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Tunable acid-sensitive ester protecting groups in oligosaccharide synthesis†

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A series of acid-cleavable ester-type protecting groups, with acid-sensitivity profiles parallel to those of 2-naphthylmethyl (NAP) or *p*-methoxybenzyl (PMB) ether, were designed and TFA in toluene was identified as a technically simple and effective deblocking cocktail for their global removal in the context of oligosaccharide synthesis.

Chemical synthesis of complex carbohydrates constitutes a powerful means to provide homogeneous glycans for their functional studies in an age of glycomics.[1] The recent emergence of new synthetic tactics and strategies has notably accelerated the preparation of carbohydrates in solution and on solid supports,[2] however the overall efficiency associated with oligosaccharide assembly remains unmatched to the efficiencies of oligonucleotide and oligopeptide synthesis. The practical applicability of automated nucleic acid and peptide synthesis is a combined result of the development of highly efficient coupling reagents and robust protecting group chemistry.[3] While there has been a collective effort to improve the efficiency of chemical glycosylations,[4] strategic development of protecting group chemistry to parallel the efficiency in preparing oligonucleotides and oligopeptides lags behind.

The protecting group strategy in modern oligosaccharide synthesis is to rely on highly acid/base-stable benzyl ethers as the dominant protecting groups for hydroxyl functionalities. A further set of orthogonal protecting groups are applied that serve their capabilities, either temporarily or permanently, in ensuring regioselectivity and stereoselectivity as well as tuning the reactivity of glycosylating agents during chemical glycosylation events.[5] Due to the introduction of a diverse set of orthogonal protecting groups, the lack of efficiency in protecting group manipulation is markedly notable at the late stage of any given oligosaccharide synthesis where multistep transformations are generally required to release the native carbohydrate molecules. This practice is cumbersome and requires considerable synthetic skill to execute successfully.[6] In contrast, standardized single-step acid- or base-mediated global deprotection procedures are widely adopted in peptide and nucleic acid chemistry, which take advantage of the identical acid- or base-sensitive profiles of permanent protecting groups used therein.[7] To address this practical efficiency gap, we envision the possibility of designing and implementing a defined set of acid-cleavable permanent protecting groups by strategically leveraging the reactivity space that exists when applying the acidic conditions used in hydrolysing oligosaccharides and chemical glycosylations. In this communication, we report the generation and application of a series of acid-cleavable ester-type protecting groups, of which the acid-sensitive profiles are tuned to be in sync with those of 2-naphthylmethyl (NAP) or *p*-methoxybenzyl (PMB) ether. The combination of these designer esters with NAP/PMB ethers allows for the establishment of a versatile permanent protecting group inventory. It also elevates the efficiency and practical applicability of late stage protecting group manipulation for hexose-containing oligosaccharide assemblies to a level that has been traditionally reserved for peptide and nucleic acid synthesis.

Protic acids such as trifluoroacetic acid (TFA), commonly used for global deblocking events in peptide chemistry,[7b] are rarely explored analogously in oligosaccharide chemistry. This is in part due to the perception that an oligosaccharide molecule is prone to acid-mediated hydrolysis,[8] but is also due to the fact that the inventory of practically useful acid-labile protecting groups in carbohydrate chemistry is restricted to acetals and silyl- and PMB-type ethers that possess limited stability in Lewis acid-mediated chemical glycosylation.[9,10] We recently discovered that NAP ether is readily cleaved from an oligosaccharide backbone with a 20 equivalent excess of HF·Py in toluene under ambient conditions without compromising a glycosidic bond, while remaining stable in the presence of a stoichiometric amount of strong Lewis acid (TMSOTf or $BF_3{\cdot}OEt_2$).[11] This unique reactivity of NAP ether towards acids suggests the possibility of using it as

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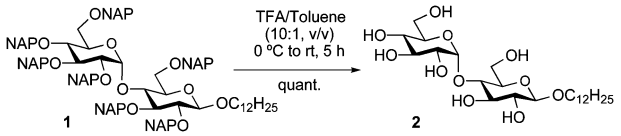
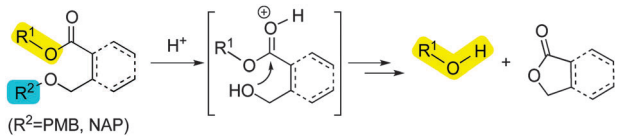


Scheme 1 Efficient global removal of NAP ethers in dodecyl maltoside **1** by TFA in toluene.

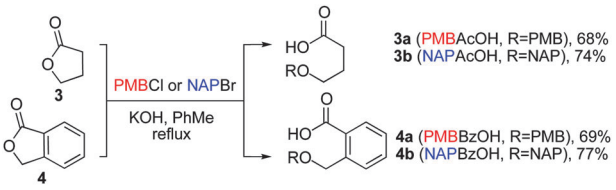
an acid-cleavable global protecting group in oligosaccharide synthesis. To test the feasibility of this approach, we screened a panel of protic acid conditions which were devoid of HF. This effort led us to identify TFA in toluene (10 : 1, v/v) as an effective cocktail to unmask all NAP ethers in an oligosaccharide, as demonstrated by their quantitative removal under ambient conditions[12] from a per-NAP protected dodecyl maltose **1** (Scheme 1).

The acidic strength of TFA in toluene (10 : 1, v/v) resides distinctly between the strengths of acids required for hexose-containing oligosaccharide hydrolysis[13] and acids for chemical glycosylations, implicating its potential as a practically useful global deblocking cocktail in oligosaccharide synthesis, reminiscent of those used in peptide chemistry.[7b] To generalize this approach, it is necessary to have a collection of readily accessible protecting groups that share analogous acid-sensitivities to NAP-ethers. Among the most widely utilized protecting groups for the hydroxyl functionality in carbohydrate chemistry are esters, including acetate, benzoate and pivaloate, which are immune to TFA in toluene (*vide infra*). As esters play indispensable roles beyond simple "protections" by balancing the reactivity of chemical glycosylating agents, as well as serving as effective stereo-directing groups for 1,2-*trans* glycosidic bond formation,[14] we set out to develop a series of designer esters that retain their essential properties as acetates and benzoates, yet can be readily released by exposure to TFA in toluene.

Our design principle (Scheme 2) for engineering acid-labile esters was inspired by early works relating to pilocarpine prodrugs, in which intramolecular cyclization of pilocarpic acid esters to form pilocarpine occurs rapidly over a broad range of pHs in aqueous media.[15] Although relayed deprotection of modified esters has been explored in synthetic carbohydrate chemistry, precedent studies have extensively focused on their development in the context of temporary protection and lack modularity and practical efficiency in their preparation.[16] We rationalize that acid-triggered 5-membered lactone formation is a versatile route for modular design of tunable acid-sensitive esters where, the acid-sensitivity difference between PMB and NAP ethers that masks the latent hydroxyl nucleophile can be strategically explored for creating two sets of otherwise-identical esters with different profiles towards acid (Scheme 2). In addition, the rate of lactone formation can be readily tuned by incorporating



Scheme 2 Design principle for tunable acid-sensitive esters.



Scheme 3 Efficient single-step preparation of PMB/NAP-modified acetic and benzoic acid homologues (**3a–b** and **4a–b** respectively).

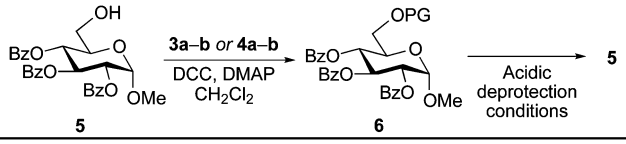
steric elements[17] that allow for the generation of analogous acetate and benzoate esters with reversed cleavage kinetics, assisted by acid rather than base (*vide infra*).

To validate our design, we first set out to establish a practical route to generate the acylating agents used for accessing the target designer esters (Scheme 3). We identified that alkylative opening of readily available[18] γ-butyrolactone **3** and phthalide **4** with PMBCl or NAPBr and KOH in toluene constitutes a highly efficient single-step method to prepare PMB- and NAP-capped 4-hydroxybutanoic acids (**3a** and **b**) as well as 2-(hydroxymethyl)benzoic acid analogues (**4a** and **b**). All target carboxylic acids can be obtained in pure forms and good yields after a simple extraction-based workup protocol (ESI† method). Carboxylic acids **3a**/**3b** and **4a**/**4b** were abbreviated as PMBAcOH/NAPAcOH and PMBBzOH/NAPBzOH respectively to highlight their structural characteristics, including their steric similarities to acetic and benzoic acid, as well as the identity of the acid-labile trigger groups. Installation of **3a**, **3b**, **4a** and **4b** into a carbohydrate backbone was readily achieved using DCC mediated esterification to afford a series of 6-*O*-designer-ester capped 1-*O*-methyl 2,3,4-tri-*O*-benzoyl glucosides **6** in excellent yields (Table 1, **5** → **6**).

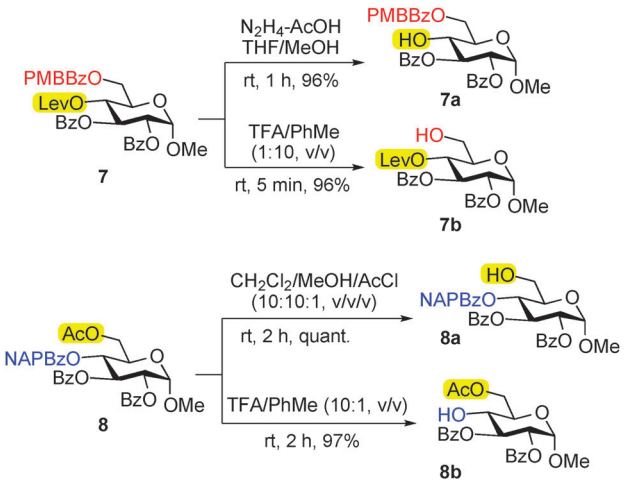
Acid-assisted cleavage of the designer esters **6** was subsequently evaluated with low and high concentrations of TFA in toluene (1 : 10 and 10 : 1, v/v respectively) under ambient conditions. Consistent with our designs, a marked difference in the sensitivity towards TFA was observed between the PMBAc and NAPAc groups. At a low concentration of TFA, PMBAc was readily removed within 1 h (Table 1, entry 1) and NAPAc remained completely stable (Table 1, entry 3a). Clean removal of NAPAc from **6c** required 8 hours exposure to a high concentration of TFA (Table 1, entry 3b), analogous to that used for NAP ether. Neither low nor high concentrations of TFA affect the stability of classical benzoyl esters embedded in **6**, or cause acyl migrations. Cleavage of the PMBBz and NAPBz groups was accelerated 12- and 5-fold (Table 1, entries 2 and 4) respectively, compared with their Ac analogues under otherwise identical conditions. This observation is in agreement with our expectation that increased steric constraint poised by the phenyl ring in 2-(hydroxymethyl)benzoyl ester promotes lactone formation.

With the acid-sensitivity profiles of designer esters elucidated, we proceeded to examine in depth their compatibility with other known esters in addition to benzoate (Scheme 4). Differentially protected glucosides **7** and **8** were prepared from 1-*O*-methyl 2,3-di-*O*-benzoyl glucoside (ESI† method). Orthogonality between PMBBz and levulinate (Lev) was demonstrated using glucoside **7**, where regiospecific removal of Lev (**7** → **7a**) and PMBBz (**7** → **7b**)

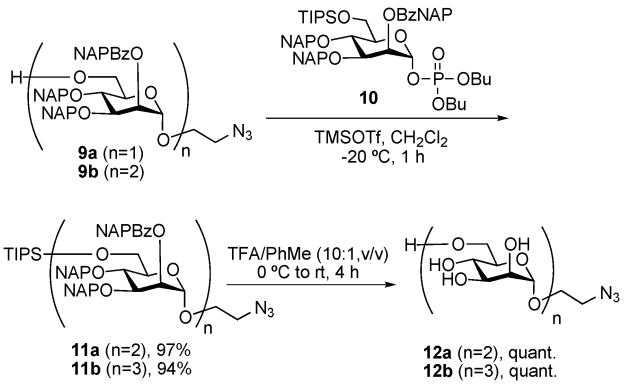
Table 1 Preparation of designer ester modified glucosides **6** and their cleavage by TFA in toluene



Entry	**5** → **6** yield	**6** → **5** conditions, yield
1	**6a** (Protecting group, PG = PMBAc), 93%	TFA/PhMe (1 : 10, v/v), rt, 1 h, 98%
2	**6b** (PG = PMBBz), 97%	TFA/PhMe (1 : 10, v/v), rt, 5 min, 98%
3	**6c** (PG = NAPAc), 95%	(a) TFA/PhMe (1 : 10, v/v), rt, 1 h, N.R. (b) TFA/PhMe (10 : 1, v/v), rt, 8 h, 94%
4	**6d** (PG = NAPBz), 97%	TFA/PhMe (10 : 1, v/v), rt, 1.5 h, 96%



Scheme 4 Evaluation of the orthogonality between designer esters (PMBBz & NAPBz) and classical esters (Ac, Lev and Bz).



Scheme 5 Application of NAPBz designer esters and NAP ethers in the stereoselective preparation of α(1–6) di- and trimannosides and their single-step global deprotection by TFA in toluene (10 : 1, v/v).

were readily achieved using hydrazine and a low concentration of TFA in toluene, respectively. Furthermore, the hydrolysis of 6-*O*-acetate in **8** was quantitatively achieved using dilute methanolic HCl in the presence of NAPBz (**8** → **8a**, Scheme 4), demonstrating that NAPBz retains the essential character of benzoate, which in comparison to acetate, is more resistant to acid- or base-catalyzed hydrolysis in water or methanol. Cleavage of 4-*O*-NAPBz in **8** by a high concentration of TFA in toluene was also equally effective, leaving 6-*O*-acetate intact (**8** → **8b**, Scheme 4).

These experiments collectively demonstrate the versatile nature of acid-sensitive designer esters that are robustly orthogonal to classical esters while retaining their essential characters, and set the stage for exploring them in complex carbohydrate assemblies. We use the preparation of linear α(1–6) mannan as a model system to illustrate the enabling features of NAPBz ester and NAP ether (Scheme 5), including their interchangeability with the classical benzoate and benzyl ether respectively and their global removal by TFA in toluene.

Glycosyl phosphate was chosen as the glycosylating agent for preparing the target di- and trimannosides **11a**/**11b**, as it requires a stoichiometric amount of strong Lewis acid (TMSOTf) for activation[19] that serves as a test ground to evaluate the stabilities of NAPBz ester and NAP ether during chemical glycosylations. The key glycosylation building block, glycosyl phosphate **10**, was readily accessible from a mannosyl tricyclic orthoester (ESI† method), along with a reducing-end mannoside **9a**. Glycosylation of **9a** with **10** provided dimannoside **11a** in excellent yield and the subsequent exposure of the primary triisopropylsilyl (TIPS) ether group in **11a** to methanolic HCl (ESI† method) quickly afforded dimannoside **9b** with the NAP-derived protecting groups intact. Combination of **9b** with **10** gave trimannoside **11b** in 94% yield. Both **11a** and **11b** are representative end products of a chemical glycosylation assembly line, where three layers of apparent orthogonality exist for hydroxyl protecting groups. This would traditionally have required three distinct chemical transformations including fluoride-mediated desilylation, Zemplen-type deacylation and hydrogenolytic debenzylation to release the native oligomannosides. By utilizing the uniform TFA-sensitive properties of NAP ether and NAPBz ester, as well as the innate acid-sensitive nature of silyl ether, conversions of **11a**/**11b** to the corresponding azide-tagged **12a**/**12b** were effectively achieved using TFA in toluene (10 : 1, v/v) in 4 hours under ambient conditions, with minimal technicalities on work up (ESI† method), to afford analytically pure compounds in quantitative yields.

In summary, we described the practical and modular generation of a series of tunable acid-sensitive ester protecting groups that are effective surrogates of acetate and benzoate. The synchronized acid-sensitive profiles of these designer esters with PMB and NAP ethers enabled the application of a single-step

TFA-mediated global deprotection protocol at the final stage of oligosaccharide assembly that emulates the synthetic efficiency traditionally reserved for peptide and nucleic acid chemistry. As glycosidic linkages between mammalian hexoses are very tolerant of TFA in toluene (10 : 1, v/v) under ambient conditions,[13] we envision that NAP ethers and NAPBz esters can readily find broad applications in complex carbohydrate assemblies by directly replacing benzyl ethers and acetates/benzoates. Further generalization of the single-step TFA-mediated global deprotection protocol in oligosaccharide chemistry would call for the development of acid-sensitive nitrogen protecting groups that are effective during chemical glycosylations. Extension of this strategy to mammalian oligosaccharides with more acid-labile glycosidic linkages (such as α-fucoside) requires the implementation of PMB ethers and PMBBz esters, which would necessitate the development of novel glycosylating agents, activatable under ''acid-neutral'' conditions.[20] The details of this work will be reported in due course.

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