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Synthesis and Characterizations of Dipeptide Derivative of Gentamicin

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Abstract

The target derivative is gentamicin linked with L-Val- L-Ala by an ester linkage. It was synthesized by esterification method, which included the reaction of hydroxyl group on (carbon No.5) of gentamicin with the acid chloride of the corresponding dipeptide. The preparation of new derivative of gentamicin involved protection of the primary and secondary amino groups of gentamicin, by ethylchloroformate (ECF) to give N-carbomethoxy gentamicin which was used for further chemical synthesis involving the free hydroxyl groups.

The prepared dipeptide (L-Val- L-Ala) by conventional solution method in the presence of DCC & HOBt then reacted with thionyl chloride to prepare acid chloride of dipeptides, then after, linked by ester linkage to N-protection gentamicin in present pyridine as base, finally deprotection the amino group of synthesized compound by using trifluoroacetic acid (TFA) in the presence anisole.

The characterization of the titled compound was performed utilizing FTIR spectroscopy, CHNO elemental analysis, and by measurements of their physical properties.

Keywords: Gentamicin, Dipeptide, Chemical synthesis.

تحضير وتوصيف مشتق ثنائي الببتيد من الجنتاميسين

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الخلاصة

المشتق المستهدف هو جنتاميسين مرتبط بـ L-Val-L-Ala بواسطة وصلة استر. تم تحضيرها عن طريقة الأسترة ، والتي شملت تفاعل مجموعة الهيدروكسيل على (الكربون رقم ٥) من جنتاميسين مع كلوريد حامض من ثنائي الببتيد المقابل ، إعداد مشتق جديد من الجنتاميسين المتضمن حماية مجموعات الأمين الأولية والثانوية للجنتاميسين ، بواسطة إيثيل كلوروفورمات (ECF) لاعطاء N- carbomethoxy gentamicin الذي تم استخدامه لمزيد من التخليق الكيميائي الذي يشمل مجموعات الهيدروكسيل الحرة. بعد ذلك يتم تحضير ثنائي الببتيد (L-Val-L-Ala) باستخدام طريقة الحل التقليدي بوجود DCC و HOBt الحالي ثم تفاعل مع كلوريد الثاينويل إلى كلوريد حامض من ثنائي الببتيد ، ثم بعد ذلك ، يربطه ارتباط استر بـ N-protection جنتاميسين في البيريدين الحالي كقاعدة ، وأخيرا تحرير المجاميع الأمينية من المركب باستخدام TFAA في anisoleالحالي.

تم تنفيذ توصيف المركبات المعنونة باستخدام التحليل الطيفي بالاشعة تحت الحمراء تحليل العناصر CHNO وعن طريق قياس خواصها الفيزيائية.

الكلمات المفتاحية : جنتاميسين ، ثنائي الببتيد ، التوليف الكيميائي.

Introduction

Gentamicin is an aminoglycoside antibiotic, used to treat many types of bacterial infections, particularly those caused by Gram-negative bacteria. Gentamicin sulphate is the sulphate salt, or a mixture of such salts, of the antibiotic substances produced by the growth of *Micromonospora purpurea*.. Gentamicin is a bactericidal antibiotic that works by binding the 30S subunit of the bacterial ribosome, interrupting protein synthesis [(1, 2)]. Like all aminoglycosides, when gentamicin is given orally it is not systemically active. This is because it is not absorbed to any appreciable extent from the small intestine. Aminoglycosides are poorly absorbed from the gastrointestinal tract and are administered parenterally [(4)]. Their high water solubility and low protein-binding facilitate distribution[(5, 6)].

Gentamicin is the only heat-stable antibiotic, hence its use during orthopedic surgery when high temperatures are required for the setting of cements (e.g. hip replacements) **[(1,3)].** Colon –specific delivery of bioactive compounds received extensive investigations, utilizing the significantly variable bio environments of the different parts of the G.I.T [(4)].

Prodrugs are bioreversible derivatives of drug molecules that undergo an enzymatic and/or chemical transformation in vivo to release the active parent drug. Strategies to improve the oral bioavailability and achieve specific targeting have been the most important developments in prodrug design during the last 5 years. [(5)]

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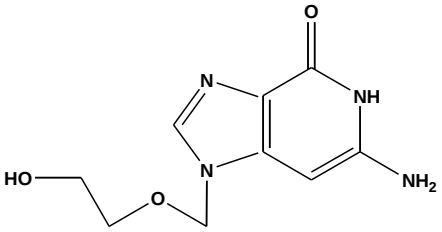
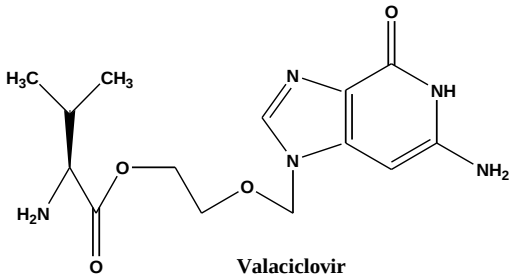
Transport of di- or tripeptides across plasma membranes in the small intestine and the kidney proximal tubules play a pivotal role in efficient absorption of protein digestion products. The absorption process is mediated actively by H^+ -coupled peptide transporters localized in the brush-border membranes of these epithelia. [6].There are two types of human peptide transporters: [7] Human Peptide Transporters 1 (hPepT1) that is present in the brush border of the small intestine and predominantly in the upper part, Human Peptide Transporters 2 (hPepT2), which are mostly abundant in the kidney [6] . The identification of two peptide transporters, PEPT1 and PEPT2, represented a major step forward toward molecular understanding of the physiological and pharmacological significance of peptide transporters [6] ,among various membrane transporters, peptide transporters are the most attractive targets in prodrug design to improve oral drug absorption[7].

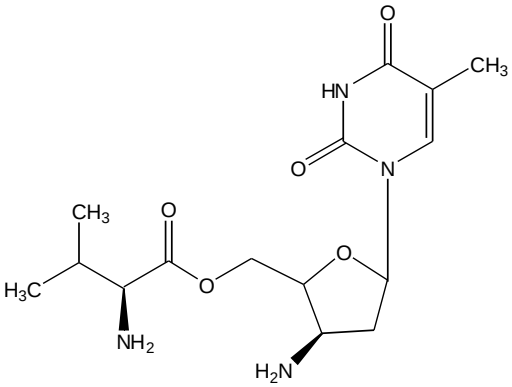
The peptide transporter is a possible route for improving the intestinal absorption of pharmacologically active amino acid analogues. As examples of compounds without a peptide bond, the aminopeptidase inhibitor arphamenine, the antiviral agent valacyclovir and 4-aminophenylacetic acid were reported to be substrates for peptide transporter [8].



Aciclovir



Valaciclovir



L-Valyl ester of AZT

Figure1. Substrates of peptide transporters

Materials and Methods

All chemicals and solvents used during synthesis were of analytical grade and used without further purification as follow ; Gentamicin Sulphate(Hyperchem, China), L-Alanine, Acetone, Boc - L – Valine, Chloroform, Ethanol (98%),Methanol (98%) Thionyl chloride , and Trifluoroacetic acid (TFA) (Fluka AG, Germany), Acetyl Chloride, Acetic acid (glacial) , Dichloromethane, methylene chloride, Hydrochloric acid (33%) Pyridine ,and Tetrahydrofuran (THF)(BDH, England), Dimethylformamide (DMF)and Triethylamine (TEA)(Fluka AG , Switzerland), Ethyl chloroformate (ECF) and Silica gel F254 aluminum sheets,(Merck, Germany), Anisole(Gillard chem.,UK), Hydroxybenzotriazole (HOBt)(Sigma-Aldrich,USA), Petroleum ether (60 º -80 º C)(BDH,UK), N,N'-Dicyclohexylcarbodiimide (DCC)(Sigma-Aldrich,USA).

Completion of reactions and the purity of compounds were ascertained by thin-layer chromatography (TLC), using silica gel GF254 (type 60) precoated aluminum sheets, Merck (Germany) exposed to UV-254nm light, or by reacting with iodine vapor, or with Ninhydrine spray reagents for detection of dipeptide, and the eluent used is Chloroform: ethanol (7:3) for

intermediate Ia & Methylene chloride: Ethanol (7:3) for others to run TLC. Melting points were determined using Stuart SMP3 melting point apparatus in open capillary tubes, and are uncorrected. Fourier Transform Infrared spectroscopy (FTIR), were recorded using (Biotech engineering management FTIR-600, UK). The elemental microanalysis of the synthesized compounds was done using (Elemental vario MICRO cube instrument, Germany).

Chemical synthesis

General method for synthesis of intermediate Ia

The primary and secondary amino groups of gentamicin were protected [9], [10], by Ethylchloroformate (ECF) to give N-carbomethoxy Gentamicin which was used for further chemical synthesis involving the free secondary hydroxyl groups.

Gentamicin Sulphate (0.0008 mol, 0.46 gm) dissolved in methylene chloride (60 ml) containing triethylamine (0.013mol, 1.8 ml) and was cooled to (-5ºC). ECF (0.0043 mol, 0.53 ml) in 40 ml of THF (tetrahydrofuran) was added. The reaction mixture was left at (- 5 ºC) for 30 min with continues stirring. The temperature of the reaction mixture was allowed to rise to room temperature gradually and the mixture was continuously and vigorously stirred for 6 hrs. The solvent was evaporated under vacuum. The residue was redissolved in methylene chloride and D.W. Acidified with 5% hydrochloric acid solution to wash out the un- reacted materials. The organic layer was separated, washed several times with saturated sodium chloride solution and then D.W. The organic layer was dried using anhydrous magnesium sulfate filtered and methylene chloride was evaporated under vacuum leaving a white precipitate re-crystallized using (70% methanol).The percentage yield, and R_f values are listed in table 1, The IR characteristic bands of N-carboethoxy- gentamicin (compound Ia) are listed in table 2, CHNO data are listed in table 3.

Chemical synthesis of compound Ib (Synthesis of Boc L-val -L-ala dipeptide)

Coupling is done by conventional solution method; (0.082 mol, 1.8 gm) of Boc – L-Valine in 10 ml of DMF were cooled to 0° C, (0.019 mol ,4.12 gm) of DCCI was added the solution was stirred with cooling [9] .A solution of (0.082 mol, 0.73 gm) alanine in 10 ml of DMF (previously cooled to 0°C) was added to the first stirred solution then kept the reaction mixture cooled to (-15 °C) for 10 min, HOBt (0.015, 2.16gm) was added with stirring. The stirring was continued for two days at 0° C. Then two days at room temp .Filtration was carried out to remove DCU precipitate formed during the reaction, the solvent was evaporated under reduced pressure to remove DMF .The residue was redissolved in chloroform (50 ml), washed with cold water (2× 20 ml). The organic layer collected, dried over anhydrous magnesium Sulphate, the volume was reduced then, diethyl ether was added to get crystals kept in deep freeze. The physical appearance, percent yield, melting point, and R_f values are listed in table 1, The IR characteristic bands are listed in figure 3, CHNO data are listed in table 3 [9] .

Synthesis of Boc L-Val –L-ala acetyl chloride (compound Ic)

This intermediate was synthesized by the conversion of compound Ib into its corresponding acid chloride by reacting with thionylchloride and as follows:

Compound Ib (0.0057 mol, 1.64 gm) was dissolved in (30) ml chloroform . Excess of thionyl chloride (0.0085mol, 0.6 ml) was added drop wise with continuous stirring at 0◦C. The temperature of the reaction was raised gradually and refluxed for three hours. The reaction mixture then cooled; the volume was reduced by evaporation to small volume to remove excess of thionyl chloride then the residue was re-dissolved in 30 ml of chloroform and re -evaporate to ensure the removal of all thionyl chloride. Brown, oily residue was obtained. This compound was used immediately for other synthesis. [11]

Chemical synthesis of Boc L-val- L-ala ester of gentamicin (compound Id)

Compound Ia (0.00066 mol, 0.5gm) was dissolved in 50 ml of (1:1) Pyridine: DMF, cooled in ice bath to 0 o C with continuous stirring.

Compound Ic (0.00066 mol, 0.19 gm) were dissolved in 30 ml of chloroform,(Prepared immediately and cooled) was added drop wise to the (previous solution) .

The reaction mixture was stirred under cooling for 5 hours, then left to stand for 12 hour at same temperature .The solvent was evaporated to small volume. 50 ml of water added to remove the pyridine HCl and the DMF [12]. Then evaporated under vacuum leaving a dark yellowish precipitate re-crystallized by dichloroethane

Chemical synthesis of compound 1;

Synthesis of L-val L-ala ester of Gentamicin; (deprotecting of amine groups of Gentamicin)

Compound (Id) (0.00005 mol, 0.05gm) was dissolved in methylene chloride (10ml) and was stirred with TFA (10 ml) for 1 hr. at 0 ºC in the presence of (1-2) drops of anisole, and

kept at room temp for 3 hrs. TLC for the reaction mixture was performed to ensure the removal of the amino–protective group.

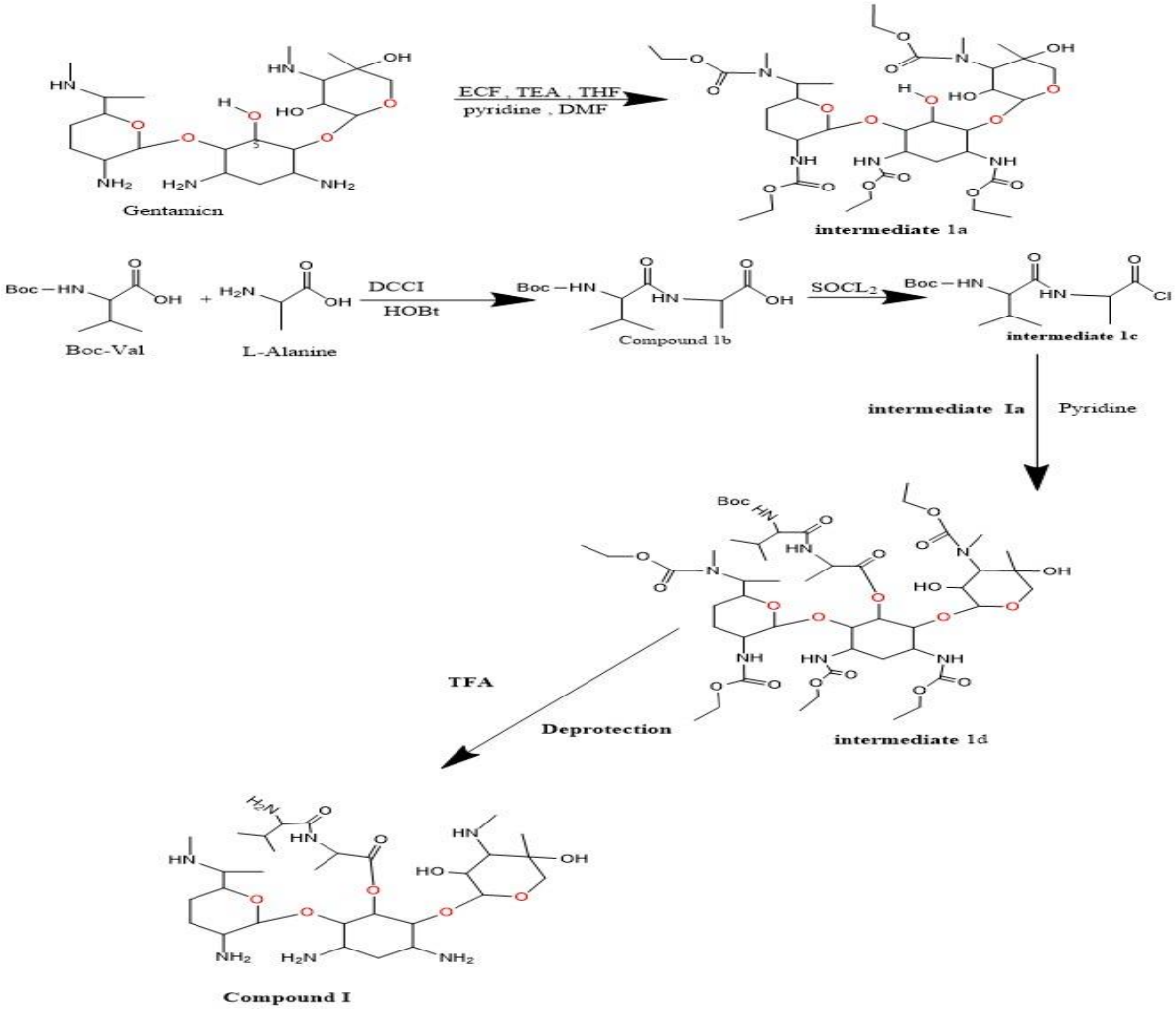
Ether (30 ml) was added to the reaction mixture and the resulting precipitate was collected. The precipitate was suspended in D.W. (30 ml) and the pH was adjusted to 5 with 5% NaHCO3. The reaction mixture was filtered and a yellow precipitate was collected, washed with D.W. (30 ml × 2), dried in an oven at 50ºC. The precipitate was triturated with ether, filtered and crystallized using petroleum ether to form yellowish crystals [11, 13] .

The physical appearance, percent yield, melting point, and R_f values are listed in table 1, CHNO data are listed in table 3, and The IR characteristic bands are listed in figure 3.

Results and Discussion

Spectral data and chemistry

The synthesis of compounds (intermediates and end products) is presented in scheme **1.**The synthesized compounds were identified and characterized by their melting points, R_f values, and physical appearance, these data are listed in the table 1, the IR data in table 2 . The Elemental Microanalysis data with their interpretations are presented in table 3.



Scheme 1.synthesis of compound I.

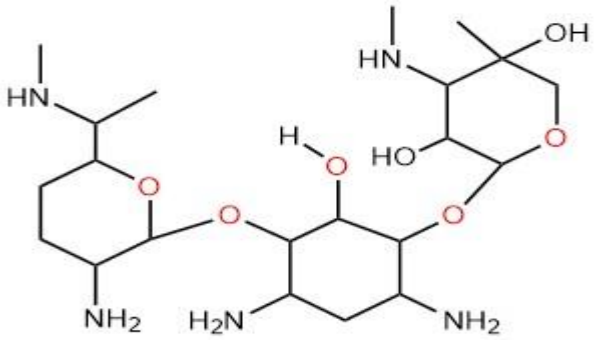
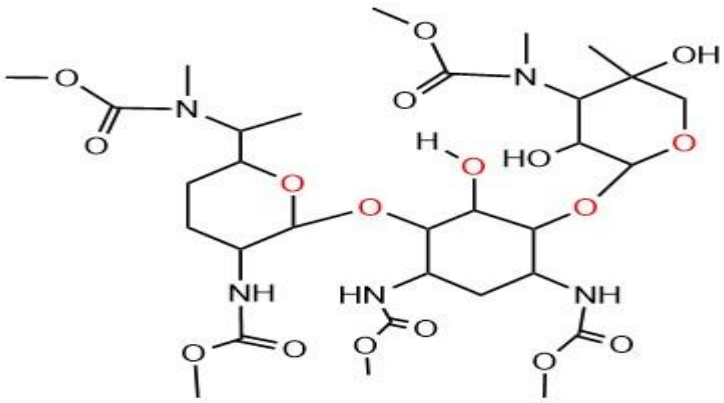
Table 1. Melting points, R_f values, and physical appearance

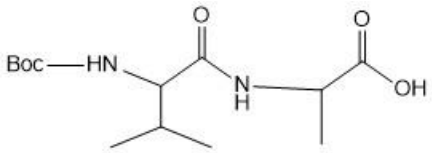
Compound	*Physical appearance*	*% yield*	*Melting point observed (ºC)*	*Rf value*
Ia	White crystals	93	161-163	(C) (0.53)
Ib	Oily	35		(B) (0.45)
I	Brown crystals	80	112-114	(B) (0..63)

B: Ethylene chloride: Methanol (8:2).

C: Chloroform: ethanol (7:3)

Table 2. FT-IR spectrum of compound

Compound	*Bands (cm^{-1})*	**Interpretatio**
	3451	band of primary NH2
 Gentamicin	3647-3562	Broad for OH stretching vibration
	3141 2964	N-H stretching of secondary amide Asymmetric C-H stretching of CH_3 & CH_2 groups
	2870	symmetric C-H stretching of aliphatic CH_3 & CH**2** groups
	1471	Asymmetric C-H bending of CH3 groups
	1604 - 1589	N-H bending of secondary amide
	1087	C-O stretching vibration of 2° alcohol
	1249-1294	C-O-C stretching vibration of ether
 **N-protected gentamicin**	3444	Broad for OH stretching vibration
	3279-3242 2964	N-H stretching of secondary amide Asymmetric C-H stretching of CH_3 & CH_2 groups
	2872	symmetric C-H stretching of aliphatic CH_3 & CH**2** groups
	1739	C=O stretching of amide
	1496	N-H bending of secondary amide

	1454	Asymmetric C-H bending of CH3 & CH2 groups
	1257	C-O stretching vibration of secondary alcohol
	1049	C-O-C stretching vibration of ether
 intermediat Ib	3336	N–H stretching of urethane.
	3298	N–H stretching of the secondary amide & O-H st. of COOH.
	2974	Asymmetric C–H stretching of methyl group.
	2875	Symmetric C–H stretching of methyl group.
	1708	C=O stretching of carboxyl group.
	1653	C=O stretching of *the* secondary amide.
	1516	N–H bending of the secondary amide.
	1456	Asymmetric C–H bending of methyl group.
	1365	Symmetric C–H bending of methyl group.
	3284	N-H stretching of 2o-amide.
	3626- 2560	broad alcoholic O-H stretching vibration
	2918	Asymmetric C-H stretching of methyl & CH2 group

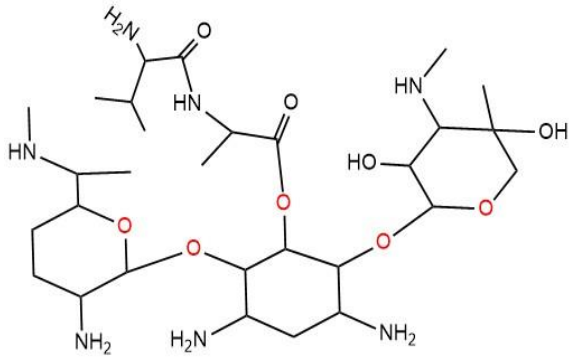
 Compound I	2848	Symmetric C-H stretching of methyl & CH2 group
	1768	C=O stretching of ester
	1681	C=O stretching of amide group
	1529	N-H bending of secondary amide
	1462	Asymmetric C–H bending of methyl group
	1432	Symmetric C–H bending of methylene group
	1203	C-O stretching of ester.

Table 3. The elemental microanalysis of the synthesized compounds

Compound	Chemical formula	Elemental microanalysis %		
	Mol.Wt.	Element	Calculated	found
Ia	C31H41N5O17 755.68	C	49.26	47.03
		H	5.46	5.72
		N	9.26	8.84
		O	35.99	38.39
Ib	C13H24N2O5 288.3396	C	54.14	
		H	8.38	
		N	9.71	
		O	27.74	
I	C29H57N7O9 647.81	C	53.76	52.3
		H	8.86	8.93
		N	15.13	14.72
		O	22.22	24.03

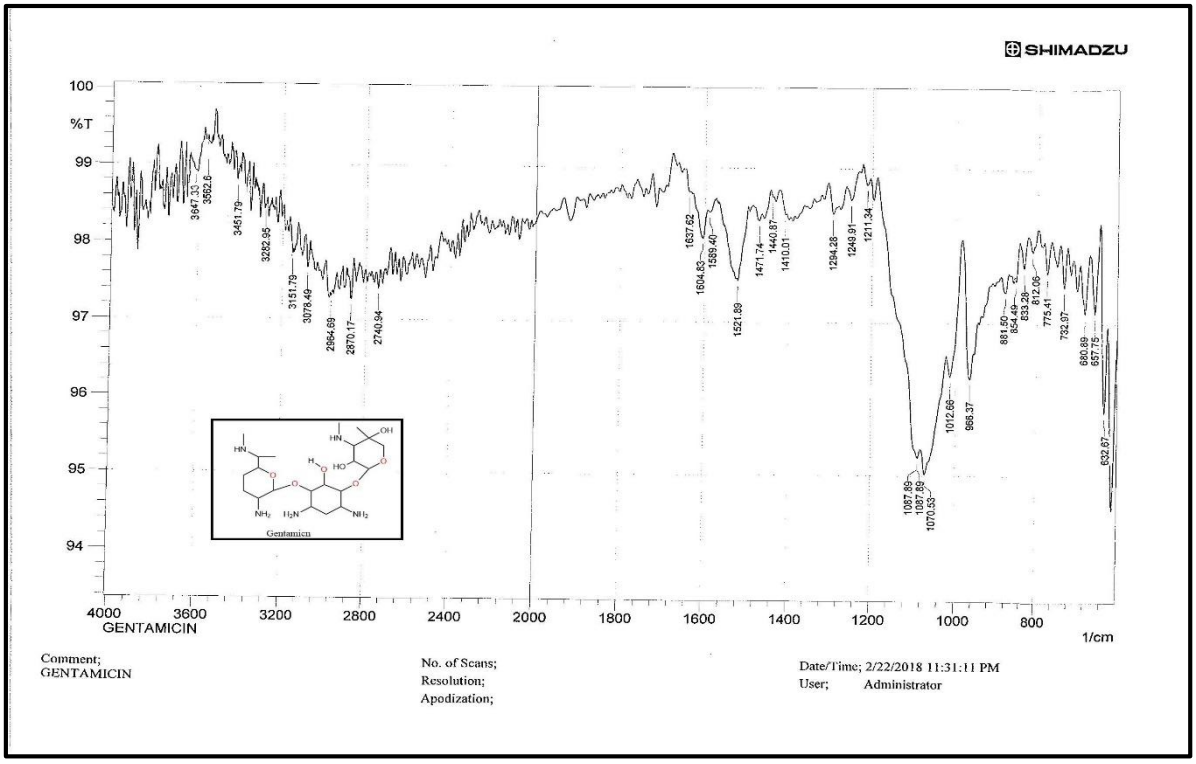


Figure1. Gentamicin

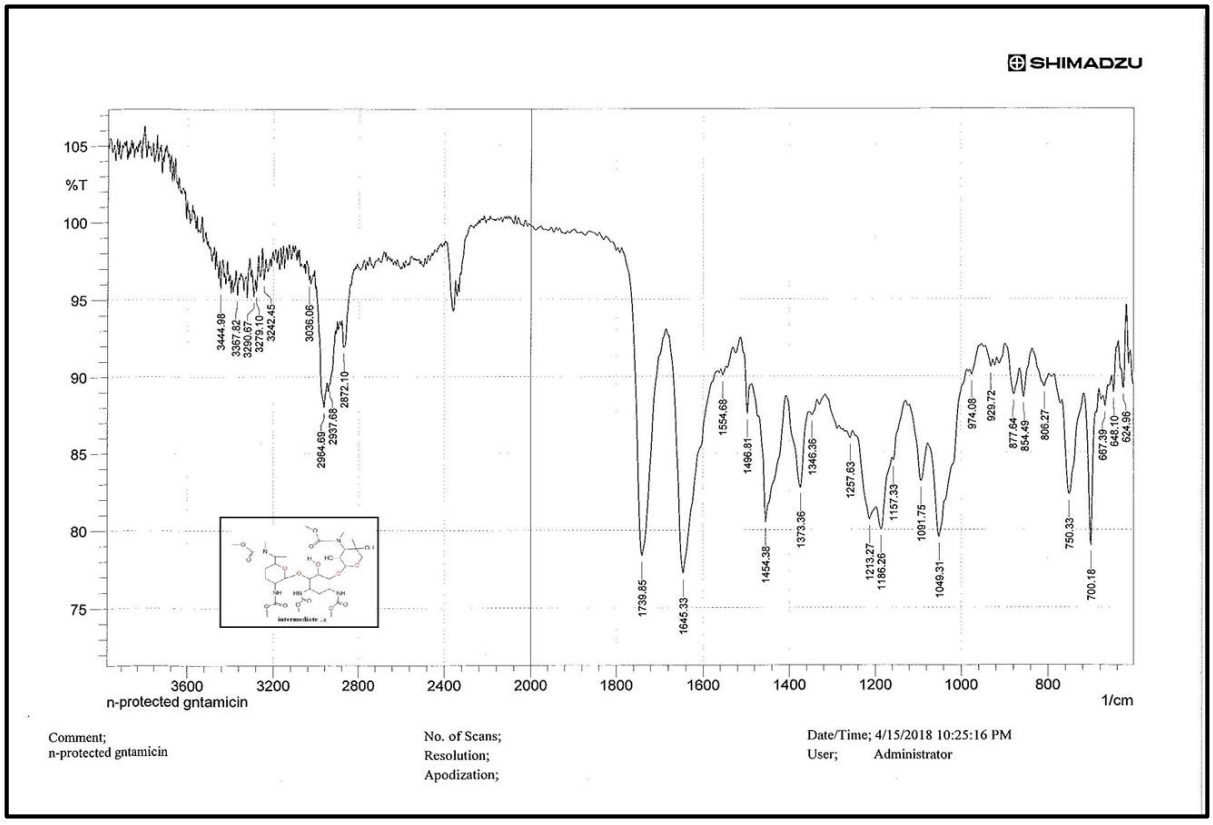


Figure 2.N-protected gentamicin

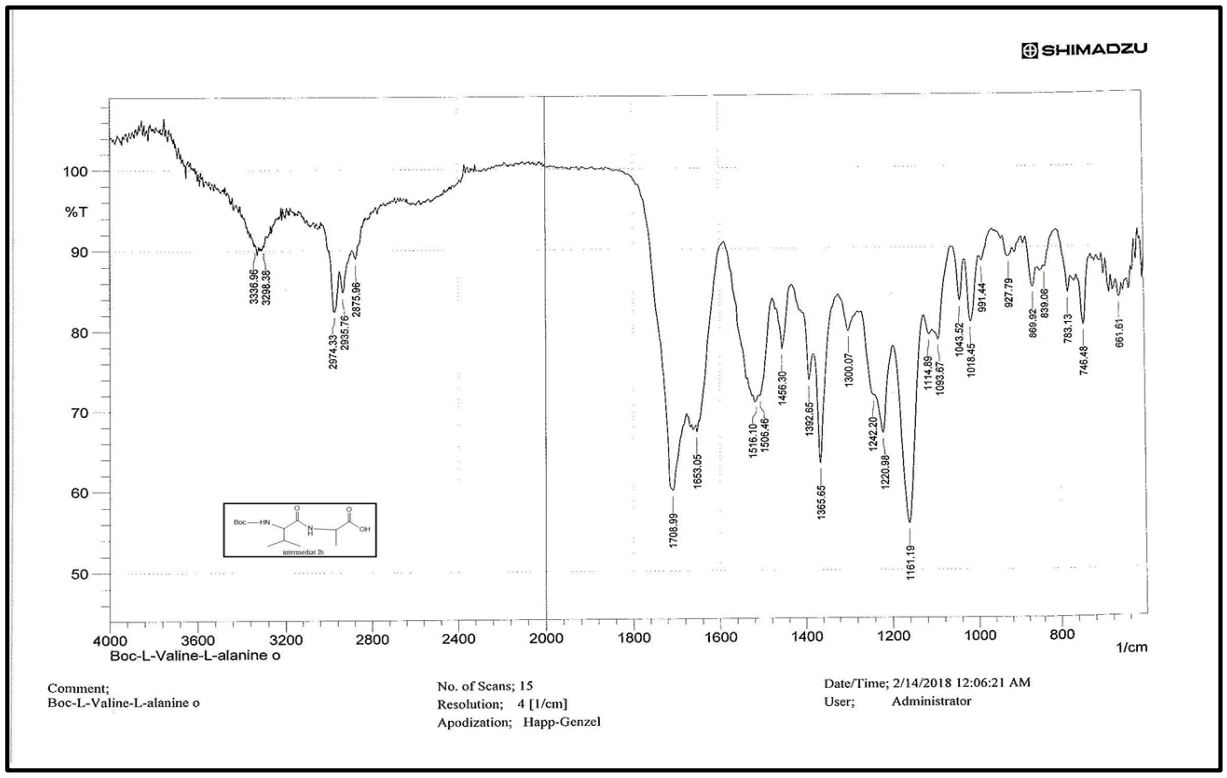


Figure 3. Intermediate Ib

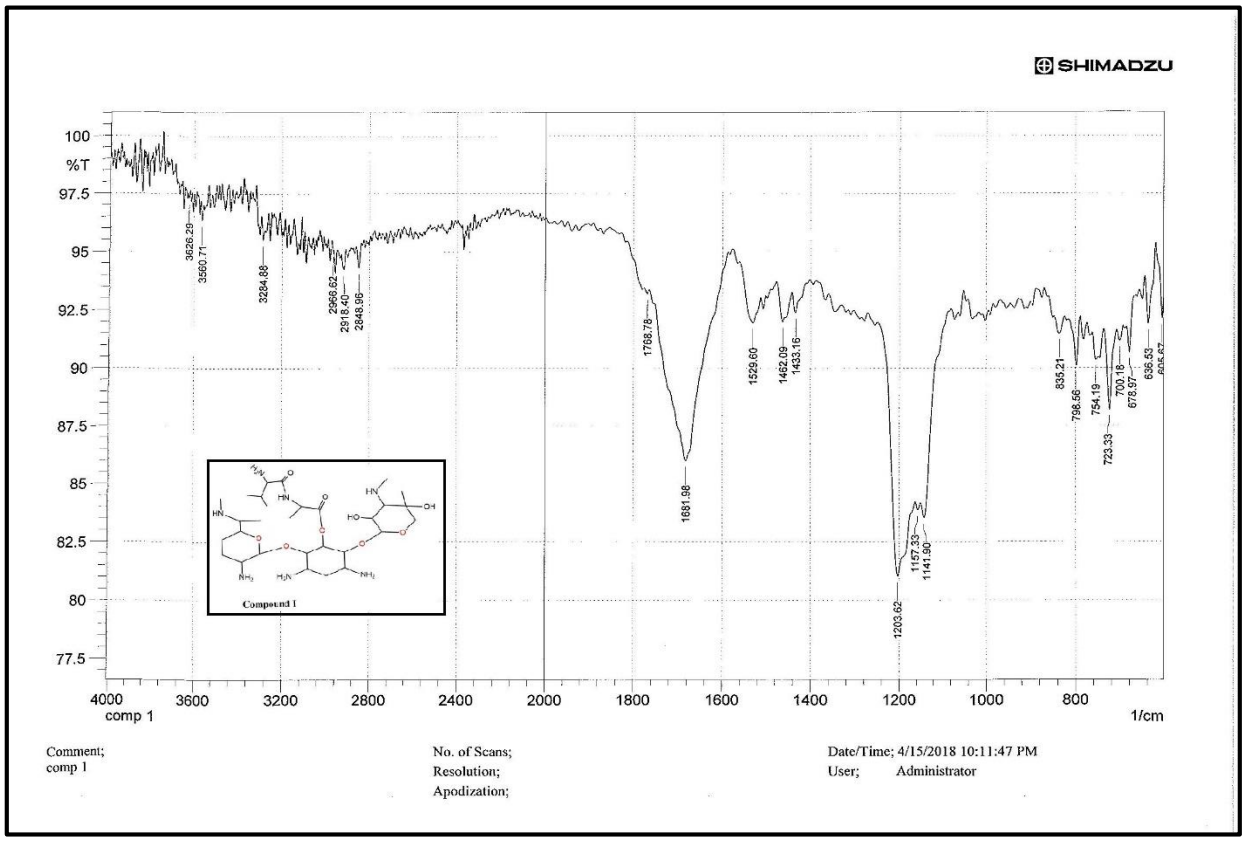


Figure 4. Compound I

Conclusion

The synthesis of the designed compound as derivative of Gentamicin was successfully achieved by following the stated procedures as previously described and resulted in the preparation of the proposed compound in reasonable yields. The spectral and CHNO analysis have confirmed their chemical structures. IR spectra were recorded and found that the characteristic bands of the compounds indicate the presence of certain groups which comply with the proposed chemical structure.

Acknowledgement

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