# Oxazine- and oxazole-fused derivatives of the alkaloid boldine and their complete structural and spectral assignments by HMQC and HMBC experiments

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**The novel heterocycles 1,11-dimethoxy-2-hydroxy-6-methyloxazolo[4,5-*k*]-5,6,6a,7-tetrahydro-4*H*-dibenzo [*de,g*]quinoline, 1,12-dimethoxy-2-hydroxy-6-methyl-9-phenyl-10*H*-oxazin[5,6-*k*]-5,6,6a,7-tetrahydro-4*H*-dibenzo[*de,g*]quinolin-10-one and 1,11-dimethoxy-2-hydroxy-6-methyl-9-phenyloxazolo[4,5-*k*]-5,6,6a,7-tetrahydro-4*H*-dibenzo[*de,g*]quinoline were prepared starting from the alkaloid boldine. The structures were confirmed and the $^{1}$H and $^{13}$C NMR spectra were completely assigned using a combination of one- and two-dimensional NMR techniques.**

**KEYWORDS:** NMR; $^{1}$H NMR; $^{13}$C NMR; boldine; oxazolo/aporphine derivatives; oxazinone/aporphine derivatives

## INTRODUCTION

We recently reported the formation of 2-phenyl-3*H*-naphth[2,1-*b*]-1,4-oxazin-3-one and 2-phenylnaphth[1,2-*d*]-oxazole by reaction of a masked oxazolinone 1,3-dipole and 1-nitroso-2-naphthol,[1] and achieved their complete structural elucidation by the use of NMR spectroscopy.[2] We have also been interested in boldine, (*S*)-(+)-2,9-dihydroxy-1,10-dimethoxyaporphine or 2,9-dihydroxy-1,10-dimethoxy-6-methyldibenzo[*de,g*]quinoline (**1**), the major alkaloid of the leaves and bark of the Chilean boldo tree (*Peumus boldus* Mol., Monimiaceae), as a biologically active substance and as a starting material for semi-synthetic transformations including substitutions on its aromatic ring carbon atoms.[3] We describe here the nitrosation of boldine in acetic acid, the subsequent reduction of the nitroso to an amino group and modification of the resulting 1,2-hydroxyamino system to generate oxazinone- and oxazole-fused boldine derivatives.

We describe the structure determination, conducted entirely by the use of NMR spectroscopy, and the complete chemical shift assignments of the $^{1}$H and $^{13}$C NMR spectra of the annellated boldine derivatives. This was achieved through the concerted application of a variety of one- and two-dimensional techniques such as COSY,[4] HMQC[5] and HMBC[6] and the incorporation of the well-documented[7] pulse field gradients (PFG).[8]

Each of these compounds contains one four-spin $^{1}$H system (two methylenes) and one three-spin system (a methylene and a methine), separated by a nitrogen heteroatom. They can be unambiguously identified from the COSY spectrum, and serve as the entry point for spectral interpretation. Furthermore, the biphenyl system in the aporphine skeleton contributes two isolated aromatic protons in compounds **2**–**6**. In compound **4** another aromatic proton is incorporated in the oxazole ring. Compounds **5** and **6** introduce an additional five-spin system corresponding to the phenyl ring substituent.

The well-resolved $^{13}$C NMR spectrum allows direct heteronuclear correlations from the HMQC spectrum to be established, but complete unequivocal assignment of the spectrum is not possible without the concerted use of the HMQC and HMBC techniques.

## RESULTS AND DISCUSSION

Treatment of boldine (**1**) with sodium nitrite in acetic acid afforded a nitrosoboldine (**2**), presumably substituted at C-3 or C-8, *ortho* to the phenol groups, although the precise regiochemistry could not be established directly. Subsequent catalytic hydrogenation gave the corresponding aminoboldine (**3**) in good yield. Reaction of aminoboldine **3** with ethyl orthoformate produced the oxazole derivative (**4**) in good yield. Similarly, the phenyloxazinone derivative (**5**)

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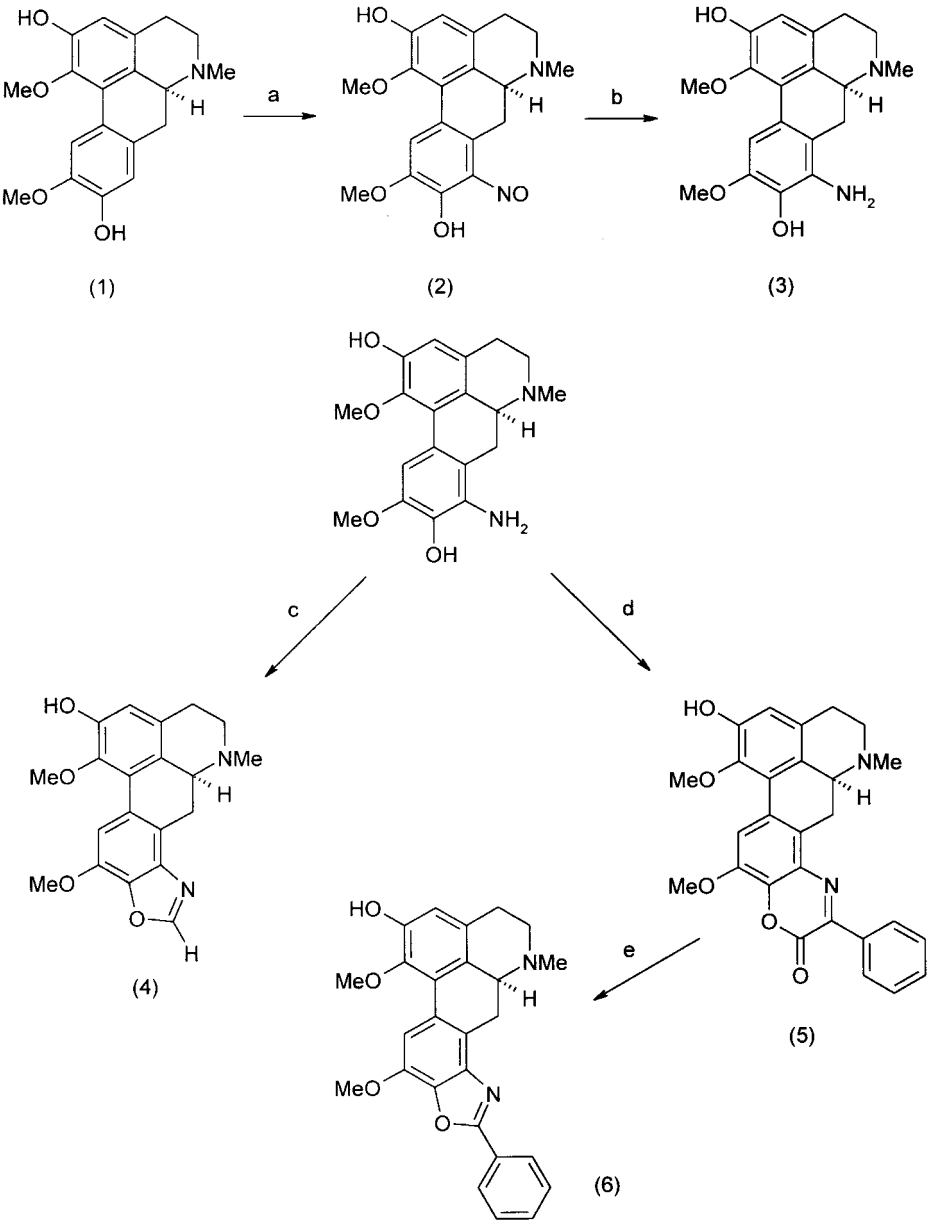
was obtained by reaction of aminoboldine **3** with methyl benzoylformate. Under basic conditions, the phenyloxazinone **5** was rapidly converted into the phenyloxazole **6** as reported recently by us for simpler model systems.[9] The synthetic scheme is shown in Scheme 1.

**Table 1.** Yields and melting-points of the boldine derivatives synthesized

| Boldine derivative | Melting-point (°C) | Yield (%) |
|---|---|---|
| **2** | 128–130 | 64 |
| **3** | 177–179 | 99 |
| **4** | 189–191 | 89 |
| **5** | 202–203 | 60 |
| **6** | 179–181 | 34 |

Assignments based on the experiments referred to above, and on previously reported assignments for benzoxazoles and -oxazinones,[2] allow us to conclude that the oxazole and oxazinone rings are fused to the aporphine skeleton at C-8/C-9, producing the novel oxazolo[4,5-*k*]-5,6,6a,7-tetrahydro-4*H*-dibenzo[*de,g*]quinoline (**4**) and 10*H*-oxazin[5,6-*k*]-5,6,6a,7-tetrahydro-4*H*-dibenzo[*de,g*]quinolin-10-one (**5**) heterocyclic systems. This, in turn, proves that the precursor of the annellated boldine derivatives is 8-aminoboldine (**3**), and that therefore electrophilic nitrosation in acetic acid occurred regioselectively at C-8 to afford **2**, unlike halogenation with *N*-halosuccinimides in trifluoroacetic acid, which occurs preferentially at C-3.[3] The complete assignments of the $^{1}H$ and $^{13}C$ NMR spectra of boldine[10,11] and some of its halogenated derivatives have been published previously.[3]




a = $NaNO_2$/AcOH, b = $H_2$/Pd/C/EtOH, c = $(EtO)_3CH$, d = MeO(CO)(CO)Ph, e = KOH/MeOH

**Scheme 1.** Synthesis of boldine heterocyclic derivatives.

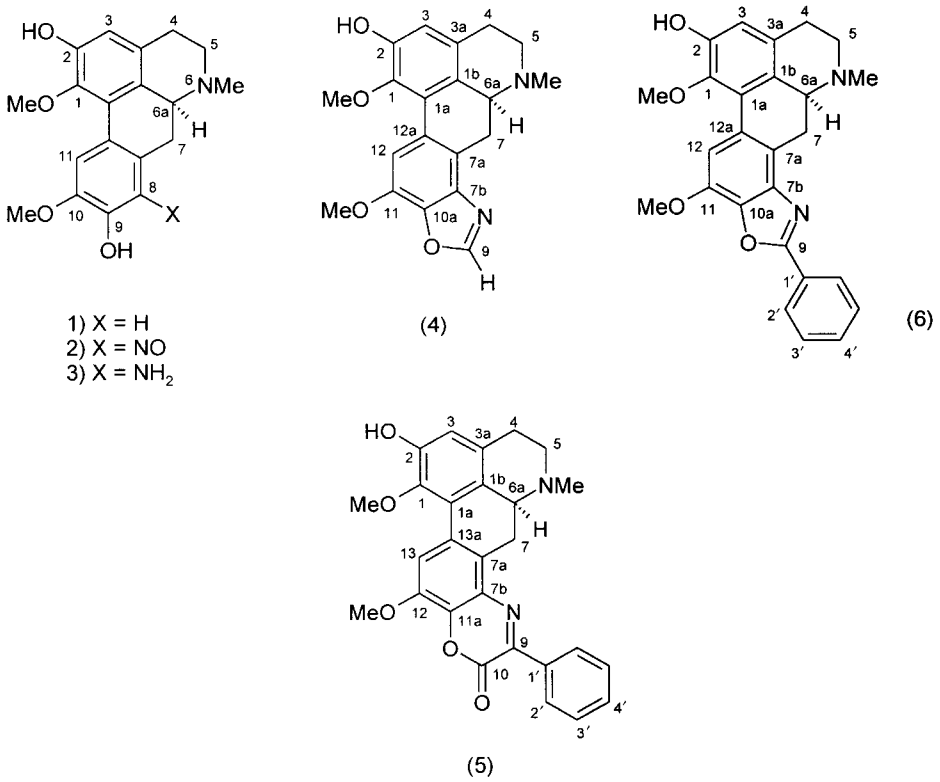



**Figure 1.** Oxazine- and oxazole-fused derivatives of boldine.

**Table 2.** $^{1}H$ and $^{13}C$ NMR of 1,11-dimethoxy-2-hydroxy-6-methyloxazolo[4,5-*k*]-5,6,6a,7-tetrahydro-4*H*-dibenzo[*de*,*g*]quinoline (**4**)

| Carbon No. | Chemical shift[a], $\delta$ [H–X, multiplicity, $J$(H,H) (Hz)] | $^{13}C$[a] | HMQC $^{1}J$(C,H) | Protons showing HMBC (coupling) |
|---|---|---|---|---|
| 1 | | 142.64 | | 3, OH-3, *O*-1-$CH_3$ |
| 1a | | 125.26 | | 3, 6a, 7, 12 |
| 1b | | 125.26 | | 3, 6a, 7, 12 |
| 2 | | 148.80 | | 3, OH-2 |
| 3 | 6.61 | 114.82 | + | OH-2 |
| 3a | | 128.69 | | 4, 5 |
| 4 | 2.51–2.94 [H-4$\alpha$/H-4$\beta$, m] | 27.98 | + | 3, 5 |
| 5 | 2.31–2.94 [H-5$\alpha$/H-5$\beta$, m] | 52.11 | + | *N*-$CH_3$ |
| 6a | 2.84 [H-6a, dd, $J$(6a, 7$\alpha$) = 10.0, $J$(6a, 7$\beta$) = 4.0][b] | 61.23 | + | 4, 5, 7, *N*-$CH_3$ |
| 7 | 2.31 [H-7$\alpha$, dd], 3.66 [H-7$\beta$, dd,] $J_{gem}$ = 14.0, $J$(7$\alpha$, 6a) = 10.0, $J$(7$\beta$, 6a) = 4.0[b] | 27.13 | + | |
| 7a | | 119.98 | | 7.12 |
| 7b | | 138.74 | | 7, 9 |
| 9 | 8.71 | 153.31 | + | |
| 10a | | 136.91 | | 9, 12 |
| 11 | | 142.14 | | 12, *O*-11-$CH_3$ |
| 12 | 8.01 | 107.50 | + | |
| 12a | | 128.26 | | 7, 12 |
| *N*-$CH_3$ | 2.44 | 43.13 | + | |
| *O*-1-$CH_3$ | 3.58 | 59.02 | + | |
| *O*-11-$CH_3$ | 3.99 | 55.50 | + | |
| OH-2 | 9.18 | | | |

[a] In ppm from TMS.

[b] $\alpha$ = Axial, $\beta$ = equatorial.

**Table 3.** $^{1}$H and $^{13}$C NMR of 1,12-dimethoxy-2-hydroxy-6-methyl-9-phenyl-10*H*-oxazin[5,6-*k*]-5,6,6a,7-tetrahydro-4-*H*-dibenzo[*de*,*g*]quinolin-10-one (**5**)

| Carbon No. | Chemical shift[a], $\delta$ [H–X, multiplicity, $J$(H,H) (Hz)] | $^{13}$C[a] | HMQC $^{1}J$(C,H) | Protons showing HMBC (coupling) |
|---|---|---|---|---|
| 1 | | 143.39 | | 3, *O*-1-$CH_3$ |
| 1a | | 125.09 | | 13 |
| 1b | | 126.03 | | 6a, 7, 13 |
| 2 | | 149.35 | | 3 |
| 3 | 6.65 | 115.82 | + | |
| 3a | | 128.83 | | 7 |
| 4 | 2.55–2.95 [H-4$\alpha$/H-4$\beta$, m] | 28.32 | + | 3 |
| 5 | 2.32–2.95 [H-5$\alpha$/H-5$\beta$, m] | 52.67 | + | *N*-$CH_3$ |
| 6a | 2.85 [H-6a, dd] $J(6a, 7\alpha) = 14.0$, $J(6a, 7\beta) = 3.8$] | 61.66 | + | 4, 5, 7, *N*-$CH_3$ |
| 7 | 2.20 [H-7$\alpha$, dd,] 4.17 [H-7$\beta$, dd], $J_{gem} = 14.0$, $J(7\alpha, 6a) = 14.0$, $J(7\beta, 6a) = 3.8$[b] | 26.70 | + | |
| 7a | | 127.26 | | 7, 13 |
| 7b | | 129.11 | | |
| 9 | | 149.99 | | 2′ |
| 10 | | 151.52 | | |
| 11a | | 135.15 | | 2′ |
| 12 | | 144.01 | | 13, *O*-12-$CH_3$ |
| 13 | 8.18 | 113.08 | + | |
| 13a | | 128.62 | | 13 |
| *N*-$CH_3$ | 2.46 | 43.69 | + | |
| *O*-1-$CH_3$ | 3.62 | 59.68 | + | |
| *O*-12-$CH_3$ | 3.96 | 56.18 | + | |
| OH-2 | 9.27 | | | |
| 1′ | | 134.61 | | 3′ |
| 2′ | 8.26 | 129.31 | + | |
| 3′ | 7.55 | 128.25 | + | |
| 4′ | 7.55 | 131.05 | + | 2′ |

[a] In ppm from TMS.
[b] $\alpha$ = Axial, $\beta$ = equatorial.

**Table 4.** $^{1}$H and $^{13}$C NMR of 1,11-dimethoxy-2-hydroxy-6-methyl-9-phenyloxazolo[4,5-*k*]-5,6,6a,7-tetrahydro-4*H*-dibenzo[*de*,*g*]quinoline (**6**)

| Carbon No. | Chemical shift[a], $\delta$ [H–X, multiplicity, $J$(H,H) (Hz)] | $^{13}$C[a] | HMQC $^{1}J$(C,H) | Protons showing HMBC (coupling) |
|---|---|---|---|---|
| 1 | | 142.65 | | *O*-1-$CH_3$ |
| 1a | | 125.30 | | 4, 6a, 7, 12 |
| 1b | | 125.30 | | 4, 5, 6a, 7, 12 |
| 2 | | 148.80 | | |
| 3 | 6.62 | 114.82 | + | OH-2 |
| 3a | | 128.68 | | 4, 5, 6a |
| 4 | 2.52–2.92 [H-4$\alpha$/H-4$\beta$, m] | 27.97 | + | 3, 5, 6a |
| 5 | 2.37–2.92 [H-5$\alpha$/H-5$\beta$, m] | 52.11 | + | *N*-$CH_3$ |
| 6a | 2.92 [H-6a, dd, $J(6a, 7\alpha) = 13.7$, $J(6a, 7\beta) = 3.6$] | 61.19 | + | 4, 5, 7, *N*-$CH_3$ |
| 7 | 2.37 [H-7$\alpha$, dd], 3.73 [H-7$\beta$, dd] $J_{gem} = 14.0$, $J(7\alpha, 6a) = 13.7$, $J(7\beta, 6a) = 3.6$[b] | 27.17 | + | |
| 7a | | 119.71 | | 7, 12 |
| 7b | | 140.41 | | 7 |
| 9 | | 161.51 | | |

**Table 4.** (*Continued*)

| Carbon No. | Chemical shift[a], $\delta$ [H–X, multiplicity, $J$(H,H) (Hz)] | $^{13}C$[a] | HMQC $^{1}J$(C,H) | Protons showing HMBC (coupling) |
|---|---|---|---|---|
| 10a | | 137.57 | | 12 |
| 11 | | 141.82 | | 12, *O*-11-$CH_3$ |
| 12 | 8.01 | 107.65 | + | |
| 12a | | 128.39 | | 7 |
| *N*-$CH_3$ | 2.48 | 43.17 | + | 4, 5, 6a |
| *O*-1-$CH_3$ | 3.60 | 59.02 | + | |
| *O*-11-$CH_3$ | 4.03 | 55.48 | + | |
| OH-2 | 9.19 | | | |
| 1′ | | 125.84 | | 3′, 4′ |
| 2′ | 8.22 | 126.76 | + | 2′ |
| 3′ | 7.62 | 128.74 | + | 3′ |
| 4′ | 7.62 | 131.30 | + | 1′ |

[a] In ppm from TMS.
[b] $\alpha$ = Axial, $\beta$ = equatorial.

**Table 5.** $^{1}H$ and $^{13}C$ NMR of boldine: 2,9-dihydroxy-1,10-dimethoxy-6-methyl-dibenzo[*de,g*] quinoline (**1**)

| Carbon No. | Chemical shift[a], $\delta$ [H–X, multiplicity, $J$(H,H) (Hz)] | $^{13}C$[a] | HMQC $^{1}J$(C,H) | Protons showing HMBC (coupling) |
|---|---|---|---|---|
| 1 | | 142.07 | | 3, *O*-1-$CH_3$ |
| 1a | | 125.68 | | 4, 7, 11, |
| 1b | | 124.99 | | 3, 4, 5, 7 |
| 2 | | 148.53 | | 3 |
| 3 | 6.50 | 113.56 | + | 4, 5 |
| 3a | | 128.23 | | 4, 5, 7 |
| 4 | 2.50–2.92[H-4$\alpha$/H-4$\beta$, m] | 27.97 | + | 3, 5 |
| 5 | 2.92–2.27[H-5$\alpha$/H-5$\beta$, m] | 52.25 | + | *N*-$CH_3$ |
| 6a | 2.77[H-6$\alpha$, m] | 61.79 | + | 4, 5, *N*-$CH_3$ |
| 7 | 2.27–2.92[H-7$\alpha$/H-7$\beta$, m] | 33.21 | + | 6a, 8 |
| 7a | | 129.04 | | 11 |
| 8 | 6.71 | 114.69 | + | 7 |
| 9 | | 145.22 | | 11, *O*-10-$CH_3$ |
| 10 | | 145.46 | | 8, *O*-10-$CH_3$ |
| 11 | 7.85 | 111.42 | + | |
| 11a | | 122.27 | | 7, 8, 11 |
| *N*-$CH_3$ | 2.39 | 43.17 | + | 5, 6a, 7 |
| *O*-1-$CH_3$ | 3.56 | 58.69 | + | |
| *O*-10-$CH_3$ | 3.78 | 55.17 | + | |
| OH-2 | 9.00 | | | 1, 3 |
| OH-9 | 9.09 | | | |

[a] In ppm from TMS.

The structures of 8-nitrosoboldine (**2**), 8-aminoboldine (**3**), 1,11-dimethoxy-2-hydroxy-6-methyloxazolo[4,5-*k*]-5,6,6a,7-tetrahydro-4*H*-dibenzo[*de,g*]quinoline (**4**), 1,12-dimethoxy-2-hydroxy-6-methyl-9-phenyl-10*H*-oxazin[5,6-*k*]-5,6,6a,7-tetrahydro-4*H*-dibenzo[*de,g*]quinolin-10-one (**5**) and 1,11-dimethoxy-2-hydroxy-6-methyl-9-phenyloxazolo[4,5-*k*]-5,6,6a,7-tetrahydro-4*H*-dibenzo[*de,g*]quinoline (**6**) are shown in Fig. 1.

In order to differentiate between both possible regioisomers (annellated at C-2/C-3 or C-8/C-9 of the aporphine skeleton), we considered the aromatic region in the HMBC spectrum of **4**. In this region only two proton signals are observed, at $\delta$ 6.61 and 8.01, the latter correlated only to quaternary (aromatic) carbon atoms. The other aromatic ring proton is correlated to three quaternary carbon atoms and one methylene carbon. These results are insufficient for the unambiguous establishment of the location of the additional heterocycle fused to the aporphine skeleton in **4**. To achieve this, it was necessary to analyze the $^{1}H$–$^{1}H$ COSY spectrum (not shown) where intense cross

peaks were found between H-7/H-7′ and H-6a (spin system AMX).

With the C-7 methylene protons clearly differentiated from those at C-4 by the connectivity of the former with the methine proton, and by the concerted use of HMQC and HMBC, it was possible to establish the long-range connectivity between C-4 and the aromatic ring proton resonating at $\delta$ 6.61. These results allowed the unambiguous assignment of this resonance to the proton at C-3, correlated with C-4, thus proving by default that the oxazole ring is located at C-8/C-9 on the aporphine skeleton. Although the chemical relationship between **4, 5** and **6**, all derivatives of 8-aminoboldine (**3**), leaves no doubt as to the position at which the oxazole or oxazine ring is fused to the aporphine system, analogous correlations between the C-4 methylene and the upfield aromatic proton singlet confirmed the structures of **5** and **6**. Similarly, the unambiguous determination of the structures of these substances proves that the nitrosation of boldine (**1**) affords 8-nitrosoboldine (**2**) as the only isolated product.

The complete spectral assignment of **1**, **4**, **5** and **6** is shown in Tables 2–5. The large vicinal coupling constant (10–14 Hz) observed for these compounds (H-6a,7$\alpha$ protons) are in good agreement with those previously described for other aporphine alkaloids.[12]

## EXPERIMENTAL

### Preparation of aminoboldine (3)

Boldine (**1**) was dissolved in acetic acid and treated at room temperature with an equimolar amount of $NaNO_2$ with stirring for 1 h. After neutralization with ammonia solution and extraction with ethyl acetate, 8-nitrosoboldine (**2**) was isolated as the only product. Compound **2**, dissolved in EtOH, was hydrogenated catalytically over Pd/C at room temperature at 50 psi for 2.5 h, to afford 8-aminoboldine (**3**) in quantitative yield as gray needles.

### Formation of the heteroannulated derivatives of boldine

Refluxing **3** in ethanol with a small excess of ethyl orthoformate for 2 days under an $N_2$ atmosphere afforded **4** in good yield. In a similar preparation with replacement of ethyl orthoformate with methyl benzoylformate, **5** was obtained as yellow needles. For the formation of **6**, it sufficed to treat **5** briefly with KOH–MeOH (5%), affording light-beige needles.

All the products were purified by column chromatography by elution with chloroform–methanol (4 : 1) and crystallization from benzene. Their yields and melting-points are reported in Table 1.

### NMR studies

The $^1$H and $^{13}$C NMR spectra were recorded using a Bruker Avance DRX 300 spectrometer operating at a $^1$H frequency of 300.13 MHz and a $^{13}$C frequency of 75.47 MHz. All measurements were performed at a probe temperature of 300 K, using solutions of **4**, **5** and **6** in DMSO-$d_6$ (20–23 mg ml$^{-1}$) containing tetramethylsilane (TMS) as an internal standard. All two-dimensional spectra were acquired with a Bruker inverse 5 mm *Z*-gradient probe. The one-dimensional carbon spectrum was obtained with a spectral width of 18 000 Hz with 3 s between transients and the 90° pulse was 10 µs. The homonuclear $^1$H–$^1$H shift-correlated 2D spectra were obtained using standard Bruker software (cosygs). The spectral widths were 3000 Hz. The spectra were collected as 512 × 512 blocks of data and were processed by sinusoidal multiplication in each dimension. Other parameters were as follows: number of increments in $t_1$, 256; number of scans, 4; and relaxation delay, 1 s. The HMQC spectra were acquired using standard Bruker software (inv4gstp). These spectra were collected with 512 × 512 data points, a data acquisition of four scans × $F_2$ and 256 increments in $t_1$. Spectral widths of 2500 Hz and 18 000 Hz were employed in the $F_2$ ($^1$H) and $F_1$ ($^{13}$C) domains, respectively. Data were processed using Qsine functions for weighting in both dimensions. The HMBC spectra were obtained using the inv4gslplrnd pulse sequence in the Bruker software and collected with 512 × 512 data points, a data acquisition of 10 scans × $F$ and 256 increments in $t_1$. The spectral widths were 2500 Hz ($F_2$) and 18 000 Hz ($F_1$) and the delays $\Delta_1$ and $\Delta_2$ were set to 3.45 and 65 ms, respectively. Data were processed using an exponential window in $F_2$ with $lb = 5$ Hz and a Qsine window in $F_1$.

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