Efficient and flexible synthesis of new photoactivatable propofol analogs

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Graphical Abstract

Keywords

Propofol; TRPA1; Diazirine; Click chemistry

Abstract

Propofol is a widely used general anesthetic, which acts by binding to and modulating several neuronal ion channels. We describe the synthesis of photoactivatable propofol analogs functionalized with an alkyne handle for bioorthogonal chemistry. Such tools are useful for detecting and isolating photolabeled proteins. We designed expedient and flexible synthetic routes to three new diazirine-based crosslinkable propofol derivatives, two of which have alkyne handles. As a proof of principle, we show that these compounds activate heterologously expressed Transient Receptor Potential Ankyrin 1 (TRPA1), a key ion channel of the pain pathway, with a similar potency as propofol in fluorescence-based functional assays. This work demonstrates that installation of the crosslinkable and clickable group on a short nonpolar spacer at the *para* position of propofol does not affect TRPA1 activation, supporting the utility of these chemical tools in identifying and characterizing potentially druggable binding sites in propofol-interacting proteins.

Propofol (2,6-diisopropylphenol) is an intravenous general anesthetic used in millions of surgical procedures worldwide.¹ In addition to the sedative effects it exerts on the central nervous system, propofol also elicits a “burning” sensation upon injection in many patients.² The γ -aminobutyric acid A (GABA_A) receptor is the principal target leading to the anesthetic properties of propofol,³ whereas Transient Receptor Potential Ankyrin 1 (TRPA1), a calcium-permeable ion channel expressed in peripheral sensory neurons, has been suggested to be the key mediator of propofol-induced pain.⁴ We recently used a photocrosslinkable propofol analog to demonstrate that this analog does indeed bind in the TRPA1 site identified by mutagenesis.⁵⁻⁷ Similar strategies have identified propofol-binding sites in the GABA_A receptor.^{8,9} These studies illustrate the power of photoaffinity labeling (PAL) to identify the binding-site location in complex biological systems, particularly where multiple binding sites may exist.^{5,6} In addition, PAL probes may elucidate new drug targets and off-target proteins, facilitating drug discovery efforts.^{10,11}

PAL finds widespread utility in drug discovery as a method to identify new macromolecular targets and as a complement to structure determination techniques like cryo-EM and X-ray crystallography to probe the location and structure of ligand binding sites. Although several photoaffinity analogs of propofol exist,^{8,9,12,13} there is a dearth of propofol probes that also contain a chemical handle to enable subsequent isolation of photolabeled protein, limiting the discovery of novel propofol-interacting proteins. Thus, it is important to expand the library of crosslinkable analogs to provide more tools to understand how propofol and similar molecules, like thymol, carvacrol and zingerone, which are plant phenols with multiple biological activities, interact with their physiological targets.

One of the ideal traits of a photoaffinity probe is structural similarity to the parent molecule. In addition, the choice of crosslinker is important; diazirines have favorable specific activities due to their activation at relatively long wavelengths, the small size of the photoreactive group, and the short half-life of the reactive carbene intermediate.¹⁴ The rapid quenching of carbenes by reaction with water molecules minimizes unspecific labeling, but also causes low yields of photolabeling. To facilitate the detection and isolation of photolabeled protein, clickable handles such as alkynes provide a convenient post-crosslinking method to incorporate an azide-containing biotin or fluorescent tag into the photoprobe.¹⁵ To the best of our knowledge, the only report of a bifunctional propofol analogue has only been demonstrated to retain biological activity for the GABA_A receptor,⁸ and has not been tested for other targets such as TRPA1 activation.

Although photoactive propofol derivatives do exist, several are unstable,¹³ or have cumbersome synthetic routes,¹² and only one has a click handle.⁸ We hypothesized that linking the propofol core with a short nonpolar aliphatic spacer to separate the diazirine moiety from the aryl ring would not only preserve the minimal pharmacophore but also facilitate synthetic expediency.^{9,13} We designed three probes that fulfill these criteria, and we synthesized them using relatively short and efficient synthetic sequences (Schemes 1-3), which could readily be applied to other similar scaffolds. Following synthesis, we used functional assays using calcium influx and binding to a fluorescent indicator as a measure of probe activation of TRPA1 to determine their promise in future crosslinking experiments.

Scheme 1. Synthesis of XPRO

Reagents and conditions: (a) Methyl vinyl ketone, Amberlyst-15, toluene, 50°C, N₂, 6 h, 80%; (b) TBSCl, imidazole, DMF, rt, N₂, 16 h, 79%; (c) NH₃, MeOH, NH₂OSO₃H, -40°C, N₂, 7 h; TEA, iodine, MeOH, 45%; (d) TBAF-acetic acid, THF, rt, N₂, 16 h, 76%.

Scheme 2. Synthesis of *o*-XPRO-Click

Reagents and conditions: (a) Methyl vinyl ketone, Amberlyst-15, toluene, 40°C, N₂, 8 h, 44%; (b) NBS, DCM, rt, N₂, 16 h, 54%; (c) PPh₃, PdCl₂(PPh₃)₂, CuI, ethynyltrimethylsilane, TEA, rt, N₂, 16 h, 59%; (d) TBSCl, imidazole, DMF, rt, N₂, 16 h, 82%; (e) NH₃, MeOH, NH₂OSO₃H, -40°C, N₂, 7 h; TEA, iodine, MeOH, 32%; (f) TBAF-acetic acid, THF, rt, N₂, 16 h, 53%.

Scheme 3. Synthesis of *p*-XPRO-Click

Reagents and conditions: (a) Hexamethylenetetramine, TFA, HCl, reflux, 9 h, 90%; (b) TBSCl, imidazole, DCM, rt, N₂, 16 h, 63%; (c) Ethynylmagnesium bromide, THF, -78°C, N₂, 16 h, 83%; (d) cat. Cp*RuCl(μ₂-SMe)₂RuCP*Cl, NHB₄BF₄, acetone, reflux, N₂, 5 h, 83%; (e) NH₃, MeOH, NH₂OSO₃H, -40°C, N₂, 7 h; TEA, iodine, MeOH, 52%; (f) TBAF-acetic acid, THF, rt, N₂, 16 h, 55%.

We designed our initial crosslinkable probe, XPRO (**5**), bearing the linker and diazirine moiety in the *para* position to minimize potential intramolecular interactions of the reactive carbene intermediate while maintaining overall hydrophobicity of the propofol starting point. Indeed, a propofol analog with a trifluoromethyl diazirine directly at the *para* position was reported to be a poor photolabel.^{9, 13} Synthesis of XPRO was achieved in an expedient 5-step sequence (Scheme 1). Initial functionalization of the *para*-position was achieved through an Amberlyst-15-mediated alkylation¹⁶ followed by protection of the phenol hydroxyl as a TBS silyl ether. The pendant methyl ketone was subsequently converted to the requisite diazirine via a two-step sequence.^{17, 18} Lastly, deprotection of the TBS ether with TBAF afforded XPRO. Overall yield for this sequence was 22%.

We next sought to generate an analog with a clickable group to enable potential labeling studies. We envisioned placing an alkyne at two possible locations within XPRO—in one analog one of the two isopropyl groups could be substituted with an alkyne (*o*-XPRO-Click) and in a second analog the alkyne could be appended proximal to the diazirine itself (*p*-XPRO-Click). At the outset, we chose to synthesize and profile both designs, since it was unclear which would have the best potency/reactivity profile.

To synthesize *o*-XPRO-Click (**12**; Scheme 2) starting from 2-isopropylphenol (**6**), Amberlyst-15 was again used to generate the alkyl ketone **7**. Subsequent bromination with NBS, Sonogashira coupling with TMS-acetylene, and phenol protection yielded the bis-silyl-protected intermediate **10**. Functionalization of the ketone using previously established conditions, followed by deprotection, generated *o*-XPRO-Click. In the case of *p*-XPRO-Click (**18**; Scheme 3), Duff formylation generated a benzaldehyde intermediate which, after phenol protection, was subjected to a Grignard reaction with ethynylmagnesium bromide. Subsequent propargylic alkylation¹⁹ followed by diazirination and deprotection yielded *p*-XPRO-Click. Moreover, *o*-XPRO-Click and *p*-XPRO-conjugation experiments with 3-Azido-7-hydroxycoumarin, whose fluorescence increases upon a successful click reaction with an alkyne,²⁰ demonstrate their ability to react with azide partners (Supplementary figure 1).

As a first assessment of the biological activity of our three new photocrosslinkable propofol analogs, we tested their ability to activate TRPA1, leveraging our previously developed cell-based calcium-influx assay.⁶ For TRPA1 production, we chose baculovirus-infected insect cells, a common heterologous expression system for eukaryotic integral membrane proteins.²¹ Cells were loaded with Fura-2, a calcium-sensitive ratiometric fluorescent dye. Ligand binding and activation causes ion channels like TRPA1 to open, allowing calcium to flow into cells and bind Fura-2, resulting in an absorption shift.²²

After measuring background fluorescence levels, addition of 60 μ M XPRO or propofol at 60 s elicited a rise in $[Ca^{2+}]_i$ in TRPA1-expressing cells, resulting in an increase in the 340/380 ratio of the Fura-2 dye (Figure 1A). Expression of TRPA1 in all samples was confirmed by activation at 180 s with 1 mM AITC, an electrophilic TRPA1 agonist.^{23, 24} Interestingly, *o*-XPRO-Click was a weaker TRPA1 agonist than propofol (Figure 1B). We hypothesize that the potency difference was due to replacement of a key structural feature of propofol, one of the 2-isopropyl group, with a smaller and non-branched alkyne. Consistent with this hypothesis, *p*-XPRO-Click, which preserves both 2-isopropyl substituents, was at least as efficient as propofol (Figure 1C).

To rule out nonspecific calcium influx due to increased hydrophobicity of XPRO relative to propofol, we also tested activation of TRPV1, a related channel found on TRPA1-expressing sensory nerves and crucial for pain sensation.²⁵ As expected,²⁶ neither propofol nor XPRO activated TRPV1 while capsaicin, which serves as a positive control for functional TRPV1 expression, was a potent TRPV1 agonist (Figure 1D). In addition, neither *o*-XPRO-Click nor *p*-XPRO-Click elicited calcium influx in TRPV1-expressing cells (Figures 1E and 1F). Taken together, calcium-influx assays demonstrate that XPRO, *o*-XPRO-Click, and *p*-XPRO-Click share similar TRPA1 potency with propofol.

Figure 1. XPRO, *o*-XRO-Click, and *p*-XPRO-Click and propofol elicit calcium influx in Sf21 insect cells heterologously expressing TRPA1 but not TRPV1. (A, B, C) Time traces showing the net change in Fura-2 340/380 fluorescence ratio (Δ Ratio) in TRPA1-expressing insect cells. Adding XPRO (A), *o*-XPRO-Click (B) or *p*-XPRO-Click (C) at 60 s caused a rise in $[Ca^{2+}]_i$. Traces showing activation of the equivalent batches of cells with 60 μ M propofol (grey traces) are included as positive controls in each panel. Addition of 1 mM allyl isothiocyanate (AITC) at 180 s provided a positive control for mouse TRPA1 expression. Data represent mean $\pm$ SEM (n=4-14). (D, E, F) Addition of 60 μ M XPRO (D), *o*-XPRO-Click (E) or *p*-XPRO-Click (F) or propofol (grey traces) at 60 s did not cause a rise in $[Ca^{2+}]_i$ in rat TRPV1-expressing insect cells. Addition of 75 nM capsaicin, a TRPV1-specific agonist, at 180 s did elicit a rise in $[Ca^{2+}]_i$, confirming TRPV1 expression in all samples. Data represent mean $\pm$ SEM for n=3-12.

In summary, the structure-function studies highlight promising photoactivatable propofol analogs to move forward in crosslinking experiments. The synthetic strategies described here are also applicable to alkyl phenols like propofol, such as carvacrol and thymol, and molecules that contain an analogous linker in the *para* position, such as zingerone, all of which include TRPA1 modulation among their biological activities.²⁷ Thus, the combination of the diazirine and alkyne functionality enables not only detection and enrichment of photolabeled biological targets but also interrogation of the propofol-interacting proteome and generation of novel ion channel modulators through click chemistry reactions.

Acknowledgments

We thank members of the Gaudet and Kahne lab for insightful discussions and technical assistance. Financial support for this work was provided by the American Heart Association (16GRNT27250119). K.A.S. was supported by an NSF Graduate Research Fellowship (DGE-1144152) and a Ford Foundation Fellowship.

Declaration of Interest

None.

Supplementary Data

¹H and ¹³C NMR spectra with synthesis methods.

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Supplementary Information

Efficient and flexible synthesis of new photoactivatable propofol analogs

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S2. UV and fluorescence characterization of diazirine and click groups

S3-10. General synthetic procedures

S11-42. ¹H and ¹³C NMR spectra

XPRO absorbance spectrum

UV spectrum data was acquired at a 10-nm wavelength step on a Flexstation 3 Plate Reader (Molecular Devices; Sunnyvale, CA).

Click reaction with *o*-XPRO-Click and 3-Azido-7-hydroxycoumarin

0.5 mM *o*-XPRO-Click was mixed with 1 mM 3-Azido-7-hydroxycoumarin in 100 mM phosphate buffer, pH 7.5 and 150 mM NaCl. A premixed solution of 0.1 mM CuSO₄ and 0.5 mM Tris(benzyltriazolylmethyl)amine (TBTA) was added, followed by addition of 5 mM sodium ascorbate. The reaction proceeded at 37°C for 1 hour and the sample was excited at 404 nm while emission was measured with a SpectraMax i3 plate reader (Sunnyvale, CA).

Click reaction with *p*-XPRO-Click and 3-Azido-7-hydroxycoumarin

0.5 mM *p*-XPRO-Click was mixed with 0-3 mM 3-Azido-7-hydroxycoumarin in phosphate-buffered saline. A premixed solution of 0.1 mM CuSO₄ and 0.5 mM TBTA was added, followed by addition of 5 mM sodium ascorbate. The reaction proceeded at 37°C for 1 hour and the sample was excited at 404 nm and emission at 480 nm was measured with increasing concentration of 3-Azido-7-hydroxycoumarin using a SpectraMax i3 plate reader (Sunnyvale, CA).

Supplementary figure 1. Characterization of diazirine and click handle of synthesized probes. (A) UV absorption spectrum of XPRO (0.2 mg mL⁻¹) in methanol showing absorbance of aromatic region and an absorption band between 320-360 nm due to the diazirine group (B) *o*-XPRO-Click conjugates with coumarin azide whose fluorescence increases upon successful click reaction (404 nm excitation). Solid cyan line indicates *o*-XPRO-Click incubated with coumarin azide under copper-catalyzed conditions. Dashed line indicates *o*-XPRO-Click incubated without coumarin azide under same conditions. Background 3-Azido-7-hydroxycoumarin was subtracted from all values (C) 0.5 mM *p*-XPRO-Click (0.5 mM) reacts with coumarin azide resulting in a fluorescence increase upon click reaction.

General synthetic procedures

Hexamethylenetetramine was purchased from TCI America (Portland, OR). Ruthenium Dichlorobis(μ -methanethioato)bis(pentamethylcyclopentadienyl)diruthenium(III) was purchased from Strem Pharmaceuticals (Newburyport, MA). All other chemicals were purchased from Sigma-Aldrich. Chemicals were used as received from commercial vendors unless otherwise noted. Crude products were chromatographed on a Teledyne Isco CombiFlash system using the indicated solvents (Lincoln, NE). ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on an Inova 500 MHz nuclear spectrometer in the Harvard Chemistry & Chemical Biology (CCB) facility. Mass spectra of synthesized compounds were acquired on an Agilent 6220 LC-TOF (Santa Clara, CA) in the Harvard Bauer Core.

Preparation of XPRO (5)

4-(4-Hydroxy-3,5-diisopropylphenyl)butan-2-one (2)

A similar procedure was adapted with minor modifications from Bunce *et al.*¹⁶ To a 5-mL toluene solution of 2,6-diisopropylphenol (2.83 mL, 15.3 mmol) **1** in a round-bottomed flask under an atmosphere of nitrogen was added 326 mg of Amberlyst-15. The slurry was stirred at room temperature while a 1-mL toluene solution of methyl vinyl ketone (0.415 mL, 5.1 mmol) was added dropwise. The vessel was heated to 50°C and allowed to proceed for 6 hours, upon which the reaction was partitioned between water and ether. The ether solution was washed with water, brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified using a Teledyne Isco CombiFlash system and the product eluted at 100% DCM in hexanes. The appropriate fractions were combined and concentrated to yield 1g (80%) of the title compound **2**.

^1H NMR (500 MHz, CDCl_3): δ 6.88 (s, 2H, H_{arom}), 4.69 (s, 1H, ArOH), 3.15 (sept, 2H, $J=6.9$ Hz, CH-i-Pr), 2.88-2.79 (m, 2H, CH_2), 2.79-2.71 (m, 2H, CH_2), 2.16 (s, 3H, $\text{H}_3\text{C}-\text{C}=\text{O}$), 1.27 (d, 12H, $J=6.9$ Hz, $\text{H}_3\text{C}-\text{i-Pr}$).

^{13}C NMR (125 MHz, CDCl_3): δ 208.7 ($\text{C}=\text{O}$), 148.3, 133.8, 132.7, 123.2 (CH_{arom}), 45.8, 30.1, 29.7, 27.2, 22.8.

HRMS calc'd for $\text{C}_{16}\text{H}_{24}\text{O}_2$: $[\text{M} + \text{H}]$ 249.1855. Found: 249.1842.

4-[4-(*tert*-Butyldimethylsilyloxy)-3,5-diisopropylphenyl]butan-2-one (3)

Dimethylformamide (DMF; 1.7 mL) was added to 385 mg of **2** (1.55 mmol, 1.0 eq) with stirring at room temperature in a round bottomed flask, followed by addition of 369 mg of imidazole (5.425 mmol, 3.5 eq). Next, 701 mg of *tert*-butyldimethylsilyl chloride (TBSCl; 4.65 mmol, 3.0 eq) was added under an atmosphere of nitrogen. After stirring at room temperature overnight, the reaction was partitioned between hexanes and water. The organic layer was washed with water and saturated NaHCO_3 , dried over Na_2SO_4 , and concentrated *in vacuo*. Following chromatography using a Teledyne Isco CombiFlash

system (15% ethyl acetate in hexanes as eluent), the appropriate fractions were combined and concentrated to yield 446 mg (79%) of **3** as an orange oil.

¹H NMR (500 MHz, CDCl₃): δ 6.85 (s, 2H, H_{arom}), 3.28 (sept, 2H, *J*=6.8 Hz, CH-*i*-Pr), 2.87-2.79 (m, 2H, CH₂), 2.79-2.71 (m, 2H, CH₂), 2.16 (s, 3H, H₃C-C=O), 1.16 (d, 12H, *J*=6.8 Hz, H₃C-*i*-Pr), 1.03 (s, 9H, *tert*-butyl), 0.18 (s, 6H, Si-CH₃).

¹³C NMR (125 MHz, CDCl₃): δ 208.7 (C=O), 147.4, 139.0, 133.7, 123.1 (CH_{arom}), 45.7, 30.1, 29.8, 26.5, 26.1, 23.4, 18.9, -3.3 (H₃C-Si).

HRMS calc'd for C₂₂H₃₈O₂Si: [M + H] 363.2719. Found: 363.2719.

3-[4-(*tert*-Butyldimethylsilyl)oxy]-3,5-diisopropylphenyl]-3-methyl-3*H*-diazirine (4)

Under an atmosphere of nitrogen, 446 mg of **3** (1.23 mmol, 1.0 eq) was dissolved in a mixture of methanol (MeOH)-ether (1:1, 4.5 mL) and cooled to -40°C. A solution of 2.0 M NH₃ in MeOH (31 mL) was added dropwise with stirring. The reaction was allowed to proceed for 6 hours at -40°C. A solution of hydroxylamine-*O*-sulfonic acid (NH₂OSO₃H; 208 mg, 1.845 mmol, 1.5 eq) in 3.6 mL of methanol was added dropwise. After 1 hour, the flask was kept at -20°C for 3 days with occasional stirring. After completion, the mixture was filtered, dried with Na₂SO₄, concentrated *in vacuo*. The crude material was dissolved in a mixture of methanol (18 mL) and 0.515 mL of triethylamine (TEA; 3.69 mmol, 3.0 eq), and cooled to -10°C. A solution of iodine (I₂; 312 mg, 1.23 mmol, 1.0 eq) in DCM (9 mL) was added dropwise while stirring. Once the solution remained persistently brown in color, the excess iodine was quenched with a 5% solution of Na₂S₂O₃ (4.5 mL), which turned the reaction orange. The mixture was diluted with water and extracted with ether. The combined organic phases were washed with water and dried over Na₂SO₄. Concentration and chromatography using a Teledyne Isco CombiFlash system (3% ethyl acetate in hexanes as eluent) yielded 205 mg (45%) of diazirine **4** as an orange oil.

¹H NMR (500 MHz, CDCl₃): δ 6.83 (s, 2H, H_{arom}), 3.28 (sept, 2H, *J*=6.9 Hz, HC-*i*-Pr), 2.43 (m, 2H, CH₂), 1.65 (m, 2H, CH₂), 1.16 (d, 12H, *J*=6.9 Hz, H₃C-*i*-Pr), 1.03 (s, 9H, *tert*-butyl), 1.00 (s, 3H, α-*azi*-CH₃) 0.18 (s, 6H, Si-CH₃).

¹³C NMR (125 MHz, CDCl₃): δ 147.3, 139.0, 133.6, 123.1 (CH_{arom}), 36.7, 29.8, 26.5, 26.1, 25.7, 23.4, 19.9, 18.8, -3.3 (H₃C-Si).

HRMS calc'd for C₂₂H₃₉N₂O₂Si: [M + H] 375.2832. Found: 375.2841.

2,6-Diisopropyl-4-[2-(3-methyl-3*H*-diazirin-3-yl)ethyl]phenol (5)

Under an atmosphere of nitrogen, 32 mg of **4** (0.0855 mmol, 1.0 eq) was deprotected with dropwise addition of tetra-*n*-butylammonium fluoride (TBAF)-acetic acid (1:2 molar ratio, 1 M TBAF in tetrahydrofuran + neat acetic acid, 0.43 mL) on ice and the reaction was

allowed to warm to room temperature overnight to obtain crude product. The mixture was diluted with water and the aqueous layer was extracted with ethyl acetate. The combined organic phases were purified using a Teledyne Isco CombiFlash system (5% ethyl acetate in hexanes as eluent) yielding 17 mg (76%) of **5** as an orange oil after concentration.

¹H NMR (500 MHz, CDCl₃): δ 6.84 (s, 2H, H_{arom}), 4.66 (s, 1H, ArOH), 3.14 (sept, 2H, *J*=6.9 Hz, HC-*i*-Pr), 2.43 (m, 2H, CH₂), 1.65 (m, 2H, CH₂), 1.27 (d, 12H, *J*=6.9 Hz, H₃C-*i*-Pr), 1.03 (s, 3H, α-*azi*-CH₃).

¹³C NMR (125 MHz, CDCl₃): δ 148.2, 133.7, 132.6, 123.2 (CH_{arom}), 36.9, 29.9, 27.2, 25.9, 22.8, 20.0.

HRMS: *m/z* calc'd for C₁₆H₂₄N₂O: [M + H] 261.1967. Found: 261.1964.

Preparation of o-XPRO-Click (12)

4-(4-hydroxy-3-isopropylphenyl)butan-2-one (7)

Under an atmosphere of nitrogen, 3 mL of 2-isopropylphenol (22.3 mmol, 3.0 eq) **6** and 3 mL of toluene were stirred in an ice-cold round-bottomed flask. Then, Amberlyst-15 (475 mg) was added. Methyl vinyl ketone (0.45 mL, 7.43 mmol, 1.0 eq) in 2 mL of toluene was added dropwise. The reaction was warmed to 40°C for 8 hours, then partitioned between ether and water. The ether solution was washed with water, brine, dried over Na₂SO₄ and concentrated under vacuum. The crude material was purified using a Teledyne Isco CombiFlash system (30% ethyl acetate in hexanes as eluent) yielding 2 g (44%) of **7** a pale-yellow oil.

¹H NMR (500 MHz, CDCl₃): δ 7.01 (s, 1H), 6.88 (dd, *J* = 8.1, 2.3 Hz, 1H), 6.68 (d, *J* = 8.0 Hz, 1H), 4.77 (bs, 1H), 3.20 (sept, *J* = 7.0 Hz, 1H), 2.94- 2.80 (m, 2H), 2.80 – 2.68 (m, 2H), 2.16 (s, 3H), 1.26 (d, *J* = 6.9 Hz, 6H)

¹³C NMR: (125 MHz, CDCl₃): δ 208.8, 151.2, 134.5, 133.1, 126.3, 126.2, 115.3, 110, 45.7, 30.1, 29.3, 27, 22.6

HRMS calc'd for C₁₃H₂₀O₂: [M + Na] 229.1204. Found: 229.1222

4-(3-bromo-4-hydroxy-5-isopropylphenyl)butan-2-one (8)

Under an atmosphere of nitrogen, 393 mg of **7** (1.905 mmol, 1.0 eq) was stirred in DCM (12 mL). 373 mg of N-bromosuccinimide (NBS; 2.1 mmol, 1.1 eq) was dissolved in 1.4 mL DMF and added dropwise to the mixture. The reaction proceeded overnight at room temperature. The mixture was then diluted with DCM, washed with water, dried over Na₂SO₄, and concentrated *in vacuo*. The crude material was purified using a Teledyne

Isco CombiFlash system (15% ethyl acetate in hexanes as eluent) yielded 300 mg (54%) of **8** as a pale-yellow oil.

¹H NMR (500 MHz, CDCl₃): δ 7.14 (s, 1H), 6.96 (dd, *J* = 8.1, 2.3 Hz, 1H), 6.68 (d, *J* = 8.0 Hz, 1H), 4.77 (bs, 1H), 3.20 (sept, *J* = 7.0 Hz, 1H), 2.94- 2.80 (m, 2H), 2.80 – 2.68 (m, 2H), 2.16 (s, 3H), 1.26 (d, *J* = 6.9 Hz, 6H)

¹³C NMR: (125 MHz, CDCl₃): δ 208.8, 151.2, 134.5, 133.1, 126.3, 126.2, 115.3, 110, 45.7, 30.1, 29.3, 27, 22.6

HRMS calc'd for C₁₃H₁₇BrO₂: [M + H] 285.0485. Found: 285.0471

4-(4-hydroxy-3-isopropyl-5-((trimethylsilyl)ethynyl)phenyl)butan-2-one (**9**)

To a dry flask was added 1g of **8** (3.5 mmol, 1.0 eq), 92 mg of triphenylphosphine (PPh₃; 0.35 mmol, 0.1 eq), 153 mg of bis(triphenylphosphine)palladium(II)dichloride (PdCl₂(PPh₃)₂; 0.22 mmol, 0.06 eq), 239 mg of copper(I) iodide (CuI; 1.26 mmol, 0.36 eq) and 1 mL of ethynyltrimethylsilane (10.5 mmol, 3.0 eq), and triethylamine (23 mL) sequentially. This flask was flushed with argon for 15 minutes. The reaction proceeded overnight at room temperature. The mixture was then diluted with water and DCM. The layers were separated, and the aqueous phase was extracted with DCM. The combined organic phases were dried over Na₂SO₄ and concentrated *in vacuo*. The crude material was purified using a Teledyne Isco CombiFlash system (15% ethyl acetate in hexanes as eluent) yielding 630 mg (59%) of **9** as a light brown oil.

¹H NMR (500 MHz, CDCl₃): δ 7.02 (s, 1H), 6.99 (s, 1H), 5.87 (s, 1H), 3.25 (sept, *J* = 6.9 Hz, 1H), 2.79 (ddd *J* = 8.5, 6.8, 1.7 Hz, 2H), 2.72 (ddd, *J* = 8.5, 7.0, 1.7 Hz, 2H), 2.15 (s, 3H), 1.23 (d, *J* = 6.8 Hz, 6H)

¹³C NMR: (125 MHz, CDCl₃): δ 208, 152.8, 134.2, 132.4, 127.8, 109, 101.9, 99.6, 45.4, 30.1, 29, 27.5, 22.4, 22.3

HRMS calc'd for C₁₈H₂₆O₂Si: [M + H] 303.1775. Found 303.1656

4-(4-((tert-butyl)dimethylsilyl)oxy)-3-isopropyl-5-((trimethylsilyl)ethynyl)butan-2-one (**10**)

Under an atmosphere of nitrogen, 0.75 mL of DMF was added to 100 mg of **9** (0.331 mmol, 1.0 eq) in a round-bottomed flask with stirring. Then 85 mg of imidazole (1.34 mmol, 3.75 eq) and 137 mg of TBSCl (0.91 mmol, 2.75 eq) were added. After proceeding overnight at room temperature, the reaction was partitioned between hexanes and water. The aqueous layer was extracted with hexanes. The combined organic layers were washed with water and saturated NaHCO₃, dried over Na₂SO₄, and concentrated *in vacuo*. Following purification using a Teledyne Isco CombiFlash system (20% ethyl

acetate in hexanes as eluent), the appropriate fractions were combined and concentrated to yield 113 mg (82%) of **10** as a light brown oil.

¹H NMR (500 MHz, CDCl₃): δ 7.08 (s, 1H), 6.98 (s, 1H), 3.28 (sept, J = 11.4, 8.2, 5.4 Hz, 1H), 2.88 – 2.76 (m, 2H), 2.73 (ddd, J = 9.0, 5.1, 1.9 Hz, 2H), 2.15 (s, 1H), 1.14 (dd, J = 7.1, 2.6 Hz, 6H), 1.05 (s, 9H), 0.24 (d, J = 3.6 Hz, 6H)

¹³C NMR (125 MHz, CDCl₃): δ 208, 151.5, 140, 133.7, 131.5, 127.1, 115.1, 101.1, 97.4, 45.3, 30.1, 29, 26.3, 26.1, 23.1, 18.7, 0, -3

HRMS calc'd for C₂₄H₄₀O₂Si₂: [M + H] 417.2640. Found: 417.2529.

4-(4-((tert-butyldimethylsilyl)oxy)-3-isopropyl-5-((trimethylsilyl)rhynyl)phenethyl)-3-methyl-3H-diazirine (11)

Under an atmosphere of nitrogen, 61 mg of **10** (0.146 mmol, 1.0 eq) was dissolved in a mixture of methanol-ether (1:1, 0.6 mL) and cooled to -40°C. A solution of 2.0 M NH₃ in MeOH (8 mL) was added dropwise with stirring over 6 hours. A solution of hydroxylamine-O-sulfonic acid (25 mg, 0.220 mmol, 1.5 eq) in 0.5 mL of methanol was added dropwise with stirring. After 1 hour, the flask was kept at -20°C for 2 days with occasional stirring. The mixture was filtered, dried with Na₂SO₄, concentrated *in vacuo*. Crude material was dissolved in a mixture of methanol (2.4 mL) and 0.062 mL of triethylamine (0.442 mmol, 3.01 eq.), and cooled to -10°C. After stirring for 15 minutes, a solution of iodine (37 mg, 0.146 mmol, 1.0 eq) in DCM (1.2 mL) was added dropwise while stirring. Once the solution remained persistently brown in color, the excess iodine was quenched with a 5% solution of Na₂S₂O₃ (0.7 mL), which turned the reaction yellow. The mixture was diluted with water and the aqueous layer was extracted with ether. The combined organic phases were washed with water and dried over Na₂SO₄. Concentration and purification using a Teledyne Isco CombiFlash system (20% ethyl acetate in hexanes as eluent) yielded 20 mg (32%) of diazirine **11** as a yellow oil.

¹H NMR (500 MHz, CDCl₃): δ 7.06 (s, 1H), 6.97 (s, 1H), 3.29 (sept, J = 6.9 Hz, 1H), 2.53 – 2.26 (m, 2H), 1.80 – 1.59 (m, 2H), 1.15 (d, J = 6.9 Hz, 1.15 (d, J = 6.9 Hz, 6H), 1.05 (s, 9H), 1.01 (s, 3H), 0.41-0.20 (m, 6H), 0.10 (s, 9H)

¹³C NMR (125 MHz, CDCl₃): 151.5, 139.9, 133.5, 131.5, 127.1, 127, 115.1, 104.1, 97.4, 36.5, 29.3, 26.3, 26.1, 25.7, 23.1, 20, 18.7, 0.01, -2.9

HRMS calc'd for C₂₄H₄₀N₂OSi₂: [M + H] 429.2752. Found: 429.2750

2-ethynyl-6-isopropyl-4-(2-(3-methyl-3H-diazirin-3-yl)ethyl)phenol (12)

Under an atmosphere of nitrogen, 20 mg of **11** (0.046 mmol, 1.0 eq) was deprotected with dropwise addition of TBAF-acetic acid (0.014 mL of 1.0 M in THF + 0.040 mL neat THF)

on ice. The reaction was allowed to warm to room temperature overnight to obtain crude product. Purification using a Teledyne Isco CombiFlash system (10% ethyl acetate in hexanes as eluent) yielded 12 mg (53%) of **12** as a yellow oil after concentration.

¹H NMR (500 MHz, CDCl₃): δ 7.03 (s, 1H), 7.00 (s, 1H), 5.79 (s, 1H), 3.46 (s, 1H), 3.28 (sept, J=6.9 Hz, 1H), 2.39 (m, 2H), 1.63 (m, 2H), 1.24 (d, J = 6.9 Hz, 6H), 1.02 (s, 3H)

¹³C NMR: (125 MHz, CDCl₃): 153, 135, 132, 129, 128, 108, 84, 79, 37, 29, 27, 26, 22, 20

HRMS calc'd for C₁₅H₁₈N₂O: [M + H] 243.1497. Found: 243.1502

Preparation of p-XPRO-Click (18)

4-hydroxy-3,5-diisopropylbenzaldehyde (13)

13 was synthesized as described in López *et al.*²⁸ A solution of 2,6-diisopropylphenol (2.0 g, 11.2 mmol, 1.0 eq), 3.15 g of hexamethylenetetramine (22.4 mmol, 2.0 eq) and 11 mL of trifluoroacetic acid (F₃CCO₂H) were mixed in a round bottomed flask and heated under reflux (90°C) overnight. The reaction mixture was cooled to room temperature and neutralized with NaHCO₃. The aqueous layer was extracted with ethyl acetate and the combined organic layers were concentrated. 3M HCl was added to the crude product and the mixture was stirred under reflux (80°C) for 3 hours. The precipitate was filtered, washed with water, and purified using a Teledyne Isco CombiFlash system (20% ethyl acetate in hexanes as eluent) to yield 2 g of **13** as a white solid (90%).

¹H NMR (500 MHz, CDCl₃): δ 9.88 (s, 1H), 7.64 (s, 2H), 5.41 (s, 1H), 3.19 (sept, J = 6.9 Hz, 2H), 1.33 (d, 12H)

¹³C NMR: (125 MHz, CDCl₃): δ 192.9, 156.9, 134.8, 129.1, 126.7, 27, 22.5

HRMS calc'd for C₁₃H₁₈O₂: [M + Na] 229.1204. Found: 229.1222.

4-(tert-butyldimethylsilyl)oxy-3,5-diisopropylbenzaldehyde (14)

Under an atmosphere of nitrogen, 204 mg of **13** (0.989 mmol, 1.0 eq) was dissolved in DCM (10 mL) and then 0.69 mL of triethylamine (4.944 mmol, 5.0 eq) and 179 mg of TBSCl (1.1867 mmol, 1.2 eq) were added. The mixture was stirred at room temperature overnight. The mixture was partitioned between hexanes and ethyl acetate, dried over Na₂SO₄, and purified using a Teledyne Isco CombiFlash system (100% hexanes as eluent) to yield 200 mg of **14** as a yellow oil (63%).

¹H NMR (500 MHz, CDCl₃): δ 9.95 (s, 1H), 7.63 (s, 2H), 3.33 (sept, J = 6.9 Hz, 2H), 1.22 (d, 12H), 1.04 (d, J = 2.4 Hz, 9H), 0.25 (s, 6H)

^{13}C NMR: (125 MHz, CDCl_3): δ 191.8, 155.4, 140.2, 130.8, 125.9, 26.8, 26, 23.1, 19, -3.2

HRMS calc'd for $\text{C}_{19}\text{H}_{32}\text{O}_2\text{Si}$ [$\text{M} + \text{H}$]: 321.2250. Found: 321.2280

1-4-((*tert*-butyldimethylsilyl)oxy)-3,5-diisopropylphenyl)prop-2-yn-1-ol (15)

Under an atmosphere of nitrogen, 680 mg of **14** (2.12 mmol, 1.0 eq) was dissolved in 2 mL of anhydrous THF and cooled to -78°C via a dry ice-acetone bath. To this solution, 7 mL of 0.5M ethynylmagnesium bromide (HCCMgBr) solution in THF was added dropwise. The reaction mixture was allowed to slowly warm to room temperature. After stirring for 3 hours at room temperature, the reaction was quenched with saturated ammonium chloride (NH_4Cl), and the mixture was partitioned between DCM and water, and the two layers were separated. The organic layer was washed with water, dried over Na_2SO_4 and concentrated *in vacuo*. Crude material was purified using a Teledyne Isco CombiFlash system (15% ethyl acetate in hexanes as eluent) to give 570 mg (83%) of **15** as a brownish oil.

^1H NMR (500 MHz, CDCl_3): δ 7.28 (s, 2H), δ 5.58 – 5.33 (m, 1H), 3.32 (sept, $J = 6.9$ Hz, 2H), 2.76-2.63 (m, 2H), 1.19 (d, 12H), 1.05 (s, 9H), 0.21 (s, 6H)

^{13}C NMR: (125 MHz, CDCl_3): 149.6, 139.4, 133, 122.1, 83.9, 74.5, 64.8, 26.8, 26.1, 23.3, 18.9, -3.29

HRMS calc'd for $\text{C}_{21}\text{H}_{34}\text{O}_2\text{Si}$ [$\text{M} + \text{Na}$]: 369.2226. Found 369.2229.

4-(4-((*tert*-butyldimethylsilyl)oxy)-3,5-diisopropylphenyl)hex-5-yn-2-one (16)

16 was synthesized according to Nishibayashi *et al.*¹⁹ Under an atmosphere of nitrogen, 7 mg of ammonium tetrafluoroborate (NH_4BF_4 ; 0.066 mmol, 0.1 eq) and 21 mg of $[\text{Cp}^*\text{RuCl}(\mu_2\text{-SMe})_2\text{RuCp}^*\text{Cl}]$ (0.033 mmol, 0.05 eq) were placed in a round-bottomed flask. Anhydrous acetone (40 mL) was added, and then the mixture was stirred at room temperature. After addition of 230 mg of **15** (0.66 mmol, 1.0 eq), the reaction flask was kept at reflux temperature for 5 hours. The reaction mixture was treated with brine (20 mL) and extracted with diethyl ether (20 mL x 3). The ether layer was dried over anhydrous Na_2SO_4 . The extract was concentrated under reduced pressure and purified using a Teledyne Isco CombiFlash system (10% ethyl acetate in hexanes as eluent) to give **16** as a light brown oil (83% yield).

^1H NMR (500 MHz, CDCl_3): δ 7.04 (s, 2H), δ 4.13 (ddd, $J = 9.2, 5.5, 2.4$ Hz, 1H), 3.29 (hept, $J = 6.9$ Hz, 2H), 2.98 (dd, $J = 16.1, 9.1$ Hz, 1H), 2.78 (dd, $J = 16.2, 5.5$ Hz, 1H), 2.28 (s, 1H), 2.16 (s, 3H), 1.17 (d, 12H), 1.03 (s, 9H), 0.19 (s, 6H)

^{13}C NMR: (125 MHz, CDCl_3): 206.2, 148.2, 139.3, 132.9, 130.9, 128.8, 122.2, 85.5, 70.9, 52.1, 38.7, 32.7, 30.7, 29, 26.7, 26.1, 23.4, 23, 18.9, -3.3

HRMS calc'd for C₂₄H₃₈O₂Si [M + H]: 387.2719. Found 387.2787.

3-(2-(4-((*tert*-butyldimethylsilyl)oxy)-3,5-diisopropylphenyl)but-3-yn-1-yl)-3-methyl-3H-diazirine (17)

Under an atmosphere of nitrogen, 160 mg of **16** (0.425 mmol, 1.0 eq) was dissolved in methanol (1.5 mL) and cooled to -40°C with a dry ice/acetonitrile bath. A solution of 2.0 M ammonia in methanol (11 mL) was added dropwise with stirring. The reaction mixture was warmed up to -30°C and stirred at this temperature for 6 hours. A solution of hydroxylamine-O-sulfonic acid (74 mg, 0.64 mmol) in 1.2 mL of methanol was added dropwise and the solution was stirred at -20°C for 2 days. After completion, the reaction mixture was evaporated under reduced pressure and dissolved in a mixture of methanol (6.4 mL) and 0.192 mL of triethylamine (1.28 mmol, 3.0 eq) and cooled to -10°C. A saturated solution of iodine (109 mg, 0.425 mmol, 1.0 eq) in dichloromethane (3.2 mL) was added dropwise until the mixture remained light-brown in color. The excess iodine was quenched with 5% solution of Na₂S₂O₃ (1.5 mL). The mixture was partitioned between water and ether. The organic phase was collected and washed with water and brine (20 mL each) and dried over Na₂SO₄. Concentration *in vacuo* and purification using a Teledyne Isco CombiFlash system (1% ethyl acetate in hexanes as eluent) afforded **17** as a light brown oil (80 mg, 52%).

¹H NMR (500 MHz, CDCl₃): δ 7.05 (s, 2H), δ 3.50 (ddd, *J* = 8.9, 6.5, 2.5 Hz, 1H), 3.29 (hept, *J* = 13.8, 6.9 Hz, 2H), 1.17 (d, 12H), 1.04 (s, 9H), 0.97 (s, 3H), 0.19 (s, 6H)

¹³C NMR: (125 MHz, CDCl₃): 148.2, 139.3, 132.9, 130.9, 122.3, 71.3.5, 44.3, 32.5, 26.7, 26.1, 23.4, 20.3, 18.9, -3.3

HRMS calc'd for C₂₄H₃₈N₂O₂Si [M + H]: 399.2832. Found: 399.2830

2,6-diisopropyl-4-(1-(3-methyl-3H-diazirin-3-yl)but-3-yn-2-yl)phenol (18)

To a round bottomed flask with 30 mg of **17** (0.075 mmol, 1.0 eq) was added 0.113 mL of TBAF (1.0 M in THF) and 0.013 mL of acetic acid under an atmosphere of nitrogen. The reaction proceeded overnight and was purified using a Teledyne Isco CombiFlash system (5% ethyl acetate in hexanes as eluent) to give **18** in 55% yield.

¹H NMR (500 MHz, CDCl₃): δ 7.04 (s, 2H), δ 4.73 (s, 1H), 3.50 (ddd, *J* = 9.0, 6.5, 2.5 Hz, 1H), 3.15 (hept, *J* = 6.9 Hz, 2H), 1.89-1.68 (m, 2H), 1.28 (d, *J* = 6.8 Hz, 12H), 1.03 (s, 3H)

¹³C NMR: (125 MHz, CDCl₃): 149.0, 133.9, 132.0, 122.4, 85.5, 71.4, 44.2, 32.5, 27.3, 24.8, 22.7, 20.4

HRMS calc'd for C₁₈H₂₄N₂O [M + H]: 285.1967. Found: 285.1981

^1H and ^{13}C NMR spectra

^1H NMR spectrum of 4-(4-Hydroxy-3,5-diisopropylphenyl)butan-2-one (**2**)

¹³C NMR spectrum of 4-(4-Hydroxy-3,5-diisopropylphenyl)butan-2-one (**2**)

KS_Progofol_Ketone_CNMR
KJS-018070 fractions 33-50

^1H NMR spectrum of **4-[4-(*tert*-Butyldimethylsilyloxy)-3,5-diisopropylphenyl]butan-2-one (3)**

¹³C NMR spectrum of **4-[4-(*tert*-Butyldimethylsilyloxy)-3,5-diisopropylphenyl]butan-2-one (3)**

KS_TBS_Ketone_CNMR
PJ05-30 fr. 32-40

¹H NMR spectrum of 3-[4-(*tert*-Butyldimethylsilyl)oxy]-3,5-diisopropylphenyl]-3-methyl-3*H*-diazirine (4)

^{13}C NMR spectrum of **3-[4-(*tert*-Butyldimethylsilyl)oxy]-3,5-diisopropylphenyl]-3-methyl-3*H*-diazirine (4)**

¹H NMR spectrum of **2,6-Diisopropyl-4-[2-(3-methyl-3H-diazirin-3-yl)ethyl]phenol (5)**

¹³C NMR spectrum of **2,6-Diisopropyl-4-[2-(3-methyl-3H-diazirin-3-yl)ethyl]phenol (5)**

KS_XPRO_CNMR

¹H NMR spectrum of **4-(4-hydroxy-3-isopropylphenyl)butan-2-one (7)**

¹³C NMR spectrum of **4-(4-hydroxy-3-isopropylphenyl)butan-2-one (7)**

Aug2115Phenol.MVKAddtn_CNMR
EMW.533A.IV-191.1500C

¹H NMR spectrum of **4-(3-bromo-4-hydroxy-5-isopropylphenyl)butan-2-one (8)**

¹³C NMR spectrum of **4-(3-bromo-4-hydroxy-5-isopropylphenyl) butan-2-one (8)**

¹H NMR spectrum of **4-(4-hydroxy-3-isopropyl-5-((trimethylsilyl)ethynyl)phenyl)butan-2-one (9)**

¹³C NMR spectrum of **4-(4-hydroxy-3-isopropyl-5-((trimethylsilyl)ethynyl)phenyl)butan-2-one (9)**

KenCNMRSONOsuccessOct215
 zzy-151002-fluorone-13C
 500C

^1H NMR spectrum of 4-(4-((tert-butyldimethylsilyl)oxy)-3-isopropyl-5-((trimethylsilyl)ethynyl)butan-2-one (10)

¹³C NMR spectrum of 4-(4-((tert-butyldimethylsilyl)oxy)-3-isopropyl-5-((trimethylsilyl)ethynyl)butan-2-one (10)

KenCNMROct515TBSProtection

CC1(C)N2C(C1)CC(C2)CCc3ccc(OC(C)(C)Si(C)(C)C)c(C#CC(C)(C)Si(C)(C)C)c3C(C)C

^{13}C NMR spectrum of 4-((tert-butyldimethylsilyl)oxy)-3-isopropyl-5-((trimethylsilyl)rhynyl)phenethyl-3-methyl-3*H*-diazirine (11)

¹H NMR spectrum of 2-ethynyl-6-isopropyl-4-(2-(3-methyl-3H-diazirin-3-yl)ethyl)phenol (12)

¹³C NMR spectrum of 2-ethynyl-6-isopropyl-4-(2-(3-methyl-3H-diazirin-3-yl)ethyl)phenol (12)

¹H NMR spectrum of **4-hydroxy-3,5-diisopropylbenzaldehyde (13)**

july1116Aldehyde
FLL-VIII-160-column-3

¹³C NMR spectrum of **4-hydroxy-3,5-diisopropylbenzaldehyde (13)**

¹H NMR spectrum of 4-(*tert*-butyldimethylsilyl)oxy)-3,5-diisopropylbenzaldehyde (14)

^{13}C NMR spectrum of 4-(*tert*-butyldimethylsilyl)oxy)-3,5-diisopropylbenzaldehyde (14)

^1H NMR spectrum of **1-4-((*tert*-butyldimethylsilyl)oxy)-3,5-diisopropylphenyl)prop-2-yn-1-ol (15)**

^{13}C NMR spectrum of **1-4-((*tert*-butyldimethylsilyl)oxy)-3,5-diisopropylphenyl)prop-2-yn-1-ol (15)**

¹H NMR spectrum of 4-(4-((*tert*-butyldimethylsilyl)oxy)-3,5-diisopropylphenyl)hex-5-yn-2-one (16)

^{13}C NMR spectrum of **4-(4-((*tert*-butyldimethylsilyl)oxy)-3,5-diisopropylphenyl)hex-5-yn-2-one (16)**

¹H NMR spectrum of **3-(2-(4-((*tert*-butyldimethylsilyl)oxy)-3,5-diisopropylphenyl)but-3-yn-1-yl)-3-methyl-3*H*-diazirine (17)**

¹³C NMR spectrum of **3-(2-(4-((*tert*-butyldimethylsilyl)oxy)-3,5-diisopropylphenyl)but-3-yn-1-yl)-3-methyl-3*H*-diazirine (17)**

^1H NMR spectrum of 2,6-diisopropyl-4-(1-(3-methyl-3H-diazirin-3-yl)but-3-yn-2-yl)phenol (18)

^{13}C NMR spectrum of **2,6-diisopropyl-4-(1-(3-methyl-3H-diazirin-3-yl)but-3-yn-2-yl)phenol (18)**