The Synthesis of *N*-Glycosyl-*N*′-pyrazolylmethylene Aminothioureas under a 4Å Molecular Sieve

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A series of novel *N*-glycosyl-*N*′-pyrazolylmethylene aminothioureas (**4a**-**4e**, **5a**-**5e**) were synthesized from *N*-glycosyl-*N*′-aminothioureas (**2a**-**2d**) and 4-formylpyrazole (**3a**-**3e**). Activated 4Å molecular sieves were adopted for dehydrated reagent to improve the reaction rate and yield. The structures of the new compounds were identified on the basis of IR, ^{1}H NMR and MS spectra. Simultaneously, the compounds were detected by fluorescence spectrophotometer and had preferable fluorescence activity, so they can be selected as a kind of novel fluorescence labeled derivative of sugar.

Keywords: Glycosylisothiocyanate; Thiourea; 4-Formylpyrazole; 4Å Molecular sieve; Fluorescence characteristic.

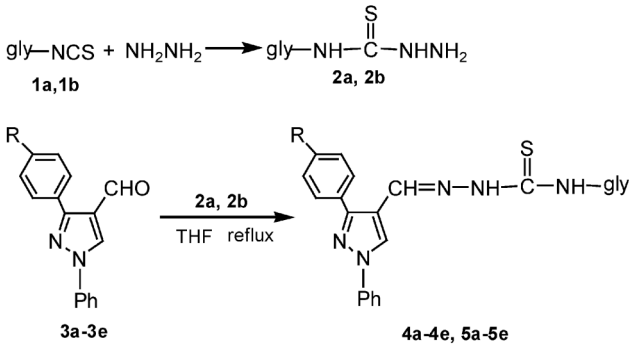
INTRODUCTION

Saccharide compounds are the main source of all organisms which maintain energy that life activities require. In fact, as the endogenesis substance of life, glucide compounds participate almost in all of the biological courses of organisms.[1] In the field of carbohydrates, glycosyl isothiocyanates are versatile synthetic intermediates that have been widely used in the synthesis of carbohydrate derivatives, mostly having thiourea structure, of synthetic, biological and pharmaceutical interest.[2] The thioureas and their derivatives have antifungal and antibacterial activity and can also modulate the plant growth.[3,4] Their broad biological activities have attracted a considerable amount of attention in recent years.[5,6] Pyrazole derivatives are a significant member in the heterocyclic family for their importance in the production of medicine and agricultural chemicals.[7] Furthermore, a number of pyrazole derivatives have been reported to exhibit strong physiological and pharmacologic activities, such as antiviral, antipyretie, analgestic, and anti-inflammatory activities.[8-11] We designed and synthesized several new thiourea derivatives by incorporating glycosyl moieties with pyrazole fragments. These derivatives might have biologic activities.

Additionally, nowadays, the research into saccharides is one of the most popular fields in chemistry and biology. As a result, as far as saccharide compounds are concerned, the means of analyzing and determining the structure and quality contained in the substance have become more and more important. Among all of the analytic methods of glycosyl compunds, the fluorescence derivative method is the primary means for its excellent sensitivity, outstanding selectivity and small quantity of sample used and so on.[12] Thus, if glucide compounds are made into appropriate fluorescence-labeled derivatives, it will be a very significant development in analyzing and determining glycosyl compounds.

In this study, we characterized the synthesis and fluorescence characteristics of new glycosyl thioureas and catalysis of a 4Å molecular sieve in the reaction. Corresponding glycosyl aminothioureas were prepared from anhydrous hydrazine and glycosyl isothiocyanate, then the title compounds were synthesized in terms of the following synthesis route (Scheme I).

Scheme I



1a, **2a**, **4a-4e**: gly=2,3,4-tri-*O*-acetyl-β-*D*-xylopyranosyl;

1b, **2b**, **5a-5e**: gly=2,3,4-tri-*O*-acetyl-α-*L*-arabinopyranosyl;

3a-3e, **4a-4e**, **5a-5e**: **a**: R=H; **b**: R=OCH_3; **c**: R=Cl; **d**: R=Br; **e**: R=NO_2

RESULTS AND DISCUSSION

In the process of the synthesis of compounds **3a**-**3e**, it was extremely difficult to directly form 4-formylpyrazole by the formylation of pyrazole, but hydrazone reacted with Vilsmeier-Haak reagent very easily to produce 4-formylpyrazole in the ratio of 1 to 2. Furthermore, the virtue of this reaction was mild conditions and high yield.

A glycosyl ring is easily opened in acidic conditions, therefore the best condition of the reaction is approximately neutral or weakly alkalescent. We selected sugar isothiocyanates (**1a**-**1d**) of high reaction activity and anhydrous hydrazine and let them react in mild conditions. *N*-glycosyl-*N*′-aminothioureas (**2a**-**2d**) were obtained, then reacted with 4-formylpyrazole (**3a**-**3e**) to give a series of novel *N*-glycosyl-*N*′-(1-phenyl-3-arylpyrazole-4-yl)methylene aminothioureas (**4a**-**4e**, **5a**-**5e**).

Regarding the synthesis of glycosylaminothiourea, we took anhydrous hydrazine to react with isothiocyanates in order to avoid the hydrolyzation of isothiocyanates. Because isothiocyanates also reacted with ethanol, the temperature of the reaction was controlled below 5 °C to prevent a side reaction.

In this paper, we attempted to use activated a 4Å molecular sieve which resulted in shorter reaction time (2-3 h) and higher yield (75%-85%). A 4Å molecular sieve must be triturated, and then activated under 500 °C before use. We show the action of an 4Å molecular sieve in the reaction of **4a** (Table 1).

From the table, we could conclude the optimum conditions of the reaction were using a 1.5 g activated 4Å molecular sieve, and in this way, the title compounds could be synthesized within only two hours to achieve high yield.

In the ^{1}H NMR spectra of compounds **2a**-**2b**, **4a**-**4e**, **5a**-**5e**, a few single peaks appearing at about δ 2.10 were attributed to hydrogen atoms of acetyl in a sugar ring; however, multiple peaks appearing at δ 3.40~5.50 were attributed to hydrogen atoms of the sugar ring. In addition, the signals of the sugar ring C_1-H displayed at about δ 5.60 and revealed a triplet-peak for coupling with both C_2-H and N-H and their coupling constants in the range of 8.8~9.6 Hz. To *D*-xylosyl thiourea derivatives and *L*-arabinosyl thiourea derivatives, the configuration of their anomeric carbon was identical. The proton (C_1-H) shifted toward downfield in contrast with other protons in the sugar ring. The reason was due to the deshielding effect of the oxygen of the hemiacetal. In the compounds of **4a**-**4e** and **5a**-**5e**, there were doublet-peaks of N-H at about δ 8.20~8.40; however, owing to the deshielding effect of the larger conjugated system formed by vicinal methyleneamino and cycle-pyrazole, the proton of N′-H shifted toward a relatively downfield position of δ 9.10~9.30. The proton of NH-CS-NH shifted toward upfield in comparison with compounds **2a**-**2b**.

Table 1. The effect of molecular sieve amount on yield of product and reaction time

Molecular sieve amount/g	Method 1		Method 2	
	time/h	yield/%	time/h	yield/%
0.5	4	65	3	75
1.0	4	65	3	82
1.5	4	65	2	85
2.0	4	65	2	83

The IR spectra of compounds **4a**-**4e** and **5a**-**5e** exhibited broad bands at 3400-3200 cm^{-1}, assigned to their NH. The strong bands appearing at 1400-1350 cm^{-1} were attributed to character absorption of NH-CS-NH. The signals at 1745 cm^{-1} were identified as CO of acetyl in the sugar ring.

The LC-MS (ESI) displayed that all the compounds had quasi-molecular ion peaks and they appeared according to the base peak, which revealed these target compounds were comparatively stable. Simultaneously, glycosyl fragment peaks and an isotopic peak cluster were exhibited in the MS.

Solid-state fluorescence spectra of compounds **4a**-**4e** and **5a**-**5e** were determined with solid powder on a quartz round plate with excitation and emission slits of 2.5/2.5 nm by spectrophotometry. The optimum excitation wavelength of compounds **4a**-**4e** and **5a**-**5e** was 350 nm. The fluorescence spectra of compounds **4** and **5** excited at optimum excitation wavelength of each compound respectively; at the same time, the emission wavelengths of compounds **4** and **5** were measured by the optimum excitation wavelength. The emission wavelengths of compounds **4a**-**4e** were 406, 453, 412, 404, 526 nm; meanwhile, the emission wavelengths of **5a**-**5e** were 468, 423, 437, 403, 529 nm. As shown in Fig. 1 and Fig. 2, the fluorescence intensity of compounds **4e** and **5e** was comparatively weak. The reason was mainly due to the nitro group in these compounds, which was a strong electron-withdrawing group and could make fluorescence highly quenched. Compounds **4a**-**4d** and **5a**-**5d** displayed

relatively good fluorescence activity. Furthermore, we could find that the fluorescence intensity of xylosyl thiourea derivatives was stronger than that of arabinosyl thiourea derivatives from Fig. 1 and Fig. 2, which might be related to the structure of the molecules. In addition, a larger conjugated system formed by a pyrazole ring and 1,3-phenyl resulted in an fluorescence property of the compounds. So, several compounds of good fluorescence activity could be utilized as labeled derivatives of sugar analysis.

EXPERIMENTAL

General Method

The ^{1}H NMR spectra were recorded on an Inova-400 (using TMS as internal standard, DMSO-d_6 as solvent). MS were determined with a HP 1100 (LC-MS). The IR spectra were obtained on a Bruker Equinox 55 FT-IR apparatus by a pressed KBr pellet. Elemental analyses were performed on a Thermo Flash EA-1112 analyzer. Melting points were taken on a Yanaco MP-S3 micromelting point apparatus. Corrected fluorescence spectra were taken on a Hitachi F-4500 fluorescence spectrophotometer. All reagents were analytical grade chemicals, which could not be utilized before not being disposed, except for special introduction. The boiling point range of petroleum ether was 60-90 °C. 4Å molecular sieves were activated under high temperature. The TLC was performed by GF_{254} and 0.5% CMC. Detection made use of UV light; the mobile phase was petroleum ether and ethyl acetate (1:2).

1. Synthesis of glycosyl isothiocyanates (1a, 1b) according to the literature[13]

1a: yield: 41%, m.p. 88-90 °C, (lit [12]: m.p. 91-92 °C);

1b: yield: 55%, syrup.

2. Preparation of *N*-amino-*N*′-glycosylthiourea (2a, 2b)

Anhydrous hydrazine (1.2 mL, 5 mmol) was added to 80 mL of ethanol below 5 °C by using an ice-water bath. The mixture was stirred. Then a solution of 5 mmol of glycosylisothiocyanates **1** or **2** in ethanol (30 mL) was added dropwise with maganetic stirring. The reaction mixture was then continuously stirred below 5 °C for at least 10 min. The precipitate was filtered off and crystallized from petroleum ether and ethyl acetate.

2a: yield: 75%. m.p. 170-172 °C. (lit [12]: m.p. 170-172 °C);

2b: yield: 73%. m.p. 128-130 °C.

3. Preparation of 1-phenyl-3-aryl-4-formylpyrazole (3a-3e) in terms of the literature[14]

4. Preparation of *N*-glycosyl-*N*′-(1-phenyl-3-arylpyrazole-4-yl)methylene aminothioureas (4a-4e, 5a-5e)

Method 1: *N*-amino-*N*′-glycosylthioureas (**2a**, **2b**) (4 mmol) and 1-phenyl-3-aryl-4-formylpyrazoles (**3a-3e**) (4 mmol) were added to THF (30 mL) and refluxed for about 4 h. The end of the reaction was detected by TLC. The reaction mixture was concentrated. The pure production was obtained by recrystallization from ethyl acetate.

Method 2: *N*-amino-*N*′-glycosylthiourea (**2a**, **2b**) (4

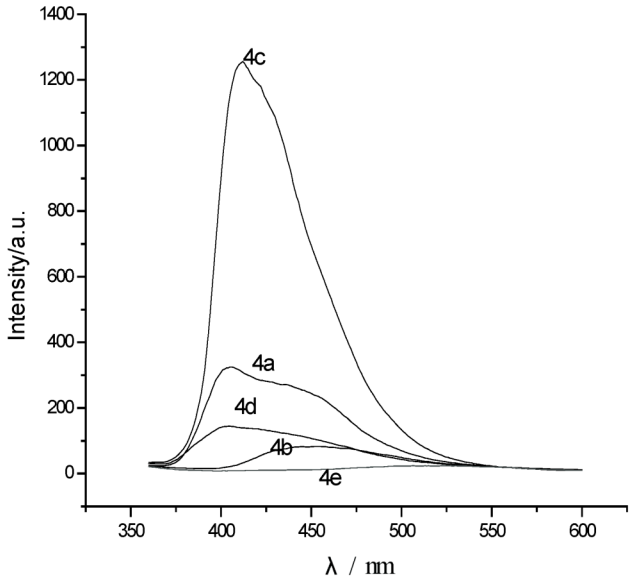


Fig. 1. Fluorescence spectra of compounds **4a-4e**.

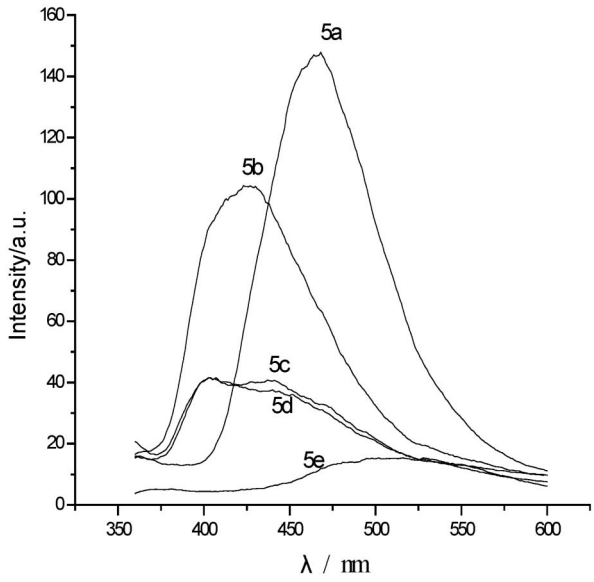


Fig. 2. Fluorescence spectra of compounds **5a-5e**.

mmol) and 1-phenyl-3-aryl-4-formylpyrazoles (**3a**-**3e**) (4 mmol) was added to THF (30 mL). Simultaneously, 0.5~2 g of activated 4Å molecular sieve was also added. The following procedure was the same as method 1.

All physical data are listed in Table 2. The data of ^{1}H NMR, IR and MS are listed in Table 3.

Table 2. Physical data and elemental analysis data and MS for compounds

No.	Molecule	Yield (%)	m.p./°C	Elemental analysis (Calcd.)/%			MS, *m/z* (%)
				C	H	N	
4a	$C_{28}H_{29}N_5O_7S$	85	226-227	58.12 (58.02)	5.06 (5.04)	12.11 (12.08)	580 ([M+H]$^+$, 100)
4b	$C_{29}H_{31}N_5O_8S$	78	241-242	57.24 (57.13)	5.11 (5.13)	11.46 (11.49)	610 ([M+H]$^+$, 100)
4c	$C_{28}H_{28}ClN_5O_7S$	88	181-182	54.69 (54.77)	4.59 (4.60)	11.43 (11.40)	614 ([M+H]$^+$, 100)
4d	$C_{28}H_{28}BrN_5O_7S$	85	169-170	50.15 (51.07)	4.30 (4.29)	10.66 (10.63)	658 ([M+H]$^+$, 100)
4e	$C_{28}H_{28}N_6O_9S$	82	155-156	53.75 (53.84)	4.53 (4.52)	13.44 (13.45)	625 ([M+H]$^+$, 100)
5a	$C_{28}H_{29}N_5O_7S$	80	159-161	58.13 (58.02)	5.03 (5.04)	12.10 (12.08)	580 ([M+H]$^+$, 100)
5b	$C_{29}H_{31}N_5O_8S$	76	149-150	57.23 (57.13)	5.12 (5.13)	11.47 (11.49)	610 ([M+H]$^+$, 100)
5c	$C_{28}H_{28}ClN_5O_7S$	90	157-158	54.67 (54.77)	4.60 (4.60)	11.43 (11.40)	614 ([M+H]$^+$, 100)
5d	$C_{28}H_{28}BrN_5O_7S$	88	150-151	51.16 (51.07)	4.28 (4.29)	10.66 (10.63)	658 ([M+H]$^+$, 100)
5e	$C_{28}H_{28}N_6O_9S$	86	164-165	53.95 (53.84)	4.51 (4.52)	13.49 (13.45)	625 ([M+H]$^+$, 100)

Table 3. ^{1}H NMR, IR, spectra data for new compounds

No.	^{1}H NMR δ	IR (KBr) ν/cm^{-1}
4a	2.05, 2.07, 2.08 (s, 9H, 3 × CH_3CO), 3.48-5.42 (m, 5H, sugar ring-H), 5.58 (t, *J* = 8.8 Hz, 1H, C_1-H), 7.35-7.86 (m, 10H, ArH), 7.87 (s, 1H, CH=N), 8.24 (d, *J* = 8.8 Hz, 1H, NH), 8.67 (s, 1H, pyrazole-H), 9.35 (br, 1H, N′H)	3318, 1738, 1371, 911
4b	2.01, 2.05, 2.09 (s, 9H, 3 × CH_3CO), 3.43 (t, *J* = 10.8 Hz, 1H, C_5-H), 3.72 (s, 3H, OCH_3), 4.15 (t, *J* = 10.8 Hz, 1H, C_5-H), 5.01-5.41 (m, 3H, sugar ring-H), 5.59 (t, *J* = 9.2 Hz, 1H, C_1-H), 7.33-7.85 (m, 9H, ArH), 7.88 (s, 1H, CH=N), 8.22 (d, *J* = 9.2 Hz, 1H, NH), 8.65 (s, 1H, pyrazole-H), 9.38 (br, 1H, N′H)	3341, 1729, 1355, 915
4c	2.03, 2.06, 2.11 (s, 9H, 3 × CH_3CO), 3.51-5.49 (m, 5H, sugar ring-H), 5.58 (t, *J* = 8.8 Hz, 1H, C_1-H), 7.40-7.90 (m, 9H, ArH), 7.94 (s, 1H, CH=N), 8.28 (d, *J* = 8.8 Hz, 1H, NH), 8.70 (s, 1H, pyrazole-H), 9.37 (br, 1H, N′H)	3294, 1743, 1389, 920
4d	2.00, 2.07, 2.09 (s, 9H, 3 × CH_3CO), 3.50-5.48 (m, 5H, sugar ring-H), 5.62 (t, *J* = 9.6 Hz, 1H, C_1-H), 7.37-7.88 (m, 9H, ArH), 7.91 (s, 1H, CH=N), 8.25 (d, *J* = 9.6 Hz, 1H, NH), 8.69 (s, 1H, pyrazole-H), 9.41 (br, 1H, N′H)	3301, 1728, 1357, 909
4e	2.01, 2.05, 2.08 (s, 9H, 3 × CH_3CO), 3.45-5.47 (m, 5H, sugar ring-H), 5.60 (t, *J* = 9.2 Hz, 1H, C_1-H), 7.35-7.85 (m, 9H, ArH), 7.88 (s, 1H, CH=N), 8.30 (d, *J* = 9.2 Hz, 1H, NH), 8.66 (s, 1H, pyrazole-H), 9.39 (br, 1H, N′H)	3310, 1739, 1376, 917
5a	1.68, 2.01, 2.18 (s, 9H, 3 × CH_3CO), 3.80-5.43 (s, 5H, sugar ring-H), 5.51 (t, *J* = 8.8 Hz, 1H, C_1-H), 7.39-7.88 (m, 10H, ArH), 7.93 (s, 1H, CH=N), 8.39 (d, *J* = 8.8 Hz, 1H, NH), 8.70 (s, 1H, pyrazole-H), 9.90 (br, 1H, N′H)	3318, 1760, 1366, 911
5b	1.70, 1.89, 2.24 (s, 9H, 3 × CH_3CO), 3.70 (s, 3H, OCH_3), 3.88-5.49 (m, 5H, sugar ring-H), 5.60 (t, *J* = 9.6 Hz, 1H, C_1-H), 7.42-7.92 (m, 9H, ArH), 7.98 (s, 1H, CH=N), 8.37 (d, *J* = 9.6 Hz, 1H, NH) 8.75 (s, 1H, pyrazole-H), 9.97 (br, 1H, N′H)	3309, 1758, 1387, 907
5c	1.65, 1.95, 2.23 (s, 9H, 3 × CH_3CO), 3.82-5.48 (m, 5H, sugar ring-H), 5.52 (t, *J* = 9.6 Hz, 1H, C_1-H), 7.40-7.89 (m, 9H, ArH), 7.99 (s, 1H, CH=N), 8.40 (d, *J* = 9.6 Hz, 1H, NH), 8.70 (s, 1H, pyrazole-H), 9.91 (br, 1H, N′H)	3321, 1767, 1374, 919
5d	1.67, 1.99, 2.19 (s, 9H, 3 × CH_3CO), 3.85-5.42 (m, 5H, sugar ring-H), 5.55 (t, *J* = 8.8 Hz, 1H, C_1-H), 7.37-7.87 (m, 9H, ArH), 7.95 (s, 1H, CH=N), 8.36 (d, *J* = 8.8 Hz, 1H, NH), 8.72 (s, 1H, pyrazole-H), 9.93 (br, 1H, N′H)	3330, 1751, 1370, 917
5e	1.72, 1.92, 2.20 (s, 9H, 3 × CH_3CO), 3.80-5.41 (m, 5H, sugar ring-H), 5.58 (t, *J* = 8.8 Hz, 1H, C_1-H), 7.39-7.88 (m, 9H, ArH), 7.96 (s, 1H, CH=N), 8.41 (d, *J* = 8.8 Hz, 1H, NH), 8.71 (s, 1H, pyrazole-H), 9.95 (br, 1H, N′H)	3319, 1764, 1359, 921

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