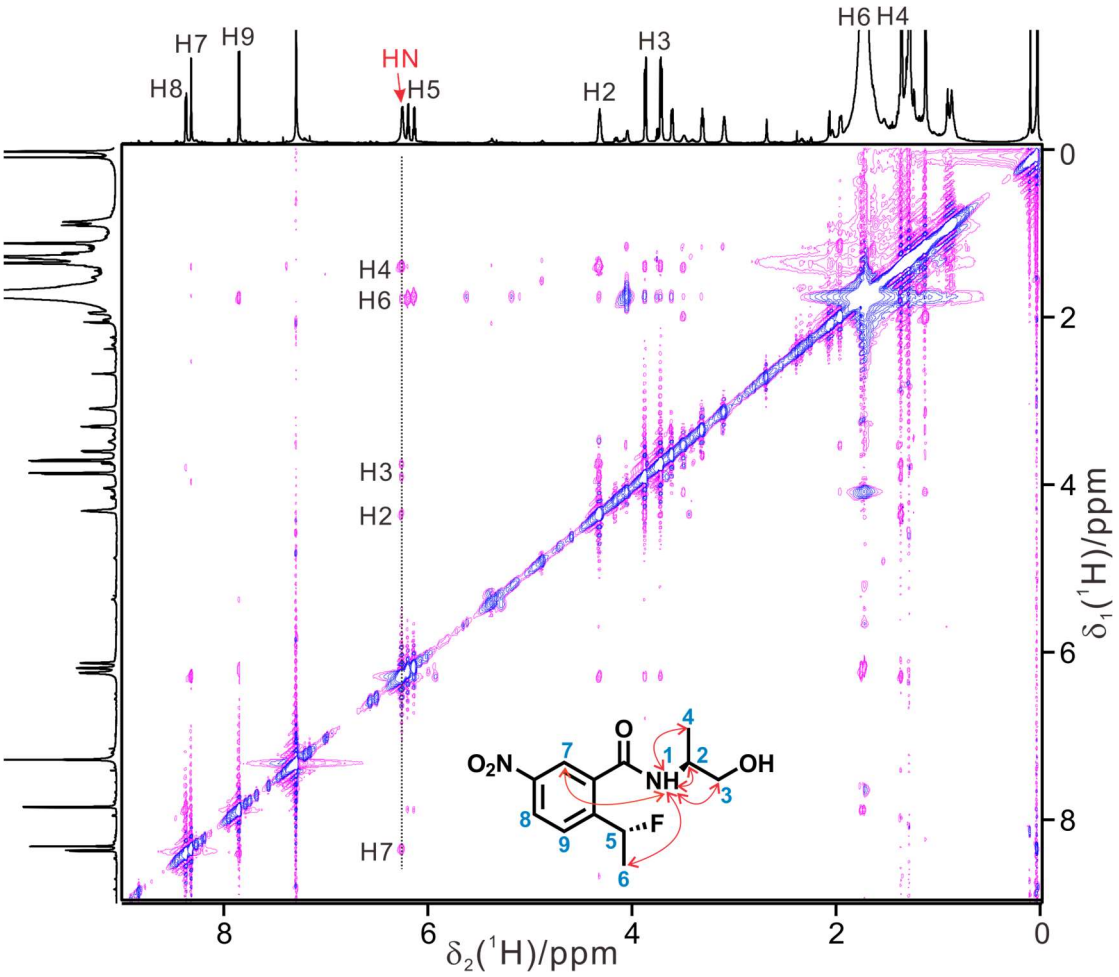


Figure S3. ^{1}H,^{1}H-NOESY spectrum was recorded for the reaction mixture of the ^{19}F-tag and the (*S*)-(+)-2-amino-1-propanol in $CDCl_3$. The NOE pattern of amide proton and other resonance signals in the amide product was shown as red arrows in the chemical structure. The resonance signal of H6 overlapped with the peak of residual water in the solvent. The sample was the same as that in Figure S2.

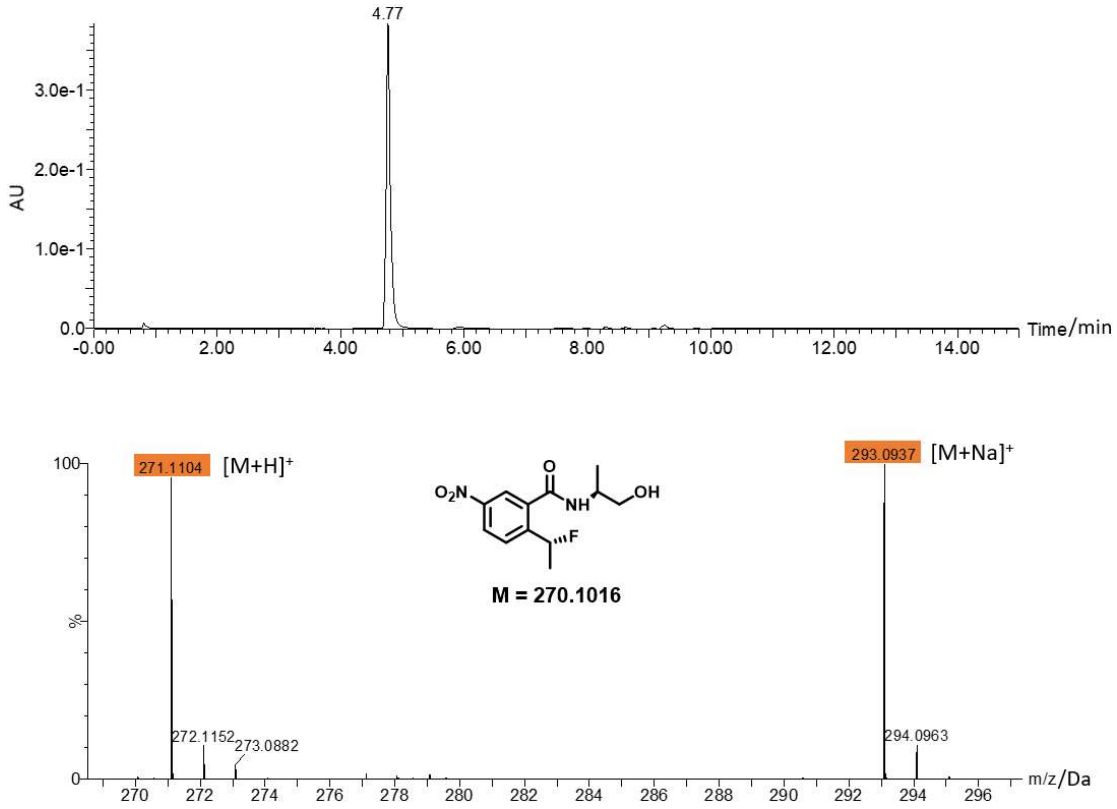


Figure S4. LC-MS spectrum was recorded for the (*S*)-(+)-2-amino-1-propanol treated by the ^{19}F-tag. The amine products were obtained through liquid chromatography equipped with a reverse phase C18 column followed by ESI-MS analysis. The chromatography profile recorded for the reaction mixture was shown in the top panel, and the molecular mass of the amide product was shown below. The sample was the same as that in Figure S2.

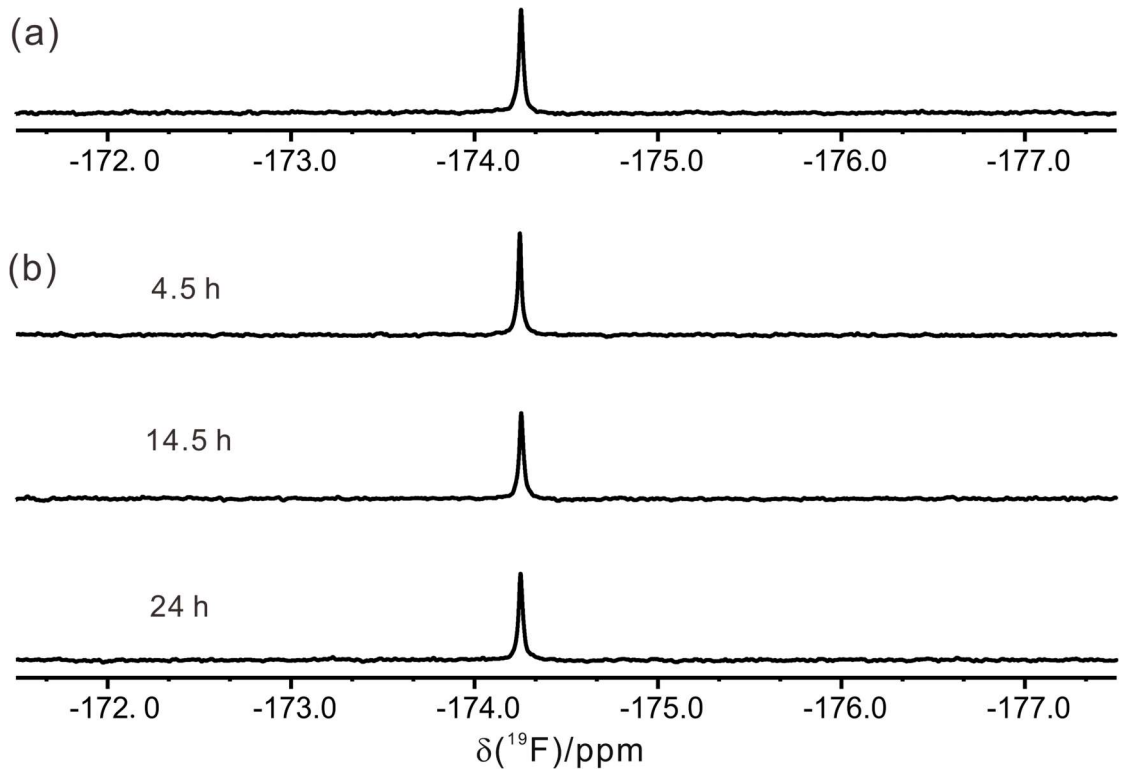


Figure S5. (a) The ^{19}F-NMR spectra of the ^{19}F-tag in $CDCl_3$. (b) Time-dependent of ^{19}F-NMR spectra recorded for the mixture of the ^{19}F-tag and (*S*)-(-)-*N*alpha-dimethylbenzylamine at 35 °C in $CDCl_3$. The concentrations of the ^{19}F-tag and (*S*)-(-)-*N*alpha-dimethylbenzylamine were 1.0 mM. The proton-decoupled ^{19}F-NMR spectra were recorded at a magnetic field with a ^{19}F frequency of 376 MHz.

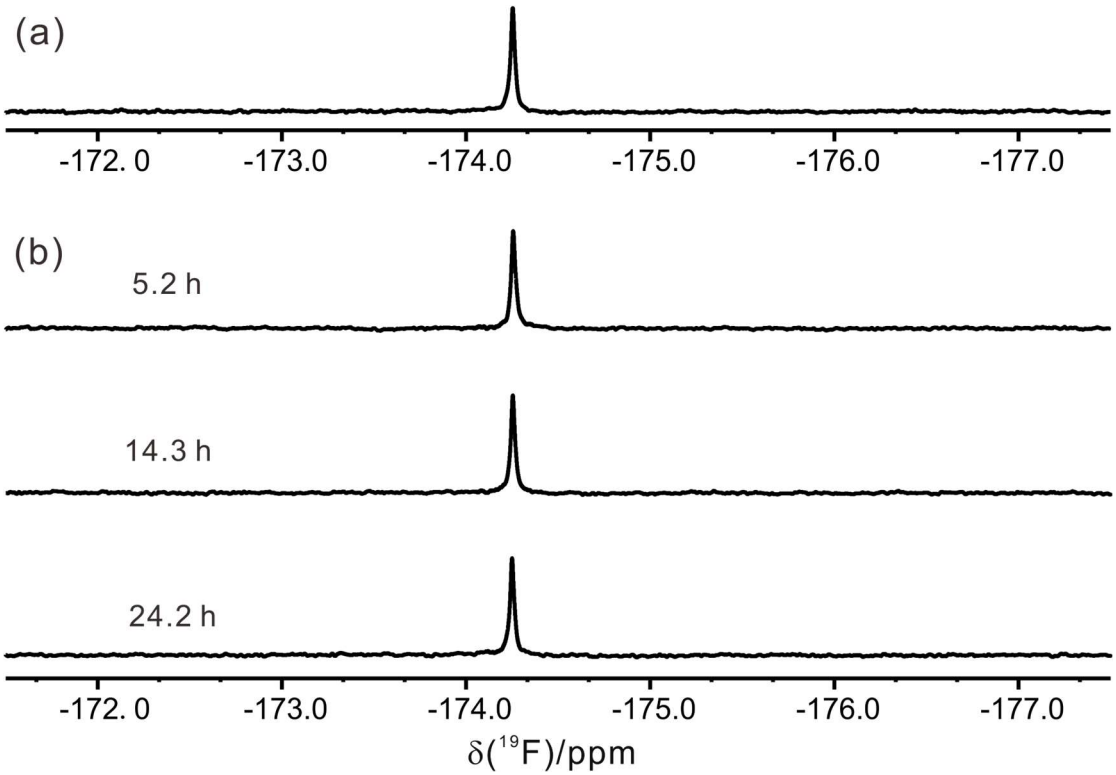


Figure S6. (a) The ^{19}F-NMR spectra of the ^{19}F-tag. (b) Time-dependent of ^{19}F-NMR spectra recorded for the mixture of the ^{19}F-tag and (*R*)-(+)-*N*alpha-dimethylbenzylamine at 35 °C in $CDCl_3$. The concentrations of the ^{19}F-tag and (*R*)-(+)-*N*alpha-dimethylbenzylamine were 1.0 mM. The proton-decoupled ^{19}F-NMR spectra were recorded at a magnetic field with a ^{19}F frequency of 376 MHz.

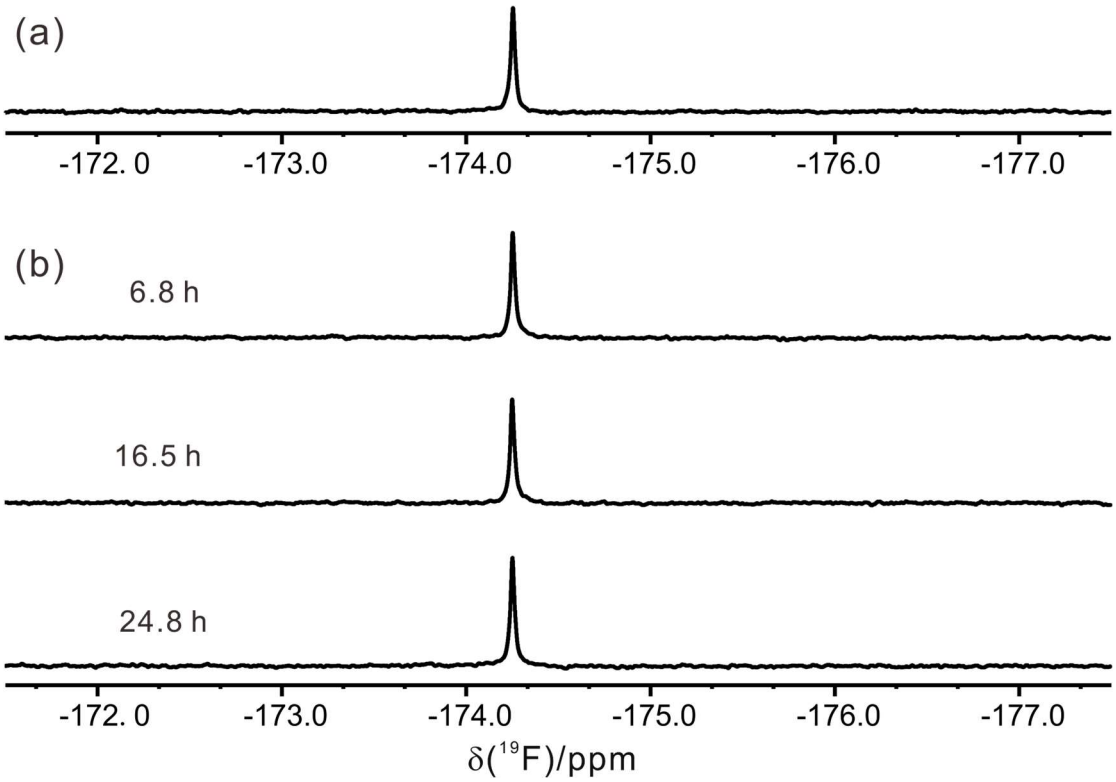
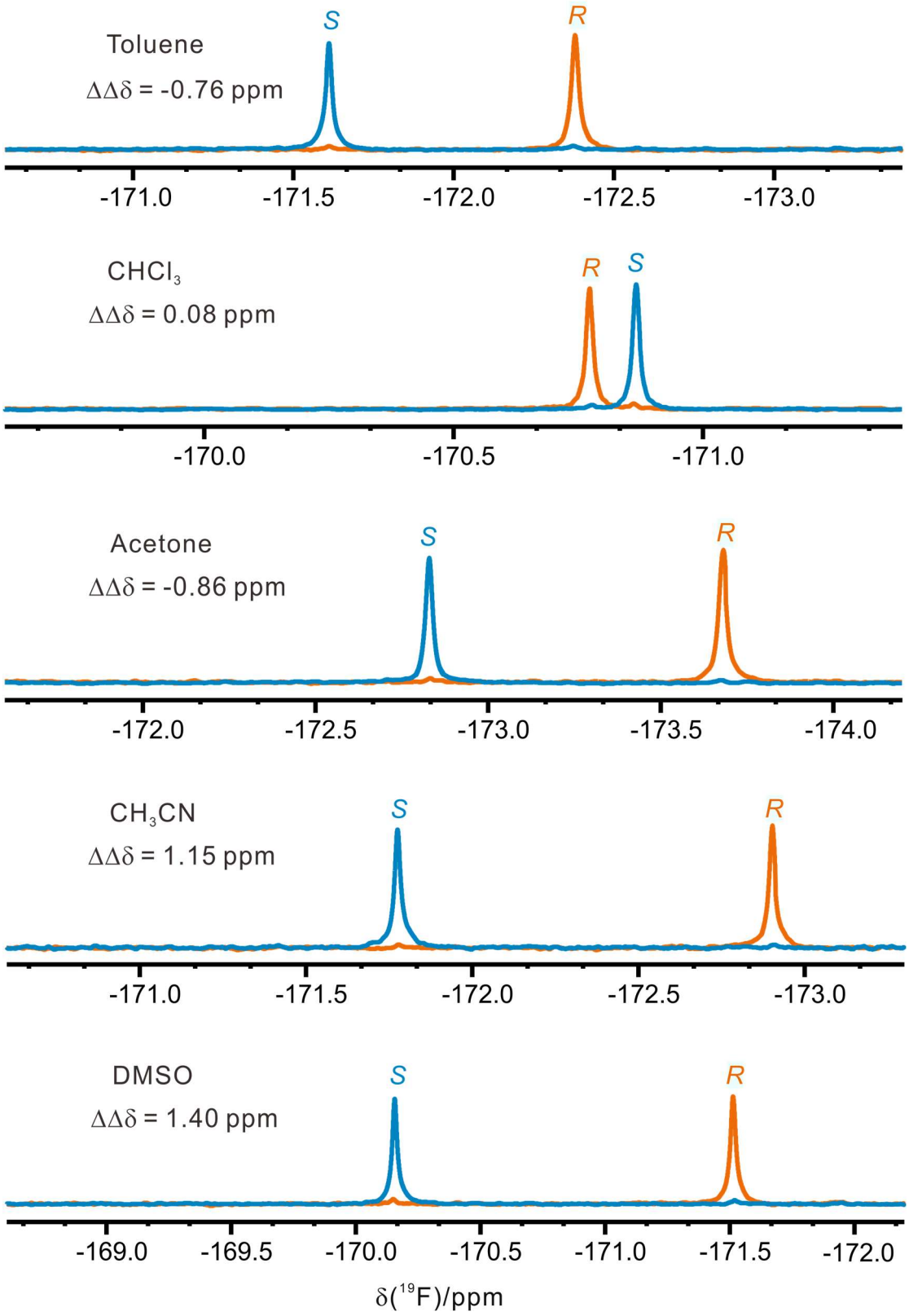
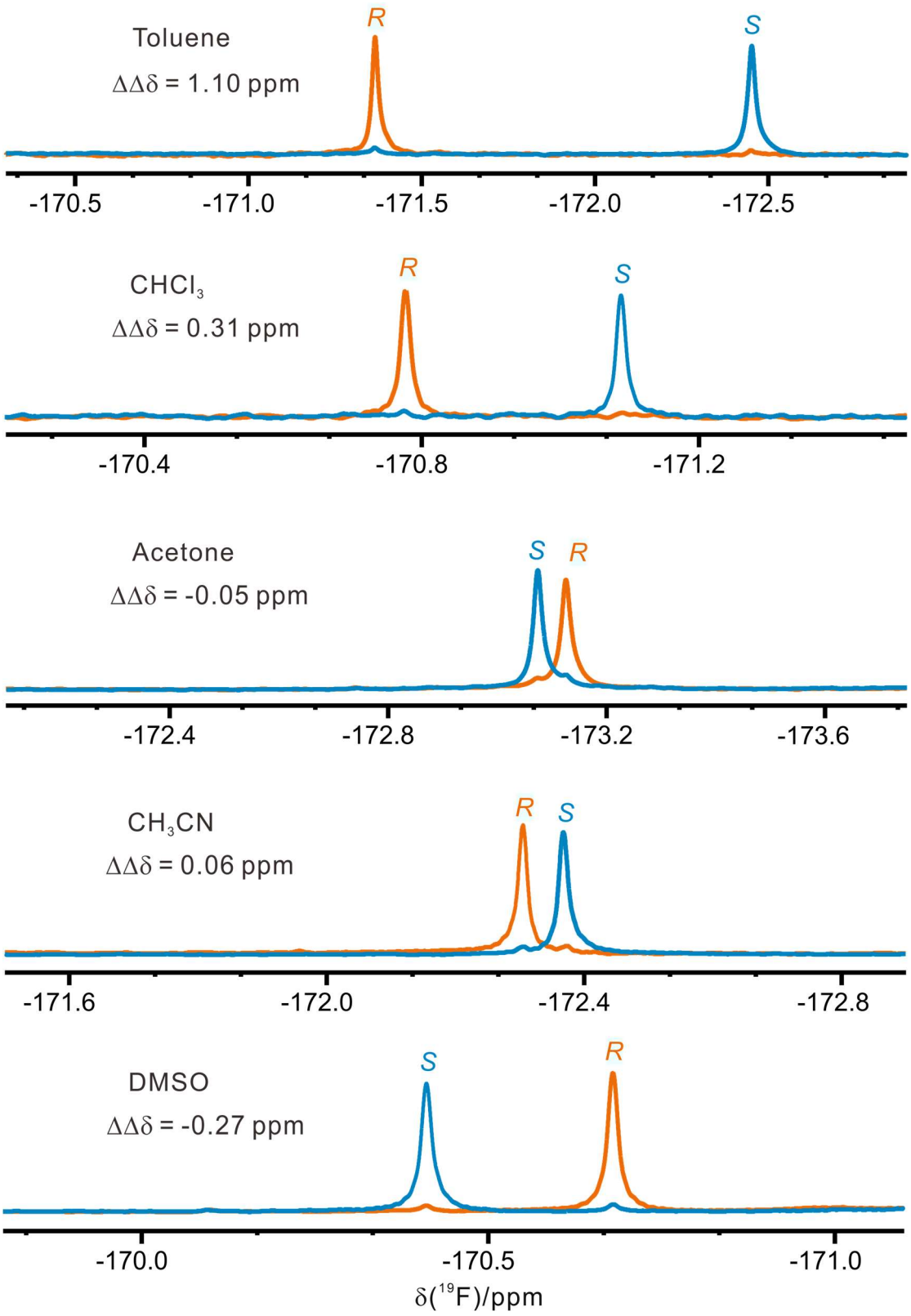


Figure S7. (a) The ^{19}F-NMR spectra of the ^{19}F-tag. (b) Time-dependent of ^{19}F-NMR spectra recorded for the mixture of the ^{19}F-tag and *N,N*-dimethylbenzylamine at 35 °C in $CDCl_3$. The concentrations of the ^{19}F-tag and *N,N*-dimethylbenzylamine was 1.0 mM. The proton-decoupled ^{19}F-NMR spectra were recorded at a magnetic field with a ^{19}F frequency of 376 MHz.

a: phenylglycinol



b: phenylpropylamine



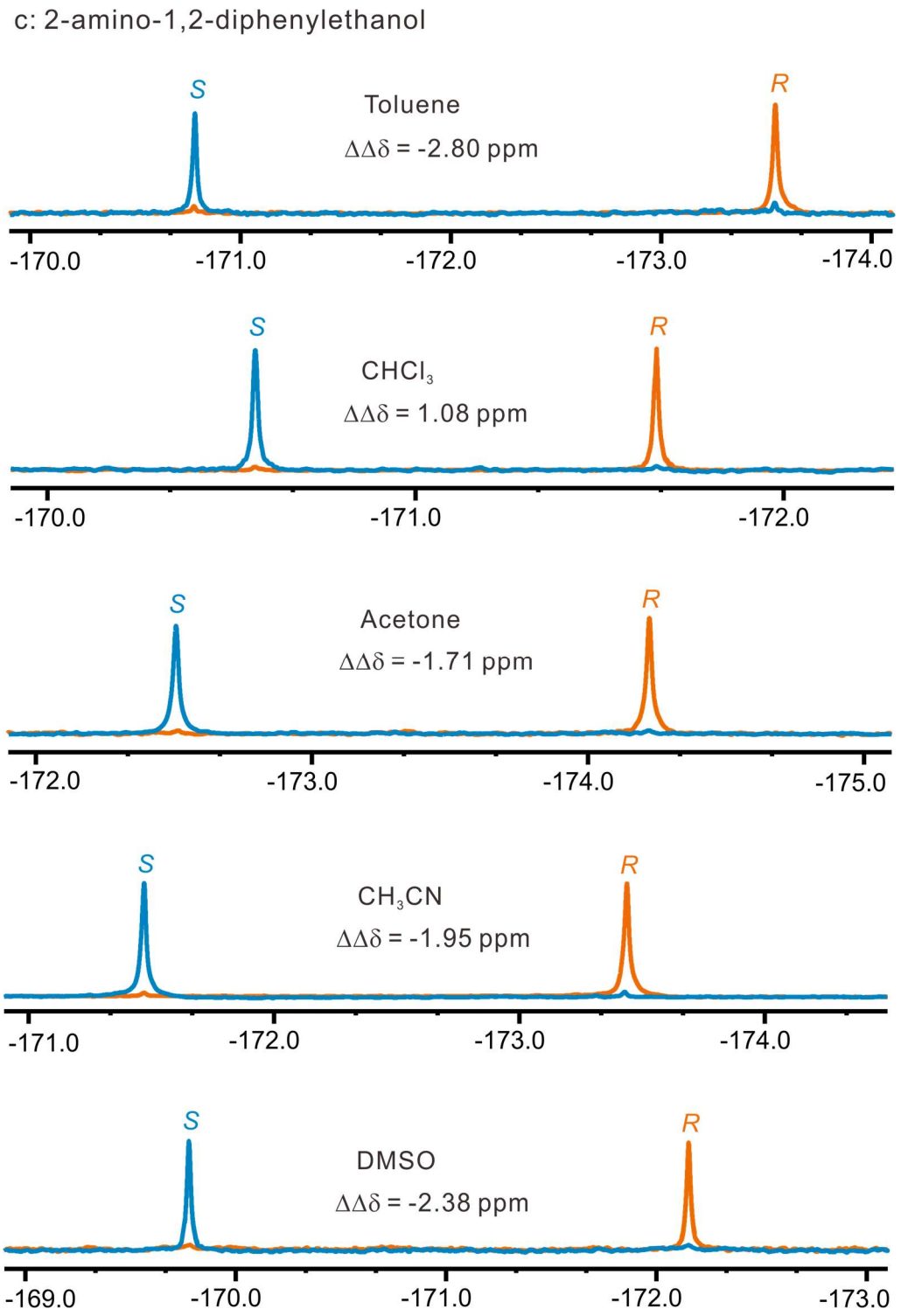


Figure S8. Comparison of chemical shift difference ($\Delta\Delta\delta = \delta^R - \delta^S$) in ^{19}F-NMR spectra of amide products formed by the ^{19}F-tag with the enantiomers of phenylglycinol (a), phenylpropylamine (b), and 2-amino-1,2-diphenylethanol (c) in different solvents. The concentration of the ^{19}F-tag was 0.5 mM and the concentrations of the enantiomeric analytes were 1.0 mM, respectively. The proton-decoupled ^{19}F-NMR spectra were recorded with a ^{19}F frequency of 376 MHz after 6 h incubation for the mixture of the ^{19}F-tag and chiral analytes at room temperature in $CDCl_3$.

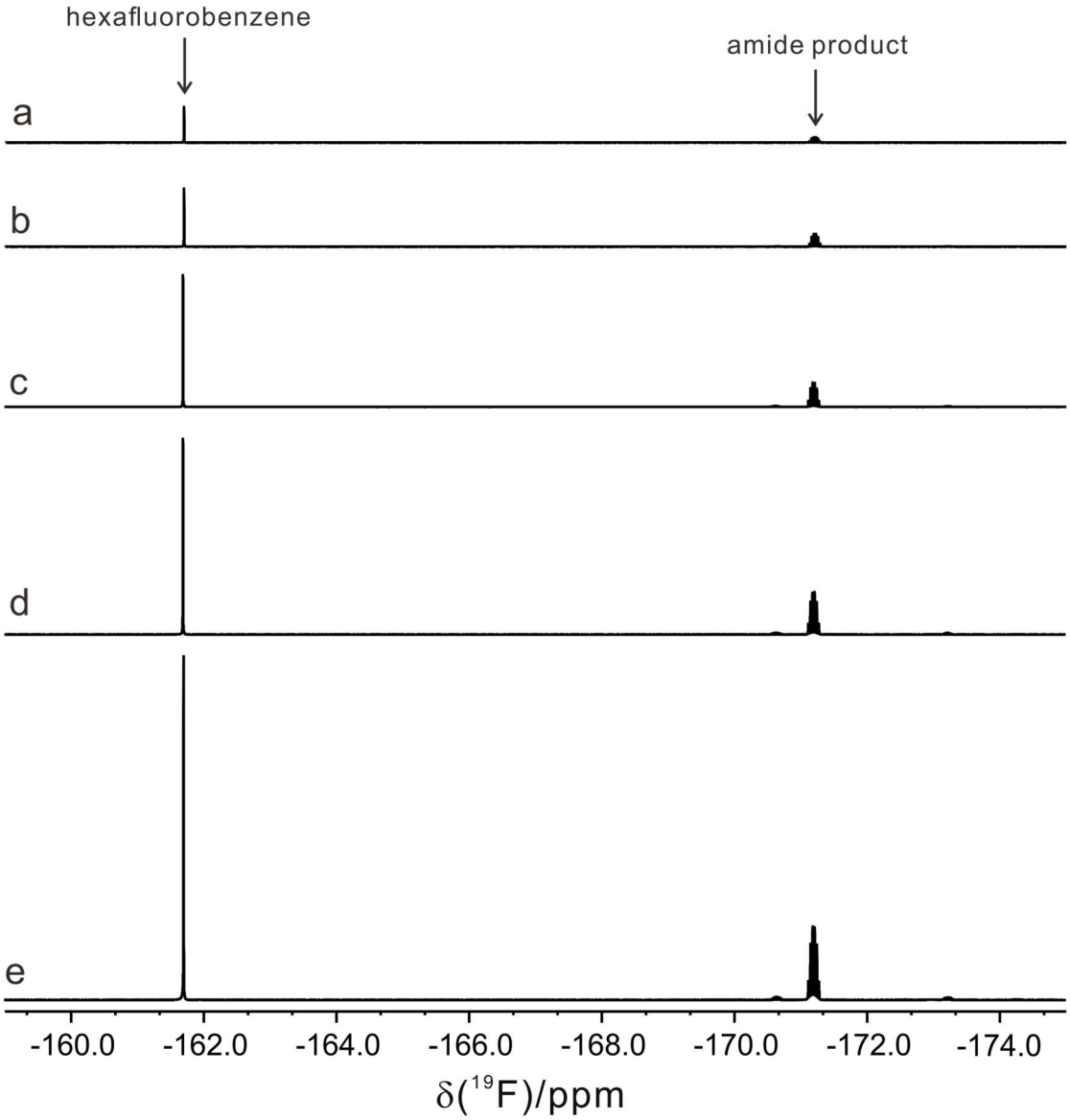


Figure S9. The amide products were quantified by ^{19}F-NMR spectra using hexafluorobenzene with different concentrations as internal standard. The concentrations of internal standard, ^{19}F-tag and (*S*)-(+)-2-amino-1-propanol were in the range of 0.01-0.3 mM, 0.1-2.0 mM and 0.2-4.0 mM, respectively. The non-proton-decoupled ^{19}F-NMR spectra were recorded with a ^{19}F frequency of 753 MHz after 6 h incubation for the mixture of the ^{19}F-tag and chiral analytes at 35 °C in $CDCl_3$.