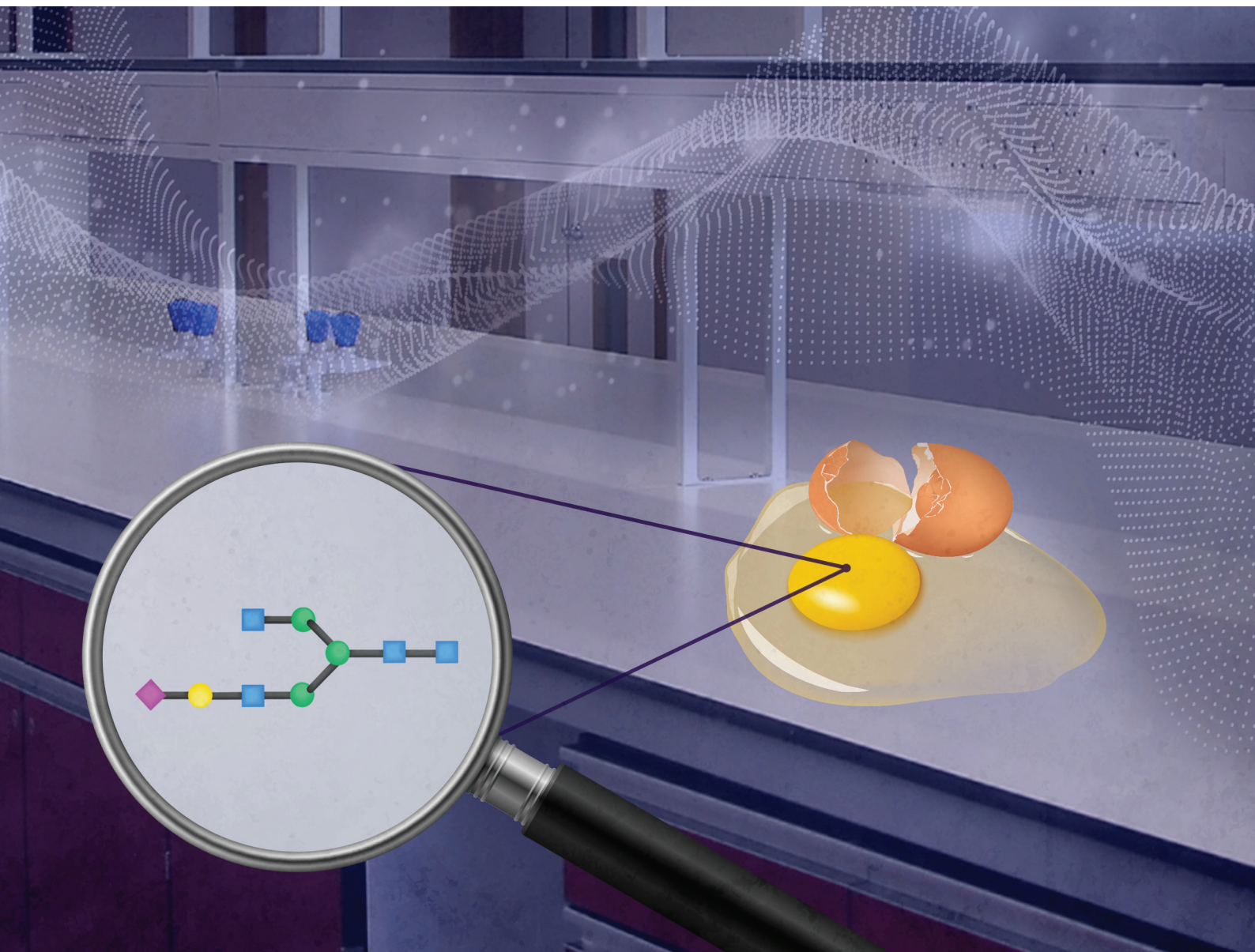


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**PAPER**

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## Egg yolk sialylglycopeptide: purification, isolation and characterization of *N*-glycans from minor glycopeptide species†

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Sialylglycopeptide (SGP) is a readily available naturally occurring glycopeptide obtained from hen egg yolk which is now commercially available. During SGP extraction, other minor glycopeptide species are identified, bearing *N*-glycan structures that might be of interest, such as asymmetrically branched and triantennary glycans. As the scale of SGP production increases, recovery of minor glycopeptides and their *N*-glycans can become more feasible. In this paper, we aim to provide structural characterization of the *N*-glycans derived from these minor glycopeptides.

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### 1. Introduction

Sialylglycopeptide (SGP) (Fig. 1) is a naturally occurring glycopeptide that can be extracted from hen egg yolks. It originates from the proteolytic cleavage of vitellogenin during yolk formation.<sup>1</sup> SGP has wide applications. Synthetically, SGP offers convenient access to *N*-glycan oxazolines, needed for glycoprotein remodelling by ENGase mediated transglycosylation.<sup>2,3</sup> Furthermore, SGP has been used as a precursor for the synthesis of constructs such as sialyl Lewis-X structures (S-Lex),<sup>4</sup> antennary polyactosamine repeats<sup>5</sup> and triantennary glycans,<sup>6,7</sup> or a combination of all the above,<sup>8</sup> providing access to *N*-glycan structures for which there is no natural source. Analytically, it has been used for testing new matrices for MALDI-MS,<sup>9</sup> for sialic acid derivatization strategies<sup>10–12</sup> and as a control for *N*-glycan release and labelling by reductive amination.<sup>13</sup>

Even though the amount of glycoconjugates present in egg yolk is only about 0.3% (w/w),<sup>14</sup> the usefulness of SGP has led to the development of different isolation protocols that have enabled to increase the purity at which SGP can be obtained.<sup>15–18</sup> However, SGP might not be the only relevant

glycopeptide target that can be extracted from egg yolks. Early reports of the isolation of SGP indicated the presence of other glycopeptides bearing monosialylated glycans,<sup>19,20</sup> some of which allegedly corresponded exclusively to the antennary extension of the  $\alpha$ -1,3 mannose arm of the *N*-glycan core SGP-A2[3]G1S1 (Fig. 1). This glycan asymmetry has been attributed to different processing in the Golgi.<sup>21</sup> Normally during SGP purification the monoantennary glycopeptide is removed by size exclusion chromatography<sup>22</sup> or digested enzymatically to afford a homogenous albeit degalactosylated glycan.<sup>23,24</sup>

Despite previous evidence for the presence of the monosialylated asymmetric glycan in egg yolk, recent publications identify it as a mixture.<sup>25</sup> Having an alternative source of asymmetric glycans would be of interest since they are difficult to synthesize and there is growing awareness of the biological implications of *N*-glycan asymmetry such as the complement-dependent cytotoxicity of monoclonal antibodies,<sup>26</sup> and influenza haemagglutinin binding.<sup>27</sup> Furthermore, asymmetric structures can be useful for the validation of mass spectrometry-based techniques.<sup>28,29</sup> Each of these examples is indicative of potential applications for these compounds.

This paper aims to present a simplified purification strategy for both A2G2S2 and A2[3]G1S1 starting from egg yolk, along with supporting evidence that confirms this structural assignment. Unexpectedly, during method development, it was possible to identify an additional glycopeptide species that corresponded to a triantennary analogue of SGP. The separation and characterization of the triantennary glycans obtained from this glycopeptide will also be presented.

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† Electronic supplementary information (ESI) available: Experimental details of analytical method, isolation, purification, characterization of *N*-glycans and *N*-glycan nomenclature. See DOI: <https://doi.org/10.1039/d2ob00615d>



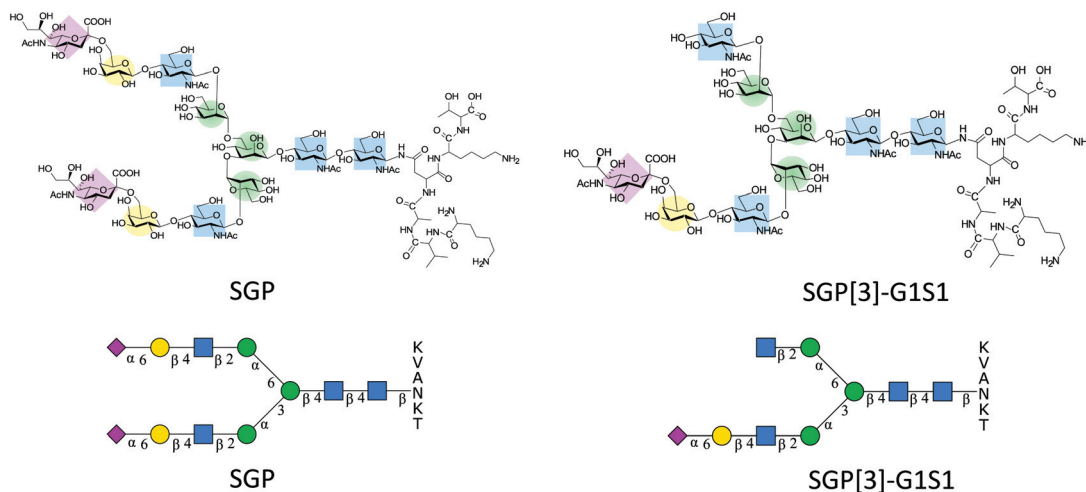


Fig. 1 Structural representation of SGP and SGP-A2[3]G1S1\* for *N*-glycan abbreviations see section 2.9 of ESI.†

## 2. Results and discussion

### 2.1 SGP extraction

Both egg yolk powder and fresh egg yolks were used to produce crude SGP preparations, the details of purification processes can be found in the materials and methods. However, as previously reported<sup>25</sup> glycosylated versions of SGP are also present when using egg yolk powder (Fig S1†), with these species most likely originating during spray drying.<sup>30</sup> SGP from the dry yolk powder was used for initial *N*-glycan analysis, but to provide a better estimation of the overall purification yield based on the weight of the crude SGP, fresh yolks were used.

### 2.2 Preliminary procainamide analysis-sialic acid derivatization

The *N*-glycans present in the crude SGP (obtained from dry egg yolk) were released with PNGase-F and then procainamide labelled producing a mixture that contains about 56% of A2G2S2 glycan (Fig. 2A), with other species identified summarized in Table 1. Sialic acid linkage characterisation was carried out on this initial mixture by subjecting the procainamide labelled glycans to ethyl esterification-amidation (EEA)<sup>31</sup> and were re-analysed by HPLC-MS to determine the mass increments due to derivatization. In brief, for each  $\alpha$ -2,6-linked

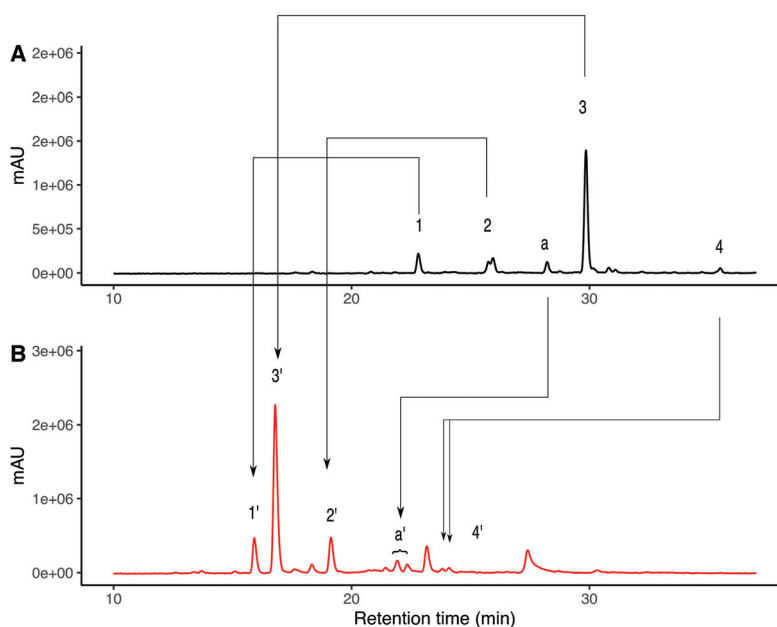


Fig. 2 UHPLC analysis for procainamide labelled *N*-glycans released from crude SGP using PNGase-F. (A) UHPLC-FLD analysis for procainamide labelled glycans species identified 1–4 are described in Table 1. (B) UHPLC-FLD analysis for ethyl esterified and amidated procainamide labelled glycans species identified 1'–4' are described in Table 2.



**Table 1** Predicted and observed  $m/z$  values of different procainamide labelled glycans from Fig. 2A

Peak	Name	SNFG representation	Predicted $m/z$	Observed $m/z$	ppm <sup>a</sup>
1	A2[3]G1S1		995.41 [M + 2H] <sup>2+</sup>	995.57 [M + 2H] <sup>2+</sup>	-161
2	A2G2S1		1076.44 [M + 2H] <sup>2+</sup>	1076.54 [M + 2H] <sup>2+</sup>	-93
3	A2G2S2		1221.98 [M + 2H] <sup>2+</sup>	1221.46 [M + 2H] <sup>2+</sup>	426
a	A2G2S2		1221.98 [M + 2H] <sup>2+</sup>	1222.34 [M + 2H] <sup>2+</sup>	524
4	A3G3S2(6)S(3)		1033.7 [M + 3H] <sup>3+</sup>	1033.7 [M + 3H] <sup>3+</sup>	-34

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<sup>a</sup> Amazon Bruker instrument accuracy quoted as  $\pm 0.5$  Da.

sialic acid, a glycan will exhibit a mass shift of +28.03 Da whereas one  $\alpha$ -2,3-linked sialic acid will produce a mass shift of  $-0.95$  Da (Scheme S1†).<sup>32</sup>

The selectivity of the mass changes due to EEA was verified using glycan standards of known sialylation (Fig S2†). ESI-MS/MS fragmentation of  $\alpha$ -2,6 linked sialic acid after EEA produced a fragment of 686.35  $m/z$  whereas  $\alpha$ -2,3 linked sialic acid produced a fragment of 656.33 which is consistent with ethyl esterification and amidation respectively<sup>33</sup> (Fig S5†). Fig. 2B shows the UHPLC separation of procainamide labelled glycans after EEA. Here, the glycans corresponding to peaks 1, 2 and 3 are converted to the EEA products giving peaks 1', 2' and 3', (Fig S6†) with mass change consistent with  $\alpha$ -2,6 linked sialic acid. However, for the triantennary glycan (peak 4) the EEA results suggest that at least one of the three sialic acids present is  $\alpha$ -2,3 linked (Table 2). Interestingly, on the underivatized UHPLC trace, there was an earlier eluting peak of the same  $m/z$  as A2G2S2 (Fig. 2A peak a). EEA allowed us to identify this species as an A2G2S2 isomer with one  $\alpha$ -2,3 linked sialic acid (Fig. 2B peak a'). The derivatized species was

detected as two peaks, suggesting  $\alpha$ -2,3 linked sialic acid can be present at either arm.

### 2.3 Large scale release and anion exchange chromatography

PNGaseF release of  $\sim 30$  mg of crude SGP obtained from fresh yolks was carried out over 2 days at 37 °C and monitored using HPAE-PAD and MALDI-MS analysis. Procainamide labelling of the released glycan mixture indicates it was 54% A2G2S2 (Fig. 3A). The released glycan mixture was separated by anion exchange chromatography producing fractions S1, S2 and S3 (Fig. 3B) which were also labelled with procainamide for HPLC-MS analysis (panels S1–S3 in Fig. 3A). ESI-MS and MS/MS of these fractions (Fig S7†) allowed for glycan composition identification, which was consistent with the preliminary *N*-glycan analysis. Even though in the original trace peak 2, A2G2S1, had a similar abundance to peak 1 A2[3]G1S1, the most abundant peak in the S1 fraction corresponded to A2[3]G1S1.

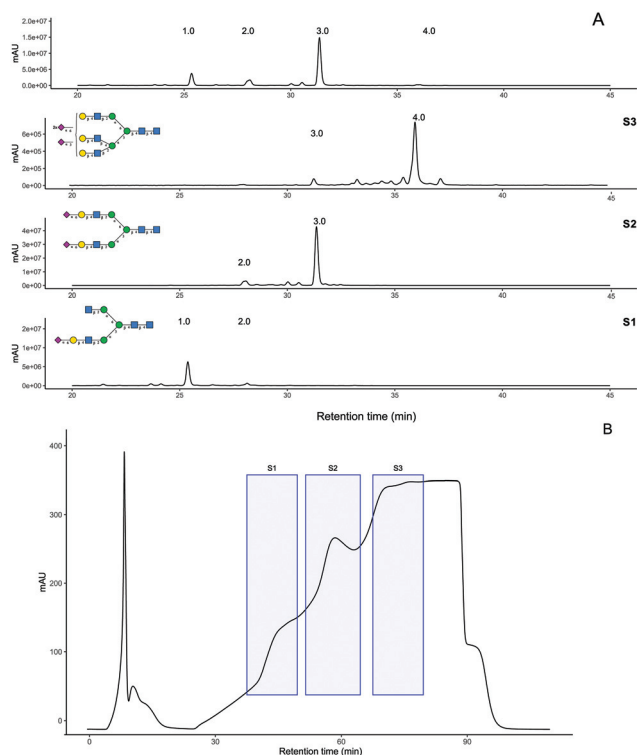
HPLC-MS analysis of the glycopeptide mixture indicates that the recovered *N*-glycans most likely originate from SPG glycoforms, with a minor contribution of naturally present free

**Table 2**  $m/z$  changes observed due to EEA for each of the peaks identified in Fig. 2B

Composition	Initial peak	Peak after EEA	Expected monoisotopic $m/z$ after EEA [M + 2H] <sup>2+</sup>	Observed monoisotopic $m/z$ after EEA [M + 2H] <sup>2+</sup>	ppm <sup>a</sup>	Expected monoisotopic $m/z$ after EEA [M + 3H] <sup>3+</sup>	Observed monoisotopic $m/z$ after EEA [M + 3H] <sup>3+</sup>	ppm
A2G1S1(6)	1	1'	1009.43	1009.53	-99	673.29	673.36	-104
A2G2S1(6)	2	2'	1090.45	1090.57	-110	727.30	727.38	-110
A2G2S1(6)S1(3)	a	a'	1235.51	1236.08	-461	824.01	824.09	-97
A2G2S2(6)	3	3'	1250.01	1250.57	-448	833.68	833.75	-84
A3G3S2(6)S(3)	4	4'				1052.09	1052.42	-314

<sup>a</sup> Amazon Bruker instrument accuracy quoted as  $\pm 0.5$  Da.





**Fig. 3** UHPLC-HILIC analysis of procainamide labelled *N*-glycans at different stages of purification (A) crude SGP before anion exchange separation. S1, S2, S3 correspond to the three regions collected during anion exchange chromatography. (B) UV-214 nm trace for anion exchange separation of released *N*-glycans from crude SGP, shaded region corresponds to the different pools of mono-sialylated S1, di-sialylated S2, and tri-sialylated S3, *N*-glycans.

*N*-glycans (Fig S8†). Triantennary *N*-glycans have been found on egg yolk proteins such as phosvitin.<sup>34–36</sup> However, recent glycopeptide analysis of the egg yolk did not identify any glycopeptides with triantennary glycans.<sup>37,38</sup> In contrast, our UHPLC-MS analysis indicates that triantennary glycans are indeed present and they are most likely to be SGP analogues *i. e.*, KVANKT glycopeptides (Fig. S9†). The detection of these glycopeptides and related *N*-glycans might be due to the larger scale of the extraction in comparison to the previously reported analytical approaches.

S1 and S2 fractions were further purified by porous graphitized chromatography (PGC), (Fig S10A†) whereas S3 was desalted by gel filtration. The resulting purified *N*-glycans were quantified using 2-amino benzamide (2AB) labelling (Fig S10B†) followed by comparison to a quantitative labelled chitotriose standard (Ludger BioQuant chitotriose). From this quantitative method, the purification process produced 8.6 mg of A2G2S2 at 94% purity and 1.3 mg A2[3]G1S1 at 93% purity (Fig S10C†).

## 2.4 Characterization of the purified *N*-glycans

### 2.4.1 Characterization of A2[3]G1S1

**2.4.1.1  $^1\text{H-NMR}$ .** After PGC purification of the S1 fraction,  $^1\text{H-NMR}$  of the isolated glycan A2[3]G1S1 was recorded and

compared to the A2G2S2 glycan that was produced in parallel (Fig S11†). The chemical shifts corresponding to the  $\text{H}_{3\text{ax}}$  and  $\text{H}_{3\text{eq}}$  of Neu5Ac indicate that both compounds have the same type of sialic acid linkage. Comparing the chemical shifts against literature data<sup>39</sup> suggests that it is an  $\alpha$ -2,6 linked sialic acid, which is also in agreement with EEA experiments. Inspection of the region corresponding to the anomeric protons provides information on the different galactosylation between structures (Fig S12A†). The A2[3]G1S1 glycan has only one doublet at 4.38 ppm corresponding to a single galactose whereas the A2G2S2 glycan (Fig S12B†) has 2 doublets at 4.36 ppm, one for each galactose present. Furthermore, the chemical shift for H1-GlcNAcs is also indicative of galactose substitution<sup>40</sup> as the anomeric signals for the antennary GlcNAcs for A2[3]G1S1 at 4.50 ppm are resolved from the anomeric proton of GlcNAc-2 at 4.55 ppm. On the other hand, for the fully galactosylated A2G2S2 glycan, the H1-GlcNAc of the antennary and the H1-GlcNAc-2 signals appear overlapped in a single multiplet at 4.51–4.52 ppm (Fig S12B†).

**2.4.1.2 Elucidation of antennary galactose position in A2G1 isomer.** To assist in the identification of which isomers of A2G1 were present in the isolated mixture, sialic acids were removed with HCl 50 mM at 65 °C before positive ion MALDI-MS/MS and UHPLC analysis. MALDI-MS was used to confirm that the sialic acid had been removed. Indeed, a peak at 1501.17  $m/z$  (242 ppm) was observed, which corresponds to the  $[\text{M} + \text{Na}]^+$  for an A2G1 glycan (Fig. 4A). The position of the antennary galactose can be obtained from MS/MS fragmentation; if the [6]-antenna is not galactosylated a  $^{2,5}\text{A}_4$  fragmentation event will produce a 462.16  $m/z$  ion. This is the case for the A2G1 glycan obtained from egg (Fig. 4B) for which a fragment of 462.08  $m/z$  (171 ppm) is observed suggesting the galactose is on the [3]-antenna and the isomer is an A2[3]G1 glycan. As further confirmation, if a galactosylated [6]-antenna is present the same  $^{2,5}\text{A}_4$  fragmentation will produce a 624.21  $m/z$  fragment (Fig S13†),<sup>41</sup> this fragment was not detected in the MS/MS experiment for this isomer. As a positive control, the A2G2 glycan (Fig. 4C) was used as a reference compound containing a galactosylated [6]-antenna, as its MS/MS produces the diagnostic 624.25  $m/z$  fragment (58 ppm) (Fig. 4D). Furthermore, the fragmentation pattern of the A2[3]G1 was preserved even after the enzymatic fucosylation using *Helicobacter pylori* fucosyltransferase (Hp  $\alpha$ -1,3FT) which will only transfer a fucose unit to the 3-OH of the galactosylated antennary GlcNAc. MS/MS analysis of the produced A2[3]G1F1 still produces a 462.10  $m/z$  ion (125 ppm), thus confirming the vacancy of the GlcNAc at the [6]-antenna. (Fig S14†). Further evidence of the placement of the galactose in the [3]-antenna position was obtained by comparing the retention times for 2AB labelled samples of A2[3]G1 against a mixture of containing both A2[6]G1 and A2[3]G1 as seen in Fig. 5. For the mixture, the later eluting peak has been assigned as A2[3]G1 according to retention time databases (<https://www.glycostore.com>) (Fig. 5A). The glycan extracted from the egg produced only a single peak and its GU value corresponded with that of the later eluting peak (Fig. 5B). Thus, providing orthogonal



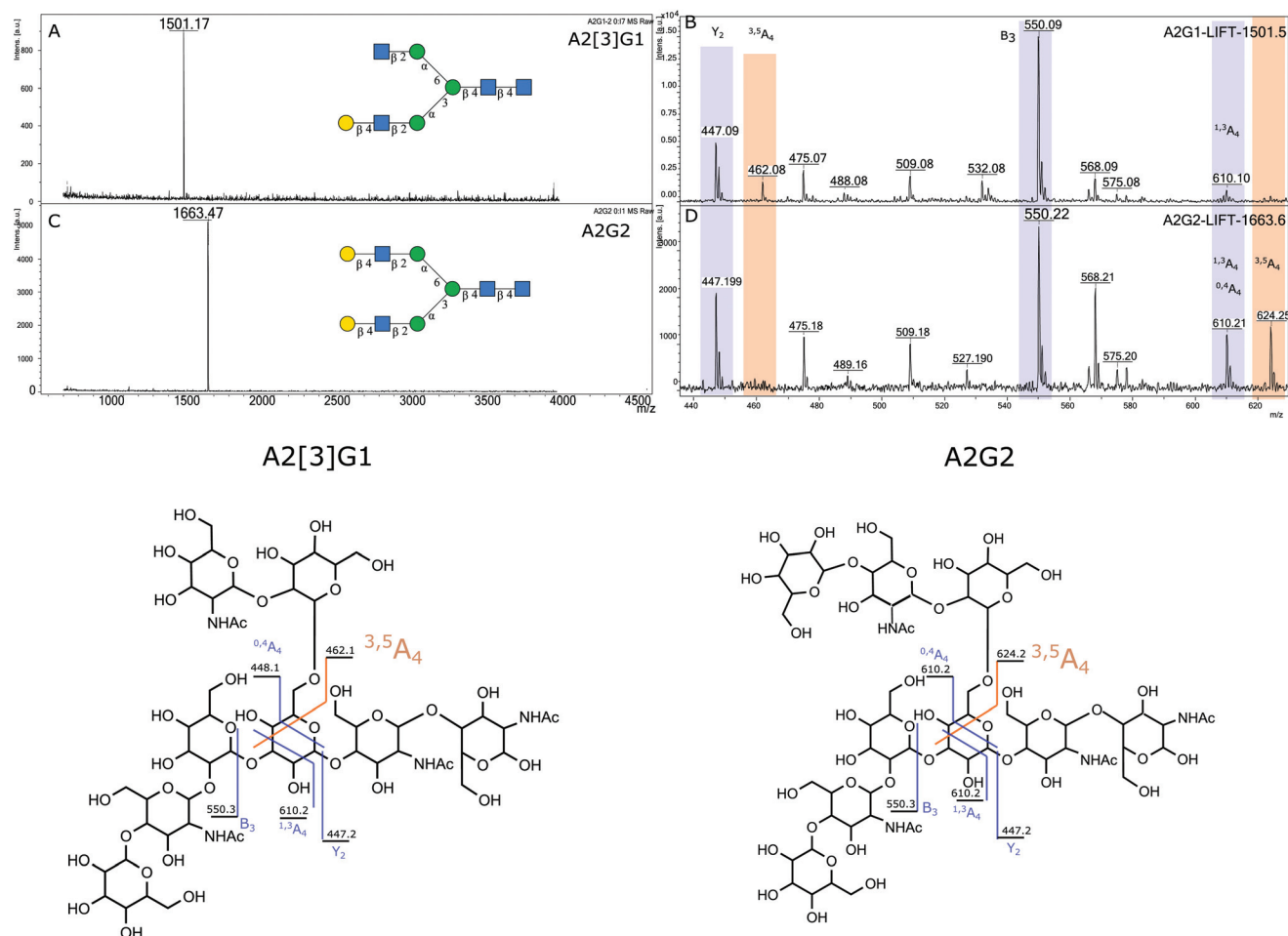


Fig. 4 Positive ion MALDI MS and MS/MS analysis for A2[3]G1 and A2G2 (A) MALDI MS for A2G1 (B) MS/MS for A2G1 (C) MALDI MS for A2G2, (D) MS/MS of A2G2.

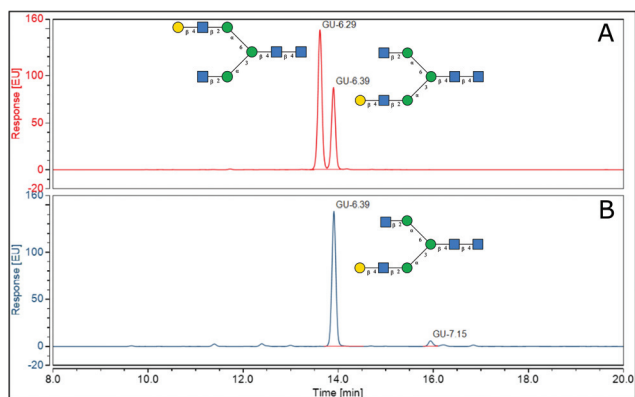


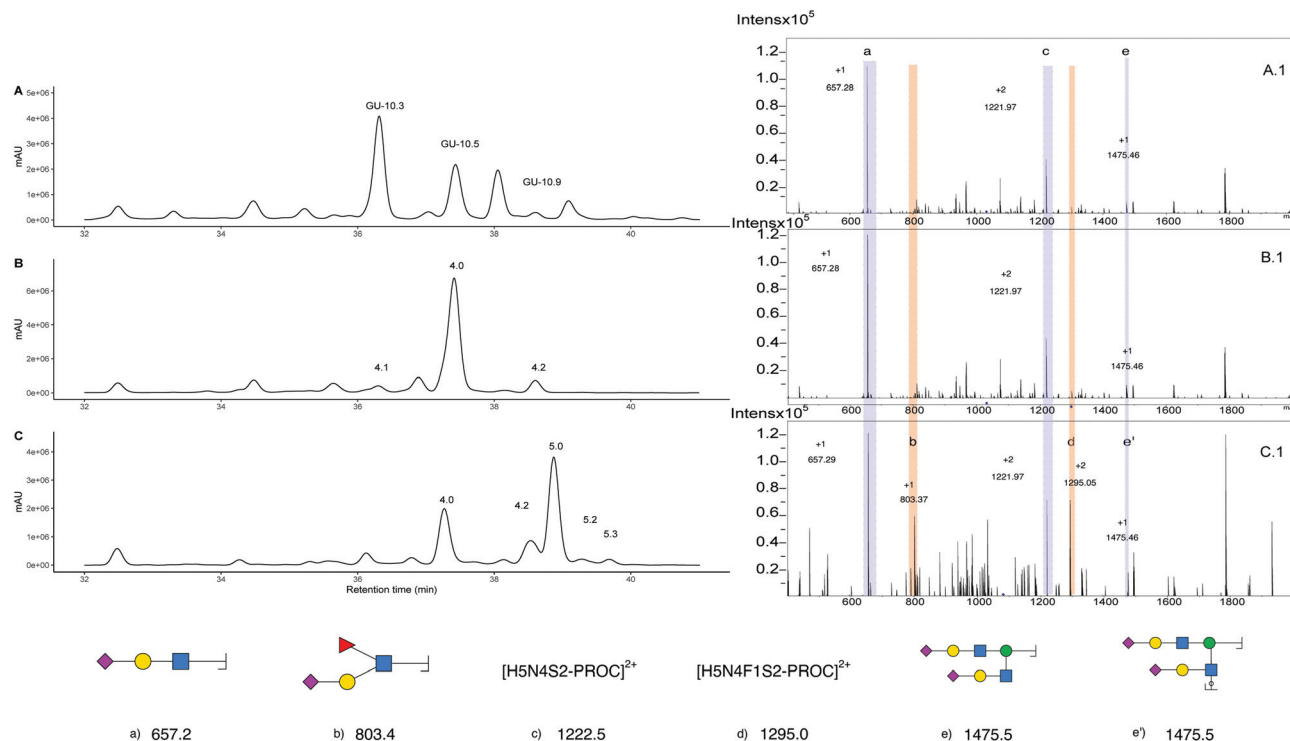
Fig. 5 UHPLC analysis of 2AB labelled *N*-glycans (A) A2[6]G1 and A2[3]G1 glycan mixture (B) A2[3]G1 glycan from egg glycopeptides.

confirmation to the MS/MS experiments. This data supports the original report<sup>19</sup> that the A2G1S1 glycan isolated from egg corresponds to A2[3]G1S1 and contrasts with recent publications which identified it as a mixture.<sup>25</sup> 2AB retention times

can also provide confirmation of the sialic acid linkage of the original glycan by comparing it against the chemoenzymatically synthesized  $\alpha$ -2,3 linked isomers. (Fig S1†) The retention time of the precursor glycan is consistent with that of  $\alpha$ -2,6 linked sialic acid,<sup>42</sup> which correlates with both the NMR and EEA findings.

**2.4.2 Characterization of A3G3S3.** Based on 2AB quantitation, the S3 fraction corresponded to a mixture of triantennary complex *N*-glycans (A3G3S3), of which the main peak accounted for 60% of the total glycans recovered in this fraction. To assist in the identification and serve as a control, procainamide labelled PNGase-F released *N*-glycans from bovine fetuin were used (Fig. 6A). The main triantennary glycan from the egg (Peak 4.0) had the same GU value as a peak from fetuin that corresponds to the tri-sialylated isomer consisting of one  $\alpha$ -2,3 linked sialic acid (GU 10.5) (Fig. 6B)<sup>43</sup> which is in agreement with the EEA experiments. The extracted ion chromatogram at 1033.70 *m/z* (Fig S16†) allowed for the identification of two additional isomers (peaks 4.1 and 4.2) that differ by the type of sialic acid linkage. When compared with fetuin they were assigned as the isomers with two (GU 10.3) and zero





**Fig. 6** UHPLC analysis of procainamide labelled triantennary *N*-glycans. (A) Procainamide labelled *N*-glycans from bovine fetuin (A.1) MS/MS *m/z* 1033.70 procainamide labelled glycan corresponding to fetuin. (B) UHPLC of procainamide labelled *N*-glycans corresponding to the S3 fraction. (B.1) ESI-MS/MS of peak 4, *m/z* 1033.70 for procainamide labelled *N*-glycans from S3 fractions. (C) UHPLC for procainamide labelled *N*-glycans from S3, after antennary fucosylation. (C.1) ESI-MS/MS *m/z* 1082.40, obtained from peak 5.0 after antennary fucosylation.

(GU 10.9)  $\alpha$ -2,3 linked sialic acids respectively. Based on the ESI-MS/MS data (Fig. 6B.1) it is unlikely that the third arm corresponds to a  $\beta$ -1,4 bisecting GlcNAc as the presence of a 1475.5 *m/z* fragment indicates that two GlcNAcs are connected to a single mannose. Triantennary *N*-glycans from bovine fetuin contain the  $\beta$ -1,4 extension of [3]-antenna. Thus, it is possible that peak 4.0 also corresponds to the same type of extension as this will be consistent with previous reports of triantennary *N*-glycans from the egg protein phosvitin.<sup>34</sup>

Further evidence of the presence of  $\alpha$ -2,3 linked sialic acid is provided by chemoenzymatic reaction with *Helicobacter pylori* Fucosyltransferase (Hp  $\alpha$ 1,3FT) and GDP-Fucose. The reaction was performed on unlabelled glycans and monitored by MALDI-MS. After one day, an aliquot of the reaction was procainamide labelled and the UHPLC-MS acquired (Fig. 6C). The newly formed peak (5.0) produces a  $[M + 3H]^{3+}$  ion of 1082.80 *m/z* that corresponds to the addition of only one fucose relative to the triantennary precursor (peak 4.0). Furthermore, MS/MS analysis (Fig. 6C.1) shows the formation of an 803.3 *m/z* fragment previously absent which is consistent with the addition of fucose to a sialylated antenna (original sialylated antenna mass 657.3 *m/z*), thus confirming the formation of the S-Lex tetrasaccharide. This enzymatic reaction is helpful for structural confirmation because the enzyme has strict requirements for the addition of fucose. For a GlcNAc to be an acceptor, it must be galactosylated at position 3 or 4. Additionally, the galactose needs to have the C6-OH vacant.<sup>44</sup>

This way, structures containing the  $\alpha$ -2,3 linked sialic acids are acceptors whereas  $\alpha$ -2,6 are not. In this case, this is observed as peak 4.2, which corresponds to a glycan containing three antennary  $\alpha$ -2,6 sialic acids, was still detected after the enzymatic reaction. Since the main glycan structure present in the egg only has one  $\alpha$ -2,3 linked sialic acid, this could allow for the control of the number of fucose units added when compared to *N*-glycans obtained from bovine fetuin which have more than one  $\alpha$ -2,3 linked sialic acid. However, even though only one fucose can be added, there is no control over which antenna is fucosylated and three products (5.1, 5.0 and 5.2) produce ions corresponding to the addition of one fucose. (Fig S17†) that cannot be distinguished from their MS/MS data (Fig S17.B†). Furthermore, the EIC at 1131.10 *m/z* helps to identify peak 5.3 as the product corresponding to the addition of two fucoses, which likely corresponds to the fucosylation of the glycan at peak 4.1. (Fig S18B and C†). Additionally, other fucosylated structures were detected after the EIC scan for the peak at 803.3 *m/z* (Fig S19A and B†) suggesting there were additional glycan structures with  $\alpha$ -2,3 linked sialic acids capable of acting as fucose acceptor (Fig S19C and D†). Purification of the triantennary acceptors is the next step in obtaining an increased yield and a purer product. Even though bovine fetuin is the most common and abundant source of triantennary glycans<sup>45</sup> there is an interest in finding alternative sources such as plasma of other vertebrates.<sup>46</sup> What makes the triantennary glycan from the egg special is the preference toward



one isomer and the possibility to be isolated alongside the more abundant SGP.

## 2.5 Comparison with other methods

Although there have been different total synthesis strategies to produce asymmetric glycans,<sup>47,48</sup> it is still a challenging process. Different semi-synthetic strategies to produce asymmetric glycans are compared in Fig. 7. Starting from SGP Kajihara *et al.*,<sup>20,49</sup> produced a library of oligosaccharides by performing partial acid hydrolysis on crude pronase digested Asn-Fmoc glycans, from which A2[3]G1S1 and A2[3]G1 can be separated. A2[6]G1S1, the non-naturally occurring isomer, was also accessible by separating out the monosialylated A2G2[6]S1, which is a hydrolytic product of the main species A2G2S2. Even though this process realises the production of a wide number of targets simultaneously, the separation of the different species generated by acid hydrolysis is not trivial. Starting with a higher purity SGP Aoyama *et al.*<sup>26</sup> produced a mixture of asymmetric monosialylated versions of SGP by partial acid hydrolysis. The monosialylated glycopeptides were separated from the unreacted precursor by reverse phase chromatography and were treated with exoglycosidases to produce A2[3]G1 and A2[6]G1 bearing glycopeptides. Although

it is an improvement on the previous method, it relies on access to large amounts of pure SGP. Enzymatic methods to introduce asymmetry have also been explored, for example, taking advantage of the branch preference of *E. coli* Beta-galactosidase and<sup>50</sup> starting from fully galactosylated A2G2, Van den Eijnden *et al.* were able to produce asymmetric *N*-glycans having galactose only on the [6]-antenna A2[6]G1. Different routes have also been explored to produce A2G2. Starting from bovine fetuin Calderon *et al.*<sup>51</sup> obtained a mixture of triantennary and bi-antennary glycans that were separated by HILIC. The A2G2 product was subjected to partial exoglycosidase digestion producing A2[6]G1 as the main product after HILIC purification. This strategy has also been used to produce asymmetric core fucosylated glycans. Starting from SGP, Asn-Glycans were produced by pronase digestion<sup>8</sup> followed by Cbz-amino group protection. Sequential exoglycosidase digestion was then used to produce a completely de-galactosylated Cbz-glycan. The removal of galactose was necessary for the introduction of  $\alpha$ -1,6 core fucose.<sup>52</sup> Galactose was then re-installed enzymatically, to produce the symmetric fully galactosylated glycan, that when digested with the *E. coli* beta-galactosidase afforded the FA2[6]G1 as the main product after HILIC purification.

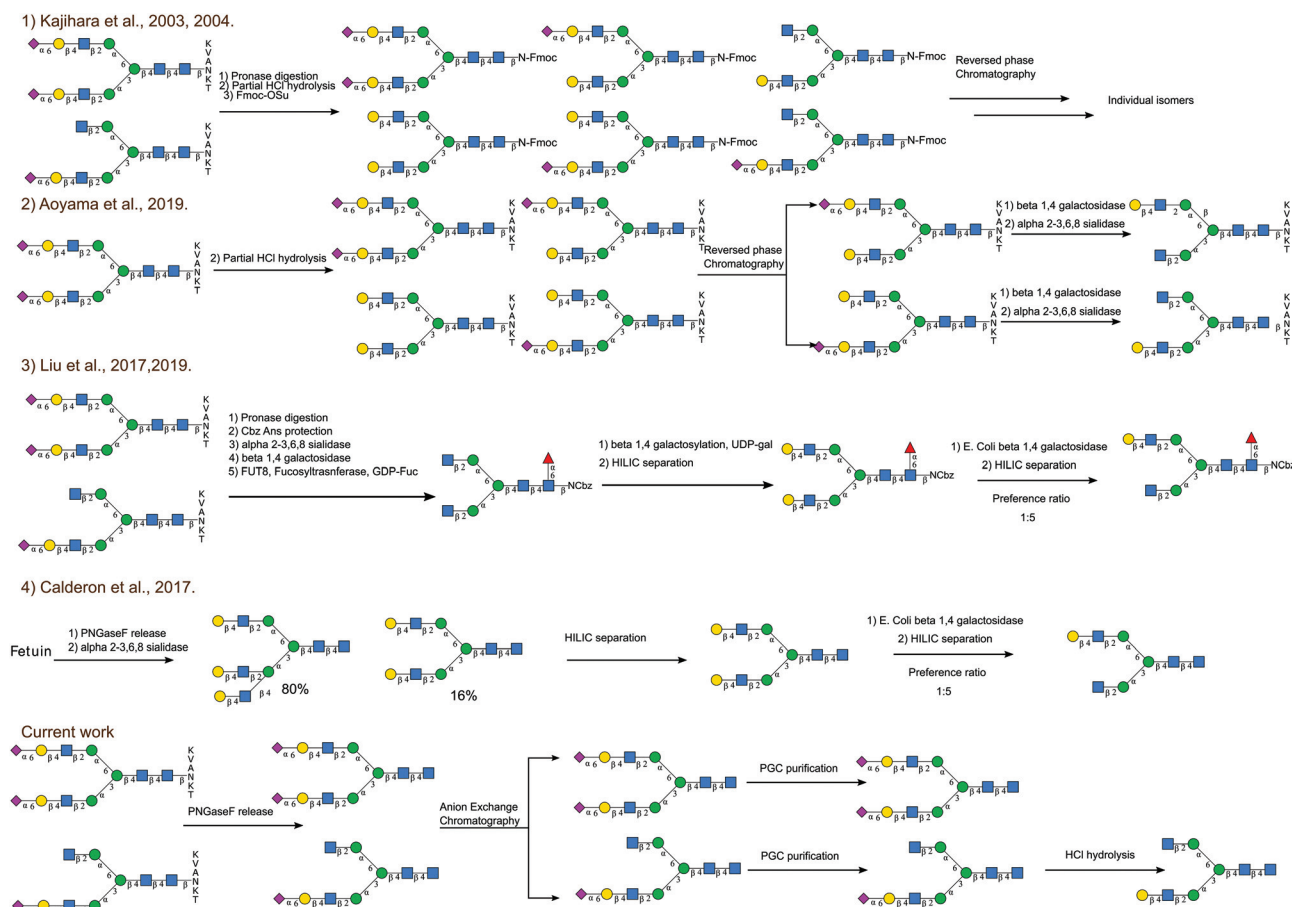


Fig. 7 Comparison of different semisynthetic approaches to produce asymmetric *N*-glycans.



The approach we present here does not require the introduction of branch asymmetry by chemical or enzymatic means, it relies on the separation of the different glycans species occurring natively according to the number of sialic acids present, and then additional HPLC purification. It allowed us, not only to recover asymmetric monosialylated glycans, namely A2[3]G1S1, but also to obtain triantennary glycans that have not been associated previously with the SGP purification process.

### 3. Conclusions

The isolation of SGP has been extensively reported in the past, and it has contributed significantly to the process of production of homogenous glycoproteins. However, we want to emphasize the additional opportunity to obtain naturally occurring asymmetric and triantennary *N*-glycans that can be concomitantly isolated alongside the most abundant glycan species. Isolating these *N*-glycans can complement existing chemoenzymatic approaches or serve as standards to verify the products of total synthetic strategies.

### Author contributions

JMMD: Conceptualisation, investigation, writing—original draft, writing—review and editing, visualization. AM: Investigation (sialic acid derivatization experiments), writing—review and editing. SRP: Resources, writing—review and editing, supervision. JLH: Writing—review and editing, visualization, supervision, funding acquisition. DIRS: Conceptualisation, writing—review and editing, supervision, project administration, funding acquisition.

### Conflicts of interest

The authors are based at Ludger Ltd which specialises in the production of commercial glycan standards for use in the field of glycomics and the analysis of biopharmaceuticals.

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