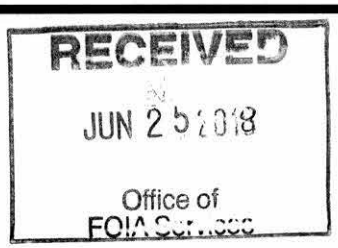
18-04459-E

foiapa

From: Mark Edwards <medwards@biosciadvisors.com>

Sent: Sunday, June 24, 2018 6:47 AM
To: foiapa
Subject: FOIA Request



I would like to request access to Exhibit 99.1 to the Form 8-K filed by Regulus Therapeutics, Inc. on 8/7/2013. Confidential treatment was sought as to certain portions when initially filed with the Commission.

In the event that confidential treatment has not expired or has been extended, I further request that you send me the expiration date(s) from the relevant CT order(s) so I will know when I should resubmit my request.

I authorize up to $61 in search and retrieval fees. Please send the exhibit(s) by PDF if possible.

Sincerely,

Mark

Mark G Edwards
Managing Director
Bioscience Advisors
2855 Mitchell Dr., Suite 103
Walnut Creek, CA 94598
medwards@biosciadvisors.com
925 954-1397

UNITED STATES
SECURITIES AND EXCHANGE COMMISSION
STATION PLACE
100 F STREET, NE
WASHINGTON, DC 20549-2465

Office of FOIA Services

July 9, 2018

Mr. Mark G. Edwards
Bioscience Advisors
2855 Mitchell Dr., Suite 103
Walnut Creek, CA 94598

RE: Freedom of Information Act (FOIA), 5 U.S.C. § 552
Request No. 18-04959-E

Dear Mr. Edwards:

This letter is in response to your request, dated June 24, 2018 and received in this office on June 25, 2018, for Exhibit 99.1 to the Form 8-K filed by Regulus Therapeutics, Inc. on August 7, 2013.

The search for responsive records has resulted in the retrieval of the enclosed exhibit (76 pages) that may be responsive to your request.

As shown on the enclosed invoice, the processing fee is $30.50 in accordance with our fee schedule. You may use our Online Payment option to pay by debit or credit card. If paying by mail, checks or money orders should be made payable to the SEC and a copy of the invoice should be mailed to our payment address: Enterprise Services Center, HQ Bldg., Room 181, AMZ-341, 6500 South MacArthur Boulevard, Oklahoma City, OK 73169. Please refer to the following link for detailed instructions on how to remit payments. http://www.sec.gov/about/offices/ofm.htm

If you have any questions, please contact me at jacksonw@sec.gov or (202) 551-8312. You may also contact me at foiapa@sec.gov or (202) 551-7900. You also have the right to seek assistance from Jeffery Ovall as a FOIA Public Liaison or contact the Office of Government Information Services (OGIS) for dispute resolution services. OGIS can be reached at 1-877-684-6448 or Archives.gov or via e-mail at ogis@nara.gov.

Sincerely,

Warren E. Jackson
FOIA Research Specialist

Enclosures

Exhibit 99.1

*****Text Omitted and Filed Separately with the Securities and Exchange Commission Confidential Treatment Requested Under 17 C.F.R. Sections 200.80(b)(4) and 240.24b-2**

EXECUTION COPY

AMENDMENT NUMBER THREE TO THE AMENDED AND RESTATED LICENSE AND COLLABORATION AGREEMENT

This Amendment Number Three (the "***Amendment***") to the Amended and Restated License and Collaboration Agreement is entered into as of the 2nd day of August, 2013 (the "***Effective Date***") by and among ALNYLAM PHARMACEUTICALS, INC., a Delaware corporation, with its principal place of business at 300 Third Street, Cambridge, Massachusetts 02142 ("***Alnylam***"), ISIS PHARMACEUTICALS, INC., a Delaware corporation, with its principal place of business at 2855 Gazelle Court, Carlsbad, California 92010 ("***Isis***", and each of Alnylam and Isis, a "***Licensor***" and together, the "***Licensors***"), and REGULUS THERAPEUTICS INC. (formerly Regulus Therapeutics LLC), a Delaware corporation, with its principal place of business at 3545 John Hopkins Court, San Diego, California 92121 ("***Regulus***").

RECITALS

WHEREAS, Isis and Alnylam each granted a license to Regulus in accordance with that certain License and Collaboration Agreement dated September 6, 2007 (the "***Original License Agreement***"), which Original License Agreement was amended and restated on January 1, 2009, and further amended on June 10, 2010 and October 25, 2011 (the "***Amended License Agreement***"); and

WHEREAS, Isis, Alnylam, and Regulus now desire to further amend the Amended License Agreement to, among other things, grant Regulus certain licenses and rights to the GalNac Process Technology, to the extent it relates to manufacturing; and to clarify Regulus' rights and restrictions on rights to transfer certain technology licensed to Regulus by Alnylam on the terms and conditions as provided below.

AGREEMENT

NOW, THEREFORE, in consideration of the mutual covenants contained herein, and other good and valuable consideration, the receipt and sufficiency of which is hereby acknowledged, Isis, Alnylam and Regulus each agrees as follows:

1. DEFINITIONS

Capitalized terms used herein and not defined elsewhere herein have the meanings set forth in the Amended License Agreement.

2. AMENDMENTS

2.1 Section 2.2(a) of the Amended License Agreement shall be deleted and replaced in its entirety by the following:

"(a) Grants. Subject to the terms and conditions of this Agreement (including but not limited to Section 2.4), each Licensor hereby grants to Regulus a worldwide, royalty-bearing, sublicenseable (in accordance with Section 2.5) license in the Field, under such Licensor's Licensed IP,

(i) to Develop miRNA Compounds and miRNA Therapeutics,

(ii) to Manufacture miRNA Compounds and miRNA Therapeutics, and

(iii) to Commercialize miRNA Therapeutics.

Subject to Section 2.4, the rights granted under clauses (i), (ii) and (iii) will be (x) exclusive with respect to miRNA Compounds which are miRNA Antagonists and miRNA Therapeutics containing such miRNA Compounds, (y) non-exclusive with respect to miRNA Compounds which are Approved Precursor Antagonists and miRNA Therapeutics containing such miRNA Compounds, and (z) non-exclusive with respect to Alnylam's Licensed IP that is GalNac ProcessTechnology."

2.2 Section 2.3(b) is hereby amended in its entirety as follows:

"(b) Regulus hereby grants to Isis a worldwide, exclusive, royalty-free, perpetual and irrevocable license, with the right to grant sublicenses, under the Regulus IP (*except* for Regulus IP (i) claiming the exact composition, i.e. specific sequence combined with chemistry, of a miRNA Mimic discovered by Regulus or (ii) that is an improvement to any GalNac Process Technology) solely to the extent necessary or useful to research, discover, develop, make, have made, use, sell, offer to sell and/or otherwise commercialize (i) single-stranded oligonucleotides or analogs thereof that are not miRNA Antagonists, Approved Precursor Antagonists, or Approved Mimics and (ii) any product containing single-stranded oligonucleotides or analogs thereof that are not miRNA Antagonists, Approved Precursor Antagonists, or Approved Mimics (the "*Isis Field*"); *provided* that in no event shall the rights granted above in any way restrict or otherwise prohibit Regulus from Researching, Developing, Manufacturing and Commercializing miRNA Mimics covered by such Regulus IP."

2.3 Section 2.5 is hereby amended by including the following Section 2.5(d) and 2.5(e):

"(d) Notwithstanding anything to the contrary in this Agreement, Regulus and its Affiliates may not transfer, sublicense, disclose or otherwise convey details of, any Alnylam Conjugate Technology, GalNac Process Technology, or Know-How or Patent

Rights licensed to Regulus by Alnylam with respect to the delivery of oligonucleotides, to any Third Party, except that subject to Third Party Rights, Regulus and its Affiliates may:

(i) with the prior written consent of Alnylam, grant a sublicense of Regulus' rights to Alnylam Conjugate Technology and/or GalNac Process Technology under Section 2.2(a)(ii), to Third Party contract manufacturing organizations for the sole purpose of manufacturing a particular miRNA Therapeutic (or component thereof) on behalf of Regulus or its Affiliate;

(ii) upon written notice to Alnylam, grant a sublicense of Regulus' rights licensed under Section 2.2(a) to Alnylam Conjugate Technology, GalNac Process Technology, or Know-How or Patent Rights licensed to Regulus by Alnylam with respect to the delivery of oligonucleotides, to a Third Party with whom Regulus or its Affiliate has entered into a bona fide Development and Commercialization collaboration with respect to a particular Development Therapeutic; provided, that (x) such sublicense shall be limited to such Development Therapeutic, and (y) Alnylam's prior written consent shall be required if such Third Party or Third Party's Affiliate is in the business of providing contract manufacturing services;

(iii) with the prior written consent of Alnylam, grant a sublicense of Regulus' rights under Section 2.2(a)(ii) to Alnylam Conjugate Technology, GalNac Process Technology, or Know-How or Patent Rights licensed to Regulus by Alnylam with respect to the delivery of oligonucleotides, to a Third Party contract manufacturing organizations for the sole purpose of manufacturing a particular Development Therapeutic that is an Approved Mimic (or component of such Development Therapeutic) on behalf of Regulus or its Affiliate; and

(iv) upon written notice to Alnylam, grant a sublicense of Regulus' rights to Alnylam Conjugate Technology, GalNac Process Technology, or Know-How or Patent Rights licensed to Regulus by Alnylam with respect to the delivery of oligonucleotides, to a Third Party with whom Regulus or its Affiliate has entered into a bona fide Development and Commercialization collaboration with respect to a particular Development Therapeutic that is an Approved Mimic; provided, that (x) such sublicense shall be limited to such Development Therapeutic, and (y) Alnylam's prior written consent shall be required if such Third Party or Third Party's Affiliate is in the business of providing contract manufacturing services.

Alnylam's prior written consent shall be required for any further sublicenses by a Sublicensee of Regulus or its Affiliates described in this Section 2.5(d) to any Third Party in the business of providing contract manufacturing services. Alnylam's prior written consent under clause (i) and (iii) of this Section 2.5(d) may be withheld by Alnylam in its sole discretion, not to be unreasonably withheld.

(e) Regulus will and hereby does grant a sublicense of Regulus' rights to Alnylam Conjugate Technology and GalNac Process Technology under Section 2.2(a) to Isis in

connection with Isis' exercise of its rights as an Opt-In Party; provided, that such sublicense shall be limited to the relevant Development Project's Development Compounds. The sublicensing restrictions in Section 2.5(d) shall also apply to Isis as an Opt-In Party solely with respect to the sublicense by Isis of any GalNac Process Technology."

2.4 Section 3.1 is hereby deleted and replaced in its entirety by the following:

"3.1 Technology Transfer to Regulus. At each meeting of the Collaboration Working Group the representatives will discuss new Know-How and Patent Rights of Isis and Alnylam that are included in such Licensor's Licensed Patents and Licensed Know-How hereunder at the level of detail necessary to enable Regulus to effectively practice such Patent Rights and Know-How."

2.5 Section 9.2 of the Amended License Agreement is hereby amended by adding the following Section 9.2(c):

"(c) GalNac Process Technology. Notwithstanding anything in this Agreement to the contrary, (i) Alnylam has the sole right in its sole discretion, to file, prosecute, maintain, defend and enforce (including but not limited to, initiating a legal action against a Third Party with respect to the infringement of) any GalNac Process Technology Patent Rights, (ii) neither Regulus nor any Commercialization Party other than Alnylam shall have any rights under this Article 9 with respect to any Patent Rights or Know-How within the GalNac Process Technology, and (iii) Regulus will provide Alnylam, sufficiently in advance for Alnylam to comment, with copies of all patent applications and other material submissions and correspondence with, to or from any patent counsel or patent authorities pertaining to any Regulus IP that is an improvement to GalNac Process Technology, and Regulus will give due consideration to the comments of Alnylam, but will in good faith determine whether or not to incorporate such comments."

2.6 Exhibit 1 to the Amended License Agreement is hereby amended by adding the following Defined Terms:

"***Alnylam Conjugate Technology***" means Alnylam's Licensed IP that relates to GalNAc conjugate technology, other than GalNac Process Technology.

"***GalNac Process Technology***" means the (i) Know-How and (ii) Patent Rights, in each case that are Controlled by Alnylam as of the Effective Date, listed on Exhibit 3, which are comprised of manufacturing technology and other technology. No more than once per calendar year Regulus may request that this definition be expanded to include any improvements to such Know-How and Patent Rights listed on Exhibit 3. Alnylam agrees to consider such a request in good faith, but shall not be obligated to expand the definition of GalNac Process Technology to include such improvements."

2.7 Section 1.51 of Exhibit 1 of the Amended License Agreement shall be deleted and replaced in its entirety by the following:

"***Licensed Know-How***" means, with respect to a Licensor, all Know-How Controlled by such Licensor on the Effective Date or during the term of this Agreement (except as otherwise expressly provided herein) that relates to (a) miRNA Compounds or miRNA Therapeutics in general, (b) specific miRNA Compounds or miRNA Therapeutics, (c) chemistry or delivery of miRNA Compounds or miRNA Therapeutics, (d) mechanism(s) of action by which a miRNA Antagonist directly prevents the production of a specific miRNA, or (e) methods of treating an Indication by modulating one or more miRNAs; provided, however, that in each case, (i) for any such Know-How that include financial or other obligations to a Third Party, the provisions of Section 2.4 will govern whether such Know-How will be included as Licensed Know-How and (ii) Licensed Know How does not include manufacturing technology (including but not limited to analytical methods), other than, in the case of Alnylam as Licensor, Know-How (to the extent related to manufacturing technology) included in the GalNac Process Technology."

2.8 Section 1.52 of Exhibit 1 of the Amended License Agreement shall be deleted and replaced in its entirety by the following:

"***Licensed Patent Rights***" means, with respect to a Licensor, (A) all Patent Rights Controlled by such Licensor on the Effective Date and listed on **Schedule 2.2(A)**, and (B) all Patent Rights Controlled by such Licensor during the term of this Agreement (except as otherwise expressly provided herein) that claim (a) miRNA Compounds or miRNA Therapeutics in general, (b) specific miRNA Compounds or miRNA Therapeutics, (c) chemistry or delivery of miRNA Compounds or miRNA Therapeutics, (d) mechanism(s) of action by which a miRNA Antagonist directly prevents the production of the specific miRNA, or (e) methods of treating an Indication by modulating one or more miRNAs; provided, however, that in each case, (i) for any such Patent Rights that include financial or other obligations to a Third Party, the provisions of Section 2.4 will govern whether such Patent Right will be included as a Licensed Patent Right and (ii) Licensed Patent Rights do not include manufacturing technology (including but not limited to analytical methods), other than, in the case of Alnylam as Licensor, Patent Rights (to the extent claiming manufacturing technology) included in the GalNac Process Technology."

2.9 The Amended License Agreement is hereby amended by including as Exhibit 3 to the Amended License Agreement, the Exhibit 3 (AlnylamGalNac Process Technology) attached hereto.

2.10 Concurrent with the Effective Date of this Amendment, and pursuant to Section 2.5(d)(i) above, Alnylam consents to a grant of a sublicense of Regulus' rights to Alnylam Conjugate Technology and Alnylam GalNac Process Technology under Section 2.2(a), [to Third Party contract manufacturing organizations, NITTO Denko Technical Corporation, Avecia Biotechnology, Inc. and Anthem Biosciences Private Ltd.,] for the sole purpose of manufacturing its [miR-122] miRNA Therapeutic (or component thereof) on behalf of Regulus or its Affiliate.

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3. MISCELLANEOUS

3.1 **Other Terms**. All other terms and conditions of the Amended License Agreement shall remain in full force and effect.

3.2 **Counterparts**. This Agreement may be executed in any number of counterparts, each of which will be deemed all original, and all of which together will constitute one and the same instrument.

[Remainder of Page Intentionally Left Blank]

IN WITNESS WHEREOF, the Parties hereby execute this Amendment Number Three to the Amended and Restated License and Collaboration Agreement as of the date first written above.

ALNYLAM PHARMACEUTICALS, INC.

By: /s/ Laurence E. Reid
Name: Laurence E. Reid, Ph.D.
Title: Chief Business Officer

ISIS PHARMACEUTICALS, INC.

By: /s/ B. Lynne Parshall
Name: B. Lynne Parshall
Title: Chief Operating Officer

REGULUS THERAPEUTICS INC.

By: /s/ Kleanthis G. Xanthopoulos, Ph.D.
Name: Kleanthis G. Xanthopoulos, Ph.D.
Title: President and CEO

Exhibit 3
[GalNac Process Technology

Patent Rights

Docket No.	Ctry	Sub Case	Case Type	Status	Application Number	Filing Date
ALN-175	US	PRO1	PRO	Pending	61/680069	6-Aug-12
	US	PRO2	PRO	Pending	61/794114	15-Mar-13

Know-How

See attached.

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GalNAc Succinate Process

Medicinal Chemistry

ALNYLAM CONFIDENTIAL INFORMATION

NOT TO BE SHARED OUTSIDE OF REGULUS

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Kilo Scale GalNAc Succinate Process

Content: Pages

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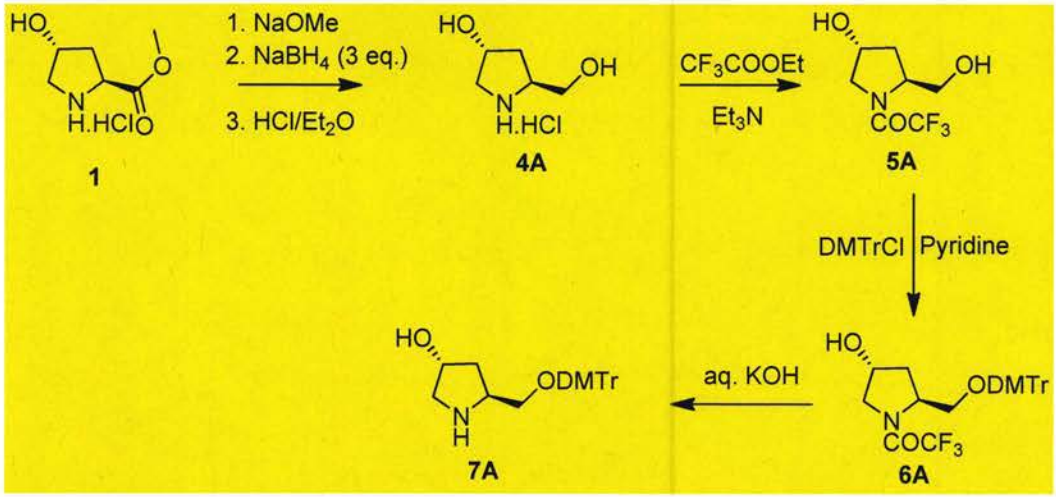
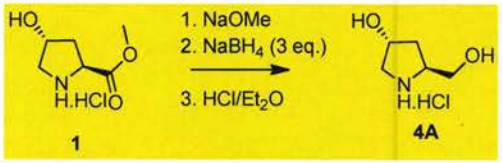
Synthesis of DMTr-Prolinol 7A

Scheme 1



Step 1. *trans*-4-Hydroxyprolinol Hydrochloride 4A:



	Reagents	MW	Qty Used	Moles	Mol. Eq.
1	Compound **1**	181.6	131.6 g	0.725	1
2	NaOMe (25 wt% in MeOH)	54.0	166 mL	0.725	1
3	$NaBH_4$	37.83	90.8 g	2.39	3.3
4	HCl in Dioxane(4M)	36.5	800 mL	3.2	4.4
5	Anhyd. MeOH		1.3 L		Solvent
6	Acetonitrile - low water		>1.5L		Solvent

Procedure 1:

The reaction was carried out in a 5L flask fitted with a gas inlet, thermometer, reflux condenser and a gas outlet placed over a reflux condenser and connected to a bubbler. 25 wt.% Solution of NaOMe in MeOH (166 mL, 0.725 mol) was added to a cooled (0 °C) and stirred solution of **1** (131.6 g, 0.725 mol) in anhyd. MeOH (1.3 L) under Ar atm followed by portion-wise addition of $NaBH_4$ (90.8 g, 2.39 mol) for ~1.5 h. Significant exothermic effect observed during the addition of the first 1/3-portion of borohydride and the rate of addition was adjusted to maintain the reaction temperature below 20-25 °C. The ice-

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water cooling bath was replaced with tap-water cooling bath and the mixture was stirred overnight while maintaining the temperature between 20-25 °C until hydrogen evolution ceased (gas bubbler monitoring). The reaction mixture was cooled to 0 °C; 4M solution of HCl in dioxane (3.2 mol, 800 mL) was added (exothermicity up to 30 °C), the cooling bath was removed and the mixture was stirred at rt for 2h. The reaction mixture was filtered through a fine-porosity glass filter and the filtrate was evaporated under reduced pressure (80 mbar, 30 °C) until bulk crystallization occurred (~ 2L of the solvent removed). Dry MeCN (1.5 L) was added, the mixture was triturated on rotary evaporator (20 °C, 2 h) filtered, crystalline residue was washed once with MeCN, and dried overnight in slow flow of nitrogen to afford 93.0 g (84%) of pure **4A**. Additional portion of 11.0 g (9%) of **4A** slowly precipitated from the mother liquor that was contaminated with NH_4Cl and other minor impurities. ^1H NMR (D_2O): 1.94-2.01 (m, 1H), 2.14-2.19 (m, 1H), 3.30-3.48 (m, 2H), 3.71-3.76 (m, 1H), 3.93-4.08 (m, 2H), 4.67-4.75 (m, 1H).

Procedure 2:

In order to optimize the above procedure in a larger scale the conversion of **1** to **4A** was carried out in 3 mole scale and the procedure is given below.

	Reagents	MW	Qty Used	Moles	Mol. Eq.
1	Compound **1**	181.62	544.86 g	3	1
2	NaOMe(25 wt% in MeOH)	54.00	686 mL	3	1
3	$NaBH_4$ (Aldrich Cat # 480886 (Granular)	37.83	300 g	7.93	2.64
4	HCl in Dioxane (4M)	36.5	3L	12	4
5	Anhyd. MeOH		5L		Solvent
6	Acetonitrile		5L		Solvent

trans-4-Hydroxyproline methyl ester hydrochloride (**1**, 544.86 g, 3 mol) was added to a 20L jacketed glass reactor fitted with a thermometer, reflux condenser and a solid addition funnel; the solid was dissolved in anhydrous methanol (5L) under Ar atm and the solution was cooled to 0° C. A solution of NaOMe in MeOH (25 wt.% , 686 mL, 3 mol) was added to the cold solution under constant stirring and constant flow of Ar; followed by portion-wise addition of $NaBH_4$ (300 g, 2.64 mol) through the solid addition funnel for ~1.5 h. The rate of addition was adjusted to maintain the reaction temperature below 20-25 °C. The reaction mixture was stirred overnight while maintaining the temperature between 25-30 °C until hydrogen evolution ceased (gas bubbler monitoring). The mixture was cooled back to 5 °C;

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4M solution of HCl in dioxane (12 mol, 3 L) was added slowly under stirring; filtered through a fine-porosity glass filter and the filtrate was evaporated under reduced pressure (8 L of the solvent removed, initial crystallization of the product was observed). Anhydrous MeCN (4 L) was added to the slurry and the mixture was stirred at rt; filtered and the crystalline product was washed once with MeCN (1 L), and dried overnight in a vacuum oven at 40 °C to afford 423.0 g (92%) of **4A**. However NMR analysis of the product indicated presence of unreacted ester **1**(<5%, *Note 1*) The solid product was dissolved in hot methanol (800 mL) and filtered under hot to remove undissolved solids (presumably NaCl). The clear filtrate was diluted with 4L of EtOAc and the solution was cooled oven an ice bath. Filtration of the crystallized product followed by drying provided the pure product **4A** (1^{st} crop 270 g + 2^{nd} crop 61 g = total 331 g, 72%) as white crystalline solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.48 (s, 1H), 8.75 (d, J = 187.3 Hz, 1H), 5.34 (dd, J = 16.9, 11.7 Hz, 2H), 4.37 (s, 1H), 3.83 – 3.59 (m, 2H), 3.61 – 3.42 (m, 1H), 3.15 (t, J = 26.0 Hz, 1H), 3.01 (t, J = 21.2 Hz, 1H), 1.98 – 1.61 (m, 2H). ^{13}C NMR (126 MHz, DMSO DMSO-d_6): δ 94.27, 68.77, 59.89, 59.86, 59.68, 52.42, 52.35, 35.39.

Note

1. (a) Three mol eq. of $NaBH_4$ may be required for the completion of the reaction. (b) The fitted filter at the bottom the reactor caused settling of solid material beneath the filter and that might have contributed to the incomplete reduction due to poor mixing beneath the filter.

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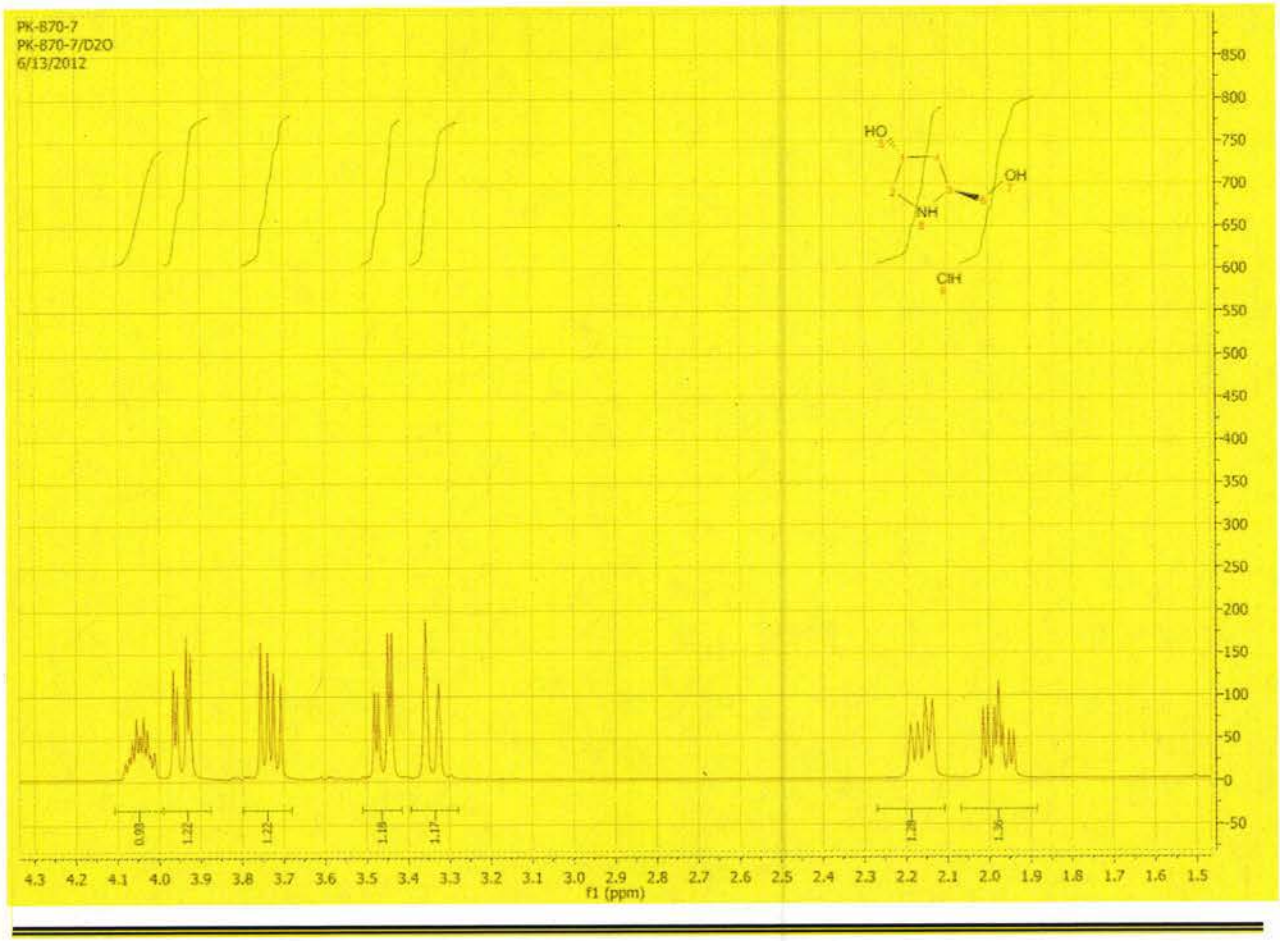
^{1}H NMR of 4A in D_2O



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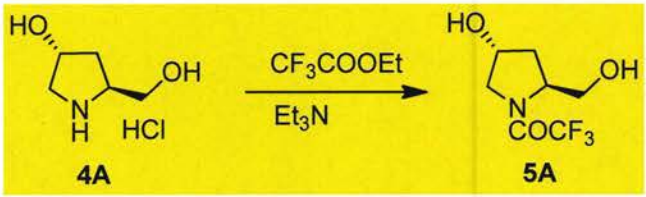
Step 2: Synthesis of compound **5A**.



	Reagents	MW	Qty Used	Moles	Mol. Eq.
1	Compound **4A**	153.61	450 g	2.94	1
2	Et_3N	101.19	1 L	7.2	2.45
3	CF_3COOEt	142.08	1000 g	7.04	2.4
4	Acetonitrile		8 L		Solvent

Procedure:

The reaction was performed in a 5 necked 15 L glass reactor fitted with an overhead stirrer over an ice bath. To a stirred solution of **4A** (450 g, 2.94 mol) in acetonitrile (8 L) were added Et_3N (1 L, 7.2 mol) and ethyl trifluoroacetate (1000 g, 7.04 mol), and stirred at room temperature overnight. The white solid (Et_3N.HCl) was filtered over sintered funnel and washed with EtOAc (4 L). The organic solution was concentrated followed by co-evaporation with toluene (2 x 2 L) and dried under reduced pressure overnight in a 22 L rotary evaporator to obtain crude **5A** as a gummy mass (~600 g).

Compound **5A** thus obtained could be used for next step without further purification.

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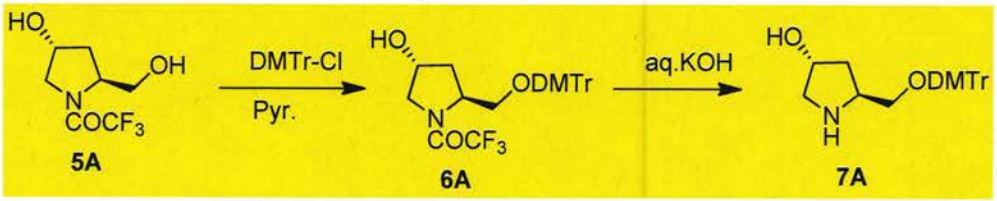
Step 3: Synthesis of compound **7A.**



	Reagents	MW	Qty Used	Moles	Mol. Eq.
1	Compound **5A**	213.15	600 g (crude)	2.94	1
2	DMTr-Cl	338.83	1000 g	2.95	1
3	KOH	56.11	330 g	5.88	2.0
4	Anhy. Pyridine		8L		Solvent
5	MeOH		5L		Solvent

Procedure:

The reaction was performed in a 5 necked 15 L glass reactor under nitrogen fitted with an overhead stirrer over an ice bath. The crude compound **5A** (2.94 mol) was dissolved in 2 L of anhyd. pyridine and transferred to the 15 L flask. Another 6 L of anhyd. pyridine was added. This solution was cooled over an ice bath to ~5 °C followed by addition of DMTr-Cl (1000 g, 2.95 mol) portion wise under nitrogen atm over 20 min and the reaction mixture was stirred at rt overnight. Completion of the reaction was confirmed by TLC. Water (12 L) was added while stirring and let it stand for 6 h. The compound was settled at the bottom of the flask as a brownish gummy mass. Water-pyridine layer was decanted using transfer pump and the aqueous layer was extracted with ethyl acetate (EtOAc, 2 x 5 L). Combined the EtOAc extract with the viscous material remained in the reactor, after decanting water layer. Concentration of the solvent gave compound **6A** as viscous oil which was used for the next step without purification.

The reaction (TFA deprotection) was performed in a 5 necked 15 L glass reactor fitted with an overhead stirrer. To a stirred solution of **6A** (2.94 mol) in MeOH (5 L) was added KOH (330 g, 5.88 mol) in 2 L of water drop wise over 30 min and stirred at room temperature for 1 h. The reaction mixture was concentrated to 3 L volume under reduced pressure; 15 L of water was added to the concentrate and allowed to stand overnight. The product was settled at the bottom as reddish brown viscous oil. Decanted the water layer using transfer pump and 6 L of dichloromethane (DCM) was added to dissolve the settled product, washed with 5 L of water and separated the organic solvent. Concentration of the solvent followed by silica gel column chromatography (4 Kg silica, eluent: hexane/ethyl acetate and ethyl acetate/MeOH, see Table below for details) purification gave compound **7A** (1000 g, 81 % from

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compound **4A**) as foamy yellowish white solid. ^{1}H NMR ($CDCl_3$): 1.35-1.42 (m, 1H), 1.65-1.70 (m, 1H), 2.33 (br s, 1H), 2.59-2.62 (m, 1H), 2.72-2.75 (m, 1H), 2.82-2.91 (m, 2H), 3.36-3.41 (m,1H), 3.71 (s, 6H), 4.1 (br s, 1H), 4.525 (d, J = 4.0 Hz, 1H).

Column Purification Condition for 7A:

Silica gel: 4 Kg (ZEO Prep 60 HYD 40-63 micron, CAS: 63237-67-4)

Number of elution	Solvent	Volume (L)
1	1% Triethylamine in Hexane	8
2	2nd elution: 2% Triethylamine in Hexane	4
3	Compound loaded with 500 mL of DCM	
4	1 % Triethylamine in 1:1 EtOAc/Hexane containing	8
5	1% Triethylamine in 1.5:2.5 EtOAc/Hexane	4
6	1% Triethylamine in 3:1 EtOAc/Hexane	4
7	1% Triethylamine in EtOAc	4
8	5 % MeOH in EtOAc	4
9	15 % MeOH in EtOAc	12
10	20 % MeOH in EtOAc	4

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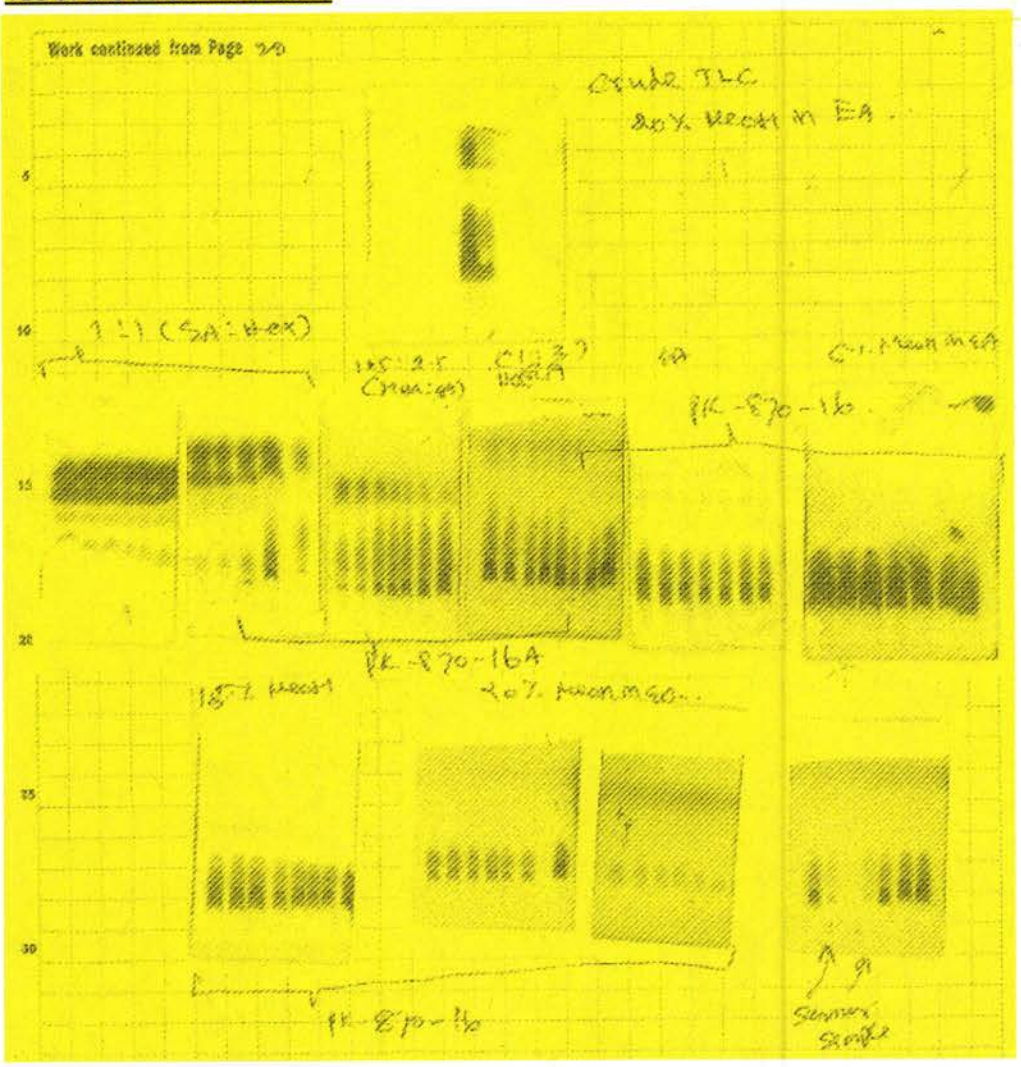
TLC of the column fractions



TLC (glass plates from Sigma-Aldrich, silica gel Matrix, HxW20cm x 20 cm) solvent condition: 20 % MeOH in EtOAc

Notes:

1. Make sure that the silica is packed in hexane containing 1% (v/v) of triethylamine to ensure the silica column is neutral or basic. After packing washing of the silica column with 1-2% Et_3N in hexane (minimum 1 column volume) is recommended.
2. The column chromatography set-up in the lab was not suitable for using excess silica, so 4kg of silica was used for the column. If use excess silica for column purification the yellow color from the product can be reduced or eliminated.
3. Only 450g out of 520g of compound **4A** from step 3 was used for this step.

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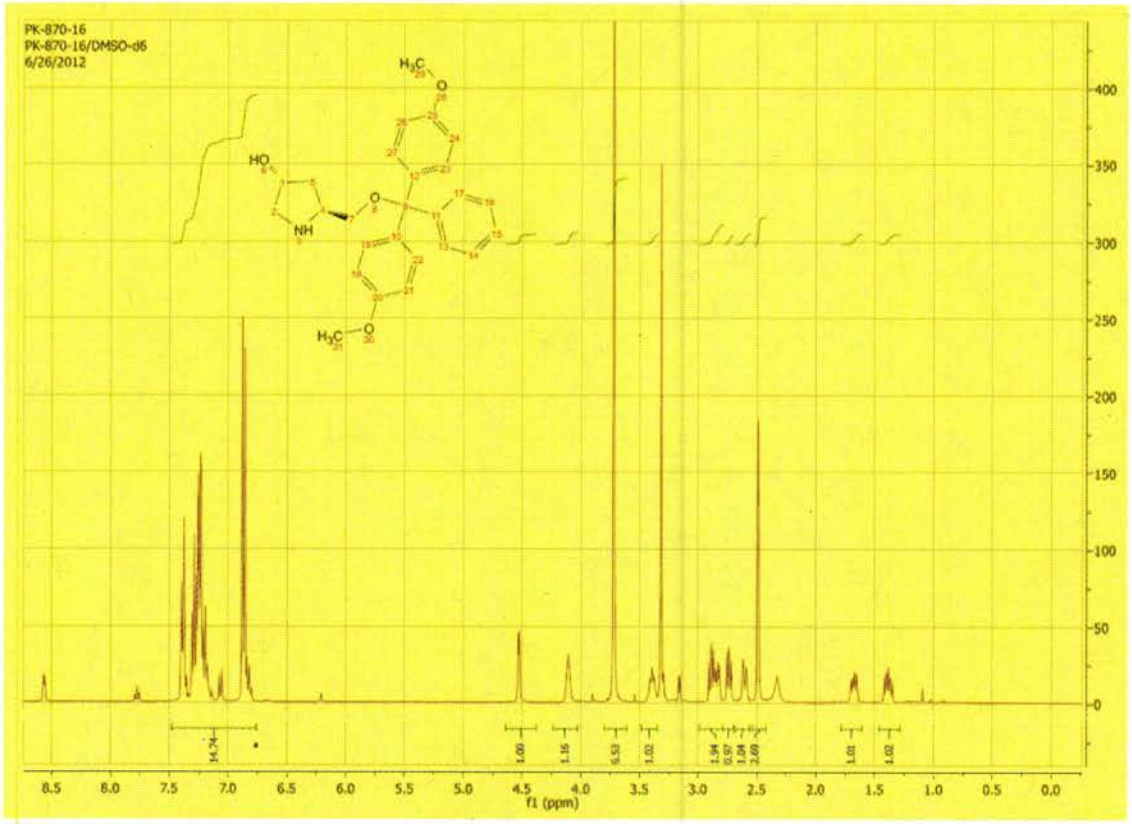
^{1}H NMR of Compound **7A** in DMSO-d_6



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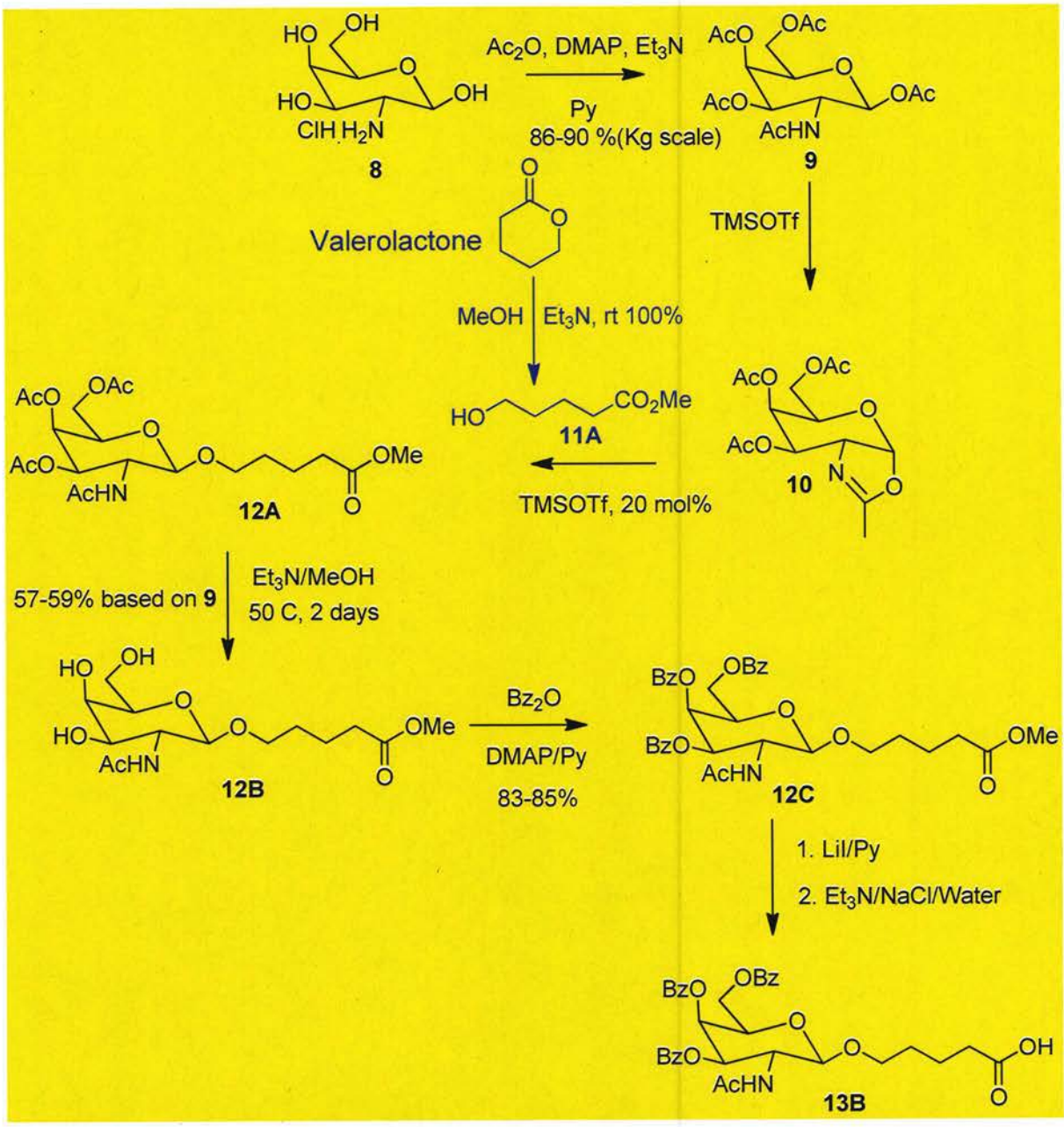
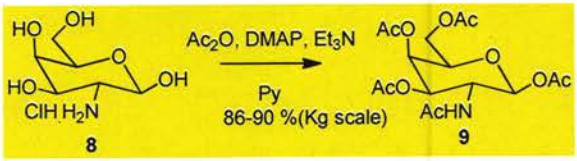
Synthesis of ^{Bz}GalNAc C_5 Acid 13B

Scheme 2



***Confidential Treatment Requested

Pentaacetyl D-(+)-2-aminogalactose (9):



	Reagents	MW	Qty Used	Moles	Mol. Eq.
1	Compound **8**	215.6	1.63 kg	7.55	1
2	DMAP	122.2	79 g	0.646	0.085
3	Triethylamine	101.2	1.05 L	7.55	1
4	Acetic Anhydride		5.3 L		Solvent
5	Anhy. Pyridine		7.2 L		Solvent
6	Toluene		8 L		Solvent

Procedure:

Anhydrous pyridine (7.2 L) was added to a stirred and cooled (ice bath) suspension of D-(+)-galactosamine hydrochloride **8** (1.63 kg, 7.55 mol) in acetic anhydride (5.3 L) in a 40 L reactor under Ar atm. DMAP (79 g), and triethylamine (1.05 L, 7.55 mol) were added consecutively, and the mixture was stirred in the ice bath overnight during which time dissolution of **8** followed by crystallization of **9** and triethylamine hydrochloride occurred along with exothermic effect up to 30 °C for the first 2 hours. The mixture was filtered through a sintered glass filter, and the residue was washed with toluene (~4 L x 2) followed by water (~3 L x 2). The crystalline residue was dried overnight on the glass filter, transferred to drying dishes and dried on air (70 °C hot plate) for two days to afford 2.53 kg (86%) of pure **9**. ^{1}H NMR (400 MHz, DMSO) δ 7.88 (d, *J* = 9.2 Hz, 1H), 5.63 (d, *J* = 8.8 Hz, 1H), 5.26 (d, *J* = 3.1 Hz, 1H), 5.05 (dd, *J* = 11.3, 3.3 Hz, 1H), 4.34 – 3.82 (m, 4H), 2.11 (s, 3H), 2.03 (s, 3H), 1.98 (s, 3H), 1.90 (s, 3H), 1.77 (s, 3H).

^{1}H NMR of Compound **9** in DMSO-d_6

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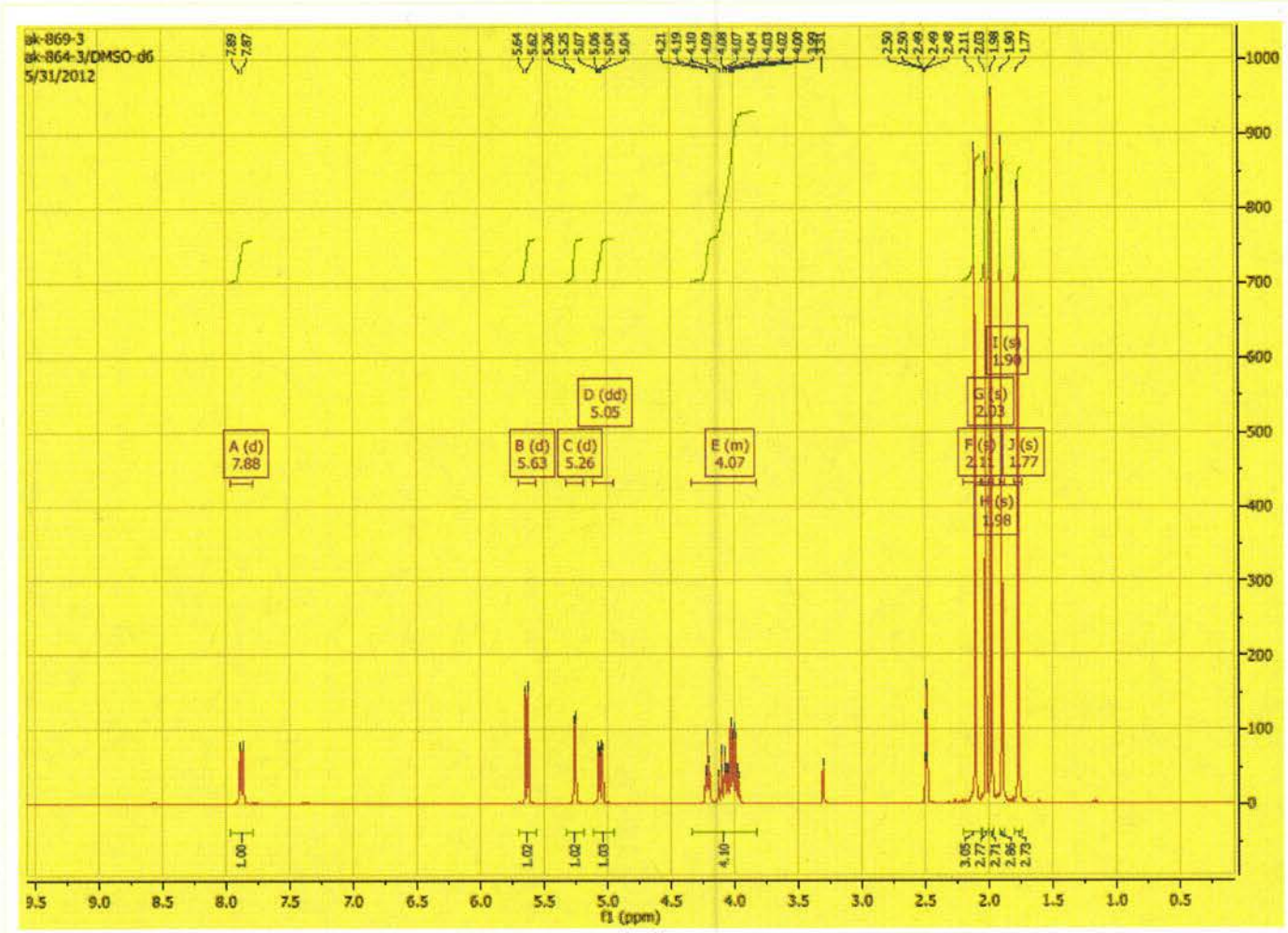

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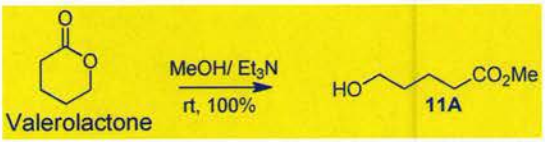
Methyl 5-hydroxypentanoate 11A:



	Reagents	MW	Qty Used	Moles	Mol. Eq.
1	Valerolactone	100.1	1.0 kg	10	1
2	Triethylamine	101.2	140 mL	1	0.1
3	Anhyd. MeOH		4 L		Solvent
4	Anhyd. Toluene		4 L		Solvent

Procedure:

The reaction was performed in a 20 L rotary evaporation flask under slow flow of argon. A solution of valerolactone (98% purity, purchased from TCI, 1.0 kg, 10 mol) and triethylamine (140 mL, 1 mol) in dry methanol (4 L) was stirred at rt for 1 h, during which time slight exothermic effect (up to 28 °C) was observed. The mixture was concentrated in vacuum (25 °C heating bath), diluted with dry toluene (4 L), and evaporated under reduced pressure, and the residue was dried on rotary evaporator at 4 mbar/25 °C followed by stirring under high vacuum (0.4-0.6 torr, rt) overnight to afford **11A** as a colorless liquid, 1.39 kg (100%), containing <1% of toluene. ^{1}H NMR (400 MHz, CD_3CN) δ 3.61 (s, 3H), 3.49 (t, J = 6.2 Hz, 2H), 2.55 (bs, 1H), 2.31 (t, J = 7.4 Hz, 2H), 1.62 (dt, J = 14.9, 7.1 Hz, 2H), 1.55 – 1.42 (m,2H).

^{1}H NMR of Compound **11A** in CD_3CN

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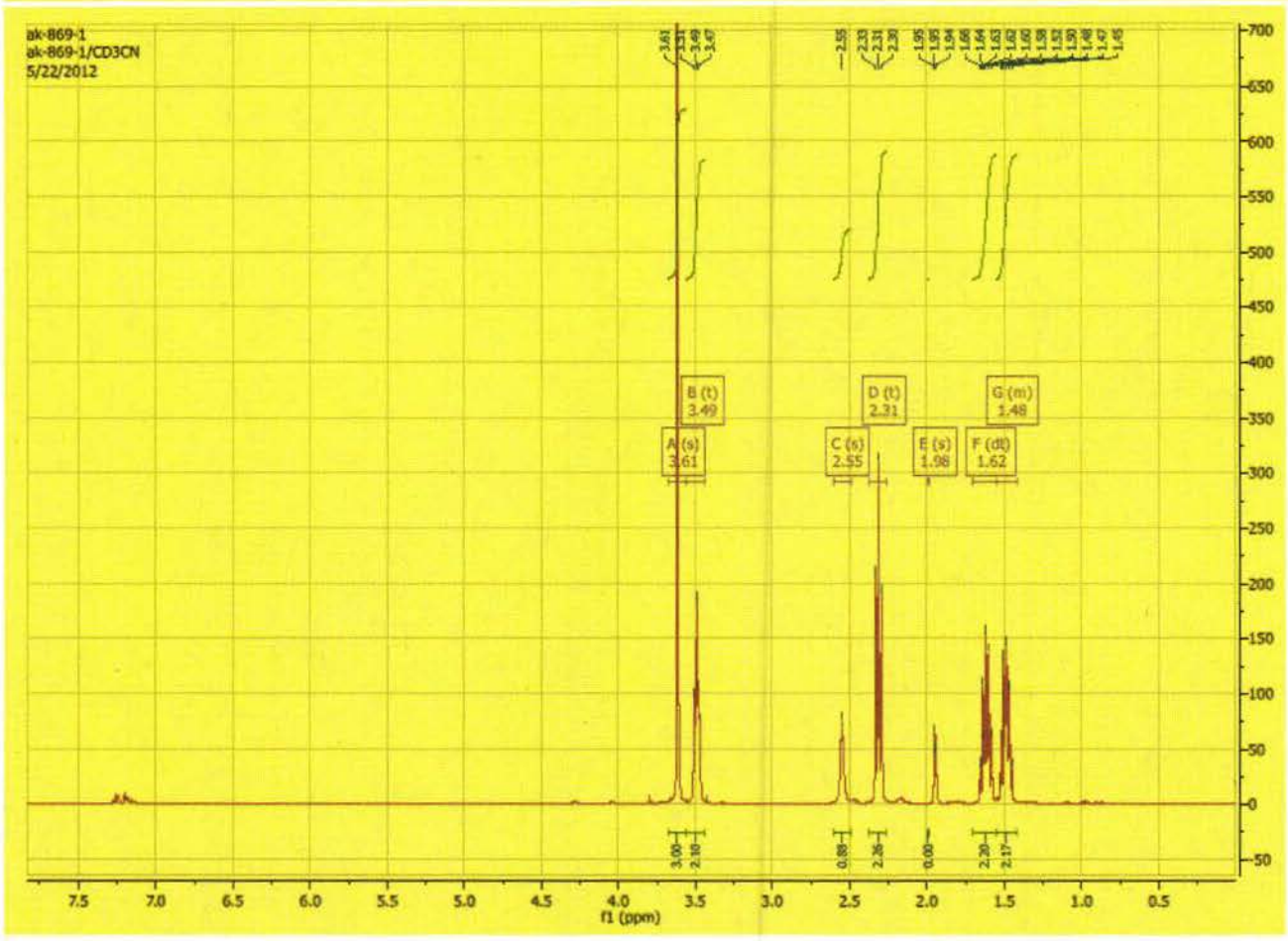

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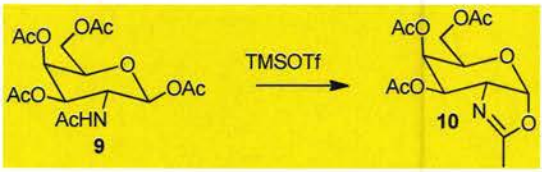
Oxazolidine intermediate 10 (crude):



	Reagents	MW	Qty Used	Moles	Mol. Eq.
1	Compound **9**	389.4	2.52 kg	6.49	1
2	TMS triflate	222.3	1.40 L	7.74	1.2
3	Sodium bicarbonate	84.0	1.64 kg	19.4	3
4	Anhy. DCE		4L		Solvent

Procedure:

The reaction was performed in a 20 L reactor under Ar atmosphere. TMSOTf (1.40 L, 7.74 mol) was added slowly (10 min) to a stirred suspension of **9** (2.52 kg, 6.49 mol) in anhyd. 1,2-dichlroethane (DCE, 4.0 L) under Ar atm. The mixture was stirred at rt overnight and transferred via transfer line to a vigorously stirred mixture of $NaHCO_3$ (1.64 kg, 19.4 mol), ice (5.5 L) and water (5.5 L) in an open 40 L reactor. The stirring was continued for 40 min, the organic layer was separated, the water layer was washed with dichloromethane (DCM 0.5L x2), and the combined organic extracts were dried over anhyd. Na_2SO_4. The mixture was filtered, the solvent was evaporated, oily residue was dried on rotary evaporator (15 mbar at 30 °C), re-dissolved in anhyd. DCE (4.0 L), and the solvent was evaporated again and the residue was dried on rotary evaporator (3 mbar, 30 °C) to afford 2.14 kg of crude **10** that was re-dissolved in anhyd. DCE (4.0 L) and the solution thus obtained was used in the next step.

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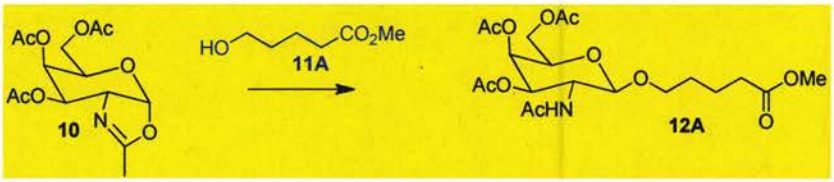
Peracetylated GalNAc glycoside 12A (crude):



	Reagents	MW	Qty Used	Moles	Mol. Eq.
1	Compound **10** (crude)	329.3	2.14 kg	<6.49	1
2	Compound **11A**	132.2	0.94 L	7.14	1.1
3	TMS triflate	222.3	250 mL	1.38	0.2
4	Sodium bicarbonate	84.0	168 g	2.0	0.3
5	Anhy. DCE		6.5 L		Solvent

Procedure:

The reaction was performed in a 20 L reactor fitted with a cooling jacket under Ar atm. TMSOTf (250 mL, 1.38 mol) was added to a stirred and cooled (12 °C) solution of crude **10** (2.14 kg, ≤6.49 mol) and the methyl 5-hydroxypentanoate (**11A**, 0.94 L, 7.14 mol) in anhyd. DCE (6.5 L). Immediate exothermic effect (up to 23 °C) observed, the cooler was turned off, and the mixture was stirred at rt for 3 h and transferred to a 22 L open flask with a vigorously stirred mixture of $NaHCO_3$ (168 g, 2.0 mol), water (3 L), and some ice. The organic phase was separated, dried over anhyd. Na_2SO_4; solvent was evaporated, and the oily residue was dried on the rotary evaporator at 12 mbar/30 °C to afford 3.09 kg of crude **12A** that was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.73 (d, J = 8.6 Hz, 1H), 5.35 (d, J = 3.1 Hz, 1H), 5.26 (dd, J = 11.2, 3.3 Hz, 1H), 4.68 (d, J = 8.4 Hz, 1H), 4.34 (t, J = 5.6 Hz, 1H), 4.21 – 4.04 (m, 3H), 3.93 (tdd, J = 17.7, 11.1, 7.0 Hz, 3H), 3.72 (s, 3H), 3.70 – 3.62 (m, 4H), 3.57 – 3.41 (m, 2H), 2.55 (t, J = 6.9 Hz, 1H), 2.32 (dt, J = 23.8, 11.7 Hz, 3H), 2.18 – 2.11 (m, 4H), 2.11 – 1.78 (m, 14H), 1.78 – 1.44 (m, 5H).

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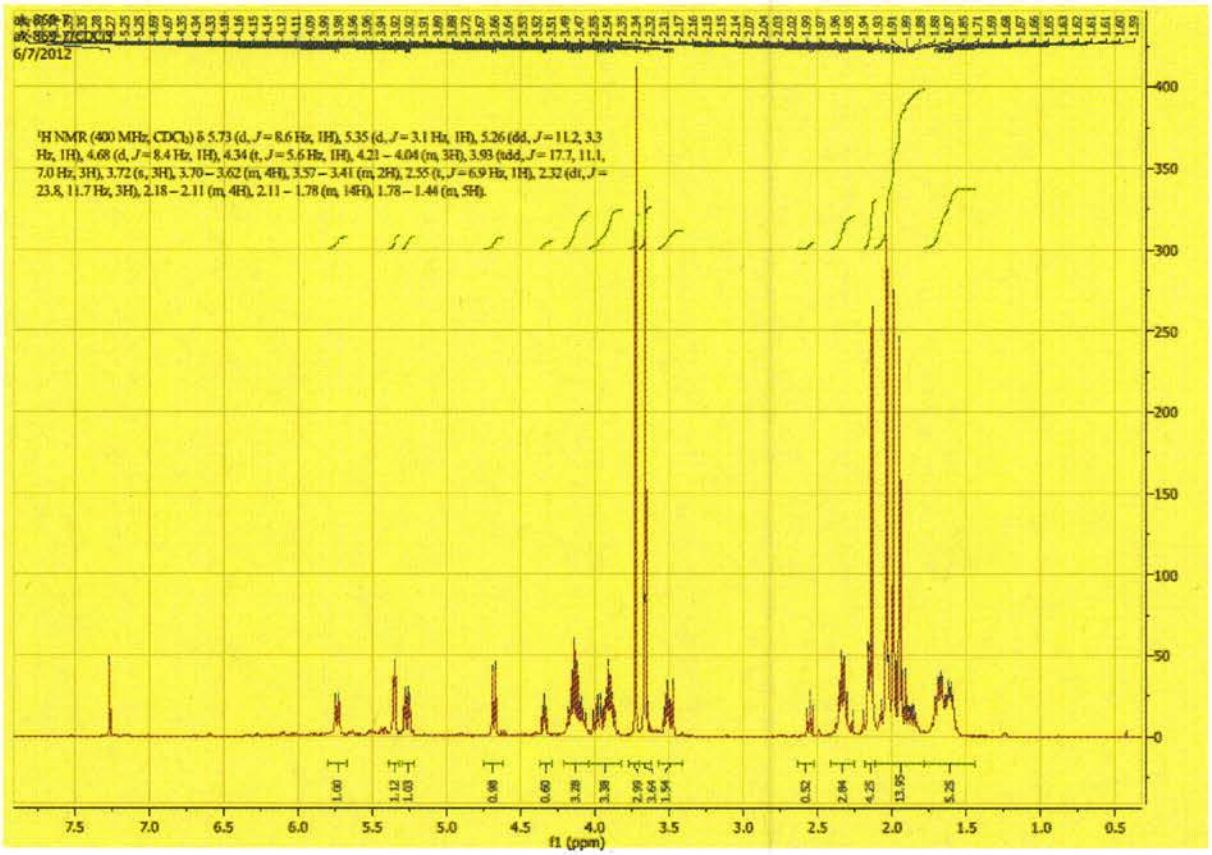
^{1}H NMR of **12A** in $CDCl_3$



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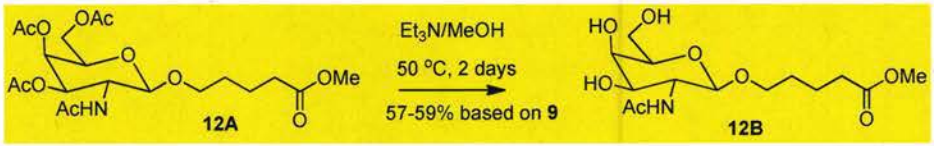
Deprotected GalNAc methyl ester 12B:



	Reagents	MW	Qty Used	Moles	Mol. Eq.
1	Compound **12A** (crude)	461.5	3.09 kg	<6.49	1
2	Triethylamine	101.2	0.90 L	6.49	1
3	MeOH		10 L		Solvent
4	Toluene		14 L		Solvent
5	Ethanol		4 L		Solvent

Procedure:

The reaction was performed in a 20 L filtration reactor fitted with a heating/cooling jacket. Crude **12A** (3.09 kg, ≤ 6.49 mol) was dissolved in anhydrous methanol (10 L) under Ar atm (*Note 1*), triethylamine (0.90 L, 6.49 mol) was added to the solution and was heated at 50 °C for 2 days under stirring. The reaction mixture was diluted with toluene (4 L) and the solution was allowed to cool to rt overnight during which time bulk crystallization occurred. The slurry was cooled to 0 °C, stirred overnight, filtered, and the solid was washed with 10% methanol in toluene (8.8 L) and dried on air (65 °C on hot plate) to afford 0.97 kg of **12B**. Filtrate was concentrated under reduced pressure till bulk precipitation begun. Dry ethanol (4.0 L) was added to the concentrate and the resulting slurry was triturated on the rotary evaporator at rt overnight, filtered, and the solid was washed with toluene-ethanol mixture (1:1, ~2 L) to afford additional 191 g of **12B** after drying on air (65 °C on hot plate). Total yield: 1.16 kg, 54% based on **9**, ~95% purity (*Note 2*). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.58 (d, J = 9.1 Hz, 1H), 4.60 – 4.47 (m, 2H), 4.44 (d, J = 4.3 Hz, 1H), 4.20 (d, J = 8.4 Hz, 1H), 3.77 – 3.60 (m, 3H), 3.56 (s, 3H), 3.54 – 3.21 (m, 6H), 2.28 (t, J = 7.4 Hz, 2H), 1.78 (d, J = 6.6 Hz, 3H), 1.59 – 1.36 (m, 4H).

Notes:

1. Regular grade methanol is sufficient if the reaction is done in the presence of triethylamine. The same reaction can also be done in the presence of sodium methoxide and the use of anhydrous methanol is essential in this case.
2. Two major impurities are:
    a. The corresponding methylglycoside (3-5 mol%) that is formed due to reverse reaction of lactonization of **11A** in the presence of TMS-triflate (Scheme 1A – Side Products). This

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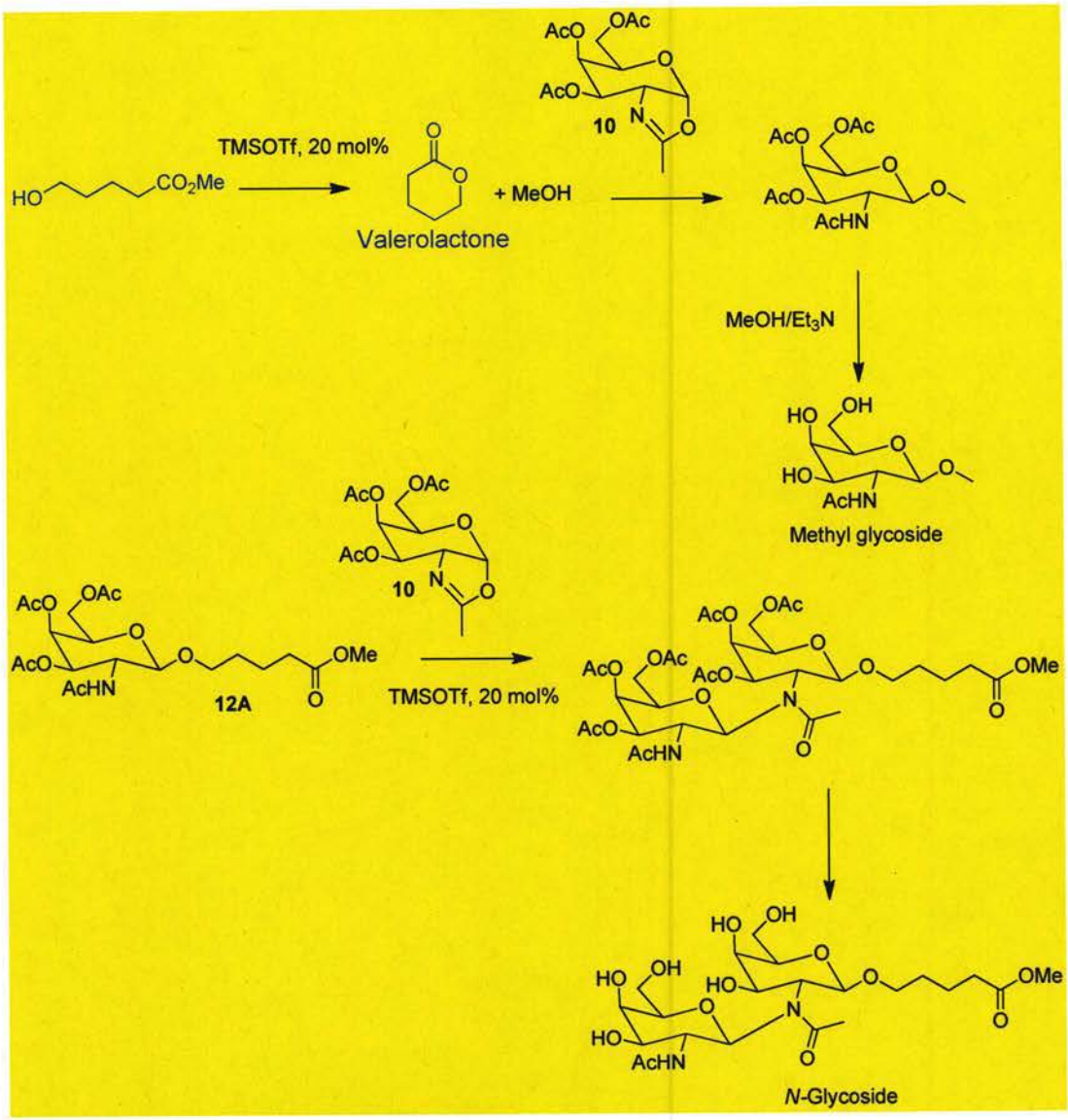
side process produces valerolactone and methanol which, in turn, reacts with **10** to give the methyl glycoside (See Scheme 1A – Formation of Side Products).

b. Formation of *N*-Glycosylated product due to the reaction of excess **10** with **12A** in the presence of TMS-triflate (See Scheme 1A – Formation of Side Products).

Scheme 2A – Formation of Side Products



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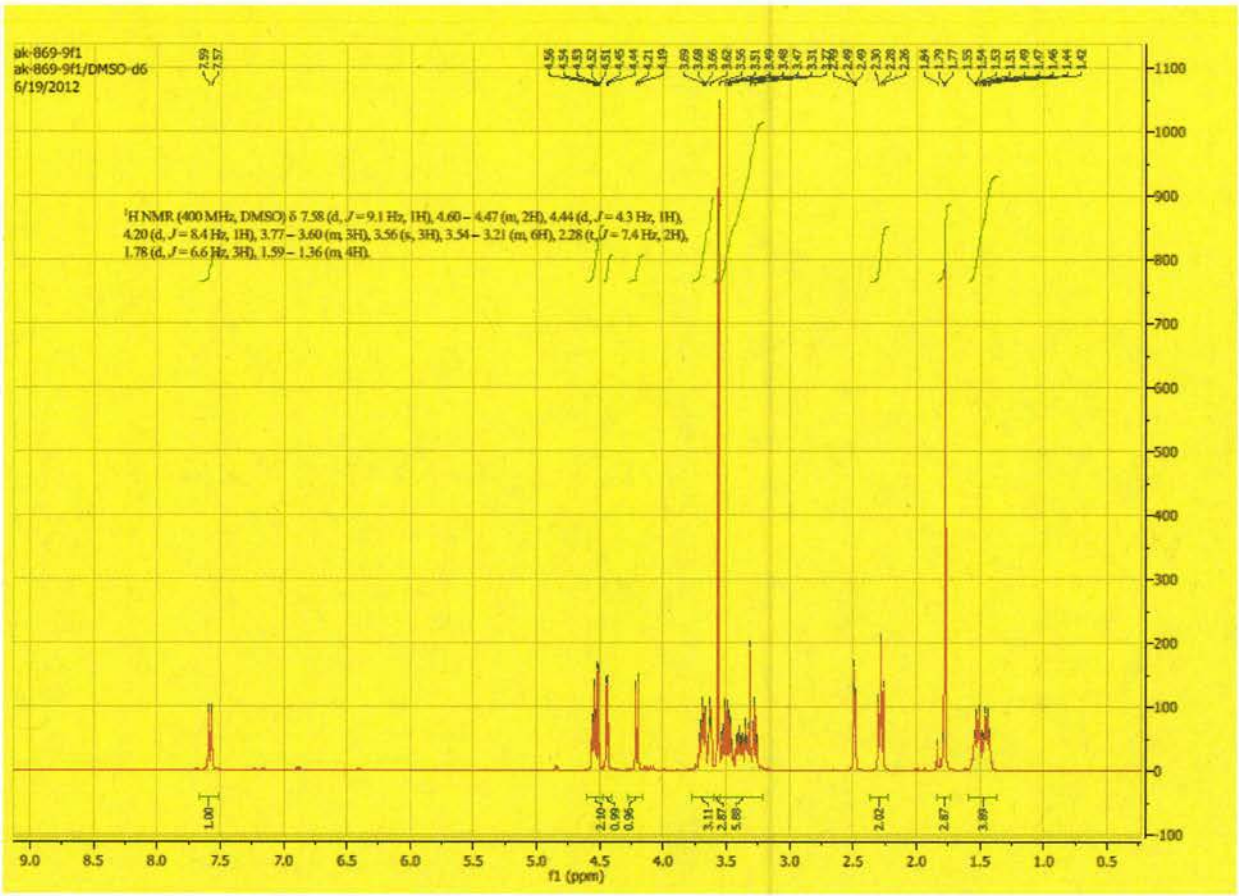
^{1}H NMR of **12B** in DMSO-d_6



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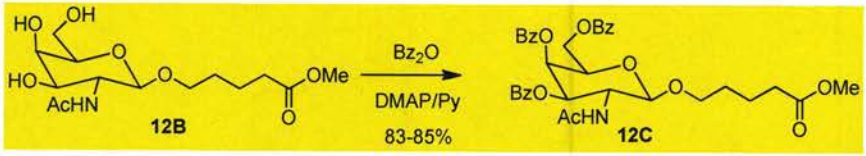
Perbenzoylated GalNAc Glycoside 12C:



	Reagents	MW	Qty Used	Moles	Mol. Eq.
1	Compound **12B** (~95%)	335.4	1.06 kg	3.15	1
2	DMAP	122.2	384 g	3.15	1
3	Benzoic anhydride	226.2	2.49 kg	11.02	3.5
4	Anhy. Pyridine		9.0 L		Solvent
5	Ethyl acetate		12 L		Solvent
6	Ethanol		14-15 L		Solvent

Procedure:

Glycoside **12B** (1.06 kg, 3.15 mol, ~95% purity) and DMAP (384 g, 3.15 mol) were dissolved in anhyd. pyridine (9.0 L) under Ar atm. Benzoic anhydride (2.49 kg, 11.02 mol) was added and the mixture was stirred overnight; water (200 mL) was added to the reaction mixture and the stirring was continued for additional 0.5 h. Pyridine was removed from the reaction mixture under reduced pressure and the residue was dried on the rotary evaporator at 10 mbar/35 °C. The residue was partitioned between water (2 L) and EtOAc (4 L) in the 20L rotary evaporator flask till complete dissolution of solids, transferred to an extraction vessel, and diluted with additional EtOAc (8 L) and water (5 L). The organic layer was separated and washed consecutively with water (10 L), sat. $NaHCO_3$ (2 x 5 L), and again water (2 x 5 L). The organic layer was separated and the solvent was evaporated under reduced pressure till viscous mass. The oily residue was re-dissolved in ethanol (3.8 L) and evaporated again under reduced pressure. The residue thus obtained was dried on the rotary evaporator at 20 mbar/35 °C for *ca* 2 h. The residue was re-dissolved in ethanol (10.6 L) at 35 °C and transferred to a 20 L filtration reactor fitted with heating/cooling jacket. Water (6.0 L) was added portion wise at 35 °C, and the mixture was allowed to cool down to 22 °C, and seeds of **12C** were added to the solution. Bulk crystallization occurred after stirring overnight; the mixture was cooled to 0 °C, stirred for additional 5h and filtered. The precipitate was pre-dried by passing air overnight, transferred to drying dishes, and dried on air (65 °C on hotplate for 2 days) till constant mass to afford 1.70 kg (84%) of **12C** (~97% purity, containing ~3% of the corresponding methyl glycoside). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.05 – 7.84 (m, 1H), 7.76 – 7.30 (m, 2H), 5.75 (d, J = 3.2 Hz, 1H), 5.35 (dd, J = 11.1, 3.3 Hz, 1H), 4.73 (d, J = 8.5 Hz, 1H), 4.54 – 4.41 (m, 1H),

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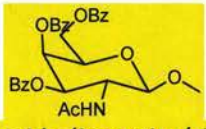
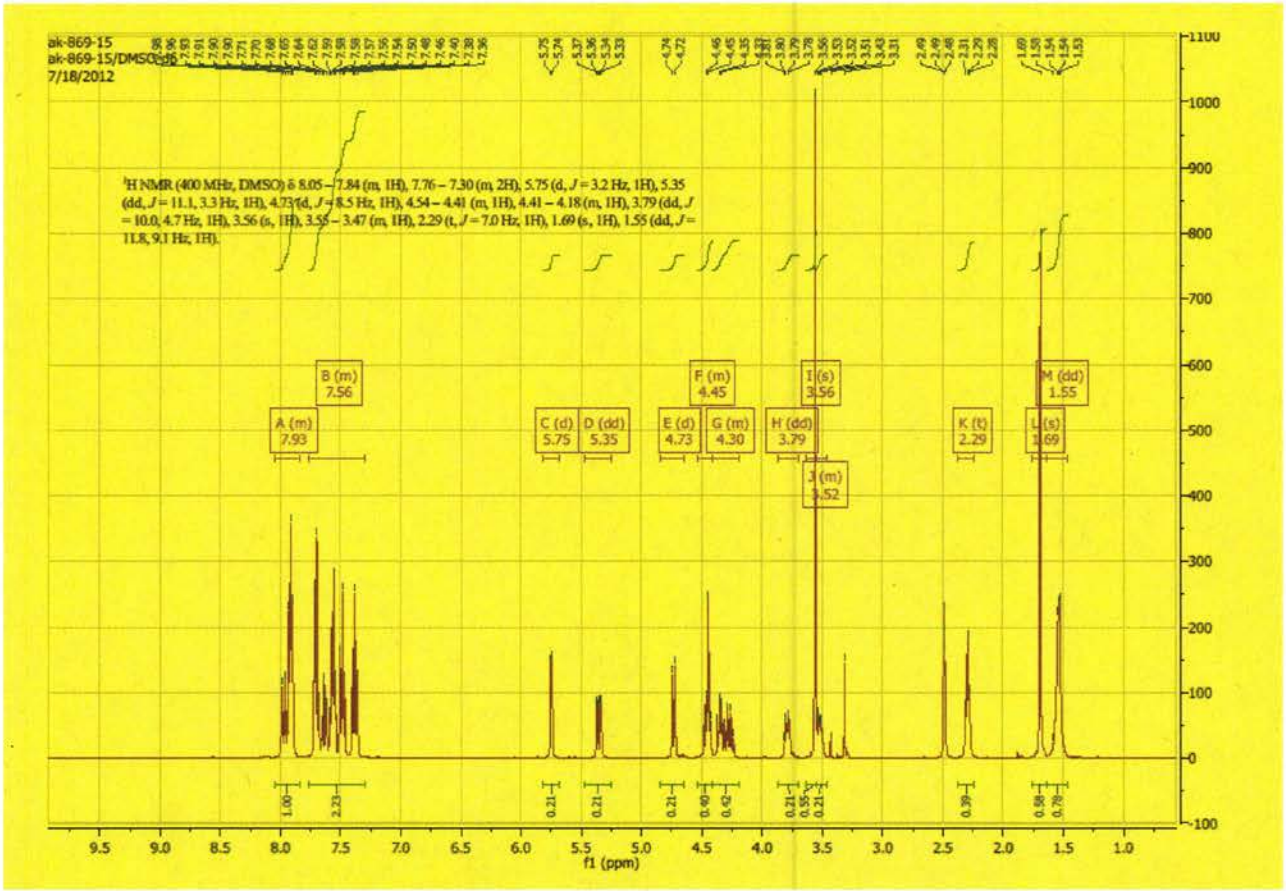
4.41 – 4.18 (m, 1H), 3.79 (dd, J = 10.0, 4.7 Hz, 1H), 3.56 (s, 1H), 3.55 – 3.47 (m, 1H), 2.29 (t, J = 7.0 Hz, 1H), 1.69 (s, 1H), 1.55 (dd, J = 11.8, 9.1 Hz, 1H).



Methyl glycoside (impurity/side product)

^{1}H NMR of **12C** in DMSO-d_6



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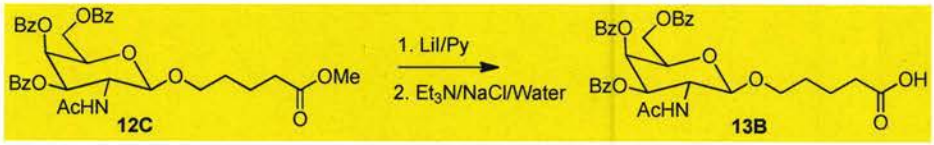
^{Bz}GalNAc C_5 Acid sodium salt 13B:



	Reagents	MW	Qty Used	Moles	Mol. Eq.
1	Compound **12C** (~97%)	647.7	1.70 kg	2.63	1
2	Lithium Iodide anhyd.	133.8	1.41 kg	10.52	4
3	Triethylamine	101.2	384 mL	2.76	1.05
4	Anhy. Pyridine		4.5 L		Solvent
5	Phosphoric acid		6.0 L		
6	Silica gel		2.5 kg		
7	Isopropanol		1 L		Solvent
8	Acetonitrile		12 L		Solvent
9	Ethyl acetate		~ 50 L (total)		Solvent
10	Hexanes		~ 20 L (total)		Solvent

Procedure:

Anhyd. LiI ("ultra-dry" - Alfa-Aesar, 1.41 kg, 10.52 mol) was added portion wise for ~30 min to a stirred solution of methyl ester **12C** (1.70 kg, 2.63 mol), in anhyd. pyridine (4.5 L) under Ar atm in a 12 L 4-neck flask fitted with gas inlet, thermometer, reflux condenser, and heating mantle. Exothermic effect up to 70 °C observed during the addition. The mixture was further heated under gentle reflux (125 °C in the flask) for 25 h, cooled to rt, and diluted with water (1.5 L). Pyridine was evaporated; the oily residue was dried on rotary evaporator at 11 mbar/35 °C, diluted with water again (2 L), evaporated and dried at 10 mbar/35 °C for 3 h. The residue (4.86 kg) was partitioned between chilled 20% H_3PO_4 (6 L) *(Note 1)*, water (2 L), and EtOAc - hexane mixture (2:1, 8 L) (*Note 2*). The organic phase was separated, and the water phase was washed again with EtOAc - hexane mixture (2:1, 3 x 3 L). Combined organic extracts were washed with 5% aq. NaCl (6 L), and dried over anhyd. Na_2SO_4. The solution (total volume ~20 L) was decanted form the drying agent and filtered through a plug of 2.5 kg silica gel (wet-loaded in EtOAc - hexane mixture (2:1) on a sintered glass 6 L filter funnel). The silica gel cake was washed with EtOAc-hexane 2:1 mixture, until no substantial amount of the acid in the filtrate was observed by TLC (45 L). The combined filtrates were evaporated and dried at 20 mbar/35 °C to afford crude acid as soft foam (1.78 kg) that was dissolved in EtOAc (4 L) and hexanes (2 L). Triethylamine (384 mL, 2.76 mol) followed by 5% NaCl solution (2.3 L) were added. The bottom aqueous layer containing triethylammonium salt of the acid **13B** was separated, and the organic layer after washing with 5% NaCl (0.5 L x 2) was discarded. The product was extracted from the combined aqueous layer into a mixture of EtOAc and isopropanol

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(5:1, 6 L); the organic layer was washed with 5% NaCl (4 L x 3), saturated NaCl (1 L x 2) and dried over anhyd. Na_2SO_4. The solvents were evaporated under reduced pressure; the residue was dried briefly on the rotary evaporator at 20 mbar/35 °C, and dissolved in acetonitrile (ACN, 6 L). The solution was filtered from inorganic precipitate through a sintered glass filter, evaporated, dried briefly on the rotary evaporator at 20 mbar/30 °C, redissolved in ACN (6 L), evaporated again, and dried under high vacuum to afford 1.58 kg (93%) of pure sodium salt **13B** (*Note 3*). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.50 (d, J = 9.1 Hz, 1H), 7.90 (dd, J = 10.9, 4.0 Hz, 4H), 7.82 – 7.28 (m, 11H), 5.75 (d, J = 3.2 Hz, 1H), 5.46 – 5.26 (m, 1H), 4.75 (dd, J = 32.1, 8.6 Hz, 1H), 4.53 – 4.20 (m, 4H), 3.74 (dt, J = 11.1, 5.7 Hz, 1H), 3.54 (dt, J = 10.6, 5.5 Hz, 1H), 2.01 – 1.82 (m, 2H), 1.71 (s, 3H), 1.65 – 1.33 (m, 4H).

Notes

1. Phosphoric acid was used in the amount necessary to neutralize remaining pyridine and to achieve pH between 3 and 4.
2. In this synthesis multiple extraction of water required due to insufficient dilution of the reaction mixture that gave rise to intermediate third layer. This may be avoided by addition of sufficient amount of EtOAc-hexane mixture.
3. HPLC was used for determining the quantity of benzoic acid present in the ^{Bz}GalNAc C_5 acid.

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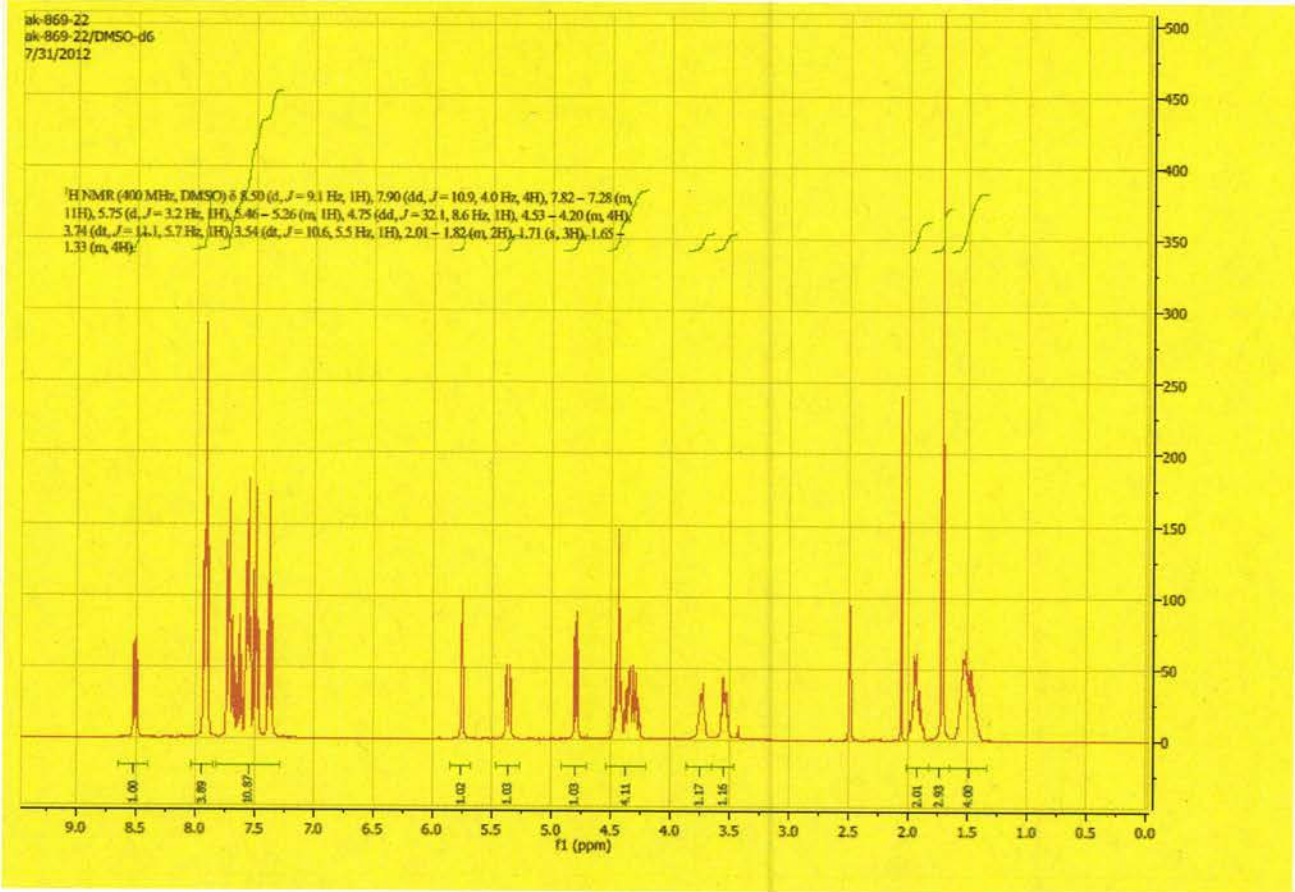
^{1}H NMR of **13B** in DMSO-d_6



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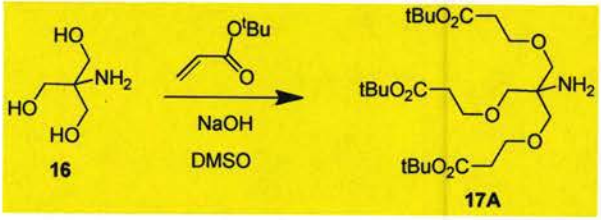
Synthesis of *Tris(tert-Butyl)-Tris amine* 17A
(*tert*-Butyl-3,3'-(2-amino-2-((3-tert-butoxy-3-oxopropoxy)methyl)propane-1,3-diyl)bis(oxy)dipropanoate)

Scheme 3



	Reagents	MW	Qty Used	Moles	Mol. Eq.
1	Tris(hydroxymethyl)aminomethane (TRIS)	121.07	500g	4.129	1
2	*tert*-Butyl Acrylate (d= 0.875)	128.08	2300 mL + 1550 mL	26.00	6.29
3	NaOH (5M in water)*	40.00	2x83 mL	-	-
4	DMSO	-	830 mL	-	Solvent
5	Ethyl acetate	48 L			Solvent
6	Hexane	24 L			Solvent
7	Toluene	4 L			Solvent
8	Methanol	1 L			Solvent

*40 g of NaOH was dissolved in 200 mL of water

Procedure:

TRIS (500 g) and DMSO (830 mL) were added under argon into a 12 L four necked flask equipped with an overhead stirrer over an ice-water bath. The reaction mixture was cooled to about 15 °C and stirred the mixture for about 10 min to dissolve TRIS completely (or until the reaction mixture become a homogeneous solution). 5 M NaOH (83 mL) was added at time and stirred the mixture for 5 min. *tert*-Butyl acrylate (2300 mL) was added to the above mixture slowly under constant stirring over a period of 1 h and maintained the reaction temperature at around ~15 °C during the addition. Slowly warmed the mixture to ambient temperature and continued stirring for 24 h under argon. TLC (eluent: 2% MeOH/EtOAc-basic $KMnO_4$ stain, *Note 1*) and mass analysis of the reaction mixture after 24 h showed the presence of large excess of the di-addiction (di-adduct) product (incomplete Michael addition).

To drive the reaction to completion an additional 83 mL of 5M NaOH was added to the reaction mixture followed by 1550 mL of *tert-b*utyl acrylate. The stirring was continued for another 24 h. The reaction mixture was transferred to a rotary evaporator and washed the flask with 2 L of EtOAc. Unreacted *tert*-butyl acrylate was removed and the residue was co-evaporated with toluene (2x2L). The residue

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obtained was dissolved in EtOAc (4 L) and washed with equal volume of water, the layers were not separating well, upon addition of 2 L of saturated brine to the mixture separated the organic and aqueous phase (*Note 2*). The washing process was repeated once again. The first aqueous layer was washed with 2 L of ethyl acetate and the combined organic layer was dried over sodium sulfate. Solvents were removed *in vacuo* and the residue (2.023 Kg, crude weight) was purified by filtration silica gel column followed by a second column chromatography as described below (*Note 3*).

Filtration column chromatography: A slurry of 3 Kg of silica gel in hexane was packed and the crude compound was loaded, eluted successively with hexane (4 L) 1:3 EtOAc/hexane (4 L) and 1:1 EtOAc/hexane (8 L) and 2.5% MeOH in EtOAc (12 L). The di-addition product (di-adduct) was completely removed. Pooled all fractions containing the desired compound **17A** (tri-adduct), evaporated (1530g-crude weight) and divided into two halves for second column chromatographic purification.

Final Purification of **17A**: The residue from the filtration column was divided into two halves for second purification.

Column conditions: ~765 g of the crude from the filtration column was dissolved in minimum amount of EtOAc/hexane and loaded on 3 Kg silica gel (3 Kg) packed in hexane. Eluted successively with hexane (4 L), 1:3 EtOAc/hexane (4 L), 3:7 EtOAc/hexane (4 L), 3:1 EtOAc/hexane (4 L); and 2.5% MeOH in EtOAc (12 L).

The column chromatography was repeated under same conditions to isolate compound **17A** from the second portion.

Pooled pure fractions were evaporated under reduced pressure to obtain the pure compound **17A** as viscous oil (1087 g, 56%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.54 (t, J = 6.0 Hz, 6H), 3.16 (s, 6H), 2.38 (t, J = 6.0 Hz, 6H), 1.39 (s, 27H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 171.06, 80.30, 73.13, 67.46, 56.42, 40.21, 40.21, 39.89 39.58, 36.50, 28.40.

Notes:

1. TLC: $KMnO_4$ stain seems to be better than Ninhydrin stain, all the impurities show-up nicely.
2. The separation of aqueous and organic phase during work-up takes longer time (due to the presence of DMSO). Addition of saturated brine helps fast separation of aqueous and organic layers.
3. Our facility is not equipped with and for larger chromatographic separation. Purification of compound **17A** is possible by single silica gel column chromatography if use sufficient qty of silica (8-10 kg) and larger column. We have purified **17A** by single silica gel column chromatography at research scale.

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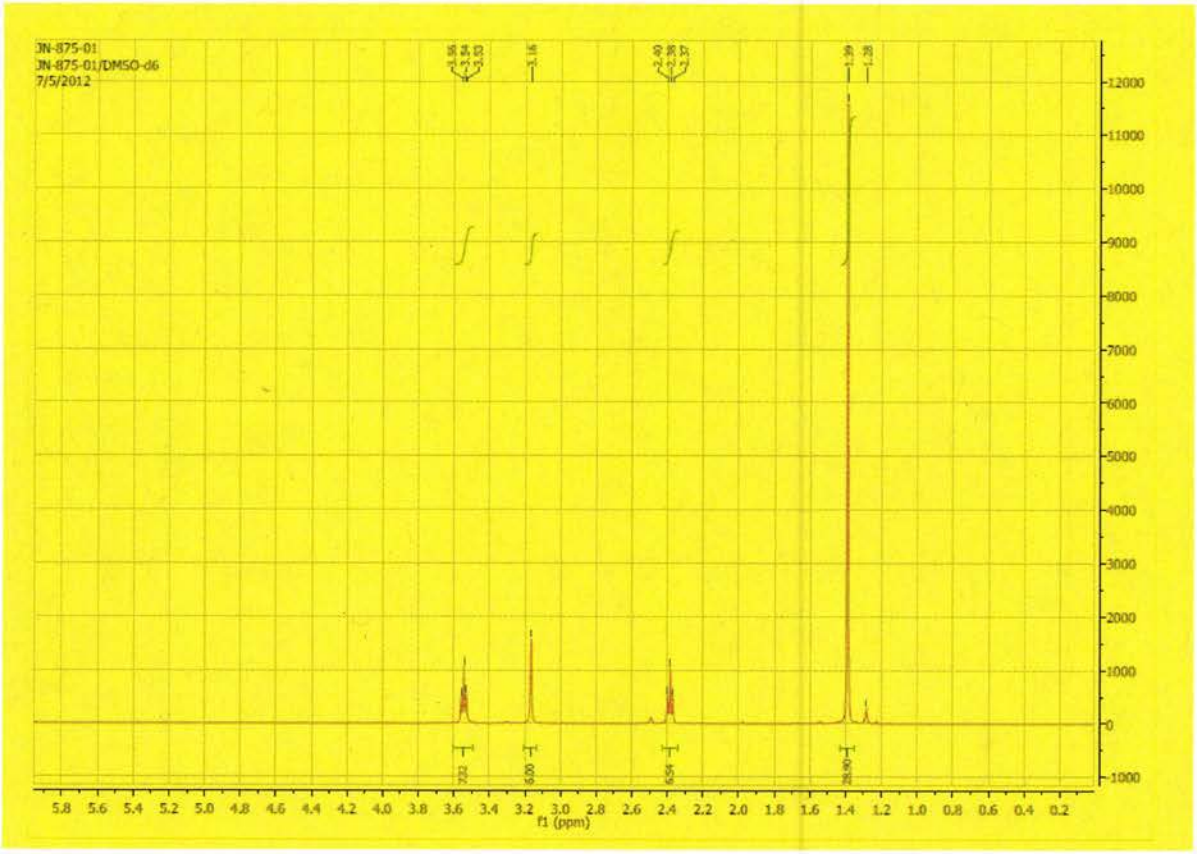
^{1}H NMR of Compound **17A** in DMSO-d_6



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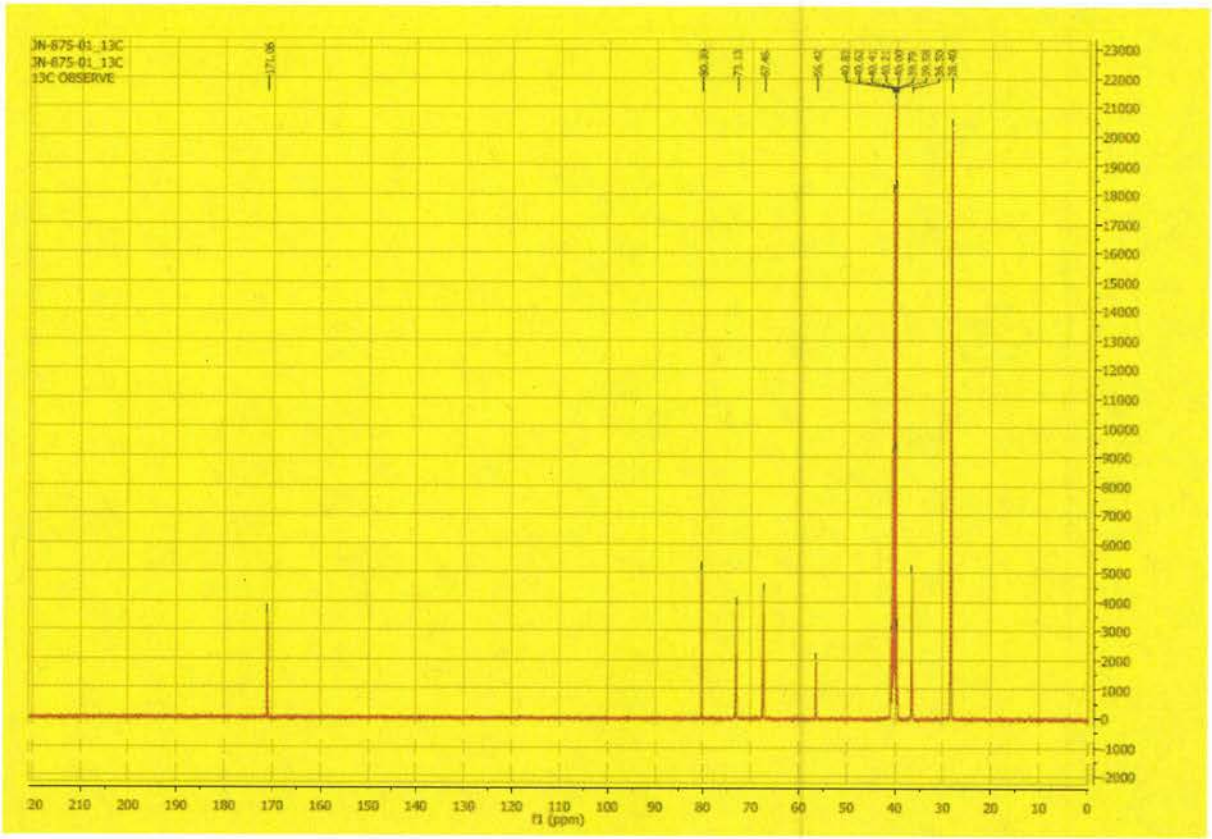
^{13}C NMR of Compound **17A** in DMSO-d_6



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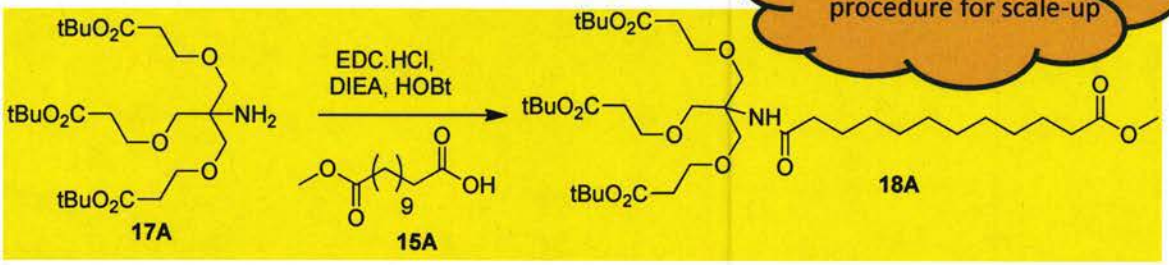
Synthesis of Tris(*tert*-Butyl) C_{12} Methyl Ester 18A

Scheme 4

Go to page 62 for alternate procedure for scale-up



	Reagents	MW	Qty Used	Moles	Mol. Eq.
1	Compound **17A**	505.33	1081.00g	2.139	1
2	Monomethyl-1,12-dodecanedioic acid (**15A**)	244.17	653g	2.673	1.25
3	EDAC.HCl	198.4	531 g	2.673	1.25
4	HOBt	135.1	433.54	3.209	1.50
5	DIEA (d 0.742)	129.1	1.12 L	6.42	3.00
6	Anhyd. DMF	-	1L	-	Solvent
7	Anhyd. DCM	-	6L	-	Solvent
8	Ethyl acetate		10 L		Solvent
9	Toluene		8 L		Solvent
10	DCM		4 L		Solvent

Procedure:

Compound **17A** (1081g, 2.139 mol), monomethyl ester **15A** (653g, 2.673 mol) and HOBt (433.5g, 3.209 mol) were dissolved in a mixture of DCM (6 L) and DMF (1L) in a 12 L four necked RB flask equipped with an overhead stirrer under argon. EDAC.HCl (531g, 2.673 mol) was added portion wise to the reaction mixture under constant stirring. Stirred the reaction mixture for 15 min (or until the reaction mixture become a homogeneous solution) and cooled over an ice-water bath to about ~10 °C. DIEA (1.12 L, 6.42 mol) was added over a period of 30 min while maintaining the temperature around ~10 °C. Slowly warmed the reaction mixture to ambient temperature and stirred under argon for two days. TLC (eluent: 35% EtOAc/Hexanes, basic $KMnO_4$ stain) checked and transferred the solutions to a 20 L rotary evaporator; removed solvents and volatiles under reduced pressure. After removing most of the dichloromethane the flask was transferred to a hood and fitted with an overhead stirrer. Water (15 L) was added with stirring and a gummy liquid separated out. The above mixture was kept for settling overnight at ambient temperature. The top layer was decanted and bottom viscous layer was dissolved in 8 L of EtOAc and washed successively with water (2 x 4 L), 10% aqueous citric acid (2 x 3.5 L),

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aq.$NaHCO_3$ solution (2 x 2.5 L), followed by saturated brine (2.5 L) wash. The organic layer was separated and dried over sodium sulfate. Solvent was removed under reduced pressure and the residue was co-evaporated with anhydrous toluene (2 L). The residue was dried under high vacuum overnight to obtain compound **18A** as a colorless *viscous* oil (1641g, 77 g more than quantitative yield- small amount of HOBt and toluene were present) which was used for the next reaction without further purification. ^1H NMR (400 MHz, DMSO-d_6): δ 6.85 (s, 1H), 3.53 (dd, J = 12.9, 6.9 Hz, 16H), 3.30 (s, 1H), 2.49 (dd, J = 3.5, 1.7 Hz, 2H), 2.37 (t, J = 6.1 Hz, 7H), 2.26 (t, J = 7.4 Hz, 2H), 2.02 (t, J = 7.3 Hz, 2H), 1.56 – 1.44 (m, 3H), 1.44 – 1.34 (m, 32H), 1.21 (s, 13H). MS calc for $C_{38}H_{69}NO_{12}$: 731.48; found 732.3 (M+H).

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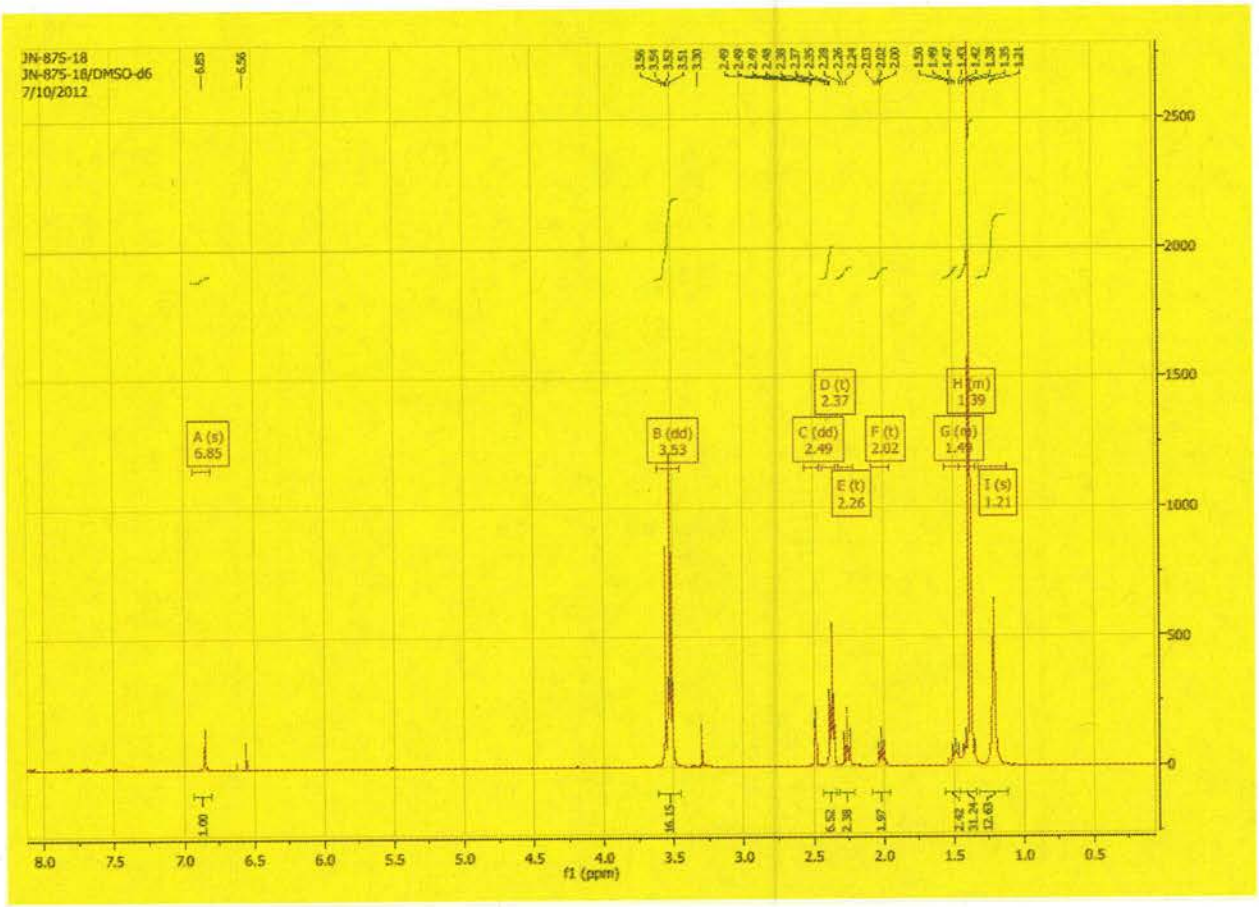
^{1}H NMR of Compound **18A** in DMSO-d_6



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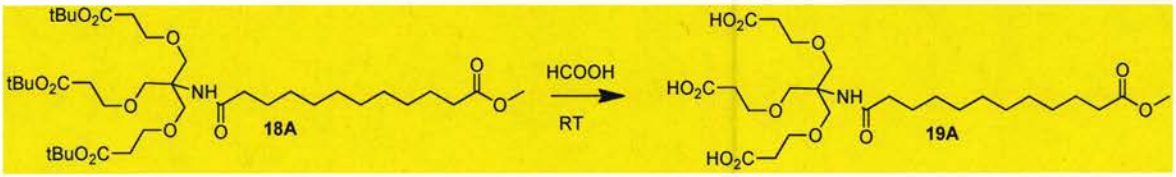
Synthesis of Tricarboxylic Acid C_{12} Methyl Ester 19A:

Scheme 5



	Reagents	MW	Qty Used	Moles	Mol Eq.
1	Compound **18A**	731.48	1560 g	2.134	1
2	Formic acid 98%	-	10 Kg (8.2 L) 5 Kg (4.1 L)	-	Solvent
3	Anhyd. Ether		4 L		Solvent
4	Ethyl acetate		6 L		Solvent
5	Toluene		9 L		Solvent
6	Hexane		6 L		Solvent

Procedure:

Compound **18A** (1560g, 2.134 mol) was charged to a 22 L four necked RB flask equipped with an overhead stirrer under argon. Formic acid (10 Kg-8.2 L) was added to the reaction flask and all reagents went in to solution within 20 min and stirred for 24 h under argon. TLC (eluent: 35% EtOAc/Hexanes-basic $KMnO_4$ stain) showed incomplete reaction and presence of unreacted **18A** (*Note 1*). An additional 4.1 L of formic acid was added and continued the stirring for another 24 hrs. TLC and mass spectra showed completion of reaction and the mixture was transferred to a 20 L rotary evaporator and volatiles were removed under reduced pressure. After the removal of formic acid the residue was co-evaporated with toluene (2x 4.5 L). Mixture of EtOAc and hexane (1:1, 8 L) was charged into the rotating flask on the rotary evaporator under slow rotation, white solid precipitated from the solution and the mixture was slowly rotated for another 2 h on the rotary evaporator (until free flowing solid separated out, *Note 2*). The solid was filtered and washed with EtOAc/hexane (1:1, 4 L) followed by anhydrous ether (4 L). The solid was transferred to a tray and air dried at 45 °C until it reaches constant weight to get the compound **19A** as a white powder (yield 1096g, 91%, *Note 3*).

*Recrystallization of **19A** from ethyl acetate afford product devoid of traces of partially deprotected tert-butyl ester. **19A** (5.0 g) was dissolved in refluxed ethyl acetate (25 mL) and the hot clear solution was decanted from the flask that contains residual solids. The flask was rinsed with small*

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amount of ethyl acetate, and the combined decanted solution was allowed to cool down to rt overnight. The precipitate was filtered and air-dried to afford 4.75 g (95%) of ***19A***.

^{1}H NMR (400 MHz, DMSO-d_6): δ 12.08 (bs, 3H), 6.89 (s, 1H), 3.65 – 3.43 (m, 7H), 2.41 (dt, J = 12.7, 6.3 Hz, 3H), 2.26 (t, J = 7.4 Hz, 1H), 2.02 (t, J = 7.3 Hz, 1H), 1.44 (ddd, J = 19.5, 13.3, 6.7 Hz, 2H), 1.21 (s, 5H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 173.41, 172.70, 172.56, 68.21, 66.75, 59.57, 51.18, 40.13, 39.92, 39.71, 39.51, 39.30, 39.09, 38.88, 35.94, 34.67, 33.33, 28.99, 28.94, 28.89, 28.75, 28.57, 28.55, 25.35, 24.51. MS calc for $C_{26}H_{45}NO_{12}$: 563.29; found 564.3 (M+H).

Notes:

1. Monitoring of the reaction by TLC is recommended and not by MS as MS analysis may be misleading due to the abundance of the tricarboxylic acid which ionizes better than partially deprotected or unreacted starting material..
2. Slow rotation in the rotary evaporator for 2 h will help to obtain free flowing white solid during the work-up with 50% EtOAc/Hexanes
3. TLC -eluent: 10% MeOH/DCM and basic $KMnO_4$ stain was used for the final product

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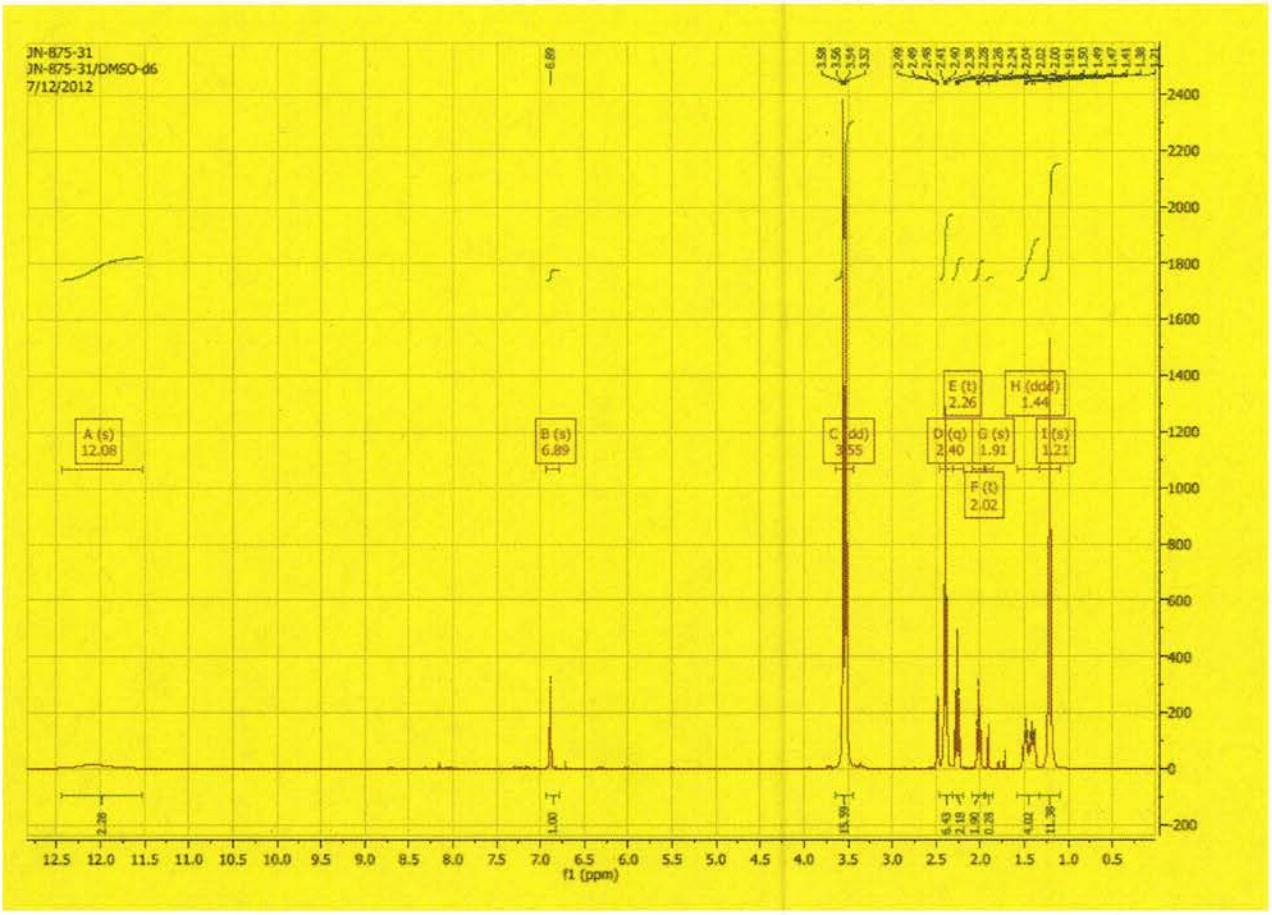
^{1}H NMR of Compound **19A** in DMSO-d_6



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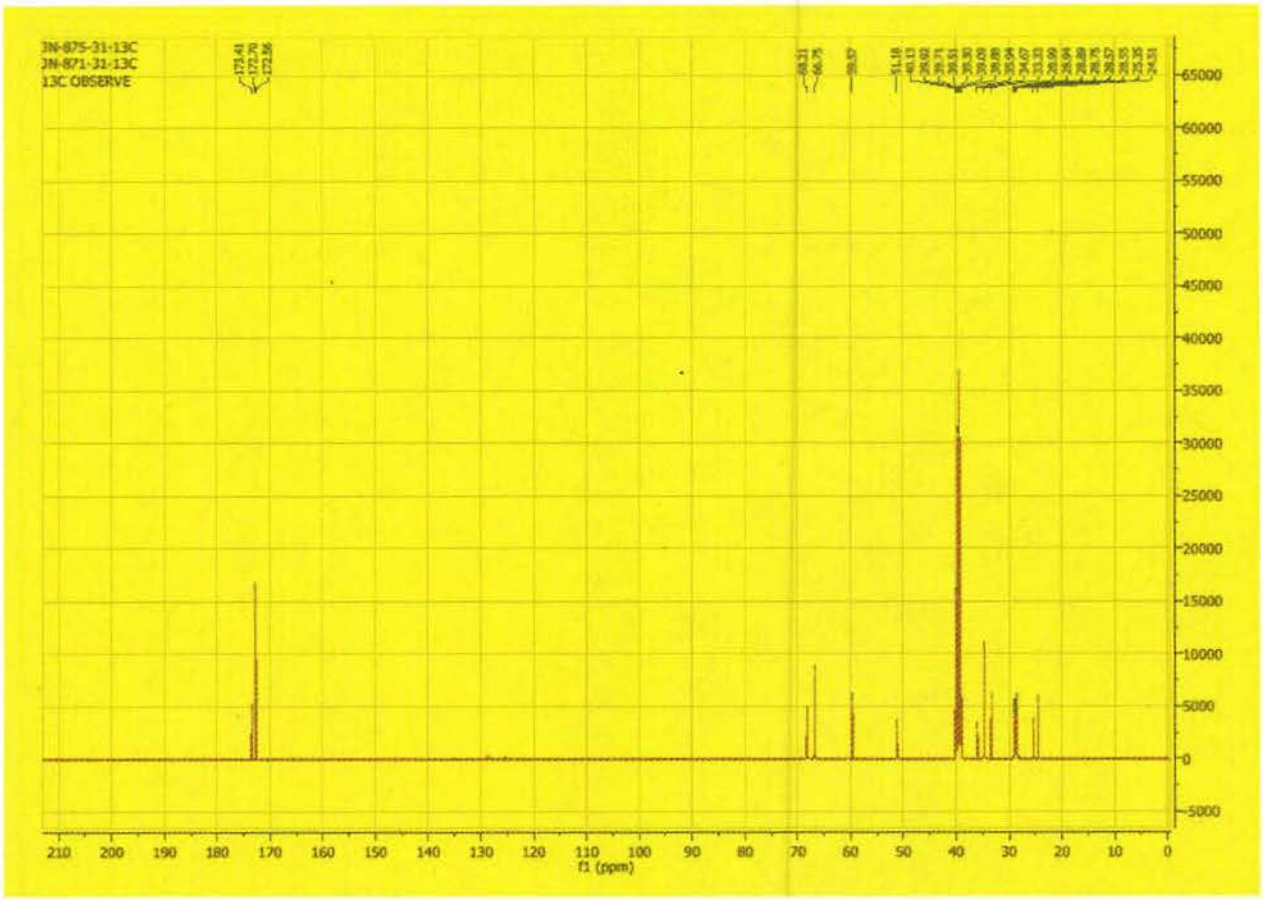
^{13}C NMR of Compound 19A in DMSO-d_6



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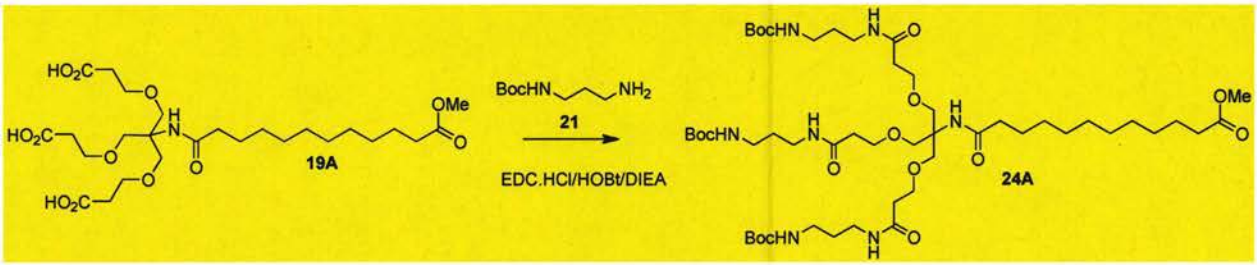
Synthesis of Tris(^{Boc}Amine) Methyl Ester 24A:

Scheme 6



	Reagents	MW	Qty Used	Moles	Mol Eq.
1	Compound **19A**	563.29	1090.00g	1.936	1.00
2	Mono Boc propanediamine **21**	174.24	1265.00g	7.260	3.75
3	EDAC.HCl	198.4	1440 g	7.260	3.75
4	HOBt	135.1	1170 g	8.667	4.50
5	DIEA (d 0.742)	129.1	2.40 L	13.55	7.00
6	Anhydrous DMF	-	2L	-	Solvent
7	Anhydrous DCM	-	9L	-	Solvent
8	Ethyl acetate	8 L			Solvent
9	Toluene	2.5 L			Solvent
10	DCM	6 L			Solvent

Procedure:

Tricarboxylic acid **19A** (1090g, 1.936 mol) and HOBt (1170g, 8.667 mol) were taken in a 22 L four necked flask equipped with an overhead stirrer under argon. 4 L of DCM and 2 L of DMF were added to the mixture with constant stirring. The reaction mixture was cooled over an ice-water bath to ~10 °C. A solution of mono Boc propanediamine **21**(1265g, 7.260 mol) in 2 L of DCM was added to the solution under stirring. Reaction became homogenous in 15 min. Slurry of EDAC.HCl (1440g, 7.260 mol) in 4 L of DCM was slowly added to the reaction mixture followed by slow addition of DIEA (2.40 L, 13.55 mol) over a period of 1.5 h to the flask under argon while maintaining the temperature ~10 °C (*Note 1*). The color of the solution turned to light brownish yellow; the mixture was slowly warmed to ambient temperature and stirred overnight under argon atm. TLC (eluent: 10% MeOH/DCM, basic $KMnO_4$ stain, *Note 2*) checked and the reaction mixture was transferred to a 20 L rotary evaporator; removed DCM and volatiles under reduced pressure (*Note 3*). The flask was transferred to a hood and fitted with an overhead stirrer. 10 L of water was added to the residue with stirring to form a milky solution. The mixture was kept overnight for settling down. Two layers were separated by overnight and decanted the top layer using a transfer pump. The bottom layer was dissolved in 8 L of EtOAc and transferred to a

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separatory funnel. The EtOAc layer was washed successively with water (2 x 4 L water + 2 L brine), aq. $NaHCO_3$ solution (2.5 L + 1 L brine), 10 % aq. citric acid solution (2 x 3.5 L + 1 L brine), water (1x 4 L + 2 L brine) and saturated brine (2.5 L). The organic layer was dried over sodium sulfate and solvent was removed under reduced pressure. The residue was co-evaporated successively with toluene (2.50 L) and dichloromethane (6 L). The residue was dried under high vacuum overnight to obtain the compound **24A** as a pale yellow gummy liquid (2026 g, 30 g more than quantitative yield, contaminated with small amount of HOBt and water, *Notes 3 & 4*). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.79 (t, J = 5.6 Hz, 1H), 6.94 (s, 1H), 6.72 (t, J = 5.3 Hz, 1H), 3.61 – 3.45 (m, 5H), 3.01 (dd, J = 12.8, 6.6 Hz, 2H), 2.89 (dd, J = 12.7, 6.5 Hz, 2H), 2.26 (t, J = 6.3 Hz, 3H), 2.03 (t, J = 7.3 Hz, 1H), 1.55 – 1.38 (m, 3H), 1.36 (d, J = 10.7 Hz, 8H), 1.21 (s, 3H). MS calc for $C_{50}H_{93}N_7O_{15}$: 1031.67; found 1032.6 (M+H).

Notes

1. Addition of DIEA to EDAC.HCl generate heat, so to maintain reaction temperature at ~10 °C ice bath was used during amine addition to the reaction mixture
2. TLC analysis: Small amount of reaction mixture was withdrawn after stirring overnight and the dichloromethane was removed by passing nitrogen through the solution. Added water to precipitate compound and decanted top layer; dissolved residue in EtOAc for TLC analysis.
3. It takes long time to remove DCM under reduced pressure from a mixture of DMF/DCM (~6 h to remove most of the DCM from the current batch at low pressure).
4. At research scale the final compound was obtained as a nice fluffy white solid under high vacuum.
5. Frothing is an issue while drying the product under vacuum on the rotary evaporator.

^{1}H NMR of **24A** in DMSO-d_6

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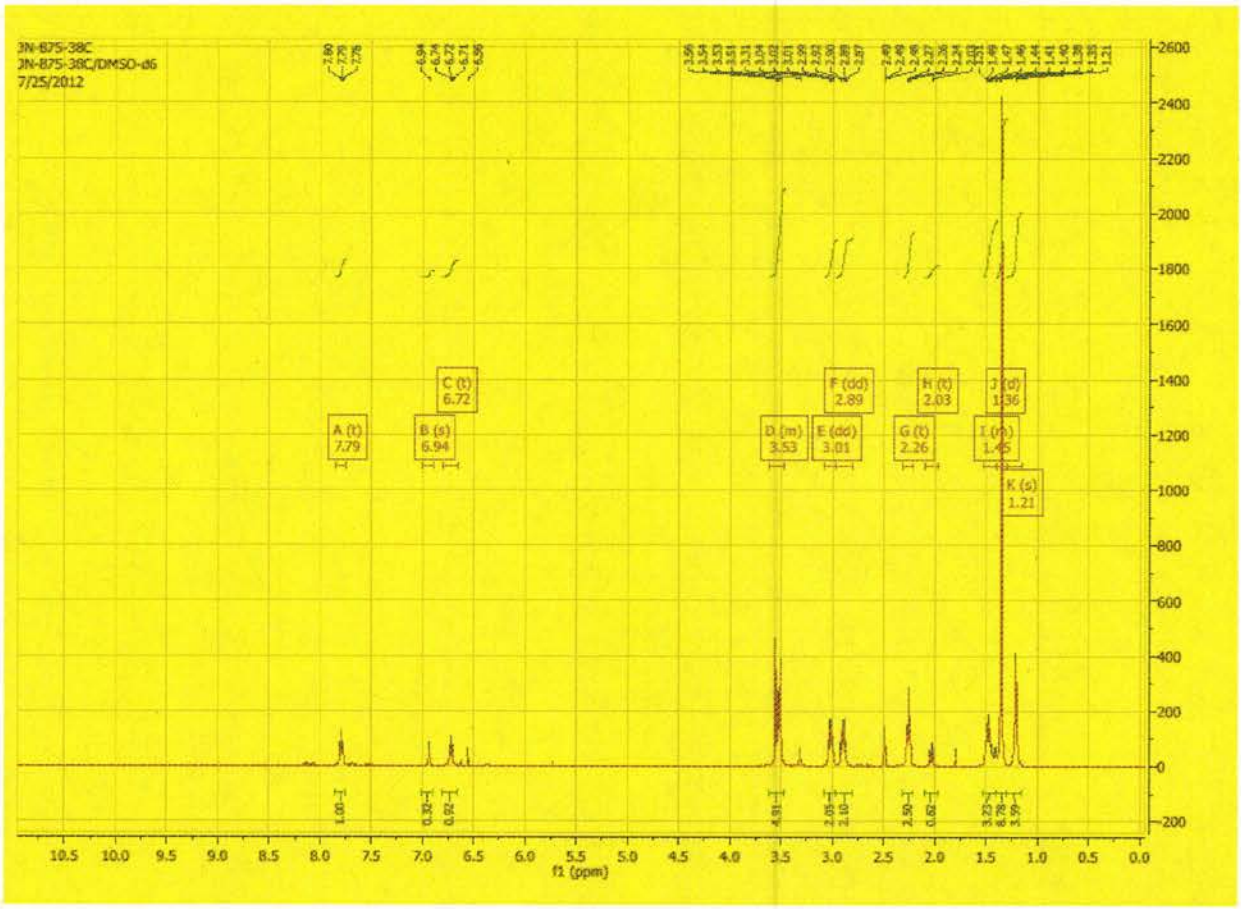

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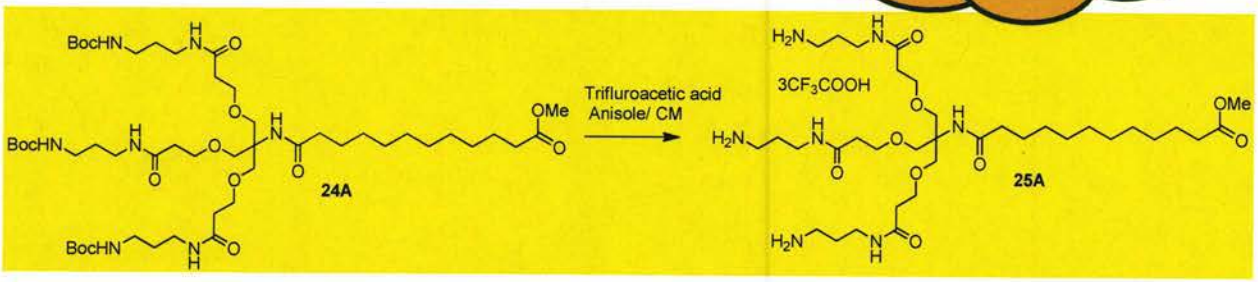
Synthesis of Triamine trifluoroacetic acid salt 25A

Scheme 7

Go to page 64 for alternate procedure for scale-up



	Reagents	MW	Qty Used	Moles	Mol Eq.
1	Tris(^{Boc}Amine) Methyl Ester **24A**	1031.67	750 g	0.727	1
2	Anisole	-	250 mL	-	-
3	Trifluoroacetic acid	-	3.5 L	-	-
4	Anhyd. DCM	-	2.5 L	-	Solvent
5	Anhyd. Toluene		4 L		Solvent
6	Methanol		1 L		Solvent
7	Anhyd. Ether		8 L		Solvent

Procedure:

Compound **24A** (1990g, gummy liquid) from the previous step was dissolved in 4 L DCM in the rotary evaporator flask. Weight of the mixture was determined and transferred the required amount of solution to another 20 L rotary evaporator flask for this reaction (*Note 1*). DCM was removed under reduced pressure and the residue was dried under high vacuum overnight. After overnight drying the residue **24A** (750g, 727 mmol) was dissolved in anhydrous DCM (2 L) and transferred to a 12 L four necked flask equipped with an overhead stirred under argon. The rotary evaporator flask was washed with 500 mL DCM and transferred that solution also to the reaction flask. Anisole (250 mL) was added to the reaction mixture followed by trifluoroacetic acid (3.5 L) with stirring. During addition of trifluoroacetic acid strong effervescence occurred due to the liberation of butylene gas, a byproduct from the reaction (*Note 2*). The reaction mixture was stirred at ambient temperature overnight. Completion of the reaction was monitored by MS analysis. The mixture was transferred to a 20 L rotary evaporator flask and volatiles were under reduced pressure. The residue was co-evaporated with anhydrous toluene (2 x 2 L) to get a pale brown gummy liquid. The flask was transferred to a hood and connected to an overhead stirrer. The residue was dissolved in anhydrous MeOH (1 L); anhydrous ether (8L) was added to the solution under constant stirring. During the addition of ether the solution turned

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milky and a gummy mass was separated out in the bottom of the flask. The flask was kept in the cold room (~4 °C) overnight to settle the entire product on the wall and bottom of the flask. The top layer was decanted and the residue containing flask was transferred to the rotary evaporator; evaporated residual solvent under reduced pressure and dried the residue under high vacuum overnight to get the compound **25A** as white fluffy solid, which upon removal of vacuum turned to a gummy colorless liquid (789g, 9 g more than quantitative yield, *Note 3*).

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.06 (t, *J* = 5.6 Hz, 1H), 7.85 (s, 3H), 6.99 (s, 1H), 3.66 – 3.42 (m, 4H), 3.21 – 3.00 (m, 2H), 2.89 – 2.68 (m, 2H), 2.28 (dt, *J* = 12.4, 6.9 Hz, 2H), 2.04 (t, *J* = 7.3 Hz, 1H), 1.76 – 1.59 (m, 2H), 1.56 – 1.35 (m, 1H), 1.21 (s, 3H). MS calc for $C_{35}H_{69}N_7O_9$: 731.52; found 732.50 (M+H).

Notes

1. Tris(Boc) derivative **24A** is a gummy liquid and it is very difficult to transfer with a spatula. It is recommended to dissolve this compound in known amount of solvent and transfer required amount to the reaction flask (rotary evaporator flask is preferred for the next reaction as it enable removal of solvent prior to the addition of trifluoroacetic acid).
2. (a) Make sure that the reaction flask out let is clear to vent high volume of butylene gas generated during the reaction. (b) The argon outlet was passed through a cold solution of 2 M aq. NaOH solution to quench any TFA coming out with the argon flow for this reaction.
3. At research scale the final compound was isolated as a nice fluffy white solid under high vacuum

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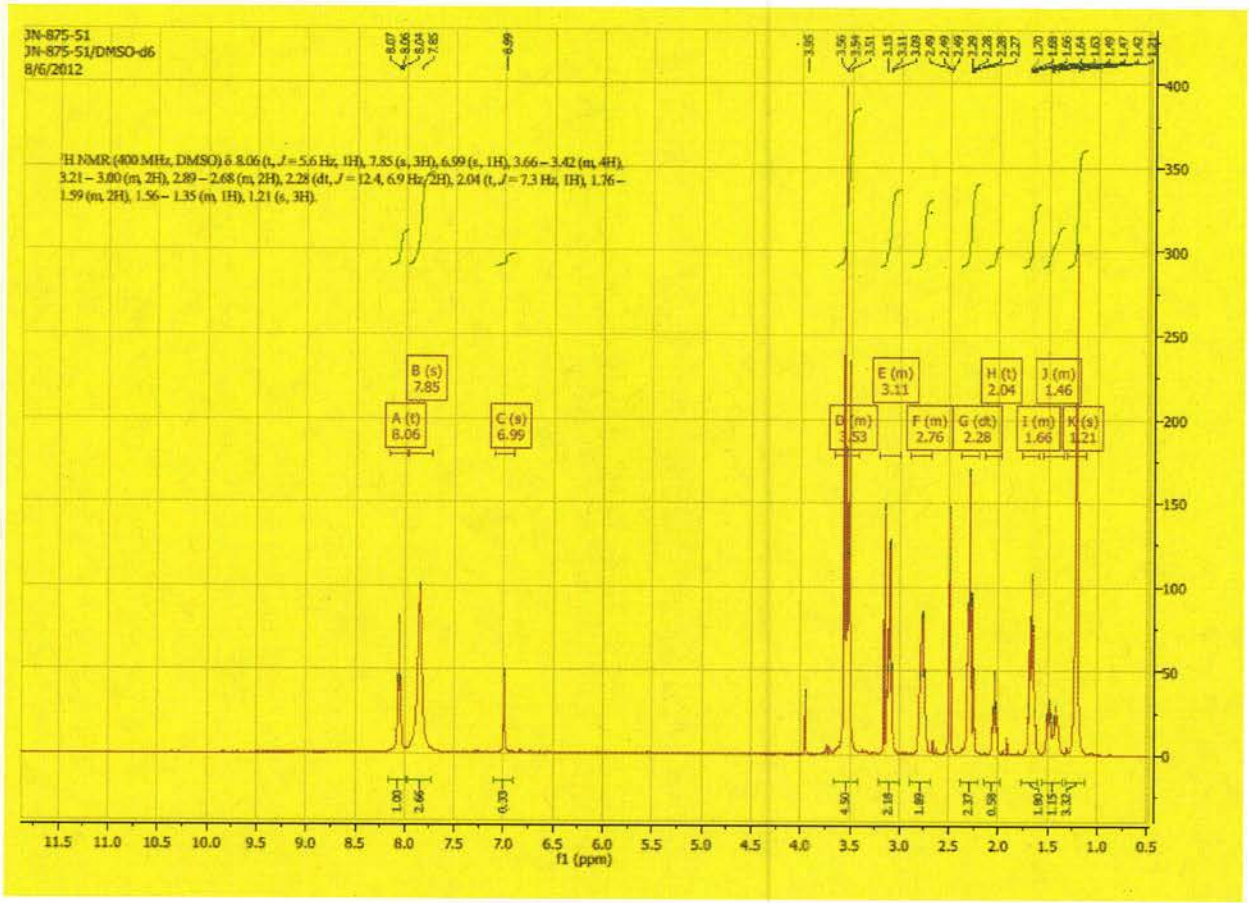
^{1}H NMR of **25A** in DMSO-d_6



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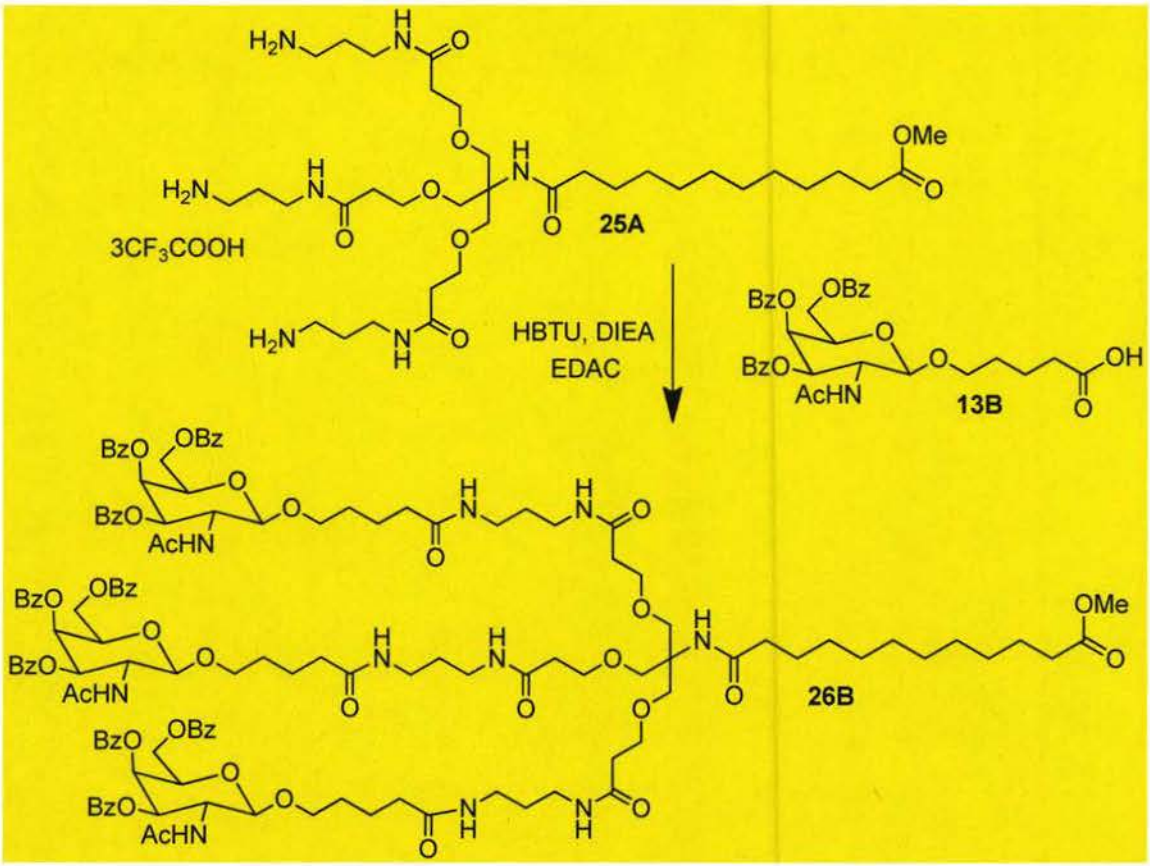
Synthesis of Tris(^{Bz}GalNAc)-Methyl Ester 26B

Scheme 8



	Reagents	MW	Qty Used	Moles	Mol Eq.t
1	Triamine trifluoroacetic acid salt **25A**	1031.67	776 g	0.722	1.00
2	^{Bz}GalNAc C_5 Acid_ sodium salt **13B**	633.92	1570g	2.476	3.42
3	EDAC.HCl	198.24	576 g	2.904	4.00
4	HOBt	135.1	490 g	3.630	5.00
5	DIEA	129.1	1260 mL	7.260	10.00
6	Anhyd. DCM	-	6 L	-	Solvent
7	Anhyd. DMF	-	7 L	-	Solvent
8	20% Phosphoric acid solution		1 L		Solvent
9	Ethyl acetate		~90 L		Solvent
10	Methanol		~15 L		Solvent

Procedure:

Triamine trifluoroacetic acid salt **25A** (776g, 0.7228 moles) was dissolved in 2 L of anhydrous DMF in a 20 L rotary evaporator flask (*Note 1*). Compound **13B** (1570g, 2.476 moles) and HOBt (490g, 3.630 moles) were charged to a 22 L four necked flask equipped with an overhead stirrer under argon. To the

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above mixture 4L of DCM and 2L of DMF were added and stirred until most of the solid went into solution (30 min, slight turbidity persists, *Note 2*). The flask was cooled over an ice-water mixture to about 10 °C. Slurry of EDAC.HCl (576g, 2.904 moles) in 2 L DMF was added followed by DIEA (1260 mL) using an addition funnel over a period of 15-20 minutes while maintaining the temperature ~10 °C. To the above mixture the solution of **25A** in DMF was added slowly and rinsed the flask with 1L of DMF and transferred this solution also to the reaction mixture. The reaction mixture was slowly warmed to ambient temperature and continued stirring overnight. The color of the solution changed from pale yellow to pale brown overnight. TLC checked and the reaction mixture was transferred to a 20 L rotary evaporator to remove most of the volatiles (*Notes 3 & 4*). The reaction mixture was divided into approximately two halves and added 12 L of ice- cold water with vigorous stirring to each of those portions. Solid was precipitated out during the addition of water and the solution was kept in the cold room overnight. Two layers separated with pale yellow solution on the top and light brown precipitate at the bottom. The top layer was decanted using a transfer pump and the residue was dissolved in EtOAc (9 L). This solution was washed successively with a mixture of 5% NaCl (5 L) and 20% H_3PO_4 (1 L), 5% NaCl (2 x 6 L), and sat. NaCl (3.5 L). The organic layer was separated, diluted with EtOAc to 20 L, and dried over anhyd. sodium sulfate. This solution was directly loaded on a filtration column of 7.5 kg of silica gel (EMD, grade 62, 60-200 mesh) wet preloaded with EtOAc in a 20 L funnel. The column was eluted with 40 L of EtOAc (1-st 12 L were pure solvent and reused), followed by 20 L of 20:1 EtOAc-methanol and 60 L of 3:1 EtOAc-methanol. EtOAc-methanol (3:1) fractions containing the product were collected, evaporated and the residue was dried at 2 mbar/35 °C overnight to afford 1.51 kg (82%) of **26B** as off-white foam.

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.00 (d, *J* = 9.3 Hz, 3H), 7.96 – 7.80 (m, 15H), 7.80 – 7.43 (m, 30H), 7.37 (t, *J* = 7.8 Hz, 6H), 6.98 (s, 1H), 5.76 (d, *J* = 3.2 Hz, 3H), 5.37 (dd, *J* = 11.1, 3.2 Hz, 3H), 4.74 (d, *J* = 8.5 Hz, 3H), 4.55 – 4.40 (m, 6H), 4.40 – 4.20 (m, 6H), 4.01 (q, *J* = 7.1 Hz, 3H), 3.89 – 3.73 (m, 3H), 3.66 – 3.42 (m, 18H), 3.34 (s, 2H), 3.03 (s, 12H), 2.35 – 2.17 (m, 8H), 2.13 – 2.00 (m, 8H), 1.70 (s, 9H), 1.61 – 1.33 (m, 21H), 1.16 (dd, *J* = 12.4, 5.2 Hz, 15H).

Notes

1. Triamine trifluoroacetic acid salt **25A** took ~2 h at 35 °C to dissolve completely.
2. Even after adding DMF slight turbidity persists but the reaction was homogenous and didn't create any problems.

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3. TLC analysis: Small amount of reaction mixture was withdrawn after stirring overnight and the dichloromethane was removed by passing nitrogen through the solution. Added water to precipitate compound and decanted top layer; dissolved residue in DCM for TLC analysis.
4. It takes long time to remove DCM under reduced pressure from a mixture of DMF/DCM (~6 h to remove most of the DCM from the current batch at low pressure). Neat DMF may be an alternate solvent for this reaction and it will ease the process.

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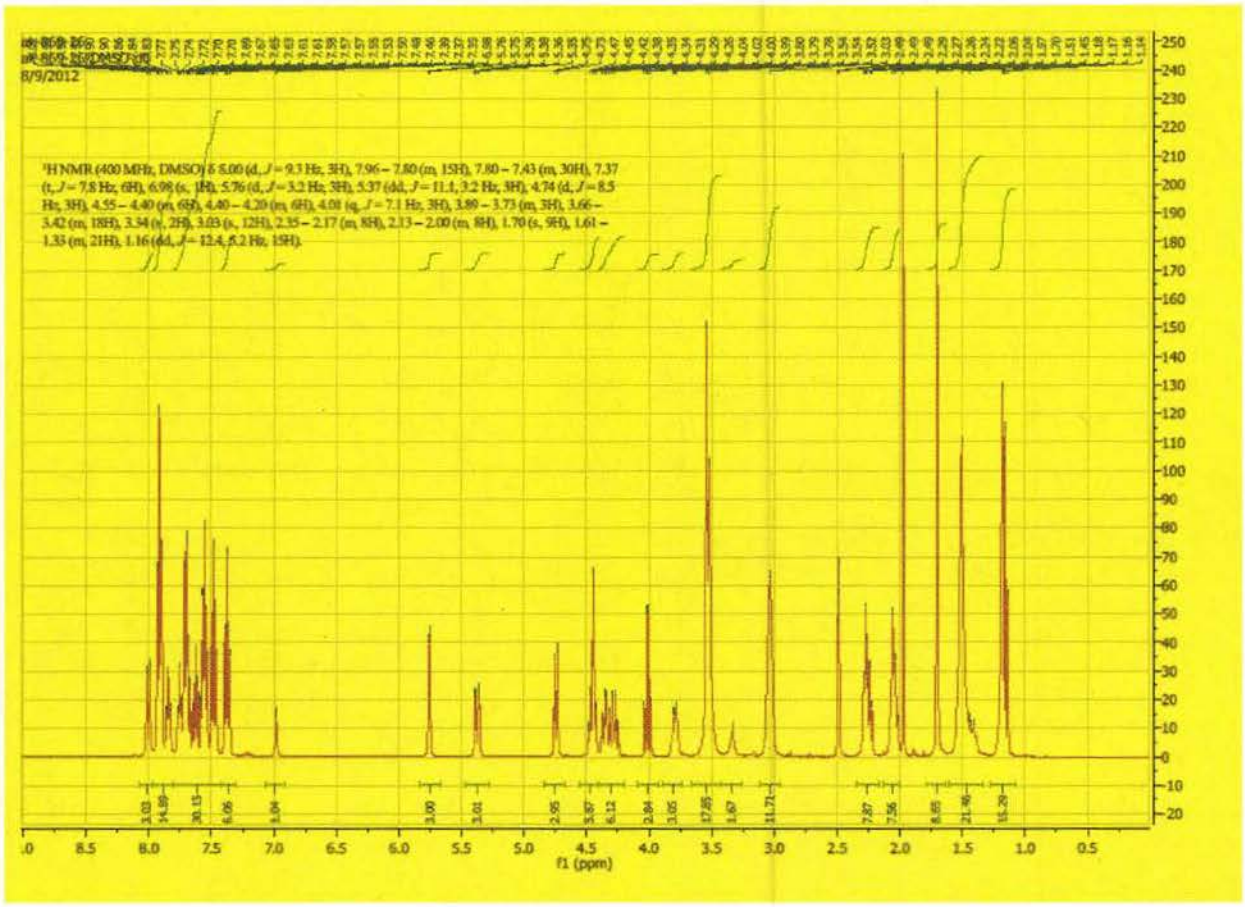
^{1}H NMR of **26B** in DMSO-d_6



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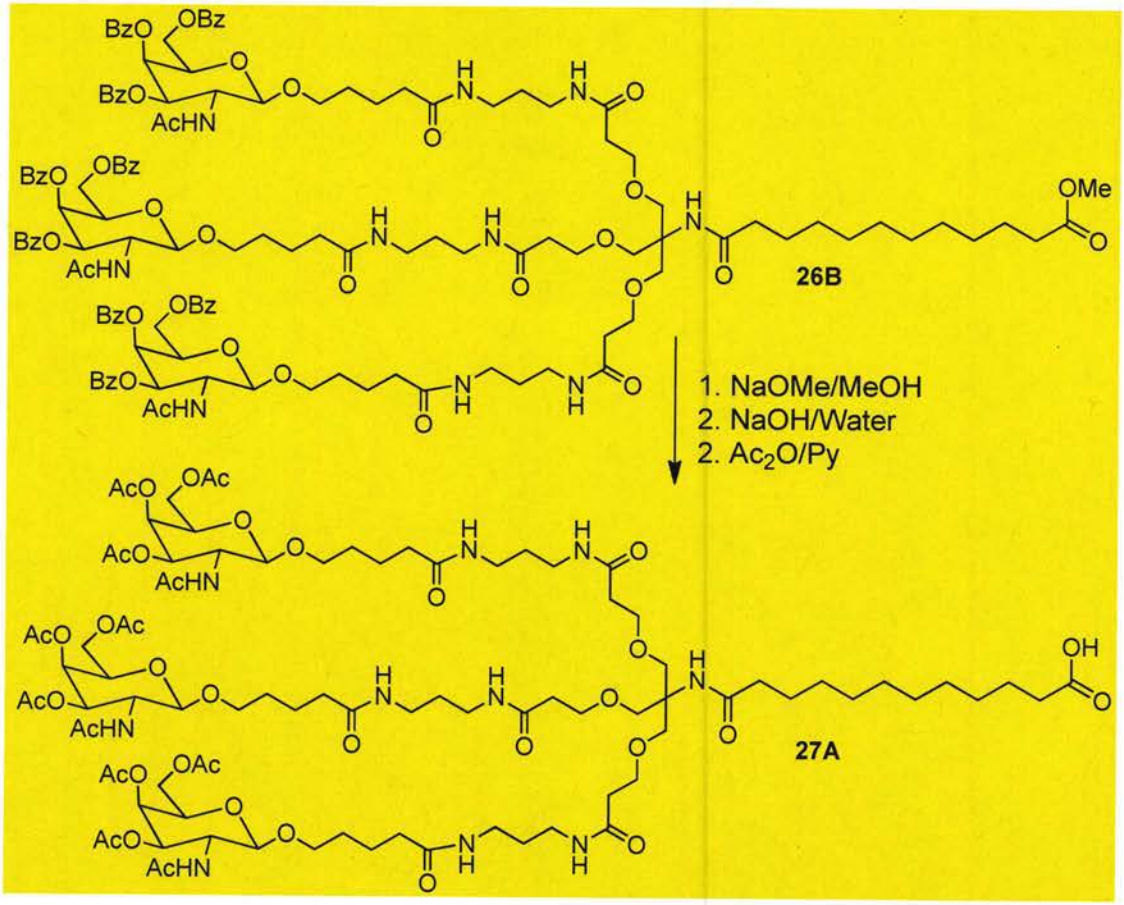
Synthesis of Tris(^{Ac}GalNAc)-Acid 27A*

Scheme 9



	Reagents	MW	Qty Used	Moles	Mol Eq.
1	Tris(^{Bz}GalNAc)-Methyl ester **26B**	2578.84	1.51 Kg	0.585	1
2	25% Sodium methoxide in methanol	-	135 mL	0.585	1
3	Triethylamine hydrochloride	137.65	337.30 g	2.45	4.18
4	Sodium hydroxide	40.00	70.00 g	1.75	3.00
5	Acetic anhydride	102.09	2.80 L	-	-
6	Anhydrous methanol	-	6.5 L	-	Solvent
7	Pyridine	-	15.5 L	-	Solvent
8	Anhyd. Pyridine	-	12 L	-	Solvent
9	Ethyl acetate		10 L		Solvent
10	Isopropanol		1.5 L		Solvent
11	Triethylamine		1 L		-

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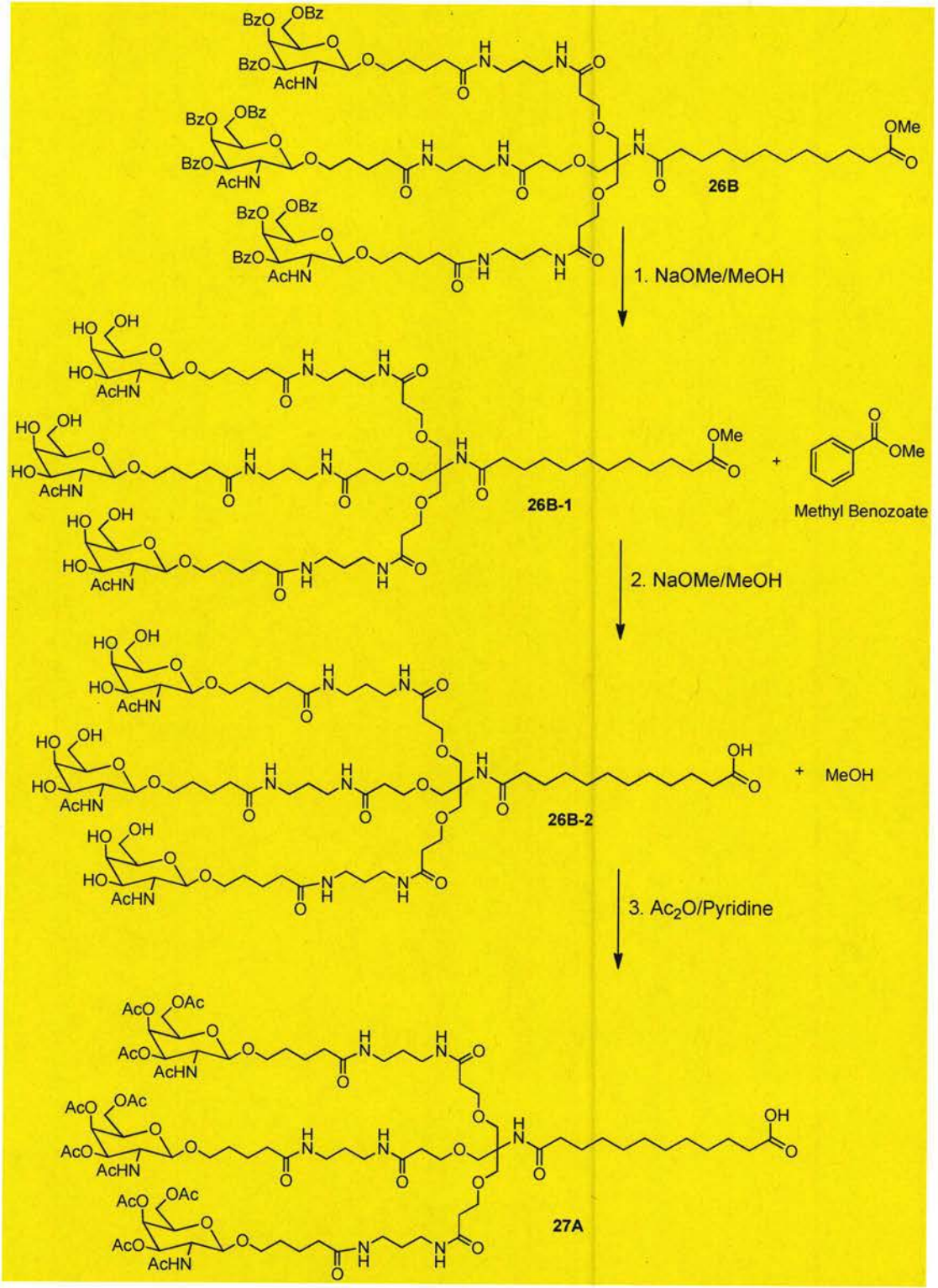
Scheme 9 (Expanded)



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27A Step 1 (*Note 1*).

To a solution of Tri-GalNAc(Bz)-Methyl ester **26B** (1.51 kg, 0.585 mol) in anhyd. methanol (6.5 L) was added 25 wt% solution of NaOMe in methanol (135 mL, 0.585 mol) under Ar atm. The mixture was stirred at ambient temperature for 2 h, neutralized with triethylamine hydrochloride (96.3 g, 0.70 mol), evaporated under reduced pressure, and the residue was partitioned between 1% aq. NaCl (7.0 L) and EtOAc (3.0 L) in a separatory funnel to extract out methyl benzoate (side product) into the organic layer (de-benzoylated product **26B-1** is highly soluble in water). The EtOAc layer was separated and the aqueous layer was washed with additional 3.0 L of ethyl acetate. Pyridine (2.0 L) was added to the aqueous extract , and the mixture was evaporated on the rotary evaporator at 40 mbar/35 °C until all traces of ethyl acetate have been removed (~2 h, *Note 2*).

27A Step 2 (*Note 3*).

The rotary evaporator flask containing water extract from the previous step was equipped with an overhead stirrer, NaOH (70.0 g, 1.75 mol) was added and the mixture was stirred overnight at ambient temperature, neutralized with triethylamine hydrochloride (241 g, 1.75 mol), diluted with 6.0 L of pyridine, and the solvents were evaporated at 13 mbar/35 °C on a 20 L rotary evaporator till viscous oily residue. The residue was re-dissolved in pyridine (6.0 L), precipitated sodium chloride (*Note 4*) was filtered off, washed with pyridine (3x500 mL), and the combined filtrates were evaporated at 20 mbar/45 °C till viscous oily residue.

27A Step 3

The residue from Step 2 was re-dissolved in anhyd. pyridine (6.0 L) under Ar atm and the solution was slowly added to a vigorously stirred mixture of anhyd. pyridine (5.6 L) and acetic anhydride (2.8 L) for about 1.5h . The mixture was stirred at ambient temperature overnight, cooled in an ice-water bath to ~4 °C and 1.5 L of ice cold water was added. Exothermic effect up to 40 °C was observed; the mixture was allowed to cool to ambient temperature (~0.5 h) and transferred to a rotary evaporator to remove the volatiles. The oily residue was dried under vacuum at 2 mbar/45 °C till constant weight (~1.82 Kg). The residue was dissolved in a stirred mixture of ethyl acetate (5.0 L) and isopropanol (1.0 L), and 25% aq. sodium chloride (6.0 L) was added (*Note 5*). The pH of the aq. phase was adjusted to 7 by slow addition of triethylamine (1.025 L). Neutralization was accompanied with precipitation of solid sodium chloride and separation of an intermediate layer, which was taken back into the organic phase by the addition of 0.5 L of isopropanol. The liquids were decanted from solid NaCl (*Note 6*) to a separatory

funnel using a transfer pump. Organic layer was separated, washed with 25% NaCl (6.0 L x 2), sat. NaCl (3.0 L), dried over anhyd. sodium sulfate, the solvents were evaporated, the foamy residue was co-evaporated with anhydrous acetonitrile (6.0 L x 2), and dried at 2 mbar/40 °C for 24 h to afford 1.11 kg (95%) of **27A**. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.00 (d, J = 9.3 Hz, 3H), 7.96 – 7.80 (m, 15H), 7.80 – 7.43 (m, 30H), 7.37 (t, J = 7.8 Hz, 6H), 6.98 (s, 1H), 5.76 (d, J = 3.2 Hz, 3H), 5.37 (dd, J = 11.1, 3.2 Hz, 3H), 4.74 (d, J = 8.5 Hz, 3H), 4.55 – 4.40 (m, 6H), 4.40 – 4.20 (m, 6H), 4.01 (q, J = 7.1 Hz, 3H), 3.89 – 3.73 (m, 3H), 3.66 – 3.42 (m, 18H), 3.34 (s, 2H), 3.03 (s, 12H), 2.35 – 2.17 (m, 8H), 2.13 – 2.00 (m, 8H), 1.70 (s, 9H), 1.61 – 1.33 (m, 21H), 1.16 (dd, J = 12.4, 5.2 Hz, 15H).

Notes

1. Step 1 was used for the deprotection of the benzoyl groups from **26B** by converting it to methyl benzoate under anhydrous condition. This will reduce (or eliminate) benzoic acid contamination from the product and makes work-up easier.
2. Addition of pyridine avoid foaming during rotary evaporation. .
3. The aq. NaOH treatment convert methyl ester **26B-1** to the corresponding carboxylic acid **26B-2**, and also deprotect if any benzoyl residue remained on the sugar moiety after Step 1.
4. NaCl partially precipitated due to saturation of the brine layer with triethylammonium acetate.
5. Addition of some 25% brine facilitates dissolution of the dry mass after rotary evaporation.
6. Residual NaCl from Step 2

** All these operations (from Steps 1-3) were performed in a 20 L rotary evaporator flask.*

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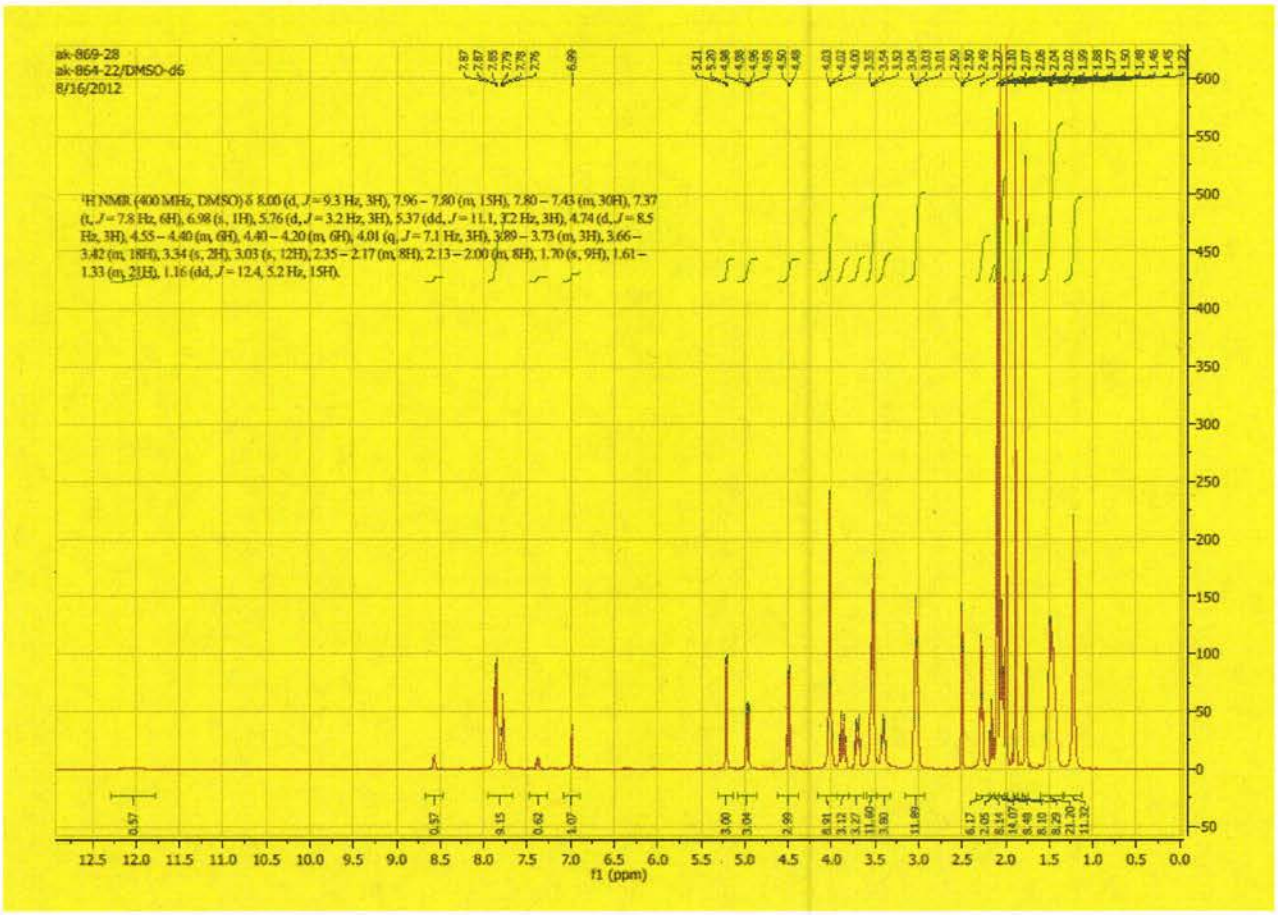
^{1}H NMR of 27A in DMSO-d_6



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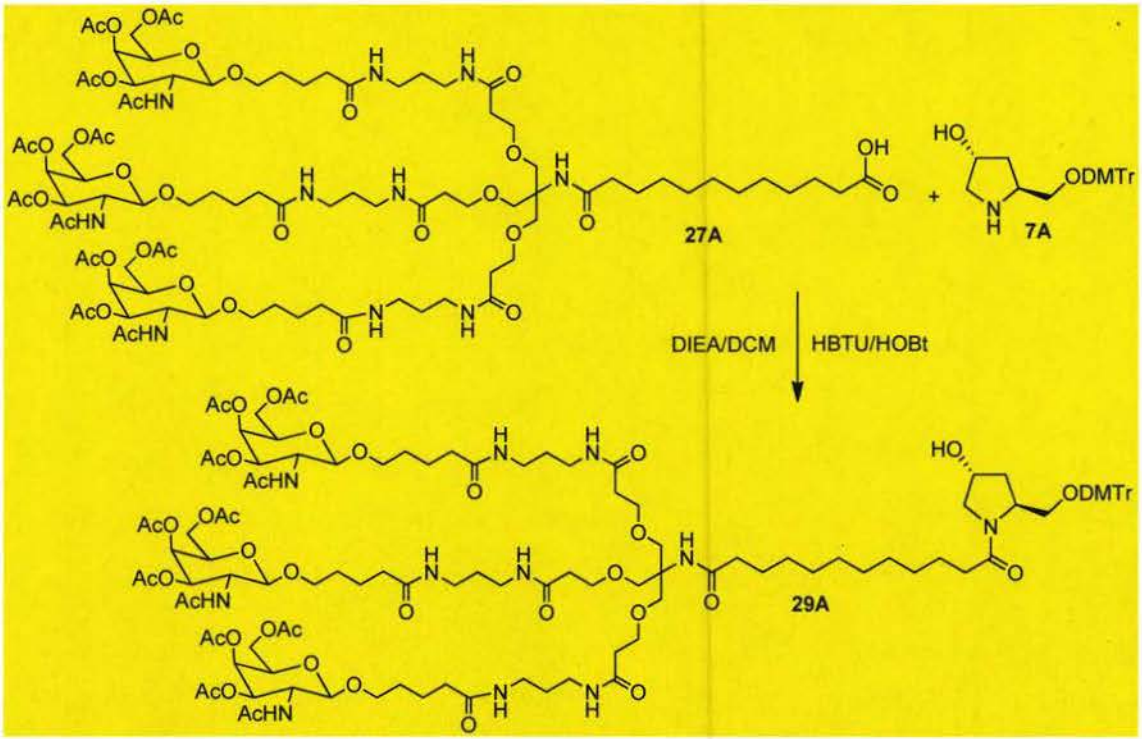
Synthesis of Hyp-Tris(^{Ac}GalNAc)29A

Scheme 10



	Reagents	MW	Qty Used	Moles	Mol Eq.
1	Tris(^{Ac}GalNAc)-acid **27A**	2006.19	1.107 Kg	0.552	1
2	Hydroxyprolinol derivative **7A**	419.51	256.00g	0.610	1.10
3	HOBt monohydrate	135.10	168.00 g	1.10	2.00
4	HBTU	379.3	250.00g	0.66	1.20
5	DIEA	129.24	286.00 mL	1.65	3.00
6	Anhyd. DCM	-	10 L	-	Solvent
7	Ethyl acetate		75 L		Solvent
8	Isopropanol		10 L		Solvent
9	Methanol		15 L		Solvent

Procedure:

To a stirred solution of triantennary GalNAc acid **27A** (1.107 kg, 0.552 mol) in anhyd. DCM (10.0 L) in a four necked 22 L flask were added hydroxyprolinol derivative **7A** (256 g, 0.61 mol), HOBt monohydrate (168 g, 1.1 mol) and HBTU (250 g, 0.66 mol) under argon atm. The mixture was stirred at ambient temperature for 15 min, DIEA (286 mL, 1.65 mol) was added, and the stirring was continued for additional 3 h. TLC was checked and the mixture was quenched with 4% aqueous $NaHCO_3$ (6.0 L), the organic layer was separated and concentrated under reduced pressure till a viscous gummy residue

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remained. The latter was partitioned between a mixture of ethyl acetate-isopropanol (5:1) (9.6 L) and 1% aq. NaCl (8.0 L) (*Note 1*), the organic layer was separated, washed with sat. NaCl solution (3.5 L), diluted to a total volume of 13 L with ethyl acetate-isopropanol (5:1) mixture, and dried over anhyd. Na_2SO_4. The dried solution was directly loaded on a filtration column of silica gel (7.2 kg, EMD 60-200 mesh) that was deactivated and wet-preloaded with 2% TEA in ethyl acetate. The column was eluted with ethyl acetate-isopropanol (5:1, 30 L) followed by ethyl acetate-methanol (2:1, 46 L) (*Note 2*). Small contaminated fraction (6 L) preceded main pure fractions were collected separately (*Note 3*). Pure fractions were evaporated in vacuum, and foamy residue was re-dissolved and co-evaporated with anhydrous acetonitrile (6 L x 2). The residue was dried overnight at 2 mbar/30 °C to afford 1.145 kg of **29A** (87%). The contaminated fraction was evaporated and purified analogously on a small filtration column of silica gel (325 g) to afford additional 46 g of the material. Total yield of **29A**: 1.19 kg, 90%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.82 (d, J = 8.9 Hz, 2H), 7.73 (t, J = 5.5 Hz, 1H), 7.37 – 7.24 (m, 1H), 7.24 – 7.11 (m, 2H), 6.97 (s, 1H), 6.93 – 6.80 (m, 1H), 5.20 (d, J = 3.3 Hz, 1H), 5.01 – 4.85 (m, 1H), 4.48 (d, J = 8.5 Hz, 1H), 4.42 – 4.23 (m, 1H), 4.13 (d, J = 3.8 Hz, 1H), 4.06 – 3.96 (m, 3H), 3.86 (dd, J = 19.9, 8.9 Hz, 1H), 3.77 – 3.64 (m, 3H), 3.62 – 3.46 (m, 5H), 3.46 – 3.28 (m, 2H), 3.15 (dd, J = 8.7, 5.0 Hz, 1H), 3.11 – 2.92 (m, 6H), 2.34 – 2.15 (m, 3H), 2.09 (s, 3H), 2.08 – 2.00 (m, 5H), 1.98 (s, 3H), 1.91 – 1.85 (m, 3H), 1.76 (s, 3H), 1.48 (dd, J = 17.3, 11.4, 6.3 Hz, 7H), 1.30 – 1.11 (m, 6H).

1. *Notes:*The residue after rotary evaporation was hardly soluble in dry ethyl acetate-isopropanol mixture alone; addition of some 1% brine facilitates dissolution.
2. Triethylamine deactivation of silica gel was done at the stage of loading onto the column. No triethylamine was added during the elution that could cause acetyl deprotection from sugar moieties in the presence of methanol. Even in the presence of 2% triethylamine in ethyl acetate during column loading slight deprotection of one of the acetyl group from the sugar moiety was detected by MALDI. Therefore, reducing percentage of triethylamine in loading solvent (EtOAc) from 2 to 1 % or even less may be safer.
3. 6 L fractions were collected and checked the TLC

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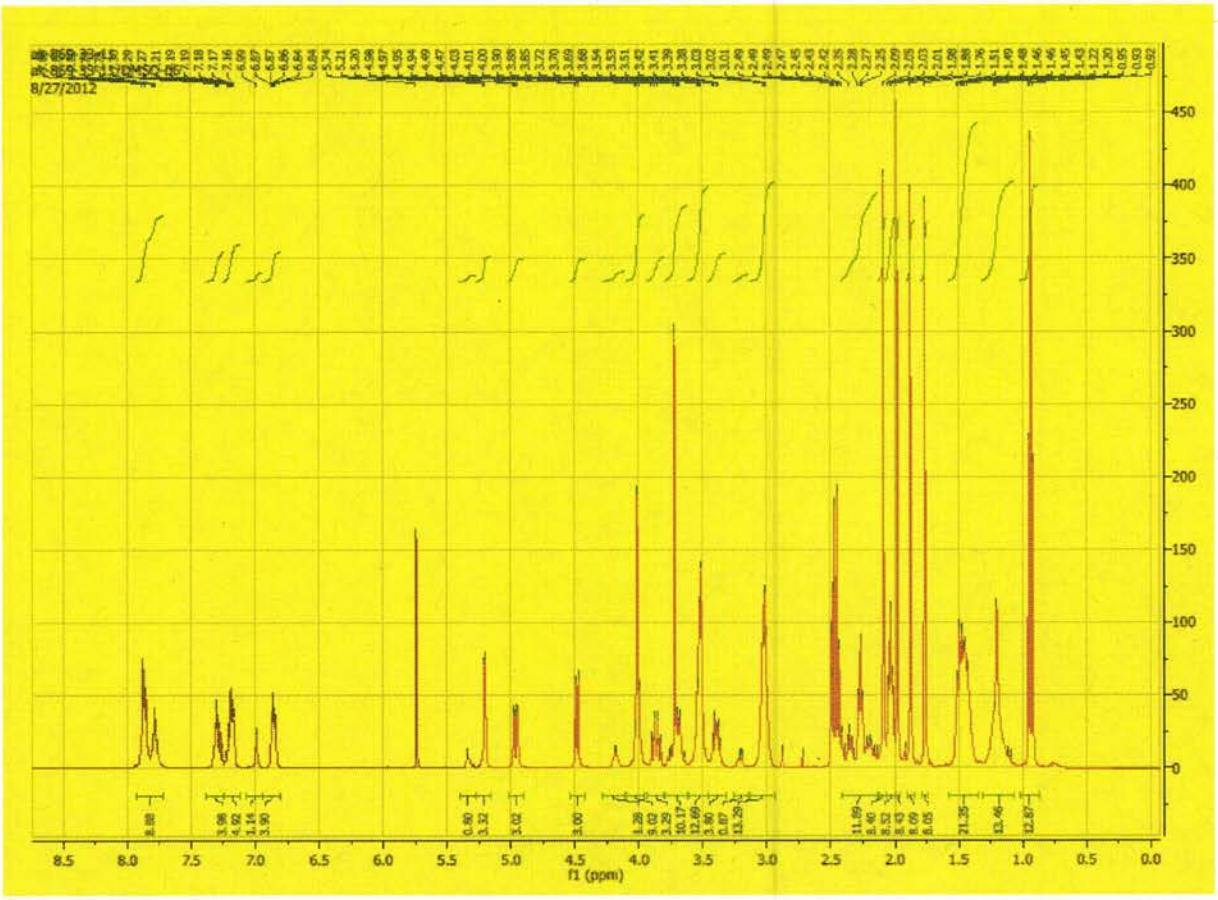
^{1}H NMR of **29A** in DMSO-d_6



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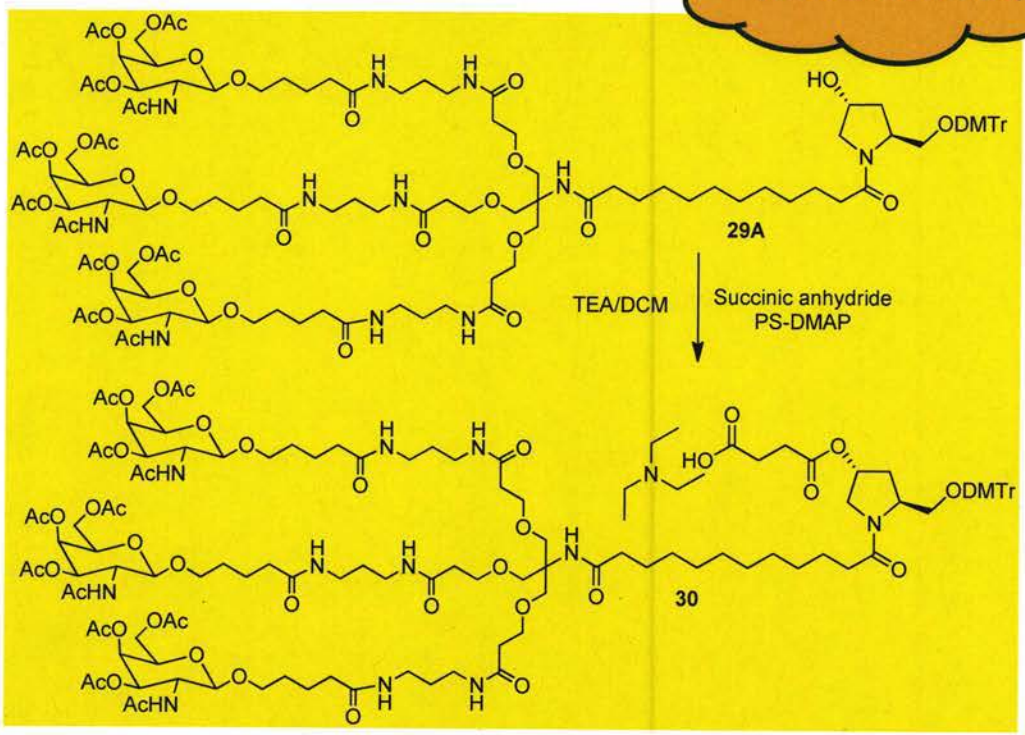
Synthesis of Hyp-Tris(^{Ac}GalNAc) Succinate 30

Scheme 11

Go to page 66 for alternate procedure for scale-up



	Reagents	MW	Qty Used	Moles	Mol Eq.
1	Hyp-AcGalNAc **29A**	2407.69	1.14 Kg	0.474	1
2	Succinic anhydride	100.07	119.00 g	1.189	2.50
3	PS-DMAP(1.57 µmol/g loading)	-	604 g	0.948	2.00
4	Triethylamine	101.1	198 mL	1.42	3.00
5	Anhyd. DCM	-	12 L	-	Solvent
6	DCM	-	2 L	-	Solvent

Procedure:

Succinic anhydride (94.8 g, 0.948 mol) and PS-DMAP (loading: 1.57 mmol/g, 604 g, 0.948 mol) were added successively to a solution of **29A** (1.14 kg, 0.474 mol) and triethylamine (198 mL, 1.42 mol) in anhyd. DCM (6.0 L) in a 12 L four necked flask equipped with an overhead stirrer under argon atm. After stirring for 24 h (*Note 1*), additional succinic anhydride (24 g, 0.24 mol) was added. The mixture was stirred for additional 24 h, filtered through wet-loaded in DCM Celite (0.5 kg), and the solids were washed thoroughly with DCM (8 L total). Combined filtrate (~14 L) was evaporated under reduced pressure to a volume of ~6 L. The mixture was transferred to a separatory funnel and triethylamine (200 mL) was added. The combined solution was washed with 5% aq. NaCl (6 L x 2), the organic layer was

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separated, and dried over anhyd. Na_2SO_4. The solvent was evaporated on a 20 L rotary evaporator under reduced pressure till soft foamy residue which was re-dissolved in 6 L of anhyd. DCM containing 100 mL of triethylamine, the solvent was evaporated again, and the foamy residue thus obtained was dried at 1 mbar/20 °C for 2 days to afford 1.19 kg (96% based on triethylamine salt) of **30** as glassy foam. ^{1}H NMR indicated presence of 4.3 mass% of residual solvents (DCM and traces of triethylamine, *Note 2*). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.93 – 7.72 (m, 1H), 7.28 (dd, J = 13.3, 6.1 Hz, 1H), 7.24 – 7.12 (m, 1H), 6.99 (s, 1H), 6.94 – 6.80 (m, 1H), 5.33 (s, 1H), 5.20 (d, J = 3.3 Hz, 1H), 4.96 (dd, J = 11.2, 3.3 Hz, 1H), 4.48 (d, J = 8.4 Hz, 1H), 4.18 (d, J = 3.4 Hz, 1H), 4.10 – 3.95 (m, 1H), 3.86 (dd, J = 19.8, 8.9 Hz, 1H), 3.79 – 3.62 (m, 1H), 3.61 – 3.45 (m, 1H), 3.45 – 3.32 (m, 1H), 3.26 – 3.15 (m, 1H), 3.13 – 2.94 (m, 1H), 2.41 – 2.13 (m, 1H), 2.09 (s, 1H), 2.03 (t, J = 6.9 Hz, 1H), 1.98 (s, 1H), 1.88 (s, 1H), 1.76 (s, 1H), 1.57 – 1.35 (m, 2H), 1.31 – 1.06 (m, 2H), 0.93 (t, J = 7.1 Hz, 1H).

Notes:

1. The reaction was monitored by TLC, MALDI and HPLC small amount of **29A** remained after 24 hrs.
2. The remaining solvents can possibly be removed by prolonged drying in high vacuum. Heating of this compound while drying is risky as it is labile. The compound is getting soft and eventually converted to oily droplets when left on humid air for several hours.

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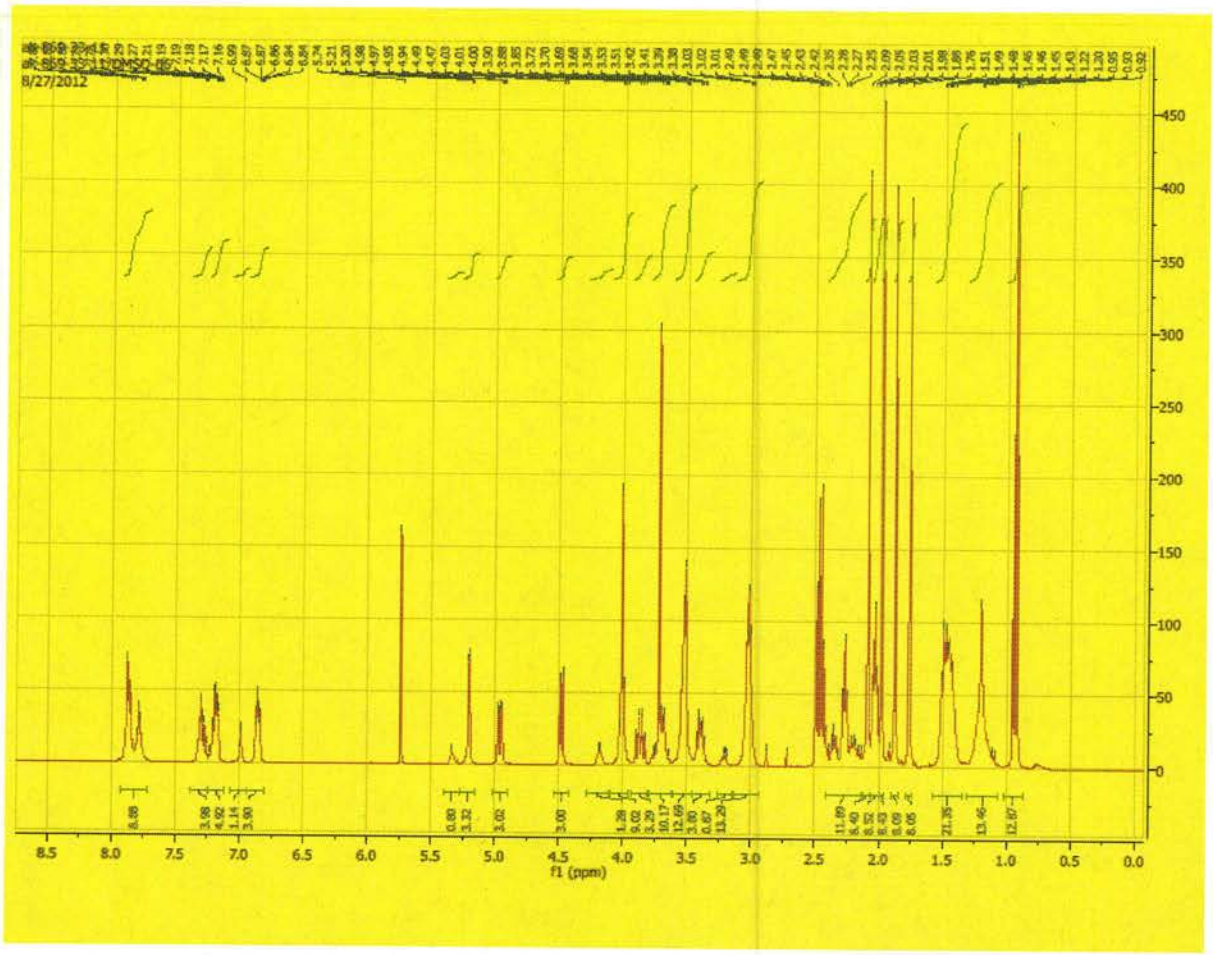
^{1}H NMR of **30** in DMSO-d_6



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Improvements and Recommended Changes for the Succinate 30 Scale-up

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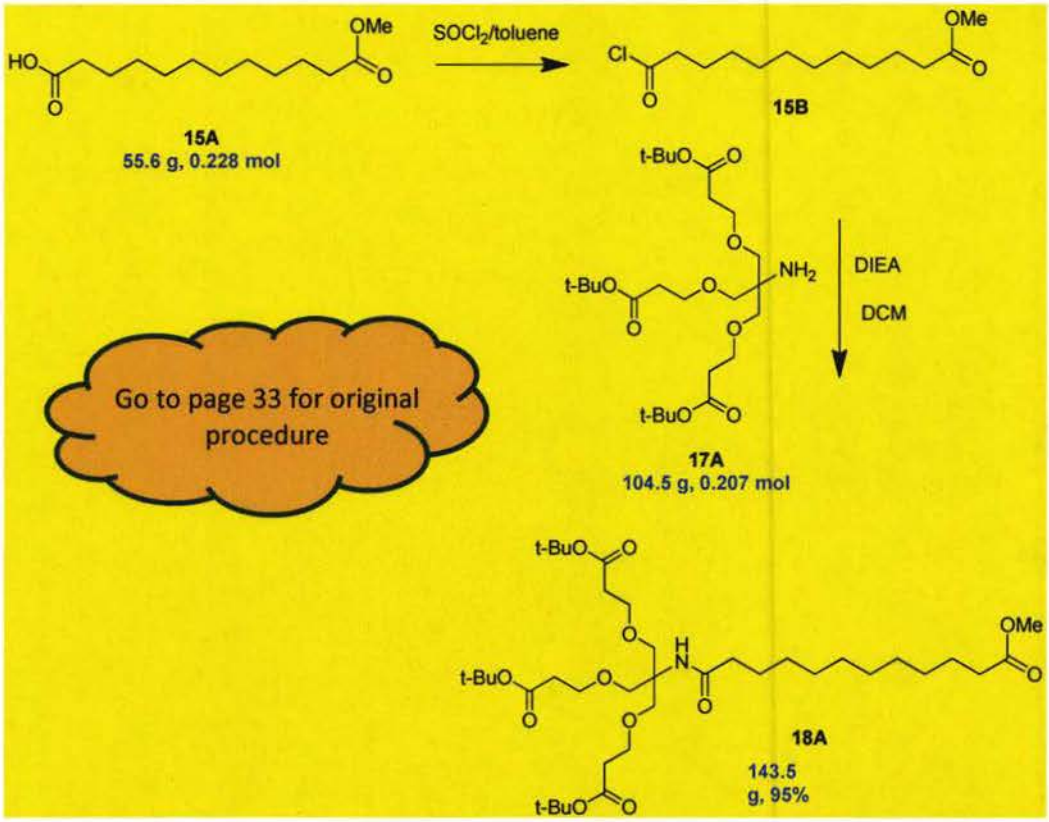
Synthesis of *Synthesis of Tris(tert-Butyl) C12 Methyl Ester* 18A – Replacing EDC/HOBt coupling with acid chloride

Scheme 12: Alternative method of making compound **18A**



Procedure

Thionyl chloride (low iron, 99%, 19.7 mL, 0.27 mol) was added to a suspension of dodecanedioic acid - monomethyl ester **15A** (55.6 g, 0.228 mol) in anhyd. toluene (200 mL) and the mixture was stirred at 60 °C until gas evolution was completed (bubbler, 3h). The mixture was cooled down to rt; solvent and volatiles were evaporated under reduced pressure, and the evaporation flask was refilled with argon. The residue (acid chloride **15B)** was diluted with anhyd. DCM (200 mL) and transferred slowly *via* cannula over 40 min in a cooled (0 °C) and stirred solution of **17A** (104.5 g, 0.207 mol) and DIEA (47 mL, 0.27 mol) in anhyd. DCM (500 mL). The stirring was continued at 0 °C for additional 30 min (*Note 1*) and the mixture was quenched by addition of sat. $NaHCO_3$ (400 mL). The organic phase was separated and

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concentrated under reduced pressure. The residue was dissolved in 1:1 mixture of ethyl acetate and hexane (600 mL) and washed consecutively with 600 mL of each: 2% aq. $NaHCO_3$, water, 5% aq. HCl, water (x2) (*Note 2*), sat. NaCl and dried over anhyd. Na_2SO_4. The solvents were removed under reduced pressure and the residue was dried by stirring at 0.4 torr overnight to afford 143.5 g (95%) of **18A**.

Notes:

1. Small amount of the starting material **17A** was found by TLC after quenching, indicating incomplete conversion. One of the following may be implemented in order to achieve complete conversion: (i) use larger excess of **15B** (up to 1.2 eq) or (ii) extend coupling time and/or warm the mixture up to rt before quenching.
2. Successive wash with 5% aq. HCl and water (twice) removed unreacted starting amine **17A**.

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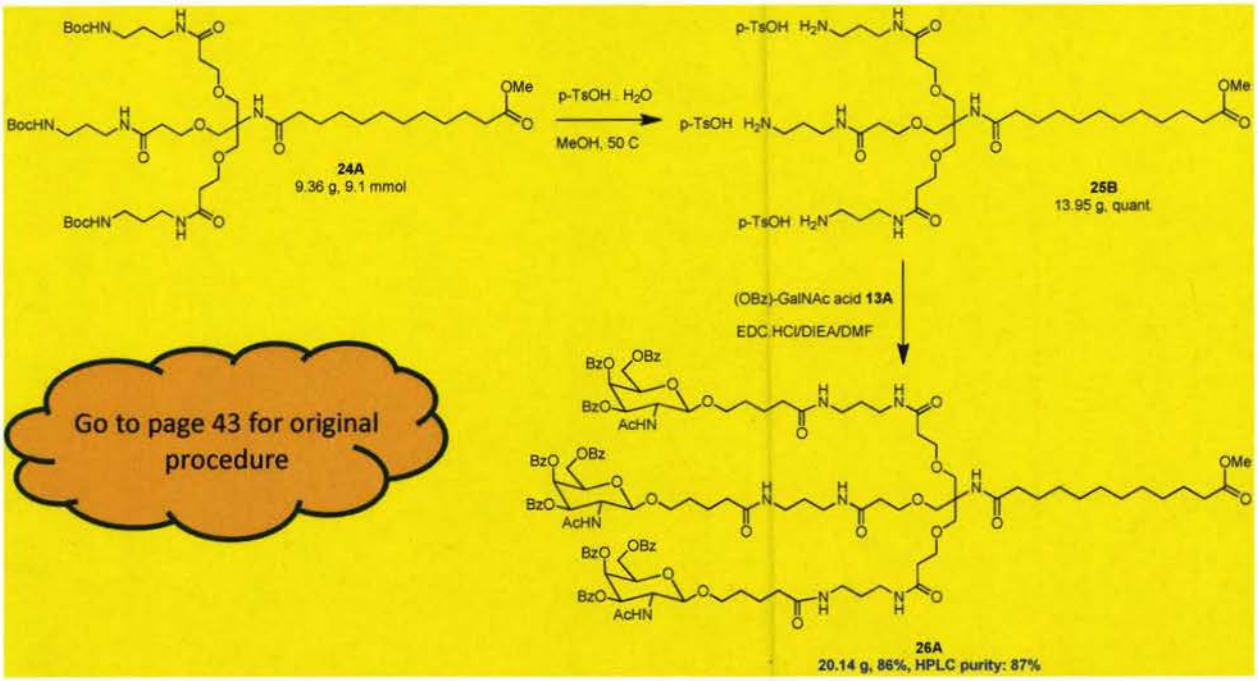
Triamine Tosic Acid Salt 25B

Scheme 13: *p*-Toluenesulfonic acid (PTSA or tosic acid) deprotection of Tris(^{Boc}Amine) Methyl Ester **24A** to the corresponding tosic acid salt **25B**



Procedure

A solution of Boc protected amine **24A** (9.36 g, 9.1 mmol) and *p*-toluenesulfonic acid monohydrate (tosic acid, 6.23 g, 32.8 mmol) in dry methanol (90 mL) was heated in a moisture-protected flask at 50 °C overnight (*Note 1*). The completion of the reaction was monitored by MS analysis. The mixture was cooled to rt, excess tosic acid was neutralized by addition of DIEA (0.96 mL, 5.5 mmol), and the mixture was diluted with toluene (180 mL). The solvents were evaporated and the foamy solid residue obtained was dried on rotary evaporator at 8 mbar/30 °C for 2 h to afford 13.95 g of **25B** containing ~5.5 mmol (1.66 g) of p-TSA-DIEA salt and traces of residual toluene.

Triantennary scaffold 26A from triamine tosic acid salt 25B:

To a cooled (10 °C) and stirred solution of ^{Bz}GalNAc C_5 acid - sodium salt **13A** (21.5 g, 32.8 mmol) and HOBt-monohydrate (5.51 g, 36 mmol) in anhyd. DMF (100 mL) was added EDC hydrochloride (7.1 g, 36 mmol) followed by DIEA (10.0 mL, 73 mmol), and the mixture was stirred at 10 °C till complete dissolution of EDC hydrochloride (~ 15 min). Concurrently, the solid from previous step containing **25B** (13.95 g, 9.1 mmol) was dissolved in anhyd. DMF (70 mL) and the solution was transferred *via* cannula to the above mixture. The mixture was allowed to warm up to rt overnight, cooled to 0 °C and diluted with

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water (600 mL). After settling of the organic components down at 0 °C overnight, the water layer was decanted from viscous organic residue, the latter was taken in EtOAc (200 mL), washed successively with a mixture of 5% NaCl (200 mL), 20% phosphoric acid (20 mL), 5% brine (x2) and saturated brine; diluted the organic layer with EtOAc to a total volume of 300 mL and dried over anhyd. Na_2SO_4. This solution was directly loaded on a short filtration column of 100 g silica gel wet-preloaded in EtOAc. The column was eluted with EtOAc (300 mL x 2) followed by 3;1 EtOAc-methanol (300 mL x 3), fractions containing the desired product were pooled, evaporated and dried at 0.4 torr overnight to afford 20.14 g (86%) of **26A**. HPLC profile (87%) was comparable to the reference batch (88%).

Note 1. Water from the tosic acid monohydrate may cause partial hydrolysis of the methyl ester. Replacing tosic acid monohydrate with anhydrous methanesulfonic acid (mesic acid) would reduce/ eliminate the ester hydrolysis during Boc deprotection. (No trial reaction has done using mesic yet).

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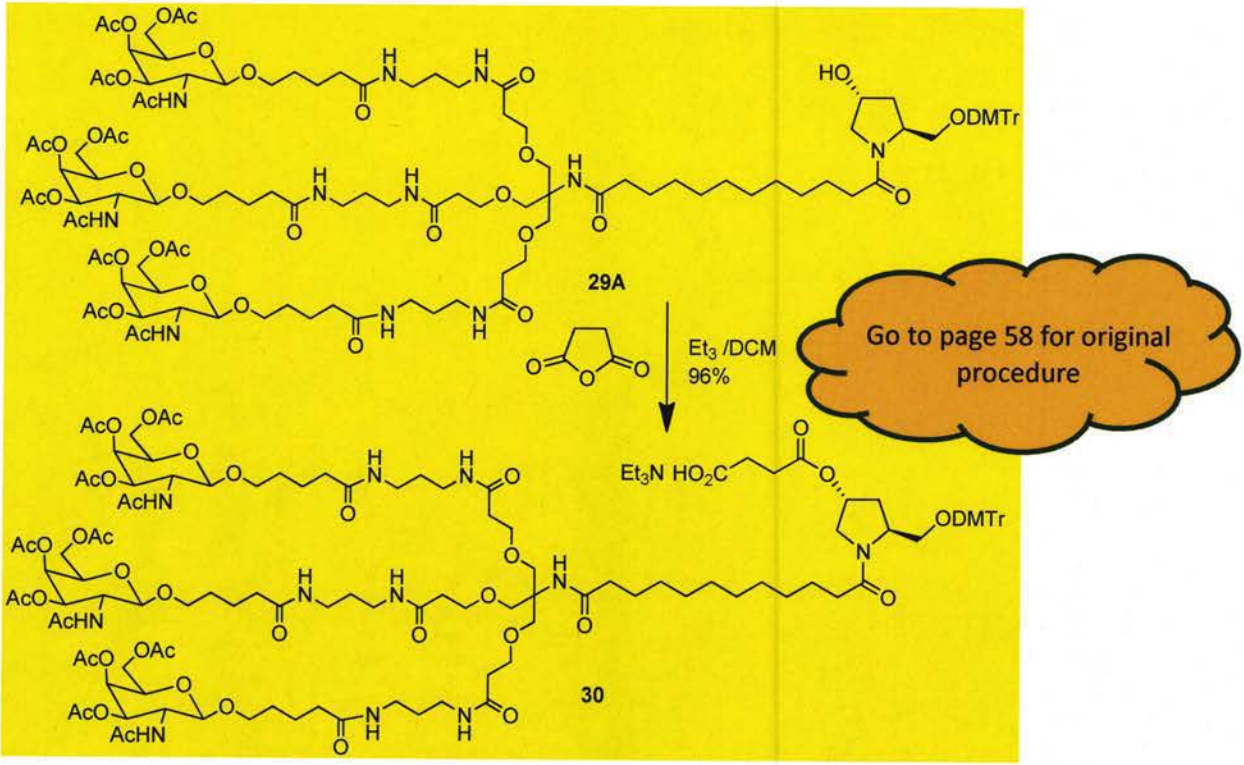
Synthesis of Hyp-Tris(^{Ac}GalNAc) Succinate 30

Scheme 14: Succinylation in the absence of DMAP or immobilized DMAP (PS-DMAP)



Go to page 58 for original procedure

Procedure:

Succinic anhydride (1.18 g, 11.8 mmol) was added to a solution of **29A** (11.4 g, 4.7 mmol) and triethylamine* (1.95 mL, 14 mmol) in anhyd. DCM (60 mL) under Ar atm. The mixture was stirred at rt for 2 days, after which time TLC indicated complete conversion. The mixture was washed twice with 5% aq. NaCl (x 2), the organic layer was dried over anhyd. Na_2SO_4 and the solvent was evaporated under reduced pressure. The residue was dried at 0.4 mbar/20 °C to afford 11.8 g (96% based on triethylamine salt) of **30.**

* The reaction proceeds in the presence of DIEA instead of TEA with similar efficiency and the product appeared to be less colored as compared to Et_3N-mediated protocol.

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Starting Material and Supplier Information

	Starting Material	Vendor
1	D-(+)-Galactosamine hydrochloride, CAS No 1772-03-8	Atomax Chemicals, www.Atomaxchem.com Cat. No: AT139938 , purity 98.6 %
2	Trimethylsilyl trifluoromethanesulfonate (TMS Triflate), CAS No 27607-77-8	Sigma Aldrich, Cat No. 91741, purity ≥98%
3	d-Valerolactone, CAS No 542-28-9	TCI-America, Cat No. V0039, purity ≥98%
4	*trans*-4-Hydroxy-L-proline methyl ester hydrochloride, CAS No 40216-83-9	CNH Technologies, Woburn, MA, USA, Cat No F-1210, purity 98%
5	Di-*tert*-butyl dicarbonate (Boc_2O), CAS No 24424-99-5	AK Scentific, Inc., www.aksci.com
6	Ethyl trifluoroacetate, CAS No 383-63-1	Alfa Aesar, Cat No. A11520
7	4,4'-Dimethoxytriphenylmethyl chloride (DMTr-Cl), CAS Number 40615-36-9	Multiple Vendors
8	Methylamine solution in absolute ethanol (33 wt. %), CAS Number 74-89-5	Sigma Aldrich, Cat No. 534102
9	Dodecanedioic acid, 1,12-dimethyl ester, CAS No 1731-79-9	TCI-America, Cat No. D2835 , purity ≥98%
10	Dodecanedioic acid, 1-methyl ester, CAS No 3903-40-0	Astatech, Inc, Bristol, PA, USA, Cat No. 59464
11	Tris(hydroxymethyl)aminomethane (TRIS), CAS No 77-86-1	Sigma Aldrich, Cat No. 154563, purity ≥ 99%
12	*tert*-Butyl acrylate, CAS Number 1663-39-4	Sigma Aldrich, Cat No. 327182, purity 98%

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13	*N*-BOC-1,3-diaminopropane, CAS No 75178-96-0	AK Scientific, Inc., www.aksci.com
14	PS-DMAP (from Biotage), Cat No. 800313	Biotage, www.biotage.com

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Analytical Methods - General:

STM103-01 denaturing AX-HPLC
STM108-01 assay of ALN-51547
STM-ADT-003-01 molar absorptivity determination of duplexes and single strands
STM-ADT-004-01 endotoxin for unconjugated and conjugated single strands
STM-ADT-008-01 non- denaturing IP-RP HPLC
STM-ADT-010-0 denaturing IP-RP HPLC

Sequence Confirmation, Methods and additional information:

STM107-01 confirmation of sequence by MS_MS
CHE10022 manual for oligonucleotide sequence verification for quality (addendum 1 for conjugates)
data 3374 amidite uU swap.xlsx (test data file for manual)
data 3374.xlsx (test data file for manual)
ms ms sequence verification for quality.ver41.xlsm (Visual basic macro file for sequence confirmation ~ 10 Mb)]

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