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Note

Synthesis and spectrum of new Schiff bases as polydentate ligands and potential antibacterial reagents

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Eight novel linear polydentate Schiff base ligands, *N*,*N*′-bis(*R*-iminoethyl)-2,6-pyridinedicarboxylic diamide (**IIa-g**), where R=2,4-dihydroxyphenyl, 2,4-dimethoxyphenyl, furanyl, thiophenic, 2-pyridinyl, 3-pyridinyl, 4-pyridinyl and *N*,*N*′-bis(2,4-dihydroxyphenyliminopropyl)-2,6-pyridinedicarboxylic diamide (**IIh**) have been synthesized by the reaction of **Ia** (**Ib**) with various aldehydes. The compounds are characterized by elemental analysis, IR, UV-Vis, LIF, MS and ^{1}H NMR. Analyzing their MS spectra found that the α-fragmentation of imine is the main fragmentation pattern. In addition, microcalorimetric test of the inhibitory capacities of these compounds on *E. coli* and *S. aureus* show that they have antibacterial activity to different extent.

Keywords: Schiff base, polydentate ligands, antibacterial reagents, pyridinedicarboxylic, diamide, aldehyde

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Research of polydentate ligands such as linear polyamine is always in the interest of chemists due to their capacities of forming various mono-, bi- or polynuclear complexes by controlling the reaction condition[1,2]. Additionally, the synthesis of aromatic aldehyde Schiff bases containing heterocycle have attracted much attention due to their diverse biological activities, such as anticancer, antimicrobial, antibacterial, antiviral activities[3-6], luminescent chemosensors, luminescent probes[7,8], *etc.*

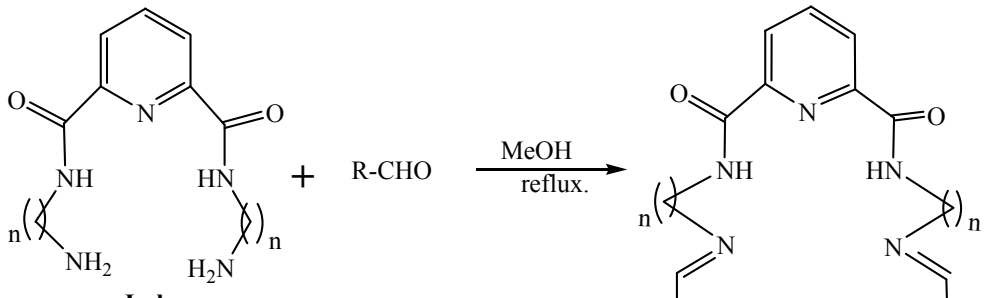
In this paper, an attempt has been made to prepare some new Schiff bases as polydentate ligands and potential antibacterial reagents. The eight novel linear polyamine ligands containing heterocycle (**Figure 1)** have been synthesized and fully characterizeed. In addition, microcalorimetric method has been employed to investigate their inhibitory capacity on *E. coli* and *S. aureus*.

Results and Discussion

The IR spectral data of these compounds (**Table I)** show a new absorption band at 1583-1645 cm^{-1} for **IIb**, **IIc**, **IId**, **IIe**, **IIf** and **IIg**, which is attributed to C═N, indicating that the intermediate (**Ia** or **Ib**) reacted with various aldehyde to form the azomethine functional group. The electron donating conjugated effect of 2,4-di-OCH_3 and N, O and S of heterocycle results in the band appearing at lower frequency. And the absorption band for **IIe**, **IIf** and **IIg** are 1583, 1635 and 1600 cm^{-1}, respectively. The band at 1637 and 1647 cm^{-1} for **IIa** and **IIh** assignable to (C=N) folds with the amide I which can be assignable to the stretching vibration of the -C=N group associated intramolecularly with the N atom of the OH group (-C=N$\cdots\cdots$H-O).

At room temperature the widths of excitated slot and emission slot are both 4 nm; and the concentration of solution is 1.0×10^{-4} mol/L (DMF as solvent). We determined the excitation and emission spectra of the eight Schiff bases. The fluorescence spectral data (**Table I**) make clear that the compound's absorbtion intensity is higher, besides **IIb**. Because the spectral data are close to **Ia** and **Ib** under the same condition and intensity is higher, as well as Schiff bases' stabilization is better; it is considered that the seven Schiff bases could be used as latent luminescent chemosensors and luminescent probes.

The ^{1}H NMR spectra of the compounds (**Table II**) exhibit all the expected signals with the desired integral values and support the postulated molecular structure. The ^{1}H NMR spectra of **IIa** and **IIh** suggest the existence of intramolecular hydrogen bond with the fact that part of the proton signal of the OH appears at δ13.56 and 13.59 ppm, respectively, and another part at δ 10.07 and 9.90. Compared with the intermediate, the signal of -NH_2 has not been observed in these compounds, showing that the two primary amine groups in **Ia** or **Ib** have reacted with the C=O group of various aldehyde to form imine groups. This is further confirmed by the downfield shift of the -CH_2- signal[9]. The proton signal of azomethine for **IIa-h** is δ 9.43, 8.59, 8.08, 8.39, 8.40, 8.85, 8.65, and 9.39 ppm, respectively. Because of the electron withdrawing effect of neighbour and opposite



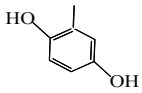
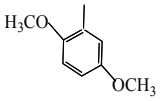
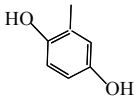
Ia-b

n = 2, **IIa**, R =2,4-dihydroxyphenyl,
IIb , R =2.4-dimethoxyphenyl,
IIc, R = furanyl,
IId, R = thiophenic,
IIe, R = 2-pyridyl,
IIf, R = 3-pyridyl,
IIg, R = 4-pyridyl,
n =3, **IIh**, R =2,4-dihydroxyphenyl

IIa-h

Figure 1

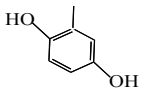
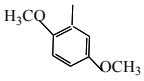
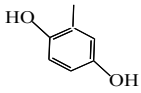
Table I — Analytical data of **IIa-h**

Compd	R	Yield(%)	UV-vis(λ_{max}/nm) (ε)	LIF(λ_{max}/nm) I%	IR(cm^{-1})
IIa		95	306(17750), 276(27370)	Ex(352.0),Em(419.2), I(65%)	3354,1641(br,amideI,C=N), 1540(amideII), 1478,1229, 746,680,645
IIb		45	310(12530), 280 (21360)	Ex(388.8),Em(438.4), I(3%)	3360,1649(amideI),1608(C=N),1540(amideII), 1455,1292, 841,67
IIc		34	270 (20510)	Ex(321.6),Em(430.4), I(80%)	3338, 1673(amide I),1645(C=N), 1532(amide II), 1447, 1380, 758, 645
IId		86	278 (25660)	Ex(310.4),Em(430.4), I(80%)	3322,1664(amideI),1625(C=N), 1536(amide II),1450,1295, 843,730,664
IIe		82	270 (16060)	Ex(308.8),Em(435.2), I(82%)	3455, 1661(amideI),1583(C=N), 1536(amideII),1380,1174, 843,641
IIf		56	272 (18130)	Ex(310.4),Em(432.0), I(80%)	3434,1676(amideI),1635(C=N), 1534(amideII), 1384, 1247, 821, 709
IIg		52	268 (10120)	Ex(310.4),Em(433.0), I(80%)	3405,1665(amideI),1600(C=N), 1536(amideII), 1379, 1234, 820, 746
IIh		76	306(12230), 276 (19910)	I(62%)	3440,1637(amideI and C=N), 1534(amideII), 1401, 1226, 841, 672

group, the azomethine proton signal of **IIf** occurs at lower field than that of **IIe** and **IIg**. The electron donating conjugated effect of OH and OCH_3 results in the downfield shift of the signal of -CH=N-, whereupon the proton signal of azomethine for **IIa** and **IIh** shifts downfield more than that of **IIb** does. This might have resulted from the stereo effect caused by intramolecular hydrogen bond.

The MS spectra data of the compounds are shown in **Table II**. This series of compounds have two kinds of MS fragmentation patterns that are *α*- and *β*-fragmentation of imine, respectively[10]. The mass

Table II — Analytical and ^{1}H NMR data of **IIa-h**

Compd	R	m.p. °C	EI-MS *m/z*	1H NMR				
				Amide H	Olefinic H	-CH_2-	Pyridine ring	R
IIa		130	492 (M+1, 10), 149 (54), 77 (60),	8.36(br)	9.43(s)	3.72, br, 8 H	8.17, br, 2H, H-3,5 7.89, br, 1H, H-4	13.42, s(e), OH; 10.07, s(e), OH; 7.19, d(e), H-4; 6.34, m(e), H-2,5
IIb		108	548 (M+1,100),192 (14), 166 (28),149 (38),136 (18), 77 (18)	9.06(s)	8.59(s)	3.82, m, 8H	8.33, d, 2H, H-3,5 8.02, t, 1H, H-4	7.77, d(e), H-4 6.36, s(e), H-3,5 2.15, s(e), 2 ×OCH_3
IIc		198	408 (M+1,100), 165 (11), 149 (18), 77 (22)	8.91(br)	8.08 (s)	3.79, br, 8H	8.30, d, 2H, H-3,5 7.99, t, 1H, H-4	7.39, s(e), H-5; 6.75, d(e), H-3; 6.42, d(e), H-4
IId		202	441 (M+2,38), 440 (M+1,100), 149 (13), 77 (17)	8.55(br)	8.39(s)	3.77, t, 8H	8.33, s, 2H, H-3,5 8.02, t, 1H, H-4	7.40, d(e), H-5; 7.31, t(e), H-3; 7.07, q(e), H-4
IIe		200	514 (M+1, 100), 191 (4), 165 (6), 149 (41), 77 (31)	8.71(br)	8.40(s)	3.88, br, 8H	8.33, d, 2H, H-3,5 8.02, t, 1H, H-4	8.60, d(e), H-3; 7.87, d(e), H-6; 7.71, t(e), H-5; 7.31, t(e), H-4
IIf		192	514 (M+1, 100), 191 (4), 165 (6), 149 (41), 77 (31)	9.42(br)	8.85 (s)	3.65, m, 4 H 3.54, m, 4H	8.14, q, 3H, pyridyl H,	8.63, t(e), H-2; 8.43, d(e), H-4; 8.22, d(e), H-6; 7.45, q(e), H-5
IIg		194	514 (M+1, 100), 191 (4), 165 (6), 149 (41), 77 (31	9.21(br)	8.65 (s)	3.65, m, 4H 3.54, m, 4H	8.33, d, 2H, H-3,5 8.02, t, 1H, H-4	8.76, 8.42, d(e), 2X A_2 B_2
IIh		>300	521 (M+2, 35), 520(M+1, 82), 164 (23),149 (54), 77 (100)	9.39(s)	8.36(s)	3.57, s, 4H 3.45, d, 4H 1.92, s, 4H	8.15, s, 3H, H-3,5	7.16, d(e), H-4; 6.25, d(e), H-5; 6.13, s(e), H-3

spectral fragmentation patterns of compound **IIa** along with its relative abundances of main daughter ions are given in **Figure 2**. From the fragmentation of **IIa**, it is noted that the relative abundance of daughter *m/z* 136 (98%) in *α*-fragmentation of imine is larger than that of corresponding daughter *m/z* 150 (50%) in *β*-fragmentation of imine. The MS spectra implicates that the *α*-fragmentation of imine is the main fragmentation pattern of compounds **IIa** and **IIe-h**. And the main fragmentation pattern of compounds **IIb, IIc** and **IId** is *β*-fragmentation.

Antibacterial activities

The plots of inhibition ratio **I***% vs* concentration ***C*** of the Schiff bases inhibiting *E. coli* and *S. aureus* showed that these compounds have the inhibitory capacity on *E. coli* and *S. aureus* metabolism growth to a certain extent. With changing concentration, the inhibitory ratios of these compounds also change, which indicated their inhibitory capacities are concentration dependent. While only the inhibitory *S.aureus* capacities of the Schiff bases **IIg** are basically linear. Their linear ***I%-C*** equations obtained from **Figure 3** and corresponding R values are: ***I%*** =-1.68741+ 0.0892 × ***C***, R=0.99569.

The *S. aureus* half inhibitory concentrations IC_{50} of **IIa-d** and **IIf-h** are: 550.2, 910.91, 500.58, 450.39, 920, 580 and 850.21 μg/mL, respectively. This reveals that the Schiff bases **IIa**, **IIc**, **IId** and **IIf** have better antibacterial activity. This is due to the existence of intramolecular hydrogen bonds forming a favourable configuration for the interaction of the inhibitor with receptor[11].

The *E. coli* half inhibitory concentrations IC_{50} of **IIa-d** and **IIf-h** are: 115.02, 390, 670.2, 560.3, 350, 356 and 110.56 μg/mL, respectively. This reveals that the Schiff bases **IIa**, and **IIh** have the best antibacterial activity. Compared with *S. aureus*, **IIa**,

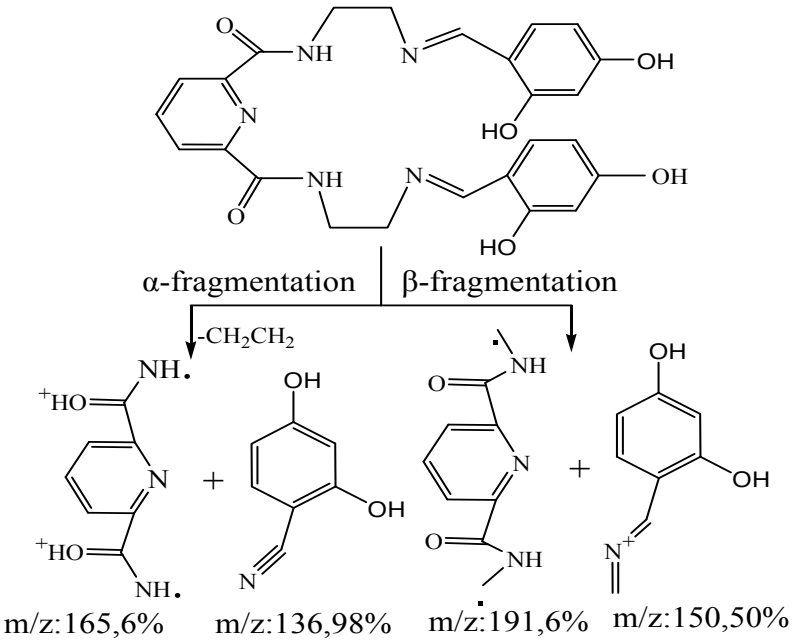


Figure 2

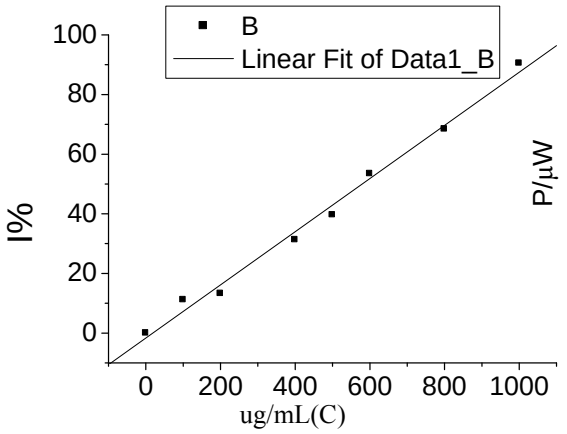


Figure 3

Figure 3 — The I%-C equations of the Schiff bases **IIg** which indicated their inhibitory capacities are concentration dependent. While only the inhibitory *S. aureus* capacities of the Schiff bases **IIg** are basically linear. The I%-C equations is: I% =-1.68741+ 0.0892 × C, R=0.99569. (R is corresponding values).

IIb, IIf, IIg and **IIh** all these compounds have better antibacterial effect on *E. coli.* It's worthy to mention that when **IIh** concentration is 200 μg/mL, inhibition is 100%. So **IIh** is a potential antibacterial reagent. The power-time curves of *E. coli* in the presence of **IIg** at the different concentrations is shown in **Figure 4**. As we can see from **Figure 4**, when the compound **IIg** is added, the maximum heat outputs are lower and the times of maximum heat output are longer than that of *E. coli* in control experiment. They reveal the same result which is also consistent with the analysis of ***I%-C*** correlation. The time of the lag-phase suggests that retarding time of bacterial growth is longer with increasing concentrations of **IIg**. It might be possible that some of the bacteria had been killed by the inhibitor so that it took longer to generate a detectable signal[12].

In summary, eight novel Schiff bases **IIa-h** have been synthesized. Their dioxapolyazo structures show that they are good polydentate ligands. The MS fragmentation pattern of **IIa-h** has been discussed. Microcalorimetric test of inhibitory capacity on *E. coli* and *S. aureus* of these Schiff bases has found that they also have antibacterial activity to different extent and the Schiff bases **IIh** have the best antibacterial activity.

Experimental Section

All chemicals and solvents used were purchased as A.R. grade from Shanghai First Chemical Industry Factory. Ethylenediamine, propylenediamine and 2-furaldehyde were purified before use. The 2,6-dimethylpyridine dicarboxylate, *N*,*N*′-bis(3-aminopropyl)-2, 6-pyridinedicarboxylic diamide **Ib** were prepared as the literature method[13]. *S. aureus* (CCTCCAB910393) and *E. coli* (CCTCCAB91112) were provided by Chinese Center for Type Culture Collection, Wuhan University, China. The LB culture

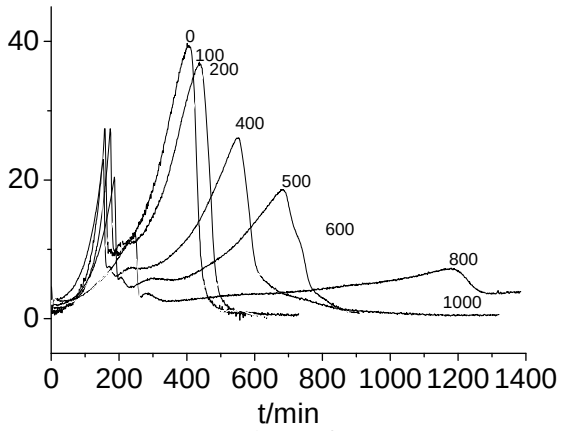


Figure 4

Figure 4 — The power-time curves of *E. coli* in the presence of **IIg** at the different concentrations. When the compound **IIg** is added, the maximum heat outputs are lower and the times of maximum heat output are longer than that of *E. coli* in control experiment. reveal the same result which is also consistent with the analysis of I%-C correlation. The time of the lag-phase suggests that retarding time of bacteria growth is longer with increasing concentrations of **IIg**. It might be that some of the bacteria had been killed by the inhibitor.

medium contained per 1000 mL (*p*H=7.0-7.2): NaCl 5 g, peptone 5 g and yeast extract 5 g (Oxiod company). The medium was sterilized at 121°C for 30 min. Sterilized DMF is used as the solvent. Melting point was determined by WC-1 Microscope melting point apparatus. The IR spectra in the 4000-400 cm^{-1} range were recorded on a Shimadzu FTIR 3000 instrument. 1D ^1H NMR spectra were acquired on Varian Mercury-UX 300 MHz spectrometer, using TMS as internal standard. The UV-Vis spectra in 200-700 nm range were recorded on a Shimadzu UV-160A spectrophotometer in DMF. The fluorescence spectra were recorded on a Shimadzu FDU-3 spectrophotometer in DMF. Mass spectra were recorded on a ZAB-3-FHF mass spectrometer. A microcalorimeter, LKB-2277 bioactivity monitor manufactured by LKB corporation of Sweden was used to obtain the metabolic power-time curves of bacteria. Two 15 mL stainless steel ampoule bottles were used.

Microcalorimetric test of bioactivity

The principle of microcalorimetry to test bioactivity of inhibitors was described in the literature[13,14].

In experiment, the microcalorimeter was thermostated at 37°C. The baseline stability for the instrument was 0.1 μW/24h. The bacteria used, *E. coli* or *S. aureus* were suspended into the LB culture medium. The compound was added from the beginning of the experiment, *i.e.* it was introduced as soon as the bacteria were inoculated in the LB culture medium. The solutions of the compounds were prepared in LB culture medium, and prepared freshly every time. The amplifier of the monitor was set at 1000 or 3000 μW.

Syntheses of *N*,*N′*-bis(2-aminoethyl)-2,6- pyridinedicarboxylic diamide, Ia. The synthetic method of **Ia** is slightly different from that of **Ib**. 2,6-dimethylpyridinedicarboxylate (0.195 g, 1 mmole) was dissolved in methanol (30 mL) and added dropwise to a stirred solution of ethylene diamine (0.6 g, 10 mmoles) in methanol (10 mL). The resulting solution was stirred 12 hr at ambient conditions. Then the precipitate was filtered off and the filtrate was refluxed for 1 hr under N_2 protection. The solvent and excess amine was removed quickly under reduced pressure to give the product **Ia** as a gray, slightly hygroscopic solid (0.24 g, 95.6%). IR: 3442, 3293, 1667, 1539, 1445, 681 cm^{-1}; ^1H NMR($CDCl_3$): δ 8.85 (br, 2H, amide), 8.26 (d, 2H, pyridyl H), 7.97 (t, 1H, pyridyl H), 3.55 (br, 4H, -CH_2-), 2.93 (br, 4H, -NH_2), 2.22 (br, 4H, -CH_2-); UV-Vis(λ_{max}/nm): 269 (3434); Fluorescence: λ_{Ex}(310.0), λ_{Em}(432.0), I(20%). Anal. Calcd. for $C_{11}H_{17}N_5O_2$: C, 52.58; H, 6.82; N, 27.87. Found: C, 52.39; H, 6.93; N, 28.15%.

General synthesis procedure of the Schiff bases (IIa-h). To 15 mL of ethanolic solution of 2.2 mmole various aldehydes, 1 mmole intermediate **Ia** or **Ib** in 15 mL ethanol was added under stirring for 4 hr at RT. The mixture was refluxed for 12 hr then concentrated solution to 15 mL and precipitate was obtained, filtered, washed with ethanol, ether and dried in vacuum to gain the product.

References

1 Dominguez-Vera J M, Moreno J M & Galvez N, *Inorg Chim Acta*, 281 (1), **1998**, 95.
2 Cao D, Lin H K, Zhou Z F, Zhu S R, Su X C & Chen Y T, *Can J Chem*, 79 (4), **2001**, 455.
3 Chohan Z H & Kausar S, *Met-Based Drugs*, 7 (1), **2000**, 17.
4 Das W A, Trousdale M D, Ren S & Lien E J, *Antiviral Res*, 44, **1999**, 201.
5 Bolos C A, Nikolov G S, Ekateriniadou L & Kyriakidis, D A, *Met-Based Drugs*, 5 (6), **1998**, 323.
6 Chohan Z H & Farooq M A, *Syn React Inor Met-Or Chem*, 31 (10), **2001**, 1853.
7 Parker D, Senanayake K & Williams J A G, *Chem Commun*,18, **1997**, 1777.

8 Bunzli J C G, in *Lanthanide Probes in Life*, *Chemical and Earth Science* Ch.7, edited by Choppin G, Bunzli J C G (Elsevier, Amsterdam), **1989**.
9 Ning Y C, *The Structure and Spectroscopy of Organic Compounds* (Science Press, Peking), **2001** (Chinese).
10 Chen Y Z, *The Principle and Application of Organic Mass Spectra* (Science Press, Peking), **2001** (Chinese).
11 Chen F E, *The Basic Principle of Design on Drugs* (Huazhong University of Science & Technology Press, Wu Han) **1995**.
12 Xie C L & Tang H K, *Thermochimica Acta*, 123, **1988**, 33.
13 Brooker S, Dunbar G S & Jameson G B, *Polyhedron*, 18, **1999**, 679.
14 Liu Y W, Liu Y, Wang C X, Qu S S & Deng F J, *Thermochimica Acta*, 351, **2000**, 51.