Switchable quaternary ammonium transformation leading to salinity-tolerance-conferring plant biostimulants

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Since their discovery by Menshutkin in 1890, quaternary ammonium salts have been synthesized by alkylating tertiary amines, with the development of new synthetic methods having received limited attention. Here we describe a photoredox-catalyzed method for synthesizing quaternary ammonium salts that involves reacting α-haloalkylammonium salts with olefins. This chemistry enables selective and switchable alkylations and alkenylations that afford a variety of structurally new quaternary ammonium salts. The key to success is attributable to the photocatalytic generation of distonic α-ammonium radicals under both oxidative and reductive quenching conditions. Furthermore, during the course of our reaction-development-based chemical-screening campaign, we serendipitously discovered that the synthesized quaternary ammonium salts confer plants with salinity tolerance. The discovery of a novel class of salinity-tolerance-conferring molecule is expected to impact agrochemical development as salinity damage increasingly becomes a global problem.

Main text

Quaternary ammonium salts are molecules with nitrogen atoms that bear four alkyl, alkenyl, alkynyl, or aryl substituents and have been widely used as antimicrobials or pharmaceuticals (Fig. 1A)[1–3]. Furthermore, quaternary ammonium salts can effectively inactivate enveloped viruses such as COVID-19[4]. Despite their importance, the synthesis of quaternary ammonium salts has predominantly relied on the classical Menshutkin reaction reported in 1890[5], in which tertiary amines react with alkyl halides. While this method is well established, it is typically reserved for the final stage of a synthetic sequence, in which the synthesis of the quaternary ammonium salts essentially involves a linear synthesis that necessitates the multistep preparation of the starting alkyl halide and/or amine. From the standpoint of divergent syntheses, existing research into quaternary ammonium salt transformations has significantly lagged behind the rich amine-transformation chemistry[6–8] that has undoubtedly accelerated the development of amine-based pharmaceuticals (Fig. 1B). In 2016, Phipps reported elegant *meta*-selective C–H borylations of benzylammonium salts[9]; however, this study mainly focused on functionalizing positions remote from the ammonium nitrogen atom. Indeed, examples of functionalization adjacent to the ammonium nitrogen moiety are limited. For example, even simple Suzuki–Miyaura or Sonogashira coupling chemistry involving α-halogenated alkenylammonium salts had not been reported until developed by us[10]. Hence, a strategy that combines the Menshutkin reaction with this new quaternary-ammonium transformation chemistry is expected to result in a significant number of compounds becoming accessible. As the development of new synthetic strategies that facilitate the expansion and development of new chemical spaces is a central issue in organic chemistry, the development of streamlined transformations of quaternary ammonium salts is expected to accelerate the discovery of novel classes of bioactive molecule.

With the above background in mind, we focused on the elusive distonic α-ammonium radical, in which the radical center is located on the carbon adjacent to the ammonium cation. In 1996, Dolbier reported reactions of distonic α-ammonium radicals, in which the treatment of an α-iodomethylammonium salt with a vinyl ether mainly afforded the tetramethylammonium salt along with the corresponding products in yields of 29% and 17% as a mixture (Fig. 1C)[11]. The bond-dissociation energy (BDE) of the α-C–H bond in a quaternary ammonium cation is 106.1 kcal/mol (as calculated at the UB3LYP/6-31+g(d) level of theory)[12]; consequently, photolytic conditions generate distonic radicals in one shot, with most of the formed radicals abstracting hydrogen atoms before they can react with an olefin. The authors clearly state: "however, except for its addition to ethyl vinyl ether, attempts to observe competitive bimolecular addition of α-ammoniomethyl radical to various other olefins, including 1-hexene, styrene, pentafluorostyrene, and α-methylstyrene, were curiously ineffective and led only to formation of tetramethylammonium tetrafluoroborate." The low yield and narrow substrate scope has hampered the use of distonic α-ammonium radicals. Moreover, while several studies were conducted to 2000[13,14], successful transformations were limited to *intra*molecular reactions; consequently, studies into distonic α-ammonium radicals effectively ceased for more than 20 years.

Herein, we report two distinctive methods for generating distonic α-ammonium radicals that rely on different photocatalytic mechanisms: oxidative quenching and reductive quenching (Fig. 1D).[15–18] These discoveries enable control over the products from the *inter*molecular reactions of distonic α-ammonium radicals and olefins. In addition, we discovered new salinity-tolerance-conferring molecules in our quaternary ammonium salt collection.

Results and discussion

Photocatalytic transformations of an α-iodomethylammonium salt through oxidative quenching: The reaction conditions for the *inter*molecular reactions of distonic α-ammonium radicals with olefins were initially screened using α-iodomethylammonium salt **1a** and 1,1-diphenylethylene (**2a**) (See SI, Table S1), which revealed that Ir-photoredox catalyst **PC1** (E^*_{ox} = –1.88 V) delivered the best outcome, with alkenylated product **3a** obtained selectively. The optimized conditions are shown in Fig 2A, left: treatment of **1a** with **2a** (1.5 equiv.) in the presence of **PC1** (2.0 mol%) and $AgBF_4$ (1.0 equiv.) for 2 h in DMSO under blue light afforded the corresponding product **3a** in 78% yield after counter-anion exchange with aqueous KPF_6. A proposed mechanism for the catalytic generation of the distonic α-ammonium radical is shown in Fig. 2B. Photoredox catalyst **PC1** is first excited by blue light. The resulting sufficiently reducing **PC1*** transfers an electron to α-iodomethylammonium salt **1a** (E = –1.39 V)[14] to form distonic α-ammonium radical **A**, which then reacts with olefin **2a** to generate radical **B**. Electron transfer from **PC1**$^{\bullet+}$ to radical **B** and subsequent deprotonation finally affords the alkenylation product **3a**. The proposed oxidative quenching mechanism is supported by Stern–Volmer experiments (Fig. 2C). Furthermore, the corresponding methoxylated product **5** was obtained from the alkenylation product when the reaction was conducted in CH_3OH (Fig. 2D), which further supports the proposed mechanism.

Photocatalytic transformation of an α-iodomethylammonium salt through reductive quenching that enables a switchable transformation: We next explored reductive quenching conditions with the aim of switching between alkenylation and alkylation chemistry[19]. Inspired by recent advancements in halogen-atom-transfer (XAT) chemistry[20], we used triethylamine as the XAT reagent (Fig 2A, right)[21]. Treatment of **1a** with **2a** in the presence of **PC2** (5.0 mol%) and NEt_3 (2.0 equiv.) for 2 h in 1:1 CH_3CN/H_2O under blue light furnished the corresponding product **4a** in 73% yield, after counter-anion exchange with aqueous KPF_6. The inclusion of triethylamine was essential for catalyst turnover, with only 6% of **4a** obtained in the absence of triethylamine. The proposed reaction mechanism for this transformation is summarized in Fig 2E. Ir catalyst **PC2** (E^*_{red} = +0.55 V) is excited using blue light to give **PC2***. Triethylamine

($E_{\mathrm{ox}} = +0.77$ V)[21] is then oxidized by the catalyst to give amino radical **C**, which then abstracts the iodine atom from **1a**. The resulting distonic α-ammonium radical **A** reacts with **2a** to give the corresponding radical **B**, which finally reacts with **PC2**$^{\bullet-}$ to afford product **4a**. Stern–Volmer experiments, in which the catalyst was only found to be quenched by triethylamine and not by **1a** or **2a**, provided evidence for this mechanism (Fig. 2F). The reaction was conducted in a deuterated solvent to obtain further insight into the conversion of radical **B** into product **4a** (Fig. 2G). Deuterated **4a-D** was obtained in 79% (^{1}H NMR yield) when the reaction was performed in CH_3CN/D_2O, which is rationalized by **PC2**$^{\bullet-}$ reducing radical intermediate **B** to form an anionic intermediate, which is then quenched with D_2O to give **4a-D**.

We next investigated the scope of these reactions using the optimized conditions for both alkenylation (conditions A) and alkylation (conditions B) (Fig 3), with the former conditions discussed first. Reactions with the bulkier triethylammonium and isopropyldimethylammonium salts gave **3b** and **3c** in yields of 62% and 74%, respectively. Heterocyclic ammonium salts were also tolerated by this reaction, with **3d**–**3f** obtained in moderate-to-good yields (**3d**: 57%, **3e**: 73%, **3f**: 39%). Olefins bearing variously substituted aryl groups were also tolerated; diarylethenes with electron-donating (methyl or methoxy) or electron-withdrawing (fluoro) groups furnished products **3g**–**3i** in high yields. A bromo substituent was also compatible and remained intact (**3j**) despite the catalyst exhibiting good reducing abilities. The unsymmetrical diarylethene led to product **3k** as a mixture of *E*/*Z* isomers. Notably, styrene, an unreactive substrate under Dolbier conditions, reacted to give **3i** in 74% yield (*E*/*Z*: 19:81); the *Z* isomer was formed as the major diastereomer because photocatalyst-accelerated *E*/*Z* isomerization was observed in solution (Fig. S7)[22]. Not only was the α-iodomethylammonium salt compatible with this reaction but the *β-bromoethyl*ammonium salt also reacted to give **3m**, albeit in relatively low yield. Other ammonium salts derived from diphenhydramine (pharmaceutical) and strychnine (natural product) were converted under these conditions to give **3n** and **3o**, with no clear side reactions observed. The doubly alkenylated product **3p** was obtained in 36% yield when the bis-iodomethylammonium salt was subjected to these reaction conditions.

We next examined the scope of the reaction under conditions B. As was observed under the previous conditions, a variety of ammonium salts were tolerated by the reaction, with **4b**–**4f** obtained in yields of 46–70%. Relatively high yields were obtained when electron-withdrawing groups were attached to the diarylethene (**4g** and **4h** vs. **4i**–**4l**) because radical intermediate **B** is more easily reduced when its aryl groups bear electron-withdrawing groups. Although the reaction proceeded sluggishly with styrene, compound **4m** was obtained in 16% yield. Conversely, the reaction proceeded more efficiently to give **4n** in 49% yield when an electron-withdrawing ester group was attached to styrene. The alkylation reaction was also applicable to other ammonium salts to afford **4o**–**4r** in moderate-to-good yields. Overall, our switchable reaction facilitates the streamlined syntheses of 2-types of diarylalkylammonium salt from the same commercially available tertiary amine in two steps. Compared with the classical linear syntheses of **3a** and **4a**, which are multistep syntheses from their respective starting materials[23], the switchable strategy presented herein accesses such compounds far more efficiently.

Serendipitous discovery of salinity-tolerance-conferring molecules in the quaternary ammonium salt collection: We conducted a chemical screening of the various structurally new quaternary ammonium salts prepared using the aforementioned methodology. We previously identified a new molecule in our C–H amination product collection that inhibits stomatal opening, which highlights the power of reaction-driven chemical screening[24]. In this study, we focused on discovering salinity-tolerance-conferring molecules in our quaternary ammonium salt collection.

Soil salinity is becoming an increasingly serious global problem; it inhibits plant growth and development, significantly reduces crop yields, and is one of the effects of climate change associated with global warming[25,26]. NaCl not only reduces water availability, it also induces toxic stress because Na^+ and Cl^- affect mRNA stability, translational regulation, and protein abundance in a manner that inhibits plant photosynthesis[27]. Strategies that increase crop yields in saline soils include creating transgenic plants with genes that "eliminate" toxic Na^+, and the use of exogenous osmolytes that accumulate naturally occurring organic osmotic solutes[27–29]. However, both transgenic plants and osmolytes inhibit growth because they constantly expend energy to manage plant stress[30–34].

While novel plant biostimulants that neither inhibit growth nor rely on genetic engineering need to be discovered and developed[35], no comprehensive studies into their specific use and subsequent mechanisms of action against salinity are currently unavailable[36]. In this study, we identified molecules that confer tolerance against salinity in the collection of quaternary ammonium salts constructed using our newly developed switchable-synthesis protocol. We screened for compounds capable of enhancing the abilities of plants to tolerate environmental stress. Among the prepared quaternary ammonium salts, exogenous compounds **3a** and **4a** appeared to confer plants with salinity tolerance (Fig. 4A and 4B) without inhibiting their growth (Fig. 4C and 4D). To demonstrate this, we sowed Arabidopsis using 100 mM NaCl medium or unstressed medium containing 0, 0.1, 1, or 5 μM of **3a** or **4a**. The seedlings were grown for 20 or 15 d, with the lengths of their primary roots measured every 4 or 2 d. We found that the primary roots and leaves of Arabidopsis seedlings grown in compound-containing media were approximately three-times longer and larger than those grown in saline medium in the absence of compound. Compounds **3a** and **4a** were effective at all concentrations, but most effective at 0.1 μM (Fig. 4A and 4B). Furthermore, we found that the root and leaves Arabidopsis seedlings grown in compound-containing media were not shorter or smaller than those grown in the compound-free medium; on the contrary, compounds **3a** and **4a** promoted Arabidopsis growth at 0.1 and 5 μM, respectively (Fig. 4C and 4D). In addition, we screened

24 analogs and discovered that four were salinity-tolerance-enhancing and growth-promoting (Fig. S8).

While these compounds are effective at very low concentrations (< 0.1 μM), the osmolyte concentrations range lies between approximately 0.1 and 1,500 mM; therefore, these compounds may cause sodium to be transported into the extracellular space or the vacuole, rather than acting as osmolytes. From a practical point of view, these compounds are useful because their effectiveness at low concentrations suggest that they are minimally environmentally persistent. Compounds **3a** and **4a** are potentially undiscovered phytohormones because they are effective at low concentrations. Investigating the mechanisms of action of these bioactive molecules may lead to the discovery of even better biostimulants.

Conclusion

We successfully developed a switchable protocol for synthesizing quaternary ammonium salts that involves the photocatalytic generation of α-ammoniomethyl radicals; we refer to this new concept as the "quaternary ammonium transformation" to underscore its significance. Traditionally, tertiary amines are converted into quaternary ammonium salts at the final stage of synthesis owing to a lack of known protocols for their transformation. In contrast, our study unequivocally demonstrated that quaternary ammonium salts can serve as key intermediates in the synthesis of various molecules, thereby enabling the rapid synthesis of divergent quaternary ammonium salts. Furthermore, novel salinity-tolerance-conferring molecules were identified in our reaction-development-based collection. This study highlights the power of our newly developed protocol for the synthesis of quaternary ammonium salts and their practical applications in the plant chemical biology field. Given the widespread nature of salinity damage that affects global agriculture, our discovery of a new protocol for transforming quaternary ammonium salts is expected to have a far-reaching impact on global food security.

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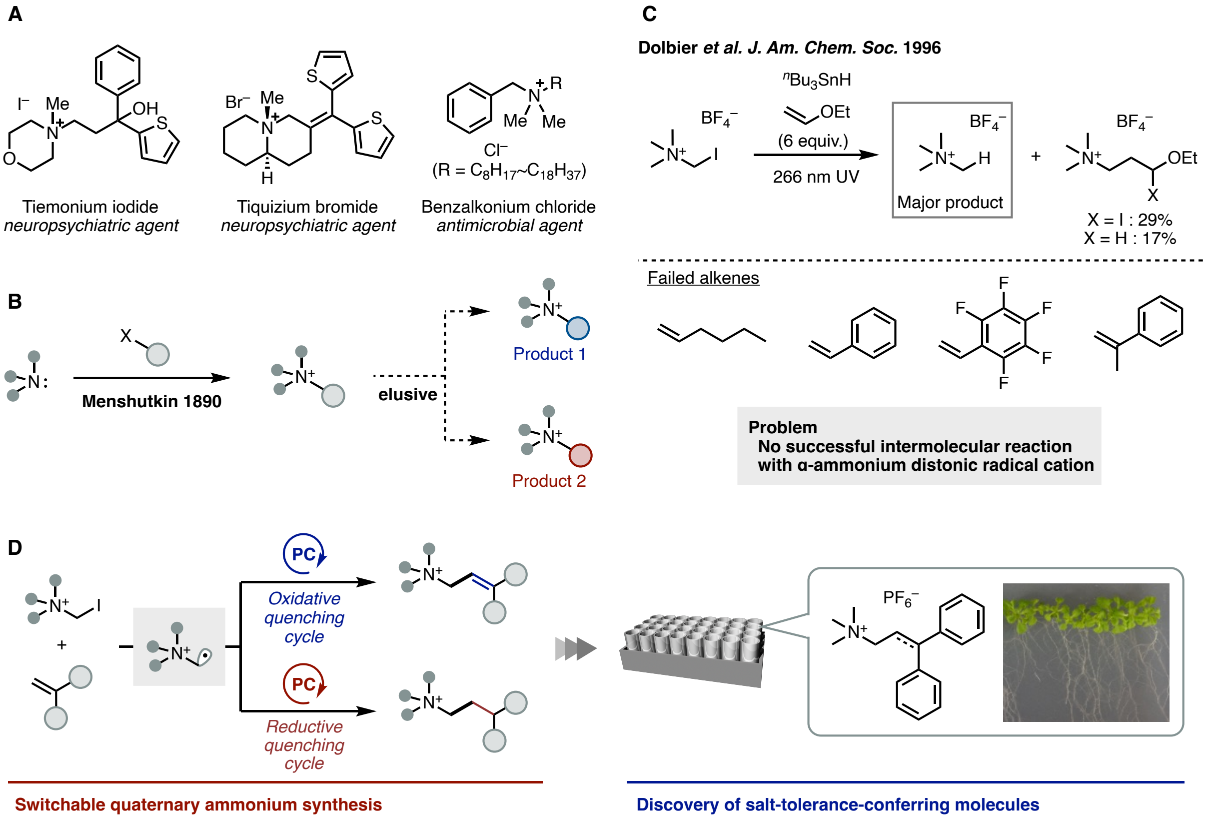


Fig. 1. Previous work and this approach for the generation of α-ammonium distonic radical. (**A**) Examples of antimicrobials or pharmaceuticals including quaternary ammonium scaffold. (**B**) Overview of quaternary ammonium salts synthesis. (**C**) Previous example of an intermolecular reaction of α-ammonium distonic radical. (**D**) This work.

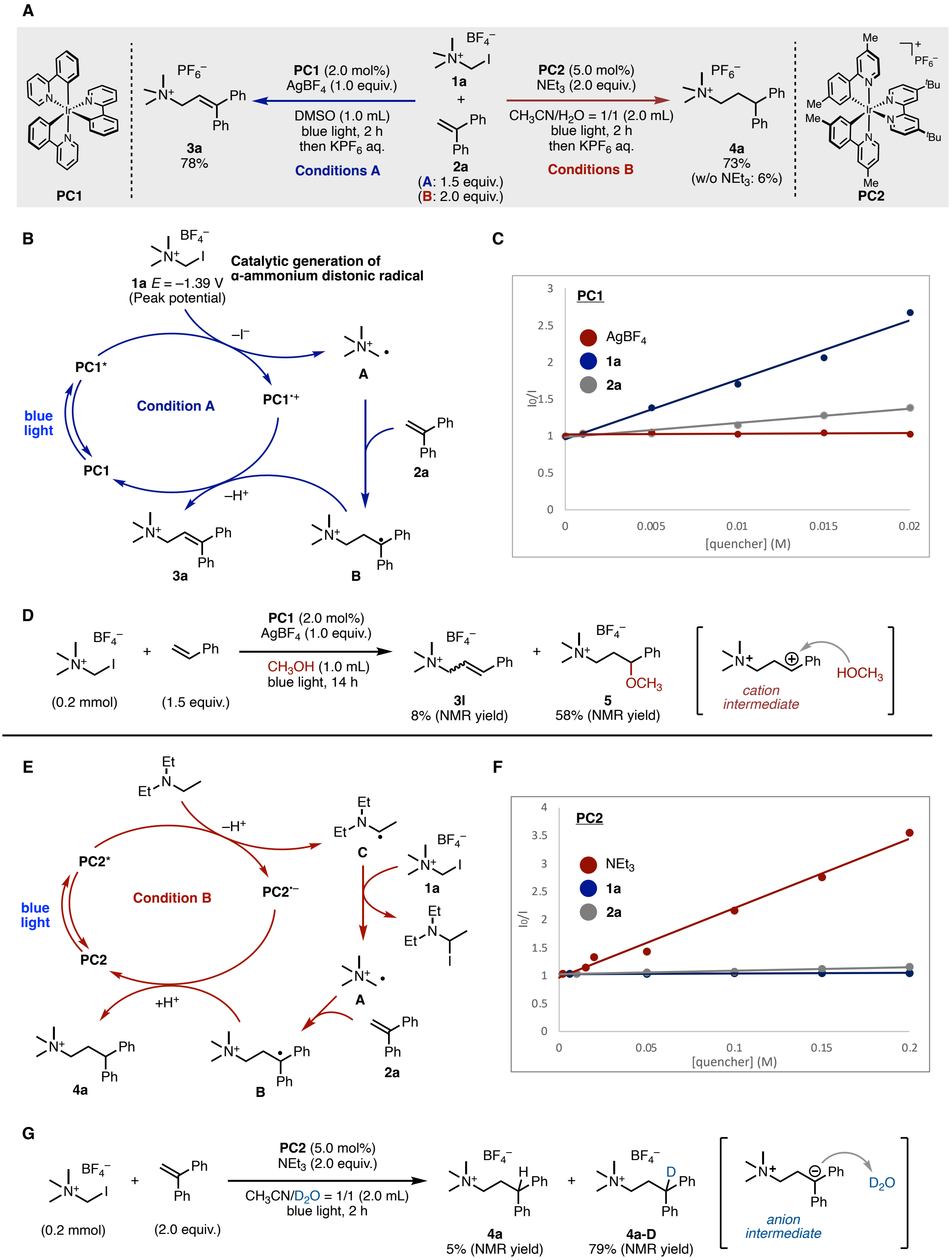


Fig. 2. Switchable synthesis of quaternary ammonium salt. (**A**) Optimized conditions. (**B**) Proposed reaction mechanism in oxidative quenching conditions. (**C**) Stern–Volmer quenching study in oxidative quenching conditions. (**D**) Cation-trapping experiment. (**E**) Proposed reaction mechanism in reductive quenching conditions. (**F**) Stern–Volmer quenching study in reductive quenching conditions. (**G**) Anion-trapping experiment.

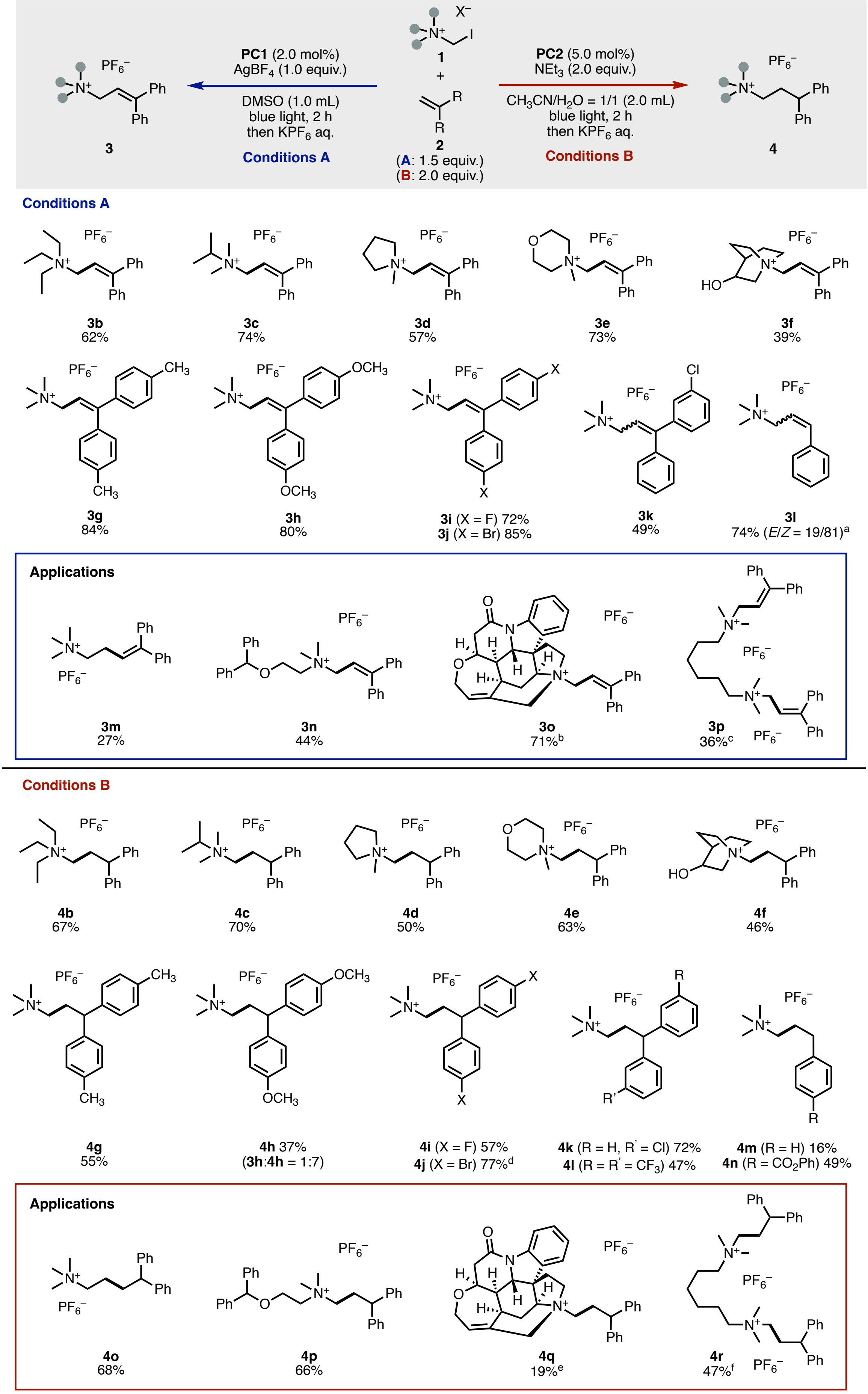

X⁻
1
+
R
R
2
(A: 1.5 equiv.)
(B: 2.0 equiv.)
PC1 (2.0 mol%)
AgBF4 (1.0 equiv.)
DMSO (1.0 mL)
blue light, 2 h
then KPF6 aq.
Conditions A
PC2 (5.0 mol%)
NEt3 (2.0 equiv.)
CH3CN/H2O = 1/1 (2.0 mL)
blue light, 2 h
then KPF6 aq.
Conditions B
3
4
Conditions A
3b 62%
3c 74%
3d 57%
3e 73%
3f 39%
3g 84%
3h 80%
3i (X = F) 72%
3j (X = Br) 85%
3k 49%
3l 74% (E/Z = 19/81)a
Applications
3m 27%
3n 44%
3o 71%b
3p 36%c
Conditions B
4b 67%
4c 70%
4d 50%
4e 63%
4f 46%
4g 55%
4h 37% (3h:4h = 1:7)
4i (X = F) 57%
4j (X = Br) 77%d
4k (R = H, R' = Cl) 72%
4l (R = R' = CF3) 47%
4m (R = H) 16%
4n (R = CO2Ph) 49%
Applications
4o 68%
4p 66%
4q 19%e
4r 47%f

Fig. 3. Scope of reaction. [a]Using styrene (0.2 mmol), **1a** (2.0 equiv.), and $AgBF_4$ (2.0 equiv.) in 1.0 mL DMA. [b]Using $AgBF_4$ (2.0 equiv.). [c]Using **2a** (6.0 equiv.) and $AgBF_4$ (2.0 equiv.). [d]Using the mixture of CH_2Cl_2 and H_2O as solvent. [e]Using $AgBF_4$ (1.0 equiv.). [f]Using **2a** (6.0 equiv.) and NEt_3 (4.0 equiv.).

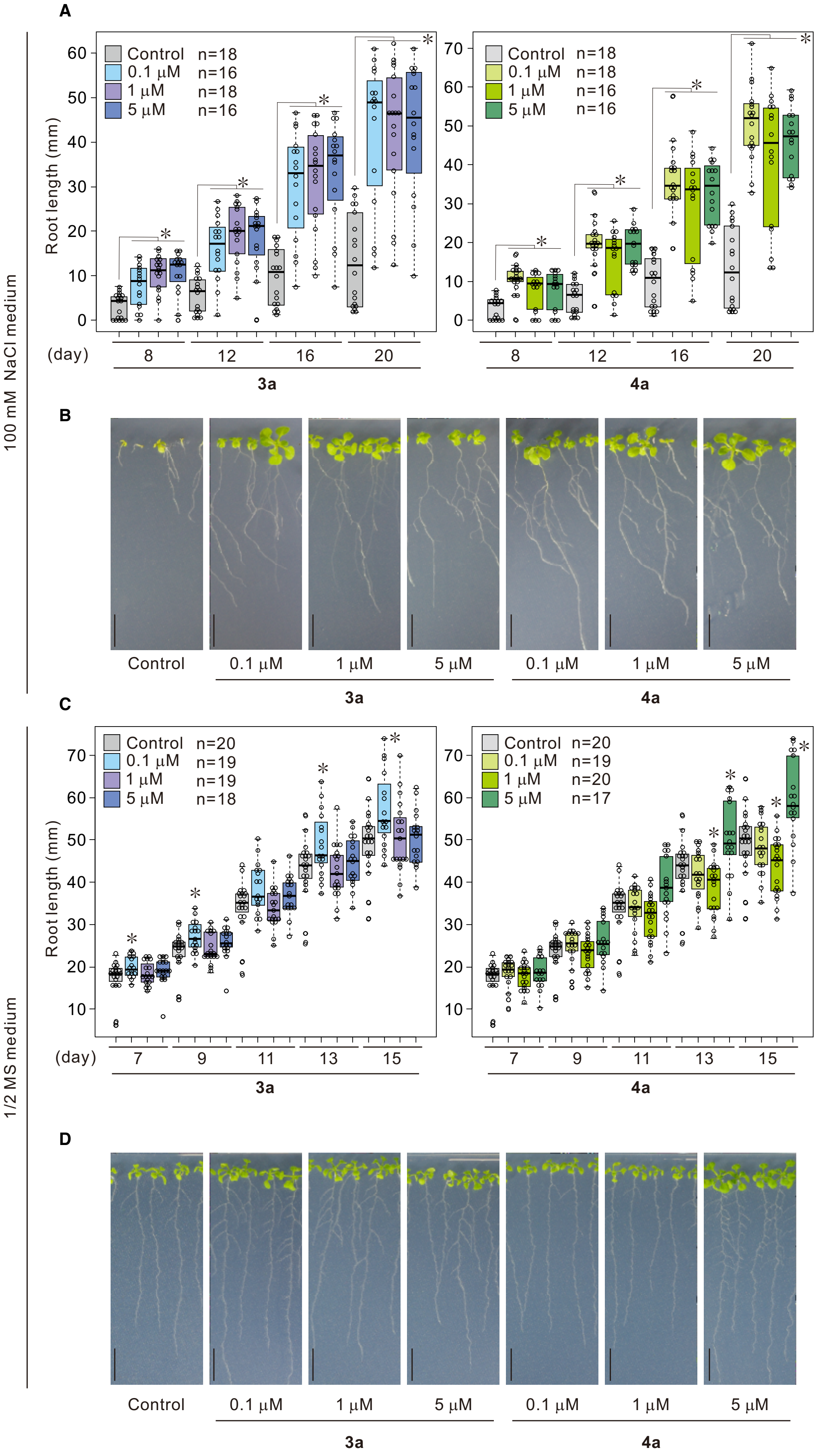
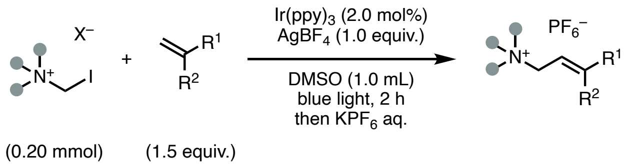
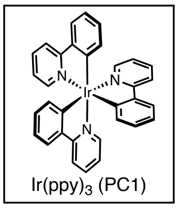

A
100 mM NaCl medium
Root length (mm)
Control n=18
0.1 µM n=16
1 µM n=18
5 µM n=16
Control n=18
0.1 µM n=18
1 µM n=16
5 µM n=16
(day)
8
12
16
20
3a
4a
*
B
Control
0.1 µM
1 µM
5 µM
0.1 µM
1 µM
5 µM
3a
4a
C
1/2 MS medium
Root length (mm)
Control n=20
0.1 µM n=19
1 µM n=19
5 µM n=18
Control n=20
0.1 µM n=19
1 µM n=20
5 µM n=17
(day)
7
9
11
13
15
3a
4a
D
Control
0.1 µM
1 µM
5 µM
0.1 µM
1 µM
5 µM
3a
4a

Fig. 4. Biological experiments on conferring salt tolerance. Root length (**A**) and images (**B**) of wild-type Arabidopsis seedlings grown on 1/2 MS medium containing 100 mM NaCl with 0.1, 1, or 5 μM the compounds **3a** or **4a**. Root length (**C**) and images (**D**) of wild-type Arabidopsis seedlings grown on 1/2 MS medium with 0.1, 1, or 5 μM compounds **3a** or **4a**. Significant differences ($P < 0.01$; indicated by asterisk) were determined using the Mann-Whitney U-test. In the box-and-whisker plots, the boxes and solid lines in the boxes represent the upper (75th) and lower (25th) quartiles and median values, respectively, and the whiskers represent the 95% confidence intervals. Experiments were independently repeated at least three times with similar results. The n values in the figure indicate the number of independent root samples used. Scale bars = 10 mm.

Methods

Procedure for the oxidative quenching conditions

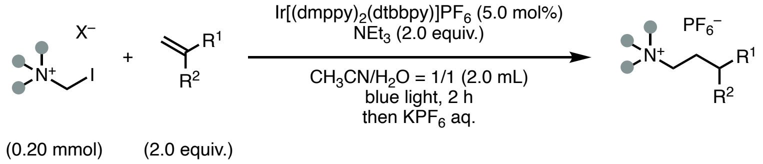


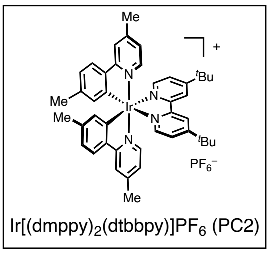


Ir(ppy)$_3$ (PC1)

Method A: A dried reaction tube with a stirring bar was charged with ammonium salt (0.20 mmol, 1.0 equiv.), AgBF$_4$ (38.9 mg, 0.20 mmol, 1.0 equiv.), and Ir(ppy)$_3$ (**PC1**, 2.6 mg, 0.0040 mmol, 2.0 mol%). The tube was filled with nitrogen by employing the usual Schlenk technique (evacuate-refill cycle). DMSO (1.0 mL) and olefin (0.30 mmol, 1.5 equiv.) were added to the tube and the mixture was stirred under blue light irradiation and a cooling fan (a 40 W Kessil PR160 blue LED was placed 1 cm side from the reaction vials) for 2 h. The crude mixture was diluted with acetone and filtered through a pad of celite, then concentrated *in vacuo*. The resulting product was dissolved in CH$_2$Cl$_2$ and washed with 10% Na$_2$S$_2$O$_3$ aqueous solution, water, for three times. Then, the organic layer was washed with saturated KPF$_6$ aqueous solution for three times to exchange the counter anion. The organic layer was dried by Na$_2$SO$_4$, filtered, and concentrated *in vacuo*. The crude product was recrystallized from CH$_2$Cl$_2$/Et$_2$O to give the pure target product.

Procedure for the reductive quenching conditions





Ir[(dmppy)$_2$(dtbbpy)]PF$_6$ (PC2)

Method B: A dried reaction tube with a stirring bar was charged with ammonium salt (0.20 mmol, 1.0 equiv.), Ir[(dmppy)$_2$(dtbbpy)]PF$_6$ (**PC2**, 9.7 mg, 0.010 mmol, 5.0 mol%). The tube was filled with nitrogen by employing the usual Schlenk technique (evacuate-refill cycle).

CH_3CN (1.0 mL), H_2O (1.0 mL), NEt_3 (40.4 mg, 0.40 mmol, 2.0 equiv.), and olefin (0.40 mmol, 2.0 equiv.) were added to the tube. The mixture was stirred under blue light irradiation and a cooling fan (a 40 W Kessil PR160 blue LED was placed 1 cm side from the reaction vials) for 2 h. The crude mixture was concentrated *in vacuo*. The resulting product was dissolved in CH_2Cl_2 and washed with water for three times. Then, the organic layer was washed with saturated KPF_6 aqueous solution for three times to exchange the counter anion. The organic layer was dried by Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was recrystallized from CH_2Cl_2/Et_2O to give the pure target product.

Procedure for biological experiments

Plant material and growth conditions

A. thaliana ecotype Columbia-0 (Col-0) plants were used. The Arabidopsis seeds were sterilized with 70% ethanol and grown on half-strength Murashige and Skoog salt (1/2 MS) agar medium [1% (w/v) sucrose, 1.2% (w/v) agar], at 22 °C under a 16 h/8 h light/dark cycle.

Measurement of root length and calculation of the growth index

Roots of seedlings were digitally photographed for measurement of length of root with some modifications, and determined using ImageJ. Growth index was calculated as the ratio of primary root length in each condition to the mean root length of the control.

Statistics

The Shapiro–Wilk test was used to assess the normality of the samples. The Mann–Whitney U test was used to assess the significant differences ($P < 0.01$; indicated by asterisk) between the two samples.

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Author Contributions

K.M. and T.H. conceived the concept and directed the project. Y.S. designed the reactions. T.K. and Y.S. performed the organic chemistry experiments. T.H. performed the biological experiments. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Competing interests

The authors declare no competing interests.

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