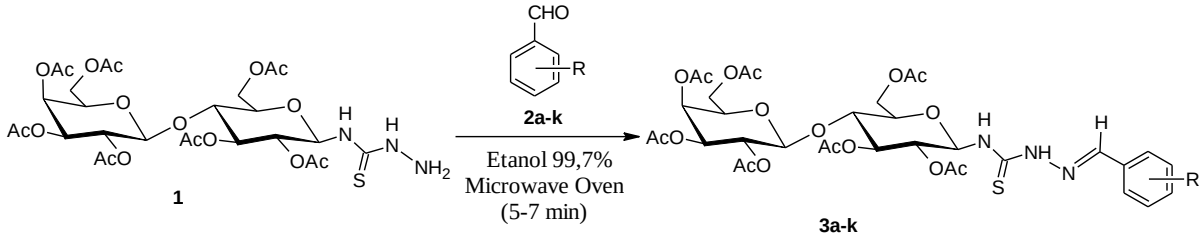
SYNTHESIS OF SOME (HEPTA-O-ACETYL-(-LACTOSYL)THIOSEMICARBAZONES CONTAINING BENZENE RING

Nguyen Dinh Thanh[*], *Hoang Thi Kim Van, Truong Thi Thu*
Faculty of Chemistry, College of Science, Hanoi National University, 19 Le Thanh Tong, Hanoi (Vietnam)

Abstract. A series of substituted benzaldehyde hepta-*O*-acetyl-β-lactosyl thiosemicarbazones were synthesized by condensation reactions of hepta-O-acetyl-β-lactosyl thiosemicarbazide with corresponding substituted benzaldehydes. Structures of thiosemicarbazones were confirmed by spectroscopic (IR, NMR) methods. The ^{1}H and ^{13}C NMR spectra of substituted acetophenone peracetylated β-lactosyl thiosemicarbazones have been recorded and discussed. The magnetic resonance signals in their NMR spectra show the relationships between the structure and positions of the substituted groups by Hammett's regression. The β anomeric configuration of these thiosemicarbazones was confirmed on the basis of the coupling constant J = 9.5–8.5 Hz between proton NH-4 of thiosemicarbazone bond and proton H-1' in lactosyl component.

Thiosemicarbazones are a class of small molecules that have been evaluated over the last 50 years as antivirals and as anticancer therapeutics, as well as for their parasiticidal action against *Plasmodium falciparum* and *Trypanasoma cruzi* which are the causative agents of malaria and Chagas's disease, respectively. The chemistry of thiosemicarbazide derivatives of saccharides is interested [1]. These compounds arouse interest as versatile intermediates for preparing various (e.g., heterocyclic) derivatives as well. Thiosemicarbazones can be used for making electrodes [2], or complexes formation of metallic ions [3]. Thiosemicarbazones exhibit various biological activities such as antituberculosis, antimicrobial, anti-inflammatory, antiviral, anticonvulsant [4].

Continuing the previous papers [5], we now report here for the first time the synthesis and characterization of hepta-*O*-acetyl-β-lactosyl thiosemicarbazide from peracetylated lactosyl isothiocyanate and its reaction with a series of aromatic aldehydes as shown in Scheme 1.



2a-k, 3a-k: R = 4-NO_2 (**a**); 4-F (**b**); 4-Cl (**c**); 2-Cl (**d**); 4-Br (**e**); 4-Me (**f**); 4-OMe (**g**); 3-OMe (**h**); 4-OH (**i**); 3-OMe-4-OH (**j**); 4-NMe_2 (**k**)

Scheme 1.

Hepta-*O*-acetyl-β-lactosyl thiosemicarbazide (**1**) was synthesized by condensation of hepta-*O*-acetyl-β-lactosyl isothiocyanate and hydrazine hydrate in absolute dioxane at 15-

[*] Corresponding author, email: nguyendinhthanh@hus.edu.vn

20°C. The IR spectrum shows the characteristic stretching vibrations for the NH and NH_2 groups in the thiosemicarbazide group (–NH–C(=S)–NH–NH_2) at 3337, 3290 and 3202 cm^{-1}, for the ester at 1746, 1234 and 1044 cm^{-1}, and for the C=S bond at 1370 cm^{-1}. The ^{1}H-NMR spectrum of hepta-*O*-acetyl-β-lactosyl thiosemicarbazide contains chemical shifts characteristic of the peracetated-β-lactosyl thiosemicarbazide. For example, the chemical shifts of the NH and NH_2 groups are observed at 9.23 ppm (NH), 8.12 ppm (NH), and4.58 ppm (N–H_c), while those of the disaccharide component are observed at 5.78-3.91 ppm. The anomeric configuration of the pyranose rings in this thiosemicarbazide is clearly established by ^{1}H-NMR spectroscopy. The β anomeric D-glucose moiety shows a characteristic signal for H-1 ($J_{1,2}$=10.0 Hz), which is consistent with the 1,2-trans relationships between protons. The α anomeric D-glucose moiety shows a characteristic signal for H-1' ($J_{1',2'}$=4.0 Hz), which is consistent with the 1,2-cis relationships between protons. The ^{13}C-NMR spectrum shows resonance signals at 95.34-61.43 ppm for the lactosyl carbons, 170.16-169.21 ppm for the seven C=O groups in the esters, 20.63-20.27 ppm for the methyl carbons of acetyl groups and 182.11 ppm for the C=S group.

Table 1. Substituted benzaldehyde (hepta-*O*-acetyl-β-lactosyl)thiosemicarbazones 3a-k

Entry	R	mp, °C	Yield, %	IR spectra (cm^{-1})				
				ν_{NH}	$\nu_{CH=N}$	$\nu_{C=O\ ester}$	$\nu_{COC\ ester}$	$\nu_{C=S}$
3a	4-NO_2	172-173	87	3325	1580	1746	1231,1056	1376
3b	4-F	159-160	70	3320	1601	1752	1238,1048	1376
3c	4-Cl	189-190	76	3279, 3215	1605	1751	1222,1046	1371
3d	2-Cl	187-188	78	3309	1615	1750	1216,1052	1375
3e	4-Br	155-156	78	3334	1605	1749	1231,1064	1367
3f	4-Me	177-178	78	3212	1615	1748	1242,1046	1373
3g	4-OMe	198-199	76	3348, 3284	1604	1737	1252, 1228,1044	1370
3h	3-OMe	196-197	73	3344, 3159	1605	1747	1241,1042	1376
3i	4-OH	161-162	78	3548*, 3340, 3275	1609		1238,1042	1372
3j	3-OMe-4-OH	176-177	76	3480*, 3337	1603	1749	1224,1051	1377
3k	4-NMe_2	187-188	77	3320	1604	1753	1252, 1220,1054	1372

Note: *) also ν_{OH}

The new substituted benzaldehyde (hepta-*O*-acetyl-β-lactosyl)thiosemicarbazones (**3a-k**) were obtained by condensation of hepta-*O*-acetyl-β-lactosyl thiosemicarbazide (**1**) with the corresponding substituted benzaldehydes (**2a-k**) in 70-87% yields (Table 1). Reactions are performed in absolute ethanol in the presence of glacial acetic acid as a catalyst by microwave-assisted method. The IR spectra show characteristic absorptions in the range of 3540–3480 cm^{-1} (ν_{OH}) and 3348–3159 cm^{-1} (ν_{NH}), 1737–1753 ($\nu_{C=O}$ ester), 1216–1252 and

1044–1056 cm^{-1} (ν_{COC} ester), 1367–1377 cm^{-1} ($\nu_{C=S}$), and 1580–1615 cm^{-1} ($\nu_{CH=N}$). The ^{1}H-NMR spectrum of hepta-*O*-acetyl-β-lactosyl thiosemicarbazones (**3a-k**) shows chemical shifts at δ 11.67–12.16 ppm (NH-1) in singlet, δ 8.41–8.88 ppm (NH-3) in doublet with coupling constants *J*=9.0–9.5 Hz, δ 7.97–8.18 ppm (CH=N) in singlet. The aromatic protons have signals at δ 6.72–8.25 ppm with the coupling constants *J* which appropriate to substituted benzene ring (Table 2). The protons in lactose moiety have chemical shifts in region δ 4.00–6.00 ppm. The β anomeric configuration of **3a-k** is confirmed on the basis of the coupling constant $J_{1,2}$=9.0 Hz, which is consistent with a 1,2-trans relationships between protons (Table 3). The ^{13}C-NMR spectrum of compound **3a-k** shows signals at δ 179.1–177.3 ppm for the carbon atom in the C=S group, δ 170.7–169.0 ppm for the carbon atoms in the C=O bond of the acetyl groups, δ 111.0–150.0 ppm for the carbon atoms in benzene ring and 20.7-20.1 ppm for the methyl carbons in the acetyl groups; the carbon atom in imine group CH=N show signals at δ 140.2–147.8 ppm (Table 4).

The ^{1}H NMR spectral data of hepta-*O*-acetyl-β-lactosyl thiosemicarbazones **1a-j** were summarized in Table 1. Proton in disaccharide component had resonance signals in region at δ 5.94–4.19 ppm. Especially, proton H-1" in second glucopyranose ring of β-lactosyl moiety resonanced in region at δ 4.88–4.77 ppm (in doublet) with coupling constant *J* = 8.0–7.0 Hz which had confirmed the β-anomeric configuration of 1,4-glycoside link. Chemical shifts of aromatic protons were in region at δ 8.49–6.77 ppm, with the coupling constants that are according to the types of substitution on benzen ring (Table 2).

Table 2. Summary of ^{1}H NMR spectral data of compounds **3**

Proton	δ (ppm)	Multiplicity	*J* (Hz)
NH-2	11.74–10.69	s	-
NH-4	8.72–8.44	d	9.5–8.5
H-1'	5.85–5.80	t	9.25–9.0
H-2'	5.21–5.13	t, dd or m	3.5–3.0,10.0–9.25
H-3'	5.33–5.20	t	9.25–9.0
H-4'	3.85–3.80	t or m	9.5–9.25
H-5'	3.92–3.86	m or ddd	1.5,6.0,8.0
H-6'a	4.31–4.29	d	11.0–10.5
H-6'b	4.07–4.06	dd or t	6.0–5.5,12.25–12.0
H-1"	4.80–4.77	d	8.0–7.0
H-2"	4.88–4.87	dd or t	8.0–3.0,10.5–10.0
H-3"	5.17–4.92	dd, t or m	3.5–3.0, 10.25–7.0
H-4"	5.24–5.239	d	3.5–3.0
H-5"	4.25–4.24	t	6.75–6.5
H-6"a	4.05–4.01	m	-
H-6"b	4.05–4.01	m	-
H-2'"	8.49–7.42	d or m	9.0–8.5 or 2.0–1.5
H-3'"	8.22–6.77	d or m	8.5–8.25 or 4.0
H-4'"	7.40–7.07	d, dd or m	4.0–1.5, 8.25–8.0
H-5'"	8.22–6.78	d or m	9.0–8.0
H-6'"	8.18–7.42	s, d, dd, or m	2.5–2.0, 9.0–8.25
MeCO	2.11–1.90	s	-
***CH*$_3$C=N**	2.56–2.29	s	-

The chemical shift were downfield at δ 11.74–10.69 ppm (in singlet) that belongs to NH-

2 proton due to anisotropic effect of imine bond, while the one was upfield at δ 8.72–8.44 ppm (doublet) that's resonance magnetic signal of NH-4. This proton coupled with disaccharide proton H-1' in glucopyranose moiety of lactosyl component with the coupling constant *J*=9.5–8.5 Hz (Table 1 and Fig. 1). The value of this coupling constant was consistent with a 1,2-trans relationships between these protons and have confirmed β-anomeric configuration of hepta-*O*-acetyl-β-lactosyl thiosemicarbazones **1** and **2** [4]. The positions of resonance signals of protons NH-2 and NH-4 in acetophenone series had linear regression expressions as follows, respectively:

$$\delta_{NH\text{-}2} = 0.245\sigma + 10.794 \ (R^2 = 0.95), \ \delta_{NH\text{-}4} = 0.213\sigma + 8.522 \ (R^2 = 0.99)$$

The reaction parameters was relative large, ρ = 0.245 and 0.213 (for NH-2 and NH-4, respectively) which indicate that the donating or withdrawing capacity of the substituents has noticeable effects to these resonance positions (Table 3, Fig. 2).

Conversely, carbon atom in imine group were affected clearly by these substituents with opposite trend: the donating ones (with σ < 0, such as 4-OH, 4-OMe, 4-Me on benzene ring of acetophenones) caused signal to be shifted to upfield region, and the withdrawing ones (with σ < 0, such as 3-NO_2-4-Cl, 4-NO_2, 3-NO_2-4-Me, 3-NO_2-4-OMe, 4-Cl, 4-Br in acetophenone series) caused the resonance of this carbon atom to be in downfield region (Table 3). These tendencies could be shown in equations as follows:

$$\delta_{>C=N} = -3.002\sigma + 149.340 \ (R^2 = 0.93)$$

the parameter ρ < 0 mean that the opposite influence of the substituents to resonance position in this case. The large values of reaction parameters ρ indicated that the electron nature of substituents in benzene ring governed remarkably the resonance positions of carbon atoms in C=N bond of these thiosemicarbazones (Table 3, Fig. 3) [3].

The ^{13}C-NMR spectra of compound **1** and **2** showed the main four-parted regions at δ 179.7–169.1 ppm, 159.7–111.3 ppm, 82.1–61.5 ppm and 21.7–20.2 ppm. Signals at δ 179.1–177.8 ppm belonged to the carbon atom in the C=S group. The acetyl groups in these compounds showed resonance at δ 171.7–169.1 ppm for carbonyl carbon atom and 21.7–20.2 ppm for the methyl carbon one. The presence of imine in molecule were confirmed by signal at δ 140.2–147.8 ppm, belong to carbon atom in imine group (Table 3) [4].

Table 3. Summary of ^{13}C NMR spectral data of compounds **1** and **2**

Carbon	δ (ppm)	Carbon	δ (ppm)
C=S	182.1–179.1	**C-1'**	81.3–80.8
***C*OCH$_3$**	170.2–169.0	**C-2'**	71.2–70.7
CH=N	150.2–146.4	**C-3'**	76.3–76.0
C-1'"	143.5–116.9	**C-4'**	73.7–73.4
C-2'"	159.3–121.9	**C-5'**	72.7–72.4
C-3'"	139.7–113.7	**C-6'**	62.0–60.9
C-4'"	160.6–123.1	**C-1"**	99.9–99.6
C-5'"	131.4–110.0	**C-2"**	69.0–68.9
C-6'"	137.9–126.6	**C-3"**	70.0–69.7
		C-4"	67.3–67.1
		C-5"	70.6–70.3
		C-6"	62.5–62.3
		COC*H*$_3$	14.5–14.2

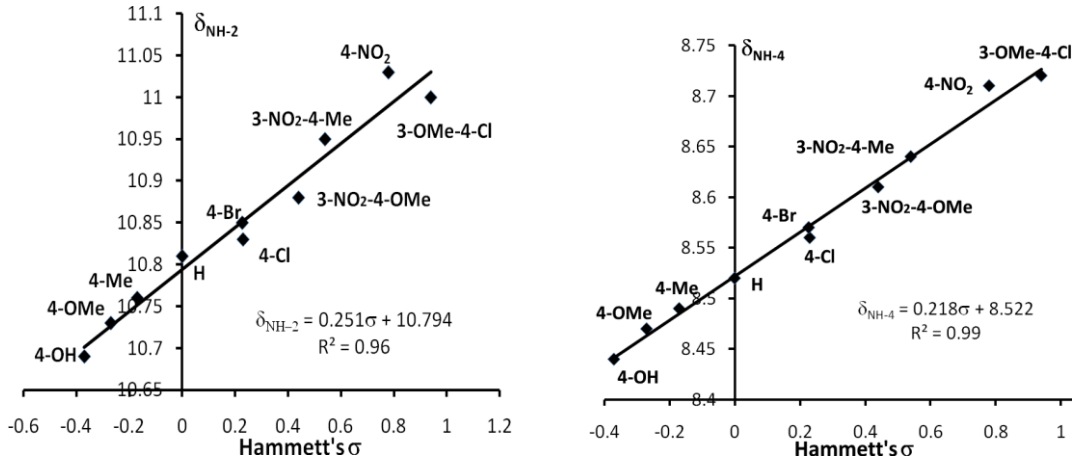


Figure 1. Linear relationships between δ_{NH-2}, δ_{NH-4} and Hammett's σ in compouds **1.**

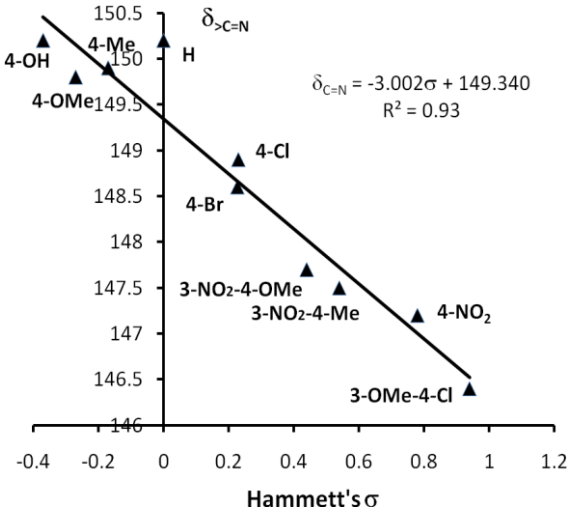


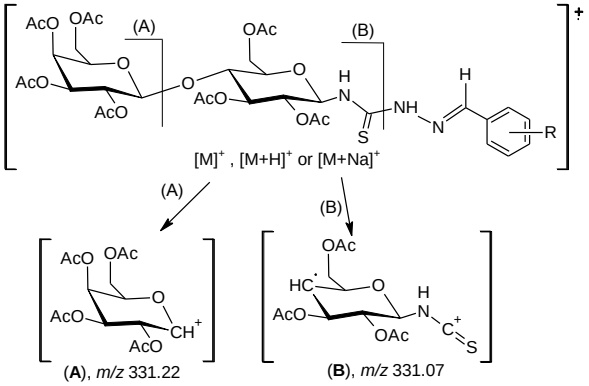
Figure 2. Relationships between $\delta_{>C=N}$ and Hammett's σ in compouds **1.**

Table 4. Hammett's relationships between chemical shifts and substituent's constans σ of compounds **1** and **2**

Entry	R	σ	δ_{NH-1}	δ_{NH-3}	$\delta_{>C=N}$
1a	3-NO_2-4-Cl	0.94	11.00	8.72	146.4
1b	4-NO_2	0.78	11.03	8.71	147.2
1c	3-NO_2-4-Me	0.54	10.95	8.64	147.5
1d	3-NO_2-4-OMe	0.44	10.88	8.61	147.7
1e	4-Cl	0.23	10.83	8.56	148.9
1f	4-Br	0.227	10.85	8.57	148.6
1g	H	0.00	10.81	8.52	150.2
1h	4-Me	-0.27	10.76	8.49	149.9
1i	4-OMe	-0.27	10.73	8.47	149.8
1j	4-OH	-0.37	10.69	8.44	150.2

The data of mass spectra of substituted benzaldehyde (hepta-*O*-acetyl-β-maltosyl)thiosemicarbazones were represented in Table 5. The calculated monoisotopic mass of the fragments which could be observed in mass spectra of these thiosemicarbazones were also listed in Table 5.

As shown in Table 5, the molecular ion peak were observed clearly in mass spectra of these thiosemicarbazones, due to the ionization method herein was electronic spray one. Fragment ion $[M+H]^+$ (all of the cases) or $[M+Na]^+$ (in case of **1m**, R=3-OMe-4-OH) or $[M–H]^+$ (incase of **1a**, R=NO_2) was usually highest intensive (100%) and was basic peak. The cleavage of β-1,4-glycoside toward galactopyranose moiety in lactosyl component were encountered in the ESI-mass spectra in this thiosemicarbazone series. The presence of fragment (**A**) with *m/z* 331.18–331.59 confirmed this tendency of fragmentation. Other fragment ion (**B**) with *m/z* 331.18–331.59 also formed by the cleavage β-1,4-glycoside toward glucopyranose moiety in lactosyl component and the cleavage which took place in thiosemicarbazide bond [3-5]. The tendency of these fragmentations was general for both monosaccharides (for example, in the case of thioureas [6,7]) and disaccharides (in these thiosemicarbazones). In the cases of halogen substituents, such as chlorine and bromine, the mass spectra showed clearly isotopic peaks M^+ and $[M+2]^+$ (Fig.1).



Another fragmentation was the cleavage that occurred with formation the fragments *m/*z 754.21+H+R (i.e., $M^+–CH_2=C=O+H$) with the mass number changed from 801.07 amu to 770.26 amu. These fragmentations took place with the elimination of ketene molecule from peaks $[M+H]^+$ and formed fragment ion (**C**). In the case of compound **1c** (R=4-F), it took place the cleavage of $CH_3COO^{\bullet}$ radical, and therefore, lead to form the fragment ion (**C***) with *m/z* 737.21+R; in this case, the value of mass number was 756.21amu. The formation of these fragment ions with, *m/*z 754.21+H+R, was the evidence which confirmed the structure of these compounds. We realized that the calculated values and the actual ones were rather consistent each other and these values depended on the substituent types (Table 1).

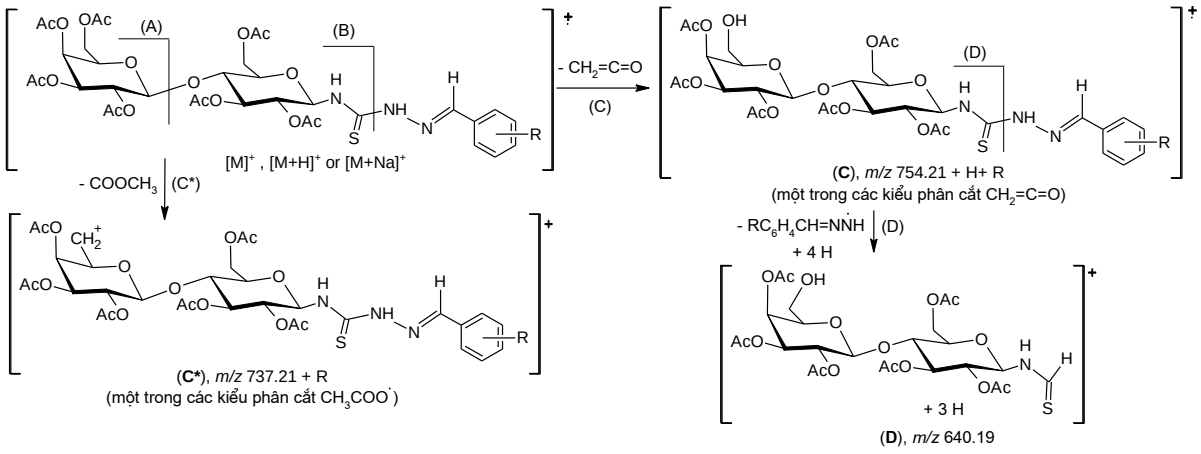


Table 5. Mass spectral data of benzaldehyde (hepta-*O*-acetyl-β-maltosyl)thiosemicarbazones

Entry	R	$[M]^+$	$[M+H]^+$ or $[M+Na]^+$ (* M–H)	Fragment (A) *m/z* 331.27	Fragment (B) *m/z* 331.07	Fragment (C) ($M–CH_2=C=O+H$)	Fragment (D)
1a	4-NO_2	842.22	840.91* *841.20*	-	-	-	-
1b	3-NO_2	842.22	843.22 *843.01*	*331.23*	*331.23*	801.21 *801.07*	*640.31*
1c	4-F	815.22	816.23 *816.07*	*331.18*	*331.18*	756.21 *756.21* (–$CH_3COO^•$)	*640.29*
1d	4-Cl	831.19/ 833.19	832.20/834.20 *832.131/834.09*	*331.30*	*331.30*	790.19 *790.18*	*640.38*
1e	2-Cl	831.19/ 833.19	832.20/834.20 *832.21/834.09*	*331.28*	*331.28*	790.19 *790.17*	*640.38*
1f	4-Br	875.14/ 877.14	876.15/878.15 *876.41/877.98*	*331.59*	*331.59*	-	*640.43*
1g	H	797.23	798.24 *798.39*	*331.48*	*331.48*	738.74 *738.50* (–$CH_3COO^•$)	*619.28*
1h	4-Me	811.25	812.25 *812.25*	*331.34*	*331.34*	770.24 *770.31*	*640.45*
1i	4-OCH_3	827.24	828.25 *828.23*	*331.27*	*331.27*	786.24 *786.26*	*640.38*
1k	3-OCH_3	827.24	828.25 *828.27*	*331.37*	*331.37*	786.24 *786.33*	*656.21*
1l	4-OH	813.23	814.23 *814.22*	*331.45*	*331.45*	772.22 *772.33*	*640.45*
1m	3-OMe-4-OH	843.24 *843.79*	866.23 *865.88*	*331.02*	*331.02*	-	-
1n	4-NMe_2	840.27	841.28 *841.27*	*331.30*	*331.30*	799.27 *799.26*	*640.18*

Note: The *m/z* values in italic were ones that observed in mass spectra.

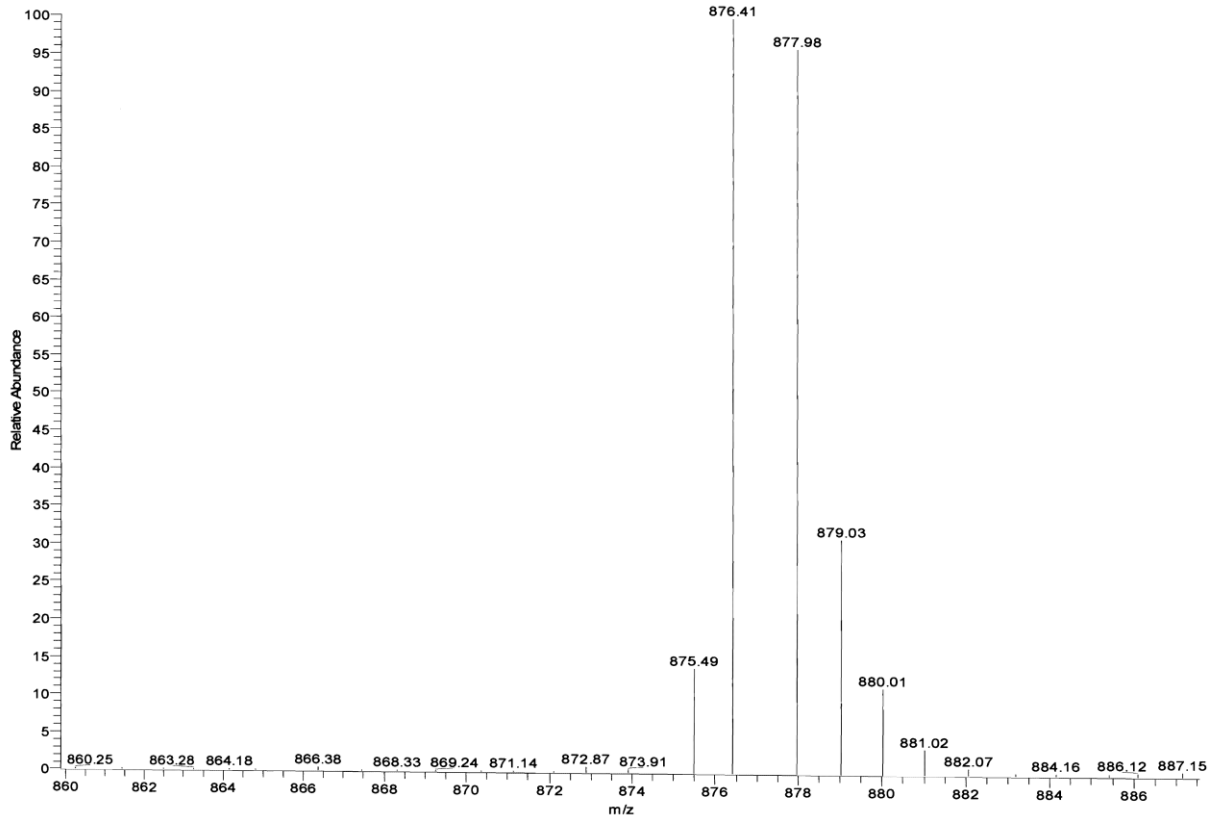
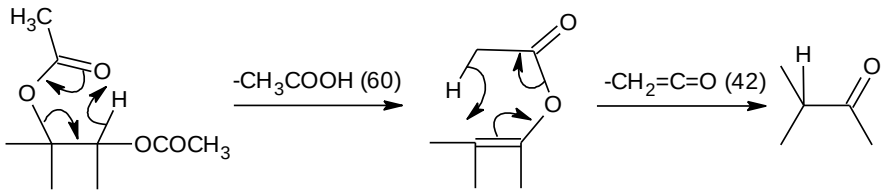


Figure 3. Mass spectrum of coumpound **1f**, R=4-Br.

The above cleavage in mass spectra of per-*O*-acetyl-β-lactosylthiousemicabazones had some features that are similar with ones of typical hexopyranose pentaacetates 3-7]. The only observable peaks in the higher mass range were due to loss of the substituents and fall, therefore, at position of fragment ions such as $[M\text{-}AcOH]^+$, $[M\text{-}AcOH,\text{-}AcO]^+$ and $[M\text{-}2AcOH,\text{-}AcO]^+$, and some peaks such as $[M\text{-}2AcOH,\text{-}C_2H_2O]^+$, $[M\text{-}3AcOH,\text{-}AcO]^+$ with intensity of 1-3% could be specified for glycosides having amino structure. A very important mode of fragmentation of these acetated compounds was the loss of acetic acid (*m/z* 60), a process well known for most esters of acetic acid, and the loss of ketene (*m/z* 42). This later process seemed to be greatly facilitated if preceded by loss of acetic acid; the resulting double bond seemed to play a significant role in the elimination of ketene, for which it could be suggest the mechanics as following [3,4]:



The cleavage of radical $RC_6H_4CH{=}NNH^{\bullet}$ from fragment ion (**C**) lead to form fragment ion (**D**) with *m/z* 640.19 (calculated value); the actual values on the mass spectra were *m/z* 640.18–640.45. Perhaps, this fragmentation direction also was general for benzaldehyde per-*O*-acetyl-β-lactosyl thiosemicarbazones.

3. Experimental

Melting points were determined on a STUART SMP3 apparatus (BIBBY STERILIN-UK). The FTIR-spectra were recorded on a Magna 760 FT-IR Spectrometer (NICOLET, USA) in KBr pellets. The ^{1}H-NMR (500.13 MHz) ,^{13}C-NMR (125.77 MHz) spectra were recorded on an AVANCE AV500 Spectrometer (BRUKER, Germany) in DMSO-d_6 solution in ppm compared to TMS as internal reference at 300K.

General synthetic method of substituted benzaldehyde (hepta-O-acetyl-β-lactosyl)thiosemicarbazones (3a-k). A mixture of hepta-*O*-acetyl-β-maltosyl thiosemicarbazide **1** (1 mmol), benzaldehyde **2** (1 mmol), glacial acetic acid (0.5 ml) in absolute ethanol (in the presence of glacial acetic acid as catalyst) or glacial acetic acid (20 ml) was heated at reflux using domestic microwave oven TIFANY 750W in 5-7 min. The solvent was evaporated to one half the original volumes. The resulting colorless crystals were filtered by suction. The crude product when recrystallized from 96% ethanol to afford the title compounds **3**.

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