Tetrahedron Vol. 42, No. 14, pp. 4073 to 4082, 1986
Printed in Great Britain.

0040-4020/86 $3.00+.00
Pergamon Journals Ltd.

4-NITROPYROCATECHOL ETHERS AS POSSIBLE PHOTOAFFINITY LABELS. PHOTOCHEMICAL REACTIONS OF 4-NITROPYROCATECHOL ETHERS OF BIOLOGICALLY ACTIVE COMPOUNDS

A. CASTELLO, J. CERVELLO, J. MARQUET*, M. MORENO-MAÑAS*, X. SIRERA.

Departamento de Química (División Orgánica).

Universidad Autónoma de Barcelona. Bellaterra. Barcelona. Spain.

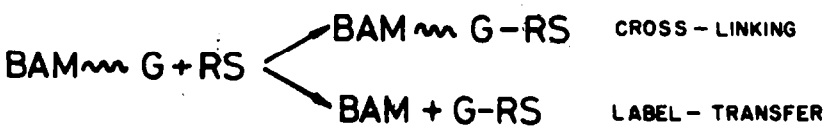
(*Received in UK* 21 *April* 1986)

Abstract.- The photochemical reactions of O- and N-(2-methoxy-4-nitro)phenoxyalkyl derivatives of estrone and of the antibiotic cycloheximide with methylamine afford clean substitutions of the methoxy group. From these experiments it is inferred that 2-methoxy-4-nitrophenyl ethers can be good photoaffinity labels.

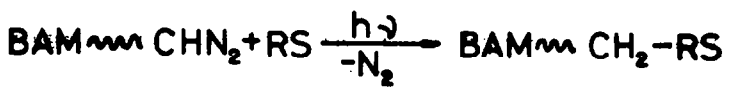
INTRODUCTION.-

Photoaffinity labelling is a useful method to identify biological receptor sites (1,2). The technique is based upon the modification of the biologically active molecule (BAM) with an auxiliary group (G) which can covalently and irreversibly bond to the receptor site (RS). Two versions are currently used (Scheme 1): in the cross-linking version, a covalent bond is formed between the receptor and the group G, the BAM not being released. The term label transfer applies when replacement of the BAM occurs instead. In any case, the success of the method depends a) on the modified BAM retaining at least part of the original activity, b) on the reaction with the receptor side being triggered at the experimenter's will (photochemical triggering being very useful), and c) on the ability to fragment the receptor and identify the fragment carrying the label.

Some auxiliary functional groups have been used for the cross-linking version of photoaffinity labelling: diazocarbonyl compounds, azides and aromatic ketones (1,2) (Scheme 2). However, both diazocarbonyl and azide groups have some drawbacks, such as lack of selectivity and thermal instability. Therefore, new groups which are more selective, thermally stable and photochemically active are being sought for photoaffinity labelling.

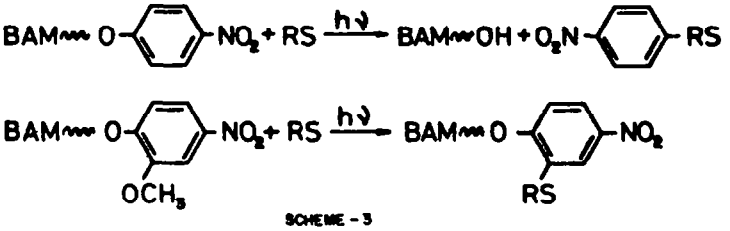


SCHEME-1

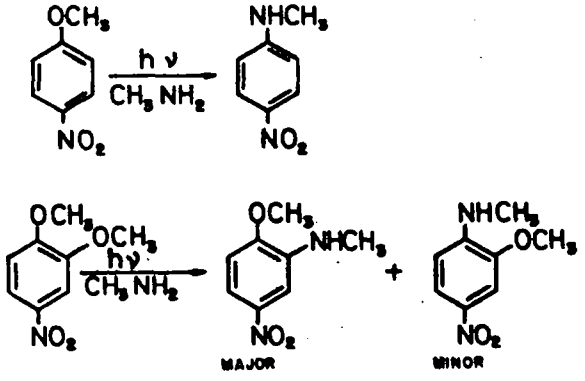


SCHEME - 2

C. Cantor (3) proposed the use of ethers of nitrophenol and nitropyrocatechol as indicated in scheme 3. The idea was based on the Nucleophilic Aromatic Photosubstitution reaction described by Havinga (4,5) and later reviewed (6-8). The key reactions described by the Dutch group are represented in scheme 4. 4-Nitroanisole and 4-nitroveratrole are inert towards amines at physiological temperatures; however, under photochemical excitation (> 300 nm), they react with methylamine as indicated in the so called nucleophilic aromatic photosubstitution. Since biological receptors contain nucleophiles and nucleophilic aromatic photosubstitution is not limited to nitrogen based nucleophiles (6-8), the use of nitrophenyl ethers is attractive for photochemical labelling purposes. The initial idea of C. Cantor has met only with limited applications in the biological side (9). However, nucleophilic aromatic photosubstitution has attracted more interest from the fundamental mechanistic side, being now founded upon a more rational basis (10-14). This type of reaction has until now been limited to examples dealing with very simple substrates, namely nitroanisoles, 4-nitroveratrole and the like. To demonstrate the validity of the method for photoaffinity labelling purposes, we present in this paper examples of nucleophilic aromatic photosubstitutions on complex molecules such as derivatives of the antibiotic cycloheximide, estrone and 9-aminoacridine. In order to make experiments valuable as models for photoaffinity labelling, we had to use mixed organic-aqueous reaction media. Finally, since the nucleophile concentration in the inmediate neighbourhood of the receptor site can be considered as high, methylamine was chosen as nucleophilic reagent since a high concentration can be secured in our reaction media; methylamine can also be considered as a model for the ϵ amino group of lysine.



SCHEME - 3

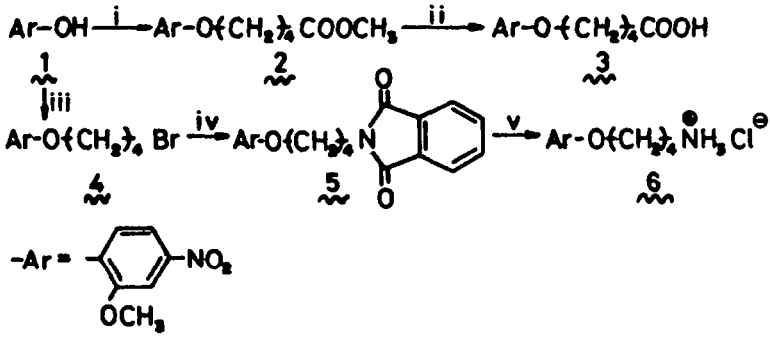


SCHEME - 4

Antibiotics (2), steroids (2), and intercalating agents (15,16) modified with photoactivatable moieties have attracted attention. Therefore, we chose cycloheximide, estrone and acridine for our experiments.

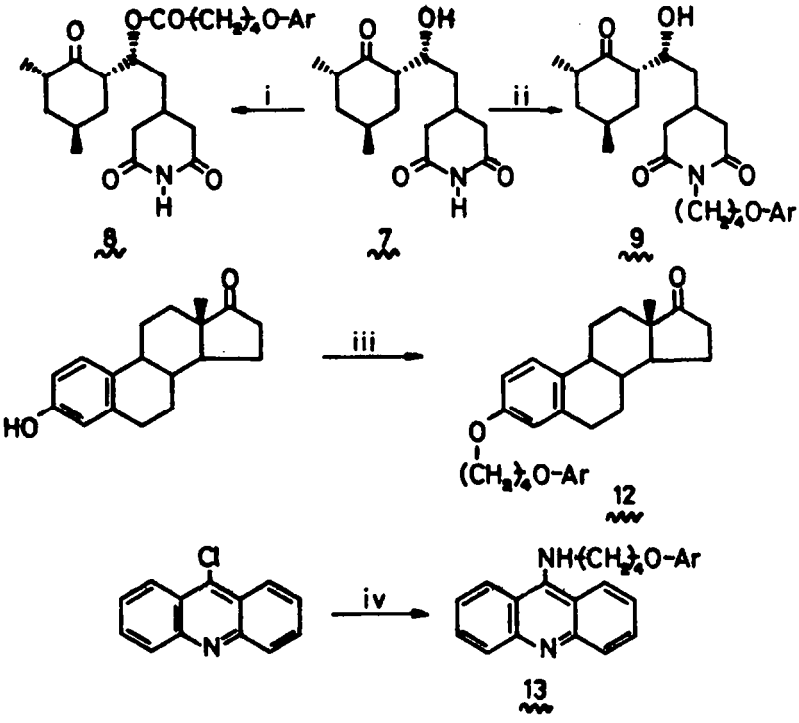
PREPARATION OF THE REAGENTS AND PHOTOACTIVATABLE DERIVATIVES

The preparation of the used reagents is described in scheme 5. Cycloheximide, 7, is an antibiotic of the glutarimide family that acts at the ribosome level (17). Two derivatives, the ester 8 and the N-alkylderivative 9, were prepared according to the procedures depicted in scheme 6.



i.- $Br(CH_2)_4COOMe$/DMF/100°; ii.- Conc. HCl/H_2O/refl.; iii.- $Br(CH_2)_4Br$/acetone refl.; iv.- Potassium phthalimide/DMF/90°; v.- 80% aq. hydrazine/EtOH/refl., then 6N HCl.

SCHEME 5



i.- $ArO(CH_2)_4COCl$/DMAP/pyridine/CH_2Cl_2/0°; ii.- 4/K_2CO_3/acetone/refl.

iii.- Na_2CO_3/methanol/4/refl.

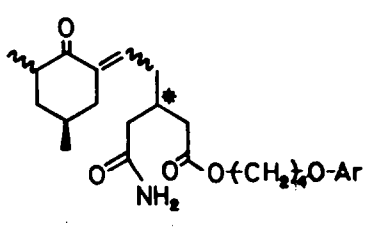
iv.- 6 as free base/phenol/125°

SCHEME 6

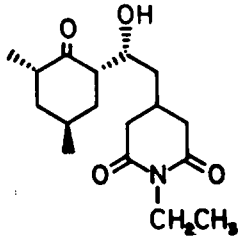
In the preparation of 9, a second derivative was isolated to which the structure of the 4-(2-methoxy-4-nitrophenoxy)butyl ester of 4-carbamoyl-3-(2-(3,5(S)-dimethyl-2-oxocyclohexylidene)ethyl)-butanoic acid, 10, was assigned. It appeared as a thick oil, probably being a mixture of isomers as indicated by the complexity of the methyl region in the PMR spectrum (note that a new chiral center was created upon ring opening). The products 10 could be obtained independently by preparing first the potassium salt of 7 and treating it with 4 in DMF. The proposed structure rests upon a multiplet at 6.21-6.66 (1H), attributed to the olefinic proton, and a multiplet between 3.95 and 4.27 (4H) compatible with both OCH_2 groups. The NCH_2 group of 9 appears at 3.84, the same as the NCH_2 of N-ethylcycloheximide, 11. Had the structure been that corresponding to the isomer containing an N-substituted amide and a free acid, the NCH_2 group would have appeared at higher field. The infrared spectrum (film) shows peaks at 1730 and a broad band with the maximum at 1680, but extending to 1650 cm^{-1}. The hydroxyl region is also compatible with a mixture of amides but not with a carboxylic acid. The elemental analysis was correct. Product 10 had to be formed through O-alkylation followed by hydrolysis.

Attempts to prepare an acylhydrazone bearing the photoactivatable group failed due to the perturbing influence of allylic interactions (A1,3-strain) (18).

The estrone ether 12 was also prepared from 4, and the acridine 13 from 6 and 9-chloroacridine (scheme 6).



10



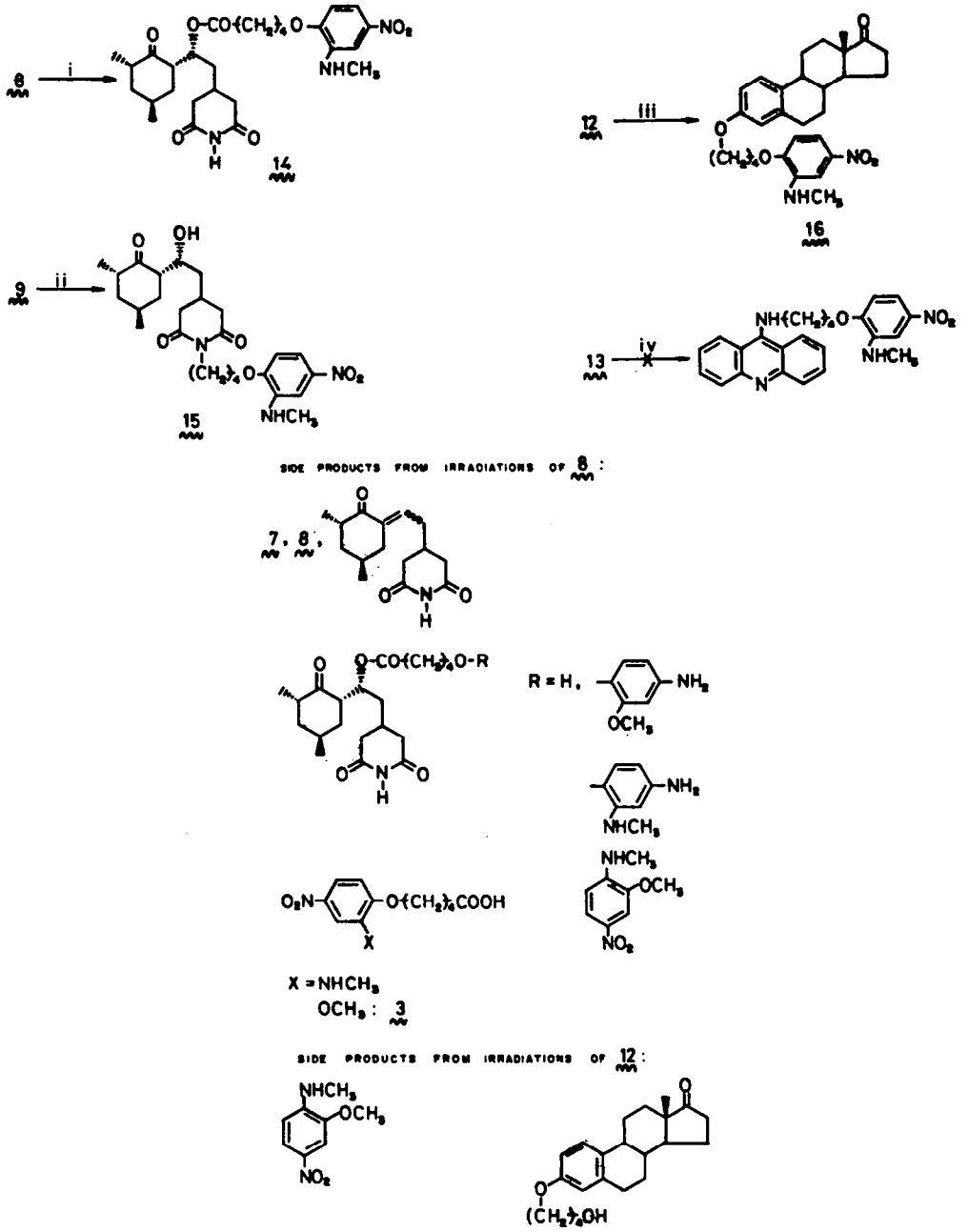
11

PHOTOCHEMICAL EXPERIMENTS

Irradiation of 8 with methylamine under the conditions indicated in scheme 7, gave a 56% yield of cycloheximide 5-(2-methylamino-4-nitrophenoxy)pentanoate, 14, the product of photosubstitution in the meta position with respect to the nitro group. This was the expected orientation according to the available experience (4-8,10-14). However, the intrinsic lability of cycloheximide and its derivatives, and also the tendency for substitution para with respect to the nitro group, gave rise to several other products in the reaction mixture. Their structures were assigned upon spectroscopic grounds and comparison, when appropiate, with authentic specimens after careful column chromatography separations. Due to the complexity of the mixture, some of them were not obtained in pure state, although the main product, 14, could be isolated, giving even correct elemental analysis. The characterized products are also indicated in scheme 7. Products lacking nitro group, were also detected, the photochemical reduction of aromatic nitro compounds by amines being documented in the chemical literature (19,20)

A similar photochemical reaction with 9 gave 15, in a 38% isolated yield, but in a impure state. The pattern observed in the methyl region of tne PMR spectrum suggested than more than one isomer was present, in agreement with the well known tendency of cycloheximide to isomerize (17,18). Note that from the viewpoint of identifying receptor sites, isomerization is an unimportant event. No search to identify other products was undertaken. An independent synthesis of 15 was also performed for identification purposes (see below).

The photochemical reaction with 12 afforded 16 in 34% yield. A pure sample had to be independently synthesized (see below) since it was very difficult to separate 16 from the starting material.



i.- $h\nu$ (> 300nm)/CH_3CN-H_2O/r.t./CH_3NH_2.HCl/borax buffer, pH 9; ii.- $h\nu$(> 300nm)/ CH_3CN-H_2O-EtOH/r.t./CH_3NH_2.HCl/borax buffer, pH 9. iii.-$h\nu$(>300nm)/DMF-H_2O/r.t./CH_3NH_2.HCl + NaOH; iv.-$h\nu$(>300nm) at 125W/MeOH/H_2O/r.t./5 h./CH_3NH_2 + NaOH

In all cases a 400W medium pressure Hg lamp was used.

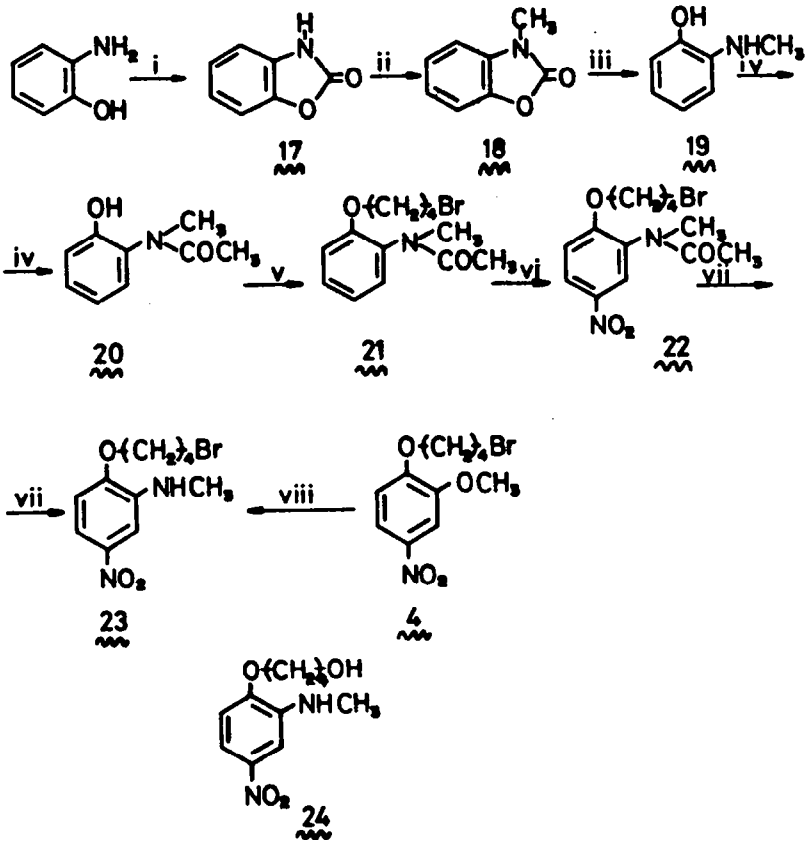
SCHEME 7

Other products formed are also indicated in scheme 7. 2-Methoxy-N-methyl-4-nitroaniline and O-(4-hydroxybutyl)estrone arise from para substitution with respect to the nitro group. The former was fully characterized by spectroscopic methods and elemental analysis. Its isomer 2-methoxy-N-methyl-5-nitroaniline was already known (5,13,14), thus allowing unambiguous assignment of structure. O-(4-Hydroxybutyl)estrone was spectroscopically characterized.

However, preliminary experiments indicate that the acridine 13 does not react under the usual photochemical conditions, being recovered unaltered. The lack of reactivity could be explained by internal quenching of the reactive excited state of the nitrophenyl ether by the 9-aminoacridine moiety. Further investigation on this subject is planned.

INDEPENDENT SYNTHESIS OF 15 AND 16

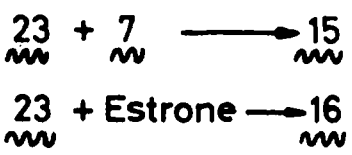
As stated above, product 14 could be isolated from the reaction mixture in 56% yield, an analytically pure sample being prepared by standard purification techniques. However, products 15 and 16 were characterized spectroscopically, but no analytically pure samples could be prepared. This forced us to prepare pure samples of both independently. Through these independent syntheses we found another example of the synthetic usefulness of the nucleophilic aromatic photosubstitution. Thus, the required amine 23 could be prepared by a cumbersome route depicted in scheme 8 in 8% overall yield. Step vii was critical since the best conditions found after some experimentation led to a 41% yield of 23 accompanied by 21% of 4-(2-methylamino-4-nitrophenoxy)-1-butanol, 24, the mixture requiring column chromatography. However, photochemical reaction of the easily available 4 with methylamine afforded a 78% yield of 23, the primary bromide function present in 4 causing no interferences.This route gives a 46% overall yield starting from veratrole (Scheme 8).



i.- NH_2CONH_2/Conc. HCl/Refl./1 h; ii.- Me_2SO_4/NaOH/H_2O/r.t.; iii.-NaOH/H_2O/Refl./1 h; iv.- Ac_2O/AcOH/Refl./0.5 h; v.- $Br(CH_2)_4Br/K_2CO_3$/acetone/refl.; vi.- HNO_3/H_2SO_4/25°/0.5 h.; vii.- H_2SO_4 50%/Refl./20 min.; viii.-$h\nu$ (>300nm)/MeOH/H_2O/r.t./CH_3NH_2.HCl NaOH.

SCHEME 8

Standard reactions of 23 with cycloheximide and with estrone gave pure samples of 15 and 16 (Scheme 9). It should be pointed out that 15 presented as a mixture of isomers as it was observed in its photochemical preparation above discussed.



i.- K_2CO_3/acetone/Refl./24 h; ii.- Na_2CO_3/MeOH/Refl./24h.

SCHEME 9

EXPERIMENTAL PART.-

All the melting points are uncorrected. PMR spectra were recorded at 80MHz on a Brucker WP80SY spectrometer using TMS as internal standard. Mass spectra were recorded on a Hewlett-Packard 5985B spectrometer, and IR spectra were recorded on a Perkin-Elmer 1310 spectrophotometer.

Benzoxazolone, 17, (21), N-methylbenzoxazolone, 18, (22), 2-methylaminophenol, 19, (22), and N-acetyl-N-methyl-2-aminophenol, 20, (22), were prepared according to described procedures.

Potassium 2-methoxy-4-nitrophenolate, 1.-

It was prepared according to the method of Pollecoff and Robinson (23). In our hands, the salt crystallized as a dihydrate with m.p. higher than 350°.

Methyl 5-(2-methoxy-4-nitrophenoxy)pentanoate, 2.

A mixture of 24.04g (0.098 mole) of 1, 19.04g (0.098 mole) of methyl 5-bromopentanoate and 200 ml of DMF was stirred at 100° for 65 hours. The orange solution containing a white precipitate was poured into ice-water. The mixture was filtered and the precipitate washed with water and dried to afford 23.03g (83% yield) of product 2, which was crystallized from methanol. It had m.p. 69-70°. Ir(KBr): 1738, 1510, 1340 cm^{-1}. PMR($CDCl_3$): δ 1.57-2.12(4H), 2.44(t, J 6Hz, 2H), 3.68(s, 3H), 3.94(s, 3H), 4.13(t, J 6Hz, 2H), 6.89(d, J 9Hz, 1H), 7.69(d, J 2.3Hz, 1H), 7.81(dd, J 9 and 2.3Hz, 1H). Calculated for $C_{13}H_{17}NO_6$: C, 55.12; H, 6.05; N, 4.94. Found: C, 55.31; H, 6.26; N, 4.74.

5-(2-Methoxy-4-nitrophenoxy)pentanoic acid, 3.

A mixture of 10.21g (0.036 mole) of 2, 32 ml of concentrated HCl and 270 ml of water was refluxed for 20 h. After cooling, the mixture was filtered to afford 9.43g (97% yield) of 3, m.p. 111-2° (from acetic acid). Ir(KBr): 3200-2600(broad), 1685, 1520, 1500, 1330 cm^{-1}. PMR(DMSO-d_6): δ 1.37-2.0(m, 4H), 2.28(t, J 6Hz, 2H), 3.86(s, 3H), 4.07(t, J 6Hz, 2H), 7.07(d, J 9Hz, 1H), 7.64(d, J 3Hz, 1H), 7.80(dd, J 9 and 3Hz, 1H), 11.95(broad, 1H). Calculated for $C_{12}H_{15}NO_6$: C, 53.53; H, 5.62; N, 5.20. Found: C, 53.51; H, 5.76; N, 5.39.

1-Bromo-4-(2-methoxy-4-nitrophenoxy)butane, 4.

A mixture of 1,4-dibromobutane (22.7g, 0.105 mole), the potassium salt 1 (5.0g, 0.021 mole) and acetone (150 ml) was refluxed for 48 hours. The solvent was evaporated and the residue partitioned between chloroform and 2N aqueous sodium hydroxide. The organic layer was washed with water, dried and evaporated to afford a solid which was partially dissolved in ether. The insoluble residue was recrystallized from acetic acid and identified as 1,4-bis-(2-methoxy-4-nitrophenoxy)butane, m.p. 171-4°. Ir(KBr): 1520, 1350 cm^{-1}. PMR($CDCl_3$ + CF_3COOH): δ 2.1(broad, 4H), 4.05(s, 6H), 4.3(broad, 4H), 7.05(d, J 9Hz, 2H), 7.85(d, J 2.5Hz, 2H), 8.05(dd, J 9 and 2.5Hz, 2H). MS: m/e 392(M,6), 224(52), 182(40), 167(23), 79(23), 55(100). Calculated for $C_{18}H_{20}N_2O_2$: C, 55.12; H, 5.10; N, 7.14. Found: C, 55.33; H, 5.13; N, 6.88.

The above ethereal solution was strongly evaporated to afford 5.24g (82% yield) of product 4, m.p. 50-1° (from ethanol) (Lit.(24), m.p. 51-2°).

N-(4-(2-Methoxy-4-nitrophenoxy))butylphthalimide, 5.

A mixture of the bromoether 4 (1.434g, 4.72 mmole), potassium phthalimide (0.87g, 4.72 mmole) and DMF(20 ml) was heated at 90° under magnetic stirring for 4 hours. The mixture was poured into ice-water (500 ml). The formed precipitate was filtered, washed with water and dried for 24 hours under high vacuum to afford 1.68g (96%) of 5, m.p. 133-5° (from ethanol). Ir(KBr): 1765, 1720, 1500, 1335 cm^{-1}. PMR($CDCl_3$): δ 1.9(broad, 4H), 3.8(broad, 2H), 3.95(s, 3H), 4.15(broad, 2H), 6.95(d, J 9Hz, 1H), 7.65-7.95(m, 6H). Calculated for $C_{19}H_{18}N_2O_6$: C, 61.62; H, 4.90; N, 7.56. Found: C, 61.44; H, 5.14; N, 7.65.

N-(2-Methoxy-4-nitrophenoxy)butylamine, 6.

A mixture of 5 (4.44g, 0.012 mole), 80% aqueous hydrazine hydrate (1.625g, 0.02 mole) and ethanol (100 ml) was refluxed for 3 hours. The solvent was evaporated and replaced with 6N HCl (50 ml) and the new mixture refluxed for 20 hours. After cooling, the formed precipitate was filtered off and the solution was evaporated to afford 1.83g (55%) of 6 hydrochloride, m.p. 175-6° (from methanol). Ir(KBr): 3250-2600(broad), 1520, 1350 cm^{-1}. PMR(D_2O): δ 1.8(m, 4H), 3.0(t, J 5Hz, 2H), 3.85(s, 3H), 4.15(t, J 5Hz, 2H), 7.0(d, J 9Hz, 1H), 7.7(d, J 2.5Hz, 1H), 7.85(dd, J 9 and 2.5Hz, 1H). Calculated for $C_{11}H_{17}ClN_2O_4$: C, 47.76; H, 6.15; N, 10.12. Found: C, 47.40; H. 5.95; N, 9.88.

Cycloheximide 5-(2-methoxy-4-nitrophenoxy)pentanoate, 8.

A mixture of the acid 3 (1.083g, 3.86 mmole), phosphorus pentachloride (0.803g, 3.85 mmole) and benzene (50 ml) was refluxed for 3 hours, after which the solvent was evaporated under vacuum. The formation of the acid chloride was monitored by infrared spectroscopy (appearance of a peak at 1800 cm^{-1}. The solution of the acid chloride in dichloromethane (10 ml) was added in 20 min. to a mixture of cycloheximide (0.867g, 3.08 mmole), 4-dimethylaminopyridine (0.192g, 1.5 mmole), anhydrous pyridine (1 ml) and dichloromethane (10 ml), cooled at 0°. When the addition was finished the mix-

ture was kept at 0° for 2 hours and then at room temperature for 12 hours, after which it was added into 1N HCl. The mixture was extracted with dichloromethane and the organic layer was washed with aqueous sodium bicarbonate, dried and evaporated. The residue was purified by passing through a silica gel column with hexane-ethyl acetate as eluent to afford 1.169g (71% yield) of 8, m.p. 114-6° (from benzene-pentane). Ir(KBr): 3280, 1715, 1700, 1685, 1505, 1320 cm^{-1}. PMR($CDCl_3$):δ 0.98(d, J 6.3Hz, 3H), 1.24(d, J 6.9Hz, 3H), 1.38-3.10(m, 19H), 3.93(s, 3H), 4.12(t, J 6.3Hz, 2H), 5.21-5.52(m, 1H), 6.89(d, J 8.6Hz, 1H), 7.76(d, J 2.7Hz, 1H), 7.88(dd, J 8.6 and 2.7Hz, 1H), 7.88(broad s, 1H). Calculated for $C_{27}H_{36}N_2O_9$: C, 60.89; H, 6.81; N, 5.26. Found: C, 61.14; H, 7.08; N, 5.33.

N-(4-(2-Methoxy-4-nitrophenoxy)-1-butyl)cycloheximide, 9.

A mixture of 0.5g (1.78 mmole) of cycloheximide, 2g of potassium carbonate, 0.648g (2.13 mmole) of the bromoderivative 4, and 40 ml of acetone was refluxed for 24 hours. After cooling, the mixture was filtered and the filtrate was evaporated. The residue was carefully chromatographed through a silica gel column with mixtures of hexane and ethyl acetate as eluent. The following compounds were isolated in order of elution: 4(31%), a dehydration product from 9 (multiplet at 6.39) (4%), 9 (31%), cycloheximide (16%) and 10 (multiplet at 6.38). Product 9 was an oil. Ir(film): 3530(broad), 1720, 1695, 1670, 1510, 1340 cm^{-1}. PMR($CDCl_3$): δ 0.97(d, J 6Hz, 3H), 1.23(d, J 7Hz, 3H), 1.34-3.0(m, 15H), 3.84(t, J 6Hz, 2H),3.94(s, 3H), 3.9-4.3(m, 3H), 6.9(d, J 9Hz, 1H), 7.73(d, J 2.5Hz, 1H), 7.89(dd, J 9 and 2.5Hz, 1H). Calculated for $C_{26}H_{36}N_2O_8$: C, 61.89; H, 7.19; N, 5.55. Found: C, 62.08; H, 7.40; N, 5.49.

4-(2-Methoxy-4-nitrophenoxy)butyl ester of 4-carbamoyl-3-(2-(3(S),5(S)-dimethyl-2-oxocyclohexylidene)ethyl)butanoic acid, 10.

A mixture of 0.258g of 85% potassium hydroxide, 2 ml of water, 1.099g (0.391 mmole) of cycloheximide and 20 ml of ethanol was stirred at room temperature for 16 hours. The solvent was evaporated and 1.188g (0.39 mmole) of 4 in 25 ml of DMF were added. The mixture was heated at 100° for 4 hours and, after cooling, it was filtered and the filtrate was strongly evaporated under vacuum. The residue was partitioned between water and chloroform. The organic layer was washed with water, dried and evaporated to afford a residue which was chromatographed through a silica gel column with mixtures of hexane and ethyl acetate as eluents. After some hydroxylic impurities, product 10 (23% yield) was eluted as an oil. Ir(film): 3500-3200, 1730, 1680, 1510, 1340 cm^{-1}. PMR($CDCl_3$):δ 0.87-1.18(two d, 6H), 1.44-2.91(m, 17H), 3.91(s, 3H), 3.95-4.27(m, 4H), 5.56(broad s, 1H, interexchanged with D_2O), 6.21-6.66(m, 1H), 6.86(d, J 8Hz, 1H), 7.70(d, J 2.3Hz, 1H), 7.85(dd, J 8 and 2.5Hz, 1H). Calculated for $C_{26}H_{38}N_2O_8$: C, 61.89; H, 7.19; N, 5.55. Found: C, 61.56; H, 7.38; N, 5.78.

N-Ethylcycloheximide, 11.

A mixture of 0.50g (1.78 mmole) of cycloheximide, 2g of potassium carbonate, 2 ml of ethyl bromide and 40 ml of acetone was refluxed for 16 hours. After cooling, the mixture was filtered and the filtrate was evaporated. The residue was chromatographed through a silica gel column with mixtures of hexane and ethyl acetate as eluents, part of the starting cycloheximide being recovered. Product 11 was an oil. Ir(film): 3500(broad), 1710, 1690, 1660 cm^{-1}. PMR($CDCl_3$): δ 0.87-1.33(two d and one t, 9H), 1.4-3.0(m, 15H), 3.84(q, J 6.9Hz, 2H), 4.06-4.38(m, 1H). Calculated for $C_{17}H_{27}NO_4$: C, 65.99; H, 8.80; N, 4.53. Found: C, 65.80; H, 8.92; N, 4.34.

Estrone 4-(2-methoxy-4-nitrophenoxy)butyl ether, 12.

A mixture of 4 (1.0g, 3.3 mmole), estrone (0.89g, 3.3 mmole), sodium carbonate (0.67g, 6.6 mmole) and methanol (40 ml) was refluxed under magnetic stirring for 24 hours. Upon cooling, a white precipitate appeared. It was filtered off and recrystallized from chloroform-hexane, eliminating the insoluble part. Product 12, (1.1g, 57% yield) had m.p. 141-3°. Ir(KBr): 1725, 1520, 1330 cm^{-1}. PMR($CDCl_3$): δ 0.9(s, 3H), 1.0-3.0(m, 19H), 4.0(s, 3H), 4.05(t, J 5.5Hz, 2H), 4.1(t, J 5.5Hz, 2H), 6.6(s, 1H), 6.7(d, J 8.5Hz, 1H), 6.9(d, J 8.5Hz, 1H), 7.2(d, J 8.5Hz, 1H), 7.8(d, J 2.4Hz, 1H), 7.9(dd, J 8.5 and 2.4Hz, 1H). MS: m/e 493(M,4), 324(26), 323(100), 224(34), 182(42), 170(26), 139(28), 115(25), 79(34), 55(88), 41(26). Calculated for $C_{29}H_{35}NO_6$: C, 70.56; H, 7.15; N, 2.84. Found: C, 70.34; H, 6.92; N, 2.56.

9-(4-(2-Methoxy-4-nitrophenoxy))butylaminoacridine, 13.

A mixture of 6 (as free base) (1.057g, 4.4 mmole), 9-chloroacridine (25) (0.94g, 4.4 mmole) and phenol (10g) was stirred at 125° for 1.5 hours. After cooling, acetone (80 ml) was added, a yellow precipitate being formed. It was filtered and washed with acetone to afford 0.793g (37%) of 13.HCl, m.p. 225-8(d)°. Ir(KBr): 3300-2600(broad), 1515, 1340 cm^{-1}. PMR(DMSO-d_6): δ 2.0(m, 4H), 3.75(s, 3H), 4.2(m, 4H), 7.1(d, J 9Hz, 1H), 7.3-8.0(m, 8H), 8.6(d, J 9Hz, 2H). MS: m/e 417(M for the free base, 44). 207(100), 180(28). Calculated for $C_{24}H_{24}ClN_3O_4$: C, 63.51; H, 5.07; N, 9.26. Found: C, 63.50; H, 5.30; N, 9.12.

The free base, 13, was obtained by shaking 13.HCl in a separatory funnel with aqueous solution of sodium carbonate and chloroform. The organic layer was washed with water, dried and evaporated to afford 13, m.p. 76-7° (from ethanol). Ir(KBr): 3420, 1520, 1350 cm^{-1}. PMR($CDCl_3$): δ 2.0(m, 4H), 3.9-4.1(m, 7H), 6.9(d, J 9Hz, 1H), 6.2-7.2(m, 10H).

Cycloheximide 5-(2-methylamino-4-nitrophenoxy)pentanoate, 14, by irradiation of 8 with methylamine.

A mixture of 8 (0.132g, 0.248 mmole), acetonitrile (180 ml) and 420 ml of a 16% solution of methylamine in water (from 64g of $MeNH_2$.HCl , 200 ml of a 0.025M solution of borax in water and 18.5 ml of 0.1M HCl complemented with water until 420 ml) (pH 9), was irradiated under stirring for 2 hours with a 400W medium pressure Hg lamp and Pyrex filter. The solution was then evaporated to eliminate most of the organic solvent, and the remaining was extracted with dichloromethane. The organic layer was washed, dried and evaporated to afford a residue (0.125g) which was carefully chromatographed under pressure through a silica gel column with hexane-ethyl acetate mixtures of growing polarity. The byproducts of scheme 7 were eluted. A mixture containing 8 and 14 was recrystallized several times from ethyl acetate-pentane to afford a sample of pure 14 (56% crude yield)

contaminated with about 1% of 8. Product 14 had m.p. 144-7°. Ir(KBr): 3410, 3250, 1720, 1695, 1520, 1320 cm^{-1}. PMR($CDCl_3$): δ 1.00(d, J 6.6Hz, 3H), 1.23(d, J 6.6, 3H), 1.33-3.08(m, 20H), 2.94(s, 3H), 4.11(m, 2H), 4.53(broad s, 1H), 5.38(m, 1H), 6.60(d, J 8.8Hz, 1H), 7.37(d, J 2.7Hz, 1H), 7.6(dd, J 8.8 and 2.7, 1H), 7.81(broad s, 1H). Calculated for $C_{27}H_{37}N_3O_8$: C, 61.00; H, 7.02; N, 7.90. Found: C, 60.94; H, 6.97; N, 7.79.

N-(4-(2-Methylamino-4-nitrophenoxy))butylcycloheximide, 15, by irradiation of 9 with methylamine.
A mixture of 9 (0.319g, 0.632 mmole), acetonitrile (180 ml), ethanol (10 ml) and 420 ml of a 16% solution of methylamine in water (from 64g of $MeNH_2$.HCl, 200 ml of a 0.025 solution of borax in water and 18.5 ml of 0.1M HCl complemented with water until 420 ml) (pH 9) was irradiated for 2.5 hours under stirring at room temperature with a 400W medium pressure Hg lamp and Pyrex filter. Upon addition of more acetonitrile (20 ml) two layers separated. The aqueous layer was extracted with dichloromethane and the combined organic extracts were dried and evaporated to afford a residue (0.315g) which upon chromatography on silica gel under pressure afforded 9 (18%) and 15 (38%) in an impure condition. The sample of 15 thus obtained exhibited the same spectroscopic behaviour than the one independently synthesized (see below).

Estrone 4-(2-methylamino-4-nitrophenoxy)butyl ether, 16, by irradiation of 12 with methylamine.
A mixture of 12 (0.10g, 0.2 mmole), methylamine hydrochloride (0.137g, 2.0 mmole), sodium hydroxide (0.080g, 2.0 mmole), water (150 ml) and DMF (400 ml), was irradiated for 4 hours under stirring at room temperature with a 125W medium pressure Hg lamp. The mixture was then poured into water (1500 ml) and extracted with chloroform. The organic layer was washed with more water (500 ml), dried and evaporated. The residue was partitioned between water and chloroform and the organic solution was strongly evaporated. By repeating three times the last procedure, the residue was found to be free of DMF. It was then chromatographed through silica gel using benzene-chloroform (3:1) as eluent, the following products being eluted:
2-Methoxy-N-methyl-4-nitroaniline, m.p. 97-8°. Ir(KBr): 3430, 1540, 1330 cm^{-1}. PMR($CDCl_3$): δ 3.0(s, 3H), 4.0(s, 3H), 6.5(d, J 8.5Hz, 1H), 7.6(d, J 2.4Hz, 1H), 7.9(dd, J 8.5 and 2.4 Hz, 1H). MS: m/e 182(M,100), 167(29), 154(49), 108(37), 96(20), 95(28), 80(30), 79(36), 78(24), 52(44), 51(27). Calculated for $C_8H_{10}N_2O_3$: C, 52.74; H, 5.53; N, 15.38. Found: C, 52.71; H, 5.35; N, 15.13.
O-(4-Hydroxybutyl)estrone: PMR($CDCl_3$): δ 0.9(s, 3H), 1.0-3.0(m, 19H), 3.6(t, J 5.5Hz, 2H), 4.1(t, J 5.5Hz, 2H), 6.6(d, J 2.4Hz, 1H), 6.7(dd, J 8.5 and 2.4Hz, 1H), 7.2(d, 8.5Hz, 1H).
A mixture of 16 and 12 in a ratio of about 9:1 (ca. 34% of 16).(See below for an independent synthesis of 16 and its full chraracterization).

1-Bromo-4-(2-(N-acetyl-N-methylamino)phenoxy)butane, 21.
A mixture of 20 (22) (1.91g, 11.6 mmole), 1,4-dibromobutane (12.52g, 58 mmole), potassium carbonate (6.44g) and acetone (75 ml) was refluxed for 66 hours. The solvent was strongly evaporated and the residue was partitioned between water and dichloromethane. The organic layer was dried and evaporated to afford a residue containing 1,4-dibromobutane which was passed through a silica gel column to give 21 (95% yield) as an oil . Ir(film): 1655 cm^{-1}. PMR($CDCl_3$): δ 1.79(s, 3H), 2.05(m, 4H), 3.16(s, 3H), 3.48(m, 2H), 4.06(m, 2H), 6.78-7.56(m, 4H). MS: m/e 301(21), 299(M,21), 148(32), 137(31), 135(33), 123(98), 121(100), 94(29), 55(20), 43(33).

1-Bromo-4-(2-(N-acetyl-N-methylamino)-4-nitrophenoxy)butane, 22.
A mixture of 1.22 ml of 60% nitric acid (16 mmole) and 2.8 ml of concentrated sulphuric acid was dropwise added in 15 minutes upon a magnetically stirred solution of 21 (4.57g, 15 mmole) in concentrated sulphuric acid (7.2 ml). The temperature was controlled by external refrigeration with water. After additional 30 minutes the mixture was poured into ice-water and extracted with dichloromethane. The organic layer was washed with water, dried and evaporated. The residue was filtered through a silica gel pad with a mixture of hexane and ethyl acetate to afford a 70% yield of 22, m.p. 62-4° (from ethanol-pentane). Ir(film): 1665, 1520, 1345 cm^{-1}. PMR($CDCl_3$): δ 1.88(s, 3H), 2.08(m, 4H), 3.21(s, 3H), 3.51(m, 2H), 4.23(m, 2H), 7.05(d, J 8.6Hz, 1H), 8.11(d, J 2.5Hz, 1H), 8.25(dd, J 8.6 and 2.5Hz, 1H). MS: m/e 346(14), 344(M,11), 193(34), 168(37), 137(95), 135(100), 55(81), 43(67). Calculated for $C_{13}H_{17}BrN_2O_4$: C, 45.23; H, 4.96; N, 8.11. Found: C, 45.53; H, 4.91; N, 8.10.

1-Bromo-4-(2-methylamino-4-nitrophenoxy)butane, 23.
A mixture of 22 (3.456g, 10 mmole) and 50% sulphuric acid (75 ml) was refluxed for 20 minutes, after which it was cooled at room temperature and poured into ice-water. 10% Aqueous potassium hydroxide was slowly added until basic pH was reached, and then the mixture was extracted with dichloromethane. The organic layer was washed, dried and evaporated to give a residue which upon chromatography on silica gel with mixtures of hexane and ethyl acetate afforded the following products:
23 (41%), m.p. 82-3°. Ir(KBr): 3420, 1520, 1330 cm^{-1}. PMR($CDCl_3$): δ 2.09(m, 4H), 2.95(s, 3H), 3.54(m, 2H), 4.14(m, 2H), 6.71(d, J 8.6Hz, 1H), 7.38(d, J 2.5Hz, 1H), 7.61(dd, J 8.6 and 2.5Hz, 1H). MS: m/e 304(18), 302(M,17), 168(33), 167(24), 137(76), 135(91), 122(29), 121(25), 109(20), 55(100), 52(22), 43(31). Calculated for $C_{11}H_{15}BrN_2O_3$: C, 43.58; H, 4.99; N, 9.24. Found: C, 43.34; H, 4.67; N, 9.38.
4-(2-Methylamino-4-nitrophenoxy)-1-butanol, 24, (21%), m.p. 93-4° (from ethyl acetate-pentane). Ir(KBr): 3440, 3410, 3500-3100(broad), 1515, 1315 cm^{-1}. PMR($CDCl_3$): δ 1.52-2.18(m, 4H), 2.4(broad, 2H), 2.92(s, 3H), 3.77(t, J 6.1Hz, 2H), 4.14(t, J 6.1Hz, 2H), 6.74(d, J 8.6Hz, 1H), 7.37(d, J 2.5Hz, 1H), 7.61(dd, J 8.6 and 2.5Hz, 1H). MS: m/e 240(M,15), 168(100), 122(25). Calculated for $C_{11}H_{16}N_2O_4$: C, 54.99; H, 6.71; N, 11.66. Found: C, 54.84; H, 6.81; N, 11.77.

1-Bromo-4-(2-methylamino-4-nitrophenoxy)butane, 23, by irradiation of 4 with methylamine.
A mixture of 4 (0.4g, 1.3 mmole), methylamine hydrochloride (1.5g, 22 mmole), sodium hydroxide (0.8g, 20 mmole), methanol (350 ml) and water (600 ml) was irradiated for 4h under stirring at room temperature with a 400W medium pressure Hg lamp and Pyrex filter. More water (300 ml) was then added to the mixture and the formed precipitate was filtered, dried and passed through an acidic

alumina column with chloroform-hexane as eluent, the following compounds being eluted:
2-Methoxy-N-methyl-4-nitroaniline (9%), m.p. 97-8°.
Starting material, 4, (13%).
Product 23, (78%), m.p. 81-2°.

N-(4-(2-Methylamino-4-nitrophenoxy)butylcycloheximide, 15, by thermal N-alkylation of 7 with 23.

A mixture of 7 (0.357g, 1.27 mmole), 23 (0.457g, 1.57 mmole), potassium carbonate (1.44g) and acetone (30 ml) was refluxed for 24 hours. The solvent was evaporated and the residue partitioned between water and dichloromethane. The organic layer was washed, dried and evaporated to give a residue which was passed through a silica gel column with hexane-ethyl acetate, a mixture of 15 and 23 being recovered (HPLC monitoring). The purification was twice repeated until 0.161g (25%) of 15 was obtained as a thick resin. Ir(film): 3520, 3430, 1720, 1700(shoulder), 1685(shoulder), 1660, 1510, 1330 cm^{-1}. PMR($CDCl_3$): δ two doublets at 1.0 and 1.22 (J ca. 6.3 Hz for each) with uneven intensity, probably indicating the presence of more than one isomer, 1.35-3.27(m, 21H), 2.97(s, 3H), 3.87(m, 2H), 4.11(m, 2H), 6.68(d, J 8.9Hz, 1H), 7.38(d, J 2.5Hz, 1H), 7.62(dd, J 8.9 and 2.5Hz, 1H). MS: m/e 503(M,2), 240(23), 168(100), 122(28), 55(21), 43(22). Calculated for $C_{26}H_{37}N_3O_7$: C, 62.01; H, 7.41; N, 8.34. Found: C, 62.24; H, 7.73; N, 7.98.

Estrone 4-(2-methylamino-4-nitrophenoxy)butyl ether, 16, by thermal etherification of estrone with 23.

A mixture of estrone (0.15g, 0.55 mmole), 23 (0.14g, 0.46 mmole), sodium carbonate (46mg) and methanol (30 ml), was refluxed under stirring for 24 hours. The mixture was poured into ice-water (400 ml) and the formed precipitate was filtered, dried and purified through a silica gel column with a mixture of ethyl acetate-hexane-chloroform (1:1:1) as eluent, to afford 207mg (85%) of 16, m.p. 127-131°. Ir(KBr): 3420, 1525, 1325 cm^{-1}. PMR($CDCl_3$):δ 0.9(s, 3H), 1.0-3.0(m, 22H), 4.0(t, J 4.5Hz, 2H), 4.1(t, J 4.5Hz, 2H), 6.6(s, 1H), 6.7(d, J 3.3Hz, 1H), 6.75(d, J 8.5Hz, 1H), 7.2(d, J 8.5Hz, 1H), 7.4(d, J 2.4Hz, 1H), 7.6(dd, J 8.5 and 2.4, 1H). MS: m/e 492(M,2), 362(20), 360(58), 325(20), 270(23), 194(100), 185(25), 148(29), 97(28), 92(45), 91(54), 69(37), 55(71), 43(42). Calculated for $C_{29}H_{36}N_2O_5$: C, 70.71; H, 7.36; N, 5.69. Found: C, 70.60; H, 7.30; N, 5.46.

ACKNOWLEDGEMENTS.- Financial support from "Comisión Asesora de Investigación Científica y Técnica" (Ministerio de Educación y Ciencia of Spain) through projects n° 0845/81 and 0343/84 is gratefully acknowledged.

References.-

1.- J.R. Knowles; Acc. Chem. Res., 5, 155, (1972).
2.- "Photoaffinity Labelling", by H. Bailey and J.R. Knowles, in Methods in Enzymology, 46, 69, (1977). W.B. Jakoby and M. Wilchek, Eds. Academic Press. New York.
3.- P.C. Jelenc, C.R. Cantor; S.R. Simon; Proc. Natl. Acad. Sci. USA, 75, 3564, (1978)
4.- M.E. Kronenberg, A. Van der Heyden, E. Havinga; Recl. Trav. Chim. Pays-Bas, 85, 56, (1966).
5.- M.E. Kronenberg, A. Van der Heyden, E. Havinga; Recl. Trav. Chim. Pays-Bas, 86, 254, (1967).
6.- J. Cornelisse, G.P. De Gunst, E. Havinga; Adv. Phys. Org. Chem., 11, 225, (1975).
7.- J. Cornelisse, E. Havinga; Chem. Rev., 75, 353, (1975).
8.- E. Havinga, M.E. Kronenberg; Pure Appl. Chem., 16, 137, (1968).
9.- H. Gozlan, V. Homburger, M. Lucas, J. Bockaert; Biochem. Pharmacol., 31, 2879, (1982).
10.- K. Mutai, R. Nakagaki, H. Tukada; Bull. Chem. Soc. Jpn., 58, 2066, (1985).
11.- H.C.H.A. van Riel, G. Lodder, E. Havinga; J. Am. Chem. Soc., 103, 7257, (1981).
12.- G.G. Wubbels, A.M. Halverson, J.D. Oxman; V.H. De Bruyn; J. Org. Chem., 50, 4499, (1985).
13.- J. Cervelló, M. Figueredo, J. Marquet, M. Moreno-Mañas, J. Bertrán; J. M. Lluch; Tetrahedron Lett., 25, 4147, (1984).
14.- A. Castelló, J. Marquet, M. Moreno-Mañas, X. Sirera; Tetrahedron Lett., 26, 2489, (1985).
15.- P.E. Nielsen; Eur. J. Biochem., 122, 283, (1982).
16.- P.E. Nielsen, J.B. Hansen, T. Thomsen, O. Buchardt; Experientia, 39, 1063, (1983).
17.- F. Johnson; Fortschr. Chem. Org. Naturst., 29, 140, (1971).
18.- A. Castelló, C. Jaime, J. Marquet, M. Moreno-Mañas; Tetrahedron, 41, 3791, (1985).
19.- a) A. Cu, A.C. Teska; J. Am. Chem. Soc., 96, 1963, (1974); b) N. Levy, M.D. Cohen; J. Chem. Soc. Perkin II, 1979, 553.
20.- J. Marquet, M. Moreno-Mañas, A. Vallribera, J. Bertrán, A. González, J.M. Lluch; submitted.
21.- S.F. Mac Donald, A.J. Chechak; Can. J. Research, 26B, 432, (1948).
22.- G. de W. Anderson, F. Bell; J. Chem. Soc., 1949, 2668.
23.- F. Pollecoff; R. Robinson; J. Chem. Soc., 113, 645, (1918).
24.- K. Mutai, K. Yokoyama, S. Kanno, K. Kobayashi; Bull. Chem. Soc. Jpn., 55, 1112, (1982).
25.- Org. Synth., Coll. Vol. 3, 53, (1955).