Supporting Information

For

Rational design of [^{13}C,D$_{14}$]*tert*-butylbenzene as a scaffold structure for designing long-lived hyperpolarized ^{13}C probes

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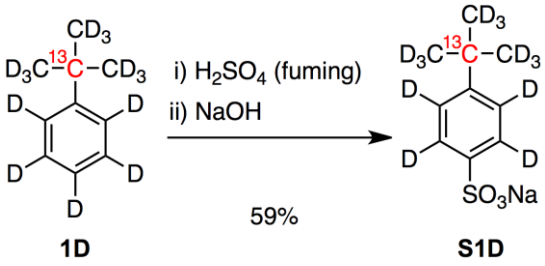
CONTENTS:

1. Synthesis

General. Reagents and solvents were purchased from standard suppliers and used without further purification. NMR spectra were measured using a JEOL ECS400 spectrometer. Acetone-d_6 in chloroform (2.15 ppm) or acetone-d_6 in methanol (2.15 ppm) was used as the internal standard for ^{2}H NMR. Chloroform-d_1 (77.0 ppm), methanol-d_4 (49.0 ppm), or methanol in D_2O (49.5 ppm) was used as the internal standard for ^{13}C NMR. Mass spectra (MS) were measured using a Bruker micrOTOF II (ESI) and a SHIMADZU GCMS-QP2010 (EI) .

Scheme S1. Synthetic scheme of [^{13}C,D_{13}]sodium *tert*-butylbenzenesulfonate (**S1D**).

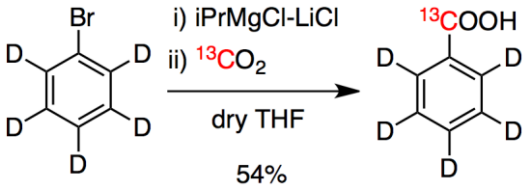


1D **S1D**

Characterization of [^{13}C]*tert*-butylbenzene (1).

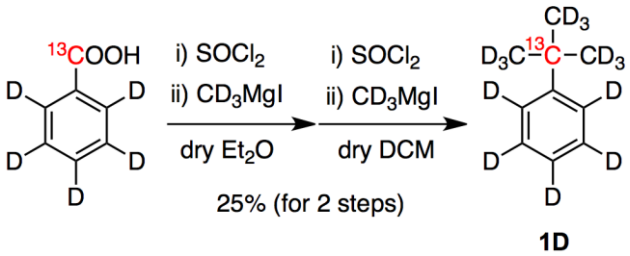
^{1}H NMR (CD_3OD, 400 MHz)□δ = 1.21 (9H, d, $^{2}J_{CH}$ = 3.6 Hz), 7.02 (1H, dd, 7.4 Hz), 7.16 (2H, dd, 7.6 Hz), 7.27-7.30 (2H, m); ^{13}C NMR (CD_3OD, 100 MHz)□δ = 30.8 ($^{1}J_{CC}$ = 35 Hz), 35.4, 126.2, 126.4, 129.0, 144.9.

Synthesis of [^{13}C,D_5]benzoic acid.



Under a nitrogen atmosphere, [D_5]bromobenzene (1.85 mL, 17.5 mmol) was added dropwise to a solution of 0.96 M iPrMgCl in THF (20 mL, 19.2 mmol) at -20 °C. The mixture was stirred at room temperature for 18.5 h. In another flask, concd. H_2SO_4 (10 mL) was added dropwise to [^{13}C]$BaCO_3$ (5.0 g, 25.2 mmol) on ice under a nitrogen atmosphere. The liberated [^{13}C]CO_2 was collected in a rubber baloon and bubbled into a stirred bromobenzene/Grignard mixture at -5 °C under a nitrogen atmosphere. The reaction was then quenched with water. After removing THF solvent, 2N aqueous HCl and $CHCl_3$ were added to the crude residue. The mixture was extracted with $CHCl_3$ three times. The combined organic phase was dried over sodium sulfate and evaporated *in vacuo* to give [^{13}C,D_5]benzoic acid as a white solid (1214 mg, 54%) Further purification was not performed in this step: ^{2}H NMR ($CHCl_3$, 61 MHz)□δ = 7.5 (3 × ^{2}H), 8.2 (2 × ^{2}H); ^{13}C NMR (CD_3OD, 100 MHz)□δ = 128.9 ($^{1}J_{CD}$ = 25 Hz), 130.3 ($^{1}J_{CD}$ = 25 Hz), 131.6 (d, $^{1}J_{CC}$ = 72 Hz), 133.5 ($^{1}J_{CD}$ = 24 Hz), 169.9; HRMS(ESI): m/z calc. for $C_6{}^{13}CHD_5O_2$ $[M-H]^-$ = 127.0642, found = 127.0637.

Synthesis of [^{13}C,D$_{14}$]*tert*-butylbenzene (1D).



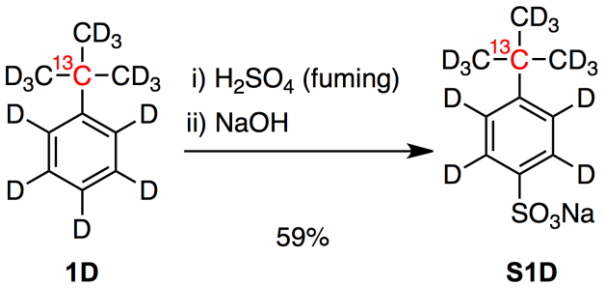
1D

$SOCl_2$ (5 mL) was added dropwise to [^{13}C,D$_5$]benzoic acid (513 mg, 4 mmol) on ice. The mixture was stirred at room temperature for 5 h. After removing $SOCl_2$ *in vacuo* on ice, the resulting oil was co-evaporated with dry Et_2O and diluted with dry Et_2O (12 mL) under a nitrogen atmosphere. The mixture was added dropwise to a solution of 1 M [D$_3$]methyl magnesium iodide in Et_2O (12 mL, 12 mmol) diluted with dry Et_2O (12 mL) at -40 °C under a nitrogen atmosphere. The mixture was stirred at room temperature for 16.5 h under a nitrogen atmosphere. Aqueous NH_4Cl was added dropwise to the mixture for quenching reaction and stirred for 10 min. The mixture was extracted with Et_2O five times. The combined organic phase was washed with brine and evaporated. After CH_2Cl_2 was added to the resulting oil, the mixture was dried over sodium sulfate and evaporated to give [^{13}C,D$_{11}$]2-phenyl-2-propanol as a brown oil including impurity (531 mg). Further purification was not performed in this step: ^{2}H NMR (MeOH, 61 MHz) δ = 1.6 (6 × ^{2}H), 7.3 (1 × ^{2}H), 7.5 (2 × ^{2}H), 7.7 (2 × ^{2}H); ^{13}C NMR (CD_3OD, 100 MHz) δ = 30.5-31.7 (m), 72.5, 125.0 ($^1J_{CD}$ = 24 Hz), 126.7 ($^1J_{CD}$ = 24 Hz), 128.4 ($^1J_{CD}$ = 24 Hz), 150.3 (d, $^1J_{CC}$ = 48 Hz). MS(EI): m/z calc. for $C_8{}^{13}CHD_{11}O$ $[M\text{-}CD_3]^+$ = 130.1, found = 130.1.

Under a nitrogen atmosphere, 1 M [D$_3$]methyl magnesium iodide in Et_2O (11.2 mL, 11.2 mmol) was added to a flask. After removing Et_2O *in vacuo*, a residue was co-evaporated with dry CH_2Cl_2 while slightly warming to complete the removal of ether. Dry CH_2Cl_2 (8.4 mL) was added to the resulting residue. In another flask, $SOCl_2$ (880 µL, 11.2 mmol) was added dropwise to a solution of [^{13}C,D$_{11}$]2-phenyl-2-propanol (531 mg including impurity from the former step) in dry CH_2Cl_2 (40 mL) on ice. The mixture was stirred on ice for 30 min. After removing $SOCl_2$ and CH_2Cl_2 *in vacuo* on ice, the resulting oil was co-evaporated with dry CH_2Cl_2 and diluted with dry CH_2Cl_2 (8.4 mL) under a nitrogen atmosphere. The mixture was added dropwise to a Grignard reagent in dry CH_2Cl_2 under a

nitrogen atmosphere on ice. The mixture was stirred at room temperature for 2 days under a nitrogen atmosphere. Ice was added into the mixture for quenching reaction. The mixture was extracted with CH_2Cl_2 three times. The combined organic phase was washed with aqueous NH_4Cl and brine. The organic phase was dried over sodium sulfate and the solvent was evaporated carefully not to loss the volatile product. The resulting oil was purified using preparative layer chromatography (eluent: hexane) to give [^{13}C,D$_{14}$]*tert*-butylbenzene (**1D**) as a clear oil (151 mg, 25% in 2 steps): ^{2}H NMR (MeOH, 61 MHz) δ = 1.3 (9 × ^{2}H), 7.2 (1 × ^{2}H), 7.3 (2 × ^{2}H), 7.5 (2 × ^{2}H); ^{13}C NMR (CD_3OD, 100 MHz) δ = 30.1-31.2 (m), 34.7, 125.7 ($^1J_{CD}$ = 23 Hz), 125.8 ($^1J_{CD}$ = 24 Hz), 128.5 ($^1J_{CD}$ = 24 Hz), 151.9 (d, $^1J_{CC}$ = 43 Hz); MS(EI): m/z calc. for $C_9{}^{13}CD_{14}$ $[M]^+$ = 149.2, found = 149.2.

Synthesis of [^{13}C,D$_{13}$]sodium *tert*-butylbenzenesulfonate (S1D).



1D **S1D**

Fuming H_2SO_4 (96 µL, 2 mmol) was added dropwise to [^{13}C,D$_{14}$]*tert*-butylbenzene (**1D**; 149 mg, 1 mmol). The mixture was shaked at room temperature for 2 h. Water (1 mL) was added to the mixture for quenching reaction. The mixture was neutralized with 1N aqueous NaOH. After removing water *in vacuo*, the resulting white solid was extracted with dry EtOH. In this step, the first extract was discarded because it included *meta*-substituted product, and the combined extacts after second extract were evaporated. Water was added to the resulting residue and then filtered to remove insoluble material. The solution was evaporated *in vacuo* to give [^{13}C,D$_{13}$]sodium *tert*-butylbenzenesulfonate (**S1D**) as a white solid (149 mg, 59%): ^{2}H NMR (MeOH, 61 MHz) δ = 1.3 (9 × ^{2}H), 7.5 (2 × ^{2}H), 7.8 (2 × ^{2}H); ^{13}C NMR (D_2O, 100 MHz) δ = 29.3-30.4 (m), 34.3, 125.3-126.6 (m), 139.9, 156.0 (d, $^1J_{CC}$ = 43 Hz); HRMS(ESI): m/z calc. for $C_9{}^{13}CD_{13}NaO_3S$ $[M\text{-}Na]^-$ = 227.1440, found = 227.1449.

2. T_1 measurement

All T_1 measurements were performed under thermal equilibriated conditions. The T_1 measurements were conducted using a JEOL JNM-ECS 400 (9.4 T), a JEOL JNM-ECA 500 (11.7 T), and JEOL JNM-ECA 600 (14.1 T) by inversion recovery method.

3. Analysis of T_1 relaxation mechanisms

The extent of contribution of each relaxation mechanism is represented as shown in eq 1.

$$\frac{1}{T_1} = \frac{1}{T_1^{CH\text{-}DD}} + \frac{1}{T_1^{CSA}} + \frac{1}{T_1^{O2}} + \frac{1}{T_1^{res}} \quad (eq\,1)$$

(i) $T_1^{CH–DD}$ calculation by measurements of T_1 and NOE (1+η)[1–6]

$^{13}C–^{1}H$ DD contribution ($T_1^{CH–DD}$) is calculated according to eq 2 by using nuclear Overhauser enhancement (NOE, 1+η) parameter in ^{13}C NMR experiment on irradiation at ^{1}H frequency .

$$T_1^{CH\text{-}DD} = T_1 \frac{1.988}{h} \quad (eq\,2)$$

The NOE (1+η) was measured on a JEOL JNM-ECS 400 (9.4 T, 25 °C) (Table S1). After measuring the ^{13}C NMR spectra with or without NOE, the value η was determined using the following equation:

$$1+\eta = (^{13}C \text{ Integral }_{\text{with NOE}})/(^{13}C \text{ Integral }_{\text{without NOE}})$$

Table S1. ^{13}C spin-lattice relaxation times (T_1) and NOE (1+η) of **1** and **1D**.[a]

	T_1 [s]	NOE(1 + η) for ^{13}C	T_1^{DD} (^{13}C-^{1}H) [s]
1	103 ± 1	2.70 ± 0.07	120 ± 5
1D	541 ± 18	ca. 1.0[b]	-[b]

[a] **1** and **1D** were dissolved in degassed toluene-d_8. T_1 values were determined by using the inversion recovery method at 25 ºC (toluene-d_8, 9.4 T). [b] $^{13}C–^{1}H$ DD contribution of **1D** was too small to be determined.

(ii) T_1^{CSA} calculation by T_1 measurements at various magnetic fields[7,8]

Chemical shift anisotropy (CSA) relaxation is dependent on external magnetic field; $1/T_1^{CSA}$ is proportional to the square of the magnetic field (B_0^2). To investigate CSA contribution (T_1^{CSA}), T_1 values of **1** and **1D** at various magnetic fields were measured accordig to previously reported methods.[7,8] T_1^{CSA} of **1** was estimated to be 1695 s (Table S2; degassed, 9.4 T, 25 °C). T_1^{CSA} of **1D** could not be estimated by the field dependent studies, because T_1^{SC} derived from ^{2}H of **1D** would be also affected by magnetic field strength.

(iii) T_1^{O2} calculation by T_1 measurements under non-degassed/degassed conditions[1,4]

Under the assumption that other relaxation mechanisms are not affected by the presence or absence of oxygen, T_1^{O2} of **1** and **1D** was estimated to be 180 and 175 s, respectively using eq 3 (9.4 T, 25 °C) (Table S2).

$$\frac{1}{T_1^{O2}} = \frac{1}{T_1\ (non\text{-}degassed)} + \frac{1}{T_1\ (degassed)} \quad (eq\ 3)$$

(iv) T_1^{res} calculation[1]

T_1^{res} indicates all other residual contributions including SR and SC. T_1^{res} was calculated from eq 1.[1]

Table S2. ^{13}C spin-lattice relaxation parameters in non-degassed toluene-d_8 at 25 °C, 9.4 T.

	T_1	T_1^{DD}	T_1^{CSA}	T_1^{res}	T_1^{O2}
1	65	120	1695	1200	180
1D	132	-[a]		541[b]	175

[a] ^{13}C–^{1}H DD contribution of **1D** was too small to be determined. [b] Sum of T_1^{CSA} and T_1^{res}.

4. DNP–NMR measurement

A 8 μL solution of Probe **S1D** (0.5 M) and OX063 trityl radical (Oxford Instruments, UK) in 50:50 glycerol-water was polarized at ~1.2 K for 3 h in a HyperSense DNP polarizer (Oxford Instruments, UK). A solution of hyperpolarized **S1D** was obtained by rapidly dissolving the frozen sample with superheated water (4 mL) and subsequently transferred into a 10-mm NMR tube inside a 400 NMR spectrometer (Agilent Technologies, USA). ^{13}C NMR acquisition was initiated 20 s after the initial dissolution to allow for complete transfer and mixing of the solution. A series of ^{13}C NMR spectra were acquired with a 5-degree pulse every 5 s at room temperature (~22 °C). ^{13}C signal intensity at each time point was normalized to the signal intensity at the first time point. Signal enhancements were calculated according to the previously described methods.[9]

5. Reference

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