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Combinatorial ionic catch-and-release oligosaccharide synthesis (combi-ICROS)†‡

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A combinatorial ionic-liquid-supported ''catch-and-release'' strategy for oligosaccharide synthesis (combi-ICROS) is reported. A series of β-(1→6) glucan di-, tri- and tetra-saccharides were synthesized in one pot to showcase the versatility of the strategy.

Methodologies that can provide rapid access to structurally defined oligosaccharides for biological screening are greatly lacking.[1,2] The challenges associated with carbohydrate synthesis, including laborious protecting group manipulation, the need for regio- and stereoselective glycosylation reactions and most notably the requirement for purification after each step, normally accomplished by chromatography, are primarily responsible for the lack of more intensive efforts. Combinatorial chemistry has become an important tool in modern drug development and although carbohydrate-based compounds hold great potential as therapeutic agents due to their involvement in a myriad of physiological and pathological processes,[3] the application of combinatorial chemistry to this class of biomolecules remains limited. Nature offers the best example of combinatorial chemistry, where glycan chains are assembled by glycosyltransferases with exquisite regio- and stereocontrol, resulting in remarkably heterogeneous glycoforms.[4]

Herein, we present a novel ionic-liquid based ''catch-and-release'' strategy for the one-pot combinatorial synthesis of oligosaccharides. This method is based on the use of covalently attached ionic-liquid tags (ITags) as soluble supports that allows fast, chromatography-free product purification and *in situ* reaction monitoring. We demonstrate the versatility and generality of this technique in the synthesis of a series of β-(1→6) glucans, which are biologically relevant fungal cell-wall components.[5]

Ionic liquids (ILs) have attracted enormous interest over the past few years because of their use in a broad number of synthetic and enzymatic applications.[6–11] The unique physical and chemical properties of ILs, which can be tuned by altering the cation or the anion structure, make this class of molecules particularly useful as new vehicles for the immobilisation of reagents.[12]

More recently, strategies based on the use of ILs as soluble functional supports, termed ITags, applied to oligosaccharide synthesis have been developed.[13–19] The IL-based approaches have shown great promise as they combine the ability to carry out reactions in solution with the additional advantage of fast, chromatography-free purification. Ionic-liquid-labeled compounds (ITag-compounds) are soluble in polar solvents used in glycosylation reactions (*i.e.* dichloromethane, acetonitrile) but in the absence of the polar solvent, the ITag-materials become insoluble in less polar solvents such as diethyl ether, isopropyl ether or hexane, which means that non-ITag-materials (excess reagents, unreacted material) can be removed from the ITag-products by simple biphasic extraction or by precipitation.

Our group has recently reported the first example of ionic-liquid-supported ''catch-and-release'' oligosaccharide synthesis (ICROS), where the ionic-liquid-purification labels (ITags) are introduced at the anomeric position of the reducing end of the oligosaccharide target.[13,14,20] The methodology was shown to be compatible with both chemical and enzymatic processes (Fig. 1).

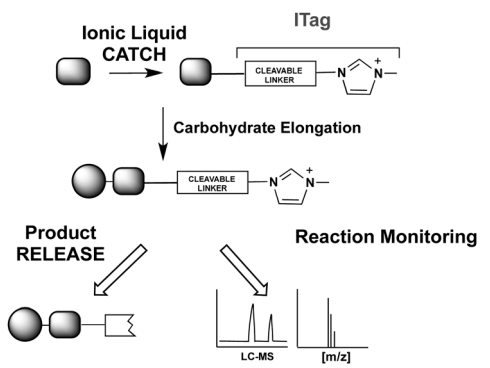

Fig. 1 ICROS methodology.

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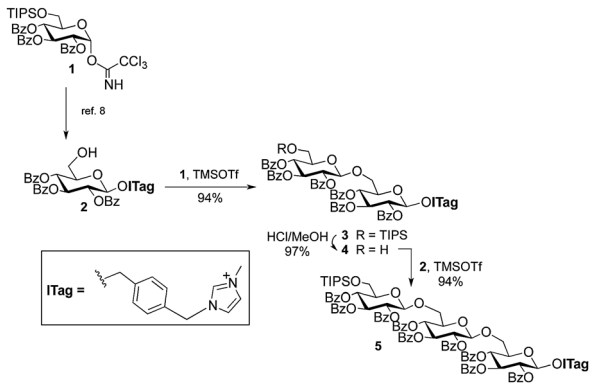


Scheme 1 Sequential ICROS.

A key feature of ICROS, in addition to the fast and chromatography-free purification, is that this strategy allows for simple and *in situ* reaction progress monitoring by mass spectroscopy (MS), HPLC and NMR, which is a great advantage over other traditional supported methodologies.[21–23]

Based on our previous investigations, we envisioned that ICROS would be ideally suited for the combinatorial synthesis of oligosaccharides in one pot.

In order to test our hypothesis, a series of β-(1→6) glucan linear mono-, di- and trisaccharides **2–5** were synthesized sequentially by ICROS and individually purified and characterized. In this instance a benzyl derived IL-tag (ITag)[15] was chosen as the cleavable purification label (Scheme 1).

ITagged-glycoside acceptor **2**, which was prepared in 2 steps from **1** in 84% yield,[15] was glycosylated with trichloroacetimidate glycosyl donor **1** in the presence of TMSOTf (0.3 equiv.) affording disaccharide **3**[15] in 94% yield, exclusively as the β-anomer due to the presence of the acyl group at C-2, which ensured neighbouring group participation. Selective 6-OH unmasking of **3** by *O*-TIPS removal using a mixture of HCl in MeOH provided pure acceptor **4** in 97% yield after simple washes. Subsequent glycosylation with **2** under the same catalytic conditions as before afforded trisaccharide **5** in 94% yield. Each individual compound was purified at each stage by simple phase extraction and fully characterized by NMR and MS (see ESI‡ for details). Compounds **2–5** were subjected to HPLC analysis and it was determined that the retention time (R_t) of the different species, while still covalently linked to the IL, was sufficiently different to be individually monitored by HPLC (Fig. 2).

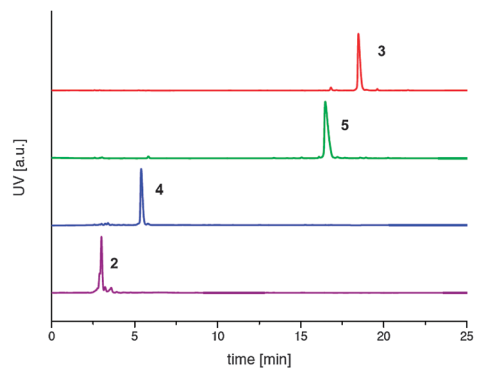
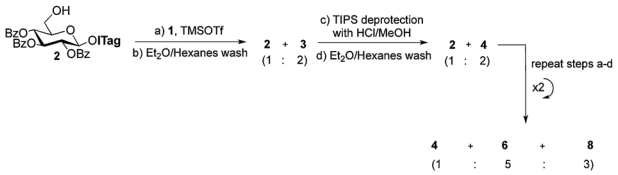


Fig. 2 HPLC traces of individual ITag-oligosaccharides.



Scheme 2 One-pot combinatorial synthesis of β-(1→6) glucans.

Encouraged by these results and having shown that purification of ITag-products from non-ITag-materials is fast and simple, we decided to apply this methodology to the one-pot combinatorial synthesis of a series of β-(1→6) glucans (Scheme 2).

To that end, glycosylation of glucoside acceptor **2** with **1** in the presence of 0.2 equiv. of TMSOTf in DCM was monitored by MALDI-TOF, HPLC and ^{1}H-NMR until a 1 : 2 ratio of ITag-compounds **2** ([M$^+$] 677.2; R_t = 2.8 min; H-1 δ 5.05 ppm, J 8.1 Hz) and **3** ([M$^+$] 1307.5; R_t = 16.8 min; H-1^A δ 5.00 ppm, J 7.9 Hz and H-1^B δ 4.90 ppm, J 8.0 Hz) was obtained.[24] The solvent was then removed and the non-ITag materials were washed away with a diethyl ether–hexane (1/1, v/v) solvent mixture. Selective *O*-TIPS removal with HCl in MeOH afforded a 1 : 2 mixture of **2** and **4** ([M$^+$] 1151.4; R_t = 5.5 min; H-1^A δ 4.92 ppm, J 8.0 Hz and H-1^B δ 4.73 ppm, J 8.0 Hz), which after purification by simple non-polar solvent washes was ready to be further glycosylated.

The reaction sequence of glycosylation with **1**, 6-OH selective deprotection and solvent washes was performed with careful monitoring by MALDI-TOF and HPLC for another two cycles (see Fig. 3) until the final mixture contained a 1 : 5 : 3 ratio of disaccharide **4**, trisaccharide **6** ([M$^+$] 1625.5; R_t = 12.4 min; H-1^A δ 4.88 ppm, J 7.9 Hz, H-1^B δ 4.98 ppm, J 8.0 Hz and H-1^C δ 4.99 ppm, J 7.9 Hz) and tetrasaccharide **8** ([M$^+$] 2099.6, R_t = 15.2 min; H-1^A δ 4.89 ppm, J 8.0 Hz, H-1^B δ 4.96 ppm, J 7.7 Hz, H-1^C δ 5.01 ppm, J 8.1 Hz and H-1^D δ 5.04 ppm, J 8.1 Hz) as the major components (as confirmed by NMR).[24]

The different size oligomers were separated at the final stage of the synthesis using size-exclusion chromatography. Thus, the mixture of compounds was subjected to purification on a Sephadex LH-20, and targets **4**, **6** and **8** were isolated in 10%, 30% and 14% yield, respectively, from starting material **2**.

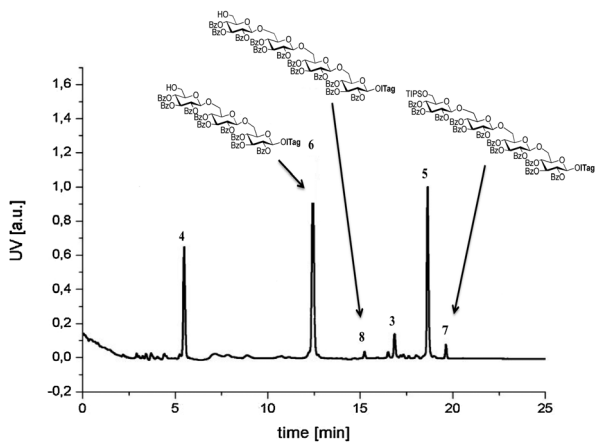
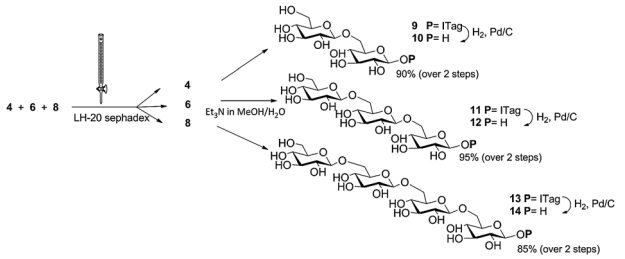


Fig. 3 Typical HPLC traces of a one-pot β-(1→6)-glucan reaction mixture between glycosylation cycles.



Scheme 3 Deconvolution of an oligosaccharide mixture.

Global deprotection of the ester groups was accomplished using NaOMe in MeOH followed by cleavage of the ionic-liquid-component in the presence of Pd/C in a mixture of water–methanol and 5% HCl_{aq} to afford the hemiacetals **10**, **12** and **14** in yields of 85–95% over 2 steps (Scheme 3).

The use of a benzyl type linker as a cleavable functionality between the glycoside and the ionic liquid provides the ITag with enough chemical stability to withstand a wide range of chemical conditions, while product release remains efficient and in a form suitable for further oligosaccharide manipulation.

In summary, the use of the ionic-liquid-supported oligosaccharide synthesis shows great promise. ICROS offers several advantages over other supported methods: (a) purification is fast and chromatography-free, since non-ITag-materials can be selectively washed away with the appropriate solvents, (b) chemoselective protecting group manipulation and (c) chemical glycosylation reactions are performed under conditions typically used for solution-phase chemistry and reaction progress can be monitored *in situ*, which offers the opportunity to better control the oligosaccharide elongation process.

We have shown here that ICROS is ideally suited for the combinatorial synthesis of small libraries of oligosaccharides and we exemplified this strategy in the preparation of a series of β-1,6-glucan oligosaccharides in one pot. Moreover, we demonstrated that HPLC in combination with MALDI-TOF and NMR can be used to efficiently monitor reaction progress *in situ* and that several ITag-species can be monitored at once in a reaction mixture, further demonstrating the versatility of the ITags, since the IL-labels not only act as purification supports but also provide a handle for MS detection.[13,14]

Overall the compounds were prepared in one pot, in a matter of days, without the use of silica gel chromatography purification in between steps. Efforts are currently underway to apply the ICROS methodology to more complex targets.

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