Supporting Information for:

First Total Synthesis of a GPI-Anchored Peptide

Jie Xue, Ning Shao and Zhongwu Guo*

Department of Chemistry, Case Western Reserve University, Cleveland, Ohio 44106-7078

Table of Contents:

I. Experimental Section	1- 3
II. Selected NMR and MS spectra	4-45

I. Experimental Section

General Methods. The NMR spectra were recorded at 200, 300 or 600 MHz respectively. Proton chemical shifts are reported in ppm (δ) downfield from tetramethylsilane (TMS), and carbon chemical shifts are reported in ppm (δ) in reference to the solvent CDCl_3 (δ 77.16). Coupling constants (J) are reported in hertz (Hz). Thin layer chromatography (TLC) was performed on silica gel GF₂₅₄ detected by charring with phosphomolibdic acid/EtOH or 5% H_2SO_4 /EtOH solutions. Molecular sieves were dried under high vacuum at 170-180 °C for 6 to 10 h before use. Commercial anhydrous solvents and other reagents were used without further purification.

(+)-2,3:4,5-Di-*O*-cyclohexylidene-*myo*-inositol [(+)-12] and (-)-2,3:4,5-Di-*O*-cyclohexylidene-*myo*-inositol [(-)-12].¹ A mixture of ($\pm$)-1,2:5,6-di-*O*-cyclohexylidene-*myo*-inositol (7.8 g, 22.9 mmol) and Bu_2SnO (6.28 g, 25.3 mmol) in 100 mL toluene was refluxed with azeotropic removal of water for 2.5 h. The reaction mixture was then concentrated to dryness under reduced pressure. To the residue were added DMF (80 mL), CsF (9.1 g, 60 mmol), and *n*-butyryl chloride (2.5 mL, 24 mmol) at -10 °C. The mixture was stirred at room temperature for 16 h, and then diluted with ethyl acetate. The solution was washed with brine, dried over Na_2SO_4 , and then concentrated to dryness. Flash column chromatography of the residue gave ($\pm$)-6-*O*-butyryl-2,3:4,5-di-*O*-cyclohexylidene-*myo*-inositol as a colorless syrup (5.6 g, 60%). ¹H NMR (CDCl_3 , 300 MHz): δ 4.96 (t, J 4.8, 1 H), 4.57 (dd, J 6.1, 4.4, 1 H), 4.34 (dd, J 8.0, 6.2, 1 H), 4.08 (dd, J 8.9, 5.2, 1 H), 3.84 (dd, J 10.4, 8.0, 1 H), 3.45 (dd, J 10.4, 8.9, 1 H), 2.54 (br, 1 H), 2.38 (t, J 7.3, 2 H), 1.30-1.75 (m, 22 H), 0.98 (t, J 7.4, 3 H).

A solution of ($\pm$)-6-*O*-butyryl-2,3:4,5-di-*O*-cyclohexylidene-*myo*-inositol (8.0 g, 19.5 mmol) dissolved in hexane-ether (10:1, 88 mL) was introduced to a 0.1 M potassium phosphate buffer (pH 7.0, 50 mL) containing crude porcine pancreatic lipase (PPL, Sigma type II, 32 g) powder. The mixture was stirred vigorously at 37 °C with a mechanical stirrer, and the reaction progress was monitored by TLC. About 30 h later, the reaction was terminated by centrifugation to separate into

two layers. The organic layer was dried over Na₂SO₄ and concentrated. Column chromatography of the residue gave (-)-**12** (2.4 g, 37%) as well as (+)-6-*O*-butyryl-2,3:4,5-di-*O*-cyclohexylidene-*myo*-inositol (2.1 g, 34%). (-)-**12**: [α]_D -16.5 (*c* 1.0, CHCl₃), Lit.¹ [α]_D -16.0 (*c* 1.0, CHCl₃).

(+)-6-*O*-Butyryl-2,3:4,5-di-*O*-cyclohexylidene-*myo*-inositol (2.1 g) obtained above was subjected to saponification with 1 M NaOMe in MeOH for 1 h at rt. After neutralization with acidic resin and condensation under vacuum, the residue was purified by silica gel column chromatography to afford (+)-**12** (1.8 g, >99%): [α]_D +22.5 (*c* 1.0, CHCl₃).

4-*O*-Allyl-1,6-anhydro-2-azido-3-*O*-benzyl-2-deoxy- β -D-glucopyranose (26).^{2,3} To the solution of D-glucal (2.0 g, 13.7 mmol) in dry acetonitrile (100 mL) was added dry 3 Å molecular sieves (3.0 g) and bis(tributylstannyl) oxide (5.6 mL, 11.0 mmol). The reaction mixture was refluxed under nitrogen for 3 h. After it was cooled down to rt, iodine (4.2 g, 16.5 mmol) was added, and the mixture was stirred at rt for 1.5 h. The solution was then concentrated under vacuum. The residue was dissolved in 50 mL of dry DMF and 7 mL of water, to which NaHCO₃ (5.8 g, 68.5 mmol) was added. After stirring at 120 °C for 3 h, the reaction mixture was separated and the organic layer was concentrated. Column chromatography of the residue gave 1,6:2,3-dianhydro- β -D-glucopyranose (1.44 g, 73%). ¹H NMR (CDCl₃, 300 MHz): δ 5.68 (d, *J* 3.0, 1 H), 4.40 (d, *J* 5.4, 1 H), 3.89 (d, *J* 7.4, 1 H), 3.73 (m, 2 H), 3.43 (m, 1 H), 3.13 (d, *J* 3.0, 1 H), 2.86 (br, 1 H).

After the above product was dissolved in dry DMF, to the solution was added allyl bromide and NaH at 0 °C. The mixture was stirred at rt for 3 h and was then concentrated under vacuum. The residue was applied to a silica gel column to afford pure 4-*O*-allyl-1,6:2,3-dianhydro- β -D-glucopyranose⁴ (70%) as a colorless syrup. ¹H NMR (CDCl₃, 300 MHz): δ 5.96 (m, 1 H), 5.70 (d, *J* 3.1, 1 H), 5.30 (m, 2 H), 4.48 (m, 1 H), 3.70 (m, 2 H), 3.62 (s, 1 H), 3.44 (m, 1 H), 3.17 (m, 1 H).

To the solution of 4-*O*-allyl-1,6:2,3-dianhydro- β -D-glucopyranose (184 mg, 1 mmol) in DMF (5 mL) and water (0.7 mL) was added sodium azide (0.33 g, 5 mmol). The mixture was stirred at 120 °C until the substrate was consumed (monitored with TLC). The reaction mixture was concentrated, and the residue was dissolved in ethyl acetate. The organic solution was washed with aqueous NaHCO₃, brine, and then dried and concentrated. Column chromatography of the residue gave 4-*O*-allyl-1,6-anhydro-2-azido-2-deoxy- β -D-glucopyranose³ as a colorless syrup (177 mg, 78 %). ¹H NMR (CDCl₃, 300 MHz): δ 5.94 (m, 1 H), 5.47 (s, 1 H), 5.28 (m, 2 H), 4.64 (d, *J* 5.3, 1 H), 4.15 (d, *J* 5.7, 1 H), 4.01 (d, *J* 7.5, 1 H), 3.88 (br, 1 H), 3.75 (dd, *J* 7.5, 5.3, 1 H), 3.36 (m, 1 H), 3.23 (m, 1 H), 2.62 (br, 1 H).

Finally, benzylation of 1,6-anhydro-2-azido-2-deoxy-4-*O*-2-propenyl- β -D-glucopyranose (0.45 g, 2.0 mmol) following the procedure described above afforded **26** as a colorless syrup (0.45 g, 80%).³ ¹H NMR (CDCl₃, 300 MHz): δ 7.36 (m, 5 H), 5.90 (m, 1 H), 5.49 (s, 1 H), 5.29 (bdd, *J* 17.3, 1.6, 1 H), 5.22 (bdd, *J* 10.3, 1.6, 1 H), 4.68 (d, *J* 11.9, 1 H), 4.64 (bd, *J* 6.0, 1 H), 4.59 (d, *J* 11.9, 1 H), 4.06 (m, 3 H), 3.76 (dd, *J* 7.1 Hz, 1 H), 3.66 (m, 1 H), 3.38 (m, 1 H), 3.27 (bs, 1 H). ¹³C NMR (CDCl₃, 50 MHz): 137.4, 134.2, 128.6, 128.1, 127.9, 117.9, 100.7, 76.4, 76.2, 74.5, 72.5, 70.5, 65.4, 59.9.

1,6-Di-*O*-acetyl-2,3,4-tri-*O*-benzyl- α -D-mannopyranoside (38).⁵ To a stirred mixture of methyl α -D-mannopyranoside (3.0 g, 15 mmol) and benzyl bromide (9.19 mL, 77 mmol) in DMF (30 mL) was added NaH (2.23 g, 93 mmol) in portions at 0 °C. The mixture was then warmed up to rt, and 17 h later, the reaction was quenched with methanol and diluted with ethyl acetate. The organic layer was isolated, washed with brine, dried over Na₂SO₄ and concentrated. After the residue was

dissolved in acetic acid and acetic anhydride (1:1, 60 mL), to this solution was added concentrated sulfuric acid (1.5 mL) dropwise at 0 °C. Half an hour later, the reaction mixture was washed with saturated NaHCO₃ solution and cold water. The organic layer was dried over Na₂SO₄ and concentrated. The residue was purified with flash chromatography on a silica gel column to afford **38** as a colorless syrup (5.6 g, 70%). [α]_D+30.0 (*c* 1.0, CHCl₃), Lit.⁵ [α]_D+31.3 (*c* 1.0, CHCl₃). ¹H NMR (300 MHz; CDCl₃): δ 7.30-7.42 (m, 15 H), 6.20 (d, *J* 1.9, 1 H), 4.96 (d, *J* 10.7, 1 H), 4.79 (d, *J* 12.6, 1 H), 4.73 (d, *J* 12.6, 1 H), 4.61 (d, *J* 10.7, 1 H), 4.60 (s, 2 H), 4.34 (m, 2 H), 3.86-4.01 (m, 3 H), 3.75 (t, *J* 2.4, 1 H), 2.07 (s, 3 H), 2.04 (s, 3 H).

Ethyl 6-O-Acetyl-2,3,4-tri-O-benzyl-1-thio- α -D-mannopyranoside (39).⁵ Ethanethiol (2.5 mL, 22.5 mmol) was added to a stirred solution of **38** (4.0 g, 7.48 mmol) in DCM (50 mL). After the reaction mixture was cooled to 0 °C, boron trifluoride etherate (1.42 mL, 11.2 mmol) added dropwise. About 3 h later, the reaction mixture was dilute with DCM and washed with aq. NaHCO₃ and brine. The organic layer was dried over Na₂SO₄ and then concentrated. The residue was purified with a silica gel column to give **39** (2.6 g, 65%). [α]_D+82.2 (*c* 1.0, CHCl₃); Lit.⁵ [α]_D+73.8 (*c* 0.91, CHCl₃). ¹H NMR (CDCl₃, 200 MHz): δ 7.28-7.43 (m, 15 H), 5.38 (d, *J* 1.3, 1 H), 4.95 (d, *J* 10.8, 1 H), 4.70 (d, *J* 3.0, 1 H), 4.59 (s, 2 H), 4.58 (d, *J* 10.8, 1 H), 4.34 (m, 2 H), 4.18 (m, 1 H), 3.83-4.00 (m, 3 H), 2.60 (m, 2 H), 1.26 (t, *J* 7.4, 3 H).

Ethyl 2,3,4-Tri-O-benzyl-1-thio- α -D-mannopyranoside (7).⁵ After a solution of **39** (2.6 g, 4.84 mmol) in methanol (20 mL) containing NaOMe (0.05 M) was stirred at rt for 1 hr, the reaction mixture was diluted with ethyl acetate and washed with water. The organic layer was dried over Na₂SO₄ and then concentrated. The residue was purified with a silica gel column to give **7** as a colorless syrup (2.15 g, 90%). [α]_D+100.4 (*c* 1.0, CHCl₃), Lit.⁵ [α]_D+88.4 (*c* 1.15, CHCl₃). ¹H NMR (CDCl₃, 300 MHz): δ 7.27-7.37 (m, 15 H), 5.31 (s, 1 H), 4.95 (d, *J* 10.9, 1 H), 4.71 (s, 2 H), 4.66 (d, *J* 10.9, 1 H), 4.63 (d, *J* 11.6, 1 H), 4.58 (d, *J* 11.6, 1 H), 4.00 (m, 2 H), 3.82-3.88 (m, 4 H), 2.58 (m, 2 H), 1.25 (t, *J* 7.4, 3 H).

3,4,6-Tri-O-benzyl-1,2-O-(methoxyethylidene)- β -D-mannopyranose (35).⁶ To a solution of **3**⁶ (2.98 g, 8.2 mmol) in benzyl chloride (12 mL) and THF (40 mL) was added solid KOH (4.5 g, 80 mmol) in portions under reflux. After stirring and refluxing for 4 h, the mixture was diluted with 50 mL water, and extracted twice with DCM (300 mL). The combined organic solution were dried over Na₂SO₄ and concentrated in vacuum. The residue was recrystallized from ethyl acetate and hexane (1:10) to give **35** as a white crystalline solid (2.65 g, 66%). ¹H NMR (CDCl₃, 300 MHz): δ 7.43-7.24 (m, 15 H), 5.37 (d, *J* 2.4, 1 H), 4.94 (d, *J* 10.7, 1 H), 4.82 (s, 2 H), 4.56-4.66 (m, 3 H), 4.43 (t, *J* 3.2, 1 H), 3.97 (t, *J* 9.3, 1 H), 3.73-3.78 (m, 3 H), 3.43-3.47 (m, 1 H), 3.32 (s, 3 H), 1.78 (s, 3 H).

References:

1. Gou, D.; Liu, Y.; Chen, C. *Carbohydr. Res.* **1992**, *234*, 51-64.
2. Xue, J.; Guo, Z. *Tetrahedron Lett.* **2001**, *42*, 6487-6489.
3. Koshida, S.; Suda, Y.; Fukui, Y.; Ormsby, J.; Sobel, M.; Kusumoto, S. *Tetrahedron Lett.* **1999**, *40*, 5725-5729.
4. Joachim, T.; Archibald, P.; L., I. *Liebigs Ann. Chem.* **1986**, *6*, 1044-1050.
5. Tennant-Eyles, R. J.; Davis, B. G.; Fairbanks, A. J. *Tetrahedron: Asymmetry*, **2000**, *11*, 231-243.
6. Ponpipom, M. M. *Carbohydr. Res.* **1977**, *59*, 311-317.

II. Selected NMR and MS Spectra

(35)

(39)

(40)

(41)

(41)

(42)

(13)

(14)

(15)

(19)

(20)

(23)

(24)

(11)

300 MHZ IN CDCL₃
06-07-2001

(28)

JX0215 CDC13

exp1 water

ACQUISITION		PROCESSING	
temp	not used	oversamp	59
solvent	CDC13	proc	ft
date	Aug 30 2001	fn	65536
		lsfrq	-2.0
sfrq	599.919		
tn	H1		
dl	1.000	AUTOMATION	
at	4.906	werr	
np	65536	wexp	autopresatpro~
sw	6679.5		ci
bs	4	wnt	wft f full sel~
ss	4	ect(1)	vsadj dssh
tof	-320.6	wbs	CHANNEL 2
tof2	2.0	dn	C13
alfa	4.0	dm	nmn
gain	30		
nt	32	dn2	N15
ct		dm2	nmn
OPTIONS		presat	y
scuba		g2lv10	0.000
jumpret	n	gt0	0.0020
shaped	n	sspl	n
watergate	n	satmode	vm
	n	satfrq	-224.4
	n	satpwr	0
il	n	satdly	58
in	n	tpwr	3.0
dp	y	dm	
hs	n	Composit

(31)

(6)

(6)

Archive directory: /export/home/guo/vnmrsys/data
 Sample directory: JX0102_02Jan2003
 File: PROTON

Pulse Sequence: s2pul

Solvent: CDCl3
 Temp. 25.0 C / 298.1 K
 INOVA-600 "Jarry"

Relax. delay 1.000 sec
 Pulse 45.0 degrees
 Acq. time 3.494 sec
 Width 7200.7 Hz
 16 repetitions

OBSERVE H1, 599.9093442 MHz
 DATA PROCESSING
 FT size 65536
 Total time 1 min, 12 sec

(47) at 25 °C

Archive directory: /export/home/guo/vnmrSYS/data
 Sample directory: JX0102-50_02Jan2003
 File: PROTON

Pulse Sequence: s2pu1

Solvent: CDCl3
 Temp: 50.0 C / 323.1 K
 INOVA-600 "farry"

Relax. delay 1.000 sec
 Pulse 45.0 degrees
 Acq. time 3.494 sec
 Width 7200.7 Hz

16 repetitions
 OBSERVE H1, 599.9003499 MHz
 DATA PROCESSING
 FT size 65536
 Total time 1 min, 12 sec

(47) at 50°C

(47)

Archive directory: /export/home/guo/vnmrSYS/data
 Sample directory: JX0113cosy_13Jan2003
 File: COSY

Pulse Sequence: COSY

Solvent: CDCl3
 Temp: 25.0 C / 298.1 K
 INOVA-600 "larry"

Relax. delay 1.000 sec
 Acq. time 0.162 sec
 Width 6317.6 Hz
 2D Width 6317.6 Hz
 8 repetitions
 256 increments

OBSERVE H1, 599.9093494 MHz
 DATA PROCESSING
 Sqr. sine bell 0.081 sec
 F1 DATA PROCESSING
 Sqr. sine bell 0.041 sec
 FT size 2048 x 2048
 Total time 41 min, 18 sec

Archive directory: /export/home/guo/vnmrSYS/data
 Sample directory: JX0514_22Jan2003
 File: PROTON

Pulse Sequence: s2pul

Solvent: CDCl₃

Temp. 25.0 C / 298.1 K

INNOVA-600 "larry"

Relax. delay 1.000 sec

Pulse 45.0 degrees

Acq. time 3.495 sec

Width 7200.7 Hz

32 repetitions

OBSERVE H1, 599.9093379 MHz

DATA PROCESSING

FT size 65536

Total time 2 min, 24 sec

Archive directory: /export/home/guo/vnmrSYS/data
 Sample directory: JX0514cosy_22Jan2003

Pulse Sequence: COSY

Solvent: CDCl₃
 Temp. 25.0 C / 298.1 K
 File: COSY
 INOVA-500 "joe"

Relax. delay 1.000 sec
 Acq. time 0.178 sec
 Width 5738.9 Hz
 2D Width 5738.9 Hz
 8 repetitions
 256 increments
 OBSERVE H1 599.9093379 MHz
 DATA PROCESSING
 S₀ sine bell 0.089 sec
 F1 DATA PROCESSING
 S₀ sine bell 0.045 sec
 FT size 2048 x 2048
 Total time 41 min, 56 sec

Archive directory: /export/home/guo/vnmrsys/data
 Sample directory: jx0514H_23Jan2003
 File: HMQC

Pulse Sequence: HMQC

Solvent: CDCl₃
 Temp. 25.0 C / 298.1 K
 INOVA-600 "larry"

Relax. delay 1.000 sec
 Acq. time 0.140 sec
 Width 7297.9 Hz
 2D Width 25641.0 Hz
 16 repetitions
 2 x 256 increments

OBSERVE H1, 599.9093379 MHz
 DECOUPLE C13, 150.8566006 MHz
 power 35 dB
 on during acquisition
 off during delay

W40 C13 modulated
 DATA PROCESSING
 Gauss apodization 0.065 sec
 F1 DATA PROCESSING
 Gauss apodization 0.018 sec
 FT size 2048 x 4096
 Total time 3 hr, 28 min, 19 sec

(50)

(1 in D₂O)

(1 in CD₃OD)

(**1** in CD₃OD)

Gradient 13C HMQC
Pulse Sequence: gHMQC

(**1** in CD₃OD)

INSTRUM_TOF	SUPARM	JXOJ66-1372-003					
ACQ_DATE	Feb 5 16:56:27	2003					
PATN_1	/Data/rchen						
AOPH_m	Linear						
TID	2000						
NUSHTOTS	2500						
SMOFTS1	0				[ns]		
SMOFTS2	0						
SMOFTS3	0						
DWAVE	MORTIS3						
ZM	0.50	(ms)					
DELAY	0 [ns]						
Usl1	19.00	[KV]					
Usef1	20.00	[KV]					
Utens	8.50	[KV]					
Rofull1	20.00	[KV]					
Refull2	20.00	[KV]					
Vdecl1	1.50	[KV]					
UdeRr	1.65	[KV]					
REPRZ	3.00	[Hz]					
ATTEN	82.0						
LML1	21676.82	0					
LML2	2526.377						
LML3	-0.040						
HITVRBO	No						
GORELY	medium						
DEFION	yes						
FLANRD	No						
ULSRND	No						
DECALA	DPCLAL	690.66					
RENVAL	RENVAL	690.00					
LBNVAL	LBNSLVA	0.28					
I22INDV	I22INDV	0.91					
CMT1	JXOJ66-1372-00303						
CMT2							