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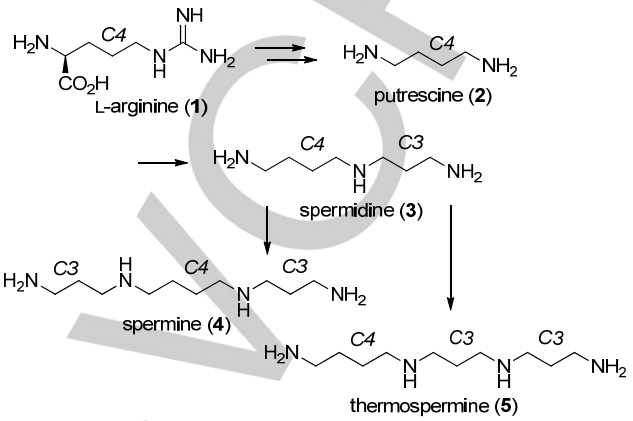
Chemical Synthesis and Biological Effect on Xylem Formation of Xylemin and Its Analogues

Hiroyoshi Takamura[+],*[a] Hiroyasu Motose[+],*[b] Taichi Otsu,[a] Shiori Shinohara,[b] Ryugo Kouno,[b] Isao Kadota,[a] and Taku Takahashi[b]

Abstract: Xylemin (**6**) and its designed structural analogues **18**–**23**, *N*-(4-aminobutyl)alkylamines, were synthesized by 2-nitrobenzenesulfonamide (Ns) strategy. Investigation of the improved synthesis of **20**–**23** resulted in the development of one-step synthesis of these analogues from the commercially available corresponding ketones. Biological assessment of the synthetic molecules elucidated that xylemin (**6**) and the analogue *N*-(4-aminobutyl)cyclopentylamine (**21**) promoted the expression level of thermospermine synthase *ACAULIS5* (*ACL5*) and enhanced xylem formation. In addition, xylemin (**6**) was found to significantly promote lateral root formation, whereas xylemin analogues **18**–**23** including **21** did not. These results indicate that the analogue **21** has the potential as a novel inhibitor of thermospermine synthesis to work specifically in xylem differentiation.



Scheme 1. Biosynthetic pathways of polyamines in plants.

Introduction

Polyamines are low-molecular cationic compounds existing in all organisms. Polyamines regulate various biological processes including RNA/DNA stabilization, transcription of RNA, protein translation, and ion channel modulation through their binding with nucleic acids and proteins.[1] Putrescine (**2**), spermidine (**3**), and spermine (**4**) are commonly found in various species and recognized as major polyamines (Scheme 1). In plants, putrescine (**2**) is synthesized from L-arginine (**1**) through multiple reaction steps.[2] Spermidine (**3**) and spermine (**4**) are synthesized from putrescine (**2**) by sequential addition of an aminopropyl group via the catalytic action of aminopropyl transferases, spermidine synthase (SPDS) and spermine synthase (SPMS), respectively. The aminopropyl group is donated by decarboxylated *S*-adenosylmethionine. These polyamines are involved in various physiological phenomena during plant development and stress response, such as cell death, alkaloid biosynthesis, and organ growth.[3]

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Thermospermine (**5**) is a structural isomer of spermine (**4**) found in bacteria and plants.[4] Thermospermine (**5**) is generated from spermidine (**3**) through adding an aminopropyl group by the action of thermospermine synthase designated ACAULIS5 (ACL5).[5] Because *ACL5* gene is well conserved in plant kingdom from algae to angiosperms, thermospermine (**5**) is a ubiquitous plant polyamine, whereas spermine (**4**) and *SPMS* gene exist only in seed plants but not in algae, bryophytes, and monilophytes.[4b] Thus, thermospermine (**5**) is a major polyamine in plants. Thermospermine-deficient *acl5* mutant of *Arabidopsis thaliana* exhibits excess xylem formation, which results in severe suppression of organ elongation.[6] Addition of exogenous thermospermine significantly suppresses xylem differentiation and recovers the defect of organ elongation in the *acl5* mutant.[5b] These studies indicate that thermospermine (**5**) is a unique plant growth regulator for limiting xylem differentiation and promoting organ growth.

Thermospermine (**5**) is not only a key regulator for plant growth but also a powerful bioactive molecule to modulate plant yield and biomass. However, its biological function has been studied only in angiosperms, especially in *Arabidopsis*. To investigate thermospermine function in various plants, we decided to develop the inhibitor of thermospermine biosynthesis. We previously reported chemical synthesis of the molecule to inhibit thermospermine biosynthesis and named this biologically active molecule xylemin.[7] Xylemin (**6**) is a spermidine analogue, *N*-(4-aminobutyl)propylamine, which loses an amino group required for the addition of the aminopropyl group in thermospermine biosynthesis (Figure 1). Xylemin (**6**) competitively inhibits biosynthesis of thermospermine (**5**) and promotes xylem differentiation in various plants.[7] Therefore, xylemin (**6**) is a novel chemical tool to analyze thermospermine function and a useful biologically active compound, which can manipulate xylem differentiation and organ elongation in plants without any genetic modification. Xylem is a source of woody

biomass which is of enormous importance for industrial applications including pulp and biofuel, therefore, xylemin (**6**) could provide a novel and sustainable method to promote woody biomass production with low environmental load. In this full paper, we report the improved synthesis of xylemin (**6**) in comparison with the previous synthetic scheme[7] and synthesis of various xylemin analogues, and biological effect of their synthetic products on xylem and root formation.

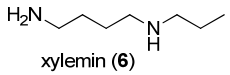
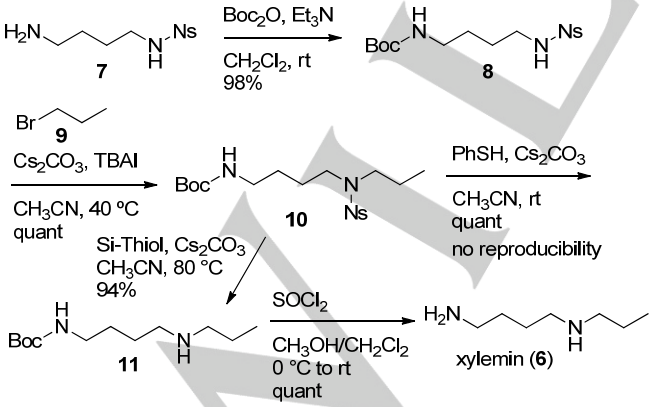


Figure 1. Structure of xylemin (**6**).

Results and Discussion

Synthesis of xylemin (6)

We commenced the synthesis of xylemin (**6**) from commercially available *N*-(4-aminobutyl)-2-nitrobenzenesulfonamide (**7**) as shown in Scheme 2. Protection of the amino group of **7** with Boc_2O/Et_3N afforded carbamate **8**.[8] Selective alkylation of the 2-nitrobenzenesulfonamide **8** was performed with 1-bromopropane (**9**)/Cs_2CO_3/tetrabutylammonium iodide (TBAI) to provide propylated product **10**.[9] The 2-nitrobenzenesulfonyl (Ns) protecting group of **10** was removed with $PhSH/Cs_2CO_3$ to give amine **11**, quantitatively. However, several experiments revealed that this reaction has no reproducibility. We observed that this reaction proceeded smoothly and thought that the problem might result from the purification step by silica gel column chromatography. Therefore, we carried out desulfonylation of **10** by using silica-supported 1-propanethiol (Si-Thiol)[10,11] to simplify the purification procedure and the desired amine **11** was obtained in 94% yield with reproducibility. Finally, the carbamate **11** was deprotected with $SOCl_2$ in CH_3OH/CH_2Cl_2[9,12] to furnish xylemin (**6**). The synthetic xylemin (**6**) was found to promote xylem differentiation by the inhibition of thermospermine synthesis, as reported in our previous communication.[7]



Scheme 2. Synthesis of xylemin (**6**). Ns = 2-nitrobenzenesulfonyl, Boc = *tert*-butoxycarbonyl, Et = ethyl, rt = room temperature, TBAI = tetrabutylammonium iodide, quant = quantitative, Ph = phenyl.

Synthesis of xylemin analogues 18–23

Because it was clarified that xylemin (**6**) works as the inhibitor of thermospermine synthesis, we next decided to synthesize xylemin structural analogues and investigate their structure–activity relationship. Shirahata and co-workers designed *N*-(3-aminopropyl)alkylamines as inhibitors of spermine synthase and evaluated their activities.[13,14] As summarized in Figure 2, *N*-(3-aminopropyl)butylamine (**12**) exhibited the inhibitory activity against spermine synthase with an IC_{50} value of 0.42 μM. Changing the alkyl chain moiety affected the activities. Thus, the activity of *N*-(3-aminopropyl)pentylamine (**13**) was decreased to an IC_{50} value of 4.2 μM and *N*-(3-aminopropyl)hexylamine (**14**) was inactive. Shirahata's group next evaluated the inhibitory activities of cycloalkylamines **15**–**17** and found that *N*-(3-aminopropyl)cyclopentylamine (**15**), *N*-(3-aminopropyl)cyclohexylamine (**16**), and *N*-(3-aminopropyl)cycloheptylamine (**17**) were active with IC_{50} values of 7.5 μM, 0.17 μM, and 1.1 μM, respectively. From these results, it was proven that the number of carbons in the substituents on nitrogen atom had influence on their inhibitory activities in both alkylamines and cycloalkylamines. By using Shirahata's results described above as a reference, we designed and decided to synthesize *N*-(4-aminobutyl)alkylamines **18**–**23** as xylemin structural analogues (Figure 3).

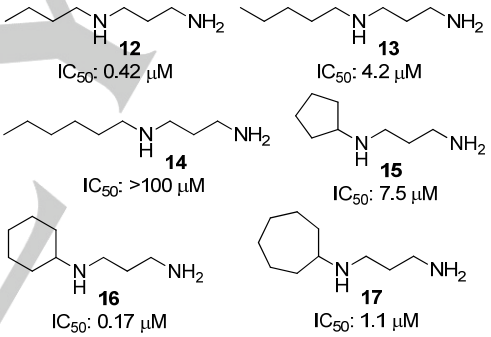


Figure 2. Inhibitory activity of *N*-(3-aminopropyl)alkylamines against spermine synthase.[13] IC_{50} = inhibitory concentration in 50% of test subjects.

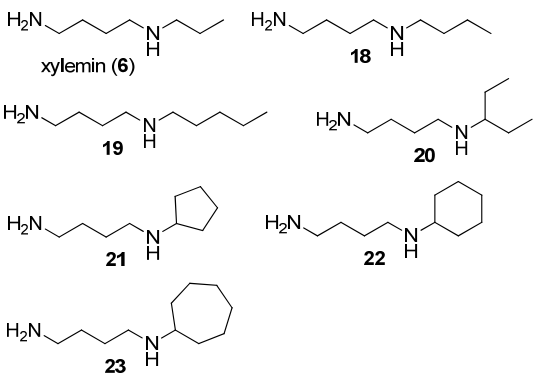
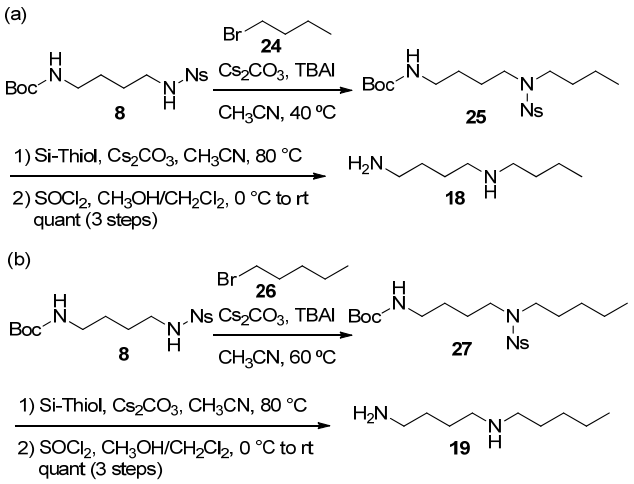


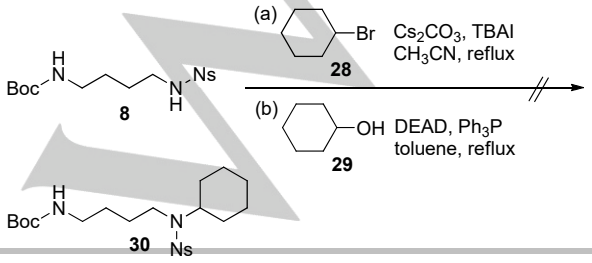
Figure 3. Structures of xylemin (**6**) and its analogues **18**–**23**.

N-(4-aminobutyl)butylamine (**18**) and *N*-(4-aminobutyl)pentylamine (**19**) were synthesized by the transformation similar to that used for the synthesis of xylemin (**6**). Thus, the butyl and pentyl substituents of amines **25** and **27** were introduced by Ns strategy (Scheme 3).[9] Subsequently, the Ns and Boc moieties were removed by Si-Thiol[10,11] and $SOCl_2/CH_3OH$,[9,12] respectively, to reach **18** and **19**.

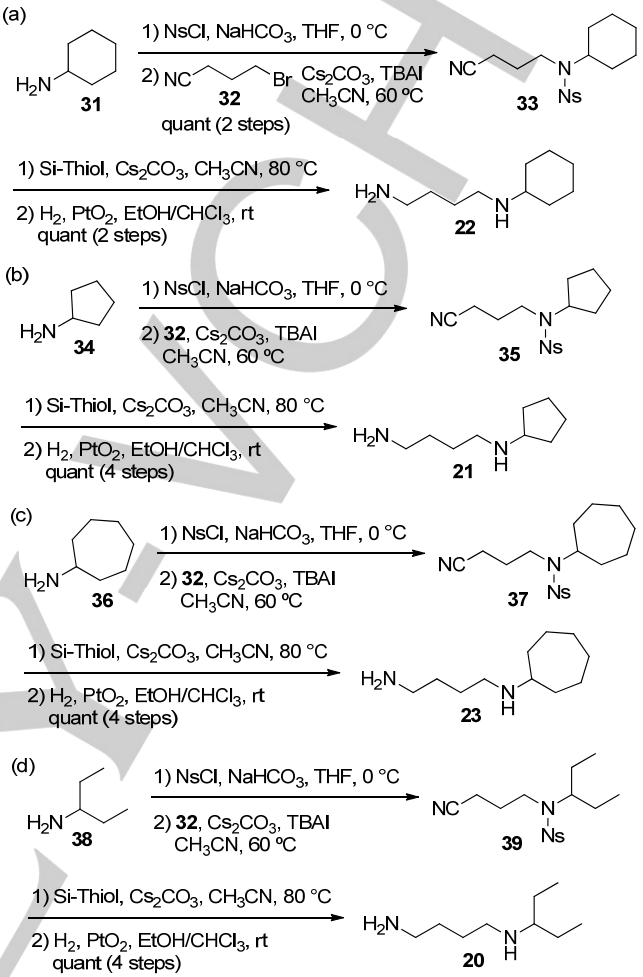


Scheme 3. Synthesis of xylemin analogues **18** (a) and **19** (b).

We next examined the synthesis of *N*-(4-aminobutyl)cyclohexylamine (**22**). When the alkylation conditions with bromocyclohexane (**28**)/Cs_2CO_3/TBAI, which were used for the synthesis of **6**, **18**, and **19**, were applied to the synthesis of cyclohexylamine **30**, the reaction did not proceed at all (Scheme 4a). Condensation of the sulfonamide **8** and cyclohexanol (**29**) under Mitsunobu conditions[15] with diethyl azodicarboxylate (DEAD)/Ph_3P in toluene at reflux resulted in failure and the starting material **8** was recovered (Scheme 4b). Therefore, we tried to investigate other synthetic transformations toward the synthesis of **22**. First, treatment of cyclohexylamine (**31**) with NsCl/$NaHCO_3$[16] afforded the corresponding nitrobenzenesulfonamide,[17] which reacted with 4-bromobutyronitrile (**32**) in the presence of Cs_2CO_3 and TBAI[9] to provide the alkylated product **33** (Scheme 5a). Deprotection of the nitrobenzenesulfonamide **33** with Si-Thiol[10,11] and subsequent hydrogenation of the nitrile group with PtO_2[17] were performed to produce the desired *N*-(4-aminobutyl)cyclohexylamine (**22**).[13] Furthermore, the synthetic sequence from **31** to **22** was successfully applied to amines **34**, **36**, and **38** to deliver the designed *N*-(4-aminobutyl)alkylamines **21**,[17] **23**, and **20** (Scheme 5b–d).



Scheme 4. Unsuccessful results for the synthesis of cyclohexylamine **30**.



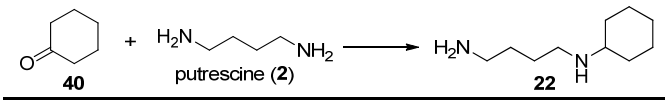
Scheme 5. Synthesis of xylemin analogues **22** (a), **21** (b), **23** (c), and **20** (d).

Improved synthesis of xylemin analogues 20–23

Although we succeeded in the synthesis of xylemin analogues **20**–**23**, these products were supplied from different amines **38**, **34**, **31**, and **36** as starting materials, respectively, which resulted in increasing the number of total steps for their synthesis. Therefore, we next examined the alternative synthetic route for obtaining **20**–**23** more concisely. We first surveyed the reaction conditions of mono-reductive amination of cyclohexanone (**40**) with putrescine (**2**) for delivering *N*-(4-aminobutyl)cyclohexylamine (**22**) in one-step as described in Table 1. Treatment of **40** with **2** (2.0 equiv) in the presence of Na_2SO_4 as dehydrating agent and AcOH followed by addition of $NaBH(OAc)_3$[18] gave trace amounts of **22** (Entry 1). When the reductive amination of **40** with **2** (10 equiv) was carried out by using $NaBH_3CN$[19] to afford **22** in 22% yield (Entry 2). Bhattacharyya et al. reported the reductive amination of aldehydes and ketones utilizing $Ti(OiPr)_4$–$NaBH_4$ reagent system.[20] These reaction conditions were successfully applied to the synthesis of various secondary amines by Brunel and co-

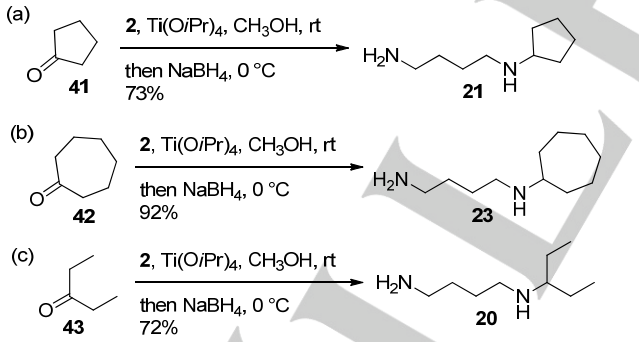
workers in 2006.[21] When these reaction conditions were used in our cases, thus, reaction of **40** with **2** (3.0 equiv) in the presence of Ti(O*i*Pr)$_4$ in CH_3OH at room temperature and subsequent reduction with $NaBH_4$ at –78 °C took place,[22] the desired product **22** was produced in 68% yield (Entry 3). Changing the reaction temperature from –78 °C to 0 °C in reduction step increased the chemical yield of **22** to 84% yield (Entry 4). Having succeeded in mono-reductive amination of cyclohexanone (**40**) with putrescine (**2**) by the combination of Ti(O*i*Pr)$_4$ as a Lewis acid and $NaBH_4$ as a reducing reagent, we carried out one-step synthesis of xylemin analogues **21**,[17] **23**, and **20** by the same reaction conditions as shown in Scheme 6.

Table 1. Mono-reductive amination of cyclohexanone (**40**) with putrescine (**2**).



Entry	Conditions[a]	Yield [%][b]
1	Na_2SO_4, AcOH, rt, then $NaBH(OAc)_3$, rt	N.D.[c]
2	MS4Å, AcOH, CH_3OH, reflux, then $NaBH_3CN$, rt	22
3	Ti(O*i*Pr)$_4$, CH_3OH, rt, then $NaBH_4$, –78 °C	68
4	Ti(O*i*Pr)$_4$, CH_3OH, rt, then $NaBH_4$, 0 °C	84

[a] Ac = acetyl, MS = molecular sieves, Pr = propyl. [b] Isolated yield. [c] N.D. = not determined. Formation of trace amounts of **22** was observed.



Scheme 6. One-step synthesis of xylemin analogues **21** (a), **23** (b), and **20** (c) via mono-reductive amination.

Effect of xylemin (6) and its analogues 18–23 on the expression of *ACL5* gene

With xylemin (**6**) and its analogues **18**–**23** in hand, we next tried to carry out the biological evaluation of these synthetic products. First, for the quantitative evaluation of biological activity of the synthetic products, we used expression level of the *ACL5* gene as a sensitive indicator of the endogenous amount of thermospermine. The expression level of *ACL5* is feedback-regulated by the amount of thermospermine (**5**); depletion of thermospermine (**5**) leads to the increased expression of *ACL5*, whereas the increase of thermospermine content causes a reduction of *ACL5* mRNA.[5b] The transcript level of *ACL5* gene was quantified in *Arabidopsis* wild-type seedlings treated with xylemin (**6**), xylemin analogues **18**–**23**, or thermospermine (**5**) by reverse transcription-quantitative polymerase chain reaction (RT-qPCR). As expected, the accumulation of *ACL5* transcripts was significantly increased in seedlings treated with xylemin (**6**) (Figure 4). Interestingly, the expression of *ACL5* was also promoted to the similar level to that in the case of xylemin (**6**) by adding the xylemin analogue **21**, whereas it was not affected when the other analogues were added to the culture medium. These results have revealed that endogenous thermospermine level is decreased by xylemin (**6**) and its analogue *N*-(4-aminobutyl)cyclopentylamine (**21**). In Shirahata's work about inhibitors of spermine synthase, *N*-(3-aminopropyl)cyclohexylamine (**16**) exhibited the inhibitory activity with an IC_{50} value similar to that of *N*-(3-aminopropyl)butylamine (**12**).[13,14] In our case of inhibitors of thermospermine synthase, *N*-(4-aminobutyl)cyclopentylamine (**21**) was active as well as xylemin (**6**), *N*-(4-aminobutyl)propylamine. It is interesting that the expression level of *ACL5* is affected by the number of carbons on the nitrogen atom, which has the similar tendency to that found in inhibitors of spermine synthase.

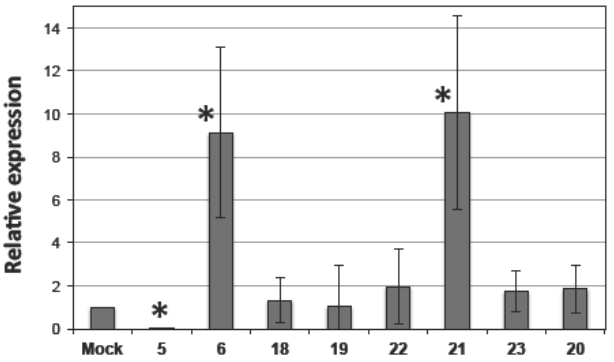


Figure 4. Effects of xylemin (**6**), its analogues **18**–**23**, and thermospermine (**5**) on the expression of thermospermine synthase gene *ACAULIS5* (*ACL5*). Wild type seedlings of *Arabidopsis thaliana* were grown for 7 days in the liquid medium without (Mock) or with **6**, **18**–**23**, or **5** at the concentration of 200 μM. Expression of *ACL5* was quantified by reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Expression of *ACTIN8* (*ACT8*) was used as a control. Columns and error bars indicate mean values and standard deviations of n = 3, respectively. Asterisks show significant difference from the value of Mock treatment ($P < 0.01$, *t*-test).

Effect of xylemin (6) and its analogues 18–23 on xylem formation

Next, we analyzed effect of xylemin (**6**) and its analogues **18**–**23** on xylem differentiation because inhibition of thermospermine synthesis by xylemin triggers excess xylem formation in *Arabidopsis*.[7] *Arabidopsis* wild-type seedlings were grown

under the presence of xylemin (**6**) and xylemin analogues **18**–**23**. Xylem formation was observed under a microscope (Figure 5) and quantification of xylem differentiation is depicted in Figure 6. The addition of xylemin (**6**) and the xylemin analogue **21** remarkably promoted xylem formation, whereas the other xylemin analogues did not affect the xylem differentiation. Thus, *N*-(4-aminobutyl)cyclopentylamine (**21**) promotes xylem differentiation by inhibiting thermospermine biosynthesis as in the case of xylemin (**6**).

Recently, crystal structure of thermospermine synthase of a leguminous plant *Medicago truncatula* (MtACL5) has been revealed.[23] The binding site of spermidine (**3**), a precursor of thermospermine (**5**), is narrow and charged negatively. Acidic amino acid residues of MtACL5 form hydrogen bonds with amino groups of spermidine (**3**). Aromatic amino acid residues generate hydrophobic interactions with **3**. The protein structure of MtACL5 is applicable to that of *Arabidopsis thaliana* ACL5, because the amino acid sequences of ACL5 are highly conserved in plants, especially within flowering plants including *Medicago* and *Arabidopsis*.[2,4b,24] Thus, relatively compact alkyl groups of xylemin (**6**) and the xylemin analogue **21** may possibly be preferred for interactions with the active site of ACL5 protein.

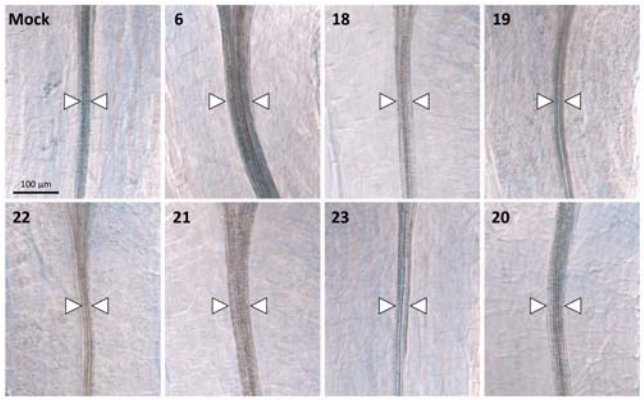


Figure 5. Effects of xylemin (**6**) and its analogues **18**–**23** on xylem differentiation. Wild type seedlings of *Arabidopsis thaliana* were grown for 7 days in the liquid medium without (Mock) or with **6** or **18**–**23** at the concentration of 200 µM. Hypocotyls were observed under a differential interference contrast microscope. The region between white triangles indicates xylem vessels, which exhibit brown color due to secondary cell wall thickening and lignin deposition.

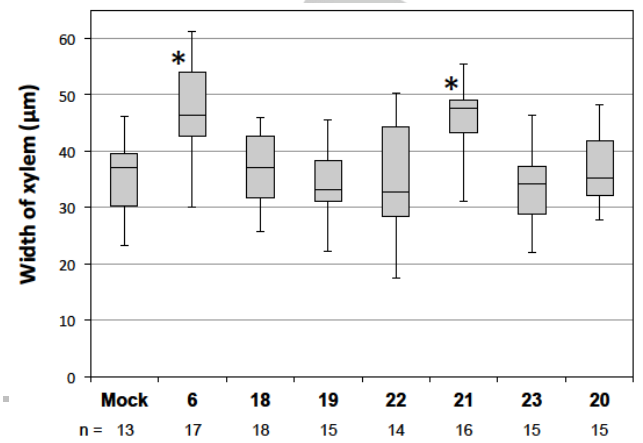


Figure 6. Quantification of xylem differentiation. The width of xylem was quantified in the hypocotyls of *Arabidopsis* wild type seedlings shown in Figure 5. Data were represented with box plots. The number of plants used for quantification (n) in each treatment was shown under horizontal axis labels. Asterisks show significant difference from the value of Mock treatment ($P < 0.0003$, *t*-test).

Effect of xylemin (6) and its analogues 18–23 on lateral root formation

Our previous study has shown that xylemin (**6**) promotes the formation of lateral roots, whereas thermospermine suppresses it.[7] Therefore, we examined effects of xylemin analogues **18**–**23** on lateral root formation (Figure 7). Xylemin (**6**) significantly promoted lateral root formation, whereas xylemin analogues **18**–**23** including the analogue **21** did not. In consideration with the similar effect of xylemin (**6**) and the analogue **21** on *ACL5* expression and xylem formation, the physiological action of **6** and **21** may differ in lateral root formation.

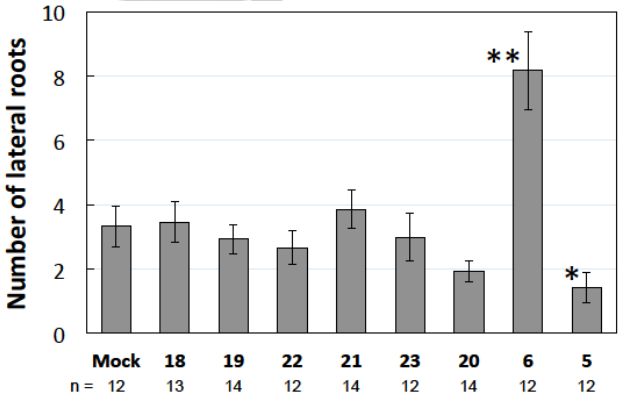


Figure 7. Effects of xylemin (**6**), its analogues **18**–**23**, and thermospermine (**5**) on lateral root formation. Wild type seedlings of *Arabidopsis thaliana* were grown for 7 days on the agar medium without (Mock) or with **6**, **18**–**23**, or **5** at the concentration of 100 µM. Seedlings were observed under a stereomicroscope. The number of lateral roots per plant was measured. The number of plants used for quantification (n) in each treatment was shown under horizontal axis labels. Columns and error bars indicate mean values and the standard errors, respectively. Single and double asterisks show significant difference from the value of Mock treatment at the P level less than 0.03 and 0.003, respectively (*t*-test).

Conclusions

First, chemical synthesis of xylemin (**6**) and its designed structural analogues **18**–**23** was examined. Xylemin (**6**) and *N*-(4-aminobutyl)alkylamines **18**–**23** were successfully synthesized by utilizing Ns strategy. In addition, improvement of the synthesis of **20**–**23** was carried out to result in the synthesis of these analogues in one-step via mono-reductive amination of the corresponding ketones, respectively. This concise synthesis by mono-reductive amination of ketones could be applied to the synthesis of polyamines including other xylemin analogues. Next, we evaluated the biological effect of synthetic molecules. Xylemin (**6**) and the xylemin analogue **21**, *N*-(4-aminobutyl)cyclopentylamine, exhibited significant biological

activity to promote xylem formation. Enhanced transcript accumulation of thermospermine synthase gene *ACL5* by the addition of **6** or **21** supports their inhibitory effects on biosynthesis of thermospermine. Other xylemin analogues (**18**–**20, 22**, **23**) did not affect xylem formation and transcript level of *ACL5*. These differential effects may be due to larger alkyl groups of the xylemin analogues **18**–**20, 22**, and **23** than **6** and **21**. Thus, the size and structure of the alkyl group are essential for the activity to enhance xylem formation. Xylemin (**6**) promoted lateral root formation, whereas the xylemin analogue **21** did not. This may reflect mild effect of **21** on xylem differentiation. Lateral roots develop from pericycle cell layer, which locates outside of vascular tissues. Pericycles in contact with inner xylem region (xylem-pole pericycles) preferentially generate lateral roots. Xylemin (**6**) enhances xylem formation, which probably causes increased number of xylem-pole pericycles and lateral roots, whereas xylem formation enhanced by the xylemin analogue **21** is not enough to increase xylem-pole pericycles. Thus, the analogue **21** could be utilized as a novel inhibitor of thermospermine synthesis, which specifically promotes xylem formation.

Experimental Section

General Methods: Reagents were used as received from commercial suppliers unless otherwise indicated. All reactions were carried out under an atmosphere of argon. Reaction solvents were purchased as dehydrated solvents and stored with active molecular sieves 4Å under argon prior to use for reactions. All solvents for work-up procedure were used as received. Analytical thin-layer chromatography (TLC) was performed with aluminium TLC plates (Merck TLC silica gel $60F_{254}$). Column chromatography was performed with Fuji Silysia silica gel BW-300 or Kanto Chemical silica gel 60N. Kugelrohr distillation was performed with Shibata Glass Tube Oven GTO-250RS. IR spectra were recorded on JASCO FT/IR-460 plus. ^{1}H and ^{13}C NMR spectra were recorded on JEOL JNM-AL400 or Varian 400-MR. Chemical shifts in the NMR spectra are reported in ppm with reference to the internal residual solvent (^{1}H NMR, $CDCl_3$ 7.26 ppm, D_2O 4.79 ppm, CD_3OD 3.31 ppm; ^{13}C NMR, $CDCl_3$ 77.0 ppm, CD_3OD 49.0 ppm, $(CD_3)_2SO$ 39.5 ppm). The following abbreviations are used to designate the multiplicities: s = singlet, t = triplet, q = quartet, quin = quintet, m = multiplet, br = broad. Coupling constants (*J*) are in hertz. High resolution mass spectra were recorded on Waters Micromass LCT (ESI–TOF–MS) or Bruker micOTOF II (ESI–TOF–MS).

Carbamate 8: To a solution of *N*-(4-aminobutyl)-2-nitrobenzenesulfonamide (**7**) (200 mg, 0.732 mmol) in CH_2Cl_2 (5.0 mL) were added Et_3N (0.12 mL, 0.878 mmol) and Boc_2O (0.20 mL, 0.878 mmol) at room temperature. The mixture was stirred at the same temperature for 2 h. The mixture was diluted with CH_2Cl_2 and washed with 1N aqueous HCl. The aqueous phase was extracted with CH_2Cl_2 twice and the combined organic phase was washed with brine and dried over Na_2SO_4. Concentration and column chromatography (hexane/EtOAc = 2:1, 1:1) gave carbamate **8** (269 mg, 98%): yellow solid; R_f = 0.27 (hexane/EtOAc = 1:1); IR (neat) 3294, 3085, 2980, 1703, 1676 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14–8.12 (m, 1 H), 7.87–7.85 (m, 1 H), 7.76–7.73 (m, 2 H), 5.36 (brs, 1 H), 4.54 (brs, 1 H), 3.13–3.06 (m, 4 H), 1.61–1.47 (m, 4 H), 1.42 (s, 9 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.0, 148.1, 133.6, 133.6, 132.8, 131.1, 125.4, 79.3, 43.4, 39.8, 28.4, 27.1, 26.8; HRMS (ESI–TOF) calcd for $C_{15}H_{23}N_3O_6SNa$ [M + Na]$^+$ 396.1205, found 396.1201.

Sulfonamide 10: To a solution of sulfonamide **8** (1.02 g, 2.73 mmol) in CH_3CN (7.8 mL) were added Cs_2CO_3 (2.69 g, 8.26 mmol), TBAI (506 mg, 1.37 mmol), and 1-bromopropane (**9**, 0.30 mL, 3.28 mmol) at room temperature. The mixture was stirred at 40 °C for 2 h. To the mixture was added 1-bromopropane (**9**, 0.30 mL, 3.28 mmol) at room temperature. The mixture was stirred at 40 °C for 1 h. The mixture was diluted with EtOAc and washed with H_2O. The aqueous phase was extracted with EtOAc three times and the combined organic phase was washed with brine and dried over Na_2SO_4. Concentration and column chromatography (hexane/EtOAc = 4:1, 1:1) gave sulfonamide **10** (1.15 g, quant): yellow oil; R_f = 0.52 (hexane/EtOAc = 1:1); IR (neat) 3412, 3094, 2971, 2934, 2876, 1699 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01–7.99 (m, 1 H), 7.68–7.66 (m, 2 H), 7.61–7.59 (m, 1 H), 4.53 (brs, 1 H), 3.30 (t, *J* = 7.6 Hz, 2 H), 3.23 (t, *J* = 7.6 Hz, 2 H), 3.10 (q, *J* = 7.6 Hz, 2 H), 1.60–1.46 (m, 6 H), 1.44 (s, 9 H), 0.85 (t, *J* = 7.2 Hz, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.9, 148.0, 133.7, 133.2, 131.4, 130.6, 124.0, 79.2, 49.1, 47.0, 40.0, 28.5, 27.3, 25.6, 21.5, 11.1; HRMS (ESI–TOF) calcd for $C_{18}H_{29}N_3O_6SNa$ [M + Na]$^+$ 438.1675, found 438.1678.

Deprotection of Sulfonamide 10 with PhSH: To a solution of sulfonamide **10** (11.3 mg, 27.2 μmol) in CH_3CN (0.8 mL) were added Cs_2CO_3 (44.3 mg, 0.136 mmol) and PhSH (10 μL, 0.101 mmol) at room temperature. The mixture was stirred at the same temperature for 3 h. The mixture was filtered through a Celite pad and washed with EtOAc. Concentration and column chromatography (CH_2Cl_2, CH_2Cl_2/CH_3OH = 5:1 including 2% Et_3N) gave amine **11** (6.8 mg, quant): yellow oil; R_f = 0.15 (CH_2Cl_2/CH_3OH = 5:1); IR (neat) 3340, 2962, 2932, 2873, 1697 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 3.04–3.03 (m, 2 H), 2.56–2.49 (m, 4 H), 1.54–1.48 (m, 6 H), 1.41 (s, 9 H), 0.95–0.89 (m, 3 H); ^{13}C NMR (100 MHz, CD_3OD) δ 158.4, 79.7, 52.6, 50.3, 41.2, 28.8, 27.7, 23.5, 12.1; HRMS (ESI–TOF) calcd for $C_{12}H_{27}N_2O_2$ [M + H]$^+$ 231.2073, found 231.2072.

Deprotection of Sulfonamide 10 with Si-Thiol: To a solution of sulfonamide **10** (700 mg, 1.68 mmol) in CH_3CN (17 mL) were added Cs_2CO_3 (1.64 g, 5.04 mmol) and Si-Thiol (1.3 mmol/g, 5.24 g, 6.81 mmol) at room temperature. The mixture was stirred at 80 °C for 5 h. The mixture was filtered through a Celite pad and washed with EtOAc. Concentration gave amine **11** (365 mg, 94%).

Xylemin (6): To a solution of carbamate **11** (92.2 mg, 0.400 mmol) in CH_3OH (2.6 mL) and CH_2Cl_2 (1.3 mL) was added $SOCl_2$ (0.260 mL, 3.60 mmol) at 0 °C. The mixture was stirred at room temperature for 5 h. Concentration gave xylemin (**6**, 131 mg, quant): yellow solid; IR (neat) 3422, 2930, 2778 cm^{-1}; ^{1}H NMR (400 MHz, D_2O) δ 3.13–3.02 (m, 6 H), 1.81–1.68 (m, 6 H), 1.00 (t, *J* = 7.6 Hz, 3 H); ^{13}C NMR (100 MHz, CD_3CD) δ 50.6, 48.1, 40.1, 25.6, 24.3, 20.7, 11.3; HRMS (ESI–TOF) calcd for $C_7H_{19}N_2$ [M + H]$^+$ 131.1548, found 131.1550.

***N*-(4-Aminobutyl)butylamine (18):** To a solution of sulfonamide **8** (30.6 mg, 81.9 μmol) in CH_3CN (1.0 mL) were added Cs_2CO_3 (80.2 mg, 0.246 mmol), TBAI (15.1 mg, 41.0 μmol), and 1-bromobutane (**24**, 11 μL, 98.3 μmol) at room temperature. The mixture was stirred at 40 °C for 3 h. To the mixture was added 1-bromobutane (**24**, 21 μL, 0.197 mmol) at room temperature. The mixture was stirred at 40 °C for 2 h. The mixture was diluted with EtOAc and washed with H_2O. The aqueous phase was extracted with EtOAc three times and the combined organic phase was washed with brine and dried over Na_2SO_4. Concentration and short column chromatography (hexane/EtOAc = 2:1) gave sulfonamide **25** (32.5 mg), which was used for the next step without further purification. To a solution of sulfonamide **25** obtained above (32.5 mg) in CH_3CN (1.0 mL) were added Cs_2CO_3 (77.0 mg, 0.236 mmol) and Si-Thiol (1.3 mmol/g,

233 mg, 0.303 mmol) at room temperature. The mixture was stirred at 80 °C for 7 h. The mixture was filtered through a Celite pad and washed with EtOAc. Concentration gave the corresponding amine (18.2 mg), which was used for the next step without further purification. To a solution of the carbamate obtained above (18.2 mg) in CH_3OH (0.8 mL) and CH_2Cl_2 (0.4 mL) was added $SOCl_2$ (48 µL, 0.652 mmol) at 0 °C. The mixture was stirred at room temperature for 5 h. Concentration gave *N*-(4-aminobutyl)butylamine (**18**, 15.0 mg, quant in three steps): yellow solid; IR (neat) 3395, 2966 cm^{-1}; ^1H NMR (400 MHz, D_2O) δ 3.12–3.07 (m, 6 H), 1.81–1.79 (m, 4 H), 1.73–1.66 (m, 2 H), 1.43 (q, J = 6.8 Hz, 2 H), 0.96 (t, J = 6.8 Hz, 3 H); ^{13}C NMR (100 MHz, CD_3OD) δ 49.9, 48.2, 40.1, 29.2, 25.6, 24.3, 20.8, 13.9; HRMS (ESI–TOF) calcd for $C_8H_{21}N_2$ $[M + H]^+$ 145.1705, found 145.1710.

***N*-(4-Aminobutyl)pentylamine (19):** To a solution of sulfonamide **8** (33.1 mg, 88.6 µmol) in CH_3CN (1.0 mL) were added Cs_2CO_3 (86.7 mg, 0.266 mmol), TBAI (16.4 mg, 44.3 µmol), and 1-bromopentane (**26**, 13 µL, 0.106 mmol) at room temperature. The mixture was stirred at 40 °C for 1 h. To the mixture was added 1-bromopentane (**26**, 39 µL, 0.315 mmol) at room temperature. The mixture was stirred at 60 °C for 1 h. The mixture was diluted with EtOAc and washed with H_2O. The aqueous phase was extracted with EtOAc three times and the combined organic phase was washed with brine and dried over Na_2SO_4. Concentration and short column chromatography (hexane/EtOAc = 4:1, 2:1) gave sulfonamide **27** (33.9 mg), which was used for the next step without further purification. To a solution of sulfonamide **27** obtained above (33.9 mg) in CH_3CN (1.0 mL) were added Cs_2CO_3 (74.6 mg, 0.229 mmol) and Si-Thiol (1.3 mmol/g, 235 mg, 0.306 mmol) at room temperature. The mixture was stirred at 80 °C for 8 h. The mixture was filtered through a Celite pad and washed with EtOAc. Concentration gave the corresponding amine (20.5 mg), which was used for the next step without further purification. To a solution of the carbamate obtained above (20.5 mg) in CH_3OH (0.8 mL) and CH_2Cl_2 (0.4 mL) was added $SOCl_2$ (52 µL, 0.713 mmol) at 0 °C. The mixture was stirred at room temperature for 5 h. Concentration gave *N*-(4-aminobutyl)pentylamine (**19**, 20.4 mg, quant in three steps): yellow solid; IR (neat) 3409, 2963 cm^{-1}; ^1H NMR (400 MHz, D_2O) δ 3.12–3.06 (m, 6 H), 1.81–1.68 (m, 6 H), 1.39–1.37 (m, 4 H), 0.95–0.91 (m, 3 H); ^{13}C NMR (100 MHz, CD_3OD) δ 49.6, 48.2, 40.1, 29.7, 27.0, 25.7, 24.3, 23.2, 14.1; HRMS (ESI–TOF) calcd for $C_9H_{23}N_2$ $[M + H]^+$ 160.1891, found 160.1886.

Nitrile 33: To a solution of cyclohexylamine (**31**, 0.10 mL, 0.877 mmol) in THF (8.8 mL) were added $NaHCO_3$ (298 mg, 3.58 mmol) and NsCl (400 mg, 1.80 mmol) at 0 °C. The mixture was stirred at the same temperature for 10 min. The reaction was quenched with 3N aqueous NaOH. The mixture was stirred at room temperature for 1 h. The mixture was diluted with EtOAc, washed with saturated 3N aqueous NaOH, 3N aqueous HCl, and brine, and then dried over Na_2SO_4. Concentration and short column chromatography (hexane/EtOAc = 4:1) gave the corresponding sulfonamide (273 mg), which was used for the next step without further purification. To a solution of the sulfonamide obtained above (273 mg) in CH_3CN (9.6 mL) were added Cs_2CO_3 (940 mg, 2.89 mmol), TBAI (178 mg, 0.482 mmol), and 4-bromobutyronitrile (**32**, 0.11 mL, 1.11 mmol) at room temperature. The mixture was stirred at 60 °C for 2 h. To the mixture was added 4-bromobutyronitrile (**32**, 0.22 mL, 2.22 mmol) at room temperature. The mixture was stirred at 60 °C for 1 h. The mixture was diluted with EtOAc and washed with H_2O. The aqueous phase was extracted with EtOAc twice and the combined organic phase was washed with brine and dried over Na_2SO_4. Concentration and column chromatography (hexane/EtOAc = 5:1, 2:1) gave nitrile **33** (340 mg, quant in two steps): yellow oil; R_f = 0.44 (hexane/EtOAc = 1:1); IR (neat) 3095, 2935, 2859, 2246, 1734 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.04–8.01 (m, 1 H), 7.73–7.59 (m, 3 H), 3.72–3.65 (m, 1 H), 3.37 (t, J = 7.6 Hz, 2 H), 2.42 (t, J = 7.0 Hz, 2 H), 2.02 (quin, J = 7.0 Hz, 2 H), 1.80–1.60 (m, 5 H), 1.44–1.26 (m, 4 H), 1.12–1.01 (m, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.0, 133.6, 133.5, 131.5, 130.7, 124.1, 118.9, 58.5, 42.7, 32.0, 27.6, 26.0, 25.2, 14.8; HRMS (ESI–TOF) calcd for $C_{16}H_{21}N_3O_4SNa$ $[M + Na]^+$ 374.1151, found 374.1144.

***N*-(4-Aminobutyl)cyclohexylamine (22):** To a solution of sulfonamide **33** (15.2 mg, 43.3 µmol) in CH_3CN (1.4 mL) were added Cs_2CO_3 (44.1 mg, 0.135 mmol) and Si-Thiol (1.3 mmol/g, 133 mg, 0.173 mmol) at room temperature. The mixture was stirred at 80 °C for 6 h. The mixture was filtered through a Celite pad and washed with EtOAc. Concentration gave the corresponding amine (6.7 mg), which was used for the next step without further purification. A mixture of the nitrile obtained above (6.7 mg) and PtO_2 (0.9 mg, 4.03 µmol) in EtOH (1.0 mL) and $CHCl_3$ (0.1 mL) was stirred for 20 h under a H_2 atmosphere at room temperature. The mixture was filtered and washed with CH_3OH. Concentration gave *N*-(4-aminobutyl)cyclohexylamine (**22**, 8.4 mg, quant in two steps).

***N*-(4-Aminobutyl)cyclopentylamine (21):** To a solution of cyclopentylamine (**34**, 20 µL, 0.202 mmol) in THF (2.0 mL) were added $NaHCO_3$ (67.9 mg, 0.808 mmol) and NsCl (89.5 mg, 0.404 mmol) at 0 °C. The mixture was stirred at the same temperature for 10 min. The reaction was quenched with 3N aqueous NaOH. The mixture was stirred at room temperature for 30 min. The mixture was diluted with EtOAc, washed with saturated 3N aqueous NaOH, 3N aqueous HCl, and brine, and then dried over Na_2SO_4. Concentration and short column chromatography (hexane/EtOAc = 4:1) gave the corresponding sulfonamide (51.8 mg), which was used for the next step without further purification. To a solution of the sulfonamide obtained above (51.8 mg) in CH_3CN (1.9 mL) were added Cs_2CO_3 (188 mg, 0.576 mmol), TBAI (35.5 mg, 96.0 µmol), and 4-bromobutyronitrile (**32**, 23 µL, 0.230 mmol) at room temperature. The mixture was stirred at 60 °C for 20 min. To the mixture was added 4-bromobutyronitrile (**32**, 46 µL, 0.460 mmol) at room temperature. The mixture was stirred at 60 °C for 1 h. The mixture was diluted with EtOAc and washed with H_2O. The aqueous phase was extracted with EtOAc twice and the combined organic phase was washed with brine and dried over Na_2SO_4. Concentration and short column chromatography (hexane/EtOAc = 4:1, 2:1) gave nitrile **35** (56.5 mg), which was used for the next step without further purification. To a solution of sulfonamide **35** obtained above (56.5 mg) in CH_3CN (5.6 mL) were added Cs_2CO_3 (163 mg, 0.501 mmol) and Si-Thiol (1.3 mmol/g, 514 mg, 0.668 mmol) at room temperature. The mixture was stirred at 80 °C for 6 h. The mixture was filtered through a Celite pad and washed with EtOAc. Concentration gave the corresponding amine (24.7 mg), which was used for the next step without further purification. A mixture of the nitrile obtained above (24.7 mg) and PtO_2 (3.6 mg, 15.7 µmol) in EtOH (3.1 mL) and $CHCl_3$ (0.3 mL) was stirred for 20 h under a H_2 atmosphere at room temperature. The mixture was filtered and washed with CH_3OH. Concentration gave *N*-(4-aminobutyl)cyclopentylamine (**21**, 35.5 mg, quant in four steps).

***N*-(4-Aminobutyl)cycloheptylamine (23):** To a solution of cycloheptylamine (**36**, 25 µL, 0.197 mmol) in THF (2.0 mL) were added $NaHCO_3$ (65.2 mg, 0.776 mmol) and NsCl (87.3 mg, 0.394 mmol) at 0 °C. The mixture was stirred at the same temperature for 10 min. The reaction was quenched with 3N aqueous NaOH. The mixture was stirred at room temperature for 30 min. The mixture was diluted with EtOAc, washed with saturated 3N aqueous NaOH, 3N aqueous HCl, and brine, and then dried over Na_2SO_4. Concentration and short column chromatography (hexane/EtOAc = 4:1) gave the corresponding sulfonamide (65.2 mg), which was used for the next step without further purification. To a solution of the sulfonamide obtained above (65.2 mg) in CH_3CN (2.2 mL) were added Cs_2CO_3 (214 mg, 0.657 mmol), TBAI (29.6 mg, 80.1 µmol), and 4-bromobutyronitrile (**32**, 26 µL, 0.263 mmol) at room temperature. The mixture was stirred at 60 °C for 1 h. To the mixture was added 4-bromobutyronitrile (**32**, 52 µL, 0.526 mmol) at room temperature. The

mixture was stirred at 60 °C for 1 h. The mixture was diluted with EtOAc and washed with H_2O. The aqueous phase was extracted with EtOAc twice and the combined organic phase was washed with brine and dried over Na_2SO_4. Concentration and short column chromatography (hexane/EtOAc = 2:1) gave nitrile **37** (78.4 mg), which was used for the next step without further purification. To a solution of sulfonamide **37** obtained above (78.4 mg) in CH_3CN (6.5 mL) were added Cs_2CO_3 (190 mg, 0.582 mmol) and Si-Thiol (1.3 mmol/g, 597 mg, 0.776 mmol) at room temperature. The mixture was stirred at 80 °C for 6 h. The mixture was filtered through a Celite pad and washed with EtOAc. Concentration gave the corresponding amine (33.3 mg), which was used for the next step without further purification. A mixture of the nitrile obtained above (33.3 mg) and PtO_2 (4.2 mg, 18.5 μmol) in EtOH (3.7 mL) and $CHCl_3$ (0.2 mL) was stirred for 20 h under a H_2 atmosphere at room temperature. The mixture was filtered and washed with CH_3OH. Concentration gave *N*-(4-aminobutyl)cycloheptylamine (**23**, 44.1 mg, quant in four steps).

***N*-(4-Aminobutyl)-1-ethylpropylamine (20):** To a solution of 1-ethylpropylamine (**38**, 25 μL, 0.215 mmol) in THF (2.2 mL) were added $NaHCO_3$ (72.2 mg, 0.860 mmol) and NsCl (95.3 mg, 0.430 mmol) at 0 °C. The mixture was stirred at the same temperature for 20 min. The reaction was quenched with 3N aqueous NaOH. The mixture was stirred at room temperature for 1 h. The mixture was diluted with EtOAc, washed with saturated 3N aqueous NaOH, 3N aqueous HCl, and brine, and then dried over Na_2SO_4. Concentration and short column chromatography (hexane/EtOAc = 4:1) gave the corresponding sulfonamide (55.4 mg), which was used for the next step without further purification. To a solution of the sulfonamide obtained above (55.4 mg) in CH_3CN (2.0 mL) were added Cs_2CO_3 (217 mg, 0.666 mmol), TBAI (31.0 mg, 83.9 μmol), and 4-bromobutyronitrile (**32**, 24 μL, 0.242 mmol) at room temperature. The mixture was stirred at 60 °C for 2 h. To the mixture was added 4-bromobutyronitrile (**32**, 48 μL, 0.484 mmol) at room temperature. The mixture was stirred at 60 °C for 1 h. The mixture was diluted with EtOAc and washed with H_2O. The aqueous phase was extracted with EtOAc three times and the combined organic phase was washed with brine and dried over Na_2SO_4. Concentration and short column chromatography (hexane/EtOAc = 2:1) gave nitrile **39** (66.3 mg), which was used for the next step without further purification. To a solution of sulfonamide **39** obtained above (66.3 mg) in CH_3CN (6.5 mL) were added Cs_2CO_3 (201 mg, 0.616 mmol) and Si-Thiol (1.3 mmol/g, 600 mg, 0.780 mmol) at room temperature. The mixture was stirred at 80 °C for 7 h. The mixture was filtered through a Celite pad and washed with EtOAc. Concentration gave the corresponding amine (29.1 mg), which was used for the next step without further purification. A mixture of the nitrile obtained above (29.1 mg) and PtO_2 (4.3 mg, 18.9 μmol) in EtOH (3.8 mL) and $CHCl_3$ (0.2 mL) was stirred for 20 h under a H_2 atmosphere at room temperature. The mixture was filtered and washed with CH_3OH. Concentration gave *N*-(4-aminobutyl)-1-ethylpropylamine (**20**, 40.3 mg, quant in four steps).

One-Step Synthesis of *N*-(4-Aminobutyl)cyclohexylamine (22) via Mono-Reductive Amination: To a solution of cyclohexanone (**40**, 0.25 mL, 2.42 mmol) in CH_3OH (15 mL) were added putrescine (**2**, 0.68 mL, 7.26 mmol) and Ti(O*i*Pr)$_4$ (0.93 mL, 3.15 mmol) at room temperature. The mixture was stirred at the same temperature for 3 h. To the mixture was added $NaBH_4$ (98.7 mg, 2.61 mmol) at 0 °C. The mixture was stirred at the same temperature for 30 min. The reaction was quenched with H_2O. The mixture was stirred at room temperature for 20 min. The mixture was filtered through a Celite pad and washed with EtOAc. The mixture was washed with 3N aqueous NaOH. The aqueous phase was extracted with EtOAc three times and the combined organic phase was washed with basic brine (pH = 11 by adding aqueous NaOH) and dried over Na_2SO_4. Concentration and kugelrohr distillation (9 mmHg, 140–155 °C) gave *N*-(4-aminobutyl)cyclohexylamine (**22**, 346 mg, 84%): colorless oil; IR (neat) 3358, 3281, 2926, 2852 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 2.65–2.57 (m, 4 H), 2.46–2.39 (m, 1 H), 1.92–1.64 (m, 5 H), 1.53–1.47 (m, 4 H), 1.34–1.06 (m, 5 H); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ 55.7, 42.6, 37.9, 28.3, 24.7, 24.1, 23.9, 22.5; HRMS (ESI–TOF) calcd for $C_{10}H_{23}N_2$ [M + H]$^+$ 171.1861, found 171.1859.

One-Step Synthesis of *N*-(4-Aminobutyl)cyclopentylamine (21) via Mono-Reductive Amination: To a solution of cyclopentanone (**41**, 0.25 mL, 2.82 mmol) in CH_3OH (18 mL) were added putrescine (**2**, 0.79 mL, 8.46 mmol) and Ti(O*i*Pr)$_4$ (1.1 mL, 3.67 mmol) at room temperature. The mixture was stirred at the same temperature for 3 h. To the mixture was added $NaBH_4$ (106 mg, 2.82 mmol) at 0 °C. The mixture was stirred at the same temperature for 30 min. The reaction was quenched with H_2O. The mixture was stirred at room temperature for 20 min. The mixture was filtered through a Celite pad and washed with EtOAc. The mixture was washed with 3N aqueous NaOH. The aqueous phase was extracted with EtOAc three times and the combined organic phase was washed with basic brine (pH = 11 by adding aqueous NaOH) and dried over Na_2SO_4. Concentration and kugelrohr distillation (9 mmHg, 115–130 °C) gave *N*-(4-aminobutyl)cyclopentylamine (**21**, 320 mg, 73%): colorless oil; IR (neat) 3370, 3279, 2950, 2860 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 3.04 (quin, *J* = 7.2 Hz, 1 H), 2.64 (t, *J* = 6.8 Hz, 2 H), 2.57 (t, *J* = 6.8 Hz, 2 H), 1.92–1.29 (m, 14 H); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ 58.1, 44.8, 37.9, 28.9, 24.0, 23.5, 22.5; HRMS (ESI–TOF) calcd for $C_9H_{21}N_2$ [M + H]$^+$ 157.1705, found 157.1698.

One-Step Synthesis of *N*-(4-Aminobutyl)cycloheptylamine (23) via Mono-Reductive Amination: To a solution of cycloheptanone (**42**, 0.30 mL, 2.54 mmol) in CH_3OH (16 mL) were added putrescine (**2**, 0.71 mL, 7.62 mmol) and Ti(O*i*Pr)$_4$ (0.97 mL, 3.30 mmol) at room temperature. The mixture was stirred at the same temperature for 3 h. To the mixture was added $NaBH_4$ (101 mg, 2.67 mmol) at 0 °C. The mixture was stirred at the same temperature for 30 min. The reaction was quenched with H_2O. The mixture was stirred at room temperature for 20 min. The mixture was filtered through a Celite pad and washed with EtOAc. The mixture was washed with 3N aqueous NaOH. The aqueous phase was extracted with EtOAc three times and the combined organic phase was washed with basic brine (pH = 11 by adding aqueous NaOH) and dried over Na_2SO_4. Concentration and kugelrohr distillation (9 mmHg, 150–165 °C) gave *N*-(4-aminobutyl)cycloheptylamine (**23**, 428 mg, 92%): colorless oil; IR (neat) 3362, 3282, 2923, 2853 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 2.65–2.55 (m, 5 H), 1.92–1.35 (m, 19 H); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ 57.9, 43.1, 37.9, 30.0, 29.0, 27.3, 27.1, 23.3; HRMS (ESI–TOF) calcd for $C_{11}H_{25}N_2$ [M + H]$^+$ 185.2018, found 185.2019.

One-Step Synthesis of *N*-(4-Aminobutyl)-1-ethylpropylamine (20) via Mono-Reductive Amination: To a solution of 3-pentanone (**43**, 0.30 mL, 2.86 mmol) in CH_3OH (18 mL) were added putrescine (**2**, 0.80 mL, 8.58 mmol) and Ti(O*i*Pr)$_4$ (1.1 mL, 3.72 mmol) at room temperature. The mixture was stirred at the same temperature for 3 h. To the mixture was added $NaBH_4$ (108 mg, 2.86 mmol) at 0 °C. The mixture was stirred at the same temperature for 30 min. The reaction was quenched with H_2O. The mixture was stirred at room temperature for 20 min. The mixture was filtered through a Celite pad and washed with EtOAc. The mixture was washed with 3N aqueous NaOH. The aqueous phase was extracted with EtOAc three times and the combined organic phase was washed with basic brine (pH = 11 by adding aqueous NaOH) and dried over Na_2SO_4. Concentration and kugelrohr distillation (9 mmHg, 110–125 °C) gave *N*-(4-aminobutyl)-1-ethylpropylamine (**20**, 325 mg, 72%): colorless oil; IR (neat) 3362, 3292, 2928, 2872 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 2.67–2.56 (m, 4 H), 2.38 (quin, *J* = 6.0 Hz, 1 H), 1.52–1.43 (m, 10 H), 0.92–0.75 (m, 7 H); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ 59.0, 43.3, 37.9, 24.1, 22.7, 22.4, 21.3, 21.1, 9.0; HRMS (ESI–TOF) calcd for $C_9H_{23}N_2$ [M + H]$^+$ 159.1861, found 159.1855.

RT-qPCR: Wild type seedlings of *Arabidopsis thaliana* (Columbia accession) were grown for 7 days in 670 µl of Murashige-Skoog liquid medium without (Mock) or with xylemin, xylemin analogues, or thermospermine at the concentration of 200 µM. Total RNA was isolated by NucleoSpin® RNA Plant RNA isolation kit (TaKaRa) according to the manufacture instruction. 0.5 µg of total RNA of each sample was reverse transcribed to cDNA by ReverTra Ace reverse transcriptase (TOYOBO) according to the manufacture protocol. Quantitative PCR was performed in Thermal Cycler Dice Real Time System Lite (TaKaRa) using KOD SYBR qPCR Mix (TOYOBO) and gene-specific primers (*ACL5*: 5'-ACCGT TAACC AGCGA TGCTT T-3' and 5'-CCGTT AACTC TCTCT TTGAT TCTTC GATCC-3', and *ACT8*: 5'-GTGAG CCAGA TCTTC ATTCG TC-3' and 5'-TCTCT TGCTC GTAGT CGACA G-3'). *ACT8* was used as a reference gene for normalization of transcript abundance. RT-qPCR was conducted in three biological replicates.

Observation and Measurement of Xylem Formation: Wild type seedlings of *Arabidopsis thaliana* (Columbia accession) were grown for 7 days in 670 µl of Murashige-Skoog liquid medium without (Mock) or with xylemin, xylemin analogues, or thermospermine at the concentration of 200 µM. Seedlings were fixed in a solution of 9:1 mixture of ethanol and acetic acid, cleared in a mixture of chloral hydrate, glycerol, and water solution (8 g : 1 mL : 2 mL), and observed under a differential interference contrast microscope (Leica DM5000B) with a CCD camera (Leica DFC500). The width of xylem in the hypocotyls was quantified by NIH ImageJ software.

Observation and Measurement of Lateral Root Formation: Wild type seedlings of *Arabidopsis thaliana* (Columbia accession) were grown vertically for 7 days on Murashige-Skoog agar medium without (Mock) or with xylemin, xylemin analogues, or thermospermine at the concentration of 100 µM. Seedlings were observed under a stereomicroscope to count the number of lateral roots.

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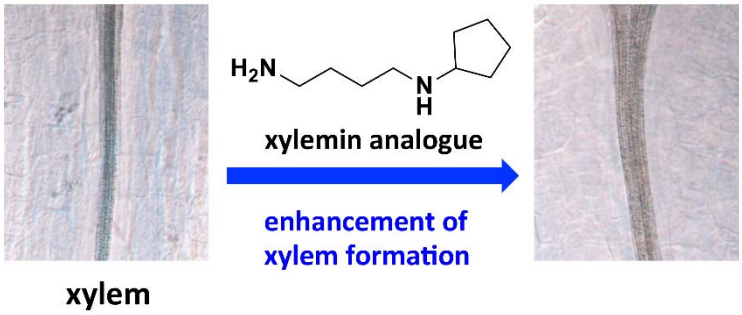
Entry for the Table of Contents



Xylemin and its six designed analogues were successfully synthesized. Evaluation of their biological effect on xylem revealed that a xylemin analogue, *N*-(4-aminobutyl)cyclopentylamine, could be a novel inhibitor of thermospermine synthesis to enhance specifically xylem formation.