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Reverse orthogonal strategy for oligosaccharide synthesis^{†,‡,§}

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Abstract

Herein, we report the invention of a novel expeditious concept for oligosaccharide synthesis. Unlike the classic orthogonal strategy based on leaving groups, the reverse approach is based on orthogonal protecting groups, herein *p*-methoxybenzyl and 4-pentenoyl, which allows for efficient oligosaccharide assembly in the reverse direction.

It is already appreciated that a chemical or enzymatic synthesis¹ could lead to natural oligosaccharides or glycoconjugates for study of their composition,² conformation,³ interaction with other molecules,⁴ and determining their biological roles.⁵ Also, only the synthetic approach can provide unnatural mimetics that are often of interest due to their therapeutic and diagnostic potential.⁶ However, even with significant progress in the recent years, chemical synthesis of oligosaccharides remains cumbersome.

According to the conventional (linear) oligosaccharide synthesis, disaccharides obtained as a result of the first glycosylation step are typically converted into the second-generation glycosyl acceptor *via* liberation of a specific hydroxyl. This requires the formal additional synthetic step that is often associated with isolation and purification of the glycosyl acceptor. The latter is then allowed to react with a glycosyl donor, resulting in the formation of a trisaccharide (Scheme 1a). The deprotection–glycosylation steps are then reiterated to yield larger saccharides. Because the reactive monosaccharide glycosyl donor is used at every step, the yields of glycosylations typically remain high.⁷ However, the requirement to perform additional deprotection steps between glycosylations is the major conceptual disadvantage of the conventional approach.

Many modern strategies that help to streamline oligosaccharide assembly by minimizing or even eliminating protecting/leaving group manipulations between coupling steps are based either on chemoselective or on selective activations of leaving groups.⁸ Amongst these, Ogawa's orthogonal concept is arguably the most advantageous.^{9,10} This technique implies the use of two orthogonal leaving groups (LG_a and LG_b) and requires a pair of orthogonal activators: A (activates LG_a selectively) and B (activates LG_b, but not LG_a, Scheme 1b). Theoretically, the orthogonal strategy allows for unlimited number of reiterations of leaving groups. In practice, however, the efficiency of glycosylations declines rapidly with the increase of the bulk of glycosyl donor: di(85%) tri(72%) tetra(66%).⁹ In our related study, STaz/SEt activation yielded the following decline in yields di(98%) tri(93%) tetra(77%) penta(59%).¹¹ Resultantly, this drawback precludes extending the

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orthogonal-like assembly and is often practical for the synthesis of relatively small oligosaccharides.^{12,13}

Herein we report a reverse orthogonal strategy that combines advantages of the linear (monosaccharide donor and high yields at each step) and orthogonal (no protecting group manipulations required) approaches. As opposed to the Ogawa's orthogonal strategy based on orthogonal leaving group, we base our approach on the orthogonal protecting groups (PG_a and PG_b, Scheme 1c). This reverse approach would allow for oligosaccharide assembly in the opposite direction to that of the leaving group-based approaches. The oligosaccharide will be elongated in a similar manner to that of the linear approach, but the reverse orthogonal strategy will significantly shorten the synthesis by combining the deprotection and glycosylation steps.

The successful execution of the reverse strategy will require at least two orthogonal protecting groups (PG_a and PG_b) that will be removed during the glycosylation and in principle the couplings can be executed with only one type of a leaving group. A concept of orthogonal protecting groups is known, but it refers to orthogonally removable protecting groups;^{14–17} for example, benzyl and benzoyl are orthogonal protecting groups. A glycosyl acceptor-based orthogonal approach has also been developed, but the term “orthogonal” used therein refers to differentiation of carbonyl vs. hydroxyl glycosyl acceptors.¹⁸ A few concepts for protecting group-based glycosylations are known, and undoubtedly served as a powerful motivation for studies described here. Kochetkov demonstrated that triphenylmethyl (trityl) ethers can be glycosylated directly with orthoesters, thiocyanates or thioglycosides.^{19,20} Approaches involving “glycodesilylation” of TMS or TBDMS-protected glycosyl acceptors have also been developed.^{21–24} None of these glycosylations, however, could be extended to the multi-step oligosaccharide synthesis due to the lack of the second (orthogonal) protecting group.

For the purpose of establishing a compatible pair of orthogonal protecting groups, we began screening a series of differently protected glycosyl acceptors. As the starting point, per-benzoylated *S*-ethyl glycoside **1a** was chosen as the glycosyl donor. Initial coupling of **1a** with 6-*O*-TBDMS-protected acceptor **2a** in the presence of a powerful promoter system for thioglycoside activation NIS/Tf OH was very disappointing, and the resulting disaccharide **3a** was isolated in a very modest yield of 11% (entry 1, Table 1). Our attempts to improve this result were unsuccessful, therefore, we began screening other temporary protecting groups. Encouragingly, Boc-protected glycosyl acceptor **2b** could be glycosylated with **1a** in the presence of SnCl₄/EtSH, followed by the addition of NIS much more efficiently. However, still a rather average yield of 50% could be achieved (entry 2). Further screening of the compatible protecting groups showed that 6-*O*-*p*-methoxybenzyl (pMB) acceptor **2c** could be glycosylated with **1a** in the presence of either SnCl₄/EtSH²⁵ followed by the addition of NIS (entry 3), or TMSI,²⁶ followed by the addition of NIS/Tf OH (entry 4).

In both reactions, disaccharide **3a** was cleanly generated with yields exceeding 80%. Conversely, NIS/TfOH-promoted glycosylation of **2c** with **1a** was rather inefficient, and only traces of disaccharide **3a** were obtained (entry 5). Further search of suitable protecting groups revealed that 6-*O*-pentenoyl (Pent) acceptor **2d** can be efficiently glycosylated with **1a** in the presence of NIS/Tf OH. Improved yield of **3a**, exceeding 80% could be achieved if water (1.0 equiv.) was added to the reaction (entry 6). Although *O*-(pent-4-enoyl) moiety has been investigated as the anomeric leaving group^{27–29} and as a precursor for the synthesis of sugar-based macrocycles,^{30,31} its use as a temporary protecting group of carbohydrates³² is still uncommon.

In our opinion, the latter two reactions created the basis for the first step of orthogonal activation: pMB is stable in the presence of NIS/TfOH as shown in entry 5, whereas pentenoyl can be easily removed followed by glycosylation of the liberated hydroxyl (entry 6). To prove the concept, we obtained 6-*O*-pMB glycosyl donor **1b** and glycosidated it with glycosyl acceptor **2d** equipped with pentenoyl. The highest yield of disaccharide **3b** (81%, entry 7) was achieved if the donor **1b** was used in excess or, alternatively, if it was added upon deprotection of **2d**. Although the mechanistic rationale is still to be understood, the use of water (1 equiv.) was found necessary to maintain high yields of this reaction.

With the intention of determining the reaction conditions for the second-stage orthogonal activation, we obtained 6-*O*-pentenoyl glycosyl donor **1c**. Disappointingly its coupling with 6-*O*-pMB acceptor **2c** in the presence of SnCl₄/EtSH and NIS was inefficient due to the rapid hydrolysis of pentenoyl group (entry 8). This was resolved by using a similar building block **1d** equipped with the SBox leaving group. Its glycosidation with the pMB acceptor **2c** was carried out in the presence of TMSI and AgOTf and the resulting disaccharide **3c** was obtained in 85% yield (entry 9).

Having established the entirely orthogonal activation conditions, we turned our attention to the synthesis of a simple oligosaccharide sequence *via* alternating reverse orthogonal activation steps. Herein, a slight excess of the glycosyl donor was used in each step. First, disaccharide **3b** was obtained from **1b** and **2d** in 81% as depicted in Scheme 2. The disaccharide **3b** was then glycosylated with SBox donor **1d** equipped with an *O*-pentenoyl group. The resulting trisaccharide **4** was obtained in 82% yield and then reacted with **1b** to afford tetrasaccharide **5** in 71% yield. Finally, tetrasaccharide **5** was coupled with **1d** and the resulting pentasaccharide **6** was isolated in 75% yield.

To expand the scope of the strategy developed we decided to investigate the glycosylation of secondary glycosyl acceptors. For this purpose we obtained glycosyl acceptors **7a** and **7b** equipped with 4-*O*-pentenoyl and 4-*O*-pMB groups respectively. Essentially the same reaction conditions developed earlier for glycosylation of primary glycosyl acceptors (Table 1) ensured successful glycosylation here as well. Thus, glycosylation of **7a** with glycosyl donor **1b** performed in the presence of NIS/Tf OH/H₂O allowed **8a** in 76% yield (Scheme 3). On the other hand, glycosylation of **7b** with glycosyl donor **1d** led to the formation of disaccharide **8b** in 65% yield. Very similar outcomes were achieved in the presence of TMSI/AgOTf or TMSI/MeOTf as activators with the major by-product recovered being the corresponding 4-OH derivative of **7b**.

In conclusion, we have discovered a simple strategy that complements both conventional linear method and expeditious orthogonal strategy for oligosaccharide synthesis. The utility of the concept has been demonstrated by a rapid assembly of pentasaccharide **6**. It should be noted that consistent yields in the range of 70–80% could be achieved regardless of the size of the glycosyl acceptor. The approach developed is nearly equally efficient for glycosylation of secondary glycosyl acceptors. It is expected that the reverse orthogonal strategy will be also beneficial for oligosaccharide assembly using polymer,^{33,34} fluororous,^{35,36} ionic liquid,^{37,38} and nanoporous gold³⁹ supports.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Scheme 1.

(a) Conventional linear synthesis involving separate glycosylation and deprotection steps. (b) Ogawa's leaving group-based orthogonal strategy. (c) New reverse orthogonal concept based on orthogonal protecting groups.

Scheme 2.

Four-step synthesis of pentasaccharide **6** *via* the reverse orthogonal strategy.

Scheme 3.

Preliminary investigation of secondary glycosyl acceptors **7a** and **7b**.

Table 1

Establishment of protecting groups and the reaction conditions for the reverse orthogonal strategy

Entry	Donor ^a	Acceptor	Promoter ^b	Product (yield)
1	1a	2a	NIS/TfOH	3a (11%)
2	1a	2b	SnCl ₄ /EtSH, ^c NIS	3a (50%)
3	1a	2c	SnCl ₄ /EtSH, ^c NIS	3a (>80%) ^d
4	1a	2c	TMSI, NIS/TfOH	3a (>80%) ^d
5	1a	2c	NIS/TfOH	3a (<5%) ^d
6	1a^e	2d	NIS/TfOH/H ₂ O ^c	3a (>80%) ^d
7	1b^e	2d	NIS/TfOH/H ₂ O ^c	3b (81%)
8	1c	2c	SnCl ₄ /EtSH, NIS	3c (<5%) ^{d,f}
9	1d	2c	TMSI/AgOTf	3c (85%)

^a In all preliminary reactions donor and acceptor were used in equimolar amounts.^b All reactions were performed in the presence of molec. sieves (3 or 4 Å).^c The use of water or EtSH (1 equiv.) was found to improve the yield.^d Estimated by TLC.^e Donor was added upon complete deprotection of the acceptor.^f Pentenoyl group of the donor was removed instead.