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Synthesis and Cytostatic Activity of Enynes, Enediynes and Dienediynes Linked to Intercalators

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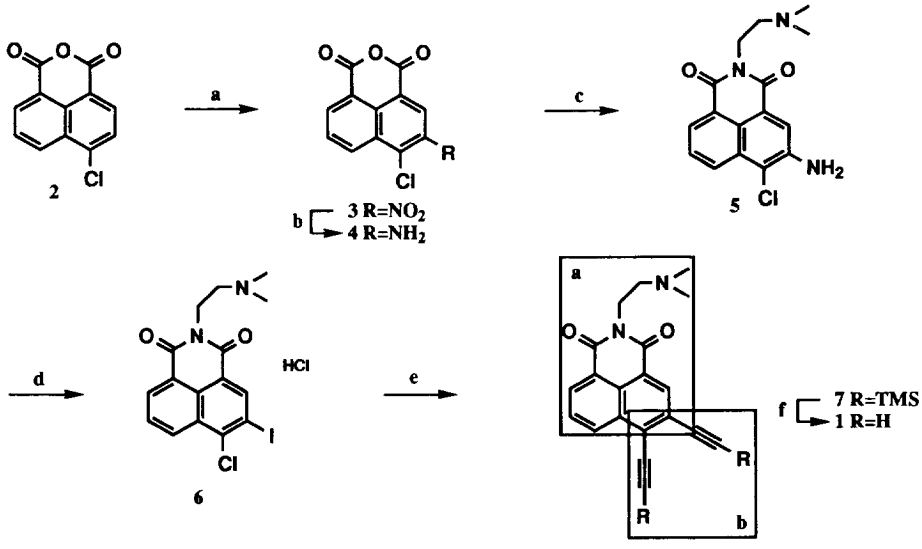
Abstract: We describe here the synthesis of several compounds that include intercalators such as naphthalimide or fluorene, linked to different systems of double and triple bonds. An unusual behaviour of the 9-fluorenylidenetriphenylphosphorane **9** with acetylenic carbonyl compounds, both aldehyde or ketone, is also reported. The isolated products are the result of a [2+2] cycloaddition between the triple bond and the C=P double bond, unreported for acetylenic aldehydes or ketones. None of the compounds described have shown any interesting cytotoxic activity.

In recent years a new type of anticancer agent, the enediyne antibiotics[1], characterized by a unique molecular structure and an interesting biological profile, has been described. Two of them, neocarzinostatin[2] and dynemicin A[3], combine properties of both the enediyne antibiotics and the intercalating agents, carrying the former a naphtoate moiety and the latter an anthraquinone chromophore in their structure. These compounds have stimulated intense synthetic studies in order to obtain highly simplified analogues that could be developed as antitumor agents[4].

During the course of research directed towards obtaining enediyne models [5], we projected the synthesis of potential antitumor compounds that possess an intercalating chromophore joined to a simple system of double and triple bonds, mimicking the enediyne unit of the above mentioned antibiotics. Therefore we have selected, in order to study the influence on the antitumor activity, intercalating moieties different from the ones of neocarzinostatin and dynemicin A, such as the naphthalimide or the fluorene ring, that are present in the structure of well-known anticancer drugs, such as Amonafide[6] and Tilorone[7].

In this context, the first compound synthesized **1** includes the naphthalimide chromophore (a) and an enediyne system (b) in which the double bond of the Z-1,2-enediyne belongs to the aromatic nucleus. The synthetic route followed to obtain compound **1** is depicted in Scheme 1.

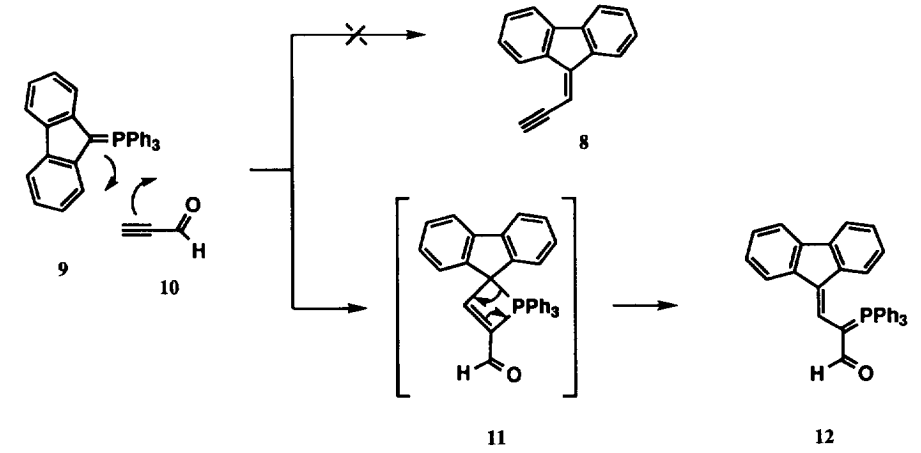
Commercially available anhydride **2** was nitrated to give **3** in 93% yield[8]. Reduction with $SnCl_2$ saturated from hydrogen chloride led to the aminoderivative **4** in 70% yield. Condensation with N,N-dimethylaminoethylamine afforded imide **5** (81%). Diazotization of the amino group of **5** and substitution by iodo gave rise to **6** as the hydrochloride (60%). Coupling of the dihalogenated compound **6** with 2 equivalents of trimethylsilylacetylene was accomplished via Pd(0)-Cu(I) catalysis[9] to provide **7** in 44% yield. Removal of the silyl groups gave enediyne **1** in 26% yield.



Scheme 1

a) HNO_3, H_2SO_4, RT. b) $SnCl_2$, HCl, 0°C. c) $(CH_3)_2N$-$(CH_2)_2$-NH_2, EtOH, RT. d) HNO_2, KI, 0°C. e) TMSC≡CH, CuI, $Pd(PPh_3)_4$, Et_3N, Δ. f) K_2CO_3, MeOH, RT.

On the other hand, using the fluorene ring as chromophore, the synthesis of enyne **8** was attempted via a Wittig reaction between the 9-fluorenylidenetriphenylphosphorane **9** and propargylic aldehyde[10] **10** (Scheme 2). Unexpectedly, a complex mixture was obtained from which only product **12** could be isolated, in low yield (27%). The structural assignment of compound **12** was based on its spectral and analytical data and confirmed by X-ray diffraction.



Scheme 2

The IR spectrum shows a strong absorption band at 1440 cm^{-1}, which can be attributed to the C-P bond stretching. The absence of bands at 3300 and 2100 cm-1 provides support for the lack of a triple bond in the structure of compound **12**. The ^{1}H NMR spectrum shows, among others, two doublets, one at δ=6.75 ppm (J_{H-P}=14.4 Hz) due to the vinylic proton and the other at δ=9.68 ppm (J_{H-P}=28.8 Hz) characteristic of the aldehydic proton; in the ^{13}C NMR the -CHO signal appears at δ=184.7 ppm (J_{C-P}=6.0 Hz). The ^{31}P NMR shows a singlet at δ=19.96 ppm characteristic of the phosphorus atom of a phosphorane[10]. The mass spectrum shows the molecular ion peak at 480 (42%), being the maximum intensity peak the one corresponding to the triphenylphosphine oxide fragment at 287 (100%).

To unambiguously identify this compound, an X-ray structure determination was performed (Figure 1). Compound **12** would appear to be the result of a [2+2] cycloaddition between the double bond of the phosphorane and the triple bond of the propargylic aldehyde via the phosphacyclobutene intermediate **11** (Scheme 2). An analogous addition has already been described by Brown et al.[12] for the reaction of dimethylacetylenecarboxylates with phosphoranes. However, the addition of phosphoranes to acetylenic bonds when a carbonyl group, either aldehyde or ketone, is present at the molecule has never been reported. In all of the other examples found in the literature, the reaction products are the Wittig ones[13].

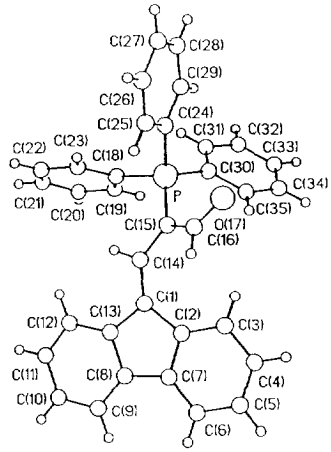
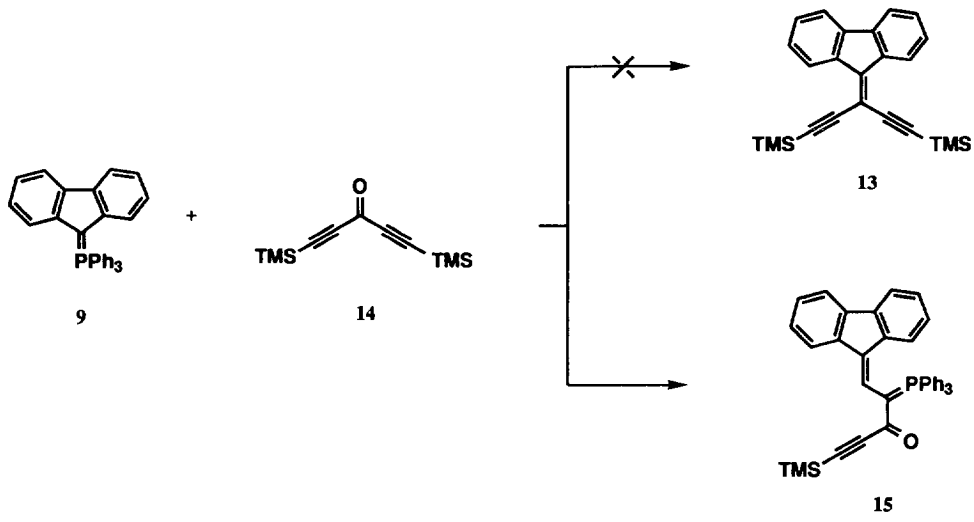


Figure 1. Crystal structure of compound **12**.

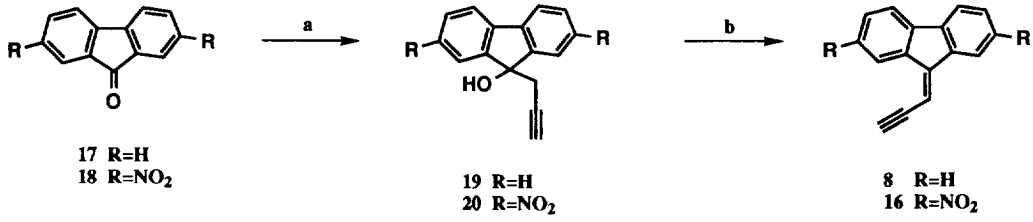
A similar result was obtained when we tried to synthesize compound **13**, which contains an enediyne group linked to the fluorene ring. Reaction of bis(trimethylsilyl)ethynylketone[14] **14** with the phosphorane **9** gave rise to compound **15** (Scheme 3) with 35% yield, which was characterized on the basis of its spectroscopic and analytical data.

The IR spectrum of **15** shows a strong absorption band at 1740 cm^{-1} due to the carbonyl group, and at 1440 cm^{-1} corresponding to the C-P stretching. The ^{1}H NMR shows, among others, a singlet at δ= -0.37 ppm characteristic of the trimethylsilyl protons and a doublet at δ=6.53 ppm (J_{H-P}=7.6 Hz) due to the vinylic proton. In the ^{13}C NMR spectrum the chemical shifts of the acetylenic bond appear at δ=79.1 and 94.2 ppm, while the carbonyl group appears at δ=168.2 ppm. The ^{31}P NMR shows a singlet at δ=20.38 ppm. The mass spectrum shows the [M^{+}-1] peak at 575 (60%) and a characteristic fragment corresponding to triphenyl-phosphine oxide at 287 (68%).



Scheme 3

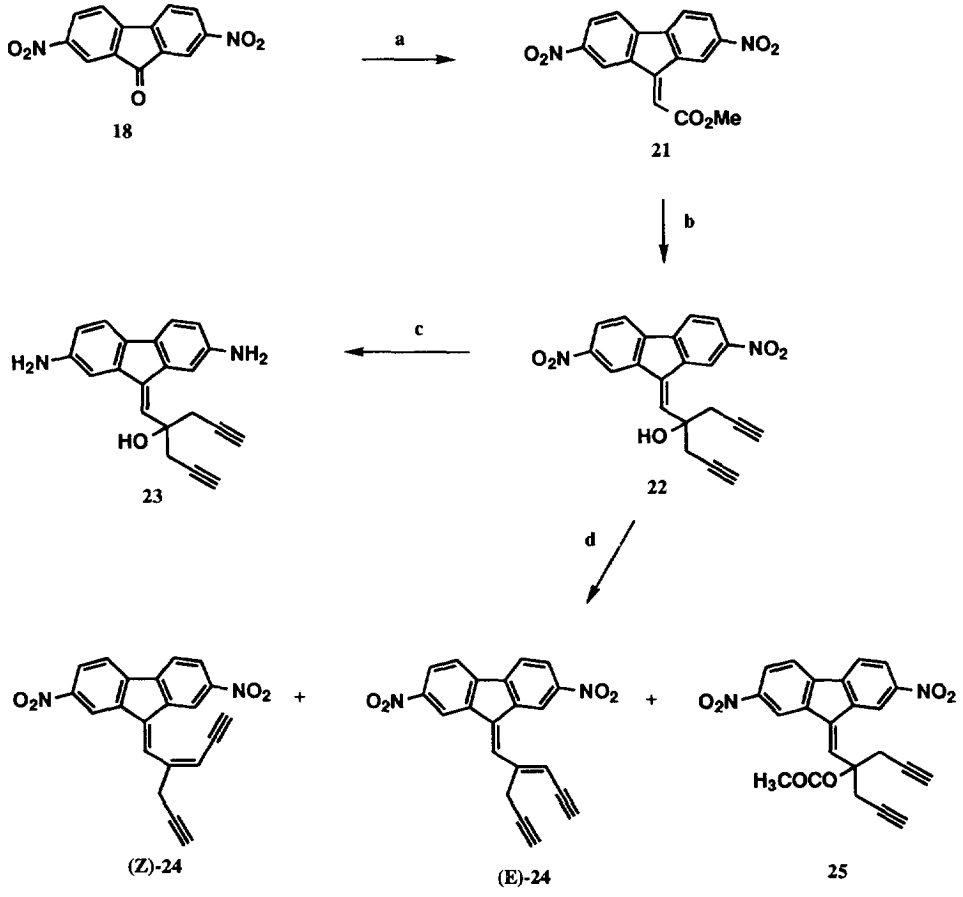
Due to the previously described results in this report, compound **8** was prepared following a new synthetic route represented in Scheme 4. Compounds **8** and **16** were synthesized in two steps, **16** being the corresponding homologue of **8** with nitro groups at positions 2 and 7 of the fluorene ring. The introduction of nitro groups at the ring is supposed to diminish the electronic density of the chromophore in order to facilitate the possible formation of a charge transfer complex with DNA[15] that will favour binding and hence lead to an increment in the activity. The first step consists of the addition of the organoaluminic, derived from propargyl bromide, to the carbonyl group of 9-fluorenone **17** and **18** to give alcohols **19**[16] and **20**, respectively. Dehydration of both alcohols by treatment with formic acid[17] gave rise to the desired compounds **8** and **16** in 49 and 28% yield respectively. Compound **8** has to be stored under -40°C, because it polymerizes at room temperature.



a) HC≡C-CH_2Br, Al, Δ-RT. b) HCOOH, RT(**8**), Δ(**16**).

Scheme 4

Other acyclic homologues structurally related to the neocarzinostatin chromophore and the C-1027 chromophore[18] containing a dienediyne system linked to position 9 of the fluorene ring were prepared as shown in Scheme 5. Treatment of 2,7-dinitro-9-fluorenone **18** with methoxycarbonylmethylidentriphenylphosphorane afforded compound **21** in 70% yield. Addition of two equivalents of the organoaluminic derived from propargyl bromide to the ester **21** led to alcohol **22** that possesses an enediyne system in its structure. In order to compare the influence of the nitro and amino groups on the antitumor activity, the aminoderivative **23** was prepared by a selective reduction of the nitro groups of **22** with Zn and $CaCl_2$[19] to yield **23** (62%). Several procedures were used to dehydrate alcohol **22** to obtain the targeted dienediyne **24**. Both reflux with formic acid and treatment with iodine/quinoleine were unsuccessful. Finally, reaction of alcohol **22** with acetic anhydride and a catalytic amount of sulfuric acid led to a mixture of the expected olefine **24** and the acetylation product **25**.



a) $Ph_3P{=}CHCO_2Me$, CH_2Cl_2, RT. b) $HC{\equiv}C\text{-}CH_2Br$, Al, Δ. c) Zn, $CaCl_2$, H_2O, EtOH, Δ. d) Ac_2O, H_2SO_4, Δ.

Scheme 5

A ^{1}H NMR-COSY experiment confirms that compound **24** is a mixture of Z+E isomers in which the signals of each isomer were not possible to be distinguished. However, the ^{13}C NMR spectrum shows two different signals for the methylene group in position α to the triple bond of each isomer at 24.8 and 27.7 ppm. Usually, the Z isomer appears at higher fields therefore tentatively the signal at 24.8 ppm can be assigned to this isomer. Accordingly, the relative proportion between the Z/E isomers was calculated on the basis of the intensity distribution of both peaks in the ^{13}C NMR spectrum. It has been found that there is a ratio of 2:1 for the Z/E isomers.

The compounds were evaluated for cytotoxic activity in a standard monolayer cell culture essay. The growth of the HT-29 cell line (human colon carcinoma) was determined at several drug concentrations. The activity of each compound was compared to Amonafide[6], a known naphthalimide intercalating agent. Results are summarized in Table 1.

Table 1

Compound	IC_{50} (mol/l)
1	3.45×10^{-5}
8	1.00×10^{-5}
16	1.56×10^{-3}
22	3.80×10^{-5}
23	1.00×10^{-4}
24	1.00×10^{-5}
25	3.20×10^{-5}
Amonafide	3.80×10^{-7}

As shown in Table 1, none of the described compounds exhibit higher antitumor activity than the model Amonafide, therefore further studies are not justified. Examination of the selectivity on hypoxic cells of the nitroderivatives (**16**, **22**, **24** and **25**) is currently being performed and will be published elsewhere.

EXPERIMENTAL

General analytical TLC was carried out on E. Merck precoated HPTLC silica gel plates (60 F_{254}) with detection by U.V. light. Column chromatography was performed using E. Merck 230-400 mesh silica gel. Melting points were determined on a Büchi 535 apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 1310; band positions are indicated in wavenumbers. ^{1}H NMR, ^{13}C NMR and ^{31}P NMR spectra were recorded on a Brüker AC-200 or a Varian Unity-300 using $CDCl_3$ or DMSO-d_6 as solvent. In both ^{1}H NMR and ^{13}C NMR, chemical shifts are reported in δ units downfield from tetramethylsilane and in ^{31}P NMR spectra from phosphoric acid. EI mass spectra were obtained on a VG Auto Spec mass spectrometer.

X-ray Crystallography: Compound **12** was recrystallized from acetonitrile. Crystal data: $C_{34}H_{25}OP$, M=480.5, crystal dimensions 0.32x0.25x0.20 mm^3, monoclinic, a=9.762(2), b=21.360(5), c=12.835(3) Å, β=110.34(2)°, V=2509.4 $Å^3$, F(000)=1008, space group $P2_1/c$, Z=4, Dc=1.272 g cm^{-1}, μ(Cu Kα)=11.4 cm^{-1}, T=294 K, 3698 reflections measured (2θ<115°) of which 3346 were unique and 2487 observed (|Fo|>4σ|Fo|). Data were measured on a Nicolet $P2_1$ diffractometer with graphite monochromated Cu Kα radiation using θ/2θ scans. Structure solution used direct methods (SHELXS-86) and refinement (SHELXTL PLUS) with anisotropic displacement parameters for all nonhydrogen atoms. Hydrogen atoms were refined with geometric constraints and isotropic displacement parameters. With a total of 351 parameters and using 2486 reflections, final residuals were R=0.046, Rw=0.049, $W^{-1}=\sigma^2(Fo)+ 3.5x10^{-4} Fo^2$, maximum residual electron density=0.19 $eÅ^{-3}$.

Propargylic aldehyde[9] **10** and Bis(trimethylsilyl)penta-1,4-diyn-3-one[13] **14** were prepared following the methods described in the literature.

4-Chloro-3-nitro-1,8-naphthalendicarboxylic anhydride (3). To a solution of 4-chloro-1,8-naphthalendicarboxylic anhydride **2** (3 g, 13 mmol) in sulfuric acid (10.5 ml), was added with stirring a mixture of sulfuric acid (2.7 ml) and nitric acid (1.9 ml) with caution. The reaction mixture was stirred for 1 h. The resulting solution was poured into ice/water (50 ml). The precipitated solid was collected by filtration and washed with water. Crystallization from toluene gave **3** as white needles: 3.6 g (93%) m. p. 220-222°C (Lit. 222-223°C)[7].

3-Amino-4-chloro-1,8-naphthalendicarboxylic anhydride (4). **3** (5 g, 18 mmol) was added at 0°C to a previously prepared solution of $SnCl_2$ (15.5 g, 81.5 mmol) in glacial acetic acid (37 ml) saturated from hydrogen chloride. The reaction mixture was stirred for 1 h. The precipitated solid was collected by filtration and washed with water. Crystallization from acetic acid gave **4** as orange needles: 3.1 g (70%) m. p. 258-259°C[(d)]. IR (KBr) ν: 3460 (NH_2), 3370 (NH_2), 1770 (CO), 1730 (CO), 1630, 1560, 1420, 1280, 1150, 1030. ^1H NMR (DMSO-d_6, 200 MHz) δ: 6.39 (s, 2H, NH_2), 7.75 (dd, 1H, J=8.5, 7.2 Hz, H_6), 8.04 (s, 1H, H_2), 8.12 (dd, 1H, J=7.2, 0.6 Hz, H_5), 8.21 (dd, 1H, J=8.5, 0.6 Hz, H_7). Anal. Calcd. for $C_{12}H_6ClNO_3$: C, 58.20; H, 2.44; N, 5.65. Found: C, 58.12; H, 2.36; N, 5.82.

5-Amino-6-chloro-2-N,N-dimethylaminoethylbenz[d,e]isoquinolein-1,3-dione (5). To a suspension of **4** (5 g, 20 mmol) in ethanol (75 ml), a solution of N,N-dimethylaminoethylamine (1.7 g, 20 mmol) in ethanol (18 ml) was added. The reaction mixture was stirred at room temperature for 24 h. The precipitated solid was collected by filtration and crystallized from ethanol to give **5** as yellow needles: 5.1 g (81%) m. p. 201-202°C. IR (KBr) ν: 3420 (NH_2), 1690 (CO), 1660 (CO), 1620, 1430, 1340, 780. ^1H NMR (DMSO-d_6, 200 MHz) δ: 2.18 (s, 6H, -$N(CH_3)_2$), 2.46 (t, 2H, J=6.8 Hz, -CH_2-$\underline{CH_2}$-$N(CH_3)_2$), 4.09 (t, 2H, J=6.8 Hz, -$\underline{CH_2}$-CH_2-$N(CH_3)_2$), 6.32 (s, 2H, NH_2), 7.75 (t, 1H, J=7.9 Hz, H_8), 8.07 (s, 1H, H_4), 8.13 (d, 1H, J=7.3 Hz, H_7), 8.20 (d, 1H, J=8.5 Hz, H_9). Anal. Calcd. for: $C_{16}H_{16}ClN_3O_2$: C, 60.47; H, 5.07; N, 13.22. Found: C, 60.23; H, 4.73; N, 13.42.

6-Chloro-2-N,N-dimethylaminoethyl-5-iodobenz[d,e]isoquinolein-1,3-dione hydrochloride (6). To a solution of **5** (5 g, 15 mmol) in HCl 22% (153 ml) at 0°C was added a solution of $NaNO_2$ (1.6 g, 23 mmol) in water (33 ml). The reaction mixture was stirred for 2 h at 0°C. The resulting precipitated was added to a solution of KI (12.4 g, 75 mmol) in water (64 ml) and stirring was continued for 1 h. A solution of $NaHSO_3$ in water was added and the solid formed was collected by filtration. Reflux in acetonitrile for 4 h gave **6** as an orange solid: 4.5 g (60%), m.p. 265-266°C[(d)]. IR (KBr) ν: 3600-3300 ($(CH_3)_2NH^+$), 2900, 2700 ($(CH_3)_2NH^+$), 1700 (CO), 1660 (CO), 1580, 1340, 1230, 780. ^1H NMR (DMSO-d_6, 200 MHz) δ: 2.88 (s, 6H, $-N(CH_3)_2$), 3.40 (t, 2H, J=6.3 Hz, $-CH_2-\underline{CH_2}-N(CH_3)_2$), 4.35 (t, 2H, J=6.3 Hz, $-\underline{CH_2}-CH_2-N(CH_3)_2$), 7.99 (t, 1H, J=7.9 Hz, H8), 8.56 (d, 1H, J=7.3 Hz, H7), 8.67 (d, 1H, J=9.5 Hz, H9), 8.69 (s, 1H, H4), 9.32 (s, 1H, $(CH_3)_2\underline{NH}^+$). Anal. Calcd. for: $C_{16}H_{15}Cl_2IN_2O_2$: C, 41.29; H, 3.22; N, 6.02. Found: C, 41.55; H, 3.53; N, 6.42.

2-N,N-Dimethylaminoethyl-5,6-bis(trimethylsilylethynyl)benz[d,e]isoquinolein-1,3-dione (7). To a suspension of **6** (3.2 g, 6.9 mmol) in triethylamine (32 ml) under N_2, CuI (0.13 g, 0.7 mmol), $Pd(PPh_3)_4$ (0.46 g, 0.4 mol) and trimethylsilylacetylene (2.8 ml, 19.3 mmol) were added. The reaction mixture was stirred at reflux temperature for 6 h. The suspension was filtered and washed with ethyl acetate. The organic layer was washed with water, dried over anhydrous $MgSO_4$ and the solvent removed in vacuo. The residual material was purified by column chromatography (hexane/ethyl acetate, 3:7) to give **7** as a white solid: 1.4 g (44%) m.p. 135-136°C. IR (KBr) ν: 2960, 2150 (-C≡C-), 1700 (CO), 1670 (CO), 1600, 1400, 1360, 1330, 1250 ($-Si(CH_3)_3$), 850 ($-Si(CH_3)_3$), 760 ($-Si(CH_3)_3$). ^1H NMR ($CDCl_3$, 200 MHz) δ: 0.31 (s, 9H, $-Si(CH_3)_3$), 0.37 (s, 9H, $-Si(CH_3)_3$), 2.50 (s, 6H, $-N(CH_3)_2$), 2.85 (t, 2H, J=6.7 Hz, $-CH_2-\underline{CH_2}-N(CH_3)_2$), 4.39 (t, 2H, J=6.7 Hz, $-\underline{CH_2}-CH_2-N(CH_3)_2$), 7.80 (t, 1H, J=7.8 Hz, H8), 8.55-8.63 (m, 3H, H4, H7, H9). ^{13}C NMR ($CDCl_3$, 50 MHz) δ: -0.1 ($-Si(CH_3)_3$), 38.0 ($-\underline{CH_2}-CH_2-N(CH_3)_2$), 45.6 ($-N(CH_3)_2$), 56.8 ($-CH_2-\underline{CH_2}-N(CH_3)_2$), 99.8, 101.8, 102.3, 109.9 (-C≡C-), 121.6, 122.6, 125.3, 126.8, 127.8, 128.8, 131.5, 132.3, 133.4 (C_{arom}), 162.9, 163.4 (CO). Anal. Calcd. for $C_{26}H_{32}N_2O_2Si_2$: C, 67.77; H, 7.00; N, 6.08. Found: C, 67.52; H, 6.89; N, 6.12.

5,6-Diethynyl-2-N,N-dimethylaminoethylbenz[d,e]isoquinolein-1,3-dione (1). To a stirred solution of **7** (1 g, 2.2 mmol) in methanol (10 ml), anhydrous K_2CO_3 (0.09 g, 0.6 mol) was added. The reaction mixture was stirred for 4 h. The solution was concentrated to dryness and the residual solid was purified by column chromatography (dichloromethane/acetone, 3:7) to give **1** as a white solid: 0.18 g (26%), m.p. 149-150°C[(d)]. IR (KBr) ν: 3250 (≡CH), 2100 (-C≡C-), 1700 (CO), 1660 (CO), 1600, 1400, 1330, 790. ^1H NMR (DMSO-d_6, 200 MHz) δ: 2.19 (s, 6H, $-N(CH_3)_2$), 2.46 (t, 2H, J=6.9 Hz, $-CH_2-\underline{CH_2}-N(CH_3)_2$), 4.07 (t, 2H, J=6.9 Hz, $-\underline{CH_2}-CH_2-N(CH_3)_2$), 4.80 (s, 1H, ≡CH), 5.35 (s, 1H, ≡CH), 7.88 (dd, 1H, J=8.3, 6.4 Hz, H8), 8.19 (s, 1H, H4), 8.41 (d, 1H, J=6.4 Hz, H7), 8.43 (d, 1H, J=8.3 Hz, H9). ^{13}C NMR (DMSO-d_6, 50 MHz) & 37.7 ($-\underline{CH_2}-CH_2-N(CH_3)_2$), 45.3 ($-N(CH_3)_2$), 58.2 ($-CH_2-\underline{CH_2}-N(CH_3)_2$), 78.4, 80.9, 87.8, 94.8 (-C≡CH), 121.5, 122.0, 123.8, 125.8, 127.5, 128.7, 130.7, 131.3, 131.6 (C_{arom}), 161.7, 162.4 (CO). M. S. m/z (%): 316 (M^+,79), 285 (6),272 (21), 258 (30), 245 (15), 228 (35), 200 (47), 188 (19), 174 (100), 149 (13), 123 (5), 100 (5), 87 (8). Anal. Calcd. for $C_{20}H_{16}N_2O_2$: C, 75.95; H, 5.06; N, 8.86. Found: C, 75.82; H, 5.15; N, 8.64.

9-(2-Formyl-2-triphenylphosphoranyliden)ethylidenfluorene (12). To a solution of propargylic aldehyde **10** (5.7 g, 101 mmol) in dry dichloromethane (150 ml) under N_2, 9-fluorenyliden-triphenylphosphorane **9** (5 g, 11.7 mmol) was added. The reaction mixture was stirred at room temperature for 15 h. The solution was concentrated to dryness, and the residual material was purified by column chromatography (dichloromethane/ethyl acetate, 97:3) followed by crystallization from acetonitrile to give **12** as yellow needles: 1.5 g (27%), m.p. 234-235°C. IR (KBr) ν: 3050, 1590, 1560, 1440 (C-P), 1300, 1280, 1100, 890, 730, 680. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.75 (d, 1H, J_{H-P}=14.4 Hz, =CH), 7.76-7.81 (m, 22H, H_{arom}), 8.40 (dd, 1H, J=8.4, 1.8 Hz, H_{arom}), 9.68 (d, 1H, J_{H-P}=28.8 Hz, -CHO). ^{13}C RMN ($CDCl_3$, 75 MHz) δ: 118.3 119.3, 119.6, 122.8, 123.3, 124.5, 125.3, 125.8, 126.0, 126.7, 127.2, 129.2, 129.3, 132.9, 133.9, 134.8, 134.9, 135.1, 136.8, 139.2, 148.4 (-C=C-), 184.7 (d, J_{C-P}=6.0 Hz, CHO). ^{31}P RMN ($CDCl_3$, 125 MHz) δ: 19.96. M.S. m/z (%): 480 (M^+,42), 287 (100), 277 (42), 262 (24), 202 (25), 183 (5). Anal. Calcd. for $C_{34}H_{24}OP$: C, 84.91; H, 5.20. Found: C, 84.70; H, 5.30.

9-(3-Oxo-2-triphenylphosphoranyliden-5-trimethylsilyl-4-pentynyliden)fluorene (15). To a stirred solution of **9** (1 g, 2.5 mmol) in toluene (30 ml), bis(trimethylsilyl)penta-1,4-diyn-3-one **14** (1.1 g, 5 mol) was added in THF/Et_2O (4:1) (100 ml). The reaction mixture was stirred at 54°C for 8 h. The solution was concentrated to dryness and the residual solid was crystallized from ethyl acetate to give **15** as red needles: 0.5 g (35%) m.p. 182-183°C. IR (KBr) ν: 1740 (CO), 1590, 1510, 1460, 1440 (C-P), 1340, 1250 (-Si(CH_3)3), 1160, 850 (-Si(CH_3)3), 765 (-Si(CH_3)3), 690. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: -0.37 (s, 9H, -Si(CH_3)3), 6.53 (d, 1H, J=7.6 Hz, =CH), 7.10-7.70 (m, 22H, H_{arom}), 8.15 (dd, 1H, J=5.5, 2.7 Hz, H_{arom}). ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 1.4 (-Si(CH_3)3), 79.1, 94.2 (-C≡C-), 104.3, 104.7, 118.7, 118.9, 123.3, 124.3, 124.5, 125.1, 125.9, 126.1, 126.2, 128.8, 129.0, 132.4, 132.5, 132.9, 133.6, 133.8, 137.5, 138.2, 139.5, 140.2 (-C=C-), 168.2 (d, J_{C-P}=9.6 Hz, CO). ^{31}P NMR ($CDCl_3$, 125 MHz) δ: 20.38. M.S. m/z (%): 575 (M^+-1,60), 298 (69), 287 (68), 283 (76), 277 (100), 239 (15), 215 (5), 201 (25), 183 (62), 152 (16), 108 (20), 77 (36). Anal. Calcd. for $C_{39}H_{33}OPSi$·1EtOAc: C, 77.69; H, 6.20. Found: C, 77.90; H, 6.00.

9-Hydroxy-9-(2-propynyl)fluorene (19). To a mixture of Al powder (1.8 g, 69 mmol) and a catalytic amount of $HgCl_2$ in Et_2O (15 ml), was added dropwise propargyl bromide (10.1 g, 69 mmol) in Et_2O (70 ml) over 20 min. The reaction mixture was refluxed for 5 h. After cooling, 9-fluorenone **17** (4.8 g, 27 mmol) was added in THF (100 ml) and stirred at room temperature for 30 min. The mixture was acidified with 10% HCl and extracted with ethyl acetate. The organic layer was dried over anhydrous $MgSO_4$ and the solvent was removed in vacuo. The residual solid was crystallized from toluene to give **19** as white needles: 3.5 g (60%) m.p. 104-105°C (Lit. 103-104°C)[14].

9-Hydroxy-2,7-dinitro-9-(2-propynyl)fluorene (20). To a mixture of Al powder (1.2 g, 46 mmol) and a catalytic amount of $HgCl_2$ in Et_2O (10 ml) was added dropwise propargyl bromide (6.8 g, 46 mmol) in Et_2O (50 ml) over 20 min. The reaction mixture is refluxed for 5 h. After cooling, 2,7-dinitro-9-fluorenone **18** (4.8 g, 27 mmol) was added in THF (100 ml) and stirred at room temperature for 30 min. The mixture was acidified with 10% HCl and extracted with ethyl acetate. The organic layer was dried over anhydrous $MgSO_4$ and the solvent was removed in vacuo. The residual material was purified by column chromatography (hexane/ethyl acetate, 6:4) to give **20** as a yellow solid: 3.5 g (63%) m.p. 189-190°C. IR (KBr) ν: 3420 (OH),

3280 (≡CH), 3100, 2120 (-C≡C-), 1620, 1590, 1530 (NO_2), 1340 (NO_2), 1080, 840, 740, 670. ^1H NMR (DMSO-d_6, 200 MHz) δ: 2.64 (t, 1H, J=2.4 Hz, ≡CH), 3.14 (d, 2H, J=2.4 Hz, -CH_2-), 6.52 (s, 1H, OH), 8.24 (d, 2H, J=8.4 Hz, H_4, H_5), 8.37 (dd, 2H, J=8.4, 2.0 Hz, H_3, H_6), 8.53 (d, 2H, J=2.0 Hz, H_1, H_8). ^{13}C NMR (DMSO-d_6, 50 MHz) δ: 29.2 (-CH_2-), 72.9, 79.4 (-C≡CH), 79.6 (-C-OH), 119.1, 122.6, 125.1, 143.2, 148.1, 151.4 (C_{arom}). M.S. m/z (%): 310 (M^+,7), 271 (100), 225 (93), 179 (40), 150 (49), 139 (18), 98 (7), 74 (12), 63 (7). Anal. Calcd. for $C_{16}H_{10}N_2O_5$: C, 61.93; H, 3.25; N, 9.00. Found: C, 61.72; H, 3.40; N, 8.82.

9-(2-Propynyliden)fluorene (8). (0.5 g, 2.3 mmol) of **19** was dissolved in formic acid (35 ml). The reaction mixture was stirred at room temperature for 2 h. The resulting solution was diluted with EtOAc and washed with water. The organic layer was dried over anhydrous $MgSO_4$ and the solvent was removed in vacuo. The residual material was purified by column chromatography (hexane/EtOAc, 7:3) to give **8** as a brown oil: 0.22 g (49%). IR (film) v: 3280 (≡CH), 2180 (-C≡C-), 1600, 1440, 1380, 1280, 770, 730. ^1H NMR ($CDCl_3$, 200 MHz) δ: 3.76 (d, 1H, J=2.8 Hz, ≡CH), 6.52 (d, 1H, J=2.8 Hz, =CH), 7.20-7.69 (m, 7H, H_{arom}), 8.53 (dd, 1H, J=6.5, 1.5 Hz, H_{arom}). Anal. Calcd. for $C_{16}H_{10}$: C, 95.04; H, 4.95; Found: C, 95.32; H, 5.29.

2,7-Dinitro-9-(2-propynyliden)fluorene (16). (0.5 g, 1.6 mmol) of **20** was dissolved in formic acid (25 ml). The reaction mixture was stirred at reflux temperature for 25 h. The resulting solution was diluted with ethyl acetate and washed with water. The organic layer was dried over anhydrous $MgSO_4$ and the solvent was removed in vacuo. The residual material was purified by column chromatography (toluene) to give **16** as a white solid: 0.13 g (28%) m.p. 209-210°C[d]. IR (KBr) v: 3100, 1590, 1530 (NO_2), 1480, 1340 (NO_2), 1080, 810, 740. ^1H NMR (DMSO-d_6, 200 MHz) δ: 3.34 (s, 1H, ≡CH), 5.62 (s, 1H, =CH), 7.52 (s, 1H, H_{arom}), 7.68 (s, 1H, H_{arom}), 7.96 (d, 2H, J=8.4 Hz, H_{arom}), 8.24 (d, 2H, J=8.4 Hz, H_{arom}). ^{13}C NMR (DMSO-d_6, 50 MHz) δ: 60.5, 106.8 (-C≡CH), 120.2, 122.5, 124.7, 125.2, 128.1, 128.8, 144.0, 144.3, 144.9, 146.0 (-C=C-). Anal Calcd. for $C_{16}H_8N_2O_4$: C, 65.81; H, 2.71; N, 9.60. Found: C, 66.07; H, 3.10; N, 9.24.

9-(1-Methoxycarbonyl)ethyliden-2,7-dinitrofluorene (21). To a suspension of **18** (5 g, 18.5 mmol) in CH_2Cl_2 (400 ml), methoxycarbonylmethylidentriphenylphosphorane (6.3 g, 18.5 mmol) was added. The reaction mixture was stirred at room temperature for 24 h. The precipitated solid was filtrated and crystallized from toluene to give **21** as yellow needles: 6 g (70%) m. p. 255-256°C. IR (KBr) v: 3120, 1710 (CO), 1615, 1530 (NO_2), 1340 (NO_2), 1300, 1230, 840, 740. ^1H NMR (DMSO-d_6, 200 MHz) δ: 3.88 (s, 3H, -CH_3), 7.51 (s, 1H, =CH), 8.22 (d, 1H, J=8.4 Hz, H_{arom}), 8.25 (d, 1H, J=8.4 Hz, H_{arom}), 8.37 (dd, 1H, J=8.4, 2.0 Hz, H_{arom}), 8.41 (dd, 1H, J=8.4, 2.0 Hz, H_{arom}), 8.90 (d, 1H, J=2.0 Hz, H_{arom}), 9.60 (d, 1H, J=2.0 Hz, H_{arom}). Anal. Calcd. for $C_{16}H_{10}N_2O_6$: C, 58.90; H, 3.09; N, 8.58. Found: C, 58.72; H, 3.16; N, 8.60.

9-[2-Hydroxy-2,2-bis-(2-propynyl)ethyliden]-2,7-dinitrofluorene (22). To a mixture of Al powder (1.4 g, 53 mmol) and a catalytic amount of $HgCl_2$ in Et_2O (10 ml), was added dropwise propargyl bromide (7.9 g, 53 mmol) in Et_2O (50 ml) over 20 min. The reaction mixture was refluxed for 5 h. After cooling, **21** (2.5 g, 7.6 mmol) was added in THF (50 ml) and refluxed for 12 h. The mixture was acidified with 10% HCl and extracted with EtOAc. The organic layer was dried over $MgSO_4$ and the solvent was removed in vacuo. The residual material was purified by column chromatography (hexane/EtOAc, 8:2) to give **21** as a yellow

solid: 0.93 g (33%) m.p. 222-223°C. IR (KBr) ν: 3510 (OH), 3290 (≡CH), 2110 (-C≡C-), 1590, 1530 (NO_2), 1340 (NO_2). ^1H NMR (DMSO-d_6, 200 MHz) δ: 2.86 (s, 2H, ≡CH), 3.33 (s, 4H, 2(-CH_2-)), 6.41 (s, 1H, OH), 7.49 (s, 1H, =CH), 8.24-8.36 (m, 4H, H_{arom}), 8.75 (s, 1H, H_{arom}), 9.87 (s, 1H, H_{arom}). ^{13}C NMR (DMSO-d_6, 50 MHz) δ: 30.6 (-CH_2-), 73.6 (-C≡<u>CH</u>), 74.2 (-C-OH), 80.4 (-<u>C</u>≡CH), 115.9, 121.8, 122.2, 123.7, 123.8, 125.0, 137.2, 141.7, 147.9 (-C=C-). M.S. m/z (%): 374 (M^+,1), 335 (37), 295 (100), 249 (25), 203 (7), 175 (10). Anal. Calcd. for $C_{21}H_{14}N_2O_5$: C, 67.20; H, 3.77; N, 7.48. Found: C, 66.90; H, 3.80; N, 7.50.

2,7-Diamino-9-[2-hydroxy-2,2-bis-(2-propynyl)ethyliden]fluorene (23). To a suspension of **22** (0.8 g, 2.1 mmol) in ethanol (16 ml), was added Zn (4.6 g, 70 mmol) and $CaCl_2$ (0.15 g) in water (3 ml). The reaction mixture was stirred at reflux temperature for 5 min. The suspension was filtered and the filtrate was concentrated to dryness. The residual material was purified by column chromatography (ethyl acetate/hexane, 7:3) followed by crystallization from toluene to give **23** as red prisms: 0.4 g (62%) m.p. 232-233°C. IR (KBr) ν: 3420, 3400, 3360, 3340 (NH_2, OH), 3300 (≡CH), 3200, 1620, 1470, 1310, 1250, 1100. ^1H NMR (DMSO-d_6, 200 MHz) δ: 2.80 (d, 4H, J=2.4 Hz, 2(-CH_2-)), 2.90 (t, 2H, J=2.4 Hz, 2(≡CH)),4.92 (s, 2H, NH_2), 4.98 (s, 2H, NH_2), 5.79 (s, 1H, OH), 6.45 (dd, 1H, J=8.0, 1.9 Hz, H_{arom}), 6.49 (dd, 1H, J=8.0, 1.9 Hz, H_{arom}), 6.57 (s, 1H, =CH), 6.77 (d, 1H, J=1.9 Hz, H_{arom}), 7.15 (d, 1H, J=8.0 Hz, H_{arom}), 7.17 (d, 1H, J=8.0 Hz, H_{arom}), 7.78 (d, 1H, J=1.9 Hz, H_{arom}). ^{13}C NMR (DMSO-d_6, 50 MHz) δ: 29.6 (-CH_2-), 72.2 (-C≡<u>CH</u>), 73.0 (-C-OH), 80.9 (-<u>C</u>≡CH), 114.2, 115.3, 118.0, 125.2, 128.1, 128.8, 128.9, 130.2, 131.6, 135.7, 137.9, 140.0, 146.1, 146.5 (-C=C-). M.S. m/z (%): 314 (M^+,27), 275 (15), 235 (29), 207 (23), 180 (8), 118 (10), 91 (100), 65 (15), 45 (6). Anal. Calcd. for $C_{21}H_{18}N_2O$: C, 80.23; H, 5.77; N, 8.80. Found: C, 79.91; H, 5.80; N, 8.52.

(Z+E)-2,7-Dinitro-9-[2-(2-propynyl)pent-2-en-4-ynyliden]fluorene (24) and 9-[2-Acetyl-2,2-bis-(2-propynyl)ethyliden]-2,7-dinitrofluorene (25). To a suspension of **24** (0.5 g, 1.3 mmol) in acetic anhydride (4 ml), was added a catalytic amount of sulfuric acid. The reaction mixture was stirred at reflux temperature for 2 h. The resulting solution was poured into ice/water (100 ml), neutralized with 30% NaOH, and extracted with ethyl acetate. The organic layer was dried over anhydrous $MgSO_4$ and the solvent was removed in vacuo. The residual material was purified by column chromatography (toluene) to give **24** as a white solid: 0.095 g (20%) m.p. 175-176°C and **25** as a yellow solid: 0.221 g (40%) m.p. 253-254°C. Data of **24**: IR (KBr) ν: 3280 (≡CH), 1590, 1530 (NO_2), 1340 (NO_2), 1260, 1080, 840, 740. ^1H NMR ($CDCl_3$, 200 MHz) δ: 2.13 (t, 1H, J=2.8 Hz, ≡CH), 2.32 (t, 1H, J=2.7 Hz, ≡CH), 2.95 (d, 1H, J=2.4 Hz, ≡CH), 3.41 (s, 2H, -CH_2-), 3.60 (d, 1H, J=2.4 Hz, ≡CH), 3.70 (d, 2H, J=2.7 Hz, -CH_2-), 6.13 (m, 1H, =CH), 6.28 (m, 1H, =CH), 7.38 (s, 1H, =CH), 7.54 (s, 1H, =CH), 7.95 (d, 2H, J=8.4 Hz, H_{arom}), 7.97 (d, 2H, J=8.4 Hz, H_{arom}), 8.32-8.39 (m, 4H, H_{arom}), 8.66 (d, 2H, J=1.9 Hz, H_{arom}), 8.73 (d, 1H, J=2.0 Hz, H_{arom}), 9.09 (d, 1H, J=2.0 Hz, H_{arom}). ^{13}C NMR ($CDCl_3$, 50 MHz) δ: 24.8 (-CH_2-), 27.7 (-CH_2-) 72.7, 74.8, 87.0, 89.4 (-C≡CH), 113.6, 115.3, 117.9, 118.0, 122.5, 122.9, 125.8, 126.1, 129.8, 132.9, 136.0, 139.6, 142.4, 143.4, 145.7, 149.9 (-C=C-). M.S. m/z (%): 356 (M^+,55), 333 (18), 263 (100), 224 (64), 187 (9), 163 (5), 130 (21), 118 (16), 91 (27). Anal. Calcd. for: $C_{21}H_{12}N_2O_4$: C, 70.60; H, 3.37; N, 7.83. Found: C, 70.53; H, 3.21; N, 7.62. Data of **25**: IR (KBr) ν: 3300 (≡CH), 1690 (CO), 1590, 1520 (NO_2), 1340 (NO_2), 1220, 1080, 840, 740. ^1H NMR ($CDCl_3$, 200 MHz) δ: 2.13 (s, 3H, -CH_3), 2.17 (s, 2H, 2(≡CH)), 3.38 (d, 2H, J=2.7 Hz, -CH_2-), 3.41 (d, 2H, J=2.7 Hz, -CH_2-), 7.22 (s, 1H, =CH), 7.94 (d, 1H, J=11.0 Hz, H_{arom}), 7.98 (d, 1H, J=11.0 Hz, H_{arom}), 8.27-8.42 (m, 2H, H_{arom}), 8.63 (d, 1H, J=2.0 Hz, H_{arom}), 9.25 (d, 1H, J=2.0 Hz, H_{arom}). ^{13}C NMR ($CDCl_3$, 50 MHz) δ:

21.2 (-CH_3), 28.2 (-CH_2-), 73.2 (-C≡CH), 78.6 (C-$OCOCH_3$), 79.9 (-C≡CH), 117.0, 122.3, 122.9, 124.9, 125.2, 125.4, 134.7, 137.1, 142.3, 142.4, 143.9, 145.7, 149.2, 153.9 (-C=C-), 169.5 (CO). M.S. m/z (%): 416 (M^+,23), 374 (10), 335 (79), 295 (100), 249 (23), 203 (1), 175 (19), 163 (5), 91 (3). Anal. Calcd. for $C_{23}H_{16}N_2O_6$: C, 66.34; H, 3.87; N, 6.72. Found: C, 66.40; H, 4.00; N, 6.50.

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