Preparation of 1,8-Naphthalimides as Candidate Fluorescent Probes of Hypoxic Cells

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A number of nitro- and dinitro-1,8-naphthalimides have been prepared as potential fluorescent probes of hypoxic cells. The susceptibility of 4-nitro-1,8-naphthalic anhydrides and -naphthalimides to nucleophilic displacement of the nitro group has been demonstrated by reaction with 1-butanethiol to yield 4-butylthio derivatives. Attempted nitration of these 4-butylthio derivatives with sodium nitrate and concentrated sulphuric acid yielded the corresponding sulphoxides in high yield.

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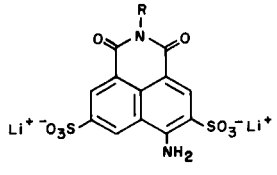
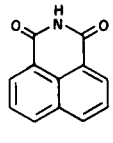
Clinical failure in the radiation treatment of solid tumours is thought to be associated with the presence of radioresistant hypoxic cells within the tumor [1]. Identification and quantitative estimation of hypoxic cells in solid tumours could improve radiation treatment of the cancer patient. However, no quick, simple and reliable method for this identification and quantitative estimation of hypoxic cells currently exists. The objective of the synthetic programme which we now report was to prepare compounds which would cause selective fluorescence only in hypoxic cells.

The rationale for the work has recently been reported [2-4] and is based on the observation that nitro compounds are selectively reduced only in hypoxic cells, through nitroso- and hydroxylamino-intermediates, to the corresponding amine [5]. We were interested therefore in the synthesis of heterocycles with nitro substituents attached to the fluorophore, thus giving a non-fluorescent product. However, hypoxia mediated reductive metabolism would lead to a fluorescent amino metabolite only in the hypoxic cells, since reduction would not occur in the oxic tumour cells.



1a $R = NHCONHNH_2$

1b $R = m\text{-}C_6H_4\text{-}SO_2CH{=}CH_2$



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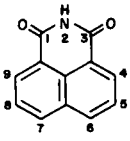

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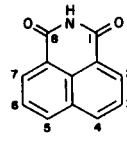

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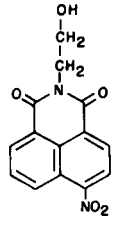

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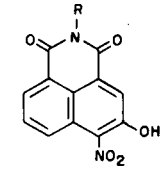

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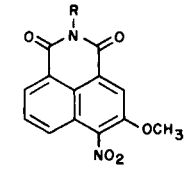
4a $R = CH_2CH_2OH$

4b $R = CH_2CH(OH)CH_2OH$

4c $R = (CH_2)_3N\langle O \rangle \cdot HCl$



5

5a $R = CH_2CH_2OH$

5b $R = CH_2CH(OH)CH_2OH$

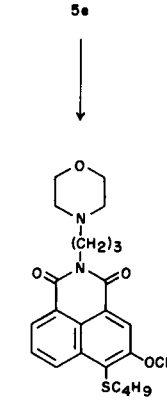
5c $R = (CH_2)_3N(CH_2CH_2OH)_2 \cdot HCl$

5d $R = (CH_2)_2NHCH_2CH_2OH \cdot HCl$

5e $R = (CH_2)_3N\langle O \rangle$

5f $R = nC_4H_9$

5g $R = CH_2N\langle \rangle \cdot HCl$



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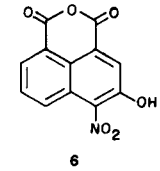

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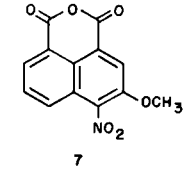

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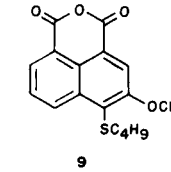

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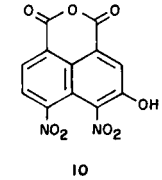

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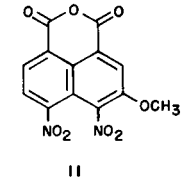

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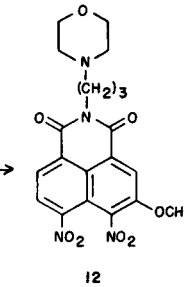

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The intense fluorescence and biological uses of Lucifer Yellow CH [6] (**1a**) and Lucifer Yellow VS [7-9] (**1b**) prompted us to investigate the synthesis and use of water soluble nitro-1,8-naphthalimides as potential fluorescent probes of hypoxic cells. The nomenclature of the structure **2a** can be a source of confusion since two systems are in use to name compounds of this type. The Chemical Abstracts nomenclature describes **2a** as a benz[*de*]isoquinoline derivative with numbering as in **2b**. The older nomenclature names the system **2a** as a 1,8-naphthalimide with numbering as in **2c**. Both the Chemical Abstracts nomenclature [10,11] and the older system [12,13] have been used recently. We shall use the naphthalimide nomenclature throughout this text.

Initially, the 4-nitro-1,8-naphthalimide (**3**) was prepared but the ready displacement of the 4-nitro substituent by a range of nucleophiles [14] led to disappointing biological results [15].

The ease with which the 4-nitro substituent could be replaced prompted us to synthesise a range of 3-hydroxy-4-nitro- **4a-c** and 3-methoxy-4-nitro-*N*-substituted-1,8-naphthalimides **5a-g** in the expectation that the electron releasing 3-substituent would reduce or prevent nucleophilic attack at the 4-position. These naphthalimides were readily obtained in high yield by refluxing the readily accessible [16] 3-hydroxy-4-nitro- (**6**) and 3-methoxy-4-nitro-1,8-naphthalic anhydride (**7**) with an equimolar quantity of the appropriate primary amine in absolute ethanol.

Preliminary biological results with the substituted naphthalimide **5e** [17] suggested that the 4-nitro-substituent was still susceptible to nucleophilic displacement.

We investigated this possibility by reacting **5e** with 1-butanethiol because thiols are known to be very powerful nucleophiles in reactions where an aryl nitro group is displaced [18,19]. In the presence of potassium carbonate and under the vigorous conditions of 100° and in dimethylformamide, the naphthalimide **8** was formed.

A similar reaction with the anhydride (**7**) yielded the corresponding nitro group displaced product **9**, which reacted with *N*-(3-aminopropyl)morpholine to yield the naphthalimide **8**. The methoxyl group is therefore not a sufficiently powerful destabiliser of the transition state to prevent the nitro displacement reaction. However, since displacement of the 4-nitro substituent could not be

Table 1

Compound Number	Yield (%)	Mp (°C)	Crystallisation Solvent	Empirical Formula	% Found (Calculated) C	H	N	^{1}H-NMR [MH$_2$] δ [ppm], TMS DMSO-d$_6$
5a	87	200-201	Aqueous Ethanol	$C_{15}H_{12}N_2O_6$	56.59 (56.96)	4.02 (3.80)	8.70 (8.86)	3.0-5.0 (br s, 1H, OH), 3.63 (t, J = 6 Hz, 2H, O-CH$_2$), 4.15 (t, J = 6 Hz, 2H, N-CH$_2$), 4.2 (s, 3H, O-CH$_3$), 8.0 (s, 3H, O-CH$_3$), 8.0 (m, 2H, 2-, 6-H), 8.42 (m, 2H, 5-, 7-H)
5b	94	212-213	Aqueous Ethanol	$C_{16}H_{14}N_2O_7$	55.51 (55.49)	4.16 (4.05)	7.88 (8.09)	2.47 (m, 1H, CH), 3.5 (d, J = 4 Hz, 2H, O-CH$_2$), 4.2 (m, 2H, N-CH$_2$), 4.25 (s, 3H, O-CH$_3$), 4.4-5.0 (br s, 2H, CH$_2$O*H*, CHO*H*), 8.10 (m, 2H, 2-, 6-H), 8.5 (m, 2H, 5-, 7-H)
5c	93	196-198	Aqueous Ethanolic HCl	$C_{20}H_{23}N_3O_7$·HCl	52.94 (52.92)	5.30 (5.29)	9.49 (9.26)	3.29 (s, 3H, 2 × OH, NH), 4.16 (m, 10H, NCH$_2$CH$_2$-CH$_2$NCH$_2$CH$_2$), 4.28 (s, 3H, OCH$_3$), 8.06 (m, 2H, 2-, 6-H), 8.52 (m, 2H, 5-, 7-H)
5d	86	220-221	Aqueous Ethanolic HCl	$C_{17}H_{19}N_3O_6$·HCl	51.50 (51.32)	4.60 (5.03)	10.42 (10.56)	3.32 (m, 6H, C*H*$_2$NHC*H*$_2$C*H*$_2$), 4.28 (s, 3H, O-CH$_3$), 4.51 (m, 2H, N-CH$_2$), 4.66-6.66 (br s, 1H, N-H), 8.06 (m, 2H, 2-, 6-H), 8.52 (m, 2H), 5-, 7-H), 8.96-9.70 (br s, 2H, OH, NH)
5e	90	124-125	95% Ethanol	$C_{20}H_{21}N_3O_6$	60.36 (60.15)	5.34 (5.26)	10.67 (10.53)	1.8 (m, 2H, 2'-CH$_2$), 2.4 (m, 4H, 2 × O-CH$_2$), 3.5 (m, 6H, 3 × N-CH$_2$), 4.15 (t, J = 6 Hz, 2H, N-CH$_2$), 4.13 (s, 3H, OCH$_3$), 8.04 (m, 2H, 2-, 6-H), 8.5 (m, 2H, 5-, 7-H)
5f	83	139-140	Aqueous Ethanol	$C_{17}H_{16}N_2O_5$	62.32 (62.20)	4.87 (4.88)	8.63 (8.54)	1.0 (d, J = 6 Hz, 3H, C-CH$_3$), 1.57 (m, 4H, CH$_2$CH$_2$), 4.08 (t, J = 6 Hz, 2H, N-CH$_2$), 4.27 (s, 3H, OCH$_3$), 8.1 (m, 2H, 2-, 6-H), 8.53 (m, 2H, 5-, 7-H)
5g	81	284-286	Aqueous Ethanolic HCl	$C_{19}H_{19}N_3O_5$·HCl	56.20 (56.23)	4.96 (4.93)	10.06 (10.36)	3.30 (m, 12H, NCH$_2$piperidine), 4.16 (s, 3H, OCH$_3$), 8.02 (m, 2H, 2-, 6-H), 8.40 (m, 2H, 5-, 7-H)
4a	73	215-216	Aqueous Ethanolic HCl	$C_{14}H_{10}N_2O_6$	55.31 (55.63)	3.25 (3.31)	9.21 (9.27)	3.0-5.0 (br s, 2H, 2 × OH), 3.55 (t, J = 6 Hz, 2H, O-CH$_2$), 4.03 (t, J = 6 Hz, 2H, N-CH$_2$), 7.67 (m, 2H, 2-, 6-H), 8.03 (m, 2H, 5-, 7-H)
4b	89	193-194	Aqueous Ethanol	$C_{15}H_{12}N_2O_7$	54.15 (54.22)	3.63 (3.61)	8.56 (8.43)	2.42 (m, 1H, CH), 3.5 (d, J = 4 Hz, 2H, O-CH$_2$), 4.2 (m, 8H, O-CH$_3$, 3 × OH, N-CH$_2$), 8.08 (m, 2H, 2-, 6-H), 8.4 (m, 2H, 5-, 7-H)
4c	76	> 300	Aqueous Ethanolic HCl	$C_{19}H_{19}N_3O_6$·HCl	53.83 (54.09)	4.63 (4.74)	10.30 (9.96)	1.82 (m, 2H, 2'-CH$_2$), 2.35 (m, 4H, 2 × OCH$_2$), 3.52 (m, 6H, 3 × N-CH$_2$), 4.15 (m, 2H, N-CH$_2$), 7.65 (m, 2H, 2-, 6-H), 8.13 (m, 3H, 5-, 7-H, OH)

Table 1 continued

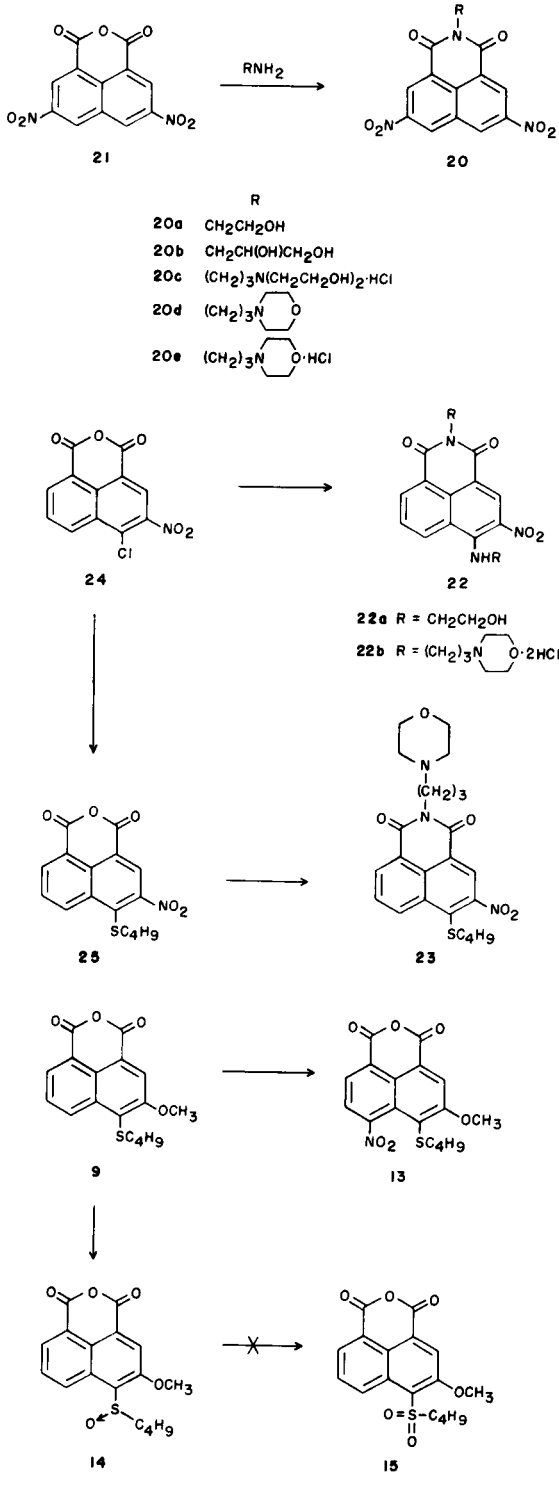
Compound Number	Yield (%)	Mp (°C)	Crystallisation Solvent	Empirical Formula	% Found (Calculated) C	H	N	^{1}H-NMR [MH$_2$] δ [ppm], TMS DMSO-d$_6$
20a	70	234-235	Ethanol	$C_{14}H_9N_3O_7$	50.81 (50.76)	2.78 (2.72)	12.56 (12.69)	3.0-5.4 (br s, 1H, OH), 3.66 (t, J = 6 Hz, 2H, OCH_2), 4.2 (t, J = 6 Hz, 2H, NCH_2), 9.13 (d, J = 2 Hz, 2H, 2-, 7-H), 9.85 (d, J = 2 Hz, 2H, 4-, 5-H)
20b	44	240-241	Aqueous DMF	$C_{15}H_{11}N_3O_8$	49.76 (49.86)	3.13 (3.05)	11.55 (11.63)	2.46 (m, 1H, C*H*OH), 3.43 (d, J = 6 Hz, 2H, OCH_2), 3.8 (s, 2H, 2 × OH), 4.15 (d, J = 6 Hz, 2H, NCH_2), 9.08 (d, J = 2 Hz, 2H, 2-, 7-H), 9.76 (d, J = 2 Hz, 2H, 4-, 5-H)
20c	74	195-196	Aqueous Ethanolic HCl	$C_{19}H_{20}N_4O_8$·HCl	48.39 (48.67)	4.62 (4.48)	11.84 (11.95)	2.2 (m, 2H, C-CH_2-C), 3.22 (m, 8H, 2 × CH_2O*H*, N-CH_2), 3.82 (m, 4H, 2 × N-CH_2), 4.21 (m, 2H, N-CH_2), 5.5-6.5 (br s, H, NH), 9.13 (d, J = 2 Hz, 2H, 2-, 7-H), 9.83 (d, J = 2 Hz, 2H, 4-, 5-H)
20d	73	225-226	Methanol-DMF	$C_{19}H_{18}N_4O_7$	54.92 (55.07)	4.32 (4.35)	13.57 (13.53)	1.80 (m, 2H, C-CH_2-C), 2.45 (m, 4H, CH_2-O-CH_2), 3.53 (m, 6H, 3 × N-CH_2), 4.10 (m, 2H, NCH_2), 9.13 (d, J = 2 Hz, 2H, 4-, 5-H), 9.91 (d, J = 2 Hz, 2H, 2-, 7-H)
20e	71	> 300	Aqueous Ethanolic HCl	$C_{19}H_{18}N_4O_7$·HCl	50.60 (50.61)	4.15 (4.22)	12.23 (12.43)	1.82 (m, 2H, C-CH_2-C), 2.44 (m, 4H, CH_2-O-CH_2), 3.73 (m, 6H, 3 × N-CH_2), 4.15 (m, 2H, NCH_2), 9.13 (d, J = 2 Hz, 2H, 4-, 5-H), 9.91 (d, J = 2 Hz, 2H, 2-, 7-H)
22a	73	234-235	Aqueous DMF	$C_{16}H_{15}N_3O_6$	56.05 (55.65)	4.24 (4.35)	11.99 (12.17)	3.4 (m, 10H, 2 × CH_2CH_2OH), 7.33 (t, J = 8 Hz, 1H, 6-H), 8.03 (d, J = 8 Hz, 1H, 5-H), 8.33 (s, 1H, 2-H), 8.35 (d, J = 8 Hz, 1H, 7-H), 8.0 (br s, 1H, N-H)
22b	38	222-224	Aqueous Ethanolic HCl	$C_{26}H_{33}N_5O_6$·2HCl	53.02 (53.42)	5.71 (5.99)	11.89 (11.98)	1.8 (m, 2H, 2'-CH_2), 2.0 (m, 2H, 2''-CH_2), 2.4 (m, 8H, 4 × O-CH_2), 3.5 (m, 12H, 6 × N-CH_2), 4.15 (m, 4H, 2 × N-CH_2), 5.2 (s, 3H, 2 ×*H*Cl, NH), 7.33 (t, J = 8 Hz, 1H, 6-H), 8.05 (d, J = 8 Hz, 1H, 5-H), 8.40 (s, 1H, 2-H), 8.48 (d, J = 8 Hz, 1H, 7-H)
23	78	124-125	Ethanol	$C_{23}H_{27}N_3O_5S$	60.51 (60.39)	6.07 (5.91)	9.36 (9.19)	0.77 (m, 3H, CH_3), 1.30 (m, 4H, CH_2C$H_2$$CH_3$), 1.87 (m, 2H, 2'-$CH_2$), 3.01 (m, 2H, SCH_2), 3.48 (m, 12H, 4 × NCH_2, 2 × OCH_2), 8.20 (t, J = 8 Hz, 1H, 6-H), 8.81 (d, J = 8 Hz, 1H, 7-H), 8.90 (s, 1H, 2-H), 9.10 (d, J = 8 Hz, 1H, 5-H)
8	83	245-246	Ethanol	$C_{24}H_{30}N_2SO_4$·HCl	60.36 (60.19)	6.42 (6.48)	7.35 (7.42)	0.79 (t, J = 6 Hz, 3H, CH_3), 1.32 (m, 4H, CH_2CH_2-CH_3), 1.89 (m, 2H, 2'-CH_2), 3.03 (t, J = 6 Hz, 2H SCH_2), 3.5 (m, 11H, 3 × NCH_2 2 × OCH_2, NH), 4.12 (s, 3H, OCH_3), 7.86 (t, J = 7 Hz, 1H, 6-H), 8.36 (s, 1H, 2-H), 8.56 (d, J = 7 Hz, 1H, 5-H), 9.06 (d, J = 7 Hz, 1H, 7-H)
12	68	204-205	Ethanol-Ethyl Acetate	$C_{20}H_{20}N_4O_8$	53.98 (54.05)	4.66 (4.50)	12.87 (12.61)	1.82 (m, 2H, C-CH_2-C), 2.4 (m, 4H, 2 × OCH_2), 3.53 (m, 6H, 3 × NCH_2), 4.15 (t, J = 6 Hz, 2H, NCH_2), 4.33 (s, 3H, OCH_3), 8.76 (m, 3H, 2-, 6-, 7-H)

achieved from the analogous 3-hydroxy-4-nitro-1,8-naphthalimide (**4c**) it appears that either the more powerful electron releasing oxy anion group destabilises the transition state or, by electrostatic repulsion, prevents its formation.

Nitration of both naphthalic anhydrides **6** and **7** yielded the corresponding dinitronaphthalic anhydrides **10** and **11** respectively. Treatment of the naphthalic anhydride **11** with *N*-(3-aminopropyl)-morpholine yielded the corresponding naphthalimide **12**.

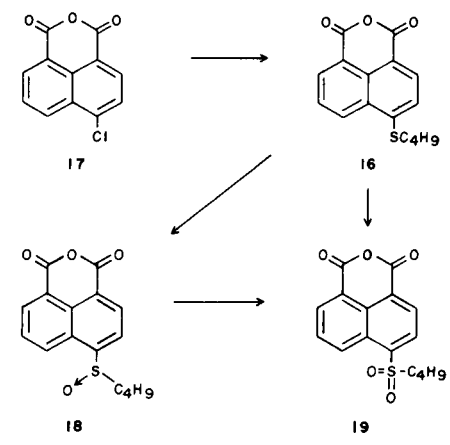
Nitration of **9** with a mixture of fuming nitric acid and concentrated sulphuric acid yielded the 5-nitronaphthalic anhydride **13** in low yield. Attempts to improve the yield using sodium nitrate in concentrated sulphuric acid at room temperature led to formation of the sulphoxide **14**. Efforts to convert the sulphoxide **14** into the corresponding sulphone **15** by addition of more sodium nitrate and use of a higher temperature were unsuccessful.

This interesting selective formation of a sulphoxide prompted us to investigate the reaction of 4-butylthio-1,8-naphthalic anhydride (**16**) previously prepared in high yield from 4-chloro-1,8-naphthalic anhydride (**17**), with sodium nitrate in concentrated sulphuric acid. Again, selective oxidation occurred in high yield to give the corresponding sulphoxide **18**. This sulphoxide **18** could not be converted to the sulphone **19** by addition of a further aliquot of sodium nitrate and use of an elevated temperature. However, the sulphone **19** was prepared in high yield using peracetic acid in the presence of manganic acetylacetonate either directly from the butylthionaphthalic anhydr-



ide **16** or from the corresponding sulphoxide **18**.

Biological testing requirements necessitated the synthesis of some 3,6-dinitro-1,8-naphthalimides **20a-e** which were prepared by treatment of 3,6-dinitro-1,8-naphthalic anhydride (**21**) [20] with an equimolar quantity of the appropriate amine in absolute ethanol.



Biological evaluation of the naphthalimides so far reported here suggested that 3-nitronaphthalimides with a 4-substituted amino- or thio- substituent could be of potential interest. We therefore prepared three compounds **22a**, **22b** and **23** for evaluation. The amines **22a** and **22b** were prepared by the method of Kadhim and Peters [14] from 4-chloro-3-nitro-1,8-naphthalic anhydride (**24**). The 4-butylthio-3-nitro-1,8-naphthalimide (**23**) was prepared by treatment of 4-butylthio-3-nitro-1,8-naphthalic anhydride (**25**), previously prepared from (**24**), with an equimolar quantity of *N*-(3-aminopropyl)morpholine in absolute ethanol.

EXPERIMENTAL

Infrared and mass spectra were recorded with a Pye Unicam SP3 - 100 infrared spectrophotometer and and A.E.I. MS 902 mass spectrometer. Proton nmr spectra were recorded using a Varian T-60, Varian CFT-20 or Bruker 220 MHz spectrometer with TMS as internal standard. Elemental analyses were performed by Butterworth Laboratories, Middlesex, UK. Melting points are uncorrected. Centrifugally accelerated radial chromatography was carried out on a Chromatotron obtained from T C Research, St. Albans, U.K.

Crystallisation solvent, yield, melting point, ^{1}H-nmr and elemental analyses for all naphthalimides are recorded in Table 1. Satisfactory mass and infrared spectra were additionally obtained.

3-Methoxy-4-nitro-1,8-naphthalimides **5**.

The appropriate primary amine (0.002 mole) was refluxed gently on a stirrer hot plate for one hour with 3-methoxy-4-nitro-1,8-naphthalic anhydride (0.002 mole) in absolute ethanol (5 ml). The hot solution was allowed to cool, the product filtered off and recrystallised. If no product formed on cooling, the solvent was removed *in vacuo* to give the product which was then recrystallised.

3-Hydroxy-4-nitronaphthalimides **4**.

The appropriate primary amine (0.0035 mole) was magnetically stirred and refluxed gently on a stirrer hot plate for two hours with 3-hydroxy-4-nitro-1,8-naphthalic anhydride (0.003 mole) in absolute ethanol (5 ml). The red solution was treated with 10 drops of concentrated hydrochloric acid and refluxed for a further 5 minutes. The hot solution or suspension was allowed to cool, the product filtered off and recrystallised. If no product was formed on cooling, the solvent was removed *in vacuo* to give the product which was then recrystallised.

4,5-Dinitro-3-methoxy-1,8-naphthalic Anhydride (**11**).

A cooled mixture of concentrated sulphuric acid (5 ml) and fuming

nitric acid (3.3 ml) was added dropwise to a stirred solution of 3-methoxy-4-nitro-1,8-naphthalic anhydride (5 g, 0.018 mole) in concentrated sulphuric acid (60 ml) while maintaining the temperature below 15°. Following addition, the reaction mixture was allowed to stir at ambient temperature for one hour, poured into crushed ice and filtered. The crude product was air dried and then recrystallised from concentrated nitric acid to yield the dinitromethoxynaphthalic anhydride (3.8 g, 66%) mp 275-276°, ir (potassium bromide): 3080, 1780, 1740, 1550, 1390, 1360, 1280, 1040 cm^{-1}; nmr (DMSO-d_6): δ 8.66 (1H, s, 2-H), 8.62 (1H, d, J = 0.6 Hz, 7-H), 8.58 (1H, d, J = 0.6 Hz, 6-H), 4.28 (3H, s, O-CH_3).

Anal. Calcd. for $C_{12}H_6N_2O_8$: C, 49.06; H, 1.89; N, 8.81. Found: C, 48.68; H, 1.90; N, 8.48.

4,5-Dinitro-3-hydroxy-1,8-naphthalic Anhydride (**10**).

A cooled mixture of concentrated sulphuric acid (2.5 ml) and fuming nitric acid (d 1.5, 1.7 ml) was added dropwise to a stirred solution of 3-hydroxy-4-nitro-1,8-naphthalic anhydride (2.5 g, 0.0096 mole) in concentrated sulphuric acid (30 ml) while maintaining the temperature below 15°. Following addition, the reaction mixture was allowed to stir for 1.5 hours at ambient temperature, poured onto crushed ice and filtered. Air drying followed by recrystallisation from aqueous acetic acid yielded the dinitrohydroxynaphthalic anhydride (1.91 g, 63%), mp 238-240°; ir (potassium bromide): 3400, 1785, 1740, 1540, 1370, 1280, 1020, 840, 760, cm^{-1}; nmr (DMSO-d_6): δ 8.46 (s, 2H, 2-H, 7-H), 8.30 (s, 1H, 6-H), 6.66 (brs, 1H, O-H).

Anal. Calcd. for $C_{12}H_4N_2O_8$: C, 47.37; H, 1.32; N, 9.21. Found: C, 46.99; H, 1.28; N, 8.84.

3-Methoxy-4-butylthio-1,8-naphthalic Anhydride (**9**).

3-Methoxy-4-nitro-1,8-naphthalic anhydride (0.55 g, 0.002 mole), anhydrous sodium acetate (0.512 g) and 1-butanethiol (0.181 g, 0.002 mole) were magnetically stirred in dry dimethylformamide (10 ml) at ambient temperature for 24 hours. The reaction mixture was poured into crushed ice and the yellow precipitate filtered off and air dried to give the crude methoxy-butylthio-naphthalic anhydride. The crude product was recrystallised from petroleum ether (boiling range 60-80°) followed by purification using centrifugally accelerated radial layer chromatography (silica gel/chloroform) to give an analytically pure sample (0.388 g, 61%), mp 63-64°; ir (potassium bromide): 2960, 2940, 1770, 1730, 1600, 1340, 1270, 1030, 790 cm^{-1}; nmr (DMSO-d_6): δ 8.90 (d, J = 1.2 Hz, 1H, 7-H), 8.40 (d, J = 1.2 Hz, 1H, 5-H), 8.25 (s, 1H, 2-H), 7.93 (t, J = 1.3 Hz, 1H, 6-H), 4.14 (s, 3H, O-CH_3), 3.03 (t, J = 1.3 Hz, 2H, S-CH_2), 1.37 (m, 4H, 2 × CH_2), 0.81 (t, J = 1.3 Hz, 3H, CH_2-CH_3).

Anal. Calcd. for $C_{17}H_{16}SO_4$: C, 64.56; H, 5.06. Found: C, 64.69; H, 4.91.

N-(3-*N*^{1}-Morpholino-1-propyl)-3-methoxy-4-butylthio-1,8-naphthalimide Hydrochloride (**8**).

(a) 3-Methoxy-4-butylthio-1,8-naphthalic anhydride (0.1 g, 32 mmoles) and *N*-(3-aminopropyl)morpholine (0.047 g, 32 mmoles) were refluxed gently in absolute ethanol (6 ml) for 5 minutes. A trace of concentrated hydrochloric acid was added to give an immediate precipitate which, after cooling, was filtered and recrystallised from absolute ethanol to yield the naphthalimide hydrochloride salt (0.125 g, 83%), mp 245-246°.

(b) *N*-(3-*N*^{1}-Morpholino-1-propyl)-3-methoxy-4-nitro-1,8-naphthalimide (0.35 g, 0.88 mmole) (**5e**), 1-butanethiol (0.082 g, 0.88 mmole) and anhydrous sodium acetate (0.6 g) were stirred and heated in dimethylformamide (5 ml) at 100° for 4 hours. The reaction mixture was cooled, poured into crushed ice and the resulting precipitate filtered and air dried. The product was purified by using centrifugally accelerated radial layer chromatography (silica gel/ethyl acetate). Evaporation of the appropriate ethyl acetate fraction *in vacuo* yielded a yellow oil which was taken up in dry diethyl ether and saturated with dry hydrogen chloride to yield an identical naphthalimide hydrochloride salt as in (a) (0.2 g, 48%), mp 245-246°.

3-Methoxy-5-nitro-4-butylthio-1,8-naphthalic Anhydride (**13**).

A cooled mixture of concentrated sulphuric acid (0.25 ml) and fuming nitric acid (d 1.5, 0.17 ml) was added dropwise to a magnetically stirred solution of 3-methoxy-4-butylthio-1,8-naphthalic anhydride (0.29 g, 0.91 mmole) in concentrated sulphuric acid (3 ml) while maintaining the temperature below 10°. The reaction mixture was stirred for one hour after addition at 0°, the ice-bath was removed and stirring continued for a further hour, after which the reaction mixture was quenched in ice to give a yellow precipitate. The precipitate was filtered, washed with water and air dried. Purification using centrifugally accelerated radial chromatography (silica gel/chloroform) yielded the analytically pure nitro naphthalic anhydride (0.085 g, 26%), mp 172-174°; ir (potassium bromide): 2975, 2950, 2875, 1780, 1740, 1540, 1380, 1340, 1270, 1050 cm^{-1}; nmr (deuteriochloroform): δ 9.77 (d, J = 6 Hz, 1H, 6-H), 9.69 (s, 1H, 2-H), 9.37 (d, J = 6 Hz, 1H, 7-H), 4.25 (s, 3H, O-CH_3), 3.1 (m, 2H, S-CH_2), 1.48 (m, 4H, C-CH_2C$H_2$$CH_3$), 0.81 (m, 3H, C-$CH_3$).

Anal. Calcd. for $C_{17}H_{15}NSO_6$: C, 56.51; H, 4.16; N, 3.88. Found: C, 56.75; H, 4.16; N, 3.65.

4-Butanesulphenyl-3-methoxy-1,8-naphthalic Anhydride (**14**).

Sodium nitrate (0.37 g) was added portionwise to a magnetically stirred solution of 3-methoxy-4-butylthio-1,8-naphthalic anhydride (1 g, 3.16 mmoles) in concentrated sulphuric acid (5 ml) at ambient temperature. The reaction mixture was stirred for one hour and quenched in ice. The precipitate formed was filtered, air dried and purified using centrifugally accelerated radial chromatography with silica gel. Elution initially with chloroform followed by ethyl acetate yielded the sulphoxide (0.52 g, 50%), mp 158-160°; on evaporation of the appropriate ethyl acetate fraction; ir (potassium bromide): 2950, 2925, 2850, 1760, 1720, 1590, 1370, 1260, 1150, 1020, 770 cm^{-1}; nmr (deuteriochloroform): δ 10.02 (d, J = 10 Hz, 1H, 7-H), 8.73 (d, J = 10 Hz, 1H, 5-H), 8.53 (s, 1H, 2-H), 8.0 (t, J = 10 Hz, 1H, 6-H), 4.23 (s, 3H, O-CH_3), 3.03 (m, 2H, S-CH_2), 1.73 (m, 4H, CH_2CH_2), 1.0 (m, 3H, CH_2-CH_3).

Anal. Calcd. for $C_{17}H_{16}SO_5$: C, 61.43; H, 4.85. Found: C, 61.54; H, 4.77.

(3-*N*^{1}-Morpholino-1-propyl)-4,5-dinitro-3-methoxy-1,8-naphthalimide (**12**).

4,5-Dinitro-3-methoxy-1,8-naphthalic anhydride (0.2 g, 0.63 mmole) and *N*-(3-aminopropyl)morpholine (0.091 g, 0.63 mmole) were refluxed gently in absolute ethanol (6 ml) for 10 minutes. The solid formed on cooling was filtered and recrystallised from a mixture of absolute ethanol and ethyl acetate to give the naphthalimide (0.19 g, 68%), mp 204-205°.

3,6-Dinitro-1,8-naphthalimides (**20**).

The appropriate primary amine (0.007 mole) was added all at once to a magnetically stirred suspension of 3,6-dinitro-1,8-naphthalic anhydride (0.007 mole) in absolute ethanol (25 ml) at room temperature. The solid was filtered off after stirring for 24 hours and recrystallised from the appropriate solvent.

4-Butylthio-1,8-naphthalic Anhydride (**16**).

4-Chloro-1,8-naphthalic anhydride (10 g, 0.043 mole), anhydrous potassium carbonate (2.5 g) and 1-butanethiol (5.8 g, 0.06 mole) were refluxed with magnetic stirring for half an hour in dry dimethylformamide (100 ml). The mixture was cooled, poured into 250 g of ice-water and the resulting yellow suspension stirred for 2 hours. The yellow product was filtered, air dried and recrystallised from a mixture of chloroform and petroleum ether (60-80°) to give the essentially pure naphthalic anhydride (8.9 g, 72%), mp 109-111°. An analytically pure sample was obtained by purification using centrifugally accelerated radial chromatography (silica gel/chloroform). Evaporation of the appropriate chloroform fraction yielded the analytically pure naphthalic anhydride, mp 114-115°; ir (potassium bromide): 2970, 2900, 1770, 1730, 1590, 1330, 1200, 770 cm^{-1}; nmr (deuteriochloroform): δ 8.5 (m, 3H, 2-, 5-, 7-H), 7.58 (m, 2H, 3-, 6-H), 3.2 (t, J = 6 Hz, 2H, S-CH_2), 1.67 (m, 4H, CH_2-CH_2), 0.98 (m, 3H, CH_3).

Anal. Calcd. for $C_{16}H_{14}SO_3$: C, 67.11; H, 4.93. Found: C, 66.92; H, 4.91.

4-Butanesulphenyl-1,8-naphthalic Anhydride (**18**).

Sodium nitrate (3.67 g) was added in portions over a 25 minute period

to a magnetically stirred solution of 4-butythio-1,8-naphthalic anhydride (10 g, 0.035 mole) in concentrated sulphuric acid (50 ml). The mixture was allowed to stir at ambient temperature for 1 hour and then poured into crushed ice (350 g). The resulting precipitate was filtered, air dried and recrystallised from ethyl acetate (charcoal) to give the sulphoxide (8.5 g, 81%), mp 177-178°; ir (potassium bromide): 3075, 2950, 2875, 1780, 1740, 1590, 1300, 1020, 790 cm^{-1}; nmr (DMSO-d_6): δ 8.5 (m, 5H, 2-, 3-, 5-, 6-, 7-H), 3.1 (m, 2H, S-CH_2), 1.5 (m, 4H, CH_2CH_2), 0.98 (m, 3H, CH_3).

Anal. Calcd. for $C_{16}H_{14}SO_4$: C, 63.56; H, 4.67. Found: C, 63.32; H, 4.64.

4-Butylsulphonyl-1,8-naphthalic Anhydride (**19**).

(a) A mixture of glacial acetic acid (4.66 ml) and hydrogen peroxide (100 vol, 1.53 ml) containing one drop of concentrated sulphuric acid was added dropwise to a magnetically stirred solution of 4-butylthio-1,8-naphthalic anhydride (1 g, 3.5 mmoles) in glacial acetic acid (50 ml) to which had been added a few grains of manganic acetylacetonate. The mixture was allowed to stir overnight at room temperature, and was then warmed cautiously on a boiling water bath (charcoal) and filtered into crushed ice to give a white precipitate. The precipitate was filtered, air dried and recrystallised from ethyl acetate (charcoal) to give the sulphone (0.85 g, 77%), mp 171-172°; ir (potassium bromide): 3100, 2975, 2875, 1780, 1740, 1300, 1130, 1030, 780 cm^{-1}; nmr (DMSO-d_6): δ 8.73 (m, 5H, 2-, 3-, 5-, 6-, 7-H), 3.6 (t, J = 6 Hz, 2H, SO_2CH_2), 1.4 (m, 4H, CH_2CH_2), 0.95 (m, 3H, CH_3).

Anal. Calcd. for $C_{16}H_{14}SO_5$: C, 60.37; H, 4.43. Found: C, 60.11; H, 4.46.

(b) A mixture of glacial acetic acid (0.7 ml) and hydrogen peroxide (100 vol, 0.23 ml) containing one drop of concentrated sulphuric acid was added dropwise to a magnetically stirred solution of the sulphoxide **18** (0.3 g, 1 mmole) in glacial acetic acid (15 ml) to which had been added a few grains of manganic acetylacetonate. The mixture was stirred overnight, quenched in ice and the resulting white precipitate filtered. Recrystallisation from ethyl acetate (charcoal) yielded an identical product to (a) above (1.26 g, 85%), mp 171-172°.

4-Butylthio-3-nitro-1,8-naphthalic Anhydride (**25**).

Aqueous potassium hydroxide (3.45 g/15 ml) was added dropwise over a 15 minute period to a magnetically stirred mixture of 4-chloro-3-nitro-1,8-naphthalic anhydride (15 g, 0.054 mole) and 1-butanthiol (5.3 g, 0.056 mole) in dimethylformamide (225 ml) at ambient temperature. Following addition, the mixture was allowed to stir for 1 hour, poured onto crushed ice and the yellow precipitate filtered off. Recrystallisation from a mixture of chloroform and petroleum ether (60-80°) gave the naphthalic anhydride (11.9 g, 67%), mp 168-170°; ir (potassium bromide): 2950, 2925, 1780, 1750, 1530, 1320, 1040, 780 cm^{-1}; nmr (DMSO-d_6): δ 9.13 (d, J = 7 Hz, 1H, 5-H), 8.95 (s, 1H, 2-H), 8.83 (d, J = 7 Hz, 1H, 7-H), 8.21 (t, J = 8 Hz, 1H, 6-H), 3.1 (m, 2H, S-CH_2), 1.48 (m, 4H, CH_2CH_2), 0.82 (m, 3H, C-CH_3).

Anal. Calcd. for $C_{16}H_{13}NSO_5$: C, 58.00; H, 3.93; N, 4.23. Found: C, 5[illegible]H, 3.76; N, 4.10.

N-(3-*N*^{1} Morpholino-1-propyl)-3-nitro-4-butylthio-1,8-naphthalimide (**23**).

3-Nitro-4-butylthio-1,8-naphthalic anhydride (1.0 g, 3 mmoles) and *N*-(3-aminopropyl)morpholine (0.42 g, 3 mmoles) were refluxed gently on a stirrer hotplate in absolute ethanol (18 ml) for 1 hour. The hot solution was treated with charcoal, refluxed gently for a few minutes and filtered to yield the naphthalimide on cooling (1.07 g, 78%), mp 124-125°.

N-(2-Hydroxyethyl)-4-(2-hydroxyethylamino)-3-nitro-1,8-naphthalimide (**22a**).

4-Chloro-3-nitro-1,8-naphthalic anhydride (1.4 g, 5 mmoles) and ethanolamine (0.92 g, 15 mmole) were refluxed on a stirrer hot plate in absolute ethanol (10 ml) for 2 hours. The orange suspension was cooled and the solid filtered off and recrystallised from aqueous dimethylformamide to give the naphthalimide (1.06 g, 73%).

N-(3-*N*^{1}-Morpholino-1-propyl)-3-nitro-4-(3-*N*-morpholino-1-propylamino)-1,8-naphthalimide Dihydrochloride (**22b**).

4-Chloro-3-nitro-1,8-naphthalic anhydride (1.3 g, 4.7 mmoles) and *N*-(3-aminopropyl)morpholine (2.02 g, 15 mmoles) were refluxed on a stirrer hot plate in absolute ethanol (13 ml) for 2 hours. The solvent was removed *in vacuo* to yield a gum which was triturated with water and the water discarded. The gum was boiled with 95% ethanol (50 ml) containing 10 drops of concentrated hydrochloric acid, treated with charcoal and filtered to give the crude dihydrochloride salt. Repeated recrystallisation from ethanolic hydrogen chloride (charcoal) yielded an analytical sample (1.0 g, 38%).

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