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Synthesis of some Glyco-amino acid congeners

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ABSTRACT

Macromolecules have been widely useful for drug delivery purpose. The fusion of drug with macromolecule could offer controlled or time specific drug delivery. Glyco amino acids are a carbohydrate derivative which occurs in nature as a subunit of oligosaccharides in cell walls of bacteria and in some antibiotic. Glyco-peptide presents an exclusive research in glycobiology for drug discovery, drug delivery and other biotechnological application. This work was on the synthesis and characterization of glyco amino acids based on monosaccharide (D-glucosamine) and amino acids as reactant. The synthesized compound was characterised by physicochemical properties and their spectral analysis. These glycosylated amino acid building blocks can be utilized in the synthesis of glycopeptides, generation of glycomimetic libraries, Preparation of glycoconjugates, glycotargetting, and inhibition of carbohydrate-protein binding.

Keywords: glycopeptides, glycomimetics, glycotargetting, glycoconjugate

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INTRODUCTION

Drug delivery talk about to technologies, approaches, systems and formulations for transporting a therapeutic compound in the body. It involves scientific site-targeting within the body. Drug delivery is an idea combined with route of administration and dosage form. These technologies modify drug's ADME properties improving product efficacy, safety, patient convenience and compliance.

The development of new formulations confirms a better pharmacological response, which improve the bioavailability, lower the doses and minimize side effects of drug. In the research of drug delivery systems, we try to advance the pharmacological activity of API on improving ADME of drug.

The high molecular weight carriers are the vital constituents of drug delivery systems in which the drug is covalently bound or fixed. Hence, the carriage of drugs to the site of action is the main function of polymeric carrier. Polymeric carrier protect the drugs to interact with other molecule. This interaction may change the chemical structure of active pharmaceutical ingredient which may cause lose its pharmaceutical action.

The mechanism of these polymers is an essential feature by which they are eliminate from the body. These polymers may be excreted rightly via renal excretion or may be excrete metabolically. Non-biodegradable macromolecules with a molecular weight lower than 50 KDa can be safely cleared from the body. The passage through the renal glomerular membrane depends on chemical structure of the molecule. The molecular weight of the substance should be under 50 KDa for clearance through renal glomerular membrane. In the case of non-biodegradable polymer, the drug can be covalently joined to the macromolecule by a linker. Some conditions are needed to degrade these polymers such as different enzyme or an acidic medium.

Other things to consider in non-biodegradable polymer design is polymer's chemical structure (degree of hydrophobicity, covalent bonding of monomers, etc.), since the degradation condition and speed and therefore, the rate and site of drug release, can be modulated depending on the chemical structure of the polymer used. Current efforts in the field of drug delivery system are the formulations in which the active constituent is released in a controlled manner over a period of time from the formulation and strategy to enhance survival of oral formulation. The designed system must avoid the host's defense mechanisms and reach to its intended site of action to achieve efficient targeted delivery.

The main purpose of polymeric transporters is to protect the drug from interacting with other molecules and carriage

to the site of action. Chemical structure can be changed by the interaction. Moreover, polymeric carriers evade the interaction of the drug with other undesirable molecules such as proteins, which could seize the drug stopping its entrance at the site of action. To obtaining the ideal release conditions the polymeric structure should be

i) biodegradable

ii) disassembled

iii) undissembled

Micro-sized polymeric carriers could be used in first two cases. Nano-sized polymers must be used for renal elimination if the arrangement of the polymer is non-biodegradable.

Smart polymers also can be used in drug delivery system. The structure of these polymer may change once they are induced by a stimulation which causes the release of drug at the appropriate time and place.

In controlled drug release, polymer does not have any specific therapeutic action. These polymer are used as excipient in the formulation. Drug are conjugated in polymer matrices for drug delivery purpose.The objective of drug polymer conjugates to improve the internalization and specificity of cell to achieve ideal release of the drug at the site of action.

When drugs are attached to the polymer with the aid of linkers the conjugation of drug and polymer play a significant role. These conjugations should be alter by certain gastric enzymes or acidic environments. This phenomenon works on nanoparticles, which have great movement in the tiniest vessels, allowing for selective drug accumulation and efficient uptake at the site of action. Depending on the preferred application to overcome physiological barriers, Nano-delivery systems must have a definite particle size, surface charge and hydrophobicity. The physiological mechanisms of these barriers prevent nanoparticles from reaching their targets, thus compromising therapeutic efficiency [1].

In current times, polymeric carriers for drugs of cancer have been widely studied. Due to certain differences in the characteristics of healthy and malignant tissue polymer can accumulate unreceptively in malevolent tissue. In general, micro molecules diffuse through the endothelial cell wall to healthy and malignant tissues. Endothelial cells of malevolent tissues have more and large opening that allows large molecules to cross endothelial cell wall of these tissues. Malevolent tissue also have poor lymphatic drainage and increased vascularity, which both lead to the retention of macromolecules. This unreceptive accumulation of macromolecules in cancer tissue, known as the EPR effect (enhanced permeability and retention effect).

Glyco-amino-acids are the sugar moieties in which sugar attached to an amino acid by covalent bond. Glycol-amino-acid contains at least one amino and one carboxyl group in their structure. Amino acid linked with saccharide through a glycosyl linkage (-O, -N) to form glycosyl amino acid. Oligopeptide linked to carbohydrate to form glycopeptide.

The glyco amino acids are synthesized from commercially obtainable or easily available mono saccharide, i.e., glucose, glucosamine, diacetone glucose, galactose etc. The large quantity of functional groups and the choice of stereo chemical linkages at the anomeric carbon has constantly challenged toward a multitude of approaches to this compounds. Monosaccharides are privileged structures with rigid molecular systems or which can be used as molecular templates to display pharmacophoric groups in distinct spatial orientations. For the generation of carbohydrate building blocks, at least one carboxyl and one amine functional group was incorporated into the sugar ring. Derivatives of amine containing sugars such as N- acetyl glucosamine and sialic acid whose nitrogen are the part of more complex functional groups rather than formally being amines are also considered amino sugars. Amino glycosides are class of antimicrobial compounds that inhibits protein synthesis in bacteria's and these compounds are conjugates of amino sugar [2].

The viability of using carbohydrate ligands to target protein receptors at sites of localization, called glycotargeting, the potential of using carbohydrates in targeted drug delivery system has been made vibrant. The use of glycoconjugates may be divided into two types-

(i) Glycoconjugates is itself act as drug

(ii) Glycoconjugates plays a role in helping the delivery of the drug.

Glycoproteins represent outstanding objects for the drug targeting; the structure can be modified with regard to the protein backbone as well the functional sugar group. Small molecule of drugs can be glycosylated which having the potential to pass into the kidneys, and be rapidly cleared. Therefore, macromolecules that allow extended circulation time and give entrance to added chemical functionality or more specific delivery is an attractive alternative.

MATERIAL AND METHODS

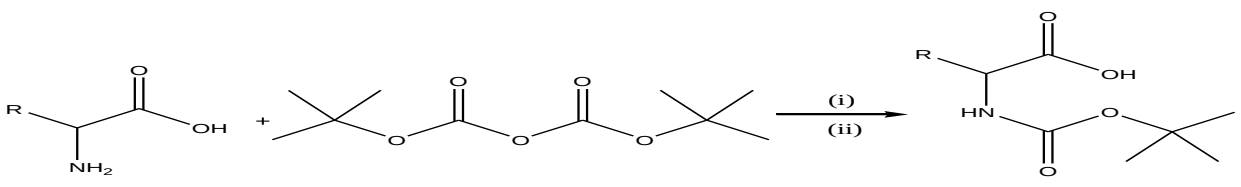
All chemicals used in the synthesis of glyco-amino acids were purchased from different resources and used without any further purification.

Synthesis of each glyco amino acid conjugates were carried out in following steps.

(A) BOC protection of amino acid:

The procedure for the preparation of BOC protected amino acids was adopted as described by Shendage D.M. (2004) [3].

Scheme: 1



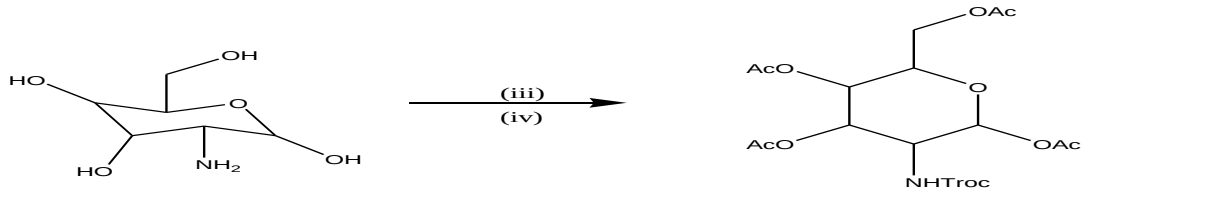
1.Amino acid 2.Boc anhydride 3.N- BOC amino acid

(i) $NaHCO_3$, THF/H_2O, 0°C, 30 min.
(ii)RT, 10-12 hrs.

(B) Protection of D-glucosamine:

The procedure for the protection of D-glucosamine was adopted as described by Mauro De Nisco (2012) [4].

Scheme: 2



(4) D-glucosamine

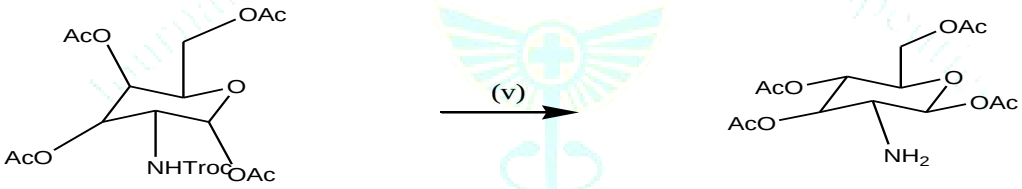
(5) 1,3,4,6-tetra-O-acetyl-2(2,2,2-trichloroethoxycarbonylamino)-D-glucopyranose

(iii) $NaHCO_3$, H_2O, trichlorethoxy carbonyl chloride, RT, 2 hrs
(iv) 1M HCl, pyridine, acetic anhydride, RT, 10-12 hrs

(C) De-protection of amine group of D-glucosamine:

Mischmetal (MM) (8.6 g, 61 mmol) was added to 250 ml of dry Tetrahydrofuran and activated with 18 ml (93 mmol) of TMSCl by refluxing under a nitrogen atmosphere for 30 min. Then, 10 mmols of compound 5 (1,3,4,6-tetra-O-acetyl-2(2,2,2-trichloroethoxycarbonylamino)-D-glucopyranose), obtained in previous step was added and refluxed until thin-layer chromatography (TLC; EtOAc–hexane) showed the completion of reaction. Before TLC, the sample was quenched with saturated Na_2CO_3. Mischmetal was filtered after the completion of reaction and the resulting filtrate was treated with 300 ml of saturated aqueous solution of sodium carbonate, stirred for 5 min, and then extracted with CH_2Cl_2 (5x300 ml) and with ethyl acetate (3x300 ml). The combined organic layers were washed with 0.2 M citric acid aqueous solution, twice with distilled water, and then dried over anhydrous magnesium sulfate. The resulting mixture was filtered and concentrated by rotavap [4].

Scheme: 3



(5) 1,3,4,6-tetra-O-acetyl-2(2,2,2-trichloroethoxyamino)-D-glucopyranose

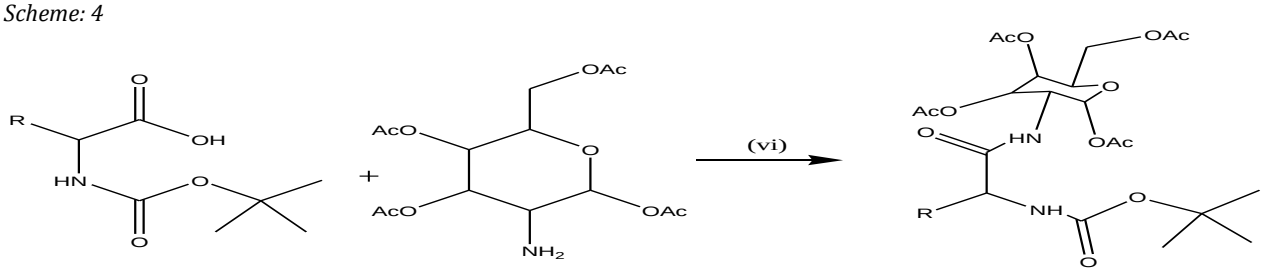
(6) 1,3,4,6-tetra-O-acetyl-D-glucosamine

(v) TMSCl, MM, dry DMF, Reflux

(D) Conjugation of BOC protected amino acids with protected D-glucosamine:

To a magnetically stirred solution of BOC amino acid (10 mmoles), in anhydrous CH_2Cl (50 ml) in an ice bath, HOBT (2.7 gm, 20 mmoles) was added in one portion. After 30 min. a solution of compound 6 (5.0 gm, 9.3 mmoles) and EDC (18 ml, 10 mmoles) in the anhydrous CH_2Cl (50 ml) was added dropwise at same temperature. The mixture was stirred for 12 hours in an ice bath and then allowed to warm slowly to room temperature. A precipitate was filtered off and the solution was washed with saturated $NaHCO_3$, brine, until neutral, and then dried over Na_2SO_4. Solvent was evaporated under reduced pressure.

Scheme: 4



Boc amino acid

O-acetyl D-glucosamine

(7) N-Boc amino acyl 1,3,4,6 tertraacetyl D-glucosamine

(vi) CH_2Cl_2, HOBT, EDC, RT, 12 hrs

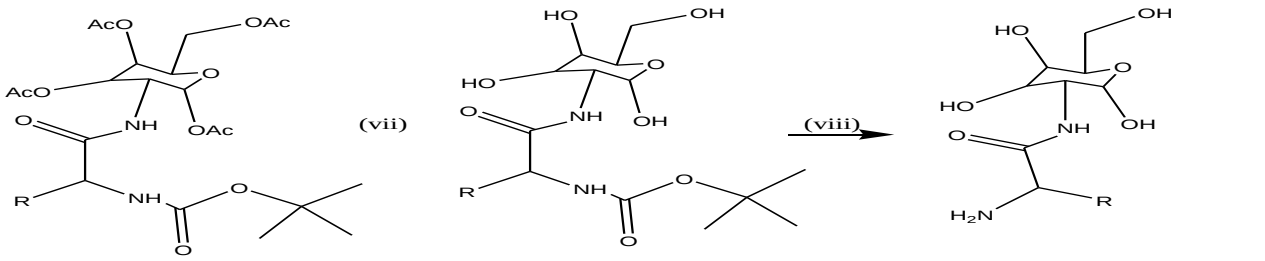
(

E) De-protection of glyco-amino acids:

(i) Compound 7 (N-BOC aminoacyl 1,3,4,6 tetra acetyl D-glucosamine), obtained from previous step was dissolved by a saturated solution of NH_3 in dry methanol (40 ml), in ice bath. Stirred for 12 hrs. Evaporation of solvent was done under controlled reduced pressure.

(ii) 10 mmols of compound (8) was dissolved in 5 equivalent of THF (4 ml) and CH_2Cl_2 (3.2 ml) and stirred for 2 hours at room temperature. Evaporation of solvent was done under controlled reduced pressure Compound 9(N-aminoacyl D-glucosamine) was obtained as final product.

Scheme: 5



(8) N-Boc aminoacyl-D-glucosamine **(9)** aminoacyl D-glucosamine

(vii) NH_3, MeOH, RT, 12 hrs
(viii) THF, CH_2Cl_2, RT, 2 hrs

RESULTS AND DISCUSSION

Glyco amino acids and their derivatives were synthesized by coupling of amino acids to the monosaccharide unit at optimized parameters of reaction. In this synthesis BOC protected amino acids were reacted with protected D-glucosamine to form different building blocks of glyco amino acids.

The synthesis of glyco amino acids involved various protection and de-protection steps to prevent side reactions. Scheme 1 and 2 schematically illustrated the procedure for the protection of different amino acids and D-glucosamine respectively. The synthesized glyco amino acids were characterized by physicochemical properties and analysis by, infrared spectroscopy, Nuclear magnetic resonance spectroscopy, and Mass spectroscopy.

The FT-IR spectra of different glyco amino acids displayed peaks around 900 and 1150 cm^{-1} of assigned saccharide structure. The peak in the range of 3475-3150 cm^{-1} showed the N-H stretch and in the range of 1700-1600 cm^{-1} showed the C=O stretch confirmed the amide bond formation. Further confirmation of structure of different glyco amino acids were done by **Mass** and **^{1}H NMR** Spectroscopy

Physicochemical parameters of synthesized Glyco-amino acids (GAAs):

The results of physicochemical properties are summarized in tables as given below:

Table 1: Physicochemical parameters of synthesized GAAs

S. No.	*Compound code*	*Amino acid*	*R*	*Appearance*	*Solubility*	*M.P. (°C)*	*Rf Value (4:1)*
1.	GAA-1	Glycine	-H	Light Brown	Methanol	140-145	0.79
2.	GAA-2	Isoleucine	$-CH(C_2H_5)\ CH_3$	White	Water, Methanol	165-170	0.65
3.	GAA-4	Leucine	$-CH_2CH(CH_3)_2$	White	Water, Methanol	Decomposed on heating	0.83
4.	GAA-5	Alanine	$-CH_3$	Pale White	Water, Methanol	120-125	0.83
5.	GAA-6	valine	$-CH(CH_3)_2$	Snow White	Water, Methanol	150-155	0.89

Spectral analysis of synthesized Glyco amino acids (GAAs):

Table 2: Molecular mass data of synthesized GAAs

S. No.	*Compound Code*	*Exact mass*	*Fragments mass*	*Fragments formula*	*Molec-ular ion peak*
1.	GAA-01	236.1	132.05	$C_4H_8N_2O_3$	252.1
2.	GAA-02	292.16	188.16	$C_8H_{16}N_2O_3$	339.4
3.	GAA-04	292.16	188.16	$C_8H_{16}N_2O_3$	305.2
4.	GAA-05	250.12	146.06	$C_5H_{10}N_2O_3$	252.1
5.	GAA-06	278.15	174.1	$C_7H_{14}N_2O_3$	287.1

Table 3: IR absorption band of synthesized GAAs

Compound Code	*Bond Type*			
	N-H	**C-H**	**C-N**	**O-H**
GAA-01	3280.09 cm^{-1}	2875.99 cm^{-1}	1065.72-1229.67 cm^{-1}	3243.44cm^{-1}
GAA-02	3218.37 cm^{-1}	2878.88 cm^{-1}	1076.33-1270.18 cm^{-1}	3218.37 cm^{-1}
GAA-04	3288.77 cm^{-1}	2877.92 cm^{-1}	1004.96-1247.03 cm^{-1}	3201.97 cm^{-1}
GAA-05	3289.74 cm^{-1}	2876.95 cm^{-1}	1007.88-1247.03 cm^{-1}	3289.74 cm^{-1}
GAA-06	3286.84 cm^{-1}	2879.85 cm^{-1}	1003.93-1259-37 cm^{-1}	3286.84 cm^{-1}

Table 4: 1H-NMR Structural characterization data of synthesized GAAs

S. No.	*Compound Code*	*NMR*
1.	GAA-01	^1H (CD_3OD) 1.368 (2H, s, NH_2), 4.967(1H,s,NH), 3.316-3.305(1H,t,HO-C-H), 3.299 (1H,s,Ar-OH).
2.	GAA-02	^1H (D_2O) 1.880 (2H,s,NH_2), 5.296 (1H,s,NH), 0.936-0.913 (3H,d,CH_3), 2.850 (1H,s,Ar-OH), 3.731-3.709 (1H,t,HO-C-H), 1.293 (2H,m,R-CH_2-R).
3.	GAA-04	^1H (D_2O) 2.826 (2H,s,NH_2), 5.3 (1H,s,NH), 2.873 (1H,s,Ar-OH), 3.781 (1H,t,HO-C-H), 0.877-0.799 (3H,t, CH_3), 1.491 (2H,q, R-CH_2-R).
4.	GAA-05	^1H (D_2O) 2.716 (2H,s,NH_2), 5.3 (1H,s,NH), 3.672-3.637 (2H,q,CH_2), 2.873 (1H,s,Ar-OH), 1.347 (3H,d,CH_3), 3.794-3.772 (1H,t,HO-C-H).
5.	GAA-06	^1H (D_2O) 2.074 (2H,s,NH_2), 5.3 (1H,s,NH), 3.693-3.613 (2H,q,CH_2), 2.855 (1H,s,Ar-OH), 0.851 (3H,d,CH_3), 3.778-3.713 (1H,t,HO-C-H).

CONCLUSION

Design of smart drug carrier is currently essential for the progressively more demanding requirement of controlled drug delivery. Polymers present advantages over further possible constitutive materials. In this respect carbohydrates are getting a particular attention because of their identification by several endogenous carbohydrate proteins and their vast potential in information storage. To a large extent attempt is devoted to design of glycopolymers as key agents in drug delivery systems. This work has describe the conventional synthesis of glyco amino acid derivatives.This synthesis was based on the use of monosaccharide and amino acids which yields glycosylated amino acid building blocks that can be utilized in the synthesis of glycopeptides.Glyco amino acids can also be used to generate libraries of glycomimetics via derivatization and oligomerization of carbohydrates. We have tried to develop a method for the synthesis of biologically relevant glyco amino acid derivatives. This method provides glycoconjugates from readily available starting material. These derivatives possess useful applications in the preparation of glycoconjugates for subsequent immobilization, glycotargetting and as a potent inhibitor of carbohydrate-protein binding.

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