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Article

A Convenient Route to Large-Scale Chemical Synthesis of *p*-Hydroxyphenylacetaldehyde Oxime and Its *p*-*β*-D-Glucopyranoside: Key Intermediates and Products in Plant Specialized Metabolism

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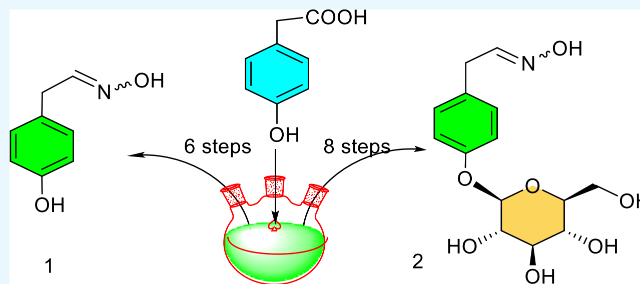
ABSTRACT: Oximes are unrecognized chameleons in general and specialized plant metabolism. *E*- and *Z*-*p*-hydroxyphenylacetaldehyde oxime are key intermediates in the biosynthesis of the cyanogenic glucoside dhurrin produced in sorghum. Nevertheless, none of the geometrical oxime isomers accumulate in the plant. Herein, we report a convenient route to the chemical synthesis of *E*- and *Z*-*p*-hydroxyphenylacetaldehyde oxime and its biologically produced *p*-*β*-D-glucopyranoside using *p*-hydroxyphenylacetic acid as a starting material. This starting material is also available in radiolabeled forms. All reaction steps proceeded with excellent yield under mild conditions, operational facility, and scalability.



■ INTRODUCTION

In plants, cytochrome P450s of the CYP79 family are known to convert certain amino acids into corresponding oximes.[1−3] Genes encoding CYP79 enzymes are reported as being present in the genomes of all hitherto analyzed species of seed plants.[3,4] Nevertheless, the oximes produced are only detected in a small minority of plant species, typically as components of emitted volatiles.[4−9] In some plant species, they are intermediates in highly channeled biosynthetic pathways and thus not detectable. This applies to *E*- and *Z*-*p*-hydroxyphenylacetaldehyde oxime, which are intermediates in the biosynthesis of the cyanogenic glucoside dhurrin, a hydrogen-cyanide-releasing defense compound.[1,3,10−17]

In sorghum (*Sorghum bicolor*), CYP79A1 catalyzes the multistep conversion of L-tyrosine into *E*-*p*-hydroxyphenylacet aldehyde oxime.[10,14−16] The *E*-isomer is a substrate for CYP71E1[17] and is, following enzyme-catalyzed conversion into the geometric *Z*-isomer,[16] converted into *p*-hydroxymandelonitrile, which by the action of UGT85B1 in the presence of the cofactor UDPG is converted into dhurrin.[17,18] Oximes are key intermediates in the biosynthesis of other plant defense compounds such as glucosinolates and camalexin[2−4] (see Figure 1).

The first steps in glucosinolate synthesis in Cruciferous plants are also catalyzed by the CYP79 family enzymes.[19] All known structures of glucosinolates have retained an *E*-configuration, implying that in this pathway, no conversion of the geometric *E*-isomer to the *Z*-isomer oximes takes place, as confirmed by catalytic in vitro studies using CYP83B1 from *Arabidopsis thaliana*.[14] Ferns also produce cyanogenic glucosides, but this takes place in the absence of CYP79 encoding genes in their genomes. Instead, the amino acid to oxime conversion is catalyzed by a flavor-dependent class B enzyme termed fern oxime synthase (FOS1) and results in formation of both structural *E*- and *Z*-oxime isomers.[20] It is noticeable that bacteria and fungi have the enzyme machinery available to use *Z*-oximes as a source of reduced nitrogen.[21−23] In addition to the established role of cyanogenic glucosides as a chemical defense system against herbivores based upon their ability to release toxic hydrogen cyanide gas upon cell disruption and hydrolysis,[3] studies have shown that cyanogenic glucosides serve as readily mobilized reservoirs of reduced carbon and nitrogen,[24−27] may act as controllers of flowering time in fruit trees,[28−30] and function as radical scavengers.[3,5] In free form, aldoximes may function as herbivore deterrents as well as attractants of moth pollinators, predatory mites, or parasitic insects.[5−9] In the symbiosis of arbuscular mycorrhizal fungi (AMF) with plants, the phenotypes may be mutualistic as well as parasitic. When parasitic, this may elicit antagonistic defense responses in the host plant. This may involve production of an abundance of *p*-hydroxyphenylacetaldoxime in the host

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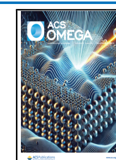

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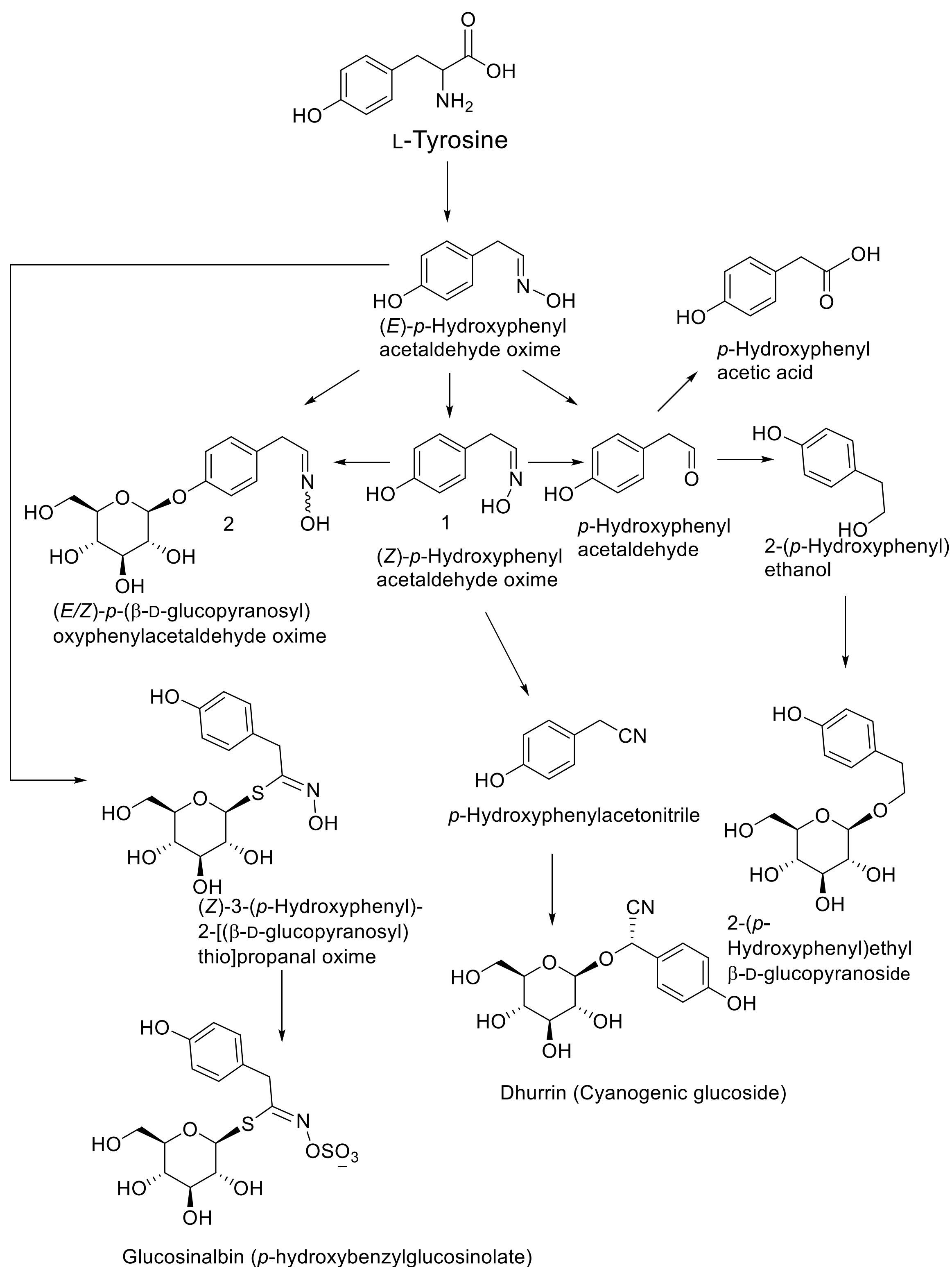

Figure 1. Involvement of *p*-hydroxyphenylacetaldehyde oxime in the biosynthesis of the cyanogenic glucoside dhurrin, the glucosinolate glucosinalbin, and as a source of other metabolic products.

plant.[31,32] In a recent paper, the glucoside of phenylacetaldoxime was demonstrated to function as a stable storage form of an insect deterrent.[33] The stored oxime glucoside provides rapid protection against future herbivory since insect herbivore species in their gut hydrolyze the glucoside linkage and release the active oxime defense compound.

In sorghum, the enzymes catalyzing dhurrin biosynthesis are organized within a dynamic enzyme complex (metabolon), channeling intermediates into the final product,[31,34] and individual components of the complex are now being studied at the single molecule level.[35] The biosynthesis of dhurrin in sorghum has been developed as a model system for studies on the biosynthesis, regulation, metabolic channeling, transport,

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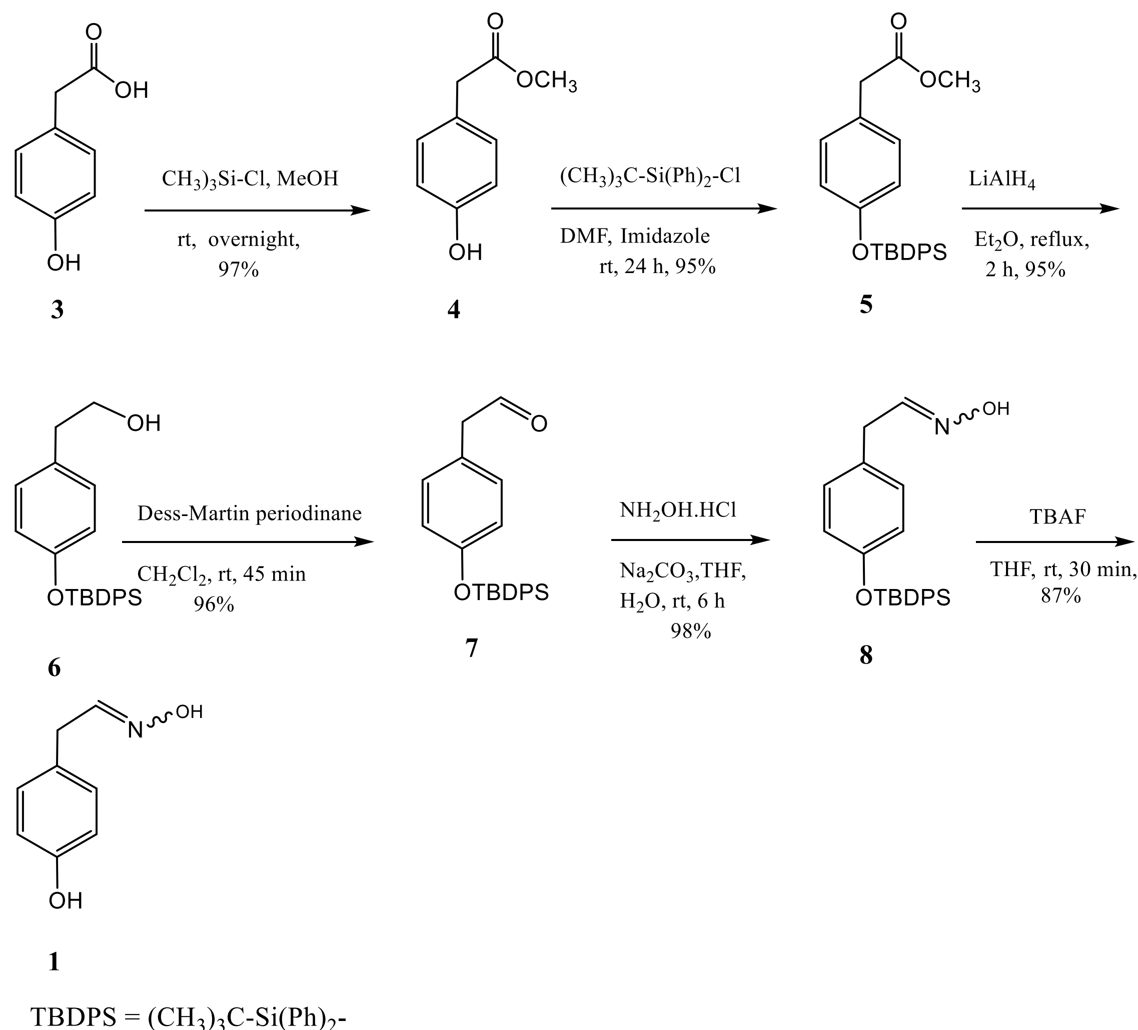
Scheme 1. Chemical Synthesis of (*E*/*Z*)-*p*-Hydroxyphenylacetaldehyde Oxime



storage, and functions of cyanogenic glucosides.[3−5,36,37] In synthetic biology approaches, the dhurrin pathway in sorghum has been used to demonstrate the possibility to transfer entire cytochrome P450-catalyzed pathways into chloroplasts and to drive the catalytic cycle of the P450s with direct ferredoxin-mediated electron transfer from photosystem I.[38−42]

In the context of these endeavors into metabolic engineering, synthetic biology, and single molecule studies, the availability of the *E*- and *Z*-isomers of *p*-hydroxyphenylacetaldehyde oxime (**1**) and the biologically derived *p*-*β*-D-glucopyranosyloxyphenylacetaldehyde oxime (**2**) for use as substrates and reference compounds became a necessity. The *p*-*β*-D-glucopyranosyloxyphenylacetaldehyde oxime is now also made available for testing as a stable easily reactivated storage molecule of the free oxime, which is a defense compound against insect herbivores,[33] whereas *p*-hydroxyphenylacetaldoxime has a role in parasitic mycorrhizal interactions with plants.[32] In this work, we describe a convenient synthetic route for the conversion of *p*-hydroxyphenylacetic acid (**3**) into these compounds.

■ RESULTS AND DISCUSSION

Chemical synthesis of (*Z*)- and (*Z*/*E*)-*p*-hydroxyphenylacetaldoxime **1** and the isomeric glucoside **2** was achieved by starting from commercially available 4-hydroxyphenylacetic acid (**3**).

Quantitative conversion of *p*-hydroxyphenylacetic acid (**3**) into its methyl ester **4** was accomplished using the trimethylsilyl chloride-MeOH system.[43] Silylation of the free phenolic OH-group of the ester **4** with *tert*-butyldiphenylchlorosilane (TBDPS) using the *N*,*N*-dimethylformamide (DMF)-imidazole method[44] afforded the silylated ester **5** in 95% yield. Reduction of the ester **5** was carried out using lithium aluminum hydride ($LiAlH_4$)[45] to provide the corresponding primary alcohol **6** in 95% yield. The alcohol **6** was selectively oxidized quantitatively to the aldehyde derivative **7** with Dess−Martin periodinane.[46] Reaction of the aldehyde with hydroxylamine hydrochloride ($NH_2OH\cdot HCl$) in the presence sodium carbonate (Na_2CO_3)[47] afforded an isomeric mixture of (*E*)- and (*Z*)-*p* (*tert*-butyldiphenylsilyloxy)phenylacetaldehyde oxime (**8**). Finally, removal of the *tert*-butyldiphenylsilyl protecting group with TBAF (tetrabutylammonium fluoride) in THF[44] afforded an isomeric mixture of (*Z*/*E*)-*p*-hydroxyphenylacetaldehyde oxime (**1**) after chromatographic purification. Recrystallization of the obtained product from $CHCl_3$/*n*-pentane afforded an isomeric mixture of *p*-hydroxyphenylacetaldoxime (*Z*/*E* ratio = 75:25). Alternatively, recrystallization of the crude product with dichloromethane/toluene afforded an almost pure Z-

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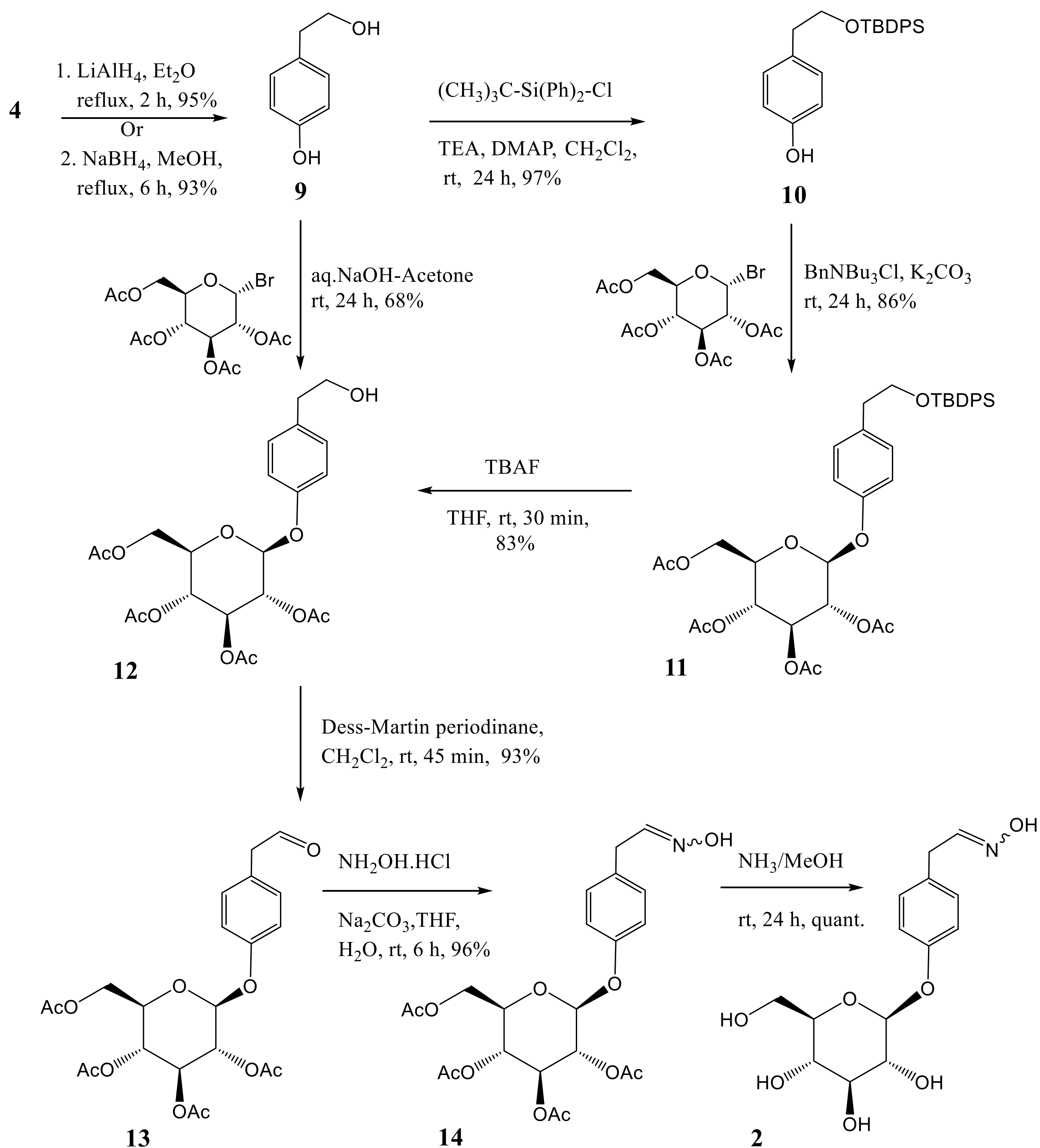
Scheme 2. Chemical Synthesis of (*E*/*Z*)-*p*-(*β*-D-Glucopyranosyloxy)phenylacetaldehyde Oxime



isomer (*Z*/*E* ratio = 94:06) without any chromatographic treatment as monitored by ^{1}H NMR spectroscopy. The spectroscopic data of both (*E*)- and (*Z*)-*p*-hydroxyphenylacetaldoxime were similar to those previously published.[47−49] For details of the synthetic procedures, see Supporting Information and methods (see Scheme 1).

The chemical synthesis of the glucoside *p*-*β*-D-glucopyranosyloxyphenylacetaldehyde oxime (**2**) is outlined in Scheme 2.

Methyl 4-hydroxyphenylacetate (**4**) obtained from 4-hydroxyphenylacetic acid (**3**) was reduced by lithium aluminum hydride ($LiAlH_4$)[45] or sodium borohydride in methanol ($NaBH_4$−MeOH system)[50,51] to afford 2-(*p*-hydroxyphenyl)ethanol (**9**) in excellent yield (>93%). Direct silylation of compound **9** under selective silylation conditions using DMAP (*N*,*N*-dimethylpyridine) as catalyst[52] resulted in the formation of 4-[2-((*tert*-butyldiphenylsilyl)oxy)ethyl]-phenol (**10**)

Glucosylation of compound **10** with 2,3,4,6-tetra-*O*-acetyl-*α*-D-glucopyranosyl bromide (commercially available as *α*-D-acetobromoglucose) under solid−liquid phase transfer catalysis using powdered potassium carbonate (K_2CO_3) and benzyl-

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tributylammonium chloride ($BnNBu_3Cl$)[53] yielded the fully-*O*-acetylated-*β*-D-glucosylated phenol-derivative **11**. Subsequent treatment of compound **11** with tetrabutylammonium fluoride (TBAF) in THF[44] resulted in the formation of 2-[4′-*β*-D-glucopyranosyl)oxyphenyl]ethanol (**12**). Compound **12** was also obtained in fairly good yield (68%) by direct glucosylation of compound **9** using 2,3,4,6-tetra-*O*-acetyl-*α*-D-glucopyranosyl bromide in basic aqueous organic media (aq. NaOH-acetone-system).[54] Dess−Martin periodinane-oxidation[46] of **12** afforded the aldehyde derivative **13**, which when reacted with hydroxylamine[47] resulted in formation of an isomeric mixture of (*Z*/*E*)-*p*-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyloxy)phenylacetaldehyde oxime (**14**). Finally, the acetyl groups were removed by treatment with NH_3−CH_3OH[55] to afford an isomeric mixture of *p*-(*β*-D-glucopyranosyloxy)hydroxyphenylacetaldehyde oxime (**2**) (*Z*/*E* ratio = 50:50).

Oximes have recently been described as the unrecognized chameleons in general and specialized plant metabolism.[4] Many of these oximes are formed from amino acids and especially tyrosine.[3,11,16,31,56] In this study, we therefore aimed to provide a robust synthetic route to *Z*- and the geometric isomers *E*/*Z*-*p*-hydroxyphenylacetaldoxime as well as their *p*-*β*-D-glucopyranoside. In all cases, chemical synthesis was started from *p*-hydroxyphenylacetic acid. This precursor is also commercially available as carboxyl-^{14}C or ring-3,5-^{3}H labeled. In this way, chemical synthesis affords the compounds as authentic reference compounds, as well as radiolabeled precursors in pathway discovery studies. All reaction steps provided excellent yields (83−98%), which is important especially in synthesis the radiolabeled compounds.

Chemical synthesis of *p*-hydroxyphenylacetaldehyde oxime (**1**) had previously been reported using different rather inefficient methodologies.[57−59] *p*-Hydroxyphenylacetaldehyde has been prepared from synephrine (2-amino-1-(4-hydroxyphenyl)-1-ethanol) following a pinacol−pinacolone type rearrangement in 85% H_3PO_4 but the yields obtained were low. The latter procedure results in specific production of the *Z*-geometric isomer.[16] A more efficient synthesis of **1** started with oxidation of 2-(*p*-hydroxyphenyl)-1-ethanol using the sulfur trioxide-pyridine complex, followed by chromatography and oximation with hydroxylamine hydrochloride.[47] A simpler synthesis has been reported,[49] where the preparation of **1** was obtained starting from commercially available *p*-hydroxyphenylacetic acid (**3**), which was consecutively esterified, reduced with DIBAL to the aldehyde, and the aldehyde allowed to react with hydroxylamine hydrochloride to yield, after column chromatography, a mixture of *E*/*Z*-oximes **1** in good overall yield.

Two approaches for the synthesis of radiolabeled *p*-hydroxyphenylacetaldehyde oxime have also been reported. One approach involved condensation of *p*-hydroxybenzaldehyde with [^{14}C]-nitromethane followed by catalytic reduction using platinum and zinc to yield [1-^{14}C]-*p*-hydroxyphenylacetaldehyde oxime.[57] The second approach involved initial enzymatic conversion of L-[UL-^{14}C]-tyrosine into [UL-^{14}C]-*p*-hydroxyphenylpyruvic acid by the action of L-amino acid oxidase in the presence of catalase. [UL-^{14}C]-*p*-hydroxyphenylpyruvic acid was then reacted with hydroxylamine to form [UL-^{14}C]-*p*-hydroxyphenylpyruvic acid oxime, which by reduction with $NaBH_4$ and decarboxylation afforded [UL-^{14}C]-*p*-hydroxyphenylacetaldehyde oxime.[58]

In summary, we demonstrate a simplified and efficient route to the synthesis of *p*-hydroxyphenylacetaldehyde oxime and its novel *p*-*β*-D-glucopyranoside. The availability of (*E*)- and (*Z*)-isomers of *p*-hydroxyphenylacetaldehyde oxime and of the glucoside *p*-*β*-D-glucopyranosyloxyphenylacetaldehyde oxime makes it possible to identify and quantify the presence of these key intermediates in biological systems and facilitates identification of enzymes using (*E*)- and/or (*Z*)-isomers of *p*-hydroxyphenylacetaldehyde oxime as a substrate or enzymes involved in their formation or further metabolism as well as their role in plant defense.

■ EXPERIMENTAL SECTION

General Experimental Procedures. All reactions were carried out under an atmosphere of argon in oven-dried glassware with magnetic stirring. Unless otherwise indicated, commercially available starting materials, reagents, solvents, and dry solvents were purchased from Sigma-Aldrich Chemicals were used without further treatment. All commercial materials were used as received, unless otherwise noted. All reactions were monitored by TLC on aluminum sheets coated with silica gel 60F254 (0.2 mm thickness, E. Merck, Darmstadt, Germany) using UV light as a visualizing agent, and the components present were detected by charring with 10% H_2SO_4 in MeOH. Column chromatographies were carried out using silica gel 60 (particle size 0.040−0.063 mm, 230−400 mesh ASTM, E. Merck). Solvent extracts were dried with anhyd. $MgSO_4$ unless otherwise specified. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE 400 spectrometer at 400 and 101 MHz, respectively, or were performed on a 600 MHz Bruker AVANCE III instrument (operating frequency of 600.13 and 125 MHz, respectively) equipped with a Bruker SampleJet sample changer (Bruker Biospin, Karlsruhe, Germany). ^{1}H and ^{13}C NMR spectra were acquired with 30° pulses and 64k data points. IconNMR version 4.2 (Bruker Biospin, Karlsruhe, Germany) was used for controlling automated sample change and acquisition of NMR data. Topspin ver. 4.0.6 (Bruker Biospin, Karlsruhe, Germany) was used for acquisition and processing of NMR data. δ-Values are relative to NMR solvent peaks used for calibration. Acetone-d_4: d_H 20.5 ppm d_C 206.68 ppm, $CDCl_3$: d_H 7.24 ppm d_C 77.23 ppm, and MeOD: d_H 3.31 ppm d_C 49.15 ppm, and coupling constants (J) are given in Hz. The multiplicities are reported as follows: singlet (s), doublet (d), doublet of doublets (dd), doublet of doublet of doublets (ddd), multiplet (m), and triplet (t). HRMS analysis was performed on a Bruker Daltonics Compact QqTOF mass spectrometer equipped with an electrospray ionization (ESI) interface (Bruker Daltonics, Bremen, Germany). Mass spectra were acquired in positive ion mode, scanning in mass range of 50−1000 m/z, using a capillary voltage of 3500 V, an end plate offset of 500 V, a drying temperature of 220 °C, a nebulizer pressure of 2.0 bar, and a drying gas flow of 8 L/min.

***tert*-Butyl-2-[(4′-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-oxyphenyl]ethyloxydiphenylsilane (11).** A mixture of compound **10** (4.0 g, 10.6 mmol), 2,3,4,6,-tetra-*O*-acetyl-*α*-D-glucopyranosyl bromide (8.8 g, 21.4 mmol), $BnNBu_3Cl$ (benzyltributylammonium chloride) (0.67 g, 2.14 mmol), K_2CO_3 (7.4 g, 53.5 mmol), and $CHCl_3$ (50 mL) was stirred at RT for 24 h. The reaction mixture was neutralized with 10% HCl, and the organic layer was separated, washed with water saturated $NaHCO_3$ and brine, dried over $MgSO_4$, and evaporated. The residue was purified by column chromatog-

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raphy on silica gel (210 g) with 40% Et_2O in *n*-pentane to produce compound **11** as white solid (6.4 g, 9.1 mmol, 86%); ^{1}H NMR (400 MHz, CD_3COCD_3): δ 7.61−7.57 (m, 4H, H-arom.), 7.44−7.37 (m, 6H, H-arom.), 7.16 (d, 2H, J = 8.7 Hz, H-arom.), 6.98 (d, 2H, J = 8.7 Hz, H-arom.), 5.40 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1′), 5.39 (t, 1H, $J_{2',3'}$ = $J_{3'4'}$ = 9.6 Hz, H-3′), 5.18 (dd, 1H,, $J_{2',3'}$ = 9.6 Hz, $J_{1',2'}$ = 8.0 Hz, H-2′), 5.12 (t, 1H, J = 9.6 Hz, H-4′), 4.27 (dd, 1H, $J_{5',6'}$ = 5.2 Hz, $J_{6a',6a'}$ =12.0 Hz, H-6′a),, 4.18 (ddd, 1H, J = 2.4, 5.2, 9.6 Hz, H-5′), 4.15 (dd, 1H, $J_{5',6'}$ = 2.4 Hz, $J_{6a',6b'}$ = 12.0 Hz, H-6′b), 3.86−3.82 (m, 2H, $-CH_2O-$), 2.85−2.80 (m, 2H, $-CH_2-$), 2.04, 2.01, 2.00, and 1.97 (4s, 12H, CH_3CO-), 1.01(s, 9H, C(CH_3)$_3$); ^{13}C NMR (101 MHz, CD_3COCD_3): δ 171.2, 170.9, 170.6, and 170.3 (4 x C, CH_3CO-), 157.2, 136.8, 136.8, 136.8, 136.8, 135.3, 135.0, 131.7, 131.7, 131.7, 131.1, 131.1, 129.1, 129.1, 129.1, 129.1, 118.0, and 118.0 (18 x C, C-arom.), 100.1 (C-1′), 73.9 (C-3′), 73.1 (C-5′), 72.6 (C-2′), 69.9 (C-4′), 66.6 (C-1), 63.3 (C-6′), 39.6 (C-2), 27.8, 27.8, and 27.8 (C(CH_3)$_3$), 21.2, 21.2, 21.1, and 21.1 (4 x CH_3CO), 20.3 (C(CH_3)$_3$). HMRS (m/z): [M + H]$^+$ calcd for $C_{38}H_{47}O_{11}Si$: 707.2843; found, 707.2845. NMR spectra are provided in Supporting Information IV.

2-[(4′-Tetra-O-acetyl-β-D-glucopyranosyl)oxyphenyl]-ethanol (12). Method A. Compound **11** (6.2 g, 8.8 mmol) was desilylated with TBAF in THF as described for compound **7**. Chromatographic purification on silica gel (130 g) using 60−100% Et_2O in *n*-pentane as eluent afforded **12** as a white solid (3.4 g, 7.3 mmol, 83%). Analytical sample was recrystallized from Et_2O.

*Method B. Direct Glucosylation of Compound **9**.* Compound **9** (2.0 g, 14.5 mmol) was added to a stirred solution of NaOH (52 mg, 13.0 mmol) in H_2O (20 mL) at 0 °C, and stirring was continued at t < 10 °C for 15 min. A solution of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (4.0 g, 9.7 mmol) in acetone (40 mL) was added dropwise, and the mixture was stirred at *RT* for 24 h. The acetone was evaporated under reduced pressure at 25 °C, the aqueous suspension was extracted several times with CH_2Cl_2 (3 × 50 mL), and the combined extracts were washed with 1 M NaOH (25 mL),H_2O (3 × 50), brine (25 mL), dried over $MgSO_4$, filtered, and concentrated to dryness. The residue was chromatographed on silica gel (130 g) using 60−100% Et_2O in *n*-pentane as an eluent to afford **12** as a white solid (3.2 g, 6.6 mmol, 68%). Analytical sample was recrystallized from Et_2O; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.13 (d, 2H, J = 8.6 Hz, H-arom.), 6.92 (d, 2H, J = 8.6 Hz, H-arom.), 5.27−5.23 (m, 2H, H-2′ and H-3′), 5.16 (t, 1H, J = 9.2 Hz, H-4′), 5.03 (d, 1H, $J_{1',2'}$ = 7.5 Hz, H-1′), 4.26 (dd, 1H, $J_{5',6'}$ = 5.3 Hz, $J_{6a',6a'}$ = 12.3 Hz, H-6′a), 4.14 (dd, 1H, $J_{5',6'}$ = 2.4 Hz, $J_{6a',6b'}$ = 12.3 Hz, H-6′b), 3.83−3.78 (m, 3H, H-5′ and − CH_2O-), 2.78 (t, 2H, J = 6.5 Hz, $-CH_2-$), 2.05, 2.03, 2.02, and 2.01 (4s, 12H, 4 x CH3CO); ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.8, 170.5, 169.6, and 169.5 (4 x C, CH_3CO-), 155.8, 133.8, 130.3, 130.3, 117.4, and 117.4 (6 x C, C-*arom.*), 99.5 (C-1′), 72.9 (C-3′), 72.2 (C-5′), 71.4 (C-2′), 68.5 (C-4′), 63.6 (C-6′), 62.2 (C-1), 38.5 (C-2), 20.9, 20.8, 20.8, and 20.8 (4 x CH_3CO); HMRS (m/z): [M + H]$^+$ calcd for $C_{22}H_{29}O_{11}$: 469.1665; found, 469.1660. NMR spectra are provided in Supporting Information IV.

4-(2′,3′,4′,6′-Tetra-O-acetyl-β-D-glucopyranosyl)-oxyphenylacetaldehyde (13). Compound **12** (1.3 g, 2.8 mmol) was oxidized with DMP as described for compound **7**. Wet CH_2Cl_2 (12.0 mL) was added slowly via a dropping funnel to a vigorously stirred solution of **12** (1.3 g, 2.8 mmol) and DMP (1.8 g, 4.2 mmol) in dry CH_2Cl_2 (7.5 mL) at *RT* over a period of 45 min. The mixture was diluted with Et_2O (10.0 mL) and concentrated into a few milliliters of solvent using a rotary evaporator. The residue was taken up in Et_2O (50.0 mL) and then washed with 10% $Na_2S_2O_3$-sat. $NaHCO_3$ (3 × 10.0 mL, 1:1 v/v), followed by H_2O (3 × 10.0 mL), brine (10.0 mL), and dried over $MgSO_4$. The solvent was evaporated under reduced pressure to afford chromatographically pure **13** (1.2, 2.6 mmol, 93%). The obtained product was used for the next step without further purification; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.70 (t, 0.5H, J = 2.2 Hz, CHO), 7.11 (d, 2H, J = 8.6 Hz, H-arom.), 6.94 (d, 2H, J = 8.6 Hz, *H*-arom.), 5.27−5.23 (m, 2H, H-2′ and H-3′), 5.14 (t, 1H, J = 9.6 Hz, H-4′), 5.05 (d, 1H, $J_{1',2'}$ = 7.5 Hz, H-1′), 4.26 (dd, 1H, $JS',6'$ = 5.3 Hz, $J_{6a',6a'}$ = 12.4 Hz, H-6′a), 4.14 (dd, 1H, $J_{5',6'}$ = 2.4 Hz, $J_{6a',6b'}$ = 12.4 Hz, H-6′b), 3.84−3.78 (m, 2H, H-5′and−CH−), 3.63 (d, 1H, J = 2.2 Hz, −CH-), 2.05, 2.03, 2.02, and 2.01 (4s, 12H, 4 x CH_3CO); ^{13}C NMR (101 MHz, $CDCl_3$): δ 199.3 (CHO), 170.8, 170.7, 169.6, and 169.5 (4 x C, CH_3CO-), 156.4, 133.8, 131.0, 130.3, 117.7, and 117.4 (6 x C, C-arom.), 99.3 (C-1′), 72.9 (C-3′), 72.3 (C-5′), 72.2 (C-2′), 71.4 (C-4′), 63. (C-6′), 38.5 (C-2), 20.9, 20.8, 20.8, and 20.8 (4 x CH_3CO); HMRS (m/z): [M + H]$^+$ calcd for $C_{22}H_{27}O_{11}$: 467.1509; found, 467.1503. NMR spectra are provided in Supporting Information IV.

(E/Z)-4-(2′,3′,4′, 6′-Tetra-O-acetyl-β-D-glucopyranosyl)-oxyphenylacetaldehyde Oxime (14). Compound **13** (1.1 g, 2.4 mmol) was reacted with $NH_2OH{\cdot}HCl$ (0.25 g, 3.6 mmol), as described for compound **6**. The residue was chromatographed on silica gel (100 g) with 0−5% EtOAc in CH_2Cl_2 as eluent to give **14** as a colorless syrup (1.1 g, 2.3 mmol, 96%); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.10, 7.62 (2s, 0.4H and 0.5H, −CH = N−OH, Z- and *E*-isomer), 7.47 (t, 0.5H, J = 6.2 Hz, −C*H* = N-, *E*-isomer), 7.13, 7.11 (2d, 2H, J_{AB} = 8.6 Hz, H-arom., *Z*- and *E*-isomer), 6.92 (d, 2H, J_{AB} = 8.5 Hz, H-arom., *Z*- and *E*-isomer), 6.82 (t, 0.5H, J = 5.4 Hz, −C*H* = N-, *Z*-isomer), 5.27−5.21 (m, 2H, H-2′ and H-3′), 5.14 (t, 1H, J = 9.4 Hz, H-4′), 5.03 (2d, 1H, $J_{1',2'}$ = 7.6 Hz, H-1′, *Z*- and *E*-isomer), 4.26 (dd, 1H, $J_{5',6'}$ = 5.2 Hz, $J_{6'a,6'a}$ = 12.2 Hz, H-6′a), 4.14 (dd, 1H, $J_{5',6'}$ = 2.0 Hz, $J_{6'a,6'b}$ = 12.2 Hz, H-6′b), 3.84−3.80 (m, 1H, H-5′), 3.66 (d, 0.95H, J = 5.4 Hz, −C*H*-, *Z*- and *E*-isomer), 3.46 (d, 1H, J = 6.2 Hz, $-CH_2$-, *Z*- and *E*-isomer), 2.05, 2.05, 2.05, 2.05, 2.02, 2.02, 2.02, and 2.01 (8s, 12H, 4 x CH_3CO); ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.8, 170.8, 170.5, 170.5, 169.6, 169.6, 169.5, and 169.5 (8 x C, CH_3CO-), 156.0, 155.9, (C-para, *Z*- and *E*-isomer), 151.0, 150.8 (CH = *N*-, *Z*- and *E*-isomer), 131.9, 131.4, 130.2, 130.2, 130.1, 130.1, 117.6, 117.6, 117.5, and 117.5 (10 x C, C-arom., *Z*- and *E*-isomer), 99.4 (C-1′), 72.9 (C-3′), 72.2 (C-5′), 71.4 (C-2′), 68.5 (C-4′), 62.2 (C-6′), 35.3, 31.1 (C-2, *Z*- and *E*-isomers), 20.9, 20.9, 20.8, 20.8, 20.8, and 20.8 (8 x CH_3CO-); HMRS (m/z): [M + H]$^+$ calcd for $C_{22}H_{28}NO_{11}$: 482.1618; found, 482.1615. NMR spectra are provided in Supporting Information IV.

(E/Z)-4-(β-D-Glucopyranosyl)oxyphenylacetaldehyde Oxime (2). Compound **14** (1.0 g, 2.1 mmol) was treated with methanolic NH_3 (50 mL, 2.0 M). The mixture was stirred at RT overnight and evaporated, and the residue was coevaporated with toluene (3 × 25 mL). The residue was suspended in CH_2Cl_2, and the solid product was filtered off and washed with CH_2Cl_2 (3 × 10 mL) to give **2** in quantitative yield (0.65 g, 2.1 mmol). An analytical sample of **2** was obtained as white crystals by crystallization in MeOH; ^{1}H

NMR (400 MHz, CD_3OD): δ 7.41 (t, 0.4H, J = 6.2 Hz, −C*H* = N-, *E*-isomer), 7.16, 7.14 (2d, 2H, J_{AB} = 8.5 Hz, H-arom., *Z*- and *E*-isomer), 7.05 (d, 2H, J_{AB} = 8.5 Hz, H-arom., *Z*- and *E*-isomer), 6.75 (t, 0.5H, J = 5.4 Hz, −C*H* = N-, *Z*-isomer), 4.88 (d, 1H, J1′,2′ = 7.4 Hz, H-1′, *Z*- and *E*-isomer), 3.89 (dd, 0.97H, $J_{5',6'}$ = 1.8 Hz, $J_{6a',6b'}$ = 12.0 Hz, H-6′b, *Z*- and *E*-isomer), 3.70 (ddd, 0.98H, $J_{5',6'}$ = 5.4 Hz, $J_{6a',6a'}$ = 12.0 Hz, H-6′a, *Z*- and *E*-isomer), 3.64 (d, 1.12H, J = 5.4 Hz, −CH-, *Z*- and *E*-isomer), 3.47−3.39 (m, 4.67H, H-2′, H-3′, H-4′,H-5′ and −CH-, *Z*- and *E*-isomers); ^{13}C NMR (101 MHz, CD_3OD): δ 158.1, 158.0, (C-para, *Z*- and *E*-isomers), 151.3, 151.2 (CH = N-, *Z*- and *E*-isomers), 132.6, 132.1, 130.9, 130.9, 130.8, 130.8, 118.2, 118.2, 118.2, and 118.2 (10C, C-arom., *Z*- and *E*-isomers), 102.6 and 102.5 (C-1′, *Z*- and *E*-isomers),78.2, 78.2, 78.1, 78.1, 75.1, 75.1, 71.5, and 71.5 (8C, C-2′, C-3′, C-4′, C-5′, *Z*- and *E*-isomers), 62.7 and 62.7 (2C, C-6′, *Z*- and *E*-isomers), 36.0, 31.7 (2C, C-2, *Z*- and *E*-isomers); HMRS (*m*/*z*): $[M + H]^+$ calcd for $C_{14}H_{20}NO_7$: 314.1195; found, 314.1197. NMR spectra are provided in Supporting Information IV.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c05014.

> General methods, detailed experimental procedures for the chemical synthesis of compounds 4−10 as well as their ^1H and ^{13}C NMR data, copies of $^1H-^{13}C$ and ^{13}C NMR-DEPT spectra for all chemically synthesized compounds, and related cited references (PDF)

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Notes

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