Synthesis and Fluorescence of *N*-Substituted-1,8-naphthalimides

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A number of novel *N*-substituted-1,8-naphthalimides have been prepared and their fluorescence yields measured in water at *p*H 7·4. The type of substitutent and the substitution pattern on the naphthalimide nucleus produce markedly different fluorescence yields, (quantum efficiencies, ϕ) varying from ϕ = 0·0037 for *N*-(3-N'-morpholino-1-propyl)-4-amino-3-methoxy-1,8-naphthalimide (**7**) to ϕ = 0·77 for *N*-(3-bromopropyl)-4-acetamido-1,8-naphthalimide (**31**).

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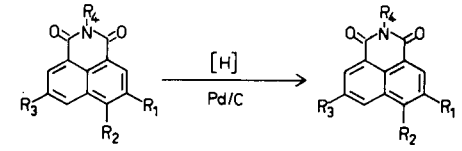
In an earlier paper we described the synthesis of a number of *N*-substituted-nitro-1,8-naphthalimides which, following metabolic reduction, may act as potential fluorescent probes of hypoxic cells [1]. The biological rationale and aspects of this work have been reported elsewhere [2-4]. However, optimisation of the fluorescence detection of metabolites in biological systems necessitated the synthesis and study of the fluorescence properties of a number of related non-nitro *N*-substituted 1,8-naphthalimides. We were particularly interested in the effects on the fluorescence of substituents having different electronic characteristics and the influence of the position of substitution on the magnitude of any effect.

Synthesis.

The *N*-substituted-1,8-naphthalimides **6**, **7**, **8** and **9** carrying an amino substituent on the aromatic nucleus and the *N*-substituted diamino-1,8-naphthalimide **10** were all prepared in high yield by the catalytic hydrogenation of the corresponding nitronaphthalimides **1**, **2**, **3**, **4** and dinitronaphthalimide **5** [1], respectively.

Additionally, the 3-aminonaphthalimide **6** was prepared in similar yield by treatment of 3-amino-1,8-naphthalic anhydride (**11**) with *N*-(3-aminopropyl)morpholine in absolute ethanol at room temperature. The anhydride **11** was conveniently prepared from 3-nitro-1,8-naphthalic anhydride (**18**) [5] by catalytic hydrogenation.

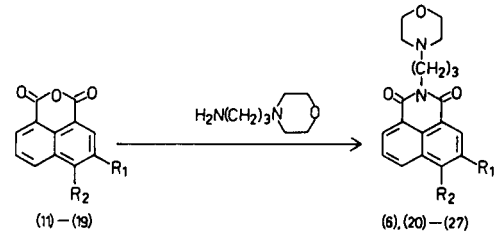
The 4-aminonaphthalimide **20** was similarly prepared from the corresponding commercially available naphthalic anhydride **12** [6] but more vigorous reaction conditions were necessary because of the deactivating, electron releasing effect of the amino group *para* to one of the anhydride carbonyl groups. Presumably, in these cases, initial attack occurs at the more reactive carbonyl group and the ring closure by attack at the deactivated carbonyl group is the step which is slowed down by the presence of the substituent.



(1)–(5) (6)–(10)

	R_1	R_2	R_3	R_4
(1)	NO_2	H	H	A
(2)	CH_3O	NO_2	H	A
(3)	NO_2	$NHCH_2CH_2OH$	H	B
(4)	NO_2	SC_4H_9	H	A
(5)	NO_2	H	NO_2	A
(6)	NH_2	H	H	A
(7)	CH_3O	NH_2	H	A
(8)	NH_2	$NHCH_2CH_2OH$	H	B
(9)	NH_2	SC_4H_9	H	A
(10)	NH_2	H	NH_2	A

A = $(CH_2)_3N$⟨O⟩ B = CH_2CH_2OH



(11)–(19) (6), (20)–(27)

	R_1	R_2	
(11)	NH_2	H	(6)
(12)	H	NH_2	(20)
(13)	OH	H	(21)
(14)	CH_3O	H	(22)
(15)	H	SC_4H_9	(23)
(16)	H	SOC_4H_9	(24)
(17)	H	H	(25)
(18)	NO_2	H	(26)
(19)	$NHCOCH_3$	H	(27)

The 3-hydroxy- **21**, 3-methoxy- **22**, 4-butylthio- **23**, 4-butanesulphenyl- **24**, 3-nitro- **26**, 3-acetamido- **27** and the unsubstituted 1,8-naphthalimide **25** were all prepared

Table 1

Starting material Wt (g) mmole	Product Wt (g) [a]	Pd/C 10% Wt (g)	Solvent ml [b]	H_2 press Atmospheres	Reaction time Hours [c]	Method
1	**6**		A			
0.6	0.413	0.2	200	3	4	A
1.6						
2	**7**		A			
1.0	0.758	0.3	150	3	2	A
2.5						
3	**8**		A:B			
0.483	0.366	0.15	50:50	5	6	A
1.4						
4	**9**		A:B			
0.594	0.416	0.15	30:30	5	9	A
1.3						
5	**10**		A:B			
1.0	0.627	0.2	50:100	5	17	A
2.4						
18	**11**		B			
5.0	4.0	0.7	150	3	4	B
20						

[a] % Yield see Table 2. [b] Solvent: A, absolute ethanol; B, dimethylformamide. [c] All reactions carried out at room temperature.

in high yield from the corresponding naphthalic anhydrides **13** [7], **14** [8,9], **15** [1], **16** [1], **18** [5], **19** and **17** by treatment with an equimolar equivalent of *N*-(3-aminopropyl)-morpholine in absolute ethanol. The 3-acetamido-1,8-naphthalic anhydride (**19**) was prepared in high yield by acetylation of the parent amine **11** with acetic anhydride in glacial acetic acid.

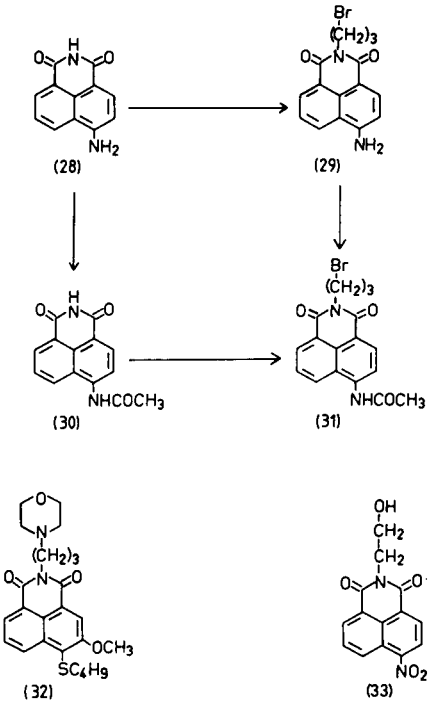
Treatment of a stirred suspension of 4-amino-1,8-naphthalimide (**28**) [6] in dimethylformamide with an equimolar equivalent of sodium methoxide followed by treatment with 1,3-dibromopropane gave the *N*-bromopropyl naphthalimide **29** in high yield. The 4-acetamidonaphthalimide **31** was obtained in almost quantitative yield by two routes: either by refluxing the amine **29** with acetic anhydride and acetic acid or by acetylation of **28** with acetic anhydride to yield 4-acetamido-1,8-naphthalimide (**30**), followed by alkylation of the imide nitrogen with 1,3-dibromopropane.

The *N*-substituted naphthalimides **32** and **33** were prepared from the corresponding naphthalic anhydrides as described in the literature [1,10].

Fluorescence.

It appears that the different types of alkyl chain attached to the imide-*N* atom considered in this work have little effect on the fluorescence emission maximum or on the relative quantum yield. Thus, the 4-aminonaphthalimides **20**, **28** and **29**, all containing different imide-*N* substitution, showed similar absorption and fluorescent properties (Table 3).

As expected, substituents on the aromatic rings have a marked effect on the fluorescence properties of the compounds. In particular the 3- or 4-nitro groups in **26** and **33**



and the 4-sulphoxide group in **24** decrease the quantum yield by at least three orders of magnitude compared with the unsubstituted example **25**. Thus, these compounds containing reducible, electron withdrawing groups exhibit almost no fluorescence. This is the desired situation for our purposes, since the biological probe is expected to be reduced only in the hypoxic cells to give fluorescent compounds. It now remained (i) to establish that the reduced products, eg. amines and sulphide, show fluorescence, and (ii) to prepare a range of naphthalimides in order to establish a relationship between structure and the wavelength of the fluorescence emission maximum and the quantum yield.

A comparison of the data (Table 3) for the 3-amino- **6** and 4-amino-naphthalimide **20** showed the quantum yield of the latter to be higher and it was speculated that the reason for this was the more marked electron 'push-pull' effect of the amino and carbonyl groups in **20**. In an attempt to improve the fluorescence yield from 3-aminonaphthalimides we prepared three derivatives with substituents *para* to the carbonyl groups. In **8** the 4-β-hydroxyethylamino substituent produced little change in quantum yield compared with **6**, though a marked change in the wavelength of the emission maximum was noted. The 4-sulphide substituent in **9** produced little effect on the fluorescence properties of the 3-aminonaph-

Table 2

Compound No	Yield %	Mp (°C)	Crystallisation Solvent	Formula	Elemental Analysis C% Calcd.	C% Found	H% Calcd.	H% Found	N% Calcd.	N% Found	^{1}H-NMR δ [ppm], TMS DMSO-d_6
6	75	179-180	Acetone	$C_{19}H_{21}N_3O_3$	67·26	66·84	6·19	6·06	12·39	12·30	1·8 (m, 2H, 2'-CH_2), 2·42 (m, 4H, 2 x OCH_2), 3·5 (m, 6H, 3 x N-CH_2), 4·15 (t, J = 6 Hz, 2H, N-CH_2), 6·0 (br s, 2H, NH_2), 7·35 (d, J = 1 Hz, 1H, 4-H), 7·60 (t, J = 8 Hz, 1H, 6-H), 8·0 (d, J = 1 Hz, 1H, 2-H), 8·2 (m, 2H, 5-, 7-H)
7	82	184-185	Ethanol	$C_{20}H_{23}N_3O_4$	65·03	64·65	6·28	6·16	11·37	11·52	1·81 (m, 2H, 2'-CH_2), 2·40 (m, 4H, 2 x O-CH_2), 3·52 (m, 6H, 3 x N-CH_2), 4·11 (m, 5H, OCH_3, N-CH_2), 7·0 (s, 2H, NH_2), 7·6 (t, J = 8 Hz, 1H, 6-H), 8·1 (s, 1H, 2-H), 8·36 (d, J = 8 Hz, 1H, 5-H), 8·76 (d, J = 8 Hz, 1H, 7-H)
8	83	150 dec	Ethanol [a]	$C_{16}H_{17}N_3O_4$	60·95	60·91	5·40	5·50	13·33	13·50	3·63 (t, J = 6 Hz, 4H, 2 x CH_2OH), 4·14 (t, J = 6 Hz, 4H, 2 x N-CH_2), 4·8 (m, 3H, NH, 2 x OH), 5·50 (s, 2H, NH_2), 7·63 (t, J = 8 Hz, 1H, 6-H), 8·06 (s, 1H, 2-H), 8·14 (d, J = 8 Hz, 1H, 5-H), 8·41 (d, J = 8 Hz, 1H, 7-H)
9	75	151-152	Ethanol [a]	$C_{23}H_{29}N_3O_3S$	64·64	64·77	6·79	6·85	9·84	9·65	0·79 (t, J = 6 Hz, 3H, CH_3), 1·30 (m, 4H, CH_2C$H_2$$CH_3$), 1·89 (m, 2H, 2'-$CH_2$), 2·41 (m, 4H, 2 x OCH_2), 2·85 (t, J = 6 Hz, 2H, S-CH_2), 3·50 (m, 6H, 3 x NCH_2), 4·20 (t, J = 6 Hz, 2H, N-CH_2), 6·5 (s, 2H, NH_2), 7·8 (t, J = 8 Hz, 1H, 6-H), 8·25 (d, J = 8 Hz, 1H, 5-H), 8·30 (s, 1H, 2-H), 8·71 (d, J = 8 Hz, 1H, 7-H)
10	68	242-243	Ethanol [a]	$C_{19}H_{22}N_4O_3$	64·39	64·21	6·26	6·07	15·81	15·92	1·82 (m, 2H, 2'-CH_2), 2·35 (m, 4H, 2 x OCH_2), 3·55 (m, 6H, 3 x NCH_2), 4·20 (t, J = 6 Hz, 2H, N-CH_2), 5·8 (s, 2H, NH_2), 7·2 (d, J = 2 Hz, 2H, 4-, 5-H), 7·8 (d, J = 2 Hz, 2H, 2-, 7-H)
20	70	175-177	Ethanol-[a] Ethyl acetate	$C_{19}H_{21}N_3O_3$	67·26	67·41	6·19	6·16	12·39	12·52	1·80 (m, 2H, 2'-CH_2), 2·43 (m, 4H, 2 x OCH_2), 3·57 (m, 6H, 3 x N-CH_2), 4·22 (t, J = 6 Hz, 2H, N-CH_2), 7·0 (d, J = 8 Hz, 1H, 4-H), 7·5 (s, 2H, NH_2), 7·8 (t, J = 8 Hz, 1H, 6-H), 8·4 (d, J = 8 Hz, 1H, 2-H), 8·7 (m, 2H, 5-, 7-H)
21	75	205-206	Methanol	$C_{19}H_{20}N_2O_4$	67·10	66·71	5·88	5·60	8·24	8·62	1·79 (m, 2H, 2'-CH_2), 2·20 (s, 1H, OH), 2·40 (m, 4H, 2 x O-CH_2), 3·56 (m, 6H, 3 x N-CH_2), 4·20 (t, J = 6 Hz, 2H, N-CH_2), 7·50 (d, J = 1 Hz, 1H, 4-H), 7·63 (m, 1H, 6-H), 7·94 (d, J = 1 Hz, 1H, 2-H), 8·11 (m, 2H, 5-, 7-H)
22	79	125-126	Ethanol	$C_{20}H_{22}N_2O_4$	67·80	67·78	6·21	6·31	7·91	7·78	1·79 (m, 2H, 2'-CH_2), 2·42 (m, 4H, 2 x O-CH_2), 3·55 (m, 9H, 3 x N-CH_2, O-CH_3), 4·22 (t, J = 6 Hz, 2H, N-CH_2), 7·65 (s, 1H, 4-H), 7·78 (m, 1H, 6-H), 8·04 (s, 1H, 2-H), 8·32 (m, 2H, 5-, 7-H)

Table 2 Continued

Compound No	Yield %	Mp (°C)	Crystallisation Solvent	Formula	Elemental Analysis C% Calcd.	C% Found	H% Calcd.	H% Found	N% Calcd.	N% Found	^{1}H-NMR δ [ppm], TMS DMSO-d_6
23	89	227-228	Ethanol	$C_{23}H_{28}N_2O_3S$,HCl	61·53	61·35	6·51	6·25	6·24	6·46	1·01 (m, 3H, CH_3), 1·28 (m, 4H, $CH_2CH_2CH_3$), 1·75 (m, 2H, 2'-CH_2), 2·2 (m, 4H, 2 x O-CH_2), 2·68 (m, 2H, S-CH_2), 3·30 (m, 6H, 3 x N-CH_2), 4·20 (m, 3H, N-CH_2, N-H), 7·8 (d, J = 8 Hz, 1H, 3-H), 7·9 (t, J = 8 Hz, 1H, 6-H), 8·4 (d, J = 8 Hz, 1H, 2-H), 8·55 (m, 2H, 5-, 7-H)
24	70	121-122	Ethanol	$C_{23}H_{28}N_2O_4S$	64·46	64·66	6·59	6·76	6·54	6·74	0·83 (m, 3H, CH_3), 1·40 (m, 4H, $SOCH_2CH_2CH_2$-), 1·83 (m, 2H, 2'-CH_2), 2·30 (m, 4H, 2 x O-CH_2), 3·29 (m, 8H, $SOCH_2$, 3 x N-CH_2), 4·15 (t, J = 6 Hz, 2H, N-CH_2), 8·5 (m, 5H, 2-, 3-, 5-, 6-, 7-H)
25	85	138-139	Ethanol	$C_{19}H_{20}N_2O_3$	70·35	70·33	6·22	6·31	8·64	8·61	1·82 (m, 2H, 2'-CH_2), 2·32 (m, 4H, 2 x O-CH_2), 3·45 (m, 6H, 3 x N-CH_2), 4·15 (t, J = 6 Hz, 2H, N-CH_2), 7·89 (t, J = 8 Hz, 2H, 3-, 6-H), 8·56 (m, 4H, 2-, 4-, 5-, 7-H)
26	69	157-159	Ethanol	$C_{19}H_{19}N_3O_5$	61·79	61·76	5·15	5·04	11·38	11·67	1·80 (m, 2H, 2'-CH_2), 2·43 (m, 4-H, 2 x O-CH_2), 3·50 (m, 6H, 3 x N-CH_2), 4·05 (m, 2H, N-CH_2), 8·06 (t, J = 8 Hz, 1H, 6-H), 8·66 (d, J = 8 Hz, 1H, 5-H), 8·91 (d, J = 8 Hz, 1H, 7-H), 9·26 (s, 2H, 2-, 4-H)
27	85	>300	Aqueous Ethanol	$C_{21}H_{23}N_3O_4$,HCl	60·36	60·59	5·75	5·33	10·06	9·82	1·90 (m, 2H, 2'-CH_2), 2·16 (s, 3H, $COCH_3$), 2·43(m, 4-H, 2 x O-CH_2), 3·45 (m, 6H, 3 x N-CH_2), 4·15 (m, 3H, N-CH_2, NH), 7·7 (t, J = 8Hz, 1H, 6-H), 8·30 (m, 2H, 5-, 7-H), 8·65 (s, 1H, 4-H), 8·75 (s, 1H, 2-H), 10·57 (s, 1H, N*H*C-OCH_3)
29	79	224-225	DMF	$C_{15}H_{13}BrN_2O_2$	54·07	53·98	3·93	4·01	8·41	8·45	2·16 (m, 2H, 2'-CH_2), 3·56 (t, J = 6 Hz, 2H, N-CH_2), 4·13 (t, J = 6 Hz, 2H, Br-CH_2), 6·76 (d, J = 8 Hz, 1H, 3-H), 7·31 (s, 2H, NH_2), 7·56 (t, J = 8 Hz, 1H, 6-H), 8·16 (d, J = 8 Hz, 1H, 2-H), 8·46 (m, 2H, 5-, 7-H)
31	95	244-246	Acetic acid	$C_{17}H_{15}BrN_2O_3$	54·41	54·47	4·00	4·08	7·47	7·53	2·20 (m, 2H, 2'-CH_2), 2·27 (s, 3H, $COCH_3$), 3·60 (t, J = 6 Hz, 2H, N-CH_2), 4·10 (t, J = 6 Hz, 2H, Br-CH_2), 7·76 (m, 1H, 6-H), 8·53 (m, 4H, 2-, 3-, 5-, 7-H), 12·33 (s, 1H, N-H)

[a] Activated charcoal used during crystallisation.

thalimide system. This was unexpected since it had been noted that the presence of a 4-butylthio group alone in **23** gave a fluorescence efficiency greater than that of 4-aminonaphthalimide. It seems likely that, in the presence of a 3-amino substituent, the effects of additional electron donating substituents in the 4-position are negligible due to steric effects causing rotation of one or both substituents out of conjugation with the aromatic ring.

A similar but more marked effect was seen in a comparison of the fluorescence properties of the 3-methoxynaphthalimide **22** (ϕ = 0·15), the 4-butylthionaphthalimide **23** (ϕ = 0·21), and the 4-butylthio-3-methoxynaphthalimide **32** (ϕ = 0·04). It seemed that a possible way around this problem was the introduction of a second amino substituent in the other benzene moiety. The 3,6-diaminonaphthalimide **10** was prepared but its fluorescence was disappointingly weak. It is clear that the effects of substituents in the two rings are not the sum of the individual effects.

The compounds carrying only a 4-amino substituent on the carbocyclic system, **28** and **29**, gave ϕ = 0·1 and 0·13

Table 3

Absorption and Fluorescence Properties of *N*-Substituted-1,8-Naphthalimides in Aqueous Phosphate Buffer *p*H 7.4, 25°

Compound number	Absorption maxima [a] λ/nm	log ε/$M^{-1}cm^{-1}$	Excitation [b] λ/nm	log ε/$M^{-1}cm^{-1}$	Emission [c] λ max/nm	φ [d]
6	345	3.93	351	3.88	600	0.020
	405	3.65	364	3.44	"	0.024
7	458	4.04	458	4.04	620	0.0037
8	361	3.80	351	3.76	450	0.022
	440	3.87				
9	351	3.89	351	3.89	600	0.019
	425	3.74	458	3.54	"	0.024
10	405	3.94	351	3.38	610	0.019
			458	3.43	"	0.021
20	432	4.08	351	3.04	550	0.12
			364	3.31	"	0.12
			458	3.95	"	0.11
21	341	3.96	351	3.88	630	0.010
	383	3.79	364	3.73	"	0.011
22	340	4.00	340	4.00	440	0.15
	380	3.84	351	3.87	"	0.15
			380	3.84	"	0.18
23	402	4.08	351	3.65	520	0.22
			364	3.73	"	0.19
			402	4.08	"	0.21
24	347	4.21	347	4.21	not detectable	<0.0001
			364	4.10	not detectable	"
25	344	4.17	344	4.17	400	0.090
			351	4.13	"	0.087
26	335	3.92	335	3.92	not detectable	<0.0001
27	343	3.98	343	3.98	460	0.085
			351	3.92	"	0.084
			364	3.76	"	0.086
28	430	3.76	430	3.76	550	0.10
			458	3.62	550	0.10
29	432	4.10	430	4.10	550	0.13
31	354	4.15	351	4.15	475	0.77
			357	4.15	"	0.74
			364	4.11	"	0.71
32	354	3.84	351	3.84	570	0.039
	400	3.92	400	3.92	"	0.053
33	354	4.08	354	4.08	not detectable	<0.0001
Lucifer Yellow CH [e]	430	4.02	430	4.02	550	0.22
			458	3.80	"	0.22

[a] Only λ > 300 nm indicated. [b] Excitation either at > 300 nm absorption maxima and/or 351-364 and 458 nm commonly used in flow cytometer applications in the Gray Laboratory [3]. [c] Corrected, to nearest 5 nm. [d] Each value from ≥ 2 determinations. [e] Lucifer Yellow CH is 4-amino-*N*-hydrazinocarbonyl-1,8-naphthalimide-3,6-disulphonate dilithium salt [Lit [11] φ 0.20-0.24].

respectively. The introduction of a 3-methoxy substituent as in **7** produced a marked decrease in fluorescence yield (ϕ = 0·0037). Interestingly, by far the highest fluorescent yield (ϕ = 0·77) in the series of naphthalimides studied was found in the 4-acetamido derivative **31**. This was unexpected since the substituent is bulky and much less electron donating than the amino group. A significant increase in fluorescence was also observed with the 3-acetamidonaphthalimide **27** (ϕ = 0·085) compared with the 3-aminonaphthalimide **6** (ϕ = 0·02).

EXPERIMENTAL

Infrared and mass spectra were recorded with a Pye-Unicam SP3-100 infrared spectrophotometer and an A. E. I. MS902 mass spectrometer. Proton nmr spectra were recorded using a Varian T-60 or Varian CFT-20 spectrometer with TMS as internal standard. Elemental analyses were performed by Butterworth Laboratories, Middlesex U. K. Melting points are uncorrected.

Crystallisation solvent, yield, melting point, ^1H nmr and elemental analyses for all naphthalimides are recorded in Table 2. Satisfactory mass and infrared spectra were additionally obtained.

Absorption and fluorescence data were obtained at 25° using a Pye-

Unicam SP8-200 spectrophotometer and a Perkin-Elmer LS-5 luminescence spectrophotometer fitted with a red sensitive R928 photomultiplier. Emission spectra were measured using 10 nm excitation and emission band widths for aerated aqueous phosphate (10 m*M*) solutions (0·5-8 μM), pH 7·4 ± 0·2 with optical densities <0·05 at excitation wavelengths in a 10 mm pathlength silica cell. Emission correction factors were determined at 10nm intervals from 250-750 nm by reference to an excitation beam diffuser plate (Perkin-Elmer, 5212-4054) [11], quinine sulphate dihydrate (Aldrich, 14,591-2) in 0·1 *M* perchloric acid [11,12] and 4-dimethylamino-4'-nitrostilbene (Kodak, 10702) in 1,2-dichlorobenzene [13]. Fluorescence quantum yields (ϕ), were determined from areas under corrected emission spectra relative to quinine sulphate dihydrate (0·4 μM) in 0·1 *M* perchloric acid (ϕ = 0·59) [11,12].

Catalytic hydrogenations were carried out by one of two methods and an example of each method is given below. The individual experimental details for each compound prepared by catalytic hydrogenation is given in Table 1.

Method A.

N-(3-*N'*-Morpholino-1-propyl)-3-amino-1,8-naphthalimide (**6**).

N-(3-*N'*-Morpholino-1-propyl)-3-nitro-1,8-naphthalimide (**1**) [1] (0·6 g, 1·6 mmole) was shaken for 4 hours with 10% palladium on charcoal (0·2 g) in absolute ethanol (200 ml) in the presence of hydrogen at 3 atmospheres at room temperature. The mixture was filtered and the filtrate evaporated *in vacuo* to yield the amine. One crystallisation yielded an analytical sample.

Method B.

3-Amino-1,8-naphthalic anhydride (**11**).

3-Nitro-1,8-naphthalic anhydride (**18**) [5] (5·0 g, 0·02 mole) was shaken with 10% palladium on charcoal (0·7 g) in dimethylformamide (150 ml) in the presence of hydrogen at 3 atmospheres for 4 hours at room temperature. The mixture was filtered into an excess of ice-water to yield the crude amine. Crystallisation from aqueous dimethylformamide yielded the amine (4·0 g, 91%), mp >300°; ir (potassium bromide): 3475, 3370, 1760, 1730, 1580, 1450, 1340, 1290, 1020, 780 cm^{-1}; nmr (DMSO-d_6): δ 6·0 (s, 2H, NH_2), 7·3 (d, J = 1 Hz, 1H, 4-H), 7·6 (m, 1H, 6-H), 7·9 (d, J = 1 Hz, 1H, 2-H), 8·0 (m, 2H, 5- and 7- H).

N-(3-*N'*-Morpholino-1-propyl)-3-amino-1,8-naphthalimide (**6**).

3-Amino-1,8-naphthalic anhydride (**11**) (1·0 g, 4·7 mmoles) was stirred magnetically with *N*-(3-aminopropyl)morpholine (0·676 g, 4·7 mmoles) in absolute ethanol (25 ml) at room temperature for 6 hours. The solvent was evaporated *in vacuo* and the residue crystallised from acetone to yield the analytically pure amine (1·2 g, 75%) which was identical to the product obtained by catalytic hydrogenation of **1**.

N-(3-*N'*-Morpholino-1-propyl)-4-amino-1,8-naphthalimide (**20**).

4-Amino-1,8-naphthalic anhydride (**12**) [14] (1·0 g, 4·6 mmoles) and *N*-(3-aminopropyl)morpholine (0·69 g, 4·8 mmoles) were refluxed in absolute ethanol (25 ml) with magnetic stirring for 2 hours. The brown solution was allowed to cool and then the solvent was evaporated *in vacuo* to leave a brown gum. Crystallisation from a mixture of ethanol and ethyl acetate (charcoal) yielded the analytically pure amine as brown prisms.

N-(3-*N'*-Morpholino-1-propyl)-3-hydroxy-1,8-naphthalimide. (**21**).

3-Hydroxy-1,8-naphthalic anhydride (**13**) [7] (1·0 g, 4·7 mmoles) and *N*-(3-aminopropyl)morpholine (0·67 g, 4·7 mmoles) were refluxed in absolute ethanol (12 ml) with magnetic stirring for half an hour. The yellow solution was allowed to cool and the precipitate crystallised from aqueous ethanol to yield the hydroxynaphthalimide. An analytical sample was obtained by recrystallisation from methanol.

N-(3-*N'*-Morpholino-1-propyl)-3-methoxy-1,8-naphthalimide (**22**).

3-Methoxy-1,8-naphthalic anhydride (**14**) [9] (0·5 g, 2·2 mmoles) and *N*-(3-aminopropyl)morpholine (0·32 g, 2·2 mmoles) were refluxed in absolute ethanol (8 ml) for 10 minutes. The methoxynaphthalimide crystallised on cooling and was filtered off and recrystallised from aqueous ethanol to give an analytical sample.

N-(3-*N'*-Morpholino-1-propyl)-4-butylthio-1,8-naphthalimide Hydrochloride (**23**).

4-Butylthio-1,8-naphthalic anhydride (**15**) [1] (1·0 g, 3·5 mmoles) and *N*-(3-aminopropyl)morpholine (0·506 g, 3·5 mmoles) were refluxed in absolute ethanol (10 ml) with magnetic stirring for half an hour. A few drops of concentrated hydrochloric acid were added, the yellow solution was boiled with charcoal, and filtered. The filtrate yielded the analytically pure butylthionaphthalimide.

N-(3-*N'*-Morpholino-1-propyl)-4-butanesulphenyl-1,8-naphthalimide (**24**).

4-Butanesulphenyl-1,8-naphthalic anhydride (**16**) [1] (0·73 g, 2·4 mmoles) and *N*-(3-aminopropyl)morpholine (0·36 g, 2·5 mmoles) were refluxed in absolute ethanol (7 ml) for 15 minutes. The hot solution was cooled and the precipitate crystallised from absolute ethanol to give the analytically pure naphthalimide.

N-(3-*N'*-Morpholino-1-propyl)-1,8-naphthalimide (**25**).

1,8-Naphthalic anhydride (**17**) (5·0 g, 25 mmoles) and *N*-(3-aminopropyl)morpholine (3·8 g, 26 mmoles) were refluxed in absolute ethanol (70 ml) for 2 hours. Absolute ethanol (50 ml) was added to the dark solution which was then boiled with charcoal and filtered hot to yield the analytically pure naphthalimide on cooling.

N-(3-*N'*-Morpholino-1-propyl)-3-nitro-1,8-naphthalimide (**26**).

3-Nitronaphthalic anhydride (**18**) [5] (2·0 g, 8·2 mmoles) and *N*-(3-aminopropyl)morpholine (1·25 g, 8·7 mmoles) were stirred in absolute ethanol (50 ml) at room temperature for 4 hours. The yellow precipitate was filtered off and crystallised from absolute ethanol (charcoal) to yield the analytically pure naphthalimide.

N-(3-*N'*-Morpholino-1-propyl)-3-acetamido-1,8-naphthalimide hydrochloride (**27**).

3-Acetamidonaphthalic anhydride (**19**) (0·5 g, 1·96 mmoles) and *N*-(3-aminopropyl)morpholine (0·29 g, 1·96 mmoles) were stirred in absolute ethanol (10 ml) at room temperature for 24 hours. The yellow precipitate was filtered off and recrystallised twice from aqueous ethanolic hydrogen chloride (charcoal) to yield the analytically pure naphthalimide.

3-Acetamido-1,8-naphthalic Anhydride (**19**).

3-Amino-1,8-naphthalic anhydride (**11**) (2·0 g, 9·4 mmoles), acetic anhydride (12 ml) and glacial acetic acid (33 ml) were refluxed with magnetic stirring for 1 hour. The reaction mixture was cooled and the acetamido naphthalic anhydride **19** was filtered off and crystallised from aqueous dimethylformamide (charcoal) to yield the analytically pure product. (2·0 g, 84%), mp >300°, ir (potassium bromide): 3220, 1760, 1730, 1550, 1370, 1290, 1140, 1000, 780 cm^{-1}; nmr (DMSO-d_6): δ 2·16 (s, 3H, $COCH_3$), 7·83 (m, 1H, 6-H), 8·40 (m, 2H, 5- and 7-H), 8·6 (s, 1H, 4-H), 8·85 (s, 1H, 2-H), 10·55 (s, 1H, N-H).

Anal. Calcd. for $C_{14}H_9NO_4$: C, 65·88; H, 3·53: N, 5·50. Found: C, 65·80; H, 3·40; N, 5·68.

N-(3-Bromopropyl)-4-amino-1,8-naphthalimide (**29**).

A magnetically stirred slurry of 4-amino-1,8-naphthalimide (**28**) (1·0 g, 4·7 mmoles) in dimethylformamide (30 ml) at room temperature was treated with a methanolic solution of sodium methoxide (5 ml, 4·7 mmoles) (1·08 g of Na in 50 ml of methanol). After stirring for 1 hour, 1,3-dibromopropane (2 ml, 19·6 mmoles) was added to the brown solution and stirring continued for a further 15 minutes. The reaction was quenched with ice-water (100 g) to yield the bromopropylnaphthalimide as a yellow solid. Crystallisation from dimethylformamide gave an analytical sample.

N-(3-Bromopropyl)-4-acetamido-1,8-naphthalimide (**31**).

(a) The bromopropylnaphthalimide (**29**) (1·0 g, 3 mmoles), acetic

anhydride (5 ml) and glacial acetic acid (10 ml) were refluxed with magnetic stirring for 45 minutes. The acetamidonaphthalimide, which crystallised on cooling, was filtered off and recrystallised from acetic acid to yield the analytically pure product.

(b) 4-acetamido-1,8-naphthalimide (**30**) (1·2 g, 4·7 mmoles) in dimethylformamide (30 ml) was treated with a methanolic solution of sodium methoxide (5 ml, 4·7 mmole) (1·08 g of Na in 50 ml of methanol) and the red-orange suspension stirred for 1 hour. 1,3-Dibromopropane (2 ml, 19·6 mmoles) was added and the suspension stirred overnight. The reaction mixture was poured into ice-water (150 g) to yield a solid which, after crystallisation from acetic acid, gave a product identical to (a) above (1·48 g, 84% yield).

4-Acetamido-1,8-naphthalimide (**30**).

4-Amino-1,8-naphthalimide (**28**) [6] (2·26 g, 10·6 mmoles), acetic anhydride (12 ml) and glacial acetic acid (33 ml) were refluxed with magnetic stirring for 1 hour. The reaction mixture was cooled and the acetamido-naphthalimide **30** was filtered off and dried *in vacuo* to give a quantitative yield, mp 332-333° [lit [14] mp 333°].

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