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Synthesis of Isotopically Labeled, Spin-Isolated Tyrosine and Phenylalanine for Protein NMR Applications

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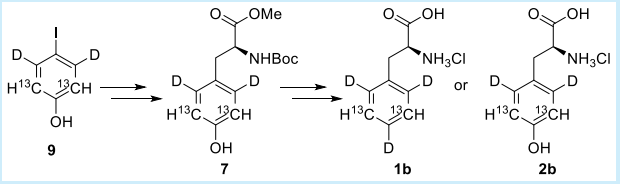
ABSTRACT: Isotopically labeled amino acids are widely used to study the structure and dynamics of proteins by NMR. Herein we describe a facile, gram-scale synthesis of compounds **1b** and **2b** under standard laboratory conditions from the common intermediate **7**. **2b** is obtained via simple deprotection, while **1b** is accessed through a reductive deoxygenation/deuteration sequence and deprotection. **1b** and **2b** provide improved signal intensity using lower amounts of labeled precursor and are alternatives to existing labeling approaches.



Protocols for the isotopic labeling of highly deuterated proteins to enable study by NMR are well established.[1a−c] Of recent interest, the ability to produce proteins containing isotopically labeled, spin-isolated aromatic amino acids has provided enhanced structural detail and enabled the mechanistic study of protein kinases.[2] In protein kinases, the knowledge of the Phe ring orientation in the conserved Asp-Phe-Gly motif (DFG) in solution is of great interest, as it correlates to the active vs inactive form.[3] The isotope pattern specificity and high levels of incorporation necessary for the success of this method are achieved via the introduction of advanced metabolic precursors that are transformed into the desired amino acid during protein expression *in situ*.[4] Phenylalanine **1a** and tyrosine **2a** are accessed via pyruvates **3** and **4** prepared from simple, commercially available isotopic building blocks and assembled in such a way that the desired isotope pattern is under complete synthetic control (Figure 1).[5]

In the course of preparing proteins incorporating spin-isolated aromatic amino acids, literature-reported pyruvates **3** and **4** were synthesized in house and several challenges were noted. The syntheses of **3** and **4** diverge from the common intermediate **5** at an early stage. The synthesis of **3** takes seven steps from **5** and requires manipulation and purification of four volatile intermediates. The preparation of **4** from **5** is carried out in six steps, the final reaction of which requires rigorous exclusion of oxygen to prevent product degradation. For the same reason, pyruvate **4** requires storage at −80 °C, which presents an additional barrier to its use.[6] In addition, up to 200 mg of **3** and **4** per liter of culture may be needed to achieve high levels of label incorporation into the protein for NMR studies using existing protocols.[4,7] In our hands, following the recently reported protocol for aromatic labeling using stereo-array isotope labeling (SAIL) amino acids,[2e] we found that **3** led to Phe **1a** incorporation at high levels (>90%) in the expressed protein at concentrations of 50 mg/mL (see the Supporting Information and Figure 2). In contrast, the total incorporation of **2a** remained ∼7-fold lower in comparison to **1a** despite the use of increasing concentrations of **4** in the expression medium (Figure 2a). A similar experience was reported by others,[8] which led us to consider an alternative strategy for the introduction of **1a** and **2a** into our labeling experiments.

While unsure of the root cause of the low incorporation, we hypothesized that incorporating amino acids **1a** and **2a** directly, rather than their precursors, might increase the labeling efficiency. Before initiating synthesis, we set the following criteria that we believed were critical to the design of

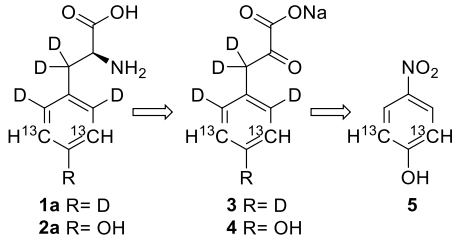


Figure 1. Spin-isolated phenylalanine **1a** and tyrosine **2a**, their corresponding pyruvate bioprecursors **3** and **4**, and the common synthetic intermediate **5**.

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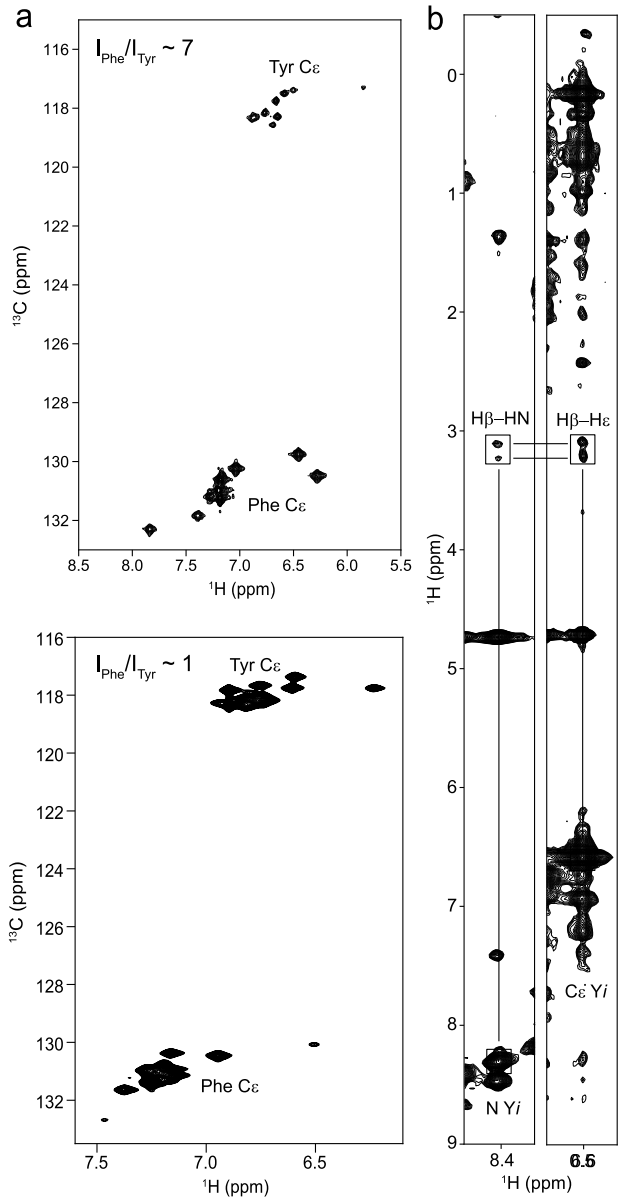
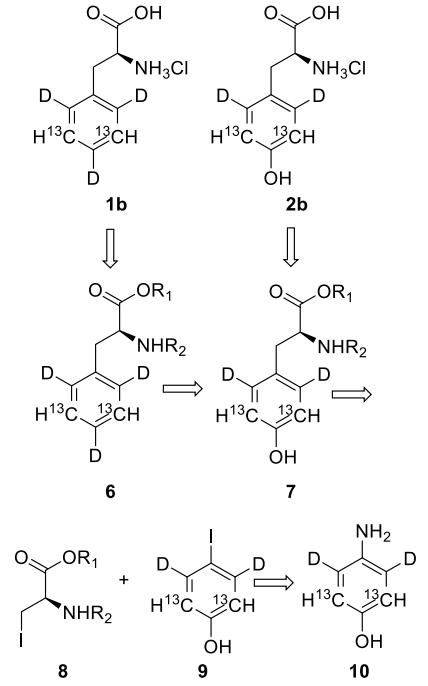


Figure 2. Aromatic ^{1}H,^{13}C-TROSY of the 36 kDa recombinant ALK extracellular domain (673−1025) prepared with a 50 mg/L culture of precursor **3** and **4** (pyruvate type) (top panel, a) and 32 kDa recombinant Src kinase domain (248−531) using 15 mg/L of precursors **1** and **2** (amino acid type) (bottom panel, a). Up to 7-fold higher Phe incorporation was found vs Tyr when **3** and **4** were used. In contrast, **1b** and **2b** gave equal and high incorporations of both Tyr and Phe. (b) Resonance assignment of Phe and Tyr in highly deuterated proteins up to 50 kDa obtained by NOESY matching the intrabenzylic proton (Hβ) using reagents **1b** and **2b** in this work.

an optimal synthetic approach to the target molecules: (1) identify a key intermediate suitable to provide both final products to minimize the number of overall synthetic steps required, (2) eliminate handling and purification of volatile intermediates, and (3) utilize existing intermediates from the synthesis of **3** and **4** when possible.

With these goals in mind, we began our retrosynthetic analysis with a regioselective reductive deuteration/deoxygenation. This would allow the production of **6** directly from **7**, addressing our desire for a single advanced intermediate capable of providing both **1** and **2** (Scheme 1). However, this

Scheme 1. Retrosynthetic Design of Spin-Isolated Amino Acids 1b and 2b from a Single Advanced Intermediate



transformation had no direct examples in the literature. We anticipated that the access to **7** could be accomplished by Negishi cross-coupling of iodoalanine **8** with iodophenol **9**. The preparation of **9** by a Sandmeyer iodination of **10**, an intermediate in the production of **4**, would allow access to the desired labeling chemistry. Though the use of **8** would produce isotopologues **1b** and **2b** with benzylic (Hβ) ^{1}H in place of ^{2}H, this change improves overall synthetic viability and maintains spin isolation while facilitating aromatic assignment by providing a probe to connect intraresidue amide and Cε via ^{13}C-edited and ^{15}N-edited NOESY experiments (Figure 2b).[9]

Before the isotopically labeled synthesis was attempted, the optimal conditions for the key reductive deuteration/deoxygenation of **7** were required. A survey of the literature revealed no examples of the desired transformation utilizing a deuterium source, although several examples of the reductive deoxygenation of Tyr or its derivatives with a proton source were found.[10a−g] However, these methods were deemed unsuitable for our purpose due to poor atom economy,[10b,c] the requirement for difficult to remove protecting groups,[10a,e−g] or challenges incorporating deuterium under standard laboratory conditions.[10d] Ultimately, this survey did suggest that Tyr triflate **11a** would provide the most direct path to the desired transformation.

While examples of aryl triflate reduction have been quite commonly reported in the literature,[11] only three of these reports provided examples of deuterium incorporation.[10c,12,13] We focused our attention on the work of Sajiki,[13] who described an operationally simple Pd/C-catalyzed reduction of aryl triflates using Mg^0 turnings in MeOH at rt. A notable rate acceleration was observed upon addition of a variety of ammonium salts, specifically 1 equiv of NH_4OAc. In the course of mechanistic experiments, CH_3OD and CD_4OD were reported to provide regioselective deuterium incorporation, suggesting that the hydroxyl proton was the source of deuterium in the reaction.

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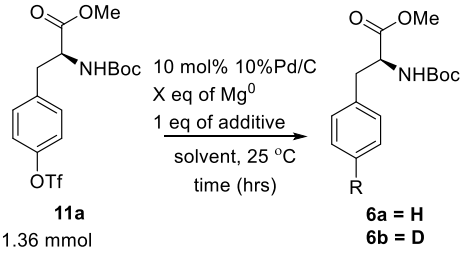
Org. Lett. 2021, 23, 6288−6292

Before employing these conditions, we opted to exchange NH_4OAc for NH_4Cl. Although both salts were reported to provide similar reaction rate enhancements, the latter was expected to be less hygroscopic than the former, reducing the chance of undesired hydrogen incorporation later. When **11a**[10e] was exposed to the reported conditions, we observed 30% conversion to **6a** after overnight stirring by UPLC-MS (entry 1, Figure 3). The reaction was quickly optimized after



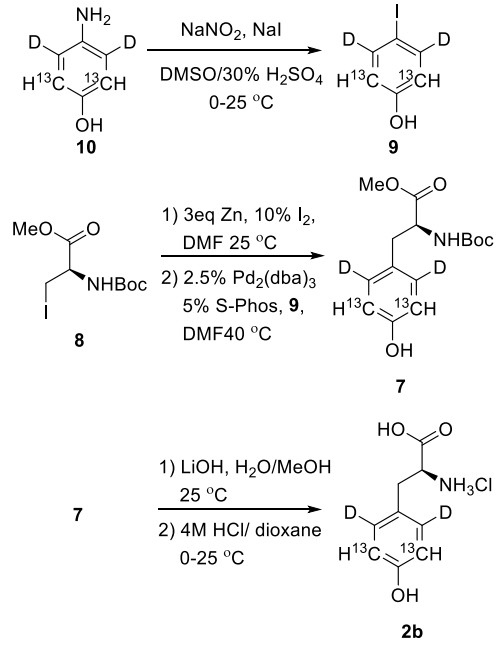
Entry	Conditions	Solvent	Time	Conversion **11:6**
1	1 eq Mg/1 eq NH_4Cl	CH_3OH	24	70:30 **6a**
2	2 eq Mg/1 eq NH_4Cl	CH_3OH	24	50:50 **6a**
3	2 eq Mg/1 eq NH_4Cl	CH_3OH	3	50:50 **6a**
4	2 eq Mg/1 eq NH_4Cl x2	CH_3OH	6	0:100 **6a**
5	2 eq Mg/1 eq ND_4Cl x2	CD_3OD	6	0:100 **6b**

Figure 3. Reaction screening to prepare **6a,b** from **11a**.

observing the effect of 2 equiv of Mg^0 resulted in an essentially complete reaction after 3 h (entries 2 and 3, Figure 3). Addition of a second bolus of 2 equiv of Mg^0 and 1 equiv of NH_4Cl after 3 h resulted in quantitative conversion to **6a** after an additional 3 h at rt (entry 4, Figure 3) producing the desired product in 92% yield. Concerned that the presence of basic $Mg(OMe)_2$ may lead to racemization, we were delighted to find that **6a** displayed the same specific rotation as a commercial standard (−4.7 and −4.4°, respectively), which was confirmed by chiral chromatography. In a final modification, **11a** was taken forward after a brief aqueous workup directly into the reduction reaction, leading to isolated **6a** in 88% overall yield for both steps from Boc-Tyr-OMe. We were gratified to find that the procedure was well-adapted to the incorporation of deuterium. Using crude **11a**, substitution of ND_4Cl and CD_3OD into the protocol produced **6b** in similar yield with a deuterium incorporation of over 90% on the basis of ^1H NMR integration (entry 5, Figure 3).

With the conditions for our key transformation secured, we turned our attention to the fully isotopically labeled synthesis (Scheme 2). Key to the success of the scheme would be conditions that did not alter the isotopic distributions already installed in **10**.[5] A Sandmeyer iodination of **10** proved unexpectedly complex, as the reported conditions had poor reproducibility with regard to yield or purity.[14] During the optimization efforts we noted that, in the time between the final addition of nitrite and the introduction of iodide, the reaction began to take on a gritty consistency, suggesting that the diazonium salt was no longer soluble in the aqueous medium. This difficulty was overcome by the use of DMSO as a cosolvent, demonstrated in Zhu's high-yielding synthesis of 2,3-trifluoromethyl-4-iodophenol,[15] circumventing these issues and giving **9** in 70% yield reproducibly with a high chemical purity after chromatography. Despite the strongly acidic conditions, we were pleased to observe no change in aromatic peak integrations between **10** and **9**. Cross-coupling of the

Scheme 2. Synthesis of Spin-Isolated Tyr 2b from Intermediate 10



Negishi reagent of **8** with **9** occurred with a slight modification of Jackson's procedure[16] using 2.5 mol % of $Pd_2(dba)_3$ and 5 mol % of S-Phos. After the Negishi reagent was prepared in DMF at 25 °C, the catalyst components and **9** were added followed by heating at 40 °C overnight. After aqueous workup and chromatography, **7** was isolated in 85% yield. The specific rotation of **7** was found to be in line (50.3°) with that of a commercial sample (49.9°), as confirmed by chiral chromatography. Interestingly, when the reaction was carried out using preformed Gen 3 S-Phos precatalyst instead of $Pd_2(dba)_3$ and S-Phos, the resulting yield dropped to 38%. We attributed this surprising result to the low basicity of the Negishi reagent that, while compatible with the free hydroxyl present in **9**, may therefore be insufficiently basic to deprotonate the precatalyst and consequently fail to produce the active catalytic species. The synthesis of **2b** was completed after a standard sequence of $LiOH{\cdot}H_2O$ ester hydrolysis[17] followed by removal of the Boc group with 4 M HCl in dioxane[18] to give the HCl salt in 96% yield over two steps. Overall, **2b** was obtained from **10** in a 57% total yield over four steps.

With the route to prepare **2b** in hand, we turned our attention to the preparation of **1b** (Scheme 3). As before, triflate **11b** was prepared from **7** under the standard conditions,[10e] subjected to a brief aqueous workup, and carried forward directly into the reduction step. An amount of 10% Pd/C, 2 equiv of Mg^0 turnings, and 1 equiv of ND_4Cl were introduced and placed under nitrogen at rt. After dilution with CD_3OD, the reaction mixture was stirred 3 h at room temperature, wherein a second bolus of 2 equiv of Mg^0 and 1 equiv of ND_4Cl were introduced, followed by a further 3 h of stirring. After an aqueous workup with 1 M citric acid and column chromatography, **6c** was isolated in 86% yield over both steps. The observed specific rotation of **6c** again compared favorably with that of the commercial standard (−4.5 and −4.4°, respectively), no loss of optical activity being demonstrated by chiral chromatography. The synthesis was completed as before with ester hydrolysis and Boc depro-

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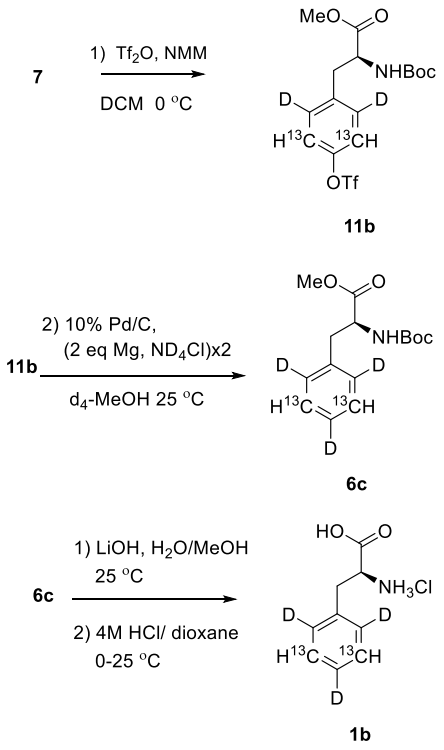
Scheme 3. Preparation of Spin-Isolated 1b from Intermediate 7



tection to give **1b** in 87% yield over the final two steps. With **10** as the starting material, **1b** was obtained in 47% total yield over six steps.

In summary, we have developed a concise, flexible, high-yielding synthesis to attain spin-isolated labeled $^{1}H\varepsilon$,$^{13}C\varepsilon$ Phe **1b** and Tyr **2b** for NMR studies. In developing this route, we were able to overcome several challenges encountered during the preparation and utilization of late-stage metabolic precursors **3** and **4**, which currently provide the best means of access to spin-isolated labeled proteins. With the previously reported aminophenol **10** as the starting material, the advanced labeled intermediate **7** is prepared in two steps, allowing access to either **1b** or **2b** in a further two or four steps, respectively. Key to the flexibility of the route were conditions allowing for the regioselective deuteration of **7** while maintaining stereochemical purity. On activation as its triflate, we demonstrated that **7** was quantitatively reduced by Mg^{0} turnings with 10% Pd/C in MeOH accelerated by ammonium salts. These conditions were readily adapted to incorporate deuterium regiospecifically at levels of above 90%. Finally, we demonstrated that **1b** and **2b** can be used to efficiently label Phe and Tyr residues in an expressed protein at concentrations of 15 mg/mL. We feel that the convenient synthesis coupled with high levels of Phe and Tyr residue labeling makes **1b** and **2b** valuable reagents to enable the future application of spin-isolated aromatic labeling in protein NMR.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.1c02084.

Complete synthetic details and characterization data of all novel compounds and NMR experimental data (PDF)

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Author Contributions

B.M.Y. and P.J.S. carried out synthetic chemistry. B.M.Y. and Z.R. designed the study. P.R., Y.C., M.S., J.D., and C.G.K. performed protein expression and protein NMR experiments. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) (a) Gardner, K. H.; Kay, L. E. Production and incorporation of ^{15}N, ^{13}C, ^{2}H (^{1}H-δ1 methyl) isoleucine into proteins for multidimensional NMR studies. *J. Am. Chem. Soc.* **1997**, *119*, 7599−7600. (b) Monneau, Y. R.; Ishida, Y.; Rossi, P.; Saio, T.; Tzeng, S.-R.; Inouye, M.; Kalodimos, C. G. Exploiting *E. coli* auxotrophs for leucine, valine, and threonine specific methyl labeling of large proteins for

NMR applications. *J. Biomol. NMR* **2016**, *65*, 99−108. (c) Schuetz, S.; Sprangers, R. Methyl TROSY spectroscopy: A versatile NMR approach to study challenging biological systems. *Prog. Nucl. Magn. Reson. Spectrosc.* **2020**, *116*, 56−84.

(2) (a) Wagner, G.; Demarco, A.; Wuthrich, K. Dynamics of aromatic amino acid residues in globular conformation of basic pancreatic trypsin-inhibitor (BPTI). *Biophys. Struct. Mech.* **1976**, *2*, 139−158. (b) Pervushin, K.; Riek, R.; Wider, G.; Wuthrich, K. Transverse relaxation-optimized spectroscopy (TROSY) for NMR studies of aromatic spin systems in ^{13}C-labeled proteins. *J. Am. Chem. Soc.* **1998**, *120*, 6394−6400. (c) Gautier, A.; Mott, H. R.; Bostock, M. J.; Kirkpatrick, J. P.; Nietlispach, D. Structure determination of the seven-helix transmembrane receptor sensory rhodopsin II by solution NMR spectroscopy. *Nat. Struct. Mol. Biol.* **2010**, *17*, 768−774. (d) Weininger, U.; Modig, K.; Akke, M. Ring Flips Revisited: ^{13}C-Relaxation Dispersion Measurements of Aromatic Side Chain Dynamics and Activation Barriers in Basic Pancreatic Trypsin Inhibitor. *Biochemistry* **2014**, *53*, 4519−4525. (e) Miyanoiri, Y.; Takeda, M.; Terauchi, T.; Kainosho, M. Recent developments in isotope-aided NMR methods for supramolecular protein complexes -SAIL aromatic TROSY. *Biochim. Biophys. Acta, Gen. Subj.* **2020**, *1864*, 129439. (f) Xie, T.; Saleh, T.; Rossi, P.; Kalodimos, C. G. Conformational states dynamically populated by a kinase determine its function. *Science* **2020**, *370*, eabc2754.

(3) Modi, V.; Dunbrack, R. L., Jr. Defining a new nomenclature for the structures of active and inactive kinases. *Proc. Natl. Acad. Sci. U. S. A.* **2019**, *116*, 6818−6827.

(4) Schorghuber, J.; Geist, L.; Platzer, G.; Feichtinger, M.; Bisaccia, M.; Scheibelberger, L.; Weber, F.; Konrat, R.; Lichtenecker, R. J. Late Metabolic Precursors for Selective Aromatic Residue Labeling. *J. Biomol. NMR* **2018**, *71*, 129−140.

(5) Lichtenecker, R. J. Synthesis of Aromatic ^{13}C/^{2}H-alpha-Ketoacid Precursors to be Used in Selective Phenylalanine and Tyrosine Protein Labelling. *Org. Biomol. Chem.* **2014**, *12*, 7551−7560.

(6) (a) Billek, G. Synthesis of phenylpyruvic acids 2. A new synthesis of 4-hydroxyphenylpyruvic acid. *Monatsh. Chem.* **1961**, *92*, 335−342. (b) Billek, G. p-Hydroxyphenylpyruvic acid. *Org. Synth.* **1963**, *43*, 49.

(7) Lichtenecker, R. J.; Weinhaupl, K.; Schmid, W.; Konrat, R. alpha-Ketoacids as Precursors for Phenylalanine and Tyrosine Labelling in Cell-based Protein Overexpression. *J. Biomol. NMR* **2013**, *57*, 327−331.

(8) Raum, H. N.; Schorghuber, J.; Dreydoppel, M.; Lichtenecker, R. J.; Weininger, U. Site-selective ^{1}H/^{2}H Labeling Enables Artifact-Free ^{1}H CPMG Relaxation Dispersion Experiments in Aromatic Side Chains. *J. Biomol. NMR* **2019**, *73*, 633−639.

(9) Rossi, P.; Xia, Y.; Khanra, N.; Veglia, G.; Kalodimos, C. G. ^{15}N and ^{13}C-SOFAST-HMQC editing enhances 3D-NOESY sensitivity in highly deuterated, selectively ^{1}H,^{13}C-labeled proteins. *J. Biomol. NMR* **2016**, *66*, 259−271.

(10) (a) Cacchi, S.; Ciattini, P. G.; Morera, E.; Ortar, G. Palladium Catalyzed Triethylammonium Formate Reduction of Aryl Triflates. A Selective Method for the Deoxygenation of Phenols. *Tetrahedron Lett.* **1986**, *27*, 5541−5544. (b) Walker, T. E.; Matheny, C.; Storm, C. B.; Hayden, H. An Efficient Chemomicrobiological Synthesis of Stable Isotope-Labeled L-Tyrosine and L-Phenylalanine. *J. Org. Chem.* **1986**, *51*, 1175−1179. (c) Wang, H.; Janowick, D. A.; Schkeryantz, J. M.; Liu, X.; Fesik, S. W. A Method for Assigning Phenylalanines in Proteins. *J. Am. Chem. Soc.* **1999**, *121*, 1611−1612. (d) Tsukamoto, H.; Suzuki, R.; Kondo, Y. Revisiting Benzenesulfonyl Linker for the Deoxygenation and Multifunctionalization of Phenols. *J. Comb. Chem.* **2006**, *8*, 289−292. (e) Iimura, S.; Wu, W. Palladium-catalyzed Borylation of L-tyrosine Triflate Derivative with Pinacolborane: Practical Route to 4-Borono-L-phenylalanine (l-BPA) Derivatives. *Tetrahedron Lett.* **2010**, *51*, 1353−1355. (f) Tobisu, M.; Yamakawa, K.; Shimasaki, T.; Chatani, N. Nickel-Catalyzed Reductive Cleavage of Aryl-oxygen Bonds in Alkoxy- and Pivaloxyarenes Using Hydrosilanes as a Mild Reducing Agent. *Chem. Commun.* **2011**, *47*, 2946−2948. (g) Xi, X.; Chen, T.; Zhang, J.-S.; Han, L.-B. Efficient and Selective Hydrogenation of C-O Bonds with a Simple Sodium Formate Catalyzed by Nickel. *Chem. Commun.* **2018**, *54*, 1521−1524.

(11) Qiu, Z. H.; Li, C. J. Transformations of Less-Activated Phenols and Phenol Derivatives via C-O Cleavage. *Chem. Rev.* **2020**, *120*, 10454−10515.

(12) Korvinson, K. A.; Akula, H. K.; Malinchak, C. T.; Sebastian, D.; Wei, W.; Khandaker, T. A.; Andrzejewska, M. R.; Zajc, B.; Lakshman, M. K. Catalytic Reductions Without External Hydrogen Gas: Broad Scope Hydrogenations with Tetrahydroxydiboron and a Tertiary Amine. *Adv. Synth. Catal.* **2020**, *362*, 166−176.

(13) (a) Sajiki, H.; Mori, A.; Mizusaki, T.; Ikawa, T.; Maegawa, T.; Hirota, K. Pd/C-Catalyzed Deoxygenation of Phenol Derivatives Using Mg Metal and MeOH in the Presence of NH_4OAc. *Org. Lett.* **2006**, *8*, 987−990. (b) Mori, A.; Mizusaki, T.; Ikawa, T.; Maegawa, T.; Monguchi, Y.; Sajiki, H. Mechanistic Study of a Pd/C-Catalyzed Reduction of Aryl Sulfonates Using the Mg-MeOH-NH_4OAc system. *Chem. - Eur. J.* **2007**, *13*, 1432−1441.

(14) (a) Dains, F. B.; Eberly, F. p-Iodophenol. *Org. Synth.* **1935**, *15*, 39. (b) Maity, S.; Das, D.; Sarkar, S.; Samanta, R. Direct Pd(II)-Catalyzed Site-Selective C5-Arylation of 2-Pyridone Using Aryl Iodides. *Org. Lett.* **2018**, *20*, 5167−5171.

(15) Zhu, G.-D.; Staeger, M. A.; Boyd, S. A. Diels-Alder Reactions of Hexafluoro-2-butyne with 2-Heterosubstituted Furans: A Facile and General Synthesis of 1,4-Disubstituted 2,3-Di(trifluoromethyl)-benzenes. *Org. Lett.* **2000**, *2*, 3345−3348.

(16) Ross, A. J.; Lang, H. L.; Jackson, R. F. Much Improved Conditions for the Negishi Cross-Coupling of Iodoalanine Derived Zinc Reagents with Aryl Halides. *J. Org. Chem.* **2010**, *75*, 245−248.

(17) Khan, R. A. Synthesis of Met-enkephalin by Solution-Phase Peptide Synthesis Methodology Utilizing Para-toluene Sulfonic Acid as N-terminal Masking of L-methionine Amino Acid. *Chem. Biol. Drug Des.* **2016**, *88*, 884−888.

(18) Han, G.; Tamaki, M.; Hruby, V. J. Fast, effcient and selective deprotection of the tert-butoxycarbonyl (Boc) group using HCl/dioxane (4 M). *J. Pept. Res.* **2001**, *58*, 338−341.

https://doi.org/10.1021/acs.orglett.1c02084
Org. Lett. 2021, 23, 6288−6292