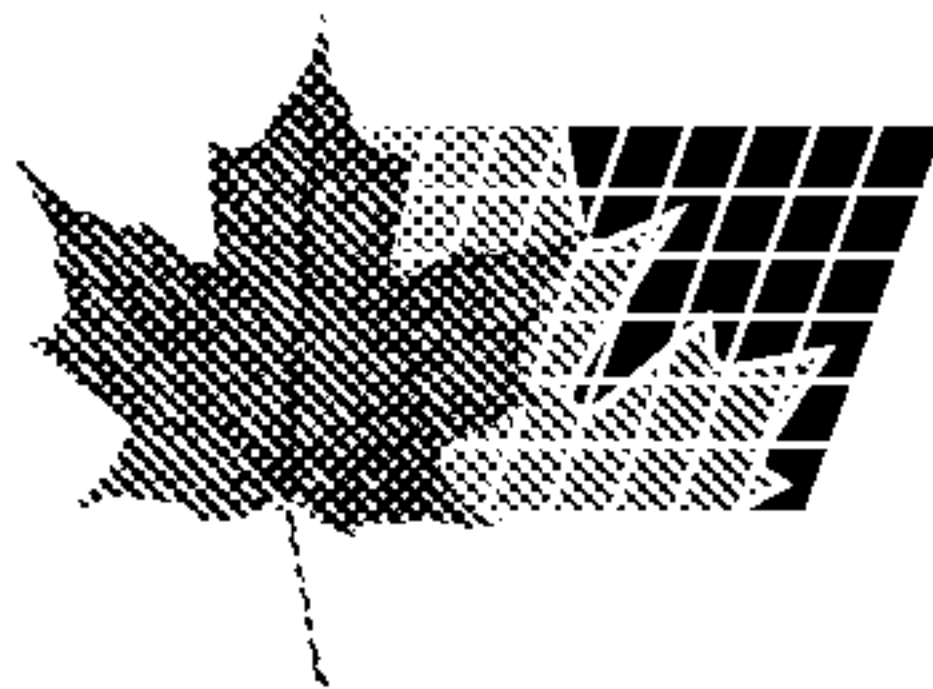




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(54) **REACTIFS POUR LE DEPISTAGE DES DROGUES**

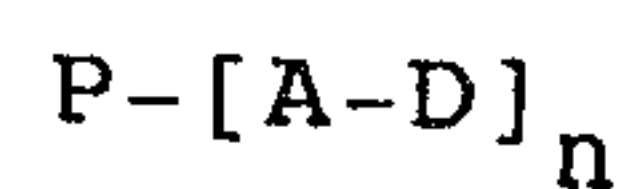
(54) **REAGENTS FOR THE DETERMINATION OF DRUGS**

(57) Novel reagents for the detection by immunoassay of drugs in body fluids, their preparation and use are disclosed. The reagents of the present invention correspond to the formula $P-[A-D]_n$ where: D is a drug derivative suitably selective for the determination of the presence of the target drug or drug metabolite, A is an activating linker-spacer group having an N-hydroxysuccinimide or isothiocyanate derived linking moiety, P is a poly(amino acid) or polymer capable of covalently bonding with A, and n is less than 1.

5

Abstract

Novel reagents for the detection by immunoassay of drugs
in body fluids, their preparation and use are disclosed.
The reagents of the present invention correspond to the
10 formula



where:

15

D is a drug derivative suitably selective for the
determination of the presence of the target drug or drug
metabolite,

20

A is an activating linker-spacer group having an
N-hydroxysuccinimide or isothiocyanate derived linking
moiety,

P is a poly(amino acid) or polymer capable of covalently
25 bonding with A, and

n is less than 1.

30

35

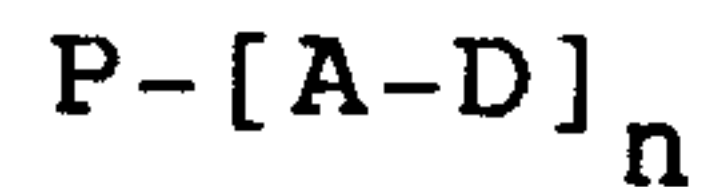
5 This invention relates to the field of the detection of
drug use by immunoassay testing of bodily fluids. More
particularly the invention relates to the search for
reagents which will provide sensitive, specific and
reproducible performance in the context of microparticle
10 based immunoassay formats. There is a widely acknowledged
need for improvements in such assays for detecting both
drugs of abuse and therapeutic agents.

 In immunoassay approaches for the detection of drugs,
15 various parent drugs, drug metabolites, as well as synthetic
drug derivatives, have been explored as haptenic starting
points for the development of one or more reagents in
various assay systems. These various compounds have
exhibited their own advantages and disadvantages when
20 employed as haptens per se, or when used in, or when further
modified for use in, specific immunoassay formats.

 The use of such compounds as haptenic ligands in
microparticle based immunoassays, i.e., linked to a
25 microparticle substrate in a variety of systems, has also
been explored. Usually, these systems have as their goal
the enhancement of the speed, specificity and, most
importantly, the detection of the ultimate interaction or
competition of the microparticle bound ligand with the
30 antibody or drug present in a sample.

 The present invention relates to novel reagents for the
detection of drug use by antibody mediated diagnostic
testing of a sample of bodily fluid. The reagents of the
35

present invention correspond to the formula:



5 where:

D is a drug derivative suitably antigenically selective for the determination of the presence of target drug or target drug metabolite,

10

A is an activating linker-spacer group having an N-hydroxysuccinimide or isothiocyanate derived linking moiety,

15

P is a poly(amino acid) or polymer capable of covalently bonding with A, and

n is less than 1.

20

Microparticle reagents fabricated from the foregoing conjugates as well as several of the activated drug derivatives, A-D, constructed for use therein, are further novel aspects of the present invention.

25

Figure 1 represents a plotting of the reaction sensitivity against the stoichiometric ratio of the A-D and P reactants used in the preparation of $P-[A-D]_n$ conjugates of the present invention as utilized in a latex microparticle reagent based agglutination assay. Reactivity
30 is plotted as the degree of agglutination on a relative scale of 0 to 4. The graph shows the results of varying ratios for the PCP (phencyclidine) activated derivative N-[1-(4-isothiocyanophenyl)ethyl]-4-[1-(1-piperidinyl)-1-cyclohexyl]benzamide and the benzoylecgonine derivative
35 [1R-(exo,exo,anti)]-8-Methyl-3-[[4-[[[[[[[(2,5-dioxo-pyrrolidin-1-yl)oxy]carbonyl]phenyl]amino](thioxomethyl)]-amino]methyl]benzoyl]oxy]bicyclo[3,2,1]octane-2-carboxylic

acid, both as the bovine serum albumin-latex microparticle reagent.

The present invention provides new and useful reagents
5 for the detection of drug use in antibody mediated testing
of bodily fluids. The reagents of the present invention
consist of a poly(amino acid) or polymer conjugate which in
turn is useful as an intermediate reagent for binding to
microparticular matrix, thereby providing an especially
10 rapid acting, sensitive and accurate reagent for
microparticle based testing such as latex agglutination.
The reagents of the present invention correspond to the
formula:



where:

D is a drug derivative suitably antigenically selective
20 for the determination of the presence of target drug or
target drug metabolite,

A is an activating linker-spacer group having an
N-hydroxysuccinimide or isothiocyanate derived linking
25 moiety,

P is a poly(amino acid) or polymer capable of covalently
bonding with A, and

30 n is less than 1.

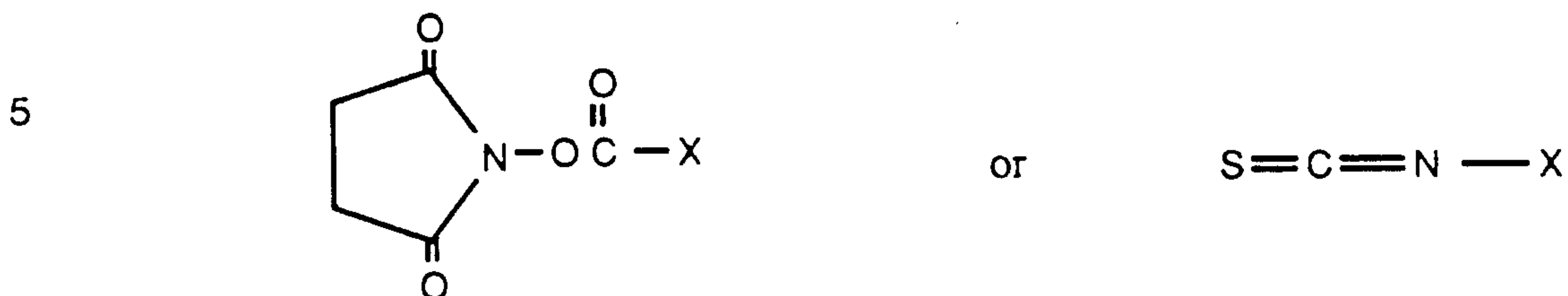
Of particular interest for the use of the subject
reagents is the conjugation to an amino group which is part
of a poly(amino acid) structure. By poly(amino acid) is
35 intended both polypeptides and proteins whether natural or
synthetic. Conjugation of the activated ester to poly(amino

acids) or to polymers containing amino groups is carried out by combining the appropriate ratio of the activated ester to poly(amino acid) or amino polymer under mild conditions, normally an organic/buffered aqueous solution of a pH range of about 6 to 9. A typically useful poly(amino acid) for the preparation of the reagents of the present invention is bovine serum albumin (BSA).

The ratio of activated drug derivative A-D to poly(amino acid) or polymer is an essential aspect of the reagents of the present invention and their high degree of sensitivity and reliability for the detection of drugs in the resulting diagnostic test. Because of their excellent antigenic reactivity, it has been found that for the activated drug derivatives of the present invention the ratio n of A-D to P must be less than 1, and typically below 0.5. Thus, allowing for the normally less than 100% yield that can be expected from the conjugation reaction of A-D with P (yields for the activated drug derivatives of the present invention with e.g. bovine serum albumin being most typically in the 50 to 75% range), the starting stoichiometric ratio of A-D to P for the reaction should be selected accordingly.

The optimal ratio n for a given activated drug derivative in accordance with the present invention can be determined readily by plotting reaction sensitivity in a desired assay format against the stoichiometric ratio of the reactants, i.e., A-D/P. Figure 1 shows a typical result of such a plotting for PCP (phencyclidine) and cocaine (benzoylecgonine) reagents in the case of a latex agglutination assay format. In accordance with standard practice, these assays are run against minimum clinically effective cutoff concentrations, i.e., as specified by the National Institute on Drug Abuse (Department of Health and Human Services), which e.g. for phencyclidine is 25 ng/ml and for cocaine is 300 ng/ml.

The activating linker-spacer group A can be a group of the formula



10 where X is an optional spacing group comprising a benzene, amide, thiourea or urea group or a straight or branched aliphatic chain of C_{1-10} , or a combination of the foregoing.

15 The drug derivative D may be a target drug itself, its metabolite or any other derivative which is sufficiently antigenically selective so as to indicate the presence of target drug or drug metabolite in a tested sample of bodily fluid, e.g., urine.

20

The activating group A is covalently linked to the drug, drug metabolite or drug derivative of choice at a sterically and reactively appropriate site which in the case of drugs of abuse reagents are preferably as follows:

25

For amphetamine and methamphetamine: at the ortho, meta or para carbon of the benzene ring, preferably para.

For THC: at the 9 carbon of the Δ^8 THC derivatives.

30 For barbiturate: at the 5 carbon of 5-substituted barbituric acid derivatives.

For phencyclidine: at the ortho, meta or para carbon of the benzene ring, preferably para.

For morphine: at the 3-hydroxyl.

35 For benzoyl ecgonine: at the meta or para carbon of the benzene ring, preferably para.

For 1,4 benzodiazepines: at the 1 nitrogen.

The reactive amino groups of P bind to A through amide (in the case of N-hydroxysuccinimide ester) or urea or thiourea (in the case of the isothiocyanates) linkage.

- 5 The subsequently purified protein conjugate is attached either covalently or hydrophobically, usually covalently to a microparticle that will be used as the antigenic reagent in a specific diagnostic test for the detection of drug.
- 10 Suitable microparticles for the covalent attachment of activated drug derivative-protein conjugates would be carboxylated styrene butadienes, carboxylated polystyrenes, acrylic acid polymers and the like. Suitable
- 15 micro-particles for the hydrophobic attachment of protein conjugate would be polystyrene, polyvinyltoluene, polydivinylbenzene, polyvinylchloride, polytertiary butylstyrene and the like. Suitable microparticles for the direct covalent attachment of the activated ester would be amino polystyrene, aminopolyvinyltoluene and the like.
- 20 Suitable magnetic microparticles for the attachment of protein conjugates would be the respective polymer impregnated with a magnetic metal such as ferric or ferrous oxides.
- 25 The size of microparticles will typically range from about 0.01 to about 1.0 microns. The size of magnetic microparticles will range from about 0.1 to about 20 microns.

30 Once the particular microparticle coupled product is formed, it can be utilized in specific diagnostic tests for the detection of drug in biological fluids. It can be used in any convenient concentration, depending upon the specific test, the mode of reading the results and the type of samples involved. In a urine latex assay visualized by the

35 naked eye the concentration by weight, of latex solids will be from about 0.1 to about 5.0%. In a urine latex assay employing an instrument to determine the degree of

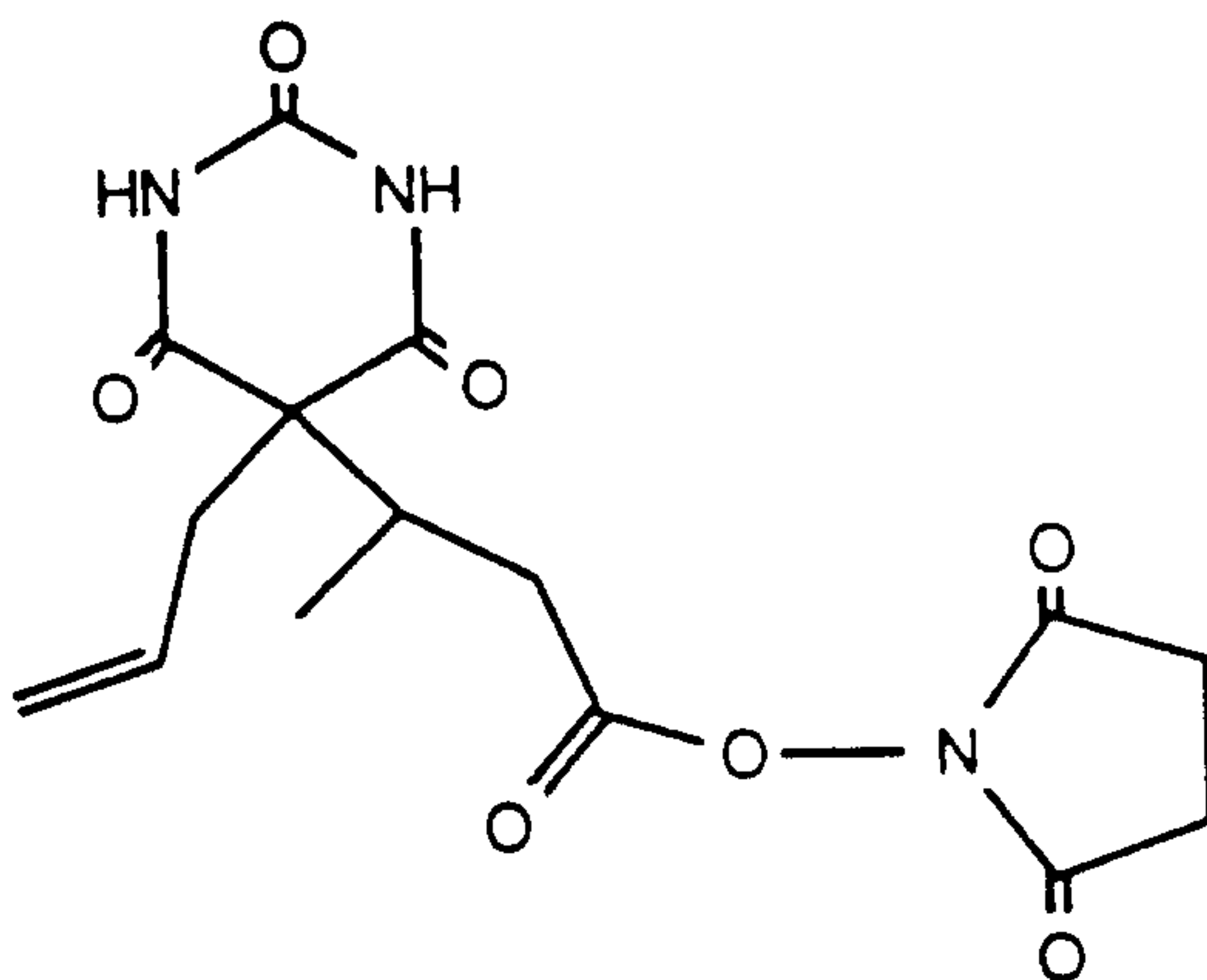
turbidity, in the range of 340 to 720 nanometers, the concentration by weight of latex solids will be from about 0.001 to about 1.0% A typical visual latex test is described in U.S. Patent No. 4,101,549.

5

For barbiturate, a preferred activated drug derivative is the N-hydroxysuccinimide ester derivative of the formula:

10

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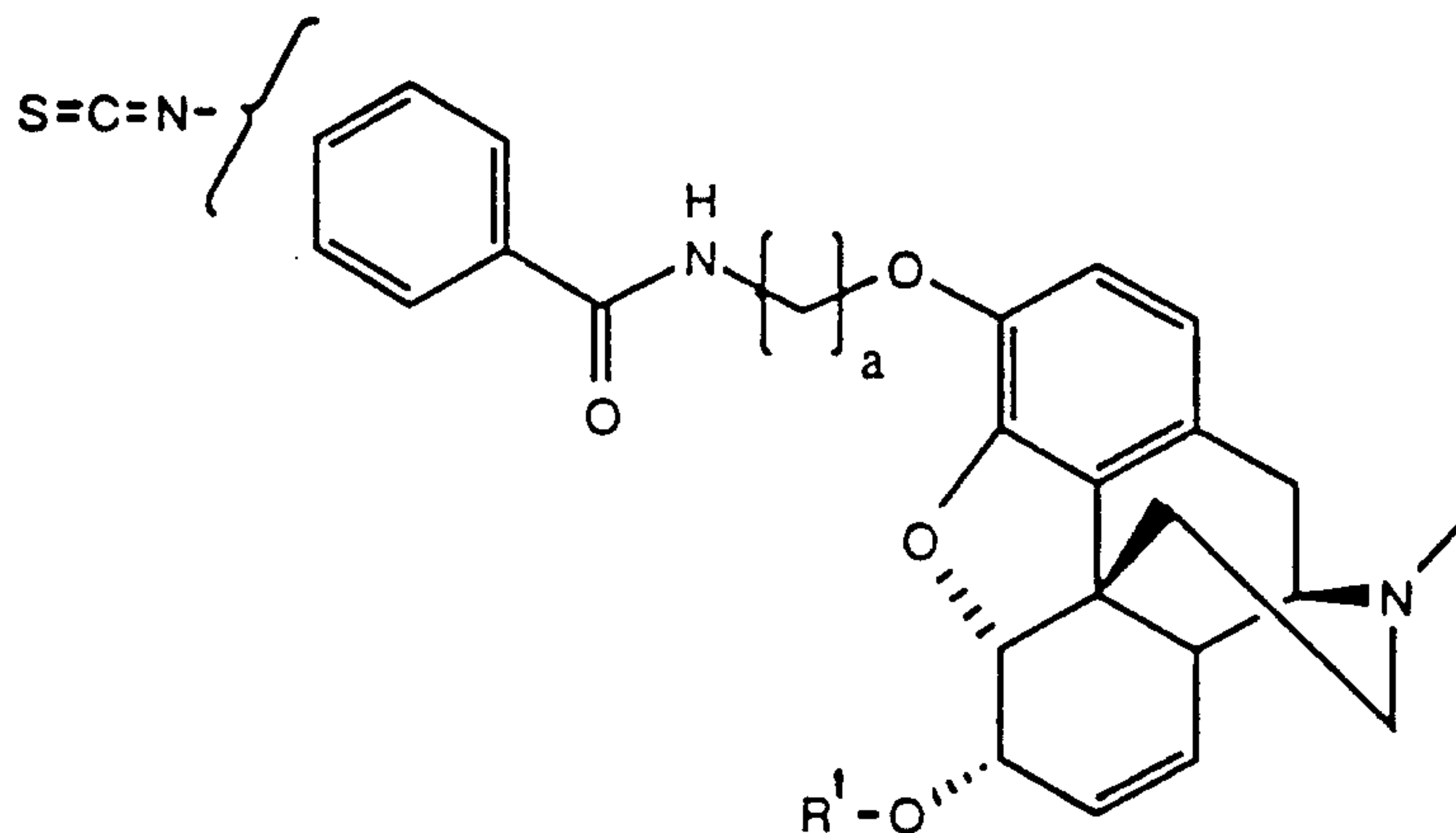
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The above derivative, along with other useful derivatives in accordance with the present invention, may be prepared by reacting the appropriate barbituric acid derivative such as disclosed in U.S. Patent No. 4,101,549, with N-hydroxysuccinimide in the presence of a carbodiimide. The preferred conjugate reagent may in turn be formed by binding the activated drug derivative to a poly(amino acid) or polymer generally through an amide linkage.

For morphine, preferred activated drug derivatives are the isothiocyanato derivatives of the formula:

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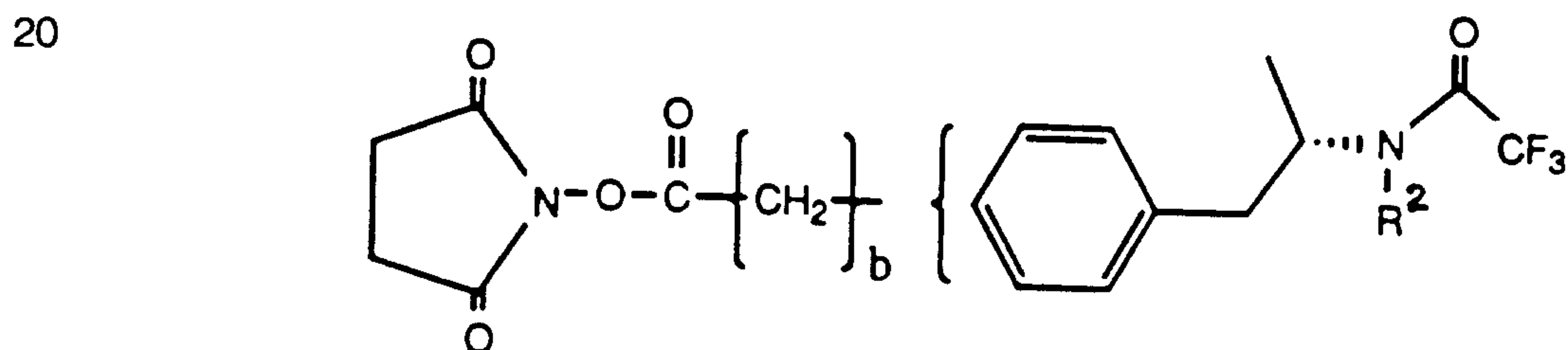
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where R^1 is hydrogen, methyl or acetyl, preferably hydrogen; a is 2 to 8, and is preferably 3; and the isothiocyanate moiety is in the meta or para position.

5 These isothiocyanates may be prepared by reacting the appropriately protected aminobenzoic acid with the desired alkylamino opiate derivative, such as disclosed in U.S. Patent No. 3,888,864. The amino group is then deprotected and derivatized to the isothiocyanate using thiophosgene
10 under mild conditions. The conjugated reagents are in turn formed by binding the activated drug derivative to a poly(amino acid) or polymer generally through a thiourea linkage.

15 For amphetamine and methamphetamine*, a preferred activated drug derivative is the N-hydroxysuccinimide ester derivative of the formula:



30 _____
* Unless otherwise specified, references herein to drugs or drug derivatives having known optical isomeric forms is to be taken as referring inclusively to the optical antipodes or racemate.

35

where: R^2 is hydrogen in the case of amphetamine and methyl in the case of methamphetamine, b is 1 to 9, preferably 3, and the alkyl chain is meta or para, preferably para.

5

The activated ester is prepared by reacting d-amphetamine (or methamphetamine) with trifluoroacetic anhydride. The resulting N-protected amphetamine is reacted with succinic anhydride in the presence of aluminum chloride
10 to give a substituted protected amphetamine which contains an acid and ketone. The ketone is reduced with palladium on charcoal in an atmosphere of hydrogen and the acid is then converted to an activated ester with N-hydroxysuccinimide in the presence of a carbodiimide.

15

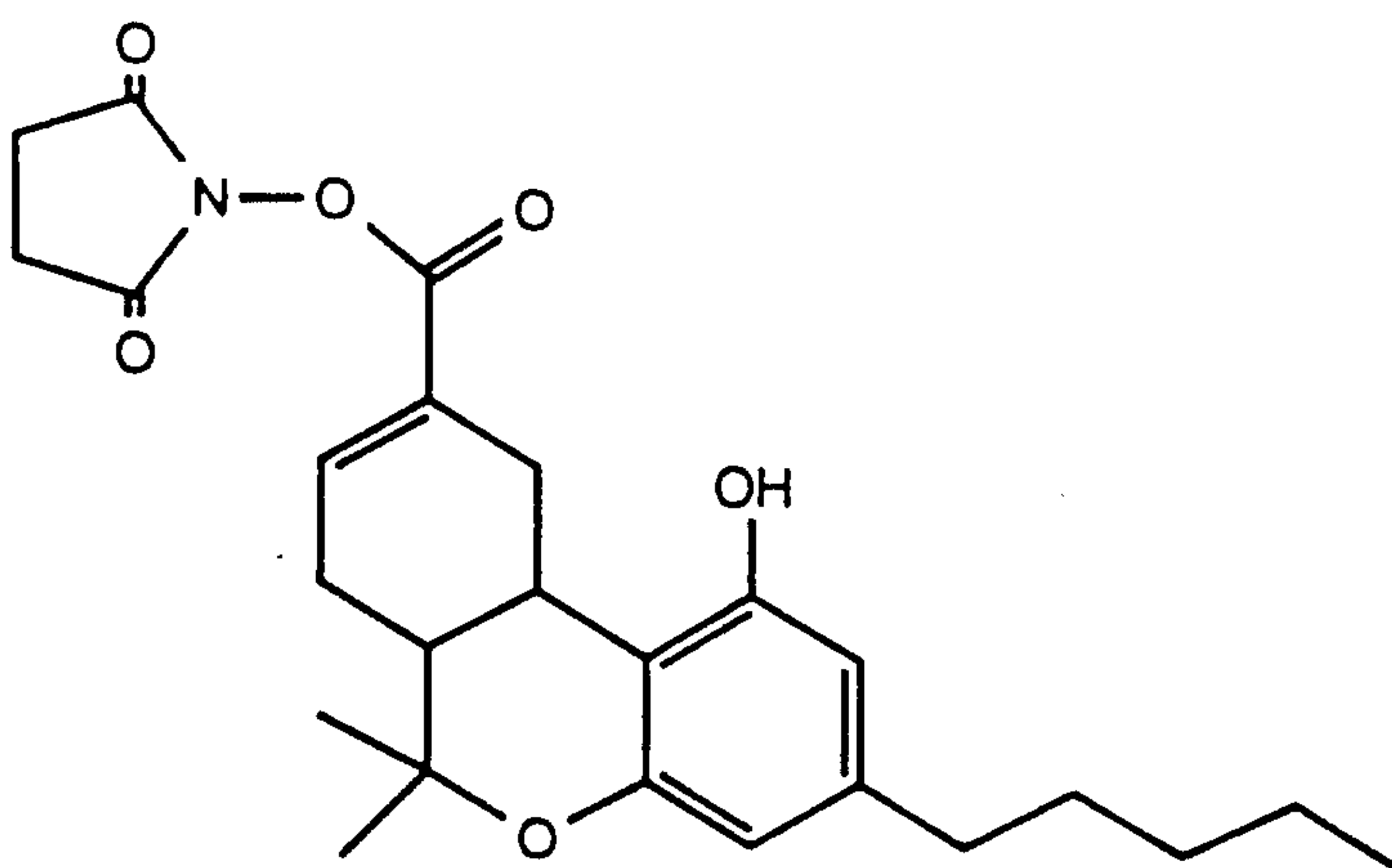
The protecting group, in this case the trifluoroacetyl, is removed prior to use in an antibody mediated assay, as for example after preparing a microparticle (e.g. latex) reagent.

20

For tetrahydrocannabinoid, a preferred activated drug derivative is the N-hydroxysuccinimide ester derivative of the formula:

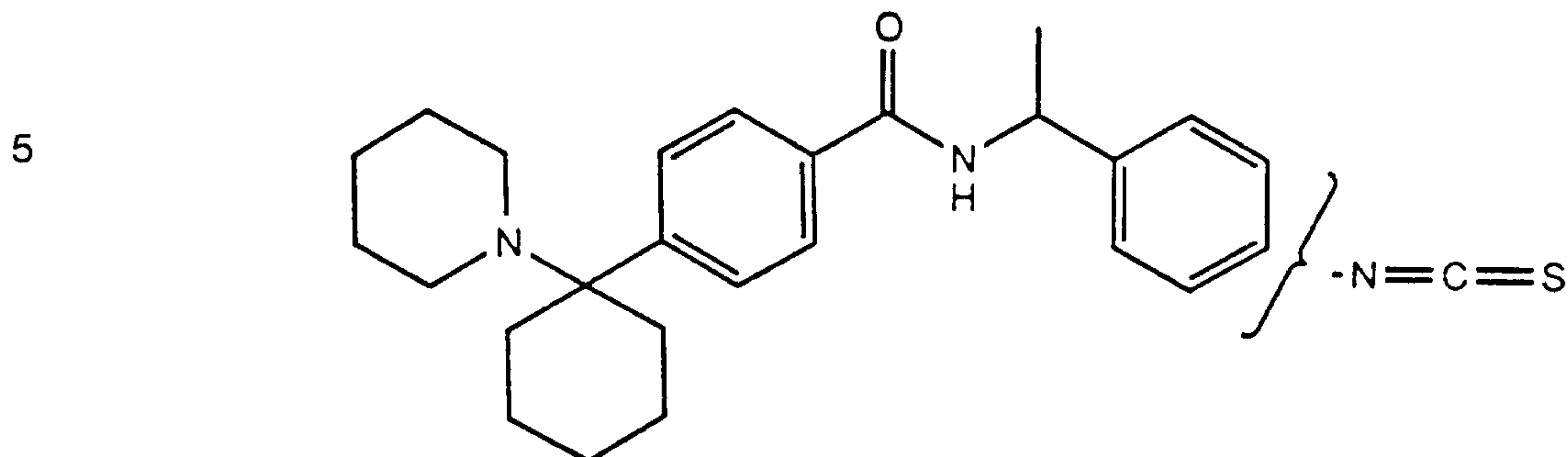
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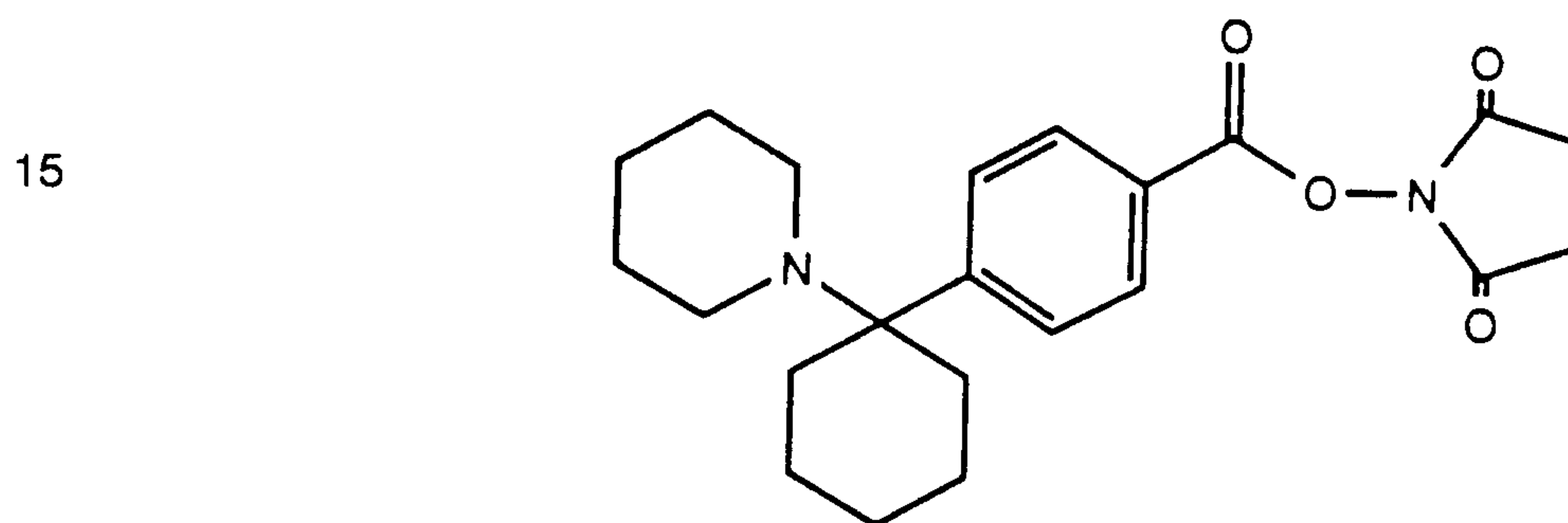


Such derivatives may be prepared by reacting the appropriate cannabinoid derivative, such as disclosed in
35 J. Org. Chem. (1986), 51, pp. 5463-5465 with N-hydroxysuccinimide in the presence of a carbodiimide.

For phencyclidine (PCP), preferred activated drug derivatives are the isothiocyanate of the formula



10 as well as the N-hydroxy succinimide ester derivative of the formula

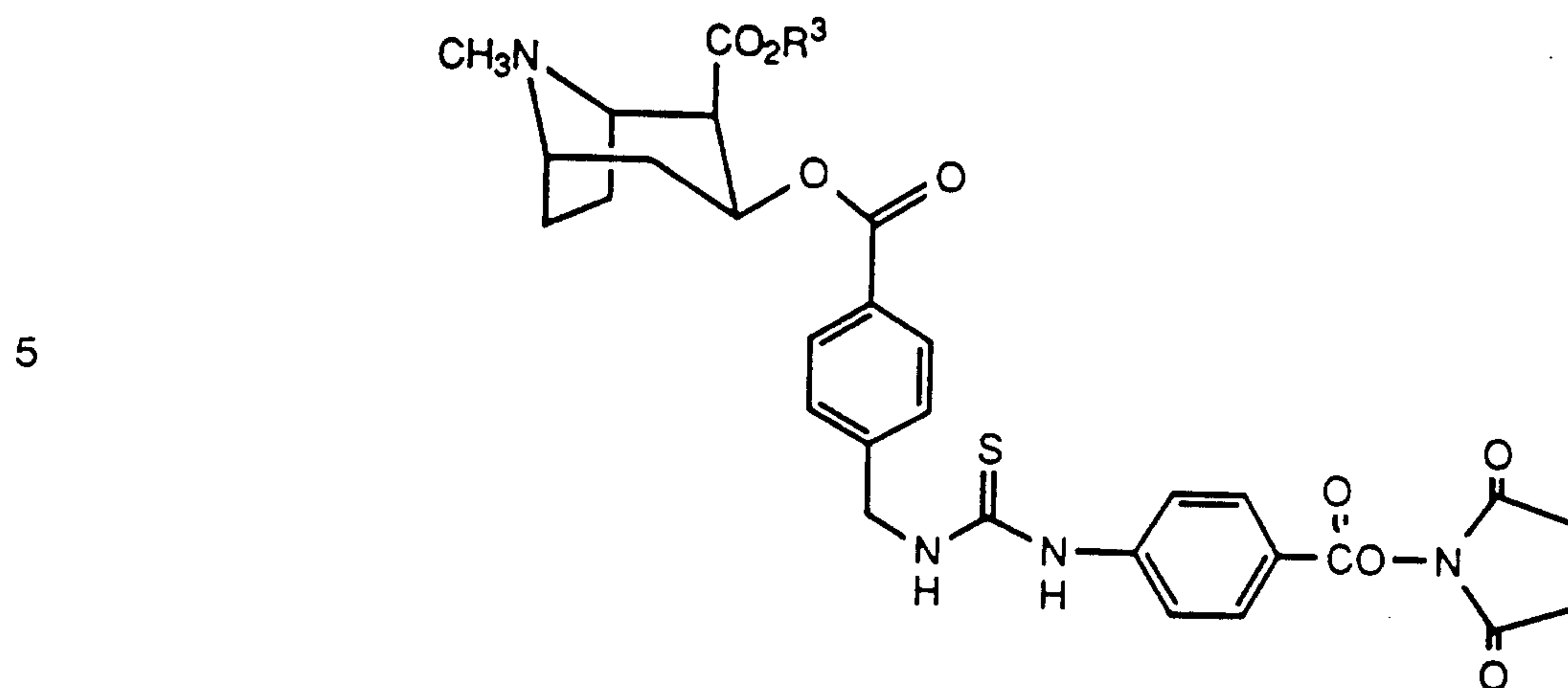


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The PCP isothiocyanate derivative may be prepared by reacting the PCP acid as disclosed in U.S. Patent No. 4,196,185, with thionyl chloride followed by reaction with
25 R-(+)p-nitro- α -methylbenzylamine hydrochloride. The nitro group is reduced with Raney nickel (H_2 , ethanol) and the resulting amino group is converted to an isothiocyanate in the same manner as described in Example 1 below.

30 The PCP N-hydroxysuccinimide ester derivative may be prepared by reacting the PCP acid as disclosed in U.S. Patent No. 4,196,185 in the same manner as described in Example 7 below.

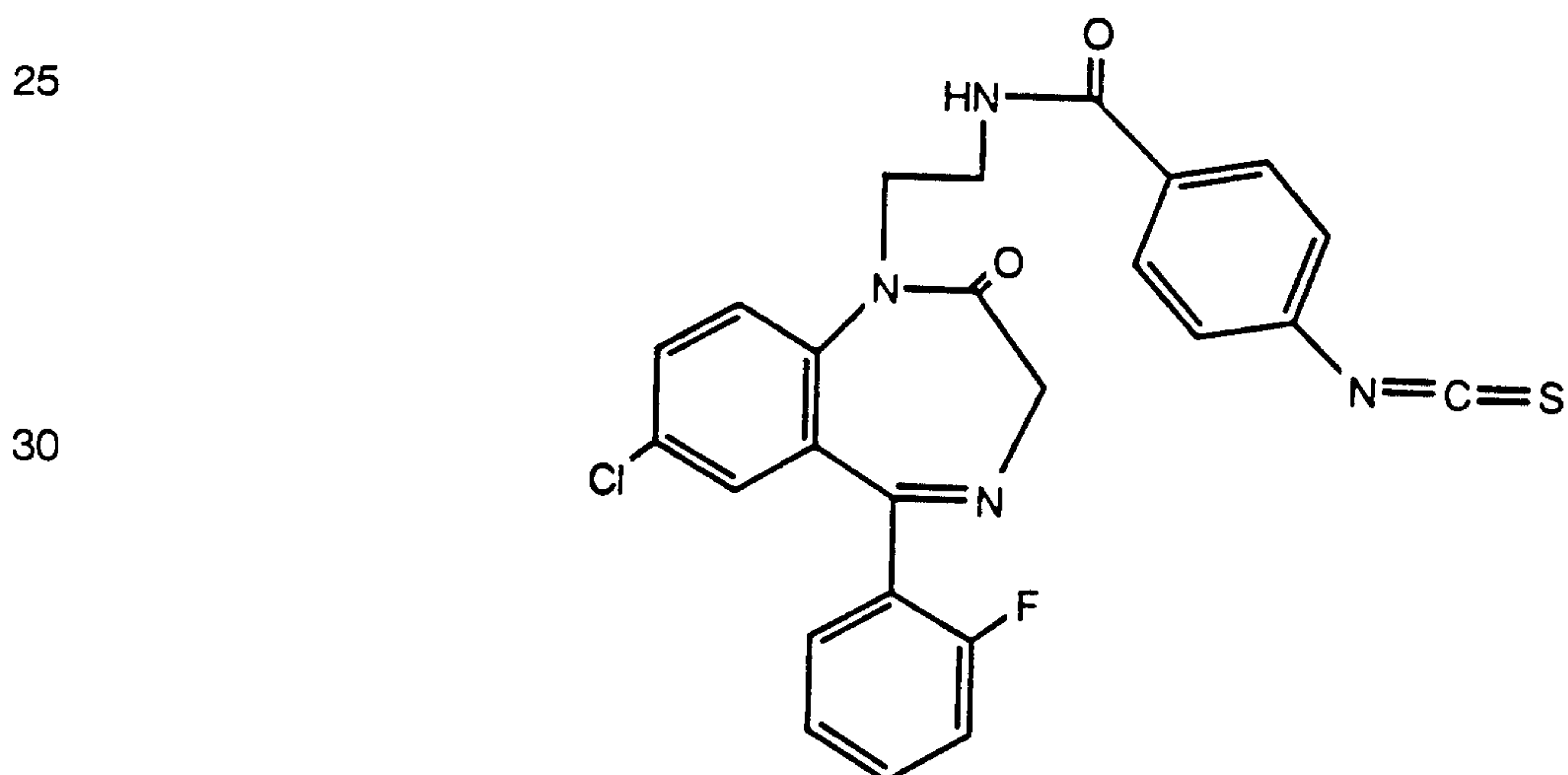
35 For benzoyl ecgonine, the preferred activated drug derivative is the N-hydroxysuccinimide ester derivative of the formula:



where R^3 is hydrogen or methyl, preferably hydrogen.

These ester derivatives may be prepared by reacting methylecgonine with the acid chloride of para-cyanobenzoic acid. The nitrile is reduced with Raney nickel (55 psi H_2 , methanol) and the methyl ester is then removed in boiling water. The amino group is then reacted with the isothiocyanate of para-aminobenzoic acid and the activated ester is prepared in the same manner as described in Example 7 below.

For benzodiazepines, a preferred activated drug derivative is the isothiocyanate derivative of the formula:



This isothiocyanate may be prepared by reacting 1-(2-aminoethyl)-7-chloro-1,3-dihydro-5-(2-fluorophenyl)-2H-1,

4-benzodiazepin-2-one as disclosed in J. Med. Chem., (1968), 11, pp. 774-777 in the same manner as described in Example 1 below where the amino group is reacted with protected para-aminobenzoic acid and carried on to the isothiocyanate.

5

Of the foregoing activated drug derivatives, the following compounds are most preferred:

For morphine: N-[3-[7,8-Didehydro-4,5-epoxy-6-
10 hydroxy-17-methylmorphinan-3-yl)oxy]propoxy]-4-isothiocyanato-
benzamide.

For barbiturate: 5-[3-[2,5-Dioxo-1-pyrrolidinyl)oxy]-1-
methyl-3-oxo=propyl]-5-(2-propenyl)2,4,6(1H,3H,5H)pyrimidi-
15 netrione.

For amphetamine: (S)-N[2-[4-[4-[(2,5-Dioxo-1-pyrro-
lidinyl)-oxy]-4-oxo-butyl]phenyl]-1-methyl-ethyl]-2-trifluoro-
acetamide.

20

For methamphetamine: (S)-N-Methyl-N[2-[4-[4-[(2,5-dioxo-
1-pyrrolidinyl)-oxy]-4-oxo-butyl]phenyl]-1-methyl-ethyl]-2-
trifluoroacetamide.

25 For tetrahydrocannabinoids: 1-[[[(6a,7,10,10a-Tetrahydro-
1-hydroxy-6,6-dimethyl-3-pentyl-6H-dibenzo)oxy][b,d]pyran-1-yl
(carbonyl)oxy]-2,5-pyrrolidinedione.

For phencyclidine: N-[1-(4-Isothiocyanophenyl)ethyl]-
30 4-[1-(1-piperidinyl)-1-cyclohexyl]benzamide.

For benzodiazepines: N-[2-[7-Chloro-5-(2-fluorophenyl)-
2,3-dihydro-2-oxo-1H-1,4-benzodiazepin-1-yl]ethyl]-4-isothio-
cyanatobenzamide.

35

For benzoylecgonine: [1R-(exo,exo,anti)]-8-Methyl-3-
[[4-[[[[[[[[(2,5-dioxopyrrolidin-1-yl)oxy]carbonyl]phenyl]amino]

(thioxomethyl)]amino]methyl]benzoyl]oxy]bicyclo[3,2,1]octane-2-carboxylic acid.

5 Preferred poly(amino acid) conjugates of the above activated drug derivatives are the bovine serum albumin (BSA) conjugates and the preferred microparticle reagent embodiment incorporates these conjugates by covalent amide bonding to the surface of carboxylated latex microparticles.

10 The invention is further explained and illustrated in the following examples. All temperatures are in degrees centigrade.

Example 1

15

Preparation of opiate reagent

Preparation of N-[3-[(7,8-Didehydro-4,5-epoxy-6-hydroxy-17-methylmorphinan-3-yl)oxy]propoxy]-4-N-(butylcarbamoyl)benzamide:
20

A stirred solution of 0.5g (1.46 mmol) of 3-O-amino-propylmorphine and 0.5g (2.1 mmol) of p-(1-t-butoxy-formamido)benzoic acid (formed by treating p-Aminobenzoic
25 acid with di-t-butyldicarbonate in the presence of 1 N NaOH) in 20 ml of methylene chloride and 5 ml of dry dimethyl formamide was treated with 0.5g (2.6 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and stirred at room temperature overnight. The reaction
30 mixture was washed with water (3 x 25 ml), dried over anhydrous sodium sulfate and concentrated in vacuo. The residue was chromatographed on 100g of silica using 15% methanol-chloroform as eluent to give 0.77g (1.37 mmol) of N-[3-[(7,8-Didehydro-4,5-epoxy-6-hydroxy-17-methylmorphinan-3-yl)oxy]propoxy]-4-(N-t-butyl-carbamoyl)-benzamide NMR
35 (CDCl₃) δ 1.64 (s, 9H, t-butyl), 2.56 (s, 3H, N-CH₃),

4.98(d,J=6Hz, 1H, ArOCH), 7.52(d,J=9H₂, 2Hz, Ar),
7.88(d,J=9Hz, 2H, Ar).

Preparation of N-[3-[(7,8-Didehydro-4,5-epoxy-6-hydroxy-17-
5 methylmorphinan-3-yl)oxy]propoxy]-4-aminobenzamide:

A stirred solution of 0.7g (1.24 mmol) of N-[3-[(7,8-Dide-
hydro-4,5-epoxy-6-hydroxy-17-methylmorphinan-3-yl)oxy]pro-
poxyl-4-N-(butylcarbamoyl)benzamide in 25 ml of methylene
10 chloride was treated with 5 ml of trifluoroacetic acid and
stirred at room temperature for 30 min. The solvent was
removed in vacuo and the residue was dissolved in methylene
chloride, washed with saturated aqueous sodium bicarbonate,
dried over anhydrous sodium sulfate, and concentrated in
15 vacuo to give 0.5 g of N-[3-[(7,8-Didehydro-4,5-epoxy-6-
hydroxy-17-methylmorphinan-3-yl)oxy]propoxyl-4-aminobenzamide.

NMR(CDCl₃) δ 2.53(s,3H,N-CH₃), 4.85 (d,J=6Hz, 1H,
ArOCH), 5.22 (d,J=10Hz, 1H, vinyl), 5.69 (d,J=10Hz, 1H,
20 vinyl), 6.45 - 6.66 (m, 4H, Ar), 7.61 (d,J=8Hz, 2H, Ar).

Preparation of N-[3-[(7,8-Didehydro-4,5-epoxy-6-hydroxy-
17-methylmorphinan-3-yl)oxy]propoxyl-4-isothiocyanatobenz-
amide:

25

A stirred solution of 0.5g (1.1 mmol) of N-[3-[(7,8-Dide-
hydro-4,5-epoxy-6-hydroxy-17-methylmorphinan-3-yl)oxy]pro-
poxyl-4-aminobenzamide in 10 ml of methylene chloride was
treated with 0.5 g of solid anhydrous sodium bicarbonate
30 followed by 150 ul (1.9 mmol) of thiophosgene and stirred at
room temperature for 30 minutes. The solvent was removed in
vacuo and the residue was chromatographed on 150g of silica
gel using 15% methanol-chloroform to give 230 mg of
N-[3-[(7,8-Didehydro-4,5-epoxy-6-hydroxy-17-methylmorphinan-3-
35 yl)oxy]propoxyl-4-isothiocyanatobenzamide as an amorphous
solid.

IR(CHCl₃) 3435(NH), 2100(NCS), 1652 (Amido), NMR(CDCl₃-
 DMSO-d₆) δ 1.92(s, 1, CH of CH₂), 2.07(t, J=6Hz, 2H, CH₂),
 3.08(d, J=19Hz, 1, CH of CH₂), 3.53(bs, 1, OH),
 4.88(d, J=6Hz, 1H, ArOCH), 5.28(d, J=10Hz, 1H, vinyl),
 5.72(d, J=10Hz, 1H, vinyl), 6.57 (d, J=6Hz, 2H, Ar), 6.69
 (d, J=6Hz, 2H, Ar), 7.28(d, J=9Hz, 2H, Ar), 7.91(d, J=9Hz, 2H,
 Ar), 7.55(bs, 1H, NH). MS, (+FAB) m/e 504(M+H)

Conjugation of N-[3-[7,8-Didehydro-4,5-epoxy-6-hydroxy-
 17-methylmorphinan-3-yl)oxy]propoxy]-4-isothiocyanatobenzamide
 (opiate) to Bovine Serum Albumin (BSA)

To a solution (175 ml) containing sodium bicarbonate (0.05
 M), 10% dimethylsulfoxide (DMSO), and BSA (5.0g) at pH 8.0,
 25° the isothiocyanate (0.004g in 14 ml of DMSO) was added
 dropwise. The reaction was stirred at 4° for 15 h at which
 point it was transferred to dialysis tubing and dialyzed
 first into an aqueous sodium bicarbonate DMSO (10%) solution
 (0.05M, pH 8, 4 x 10 volumes) and then into a sodium
 bicarbonate buffer (0.05M, pH 8, 7 x 10 volumes). After
 dialysis the protein concentration was measured and adjusted
 to 0.005g per ml.

Example 2

25

Preparation of sensitized latex containing the opiate-BSA
 conjugate - general procedure.

To a stirred latex (SeradynTM carboxy modified polystyrene
 0.888 microns) suspension (180 ml, 10% solids),
 N-hydroxybenzotriazole (NHB) (0.6g in 24 ml of aqueous
 dimethylformamide) and 1-cyclohexyl-3-(2-morpholinoethyl)-
 carbodiimide metho p-toluenesulfonate (CMC) (2.0g in 32 ml
 of water) were added. The suspension was stirred for 3 h at
 4° then diluted to 2% latex and extensively washed with
 water by tangential flow filtration. To the washed 2% latex
 were added 900 ml of the opiate-BSA solution from Example

1. The reaction was stirred overnight at 4° and was again extensively washed with water by tangential flow filtration. The washed latex was then adjusted to a 3% latex solids (by weight) concentration.

5

Example 3

Opiate Test

10 Preparation of reaction buffer for test:

This diluent consists of the following aqueous solution at pH 7.5:

- 15 1. Pipes [0.20M, 1,4-piperazine bis(ethanesulfonic acid)]
2. PVP [0.50%, Polyvinylpolypyrrolidone]
3. PEG 8000 [1.50%, Poly(ethylene glycol)]
4. Sodium Chloride [10%]
5. Sodium Azide [0.1%]

20

Preparation of antiserum buffer for test:

Purified mouse monoclonal antibody against opiates is diluted in an appropriate buffer system. This diluent
25 consists of the following in aqueous solution in pH 7.0:

1. Pipes (0.05M)
2. BSA (0.25%)
3. Sodium Chloride (0.15%)
30 4. Sodium Azide (0.15%)

Test Methodology:

Ten microliters of opiate free urine is dispensed into a
35 mixing well of a capillary agglutinography slide followed by
50 microliters each of antiserum buffer, reaction buffer and
latex (example 2). The added liquids are stirred for three

seconds and the solution is moved to allow contact with the capillary. The liquid will move through the capillary and fill the viewing area at the end of the capillary in three to six minutes. The liquid in the viewing area will appear
5 as fine floccules (agglutinated) or milky. For negative samples agglutination should be very strong with very little cloudiness.

The dilution of a particular antiserum which is chosen for
10 the test is the one which has the highest dilution that will still produce strong agglutination in the viewing area. When various amounts of opiates are dissolved in opiate free urine in the test systems, no agglutination occurs. The amount of opiate required to inhibit the agglutination will
15 vary from 100 nanograms per ml or greater depending on the concentration of antiserum used and the strength of antiserum produced. Thus, for the system described above a urine containing 300 nanograms of opiate per ml is
sufficient to partially inhibit agglutination and a urine
20 containing 600 nanograms of opiate per ml is sufficient to completely inhibit agglutination.

The following table reports the type of cross-reactivity that is observed in the system described above:
25

30

35

20 1 1722

- 18 -

		Concentration (ng/ml) equivalent to 300 ng/ml of Morphine	% Cross reactivity
5	Codeine	250	120
	Dihydrocodeine bitartrate	500	60
	Dihydromorphine	400	75
	Ethylmorphine HCl	400	75
	Hydrocodone bitartrate	800	38
10	Hydromorphone HCl	800	38
	Meperidine	50,000	<0.6
	Morphine 3-glucuronide	800	38
	N-Norcodeine HCl	100,000	<0.3
	Oxycodone	50,000	<0.6
15	Thebaine	1,600	19

The following table reports the type of specificity and accuracy that is observed with the above described assay when used with samples positive for opiates:

20

		Gas Chromatography- Mass Spectroscopy +
25	Agglutination	+ 54
	Test	- 0

30

Example 4

Preparation of barbiturate reagent

Preparation of 5-[3-[(2,5-Dioxo-1-pyrrolidinyl)oxy]-1-methyl-
35 3-oxopropyl]-5-(2-propenyl)-2,4,6(1H,3H,5H)pyrimidinetrione:

To a solution of 5-allyl-5-(1-carboxyisopropyl)barbituric acid (5g, 19.6 mmol) in methylene chloride (150 ml) and dimethylformamide (25 ml), N-hydroxysuccinimide (2.5g, 21.7 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (4.5g, 23.4 mmol) were added. The reaction was stirred overnight at room temperature and was then concentrated in vacuo. The residue was dissolved in methylene chloride washed three times with hydrochloric acid (0.2N, 200 ml), once with water and once with sodium bicarbonate. During the washings, a precipitate formed which was filtered to yield 2.4 grams of product. The aqueous phase was extracted with ethylacetate and all organic layers were combined, dried (Na_2SO_4), filtered and solvents evaporated to yield an additional 1.0g of product.

IR(KBr), 3245(Broad NH), 1810-1685($\text{C}=\text{O}$) cm^{-1} ,
 ^1H NMR($\text{DMSO}-d_6$), δ 0.97(d, $J=6\text{Hz}$, 3H, CH_3),
2.55-2.78(m, 4H, CH_2), 2.82 (s, 4H, CH_2), 5.11(t, 2H,
20 CH_2 , Vinyl), 5.52(m, 1H, CH, Vinyl), 11.68(bs, 2H, NH)
MS, (+)FAB, m/e 352($\text{M}+\text{H}$)

Anal. Calc. for C, 51.28; H, 4.87; N, 11.96;

Anal. found for C, 51.06; H, 4.91; N, 11.94.

25 Conjugation of 5-[3-[(2,5-Dioxo-1-pyrrolidinyl)oxy]-1-methyl-3-oxopropyl]-5-(2-propenyl)-2,4,6(1H,3H,5H)pyrimidine-trione (barbiturate) to Bovine Serum Albumin (BSA)

To a solution (400 ml) containing sodium bicarbonate (0.05
30 M), 10% dimethylsulfoxide (DMSO), and BSA (6.0g) at pH 8.5, 25° the N-hydroxysuccinimide ester (0.008g in 6.6 ml of DMSO) was added dropwise. The reaction was stirred at 4° for 15 h at which point it was transferred to dialysis tubing and dialyzed first into an aqueous sodium
35 bicarbonate-DMSO (10%) solution (0.05M, pH 8, 4 x 10 volumes) and then into a sodium phosphate buffer (0.05M, pH

7, 7 x 10 volumes). After dialysis the protein concentration was measured and adjusted to 0.005g per ml.

Example 5

5

Preparation of sensitized latex containing the barbiturate-BSA conjugate general procedure.

To a stirred latex (SeradynTM carboxy modified polystyrene 0.888 microns) suspension (180 ml, 10% solids), N-hydroxybenzotriazole (NHB) (0.6g in 24 ml of aqueous dimethylformamide) and 1-cyclohexyl-3-(2-morpholino-ethyl)carbodiimide metho p-toluenesulfonate (CMC) (2.0g in 32 ml of water) were added. The suspension was stirred for 3 h at 4° then diluted to 2% latex and extensively washed with water by tangential flow filtration. To the washed 2% latex were added 900 ml of the barbiturate-BSA solution from Example 4. The reaction was stirred overnight at 4° and was again extensively washed with water by tangential flow filtration. The washed latex was then adjusted to a 3% latex solids (by weight) concentration.

Example 6

25

Barbiturate Test

Preparation of reaction buffer for test:

This diluent consists of the following in aqueous solution at pH 7.5:

1. Hepes [0.20M, 4-(2-hydroxyethyl)-1-piperazineethane-sulfonic acid]
2. PVP [0.50%, Polyvinylpolypyrrolidone]
- 35 3. PEG 8000 [1.00%, Poly(ethylene glycol)]
4. Sodium Chloride [1.5%]
5. Sodium Azide [0.1%]

Preparation of antiserum for test:

Rabbit antiserum against barbiturates is diluted in an appropriate buffer system. This diluent consists of the following in aqueous solution at pH 8.0:

1. Hepes, (0.05M)
2. BSA (0.25%)
3. Sodium Chloride (0.15%)
- 10 4. Sodium Azide (0.15%)

Test Methodology:

Ten microliters of barbiturate free urine is dispensed into a mixing well of a capillary agglutinography slide followed by 50 microliters each of antiserum buffer, reaction buffer and latex (example 5). The added liquids are stirred for three seconds and the solution is moved to allow contact with the capillary. The liquid will move through the capillary and fill the viewing area at the end of the capillary in three to six minutes. The liquid in the viewing area will appear as fine floccules (agglutinated) or milky. For negative samples agglutination should be very strong with very little cloudiness.

25

The dilution of a particular antiserum which is chosen for the test is the one which has the highest dilution that will still produce strong agglutination in the viewing area. When various amounts of barbiturates are dissolved in barbiturate free urine in the test systems, no agglutination occurs. The amount of barbiturate required to inhibit the agglutination will vary from 100 nanograms per ml or greater depending on the concentration of antiserum used and the strength of antiserum produced. Thus, for the system described above a urine containing 200 nanograms of barbiturate per ml is sufficient to partially inhibit agglutination and a urine containing 400 nanograms of

30

35

barbiturate per ml is sufficient to completely inhibit agglutination.

The following table reports the type of cross-reactivity that is observed in the system described above:

		Concentration (ng/ml) equivalents to 200 ng/ml of secobarbital	% Cross- reactivity
10	Allobarbital	200	100
	Allylcyclopentylbarbituric acid	25	800
	Amobarbital	200	100
	Aprobarbital	200	100
	Barbital	100	200
15	Butabarbital	250	80
	p-Hydroxyphenobarbital	700	29
	Methobarbital	25,000	0.9
	Pentobarbital	500	40
	Phenobarbital	700	29

20

The following table reports the type of specificity and accuracy that is observed with the above described assay when used with samples positive for barbiturates:

25

Gas Chromatography-
Mass Spectroscopy

+

30

Agglutination +

48

Test

—

0

35

Example 7Preparation of amphetamine reagent

5 Preparation of (S)-N-(1-methyl-2-phenylethyl)fluoro-
acetamide(1):

To a mixture of d-amphetamine sulfate (20.0g, 0.108 mol) in
triethylamine (75 ml) that was purged with argon and cooled
10 in an ice bath trifluoroacetic anhydride (31 ml) was added
dropwise over 15 min. The reaction was stirred at ambient
temperature overnight. The solvents were then evaporated in
vacuo and the residue was dissolved in methylene chloride
(200 ml), washed with 5% aqueous tartaric acid (3 x 250 ml),
15 dried (Na_2SO_4), filtered and concentrated in vacuo to
yield a yellow oil. The material was crystallized in a
hexane ether mixture to yield 14g of the product.

^1H NMR (CDCl_3) δ 1.17 (d J=6 Hz, 3H, CH_3), 2.78 (t,
20 J=5Hz, 2H, CH_2), 4.22 (m, J=6Hz, 1H, CH), 6.00 (bs, 1H,
NH), 7.06 (d, J=6 Hz, 2H, Ar), 7.22 (m, 3H, Ar)

Preparation of (S)-4-[2-methyl-2-[(trifluoroacetyl)amino]-
ethyl]-p-oxo-benzenebutanoic acid (2):

25

To a stirred solution of (1), (12.0g, 0.05 mol) in methylene
chloride (210 ml) under argon, succinic anhydride (8.0g,
0.08 mol) was added. The reaction was cooled in an ice bath
and then treated with aluminum chloride (28.0g, 0.21 mol),
30 portionwise over 5 min. The reaction was stirred at 0-5°
for 2 h and then at room temperature overnight.

Hydrochloric acid (3N, 120 ml) was then slowly added and
then solution was stirred for an additional one hour. The
methylene chloride was removed in vacuo and the aqueous
35 layer was extracted with ethyl acetate. The organic layer
was dried (Na_2SO_4), filtered and the solvents evaporated
to yield a tan residue which upon trituration with ether

gave 10.5g of product.

IR(CHCl_3) 3300(NH), 1700 (Acid + Amide), 1555 (Amide) cm^{-1} , ^1H NMR(CDCl_3 + $\text{DMSO}-d_6$) δ 1.21(d, J=7Hz, 3H, CH_3), 2.73 (t, J=6Hz, 2H, CH_2), 2.82(m, 1H, H of CH_2), 2.95(m, 1H, H of CH_2), 3.27 (t, J=6Hz, 2H, CH_2), 4.25(1H, CH), 7.27(d, J=8 Hz, 2H, Ar), 7.92(d, J8Hz, 2H, Ar), 7.57(bs, 1H, NH). Ms, m/e 331(M+)

10 Preparation of (S)-4-[2-methyl-2-[trifluoroacetyl]amino]-ethyl]benzenebutanoic acid (3):

A mixture of 2 (9.2g, 0.027 mol), and 10% palladium on charcoal (4.0g) in acetic acid (400 ml) was hydrogenated at 15 50 psi for 24 h. The catalyst was filtered off, the filtrate was concentrated in vacuo and the residue was triturated with ether to yield 7.0g of a white product. IR(CHCl_3) 3425(NH), 1718(Acid), 1532(Amide), 1170(CF_3) cm^{-1} , ^1H NMR(CDCl_3) δ 1.16(d, J=7Hz, 3H, CH_3), 1.92(m, 2H, CH_2), 2.34(bs, 2H, AR- CH_2), 2.63 (m, 2H, CH_2), 2.79(m, 2H, CH_2), 4.28(m, 1H, CH), 5.94(bs, 1H, NH), 20 6.97(d, J=8Hz, 2H, AR), 7.03(d, J=8Hz, 2H, AR). MS, m/e 299(M- H_2O)

25 Preparation of (S)-N-[2-[4-[4-[(2,5-Dioxo-1-pyrrolidinyl)-oxy]-4-oxo=butyl]phenyl]-1-methyl-ethyl]-2-trifluoroacetamide (4)

To a stirred solution of 3 (5.4g, 0.019 mol) in methylene 30 chloride (150 ml), tetrahydrofuran (150 ml) and dimethylformamide (50 ml), N-hydroxysuccinimide (2.7g, 0.023 mol) and 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (6.0g, 0.031 mol) was added. The reaction was stirred at room temperature overnight, and then was 35 concentrated in vacuo. The resulting residue was dissolved in methylene chloride, filtered and purified by silica gel chromatography (7% ether-methylene chloride as eluent) to

give a yellow oil which crystallized in a ether to yield 3.7g of a white product.

IR(CHCl_3), 3425(NH), 1812-1741(Imide), 1725(Amide),
 5 1170(CF_3) cm^{-1} . ^1H NMR(CDCl_3) δ 1.21(d, $J=7\text{Hz}$, 3H, CH_3), 2.07(m, 2H, CH_2), 2.60(t, $J=7\text{Hz}$, 2H, CH_2), 2.72 (t, $J=7\text{Hz}$, 2H, CH_2), 2.80 -2.90(m, 6H, CH_2), 4.27(m, 1H, CH), 6.10(bs, 1H, NH), 7.09(d, $J=8\text{Hz}$, 2H, Ar), 7.15(d, $J=8$ Hz, 2H, Ar).

10

MS, (+)FAB, m/e 415(M+H), m/e 437(M + Na).

Anal. Calc. for C, 55.07; H, 5.11; N, 6.76

Anal. found for C, 54.94; H, 5.22; N, 6.76

15

Conjugation of (S)-N-[2-[4-[4-[(2,5-Dioxo-1-pyrrolidinyl)-oxy]-4-oxobutyl]phenyl]-1-methyl-ethyl]-2-trifluoroacetamide (amphetamine) to Bovine Serum Albumin (BSA):

To a solution (400 ml) containing sodium bicarbonate (0.05
 20 M), 10% dimethylsulfoxide (DMSO), and BSA (6.0g) at pH 8.0, 25° the N-hydroxysuccinimide ester (4) (0.035g in 32.6 ml of DMSO) was added dropwise. The reaction was stirred at 4° for 15 h and dialyzed as in Example 4. After dialysis the protein concentration was measured and adjusted to 0.0005g
 25 per ml.

Example 8

Preparation of sensitized latex containing the
 30 amphetamine-BSA conjugate-general procedure.

To a stirred latex (SeradynTM carboxy modified polystyrene 0.888 microns) suspension (180 ml, 10% solids), N-hydroxybenzotriazole (NHB) (0.6g in 24 ml of aqueous
 35 dimethylformamide) and 1-cyclohexyl-3-(2-morpholinoethyl) carbodiimide metho p-toluenesulfonate (CMC) (2.0g in 32 ml of water) were added. The suspension was stirred for 3 h at

4° then diluted to 2% latex and extensively washed with water by tangential flow filtration. To the washed 2% latex were added 900 ml of the amphetamine-BSA solution from Example 7. The reaction was stirred overnight at 4° and was
5 again extensively washed with water by tangential flow filtration. The washed latex was then taken off the filtration apparatus and pelleted by centrifugation. The pellets were resuspended in 3000 ml of sodium carbonate (pH 11.4, 0.10M) and allowed to stir at room temperature for
10 24 h. The mixture was again pelleted by centrifugation, resuspended in 2000 ml of phosphate buffer (pH 6, 0.05M), put back on the tangential flow filtration system and extensively washed with water. The washed latex was then adjusted to 3.0% latex solids (by weight) concentration.

15

Example 9

Amphetamine Test

20 Preparation of reaction buffer for test:

This diluent consists of the following in aqueous solution at pH 7.5:

- 25 1. Pipes [0.20M, 1,4-piperazine bis(ethanesulfonic acid)]
2. PVP [0.80%, Polyvinylpolypyrrolidone]
3. PEG 8000 [1.50%, Poly(ethylene glycol)]
4. Sodium Chloride [20%]
5. Sodium Azide [0.1%]

30

Preparation of antiserum buffer for test:

Sheep antiserum against amphetamines is diluted in an appropriate buffer system. This diluent consists of the
35 following in aqueous solution at pH 7.2:

1. Hepes, [0.05M,
4-(2-hydroxyethyl)-piperazine-ethanesulfonic acid]
2. BSA (0.25%)
3. Sodium Chloride (0.15%)
- 5 4. Sodium Azide (0.15%)

Test Methodology

Ten microliters of amphetamine free urine is dispensed into
10 a mixing well of a capillary agglutinography slide followed
by 50 microliters each of antiserum buffer, reaction buffer
and latex (example 8). The added liquids are stirred for
three seconds and the solution is moved to allow contact
with the capillary. The liquid will move through the
15 capillary and fill the viewing area at the end of the
capillary in three to six minutes. The liquid in the
viewing area will appear as fine floccules (agglutinated) or
milky. For negative samples agglutination should be very
strong with very little cloudiness.

20

The dilution of a particular antiserum which is chosen for
the test is the one which has the highest dilution that will
still produce strong agglutination in the viewing area.
When various amounts of amphetamine are dissolved in
25 amphetamine free urine in the test systems, no agglutination
occurs. The amount of amphetamine required to inhibit the
agglutination will vary from 250 nanograms per ml or greater
depending on the concentration of antiserum used and the
strength of antiserum produced. Thus, for the system
30 described above a urine containing 1000 nanograms of
amphetamine per ml is sufficient to partially inhibit
agglutination and a urine containing 1500 nanograms of
amphetamines per ml is sufficient to completely inhibit
agglutination.

35

The following table reports the type of cross-reactivity
that is observed in the system described above:

	Concentration (ng/ml) equivalent to 300 ng/ml of Morphine	% Cross reactivity
5 p-Hydroxyamphetamine	5,000	20
Tyramine HCl	100,000	0.01

The following table reports the type of specificity and accuracy that is observed with the above described assay when
10 used with samples positive for amphetamine:

		Gas Chromatography- Mass Spectroscopy +
15 Agglutination	+	45
Test	-	1*

20 * The one discrepant sample was shown to contain drug at a concentration close to the cut-off (1,000 ng/ml) of the assay.

25 Example 10

Preparation of tetrahydrocannabinoid reagent

Preparation of 1-[[[(6a, 7, 10, 10a-Tetrahydro-1-hydroxy-
30 6,6-dimethyl-3-pentyl-6H-dibenzo[b,d]pyran-1-yl(carbonyl]oxy]-2,5-pyrrolidinedione:

To a solution of 1.26g (3.67 mmole) of 11-nor- Δ^8 -THC-9-carboxylic acid¹ in 50 ml of 1:1 ethylacetate/methylene
35 chloride were added 1.68g (14.7 mmole) of N-hydroxy-succinimide and 2.10g (11.0 mmole) of 1-(3-dimethylaminopropyl)3-ethylcarbodiimide hydrochloride. The reaction

- was stirred for 16 hours at room temperature. The reaction mixture was washed with 1N hydrochloric acid (3 x 100 ml), water (3 x 100 ml) and saturated brine (100 ml). The organic phase was dried over Na_2SO_4 , concentrated under reduced pressure and chromatographed over silica gel. Eluting with 40% ethylacetate in hexane afforded 1.41g (87%) of the desired N-hydroxysuccinimide ester. Satisfactory NMR, IR and MS were obtained.
- 10 IR(KBr) 3415(OH), 1802, 1760, 1738 (C=O) cm^{-1} , ^1H NMR (CDCl_3) δ 0.88(t, J=6Hz, 3H, CH_3), 1.13(s, 3H, CH_3), 1.40 (s, 3H, CH_3), 2.86(bs, 4H, CH_2), 6.11(s, 1H, Ar), 6.27 (s, 1H, Ar), 7.32(d, J=4Hz, 1H, vinyl). MS, m/e 441(M+)
- 15 Conjugation of 1-[[[(6a, 7, 10, 10a-Tetrahydro-1-hydroxy-6,6-dimethyl-3-pentyl-6H-dibenzo)oxy][b,d]pyran-1-yl(carbonyl)oxy]-2,5-pyrrolidinedione (cannabinoid) to Bovine Serum Albumin (BSA):
- 20 To a solution (300 ml) containing sodium bicarbonate (0.05 M), 10% dimethylsulfoxide (DMSO), and BSA (4.5g) at pH 8.5, 25° the N-hydroxysuccinimide ester (0.006g in 6.2 ml of DMSO) was added dropwise. The reaction was stirred at 4° for 15 h and dialyzed as in Example 4. After dialysis the protein concentration was measured and adjusted to 0.005g per ml.

Example 11

- 30 Preparation of sensitized latex containing the cannabinoid-BSA conjugate - general procedure.

To a stirred latex (SeradynTM carboxy modified polystyrene 0.888 microns) suspension (180 ml, 10% solids),

35 N-hydroxybenzotriazole (NHB) (0.6g in 24 ml of aqueous dimethylformamide) and 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho p-toluenesulfonate (CMC) (2.0g in 32 ml of

water) were added. The suspension was stirred for 3 h at 4° then diluted to 2% latex and extensively washed with water by tangential flow filtration. To the washed 2% latex were added 900 ml of the opiate-BSA solution from Example 10.

- 5 The reaction was stirred overnight at 4° and was again extensively washed with water by tangential flow filtration. The washed latex was then adjusted to a 2.2% latex solids (by weight) concentration.

10

Example 12THC Test

Preparation of reaction buffer for test:

15

This diluent consists of the following aqueous solution at pH 6.5:

1. Hepes [0.05M,
- 20 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid]
2. PVP [0.50%, Polyvinylpyrrolidone]
3. PEG 8000 [1.00%, Poly(ethylene glycol)]
4. Sodium Chloride [0.5%]
5. Sodium Azide [0.1%]

25

Preparation of antiserum buffer for test:

Purified mouse monoclonal antibody against cannabinoids is diluted in an appropriate buffer system. This diluent
30 consists of the following in aqueous solution in pH 7.0:

1. Hepes (0.05M)
2. BSA (0.25%)
3. Sodium Chloride (0.15%)
- 35 4. Sodium Azide (0.15%)

Test Methodology:

- Ten microliters of cannabinoid free urine is dispensed into a mixing well of a capillary agglutinography slide followed
5 by 50 microliters each of antiserum buffer, reaction buffer and latex reagent (example 11). The added liquids are stirred for three seconds and the solution is moved to allow contact with the capillary. The liquid will move through the capillary and fill the viewing area at the end of the
10 capillary in three to six minutes. The liquid in the viewing area will appear as fine floccules (agglutinated) or milky. For negative samples agglutination should be very strong with very little cloudiness.
- 15 The dilution of a particular antiserum which is chosen for the test is the one which has the highest dilution that will still produce strong agglutination in the viewing area. When various amounts of cannabinoids are dissolved in cannabinoid free urine in the test systems, no agglutination
20 occurs. The amount of cannabinoid required to inhibit the agglutination will vary from 50 nanograms per ml or greater depending on the concentration of antiserum used and the strength of antiserum produced. Thus, for the system described above a urine containing 100 nanograms of
25 cannabinoid per ml is sufficient to partially inhibit agglutination and a urine containing 600 nanograms of cannabinoid per ml is sufficient to completely inhibit agglutination.
- 30 The following table reports the type of cross-reactivity that is observed in the system described above:

	Concentration (ng/ml) equivalent to 300 ng/ml of Δ^8 THC	% Cross reactivity
5		
11-Hydroxy-cannabinol	2,000	5.0
Cannabinol	800	12.5
Δ^9 -THC	10,000	1.0
10 11-Hydroxy- Δ^9 -THC	2,000	5.0
8 β -11-Dihydroxy- Δ^9 -THC	4,000	2.5
8 β -Hydroxy- Δ^9 -THC	4,000	2.5

15 The following table reports the type of specificity and accuracy that is observed with the above described assay when used with samples positive for cannabinoids:

20		Gas Chromatography- Mass Spectroscopy
		+
	Agglutination	+ 43
25	Test	- 2*

30 * The two discrepant samples were shown to contain drug near the cut-off (100 ng/ml) of the assay.

We claim:

1. A reagent of the formula

5 $P-[A-D]_n$

where:

D is a drug derivative antigenically selective for the
10 determination of the presence of target drug or target drug
metabolite,

A is an activating linker-spacer group having an N-
hydroxysuccinimide or isothiocyanate derived linking
15 moiety,

P is a poly(amino acid) or polymer covalently bonded
to A, and

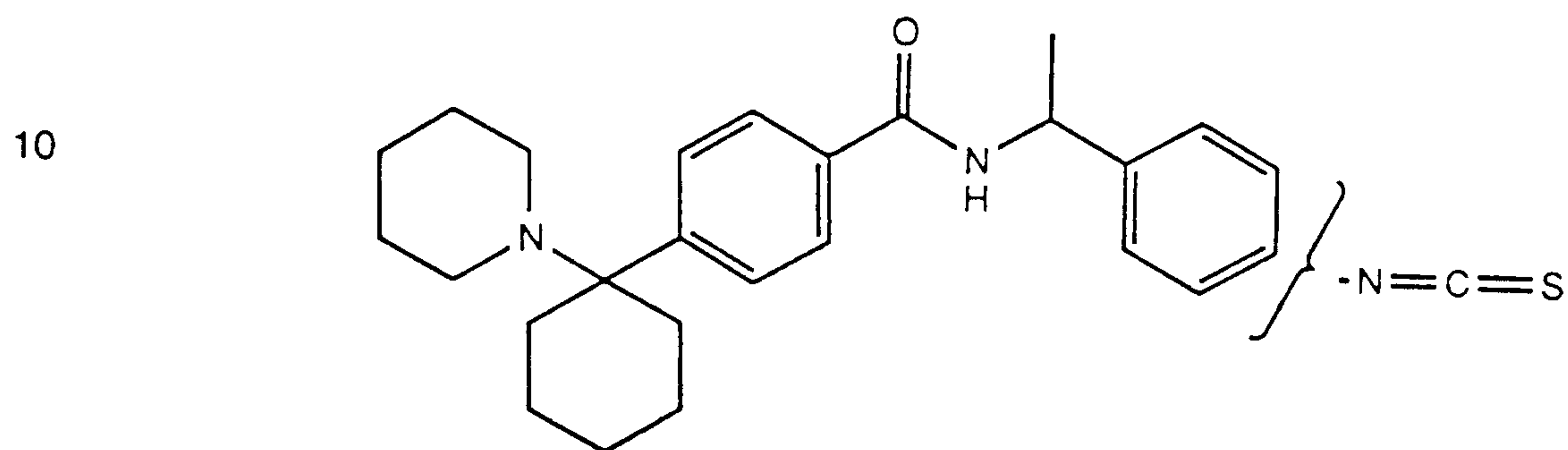
20 n is more than 0 and less than 1.

25 2. The reagent of claim 1 wherein the part [A-D] of the
reagent is an activated drug derivative selected from the
group consisting of a Δ^8 THC derivative with A as a
substituent at the 9-carbon thereof, a 5-substituted
barbituric acid derivative with A as a second substituent
30 at the 5-carbon thereof, a phencyclidine derivative with A
as a substituent on the benzene thereof, a morphine
derivative with A as a substituent on the 3-hydroxyl
thereof, a benzoyl ecgonine derivative with A as

a substituent on the benzene thereof, a 1,4 benzodiazepine derivative with A as a substituent on the 1 nitrogen thereof, and an amphetamine derivative with A as a substituent on the benzene thereof.

5

3. The reagent according to claim 2 wherein A-D is a compound of the formula:

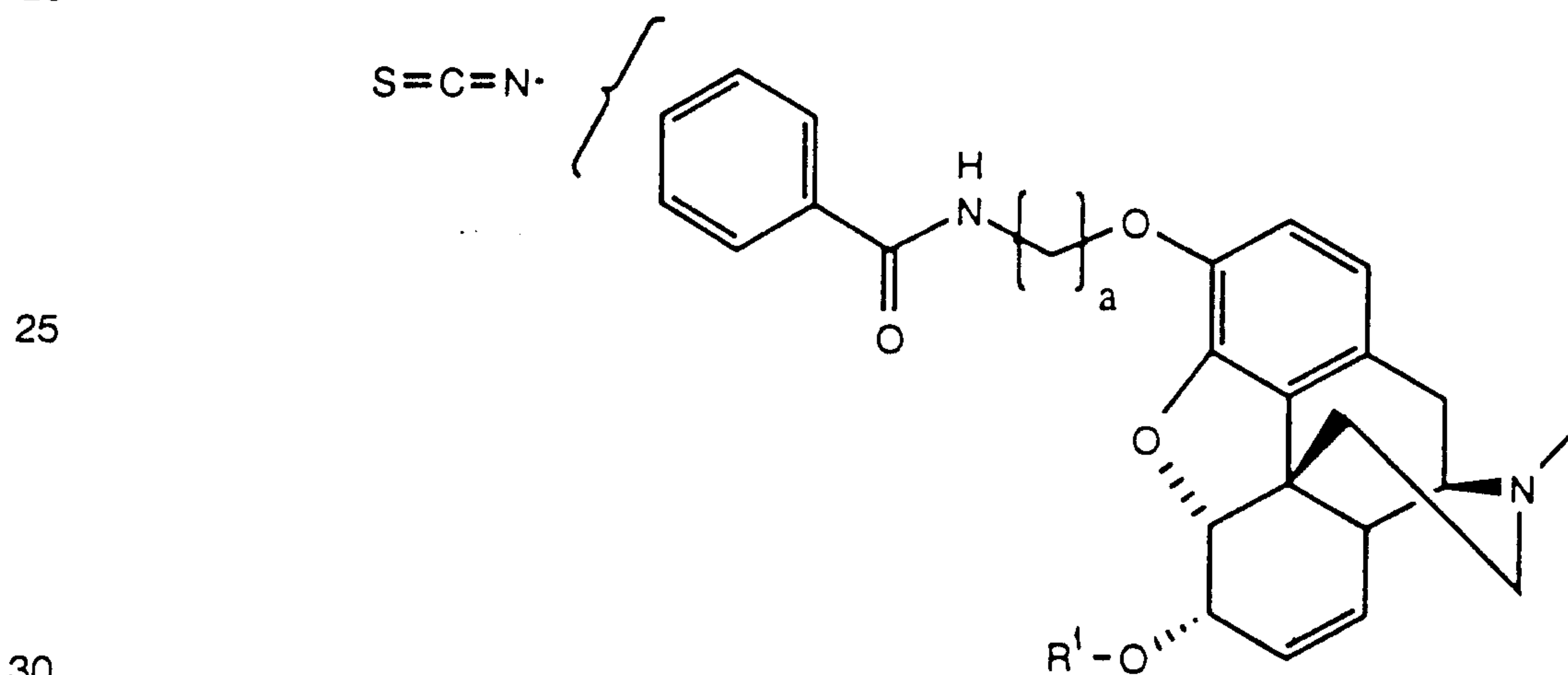


15

and the isothiocyanate is meta or para.

4. The reagent according to claim 2 wherein A-D is a compound of the formula:

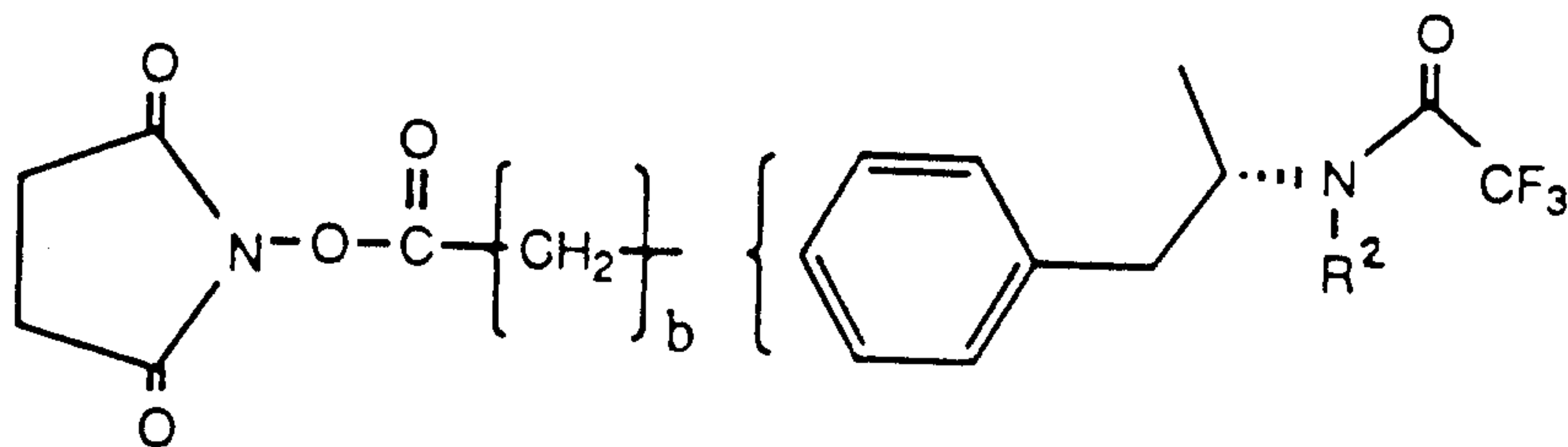
20



where R_1 is hydrogen, methyl or acetyl, a is 2 to 8, and the isothiocyanate is meta or para.

5. The reagent according to claim 2 wherein A-D is a compound of the formula:

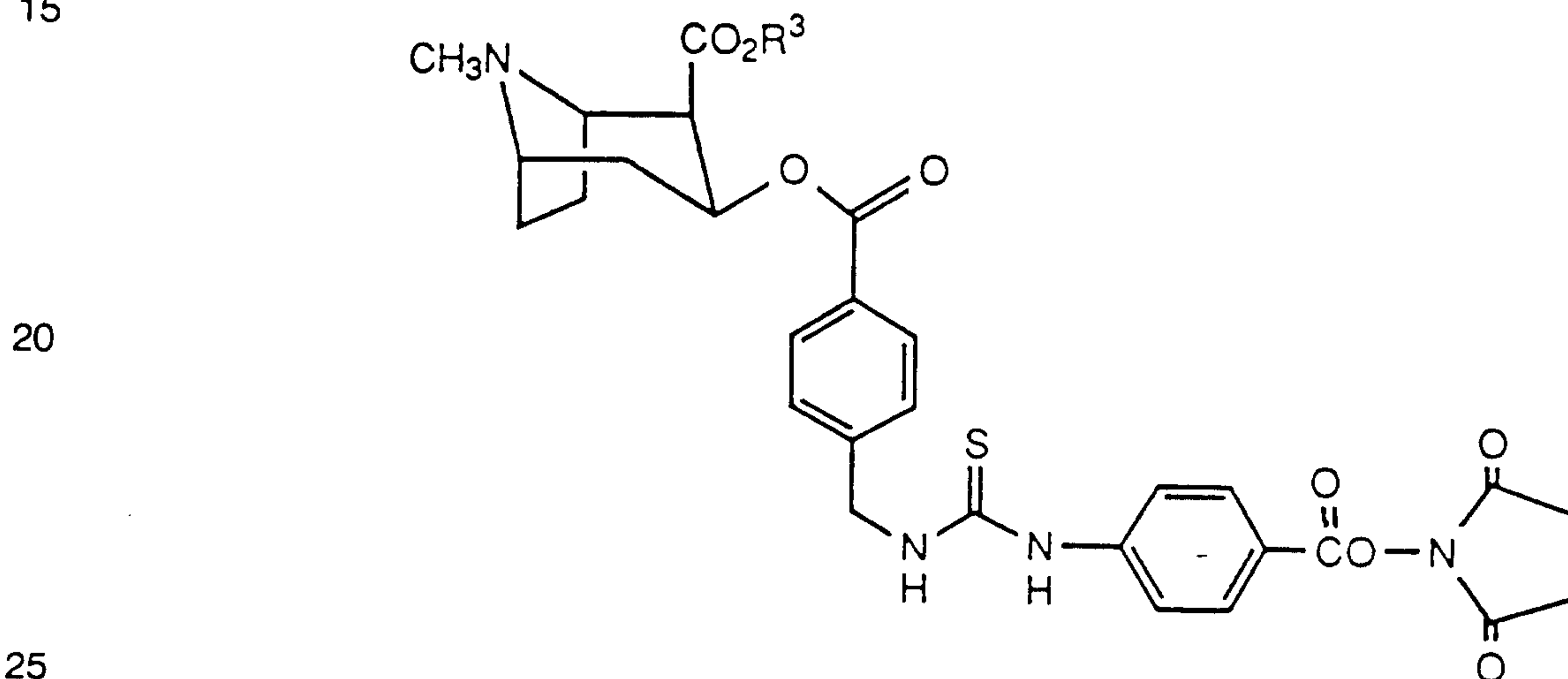
5



where R² is hydrogen or methyl, b is 1 to 9, and the alkyl
 10 group is bound to the benzene ring in the meta or para-
 position.

6. The reagent according to claim 2 wherein A-D is a
 compound of the formula:

15



20

25

and R³ is hydrogen or methyl.

7. The reagent according to claim 2, wherein A-D is a
 compound selected from the group consisting of:

30

N-[3-[7,8-Didehydro-4,5-epoxy-6-hydroxy-17-methylmorphinan
 -3-yl)oxy]propoxy]-4-isothiocyanatobenzamide,

5-[3-[2,5-Dioxo-1-pyrrolidinyl)oxy]-1-methyl-3-oxo-propyl]
 35 -5-(2-propenyl)2,4,6(1H,3H,5H) pyrimidinetrione,

(S)-N[2-[4-[4-[(2,5-Dioxo-1-pyrrolidinyl)-oxy]-4-oxo-butyl]phenyl]-1-methyl-ethyl]-2-trifluoroacetamide,

(S)-N-Methyl-N[2-[4-[4-[(2,5-Dioxo-1-pyrrolidinyl)-oxy]-4-oxo-butyl]phenyl]-1-methyl-ethyl]-2-trifluoroacetamide,

N-[1-(4-Isothiocyanophenyl)ethyl]-4-[1-(1-piperidinyl)-1-cyclohexyl]benzamide,

10 N-[2-[7-Chloro-5-(2-fluorophenyl)-2,3-dihydro-2-oxo-1H-1,4-benzodiazepin-1-yl]ethyl]-4-isothiocyanatobenzamide,

[1R-(exo,exo,anti)]-8-Methyl-3-[[4-[[[[[[[(2,5-dioxo-pyrrolidin-1-yl)oxy]carbonyl]phenyl]amino](thioxomethyl)]amino]methyl]benzoyl]oxy]bicyclo[3,2,1]octane-2-carboxylic acid,

15 1[4-[1-(1-Piperidinyl)-1-cyclohexyl]benzoyloxy]-2,5-pyrrolidinedione, and

20 1-[[[(6a,7,10,10a-Tetrahydro-1-hydroxy-6,6-dimethyl-3-pentyl-6H-dibenzo)oxy][b,d]pyran-1-yl(carbonyl)oxy]-2,5-pyrrolidinedione.

8. A microparticulate immunoassay reagent comprising the reagent of claim 1, 2 or 7 bound to microparticles by the hydrophobic or covalent bonding of the poly(amino acid) or polymer, P, to the surface of said microparticles.

9. The microparticulate reagent of claim 8, wherein P is covalently bound to latex microparticles.

10. The reagent of claim 9, wherein P is bovine serum albumin.

11. A compound selected from the group consisting of:

N-[3-[7,8-Didehydro-4,5-epoxy-6-hydroxy-17-methyl-morphinan-3-yl)oxy]propoxy]-4-isothiocyanatobenzamide,

5

5-[3-[2,5-Dioxo-1-pyrrolidinyl)oxy]-1-methyl-3-oxo-propyl]-5-(2-propenyl)2,4,6(1H,3H,5H)pyrimidimetrione,

(S)-N[2-[4-[4-[(2,5-Dioxo-1-pyrrolidinyl)-oxy]-4-oxo-butyl]phenyl]-1-methyl-ethyl]-2-trifluoroacetamide,

10

(S)-N-Methyl-N[2-[4-[4-[(2,5-Dioxo-1-pyrrolidinyl)oxy]-4-oxo-butyl]phenyl]-1-methyl-ethyl]-2-trifluoroacetamide,

15

N-[1-(4-Isothiocyanophenyl)ethyl]-4-[1-(1-piperidinyl)-1-cyclohexyl]benzamide,

N-[2-[7-Chloro-5-(2-fluorophenyl)-2,3-dihydro-2-oxo-1H-1,4-benzodiazepin-1-yl]ethyl]-4-isothiocyanatobenzamide,

20

[1R-(exo,exo,anti)]-8-Methyl-3-[[4-[[[[[[[(2,5-dioxo-pyrrolidin-1-yl)oxy]carbonyl]phenyl]amino](thioxomethyl)]amino]methyl]benzoyl]oxy]bicyclo[3,2,1]octane-2-carboxylic acid,
and

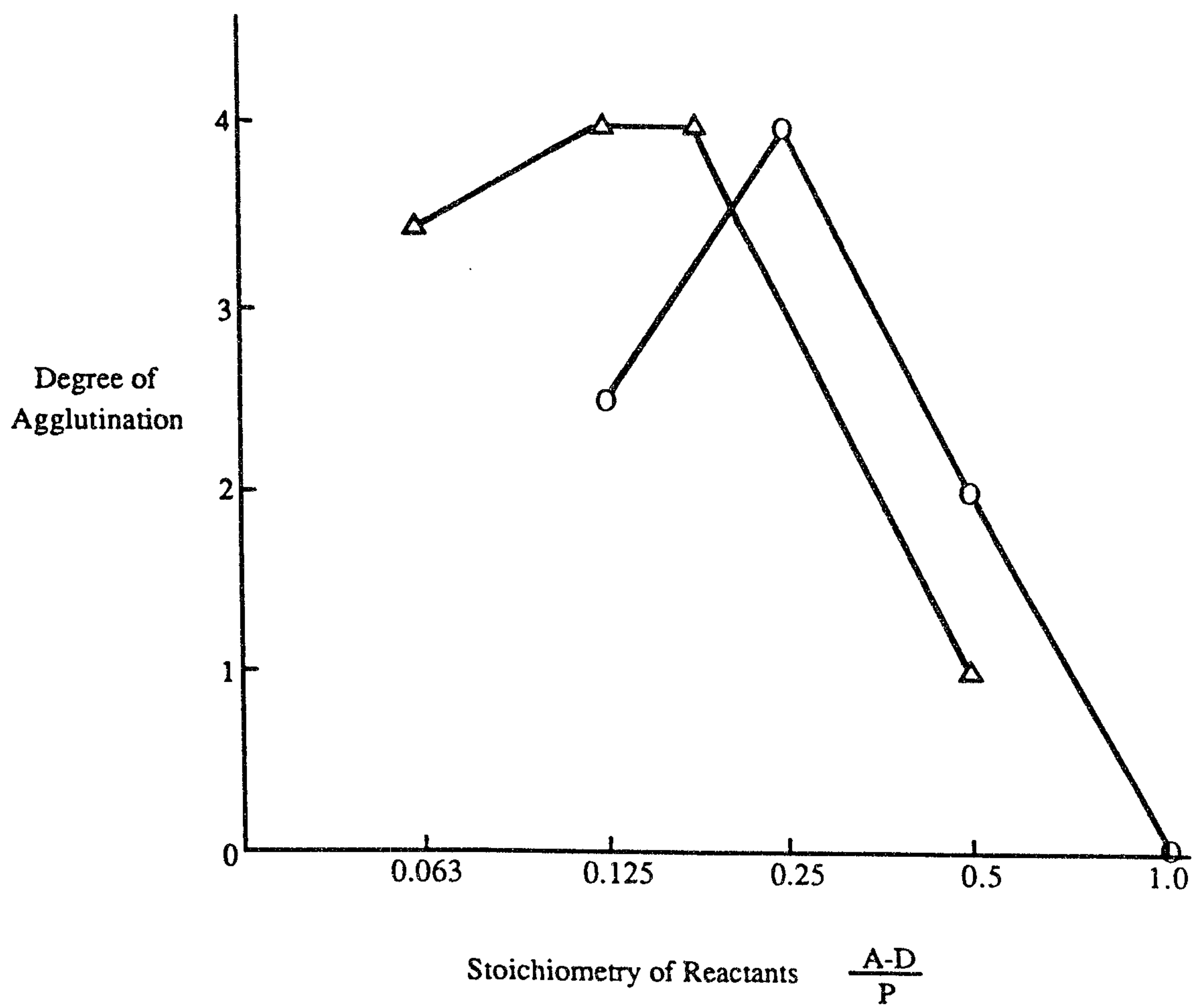
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1-[4-[1-(1-Piperidinyl)-1-cyclohexyl]benzoyloxy]-2,5-pyrrolidinedione.

30

35

FIGURE 1



O = Benzoylecgonine Reagent

Δ = Phencyclidine Reagent