PHARMACEUTICAL COMPOSITIONS FOR PREVENTION OF OVERDOSE OR ABUSE

FIELD OF INVENTION

[001] Accidental and intentional overdose with prescription and over the counter drugs is a serious health problem with thousands of fatalities occurring each year as a result. The present invention relates to pharmaceutical compositions comprised of a chemical moiety attached to an active agent in a manner that substantially decreases the potential of the active agent to cause overdose or to be abused. When delivered at the proper dosage the pharmaceutical composition provides therapeutic activity similar to that of the parent active agent. However, when the composition is delivered at higher doses the potential for overdose or abuse is reduced due to the limited bioavailability of the active agent as compared to the active agent delivered as free drug.

BACKGROUND

[002] Drug overdose is a significant and growing problem. It can occur accidentally, as when a child swallows pills without understanding the consequences, or intentionally as with suicide attempts. In addition, accidental overdose due to an unusually potent batch of a street drug in illicit drug users is quite common. Common examples of drugs that are seen in overdose cases include the ubiquitous over-the-counter analgesics acetaminophen (paracetamol) and aspirin. While the former is the preferred drug among adolescents in cases of deliberate self poisonings (Lifshitz et al., Isr. Med. Assoc. J., 4(4): 252-4 (2002), aspirin is perhaps more dangerous because there is no antidote (Jones, Am. J. Ther. 9(3):245-57 (2002).

[003] In the elderly population, drugs most often implicated in poisonings include psychotherapeutic drugs, cardiovascular drugs, analgesics and anti-inflammatory drugs, oral hypoglycemics and theophylline (Klein-Schwartz et al., Drugs Aging 1(1):67-89 (1991). It is important to realize that in many cases where death due to overdose is averted, there appears to be extensive morbidity associated with overdoses (Warner-Smith et al., Addition 97(8):963-7 (2002).

[004] The Drug Abuse Warning Network (DAWN) reported in June 2003 on the most recent trends in emergency department (ED) visits related to drug abuse. Data was presented for 8-year trends from 1994 to 2001. The following summaries were provided:

- In 2001, there were over 638,000 ED visits related to drug abuse in the conterminous U.S. This translates to 252 visits per 100,000 populations or 0.6 percent of all ED visits.

- Seven categories of drugs accounted for 85% of the ED mentions in 2001. The ED visits related to drug abuse most frequently involved alcohol, (34% of mentions), marijuana (17%), benzodiazepines (16%), narcotic analgesic combinations (16%), heroin (15%), other analgesics/combinations (12%), and antidepressants (10%).

- ED mentions of benzodiazepines increased 14 percent from 2000 to 2001 (from 91,078 to 103,972), as did the top 2 benzodiazapines, alprazolam (up 16%) and benzodiazepines-NOS (up 35%). The latter includes benzodiazepines not identified by name.

- ED mentions of narcotic analgesics/combinations rose 21 percent (from 82,373 to 99,317) from 2000 to 2001.

- Narcotic analgesics not identified by name were mentioned most frequently (narcotic analgesics-NOS, 32,196 mentions, up 24% from 2000 to 2001), followed by those containing hydrocodone (21,567), oxycodone (18,409, up 70%), and methadone (10,725, up 37%). Narcotic analgesics/combinations containing propoxyphene (5,361), codeine (3,720, down 30%), and morphine (3,403) were much less frequent and not increasing.

[005] Emergency department reporting for a number of drugs rose substantially from 1994 to 2000. These include: amphetamines (10,118 to 18,555, up 83.4%), anticonvulsants, including carbamazepine (9,358 to 14,642, up 56.5%), muscle relaxants, including carisoprodol (12,223 to 19,001, up 55.5%), psychotherapeutic drugs, including SSRI antidepressants, tricyclic antidepressants, and other antidepressants (190,467 to 220,289, up 15.7%). Anxiolytics, sedatives, and hypnotics, including benzodiazepines (74,637 to 103,972, up 27.7%) and narcotic

analgesics including codeine, hydrocodone, methadone, oxycodone, propoxyphene and others (44,518 to 99,317, up 123.1%).

[006] Other drugs for which the number of ED mentions did not rise but were still responsible for over 10,000 visits include respiratory agents, including antihistamines (12,238), antipsychotics including risperidone (20,182), nonsteroidal anti-inflammatory agents, including ibuprofen and naproxen (22,663) and acetaminophen (42,044). Aspirin and salicylates-NOS accounted for 8,499 ED visits in 2001.

[007] The commercial drugs benzodiazapines (16%), narcotic analgesics other than heroin (16%), non-narcotic analgesics (12%), and antidepressants (10%) accounted for 54% of ED visits in 2001.

[008] Amphetamine is commonly administered as the sulfate salt in single oral doses of 5-15 mg. When abused amphetamine is typically either orally or intravenously used in amounts up to 2000 mg per day by addicts. A normal dosage of amphetamine typically provides blood concentrations which peak at 35 ng/mL, 2 hours following a single oral dose of 10 mg (half-life 11-13 hours). Following the oral administration of 30 mg of amphetamine, an average peak plasma level of about 111 ng/mL may be observed at 2.5 hours. After 4.5 hours, the level may drop to about 84 ng/mL. After oral ingestion of amphetamine, absorption is complete in 4-6 hours. Concentration in blood or plasma following a therapeutic dose is low because of the large volume of distribution. Contrarily, a steady-state blood level of 2000-3000 ng/mL has been observed in addicts who orally consume an average of 1000 mg per day of amphetamine. While peripheral effects such as increased heart rate start at blood levels of 20 ng/mL, rapid tolerance from intravenous use develops.

[009] Similarly, methamphetamine used in the treatment of obesity in single oral doses of 2.5-15 mg. After the administration of a single dose of 10 mg of methamphetamine, a maximum blood concentration of 30 ng/mL may be observed at one hour. A 12.5 mg dose may produced an average peak blood level of about 20 ng/mL at 2.5 hours, about 16 ng/mL at 6 hours, and about 10 ng/mL at 24 hours. Methamphetamine urine concentrations after the administration of 10 mg are typically 500-4,000 ng/mL during the first 24 hours. It has been reported that the

methamphetamine concentration of methamphetamine abusers is 2,400-33,300 ng/mL (average 14,200 ng/mL) and amphetamine concentrations of 1,000-9,000 ng/mL. (average 1,800 ng/mL). The estimated lethal dose is 100 mg in children and 1 g in adults.

[010] Oxycodone is an ingredient of Percodan, Percocet, Roxicet, and Tylox. It is a semisynthetic narcotic analgesic that is derived from thebaine. Available in oral formulations often in combination with aspirin, phenacetin and caffeine. Typical adult dose is 2.5 – 5 mg as the hydrochloride or terephthalate salt every 6 hours. Although it is typically used for the relief of moderate to moderately severe pain, it can also produce drug dependence of the morphine type. Therapeutic plasma concentration is 10–100 ng/mL and the toxic plasma concentration is greater than 200 ng/mL.

[011] Hydrocodone is an opioid analgesic and antitussive and occurs as fine, white crystals or as crystalline powder. Hydrocodone is a semisynthetic narcotic analgesic prepared from codeine with multiple actions qualitatively similar to those of codeine. It is mainly used as an antitussive in cough syrups and tablets in sub-analgesic doses (2.5 – 5 mg). Additionally, it is used for the relief of moderate to moderately severe pain. Hydromorphone is administered orally in 5 - 10 mg doses four times daily. Therapeutic plasma concentration is 1 – 30 ng/mL and the toxic plasma concentration is greater than 100 ng/mL.

[012] Others have sought to prevent the potential harmful effects of overdose through various formulations. For example, opioids have been combined with antagonists in particular formulations designed to counteract the opioid if the formulation is disrupted before oral administration or is given parenterally. Extended release Concerta (methylphenidate) has been formulated in a paste to preclude administration by snorting or injection. Compositions have been coated with emetics in a quantity that if administered in moderation as intended no emesis occurs, however, if excessive amounts are consumed emesis is induced therefore preventing overdose. These methods, as well as conventional control release formulations, are insufficient and can be easily circumvented. Consequently, improved methods are

needed to make drugs with reduced potential for overdose that are resistant to manipulation.

Brief Description of the Figures

[013] Figure 1. Synthesis of amino acid amphetamine conjugates.

[014] Figure 2. Synthesis of lysine amphetamine conjugate.

[015] Figure 3. Synthesis of serine amphetamine conjugate.

[016] Figure 4. Synthesis of phenylalanine amphetamine conjugate.

[017] Figure 5. Synthesis of triglycine amphetamine conjugate.

[018] Figure 6. Plasma concentrations of *d*-amphetamine from individual animals orally administered *d*-amphetamine or L-lysine-*d*-amphetamine.

[019] Figure 7. Plasma concentrations of *d*-amphetamine following oral administration of *d*-amphetamine sulfate or L-lysine-*d*-amphetamine (1.5mg/kg d-amphetamine base) to rats (ELISA analysis).

[020] Figure 8. Plasma concentrations of *d*-amphetamine following oral administration of *d*-amphetamine sulfate or L-lysine-*d*-amphetamine (3 mg/kg *d*-amphetamine base) to rats (ELISA analysis).

[021] Figure 9. Plasma concentrations of *d*-amphetamine following oral administration of *d*-amphetamine sulfate or L-lysine-*d*-amphetamine (6 mg/kg *d*-amphetamine base) to rats (ELISA analysis).

[022] Figure 10. Plasma concentrations of *d*-amphetamine at 30-minutes post-dose for escalating doses of L-lysine-*d*-amphetamine or *d*-amphetamine sulfate (ELISA analysis).

[023] Figure 11. Plasma concentrations of *d*-amphetamine following oral administration of L-lysine-*d*-amphetamine or *d*-amphetamine sulfate (60 mg/kg *d*-amphetamine base) to rats (ELISA analysis).

[024] Figure 12. Plasma concentrations of *d*-amphetamine following intranasal administration of L-lysine-*d*-amphetamine or *d*-amphetamine sulfate (3 mg/kg *d*-amphetamine base) to rats (ELISA analysis).

[025] Figure 13. Plasma concentrations of *d*-amphetamine following bolus intravenous administration of L-lysine-*d*-amphetamine or *d*-amphetamine sulfate (1.5mg/kg *d*-amphetamine base) to rats (ELISA analysis).

[026] Figure 14. Plasma concentrations of *d*-amphetamine levels following oral administration of Dexadrine Spansule capsules, crushed Dexadrine Spansule capsules, or L-lysine-*d*-amphetamine (3 mg/kg *d*-amphetamine base) to rats (ELISA analysis).

[027] Figures 15A-B. Plasma concentrations of *d*-amphetamine in ng/mL (Figure 15A), and in uM (Figure 15B), following oral administration of L-lysine-*d*-amphetamine or *d*-amphetamine sulfate (1.5mg/kg *d*-amphetamine base) to rats (LC/MS/MS analysis).

[028] Figures 16A-B. Plasma concentrations of *d*-amphetamine in ng/mL (Figure 16A), and in uM (Figure 16B), following oral administration of L-lysine-*d*-amphetamine or *d*-amphetamine sulfate (3 mg/kg *d*-amphetamine base) to rats (LC/MS/MS analysis).

[029] Figures 17A-B. Plasma concentrations of *d*-amphetamine in ng/mL (Figure 17A), and in uM (Figure 17B), following oral administration of L-lysine-*d*-amphetamine or *d*-amphetamine sulfate (6 mg/kg *d*-amphetamine base) to rats (LC/MS/MS analysis).

[030] Figures 18A-B. Plasma concentrations of *d*-amphetamine in ng/mL (Figure 18A), and in uM (Figure 18B), following oral administration of L-lysine-*d*-amphetamine or *d*-amphetamine sulfate (12 mg/kg *d*-amphetamine base) to rats (LC/MS/MS analysis).

[031] Figures 19A-B. Plasma concentrations of *d*-amphetamine in ng/mL (Figure 19A), and in uM (Figure 19B), following oral administration of or *d*-amphetamine sulfate (60 mg/kg *d*-amphetamine base) to rats (LC/MS/MS analysis).

[032] Figure 20. Comparative bioavailability (C_{max}) of L-lysine-*d*-amphetamine and *d*-amphetamine in proportion to escalating human equivalent doses in rats (mg/kg *d*-amphetamine base).

[033] Figure 21. Comparative bioavailability (AUC_{inf}) of L-lysine-*d*-amphetamine and *d*-amphetamine in proportion to escalating doses in rats (mg/kg *d*-amphetamine base).

[034] Figure 22. Comparative Bioavailability (AUC_{inf}) of L-lysine-*d*-amphetamine and *d*-amphetamine in proportion to escalating human equivalent doses in rats (mg/kg *d*-amphetamine base).

[035] Figure 23. Plasma concentrations of *d*-amphetamine following intranasal administration of L-lysine-*d*-amphetamine or *d*-amphetamine sulfate (3 mg/kg *d*-amphetamine base) to rats (LC/MS/MS analysis).

[036] Figure 24. Plasma concentrations of *d*-amphetamine and L-lysine-*d*-amphetamine in ng/mL (Figure 24A), and in μM (Figure 24B), following intranasal administration of L-lysine-*d*-amphetamine or *d*-amphetamine sulfate (3 mg/kg *d*-amphetamine base) to rats (LC/MS/MS analysis.

[037] Figure 25. Plasma concentrations of *d*-amphetamine following bolus intravenous administration of L-lysine-*d*-amphetamine or *d*-amphetamine sulfate (1.5 mg/kg *d*-amphetamine base) to rats (LC/MS/MS analysis).

[038] Figures 26A-B. Plasma concentrations of *d*-amphetamine in ng/mL (Figure 26A), and in μM (Figure 26B), following intranasal administration of L-lysine-*d*-amphetamine or *d*-amphetamine sulfate (3 mg/kg *d*-amphetamine base) to rats (LC/MS/MS analysis).

[039] Figure 27. Mean plasma concentration time profile of L-lysine-*d*-amphetamine following 30-min intravenous infusion (2 mg/kg) or oral administration of L-lysine-*d*-amphetamine (2 mg/kg) in conscious male beagle dogs (n=3).

[040] Figure 28. Plasma concentration time profile of *d*-amphetamine following 30-min intravenous infusion or oral administration of L-lysine-*d*-amphetamine (2 mg/kg) in conscious male beagle dogs (n=3).

[041] Figures 29A-B. Mean plasma concentration time profile of L-lysine-*d*-amphetamine and *d*-amphetamine levels in ng/ml (Figure 29A), and in uM (Figure 29B), following 30-min intravenous infusion (2 mg/kg) in conscious male beagle dogs (n=3).

[042] Figures 30A-B. Mean plasma concentration time profile of L-lysine-*d*-amphetamine and *d*-amphetamine levels in ng/ml (Figure 30A), and in nM (Figure

30B), following oral administration of L-lysine-*d*-amphetamine (2 mg/kg) in conscious male beagle dogs (n=3).

[043] Figures 31A-B. Individual plasma concentration time profile of L-lysine-*d*-amphetamine following intravenous administration (Figure 31A) or oral administration (Figure 31B) of L-lysine-*d*-amphetamine in conscious male beagle dogs. The oral formulation used comprises solution and 0.2 mg/mL in water.

[044] Figures 32A-B. Individual plasma concentration time profile of *d*-amphetamine following intravenous administration (Figure 32A) or oral administration (Figure 32B) of L-lysine-*d*-amphetamine in conscious male beagle dogs.

[045] Figure 33. Plasma concentrations of *d*-amphetamine following oral administration of L-lysine-*d*-amphetamine or *d*-amphetamine sulfate (1.8 mg/kg *d*-amphetamine base) to male dogs.

[046] Figure 34. Plasma concentrations of *d*-amphetamine following oral administration of L-lysine-*d*-amphetamine or *d*-amphetamine sulfate (1.8 mg/kg *d*-amphetamine base) to female dogs.

[047] Figure 35. Mean blood pressure following intravenous bolus injection of increasing amounts of L-lysine-*d*-amphetamine or *d*-amphetamine in male and female dogs.

[048] Figure 36. Left ventricular blood pressure following intravenous bolus injection of increasing amounts of L-lysine-*d*-amphetamine or *d*-amphetamine in male and female dogs.

[049] Figure 37. Locomotor activity of rats following oral administration of L-lysine-*d*-amphetamine or *d*-amphetamine (5 hour time-course).

[050] Figure 38. Locomotor activity of rats following oral administration of L-lysine-*d*-amphetamine or *d*-amphetamine (12 hour time-course).

[051] Figure 39. Locomotor activity of rats following intranasal administration of L-lysine-*d*-amphetamine or *d*-amphetamine (1 hour time-course).

[052] Figure 40. Locomotor activity of rats following intranasal administration (with carboxymethylcellulose) of L-lysine-*d*-amphetamine or *d*-amphetamine (2 hour time-course).

[053] Figure 41. Locomotor activity of rats following intravenous administration of L-lysine-*d*-amphetamine or *d*-amphetamine (3 hour time-course).

[054] Figure 42. Intranasal bioavailability of abuse-resistant amphetamine amino acid-, di-, and tri-peptide conjugates (ELISA analysis).

[055] Figure 43. Oral bioavailability of abuse-resistant amphetamine amino acid-, di-, and tri-peptide conjugates (ELISA analysis).

[056] Figure 44. Intravenous bioavailability of an abuse-resistant amphetamine tri-peptide conjugate (ELISA analysis).

[057] Figure 45. Intranasal bioavailability of an abuse-resistant amphetamine amino acid conjugate (ELISA analysis).

[058] Figure 46. Oral bioavailability of an abuse-resistant amphetamine amino acid conjugate (ELISA analysis).

[059] Figure 47. Intravenous bioavailability of abuse-resistant amphetamine amino acid-, di-, and tri-peptide conjugates (ELISA analysis).

[060] Figure 48. Intranasal bioavailability of an abuse-resistant amphetamine amino tri-peptide conjugate (ELISA analysis).

[061] Figure 49. Intranasal bioavailability of abuse-resistant amphetamine amino acid-, and di-peptide conjugates (ELISA analysis).

[062] Figure 50. Intranasal bioavailability of an abuse-resistant amphetamine di-peptide conjugate containing D- and L- amino acid isomers (ELISA analysis).

[063] Figs. 51A-B. Plasma concentrations of *d*-amphetamine and L-lysine-*d*-amphetamine in ng/mL for the serum levels (Fig. 51A), and in ng/g for brain tissue (Fig. 51B), following oral administration of L-lysine-*d*-amphetamine or *d*-amphetamine sulfate (5mg/kg *d*-amphetamine base) to rats. Serum and brain tissue *d*-amphetamine and L-lysine-*d*-amphetamine concentrations were measured by LC/MS/MS (compound indicated in parenthesis).

[064] Figure 52. illustrates preparation of Galacto-Hydrocodone.

[065] Figure 53. Oral bioavailability of abuse-resistant hydrocodone carbohydrate conjugates, measured as free hydrocodone (with measured plasma levels by ELISA).

[066] Figure 54. illustrates preparation of Ribo-Hydrocodone.

[067] Figure 55. Intranasal bioavailability of abuse-resistant hydrocodone carbohydrate conjugate, measured as free hydrocodone (with measured plasma levels by ELISA).

[068] Figure 56. illustrates preparation of Leu-Hydrocodone.

[069] Figure 57. illustrates preparation of Ala-Pro-Hydrocodone.

[070] Figure 58. illustrates the preparation of Gly-Gly-Leu-Hydrocodone.

[071] Figure 59. illustrates preparation of Gly-Gly-Gly-Gly-Leu**[SEQ ID NO: 1]**-Hydrocodone.

[072] Figure 60. Intranasal bioavailability of abuse-resistant hydrocodone amino acid , di- and tri-peptide conjugates, measured as free hydrocodone.

[073] Figure 61. Analgesic effect of abuse-resistant hydrocodone tri-peptide conjugate following intranasal administration, measured as free hydrocodone.

[074] Figure 62. Analgesic effect of abuse-resistant hydrocodone tri- and penta-peptide conjugates following subcutaneous administration, measured as free hydrocodone.

[075] Figure 63. Analgesic effect of abuse-resistant hydrocodone penta-peptide conjugate following intransal administration, measured as free hydrocodone.

[076] Figure 64. Intranasal bioavailability of abuse-resistant hydrocodone tri- and penta-peptide conjugates, measured as free hydrocodone.

[077] Figure 65. Intranasal bioavailability of abuse-resistant hydrocodone tri- and penta-peptide conjugates, measured as free hydrocodone.

[078] Figure 66. Intranasal bioavailability of abuse-resistant hydrocodone an amino acid-carbohydrate peptide conjugate, measured as free hydrocodone.

[079] Figure 67. Analgesic effect of abuse-resistant hydrocodone penta-peptide conjugate following intravenous administration, measured as free hydrocodone.

[080] Figure 68. Intranasal bioavailability of an abuse-resistant hydrocodone tri-peptide conjugate, measured as free hydrocodone.

[081] Figure 69. Intranasal bioavailability of an abuse-resistant hydrocodone penta-peptide conjugate, measured as free hydrocodone.

[082] Figure 70. Intranasal bioavailability of an abuse-resistant hydrocodone tri-peptide conjugate, measured as free hydrocodone.

[083] Figure 71. Intranasal bioavailability of abuse-resistant hydrocodone tri- and penta-peptide conjugates, measured as free hydrocodone.

[084] Figure 72. Intranasal bioavailability of abuse-resistant hydrocodone penta-peptide conjugates, measured as free hydrocodone.

[085] Figure 73. Intranasal bioavailability of an abuse-resistant hydrocodone penta-peptide conjugate, measured as free hydrocodone.

[086] Figure 74. Intravenous bioavailability of an abuse-resistant hydrocodone tri-peptide conjugate, measured as free hydrocodone.

[087] Figure 75. Intranasal bioavailability of an abuse-resistant hydrocodone tri-peptide conjugate, measured as free hydrocodone.

[088] Figure 76. Oral bioavailability of an abuse-resistant hydrocodone penta-peptide conjugate, measured as free hydrocodone.

[089] Figure 77. Intranasal bioavailability of an abuse-resistant hydrocodone tri-penta-peptide conjugate, measured as free hydrocodone.

[090] Figure 78. Intranasal bioavailability of an abuse-resistant hydrocodone penta-peptide conjugate, measured as free hydrocodone.

[091] Figure 79. Intranasal bioavailability of abuse-resistant hydrocodone penta-peptide conjugates, measured as free hydrocodone.

[092] Figure 80. Intranasal bioavailability of an abuse-resistant hydrocodone tri-peptide conjugate containing D-and L-isomers, measured as free hydrocodone.

[093] Figure 81. Intranasal bioavailability of an abuse-resistant hydrocodone penta-peptide conjugate, measured as free hydrocodone.

[094] Figure 82. Intranasal bioavailability of an abuse-resistant hydrocodone penta-peptide conjugate, measured as free hydrocodone.

[095] Figure 83. Intranasal bioavailability of an abuse-resistant hydrocodone penta-peptide conjugate, measured as free hydrocodone.

[096] Figure 84. Intranasal bioavailability of abuse-resistant hydrocodone penta-peptide conjugates, measured as free hydrocodone.

[097] Figure 85. Intranasal bioavailability of an abuse-resistant hydrocodone penta-peptide conjugate, measured as free hydrocodone.

[098] Figure 86. illustrates preparation of 1,2:3,4-di-O-isopropylidene-D-galactopyranose.

[100] Figure 87. Oral bioavailability of abuse-resistant hydrocodone glyco-peptide conjugates, measured as free hydrocodone.

[101] Figure 88. Oral bioavailability of an abuse-resistant hydrocodone amino acid-crabohydrate conjugate, measured as free hydrocodone.

[102] Figure 89. illustrates nucleosides and conjugation sites.

[103] Figure 90. Oral bioavailability in rats for hydrocodone vs. EEFFFI**[SEQ ID NO: 2]**-HC at a dose (1 mg/kg) approximating a therapeutic human dose equivalent measured as free hydrocodone.

[104] Figure 91. Oral bioavailability in rats for hydrocodone vs. EEFFF**[SEQ ID NO: 3]**-HC at a dose (1mg/kg) approximating a therapeutic human dose equivalent measured as free hydrocodone.

[105] Figure 92. Oral bioavailability in rats for hydrocodone vs. YYI-HC at a dose (1 mg/kg) approximating a therapeutic human dose equivalent measured as free hydrocodone.

[106] Figure 93. Oral bioavailability in rats for hydrocodone vs. DDI-HC at a dose (1 mg/kg) approximating a therapeutic human dose equivalent measured as free hydrocodone.

[107] Figure 94. Oral bioavailability in rats for hydrocodone vs. YYFFI**[SEQ ID NO: 6]**-HC at a dose (1mg/kg) approximating a therapeutic human dose equivalent measured as free hydrocodone.

[108] Figure 95. Oral bioavailability in rats for hydrocodone vs. EEFFI**[SEQ ID NO: 5]**-HC at a dose (5 mg/kg) approaching a human overdose equivalent measured as free hydrocodone.

[109] Figure 96. Oral bioavailability in rats for hydrocodone vs. YYI-HC at a dose (5 mg/kg) approaching a human overdose equivalent measured as free hydrocodone.

[110] Figure 97. Oral bioavailability in rats for hydrocodone vs. DDI-HC at a dose (5mg/kg) approaching a human overdose equivalent measured as free hydrocodone.

[111] Figure 98. Oral bioavailability in rats for hydrocodone vs. YYFFI**[SEQ ID NO: 6]**-HC at a dose (5 mg/kg) approaching a human overdose equivalent measured as free hydrocodone.

[112] Figure 99. Decrease in bioavailability of EEFFF**[SEQ ID NO: 3]**-HC as compared to hydrocodone by the intranasal route of administration measured as free hydrocodone.

[113] Figure 100. Decrease in bioavailability of YYI-HC as compared to hydrocodone by the intranasal route of administration measured as free hydrocodone.

[114] Figure 101. Decrease in bioavailability of DDI-HC as compared to hydrocodone by the intranasal route of administration measured as free hydrocodone.

[115] Figure 102. Decrease in bioavailability of YYFFI**[SEQ ID NO: 6]**-HC as compared to hydrocodone by the intranasal route of administration measured as free hydrocodone.

[116] Figure 103. Decrease in bioavailability of EEFFI**[SEQ ID NO: 5]**-HC as compared to hydrocodone by the intravenous route of administration measured as free hydrocodone.

[117] Figure 104. Decrease in bioavailability of EEFFF**[SEQ ID NO: 3]**-HC as compared to hydrocodone by the intravenous route of administration measured as free hydrocodone.

[118] Figure 105. Decrease in bioavailability of YYI-HC as compared to hydrocodone by the intravenous route of administration measured as free hydrocodone.

[119] Figure 106. Decrease in bioavailability of YYFFI**[SEQ ID NO: 6]**-HC as compared to hydrocodone by the intravenous route of administration measured as free hydrocodone.

[120] Figure 107. Oral bioavailability of hydrocodone plus hydromorphone (concentration vs. time) following administration of hydrocodone bitratrate or YYFFI**[SEQ ID NO: 6]**-HC at 1 mg/kg (equimolar doses with equivalent content of hydrocodone base) in rats, measured as free hydrocodone.

[121] Figure 108. Oral bioavailability of hydrocodone (concentration vs. time) following administration of hydrocodone bitratrate or YYFFI**[SEQ ID NO: 6]**-HC at 1 mg/kg (equimolar doses with equivalent content of hydrocodone base) in rats, measured as free hydrocodone.

[122] Figure 109. Oral bioavailability of hydromorphone (concentration vs. time) following administration of hydrocodone bitratrate or YYFFI**[SEQ ID NO: 6]**-HC at 1 mg/kg (equimolar doses with equivalent content of hydrocodone base) in rats, measured as free hydrocodone.

[123] Figure 110. Oral bioavailability of hydrocodone plus hydromorphone (concentration vs. time) following administration of hydrocodone bitratrate or YYFFI**[SEQ ID NO: 6]**-HC at 2 mg/kg (equimolar doses with equivalent content of hydrocodone base) in rats, measured as free hydrocodone.

[124] Figure 111. Oral bioavailability of hydrocodone (concentration vs. time) following administration of hydrocodone bitratrate or YYFFI**[SEQ ID NO: 6]**-HC at 2 mg/kg (equimolar doses with equivalent content of hydrocodone base) in rats, measured as free hydrocodone.

[125] Figure 112. Oral bioavailability of hydromorphone (concentration vs. time) following administration of hydrocodone bitratrate or YYFFI**[SEQ ID NO: 6]**-HC at 2 mg/kg (equimolar doses with equivalent content of hydrocodone base) in rats, measured as free hydrocodone.

[126] Figure 113. Oral bioavailability of hydrocodone plus hydromorphone (concentration vs. time) following administration of hydrocodone bitratrate or YYFFI**[SEQ ID NO: 6]**-HC at 5 mg/kg (equimolar doses with equivalent content of hydrocodone base) in rats, measured as free hydrocodone.

[127] Figure 114. Oral bioavailability of hydrocodone (concentration vs. time) following administration of hydrocodone bitratrate or YYFFI**[SEQ ID NO: 6]**-HC at 5 mg/kg (equimolar doses with equivalent content of hydrocodone base) in rats, measured as free hydrocodone.

[128] Figure 115. Oral bioavailability of hydromorphone (concentration vs. time) following administration of hydrocodone bitratrate or YYFFI**[SEQ ID NO: 6]**-HC at 5 mg/kg

(equimolar doses with equivalent content of hydrocodone base) in rats, measured as free hydrocodone.

[129] Figure 116. Oral bioavailability of hydrocodone plus hydromorphone (concentration vs. time) following administration of hydrocodone bitratrate or YYFFI**[SEQ ID NO: 6]**-HC at 25 mg/kg (equimolar doses with equivalent content of hydrocodone base) in rats, measured as free hydrocodone.

[130] Figure 117. Oral bioavailability of hydrocodone (concentration vs. time) following administration of hydrocodone bitratrate or YYFFI**[SEQ ID NO: 6]**-HC at 25 mg/kg (equimolar doses with equivalent content of hydrocodone base) in rats, measured as free hydrocodone.

[131] Figure 118. Oral bioavailability of hydromorphone (concentration vs. time) following administration of hydrocodone bitratrate or YYFFI**[SEQ ID NO: 6]**-HC at 25 mg/kg (equimolar doses with equivalent content of hydrocodone base) in rats, measured as free hydrocodone.

[132] Figure 119. Oral bioavailability ($AUC_{0\text{-}4h}$) of hydrocodone plus hydromorphone (concentration vs. dose) in proportion to dose following administration of hydrocodone bitratrate or YYFFI**[SEQ ID NO: 6]**-HC at escalating doses (1, 2, 5, and 25 mg/kg - equimolar doses with equivalent content of hydrocodone base) in rats, measured as free hydrocodone.

[133] Figure 120. Oral bioavailability ($AUC_{0\text{-}4h}$) of hydrocodone plus hydromorphone in proportion to human equivalent doses (HED) following administration of hydrocodone bitratrate or YYFFI**[SEQ ID NO: 6]**-HC at escalating doses (1, 2, 5, and 25 mg/kg - equimolar doses with equivalent content of hydrocodone base) in rats, measured as free hydrocodone.

[134] Figure 121. Oral bioavailability (C_{max}) of hydrocodone plus hydromorphone (concentration vs. dose) in proportion to dose following administration of hydrocodone bitratrate or YYFFI**[SEQ ID NO: 6]**-HC at escalating doses (1, 2, 5, and 25 mg/kg - equimolar doses with equivalent content of hydrocodone base) in rats, measured as free hydrocodone.

[135] Figure 122. Oral bioavailability (C_{max}) of hydrocodone plus hydromorphone in proportion to human equivalent doses (HED) following administration of

hydrocodone bitratrate or YYFFI**[SEQ ID NO: 6]**-HC at escalating doses (1, 2, 5, and 25 mg/kg - equimolar doses with equivalent content of hydrocodone base) in rats, measured as free hydrocodone.

[136] Figure 123. Intravenous bioavailability of hydrocodone plus hydromorphone and YYFFI**[SEQ ID NO: 6]**-HC (concentration vs. time) following administration of hydrocodone bitratrate or YYFFI**[SEQ ID NO: 6]**-HC at 1 mg/kg (equimolar doses with equivalent content of hydrocodone base) in rats, measured as free hydrocodone.

[137] Figure 124. Intravenous bioavailability of hydrocodone (concentration vs. time) following administration of hydrocodone bitratrate or YYFFI**[SEQ ID NO: 6]**-HC at 1 mg/kg (equimolar doses with equivalent content of hydrocodone base) in rats, measured as free hydrocodone.

[138] Figure 125. Intravenous bioavailability of hydromorphone (concentration vs. time) following administration of hydrocodone bitratrate or YYFFI**[SEQ ID NO: 6]**-HC at 1 mg/kg (equimolar doses with equivalent content of hydrocodone base) in rats, measured as free hydrocodone.

[139] Figure 126. Intranasal bioavailability of hydrocodone plus hydromorphone (concentration vs. time) following administration of hydrocodone bitratrate or YYFFI**[SEQ ID NO: 6]**-HC at 1 mg/kg (equimolar doses with equivalent content of hydrocodone base) in rats, measured as free hydrocodone.

[140] Figure 127. Intranasal bioavailability of hydrocodone (concentration vs. time) following administration of hydrocodone bitratrate or YYFFI**[SEQ ID NO: 6]**-HC at 1 mg/kg (equimolar doses with equivalent content of hydrocodone base) in rats, measured as free hydrocodone.

[141] Figure 128. Intranasal bioavailability of hydromorphone (concentration vs. time) following administration of hydrocodone bitratrate or YYFFI**[SEQ ID NO: 6]**-HC at 1 mg/kg (equimolar doses with equivalent content of hydrocodone base) in rats, measured as free hydrocodone.

[142] Figure 129. Oral bioavailability of hydrocodone plus hydromorphone (concentration vs. time) following administration of hydrocodone bitratrate or

YYFFI**[SEQ ID NO: 6]**-HC at 1 mg/kg (equimolar doses with equivalent content of hydrocodone base) in rats, measured as free hydrocodone.

[143] Figure 130. Oral bioavailability of hydrocodone (concentration vs. time) following administration of hydrocodone bitratrate or YYFFI**[SEQ ID NO: 6]**-HC at 1 mg/kg (equimolar doses with equivalent content of hydrocodone base) in rats, measured as free hydrocodone.

[144] Figure 131. Oral bioavailability of hydromorphone (concentration vs. time) following administration of hydrocodone bitratrate or YYFFI**[SEQ ID NO: 6]**-HC at 1 mg/kg (equimolar doses with equivalent content of hydrocodone base) in rats, measured as free hydrocodone.

[145] Figure 132. Oral bioavailability of hydrocodone plus hydromorphone (concentration vs. time) following administration of hydrocodone bitratrate or YYFFI**[SEQ ID NO: 6]**-HC at 2 mg/kg (equimolar doses with equivalent content of hydrocodone base) in rats, measured as free hydrocodone.

[146] Figure 133. Oral bioavailability of hydrocodone (concentration vs. time) following administration of hydrocodone bitratrate or YYFFI**[SEQ ID NO: 6]**-HC at 2 mg/kg (equimolar doses with equivalent content of hydrocodone base) in rats, measured as free hydrocodone.

[147] Figure 134. Oral bioavailability of hydromorphone (concentration vs. time) following administration of hydrocodone bitratrate or YYFFI**[SEQ ID NO: 6]**-HC at 2 mg/kg (equimolar doses with equivalent content of hydrocodone base) in rats, measured as free hydrocodone.

[148] Figure 135. Oral bioavailability of hydrocodone plus hydromorphone (concentration vs. time) following administration of hydrocodone bitratrate or YYFFI**[SEQ ID NO: 6]**-HC at 5 mg/kg (equimolar doses with equivalent content of hydrocodone base) in rats, measured as free hydrocodone.

[149] Figure 136. Oral bioavailability of hydrocodone (concentration vs. time) following administration of hydrocodone bitratrate or YYFFI**[SEQ ID NO: 6]**-HC at 5 mg/kg (equimolar doses with equivalent content of hydrocodone base) in rats, measured as free hydrocodone.

[150] Figure 137. Oral bioavailability of hydromorphone (concentration vs. time) following administration of hydrocodone bitratrate or YYFFI**[SEQ ID NO: 6]**-HC at 5 mg/kg

(equimolar doses with equivalent content of hydrocodone base) in rats, measured as free hydrocodone.

[151] Figure 138. Oral bioavailability of hydrocodone plus hydromorphone (concentration vs. time) following administration of hydrocodone bitratrate or YYFFI**[SEQ ID NO: 6]**-HC at 25 mg/kg (equimolar doses with equivalent content of hydrocodone base) in rats, measured as free hydrocodone.

[152] Figure 139. Oral bioavailability of hydrocodone (concentration vs. time) following administration of hydrocodone bitratrate or YYFFI**[SEQ ID NO: 6]**-HC at 25 mg/kg (equimolar doses with equivalent content of hydrocodone base) in rats, measured as free hydrocodone.

[153] Figure 140. Oral bioavailability of hydromorphone (concentration vs. time) following administration of hydrocodone bitratrate or YYFFI**[SEQ ID NO: 6]**-HC at 25 mg/kg (equimolar doses with equivalent content of hydrocodone base) in rats, measured as free hydrocodone.

[154] Figure 141. Oral bioavailability (AUC_{0-4}) of hydrocodone plus hydromorphone (concentration vs. dose) in proportion to dose following administration of hydrocodone bitratrate or YYFFI**[SEQ ID NO: 6]**-HC at escalating doses (1, 2, 5, and 25 mg/kg - equimolar doses with equivalent content of hydrocodone base) in rats, measured as free hydrocodone.

[155] Figure 142. Oral bioavailability (AUC_{0-4}) of hydrocodone plus hydromorphone in proportion to human equivalent doses (HED) following administration of hydrocodone bitratrate or YYFFI**[SEQ ID NO: 6]**-HC at escalating doses (1, 2, 5, and 25 mg/kg - equimolar doses with equivalent content of hydrocodone base) in rats, measured as free hydrocodone.

[156] Figure 143. Oral bioavailability (C_{max}) of hydrocodone plus hydromorphone (concentration vs. dose) in proportion to dose following administration of hydrocodone bitratrate or YYFFI**[SEQ ID NO: 6]**-HC at escalating doses (1, 2, 5, and 25 mg/kg - equimolar doses with equivalent content of hydrocodone base) in rats, measured as free hydrocodone.

[157] Figure 144. Oral bioavailability (C_{max}) of hydrocodone plus hydromorphone in proportion to human equivalent doses (HED) following administration of

hydrocodone bitratrate or YYFFI[**SEQ ID NO: 6**]-HC at escalating doses (1, 2, 5, and 25 mg/kg - equimolar doses with equivalent content of hydrocodone base) in rats, measured as free hydrocodone.

[158] Figure 145. Intravenous bioavailability of hydrocodone plus hydromorphone and YYFFI[**SEQ ID NO: 6**]-HC (concentration vs. time) following administration of hydrocodone bitratrate or YYFFI[**SEQ ID NO: 6**]-HC at 1 mg/kg (equimolar doses with equivalent content of hydrocodone base) in rats, measured as free hydrocodone.

[159] Figure 146. Intravenous bioavailability of hydrocodone (concentration vs. time) following administration of hydrocodone bitratrate or YYFFI[**SEQ ID NO: 6**]-HC at 1 mg/kg (equimolar doses with equivalent content of hydrocodone base) in rats, measured as free hydrocodone.

[160] Figure 147. Intravenous bioavailability of hydromorphone (concentration vs. time) following administration of hydrocodone bitratrate or YYFFI[**SEQ ID NO: 6**]-HC at 1 mg/kg (equimolar doses with equivalent content of hydrocodone base) in rats, measured as free hydrocodone.

[161] Figure 148. Intranasal bioavailability of hydrocodone plus hydromorphone (concentration vs. time) following administration of hydrocodone bitratrate or YYFFI[**SEQ ID NO: 6**]-HC at 1 mg/kg (equimolar doses with equivalent content of hydrocodone base) in rats, measured as free hydrocodone.

[162] Figure 149. Intranasal bioavailability of hydrocodone (concentration vs. time) following administration of hydrocodone bitratrate or YYFFI[**SEQ ID NO: 6**]-HC at 1 mg/kg (equimolar doses with equivalent content of hydrocodone base) in rats, measured as free hydrocodone.

[163] Figure 150. Intranasal bioavailability of hydromorphone (concentration vs. time) following administration of hydrocodone bitratrate or YYFFI[**SEQ ID NO: 6**]-HC at 1 mg/kg (equimolar doses with equivalent content of hydrocodone base) in rats, measured as free hydrocodone.

[164] Figure 151. depicts oxycodone.

[165] Figure 152. depicts oxycodone with lysine branched peptides.

[166] Figure 153. depicts a glycosylated oxycodone.

[167] Figure 154. depicts formation of an enol ether with serine.

[168] Figure 155. depicts niacin and biotin.

[169] Figure 156. Oral bioavailability of abuse-resistant oxycodone disubstituted tripeptide conjugates, measured as free oxycodone.

[170] Figure 157. Oral bioavailability of abuse-resistant oxycodone disubstituted tripeptide conjugates, measured as free oxycodone.

[171] Figure 158. Oral bioavailability of abuse-resistant oxycodone disubstituted tripeptide conjugates, measured as free oxycodone.

[172] Figure 159. Oral bioavailability of abuse-resistant oxycodone disubstituted tripeptide conjugates, measured as free oxycodone.

[173] Figure 160. Oral bioavailability of abuse-resistant oxycodone disubstituted tripeptide conjugates, measured as free oxycodone.

[174] Figure 161. Oral bioavailability of abuse-resistant oxycodone disubstituted tripeptide conjugates, measured as free oxycodone.

[175] Figure 162. Oral bioavailability of abuse-resistant oxycodone disubstituted tripeptide conjugates, measured as free oxycodone.

[176] Figure 163. Oral bioavailability of abuse-resistant oxycodone disubstituted tripeptide conjugates, measured as free oxycodone.

[177] Figure 164. Oral bioavailability of abuse-resistant oxycodone disubstituted tripeptide conjugates, measured as free oxycodone.

[178] Figure 165. Oral bioavailability of abuse-resistant oxycodone disubstituted tripeptide conjugates, measured as free oxycodone.

[179] Figure 166. Oral bioavailability of abuse-resistant oxycodone disubstituted tripeptide conjugates, measured as free oxycodone.

[180] Figure 167. Oral bioavailability of abuse-resistant oxycodone disubstituted tripeptide conjugates, measured as free oxycodone.

[181] Figure 168. Oral bioavailability of abuse-resistant oxycodone disubstituted tripeptide conjugates, measured as free oxycodone.

[182] Figure 169. Oral bioavailability of abuse-resistant oxycodone disubstituted tripeptide conjugates, measured as free oxycodone.

[183] Figure 170. Oral bioavailability of abuse-resistant oxycodone disubstituted tripeptide conjugates, measured as free oxycodone.

[184] Figure 171. Oral bioavailability of abuse-resistant oxycodone disubstituted tripeptide conjugates, measured as free oxycodone.

[185] Figure 172. Oral bioavailability of abuse-resistant oxycodone disubstituted tripeptide conjugates, measured as free oxycodone.

[186] Figure 173. Oral bioavailability of abuse-resistant oxycodone disubstituted tripeptide conjugates, measured as free oxycodone.

[187] Figure 174. Oral bioavailability of abuse-resistant oxycodone disubstituted tripeptide conjugates, measured as free oxycodone.

[188] Figure 175. Intranasal bioavailability of abuse-resistant oxycodone disubstituted tripeptide conjugates, measured as free oxycodone.

[189] Figure 176. Intranasal bioavailability of abuse-resistant oxycodone disubstituted tripeptide conjugates, measured as free oxycodone.

[190] Figure 177. Intranasal bioavailability of abuse-resistant oxycodone disubstituted tripeptide conjugates, measured as free oxycodone.

[191] Figure 178. Intravenous bioavailability of abuse-resistant oxycodone disubstituted tripeptide conjugates, measured as free oxycodone.

[192] Figure 179. Intranasal bioavailability of abuse-resistant oxycodone disubstituted tripeptide conjugates, measured as free oxycodone.

[193] Figure 180. Intranasal bioavailability of abuse-resistant oxycodone disubstituted tripeptide conjugates, measured as free oxycodone.

[194] Figure 181. Intranasal bioavailability of abuse-resistant oxycodone disubstituted tripeptide conjugates, measured as free oxycodone.

[195] Figure 182. Intranasal bioavailability of abuse-resistant oxycodone disubstituted tripeptide conjugates, measured as free oxycodone.

[196] Figure 183. Intranasal bioavailability of abuse-resistant oxycodone disubstituted tripeptide conjugates, measured as free oxycodone.

[197] Figure 184. Intranasal bioavailability of abuse-resistant oxycodone disubstituted tripeptide conjugates, measured as free oxycodone.

[198] Figure 185. Intranasal bioavailability of abuse-resistant oxycodone disubstituted tripeptide conjugates, measured as free oxycodone.

[199] Figure 186. Intranasal bioavailability of abuse-resistant oxycodone disubstituted tripeptide conjugates, measured as free oxycodone.

[200] Figure 187. Intranasal bioavailability of abuse-resistant oxycodone disubstituted tripeptide conjugates, measured as free oxycodone.

[201] Figure 188. Intranasal bioavailability of abuse-resistant oxycodone disubstituted tripeptide conjugates, measured as free oxycodone.

[202] Figure 189. Intranasal bioavailability of abuse-resistant oxycodone disubstituted tripeptide conjugates, measured as free oxycodone.

[203] Figure 190. Intranasal bioavailability of abuse-resistant oxycodone disubstituted tripeptide conjugates, measured as free oxycodone.

[204] Figure 191. Intranasal bioavailability of abuse-resistant oxycodone disubstituted tripeptide conjugates, measured as free oxycodone.

[205] Figure 192. Intranasal bioavailability of abuse-resistant oxycodone disubstituted tripeptide conjugates, measured as free oxycodone.

[206] Figure 193. Oral bioavailability in rats of oxycodone vs. $P2L_{(2)}$-Oxycodone at a dose (2.5 mg/kg) approximating a therapeutic human dose equivalent measured as free oxycodone.

[207] Figure 194. Decrease in bioavailability of $P2L_{(2)}$-Oxycodone as compared to oxycodone by the intranasal route of administration- dose 2.5 mg/kg measured as free oxycodone.

[208] Figure 195. Decrease in bioavailability of $P2L_{(2)}$-Oxycodone as compared to oxycodone by the intravenous route of administration- dose 0.5 mg/kg measured as free oxycodone.

DETAILED DESCRIPTION OF THE INVENTION

[209] The present invention relates to changing the pharmacokinetic and pharmacological properties of active agents through covalent modification. Covalent attachment of a chemical moiety to an active agent can change the rate and extent of absorption, metabolism, distribution, and elimination of the active agent. When administered at a normal therapeutic dose the bioavailablility (area under the time-versus-concentration curve; AUC) of the active agent is similar to that of the parent active agent compound. As the oral dose is increased, however, the bioavailability of

the covalently modified active agent relative to the parent active agent begins to decline. At suprapharmacological doses the bioavailability of the active agent conjugate is substantially decreased as compared to the parent active agent. The relative decrease in bioavailability at higher doses abates the euphoria obtained when doses of the active agent conjugate are taken above those of the intended prescription. This in turn diminishes the abuse potential, whether unintended or intentionally sought.

[210] Persons that abuse prescription drugs commonly seek to increase their euphoria by snorting or injecting the drugs. These routes of administration increase the rate and extent of drug absorption and provide a faster, nearly instantaneous, effect. This increases the amount of drug that reaches the central nervous system where it has its effect. In a particular embodiment of the invention the bioavailability of the covalently modified active agent is substantially decreased by the intranasal and intravenous routes as compared to the parent active agent. Thus the illicit practice of snorting and shooting the drug loses its advantage.

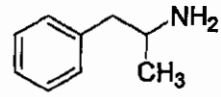
[211] In accordance with the present invention and as used herein, the following terms are defined with the following meanings, unless explicitly stated otherwise. For additional methods of attaching active agents to carriers, see application number U.S. 10/156,527, and/or PCT/US03/05524, and/or PCT/US03/05525 and/or PCT/US04/17204 each of which is hereby incorporated by reference in its entirety.

[212] The invention utilizes covalent modification of an active agent to decrease its potential for causing overdose or being abused. The active agent is covalently modified in a manner that decreases its pharmacological activity, as compared to the unmodified active agent, at doses above those considered therapeutic, e.g., at doses inconsistent with the manufacturer's instructions. When given at lower doses, such as those intended for therapy, the covalently modified active agent retains pharmacological activity similar to that of the unmodified active agent. The covalent modification of the active agent may comprise the attachment of any chemical moiety through conventional chemistry.

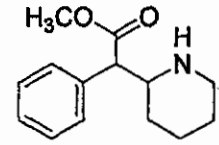
[213] Compounds, compositions and methods of the invention provide reduced potential for overdose, reduced potential for abuse or addiction and/or improve the

active agent's characteristics with regard to high toxicities or suboptimal release profiles. Without wishing to be limited to the below theory, we believe that in some instances (e.g., with amphetamines) overdose protection results from a natural gating mechanism at the site of hydrolysis that limits the release of the active agent from the prodrug at greater than therapeutically prescribed amounts. Therefore, abuse resistance is provided by limiting the "rush" or "high" available from the active agent released by the prodrug and limiting the effectiveness of alternative routes of administration.

[214] "Amphetamine" shall mean any of the sympathomimetic phenethylamine derivatives which have central nervous system stimulant activity, such as but not limited to, amphetamine, methamphetamine, p-methoxyamphetamine, methylenedioxyamphetamine, 2,5-dimethoxy-4-methylamphetamine, 2,4,5-trimethoxyamphetamine and 3,4-methylenedioxymethamphetamine.



Amphetamine



Methylphenidate

[215] Other embodiments of amphetamine are described according to the following abbreviations.

L-lysine-*d*-amphetamine = Lys-Amp, Lys-Amph, Lysine-Amphetamine, KAMP, K-amphetamine, or: = 2,6-diaminohexanoic acid-(1-methyl-2-phenylethyl)-amide

Phe-Amp = Phenylalanine-Amphetamine, FAMP,

= 2-amino-3-phenylpropanoic acid-(1-methyl-2-phenylethyl)-amide,

Ser-Amp = Serine-Amphetamine, SAMP

= 2-amino-3-hydroxylpropanoic acid-(1-methyl-2-phenylethyl)-amide, Gly_3-Amp = GGG-Amphetamine, GGGAMP

=2-Amino-N-({[(1-methyl-2-phenyl-ethylcarbomyl)-methyl]-carbomyl}- methyl) - acetamide

[216] Throughout this application the use of "opioid" is meant to include any drug that activates the opioid receptors found in the brain, spinal cord and gut. There are

three broad classes of opioids: naturally occurring opium alkaloids, such as morphine (the prototypical opioid) and codeine; semi-synthetics such as heroine, oxycodone and hydrocodone that are produced by modifying natural opium alkaloids and have similar chemical structures; and pure synthetics such as fentanyl and methadone that are not produced from opium and may have very different chemical structures than the opium alkaloids. Other opioids include hydroxymorphone, oxymorphone, methadone, levorphanol, dihydrocodeine, meperidine, diphenoxylate, sufentanil, alfentanil, propoxyphene, pentazocine, nalbuphine, butorphanol, buprenorphine, meptazinol, dezocine, and pharmaceutically acceptable salts thereof.

[217] Throughout this application the use of "oxyocodone" is meant to include a narcotic alkaloid (chemical formula $C_{18}H_{21}NO_4$) and its derivatives such as the hydrochloride salt of oxycodone. Oxycodone is related to codeine and is used as an analgesic and/or a sedative. Oxycodone is a powerful and potentially addictive opioid analgesic synthesized from thebaine. It is similar to codeine, but is more potent and has a higher dependence potential. It is effective orally and is often marketed in combination with aspirin (Percodan®) or acetaminophen (Percocet®) for the relief of pain. It is also sold in a sustained-release form under the trade name Oxycontin®. All of these deriviatives or combinations of oxycodone are encompassed by the present invention.

[218] Throughout this application the use of "hydrocodone" is meant to include a semisynthetic narcotic analgesic and antitussive prepared from codeine with multiple actions qualitatively similar to those of codeine. It is commonly used for the relief of moderate to moderately severe pain. Trade names include Anexsia®, Hycodan®, Hycomine®, Lorcet®, Lortab®, Norco®, Tussionex®, Tylox®, and Vicodin®. Derivatives of hydrocodone, such as hydrocodone bitartrate and hydrocodone polistirex, are encompassed by the present invention.

[219] Throughout this application the use of "peptide" is meant to include a single amino acid, a dipeptide, a tripeptide, an oligopeptide, a polypeptide, or the carrier peptide. Oligopeptide is meant to include from 2 amino acids to 70 amino acids. Further, at times the invention is described as being an active agent attached to an

amino acid, a dipeptide, a tripeptide, an oligopeptide, or polypeptide to illustrate specific embodiments for the active agent conjugate. Preferred lengths of the conjugates and other preferred embodiments are described herein.

[220] Throughout this application the use of "chemical moiety" is meant to include at least amino acids, peptides, glycopeptides, carbohydrates, lipids, nucleosides, or vitamins.

[221] "Carbohydrates" includes sugars, starches, cellulose, and related compounds. e.g., $(CH_2O)_n$, wherein n is an integer larger than 2 or $C_n(H_2O)_{n-1}$, with n larger than 5. More specific examples include for instance, fructose, glucose, lactose, maltose, sucrose, glyceraldehyde, dihydroxyacetone, erythrose, ribose, ribulose, xylulose, galactose, mannose, sedoheptulose, neuraminic acid, dextrin, and glycogen.

[222] A "glycoprotein" is a compound containing carbohydrate (or glycan) covalently linked to protein. The carbohydrate may be in the form of a monosaccharide, disaccharide(s). oligosaccharide(s), polysaccharide(s), or their derivatives (e.g. sulfo- or phospho-substituted).

[223] A "glycopeptide" is a compound consisting of carbohydrate linked to an oligopeptide composed of L- and/or D-amino acids. A glyco-amino-acid is a saccharide attached to a single amino acid by any kind of covalent bond. A glycosyl-amino- acid is a compound consisting of saccharide linked through a glycosyl linkage (O-, N- or S-) to an amino acid.

[224] A "composition" as used herein, refers broadly to any composition containing a described molecule conjugates. The composition may comprise a dry formulation, an aqueous solution, or a sterile composition. Compositions comprising the molecules described herein may be stored in freeze-dried form and may be associated with a stabilizing agent such as a carbohydrate. In use, the composition may be deployed in an aqueous solution containing salts, *e.g.*, NaCl, detergents, *e.g.*, sodium dodecyl sulfate (SDS), and other components.

[225] A "controlled substance" is a substance subject to federal regulation of its manufacture, sale, or distribution because of the potential for, or proved evidence of, abuse; because of its potential for psychic or physiological dependence; because it constitutes a public health risk; because of the scientific evidence of its

pharmacologic effect; or because of its role as a precursor of other controlled substances.

[226] Important note regarding stereochemistry: This patent is meant to cover all compounds discussed regardless of absolute configurations. Thus, natural, L-amino acids are discussed but the use of D-amino acids are also included.

[227] The following abbreviations may be in this application:

BOC = t-butyloxycarbonyl

CMC = carboxymethylcellulose

DIPEA = di-isopropyl ethyl amine

mp = melting point

NMR = nuclear magnetic resonance

OSu = hydroxysuccinimido ester

Nia = Niacin

Bio = Biotin

[228] The attached chemical moiety may be any chemical substance that decreases the pharmacological activity until the active agent is released. Preferably the chemical moiety is a single amino acid, dipeptide or tripeptide, tetrapeptide, pentapeptide, or hexapeptide. The active agent binds to specific sites to produce various effects (Hoebel, *et al.,* 1989). The attachment of certain chemical moieties can therefore diminish or prevent binding to these biological target sites. Preferably, absorption of the composition into the brain is prevented or substantially diminished and/or delayed when delivered by routes other than oral administration.

[229] The attached chemical moiety may further comprise naturally occurring or synthetic substances. This would include but is not limited to the attachment of an active agent to one or more amino acids, peptides, lipids, carbohydrates, glycopeptides, nucleic acids or vitamins. These chemical moieties could be expected to affect delayed release in the gastrointestinal tract and prevent rapid onset of the desired activity, particularly when delivered by parenteral routes. (Hoebel, B. G., L. Hernandez, et al. (1989). "Microdialysis studies of brain norepinephrine, serotonin, and dopamine release during ingestive behavior. Theoretical and clinical implications." Ann N Y Acad Sci 575: 171-91).

[230] For each of the embodiments recited herein, the amino acid or peptide may comprise of one or more of the naturally occurring (L-) amino acids: alanine, arginine, asparagine, aspartic acid, cysteine, glycine, glutamic acid, glutamine, histidine, isoleucine, leucine, lysine, methionine, proline, phenylalanine, serine, tryptophan, threonine, tyrosine, and valine. In another embodiment the amino acid or peptide is comprised of one or more of the naturally occurring (D) amino acids: alanine, arginine, asparagine, aspartic acid, cysteine, glycine, glutamic acid, glutamine, histidine, isoleucine, leucine, lysine, methionine, proline, phenylalanine, serine, tryptophan, threonine, tyrosine, and valine. In another embodiment the amino acid or peptide is comprised of one or more unnatural, non-standard or synthetic amino acids such as, aminohexanoic acid, biphenylalanine, cyclohexylalanine, cyclohexylglycine, diethylglycine, dipropylglycine, 2,3-diaminoproprionic acid, homophenylalanine, homoserine, homotyrosine, naphthylalanine, norleucine, ornithine, pheylalanine(4-fluoro), phenylalanine(2,3,4,5,6 pentafluoro), phenylalanine(4-nitro), phenylglycine, pipecolic acid, sarcosine, tetrahydroisoquinoline-3-carboxylic acid, and tert-leucine. In another embodiment the amino acid or peptide comprises of one or more amino acid alcohols. In another embodiment the amino acid or peptide comprises of one or more N-methyl amino acids.

[231] In another embodiment, the specific carriers are utilized as a base short chain amino acid sequence and additional amino acids are added to the terminus or side chain. In another embodiment, the above amino acid sequence may have one more of the amino acids substituted with one of the 20 naturally occurring amino acids. It is preferred that the substitution be with an amino acid which is similar in structure or charge compared to the amino acid in the sequence. For instance, isoleucine (Ile)[I] is structurally very similar to leucine (Leu)[L], whereas, tyrosine (Tyr)[Y] is similar to phenylalanine (Phe)[F], whereas serine (Ser)[S] is similar to threonine (Thr)[T], whereas cysteine (Cys)[C] is similar to methionine (Met)[M], whereas alanine (Ala)[A] is similar to valine (Val)[V], whereas lysine (Lys)[K] is similar to arginine (Arg)[R], whereas asparagine (Asn)[N] is similar to glutamine (Gln)[Q], whereas aspartic acid (Asp)[D] is similar to glutamic acid (Glu)[E], whereas

histidine (His)[H] is similar to proline (Pro)[P], and glycine (Gly)[G] is similar to tryptophan (Trp)[W]. In the alternative the preferred amino acid substitutions may be selected according to hydrophilic properties (i.e. polarity) or other common characteristics associated with the 20 essential amino acids. While preferred embodiments utilize the 20 natural amino acids for their GRAS characteristics, it is recognized that minor substitutions along the amino acid chain which do not effect the essential characteristics of the amino are also contemplated.

[232] In one embodiment the carrier range is between one to 12 chemical moieties with one to 8 moieties being preferred. In another embodiment the number of chemical moieties attached is selected from 1, 2, 3, 4, 5, 6, or 7, etc. In another embodiment of the invention the molecular weight of the carrier portion of the conjugate is below about 2,500, more preferably below about 1,000 and most preferably below about 500.

[233] The compositions and methods of the invention may be applied to various therapeutically valuable active agents (e.g., drugs) and include, for example, stimulants such as amphetamines, anticonvulsants, muscle relaxants, antidepressants, anxiolytics, benzodiazepines, sedatives, hypnotics, narcotics, steroids, respiratory agents, including antihistamines, antipsychotics including risperidone, and nonsteroidal anti-inflammatory agents.

[234] Exemplary narcotics include opioids, hydrocodone, oxycodone, morphine, codeine, hydroxymorphone, oxymorphone, methadone, fentanyl, levorphanol, dihydrocodeine, meperidine, diphenoxylate, sufentanil, alfentanil, propoxyphene, pentazocine, nalbuphine, butorphanol, buprenorphine, meptazinol, dezocine or pharmaceutically acceptable salts thereof.

[235] Exemplary benzodiazepines include alprazolam, chlordiazepoxide, clonazepam, clorazepate, diazepam, estazolam, flurazepam, halazepam, lorazepam, midazolam, oxazepam, quazepam, temazepam, or triazolam.

[236] Exemplary nonsteroidal anti-inflammatory agents include ibuprofen, naproxen or indomethacin, aspirin or a salicylic acid derivative, or acetaminophen.

[237] Exemplary anti-depressants include citalopram, fluoxetine, norfluoxetine, fluvoxamine, paroxetine, sertraline, amitriptyline, desipramine, doxepin,

imipramine, nortryiptyline, bupropion, mirtazapine, nefazodone, trazodone, or venlafaxine.

[238] Exemplary anti-psychotics include clozapine, haloperidol, olanzapine, quetiapine, or risperidone.

[239] Exemplary amphetamines include amphetamine, mmethamphetamine, p-methoxyamphetamine, methylenedioxyamphetamine, 2,5-dimethoxy-4-methylamphetamine, 2,4,5-trimethoxyamphetamine and 3,4-methylenedioxymethamphetamine.

[240] The compositions and methods of the invention provide active agents which when bound to the chemical moiety provide safer and/or more effective dosages for the above recited active agent classes through improved bioavailability curves and/or safer C_{max} and/or reduce area under the curve for bioavailability, particularly for abused substances taken in doses above therapeutic levels. As a result, the compositions and methods of the invention may provide improved methods of treatment for attention deficit hyperactivity, attention deficit hyperactivity disorder (ADHD), attention deficit disorder (ADD), cognitive decline associated with acquired immunodeficiency syndrome (AIDS) or AIDS-related complex, depression, anxiety and anxiety related disorders, psychosis, nicotine addiction, narcotic addiction, alcoholism, narcolepsy, and/or analgesia.

[241] In one embodiment the chemical moiety is comprised of an amino acid or a polypeptide. Preferred amino acid and peptide chemical moieties include, for example, Lys, Ser, Ala, Phe, Ile, Pro-Pro-Leu, Pro-Pro-Ile, Val-Val, Lys-Lys, Gly-Gly-Ile, Phe-Phe-Ile, Phe-Phe-Leu, Thr-Thr-Val, Tyr-Tyr-Val, Tyr-Tyr-Phe, Glu-Glu-Val, Asp-Asp-Val, Lys-Lys-Val, Glu-Glu-Phe-Phe-Ile**[SEQ ID NO: 5]**, Glu-Glu-Phe-Phe-Phe**[SEQ ID NO: 3]**, Tyr-Tyr-Ile, Asp-Asp-Ile, Tyr-Tyr-Phe-Phe-Ile**[SEQ ID NO: 6]**, Tyr-Tyr-Lys-Tyr-Tyr**[SEQ ID NO: 7]**, Phe-Phe-Lys-Phe-Phe**[SEQ ID NO: 8]**, $(Lys\text{-}Lys\text{-}Gly\text{-}Gly)_2$**[SEQ ID NO: 9]**, and $[(l)\text{-}Lys\text{-}(d)\text{-}Lys\text{-}Leu]_2$. In some embodiments, the active agent is disubstituted with one or more of the preceding chemical moieties.

[242] Another embodiment of the invention is a composition for preventing overdose comprising an active agent which has been covalently bound to a chemical moiety.

[243] Another embodiment of the invention is a composition for safely delivering an active agent comprising providing a therapeutically effective amount of said active agent which has been covalently bound to a chemical moiety wherein said chemical moiety reduces the rate of absorption of the active agent as compared to delivering the unbound active agent.

[244] Another embodiment of the invention is a composition for reducing drug toxicity comprising providing a patient with an active agent which has been covalently bound to a chemical moiety wherein said chemical moiety increases the rate of clearance of an active agent when given at doses exceeding those within the therapeutic range of said active agent.

[245] Another embodiment of the invention is a composition for reducing drug toxicity comprising providing a patient with an active agent which has been covalently bound to a chemical moiety wherein said chemical moiety provides a serum release curve which does not increase above said active agent toxicity level when given at doses exceeding those within the therapeutic range of said active agent.

[246] Another embodiment of the invention is a composition for reducing bioavailability of active agent comprising active agent covalently bound to a chemical moiety wherein said bound active agent maintains a steady-state serum release curve which provides a therapeutically effective bioavailability but prevents spiking or increase blood serum concentrations compared to unbound active agent when given at doses exceeding those within the therapeutic range of said active agent.

[247] Another embodiment of the invention is a composition for preventing a C_{max} spike for active agent while still providing a therapeutically effective bioavailability curve comprising an active agent which has been covalently bound to a chemical moiety.

[248] Another embodiment of the invention is a composition for preventing a toxic release profile in a patient comprising active agent covalently bound to a chemical moiety wherein said bound active agent maintains a steady-state serum release curve

which provides a therapeutically effective bioavailability but prevents spiking or increase blood serum concentrations compared to unbound active agent.

[249] Another embodiment of the invention is a compound of Formula I:

$$A\text{-}X_n\text{-}Z_m$$

wherein A is active agent as defined herein; X is a chemical moiety as defined herein and n is between 1 and 50 and increments thereof; and Z is a further chemical moiety different from X which acts as an adjuvant and m is between 1 and 50 and increments thereof. In another embodiment n is between 1 and 10 and m is 0. It should be recognized that the compounds of this formula may be used alone or in combination with any of the recited embodiments of the invention.

[250] Embodiments of the invention provide compositions which allow the active agent to be therapeutically effective when delivered at the proper dosage but reduces the rate of absorption or extent of bioavailability of the active agent when given at doses exceeding those within the therapeutic range of the active agent. Embodiments of the invention also provide compositions wherein the covalently bound chemical moiety increases the rate of clearance of active agent when given at doses exceeding those within the therapeutic range of the active agent.

[251] In another embodiment the compositions have substantially lower toxicity compared to unbound active agent. In another embodiment the compositions reduce or eliminate the possibility of overdose by oral administration. In another embodiment the compositions reduce or eliminate the possibility of overdose by intranasal administration. In another embodiment the compositions reduce or eliminate the possibility of overdose by injection.

[252] In another embodiment, the conjugates of the invention may further comprise a polymer blend which comprises at least one hydrophilic polymer and at least one water-insoluble polymer. The polymer may be used according to industry standard to further enhance the sustained release properties of the active agent conjugate without reducing the abuse resistance. For instance, a composition might include: about 75% to about 95% active agent conjugate by weight, from about 0.5% to about 10% of a hydrophilic polymer (e.g. hydroxypropyl methylcellulose), from about 0.5% to about 2.5% of a water-insoluble polymer (e.g. acrylic resin), from

about 0.4% to about 1.5% of additives (e.g. magnesium stearate), and from about 0.01% to about 1% colorant by weight. Hydrophilic polymers suitable for use in the sustained release formulation include: one or more natural or partially or totally synthetic hydrophilic gums such as acacia, gum tragacanth, locust bean gum, guar gum, or karaya gum, modified cellulosic substances such as methylcellulose, hydroxomethylcellulose, hydroxypropyl methylcellulose, hydroxypropyl cellulose, hydroxyethylcellulose, carboxymethylcellulose; proteinaceous substances such as agar, pectin, carrageen, and alginates; and other hydrophilic polymers such as carboxypolymethylene, gelatin, casein, zein, bentonite, magnesium aluminum silicate, polysaccharides, modified starch derivatives, and other hydrophilic polymers known to those of skill in the art or a combination of such polymers.

[253] These hydrophilic polymers gel and would dissolve slowly in aqueous acidic media thereby allowing the active agent conjugate to diffuse from the gel in the stomach. When the gel reaches the intestines it would dissolve in controlled quantities in the higher pH medium to allow sustained release. Preferred hydrophilic polymers are the hydroxypropyl methylcelluloses such as those manufactured by The Dow Chemical Company and known as Methocel ethers, such as Methocel E10M.

[254] Other formulations may further comprise pharmaceutical additives including, but not limited to: lubricants such as magnesium stearate, calcium stearate, zinc stearate, powdered stearic acid, hydrogenated vegetable oils, talc, polyethylene glycol, and mineral oil; colorants; binders such as sucrose, lactose, gelatin, starch paste, acacia, tragacanth, povidone polyethylene glycol, Pullulan and corn syrup; glidants such as colloidal silicon dioxide and talc; surface active agents such as sodium lauryl sulfate, dioctyl sodium sulfosuccinate, triethanolamine, polyoxyethylene sorbitan, poloxalkol, and quarternary ammonium salts; preservatives and stabilizers; excipients such as lactose, mannitol, glucose, fructose, xylose, galactose, sucrose, maltose, xylitol, sorbitol, chloride, sulfate and phosphate salts of potassium, sodium, and magnesium; and/or any other pharmaceutical additives known to those of skill in the art. Colorants include, but are not limited to, Emerald Green Lake, FD&C Red No. 40, FD&C Yellow No. 6, D&C Yellow No.

10, or FD&C Blue No. 1 and other various certified color additives (See 21 CFR, Part 74). In one preferred embodiment, a sustained release formulation further comprises magnesium stearate and Emerald Green Lake.

[255] An active agent conjugate, which is further formulated with excipients may be manufactured according to any appropriate method known to those of skill in the art of pharmaceutical manufacture. For instance, the active agent conjugate and a hydrophilic polymer may be mixed in a mixer with an aliquot of water to form a wet granulation. The granulation may be dried to obtain hydrophilic polymer encapsulated granules of active agent-conjugate. The resulting granulation may be milled, screened, then blended with various pharmaceutical additives, water insoluble polymer, and additional hydrophilic polymer. The formulation may then tableted and may further be film coated with a protective coating which rapidly dissolves or disperses in gastric juices.

[256] However, it should be noted that the active agent conjugate controls the release of active agent into the digestive tract over an extended period of time resulting in an improved profile when compared to immediate release combinations and reduces and/or prevents abuse without the addition of the above additives. In a preferred embodiment no further sustained release additives are required to achieve a blunted or reduced pharmacokinetic curve (e.g. reduced euphoric effect) while achieving therapeutically effective amounts of active agent release.

[257] The compounds of the invention can be administered by a variety of dosage forms. Any biologically-acceptable dosage form known to persons of ordinary skill in the art, and combinations thereof, are contemplated. Examples of such dosage forms include, without limitation, chewable tablets, quick dissolve tablets, effervescent tablets, reconstitutable powders, elixirs, liquids, solutions, suspensions, emulsions, tablets, multi-layer tablets, bi-layer tablets, capsules, soft gelatin capsules, hard gelatin capsules, caplets, lozenges, chewable lozenges, beads, powders, granules, particles, microparticles, dispersible granules, cachets, douches, suppositories, creams, topicals, inhalants, aerosol inhalants, patches, particle inhalants, implants, depot implants, ingestibles, injectables (including subcutaneous, intramuscular, intravenous, and intradermal), infusions, health bars, confections,

animal feeds, cereals, yogurts, cereal coatings, foods, nutritive foods, functional foods and combinations thereof.

[258] However, the most effective means for delivering the abuse-resistant compounds of the invention is orally, to permit maximum release of the active agent to provide therapeutic effectiveness and/or sustained release while maintaining abuse resistance. When delivered by the oral route the active agent is released into circulation, preferably over an extended period of time as compared to active agent alone.

[259] Formulations of the invention suitable for oral administration can be presented as discrete units, such as capsules, caplets or tablets. These oral formulations also can comprise a solution or a suspension in an aqueous liquid or a non-aqueous liquid. The formulation can be an emulsion, such as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The oils can be administered by adding the purified and sterilized liquids to a prepared enteral formula, which is then placed in the feeding tube of a patient who is unable to swallow.

[260] Soft gel or soft gelatin capsules may be prepared, for example by dispersing the formulation in an appropriate vehicle (vegetable oils are commonly used) to form a high viscosity mixture. This mixture is then encapsulated with a gelatin based film using technology and machinery known to those in the soft gel industry. The industrial units so formed are then dried to constant weight.

[261] Chewable tablets, for example may be prepared by mixing the formulations with excipients designed to form a relatively soft, flavored, tablet dosage form that is intended to be chewed rather than swallowed. Conventional tablet machinery and procedures, that is both direct compression and granulation, i.e., or slugging, before compression, can be utilized. Those individuals involved in pharmaceutical solid dosage form production are versed in the processes and the machinery used as the chewable dosage form is a very common dosage form in the pharmaceutical industry.

[262] Film coated tablets, for example may be prepared by coating tablets using techniques such as rotating pan coating methods or air suspension methods to deposit a contiguous film layer on a tablet.

[263] Compressed tablets, for example may be prepared by mixing the formulation with excipients intended to add binding qualities to disintegration qualities. The mixture is either directly compressed or granulated then compressed using methods and machinery known to those in the industry. The resultant compressed tablet dosage units are then packaged according to market need, i.e., unit dose, rolls, bulk bottles, blister packs, etc.

[264] The invention also contemplates the use of biologically-acceptable carriers which may be prepared from a wide range of materials. Without being limited thereto, such materials include diluents, binders and adhesives, lubricants, plasticizers, disintegrants, colorants, bulking substances, flavorings, sweeteners and miscellaneous materials such as buffers and adsorbents in order to prepare a particular medicated composition.

[265] Binders may be selected from a wide range of materials such as hydroxypropylmethylcellulose, ethylcellulose, or other suitable cellulose derivatives, povidone, acrylic and methacrylic acid co-polymers, pharmaceutical glaze, gums, milk derivatives, such as whey, starches, and derivatives, as well as other conventional binders known to persons skilled in the art. Exemplary non-limiting solvents are water, ethanol, isopropyl alcohol, methylene chloride or mixtures and combinations thereof. Exemplary non-limiting bulking substances include sugar, lactose, gelatin, starch, and silicon dioxide.

[266] Preferred plasticizers may be selected from the group consisting of diethyl phthalate, diethyl sebacate, triethyl citrate, cronotic acid, propylene glycol, butyl phthalate, dibutyl sebacate, castor oil and mixtures thereof, without limitation. As is evident, the plasticizers may be hydrophobic as well as hydrophilic in nature. Water-insoluble hydrophobic substances, such as diethyl phthalate, diethyl sebacate and castor oil are used to delay the release of water-soluble vitamins, such as vitamin B6 and vitamin C. In contrast, hydrophilic plasticizers are used when water-insoluble vitamins are employed which aid in dissolving the encapsulated film, making channels in the surface, which aid in nutritional composition release.

[267] It should be understood that in addition to the ingredients particularly mentioned above, the formulations of this invention can include other suitable

agents such as flavoring agents, preservatives and antioxidants. Such antioxidants would be food acceptable and could include vitamin E, carotene, BHT or other antioxidants known to those of skill in the art.

[268] Other compounds which may be included by admixture are, for example, medically inert ingredients, e.g. solid and liquid diluent, such as lactose, dextrose, saccharose, cellulose, starch or calcium phosphate for tablets or capsules, olive oil or ethyl oleate for soft capsules and water or vegetable oil for suspensions or emulsions; lubricating agents such as silica, talc, stearic acid, magnesium or calcium stearate and/or polyethylene glycols; gelling agents such as colloidal clays; thickening agents such as gum tragacanth or sodium alginate, binding agents such as starches, arabic gums, gelatin, methylcellulose, carboxymethylcellulose or polyvinylpyrrolidone; disintegrating agents such as starch, alginic acid, alginates or sodium starch glycolate; effervescing mixtures; dyestuff; sweeteners; wetting agents such as lecithin, polysorbates or laurylsulphates; and other therapeutically acceptable accessory ingredients, such as humectants, preservatives, buffers and antioxidants, which are known additives for such formulations.

[269] For oral administration, fine powders or granules containing diluting, dispersing and/or surface-active agents may be presented in a draught, in water or a syrup, in capsules or sachets in the dry state, in a non-aqueous suspension wherein suspending agents may be included, or in a suspension in water or a syrup. Where desirable or necessary, flavoring, preserving, suspending, thickening or emulsifying agents can be included.

[270] Liquid dispersions for oral administration may be syrups, emulsions or suspensions. The syrups may contain as carrier, for example, saccharose or saccharose with glycerol and/or mannitol and/or sorbitol. In particular a syrup for diabetic patients can contain as carriers only products, for example sorbitol, which do not metabolize to glucose or which metabolize only a very small amount to glucose. The suspensions and the emulsions may contain a carrier, for example a natural gum, agar, sodium alginate, pectin, methylcellulose, carboxymethylcellulose or polyvinyl alcohol.

[271] The dose range for adult human beings will depend on a number of factors including the age, weight and condition of the patient and the administration route. Tablets and other forms of presentation provided in discrete units conveniently contain a daily dose, or an appropriate fraction thereof, of one of the present compounds. For example, units may contain from 5 mg to 500 mg, but more usually from 10 mg to 250 mg, of one of the present compounds.

[272] It is also possible for the dosage form to combine any forms of release known to persons of ordinary skill in the art. These include immediate release, extended release, pulse release, variable release, controlled release, timed release, sustained release, delayed release, long acting, and combinations thereof. The ability to obtain immediate release, extended release, pulse release, variable release, controlled release, timed release, sustained release, delayed release, long acting characteristics and combinations thereof is known in the art.

[273] Compositions of the invention may be administered in a partial, i.e., fractional dose, one or more times during a 24 hour period, a single dose during a 24 hour period of time, a double dose during a 24 hour period of time, or more than a double dose during a 24 hour period of time. Fractional, double or other multiple doses may be taken simultaneously or at different times during the 24 hour period. The doses may be uneven doses with regard to one another or with regard to the individual components at different administration times.

[274] Likewise, the compositions of the invention may be provided in a blister pack or other such pharmaceutical package. Further, the compositions of the present inventive subject matter may further include or be accompanied by indicia allowing individuals to identify the compositions as products for a prescribed treatment. The indicia may further additionally include an indication of the above specified time periods for administering the compositions. For example the indicia may be time indicia indicating a specific or general time of day for administration of the composition, or the indicia may be a day indicia indicating a day of the week for administration of the composition. The blister pack or other combination package may also include a second pharmaceutical product.

[275] It will be appreciated that the pharmacological activity of the compositions of the invention can be demonstrated using standard pharmacological models that are known in the art. Furthermore, it will be appreciated that the inventive compositions can be incorporated or encapsulated in a suitable polymer matrix or membrane for site-specific delivery, or can be functionalized with specific targeting agents capable of effecting site specific delivery. These techniques, as well as other drug delivery techniques are well known in the art.

[276] In another embodiment of the invention, the solubility and dissolution rate of the composition is substantially changed under physiological conditions encountered in the intestine, at mucosal surfaces, or in the bloodstream. In another embodiment the solubility and dissolution rate substantially decrease the bioavailability of the said pharmaceutical, particularly at doses above those intended for therapy. In another embodiment the decrease in bioavailability occurs upon oral administration. In another embodiment the decrease in bioavailability occurs upon intranasal administration. In another embodiment the decrease in bioavailability occurs upon intravenous administration.

[277] Another particular embodiment of the invention provides that when the covalently modified active agent is provided for oral dosing in the form (e.g., a tablet or capsule) it is resistant to manipulation. Crushing of the tablet or disruption of the capsule does not substantially increase the rate and amount of active agent absorbed when compositions of the invention are ingested.

[278] For each of the described embodiments one or more of the following characteristics may be realized. The toxicity of the compound is substantially lower than that of the unbound active agent. The covalently bound chemical moiety reduces or eliminates the possibility of overdose by oral administration. The covalently bound chemical moiety reduces or eliminates the possibility of overdose by intranasal administration. The covalently bound chemical moiety reduces or eliminates the possibility of overdose by injection.

[279] The invention further provides methods for altering active agent in a manner that decreases their potential for abuse. Methods of the invention provide various ways to regulate pharmaceutical dosage through covalent attachment of active agent

to different chemical moieties. One embodiment provides a method of preventing overdose comprising administering to an individual an active agent which has been covalently bound to a chemical moiety.

[280] Another embodiment provides a method of safely delivering an active agent comprising providing a therapeutically effective amount of an active agent which has been covalently bound to a chemical moiety wherein the chemical moiety reduces the rate of absorption of active agent as compared to delivering the unbound active agent.

[281] Another embodiment provides a method of reducing drug toxicity comprising providing a patient with an active agent which has been covalently bound to a chemical moiety wherein the chemical moiety increases the rate of clearance of a pharmacologically active active agent when given at doses exceeding those within the therapeutic range of active agent.

[282] Another embodiment provides a method of reducing drug toxicity comprising providing a patient with an active agent which has been covalently bound to a chemical moiety wherein the chemical moiety provides a serum release curve which does not increase above the active agent's toxicity level when given at doses exceeding those within the therapeutic range for the unbound active agent.

[283] Another embodiment provides a method of reducing bioavailability of an active agent comprising providing active agent covalently bound to a chemical moiety wherein the bound active agent maintains a steady-state serum release curve which provides a therapeutically effective bioavailability but prevents spiking or increase blood serum concentrations compared to unbound active agent when given at doses exceeding those within the therapeutic range for the unbound active agent. Another embodiment provides a method of preventing a C_{max} spike for active agent while still providing a therapeutically effective bioavailability curve comprising providing an active agent which has been covalently bound to a chemical moiety. In another embodiment, methods of the invention provide bioavailability curves similar to those found in Figures 1-195.

[284] Another embodiment provides a method for preventing a toxic release profile in a patient comprising administering to a patient an active agent covalently bound

to a chemical moiety wherein said bound active agent maintains a steady-state serum release curve which provides a therapeutically effective bioavailability but prevents spiking or increase blood serum concentrations compared to unbound active agent.

[285] Another embodiment of the invention is a method for reducing or preventing abuse of a pharmaceutical composition, comprising providing, administering, or prescribing said composition to a human in need thereof, wherein said composition comprises a chemical moiety covalently attached to an active agent such that the pharmacological activity of active agent is substantially decreased when the composition is used in a manner inconsistent with the manufacturer's instructions. Another embodiment of the invention is a method for reducing or preventing abuse of a pharmaceutical composition, comprising consuming said composition, wherein said composition comprises a chemical moiety covalently attached to an active agent such that the pharmacological activity of the active agent is substantially decreased when the composition is used in a manner inconsistent with the manufacturer's instructions.

[286] Another embodiment of the invention is a method of preventing overdose of a pharmaceutical composition, comprising providing, administering, or prescribing said pharmaceutical composition to a human in need thereof, wherein said composition comprises a chemical moiety covalently attached to an active agent in a manner that substantially decreases the potential of overdose from active agent. Another embodiment of the invention is a method of preventing overdose of a pharmaceutical composition, comprising consuming said pharmaceutical composition, wherein said composition comprises a chemical moiety covalently attached to active agent in a manner that substantially decreases the potential of overdose from the active agent.

[287] Another embodiment of the invention is a method for reducing or preventing the euphoric effect of a pharmaceutical composition, comprising providing, administering, or prescribing said composition to a human in need thereof, wherein said composition comprises a chemical moiety covalently attached to an active agent such that the pharmacological activity of active agent is substantially decreased when the composition is used in a manner inconsistent with the manufacturer's

instructions. Another embodiment of the invention is a method for reducing or preventing the euphoric effect of a pharmaceutical composition, comprising consuming said composition, wherein said composition comprises a chemical moiety covalently attached to an active agent such that the pharmacological activity of active agent is substantially decreased when the composition is used in a manner inconsistent with the manufacturer's instructions.

[288] Another embodiment of the invention is any of the preceding methods wherein said pharmaceutical composition is adapted for oral administration, and wherein said active agent is resistant to release from said chemical moiety when the composition is administered parenterally, such as intranasally or intravenously. Preferably, said active agent may be released from said chemical moiety in the presence of acid and/or enzymes present in the stomach, intestinal tract, or blood serum. Optionally, said composition may be in the form of a tablet, capsule, oral solution, or oral suspension.

[289] Another embodiment of the invention is any of the preceding methods wherein said chemical moiety is an amino acid, oligopeptide, polypeptide, carbohydrate, glycopeptide, nucleic acid, or vitamin. Preferably, said chemical moiety is an amino acid, oligopeptide, or polypeptide. Where the chemical moiety is a polypeptide, preferably said polypeptide comprises fewer than 70 amino acids, fewer than 50 amino acids, fewer than 10 amino acids, or fewer than 6 amino acids.

[290] Another embodiment of the invention is any of the preceding methods wherein said covalent attachment comprises an ester or carbonate bond. Another embodiment of the invention is any of the preceding methods wherein said active agent covalently attaches to a chemical moiety through a ketone and/or hydroxyl in a pharmaceutically acceptable oral dosage form.

[291] Another embodiment of the invention is any of the preceding methods wherein said composition yields a therapeutic effect without substantial euphoria. Preferably, said active agent provides a therapeutically bioequivalent AUC when compared to active agent alone but does provide a C_{max} which results in euphoria.

[292] Another embodiment of the invention is a method for reducing or preventing abuse of a pharmaceutical composition, comprising orally administering said

composition to a human in need thereof, wherein said composition comprises an amino acid or peptide covalently attached to active agent such that the pharmacological activity of active agent is substantially decreased when the composition is used in a manner inconsistent with the manufacturer's instructions.

[293] Another embodiment is a method of preventing overdose of a pharmaceutical composition, comprising orally administering said pharmaceutical composition to a human in need thereof, wherein said composition comprises an amino acid or peptide covalently attached to active agent in a manner that substantially decreases the potential of active agent to result in overdose.

[294] Another embodiment is a method for reducing or preventing the euphoric effect of a pharmaceutical composition, comprising orally administering said composition to a human in need thereof, wherein said composition comprises an amino acid or peptide covalently attached to active agent such that the pharmacological activity of active agent is substantially decreased when the composition is used in a manner inconsistent with the manufacturer's instructions.

[295] For each of the recited methods of the invention the following properties may be achieved through bonding active agent to the chemical moiety. In one embodiment, the toxicity of the compound may be substantially lower than that of the active agent when delivered in its unbound state or as a salt thereof. In another embodiment, the possibility of overdose by oral administration is reduced or eliminated. In another embodiment, the possibility of overdose by intranasal administration is reduced or eliminated. In another embodiment, the possibility of overdose by injection administration is reduced or eliminated.

[296] Another embodiment of the invention provides methods of treating various diseases or conditions comprising administering compounds or compositions of the invention which further comprise commonly prescribed active agents for the respective illness or diseases wherein the amphetamine is covalently attached to a chemical moiety. For instance, one embodiment of the invention comprises a method of treating attention deficit hyperactivity comprising administering to a patient amphetamine covalently bound to a chemical moiety. Another embodiment provides a method of treating attention deficit hyperactivity disorder (ADHD)

comprising administering to a patient compounds or compositions of the invention, such as amphetamine covalently bound to a chemical moiety. Another embodiment provides a method of treating attention deficit disorder (ADD) comprising administering to a patient compounds or compositions of the invention, amphetamine covalently bound to a chemical moiety.

[297] Another embodiment of the invention provides a method of treating cognitive decline associated with acquired immunodeficiency syndrome (AIDS) or AIDS-related complex comprising administering to a patient compounds or compositions of the invention.

[298] Another embodiment of the invention provides a method of treating depression comprising administering to a patient compounds or compositions of the invention. Another embodiment of the invention provides a method of treating anxiety and anxiety related disorders comprising administering to a patient compounds or compositions of the invention. Another embodiment of the invention provides a method of treating psychosis comprising administering to a patient compounds or compositions of the invention.

[299] Another embodiment of the invention provides a method of treating nicotine addiction comprising administering to a patient compounds or compositions of the invention. Another embodiment of the invention provides a method of treating narcotic addiction comprising administering to a patient compounds or compositions of the invention. Another embodiment of the invention provides a method of treating alcoholism comprising administering to a patient compounds or compositions of the invention.

[300] Another embodiment of the invention provides a method of treating narcolepsy comprising administering to a patient compounds or compositions of the invention. Another embodiment of the invention provides a method of providing analgesia comprising administering to a patient compounds or compositions of the invention.

[301] In order to facilitate a more complete understanding of the invention, Examples are provided below. However, the scope of the invention is not limited to

specific embodiments disclosed in these Examples, which are for purposes of illustration only.

Examples

[302] The invention is illustrated by pharmacokinetic studies with amphetamine, hydrocodone, and oxycodone that have been covalently modified by attachment to various moieties such as an individual amino acid, specific short chained amino acid sequences such as di-, tri-, and pentapeptides, or carbohydrates such as ribose, etc. Studies include pharmacokinetic evaluations of the various drug conjugates administered by the oral, intranasal, and intravenous routes. Collectively the compounds demonstrate that active agents may be modified by covalent attachment to various moieties and retain their therapeutic value at normal doses while preventing potential overdose by oral administration and prevention of abuse through intranasal and intravenous administration.

CARRIER BOUND AMPHETAMINE

[303] Examples 1 through 32 demonstrate the use and effectiveness of an chemical moiety conjugated to an active agent for reducing the potential for overdose while maintaining its therapeutic value wherein the amino acid lysine (K) is conjugated to the active agent amphetamine (K-amphetamine). However, the example is illustrative of the attachment of amphetamine to any variety of chemical moieties. Further, examples of amphetamine attachment include for instance and may be synthesized through similar procedures described in examples 1- 32 and throughout the specification.

A. Synthesis of Amphetamine Compositions

Example 1. General synthesis of amino acid-amphetamine conjugates

[304] Amino acid conjugates were synthesized by the general method described in Figs. 1-5.

Example 2. Synthesis of L-lysine-*d*-amphetamine

[305] L-lysine-*d*-amphetamine was synthesized (see Fig. 2) by the following method:

a. Coupling

Reagents	MW	Weight	mmoles	Molar Equivalents
d-amphetamine freebase	135.2	4.75 g	35.13	1
Boc-Lys(Boc)-OSu	443.5	15.58 g	35.13	1
Di-iPr-Et-Amine	129	906 mg	7.03	0.2, d=0.74, 1.22 mL
1,4-Dioxane	-	100 mL	-	-

[306] To a solution of Boc-Lys(Boc)-OSu (15.58 g, 35.13 mmol) in dioxane (100 mL) under an inert atmosphere was added *d*-amphetamine freebase (4.75 g, 35.13 mmol) and DiPEA (0.9 g, 1.22 mL, 7.03 mmol). The resulting mixture was allowed to stir at room temperature overnight. Solvent and excess base were then removed using reduced pressure evaporation. The crude product was dissolved in ethyl acetate and loaded on to a flash column (7 cm wide, filled to 24 cm with silica) and eluted with ethyl acetate. The product was isolated; the solvent reduced by rotary evaporation and the purified protected amide was dried by high-vac to obtain a white solid. ^{1}H NMR (DMSO-d_6) δ 1.02-1.11 (m, 2H, Lys γ-CH_2), δ 1.04 (d, 3H, Amp α-CH_3), δ 1.22-1.43 (m, 4H, Lys-β and δ-CH_2), δ 1.37 (18H, Boc, 6x CH_3), δ 2.60-2.72 (2H, Amp CH_2), δ 3.75-3.83, (m, 1H, Lys α-H) δ 3.9-4.1 (m, 1H, Amp α-H), δ 6.54-6.61 (d, 1H, amide NH), δ 6.7-6.77 (m, 1H, amide NH), δ 7.12-7.29 (m, 5H, ArH), δ 7.65-7.71 (m, 1, amide NH); mp = 86-88 °C.

b. Deprotection

Reagents	MW	Weight	mmoles	Molar Equivalents
4M HCl in dioxane	4 mmol/mL	50 mL	200	6.25
Boc-Lys(Boc)-Amp	463.6	14.84 g	32	1
1,4-Dioxane	-	50 mL	-	-

[307] The protected amide was dissolved in 50 mL of anhydrous dioxane and stirred while 50 mL (200 mmol) of 4M HCl/dioxane was added and stirred at room temperature overnight. The solvents were then reduced by rotary evaporation to afford a viscous oil. Addition of 100 mL MeOH followed by rotary evaporation resulted in a golden colored solid material that was further dried by storage at room

temperature under high vacuum. ^{1}H NMR (DMSO-d_6) δ 0.86-1.16 (m, 2H, Lys γ-CH_2), δ 1.1 (d, 3H, Amp α-CH_3), δ 1.40-1.56 (m, 4H, Lys-β and δ-CH_2), δ 2.54-2.78 (m, 2H, Amp CH_2, 2H, Lys ε-CH_2), 3.63-3.74 (m, 1H, Lys α-H), δ 4.00-4.08 (m, 1H, Amp α-H), δ 7.12-7.31 (m, 5H, Amp ArH), δ 8.13-8.33 (d, 3H, Lys amine) δ 8.70-8.78 (d, 1H, amide NH); mp = 120-122 °C.

Example 3. Synthesis of Ser-Amp

[308] Ser-Amp was synthesized by a similar method (see Fig. 3) except the amino acid starting material was Boc-Ser(O-tBu)-OSu and the deprotection was done using a solution of trifluoroacetic acid instead of HCl.

Example 4. Synthesis of Phe-Amp

[309] Phe-Amp was synthesized by a similar method (see Fig. 4) except the amino acid starting material was Boc-Phe-OSu.

Example 5. Synthesis of Gly_3-Amp

[310] Gly_3-Amp was synthesized by a similar method (see Fig. 5) except the amino acid starting material was Boc-GGG-OSu.

B. Pharmacokinetics of L-lysine-*d*-amphetamine

ELISA Analysis

Example 6. Pharmacokinetics of L-lysine-*d*-amphetamine compared to *d*-amphetamine sulfate

[311] Male Sprague-Dawley rats were provided water ad libitum, fasted overnight and dosed by oral gavage L-lysine-*d*-amphetamine or *d*-amphetamine sulfate. In all studies doses contained equivalent amounts of *d*-amphetamine base. Plasma d-amphetamine concentrations were measured by ELISA (Amphetamine Ultra, 109319, Neogen, Corporation, Lexington, KY). The assay is specific for *d*-amphetamine with only minimal reactivity (0.6%) of the major d-amphetamine metabolite (para-hydroxy-*d*-amphetamine) occurring. L-lysine-*d*-amphetamine was also determined to be essentially unreactive in the ELISA (<1%).

[312] Mean (n=4) plasma concentration curves of *d*-amphetamine or L-lysine-*d*-amphetamine are shown in Fig. 6. Extended release was observed in all four L-lysine-*d*-amphetamine dosed animals and C_{max} was substantially decreased as compared to animals dosed with d-amphetamine sulfate. Plasma *d*-amphetamine

concentrations of individual animals for *d*-amphetamine or L-lysine-*d*-amphetamine are shown in Table 1. The mean plasma *d*-amphetamine concentrations are shown in Table 2. The time to peak concentration for L-lysine-*d*-amphetamine was similar to that of *d*-amphetamine. Pharmacokinetic parameters for oral administration of *d*-amphetamine or L-lysine-*d*-amphetamine are summarized in Table 3.

Table 1. Plasma Concentrations of *d*-amphetamine from Individual Animals Orally Administered *d*-amphetamine or L-lysine-*d*-amphetamine (3 mg/kg *d*-amphetamine base).

Time	*d*-amphetamine (ng/ml)				L-lysine-*d*-amphetamine (ng/ml)			
(hours)	Rat #1	Rat #2	Rat #3	Rat #4	Rat #1	Rat #2	Rat #3	Rat #4
0.5	144	157	101	115	52	62	74	44
1	152	78	115	78	48	72	79	57
1.5	85	97	117	95	42	62	76	53
3	34	45	72	38	61	60	71	43
5	20	14	12	15	49	33	44	22
8	3	3	2	2	15	14	12	8

Table 2. Mean Plasma Concentrations of *d*-amphetamine Following Oral Administration of *d*-amphetamine or L-lysine-*d*-amphetamine.

Hours	Plasma *d*-amphetamine Concentrations (ng/ml)					
	d-amphetamine			L-lysine-*d*-amphetamine		
	Mean	+/- SD	CV	Mean	+/- SD	CV
0.5	129	25	20	58	13	22
1	106	35	33	64	14	22
1.5	99	13	14	58	14	25
3	47	17	36	59	11	19
5	15	4	24	37	12	32
8	2	1	35	12	3	24

Table 3. Pharmacokinetic Parameters of *d*-amphetamine Following Oral Administration of *d*-amphetamine or L-lysine-*d*-amphetamine.

Drug	AUC (0-8 h) ng/ml h	Percent Amphetamine	*Cmax* (ng/ml)	Percent Amphetamine	Mean Peak (ng/ml)	Percent Amphetamine
Amphetamine	341 +/- 35	100	111 +/- 27	100	129	100
Lys-Amp	333 +/- 66	98	61 +/- 13	55	64	50

[313] Example 6 illustrates that when lysine is conjugated to the active agent amphetamine the peak levels of amphetamine are decreased while bioavailability is maintained approximately equal to amphetamine. The bioavailability of amphetamine released from L-lysine-*d*-amphetamineis similar to that of amphetamine sulfate at the equivalent dose, thus L-lysine-*d*-amphetamine maintains its therapeutic value. The gradual release of amphetamine from L-lysine-*d*-amphetamine and decrease in peak levels reduce the possibility of overdose.

Example 7. Oral bioavailability of L-lysine-*d*-amphetamine at various doses approximating a range of therapeutic human doses

[314] Mean (n=4) plasma concentration curves of *d*-amphetamine vs. L-lysine-*d*-amphetamine are shown for rats orally administered 1.5, 3, and 6 mg/kg in Figs. 7, 8 and 9, respectively. Extended release was observed at all three doses for L-lysine-*d*-amphetamine dosed animals. The mean plasma concentrations for 1.5, 3, and 6 mg/kg are shown in Tables 4, 5 and 6, respectively. Pharmacokinetic parameters for oral administration of *d*-amphetamine vs. L-lysine-*d*-amphetamine at the various doses are summarized in Table 7.

Table 4. Mean Plasma Concentrations of *d*-amphetamine vs. L-lysine-*d*-amphetamine Following Oral Admistration (1.5 mg/kg)

Hours	Plasma Amphetamine Concentrations (ng/ml)					
	d-amphetamine			L-lysine-*d*-amphetamine		
	Mean	+/- SD	CV	Mean	+/- SD	CV
0	0	0	0	0	0	0
0.25	103	22	21	31	11	37
0.5	126	20	16	51	23	45
1	101	27	27	68	23	34
1.5	116	28	24	72	10	14
3	66	13	20	91	5	5
5	40	7	18	75	16	22
8	17	2	15	39	13	34

Table 5. Mean Plasma Concentrations of *d*-amphetamine vs. L-lysine-*d*-amphetamine Following Oral Admistration (3 mg/kg)

Hours	Plasma Amphetamine Concentrations (ng/ml)					
	d-amphetamine			L-lysine-*d*-amphetamine		
	Mean	+/- SD	CV	Mean	+/- SD	CV
0	0			0		
0.25	96	41	43	51	49	97
0.5	107	49	46	36	35	96
1	121	17	14	81	44	54
1.5	120	33	27	97	32	33
3	91	30	33	88	13	15
5	62	22	36	91	21	23
8	19	6	33	46	16	34

Table 6. Mean Plasma Concentrations of *d*-amphetamine vs. L-lysine-*d*-amphetamine Following Oral Admistration (6 mg/kg).

Hours	Plasma Amphetamine Concentrations (ng/ml)					
	d-amphetamine			L-lysine-*d*-amphetamine		
	Mean	+/- SD	CV	Mean	+/- SD	CV
0	0			0		
0.25	204	14	7	74	38	51
0.5	186	9	5	106	39	37
1	167	12	7	133	33	24
1.5	161	24	15	152	22	15
3	111	29	26	157	15	10
5	78	9	11	134	18	13
8	35	5	15	79	12	15

Table 7. Pharmacokinetic Parameters of *d*-amphetamine Following Oral Administration of *d*-amphetamine or L-lysine-*d*-amphetamine.

Parameter	1.5 mg/kg		3 mg/kg		6 mg/kg	
	d-amphetamine	L-lysine-*d*-amphetamine	*d*-amphetamine	L-lysine-*d*-amphetamine	*d*-amphetamine	L-lysine-*d*-amphetamine
AUC (ng/ml h)	481	538	587	614	807	1005
Percent	100	112	100	105	100	125
Cmax (ng/ml)	133	93	587	614	807	1005
Percent	100	70	100	105	100	125
Tmax (hours)	0.938	3.5	1	1.56	0.563	2.625
Percent	100	373	100	156	100	466

Example 8. Oral bioavailability of L-lysine-*d*-amphetamine at various doses approximating a range of therapeutic human doses compared to a suprapharmacological dose

[315] Male Sprague-Dawley rats were provided water ad libitum, fasted overnight and dosed by oral gavage with 1.5, 3, 6, 12, and 60 mg/kg of amphetamine sulfate or L-lysine-*d*-amphetamine containing the equivalent amounts of *d*-amphetamine. Concentrations of *d*-amphetamine were measured by ELISA.

[316] It has been demonstrated that when lysine is conjugated to the active agent *d*-amphetamine the levels of *d*-amphetamine at 30 minutes post-administration are decreased by approximately 50% over a dose range of 1.5 to 12 mg/kg. However, when a suprapharmcological dose (60 mg/kg) is given the levels of *d*-amphetamine from L-lysine-*d*-amphetamine only reached 8% of those seen for *d*-amphetamine sulfate (Tables 8 and 9, Fig. 10). The substantial decrease in oral bioavailability at a high dose greatly reduces the abuse potential of L-lysine-*d*-amphetamine.

Table 8. Levels of *d*-amphetamine vs. Dosage at 0.5 h Post Dosing with *d*-amphetamine Sulfate.

Dose mg/kg	1.5	3	6	12	60
ng/ml 0.5 h	109 +/- 59	196 +/- 72	294 +/- 202	344 +/- 126	3239 +/- 73
Percent	100	100	100	100	100

Table 9. Levels of *d*-amphetamine vs. Dosage at 0.5 h Post Dosing with L-lysine-*d*-amphetamine.

Dose mg/kg	1.5	3	6	12	60
ng/ml 0.5 h	45 +/- 10	86 +/- 26	129 +/- 46	172 +/- 113	266 +/- 18
Percent	41	44	44	50	8

Example 9. Decreased oral bioavailability of L-lysine-*d*-amphetamine at a high dose

[317] An additional oral PK study illustrated in Fig. 11 shows the *d*-amphetamine blood levels of a 60 mg/kg dose over an 8 h time course. In the case of *d*-amphetamine blood levels quickly reached a very high level and 8 of 12 animals either died or were sacrificed due to acute symptoms of toxicity. Blood levels (Tables 10-11) of animals administered L-lysine-*d*-amphetamine, on the other hand, did not peak until 5 hours and reached only a fraction of the levels of the animals receiving amphetamine (note: valid data past 3 h for *d*-amphetamine could not be determined due to death and sacrifice of animals).

Table 10. Mean Plasma Concentrations of *d*-amphetamine vs. L-lysine-*d*-amphetamine Following Oral Administration of a High Dose (60 mg/kg).

Hours	Plasma Amphetamine Concentrations (ng/ml)					
	d-amphetamine			L-lysine-*d*-amphetamine		
	Mean	+/- SD	CV	Mean	+/- SD	CV
0	NA	NA	NA	NA	NA	NA
0.25	2174	907	42	35	17	48
0.5	2643	578	22	81	33	41
1	2828	1319	47	212	30	14
1.5	2973	863	29	200	79	40
3	2944	95	3	440	133	30
5	NA	NA	NA	565	100	18
8	NA	NA	NA	410	206	50

Table 11. Pharmacokinetic Parameters of *d*-amphetamine vs. L-lysine-*d*-amphetamine

Drug	AUC ng/ml h	Percent *d*-amphetamine	Cmax (ng/ml)	Percent *d*-amphetamine	Mean Peak (ng/ml)	Percent *d*-amphetamine
d-mphetamine	8,130	100	3623	100	2973	100
L-lysine-*d*-amphetamine	3,143	39	582	16	565	19

Example 10. Oral Bioavailability of *d*-amphetamine following administration of an extended release formulation (intact or crushed) or L-lysine-d-amphetamine

[318] Doses of an extended release formulation of *d*-amphetamine sulfate (Dexadrine Spansule capsules) were orally administered to rats as intact capsules or as crushed capsules and compared to a dose of L-lysine-d-amphetamine containing an equivalent amount of *d*-amphetamine base (Fig. 14). The crushed capsules showed an increase in C_{max} and AUC_{inf} of 84 and 13 percent, respectively, as compared to intact capsules (Tables 12-13). In contrast, C_{max} and AUC_{inf} of *d*-amphetamine following administration of L-lysine-*d*-amphetamine were similar to that of the intact capsule illustrating that extended release is inherent to the compound itself and can not be circumvented by simple manipulation.

Table 12. Time-course Concentrations of *d*-amphetamine Following Oral Administration of Extended Release Dexadrine Spansule Capsules or Crushed Extended Release Dexadrine Spansule Capsules or L-lysine-d-amphetamine at Doses Containing 3 mg/kg *d*-Amphetamine Base.

Hours	Plasma Concentration (ng/ml)		
	Intact Spansule Capsule	Crushed Spansule Capsule	L-lysine-*d*-amphetamine
0	0	0	0
0.25	32	46	3
0.5	33	85	5
1	80	147	34
1.5	61	101	60
3	64	66	76
5	46	39	66
8	34	12	38

Table 13. Time-course Concentrations of *d*-amphetamine Following Oral Administration of Extended Release Dexadrine Spansule Capsules or Crushed Extended Release Dexadrine Spansule Capsules or L-lysine-d-amphetamine at Doses Containing 3 mg/kg *d*-Amphetamine Base.

Parameter	Intact Spansule Capsule	Crushed Spansule Capsule	L-lysine-*d*-amphetamine
AUC_{0-8h} (ng.h/ml)	399	449	434
Percent	100	113	109
C_{max} (ng/ml)	80	147	76
Percent	100	184	95
T_{max} (hours)	1	1	3
Percent	100	100	300

[319] Example 10 illustrates the advantage of the invention over conventional controlled release formulations of *d*-amphetamine.

Example 11. Decreased intranasal bioavailability of L-lysine-*d*-amphetamine vs. amphetamine

[320] Male Sprague-Dawley rats were dosed by intranasal administration with 3 mg/kg of amphetamine sulfate or L-lysine-*d*-amphetamine hydrochloride containing the equivalent amounts of *d*-amphetamine. L-lysine-*d*-amphetamine did not release any significant amount of *d*-amphetamine into circulation by IN administration. Mean (n=4) plasma amphetamine concentration curves of amphetamine vs. L-lysine-*d*-amphetamine are shown in Fig. 12. Pharmacokinetic parameters for IN administration of L-lysine-*d*-amphetamine are summarized in Table 14.

Table 14. Pharmacokinetic Parameters of Amphetamine vs. L-lysine-*d*-amphetamine by IN Administration.

Drug	AUC (0-1.5 h) ng/ml h	Percent *d*-amphetamine	C*max* (ng/ml)	Percent *d*-amphetamine
Amphetamine	727	100	1,377	100
L-lysine-*d*-amphetamine	4	0.5	7	0.5

[321] Example 11 illustrates that when lysine is conjugated to the active agent *d*-amphetamine the bioavailability by the intranasal route is substantially decreased thereby diminishing the ability to abuse the drug by this route.

Example 12. Intravenous bioavailability of amphetamine vs. L-lysine-*d*-amphetamine

[322] Male Sprague-Dawley rats were dosed by intravenous tail vein injection with 1.5 mg/kg of *d*-amphetamine or L-lysine-*d*-amphetamine containing the equivalent amount of amphetamine. As observed with IN dosing, the conjugate did not release a significant amount of *d*-amphetamine. Mean (n=4) plasma concentration curves of amphetamine vs. L-lysine-*d*-amphetamine are shown in Fig. 13. Pharmacokinetic parameters for IV administration of L-lysine-*d*-amphetamine are summarized in Table 15.

Table 15. Pharmacokinetic Parameters of *d*-amphetamine vs. L-lysine-*d*-amphetamine by IV Administration.

Drug	AUC (0-1.5 h) ng/ml h	% Amphetamine	Cmax (ng/ml)	% Amphetamine
Amphetamine	190	100	169	100
K-amphetamine	6	3	5	3

[323] Example 12 illustrates that when lysine is conjugated to the active agent amphetamine the bioavailability of amphetamine by the intravenous route is substantially decreased, thereby diminishing the ability to abuse the drug by this route.

LC/MS/MS Analysis

Example 13. Oral Bioavaialability of L-lysine-*d*-amphetamine compared to *d*-amphetamine at escalating doses.

[324] As shown in Figs. 15-19, the fraction of intact L-lysine-*d*-amphetamine absorbed following oral administration in rats increased non-linearly in proportion to escalating doses from 1.5 to 12 mg/kg (*d*-amphetamine base). The fraction absorbed at 1.5 mg/kg was only 2.6 percent whereas it increased to 24.6 percent by 12 mg/kg. The fraction absorbed fell to 9.3 percent at the high dose of 60 mg/kg. T_{max} ranged from 0.25 to 3 hours and peak concentrations occurred earlier than for *d*-amphetamine in L-lysine-*d*-amphetamine dosed rats. L-lysine-*d*-amphetamine was

cleared more rapidly than *d*-amphetamine with nearly undetectable concentrations by 8 hours at the lowest dose.

[325] T_{max} for *d*-amphetamine from L-lysine-*d*-amphetamine ranged from 1.5 to 5 hours as compared to 0.5 to 1.5 following administration of *d*-amphetamine sulfate. The difference in time to reach maximum concentration was greater at higher doses. C_{max} of *d*-amphetamine following oral delivery of L-lysine-*d*-amphetamine was reduced by approximately half as compared to C_{max} following *d*-amphetamine sulfate administration at doses of 1.5 to 6 mg/kg, approximating human equivalent doses (HEDs) in the therapeutic range (HED *d*-amphetamine sulfate; 19.9 to 39.9 mg). HEDs are defined as the equivalent dose for a 60 kg person in accordance to the body surface area of the animal model. The adjustment factor for rats is 6.2. The HED for a rat dose of 1.5 mg/kg of *d*-amphetamine, for example, is equivalent to 1.5/6.2 x 60 = 14.52 *d*-amphetamine base; which is equivalent to 14.52/.7284 = 19.9 mg *d*-amphetamine sulfate, when adjusted for the salt content.

[326] At doses above HEDs in the targeted therapeutic range (12 and 60 mg/kg; HED *d*-amphetamine sulfate 79.8 and 399 mg), C_{max} was reduced by 73 and 84 percent, respectively, as compared to *d*-amphetamine sulfate. AUCs of *d*-amphetamine following oral administration of L-lysine-*d*-amphetamine were similar to those of *d*-amphetamine sulfate at lower doses. As observed with C_{max}, however, the AUCs for *d*-amphetamine from L-lysine-*d*-amphetamine were substantially decreased compared to those of *d*-amphetamine sulfate at higher doses with the AUC_{inf} reduced by 76% at the highest dose (60 mg/kg; HED 399 mg *d*-amphetamine sulfate.

[327] In summary, oral bioavailability of *d*-amphetamine from L-lysine-*d*-amphetamine decreased to some degree at higher doses in rats. However, pharmacokinetics with respect to dose were nearly linear for L-lysine-*d*-amphetamine at doses from 1.5 to 60 mg/kg (HED *d*-amphetamine sulfate; 19.9 to 797.2 mg) with the fraction absorbed ranging from 52 to 81 percent (extrapolated form 1.5 mg/kg dose). Pharmacokinetics of *d*-amphetamine sulfate was also nearly linear at lower doses of 1.5 to 6 mg/kg (HED; 19.9 to 79.7) with the fraction absorbed ranging form 62 to 84. In contrast to L-lysine-*d*-amphetamine, however,

parameters were disproportionately increased at higher doses for *d*-amphetamine sulfate with the fraction absorbed calculated as 101 and 223 percent (extrapolated form 1.5 mg/kg dose), respectively, for the suprapharmacological doses of 12 and 60 mg/kg (HED *d*-amphetamine sulfate; 159.4 and 797.2 mg).

[328] The results suggest that the capacity for clearance of *d*-amphetamine when delivered as the sulfate salt becomes saturated at the higher doses whereas the gradual hydrolysis of L-lysine-*d*-amphetamine precludes saturation of *d*-amphetamine elimination at higher doses. The difference in proportionality of dose to bioavailability (Cmax and AUC) for *d*-amphetamine and L-lysine-*d*-amphetamine is illustrated in Figs. 20-22. The pharmacokinetic properties of L-lysine-*d*-amphetamine as compared to *d*-amphetamine at the higher doses decrease the ability to escalate doses. This improves the safety and reduces the abuse liability of L-lysine-*d*-amphetamine as a method of delivering *d*-amphetamine for the treatment of ADHD or other indicated conditions.

Example 14. Intranasal Bioavailability of L-lysine-*d*-amphetamine compared to *d*-amphetamine

[329] As shown in Figs. 23-24, bioavailability of *d*-amphetamine following bolus intranasal administration of L-lysine-*d*-amphetamine was approximately 5 percent of that of the equivalent *d*-amphetamine sulfate dose with AUC_{inf} values of 56 and 1032, respectively. C_{max} of *d*-amphetamine following L-lysine-*d*-amphetamine administration by the intranasal route was also about 5 percent of that of the equivalent amount of *d*-amphetamine sulfate with values of 78.6 ng/mL and 1962.9 ng/mL, respectively. As with intravenous administration, T_{max} of *d*-amphetamine concentration was delayed substantially for L-lysine-*d*-amphetamine (60 minutes) as compared to T_{max} of *d*-amphetamine sulfate (5 minutes), again reflecting the gradual hydrolysis of L-lysine-*d*-amphetamine. A high concentration of intact L-lysine-*d*-amphetamine was detected following intranasal dosing suggesting that the large decrease in bioavailability of *d*-amphetamine was due to minimal hydrolysis of L-lysine-*d*-amphetamine when delivered by this route. It appears that only minimal amounts of *d*-amphetamine can be delivered by intranasal administration of L-lysine-*d*-amphetamine.

Example 15. Intravenous Bioavaialability of L-lysine-*d*-amphetamine compared to *d*-amphetamine

[330] As shown in Figs. 25-26, bioavailability of *d*-amphetamine following bolus intravenous administration of L-lysine-*d*-amphetamine was approximately one-half that of the equivalent *d*-amphetamine sulfate dose with AUC_{inf} values of 237.8 and 420.2, respectively. C_{max} of *d*-amphetamine following L-lysine-*d*-amphetamine administration was only about one-fourth that of the equivalent amount of *d*-amphetamine with values of 99.5 and 420.2, respectively. T_{max} of *d*-amphetamine concentration was delayed substantially for L-lysine-*d*-amphetamine (30 minutes) as compared to T_{max} of *d*-amphetamine sulfate (5 minutes), reflecting the gradual hydrolysis of L-lysine-*d*-amphetamine. In conclusion, the bioavailability of *d*-amphetamine by the intravenous route is substantially decreased and delayed when given as L-lysine-*d*-amphetamine. Moreover, bioavailability is less than that obtained by oral administration of the equivalent dose of L-lysine-*d*-amphetamine.

Summary of LC/MS/MS Bioavailability Data in Rats

[331] The following tables summarize the bioavailability data collected in the experiments discussed in examples 13-15. Tables 15-17 summarize the pharmacokinetic parameters of *d*-amphetamine following oral, intransal, or bolus intravenous administration of d-amphetamine or L-lysine-*d*-amphetamine.

Table 15. Pharmacokinetic Parameters of *d*-amphetamine Following Oral Administration of L-lysine-*d*-amphetamine or d-amphetamine at Escalating Doses.

Route	Drug	Dose (mg/kg)	Cmax (ng/mL)	Tmax (h)	AUC(0-8) (ng•mL/h)	AUC(Inf) (ng•mL/h)	F (%)	AUC/Dose (ng.h.kg/mL/mg)	Cmax/Dose ng.kg/mL/mg
Oral	L-lysine-*d*-amphetamine	1.5	59.6	3	308	331	61	220.7	39.7
Oral	*d*-amphetamine	1.5	142.2	0.5	446	461	84	307.3	94.8
Oral	L-lysine-*d*-amphetamine	3	126.9	1.5	721	784	72	261.3	42.3
Oral	*d*-amphetamine	3	217.2	1.5	885	921	84	307.0	72.4

Route	Drug	Dose (mg/kg)	Cmax (ng/mL)	Tmax (h)	AUC(0-8) (ng•mL/h)	AUC(inf) (ng•mL/h)	F (%)	AUC/Dose (ng.h.kg/mL/mg)	Cmax/Dose ng.kg/mL/mg
Oral	L-lysine-*d*-amphetamine	6	310.8	3	1,680	1,797	82	299.5	51.8
Oral	*d*-amphetamine	6	815.3	0.25	1,319	1,362	62	227.0	135.9
Oral	L-lysine-*d*-amphetamine	12	412.6	5	2,426	2,701	62	225.1	34.4
Oral	*d*-amphetamine	12	1,533.1	0.25	4,252	4,428	101	369.0	127.8
Oral	L-lysine-*d*-amphetamine	60	2,164.3	5	9995.1	11,478	52	191.3	36.1
Oral	*d*-amphetamine	60	13,735	1	32,323	48,707	223	811.8	228.9

Table 16. Pharmacokinetic Parameters of *d*-amphetamine Following Bolus Intravenous Administration of L-lysine-*d*-amphetamine.

Route	Drug	Dose (mg/kg)	Cmax (ng/mL)	Tmax (h)	AUC(0-24) (ng•mL/h)	AUC(Inf) (ng•mL/h)
IV	L-lysine-*d*-amphetamine	1.5	99.5	0.5	237.8	237.9
IV	*d*-amphetamine	1.5	420.2	0.083	546.7	546.9

Table 17. Pharmacokinetic Parameters of *d*-amphetamine Following Intranasal Administration of L-lysine-*d*-amphetamine.

Route	Drug	Dose (mg/kg)	Cmax (ng/mL)	Tmax (h)	AUC(0-1) (ng•mL/h)	AUC(inf) (ng•mL/h)
IN	L-lysine-*d*-amphetamine	10.16	78.6	1	56	91
IN	*d*-amphetamine	4.12	1962.9	0.083	1032	7291

[332] Tables 18-20 summarize the pharmacokinetic parameters of L-lysine-*d*-amphetamine following oral, bolus intravenous, or intransal administration of L-lysine-*d*-amphetamine.

Table 18. Pharmacokinetic Parameters of L-lysine-*d*-amphetamine Following Oral Administration of L-lysine-*d*-amphetamine at Escalating Doses.

Dose	Drug	Dose (mg/kg)	Cmax (ng/ml)	Tmax (ng/ml)	AUC(0-8) (ng•ml/h)	AUC(inf) (ng•ml/h)	F (%)
Oral	L-lysine-*d*-amphetamine	1.5	36.5	0.25	59.4	60	2.6
Oral	L-lysine-*d*-amphetamine	3	135.4	1.5	329.7	332.1	7.2
Oral	L-lysine-*d*-amphetamine	6	676.8	0.25	1156.8	1170.8	12.8
Oral	L-lysine-*d*-amphetamine	12	855.9	1	4238.6	4510.4	24.6
Oral	L-lysine-*d*-amphetamine	60	1870.3	3	8234.3	8499.9	9.3

Table 19. Pharmacokinetic Parameters of L-lysine-*d*-amphetamine Following Bolus Intravenous Administration of L-lysine-*d*-amphetamine.

Route	Drug	Dose (mg/kg)	Cmax (ng/mL)	Tmax (h)	AUC(0-24) (ng•mL/h)	AUC(inf) (ng•mL/h)
IV	L-lysine-*d*-amphetamine	1.5	4513.1	0.083	2,282	2,293

Table 20. Pharmacokinetic Parameters of L-lysine-*d*-amphetamine Following Intranasal Administration of L-lysine-*d*-amphetamine.

Route	Drug	Dose (mg/kg)	Cmax (ng/mL)	Tmax (h)	AUC(0-1) (ng•mL/h)	AUC(inf) (ng•mL/h)
IN	L-lysine-*d*-amphetamine	3	3345.1	0.25	2,580	9,139

[333] Tables 21 and 22 summarize the percent bioavailability of *d*-amphetamine following oral, intranasal, or intravenous administration of L-lysine-*d*-amphetamine as compared to *d*-amphetamine sulfate.

Table 21. Percent Bioavailability (AUC_{inf}) of *d*-amphetamine Following Administration of L-lysine-*d*-amphetamine by Various Routes as Compared to Bioavailability Following Administration of *d*-amphetamine Sulfate.

Dose (mg/kg) *d*-amphetamine base	1.5	3	6	12	60
HED	19.9	39.9	79.7	159.4	797.2
Oral	72	85	132	61	24
IV	43	NA	NA	NA	NA
IN	NA	1	NA	NA	NA

Table 22. Percent Bioavailability (C_{max}) of *d*-amphetamine Following Administration of L-lysine-*d*-amphetamine by Various Routes as Compared to Bioavailability Following Administration of *d*-amphetamine Sulfate.

Dose (mg/kg) *d*-amphetamine base	1.5	3	6	12	60
HED	19.9	39.9	79.7	159.4	797.2
Oral	42	58	38	27	16
IV	24	NA	NA	NA	NA
IN	NA	4	NA	NA	NA

[334] Tables 23-28 summarize the time-course concentrations of *d*-amphetamine and L-lysine-*d*-amphetamine following oral, intranasal or intravenous administration of either *d*-amphetamine or L-lysine-*d*-amphetamine.

Table 23. Time-course Concentrations of *d*-amphetamine Following Bolus Intravenous Administration of L-lysine-*d*-amphetamine or *d*-amphetamine Sulfate at Doses Containing 1.5 mg/kg *d*-amphetamine Base.

Time (hours)	Concentration (ng/ml)	
	L-lysine-*d*-amphetamine	*d*-amphetamine sulfate
0	0	0
0.083	52.8	420.2
0.5	99.5	249.5
1.5	47.1	97.9
3	21.0	38.3
5	9.0	13.2

Time (hours)	Concentration (ng/ml)	
	L-lysine-*d*-amphetamine	*d*-amphetamine sulfate
8	3.7	4.3
24	0.1	0.2

Table 24. Time-course Concentrations of L-lysine-*d*-amphetamine Following Bolus Intravenous Administration of L-lysine-*d*-amphetamine at a Dose Containing 1.5 mg/kg *d*-amphetamine Base.

Time (hours)	Concentration (ng/ml)
	L-lysine-*d*-amphetamine
0	0
0.083	4513.1
0.5	1038.7
1.5	131.4
3	19.3
5	17.9
8	8.7
24	11.5

Table 25. Time-course Concentrations of *d*-amphetamine Following Oral Administration of L-lysine-*d*-amphetamine at Various Doses (mg/kg *d*-amphetamine base).

Time (hours)	Concentration (ng/ml)				
	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg	60 mg/kg
0	0	0	0	0	0
0.25	20.5	25.3	96	54.3	90.9
0.5	34	40.9	140.2	96	175.1
1	46.7	95.1	225.9	233.3	418.8
1.5	40.7	126.9	268.4	266	440.7
3	59.6	105	310.8	356.8	1145.5
5	38.6	107.6	219.5	412.6	2164.3
8	17.1	48	86	225.1	1227.5

Table 26. Time-course Concentrations of *d*-amphetamine Following Oral Administration of *d*-amphetamine Sulfate at Various Doses (mg/kg *d*-amphetamine Base).

Time (hours)	Concentration (ng/ml)				
	1.5 mg/kg	3 mg/kg	6 mg/kg	12 mg/kg	60 mg/kg
0	0	0	0	0	0
0.25	107.1	152.6	815.3	1533.1	6243.6
0.5	142.2	198.4	462.7	1216	7931.6
1	105.7	191.3	301.3	828.8	13735.2
1.5	129.5	217.2	314	904.8	11514.9
3	52.6	135.3	134.6	519.9	NA
5	29.5	73.5	77.4	404.3	NA
8	11.5	25.7	31.8	115.4	NA

Table 27. Time-course Concentrations of *d*-amphetamine Following Intranasal Administration of L-lysine-*d*-amphetamine or *d*-amphetamine Sulfate at Doses Containing 3 mg/kg *d*-amphetamine Base.

Time (hours)	Concentration (ng/ml)	
	L-lysine-*d*-amphetamine	*d*-amphetamine sulfate
0	0	0
0.083	31.2	1962.9
0.25	45.3	1497.3
0.5	61.3	996.2
1	78.6	404.6
AUC	56	1032.3

Table 28. Time-course Concentrations of L-lysine-*d*-amphetamine Following Intranasal Administration of L-lysine-*d*-amphetamine at a Dose Containing 3 mg/kg *d*-amphetamine Base.

Time (h)	Conc. (ng/ml) L-lysine-*d*-amphetamine
0	0
0.083	3345.1
0.25	3369.7
0.5	2985.8
1	1359.3

Example 19. LC/MS/MS analysis of Bioavailability in Dogs

[335] Example Experimental Design:

[336] This was a non-randomized, two-treatment crossover study. All animals were maintained on their normal diet and were fasted overnight prior to each dose administration. L-lysine-d-amphetamine dose was based on the body weight measured on the morning of each dosing day. The actual dose delivered was based on syringe weight before and after dosing. Serial blood samples were obtained from each animal by direct venipuncture of a jugular vein using vacutainer tubes containing sodium heparin as the anticoagulant. Derived plasma samples were stored frozen until shipment to the Quest Pharmaceutical Services, Inc. (Newark, DE). Pharmacokinetic analysis of the plasma assay results was conducted by Calvert. Animals were treated as follows:

# of Dog/Sex	Route of Administration	Treatment	Dose Concn (mg/mL)	Dose Vol (mL/kg)	Dose Level (mg/kg)
3M	PO	1	0.2	10	2
3M	IV	2	1	2	2

The mg units in the dose concentration and dose level refer to the free base form of test article.

Administration of the Test Article:

[337] *Oral:* The test article was administered to each animal via a single oral gavage. On Day 1, animals received the oral dose by gavage using an esophageal tube attached to a syringe. Dosing tubes were flushed with approximately 20 mL tap water to ensure the required dosing solution was delivered.

[338] *Intravenous:* On Day 8, animals received L-lysine-*d*-amphetamine as a single 30-minute intravenous infusion into a cephalic vein.

[339] ***Sample Collection:***

[340] *Dosing Formulations:* Post-dosing, remaining dosing formulation was saved and stored frozen.

[341] *Blood*: Serial blood samples (2 mL) were collected using venipuncture tubes containing sodium heparin. Blood samples were taken at 0, 0.25, 0.5, 1, 2, 4, 8, 12,

24, 48, and 72 hours post-oral dosing. Blood samples were collected at 0, 0.167, 0.33, 0.49 (prior to stop of infusion), 0.583, 0.667, 0.75, 1, 2, 3, 4, 8, 12, and 23 hours post-intravenous infusion start. Collected blood samples were chilled immediately.

[342] *Plasma:* Plasma samples were obtained by centrifugation of blood samples. Duplicate plasma samples (about 0.2 mL each) were transferred into prelabeled plastic vials and stored frozen at approximately -70°C.

[343] ***Sample Assay:***

[344] Plasma samples were analyzed for L-lysine-*d*-amphetamine and *d*-amphetamine using a validated LC-MS/MS method with an LLOQ of 1 ng/mL for both analytes.

[345] Microsoft Excel (Version 6, Microsoft Corp., Redmond, WA) was used for calculation of mean plasma concentration and graphing of the plasma concentration-time data. Pharmacokinetic analysis (non-compartmental) was performed using the WinNonlin® software program (Version 4.1, Pharsight, Inc. Mountain View, CA). The maximum concentration, C_{max}, and the time to C_{max}, T_{max}, were observed values. The area under the plasma concentration-time curve (AUC) was determined using linear-log trapezoidal rules. The apparent terminal rate constant (λz) was derived using linear least-squares regression with visual inspection of the data to determine the appropriate number of points (minimum of 3 data points) for calculating λz. The AUC(0-inf) was calculated as the sum of AUC(0-t) and Cpred/λz, where Cpred was the predicted concentration at the time of the last quantifiable concentration. The plasma clearance (CL/F) was determined as the ratio of Dose/AUC (0-inf). The mean residence time (MRT) was calculated as the ratio of AUMC(0-inf)/AUC (0-inf), where AUMC(0-inf) was the area under the first moment curve from the time zero to infinity. The volume of distribution at steady state (V_{ss}) was estimated as CL*MRT. Half-life was calculated as $\ln 2/\lambda z$. The oral bioavailability (F) was calculated as the ratio of AUC(0-inf) following oral dosing to AUC(0-inf) following intravenous dosing. Descriptive statistics (mean and standard deviation) of the pharmacokinetic parameters were calculated using Microsoft Excel.

[346] The objectives of this study were to characterize the pharmacokinetics of L-lysine-*d*-amphetamine and *d*-amphetamine following administration of L-lysine-*d*-amphetamine in male beagle dogs. As shown in Fig. 27, in a cross-over design, L-lysine-*d*-amphetamine was administered to 3 male beagle dogs orally (2 mg/kg) and intravenously (2 mg/kg, 30-minute infusion). Blood samples were collected up to 24 and 72 hour after the intravenous and oral does, respectively. Plasma samples were analyzed using a LC-MS/MS assay which provided an LLOQ of 1 ng/mL for both analytes.

[347] The mean L-lysine-*d*-amphetamine and *d*-amphetamine plasma concentration-time profiles following an intravenous or oral dose of L-lysine-*d*-amphetamine are presented in Figs. 29 and 30, respectively. Comparative profiles of L-lysine-*d*-amphetamine to *d*-amphetamine following both routes are depicted in Figs. 27-28. Individual plots are depicted in Figs. 31-32. The pharmacokinetic parameters are summarized in Tables 29-37.

[348] Following a 30-minute intravenous infusion of L-lysine-*d*-amphetamine, the plasma concentration reached a peak at the end of the infusion. Post-infusion L-lysine-*d*-amphetamine concentration declined very rapidly in a biexponential manner, and fell below the quantifiable limit (1 ng/mL) by approximately 8 hours post-dose. Results of non-compartmental pharmacokinetic analysis indicate that L-lysine-*d*-amphetamine is a high clearance compound with a moderate volume of distribution (Vss) approximating total body water (0.7 L/kg). The mean clearance value was 2087 mL/h•kg (34.8 mL/min•kg) and was similar to the hepatic blood flow in the dog (40 mL/min•kg). Consequently, L-lysine-*d*-amphetamine is a moderate to high hepatic extraction compound with significant first pass effects (including the conversion to *d*-amphetamine) following oral administration.

[349] L-lysine-*d*-amphetamine was rapidly absorbed after oral administration with T_{max} at 0.5 hours in all three dogs. Mean absolute oral bioavailablity was 33%. Since significant first pass effects are expected for L-lysine-*d*-amphetamine, a 33% bioavailability suggests that L-lysine-*d*-amphetamine is very well absorbed in the dog. The apparent terminal half-life was 0.39 hours, indicating rapid elimination, as observed following intravneous administration.

[350] Plasma concentration-time profiles of d-amphetamine following intravenous or oral administration of L-lysine-*d*-amphetamine were very similar, with C_{max}, T_{max} and AUC values for both routes essentially the same. At a 2 mg/kg oral dose of L-lysine-*d*-amphetamine, the mean C_{max} of *d*-amphetamine was 104.3 ng/mL. The half-life of *d*-amphetamine was 3.1 to 3.5 hours, much longer when compared to L-lysine-*d*-amphetamine.

[351] In this study, L-lysine-*d*-amphetamine was infused over a 30 minute time period. Due to rapid clearance of L-lysine-*d*-amphetamine it is likely that bioavailability of *d*-amphetamine from L-lysine-*d*-amphetamine would decrease if a similar dose were given by intravenous bolus injection. Even when given as an infusion the bioavailability of *d*-amphetamine from L-lysine-*d*-amphetamine did not exceed that of a similar dose given orally and the time to peak concentration was substantially delayed. This data further supports that L-lysine-*d*-amphetamine affords a decrease in the abuse liability of *d*-amphetamine by intravenous injection.

Table 29. Pharmacokinetic Parameters of L-lysine-*d*-amphetamine in Male Beagle Dogs Following Oral or Intravenous Administration of L-lysine-*d*-amphetamine (1 mg/kg d-amphetamine base).

Route	Dose (mg/kg)	C_{max} (ng/mL)	T_{max}[a] (h)	AUC(inf) (ng•h/mL)	$t_{1/2}$ (h)	MRT (h)	CL/F (mL/h•kg)	V_{ss} (mL/kg)	F (%)
IV	1	1650	0.49	964	0.88	0.33	2087	689	NA
	(0.00)	(178)	(0.49-0.49)	(97.1)	(0.2)	(0.03)	(199)	(105.9)	
Oral	1	328.2	0.5	319	0.39	0.81	6351	NA	33
	(0.00)	(91.9)	(0.5-0.5)	(46.3)	(0.1)	(0.19)	(898.3)		(1.9)

[a]: median (range)

Table 30. Pharmacokinetic Parameters of *d*-amphetamine in Male Beagle Dogs Following Oral or Intravenous Administration of L-lysine-*d*-amphetamine (1 mg/kg d-amphetamine base).

Route	Dose (mg/kg)	C_{max} (ng/mL)	T_{max}[a] (h)	AUC(inf) (ng•h/mL)	$t_{1/2}$ (h)
IV	2	113.2	1.0	672.5	3.14
	(0.00)	(3.2)	(0.67 – 2.0)	(85.7)	(0.4)
Oral	2	104.3	2.0	728.0	3.48

Route	Dose (mg/kg)	C_{max} (ng/mL)	T_{max}[a] (h)	AUC(inf) (ng•h/mL)	$t_{1/2}$ (h)
	(0.00)	(21.8)	(2 – 2)	(204.9)	(0.4)

[a]: median (range)

Table 31. Pharmacokinetics of L-lysine-*d*-amphetamine in Male Beagle Dogs Following Intravenous Administration of L-lysine-*d*-amphetamine (1 mg/kg d-amphetamine base).

Dose Route : 30-min iv Infusion **Dose** : 2 mg/kg/h (free form)

Dog ID	C_{max} (ng/mL)	T_{max}[a] (h)	AUC(0-t) (ng•h/mL)	AUC(inf) (ng•h/mL)	$t_{1/2}$ (h)	CL (mL/h/kg)	Vss (mL/kg)	MRT (h)
1	1470.3	0.49	898.2	900.2	0.72	2222	807.4	0.36
2	1826.4	0.49	1072.3	1076.1	ND[b]	1859	603.4	0.32
3	1654.2	0.49	914.1	916.9	1.05	2181	656.0	0.30
Mean	1650	0.49	961.5	964.4	0.88	2087	689.0	0.33
SD	178	0.49-0.49	96.0	97.1	0.2	199	105.9	0.03

[a]: median (range); [b]: not determined

Abbreviations of pharmacokinetic parameters are as follows: C_{max}, maximum observed plasma concentration; AUC(0-t), total area under the plasma concentration versus time curve from 0 to the last data point; AUC(0-inf), total area under the plasma concentration versus time curve; $t_{1/2}$, apparent terminal half-life; CL, clearance following iv administration; MRT, mean residence time; Vss, volume of distribution at steady state.

Table 32. Pharmacokinetic Parameters of L-lysine-*d*-amphetamine in Male Beagle Dogs Following Oral Administration of L-lysine-*d*-amphetamine (1 mg/kg d-amphetamine base).

Dose Route : Oral **Dose** : 2 mg/kg (free form)

Dog ID	C_{max} (ng/mL)	T_{max}[a] (h)	AUC(0-t) (ng•h/mL)	AUC(inf) (ng•h/mL)	$t_{1/2}$ (h)	CL/F (mL/h/kg)	MRT (h)	F (%)
1	350.2	0.5	275.3	277.1	0.24	7218	0.68	30.8
2	407.2	0.5	367.8	368.7	0.48	5424	0.74	34.3
3	227.4	0.5	310.8	312.0	0.45	6410	1.03	34.0
Mean	328.2	0.5	318.0	319.3	0.39	6351	0.81	33.0
SD	91.9	0.0	46.7	46.3	0.1	898.3	0.19	1.9

[a]: median (range)

Abbreviations of pharmacokinetic parameters are as follows: C_{max}, maximum observed plasma concentration; T_{max}, time when C_{max} observed; AUC(0-t), total area under the plasma concentration versus time curve from 0 to the last data point; AUC(0-inf), total area under the plasma concentration versus time curve; $t_{1/2}$, apparent terminal half-life; CL/F, oral clearance; MRT, mean residence time; F, bioavailability.

Table 33. Pharmacokinetics of L-lysine-*d*-amphetamine in Male Beagle Dogs Following Intravenous Administration of L-lysine-*d*-amphetamine (1 mg/kg d-amphetamine base).

Dose Route : 30-min iv Infusion **Dose** : 2 mg/kg of L-lysine-*d*-amphetamine (free form)

Dog ID	C_{max} (ng/mL)	T_{max}[a] (h)	AUC(0-t) (ng•h/mL)	AUC(inf) (ng•h/mL)	$t_{1/2}$ (h)
1	111.2	2.0	751.9	757.6	3.35
2	116.8	0.67	668.5	673.7	3.43
3	111.4	1.0	557.8	586.1	2.65
Mean	113.2	1.00	659.4	672.5	3.14
SD	3.2	0.67-2.0	97	85.7	0.4

[a]: median (range)

Abbreviations of pharmacokinetic parameters are as follows: C_{max}, maximum observed plasma concentration; T_{max}, time when C_{max} observed; AUC(0-t), total area under the plasma concentration versus time curve from 0 to the last data point; AUC(0-inf), total area under the plasma concentration versus time curve; $t_{1/2}$, apparent terminal half-life; CL/F, oral clearance; MRT, mean residence time; F, bioavailability.

Table 34. Pharmacokinetics of L-lysine-*d*-amphetamine in Male Beagle Dogs Following Oral Administration of L-lysine-*d*-amphetamine (1 mg/kg d-amphetamine base).

Dose Route : Oral **Dose** : 2 mg/kg of L-lysine-*d*-amphetamine (free form)

Dog ID	C_{max} (ng/mL)	T_{max}[a] (h)	AUC(0-t) (ng•h/mL)	AUC(inf) (ng•h/mL)	$t_{1/2}$ (h)
1	102.1	2.0	686.34	696.89	3.93
2	127.2	2.0	937.57	946.62	3.44
3	83.7	2.0	494.61	540.38	3.06
Mean	104.3	2.0	706.2	728.0	3.48
SD	21.8	2.0 –2.0	222.1	204.9	0.4

[a]: median (range)

Abbreviations of pharmacokinetic parameters are as follows: C_{max}, maximum observed plasma concentration; T_{max}, time when C_{max} observed; AUC(0-t), total area under the plasma concentration versus time curve from 0 to the last data point; AUC(0-inf), total area under the plasma concentration versus time curve; $t_{1/2}$, apparent terminal half-life; CL/F, oral clearance; MRT, mean residence time; F, bioavailability.

Table 35. Pharmacokinetics of *d*-amphetamine in Male Beagle Dogs Following Oral Administration of L-lysine-*d*-amphetamine or d-amphetamine sulfate (1.8 mg/kg d-amphetamine base).

Time	Mean Plasma Concentration		Standard Deviation (SD)		Coefficient of Variation (CV)	
(hours)	*d*-amphetamine	L-lysine-*d*-amphetamine	*d*-amphetamine	L-lysine-*d*-amphetamine	*d*-amphetamine	L-lysine-*d*-amphetamine
0	0	0	0	0	0	0
1	431.4	223.7	140.7	95.9	32.6	42.9
2	360	291.8	87.6	93.6	24.3	32.1
4	277.7	247.5	68.1	66	24.5	26.7
6	224.1	214.7	59.3	62.1	26.5	28.9
8	175.4	150	66.7	40.1	38.0	26.7
12	81.4	47.6	58.7	19	72.1	39.9
16	33	19.6	28.1	9	85.2	45.9
24	7.2	4.5	4.5	1.7	62.5	37.8

Table 36. Pharmacokinetics of *d*-amphetamine in Female Beagle Dogs Following Oral Administration of L-lysine-*d*-amphetamine or d-amphetamine sulfate (1.8 mg/kg d-amphetamine base).

Time	Mean Plasma Concentration		Standard Deviation (SD)		Coefficient of Variation (CV)	
(hours)	*d*-amphetamine	L-lysine-*d*-amphetamine	*d*-amphetamine	L-lysine-*d*-amphetamine	*d*-amphetamine	L-lysine-*d*-amphetamine
0	0	0	0	0	0	0
1	217.8	308.8	141.7	40.7	65.1	13.2
2	273.5	308	113.7	29.6	41.6	9.6
4	266	260.9	132.7	37.3	49.9	14.3
6	204.7	212.1	84.5	38.7	41.3	18.2
8	160.1	164.3	72.7	43.5	45.4	26.5
12	79.4	68.7	41.3	31	52.0	45.1
16	25.5	22.3	13.4	4.7	52.5	21.1
24	5.6	5.4	4.1	1.9	73.2	35.2

Table 37. Pharmacokinetic Parameters of *d*-amphetamine in Male and Female Beagle Dogs Following Oral Administration of L-lysine-*d*-amphetamine or *d*-amphetamine sulfate (1.8 mg/kg *d*-amphetamine base).

Parameter	Males		Females	
	Compound		Compound	
	d-amphetamine	L-lysine-*d*-amphetamine	*d*-amphetamine	L-lysine-*d*-amphetamine
AUCinf	3088.9	2382.2	2664.5	2569.9
Percent	100	77	100	96
Cmax	431.4	291.8	308.8	273.5
Percent	100	67	100	89
Tmax(hours)	1	2	1	2
Percent	100	200	100	200

Example 20. Delayed Cardiovascular Effects of L-lysine-*d*-amphetamine as Compared to *d*-amphetamine Following Intravenous Infusion

[352] Systolic and diastolic blood pressure (BP) are increased by *d*-amphetamine even at therapeutic doses. Since L-lysine-*d*-amphetamine is expected to release *d*-amphetamine (albeit slowly) as a result of systemic metabolism, a preliminary study was done using equimolar doses of *d*-amphetamine or L-lysine-*d*-amphetamine to 4 dogs (2 male and 2 female). The results suggest that the amide prodrug is inactive and that slow release of some *d*-amphetamine, occurs beginning 20 minutes after the first dose. Relative to *d*-amphetamine, however, the effects are less robust. For example, the mean blood pressure is graphed in Fig. 35. Consistent with previously published data (Kohli and Goldberg, 1982), small doses of *d*-amphetamine were observed to have rapid effects on blood pressure. The lowest dose (0.202 mg/kg, equimolar to 0.5 mg/kg of L-lysine-*d*-amphetamine) produced an acute doubling of the mean BP followed by a slow recovery over 30 minutes.

[353] By contrast, L-lysine-*d*-amphetamine produced very little change in mean BP until approximately 30 minutes after injection. At that time, pressure increased by about 20-50%. Continuous release of *d*-amphetamine is probably responsible for the slow and steady increase in blood pressure over the remaining course of the experiment. Upon subsequent injections, *d*-amphetamine is seen to repeat its effect

in a non-dose dependent fashion. That is, increasing dose 10-fold from the first injection produced a rise to the same maximum pressure. This may reflect the state of catecholamine levels in nerve terminals upon successive stimulation of *d*-amphetamine bolus injections. Note that the rise in mean blood pressure seen after successive doses of L-lysine-*d*-amphetamine (Fig. 35) produces a more gradual and less intense effect. Similar results were observed for left ventricular pressure (Fig. 36). These results further substantiate the significant decrease in *d*-amphetamine bioavailability by the intravenous route when given as L-lysine-*d*-amphetamine. As a result the rapid onset of the pharmacological effect of *d*-amphetamine that is sought by persons injecting the drug is eliminated.

Table 38. Effects of L-lysine-*d*-amphetamine on Cardiovascular Parameters in the Anesthetized Dog – Mean Values (n=2)

TREATMENT	TIME	SAP	% Change	DAP	% Change	MAP	% Change	LVP	% Change
0.9% Saline	0	81	0	48	0	61	0	87	0
1 ml/kg	30	87	7	54	11	67	10	87	0
L-lysine-*d*-amphetamine	0	84	0	51	0	64	0	86	0
0.5 mg/kg	5	87	4	52	3	66	3	87	2
	15	93	11	51	1	67	5	95	11
	25	104	25	55	8	73	15	105	22
	30	107	28	58	14	77	21	108	26
L-lysine-*d*-amphetamine	0	105	0	55	0	74	0	108	0
1.0 mg/kg	5	121	15	63	15	85	15	120	11
	15	142	35	73	33	100	35	140	29
	25	163	55	97	75	124	68	162	50
	30	134	28	73	32	98	32	144	33
L-lysine-*d*-amphetamine	0	132	0	71	0	95	0	144	0
5.0 mg/kg	5	142	7	71	0	99	4	151	5
	15	176	33	98	39	130	37	184	28
	25	126	-5	69	-3	96	1	160	11
	30	132	0	70	-1	99	4	163	13

SAP - systolic arterial pressure (mmHg) MAP - mean arterial pressure (mmHg)
DAP - diastolic arterial pressure (mmHg) LVP - left ventricular pressure (mmHg)
% Change- percent change from respective Time 0.

Table 39. Effects of d-Amphetamine on Cardiovascular Parameters in the Anesthetized Dog – Mean Values (n=2)

TREATMENT	TIME	SAP	% Change	DAP	% Change	MAP	% Change	LVP	% Change
0.9% Saline	0	110	0	67	0	84	0	105	0
1 ml/kg	30	108	-2	65	-3	82	-2	101	-3
d-amphetamine	0	111	0	67	0	84	0	104	0
0.202 mg/kg	5	218	97	145	117	176	109	214	107
	15	168	52	97	45	125	49	157	52
	25	148	34	87	30	110	31	142	37
	30	140	26	80	20	103	23	135	30
d-amphetamine	0	139	0	78	0	101	0	133	0
0.404 mg/kg	5	240	73	147	88	187	85	238	79
	15	193	39	112	44	145	43	191	43
	25	166	19	92	17	122	20	168	26
	30	160	16	87	11	117	16	163	22
d-amphetamine	0	158	0	87	0	115	0	162	0
2.02 mg/kg	5	228	44	128	48	169	47	227	40
	15	196	24	107	23	142	23	200	24
	25	189	20	102	17	135	17	192	19
	30	183	16	98	13	129	12	187	16

SAP - systolic arterial pressure (mmHg) MAP - mean arterial pressure (mmHg)
DAP - diastolic arterial pressure (mmHg) LVP - left ventricular pressure (mmHg)
% Change- percent change from respective Time 0.

Example 21. Pharmacodynamic (Locomotor) Response to Amphetamine vs. L-lysine-*d*-amphetamine by Oral Administration

[354] Male Sprague-Dawley rats were provided water ad libitum, fasted overnight and dosed by oral gavage with 6 mg/kg of amphetamine or L-lysine-*d*-amphetamine containing the equivalent amount of *d*-amphetamine. Horizontal locomotor activity (HLA) was recorded during the light cycle using photocell activity chambers (San Diego Instruments). Total counts were recorded every 12 minutes for the duration of the test. Rats were monitored in three separate experiments for 5, 8, and 12 hours, respectively. Time vs. HLA counts for *d*-amphetamine vs. L-lysine-*d*-amphetamine is shown in Figs. 37-38. In each experiment the time until peak activity was delayed and the pharmacodynamic effect was evident for an extended

period of time for L-lysine-*d*-amphetamine as compared to *d*-amphetamine. The total activity counts for HLA of Lys-Amp dosed rats were increased (11–41%) over those induced by *d*-amphetamine in all three experiments (Tables 40 and 41).

Table 40. Locomotor Activity of Rats Orally Administered *d*-amphetamine vs. L-lysine-*d*-amphetamine (5 Hours)

Test Material	Total Activity Counts	Total Activity Counts Above Baseline	Peak of activity (Counts per 0.2 h)	Time of Peak (Counts per 0.2 h)	Time of Last Count Above 200 per 0.2 h
Vehicle	4689	4174	80	1.4	-
L-lysine-*d*-amphetamine	6417	5902	318	1.8	5h
d-amphetamine	515	0	291	0.6	2.6h

Table 41. Locomotor Activity of Rats Orally Administered Amphetamine vs. L-lysine-*d*-amphetamine (12 Hours)

Test Material	Total Activity Counts	Total Activity Counts Above Baseline	Peak of activity (Counts per 0.2 h)	Time of Peak (Counts per 0.2 h)	Time of Last Count Above 100 per 0.2 h
Vehicle	936	0	81	7.2	-
L-lysine-*d*-amphetamine	8423	7487	256	1.8	8.6 h
d-amphetamine	6622	5686	223	0.6	6.4 h

Example 22. Pharmacodynamic Response to Amphetamine vs. L-lysine-*d*-amphetamine by Intranasal Administration

[355] Male Sprague-Dawley rats were dosed by intranasal administration with 1.0 mg/kg of amphetamine or L-lysine-*d*-amphetamine containing the equivalent amount of *d*-amphetamine. In a second set of similarly dosed animals carboxymethyl cellulose (CMC) was added to the drug solutions at a concentration of 62.6 mg/ml (approximately 2-fold higher than the concentration of L-lysine-*d*-amphetamine and 5-fold higher than the *d*-amphetamine content). The CMC drug mixtures were suspended thoroughly before each dose was delivered. Locomotor activity was monitored using the procedure described in the section titled example 7. As shown in Figs. 39-40, the activity vs. time (1 hour or 2 hours) is shown for amphetamine/CMC vs. L-lysine-*d*-amphetamine and compared to that of

amphetamine vs. L-lysine-*d*-amphetamine CMC. As seen in Fig. 39, addition of CMC to L-lysine-*d*-amphetamine decreased the activity response of IN dosed rats to levels similar to the water/CMC control, whereas no effect was seen on amphetamine activity by the addition of CMC. The increase in activity over baseline of L-lysine-*d*-amphetamine with CMC was only 9% compared to 34% for Lys-Amp without CMC when compared to activity observed for *d*-amphetamine dosed animals (Table 42). CMC had no observable affect on *d*-amphetamine activity induced by IN administration.

Table 42. Locomotor Activity of Intranasal *d*-amphetamine vs. L-lysine-*d*-amphetamine with and without CMC

Drug	n	Total Activity Counts (1h)	Total Activity Counts Above Baseline	Percent *d*-amphetamine
d-mphetamine	3	858	686	100
d-amphetamine CMC	3	829	657	100
L-lysine-*d*-amphetamine	4	408	237	35
L-lysine-*d*-amphetamine CMC	4	232	60	9
Water	1	172	0	0
Water CMC	1	172	0	0

Example 23. Pharmacodynamic Response to Amphetamine vs. L-lysine-*d*-amphetamine by Intravenous (IV) Administration

[356] Male Sprague-Dawley rats were dosed by intravenous administration with 1.0 mg/kg of *d*-amphetamine or L-lysine-*d*-amphetamine containing the equivalent amount of amphetamine. The activity vs. time (3 hours) is shown for *d*-amphetamine vs. L-lysine-*d*-amphetamine (Fig. 41). The activity induced by L-lysine-*d*-amphetamine was substantially decreased and time to peak activity was delayed. The activity expressed as total activity counts over a three hour period of time is shown in Fig. 41. The increase in activity over baseline of L-lysine-*d*-amphetamine was 34% for L-lysine-*d*-amphetamine when compared to activity observed for *d*-amphetamine dosed animals (Table 43).

Table 43. Total activity counts after *d*-amphetamine vs. L-lysine-*d*-amphetamine

Drug	n	Total Activity Counts 3h	Above Baseline	Percent *d*-amphetamine
d-amphetamine	3	1659	1355	100
L-lysine-*d*-amphetamine	4	767	463	34
Water	1	304	0	0

Following Intravenous (IV) Administration.

Example 24. Decrease in toxicity of orally administered L-lysine-*d*-amphetamine

[357] Three male and three female Sprague Dawley rats per group were given a single oral administration of L-lysine-*d*-amphetamine at 0.1, 1.0, 10, 60, 100 or 1000 mg/kg (Table 44). Each animal was observed for signs of toxicity and death on Days 1-7 (with Day 1 being the day of the dose) and one rat/sex/group was necropsied upon death (scheduled or unscheduled).

Table 44. Dosing Chart Oral Administration of L-lysine-*d*-amphetamine Toxicity Testing.

Groups	**No. of Animals**		**Test Article**	**Dosages (mg/kg)**	**Concentrations (mg/mL)**
	M	**F**			
1	3	3	L-lysine-*d*-amphetamine	0.1	0.01
2	3	3	L-lysine-*d*-amphetamine	1.0	0.1
3	3	3	L-lysine-*d*-amphetamine	10	1.0
4	3	3	L-lysine-*d*-amphetamine	60	6.0
5	3	3	L-lysine-*d*-amphetamine	100	10
6	3	3	L-lysine-*d*-amphetamine	1000	100

[358] Key observations of this study include:

- All animals in Groups 1-3 showed no observable signs throughout the conduct of the study.
- All animals in Groups 4-6 exhibited increased motor activity within two hours post-dose and which lasted into Day 2.

- One female rat dosed at 1000 mg/kg was found dead on Day 2. Necropsy revealed chromodacryorrhea, chromorhinorrhea, distended stomach (gas), enlarged adrenal glands, and edematous and distended intestines.
- A total of 4 rats had skin lesions of varying degrees of severity on Day 3.
- One male rat dosed at 1000 mg/kg was euthanatized on Day 3 due to open skin lesions on the ventral neck.
- All remaining animals appeared normal from Day 4 through Day 7.

[359] Animals were observed for signs of toxicity at 1, 2 and 4 h post-dose, and once daily for 7 days after dosing and cage-side observations were recorded. Animals found dead, or sacrificed moribund were necropsied and discarded. A total of one animal/sex/group was necropsied upon scheduled or unscheduled death.

[360] Cage-side observations and gross necropsy findings are summarized in Table 5. The data are not sufficient to establish a lethal dose, however, the study indicates that the lethal oral dose of L-lysine-*d*-amphetamine is above 1000 mg/kg, because only one death occurred out of a group of six animals. Although a second animal in this dose group was euthanatized on Day 3, it was done for humane reasons and it was felt that this animal would have fully recovered. Observations suggested drug-induced stress in Groups 4-6 that is characteristic of amphetamine toxicity (NTP, 1990; NIOSH REGISTRY NUMBER: SI1750000; Goodman et. al., 1985). All animals showed no abnormal signs on Days 4-7 suggesting full recovery at each treatment level.

[361] The lack of data to support an established lethal dose is believed to be due to a putative protective effect of conjugating amphetamine with lysine. Intact L-lysine-*d*-amphetamine has been shown to be inactive, but becomes active upon metabolism into the unconjugated form (*d*-amphetamine). Thus, at high doses, saturation of metabolism of L-lysine-*d*-amphetamine into the unconjugated form may explain the lack of observed toxicity, which was expected at doses greater than 100 mg/kg, which is consistent with *d*-amphetamine sulfate (NTP, 1990). The formation rate of *d*-amphetamine and the extent of the formation of amphetamine may both attribute

to the reduced toxicity. Alternatively, oral absorption of L-lysine-*d*-amphetamine may also be saturated at such high concentrations, which may suggest low toxicity due to limited bioavailability of L-lysine-*d*-amphetamine.

Example 25. *In Vitro* Assessment of L-lysine-*d*-amphetamine Pharmacodynamic Activity.

[362] It was anticipated that the acylation of amphetamine, as in the amino acid conjugates discussed here, would significantly reduce the stimulant activity of the parent drug. For example, Marvola (1976) showed that N-acetylation of amphetamine completely abolished the locomotor activity increasing effects in mice. To confirm that the conjugate was not directly acting as a stimulant, we tested (Novascreen, Hanover, MD) the specific binding of Lys-Amp (10^{-9} to 10^{-5} M) to human recombinant dopamine and norepinephrine transport binding sites using standard radioligand binding assays. The results (see Table 45) indicate that the Lys-Amp did not bind to these sites. It seems unlikely that the conjugate retains stimulant activity in light of these results. (Marvola, M. (1976). "Effect of acetylated derivatives of some sympathomimetic amines on the acute toxicity, locomotor activity and barbiturate anesthesia time in mice." Acta Pharmacol Toxicol (Copenh) 38(5): 474-89).

Table 45. Results From Radioligand Binding Experiments with L-lysine-*d*-amphetamine

Assay	Radioligand	Reference Compound	Ki (M) for Ref. Cpd.	Activity*
NE Transporter	[3H]-Nisoxetine	Desipramine	4.1×10^{-9}	No
DA Transporter	[3H]-WIN35428	GBR-12909	7.7×10^{-9}	No

*No activity is defined as producing between -20% and 20% inhibition of radioligand binding (Novascreen).

Example 26. *In Vitro* Assessment "Kitchen Tests" to Release Amphetamine.

[363] It was anticipated that attempts would be made by illicit chemists to treat the compound with various easily accessible physical and chemical methods by which to release free amphetamine from the conjugate. An abuse-resistant preparation would have the additional feature of not releasing *d*-amphetamine when exposed to

water, acid (vinegar), base (baking powder and baking soda), and heat. In several tests with L-lysine-*d*-amphetamine and GGG-Amp, no amphetamine was detected after the following treatments:

	Vinegar	Tap Water	Baking Powder	Baking Soda
L-lysine-*d*-amphetamine	0%	0%	0%	0%
Gly_3-Amp	0%	0%	0%	0%

Samples were heated to boiling for 20-60 minutes in each test.

Example 27. Bioavailability of Various Amino Acid-Amphetamine Compounds Administered by Oral, Intranasal, and Intravenous Routes.

[364] *Oral Administration.* Male Sprague-Dawley rats were provided water ad libitum, fasted overnight, and dosed by oral gavage with amphetamine or amino acid-amphetamine conjugates containing the equivalent amount of amphetamine.

[365] *Intranasal Administration.* Male Sprague-Dawley rats were dosed by intranasal administration with 1.8 mg/kg of amphetamine or lysine-amphetamine containing the equivalent amount of amphetamine.

[366] The relative *in vivo* performance of various amino acid-amphetamine compounds is shown in Figs. 42-50 and summarized in Table 46. Intranasal bioavailability of amphetamine from Ser-Amp was decreased to some degree relative to free amphetamine. However, this compound was not bioequivalent with amphetamine by the oral route of administration. Phenylalanine was bioequivalent with amphetamine by the oral route of administration, however, little or no decrease in bioavailability by parenteral routes of administration was observed. Gly_3-Amp had nearly equal bioavailability (90%) by the oral route accompanied by a decrease in C*max* (74%). Additionally, Gly_3-Amp showed a decrease in bioavailability relative to amphetamine by intranasal and intravenous routes.

Table 46. Percent Bioavailability of Amino Acid Amphetamine Compounds Administered by Oral, Intranasal or Intravenous Routes

Drug	Oral		Intranasal		Intravenous	
	Percent AUC	Percent *Cmax*	Percent AUC	Percent *Cmax*	Percent AUC	Percent *Cmax*
Amphetamine	100	100	100	100	100	100
E-Amp	73	95	NA	NA	NA	NA
EE-Amp	26	74	NA	NA	NA	NA
L-Amp	65	81	NA	NA	NA	NA
S-Amp	79 / 55	62 / 75	76	65	NA	NA
GG-Amp	79	88	88	85	NA	NA
GGG-Amp	111 / 68	74 / 73	32	38	45	46
F-Amp	95	91	97	95	87	89
EEF-Amp	42	73	39	29	NA	NA
FF-Amp	27	64	NA	NA	NA	NA
Gulonate-Amp	1	1	0.4	0.5	3	5
K-Amp	98	55	0.5	0.5	3	3
KG-Amp	69	71	13	12	NA	NA
*d*KK-Amp	16	7	2	2	NA	NA
LE-Amp	40	28	6	6	NA	NA
H-Amp	16	21	22	42	NA	NA

C. Methods of In Vivo Testing of Abuse resistant Amphetamine Conjugates

Example 28. Decreased Oral C_{max} of *d*-Amphetamine Conjugates.

[367] Male Sprague-Dawley rats were provided water ad libitum, fasted overnight and dosed by oral gavage with amphetamine conjugate or *d*-amphetamine sulfate. All doses contained equivalent amounts of *d*-amphetamine base. Plasma *d*-amphetamine concentrations were measured by ELISA (Amphetamine Ultra, 109319, Neogen, Corporation, Lexington, KY). The assay is specific for *d*-amphetamine with only minimal reactivity (0.6%) of the major *d*-amphetamine metabolite (para-hydroxy-*d*-amphetamine) occurring. Plasma *d*-amphetamine and L-lysine-*d*-amphetamine concentrations were measured by LC/MS/MS where indicated in examples.

Example 29. Decreased Intranasal Bioavailability (AUC and C_{max}) of *d*-Amphetamine Conjugates.

[368] Male Sprague-Dawley rats were provided water ad libitum and doses were administered by placing 0.02 ml of water containing amphetamine conjugate or *d*-amphetamine sulfate into the nasal flares. All doses contained equivalent amounts of *d*-amphetamine base. Plasma *d*-amphetamine concentrations were measured by ELISA (Amphetamine Ultra, 109319, Neogen, Corporation, Lexington, KY). The assay is specific for *d*-amphetamine with only minimal reactivity (0.6%) of the major *d*-amphetamine metabolite (para-hydroxy-*d*-amphetamine) occurring. Plasma *d*-amphetamine and L-lysine-*d*-amphetamine concentrations were measured by LC/MS/MS where indicated in examples.

Example 30. Decreased Intravenous Bioavailability (AUC and C_{max}) of *d*-Amphetamine Conjugates.

[369] Male Sprague-Dawley rats were provided water ad libitum and doses were administered by intravenous tail vein injection of 0.1 ml of water containing amphetamine conjugate or *d*-amphetamine sulfate. All doses contained equivalent amounts of *d*-amphetamine base. Plasma *d*-amphetamine concentrations were measured by ELISA (Amphetamine Ultra, 109319, Neogen, Corporation, Lexington, KY). The assay is specific for *d*-amphetamine with only minimal reactivity (0.6%) of the major *d*-amphetamine metabolite (para-hydroxy-*d*-amphetamine) occurring. Plasma *d*-amphetamine and L-lysine-*d*-amphetamine concentrations were measured by LC/MS/MS where indicated in examples.

Example 31. Attachment of Amphetamine to Variety of Chemical Moieties

[370] The above examples demonstrate the use of an amphetamine conjugated to a chemical moiety, such as an amino acid, which is useful in reducing the potential for overdose while maintaining its therapeutic value. The effectiveness of binding amphetamine to a chemical moiety was demonstrated through the attachment of amphetamine to lysine (K), however, the above examples are meant to be illustrative only. The attachment of amphetamine to any variety of chemical moieties (i.e. peptides, glycopeptides, carbohydrates, nucleosides, or vitamins) may be accomplished through similar procedures described throughout the Examples. For

instance the below moieties may be attached to amphetamine using methods similar to those described in Example 2.

Amphetamine Synthetic Examples

Synthesis of Gly_2-Amp

Gly_2-Amp was synthesized by a similar method except the amino acid starting material was Boc-Gly-Gly-OSu.

Synthesis of Glu_2-Phe-Amp

Glu_2-Phe-Amp was synthesized by a similar method except the amino acid starting material was Boc-Glu(OtBu)-Glu(OtBu)-OSu and the starting drug conjugate was Phe-Amp (see Phe-Amp synthesis).

Synthesis of His-Amp

His-Amp was synthesized by a similar method except the amino acid starting material was Boc-His(Trt)-OSu.

Synthesis of Lys-Gly-Amp

Lys-Gly-Amp was synthesized by a similar method except the amino acid starting material was Boc-Lys(Boc)-OSu and the starting drug conjugate was Gly-Amp (see Gly-Amp synthesis).

Synthesis of Lys-Glu-Amp

Lys-Glu-Amp was synthesized by a similar method except the amino acid starting material was Boc-Lys(Boc)-OSu and the starting drug conjugate was Glu-Amp.

Synthesis of Glu-Amp

Glu-Amp was synthesized by a similar method except the amino acid starting material was Boc-Glu(OtBu)-OSu.

Synthesis of (d)-Lys-(l)-Lys-Amp

(d)-Lys-(l)-Lys-Amp was synthesized by a similar method except the amino acid starting material was Boc-(d)-Lys(Boc)-(l)-Lys(Boc)-OSu.

Synthesis of Gulonic acid-Amp

Gul-Amp was synthesized by a similar method except the carbohydrate starting material was gulonic acid-OSu.

Example 32. Lack of detection of L-lysine-*d*-amphetamine in Brain Tissue Following Oral Administration

[371] Male Sprague-Dawley rats were provided water ad libitum, fasted overnight and dosed by oral gavage with L-lysine-*d*-amphetamine or *d*-amphetamine sulfate. All doses contained equivalent amounts of *d*-amphetamine base. As shown in Figs. 51A-B, similar levels of *d*-amphetamine were detected in serum as well as in brain tissue following administration of *d*-amphetamine sulfate or L-lysine-*d*-amphetamine. The conjugate L-lysine-*d*-amphetamine, however, was present in appreciable amounts in serum but was not detected in brain tissue indicating that the conjugate does not cross the blood brain barrier to access the central nervous system site of action.

CARRIER BOUND NARCOTICS

Examples 33 through 83 Hydrocodone

Applicability of abuse resistance for the narcotic analgesics demonstrated through the use of hydrocodone.

[372] Examples 33 through 83 illustrate the applicability of a number of peptide-active agent compositions in reducing the potential for overdose while maintaining their therapeutic value wherein the peptides are conjugated to the active agent hydrocodone (HC). Exemplary compounds which were substituted at the 6 position of hydrocodone are termed EEFFI-HC**[SEQ ID NO: 5]**, EEFFF-HC**[SEQ ID NO: 3]**, YYI-HC, DDI-HC, and YYFFI**[SEQ ID NO: 6]**-HC.

[373] Oral, intranasal, and intravenous bioavailability studies of hydrocodone and hydrocodone conjugates were conducted in male Sprague-Dawley rats. Doses of hydrocodone bitartrate and hydrocodone conjugates containing equivalent amounts of hydrocodone were administered in deionized water. Oral administration was in 0.5 ml by gavage needle (with the exception of YYI-HC, which was delivered as a solid in gelatin capsules). Intranasal doses were administered by placing 20 microliters into the nasal flares of rats anesthetized with isoflurane. Intravenous administration was in 0.1 ml by tail vein injection. Plasma was collected by retroorbital sinus puncture under isoflurane anesthesia. Hydrocodone and

hydromorphone (major active metabolite) concentrations were determined by LC/MS/MS.

[374] The below examples are illustrative only and the below amino acid sequences attached to hydrocodone is not meant to be limiting. As such, synthesis and attachment of hydrocodone may be accomplished for instance view the following exemplary methods.

Hydrocodone Synthetic Examples Carbohydrates

Example 33. Galacto-Hydrocodone

Figure 52 illustrates preparation of Galacto-Hydrocodone.

Reagents	MW	Weight	mmoles	Molar Equivalents
1. Hydrocodone	299	0.223g	0.75	1.0
1. $LiN(TMS)_2$ in THF	1M	1.13ml	1.13	1.5
1. DMF	-	5ml	-	-
2. Galactose Chloroformate	-	-	1.49	2.0
2. DMF	-	3ml	-	-
3. 1M HCl	1M	30ml	-	-
3. Acetone	-	20ml	-	-

Galacto-Hydrocodone

[375] To a solution of hydrocodone in DMF was added $LiN(TMS)_2$ in THF via syringe. The solution was stirred at ambient temperatures for 5 minutes then the chloroformate of galactose in DMF was added via syringe. The resulting solution was stirred at ambient temperatures for 2 hours. A TLC was taken (9:1 $CHCl_3$:MeOH; UV and 5% H_2SO_4 in MeOH; $R_{f(product)}$ = ~0.5). Reaction was neutralized to pH 7 with 6M HCl. Solvent was removed. Final product was purified using preparative TLC (0-10% MeOH in $CHCl_3$). Solid was collected as a white powder (0.180g, 41% yield): ^1H NMR (DMSO-d_6) δ 1.28 (2s, 6H), 1.37 (s, 3H), 1.44 (3, 3H), 1.49 (m, 2H), 1.88 (dt, 1H), 2.08 (m, 2H), 2.29 (s, 4H), 2.40 (m, 2H), 2.90 (d, 1H), 3.09 (s, 1H), 3.73 (s, 3H), 3.99 (dd, 1H), 4.14 (t, 1H), 4.26 (dt, 2H), 4.39 (d, 1H), 4.63 (d, 1H), 4.95 (s, 1H), 5.48 (d, 1H), 5.68 (d, 1H), 6.65 (d,1H), 6.74 (d, 1H); MS Calculated mass = 585.6 Found = 586.4 (M+H).

[376] To the protected galactose intermediate was added 30ml of 1M HCl and 20ml acetone. The resulting solution was stirred at ambient temperatures for 3

hours. Solvent was removed and final product dried under vacuum. Solid was collected as a white solid: MS Calculated mass = 505.5 Found = 506.4 (M+H).

[377] Figure 53 depicts oral bioavailability of abuse-resistant hydrocodone carbohydrate conjugates, measured as free hydrocodone (with measured plasma levels by ELISA).

Example 34. Ribo-Hydrocodone

Figure 54 illustrates preparation of Ribo-Hydrocodone.

Reagents	MW	Weight	mmoles	Molar Equivalents
1. Hydrocodone	299	0.733g	2.45	1.0
1. $LiN(TMS)_2$ in THF	1M	3.68ml	3.68	1.5
1. DMF	-	8ml	-	-
2. Ribose Chloroformate	-	-	4.90	2.0
2. DMF	-	3ml	-	-
3. 1M HCl	1M	10ml	-	-

Ribo-Hydrocodone

[378] To a solution of hydrocodone in DMF was added $LiN(TMS)_2$ in THF via syringe. The solution was stirred at ambient temperatures for 5 minutes then the chloroformate of ribose in DMF was added via syringe. The resulting solution was stirred at ambient temperatures for 2 hours. A TLC was taken (9:1 $CHCl_3$:MeOH; UV and 5% H_2SO_4 in MeOH; $R_{f(product)}$ = ~0.5). Reaction was neutralized to pH 7 with 1M HCl. Solvent was removed. Crude product was taken up in $CHCl_3$ (50ml), washed with water (3 X 50ml), dried over $MgSO_4$, filtered and solvent removed. Final product was purified using preparative HPLC (10mM CH_3COONH_4 / MeCN; 0-20min: 80/20 → 0/100). Solid was collected as a clear, colorless glass (0.095g, 7% yield): ^1H NMR (DMSO-d_6) δ 1.26 (s, 3H), 1.39 (s, 3H), 1.50 (m, 2H), 1.89 (s, 4H), 2.08 (m, 2H), 2.29 (s, 4H), 2.40 (m, 2H), 2.88 (d, 1H), 3.08 (m, 1H), 3.25 (s, 3H), 3.73 (s, 3H), 4.12 (m, 2H), 4.28 (t, 1H), 4.58 (d, 1H), 4.72 (d, 1H), 4.97 (s, 1H), 4.98 (s, 1H), 5.70 (s, 1H), 6.66 (d, 1H), 6.75 (d, 1H). MS Calculated mass = 529.2 Found = 530.4 (M+H).

[379] To the protected ribose intermediate was added 10ml of 1M HCl. The resulting solution was stirred at ambient temperatures for 2 hours. Solvent was removed and final product dried under vacuum. Solid was collected as a waxy,

slightly yellow solid (0.092g, quant.): ^{1}H NMR (DMSO-d_6) δ 1.51 (t, 1H), 1.83 (d, 1H), 2.41 (dt, 1H), 2.27 (t, 1H), 2.63 (dd, 1H), 2.80 (s, 3H), 2.96 (m, 2H), 3.20 (m, 1H), 3.75 (s, 3H), 3.82-4.34 (br m, 12H), 5.15 (s, 1H), 5.72 (s, 1H), 6.75 (d, 1H), 6.88 (d, 1H), 11.37 (br s, 1H).

[380] Figure 55 illustrates intranasal bioavailability of abuse-resistant hydrocodone carbohydrate conjugate, measured as free hydrocodone (with measured plasma levels by ELISA).

Single Amino Acids

Example 35. Leu-Hydrocodone

Figure 56 illustrates preparation of Leu-Hydrocodone.

Reagents	MW	Weight	mmoles	Molar Equivalents
1. Hydrocodone	299	1.00g	3.34	1.0
1. LiN(TMS)$_2$ in THF	1M	10.5ml	10.5	3.15
1. THF	-	25ml	-	-
2. Boc-Leu-OSu	328	3.28g	10.0	3.0

Leu-Hydrocodone

[381] To a solution of hydrocodone in THF was added LiN(TMS)$_2$ in THF via syringe. The solution was stirred at ambient temperatures for 5 minutes then Boc-Leu-OSu was added. The resulting reaction mixture was stirred at ambient temperatures for 18 hours. Reaction was neutralized to pH 7 with 6M HCl. Solvent was removed. Crude material was taken up in $CHCl_3$ (100ml), washed with sat. $NaHCO_3$ (3X100ml), dried over $MgSO_4$, filtered, and solvent removed. Solid was collected as a yellow powder (1.98g, 95% yield): ^{1}H NMR (DMSO-d_6) δ 0.86 (dd, 6H), 1.31 (s, 9H), 1.46 (s, 2H), 1.55 (m, 2H), 1.69 (m, 1H), 1.87 (dt, 1H), 2.07 (dt, 2H), 2.29 (s, 3H), 2.43 (m, 2H), 2.93 (d, 1H), 3.11 (s, 1H), 3.72 (s, 3H), 3.88 (dt, 1H), 4.03 (dt, 1H), 4.87 (s, 1H), 5.51 (d, 1H), 6.65 (d, 1H), 6.73 (d, 1H), 6.90 (s, 1H).

[382] To the Boc-Leu-Hydrocodone was added 25ml of 4N HCl in dioxane. The resulting mixture was stirred at ambient temperatures for 18 hours. Solvent was removed and final product dried under vacuum. Solid was collected as a slightly yellow solid (1.96g, 97% yield): ^{1}H NMR (DMSO-d_6) δ 0.94 (d, 6H), 1.52 (m, 1H),

1.75-1.90 (m, 4H), 2.22 (dt, 1H), 2.34 (dt, 1H), 2.64 (q, 1H), 2.75 (s, 3H), 2.95-3.23 (m, 4H), 3.74 (s, 3H), 3.91 (d, 1H), 4.07 (s, 1H), 5.10 (s, 1H), 5.72 (d, 1H), 6.76 (d, 1H), 6.86 (d, 1H), 8.73 br s, 3H).

Example 36. Glu-Hydrocodone

Synthesis of Glu-Hydrocodone

[383] Glu-Hydrocodone was prepared by a similar method to Example 35 except the amino acid starting material was Boc-Glu(OtBu)-OSu.

Example 37. Ile-Hydrocodone

Synthesis of Ile-Hydrocodone

[384] Ile-Hydrocodone was prepared by a similar method to Example 35 except the amino acid starting material was Boc-Ile-OSu.

Dipeptides

Figure 57 illustrates preparation of Ala-Pro-Hydrocodone.

Example 38. Ala-Pro-Hydrocodone

Reagents	MW	Weight	mmoles	Molar Equivalents
Pro-Hydrocodone	468	0.25g	0.53	1.0
Boc-Ala-OSu	286	0.33g	1.2	2.26
NMM	101	0.50ml	5.38	10.2
DMF	-	10ml	-	-

Ala-Pro-Hydrocodone

[385] To a solution of Pro-Hydrocodone in DMF was added NMM followed by Boc-Ala-OSu. The solution was stirred at ambient temperatures for 18hours. Solvent was removed. Crude material was purified using preparative HPLC (Phenomenex Luna C18, 30X250mm, 5μM, 100Å; Gradient: 100 water/0 0.1% TFA-MeCN → 0/100; 30ml/min.). Solid was collected as a slightly yellow powder (0.307g, 85% yield): ^{1}H NMR (DMSO-d_6) δ 1.16 (d, 3H), 1.35 (s, 9H), 1.51 (m, 2H), 1.86-2.10 (m, 6H), 2.50 (m, 1H), 2.54 (m, 1H), 2.69 (m, 1H), 2.88 (s, 3H), 3.02 (dd, 1H), 3.26 (d, 1H), 3.55 (m, 1H), 3.67 (m, 1H), 3.72 (s, 3H), 3.80 (s, 1H), 4.25 (m, 1H), 4.43 (d, 1H), 5.01 (s, 1H), 5.59 (d, 1H), 6.75 (d, 1H), 6.88 (d, 1H), 6.99 (t, 1H), 9.91 (br s, 1H).

[386] To the Boc-Ala-Pro-Hydrocodone (0.100g) was added 10ml of 4N HCl in dioxane. The resulting mixture was stirred at ambient temperatures for 18 hours. Solvent was removed and final product dried under vacuum. Solid was collected as a slightly yellow solid (0.56g, 71% yield): ^{1}H NMR (DMSO-d_6) δ 1.38 (s, 3H), 1.48 (t, 1H), 1.80-2.29 (m, 8H), 2.65 (m, 1H), 2.80 (s, 3H), 2.96 (m, 3H), 3.23 (m, 2H), 3.76 (s, 3H), 3.92 (s,1H), 4.22 (s, 1H), 4.53 (s, 1H), 5.00 (s, 1H), 5.84 (d, 1H), 6.77 (d, 1H), 6.86 (d, 1H), 8.25 (br s, 3H).

Example 39. Glu-Glu-Hydrocodone

Synthesis of Glu-Glu-Hydrocodone

[387] Glu-Glu-Hydrocodone was prepared by a similar method to Example 38 except the amino acid starting material was Boc-Glu(OtBu)-OSu and the conjugate starting material was Glu-Hydrocodone.

Example 40. (pyro)Glu-Glu-Hydrocodone

Synthesis of (pyro)Glu-Glu-Hydrocodone

[388] The compound (pyro)Glu-Glu-Hydrocodone was prepared by a similar method to Example 38 except the amino acid starting material was Boc-pyroglutamic acid-OSu and the conjugate starting material was Glu-Hydrocodone.

Tripeptides

Figure 58 illustrates the preparation of Gly-Gly-Leu-Hydrocodone.

Example 41. Gly-Gly-Leu-Hydrocodone

Reagents	MW	Weight	mmoles	Molar Equivalents
Leu-Hydrocodone	484	2.21g	4.56	1.0
Boc-Gly-Gly-OSu	329	3.00g	9.12	2.0
NMM	101	5.0ml	45.6	10
DMF	-	100ml	-	-

Gly-Gly-Leu-Hydrocodone

[389] To a solution of Leu-Hydrocodone in DMF was added NMM followed by Boc-Gly-Gly-OSu. The solution was stirred at ambient temperatures for 18hours. Solvent was removed. Crude material was purified using preparative HPLC (Phenomenex Luna C18, 30X250mm, 5μM, 100Å; Gradient: 90 water/10 0.1% TFA-MeCN → 0/100; 30ml/min.). Solid was collected as a slightly yellow powder

(2.08g, 73% yield): ^{1}H NMR (DMSO-d_6) δ 0.88 (dd, 6H), 1.38 (s, 9H), 1.53-1.72 (m, 5H), 1.89 (d, 1H), 2.15 (m, 1H), 2.67 (m, 2H), 2.94 (s, 3H), 3.05 (m, 2H), 3.25 (m, 2H), 3.56 (d, 3H), 3.76 (s, 6H), 3.98 (s, 1H), 4.35 (q, 1H), 5.04 (s, 1H), 5.59 (d, 1H), 6.77 (d, 1H), 6.85 (d, 1H), 7.04 (t, 1H), 8.01 (t, 1H), 8.30 (d, 1H), 9.99 (br s, 1H).

[390] To the Boc-Gly-Gly-Leu-Hydrocodone (2.08g) was added 50ml of 4N HCl in dioxane. The resulting mixture was stirred at ambient temperatures for 18 hours. Solvent was removed and final product dried under vacuum. Solid was collected as a slightly yellow solid (1.72g, 86% yield): ^{1}H NMR (DMSO-d_6) δ 0.89 (dd, 6H), 1.50-1.87 (m, 5H), 2.26 (m, 2H), 2.66 (m, 2H), 2.82-2.97 (m, 5H), 3.21 (m, 2H), 3.60 (m, 4H), 3.88 (m, 5H), 4.37 (m, 1H), 5.04 (s, 1H), 5.60 (s, 1H), 6.79 (d, 2H), 8.07 (br s, 3H), 8.54 (br s, 1H), 8.66 (br s, 1H), 11.29 (br s, 1H).

Example 42. Glu-Glu-Glu-Hydrocodone

Synthesis of Glu-Glu-Glu-Hydrocodone

[391] Glu-Glu-Glu-Hydrocodone was prepared by a similar method to Example 41 except the amino acid starting material was Boc-Glu(OtBu)-Glu(OtBu)-OSu and the conjugate starting material was Glu-Hydrocodone.

Example 43. Pro-Pro-Leu-Hydrocodone

Synthesis of Pro-Pro-Leu-Hydrocodone

[392] Pro-Pro-Leu-Hydrocodone was prepared by a similar method to Example 41 except the amino acid starting material was Boc-Pro-Pro-OSu.

Example 44. Leu-Leu-Leu-Hydrocodone

Synthesis of Leu-Leu-Leu-Hydrocodone

[393] Leu-Leu-Leu-Hydrocodone was prepared by a similar method to Example 41 except the amino acid starting material was Boc-Leu-Leu-OSu.

Example 45. Pro-Pro-Ile-Hydrocodone

Synthesis of Pro-Pro-Ile-Hydrocodone

[394] Pro-Pro-Ile-Hydrocodone was prepared by a similar method to Example 41 except the amino acid starting material was Boc-Pro-Pro-OSu and the conjugate starting material was Ile-Hydrocodone.

Example 46. Leu-Pro-Leu-Hydrocodone

Synthesis of Leu-Pro-Leu-Hydrocodone

[395] Leu-Pro-Leu-Hydrocodone was prepared by similar methods except the amino acid starting material was Boc-Leu-Pro-OSu.

Example 47. Lys-Lys-Ile-Hydrocodone

Synthesis of Lys-Lys-Ile-Hydrocodone

[396] Lys-Lys-Ile-Hydrocodone was prepared by similar methods except the amino acid starting material was Boc-Lys(Boc)-Lys(Boc)-OSu and the conjugate starting material was Ile-Hydrocodone.

Example 48. Glu-Glu-Ile-Hydrocodone

Synthesis of Glu-Glu-Ile-Hydrocodone

[397] Glu-Glu-Ile-Hydrocodone was prepared by similar methods except the amino acid starting material was Boc-Glu(OtBu)-Glu(OtBu)-OSu and the conjugate starting material was Ile-Hydrocodone.

Example 49. Tyr-Tyr-Ile-Hydrocodone

Synthesis of Tyr-Tyr-Ile-Hydrocodone

[398] Tyr-Tyr-Ile-Hydrocodone was prepared by similar methods except the amino acid starting material was Boc-Tyr(tBu)-Tyr(tBu)-OSu and the conjugate starting material was Ile-Hydrocodone.

Pentapeptides

Example 50. Gly-Gly-Gly-Gly-Leu**[SEQ ID NO: 1]**-Hydrocodone

Figure 59 illustrates preparation of Gly-Gly-Gly-Gly-Leu**[SEQ ID NO: 1]**-Hydrocodone.

Reagents	MW	Weight	mmoles	Molar Equivalents
Gly-Gly-Leu-Hydrocodone	**599**	**0.580g**	**0.970**	**1.0**
Boc-Gly-Gly-OSu	**329**	**0.638g**	**1.94**	**2.0**
NMM	**101**	**1.06ml**	**9.70**	**10**
DMF	**-**	**20ml**	**-**	**-**

Gly-Gly-Gly-Gly-Leu[SEQ ID NO: 1]-Hydrocodone

[399] To a solution of Gly-Gly-Leu-Hydrocodone in DMF was added NMM followed by Boc-Gly-Gly-OSu. The solution was stirred at ambient temperatures for 18hours. Solvent was removed. Crude material was purified using preparative HPLC (Phenomenex Luna C18, 30X250mm, 5μM, 100Å; Gradient: 85 water/15

0.1% TFA-MeCN → 50/50; 30ml/min.). Solid was collected as a slightly yellow powder (0.304g, 37% yield).

[400] To the Boc-Gly-Gly-Gly-Gly-Leu**[SEQ ID NO: 1]**-Hydrocodone (0.304g) was added 25ml of 4N HCl in dioxane. The resulting mixture was stirred at ambient temperatures for 18 hours. Solvent was removed and final product dried under vacuum. Solid was collected as a slightly yellow solid (0.247g, 97% yield): ^{1}H NMR (DMSO-d_6) δ 0.87 (m, 6H), 1.23 (s, 1H), 1.51-1.86 (m, 4H), 2.18 (m, 1H), 2.71 (m, 2H), 2.77 (s, 3H), 2.96 (m, 2H), 3.17 (m, 2H), 3.61 (s, 3H), 3.81-3.84 (m, 10H), 4.22 (m, 1H), 4.36 (m, 1H), 5.09 (m, 1H), 5.59 (d, 1H), 6.74 (dd, 2H), 8.16 (br s, 4H), 8.38 (br s, 1H), 8.74 (br s, 1H), 11.42 (br s, 1H).

Example 51. Glu_5**[SEQ ID NO: 11]**-Hydrocodone

Synthesis of Glu_5[SEQ ID NO: 11]-Hydrocodone

[401] Glu_5**[SEQ ID NO: 11]**-Hydrocodone was prepared by a similar method to Example 50 except the amino acid starting material was Boc-Glu(OtBu)-Glu(OtBu)-OSu and the conjugate starting material was Glu_3-Hydrocodone.

Example 52. Glu_2-Gly_2-Ile**[SEQ ID NO: 12]**-Hydrocodone

Synthesis of Glu_2-Gly_2-Ile[SEQ ID NO: 12]-Hydrocodone

[402] Glu_2-Gly_2-Ile**[SEQ ID NO: 12]**-Hydrocodone was prepared by a similar method to Example 50 except the amino acid starting material was Boc-Glu(OtBu)-Glu(OtBu)-OSu and the conjugate starting material was Gly_2-Ile-Hydrocodone.

Example 53. Glu_2-Gly_2-Leu**[SEQ ID NO: 13]**-Hydrocodone

Synthesis of Glu_2-Gly_2-Leu[SEQ ID NO: 13]-Hydrocodone

[403] Glu_2-Gly_2-Leu**[SEQ ID NO: 13]**-Hydrocodone was prepared by a similar method to Example 50 except the amino acid starting material was Boc-Glu(OtBu)-Glu(OtBu)-OSu and the conjugate starting material was Gly_2-Leu-Hydrocodone.

Example 54. Gly_4-Ile**[SEQ ID NO: 14]**-Hydrocodone

Synthesis of Gly_4-Ile[SEQ ID NO: 14]-Hydrocodone

[404] Glu_4-Ile**[SEQ ID NO: 14]**-Hydrocodone was prepared by a similar method to Example 50 except the amino acid starting material was Boc-Gly-Gly-OSu and the conjugate starting material was Gly_2-Ile-Hydrocodone.

Example 55. Glu_2-Phe_3[SEQ ID NO: 3]-Hydrocodone

Synthesis of Glu_2-Phe_3[SEQ ID NO: 3]-Hydrocodone

[405] Glu_2-Phe_3[SEQ ID NO: 3]-Hydrocodone was prepared by a similar method to Example 50 except the amino acid starting material was Boc-Glu(OtBu)-Glu(OtBu)-OSu and the conjugate starting material was Phe_3-Hydrocodone.

Example 56. Lys_2-Gly_2-Ile[SEQ ID NO: 15]-Hydrocodone

Synthesis of Lys_2-Gly_2-Ile[SEQ ID NO: 15]-Hydrocodone

[406] Lys_2-Gly_2-Ile[SEQ ID NO: 15]-Hydrocodone was prepared by a similar method to Example 50 except the amino acid starting material was Boc-Lys(Boc)-Lys(Boc)-OSu and the conjugate starting material was Gly_2-Ile-Hydrocodone.

Example 57. Lys_2-Pro_2-Ile[SEQ ID NO: 16]-Hydrocodone

Synthesis of Lys_2-Pro_2-Ile[SEQ ID NO: 16]-Hydrocodone

[407] Lys_2-Pro_2-Ile[SEQ ID NO: 16]-Hydrocodone was prepared by a similar method to Example 50 except the amino acid starting material was Boc-Lys(Boc)-Lys(Boc)-OSu and the conjugate starting material was Pro_2-Ile-Hydrocodone.

Example 58. Tyr_2-Gly_2-Ile[SEQ ID NO: 17]-Hydrocodone

Synthesis of Tyr_2-Gly_2-Ile[SEQ ID NO: 17]-Hydrocodone

[408] Tyr_2-Gly_2-Ile[SEQ ID NO: 17]-Hydrocodone was prepared by a similar method to Example 50 except the amino acid starting material was Boc-Tyr(tBu)-Tyr(tBu)-OSu and the conjugate starting material was Gly_2-Ile-Hydrocodone.

Example 59. Gly_2-Pro_2-Ile[SEQ ID NO: 18]-Hydrocodone

Synthesis of Gly_2-Pro_2-Ile[SEQ ID NO: 18]-Hydrocodone

[409] Gly_2-Pro_2-Ile[SEQ ID NO: 18]-Hydrocodone was prepared by a similar method to Example 50 except the amino acid starting material was Boc-Gly_2-OSu and the conjugate starting material was Pro_2-Ile-Hydrocodone.

Example 60. Asp_2-Phe_2-Ile[SEQ ID NO: 19]-Hydrocodone

Synthesis of Asp_2-Phe_2-Ile[SEQ ID NO: 19]-Hydrocodone

[410] Asp_2-Phe_2-Ile[SEQ ID NO: 19]-Hydrocodone was prepared by a similar method to Example 50 except the amino acid starting material was Boc-Asp(OtBu)-Asp(OtBu)-OSu and the conjugate starting material was Phe_2-Ile-Hydrocodone.

Example 61. Glu_2-Asp_2-Ile**[SEQ ID NO: 20]**-Hydrocodone

Synthesis of Glu_2-Asp_2-Ile[SEQ ID NO: 20]-Hydrocodone

[411] Glu_2-Asp_2-Ile**[SEQ ID NO: 20]**-Hydrocodone was prepared by a similar method to Example 50 except the amino acid starting material was Boc-Glu(OtBu)-Glu(OtBu)-OSu and the conjugate starting material was Asp_2-Ile-Hydrocodone.

Example 62. Lys_2-Asp_2-Ile**[SEQ ID NO: 21]**-Hydrocodone

Synthesis of Lys_2-Asp_2-Ile[SEQ ID NO: 21]-Hydrocodone

[412] Lys_2-Asp_2-Ile**[SEQ ID NO: 21]**-Hydrocodone was prepared by a similar method to Example 50 except the amino acid starting material was Boc-Lys(Boc)-Lys(Boc)-OSu and the conjugate starting material was Asp_2-Ile-Hydrocodone.

Example 63. Tyr_2-Glu_2-Ile**[SEQ ID NO: 22]**-Hydrocodone

Synthesis of Tyr_2-Glu_2-Ile[SEQ ID NO: 22]-Hydrocodone

[413] Tyr_2-Glu_2-Ile**[SEQ ID NO: 22]**-Hydrocodone was prepared by a similar method to Example 50 except the amino acid starting material was Boc-Tyr(tBu)-Tyr(tBu)-OSu and the conjugate starting material was Glu_2-Ile-Hydrocodone.

Example 64. Asp_4-Ile**[SEQ ID NO: 23]**-Hydrocodone

Synthesis of Asp_4-Ile[SEQ ID NO: 23]-Hydrocodone

[414] Asp_4-Ile**[SEQ ID NO: 23]**-Hydrocodone was prepared by a similar method to Example 50 except the amino acid starting material was Boc-Asp(OtBu)-Asp(OtBu)-OSu and the conjugate starting material was Asp_2-Ile-Hydrocodone.

Example 65. Glu_2-Phe_2-Ile**[SEQ ID NO: 5]**-Hydrocodone

Synthesis of Glu_2-Phe_2-Ile[SEQ ID NO: 5]-Hydrocodone

[415] Glu_2-Phe_2-Ile**[SEQ ID NO: 5]**-Hydrocodone was prepared by a similar method to Example 50 except the amino acid starting material was Boc-Glu(OtBu)-Glu(OtBu)-OSu and the conjugate starting material was Phe_2-Ile-Hydrocodone.

Example 66. Lys_2-Glu_2-Ile**[SEQ ID NO: 24]**-Hydrocodone

Synthesis of Lys_2-Glu_2-Ile[SEQ ID NO: 24]-Hydrocodone

[416] Lys_2-Glu_2-Ile**[SEQ ID NO: 24]**-Hydrocodone was prepared by a similar method to Example 50 except the amino acid starting material was Boc-Lys(Boc)-Lys(Boc)-OSu and the conjugate starting material was Glu_2-Ile-Hydrocodone.

Example 67. Tyr_2-Phe-Pro-Ile**[SEQ ID NO: 10]**-Hydrocodone

Synthesis of Tyr_2-Phe-Pro-Ile[SEQ ID NO: 10]-Hydrocodone

[417] Tyr_2-Phe-Pro-Ile**[SEQ ID NO: 10]**-Hydrocodone was prepared by a similar method to Example 50 except the amino acid starting material was Boc-Tyr(tBu)-Tyr(tBu)-OSu and the conjugate starting material was Phe-Pro-Ile-Hydrocodone.

YYFFI-**[SEQ ID NO: 6]**HC

Example 68. Tyr-Tyr-Phe-Phe-Ile**[SEQ ID NO: 6]**-(6-*O*)-Hydrocodone

Preparation of Tyr-Tyr-Phe-Phe-Ile[SEQ ID NO: 6]-(6-*O*)-hydrocodone

[418] Hydrocodone bitartrate (48.38g) was stirred in 500ml 1N NaOH for 5 minutes. Suspension was split into 2 batches and extracted using $CHCl_3$ (2 X 250ml), organics were dried using $MgSO_4$ and filtered. Solvent was removed and product was obtained as a white powder (29.05g).

[419] To a solution of hydrocodone freebase (7.12g) in tetrahydrofuran (THF) (300ml) was added $LiN(TMS)_2$ in THF (1M, 36.0ml) via syringe. The solution was stirred at ambient temperatures for 10 minutes then Boc-Ile-OSu (11.7g) was added. The resulting reaction mixture was stirred at ambient temperatures for 3 hours. Reaction was neutralized to pH 7 with 1M HCl and stirred for 10 minutes. Solvent was removed. Crude material was taken up in diethyl ether (100ml), washed with sat. $NaHCO_3$ (3X100ml), dried over $MgSO_4$, filtered, and solvent was removed. Solid was collected as a yellow powder (11.1g).

[420] To the Boc-Ile-Hydrocodone (11.1g) was added 125ml of 4N HCl in dioxane. The resulting mixture was stirred at ambient temperatures for 1 hour. Solvent was removed and final product dried under vacuum. Solid was collected as a slightly yellow powder (10.43g).

[421] To a suspension of Boc-Phe-Phe-OH (10.0g) and N-hydroxysuccinimide (NHS) (3.06g) in acetone (300ml) was added dicyclohexylcarbodiimide (DCC) (4.99g). The solution was stirred at ambient temperatures under argon for 18hrs. Solid dicyclohexylurea (DCU) was filtered away and washed with acetone. Solvent was removed from filtrate. Crude material was recrystallized using a system of acetone and hexane. Solvent was filtered off and the solid was collected as a white powder (12.2g).

[422] To a solution of Ile-HC·2HCl (6.00g) in N,N-dimethylformamide (DMF) (150ml) was added 4-methyl morpholine (NMM) (6.79ml) followed by Boc-Phe-Phe-OSu (6.93g). The solution was stirred at ambient temperatures for 18 hours. Solvent was reduced to approximately ¼ total volume, added to sat. $NaHCO_3$ (~100ml), and stirred for 30 minutes. The precipitate was filtered and washed thoroughly with water. Solid material was dried in vacuum, dissolved in a small amount of ethyl acetate, and filtered. Product was obtained as a slightly yellow powder (8.39g).

[423] To Boc-Phe-Phe-Ile-HC (2.99g) was added 50ml 4N HCl in dioxane. The resulting suspension was stirred at ambient temperatures for 1 hour. Solvent was removed and product was dried. Product was obtained as a yellow solid (2.60g).

[424] To a solution of Boc-Tyr(tBu)-OH (1.00g) in 15ml DMF was added O-(N-succinimidyl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TSTU) (0.892g) and NMM (0.65ml). After 10 minutes of activation, H-Tyr(tBu)-OH (0.844g) in 40ml DMF:dioxane:water (2:2:1) was added. The resulting suspension was stirred at ambient temperature for 4 hours. After this time, water (15ml) was added and the resulting solution was stirred at ambient temperature for 30 minutes. The solvent volume was reduced to ¼ and extracted with ethyl acetate (250ml), washed with 5% acetic acid in water (2 x 150ml), water (3 x 150ml), and brine (150ml). The organic layer was dried over $MgSO_4$, filtered, and solvent removed. Crude product was purified using recrystallization with IPAC/hexane solvent system. Final product was isolated as a white solid (1.025g).

[425] To a suspension of Boc-Tyr(tBu)-Tyr(OtBu)-OH (7.32g) and NHS (1.54g) in acetone (150ml) was added DCC (2.51g). The solution was stirred at ambient temperatures under argon for 18hrs. Solid DCU was filtered away and washed with acetone. Solvent was removed from filtrate. Crude material was washed with warm hexane. Solid was collected as a white powder (6.65g).

[426] To a solution of Phe-Phe-Ile-HC·2HCl (2.63g) in DMF (100ml) was added NMM (3.70ml) followed by Boc-Tyr(tBu)-Tyr(tBu)-OSu (4.41g). The solution was stirred at ambient temperatures for 18 hours. Solvent was reduced to approximately ¼ total volume, added to sat. $NaHCO_3$ (~100ml), and stirred for 30 minutes. The

precipitate was filtered and washed thoroughly with water. Solid material was dried in vacuum and purified by reverse phase HPLC (2.77g). Product was deprotected using 4N HCl in dioxane (~50ml).

[427] To a solution of Phe-Phe-Ile-HC·2HCl (5.00g) in DMF (250ml) was added NMM (3.52ml) followed by Boc-Tyr(tBu)-Tyr(tBu)-OSu (4.61g). The solution was stirred at ambient temperatures for 6 hours. Solvent was reduced to approximately ¼ total volume, added to sat. $NaHCO_3$ (~500ml), and stirred for 30 minutes. The precipitate was filtered and washed thoroughly with water. Solid material was dried in vacuum overnight, dissolved in methanol, and any remaining solid material was filtered. The solvent was evaporated from the filtrate and the product was recrystallized using ethanol (~60ml). The precipitate was filtered and dried in vacuum overnight. Product was collected as a pale brown powder (4.57g).

[428] Boc-Tyr(OtBu)-Tyr(OtBu)-Phe-Phe-Ile-HC (3.53g) was deprotected using 4N HCl in dioxane (~100ml). This material was stirred at ambient temperatures for ~1hour. The solvent was evaporated and the product was collected as a slightly yellow powder (3.64g).

[429] Figures 60 through 85 demonstrate plasma levels measured by ELISA of various compounds described in Examples 35 through 68.

Glycopeptides

Figure 86 illustrates preparation of 1,2:3,4-di-O-isopropylidene-D-galactopyranose.

Reagents	MW	Weight	mmoles	Molar Equivalents
1,2:3,4-di-*O*-isopropylidene-D-galactopyranose	260	1.00g	3.85	1
20% Phosgene in toluene	-	20ml	-	-

Chloroformate of 1,2:3,4-di-*O*-isopropylidene-D-galactopyranose

[430] To a stirring solution of 20% phosgene in toluene under an inert atmosphere was added 1,2:3,4-di-*O*-isopropylidene-D-galactopyranose via syringe. The resulting clear, colorless solution was stirred at ambient temperature for 30 minutes. After stirring, Ar(g) was bubbled through the solution for approximately 20 minutes to remove any excess phosgene. Solvent was then removed and product dried under

vacuum for 18 hours. Product was used without further purification or characterization.

Example 69. Galactose-CO-Leu-Hydrocodone

Synthesis of Galactose-CO-Leu-Hydrocodone

[431] To the chloroformate of galactose (1.5eq) in dimethylformamide (DMF) (2ml/mmol) was added Leu-Hydrocodone (1eq) and 4-methylmorpholine (NMM) (6eq). The reaction was stirred at ambient temperatures for 18 hours. Reaction was quenched by the addition of water, solvents were removed and crude product was isolated by purification with reverse-phase HPLC.

[432] Product was deprotected using 1:1 1M HCl : THF (1ml/0.1mmol) in 3 hours. Product was re-purified by reverse-phase HPLC.

Example 70. Galactose-CO-Pro2-Ile-Hydrocodone

Synthesis of Galactose-CO-Pro_2-Ile-Hydrocodone

[433] Galactose-CO-Pro_2-Ile-Hydrocodone was prepared in a manner similar to Example 69 except Pro_2-Ile-Hydrocodone was used as the conjugated starting material.

Example 71. Galactose-CO-Pro2-Leu-Hydrocodone

Synthesis of Galactose-CO-Pro_2-Leu-Hydrocodone

[434] Galactose-CO-Pro_2-Leu-Hydrocodone was prepared in a manner similar to Example 69 Pro_2-Leu-Hydrocodone was used as the conjugated starting material.

[099] Figure 87 illustrates oral bioavailability of abuse-resistant hydrocodone glyco-peptide conjugates, measured as free hydrocodone.

Example 72. Gulonic acid-Ile-Hydrocodone

Synthesis of Gulonic acid-Ile-Hydrocodone

[435] Gulonic acid-Ile-Hydrocodone was prepared in a manner similar to Example 69 except Ile-Hydrocodone was used as the conjugated starting material and Gulonic acid-OSu was used as the carbohydrate starting material.

[436] Figure 88 illustrates Oral bioavailability of an abuse-resistant hydrocodone amino acid-carbohydrate conjugate, measured as free hydrocodone.

D-amino acids

Example 73. (d)-Lys-(l)-Lys-Ile-Hydrocodone

Preparation of (d)-Lys-(l)-Lys-Ile-Hydrocodone

[437] To a solution of Ile-Hydrocodone in DMF was added NMM followed by Boc-(d)-Lys(Boc)-(l)-Lys(Boc)-OSu. The solution was stirred at ambient temperatures for 18hours. Solvent was removed. Crude material was purified using preparative HPLC (Phenomenex Luna C18, 30X250mm, 5μM, 100Å; Gradient: 90 water/10 0.1% TFA-MeCN → 0/100; 30ml/min.). Solid was collected as a slightly yellow powder. To the Boc-(d)-Lys(Boc)-(l)-Lys(Boc)-Hydrocodone was added 4N HCl in dioxane. The resulting mixture was stirred at ambient temperatures for 18 hours. Solvent was removed and final product dried under vacuum. Solid was collected as a slightly yellow solid.

Nucleosides

[438] Figure 89 illustrates nucleosides and conjugation sites. Examples 74 through 83 are also described through figures 90 through 128 (with plasma levels measured by LC/MS/MS).

Example 74. Oral bioavailability of peptide-hydrocodone conjugates at a dose (1 mg/kg) approximating a therapeutic human dose and at an elevated dose

[439] Example 74 illustrates that when the peptides EEFFI**[SEQ ID NO: 5]** (Table 46, figure 90), EEFFF**[SEQ ID NO: 3]** (Table 47, figure 91), YYI (Table 48, figure 92), DDI (Table 49, figure 93), and YYFFI**[SEQ ID NO: 6]** (Table 50, figure 94) are conjugated to the active agent hydrocodone oral bioavailability is maintained or increased over an equivalent hydrocodone dose when the dose is administered as 1 mg/kg. This dose is the equivalent of a human dose of 10 to 14 mg for an individual weighing 70 kg (148 lbs) according to Chou et al. However, when administered orally at 5 mg/kg peak levels and bioavailability of EEFFI**[SEQ ID NO: 5]**-HC (Table 51, figure 95), YYI-HC (Table 52, figure 96), DDI-HC (Table 53, figure 97) and YYFFI**[SEQ ID NO: 6]**-HC (Table 54, figure 98) are substantially decreased. A 5 mg/kg dose in rats approximates an 80 mg human equivalent dose (HED) of hydrocodone bitartrate; a dose that would be likely to be harmful to a naïve patient in immediate release form with the potential for fatal overdose. Human equivalent

doses are defined as the equivalent dose for a 60 kg person adjusted for the body surface area of the animal model. The adjustment factor for rats is 6.2. The HED for

a rat dose of 5 mg/kg of hydrocodone base, for example, is equivalent to 48.39 mg (5/6.2 x 60) hydrocodne base; which is equivalent to 79.98 (48.39/.605) mg hydrocodone bitartrate, when adjusted for the salt content.

[440] Thus the peptide-hydrocodone conjugates maintain their therapeutic value at the lower dose (1 mg/kg), whereas when given at a dose above a safe level (5 mg/kg) bioavailability is decreased as compared to hydrocodone, thus diminishing the potential for overdose by oral ingestion. The decrease in bioavailability of hydrocodone from peptide hydrocodone conjugates relative to hydrocodone ranged from 9 to 70 percent (Table 55).

Table 46. Oral Pharmacokinetics of Hydrocodone vs. EEFFI**[SEQ ID NO: 5]**-HC (1 mg/kg dose).

Drug	Hours					AUC (ng/ml h)	Percent	Cmax	Percent
	0.5	1.5	3	5	8	0-8 h	HC	ng/ml	HC
Hydrocodone Bitartrate	9.5	4.5	1.9	0	2	19.1	100	9.5	100
EEFFI**[SEQ ID NO: 5]**-HC	12.9	5.2	4.2	0	1.6	25.8	135	12.9	136

hydrocodone plus hydromorphone (ng/ml)

Table 47. Oral Pharmacokinetics of Hydrocodone vs. EEFFF**[SEQ ID NO: 3]**-HC (1 mg/kg dose).

Drug	Hours					AUC (ng/ml h)	Percent	Cmax	Percent
	0.5	1.5	3	5	8	0-8 h	HC	ng/ml	HC
Hydrocodone Bitartrate	9.5	4.5	1.9	0	2	19.1	100	9.5	100
EEFFF**[SEQ ID NO: 3]**-HC	11.3	4.1	1.2	1.2	1.2	20.7	108	11.3	119

hydrocodone plus hydromorphone (ng/ml) **[SEQ ID NO: 3]**

Table 48. Oral Pharmacokinetics of Hydrocodone vs. YYI-HC (1 mg/kg dose).

Drug	Hours					AUC (ng/ml h)	Percent	Cmax	Percent
	0.5	1.5	3	5	8	0-8 h	HC	ng/ml	HC
Hydrocodone Bitartrate	9.2	5.9	2.3	1.9	2	26.1	100	9.2	100
YYI-HC	9.2	4.3	1.5	1.1	1.8	20.4	78	9.2	100

hydrocodone plus hydromorphone (ng/ml)

Table 49. Oral Pharmacokinetics of Hydrocodone vs. DDI-HC (1 mg/kg dose).

Drug	Hours					AUC (ng/ml h)	Percent	Cmax	Percent
	0.5	1.5	3	5	8	0-8 h	HC	ng/ml	HC
Hydrocodone Bitartrate	8.6	3	1.1	0	1.4	14	100	8.6	100
DDI-HC	14.9	5	0	0	0	17.4	124	14.9	173

hydrocodone plus hydromorphone (ng/ml)

Table 50. Oral Pharmacokinetics of Hydrocodone vs. YYFFI**[SEQ ID NO: 6]**-HC (1 mg/kg dose).

Drug	Hours						AUC (ng/ml h) 0-8 h	Percent HC	Cmax ng/ml	Percent HC
	0.5	1.0	1.5	3	5	8				
Hydrocodone Bitartrate	8.6	4.5	3	1.1	0	1.4	13.6	100	8.6	100
YYFFI**[SEQ ID NO: 6]**-HC	7	3.7	4.3	1.4	1.1	0	14.9	110	7	81

hydrocodone plus hydromorphone (ng/ml)

Table 51. Oral Pharmacokinetics of Hydrocodone vs. EEFFI**[SEQ ID NO: 5]**-HC (5 mg/kg dose).

Drug	Hours					AUC (ng/ml h) 0-8 h	Percent HC	Cmax ng/ml	Percent HC
	0.5	1.5	3	5	8				
Hydrocodone Bitartrate	93	5.3	39	5	6.5	167	100	93	100
EEFFI**[SEQ ID NO: 5]**-HC	44	6.5	5.7	4.2	4.5	68	41	44	47

hydrocodone plus hydromorphone (ng/ml)

Table 52. Oral Pharmacokinetics of Hydrocodone vs. YYI-HC (5 mg/kg dose).

Drug	Hours					AUC (ng/ml h) 0-8 h	Percent HC	Cmax ng/ml	Percent HC
	0.5	1.5	3	5	8				
Hydrocodone Bitartrate	37	13	12	3	0	71	100	37	100
YYI-HC	15	6.3	3.3	1.6	2.7	33	46	15	41

hydrocodone plus hydromorphone (ng/ml)

Table 53. Oral Pharmacokinetics of Hydrocodone vs. DDI-HC (5 mg/kg dose).

Drug	Hours					AUC (ng/ml h) 0-8 h	Percent HC	Cmax ng/ml	Percent HC
	0.5	1.5	3	5	8				
Hydrocodone Bitartrate	73	42	6.7	1.2	3.8	128	100	73	100
DDI-HC	115	19	11	4	3.1	145	113	115	158

hydrocodone plus hydromorphone (ng/ml)

Table 54. Oral Pharmacokinetics of Hydrocodone vs. YYFFI**[SEQ ID NO: 6]**-HC (5 mg/kg dose).

Drug	Hours						AUC (ng/ml h) 0-8 h	Percent HC	Cmax ng/ml	Percent HC
	0.5	1.0	1.5	3	5	8				
Hydrocodone Bitartrate	73	62	42	6.7	1.2	3.8	123	100	73	100
YYFFI**[SEQ ID NO: 6]**-HC	46	33	34	13	8.3	4.5	105	86	46	63

hydrocodone plus hydromorphone (ng/ml)

Table 55. Decrease in Oral Bioavailability at 5 mg/kg vs. Therapeutic Dose of 1 mg/kg.

Drug	**Bioavailability 1 mg/kg**		**Bioavailability 5 mg/kg**		**Percent Decrease 1 mg/kg vs. 5 mg/kg**	
	AUC	Cmax	AUC	Cmax	AUC	Cmax
YYI-HC	78	100	46	40	41	60
DDI-HC	124	174	113	158	9	9
YYFFI**[SEQ ID NO: 6]**-HC	109	81	86	62	15	23
EEFFI**[SEQ ID NO: 5]**-HC	135	136	41	47	70	65

Example 75. Bioavailability of peptide-HC conjugates by the intranasal route

[441] Example 75 illustrates that when the peptides EEFFF**[SEQ ID NO: 3]** (Table 56, figure 99), YYI (Table 57, figure 100), DDI (Table 58, figure 101) and YYFFI**[SEQ ID NO: 6]** (Table 59, figure 102) are conjugated to the active agent hydrocodone the bioavailability by the intravenous route is substantially decreased thereby diminishing the possibility of overdose when the drug is administered by snorting.

Table 56. Intranasal Pharmacokinetics of Hydrocodone vs. EEFFF**[SEQ ID NO: 3]**-HC (1 mg/kg dose).

	Minutes				AUC (ng/ml h)	Percent	Cmax	Percent
Drug	5	15	30	60	0-1 h	HC	ng/ml	HC
Hydrocodone Bitartrate	262	259	142	47	152	100	262	100
EEFFF**[SEQ ID NO: 3]**-HC	34	21	24	15	21	14	34	13

hydrocodone plus hydromorphone (ng/ml)

Table 57. Intranasal Pharmacokinetics of Hydrocodone vs. YYI-HC (1 mg/kg dose).

	Minutes				AUC (ng/ml h)	Percent	Cmax	Percent
Drug	5	15	30	60	0-1 h	HC	ng/ml	HC
Hydrocodone Bitartrate	446	553	244	103	288	100	553	100
YYI-HC	31	17	12	2	12	4	31	6

hydrocodone plus hydromorphone (ng/ml)

Table 58. Intranasal Pharmacokinetics of Hydrocodone vs. DDI-HC (1 mg/kg dose).

	Minutes				AUC (ng/ml h)	Percent	Cmax	Percent
Drug	5	15	30	60	0-1 h	HC	ng/ml	HC
Hydrocodone Bitartrate	446	553	244	103	288	100	553	100
DDI-HC	281	121	64	16	88	31	281	51

hydrocodone plus hydromorphone (ng/ml)

Table 59. Intranasal Pharmacokinetics of Hydrocodone vs. YYFFI**[SEQ ID NO: 6]**-HC (1 mg/kg dose).

	Minutes				AUC (ng/ml h)	Percent	Cmax	Percent
Drug	5	15	30	60	0-1 h	HC	ng/ml	HC
Hydrocodone Bitartrate	446	553	244	103	288	100	553	100
YYFFI**[SEQ ID NO: 6]**-HC	28	27	16	21	20	100	28	5

hydrocodone plus hydromorphone (ng/ml)

Example 76: Bioavailability of peptide-HC conjugates by the intravenous route

[442] Example 76 illustrates that when the peptides EEFFI**[SEQ ID NO: 5]** (Table 60, figure 103), EEFFF**[SEQ ID NO: 3]** (Table 61, figure 104), YYI (Table 62, figure 105) and YYFFI**[SEQ ID NO: 6]** (Table 63, figure 106) are conjugated to the active agent hydrocodone the bioavailability by the intravenous route is substantially decreased thereby diminishing the possibility of overdose when the drug is administered by this unintended route.

Table 60. Intravenous Pharmacokinetics of Hydrocodone vs. EEFFI**[SEQ ID NO: 5]**-HC (1 mg/kg dose).

	Minutes				AUC (ng/ml h)	Percent	Cmax	Percent
Drug	5	15	30	60	0-1 h	HC	ng/ml	HC
Hydrocodone Bitartrate	179	204	201	132	173	100	179	100
EEFFI**[SEQ ID NO: 5]**-HC	89	76	78	66	66	38	89	44

hydrocodone plus hydromorphone (ng/ml)

Table 61. Intravenous Pharmacokinetics of Hydrocodone vs. EEFFF**[SEQ ID NO: 3]**-HC (1 mg/kg dose).

	Minutes				AUC (ng/ml h)	Percent	Cmax	Percent
Drug	5	15	30	60	0-1 h	HC	ng/ml	HC
Hydrocodone Bitartrate	179	204	201	132	173	100	179	100
EEFFF**[SEQ ID NO: 3]**-HC	135	77	140	85	107	62	135	75

hydrocodone plus hydromorphone (ng/ml)

Table 62. Intravenous Pharmacokinetics of Hydrocodone vs. YYI-HC (1 mg/kg dose).

	Minutes				AUC (ng/ml h)	Percent	Cmax	Percent
Drug	5	15	30	60	0-1 h	HC	ng/ml	HC
Hydrocodone Bitartrate	238	182	136	77	138	100	238	100
YYI-HC	9	13	13	3	10	7	13	6

hydrocodone plus hydromorphone (ng/ml)

Table 63. Intravenous Pharmacokinetics of Hydrocodone vs. YYFFI**[SEQ ID NO: 6]**-HC (1 mg/kg dose).

	Minutes				AUC (ng/ml h)	Percent	Cmax	Percent
Drug	5	15	30	60	0-1 h	HC	ng/ml	HC
Hydrocodone Bitartrate	238	182	136	77	138	100	238	100

YYFFI**[SEQ ID NO: 6]**-HC	171	28	22	18	40	29	171	72

hydrocodone plus hydromorphone (ng/ml)

Example 77. Hydrocodone conjugates.

[443] Bioavailability (AUC and Cmax) of various peptide-hydrocodone conjugates relative to that of hydrocodone bitartrate are shown in Table 64. The invention is well illustrated by the *in vivo* performance of YYFFI**[SEQ ID NO: 6]**-HC (Figues 107 through 128). At the relatively low doses of 1 and 2 mg/kg (human equivalent doses (HEDs) of 16 and 32 mg hydrocodone bitartrate) YYFFI**[SEQ ID NO: 6]**-HC showed comparable bioavailability to that of hydrocodone bitartrate (Table 65, Figures 129 through 134). At the elevated doses of 5 and 25 mg/kg bioavailability of hydrocodone and hydromorphone were substantially decreased as compared to that of hydrocodone (Table 66, Figures 135 through 150). These doses (HED of 80 and 400 mg hydrocodne bitartrate) are equivalent to amounts well above the available prescription doses of hydrocodone bitartrate which range from 2.5 to 10 mg. When delivered by the parentaral routes of intravenous and intranasal administration a substantial decrease in bioavailability of hydrocodone and hydromorphone from YYFFI**[SEQ ID NO: 6]**-HC as compared to hydrocodone bitratrate was observed. These examples establish that covalent modification of an opiod via attachment of a peptide provides a method of delivering bioequivalent doses when given at doses approximating a normal prescribed dose. When administered by parenteral routes or at oral doses in excess of the intended prescription the bioavailability is substantially decreased. Collectively, the examples clearly illustrate the utility of the invention for decreasing the abuse potential of opiods.

Table 64. Mean hydrocodone concentrations following oral administration of hydrocodone bitartrate or YYFFI**[SEQ ID NO: 6]**-HC at escalating doses.

Hours	Dose[1] / Concentration (ng/ml)							
	1 mg/kg		2 mg/kg		5 mg/kg		25 mg/kg	
	HC[2]	YYFFI**[SEQ ID NO: 6]**-HC[3]	HC[2]	YYFFI**[SEQ ID NO: 6]**--HC[3]	HC[2]	YYFFI**[SEQ ID NO: 6]**--HC[3]	HC[2]	YYFF**[SEQ ID NO: 6]**-I-HC[3]
0	0	0	0	0	0	0	0	0
0.1	114.0	20.3	60.3	35.2	628.7	26.6	408.9	41.4
0.5	14.3	17.9	15.6	23	74.3	22.5	153.9	23.3
1.0	7.0	10.4	12.9	14.4	80.8	15.1	86.2	31.0
2.0	2.6	2.8	3.4	9.8	18.4	10.3	83.3	43.9
4.0	1.0	1.2	1.3	3.3	4.9	3.6	57.8	25.0

1 - hydrocodone base content
2 - hydrocodone bitartrate
3 - YYFFI**[SEQ ID NO: 6]**-HC HCl

Table 65. Hydrocodone pharmacokinetic parameters following oral administration of hydrocodone bitartrate or YYFFI**[SEQ ID NO: 6]**-HC at escalating doses.

Parameter	Dose[1] / Concentration (ng/ml)							
	1 mg/kg		2 mg/kg		5 mg/kg		25 mg/kg	
	HC[2]	YYFFI**[SEQ ID NO: 6]**-HC[3]	HC[2]	YYFFI**[SEQ ID NO: 6]**-HC[3]	HC[2]	YYFFI**[SEQ ID NO: 6]**-HC[3]	HC[2]	YYFFI**[SEQ ID NO: 6]**-HC[3]
AUC	45.1	26.3	38.2	48	234	47	419.0	135.0
Percent HC + HM[4]	100	58	100	126	100	20	100	32
Cmax	114.0	20.3	60.3	35.2	628.7	26.6	408.9	41.4
Percent HC + HM[4]	100	18	100	58	100	4	100	10

1 - hydrocodone base content
2 - hydrocodone bitartrate
3 - YYFFI**[SEQ ID NO: 6]**-HC HCl
4 – percent relative to parameter following administration of hydrocodone bitartrate

Table 66. Mean hydromorphone concentrations following oral administration of hydrocodone bitartrate or YYFFI**[SEQ ID NO: 6]**-HC at escalating doses.

Hours	Dose[1] / Concentration (ng/ml)							
	1 mg/kg		2 mg/kg		5 mg/kg		25 mg/kg	
	HC[2]	YYFFI**[SEQ ID NO: 6]**-HC[3]	HC[2]	YYFFI**[SEQ ID NO: 6]**-HC[3]	HC[2]	YYFFI**[SEQ ID NO: 6]**-HC[3]	HC[2]	YYFFI**[SEQ ID NO: 6]**-HC[3]
0	0	0	0	0	0	0	0	0
0.1	1.95	0.27	7.61	1.13	9.03	0.49	44.36	8.00
0.5	3.22	2.87	18.10	8.74	13.46	10.41	62.24	10.35
1.0	2.69	2.39	9.23	3.63	10.36	4.82	29.89	12.70
2.0	2.11	2.24	2.31	3.41	6.68	3.17	31.62	16.22
4.0	0.64	1.02	0.59	0.88	2.00	1.07	40.86	8.98

1 - hydrocodone base content
2 - hydrocodone bitartrate
3 - YYFFI**[SEQ ID NO: 6]**-HC HCl

Table 67. Hydromorphone pharmacokinetic parameters following oral administration of hydrocodone bitartrate or YYFFI**[SEQ ID NO: 6]**-HC at escalating doses.

Parameter	Dose[1] / Concentration (ng/ml)							
	1 mg/kg		2 mg/kg		5 mg/kg		25 mg/kg	
	HC[2]	YYFFI**[SEQ ID NO: 6]**-HC[3]	HC[2]	YYFFI**[SEQ ID NO: 6]**-HC[3]	HC[2]	YYFFI**[SEQ ID NO: 6]**-HC[3]	HC[2]	YYFFI**[SEQ ID NO: 6]**-HC[3]
AUC	7.8	7.5	21.0	12.9	28.1	14.3	149	49
Percent HM[4]	100	97	100	61	100	51	100	33
Cmax	3.2	2.9	18.1	8.7	13.5	10.4	44.4	16.2
Percent HM[4]	100	89	100	48	100	77	100	37

1 - hydrocodone base content
2 - hydrocodone bitartrate
3 - YYFFI**[SEQ ID NO: 6]**-HC HCl
4 – percent relative to parameter following administration of hydrocodone bitartrate

Table 68. Mean hydrocodone plus hydromorphone concentrations following oral administration of hydrocodone bitartrate or YYFFI**[SEQ ID NO: 6]**-HC at escalating doses.

Hours	Dose[1] / Concentration (ng/ml)							
	1 mg/kg		2 mg/kg		5 mg/kg		25 mg/kg	
	HC[2]	YYFFI**[SEQ ID NO: 6]**-HC[3]	HC[2]	YYFFI**[SEQ ID NO: 6]**-HC[3]	HC[2]	YYFFI**[SEQ ID NO: 6]**-HC[3]	HC[2]	YYFFI**[SEQ ID NO: 6]**-HC[3]
0	0	0	0	0	0	0	0	0

0.1	116	20.6	67.9	36.3	637.7	27.1	453.3	49.4
0.5	17.5	20.;8	33.7	31.7	87.8	32.9	216.1	33.7
1.0	9.7	12.8	22.1	18.0	91.2	19.9	116.1	43.7
2.0	4.7	5.0	5.7	13.2	25.1	13.5	114.9	60.1
4.0	1.6	2.2	1.9	4.2	6.9	4.7	98.7	34.0

1 - hydrocodone base content
2 - hydrocodone bitartrate
3 - YYFFI**[SEQ ID NO: 6]**-HC HCl

Table 69. Hydrocodone plus hydromorphone pharmacokinetic parameters following oral administration of hydrocodone bitartrate or YYFFI**[SEQ ID NO: 6]**-HC at escalating doses.

Parameter	Dose[1] / Concentration (ng/ml)							
	1 mg/kg		2 mg/kg		5 mg/kg		25 mg/kg	
	HC[2]	YYFFI**[SEQ ID NO: 6]**-HC[3]	HC[2]	YYFFI**[SEQ ID NO: 6]**-HC[3]	HC[2]	YYFFI**[SEQ ID NO: 6]**-HC[3]	HC[2]	YYFFI**[SEQ ID NO: 6]**-HC[3]
AUC	53	34	59	61	312	62	569	193
Percent HC[4]	100	64	100	103	100	20	100	34
Cmax	116	20.8	67.9	36.3	638	32.9	453	49.4
Percent HC[4]	100	18	100	53	100	5	100	11

1 - hydrocodone base content
2 - hydrocodone bitartrate
3 - YYFFI**[SEQ ID NO: 6]**-HC HCl
4 – percent relative to parameter following administration of hydrocodone bitartrate

Table 70. Mean hydrocodone plus hydromorphone, hydrocodone, and hydromorphone, concentrations following intravenous administration of hydrocodone bitartrate or YYFFI**[SEQ ID NO: 6]**-HC at 1 mg/kg (hydrocodone base content).

Hours	Concentration (ng/ml)					
	HC + HM		HC		HM	
	HC[1]	YYFFI**[SEQ ID NO: 6]**-HC[2]	HC[1]	YYFFI**[SEQ ID NO: 6]**-HC[2]	HC[1]	YYFFI**[SEQ ID NO: 6]**-HC[2]
0	0	0	0	0	0	0
0.1	208.9	22.6	42.97	8.75	251.9	31.3
0.5	83.7	13.5	16.09	1.44	99.8	14.9
1.0	38.4	13.0	3.65	0.92	42.1	13.9
2.0	12.4	13.1	1.77	0.41	14.2	13.5
4.0	2.9	8.5	0.70	0.33	3.6	8.8

1 - hydrocodone bitartrate
2 - YYFFI**[SEQ ID NO: 6]**-HC HCl

Table 71. Hydrocodone plus hydromorphone, hydrocodone, and hydromorphone pharmacokinetic parameters following intravenous administration of hydrocodone bitartrate or YYFFI**[SEQ ID NO: 6]**-HC at1 mg/kg (hydrocodone base content).

Parameter	Concentration (ng/ml)					
	HC + HM		HC		HM	
	HC[1]	YYFFI**[SEQ ID NO: 6]**-HC[2]	HC[1]	YYFFI**[SEQ ID NO: 6]**-HC[2]	HC[1]	YYFFI**[SEQ ID NO: 6]**-HC[2]
AUC	140.0	50.0	24.10	4.50	164	54
Percent [1]	100	36	100	19	100	33
Cmax	208.9	22.6	43.0	8.7	252	31.3

Percent [3]	100	10.8	100	20.2	100	12.4

1 - hydrocodone bitartrate
2 - YYFFI**[SEQ ID NO: 6]**-HC HCl
3 – percent relative to parameter following administration of hydrocodone bitartrate

Table 72. Mean hydrocodone plus hydromorphone, hydrocodone, and hydromorphone, concentrations following intranasal administration of hydrocodone bitartrate or YYFF**[SEQ ID NO: 6]**I-HC at 1 mg/kg.

Minutes	Concentration (ng/ml)					
	HC + HM		HC		HM	
	HC[1]	YYFFI**[SEQ ID NO: 6]**-HC[2]	HC[1]	YYFFI**[SEQ ID NO: 6]**-HC[2]	HC[1]	YYFFI**[SEQ ID NO: 6]**-HC[2]
0	0	0	0	0	0	0
5	446	28	441	28	4.4	bql[3]
15	553	27	543	27	10.6	bql[4]
30	244	16	227	16	17.1	bql[5]
60	103	21	96	21	7.2	bql[6]

1 - hydrocodone bitartrate
2 - YYFFI**[SEQ ID NO: 6]**-HC HCl

Table 73. Hydrocodone plus hydromorphone, hydrocodone, and hydromorphone pharmacokinetic parameters following intravenous administration of hydrocodone bitartrate or YYFFI**[SEQ ID NO: 6]**-HC at1 mg/kg (hydrocodone base content).

Parameter	Concentration (ng/ml)					
	HC + HM		HC		HM	
	HC[1]	YYFFI**[SEQ ID NO: 6]**-HC[2]	HC[1]	YYFFI**[SEQ ID NO: 6]**-HC[2]	HC[1]	YYFFI**[SEQ ID NO: 6]**-HC[2]
AUC	288.0	20.0	74.70	10.30	7.0	NA
Percent [3]	100	6.9	100	13.8	100	NA
Cmax	553.0	28.0	543.0	28.0	17	NA
Percent [3]	100	5.1	100	5.2	100	NA

1 - hydrocodone bitartrate
2 - YYFFI**[SEQ ID NO: 6]**-HC HCl
3 – percent relative to parameter following administration of hydrocodone bitartrate

[444] Summary of *in vivo* testing of abuse resistant hydrocodone conjugates. *In vivo* testing of hydrocodone conjugates demonstrates for instance decreased intranasal analgesic response, decreased intravenous analgesic response, decreased subcutaneous analgesic response, decreased oral C_{max}, decreased intranasal bioavailability (AUC and C_{max}), and decreased intravenous bioavailability (AUC and C_{max}) of hydrocodone conjugates and is described in further detail below.

Example 78. Decreased Intranasal Analgesic Response to Hydrocodone Conjugates

[445] Male Sprague-Dawley rats were dosed by placing 0.02 ml of water containing hydrocodone conjugate or hydrocodone bitartrate into the nasal flares. All doses contained equivalent amounts of hydrocodone base. The time (seconds) until paw lick latency was used a measure of the analgesic effect. Rats were habituated to determine baseline response. Hot plate tests were conducted at 55°C. A limit of 45 seconds was used in all testing to avoid tissue damage. All animals were humanely sacrificed following the end of testing. The paw lick latency (analgesic effect)-time curves shown in figures 112 and 114 indicate the decrease in analgesia produced by the hydrocodone conjugates as compared to an equimolar (hydrocodone base) dose of hydrocodone bitartrate. The analgesic response as determined by the hot plate test is a pharmacodynamic measurement of the pharmacological effect of hydrocodone. These examples illustrate that hydrocodone conjugates decrease the analgesic effect by the intranasal route of administration as compared to hydrodone bitartrate.

Example 79. Decreased Intravenous Analgesic Response to Hydrocodone Conjugates

[446] Male Sprague-Dawley rats were dosed by tail vein injection of 0.1 ml of water containing hydrocodone conjugates or hydrocodone bitartrate. All doses contained equivalent amounts of hydrocodone base. The time (seconds) until paw lick latency was used a measure of the analgesic effect. Rats were habituated to determine baseline response. Hot plate tests were conducted at 55°C. A limit of 45 seconds was used in all testing to avoid tissue damage. All animals were humanely sacrificed following the end of testing. The paw lick latency (analgesic effect)-time curve shown in figure 67 indicates the decrease in analgesia produced by a hydrocodone conjugate as compared to an equimolar (hydrocodone base) dose of hydrocodone bitartrate. The analgesic response as determined by the hot plate test is a pharmacodynamic measurement of the pharmacological effect of hydrocodone. This example illustrates that a hydrocodone conjugate decreased the analgesic effect by the intravenous route of administration as compared to hydrodone bitartrate.

Example 80. Decreased Subcutaneous Analgesic Response to Hydrocodone Conjugates

[447] Male Sprague-Dawley rats were dosed by subcutatenous injection of 0.1 ml of water containing hydrocodone conjugates or hydrocodone bitartrate. All doses contained equivalent amounts of hydrocodone base. The time (seconds) until paw lick latency was used a measure of the analgesic effect. Rats were habituated to determine baseline response. Hot plate tests were conducted at 55°C. A limit of 45 seconds was used in all testing to avoid tissue damage. All animals were humanely sacrificed following the end of testing. The paw lick latency (analgesic effect)-time curve shown in figure 62 indicates the decrease in analgesia produced by a hydrocodone conjugate as compared to an equimolar (hydrocodone base) dose of hydrocodone bitartrate. The analgesic response as determined by the hot plate test is a pharmacodynamic measurement of the pharmacological effect of hydrocodone. This example illustrates that a hydrocodone conjugate decreased the analgesic effect by the subcutaneous route of administration as compared to hydrodone bitartrate.

Example 81. Decreased Oral C_{max} of Hydrocodone Conjugates

[448] Male Sprague-Dawley rats were provided water ad libitum, fasted overnight and dosed by oral gavage with hydrocodone conjugates or hydrocodone bitartrate. All doses contained equivalent amounts of hydrocodone base. Plasma hydrocodone concentrations were measured by ELISA (Hydromorphone, 106619-1, Neogen, Corporation, Lexington, KY). The assay is specific for hydromorphone (the major hydrocodone metabolite, 100% reactive) and hydrocodone (62.5% reactive). The plasma concentration-time curves of various hydrocodone conjugates vs. hydrocodone bitratrate are shown in figures 53, 76, 84, and 85. These examples illustrate that hydrocodone conjugates decrease the peak level (C_{max}) of hydrocodone plus hydromorphone as compared to that produced by equimolar (hydrocodone base) doses of hydrocodone bitartrate when given by the oral route of administration.

Example 82. Decreased Intranasal Bioavailability (AUC and C_{max}) Hydrocodone Conjugates

[449] Male Sprague-Dawley rats were provided water ad libitum and doses were administered by placing 0.02 ml of water containing hydrocodone conjugates or hydrocodne bitartrate into the nasal flares. All doses contained equivalent amounts of hydrocodone base. Plasma hydrocodone concentrations were measured by ELISA (Hydromorphone, 106619-1, Neogen, Corporation, Lexington, KY). The assay is specific for hydromorphone (the major hydrocodone metabolite, 100% reactive) and hydrocodone (62.5% reactive). The plasma concentration-time curves of various hydrocodone conjugates vs. hydrocodone bitartrate are shown in figures 55, 60, 64-66, 69-73, 75, 77-85. These examples illustrate that hydrocodone conjugates decrease the peak level (C_{max}) and total absorption (AUC) of hydrocodone plus hydromorphone as compared to those produced by equimolar (hydrocodone base) doses of hydrocodone bitartrate when given by the intranasal route of administration.

Example 83. Decreased Intravenous Bioavailability (AUC and C_{max}) Hydrocodone Conjugates

[450] Male Sprague-Dawley rats were provided water ad libitum and doses were administered by intravenous tail vein injection of 0.1 ml of water containing hydrocodone conjugates or hydrocodone bitartrate. All doses contained equivalent amounts of *d*-amphetamine base. Plasma hydrocodone concentrations were measured by ELISA (Hydromorphone, 106619-1, Neogen, Corporation, Lexington, KY). The assay is specific for hydromorphone (the major hydrocodone metabolite, 100% reactive) and hydrocodone (62.5% reactive). The plasma concentration-time curves of a hydrocodone conjugate vs. hydrocodone bitartrate is shown in figure 74. This example illustrates that a dose of hydrocodone conjugate decreases the peak level (C_{max}) and total absorption (AUC) of hydrocodone plus hydromorphone as compared to those produced by an equimolar (hydrocodone base) dose of hydrocodone bitartrate when given by the intranasal route of administration.

Examples 84 through 118 Oxycodone

[451] Examples 84 through 118 illustrate the compounds and compositions for reducing the potential for overdose and abuse while maintaining therapeutic value wherein the active agent oxycodone (OC) is covalently attached to a chemical moiety. The compound which is di-substituted at the 6 and 14 position of oxycodone is termed PPL(2)-OC.

[452] Oral, intranasal, and intravenous bioavailability studies of oxycodone and oxycodone conjugates were conducted in male Sprague-Dawley rats. Doses of oxycodone hydrochloride and oxycodone conjugates containing equivalent amounts of oxycodone were administered in deionized water. Oral administration was in 0.5 ml by gavage needle. Intranasal doses were administered by placing 20 microliters into the nasal flares of rats anesthetized with isoflurane. Intravenous administration was in 0.1 ml by tail vein injection. Plasma was collected by retroorbital sinus puncture under isoflurane anesthesia. Oxycodone and oxymorphone (major active metabolite) concentrations were determined by LC/MS/MS.

[453] The below examples are illustrative only and PPL(2)-OC is not meant to be limiting. As such, synthesis and attachment of oxycodone may be accomplished for instance view the following exemplary methods. Additionally, Examples 84 through 96 describe methods for attaching amino acid or various length peptides to oxycodone.

Oxycodone Synthetic Examples

Example 84: Synthesis of $[Boc\text{-}X]_2$-Oxycodone

[454] To a solution of oxycodone free base (2.04 g, 6.47 mmol) in THF (~35 ml) was added $LiN(TMS)_2$ (19.41 ml, 19.41 mmol) and stirred for ~30 mins. To this was added solid Boc-X-OSu (X = amino acid, 21 mmol) at one time and the reaction mixture was stirred at room temperature overnight. The solution was neutralized with 1N HCl and the THF was removed under reduced pressure. The residue was diluted with EtOAc (200 mL), satd. $NaHCO_3$ (150 mL) was added and stirred for 1h. EtOAc part was washed with NaHCO3 and brine. Dried over Na_2SO_4 and evaporated to dryness. Compound was obtained by purification over silica gel column (30% EtOAc/Hexane).

Deprotection of [Boc-X]$_2$-Oxycodone:

[455] General method of deprotection: The above compound was reacted with 4N HCl/ dioxane (25 mL/gm) at room temperature for 4h. Solvent was evaporated and dried over vacuum to give X_2-Oxycodone·3HCl.

Examples:

1. (Val)$_2$-Oxycodone
2. (Ile)$_2$-Oxycodone
3. (Leu)$_2$-Oxycodone
4. (Lys)$_2$-Oxycodone
5. (Phe)$_2$-Oxycodone
6. (Glu)$_2$-Oxycodone

Example 85. Synthesis of [Boc-Z-Y-X]$_2$-Oxycodone [X, Y and Z are amino acids]

[456] To a solution of X_2-Oxycodone · 3HCl (1 mmol) in DMF (15-20 mL) were added NMM (10-12 eqv) and Boc-Z-Y-OSu (2.6 eqv). The reaction mixture was stirred at RT overnight. Solvent was evaporated under reduced pressure. To the residue was added satd. $NaHCO_3$ (~30 mL) and stir for 1-2h. The white/ pale yellow residue was filtered, thoroughly washed with water and dried in the vacuum oven at room temperature.

Deprotection of [Boc-X-Y-Z]$_2$-Oxycodone:

[457] Deprotection is same as general method mentioned above. For 100-200 mg of tripeptide derivative 10-15 ml 4N HCl/dioxane is used. Deprotection is done overnight to give [X-Y-Z]$_2$-Oxycodone·3HCl.

Deprotection of tripeptide derivatives containing Threonine and Serine:

[458] First the tripeptide derivatives are dissolved 95% TFA (5% water) and stirred for 4h at room temperature. Solvent is evaporated, the residue is co-evaporated with toluene twice and dried over vacuum. 4N HCl/dioxane is added and stirred overnight. Residue was evaporated to dryness and dried over vacuum.

Examples:

1. (Glu-Asp-Val)$_2$-Oxycodone
2. (Ile-Tyr-Val)$_2$-Oxycodone
3. (Tyr-Pro-Val)$_2$-Oxycodone
4. (Gly-Leu-Val)$_2$-Oxycodone
5. (Phe-Val-Val)$_2$-Oxycodone
6. (Ser-Thr-Val)$_2$-Oxycodone
7. (Lys-Ser-Val)$_2$-Oxycodone

Example 86. Synthesis of [Boc-X]-O^6-Oxycodone:

[459] To a solution of oxycodone (10 mmol) in THF (50 mL) was added $LiN(TMS)_2$ (10.5 mmol) at 0oC. After 20 mins was added Boc-X-OSu (11 mmol) and then the reaction mixture was stirred at room temperature overnight. The solution was cooled down to 0oC and neutralized with 1N HCl. The organic solvent was evaporated and to the residue were added EtOAc (200 mL) and saturated aq. $NaHCO_3$ (150 mL) and stirred for 1h. The EtOAc portion was washed with water, brine, dried over Na_2SO_4 and evaporated to dryness. The residue was purified over silica gel (70% EtOAc-Hexane) to give the title compound.

Deprotection of Boc-X-O^6-Oxycodone:

[460] A solution of [Boc-X]-Oxycodone in 4N HCl/ dioxane (10 ml/mmol) was stirred at room temperature 4h. Solvent was evaporated under reduced pressure and the residue was dried under vacuum to give X-O^6-Oxycodone·2HCl.

Examples:

1. Val-Oxycodone
2. Ile-Oxycodone
3. Leu-Oxycodone

Example 87. Synthesis of Boc-Z-Y-X-O^6-Oxycodone

[461] To a solution of X-O^6-Oxycodone·2HCl (1 mmol) in DMF were added NMM (10 mmol) and Boc-Z-Y-OSu (1.2 mmol). The reaction mixture was stirred at room temperature overnight. Solvent was evaporated to the residue was added saturated $NaHCO_3$ solution and stirred for 1h. The precipitate was filtered, thoroughly washed with water and dried to give the title compound.

Deprotection of Boc-Z-Y-X-O^6-Oxycodone:

[462] Deprotection is same as general method mentioned above to give Z-Y-X-O^6-Oxycodone·2HCl.

Examples:

1. Pro-Glu-Val-Oxycodone
2. Glu-Leu-Val-Oxycodone
3. Glu-Tyr-Val-Oxycodone

Example 88. Synthesis of Boc-X-O^6-Oxycodone-O^{14}-Ac:

[463] To a solution of [Boc-X]-O^6-Oxycodone (1mmol) in pyridine (15 mL) were added DMAP (75 mg), triethyl amine (1.5 mmol) and Ac_2O (8 mmol). The reaction mixture was heated at 65°C for 3 days. The dark brown solution was cooled down to room temperature and MeOH (5 mL) was added and stirred for 1h. The solvent was evaporated, co-evaporated with toluene. The residue was taken in EtOAc (50 mL), washed with satd. $NaHCO_3$, brine, dried over Na_2SO_4 and evaporated to dryness. The residue was purified over silila gel to give the title compound.

Example 89. Synthesis of Boc-X- O^6-Oxycodone-O^{14}-CO_2Et:

[464] To a solution of [Boc-X]-O^6-Oxycodone (1 mmol) in THF (10 mL) was added $LiN(TMS)_2$ (1.05 mmol) at 0°C. After 20 mins, ethyl chloroformate (1.1 mmol) was added and reaction mixture was slowly brought to room temperature and stirred at room temperature for 1h. The solution was poured into 2% aqueous acetic acid (ice cold) and extracted with EtOAc. The EtOAc part was washed with water, aq. $NaHCO_3$, brine, dried over Na_2SO_4 and evaporated to dryness. The residue was purified over silica gel to give the title compound.

Deprotection of Boc-X-O^6-Oxycodone-O^{14}-R (R=Ac, CO_2Et):

[465] Deprotection is same as general method mentioned above to give X-O^6-Oxycodone-O^{14}-R·2HCl (R=Ac, CO_2Et).

Examples:

1. (Val)-Oxycodone-(CO_2Et)
2. (Val)-Oxycodone-(OAc)

Example 90. Synthesis of Boc-Z-Y-X-O^6-Oxycodone-O^{14}-R (R=Ac, CO_2Et):

[466] To a solution of X-O^6-Oxycodone-O^{14}-R·2HCl (1 mmol, R=Ac, CO_2Et) in DMF were added NMM (10 mmol) and Boc-Z-Y-OSu (1.2 mmol). The reaction mixture was stirred at room temperature overnight. Solvent was evaporated to the residue was added saturated $NaHCO_3$ solution and stirred for 1h. The precipitate was filtered, thoroughly washed with water and dried to give the title compound.

Deprotection of Boc-Z-Y-X-O^6-Oxycodone-O^{14}-R (R=Ac, CO_2Et):

[467] Deprotection is same as general method mentioned above. Deprotection is done overnight to give Z-Y-X-O^6-Oxycodone-O^{14}-R·2HCl.

Examples:

1. (Ile-Tyr-Val)-Oxycodone-(CO_2Et)
2. (Ile-Tyr-Val)-Oxycodone-(OAc)

Example 91. Synthesis of Boc-X-O^6-Oxycodone-O^{14}-Y-Boc:

[468] To a solution of Boc-X-Oxycodone (1mmol) in THF (10 mL) was added $LiN(TMS)_2$ (1.1 mmol) at 0°C and the solution was stirred for 30 mins then Boc-Y-OSu (1.25 mmol) was added. The reaction mixture was stirred at room temperature overnight. The solution was cooled down to 0°C, neutralized with 1N HCl and the organic part was evaporated. To the residue were added EtOAc (50 mL) and satd. $NaHCO_3$ (50 ml), stirred for 1h. The organic part was washed with water, brine, dried over Na_2SO_4 and evaporated to dryness. The residue was purified over silica gel to give the title compound.

Deprotection of Boc-X-O^6-Oxycodone-O^{14}-Y-Boc:

[469] Boc-X-O^6-Oxycodone-O^{14}-Y-Boc was deprotected following the general method for deprotection mentioned above to give X-O^6-Oxycodone-O^{14}-Y·3HCl.

Example:

Val-Oxycodone-Gly

Example 92. Synthesis of Boc-A-B-X-O^6-Oxycodone-O^{14}-Y-B-A-Boc (A,B,X,Y = amino acids):

[470] To a solution of X-O^6-Oxycodone-O^{14}-Y·3HCl (1 mmol) and NMM (10 mmol) in DMF (10 mL) was added Boc-A-B-OSu (2.5 mmol) and the reaction mixture was stirred at room temperature overnight. Solvent was evaporated under reduced pressure and to the residue satd. $NaHCO_3$ (15mL) was added and stirred for 1h. The precipitate was filtered off and the residue was washed thoroughly with water and dried.

Deprotection of Boc-A-B-X-O^6-Oxycodone-O^{14}-Y-B-A-Boc:

[471] Deprotection is same as general method mentioned above. Deprotection is done overnight to give A-B-X-O^6-Oxycodone-O^{14}-Y-B-A·3HCl.

Examples:

1. (Ile-Tyr-Val)-Oxycodone-(Gly-Tyr-Ile)
2. (Leu-Tyr-Val)-Oxycodone-(Gly-Tyr-Leu)

Example 93. Synthsis of Boc-X-O^6-Oxycodone-O^{14}-Y-Cbz:

[472] To a solution of Boc-X-Oxycodone (1mmol) in THF (10 mL) was added $LiN(TMS)_2$ (1.1 mmol) at 0°C and the solution was stirred for 30 mins then Cbz-Y-OSu (1.25 mmol) was added. The reaction mixture was stirred at room temperature overnight. The solution was cooled down to 0°C, neutralized with 1N HCl and the organic part was evaporated. To the residue were added EtOAc (50 mL) and satd. $NaHCO_3$ (50 ml), stirred for 1h. The organic part was washed with water, brine, dried over Na_2SO_4 and evaporated to dryness. The residue was purified over silica gel to give the title compound.

Deprotection of Boc-X-O^6-Oxycodone-O^{14}-Y-Cbz·2HCl:

[473] Boc-X-O^6-Oxycodone-O^{14}-Y-Cbz was deprotected following the general method for deprotection mentioned above to give X-O^6-Oxycodone-O^{14}-Y-Cbz·2HCl.

Example 94. Synthesis of Boc-A-B-X-O^6-Oxycodone-O^{14}-Y-Cbz:

[474] To a solution of X-O^6-Oxycodone-O^{14}-Y-Cbz·2HCl (1 mmol) and NMM (10 mmol) in DMF (10 mL) was added Boc-A-B-OSu (1.1 mmol) and the reaction mixture was stirred at room temperature overnight. Solvent was evaporated under reduced pressure and to the residue satd. $NaHCO_3$ (20 mL) was added and stirred vigorously for 2-3h. The precipitate was filtered off and the residue was washed thoroughly with water and dried.

Example 95. Synthesis of Boc-A-B-X-O^6-Oxycodone-O^{14}-Y-NH2:

[475] To a suspension of Boc-A-B-X-O^6-Oxycodone-O^{14}-Y-Cbz and Pd/C (25 Wt%) in EtOH (20 ml/gm) and cyclohexene (10 ml/gm) was heated under reflux for 30 mins. The reaction mixture was cooled down to room temperature and filtered. The filtrate was evaporated to dryness to give the title compound.

Example 96. Synthesis of Boc-A-B-X-O^6-Oxycodone-O^{14}-Y-C-D-Boc (A,B,C,D,X,Y = amino acids):

[476] To a solution of Boc-A-B-X-O^6-Oxycodone-O^{14}-Y-NH_2 (1 mmol) in DMF (10 mL) were added NMM (5 mmol) and Boc-D-C-OSu (1.1 mmol) and the reaction mixture was stirred at room temperature overnight. Solvent was evaporated under

reduced pressure and to the residue satd. $NaHCO_3$ was added and stirred for 1h. The white precipitate was filtered, washed with water and dried.

Deprotection of Boc-A-B-X-O^6-Oxycodone-O^{14}-Y-C-D-Boc:

[477] Deprotection is same as general method mentioned above. Deprotection is done overnight to give A-B-X-O^6-Oxycodone-O^{14}-Y-C-D·3HCl.

Examples:

1. (Ile-Tyr-Val)-Oxycodone-(Val-Glu-Gly)
2. (Leu-Tyr-Val)-Oxycodone-(Val-Glu-Gly)

Mono-Substituted Single Amino Acids (Enol Ester)

[478] Figure 151 depicts oxycodone.

Example 97. Phe-Oxycodone

[479] To a solution of oxycodone-freebase (1.0eq) in tetrahydrofuran (THF) (10ml/mmol) was added $LiN(TMS)_2$ (3.5eq). After 5 minutes, Boc-Phe-OSu (3.5eq) was added. The reaction was stirred at ambient temperatures for 18 hours, quenched with water and solvents removed. Crude protected product was purified using reverse-phase HPLC. Deprotection occurred with 4N HCl in dioxane (20ml/mmol) to obtain Phe-Oxycodone.

Example 98. Synthesis of Ile-Oxycodone

[480] Ile-Oxycodone was prepared in a similar manner to Example 97 except Boc-Ile-OSu was used as the amino acid starting material.

Mono-Substituted Tripeptides (Enol Ester)

Example 99. Pro_2-Leu-Oxycodone

[481] To a solution of Leu-Oxycodone (1.0eq) in dimethylformamide (10ml/0.1mmol) was added 4-methylmorpholine (10eq) and Boc-Pro-Pro-OSu (2eq). The reaction was stirred at ambient temperatures for 18 hours, quenched with water, and solvents removed. Crude protected product was purified using reverse phase HPLC. Deprotection occurred using 4N HCl in dioxane (20ml/mmol) to obtain Pro_2-Leu-Oxycodone.

Example 100. Synthesis of Pro_2-Ile-Oxycodone

[482] Pro_2-Ile-Oxycodone was prepared in a similar manner to Example 99 except Ile-Oxycodone was used as the conjugated starting material.

Example 101. Oxycodone Disubstituted Tripeptides

General Synthetic Procedure

Synthesis of [Boc-Val]$_2$-OC:

[483] To a solution of OC (2.04 g, 6.47 mmol) in tetrahydrofuran (THF) (~35 ml) was added LiN(TMS)$_2$ (19.41 ml, 19.41 mmol) and stirred for ~30 mins. To this was added solid Boc-Val-OSu (6.72 g, 21 mmol) at one time and the reaction mixture was stirred at room temperature overnight. The solution was neutralized with 1N HCl and the THF was removed under reduced pressure. The residue was diluted with ethyl acetate (EtOAc) (200 mL), satd. $NaHCO_3$ (150 mL) was added and stirred for 1h. EtOAc part was washed with $NaHCO_3$ and brine. Dried over Na_2SO_4 and evaporated to dryness. Crude product was purified with either silica gel column. (30% EtOAc/Hexane).

[484] **Deprotection**: For the deprotection of 2.5 g of [Boc-Val]$_2$-OC , 75-80 mL of 4N HCl/dioxane was used. Reaction was complete within 3-4 hours. Evaporate dioxane and dry over vacuum at lease for 24 h.

[485] **Coupling**: To a solution of Val$_2$-OC·3HCl (250 mg, 0.4 mmol) in DMF (10-12 ml) were added NMM (10-12 eqv) and Boc-X-Y-OSu (2.6 eqv). The reaction mixture was stirred at RT overnight. Solvents were evaporated under reduced pressure. To the residue was added satd. $NaHCO_3$ (~30 mL) and stirred for 1h. The white/ pale yellow residue was filtered, thoroughly washed with water and dried in the vacuum oven at RT.

[486] **Deprotection:** Deprotection was same as above method. For 100-200 mg of tripeptide derivative 10-15 ml 4N HCl/dioxane was used. Deprotection lasts 18 hours.

[487] **Deprotection of tripeptide derivatives containing Threonine and Serine:** Tripeptide derivatives were dissolved in 95% TFA (5% water) and stirred for 4h at room temperature. Solvent was evaporated and the residue was co-evaporated with toluene twice and dried over vacuum. 4N HCl/dioxane was added and stirred overnight. Product was evaporated to dryness and dried over vacuum.

Example 102. Oxycodone Branched Amino Acid Chains

General Synthesis

[488] Figure 152 depicts oxycodone with lysine branched peptides.

Example 103. $(Lys)_2$-Oxycodone

[489] Method was similar to other single amino acid derivatives except Boc-Lys(Boc)-OSu was used as the amino acid starting material.

Example 104. XX-Lys(XX)-Oxycodone

[490] To a solution of $(Lys)_2$-Oxycodone (1.0eq) in dimethylformamide (1ml/mmol) was added 4-methylmorpholine (5.5eq) followed by Boc-XX_2-OSu (4.1). Reaction was stirred at ambient temperature for 24 hours. Solvents were removed and crude product was purified by reverse phase HPLC.

Example 105. Synthesis of $[Gly_2\text{-}Lys(\text{-}Gly_2)]_2$**[SEQ ID NO: 4]**-Oxycodone

[491] $[Gly_2\text{-}Lys(\text{-}Gly_2)]_2$**[SEQ ID NO: 4]**-Oxycodone was prepared in a manner similar to Example 104 except Boc-Gly_2-OSu was used as the amino acid starting material.

Example 106. Oxycodone D-amino acids

General Synthesis

[492] Disubstituted D-amino acid tripeptides were prepared in a manner similar to disubstituted tripeptide conjugates except the amino acid starting material used the unnatural D-amino acids.

[(l)-Lys-(d)-Lys-Leu]$_2$-Oxycodone

[493] To a solution of $(Leu)_2$-Oxycodone (1.0eq) in dimethylformamide (1ml/mmol) was added 4-methylmorpholine (10eq) followed by Boc-(l)-Lys(Boc)-(d)-Lys(Boc)-OSu (3eq). Reaction was stirred at ambient temperature for 24 hours. Solvents were removed and crude product was purified by reverse phase HPLC.

Example 107. Synthetic Amino Acids

[494] Synthesis of [Boc-Z]$_2$-OC [where Z can equal cyclohexylalanine (Cha), dipropylglycine (Dpg), tert-Leucine (Tle) or any other synthetic amino acid]

To a solution of OC (6.47 mmol) in THF was added $LiN(TMS)_2$ (19.41 mmol) and stirred for ~30 mins. To this was added solid Boc-Z-OSu (21 mmol) at one time and

the reaction mixture was stirred at room temperature overnight. The solution was neutralized with 1N HCl and the THF was removed under reduced pressure. The

residue was diluted with ethyl acetate (EtOAc), satd. $NaHCO_3$ was added and stirred for 1h. EtOAc part was washed with $NaHCO_3$ and brine. Dried over Na_2SO_4 and evaporated to dryness. Crude product was purified with either silica gel column. (30% EtOAc/Hexane).

Example 108. Non-Standard Amino Acids (Naturally occurring, not the standard 20)

[495] Synthesis of [Boc-N]$_2$-OC [where N can equal norleucine (Nle), homophenylalanine (hPhe) or any other non-standard amino acid]

[496] To a solution of OC (6.47 mmol) in THF was added $LiN(TMS)_2$ (19.41 mmol) and stirred for ~30 mins. To this was added solid Boc-N-OSu (21 mmol) at one time and the reaction mixture was stirred at room temperature overnight. The solution was neutralized with 1N HCl and the THF was removed under reduced pressure. The residue was diluted with ethyl acetate (EtOAc), satd. $NaHCO_3$ was added and stirred for 1h. EtOAc part was washed with $NaHCO_3$ and brine. Dried over Na_2SO_4 and evaporated to dryness. Crude product was purified with either silica gel column. (30% EtOAc/Hexane).

Other Oxycodone Conjugates

Example 109. Glycopeptides

[497] Using galactose and a number of tripeptides, glycopeptides will be produced.

Initial Glycopeptides to be Produced

1. (Gal-Gly$_2$-Ile)$_2$-OC
2. (Gal-Pro$_2$-Ile)$_2$-OC
3. (Gal-Gly$_2$-Leu)$_2$-OC
4. (Gal-Pro$_2$-Leu)$_2$-OC

Example 110. Glycosylation of Oxycodone

[498] Figure 153 depicts a glycosylated oxycodone.

[499] A glycosylation reaction of Oxycodone with a carbohydrate will be attempted. The linkage produced would essentially be an enol ether which are difficult to cleave chemically yet glycosidic bonds are commonly broken down *in vivo*. Either site or both may be conjugated.

Example 111. Formation of an Enol Ether with Serine

[500] Figure 154 depicts formation of an enol ether with serine.

[501] Using serine and OC, an enol ether conjugate will be produced. This conjugate would be stable to most hydrolysis conditions. Only the enol ether would be formed in this reaction.

Example 112. Vitamins

[502] Figure 155 depicts niacin and biotin.

[503] Vitamins can be used to cap or further functionalize the peptide chain. Niacin and biotin will be conjugated to four different dipeptides.

Conjugates to Prepare

1. (Nia-Gly_2-Ile)$_2$-OC
2. (Nia-Gly_2-Leu)$_2$-OC
3. (Bio-Gly_2-Ile)$_2$-OC
4. (Bio-Gly_2-Leu)$_2$-OC

Figures 156-192 demonstrate plasma levels of oxycodone measured by ELISA.

Example 113. Decreased oral C_{max} of Oxycodone Conjugates

[504] Male Sprague-Dawley rats were provided water ad libitum, fasted overnight and dosed by oral gavage with oxycodone conjugates or oxycodone HCl. All doses contained equivalent amounts of oxycodone base. Plasma oxycodone concentrations were measured by ELISA (Oxymorphone, 102919, Neogen, Corporation, Lexington, KY). The assay is specific for oxymorphone (the major oxycodone metabolite) and oxycodone. Plasma concentration-time curves are shown in figures 156-174. These examples illustrate that doses of oxycodone conjugates decrease the peak level (C_{max}) of oxycodone plus oxymorphone as compared to that produced by equimolar (oxycodone base) doses of oxycodone HCl when given by the oral route of administration.

Example 114. Oral bioavailability of a peptide-oxycodone conjugates at a dose (2.5 mg/kg) approximating a therapeutic human dose

[505] This example illustrates that when the peptide PPL (Table 74, figure 193) is conjugated (disubstituted at the 6 and 14 positions) to the active agent oxyocodone oral bioavailability is maintained as compared to an equimolar oxyocodone dose when the dose administered is 1 mg/kg. This dose is the equivalent of a human dose of 25 to 35 mg for an individual weighing 70 kg (148 lbs) according to Chou et al.

Table 74. Oral Pharmacokinetics of Oxycodone vs. $P2L_{(2)}$-OC (2.5 mg/kg dose).

Drug	Hours					AUC (ng/ml h) 0-8 h	Percent OC	Cmax ng/ml	Percent OC
	0.5	1.5	3	5	8				
Oxycodone Bitartrate	145	27	11	2	1	168	100	145	100
PPL(2)-OC	124	78	46	1	3	278	165	124	86

oxycodone plus oxymorphone

Example 115 Bioavailability of $P2L_{(2)}$-oxycodone by the intranasal route

[506] This example illustrates that when PPL(2) is conjugated to the active agent oxycodone the bioavailability by the intranasal route is substantially decreased thereby diminishing the possibility of overdose (Table 75, figure 194).

Table 75. Intranasal Pharmacokinetics of Oxyocodone vs. $P2L_{(2)}$-OC (1 mg/kg dose).

Drug	Minutes				AUC (ng/ml h) 0-1 h	Percent OC	Cmax ng/ml	Percent OC
	5	15	30	60				
Oxycodone Bitartrate	2128	1003	688	278	428	100	2128	100
PPL(2)-OC	1380	499	390	98	261	61	1380	65

oxycodone plus oxymorphone

Example 116 Bioavailability of $P2L_{(2)}$-oxycodone by the intravenous route

[507] This example illustrates that when $P2L_{(2)}$ is conjugated to the active agent oxycodone the bioavailability by the intravenous route is substantially decreased thereby diminishing the possibility of overdose (Table 76, figure 195).

Table 76. Intravenous Pharmacokinetics of Oxyocodone vs. $P2L_{(2)}$-OC (1 mg/kg dose).

Drug	Minutes				AUC (ng/ml h) 0-1 h	Percent OC	Cmax ng/ml	Percent OC
	5	15	30	60				
Oxycodone Bitartrate	99	104	94	51	82	100	99	100
PPL(2)-OC	22	19	19	43	24	29	43	43

oxycodone plus oxymorphone

Summary of *in vivo* testing of abuse resistant oxycodone conjugates.

[508] In vivo testing of oxycodone conjugates demonstrates for instance decreased oral C_{max}, decreased intranasal bioavailability (AUC and C_{max}), and decreased intravenous bioavailability (AUC and C_{max}) and is described in further detail below.

Example 117. Decreased Intranasal Bioavailability (AUC and C_{max}) of Oxycodone Conjugates

[509] Male Sprague-Dawley rats were provided water ad libitum and doses were administered by placing 0.02 ml of water containing oxycodone conjugates or

oxycodone bitartrate into the nasal flares. All doses contained equivalent amounts of oxycodone base. Plasma oxycodone concentrations were measured by ELISA (Oxymorphone, 102919, Neogen, Corporation, Lexington, KY). The assay is specific for oxymorphone (the major oxycodone metabolite) and oxycodone. Plasma concentration-time curves of various oxycodone conjugates vs. oxycodone HCl are shown in figures 175-192. These examples illustrate that oxycodone conjugates decrease the peak level (C_{max}) and total absorption (AUC) of oxycodone plus oxymorphone as compared to those produced by equimolar (oxycodone base) doses of oxycodone HCl when given by the intranasal route of administration.

Example 118. Decreased Intravenous Bioavailability (AUC and C_{max}) of Oxycodone Conjugates

[510] Male Sprague-Dawley rats were provided water ad libitum and doses were administered by intravenous tail vein injection of 0.1 ml of water containing oxycodone conjugates or oxycodone HCl. All doses contained equivalent amounts of oxycodone base. Plasma oxycodone concentrations were measured by ELISA (Oxymorphone, 102919, Neogen, Corporation, Lexington, KY). The assay is specific for oxymorphone (the major oxycodone metabolite) and oxycodone. Plasma concentration-time curves of an oxycodone conjugate vs. oxycodone HCl is shown in figure 195. This example illustrates that an oxycodone conjugate decreases the peak level (C_{max}) and total absorption (AUC) of oxycodone plus oxymorphone as compared to those produced by an equimolar (oxycodone base) dose of oxycodone HCl when given by the intravenous route of administration.

	oral 2mg/kg		intranasal 2mg/kg	
	% AUC	% Cmax	% AUC	% Cmax
$[Gly\text{-}Glu\text{-}Val]_2$-OC	93	61	29	48
$[Pro\text{-}Glu\text{-}Val]_2$-OC	90	82	34	46
$[Glu\text{-}Pro\text{-}Val]_2$-OC	142	134	56	65
$[Ser\text{-}Gly\text{-}Val]_2$-OC	90	92	64	73
$[Glu\text{-}Tyr\text{-}Val]_2$-OC	115	103	18	20
$[Gly\text{-}Tyr\text{-}Val]_2$-OC	92	99	56	54
$[Ile\text{-}Tyr\text{-}Val]_2$-OC	71	82	3	4
$[Leu\text{-}Tyr\text{-}Val]_2$-OC	131	120	4	5

OC = Oxycodone

[511] Collectively, examples 33 through 118 illustrate the application of the invention for reducing the overdose potential of narcotic analgesics. These examples establish that an active agent can be covalently modified by attachment of a chemical moiety in a manner that maintains therapeutic value over a normal dosing range, while substantially decreasing if not eliminating the possibility of overdose by oral, intranasal, or intravenous routes of administration with the active agent.

Claims:

1. A method of preventing overdose comprising administering to an individual a pharmaceutical which has been covalently bound to a chemical moiety.
2. A method of safely delivering a pharmaceutical comprising providing a therapeutically effective amount of said pharmaceutical which has been covalently bound to a chemical moiety wherein said chemical moiety reduces the rate of absorption or extent of bioavailability of the pharmaceutical as compared to delivering the unbound pharmaceutical.
3. A method of reducing drug toxicity comprising providing a patient with a pharmaceutical which has been covalently bound to a chemical moiety wherein said chemical moiety increases the rate of clearance of a pharmacologically active agent when given at doses exceeding those within the therapeutic range of said active agent.
4. A method of reducing drug toxicity comprising providing a patient with a pharmaceutical which has been covalently bound to a chemical moiety wherein said chemical moiety provides a serum release curve which does not increase above said pharmaceutical toxicity level when given at doses exceeding those within the therapeutic range of said active agent.
5. A method of reducing bioavailability of a pharmaceutical comprising providing a pharmaceutical covalently bound to a chemical moiety wherein said bound pharmaceutical maintains a steady-state serum release curve which provides a therapeutically effective bioavailability but prevents spiking or increase blood serum concentrations compared to unbound pharmaceutical when given at doses exceeding those within the therapeutic range of said active agent.
6. A method of preventing a C_{max} spike for a pharmaceutical while still providing a therapeutically effective bioavailability curve comprising providing a pharmaceutical which has been covalently bound to a chemical moiety.
7. A method for preventing a toxic release profile in a patient comprising administering to a patient a pharmaceutical covalently bound to a chemical

moiety wherein said bound pharmaceutical maintains a steady-state serum release curve which provides a therapeutically effective bioavailability but prevents spiking or increase blood serum concentrations compared to unbound pharmaceutical.

8. The method of any of the preceding claims wherein the toxicity of the compound is substantially lower than that of the unbound pharmaceutical.
9. The method of any of the preceding claims wherein said covalently bound chemical moiety reduces or eliminates the possibility of overdose by oral administration.
10. The method of any of the preceding claims wherein said covalently bound chemical moiety reduces or eliminates the possibility of overdose by intranasal administration.
11. The method of any of the preceding claims wherein said covalently bound chemical moiety reduces or eliminates the possibility of overdose by injection.
12. The method of any of the preceding claims wherein the chemical moiety is a single amino acid, a dipeptide, a tripeptide, a short chain of amino acids, a polypeptide, a carbohydrate, a glycopeptide, or a vitamin.
13. The method of any of the preceding claims wherein said covalently bound chemical moiety is comprised of an amino acid.
14. The method of any of the preceding claims, wherein the pharmaceutical carrier is GluGluPhePheIle, GluGluPhePhePhe, TyrTyrIle, AspAspIle, TyrTyrPhePheIle, or GluGluPhePheIle.
15. The method of any of the preceding claims wherein said covalently bound chemical moiety is comprised of a peptide of two or more amino acids.
16. The method of any of the preceding claims wherein the pharmaceutical carrier is Lys.
17. The method of any of the preceding claims wherein said covalently bound chemical moiety is comprised of a glycopeptide.
18. The method of any of the preceding claims wherein said covalently bound chemical moiety is comprised of a carbohydrate.

19. The method of any of the preceding claims, wherein the pharmaceutical is a stimulant, an anticonvulsant, a muscle relaxant, an antidepressant, an anxiolytic, a sedative, a hypnotic, a narcotic, a respiratory agent, an antipsychotic, or a nonsteroidal anti-inflammatory agent.
20. The method of any of one of claims 1-13, 15, 16 or 19 wherein said active agent is a stimulant.
21. The method of claim 20 wherein said stimulant is an amphetamine.
22. The method of claim 21 wherein said amphetamine is dextroamphetamine or methylphenidate.
23. The method of claim 21, wherein the carrier is lysine.
24. The method of claim 23 wherein amphetamine is covalently bound to a single lysine.
25. The method of claim 23 wherein said lysine is bound to an additional amino acid.
26. The method of claims 1-19 wherein said active agent is a narcotic.
27. The method of claim 26, wherein the narcotic is codeine, hydrocodone, methadone, oxycodone, or propoxyphene.
28. The method of claim 27 wherein hydrocodone is covalently bound to a short chain of amino acids.
29. The method of claim 28, wherein the short chain of amino acids is GluGluPhePheIle, GluGluPhePhePhe, TyrTyrPhePheIle, GluGluPhePheIle, TyrTyrIle, or AspAspIle.
30. The method of claim 27 wherein oxycodone is covalently bound to a short chain of amino acids.
31. The method of claim 30, wherein the short chain of amino acids is GluGluPhePheIle, GluGluPhePhePhe, TyrTyrPhePheIle, GluGluPhePheIle, TyrTyrIle, or AspAspIle.
32. The method of claims 1-7, wherein the bioavailability curve provided is similar to those of Figures 1-29 when tested in rats.
33. The method of claims 1-7, wherein the bioavailability curve is provided is similar to those of Figures 1-29.

34. A composition for preventing overdose comprising a pharmaceutical which has been covalently bound to a chemical moiety.

35. A composition for safely delivering a pharmaceutical comprising providing a therapeutically effective amount of said pharmaceutical which has been covalently bound to a chemical moiety wherein said chemical moiety reduces the rate of absorption of the pharmaceutical as compared to delivering the unbound pharmaceutical.

36. A composition for reducing drug toxicity comprising providing a patient with a pharmaceutical which has been covalently bound to a chemical moiety wherein said chemical moiety increases the rate of clearance of a pharmacologically active agent when given at doses exceeding those within the therapeutic range of said active agent.

37. A composition for reducing drug toxicity comprising providing a patient with a pharmaceutical which has been covalently bound to a chemical moiety wherein said chemical moiety provides a serum release curve which does not increase above said pharmaceutical toxicity level when given at doses exceeding those within the therapeutic range of said active agent.

38. A composition for reducing bioavailability of a pharmaceutical comprising a pharmaceutical covalently bound to a chemical moiety wherein said bound pharmaceutical maintains a steady-state serum release curve which provides a therapeutically effective bioavailability but prevents spiking or increase blood serum concentrations compared to unbound pharmaceutical when given at doses exceeding those within the therapeutic range of said active agent.

39. A composition for preventing a C_{max} spike for a pharmaceutical while still providing a therapeutically effective bioavailability curve comprising a pharmaceutical which has been covalently bound to a chemical moiety.

40. A composition for preventing a toxic release profile in a patient comprising pharmaceutical covalently bound to a chemical moiety wherein said bound pharmaceutical maintains a steady-state serum release curve which provides

a therapeutically effective bioavailability but prevents spiking or increase blood serum concentrations compared to unbound pharmaceutical.

41. A method of treating attention deficit hyperactivity comprising administering to a patient a composition according to the methods of claims 34-40.

42. A method of treating attention deficit hyperactivity disorder (ADHD) comprising administering to a patient a composition according to the methods of claims 34-40.

43. A method of treating attention deficit disorder (ADD) comprising administering to a patient a composition according to the methods of claims 34-40.

44. A method of treating cognitive decline associated with acquired immunodeficiency syndrome (AIDS) or AIDS-related complex comprising administering to a patient a composition according to the methods of claims 34-40.

45. A method of treating depression comprising administering to a patient a composition according to the methods of claims 34-40.

46. A method of treating anxiety and anxiety related disorders comprising administering to a patient a composition according to the methods of claims 34-40.

47. A method of treating psychosis comprising administering to a patient a composition according to the methods of claims 34-40.

48. A method of treating nicotine addiction comprising administering to a patient a composition according to the methods of claims 34-40.

49. A method of treating narcotic addiction comprising administering to a patient a composition according to the methods of claims 34-40.

50. A method of treating alcoholism comprising administering to a patient a composition according to the methods of claims 34-40.

51. A method of treating narcolepsy comprising administering to a patient a composition according to the methods of claims 34-40.

52. A method of providing analgesia comprising administering to a patient a composition according to the methods of claims 34-40.

53. A composition comprising a stimulant covalently bound to an amino acid.
54. The composition of claim 53, wherein said amino acid is lysine.
55. The composition claim 54 wherein said stimulant is amphetamine
56. The composition of claim 55, wherein said amphetamine is dextroamphetamine or methylphenidate.
57. The composition of claim 55, wherein the pharmaceutical is amphetamine and the carrier is lysine.
58. The composition of claim 57 wherein said lysine is bound to additional amino acids.
59. The composition of claim 58 wherein the lysine is a chain less than five lysines.
60. The composition of claim 59 wherein a single lysine is bound to amphetamine.
61. The composition comprising a narcotic covalently bound to a chemical moiety.
62. The composition of claim 61, wherein the narcotic is codeine, hydrocodone, methadone, oxycodone, or propoxyphene.
63. A composition of claim 62 comprising hydrocodone covalently bound to a short chain of amino acids.
64. A composition of claim 62 wherein comprising oxycodone covalently bound to a short chain of amino acids.
65. The composition of claim 63 or 64, wherein the short chain of amino acids is GluGluPhePhePheIle, GluGluPhePhePhe, TyrTyrIle, AspAspIle, TyrTyrPhePheIle, GluGluPhePheIle, TyrTyrIle, or AspAspIle.
66. A composition comprising an active agent covalently bound to a chemical moiety.
67. A pharmaceutical composition of claim 66 wherein said covalently bound chemical moiety reduces or eliminates the possibility of overdose.
68. A pharmaceutical composition of claim 66 wherein said active agent is therapeutically effective when delivered at the proper dosage.

69. A pharmaceutical composition of claim 66 wherein said covalently bound chemical moiety reduces the rate of absorption or extent of bioavailability of a pharmacologically active agent when given at doses exceeding those within the therapeutic range of said active agent.

70. A pharmaceutical composition of claim 66 wherein said covalently bound chemical moiety increases the rate of clearance of a pharmacologically active agent when given at doses exceeding those within the therapeutic range of said active agent.

71. A pharmaceutical composition of claim 66 wherein said covalently bound chemical moiety reduces the bioavailability of a pharmacologically active agent when given at doses exceeding those within the therapeutic range of said active agent.

72. A pharmaceutical composition of claim 66 wherein the toxicity of the compound is substantially lower than that of the said active agent.

73. A pharmaceutical composition of claims 66-72 wherein said covalently bound chemical moiety reduces or eliminates the possibility of overdose by oral administration.

74. A pharmaceutical composition of claims 66-72 wherein said covalently bound chemical moiety reduces or eliminates the possibility of overdose by intranasal administration.

75. A pharmaceutical composition of claims 66-72 wherein said covalently bound chemical moiety reduces or eliminates the possibility of overdose by injection.

76. A pharmaceutical composition of claims 66-72 wherein said covalently bound chemical moiety is comprised of an amino acid.

77. A pharmaceutical composition of claims 66-72 wherein said covalently bound chemical moiety is comprised of two or more amino acids.

78. A pharmaceutical composition of claims 66-72 wherein said covalently bound chemical moiety is comprised of a glycopeptide.

79. A pharmaceutical composition of claims 66-72 wherein said covalently bound chemical moiety is comprised of a carbohydrate.

80. A pharmaceutical composition of claims 66-72 wherein said active agent is a stimulant.

81. A pharmaceutical composition of claim 80 wherein said stimulant is an amphetamine.

82. The pharmaceutical composition of 81 wherein said amphetamine is dextroamphetamine or methylphenidate.

83. A pharmaceutical composition of claims 66-79 wherein said active agent is a narcotic analgesic.

84. A pharmaceutical composition of claim 83 wherein said narcotic is hydrocodone, oxycodone, morphine, codeine, hydroxymorphone, oxymorphone, methadone, fentanyl, levorphanol, dihydrocodeine, meperidine, diphenoxylate, sufentanil, alfentanil, propoxyphene, pentazocine, nalbuphine, butorphanol, buprenorphine, meptazinol, dezocine or pharmaceutically acceptable salts thereof.

85. A pharmaceutical composition of claims 66-79 wherein said active agent is a benzodiazepine.

86. A pharmaceutical composition of claim 85 wherein said benzodiazepine is alprazolam, chlordiazepoxide, clonazepam, clorazepate, diazepam, estazolam, flurazepam, halazepam, lorazepam, midazolam, oxazepam, quazepam, temazepam, or triazolam.

87. A pharmaceutical composition of claims 66-79 wherein said active agent is a nonsteroidal anti-inflammatory agent.

88. A pharmaceutical composition of claim 87 wherein said nonsteroidal anti-inflammatory agent is ibuprofen, naproxen or indomethacin.

89. A pharmaceutical composition of claims 66-79 wherein said active agent is aspirin or a salicylic acid derivative.

90. A pharmaceutical composition of claims 66-79 wherein said active agent is acetaminophen.

91. A pharmaceutical composition of claims 66-79 wherein said active agent is an anti-depressant.

92. A pharmaceutical composition of claim 91 wherein said anti-depressant is citalopram, fluoxetine, norfluoxetine, fluvoxamine, paroxetine, sertraline, amitriptyline, desipramine, doxepin, imipramine, nortryiptyline, bupropion, mirtazapine, nefazodone, trazodone, or venlafaxine.

93. A pharmaceutical composition of claims 66-79 wherein said active agent is an anti-psychotic.

94. A pharmaceutical composition of claim 93 wherein said anti-psychotic is clozapine, haloperidol, olanzapine, quetiapine, or risperidone.

95. A composition of claims 12-13 wherein the said amino acid or peptide is comprised of one or more of the naturally occurring (L-) amino acids: alanine, arginine, asparagine, aspartic acid, cysteine, glycine, glutamic acid, glutamine, histidine, isoleucine, leucine, lysine, methionine, proline, phenylalanine, serine, tryptophan, threonine, tyrosine, and valine.

96. A composition of claims 12-13 wherein the said amino acid or peptide is comprised of one or more of the naturally occurring (D-) amino acids: alanine, arginine, asparagine, aspartic acid, cysteine, glycine, glutamic acid, glutamine, histidine, isoleucine, leucine, lysine, methionine, proline, phenylalanine, serine, tryptophan, threonine, tyrosine, and valine.

97. A composition of claims 12-13 wherein the said amino acid or peptide is comprised of one or more unnatural, non-standard or synthetic amino acids, including: aminohexanoic acid, biphenylalanine, cyclohexylalanine, cyclohexylglycine, diethylglycine, dipropylglycine, 2,3-diaminoproprionic acid, homophenylalanine, homoserine, homotyrosine, naphthylalanine, norleucine, ornithine, pheylalanine(4-fluoro), phenylalanine(2,3,4,5,6 pentafluoro), phenylalanine(4-nitro), phenylglycine, pipecolic acid, sarcosine,
tetrahydroisoquinoline-3-carboxylic acid, and tert-leucine.

98. A composition of claims 12-13 wherein the said amino acid or peptide is comprised of one or more amino acid alcohols.

99. A composition of claims 12-13 wherein the said amino acid or peptide is comprised of one or more N-methyl amino acids.

100. A composition of claims 95-99 wherein the said peptide is comprised of a mixture of any of the said amino acids.

101. A method of claims 1-13 wherein the solubility and dissolution rate of the composition is substantially changed under physiological conditions encountered in the intestine, at mucosal surfaces, or in the bloodstream.

102. A method of claim 101 wherein the said change in solubility and dissolution rate substantially decrease the bioavailability of the said pharmaceutical, particularly at doses above those intended for therapy.

103. A method of claim 102 wherein said decrease in bioavailability occurs upon oral administration.

104. A method of claim 102 wherein said decrease in bioavailability occurs upon intranasal administration.

105. A method of claim 102 wherein said decrease in bioavailability occurs upon intravenous administration.

106. A method for reducing or preventing abuse of a pharmaceutical composition, comprising providing said composition to a human in need thereof, wherein said composition comprises a chemical moiety covalently attached to an active agent such that the pharmacological activity of the active agent is substantially decreased when the composition is used in a manner inconsistent with the manufacturer's instructions.

107. A method for reducing or preventing abuse of a pharmaceutical composition, comprising administering said composition to a human in need thereof, wherein said composition comprises a chemical moiety covalently attached to an active agent such that the pharmacological activity of the active agent is substantially decreased when the composition is used in a manner inconsistent with the manufacturer's instructions.

108. A method for reducing or preventing abuse of a pharmaceutical composition, comprising prescribing said composition to a human in need thereof, wherein said composition comprises a chemical moiety covalently attached to an active agent such that the pharmacological activity of the active agent is

substantially decreased when the composition is used in a manner inconsistent with the manufacturer's instructions.

109. A method for reducing or preventing abuse of a pharmaceutical composition, comprising consuming said composition, wherein said composition comprises a chemical moiety covalently attached to an active agent such that the pharmacological activity of the active agent is substantially decreased when the composition is used in a manner inconsistent with the manufacturer's instructions.

110. A method of preventing overdose of a pharmaceutical composition, comprising providing said pharmaceutical composition to a human in need thereof, wherein said composition comprises a chemical moiety covalently attached to an active agent in a manner that substantially decreases the potential of overdose from the active agent.

111. A method of preventing overdose of a pharmaceutical composition, comprising administering said pharmaceutical composition to a human in need thereof, wherein said composition comprises a chemical moiety covalently attached to an active agent in a manner that substantially decreases the potential of overdose from the active agent.

112. A method of preventing overdose of a pharmaceutical composition, comprising prescribing said pharmaceutical composition to a human in need thereof, wherein said composition comprises a chemical moiety covalently attached to an active agent in a manner that substantially decreases the potential of overdose from the active agent.

113. A method of preventing overdose of a pharmaceutical composition, comprising consuming said pharmaceutical composition, wherein said composition comprises a chemical moiety covalently attached to an active agent in a manner that substantially decreases the potential of overdose from the active agent.

114. A method for reducing or preventing the euphoric effect of a pharmaceutical composition, comprising providing said composition to a human in need thereof, wherein said composition comprises a chemical moiety covalently

attached to an active agent such that the pharmacological activity of the active agent is substantially decreased when the composition is used in a manner inconsistent with the manufacturer's instructions.

115. A method for reducing or preventing the euphoric effect of a pharmaceutical composition, comprising administering said composition to a human in need thereof, wherein said composition comprises a chemical moiety covalently attached to an active agent such that the pharmacological activity of the active agent is substantially decreased when the composition is used in a manner inconsistent with the manufacturer's instructions.

116. A method for reducing or preventing the euphoric effect of a pharmaceutical composition, comprising prescribing said composition to a human in need thereof, wherein said composition comprises a chemical moiety covalently attached to an active agent such that the pharmacological activity of the active agent is substantially decreased when the composition is used in a manner inconsistent with the manufacturer's instructions.

117. A method for reducing or preventing the euphoric effect of a pharmaceutical composition, comprising consuming said composition, wherein said composition comprises a chemical moiety covalently attached to an active agent such that the pharmacological activity of the active agent is substantially decreased when the composition is used in a manner inconsistent with the manufacturer's instructions.

118. The method of claims 43-54, wherein said pharmaceutical composition is adapted for oral administration, and wherein said active agent is resistant to release from said chemical moiety when the composition is administered parenterally, such as intranasally or intravenously.

119. The method of claims 55, wherein said active agent is released from said chemical moiety in the presence of acid and/or enzymes present in the stomach, intestinal tract, or blood serum.

120. The method of claim 56, wherein said composition is the form of a tablet, capsule, oral solution, or oral suspension.

121. The method of claims 43-54, wherein said chemical moiety is an amino acid, oligopeptide, polypeptide, carbohydrate, glycopeptide, nucleic acid, or vitamin.

122. The method of claim 58, wherein said chemical moiety is an amino acid, oligopeptide, or polypeptide.

123. The method of claim 59, wherein said polypeptide comprises fewer than 70 amino acids.

124. The method of claims 60, wherein said polypeptide comprises fewer than 50 amino acids.

125. The method of claim 61, wherein said polypeptide comprises fewer than 10 amino acids.

126. The method of claim 62, wherein said polypeptide comprises fewer than 6 amino acids.

127. The method of claim 59, wherein said polypeptide comprises the amino acid sequence Glu-Glu-Phe-Phe-Ile.

128. The method of claim 59, wherein said polypeptide comprises the amino acid sequence Glu-Glu-Phe-Phe-Phe.

129. The method of claim 59, wherein said polypeptide comprises the amino acid sequence Tyr-Tyr-Ile.

130. The method of claim 59, wherein said polypeptide comprises the amino acid sequence Asp-Asp-Ile.

131. The method of claim 59, wherein said polypeptide comprises the amino acid sequence Tyr-Tyr-Phe-Phe-Ile.

132. The method of claim 59, wherein said polypeptide comprises the amino acid sequence Glu-Glu-Phe-Phe-Ile.

133. The method of claim 59, wherein said polypeptide comprises the amino acid sequence Tyr-Tyr-Ile.

134. The method of claims 43-54, wherein said covalent attachment comprises an ester or carbonate bond.

135. The method of claims 43-54, wherein said covalent attachment comprises a ketone and/or a hydroxyl functionality.

136. The method of claims 43-54, wherein said composition yields a therapeutic effect without substantial euphoria.

137. The method of claim 73, wherein said active agent provides a therapeutically bioequivalent AUC when compared to active agent alone but does provide a C_{max} which results in euphoria.

138. A method for reducing or preventing abuse of a pharmaceutical composition, comprising orally administering said composition to a human in need thereof, wherein said composition comprises a peptide covalently attached to hydrocodone such that the pharmacological activity of hydrocodone is substantially decreased when the composition is used in a manner inconsistent with the manufacturer's instructions.

139. A method of preventing overdose of a pharmaceutical composition, comprising orally administering said pharmaceutical composition to a human in need thereof, wherein said composition comprises a peptide covalently attached to hydrocodone in a manner that substantially decreases the potential of hydrocodone to result in overdose.

140. A method for reducing or preventing the euphoric effect of a pharmaceutical composition, comprising orally administering said composition to a human in need thereof, wherein said composition comprises a peptide covalently attached to hydrocodone such that the pharmacological activity of hydrocodone is substantially decreased when the composition is used in a manner inconsistent with the manufacturer's instructions.

141. A method for reducing or preventing abuse of a pharmaceutical composition, comprising orally administering said composition to a human in need thereof, wherein said composition comprises a peptide covalently attached to oxycodone such that the pharmacological activity of oxycodone is substantially decreased when the composition is used in a manner inconsistent with the manufacturer's instructions.

142. A method of preventing overdose of a pharmaceutical composition, comprising orally administering said pharmaceutical composition to a human in need thereof, wherein said composition comprises a peptide covalently

attached to oxycodone in a manner that substantially decreases the potential of oxycodone to result in overdose.

143. A method for reducing or preventing the euphoric effect of a pharmaceutical composition, comprising orally administering said composition to a human in need thereof, wherein said composition comprises a peptide covalently attached to oxycodone such that the pharmacological activity of oxycodone is substantially decreased when the composition is used in a manner inconsistent with the manufacturer's instructions.

144. A method for reducing or preventing abuse of a pharmaceutical composition, comprising orally administering said composition to a human in need thereof, wherein said composition comprises a peptide covalently attached to amphetamine such that the pharmacological activity of amphetamine is substantially decreased when the composition is used in a manner inconsistent with the manufacturer's instructions.

145. A method of preventing overdose of a pharmaceutical composition, comprising orally administering said pharmaceutical composition to a human in need thereof, wherein said composition comprises a peptide covalently attached to amphetamine in a manner that substantially decreases the potential of amphetamine to result in overdose.

146. A method for reducing or preventing the euphoric effect of a pharmaceutical composition, comprising orally administering said composition to a human in need thereof, wherein said composition comprises a peptide covalently attached to amphetamine such that the pharmacological activity of amphetamine is substantially decreased when the composition is used in a manner inconsistent with the manufacturer's instructions.

147. The method of claims 138-146, wherein said peptide comprises the amino acid sequence selected from the group consisting of Lys, Ser, Phe, Glu-Glu-Phe-Phe-Ile, Glu-Glu-Phe-Phe-Phe, Tyr-Tyr-Ile, Asp-Asp-Ile, Tyr-Tyr-Phe-Phe-Ile, Glu-Glu-Phe-Phe-Ile, or Tyr-Tyr-Ile.

148. The method of claim 147, wherein said peptide comprises the amino acid sequence Lys.

149. The method of claim 147, wherein said peptide comprises the amino acid sequence Tyr-Tyr-Phe-Phe-Ile.

150. The method of claim 147, wherein said peptide comprises the amino acid sequence Phe-Phe-Ile.

151. A compound comprising hydrocodone covalently attached to a peptide.

152. A compound comprising oxycodone covalently attached to a peptide.

153. A compound comprising amphetamine covalently attached to a peptide.

154. A compound comprising hydrocodone covalently attached to a peptide comprising the amino acid sequence Tyr-Tyr-Phe-Phe-Ile.

155. A compound comprising oxycodone covalently attached to a peptidecomprising the amino acid sequence Phe-Phe-Ile.

156. A compound comprising amphetamine covalently attached to Lys.

157. A method of treating acute or chronic pain comprising administering to a patient a composition of claims 34-100.

158. A method of treating acute or chronic pain comprising administering to a patient a compound of claims 151-156.

SEQUENCE LISTING

<110> MICKLE, Travis
KRISHNAN, Suma
MONCRIEF, James Scott
LAUDERBACK, Christopher
MILLER, Christal

<120> Pharmaceutical Compositions for Prevention of Overdose or Abuse

<130> 54719.000106

<140> PCT/US04/32131
<141> 2004-09-30

<150> 60/507,012
<151> 2003-09-30

<150> 60/567,800
<151> 2004-05-05

<150> 60/567,802
<151> 2004-05-05

<150> 60/568,011
<151> 2004-05-05

<160> 26

<170> PatentIn version 3.2

<210> 1
<211> 5
<212> PRT
<213> artificial sequence

<220>
<223> synthetic carrier

<400> 1

Gly Gly Gly Gly Leu
1 5

<210> 2

<211> 6
<212> PRT
<213> artificial sequence

<220>
<223> synthetic carrier

<400> 2

Glu Glu Phe Phe Phe Ile
1 5

<210> 3
<211> 5
<212> PRT
<213> artificial sequence

<220>
<223> synthetic carrier

<400> 3

Glu Glu Phe Phe Phe
1 5

<210> 4
<211> 6
<212> PRT
<213> artificial sequence

<220>
<223> synthetic carrier

<400> 4

Gly Gly Lys Lys Gly Gly
1 5

<210> 5
<211> 5
<212> PRT
<213> artificial sequence

<220>
<223> synthetic carrier

<400> 5

Glu Glu Phe Phe Ile
1 5

<210> 6
<211> 5
<212> PRT
<213> artificial sequence

<220>
<223> synthetic carrier

<400> 6

Tyr Tyr Phe Phe Ile
1 5

<210> 7
<211> 5
<212> PRT
<213> artificial sequence

<220>
<223> synthetic carrier

<400> 7

Tyr Tyr Lys Tyr Tyr
1 5

<210> 8
<211> 5
<212> PRT
<213> artificial sequence

<220>
<223> synthetic carrier

<400> 8

Phe Phe Lys Phe Phe
1 5

<210> 9
<211> 4
<212> PRT
<213> artificial sequence

<220>
<223> synthetic carrier

<400> 9

Lys Lys Gly Gly
1

<210> 10
<211> 5
<212> PRT
<213> artificial sequence

<220>
<223> synthetic carrier

<400> 10

Tyr Tyr Phe Pro Ile
1 5

<210> 11
<211> 5
<212> PRT
<213> artificial sequence

<220>
<223> synthetic carrier

<400> 11

Glu Glu Glu Glu Glu
1 5

<210> 12
<211> 5
<212> PRT
<213> artificial sequence

<220>
<223> synthetic carrier

<400> 12

Glu Glu Gly Gly Ile
1 5

<210> 13
<211> 5
<212> PRT
<213> artificial sequence

<220>
<223> synthetic carrier

<400> 13

Glu Glu Gly Gly Leu
1 5

<210> 14
<211> 5
<212> PRT
<213> artificial sequence

<220>
<223> synthetic carrier

<400> 14

Gly Gly Gly Gly Ile
1 5

<210> 15
<211> 5
<212> PRT

<213> artificial sequence

<220>
<223> synthetic carrier

<400> 15

Lys Lys Gly Gly Ile
1 5

<210> 16
<211> 5
<212> PRT
<213> artificial sequence

<220>
<223> synthetic carrier

<400> 16

Lys Lys Pro Pro Ile
1 5

<210> 17
<211> 5
<212> PRT
<213> artificial sequence

<220>
<223> synthetic carrier

<400> 17

Tyr Tyr Gly Gly Ile
1 5

<210> 18
<211> 5
<212> PRT
<213> artificial sequence

<220>
<223> synthetic carrier

<400> 18

Gly Gly Pro Pro Ile
1 5

<210> 19
<211> 5
<212> PRT
<213> artificial sequence

<220>
<223> synthetic carrier

<400> 19

Asp Asp Phe Phe Ile
1 5

<210> 20
<211> 5
<212> PRT
<213> artificial sequence

<220>
<223> synthetic carrier

<400> 20

Glu Glu Asp Asp Ile
1 5

<210> 21
<211> 5
<212> PRT
<213> artificial sequence

<220>
<223> synthetic carrier

<400> 21

Lys Lys Asp Asp Ile

1 5

<210> 22
<211> 5
<212> PRT
<213> artificial sequence

<220>
<223> synthetic carrier

<400> 22

Tyr Tyr Glu Glu Ile
1 5

<210> 23
<211> 5
<212> PRT
<213> artificial sequence

<220>
<223> synthetic carrier

<400> 23

Asp Asp Asp Asp Ile
1 5

<210> 24
<211> 5
<212> PRT
<213> artificial sequence

<220>
<223> synthetic carrier

<400> 24

Lys Lys Glu Glu Ile
1 5