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

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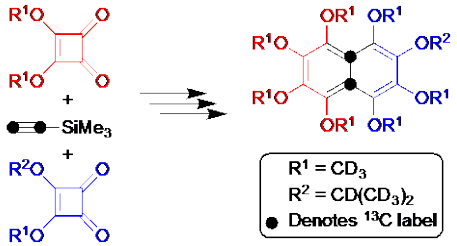
Supporting Information Placeholder



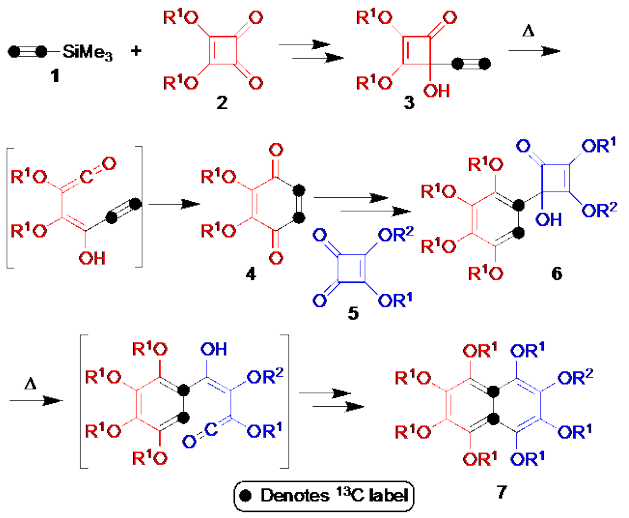
ABSTRACT: The synthesis of an octa-alkoxy substituted isotopically labeled naphthalene derivative, shown to have excellent properties in singlet NMR experiments, is described. This highly substituted naphthalene system, which incorporates an adjacent ^{13}C spin-pair, is readily accessed from a commercially 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,[1–3] 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.[7–9] Nuclear hyperpolarization, generated by methods such as dynamic nuclear polarization (DNP) gives rise to greatly increased NMR signal intensities;[10–14] theoretically ^{13}C NMR signals can be enhanced by a factor of 10^5 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.[15–19] 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.[9] 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 ~ 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.[18,19] 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 ^1H and ^{19}F. **(3)** Nuclei such as ^2H with quadrupole moments, while preferable to ^1H, 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.

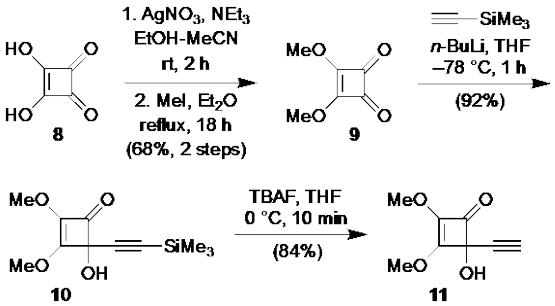
Based upon these design criteria, we considered that a naphthalene **7**, with a central ^{13}C spin-pair and fully deuterated side-chains (R^1 and R^2) would provide a suitable candidate (Scheme 1). Such an aromatic system is rigid, incorporates a local inversion center and all other spin-active nuclei 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 alkynyl- and arylcyclobutenone 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**.

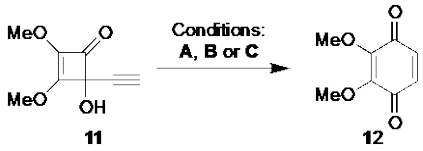
Scheme 2. Synthesis of cyclobutenone **11** as a precursor for the first cyclobutenone rearrangement.



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).[30] Thus, for the unlabeled synthesis, treatment of squaric acid (**8**) with $AgNO_3$ and Et_3N, followed by reaction with CH_3I in refluxing Et_2O for 18 h, afforded dimethyl squarate (**9**) in 68% yield. Alkynylation 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 baseline 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 arylcyclobutenone 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[a] 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%

[a] Isolated yields of purified compounds are quoted.

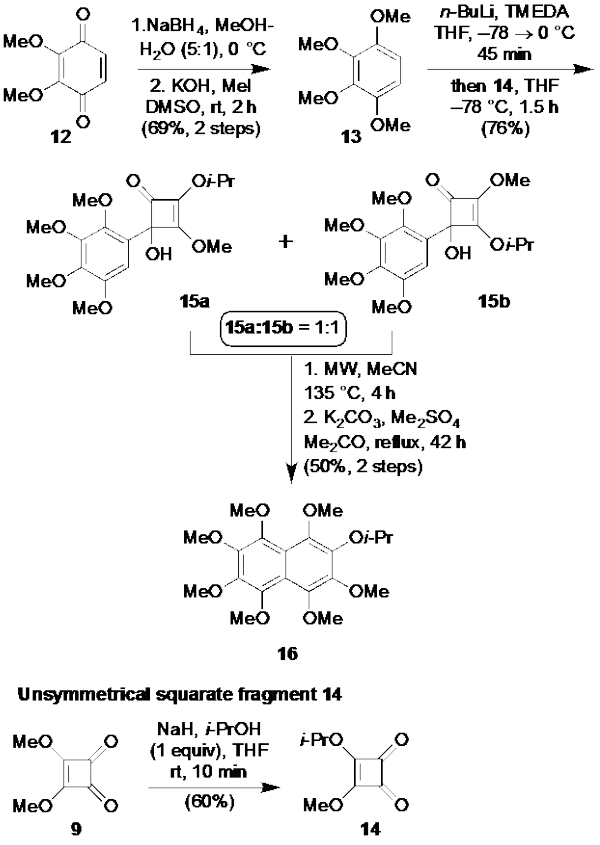
Reduction of quinone **12** by treatment with $NaBH_4$ afforded the dihydroquinone (Scheme 3), which was immediately dimethylated under basic conditions to afford the tetramethoxybenzene **13** in 69% over the two steps.

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[31] coupling sequence, affording an inseparable mixture of regioisomers **15a** and **15b** (~1:1, ^1H 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; $NaBH_4$ and $Na_2S_2O_4$ were both incompatible, ultimately leading to degradation of the products. Consequently, following the thermal rearrangement, the crude reaction mixture was immediately treated with K_2CO_3 and Me_2SO_4 in refluxing acetone, allowing isolation of naphthalene **16** in 50% yield over the two steps.

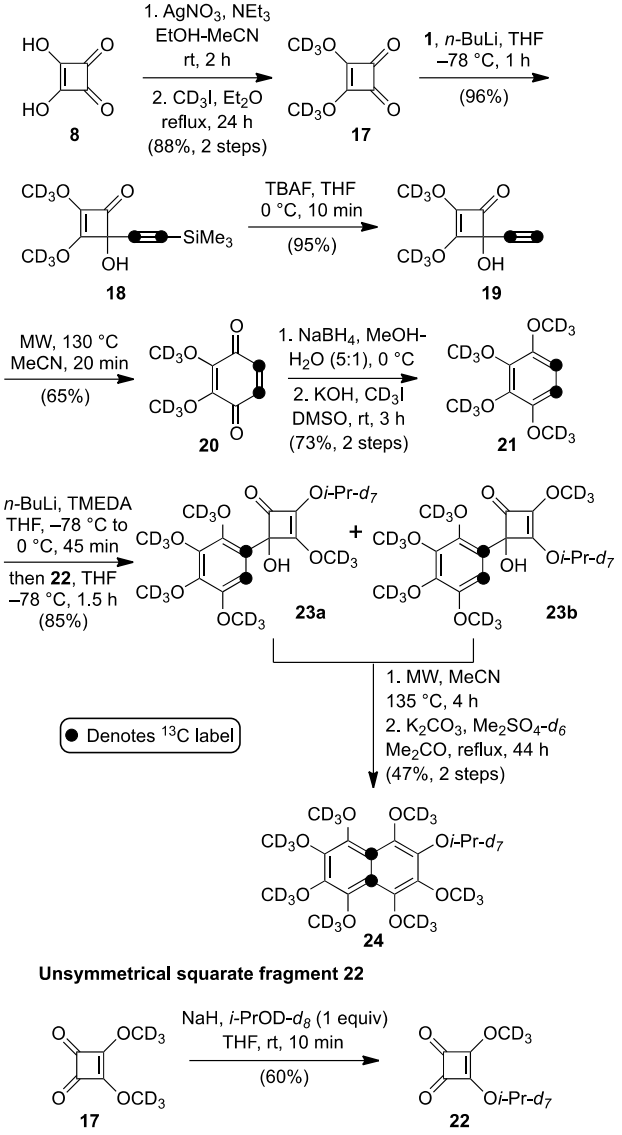
Scheme 3. Synthesis of unlabeled naphthalene system **16**.



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 ^{13}C NMR spectrum of **16** (acetone-d_6). 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]

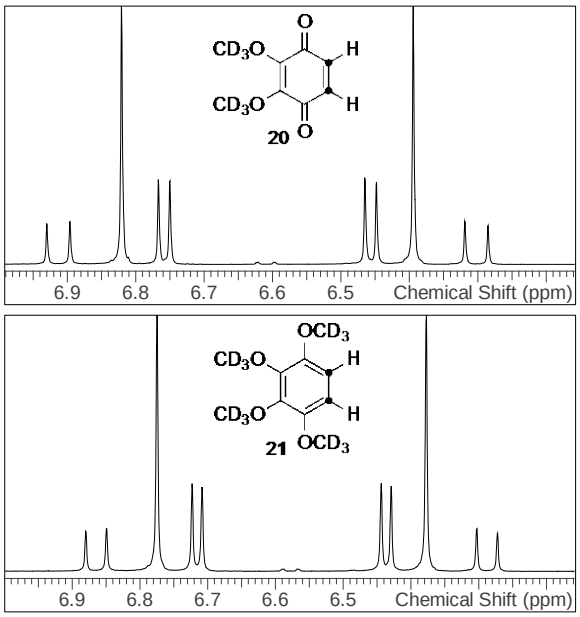
With an optimized route to the unlabeled naphthalene system **16** established, the labeled synthesis was subsequently performed (Scheme 4). Alkylation of squaric acid (**8**) with CD_3I via the di-silver salt afforded perdeuterated squarate **17** in 88% yield over the two steps. Introduction of the ^{13}C spin-pair proceeded smoothly via deprotonation of ethynyltrimethylsilane-$^{13}C_2$ (**1**) with *n*-BuLi and subsequent reaction with squarate **17**, to afford cyclobutenone **18** in 96% yield. Silyl deprotection of **18** 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 ^{13}C labels and the large difference between $^1J_{CH}$ and $^2J_{CH}$ 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 CD_3I, 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 ^{13}C labels, a rare occasion is presented in which the coupling constants (J_{HH}, J_{CC}, $^1J_{CH}$ and $^2J_{CH}$) 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 ^{13}C 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 alkoxy substituents of the left-hand fragment.

Figure 1. ^1H NMR spectra for compounds **20** and **21** (400 MHz, $CDCl_3$).



The unsymmetrical squarate **22** was prepared from squarate **17** in 60% yield, using *iso*-propanol-d_8 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 Me_2SO_4-d_6, 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 ^{13}C 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 commercially available starting materials in 10 linear steps (11 steps in total) with 15% overall yield for the linear sequence.

ASSOCIATED CONTENT

Supporting Information

Experimental details and procedures, compound characterization data, copies of ^1H, ^2H and ^{13}C 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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Author Contributions

The manuscript was written through contributions of all authors.

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