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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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Abstract

Dynamic nuclear polarization (DNP) is a technique to polarize the nuclear spin population. As a result of the hyperpolarization, NMR sensitivity of the nuclei in molecules can be enhanced dramatically. Recent application of the hyperpolarization technique has led to advances in biochemical and molecular studies. A major problem is the short lifetime of the polarized nuclear spin state. Generally, in solution, the polarized nuclear spin state decays to a thermal spin equilibrium, resulting in loss of the enhanced NMR signal. This decay is correlated directly with the spin-lattice relaxation time T_1. Here we report [^{13}C,D_{14}]*tert*-butylbenzene as a new scaffold structure for designing hyperpolarized ^{13}C probes. Thanks to the minimized spin-lattice relaxation (T_1) pathways, its water-soluble derivative showed a remarkably long ^{13}C T_1 value and long retention of the hyperpolarized spin state.

Graphical Abstract

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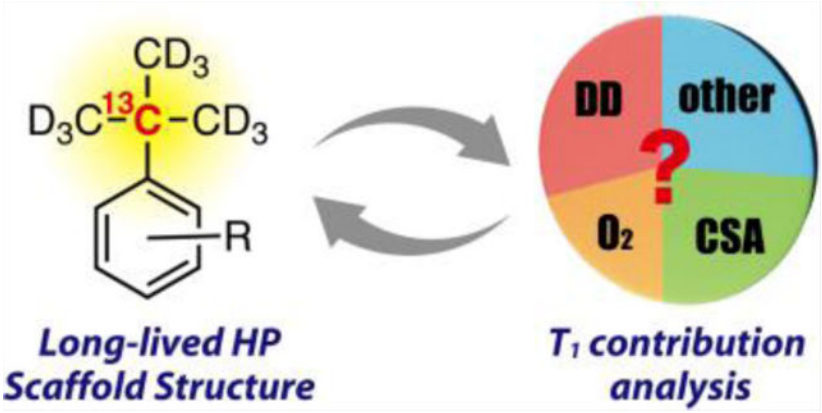
Conflicts of interest

There are no conflicts to declare.



Here we report [^{13}C,D$_{14}$]*tert*-butylbenzene as a new scaffold structure for designing hyperpolarized ^{13}C probes. Thanks to the minimized spin-lattice relaxation (T_1) pathways, its water-soluble derivative showed a remarkably long ^{13}C T_1 value and long retention of the hyperpolarized spin state.

Keywords

dynamic nuclear polarization; hyperpolarization; nuclear magnetic resonance; spin-lattice relaxation time; chemical probe

Dynamic nuclear polarization (DNP) is a technique to polarize the nuclear spin population.[1–3] As a result of the hyperpolarization, NMR sensitivity of the nuclei in molecules can be enhanced dramatically. Recent application of the hyperpolarization technique has led to advances in biochemical and molecular studies.[4] Hyperpolarized (HP) molecules have enabled highly sensitive and real-time monitoring of metabolic fluxes[4,5] and chemical status[6] in vivo. DNP has not only been applied to these biomedical studies, but also to molecular interaction analysis and chemical reaction monitoring.[7] Such extensive research has demonstrated the high potential of HP-NMR techniques.

A major problem is the short lifetime of the polarized nuclear spin state. Generally, in solution, the polarized nuclear spin state decays to a thermal spin equilibrium, resulting in loss of the enhanced NMR signal. This decay is correlated directly with the spin-lattice relaxation time T_1. In solution, the T_1 value of ^{13}C in small molecules is typically from a few to tens of seconds.[4] This short lifetime has hampered the flexible design of HP chemical probes.

To overcome the decay problem and to generate a series of HP probes systematically, we have proposed a scaffold strategy.[8–11] A representative scaffold structure is [^{15}N,D]trimethylphenylammonium (TMPA), which comprises a long-lived HP signalling moiety (–^{15}N(CD$_3$)$_3$) and an aromatic ring for facile chemical modifications (Figure 1). We have successfully demonstrated that a series of HP probes could be developed from the [^{15}N,D]TMPA by introducing a molecular sensing unit R.[9,10] However, in principle, the ^{15}N NMR sensitivity is lower than that of ^{13}C nuclei because of its smaller gyromagnetic

ratio. Therefore, a ^{13}C HP scaffold structure with a long T_1 value has been highly sought after.[11]

Here, we report a ^{13}C scaffold structure, [^{13}C,D_{14}]*tert*-butylbenzene, that can be used to design long-lived HP ^{13}C probes.

There are two approaches to achieving a long-lived HP spin state. One is to design a molecule with long T_1, because the HP spin state is directly correlated with T_1.[4] The other is to use a nuclear singlet state with a magnetically equivalent spin −1/2 pair.[12] Both approaches are attractive and have advanced long-lived hyperpolarization.[8–10,13–18] In the present study, we adopt the more straightforward former approach.

T_1 relaxation is affected by several factors, such as dipole–dipole (DD) interactions, chemical shift anisotropy (CSA), spin–rotation, scalar coupling.[19,20] For ^{13}C small organic molecules, especially in high magnetic field, DD and CSA relaxations are dominant factors.[4,6]

To minimize the CSA relaxation, sp^3 carbon with symmetrical environment is preferable. The *tert*-butyl carbon has an sp^3 hybridization. This carbon centre is also attractive for HP applications because it experiences only a small DD-induced T_1 relaxation because of the absence of neighbouring ^1H. Indeed, *tert*-butanol and 1,1-cyclopropane dimethanol have been shown to have long T_1 values and have been proposed as potential HPMRI probes.[21,22]

We then designed [^{13}C]*tert*-butylbenzene (**1**) as a potential ^{13}C scaffold structure. Molecule **1** is composed of a ^{13}C *tert*-butyl group attached to an aromatic ring for feasible chemical modification to incorporate functional groups such as sensing moieties. **1** was synthesized from [^{13}C]benzoic acid in 2 steps. The ^{13}C T_1 value of **1** was 76 ± 1 s (CD_3OD, 9.4 T, 25 °C). The ^{13}C T_1 of [^{13}C]benzoic acid, which was another candidate for a ^{13}C scaffold utilizing a ^{13}C carboxyl group as a HP unit, was 33.9 ± 0.3 s (CD_3OD, 9.4 T, 25 °C), suggesting an advantage in utilizing sp^3 carbon as a long-lived HP unit.

To extend the ^{13}C T_1 of **1** further, we then prepared [^{13}C,D_{14}]*tert*-butylbenzene (**1D**, Figure 2), wherein all ^1H were replaced with ^2H (D) to minimize ^{13}C-^1H DD relaxation. This **1D** was synthesized from [D_5]bromobenzene in 3 steps (Scheme 1). The ^{13}C T_1 of **1D** reached over 100 s (141 ± 3 s, CD_3OD, 9.4 T, 25 °C), which was 1.3 times longer than that of deuterated *tert*-butanol (106 s).

T_1 relaxation of **1D** was investigated further by decomposing it to each relaxation mechanism: ^{13}C–^1H DD ($T_1^{CH\text{-}DD}$), CSA (T_1^{CSA}), dissolved oxygen (T_1^{O2}), and residual factors (T_1^{res}). Total T_1 can be described using these decomposed T_1 values as equation 1. These T_1 contributions were determined according to previous reports (experimental details, see: supporting information).[19,20,23–28]

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

The estimated T_1 contributions in **1** and **1D** are summarized in Figure 3. As shown in equation 1, a larger $1/T_1{}^{X}$ indicates the larger contribution of the relaxation mechanism X to shorten the apparent T_1. These data include three important implications. First, ^{13}C–^{1}H DD relaxation of **1D** is almost completely suppressed by full deuteration, while **1** suffers from non-negligible ^{13}C–^{1}H DD relaxation (red bar). Second, the sp^3 carbon of **1** and **1D** experience low CSA relaxation, as designed. Third, as a result of sufficient minimization of ^{13}C–^{1}H DD and CSA relaxation mechanisms, the relaxation from dissolved oxygen ($T_1{}^{O2}$) becomes the major contributor for T_1 relaxation (blue bar). In the case of **1D**, $T_1{}^{O2}$ governs the majority of the apparent T_1 relaxation, resulting in the considerably longer T_1 value of **1D** in degassed condition (541 ± 18 s, toluene-d8, 9.4 T, 25 °C). These data support the validity of our design and the potential of **1D** as a long-lived HP scaffold.

Furthermore, we tried to achieve a T_1 value longer than **1D** based on the understanding of T_1 contributions of **1D** shown in Figure 3. Considering that the dissolved oxygen is a dominant relaxation factor of **1D**, it was considered that a longer T_1 could be achieved by changing the solvent to water in which the oxygen concentration is lower than that in organic solvents.[29] We then attempted to add the water solubility into **1D**.

In order to increase the water solubility of **1D**, sulfonated **1D** (**S1D**, Figure 4a) was designed. Thanks to the rich chemical functionality of conjugated aromatic systems, a variety of substitutions can be introduced to the benzene ring of **1D**. In this case, **1D** was diversified by aromatic sulfonation with fuming sulphuric acid to produce **S1D** (Scheme S1) as an HP ^{13}C probe with excellent water solubility (> 0.4 mol/L, r.t.).

The probe **S1D** showed a remarkably long T_1 in water. The ^{13}C T_1 exceeded 200 s in D_2O (209 ± 7 s, 9.4 T, 37 °C), which was much longer than that of the original (**1D**) in organic solvent despite the increased molecular weight. It was shortened in 90% H_2O, but still maintained a long T_1 (147 ± 1 s, 9.4 T, 37 °C). These values were longer than those for [D_9]*tert*-butanol (140 ± 7 in D_2O, 77± 1 s in H_2O, 9.4 T, 37 °C).

Finally, **S1D** was applied for a DNP-NMR study in water. **S1D** was hyperpolarized efficiently by dissolution DNP. The liquid state ^{13}C NMR signal of the probe dissolved in water was enhanced by approximately 29,000-fold (%P_{13C} = 23.5%, T = 298 K, B_0 = 9.4 T) compared with the thermally polarized signal, allowing detection of ^{13}C NMR signal by 1 scan. Figure 4b shows the time course stack of ^{13}C NMR spectra of HP **S1D**. Because of the long T_1 value, **S1D** could retain a hyperpolarized spin state for a relatively long time, allowing observation of the HP ^{13}C NMR signal for over 10 min under our experimental conditions. These results strongly indicate that this approach toward T_1 extension based on an analysis of the T_1 contribution is a useful approach to the design of HP probes.

In summary, we developed a new HP ^{13}C scaffold [^{13}C,D_{14}]*tert*-butylbenzene (**1D**). The significance of this scaffold can be summarized as follows. First, it satisfies the requirement of a long T_1. The **1D** scaffold was designed by minimizing each relaxation mechanism to achieve longer T_1 value. Notably, **S1D**, developed from **1D**, afforded a remarkably long ^{13}C T_1 (209 s in D_2O and 147 s in 90%H_2O, non-degassed, 9.4 T, 37 °C). To our knowledge, this is the longest among the water-soluble ^{13}C small organic molecules used for HP-NMR

research. Second, it satisfies a facile functionalization of HP probes by feasible chemical modification. As we reported in the present and the previous papers, thanks to the rich organic chemistry of aromatic compounds, a variety of HP probes may potentially be developed using this ^{13}C HP scaffold. Development of water soluble **S1D** from **1D** scaffold is a good example of this versatility. Third is higher sensitivity. The present ^{13}C scaffold is more sensitive than the previous ^{15}N scaffold. Although further optimization may be necessary for practical applications, these advantages indicate the high potential of **1D** as a new scaffold structure for designing a series of HP ^{13}C MR probes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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References

[1]. Lee JH, Okuno Y, Cavagnero S, J. Magn. Reson 2014, 241, 18–31. [PubMed: 24656077]

[2]. Nikolaou P, Goodson BM, Chekmenev EY, Chem. Eur. J 2015, 21, 3156–3166. [PubMed: 25470566]

[3]. Ardenkjær Larsen JH, Fridlund B, Gram A, Hansson G, Hansson L, Lerche MH, Servin R, Thaning M, Golman K, Proc. Natl. Acad. Sci. U.S.A 2003, 100, 10158–10163. [PubMed: 12930897]

[4]. Keshari KR, Wilson DM, Chem. Soc. Rev 2014, 43, 1627–1659. [PubMed: 24363044]

[5]. Kurhanewicz J, Vigneron DB, Brindle K, Chekmenev EY, Comment A, Cunningham CH, Deberardinis RJ, Green GG, Leach MO, Rajan SS, Rizi RR, Ross BD, Warren WS, Malloy CR, Neoplasia 2011, 13, 81–97. [PubMed: 21403835]

[6]. Meier S, Jensen PR, Karlsson M, Lerche MH, Sensors 2014, 14, 1576–1597. [PubMed: 24441771]

[7]. a)Keshari KR, Kurhanewicz J, Macdonald JM, Wilson DM, Analyst 2012, 137, 3427–3429.; [PubMed: 22655289] b)Lee Y, Heo GS, Zeng H, Wooley KL, Hilty C, J. Am. Chem. Soc 2013, 135, 4636–4639. [PubMed: 23461287]

[8]. Doura T, Hata R, Nonaka H, Ichikawa K, Sando S, Angew. Chem. Int. Ed 2012, 51, 10114–10117.

[9]. Nonaka H, Hata R, Doura T, Nishihara T, Kumagai K, Akakabe M, Tsuda M, Ichikawa K, Sando S, Nat. Commun 2013, 4, 2411. [PubMed: 24022444]

[10]. Nonaka H, Hirano M, Imakura Y, Takakusagi Y, Ichikawa K, Sando S, Sci. Rep 2017, 7, 40104. [PubMed: 28067292]

[11]. Nishihara T, Kameyama Y, Nonaka H, Takakusagi Y, Hyodo F, Ichikawa K, Sando S, Chem. Asian. J 2017, 12, 949–953. [PubMed: 28328174]

[12]. Pileio G, Hill-Cousins JT, Mitchell S, Kuprov I, Brown LJ, Brown RCD, Levitt MH, J. Am. Chem. Soc 2012, 134, 17494–17497. [PubMed: 23067426]

[13]. Allouche-Arnon H, Lerche MH, Karlsson M, Lenkinski RE, Katz-Brull R, Contrast Media Mol. Imaging 2011, 6, 499–506. [PubMed: 22144028]

[14]. Vuichoud B, Milani J, Bornet A, Melzi R, Jannin S, Bodenhausen G, J. Phys. Chem. B 2014, 118, 1411–1415. [PubMed: 24397585]

[15]. Rodrigues TB, Serrao EM, Kennedy BWC, Hu D, Kettunen MI, Brindle KM, Nat. Med 2014, 20, 93–97. [PubMed: 24317119]

[16]. Tayler MCD, Marco-Rius I, Kettunen MI, Brindle KM, Levitt MH, Pileio G, J. Am. Chem. Soc 2012, 134, 7668–7671. [PubMed: 22509846]

[17]. Stevanato G, Hill-Cousins JT, Håkansson P, Roy SS, Brown LJ, Brown RCD, Pileio G, Levitt MH, Angew. Chem. Int. Ed 2015, 54, 3740–3743.

[18]. Theis T, Ortiz GX Jr., Logan AWJ, Claytor KE, Feng Y, Huhn WP, Blum V, Malcolmson SJ, Chekmenev EY, Wang Q, Warren WS, Sci. Adv 2016, 2, e1501438. [PubMed: 27051867]

[19]. Levy GC, Acc. Chem. Res 1973, 6, 161–169.

[20]. Breitmaier E, Spohn KH, Berger S, Angew. Chem. Int. Ed 1975, 14, 144–159.

[21]. Cheng T, Mishkovsky M, Junk MJN, Münnemann K, Comment A, Macromol. Rapid Commun 2016, 37, 1074–1078. [PubMed: 27184565]

[22]. von Morze C, Larson PE, Hu S, Yoshihara HA, Bok RA, Goga A, Ardenkjaer-Larsen JH, Vigneron DB, Magn. Reson. Imaging 2014, 72, 1599–1609.

[23]. Kuhlmann KF, Grant DM, J. Am. Chem. Soc 1968, 90, 7355–7357.

[24]. Levy GC, Cargioli JD, Anet FAL, J. Am. Chem. Soc 1973, 95, 1527–1535.

[25]. Giannini DD, Armitage IM, Pearson H, Grant DM, Roberts JD, J. Am. Chem. Soc 1975, 97, 3416–3419.

[26]. Gust D, Pearson H, Armitage IM, Roberts JD, J. Am. Chem. Soc 1976, 98, 2723–2726.

[27]. Alger TD, Hamill WD Jr., Pugmire RJ, Grant DM, Silcox GD, Solum M, J. Phys. Chem 1980, 84, 632–636.

[28]. Wong TC, Ang TT, Guziec FS, Moustakis CA, J. Magn. Reson 1984, 57, 463–470.

[29]. a)Quaranta M, Murkovic M, Klimant I, Analyst 2013, 138, 6243–6245; [PubMed: 23803965] b)Sato T, Hamada Y, Sumikawa M, Araki S, Yamamoto H, Ind. Eng. Chem. Res 2014, 53, 19331–19337.

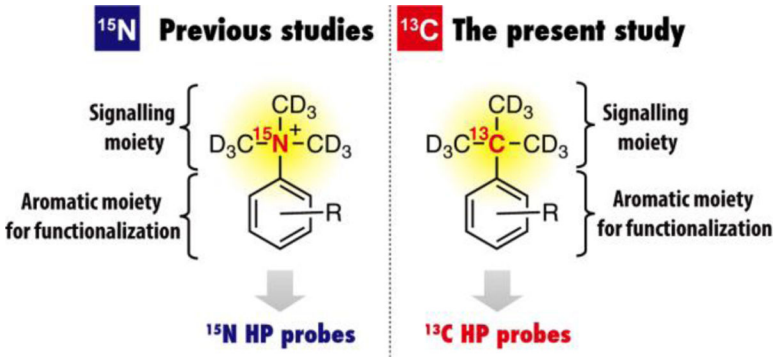


Figure 1.
Schematic illustration of ^{15}N and ^{13}C scaffold structures reported in previous and this study, respectively.

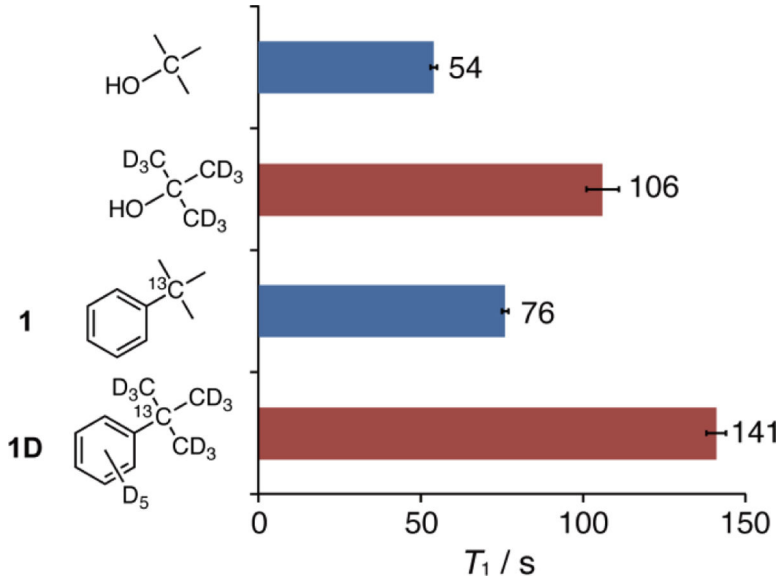


Figure 2.

T_1 of *tert*-butanol and *tert*-butylbenzene (**1** and **1D**). ^{13}C T_1 were determined by using inversion recovery method (9.4 T, 25 °C, CD_3OD, *tert*-butanol = 1.0 M, [D_9]*tert*-butanol = 1.5 M, **1** = 0.05 M, **1D** = 0.05 M)

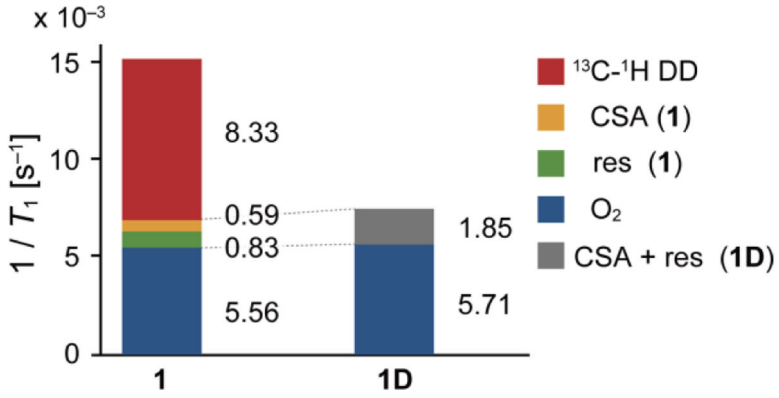


Figure 3.

Stacked bar graph of $1/T_1^{X}$ of **1** and **1D** (50 mM, toluene-d_8, 25 °C, 9.4 T). $1/T_1^{X}$ values were determined experimentally (see, supporting information).

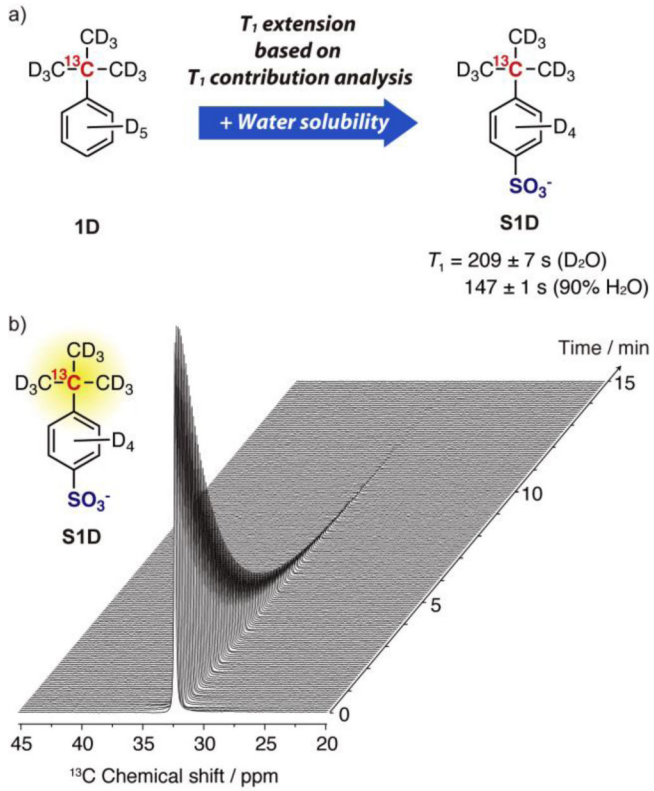
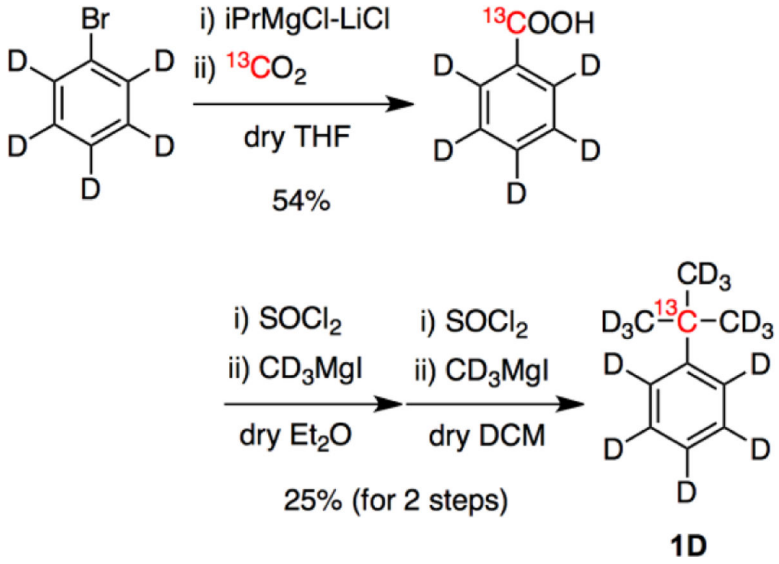


Figure 4.

(a) Molecular design of **S1D**. T_1s in D_2O and 90% H_2O were determined by inversion recovery method at 50 mM **S1D**, 37 °C, 9.4 T. (b) ^{13}C NMR spectra of the hyperpolarized probe **S1D** (2 mM in H_2O), stacked from 0 to 15 min (every 2 s, pulse angle 5°).



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