PREPARATION OF A FENTANYL LIGAND FOR THE OPIATE RECEPTOR (U) PITTSBURGH UNIV PA SCHOOL OF MEDICINE L B WINGARD ET AL. NOV 87 CRDEC-CR-88008
PREPARATION OF A FENTANYL LIGAND FOR THE OPIATE RECEPTOR

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The findings in the report shall be presented to the Commanding General, Department of the Army, for action.
Preparation of a Fentanyl Ligand for the Opiate Receptor

The objective of this task was to prepare the radiolabeled and nonradiolabeled photoaffinity label fentanyl derivative N-[1-[2-(4-azidophenyl)ethyl]-4-piperidinyl]-N-phenyl-[2,3,4-H]propanamide. The compound was prepared for use by the U.S. Army in the study of opiate receptors. Several grams of the nonlabeled and a few micrograms of the radiolabeled analog were synthesized. The route of the synthesis was first worked out using nonradiolabeled compounds. The addition of the radiolabel was incorporated as the final step in the synthesis. Alternate purification methods need to be considered for optimization of the yield of pure radiolabeled product.
The work described in this report was authorized under Contract No. DAAA15-85-D-0019. This work was started in June 1986 and completed in September 1986.

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This report has been approved for release to the public.

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The authors thank Dr. Michael Casner for carrying out some of the synthesis work and Dr. Ian Campbell for conducting preliminary experiments to assess alternate methods for purification of the final radiolabeled product. Alvin Marcus very kindly ran the mass spectroscopic determinations. Dr. Aksel Bothner-by arranged to have the nuclear magnetic resonance (NMR) measurements made at the 620 MHz NMR facility (NIH RR00292).
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PREPARATION OF A FENTANYL LIGAND FOR THE OPIATE RECEPTOR

1. APPROACHES FOR SYNTHESIS

The desired product is shown as compound 1. The * indicates the location of the tritium (\(^{3}H\)) on the radiolabeled form.

![Chemical structure of compound 1](image)

The general approach that was used was to prepare \(N\)-[1-[2-(4-nitrophenyl)ethyl]-4-(phenylamino)piperidine] using published procedures, convert the nitro group to an azide, and then add the side chain with or without the radiolabel by acylation of the secondary amine. However, there was considerable uncertainty from the literature in regard to whether these steps could be carried out without also encountering unwanted side reactions. Therefore, several alternative approaches were discussed and some were tested experimentally.

Published procedures (1,2) were available for that part of the synthesis shown in Figure 1. Several approaches were considered for converting the nitro group to an azide and for attaching the propionate to 4.

One approach involved the conversion of 4, by acylation with acryloyl chloride to give \(N\)-[1-[2-(4-nitrophenyl)ethyl]-4-piperidinyl-N-phenyl-acrylamide] 5. In principle 5 could then be reduced with tritium gas in the presence of a palladium catalyst to form the radiolabeled 6. Both the double bond and the nitro group would need to undergo the tritium reduction. After suitable purification, the radiolabeled 6 would then need to undergo diazotization and subsequent reaction with sodium azide to form the desired product, 1. The structures of 5 and 6 are described on page 6.

The approach via 5 and 6 was discussed extensively with the custom synthesis and tritiation personnel at both New England Nuclear Co. and Amersham Co., since the tritiation could not be done at the University. The conclusions from those discussions did not make the approach via 5 and 6 look very promising. The cost of the commercial tritiation was estimated by these companies to require at least $2,000. This included the cost of 50 Ci of tritium gas to preload the


Figure 1. Portions of Synthesis Based on References (1,2).
catalyst. The time required by the commercial firm would be 5-6 weeks, with no purification of the resulting product mixture included in the cost or in the time scale. The custom synthesis personnel were uncertain that both the nitro group and the double bond reductions would proceed; and further they cautioned that radiolytic decomposition of the product could be significant. And finally, the custom synthesis personnel could not propose a simple method to tell whether or not both of the reductions had occurred as planned. Two purifications with radiolabeled material would have been required, once after the tritium reductions and again after the diazotization-azide reactions. And finally, there was a slight possibility of a 1,6-sigmatropic hydrogen shift in 5, as shown below, which might lead to degradation of the compound. Therefore, the tritium gas double reduction approach was not used.

An alternative method of converting the nitro group on 4 to an acrylamide followed by addition of an acrylamide side chain also did not appear to be feasible. The subsequent tritium gas reduction of the acrylamide double bond would most likely have caused reaction of at least one of the highly labile azide group. Moreover, the initial reduction of the nitro group and subsequent azide treatment in 4 would most likely have required protection of the secondary amine (reserved for
the acrylamide attachment) to prevent reaction at the secondary amine as well; however it is shown later that the conversion of the nitro group to the azide could be carried out without having to protect the secondary amine.

These problems in principle could be overcome by an approach that a) put the radiolabel on at the last step, thus requiring only one purification with the radiolabel attached, and b) that did not necessitate a tritium gas reduction.

After extensive discussion in this laboratory and with CRDEC personnel, the approach in Figure 2 was selected. This involved the conversion of 4 to 7, to protect the secondary amine, and then reduction of the nitro group to produce 8 followed by conversion to the azide (9). Hydrolytic cleavage of the amide would remove the protective group from the secondary amine to produce 1-[2-(4-azidophenyl)ethyl]-4-(phenylamino)-piperidine (10), which could be acylated with a radiolabeled propionylating reagent to give the final product (1).

It was planned to use N-succinimidy-[2,3-3H] propionate as the acylating reagent since it was commercially available at about $498/5 mCi and specific activity 80-100 Ci/mmol. A more reactive propionylating agent, radiolabeled propionyl chloride, would have required a $7,000 custom synthesis for 1-5 Ci/mmol at 95% purity. It was also planned a) to prepare the non-radiolabeled analog 9 of the desired product 1 for use at CRDEC and b) to test the final attachment chemistry for the radiolabeled group (10 + 11 to give 1) first with non-radiolabeled compounds (10 + 12 to give 9).

It turned out that the hydrolysis of 9 to produce 10 was extremely difficult to carry out. Therefore a method was developed to go directly from 4 to the primary amine and then to the azide, 10, as shown in Figure 3.
Figure 2. Approach selected for use. Radiolabel shown as * in 11 and 12.
Figure 3. Pathway used for converting 4 to 10.
2. EXPERIMENTAL PROCEDURES

2.1 Materials

p-Nitrophenyl acetic acid, 4-piperidone monohydrate hydrochloride, thionyl chloride, sodium azide, aniline, sodium borohydride, N-hydroxysuccinimide, and propionic anhydride were purchased from Aldrich Chemical Co. The other chemicals were reagent grade from Fisher. N-succinimidyl-[2,3-3H]propionate with a specific activity of 107 mCi/µmol was purchased from Amersham Co. Tetrahydrofuran was distilled over lithium aluminum hydride. Toluene and benzene were distilled over sodium and kept over 4 Å molecular sieves.

2.2 Procedures

Liquid scintillation counting was carried out using Aquasol as the counting fluor and a Beckman Model 1801 spectrometer with H-number quench correction. Radiochromatogram scanning of the TLC plates was done with a Packard scanner.

\(^1H\) NMR spectra were measured in deuterochloroform with TMS as the internal standard using the 620 MHz NMR facility at Carnegie Mellon University. Infrared spectra were obtained with a Digilab FTS 15/80 Fourier Transform Infrared Spectrometer. Low resolution mass spectra were recorded on a double focussing VG 7070G instrument at the University of Pittsburgh Chemistry Department.

Purifications were achieved by column chromatography on silica gel (70-230 mesh) or by repeated crystallization. Melting points were determined on a Thomas Hoover Unimelt capillary melting point apparatus. Solvents containing organic fractions were dried over anhydrous sodium sulphate.
3. RESULTS AND DISCUSSION

Several of the Fig. 1 reactions did not give the reported yield of pure products when carried out as described in references (1) and (2). Furthermore, the literature crystallization method for purifying the intermediate compounds, i.e. recrystallization from ethyl ether at 
70°C, did not prove satisfactory. Alternate methods using ethyl acetate, ethyl acetate/petroleum ether, and cyclohexane had to be developed to obtain the purified products. The detailed experimental conditions and the results of the characterization measurements for each compound are given in the last section of the report.

Compound 4 was converted in several aliquots to give 16 g of pure
7 of m.p. 119-120°C. Catalytic hydrogenation of 7 over palladium, following the published procedure, gave quantitative conversion of 7 to 8, with a product m.p. of 150-152°C. Diazotization of 8 with sodium nitrite and 2N HCl at 0°C followed by the addition of sodium azide produced 9, as a colorless material after crystallization from ethyl acetate/ethanol.

Purified 9 had a decomposition point at 195°C and exhibited intense infrared absorption at 2110 cm⁻¹ (indicative of the azide group) and at 1638 cm⁻¹ (indicative of the amide carbonyl). Mass spectroscopic analysis of 9 showed a molecular ion peak at 377 mass units and another peak at 349 mass units. The loss of 28 mass units from the molecular ion was indicative of azide breakdown to release nitrogen to yield a nitrenium ion. Proton NMR spectra were obtained for 7, 8, and 9; and the spectra agreed with the assigned structures. Compound 9 was stable, in that no decomposition was observed (tested by TLC) over several weeks storage at 5°C while protected from light.

The hydrolysis of 9, to remove the non-radiolabeled propionyl group and form 10, while keeping the azide function intact, proved to be very difficult to carry out. Both acidic and basic reagents, including various concentrations of hydrochloric acid or sodium hydroxide, ammonia, HF, and Triton B, were tried. The procedure was complicated by two factors: 1) the mobilities of the components of the reaction mixture seemed peculiar on TLC (this is discussed later in more detail) and 2) in some of the trials with acidic reagents a new compound that differed from 8, 9, or 10 was formed, as seen by TLC. This new compound did not show an azide peak on IR but did show NH and amide carbonyl absorptions. It was postulated that the new compound might have been the result of internal NH insertion between the azide and the N-phenyl to give 13. No attempt was made to isolate and characterize 13; however, there is precedence for insertion of an azide-derived nitrene into phenyl rings to give an NH linkage (3).

In view of the difficulties in hydrolyzing 9 to form 10, several alternative approaches were tried. One approach was the direct substitution of the nitro group of 4 with sodium azide in DMSO to give 10. Such a procedure had been reported earlier for a similar transformation (4); but this approach was unproductive for our system, even with heating to 70°C. As a second alternative approach to form 10, it was thought that the trifluoroacetyl function would be more desirable than the propionyl function as a protective group for the secondary amine since the trifluoroacetyl group should be more amenable to cleavage than the propionyl group. Accordingly, 4 was reacted with trifluoroacetic anhydride to give the trifluoroacetyl derivative of 7. A similar trifluoroacetate analog of 8 was prepared by catalytic hydrogenation. Conversion to the azide, and treatment of the trifluoroacetate analog of 9 with mild aqueous ammonia was expected to remove the protecting group and give the desired 10. However, this trifluoroacetate approach was not characterized fully because a more simple procedure was devised to go from 4 to 10 without the need to protect the secondary amine.

Compound 4 was catalytically reduced with hydrogen over palladium at greater than 80% yield to produce 14 (Figure 3). Earlier it was thought that diazotization of 14 would lead to diazotization of the primary aromatic amine and simultaneous nitrosation of the secondary amine. However, conditions were established to convert 14 to 10 without affecting the secondary amine by carrying out the diazotization and reaction with sodium azide over a very short time. Thus, 10 was obtained as a pure, colorless, crystalline material that showed the azide absorption band at 2118 cm\(^{-1}\) by IR and had the expected molecular ion peak at 321 mass units by mass spectrometry. The proton NMR data also were compatible with the assigned structure for 10.

There was a curious reversal of mobilities (mentioned earlier) of 10 and 9 on TLC. With a silica gel plate and chloroform containing 6% methanol as the mobile phase, the R\(_f\) values were 0.62 and 0.39 for 10 and 9, respectively. This is the reverse of what would be expected, based on the relative polarities of the two compounds. The reversal

in $R_f$ could be explained if $10$ formed an internal hydrogen bond between the secondary amine and the azide (shown below in Figure 4); whereas such a possibility is not present in $9$.

The apparent internal hydrogen bond formation in Figure 4 suggests that fentanyl type compounds may be able to assume a folded conformation in which the ends of the molecule are brought into close proximity. This agrees with the idea presented elsewhere (2) that fentanyl compounds assume a morphine-like conformation in order to exert their morphinomimetic activity.

With the successful preparation of $10$, it was then necessary to synthesize the non-radiolabeled $N$-succinimidyl propionate, $12$, and to develop the reaction conditions for coupling $12$ and $10$ to give the non-radiolabeled analog, $9$, of the desired radiolabeled product, $1$. Compound $12$ was prepared from $N$-succinimide and propionic acid, under the catalytic influence of dicyclohexylcarbodiimide. The recrystallized, pure material (m.p. 33-34°C) had the required IR and NMR characteristics. Several test experiments were performed to establish the conditions for the maximum conversion of $10$ to $9$. The use of compounds $11$ and $12$ is described in the literature for the acylation of proteins in aqueous solution; but no information is available as to acylation conditions with this reagent in organic solvents. Toluene was used as the solvent since the radiolabeled material, $11$, is available commercially only in toluene. The quantitative conversion (monitored by TLC) of $10$ to $9$ could be achieved with an equimolar solution of $10$ and $12$ in toluene with the mixture maintained at 90°C for 3 hr. The reaction went poorly at room temperature, possibly because of the internal hydrogen bonding (see Figure 4). The azide group was surprisingly stable during the 3 hr reaction at 90°C, as determined by IR measurements of the reactants and products and a control experiment with $10$ maintained at 90°C for 3 hr.

The final reaction of $10$ with $11$ to form the radiolabeled product, $1$, was carried out in sealed glass tubes at 90°C for 3 hr. Two runs were made, each with 5 mCi (in 5 ml) of $11$ at 107 Ci/mmol specific activity. The total quantities used for the reactions were $10$ mCi ($0.094$ u moles; MW 171) of $11$ and $0.097$ u moles of $10$. At 106.
yield, there would be formed 0.094 mmol or 35.4 μg of 1 (MW 377) at an assumed specific activity of 107 mCi/mmol.

After the reaction of 10 and 11, the solution from the two runs were combined and layered onto preparative silica gel plates. The plates were developed with methylene chloride containing 5% methanol. After development and radiochromatogram scanning, the silica gel bands containing the product were scraped off and pooled. Some of the labelled material could be extracted from the silica with methylene chloride containing 5% methanol; however, the isolation of the radiolabelled material from the silica gel proved to be difficult. Even after repeated extraction and centrifugation to remove the silica gel, complete isolation of radioactive material from the silica gel was not possible. The isolated labelled product amounted to a total of 1.365 mCi. Assuming a specific activity of 107 mCi/mmol, this amounted to 4.81 μg (13.6% yield) of 1. The first several extractions contained 0.975 mCi (3.44 μg) of 1 and was the material forwarded to the CRDEC.

The low level of recovery of 1 might have been due 1) to transesterification of the silica gel surface by 1 during development of the chromatogram or 2) to the poor ability of the extraction procedure to dislodge 1 from the silica gel. Even after repeated extraction, the residual silica gel contained appreciable radioactive material. Discussion with Amersham personnel led to the conclusion that preparative HPLC would be a more desirable method for such purification.

Test runs showed that 10 and 9 could be separated by HPLC using a C-18 column with 50% aqueous acetonitrile as the mobile phase. Therefore, it was planned to use HPLC for the final purification of 1 in the future. However, the project was terminated at this point because the funds were all expended.

The following materials were forwarded to the U.S. Army Chemical Research, Development and Engineering Center during the course of this task 02:

a. 1 g of 9 (amino derivative)
b. 0.45 g of 9 (non-radiolabeled form of 1)
c. 3.4 μg (0.37 mCi) of 1

The results of the synthesis and opiate testing are being written up in one or more manuscripts for publication in scientific journals.
4. SPECTROSCOPIC CHARACTERIZATION OF STRUCTURES OF INTERMEDIATES

4.1 NMR SPECTRA

The NMR spectra of several intermediates were obtained in order to complete the structural assignment. Several of these compounds had been reported earlier in the literature (1,2). However, no NMR spectra were presented in these references.

The NMR spectrum of 1,1,4(4-nitrophenyl)-1,2-4-piperidone (figure 3) presented simple features which are attributable to first order analyses. The aromatic ortho protons appeared at 8.072, showing the effect of the nitro function. The remaining two aromatic protons appeared at 7.34. The benzylic protons appeared as a sharp singlet at 3.94. Due to the symmetrical nature of the piperidine ring, the three protons of the piperidine ring protons are simplified. The ortho protons appeared as a doublet at 7.78 with large coupling constants (terminal coupling), each further split into a triplet due to coupling to adjacent methylene protons. Similarly, the meta protons appeared as triplets of doublets at 2.46.

The spectrum of compound 3 (figure 6) further attests to its structure. The ortho aromatic protons (with respect to nitro functions) appeared at 9.20 (2H); and the remaining 2 aromatic protons on the aromatic ring containing the nitro function are seen at 8.45. The introduction of the amine function is shown by the appearance of ortho proton at 6.05 (2H), meta proton at 7.18 (2H), and the para proton at 6.75 (2H). The chemical shifts are consistent with the values calculated according to Shonley's "electronic" relationship. The benzylic proton appeared as a sharp singlet at 3.95. However, the introduction of the amine function to the piperidine ring makes the ring asymmetrical; and hence the piperidine ring protons present a complex coupled relationship.

Compound 4 (figure 7) continues to exhibit the same features for the aromatic proton with a 2 proton signal at 9.18 and another 2 proton signal at 8.40 for the aromatic ring containing the nitro function. The amine ring presents the ortho protons at 6.05 (2H), meta protons at 7.18 (2H), and the para proton at 6.75. The benzylic proton appeared at 3.95 and the adjacent methylene, attached to the piperidine ring nitrogen, appeared at 7.63.

The propionylated derivative compound 5 (figure 8) presents similar features as compound 4 with the addition of a propionyl group. The propionyl methyl appeared as a triplet at 1.62 (3H) and the nitrogen as a quartet at 1.95 (3H).

The reduction of the nitro function to yield compound 8 is evidenced in its spectrum (figure 9). The amine proton function signal at 7.63. However, the triplet and quartet for the propionyl methylene signal appeared at 1.99 (3H) and 1.85 (3H).

The conversion of aromatic amine function in compound 8 into a piperazine function in compound 9 (figure 13) brought about changes in the
ortho and meta aromatic proton signals. The presence of the triplet and quartet at -1.02 and -1.95 as indicative of the propionyl function still remaining intact during the conversion of the aromatic amine into the azide function.

Compound 10 (Figure 11) presents similar features to compound 9. The aromatic signals are quite comparable. However, the absence of familiar triplet and quartet signals for the propionyl group is indicative of its removal. These signals reappear (Figure 12) when 10 is converted into 9 by treatment with II. Incidentally, the non-radiolabeled form of II (i.e. 12) made during this study had the following signals (Figure 13): -1.74 (3H, triplet), -2.66 (4H, quartet), and -2.88 (4H) in agreement with the structure of compound 12.

Complete assignment of the chemical shifts for the piperidine ring protons would be possible only by selective deuteration experiments and by temperature dependent spectral measurements. These special experiments were not done because the need did not appear to justify the effort and expense.

4.2 FTIR Spectra and Ultra Violet Spectra Characterization of the Intermediates

The infrared spectra were taken as KBr pellets on a Digilab FTS 15-80 FTIR Spectrometer. The spectral frequencies provide convenient evidence to document the changes in the functional groups brought out by the synthetic transformations. For instance, the presence as well as the removal of the propionyl functionality crucial to the synthetic scheme can be followed by the appearance and absence of the tertiary amide function around 1650 cm⁻¹. Similarly the success of the reduction of the aromatic nitro function to yield compounds 8 and 14 can be followed by the disappearance of the typical aromatic nitro group absorptions around 1530 and 1350 cm⁻¹ and by the appearance of primary amino N-H stretching frequencies in the region 3500-3300 cm⁻¹. The azide functionality in the intermediates 9 and 10 is characterized by the appearance of a characteristic strong band around 2100 cm⁻¹. The relevant infrared frequencies for the individual intermediates are given in the experimental section.

It is interesting to note that in the tert-butyl piperidyl derivative and b, the tertiary amide absorption is shifted to 1690 cm⁻¹ from the normal value of 1650 cm⁻¹. This clearly shows the influence of the tert-butyl group on the amide carbonyl absorption. The lone pair of electrons of the nitrogen atom are drawn towards the carbon containing the tert-butyl group. The contribution of the positive form of the amide is reduced, shifting the frequency toward higher wave numbers.

The UV spectral data are of particular interest in following the synthetic transformations of the amine. Generally one can follow the changes in the functional groups of the aromatic ring by intermediate intermediate and starting materials. Generally, a absorption band at 285 nm is observed [21], all of which is to be taken into consideration in the interpretation of the spectral data.
into an amino group shifts the prominent $\pi^-\pi^*$ absorption to 234 nm. Similarly the azide intermediate 9 still has absorption at 232 nm. This shows that the electronic demands of the $N_3$ as well as that of the primary amino function are similar in this series of compounds. Compound 7 shows a peculiar behavior. The $\pi^-\pi^*$ and $n^-\pi^*$ absorptions are combined into a single band at 272 nm. One explanation is that the propionyl group caused conformational changes that brought the two aromatic rings closer together, thus producing the spectral shifts.
NMR spectrum of 1-[(4-nitrophenyl)acetamido]-4-phenylpiperidine (Compound 3)
Figure 8. NMR spectrum of N-[1-2-(4-nitrophenyl)ethyl]-4-piperidinyl]-N-phenyl-propanamide; (compound 7)
Figure 9: NMR spectrum of N-{1-[2-(4-aminophenyl)ethyl]-4-piperidine}-N-phenvl-propanamide (compound A)
Figure 10. NMR spectrum of N-[1-[2-(4-azidophenyl)ethyl]-4-piperidinyl]-N-phenyl-propanamide (compound 9).
Figure 11. NMR spectrum of 1-[2-(4-azidophenyl)ethyl]-4-(phenylamino)-piperidine; (compound 1).
Figure 12. NMR spectrum of 9 prepared by propionylation of 10 with 12.
5. EXPERIMENTAL DETAIL

5.1 (4-Nitrophenyl)acetyl chloride

4-Nitrophenyl acetic acid (54 g, 0.3 moles) was dissolved in 500 ml of dry benzene and treated with 49 g (0.4 moles) of thionyl chloride. The solution was refluxed for 1 hour with exclusion of moisture. The benzene solvent was removed in a rotary evaporator. Excess unreacted thionyl chloride was removed by azeotropic distillation with dry benzene. The dry residue was crystallized from benzene/cyclohexane to give 28.1 g of the acid chloride, m.p. 45-46°C.

5.2 1-[(4-Nitrophenyl)acetyl]-4-piperidone

(4-Nitrophenyl)acetyl chloride (2.5 g, 12.5 mmoles) was introduced into a mixture of methylene chloride (30 mL) and a solution of sodium bicarbonate (3.2 g) in water (30 mL). The reaction mixture was kept stirring at room temperature; and powdered 4-piperidone monohydrate hydrochloride (1.92 g, 12.5 mmoles) was added. After 1 hour, the methylene chloride layer was separated. The aqueous phase was extracted with 2 x 30 mL methylene chloride. The combined methylene chloride layers were washed with 2 N HCl (10 ml), dried, and evaporated to furnish crude product. Purification was achieved by column chromatography to yield pure crystalline material 2.32 g (75%) m.p. 103-104°C. IR (KBr): 1627 (tertiary amide), 1560 and 1350 cm⁻¹ (NO₂ group). NMR: 3.78 (t, 4H), 2.46 (t, 4H), 3.94 (s, 2H). 7.46 (m, 2H), 8.22 (m, 2H).

5.3 1-[(4-Nitrophenyl)acetyl]-4-(phenylaniline)-piperidine

Crude 1-[(4-nitrophenyl)acetyl]-4-piperidone prepared from 25 g of p-nitrophenyl acetyl chloride and 19.2 g of 4-piperidone hydrate hydrochloride (each 124 mmoles) was dissolved in 450 ml of dry toluene. Aniline (11.1 ml, 122 mmoles) and p-toluene sulfonic acid (50 mg) was added and the mixture was heated under reflux under N₂ atmosphere for 5 1/2 hours, with a Dean-Stark water separator. The toluene solution was decanted from some tarry material and evaporated to dryness in a rotary evaporator. The crude material was dissolved in 200 ml of methanol and cooled in ice. Sodium borohydride (7.07 g, 186 mmoles) was added in portions: and the mixture stirred for 1 hour. 200 ml of water was added; and the organic product was extracted with 4 x 100 ml methylene chloride. After drying, filtering, and evaporation, 36.1 g of crude product was obtained. Recrystallization from ether/methanol at -70°C was quite unsatisfactory. The material was layered on silica gel and sequentially eluted with 200 ml portions of petroleum ether, petroleum ether/ethyl acetate (9:1), and petroleum ether/ethyl acetate (4:3). The last two fractions yielded pure product, which was recrystallized from ethyl acetate. Yellow crystals, 9.2 g (26%): m.p. 160,161°C. IR (KBr): 3360 (NH), 1640 (amide), 1519 and 1346 (NO₂) cm⁻¹. NMR: 3.85 (benzyl, 5H), 6.52 (m, 2H), 6.75 (m, 1H), 7.46 (m, 2H) and 8.20 (m, 2H). The piperidine ring protons appeared as complex signals of 1.3 3.7.
5.4 \((4\text{Nitrophenyl})\text{ethyll} \cdot 4\text{piperidinyl}\) N-phenyl propanamide.

A solution of 3 (8.0 g, 20 mmoles) of \(4\text{Nitrophenyl} \cdot 4\text{piperidinyl}\) N-phenyl propanamide was kept stirring under nitrogen atmosphere. To this solution 3 M diborane in tetrahydrofuran was slowly added and the solution was heated under reflux with stirring for 2 hours. After cooling, methanol (20 mL) was slowly added, and the reaction mixture was kept stirring for 1/2 hour. The solvent was removed at room pressure, treated with water (80 mL) and 2 N HCl (95 mL). The solution was heated under reflux for 1 hour. The reaction mixture was alkaline with 2 N NaOH; and the organic material was extracted with methylene chloride. The methylene chloride layer was washed with brine, dried, and evaporated to give crude product. This was purified by chromatography over silica gel; elution was with methylene chloride containing 3% methanol. 6.5 g of yellow crystalline material was obtained which was recrystallized from ethyl acetate/cyclohexane to give 4.52 g of pure product (54%) m.p. 90-92°C. IR (KBr): 3406 (NH), 1508 and 1339 (NO) cm\(^{-1}\), note absence of amide carbonyl. NMR: 2.68 (m, 2H), 2.98 (m, 2H), 6.62 (m, 2H), 6.70 (m, 2H), 7.18 (m, 2H), 7.40 (m, 2H), 8.18 (m, 2H).

5.5 \((2\text{-(4\text{Nitrophenyl})ethyl} \cdot 4\text{piperidinyl})\) N-phenyl propanamide.

Compound 4 (4.0 g, 12.3 mmole) was taken in 30 mL dry toluene. Propionic anhydride (3.2 g, 24.6 mmole) was added, and the solution was heated under reflux under nitrogen atmosphere for 3 hours. Aqueous ammonia solution (2 mL, 30%) was added and the reaction mixture stirred for 10 minutes. Saturated sodium bicarbonate solution (100 mL) was added; and the aqueous organic matter was extracted with 4 x 50 mL methylene chloride. Usual work up gave crude material (5.2 g), which was recrystallized from ethyl acetate/petroleum ether. Pale yellow crystals (4.1 g, 87%). IR (KBr): 1651 (amide), 1516 and 1342 (NO) cm\(^{-1}\). NMR: 1.02 (t, 3H), 1.95 (q, 2H), 6.95 (m, 2H), 7.08 (m, 2H), 7.23 (m, 2H), 7.45 (m, 3H).

5.6 \((2\text{-(4\text{Aminophenyl})ethyl} \cdot 4\text{piperidinyl})\) N-phenyl propanamide.

Compound 7 (3.80 g, 9.92 mmole) was dissolved in 150 mL absolute ethanol. Pd on carbon (5%, 300 mg) was added, and the solution was hydrogenated at 45 psi over hydrogen in a 50 mL reactor. The solution was filtered through Celite and evaporated under vacuum. The crude material was recrystallized from ethyl acetate/petroleum ether 1.5 g of white crystalline product (91%) m.p. 150-152°C was obtained. IR (KBr): 3480 and 3360 (p. ar. NH), 1647 cm\(^{-1}\) (amide), 1.02 (t, 3H), 1.95 (q, 2H), 7.05 (m, 2H), 7.65 (m, 2H), 7.93 (m, 2H), 8.19 (m, 3H).

5.7 \((2\text{-(4\text{Aminoethyl})ethyl} \cdot 4\text{piperidinyl})\) N-phenyl propanamide.

Compound 8 (2.1 g, 8.08 mmole) was dissolved in 50 mL absolute ethanol (50 mL) in water (25 mL) was added. Palmitic acid (0.5 g) was added to this solution of arg (40 mL) was added. After being stirred at room temperature for 1 hour, the reaction mixture was filtered through Celite. The filtrate was evaporated to give a crude product 2.2 g. After purification by chromatography on silica gel, 1.8 g of pure product (85%) was obtained. IR (KBr): 3420 (NH), 1516 and 1342 (NO) cm\(^{-1}\). NMR: 1.70 (m, 1H), 2.60 (m, 1H), 3.99 (m, 1H), 7.03 (m, 2H), 8.30 (m, 3H).
solution of sodium azide (0.66 g, 10 mmole) in water (3 ml). Considerable frothing and gas evolution was noticed, and a white precipitate appeared. The organic matter was extracted with methylene chloride; the methylene chloride was washed with water, dried, and evaporated with a stream of nitrogen. The crude material (1.97 g) was recrystallized from ethyl acetate/ethanol. Colorless plates (1.10 g, 67% yield) were obtained. IR (KBr): 2110 (N=N), 1640 cm⁻¹ (amide). \textbf{NMR:} \textbf{1H:} 1.01 (t, 3H), 1.98 (q, 2H), 6.96 (d, 2H), 7.03 (d, 2H), 7.22 (t, 2H), 7.42 (m, 3H). \textbf{MS:} 377 (M⁺).

5.8 Attempted Depropionylation of Compound 9

Several reaction conditions were tried in order to remove the propionyl function of 9. The desired hydrolysis could not be effected by basic conditions or by acidic conditions at ambient temperatures. When 2 N HCl was used at 100° for 1 hour, the major product isolated by preparative TLC appeared to be an internal nitrene insertion product. This product was different from compound 8 and had the following spectral properties. IR (KBr): 1643 (amide), no azide peak, 3364, 3348 (NH) cm⁻¹. \textbf{NMR:} \textbf{1H:} 1.02 (t), 1.94 (q), 6.65 (d), 6.84 (d), 7.09 (m), 7.40 (m) in addition to piperidine ring protons. This material, although quite interesting, was not pursued further as it did not meet the objective of this investigation.

5.9 1-[2(4-Azidophenyl)ethyl]-4-(phenylamino)piperidine, 10

Compound 4 was catalytically hydrogenated in ethanol in the presence of Pd on carbon (5%) at 45 psi to yield 14 in 94% yield. A solution of 14 (300 mg, 1.0 mmole) in 2 ml 2 N HCl was cooled in an ice bath and treated with a solution of 69 mg (1.0 mmole) in 1 ml cold water quickly with stirring. After 5 min., 20 mg of urea in 0.5 ml water was added followed by a solution of sodium azide (110 mg, 1.69 mmole) in 1 ml water. The solution was made basic and extracted with methylene chloride. The organic layer was washed with water, dried and evaporated by a stream of nitrogen. 210 mg of colorless product (65%) was obtained. The product appeared homogenous by TLC. It could be recrystallized from ethyl acetate/ethanol to furnish colorless plates, dec 152°. IR (KBr): 2118 cm⁻¹ (N=N). \textbf{NMR:} In addition to piperidine ring protons and methylenes, the aromatic ring protons appeared at 6.92, 7.18, 7.35. \textbf{MS:} 321 (M⁺).

5.10 N-Succinimidyl Propionate, 12

This was prepared following a published procedure (5). N-Hydroxysuccinimide (5.4 g, 56 mmole), propionic acid (4 ml, 55 mmoles), and succinimide (5.8 g, 56 mmole) were kept stirring with 200 g dry ethyl acetate for 18 hours at ambient temperature. The precipitated diethyl urea was removed by filtration. The filtrate was evaporated to dryness. The residual oil was twice recrystallized from ethyl acetate and petroleum ether. \textbf{Colorless solid:} 5.25 g (55%). IR (KBr): 1726, 1792, 1930 cm⁻¹. \textbf{NMR:} 1.75 (t, 2H), 2.52 (q, 2H), 7.51 (s, 4H).

5.11 Propionylation of 10 (2.1 g)

Compound 10 (10 mg) and N-hydroxysuccinimide (5 mg) was taken in 5 ml dry toluene and kept at room temperature until completion of the reaction. The reaction mixture was filtered and the filtrate was separated from N-hydroxysuccinimide by preparative TLC using chloroform containing 6% methanol. IR, NMR and Rf values were in agreement with the desired propionylation.

5.12 11 (2.14 Arid phenol) and 4-propionyl-10 (2.1 g)

Five ml lots of 11 in 5 ml toluene at 10 mg each, specific activity (0.0094 , moles total) was taken in two pyrex glass tubes. Compound 10 (31.7 g total, 0.097 mol) was divided equally between the tubes. The reaction mixture was concentrated to 1 ml by passage of nitrogen gas. The tubes were sealed and kept at 90° for 3 hours with occasional shaking. The tubes were opened and the material in each tube was layered on two 5 x 20 cm preparative silica gel plates. After radiochromatogram scanning, the band containing the radioactive material, appearing at the required Rf value, were pooled and extracted with methylene chloride containing 3% methanol.

Considerable difficulty was experienced in completely extracting the radiolabeled material. Centrifugation was employed to settle the silica gel particles in methylene chloride after each extraction. The pooled methylene chloride extracted fractions had a total of 1.865 g. Assuming a specific activity of 107 (10 mg), this amounted to 4.82 g (13.6% yield).
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