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Synthesis and Protection of Nitrogen Containing Heterocycles

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SYNTHESIS AND PROTECTION OF NITROGEN CONTAINING HETEROCYCLES

by

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in

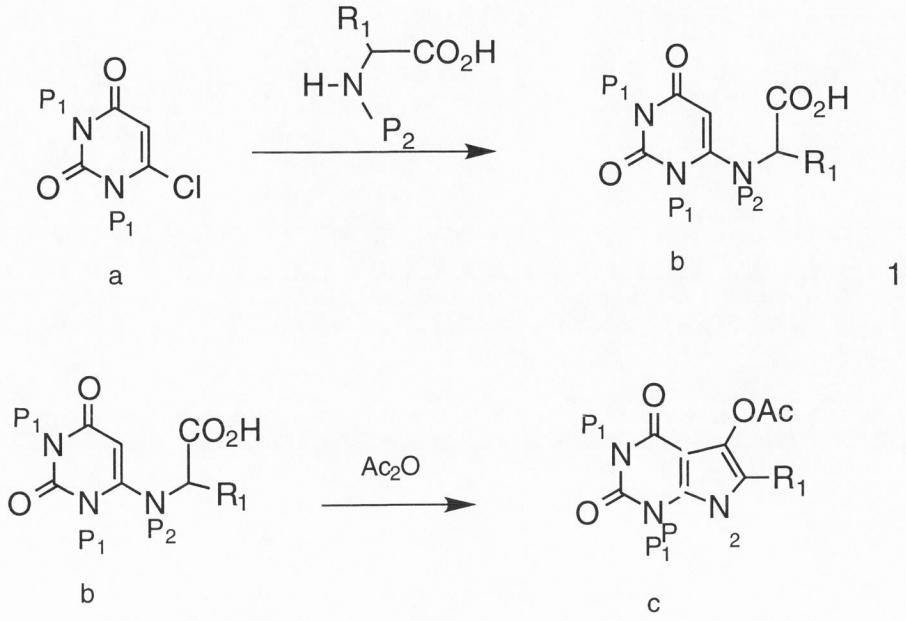
Chemistry

Synthesis of precursors to pyrrolo[2,3-d]pyrimidine ring systems

Introduction

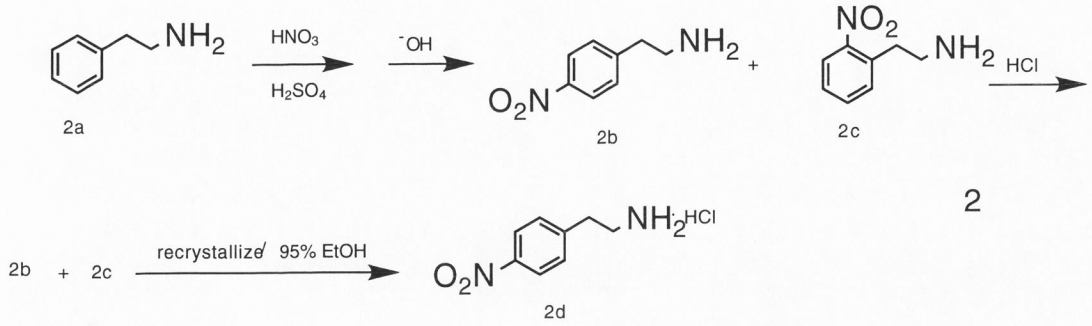
The pyrrolo[2,3-d] pyrimidine ring systems are present in a variety of antibacterial/anticancer compounds. A scarcity of natural sources has made necessary the need for researchers to find more efficient methodologies to synthesize such compounds.[1] The Edstrom research group is currently exploring new routes to these compounds, varying the protecting groups and the precursors used to make them.

The synthesis of pyrrolo[2,3-d]pyrimidine ring systems is carried out by substitution at the 6 carbon position on barbituric acid to produce 6-chlorouracil (equation 4) followed by addition of the amine (2d) with the para-nitrophenylethyl protecting group ($P_{1)}$ in place at the nitrogen atom in the pyrrolo ring (equation 1). The pyrrolo backbone was synthesized by nitrating phenylethylamine (equation 2) followed by monoalkylation to produce 3b. Finally ring closure takes place providing the heterocyclic system c. The advantage of Edstrom and Wei's method, is that P_2 is in place during ring closure and is removed by relatively weak basic conditions. The choice of protecting groups is critical, and represents a formidable branch of the group's work.



Results

The choice of the paranitrophenethyl protecting group (equation 2) is the result of previous research performed by Edstrom and Wei in which they ran into difficulties removing the benzyl group used to protect the molecule at the nitrogen atoms during ring closure and subsequent addition reactions.[1] Equation 2 is a nitration in which the ortho and para positions on the phenyl ring are both substituted, the para isomer is the sought after product.[2] The two constitutional isomers are produced in a nearly equal ratio, which is the dominant cause for the low overall yield of the para isomer. Recrystallization produced 29.2% yield, a second recrystallization raised that figure to 38.0% of 2d. NMR spectra of each crop taken separately indicate identical purity with regard to the ortho isomer.

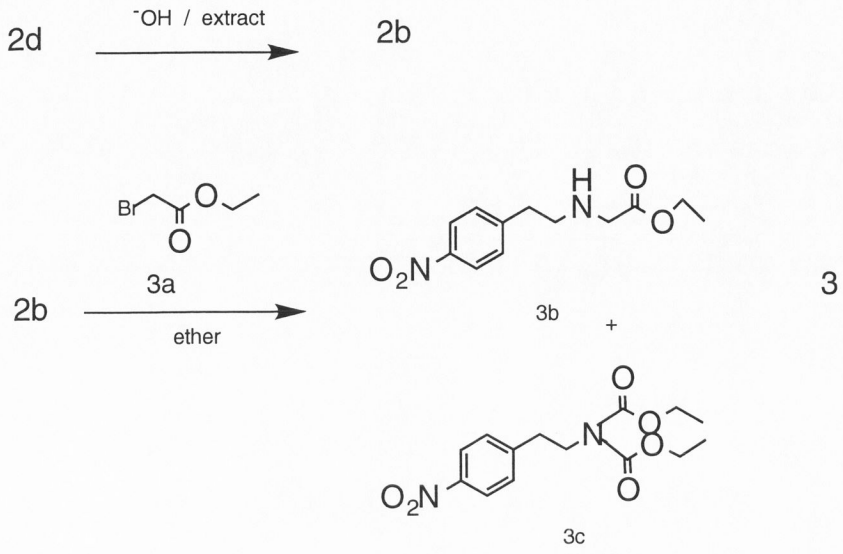


Equation 3 presented two main challenges. One is lowering the ratio of dialkylation that was found to occur readily in previous experiments. The other is finding an efficient technique with which to isolate the monoalkylated product. The first complication was dealt with by lowering the stoichiometric ratio of ethylbromoacetate by one-half with regard to the mass the free amine. The separation was first attempted using bulb to bulb distillation under reduced pressure (.001 torr). Droplets of distillate began to appear after the mother liquid reached a temperature of 140 C. NMR taken of each flask separately indicate no reasonable amount of separation occurred. The spectra did indicate that both compounds retain their composition such a high temperature, keeping separation by distillation a viable option for future experiments.

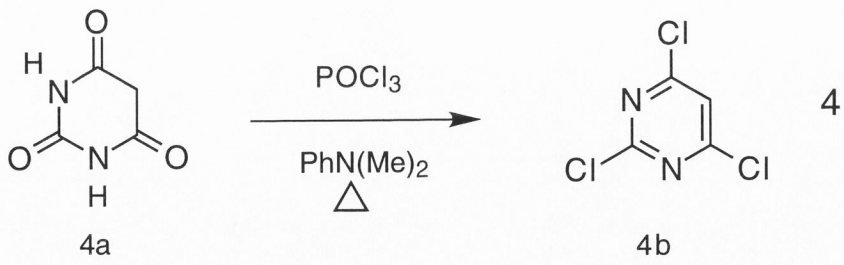
Reaction 3 was performed using ether and benzene as the solvents in separate reaction vessels under identical conditions, giving identical results. Ether was chosen for subsequent experiments because of the cost and toxicity of benzene.

Column chromatography yielded 25.0% pure monoalkylated product based on the amine and a ratio of 3.73:1 mmol of mono to dialkyl product. A small amount of product was visible in the column, however a TLC taken from subsequent elutions indicate the concentration of product to be negligible. NMR spectra of the final

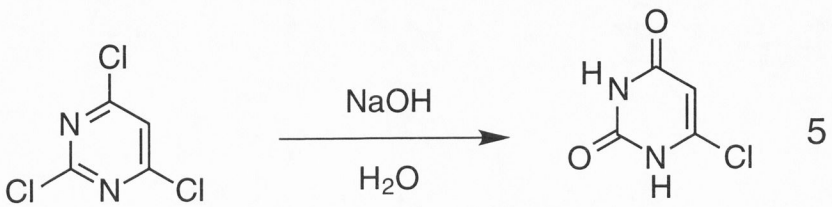
product indicate the presence (>4%) of ortho nitro substitution of the phenyl ring remaining from equation 2.



Equation 4 represents a substitution reaction in which barbituric acid (4a) is converted into 2,4,6 Tricholoropyrimidine (4b). The reagents are refluxed at 120 C and followed by the removal of excess Phosphorous trioxychloride by distillation. The reaction is heat sensitive and care must be taken during reflux and distillations.



4b is then converted to 6-chlorouracil (5a) and was obtained in 66.9% yield. 49.1% overall yield (including eq.4 and 5).



4b 5a 5

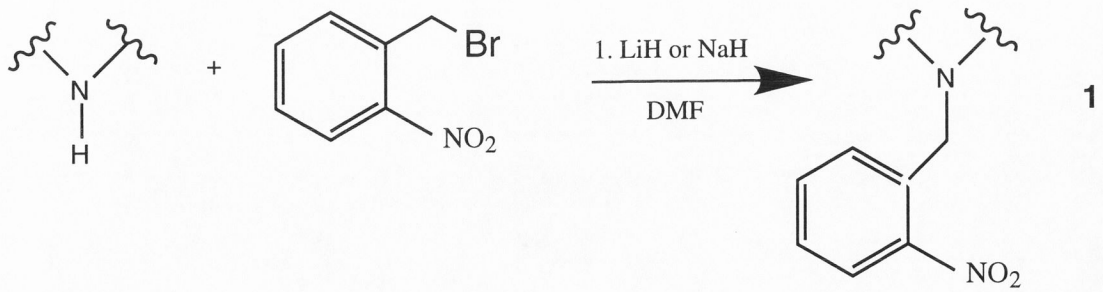
The two heterocyclic skeletons were obtained with the paranitrophenylethyl (PNPE) protecting group at nitrogen 1 intact. The method is efficient and has given the group an additional route to pyrrolo[2,3-d]pyrimidinesand related heterocyclic systems.

Ortho-nitrobenzyl - A Photo-Cleavable Protecting Group

This section summarizes much of the reasearch devoted to the development of ortho-nitrobenzyl (ONB) as a photocleavable protecting group. The goal of this project is to develop effective methods for ONB protection such that it may be utilized in multistep synthesis involving heterocyclic compounds. Alkylation of ONB and subsequent deprotection was investigated on a variety of substrates including 2-pyridinol, pyrrole, and substituted derivatives.

The characteristic alkylation reaction shown below in equation 1, involves removing the proton on the nitrogen atom targeted for protection, via lithium or sodium hydride. Lithium hydride was found to be the preferred base over sodium hydride in the majority of reactions because upon analysis of the reaction vessel contents by TLC, alkylation steps involving sodium hydride indicated the absence of o-nitrobenzyl bromide, even in the presence of an initial excess; leading to the assumption that the benzyl bromide was effectively decomposed in the strong basic environment of created by the sodium hydride. Thus, the weaker less soluble lithium hydride was used and a significant improvement in the yields of the alkylation step was evident. There still appears to be problems with optimizing the relative basicity of the reaction mixture. In some cases the base appears to be too weak, as starting material is recovered when using LiH and as mentioned above, the alkyl bromide is decomposed when subjected to significantly stronger base, NaH. Stabilization of the anion created via the removal of the proton is likely source of difficulty in the alkylation step. All alkylation reactions were carried out using the aprotic solvent DMF. The success of

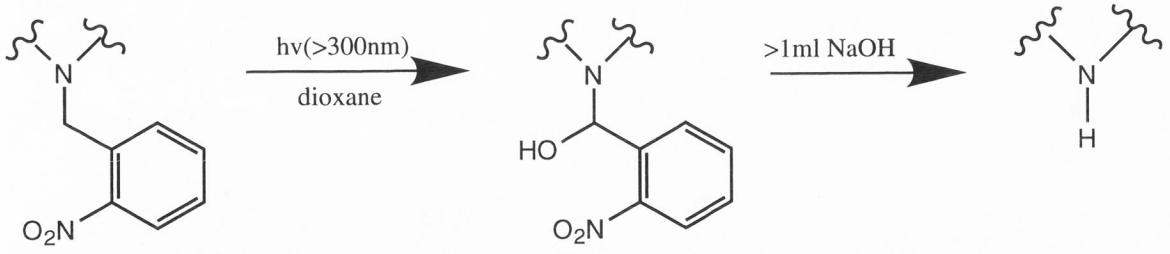
the alkylation step proved to highly related to the type of substitution on the heterocycle. The presence of a strong electron withdrawing group substituted on the pyrolles, highly correlated with success of alkylation. This observation can be rationalized by noting that the strong anion created in the deprotenation step is stabilized by delocalization of the negative charge. Thus, stabilization of the anion is necessary for successful alkylation with alkyl bromide. Attempts to ONB protect several compounds were abandon because this step proved to be troublesome, as the reactions yielded a variety of side products. On additional source of stabilization for the intermediate anion could be the solvent used in theses reactions. Our requirements for a solvent include: (1) the hydride bases LiH, NaH must soluble, (2) it must be easily removed, (3) it must be aprotic, and (4) it must not too strongly solvate the anion. One possible to solution could be to reduce the temperatures of the reaction environment to < - 10 C. Another solution could be to use DMSO , which has higher dipole (μ=3.96) than DMF (μ=3.82) and could serve as significant source of charge distribution for the nucleophile and is removed under conditions similar to DMF. Searching for and experimenting with different solvents with requirements above could improve the alkylation step.



1

The method of removal of ONB from protected substrates is shown below (equation 2). Photolysis times of various substrates ranged from 14 to 48 hours. The preferred solvent for this reaction proved to be dioxane. Several reactions were tried in ethanol, but photolysis yielded approximately .5:.5 ratio of protected to unprotected substrate by TLC inspection.

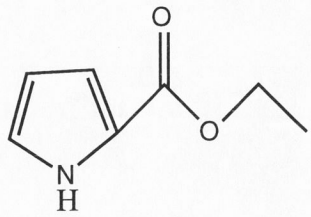
The main challenge following protected substrate exposure in the photoreactor is the isolation of the unprotected product. Reaction mixtures turned dark brown after exposure due to the by product of deprotection. This material proved to be difficult to separate from the desired deprotected product. Successful separation is made possible by addition of dichloromethane and heating the mixture slightly. A plug using silica is then prepared and the mixture run through. This procedure proved to be highly effective. The intermediate nitrosohemiaminal shown in equation is easily removed by treatment with a catalytic amount of NaOH.



2

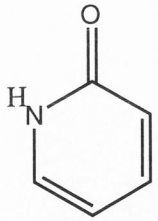
Substrates-(see attached tables 1 and 2 for condensed summary)

Substrate: Ethyl-indole-2-carboxylase



The protection/deprotection of this compound proved to be successful with yields consistently high for both the alkylation and deprotection step. The success is attributed to the electron withdrawing ability of the ester. TLC analysis following the alkylation mixture indicated little or no side products. The ONB protected product is recrystallized from 50% ethyl acetate/hexane with no further purification necessary. TLC analysis following photolysis indicated a mixture of products that were removed upon addition of > 1ml 33% NaOH. A plug prepared with silica and using ethyl acetate/hexane solvent system yielded pure by TLC, unprotected substrate. This compound thus, proved to be ideal for ONB protection and the protocol used can be carried over to other systems.

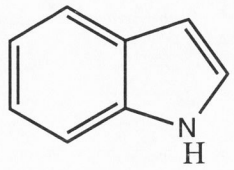
Substrate: 2-pyridinol



The alkylation of this compound (equatiis successful with yields over 90% for the ONB protected product. The photolysis or ONB removal proved to be troublesome (equation 2). TLC analysis indicated a large number of side products and low

concentration of the desired product. The treatment of the post photolysis mixture with NaOH did little to improve the contents of the mixture and efforts to improve the deprotection step included using ethanol instead of dioxane, but these efforts were of little avail.

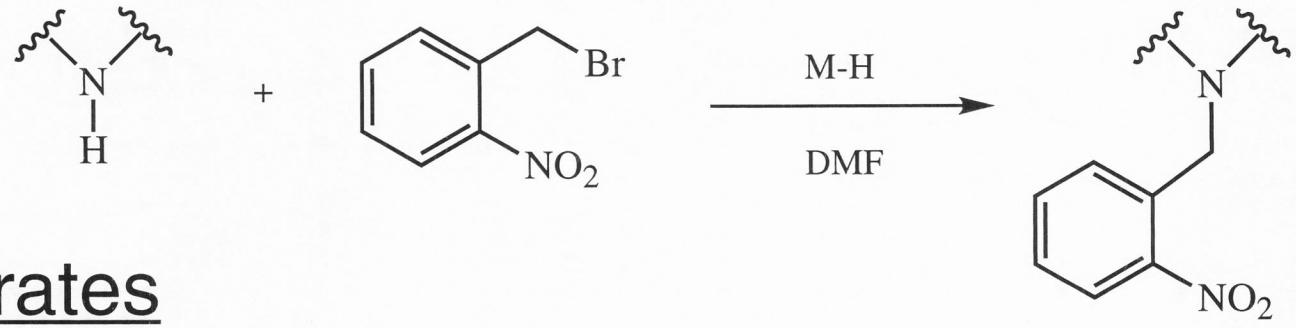
Substrate: indole



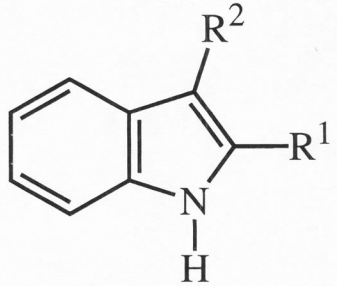
Intermediate success was acheived for ONB protection of the indole, as a decent amount of starting material remained after reaction 1. The resulting mixture is a dark yellow oil and the ONB protected product was isolated via column chromatography. The nature of the products, being starting material and protected product indicates that additional success could be reached by adjusting the solvent according the discussion earlier in this paper. Because the deprotenation step is suspected to be incomplete a solvent that would better distribute the excess charge of the intermediate anion may improve the relative completeness of the deprotenation. The photolysis of ONB proved to be problematic with this compound. Indole was detected via TLC analysis, but an array of side products were detected and the yield of unprotected indole was suspected to be unacceptably low. Treatment of the post photolysis mixture with NaOH was unsuccessful in eliminating the number of side products detected.

TABLE 1.

Alkylation of substrates



Substrates



Method A: NaH (1.1 eq) DMF, 0 -> 25 °C
B: LiH (8.0 eq) DMF, rt

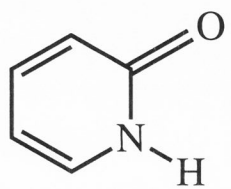
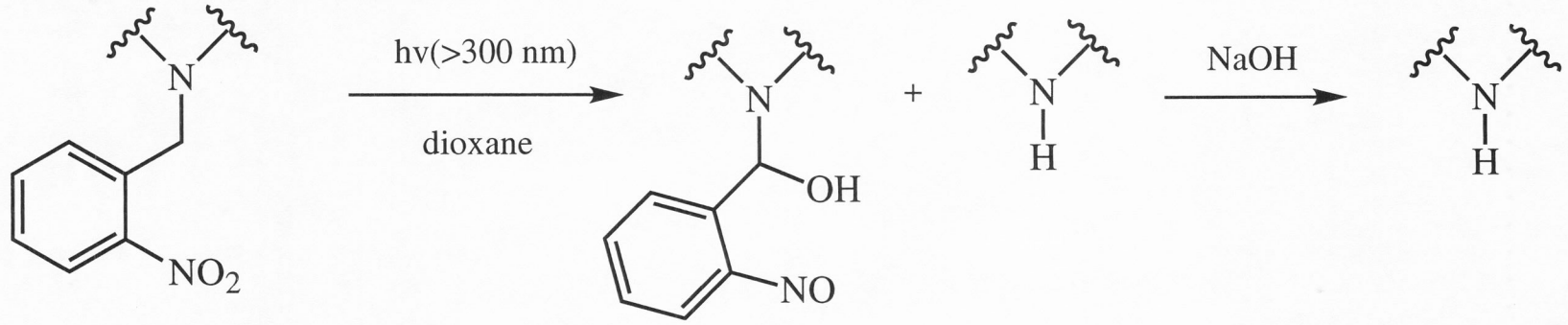
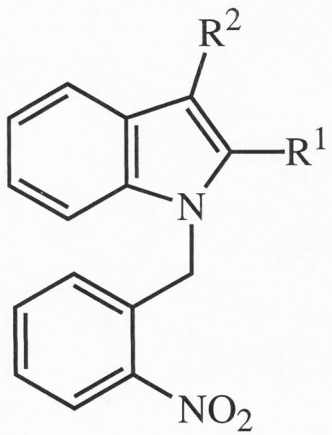
	Method A	Method B
$R^1 = CO_2Et$, $R^2 = H$	42%	85%
$R^1 = H$, $R^2 = CHO$	73%	81%
$R^1 = H$, $R^2 = H$	67%	53%
	xx	90%

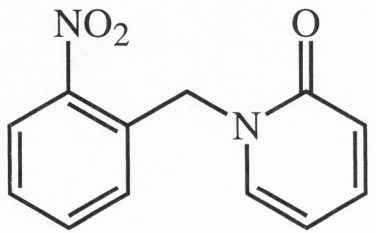
TABLE 2.

Photolysis of Substrates





Substrate	Photolysis Time	yield
$R^1 = CO_2Et$, $R^2 = H$	25h	72%
$R^1 = H$, $R^2 = CHO$	48h	95%
$R^1 = H$, $R^2 = H$	25h	xx



xx	xx

Experimental Section

4-Nitrophenylethylamine[2] (2b). A three valved flask equipped with an overhead stirring bar was bathed in an ice/salt bath to which 100g of concentrated H_2SO_4 was added and allowed to cool. 30.02g (248 mmol) of phenylethylamine were then added through an addition funnel dropwise over a period of 15 minutes. Precipitate did appear and the solution was stirred vigorously for 1 hour until homogeneity was achieved. 11.1ml of 90% HNO_3 was added dropwise by pipet, and a brown solution resulted, and was allowed to stir overnight. The reaction flask was cooled in an ice/ salt bath during which an aqueous solution of 40% NaOH was added slowly to the flask until the solution reached a pH of 11. The solution turned bright orange upon addition of the NaOH. The resulting solution was extracted with ether (4*150ml). The organic layers were combined and dried over Na_2SO_4 for 1.5 hrs, concentrated and brought to a pH of 1 using concentrated HCl, a reddish, brown thick solution resulted and was stoppered and left overnight. Water was removed and the resulting mixture was recrystallized from 95% ethanol which resulted in 14.67g of orange crystals. ^1H NMR ($CDCl_3$ 40–0MHz) delta; 8.10 (2H,d), 7.35 (2H,m), 3.00 (2H,q), 2.83 (2H,t), 1.97 (2H,t)

Ethyl-3-amino-5-paranitrophenylpentanoate (3b) 9.18g of 2d were dissolved in 75ml of H_2O and basified with 30% NaOH to pH 12. The resulting solution was extracted with CH_2Cl_2 (3*50ml). The organic layers were then combined, dried, concentrated, and put under .75 torr for 45 minutes the resulting weight of the free base was 8.22g (49.5 mmol). 2d was added to a round bottom 1L flask to which 400ml of ether and 4.15g ethylbromoacetate was then added dropwise through and addition funnel over a period of 15 min. The reaction flask was kept under N_2 atmosphere and left stirring for 80 hrs. White precipitate was visible in the yellow solution, precipitate filtered off and solution extracted with H_2O (3*75ml), concentrated and put on vacuum pump (.75-1torr) for 1.5 hrs. Column (29 by 2 in.) prepared using Merck ??? silica gel mixed with 50% EtOAc and hexanes. Solution slowly added dropwise into the column and two yellow bands became visible, all elutions collected and column progress monitored by TLC. The top band was rinsed with 100% EtOAc, elutions combined and concentrated yielding 3.11g of product (24.9%). ^1H NMR ($CDCl_3$ 400MHz) δ 8.18 (2H,d), 7.42 (2H,d), 4.24 (2H,q), 3.45 (2H,s), 2.96 (3H,m), 1.89 (2H,s), 1.30 (3H,t)

2,4,6 Trichloropyrimidine (4b) [3] 52.4g (409 mmol) of Barbituric acid, 88.5ml of $PhN(Me)_2$, and 150ml of $POCl_3$ were added to a three necked flask equipped with an overhead stirrer and condenser equipped with a drying tube for reflux. The flask was placed in an oil bath and the temperature brought to 120 C the solution was refluxed for 20 min. The flask was then cooled and excess $POCl_3$ was distilled under reduced

pressure (water aspirator). The vapor temp upon first distillate was 25 C. The resulting solution was poured slowly over 600ml of a water and ice mixture, the flask was warm to touch after 5 min. The resulting solution was extracted with ether (3*150ml) the organic layers were combined, concentrated and distilled under 1 torr, 55.1g clear liquid recovered (73.5% yield). ^{1}H NMR (DMSO 400MHz) δ 7.24 (1H,s)

5-Chlorouracil (5a) [3] 55.1g (300 mmol) of 4b and 380ml of H_2O were added to a 1L. In a separate beaker a solution of 48.11g of NaOH and 100ml of H_2O were prepared and added slowly added to the reaction flask. The solution was refluxed for 70 min and periodically shaken vigorously to obtain a homogeneous, clear solution. The flask was then cooled and 111ml of concentrated HCl was slowly added, the flask was cooled in an ice bath, capped and refrigerated overnight. The solution turned cloudy immediately upon cooling. White crystals were filtered and recrystallized with H_2O. Filtration and drying provided 29.43g of white crystals (66.94% yield). ^{1}H NMR (DMSO 400MHz) δ 12.01 (1H,s), 11.25 (1H,s)

ethyl-2-carboxylate-1-ONB indole

Ethyl-indole-2-carboxylate (.415g, 2.19mmol) was placed in reaction flask and dissolved in, ml DMF. 4 equivalents (.0841g, 10.58 mmol) of lithium hydride were added and the flask was put under a nitrogen environment. The flask was then stirred at room temperature for 15 minutes. Septum was removed and 1.1 equivalents o-nitro-benzyl was added (.521g, 2.41mmol) and the flask was allowed to stir under nitrogen environment overnight. Flask contents were then poured into 30ml brine and washed with (3*30)ml portions of CH_2Cl_2. Organic layers were combined and dried over sodium sulfate for 2hrs. CH_2Cl_2 and DMF removed and the resulting yellow material was recrystallized from 50% ethyl acetate/hexanes, providing .6059g , 85.3% yield, of product pure by TLC.

Photolysis of ethyl-2-carboxylate-1-ONB indole

Starting material was placed in a 50ml test tube to which 37ml of dioxane was added. Top of tube was secured by a septum and flask contents were placed in N_2 environment and then placed in a Rayonet Photoreactor for approximately 24 hrs. TLC analysis of tube contents indicated no starting material remained. >1 ml 33% NaOH was added to flask and mixture was allowed to stand at room temperature for 1 hour. TLC at this time indicated pure unprotected ethyl-2-carboxylate indole. A plug was then prepared using a small funnel and silica gel with ethyl acetate/hexane. Separation and subsequent recrystallization yielded .147g, 72% yield of product.

1-ONB-2-pyridinol

2-pyridinol (.400g, 4.17 mmol) was placed in 12 ml of DMF and LiH (.1486g, 18.7mmol) was added. The flask was allowed to stir under N_2 environment for 15 min and then septum was removed and 1.2 equivalents (.999g, 4.62mmol) of o-

nitrobromobenzyl was then added and the flask contents were stirred at room temperature under N_2 for 6 hours. Flask contents were poured into 20ml of brine and extracted with (3*25)ml ethyl acetate. Combined organic layers were then dried overnight with sodium sulfate. Subsequent removal of solvents and recrystallization with ethyl acetate/hexane yielded .8532g of product pure by TLC (90% yield).

Photolysis of 1-ONB-2-pyridinol

1-ONB-2-pyridinol (.0506g, .273mmol) was placed in a test tube to which 5 ml dioxane was added and tube was placed under N_2 environment. Tube was placed in Rayonet Photoreactor until all starting material was gone via TLC analysis. >1ml of 33% NaOH was added and mixture was allowed to stand for 1 hour. TLC analysis of flask contents indicated many side products with an estimated 10% concentration of product. Addition of more NaOH did not improve the TLC.

1-ONB-indole

Indole (.261g, 2.23mmol) was placed in 10ml DMF and 10 equivalents of LiH (.1764g, 22.2mmol) were added and flasked was stirred for 15 min at room temperature under N_2. Septum was removed and 1.1 equivalents of o-nitro-bromobenzyl was added. Mixture was stirred at room temperature overnight under N_2 environment. Reaction contents were poured over 25ml of brine ice mixture and extracted with (3*25)ml portions of ethylacetate. Organic layers were combined and dried over sodium sulfate for 3 hours. Solvents were then removed and yellow oil resulted. TLC indicated a mixture of starting material and ONB protected product. Separation via column chromatography using silica gel (Merck 6nm, 230-400 mesh) and 2% ethylacetate/hexanes yielded .3243g (58%)protected product.

Photolysis of 1-ONB-indole

1-ONB-indole (.0517g, .205mmol) was placed in test tube under N_2 environment with 5ml dioxane. Test tube was placed in Rayonet Photoreactor for approximately 25 hours. TLC analysis indicated many sided products along with indole in an estimated 50% concentration. Drops > 1ml of NaOH were added to reaction and mixture and no purification was observed.

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