Carbohydrate Research 351 (2012) 121–125

Contents lists available at SciVerse ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Note

Glycosylation of α-amino acids by sugar acetate donors with $InBr_3$. Minimally competent Lewis acids

Mark R. Lefever, Lajos Z. Szabò, Bobbi Anglin, Michael Ferracane, Joanna Hogan, Lauren Cooney, Robin Polt *

Carl S. Marvel Laboratories, Department of Chemistry & Biochemistry, The University of Arizona, Tucson, AZ 85721, United States

ARTICLE INFO

Article history:
Received 27 July 2011
Received in revised form 8 January 2012
Accepted 18 January 2012
Available online 28 January 2012

Keywords:
Glycosylation
O-linked glycopeptides
Serine
Threonine
Indium tribromide

ABSTRACT

A simplified method for the preparation of Fmoc-serine and Fmoc-threonine glycosides for use in O-linked glycopeptide synthesis is described. Lewis acids promote glycoside formation, but also promote undesired reactions of the glycoside products. Use of 'minimally competent' Lewis acids such as $InBr_3$ promotes the desired activation catalytically, and with greatly reduced side products from sugar peracetates.

Glycoside construction remains as a tedious process that has not yielded to general methods. Intense efforts have been expended to develop synthetic methods for glycosides,[1] largely due to increased understanding of glycobiology, and subsequent demand for glycopeptides. The ideal methodology should produce high yields in a stereoselective manner and tolerate diverse functionality; it should be economical, environmentally friendly, easily reproduced, and scalable; it should use inexpensive, stable, and readily accessible glycosyl donors. Many existing methods produce high yields of the desired anomers, but often require the production of labile glycosyl donors, or very reactive (e.g., unstable) promoters.[2] All of these methods require scrupulously dry conditions.

Efficient production of O-linked glycopeptides requires the availability of acetate-protected glycosides of Fmoc serine and threonine, but direct approaches to these precursors have been problematic, principally due to difficulties in purification.[1a,10] The first example of this approach was provided by Kihlberg and coworkers.[1a] This study describes the glycosylation of Fmoc-L-Ser-OBn, Fmoc-L-Ser-OH, (Table 1) and simple alcohols (Table 2) with sugar peracetates in the presence of $Sc(OTfl)_3$ or In(III) salts (Figs. 1 and 2). Both $Sc(OTfl)_3$ and $InBr_3$ proved to be excellent glycosylation promoters. The *trans*- or β-glycosides were produced in moderate to excellent yields.[11,12] For simple alcohol acceptors anomeric ratios depended on reaction times and solvent polarity.

Various In^3 salts displayed striking differences in reactivity. Under reaction conditions utilizing halogenated solvents CH_2Cl_2 or $ClCH_2CH_2Cl$, $In(OAc)_3$, $InCl_3$, InF_3, and InI_3 salts proved to be ineffective as glycosylation promoters, whereas $InBr_3$ effected nearly quantitative conversion of Fmoc-L-Ser-OBn to the β-glycoside within minutes. Upon addition of catalytic amounts of HBr, or use of CH_2Br_2 as a solvent, $InCl_3$ became effective. A thorough investigation of $InBr_3$ mediated reactions showed this Lewis acid to be a superior promoter of O-glycosylation. All glycosyl donors tested afforded the desired Fmoc amino acid O-glycosides in moderate to excellent yields. $InBr_3$ promoted reactions of the Fmoc serine benzylesters provided greater than 90% isolated yield of the desired β-glycoside. The complementary $InBr_3$- or $Sc(OTfl)_3$-promoted reactions with Fmoc-Ser-OH gave somewhat lower yields which mirrored each other. Both microwave heated and traditional oil bath reflux glycosylations promoted by $InBr_3$ allowed for the use of *catalytic quantities* of the Lewis acid.

Of many Lewis acids tested, $Sc(OTfl)_3$ and $InBr_3$ proved to be the most effective at promoting glycosylation with sugar peracetates. In the case of the more active promoter $Sc(OTfl)_3$ optimal yields required strict control of reaction conditions. The $InBr_3$ promoted glycosylations on the other hand, were much milder, allowing reactions to be run for longer times at higher temperatures without incurring significant side product formation. This suggests that $InBr_3$ possesses sufficient *Lewis acid competency* to activate the anomeric acetate with neighboring group participation from the 2-position, but lacks the degree of reactivity associated with side product formation via Brønsted acid catalysis (Scheme 1). This

* Corresponding author. Tel.: +1 520 621 6322; fax: +1 520 621 8407.
E-mail address: polt@u.arizona.edu (R. Polt).

0008-6215/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2012.01.008

Table 1
Glycosylation of Fmoc-protected L-serine and L-serine benzyl ester with β-peracetate donors and Sc(III) or In(III) salts

Reaction	Donor	R	Promoter	Solvent	Time (min)	Temp (°C)	Isolated yield
1	β-Glc	Bn	1.0 equiv $Sc(OTfl)_3$	$PhCH_3$ CH_2Cl_2	3	80	85%
2	β-Xyl	Bn	1.0 equiv $Sc(OTfl)_3$	$PhCH_3$ CH_2Cl_2	3	80	90%
3	β-Lact	Bn	1.0 equiv $Sc(OTfl)_3$	$PhCH_3$ CH_2Cl_2	3	80	81%
4	β-Glc	H	1.0 equiv $Sc(OTfl)_3$	$ClCH_2CH_2Cl$	5	80	71%
5	β-Xyl	H	1.0 equiv $Sc(OTfl)_3$	$ClCH_2CH_2Cl$	5	80	68%
6	β-Lact	H	1.0 equiv $Sc(OTfl)_3$	$ClCH_2CH_2Cl$	5	80	74%
7	β-Glc	Bn	1.0 equiv $InBr_3$	$PhCH_3$ CH_2Cl_2	2	80	90%
8	β-Glc	Bn	0.5 equiv $InBr_3$	$PhCH_3$ CH_2Cl_2	5	80	93%
9	β-Glc	Bn	0.2 equiv $InBr_3$	$PhCH_3$ CH_2Cl_2	12	80	92%
10	β-Glc	Bn	0.1 equiv $InBr_3$	$PhCH_3$ CH_2Cl_2	20	80	93%
11	β-Glc	Bn	0.05 equiv $InBr_3$	$PhCH_3$ CH_2Cl_2	45	80	93%
12	β-Glc	Bn	0.01 equiv $InBr_3$	$PhCH_3$ CH_2Cl_2	90	80	92%
13	β-Glc	Bn	0.5 equiv $InCl_3$/HBr	$PhCH_3$ CH_2Cl_2	5	80	91%
14	β-Glc	H	0.5 equiv $InCl_3$	CH_2Br_2	5	100	84%
15	β-Glc	H	0.1 equiv $InBr_3$	$PhCH_3$ CH_2Br_2	5	80–100	42% (no chrom.)
16	β-Lact	H	0.15 equiv $InBr_3$	$PhCH_3$ CH_2Br_2	300	80	61% (no chrom.)

Table 2
Glycosylation of simple alcohols with β-glucose peracetate and $Sc(OTfl)_3$ or $InBr_3$

	R	Promoter	Solvent	Time (min)	Isolated yield	α:β (HPLC)
1	Me	1.0 equiv $Sc(OTfl)_3$	$PhCH_3$	1.00	75%	47:53
2	Me	1.0 equiv $Sc(OTfl)_3$	$PhCH_3$	5.00	32%	92:8
3	Me	1.0 equiv $Sc(OTfl)_3$	$PhCH_3$	15.00	—	—
4	Me	1.0 equiv $Sc(OTfl)_3$	DCE	1.00	42%	95:5
5	Me	1.0 equiv $Sc(OTfl)_3$	DCE	5.00	27%	90:10
6	Me	1.0 equiv $Sc(OTfl)_3$	DCE	15.00	—	
7	Me	0.5 equiv $InBr_3$	$PhCH_3$	1.00	96%	α
8	Me	0.5 equiv $InBr_3$	$PhCH_3$	5.00	95%	α
9	Me	0.5 equiv $InBr_3$	$PhCH_3$	15.00	95%	α
10	Me	0.5 equiv $InBr_3$	DCE	1.00	96%	α
11	Me	0.5 equiv $InBr_3$	DCE	5.00	97%	α
12	Me	0.5 equiv $InBr_3$	DCE	15.00	97%	α
13	Et	1.0 equiv $Sc(OTfl)_3$	$PhCH_3$	1.00	71%	9:91
14	Et	1.0 equiv $Sc(OTfl)_3$	$PhCH_3$	5.00	69%	19:81
15	Et	1.0 equiv $Sc(OTfl)_3$	$PhCH_3$	15.00	66%	51:49
16	Et	1.0 equiv $Sc(OTfl)_3$	DCE	1.00	62%	6:94
17	Et	1.0 equiv $Sc(OTfl)_3$	DCE	5.00	60%	25:75
18	Et	1.0 equiv $Sc(OTfl)_3$	DCE	15.00	58%	62:38
19	Et	0.5 equiv $InBr_3$	$PhCH_3$	1.00	83%	2:98
20	Et	0.5 equiv $InBr_3$	$PhCH_3$	5.00	84%	4:96
21	Et	0.5 equiv $InBr_3$	$PhCH_3$	15.00	81%	10:90
22	Et	0.5 equiv $InBr_3$	DCE	1.00	82%	5:95
23	Et	0.5 equiv $InBr_3$	DCE	5.00	83%	25:75

Table 2 (*continued*)

	R	Promoter	Solvent	Time (min)	Isolated yield	α:β (HPLC)
24	Et	0.5 equiv $InBr_3$	DCE	15.00	85%	57:43
25	*i*-Pr	1.0 equiv $Sc(OTfl)_3$	$PhCH_3$	1.00	72%	20:80
26	*i*-Pr	1.0 equiv $Sc(OTfl)_3$	$PhCH_3$	5.00	70%	52:48
27	*i*-Pr	1.0 equiv $Sc(OTfl)_3$	$PhCH_3$	15.00	65%	80:20
28	*i*-Pr	1.0 equiv $Sc(OTfl)_3$	DCE	1.00	65%	13:87
29	*i*-Pr	1.0 equiv $Sc(OTfl)_3$	DCE	5.00	64%	19:81
30	*i*-Pr	1.0 equiv $Sc(OTfl)_3$	DCE	15.00	61%	57:43
31	*i*-Pr	0.5 equiv $InBr_3$	$PhCH_3$	1.00	90%	2:98
32	*i*-Pr	0.5 equiv $InBr_3$	$PhCH_3$	5.00	88%	6:94
33	*i*-Pr	0.5 equiv $InBr_3$	$PhCH_3$	15.00	89%	15:85
34	*i*-Pr	0.5 equiv $InBr_3$	DCE	1.00	88%	21:79
35	*i*-Pr	0.5 equiv $InBr_3$	DCE	5.00	90%	64:36
36	*i*-Pr	0.5 equiv $InBr_3$	DCE	15.00	89%	84:16
37	C_6H_{11}	1.0 equiv $Sc(OTfl)_3$	$PhCH_3$	1.00	67%	28:72
38	C_6H_{11}	1.0 equiv $Sc(OTfl)_3$	$PhCH_3$	5.00	66%	53:47
39	C_6H_{11}	1.0 equiv $Sc(OTfl)_3$	$PhCH_3$	15.00	63%	80:20
40	C_6H_{11}	1.0 equiv $Sc(OTfl)_3$	DCE	1.00	55%	14:86
41	C_6H_{11}	1.0 equiv $Sc(OTfl)_3$	DCE	5.00	54%	37:63
42	C_6H_{11}	1.0 equiv $Sc(OTfl)_3$	DCE	15.00	51%	70:30
43	C_6H_{11}	0.5 equiv $InBr_3$	$PhCH_3$	1.00	79%	5:95
44	C_6H_{11}	0.5 equiv $InBr_3$	$PhCH_3$	5.00	76%	10:90
45	C_6H_{11}	0.5 equiv $InBr_3$	$PhCH_3$	15.00	77%	23:77
46	C_6H_{11}	0.5 equiv $InBr_3$	DCE	1.00	77%	46:54
47	C_6H_{11}	0.5 equiv $InBr_3$	DCE	5.00	75%	86:14
48	C_6H_{11}	0.5 equiv $InBr_3$	DCE	15.00	77%	87:13

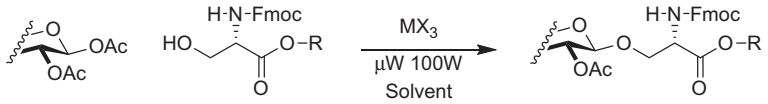


Figure 1. Glycosylation with β-peracetate donors.

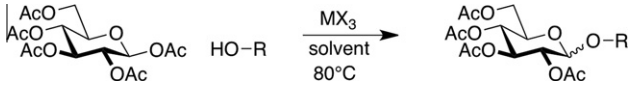


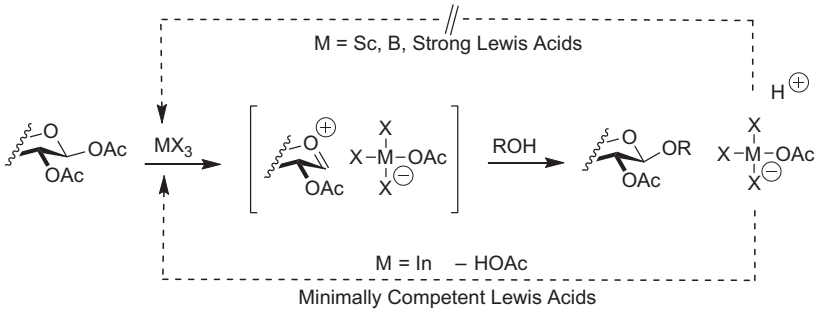
Figure 2. Glycosylation of simple alcohols with β-glucose peracetate.

minimal competency is also reflected in the promoter's ability function catalytically. Similar patterns of Lewis acid reactivity have been demonstrated in Denmark's studies.[3,4]

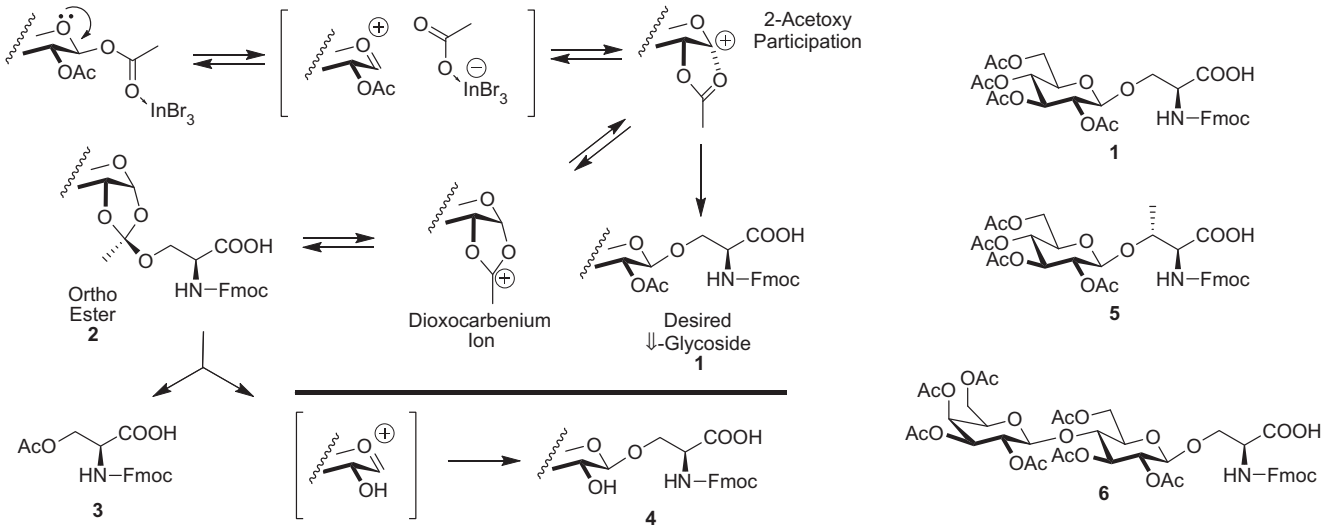
The results are consistent with the mechanism in Scheme 2. The product profiles of reactions involving Fmoc-serine glycosyl acceptors provide compelling evidence for an orthoester intermediate.[5] Although in none of the examples were the orthoesters isolated, evidence for the existence of this intermediate was provided by the isolation of acylated serine **3**, and the deacylated glycosides **4**, all presumably arising from the orthoester **2**. The lower yields observed with the free acid derivatives can be rationalized by the work of Szabo and Polt showing that the amino acid carboxylic acid can react with both the anomeric center and the carbonyl carbon of the participating group in the dioxocarbenium ion.[1b]

The anomeric α-acetates are of limited use in this reaction.[2c] Earlier studies of Lewis acid promoted glycosylations by Moraru also showed that the α-anomers were unreactive.[2a] This suggests that the orthoester **2** (Scheme 2) may not be an important intermediate in the glycosylation pathway under the described conditions.[6] This conclusion is consistent with the observation of complete selectivity for the β-product in the case of Fmoc-serine glycosyl acceptors. Lewis acid activation of anomeric acetates has been used quite effectively for 2-*deoxy*-2-iodo-sugars[7] due to the relatively electropositive iodine that is participating, but not as deactivating as the more electron-withdrawing *trans*-1,2-diacetate.[8] Other donors, such as lactose peracetate and acceptors, such as Fmoc-L-threonine have been used to provide good yields of compounds **5** and **6** as well. Other halogenated solvent systems, such as neat $CHCl_3$ and CCl_4, have also been used to good effect.

The $InBr_3$-catalyzed reaction has been accomplished with several 2° alcohols (Table 2), including the production of the Fmoc-L-threonine-β-D-glucoside, (Table 1, entry 15).[9] It is important to emphasize that the $InBr_3$ catalytic system is extremely moisture tolerant, in addition to demonstrating reduced sensitivity to overheating or extended reaction times. The free acids of Fmoc-Ser-OH and Fmoc-Thr-OH have been converted to their corresponding glucoside peracetates in good yields, high purity, and in one step without chromatography.[7,9,10]



Scheme 1. Minimally competent Lewis acids such as $InBr_3$ can dissociate (lower pathway) from the displaced acetate to form acetic acid and regenerate the Lewis acid catalyst. Stronger Lewis acids remain associated with the acetate (upper pathway) to produce a Brønsted acid, and generally require a full equivalent of the Lewis acid.



Scheme 2. Participation by the 2-acetoxy group produces an intermediate that largely undergoes desired *trans* β-glycoside formation. Formation of an orthoester can decompose in 3 ways, leading to the desired glycoside **1**, or acyl transfer to the serine acceptor (cf. **4**), which produces a 2-hydroxyl sugar oxonium ion that rapidly glycosylates another acceptor in situ. Lactose β-peracetate and Fmoc-L-threonine have been used as well to provide **5** and **6** in good yields and without the use of molecular sieves or chromatography.

The very reactive and non-bulky acceptor CH_3OH leads to α-glycosides (Table 2, entries 1–12), and prolonged heating leads to higher proportions of the α-glycoside, especially in the presence of the stronger Lewis acid $Sc(OTfl)_3$ that is not '*minimally competent*,' requiring a full equivalent of this Lewis acid promotor such as $BF_3{\cdot}Et_2O$, $SnCl_4$, or AgOTfl.[1a,10–12] These observations are quite consistent with what is known about the anomeric effect, and it is not surprising that the axial glycosides ultimately predominate as reaction times are extended. Further studies of minimally competent Lewis acid promotors with more complex donors and other acceptors are probably warranted.

Acknowledgments

We thank the Office of Naval Research (N00014-05-1-0807 & N00014-02-1-0471), the National Science Foundation (CHE-607917) and the National Institutes of Health (NINDS-NS-052727) for Support.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2012.01.008.

References

1. (a) Elofsson, M.; Walse, B.; Kihlberg, J. *Tetrahedron Lett.* **1991**, *32*, 7613–7616; (b) Szabo, L.; Polt, R. *Carbohydr. Res.* **1994**, *258*, 293–297; (c) Paulsen, H.; Peters, S.; Bielfeldt, T.; Meldal, M.; Bock, K. *Carbohydr. Res.* **1995**, *268*, 17–34.
2. (a) Seibel, J.; Hillringhaus, L.; Moraru, R. *Carbohydr. Res.* **2005**, *340*, 507–511; (b) Wei, G.; Lv, X.; Du, Y. *Carbohydr. Res.* **2008**, *343*, 3096–3099; (c) Keyari, C. M.; Polt, R. *J. Carbohydr. Chem.* **2010**, *29*, 181–206. , and references therein; (d) Yao, N.; Fung, G.; Malekan, H.; Ye, L.; Kurth, M. J.; Lam, K. S. *Carbohydr. Res.* **2010**, *345*, 2277–2281.
3. Denmark, S. E.; Beutner, G. L.; Wynn, T.; Eastgate, M. D. *J. Am. Chem. Soc.* **2005**, *127*, 3774–3789.
4. Denmark, S. E.; Wynn, T. *J. Am. Chem. Soc.* **2001**, *123*, 6199–6200.
5. (a) Garegg, P. J.; Kvarnstrom, I. *Acta Chem. Scand. B* **1976**, *30*, 655–658; (b) Wallace, J. E.; Schroeder, L. R. *J. Chem. Soc., Perkin Trans. 2* **1977**, 795–802; (c) Banoub, J.; Bundle, D. R. *Can. J. Chem.* **1979**, *57*, 2091–2097; (d) Garegg, P. J.; Konradsson, P.; Kvarnstrom, I.; Norberg, T.; Svensson, S. C. T.; Wigilius, B. *Acta Chem. Scand. B* **1985**, *39*, 569–577; (e) Polt, R.; Szabò, L.; Treiberg, J.; Li, Y.; Hruby, V. J. *J. Am. Chem. Soc.* **1992**, *114*, 10249–10258.
6. A 250 mL round bottom flask equipped with a magnetic stirrer and a reflux condenser was charged with 17.8 g (45.6 mMol) β-glucose peracetate, 4.98 g (15.2 mMol) Fmoc-L-serine, and 808 mg (1.52 mMol) $InBr_3$. Next, 80 mL dry $PhCH_3$ and 8 mL dry (filtered through a plug of SiO_2) CH_2Br_2 were added, and the reaction was placed in an oil bath at 65–70 °C for 20 min until the starting materials had dissolved forming a pink solution. The oil bath was heated to 110 °C and the reaction run for 3–4 h until the Fmoc-serine had disappeared by TLC. The reaction was cooled, and the solvent evaporated to form a glassy solid, which was redissolved in 250 mL dry EtOAc, washed 3× with 75 mL saturated $NaHCO_3$, then extracted 3× with 75 mL deionized H_2O, alternating between washing and extracting each time. The neutral extractions were combined, and

the organic phase and basic washes were discarded after verifying no product was present by TLC. The combined neutral extractions were acidified to pH 2–3 with HCl, and the resulting precipitate recrystallized from EtOAc/hexanes or *i*PrOAc/hexanes to provide 5.0 g (67 %) of Fmoc-L-serine-β-D-glucoside-peracetate **1**, mp 160–162 °C. See Supplementary data for HPLC traces and NMR spectra.

7. (a) Handa, M.; Smith, W. J.; Roush, W. R. *J. Org. Chem.* **2008**, *73*, 1036–1039; (b) Chong, P. Y.; Roush, W. R. *Org. Lett.* **2002**, *4*, 4523–4526.
8. Ogawa, T.; Beppu, K.; Nakabayashi, S. *Carbohydr. Res.* **1981**, *93*, C6–9.
9. A 50 mL triple-walled Pyrex® tube with a threaded Teflon® plug was charged with 3.90 g β-glucoseperacetate, 1.14 g Fmoc-L-threonine and 118 mg $InBr_3$, 1 mL CH_2Br_2 and 9 mL $PhCH_3$. The tube was tightly sealed with the Teflon plug, and the reaction mixture was heated in an Emerson commercial 1000 watt microwave oven (purchased at home depot) for 30 s increments at 50% power setting. After each increment of heating the reaction was shaken, temperature was monitored, and an aliquot removed for TLC or HPLC analysis. The temperature never exceeded 100 °C. After several 30 second heating increments 4–5% of the original Fmoc-L-threonine was evident by HPLC analysis. The mixture was evaporated to a viscous oil and redissolved in 50 mL dry EtOAc. This solution was gently washed (to avoid formation of an emulsion) with 15 mL saturated $NaHCO_3$– very little product was in this basic wash layer. The EtOAc solution was extracted with 15 mL deionized H_2O– this neutral extract contained the glycoside product. This washing and extraction process was repeated 4×, and the neutral washings were combined. The combined neutral washings were stirred vigorously and acidified to pH 3 with 1 N HCl while stirring continued. The resulting white precipitate was collected and dried in vacuo. Recrystallization from 10 mL EtOAc and 5 mL hexanes provided 0.95 g of Fmoc-L-threonine-β-D-glucoside-peracetate **5**, mp 180–182 °C. See Supplementary data for HPLC traces and NMR spectra.
10. Salvador, L. A.; Elofsson, M.; Kihlberg, J. *Tetrahedron* **1995**, *51*, 5643–5656.
11. Garg, H. G.; Hasenkamp, T.; Paulsen, H. *Carbohydr. Res.* **1986**, *151*, 225–232.
12. Mitchell, S. A.; Pratt, M. R.; Hruby, V. J.; Polt, R. *J. Org. Chem.* **2001**, *66*, 2327–2342.