COMMUNICATION

Iodine and its interhalogen compounds: versatile reagents in carbohydrate chemistry. XIV.[1] Glycosylated amino acid synthesis

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A practical procedure for glycosylated amino acid synthesis using iodine-promoted glycosylation of various protected serine and threonine with a selection of thioglycoside and glycosyl halide donors is described.

Introduction

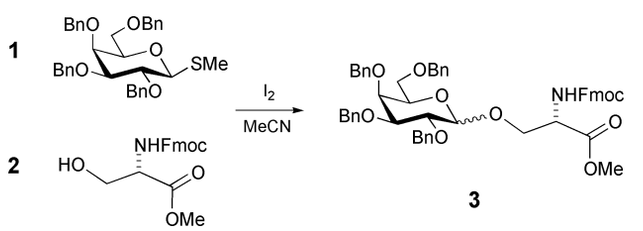
Glycosylation is increasingly recognised as central to the function of glycoproteins,[2] having a profound impact on the structure, dynamics and recognition of this major class of biopolymer.[3] There is currently substantial interest in understanding the chemistry and biology of glycosylation processes, with a view to therapeutic intervention, for instance.[4] Many new opportunities present themselves as a result of recent developments in glycopeptide synthesis,[5,6] with the use of synthetic glycopeptides as cancer vaccines being a particularly successful recent example.[7] A number of target glycopeptide syntheses have been reported of late, including a highly glycosylated structure with numerous Tn-antigen moieties corresponding to human glycophorin A(N),[8] glycosylated 5-hydroxy-L-norvaline-based peptides related to type II collagen,[9] a heptacosapeptide carrying a sialyl T trisaccharide corresponding to the B-chain of human alpha 2HS glycoprotein,[10] the antimicrobial diptericin glycopeptide prepared by a native chemical ligation approach,[11] and sialyl Lewis a containing partial sequences of the P-selectin ligand PSGL-1.[12]

For some time we have been investigating iodine and related compounds with a view to minimising the number of reagents routinely necessary for a variety of reactions in carbohydrate chemistry, including acetal protection/deprotection, acyl transfer reactions, glycosyl halide formation and glycosylation.[1] Only a limited number of glycosylated amino acid building blocks suitable for glycopeptide synthesis are commercially available, hence there is a requirement for the development of efficient methods for preparation of glycosylated amino acids.[5,6] Herein we report our observations on iodine-promoted glycosylation of appropriately protected serine and threonine building blocks.

Results and discussion

Reaction of 'armed'[13] benzylated thiogalactoside (**1**) with *N*-Fmoc serine methyl ester (**2**) in the presence of iodine was found to be an effective reaction (Table 1), affording glycosides (**3**) in excellent yield. For reasons of acceptor solubility,[14] and in order to achieve practical reaction rates, couplings were routinely conducted in acetonitrile and with an excess of acceptor, which enabled more straightforward product purification since generation of the hemi-acetal by-product is substantially reduced. As expected, in the presence of base (anhydrous K_2CO_3 to remove HI), iodine-promoted glycosylation of alcohol **2** with thioglycoside **1** in the participating solvent acetonitrile [15] gave predominantly **3**β. In contrast, the same reaction in the absence of base gave predominantly **3**α; formation of the latter was accompanied by consumption of **3**β, which formed first, as judged by TLC time course analysis. Also evident during the reaction was epimerisation of β-thioglycoside donor **1**.[16] Reaction at lower temperature (−5 °C) favoured formation of **3**α even in the presence of base, whilst at −18 °C the α : β was a very respectable 5 : 1 in favour of the thermodynamic product (Table 1). Attempts to further enhance α-selectivity through the use of solvents known to favour α-glycoside formation (*e.g.* toluene–ether or toluene–dioxane mixture)[17] were precluded either by poor acceptor solubility on cooling, or by competing degradation of donor at ambient temperature.

Table 1 Iodine-promoted glycosylation with 'armed' thiogalactoside (**1**)



Reaction conditions	% Yield of **3**	α : β
55 min, 0 °C[a]	88	1 : 3.5
30 min, 0 °C	82	2.5 : 1
2.5 h, −5 °C[a]	82	2.5 : 1
2.5 h, −18 °C to −5 °C	82[b]	5 : 1[b]

[a] Reaction carried out in the presence of 1.5 mol equiv. anhydrous K_2CO_3. [b] Essentially the same results were obtained using the α-anomer of **1**.

Whilst there are clearly a number of competing effects, data in Table 1 suggest that it should prove possible to conduct iodine-mediated glycosylation reactions under either kinetic or thermodynamic control, depending mainly on the presence or absence of base, respectively. Presumably the key to such thermodynamically controlled reactions is the *in situ* generation of stoichiometric HI, which effects *O*-glycoside epimerisation whilst showing no apparent tendency to remove benzyl ether protecting groups.

Reaction of 'disarmed'[13] acetylated galactosyl bromide (**4**) with *N*-Fmoc serine methyl ester (**2**) in the presence of iodine was also found to be an effective reaction (Table 2), giving the expected product, **5**β, in excellent yield when acceptor was present in excess. When a more conventional approach was adopted, with donor in excess, an appreciable amount of **5**α was also formed. This presumably reflects the ability of HI to also effect epimerisation of 'disarmed' glycoside **5**β, which was observed by TLC to be the first product formed in this reaction.

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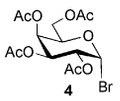
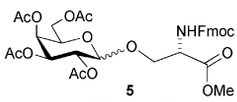
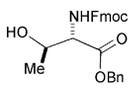
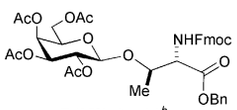
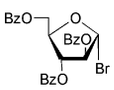
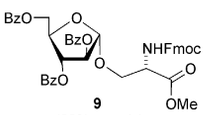
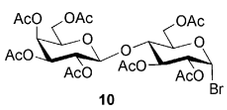
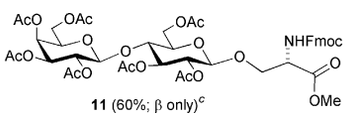
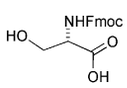
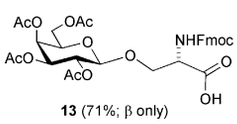
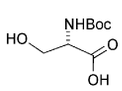
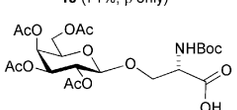
Table 2 Iodine-promoted glyco-amino acid synthesis

Glycosyl donor	Glycosyl acceptor (mol equiv.)	Reaction conditions	Product (yield, stereocontrol)
 **4**	**2** (2.5)	18 h, rt	 **5** (90%; β only)
4	**2** (0.8)	24 h, rt	**5** (60%; α : β, 1 : 3)[a]
4	 **6** (1.5)	5 h, rt	 **7** (65%; β only)[b]
 **8**	**2** (2.0)	1 h, rt	 **9** (88%; α only)
 **10**	**2** (2.0)	1 h, rt	 **11** (60%; β only)[c]
4	 **12** (2.5)	5 h, rt	 **13** (71%; β only)
4	 **14** (2.5)	5 h, rt	 **15** (81%, β only)

[a] Acceptor had been consumed after 5 h, when formation of **5**β only was evident. [b] Extended reaction (>24 h) resulting in the appearance of **11**α. [c] Unoptimised; reaction was terminated after 1 h to avoid product epimerisation.

The iodine-promoted glycosylation procedure described is also effective for galactosylation of protected *N*-Fmoc threonine benzyl ester **6**, giving **7**β, and for glycosylation of *N*-Fmoc serine methyl ester **2** with glycosyl halides derived from benzoylated arabinofuranose **8** giving **9**α, and acetylated lactose **10** giving **11**β (Table 2). Direct glycosylation of commercially available *N*-Fmoc serine **12** possessing a free carboxy group[14] affords the corresponding glycoside **13**β, in excellent yield, the same reaction also being effective for the corresponding *N*-Boc serine derivative **13** giving **14**β. These latter two reactions result in materials suitable for use directly in peptide and combinatorial chemistry, without the need for cleavage of a carboxy protecting group.

Conclusions

In summary, iodine-promoted glycosylation of appropriately protected amino acids provides straightforward access to a variety of building blocks for target glycopeptide synthesis and combinatorial chemistry. The procedures described are simple to conduct, are high yielding and, in spite of the *in situ* generation of HI, are compatible with potentially acid-labile groups, including inter-sugar glycosidic linkages, furanosyl glycosides and Boc protecting groups. The literature shows a highly variable success rate for amino acid glycosylation methods; the procedures described herein are competitive with established procedures.[5,6]

Experimental

All new compounds gave satisfactory spectroscopic and analytical data; glycosides **5**β[18] and **13**β[14,19] have been reported previously.

Typical glycosylation procedure

Glycosyl donor (0.25 mmol) and acceptor (1.5–2.5 mol equiv.) were dissolved in acetonitrile (1–2 ml), in the presence/absence of anhydrous potassium carbonate (1.5 mol equiv. with respect to donor). The mixture was cooled to an appropriate temperature and iodine (1.5 mol equiv. with respect to donor) was added. The reaction was monitored by TLC until complete and then quenched by addition of saturated aqueous sodium thiosulfate solution. Following a conventional work-up, products were isolated by column chromatography.

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