Fischer indole synthesis in water: simple, efficient preparation of naltrindole, naltriben and analogs†

Romain A. Duval[a,b] and John R. Lever*[a,b,c]

Received 6th October 2009, Accepted 17th November 2009
First published as an Advance Article on the web 7th January 2010
DOI: 10.1039/b920864j

Naltrindole, naltrindole analogs and the benzofuran congener naltriben have been prepared by Fischer syntheses using mildly acidic, purely aqueous conditions. The preparation of naltrindole and several analogs was accomplished under almost neutral conditions using just the hydrochloride salts of naltrexone and various electron-rich and electron-poor phenylhydrazines in boiling water. The products were obtained by simple filtration in good to excellent yields and with high purities in the majority of cases. The route is suited to gram-scale synthesis, does not require the use of organic solvents, minimizes the use of corrosive acids, and is simple, efficient and environmentally friendly. Naltriben was prepared efficiently from the hydrochloride salts of naltrexone and *O*-phenylhydroxylamine but more forcing conditions, 6.0 N HCl, were required. A limitation to the method is the failure of Fischer cyclization between naltrexone and nitro-substituted phenylhydrazines under aqueous conditions.

Introduction

Since their development by the Portoghese group, naltrindole[1] (NTI, **1**) and its benzofuran congener naltriben[2] (NTB, **2**) have become valuable tools for studies of opioid receptor pharmacology (Fig. 1). These potent and selective δ opioid receptor antagonists were designed from the peptide enkephalin using the message-address concept.[1,2] Tritiated NTI is routinely used for δ opioid receptor binding assays *in vitro*,[3] while tritiated NTI analogs and tritiated NTB have been used for *in vivo* studies of cerebral δ opioid receptors in rodents.[4] Moreover, a number of NTI analogs have been labeled with positron- and single photon-emitting radionuclides for non-invasive imaging.[5] In fact, carbon-11 labeled *N*1′-methyl-NTI[6] (Fig. 1) is in use for clinical positron emission tomography (PET) imaging studies of the δ opioid receptors expressed by normal brain[7a] and heart,[7b] as well as the δ sites expressed by the primary tumors of certain breast[8a] and lung cancer patients.[8b] In our laboratories, an area of current interest is the development of radiometal-labeled, macrocyclic conjugates of NTI (Fig. 1) for studies of the peripheral δ opioid receptors expressed by some normal organs and certain cancers.[9] Although small samples of NTI and NTB for pharmacology studies are commercially available, continued advances in the field would be facilitated by simple, scalable and environmentally friendly methods for preparation of gram quantities of NTI, NTB and analogs for synthetic elaborations and pharmacological evaluations.[10]

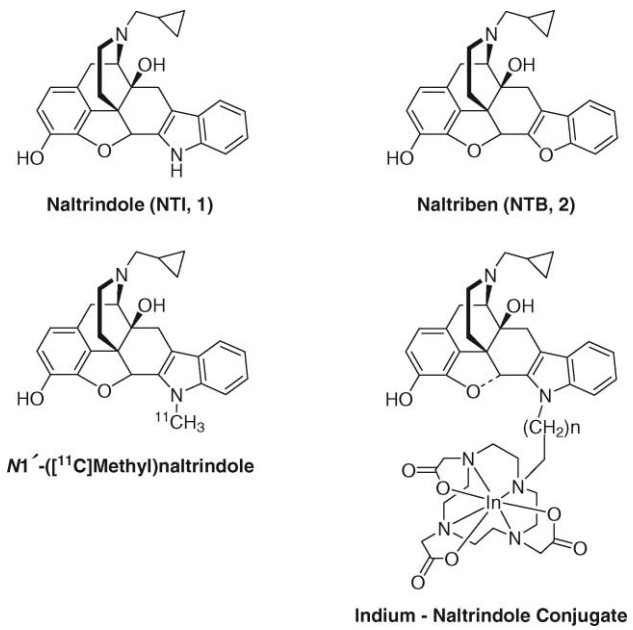


Fig. 1 Naltrindole, naltriben and selected analogs.

NTI and derivatives having a variety of substituents have been obtained in moderate to good yields by Fischer cyclizations between naltrexone (NTX), a ketonic 4,5α-epoxymorphinan, and various phenylhydrazines as exemplified in Scheme 1.[1,4a,10] Similarly, NTB and analogs have been obtained by using NTX and *O*-phenylhydroxylamines.[2,10] These reactions use strongly acidic conditions, such as concentrated HCl or methanesulfonic acid, and take place at elevated temperatures in organic solvents, such as refluxing ethanol or acetic acid. A milder variant using

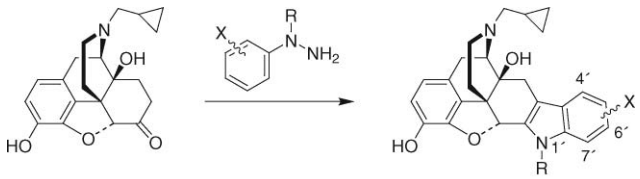
[a]*Department of Radiology and the Radiopharmaceutical Sciences Institute, University of Missouri, Columbia, Missouri, 65212. E-mail: leverj@health.missouri.edu; Fax: (573) 814-6551; Tel: (573) 814-6000 ext. 53686*
[b]*Research Service, Harry S. Truman Veterans Administration Medical Center, Columbia, Missouri, 65201*
[c]*Department of Medical Pharmacology and Physiology, University of Missouri, Columbia, Missouri, 65212*

† Electronic supplementary information (ESI) available: Further experimental details, spectroscopic analyses, and reversed-phase HPLC conditions, retention times and capacity factors. See DOI: 10.1039/b920864j

Table 1 Exploratory screening of conditions for Fischer synthesis of NTI (**1**)

[a]Reactants	[b]Conditions	[c]Product(s)	[c]Conversion (%)
NTX·HCl, Phenylhydrazine.HCl	Amberlyst-15®, toluene	[d]NTX-phenylhydrazone	~100
NTX·HCl, Phenylhydrazine.HCl	Amberlyst-15®, CH_3OH	NTI	~100
NTX·HCl, Phenylhydrazine.HCl	0.01 - 6 N HCl	NTI	~100
NTX·HCl, Phenylhydrazine·HCl	H_2O	NTI	~100
NTX base, Phenylhydrazine·HCl	H_2O	NTI	~ 50
		[d]NTX-phenylhydrazone	~ 50

[a] NTX·HCl (10 mg, 25 μmol) and phenylhydrazine·HCl (1.2 equiv). [b] Reflux, 1.5 h. [c] Product assignments and estimates of conversion are from visual inspection of analytical silica gel TLC of reaction mixtures as compared to standard samples of reactants and products. TLC used cyclohexane/EtOAc (25/75) containing 1% triethylamine: R_f 0.41, NTX; R_f 0.29, NTI; R_f 0.51, NTX-phenylhydrazone. [d] NTX-phenylhydrazone was identified in the crude mixtures by HRMS (ESI): calcd for $C_{26}H_{30}N_3O_3$ $[M+H]^+$ 432.2287; found 432.2271.



1, R = H, X = H
3, R = CH_3, X = H
4, R = H, X = 5′-OCH_3
5, R = H, X = 7′-Cl
6, R = H, X = 7′-F
7, R = CH_2CH_3, X = 5′,7′-diBr
8, R = H, X = 5′-NO_2

Scheme 1 Fischer synthesis of naltrindole and analogs from naltrexone.

just 2 equivalents of *p*-toluenesulfonic acid in ethanol at reflux has been reported for the Fischer synthesis of several NTI analogs.[10c,d] Overall, fairly harsh conditions are typical for the classical Fischer indole synthesis.[11]

Recently, a variety of synthetic modifications have been introduced to increase the practicality, and to lessen the environmental impact, of the Fischer cyclization. This is particularly important from the process chemistry viewpoint.[12] Hazardous materials have been reduced by use of Brønsted or Lewis acids in ionic liquids, followed by filtration or sublimation for product isolation.[13] Mild solid acids, including zeolites[14a] and montmorillonite clays,[14b,c] have been used as catalysts for some Fischer reactions, although organic solvents, such as acetic acid, dihydropyran and aqueous dimethylacetamide, are employed. Zinc chloride has been used successfully in truly catalytic quantities during microwave-assisted Fischer reactions conducted in biodegradable triethyleneglycol.[15a] The reusable catalysts, Amberlyst-15®[15b] and bismuth nitrate,[15c] have also been used to promote Fischer indole synthesis in organic solvents.

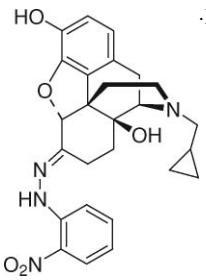
Here we report "green" Fischer syntheses of NTI and several analogs using essentially neutral and purely aqueous media at reflux. Aqueous mineral acids, however, were required for efficient synthesis of NTB. The majority of reactions led to the targets in good to excellent yields, and furnished compounds of high purity after simple filtration or centrifugation. These economical and efficient methods do not require the use of organic solvents, minimize the use of corrosive acids, and provide an environmentally friendly synthetic approach to this important class of opioid receptor ligands.

Results and discussion

We explored several conditions on a small scale for Fischer synthesis of NTI (**1**) from NTX·HCl and phenylhydrazine·HCl using various acids and solvents at reflux for 1.5 h (Table 1, Scheme 1). Initial studies using the strongly acidic resin Amberlyst-15® in refluxing toluene led quantitatively to a non-polar compound that remained stable even during extended reaction times (ca. 10 h). This material was isolated by filtration, and characterized as the phenylhydrazone of NTX by mass spectroscopy. Interestingly, the use of Amberlyst-15® in refluxing methanol for 1.5 h gave quantitative conversion to NTI·HCl. Although Amberlyst-15® has some advantages as a reusable heterogeneous catalyst, the use of water alone and dilute mineral acids proved simpler and just as efficient. In model reactions, treating NTX·HCl with phenylhydrazine·HCl in aqueous HCl ranging from 0.01 to 6.0 N at reflux for 1.5 h led quantitatively to NTI·HCl by TLC. A 90% yield of NTI·HCl, pure by reversed-phase HPLC, was obtained on a 1.3 mmol scale by using 0.1 N HCl followed by filtration. Similar aqueous conditions, 4% H_2SO_4 at reflux, have previously been employed for Fischer synthesis of various pharmacologically active tryptamines,[16a,b] and indole analogs of the antipsychotic butyrophenones.[16c] Next, we investigated the degree of acidity required for NTI synthesis in water. The minimum amount of HCl necessary was found to be just two molar equivalents, corresponding to that provided by the hydrochloride salts of the reactants. Quantitative conversion of NTX·HCl and phenylhydrazine·HCl to NTI·HCl in boiling water was observed by TLC over 1.5 h. By contrast, treating the free base of NTX with phenylhydrazine·HCl under the same conditions for 2.5 h led to a roughly equimolar mixture of NTI and a compound tentatively assigned by mass spectroscopy of the crude mixture as the phenylhydrazone of NTX. Presumably, the phenylhydrazone is formed quantitatively, but one equivalent of HCl is not sufficient to promote isomerization to the ene-hydrazine tautomer necessary for [3,3]-sigmatropic rearrangement to the indole.[11]

The novel synthesis of NTI using just the hydrochloride salts of the reactants in boiling water is particularly mild, pH ~ 5, and proved efficient and convenient on a preparative scale. We obtained 3.6 g (96%) of pure NTI·HCl (8 mmol) after bringing an equimolar mixture of the salts to reflux and stirring for

Table 2 Preparation of NTI, NTI congeners and NTB in aqueous media at reflux

Reactants	Product	Conditions	% Purity	% Yield Aqueous Conditions	% Yield Classical Conditions
NTX·HCl, Phenylhydrazine·HCl	NTI	H_2O, precipitation	99	97	71[a]
	NTI·HCl **1**	H_2O, filtration, 4 °C	99	96	
NTX·HCl, 1-Methyl-1-phenylhydrazine	*N*1′-Me-NTI·HCl **3**	HCl 0.1 N, centrifugation, 4 °C	99	73	58[a]
		HCl 0.1 N, evaporation	98	97	
NTX·HCl, *O*-Phenylhydroxylamine·HCl	NTB·HCl **2**	HCl 6.0 N, filtration, 4 °C	98	93	80[e]
NTX·HCl, *p*-Methoxyphenylhydrazine·HCl	5′-OMe-NTI·HCl **4**	H_2O, centrifugation, 4 °C	95	61	56[a]
NTX·HCl, *o*-Chlorophenylhydrazine·HCl	7′-Cl-NTI·HCl **5**	HCl 0.1 N, filtration, 4 °C	90	78[c]	77[b]
NTX·HCl, *o*-Fluorophenylhydrazine·HCl	7′-F-NTI·HCl **6**	H_2O, centrifugation, 4 °C	71	53[c]	50[a]
NTX·HCl, *N*-Ethyl-2,4-dibromophenylhydrazine·HCl	*N*1′-Et-5′,7′-dibromo-NTI·HCl **7**	H_2O, extraction and chromatography	98	27	13[d]
NTX·HCl, *o*-Nitrophenylhydrazine	 ·HCl **9**	HCl 0.1 N, filtration	96	75	---

[a] Ref. 1. [b] Present work. [c] Molar yield calculated based on HPLC data as if purification to homogeneity were performed. Isolated yields of stated purity reported for other cases. [d] Ref. 4a. [e] Ref. 2.

45 min, followed by cooling and simple filtration of the product that has low solubility in water (Table 2). Alternatively, the free base of NTI could be prepared, with similar results, by careful addition of solid potassium carbonate to precipitate the product from the hot reaction medium. The method can also be applied to *N*-substituted phenylhydrazines as long as sufficient acid is available. Treatment of NTX·HCl with 1-methyl-1-phenylhydrazine in refluxing HCl (0.1 N) for 90 min, followed by chilling and centrifugation at 4 °C, gave *N*1′-methyl-NTI (**3**) in 73% yield with high purity (Table 2, Scheme 1). Alternatively, **3** was obtained in nearly quantitative yield and with high purity after evaporation of the reaction medium (Table 2). In marked contrast, reaction of NTX·HCl with the free base of 1-methyl-1-phenylhydrazine in plain water was not complete even after extended times. Next, we attempted to obtain NTB (**2**, Fig. 1) from NTX·HCl and phenylhydroxylamine·HCl under aqueous conditions. NTB synthesis in water or dilute HCl proved sluggish. However, NTB·HCL was obtained in 90% yield with 98% purity by using boiling 6.0 N HCl followed by cooling and filtration. This procedure proved more convenient than the use of refluxing methanesulfonic acid for NTB synthesis as previously described.[2]

To gain insight into the scope and limitations of such aqueous Fischer syntheses, we treated various aryl-substituted phenylhydrazines with NTX·HCl (Table 2, Scheme 1). Treatment of electron-rich *p*-methoxyphenylhydrazine·HCl with NTX·HCl in boiling water gave 5′-OMe-NTI·HCl (**4**) in 61% isolated yield with 95% purity. The yield proved similar to the 56% reported when boiling acetic acid was used,[1] but without the need for organic solvents or a discrete purification step.

We then examined the reactivity of electron-deficient phenylhydrazines (Table 2). In the halogen series, *o*-chlorophenylhydrazine·HCl reacted with NTX·HCl in boiling water to give 7′-Cl-NTI·HCl (**5**) in 74% yield with 90% purity after filtration. This derivative has not been described previously to our knowledge, so we also used typical Fischer conditions for synthesis. Refluxing NTX·HCl and *o*-chlorophenylhydrazine·HCl in methanol/HCl (g) for 4.5 h, followed by workup and flash chromatography, provided **5** in 54% yield. Thus, synthesis in water and isolation by filtration provides a simpler alternative that gives fairly comparable results. 7′-F-NTI (**6**) was formed as the major product resulting from refluxing NTX·HCl and *o*-fluorophenylhydrazine·HCl in water. However, evaporation followed by HPLC analysis showed only 42% purity and a calculated yield of 49%. Purity was increased to a more acceptable 71% by chilling the mixture and isolating the product by centrifugation in 53% yield. Using methanol/HCl (g) at reflux, **6** was obtained previously in 50% yield after recrystallization.[1]

We then explored Fischer cyclization between NTX·HCl and *N*-ethyl-2,4-dibromophenylhydrazine·HCl. This synthesis was best conducted under dilute conditions, and the poorly reactive dihalophenylhydrazine had to be used in two-fold excess to achieve significant conversion. Nonetheless, *N*1′-ethyl-5′,7′-dibromo-NTI·HCl (**7**) was obtained after chromatography in 27% yield with >98% purity. Although rigorous purification was required and the isolated yield was low, the results still compare

favorably to the 13% yield reported for preparation of **7** under harsher, classical conditions (concentrated HCl/glacial HOAc) for use as the precursor to [^{3}H]-*N*1′-Et-NTI·HCl.[4a]

Fischer synthesis of even simple nitroindoles is difficult, and requires forcing conditions such as polyphosphoric acid or 85% phosphoric acid/toluene at elevated temperatures.[17] Notably, Portoghese and colleagues were able to prepare 5′-NO_2-NTI (**8**) in 55% yield using concentrated HCl/glacial HOAc at reflux.[1] Treatment of NTX·HCl with *p*-nitrophenylhydrazine failed to provide **8** under a variety of aqueous conditions (0.1 - 12 N HCl, reflux). We then tested the reactivity of NTX·HCl with *o*-nitrophenylhydrazine in boiling 0.1 N HCl and observed a clean reaction by TLC and HPLC. However, the product obtained by filtration and drying was not 7′-NO_2-NTI (R = H, X = 7′-NO_2; Scheme 1), but *o*-nitrophenylhydrazone **9** which was obtained in 71% yield with 96% purity (Table 2). Although phenylhydrazones are intermediates in the Fischer cyclization, this compound could not be forced to cyclize after extended reaction times in aqueous acid. Apparently, reactivity is diminished predominantly by electronic rather than steric effects under aqueous conditions since neither *p*- nor *o*-nitro groups permitted cyclization. Thus, nitro-substituted NTI analogs are best prepared under the classical, harsher conditions.

Conclusion

We have described novel Fischer indole syntheses of NTI, indole ring-substituted NTI analogs, and the benzofuran congener NTB under mildly acidic, purely aqueous conditions. Two equivalents of HCl were required to promote the condensation and rearrangement steps of the Fischer reaction, allowing synthesis of NTI and analogs using just the hydrochloride salts of NTX and the various phenylhydrazines in boiling water. In one case where the HCl salt of the phenylhydrazine was not available, the reaction took place efficiently in dilute HCl (0.1 N). Notably, these mild conditions sufficed for both electron-rich and electron-poor phenylhydrazines, but failed to furnish the corresponding indoles when the phenylhydrazine was substituted with strongly electron-withdrawing nitro groups. With the exception of poorly reactive *N*-ethyl-2,4-dibromophenylhydrazine·HCl, the phenylhydrazines were used in only 2 - 10% molar excess relative to NTX·HCl. Thus, convergent synthesis of more elaborate NTI analogs from NTX using only a slight excess of complex phenylhydrazines from custom synthesis seems feasible. The aqueous Fischer reaction between NTX·HCl and phenylhydroxylamine·HCl to produce NTB·HCl was best accomplished using boiling 6.0 N HCl. The synthesis of NTB analogs from substituted phenylhydroxylamines may also be possible, but was not explored in our study. The Fischer products were easily obtained by filtration or centrifugation due to their insolubility in water with respect to the reactants and byproducts (e.g., NH_4Cl). In the majority of cases, product yields and purities were good to excellent. These mild aqueous conditions provide a simple, efficient, scalable and environmentally friendly alternative to the harsher Fischer conditions typically used for the preparation of NTI, NTB and analogs. Moreover, the use of only the acid salts of Fischer reaction components in water may have a degree of general applicability, and could circumvent problems encountered under the standard conditions when the product proves acid-sensitive.[18]

Experimental

General information

Reagents and solvents were the best grades available, and were used as received. Naltrexone·HCl was obtained from Mallinckrodt, Inc. Naltrindole hydrochloride and naltriben methanesulfonate were purchased as reference standards from Sigma-Aldrich, Inc. Known, but commercially unavailable, naltrindole derivatives were synthesized for reference as described previously.[1,4a] TLC was done on Macherey-Nagel silica gel 60 UV254 (250 μm) plates. Flash chromatography was conducted with EM Science 9385 silica gel 60 (230 - 400 mesh) under N2 pressure. Reversed-phase column chromatography was performed under N2 pressure using C18 (35-75 μm) from Analtech. High resolution mass spectroscopy (HRMS) using electrospray (ESI) mode was done at the University of Minnesota. ^{1}H NMR spectra were obtained on Bruker spectrometers at 300 MHz. Chemical shifts are parts per million (δ) relative to residual solvent ($CHCl_3$, 7.24 ppm), or to TMS (0.00 ppm) as an internal standard. Elemental analyses were performed by Atlantic Microlab, Inc.

17-(Cyclopropylmethyl)-6,7-didehydro-4,5α-epoxy-3,14-dihydroxy-indolo[6,7:2′,3′] morphinan (naltrindole, NTI, 1). Procedure A (NTI free base). NTX·HCl (3.00 g, 7.94 mmol) and phenylhydrazine·HCl (1.18 g, 8.08 mmol, 1.02 equiv) were dissolved in water (60 mL) with stirring at ambient temperature. After approximately 5 min a heavy precipitate, presumably the phenylhydrazone, was formed. The mixture was then brought to boiling and a clear yellow solution was obtained. After reflux for 45 min, solid K_2CO_3 (2.00 g, 14.5 mmol) was added cautiously and in small portions. The mixture was then cooled to ambient temperature, and a yellow paste was obtained by filtration. Trituration with water followed by drying *in vacuo* at 45 °C gave NTI·0.7 H_2O (3.32 g, 98%) as a beige powder of 99% purity by reversed-phase HPLC. Characteristic ^{1}H NMR signals[1] ($CDCl_3$, 300 MHz): δ 8.15 (s, 1H), 7.39 (d, J = 7.8 Hz, 1H), 7.25 (d, J = 7.8 Hz, 1H), 7.12 (dd, J = 7.2 Hz, J = 7.8 Hz, 1H), 7.0 (dd, J = 7.2 Hz, J = 7.8 Hz, 1H). HRMS (ESI) calcd for $C_{26}H_{27}N_2O_3$ $[M+H]^+$ 415.2022; found: 415.2002. Anal. Calcd for $C_{26}H_{26}N_2O_3 \cdot 0.7\ H_2O$: C, 73.11; H, 6.47; N, 6.56. Found: C, 73.15; H, 6.19; N, 6.63.

Procedure B (NTI·HCl). As described above, NTX·HCl (3.00 g, 7.94 mmol) and phenylhydrazine·HCl (1.18 g, 8.08 mmol) in water (60 mL) were brought to reflux for 45 min. The mixture was cooled to ambient temperature, and then chilled at 4 °C for 1 h. The resulting pale yellow solid was filtered, triturated with ice-cold water, and then dried *in vacuo* at 45 °C to give NTI·HCl·1.8 H_2O (3.57 g, 93%) as a pale beige powder of > 99% purity by reversed-phase HPLC. Anal. Calcd for $C_{26}H_{27}ClN_2O_3$ + 1.8 H_2O: C, 64.60; H, 6.38; N, 5.80. Found: C, 64.58; H, 6.26; N, 6.17.

17-(Cyclopropylmethyl)-6,7-dehydro-4,5α-epoxy-3,14-dihydroxy-1′-methyl-indolo [6,7:2′,3′] morphinan hydrochloride (*N*1′-methylNTI·HCl, 3). Procedure A (isolation by centrifugation).

A mixture of NTX·HCl (0.15 g, 0.40 mmol) and 1-methyl-1-phenylhydrazine (50 μL, 0.052 g, 0.42 mmol) was dissolved in 0.1 N HCl (3 mL) using a 15 mL plastic centrifuge tube as the reaction vessel. After heating in a boiling water bath for 1.5 h, the mixture was cooled to ambient temperature and then chilled at 4 °C for 30 min. After centrifugation (10,000 rpm, 4 °C; 5 min), the supernatant was discarded and the precipitate dried *in vacuo* at 45 °C to furnish *N*1′-Me-NTI·HCl (0.14 g, 73%) as a beige powder of 99% purity by reversed-phase HPLC. Characteristic ^{1}H NMR signals[1] for the free base ($CDCl_3$, 300 MHz): δ 7.40 (d, $J = 7.8$ Hz, 1H), 7.23 (d, $J = 8.2$ Hz, 1H), 7.17 (ddd, $J = 1.0$ Hz, $J = 6.9$ Hz, $J = 8.2$ Hz, 1H), 7.0 (ddd, $J = 1.0$ Hz, $J = 6.9$ Hz, $J = 7.8$ Hz, 1H), 3.80 (s, 3H). HRMS (ESI) calcd for $C_{27}H_{29}N_2O_3$ $[M+H]^+$ 429.2173; found: 429.2162. **Procedure B (isolation by evaporation)**. As described above, NTX·HCl (0.15 g, 0.40 mmol) and 1-methyl-1-phenylhydrazine (50 μL, 0.052 g, 0.42 mmol) in 0.1 N HCl (3 mL) were heated in a boiling water bath for 1.5 h, and then cooled to ambient temperature. The entire reaction mixture was evaporated to dryness under reduced pressure, and then dried *in vacuo* at 45 °C to furnish *N*1′-Me-NTI·HCl (0.20 g, 97%) as a beige powder of 98% purity by reversed-phase HPLC.

17-(Cyclopropylmethyl)-6,7-didehydro-4,5α-epoxy-3,14-dihydroxy-benzo[*b*]furano [6,7:2′,3′] morphinan hydrochloride (naltriben hydrochloride, NTB·HCl, 2). A mixture of NTX·HCl (1.13 g, 3.00 mmol) and *O*-phenylhydroxylamine·HCl (0.46 g, 3.16 mmol, 1.05 equiv) in 6.0 N HCl (30 mL) was refluxed for 4 h. The reaction was cooled to ambient temperature, and then refrigerated at 4 °C overnight. The precipitate was collected by filtration, and washed with ice-cold 6.0 N HCl until the filtrate was colorless. The solid was dried *in vacuo* at 45 °C to furnish NTB·HCl (1.26 g, 93%) as a pale brown powder of 98% purity by reversed-phase HPLC. Characteristic ^{1}H NMR signals[2] for the free base ($CDCl_3$, 300 MHz): δ 7.42 (d, $J = 8.1$ Hz, 1H), 7.37 (d, $J = 7.4$ Hz, 1H), 7.25 (ddd, $J = 1.3$ Hz, $J = 7.2$ Hz, $J = 8.1$ Hz, 1H), 7.15 (ddd, $J = 0.9$ Hz, $J = 7.2$ Hz, $J = 7.4$ Hz, 1H). HRMS (ESI) calcd for $C_{26}H_{26}NO_4$ $[M+H]^+$ 416.1862; found: 416.1869.

17-(Cyclopropylmethyl)-6,7-didehydro-4,5α-epoxy-3,14-dihydroxy-7′-chloro-indolo[6,7:2′,3′] morphinan (7′-chloronaltrindole, 7′-Cl-NTI, 5). Procedure A (classical Fischer conditions, free base). A mixture of NTX·HCl (0.75 g, 2.0 mmol) and 2-chlorophenylhydrazine·HCl (0.54 g, 3.0 mmol) was dissolved in MeOH (20 mL) that had been treated with HCl (g) to 10% by weight. After reflux under nitrogen for 4.5 h, the reaction mixture was cooled, and solvent removed under reduced pressure. The residue was dissolved in MeOH, and then partitioned between EtOAc and saturated Na_2CO_3. The combined organic extracts were dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography using a gradient of hexane/EtOAc (70/30) containing 2 - 10% MeOH and 2% triethylamine to give 0.72 g (77%) of 7′-Cl-NTI monohydrate as a yellow powder (mp 240 °C, dec). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.52 (s, 1H), 7.27 (d, $J = 9.0$ Hz, 1H), 7.09 (d, $J = 7.5$ Hz, 1H), 6.91 (t, $J = 7.7$ Hz, 1 H), 6.62 (d, $J = 8.1$ Hz, 1H), 6.54 (d, $J = 8.1$ Hz, 1H), 5.67 (s, 1H), 5.23 (br. s, 1H), 3.37 (d, $J = 6.1$ Hz), 3.13 (d, $J = 18.5$ Hz, 1H), 2.86 (d, $J = 15.5$ Hz, 1H), 2.81 - 2.73 (m, 2H), 2.63 (d, $J = 15.5$ Hz, 1H), 2.49 - 2.25 (m, 4H), 1.77 (d, $J = 11.8$ Hz, 1H) 1.26 (br s, 1 H), 0.89 (m, 1H), 0.57 (d, $J = 7.3$ Hz, 2H), 0.17 (d, $J = 4.6$ Hz, 2H). Anal. Calcd for $C_{26}H_{25}ClN_2O_3 \cdot H_2O$: C, 66.88; H, 5.82; N, 6.00. Found: C, 67.29; H, 5.54; N, 5.93. **Procedure B (aqueous Fischer conditions, HCl salt):** A mixture of NTX·HCl (0.15 g, 0.40 mmol) and 2-chlorophenylhydrazine·HCl (0.080 g, 0.44 mmol) was dissolved in water (3.0 mL) using a 15 mL plastic centrifuge tube as the reaction vessel. After heating in a boiling water bath for 1.5 h, the mixture was cooled to ambient temperature and then chilled at 4 °C for 30 min to give a brown precipitate. After centrifugation (10,000 rpm, 4 °C; 5 min), the supernatant was discarded and the precipitate suspended in ice-cold 0.1 N HCl (2 mL), vortexed thoroughly, and then centrifuged again. This process was repeated three times, and the solid was then dried *in vacuo* at 45 °C to furnish 7′-Cl-NTI·HCl·0.4 H_2O (0.16 g) as a brown powder of 90% purity by reversed phase HPLC in an effective 78% molar yield when adjusted for purity. ^{1}H NMR data obtained for the free base as reported in Procedure A above. HRMS (ESI) calcd for $C_{26}H_{26}ClN_2O_3$ $[M+H]^+$ 449.1626; found: 449.1618. Anal. Calcd for $C_{26}H_{25}ClN_2O_3 \cdot 0.4\ H_2O$: C, 68.46; H, 5.70; N, 6.14, Cl, 7.77. Found: C, 68.43; H, 6.25; N, 6.46, Cl, 7.92.

17-(Cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxymorphinan-6-one, (*E*)-2-nitrophenylhydrazone hydrochloride (naltrexone *o*-nitrophenylhydrazone·HCl, 9). A mixture of NTX·HCl (0.15 g, 0.40 mmol) and *o*-nitrophenylhydrazine (0.065 g, 0.41 mmol, 1.03 equiv) in 0.1 N HCl (3 mL) was refluxed for 1.5 h, and then cooled to ambient temperature. The bright orange paste was collected by filtration, and washed with 0.1 N HCl until the filtrate was pale yellow. The solid was dried *in vacuo* at 45 °C to furnish the monohydrate of the title compound (0.16 g, 75%) as a yellow powder of 96% purity by reversed-phase HPLC. ^{1}H NMR (free base, $CDCl_3$, 300 MHz): δ 12.74 (s, 1H), 8.11 (dd, $J = 1.2$ Hz, $J = 7.6$ Hz, 1H), 7.77 (dd, $J = 1.2$ Hz, $J = 8.5$ Hz, 1H), 7.47 (ddd, $J = 1.2$ Hz, $J = 7.0$ Hz, $J = 7.6$ Hz, 1H), 6.77 (ddd, $J = 1.2$ Hz, $J = 7.0$ Hz, $J = 8.5$ Hz, 1H), 6.69 (d, $J = 8.1$ Hz, 1H), 6.59 (d, $J = 8.1$ Hz, 1H), 5.49 (bs, 1H), 5.36 (s, 1H), 5.13 (bs, 1H), 3.15 (d, $J = 6.1$ Hz, 1H), 3.03 (d, $J = 18.5$ Hz, 1H), 2.94 (m, 1H), 2.70 (dd, $J = 4.3$ Hz, $J = 11.9$ Hz, 1H), 2.58 (dd, $J = 6.1$ Hz, $J = 18.5$ Hz, 1H), 2.46 (m, 1H), 2.39 (d, $J = 6.6$ Hz, 2H), 2.33 (dd, $J = 4.9$ Hz, $J = 11.9$ Hz, 1H), 2.16 (m, 1H), 1.71 (m, 1H), 1.56 (m, 2H), 0.85 (m, 1H), 0.54 (m, 2H), 0.13 (m, 2H). HRMS (ESI) calcd for $C_{26}H_{29}N_4O_5$ $[M+H]^+$ 477.2138; found: 477.2154. Anal. Calcd for $C_{26}H_{28}ClN_4O_5 \cdot H_2O$: C, 58.92; H, 5.71; N, 10.57. Found: C, 58.78; H, 5.68; N, 10.51.

Acknowledgements

We thank Dr. Yong-de Lu for preparation of 7′-Cl-NTI using classical Fischer conditions. We thank the National Cancer Institute (P50 CA 103130: Center for Single Photon-Emitting Cancer Imaging Agents) for support of this research, and for a postdoctoral fellowship (RAD) through the Career Development Core. We also acknowledge resources provided by Harry S. Truman Memorial Veterans' Hospital, the University of Missouri Life Sciences Mission Enhancement Program, and NSF CHE-95-31247 and NIH 1S10RR11962-01 awards for NMR instrumentation.

References

1 P. S. Portoghese, M. Sultana and A. E. Takemori, *J. Med. Chem.*, 1990, **33**, 1714–1720.

2 P. S. Portoghese, H. Nagase, K. E. Maloney-Huss, C.-E. Lin and A. E. Takemori, *J. Med. Chem.*, 1991, **34**, 1715–1720.

3 (*a*) C. R. Dorn, C. S. Markos, M. S. Dappen and B. S. Pitzele, *J. Labelled Compd. Radiopharm.*, 1992, **31**, 375–380; (*b*) M. S. Yamamura, R. Horvath, G. Tóth, F. Ötvös, E. Malatynska, R. J. Knapp, F. Porreca and V. J. Hruby, *Life Sci.*, 1992, **50**, PL119–124; (*c*) G. Tóth, F. Ötvös and S. Hosztafi, *Helv. Chim. Acta*, 1993, **76**, 2274–2278; (*d*) P. C. Contreras, L. Tam, E. Drower and M. F. Rafferty, *Brain Res.*, 1993, **604**, 160–164.

4 (*a*) J. R. Lever and S. M. Johnson, *J. Labelled Compd. Radiopharm.*, 1997, **39**, 115–122; (*b*) J. R. Lever and U. Scheffel, *Eur. J. Pharmacol.*, 1998, **350**, 335–344; (*c*) J. R. Lever, S. L. McCallister, S. A. Chapman, P. A. Rauseo, R. L. Allmon, W. B. Mathews and U. Scheffel, *Soc. Neurosci. Abstr.*, 2003, **33**, 802.1.

5 For a review on opioid receptor radioligands for imaging, see: J. R. Lever, *Curr. Pharm. Des.*, 2007, **13**, 33–49.

6 J. R. Lever, C. M. Kinter, H. T. Ravert, J. L. Musachio, W. B. Mathews and R. F. Dannals, *J. Labelled Compd. Radiopharm.*, 1995, **36**, 137–145.

7 (*a*) I. Madar, J. R. Lever, C. M. Kinter, U. Scheffel, H. T. Ravert, J. L. Musachio, W. B. Mathews, R. F. Dannals and J. J. Frost, *Synapse*, 1996, **24**, 19–28; (*b*) P. S. R. Villemagne, R. F. Dannals, H. T. Ravert and J. J. Frost, *Eur. J. Nucl. Med. Mol. Imaging*, 2002, **29**, 1385–1388.

8 (*a*) P. M. Villemagne, D. A. Bluemke, R. F. Dannals, H. T. Ravert and J. J. Frost, *J. Nucl. Med.*, 2002, **43S**, 280P; (*b*) I. Madar, B. Bencherif, J. Lever, R. F. Heitmiller, S. C. Yang, M. Brock, J. Brahmer, H. Ravert, R. Dannals and J. J. Frost, *J. Nucl. Med.*, 2007, **48**, 207–213.

9 R. A. Duval, R. L. Allmon and J. R. Lever, *J. Med. Chem.*, 2007, **50**, 2144–2156.

10 For examples of NTI and NTB analog synthesis and pharmacological studies, see: (*a*) M. Spetea, S. T. Nevin, S. Hosztafi, A. Z. Rónai, G. Tóth and A. Borsodi, *Neurochem. Res.*, 1998, **23**, 1211–1216; (*b*) S. Ananthan, C. A. Johnson, R. L. Carter, S. D. Clayton, K. C. Rice, H. Xu, P. Davis, F. Porreca and R. B. Rothman, *J. Med. Chem.*, 1998, **41**, 2872–2881; (*c*) A. Coop, J. Pinto, L. Wang, K. McCullough, R. B. Rothman, C. Dersch, A. E. Jacobson and K. C. Rice, *Bioorg. Med. Chem. Lett.*, 1999, **9**, 3435–3438; (*d*) A. Coop, A. E. Jacobson, M. D. Aceto, L. S. Harris, J. R. Traynor, J. H. Woods and K. C. Rice, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 2449–2451; (*e*) J. Schütz, C. M. Dersch, R. Horel, R., M. Spetea, M. Koch, R. Meditz, E. Greiner, R. B. Rothman and H. Schmidhammer, *J. Med. Chem.*, 2002, **45**, 5378–5383; (*f*) S. Sakami, M. Maeda, K. Kawai, T. Aoki, K. Kawamura, H. Fujii, K. Hasebe, M. Nakajima, T. Endo, S. Ueno, T. Ito, J. Kamei and H. Nagase, *J. Med. Chem.*, 2008, **51**, 4404–4411.

11 For reviews of the Fischer indole synthesis, see: (*a*) B. Robinson, *The Fischer Indole Synthesis*, John Wiley and Sons, New York, NY, 1982; (*b*) G. W. Gribble, *Contemp. Org. Synth.*, 1994, **1**, 145–172.

12 G. R. Humphrey and J. T. Kuethe, *Chem. Rev.*, 2006, **106**, 2875–2911.

13 (*a*) R. Calderon-Morales, V. Tambyrajah, P. R. Jenkins, D. L. Davies and A. P. Abbott, *Chem. Commun.*, 2004, 158–159; (*b*) D.-Q. Xu, W.-Y. Yang, S.-P. Luo, B.-T. Wang, J. Wu and Z.-Y. Xu, *Eur. J. Org,. Chem.*, 2007, 1007–1012; (*c*) D.-Q. Xu, J. Wu, S.-P. Luo, J.-X. Zhang, J.-Y. Wu, X.-H. Du and Z.-Y. Xu, *Green Chem*, 2009, **11**, 1239–1246.

14 (*a*) S. B. Mhaske and N. P. Argade, *Tetrahedron*, 2004, **60**, 3417–3420; (*b*) A. Dhakshinamoorthy and K. Pitchumani, *Appl. Catal., A*, 2005, **292**, 305–311; (*c*) P. R. Singh, M. P. Surpur and S. B. Patil, *Tetrahedron Lett.*, 2008, **49**, 3335–3340.

15 (*a*) T. M. Lipińska and S. J. Czarnocki, *Org. Lett.*, 2006, **8**, 367–370; (*b*) L. Martarello, D. Joseph and G. Kirsch, *J. Chem,. Soc. Perkin Trans. I*, 1995, 2941–2944; (*c*) A. Sudhakara, H. Jayadevappa, H. N. H. Kumar and K. M. Mahadevan, *Lett. Org. Chem*, 2009, **6**, 159–164.

16 (*a*) C.-Y. Chen, C. H. Senanayake, T. J. Bill, R. D. Larsen, T. R. Verhoeven and P. J. Reider, *J. Org. Chem.*, 1994, **59**, 3738–3741; (*b*) C. F. Masaguer, E. Formoso, E. Raviña, H. Tristán, M. I. Loza, E. Rivas and J. A. Fontenla, *Bioorg. Med. Chem. Lett*, 1998, **8**, 3571–3576; (*c*) A. M. Schmidt and P. Eilbracht, *J. Org. Chem.*, 2005, **70**, 5528–5535.

17 (*a*) S. M. Parmerter, A. G. Cook and W. B. Dixon, *J. Am. Chem. Soc.*, 1958, **80**, 4621–4622; (*b*) A. R. Katritzky, S. Rachwal and S. Bayyuk, *Org. Prep. Proc. Int.*, 1991, **23**, 357–363.

18 (*a*) P. R. Brodfuehrer, B.-C. Chen, T. R. Sattelberg, P. R. Smith, J. P. Reddy, D. R. Stark, S. L. Quinlan, J. G. Reid, J. K. Thottathil and S. Wang, *J. Org. Chem.*, 1997, **62**, 9192–9202; (*b*) K. R. Campos, J. C. S. Woo, S. Lee and R. D. Tillyer, *Org. Lett.*, 2004, **6**, 79–82.