

# Modification and Characterization of Lactoferrin Iron Free with Methylimidazolium N-ethylamine Ionic Liquid as Potential Drugs Anti SARS-CoV-2 †

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† Presented at the 2nd International Electronic Conference on Processes: Process Engineering—Current State and Future Trends (ECP 2023), 17–31 May 2023; Available online: <https://ecp2023.sciforum.net/>.

**Abstract:** Methylimidazolium N-ethylamine amine (MIE-NH<sub>2</sub>) is synthesized successfully with excellent yield in the high performances and green chemical process, using N-methylimidazole and tert-butyl N-(2-bromoethyl) carbamate as starting materials. Following the mechanism of reductive amination, using this ionic liquid as suitable ligand for modification N-glycans contains carbonyl group of the oligosaccharides, and the activity of an ionic liquid is disclosed by mass spectrometric techniques. This work illustrates that methylimidazolium N-ethylamine as an ionic liquid linked to carbohydrate including N-glycans in Lactoferrin and its derivatives, for example lactoferrin (BL iron free) has been selected as examples of glycoproteins. The detection of profiling linked of oligosaccharides and glycoproteins is performed using UPLC/ESI-QTOF and by MALDI-TOF mass spectrometry. Moreover, the ionic synthesis with active amino-group and employed as a multifunctional of modification of the oligosaccharide, and using the products as applicable small molecules therapeutics linked to GlcNAc and its derivatives. Modifying glycoproteins by adding IL-MIE-NH<sub>2</sub>, it has improving ESI ionization efficiency and providing labelling results of N-glycans, even better than 2-AB derivatives. Relevantly, this ionic liquid is applicable as advancement and development in catalytic methods, N-glycosylation and modification of small molecules as potential drugs for anti-viral and microbes' infections.

**Keywords:** ionic liquid 1; infections 2; lactoferrin 3; N-glycans 4; mass spectrophotometry 5

**Citation:** Senan, A.M.; Akkoc, S.; Reem, A. Modification and Characterization of Lactoferrin Iron Free with Methylimidazolium N-ethylamine Ionic Liquid as Potential Drugs Anti SARS-CoV-2. *Eng. Proc.* **2023**, *5*, x. <https://doi.org/10.3390/xxxxx> Published: 17 May 2023



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## 1. Introduction

Lactoferrin (Lf-iron free) is a transferrin protein, and the most abundant glycoprotein in human and ruminant milk resources. Lactoferrin are containing 1–4 glycans with single-chain polypeptides of about 80,000 Da. Lactoferrin is a multifunctional glycoprotein involving N-glycans have active with functional groups and depending on the species, and it makes an important contribution to the host that defines the system [1–3]. In addition, lactoferrin carries many important biological functions, including N-glycans bonding to iron or other metals, being bioactive in cell proliferation and differentiation, as an anti-parasitic protein, an anti-bacterial, and anti-viral. These functions differ of lactoferrin considerable attention as primary nutritional contribution to iron-binding by role of glycosylation [4,5]. Ionic liquids containing N-active group, that's make the critical role of carbonyl groups of glycan binding in many biological processes very easily. The chain of

saccharides-glycan moieties in lactoferrin is likely to contribute significantly to the N-ionic liquids roles by carbonyl of saccharides. Despite the high amino group of ionic liquid sequence homology in different with excellent results, which exhibits as a unique N-glycosylation for heterogeneity of the biological properties and Lactoferrin is chosen as a good example sources of N-glycans [6,7].

Exploring and identifying the new characterization of novel ionic liquid provides reacting with oligosaccharides of glycoproteins. Several interesting studies are encouraging for discovers the new application of ionic liquids as potential drugs antimicrobial include antiviral. Study the activity of glycoproteins is the assessment of the contributions of individual glycans to the observed bioactivities. This work examines how the study of N-link glycosylation in Lactoferrin which reacted with ionic liquid MIE-NH<sub>2</sub> increasing the understanding of ionic liquid functionality [2,8,9].

## 2. Experimental Section

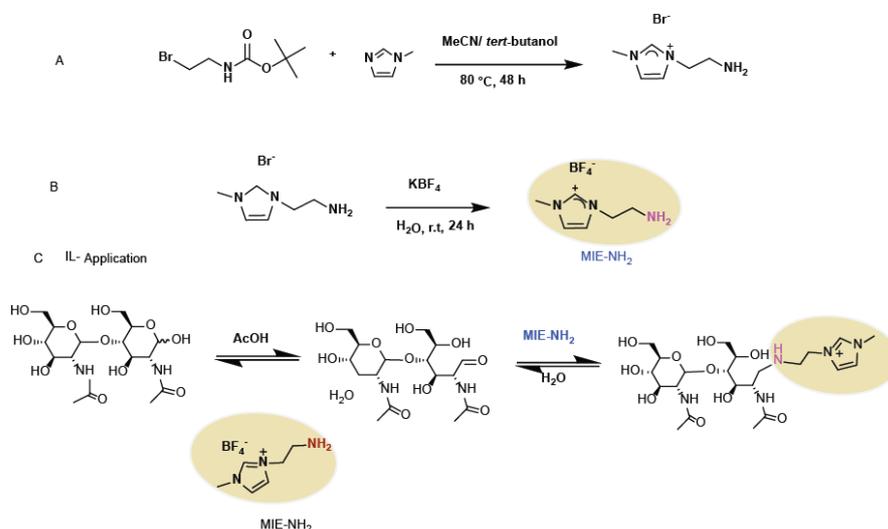
### 2.1. Materials

Tert-butyl N-(2-bromoethyl) carbamate and N-methylimidazole were purchased from J&K (Shanghai, China). Acetonitrile and solvent used for HPLC was purchased from Merck (Ankara, Turkey). Lactoferrin free iron and all other chemicals used in this study were bought at the highest grade from commercial suppliers without further purification or modification.

### 2.2. Synthesis of Ionic Liquid-[1-(2-aminoethyl)-3-methyl-1H-imidazole-3-ium] MIE-NH<sub>2</sub>[BF<sub>4</sub><sup>-</sup>]

Tert-butyl N-(2-bromoethyl) carbamate (225 mg, 1.1 mmol) was reacted with N-methylimidazole (82 mg, 1 mmol) in anhydrous mixture solvent of CH<sub>3</sub>CN and t-BuOH [5 mL (3/2, v/v)], reaction mixture refluxing at 80 °C for 2 days. Removing unreacted materials by washing with ethyl acetate three times and the product was dried under reduced pressure and obtained the light yellow viscous liquid (Scheme 1 step 1) [10–13].

The viscous liquid (1) IL-Br-1 (289 mg g, 85.7 mmol) was generated by stirring with KBF<sub>4</sub> (1.1 equiv.) in water solution for 24 h at room temperature. Then, the reaction mixture was filtered and vacuum distilled and washed the product by dichloromethane and ethyl acetate, respectively. The product was vacuum dried by rotary evaporator at 55 °C to remove the traces of dichloromethane and ethyl acetate. After drying for 6 h under vacuum at 80 °C, the expected ionic liquid [MIE-NH<sub>2</sub>][BF<sub>4</sub><sup>-</sup>] was obtained (Scheme 1 step 2) [10,14,15].



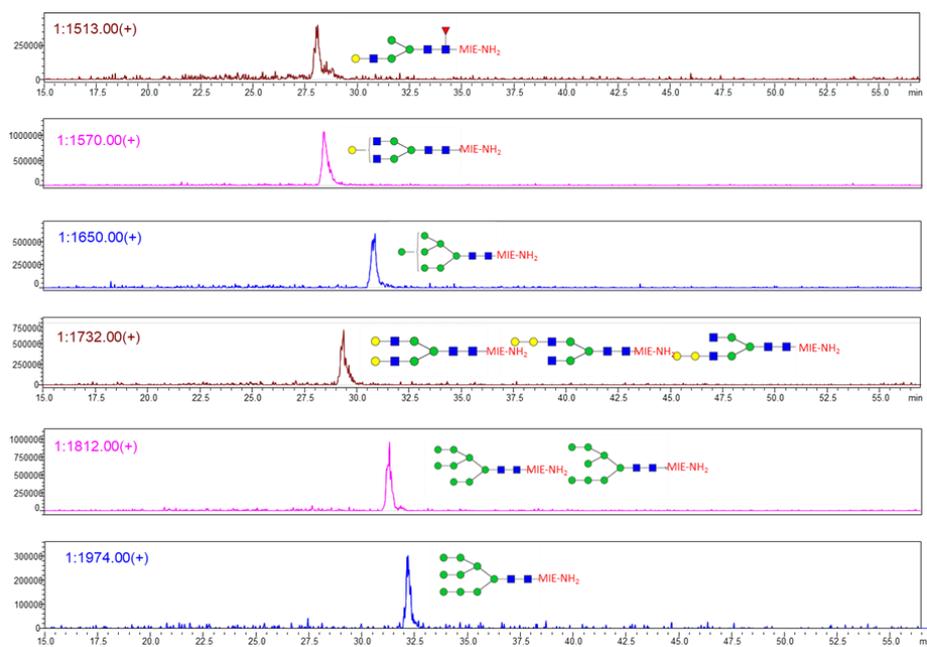
**Scheme 1.** Synthetic route of methylimidazolium N-ethylamine and derivation of aminoglycosides with GlcNAc.

### 2.3. Derivatization N-glycans of Lactoferrin with ILS-NH<sub>2</sub>

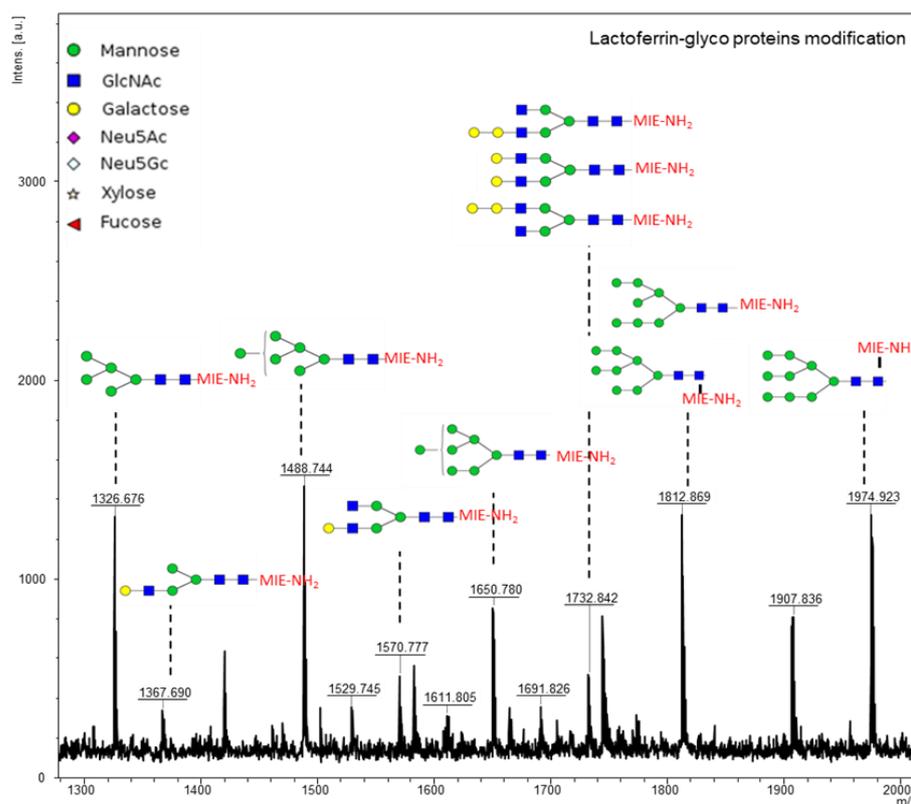
Derivatization solution contains 70 mM MIE-NH<sub>2</sub> and 0.1 M sodium cyanoborohydride in dimethyl sulfoxide/acetic acid solution (7:3, v/v) was added to sample of Lactoferrin until completely dissolved. The derivatization mixture was mixing by ultrasonic about 30 mints and incubated at 70–90 °C for 4 h.

### 3. Results and Discussion

The glycoproteins in bovine lactoferrin were chosen as substrates to prepare N-glycans with high structure including saccharides. The high proportion of glycosylation of verifies the methodology of MIE-NH<sub>2</sub>, it was using for labelling of N-glycans [10]. According to our previous studies, lactoferrin iron free (LF-iron free) is a well-known as a good resource of glycoprotein and founded proximity 42 types of N-glycans with five potential sits N-glycosylation, the different N-glycans with all structures sites [16]. The lactoferrin modified by using ionic liquid, there are 14 of different MIE-NH<sub>2</sub> derivative lactoferrin-N-glycans were deduced according to UPLC profile and MS spectrum see in Figure 1. The corresponding structures of Lactoferrin-MIE-NH<sub>2</sub> were assigned as shown in Figures 1 and 2. The results of the detection in the Figure 1 suggested the possible structures of compounds were modified by IL-MIE-NH<sub>2</sub> and this result confirmed by MALDI ToF analysis. The *m/z* values of structures either with mono-charge or di-charge was calculated related to the signals of MIE-NH<sub>2</sub> linked to N-glycans was observed. In extracted ion chromatogram of the products of N-glycans linked to MIE-NH<sub>2</sub> from Lactoferrin by HPLC and two peaks exhibited the same *m/z* value of new products 1716.50 which assigned and identified with theoretical *m/z* = 1716.70 [m<sup>+</sup>). In this case, we suggested the new product is MIE-NH<sub>2</sub> linked monofucosylated monogalactosylated bi antennary complex N-glycan isoforms [10,17]. For example, from LC-MS analysis, it was founded the peak of 13.7 min was assigned as MIE-NH<sub>2</sub>-FA2G1 and the peak at 14.6 min was derivate as MIE-NH<sub>2</sub>-A2G1F. This work demonstrated the catalytic mechanism of the derivatization of Lactoferrin-N-glycans with ionic liquid MIE-NH<sub>2</sub> follows the reductive amination [10,16]. The free aldehyde realized in the acidic medium and by reducing ligand as sodium cyanoborohydride, which possess significant converted the carbonyl to an imine by the NH<sub>2</sub> group of MIE-NH<sub>2</sub> (Scheme 1B) [10].



**Figure 1.** LCMS result of derivatization of different N-Glycans from lactoferrin and linked with MIE-NH<sub>2</sub>.



**Figure 2.** MALDI-TOF-MS results confirm the modulation of N-glycans from lactoferrin linked with MIE-NH<sub>2</sub>.

Recent scientific research focused on the progress in protein-based nano medicine, albumin-paclitaxel as nanoparticles have introduced in novel therapeutics and used for treatment of cancer and viral infections. However, specific drug targeting of SARS-CoV-2 is almost challenging and absence until now, premature drug release and supports the poor pharmaceuticals stores for resistance COVID-19 and its mutations. Therefore, some studies with alternative protein-based nano medicines have opening the eyes to use ionic liquids for extend and developing a novel of small molecules form glycoproteins. Regarding to this challenging, lactoferrin (Lf-iron free) offers a promising bioactive well as potentials therapeutic and drug nano carrier. In this work, we focused in the major pharmacological actions of modified glycoproteins form lactoferrin with ionic liquids to produced new molecules including antiviral, anti-cancer, and/ or improve the immunology.

For enhancing the efficacy of glycoproteins as potential drugs anti COVID-19 it was functionalization of N-glycans with emphasis of lactoferrin. Besides this technique wide application in small molecules therapeutics, we depended on the recent advances of ionic liquids-Lf-based small molecules as efficient platforms for delivering novel drugs an antiviral drugs, particularly for treating the SARS-CoV-2 infections.

#### 4. Conclusions

This conclusion confirmed the modification and application of ionic liquid methyl imidazole ethyl amine ionic liquid MIE-NH<sub>2</sub> derivatization of lactoferrin (Lf-iron free). The detection N-glycans from lactoferrin-glycoprotein promoted to use the ionic liquid methyl imidazole ethyl amine IL-MIE-NH<sub>2</sub> (IL-MIE-NH<sub>2</sub>) for producing small molecules ionic liquids which could provide novel drugs antiviral, this study exploded the available strategy for modifying glycoproteins by ionic liquids follows the reductive amination mechanism, this study suggested new drugs by modifying carbohydrates ionic liquid with potential bioactive, the separation of MIE-NH<sub>2</sub> shows the highly selectivity of the

carbonyl group of sugars which could be accomplished by the hydrophilic interaction chromatography. This study suggested that new small molecules of lactoferrin containing methyl imidazole ethylamine promote antimicrobial, and antiviral including SARS-CoV 2 treatment.

**Author Contributions:** Methodology, writing—review and analysis by A.M.S. and S.A.; software, and editing, A.R.; supervision, A.M.S.; project administration, A.M.S. All authors have read and agreed to the published version of the manuscript.

**Funding:** TUBITAK 2221 Programme 2022, the scientific and technological research council of Türkiye.

**Acknowledgments:** Authors would like to thank TUBITAK 2221 Programme 2022, the scientific and technological research council of Türkiye.

**Conflicts of Interest:** The authors declare no conflict of interest.

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