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Synthesis of ^{13}C and ^{2}H Labeled Vinyl Pyruvate and Hyperpolarization of Pyruvate

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Abstract: The hyperpolarization of nuclear spins has enabled unique applications in chemistry, biophysics, and particularly metabolic imaging. Parahydrogen-induced polarization (PHIP) offers a fast and cost-efficient way of hyperpolarization. Nevertheless, PHIP lags behind dynamic nuclear polarization (DNP), which is already being evaluated in clinical studies. This shortcoming is mainly due to problems in the synthesis of the corresponding PHIP precursor molecules. The most widely used DNP tracer in clinical studies, particularly for the detection of prostate cancer, is 1-^{13}C-pyruvate. The ideal derivative for PHIP is the deuterated vinyl ester because the spin physics allows for 100% polarization. Unfortunately, there is no efficient synthesis for vinyl esters of β-ketocarboxylic acids in general and pyruvate in particular. Here, we present an efficient new method for the preparation of vinyl esters, including ^{13}C labeled, fully deuterated vinyl pyruvate using a palladium-catalyzed procedure. Using 50% enriched parahydrogen and mild reaction conditions, a ^{13}C polarization of 12% was readily achieved; 36% are expected with 100% pH_2. Higher polarization values can be potentially achieved with optimized reaction conditions.

Introduction

Nuclear magnetic resonance (NMR) and magnetic resonance imaging (MRI) are among the most powerful analytical methods in chemistry, physics, and medicine.[1–3] It is a well-known fact, however, that both methods exploit only a very small part of their actual potential. The sensitivity is of the order of a few parts per million due to the low spin polarization of the magnetically active nuclei. The need for more sensitive MR is addressed by hardware improvements that include the construction of systems with higher magnetic fields and the use of cryo-probes.[4,5] These strategies, however, are expensive and limited by a sensitivity gain of one order of magnitude. Hyperpolarization (HP), on the other hand, is a more effective approach that allows amplifying the signal of selected molecules by a factor of 10^4–10^6, surpassing any current hardware technology. This increase in sensitivity has opened up entirely new applications such as visualizing metabolic processes beyond the conventional anatomical MRI imaging. It is therefore not surprising that hyperpolarization has experienced a steep upswing within the last decade. Nevertheless, there are still restrictions that prevent the general use of these methods.

Dynamic nuclear polarization (DNP)[6,7] is the most elaborate HP method with applications from proteomics[8] to real-time in vivo metabolomics.[9,10] The rapid development of dissolution DNP (dDNP) was triggered by the discovery of fast in vivo conversion of hyperpolarized 1-^{13}C-pyruvate (1-^{13}C-Pyr) to 1-^{13}C-lactate (1-^{13}C-Lac) in some malignant tumors and inflammations, known as the Warburg effect.[11] Administration of hyperpolarized 1-^{13}C-Pyr and monitoring metabolic conversion by MRI in vivo is considered to be a promising modality to detect early stages of prostate and breast cancer.[12] However, the main drawback of dDNP (disregard the high price) is its relatively low hyperpolarization throughput, which for commercial systems is ~1 h per sample while the lifetime of HP 1-^{13}C-pyruvate is only ~1 minute.

Parahydrogen-induced polarization (PHIP) is another quickly developing HP method.[13,14] Here, the spin order of parahydrogen (pH_2) is used to enhance the MR signal of dedicated contrast agents. pH_2 and orthohydrogen (oH_2) are the two nuclear magnetic spin isomers of H_2. pH_2 is a singlet state and asymmetric to the exchange of two nuclear spins, while oH_2 is a triplet spin state and symmetric under nuclear spin exchange. Following the Boltzmann distribution, at room temperature, 75% of H_2 is in the ortho state, and 25% in pH_2. At 77 K (liquid nitrogen), the pH_2 fraction is about 50%, and at 21 K (boiling

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point of H_2), it is >99%. pH_2 can be produced in bulk and stored for days, making it a convenient and readily available source of nuclear spin order.[15–17]

The spin order of pH_2 can be used to enhance the ^{13}C[18–21] or ^{15}N[22–24] magnetization of a contrast agent either by temporary contact (signal amplification by reversible exchange, SABRE)[25] or by pairwise addition of pH_2 to an unsaturated precursor (catalytic hydrogenation).[26] The fact that many biologically interesting compounds do not have an unsaturated precursor has long hindered the development of PHIP. This restriction, however, was mitigated by adding an unsaturated side group (unsaturated auxiliary group, UAG) to the desired contrast agents.[27–29] After hydrogenation of the side group and polarization transfer to the main molecule, the auxiliary group was cleaved and the desired contrast agents resulted.

To date, mainly allyl-, propargyl- and vinyl esters have been used as a sidearm or "unsaturated auxiliary group" (UAG).[30] 1-^{13}C-Pyr or its precursors were polarized to a maximum value of 6.2% by sidearm hydrogenation (PHIP-SAH) of allyl-pyruvate using magnetic field cycling[31,32] and below 1% using INEPT-type sequences[33] and 10.8% by SABRE.[34] The practicability of the PHIP-SAH method was demonstrated in cells[35] and animal models in vivo.[31] However, the polarization yield is still lower for allyl or propargyl precursors than for vinyl precursors.[31,36]

The transfer of pH_2-derived spin order into the polarization of a third nucleus is most efficient if there are no other nuclei interacting with pH_2,[21,37] or if the interactions can be canceled.[38] In these cases, the spin system is effectively a three spin-$^1/_2$ system, and X-nuclei polarization of 100% can be achieved.

Such a spin system can be approximated by deuteration of all non-participating protons and selective labeling of the X-nuclei of choice (e.g. ^{13}C or ^{15}N). Vinyl esters are particularly suited as UAGs because desired three-spin system is easily realized if the vinyl group is fully deuterated (disregarding couplings of the ^{13}C to other nuclei in the main molecule). Also important is that the added hydrogens are only 3 and 4 bonds away from the carbonyl ^{13}C atom, which facilitates polarization transfer. This method has been applied to prepare ^{13}C polarization levels exceeding 50% for ethyl acetate-*d6* from fully deuterated vinyl acetate.[21]

Encouraged by these results, we set out to synthesize 1-^{13}C-vinyl-pyruvate-*d6* (1-^{13}C-VP-*d6*), which is the precursor of the probably most promising metabolic tracer for hyperpolarized MRI as mentioned earlier. After parahydrogenation of the vinyl group, followed by spin order transfer to 1-^{13}C and ester hydrolysis, hyperpolarized 1-^{13}C-Pyr should be formed.

Vinyl esters are synthesized on an industrial scale as monomers for the production of polymers. The most important process is the reaction of ethylene with acetic acid in the presence of air and a Pd metal catalyst. Vinyl esters of higher carboxylic acids or substituted carboxylic acids are more difficult to prepare and usually are synthesized by transvinylation of the corresponding carboxylic acid with vinyl acetate and a transition metal catalyst.[39] Hg(II) salts,[40] Pd(II) salts,[41] Pd(II) complexes,[42–45] Au(I) complexes[46] and Ru(III) complexes[47] have been used towards this end. However, high temperatures and a large excess of the vinylation reagent are necessary to achieve reasonable yields, and the separation from unreacted vinylation reagents, polymerization or other by-products are major problems in most procedures. Even more problematic is the synthesis of vinyl esters of α- and β-ketocarboxylic acids, which are base sensitive or susceptible to decarboxylation under acidic conditions.

Two procedures have been published describing the synthesis of pyruvic acid vinyl ester. Both procedures start with pyruvic acid. The first reaction uses the known palladium-catalyzed trans vinylation procedure with a large excess of vinyl acetate (Figure 1a). However, unlike aliphatic carboxylic acids, pyruvic acid (α-ketocarboxylic acid) gives only a 6% yield.[30] The strongly basic reaction conditions favor dimerization and oligomerization of the product via Aldol reactions, reducing the yield and giving rise to a reaction mixture, which is difficult to purify. Consequently, the second published synthesis tries to avoid these side reactions by transforming the keto group (position 1 in pyruvic acid) into the diethyl acetal, and by applying an optimized transvinylation procedure.[48] While improving the transvinylation process, the two additional steps, the acetalization of pyruvic acid in the first step, and the hydrolysis of the acetal after transvinylation, reduce the overall yield to 8.2%. The product was contaminated with ethyl pyruvate (EP) but was used for successful hyperpolarization experiments.[48] The first synthesis of 1-^{13}C-VP-*d6* was described in our recent patent.[49]

Except for VP, only one α-ketocarboxylic acid vinyl ester is known,[50] and the synthetic procedure is not suitable for large-scale preparations, or for the synthesis of labeled compounds. Vinyl esters of 1,3-ketocarboxylic acids cannot be synthesized by the usual vinylation or transvinylation methods at all (see above). Only vinyl acetoacetate has been synthesized, however, by high-temperature pyrolysis in moderate yields.[51,52]

Here, we present a new strategy for the synthesis of ketocarboxylic acid vinyl esters (Figure 1b) by using different ^{13}C

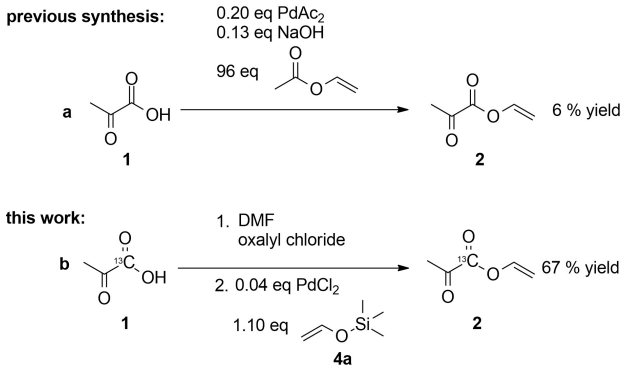

Figure 1. Synthesis of vinyl pyruvate. (a) Preparation by Chukanov et al.,[30] where the vinyl group is transferred from vinyl acetate to pyruvate (transvinylation) in the presence of palladium(II). (b) Our new synthesis uses a vinyloxy silane (**4**) as a vinyl source. Significantly improved yields of vinyl pyruvate were achieved. The method was easily modified to obtain also isotopically labeled molecules. DMF = dimethylformamide.

and ^{2}H (D) labeled pyruvate vinyl esters as an example (Figure 2). We achieved 67% yield for 1-^{13}C-VP and 45% for 1-^{13}C-VP-*d6*. Other isotopomers were synthesized as well. We successfully conducted PHIP of these agents and accomplished a ^{13}C-polarization of 12% for 1-^{13}C-EP-*d6* after hydrogenation with 50% pH_2. With 100% enriched pH_2 the polarization yield is expected to triple to more than 30%.

Results and Discussion

Synthesis

The preparation starts by transforming the pyruvic acid into the corresponding acid chloride (**3a**–**c**) using the Vilsmeier-Haack reagent[53] with oxalyl chloride and catalytic amounts of N,N-dimethylformamide. Alternative procedures described in the literature provided lower yields and side products that were difficult to remove.[54–57] The acid chloride was then used as a raw product in the next reaction step. As vinylating agents, vinyloxy silanes (**4a**–**c**) were used in the presence of a palladium catalyst. The resulting vinyl esters (**2a**–**e**) could be isolated by flash column purification. Vinyloxy-trimethylsilane is very moisture sensitive but more reactive than more hindered derivatives. Vinyloxy-dimethyl-isopropylsilane turned out to be a good compromise between stability and reactivity.

Several transition metal catalysts were tested. Only $HgCl_2$ and especially $PdCl_2$ gave satisfying yields, with $PdCl_2$ being superior to $Pd(OAc)_2$. Dichloromethane (DCM) was the solvent of choice (for details see Supporting Information). Even though tetrahydrofuran (THF) and acetonitrile (MeCN) gave slightly better yields, DCM can be removed much more easily and gently, preventing the decomposition of the sensitive vinyl ester. In addition, residues of DCM in the crude product proved to be unproblematic for column chromatography while traces of acetonitrile decreased the purity of the isolated fractions due to its strong elution properties.

According to this scheme, VP-*H6* (**2a**) and 1-^{13}C-VP-*H6* (**2b**) were prepared for the first time with high yields and high purity in just two consecutive steps (Figure 3). All reactions were carried out starting with 1 g of (^{13}C labeled and unlabeled) pyruvic acid. The isolated yield of vinyl

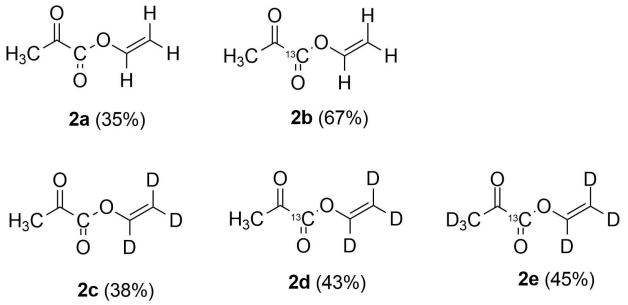

Figure 2. Synthesized isotopomers of vinyl pyruvate (VP) and the corresponding yields: VP (2a), 1-^{13}C-VP (2b), VP-*d3* (2c), 1-^{13}C-VP-*d3* (2d), 1-^{13}C-VP-*d6* (2e).

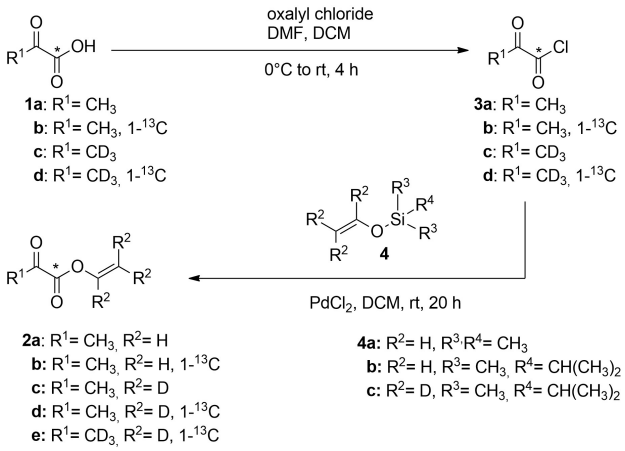

Figure 3. General reaction scheme for the synthesis of vinyl pyruvates (VP). First, the carboxylic acid (**1a**–**c**) was converted to an acid chloride (**3a**–**c**). Then catalytic amounts of $PdCl_2$ were added and vinyloxy silane (**4a**–**c**) was used as a vinylating agent to produce VP (**2a**–**e**). The C-1 position is marked with a star. DCM = dichloromethane, DMF = dimethylformamide.

pyruvate (**2b**) was 67%. The raw yield was determined by NMR to be up to 90%. Vinyl pyruvates are very sensitive to acidic and particularly basic conditions. The isolated yields, therefore, depended decisively on the workup procedure (see Supporting Information). Because of the very mild reaction conditions, the yields compared to established procedures were improved by a factor of eight.[30] Even if a significant amount of VP (**2**) was lost during the workup procedure, the isolated VP (**2**) exhibited a higher purity than reported.[48] The procedure is especially suitable for small batches in the range of hundreds of milligrams to several grams, making it ideal for producing small amounts of isotopically labeled substances starting from the corresponding acids.

For hyperpolarization experiments, the ^{13}C-labeled and fully deuterated 1-^{13}C-VP-*d6* (**2e**, 1-^{13}C-VP-*d6*, Figure 4) is the most promising precursor (see above). 1-^{13}C-Pyr-*d4* (**1c**, Figure 3) and dimethylisopropyl(vinyloxy) silane-*d3* (**4c**) were used as starting compounds to synthesize 1-^{13}C-VP-*d6*. Based on a procedure reported by Denmark et al.,[58] we developed a synthetic route to obtain deuterated vinyloxysilane **4c** using tetrahydrofuran-*d8* (THF-*d8*, **5**) as a deuterium source. THF-*d8* (**5**) was cleaved with *n*-butyllithium (*n*-BuLi) and the generated enolate (**6**) of acetaldehyde was trapped with a trialkylsilyl chloride to obtain the deuterated trialkyl(vinyloxy)silane (Figure 4). It is noteworthy that a considerable kinetic isotope effect was observed in the cleavage of THF-*d8* and the reaction times with *n*-BuLi had to be extended. The silane (**4c**) was subsequently reacted with the pyruvyl chloride to the target product **1c**. Since trimethyl(vinyloxy)silane (**4a**) is very sensitive to water, and because its boiling point (75 °C) is close to the one of THF (67 °C), separation was not possible on a small scale. We, therefore, used dimethylisopropyl(vinyloxy)silane (**4b**–**c**) (b.p. 137 °C),

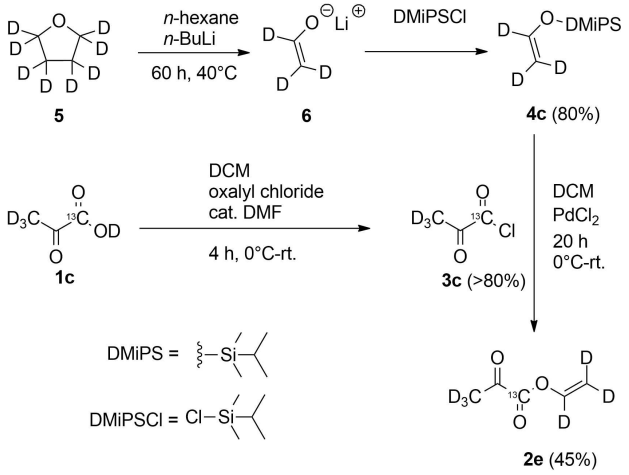

Figure 4. Synthesis of 1-^{13}C-VP-*d6*. 1-^{13}C-VP-*d6* **2e** was prepared using dimethyl isopropyl (vinyloxy)silane as vinyl source. Purification was performed after the last reaction step.

which was obtained in high yields and purity as *d3*-derivate (**4c**) after a simple workup procedure.

From 1 g (10.9 mmol) of 1-^{13}C-Pyr-*d4* (**1c**), we obtained 572 mg 4.73 mmol) of 1-^{13}C-VP-*d6* (**2e**) which was further investigated in PHIP-SAH experiments. The total yield over two steps was determined to be 45 % relative to 1-^{13}C-Pyr-*d4*.

Hyperpolarization

Parahydrogenation and subsequent spin order transfer and cleavage of the sidearm (Figure 5) were carried out at a probe temperature of 330 K, in chloroform-*d*, at a magnetic field of 9.4 T, with 50 % pH_2[59] and a hydrogenation time of $t_{h2} = 20$ s (see experimental section for more details).

Upon hydrogenation of 1-^{13}C-VP-*d6*, 1-^{13}C-EP-*d6* was produced (Figure 6a). As expected, the ^{1}H spectrum exhibited two

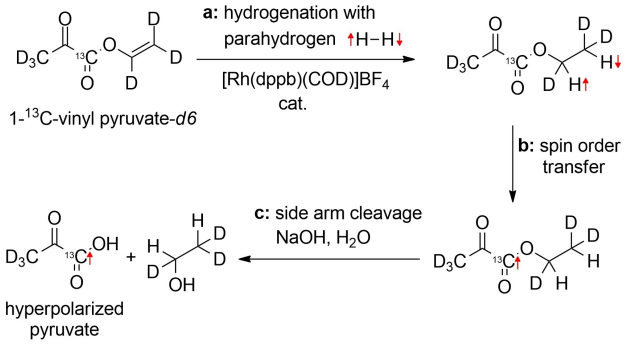

Figure 5. Pyruvate PHIP-SAH hyperpolarization. (a) Parahydrogen was added to 1-^{13}C-VP-*d6* in chloroform-d and in the presence of homogeneous catalyst [Rh(dppb)(COD)BF_4] (dppb = 1,4-Bis(diphenylphosphino)butane). (b) Then using the spin order transfer method pH_2 spin order is converted to the net magnetization of 1-^{13}C nucleus of pyruvate. (c) And finally, an aqueous NaOH solution is added to cleave the sidearm. As a result, hyperpolarized 1-^{13}C-Pyr in the aqueous phase is produced.

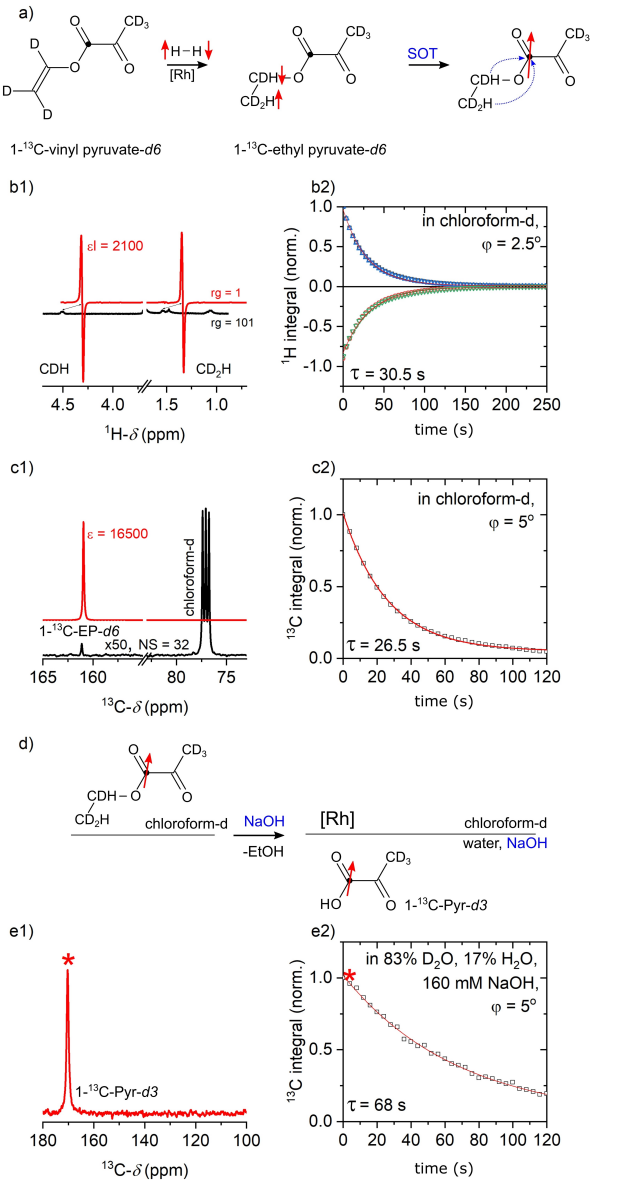

Figure 6. Hyperpolarization of 1-^{13}C-ethyl pyruvate-*d6* and 1-^{13}C-pyruvate-*d3*. (a) Schematic view of the addition of pH_2 (red arrows) to 1-^{13}C-VP-*d6* yielding 1-^{13}C-EP-*d6* and subsequent spin order transfer (SOT) using the J-coupling network to obtain ^{13}C polarization (red arrow). (b) Hyperpolarized ^{1}H-PASADENA (red) and corresponding thermal spectrum (black) acquired with receiver gains (rg) 1 and 101. The average line enhancement was $\varepsilon l = 2100$ at 9.4 T (the black thermal signal was moved 0.2 ppm to the left for better visualization). The decaying magnetization was sampled with a train of small flip angle pulses and a monoexponential function was fitted to the data, yielding an observable ZZ-spin order decay $\tau = 30.5$ s. (c) After the SOT to ^{13}C, a signal enhancement of 16500 or 12 % polarization of 1-^{13}C-EP-*d6* was obtained (Figure 6, parameters $\tau_1 = \tau_3 = 84$ ms and $\tau_2 = 36$ ms). The observable signal decay was $\tau = 26$ s. The thermal spectrum was enlarged 50 fold and measured with the same acquisition parameters using 32 scans and a repetition time of 200 s. (d) Scheme of two-phase separation and sidearm cleavage as a result of the addition of NaOH aqueous solution. (e1) ^{13}C NMR spectrum of aqueous phase showing strong ^{13}C-signal of hyperpolarized pyruvate after cleavage, the first in a row used to sample the decaying polarization (e2). The observable lifetime of 1-^{13}C-Pyr-*d3* was extended to $\tau = 68$ s in aqueous solution. Note that all three hyperpolarization decays (b2, c2, e2) were measured with repetition time TR = 4 s and an excitation angle of 5° (2.5° used for PASADENA is equivalent to 5° used for magnetization). Corresponding corrected hyperpolarization lifetime values do not significantly differ from observed values: 30.9 s, 27.2 s and 72.7 s.

pairs of hyperpolarized, antiphase lines corresponding to methylene (CDH) and methyl (CD_2H) sites 1-^{13}C-EP-*d6* after a hard 45° excitation (Figure 6b1, PASADENA experiment). The average enhancement of each line was $\varepsilon l = 2100$, corresponding to a multiplet polarization mP = 12.2%. The multiplet polarization mP of two spins corresponds to $\frac{\hat{1}}{4} - \mathrm{mP} \cdot \hat{I}_z^1 \hat{I}_z^2$ density matrix and therefore also referred to as ZZ-spin order. The maximum multiplet polarization of two protons that can be achieved theoretically by the addition of 50% pH_2 is $P^{1H} = 33.3\%$,[60] only ~2.7 times higher than what was achieved experimentally. The main polarization losses are usually considered singlet-triplet mixing on the catalyst and relaxation.[61,62]

Using a series of small flipping angles $\varphi = 5^o$ with a repetition time TR = 4 s, we quantified the lifetime of the multiplet polarization to $T_{HP}^{ZZ} = 30.9$ s (observed value 30.5 s, Figure 6b2). Using this constant to estimate the polarization at the beginning of the hydrogenation period (Eq. (1)) yielded an initial polarization of 18.8% - accounting for some, but not the complete difference from the theoretical maximum of 33.3%.

$$P = exp\left[-t_{h2}/T_{HP}^{ZZ}\right] \cdot P_{max} \quad (1)$$

Using "efficient spin order transfer to heteronuclei via relayed INEPT chains" (ESOTHERIC)[21] allowed us to achieve ≈12% ^{13}C-polarization of 1-^{13}C-EP-*d6*, a 16500 fold enhancement with respect to the thermal equilibrium at 9.4 T. By using 100% pH instead of 50%, these numbers are expected to triple.

The lifetime of the polarization in chloroform-*d* at 9.4 T was measured to ≈27.2 s (26.5 s observed value, Figure 6c), less than expected for example in water, and may be caused by the detrimental effect of Cl-containing solvents as recently reported[63] Here, it was essential to clean the high-pressure tube with concentrated nitric acid before the experiment; – otherwise, T_1 was found to decrease further (down to ~15 s). This effect may be attributed to a grey film that appeared on the surface of the tube if chloroform was used for flushing instead, and which did not dissolve in organic solvents like acetone, chloroform, or ethanol.

For any in vivo application of hyperpolarized contrast agents, it is necessary to extract the hyperpolarized contrast agent from the solution of the organic solvent and the metal-organic catalyst. Here, we used the established two-phase approach described before.[31] After hydrogenation and SOT at 9.4 T, the sample was flushed into another NMR tube outside of the magnet, containing water (83% D_2O and 17% H_2O) and 167 mM NaOH. This tube was shaken and inserted into the NMR spectrometer again. We did this outside to mimic an envisaged experiment where hyperpolarized media will be manipulated manually and administrated outside of the magnet. For the anticipated in vivo applications, this process must be automated and thoroughly optimized to assure high-quality standards of administrable material.

A single, strong ^{13}C resonance was observed at 170.3 ppm, the expected frequency of pyruvate. No signal was observed at 161 ppm, the expected resonance of 1-^{13}C-EP.[27,31] More interestingly, the lifetime of the hyperpolarized pyruvate signal was found to increase to 72.7 s (68 s observed, Figure 6e) – expected for pyruvate in aqueous solution, and sufficiently long to reduce relaxation during administration.

Conclusion

We present a significantly improved synthesis of vinyl esters of α-ketocarboxylic acids, particularly vinyl pyruvate. In contrast to previous methods, which depend on a very large excess of vinyl acetate and the addition of a base, we use equimolar amounts of vinyloxysilylanes as the vinyl source for transvinylation. The reaction proceeds at room temperature under $PdCl_2$ catalysis and under neutral or slightly acidic conditions, which prevents dimerization and oligomerization of the base-sensitive vinyl esters. The high yields and simple workup are particularly suitable for the preparation of isotopically labeled VP. Five different deuterium and ^{13}C labeled VP isotopomers were prepared, including the fully deuterated and 1-^{13}C labeled vinyl pyruvate (45% yield). Note, that when this manuscript was in the revision state, an alternative synthesis for 1-^{13}C-VP-*d6* was reported with a yield of 3% starting from 1-^{13}C-Pyr-*d4*. The synthesis avoids base-catalyzed side reactions by using a photocleavable protecting group.[64] 1-^{13}C-VP-*d6* is ideally suited for the preparation of hyperpolarized pyruvate via the PHIP-SAH method. Hyperpolarization yields as high as 12% were obtained with 50% parahydrogen, which corresponds to a gain of factor 16 500 in the intensity of the 1-^{13}C signal at 9.4 T magnet. With 100% pH_2, a polarization of 36% is expected. Polarization rates in this range, to date, were only obtained by the much more expensive and elaborate DNP method. DNP-hyperpolarized pyruvate has been used to monitor the metabolism of pyruvate to lactate by MRI in vivo. The conversion of pyruvate to lactate and other metabolites is faster in prostate and breast cancer cells (Warburg effect). This molecular imaging method has high prospects for the early detection of cancer before structural or anatomical changes are visible in conventional MRI. Our synthetic method now provides access to the suitable precursor for the convenient and cost-effective PHIP method, which allows hyperpolarization of pyruvate at a much-reduced cost, with less sophisticated instrumentation and significantly higher throughput and thus brings hyperpolarization closer to clinical applications.

Experimental Section

General Experimental Information: All solvents used for the synthesis were purchased from commercial sources and filtered over silica and dried before use. All reagent-grade chemicals were purchased from commercial sources and used without further purification. Isolera One (Biotage) with a pre-packed column (PF-DLE-F0040, Interchim) was used for chromatography. ^{1}H and ^{13}C NMR spectra were obtained using AC 200 MHz and DRX 500 MHz high-resolution NMR spectrometers with 5 mm TXI probes

(Bruker) to control the synthesis and purity. We used AVANCE NEO 400 MHz WB with 5 mm BBFO probe (Bruker) for PHIP experiments. High-resolution mass spectra were recorded with an AccuTOF GCv4G (JEOL). Detailed information about chemicals, the synthesis, and methods used as well as further analytical data is available in Supporting Information.

PHIP-SAH experiments. We used a liquid nitrogen pH_2 generator[59] to prepare 50% pH_2 hydrogen gas and the pH_2 delivery system described before.[37,60] To hyperpolarize ethyl pyruvate we mixed 2 mM of 1-^{13}C-VP-*d6* and 2 mM of [1,4-Bis-(diphenylphosphino)-butan]-(1,5-cyclooctadiene)-rhodium(I)-tetrafluoroborate ([Rh], CAS 79255-71-3, Sigma Aldrich) in chloroform-*d* (CAS 865-49-6, Sigma Aldrich). Then we filled a clean medium wall high-pressure 5 mm NMR tube (524-PV-7, Wilmad-LabGlass) with 350 µL of this solution. The NMR tube was placed into the NMR spectrometer, and allowed to reach a temperature of 330 K; the waiting time was 2–3 minutes. Then the pH_2 bubbling system was activated, pH_2 pressure in the tube was increased to 7 bar and the sample was flushed with pH_2 for 20 seconds. Finally, the bubbling was stopped and SOT was executed. For a two-phase separation, we mixed 500 µL D_2O (Deutero GmbH) and 100 µL of 1 mol/L sodium hydroxide solution in a standard 5 mm NMR tube. After hydrogenation with pH_2 and subsequent SOT, the sample was pneumatically shuttled to the NMR tube and shaken vigorously. This tube and an empty high-pressure tube were exchanged, and the signal decay of the ^{13}C signal in acquous phase was measured. More details are given in the Supporting Information.

Synthesis of vinyl pyruvates (2a–e). To obtain the vinyl esters of pyruvic acid, 10 mL DCM was placed in a three-neck round bottom flask under N_2 atmosphere. The flask was cooled to 0 °C and 1.0 equivalent of oxalyl chloride was added. Afterward, 1.0 equivalents of pyruvic acid and DMF (13 drops for 1 g pyruvic acid) were added as a solution in 5 mL DCM in batches of 5 mL every 5 min. The solution was stirred for 2 h at 0 °C and another 2 h at rt. The solution was degassed under Schlenk conditions and its volume was reduced to 10 mL to obtain a solution of pyruvoyl chloride. Palladium(II)chloride (80.5 mg, 0.454 mmol) was placed in a round bottom flask under an N_2 atmosphere and brought into suspension with 10 mL DCM. The suspension was cooled to 0 °C and 1.3 equivalents of (vinyloxy)silane (**4a–c**) were added. The mixture was stirred for 0.5 h and the freshly prepared solution of the pyruvoyl chloride was added dropwise over 0.5 h. With no further cooling, the solution was stirred for 20 h. Small amounts of hydroquinone were added and the solution was concentrated i. vac. to a volume of 3 mL, which was then purified via flash column chromatography on silica using DCM: *n*-pentane (20:80) (R_f = 0.6). The product was obtained as a slightly yellow oil. (for further details see Supporting Information).

Vinyl pyruvate (2a): yield: 443 mg (34%), purity: 93% (7% EE) **^{1}H NMR** (500 MHz, $CDCl_3$, 298 K): δ = 7.28 (dd, ^{3}J = 13.84 Hz, ^{3}J = 6.17 Hz, 1 H, *H*-4), 5.17 (dd, ^{3}J = 13.84 Hz, ^{2}J = 2.17 Hz, 1 H, *H*-5), 4.83 (dd, ^{3}J = 6.17 Hz, ^{2}J = 2.17 Hz, 1 H, *H*-5), 2.52 (s, 3 H, CH_3) ppm. **^{13}C NMR** (500 MHz, $CDCl_3$, 298 K): δ = 190.56 (*C*-2), 157.51 (*C*-2), 140.68 (*C*-5), 101.40 (*C*-4), 26.77 (*C*-3) ppm.

1-^{13}C-Vinyl pyruvate (2b): yield: 859 mg (67%), purity: 76% (9% DCM, 15% EE) **^{1}H NMR** (500 MHz, $CDCl_3$, 298 K): δ = 7.27 (ddd, ^{3}J = 13.83 Hz, ^{3}J = 6.18 Hz, ^{3}J = 2.40 Hz, 1 H, *H*-4), 5.12 (ddd, ^{3}J = 13.82, ^{2}J = 2.14 Hz, ^{4}J = 0.50 Hz, 1 H, *H*-5), 4.82 (dd, ^{3}J = 6.17 Hz, ^{2}J = 2.17 Hz, 1 H, *H*-5), 2.52 (d, ^{3}J = 1.57 Hz, 3 H, CH_3) ppm.**^{13}C NMR** (500 MHz, $CDCl_3$, 298 K): δ = 190.53 (d, C-2), 157.50 (C-1), 140.66 (C-5), 101.39 (d, C-4), 26.80 (d, C-3) ppm.

Vinyl pyruvate-*d3* (2c): yield: 497 mg (38%), purity: 91% (5% DCM, 4% EE) **^{1}H NMR** (500 MHz, $CDCl_3$, 298 K): δ = 2.52 (s, 3 H, CH_3) ppm. **^{13}C NMR** (500 MHz, CDCl3, 298 K): δ = 190.54 (*C*-2), 157.48 (*C*-1), 26.75 (*C*-3).

1-^{13}C-Vinyl pyruvate-*d3* (2d): yield: 566 mg (43%), purity:95% (1% DCM, 4% EE) **^{1}H NMR** (500 MHz, $CDCl_3$, 298 K): δ = 2.52 (d, ^{3}J = 1.59 Hz, 3 H, CH_3) ppm. **^{13}C NMR** (500 MHz, $CDCl_3$, 298 K): δ = 190.40 (d, *C*-2), 157.48 (*C*-1), 140.43 (t, *C*-5), 100.73 (t, *C*-4), 26.60 (d, *C*-3) ppm.

1-^{13}C-Vinyl pyruvate-*d6* (2e): yield: 572 mg (45%), purity: 76% (6% DCM, 18% EE) **^{2}H NMR** (500 MHz, $CDCl_3$, 298 K): δ = 7.31 (s, 1 D, D-4), 5.20 (s, 1 D, D-5), 4.86 (s, 1 D, D-5), 2.50 (s, 3 D, D-3) ppm. **^{13}C NMR** (500 MHz, $CDCl_3$, 298 K): δ = 190.61 (d, C-2), 157.32 (s, C-1), 140.20 (t, C-5), 100.63 (t, C-4), 26.05 (d, C-3) ppm.

Synthesis of dimethylisopropyl(vinyloxy)silane (4b–c): To obtain the deuterated vinyloxysilane 1.0 equivalent of *n*-BuLi was added dropwise to 4.0 equivalents of cooled (0 °C) THF to synthesize **4b** or deuterated THF-*d8* for **4c**. After 0.5 h, the solution was heated to 40 °C and stirred for 20 h for THF and 5 days for THF-*d8*. The resulting solution was again cooled to 0 °C and dimethylisopropylsilyl chloride (13.0 mL, 82.7 mmol) was added slowly. After 2 h of stirring, the solvent was removed i. vac. yielding a slurry containing lithium chloride. Diethyl ether was added and the organic phase was washed three times with half saturated sodium hydrogen carbonate solution. The resulting combined aqueous solution was extracted once with diethyl ether. The solvent was removed i. vac. and the product (**4b** or **4c**) was obtained as a clear liquid.

Dimethylisopropyl(vinyloxy)silane (4b): yield: 6.59 g (57%), purity: ~90% (5% EE) **^{1}H NMR** (500 MHz, $CDCl_3$, 298 K): δ = 6.51 (dd, ^{3}J = 13.61 Hz, ^{3}J = 5.85 Hz, 1 H, *H*-1), 4.43 (dd, ^{3}J = 13.61 Hz, ^{2}J = 0.76 Hz, 1 H, *H*-2), 4.11 (dd, ^{3}J = 5.85 Hz, ^{2}J = 0.76 Hz, 1 H, *H*-2), 0.98 (m, 6 H, $CH(CH_3)_2$), 0.14 (s,6 H, $Si(CH_3)_2$) ppm. **^{13}C NMR** (500 MHz, $CDCl_3$, 298 K): δ = 146.17 (*C*-1), 94.42 (*C*-2), 16.57 (*C*-3,4), 14.43 (*C*-5), −4.41 (*C*-6,7).

Dimethylisopropyl(vinyloxy)silane-*d3* (4c): yield: 5.21 g (73%), purity: ~80% (8% EE) **^{1}H NMR** (500 MHz, $CDCl_3$, 298 K):δ = 1.32 (m, 1 H, *H*-5), 0.98 (m, 6 H, $CH(CH_3)_2$), 0.15 (s, 6 H, $SiC(CH_3)_2$) ppm. **^{13}C NMR** (500 MHz, $CDCl_3$, 298 K):δ = 16.75 (*C*-3,4), 14.60 (*C*-5), −4.23 (*C*-6,7) ppm.

Author Contributions

AB, ANP, JBH, RH: conceptualization, writing – original draft. AB, RH conceptualization of VP synthesis. AB, RH and TS investigation of VP synthesis. FS analysis of NMR during VP synthesis. ANP, FE investigation of EP hyperpolarization. ANP, RH, FS, and JBH: supervision, funding acquisition. All authors contributed to discussions and helped interpreting the results and have given approval to the final version of the manuscript.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

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