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Chemical synthesis of *scyllo*-inosamine and catabolism studies in *Sinorhizobium meliloti*

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ABSTRACT

Rhizopines such as *scyllo*-inosamine (SIA) and L-3-*O*-methyl-*scyllo*-inosamine (3-*O*-MSI) play an intricate role as nutritional mediators during the establishment of the symbiotic relationship between legumes and rhizobia. The mechanism of action is not well understood. One challenge is the availability of rhizopines, which occur in only minute amounts in plant nodules. We herewith report an efficient synthesis of *scyllo*-inosamine and its biochemical activity in specific bacteria. SIA was prepared in 7 steps and 32% overall yield from readily available *myo*-inositol. The chemically synthesized SIA was tested to determine whether it can serve as sole carbon and nitrogen source for *Sinorhizobium meliloti* wild-type strain L5-30 and for strains carrying mutations in the rhizopine degradation (*moc*) genes. The analysis of the phenotype of the mutant strains revealed that the *moc* genes previously shown to be essential for the breakdown of the rhizopines isolated from root nodules are also essential for the utilization of the chemically synthesized SIA.

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1. Introduction

The cyclitol *myo*-inositol (MI, **1**, Fig. 1) and its derivatives play important roles in the cellular metabolism of animals, plants, and microorganisms.[1] For example, in biological membranes, phosphoinositides serve either as precursors for the second messenger inositol 1,4,5-triphosphate or act directly as ligands for protein signaling.[2] In plant cells, *myo*-inositol serves as precursor for cell wall carbohydrates and has been found to be conjugated with the phytohormone auxine.[1] Various methyl ethers of inositols are commonly found in tissues of different plant species and have been shown to specifically accumulate under different ecological conditions, such as water stress. Inositol also serves as phosphate storage compound when esterified with orthophosphate yielding phytic acid.[1]

In microbiology, *myo*-inositol is of interest because it can serve as sole carbon source for many bacteria, including *Aerobacter aerogenes* (now *Klebsiella pneumoniae*),[3] *Salmonella*,[4] *Bacillus subtilis*,[5] and *Rhizobium* species.[6–8] Moreover, diaminocyclitols are components of aminoglycoside antibiotics, such as streptomycin, hygromycin B, and spectinomycin.[9]

We have a long standing interest in two specific inosamine derivatives, *scyllo*-inosamine (SIA, **4a**) and L-3-*O*-methyl-*scyllo*-inosamine (3-*O*-MSI, **5**). Both compounds are relevant in the symbiotic association between bacteria and legume plants such as alfalfa, pea, and bean. Bacteria of the genus *Rhizobium* and *Sinorhizobium* colonize the roots of legume plants and induce the formation of root nodules. Once inside, bacterial nitrogenase fixes atmospheric nitrogen which benefits the plant as a nutrient. SIA and 3-*O*-MSI were first isolated from the nodules of alfalfa plants (*Medicago sativa*) that were induced by one particular *Sinorhizobium meliloti* strain, L5-30.[10] It is believed that SIA and 3-*O*-MSI can serve as proprietary growth substrate for the rhizobial strain inducing the nodules. In analogy to agrobacterial opines, these chemicals have been named 'opine-like compounds' or 'rhizopines',[10–12] but spectral analysis for the proposed structures of the compounds extracted from nodules was not given in the original literature. Rhizopines have been implicated to give a competitive advantage in nodulation for strains that are able to synthesize and catabolize rhizopines.[13–16]

Bacterial genes required for the synthesis (*mos*) and catabolism (*moc*) of rhizopines have been cloned and sequenced.[11,17–19] Some of the rhizopine degradation genes show homology to *myo*-inositol degradation genes, but the exact function of gene products encoded by *mos* and *moc* genes remains unknown.[7,19] This is in part due to the limited availability of rhizopines.

Since 1948, only three publications address the chemical synthesis of *scyllo*-inosamine (SIA),[20–22] while Yokoyama et al.[24] used an aminotransferase to afford unknown amounts of SIA. Several investigators prepared *N*-acyl and hexaacetyl derivatives.[20–23,25–29] The first synthesis of the 3-methoxyderivative (3-*O*-MSI) was

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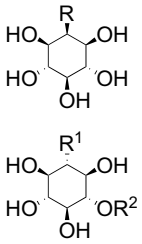


1 R=OH *myo*-inositol (MI)
2a R=NH_2 *myo*-inosamine, inosamine-SA
2b R=NHAc *N*-acetyl-*myo*-inosamine

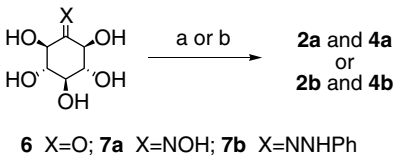
3 R^1=OH, R^2=H, *scyllo*-inositol
4a R^1=NH_2, R^2=H, *scyllo*-inosamine, SIA, inosamine-SB
4b R^1=NHAc, *N*-acetyl-*scyllo*-inosamine
4c R^1=NH_3Cl, R^2=H, *scyllo*-inosamine hydrochloride
5 R^1=NH_2, R^2=CH_3, 3-*O*-methyl-*scyllo*-inosamine, 3-*O*-MSI

Figure 1. *scyllo*-Inosamine and related derivatives.

reported in 2004.[30] Unfortunately, spectroscopic evidence for these inosamines has been sparse. Carter, who coined the term 'inosamine' to describe 'analogues of inositol', utilized oxime and phenylhydrazine intermediates (**7**, Scheme 1).[21] They were derived from *scyllo*-inosose (**6**) and reduced with Raney nickel to produce an epimeric mixture of *myo*(**2a**)- and *scyllo*(**4a**)-inosamine in 70% yield. Extraction and recrystallization methods allowed for the isolation of pure SIA, but in only 21% yield. Carter et al. did not identify the configuration of the new C–N bond and arbitrarily named their products 'inosamine-SA' and 'inosamine-SB'. The stereochemistry of the latter as SIA was not assigned until Anderson and Lardy[20] carried out a catalytic, stereoselective reduction of the oxime with sodium amalgam affording a mixture of both epimers as *N*-acetyl derivatives (**2b**/**4b**, 27: 73) in 57% yield.[31] After deprotection and purification pure SIA was isolated as a hemihydrate, decreasing the overall yield to 28% from the oxime. The stereochemistry of 'inosamine-SB' was inferred through comparison with preferred '*cis* alcohols' obtained from the reduction of *scyllo*-inosose with hydrogen over platinum.[32] The stereochemistry of the product from this procedure was never established with modern analytical methods, yet some biochemical studies using SIA relied on this reduction.[33,34]

A more recent report by Delgado and co-workers[22] utilized epoxide **8** as key intermediate to afford SIA in four steps via ring opening with sodium azide (Scheme 2). The epoxide itself was obtained in 9 steps from methyl-α-D-glucopyranoside; a Ferrier rearrangement converted the carbohydrate to a carbocycle. SIA was prepared in 24% over 13 steps. The last step involved the deprotection of five benzyl groups with BCl_3. Such reaction conditions are problematic without subsequent purification because polar side products are difficult to separate from the final inosamine.

We herewith describe a practical method that affords pure SIA in 7 steps and 34% overall yield from *myo*-inositol. Moreover, we provide relevant spectroscopic characterization data for the key intermediates and the final product. Our approach entails the overall stereoselective conversion of the *myo* to the *scyllo* configuration. The quantities obtained were sufficient to carry out *in vitro* catabolic studies using different *S. meliloti* strains, including those with transposon insertions in the rhizopine catabolic genes. We show that the same *moc* genes that are essential for the breakdown of the rhizopine isolated from root nodules are also essential for the utilization of the chemically synthesized SIA.



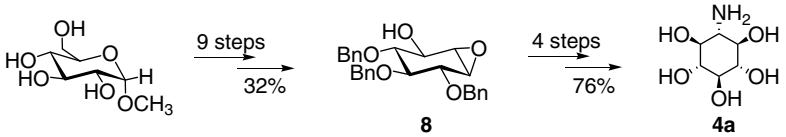
6 X=O; **7a** X=NOH; **7b** X=NNHPh

Scheme 1. Reduction of oxime and phenylhydrazine intermediates. Reagents and condition: (a) Na/Hg; (b) H_2, Raney Ni, 100–150 atm.

2. Results and discussion

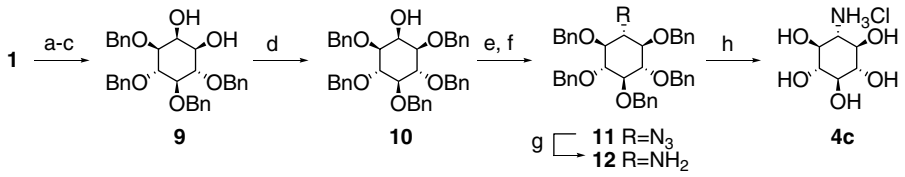
myo-Inositol (**1**) served as inexpensive starting material (Scheme 3). Protection of the cis diol,[35,36] subsequent benzylation,[36] and acetonide removal[36] furnished diol **9**. Selective benzylation using dibutyltin oxide[37,38] yielded alcohol **10,** which was converted to azide **11** via its triflate.[39] Initial efforts entailed the conversion of the azide to its amine **12** followed by treatment with BBr_3 and K_2CO_3. However, after testing a variety of catalytic hydrogenation procedures, we optimized the reaction conditions. The use of medium pressure hydrogen in combination with Pd or $Pd(OH)_2$ on carbon under acidic conditions provided a practical one-step method for the reduction of the azide and the removal of five benzyl groups to afford pure *scyllo*-inosamine as HCl salt (**4c**) in 87% yield. (These hydrogenation conditions are also applicable to amine **12.**) A small amount of product (**4c**) was converted into the hexaacetate using standard conditions. SIA and its acetate were fully characterized via mp, ^1H NMR, ^{13}C NMR, IR, and EA.

In order to test the chemically synthesized *scyllo*-inosamine for its biological efficacy, we used various *S. meliloti* Moc^+ and Moc^- wild-type and transposon-induced mutant strains for a catabolism assay. The growth of the different strains in liquid, chemically defined medium with 0.2% *scyllo*-inosamine as sole carbon (C) and nitrogen (N) source, was determined by measuring the absorption of all cultures at 600 nm. The Moc^+ wild-type strain L5-30 grew to an optical density of 1.4 with *scyllo*-inosamine as sole C- and N-source, whereas, as expected, the Moc^- wild-type strain 1021 did not grow at all (Fig. 2A). The same mutant strains that were reported earlier as being unable to degrade the rhizopine purified from nodules,[7,19] namely the *mocA* (M20), *mocB* (M21), *mocC* (M11), and *mocR* (M22, M23) mutants, were also not able to grow in minimal medium with the chemically synthesized *scyllo*-inosamine as sole C- and N-source (Fig. 2A). Mutant strains with insertions in *mocD* (M24, M25), *mocE* (M30), and *mocF* (M32) grew to almost the same final optical density as the wild type, whereas



8 **4a**

Scheme 2. Synthesis of *scyllo*-inosamine from methyl-α-D-glucopyranoside.



Scheme 3. Synthesis of *scyllo*-inosamine from *myo*-inositol. Reagents and conditions: (a) 2,2-dimethoxypropane, *p*-TsOH, DMSO, NEt_3, 74%; (b) BnBr, NaOH, 95%; (c) HCl, CH_3OH, 89%; (d) Bu_2SnO, Bu_4NI, BnBr, CH_3CN, 83%; (e) Tf_2O, pyr, CH_2Cl_2, 93%; (f) NaN_3, DMF, 81%; (g) Ph_3P, THF, H_2O, 96%; (h) H_2/Pd, $MeOH/CH_2Cl_2/H_2O$, HCl, 56 psi, 82%.

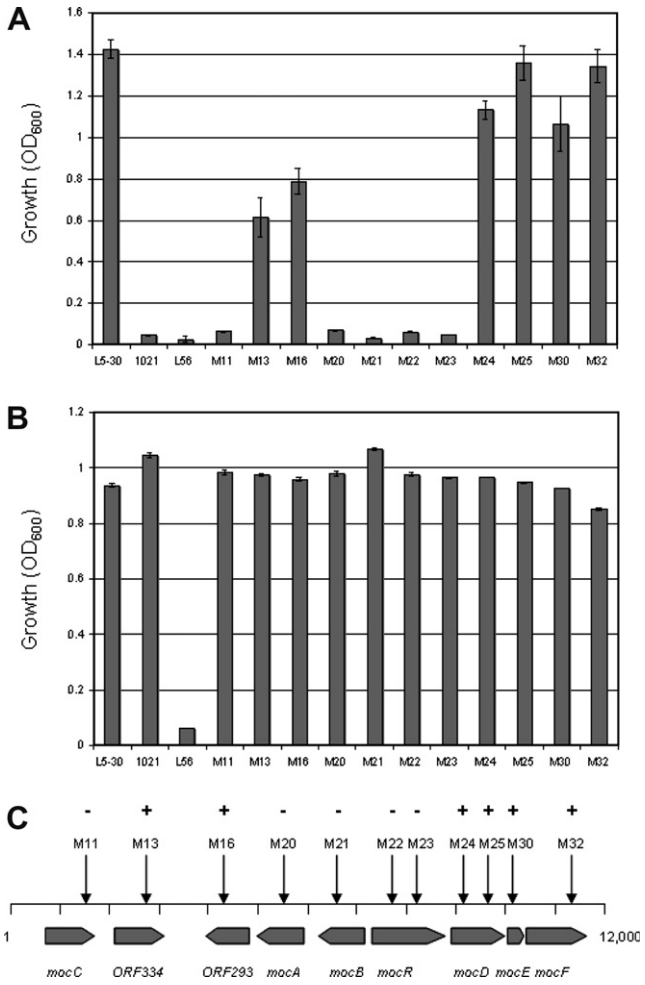


Figure 2. Growth of *Sinorhizobium meliloti* wild-type and mutant strains in minimal medium with (A) 0.2% *scyllo*-inosamine as sole carbon and nitrogen source and (B) 0.2% *myo*-inositol as carbon and 0.1% KNO_3 as nitrogen source. The growth was determined spectrophotometrically at 600 nm (OD_{600}). Strain designations are as follows: L5-30, Moc^+ wild-type; 1021, Moc^- wild-type; L56, *idhA*$^-$; M11, *mocC*$^-$; M13, Tn*5* insertion in ORF334; M16, Tn*5* insertion in ORF 293; M20, *mocA*$^-$; M21, *mocB*$^-$; M22, *mocR*$^-$; M23, *mocR*$^-$; M24, *mocD*$^-$; M25, *mocD*$^-$; M30, *mocE*$^-$; M32, *mocF*$^-$. Bars represent the average values from duplicate cultures of one typical experiment. Error bars denote standard deviations. (C) Structure of the *moc* operon in *Sinorhizobium meliloti* L5-30. The horizontal arrows indicate the location of the open reading frames in the *moc* region. The locations of the Tn*5* insertions are marked with vertical arrows, and labeled with the strain designations. The *scyllo*-inosamine catabolic phenotype is indicated by + (catabolism) and – (no catabolism).

mutants with insertions in ORF334 (M13) and ORF293 (M16) grew to around half of the optical density as the wild-type strain. The L56 mutant strain, which carries a transposon insertion in the *idhA* gene of *S. meliloti* L5-30,[7] did not grow with the *scyllo*-inosamine as sole C- and N-source. As control, the strains were grown on 0.2% *myo*-inositol as sole carbon source. As expected, all strains but the mutant L56 grew with *myo*-inositol as sole C-source (Fig. 2B). As an additional control, we found that all strains grew with 0.2% proline as sole C- and N- source (data not shown). Thus, our results confirm that the *mocABCR* genes are essential for the degradation of *scyllo*-inosamine.

The location of the Tn*5* transposons in the mutant strains used for the catabolism assay had been mapped previously by restriction enzyme analysis.[7,19] Under best circumstances, mapping can determine the location of transposon insertions with accuracy of ±50 base pairs. For this study, it was desirable to confirm the exact locations of the Tn*5* transposons in the different *moc* genes (Table 1). We constructed oligonucleotides (Table 2) based on the published DNA sequence of the rhizopine catabolism genes (Genbank Accession Nos. X78503 and AF076471) and used them as primers in PCR. The purified PCR products were subjected to DNA sequencing, and for each mutant strain the location inside the targeted open reading frame was confirmed (Fig. 2C).

Table 1
Sinorhizobium meliloti wild-type and mutant strains, shown with the exact locations of the transposon insertions in the inositol dehydrogenase (*idhA*) and the rhizopine catabolism (*moc*) genes

Strain	Phenotype	Tn*5* insertion *n* bp after start/total gene length in bp
1021	*Sinorhizobium meliloti* wild-type, streptomycin resistant derivative of 2011, Mos^-, Moc^-,[40]	No transposon insertion
L5-30	*S. meliloti* wild-type, Sm^R, Mos^+, Moc^+,[41]	No transposon insertion
L56	*S. meliloti* L5-30 *idhA*::Tn*5* Mutant, Sm^R, Km^R,[7]	387/993
M11	*S. meliloti* L5-30 *mocC*::Tn*5*, Sm^R, Km^R	841/978
M13	*S. meliloti* L5-30 ORF334::Tn*5*, Sm^R, Km^R	633/1005
M16	*S. meliloti* L5-30 ORF293::Tn*5*, Sm^R, Km^R	541/882
M20	*S. meliloti* L5-30 *mocA*::Tn*5*, Sm^R Km^R	418/954
M21	*S. meliloti* L5-30 *mocB*::Tn*5*, Sm^R, Km^R	539/930
M22	*S. meliloti* L5-30 *mocR*::Tn*5*, Sm^R, Km^R	452/1482
M23	*S. meliloti* L5-30 *mocR*::Tn*5*, Sm^R, Km^R	910/1482
M24	*S. meliloti* L5-30 *mocD*::Tn*5*, Sm^R, Km^R	263/1086
M25	*S. meliloti* L5-30 *mocD*::Tn*5*, Sm^R, Km^R	667/1086
M30	*S. meliloti* L5-30 *mocE*::Tn*5*, Sm^R, Km^R	133/318
M32	*S. meliloti* L5-30 *mocF*::Tn*5*, Sm^R, Km^R	972/1227

Table 2
Oligonucleotides used as primers in this study

Primer	Sequence
idhA Forward	5′ GCC GAC GGA TAC TCA TGC CGA TCT C 3′
mocA Forward	5′ CCT TAA TTT GGC GGG CAA TGG GAT 3′
mocB Forward	5′ CAA GTT CGG AAC CCT GCT AC 3′
mocC Forward	5′ GCG GGA TGT GGA TGC AGA ATT TGA 3′
mocD Forward	5′ CGA GAT TAC AGT CTG CTT GGT CG 3′
mocF Forward	5′ GAG CCG TAT TGT GAT CGT TGG CGC G 3′
mocR Forward	5′ CAT CTC AAG GCT GAC ACA CG 3′
ORF293 Forward	5′ GTT GAG AAA AGC GAC AGC G 3′
ORF334 Forward	5′ CGC AAT CGC CGT TGA GAA GGT TAT 3′
Tn*5*Ext	5′ GAA AGT TAC CAT GTT AGG AGG TC 3′

In conclusion, we present an efficient chemical method for the preparation of *scyllo*-inosamine in 7 steps and 32% overall yield. Moreover, we provide detailed spectroscopic data for the characterization of SIA and combine these chemical data with biological results to unequivocally prove that the *S. meliloti mocABCR* genes are necessary for the utilization of SIA as sole carbon and nitrogen source.

3. Experimental

Commercial chemicals and reagents in 98+ purity were used without further purification. Solvents were purchased as reagent grade. Dichloromethane, DMF, and methanol were purified and dried using standard procedures. Melting points were measured in open capillaries using a Thomas–Hoover Unimelt instrument. NMR spectra were recorded using a 400 MHz Jeol Eclipse nuclear magnetic resonance instrument. IR spectra were obtained from Bruker Equinox 55 and Perkin Elmer 1710 Fourier Transform Infrared Spectrometers. Elemental analyses were carried out by Numega Resonance Labs, Inc. in San Diego, CA.

3.1. 1,3,4,5,6-Penta-*O*-benzyl-2-*O*-deoxy-2-azido-*scyllo*-inositol (11)

Azide **11** was prepared from alcohol **10** using modified literature procedures.[35–38] To a solution of 1,3,4,5,6-penta-*O*-benzyl-*myo*-inositol **10** (3.45 g, 5.47 mmol) in anhydrous dichloromethane (200 mL) was added anhydrous pyridine (1.33 mL, 16.41 mmol) at −60 °C under argon. Triflic anhydride (2.27 mL, 13.67 mmol) was added dropwise to the solution, and the reaction mixture was allowed to warm to room temperature. TLC (hexane/ethyl acetate, 2:1) revealed the reaction completed after 26 h. After quenching with water, the reaction mixture was extracted with CH_2Cl_2, and subsequently washed with water, saturated aqueous $NaHCO_3$, and brine. The organic layer was dried ($MgSO_4$) and concentrated to afford a thick oil (3.89 g, 93%) which was used without purification for the next step. The crude triflate (3.75 g, 4.92 mmol) and sodium azide (5.11 g, 78.65 mmol) were dissolved in dry DMF (300 mL). The reaction mixture was stirred vigorously at 80 °C for 5.5 h while monitoring by TLC (heptane/ethyl acetate, 4:1). The undissolved sodium azide was filtered off and the residue concentrated. An excess of water was added and the mixture was extracted with CH_2Cl_2. The organic layer was washed with a 5% sodium chloride solution, dried with $MgSO_4$, and concentrated to yield 95% of crude yellow solid. Recrystallization from hot methanol yielded pure azide **11** (2.60 g, 81%) which was previously described as oil.[22] mp 95–96 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.42–7.2 (m, 25H), 4.93–4.88 (m, 10H), 3.63–3.49 (m, 4H), 3.38 (app t, J = 9.16 Hz, 2H); ^{13}C, NMR ($CDCl_3$, 100 MHz) δ 138.37, 138.34, 137.9, 128.62, 128.58, 128.4, 128.1, 127.98, 127.91, 127.88, 83.4, 82.7, 81.2, 76.2, 76.11, 76.08, 67.1; IR (KBr) 3062, 2904, 2200, 2102; Anal. calcd for $C_{41}H_{41}N_3O_5$: C, 75.09; H, 6.30; N, 6.41. Found: C, 75.10; H, 6.11; N, 6.34.

3.2. 1,3,4,5,6-Penta-*O*-benzyl-2-*O*-deoxy-2-amino-*scyllo*-inositol (12)

Azide **11** (1.10 g, 1.677 mmol) and triphenylphosphine (0.484 g, 1.845 mmol) were dissolved in THF (100 mL). The solution was stirred at room temperature for 2 h and water (5 mL) was added. The reaction mixture was refluxed at 80 °C for 24 h until completion as monitored by TLC (hexane/ethyl acetate, 4:1). The reaction mixture was concentrated and the residue was extracted with ethyl acetate. The organic layer was dried ($MgSO_4$) and concentrated to obtain the crude product which was purified by column chromatography (hexane/ethyl acetate, 2:1) to afford 1.02 g (96%) of amine **12**. Mp 120–121 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.44–7.34 (m, 25H), 5.21–4.80 (m, 10 H), 3.74–3.73 (m, 3H), 3.46 (m, 2H), 3.05 (t, J = 9.88 Hz, 1H), 1.86 (br s, NH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 138.6, 138.5, 128.8, 128.7, 128.6, 128.1, 128.03, 128.0, 127.9, 127.8, 84.5, 83.7, 83.3, 76.2, 76.0, 75.9, 55.6. Anal. calcd for $C_{41}H_{43}NO_5$: C, 78.19; H, 6.88; N, 2.22. Found: C, 77.83; H, 6.77; N, 2.41.

3.3. *scyllo*-Inosamine hydrochloride (4c)

To the suspension of 10% Pd/C (200 mg, 0.188 mmol) in MeOH/H_2O (2:1, 9 mL) was added a solution of azide **11** (794 mg, 1.212 mmol) in CH_2Cl_2 (9 mL). Concentrated HCl (1 mL) was added to the mixture which was hydrogenated at 56 psi for 48 h. The reaction mixture was filtered through Celite and rinsed with MeOH/H_2O (4:1). The filtrate was concentrated and the residue was partitioned between H_2O and EtOAc layers. The aqueous layer was concentrated to give 215 mg (82%) of a white solid. A white precipitate was collected. Using the same procedure hydrogenation of 49 mg of amine **12** gave 13 mg (76%) of *scyllo*-inosamine hydrochloride **4c**. Mp 290 °C (dec.); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.51 (app t, J = 9.72 Hz, 2H), δ 3.39 (app t, J = 9.16 Hz, 2H), δ 3.32 (t, J = 9.16 Hz, 1H), δ 3.07 (t, J = 10.44 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 74.4, 74.2, 70.02, 55.90; IR (KBr) 3400, 3045, 1929, 1626, 1576, 1505, 1347, 1036; Anal. calcd for $C_6H_{14}ClNO_5$: C, 33.42, H, 6.54, N, 6.50. Found: C, 33.45, H, 6.23; N, 6.76. A small sample was reacted with acetic anhydride in dry pyridine at room temperature for 48 h to afford the corresponding hexaacetate. Mp 299 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.71 (d, J = 9.88 Hz, 1H), δ 5.28 (app t, J = 9.90 Hz, 2H), δ 5.17 (t, J = 9.88 Hz, 1H), δ 5.05 (app t, J = 10.26 Hz, 2H), δ 4.42 (app dd, J_1 = 10.48 Hz, J_2 = 21.06 Hz, 1H), δ 2.02 (s, 6H), δ 2.00 (s, 6H), δ 1.99 (s, 3H), δ 1.89 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.86, 170.16, 169.76, 169.36, 70.54, 70.48, 70.42, 51.35, 23.13, 20.58, 20.53, 20.50; IR (KBr) 3426, 2939, 1733, 1687, 1521, 1381, 1234, 1037; Anal. calcd for $C_{18}H_{25}NO_{11}$: C, 50.12; H, 5.84; N, 3.25. Found: C, 50.51; H, 5.80; N, 3.19.

3.4. Bacterial strains, media, and growth conditions

Bacterial strains used are shown in Table 1. The complete medium used was TY[42] and the defined medium was Minimal M medium.[19] Antibiotics were used in the following concentrations: streptomycin (Sm), 250 μg/ml, kanamycin (Km), 200 μg/ml.

3.5. Catabolism assays

Sinorhizobium meliloti precultures were grown in TY for 48 h and then inoculated 1:100 into glass tubes containing 2 ml minimal medium with either 0.2% *myo*-inositol as sole carbon (C) source and 0.1% KNO_3 as nitrogen (N) source or 0.2% proline or 0.2% *scyllo*-inosamine as sole C- and N- source. The cultures were shaken (200 rpm) at 28 °C. After 72 h, the bacterial growth was determined by measuring the absorbance at 600 nm (OD_{600}) with a Beckman DU 640 spectrophotometer.

3.6. Determination of transposon insertions sites by PCR and DNA sequencing

The exact insertion site of the Tn*5* insertions in the mutant strains was determined via PCR. As template, 1 microliter of a liquid bacterial culture was used in 50-μl reaction mixtures containing (as final concentrations): 1× Red-Taq PCR Buffer (Sigma–Aldrich Corp., St. Louis, MO), a 0.2 mM concentration of each deoxyribonucleotide, 50 pmol of each forward and the T*n*5Ext primer (see Table 2), and 1.5 U of Red-Taq DNA polymerase (Sigma–

Aldrich Corp., St. Louis, MO). PCR amplification was carried out in a Mastercycler gradient DNA thermal cycler (Eppendorf, Westbury, NY), with an initial denaturation step for 5 min at 95 °C; 30 cycles at 95 °C for 1 min, 50 °C for 1 min, and 72 °C for 2 min for elongation; and a final elongation step at 74 °C for 7 min. The PCR products were purified using a Promega Wizard SV Gel and PCR Clean-up System (Promega, Madison, Wisconsin). DNA sequencing was performed by the Cornell Biotechnology Resource Center (USA) using the T*n*5Ext Primer.

Note added in proof

Since the submission of our manuscript another publication reported the synthesis of SI in 23% yield using 5 steps from the monoorthoformate of *myo*-inositol. [43]

Acknowledgments

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