# An Unexpected Result in the Condensation of 2-Amino-6-bromopyridine with Ethyl 4-Chloroacetoacetate: Four Dihalo-substituted Pyrido[1,2-*a*]pyrimidin-4-ones as Reaction Products



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We report here the condensation of 2-amino-6-bromopyridine with ethyl 4-chloroacetoacetate in polyphosphoric acid. In this reaction, a mixture of the four possible dihalo-4*H*-pyrido[1,2-*a*]pyrimidin-4-ones **1-4** was obtained.

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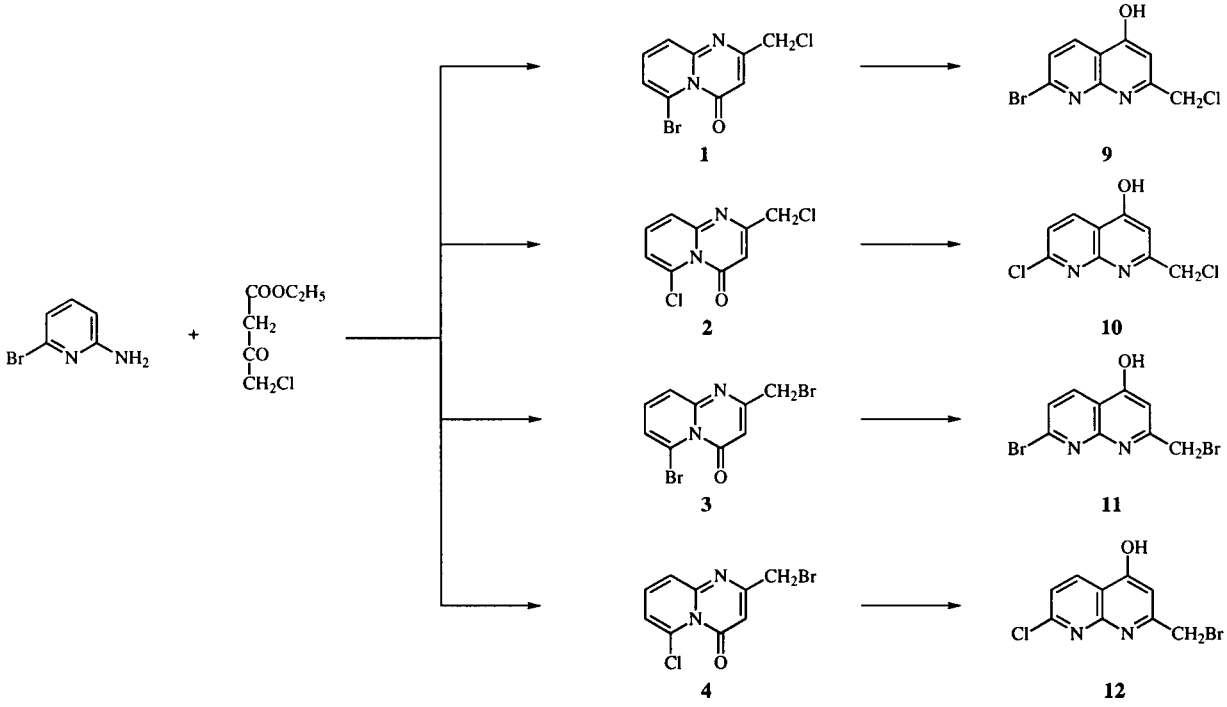
In the course of our work on the chemistry of 1,8-naphthyridine derivatives with potential biological activity, we have investigated extensively the synthesis of 4*H*-pyrido-[1,2-*a*]pyrimidin-4-ones, prepared by condensation of suitable 2-aminopyridines with β-ketocarboxylic acid in polyphosphoric acid [1-5]. Pursuing our interest in this field, we attempted to prepare compound **1** (Scheme 1) by condensation of 2-amino-6-bromopyridine [6] with ethyl 4-chloroacetoacetate. After crystallization, thin layer chromatography of the reaction product performed on silica gel plates using different eluants, a single spot was observed. In contrast, the $^1$H nmr spectrum showed various signals corresponding to a mixture of products. Attempts to separate the reaction mixture by gas chromatography under different conditions failed, as decomposition occurred.

In the light of these results, the reaction mixture was examined by reversed phase liquid chromatography/elec-

Scheme 1



trospray mass spectrometry: these analyses yielded an uv and total ion current chromatograms with four mayor peaks (Figures 1a and 1b). The positive electrospray mass spectra associated with the four total ion current peaks are shown in Figures 2a-d. The spectrum associated with the peak centered at the retention time of 6.66 minutes exhibits m/z 229 $[M + H]^+$ (Figure 2a), while the spectrum associated with the peak at the retention time of 7.65 is dominated by m/z 319 $[M + H]^+$ (Figure 2d). Furthermore, the spectra associated with the peaks centered at the retention times of 6.96 and 7.35 are both dominated by m/z 273 $[M + H]^+$ (Figures 2b and 2c): the presence of the same ion in two different chromatographic peaks strongly suggested isomeric structures which eluted at different times but had the same molecular weight.

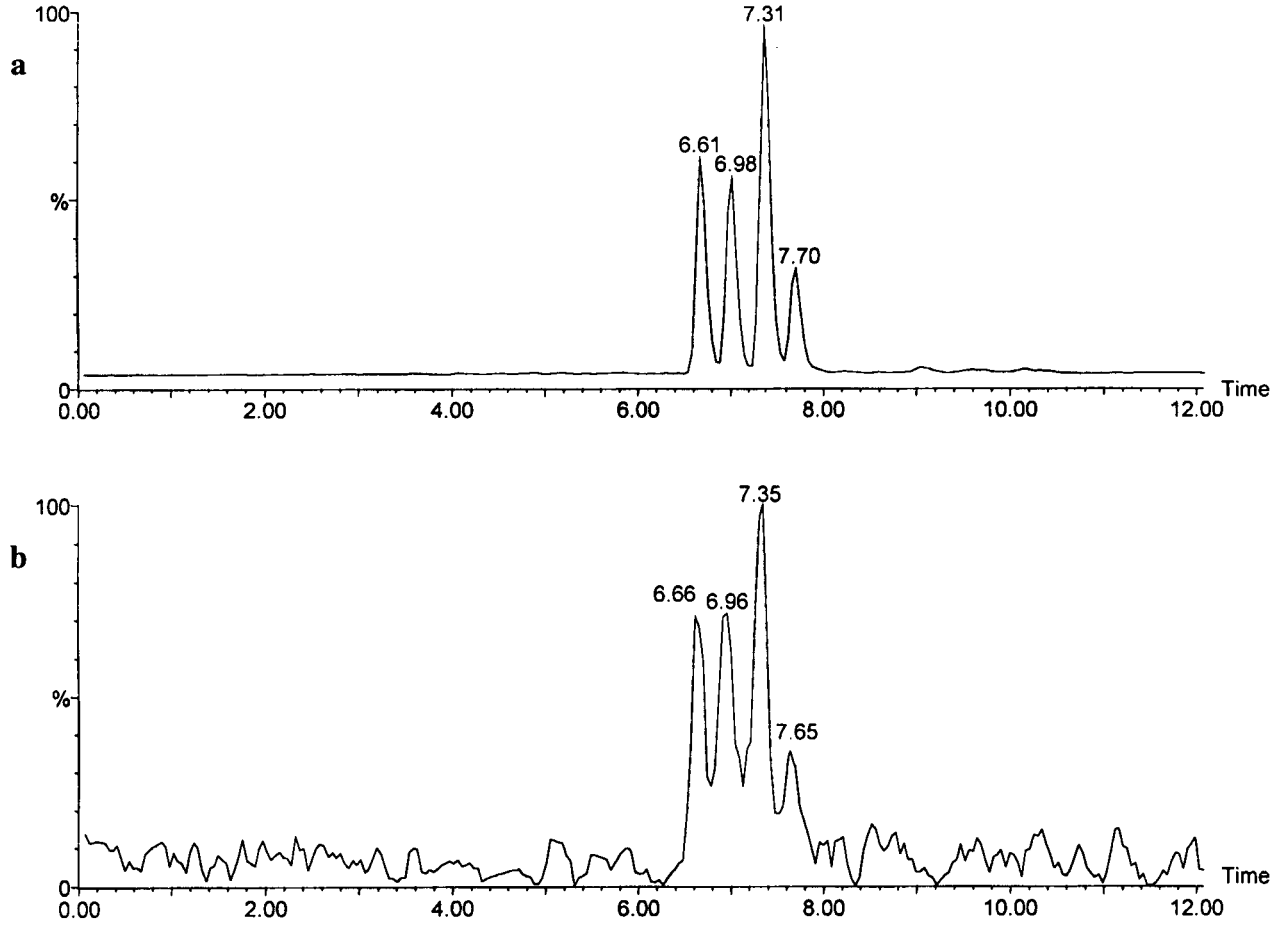



Figure 1. The uv (**a**) and total ion current (**b**) chromatogram of the reaction mixture resulting from condensation of 2-amino-6-bromopyridine with ethyl 4-chloroacetoacetate.

In order to assign these peaks unequivocally to their chemical structures, the four major peaks observed in the reversed phase liquid chromatography/electrospray mass spectrometry were isolated and purified by preparative reversed phase liquid chromatography and subjected to $^1H$ nmr analysis.

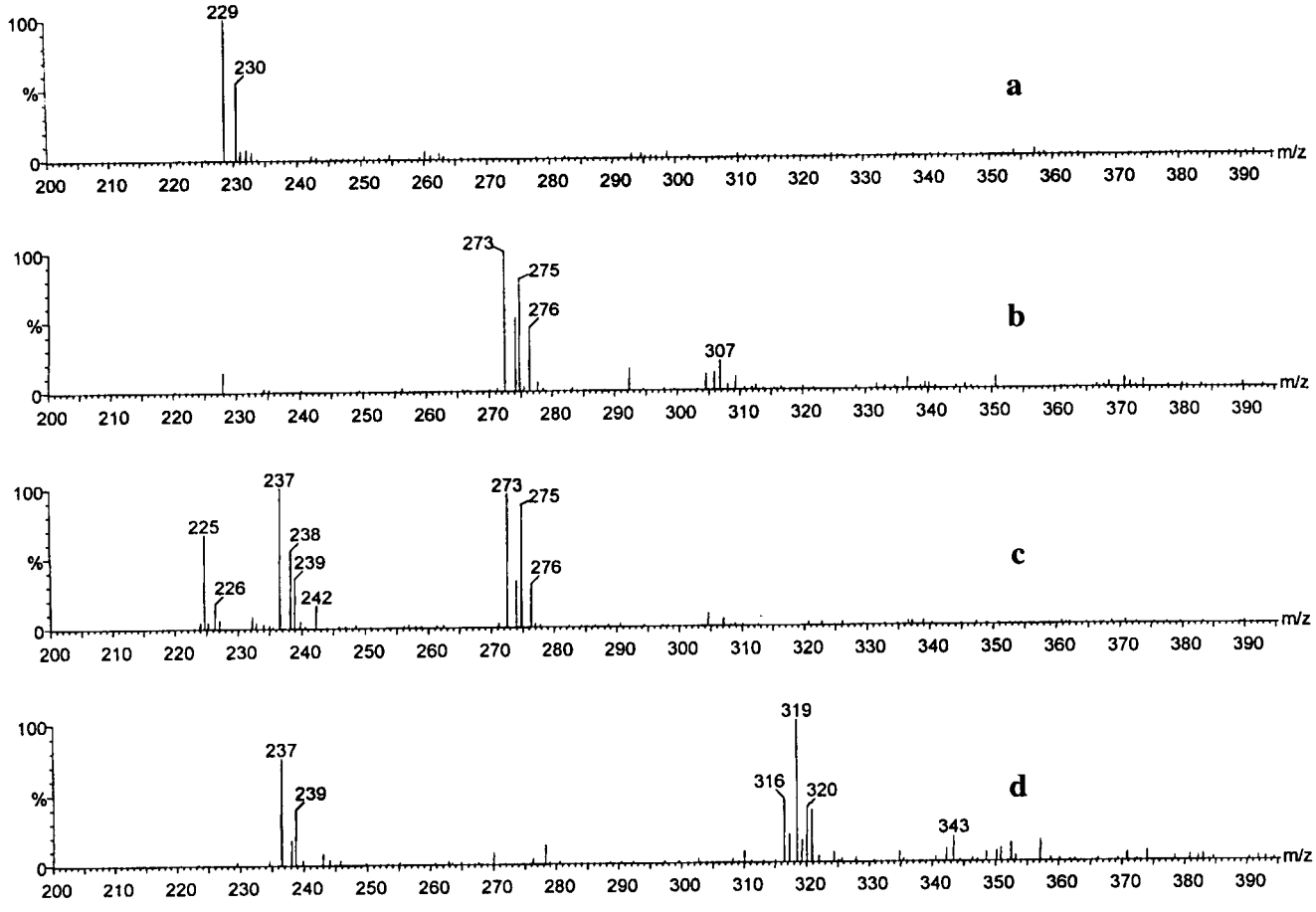


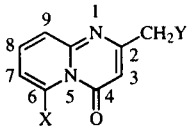

Figure 2. Positive electrospray mass spectra (**a-d**) associated with the four total ion current peaks of Figure 1b, centered at the retention times of 6.66, 6.96, 7.35 and 7.65 minutes, respectively. Spectrum **a** shows a protonated ion at m/z 229 $[M + H]^+$ and the characteristic 2 Cl pattern which coincide with compound **2**. Spectrum **d** shows m/z 319 $[M + H]^+$ and the characteristic 2 Br pattern which is in agreement with structure **3**. The two spectra **b** and **c**, show the same protonated molecule, m/z 273 and the characteristic 1 Cl + 1 Br pattern which coincides with structures **4** and **1**, as further demonstrated by $^1H$ nmr spectroscopy.

Both electrospray mass spectrometry and $^1H$ nmr showed that the peak centered at the retention time of 6.66 (m/z 229 $[M + H]^+$) could be attributed to the dichloro derivative **2** (Scheme 1), while the peak at the retention time of 7.65 (m/z 319 $[M + H]^+$) corresponded to the dibromo derivative **3**. Furthermore, the $^1H$ nmr showed that the isomeric structures associated with the peaks at the retention times of 6.96 and 7.35 (m/z 273 $[M + H]^+$) could be attributed respectively to the chloro-bromo-substituted derivatives **4** and **1** (Scheme 1), which differ from each other structurally in the position of the two halogen atoms on the heterocyclic rings.

In particular, the structures of compounds **1** and **4** were easily assigned by $^1H$ nmr from a comparison of the spectral data of derivatives **3** and **2** (see Table 1). The $H_7$, $H_8$, and $H_9$ protons of the dihalo-substituted pyrido[1,2-*a*]-pyrimidin-4-ones **1-4** appeared as an ABX system and the spectral parameters were refined by the program LAOCOON 5. As expected for these kinds of structures, the chemical shifts of the protons adjacent to the halogen atom in the 6 position depend on the nature of the halogen atom itself; thus, the close analogy (Table 1) between the chemical shifts of $H_7$, $H_8$, and $H_9$ of compounds **4** and **2**, as well as that between the chemical shifts of the same protons of compounds **1** and **3**, made it possible to assign the Cl atom as the 6-substituent for compounds **4** and **2** and the Br atom as the 6-substituent for compounds **1** and **3**. Furthermore, the same type of analogy between the chemical shifts of 2-$CH_2$ and $H_3$ of compounds **1**, **2** and **3**, **4** (Table 1) made it possible to assign the Cl and Br atoms respectively as the substituents on the 2-$CH_2$ side chain of these structures.

Table 1

$^1$H NMR Spectral Data of Compounds **1-4**

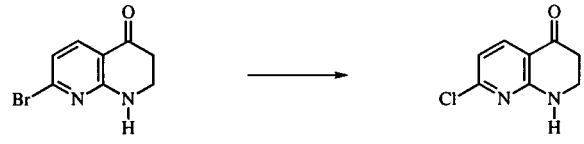


| Compound | **1** | **2** | **3** | **4** |
|---|---|---|---|---|
| X | Br | Cl | Br | Cl |
| Y | Cl | Cl | Br | Br |
| $H_3$ | 6.57 | 6.55 | 6.48 | 6.47 |
| $H_7$ | 7.26 | 7.01 | 7.26 | 7.00 |
| $H_8$ | 7.39 | 7.50 | 7.40 | 7.50 |
| $H_9$ | 7.47 | 7.44 | 7.49 | 7.44 |
| 2-$CH_2$ | 4.46 | 4.46 | 4.30 | 4.29 |
| $J_{2\text{-}CH_2,3}$ | 0.5 | 0.5 | 0.0 | 0.0 |
| $J_{7,8}$ | 7.15 | 7.19 | 7.17 | 7.18 |
| $J_{7,9}$ | 1.33 | 1.36 | 1.38 | 1.36 |
| $J_{8,9}$ | 8.99 | 9.03 | 8.99 | 9.00 |

The structures of compounds **2** and **3** were also unequivocally demonstrated by chemical evidence: the physical data of compounds **2** and **3** (see Table 2) were found to be practically identical to those of authentic samples of **2** [4] and **3**, respectively prepared by condensation of 2-amino-6-chloro- and 2-amino-6-bromopyridine with the appropriate β-ketocarboxylic ester in polyphosphoric acid.

It is important to point out that results analogous to those reported in this paper had previously been obtained by the treatment of 7-bromo-2,3-dihydro-1,8-naphthyridin-4(1*H*)-one **5** (Scheme 2) with hydrochloric acid, leading in good yield to the formation of the corresponding 7-chloro derivative **6** [7].

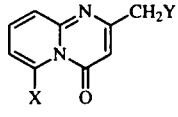
Scheme 2



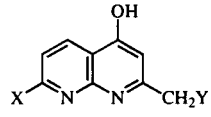
**5** **6**

Furthermore, in order to confirm the assignment of structures **1-4**, the reaction mixture was also submitted to isomerization (Scheme 1). The conditions used for isomerization were chosen bearing in mind our previous studies concerning the isomerization of some pyrido[ 1,2-*a*]-pyrimidin-4-ones to the corresponding 1,8-naphthyridine derivatives under reflux in diphenyl ether for several hours [2]. It was found that, under these strong conditions, the pyrido[1,2-*a*]pyrimidin-4-one **7** was converted to the 2,7-dimethyl-4-hydroxy-1,8-naphthyridine **8** with the loss

Table 2



**1-4**



**10, 11**

| Compound | X | Y | Yield % | mp °C [a] | Empirical Formula | MS m/z $[M+H]^+$ | Elemental Analyses Calcd./Found | | |
|---|---|---|---|---|---|---|---|---|---|
| **1** [b] | Br | Cl | 35 | 158 dec | $C_9H_6N_2OBrCl$ | 273 | 39.52 | 2.21 | 10.24 |
| | | | | | | | 39.35 | 2.48 | 10.55 |
| **2** [b] | Cl | Cl | 21 | [4] | $C_9H_6N_2OCl_2$ | 229 | | | |
| **3** [c] | Br | Br | 63 | 147-149 dec [d] | $C_9H_6N_2OBr_2$ | 319 | 33.99 | 1.90 | 8.81 |
| | | | | | | | 33.61 | 2.21 | 8.54 |
| **3** [b] | Br | Br | 10 | | | | | | |
| **4** [b] | Cl | Br | 18 | 118-120 dec | $C_9H_6N_2OBrCl$ | 273 | 39.52 | 2.21 | 10.24 |
| | | | | | | | 39.18 | 2.12 | 9.95 |
| **10** [e] | Cl | Cl | 72 | >320 dec [f] | $C_9H_6N_2OCl_2$ | 229 | 47.19 | 2.64 | 12.23 |
| | | | | | | | 47.01 | 2.93 | 12.11 |
| **11** [e] | Br | Br | 72 | >320 dec [f] | $C_9H_6N_2OBr_2$ | 319 | 33.99 | 1.90 | 8.81 |
| | | | | | | | 33.64 | 2.18 | 8.98 |

[a] Recrystallization solvents; [b] Separated from the reaction mixture by preparative reversed phase liquid chromatography; [c] By reaction of 2-amino-6-bromopyridine with ethyl 4-bromoacetoacetate; [d] Petroleum ether 100/140°; [e] By isomerization of **2** or **3**; [f] Ethanol.

of the halogen atom (Scheme 3). Thus, in order to avoid this undesirable result, the mixture of the pyrido[1,2-*a*]-pyrimidin-4-ones **1-4** was isomerized under milder conditions, *i.e.* in diphenyl ether at 220° for 10 minutes, to yield the corresponding 1,8-naphthyridines **9-12**, as unequivocally confirmed by reversed phase liquid chromatography/electrospray tandem mass spectrometry.

Scheme 3


**7** **8**

In particular, to establish the time windows during which a particular ion is selected for tandem mass spectrometry, reversed phase liquid chromatography/electrospray mass spectrometry analyses were performed, which yielded the uv and total ion current chromatograms in Figures 3a and 3b, respectively. The positive electrospray mass spectra associated with the four total ion current peaks are given in Figures 4a-d. The spectrum in Figure 4a, associated with the peak at the retention time of 11.56 minutes, shows a protonated ion at m/z 229 $[M + H]^+$ and the characteristic 2 Cl pattern which coincides with compound 10 (Scheme 1), while the spectrum in Figure 4d, associated with the peak at the retention time of 13.77 minutes, shows m/z 319 $[M + H]^+$ and the characteristic 2 Br pattern which is in agreement with structure **11** (Figure 3). On the other hand, the two spectra in Figures 4b and 4c, associated with the peaks at the retention times respectively of 12.49 and 12.88 minutes, show the same protonated molecule, m/z 273, and the characteristic 1 Cl + 1 Br pattern which coincides with structures **9** and **12** (Scheme 1). To distinguish between these structures, it was necessary to perform reversed phase liquid chromatography/electrospray tandem mass spectrometry measurements which yielded the tandem mass spectra in Figures 5a and 5b. The first spectrum (Figure 5a) is associated with the total ion current peak at the retention time of 12.49 minutes, while the second spectrum (Figure 5b) is associated with the peak at the retention time of 12.88 minutes. The major fragment ion in the first spectrum is observed at m/z 194 $[273\text{-Br}]^+$, while the mayor fragment ion in the second spectrum is observed at m/z 238 $[273\text{-Cl}]^+$; however, the marker fragment ion which distinguishes the two isomers is observed in the second spectrum at m/z 181 $[273\text{-CHBr}]^+$ which can only be derive from structure **12**. These data confirm that isomer **12** elutes at the retention time of 12.88 minutes while isomer **9** elutes at the retention time of 12.49 minutes.

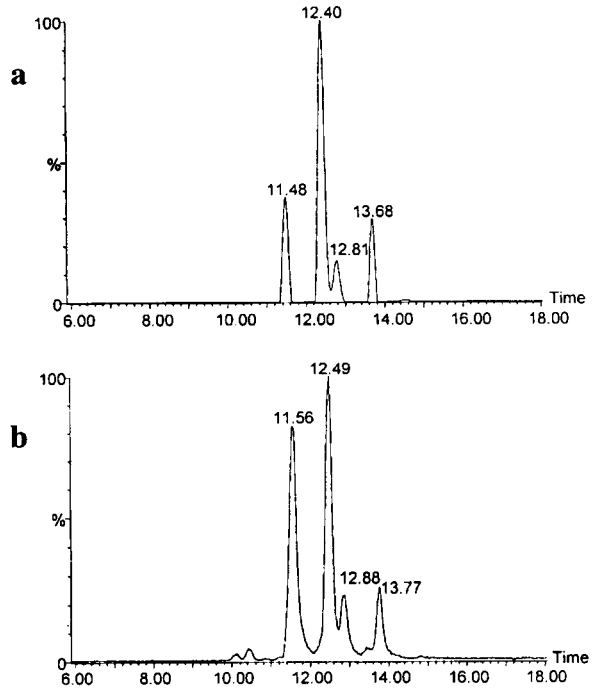



Figure 3. The uv (**a**) and total ion current (**b**) chromatogram of the reaction mixture resulting from isomerization of 4*H*-pyrido[1,2-*a*]pyrimidin-4-ones derivatives **1-4**.

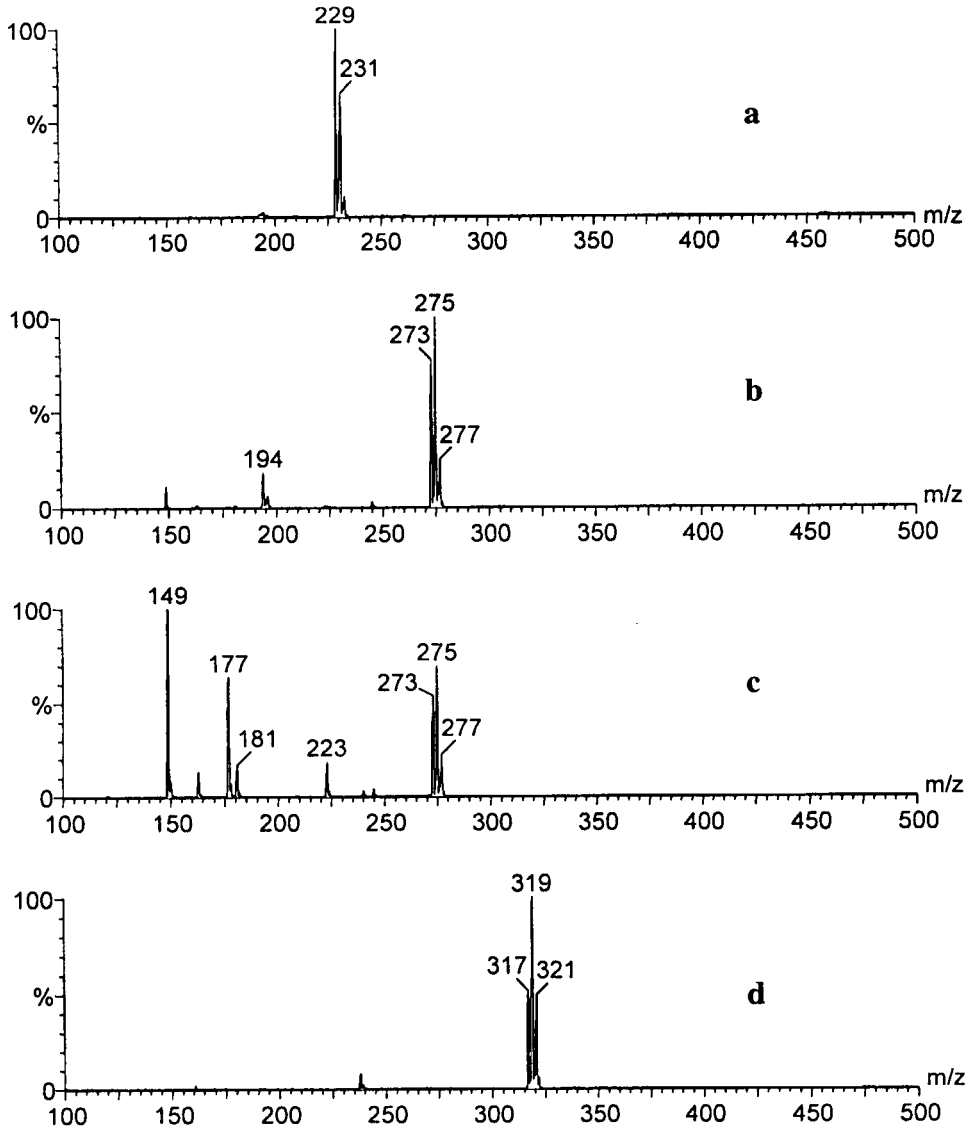



Figure 4. Positive electrospray mass spectra (**a-b**) associated with the four total ion current peaks of Figure 3b, centered at the retention times of 11.56, 12.49, 12.88 and 13.77 minutes, respectively. Spectrum **a** shows a protonated ion at m/z 229 $[M + H]^+$ and the characteristic 2 Cl pattern which coincides with compound **10**. Spectrum **d** shows m/z 319 $[M + H]^+$ and the characteristic 2 Br pattern which is in agreement with structure **11**. The two spectra **b** and **c**, show the same protonated molecule, m/z 273 and the characteristic 1 Cl + 1 Br pattern which coincides with structures **9** and **12**.

Furthermore, pure samples of compounds **2** and **3** were subjected to isomerization under the above conditions to yield 1,8-naphthyridines **10** and **11**, respectively. The $^1H$ nmr spectra of compounds **10** and **11** (see Table 3) showed two doublets and a singlet due to the 1,8-naphthyridine nucleus, together with another singlet attributable to the 2-$CH_2$ side chain.

Lastly, it is important to note that in the above reported condensation between 2-amino-6-bromopyridine with ethyl 4-chloroacetoacetate, the presence of a $CH_2Cl$ group as the substituent on the β-ketocarboxylic ester, favored only the formation of pyridopyrimidone derivatives (compounds **1-4**). These results are in agreement with our previous reports for similar condensation reactions in which other substituents ($CH_3$, $CF_3$, *etc.*) are present on the β-ketocarboxylic ester [5].

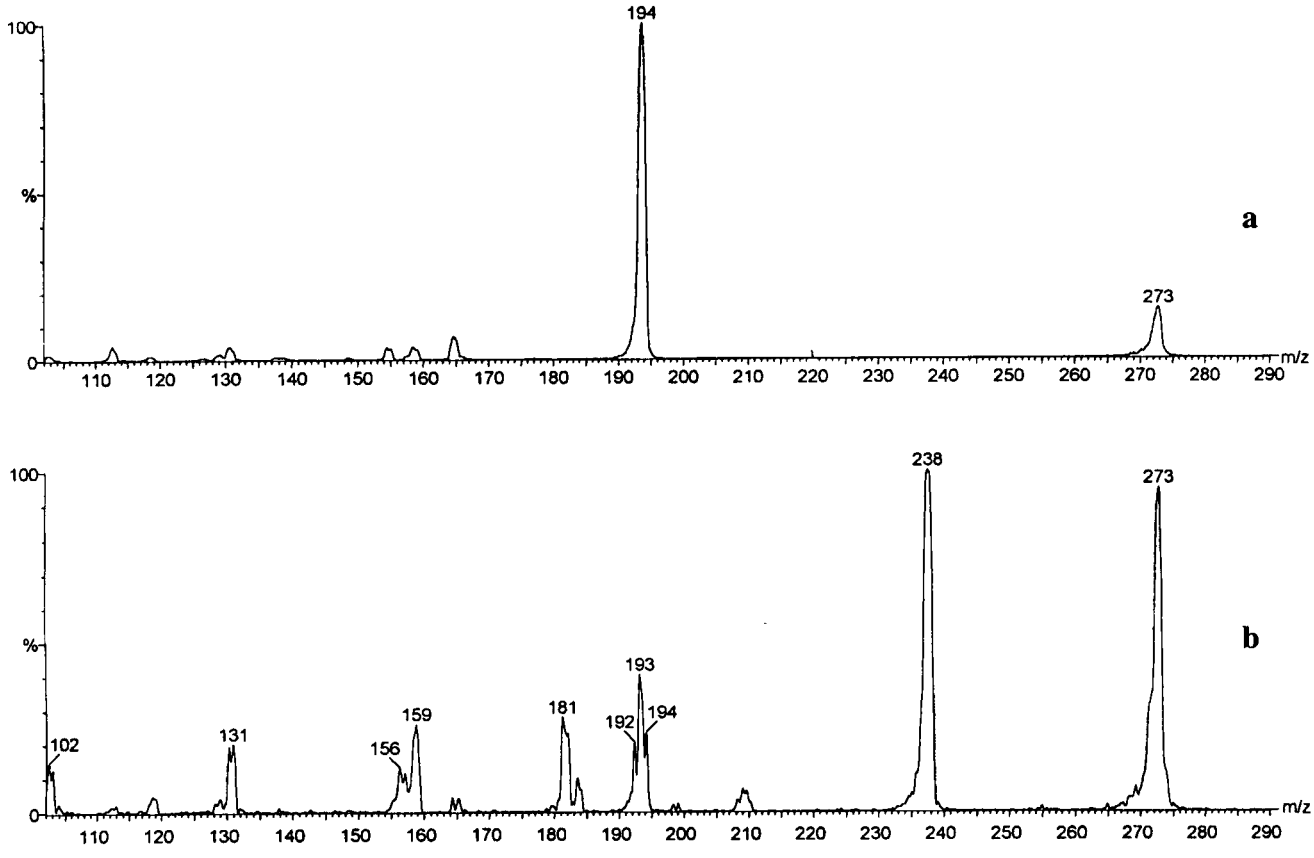
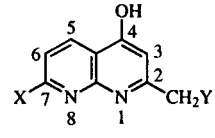



Figure 5. Tandem mass spectra (**a**, **b**) associated with the two total ion current peaks of Figure 3b, centered at the retentioin times of 12.49 and 12.88 minutes, respectively. The marker fragment ion which distinguishes the two isomers (**9**, **12**) is observed in spectrum **b** at m/z 181 [273-CHBr]$^+$, which can only derive from structure **12**.

Table 3

$^1$H NMR Spectral Data of Compounds **10** and **11**




| Compound | **10** | **11** |
|---|---|---|
| X | Cl | Br |
| Y | Cl | Br |
| $H_3$ | 6.36 | 6.34 |
| $H_5$ | 8.52 | 8.39 |
| $H_6$ | 7.27 | 7.42 |
| 2-$CH_2$ | 4.53 | 4.41 |
| $J_{5,6}$ | 8.23 | 8.27 |

## EXPERIMENTAL

Chemistry.

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. The ir spectra for comparison of compounds were taken as paraffin oil mulls or as liquid film on a Mattson 1000 FTIR spectrometer. The $^1$H nmr spectra of all compounds were obtained with a Bruker AC-200 instrument operating at 200 MHz, in a *ca* 2% solution of deuteriochloroform or dimethyl-$d_6$ sulfoxide, using tetramethylsilane or sodium trimethylsilylpropanesulfonate as the internal standard, respectively. Reversed phase liquid chromatography/electrospray mass spectrometry analyses and reversed phase liquid chromatography/electrospray tandem mass spectrometry analyses were performed on a triple quadrupole mass spectrometer (QUATTRO II, MICROMASS, UK) equipped with an electrospray ion source and coupled to an HP1100 liquid chromatography binary pump equipped with a diode array (wavelength, 190-900 nm) supplied by Hewlett Packard (Palo Alto, USA). The tandem mass spectrometry analyses were effected using argon collision gas and 30eV collision energy.

Gas chromatography analyses were performed on a Carlo Erba model 4200 apparatus with a flame ionization detector, using a 1.6 m x 2.6 mm column packed with SE30 3% on chromosorb W silanised 80/100 mesh.

Elemental analyses were carried out by our analytical laboratory and were consistent with theoretical values to within ±0.4%. Analytical thin layer chromatography was carried out on 0.25 mm layer silica gel plates containing a fluorescent indicator; spots were detected under uv light (254 nm).

4*H*-Pyrido[1,2-*a*]pyrimidin-4-ones Derivatives **1-4**.

A mixture of 2-amino-6-bromopyridine (1.73 g, 10 mmoles) and ethyl 4-chloroacetoacetate (1.97 g, 12 mmoles) in 30 g of polyphosphoric acid was heated at 100° for 4 hours. After cooling, the resulting mixture was poured over crushed ice, treated with 10% aqueous sodium hydroxide until *p*H 6 and then extracted with chloroform. The organic layer was washed with water, dried over magnesium sulphate and evaporated under reduced pressure to yield 2.83 g of a crude mixture consisting almost exclusively of four main compounds, as shown by reversed phase liquid chromatography/electrospray mass spectrometry analyses (see Figures 1 and 2). The liquid chromatography conditions were: Supelcosil ABZ 5μ column (4.6 mm x 15 cm); eluant A: water, eluant B: methanol; gradient from 30% to 100% B over 30 minutes; 1 ml/minute flow rate, which was split to allow 20 μl flow into the ion source; uv detection 254 nm.

An analytical sample of the mixture of compounds was then separated by preparative reversed phase liquid chromatography, performed on a Beckman System Gold apparatus under the following conditions: Beckman Ultrasphere Ods 5μ column (10 mm x 25 cm); eluant A: water, eluant B: methanol; gradient from 20% to 80% B over 30 minutes; flow 5 ml/minute; uv detection 254 nm. The appropriate fractions corresponding to the retention times respectively of 17.49, 18.12, 18.48 and 19.04 minutes, were combined, evaporated, lyophilized and pump-dried to yield compounds **1-4** as pure solids (Table 1 and 2).

6-Chloro-2-chloromethyl-4*H*-pyrido[1,2-*a*]pyrimidin-4-one **2**.

Compound **2** was prepared as previously reported by us [4] by the reaction of 2-amino-6-chloropyridine and ethyl 4-chloroacetoacetate in polyphosphoric acid.

6-Bromo-2-bromomethyl-4*H*-pyrido[1,2-*a*]pyrimidin-4-one **3**.

A mixture of 2-amino-6-bromopyridine [6] (1.73 g, 10 mmoles) and ethyl 4-bromoacetoacetate [8] (2.46 g, 12 mmoles) in 30 g of polyphosphoric acid was treated as for the preparation of the mixture of compounds **1-4** (see above) to yield derivative **3** (2.8 g), which was purified by crystallization (Table 2).

Isomerization of 4*H*-Pyrido[1,2-*a*]pyrimidin-4-one Derivatives **1-4** to 1,8-Naphthyridines **9-12**.

The mixture of compounds **1-4** (0.50 g) was dissolved in diphenyl ether (10 ml) and then heated at 220° for 10 minutes. After cooling, the resulting precipitate was collected, washed with light petroleum, and proved to consist almost exclusively of a mixture of the 1,8-naphthyridines **9-12**, as unequivocally confirmed by reversed phase liquid chromatography/electrospray tandem mass spectrometry (see Figures 3-5). The liquid chromatography conditions were the same as those used in the case of compounds **1-4**. The uv chromatogram (Figure 3a) shows that 1,8-naphthyridines **9-12** are present in the reaction mixture in a ratio respectively of 4.5, 1.7, 1.3 and 0.7.

7-Chloro-2-chloromethyl-4-hydroxy-1,8-naphthyridine **10** and 7-Bromo-2-bromomethyl-4-hydroxy-1,8-naphthyridine **11**.

A mixture of compounds **2** or **3** (5 mmoles) in 10 ml of diphenyl ether was heated at 220° for 10 minutes. After cooling, the resulting crude precipitate was collected, washed with light petroleum, and crystallized from ethanol to yield pure naphthyridines **10** or **11** (Tables 2 and 3).

Acknowledgements.

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