On the Relocation of the Amino Functionality to the Ethyl Side Chain in Aminoglutethimide: Synthesis and Aromatase-inhibitory Activity of 3-(2'-*N*,*N*-Diethylamino)ethyl-3-phenylpiperidine-2,6-dione

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Aminoglutethimide [3-(4-aminophenyl)-3-ethylpiperidine-2,6-dione] has been used clinically in the treatment of metastatic breast carcinoma. Inhibition of tumor growth is due to interference with estrogen biosynthesis. However, its action is not specific and its metabolism gives rise to toxic and non-inhibitory metabolites. We sought to explore the impact of relocating the amino group in aminoglutethimide to the ethyl side chain. To that end, we now report the synthesis and aromatase-inhibitory activity of 3-(2'-*N*,*N*-diethylamino)ethyl-3-phenylpiperidine-2,6-dione **5**. The introduction of the amino functionality on the ethyl group is accomplished *via* reductive amination of aldehyde **8**, prepared in three steps from benzyl cyanide. The synthetic route presented can be used for the preparation of related derivatives of aminoglutethimide.

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Introduction.

Aminoglutethimide [3-(4-aminophenyl)-3-ethylpiperidine-2,6-dione] **1** was first introduced into clinical use in 1958 as an anticonvulsant drug but was withdrawn in 1966 after it was found to exert adverse effects on adrenal steroidogenesis during prolonged treatment. The drug was subsequently shown to suppress the enzymatic conversion of cholesterol to pregnenolone, mediated by the enzyme complex desmolase [1]. Aminoglutethimide is now known to inhibit steps in other steroidogenic pathways, including the aromatization of androst-4-ene-3,17-dione and testosterone to estrone and estradiol, respectively, mediated by the enzyme aromatase [2]. The use of aromatase inhibitors is, today, a well-established strategy for the treatment of metastatic hormone-dependent breast cancer. It is within this context that aminoglutethimide is clinically useful, showing positive results in the treatment of ovariectomized and postmenopausal women [3]. Because peripheral aromatization of adrenal androgens is the principal pathway of estrogen production in postmenopausal and ovariectomized women [4], aminoglutethimide and other aromatase inhibitors represent a powerful treatment modality for hormone-dependent breast cancer in these patients.

Aminoglutethimide therapy is not without its drawbacks, however. Studies on the metabolism of **1** have shown that the principal metabolite results from *N*-acetylation of the free amino group [5,6]. This metabolic pathway is detrimental to the patient in that the metabolite is not only an ineffective enzyme inhibitor, but is also toxic to normal cells resulting in a host of side effects which range in severity from drowsiness and lethargy to gastrointestinal complications and hypothyroidism.

The mode of interaction of aminoglutethimide with these enzyme complexes is not completely understood, however, research has revealed certain essential structure/activity relationships. The location of the amino group within the molecular framework has been found to exert a profound effect on the inhibitory activity of aminoglutethimide and its analogs [1]. Early work by Paul and coworkers [7] showed that the absence of an amino group results in the loss of inhibitory activity. *N*-Aminoglutethimide **2** is a desmolase inhibitor but exhibits no activity towards aromatase. The *meta* isomer **4** is a weak aromatase inhibitor while the 5-amino isomer **3** inhibits neither enzyme system. The basic character of the amino group also plays an important role in the activity of the molecule [8,9]. Foster [10] demonstrated by the synthesis of an aromatase-specific 4-pyridyl derivative, that increasing the basicity of the amino group results in enhanced aromatase selectivity. However, the inhibitory strength of this derivative decreased sharply upon incorporation of fluorine into the pyridyl ring indicating that basic strength affects inhibitory potency as well as selectivity. In a 1986 survey article, Nicholls [6] reported studies that suggest a critical distance of 6.3-6.8 Å between the C-6 keto group and the amino functionality in aminoglutethimide and its active derivatives, a value equivalent to the distance between the C-17 oxygen and the C-19 methyl of testosterone as determined by X-ray crystallography. It was proposed that this structural similarity allows aminoglutethimide and its active analogs to interact with aromatase at appropriate binding sites, competitively inhibiting the aromatization of testosterone and androstenedione.

In continuing our efforts toward the development of aminoglutethimide derivatives of increased inhibitory potency [11], we decided to investigate the impact of relocating the amino group to the ethyl side chain. We carried out preliminary molecular modeling studies on 3-(2'-*N*,*N*-diethylamino)ethyl-3-phenylpiperidine-2,6-dione **5** and, upon minimization, found a distance of 6.8 Å between the

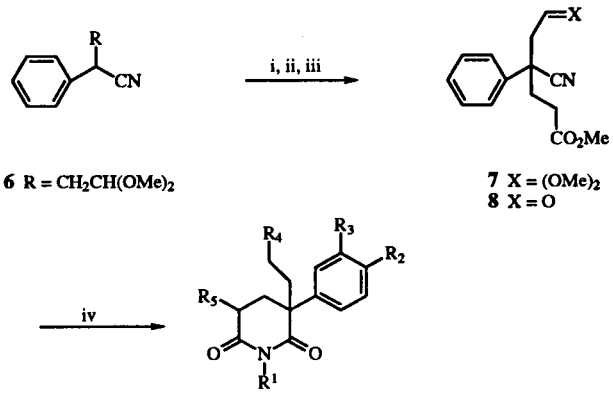
C-6 keto group and the diethylamino moiety, a value consistent with those summarized by Nicholls [6]. An added feature of **5** is the presence of a tertiary amino group which should prove both more basic and more resistant towards *N*-acetylation than the corresponding primary amine. We now wish to report the synthesis of **5** and its aromatase-inhibitory activity.

Results and Discussion.

In our proposed synthetic sequence (Scheme 1) the introduction of the amino functionality on the ethyl group could be accomplished *via* reductive amination of an aldehyde precursor **8**, prepared *via* Michael addition of **6** to methyl acrylate. The first step would necessarily involve alkylation of benzyl cyanide with 2-bromo-1,1-dimethoxyethane to provide cyanoacetal **6**, in which the terminal carbon atom is in the aldehyde oxidation state, protected as its acetal. Sukata [12] showed that it was possible to selectively monoalkylate benzyl cyanide using an alkali metal hydroxide impregnated on alumina. Alkylation with 2-bromo-1,1-dimethoxyethane was attempted using this method, but the reaction was found to proceed at a very slow rate (<10% yield of **6** after 28 hours) even at 110°. In an effort to improve the kinetics of the reaction, a halide exchange with iodide (Finkelstein reaction) was performed but without visible improvement in reaction rate. Use of sodium hydride as base in refluxing toluene afforded the cyano acetal **6** in slightly better yield (30%) after 28 hours. This still was not satisfactory. It is known that enolate anions tend to form aggregates with alkali metal counterions in non-polar media such as benzene or toluene [13]. However, toluene had been chosen more for its boiling point than for its ion-solvating ability since the reaction appeared to require high temperature to be kinetically feasible. It was hoped that a more polar solvent would improve ion salvation, thus reducing aggregation and increasing the availability of free anion. DMSO specifically was chosen for its polarity, dielectric constant and high boiling point. Alkylation in DMSO resulted in 61% yield in 10 minutes at 55°. This was a dramatic improvement over the previous reaction, doubling the product yield at nearly half the temperature in 1/168 of the time.

Introduction of the C-4-C-6 unit of piperidine-2,6-dione was accomplished smoothly *via* Michael addition of **6** to methyl acrylate in THF at 50°. This afforded the ester acetal **7** in 72% yield. Acetal cleavage using trifluoroacetic acid provided the aldehyde **8** in 68% yield which was then subjected to reductive amination using the Borch [14] procedure to furnish the desired aminocyanoester **9**. Only modest conversion was observed at first, but this was remedied using 4 Å molecular sieves (Davisil) to remove the water liberated by the amination reaction and provide a driving force. Finally, treatment of **9** with glacial acetic and concentrated sulfuric acids at 100° for 2 hours afforded the target product **5** as a solid which was recrystallized from methanol/water.

Scheme 1



1 $R_1 = R_3 = R_4 = R_5 = H$, $R_2 = NH_2$
2 $R_1 = NH_2$, $R_2 = R_3 = R_4 = R_5 = H$
3 $R_1 = R_2 = R_3 = R_4 = H$, $R_5 = NH_2$
4 $R_1 = R_2 = R_4 = R_5 = H$, $R_3 = NH_2$
5 $R_1 = R_2 = R_3 = R_5 = H$, $R_4 = N(Et)_2$

i) Me acrylate, Triton B®, THF, 50°, ii) 50% (v/v) $TFA_{(aq)}$, CH_2Cl_2, 25°; iii) $NH(Et)_2$, $NaBH_3CN$, MeOH, 25°; iv) glacial HOAc, conc H_2SO_4, 100°.

The inhibitory activity of **5** against aromatase was tested in human placental microsomes [15]. As may be seen from Table 1, substrate aromatization was suppressed by 31% at 100 n*M* concentration. This activity is well below the corresponding value for the parent aminoglutethimide (90% at 86 n*M*), comparatively similar to **4** (40% inhibition at 86 n*M*) and markedly superior to **3** (0% inhibition). Syntheses of other analogues of **5** are currently in progress.

Table 1
Aromatase Activity:
% Labeled Substrate Conversion with Increasing [5]

Concentration of **5** (n*M*)	0	50	100	250	500	1000	2000	5000
% Labeled Substrate Conversion [a]	100	78	69	69	66	69	86	60

[a] The reagents and conditions for the inhibitory activity assay have been described elsewhere [15].

EXPERIMENTAL

Tetrahydrofuran (THF) was distilled from sodium/benzophenone immediately prior to use. Methanol and dimethyl sulfoxide (DMSO) were distilled from calcium hydride and stored over molecular sieves (Davisil, 4 Å). Reactions utilizing these solvents were conducted under a positive pressure of nitrogen gas. Masses were measured on an Ohaus AS120S analytical balance.

Dry column chromatography was performed using Florisil®, 60-100 mesh (U.S. Silica Company). Preparative tlc was performed on E. Merck Silica Gel 60 F_{254} plates. The ^1H and ^{13}C nmr spectra were recorded in deuteriochloroform at 300 and 75 MHz, respectively and are referenced against internal tetramethylsilane. The ^{13}C spectra were broadband decoupled from hydrogen nuclei. Low resolution EI mass spectra were recorded with an ionization voltage of 70 eV; selected peaks are reported as m/z (% intensity relative to base peak). Elemental analyses were performed at the School of Chemistry, University of Birmingham, U.K. High resolution mass spectrum was recorded at the Center for Mass Spectrometry, Department of Chemistry, University of Nebraska, Lincoln, NE.

4,4-Dimethoxy-2-phenylbutanenitrile (**6**).

To a mixture of sodium hydride (50% dispersion in oil, 0.966 g, 20.0 mmoles) in dry DMSO (50 ml) was added slowly dropwise, benzyl cyanide (2.8 ml, 24 mmoles). This mixture was allowed to stir at 55° forming a dark red solution. After 1 hour, bromoacetaldehyde dimethyl acetal (2.4 ml, 20 mmoles) was added forming a dark blue solution which was allowed to stir at 55° for 10 minutes only. The reaction mixture was decanted hot into a dilute solution of hydrochloric acid (2% v/v, 140 ml) forming a milky orange emulsion which was extracted with methylene chloride (4 x 20 ml). Organic layers were pooled, dried over magnesium sulfate, filtered, and concentrated under reduced pressure to give a reddish oil. Distillation *in vacuo* afforded **6** (2.50 g, 61%) as a colorless oil, bp 107-110° (0.6 mm Hg); ^1H nmr: δ 2.05-2.27 (m, 2H), 3.34 (s, 3H) 3.38 (s, 3H), 3.92 (dd, J = 6.6 Hz, J = 9.2 Hz, 1H), 4.46 (dd, J = 5.0 Hz, J = 6.7 Hz, 1H), 7.32-7.40 (m, 5H); ^{13}C nmr: δ 33.1, 38.5, 53.3, 53.8, 101.8, 120.5, 127.3, 128.2, 129.2, 135.4; ms: m/z 142 (M^+-63, 11), 116 (39), 89 (15), 77 (9), 75 (100), 58 (12), 47 (25), 43 (14), 39 (10), 31 (21).

Anal. Calcd. for $C_{12}H_{15}NO_2$: C, 70.22; H, 7.37. Found: C, 70.50; H, 7.14.

Methyl 4-Cyano-6,6-dimethoxy-4-phenylhexanoate (**7**).

To a stirred solution of **6** (1.017 g, 5.0 mmoles) in dry THF (10 ml) at 25°, was added Triton B® (0.25 ml, 0.5 mmole) immediately forming a dark red solution. This mixture was stirred for 10 minutes at 25° after which methyl acrylate (1.4 ml, 15 mmoles) was added and the mixture stirred at 50° for an additional 30 minutes. The reaction was quenched by addition of water (20 ml) and 2 drops of 2.4 *M* hydrochloric acid forming a yellow emulsion which was extracted with diethyl ether (3 x 20 ml). Organic layers were pooled, dried over magnesium sulfate, filtered and concentrated under reduced pressure to give a viscous amber oil. Dry column chromatography (Florisil®, ethyl acetate, column d = 4 cm, h = 1.7 cm) afforded **7** (1.03 g, 72%) as an orange oil; ^1H nmr: δ 2.04-2.56 (m, 6H), 3.17 (s, 3H), 3.31 (s, 3H), 3.60 (s, 3H), 4.22 (dd, J = 3.9 Hz, J = 7.0 Hz, 1H), 7.32-7.46 (m, 5H); ^{13}C nmr: δ 29.8, 35.9 43.4, 44.3, 51.7, 52.9, 53.7, 101.8, 121.2, 125.9, 128.2, 129.1, 136.8, 172.6; ms: m/z 196 (2), 172 (2), 142 (7), 115 (9), 89 (9), 75 (100), 58 (8), 47 (15), 43 (9), 31 (13).

Anal. Calcd. for $C_{16}H_{21}NO_4$: C, 65.96; H, 7.27. Found: C, 66.30; H, 7.61.

Methyl 4-Cyano-6-oxo-4-phenylhexanoate (**8**).

To a stirred solution of **7** (1.556 g, 5.3 mmoles) in dichloromethane (24 ml) at 0° was added slowly, dropwise an aqueous solution of trifluoroacetic acid (50% v/v, 12 ml, 80 mmoles). After 5 minutes of stirring at 0°, the mixture was warmed to room temperature and allowed to stir for an additional 67 hours. The organic layer was separated and the aqueous layer extracted with methylene chloride (2 x 20 ml). Combined organic phases were washed with saturated sodium bicarbonate soln. (3 x 20 ml), dried over magnesium sulfate, filtered and concentrated to give **8** (0.879 g, 68%). An aliquot (74 mg) was further purified by preparative tlc (silica, ethyl acetate:hexane 1:1) to provide an analytical sample; ^1H nmr: δ 1.99-2.55 (m, 4H), 3.08 (m, 2H), 3.60 (s, 3H), 7.22-7.46 (m, 5H), 9.53 (m, 1H); ^{13}C nmr: δ 29.6, 35.3, 42.7, 51.8, 52.0 120.5, 125.8, 128.6, 129.3, 135.9, 172.1, 197.0 ms: m/z 217 (M^+-28, 30), 196 (42), 190 (37), 185 (50), 144 (38), 143 (49), 131 (58), 130 (67), 129 (70), 115 (65), 103 (100), 77 (47), 74 (61), 59 (32), 51 (37).

Anal. Calcd. for $C_{14}H_{15}NO_3$: C, 68.56; H, 6.16. Found: C, 68.90; H, 6.22.

Methyl 4-Cyano-6-(*N*,*N*-diethylamino)-4-phenylhexanoate (**9**).

To a stirred mixture of **8** (0.596 g, 2.4 mmoles) and approximately 164 Å molecular sieves (Davisil) in dry methanol (17 ml) at 25° was added diethylamine (0.30 ml, 2.9 mmoles). This was followed by immediate addition of a solution of sodium cyanoborohydride (0.112 g, 1.7 mmoles) in dry methanol (3 ml). The mixture was allowed to stir for 15 minutes at 25° after which it was filtered, with the aid of an additional 25 ml of methanol, through a 60 ml sintered glass funnel containing Celite® 521. Filtrate was concentrated to afford a yellow-orange residue which was partitioned between chloroform (25 ml) and water (25 ml). The organic layer was separated and the aqueous layer extracted with chloroform (3 x 25 ml). Combined organic phases were dried over magnesium sulfate, filtered and concentrated to give **9** (0.639 g, 88%) as a viscous yellow oil which was used without further purification; ^1H nmr: δ 0.94-0.99 (t, J = 7.2 Hz, 6H), 2.00-2.68 (m, 12H), 2.48 (q, J = 7.2 Hz, 4H), 3.61 (s, 3H), 7.30-7.47 (m, 6H).

3-(2'-(*N*,*N*-Diethylamino)ethyl)-3-phenyl-piperidine-2,6-dione (**5**).

A mixture of **9** (0.482 g, 1.6 mmoles), concentrated sulfuric acid (0.799 g) and glacial acetic acid (1.607 g) was stirred with moisture exclusion at 100° for 2 hours. The cooled solution was poured onto ice, adjusted to *p*H 7 with 2*N* sodium hydroxide, and extracted with chloroform (4 x 20 ml). The combined organic phases were dried over magnesium sulfate, filtered, and concentrated to give a viscous yellow oil which solidified on standing. The resulting solid was recrystallized from methanol/-water to afford **5** (0.160 g, 35%) as short white needles, mp 122-123°; ^1H nmr: δ 0.97 (t, J = 7.2 Hz, 6H), 2.14-2.62 (m, 12H), 2.50 (q, J = 7.2 Hz, 4H), 7.25-7.40 (m, 5H), 7.97 (br s, 1H); ^{13}C nmr: δ 11.2, 28.7, 29.4, 36.1, 46.6, 47.9, 50.3, 126.2, 127.9 129.3, 138.7, 172.6, 175.4; hrms: Calcd. for $C_{17}H_{24}N_2O_2$: 288.1838. Found: 288.1834.

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