Chiral synthesis of (+)-8′-demethyl abscisic acid[1]

Patricia A. Rose, Bo Lei, Angela C. Shaw, M. K. Walker-Simmons, Scott Napper, J. Wilson Quail, and Suzanne R. Abrams

Abstract: An enantioselective synthesis of (+)-8′-demethyl ABA (**2**) is described. The chiral intermediate **7** was prepared by yeast reduction of a substituted monoprotected cyclohexa-2,5-dien-1,4-dione (**9**) synthesized through a phenol oxidation. The scope and limitations of the phenol oxidation is described. 8′-Demethyl ABA shows ABA-like activity in wheat embryo germination inhibition, showing that the 8′-methyl group is not essential for biological activity.

Key words: abscisic acid, phenol oxidation, yeast reduction.

Résumé : On décrit une synthèse énantiosélective de l'acide (+)-8′-déméthyl-abscisique (AAB) (**2**). On a préparé l'intermédiaire chiral **7** par réduction, à l'aide de levures, d'une cyclohexa-2,5-diène-1,4-dione substituée monoprotégée (**9**) obtenue par oxydation d'un phénol. On décrit la généralité et les limitations de l'oxydation des phénols. L'acide 8′-déméthyl-abscisique présente une activité semblable à celle de l'AAB comme inhibiteur de la germination des embryons de blé; ce résultat démontre que le groupe 8′-méthyle n'est pas essentiel à l'activité biologique.

Mots clés : acide abscisique (AAB), oxydation des phénols, réduction par des levures.

[Traduit par la rédaction]

Introduction

The plant hormone (+)-(S)-abscisic acid (ABA, **1**) regulates a wide range of processes in plants, including transpiration through controlling stomatal aperture and responses to environmental stress. In developing seeds, ABA affects accumulation of proteins, acquisition of desiccation tolerance, maintenance of dormancy, and inhibition of germination (for reviews on ABA action in plants, see ref. 1). We have used optically active analogs of the hormone to probe the mechanisms by which ABA triggers different physiological effects such as germination inhibition and induction of genes in plants (2, 3). Enantiomerically pure molecules are required for these investigations, as previous studies have shown that the enantiomers of ABA can have different effects in the same tissue, and optically pure analogs can be either ABA agonists or antagonists depending upon the enantiomer tested (4).

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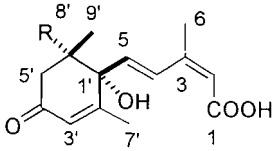
In this phase of our research we undertook to synthesize and test the optically pure ABA analog **2**, which lacks one of the geminal methyl groups of ABA, the 8′-carbon atom (following the conventional ABA numbering system). The aim was to provide analogs that could be used for differential screening to relate physiological and molecular effects in plant tissues. Methyl groups on the six-membered ring are important for activity. Racemic 8′-demethyl ABA had earlier been prepared and, in an assay looking at the growth inhibition of rice seedlings, was found to have moderate activity (5). Both enantiomers of the analog missing the 7′-methyl group are completely inactive in germination inhibition of excised wheat embryos, while the optically active analogs missing both of the geminal methyls have some activity (3).

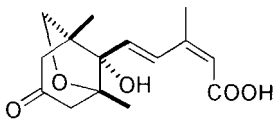
In addition, the analog **2** is a probe for differentiating between the activity conferred by ABA and its oxidized metabolites. The major pathway of ABA metabolism is through oxidation of the 8′-methyl group, transiently affording 8′-hydroxy ABA, **3**, which then cyclizes through an internal Michael addition to phaseic acid (**4**). While **4** is generally considered to be inactive (6), a method for trapping the open form **3** has recently been developed, and it was found to be as active as ABA in increasing very long chain fatty acid production in Brassica embryos (7).

We had previously developed the synthesis of (+)-2′,3′-dihydroABA (**5**) (8), which is oxidized in a plant cell culture at the 8′-carbon to give 8′-hydroxy-2′,3′-dihydroABA, **6**. This metabolite cannot cyclize to form phaseic acid. Analog **5** was found to be as active as ABA in germination inhibition of wheat embryos (2) and in inducing freezing tolerance in plant cells; however, its metabolic product **6** was inactive (9). Compound **6** appeared to exist in equilibrium with the hemi-ketal formed through the 4′-carbonyl. The lack of activity of the metabolite may arise from inactivity of the predominant ring-

closed form. Compound **5** did not conclusively answer the question of whether ABA or 8′-hydroxy ABA was the active plant hormone in the wheat embryo assay. 8′-Demethyl ABA **2** cannot be oxidized to an 8′-hydroxy ABA intermediate, as it is missing the 8′-methyl group, making it a useful tool for differentiating between the activity of ABA and its metabolites.



1 R = CH_3
2 R = H
3 R = CH_2OH



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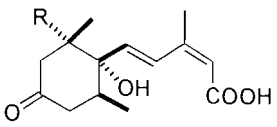
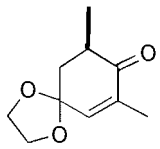


5 R = CH_3
6 R = CH_2OH



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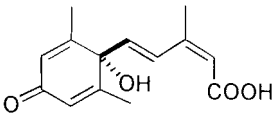

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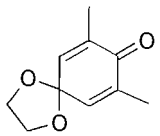

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We report here an enantioselective synthesis of (+)-8′-demethyl ABA, **2**, its activity in inhibiting wheat embryo germination, and its effect in reducing transpiration in wheat seedlings.

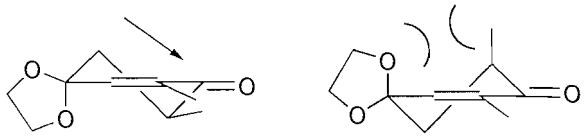
Results and discussion

Abscisic acid type compounds are readily synthesized by reaction of the appropriate cyclohexenone derivatives with side chains containing lithium acetylides. The dienoic side chain is generated by reduction of the propargylic alcohol with RedAl. Standard functional group manipulations afford a wide variety of analogs (10).

For the synthesis of 8′-demethyl ABA **2**, we decided to prepare chiral ring synthon **7** with the intention that the new chiral centre, generated through lithium acetylide addition to the carbonyl, would be formed stereoselectively. The preferred conformation for **7** presumably has the methyl group equatorial, to avoid 1,3-diaxial interactions between an axial methyl group and the ketal (Fig. 1). In the preferred conformation, the less hindered side of attack should be from the same face as the methyl group.

We synthesized the key intermediate **7** from 2,6-dimethylphenol in two steps: the first a phenol oxidation and the second a stereoselective yeast reduction. We had previously modified a phenol oxidation procedure for the synthesis of an achiral cyclohexadienone analog of ABA, **8** (11). The key intermediate for this synthesis, **9**, was prepared directly through an oxidation of 2,6-dimethyl phenol. The oxidation

Fig. 1. Conformations of enone **7**.



7, equatorial methyl **7**, axial methyl

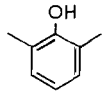
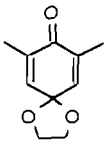
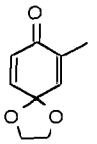
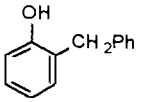
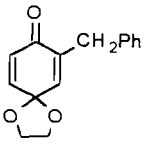
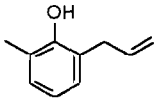
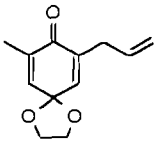
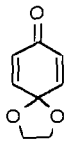
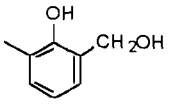
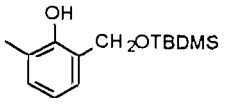
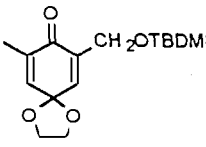
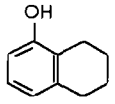
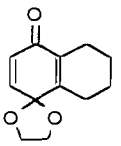
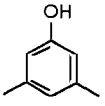
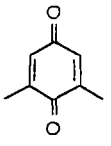
was a modification of Pelter's iodobenzene diacetate oxidation of phenols in methanolic solution (12), which affords quinone mono-dimethyl ketals. Replacing methanol with ethylene glycol and adding hexane to form a biphasic reaction mixture directly gave the more stable ethylene ketal in good yield.

As an aside, because the ethylene ketal is more convenient for synthetic purposes, we explored the scope of this reaction. This procedure worked for a number of phenols (entries 1–5, 7, Table I) in moderate yield. Unsubstituted phenol underwent oxidation to give the desired product (as determined by GC–MS), but the product was not stable to the work-up conditions (entry 5). Oxidation of *meta*-substituted phenols showed the steric limitations of this reaction. The introduction of one *meta* substitutent lowered the yield of ketalized product significantly (entry 8), whereas 3,5-dimethyl phenol (entry 9) produced only the quinone. Presumably, steric hinderance of the *meta* substituents hinders the formation of the 1,3-dioxolane ring. Phenols substituted with electron-withdrawing groups at either the *ortho* (difluoromethyl group) or *meta* (trifluoromethyl group) positions were unreactive, with only starting materials being recovered. The introduction of manipulable functional groups to the ring system can be accomplished, however, through an allyl group (entry 4) or a protected hydroxy methyl group (entry 7). Although 2-hydroxymethyl-6-methylphenol decomposed under the reaction conditions (entry 6), oxidation of its *tert*-butyldimethylsilyl ether gave the ketalized product in 67% yield (entry 7). This is a facile reaction that provides highly functionalized molecules in one step, and should prove to be useful in the synthesis of natural products and their analogs.

The second step in the synthesis of 8′-demethyl ABA was an enantioselective yeast reduction of the quinone monoketal **9** to introduce the chiral centre. Yeast reductions have previously been carried out under non-deketalizing conditions, and have been shown to reduce electron-deficient double bonds in reasonable yields and with good stereoselectivity (13). We were able to reduce quinone monoketal **9** in 50% yield (92% ee) to enone **7**, with little epimerization of the newly formed chiral centre, hydrolysis of the ketal, or overreduction of the second double bond.

The remaining steps in the synthesis of 8′-demethyl ABA closely follow those previously reported for the synthesis of ABA (14), with the exception of combining the alkyne addition and subsequent reduction of the triple bond into one step (see Fig. 2). Enone **7** was reacted with the lithium anion of *cis*-(5-*tert*-butyldimethylsiloxy-3-methylpent-3-enyne) (8), **10**, to give one diastereomer and after the alkyne addition was complete, Redal® was added directly to the reaction mixture, reducing the triple bond to a *trans* double bond, as well as cleaving the silyl protecting group, to give dienol **11** in a one-

Table 1. Iodobenzene diacetate oxidation of phenols in ethylene glycol–hexane.

Entry	Phenol	Product	Yield (%)
1			63
2			56
3			35
4			55
5			45[a]
6		Decomposed	
7			67
8			27
9			63

[a]Yield based on GC analysis.

pot reaction in 38% yield. Yields for the Redal® reduction of the isolated alkyne were very low when the reaction was carried out independently. The bulky protecting group on the alcohol of the pentenyne chain was found to be necessary for obtaining high diastereoselectivity when reacted with chiral enone **7**. Addition of the lithium dianion of the unprotected alcohol gave a mixture of both *cis* and *trans* addition products. Assuming addition is only occurring at the less hindered face of the ketone (Fig. 1), either the remote bulky protecting group is contributing to the increased diastereoselectivity, or possibly the lithium anion of the unprotected alcohol is chelating with the oxygens on the ketal to facilitate addition from either face.

Dienol **11** was deketalized in 83% yield under standard conditions to give enone **12**, which was then oxidized to the aldehyde with MnO_2, and then to the ester (15) to give the methyl ester of 8′-demethyl ABA, **13**, in 35% yield over the two steps. Other oxidants gave unwanted isomerization of the *cis* double bond. Methyl ester **13** could be analyzed on a Chiralcel OD HPLC column, showing that no racemization of the 6′-methyl group of ketone **7** had occurred during addition of the side chain, and, using preparative HPLC, the compound was purified to greater than 99% ee. The methyl ester was cleaved using porcine liver esterase (to avoid possible aromatization of the ring under alkaline hydrolysis conditions) to give the desired (+)-8′-demethyl abscisic acid, **2**.

The stereoselectivity of the yeast reduction and diastereoselectivity of alkynylation were proven through an X-ray crystal structure of Mosher's ester **14**, made from an intermediate of which we had made quantities while trying to improve reduction of the triple bond. Compound **14** was prepared by reacting enone **7** with the lithium anion of **10** to give one diastereomer, **15**, in 83% yield (see Fig. 3). Alkyne **15** was deprotected in succession with Bu_4NF, to remove the silyl group, and then with pTsOH to remove the ketal, to form acetylenic alcohol **16**. The C1-hydroxy group of **16** was then coupled with *R*-(+)-α-methoxy-α-(trifluoromethyl)phenyl acetic acid using DCC to form the crystalline Mosher's ester. The X-ray structure is displayed in the PLUTON92 (16) drawing in Fig. 4 and shows the chiral center of enone **7** (generated by the yeast reduction) to be *R*, and relationship of the side-chain and 9′-methyl group of **2** to be *cis*, as shown.

Biological results

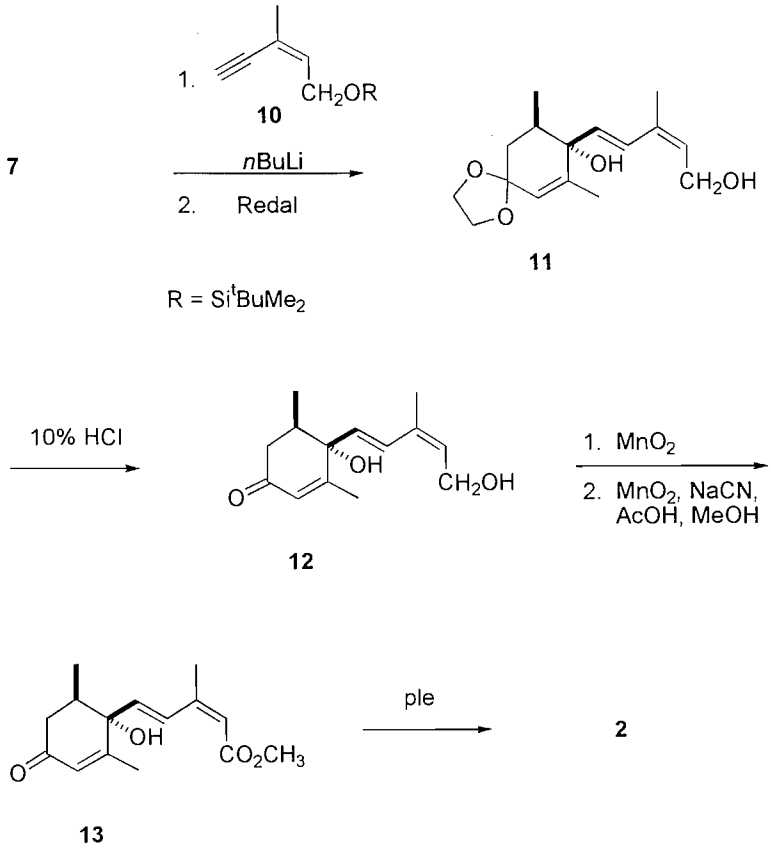
The effectiveness of compound **2** compared to (+)-ABA (**1**) as a germination inhibitor was determined with isolated wheat (cultivar Brevor) embryos. The compounds were tested over a range of concentrations and the concentrations required to inhibit embryo germination by 50% were 0.05 μM for (+)-ABA and 0.5 μM for (+)-8′-demethyl ABA. In a wheat seedling transpiration assay, 8 h after applying 100 μM (+)-ABA as a root drench, the transpiration rate of the seedlings was reduced by 80%. 8′-Demethyl ABA applied at 100 μM concentration reduced transpiration by 45% under the same conditions. This strong activity of an analog that has no 8′-methyl group available for oxidation suggests that ABA itself is the active hormone in germination inhibition, and transpiration reduction. These results do not preclude 8′-hydroxy ABA from also being biologically active, and investigations are ongoing to determine the biological activity of 8′-hydroxy ABA in wheat.

Experimental

General

Melting points were determined with a microscope hot stage

Fig. 2. Synthesis of (+)-8′-demethyl ABA **2**.



apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AMX-500 spectrometer, employing $CDCl_3$ as solvent with $CHCl_3$ as reference, unless otherwise specified. For clarity, the conventional ABA numbering system is employed in assignments of peaks in the ^{1}H NMR spectra. IR spectra were obtained with a Perkin Elmer 237B instrument or a Perkin-Elmer Paragon 1000 FTIR. Optical rotations were obtained from a Perkin–Elmer 141 polarimeter. Flash column chromatography was performed using E. Merck silica gel 60 (230 – 400 mesh). High-resolution electron impact (HREIMS) mass spectra were recorded with a VG 70-250SEQ double-focusing hybrid spectrometer. Elemental analyses were carried out by the Microanalytical Laboratory of the University of Saskatchewan. Commercially available compounds were used in this work without further purification. Tetrahydrofuran was distilled over sodium and benzophenone (indicator). Fleischmann's brand bakers' yeast was used in the yeast reduction reaction. The wheat embryo germination assay was carried out as previously reported (8). The transpiration assay was carried out as previously reported (17).

X-ray analysis of 14

Crystal data: $C_{24}F_3O_5H_{25}$, M_r=450.45, monoclinic, $P2_1$, a = 11.91(2), b = 7.612(3), c = 12.50(1) Å, β=105.71(1)°, V = 1091(2) Å^3, Z = 2, $F(000)$ = 472, D_x = 1.372 Mg m^{-3}, μ = 0.11 mm^{-1}, crystal dimensions 0.68 × 0.12 × 0.09 (mm). Intensity data were measured at 123(2) K on an Enraf–Nonius CAD-4 diffractometer, using graphite monochromatized MoK_α (λ = 0.71073 Å) radiation. Intensity data were collected with ω scans to a maximum 2Θ angle of 49.7°. The unit-cell dimensions were obtained by a least-squares fit of 25 centered reflections in the range 17 ≤ 2Θ ≤ 29°. The scan width, Δω, for each reflection was (1.20 + 0.35 tan Θ)° with a scan speed of 0.40–2.74° min^{-1}. Three standard reflections were monitored every two hours for intensity and two standard reflections were monitored every 200 reflections for orientation. A total of 2130 reflections were measured, of which 2022 were unique (R_{merge} = 0.046) with index range h −14/+13, k 0/+8, l 0/+14, and 1328 were observed (I)2σ(I)). Intensities were corrected for Lorentz and polarization factors, but no correction was made for absorption.

The structure was solved by direct methods and refined using full-matrix least-squares techniques, minimizing the function to R = 0.112 and R_w = 0.103, S = 3.40. Final $(\Delta/\sigma)_{max}$ was 0.000. In the final difference map $\Delta\rho_{max}$ = 0.79 e Å^{-3} and $\Delta\rho_{min}$ = −0.92 e Å^{-3}. The function minimized was Σwith w = 1.0/(σ^2(F) + 0.0001 F^2). Because the crystal was weakly diffracting and the number of observed reflections was too small for anisotropic refinement, isotropic temperature factors were used for all non-hydrogen atoms. Once the non-hydrogen atoms were refined, H-atoms were placed in calculated positions on the corresponding C and O atoms (d(C—H) = 1.00, d(O—H) = 0.80 Å) and not refined. The U_{iso} of each hydrogen atom was assigned as equal to the U_{iso} of the attached atom plus 0.01. The configuration of the molecule was determined from the known configuration of Mosher's acid. All calcula-

Fig. 3. Synthesis of Mosher's ester **14**.

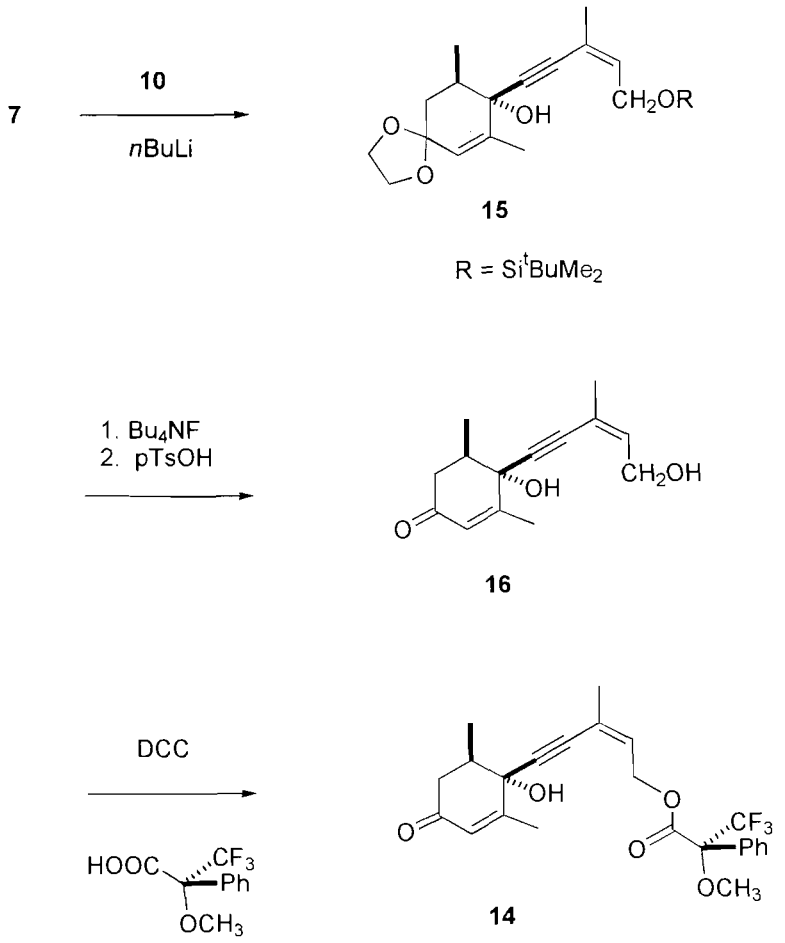
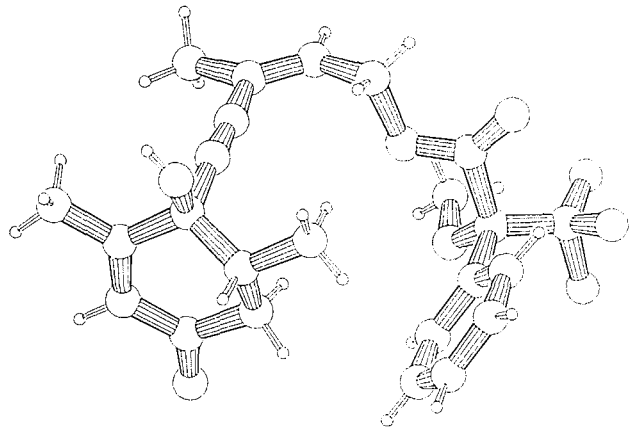


Fig. 4. PLUTON92 drawing of Mosher's ester **14**.



tions were performed with the NRCVAX crystallographic program package (18). The atomic scattering factors were taken from the International Tables for X-ray Crystallography (1974) (19).

Detailed atomic coordinates, bond distances and angles have been deposited at the Cambridge Crystallographic Data Center. These data can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, U.K.

General procedure for the preparation of quinone monoketals

2,6-Dimethyl-4,4-ethylenedioxycyclohexa-2,5-dienone (9) Iodobenzene diacetate (20.29 g, 0.063 mol) was suspended in hexane (250 ml), mechanically stirred, and cooled with an external ice bath. To the suspension was added 2,6-dimethyl phenol (3.66 g, 0.030 mol) in anhydrous ethylene glycol (20 mL). The ice bath was removed and the mixture stirred for 2 h before being quenched with H_2O (200 mL). The two phases were separated and the aqueous layer was extracted with ether (200 mL). The organic layers were combined and washed with 5% $NaHCO_3$ and saturated NaCl solution, dried over $MgSO_4$, and concentrated. Distillation under vacuum removed most of the iodobenzene by-product and the residue was purified by flash chromatography (5% EtOAc – hexane) to give 3.38 g (63%) of product as a solid. Recrystallization from ether–hexane gave a white crystal, mp 47–49°C. IR ($CHCl_3$) ν_{max} cm^{-1}: 1715 (C=O), 1630 (C=C). ^1H NMR, δ: 6.39 (s, 2H, H-3, H-5), 4.18 (s, 4H, OCH_2CH_2O), 1.86 (s, 6H, Me). ^{13}C NMR, δ: 186.4, 138.3 (2C), 135.5 (2C), 98.8, 65.3 (2C), 15.4 (2C). HRE-IMS: (M^+) at *m/z* 180.0786 ($C_{10}H_{12}O_3$ requires 180.0786).

2-Methyl-4,4-ethylenedioxycyclohexa-2,5-dienone (Table 1, entry 2): IR(neat) ν_{max} cm^{-1}: 1720 (C=O), 1680, 1650 (C=C). ^1H NMR, δ: 6.58 (*dd*, 1H, *J* = 10.0, 3.1 Hz, H-5), 6.38

(m, 1H, H-3), 6.13 (d, 1H, J = 10.0 Hz, H-6), 4.11 (s, 4H, OCH_2CH_2O), 1.85 (d, 3H, J = 1.6 Hz, vinyl Me); ^{13}C NMR, δ: 185.9, 142.9, 138.6, 136.0, 128.9, 99.0, 65.7 (2C), 15.4; HRE-IMS: [M^+] at m/z 166.0644 ($C_9H_{10}O_3$ requires 166.0630).

4,4-Ethylenedioxycyclohexa-2,5-dienone (entry 5): IR(neat) ν_{max} cm^{-1}: 1680 (C=O). ^1H NMR, δ: 6.60 (d, 2H, J = 10.1 Hz, H-3, H-5), 6.14 (d, 2H, J = 10.1 Hz, H-2, H-6), 4.12 (s, 4H, OCH_2CH_2O); ^{13}C NMR, δ: 185.3, 143.2, 129.0, 98.2, 65.8; HREIMS: [M^+] at m/z 152.0462 ($C_8H_8O_3$ requires 152.0734).

2-Benzyl-4,4-ethylenedioxycyclohexa-2,5-dienone (entry 3): IR(neat) ν_{max} cm^{-1}: 1680 (C=O), 1650 (C=C). ^1H NMR, δ: 7.29–7.17 (m, 5H, phenyl H), 6.58 (dd, 1H, J = 10.0, 3.1 Hz, H-5), 6.16 (d, 1H, J = 10.0 Hz, H-6), 6.12 (m, 1H, H-3), 4.07-4.03 (m, 4H, OCH_2CH_2O), 3.59 (d, 2H, J = 0.8 Hz, CH_2); ^{13}C NMR, δ: 185.2, 142.9, 139.4, 139.2, 137.9, 129.4, 128.9, 128.6, 126.4, 99.1, 65.7, 34.9; HREIMS: [M^+] at m/z 242.0934 ($C_{15}H_{14}O_3$ requires 242.0943.)

2-Allyl-6-Methyl-4,4-ethylenedioxycyclohexa-2,5-dienone (entry 4): IR(neat) ν_{max} cm^{-1}: 1685 (C=O), 1650 (C=C). ^1H NMR, δ: 6.36 (m, 1H, CH=CH*H*), 6.32 (m, 1H, CH=C*H*H), 5.78 (m, 1H, C*H*=CHH), 5.15 (m, 1H, H-3), 5.07 (m, 1H, H-5), 4.08 (s, 4H, OCH_2CH_2O), 3.00 (dd, 2H, J = 6.8, 1.1 Hz, CH_2), 1.85 (d, 3H, J = 1.4 Hz, CH_3); ^{13}C NMR, δ: 185.8, 138.4, 138.3, 138.0, 135.9, 134.4, 117.6, 99.1, 65.6 (2C), 32.9, 15.7; HREIMS: [M^+] at m/z 206.0946 ($C_{12}H_{14}O_3$ requires 206.0943).

*2-(*tert*-Butyldimethylsiloxymethyl)-6 methylphenol*
Hydroxymethyl-6-methylphenol (20) (3.1 g, 0.022 mol) was dissolved in 30 mL CH_2Cl_2 and cooled to 0°C. *tert*-Butyldimethylsilylchloride (1.2 equiv. 4.0 g) and imidazole (1.2 equiv. 1.8 g) were added, and the reaction vessel removed from the ice bath. After 1 h, the mixture was filtered and the filtrate washed with 1 M HCl and brine solution, and then dried over Na_2SO_4. The crude material was purified by flash chromatography (3% ether in hexane) yielding 3.9 g (69% yield) of product. IR ν_{max} cm^{-1}: 3350 (O-H). ^1H NMR, δ: 8.15 (s, 1H, O-H), 7.05 (d, 1H, J = 7.3 Hz, phenyl H), 6.79 (d, 1H, J=7.1 Hz, phenyl H), 6.72 (*t*, 1H, J=7.3 Hz, phenyl H), 4.89 (s, 2H, CH_2OSi), 2.25 (s, 3H, $PhCH_3$), 0.94 (s, 9H, $SiC(CH_3)_3$), 0.15 (s, 6H, $Si(CH_3)_2$). HRE-IMS: [M^+] at m/z 252.1544 ($C_{14}H_{22}O_2Si$ requires 252.1546).

*2-(*tert*-Butyldimethylsiloxymethyl)-4,4-ethylenedioxy-6-methyl-cyclohexa-2,5-diene-1-one (entry 7):* IR ν_{max} cm^{-1}: 1645 (C=O). ^1H NMR, δ: 6.61 (m, 1H, =CH), 6.40 (m, 1H, =CH), 4.39 (d, 2H, J = 1.6 Hz), 4.12 (m, 4H), 1.84 (d, 3H, J = 1.3 Hz), 0.90 (s, 9H, $SiC(CH_3)_3$), 0.07 (6H, s, $Si(CH_3)_2$). ^{13}C NMR, δ: 185.6 (C=O), 139.36, 138.7, 137.1, 135.7, 99.8, 65.3, 60.0, 26.1, 26.1, 18.5, 15.3, −9.5. HREIMS: [M^+] at m/z 310.1607 ($C_{16}H_{26}O_4Si$ requires 310.1600).

4,4-Ethylenedioxy-5,6,7,8-tetrahydronaphtha-2,5-dienone (entry 8): IR(neat) ν_{max} cm^{-1}: 1675 (C=O), 1645, 1620 (C=C). ^1H NMR, δ: 6.63 (d, 1H, J = 10.0 Hz, H-5), 6.10 (d, 1H, J = 10.0 Hz, H-6), 4.15 (m, 4H, OCH_2CH_2O), 2.28 (m, 4H, CH_2), 1.65 (m, 4H, CH_2). ^{13}C NMR, δ: 185.2, 150.3, 142.4, 134.6, 127.7, 99.8, 66.2 (2C), 22.8, 22.0, 21.4 (2C). HREIMS: [M^+] at m/z 206.0939 ($C_{12}H_{14}O_3$ requires 206.0943).

Synthesis of 8′-demethylABA

*(6*R*)-2,6-Dimethyl-4,4-ethylenedioxycyclohexa-2-enone (**7**)*
Sucrose (25 g) was dissolved in 1.0 L of 0.1 M HEPES buffer (pH 6.5) and warmed to 45°C. Yeast (50 g) was added and the mixture was shaken for 30 min. 2,6-Dimethyl-4,4-ethylene-dioxycyclohexa-2,5-dienone (2.13 g) in ethanol (10 mL) was added dropwise. The fermentation mixture was shaken at room temperature and the reduction monitored by GC. After the reaction was completed (typically 3–4 h), 200 mL ether was added and the mixture was filtered through a pad of Celite®. Alternatively, centrifugation for 20 min at 10 000 rpm effectively removed the yeast cells. The filtrate was extracted with large quantities of ether (3 × 400 mL) to avoid emulsions. The combined ether extracts were dried over $MgSO_4$ and con-centrated. Column chromatography (5% EtOAc – hexane) gave 1.08 g (50% yield) product as a colorless liquid. $[\alpha]_D^{20}$ +31.8 ($CHCl_3$, *c* 2.0). IR ($CHCl_3$) ν_{max} cm^{-1}: 1682 (C=O). ^1H NMR, δ: 6.32 (m, 1H, H-3), 3.87–4. 08 (m, 4H, OCH_2CH_2O), 2.73 (m, 1H, H-6), 2.15 (ddd, 1H, J = 2.1, 4.8, 13.3 Hz, H-5ax), 1.98 (t, 1H, J = 13.0 Hz, H-5eq), 1.77 (d, 3H, J = 1.5 Hz, $=CCH_3$), 1.14 (d, 3H, J = 7 Hz, CH_3). ^{13}C NMR, δ: 201.37 (C=O), 140.63 (C=), 137.20 (C=), 104.59, 65.06, 64.73, 41.56, 39.50, 15.77, 15.01. HREIMS: (M^+) at m/z 182.0950 ($C_{10}H_{14}O_3$ requires 182.0950).

*(+)-8-(1*E*,3*Z*)-(8*R*,9*R*)-8-(5-Hydroxy-3-methylpent-1,3-die-nyl)-7,9-dimethyl-1,4-dioxaspiro[4.5]dec-6-en-8-ol, **11***
A solution of known alkyne **10** (8) (2.1 g, 9.9 mmol) in dry THF (40 mL) was cooled in a Dry Ice – acetone bath under an argon atmosphere. *n*-Butyllithium (Aldrich, 0.63 M in hexane, 5.6 ml, 8.9 mmol) was added dropwise with stirring. A solution of ketal **7** (1.29 g , 7.1 mmol) in dry THF (20 mL) was added dropwise. After the addition was complete, the reaction solution was allowed to warm to room temperature (over 45 min). The reaction mixture was then cooled in an ice bath, and sodium bis(2-methoxyethoxy) aluminium hydride (Redal®, Aldrich, 3.4 M in toluene, 4.1 mL, 14.2 mmol) in THF (20 mL) was added dropwise. The reaction solution was allowed to warm to room temperature. After 1.5 h, the reaction was cooled to 0°C and water was added carefully to destroy the excess reducing agent. The THF was removed and the aqueous layer extracted with ether three times. The pooled organics were dried over Na_2SO_4 and concentrated. Purification by flash chromatography eluting with ether afforded alcohol **11** (760 mg, 38%). IR ($CHCl_3$) ν_{max} cm^{-1}: 3600 (O-H). ^1H NMR (C_6D_6), δ: 6.80 (d, 1H, J = 15.7 Hz, H-5), 5.63 (d, 1H, J = 15.8 Hz, H-4), 5.52 (s 1H, H-3′), 5.41 (*t*, 1 H, J = 5.8 Hz, H-2), 4.1 (*t*, 2H, J = 5.8 Hz, H-1), 3.61–3.46 (m, 4H, OCH_2), 2.20 (m, 1H, H-6′), 1.90 (d, 1H, J = 13.5 Hz, H-5′), 1.80 (dm, 1H, J = 13.7 Hz, H-5′), 1.65 (s, 3H, $=CCH_3$), 1.63 (s, 3H, $=CCH_3$), 0.90 (d, 3H, J = 7.8 Hz, CH_3). ^{13}C NMR (C_6D_6), δ: 144.16, 133.68, 129.79, 127.50, 125.57, 106.15, 77.41, 64.62, 64.34, 58.40, 40.29, 40.18, 20.52, 17.59, 15.58. HREIMS: [M^+] at m/z 280.1693 ($C_{16}H_{24}O_4$ requires 280.1675).

*(+)-(1*E*,3*Z*)-(4*R*,5*S*)-4-Hydroxy-4-(5-hydroxy-3-methylpent-1,3-dienyl)-3,5-dimethylcyclohex-2-enone, **12***
Compound **11** (106 mg), 10% HCl (10 mL), and THF (10 mL) were stirred at room temperature for 1.5 h. The mixture was diluted with water and extracted into ether (3 × 30 ml). The

combined organics were washed with brine, dried over Na_2SO_4, and concentrated. The crude material (74 mg, 83%) was carried through to the next step. $[\alpha]_D^{20}$ +246.4 (MeOH, *c* 0.84). IR (neat) ν_{max} cm^{-1}: 3380 (br O-H), 1705 (C=O). ^1H NMR, δ: 6.69 (d, 1H, *J* = 15.8 Hz, H-5), 5.89 (s, 1H, H-3′), 5.66 (d, 1H, *J* = 15.7 Hz, H-4), 5.62 (t, 1 H, *J* = 6.9 Hz, H-2), 4.28 (d, 2H, *J* = 6.9 Hz, H-1), 2.41 (ddd, 1H, *J* = 0.9, w coupling to H-3′, 4.2, 16.8 Hz, H-5′eq), 2.31–2.38 (m, 1H. H-6′), 2.21 (*dd*, 1H, *J* = 13.2, 16.8 Hz, H-5′ax), 1.92 (d, 3H, *J* = 1.1 Hz, =CCH_3), 1.84 (d, 3H, *J* = 0.8 Hz, =CCH_3), 0.99 (d, 3H, *J* = 6.7 Hz, H-9′). ^{13}C NMR, δ: 198.15, 165.24, 134.13, 129.63, 128.14, 128.05, 127.41, 77.48, 58.34, 43.06, 41.24, 20.61, 18.46, 15.04. HREIMS: [M^+] at *m/z* 236.1393 ($C_{14}H_{20}O_3$ requires 236.1412). Anal. calcd. for $C_{14}H_{20}O_3$: C 71.14, H 8.54; Found: C 71.02, H 8.56.

(+)-(1E,3Z)-(4R,5R)-4-Hydroxy-4-(4-carbomethoxy-3-methylbutyl-1,3-dienyl)-3,5-dimethylcyclohex-2-enone, ***13***

A mixture of MnO_2 (15 equiv. 4.1 mmol, 352 mg) and diene **12** (64 mg, 0.27 mmol) in 15 mL of acetone was stirred at room temperature for 1.5 h. The suspension was filtered and the cake of MnO_2 washed with excess ether. After concentration, 50 mg of aldehyde was recovered and directly reacted in the following manner. To the aldehyde (50 mg, 0.21 mmol) in MeOH (10 mL) was added sequentially MnO_2 (3.2 mmol, 274 mg), NaCN (0.65 mmol, 25 mg), and AcOH (0.21 mmol, 12 mL). The reaction was stirred at room temperature for 1.5 h. The suspension was filtered and washed with MeOH. After concentration, the residue was partitioned between Et_2O and H_2O and the organic layer washed with saturated NaCl solution, dried over Na_2SO_4, and concentrated. The crude product was purified on a Chromatotron eluting with ether, giving 25 mg (35% yield over the two steps) of the desired ester. Analysis on chiral HPLC (semiprep Chiralcel OD column, 10% IPA in hexane, flow rate of 4 mL/min) showed the product to be a 87:13 mixture of enantiomers. Preparative separation of the major enantiomer gave ester **13** with greater than 99% ee. $[\alpha]_D^{20}$ +359.4 (MeOH, *c* 1.38). IR ($CHCl_3$) ν_{max} cm^{-1}: 3450 (br O-H), 3600 (sharp OH), 1660 (C=O, enone), 1705 (C=O, ester). ^1H NMR, δ: 7.81 (d, 1H, *J* = 16.2 Hz, H-5), 6.00 (d, 1H, *J* = 16.2 Hz, H-4), 5.92 (s, 1H, =CH), 5.73 (s, 1H, =CH), 3.68 (s, 3H, $COOCH_3$), 2.35–2.45 (m, 2H), 2.19–2.26 (m, 2H), 1.99 (d, 3H, *J* = 1.0 Hz, =CCH_3), 1.95 (d, 3H, *J* = 1.2 Hz, =CCH_3), 1.00 (d, 3H, *J* = 6.5 Hz, H-9′). ^{13}C NMR, δ: 197.83, 166.39, 164.62, 149.24, 133.72, 129.29, 127.70, 118.42, 77.53, 51.21, 43.01, 41.22, 21.10, 18.40, 15.10. HREIMS: [M^+] at *m/z* 264.1368 ($C_{15}H_{20}O_4$ requires 264.1362).

(+)-(1E,3Z)-(4R,5R)-4-Hydroxy-4-(4-carboxy-3-methylbutyl-1,3-dienyl)-3,5-dimethylcyclohex-2-enone, ***2***

Optically pure ester **13** (28.7 mg, 0.11 mmol) was dissolved in methanol (12 drops) and potassium phosphate buffer (0.1 M, pH 7.5, 2.5 mL), porcine liver esterase (EC 3.1.1.1, Sigma E-3128, 200 mL), and KOH solution (1 M, added dropwise to adjust the pH to 8.0) were added and the solution stirred overnight. 10% HCl was added until the pH was lower than 3 and the mixture was repeatedly extracted with ethyl acetate (6 × 10 mL) to obtain the product from the resultant emulsion. The combined ethyl acetate phases were extracted with saturated aqueous $NaHCO_3$ (3 × 25 mL). The aqueous phases were acidified with HCl and extracted with ethyl acetate (3 × 25 mL). The ethyl acetate extracts were washed with brine and dried over Na_2SO_4. Evaporation gave 24 mg (89% yield) of pure product. $[\alpha]_D^{20}$ +430.3 (MeOH, *c* 2.30). IR ($CHCl_3$) ν_{max} cm^{-1}: 3000–3500 (O-H), 1675 (C=O), 1650 (C=O). ^1H NMR (CD_3OD), δ: 7.72 (d, 1H, *J* = 16.2 Hz, H-5), 6.09 (d, 1H, *J* = 16.2 Hz, H-4), 5.91 (s, 1H. =CH), 5.74 (s, 1H, =CH), 4.86 (*bs*, 1H, -OH), 3.29-2.25 (m, 3H, H5 and H6), 2.02 (d, 3H, *J* = 1.0 Hz, =CCH_3), 1.95 (d, 3H, *J* = 1.2 Hz, =CCH_3), 1.03 (d, 3H, *J* = 6.5 Hz, H-9′); ^{13}C NMR (DMSO-d_6): δ: 201.02, 169.38, 169.19, 150.89, 130.47, 128.13, 121.65, 119.72, 78.37, 43.81, 42.57, 21.17, 19.14, 15.45. HREIMS: [M^+] at *m/z* 250.1198 ($C_{14}H_{18}O_4$ requires 250.1205).

Synthesis of Mosher's ester 14

*(+)-8-(4Z)-(8S,9R)-8-(5-*tert*-Butyldimethylsiloxy-3-methylpent-3-en-1-ynyl)-7,9-dimethyl-1,4-dioxaspiro[4.5]dec-6-en-8-ol,* ***15***

A solution of known alkyne **10** (8) (2.1 g, 10.2 mmol) in dry THF (40 mL) was cooled in a Dry Ice – acetone bath under an argon atmosphere. *n*-Butyllithium (Aldrich, 0.63 M in hexane, 6.4 mL, 10.2 mmol) was added dropwise with stirring. The reaction solution was kept at −78°C for 1 h. A solution of enone **7** (930 mg, 5.1 mmol) in dry THF (20 mL) was added dropwise. After the addition was complete, the reaction solution was allowed to warm to room temperature (over 45 min), quenched with water, and extracted into ether. The pooled organics were washed with brine and dried over Na_2SO_4. Separation by flash chromatography eluting with ether–hexane (30:70) gave alkyne **15** in 83% yield (1.65 g). Alkyne **15** showed the following spectral properties: $[\alpha]_D^{20}$ +122.2 (MeOH, *c* 1.70). IR (neat) ν_{max} cm^{-1}: 3440 (O-H). ^1H NMR (C_6D_6), δ: 5.78 (dt, 1 H, *J* = 1, 6.2 Hz, H-2), 5.42 (s, 1H, H-3′), 4.48 (m, 2H, H-1), 3.42-3.57 (m, 4H, OCH_2), 2.13 (d, 1H, *J* = 13.3 Hz, H-5′ax), 1.89 (s, 3H, =CCH_3), 1.85 (dt, 1H, *J* = 2.1, 13.4 Hz, H-5′eq), 1.69 (s, 1H, OH), 1.64 (s, 3H, =CCH_3), 1.21 (d, 3H, *J* = 6.7 Hz, H-9′), 0.99 (s, 9H, Si^{t}Bu), 0.13 (s, 3H, $SiCH_3$). ^{13}C NMR (C_6D_6), δ: 142.39, 138.11, 125.30, 118.06, 105.94, 93.69, 84.60, 73.73, 64.60, 64.31, 62.61, 40.47, 39.85, 26.13, 22.84, 18.47, 17.72, 16.33, −4.98. HREIMS: [M^+] at *m/z* 392.2402 ($C_{22}H_{36}O_4Si$ requires 392.2383). Anal. calcd. for $C_{22}H_{36}O_4Si$: C 67.31, H 9.25; found: C 67.13, H 9.16.

(+)-4Z-(4S,5R)-4-Hydroxy-4-(5-hydroxy-methylpent-3-en-1-ynyl)-3, 5-dimethylcyclohex-2-enone, ***16***

To an ice-cooled solution of alkyne **15** (900 mg, 0.2.3 mmol) in dry THF (50 mL) under argon was added, dropwise, tetrabutylammonium fluoride (1.0 M in THF, 3.45 m, 3.45 mmol). The reaction solution was allowed to warm to room temperature. After 0.75 h, water was added and the aqueous layer extracted three times with ether. The pooled organics were dried over Na_2SO_4 and concentrated. The crude product was directly treated with 10% aqueous HCl (25 mL) in THF (25 mL), and was stirred at room temperature for 0.5 h. After addition of water, the mixture was extracted three times with ether, and the combined organic extracts were washed with brine, dried over Na_2SO_4, and purified by flash chromatography eluting with ether. Acetylenic alcohol **16** was produced in 87% yield (470 mg) over two steps. IR (neat) ν_{max} cm^{-1}: 3605 (s, OH), 3400 (br, O-H). ^1H NMR, δ: 5.92 (dt, 1 H, *J* = 1.4, 6.8 Hz, H-2), 5.81 (s, 1H, H-3′), 4.26 (dd, 2H, *J* = 0.8, 6.8 Hz, H-

1), 2.74 (bs, 1H, -OH), 2.43 (m, 3H), 2.11 (d, 3H, J = 1.1 Hz, $=CCH_3$), 1.87 (d, 3H, J = 1.0 Hz, $=CCH_3$), 1.20 (d, 3H, J = 6.0 Hz, H-9′). ^{13}C NMR, δ: 198.62, 163.86, 136.91, 126.31, 120.17, 90.62, 85.57, 72.75, 60.85, 43.42, 41.01, 22.96, 18.76, 15.91. HREIMS: [M^+] at m/z 234.1234 ($C_{14}H_{18}O_3$ requires 234.1256).

Mosher's ester, ***14***

Acetyleneic alcohol **16** (0.36 mmol, 84 mg), DCC (0.36 mmol, 74 mg), R-(+) α-methoxy α-(trifluoromethyl)phenyl acetic acid (0.32 mmol, 76 mg), and DMAP (0.04 mmol, 4 mg) were added to 5 mL of CH_2Cl_2 and stirred at room temperature overnight. The CH_2Cl_2 was washed with water and 10% HCl, dried over Na_2SO_4, and concentrated. Purification on a Chromatotron, eluting with 75:25 ether–hexane gave 34 mg recovered starting material and 78 mg of product (81% based on recovered starting material). Analysis of enantiotopic purity by chiral HPLC (Semiprep Chiralpak AS column, 25% IPA in hexane, flow rate 2 ml/min) showed an 85:15 ratio of diastereomers after recrystallization from ether–hexane, which could be purified to >99% ee by preparative chiral HPLC; mp 129–130. $[\alpha]_D^{20}$ +227.3 ($CHCl_3$, c 2.39). IR ($CHCl_3$) ν_{max} cm^{-1}: 3600 (OH), 1750 (C=O, ester), 1665 (C=O, enone). ^1H NMR, δ: 7.48 (m, 2H, aromatic), 7.39 (m, 2H, aromatic), 5.88 (dt, 1H, J = 1.2, 7.3 Hz, H-2), 5.81 (s, 1H, =CH), 4.96 (dd, 1H, J = 7.3, 12.1 Hz, H-1), 4.91 (dd, 1H, J = 7.3, 12.1 Hz, H-1), 3.52 (s, 3H, OCH_3), 2.61 (bs, 2H, OH), 2.33–2.46 (m, 3H), 2.09 (d, 3H, J = 1.0 Hz, $=CCH_3$), 1.89 (s, 3H, $=CCH_3$), 1.19 (d, 3H, J = 5.8 Hz, H-9′). ^{13}C NMR, δ: 198.03, 166.45, 162.74, 132.12, 130.18, 129.99, 129.70, 128.56, 128.45, 127.27, 126.66, 124.52, 122.06, 92.14, 84.98, 72.91, 63.99, 55.47, 43.38, 40.95, 22.93, 18.58, 15.83. HREIMS: [M^+] at m/z 450.1612 ($C_{24}H_{25}O_3F_3$ requires 450.1654).

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