Organic & Biomolecular Chemistry

Cite this: *Org. Biomol. Chem.*, 2012, **10**, 2373

www.rsc.org/obc

COMMUNICATION

Direct *O*-glycosidation of resin bound thioglycosides†

Son Hong Nguyen, Adam H. Trotta, John Cao, Timothy J. Straub and Clay S. Bennett*

Received 8th November 2011, Accepted 19th December 2011
DOI: 10.1039/c2ob06883d

The application of the safety-catch linker concept to solid-phase glycoconjugate synthesis is described. The process allows for direct conjugation of resin bound glycans to complex aglycones during cleavage. Large excesses of either coupling partner are not required, and even very hindered alcohols serve as acceptors in the reaction.

The introduction or alteration of carbohydrate chains on a small molecule can have a profound effect on its biological activity.[1] Such effects can include altering the molecule's target,[2] reducing toxicity,[3] or increasing potency.[4] As a consequence, glycodiversification holds enormous promise for the discovery of novel therapeutics. This approach is currently underutilized, however, due to limitations that preclude the routine, parallel, and automated synthesis of glycoconjugate libraries.

A central focus of our group's research is developing methods for the automated synthesis of small molecule glycoconjugate libraries. Over the past decade, solid-phase[5] and fluorous-phase[6] oligosaccharide synthesis have emerged as methods of choice for automated carbohydrate synthesis. Solid-phase synthesis in particular has received a significant amount of attention, including the development of combinatorial approaches to oligosaccharide library synthesis.[7] While this approach has proven its utility for carbohydrate library construction, its application to the synthesis of small molecule glycoconjugate libraries has been comparatively underexplored. This is due to a scarcity of methods for attaching aglycones to the reducing end of an immobilized glycan, either during solid-phase synthesis,[8] or upon cleavage from the resin. As a result, glycoconjugate synthesis requires multiple solution-phase manipulations, a process that is not readily amenable to automation or large-scale library synthesis (Fig. 1A).

We envisioned that a method for directly transferring an immobilized oligosaccharide to an aglycone during cleavage would significantly reduce the number of solution-phase manipulations necessary to obtain the desired target (Fig. 1B). For such an approach to be effective, it requires a linker analogous to the "safety-catch" linkers developed for solid-phase peptide synthesis.[9] In other words, the linker must be orthogonal to all solid-phase manipulations, and selectively activated at the end of the synthesis. In this context a thioglycoside linker appeared to be an ideal choice.

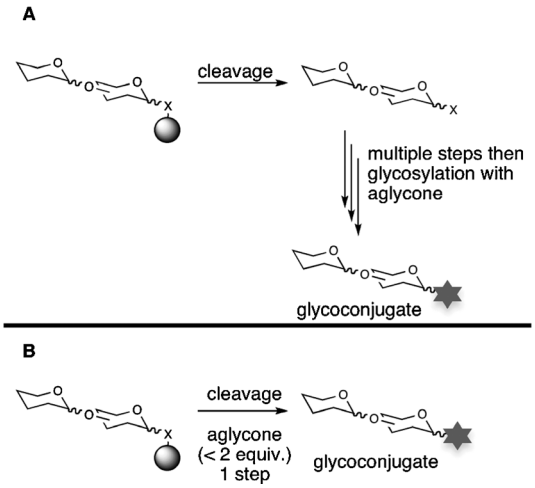


Fig. 1 **A**. Standard approaches to glycoconjugate construction using solid-phase oligosaccharide synthesis require multiple solution-phase transformations. **B**. The use of a safety-catch linker for direct glycoconjugate synthesis upon cleavage described in this work.

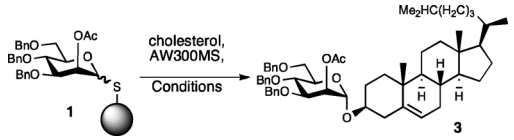
Thioglycosides are orthogonal to the Lewis acidic conditions used to activate many donors in oligosaccharide synthesis, and can themselves be selectively activated with a variety of thiophilic reagents.[10] Surprisingly, although thioglycoside-based linkers have been known for quite some time,[11] reports of activating them for transfer to an aglycone have been limited to examples that used a large excess (>20 equivalents) of simple acceptors.[12] While such approaches are acceptable for solvolysis, the use of a large excess of acceptor is impractical when valuable molecules, such as complex natural products, are to serve as aglycones. To address this issue we decided to examine thiophilic promoters for the ability to activate carbohydrates immobilized through a thioglycoside linker for transfer to a small excess (<2 equivalents) of a complex aglycone.

In our initial screening we examined mannose-bearing resin **1** (Table 1). We chose JandaJel as a solid support because this resin had been reported to work particularly well in solid-phase oligosaccharide synthesis.[7c] To determine the optimal reagent combination, we examined different promoters for the ability to

Department of Chemistry, Tufts University, Medford, MA 02155, USA. E-mail: clay.bennett@tufts.edu; Fax: +1 617-627-0621; Tel: +1-617-627-0621

†Electronic supplementary information (ESI) available: Synthesis of **1** and **14**, experimental procedures and spectroscopic data. See DOI: 10.1039/c2ob06883d

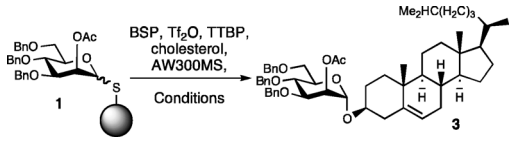
Table 1 Preliminary reagent screening



Entry	Promoter[a]	Donor : acceptor ratio	Solvent	*T*	Yield (%)
1	*N*-(phenylthio)-ε-caprolactam/Tf_2O	1 : 1.5	CH_2Cl_2	−70 °C to rt	20
2	Ipy_2BF_4	1 : 1.5	CH_2Cl_2	−35 °C	13
3	*N*-fluoro-2,6-dichloropyridinium triflate	1 : 1.3	CH_2Cl_2	−35 °C	NR
4	NIS/TfOH	1 : 1	CH_2Cl_2	rt	NR
5	**benzenesulfinyl piperidine/Tf_2O**	**1 : 2**	**CH_2Cl_2**	**−40 °C to rt**	**40**

[a] Ipy_2BF_4 = bis(pyridine)iodonium(I)tetrafluoroborate, NIS = *N*-iodosuccinimide, AW300MS = acid washed 3 Å molecular sieves.

Table 2 BSP/TF_2O optimization



Entry	Donor : acceptor ratio	Solvent	Resin	*T*	Yield (%)
1	1 : 2	CH_2Cl_2	JandaJel	−40 °C to rt	40
2	2 : 1	CH_2Cl_2	JandaJel	−40 °C to rt	42
3	1 : 2	CH_2Cl_2	JandaJel	−60 °C to rt	30
4	2 : 1	CH_2Cl_2	JandaJel	−60 °C to rt	21
5	2 : 1	DCE	JandaJel	−40 °C to rt	16
6	2 : 1	toluene	JandaJel	−40 °C to rt	50
7	**1 : 1.5**	**toluene**	**JandaJel**	**−60 °C to rt**	**71%**
8	1 : 1.5	toluene	Merrifield resin	−60 °C to rt	31%

[a]TTBP = 2,4,6-tri-*tert*-butylpyrimidine, DCE = 1,2-dichloroethane, AW300MS = acid washed 3 Å molecular sieves.

transfer the immobilized sugar to cholesterol (**2**), a model acceptor. A number of different promoter systems were screened for the ability to catalyze the glycosylation reaction.[13–16] From this study we identified the benzenesulfinyl piperidine/triflic anhydride (BSP/Tf_2O) promoter as the best candidate for further optimization (Table 1, entry 5).[17]

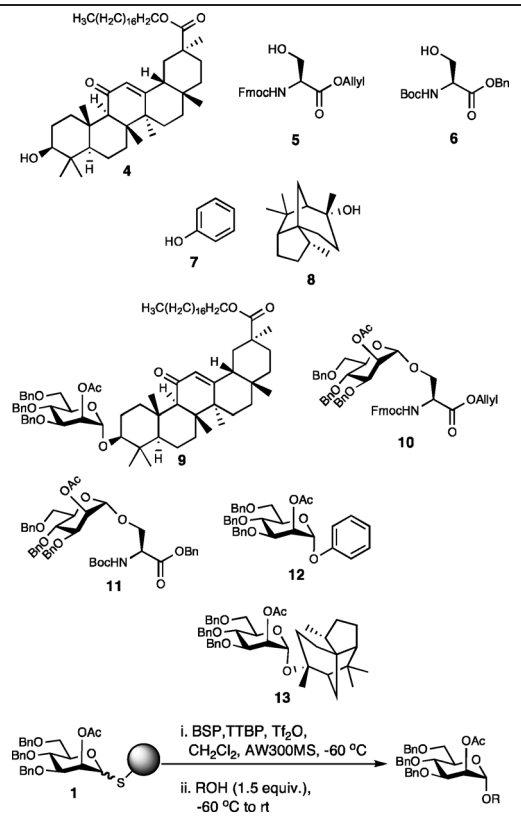
Having identified BSP/Tf_2O as the optimal promoter for glycan transfer, we next turned our attention to optimizing the reaction conditions. Using cholesterol as an acceptor we found that toluene was the optimal solvent for the reaction (Table 2, entry 6). Further optimization revealed that initiating the reaction at lower temperature (−60 °C) and slowly warming to room temperature provided the product in 71% yield (Table 2, entry 7). Finally, attempts to use the less expensive Merrifield resin as a replacement for JandaJel led to a dramatic decrease in yield (Table 2, entry 8), presumably due to its poorer swelling properties. Accordingly, all subsequent studies were carried out using JandaJel as the solid support.

We examined the scope of the reaction with several acceptors, representing model aglycones. When stearyl glycyrrhetinate (**4**) was used as an acceptor in toluene, the reaction failed to provide any product. Reasoning that this may be due to poor solubility of **4** in toluene at low temperature, we next examined its reactivity in CH_2Cl_2. Under these conditions we found we were able to isolate the desired glycoconjugate in 46% yield (Table 3, entry 1). The lower yield was presumably a result of hindrance about the neopentyl center of the acceptor. Indeed, less hindered alcohols provided the product in much higher yields (Table 3, entries 2–4). Pleasingly, both Fmoc and Boc amino acids **5** and **6** were effective acceptors, implying that this approach could have utility in generating libraries of glycopeptides. Finally, the very hindered tertiary alcohol acceptor cedrol **8** provided the product in low yield (Table 3, entry 5), in accordance with our observation that more hindered acceptors are less efficient in the reaction. In all cases, the products were all α-anomers due to participation of the C-2 acetate.

We next examined disaccharide **14** as a model of a more hindered oligosaccharide donor (Table 4). Pleasingly, the increased steric encumbrance at C-2 in **14** did not have a deleterious effect on the reaction, and yields were similar to those observed with the monosaccharide acceptors (Table 4). Additionally, in all cases the reaction afforded the product exclusively as the α-anomer.

In conclusion we have demonstrated that it is possible to transfer carbohydrates immobilized to a solid phase through a through a thiol linker directly to complex molecule aglycones.

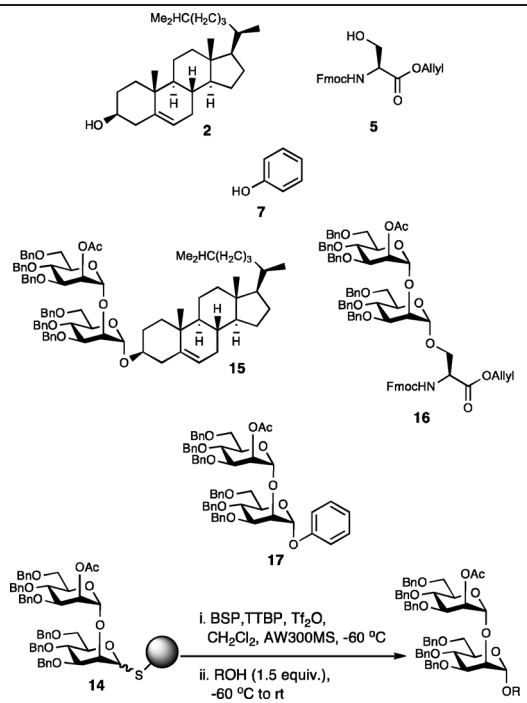
Table 3 Scope of reaction with monosaccharide resin **1**



Entry	Acceptor	Product	Yield (%)
1	**4**	**9**	46
2	**5**	**10**	69
3	**6**	**11**	65
4	**7**	**12**	75
5	**8**	**13**	20

TTBP = 2,4,6-tri-*tert*-butylpyrimidine, AW300MS = acid washed 3 Å molecular sieves.

Table 4 Scope of the reaction with disaccharide resin **14**



Entry	Acceptor	Product	Yield (%)
1	**2**	**15**	66
2	**5**	**16**	60
3	**7**	**17**	75

TTBP = 2,4,6-tri-*tert*-butylpyrimidine, AW300MS = acid washed 3 Å molecular sieves.

This approach is similar to the "safety-catch" linker approach used in solid-phase peptide synthesis. Large excesses of either coupling partner are not required, and even extremely hindered alcohols serve as acceptors. To our knowledge this is the first report of direct transfer of resin bound glycans to a slight excess of complex small molecule acceptors during cleavage. We anticipate that this technology will greatly facilitate the synthesis of glycoconjugate libraries by reducing the number of solution phase manipulations necessary for target synthesis. The scope and limitations of the approach, as well as its application to the construction of glycorandomized libraries of bioactive natural products, is currently under investigation in our lab.

Acknowledgements

Support for this research was provided in part by a Tufts University Faculty Research Fund Award.

Notes and references

1 (*a*) J. M. Langenhan, B. R. Griffith and J. S. Thorson, *J. Nat. Prod.*, 2005, **68**, 1696–1711; (*b*) R. W. Gantt, P. Peltier-Pain and J. S. Thorson, *Nat. Prod. Rep.*, 2011, **28**, 1811–1853.

2 (*a*) H.-Y. L. Wang, Y. Rojanasakul and G. A. O'Doherty, *ACS Med. Chem. Lett.*, 2011, **2**, 264–269; (*b*) P. Peltier-Pain, S. C. Timmons, A. Grandemange, E. Benoit and J. S. Thorson, *ChemMedChem*, 2011, **6**, 1347–1350.

3 K. Deng, M. M. Adams and D. Y. Gin, *J. Am. Chem. Soc.*, 2008, **130**, 5860–5861.

4 M. Yang, M. R. Proctor, D. N. Bolam, J. C. Errey, R. A. Field, H. J. Gilbert and B. G. Davis, *J. Am. Chem. Soc.*, 2005, **127**, 9336–9337.

5 (*a*) O. J. Plante, E. R. Palmacci and P. H. Seeberger, *Science*, 2001, **291**, 1523–1527; (*b*) P. H. Seeberger, *Chem. Soc. Rev.*, 2008, **37**, 19–28; (*c*) M. Weishaupt, S. Eller and P. H. Seeberger, *Methods Enzymol.*, 2010, **478**, 463–484.

6 F. A. Jaipuri and N. L. Pohl, *Org. Biomol. Chem.*, 2008, **6**, 2686–2691.

7 (*a*) R. Liang, L. Yan, J. Loebach, M. Ge, Y. Uozumi, K. Sekanina, N. Horan, J. Gildersleeve, C. Thompson, A. Smith, K. Biswas, W. C. Still and D. Kahne, *Science*, 1996, **274**, 1520–1522; (*b*) G. A. Elsayed, T. Zhu and G.-J. Boons, *Tetrahedron Lett.*, 2002, **43**, 4691–4694; (*c*) T. Ako, S. Diakoku, I. Ohtsuka, R. Kato and O. Kanie, *Chem.–Asian J.*, 2006, **1**, 798–813; (*d*) S. Komba and S. Machida, *J. Carbohydr. Chem.*, 2009, **28**, 369–393; (*e*) P. Pornsuriyasak, S. C. Ranade, A. Li, M. C. Parlato, C. R. Sims, O. V. Shulga, K. J. Stine and A. V. Demchenko, *Chem. Commun.*, 2009, 1834–1836.

8 For an example where aglcyones are conjugated to glycans attached to solid supports through the non-reducing end see: D. Crich and M. Smith, *J. Am. Chem. Soc.*, 2002, **124**, 8867–8869.

9 (*a*) G. W. Kenner, J. C. McDermott and R. C. Sheppard, *J. Chem Soc., Chem Commun.*, 1971, 636–637; (*b*) B. J. Backes, A. A. Virgilio and

J. A. Ellman, *J. Am. Chem. Soc.*, 1996, **118**, 3055–3056; (*c*) B. J. Backes and J. A. Ellman, *J. Org. Chem.*, 1999, **64**, 2322–2330.
10 For a recent review on methods to activate sugars for glycosylation reactions see: X. Zhu and R. R. Schmidt, *Angew. Chem., Int. Ed.*, 2009, **48**, 1900–1934.
11 S.-H. L. Chiu and L. Anderson, *Carbohydr. Res.*, 1976, **50**, 227–238.
12 (*a*) J. Rademann and R. R. Schmidt, *Tetrahedron Lett.*, 1996, **37**, 3989–3990; (*b*) T. Wunberg, C. Kallus, T. Opatz, S. Henke, W. Schmidt and H. Kunz, *Angew. Chem., Int. Ed.*, 1998, **37**, 2503–2505.
13 *N*-(Phenylthiol)-ε-caprolactam: S. G. Durón, T. Polat and C.-H. Wong, *Org. Lett.*, 2004, **6**, 839–841.
14 Ipy_2BF_4: K.-T. Huang and N. Winssinger, *Eur. J. Org. Chem.*, 2007, 1887–1890.
15 *N*-Fluoro-2,6-dichloropyridinium triflate: H. Tsukamoto and Y. Kondo, *Tetrahedron Lett.*, 2003, **44**, 5247–5249.
16 NIS/Tf_2O: G. H. Veeneman, S. H. van Leeuwen and J. H. van Boom, *Tetrahedron Lett.*, 1990, **31**, 1331–1334.
17 D. Crich and M. Smith, *J. Am. Chem. Soc.*, 2001, **123**, 9015–9020.

