Rhizopine Synthesis – Potential Mediators for Nitrogen Fixation in Legume Plants

Venkat Reddy Guduru

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RHIZOPINE SYNTHESIS - POTENTIAL MEDIATORS FOR NITROGEN FIXATION IN LEGUME PLANTS

by

Venkat Reddy Guduru

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Venkat Reddy Guduru
This research project focused on novel and efficient synthetic approaches of inosamine derivatives (rhizopines). The preparation of specific rhizopines would help elucidate the microbial pathway of rhizopine catabolism and degradation, and determine the role of corresponding genes during nitrogen fixation. We have investigated regio- and stereoselective transformations of *myo*-inositol derivative 28 utilizing an organostannane as key intermediate towards rhizopine 1a. The synthesis involved several protection and deprotection strategies to control competing reactivities of six hydroxyl groups of *myo*-inositol. Rhizopine 1a was prepared efficiently as a single isomer in 8 steps and 13% yield by controlling reactions regio- and stereoselectively.
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INTRODUCTION

Nitrogen fixation is a very important process for soil fertility because nitrogen availability in agricultural soils is often the limiting factor for crop productivity. This problem will become more severe in the 21st century, as agricultural production must keep pace with an increase in world population. Biological nitrogen fixation (BNF), through symbiotic, associative and free-living microbial systems, already contributes a major and sustainable input into agriculture. Enhancing these systems through research and development could provide an ecologically acceptable alternative to the increased application of nitrogen fertilizers in the world.

Among various soil microorganisms, nitrogen-fixing bacteria have the ability of converting atmospheric nitrogen into nitrogen fertilizer and often live in symbiosis with legume plants. In the root area (the rhizosphere) the plant releases molecules known as rhizopines, which act as nutrients for the bacteria. In return, the bacteria fix nitrogen for the plant (symbiotic process, Figure 1).

![Figure 1: Symbiotic process in the rhizosphere](image)
These rhizopines have been proposed as nutritional mediators in nitrogen-fixing symbioses to benefit growth in legume plants. They have been isolated in only minute amounts via extraction from plant nodules (ca 10 ng can be extracted from 10 g of dried nodules)\(^2\) and have not been chemically synthesized in sufficient amounts so far. In addition, rhizopines are thought to be involved in nitrogen fixation and their role has not yet been identified clearly. Therefore, the ready availability of rhizopines could aid biological studies, and organic synthesis can address this need.

Among others, the two rhizopines scyllo-inosamine 1a and L-3-O-methyl-scyllo-inosamine 1b (3-O-MSI) have been proposed as nutritional mediators for nitrogen-fixing symbiosis.\(^3\) The compound myo-inositol (Figure 2), which is inexpensive and commercially available, was chosen as starting material for the synthesis of scyllo-inosamine 1a. Future goals of this project could extend to the synthesis of additional rhizopine stereoisomers for structure-activity correlation studies. Such compounds could help in structural elucidation during symbiosis. Rhizopines 1a and 1b are highly functionalized and contain six stereogenic centers. The most significant challenge is to control reactions stereo- and regioselectively in order to obtain the desired molecules as single isomers.
BACKGROUND

Few types of soil microorganisms such as *Rhizobium, Sinorhizobium, Azorhizobium* are known to convert atmospheric nitrogen to ammonia, enriching the soil nutrients by living in symbiosis with legume plants. In recent years, there has been increasing interest in this field because it could also address environmental and ecological problems caused by extensive use of fertilizers. Adverse effects of fertilizers include water pollution by nitrates, production of smog and haze, destruction of the ozone layer and hence global warming, and farm run-offs into rivers and streams and finally into oceans. The latter causes explosive growth of algae that steal O₂ from other organisms creating the so called “Dead Zone.”

The interaction between plants and nitrogen-fixing bacteria requires a specific recognition in the root area, the rhizosphere, a nutritionally enriched area around the plant roots where a variety of compounds are deposited by plants. Numerous microbes can be found in the rhizosphere, competing among themselves for the limited supply of nutrients released by the plants, including rhizopines (opines released into the rhizosphere) which serve as nutrients for the bacteria. In return, nitrogen-fixing bacteria, which have evolved their own advantageous strategies to obtain carbon compounds in exchange, fix nitrogen for the plant.

The symbiotic interactions between the plant and bacteria are equally advantageous for both participants. Rhizopine production by the plant provides the bacteria with required
nutrients favoring its propagation and survival in the rhizosphere. In return, the plant
obtains ammonia for its growth.\(^4\)

Murphy \textit{et al}\(^4\) reported that "a nodule-specific, opine-like compound was isolated from
alfalfa (\textit{Medicago sativa}) nodules induced by \textit{Rhizobium meliloti} strain L5-30. This
compound was described as opine-like by analogy with \textit{Agrobacterium}. This compound
has been identified as L-3-O-methyl-scyllo-inosamine (3-O-MSI).” To distinguish these
nodule-specific, opine-like compounds from \textit{Agrobacterium} opines, Murphy \textit{et al}\(^4\) have
introduced the generic name rhizopine for such compounds.

Rhizopines found in the rhizosphere of the soil are opine-like compounds. Opines are
compounds such as amino acids, carbohydrates, inositols, and inosamines that act as
either carbon or nitrogen sources for bacteria.\(^3\) Opines are produced by the plant in
response to a stimulus from the pathogen.\(^7\) The nature of these opines, and their
utilization by microorganisms as either nitrogen and/or carbon sources, is strain specific.\(^3\)

Even though rhizopines play an important biological role many rhizopines have not been
chemically synthesized and studied. Our immediate goal is to obtain sufficient amounts
of specific rhizopines, \textit{scyllo}-inosamine \textit{1a} and L-3-O-methyl-\textit{scyllo}-inosamine \textit{1b}.

In 1987, Murphy \textit{et al}\(^6\) reported that "\textit{scyllo}-inosamine (SI) was chemically synthesized
(M.E.T., unpublished data);” However neither yields nor characterization data were
included for compound \textit{1a}. Carter \textit{et al}\(^8\) synthesized \textit{scyllo}-inosamine \textit{1a} in 1948 along
with its isomers, myo-inosamine 4 and epi-inosamine 5 (Scheme 1) from phenylhydrazone 2 and oxime 3 via hydrogenation. The mixture was separated by fractional recrystallization.

Scheme 1: Synthesis of scyllo-inosamine and its stereoisomers

Each compound was characterized as its ester derivative (hexaacetyinosamine 6, N-acetylinosamine 7, and inosamine hydrochloride 8) by means of melting points and elemental analyses (Scheme 2).

Scheme 2: Synthesis of scyllo-inosamine derivatives
In 1962, Nakajima et al. synthesized scyllo-inosamine 1a together with seven isomers (Scheme 3) from diol 9. The product was converted to hexaacetyl-scyllo-inosamine 6 and then characterized by melting point, IR, and elemental analysis.

Scheme 3: Synthesis of scyllo-inosamine and its stereoisomers

In 1966, Suami et al. synthesized peracylated product 6 from tetraacylated-myo-inositol 12 (Scheme 4), which underwent selective equatorial benzylation of alcohol 13, followed by mesylation. The mesylate of the axial OH group was substituted by azide followed by the conversion of the benzoyl group to an acetate. Azide 15 was reduced with H$_2$/Pt to provide hexaacetyl-scyllo-inosamine 6, a derivative of scyllo-inosamine 1a.

Scheme 4: Synthesis of scyllo-inosamine derivatives
More recently, Krief et al\textsuperscript{11} reported the first synthesis of 3-O-MSI 1b as its ammonium chloride salt. Starting with the protection of myo-inositol (16) they took advantage of the conformational rigidity of the orthoformate moiety (17, Scheme 5). In intermediate 17 two hydroxyl groups are oriented axially and sequential alkylation of these axial hydroxyl groups was carried out with methyl iodide and benzyl bromide in DMF, respectively.

Scheme 5: Synthesis of L-3-O-methyl-scyllo-inosamine 1b

Oxidation of alcohol 19 with tetrapropylammonium perruthenate (TPAP) in the presence of N-methylmorpholine N-oxide gave the desired ketone 21 (Scheme 6), along with alcohol 20 in an 80/20 ratio. Reductive amination of the crude reaction mixture (20 and 21) with ammonium acetate and sodium cyanoborohydride, yielded the required amine (22). Compound 22 was then separated from alcohol 20 by taking advantage of the insolubility of its ammonium salt in ether. The pure amine was finally obtained in 45% yield after a basic workup.
Scheme 6: Synthesis of L-3-O-methyl-scyllo-inosamine 1b

Debenzylation of 22 using sodium in liquid ammonia and acid hydrolysis (HCl) yielded 3-O-methyl-scyllo-inosamine hydrochloride 24 in its racemic form (Scheme 7).

Scheme 7: Synthesis of L-3-O-methyl-scyllo-inosamine 1b
OBJECTIVE

Even though rhizopines such as scyllo-inosamine 1a and 3-O-MSI 1b have been synthesized and described in the literature, their preparations often lack selectivity and thus generate isomeric side products. In addition many reports lack vital spectral data to corroborate the results. Our goal is to synthesize rhizopines in sufficient amounts with high regio- and stereoselectivity and to fully characterize them.
RESULTS AND DISCUSSION

Our synthetic procedure involves protection, deprotection and several functional group manipulation steps of the six secondary hydroxyl groups of myo-inositol (Figure 3). This approach involves the control of chemical conversions regio- and stereoselectively, which is a critical point for the success of this project.

The synthesis started with commercially available myo-inositol, which is a meso-isomer of 1,2,3,4,5,6-hexahydroxycyclohexane and contains five equatorial hydroxyl groups and one axial one. Inositols are cyclohexanols and a wide variety occur in nature, such as myo-, neo-, chiro-, and scyllo-inositol (Figure 3). Synthetic isomers cis-, epi-, allo-, and muco-inositol are also known and shown below.\(^\text{12,13}\)

\[\text{myo-inositol} \quad \text{neo-inositol} \quad \text{allo-inositol} \]
\[\text{(-)-L-chiro-inositol} \quad \text{(+)D-chiro-inositol} \quad \text{scyllo-inositol} \]
\[\text{epi-inositol} \quad \text{cis-inositol} \quad \text{muco-inositol} \]

Figure 3: Natural and unnatural isomers of inositol\(^\text{12}\)
Inositols are closely related to inosamines and are therefore suitable starting materials. *myo*-Inositol (16) widely occurs in nature, is commercially available at low cost, and has many similarities to our target molecule, which made it an attractive choice as a starting material. The configuration of *myo*-inositol favors a conformation with only one axial hydroxyl group. Our synthesis requires the inversion of that axial hydroxyl group and substitution with an amino group to obtain the desired product, *scyllo*-inosamine 1a. The overall approach is depicted in the following scheme (Scheme 8). Inositol 16 contains six hydroxyl groups with similar reactivities, and as a result we had to utilize protection and deprotection protocols for the selective manipulation of functional groups. As shown in the scheme below, we protected the two vicinal *cis* hydroxyl groups of *myo*-inositol 16, yielding the corresponding acetonide 25. Further benzyl protection and the removal of the acetal yielded diol 27.

Scheme 8: Overall synthetic strategy for *scyllo*-inosamine 1a
Dibutyltin oxide was used in the next step to form selectively an equatorial benzyl ether 28, followed by conversion to its trifluoromethane sulfonate. Nitrogen was introduced via azide substitution. Intermediate 30 was reduced to the free amine 31, which was then deprotected to afford target molecule 1a.

**Protection of cis-diol**

For the very first step, we followed the procedures by Gigg et al.\textsuperscript{14,15} Achiral myo-inositol was treated with dimethoxypropane in order to protect the two adjacent cis-hydroxyl groups which yielded the corresponding chiral racemic cis-isopropylidene acetal 25 (1,2-O-isopropylidene-myoinositol, Scheme 9). cis-Acetonides are thermodynamically more stable than trans-acetonides.\textsuperscript{16} Therefore adjacent cis-hydroxyl groups react preferentially, yielding isopropylidene myo-inositol 25.

\[\text{Scheme 9: Synthesis of acetonide 25}\]

Mechanism involved is:
The two procedures by Gigg et al\textsuperscript{14,15} were similar with regards to reagents and solvents, but involved different conditions. In their first procedure,\textsuperscript{14} a mixture of myo-inositol and toluene-$p$-sulphonic acid in dry dimethyl sulfoxide (DMSO) and 2,2-dimethoxypropane (DMP) was stirred for 3 hrs at 110°C. Excess of solvent and reagents were removed by distillation. The residue was triturated and provided the precipitated acetonide 25 in 73\% yield.

In Gigg et al's later procedure,\textsuperscript{15} all the reagents including myo-inositol were dissolved in dry DMSO and stirred at 100°C until a clear solution was obtained (~ 30 min). The solution was cooled and triethylamine and ethanol were added, followed by ether. The mixture was stirred for 4 hrs at room temperature. The crystalline product was collected through filtration to give acetonide 25 (32\%). Gigg's later procedure\textsuperscript{15} was adopted as it allowed direct crystallization of the product avoiding the need to remove DMSO (high boiling point, 187°C) by distillation. The reaction yield was improved from 32\% to 41\% by increasing the reaction time from 4 hrs to 24 hrs. The amount of ether used in the reaction was also relevant. The volume was quadrupled, which helped precipitate the product as a white solid. Acetonide 25 was characterized by m.p, $^1$H NMR, and $^{13}$C NMR and compared with literature data. However, this shorter improved procedure still gave lower yields than that of the lengthy first preparation (73\%).
Protecting group manipulation

The remaining four hydroxyl groups of 1,2-O-isopropylidene-myoinositol were reacted with BnBr under basic conditions to give the fully protected myo-inositol 26 as outlined by Gigg and Warren\textsuperscript{14} (Scheme 10).

![Scheme 10: Synthesis of tetrabenzylated myo-inositol 27](image_url)

The reaction was monitored by normal phase silica thin layer chromatography (TLC) that showed complete conversion of starting material into fully benzylated product along with traces of partially benzylated product and dibenzyl ether as side products. The reaction progress was also observed by the color change from white to thick yellow oily semi solid material that formed after stirring the reaction mixture for 24 hrs at 120°C. Crude compound 26 was purified on a neutral alumina column using toluene/ether 3:1 solvent mixture as eluent as outlined in the literature.\textsuperscript{14} But the removal of toluene (110°C) with the rotary evaporator and the use of expensive alumina were a disadvantage. Therefore, the purification procedure was modified using silica gel and lower boiling point solvents such as hexane and ethyl acetate to provide the fully benzylated-1,2-isopropylidene-myoinositol 26. After column chromatography, compound 26 was free from partially benzylated compound but was contaminated with traces of dibenzylether, which was removed in the next step (deprotection step) by triturating with light petroleum ether. Purified tetrabenzylated acetonide 26 was treated with concentrated HCl to deprotect the
acetal. The product, tetrabenzylated cis-diol 27, was obtained as a white solid, which was further purified by recrystallization from MeOH (Scheme 10). The 3,4,5,6-tetra-O-benzyl-myoinositol (27) was first synthesized by Angyal and Tate\(^\text{17}\) by benzylation of 1,2-O-cyclohexylidene-myoinositol (32, Scheme 10). However, we have prepared compound 27 by employing the isopropylidene derivative of myoinositol (25), for which Gigg and Warren developed an easy and improved synthesis (82%). In Angyal and Tate’s procedure\(^\text{17}\) they first prepared cyclohexylidene-myoinositol derivative (32) in extremely low yield (2%), which was used as seeds (seedling procedure) to improve the yield for a 2\(^{\text{nd}}\) time. The procedure also involved Dean-stark distillation and longer reaction times (30 hours). Whereas for the Gigg and Warren procedure the reaction mixture was stirred at 100°C for 1 hr, excess acid was neutralized, ether added, and the mixture was stirred overnight when the acetonide 25 precipitated. The product was filtered and directly used in the following benzylation reaction to obtain compound 26. Compounds 26 and 27 were characterized by m.p, \(^1\)H NMR, and \(^{13}\)C NMR and compared with literature data.\(^\text{18,19}\)

**Regioselective benzylation**

Regioselective benzylation was a key step in our synthesis allowing the selective protection of the equatorial hydroxyl group over the axial one in diol 27 (Figure 4). A thorough literature review provided several procedures for the regioselective alkylation\(^\text{17,18}\) and allylation reaction.\(^\text{20}\)
Angyal et al.\textsuperscript{17} carried out the selective benzylation of diol 27 using benzyl chloride (BnCl) and KOH as a base in anhydrous benzene at 100°C for 1.5 hours (Equation 1). The desired equatorially protected 1,3,4,5,6-penta-O-benzyl-myoinositol (28) was obtained in moderate yield (52%) along with the axially protected isomer 1,2,4,5,6-penta-O-benzyl-myoinositol (33, 1%).

Equation 1: Synthesis of alcohol 28 with its stereoisomer 33

Koto et al.\textsuperscript{18} performed the same reaction by stirring at 130°C for 3 hrs using LiOH as a base (Equation 2). The yield for product 28 was somewhat higher (67%) but the reaction still formed undesired axially protected product 33 (2%); starting material was also recovered 27 (8%).

Equation 2: Synthesis of alcohol 28 with its stereoisomer 33
Nashed et al\textsuperscript{20} carried out the equatorial allylation reaction via a dibutylstannylene derivative on allyl-2,6-di-O-benzyl-\(\alpha\)-D-galactopyranoside 34 in two steps (Scheme 11). Initially they prepared stannylene intermediate 35 in DMF to which allyl iodide was added and the reaction mixture was heated for 1 hr yielding exclusive equatorially benzylated galactopyranoside product (36, 79\%).

\[
\begin{align*}
\text{Scheme 11: Equatorial allylation of compound 34} \\
&\text{The same authors applied the above procedure to diol 27 (Scheme 12),}^{21} \text{ affording the equatorially protected alcohol 28 (67\%).}
\end{align*}
\]

A high degree of selectivity is achieved during the alkylation and allylation reactions involving dibutylstannylene derivatives.\textsuperscript{22} Vicinal cis-diols in six membered derivatives undergo preferential reaction on the equatorial hydroxyl group. We attempted both procedures of Nashed et al\textsuperscript{20,21} but were unsuccessful. We also tried modified reaction conditions by varying the temperature and solvent (vide infra).
The key stannylene intermediate is prepared from dibutyltin oxide (DBTO), which is commercially available at low-cost and a widely used industrial compound.\textsuperscript{23} It exists as an amorphous polymeric solid insoluble in inert solvents and is produced during the hydrolysis of dialkyltin halides (Figure 5).\textsuperscript{23}

\[
\begin{align*}
R_2\text{Sn}X_2 & \quad \rightarrow \quad R_2\text{Sn}(X)\text{OSn}(X)R_2 \\
R_2\text{SnO} & \quad \rightarrow \quad R_2(X)\text{SnOSnR}_2(\text{OH})
\end{align*}
\]

Figure 5: Hydrolysis of dialkyltin halide and amorphous polymeric solid structure of DBTO.\textsuperscript{23}

The best-known application of DBTO in organic chemistry is the generation and reaction of stannynes from polyhydroxy compounds.\textsuperscript{23} Generally dibutylstannynes are synthesized in almost quantitative yields by heating stoichiometric amounts of DBTO and polyols in a solvent such as benzene, toluene or acetonitrile with concomitant removal of water.\textsuperscript{24}

To understand the stannylene intermediate formation better, we tried a series of test reactions using myo-inositol as starting material with DBTO in methanol, toluene, and DMSO (Scheme 13). Formation of the stannylene intermediate was confirmed through GC/MS, but there was only partial conversion. However, one of the major obstacles when
using myo-inositol was its poor solubility in most organic solvents except hot MeOH (ca. 100 mg in 400 ml) and hot DMSO (even small amount of DMSO can dissolve it).

Scheme 13: Equatorial benzylation of compound 16

After further literature review, we adapted Gigg's procedure\textsuperscript{25} (BnBr, DBTO, CH\textsubscript{3}CN, molecular sieves, Bu\textsubscript{4}NI, 120°C, 18 hr) but with a different work up. Diol 27 and Bu\textsubscript{2}SnO (DBTO) were reacted in excess refluxing acetonitrile in a soxhlet apparatus using molecular sieves (4Å). Tetrabutylammonium iodide (1 equivalent) was employed to speed up the reaction (Scheme 14). The formation of an ion pair RO\textsuperscript{+}NBU\textsubscript{4} with a large cation-anion separation reduces the reaction time by enhancing the reactivity of the alkoxide.\textsuperscript{26} Alcohol 28 was obtained in high yield (79%) and characterized by m. p, \textsuperscript{1}H NMR, and \textsuperscript{13}C NMR and compared with literature data.\textsuperscript{18,19}

Scheme 14: Regioselective benzylation of compound 27
It should be noted that while acylation reactions proceed without any catalyst, alkylations are much slower and are accelerated in the presence of tetrabutylammonium halides (0.5 equivalent).\(^{27}\)

Possible explanations for regioselectivity

Synthetic applications of stannylenes (R\(_2\)Sn(OR')\(_2\), organotin derivatives) were investigated by Moffat,\(^{28}\) Ogawa,\(^{29}\) David and Hanessian,\(^{22}\) who showed the inherent differences in the nucleophilicities of two cis-hydroxyl groups and the effect of steric hindrance. Extensive studies\(^{22}\) on chiral carbohydrate-derived stannylenes indicate that the stannylenes are dimeric in all physical states, except perhaps in polar solvents. Also stannylene derivatives, as well as trialkyltin alkoxides undergo regioselective acylation, alkylation and oxidation, though the precise origin of the regioselectivity is still unclear. One hypothesis states that one of the two oxygens involved in the stannylene ring is more
nucleophilic than the other. Understanding dimeric structures of tin complexes may provide a plausible explanation for the regioselectivity (Figure 6).\textsuperscript{22}

![Dimeric structure of stannylene](image)

Figure 6: Dimeric structure of stannylene\textsuperscript{22}

In a single monomeric unit of the dimer, the apically bound O-atoms (O\textsuperscript{1} and O\textsuperscript{1'} ) are regioselectively acylated and alkylated because they are not involved in the Sn\textsubscript{2}O\textsubscript{2} parallelogram (Figure 6). Such nucleophilic enhancement could result in electron flow from the tin towards the apically bound oxygen atoms. Feshin \textit{et al}\textsuperscript{30} have shown that Sn is a better transmitter of electronic effects than carbon.

The next logical question would be, which of the two oxygen atoms of the diol would preferentially bind apically in stannylene formation and hence undergo alkylation. Naturally, the more electronegative oxygen would bind apically, as it is known that in trigonal bipyramid complexes (Figure 7), electronegative ligands are more stable when occupying apical positions.\textsuperscript{31}
Another hypothesis is based on kinetic and thermodynamic criteria which can predict the reactivity of an equatorial hydroxyl group. M. S. Shashidhar et al. mentioned in their review that the OH group in the equatorial position is more reactive and the resulting products are thermodynamically more stable. Thus the conversion to a stannylene may emphasize small electronegativity differences between the two oxygen atoms of a diol, though steric factors may also play a key role.

**Triflate as a good leaving group for $S_N^2$ reaction**

The inversion of configuration of secondary alcohol 28 (nucleophilic substitution reaction at sp$^3$-carbon center) is necessary to obtain the desired scyllo-configuration. However, because the OH group is not a good leaving group it must be converted to a mesylate, tosylate, or triflate. It is important to consider the steric and electronic requirements of both the nucleophile and the electrophile involved when selecting reagents for $S_N^2$ reactions. We tried three methods to convert the hydroxyl group, namely tosylation, mesylation and triflation (Scheme 15).

We followed Rucker’s procedure for the conversion of hydroxyl group into tosylate.
with TsCl (Path a, Scheme 15, p-TsCl, Pyridine, Et$_3$N, dry DCM, rt, 3 days) and also tried various conditions shown in Table 1. Unfortunately, starting material was recovered. We suspected that the failure was due to steric hindrance of the OH group, flanked by the two bulky benzyl groups.

Scheme 15: Conversion of alcohol to a good leaving group

Table 1: Tosylation of alcohol 28 (Path a, Scheme 15) under different reaction conditions

<table>
<thead>
<tr>
<th>Trials</th>
<th>Type of base</th>
<th>Temperature</th>
<th>Time</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Pyridine/Et$_3$N</td>
<td>RT*</td>
<td>3 days</td>
<td>Recovered starting material</td>
</tr>
<tr>
<td>2.</td>
<td>Dry pyridine</td>
<td>RT</td>
<td>5 days</td>
<td>Recovered starting material</td>
</tr>
<tr>
<td>3.</td>
<td>Pyridine</td>
<td>RT</td>
<td>3 days</td>
<td>Recovered starting material</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40°C</td>
<td>1 day</td>
<td>Recovered starting material</td>
</tr>
<tr>
<td>4.</td>
<td>NaH</td>
<td>RT</td>
<td>2 days</td>
<td>Recovered starting material</td>
</tr>
<tr>
<td></td>
<td></td>
<td>70°C</td>
<td>2 days</td>
<td>Recovered starting material</td>
</tr>
<tr>
<td>5.</td>
<td>t-BuOK</td>
<td>RT</td>
<td>3 days</td>
<td>Recovered starting material</td>
</tr>
</tbody>
</table>

*RT = room temperature. All reactions were carried out in dry CH$_2$Cl$_2$ under Argon.*
Second, since the mesyl group is smaller, we tried reactions with methanesulfonyl chloride, following the procedure by Suzuki and co-workers. In addition, we tested various conditions shown in Table 2. Again, none of the reactions worked and starting material was recovered.

Table 2: Mesylation of alcohol 28 (Path b, Scheme 15) under different reaction conditions

<table>
<thead>
<tr>
<th>Trials</th>
<th>Type of base</th>
<th>Temperature</th>
<th>Time</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Dry pyridine</td>
<td>RT</td>
<td>2 days$^{34}$</td>
<td>Recovered starting material</td>
</tr>
<tr>
<td>2.</td>
<td>Dry Et$_3$N</td>
<td>RT</td>
<td>3 days</td>
<td>Recovered starting material</td>
</tr>
<tr>
<td>3.</td>
<td>NaH</td>
<td>70°C</td>
<td>2 days</td>
<td>Recovered starting material</td>
</tr>
<tr>
<td>4.</td>
<td>t-BuOK</td>
<td>RT</td>
<td>3 days</td>
<td>Recovered starting material</td>
</tr>
</tbody>
</table>

All reactions were carried out in dry CH$_2$Cl$_2$ under Argon.

We then followed the work by Paulsen and Roben for the triflation of alcohol 28. Triflates are more reactive and excellent leaving groups. Again, due to the probable involvement of steric hindrance, triflation also didn’t work. Table 3 shows the various reaction conditions we tried.
Table 3: Triflation of alcohol 28 (Path c, Scheme 15) with different reaction conditions

<table>
<thead>
<tr>
<th>Trials</th>
<th>Type of base</th>
<th>Temperature</th>
<th>Time</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Dry pyridine (3.5 eq)</td>
<td>-35°C</td>
<td>12 hr</td>
<td>Recovered starting material</td>
</tr>
<tr>
<td>2.</td>
<td>Et₃N (4 eq)</td>
<td>-40°C</td>
<td>4 hr</td>
<td>Recovered starting material</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-20°C</td>
<td>1 day</td>
<td>Recovered starting material</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0°C - 10°C</td>
<td>42 hr</td>
<td>Recovered starting material</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RT</td>
<td>1 day</td>
<td>Recovered starting material</td>
</tr>
<tr>
<td>3.</td>
<td>Pyridine (10 eq)</td>
<td>RT</td>
<td>2 days</td>
<td>Recovered starting material</td>
</tr>
<tr>
<td>4.</td>
<td>Dry pyridine (3.5 eq)</td>
<td>-60°C to RT</td>
<td>8 hr</td>
<td>Product (83%)</td>
</tr>
</tbody>
</table>

All reactions were carried out in dry CH₂Cl₂ under Argon.

We followed the work by Lowe and Mcphee’s for the preparation of the triflate derivative of pentabenzylated-myoinositol 29. The reaction was carried out at much lower temperatures (-60°C) and highly reproducible (Scheme 16).

![Scheme 16: Synthesis of triflate 29 and azide 30]

Previously, the same reaction was unsuccessful at -35°C (Table 3), but surprisingly worked fine at -60°C according to Lowe and Mcphee’s procedure. The success of this reaction could possibly be due to the lower reactivity of pyridine at lower temperatures (-60°C). Pyridine is known to form a salt with triflic anhydride and decreased reaction
temperatures may have eliminated or slowed down this side reaction. Hydrolysis of the triflate 29 back to the starting material could also be a possible explanation for failed reactions at higher temperature.

Eventually, the hydroxyl group of alcohol 28 was converted into triflate as a good leaving group with triflic anhydride (Scheme 16) in very good yields. Triflate derivative 29 was purified by silica gel column chromatography to give a thick yellow oil (83%), stable at room temperature. Compound 29 was characterized by $^1$H NMR, $^{13}$C NMR, and IR. To our surprise the triflate carbon (CF$_3$) was absent in the spectrum but later corroborated by $^{19}$F NMR. Comparison to literature data$^{36,37}$ was satisfactory.

Because tosylation, mesylation, and triflation did not work out initially we searched for alternative routes. For example Mitsunobu displacement,$^{38}$ (Equation 3) allowing for the conversion of alcohols to azides directly, has become an important method for the inversion of configuration in $S_N^2$ reactions. These reactions are often more effective and avoid side reactions such as eliminations.

\[
\begin{align*}
\text{OH} & \quad \xrightarrow{\text{Ph$_3$P, DEAD}} \quad \text{Nu} \\
R_1R_2 & \quad \text{NuH, THF, rt} \quad \xrightarrow{\text{Ph$_3$P=O}} \quad R_1R_2
\end{align*}
\]

(Nu = nucleophile, DEAD = diethylazodicarboxylate)

Equation 3: Mitsunobu conditions

For example, Birendra and Pramanik et al$^{39}$ carried out azide substitution reactions on secondary alcohols at room temperature in dry THF. In their procedure Ph$_3$P, diethylazodicarboxylate (DEAD), and diphenylphosphoryl azide (DPPA) were used. We
applied their reaction conditions to intermediate 28 many times (Path d, Scheme 17). Modifications with varying temperatures and solvents such as DMF, toluene, and THF gave only starting material.

Scheme 17: Synthesis of azide 30 via Mitsunobu conditions

Another unsuccessful attempt (Path e, Scheme 17) for the direct conversion of the alcohol to the azide was made using a more recent method by Thompson et al. DPPA and 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) in dry toluene afforded the desired azide substitution product in simple alcohols proceeding via a phosphate intermediate. The failure of Mitsunobu conditions for our conversion could be due to the steric environment of the alcohol. Primary alcohols react preferentially over sterically hindered secondary alcohols.

In summary, Mitsunobu conditions could not be applied to our substrate while triflation was highly temperature dependent and succeeded only at -60°C. Triflate 29 was reacted with NaN₃ in DMF at 80°C in good yield (83%, Scheme 16). The product, 1,3,4,5,6-penta-O-benzyl-2-deoxy-2-azido-scyllo-inositol (30), is a new compound, and was purified by recrystallization from MeOH as a white solid. Compound 30 was fully characterized by m.p, ¹H NMR, ¹³C NMR, ¹H - ¹³C HETCOR NMR, IR and elemental analysis.
Reduction of azide to amine

The next step involved the reduction of the azide functional group to an amine. Following the standard protocol, we used PPh₃ in THF at room temperature for two hours, followed by the addition of water refluxing at 80°C for 24 hours (Equation 4). Conversion took place via iminophosphorane intermediate 42 and gave the corresponding amine 31 and triphenylphosphine oxide as a byproduct.

Equation 4: Synthesis of pentabenzylated amine 31

Pentabenzylated amine 31 is also a new compound, and was purified via silica gel column chromatography affording a white solid. Subsequent recrystallization from hexane provided the product in excellent yield (93%) and it was characterized by m.p, ¹H NMR, ¹³C NMR, ¹H - ¹³C HETCOR NMR, IR, and elemental analysis. The conversion of azide to amine was confirmed by ¹³C NMR and IR data. In ¹³C NMR, the peak for the carbon attached to the azide at 67.05 ppm moved to 55.5 ppm. The disappearance of a sharp peak at 2106 cm⁻¹ proved in the IR spectrum the loss of azide functionality, and the appearance of two small peaks (double peak) at 3378 and 3317 cm⁻¹ confirmed the primary amine.
Deprotection leads to the target molecule

All that remained to be done was the removal of the protecting groups. The benzyl ethers can be removed with BBr$_3$ under mild conditions.$^{44}$ It acts as a Lewis acid and the coordination to ethereal oxygens aids C-O bond cleavage. The generated alkyl bromide and alkoxyboranes are hydrolyzed during workup (Scheme 18).$^{44}$

\[
\begin{align*}
R^1\text{-O-}R^2 & \quad + \quad \text{BBr}_3 \\
\quad & \quad \rightarrow \quad \left[ R^1\text{-O-}R^2 \quad \text{BBr}_3 \right] \\
\quad & \quad \rightarrow \quad R^1\text{OBBr}_2 \quad + \quad R^2\text{Br} \\
R^1\text{OBBr}_2 \quad + \quad 3 \text{H}_2\text{O} & \quad \rightarrow \quad R^1\text{OH} \quad + \quad \text{H}_3\text{BO}_3 \quad + \quad 2\text{HBr}
\end{align*}
\]

\(R^1, R^2 = \text{alkyl substituents}\)

Scheme 18: Mechanism of benzyl ether deprotection by BBr$_3$

This deprotection reaction (Equation 5) was carried out according to the procedure by Suzuki and co-workers.$^{34}$ It was run under argon at low temperature (-78°C) because BBr$_3$ is highly reactive, moisture sensitive, and decomposes in air with evolution of HBr. Moreover, it reacts violently with protic solvents such as water and alcohols.

Equation 5: Synthesis of target molecule 1a

The reaction was monitored by TLC, which showed the highly polar product on the base line. The reaction was quenched with methanolic K$_2$CO$_3$, accompanied by a yellow to pale yellow color of the solution. The desired product was separated from inorganic salts such as KBr, K$_3$BO$_3$, K$_2$CO$_3$ and H$_3$BO$_3$ by using cold ethanol, in which these salts do...
not dissolve. The target molecule obtained was a light brown solid and characterized by $^1$H NMR, and $^{13}$C NMR. Although the preparation of scyllo-inosamine was mentioned in the literature, no spectral data has been published.\textsuperscript{6}

Suzuki and Chida et al\textsuperscript{34} also reported that a Lewis acid like BBr\textsubscript{3} not only deprotects the benzyl ether group but also reduces azides to the corresponding amines. When these conditions were applied to azide 43 deprotection as well as reduction occurred in 63\% yield (Equation 6).


g463

\textbf{Equation 6: Deprotection as well as reduction of azide 43 by BBr\textsubscript{3}}

Aryl azides are known to undergo reduction to amines by the action of Bronsted-Lowry acids (HBr or HI) or Lewis acids (AlBr\textsubscript{3} or AlEt\textsubscript{3}) followed by hydrolysis.\textsuperscript{45} To minimize the number of steps in the synthesis of scyllo-inosamine 1a, we attempted to apply their procedure,\textsuperscript{34} describing the deprotection of benzyl ethers and azide reduction with BBr\textsubscript{3} (Scheme 19).

\textbf{Scheme 19: Synthesis of azido inositol 45}
However, reaction of pentabenzylated-azido-scyllo-inositol 30 using the same conditions only removed the benzyl ethers, affording azido-scyllo-inositol 45. By dissolving the crude product in MeOH, it could be isolated from its byproducts in high yield (91%). The product was characterized by $^1$H NMR, $^{13}$C NMR, and IR spectroscopy. The presence of the azide was confirmed in the $^{13}$C NMR by the peak at 68.02 ppm and a strong peak at 2115 cm$^{-1}$ in the IR. Kohne and Praefcke$^{46}$ had already synthesized this compound from penta acylated azido-myoinositol 15.

Alternative approaches

Because of the initial difficulties in converting the alcohol 28 to the triflate 29 we prepared another alcohol precursor, i.e. derivative 46. The axial OH group of the MOM-protected tetrabenzylated myoinositol 46 could be converted into a good leaving group, followed by azidation, reduction, and deprotection (Scheme 20).

Scheme 20: Overall proposed approach via MOM protected alcohol 46
We protected the equatorial hydroxyl group of compound 27 using David and Thieffry's procedure, who converted carbohydrate substrate 50 to its MOM derivative (Equation 7).

Equation 7: Synthesis of equatorially MOM protected compound 51

The reaction procedure was similar to the procedure for the preparation of compound 28 (Scheme 14). Substrate 27 and dibutyltin oxide were refluxed in toluene at 120°C for 16 hours with azeotropically removal of water yielding the corresponding stannylene intermediate. The MOM protection was carried out at room temperature in the presence of molecular sieves (Equation 8). Silica gel column chromatography provided pure product 46

Equation 8: Synthesis of MOM protected alcohol 46

as a brown solid (72%). The possible use of this product in the making of the triflate derivative has not yet been explored. Although this reaction was successful when using a freshly opened MOMCl bottle, this result could not be reproduced in subsequent attempts. Failure of the reaction could be due to the deterioration of MOMCl. Hence additional
work will have to be done on this reaction. Simultaneously, we thought about another route that involved an oxidation and reductive amination approach (Scheme 21).

Scheme 21: Overall proposed approach via oxidation and reductive amination

Numerous methods are available for the oxidation of alcohol 28 to its ketone, such as the procedures by Reckendorf\textsuperscript{48} (DMSO, P\textsubscript{2}O\textsubscript{5}, 65°C, 80%), Angyal et al\textsuperscript{17} (AcOH, CrO\textsubscript{3}, 100°C, 49%), Lowe et al\textsuperscript{36} (Acetone, Na\textsubscript{2}Cr\textsubscript{2}O\textsubscript{7}.2H\textsubscript{2}O, water, rt, 74%), and Offer et al\textsuperscript{49} (Acetone, Na\textsubscript{2}Cr\textsubscript{2}O\textsubscript{7}, H\textsubscript{2}SO\textsubscript{4}, rt, 83%). Martin et al\textsuperscript{50} had already prepared this pentabenzylated ketone 52 from alcohol 28 by following the Swern oxidation method (DCM, (COCl)\textsubscript{2}, DMSO, -78°C, Et\textsubscript{3}N, 84%). We tested the use of pyridinium chlorochromate by Savoia’s procedure\textsuperscript{51} (PCC-Al\textsubscript{2}O\textsubscript{3} anhydrous DCM, rt, 18 hours, 27%) and pyridinium dichromate following Czerniacki’s procedure\textsuperscript{52} (PDC, AcOH, 4Å molecular sieves, DCM, rt, 60 hours, 82%, Scheme 22). Czerniacki’s procedure worked best. AcOH accelerated the rate of oxidation, and molecular sieves served as a catalyst by favoring the cleavage of the C-H bond on the alcoholic carbon.\textsuperscript{53} Ketone 52 was purified by washing through silica gel with an aprotic solvent like ethyl acetate. Recrystallization
from ethyl acetate yielded a white solid (82%), which was characterized by m.p, \(^1\)H NMR, \(^{13}\)C NMR, IR, and compared with literature data.\(^{36}\)

Scheme 22: Synthesis of amine 53 via oxidation and reductive amination

The next step entailed the reductive amination of ketone 52, converting it directly to the corresponding secondary alkyl amine in a one pot procedure without isolating the intermediate.\(^{54}\) However, this reaction can theoretically produce two stereoisomers. The iminium ion intermediate (Scheme 22) has an sp\(^2\)-carbon (trigonal geometry) on which the incoming nucleophile (hydride ion) could attack from either the top or the bottom side, forming amines 31 and 53 respectively. Ketone 52 was treated with sodium cyanoborohydride and ammonium acetate in the presence of molecular sieves at room temperature for 24 hrs under argon atmosphere.\(^{55}\) While monitoring the reaction, the amine was visualized by TLC as a yellow spot with vanillin dip, and as a blue spot in the phosphomolybdic acid dip. Pentabenzylated amine 53 was obtained as the sole product, which is the epimer of the desired compound 31. We isolated the new isomer 53 in excellent yield (92%) with traces of pentabenzylated-\(\text{myo}\)-inositol 28 (1%) after column chromatography.
In the above reaction, excess amount of ammonium acetate should be used because it has low solubility in methanol. Borch et al. mentioned that the cyanoborohydride reduction of aldehydes, ketones, and imines is pH sensitive. Manipulating pH accordingly provides an excellent means for controlling the reaction. They also mentioned that the optimum pH to obtain the complete reduction of ketones to alcohols is ~3 and for reductive aminations is ~ 6-8. However, the only explicit requirement appears to be the presence of enough proton source to generate a positively charged >C=\(^\text{N}<\) moiety. Molecular sieves were used to absorb the water generated in the reaction, which enhanced the yield of the reaction and more specifically for those ketones, which form imines slowly. The weak acidity of MeOH used in the reaction as a solvent or acetic acid produced in the ammonium acetate equilibrium reaction (Equation 9) could be the cause for the formation of 1% of alcohol in the reductive amination, which is a pH dependent reaction.

\[
\begin{align*}
\text{CH}_3\text{COONH}_4 & \rightleftharpoons \text{CH}_3\text{COOH} + \text{NH}_3 \\
\text{Equation 9: Ammonium acetate equilibrium reaction}
\end{align*}
\]

Nucleophilic attack at the sterically hindered ketone and formation of an axial or equatorial product was elegantly explained by Barton based on steric hindrance caused by the bulky groups around the ketone. The formation of the major product in strongly hindered cyclic ketones is due to the approach of the reagent to the less hindered side of the sp\(^2\) carbon. The more stable equatorial product dominates in unhindered ketones whereas in hindered ketones the axial product is the major product. In general it is believed that the incoming reagent tends to attack the carbonyl group in the axial direction leading to the equatorial product. However, in hindered ketones axial approach...
may be impeded by steric factors thus favoring equatorial approach of the reagent and formation of the axial product.\(^{58}\) The stereochemistry of the product in the borohydride reduction of ketone 52 is most likely due to steric hindrance of nearby substituents favoring an equatorial attack by the nucleophile.

The axial position of the amine functional group in pentabenzylated amine 53 was confirmed by comparing \(^1\)H NMR data using the coupling constant values of its epimer 31 in which the amine functional group is in an equatorial position. The coupling constants between H\(_a\) and H\(_b\) of compound 53 (J = 2.2 Hz, typical of an axial-equatorial interaction) and H\(_a\) and H\(_b\) of compound 31 (J = 9.6 Hz, typical of a diaxial interaction) provide the evidence for the axial orientation of the protons based on the Karplus diagram (Figure 8).\(^{59}\)

Figure 8: Karplus curve depicting the J value of axial and equatorial protons based on dihedral angle\(^{59}\)
SUMMARY AND CONCLUSIONS

Commercially available myo-inositol was chosen as starting material for the synthesis of scyllo-inosamine 1a in 8 steps and 13% overall yield. Among others, we used known reactions as well as modified and improved literature procedures. The conversion of diol 27 via the corresponding stannylene intermediate was crucial for the regio- and stereoselective preparation of scyllo-inosamine. We prepared new compounds 30, 31, 46, and 53 en route and have successfully isolated rhizopine 1a in one isomeric form by controlling chemical conversions regio- and stereoselectively.

This project has the potential to avoid further negative environmental impact associated with intensive use of chemical fertilizers and might improve agricultural productivity of legume plants. Our synthetic method would also provide an avenue for the synthesis of new and known biologically active cyclitol derivatives. For instance, streptamine derivatives have been identified as aminoglycoside subunits in clinically useful antibiotics such as neomycin, kanamycin, gentamycin, sisomycin, and streptomycin.
FUTURE WORK

This research produced four new compounds (30, 31, 46, and 53) that could serve as useful intermediates in the synthesis of additional rhizopine stereoisomers and derivatives (Scheme 23 and 24). We are interested in applying the above key intermediates to the synthesis of additional rhizopine stereoisomers and derivatives as shown in the following proposed schemes. After the deprotection of benzyl groups from the key intermediate 31, inosamine 1a would be further protected by triethylformate and benzyl bromide consecutively affording compound 54. The hydroxyl group of the orthoformate 54 would be converted into amines 55 or 56 after oxidation and subsequent reductive amination.

Scheme 23: Proposed synthesis of inosodiamines
The incoming nucleophile (hydride on) could attack from either top or the bottom side on the sp\(^2\) carbon in the iminium ion intermediate and could afford two epimers, which would be further deprotected to give compounds 55 and 56. Applying the same strategies to 53 could provide additional stereoisomers (56 and 57).

Benzylation and deprotection of the MOM group of intermediate, 46, would afford the pentabenzylated alcohol 58 (Scheme 24). The hydroxyl group of alcohol 58 would be converted into the trifluoromethane sulfonate and substituted to provide azide 59. Deprotection of the benzyl groups as well as reduction of the azide would provide inosamine isomer 60 after catalytic hydrogenolysis.

Scheme 24: Proposed synthesis of inosamine isomer

There are possible applications for key intermediates from this project in the synthesis of closely related natural products, e.g. aminocyclitol antibiotics (Figure 9). The availability of synthetic inosamine intermediates will help biochemist to study and understand the role of chemical messenger molecules during plant-microbe symbiosis.
Figure 9: Aminocyclitol antibiotics
EXPERIMENTAL

Reagents and solvents

All the reagents and solvents used in this project were obtained from Aldrich, Acros, Lancaster, TCI Chemical Companies. All chemicals were of more than 98% purity and were used without further purification. All solvents used were of reagent grade. Dichloromethane, and triethylamine were further purified by distillation from P₂O₅ and KOH, respectively.

Characterization of compounds and instrumentation

Melting points, reported in degree Celsius, were determined in open capillaries using a Thomas-Hoover Unimelt instrument. ¹H NMR and ¹³C NMR spectra were obtained with a 400 MHz Jeol Eclipse nuclear magnetic resonance spectrometer using deuterated water, chloroform, methanol, or dimethyl sulfoxide. NMR spectral data were assigned according to the Pretsch text. Infrared (IR) spectra were recorded on a Bruker Equinox 55 and Perkin Elmer 1710 Fourier Transform Infrared Spectrometers.

(±)-1,2-O-Isopropylidene-myco-inositol (25)

Prepared from commercially available myo-inositol by the literature procedure. A mixture of myo-inositol (2.0 g, 0.011 mol) and toluene-p-sulphonic acid (15 mg, 0.078 mmol, 7.01 eq) in dry dimethyl sulfoxide (7.5 ml) and 2,2-dimethoxypropane (3.22 g, 3.8 ml, 0.033 mol, 3 eq) was stirred at 110°C. Excess of dimethoxypropane, DMSO were
removed by distillation, then myo-inositol (2 g) was added and the solution was heated for 1.5 hrs at 120°C again. Potassium carbonate (15 mg) was added and DMSO was evaporated off. The brown syrupy residue was triturated with ethanol to give a crystalline solid, which was suspended in boiling ethanol (40 ml) for half an hour. The solution was filtered from the residue of unchanged starting material and the filtrate was concentrated. Solid compound was precipitated out after 18 hrs (74%). Isopropylidene-myoinositol (25) was also prepared by following another literature procedure.15 A mixture of myo-inositol (10.0 g, 0.055 mol) and toluene-p-sulphonic acid (100 mg, 0.50 mmol, 0.009 eq) in dry dimethyl sulfoxide (32.3 g, 32.0 ml, 0.414 mol, 7.50 eq) and 2, 2-dimethoxypropane (14.42 g, 17.0 ml, 0.138 mol, 2.50 eq) was stirred at 110°C until a clear solution was obtained. The solution was then cooled at RT and triethylamine (1 ml), and EtOH (40 ml) were added, followed by ether (200 ml). The reaction mixture was stirred at RT for 20 hrs. The white product was collected, washed with ether/methanol (5:1, 40 ml) and then ether, and was dried over anhydrous MgSO₄ to give compound 3 (41%). m.p: 178-179°C (lit: 182-184°C); ^1H NMR (D₂O, 400 MHz) δ 4.47 (pp t, J = 4.4 Hz, 1H), 4.05 (dd, J₁ = 4.76 Hz, J₂ = 7.68 Hz, 1H), 3.83 (dd, J₁ = 4.04 Hz, J₂ = 9.88 Hz, 1H), 3.63-3.54 (m, 2H), 3.24 (app t, J = 9.88 Hz, 1H), 1.52 (s, 3H), 1.38 (s, 3H); ^13C NMR (D₂O, 100 MHz) δ 110.6, 78.7, 76.3, 74.8, 72.8, 72.4, 69.6, 27.5, 25.3; IR (on KBr pellet) 3416 cm⁻¹ (s, -OH), 2912 cm⁻¹ (aliphatic C-H), 1070 cm⁻¹ (C-O-C).
(±)-1,2-0-Isopropylidene-3,4,5,6-tetra-O-benzyl-myoinositol (26)

Prepared from 1,2-0-isopropylidene-myoinositol (25) following a literature procedure.\textsuperscript{14} Isopropylidene-myoinositol (25) (50.0 mg, 0.20 mmol) was treated with powdered sodium hydroxide (220 mg, 5.50 mmol, 27.7 eq) in benzyl bromide (550 ml, 4.80 mmol, 24.0 eq) at 120°C with vigorous stirring for 22 hrs; reaction was monitored by silica gel TLC. The solution was diluted with ether (30-50 ml), washed with water, and dried over anhydrous MgSO\textsubscript{4}, and the solvents were evaporated off. The residue was dissolved in hexane/ethyl acetate (4:1) and purified via silica gel column chromatography. Elution with the same solvent mixture provided the (±)-1,2-0-isopropylidene-3,4,5,6-tetra-O-benzyl-myoinositol (4) free from partially benzylated-myoinositol in 87% yield. \textsuperscript{1}H NMR (CDCl\textsubscript{3}, 400 MHz) δ 7.26-7.35 (m, aromatic H), 4.71-4.87 (m, 8H), 4.26 (dd, J\textsubscript{1} = 4.04 Hz, J\textsubscript{2} = 5.52 Hz, 1H), 4.10 (app t, J = 6.6 Hz, 1H), 3.94 (app t, J = 8.78 Hz, 1H), 3.78 (dd, J\textsubscript{1} = 6.96 Hz, J\textsubscript{2} = 9.52 Hz, 1H), 3.69 (dd, J\textsubscript{1} = 3.68 Hz, J\textsubscript{2} = 8.8 Hz, 1H), 3.41 (app t, J = 9.16 Hz, 1H), 1.51 (s, 3H), 1.35 (s, 3H); \textsuperscript{13}C NMR (CDCl\textsubscript{3}, 100 MHz) δ 138.7, 138.6, 138.3, 128.51, 128.48, 128.44, 128.36, 128.2, 128.13, 128.06, 127.9, 127.8, 127.7, 127.6, 109.9, 82.6, 82.2, 80.9, 79.2, 77.2, 75.5, 75.4, 74.7, 74.0, 73.4, 27.9, 25.9; IR (DCM solution) 3032 cm\textsuperscript{-1} (m, aromatic C-H), 2873 cm\textsuperscript{-1} (aliphatic C-H), 1071 cm\textsuperscript{-1} (C-O-C).
The following procedure was adopted from Gigg et al.’s procedure.\(^{14}\) Compound 26 was added to a mixture of methanol (7.75 ml) and hydrochloric acid (0.62 ml) and the mixture was heated under reflux for 15 min. Sodium hydrogen carbonate (0.05 g) was added and methanol was removed \textit{in vacuo}. The residue was extracted with chloroform and the solution was filtered, dried over anhydrous MgSO\(_4\) and chloroform was evaporated. The residue was triturated with petroleum ether to dissolve the dibenzyl ether and the solid product was obtained by filtration, dried on vacuum, and recrystallized from MeOH. m.p: 107°C (lit: 112-115°C); \(^1\)H NMR (CDCl\(_3\), 400 MHz) \(\delta\) 7.30-7.33 (m, aromatic H), 4.72-4.96 (m, 8H), 4.20 (app t, \(J = 2.74\) Hz, 1H), 3.98 (t, \(J = 9.52\) Hz, 1H), 3.84 (t, \(J = 9.52\) Hz, 1H), \(\delta\) 3.46-3.51 (m, 3H), 2.31 (bs, OH); \(^{13}\)C NMR (CDCl\(_3\), 100 MHz) \(\delta\) 138.8, 138.7, 138.6, 138.0, 128.70, 128.67, 128.56, 128.53, 128.12, 128.04, 128.0, 127.9, 127.8, 83.4, 81.7, 81.5, 80.1, 76.1, 75.9, 75.7, 72.8, 71.9, 69.4; IR (NaCl) 3416 cm\(^{-1}\) (s, -OH), 3063 cm\(^{-1}\) (m, aromatic C-H), 2912 cm\(^{-1}\) (aliphatic C-H), 1454 cm\(^{-1}\) (C=C), 1070 cm\(^{-1}\) (C-O-C).

\(1,3,4,5,6\)-Penta-O-benzyl-myo-inositol (28)

This procedure was adopted from Gigg et al.\(^{25}\) (\(\pm\))-3,4,5,6-Tetra-O-benzyl-myo-inositol (27) (100 mg, 0.185 mmol), dibutyltin oxide (69.0 mg, 2.78 mmol, 1.50 eq), tetrabutylammonium iodide (76.3 mg, 0.185 mmol, 1 eq), benzyl bromide (102 mg, 71.0 µL, 0.60 mmol, 3.24 eq) were dissolved in acetonitrile (20.0 ml) and the reaction mixture
was heated under reflux for 18 hrs with a Soxhlet apparatus that contained molecular
sieves 4Å (4.00 g). The reaction was monitored by silica gel TLC (4:1
chloroform/acetone). The reaction was quenched with a saturated NaHCO$_3$
solution (20.0
ml) and the solvent was evaporated including some water. The residue was extracted with
hexane/ethyl acetate (1:1) and dried over anhydrous MgSO$_4$ and solvent was removed in
vacuo and recrystallized from hexane (79%). m.p: 120-121°C (lit: 122-124°C); $^1$H NMR
(CDC$_3$, 400 MHz) $\delta$ 7.28-7.32 (m, aromatic H), 4.89 (d ABq, $J = 10.64$ Hz 2H), 4.85 (s, 2H),
4.84 (d ABq, $J = 10.64$ Hz 2H), 4.70 (s, 4H), 4.22 (t, $J = 2.56$Hz, 1H), 3.99 (t, J =
9.54 Hz, 2H), 3.45 (t, J = 9.54 Hz, 1H), 3.38 (dd, $J_1 = 2.56$ Hz, $J_2 = 9.52$ Hz, 2H), 2.45 (s, 3H)
(OH); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 138.8, 138.7, 138.1, 128.6, 128.5, 128.2, 128.0,
127.7, 83.3, 81.3, 79.9, 76.1, 72.9, 67.6; IR (NaCl) 3458 cm$^{-1}$ (s b, OH), 3030 cm$^{-1}$ (m, aromatic C-H), 2873 cm$^{-1}$ (aliphatic C-H), 1454 cm$^{-1}$ (C=C), 1071 cm$^{-1}$ (C-O-C).

1,3,4,5,6-Penta-O-benzyl-2-O-(trifluoromethylsulfinyl)-myo-inositol (29)

Preparation of compound 29 follows Lowe and McPhee’s procedure.$^{36}$ A solution of 1,3,
4,5,6-penta-O-benzyl-myosinositol (28) (100 mg, 0.158 mmol) in anhydrous
dichloromethane (1.56 ml) containing anhydrous pyridine (38.3 microL, 37.5 mg, 0.474
mmol, 3 eq) was allowed to cool to $-60^\circ$C before the dropwise addition of triflic
anhydride (47.1 microL, 78.9 mg, 0.279 mmol, 1.77 eq) in a two-necked round bottom
flask under argon atmosphere. After the completion of the addition of the triflic
anhydride the reaction mixture was allowed to warm up to ambient temperature and was
stirred for 7 hrs. TLC analysis (2:1 hexane/ethyl acetate) showed the presence of product at $R_f$ 0.52 and disappearance of starting material spot. Then the reaction mixture was quenched with water (0.65 ml), diluted with regular CH$_2$Cl$_2$ (1.56 ml) and washed successively with saturated aq. NaHCO$_3$, water, and brine. The combined organic layers were dried over anhydrous MgSO$_4$, and all the solvent was removed using a rotary evaporator and vacuum to obtain compound 29 as orange syrup (83%). $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$ 7.34-7.39 (m, aromatic H), 5.35 (t, $J = 2.18$Hz, 1H), 4.95 (d ABq, $J = 10.62$ Hz 2H), 4.92 (s, 2H), 4.88 (d ABq, $J = 10.62$ Hz 2H), 4.84 (d ABq, $J = 11.54$ Hz 2H), 4.66 (d ABq, $J = 11.54$ Hz 2H), 3.95 (t, $J = 9.54$ Hz, 2H), 3.52-3.57 (m, 3H); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 138.41, 138.37, 137.1, 128.7, 128.6, 128.5, 128.24, 128.20, 128.0, 127.9, 84.1, 82.8, 80.9, 77.0, 76.3, 76.2, 73.2; $^{19}$F NMR (CDCl$_3$, 375 MHz) $\delta$ -74.0 (s, 3F); IR (DCM solution) 3032 cm$^{-1}$ (m, aromatic C-H), 2873 cm$^{-1}$ (aliphatic C-H), 1454 cm$^{-1}$ (C=C), 1410.6 cm$^{-1}$ (s, S=O), 1208.5 cm$^{-1}$ (s, C-F), 1141.3 cm$^{-1}$ (s, S-O), 1071 cm$^{-1}$ (C-O-C).

1,3,4,5,6-Penta-O-benzyl-2-deoxy-2-azido-seyro-inositol (30)

The following procedure is a modification of the method of Paulsen and Roben. Pure triflate 29 (100 mg, 0.131 mmol) was dissolved in dry $N, N$-dimethyl formamide (5 ml) followed by NaN$_3$ (142.4 mg, 2.19 mmol, 16.7 eq) and this suspension was vigorously stirred at 80°C for 5 hrs. The reaction was monitored by TLC (4:1 hexane/ethyl acetate). After the completion of the reaction, the undissolved NaN$_3$ was filtered off. All the solvent was removed on a rotary evaporator, and excess water (150 ml) was added, and
diluted with CH₂Cl₂ (50 ml). The organic layer was separated and washed with 5% NaCl solution (50 ml), dried over anhydrous MgSO₄, and concentrated to obtain a pale yellow color solid. Product 30 was recrystallized from methanol (83%). m.p: 87-90°C; ¹H NMR (CDCl₃, 400 MHz) δ 7.27-7.35 (m, aromatic H), 4.87 (s, 2H), 4.85 (s, 4H), 4.84 (s, 4H), 3.46-3.59 (m, 4H), 3.35 (t, J = 9.13 Hz, 2H); ¹³C NMR (CDCl₃, 100 MHz) δ 138.34, 138.32, 137.9, 128.6, 128.5, 128.3, 128.0, 127.94, 127.87, 127.82, 83.4, 82.6, 81.2, 76.10, 76.06, 76.03, 67.1; IR (NaCl) 3064 cm⁻¹ (m, aromatic C-H), 2906 cm⁻¹ (aliphatic C-H), 2106.1 cm⁻¹ (v.s, -N₃), 1454 cm⁻¹ (C=C), 1071 cm⁻¹ (C-O-C); E.A. Calculated: C, 75.09; H, 6.30; N, 6.41, Found: C, 75.10; H, 6.11; N, 6.34.

1,3,4,5,6-Penta-O-benzyl-2-deoxy-2-amino-scyllio-inositol (31)

This procedure follows the literature.⁴³ Pentabenzylated azide 30 (200 mg, 0.305 mmol), triphenylphosphine (88.09 mg, 0.335 mmol, 1.1 eq) were dissolved in THF (18 ml) and the solution was stirred at room temperature for 2 hrs. Water was then added to that solution. The reaction mixture was refluxed at 80°C for 24 hrs, and monitored by TLC (4:1 hexane/ethyl acetate) in PMA dip. After the completion of the reaction, the reaction mixture was concentrated on the rotary evaporator. The residue was diluted with ethyl acetate (20 ml) and dried over anhydrous NaSO₄. The solution was concentrated to give a pale yellow solid product. The crude yellow solid was chromatographed (2:1 H/EtOAc) to obtain pure 1,3,4,5, 6-penta-O-benzyl-2-deoxy-2-amino-scyllio-inositol (31) as a white solid. Recrystallization was done from hexane (93%). m.p: 110-111°C; ¹H NMR (CDCl₃,
400 MHz) δ 7.31-7.38 (m, aromatic H), 5.03 (d ABq, J = 10.64 Hz, 2H), 4.97 (d ABq, J = 10.60 Hz, 2H), 4.95 (s, 2H), 4.90 (d ABq, J = 10.60 Hz, 2H), 4.74 (d ABq, J = 10.82 Hz, 2H), 3.67-3.66 (m, 3H), 3.41 (b t, J = 7.34 Hz, 2H), 2.98 (t, J = 9.88 Hz, 1H), 1.90 (bs, -NH₂); ¹³C NMR (CDCl₃, 100 MHz) δ 138.54, 138.50, 138.48, 128.7, 128.6, 128.0, 127.9, 127.8, 84.4, 83.7, 83.1, 76.1, 75.94, 75.93, 55.5; IR (NaCl) 3378 and 3317 cm⁻¹ (double peak, NH₂), 3060 cm⁻¹ (m, aromatic C-H), 2901 cm⁻¹ (aliphatic C-H), 1061 cm⁻¹ (C-O-C); E.A. Calculated: C, 78.19; H, 6.88; N, 2.22, Found: C, 77.83; H, 6.77; N, 2.41.

2-Amino-2-deoxy-scyllo-inositol (1a)

Procedure a) This procedure was adapted from Suzuki and Chida’s procedure.³⁴ BBr₃ (50 mg, 0.0795 mmol) was slowly added to a solution of the pentabenzylated amine 31 (0.953 ml, 0.953 mmol, 12 eq) in anhydrous CH₂Cl₂ (7 ml) at -78°C under argon. The reaction mixture was stirred at -78°C for 30 min and at 0°C for 20 min. Then K₂CO₃ (548 mg, 3.975 mmol, 50 eq) and MeOH (2 ml) were added to this mixture at 0°C. Reaction was monitored by TLC (2:1 hexane/ethyl acetate) using PMA dip. After stirring at 0°C for 30 min, insoluble salts were filtrated off and washed with small amount of ethanol. Then the filtrate was diluted with CH₂Cl₂ and extracted with water. The combined aqueous layer was concentrated in vacuo to give brown solid residue, which was dissolved in cold ethanol and separated from undissolved inorganic salts as light brown solid (71%). Procedure b): reaction conditions and work up were same other than purification. Once the crude product was extracted with water, it was dissolved in 0.1M
KOH solution to purify on reverse phase column chromatography (C-18 silicca gel) using Propanol/water/AcOH (4:1:1) solvent mixture. Then followed by normal phase column chromatography (silica, MeOH/CHCl3, 1:2) produced white solid. m.p: 110-130°C (crude product); 1H NMR (D2O, 400 MHz) δ 3.51 (dt, J = 1.56Hz, 2H), 3.30- 3.42 (m, 3H), 3.07 (dt, J = 2.24 Hz, 1H); 13C NMR (D2O, 100 MHz) δ 74.4, 73.3, 70.0, 55.9; IR (on KBr pellet) 3445 cm⁻¹ (s, -OH), 3445 and 3343 cm⁻¹ (very small double peak, NH2), 2938 cm⁻¹ (aliphatic C-H), 1475 cm⁻¹ (C-N), 1035 cm⁻¹ (C-O).

2-Azido-2-deoxy-scyllo-inositol (45)

The procedure used was the same as described in the literature.34 BBr3 (0.915 ml, 0.915 mmol, 12 eq) was slowly added to a solution of the pentabenzylated azide 30 (50 mg, 0.0763 mmol) in anhydrous CH2Cl2 (7 ml) at -78°C under argon. The reaction mixture was stirred at -78°C for 30 min and at 0°C for 20 min. Then K2CO3 (526 mg, 3.815 mmol, 50 eq) and MeOH (2 ml) were added to this mixture at 0°C. Reaction was monitored by TLC (2:1 H/EtOAc). After stirring at 0°C for 30 min, insoluble salts were filtered and washed with small amount of EtOH. The filtrate was diluted with CH2Cl2 and extracted with water. The combined aqueous layer was concentrated in vacuo to give a brown solid residue, which was dissolved in MeOH to separate from undissolved inorganic salts as light brown solid (91%). m.p: 90-120°C; 1H NMR (D2O, 400 MHz) δ 3.73 (app s, 1H), 3.66 (app s, 2H), 3.01 (app s, 2H), 2.83 (app s, 1H); 13C NMR (D2O, 100 MHz) δ 74.8, 73.9, 73.2, 68.0; IR (on KBr pellet) 3376 cm⁻¹ (s, -OH), 2927 cm⁻¹ (aliphatic C-H), 2115 cm⁻¹ (-N3), 1632 cm⁻¹ (C-N), 1100 cm⁻¹ (C-O).
We followed the work by David and Thieffry. A mixture of tetrabenzylated myo-inositol (27) (100 mg, 0.185 mmol) and dibutyltin oxide (47 mg, 0.185 mmol, 1 eq) in toluene (25 ml) was refluxed at 125°C for 32 hrs with azeotropically removing water. Reaction was warmed to room temperature, then MOMCl 16.38 mg, 0.203 mmol, 1.1 eq) and Bu₄NI (68.3 mg, 0.185 mmol, 1eq) were added to the reaction mixture along with molecular sieves (100 mg) and stirred for 18 hrs. After completion of the reaction, all the solvent was evaporated and product was isolated as a brown solid (71%) after column chromatography (4:1 chloroform/acetone). Note: this result could not be reproduced in subsequent attempts due to the deterioration of MOMCl. ¹H NMR (CDCl₃, 400 MHz) δ 7.28-7.37 (m, aromatic H), 5.35 (app t, J = 2.38 Hz, 1H), 4.91 (s, 1H), 4.88 (d, J = 3.68 Hz, 3H), 4.82 (d, J = 4.4 Hz, 2H), 4.79 (d, J = 4.76 Hz, 2H), 4.63 (d, J = 10.96 Hz, 2H), 3.89 (app t, J = 9.52 Hz, 2H), 3.45-3.52 (m, 3H), 3.03 (s, 3H).

The following procedure was adapted from Czemecki et al.'s procedure. Pentabenzylated alcohol 28 (100 mg, 0.158 mmol), pridinium dichromate (297 mg, 0.793 mmol, 5 eq) and AcOH (44.8 µL, 47.6 mg, 0.793 mmol, 5 eq), powdered molecular sieves (209.8 mg) were dissolved in CH₂Cl₂ (6 ml), then the reaction mixture was stirred...
at room temperature for 60 hr, and monitored by TLC (2:1 hexane/ethyl acetate). After
completion, the reaction mixture was stirred with celite (36 mg) for about 20 min.,
filtered and the solvent evaporated on a rotary evaporator with toluene to remove
pyridine and AcOH. The resulting dark brown residue was dissolved in ethyl acetate and
filtered through silica gel and evaporated to obtain the pure product 52 as white solid.
Recrystallized from ethyl acetate (82%). m.p: 160-161°C; $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$
7.27-7.40 (m, aromatic H), 4.87-4.91 (m, 6H), 4.76 (d, J = 10.60 Hz, 2H), 4.54 (d, J =
11.32 Hz, 2H), 4.15 (d, J = 9.92 Hz, 2H), 3.86 (t, J = 9.16 Hz, 1H), 3.62 (t, J = 9.54 Hz,
2H); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 202.3, 138.3, 138.2, 137.4, 128.6, 128.5, 128.23,
128.17, 128.07, 128.00, 127.86, 83.9, 82.3, 81.6, 76.2, 76.1, 73.5; IR (NaCl) 3060 cm$^{-1}$
(m, aromatic C-H), 2901 cm$^{-1}$ (aliphatic C-H), 1728 cm$^{-1}$ (s, C=O), 1454 cm$^{-1}$ (C=C),
1061 cm$^{-1}$ (C-O-C).

1,3,4,5,6-Penta-O-benzyl-2-deoxy-2-amino-myoinositol (53)
The following procedure was adapted from Ryckman and Stevens’s procedure.$^{55}$
Powdered 4Å molecular sieves (22 mg) were added to CH$_2$Cl$_2$ (10 ml) and MeOH (10 ml)
containing pentabenzylated ketone 52 (50 mg, 0.0796 mmol). While stirring ammonium
acetate (61.35 mg, 0.796 mmol, 10 eq) was added. After the addition of sodium
cyanoborohydride (5 mg, 0.0796 mmol, 1 eq), the reaction mixture was stirred at room
temperature for 24 hrs under argon. Reaction was monitored by TLC (2:1 H/EtOAc).
After the completion, reaction mixture was filtered, concentrated on the rotary evaporator,
diluted with water (3 ml), basified with 15% NaOH solution, and extracted with ether (40
ml). The combined organic layers were dried over anhydrous MgSO₄, and then concentrated in vacuo to obtain 1,3,4,5,6-penta-O-benzyl-2-deoxy-2-amino-\textit{myo}-inositol (53) as a white solid. The crude mixture was purified on a silica gel column (2:1 hexane/ethyl acetate) and yielded pure compound 53 (90%) along with traces of pentabenzylated alcohol (28) (1%). m.p: 110°C; \textsuperscript{1}H NMR (CDCl₃, 400 MHz) δ 7.34-7.39 (m, aromatic H), 4.89 (d ABq, J = 10.64 Hz, 2H), 4.89 (s, 2H), 4.84 (d ABq, J = 10.64 Hz, 2H), 4.71 (d ABq, J = 11.72 Hz, 2H), 4.67 (d ABq, J = 11.72 Hz, 2H), 4.19 (t, J = 9.56 Hz, 2H), 3.74 (t, J = 3.30 Hz, 1H), 3.45 (t, J = 9.16 Hz, 1H), 3.42 (dd, J₁ = 3.32 Hz, J₂ = 9.52 Hz, 2H), 1.74 (bs, -NH₂); \textsuperscript{13}C NMR (CDCl₃, 100 MHz) δ 139.1, 139.0, 138.5, 128.5, 128.42, 128.41, 128.2, 127.8, 127.77, 127.72, 127.6, 127.5, 83.8, 81.4, 80.7, 75.9, 75.8, 72.7, 49.7; IR (NaCl) 3396 and 3331 cm\textsuperscript{-1} (double peak, NH₂), 3030 cm\textsuperscript{-1} (m, aromatic C-H), 2873 cm\textsuperscript{-1} (aliphatic C-H), 1454 cm\textsuperscript{-1} (C=C), 1071 cm\textsuperscript{-1} (C-O-C); E.A. Calculated: C, 78.19; H, 6.88; N, 2.22, Found: C, 77.82; H, 6.72; N, 2.28.
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APPENDIX

$^1$H NMR, $^{13}$C NMR, IR, and $^1$H-$^{13}$C HETCOR spectral data
\[
\begin{align*}
&\text{H}_3\text{C} \quad \text{O} \\
&\text{O} \\
&\text{Bn} \quad \text{O} \\
&\text{Bn} \\
&\text{O} \\
&\text{OBn} \\
\end{align*}
\]

\text{26}

\text{({}^1\text{H} \text{NMR in CDCl}_3)}

\[
\begin{align*}
&\text{H}_3\text{C} \quad \text{O} \\
&\text{O} \\
&\text{Bn} \quad \text{O} \\
&\text{Bn} \\
&\text{O} \\
&\text{OBn} \\
\end{align*}
\]

\text{26}

\text{({}^{13}\text{C} \text{ NMR in CDCl}_3)}
$\text{27} \quad 1^H \text{NMR in CDCl}_3$

$\text{27} \quad ^{13}\text{C NMR in CDCl}_3$
29
(\(^1\)H NMR in CDCl\(_3\))

29
(\(^{13}\)C NMR in CDCl\(_3\))
30
(1H NMR in CDCl₃)

30
(13C NMR in CDCl₃)
**$^{1}H$ NMR in CDCl$_3$**

![NMR spectrum of compound 31](image)

**$^{13}C$ NMR in CDCl$_3$**

![NMR spectrum of compound 31](image)
31
(\(^1H^{13}C\) HETCOR NMR)

31
(IR)
$^{1}H$ NMR in D$_2$O

$^{13}C$ NMR in D$_2$O
**46**

(1H NMR in CDCl₃)

**52**

(1H NMR in CDCl₃)
\begin{equation}
\begin{align*}
\text{BnO} & \quad \text{O} \quad \text{Bn} \\
\text{BnO} & \quad \text{O} \quad \text{Bn} \\
\text{BnO} & \quad \text{O} \quad \text{Bn}
\end{align*}
\end{equation}

52

(\textsuperscript{13}C NMR in CDCl\textsubscript{3})

\begin{center}
\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1}
\end{figure}
\end{center}

\begin{center}
\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure2}
\end{figure}
\end{center}
\[ \text{H NMR in CDCl}_3 \]

\[ \text{C NMR in CDCl}_3 \]

X: parts per million; 13C.

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