Supporting Information

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Materials and Methods

LC-MS. Five-day-old Col-0 seedlings were incubated for ≈48 h in 0.5× MS media (50 ml) supplemented with 5 μM of DMSO, **533**, or **602**. Seedlings were collected and washed extensively with water to minimize traces of the external compound. Washed seedlings were extracted in a solution containing 95% ACN, 4.9% H_2O, and 0.1% TFA. The extract was dried and resuspended in 100 μl of DMSO. The extracts were analyzed by liquid chromatography (LC) on an Agilent 1100 HPLC using a Phenomenex Synergi Fusion-RP 80 column (4 μm, 150 mm × 4.60 mm) at a flow rate of 0.5 ml/min, coupled to an electrospray ionization (ESI) XCT ion trap mass spectrometer (Agilent) in positive- and negative-ion modes under the addition of 20 mM ammonium acetate in 65% acetonitrile (100 μl/min). A linear gradient of acetonitrile/0.1% formic acid (40–100%) in water/0.1% formic acid was used. The positive ion–ESI mass spectrum of **602** and **533** standards were (m/z) = 304.8 ($[M-H]^+$) and (m/z) = 311 ($[M-H]^+$), respectively. The negative ion–ESI mass spectrum of 2,4-D and conjugated **5617690** standards were (m/z) = 219 ($[M-H]^-$) and (m/z) = 213.5 ($[M-H]^-$), respectively.

LC-MS Analysis for Proauxin Stability. *Sample Preparation.* Compounds 5336619 (**533**) and 6027864 (**602**) were analyzed for their stability to both acidic and basic conditions. The test compounds **533** and **602** were used as received from Chembridge without further purification and dissolved in 100% DMSO (Sigma Aldrich) to 100 mM stock solutions. The samples were prepared directly in Agilent 2.0-ml LC-MS vials, sealed with magnetic stirring and maintained at 25°C. For acid digestion, 10 μl of **533** stock solution was added to 90 μl of 1.0M HCl (pH ≈0) in four replicates corresponding to time points 0, 30, 60, and 120 min. A control sample of 10 μl of **533** stock solution was added to 90 μl H_2O (deionized). At the appropriate time point, the four samples were neutralized with 90 μl of 1.0 M NaOH (pH ≈14) and an additional 10 μl of DMSO. The final concentration of **533** in the quenched reaction mixture was 5 mM. The control sample was diluted with an additional 90 μl of H_2O and 10 μl of DMSO. LC-MS analysis samples were prepared by diluting these five samples with 300 μl of 1:1 MeCN-H_2O, where the final theoretical concentration of **533** was 2 mM.

For base digestion, 10 μl of **533** stock solution was added to 90 μl of 1.0 M NaOH (pH ≈14) in four replicates corresponding to time points 0, 30, 60, and 120 min. A control sample of 10 μl of **533** stock solution was added to 90 μl of H_2O (deionized). At the appropriate time point, the four samples were neutralized with 90 μl of 1.0 M HCl (pH ≈0) and an additional 10 μl of DMSO. The final concentration of **533** in the quenched reaction mixture was 5 mM. The control sample was diluted with an additional 90 μl of H_2O and 10 μl of DMSO. LC-MS analysis samples were prepared by diluting these five samples with 300 μl of 1:1 MeCN-H_2O, where the final theoretical concentration of **533** was 2 mM. The same acid and base digestion treatment and sample preparation was carried out for **602**.

LC-MS analysis. This analysis was performed on an Agilent 1100 high-performance liquid chormatograph with and Agilent LCT Trap MS operating in ESI± mode. The column was a Phenomenex Gemini Column (hybrid C_{18}). The Sample injection volume was 10 μl, with gradient elution in H_2O (A) containing 0.1% formic acid and MeCN (B) containing 0.1% formic acid as follows: 95:5 (A:B) for 3 min (t = 3 min), to 0:100 (A:B) over 10 min (t = 13 min), holding 0:100 (A:B) for 10 min (t = 23 min), and equilibrating to 95:5 (A:B) for 2 min (t = 25 min), for a total analysis time of 25 min per sample. The flow rate was 0.5 ml/min, and the column temperature was maintained at 30°C. The MS operated in alternating positive and negative ESI mode. UV-Vis was monitored via a diode array detector, and LC traces were reported at 254 nM (246–264 nM) over the entire time course. The samples ionized most strongly with ESI+ ionization for both **533** (rt = 16.1 min) and **602** (rt = 16.4 min).

Pull-Down Experiments. For the pull-down assays, GST-IAA7 was expressed in *Escherichia coli* and purified on glutathione-agarose beads. *TIR1-myc* seedlings were grown for 8 days in liquid 0.5X MS media supplemented with 1% sucrose. *TIR1-myc* expression was induced by treatment with 30 μM Dex for 24 h. Before harvest, seedlings were incubated for 2 h in 50 μM of the indicated compounds or an equivalent volume of DMSO. After treatment, the tissue was ground to a powder under liquid nitrogen and vortexed vigorously in extraction buffer (50 mM Tris [pH 7.5], 150 mM NaCl, 10 vol/vol glycerol, 0.1% Nonidet P-40 complete protease inhibitor [Roche], 50 μM MG-132). Cellular debris was removed by centrifugation, and total protein concentration was determined by Bradford assays. Each pull-down reaction included 1 mg of total protein extract and 50 μl of GST-IAA7 beads in a total volume of 500 μl. The pull-downs were kept at 4°C for 30 min with rocking. After a brief spin, each reaction was transferred to Micro Bio-Spin chromatography columns (BioRad). Unbound protein was removed by centrifugation, and the GST-IAA7 beads were rinsed with wash buffer (extraction buffer without protease inhibitors or MG-132). GST-IAA7 was eluted from the columns in wash buffer containing reduced glutathione (Sigma). Eluted proteins were resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis in duplicate. One gel was stained with Coomassie and dried. Proteins from the other gel were transferred to nitrocellulose and probed with anti-myc antibodies (Covance).

Auxin Mutant Isolation and Hypocotyl Measurements. *tir1–1* and *axr1–3* seedlings were characterized previously(1, 2). *afb1–3* and *afb2–3* seeds were obtained from the Salk Institute Genomic Analysis Laboratory, (3), and homozygous lines were identified using the T-DNA–specific LB02 primer TTGGGTGATGGTTCACGTAGTGGGCCATCG and following gene-specific primers: AFB1–3F: CGATTCCCACCTAAGGTGTTGGAACA; AFB1–3R: ACTCCATTCTCAGGATTCTTGTG; AFB2–3F: GTGTTCTTGACTTCCTATGGTCTA; and AFB2–3R: CAGCTGTGTTGCCTCCACCGAGTAA. Sequencing revealed that the T-DNA insertion in *afb1–3* lies within the second exon at 878bp. The T-DNA insertion within *afb2–3* is positioned 37 bp upstream of the translational start point. Full phenotypic characterization of these mutant lines is not yet completed.

To investigate the hypocotyl elongation of seedlings with a defect in the auxin response, surface-sterilized seeds were spotted on 0.5X Murashige and Skoog media (Caisson Laboratories) supplemented with 1% (wt/vol) sucrose, the appropriate hormone, and 1% (wt/vol) agar and stratified for 3 days in the dark at 4°C. Seedlings were grown for 5 days under the same conditions as outlined above. Pictures of hypocotyls were taken with a Nikon SMZ1500 and measured using ImageJ version 1.35s.

Primers Used for qPCR. IAA5 (At1g15580): 5′-ACCGGCGAAAAAGAGTCAAG-3′ and 5′-AGACTGTTCTTTCTCCGGTACGA- 3′; IAA19 (AT3G15540): 5′- TGCTCTTGATA-

AGCTCTTCGGTT-3′ and 5′-AACGTATTCGCAGTTGT-CACCAT-3′. At5g15400 was used to normalize the expression as described in ref. 4.

Lipinski's Rule Information. Lipinski's rules evaluate the molecular properties important for a small molecule's pharmacokinetics, in terms of adsorption, distribution, metabolism, and excretion (ADME). Generally, successful drugs have molecular weights <500 g/mol, log *P* values <5, five or fewer hydrogen bond donor atoms, and fewer than 10 hydrogen bond acceptors. Within a 95% confidence limit, all drugs meet three of the four criteria described by the "Rule of 5." Because of the significant difference in pH between the apoplast and the intracellular cytoplasm in plants, we added pK_a and logD values to our molecular descriptor set (calculations from ChemAxon's Marvin and Calculator Plugins; available from www.chemaxon.com/demosite/marvin/index.html).

With the exception of NAA, which had a higher clogD value and is known to efficiently diffuse into the cells independently of influx transporters (Tables S3 and S4) (5), the remaining control compounds fall short of the accepted criteria for facile passive diffusion and must depend on active transporters; for calculated diffusion rates of NAA and 2,4-D, see (5). Interestingly, the predicted low lipophilic nature of picloram is in contrast to its effective induction of hypocotyl elongation. Thus, picloram is likely to be transported by distinct carriers or mechanisms differing substantially from 2,4-D and NAA (Tables S3 and S4).

1. Lincoln C, Britton JH, Estelle M (1990) Growth and development of the axr1 mutants of *Arabidopsis*. *Plant Cell* 2:1071–1080.
2. Ruegger M, *et al.* (1998) The TIR1 protein of *Arabidopsis* functions in auxin response and is related to human SKP2 and yeast grr1p. *Genes Dev* 12:198–207.
3. Alonso JM, *et al.* (2003) Genome-wide insertional mutagenesis of Arabidopsis Thaliana. *Science* 30:653–657.
4. Nemhauser JL, Mockler TC, Chory J (2004) Interdependency of brassinosteroid and auxin signaling in *Arabidopsis*. *PLoS Biol* 2:e258.
5. Delbarre A, Muller P, Imhoff V, Guern J (1996) Comparison of mechanisms controlling uptake and accumulation of 2,4-dichlorophenoxy acetic acid, naphthalene-1-acetic acid, and indole-3-acetic acid in suspension-cultured tobacco cells. *Planta* 198:532–541.

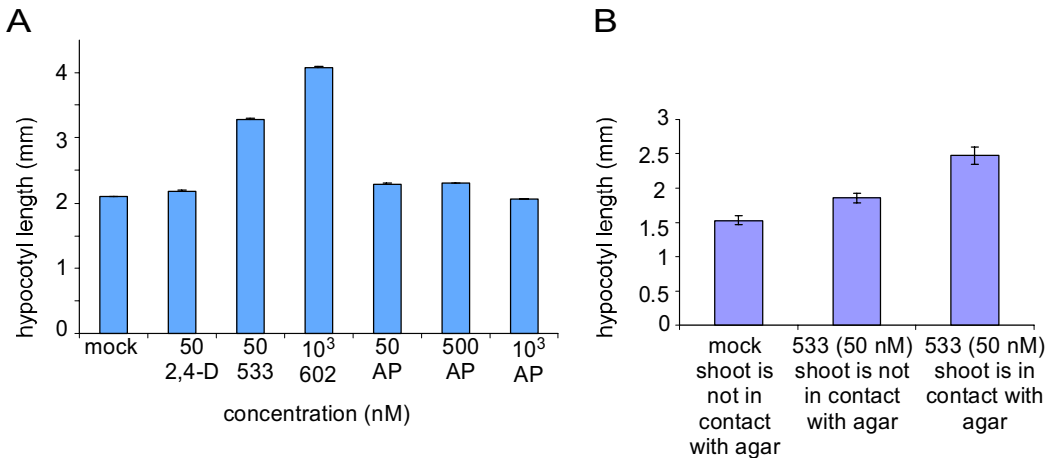
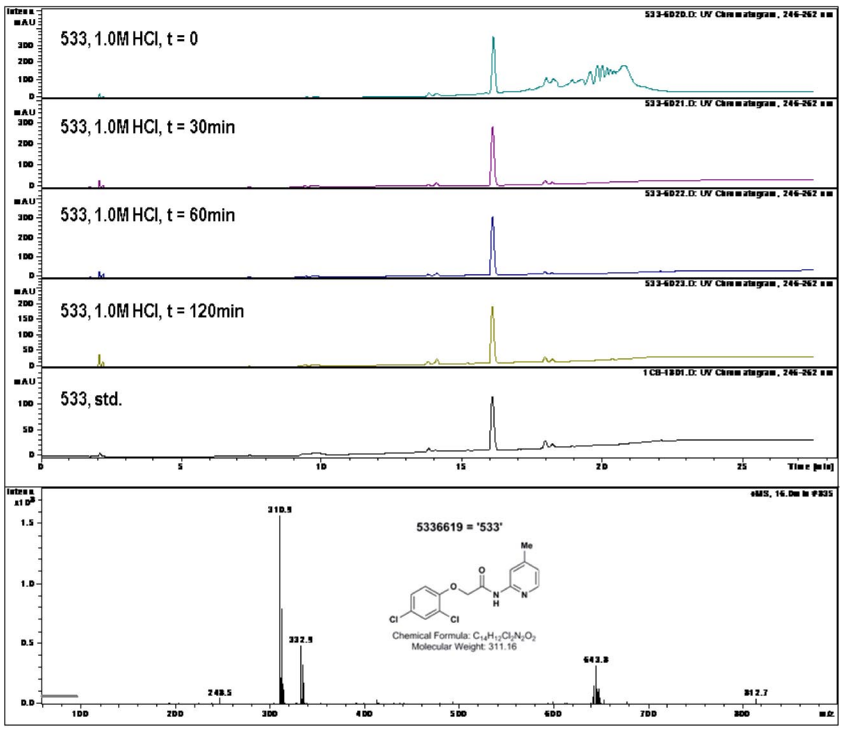


Fig. S1. Hypocotyl assay on different chemicals. (*A*) The hydrolyzed product of **533** and **602**, 2-amino-4-picoline, had no effect on hypocotyl length. (*B*) The **533** bipartite auxin conjugate induced hypocotyl elongation more efficiently when in direct contact with the shoot. Seedlings grown in horizontal plates were compared with seedlings grown in vertically oriented plates. Error bars indicate SE.

A



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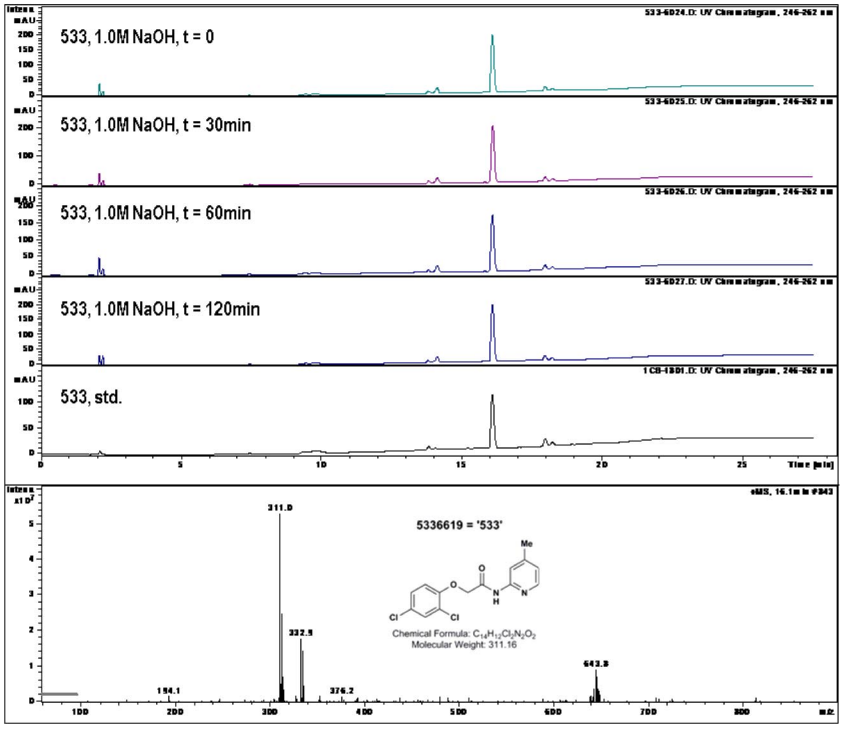
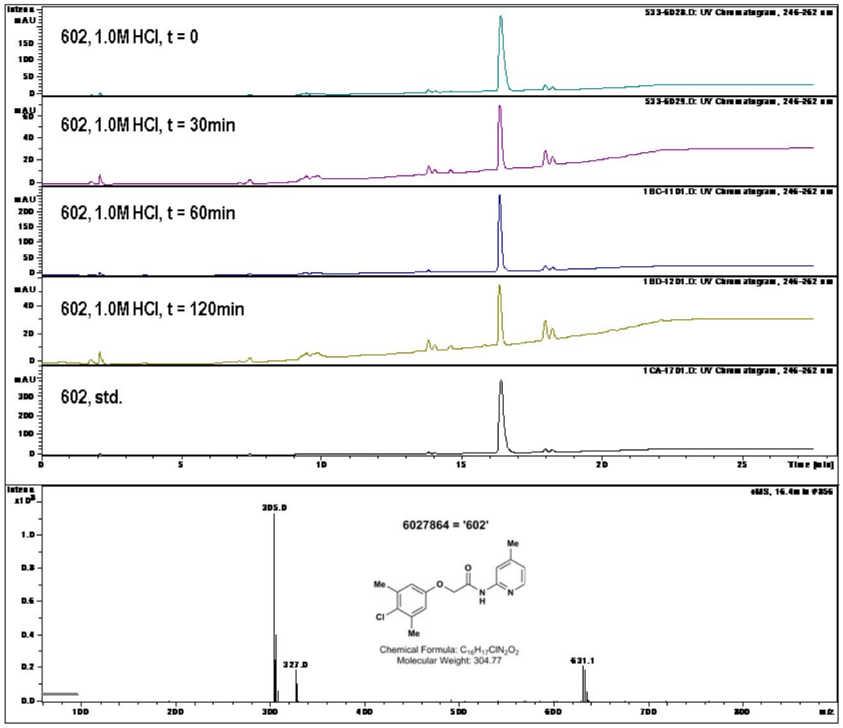


Fig. S2. Proauxin stability studies by LC-MS: (*A*) **533** acid digestions; (*B*) **533** base digestions; (*C*) **602** acid digestions; and (*D*) **602** base digestions. Analysis of the LC-MS data were consistent for the stability of both **533** and **602** to strong acid or base over a 2-h period (for details, see the text). In both trials, the only discernible peak was that of the corresponding starting material. Neither the unconjugated free carboxylic acid nor the aminopicoline side product was observed by UV-Vis or MS analysis.

C



D

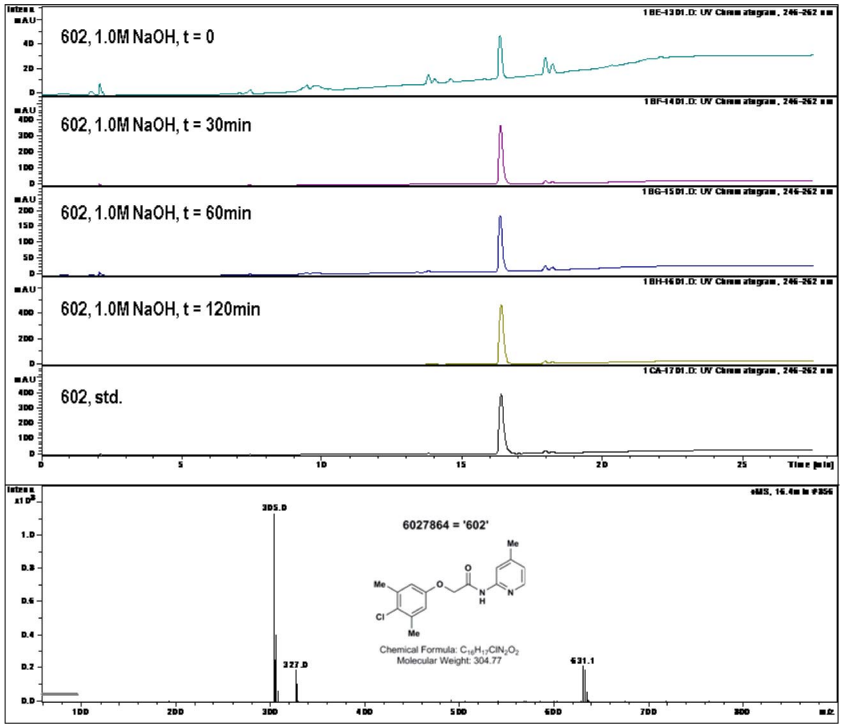


Fig. S2. (continued).

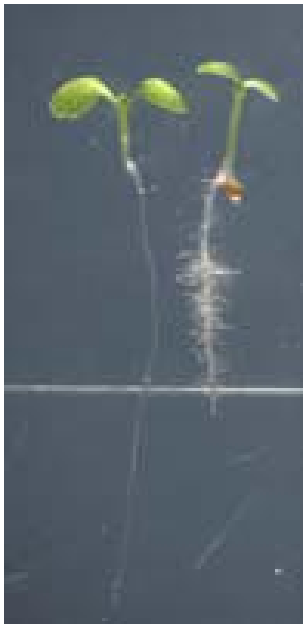


Fig. S3. The effect of **602-UC**. Six-day-old seedlings were grown in the absence or presence of the chemical. A representative seedling is shown. Note the mild induction of hypocotyl length, the inhibition of root length, and induction of root hair length.

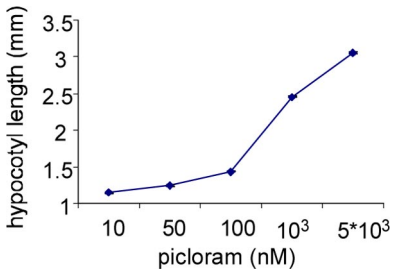


Fig. S4. Hypocotyl lengths of 6-day-old seedlings grown in the presence of picloram. Bars: SE.

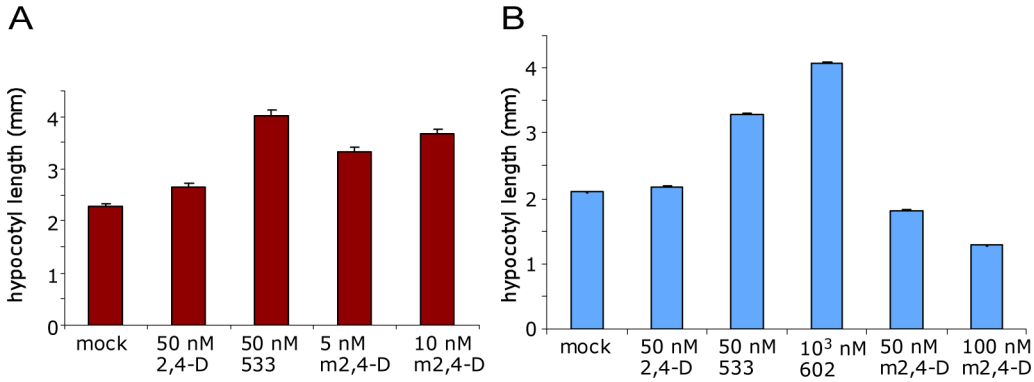


Fig. S5. Hypocotyl lengths of 5-day-old seedlings grown in the presence of 2,4-D, **533**, **602**, and methyl-2,4-D (m2,4-D). (*A* and *B*) Two independent experiments testing low and high m2,4-D concentrations, respectively. Error bars indicate SE.

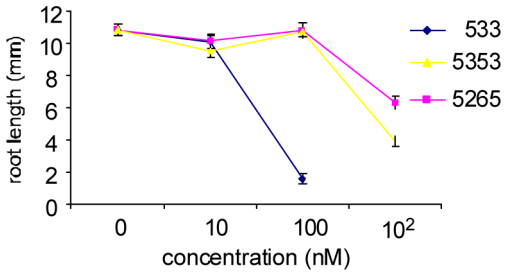


Fig. S6. Root inhibition by bipartite proauxins with different predicted hydrolysis rates. Note that the bipartite proauxins with lower predicted hydrolysis rate compared with **533**, such as **5353** and **5265**, were active at higher concentrations. Error bars indicate SE.

Fig. S7. Auxin-mediated elongation response was diminished in the presence of the brassinosteroid biosynthesis inhibitor BRZ. Six-day-old seedlings are shown.

Other Supporting Information Files

Table S1
Table S2
Table S3
Table S4