Synthesis and Fluorescence Properties of Novel Benzo[*a*]phenoxazin-5-one Derivatives

Jiang Long, Yong-mei Wang, Teruo Matsuura and Ji-ben Meng*

Department of Chemistry, Nankai University, Tianjin, P. R. China, 300071

Received January 20, 1999

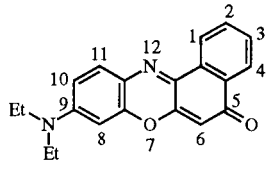
Thirteen, benzo[*a*]phenoxazin-5-one derivatives **3a-m** were synthesized from 4-nitrosoaniline hydrochlorides **1a-m** and ethyl 1,3-dihydroxynaphthoate **2** and their fluorescence properties were discussed in terms of the electronic effect of substituents. A coupling reaction was carried out with 6-carbethoxy-9-*N*-(2-hydroxyethyl)-*N*-methylamino-5*H*-benzo[*a*]phenoxazin-5-one (**3k**) and acetyl-DL-alanine to afford *N*-[(6-carbethoxy-5-oxo-5*H*-benzo[*a*]phenoxazin)-9-yl]-*N*-methylaminoethylene acetyl-DL-alanine ester (**4**).

J. Heterocyclic Chem., **36**, 895 (1999).

Introduction.

The application of near-infrared fluorogenic labels is a more selective and adaptable approach to evaluate various properties of biomolecules (such as DNA, Protein, *etc.*) [1,2,3]. There are few fluorogenic labels commercially available, which can be operated at the far-visible and the near-infrared spectral regions (600-1000 nm) which are areas of low interference. To develop novel near-infrared fluorogenic labels, our group prepared a series of Nile Red analogues, benzo[*a*]phenoxazin-5-one derivatives, and carried out the reaction with acetyl-DL-alanine as a model for labeling proteins.

Scheme 1



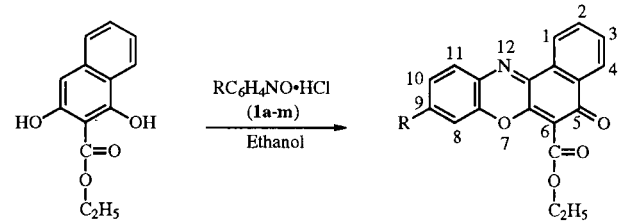
Nile Red

Results and Discussion.

The addition of *N*-methyl-4-nitrosoaniline hydrochloride (**1a**) and a series of *N,N*-disubstituted amino-4-nitrosoaniline hydrochlorides **1b-1l** to a solution of ethyl 1,3-dihydroxynaphthoate (**2**) in ethanol gave 6-carbethoxy-9-*N*-methylamino-5*H*-benzo[*a*]phenoxazin-5-one (**3a**) and 6-carbethoxy-9-*N,N*-disubstitued amino-5*H*-benzo[*a*]phenoxazin-5-ones **3b-3l** respectively. A morpholino derivative, 6-carbethoxy-9-(4-morpholino)-5*H*-benzo[*a*]phenoxazin-5-one **3m** was prepared from the reaction of *N*-(4-nitrosophenyl)morpholine hydrochloride (**1m**) and **2** in the same manner (Scheme 2).

The maxima of the absorption, excitation and fluorescence spectra of 5*H*-benzo[*a*]phenoxazin-5-one derivatives **3a-3m** are shown together with those of Nile Red in Table 1. In general, these compounds have a 5-10% decrease in fluorescence intensity in comparision with that of Nile Red. The shift in the wave length of absorption, excitation and emission maxima due to their structural change was generally as expected. When an alkyl group on the 9-amino substituent was methyl or ethyl group, the increase in the size of another alkyl group caused the maximum to shift to a higher wave length. When both of the alkyl groups in the 9-amino substituent are lengthened symmetrically, the maximum shifted

Scheme 2



2 **3a-m**

a. R = –NH–CH$_3$

b. R = –N(CH$_3$)–CH$_3$

c. R = –N(CH$_3$)–C$_2$H$_5$

d. R = –N(CH$_3$)–*n*-C$_3$H$_7$

e. R = –N(CH$_3$)–*n*-C$_4$H$_9$

f. R = –N(CH$_3$)–*n*-C$_5$H$_{11}$

g. R = –N(C$_2$H$_5$)–C$_2$H$_5$

h. R = –N(C$_2$H$_5$)–*n*-C$_3$H$_7$

i. R = –N(C$_2$H$_5$)–*n*-C$_4$H$_9$

j. R = –N(*n*-C$_3$H$_7$)–*n*-C$_3$H$_7$

k. R = –N(CH$_3$)–CH$_2$CH$_2$OH

l. R = –N(CH$_2$CH$_2$OH)–CH$_2$CH$_2$OH

m. R = morpholino

toward the red region in agreement with the higher electron-releasing inductive effect of the longer chains. If the alkyl groups in the 9-amino substutent were replaced with one or two 2-hydroxyethyl group, the maximum shifted to the violet region 10 nm or so. When a heterocyclic group, morpholino group, was introduced to the 9-position, the absorption maximum shifted to the violet region markedly (30 nm), but other properties were unchanged (Table 1).

Table 1

The Maxima of the Absorption [a], Excitation and Fluorescence [b] Spectra of **3a-3m** and **4**

Compound	λmax/nm	λex/nm	λem/nm
Nile Red	553	572	618
3a	549	571	615
3b	553	576	619
3c	561	582	622
3d	561	581	621
3e	562	582	622
3f	564	589	622
3g	566	586	623
3h	568	583	622
3i	569	583	624
3j	569	585	625
3k	558	578	620
3l	556	576	621
3m	528	560	617
4	558	578	622

[a] Concentration was 10^{-5} *M* in methanol; [b] Concentration was 10^{-6} *M* in methanol.

In fluorogenic labeling a fluorescent molecule normally is bound either covalently or noncovalently to biomolecules. The fluorescence emitted is then recorded. In order to allow labeling of biomolecules such as proteins covalently, a fluorescent molecule must undergo a reaction with functional groups (such as carboxyl group, amino group, *etc.*). A model coupling reaction was undertaken between 9-*N*-(2-hydroxyethyl)-*N*-methylamino-6-carbethoxy-5*H*-benzo[*a*]phenoxazin-5-one **3k** and acetyl-DL-alanine in dichloromethane to provide *N*-[(6-carbethoxy-5-oxo-5*H*-benzo[*a*]phenoxazin)-9-yl]-*N*-methylaminoethylene acetyl-DL-alanine ester **4** which exhibited virtually the same fluorescence property as that of **3k** (Scheme 3). The results imply that a series of compounds **3** may be utilized as a fluorogenic label for proteins.

Among these benzo[*a*]phenoxazin-5-one derivatives **3a-3m**, it was found that some of the compounds crystallized with solvent of crystallization, possibly due to the formation of inclusion compounds. The occurrence of this phenomenon was deduced from elemental analysis and spectral determination. Thus, the solvent of crystallization was water for **3a**, **3e**, **3h**, **3i**, and **3j**, chloroform for **3g**, dichloromethane for **3k** and methanol for **3l**. We have no reasonable interpretation for this phenomenon, since attempts to obtain single crystals suitable for X-ray Crystallographic analysis were unsuccessful.

EXPERIMENTAL

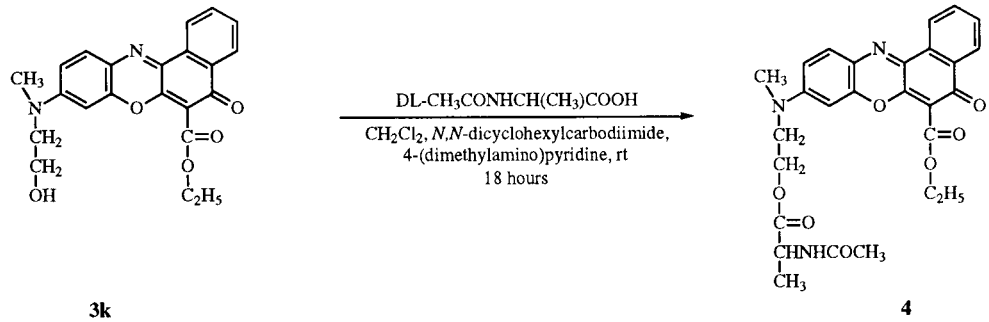
Melting points were determined with a Yanagimoto MP-35 melting point apparatus and reported uncorrected. The ^{1}H nmr spectra were recorded on a Bruker AC-P200 spectrometer at 200 MHz using tetramethylsilane as the internal standard. Coupling constants are given in Hertz. The mass spectra were recorded on a 7070E-HE spectrometer operating in electron impact mode at 70 eV. The ir spectra were recorded on a Bio-Rad FTS135 spectrophotometer. The uv-visible spectra were obtained using a Shimadzu UV-240 spectrometer. Fluorescence spectra were recorded on an RF-540 Shimadzu spectrofluorophotometer.

N,*N*-Dipropylaniline and 4-phenylmorpholine were purchased from ACROS. The 4-nitrosoaniline hydrochlorides **1a-m** and ethyl 1,3-dihydroxynaphthoate **2** were prepared in the manner reported in the literature [4,5].

6-Carbethoxy-9-*N*-methylamino-5*H*-benzo[*a*]phenoxazin-5-one (**3a**).

To a boiling solution of ethyl 1,3-dihydroxynaphthoate (**2**) (10 g, 4.3 mmoles) in absolute ethanol (20 ml) was added *N*-methyl-4-nitrosoaniline hydrochloride (**1a**) (1.2 g, 7.0 mmoles) in small portions over a 1.5 hour period and refluxed for another 3 hours. The ethanol was removed by a rotary evaporator. The residue was dissolved in dichloromethane (20 ml) and washed with water until the water layer became colorless. The organic phase

Scheme 3



3k **4**

was dried over anhydrous sodium sulfate for 2 hours. The solvents were evaporated after removal of the drying agent. The crude product was purified by column chromatography (methanol:benzene = 1:30, v/v) to yield a bluish-purple solid. Recrystallization from dichloromethane and *n*-pentane (1:1, v/v) gave green needles (0.45 g, 30%), mp 240-241°; ir (potassium bromide): ν OH 3369 cm^{-1} (water included), ν CO 1716, 1612 cm^{-1}; ^1H nmr (deuteriochloroform): δ 1.46 (t, 3H, OCH_2CH_3, J = 7.1 Hz), 3.02 (s, 3H, NCH_3), 4.53 (q, 2H, OCH_2CH_3, J = 7.2 Hz), 6.51 (s, 1H, 8-H), 6.90 (br, 1H, 10-H), 7.62-7.89 (m, 3H, 11-, 2-, and 3-H), 8.30 (d, 1H, 1-H, J = 7.3 Hz), 8.65 (d, 1H, 4-H, J = 7.3 Hz); ms: m/z 348 (M^+), 276 ($M-CO_2-C_2H_4$).

Anal. Calcd. for $C_{20}H_{16}N_2O_4 \cdot 0.5H_2O$: C, 67.22; H, 4,79; N, 7.83. Found: C, 67.59; H, 4.41; N, 8.11.

The following compounds were prepared by following the above procedure from the appropriate reactants.

6-Carbethoxy-9-dimethylamino-5*H*-benzo[*a*]phenoxazin-5-one (**3b**).

This compound was prepared from ethyl 1,3-dihydroxynaphthoate (**2**) (1.0 g, 4.3 mmoles) and *N,N*-dimethyl-4-nitrosoaniline hydrochloride (**1b**) (1.2 g, 6.5 mmoles). The crude product was purified by column chromatography (diethyl ether:chloroform = 1:3, v/v). Recrystallization from chloroform gave a red solid (0.50 g, 32%), mp 180-181°; ir (potassium bromide): ν CO 1720, 1640 cm^{-1}; ^1H nmr (deuteriochloroform): δ 1.44 (t, 3H, OCH_2CH_3, J = 7.1 Hz), 3.02 [s, 3H, $N(CH_3)_2$], 3.09 [s, 3H, $N(CH_3)_2$], 4.50 (q, 2H, OCH_2CH_3, J = 6.7 Hz), 6.48 (d, 1H, 8-H, J = 2.2 Hz), 6.71 (dd, 1H, 10-H, J = 10.8, 2.2 Hz), 7.61-7.74 (m, 3H, 11-, 2-, and 3-H), 8.29 (d, 1H, 1-H, J = 7.5 Hz), 8.61 (d, 1H, 4-H, J = 7.6 Hz); ms: m/z 362 (M^+), 290 ($M^+-CO_2-C_2H_4$).

Anal. Calcd. for $C_{21}H_{18}N_2O_4$: C, 69.60; H, 5.01; N, 7.73. Found: C, 70.31; H, 5.09; N, 7.51.

6-Carbethoxy-9-*N*-ethyl-*N*-methylamino-5*H*-benzo[*a*]phenoxazin-5-one (**3c**).

This compound was prepared from ethyl 1,3-dihydroxynaphthoate (**2**) (1.0 g, 4.3 mmoles) and *N*-ethyl-*N*-methyl-4-nitrosoaniline hydrochloride (**1c**) (1.5 g, 7.5 mmoles). The crude product was purified by column chromatography (benzene:chloroform:diethyl ether = 20:10:3, v/v). Recrystallization from chloroform and *n*-pentane (1:5, v/v) gave green needles (0.51 g, 31%), mp 168-169°; ir (potassium bromide): ν CO 1730, 1637 cm^{-1}; ^1H nmr (deuteriochloroform): δ 1.23 (t, 3H, NCH_2CH_3, J = 6.9 Hz), 1.45 (t, 3H, OCH_2CH_3, J = 7.2 Hz), 3.08 (s, 3H, NCH_3), 3.52 (q, 2H, NCH_2CH_3, J = 7.0 Hz), 4.51 (q, 2H, OCH_2CH_3, J = 7.2 Hz), 6.60 (s, 1H, 8-H), 6.89 (d, 1H, 10-H, J = 7.3 Hz), 7.65-7.80 (m, 3H, 11-, 2-, and 3-H), 8.32 (d, 1H, 1-H, J = 7.3 Hz), 8.64 (d, 1H, 4-H, J = 7.2 Hz); ms: m/z 376 (M^+), 304 ($M^+-CO_2-C_2H_4$).

Anal. Calcd. for $C_{22}H_{20}N_2O_4$: C, 70.20; H, 5.36; N, 7.44. Found: C, 70.68; H, 5.13; N, 7.20.

6-Carbethoxy-9-*N*-methyl-*N*-*n*-propylamino-5*H*-benzo[*a*]phenoxazin-5-one (**3d**).

This compound was prepared from ethyl 1,3-dihydroxynaphthoate (**2**) (1.0 g, 4.3 mmoles) and *N*-methyl-*N*-propyl-4-nitrosoaniline hydrochloride (**1d**) (2.1 g, 9.8 mmoles). The crude product was purified by column chromatography (benzene:chloroform = 2:1, v/v). Recrystallization from chloroform and *n*-pentane (1:3, v/v) gave green needles (0.62 g, 37%), mp 132-133°; ir (potassium bromide): ν CO 1714, 1633 cm^{-1}; ^1H nmr (deuteriochloroform): δ 0.96 (t, 3H, $NCH_2CH_2CH_3$, J = 7.3 Hz), 1.45 (t, 3H, OCH_2CH_3, J = 7.2 Hz), 1.67 (sex, 2H, $NCH_2CH_2CH_3$, J = 7.4 Hz), 3.08 (s, 3H, NCH_3), 3.38 (t, 2H, $NCH_2CH_2CH_3$, J = 7.4 Hz), 4.48 (q, 2H, OCH_2CH_3, J = 6.8 Hz), 6.50 (d, 1H, 8-H, J = 2.2 Hz), 6.76 (dd, 1H, 10-H, J = 12, 2.8 Hz), 7.24-7.72 (m, 3H, 11-, 2-, and 3-H), 8.30 (dd, 1H, 1-H, J = 9.0, 1.9 Hz), 8.63 (dd, 1H, 4-H, J = 8.7, 1.9 Hz); ms: m/z 390 (M^+), 361 ($M^+-C_2H_5$).

Anal. Calcd. for $C_{23}H_{22}N_2O_4$: C, 70.75; H, 5.67; N, 7.17. Found: C, 70.22; H, 5.45; N, 7.14.

6-Carbethoxy-9-*N*-*n*-butyl-*N*-methylamino-5*H*-benzo[*a*]phenoxazin-5-one (**3e**).

This compound was prepared from ethyl 1,3-dihydroxynaphthoate (**2**) (1.0 g, 4.3 mmoles) and *N*-*n*-butyl-*N*-methyl-4-nitrosoaniline hydrochloride (**1e**) (1.5 g, 6.6 mmoles). The crude product was purified by column chromatography (benzene:chloroform:diethyl ether = 20:10:1). Recrystallization from chloroform and *n*-pentane (1:5, v/v) gave red solid (0.34 g, 29%), mp 120-121°; ir (potassium bromide): ν OH 3369 cm^{-1} (water included), ν CO 1716, 1638 cm^{-1}; ^1H nmr (deuteriochloroform): δ 0.96 [t, 3H, $N(CH_2)_3CH_3$, J = 6.8 Hz], 1.25-1.62 (m, 5H, OCH_2CH_3, $NCH_2CH_2CH_2CH_3$), 1.65-1.79 (m, 2H, $NCH_2CH_2CH_2CH_3$), 3.07 (s, 3H, NCH_3), 3.43 (t, 2H, $NCH_2(CH_2)_2CH_3$, J = 7.1 Hz], 4.50 (q, 2H, OCH_2CH_3, J = 6.7 Hz), 6.52 (d, 1H, 8-H, J = 2.1 Hz), 6.89 (dd, 1H, 10-H, J = 12.0, 2.2 Hz), 7.61-7.72 (m, 3H, 11-, 2-, and 3-H), 8.32 (d, 1H, 1-H, J = 9.0 Hz), 8.64 (d, 1H, 4-H, J = 9.1 Hz); ms: m/z 404 (M^+), 361 ($M^+-C_3H_7$).

Anal. Calcd. for $C_{24}H_{24}N_2O_4 \cdot 1/4H_2O$: C, 70.48; H, 6.03; N, 6.84. Found: C, 70.58; H, 5.73; N, 7.06.

6-Carbethoxy-9-*N*-*n*-amyl-*N*-methylamino-5*H*-benzo[*a*]phenoxazin-5-one (**3f**).

This compound was prepared from ethyl 1,3-dihydroxynaphthoate (**2**) (1.0 g, 4.3 mmoles) and *N*-*n*-amyl-*N*-methyl-4-nitrosoaniline hydrochloride (**1f**) (1.5 g, 7.5 mmoles). The crude product was purified by column chromatography (benzene:chloroform:diethyl ether = 20:10:3). Recrystallization from chloroform and *n*-pentane (1:5, v/v) gave a green needles (0.55 g, 30%), mp 89-90°; ir (potassium bromide): ν CO 1710, 1635 cm^{-1}; ^1H nmr (deuteriochloroform): δ 0.91 [t, 3H, $N(CH_2)_4CH_3$], 1.20-1.42 (m, 4H, $NCH_2CH_2CH_2CH_2CH_3$), 1.45 (t,3H, OCH_2CH_3, J = 7.2 Hz), 1.71 (m, 2H, $NCH_2CH_2CH_2CH_2CH_3$), 3.05 (s, 3H, NCH_3), 3.38 [t, 2H, $NCH_2(CH_2)_3CH_3$, J = 7.0 Hz], 4.50 (q, 2H, OCH_2CH_3, J = 7.2 Hz), 6.60 (d, 1H, 8-H, J = 2.2 Hz), 6.80 (dd, 1H, 10-H, J = 12.1, 2.2 Hz), 7.60-7.82 (m, 3H, 11-, 2-, and 3-H), 8.30 (d, 1H, 1-H, J = 8.1 Hz), 8.61 (d, 1H, 4-H, J = 8.3 Hz); ms: m/z 418 (M^+).

Anal. Calcd. For $C_{25}H_{26}N_2O_4$: C, 71.75, H, 6.26; N, 6.69. Found: C, 71.96; H, 6.09; N, 6.63.

6-Carbethoxy-9-diethylamino-5*H*-benzo[*a*]phenoxazin-5-one (**3g**).

This compound was prepared from ethyl 1,3-dihydroxynaphthoate (**2**) (1.0 g, 4.3 mmoles) and *N,N*-diethyl-4-nitrosoaniline hydrochloride (**1g**) (1.5 g, 7.0 mmoles). The crude product was purified by column chromatography (benzene:chloroform:diethyl ether = 20:10:3). Recrystallization from chloroform and *n*-pen-

tane (1:1, v/v) gave green needles (0.64 g, 38%), mp 144-145°; ir (potassium bromide): ν CO 1706, 1619 cm^{-1}, ν C-Cl 746 (chloroform included); ^1H nmr (deuteriochloroform): δ 1.25 [t, 6H, $N(CH_2CH_3)_2$, J = 7.1 Hz], 1.44 (t, 3H, OCH_2CH_3, J = 7.2 Hz), 3.44 (q, 4H, $N(CH_2CH_3)_2$, J = 7.0 Hz), 4.52 (q, 2H, OCH_2CH_3, J = 7.2 Hz), 6.51 (s, 1H, 8-H), 6.89 (d, 1H, 10-H, J = 11.2 Hz), 7.24-7.71 (m, 3H, 11-, 2-, and 3-H), 8.29 (d, 1H, 1-H, J = 9.0 Hz), 8.60 (d, 1H, 4-H, J = 9.1 Hz); ms: m/z 390 (M^+), 375 (M^+-CH_3).

Anal. Calcd. for $C_{23}H_{22}N_2O_4 \cdot CHCl_3$: C, 56.54, H, 4.55; N, 5.49. Found: C, 56.95; H, 4.61; N, 5.67.

6-Carbethoxy-9-*N*-ethyl-*N*-*n*-propylamino-5*H*-benzo[*a*]phenoxazin-5-one (**3h**).

This compound was prepared from ethyl 1,3-dihydroxynaphthoate (**2**) (1.0 g, 4.3 mmoles) and *N*-ethyl-*N*-*n*-propyl-4-nitrosoaniline hydrochloride (**1h**) (1.5 g, 6.6 mmoles). The crude product was purified by column chromatography (benzene:chloroform:diethyl ether = 20:10:3). Recrystallization from methanol and water (3:1, v/v) gave green needles (0.61 g, 35%), mp 143-144°; ir (potassium bromide): ν OH 3394 cm^{-1} (water included), ν CO 1724, 1637 cm^{-1}; ^1H nmr (deuteriochloroform): δ 0.97 (t, 3H, $NCH_2CH_2CH_3$, J = 7.1 Hz), 1.25 (t, 3H, NCH_2CH_3, J = 6.9 Hz), 1.41 (t, 3H, OCH_2CH_3, J = 7.2 Hz) 1.68-1.80 (m, 2H, $NCH_2CH_2CH_3$), 3.34 (t, 2H, $NCH_2CH_2CH_3$, J = 7.6 Hz), 3.48 (q, 2H, NCH_2CH_3, J = 7.0 Hz), 4.51 (q, 2H, OCH_2CH_3, J = 7.2 Hz), 6.55 (t, 1H, 8-H, J = 13 Hz), 6.89 (q, 1H, 10-H, J = 13 Hz), 7.65-7.80 (m, 3H, 11-, 2-, and 3-H), 8.32 (d, 1H, 1-H, J = 7.7 Hz), 8.64 (d, 1H, 4-H, J = 7.5 Hz); ms: m/z 404 (M^+), 375 (M^+-C_2H_5).

Anal. Calcd. for $C_{24}H_{24}N_2O_4 \cdot 1/2H_2O$: C, 69.72; H, 6.09; N, 6.78. Found: C, 69.80; H, 5.37; N, 7.18.

6-Carbethoxy-9-*N*-*n*-butyl-*N*-ethylamino-5*H*-benzo[*a*]phenoxazin-5-one (**3i**).

This compound was prepared from ethyl 1,3-dihydroxynaphthoate (**2**) (1.0 g, 4.3 mmoles) and *N*-*n*-butyl-*N*-ethyl-4-nitrosoaniline hydrochloride (**1i**) (1.5 g, 6.2 mmoles). The crude product was purified by column chromatography (diethyl ether:petroleum ether = 1:3). Concentration of the eluent gave a dark purple solid (0.49 g, 27%), mp 99-100°; ir (potassium bromide): ν OH 3392 cm^{-1} (water included), ν CO 1725, 1631 cm^{-1}; ^1H nmr (deuteriochloroform): δ 0.97 [t, 3H, $N(CH_2)_3CH_3$, J = 7.2 Hz), 1.24 (t, 3H, NCH_2CH_3, J = 7.0 Hz), 1.44 (t, 3H, OCH_2CH_3, J = 7.1 Hz), 1.60-1.82 (m, 2H, $NCH_2CH_2CH_2CH_3$), 3.37 (t, 2H, $NCH_2CH_2CH_2CH_3$, J = 8.1 Hz), 3.47 (q, 2H, NCH_2CH_3, J = 7.2 Hz), 4.51 (q, 2H, OCH_2CH_3, J = 7.2 Hz), 6.56 (s, 1H, 8-H), 6.91 (q, 1H, 10-H, J = 9.3 Hz), 7.63-7.72 (m, 3H, 11-, 2-, and 3-H), 8.29 (d, 1H, 1-H, J = 7.3 Hz), 8.62 (d, 1H, 4-H, J = 7.2 Hz); ms: m/z 418 (M^+), 375 (M^+-C_3H_7).

Anal. Calcd. for $C_{25}H_{26}N_2O_4 \cdot 1/2H_2O$: C, 70.23; H, 6.36; N, 6.55. Found: C, 70.52; H, 6.54; N, 6.85.

6-Carbethoxy-9-dipropylamino-5*H*-benzo[*a*]phenoxazin-5-one (**3j**).

This compound was prepared from ethyl 1,3-dihydroxynaphthoate (**2**) (1.0 g, 4.3 mmoles) and *N*,*N*-dipropyl-4-nitrosoaniline hydrochloride (**1j**) (1.1 g, 4.5 mmoles). The crude product was purified by column chromatography (benzene:chloroform:diethyl ether = 20:10:1). Recrystallization from chloroform and *n*-pentane (1:5, v/v) gave green needles (0.60 g, 33%), mp 144-146°; ir (potassium bromide): ν OH 3397 cm^{-1} (water included), ν CO 1728, 1638 cm^{-1}; ^1H nmr (deuteriochloroform): δ 0.97 [t, 6H, $N(CH_2CH_2CH_3)_2$, J = 7.3 Hz], 1.44 (t, 3H, OCH_2CH_3, J = 7.2 Hz), 1.72 [sex, 4H, $N(CH_2CH_2CH_3)_2$, J = 7.5 Hz], 3.34 [t, 4H, $N(CH_2CH_2CH_3)_2$, J = 7.5 Hz], 4.51 (q, 2H, OCH_2CH_3, J = 7.2 Hz), 6.47 (d, 1H, 8-H, J = 2.7 Hz), 6.79 (dd, 1H, 10-H, J = 11, 2.5 Hz), 7.60-7.82 (m, 3H, 11-, 2-, and 3-H), 8.29 (d, 1H, 1-H, J = 7.1 Hz), 8.64 (d, 1H, 4-H, J = 7.2 Hz); ms: m/z 418 (M^+), 389 (M^+-C_2H_5).

Anal. Calcd. for $C_{25}H_{26}N_2O_4 \cdot 1/4H_2O$: C, 70.98; H, 6.31; N, 6.62. Found: C, 70.84; H, 6.56; N, 6.42.

6-Carbethoxy-9-*N*-(2-hydroxyethyl)-*N*-methylamino-5*H*-benzo[*a*]phenoxazin-5-one (**3k**).

This compound was prepared from ethyl 1,3-dihydroxynaphthoate (**2**) (1.0 g, 4.3 mmoles) and *N*-(2-hydroxyethyl)-*N*-methyl-4-nitrosoaniline hydrochloride (**1k**) (1.5 g, 6.5 mmoles). The crude product was purified by column chromatography (methanol:benzene = 1:10). Recrystallization from dichloromethane gave green needles (0.40 g, 24%), mp 188-190°; ir (potassium bromide): ν OH 3405 cm^{-1} (br), ν CO 1772, 1633 cm^{-1}, ν C-Cl 752 cm^{-1} (dichloromethane included); ^1H nmr (dimethyl-d_6 sulfoxide): δ 1.33 (t, 3H, OCH_2CH_3, J = 7.2 Hz), 3.13 (s, 3H, NCH_3), 3.61 (s, 4H, NCH_2CH_2OH), 4.39 (q, 2H, OCH_2CH_3, J = 7.2 Hz), 6.66 (d, 1H, 8-H, J = 2.2 Hz), 6.99 (dd, 1H, 10-H, J = 10, 2.1 Hz), 7.67-7.90 (m, 3H, 11-, 2-, and 3-H), 8.19 (d, 1H, 1-H, J = 9.3 Hz), 8.64 (d, 1H, 4-H, J = 9. 1 Hz); ms: m/z 392 (M^+).

Anal. Calcd. for $C_{22}H_{20}N_2O_5 \cdot CH_2Cl_2$: C, 57.87; H, 4.64; N, 5.87. Found: C, 57.58; H, 4.54; N, 5.57.

6-Carbethoxy-9-bis(2-hydroxyethyl)amino-5*H*-benzo[*a*]phenoxazin-5-one (**3l**).

A mixture of *N*,*N*-bis(2-hydroxyethyl)-4-nitrosoaniline hydrochloride (**1l**) (0.14 g, 0.7 mmole) and ethyl 1,3-dihydroxynaphthoate (**2**) (0.1 g, 0.4 mmole) in dried dimethylformamide (20 ml) was refluxed for 2 hours. The dimethylformamide was removed under diminished pressure.The crude product was purified by column chromatography (acetone:chloroform = 1:1). Recrystallization from methanol and water (3:1, v/v) gave green needles (0.20 g, 60%), mp 222-223°; ir (potassium bromide): ν OH 3393 cm^{-1} (br), ν CO 1704, 1632 cm^{-1}; ^1H nmr (dimethyl-d_6 sulfoxide): δ 1.12-1.22 (m, 2H), 1.33 (t, 3H, OCH_2CH_3, J = 7.2 Hz), 3.32 (s, 3H, CH_3OH, included), 3.62 [s, 8H, $N(CH_2CH_2OH)_2$], 4.38 (q, 2H, OCH_2CH_3, J = 7.0 Hz), 6.71 (d, 1H, 8-H, J = 2.2 Hz), 7.02 (d, 1H, 10-H, J = 10.2 Hz), 7.65-7.82 (m, 3H, 11-, 2-, and 3-H), 8.12 (d, 1H, 1-H, J = 9.0 Hz), 8.55 (d, 1H, 4-H, J = 9.2 Hz); ms: m/z 422 (M^+), 391 (M^+-CH_2OH).

Anal. Calcd. for $C_{23}H_{22}N_2O_6 \cdot CH_3OH$: C, 63.43; H, 5.77; N, 6.16. Found: C, 63.49; H, 5.52; N, 5.57.

6-Carbethoxy-9-(4-morpholino)-5*H*-benzo[*a*]phenoxazin-5-one (**3m**).

This compound was prepared from ethyl 1,3-dihydroxynaphthoate (**2**) (1.0 g, 4.3 mmoles) and *N*-(4-nitrosophenyl)morpholine hydrochloride (**1m**) (1.5 g, 7.5 mmoles). The crude product was purified by column chromatography (benzene:chloroform:diethyl ether = 20:10:3). Concentration of the eluent gave purple solid (0.70 g, 40%), mp 210-212° dec; ir (potassium bromide): ν CO 1717, 1637 cm^{-1}; ^1H nmr (deuteriochloroform): δ 1.44 (t, 3H, OCH_2CH_3, J = 7.3 Hz), 3.36 (t, 4H, morpholino, J = 5.0 Hz), 3.87 (t, 4H, morpholino, J = 5.0 Hz), 4.51 (q, 2H, OCH_2CH_3, J =

7.2 Hz), 6.73 (d, 1H, 8-H, J = 2.2 Hz), 6.91 (dd, 1H, 10-H, J = 9.5, 2.2 Hz), 7.66-7.82 (m, 3H, 11-, 2-, and 3-H), 8.32 (dd, 1H, 1-H, J = 7.1, 1.9 Hz), 8.64 (dd, 1H, 4-H, J = 7.2, 1.9 Hz); ms: m/z 404 (M^+), 332 (M^+-CO_2-C_2H_4).

Anal. Calcd. for $C_{23}H_{20}N_2O_5$: C, 68.31, H, 4.98; N, 6.92. Found: C, 67.92; H, 4.67; N, 6.46.

N-[(6-Carbethoxy-5-oxo-5*H*-benzo[*a*]phenoxazin)-9-yl]-*N*-methylaminoethylene Acetyl-DL-alanine Ester (**4**).

A mixture of 6-carbethoxy-9-*N*-hydroxyethyl-*N*-methylamino-5*H*-benzo[*a*]phenoxazin-5-one (**3k**) (0.2 g, 0.51 mmole), acetyl-DL-alanine (0.1 g, 0.76 mmole), *N*,*N*-dicyclohexylcarbodiimide (0.2 g, 0.98 mmole) and a catalytic quantity of 4-dimethylaminopyridine in dried dichloromethane (50 ml) was stirred at ambient temperature for 18 hours. The filtrate was concentrated after removal of dicyclohexylurea by filtration. The crude product was purified by column chromatography (acetone:benzene = 1:4) to yield a bluish solid (0.20 g, 77%), mp 180-182°; ir (potassium bromide): ν NH 3368 cm^{-1}; ν CO 1750, 1719, 1675, 1623 cm^{-1}; ^1H nmr (deuteriochloroform): δ 1.34 (d, 3H, $CH_3CH(NHCOCH_3)CO$-, J = 7.2 Hz), 1.46 (t, 3H, OCH_2CH_3, J = 7.0 Hz), 1.99 (s, 3H, -$COCH_3$), 3.13 (s, 3H, NCH_3), 3.75 (t, 2H, -NCH_2CH_2O-, J = 5.0 Hz), 4.37 (t, 2H, -NCH_2CH_2O-, J = 4.4 Hz), 4.51 (q, 2H, OCH_2CH_3, J = 7.2 Hz), 6.30 [br, 1H, $CH_3CH(NHCOCH_3)CO$-], 6.56 (d, 1H, 8-H, J = 2.2 Hz), 6.89 (d, 1H, 10-H, J = 9.0 Hz), 7.34 (s, 1H, NH), 7.63-7.80 (m, 3H, 11-, 2-, and 3-H), 8.34 (d, 1H, 1-H, J = 7.3 Hz), 8.74 (d, 1H, 4-H, J = 7.2 Hz); ms: m/z 505 (M^+).

Anal. Calcd. for $C_{27}H_{27}N_3O_7$: C, 64.15; H, 5.38; N, 8.31. Found: C, 63.97; H, 5.21; N, 8.03.

Acknowledgment.

This study was supported by the National Natural Science Foundation of China and Doctoral Programme from the State Education Commission Foundation of China.

REFERENCES AND NOTES

[*] Author to whom correspondence should be addressed.

[1] M. S. J. Briggs, I. Bruce, J. N. Miller, C. J. Moody, A. C. Simmonds and E. Swann, *J. Chem. Soc., Perkin Trans., 1,* 1051 (1997).

[2] G. Patonary and M. D. Antoine, *Anal. Chem.,* **63**, 321A (1991).

[3] F. V. Bright, *Anal. Chem.,* **60**, 1031A (1988).

[4] M. L. Crossley, P. F. Dreisbach, C. M. Hofmann and R. P. Parker, *J. Am. Chem. Soc.,* **74**, 573 (1952)

[5] K. Meyer and H. S. Bloch, *Org. Synth. Coll.*, Vol. **3**, 132.