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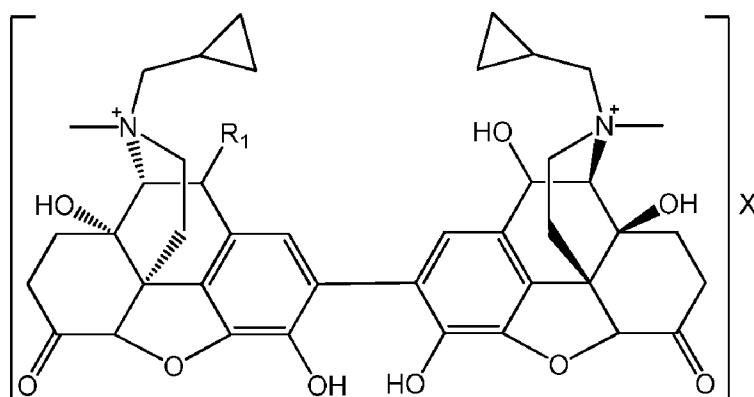
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(54) Title: HYDROXY-(R)-2,2'-BISMETHYLNAL TREXONES AND USES THEREOF



(I)

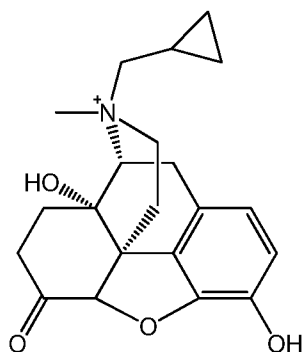
(57) Abstract: The present application relates to compounds of Formula (I): wherein R_1 and X are as defined herein, as well as methods for synthesis and use thereof. The present application also relates to 10'-hydroxy-(R),(R)-2,2'-bismethylnaltrexone, as well as methods for synthesis and use thereof.

HYDROXY-(R),(R)-2,2'-BISMETHYLNALTREXONES AND USES THEREOF**RELATED APPLICATIONS**

[001] This application claims priority to United States Provisional Application No. 62/027,852, filed on July 23, 2014, the entire content of which is incorporated in its entirety herein.

BACKGROUND

[002] Methylnaltrexone (MNTX) is a quaternary derivative of the opioid antagonist naltrexone.



(MNTX)

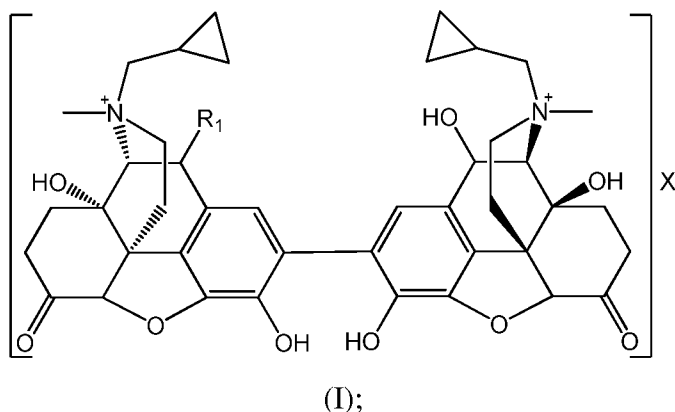
MNTX was first reported by Goldberg *et al.* (see, *e.g.*, U.S. Patent No. 4,176,186) and exists as a salt, for example, a bromide salt. Addition of the methyl group to the ring nitrogen of naltrexone forms a charged compound, which is believed to have greater polarity and less liposolubility than naltrexone, thus preventing MNTX from crossing the blood-brain barrier in humans. As a consequence, MNTX exerts its effects in the periphery rather than in the central nervous system with the advantage that it does not counteract the analgesic effects of opioids on the central nervous system. Thus, MNTX is able to reduce the side effects of opioids (*e.g.*, nausea, emesis, dysphoria, pruritus, urinary retention, bowel hypomotility, constipation, gastric hypomotility, delayed gastric emptying and immune suppression) with minimal or no effect on the analgesic effect. See, *e.g.*, U.S. Patent Nos. 4,176,186, 4,719,215, 4,861,781, 5,102,887, 5,972,954, 6,274,591, 6,559,158, and 6,608,075, and U.S. Patent Application Publication Nos. 2003/0022909, 2004/0266806, 2004/0259899 and 2005/0004155.

[003] MNTX is a chiral molecule, wherein the quaternary nitrogen can possess either the (R) or (S) configuration. For example, (R)-MNTX refers to a molecule of MNTX having (R) stereochemistry at the quaternary nitrogen, while (S)-MNTX refers to a molecule of MNTX having (S) stereochemistry at the quaternary nitrogen. It may be desired to isolate and/or purify a single stereoisomer of an agent, particularly for pharmaceutical use. For example, methylnaltrexone bromide currently approved for use by the FDA is identified as (R)-N-(cyclopropylmethyl) noroxymorphone methobromide (or (R)-MNTX). See, *e.g.*, RELISTOR product label.

SUMMARY OF THE INVENTION

[004] Pharmaceutical compositions typically require a high level of purity to meet regulated standards for drug quality and purity. In the synthesis of MNTX, impurities such as the molecules described herein can be formed.

[005] In one aspect, provided herein is a compound of Formula (I):



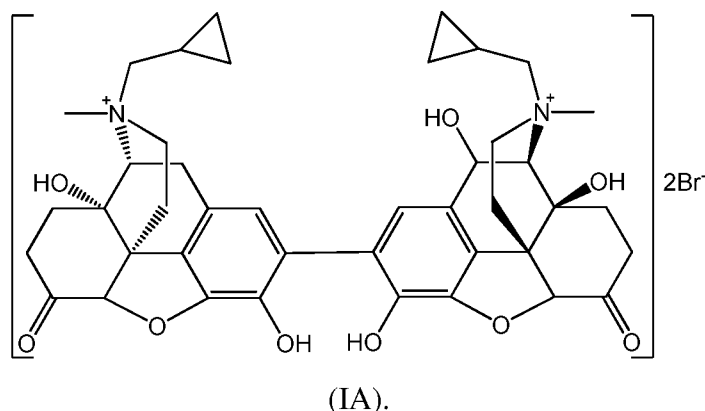
wherein R_1 is selected from hydrogen and hydroxy; and X is a counterion moiety with a net charge of -2.

[006] In one embodiment, X comprises two counterions, each with a charge of -1. In one embodiment, X comprises a halide. In one embodiment, X is $2Br^-$.

[007] In one embodiment, the compound is isolated with at least 75% purity. In one embodiment, the compound is isolated with at least 80% purity. In one embodiment, the compound is isolated with at least 85% purity. In other related embodiments, the compound is isolated with at least 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% purity.

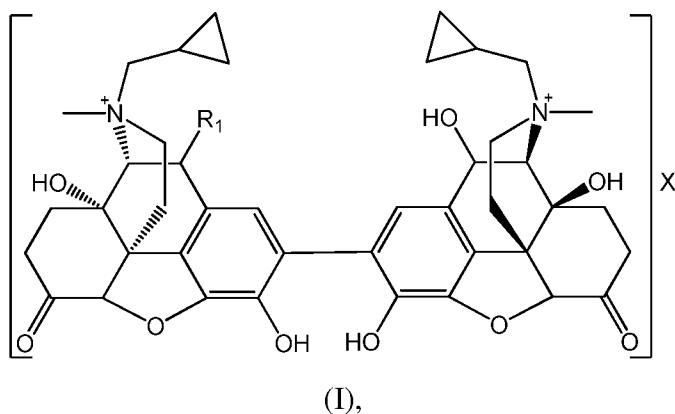
[008] In one embodiment, the compound is a solid.

[009] In one aspect, provided herein is a compound of Formula (IA):



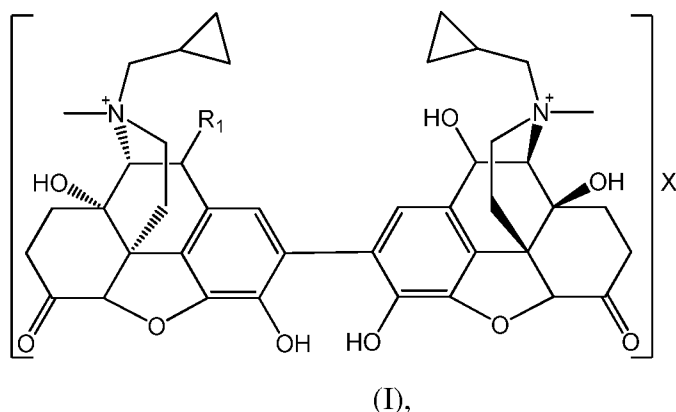
[0010] In one embodiment, provided herein is a composition comprising a compound described herein, wherein the composition comprises less than about 20% total impurities. In one embodiment, provided herein is a composition comprising a compound described herein, wherein the composition comprises less than about 10% total impurities. In one embodiment, provided herein is a composition comprising a compound described herein, wherein the composition comprises less than about 5% total impurities. In other related embodiments, the composition comprises less than about 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% total impurities.

[0011] In one aspect, provided herein is a method of synthesizing a compound of Formula (I):



the method comprising coupling 10-OH-(R)-MNTX with a compound selected from (R)-MNTX and 10-OH-(R)-MNTX such that a compound of Formula (I) is formed.

[0012] In one aspect, provided herein is a method of determining the presence and/or amount of a compound of Formula (I):



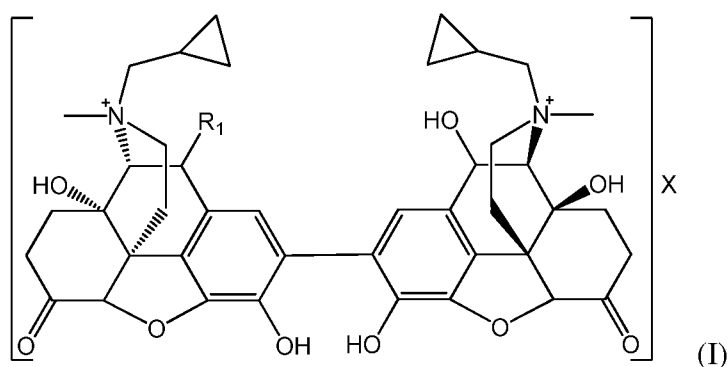
in a sample, the method comprising: injecting a reference solution of a compound of Formula (I) into an HPLC column under a set of conditions to obtain a first HPLC chromatogram wherein the amount and/or chemical identity of the compound of Formula (I) present in the reference solution is known; injecting a sample solution into the HPLC column under the same set of conditions to obtain a second HPLC chromatogram; and comparing the first HPLC chromatogram with the second HPLC chromatogram to determine the presence and/or amount of the compound of Formula (I).

[0013] In one embodiment, the sample is a sample comprising (R)-MNTX.

[0014] In one embodiment, the presence of a compound of Formula (I) in the sample solution is determined by comparing retention times of peaks in the first HPLC chromatogram with the retention times of peaks in the second HPLC chromatogram.

[0015] In one aspect, provided herein is a method for determining the purity of a sample, the method comprising:

injecting a reference solution comprising a compound of Formula (I):



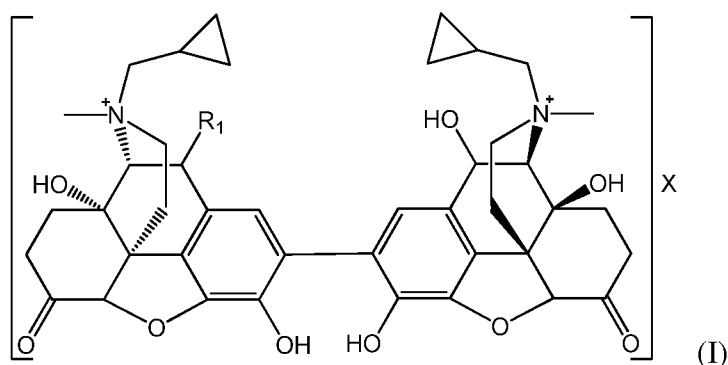
into an HPLC column under a set of conditions to obtain a first HPLC chromatogram wherein the amount and/or chemical identity of the compound of Formula (I) present in the reference solution is known; injecting a sample solution into the HPLC column under the same set of conditions to obtain a second HPLC chromatogram; and comparing the first HPLC chromatogram with the second HPLC chromatogram to determine the presence and/or amount of the compound of Formula (I) in the sample.

[0016] In one embodiment, the sample is a sample comprising (R)-MNTX.

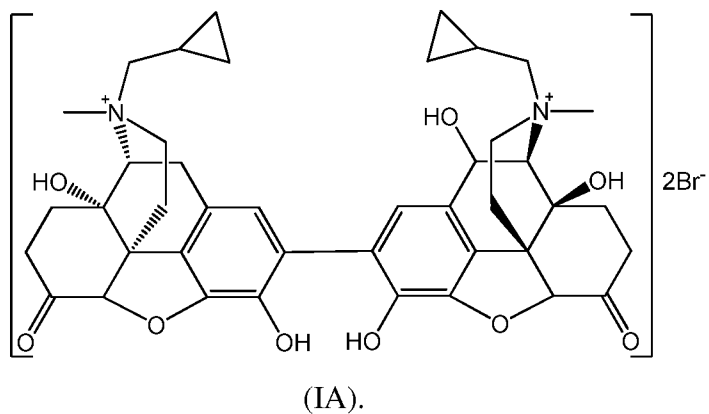
[0017] In one embodiment, the presence of the compound of Formula (I) in the sample indicates an impure sample.

[0018] In one embodiment, the presence of a compound of Formula (I) in the sample solution is determined by comparing retention times of peaks in the first HPLC chromatogram with the retention times of peaks in the second HPLC chromatogram.

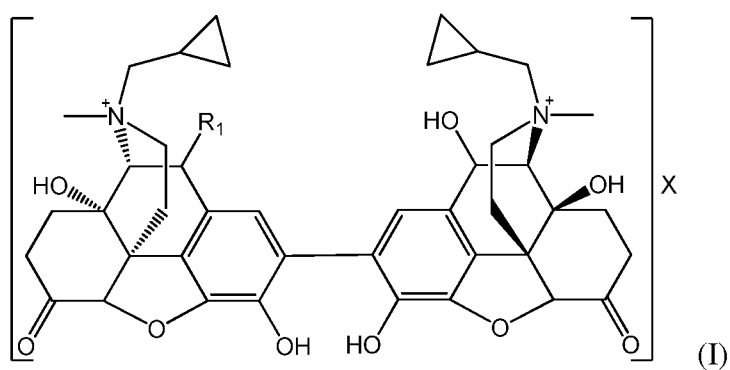
[0019] In one aspect, the present invention provides a composition comprising methylnaltrexone, wherein the composition comprises $\leq 0.2\%$ of a compound of Formula (I):



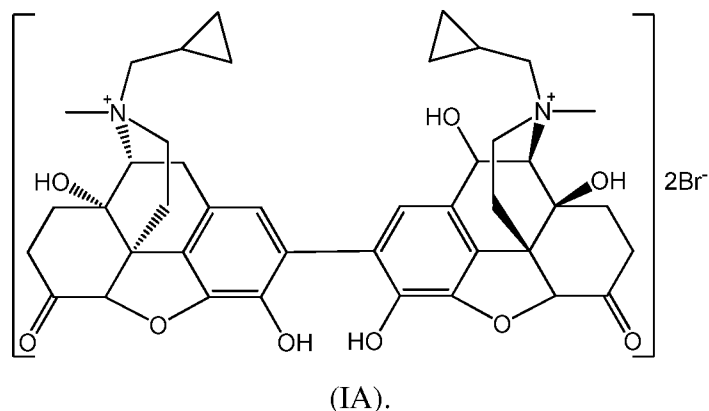
[0020] In another aspect, the present invention provides a composition comprising methylnaltrexone, wherein the composition comprises $\leq 0.2\%$ of a compound of Formula (IA):



[0021] In yet another aspect, the present invention provides a pharmaceutical composition comprising methylnaltrexone, wherein the pharmaceutical composition comprises $\leq 0.2\%$ of a compound of Formula (I):



[0022] In yet another aspect, the present invention provides a pharmaceutical composition comprising methylnaltrexone, wherein the pharmaceutical composition comprises $\leq 0.2\%$ of a compound of Formula (IA):

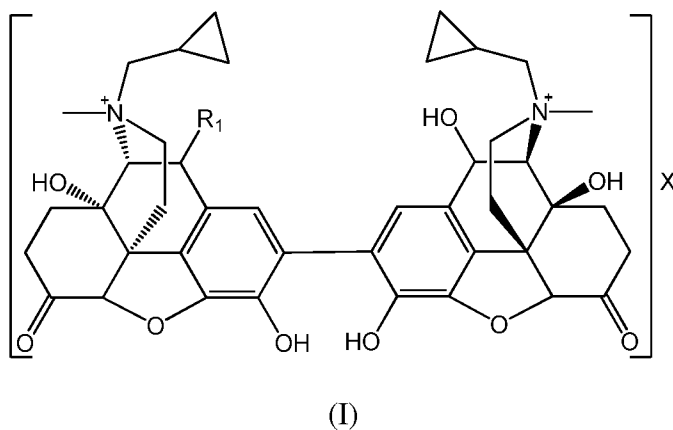


[0023] Other embodiments are disclosed *infra*.

DETAILED DESCRIPTION OF THE INVENTION

[0024] The present teachings provide hydroxy-(R),(R)-2,2'-bis-methylnaltrexones, such as 10' hydroxy-(R),(R)-2,2'-bis-methylnaltrexone, also referred to as 10'-OH-(R),(R)-2,2'-bis-MNTX, and related products and methods (*e.g.*, synthetic methods).

[0025] Accordingly, in some embodiments, the present teachings are directed to compounds of Formula (I):



wherein R_1 is selected from hydrogen and hydroxy; and X is a counterion moiety with a net charge of -2.

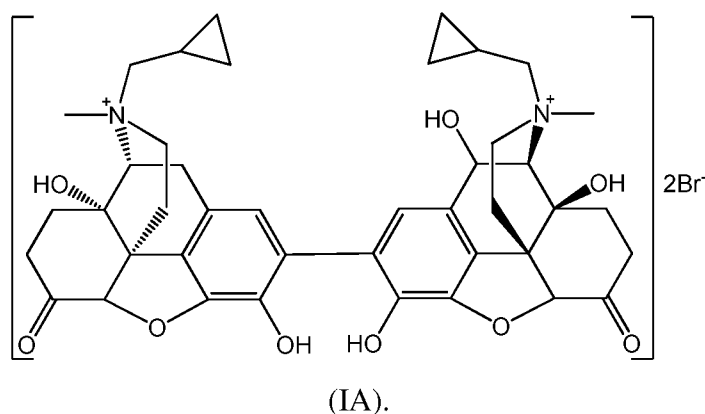
[0026] The term “counterion moiety” as used herein, refers to an ion or group of ions that exists in the formulation for the purpose of maintaining electrical neutrality of the compound.

The counterion moiety can comprise, for example, halides (*e.g.*, iodide, bromide, chloride or fluoride), sulfates, phosphates, nitrates, or anionic-charged organic species (*e.g.*, sulfonates, such as mesylate, besylate, tosylate, triflate, and the like; carboxylates, such as formate, acetate, citrate, and the like). In one embodiment, the counterion moiety comprises a halide, such as bromide. In some embodiments, X is 2Br^- .

[0027] In some embodiments, X comprises two counterions, each with a charge of -1. In such embodiments, each counterion can be the same (*e.g.*, 2Br^-) or can be different (*e.g.*, Br^- Cl^-). In other embodiments, X comprises a single counterion with a net charge of -2 (*e.g.*, SO_4^{2-}).

[0028] In some embodiments, R_1 is hydrogen. That is, in some embodiments, the compound of Formula (I) is 10'-OH-(R),(R)-2,2'-bis-MNTX. In other embodiments, R_1 is hydroxy. That is, in some embodiments, the compound of Formula (I) is 10,10'-bis -OH-(R),(R)-2,2'-bis-MNTX.

[0029] In some embodiments, the present teachings provide a compound of Formula (IA):



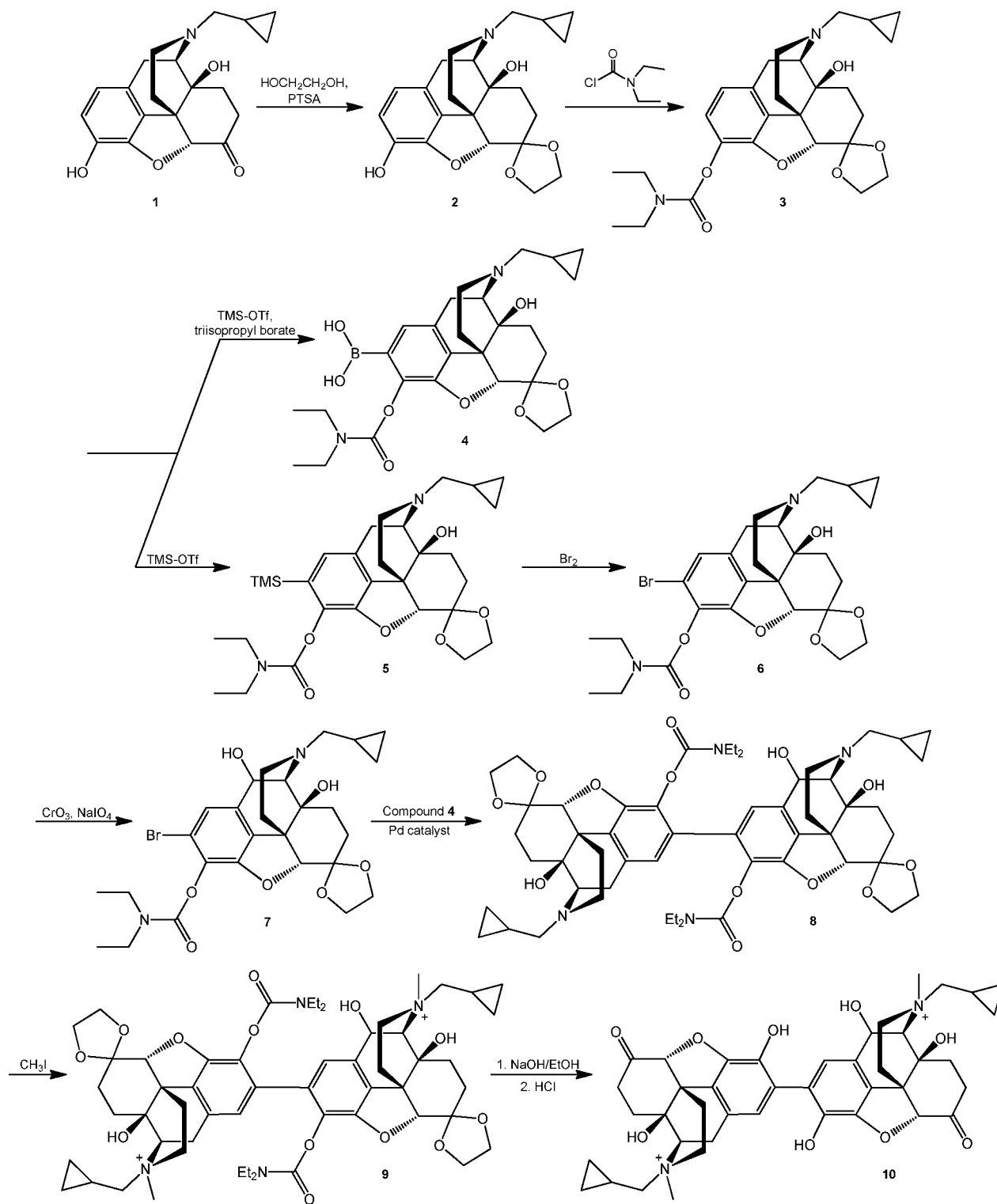
[0030] In some embodiments, the present teachings provide a composition comprising a compound of Formula (I) (for example a compound of Formula (IA)) having a purity of at least about 5%, at least about 10%, at least about 15%, at least about 25%, at least about 50%, at least about 75%, at least about 85%, at least about 95%, or at least about 97%. In particular embodiments, the composition comprises a compound of Formula (I) (for example a compound of Formula (IA)) having a purity of at least about 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%. In some embodiments, the present teachings provide a composition comprising a compound of Formula (I) (for example a compound of Formula (IA)), wherein the composition has less than about 20% total impurities, *e.g.*, less than about 15%, less than

about 10%, less than about 5%, or less than about 3% total impurities. In other related embodiments, the composition comprises less than about 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% total impurities. In some embodiments, the present teachings provide a composition comprising a compound of Formula (I) for use as a reference or standard in various analytical methods (*e.g.*, HPLC).

[0031] Embodiments are also directed to a composition for oral administration, wherein the composition includes methylnaltrexone and an amphiphilic pharmaceutically acceptable excipient, and wherein the composition comprises less than about 0.5% of a compound of Formula I or IA. Exemplary compositions comprising methylnaltrexone and an amphiphilic pharmaceutically acceptable excipient are described, for example, in U.S. Patent Nos. 8,524,276 and 8,956,651, each of which is incorporated herein by reference in its entirety. In some embodiments, the composition comprises less than about 0.4% of a compound of Formula I or IA. In some embodiments, the composition comprises less than about 0.3% of a compound of Formula I or IA. In some embodiments, the composition comprises less than about 0.2% of a compound of Formula I or IA. In some embodiments, the composition comprises about 0.5%, 0.4%, 0.3% or 0.2% of a compound of Formula I or IA. Detection of a compound of Formula I or IA in such compositions can be carried out by standard quantitative methods, including mass spectrometry, gas chromatography, liquid chromatography (*e.g.*, HPLC).

[0032] The present teachings also provide methods for the synthesis of compounds of Formula (I). Such methods include coupling a 10-OH-(R)-MNTX with an (R)-MNTX or another 10-OH-(R)-MNTX. Scheme 1, below, shows an exemplary synthesis of 10'-OH-(R),(R)-2,2'-bis-MNTX. Briefly, the phenolic ring of a first (R)-MNTX molecule, which has been modified with a 10-hydroxy group, may be coupled with another phenolic ring of a second (R)-MNTX molecule in the presence of a catalyst (and optionally an oxidizing agent) in amounts sufficient to produce 10'-OH-(R),(R)-2,2'-bis-MNTX.

Scheme 1



[0033] The above synthesis can be modified, *e.g.*, by substituting the hydrogen at the 10 position of compound 4 with a hydroxy, in order to form 10,10'-bis-OH-(R),(R)-2,2'-bis-MNTX.

[0034] In some embodiments, methods for the synthesis of compounds of Formula (I) are provided, comprising coupling the phenolic ring of a naltrexone molecule that has been modified with a 10-hydroxy group with another phenolic ring of a second naltrexone molecule, which may or may not be modified with a 10-hydroxy group, under similar coupling conditions. The methods can further comprise alkylating the resulting naltrexone dimer at the N-substituted tertiary amines with a methylating agent to form a compound of Formula (I).

[0035] Because 10'-OH-2,2'-bis-MNTX contains two chiral quaternary amines, it may possess a number of different stereochemical configurations (*e.g.*, 10'-OH-(R),(R)-2,2'-bis-MNTX, 10'-OH-(S),(S)-2,2'-bis-MNTX, 10'-OH-(S),(R)-2,2'-bis-MNTX or 10'-OH-(R),(S)-2,2'-bis-MNTX) based on the stereochemistry of the individual MNTX molecules which are coupled to form the 2,2'-bis-MNTX dimer. In some embodiments, methods described herein utilize MNTX with at least 99.5% in the (R) configuration as the starting material, such that the 10'-OH-(R),(R)-2,2'-bis-MNTX isomer is the major isomer formed. It is believed that, while other isomers of 10'-OH-2,2'-bis-MNTX may be formed, they would be present in very low or negligible quantities. However, it is to be understood that, should other isomers be desired, such isomers can be produced, *e.g.*, using MNTX in the (S) configuration as the starting material. The synthesis of (R)-MNTX is described in U.S. Patent Application Publication No. 20070099946, incorporated by reference in its entirety herein. The synthesis of (S)-MNTX is described in U.S. Patent Application Publication No. 20070265293, incorporated by reference in its entirety herein.

[0036] In some embodiments, the methods comprise a coupling reaction, such as a Suzuki coupling of aryl groups. See, *e.g.*, Suzuki *et al.*, Synthetic Communications, 11(7): 513-519 (1981), incorporated by reference in its entirety herein, among others. The coupling reaction can involve use of a catalyst (*e.g.*, a palladium or nickel catalyst) that is capable of catalyzing the formation of a covalent bond between two molecules, such as two aryl molecules. In some embodiments, the catalyst is a palladium catalyst. Examples of palladium catalysts include, but are not limited to, palladium phosphines. Palladium catalysts, *e.g.*, palladium phosphines, may be directly added to a reaction mixture or may be generated *in situ*. For example, Pd(OAc)₂ may be added in the presence of a ligand (*e.g.*, a phosphine ligand), such that the palladium is complexed to the ligand before or during the coupling reaction. In some embodiments, the catalyst is present in the reaction in a sub-stoichiometric amount (*e.g.*, catalytic amount) relative to the substrate (*e.g.*, modified MNTX or naltrexone). For

example, the catalyst may be present in 25 mol % or less, *e.g.*, 10 mol % or less, relative to the amount of MNTX or naltrexone. For example, the catalyst may be present in 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1 % mol or less, relative to the amount of MNTX or naltrexone. In one embodiment, the catalyst is Pd(PPh₃)₄ and is present in the reaction in an amount between about 2 and about 5 mol %. Catalysts are well known to those of ordinary skill in the art and are described extensively in both the patent literature and in chemistry text books. Appropriate catalysts can be selected by one of ordinary skill in the art without undue experimentation.

[0037] It is also to be understood that, although Suzuki coupling is described in detail herein, other methods for coupling two aromatic compounds may also be used. For example, aromatic compounds may be coupled using oxidative coupling or Stille coupling, or coupling reactions reported by Negishi, Hiyama or Kumada. See, *e.g.*, Smith, M. and March, J. March's Advanced Organic Chemistry, Ed. 6, John Wiley & Sons, (2007) or www.organic-chemistry.org for details, the contents of each of which is incorporated by reference in its entirety herein.

[0038] As used herein, the term "coupling" includes a reaction directly between the two aromatic groups, as well as reactions that require pre-modification of the aromatic groups. That is, in some embodiments, the 10-OH-(R)-MNTX molecules (and/or (R)-MNTX molecule) are modified prior to coupling. For example, in some embodiments, substituents are added to the 2 positions of both aromatic species involved in the coupling reaction (that is, the 10-OH-(R)-MNTX molecule and the (R)-MNTX molecule *or* the two 10-OH-(R)-MNTX molecules). In the case of a Suzuki coupling, for example, one aromatic species can be modified with a boronic acid (-B(OH)₂) and the other with a halide, *e.g.*, -Br. Similarly, in the case of a Stille coupling, one aromatic species can be modified with a stannate (-Sn(R')₃ where each R' is typically a small alkyl moiety). This can, *e.g.*, facilitate coupling at a specific position on each ring, as well as minimize unwanted byproducts (*e.g.*, bis-(R)(R)-MNTX).

[0039] The reaction may be performed in any organic solvent, mixture of organic solvents, or mixture of organic solvent(s) and water. In some embodiments, the reaction may be performed in acidic conditions. In some embodiments, the reaction may be performed in basic conditions.

[0040] In some cases, methods of the present teachings further include a methylation reaction, such as methylation of the N-substituted tertiary amines of a naltrexone dimer. The methylation reaction can involve use of a methylating agent, such as methyl bromide. As used herein, the term “methylating agent” refers to a reactive species having electrophilic properties that is capable of introducing a methyl group at the nitrogen atom of naltrexone, so as to form a covalent bond therewith. Illustrative methylating agents can be represented by the formula CH_3Z , wherein “Z” is a leaving group which, upon its departure, enables a covalent bond to be formed between the methyl group and the nitrogen atom of naltrexone, forming MNTX. Methylating agents and leaving groups are well known to those of ordinary skill in the art and are described extensively in both the patent literature and in chemistry text books. Suitable Z groups include, but are not limited to, fluoro, chloro, bromo, iodo, - OSO_2CF_3 , - OSO_2OCH_3 , - OSO_2CH_3 , - $\text{OSO}_2\text{C}_6\text{H}_4\text{-p-CH}_3$, - $\text{OSO}_2\text{C}_6\text{H}_4\text{-p-Br}$.

[0041] The method can further involve purification of compounds of Formula (I) using at least one purification technique, such as chromatography or crystallization. The chromatography can be reverse-phase chromatography, regular-phase chromatography, and/or ion exchange chromatography. In some embodiments, the regular-phase chromatography may involve use of an alumina or silica gel column. In some cases, methods of the invention may involve use of a reverse phase HPLC column followed by ion exchange chromatography. For reverse phase chromatography, the HPLC column may be eluted using a mobile phase comprising water, methanol, trifluoroacetic acid, or mixtures thereof. The crystallization solvent can be an organic solvent, a mixture of organic solvents, or a mixture of organic solvent(s) plus water. An exemplary solvent is an alcohol, such as methanol. Methods for both chromatography and crystallization are known in the art. In some cases, the HPLC column is a reverse phase column and the column is eluted using a mobile phase which includes water, methanol, trifluoroacetic acid, or mixtures thereof.

[0042] In some embodiments, the present teachings are directed to methods for determining the presence and/or amount of a compound of Formula (I) in a sample (*e.g.*, a sample comprising (R)-MNTX). In one embodiment, the method includes injecting a reference solution of a compound of Formula (I) into an HPLC column under a set of conditions to obtain a first HPLC chromatogram wherein the amount and/or chemical identity of the compound of Formula (I) present in the reference solution is known; injecting a sample solution into the HPLC column under the same set of conditions to obtain a second HPLC

chromatogram; and comparing the first HPLC chromatogram with the second HPLC chromatogram to determine the presence and/or amount of the impurity.

[0043] In one embodiment, the presence of a compound of Formula (I) in the sample solution may be determined by comparing retention times of peaks in the first HPLC chromatogram with the retention times of peaks in the second HPLC chromatogram. For example, a reference solution comprising 10'-OH-(R),(R)-2,2'-bis-MNTX may produce a chromatogram with a peak corresponding to 10'-OH-(R),(R)-2,2'-bis-MNTX and having a particular retention time. A sample solution will produce a resulting chromatogram, which is then studied to determine if a peak exists at the same retention time as the peak corresponding to 10'-OH-(R),(R)-2,2'-bis-MNTX in the HPLC chromatogram of the standard solution. The existence of such a peak can indicate that 10'-OH-(R),(R)-2,2'-bis-MNTX is present in the sample.

[0044] In another embodiment, the amount of a compound of Formula (I) in the sample solution may be determined by comparing the area of peaks in the first HPLC chromatogram with the area of peaks in the second HPLC chromatogram. For example, the amount (in grams) of the compound of Formula (I) in the sample solution (W_2) can be calculated as follows:

$$W_2 = A_2/A_1 \times W_1$$

wherein W_1 is the amount (in grams) of the compound in the reference solution; A_1 is the area under the curve of the first HPLC chromatogram; and A_2 is the area under the curve (having the same retention time as A_1) of the second HPLC chromatogram.

[0045] The reference solution may be formed by dissolving a sample (*e.g.*, solid sample of known weight) of the compound of Formula (I) in a first solvent (*e.g.*, a known amount of solvent), and the sample solution may be formed by dissolving a sample (*e.g.*, a solid sample of unknown purity) in a second solvent. In some embodiments, the first solvent and the second solvent are the same type of solvent (*e.g.*, both methanol). In some embodiments, the reference solution may contain one or more additional compounds, wherein the amount and/or identity of the additional compounds is also known. In one embodiment, the sample (*e.g.*, sample solution) includes (R)-MNTX. However, it should be understood that the invention may encompass other samples suspected of containing compounds of Formula (I).

[0046] In some embodiments, the present teachings are directed to methods for determining the purity of a sample, *e.g.*, a sample of (R)-MNTX, using the compounds of the present

invention. In some embodiments, the method includes injecting a reference solution comprising a compound of Formula (I) into an HPLC column under a set of conditions to obtain a first HPLC chromatogram wherein the amount and/or chemical identity of the compound of Formula (I) present in the reference solution is known; injecting a sample solution into the HPLC column under the same set of conditions to obtain a second HPLC chromatogram, and comparing the first HPLC chromatogram with the second HPLC chromatogram to determine the presence and/or amount of the compound of Formula (I) in the sample.

[0047] In other embodiments, the method includes injecting a solution in which the sample is dissolved into an HPLC column; obtaining an HPLC chromatogram of the solution; and determining the amount in the sample of a compound known to have the structure of Formula (I) from the HPLC chromatogram. The amount in the sample of the compound of Formula (I) may be determined by (i) identifying a peak on the chromatogram that corresponds to a peak on a control chromatogram, (ii) identifying a peak on the chromatogram that corresponds to a relative retention time of a compound known to have the structure of a compound of Formula (I) and/or (iii) identifying a peak on the chromatogram that corresponds to a known amount of a spike of the compound known to have the structure of a compound of Formula (I).

[0048] In one embodiment, the presence of a compound of Formula (I) in the sample solution is determined by comparing retention times of peaks in the first HPLC chromatogram with the retention times of peaks in the second HPLC chromatogram, as described in more detail above. In other embodiments, the presence of a compound of Formula (I) in the sample solution includes visual inspection for the presence or absence of peaks at relative retention times corresponding to a compound of Formula (I). In some embodiments, the presence of a peak at a relative retention time corresponding to a compound of Formula (I) indicates an impure sample. The relative retention times for 10'-OH-(R),(R)-2,2'-bis-MNTX, (R)-MNTX and (S)-MNTX are 1.10, 1.00, and 0.89, respectively, as determined by HPLC Method II, the method described in U.S. Patent Application Publication No. 20070099946, incorporated by reference in its entirety herein.

[0049] Where purity of a solid is concerned, purity is the weight percent of the compound of Formula (I) versus that of other compounds in the solid. Where purity of a solution is concerned, purity is the weight percent of the compound of Formula (I) versus that of other compounds in the solution. As described above, purity may be determined by HPLC,

wherein the percent purity of a compound refers to the weight percent of the compound present in solution relative to the weight percent of other compounds present in the same solution.

[0050] In some embodiments, the present teachings provide methods for determining the amount and/or presence of 10'-OH-(R),(R)-2,2'-bis-MNTX and/or 10,10'-bis-OH-(R),(R)-2,2'-bis-MNTX in a sample comprising MNTX, (*e.g.*, (R)-MNTX). The sample may be a sample of freshly manufactured material or the sample may be one stored for a defined period of time. In one embodiment, a sample of (R)-MNTX may be stored and periodically analyzed using methods described herein to determine the presence and/or amount of compounds of Formula (I) in the sample. The sample solution may also comprise (R)-MNTX, (S)-MNTX, (R),(R)-2,2'-bis-MNTX, (R),(S)-2,2'-bis-MNTX, (S),(S)-2,2'-bis-MNTX or mixtures thereof. In one embodiment, the sample solution comprises (R)-MNTX and/or (S)-MNTX. In another embodiment, the sample solution comprises a mixture of (R)-MNTX and (S)-MNTX. In other embodiments, the sample solution consists essentially of MNTX (and the solvent in which it is dissolved).

[0051] The methods disclosed herein may further comprise documenting in a written form the chemical identity of the compound and the amount of the compound as an impurity in the material.

[0052] Also provided herein are kits containing a compound of Formula (I). For example, a kit including a compound of Formula (I) may be provided for the purpose of producing standards for analytical methods, such as HPLC. In one embodiment, the kit may comprise a compound of Formula (I) and indicia in or on the kit indicating that a compound of Formula (I) is present in the kit. In some cases, the indicia specifies the purity of a compound of Formula (I). The indicia may also specify the chemical structure of the compound by providing a chemical formula or a structural drawing. In some cases, the kit can include other compounds, for example, (R)-MNTX, (S)-MNTX, 2,2'-bis-MNTX or one or more other compounds. The indicia may specify that other compounds are present in the kit, and/or may specify the purity of the other compounds or the chemical structure of the other compounds by providing a chemical formula or a structural drawing.

[0053] In some cases, the kit comprises a solution of a compound of Formula (I). In some cases, the kit comprises a solid sample of a compound of Formula (I). A "kit," as used herein, typically defines a package or an assembly including one or more of the compositions

of the invention, and/or other materials associated with the invention, such as solvents. Each of the compositions of the kit may be provided in liquid form (*e.g.*, in solution), or in solid form. In certain cases, some of the compositions may be constitutable or otherwise processable, for example, by the addition of a suitable solvent or other species, which may or may not be provided with the kit. Examples of other compositions or components associated with the invention include, but are not limited to, solvents, surfactants, diluents, salts, buffers, emulsifiers, chelating agents, antioxidants, drying agents, needles, syringes, packaging materials, tubes, bottles, flasks, beakers, dishes, frits, filters, rings, clamps, wraps, patches, containers, and the like, for example, for using, modifying, assembling, storing, packaging, preparing, mixing, diluting, and/or preserving the compositions components for a particular use, for example, to a sample.

[0054] A kit of the invention may, in some cases, include instructions in any form that are provided in connection with the compositions of the invention in such a manner that one of ordinary skill in the art would recognize that the instructions are to be associated with the compositions of the invention. For instance, the instructions may include instructions for the use, modification, mixing, diluting, preserving, assembly, storage, packaging, and/or preparation of the compositions and/or other compositions associated with the kit. In some cases, the instructions may also include instructions for the delivery of the compositions, for example, for a particular use, *e.g.*, to a sample. As used herein, “instructions” can define a component of instructional utility (*e.g.*, directions, guides, warnings, labels, notes, FAQs or “frequently asked questions,” etc.), and typically involve written instructions on or associated with the invention and/or with the packaging of the compounds of the invention. Instructions can also include instructional communications in any form (*e.g.*, oral, electronic, audible, digital, optical, visual, etc.), provided in any manner such that a user will clearly recognize that the instructions are to be associated with compounds of the invention.

EXEMPLIFICATION

Example 1: Synthesis of 10'-OH-(R),(R)-2,2'-bis-MNTX

[0055] (R)-Methylnaltrexone (30 g, 0.069mol), prepared, *e.g.*, by methods known in the art, and NaHCO₃ (12 g, 0.4 wt. equiv.) were dissolved in water (3.0 L, 100 vol). The resultant solution was heated to 40°C with stirring and progress of the reaction was monitored by HPLC as illustrated in Table 2.

Table 2:

Time (days)	% Formula (IA)
0.67	0.54
3	1.62
6	2.33
8	2.84
10	3.19

[0056] The reaction was stopped after 10 days at 40 °C and the batch was cooled to 20-25 °C and 10'-OH-(R),(R)-2,2'-bis-MNTX was isolated by means of preparative HPLC as described below.

Example 2: Purification of 10-OH-(R),(R)-2,2'-bis-MNTX

[0057] The sample for purification was prepared by adding 30 mL of solvent (10% methanol/90% water) to the material isolated in Example 1, sonicating for 10 min, and centrifuging. The supernatant was collected and acidified with acetic acid to pH 4. The resulting solution was filtered through a 0.45 mm glass filter disk and purified on a Gilson preparative HPLC system (Xterra Prep MS, C18, OBD, 10u column) with a dual wavelength UV detector (at 225 nm and 240 nm) and a Model 215 injector/fraction collector. Instrumental conditions are summarized below.

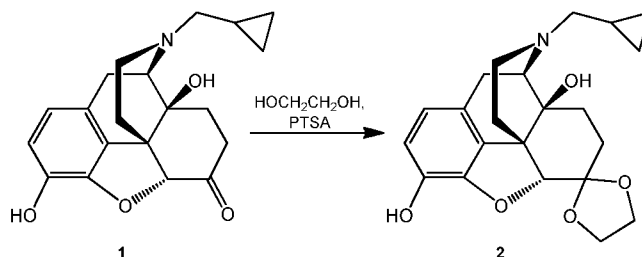
Mobile phase A: 95:5 Water: Methanol + 0.1% TFA

Mobile phase B: Methanol + 0.1% TFA

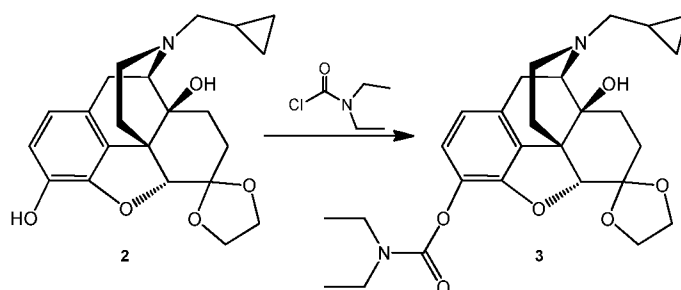
Gradient	
<i>Time</i>	<i>%B</i>
0.00	0
0.10	0
22.50	10.26
23.00	60.0
28.00	60.0
28.50	0

Temp.: 50°C; Flow: 30.0 mL/min; Inj. Vol.: 1.0 mL

[0058] Clean fractions were combined and concentrated at 40°C *in vacuo* to give an oil which was then repeatedly azeotroped with EtOAc to provide a solid (100 mg) having approximately 84% purity.

Example 3: Synthesis of 10'-OH-(R),(R)-2,2'-bis-MNTXKetalization of Naltrexone HCl

[0059] The ketone of the Naltrexone HCl was protected as cyclic ketal with ethylene glycol using p-toluenesulfonic acid (PTSA) as a catalyst. The naltrexone HCl (70 g) was taken up in a mixture of ethylene glycol (316 g) and toluene (700 mL). To this stirred mixture, catalytic amount of PTSA was added and refluxed in a dean-stark apparatus overnight (20h) to remove the water. The progress of the reaction was periodically monitored using HPLC. Reaction was stopped at the disappearance of starting material on LC. Upon completion of reaction, the layers were separated. To the bottom layer, water was added (18 vol) and cooled to 0°C. The pH of the solution was then adjusted to 9-10 by the addition of sodium carbonate. The resulting solid was stirred at room temperature for 1h, filtered, washed with water and dried on the frit for 1h followed by *in vacuo* at 35°C for 18h to give 62 g (87%) of the ketal **2**. NMR is consistent with the reported values.

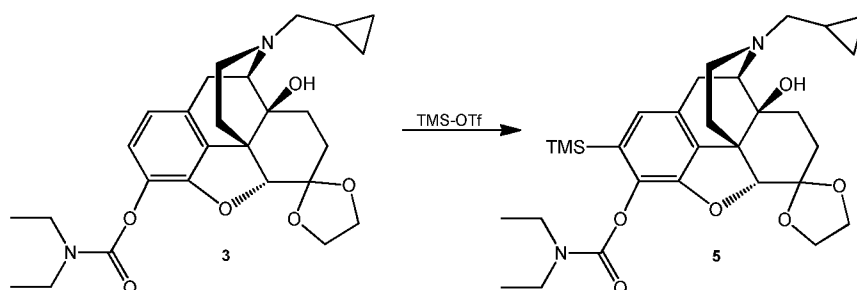
Carbamate Protection

[0060] The phenol-ketal **2** was protected as N,N-diethylamino carbamate using corresponding carbamoyl chloride. N,N-diethyl carbamoyl chloride was added to a mixture of the protected ketal **2** (50 g) and pyridine (250 mL) and the batch was heated to 110°C for 30h. Conversion to the carbamate **3** was periodically monitored by LC. Upon completion of reaction, the mixture was cooled and concentrated in a rotavap to remove pyridine. To this residue, water was added and the pH was adjusted to ~9 with saturated bicarbonate solution.

The product was then extracted into ethyl acetate (3 × 200 mL). The combined organic extracts were washed with water, brine and dried over anhydrous sodium sulfate.

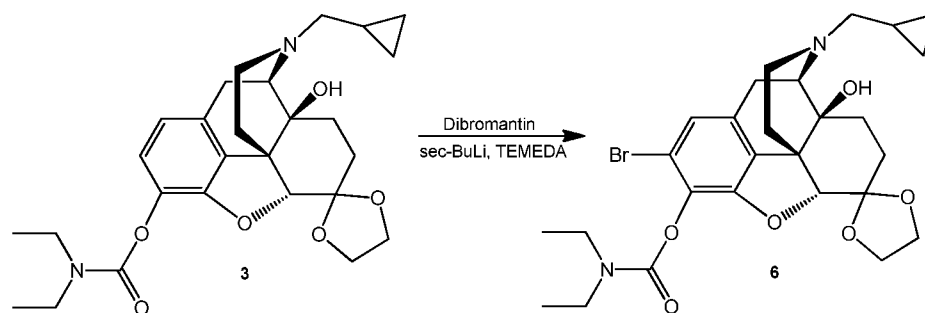
[0061] Concentration of the ethyl acetate solution provided a light tan solid. The crude product was purified by column chromatography using (0-5% MeOH/DCM). Concentration of pure fractions provided 45 g (71%) of compound **3** as a white solid. NMR analysis was consistent with the structure.

Silylation using *ortho*-Lithiation:



[0062] The protected carbamate **3** (1 g scale) was then subjected to *ortho* lithiation. Addition of LDA to a mixture of compound **3** and TMSOTf gave rise to compound **5**. Optionally, *sec*-BuLi was used to *ortho* lithiate the aromatic ring at -78 °C followed by reaction with TMSOTf to provide compound **5**. LC/MS data indicates the formation of the TMS product.

Bromination of the carbamate compound:



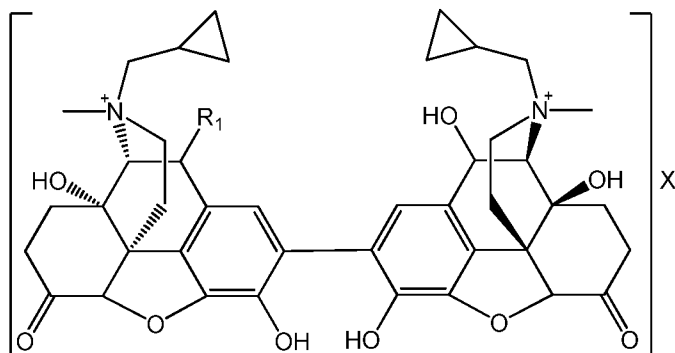
[0063] Bromination of the carbamate compound **3** was also performed using 1,3-dibromo-5,5-dimethylhydantoin (dibromatin) as a bromine source and *sec*-BuLi/TMEDA. Reaction produced the desired bromide intermediate **6**. The crude material was purified by column chromatography, producing intermediate **6** with a 35% yield. The bromination reaction was performed on a 10-gram scale.

EQUIVALENTS

[0064] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures described herein. Such equivalents are considered to be within the scope of the present invention and are covered by the following claims. The contents of all references, patents, and patent applications cited throughout this application are hereby incorporated by reference. The appropriate components, processes, and methods of those patents, applications and other documents may be selected for the present invention and embodiments thereof.

CLAIMS

1. A compound of Formula (I):



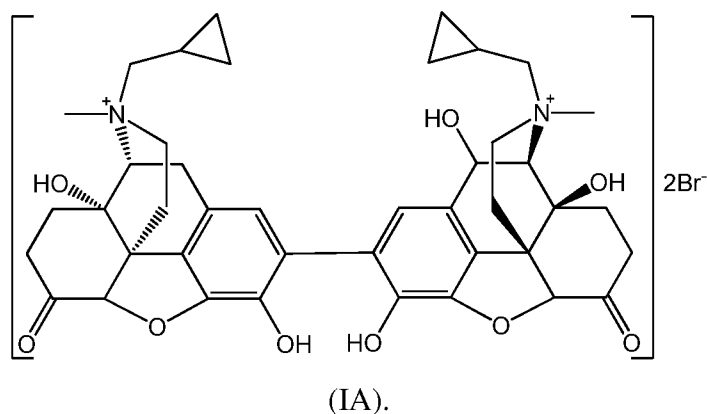
(I);

wherein R₁ is selected from hydrogen and hydroxy; and

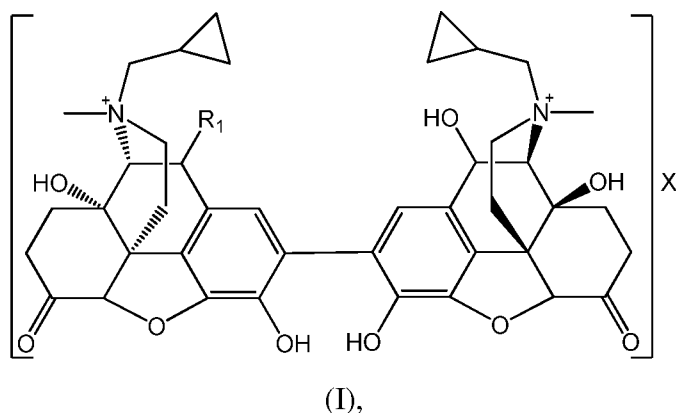
X is a counterion moiety with a net charge of -2.

2. The compound of claim 1, wherein X comprises two counterions, each with a charge of -1.
3. The compound of claim 1, wherein X comprises a halide.
4. The compound of claim 1, wherein X is 2Br⁻.
5. The compound of any one of claims 1-4, wherein the compound is isolated with at least 75% purity.
6. The compound of any one of claims 1-4, wherein the compound is isolated with at least 80% purity.
7. The compound of any one of claims 1-4, wherein the compound is isolated with at least 85% purity.
8. The compound of any one of claims 1-7, wherein the compound is a solid.

9. A compound of Formula (IA):

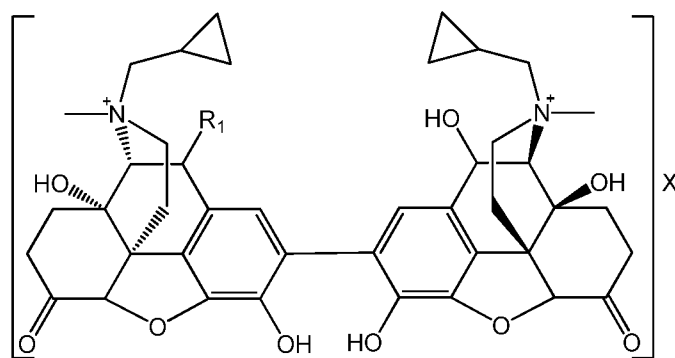


10. A composition comprising a compound of any one of claims 1-4, wherein the composition comprises less than about 20% total impurities.
11. A composition comprising a compound of any one of claims 1-4, wherein the composition comprises less than about 10% total impurities.
12. A composition comprising a compound of any one of claims 1-4, wherein the composition comprises less than about 5% total impurities.
13. A method of synthesizing a compound of Formula (I):



the method comprising coupling 10-OH-(R)-MNTX with a compound selected from (R)-MNTX and 10-OH-(R)-MNTX such that a compound of Formula (I) is formed.

14. A method of determining the presence and/or amount of a compound of Formula (I):



(I),

in a sample, the method comprising:

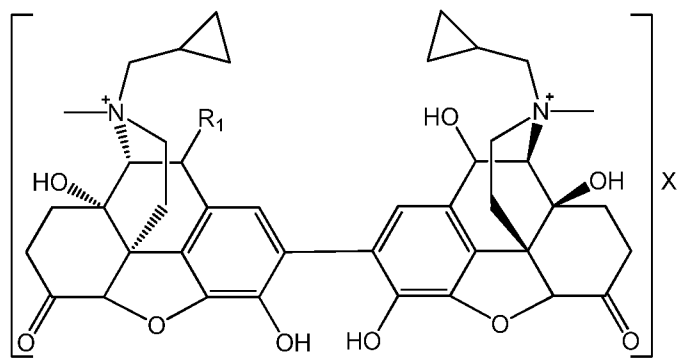
injecting a reference solution of a compound of Formula (I) into an HPLC column under a set of conditions to obtain a first HPLC chromatogram wherein the amount and/or chemical identity of the compound of Formula (I) present in the reference solution is known;

injecting a sample solution into the HPLC column under the same set of conditions to obtain a second HPLC chromatogram; and

comparing the first HPLC chromatogram with the second HPLC chromatogram to determine the presence and/or amount of the compound of Formula (I).

15. The method of claim 14, wherein the sample is a sample comprising (R)-MNTX.
16. The method of claim 14, wherein the presence of a compound of Formula (I) in the sample solution is determined by comparing retention times of peaks in the first HPLC chromatogram with the retention times of peaks in the second HPLC chromatogram.

17. A method for determining the purity of a sample, the method comprising:
 injecting a reference solution comprising a compound of Formula (I):



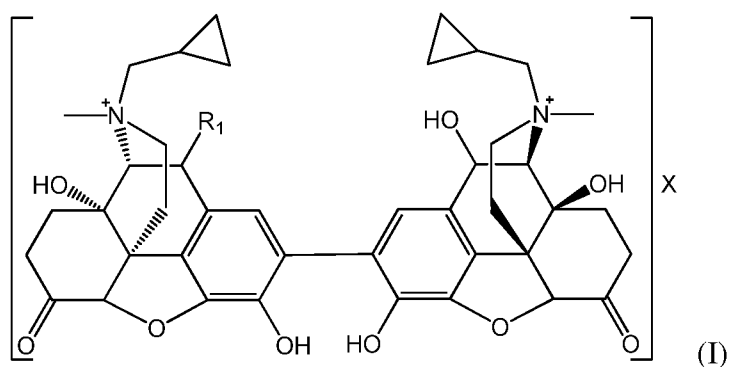
into an HPLC column under a set of conditions to obtain a first HPLC chromatogram wherein the amount and/or chemical identity of the compound of Formula (I) present in the reference solution is known;

injecting a sample solution into the HPLC column under the same set of conditions to obtain a second HPLC chromatogram; and

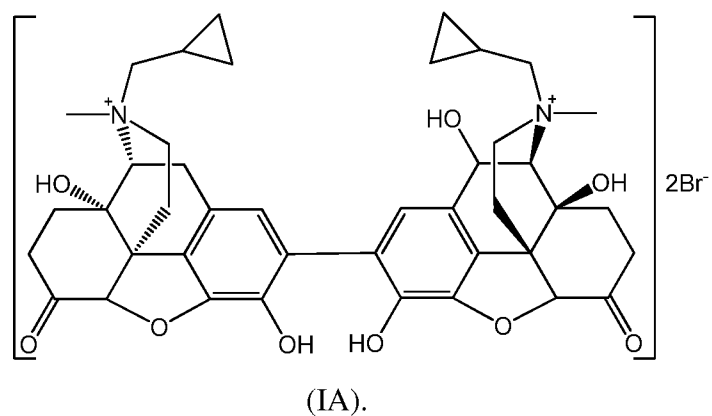
comparing the first HPLC chromatogram with the second HPLC chromatogram to determine the presence and/or amount of the compound of Formula (I) in the sample.

18. The method of claim 17, wherein the sample is a sample comprising (R)-MNTX.
19. The method of claim 18, wherein the presence of the compound of Formula (I) in the sample indicates an impure sample.
20. The method of claim 17, wherein the presence of a compound of Formula (I) in the sample solution is determined by comparing retention times of peaks in the first HPLC chromatogram with the retention times of peaks in the second HPLC chromatogram.

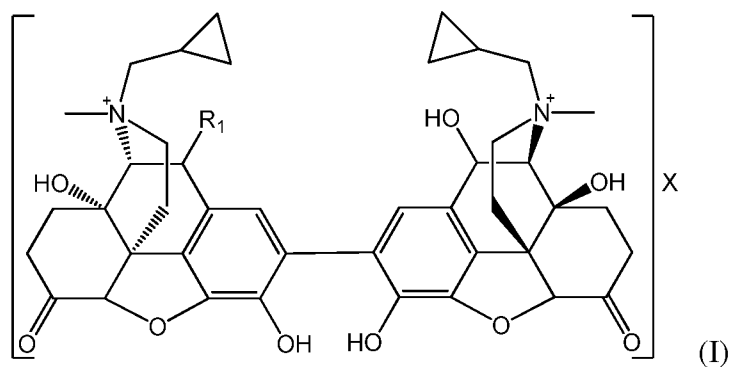
21. A composition comprising methylnaltrexone, wherein the composition comprises $\leq 0.2\%$ of a compound of Formula (I):



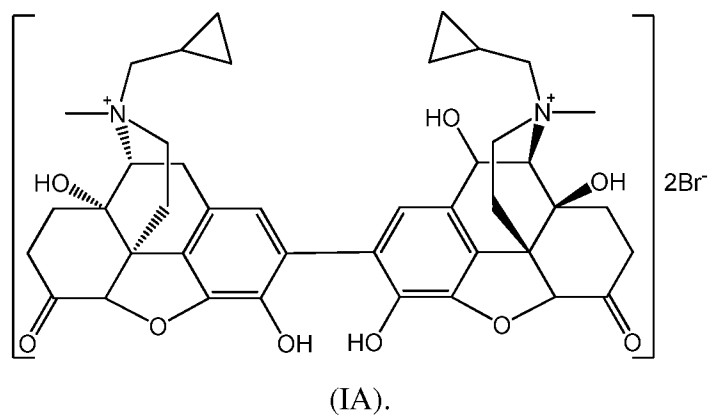
22. A composition comprising methylnaltrexone, wherein the composition comprises $\leq 0.2\%$ of a compound of Formula (IA):



23. A pharmaceutical composition comprising methylnaltrexone, wherein the pharmaceutical composition comprises $\leq 0.2\%$ of a compound of Formula (I):



24. A pharmaceutical composition comprising methylnaltrexone, wherein the pharmaceutical composition comprises $\leq 0.2\%$ of a compound of Formula (IA):



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US15/41848

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C07D 489/02 (2015.01)

CPC - C07D 489/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8) - C07D 489/02, 489/04; A61K 31/485 (2015.01)

CPC - C07D 489/08; G01N 30/24

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, INPADOC Data); ProQuest; Ebsco; Google; Google Scholar; SureChem; PubMed; PubChem; methyl naltrexone; (R)-methyl naltrexone; (R)-MNTX; (R)-N-(cyclopropylmethyl)noroxymorphone methobromide; (R),(R)-2,2'-bis-methyl naltrexone; hydroxyl-(R)-2-methyl naltrexone; 10-OH-(R)-MNTX

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2013/0296570 A1 (PROGENICS PHARMACEUTICALS) 7 November 2013; paragraphs [0005]-[0010], [0013]-[0017], [0028]-[0032].	1-4, 5/1-4, 6/1-4, 7/1-4, 9, 10/1-4, 11/1-4, 12/1-4 and 13-24
Y	US 2011/0190331 A1 (AVEY AA et al.) 4 August 2011; Table 6; paragraph [0054].	1-4, 5/1-4, 6/1-4, 7/1-4, 9, 10/1-4, 11/1-4, 12/1-4 and 13-24

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

29 September 2015 (29.09.2015)

Date of mailing of the international search report

28 OCT 2015

Name and mailing address of the ISA/

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Shane Thomas

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US15/41848

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 8
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.