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Ionic-liquid Supported Rapid Synthesis of N-glycan Core Pentasaccharide in 10g Scale

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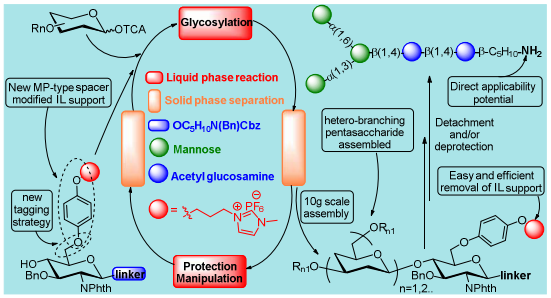
Wei Li, Yu Gao, Qing Li and Zhong-Jun Li*

A new and efficient Ionic Liquid-Supported Oligosaccharide Synthesis (ILSOS) strategy for N-linked core pentasaccharide in 10g scale is reported. This new ILSOS includes new spacer for IL support, new tagging strategy, fast, efficient and orthogonal removal of ionic-liquid support, producing N-linked core pentasaccharide with direct applicability potential in short time, high yield and large gram scale.



Introduction

The importance of structurally defined oligosaccharides with specified biological activities is well known.[1-4] Great efforts have been made to acquire these molecules efficiently and conveniently over the decades. During the preparation process, the purifications after each protection-deprotection reaction and/or glycosylation are likely the most laborious and costly steps[5-7]. Several strategies have been developed to simplify such process. Wong and co-workers developed a computer programmed one pot oligosaccharide synthesis strategy which avoids the need for protecting group manipulations between glycosylation couplings.[8-10] In addition, several one pot synthesis strategies, like iterative one-pot method were developed.[11-13] To simplify the purification after glycosylation coupling, supported synthesis strategies were investigated, which mainly include solid phase approach,[14-16] fluorous-assisted separation method[17-21] and Ionic Liquid Supported Oligosaccharides Synthesis (ILSOS).[22-30]

In recent years, several groups have successfully developed and applied ILSOS strategy in homo-linear oligosaccharides assemblies:[22] Pathak et al. synthesized homolinear α(1→6) linked tetra- and octamannoside using ester-linker IL support via fragment coupling.[23,24] Our group has systematically explored an ILSOS methodology including trichloroacetimidate glycosylation method, acceptor-tagging strategy and centrifugal purification via DCM-iPr_2O solvent system, and successfully synthesized a homolinear α(1→2) linked nonamannoside using a DOX-linker modified IL support.[25] Similar strategy was also utilized in Galan group's work,[26,27] Beau's group[28] and Itoh's group.[29] Besides, Li's group has successfully assembled a homolinear β(1→6) linked hexaglucosamine and a glycopeptide applying a PMB-type linker-modified IL support.[30] These reports have confirmed the validity of ILSOS strategy, in which the rapid glycosylation via active trichloroacetimidates, rational acceptor-tagged strategy, stable ether-type linker modified IL support and efficient centrifugal purification in DCM-iPr_2O solvent system were prevailingly adopted.

However, there are still drawbacks of current ILSOS strategy(**Figure 1a**): 1)the feasibility in preparation of commonly existing hetero-branching oligosaccharides instead of simple homo-linear ones needs to be broadened; 2)the removal of the IL support was not ideal: The DOX-type support was removed cumbersomely via long time hydrogenation,[25-27] while the PMB-type support was detached in acidic condition[30] which challenges some glycosides' stability;[31] 3) ILSOS' ability to assemble oligosaccharides with direct applicability potential needs to be improved: Current ILSOS' deprotection processes leave a free reductive hydroxyl group, which proceeds through multiple steps to forms linkage to other molecules/structures (like microarray and/or glyco-conjugates) for advanced biological chemical studies,[32-34] thus constraining synthesized oligosaccharides' further applications; 4) ILSOS' scalability needs to be proven: Scaled up ILSOS needs to be investigated to afford enough amount of target oligosaccharide molecules for further advanced researches mentioned above.

The State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, Beijing 100191, China.
E-mail: zjli@bjmu.edu.cn

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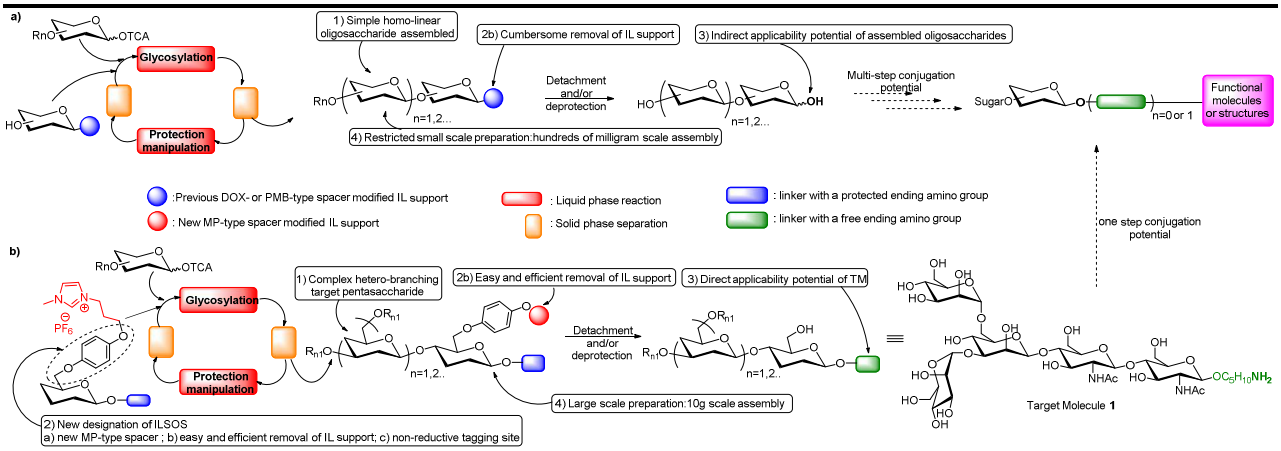

Figure 1. Drawbacks of current ILSOS (**a**) and scheme of new ILSOS designation in the synthesis of **1**(**b**).

Correspondingly (**Figure 1b**), we designed and investigated a new ILSOS in the 1) successful rapid assembly of a complex hetero-branched pentasaccharide **1**, which is the core structure of N-glycans of great biological importance.[35-39] 2) To avoid the cumbersome or challenging removal of IL support, a new spacer-modified IL support was designed, which was efficiently removed after the assembly process. 3)To prepare target molecules with direct applicability potential, a non-reductive site (the 6-OH on reducing end unit) was tagged with IL support, allowing the reductive hydroxyl group being conjugated to a linker with a protected ending amino group, which was released and could directly form an amide bond with other molecules after global deprotection. 4) Multigram scale assembly of **1** by ILSOS was practiced to investigate the scalability of ILSOS.

Results and discussion

Retrosynthetic analysis:

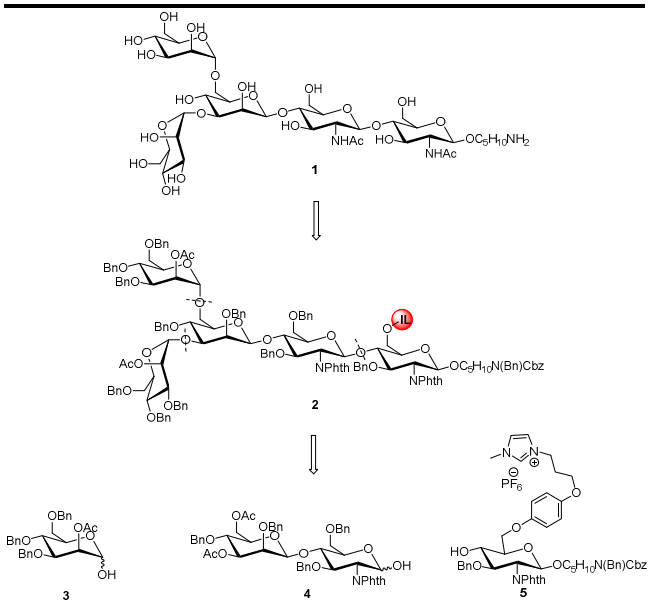

Figure 2. Retrosynthetic analysis of TM and synthesis design.

The fully protected and tagged target pentasaccharide was designed as **2**, which was prepared by assembling the branching mannosyl moiety **3**, the medium disaccharide block **4** and the tagged unit **5**. Donor **3** was prepared according to previous report (Detailed preparation process seen in *supporting information*).[40] Disaccharide **4**, as the key building block containing a β-mannoside, was prepared conventionally via a new route in large scale. Tagged unit **5** was the key block to develop a new ILSOS strategy which mainly included a new methoxyphenyl(MP-)[41-45] type spacer modified IL support and new tagging strategy on the 6-OH of the reductive end unit.

Large scale preparation of disaccharide block 4

Among the many reports of synthesizing N-glycans or its core pentasaccharide, the construction of β-mannoside was always a key point.[46-50] Among commonly adopted β-mannosylation methods, direct construction of β-mannoside from 4,6-benzylidened mannosyl donor requires strict conditions (low temperature, extra unhydrous conditions) and chemical equivalence of relatively expensive steric bases like TTBP or TTDMP.[51-54] Such reaction conditions would be costly and difficult to control especially in large scale preparation; Mannosyl halide method involves large amount of heavy metal salts;[55] And the intramolecular aglycon delivery (IAD) strategy requires a regioselective oxidation of the protecting group on 2'-OH,[56,57] which was not suitable in our case.

However, the 2'-OH inversion strategy[58-61] has shown its advantages in preparing building blocks containing β-mannoside as it involves environment-friendly reagents, exhibits excellent tolerance with various protection groups and proceeds in mild conditions, thus being adopted for large scale preparation of disaccharide **4** to meet the requirements of scalability investigation of ILSOS. Herein, a highly stable, efficient, convenient and economical route was designed and practiced to prepare disaccharide **4** in about 20g scale in one preparation trial (**Figure 3**)

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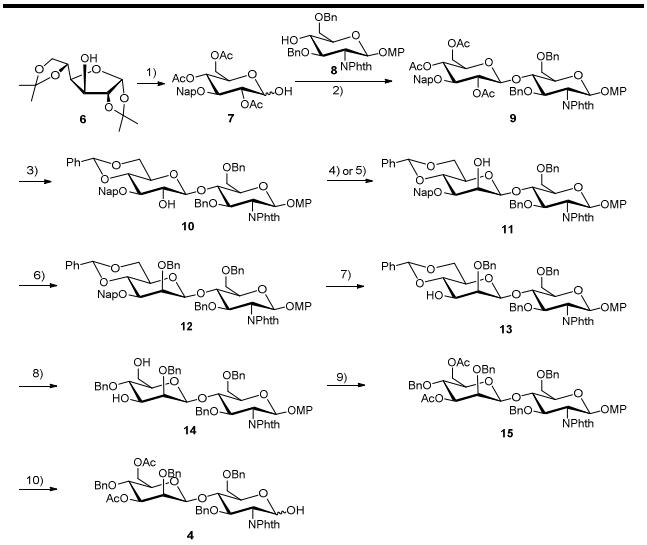

Figure 3. Large scale preparation of disaccharide **4.**
1) i) NapBr, NaH(60%), DMF; ii) TsOH, 75% $MeCN_{a.q.}$, reflux; iii) Ac_2O, Pyr; iv)$H_2NC_3H_6NMe_2$, THF, 90% in 4 steps; 2) i) $CNCCl_3$, DBU, DCM; ii) TMSOTf, DCM, 98%; 3) i) MeONa, MeOH, DMF; ii) $PhCH(OMe)_2$, TsOH, DMF, 93% in 2 steps; 4) Ac_2O, DMSO, then $NaBH_4$, MeOH, DCM, 90%, **10**:**11**=30:70; 5) Tf_2O, Pyr, DCM, then $NaNO_2$, DMF, 48%, 2g scale; 6) BnBr, NaH(60%), DMF, 93%; 7)DDQ, DCM, MeOH, 89% ; 8)BH_3.THF, Bu_2BOTf, DCM, 84%; 9) Ac_2O, Pyr, quant.; 10)CAN, 80% $MeCN_{a.q.}$, 95%.

Commercial 1,2;5,6-Di-*O*-isopropylidene-α-D-glucofuranose **6** was converted into **7** in 90% yield over four continuous steps by only one final flash column chromatography. Following coupling of **7** and **8** to give disaccharide **9** was almost quantitative. **9** proceeded a continuous de-acetylation and benzylidenation to give **10** in 93% yield. For the key inversion of **10** to **11** with the inversion of 2'-OH, "oxidation-reduction" method[58-61] and SN_2 inversion[62] were tried and compared. The SN_2 replacement method showed low yield and limited scalability (48%, 2g scale at most in our trial). The oxidation-reduction method gave high yield (90% in 2 steps), but the stereo-outcome of reduction by $NaBH_4$ in a co-solvent of MeOH/DCM was moderate (**10**:**11**=30:70).[54] Because substrate **10** could be recovered and recycled, such an oxidation-reduction method was finally adopted. The followed benzylation (giving **12** in 93%), oxidative Nap-deprotection (giving **13** in 89%)[63, 64] and regioselective ring-opening[65, 66] of benzylidene **13** all went smoothly to give intermediate **14**. Then **14** was converted into **4** after two efficient manipulations. In this route, all steps proceeded in mild conditions and were repeatable. The required reagents were commercially available at low cost. Finally, about 20g desired disaccharide **4** was prepared in one preparation trial with an overall yield of 34% apart from recycling **10**.

Establishment of a new ILSOS strategy—tagging unit 5

Disaccharide **4** was the key block for the synthesis of target pentasaccharide, and unit **5** was the key for the development of the new ILSOS. The new ILSOS was established in a "catch and release" mode (Figure **4**): tagging process to synthesize unit **5** ("Catch" step) and efficient removal of the new IL support ("Release" step).

Phenol **17** (detailed preparation process seen in *supporting information*) and the 6-OH on **16** (detailed preparation process seen in supporting information) was condensed by a Mitsunobu reaction[58], giving ether **18** in 76% yield. Then **18** was substituted by 1-methyl imidazole to complete the "Catch" step, giving supported **5** in 95% ILSOS separation yield. Such substitution reaction was amenable to large scale synthesis, and unit **5** was prepared in 5g scale for the following exploration. A model detachment reaction ("Release" step) of **5** by CAN[41-45] was completed within 5min to recover **16** in 95% chromatography yield, which was much faster compared with previously reported detaching processes.[25-27] By far, the new ILSOS strategy was fundamentally established.

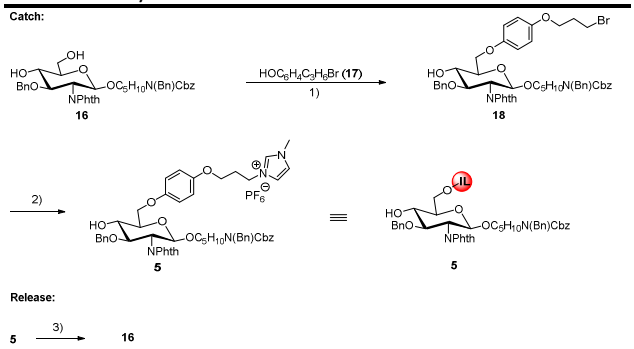

Figure 4. Establishment of the new ILSOS**.**
1) Ph_3P, DIAD, THF, reflux, 76%; 2) Me-Im, KPF_6, MeCN, reflux, 95%; 3) CAN, 80% $MeCN_{a.q.}$, 5min, 95**%**.

Rapid assembly of target pentasaccharide via new ILSOS

After large scale preparation of disaccharide block **4** and successful establishment of the new ILSOS final assembly of the target pentasaccharide was carried out (**Table 1**). Disaccharide donor **4** was converted to a trichloroacetimidate intermediate and used in coupling acceptor **5** (about 4g scale), giving a supported trisaccharide **19** in 96% yield (about 6g scale). Acetyl groups were removed by MeONa in a co-solvent of MeOH and DCM (3h), giving a trisaccharide acceptor **20** quantitatively for the next coupling. Mannosyl donor **3** was treated with $CNCCl_3$/DBU, and coupled with **23** (about 6g scale) smoothly to give the fully protected and tagged pentasaccharide **2** with high yield (98%, 10g scale). In both glycosylation couplings, TfOH was a better promoter than TMSOTf in avoiding the formation of TMS-byproduct of acceptors,[60,61] which was certified in the sugar chain extending coupling of **5** promoted by TMSOTf (For NMR results comparison, see Figure **S1-S4**).

After successful assembly of pentasaccharide **2**, IL support was removed orthogonally by previously experimented oxidation method, giving an un-tagged pentasaccharide **21** in

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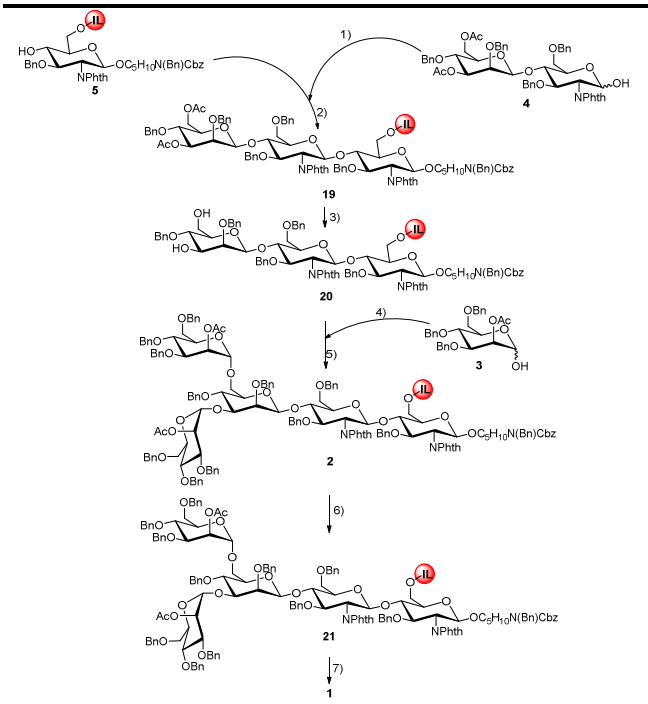
72% chromatography yield. Reason of such relative low recovery of release process was analyzed in the following HPLC analysis paragraph. Compound **21** proceeded through a reported four-step global deprotection process to give the unprotected target pentasaccharide **1** in 70% yield[69, 70]. Besides, abundantly assembled **21** can also be directly used in the preparation of other N-glycan derivatives.[71-73]

Table 1. Rapid synthesis of target pentasaccharide



1): $CNCCl_3$, DBU, DCM, 91%; 2): TfOH, DCM, 96%; 3)MeONa, MeOH/DCM, 99%; 4): $CNCCl_3$, DBU, DCM, 96%; 5):TfOH, DCM, 98%; 6): CAN, 80% $MeCN_{a.q.}$, 10min, 72%; 7): i)$H_2NC_2H_4NH_2$, EtOH, reflux; ii) Ac_2O, Pyr; iii) MeONa, MeOH/ DMF; iv) $Pd(OH)_2$, MeOH, 70%.

n	operations[a]	product	time[b]	recovery	purity[c]	scale
1	B	**5**	40	95%	99%	>5g
3	A then B	**19**	90+40	96%	95%	>7g
	C	**20**	180	>99%	90%	>6g
5	A then B	**2**	90+40	98%	80%	**10g**
	D	**21**	10+180	72%	/	>6g
total			670	64.4%		

[a] A:glycosylation. B: purification. C: removal of acetyl group. D: detachment of IL support. [b] Typical time of glycosylation is about 90min (60min of preparation and 30min of coupling); Typical time of centrifugal purification is 40min. The time of removing acetyl protection groups is about 3h. The time of detaching IL support is 10min and typical flash chromatography time is about 3h. [c] purity was roughly estimated deducted from baseline inequality and column's death volume.

HPLC analysis of the new ILSOS

In our ILSOS exploration, we assumed that inseparable byproducts like large polar inorganics, supported byproducts would accumulate along with the assembly process. Because ILSOS is a rapid synthesis method which excludes conventional chromatographic purification, the results of its easily practiced precipitation-centrifugation separation process, *i.e.* the purity of separated supported oligomers requires to be measured by precise method. As a precise purity analysis method, HPLC analysis was applied. Results were shown in **Figure 5**:

Several apparent tendencies were observed in the HPLC result above:

a): Purity of supported oligomers indeed decreased as the increase of supported sugar units (from 1 unit to 5 units), which was the most intuitive conclusion from these spectra. To be more detailed, decreased purity of supported oligomers mainly attributed to the accumulation of large polar impurities which occupied shorter retention time (apart from the column's death volume which shared a common negative peak and the peaks around) compared with supported oligomer's peak in each spectrum. Such large polar impurities were likely inferred as inorganic salts and/or supported byproducts since each supported oligomer is a large polar organic salt essentially. Besides, such impurity accumulation assumption provided a likely explanation of the

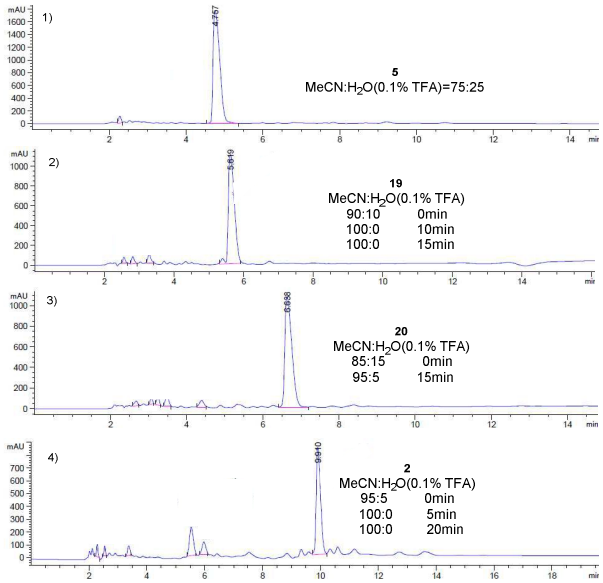


Figure 5. HPLC analysis of the assembly process via new ILSOS

Generally, higher proportion of MeCN in solvent gradient and longer analysis time were required in supported oligomers occupying more sugar units. General HPLC conditions seen in *Experimental section*. Detailed gradients and retention for each compound: 1) for compound **5**, isocratic elution ($MeCN:H_2O$(0.1% TFA) = 75:25), retention time(RT) 4.757min; 2) for compound **19**, gradient elution ($MeCN:H_2O$ (0.1% TFA) = 90:10~100:0 (0-10min), 100:0 (10-15min)), RT 5.619min; 3) for compound **20**, gradient elution ($MeCN:H_2O$ (0.1% TFA) = 85:15~95:5 (0-15min)), RT 6.638min; 4) for compound **2**, gradient elution($MeCN:H_2O$ (0.1% TFA) = 95:15~100:0 (0-5min), 100:0 (5-20min)), RT 9.910min.

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relatively low recovery of **21** released from **2**. The purity of **2** was already decreased due to impurity accumulation, so the actual release process was likely more efficiently practiced than the apparent moderate detachment (72% recovery).

b): Baseline inequality phenomena were commonly observed not only in the HPLC spectra of the new ILSOS, which was also observed in our other unpublished ILSOS works. Based on the analysis of a), such inequality was likely caused by the systematically accumulated large polar impurities, for baseline inequality was more obviously recorded in larger supported oligomer's spectra. At least, there was a strong positive correlation between impurity accumulation and baseline inequality, which could be a possible mechanism explanation of HPLC results. Besides, ionic liquid support itself as an organic salt could possibly contribute to such baseline inequality in part.

c): Based on the analysis above, the purity of each oligomer just couldn't be regularly precisely calculated by HPLC because of the uncertainty between baseline inequality and suspicious signal peaks, as well as the exaggerated response factor difference between supported oligomers and impurities, especially the inorganic impurities which acquired few or none aromatic rings. So only a rough purity estimation could be proceeded on the deduction from baseline inequality, column's death column *etc.* Rough estimated purity of each oligomer was listed in **Table 1**, and final purity of supported **2** was about 80%.

By far, the overall yield, consumed time, assembly scale and estimated purity of core pentasaccharide **21** were summarized in **Table 1**. Released **21** was prepared in 670min with an overall 64.4% yield, a final purity of about 80% (for supported oligomer **2**), and in about 10g scale, conclusively.

Conclusion

In summary, we have developed a new ILSOS strategy to assemble an N-glycan core pentasaccharide of direct applicability potential based on our previously established ILSOS method. Apart from the advantages of efficiency and rapidness, the new ILSOS allows the efficient and orthogonal removal of IL support and large scale preparation of complex hetero-branching oligosaccharides. The combination of this new ILSOS with orthogonal protection strategy[74] could find application in constructing structurally complex sugar molecules like N-glycans in a more efficient way and on a larger scale.

Experimental section

General experimental methods

All chemicals were purchased as reagent grade and used without further purification, unless otherwise noted. Dichloromethane was distilled over calcium hydride. Analytical TLC was performed on silica Gel 60 F_{254} pre-coated on glass plates, with detection by fluorescence and/or by staining with 5% concentrated sulfuric acid in EtOH. Column chromatography was performed employing silica gel (300-400 mesh). ^{1}H NMR and ^{13}C NMR spectra were recorded on Advance spectrometers. Chemical shifts (in ppm) were referenced with tetramethylsilane (δ = 0 ppm) for ^{1}H NMR and $CDCl_3$ (δ = 77.00 ppm) for ^{13}C NMR in deuterated chloroform. High-resolution mass spectrometry was performed on FTICR mass spectrometer.

General ILSOS separation procedure

After filtration and concentration of the reaction mixture, the residue was dissolved in small volume of CH_2Cl_2 (about 2mL/g), followed by addition of 5-10 equiv. volume of isopropyl ether in shaking until no more visible precipitate was generated, which indicated the full precipitation of each oligomer. After centrifugation (3000 r/min, 10 min, 3 times), the precipitate was collected and became a sticky syrup.

General HPLC condition:

HPLC analyses were performed on an Agilent 1260 infinite analyzer with an Agilent ZORBRAX SB-C18 column (5μm, 4.6×250mm), detection wavelength of 220nm, mobile phase of MeCN/H_2O (0.1% TFA) and a constant flow rate of 1mL/min.

Selected synthetic processes and compound data

5-aminopentyl α-D-mannopyranosyl-(1→3)-[α-D-mannopyranosyl-(1→6)]-β-D-mannopyranosyl-(1→4)-2-*deoxy*-2-acetimido-β-D-glucopyranosyl-(1→4)-2-*deoxy*-2-acetimido-β-D- glucopyranoside (1) The global deprotection of **21** to give **1** was proceeded via 4 conventional steps: 1) To a solution of **21** (500 mg, 0.2 mmol, 1.0 equiv.) in EtOH (10 mL) was added ethylenediamine (337 μL, 5.06 mmol, 25.0 equiv.). The reaction was refluxed at 100 °C for 12h. After cooled down to RT, the solution was neutralized by adding AcOH, and concentrated; 2) Residue from 1) was dissolved in pyridine (10 ml), followed by adding Ac_2O (5 ml) and a catalytic amount of DMAP. The mixture was stirred *o.n.*. MeOH was added to quench acetylation. The solution was concentrated. Residue was dissolved in DCM, washed with 1N $HCl_{a.q}$, *sat.* $NaHCO_{3a.q.}$ and brine sequentially. Organic layer was collected, dried over Na_2SO_4, concentrated and purified via flash column chromatography on silica gel (PE:EA=1:2), giving a light yellow syrup; 3) the syrup from 2) was dissolved in MeOH/DMF (*v/v*=1:1, 10mL), followed by adding MeONa (0.1 M in MeOH), adjusting solution's pH between 8~10. The reaction was kept at 60 °C for 12h with TLC (PE:EA = 1:4) showing a single spot on the plate. 1N $HCl_{a.q.}$ was added to neutralize MeONa and the solution was concentrated, re-dissolved in DCM, filtered and concentrated; 4) Residue from 3) was co-evaporated with toluene three times, dissolved in MeOH, mixed with $Pd(OH)_2$/C, and hydrogenated under 4 atm of H_2 for 16h at RT. The mixture was filtered. Filtrate was concentrate and purified by size-exclusion chromatography (Sephadex LH-20) with dH_2O to give a white solid as **1** (136 mg, 70%). ^{1}H NMR, ^{13}C NMR and MS results were in accordance with previous report, see reference (DOI: 10.1002/chem.201001295).[75]

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N-Benzyl-N'-benzyloxycarbonyl-5-aminopentyl 2-*O*-acetyl-3,4,6-*O*-tri-benzyl-α-D-mannopyranosyl-(1→3)-[2-*O*-acetyl-3,4,6-*O*-tri-benzyl-α-D-mannopyranosyl-(1→6)-]-2,4-*O*-di-benzyl-β-D-mannopyranosyl-(1→4)-3,6-*O*-di-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3-*O*-benzyl-2-deoxy-2-phthalimido-6-{4-[3-(1-Methylimdazoliumhexafluorophospho)propyloxy]phenyl}-β-D-glucopyranoside (2) a) Donor **3** (6.30g, 12.79mmol, 3.50 equiv.) was dissolved in dry DCM (50 mL) in ice bath, followed by adding $CNCCl_3$ (6 mL) and DBU (0.6 mL). The reaction was stirred for 15min, directly concentrated and purified via a short column on silica gel (PE:EA=5:1), giving a yellow syrup (7.8 g, 96%), which was dried in vacuo for next coupling; b) Acceptor **20** (6.88g, 3.65mmol, 1.0 equiv.) was dried in vacuo, mixed with prepared intermediate in a), and dissolved in dry DCM (40 mL) in ice bath. After stirred for 10min, TfOH (32μL, 0.36mmol, 0.1 equiv.) was slowly added. The reaction was kept for 30min with TLC (DCM:MeOH=10:1) showing the absence of **20**. The reaction was warmed to RT, neutralized by adding Et_3N, and Purified via ***General ILSOS separation Procedure***, giving a yellowish syrup as **2** (10.15 g, 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.68 (s, 1H), 7.76-7.46 (m, 7H), 7.45-7.00 (m, 68H), 7.00-6.55 (m, 15H), 5.49 (s, 1H), 5.31 (s, 1H), 5.21 (s, 1H), 5.17-5.03 (m, 3H), 5.00-4.72 (m, 8H), 4.70-4.24 (m, 23H), 4.20 (d, 2H), 4.15 (d, 2H), 4.10 (s, 1H), 4.05 (s, 3H), 4.00-3.72 (m, 18H), 3.72-3.52 (m, 10H), 3.48 (d, 4H), 3.36 (d, 1H), 3.31 (s, 1H), 3.13 (s, 6H), 2.93-2.76 (m, 2H), 2.27 (s, 2H), 2.09 (s, 3H), 1.83 (s, 3H), 1.26-1.15 (m, 4H), 0.88 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.09, 169.84, 153.01, 152.52, 138.79, 138.63, 138.59, 138.52, 138.32, 138.04, 138.02, 137.89, 137.85, 137.82, 137.79, 137.73, 136.85, 136.81, 136.78, 136.61, 136.59, 133.91, 133.81, 133.67, 131.56, 131.16, 129.80, 128.66, 128.53, 128.45, 128.38, 128.30, 128.27, 128.25, 128.21, 128.10, 127.94, 127.89, 127.80, 127.75, 127.67, 127.59, 127.48, 127.34, 126.96, 123.49, 123.35, 123.14, 122.62, 122.15, 118.97, 116.03, 115.13, 101.95, 99.65, 98.29, 98.15, 97.56, 81.06, 79.58, 78.13, 77.91, 77.69, 77.35, 75.03, 74.99, 74.72, 74.68, 74.52, 74.23, 74.17, 74.06, 73.65, 73.50, 73.33, 73.21, 72.40, 71.87, 71.22, 69.12, 68.72, 68.69, 68.36, 68.16, 67.05, 66.48, 64.26, 60.44, 56.52, 55.71, 52.43, 50.36, 47.29, 47.02, 46.03, 36.38, 29.61, 28.82, 27.55, 27.16, 22.92, 21.06, 20.79, 14.25, 8.74, 7.31. HRMS *m/z* calcd. 2685.1460 $[M\text{-}PF_6]^+$ for $C_{160}H_{166}N_5O_{33}$, found 2685.1705. HPLC gradient elution: MeCN:H_2O (0.1% TFA)= 95:5~100:0 (0-5min), 100:0 (5-20min); purity: 80%.

N-Benzyl-N'-benzyloxycarbonyl-5-aminopentyl 3-*O*-benzyl-2-*deoxy*-2-phthalimido-6-*O*-{4-[3-(1-Methylimdazoliumhexafluorophospho)propyloxy]phenyl}-β-D-glucopyranoside (5) To a solution of **18** (4.89 g, 5.31 mmol, 1.0 equiv.) in MeCN (50 mL) was added 1-Methylimidazole (1.27 mL, 15.93 mmol, 3.0 equiv.) and KPF_6(1.17 g, 6.37 mmol, 1.2 equiv.). The reaction was refluxed *o.n.* with TLC (PE:EA=1:1) showing the absence of **18**. After cooling down, the reaction was concentrated, re-dissolved in DCM, filtered and concentrated. Residue was purified via ***General ILSOS separation Procedure,*** giving a yellowish syrup as **5** (5.39 g, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.46 (s, 1H), 7.71-7.57 (m, 4H), 7.35-7.20 (m, 10H), 7.18 (s, 1H), 7.14 (d, 1H),7.09 (s, 1H), 7.06-7.02 (m, 2H), 6.98-6.92 (m, 3H), 6.84 (d, 2H), 6.75 (d, 2H),5.13 (d, 1H), 5.09 (s, 2H),4.73 (d, 1H), 4.53 (d, 1H), 4.31-4.28 (m, 4H), 4.27-4.21 (m, 2H), 4.20-4.08 (m, 2H), 3.89 (t, 2H), 3.83 (d, 1H), 3.80 (s, 3H), 3.77-3.71 (m, 2H), 3.68 (s, 1H), 3.30 (s, 1H), 2.90-2.81 (m, 2H), 2.31-2.21 (m, 2H), 1.37-1.19 (m, 4H), 1.02-0.88 (m, 2H). ^{13}C NMR (100 MHz, CDCl3) δ: 153.39, 152.71, 138.24, 136.32, 134.04, 128.64, 128.35, 128.01, 127.87, 127.65, 127.39, 123.59, 122.65, 116.04, 115.51, 98.55, 79.32, 77.35, 74.64, 74.29, 72.69, 67.19, 64.48, 55.74, 47.36, 36.33, 29.56, 23.10. HRMS *m/z* calcd. 923.4226 $[M\text{-}PF_6]^+$ for $C_{54}H_{59}N_4O_{10}$, found 923.4207. HPLC isocratic elution: MeCN:H_2O (0.1% TFA) = 75:25 (0-15min); purity: 99%.

N*-Benzyl-*N'*-benzyloxycarbonyl-5-aminopentyl 3,6-*O*-di-acetyl-2,4-*O*-dibenzyl-β-D-mannopyranosyl-(1→4)-3,6-*O*-di-benzyl-2-*deoxy*-2-phthalimido-β-D-glucopyranosyl-(1→4)-3-*O*-benzyl-2-*deoxy*-2-phthalimido-6-*O*-{4-[3-(1-Methylimdazoliumhexafluorophospho)propyloxy]phenyl}-β-D-glucopyranoside (19)** a) Donor **4** (5.41g, 5.88 mmol, 1.6 equiv.) was dissolved in dry DCM (30mL) in ice bath, followed by adding CNCCl3 (3 mL) and DBU (0.2 mL). The reaction was kept for 30min with TLC (PE:EA=2:1) showing the absence of **4**. The reaction was directly concentrated and purified via a short column on silica gel (PE:EA=3:1), giving a yellow syrup (5.71g, 91%), which was dried in vacuo for next coupling; b) Acceptor **5** (4.0 g, 3.74 mmol, 1.0 equiv.) was dried in vacuo, mixed with previously prepared intermediate in a) and dissolved in dry DCM (50mL) in ice bath. After stirred for 10min, TfOH (34 μL, 0.37 mmol, 0.1 equiv.) was slowly added. The reaction was kept for 30min with TLC (PE:EA =10:1) showing the absence of **5**. The reaction was neutralized by adding Et_3N and concentrated. Residue was purified via ***General ILSOS separation Procedure, giving a yellowish syrup as **19** (7.21 g, 96%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.65 (s, 1H), 7.79-7.59 (m, 4H), 7.49 (m, 4H), 7.40-7.16 (m, 30H), 7.14-7.02 (m, 3H), 6.96 (d, 2H), 6.86-6.63 (m, 13H), 5.26 (d, 1H), 5.07 (s, 2H), 4.95 (d, 1H), 4.87 (d, 2H), 4.81 (d, 1H), 4.75 (dd, 1H), 4.70-4.60 (m, 3H), 4.52 (dt, 5H), 4.33 (dd, 6H), 4.25-3.98 (m, 10H), 3.96-3.71 (m, 9H), 3.83 (s, 3H), 3.67-3.52 (m, 2H), 3.48 (dd, 1H), 3.37-3.33 (m, 2H), 3.15 (td, 4H), 2.91-2.74 (m, 1H), 2.35-2.22 (m, 2H), 1.93 (s, 3H), 1.84 (s, 3H), 1.27-1.21 (m, 4H), 0.88 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 170.85, 170.23, 153.06, 152.60, 138.92, 138.67, 138.54, 137.97, 137.88, 137.84, 136.52, 134.00, 133.73, 131.62, 128.65, 128.56, 128.52, 128.37, 127.98, 127.92, 127.84, 127.78, 127.62, 127.31, 126.97, 123.52, 122.63, 116.00, 115.24, 101.03, 98.23, 97.62, 79.24, 77.35, 76.11, 76.00, 74.87, 74.76, 74.70, 74.64, 74.57, 73.73, 73.40, 73.27, 73.16, 68.41, 68.25, 64.37, 63.23, 56.53, 55.78, 52.47, 47.31, 47.04, 36.33, 29.63, 28.87, 21.02, 20.73, 8.75, 7.28. HRMS *m/z* calcd 1820.7586 $[M\text{-}PF_6]^+$ for $C_{106}H_{110}N_5O_{23}$, found 1820.7593. HPLC gradient elution: MeCN:H_2O (0.1% TFA) = 90:10~100:0 (0-10min), 100:0 (10-15min); purity: 95%.

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***N*-Benzyl-*N'*-benzyloxycarbonyl-5-aminopentyl2,4-*O*-di-benzyl-β-D-mannopyranosyl-(1→4)-3,6-*O*-di-benzyl-2-*deoxy*-2-phthalimido-β-D-glucopyranosyl-(1→4)-3-*O*-benzyl-2-*deoxy*-2-phthalimido-6-*O*-{4-[3-(1-Methylimdazoliumhexafluorophospho)propyloxy] phenyl}-β-D-glucopyranoside (20)** To a solution of **19** (6.67g, 3.39mmol, 1.0 equiv.) in MeOH/DCM (*v/v*=2:1, 60 mL) was added MeONa (0.1 M in MeOH), adjusting solution's pH between 8~10. The reaction was kept for 3h with TLC (DCM:MeOH=10:1) showing the completion of de-acetylation. 1N $HCl_{a.q.}$ was added to neutralize MeONa. The solution was concentrated, re-dissolved in DCM, filtered and concentrated, giving a yellow syrup as **20** (6.88 g, quant.). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.62 (s, 1H), 7.80 (d, 1H), 7.68 (dd, 3H), 7.53 (s, 4H), 7.39-7.14 (m, 28H), 7.13-7.03 (m, 2H), 6.98 (d, 2H), 6.89 (s, 5H), 6.75 (dt, 6H), 5.28 (d, 1H), 5.07 (s, 1H), 4.99-4.92 (m, 1H), 4.92-4.85 (m, 2H), 4.80 (d, 1H), 4.62 (d, 1H), 4.58-4.44 (m, 5H), 4.36-4.26 (m, 4H), 4.26-4.14 (m, 4H), 4.08 (d, 2H), 4.02-3.97 (m, 1H), 3.90 (s, 2H), 3.87-3.71 (m, 7H), 3.68 (d, 2H), 3.60 (d, 3H), 3.48 (dd, 2H), 3.39 (dd, 3H), 3.12 (dd, 4H), 2.88-2.75 (m, 2H), 2.25 (s, 2H), 1.30-1.11 (m, 4H), 0.95-0.80 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 152.99, 152.61, 138.64, 138.43, 138.33, 138.29, 137.64, 136.50, 134.28, 134.22, 133.75, 131.58, 131.57, 128.70, 128.64, 128.57, 128.49, 128.29, 128.13, 128.08, 128.04, 128.01, 127.94, 127.93, 127.87, 127.81, 127.77, 127.65, 127.30, 127.17, 127.08, 126.98, 123.56, 123.22, 122.61, 115.94, 115.25, 101.25, 98.24, 97.56, 78.88, 78.43, 77.35, 76.58, 75.30, 74.75, 74.70, 74.60, 74.25, 73.69, 73.48, 68.04, 67.09, 64.35, 62.35, 56.42, 55.76, 52.48, 52.45, 52.42, 47.30, 46.86, 36.40, 30.38, 29.63, 28.85, 22.96, 8.75, 7.32. HRMS *m/z* calcd 1736.7375 $[M\text{-}PF_6]^+$ for $C_{102}H_{106}N_5O_{21}$, found 1736.7381. HPLC gradient elution: MeCN:H_2O (0.1% TFA) = 85:15-95:5 (0-15min); purity: 90%.

***N*-Benzyl-*N'*-benzyloxycarbonyl-5-aminopentyl 2-*O*-acetyl-3,4,6-*O*-tri-benzyl-α-D-mannopyranosyl-(1→3)-[2-*O*-acetyl-3,4,6-*O*-tri-benzyl-α-D-mannopyranosyl-(1→6)]-2,4-*O*-dibenzyl-β-D-mannopyranosyl-(1→4)-3,6-*O*-di-benzyl-2-*deoxy*-2-phthalimido-β-D-glucopyranosyl-(1→4)-3-*O*-benzyl-2-*deoxy*-2-phthalimido-β-D- glucopyranoside (21)** To a solution of **2** (10.09 g, 3.57 mmol, 1.0 equiv.) in 80% $MeCN_{a.q.}$ (100 mL) was added CAN (7.82 g, 14.27 mmol, 4.0 equiv.). The reaction was kept for 10min with TLC (DCM:MeOH=10:1) showing the absence of substrate. The reaction was diluted with DCM, washed with brine, dried over Na_2SO_4, concentrated and purified via column chromatography on silica gel (PE:EA=5:1), giving a yellow syrup as **21** (6.35 g, 72%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.76 (d, 1H), 7.72-7.60 (m, 4H), 7.53 (s, 4H), 7.42 (d, 2H), 7.37-7.04 (m, 67H), 6.91 (s, 2H), 6.83 (d, 2H), 6.73 (s, 3H), 6.67 (d, 1H), 6.62 (t, 2H), 5.49 (s, 1H), 5.32 (s, 1H), 5.26 (d, 1H), 5.08 (t, 4H), 4.92 (s, 1H), 4.89-4.74 (m, 7H), 4.68 (d, 2H), 4.63-4.50 (m, 7H), 4.43 (m, 11H), 4.33 (d, 3H), 4.23-4.13 (m, 3H), 4.13-4.06 (m, 3H), 4.06-3.94 (m, 4H), 3.94-3.87 (m, 3H), 3.86-3.76 (m, 5H), 3.75-3.68 (m, 2H), 3.68-3.53 (m, 9H), 3.52-3.34 (m, 4H), 3.25-3.11 (m, 3H), 2.89 (s, 1H), 2.79 (s, 1H), 2.09 (s, 3H), 1.80 (s, 3H), 1.70 (s, 2H), 1.30-1.12 (m, 4H), 1.01-0.82 (m, 2H). ^{13}C NMR (100 MHz, CDCl3) δ: 170.17, 169.86, 138.88, 138.76, 138.71, 138.64, 138.43, 138.14, 138.08, 137.99, 137.91, 133.81, 133.79, 133.69, 133.67, 133.65, 133.63, 131.92, 131.65, 131.58, 128.69, 128.58, 128.52, 128.49, 128.45, 128.33, 128.30, 128.26, 128.19, 128.07, 128.00, 127.92, 127.89, 127.82, 127.74, 127.59, 127.48, 127.45, 127.20, 127.00, 126.90, 123.45, 123.33, 123.32, 123.27, 123.20, 123.14, 101.99, 99.81, 98.38, 98.28, 97.58, 81.41, 79.72, 78.20, 77.95, 77.81, 77.35, 76.98, 76.42, 76.11, 75.11, 75.01, 74.91, 74.69, 74.60, 74.47, 74.31, 74.17, 73.62, 73.41, 73.22, 72.39, 71.97, 71.90, 71.31, 69.43, 69.25, 68.81, 68.25, 67.17, 66.57, 61.01, 56.54, 55.84, 28.93, 22.99, 21.14, 20.84. HRMS *m/z* calcd 2488.0619 $[M+NH_4]^+$ for $C_{147}H_{155}N_4O_{32}$, found 2488.0689.

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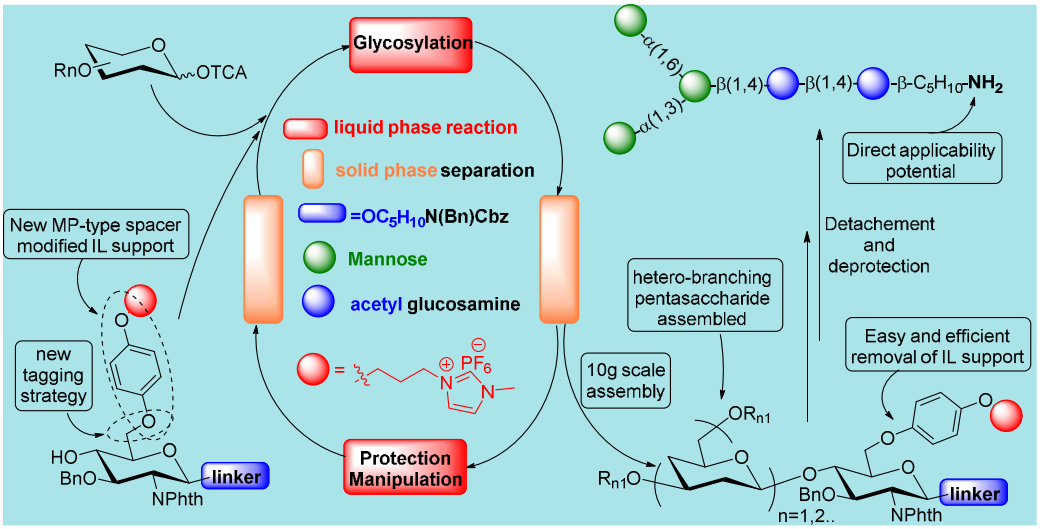

Hetero-branched N-glycan core pentasaccharide was rapidly assembled on new ionic liquid support in 10g scale.