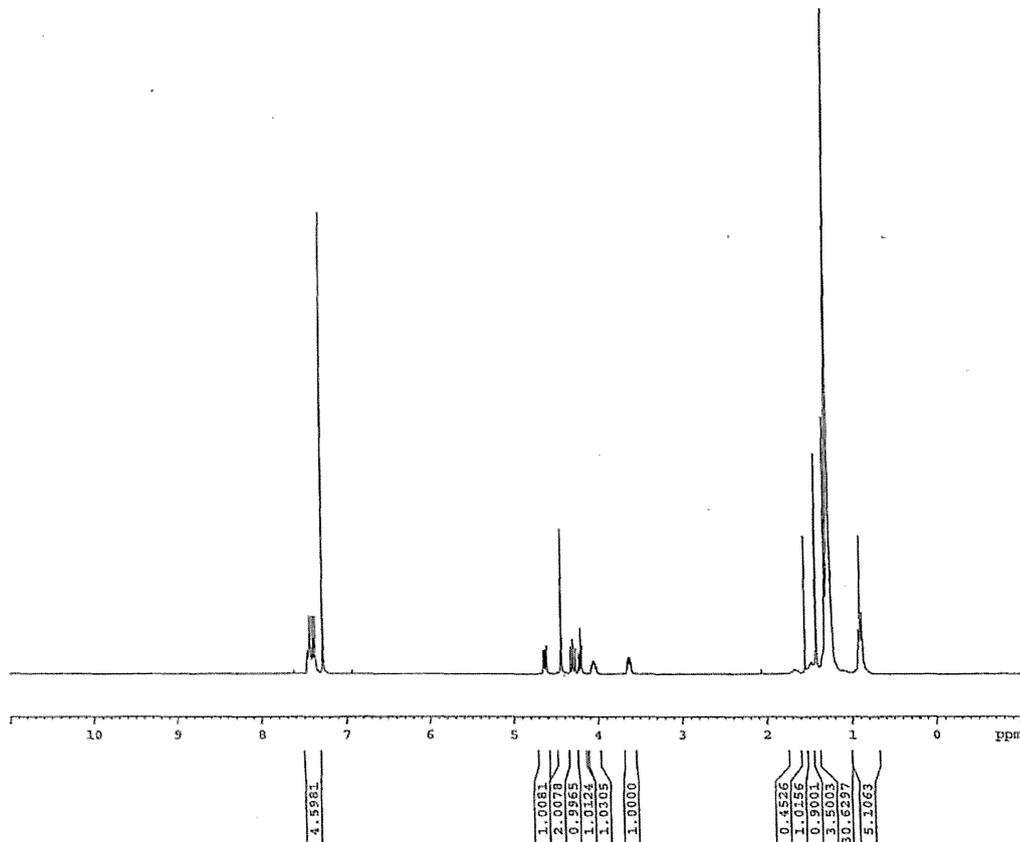


Attachment 17

AMRI SRC AV-300
location; 10
Compound B



Name Di Xu
Date 3/26/2014
NB# 09DX025



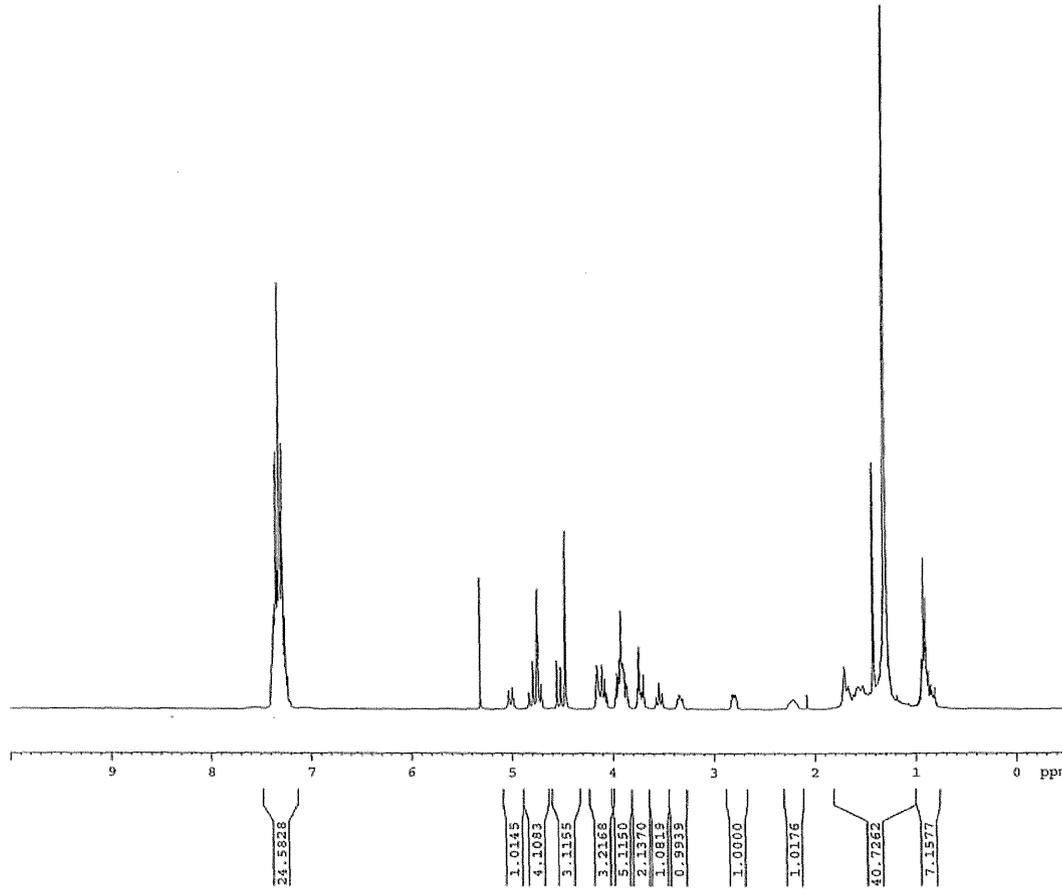
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Date 20140326
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DE 6.00 usec
TE 300.0 K
D1 1.00000000 sec
TDC 1

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AMRI SRC AV-300
 Location; 10
 Compound 25



Name Geelka
 Date 03/18/2014
 NB# 09GB29B



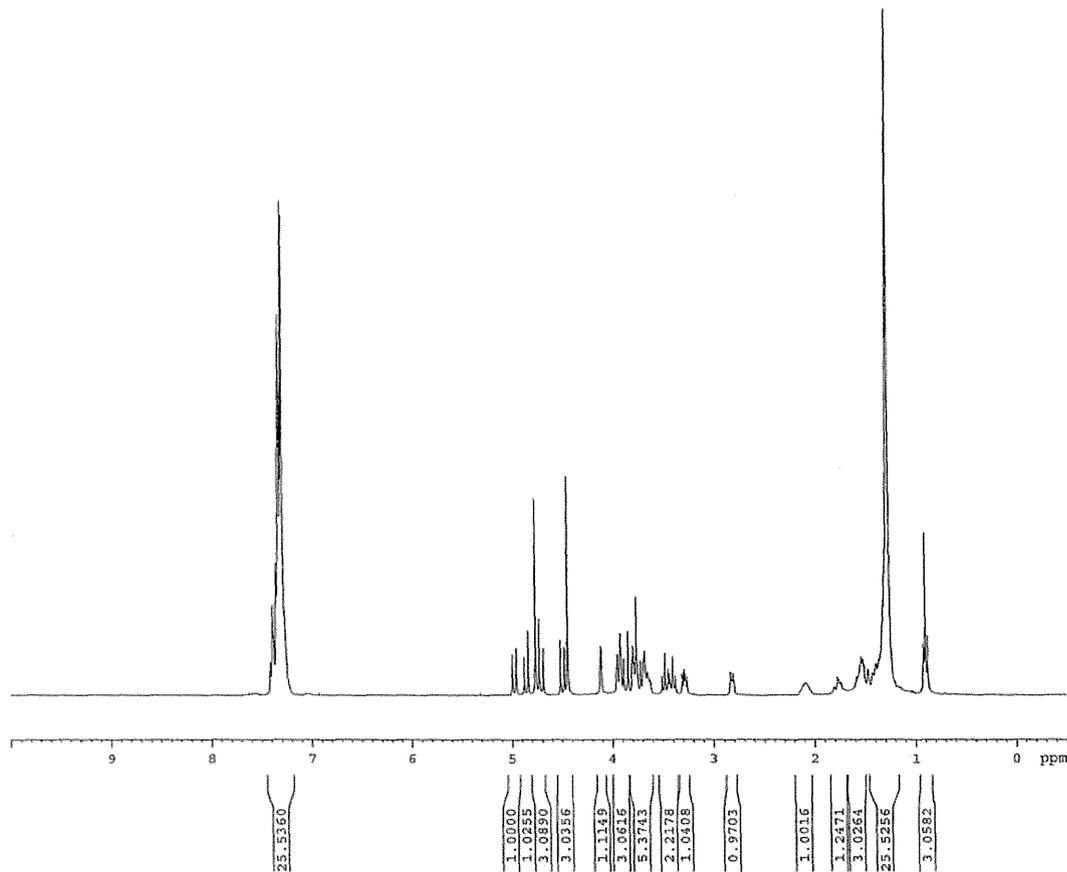
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 DE 6.00 usec
 TE 300.0 K
 D1 1.00000000 sec
 TDO 1

===== CHANNEL f1 =====
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 PL1 2.00 dB
 SFO1 300.1418534 MHz
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AMRI SRC AV-300
 location; 10
 Compound 26

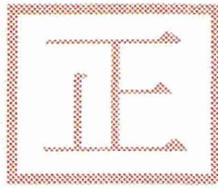


Name Geetha
 Date 03/26/2014
 NB# 09GB032B



NAME 09GB032B
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 PROCNO 1
 Date_ 20140326
 Time 15.12
 INSTRUM spect
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 PULPROG zg30
 TD 32768
 SOLVENT CDC13
 NS 16
 DS 2
 SWH 6172.839 Hz
 FIDRES 0.188380 Hz
 AQ 2.6542580 sec
 RG 362
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 DE 6.00 usec
 TE 300.0 K
 D1 1.00000000 sec
 TD0 1

===== CHANNEL f1 =====
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 P1 12.75 usec
 PL1 2.00 dB
 SFO1 300.1418534 MHz
 SI 16384
 SF 300.1400000 MHz
 WDW EM
 SSB 0
 LB 0.30 Hz
 SB 0
 PC 1.00



最終報告書

表題： アルファガラクトシルセラミド類縁体の合成方法の確立

案件番号： 9067

担当者： Lei Liu, Ph.D. (Albany Molecular Research, Inc)

承認者： Jianxie Chen, Ph.D. (Albany Molecular Research, Inc)

責任者： 藤井 亮介  (キシダ化学(株) 創薬推進部 探索支援 G)

平成 26 年 3 月 31 日

キシダ化学株式会社

本報告書は表紙を含む 56 頁

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要 約

1) スループットならびに収量の向上

個々の合成段階において、当初の手法と比較して向上させた収量は Preparation A の 1 と 2 (Scheme2) を除き、再現可能であった。Compound 1 30g (当初の手法の 2 倍スケール) での反応から開始した。反応は TLC により追跡したが、反応速度は遅く 1 日経過した後も原料が残存していた。この現象は次の 100g スケールの反応においても観察された。様々な反応条件を追求する別の理由としてはピリジンを溶媒として使用することを避けることが挙げられる。大スケールにおけるピリジン除去は長い時間を要するからである。DABCO とジクロロメタンがその反応において使用された。この条件下では、反応は 4 時間で終了した。次の段階においては、コスト低減のため Et₃N を添加した。Step3 では当量数の代わりに使用する p-TsOH の量は反応液中の pH に基づいて決定されるべきであることを見出した。様々な反応条件にて置き換えた結果、3 つの合成段階において収率は 36% から 58% に向上した。

2) 精製方法の置換え (例. カラムクロマトグラフィーから再結晶等)

固体である compound 5 について再結晶の条件検討を実施した。再結晶条件の小スケールの予備試験を実施した上、大スケールで実施した。こちらの条件検討についてはスケールアップの検討過程において引き続き継続するものとする。

3) 全ステージにおけるインプロセス・コントロール手法の確立

HPLC 分析による in-process コントロールを試験した。特定の HPLC カラムによる手法との適合性の問題により、いくつかの中間体は正確に測定することができなかった。加えて、当初の合成実施を通して使用可能な中間体を使用せず、必要のない限り (例えば、Scheme2 における compound 6 のための Step5 では HPLC 分析により出発物質の変換が測定できる) IPC データは HPLC 分析に依存しなかった。TLC と ¹H-NMR 分析は反応をモニタリングする主な分析ツールである。手元にある中間体を用いて、全ての中間体ならびに最終化合物の HPLC 分析手法の構築は引き続き検討する。

4) HPLC あるいは GC による、インプロセスならびに分離されたサンプルの分析

IPC サンプル分析については上述のコメントを参照されたい。サンプルの分離精製のため、いくつかの中間体は HPLC により分析した。化合物と現在の手法との適合性に問題があるため、純度については適切な手法を構築した後、確定されるべきものである。GC 分析については追って確認する。

5) プロセスの全てのステップにおける乾燥による濃縮、硫化マグネシウム処理、適合性の低い溶媒の使用を回避する手法の構築

こちらについてはプロジェクト全体を通じて検討してきた。これまでのところ、Scheme2 の Step1 において、クロロフォルムをジクロロメタンで置き換えることに成功した。今後、特段に必要という場合を除き、可能な限りにおいて硫酸マグネシウム処理を除く条件を検討する。

6) 安全性を確保できるポイントを決定するための耐久性試験

スキーム 2 の Step 1, 2, 3 および 5 についてストレス反応を行った。それにより、Step 5 の反応は、これ以上加熱することができないことがわかった。その際の反応時間は、200 g スケールにおいて 27 時間で、出発物質の compound 5 の量は、5% 未満だった (HPLC 解析による AUC をもとに)。この反応は、より長時間モニターされる。より多くの量の中間体存在下で、その他のステ

ップ (特に、スキーム 2 の Step 8 および Step 10、また、スキーム 1 の Step 5、スキーム 4 の Step 1) の hold point を追跡する予定である。

7) プロセスの安定性に影響を与えるファクターの理解とコントロール
我々は、いくつかの反応ステップがプロセスとスケールアップにインパクトを持つことを見出した (例えば: スキーム 2 における Compound A 合成の Step 8 および Step 10、スキーム 3 における Compound B 合成の Step 1 および Step 2、スキーム 4 における RCI-56 合成の Step 1 および Step 3)。スキーム 2 の Step 8 は、より大きなスケールのクロマトグラフィーによって分離困難なステレオアイソマーを精製する。スキーム 2 の Step 10 は、Tebbe 反応を含み、高価でありかつ粘性の高い個体になってしまう。代替反応または条件、例えば: Petasis dimethyltitanocene (DMT) 反応および Witting 反応を検討すべきである。現在の反応条件において、スキーム 3 の Step 1 と Step 2 は、20 g スケールで 48% の収率が得られる。これらのステップは、収率チェックのためスケールアップされる。スキーム 4 の Step 1 では、Compound B を複数回投入している。我々は、Compound B の量を減らすためにこのステップの最適化を図る予定である。スキーム 4 の Step 3 は、オリジナルの反応条件下では、手間のかかる反応である。Pd/C または Pd(OH)₂/C による加圧水素化反応を用いた他の反応条件を検討中である。

8) 必要に応じ、分析の際の標準物質ならびにマーカーの準備
各ステップの中間体は、分析のレファレンススタンダードとして残してある。

9) 個々のオペレーションの安全性評価
安全なオペレーションの検討を行った。特に、スキーム 2 における Compound A 合成の Step 11、つまり、hydroboration 酸化反応、は、hydrogen peroxide の使用において (的確にクエンチしてはいるが) 安全性を確認した。さらに、他の塩基、例えば、potassium tert-butoxide は、NaH (スキーム 2 およびスキーム 4 のいくつかのステップに関与) を代替するよう検討予定である。

10) 安全性を確保できるポイントの決定 (反応混合液と中間体の両方において)
我々は、ほとんどの反応における Safe hold point を決定した。このプロジェクトの短い期間内においては、各中間体の安定性は、未確認である。

11) 約 50 グラムスケールにおける最終プロセスの提示
これは継続中のプロジェクトである。10g の Compound A が次週までに得られる予定。つまり、RCI-56 を 50g 得るためにプロセスの改善を継続する。

要約すると、我々は、RCI-56 を得るプロセスにはほぼ慣れた状況にあり、スケールアップのためのプロセス開発を行った。さらなる開発のための作業は、50g の RCI-56 を提供するため、次週以降に実施予定である。

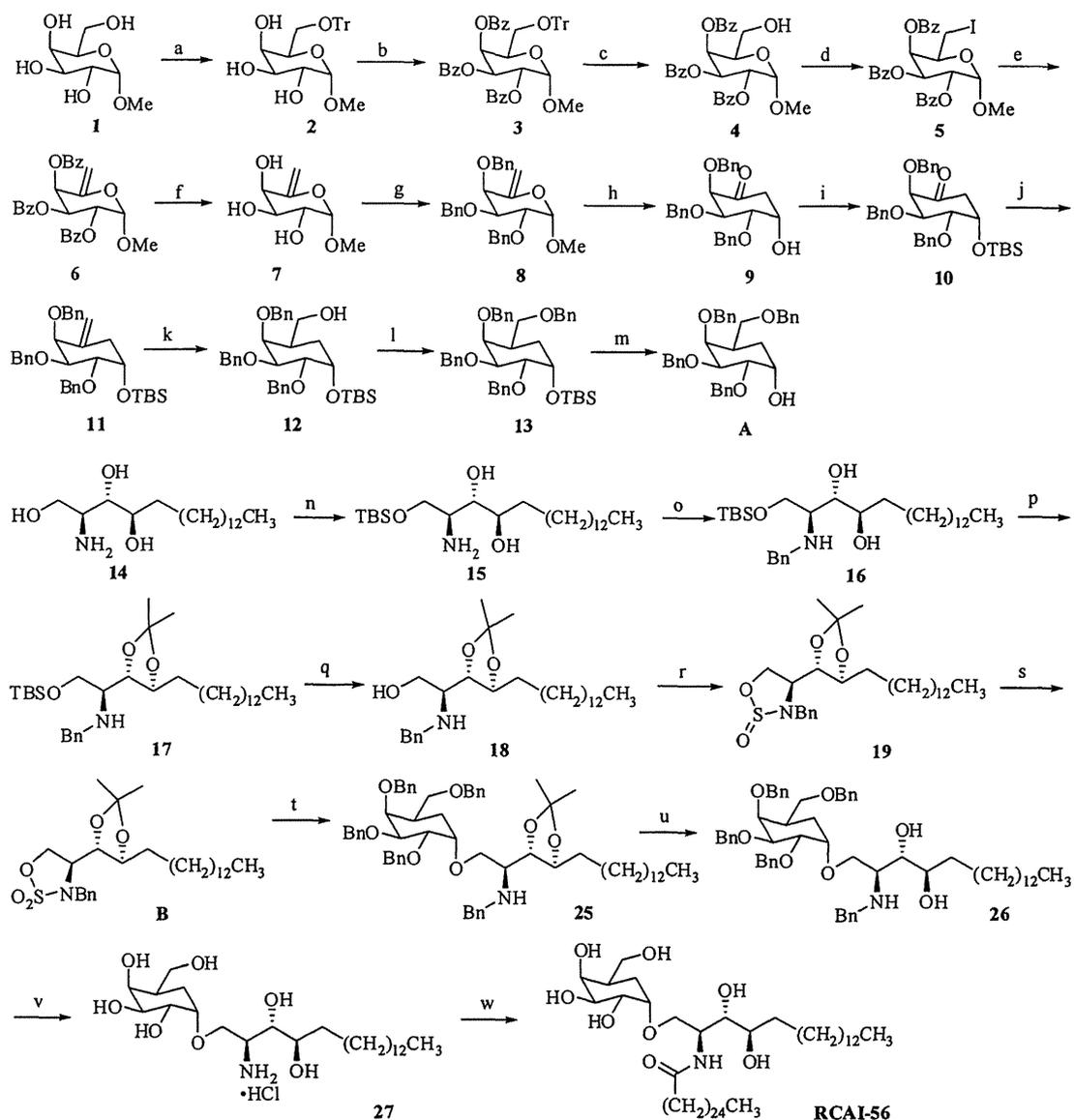
1.0 Delivery Summary

A request was made to Albany Molecular Research, Inc. (AMRI) by Riken, Japan (Riken) to conduct familiarization of the synthesis of compound **RCAI-56**, preparation of 10 g of compound **A**, and preparation of 50 g of **RCAI-56** with limited process development. The project was initiated at AMRI at the Syracuse site in January 6, 2014.

Date Started: January 2014
Date of the Report: March 23, 2014
Chemists: Geetha Banda; Jianxie Chen, Ph.D.; Lei Liu, Ph.D.;
Mary Beth MacBain; and Di Xu

Riken Contacts: Yasuyuki Ishii, Ph.D.

Scheme 1. Final Route of Synthesis of RCAI-56



Conditions: (a) TrCl, DABCO, DCM, rt, overnight; (b) Et₃N, BzCl, DCM, rt, overnight; (c) *p*TsOH, MeOH, DCM, rt, overnight; (d) Imidazole, PPh₃, I₂, toluene, 70 °C, 2 h; (e) DBU, THF, 68 °C, 27 h; (f) NaOMe, MeOH, rt, overnight; (g) NaH, BnBr, *n*Bu₄NI, THF, DMF, 0 °C to rt, overnight; (h) PdCl₂, 1,4-dioxane, water, 60 °C, 6 h; (i) 2,6-lutidine, TBSOTf, DCM, -18 °C to -28 °C, 2 h; (j) Tebbe reagent, pyridine, THF, -40 °C to rt, 4 h; (k) BH₃·THF, THF, 0 °C to rt, 2 h; 2 M NaOH, 30% H₂O₂, 0 °C to 10 °C, 1 h; (l) NaH, BnBr, *n*Bu₄NI, THF, DMF, 0 °C to rt, overnight; (m) TBAF, THF, rt, 40 h; (n) TBDMSCl, imidazole, THF, rt, overnight; (o) PhCHO, NaBH(OAc)₃, THF, rt, overnight; (p) DMP, *p*TsOH, rt, 5 h; (q) TBAF, THF, rt, 2 h; (r) Et₃N, SOCl₂, DCM, -35 °C to -45 °C, 1 h; (s) NaIO₄, RuCl₃·H₂O, CH₃CN, water, 0 °C, 1 h; (t) NaH, A, THF, DMF, 0 °C to 70 °C, 32 h; (u) *p*TsOH, MeOH, DCM, 60 °C, overnight; (v) Pd/C, HCl, H₂, 50 psi, ethanol, 24 h; (w) Et₃N, C, DCM, MeOH, THF, 0 °C, 1 h

2.0 Introduction

The preparations of compounds **A** (Scheme 2), **B** (Scheme 3), and **RCAI-56** (Scheme 4), were carried out mostly by following the procedure provided by Riken, but there were steps where it was deemed necessary to conduct a truncated development work. This involved development of either the reaction conditions or the isolation protocol.

We have currently almost completed the familiarization of the chemistry toward **RCAI-56** with relatively reproducible yields at most of the steps with exception in the formation of compound **9** due to the close-eluted other isomer. The first two steps on the preparation of compound **B** were changed because the reaction of protecting amine with benzyl group which was the first step in the original scheme had failed to work. We are currently on Step 3 of Scheme 4.

We successfully developed process procedures in some of the steps on a large scale. While currently carrying the intermediates through, based on the experience from the almost completed familiarization, optimizations and developments in some of the steps will continually be conducted. We are confident that 10 g of compound **A** and 50 g of **RCAI-56** will be prepared and delivered.

According to the list of the subjects needed to be conducted in the proposal, we have performed the work with the consideration. During the short time of the familiarization of the chemistry and medium scale reactions, most of the items have been achieved. While the project is on-going, some of the items are being looked at and some work is being planned for the future. Below are the details.

- Improve throughput/yield whenever possible:

For Scheme 2:

The yields from each of the steps were reproducible throughout from the original procedure except the Step 1 and 2 of preparation **A** (Scheme 2). We initially ran the reaction on 30 g of compound **1**, which was double scale to that in the provided procedure. The reaction was monitored by TLC analysis which indicated that the reaction was sluggish and the starting material persistently remained after 1 day. This was observed on the next 100 g scale reaction, in which starting material remained even more shown by TLC analysis. Another reason to seek different reaction condition was to avoid pyridine which was used in the reaction as a solvent, since removing pyridine usually takes time and volumes on large scales. DABCO and dichloromethane were then used in the reaction. By using this condition, the reaction went to completion after 4 hours. In the following step, Et₃N was added due to it being less expensive. In Step 3, we found that amount of *p*-TsOH to use had to be based on pH of the reaction instead of how many equivalents. The yield, after replacing with different reaction conditions, was improved from 36% to 58% in three steps.

Table 1. Yields Comparison on Steps in Scheme 2

Steps	Yields from the Original Procedure	Our Current Yields (Average)	Comments
Step 1, 2, and 3	36%	58%	Changed original reaction conditions.
Step 4 and 5	76%	80%	Currently use HPLC analysis for IPC on Step 5.
Step 6	87%	92%	
Step 7	68%	86%	
Step 8	89%	64%	On our 76 g scale reactions, about 14 g of desired α isomer remained as a mixture with β isomer. If it was isolated, the yield would be about 80%.
Step 9	85%	88%	
Step 10	80%	83%	
Step 11	77%	76%	
Step 12	95%	88%	
Step 13	80%	80%	
Overall	5.7%	9.2%	

For Scheme 3:

The Step 1, reductive amination, was tried by following the procedure described in the journal (*JOC*, **2004**, *69*, 7694–7699) using the common borane reducing reagent, NaBH₄. There were none or trace product formed. Therefore, we chose to protect primary hydroxyl group in the first step and then attach benzyl group to the amine group. On the 20 g scale of starting material, phytosphingosine, the yield for the over two steps was 48%, which was comparable to the yield from the two steps to compound **16** in the original procedure. We are currently working on a larger scale to examine the repeatability.

Table 2. Yields Comparison on Steps in Scheme 3

Steps	Yields from the Original Procedure	Our Current Yields (Average)	Comments
Step 1 and 2	41%	48%	Changed original reaction conditions and intermediate
Step 3	62%	71%	
Step 4	N/A%	96%	
Step 5 and 6	92%	83%	

For Scheme 4:

The reaction of Step 3, deprotection of benzyl group, in Scheme 4 is current in progress. After that, the familiarization of the synthesis will be completed after the final step, coupling with compound C.

Table 3. Yields Comparison on Steps in Scheme 4

Steps	Yields from the Original Procedure	Our Current Yields (Average)	Comments
Step 1	62%	70%	
Step 2	89%	77%	
Step 3	N/A	N/A	On-going
Step 4	N/A	N/A	

- Whenever possible, replace column chromatographic purifications by recrystallization, reslurry, etc.:

Compound 5 has been noticed as the solid. Purification of solid compound 5 by recrystallization was examined. The preliminary recrystallization condition will be further examined and implemented on the large scale. We will continue to seek possibility of purification other than chromatography for other compounds during scale-up.

- Establish in-process control for all stages:

In-process control by HPLC analysis was examined. Due to the compatibility with the certain HPLC columns and method, some of the intermediates were not able to show properly. Besides, through the first time run-through the chemistry and without all the intermediates available, the data of IPC was not the depended on the HPLC analysis unless there was a necessity, for example in Step 5 for compound 6, Scheme 2, in which the HPLC analysis showed the conversion of starting material. TLC and ¹H NMR analyses were the main analysis tools to monitor the reactions. With all the intermediates in hands now, developing HPLC method(s) for all the intermediates and final product will be performed.

- Analyze the in-process and isolated samples by HPLC or GC:

For analyzing IPC samples, see the comment above. For the isolated samples, some of the intermediates were analyzed by HPLC analysis. Due to the compatibility of the compounds and current methods, the purity is needed to confirm after the suitable method(s) are developed. GC analysis will be checked.

- Avoid concentration to dryness, magnesium sulfate treatment, use of unfriendly solvents from all steps of the process:

We have considered these in the project through. So far, dichloromethane has replaced chloroform in Step 1 of Scheme 2. We will try to eliminate magnesium sulfate treatment as much as possible unless it is necessary.

- Conduct stress tests to determine safe hold points:

We have stressed reaction on Step 1, 2, 3, and 5 in Scheme 2. We found that the reaction of Step 5 can not be heated for longer; reaction time for 200 g scale was 27 hours with starting material, compound **5**, less than 5% (AUC) by HPLC analysis. The reaction will be continued to monitor on larger scale. With more amounts of intermediates, we will continue to look into the hold points on other steps, especially Step 8 and Step 10 in Scheme 2, Step 5 in Scheme 3, and Step 1 in Scheme 4.

- Understand and control factors that impact process robustness:

We have identified some steps which could impact the process and scale-up, such as Step 8 and Step 10 in the synthesis of compound **A** (Scheme 2), Step 1 and 2 in the synthesis of compound **B** (Scheme 3), and Step 1 and 3 in the synthesis of **RCAI-56** (Scheme 4). More development work is needed in these steps.

Step 8 in Scheme 2 generated stereoisomers which are difficult to separate by chromatography on large scale, besides it is unknown if the stereoisomer ratio changes with the scale.

Step 10 in Scheme 2 involved Tebbe reagent which is expensive and the work-up of the reaction generated sticky solid. Alternative reagent or conditions, such as Petasis's dimethyltitanocene (DMT) and Wittig, are thought to try in the future.

Step 1 and Step 2 in Scheme 3 under current reaction condition provided 48% overall yield on 20 g scale. The steps are being scaled up and will check if the yields hold up.

Step 1 in Scheme 4 employed multiple addition of compound **B** during reaction. We have planned to optimize this step to reduce the amount of compound **B** once we have more precursors available.

Step 3 in Scheme 4 is a tedious reaction under original reaction condition. Other reaction conditions using Pd/C or Pd(OH)₂/C under pressurized hydrogenation conditions are being examined.

- Prepare analytical reference standards and markers as necessary:

Intermediates from each step have been retained as analytical references.

- Evaluate safety of each operation:

Safe operations have been considered. In particular, Step 11 in the synthesis of compound **A** (Scheme 2), hydroboration-oxidation, will be re-evaluated due to use of hydrogen peroxide although it was properly quenched during work up. Also, other bases, such as potassium *tert*-butoxide, have been planned to examine to replace NaH in a couple of steps in Scheme 2 and Scheme 4.

- Determine safe hold points (both reaction mixtures and intermediates):

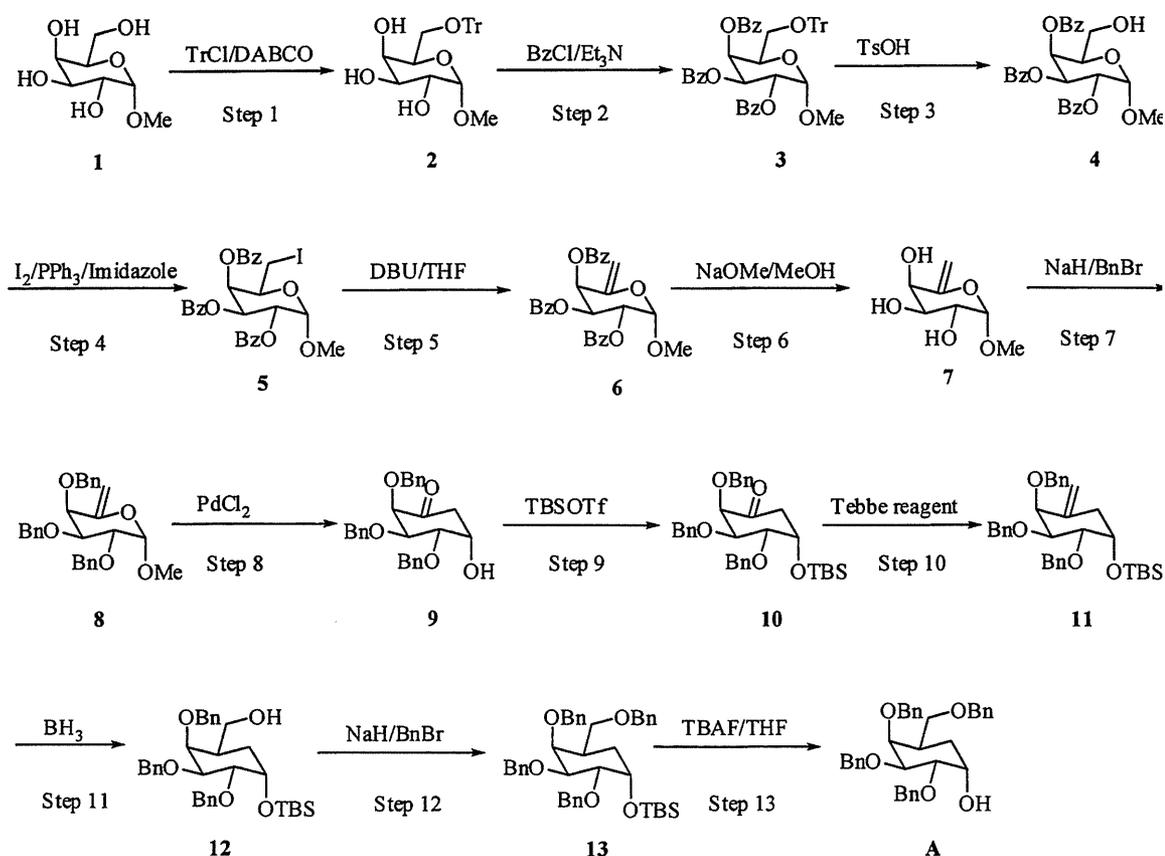
We have identified safe hold points for most of the reactions. Within the short period of the project so far, the stability of the intermediates have not been examined.

- Demonstrate the final process on approximately 50 g scale:

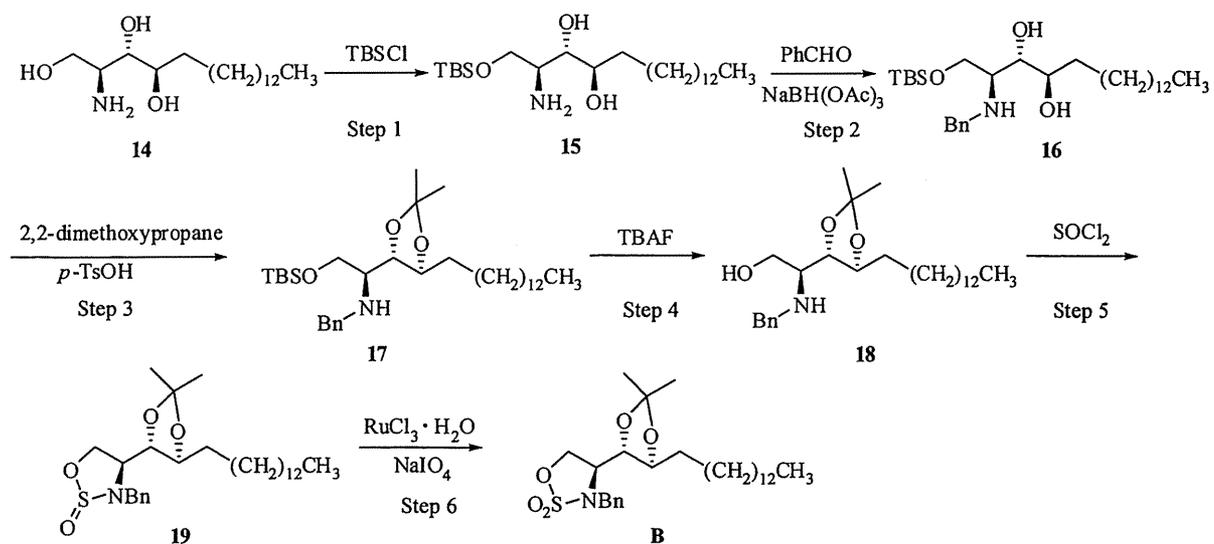
The project is ongoing. We are projecting to have the 10 g of compound A prepared by next week. In the meantime, we are continuing to work on the improvement of the process for making 50 g of **RCAI-56**.

In summary, we are close to finishing the familiarization of the chemistry toward **RCAI-56** and have developed process procedures on larger scale for some of the steps. More development work will be carried out in the coming weeks before further scale-up toward the completion of the delivery of 50 g of **RCAI-56**.

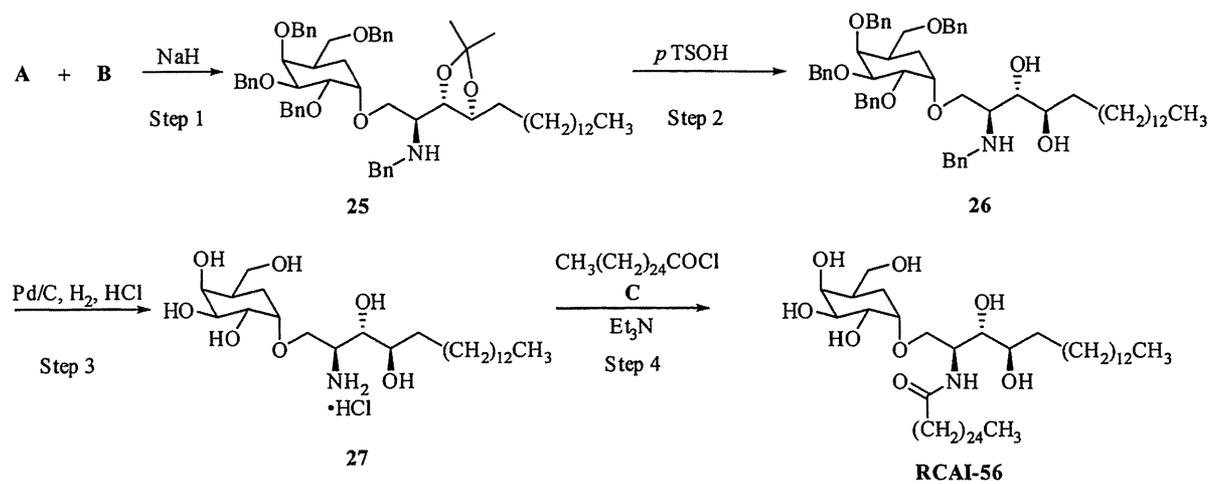
Scheme 2. Synthesis of Compound A



Scheme 3. Synthesis of Compound B



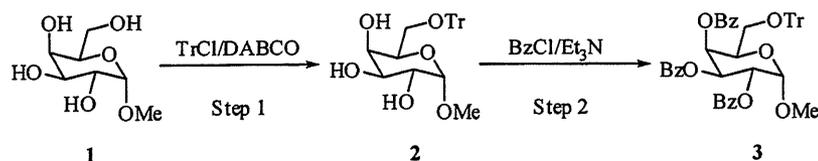
Scheme 4. Synthesis of Compound RCI-56



3.0 Results and Discussion

3.1 Synthesis of Compound A

Step 1 to Step 2



The two-step reaction was carried out in one pot, without work-up after Step 1. The original procedure for the Step 1 was run on 30 g and 100 g scales in pyridine (16.6 vol) as solvent and 2.2 equivalents of trityl chloride. After 20 to 40 hours, the reaction was not complete by TLC analysis. There was more starting material 1 remaining in a larger scale. The reactions were continued by adding 5 equivalents of benzoyl chloride into the Step 1 reaction mixture. The reactions were complete after overnight, at which time TLC analysis was indicated. The reaction was worked up by extensive aqueous washes to remove pyridine.

The preparation of compound 2 was examined under different conditions. The results are shown in Table 4. ¹H NMR analysis in solvents such as MeOH-*d*₄, DMSO-*d*₆, CDCl₃, cannot differentiate the starting material and the product. Therefore, the reactions were taken to the next step without knowing how much starting material remained. The reaction in 2 equivalents of DABCO, 2 equivalents of trityl chloride, and 20 vol of dichloromethane showed a complete conversion of the starting material 1 besides there was no pyridine involved.

Table 4. Examination of Preparation of Compound 2

Experiment	Conditions	Comments
24LL008A	1 (10.0 g, 1 equiv), TrCl (1.08 equiv), Pyridine (5 vol), 100 °C, 6 h	<ul style="list-style-type: none">• After 6 h, TLC analysis still showed 1.• Reaction mixture was used in the next step without workup (24LL008B).
24LL009A1	1 (1.0 g, 1 equiv), TrCl (2.0 equiv), Pyridine (10 vol), DMAP (0.2 equiv), 50 °C, 20 h	<ul style="list-style-type: none">• After 20 h, TLC analysis still showed 1.• Reaction mixture was used in the next step without workup (24LL009A2).
24LL009B1	1 (1.0 g, 1 equiv), TrCl (2.0 equiv), TEA (3.0 equiv), DMAP (0.2 equiv), DCM (20 vol), rt, 20 h	<ul style="list-style-type: none">• After 20 h, TLC analysis still showed 1.• Reaction mixture was used in the next step without workup (24LL009B2).
24LL009C1	1 (1.0 g, 1 equiv), TrCl (2.0 equiv), DABCO (2.0 equiv), DCM (20 vol), rt, 6 h	<ul style="list-style-type: none">• After 6 h, TLC analysis showed almost no 1 remaining.• Reaction mixture was used in the next step without workup (24LL009C2).
24LL009D1	1 (1.0 g, 1 equiv), TrCl (2.0 equiv), pyridine (5.0 equiv), DMAP (0.2 equiv), ACN (20 vol), 70 °C, 20 h	<ul style="list-style-type: none">• After 20 h, TLC analysis still showed 1.• Reaction mixture was used in the next step without workup (24LL009D2).
24LL009F1	1 (1.0 g, 1 equiv), TrCl (2.0 equiv), pyridine (5.0 equiv), DMAP (0.2 equiv), ACN (20 vol), rt, 20 h	<ul style="list-style-type: none">• After 20 h, TLC analysis still showed 1.• Reaction mixture was used in the next step without workup (24LL009F2).

After the reactions listed in Table 4 were taken to benzoyl protections under different conditions, TLC and ¹H NMR analyses in all of the reactions except lot 24LL009C2 showed additional spot or peaks other than from product **2**. The results are shown in Table 5. The additional spot or peaks were possibly from the tetra-benzoyl by-product which was from the unreacted starting material **1**. ¹H NMRs of each crude reaction mixture was analyzed and the mole ratio of **3** and the major by-product are listed in Table 6. The ratio reflected the amount of starting material **1** remaining in Step 1.

Table 5. Examination of Preparation of Compound 3

Experiment	Conditions	Comments
24LL008B	2 (reaction mixture from 24LL008A), BzCl (5 equiv), rt, 20 h	TLC showed no compound 1 remaining, but product and one adjacent spot.
24LL009A2	2 (reaction mixture from 24LL009A1), BzCl (5 equiv), rt, 20 h	TLC showed no compound 1 remaining, but product and one adjacent spot.
24LL009B2	2 (reaction mixture from 24LL009B1), TEA (6.0 equiv), BzCl (5 equiv), rt, 20 h	TLC showed no compound 1 remaining, but product and one adjacent spot.
24LL009C2	2 (reaction mixture from 24LL009C1), TEA (6.0 equiv), BzCl (5 equiv), rt, 20 h	TLC showed no compound 1 remaining and one major product spot.
24LL009D2	2 (reaction mixture from 24LL009D1), pyridine (5 equiv), BzCl (5 equiv), rt, 20 h	TLC showed no compound 1 remaining, but product and one adjacent spot.
24LL009F2	2 (reaction mixture from 24LL009D1), pyridine (5 equiv), BzCl (5 equiv), rt, 20 h	TLC showed no compound 1 remaining, but product and one adjacent spot.

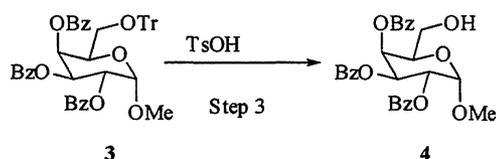
Table 6. Mole Ratio of Compound 3 and the Major By-product by ¹H NMR Analysis

Ratio of 3 and the major by-product	24LL008B	24LL009A2	24LL009B2	24LL009C2	24LL009D2	24LL009F2	19MEM003*
	1:3.9	1:0.43	1:0.47	1:0	1:0.14	1:0.12	1:0.35

* Starting material was the reaction mixture in which 100 g of 1 in 16.6 volumes of pyridine and 2.2 equivalents of trityl chloride was stirred at room temperature for 40 hours.

The reaction condition in 24LL009C1 and 24LL009C2 was applied to the reaction on 30 g, 400 g, and 800 g of compound 1. The similar results were obtained with complete conversion and less laborious work-up.

Step 3



Deprotection of trityl group was carried out by using *p*-TsOH•H₂O. Initially 0.3 equivalents of *p*-TsOH•H₂O were used, but the reaction was not complete after overnight. With additional *p*-TsOH•H₂O to pH ≈2, the reaction was complete in a few hours. The reaction was then examined at different pH. The results are shown in Table 7, in which indicated that pH ≈2 is the key for the reaction using *p*-TsOH•H₂O. There was a major impurity spot generated in the reaction using ferrous chloride.

Table 7. Examination of Preparation of Compound 4

Experiment	Conditions	Comments
24LL010A	3 (0.9 g, 24LL009C2), DCM (2 mL), MeOH (16 mL), <i>p</i> -TsOH•H ₂ O (0.2–0.6 equiv), rt, 20 h	<ul style="list-style-type: none"> 0.2 equiv of <i>p</i>-TSA was added initially and pH ≈3. 2 h TLC analysis showed no reaction. Additional 0.2 equiv of <i>p</i>-TSA was added to pH ≈2–2.5. 1 h TLC showed some product. Another 0.2 equiv of <i>p</i>-TSA was added to pH ≈1.5–2. 5 h TLC showed almost complete and the reaction was stirred overnight. ¹H NMR analysis was consistent with the product.
24LL010B	3 (0.9 g, 24LL009C2), DCM (2 mL), MeOH (16 mL), <i>p</i> -TsOH•H ₂ O (1–3 equiv), rt, 20 h	<ul style="list-style-type: none"> 1 equiv of <i>p</i>-TSA was added initially and pH ≈1.5. 1 h TLC analysis showed some product. Additional 2 equiv of <i>p</i>-TSA was added to pH ≈1. The reaction was stirred overnight. ¹H NMR analysis was not as clean as 24LL010A.
24LL010C	3 (0.9 g, 24LL009C2), DCM (2 mL), MeOH (16 mL), <i>p</i> -TsOH•H ₂ O (10 equiv), rt, 20 h	<ul style="list-style-type: none"> 10 equiv of <i>p</i>-TSA was added initially and pH <0.5. 1.5 h TLC analysis showed some product. The reaction was stirred overnight. ¹H NMR analysis was not as clean as 24LL010B and 24LL010A
24LL011	3 (1.15 g, 24LL009C2), DCM (12 mL), FeCl ₃ (2 equiv), rt, 2 h	<ul style="list-style-type: none"> TLC analysis showed there was no starting material remaining, but small amount of product and a major impurity spot below starting material.

The results also indicated that the reaction would not be expedited using more *p*-TsOH•H₂O, but more impurities formed after prolonging the reaction time.

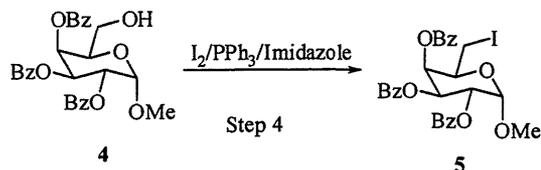
Using aqueous work up to remove *p*-TsOH was examined in the first reaction and it was found that the product generated other by-product which possibly was a benzoyl-migrated product. Therefore, without any workup after the reaction was complete the reaction mixture was directly concentrated to remove solvent before column purification.

The over-three-step results from the reaction on 100 g, 30 g, 400 g, and 800 g of compound 1 are tabulated in Table 8.

Table 8. Preparation of Compound 4

Lot Number	Input of Compound 1	Output of Compound 4	Yield (%)	Comments
09GB003	30 g	16 g	20	Step 1 and Step 2: Original condition; Step 3: Using aqueous NaHCO ₃ work up
14KL0078	100 g	65 g	25	Step 1 and Step 2: Original condition; Step 3: Original condition
19MEM008	30 g	60 g	52	Optimized conditions in all three steps
14KL0083	400 g	665	64	Optimized conditions in all three steps

Step 4

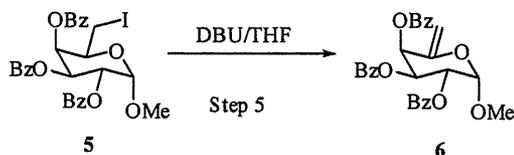


The reaction was carried out by following the provided procedure using Imidazole (3.1 equiv), PPh₃ (1.5 equiv), and I₂ (1.5 equiv) in toluene (18 vol). The reaction was completed after 2 hours at 70 °C. The reaction was worked up by quenching with saturated Na₂S₂O₃ solution and extracting with EtOAc. There were over 40 volumes of aqueous and organic solvents were used during workup. The crude product was purified by column chromatography. Since solid product was obtained from a larger scale, recrystallization can be examined in the future for the purification.

Table 9. Preparation of Compound 5

Lot Number	Input of Compound 4	Output of Compound 5	Yield (%)	Comments
09GB004	15.5 g	17.6 g	96	<ul style="list-style-type: none">• Purification by flash silica gel chromatography, a foaming solid.• >99% (AUC) by HPLC at 254 nm.
19MEM010	30 g	36 g	99	<ul style="list-style-type: none">• Purification by flash silica gel chromatography, a foaming solid.• 96.6% (AUC) by HPLC at 254 nm.
19MEM016	665 g	518 g	64	<ul style="list-style-type: none">• Purification by flash silica gel chromatography, lot 14KL0130A, a white solid.• Another 60 g, lot 14KL0130B, a waxy solid.• The yield was lower than usual due to low potency of the starting material which was an oil. The yield of over 4 steps was 41% which was comparable to what was expected.

Step 5



By following the provided procedure, DBU (9.1 equiv) in THF (13 vol) was used in the reaction. On a 15 g scale, the reaction was not complete after 24 hours by HPLC analysis and there was about 20% starting material remaining. The reaction was worked up and