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Synthesis and structural studies of Glucosylimino-1,2,4,5-Tetrazine

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ABSTRACT

Series of 3-tetra-O-acetyl–β-D-glucopyranosylimino, 6 substituted -1,2,4,5-tetrazine (IV) have been synthesized by the reaction of aryl dihydroformazanes (III) and tetra-O-acetyl-β-D-glucosylimino-isocyanodichloride in refluxing chloroform medium. The aryl dihydroformazans (III) were prepared by the interaction of acid hydrazides (I) and hydrazine hydrate (II) in refluxing ethanol medium in 1:1 proportion. The structures of tetrazines (IV) were confirmed on the basis of elemental analysis and IR, PMR, ^{13}C and mass spectral analysis.

Keywords: Tetrazine, isocynodichloride, dihydroformazans.

INTRODUCTION

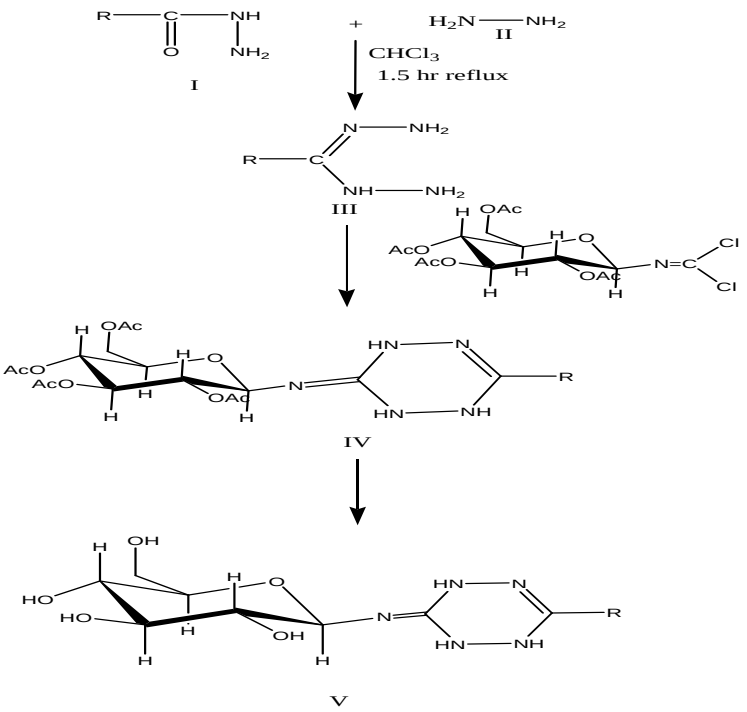
Natural products show a broad spectrum of biological activities with a high potential for medicinal applications. Carbohydrates are also structurally important component of such numerous biologically active natural products. Some of its N and S linked sugar derivatives are exhibiting antifungal [1], antitumor [2], anticancer [3], antiviral [4], antimalarial activity [5] and antilukemic activity [6]. Recently, ortho carbonyl glycosides used for the treatment of cancer by boron neutron capture therapy [7].

Various routes [8-16] have been reported in the literature for the synthesis of different 1,2,4,5-tetrazines. Majority of them [17-18] are 3-6-symmetrically disubstituted tetrazines obtained from the treatment of nitrile derivatives with hydrazines or aldehyde with substituted hydrazines. Tetrazines possess a wide range of antiviral and antitumor properties and have been widely used as pesticides and herbicides [19]. Recently, new 1,2,4,5-tetrazines have been successfully synthesized by using N-aryl isocynodichloride [20-21].

Such significant and diversified pharmaceutical values of glycosides have focused our interest on the studies towards the monosaccharide moiety. Our present work relates to synthesis of several N-linked glucose derivatives via one of the most powerful intermediate glucosylimino isocyanodichloride. The glucosylimino isocyanodichloride was firstly synthesized in this laboratory.

In our research interest we explored the application of glucosyl isothiocynate and its dichloro derivative in the synthesis of heterocyclic system with glucosidic base [22]. Here we explored the route for the synthesis of tetrazine molecules by using glucosylimino isocynodichloride as one of the reagent for the synthesis of heterocyclic system.

Reaction scheme



Ia, IIa, IIIa, IVa where R = p-Nitrophenyl,
Ib, IIb, IIIb, IVb, where R = Phenyl,
Ic IIc IIIc IVc where R = Phenylacetyl,
Id ,IId ,IIId, IVd where R = p-Chlorophenyl,
Ie IIe IIIe IVe where R = o-Chlorophenyl,
If IIf IIIf IVf where R = o-Hydroyphenyl,
Ig IIg IIIg IVg where R = Pyridyl,
Ih IIh IIIh IVh where R = m-Chlorophenyl
Ik IIk IIIk IVk where R = o-Nitrophenyl.

MATERIALS AND METHODS

Experimental:

All melting points are uncorrected and were obtained in capillary using paraffin bath. FT-IR spectra were recorded using KBr disk on Perkin Elmer FT-IR KBr spectrophotometer and ^{1}HNMR on Brucker Advance II 400 NMR spectrometer using DMSO, $CDCl_3$ as solvent. Purity of the compound is checked on silica gel G plate using iodine vapors as a visualizing agent. All aryl dihydroformazans were prepared by the extension of the known procedure [23].

1. Preparation of 3-Tetra-O-acetyl–β-D-glucopyransoylimino, 6(4-nitrophenyl) 1,2,4,5-tetrazine (IVa).

3-tetra-O-acetyl–β-D-glucopyransoylimino,6(4-nitrophenyl) 1,2,4,5-tetrazine (IVa) was prepared by refluxing 4-nitrophenyl dihydroformazan (0.001mole) with glucosylimino isocyanodichloride (0.001mole) in chloroform medium for 4 hours. After completion of reaction, the reaction mixture was cooled; the solvent was distilled off to obtained pale yellow solid residue (IVa). It was recrystallized from ethanol (70%). m.p.132^{0}C, yield 81%. **4a**. IR: NH 3350cm^{-1}, C=O 1715cm^{-1}, -N-N- 1280cm^{-1}, C-N 1160 cm^{-1}, C=C 1560cm^{-1}, CH_2 bend.1400cm^{-1}. ^{1}HNMR: 12H singlet 2.31δppm acetyl group, 1H doublet 6.33δppm β anomeric proton, 4H d-d 7.6-8.2δppm aromatic ring, glucosyl ring protons 4.1-5.4δppm, 2H 2.03-2.06δppm –NH- proton, 1H singlet 7.2δppm –NH- proton. Mass: (m/e) (M-1)$^+$ 551. ^{13}CNMR:-$CDCl_3$ 77.06-77.38δppm, $4CH_3$ 20.48-20.91δppm, glucosyl ring carbon $C_6^{'}$ 61.43δppm, $C_5^{'}$ 76.74δppm, $C_2^{'}$ 69.16δppm, $C_3^{'}$ 69.16δppm, $C_4^{'}$ 67.84δppm, β anomeric carbon $C_1^{'}$ 89.05δppm, C_2 and C_6 of aromatic ring 129δppm, C_3 and C_5 of aromatic ring 123δppm, C_4 of aromatic ring 151δppm, carbon from tetrazine ring 168.82δppm carbon from (C=N) 168.44-170.71δppm. (Found: C 47.63; H 4.32; N 14.86; Calc.for $C_{22}H_{26}N_6O_{11}$:

C 48.00; H 4.76; N 15.27). **4b**.m.p.103^{0}C, yield 76%. IR: NH 3345cm^{-1}, C=O 1720cm^{-1}, -N-N- 1280cm^{-1}, C-N 1150cm^{-1}, C=C 1560cm^{-1}, CH_2 bend.1400cm^{-1}. ^{1}HNMR: 12H singlet 2.31δppm acetyl group, 1H doublet 6.33δppm anomeric proton, 5H multiplet 8.2δppm aromatic ring, glucosyl ring proton 4.1-5.4δppm, 2H 2.03-2.06δppm –NH- proton, 1H singlet 7.2δppm -NH- proton. Mass: (m/e) (M-2)$^+$ 507. ^{13}CNMR: $CDCl_3$ 77.06-77.38δppm, $4CH_3$ group 20.48-20.91δppm, glucosyl ring carbon C_6' 61.43δppm, C_5' 76.74δppm, C_2' 69.16δppm, C_3' 69.16δppm, C_4' 67.84δppm, β anomeric carbón C_1' 89.05δppm, C_2 and C_6 of aromatic ring 128.55δppm, C_3 and C_5 of aromatic ring 128.00δppm, C_1 of aromatic ring 136.25δppm, carbon of CH_2 41.00δppm, carbon of tetrazine ring 168.82δppm, carbon from C=N 168.44-170.71δppm. (Found: C 54.83; H 4.92; N 13.38; Calc.for $C_{22}H_{27}N_5O_{11}$: C 55.27; H 5.38; N 13.85). m.p. 122^{0}C, yield 79%. **4c**. IR: NH 3350cm^{-1} , C=O 1720cm^{-1}, -N-N- 1270cm^{-1}, C-N 1160cm^{-1}, C=C 1555cm^{-1}, CH_2 bend.1400cm^{-1}. ^{1}HNMR: 12H singlet 2.31δppm acetyl group, 1H doublet 6.33δppm anomeric proton, 4H d-d 7.6-8.2δppm aromatic ring, glucosyl ring proton 4.1-5.4δppm, 2H 2.03-2.06δppm –NH- proton, 1H singlet 7.2δppm –NH- proton. Mass: (m/e)(M)$^+$ 521. ^{13}CNMR: $CDCl_3$ 77.06-77.38δppm, $4CH_3$ 20.48-20.91δppm, glucosyl ring carbon C_6' 61.43δppm, C_5' 76.74δppm, C_2' 69.16δppm, C_3' 69.16δppm, C_4' 67.84δppm, β anomeric carbon C_1' 89.05δppm, C_2 and C_6 of aromatic ring 129δppm, C_3 and C_5 of aromatic ring 123δppm, C_4 of aromatic ring 151δppm, carbon from tetrazine ring 168.82δppm carbon from C=N 168.44-170.71δppm. (Found: C 47.63; H 4.32; N 14.86; Calc.for $C_{22}H_{26}N_6O_{11}$: C 48.00; H 4.76; N.15.27).**4d**.m.p.126^{0}C, yield 77%. IR: NH 3350cm^{-1} , C=O 1725cm^{-1}, -N-N- 1280cm^{-1}, C-N 1150cm^{-1}, C=C 1560cm^{-1}, CH_2 bend.1400cm^{-1}. ^{1}HNMR: 12H singlet 2.31δppm acetyl group, 1H doublet 6.33δppm β anomeric proton, 4H d-d 7.6-8.2δppm aromatic ring, glucosyl ring proton 4.1-5.4δppm, 2H 2.03-2.06δppm –NH- proton, 1H singlet 7.2δppm –NH- proton. Mass: (m/e)(M-1)$^+$ 539. ^{13}CNMR: $CDCl_3$ 77.06-77.38δppm, $4CH_3$ 20.48-20.91δppm, glucosyl ring carbon C_6' 61.43δppm, C_5' 76.74δppm, C_2' 69.16δppm, C_3' 69.16δppm, C_4' 67.84δppm, β anomeric carbon C_1' 89.05δppm, C_2 and C_6 of aromatic ring 129δppm, C_3 and C_5 of aromatic ring 123δppm, C_4 of aromatic ring 151δppm, carbon from tetrazine ring 168.82δppm carbon from (C=N) 168.44-170.71δppm. (Found: C 47.63; H 4.32; N 14.86; Calc.for $C_{22}H_{26}N_6O_{11}$: C 48.00; H 4.76; N 15.27).**4e**.m.p.132^{0}C, yield 81%. IR: NH 3350cm^{-1} , C=O 1715cm^{-1}, -N-N- 1280cm^{-1}, C-N 1160 cm^{-1}, C=C 1560cm^{-1}, CH_2 bend.1400cm^{-1}. ^{1}HNMR: 12H singlet 2.31δppm acetyl group, 1H doublet 6.33δppm β anomeric proton, 4H d-d 7.6-8.2δppm aromatic ring, glucosyl ring proton 4.1-5.4δppm, 2H 2.03-2.06δppm –NH- proton, 1H singlet 7.2δppm –NH- proton. Mass: (m/e)(M)$^+$ 539. ^{13}CNMR: $CDCl_3$ 77.06-77.38δppm, $4CH_3$ 20.48-20.91δppm, glucosyl ring carbon C_6' 61.43δppm, C_5' 76.74δppm, C_2' 69.16δppm, C_3' 69.16δppm, C_4' 67.84δppm, β anomeric carbon C_1' 89.05δppm, C_2 and C_6 of aromatic ring 129δppm, C_3 and C_5 of aromatic ring 123δppm, C_4 of aromatic ring 151δppm, carbon from tetrazine ring 168.82δppm carbon from C=N 168.44-170.71δppm. (Found: C 47.63; H 4.32; N 14.86; Calc.for $C_{22}H_{26}N_6O_{11}$: C 48.00; H 4.76; N.15.27).**4f**.m.p.112^{0}C, yield 74%. IR: NH 3350cm^{-1} , C=O 1725cm^{-1}, -N-N- 1275cm^{-1}, C-N 1155cm^{-1}, C=C 1565cm^{-1}, CH_2 bend.1400cm^{-1}. ^{1}HNMR: 12H singlet 2.31δppm acetyl group, 1H doublet 6.33δppm β anomeric proton, 4H d-d 7.6-8.2δppm aromatic ring, glucosyl ring proton 4.1-5.4δppm, 2H 2.03-2.06δppm –NH- proton, 1H singlet 7.2δppm –NH- proton. Mass: (m/e)(M)$^+$ 521. ^{13}CNMR: $CDCl_3$ 77.06-77.38δppm, $4CH_3$ 20.48-20.91δppm, glucosyl ring carbon C_6' 61.43δppm, C_5' 76.74δppm, C_2' 69.16δppm, C_3' 69.16δppm, C_4' 67.84δppm, β anomeric carbon C_1' 89.05δppm, C_2 and C_6 of aromatic ring 129δppm, C_3 and C_5 of aromatic ring 123δppm, C_4 of aromatic ring 151δppm, carbon from tetrazine ring 168.82δppm carbon from C=N 168.44-170.71δppm. (Found: C 47.63; H 4.32;N 14.86; Calc.for $C_{22}H_{26}N_6O_{11}$: C 48.00; H 4.76; N.15.27).

2. Preparation of 3–β-D-glucopyransoylimino, 6(4-nitrophenyl) 1, 2, 4, 5-tetrazine (Va).

3-β-D-Glucopyranosylamino,6-(4-nitrophenyl)-1,2,4,5-tetrazine (Va) was prepared by stirring the 3-tetra-O-acetyl-β-D-glucopyranosylamino-6(4-nitrophenyl)-1,2,4,5-tetrazine (IVa) in methanolic ammonia for about 24hour.**5a**.m.p.136^{0}C, yield 68%. IR: υNH 3300cm^{-1}, υC-N 1150cm^{-1}, υC=C 1560cm^{-1}. ^{1}HNMR: 1H doublet 6.33δppm β anomeric proton, 4H d-d 8.2-8.3δppm aromatic ring proton, glucosyl ring proton 3.3-4.6δppm, 1H singlet 7.4δppm–NH. ^{13}CNMR: $CDCl_3$ 77.06-77.38δppm, glucosyl ring carbon C_6' 61.43δppm, C5' 76.74δppm, C_2' 69.16δppm, C_4' 67.84δppm, β anomeric carbon C_1' 89.05δppm, C_2 and C_6 of aromatic ring 129δppm, C_3 and C_5 of aromatic ring 123δppm, C_4 of aromatic ring 151δppm, carbon from tetrazine ring 168.82δppm. (Found: C 43.48; H 4.43; N 21.66; Cal.for $C_{14}H_{18}N_6O_7$ C 43.98; H 4.75; N 21.98).**5b**.m.p.153^{0}C, yield 75%. IR: υNH 3300cm^{-1}, υ-N-N-1200cm^{-1}, υC-N 1150cm^{-1}, υC=C 1560cm^{-1}, υCH_2 bend.1400cm^{-1}. ^{1}HNMR: 1H doublet 6.3δppm β anomeric proton, 5H multiplate 7.2-7.3δppm aromatic ring protons, glucosyl ring protons 3.3-4.46δppm, 1H singlet 7.4δppm NH. ^{13}CNMR: $CDCl_3$ 77.06-77.38δppm, glucosyl ring carbon C_6' 61.43δppm, C_5' 76.74δppm, C_2' 69.16δppm, C_3' 69.16δppm, C_4' 67.84δppm, β anomeric carbon C_1' 89.05δppm, C_2 and C_6 of aromatic ring 129.31δppm, C_3 and C_5 of aromatic ring 128.97δppm, C_4 of aromatic ring127δppm, carbon of CH_2 41.00δppm, carbon of tetrazine ring 168.82δppm. (Found: C 50.85; H 5.83; N 19.67; Cal.for $C_{14}H_{18}N_6O_7$: C 51.28; H 6.02; N 19.93). **5c**.m.p.161^{0}C. IR: υNH 3310cm^{-1}, υ-N-N- 1220cm^{-1}, υC-N 1150cm^{-1}, υC=C 1560cm^{-1}, υCH_2 bend.1400cm^{-1}. **5d**.m.p.168^{0}C. IR: υNH

3315cm^{-1}, υ-N-N- 1200cm^{-1}, υC-N 1155cm^{-1}, υC=C 1560cm^{-1}, υCH$_2$ bend.1400cm^{-1}. **5e**.m.p.157^{0}C. IR: υNH 3300cm^{-1}, υ-N-N- 1220cm^{-1}, υC-N 1140cm^{-1}, υC=C 1560cm^{-1}, υCH$_2$ bend.1400cm^{-1}.

RESULTS AND DISCUSSION

For the synthesis of compound (4a-4g) and (5a-5g) the scheme shown above were followed. The tetra-O-acetyl-β-D-glucopyranosylimino isocyanodichloride was prepared by the excess chlorination of tetra-O-acetyl-β-D-glucopyranosyl isothiocynate. The tetra-O-acetyl-β-D-glucopyranosyl isothiocynate was prepared by the known procedure. The tetra-O-acetyl-β-D-glucopyranosylimino isocyanodichloride gave negative lead plumbite test indicating the absence of C=S bond. It was charred when treated with conc.H_2SO_4 indicating the presence of glucosyl moiety. The PMR spectrum of the compound clearly indicated the signals for the acetyl protons, other glucosyl proton and doublet of β-glucopyranosyl proton. In the typical synthesis of (IVa) was synthesized by refluxing tetra-O-acetyl-β-D-glucopyranosylimino isocyanodichloride with 4-nitrophenyl dihydraformazan in 1:1 proportion for 4 hour in chloroform medium. After completion of reaction, the crude product was recrystallized from 70% of ethanol. It was charred when treated with conc. H_2SO_4 indicating the presence of glucosyl moiety. In IR, it has higher C=O stretching frequency (typically 1740) than comparable C=O (typically 1710) because of the inductive effect of ester. The IR spectra do not show the band for stretching frequency of the hydroxyl group confirming the formation of acylated glucosides. The IR spectrum displayed the band due to υNH, υC=O and υC=C groups. The PMR spectrum of the compound indicated the signals of acetyl proton, NH protons, aromatic protons and pyranosyl protons as well as doublet due to β-glucopyranosyl proton. The mass spectrum of the compound showed presence of $(M-1)^+$ peak at m/e 551. Other important fragment peaks were located at 523, 413, 353, 171 etc. the elemental analysis of the product indicated the molecular formula $C_{22}H_{26}N_6O_{11}$.

On the basis of all the above facts the compound (IVa) was assigned structure as 3-tetra-O-acetyl–β-D-glucopyransoylimino-6(4-nitrophenyl)-1,2,4,5-tetrazine. Similarly all other compounds were prepared by extending the reaction of tetra-O-acetyl-β-D-glucopyranosylimino isocyanodichloride with other aryl dihydroformazans (IIIb-IIIg).

The compound (Va) was prepared by the deacetylation of compound (IVa) via methanolic ammonia. The product was charred on treatment with conc. H_2SO_4 indicating the presence of glucosyl ring. The IR spectrum indicated the broad band of –OH group at 3300cm^{-1}.and the signals of carbonyl C=O band at 1740cm^{-1} were disappeared. Also in PMR spectra of the product the signal of acetyl protons (2.31) were absent. The PMR of the product showed the double doublet at 5.36ppm indicating the presence of β glucosyl ring. All the above facts favored the complete deacetylation of product (IV). The elemental analysis of product indicated the molecular formula as $C_{14}H_{18}N_6O_7$.

On the basis of all above fact the compound (Va) was assigned the structured of 3–β-D-glucopyransoylimino, 6(4-nitrophenyl) 1,2,4,5-tetrazine. Similarly all the other compounds were prepared (Vb-Vg) by the deacetylation of (IVb-IVg) respectively.

CONCLUSION

Here we explored the route for the synthesis of asymmetrical and symmetrical tetrazine molecules by using glucosylimino isocynodichloride as one of the reagent for the synthesis of heterocyclic system.

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