One-Pot Glycosylation (OPG) for the Chemical Synthesis of Oligosaccharides

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Abstract: This review provides a comprehensive survey of the “one pot glycosylation” (OPG) strategy for the chemical synthesis of oligosaccharides, covering literatures from the first example reported by Kahne and Raghavan in 1993 through May 2003. The essence of the OPG is to distinguish the reactivity difference of a pair of the glycosylation donors or acceptors so as to carry out two glycosylation steps sequentially without purification of the first-step coupling product. Accordingly, the literature reports are grouped based on the major stereoelectronic factors causing the reactivity differences, those include the “armed-disarmed effect”, “orthogonality of leaving groups”, “distinguishable acceptors”, and “the hybrid”. “The hybrid” OPG procedure takes advantage of a combination of the reactivity disparity of a set of the armed-disarmed donors, orthogonal leaving groups, as well as acceptors so as to proceed three or more steps of glycosylation sequentially in one pot. Relevant conception and exploitation of the reactivity differences of the donors and acceptors in the synthesis of oligosaccharides, which finally evolve the OPG or advance parallelly, are briefly described at the beginning.

1. INTRODUCTION

The need for oligosaccharides and glycoconjugates of defined composition to serve as molecular tools for biological and medicinal studies have driven impetuously the field of oligosaccharide synthesis especially since 1980s. The flourishing alternatives thus developed for glycosylation and protection, sometimes seemingly overgrown, have in facts laid a ground for the assembly of oligosaccharides in unprecedented efficient fashions [1-8]. Among the most promising ones is the “one-pot glycosylation” (OPG) strategy. An OPG carries out two or more glycosylation steps sequentially without the requirement of protecting group manipulation and intermediate isolation in between, thus removes the major burden in the conventional chemical synthesis of oligosaccharides in contrast to the natural enzymatic way. To realize the OPG, the reactivity difference of the donors/acceptors involved must be well distinguished by a set of glycosylation conditions, and these conditions sequentially performed in one pot need to be compatible. Such a high demanding was first fulfilled by Kahne and Raghavan, seems occasionally, in 1993 [9]. Through May 2003, nearly 50 fine designed OPGs have been published, those from the group of Wong have been accounted [10]. And we present here a comprehensive treatment of this topic.

According to the major factors creating the reactivity disparity of glycosylation donors/acceptors, the OPGs are divided into four groups in the present review, namely, “OPG steered by armed-disarmed donors”, “OPG steered by orthogonal leaving groups”, “OPG steered by distinguishable acceptors”, and “the hybrid OPG”. Within each group, a chronological order is largely followed. Before going to these topics, relevant conception and exploitation of the reactivity differences of the donors and acceptors in the synthesis of oligosaccharides, which finally evolve the OPG strategy or develop parallelly, are briefly described.

2. RELEVANT CONCEPTS AND PROCEDURES

2.1 Historical Perception on the Reactivity of Glycosylation Donors/Acceptors

In the seminal review of 1982, Paulsen concluded the observations during oligosaccharide synthesis as such: “The reactivities at the anomeric center of the glycosyl component and of the OH group of the reaction partner depend very strongly on the type of blocking pattern of the two compounds. A variation of the blocking pattern can exert a decisive influence on the coupling step. Conformation and steric influences, and the molecular sizes of the two partners are also significant” [1].

2.2 “Armed-Disarmed” Concept

The previous view on the reactivity difference of glycosyl donors resulting from the blocking pattern was enlarged by Fraser-Reid and coworkers, in 1988, to such a level that it could be thoroughly distinguished and put into a chemoselective glycosylation. [11]. Thus, n-pentenyl glycoside 1, “armed” with the electron-donating benzyl groups, was selectively activated with ICP (iodonium dicollidine perchlorate) to couple with n-pentenyl glycoside 2, which is “disarmed” with the electron-withdrawing acetyl groups, providing disaccharide 3 in 62% yield without detection of the self coupling products of 2 (Scheme 1). The resulting “disarmed” 3 is obviously a donor capable of further glycosylation under stronger conditions.

Fraser-Reid et al. rationalized the armed-disarmed effect as such: “Reaction of a glycosyl donor with an appropriate electrophile gives a positively charged intermediate which is less favorable when there is an adjacent electron-withdrawing group (for example OCOR, as in a disarmed
donor) than when there is an adjacent alkoxy group (as in the armed counterpart). The latter therefore reacts faster and if, in the reaction medium, there is a disarmed species carrying a free hydroxyl group, a pathway based on Le Chatelier’s principle can be envisaged that leads to products of cross-coupling with (virtually) none of the self-coupled analogue” [12]. Based on these mechanistic rationales, the armed-disarmed concept, first recognized in n-pentenyl glycosides, was rapidly extended to other types of glycosyl donors, such as thioglycosides [13] and glycals [14]. And the torsional effect caused by cyclic acetal protections was also realized [15].

2.3 “Two-Stage Activation” and “Orthogonal Glycosylation” Strategies

After successful practice with thioglycosides and glycosyl fluorides, Nicolaou and coworkers have developed, since 1984, the “two-stage activation” strategy for the efficient synthesis of oligosaccharides. [7, 16]. This reiterative procedure utilizes the differential and orthogonal reactivities of glycosyl fluorides, phenylthioglycosides, and silyl ethers to achieve a sequential growth of the oligosaccharide chain (Scheme 2).

The “orthogonal glycosylation” strategy described by Kanie, Ito, and Ogawa, in 1994, activated the glycosyl fluorides (Cp₂HfCl₅, AgClO₄, -78 °C - rt) and thioglycosides (NIS, AgOTf, -50 °C - rt) for glycosylation in an orthogonal fashion, so that the requirement in the “two-stage activation” for conversion of thioglycosides into fluorides for the next step glycosylation was joyfully removed [17].

In complementary to the “orthogonal” effect, leaving groups of a similar type but with different steric or electronic nature were also found possible for chemoselective glycosylations, such as glycosylations between phenyl-selenoglycosides and thioglycoside [18] and between thioglycosides with different substituents on the anomic sulfur, termed “active-latent” donors [19]. In a broad sense, “orthogonal effect” covers any distinguishable effects caused by the leaving groups of the glycosyl donors on the glycosylation selectivity.

2.4 “Two-Directional Glycosylation” Strategy

The reactivity difference between hydroxyl groups of the acceptors, caused by stereoelectronic effects, has enabled the regioselective glycosylation to be a quite common practice [20]. Furthermore, several protected forms, such as trityl [21] and silyl ethers [22], which are normally inert toward glycosylation, have been found to be capable of direct glycosidation under some conditions. Thus an additional level for reactivity manipulation in the oligosaccharide synthesis is provided. Utilizing the reactivity disparity of both the donors and acceptors, Boons and Zhu developed a “two directional glycosylation” strategy [22-24]. A remarkable example is shown in Scheme 3 [24]. The protected hexasaccharide derivative of the tumor-associated antigen Globo-H (14) was assembled from six well-designed building blocks (4-9) via five successive steps of glycosylation. Not a single step for protecting group transformation was needed. Whereupon, the reactivity disparity between the coupling pair of donors (ethyl thioglycoside 4/phenyl thioglycoside 5, thioglycoside 10/fluoride 8) and acceptors (primary alcohol 6/secondary alcohol 7, alcohol 11/TES ether 9) were fully distinguished.

3. OPG For the Chemical Synthesis of Oligosaccharides

The above mentioned “armed-disarmed”, “two-stage activation”, “orthogonal glycosylation”, as well as the hybrid “two-directional glycosylation” strategies are, in essence, a matter of reactivity tuning, so as to perform two or more steps of glycosylation successively without the need of protecting group manipulation in between. OPG strategy advances one step further where a set of compatible conditions are used for the sequential glycosylation so that the purification of the coupling intermediates is no longer required.

3.1 OPG Steered by Armed-Disarmed Donors

Application of the armed-disarmed concept for an OPG was first established by Ley and coworkers in 1994 (Scheme 4) [25]. Coupling of the armed thioglycoside 15 with disarmed 16 (NIS/TIOH) provided disaccharide 17 as the sole coupling product (as monitored by TLC), without detection of the self-coupling products of 16. Upon addition of 18 and an additional portion of the promoters, the nascent
disarmed 17 was activated and coupled with 18 to give trisaccharide 19 in a satisfactory 62% yield, equal to a per glycosylation yield of 79%.

In 1997, the OPG of armed-disarmed thioglycosides was successfully employed in our total synthesis of the complex resin glycoside, tricolorin A (Scheme 5) [26]. The armed thioglycoside 21 was selectively activated at –15 °C (NIS/TfOH) to glycosylate the disarmed 20, the resulting 22 was then activated in the presence of an additional portion of NIS/TfOH at room temperature to couple with the newly added acceptor 23, affording 24 in 43% isolated yield.

Similar protocol was also applied by Kondo et al., in 1998, in the synthesis of Le oligosaccharide derivatives (28) (Scheme 6) [27].

In 1999, Wong and coworkers contributed a general procedure for the quantitative measurement of the relative reactivities of the p-methylphenyl thioglycoside (STol) donors which are either fully protected or have one hydroxyl group exposed [28]. Thus, the structural effects of different monosaccharide cores and different protecting groups on the reactivity of each thioglycoside donors, which are previously obscure or simply classified as armed-disarmed, were accurately characterized and quantified. Furthermore, they compiled the relative reactivity values (RRVs) into a computer program, Optimer\textsuperscript{TM}, which can suggest optimal combinations of glycosyl building blocks for an OPG synthesis of the target oligosaccharides.

A concept-prove assembly of 33 linear or branched oligosaccharides based on RRVs was then provided. [29]. A representative synthesis is shown in Scheme 7. The thioglycoside donor 29 (RRV = 7.2 x 10^7), thioglycoside donor/acceptor 30 (RRV = 142.9), and acceptor 32 (RRV = 0) were confidently chosen for the OPG synthesis of trisaccharide 33.

Wong and coworkers have been expending the RRV database while achieving programmable OPG synthesis of biologically significant oligosaccharides [10]. An OPG strategy for a rapid assembly of the Globo H hexasaccharide

Scheme 3. A “two-directional glycosylation” strategy toward the synthesis of a hexasaccharide derivative of the tumor-associated antigen Globo-H.
is shown in Scheme 8 [30]. The flow of the OPG synthesis toward hexasaccharide 41 by thio-monosaccharides 34-36 and 39 and acceptor 40 was only interrupted at 37, where a selective deprotection of the Lev group (37 → 38) was required for the following glycosylation.

Disaccharides and oligosaccharides could be viewed as monosaccharides with sugar substituents, therefore, have their own RRVs and can be used as components in the OPG. In a synthetic route toward Lewis Y oligosaccharide hapten, a colon-rectal cancer antigen determinant, Wong et al., realized a OPG synthesis of the hexasaccharide 45 employing monosaccharide donor 42, disaccharide donor/acceptor 43, and disaccharide acceptor 44 (Scheme 9) [31]. The RRV of 42 is only six times larger than the RRV of 43, nevertheless, a selective activation of 42 was achieved at –70 °C to bis-glycosylate 43, the thio-function of which was kept latent until the next glycosylation with 44.

Recently, Wong and coworkers added thioglycoside building blocks for the sialylated oligosaccharides [32] and the N-acetyllactosamine oligomers [33] to the Optimer database. Shown in Scheme 10 is an OPG synthesis of the acetyllactosamine trimer (49).

Adding stronger or more equivalents of the promoters or raising the reaction temperature after the completion of the first glycosylation is the usual way to force the second glycosylation to take place in the reactivity-based OPG. Oscarson and coworkers demonstrated that changing a reaction medium could also have the OPG done (Scheme 11) [34]. Thus, the reactivity-based selective coupling of the thioglycosides 50 and 51 was achieved in Et₂O (NIS/AgOTf), but not in CH₂Cl₂, where a complex mixture was produced due to the activation of both components. Adding acceptor 52 and additional promoters in CH₂Cl₂, but not in Et₂O, realized the second glycosylation, affording 53 in high yield. Such a technique was later successfully used in the OPG syntheses of the glucosamine oligosaccharides by Baasov et al. (Scheme 12) [35]. In combination with the armed-disarmed glycosylation with Troc and Phth protected...
thioglycosides, they were able to realize a three-step OPG synthesis of the glucosamine tetrasaccharide 58.

A new promoter system for the OPG of armed-disarmed thioglycosides was reported by Mukaiyama et al. in 2000 (Scheme 13) [36]. The selective activation of the armed thioglycoside 59 was achieved by TrB(C\text{C}_{6}F_{5})_{4}/PhthNSEt. Subsequent addition of NIS and acceptor 61 forced the second glycosylation to afford the trisaccharide 62.

Scheme 6. OPG synthesis toward Le\text{\textasciitilde} oligosaccharides using armed-disarmed thioglycosides (26 vs. 25).

Scheme 7. A representative RRVs-based OPG synthesis using thioglycosides (29 vs. 30).

Scheme 8. Programmable reactivity-based OPG toward the Globo H hexasaccharide using thioglycosides.
Although the armed-disarmed steered, or more broadly “reactivity-based” OPG has been largely relied on thioglycosides. In 1998, Ley and co-workers have noticed the capability of armed-disarmed glycosyl fluorides for OPG synthesis of oligosaccharides (Scheme 14) [37].

Recently, Mukaiyama and Chiba developed armed-disarmed glycosyl \( p \)-trifluoromethylbenzylthio-\( p \)-trifluoromethylphenyl formimidates (glycosyl thioformimidates) for OPG synthesis (Scheme 15) [38]. The first step coupling between armed 67 and disarmed 68 was performed in the presence of a catalytic amount of TfOH at \(-78^\circ C\), and the subsequent second glycosylation took place upon addition of the acceptor 69 and raising the temperature gradually to \(0^\circ C\).

### 3.2 OPG steered by the Orthogonal Leaving Groups

In 1994, Takahashi and coworkers firstly transformed the “orthogonal glycosylation” procedure into an OPG fashion (Scheme 16) [39]. Thus, glycosyl bromide 71, imidate 72, or fluoride 73 was activated in the presence of AgOTf or BF\(_3\)OEt\(_2\) to couple with thioglycoside acceptor 74, the resulting thio-disaccharide was then activated under the action of the existing AgOTf or BF\(_3\)OEt\(_2\) in combination.

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**Scheme 9.** Programmable reactivity-based OPG synthesis toward the Le\(^+\) hapten using thioglycosides.

**Scheme 10.** Reactivity-based OPG synthesis of N-acetyllactosamine oligomers using thioglycosides.

**Scheme 11.** Reactivity-based OPG synthesis controlled by varying solvents.
with an additional portion of NIS to couple with the newly added acceptor 75, leading to the corresponding trisaccharide (76, 77). Shortly, Takahashi et al. applied this OPG protocol to a successful assembly of the phytoalexin hexaglucan (Scheme 17) [40].

At the same year, Chenault and Castro reported an OPG protocol with orthogonal isopropenyl glycoside (82) and n-pentenyl glycoside (83). However, the overall yields were not satisfactory (Scheme 18) [41].

Ley and coworkers paired selenoglycosides and thioglycosides for OPG, while the orthogonality of these

Scheme 13. OPG using armed-disarmed thioglycosides with TrB(C_6F_5)_4/PhthNSEt/NIS as the promoter system.

Scheme 14. OPG synthesis using armed-disarmed glycosyl fluorides (63/64).

Scheme 15. OPG using armed-disarmed glycosyl thioformimidates (67 vs. 68).
pairs might require additional enforcement by the structural effects (e. g., the armed-disarmed effect) (Scheme 19) [42].

The OPG with orthogonal glycosyl imidates and thioglycosides might be more attractive for a practical synthesis due to the popularity of these two types of donors. Thus, we were able to prepare a group of natural diosgenyl saponins in a highly efficient parallel manner. [43, 44]. A representative is shown in Scheme 20. The first step coupling between imidate donor 90 and thioglycoside acceptor 91 required only 0.1 equivalent of TMSOTf at –70 °C, while addition of 1.0 equivalent of NIS and the acceptor 92 promoted the second glycosylation.

Glycosyl phenylcarbonates have also been paired with thioglycosides by Mukaiyama et al to achieve the OPG synthesis of oligosaccharides (Scheme 21) [45, 46]. However, the overall yields (62%-85%) were lower compared to those obtained by OPG using glycosyl imidates in the first step. In addition, only 1→6 linkages were made by this protocol. Mukaiyama and coworkers then also modified the OPG with glycosyl fluorides and thioglycosides. The first step glycosylation with the fluoride donors was promoted by 20 mol% TfOH in the coexistence of 5 Å MS in Et2O, generating the corresponding α anomers predominantly in the absence of a neighboring control (Scheme 22) [47]. These two OPG protocols were proved...
successful in the synthesis of the F1 α antigen (Scheme 23) [48].

3.3 OPG Steered by Distinguishable Acceptors

In 1997, Boons and Zhu published an excellent OPG protocol basing on the reactivity disparity between hydroxyl groups of a glycosyl donor (109) and an acceptor (110) (Scheme 24) [49]. In the right flask, the “disarmed” primary OH of the thioglycoside donor 109 is less active than the “armed” primary OH of the acceptor 110, therefore coupling was managed to provide 112, leaving the “disarmed” OH free. Meanwhile in the left flask, an “armed-disarmed” coupling of thioglycosides 107 and 108 was carried out to give 111. Mixing the mixtures in the two flasks followed by addition of another portion of the promoters (NIS/TfOH) afforded hexasaccharide 113 in a remarkable 53% isolated yield.

Scheme 19. OPG with orthogonal selenoglycoside (86) vs. thioglycoside (87).

Scheme 20. OPG synthesis of a diosgenyl saponin with orthogonal imidate (90) vs. thioglycoside (91).
Valverde et al. employed the kinetic effect of an intramolecular vs. intermolecular coupling to achieve the OPG synthesis of the trisaccharide 117 (Scheme 25) [50]. Thus the intramolecular glycosidation of 114 took place regioselectively on the 6-OH, then the second glycosidation of the 3-OH with the newly added donor 116 provided 117.

Recently, Ning and Wang reported a facile OPG in the synthesis of the unique trisaccharide fragment of the Motif E of the *Mycobacterium tuberculosis* Cell Wall (Scheme 26) [51]. The primary 6-OH of acceptor 118 was much active than the secondary 5-OH, therefore steering the OPG via a sequential addition of the two imidate donors (119 and 120) of a similar type.

### 3.4 The Hybrid OPG

The first ever OPG protocol was reported by Kahne and Raghavan in 1993, which utilized the reactivity disparity of both the donors (sulfoxide 123 > sulfoxide 122) and the acceptors (124 with OH > 123 with TMS ether) (Scheme 27) [9]. Thus, the sequential two-step OPG needs not to be controlled by a sequential addition of the coupling partner. Instead, three reactants (122-124) were mixed, upon addition of the promoter (TIOH), coupling between 123 and 124 took place faster, generating 125, then a slow coupling between 122 and 125 produced 126. A molar ratio of 3 : 2 : 1 for 122 : 123 : 124 was thus arranged that the decomposed donors 122 and 123 during the coupling were compensated.

Ley and coworkers combined the orthogonality and the armed-disarmed effect of donors for achieving the multi-step linear OPG synthesis of mannose type oligosaccharides [52-55]. A three-step OPG synthesis of the tetrasaccharide 133 was achieved in an unoptimized 10% yield, employing the orthogonal pairs of the fluoride 127/thioglycoside 128 and the intermediate selenoglycoside 131/thioglycoside 132 and the armed-disarmed pair of the intermediate selenoside 129/selenoside 130 for the successive glycosylation (Scheme 28) [52]. Utilizing the similar strategy, a four-step OPG synthesis was also realized for the synthesis of the pentasaccharide 140 from five monosaccharide building blocks (fluorides 63, 64, selenosides 135, 137, and acceptor 139) (Scheme 29) [55].

![Scheme 21. OPG synthesis of 1→6 linked trisaccharides with orthogonal phenylcarbonates (94) vs. thioglycosides (95).](image)

*Current Organic Chemistry, 2005, Vol. 9, No. 2 Yu and Cao*

In 1999, Takahashi and coworkers reported an OPG protocol for branched synthesis of oligosaccharides employing the combination of a pair of orthogonal bromide and thioglycoside donors and an acceptor with a pair of distinguishable OHs [56]. A representative example is depicted in Scheme 30. To the mixture of bromide 71 (1.05 eq), thioglycoside 142 (2.0 eq), and acceptor 141 (1.0 eq), AgOTf was added first to promote the coupling of 71 with
the active 6-OH, following addition of NIS/TfOH then activated to couple with the remaining 3-OH.

Employing this strategy, together with the previous linear OPG with orthogonal donors, Takahashi et al. were able to build a library of 72 trisaccharides on a Quest 210\textsuperscript{TM} manual synthesizer [57]. The branched and linear type OPG were then combined in one pot for the synthesis of the heptaglucan of the phytoalexin elicitor [58]. A remarkable six-step OPG synthesis of this interesting glucan was achieved on the manual synthesizer (Scheme 31) [59]. This is so far the OPG assembly coupling the highest number of saccharide building blocks. A little earlier, Muaiyama et al. employed four blocks for an OPG synthesis of this heptaglucan [60].

In 1999, we managed the synthesis of a bidesmosidic triterpene saponin using a novel hybrid OPG protocol (Scheme 32) [61]. Where the trityl ester (on 151) remained latent during the glycosylation of the 3-OH with imidate 152 at -60 °C, but conducted ready cleavage upon raising the temperature to rt. The resulting carboxylic acid 154 was then glycosylated with imidate 155 at 0 °C without additional

Scheme 24. OPG utilizing the reactivity difference between the OHs of donor (109) and acceptor (110).

Scheme 25. OPG utilizing the reactivity difference of the OHs for an intramolecular vs. intermolecular coupling.

Scheme 26. OPG synthesis of the Motif E trisaccharide based on the reactivity difference of the two OHs of the acceptor (118).
promoter. The trityl ether (on 155) remained intact during the coupling, was directly glycosylated by thioglycoside 159, prepared in another flask, to give the target molecule 160 in a satisfactory 62% yield. The use of two (or more) flasks provides another manual choice for controlling the OPG sequence.

Nearly 15 years, since the discovery of the armed-disarmed effect of donors, brings Fraser-Reid and
collaborators, in 2002, to a novel concept of reciprocal donor-acceptor selectivity (RDAS) [62]. In a RDAS-based OPG shown in Scheme 33, a 1 : 1.3 : 1.3 mixture of diol 163, n-pentenyl donor 162, and n-pentenyl orthoester donor 161 was treated with NIS (2.5 eq)/BF$_3$OEt$_2$ (cat.), trisaccharide 164 and disaccharide 165 was obtained as the only products in 37% and 37% yields, respectively. The results indicate a reciprocal choice of donor 161 for the 3-OH and donor 162 for the 2-OH. However, a rational of these selectivities awaits further insight.

**CONCLUSION**

The last decade has witnessed the advent to fully development of the OPG procedures for the chemical synthesis of oligosaccharides. The influence of the armed-disarmed protecting groups, together with other structural
factors, on the reactivity of glycosyl donors, are able to be quantified, thus a reactivity-based OPG could be programmable. The exploration of orthogonal leaving groups and the distinguishable acceptors has brought additional levels for sequential control of the OPG synthesis, thus multi-step OPGs become common practice. Addition sequence, temperature, solvent, as well as using separate flasks then mixing, provide external control for the OPG sequence. While the reciprocal donor-acceptor selectivity (RDAS) awaits further insight. The six-step OPG synthesis of the heptaglucan of phytoalexin elicitor on a manual synthesizer represents a height has been reached, and foresees the future trend, that the OPG procedure, which is performed in solution phase, might be an alternative for automation of the oligosaccharide synthesis, comparable to the solid phase methods for peptide and nucleotide synthesis.
Scheme 33. OPG using donors/acceptors with reciprocal selectivity.

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