Synthesis of an Isotopically Labeled Naphthalene Derivative that Supports a Long-Lived Nuclear Singlet State

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Supporting Information Placeholder

ABSTRACT: The synthesis of an octa-alkoxy substituted isotopically labeled naphthalene derivative, shown to have excellent properties in singlet NMR experiments, is described. This highly substituted naphthalene system, which incorporates an adjacent $^{13}$C spin-pair, is readily accessed from a commercially available $^{13}$C$_2$-labeled building block via sequential thermal alkynyl- and arylcyclobutenone rearrangements. The synthetic route incorporates a simple desymmetrization approach leading to a small difference in the chemical shifts of the $^{13}$C spin-pair; a design constraint crucial for accessing nuclear singlet order.

Singlet NMR has potential as a diagnostic tool with a number of potential applications including the study of molecular diffusion and motion,\(^1\text{-}\(^3\)\) protein-ligand binding,\(^4\) analysis of intrinsically disordered protein domains\(^5\) and metabolomics.\(^6\) Furthermore, the combination of nuclear hyperpolarization and singlet NMR offers opportunities to develop novel MR imaging techniques.\(^7\text{-}\(^8\)\) Nuclear hyperpolarization, generated by methods such as dynamic nuclear polarization (DNP) gives rise to greatly increased NMR signal intensities,\(^9\text{-}\(^14\)\) theoretically $^{13}$C NMR signals can be enhanced by a factor of $10^6$ compared with conventionally thermally polarized nuclei. However, this technique has been limited by the short lifetime of hyperpolarized magnetization, which decays with the spin-lattice relaxation time-constant, $T_1$. Nuclear singlet order is immune to many of the relaxation mechanisms responsible for $T_1$ and decays with a time-constant $T_S$ which can often be far larger than $T_1$.\(^15\text{-}\(^19\)\) As a result, nuclear singlet order provides a means to 'store' nuclear hyperpolarization for extended periods of time, paving the way for a variety of applications.\(^7\) We have recently reported an octa-alkoxy substituted naphthalene derivative, incorporating a $^{13}$C spin-pair, which supports a long-lived nuclear singlet state in both low ($T_S > 1$ h; 0.4 T; acetone-$d_6$) and high magnetic field ($T_S \sim 950$ s; 9.4 T; acetone-$d_6$).\(^20\) Herein, we report the synthetic approach to this target.

Two key aspects of the design of molecular systems that support long-lived singlet states are the ability to access the singlet state, and attenuating the rate of relaxation of the singlet state occurring through different mechanisms.\(^3\text{-}\(^5\)\) The criteria for a suitable molecule can be summarized as follows:

\(^20\) (1) Fundamentally, the molecule must incorporate a strongly coupled spin-1/2 pair with which the singlet state can be created. (2) There should be no spin-active nuclei in close proximity (through bond and through space) to the spin-pair, especially isotopes with strong magnetism such as $^1$H and $^{19}$F. (3) Nuclei such as $^2$H with quadrupole moments, while preferable to $^1$H, should also be physically remote from the spin-pair. (4) The local molecular environment of the spin-pair should exhibit inversion symmetry. (5) The molecule as a whole must provide either a small chemical shift difference between the members of the spin-pair, or different spin-spin couplings between the members of the spin-pair and other magnetic nuclei. (6) The local molecular environment of the spin-pair should be conformationally inflexible. (7) The spin-pair should be shielded against close approach of paramagnetic molecules, such as molecular oxygen.

Based upon these design criteria, we considered that a naphthalene, with a central $^{13}$C spin-pair and fully deuterated side-chains ($R^1$ and $R^2$) would provide a suitable candidate (Scheme 1). Such an aromatic system is rigid, incorporates a local inversion center and all other spin-active nuclei are at least four bonds away from the spin-pair as well as an optimal distance through space. A small chemical shift difference may be provided by asymmetric substitution.

Scheme 1. Synthesis plan.
An additional challenge posed for the synthesis of such molecules lies in the availability of isotopically labeled starting materials. Starting materials incorporating $^{13}$C atoms are generally limited to small-molecule building blocks and as such syntheses must be designed around these. We have previously utilized commercially available ethynyltrimethylsilane-$^{13}$C$_2$ (1) to synthesize a series of acetylene-based compounds that support long-lived nuclear singlet states,$^{9,21}$ and considered this fragment to be convenient for construction of the requisite naphthalene system 7. Our approach was designed around sequential thermal alkynylation and aroylcyclobutone rearrangements to construct each ring of the bis-aromatic framework.$^{22-29}$ By this method the central $^{13}$C pair would be derived from the acetylene building block 1 and asymmetry could be easily introduced using an unsymmetrically-substituted squarate fragment 5.

**Scheme 2.** Synthesis of cyclobutenone 11 as a precursor for the first cyclobutenone rearrangement.

The synthetic route was optimized using unlabeled materials, primarily to reduce costs as well as to simplify analysis of the intermediates. The left-hand side of the naphthalene ring system was constructed first, beginning with squaric acid (8, Scheme 2). One of the primary requirements for the target naphthalene system was perdeuteration of the alkoxy substituents for reasons described above. Ultimately, this would be achieved through alkylation of the di-silver salt of squaric acid using deuterated iodomethane (99.5 atom % D). Thus, for the unlabeled synthesis, treatment of squaric acid (8) with AgNO$_3$ and Et$_3$N, followed by reaction with CH$_3$I in refluxing Et$_2$O for 18 h, afforded dimethyl squarate (9) in 68% yield. Alkylation of dimethyl squarate (9) with the lithium salt of ethynyltrimethylsilane proceeded smoothly to afford cyclobutenone 10 in 92% yield. Subsequent silyl-deprotection of 10 with TBAF gave cyclobutenone 11 in 84% yield, providing the substrate for the first thermal rearrangement.

Thermal rearrangement of cyclobutenone 11 was initially conducted under reflux in toluene, affording quinone 12 as the only isolated product in 56% yield after 2 h heating (Table 1, Entry 1). The remaining material consisted of intractable base-line components. We considered that the moderate yield of the quinone 12 could be attributed to prolonged heating under reflux and consequently, alternative reactor technologies were explored (Table 1). Harrowven and co-workers have recently demonstrated a series of highly efficient aroylcyclobutenone rearrangements in a flow reactor proceeding with short residence times and excellent yields.$^{28}$ On application of similar conditions to the rearrangement of acetylenyl-substituted cyclobutenone 11, the yield of quinone 12 was improved to 62% (Table 1, Entry 2). Further improvement was achieved under microwave irradiation, delivering quinone 12 in 72% yield after 20 min in MeCN at 130 °C (Table 1, Entry 3).

**Table 1.** Optimization of the rearrangement of cyclobutenone 11.

| Entry | Conditions | Yield of 12 |
|-------|------------|-------------|
| 1     | A: PhMe, reflux, 2 h | 56%         |
| 2     | B: Flow reactor, dioxane, 130 °C, t$_R$ = 30 min | 62%         |
| 3     | C: Microwave irradiation, MeCN, 130 °C, 20 min | 72%         |

*Isolated yields of purified compounds are quoted.

Reduction of quinone 12 by treatment with NaBH$_4$ afforded the dihydroquinone (Scheme 3), which was immediately dimethylated under basic conditions to afford the tetramethoxybenzene 13 in 69% over the two steps.

The second ring required an unsymmetrical squarate fragment 14, obtained in 60% yield by reaction of sodium isopropoxide with dimethyl squarate (9). The relatively high yield of 14 is perhaps quite surprising as we had anticipated rapid equilibration to a statistical mixture of both symmetrical esters and the unsymmetrical ester 14. Indeed, on extension of the reaction time to 20 min, the yield of squarate 14 was reduced to 51%. In any case this method of desymmetrization would again permit the introduction of the required perdeuterated iso-propoxy side-chain during the labeled synthesis.

The squarate and tetramethoxybenzene fragments, 14 and 13, were combined through an *ortho*-lithiation$^{13}$ coupling sequence, affording an inseparable mixture of regioisomers 15a and 15b (~1:1, $^1$H NMR) in 76% overall yield. Upon thermal rearrangement under microwave irradiation the mixture of isomers 15a and 15b converged upon a common, naphthalene-1,4-diol intermediate. Despite the reaction solution being purged with N$_2$ gas prior to heating, small amounts of the naphthalene-1,4-dione were present in the crude product. At-
tempts to reduce the quinone present in the crude reaction mixture proved unsuccessful; NaBH₄ and Na₂S₂O₄ were both incompatible, ultimately leading to degradation of the products. Consequently, following the thermal rearrangement, the crude reaction mixture was immediately treated with K₂CO₃ and Me₂SO₄ in refluxing acetone, allowing isolation of naphthalene 16 in 50% yield over the two steps.

Scheme 3. Synthesis of unlabeled naphthalene system 16.

Gratifyingly, the asymmetry of naphthalene 16, resulting from the presence of a single iso-propoxy group, achieved a suitable chemical shift difference of 0.08 ppm between the two central carbons in the ¹³C NMR spectrum of 16 (acetone-d₆). Such near-equivalence of the spin-pair in the labeled system is necessary for a long-lived singlet state that is stable in high magnetic field, while the small measure of asymmetry enables initial creation of the singlet state.²¹

With an optimized route to the unlabeled naphthalene system 16 established, the labeled synthesis was subsequently performed (Scheme 4). Alkylation of squaric acid (8) with CD₃I via the disilver salt afforded perdeuterated squarate 17 in 88% yield over the two steps. Introduction of the ¹³C spin-pair proceeded smoothly via deprotonation of ethynyltrimethylsilane-¹³C₂ (1) with n-BuLi and subsequent reaction with squarate 17, to afford cyclobutenone 19 in 96% yield. Silyl deprotection of 19 with TBAF gave cyclobutenone 19 in 95% yield. The ¹H NMR spectrum of 19 displays some interesting second order effects, with the signal for the alkyne proton appearing as a well-defined X portion of an ABX spin-system.³² The small chemical shift difference between the ¹³C labels and the large difference between ¹JCH and ²JCH for this system presents a case in which all six spectral lines are clearly visible (see Supporting Information).

Scheme 4. Synthesis of isotopically labeled naphthalene 24.

Cyclobutenone 19 was submitted to the previously optimized conditions for thermal rearrangement, affording quinone 20 in 65% yield. Reduction of quinone 20 and subsequent alkylation of the intermediate hydroquinone, using CD₃I, delivered the labeled tetra-alkoxybenzene 21 in 73% yield over the two steps. Quinone 20 and tetra-alkoxybenzene 21 also both display interesting ¹H NMR spectra with well-resolved signals for the XX' portion of an AA'XX' spin-system, arising from magnetic nonequivalence (Figure 1).³² As a consequence of the adjacent ¹³C labels, a rare occasion is presented in which the coupling constants (¹JHH, ¹JCC, ¹JCH and ²JCH) of these AA'XX' systems can be easily determined. The corresponding AA' portions of the spectra for 20 and 21 were observed as singlets due to proton decoupling during ¹³C NMR data acquisition. Furthermore, as anticipated the ¹H NMR spectrum of tetra-alkoxybenzene 21 confirmed very high levels of deuterium incorporation (>99%, ¹H NMR) into the alkyoxy substituents of the left-hand fragment.

Figure 1. ¹H NMR spectra for compounds 20 and 21 (400 MHz, CDCl₃).
The unsymmetrical squarate 22 was prepared from squarate 17 in 60% yield, using iso-propanol-d8 to achieve perdeuteration of the fragment. The left-hand and right-hand fragments were coupled as described above, affording a mixture of regioisomers 23a and 23b in a 85% overall yield. Following thermal rearrangement of the mixture of regioisomers 23a and 23b, sequential alkylation of the intermediate hydroquinone, using MesSO-d6, afforded isotopically labeled naphthalene 24 in 47% yield for the two steps.

In summary, we have synthesized an isotopically labeled naphthalene derivative 24, incorporating an adjacent 13C spin-pair and perdeuterated alkoxy substituents. As reported elsewhere, this compound supports a long-lived nuclear singlet state with a lifetime exceeding 1 hour in room-temperature solution.20 The target naphthalene 24 was synthesized on a 1–4 mmol scale from compounds 6, 8, 10, 11, 12, 16, 17, and 23a and 23b.

ASSOCIATED CONTENT
Supporting Information
Experimental details and procedures, compound characterization data, copies of 1H, 2H and 13C NMR spectra for all new compounds. This materials is available free of charge via the Internet at http://pubs.acs.org.

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