Compositions and methods for synthetic amphiphile-induced changes in plant root morphology

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Gokel et al.

COMPOSITIONS AND METHODS FOR SYNTHETIC AMPHIPHILE-INDUCED CHANGES IN PLANT ROOT MORPHOLOGY

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Field of Classification Search

See application file for complete search history.

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FOREIGN PATENT DOCUMENTS
WO 2008062035 A2 5/2008
WO 2013040206 A1 7/2013
WO 2014049546 A2 4/2014

OTHER PUBLICATIONS

ABSTRACT
The disclosure provides a method for increasing the lateral root development of a plant by exposing said plant to a composition containing a synthetic amphiphile. By increasing the number of lateral roots, the surface area of the root structure is increased, making the plants better able to survive such stresses as drought or low nutrients.

4 Claims, 13 Drawing Sheets
Arabidopsis thaliana (Col-0)

Figure 2

C_{14} Benzyl Hydraphile (20µM)
PNS Media
Figure 3

C_{14} benzylhydraphile (2) effect on Arabidopsis thaliana plants

50µM

20µM
Figure 4

Root Density of *A. thaliana* in Presence and Absence of Hydraphiles

Laterale roots/mm primary root
Lateral root density with C8 and C10 LE

Figure 7

Lateral roots/mm primary root

[Lariat ether (μM)]

R² = 0.9442

R² = 0.9663
Heptapeptide: (Xxx)_3-Pro-(Xxx)_3

[Chemical structure diagram]

C-terminal anchor

diacid linker

Figure 8
Figure 12

Number of Lateral Roots

Compound Number

C_{18}

C_{12}
Figure 13

% CI Released from Liposomes

Compound Number
COMPOSITIONS AND METHODS FOR SYNTHETIC AMPHiphile-INDUCED CHANGES IN PLANT ROOT MORPHOLOGY

CROSS-REFERENCE TO RELATED APPLICATIONS


STATEMENT OF FEDERALLY SPONSORED RESEARCH

This invention was made with Government support under Grant No. CHE 1307324 awarded by the National Science Foundation. The Government has certain rights in the invention.

BACKGROUND

The present disclosure relates to methods for inducing changes in plant root morphology. For example, certain methods are directed to increasing the development of lateral root growth while decreasing the development of primary roots.

Natural protein channels function in many organisms to facilitate the transport of ions across cellular boundaries. Channels are known to play critical roles in bacteria, in fungi, in mammalian cells, and in plants. The cells of each of these organisms are bounded by a barrier membrane or cell wall but the nature of these boundary layers can be dramatically different. Even in simple organisms such as bacteria, the boundary layers of Gram-positive and Gram-negative bacteria are very different and a further variation occurs in Mycobacteria. The difference between the boundary layers of mammals and plants is even more dramatic. A similarity among all of these cell types is the need for various molecular and ionic species to pass through the membranes so that ionic balance can be maintained, so that nutrients can enter, and so waste products can exit. Plants in particular have a cell wall that creates challenges in terms of penetration. Natural protein channels typically mediate this process.

Hydrophilic are synthetic amphiphiles that emulate channel function in bacteria. At certain concentrations, they are toxic to bacteria, by a mechanism that disrupts ion homeostasis. Recently, it was shown that hydrophilic enhance the efficacy of antibiotic function against bacteria when co-administered with appropriate pharmaceuticals.


SUMMARY

Certain embodiments provide for methods of treating a plant by adding synthetic amphiphiles to a plant’s growth media at defined concentrations. In certain embodiments, treatment at certain concentrations results in changes in plant root morphology, such as decreased primary root length and/or increased lateral root density. In certain embodiments, such as at higher concentrations, treatment inhibits the growth of the plant.

Certain embodiments are drawn to methods for altering the root morphology of a plant comprising treating one or more plants with a composition comprising a synthetic amphiphile. In certain embodiments, the plant is grown on a solid plant growth media that comprises the synthetic amphiphile. In certain other embodiments, the plant is supplied with an aqueous solution that comprises the synthetic amphiphile. In certain embodiments, the plant is contacted with the aqueous solution. In certain embodiments, the plant is contacted with the aqueous solution on its roots. In certain embodiments, the alteration in the plant root morphology is a decrease in the primary root length, an increase in the lateral root density of the plant, or both a decrease in the primary root length and an increase in the lateral root density, compared to the average primary root length and/or lateral root density of a statistically significant control population of plants that has not been treated with a synthetic amphiphile. In certain embodiments, the treated plant exhibits at least a 2-fold, 3-fold, 4-fold, 5-fold, or more increase in lateral root density compared to the average lateral root density of a statistically significant control population that has not been treated with the synthetic amphiphile. In certain embodiments, a treated plant is selected based on the alteration in root morphology. In certain embodiments, the treated plant is selected for an increase in the lateral root density of the plant or selected for both an increase in the lateral root density and a decrease in the primary root length. In certain embodiments, the treated plant is selected for at least a 2-fold, 3-fold, 4-fold, 5-fold, or more increase in lateral root density. In certain embodiments, the method further comprises growing a selected plant following its selection.

In certain embodiments, the amount of the synthetic amphiphile is an amount that is lower than the amount at which a toxic effect is apparent for certain plants. For example, in certain embodiments, the concentration of the synthetic amphiphile in the composition is less than about 50 μM. For example, the concentration of the synthetic amphiphile in the composition is from about 1.0 μM to about 50 μM, or from about 0.1 μM to about 50 μM, or from about 0.01 μM to about 50 μM, or from about 0.001 μM to about 50 μM. For example, the concentration of synthetic amphiphile in the composition is less than about 25 μM. For example, the concentration of the synthetic amphiphile in the composition is from about 1.0 μM to about 25 μM, or from about 0.1 μM to about 25 μM, or from about 0.01 μM to about 25 μM, or from about 0.001 μM to about 25 μM.

In certain embodiments, the amount of synthetic amphiphile is an amount that is higher than the amount at which a toxic effect is apparent for certain plants. For example, the concentration of the synthetic amphiphile in the composition is greater than about 25 μM. For example, the concentration of the synthetic amphiphile in the composition is greater than about 50 μM. For example, the concentration of the synthetic amphiphile in the composition is greater than about 75 μM. For example, the concentration of the synthetic amphiphile in the composition is from about 25 μM to about 100 μM. For example, the concentration of the synthetic amphiphile in the composition is from about 25 μM to about 100 μM. For example, the concentration of the synthetic amphiphile in the composition is from about 25 μM to about 100 μM.
about 75 \mu M. For example, the concentration of the synthetic amphiphile in the composition is from about 50 \mu M to about 75 \mu M. For example, the concentration of the synthetic amphiphile in the composition is from about 75 \mu M to about 100 \mu M.

In certain embodiments, the primary root length is decreased by at least 90% compared to the average primary root length of a statistically significant control population that has not been treated with the synthetic amphiphile. In certain embodiments, the plant with altered root morphology is a crop plant and wherein the plant exhibits increased yield compared to the average yield of a statistically significant control population that has not been treated with the synthetic amphiphile. In certain embodiments, the plant with altered root morphology exhibits improved growth in low nutrient growth conditions compared to the average growth of a statistically significant control population that has not been treated with the synthetic amphiphile. In certain embodiments, the plant with altered root morphology exhibits improved tolerance to stress conditions selected from the group consisting of drought, flooding, high salt growth conditions, extreme cold, and extreme heat, compared to the average tolerance of a statistically significant control population that has not been treated with the synthetic amphiphile.

Certain embodiments are drawn to methods for inhibiting plant growth comprising treating one or more plants with a composition comprising a synthetic amphiphile at a concentration higher than the concentration that increases lateral root density and decreases primary root length. In certain embodiments, the plant is contacted with the composition on its roots, stems, and/or leaves. In certain embodiments, the plant is contacted with the composition on its leaves. For example, in certain embodiments the concentration of the synthetic amphiphile in the composition is greater than about 25 \mu M. For example, in certain embodiments the concentration of the synthetic amphiphile in the composition is greater than about 50 \mu M. For example, in certain embodiments the concentration of the synthetic amphiphile in the composition is greater than about 75 \mu M. For example, in certain embodiments the concentration of the synthetic amphiphile in the composition is from about 25 \mu M to about 100 \mu M or from about 50 \mu M to about 100 \mu M or from about 75 \mu M to about 100 \mu M. For example, in certain embodiments the concentration of the synthetic amphiphile in the composition is from about 25 \mu M to about 75 \mu M or from about 50 \mu M to about 75 \mu M.

In certain embodiments, the synthetic amphiphile comprises a hydraphile structure of Formula I:

In certain embodiments, the synthetic amphiphile comprises a lariat ether structure of Formula II:

In certain embodiments, the synthetic amphiphile comprises a lariat ether amide structure of Formula III:

In certain embodiments, the synthetic amphiphile comprises a peptide-based synthetic amphiphile structure of Formula IV:

wherein:
R^1 and R^2 are straight-chained, branched-chained, cyclic alkyl, aralkyl, or substituted or unsubstituted aryl having from 1-24 carbons and R^3 and R^4 can be the same or different;
R³ represents the side chains of amino acids in a peptide sequence having 4-10 amino acids, which can be the same or different;
R⁴ is the side chain of an amino acid that is the C-terminal amino acid;
R⁵ is a straight-chained, branched-chained, cyclic alkyl, aralkyl, or substituted or unsubstituted aryl having from 1-18 carbons;
Y is substituted or unsubstituted carbon, nitrogen, sulfur, oxygen or absent;
Z is the connecting element between the C-terminal amino acid and the element defined by R⁵, typically, O, N, or S; and
n is an integer from 3-10.
In certain embodiments, the synthetic amphiphile comprises a peptide-based synthetic amphiphile structure of Formula V:

wherein n can be 5 to 21. In certain embodiments, the growth medium can comprise concentrations up to 50 μM or more before toxicity is the predominant effect.

In certain embodiments, methods comprise supplementing a plant with growth medium containing a synthetic lariat ether amide having Formula III:

wherein n is 4 to 20 (overall side arm lengths are C₈ to C₂₂).
In certain embodiments, the growth medium can comprise concentrations up to 50 μM or more before toxicity is the predominant effect.
In certain embodiments, methods comprise supplementing a plant with growth medium containing a synthetic anion transporter having Formula IV:

Wherein:
R¹ and R² are straight-chained, branched-chained, cyclic alkyl, aralkyl, or substituted or unsubstituted aryl having from 1-24 carbons and R¹ and R² can be the same or different;
R³ represents the side chains of amino acids in a peptide sequence having 4-10 amino acids, which can be the same or different;
R⁴ is the side chain of an amino acid that is the C-terminal amino acid;
R⁵ is a straight-chained, branched-chained, cyclic alkyl, aralkyl, or substituted or unsubstituted aryl having from 1-18 carbons;
Y is substituted or unsubstituted carbon, nitrogen, sulfur, oxygen, or absent;
Z is the connecting element between the C-terminal amino acid and the element defined by R⁵, typically, O, N, or S; and
n is an integer from 3-10.

In certain embodiments, methods comprise supplementing a plant with growth medium containing a synthetic anion transporter having Formula V:

FIG. 13 shows a graphical representation of ion release C₁₈H₃₇ from liposomes.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 illustrates the chemical structures of lariat ethers, lariat ether amides, and hydaphiles, which are exemplified in the present disclosure.

FIG. 2 shows (left panel) normal root development of Arabidopsis thaliana when grown in plant nutrient media plus sucrose (PNS). The right panel shows the change in root morphology leading to a shorter primary root and new lateral roots when C₁₄ benzyl hydrphilic is added at a concentration of 20 μM to PNS media.

FIG. 3 shows the growth of Arabidopsis thaliana in the presence of C₁₄ benzyl hydrphilic, added to the growth media at concentrations of 20 μM (left) and 50 μM. Growth appears normal at 20 μM but stunted at 50 μM.

FIG. 4 is a graphical comparison of Arabidopsis thaliana germination in the presence of growth media to which various substances have been added.

FIG. 5 is a representative synthetic scheme for the preparation of C₁₂ benzyl hydrphilic.

FIG. 6 is a representative synthetic scheme for the preparation of N,N-di-n-octyl-4,13-diazao-18-crown-6.

FIG. 7 is a graphical representation of A. thaliana lateral root density with increasing concentrations (0 μM to 50 μM) of C₈ and C₁₀ lariat ether. Linear increase in lateral root density is observed with increasing concentration of C₈ and C₁₀ lariat ethers.

FIG. 8 illustrates the chemical structure of representative synthetic anion transporters (SATs).

FIG. 9 shows (left panel) normal root development of Arabidopsis thaliana when grown in plant nutrient media plus sucrose (PNS). The right panel shows the change in root morphology leading to a shorter primary root and new lateral roots when (C₁₄H₂₂)₈ NCOCH₂CH₃-CO-GGGPGSGS-OC₈H₄ is added at a concentration of 50 μM to PNS media.

FIG. 10 shows a comparison of primary root lengths in pairs of compounds having C₁₂ and C₁₄ N-terminal anchors and succinyl linkers. The pairs have the following heptapeptide sequences: 6,7 (GGGPGGG); 10,11 (AAAPGGG); 15,16 (GGGPGSGS); 19,20; [GGGPG(t-Bu)GSG(t-Bu)]; and 22,23 [GGGPG(t-Bu)]. The dashed line indicates the primary root length of the controls.

FIG. 11 shows a representative synthetic scheme for the preparation of (C₁₈H₃₇)₈ NCOCH₂CH₃-CO-GGGPGSGS-OC₈H₄ synthetic anion transporter (SAT).

FIG. 12 is a comparison of the number of lateral roots observed in pairs of compounds having C₁₂ and C₁₄ N-terminal anchors and succinyl linkers. The pairs have the following heptapeptide sequences: 6,7 (GGGPGGG); 10,11 (AAAPGGG); 15,16 (GGGPGSGS); 19,20; (GGGPG(t-Bu)GSG(t-Bu)); and 22,23 [GGGPG(t-Bu)]. The dashed line indicates the lateral root number of the controls.

FIG. 13 shows a graphical representation of ion release from liposomes.

DETAILED DESCRIPTION

It is to be noted that the term “a” or “an” entity refers to one or more of that entity; for example, “a hydrphile” is understood to represent one or more hydrphile compounds. As another example, “a synthetic anion transporter” is understood to represent one or more synthetic anion transporter (SAT) compounds. As such, the terms “a” (or “an”), “one or more,” and “at least one” can be used interchangeably herein.

Furthermore, “and/or” where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term and/or as used in a phrase such as “A and/or B” herein is intended to include “A or B,” “A and B,” “A” (alone), and “B” (alone). Likewise, the term “and/or” as used in a phrase such as “A, B, and/or C” is intended to encompass each of the following embodiments: A, B, C; A and B; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

It is understood that wherever aspects are described herein with the language “comprising...” otherwise analogous aspects described in terms of “consisting of” and/or “consisting essentially of” are also provided.
Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure is related. Units, prefixes, and symbols are denoted in their Système International de Unites (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range. The headings provided herein are not limitations of the various aspects or aspects of the disclosure, which can be had by reference to the specification as a whole.

Overview

Disclosed herein are methods for altering root morphology in plants by growing plants in nutrient media containing synthetic amphiphiles. Lateral roots provide a greater surface area for water and nutrient infusion and often develop when a plant is under stress, such as in drought, flooding, high salt growth conditions, extreme cold, and other challenging conditions.

Under normal conditions of temperature, light, moisture, and nutrients, certain plants grow with a single primary root and few, if any, lateral roots. It has been discovered that when the plant is supplemented with a solution or media containing certain concentrations of synthetic amphiphile molecules, a significant change is observed in the root morphology, which alters from a single primary root to multiple lateral roots and a primary root of diminished length.

Representative examples of three types of synthetic amphiphiles are: hydraphiles comprising the chemical structure of Formula I; lariat ethers comprising the chemical structure of Formula II; and lariat ether amides comprising the chemical structure of Formula III (FIG. 1). Other representative examples are synthetic anion transporters (“SAs”). Examples of these compounds have been added in the growth media in plant model systems in order to treat the plants with synthetic amphiphiles and resulted in changes in root morphology.

The effect treating a plant with a composition comprising a synthetic amphiphile on root morphology or other physical characteristics or attributes can be quantified. For example, a change in primary root length can be determined by measuring the difference, if any, in primary root length of a plant treated with the composition compared to the average of a control sample of plants of the same type grown under identical conditions in the absence of the composition. A change in lateral root density can be determined by comparing the lateral root density of a plant treated with the composition to the average lateral root density of a sample grown under identical conditions in the absence of the composition. Lateral root density can be determined by dividing the number of lateral roots by the length of the primary root. In certain embodiments for any quantitated characteristic or attribute, the average value of the control is the average of a statistically significant population of control plants. For example, in certain illustrative embodiments disclosed herein, three trials of about 20 plants each were used to determine the average of the control.

Hydraphiles

Synthetic amphiphiles known as hydraphiles that contain three macrocyclic polyether rings are known to affect plant root morphology as disclosed in Patel, M. B., Stavri, A., Curvey, N. S., Gökbel, G. W.; Hydraphile synthetic ion channels alter root architecture in Arabidopsis thaliana. Chemical Communications. 2014, 50, 11562-11564. These hydraphiles are compounds that insert into bilayer membranes and form ion-conducting channels.

Certain aspects are drawn to treating a plant by the addition of any one of the synthetic hydraphiles disclosed. In certain embodiments, the plant is supplemented with a composition comprising the hydraphile. In certain embodiments, the composition comprising the hydraphile is water and/or an aqueous solution. In certain embodiments, the composition comprising the hydraphile is a solid plant growth medium. An illustrative example is a plant nutrient media containing 0.5% sucrose and 0.6% agar. In certain embodiments, the hydraphile is dissolved, sulfused, etc., as appropriate for the composition within which it is contained, which can be readily determined by one of ordinary skill in the art. In certain embodiments, the addition of a hydraphile to a plant causes a change in plant root morphology such as a change in primary root length and/or a change in lateral root density. In certain embodiments, the addition of a hydraphile causes a decrease in primary root length and/or an increase in lateral root density. In certain embodiments, addition inhibits growth of the plant.

Certain illustrative hydraphile embodiments are shown in Table 1. Table 1 shows the effect of three benzyl hydraphiles (of Formula I), compounds 1-3 (having n=8, n=14, and n=16, respectively), on primary root development.

<p>| TABLE 1 |</p>
<table>
<thead>
<tr>
<th>Additive effects on A. thaliana primary root development*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. thaliana (Col-0) plant</td>
</tr>
<tr>
<td>Plant nutrient + sucrose (PNS)</td>
</tr>
<tr>
<td>DMSO (0.2 vol-%)</td>
</tr>
<tr>
<td>C₈ hydraphile (1) 20 μM</td>
</tr>
<tr>
<td>C₈ hydraphile (1) 50 μM</td>
</tr>
<tr>
<td>C₁₄ hydraphile (2) 20 μM</td>
</tr>
<tr>
<td>C₁₄ hydraphile (2) 50 μM</td>
</tr>
<tr>
<td>C₁₄ hydraphile (3) 10 μM</td>
</tr>
<tr>
<td>C₁₄ hydraphile (3) 20 μM</td>
</tr>
<tr>
<td>C₁₄ hydraphile (3) 50 μM</td>
</tr>
</tbody>
</table>

*The standard deviations reflect a minimum of three trials of 21-27 plants per trial.

The data in Table 1 are expressed as the measured primary root length in millimeters (middle column) and as the percent the primary root length diminished under the specified conditions. Compounds 2 and 3 had a concentration dependent effect on primary root length. In the presence of 20 μM and 50 μM compound 2, the primary root length decreased to 14±2 mm and 0.7±0.2 mm, respectively. The primary root development was decreased by 63% with 20 μM compound 2. When the concentration of compound 2 was increased to 50 μM, the primary root development was completely inhibited; it was decreased by 98%. In the presence of 10 μM and 20 μM C₁₄ benzyl hydraphile (compound 3), the primary root length was decreased to 23±0.4 mm and 15±0.5 mm, respectively. In the presence of 50 μM compound 3, the primary root length decreased to 4±0.2 mm, a 90% decrease in root length. Increasing concentrations of compounds 2 and 3 resulted in decreased primary root length of the A. thaliana samples studied. Both C₁₄ and C₁₆ benzyl hydraphiles inhibit the primary root development at 50 μM concentration. Here, the primary root length of the A. thaliana plant in presence of 50 μM hydraphile is so short that the growth and development of the plant is minimal and also causes chlorosis of the leaves. This could result in inhibition of plant growth.
Further studies were conducted to determine the effect of benzylic hydraphiles (of Formula I) on the lateral root density of *A. thaliana*. The average number of lateral roots observed for plant nutrient media plus sucrose (PNS) and for PNS in the presence of DMSO control was 5±1. The lateral root density for PNS was 0.14±0.02 and the same for 0.2 volume-% DMSO (0.13±0.01).

FIG. 2 illustrates root development in the presence of PNS and PNS+C14. The number of lateral roots observed for 20 μM C16 benzylic hydraphile (2) was 6.2±0.3, whereas the lateral root density observed was 0.48±0.08. This is a 4-fold increase in lateral root density caused by the presence of compound 2.

FIG. 3 illustrates root development in the presence of C14 at different concentrations. At 50 μM C14 benzylic hydraphile, there is a decrease in the primary root length by 98% and an inhibition of lateral root development causing chlorosis of the leaves. This indicates the inhibition of growth and development of plants, which can be due to the toxicity of the hydraphile at this concentration.

FIG. 4 is a graphical comparison of the root development in the presence of different growth media showing that the lateral root densities observed with C8 benzylic hydraphile (1) at 20 μM and 50 μM were 0.15±0.01 and 0.18±0.03, respectively. Within experimental error, C8 benzylic hydraphile did not affect lateral root density.

Without being bound by theory, it is thought that the C8 benzylic hydraphile (1) is not an efficient pore-former, although it can function as an ion transporter. It did not conduct sodium cation efficiently in a study of hydraphiles having various chain lengths ("n" in FIG. 1) as described in the Weber, M. E., Schlesinger, P. H., Gokel, G. W.; Dynamic Assessment of Bilayer Thickness by Varying Phospholipid and Hydraphile Synthetic Chain Lengths. *Journal of the American Chemical Society* 2005, 127(2), 636-642. In this case, the C8 benzylic hydraphile was used as a control. A possible mechanism for the action of hydraphiles is that they affect ion transport and/or compete with endogenous pore-formers. Without being bound by theory, if C8 benzylic hydraphile was inactive and significant changes in root morphology were observed with C14 benzylic hydraphile (2), it would correlate to the known ion transport or poreformation abilities of these two compounds.

In the presence of 20 μM and 50 μM concentrations of C8 benzylic hydraphile (compound 1), the average primary root length was 34±7 mm and 31±6 mm, respectively as recorded in Table 1. The change in primary root length observed with C8 benzylic hydraphile (1) was within experimental error of PNS alone (38 mm) and PNS+DMSO (35 mm) controls. Within experimental error, C8 benzylic hydraphile did not affect the development of primary roots.

As the pore forming and ion transport abilities of benzylic hydraphiles depend significantly on the spacer chain lengths that define the molecule’s overall length, the failure of C8 benzylic hydraphile to alter root architecture, is consistent with a mechanism that involves ion transport, although the plant growth could be indifferent to 1 for a variety of reasons. The comparison of the effect of C8 and C14 hydraphiles is useful because the two compounds have identical component structures and differ chemically mainly in length and molecular weight. The difference in the effect on root architecture of C8 benzylic hydraphile (1), which does not form ion channels, and C14 benzylic hydraphile (2), which is an excellent ion transporter, is consistent with the channel function of these synthetic amphiphiles. The effect of the ion-transporting hydraphiles in this case is similar to that of the auxins, which affect channel function and both the growth and development of the plant.

**Larit Ethers and Larit Ether Amides.**

Larit ethers are synthetic amphiphiles that are known to insert in bilayer membranes and transport cations. Recently, it was found that larit ethers enhance the efficacy of antibiotic function against bacteria when co-administered with appropriate pharmaceuticals.

Certain aspects are drawn to treating a plant by the addition of any one of the larit ethers or larit ether amides disclosed herein to a plant. In certain embodiments, a plant is supplemented with a composition comprising the larit ether or larit ether amide. In certain embodiments, a plant is contacted with a composition comprising the larit ether or larit ether amide in either a solid plant growth medium. An illustrative example is a plant nutrient media containing 0.5% sucrose and 0.6% agar. In certain embodiments, the larit ethers or larit ether amides is dissolved, sulfused, etc., as appropriate for the composition within which it is contained, which can be readily determined by one of ordinary skill in the art. In certain embodiments, the addition of a larit ether or larit ether amide to a plant causes a change in plant root morphology such as a change in primary root length and/or a change in lateral root density. In certain embodiments, the addition of a larit ether or larit ether amide causes a decrease in primary root length and/or an increase in lateral root density. In certain embodiments, addition inhibits growth of the plant.

Certain illustrative embodiments are shown in Table 2. Larit ethers and larit ether amides were added to plant nutrient plus sucrose broth to determine the effect on growth or root morphology. Table 2 shows the results of those studies.

### Table 2

<table>
<thead>
<tr>
<th>Conc. (μM)</th>
<th>Primary root length</th>
<th>Percent decrease</th>
<th>Number lateral roots</th>
<th>Lateral root density</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNS</td>
<td>n/a</td>
<td>40.6</td>
<td>n/a</td>
<td>6.5</td>
</tr>
<tr>
<td>DMSO 0.2%</td>
<td>37.9</td>
<td>6.7</td>
<td>6.3</td>
<td>0.17</td>
</tr>
<tr>
<td>Larit ethers&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C6</td>
<td>20</td>
<td>38.3</td>
<td>5.9</td>
<td>6.6</td>
</tr>
<tr>
<td>C6</td>
<td>50</td>
<td>33.0</td>
<td>18.8</td>
<td>4.6</td>
</tr>
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<td>C8</td>
<td>20</td>
<td>6.8</td>
<td>83.4</td>
<td>5.1</td>
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<tr>
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<td>50</td>
<td>1.6</td>
<td>96.1</td>
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<td>C10</td>
<td>20</td>
<td>3.5</td>
<td>91.4</td>
<td>4.4</td>
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</tr>
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<td>C12</td>
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<td>41.5</td>
<td>0</td>
<td>6.9</td>
</tr>
<tr>
<td>C14</td>
<td>50</td>
<td>20.9</td>
<td>48.4</td>
<td>4.9</td>
</tr>
<tr>
<td>Larit ether amides&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C6</td>
<td>50</td>
<td>38.6</td>
<td>4.9</td>
<td>5.6</td>
</tr>
<tr>
<td>C7</td>
<td>20</td>
<td>22.9</td>
<td>45.3</td>
<td>4.5</td>
</tr>
<tr>
<td>C7</td>
<td>50</td>
<td>7.3</td>
<td>82.5</td>
<td>4.2</td>
</tr>
<tr>
<td>C8</td>
<td>50</td>
<td>31.2</td>
<td>23.2</td>
<td>5.2</td>
</tr>
<tr>
<td>C9</td>
<td>20</td>
<td>9.2</td>
<td>78.2</td>
<td>1.6</td>
</tr>
<tr>
<td>C9</td>
<td>50</td>
<td>15.7</td>
<td>62.6</td>
<td>2.0</td>
</tr>
<tr>
<td>C10</td>
<td>50</td>
<td>3.1</td>
<td>92.4</td>
<td>0.6</td>
</tr>
<tr>
<td>C12</td>
<td>50</td>
<td>39.9</td>
<td>1.73</td>
<td>7.0</td>
</tr>
</tbody>
</table>
TABLE 2 - continued
Effect of lariat ethers or lariat ether amides on the growth of A. thaliana when added to PNS growth media.

<table>
<thead>
<tr>
<th>Concentration (μM)</th>
<th>Primary root length (%)</th>
<th>Percent decrease in root length</th>
<th>Number of lateral roots</th>
<th>Lateral root density</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14</td>
<td>20</td>
<td>36.9</td>
<td>11.8</td>
<td>5.1</td>
</tr>
<tr>
<td>C18</td>
<td>50</td>
<td>35.6</td>
<td>14.9</td>
<td>4.2</td>
</tr>
<tr>
<td>C24</td>
<td>50</td>
<td>41.9</td>
<td>0</td>
<td>8.6</td>
</tr>
</tbody>
</table>

*92% by volume.

In the series of lariat ethers and lariat ether amides shown, only the C14 and C18 lariat ethers shows a significant change in root morphology when administered at 20 μM. Some variation is observed when the concentration of additive is 50 μM, but this concentration borders on being toxic to the plant.

FIG. 7 shows the relationship between lateral root density and lariat ether concentration. The two compounds studied were 4,13-diaza-18-crown-6 derivatives (C14 and C18 lariat ethers) having linear C14 or C18 alkyl side arms. The two compounds were administered to the growth medium in concentrations of 5, 15, 25, 35, 45, 50 μM. For both compounds, the calculated value for lateral roots divided by length (in mm) of the primary root was graphed on the ordinate as a function of concentration. The two data sets were modeled by linear regression which shows a straight line relationship in both cases. The “goodness of fit” or correlation parameter (R²) for both C14 and C18 for C18 10.45 and 0.94 for C18. Synthetic Anion Transporters.

Synthetic anion transporters (“SATs”) that are present in this disclosure are amphiphiles that facilitate the transport of anions across bilayer membranes. These compounds have four structural modules. They are (1) a hydrophobic anchor chain, (2) a linker element that is typically a diacid, (3) a peptide, and (4) a C-terminal residue appended to the carboxyl group.

Table 3 illustrates chemical structures of the synthetic anion transporter (“SAT”) synthetic amphiphiles. Table 3 shows several representative compounds. Further, a representative synthetic scheme for the SAT compounds is shown in FIG. 11.

TABLE 3
Structures of Compounds 4-24

<table>
<thead>
<tr>
<th>No.</th>
<th>Twin anchors</th>
<th>Linker</th>
<th>Peptide</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>n-C4H13</td>
<td>-COCH3CH2CO-</td>
<td>GGGPGGG</td>
</tr>
<tr>
<td>5</td>
<td>n-C4H13</td>
<td>-COCH3CH2CO-</td>
<td>GGGPGGG</td>
</tr>
<tr>
<td>6</td>
<td>n-C4H13</td>
<td>-COCH3CH2CO-</td>
<td>GGGPGGG</td>
</tr>
<tr>
<td>7</td>
<td>n-C4H13</td>
<td>-COCH3CH2CO-</td>
<td>GGGPGGG</td>
</tr>
<tr>
<td>8</td>
<td>n-C4H13</td>
<td>-COCH3CH2CO-</td>
<td>GGGPGGG</td>
</tr>
<tr>
<td>9</td>
<td>n-C4H13</td>
<td>-COCH3CH2CO-</td>
<td>GGGPGGG</td>
</tr>
<tr>
<td>10</td>
<td>n-C4H13</td>
<td>-COCH3CH2CO-</td>
<td>GGGPGGG</td>
</tr>
<tr>
<td>11</td>
<td>n-C4H13</td>
<td>-COCH3CH2CO-</td>
<td>GGGPGGG</td>
</tr>
<tr>
<td>12</td>
<td>n-C4H13</td>
<td>-COCH3CH2CO-</td>
<td>GGGPGGG</td>
</tr>
<tr>
<td>13</td>
<td>n-C4H13</td>
<td>-COCH3CH2CO-</td>
<td>GGGPGGG</td>
</tr>
<tr>
<td>14</td>
<td>n-C4H13</td>
<td>-COCH3CH2CO-</td>
<td>GGGPGGG</td>
</tr>
<tr>
<td>15</td>
<td>n-C4H13</td>
<td>-COCH3CH2CO-</td>
<td>GGGPGGG</td>
</tr>
<tr>
<td>16</td>
<td>n-C4H13</td>
<td>-COCH3CH2CO-</td>
<td>GGGPGGG</td>
</tr>
<tr>
<td>17</td>
<td>n-C4H13</td>
<td>-COCH3CH2CO-</td>
<td>GGGPGGG</td>
</tr>
<tr>
<td>18</td>
<td>n-C4H13</td>
<td>-COCH3CH2CO-</td>
<td>GGGPGGG</td>
</tr>
<tr>
<td>19</td>
<td>n-C4H13</td>
<td>-COCH3CH2CO-</td>
<td>GGGPGGG</td>
</tr>
<tr>
<td>20</td>
<td>n-C4H13</td>
<td>-COCH3CH2CO-</td>
<td>GGGPGGG</td>
</tr>
<tr>
<td>21</td>
<td>n-C4H13</td>
<td>-COCH3CH2CO-</td>
<td>GGGPGGG</td>
</tr>
<tr>
<td>22</td>
<td>n-C4H13</td>
<td>-COCH3CH2CO-</td>
<td>GGGPGGG</td>
</tr>
</tbody>
</table>

TABLE 4

<table>
<thead>
<tr>
<th>Compound</th>
<th>Primary root length (mm)</th>
<th>Number of lateral roots</th>
<th>Lateral root density</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>40.4 ± 3.8</td>
<td>61 ± 0.8</td>
<td>0.15 ± 0.01</td>
</tr>
<tr>
<td>5</td>
<td>3.9 ± 0.6</td>
<td>3.4 ± 0.6</td>
<td>0.85 ± 0.12</td>
</tr>
<tr>
<td>6</td>
<td>30.5 ± 0.6</td>
<td>4.4 ± 0.8</td>
<td>0.15 ± 0.03</td>
</tr>
</tbody>
</table>

Certain aspects are drawn to treating a plant by the addition of any one of the SATs disclosed herein to a plant. In certain embodiments, a plant is supplemented with a composition comprising the SAT. In certain embodiments, a plant is contacted with a composition comprising the SAT. In certain embodiments, the composition comprising the SAT is water and/or an aqueous solution. In certain embodiments, the composition comprising the SAT is a solid plant growth medium. An illustrative example is a plant nutrient medium containing 0.5% sucrose and 0.6% agar. In certain embodiments, the SAT is dissolved, sulfuized, etc., as appropriate for the composition within which it is contained, which can be readily determined by one of ordinary skill in the art. In certain embodiments, the addition of an SAT to a plant causes a change in plant root morphology such as a change in primary root length and/or a change in lateral root density. In certain embodiments, the addition of an SAT causes a decrease in primary root length and/or an increase in lateral root density. In certain embodiments, addition inhibits growth of the plant.

Illustrative of certain embodiments, approximately 60 plants were grown on PNS media (no additives) and root lengths and the number of lateral roots were recorded for each. The data points were averaged to obtain the following baseline values: primary root length=40.4±3.8 mm and number of lateral roots=61±0.8, respectively. The lateral root density was obtained by dividing the number of lateral roots by the length (in mm) of the primary root. The control value was (61/40.4)=0.15.

Test compounds were added to the growth medium as 0.2% by volume DMSO. A control for DMSO (60 plants) showed no effect was observed on growth or root morphology compared to the PNS control absent DMSO (data not shown). Each SAT was added to a concentration of 50 μM in the PNS/agar growth medium.

2,4-Dichlorophenoxyacetic acid (2,4-D) is a well-known, broad leaf herbicide was used as a positive control. 2,4-D was present in the PNS medium at a concentration of 100 nM and plants were grown as noted above. 2,4-D significantly decreases both primary root length and lateral root density to 3.9±0.6 and 3.4±0.6, respectively. The calculated lateral root density in this case was 0.85.
TABLE 4—continued

<table>
<thead>
<tr>
<th>Compound Number</th>
<th>Primary root length (mm)</th>
<th>Number of lateral roots</th>
<th>Lateral root density</th>
</tr>
</thead>
<tbody>
<tr>
<td>(50 μM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compound 7</td>
<td>30.6 ± 4.3</td>
<td>3.9 ± 0.7</td>
<td>0.13 ± 0.01</td>
</tr>
<tr>
<td>Compound 8</td>
<td>28.4 ± 5.4</td>
<td>6.0 ± 2.2</td>
<td>0.23</td>
</tr>
<tr>
<td>Compound 9</td>
<td>40.5 ± 3.4</td>
<td>6.8 ± 1.3</td>
<td>0.17 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>AAAPGGG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compound 10</td>
<td>28.4</td>
<td>3.4</td>
<td>0.12</td>
</tr>
<tr>
<td>Compound 11</td>
<td>39.4 ± 6.5</td>
<td>6.1 ± 1.2</td>
<td>0.16 ± 0.01</td>
</tr>
<tr>
<td>Compound 12</td>
<td>45.2 ± 8.0</td>
<td>6.5 ± 0.9</td>
<td>0.15 ± 0.01</td>
</tr>
<tr>
<td>Compound 13</td>
<td>36.9 ± 7.5</td>
<td>5.2 ± 2.0</td>
<td>0.18 ± 0.02</td>
</tr>
<tr>
<td>Compound 14</td>
<td>40.3 ± 10.1</td>
<td>7.0 ± 1.7</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>GGPPGSG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compound 15</td>
<td>9.0 ± 1.2</td>
<td>4.5 ± 0.4</td>
<td>0.51 ± 0.03</td>
</tr>
<tr>
<td>Compound 16</td>
<td>46.0 ± 12.0</td>
<td>8.7 ± 4.9</td>
<td>0.17 ± 0.06</td>
</tr>
<tr>
<td>Compound 17</td>
<td>40.9 ± 10.8</td>
<td>6.2 ± 2.4</td>
<td>0.15 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>GGPPGSG(t-Bu)G(t-Bu)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compound 18</td>
<td>30.2 ± 7.3</td>
<td>5.8 ± 0.7</td>
<td>0.20 ± 0.03</td>
</tr>
<tr>
<td>Compound 19</td>
<td>28.3</td>
<td>5.9</td>
<td>0.15</td>
</tr>
<tr>
<td>Compound 20</td>
<td>35.5 ± 2.5</td>
<td>5.0 ± 0.2</td>
<td>0.17 ± 0.02</td>
</tr>
<tr>
<td>Compound 21</td>
<td>29.3 ± 5.9</td>
<td>5.2 ± 0.3</td>
<td>0.21 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>GGPPGSG(t-Bu)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compound 22</td>
<td>47.3 ± 0.5</td>
<td>7.0 ± 0.5</td>
<td>0.17 ± 0.08</td>
</tr>
<tr>
<td>Compound 23</td>
<td>48.2 ± 0.7</td>
<td>8.2</td>
<td>0.17</td>
</tr>
<tr>
<td>Compound 24</td>
<td>47.7 ± 1.7</td>
<td>6.8</td>
<td>0.15 ± 0.01</td>
</tr>
</tbody>
</table>

The most extensively studied SAT compounds have a GGPPGSG (Gly-Gly-Gly-Pro-Gly-Gly-Gly) heptapeptide sequences. These compounds were designed to be chloride ion transporters. Planar bilayer conductance data confirmed this capability and function. Compounds 4-10 have the GGPPGSG peptide sequence, but differ both in the anchor and linker chains. The primary root length and number of lateral roots for 9 is within experimental error of the PNS control. The average primary root length for 4-8 is 30 mm, which compares with 40.4 mm for the PNS control.

The linker in 4-7 are succinic acid (Y of Formula IV is absent) and diglycic acid (Y = O) in 8 and 9. Direct comparisons can be made between compounds 4 and 8, which have n-hexyl N-terminal anchors and 7 and 9 which have n-octadecyl twin tails respectively. Thus, the structural differences in these compound pairs reside in the linkers (O present vs. O absent). Table 4 shows that 9 is essentially identical control but 7 shows diminished primary root length and lateral root number. Since both variables are reduced in 7 compared to 9, no net effect is observed on lateral root density. Compounds 4-8 caused diminished growth rate.

The heptapeptide sequence in compounds 10-14 is AAAPGGG (Ala-Ala-Ala-Pro-Gly-Gly-Gly). Compounds 10 and 11 have succinyl linkers (Y is absent in Figure IV) and 12 and 13 are linked by diglycic acid diamide. A different linker is present in 11, which has the structure (C6H13)2NCOCH2CH2CO-AAAAAPGGG-OC7. The linker here is thioglycic acid (Formula IV, Y = S). In short, no significant deviation from control was observed with 11-14 despite variations in linker and anchor chain length. Compound 9 can be compared directly to 13. Their structures are identical except for the GGPPGSG (9) vs. AAAPGGG (13) peptide sequences. Neither compound differs from the control in its biological effect on A. thaliana.

Compound 10, however, which has the structure (C12H25)2NCOCH2CH2CO-AAAAAPGGG-OC7, showed primary root length comparable to that observed for 4-8, but an even smaller number of lateral roots. SATs 10 and 11 are identical except for the N-terminal anchor chains, which are n-dode-
same suggests that it is the peptide sequence that is important rather than the N-terminal chain length. However, compound 10 reduces primary root length while its partner, 8, shows no effect, suggesting that the peptide sequence itself cannot account for the difference.

The most striking results were observed with the 15,16 pair. In both cases, the heptapeptide sequence is GGPGSGS. The N-terminal anchor chains are C12 in 15 and C18 in 16. The former shows a dramatic reduction in primary root length and the latter an increase outside of experimental error relative to control. In a previous study, it was found that the amide hydrogens of amino acids G and G were the key C1-"binding" donors when studied by NMR in a micellar matrix. (Cook, G. A.; Pawlowski, R.; Aburi, M.; Smith, P. E.; Prakash, O.; Tomich, J. M.; Gokel, G. W., NMR structure and dynamic studies of anion-binding, channel-forming heptapeptide, J. Am. Chem. Soc. 2006, 128, 1633-8). At present, there is no direct evidence that C1— or any ion—binding is critical to the effect these compounds have on plants. Notwithstanding, the difference in effect on A. thaliana by 15 and 16 is dramatic and striking.

The results shown in FIG. 12 parallel those of FIG. 10, i.e., they show the effect of the same compounds on the number of lateral roots observed when administered to A. thaliana. As with primary root length, compounds 16, 22, and 24 show enhancements relative to controls. As with primary root length, 11 shows no effect on lateral root number. In contrast, compounds 6, 7, 18, and 19 showed similar, reduced primary root length, but the number of lateral roots is unaltered by the presence of 18 and 19. The lateral root number is diminished by the presence of 7 and 10 by an approximately equal amount and 15 does not show such a dramatic effect as is apparent in primary root length. FIG. 12 shows a comparison of the number of lateral roots observed in pairs of compounds having C12 and C18 N-terminal anchors and succinyl linkers. The pairs have the following heptapeptide sequences: 6,7 (GGPGPGG); 10,11 (AAAPGG); 15,16 (GGPGPSG); 18,19; [GGPGPS(Bu) GS(t-Bu)]; and 22,23 [GGPGPS(t-Bu)G]. The dashed line indicates the lateral root number of the controls.

A possible explanation for the activity of SAT compounds in A. thaliana is that they can alter ionic concentrations in the growing plant. FIG. 6 shows a survey of the ability of various SAT compounds to release ions from synthetic vesicles. It is well known that changes in ion concentrations can affect plant growth and development.

It has been discovered that synthetic amphiphiles are effective in promoting lateral root growth at lower concentrations but diminish the growth of the primary roots. The concentration ranges of the synthetic anion transporters (SATs) for promoting lateral root growth in plants vary depending on the plant species and/or the particular synthetic amphiphiles employed. Furthermore, in another aspect, a composition having a high synthetic amphiphile concentration, such as a high synthetic anion transporter concentration, can be employed as a herbicide for undesired plants, such as by spraying a composition comprising such composite onto the leaves of growing plants.

In certain embodiments, a plant is treated by contacting it with water and/or an aqueous solution comprising a synthetic amphiphile and/or synthetic anion transporter disclosed herein. In certain embodiments, the concentration in water is about 0.001 μM, 0.01 μM, 0.1 μM, 1 μM, 5 μM, 10 μM, 15 μM, 20 μM, 25 μM, 30 μM, 40 μM, 50 μM, 60 μM, 75 μM, or 100 μM. In certain embodiments, the concentration in water is about 0.01 μM to about 1 μM, 5 μM, 10 μM, 15 μM, 20 μM, 25 μM, 30 μM, 40 μM, 50 μM, 60 μM, 75 μM, or 100 μM. In certain embodiments, the concentration in water is about 0.1 μM to about 1 μM, 5 μM, 10 μM, 15 μM, 20 μM, 25 μM, 30 μM, 40 μM, 50 μM, 60 μM, 75 μM, or 100 μM. In certain embodiments, the concentration in water is about 1 μM to about 5 μM, 10 μM, 15 μM, 20 μM, 25 μM, 30 μM, 40 μM, 50 μM, 60 μM, 75 μM, or 100 μM. In certain embodiments, the concentration in water is about 5 μM to about 10 μM, 15 μM, 20 μM, 25 μM, 30 μM, 40 μM, 50 μM, 60 μM, 75 μM, or 100 μM. In certain embodiments, the concentration in water is about 10 μM to about 25 μM, 30 μM, 40 μM, 50 μM, 60 μM, 75 μM, or 100 μM. In certain embodiments, the concentration in water is about 25 μM to about 50 μM, 75 μM, or 100 μM. In certain embodiments, the concentration in water is about 50 μM to about 75 μM, 100 μM.
μM, 75 μM, or 100 μM. In certain embodiments, the concentration in the solid media is about 5.0 μM to about 10 μM, 15 μM, 20 μM, 25 μM, 30 μM, 40 μM, 50 μM, 60 μM, 75 μM, or 100 μM. In certain embodiments, the concentration in the solid media is about 10 μM to about 15 μM, 20 μM, 25 μM, 30 μM, 40 μM, 50 μM, 60 μM, 75 μM, or 100 μM. In certain embodiments, the concentration in the solid media is about 25 μM to about 30 μM, 40 μM, 50 μM, 60 μM, 75 μM, or 100 μM. In certain embodiments, the concentration in the solid media is about 50 μM to about 60 μM, 70 μM, 75 μM, 80 μM, 90 μM or 100 μM.

The concentration range can vary depending on the plant species and/or the particular synthetic hydraphile employed. When n is 14 or 16, hydraphile, C₄₋₁₆, respectively, achieves high inhibition to primary root development (in other words, increases lateral root density) at concentrations up to about 25 μM. At concentrations of from about 50 μM to about 100 μM of C₄₋₁₆ hydraphile, the primary root development is inhibited by greater than 90%, which exhibits toxic effects on development and growth of the plant.

The concentration range can vary depending on the plant species and/or the particular synthetic lariat ether employed. When n is 7 or 9, lariat ether, C₇₋₉, respectively, achieves high inhibition against the primary root development and fosters lateral root growth at the lower end of the concentration range. At concentrations ranging from about 25 μM or higher of C₇₋₉ lariat ethers, the primary root development is inhibited by greater than 90%, having toxic effects on the development and growth of the plant.

The concentration range can vary depending on the plant species and/or the particular synthetic lariat ether amide employed. At concentrations ranging from about 25 μM or higher of C₇₋₉ lariat ether amides, the primary root development is inhibited by greater than 90%, having toxic effects on the development and growth of the plant.

The compound defined by Formula IV can be administered at certain concentration ranges. The concentration range can vary depending on the plant species and/or the particular synthetic amphiphile employed. When the structure is that shown as Formula V herein, administration of the compound to plant growth media in which Arabidopsis thaliana is grown achieves high inhibition of primary root development (in other words, increases lateral root density) at concentrations up to 50 μM.

In certain embodiments described anywhere herein, the roots of the plant are contacted with a composition comprising the synthetic amphiphiles and/or synthetic anion transporters, for example when inducing alteration in root morphology is desired. In certain embodiments described anywhere herein, the leaves and/or stem of the plant are contacted with a composition comprising the synthetic amphiphiles and/or synthetic anion transporters, for example, when used as an herbicide.

Although synthetic amphiphiles are effective in promoting lateral root growth at lower concentrations they also diminish the growth of the primary roots at higher concentrations and eventually reach toxic concentrations. The concentration ranges of the synthetic amphiphiles for promoting lateral root growth in plants can vary depending on the plant species and/or the particular synthetic amphiphiles employed. Furthermore, in another aspect, a composite having a high concentration of synthetic amphiphiles can be employed as a herbicide for undesired plants, such as by spraying such composite onto the leaves of growing plants.

The synthetic amphiphiles (all three types having Formulas I, II, and III) and the synthetic anion transporters exemplified by, but not limited to, compound numbers 4-24 can be added to the growth solution by any route, protocol, means, etc., appropriate for its administration and embodiments are not limited to any particular route, protocol, means, etc. of addition. Likewise, the lariat ethers or hydraphiles can be added to the plant growth media having various compositions. All types of plant growth solutions media can be adopted. A representative plant growth media is an aqueous solution containing K₂PO₄, KNO₃, MgSO₄, Ca(NO₃)₂, ferric EDTA (1000x), and micronutrients (1000x). A typical pH of the plant nutrient medium is 5.5.

Disclosed herein are also methods of synthesis for amphiphiles. FIG. 5 illustrates an exemplary synthetic scheme for hydraphiles (Formula I). FIG. 6 is an illustrative example for the synthesis of the chemical structures of N,N'-bis(n-octyl)-4,13-diaza-18-crown-6 (Formula II) and N,N'-bis(2-oxo-n-octyl)-4,13-diaza-18-crown-6 (Formula III).

The following examples of specific aspects are offered for illustrative purposes only, and are not intended to limit the scope of the present disclosure in any way.

EXAMPLES

Example 1. Preparation of Plant Nutrient Media (PN)

Plant Nutrient Media (PN).

To make 500 mL PN media, 485 mL doubly distilled (‘dd’) H₂O was first added to an autoclave beaker. The following salts and nutrients were added to a beaker and mixed well: 5 mL of 250 mM of KPO₄ (pH 5.5), 2.5 mL of 1M KNO₃, 1 mL of 1M MgSO₄, 1 mL of 1M Ca(NO₃)₂, 0.5 mL of Ferric EDTA (1000x or 49 mM) and 0.5 mL of Micronutrients (1000x). In a 1 L autoclaved bottle, 0.6% (3 g) of bacteriological agar was added, followed by 500 mL solution prepared above. The media was autoclaved using the Liquid-30 program.

Micronutrients (1000x) contained 70 mM H₃BO₃, 14 mM MnCl₂·4H₂O, 0.5 mM CuSO₄·5H₂O, 1 mM ZnSO₄·7H₂O, 0.2 mM Na₂MoO₄, 10 mM NaCl, and 0.01 mM COCl₂·6H₂O.

Pouring Plates.

Plant Nutrient Media Plus Sucrose (PNS).

PNS plates were used for plating sterile Arabidopsis thaliana (Columbia-0) seeds. A sterile stock of PNS agar was maintained. Sterile sucrose was added to PN media to make PNS media. To make PNS media containing 0.5% sucrose, 5 mL of 50% sucrose (sterile filtered) was added to 500 mL of PN agar and mixed well.

To Make Plates from PNS Stock.

The stored PNS media was liquefied using a water bath and allowed to cool to 55°C. The type of plates, compounds, and concentrations to be used were confirmed while the media cooled. The plates were labeled on the cover and the bottom with all the relevant information. 50 mL sterile centrifuge tubes were used to measure 29,940 μL (29.94 mL) PNS media. A fresh sterile 50 mL tube was used for each plate. The desired amount and concentration of compound was added to the PNS media, mixed by inverting the tube and poured in the plate. Total volume of media and compound in each plate was kept constant at 30 mL. A maximum of 60 μL (0.2% by volume) compound was added per plate. For example: 60 μL of 25 mM compound was added to 29.94 mL PNS media to get final concentration of 50 μM compound in PNS media. Plates were incubated at room temperature for 2 hours before plating seeds on them.
Sterilizing and Plating *A. thaliana* Seeds.

Hands and bench area were washed thoroughly before beginning. Sterilizing solution was prepared in a sterile 15 ml centrifuge tube by mixing 5 ml of 30% bleach and 5 ml of 10% Triton X-100. *A. thaliana* Col-0 seeds were added to sterile 1.5 ml micro-centrifuge tube. 500 μl of sterilizing solution (30% bleach+0.01% Triton X-100) was added to the seeds, vortexed for 3 seconds and incubated at room temperature for 8 minutes. After 8 minutes, bleach was removed and 1 ml autoclaved MilliQ water was added. Seeds were briefly vortexed, allowed to settle, and the water was removed. The seeds were washed two more times with autoclaved MilliQ water. Seeds were finally suspended in 200 μl of sterile 0.1% agar. These seeds were plated (≥20 seeds per plate) on PNS media using sterilized Pasteur pipettes. Seeds were not allowed to touch each other or the boundaries of the plates. The plates were covered with the lid and sealed with surgical tape. Plates were placed in the Intellus Environment Controller, under continuous white light at 23°C for 11 days.

Measuring Root and Collecting Data.

*A. thaliana* Col-0 seeds (>20 in each trial), the most common strain of this plant, were sterilized and plated on plant nutrient and 0.5% sucrose (PNS) media, containing 0.6% agar. Reference to this type of media can be found in Haughn, G. W., Somerville, C., Sulfonylurea-resistant mutants of Arabidopsis thaliana. Molecular and General Genetics, 1986, 204(3), 430-434 and Gamborg, O. L., Murashige, T., Thorpe, T. A., Vasil, I. K., Plant tissue culture media. *In Vitro* 1976, 12(7), 473-478. The plates were incubated using an Intellus Environment Controller, under continuous white light at 23°C for 11 days. At 11 days, the agar around each plant was disturbed and the plant was pulled out from the agar, using sterile forceps. The length of primary roots for each plant was measured (data reported in millimeters) using a ruler. The plant was then transferred to a petri dish containing water, under a dissecting microscope. All the lateral roots, including emerging and developed roots were counted. The number of lateral roots, emerging from the primary root was counted under a dissecting microscope.

Example 2. Measuring Germinated Col-0 Seeds

*A. thaliana* Col-0 seeds (>20 in each trial), the most common strain of this plant, were sterilized and plated on plant nutrient and 0.5% sucrose (PNS) media, containing 0.6% agar. The plates were incubated using an Intellus Environment Controller, under continuous white light at 23°C. The number of seeds germinated were observed under dissecting microscope and counted. Results were recorded every 12 hours for 3 days. The plates were returned to the incubator for root development.

Example 3

N,N'-Dioctanoyl-4,13-diazia-18-crown-6. This compound was prepared by methods known in the art. To a mixture of 4,13-diazia-18-crown-6 (1.2 g, 4.6 mmol) and Na₂CO₃ (1.4 g, 13 mmol) in benzene (35 ml) was added octanoyl chloride (1.6 g, 9.8 mmol) in benzene (25 ml) over 30 min and then stirred ambient temperature for an additional 16 h. The mixture was filtered and the solvent evaporated in vacuo. The lariat ether amide was obtained crystallization from hexanes in 71% yield as a white solid (mp 54.5-55°C).

Example 4

N,N'-Di-octyl-4,13-diazia-18-crown-6. This compound was prepared by methods known in the art. 4,13-Diazia-18-

crown-6 was acylated with octanoyl and the diamide was reduced with BH₃·THF. Short path distillation afforded the lariat ether (63%) as a colorless oil (bp 181-190°C, 0.04 torr).

Example 5

N,N'-Didecanoyl-4,13-diazia-18-crown-6 was synthesized by a method known in the art and as described above in Example 3 above using decanoyl chloride. The lariat ether amide was obtained (2.48 g, 100%) as a white solid (mp 65.5-67°C).

Example 6

N,N'-Didecyl-4,13-diazia-18-crown-6. To a stirred solution of BH₃·THF (16 ml, 1.0 M) was added the diamide of Example 5 (1.2 g, 2.1 mmol). The reaction was heated at reflux (24 h) and then quenched by dropwise addition of 6.0 M HCl. The reaction was heated for an additional 3 h, cooled, the pH was adjusted 10 with NaOH pellets, and the product extracted with CHCl₃. The organs were dried with MgSO₄ and concentrated in vacuo. Bulk to bulk distillation (bp 205-215°C C.0.07 mm) afforded the lariat ether in 96% yield as a colorless solid, mp 34.5-36.5°C.

Example 7

Synthesis of C₁₀ Benzyl Hydrazilphile.

PhCH₂OCO(CH₂)₇COOH (decanedioic acid monobenzyl ester). This compound was prepared similarly to PhCH₂OCO(CH₂)₇COOH. Dodecanedioic acid (2.90 g, 14.3 mmol), 10% KOH (0.85 g, 15.1 mmol) in MeOH, Bu₄NBr (0.48 g, 1.49 mmol), and PhCH₂Br (2.47 ml, 20.8 mmol) afforded a white solid (2.76 g, 60%).

PhCH₂OCO(CH₂)₇CO-NH-N(CH₃)₂SO₂H (18C-18 crown ether). This compound was prepared similarly to PhCH₂OCO(CH₂)₇CO-NH-N(CH₃)₂SO₂H. Dodecanedioic acid (2.23 g, 7.62 mmol), HBTU (2.89 g, 7.62 mmol), Et(i-Pr)₂N (7.85 ml, 45.1 mmol), and diaza-crown-6 (0.0 g, 9.34 mmol) afforded a colorless oil (2.50 g, 90%). ¹H-NMR: 0.99 (16H, bs), 1.31 (8H, bs), 2.03 (8H, m), 3.18-3.36 (24H, m), 6.89-7.07 (10H, m). ¹³C-NMR: 24.11, 24.49, 28.22, 28.32, 28.50, 28.53, 32.19, 33.33, 33.73, 46.21, 46.29, 48.03, 48.11, 65.02, 68.94, 69.07, 69.32, 69.63, 69.77, 69.91, 70.04, 127.27, 127.69, 135.49, 172.25, 172.27, 172.39.

H₂O₂(C₇H₆)CO-N(CH₃)₂SO₂H (18C-18 crown ether). This compound was prepared similarly to H₂O₂(C₇H₆)CO-N(CH₃)₂SO₂H. PhCH₂OCO(CH₂)₇CO-NH-N(CH₃)₂SO₂H (18C-18 crown ether). This compound was prepared similarly to PhCH₂OCO(CH₂)₇CO-NH-N(CH₃)₂SO₂H. HCl₂OOC(CH₂)₇CO-N(CH₃)₂SO₂H (18C-18 crown ether). This compound was prepared similarly to HCl₂OOC(CH₂)₇CO-N(CH₃)₂SO₂H.
Example 8

PhCH<sub>3</sub>::<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>N</sub>:<sub>
Example 11

Boc-PGGG-OCCH$_3$ Ph

Boc-L-Proline (1.43 g, 6.7 mmol), TsOH GGG-O—CH$_2$Cl (6.7 mmol), and Et$_3$N (2.60 g, 25 mmol) were dissolved in CH$_2$Cl$_2$ (40 mL) and cooled to 0°C. EDCI (1.34 g, 7 mmol) was added and reaction was stirred at ambient temperature for 3 days. Solvent was evaporated and residue was dissolved in EtOAc (50 mL) and washed with saturated aqueous NH$_4$Cl (25 mL) and brine (25 mL), dried over MgSO$_4$ and evaporated. The crude oil product was purified by chromatography (SiO$_2$, 5% MeOH—CH$_2$Cl$_2$) and afforded colorless crystals (2.25 g, 71%, mp 54-55°C).

1H-NMR (CDCl$_3$): 1.42 (9H, s, CH$_3$COOCH$_3$), 1.80-2.20 (4H, m, ProNCH$_2$CH$_2$CH$_2$), 3.35-3.55 (2H, m, ProNCH$_2$CH$_2$CH$_2$), 3.85-4.20 (7H, m, GGG-O—CH$_2$Cl), 5.15 (2H, s, PhCH$_2$O), 7.05 (2H, bs, GGG-O—CH$_2$Cl), 7.30-7.35 (5H, m, H$_5$), 7.80 (11H, bs, GGG-O—CH$_2$Cl). The reaction mixture was further purified by column chromatography (SiO$_2$, 5% MeOH—CH$_2$Cl$_2$), 5.15 (2H, s, PhCH$_2$O), 7.05 (2H, bs, GGG-O—CH$_2$Cl), 7.30-7.35 (5H, m, H$_5$), 7.80 (11H, bs, GGG-O—CH$_2$Cl).

The white solid (1.26 g, 89%) was obtained and recrystallized from EtOAc to give a white solid (0.80 g, 96%), mp 156-164°C. 1H-NMR CD$_2$OD: 0.90 (6H, t, J=6.9 Hz, CH$_3$CO), 1.29 (60H, pseudo-s, CH($\_3$)$_{12}$H$_6$CH$\_2$CH$_2$N), 1.57 (4H, bs, CH$_2$CH$_2$N—CH$\_2$CH$_2$N), 3.21 (2H, t, J=7.8 Hz, CH$_2$CH$_2$N—CH$\_2$CH$_2$N), 3.35 (2H, t, J=7.8 Hz, CH$_2$CH$_2$N), 3.93 (2H, s, s, CH$_2$NCH$_2$), 3.94 (2H, s, s, CH$_2$NCH$_2$), 3.97 (2H, s, s, CH$_2$NCH$_2$), 4.12 (2H, s, s, CH$_2$NCH$_2$), 4.20 (2H, s, s, CH$_2$NCH$_2$), 4.20 (2H, s, s, CH$_2$NCH$_2$)

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The white solid (1.26 g, 89%) was obtained and recrystallized from EtOAc to give a white solid (0.80 g, 96%), mp 156-164°C. 1H-NMR CD$_2$OD: 0.90 (6H, t, J=6.9 Hz, CH$_3$CO), 1.29 (60H, pseudo-s, CH($\_3$)$_{12}$H$_6$CH$\_2$CH$_2$N), 1.57 (4H, bs, CH$_2$CH$_2$N—CH$\_2$CH$_2$N), 3.21 (2H, t, J=7.8 Hz, CH$_2$CH$_2$N—CH$\_2$CH$_2$N), 3.35 (2H, t, J=7.8 Hz, CH$_2$CH$_2$N), 3.93 (2H, s, s, CH$_2$NCH$_2$), 3.94 (2H, s, s, CH$_2$NCH$_2$), 3.97 (2H, s, s, CH$_2$NCH$_2$), 4.12 (2H, s, s, CH$_2$NCH$_2$), 4.20 (2H, s, s, CH$_2$NCH$_2$), 4.20 (2H, s, s, CH$_2$NCH$_2$).
from the present disclosure as come within known or customary practice within the art to which the invention pertains and as can be applied to the essential features herein before set forth.

What is claimed is:

1. A method for increasing the lateral root density of a plant, the method comprising treating one or more plants with a composition comprising a lariat ether amide with the structure of formula III:

   ![Diagram of molecular structure]

   \[ \text{H}_n\text{C(H}_2\text{C)}_n \]
   \[ \text{O} \]
   \[ \text{O} \]
   \[ \text{O} \]
   \[ \text{O} \]
   \[ \text{N} \]
   \[ \text{O} \]
   \[ \text{O} \]
   \[ \text{O} \]
   \[ \text{N} \]
   \[ \text{O} \]
   \[ \text{O} \]
   \[ \text{O} \]
   \[ \text{O} \]
   \[ \text{(CH}_2\text{)}_n\text{CH}_3 \]

wherein \( n \) is 4 to 20, and

wherein the increase in the lateral root density of the plant is in comparison to the average lateral root density of a statistically significant control population of plants that has not been treated with the lariat ether amide.

2. The method of claim 1, further comprising selecting a treated plant based on the increase in lateral root density.

3. The method of claim 1, wherein the concentration of the lariat ether amide in the composition is from about 0.01 \( \mu \text{M} \) to about 1.0 \( \mu \text{M} \).

4. The method of claim 1, wherein the concentration of the lariat ether amide in the composition is from about 1.0 \( \mu \text{M} \) to about 25 \( \mu \text{M} \).

* * * * *