Improved synthesis of *O*-linked, and first synthesis of *S*- linked, carbohydrate functionalised *N*-carboxyanhydrides (glycoNCAs)†

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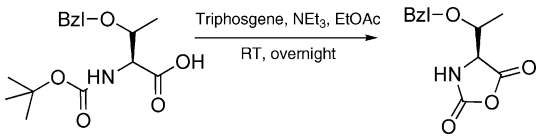
An improved method for the synthesis of glycosylated *N*-carboxyanhydrides, which are monomers for glycopeptide synthesis, is presented.

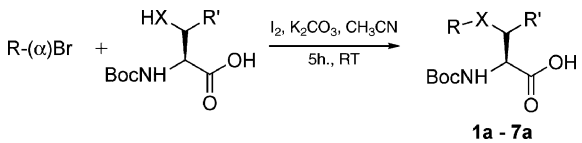
α-Amino acid *N*-carboxyanhydrides (NCAs) have been used extensively as monomers for the synthesis of high molecular weight homopolypeptides.[1] Addition of a suitable initiator (base or nucleophile) leads to ring-opening polymerisation with the expulsion of a molecule of CO_2. In recent years it has been shown that this polymerisation can be controlled by either transition metal complexes,[2] ammonium ions,[3] or by using ultra pure monomers[4] and inert conditions,[5] leading to well-defined polymers of predictable degree of polymerization and narrow polydispersity. The materials produced are both biocompatible and biodegradable, leading to a plethora of potential biomedical applications.[6] Currently there is a great interest in the synthesis and properties of carbohydrate bearing polymers, known as "glycopolymers"[7] (and also synthetic glycoproteins.[8]) These have applications in drug delivery,[9] lectin binding assays[10] and nanotechnology. However, glycopolypeptides synthesized by the NCA method[11] have not received the same attention as glycopolymers synthesised from acrylates, methacrylates, *etc.* This may be due to the extreme sensitivity of NCAs to all nucleophilic functionalities (including water), which makes synthesis, handling and storage difficult.

There is currently only one method described in the literature for the synthesis of carbohydrate bearing NCAs (glycoNCAs).[12] *N*-Carbobenzoxy-L-serine benzyl ester and an acetobromosugar were coupled by a Koenigs–Knorr reaction with mercuric cyanide, followed by removal of both protecting groups by hydrogenation. The amino acids were then phosgenated to give the first glycoNCAs with yields between 15 and 40%. This atom inefficient strategy requires revision to take advantage of modern synthetic carbohydrate chemistry. In particular, the use of highly toxic and environmentally damaging mercury salts[13] as promoters is no longer necessary and atom efficiency can be improved by removing the requirement to protect the carboxylic acid functionality in amino acids.[14] In this work, our strategy was to prepare glycosylated *N*-Boc amino acids then use the well-known cyclisation of these to their corresponding NCAs[15] (Scheme 1).



Scheme 1 Cyclisation of *N*-Boc (*O*-Bzl) L-threonine to the corresponding NCA.

To access the *N*-Boc glycosylated amino acids we utilised the method of Field *et al.*,[16] which employs acetobromosugars as glycosyl donors and iodine as the Lewis acid promoter (Scheme 2 and Table 1). Iodine is a much more benign promoter than mercuric cyanide and is compatible with the Boc group, unlike more commonly used Lewis acids such as BF_3[17] and $AlCl_3$.[18] Purification was achieved by flash column chromatography. In all the cases, the β-anomer was formed exclusively (single anomeric peak in ^{13}C NMR, see ESI†) due to neighbouring group participation from the equatorial 2-*O*-acetate group. The generality of this method is demonstrated by the synthesis of *S*-linked glycosides **6a** and **7b** from L-cysteine.



Scheme 2 Synthesis of *N*-Boc glycosylated amino acids by iodine promoted glycosylation. R, R′ and X are defined in Table 1.

Treatment of these compounds under the conditions shown in Scheme 1 did not result in NCA formation. The reasons for this remain unclear, but the IR absorption band for N–H shifted from 3372 cm^{-1} for BocThr(bzl) to 3439 cm^{-1} for BocThr(GluAc)

Table 1 Iodine promoted glycosylation of various *N*-Boc glycosylated amino acids

Entry	R [a,b]	X	R′	Yield (%)[c]
1a	Ac_4Gal	O	Me	59
2a	Ac_4Glu	O	Me	55
3a	Ac_4Gal	O	H	57
4a	Ac_4Glu	O	H	54
5a	Ac_7Lac	O	H	43
6a	Ac_4Glu	S	H	47
7a	Ac_4Gal	S	H	49

[a] Acetobromosugar used. [b] Ac_4Gal = (2,3,4,6)-tetraacetyl galactopyranose; Ac_4Glu = (2,3,4,6)-tetraacetyl glucopyranose; Ac_7Lac = (2,3,6,2′,3′,4′,6′)-heptaacetyl lactose. [c] Isolated yield following column chromatography (silica, hexane–THF 1 : 0 → 3 : 7).

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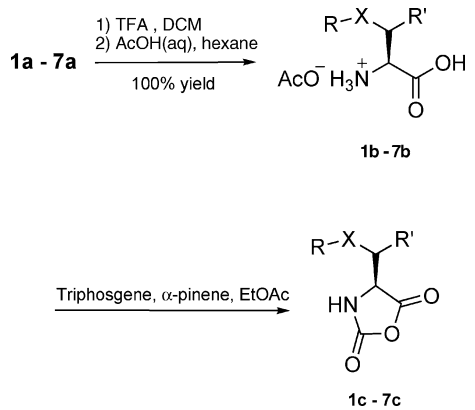
suggesting the involvement of this group in hydrogen bonding, presumably to an acetate carbonyl. Such an interaction may hinder cyclisation of the Boc-protected amine. This obstacle was overcome by cleaving selectively the Boc group by treatment with TFA in DCM, which proceeds rapidly at room temperature without anomerisation (Scheme 3).[19] Further purification was not required as the side products are gaseous, expediting the process.



Scheme 3 Synthesis of glycoNCAs, **1c–7c**, from their *N*-Boc glycosylated amino acids, **1a–7a**. R, R′ and X as for **1a–7a** (see Table 1).

The resulting glycosylated amino acids were then treated with triphosgene[20] in the presence of α-pinene,[21] an unsaturated terpene that scavenges residual HCl, to give the glycosylated NCAs **1c–7c** in moderate to good yields following recrystallisation (Table 2; the yields are compromised by the inherent instability of NCAs). Recent work has shown that the precision polymerisation of NCAs requires a further, more rigorous purification step[4,5] (involving an aqueous extraction) to remove the small, but ever-present residual impurities (see ESI†). This is best done immediately before polymerisation, so was not undertaken in the present work. Of particular note here is the syntheses of **6c** and **7c**, which represent the first synthesis of an *S*-linked glycoNCA. *S*-Linked sugars show higher stability towards glycosidases than *O*-linked analogues,

Table 2 Overall yields of glycosylated *N*-carboxyanhydrides

Entry	R	X	R′	Yield (%)[a]
1c	Ac_4Gal	O	Me	51
2c	Ac_4Glu	O	Me	48
3c	Ac_4Gal	O	H	50
4c	Ac_4Glu	O	H	49
5c	Ac_7Lac	O	H	37
6c	Ac_4Glu	S	H	30
7c	Ac_4Gal	S	H	26

[a] Isolated yield following recrystallisation.

thus these *S*-linked glycoNCAs may lead to new and interesting materials that are models of biologically occurring glycoproteins.

In summary we have applied modern synthetic carbohydrate chemistry to update and improve the synthesis of glycoNCAs and shown that it can be used to create a diverse set of functional monomers. We anticipate that this will allow access to a range of glycosylated polypeptides that could be employed in a wide range of applications, including drug delivery and tissue engineering.

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