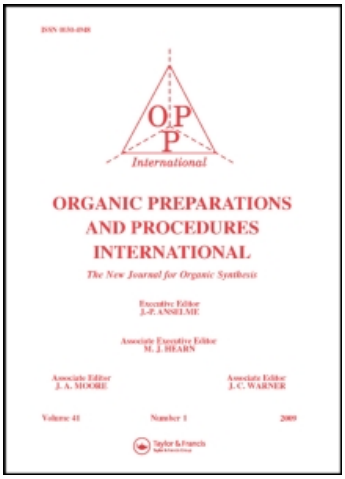

Organic Preparations and Procedures International
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t902189982

SYNTHESIS OF FUNCTIONALIZED N-ALKYLMALEIMIDES AS POTENTIAL THIOL-DIRECTED ENZYME INHIBITORS

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To cite this Article Ramirez, Fausto , Okazaki, Hiroshi , Marecek, James F. and Levy, Harvey M.(1981) 'SYNTHESIS OF FUNCTIONALIZED N-ALKYLMALEIMIDES AS POTENTIAL THIOL-DIRECTED ENZYME INHIBITORS', Organic Preparations and Procedures International, 13: 3, 217 — 223
To link to this Article: DOI: 10.1080/00304948109356129
URL: http://dx.doi.org/10.1080/00304948109356129

ORGANIC PREPARATIONS AND PROCEDURES INT. 13(3-4), 217-223 (1981)

SYNTHESIS OF FUNCTIONALIZED N-ALKYLMALEIMIDES AS POTENTIAL THIOL-DIRECTED ENZYME INHIBITORS

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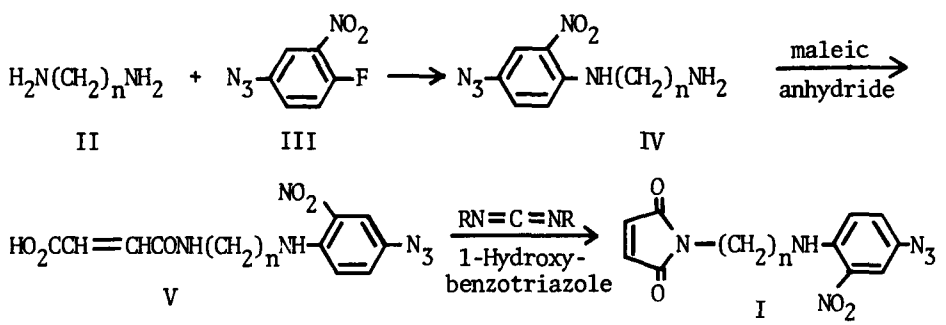
N-Ethylmaleimide, (NEM) has been extensively used to modify thiol groups in proteins.[1] The nitrophenylazide group is useful in affinity labeling of proteins,[2,3] since the photolysis of this group generates nitrene intermediates ($ArN_3 \xrightarrow{h\nu} Ar\ddot{N} + N_2$) which are quite reactive toward certain amino acid residues in proteins.[4] Hence, compounds containing the maleimide and nitrophenylazide moieties in the same molecule are potential light-dependent proximity probes for thiol-containing enzymes. This paper describes the synthesis of a series of compounds I (Scheme 1) containing the photosensitive nitroazidoanilino-group connected to maleimide by an alkyl chain of variable length.



a) n = 2 b) n = 3 c) n = 4

SCHEME 1

The acylation of simple amines by maleic anhydride is a well-known procedure,[5] and there is abundant literature[6-12] on the formation of isomaleimides from N-substituted maleamic acids at temperatures below 80°. However, the conversion of isomaleimides into the corresponding N-substituted maleimides requires severe heating, preferably in the presence of nucleophilic reagents. Evidently, these conditions are undesirable with sensitive maleamic acids of the type shown in formula V. A solution to this problem is provided by the 1-hydroxybenzotriazole/carbodiimide procedure[13] originally developed by König and Geiger[14] in peptide synthesis.

The monoarylation of polymethylenediamines, II, by 4-fluoro-3-nitrophenylazide (III) does not require protection of one of the two amino groups, and is achieved by the slow addition of azide III to an excess of diamine in ethanol solution (Scheme 1). The resulting diaminonitroazides, IV, are readily acylated by maleic anhydride in a dichloromethane medium, to yield the corresponding maleamic acids, V. We studied the action of dicyclohexylcarbodiimide on the maleamic acids, V, at 25°, in the absence and in the presence of 1-hydroxybenzotriazole. The mixed carbodiimide/triazole reagent led, indeed, to higher yields of purer products. However, even utilizing the optimum experimental conditions, the yields of the maleimides, I, never exceeded 50% of the theoretical value.

The structure of the azidomaleimides I is supported by elemental analyses of some members of the series and by ^{1}H NMR, infrared and ultraviolet spectroscopy. The two vinyl hydrogens of the maleimide moiety are magnetically equivalent; e.g. in compound Ib they give rise to a singlet at τ 3.23 ppm (in $CDCl_3$), and in compound Ic, to a singlet at τ 3.10 (in pyridine-d_5). The infrared spectra of I are unexceptional in the carbonyl region, in both Nujol mull and solutions. The three compounds exhibit the characteristic single band due to the asymmetric stretching vibration of

organic azides at 2125 ± 5 cm^{-1} in dichloromethane solutions[15]. The Nujol mull of the maleimides shows this azide band as a doublet. The observation of a double asymmetric stretching vibration in several organic azides has been reported, and has been tentatively ascribed to Fermi resonance.[15] Since the doublet is observed only in the solid state, it is conceivable that the phenomenon may be associated with different conformations of the molecule in the solid.

The maleimides, I, are quite sensitive to the usual laboratory illumination and must be prepared and handled under subdued red light. The compounds are moderately soluble in acetonitrile and more soluble in pyridine; they remain in solution in 10:90 pyridine/water, v/v, which is, therefore, a convenient vehicle for incorporation of the probe into the protein. No changes are observed when pyridine solutions of compound Ib are kept 18 hrs at 25° in the dark.

TABLE. Ultraviolet and Infrared Data of Maleimides I

	Ultraviolet Spectrum[a]		Infrared Spectrum			
	λ_1(ε max)	λ_2(ε max)	C=O		N_3	
I			CH_2Cl_2	Nujol	CH_2Cl_2	Nujol
n = 2	260(23,360)	455(5,150)	1710	1700	2130	2140 2110
n = 3	260(24,070)	462(5,500)	1710	1700	2120	2135 2110[b]
n = 4	260(23,160)	463(5,500)	1710	1710	2125	2120 2090

a) In acetonitrile b) Shoulder.

EXPERIMENTAL

Due to the instability of compounds I, and of their precursors, satisfactory elemental analyses could not be obtained in all cases.

4-Fluoro-3-nitrophenylazide (III).- The fluoroazide III, mp 51-52° (from petroleum ether) was prepared from 4-fluoro-3-nitroaniline in 70% yield by

the procedure of Knowles et al.[4] Freshly prepared fluoroazide was used in all experiments.

4-(3'-aminopropyl)amino-3-nitrophenylazide (IVb).- A solution of the fluoroazide (III, 4.11 g, 22.6 mmol) in 95% ethanol (100 mL) was added dropwise (ca. 1 h) to a stirred solution of distilled 1,3-diaminopropane (8.36 g, 112.8 mmol) in 95% ethanol (100 mL) at 25°. The course of the reaction was followed by TLC on silica gel plates using CH_2Cl_2/CH_3OH, 9/1, v/v, as solvent. After 12 h at 25°, the solution was filtered to remove a small amount of by-product, and the filtrate evaporated (40°, 20 mm). The residue was kept 12 h at 25° (0.1 mm) to remove most of the excess diamine then was treated with 5% aq. $NaHCO_3$ (200 mL). The mixture was extracted with dichloromethane (3 x 200 mL), and the dried extracts were evaporated (40°, 2 mm and 0.2 mm). The solid red diaminoazide IVb (4.4 g, 82% yield, mp 44-46°) exhibited one spot on TLC and was immediately utilized for the next step of the synthesis. The analytical sample, mp 45-46° was obtained from toluene/diethyl ether, 1/1.

Anal. Calcd. for $C_9H_{12}O_2N_6$: C, 45.7; H, 5.12; N, 35.6.
Found: C, 46.1; H, 5.20; N, 35.4.

4-(2'-aminoethyl)amino-3-nitrophenylazide (IVa), mp 65-68°, was obtained from 1,2-diaminoethane in 75% yield by an analogous procedure. Since this diaminoazide is sparingly soluble in dichloromethane, the solid obtained in the $NaHCO_3$ treatment was collected by filtration and was dried 16 hrs at 25° (0.2 mm).

4-(4'-aminobutyl)amino-3-nitrophenylazide (IVc), was obtained as a red oil from 1,4-diaminobutane in 82% yield, by a procedure analogous to that described for the propylamino analog.

N-[3-(2'-nitro-4'azidophenyl)amino] propylmaleamic Acid (Vb).- A solution of reagent-grade maleic anhydride (1.82 g, 18.6 mmol) in dichloromethane

(100 mL) was added to a stirred mixture of the diaminoazide (IVb, 4.4 g, 18.6 mmol) and dichloromethane (200 mL) at 25°. The mixture was stirred 12 hr at 25°, and the insoluble red maleamic acid Vb was filtered, and recrystallized from 95% ethanol (80 mL/g). The maleamic acid (4.4 g, 88%, mp 139-140° with decomposition) was immediately submitted to the next step of the synthesis.

Anal. Calcd. for $C_{13}H_{14}O_5N_6$: C, 46.7; H, 4.22; N, 25.1.
Found: C, 46.6; H, 4.37; N, 25.3.

N-[2-(2'-nitro-4'azidophenyl)amino] ethylmaleamic acid (Va), was obtained in 84% yield by an analogous procedure; it had mp 140-145° (dec.) after recrystallization from 95% ethanol.

N-[4-(2'-nitro-4'-azidophenyl)amino] butylmaleamic acid (Vc), was obtained in 94% yield by an analogous procedure; it had mp 115-120° (dec.).

N-[3-(2'-nitro-4'-azidophenyl)amino] propylmaleimide (Ib).- 1-Hydroxybenzotriazole (0.98 g, 7.2 mmol) was added to a suspension of the maleamic acid (Vb, 2.42 g, 7.2 mmol) in dichloromethane (200 mL) at 25°. The mixture was stirred for 5 min and was treated with dicyclohexylcarbodiimide (1.64 g, 1.1 molequiv). The course of the cyclization was followed by TLC on silica gel plates using dichloromethane as solvent. After 24 hr at 25°, the mixture was filtered to remove dicyclohexyl urea. The filtrate was evaporated at 30° (20 mm) and the residue was triturated with petroleum ether to remove any remaining carbodiimide. The crude maleimide Ib was dissolved in dichloromethane (20 mL) and placed on a 4.7 x 35 cm column containing 150 g of silica gel 60. The column was eluted with ca 1.5 L of dichloromethane to remove a yellow by-product, followed by ca 2.5 L of dichloromethane to obtain the red maleimide. The solution was evaporated at 40° (20 mm) and the residue was dried at 25° (0.1 mm). The maleimide (1.1 g, 48% yield) melted with decomposition near 200° before and after

recrystallization from dichloromethane/hexane, 3/2.

Anal. Calcd. for $C_{13}H_{12}O_4N_6$: C, 49.3; H, 3.83; N, 26.5.
Found: C, 49.1; H, 3.78; N, 26.3.

N-[2-(2'-nitro-4'-azidophenyl)amino] ethyl maleimide (Ia), was prepared in 47% yield after chromatography, following the same procedure as for Ib. Compound Ia melted with decomposition near 150°.

Anal. Calcd. for $C_{12}H_{10}O_4N_6$: C, 47.7; H, 3.33; N, 27.8.
Found: C- 47.8; H, 3.58; N, 27.6.

N-[4-(2'-nitro-4'-azidophenyl)amino] butylmaleimide (Ic), was prepared in 31% yield by a similar procedure. However, the compound appeared to be less stable than the other analogs, and the final purification step was as follows. The crude maleimide Ic was dissolved in CH_2Cl_2 and any insoluble material removed by filtration. The product was precipitated by the addition of petroleum ether, filtered, washed with petroleum ether and dried at 25° (0.2 mm) for several hours. TLC on silica gel using $CH_2Cl_2/C_6H_5CH_3$, 1/1, v/v as solvent showed only one spot. The purity of this sample was also supported by the values of the UV molecular extinction coefficients (ε), (Table). The physical constants of the sample did not change after preparative TLC on 2 mm silica gel plates using dichloromethane as solvent.

Acknowledgement. - This research was supported by Grant GM20672 from the National Institute of General Medical Sciences (to F.R.) and by a Grant from the Muscular Dystrophy Association (to H.M.L.).

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(Received December 8, 1980; in revised form January 27, 1981)