

Optimized synthesis of aminoxy-peptides as glycoprobe precursors for surface-based sugar–protein interaction studies

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Received 12 June 2007; revised 27 June 2007; accepted 28 June 2007
Available online 5 July 2007

Abstract—An improved procedure for solid phase coupling of Boc-aminoxyacetic acid to peptides is described. By avoiding base-containing activation mixtures which cause over-acylation, it practically suppresses this unwanted side reaction and leads to near quantitative yields of Aoa-peptides, useful as glycoprobe precursors in glycomic studies.

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With the ever-increasing awareness of the importance of protein glycosylation as a key player in inter- and intracellular communication,^{1–4} the need for powerful chemical tools to document sugar–protein cross-talk is rising. One reason for such interest in carbohydrate–protein interactions is their implication in the targeting of enveloped viruses such as HIV, influenza-, and coronavirus,^{5,6} an understanding of which will facilitate the design of carbohydrate-binding agents capable of neutralizing viral fusion and transmission. Different biophysical techniques have been used to monitor sugar–protein interactions,^{7,8} including NMR, X-ray crystallography, and more recently, surface plasmon resonance (SPR). The latter is fast gaining recognition because of its sensitivity, low sample consumption, and capability for real-time monitoring. In this technique, one of the two interacting entities (protein or sugar) is immobilized onto the surface of a sensor chip, the other one is flown across and the resulting read-out enables both quantitation and kinetic analysis of the interaction

Among the two immobilization approaches possible, the sugar-on-chip option has demonstrable advantages^{9,10} but requires a sugar in highly purified form and attached to the chip surface in a chemically well-defined manner. While the synthesis of complex carbohydrate structures is a fast expanding field,^{11–15} the structural diversity encountered in nature cannot yet be fully met in the laboratory. Thus, some glycans (e.g., bacterial polysaccharide repeating units, elongated mucin-type glycans or complex *N*-glycans) cannot be efficiently produced for lack of suitable synthetic chemistries or glycosyltransferases/glycosidases,¹⁶ and must be purified from natural sources. As the amount of material available is scarce, the immobilization chemistry to the sensor chip surface must be optimal to avoid losses of precious material. Although a direct aldehyde coupling has been described,¹⁷ its efficiency is questionable.

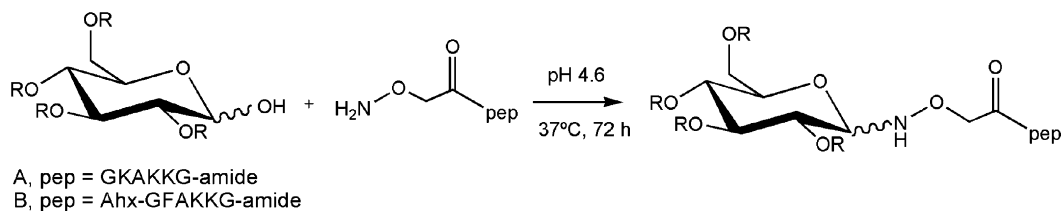
Recently, we reported a practically universal approach to carbohydrate immobilization on carboxymethyl dextran chips via standard peptide-bond chemistry, subsequent to chemical ligation of the sugar to a tailor-made peptide module.¹⁸ A chemospecific oxime linkage between the reducing end of the first monosaccharide and the peptide is achieved (Scheme 1) through the introduction of an *N*-terminal aminoxyacetic acid (Aoa) residue in the latter.

Over the last decade, oxime chemistry has been proven as one of the most successful approaches to peptide

Abbreviations: Boc, *tert*-butyloxycarbonyl; DIEA, *N,N*-diisopropylethylamine; DIC, *N,N*-diisopropylcarbodiimide; Fmoc, 9-fluorenylmethoxycarbonyl; HBTU, 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; TFA, trifluoroacetic acid; TIS, triisopropylsilane.

Keywords: Glycoprobes; Surface-based interaction studies; Aminoxy-peptide; Coupling reagent.

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Scheme 1. Illustration of oxime ligation between an N-terminal Aoa-containing peptide and a carbohydrate ligand. See Ref. 18 for further details.

chemical ligation.^{19,20} Moreover, applications to sugar-peptide conjugation have also been described.^{21,22} Despite the obvious advantages of this chemistry,²³ it has as a main drawback that over-acylation of the NH–O nitrogen leads to undesired heterogeneity.²⁴ To resolve this problem, deprotection of Aoa²⁵ or use of *N*-trityl protection²⁶ has been advocated; however, neither of these two approaches utilizes commercially available reagents.

In this work, we have explored the feasibility of minimizing Aoa over-acylation using Boc-protected Aoa

and conventional coupling chemistry. Our study is based on two starting peptide substrates, the hexapeptide GFAKKG-amide¹⁸ (A) and a version N-terminally elongated with an ϵ -amino hexanoic acid (Ahx) spacer, Ahx-GFAKKG-amide²⁷ (B), both acylated with Boc-Aoa-OH²⁸ under different conditions (Fig. 1, Table 1).

In line with previous work,²⁴ we reasoned that the presence of base at the coupling step would favor over-acylation. Indeed, as shown on panels A(i) and B(i), otherwise efficient HBTU-mediated coupling conditions

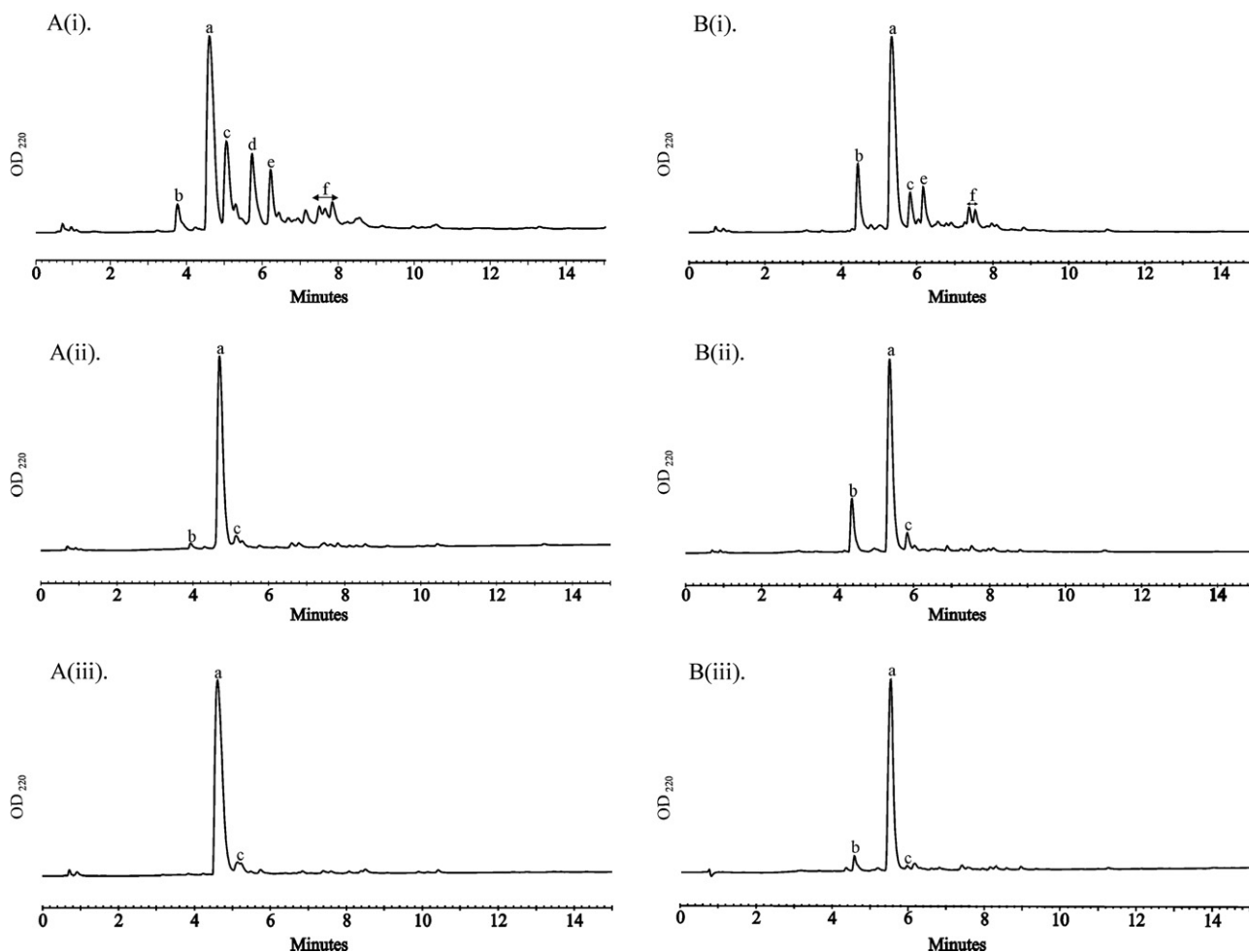


Figure 1. HPLC analysis of different conditions for Aoa-GFAKKG-amide [entries A(i–iii)] and Aoa-Ahx-GFAKKG-amide [entries B(i–iii)] synthesis. (i) Boc-Aoa-OH/HBTU/DIEA (3:3:6 equiv), 40 min; (ii) Boc-Aoa-OH/DIC (10:10 equiv), 60 min; (iii) Boc-Aoa-OH/DIC (8:8 equiv), 10 min. Peak assignments: a: target Aoa-peptides; b: starting peptide; c: diacylated peptide; d: triacylated peptide; e: N-terminal guanidine byproduct (uronium capping); f: oligomeric impurities. HPLC conditions: Phenomenex Luna C8 column; elution with linear 0–30% [panels A(1–iii)] and 0–40% [panels B(1–iii)] gradient of acetonitrile (+0.036% TFA) into water (+0.045% TFA) over 15 min; flow rate: 1 mL/min.

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