



[12] 发明专利申请公开说明书

[21] 申请号 02811464.7

[43] 公开日 2004 年 7 月 21 日

[11] 公开号 CN 1514843A

[22] 申请日 2002. 6. 7 [21] 申请号 02811464. 7

[30] 优先权

[32] 2001. 6. 8 [33] US [31] 60/297,059

[86] 国际申请 PCT/US2002/017859 2002. 6. 7

[87] 国际公布 WO2002/100888 英 2002. 12. 19

[85] 进入国家阶段日期 2003. 12. 8

[71] 申请人 研究及应用科学协会股份有限公司

地址 法国巴黎

[72] 发明人 迈克尔·D·卡勒 董正新

孙·H·金 雅克·皮埃尔·莫罗

[74] 专利代理机构 隆天国际知识产权代理有限公司

代理人 郑特强 杨淑媛

权利要求书 76 页 说明书 110 页

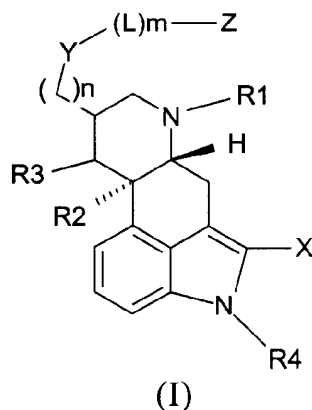
[54] 发明名称 生长激素释放抑制因子 - 多巴胺嵌合类似物

[57] 摘要

本发明公开了一系列的生长激素释放抑制因子 - 多巴胺嵌合体类似物, 其在活体内既保留了生长激素释放抑制因子的活性也保留了多巴胺的活性。一个例子是 6 - 正丙基 - 8 β - 麦角灵基甲基硫代乙酰基 - D - Phe - 环 [Cys - Tyr - D - Trp - Lys - Abu - Cys] - Thr - NH₂。

1. 式(I)化合物或其药学上可接受的盐,

5



其中:

X 是 H, Cl, Br, I, F, -CN 或 C₁₋₅ 烷基;

10 R1 是 H, C₁₋₄ 烷基, 烯丙基, 链烯基或 -CN;

R2 和 R3 分别独立地为 H 或不存在, 条件是当 R2 和 R3 不存在时, 它们所连接的炭原子之间有双键;

R4 是 H 或 -CH₃;

15 Y 是 -O-, -C(O)-, -S-, -S-(CH₂)_s-C(O)-, -S(O)-, -S(O)₂-, -SC(O)-, -OC(O)-, -N(R5)-C(O)- 或 -N(R6)-;

R5, R6, R7 和 R8 分别独立地为 H 或 C₁₋₅ 烷基;

R6 是 H 或 C₁₋₅ 烷基;

m 是 0 或 1;

n 是 0-10;

20 当 Y 是 -S-, -S(O)-, -S(O)₂-, -O- 或 -N(R6)- 时, L 是 -(CH₂)_p-C(O)-;

当 Y 是 -N(R6)-, -O- 或 -S- 时; L 是 -C(O)-(CR₇R₈)_q-C(O)-;

当 Y 是 -C(O)-, -SC(O)-, -OC(O)-, -S-(CH₂)_s-C(O)- 或 -N(R5)-C(O)- 时, L 是 -(Doc)_t-;

p 是 1-10;

25 q 是 2-4;

s 是 1-10;

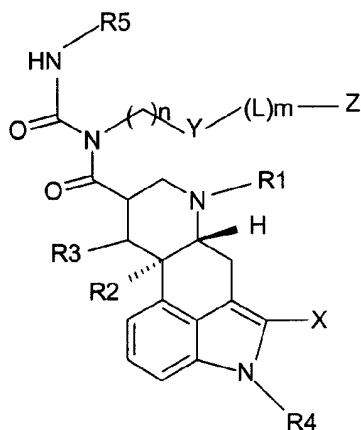
t 是 1-10; 且

Z 是生长激素释放因子类似物或含有 -H, -OH, (C₁-C₆)烷氧基, 芳基烷氧基, -NH₂ 或 -NR₉R₁₀, 其中 R₉ 和 R₁₀ 分别独立地为 H 或 C₁₋₅ 烷基。

2. 式(II)化合物或其药学上可接受的盐,

5

10



(II)

其中:

15

X 是 H, Cl, Br, I, F, -CN 或 C₁₋₅ 烷基;

R₁ 是 C₁₋₄ 烷基, H, 烯丙基, 链烯基或 -CN;

R₂ 和 R₃ 分别独立地为 H 或不存在, 条件是当 R₂ 和 R₃ 不存在时, 它们所连接的碳原子之间有双键;

R₄ 是 H 或 -CH₃;

20

R₅ 是 C₁₋₅ 烷基基团, 或式 -(CH₂)_rN(CH₃)_q 的基团;

Y 是 -O-, -C(O)-, -S-, -SC(O)-, -OC(O)-, -N(R₆)-C(O)-, -N(R₇)-, 或 -N(R₈)-(CH₂)_s-C(O)-;

R₆, R₇, R₈, R₉ 和 R₁₀ 分别独立地为 H 或 C₁₋₅ 烷基;

当 Y 是 -S-, -O- 或 -N(R₇)-时, L 是 -(CH₂)_p-C(O)-;

25

当 Y 是 -N(R₇)-, -O- 或 -S- 时, L 是 -(CO)-(CR₉R₁₀)_q-C(O)-;

当 Y 是 -C(O)-, -SC(O)-, -OC(O)-, -N(R₈)-(CH₂)_s-C(O)- 或 -N(R₆)-C(O)- 时, L 是 -(Doc)_t-;

m 是 0 或 1;

n 是 2-10;

q 是 2-4;

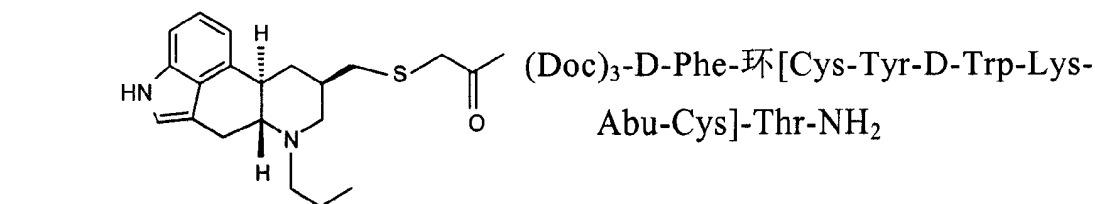
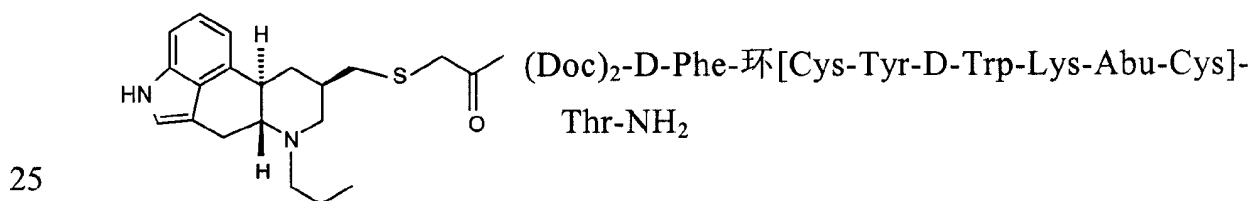
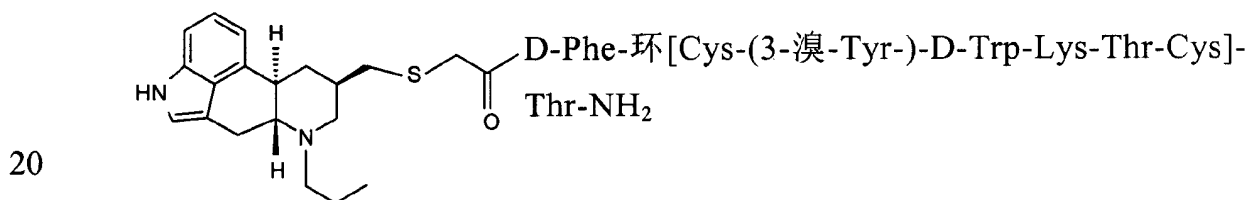
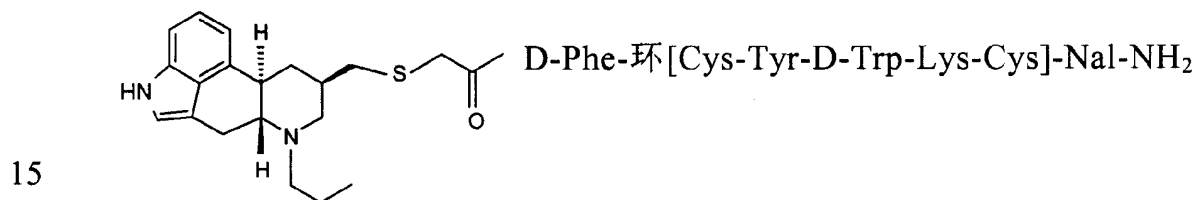
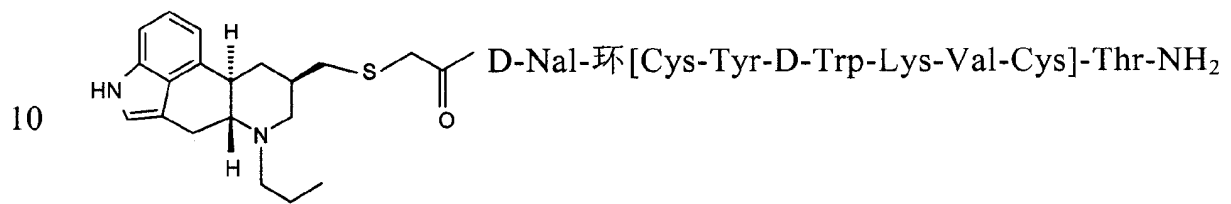
p 是 1-10;

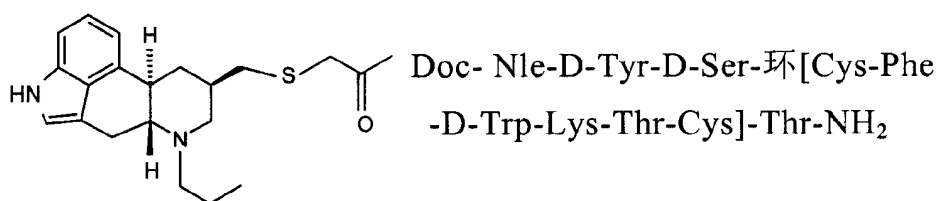
s 是 1-10;

t 是 1-10; 和

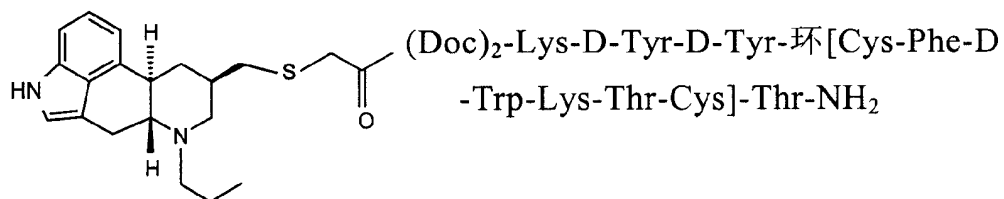
5 Z 是生长激素释放因子类似物或含有 -H, -OH, (C₁-C₆) 烷氧基, -NH₂ 或 -NR₉R₁₀。

3. 下式化合物或其药学上可接受的盐:

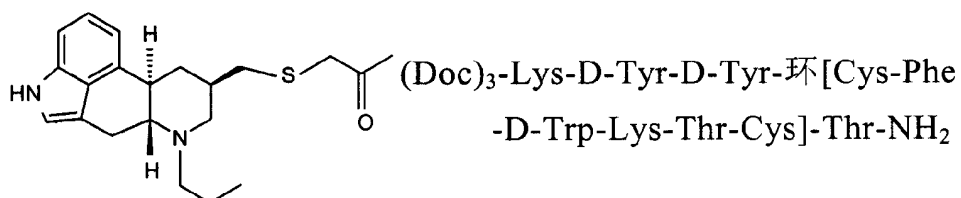




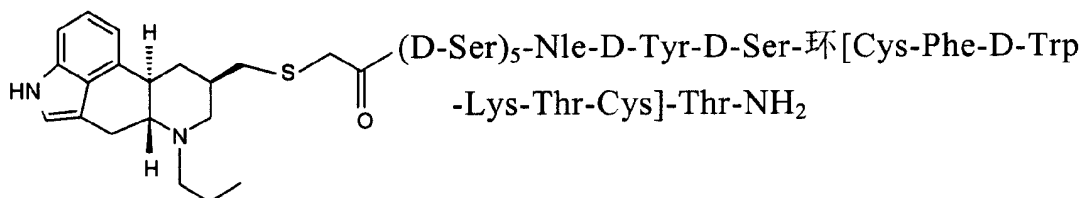
5



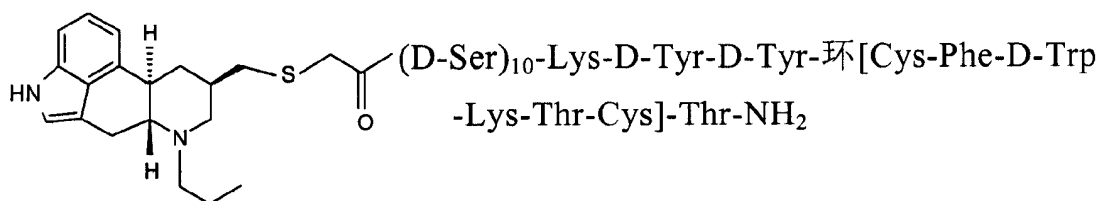
10



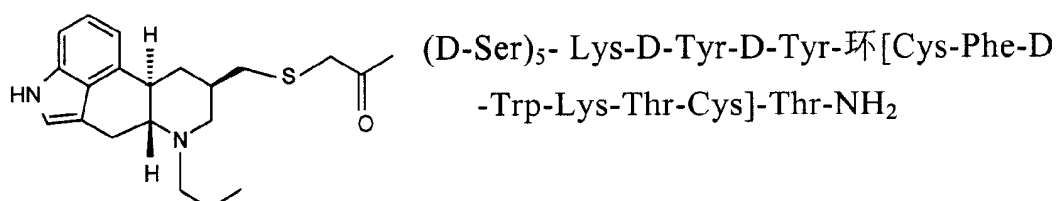
15



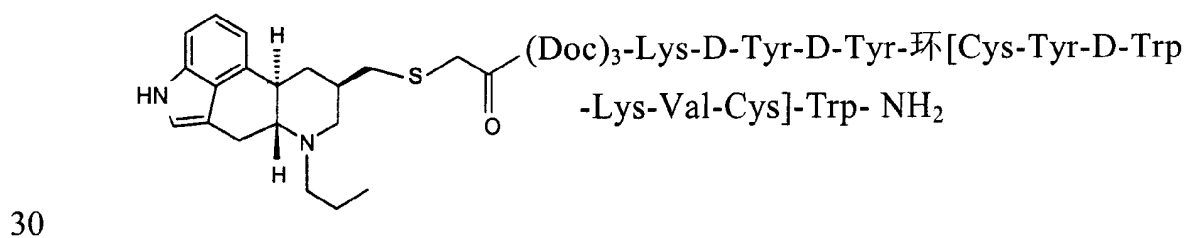
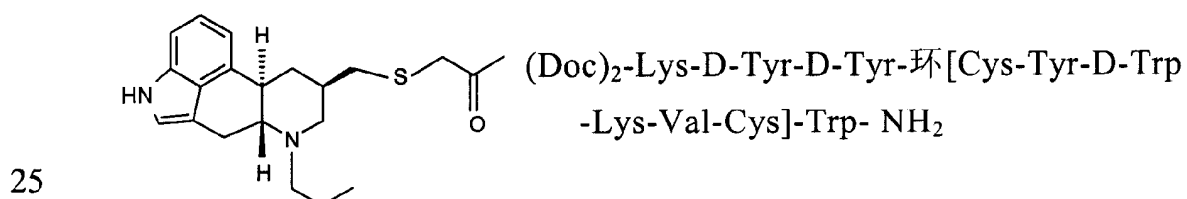
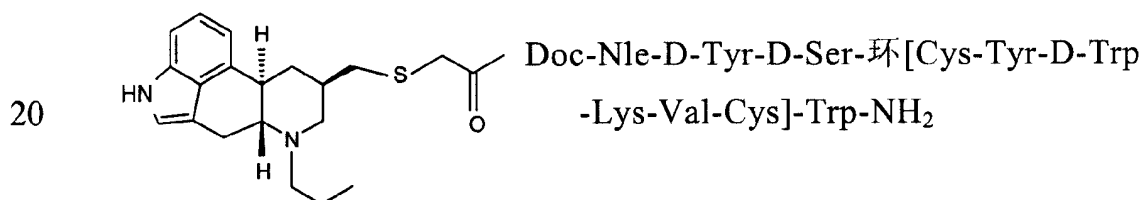
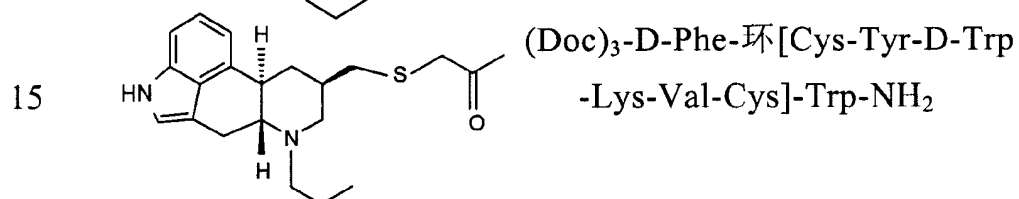
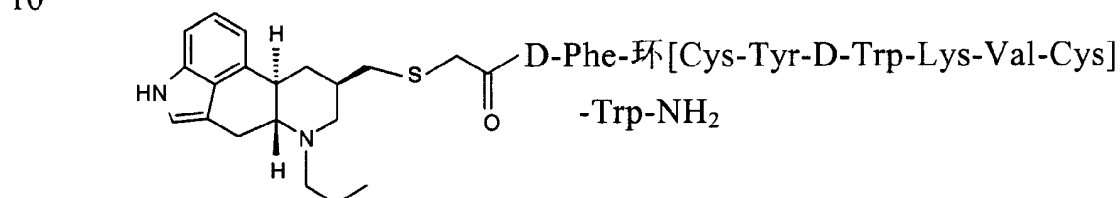
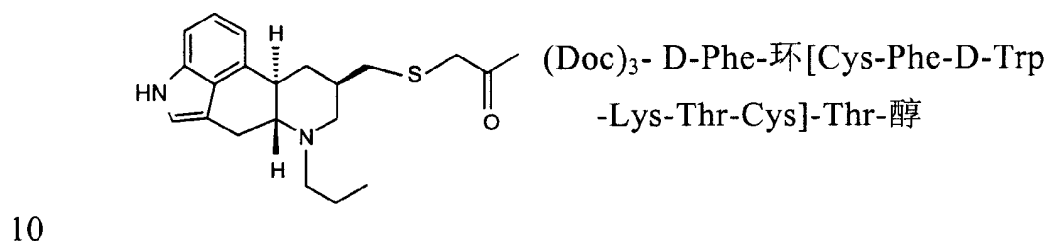
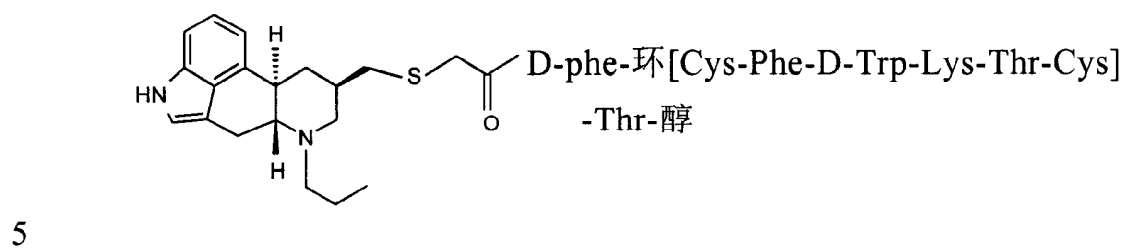
20

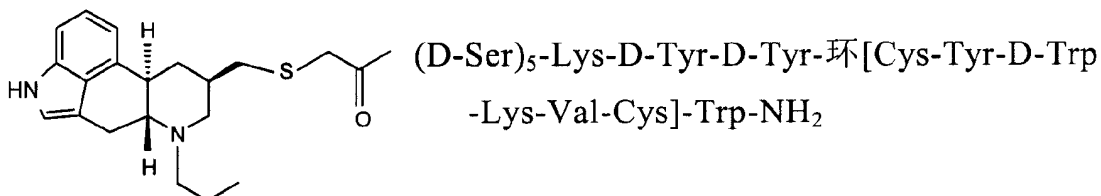
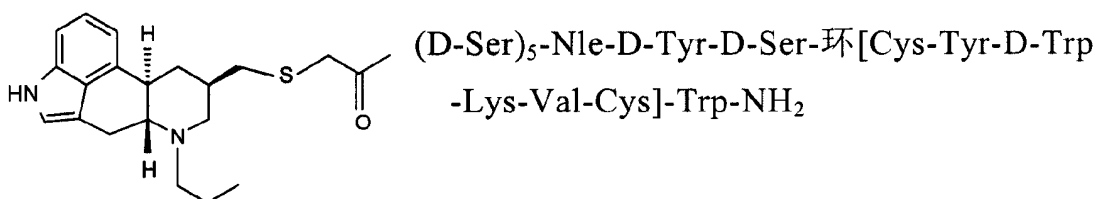


25

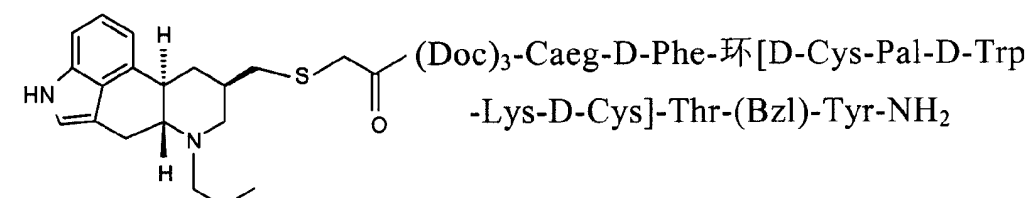
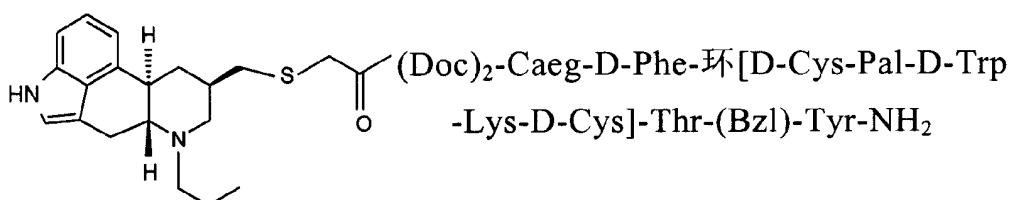
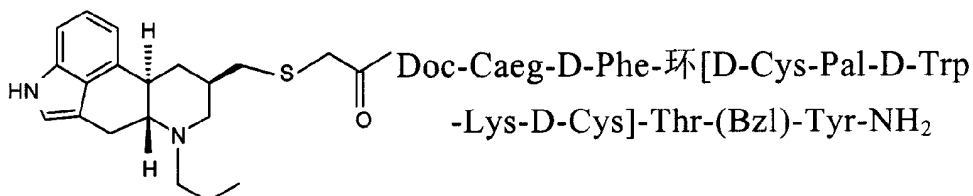
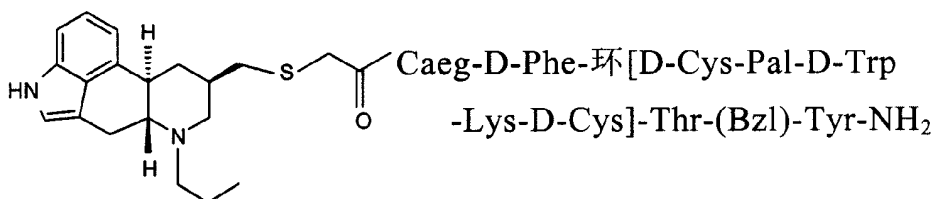


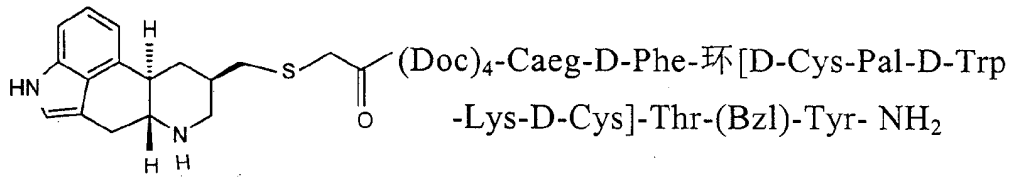
30



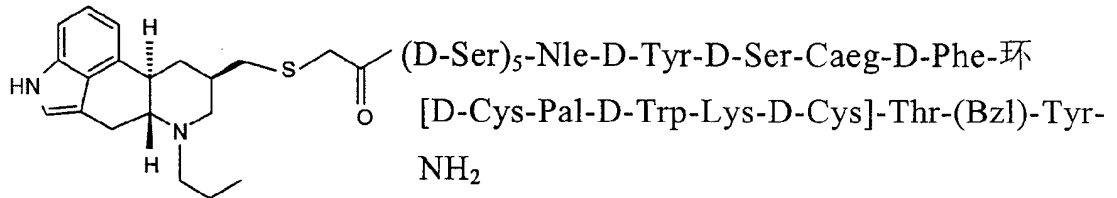


Caeg= N-(2-氨基乙基)-N-(2-胞嘧啶基-1-氧代-乙基)-甘氨酸

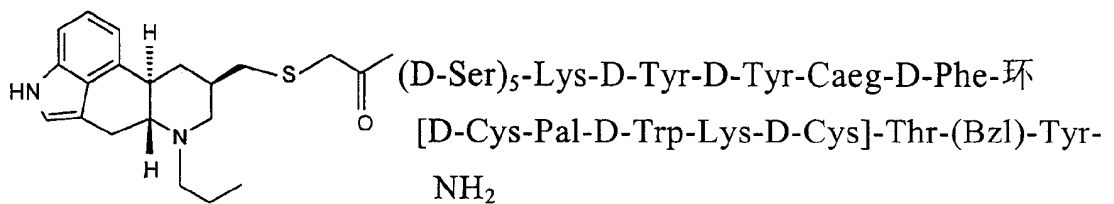




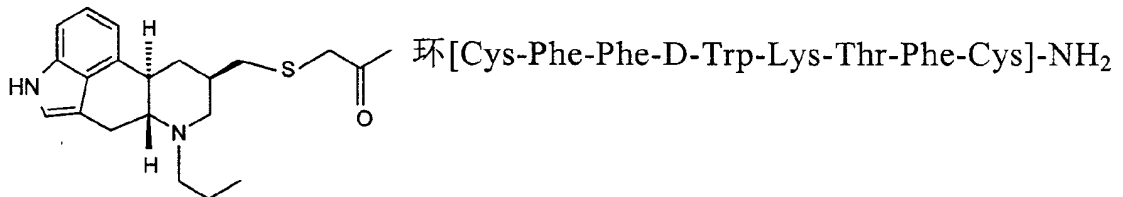
5



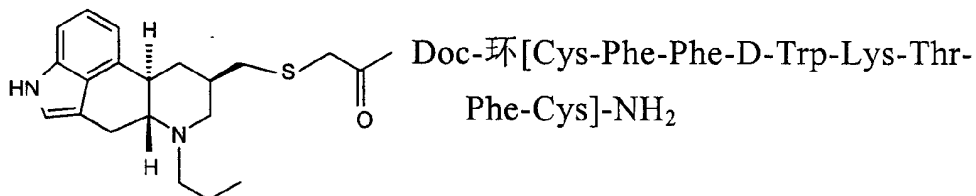
10



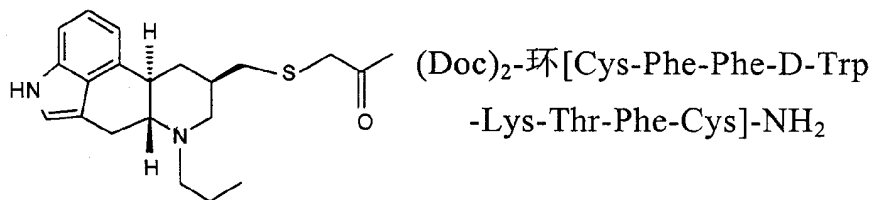
15



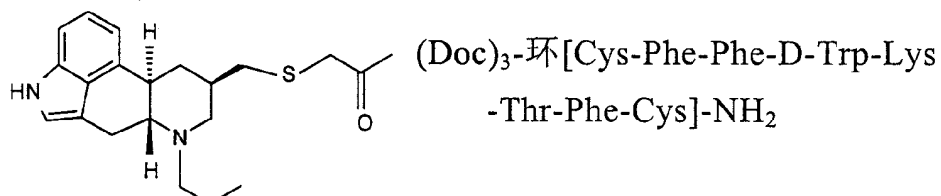
20

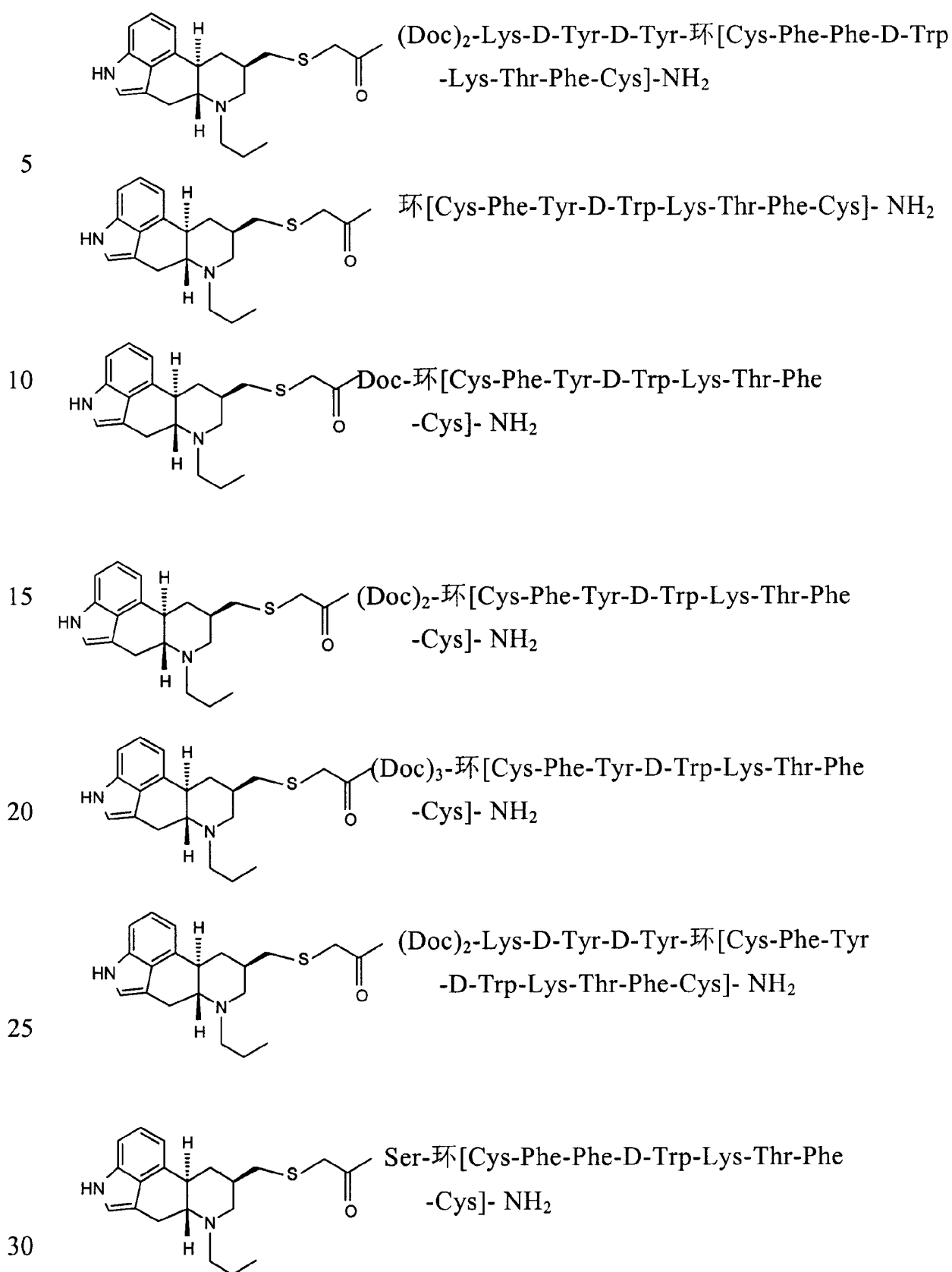


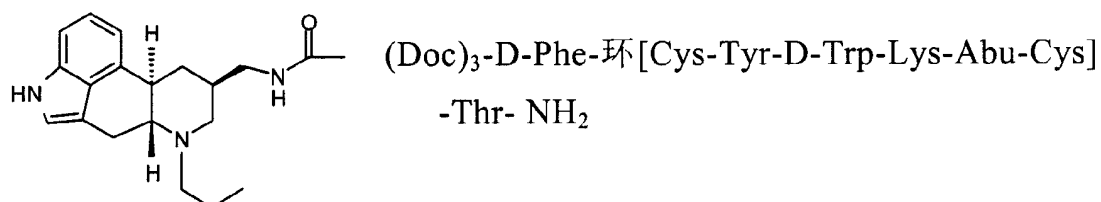
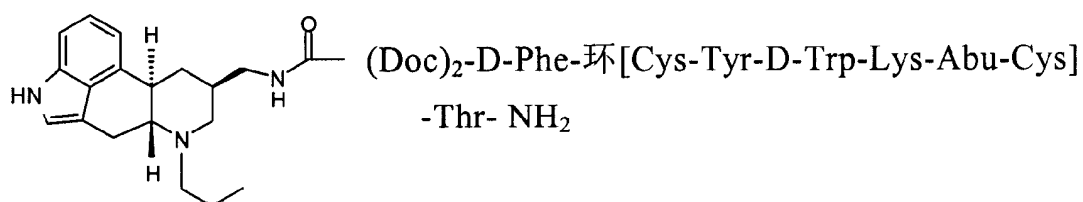
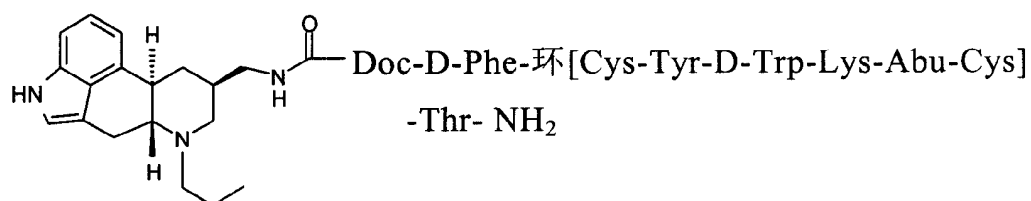
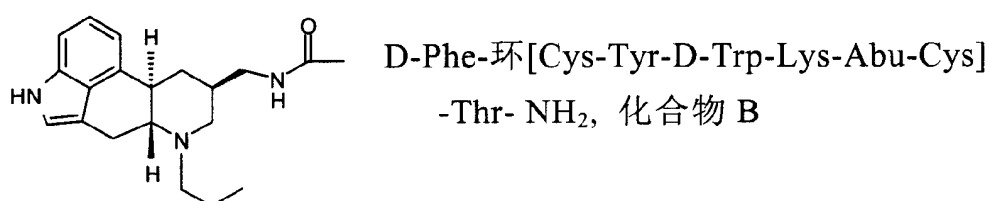
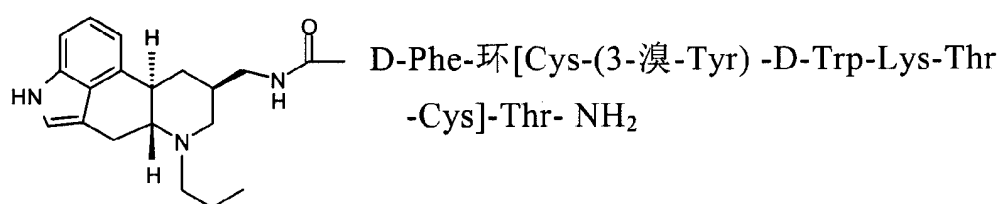
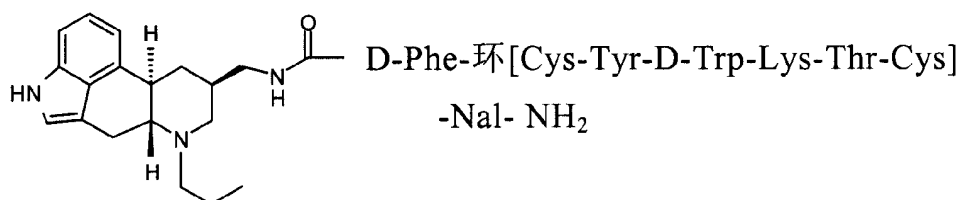
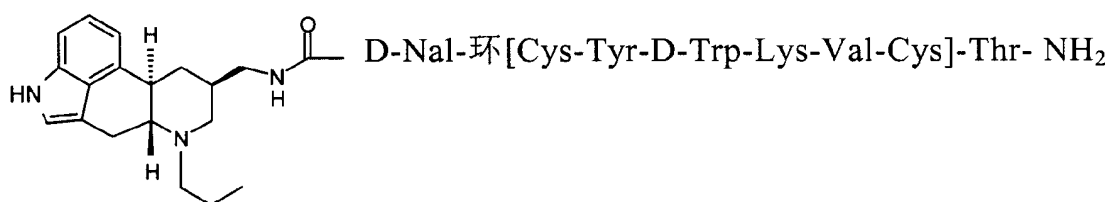
25

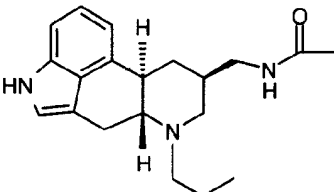
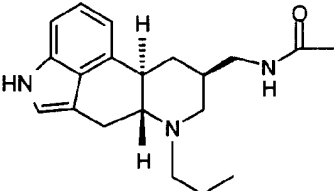
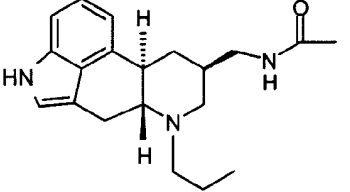
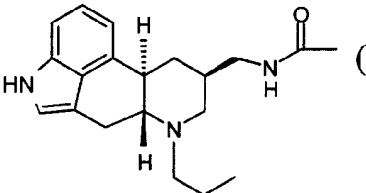
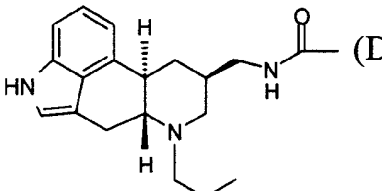
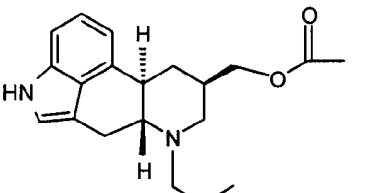
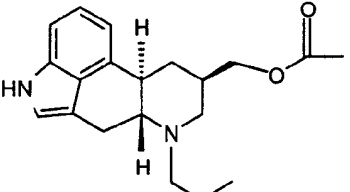


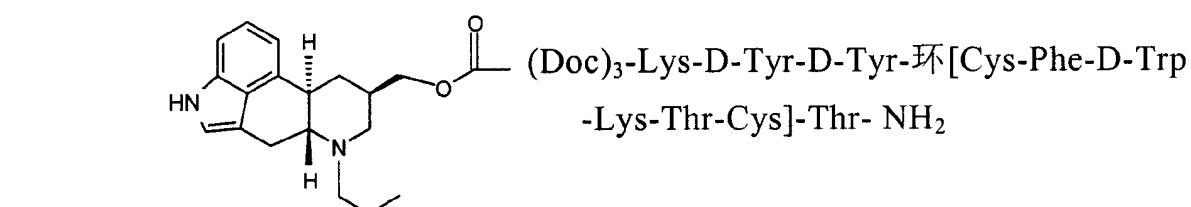
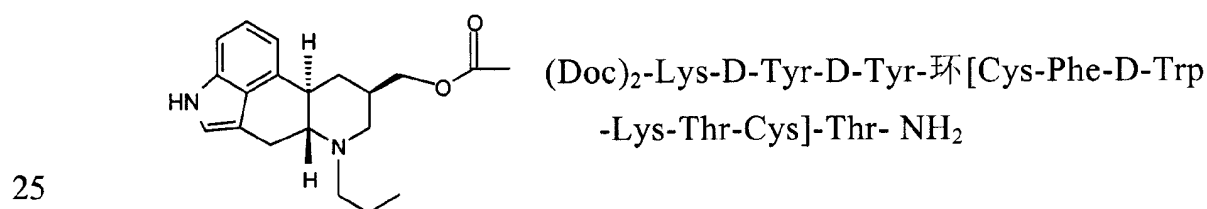
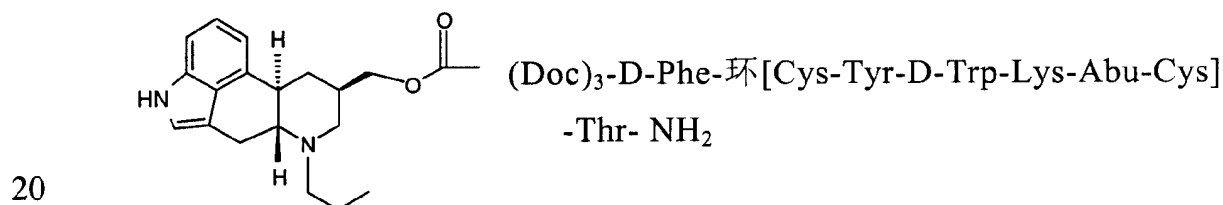
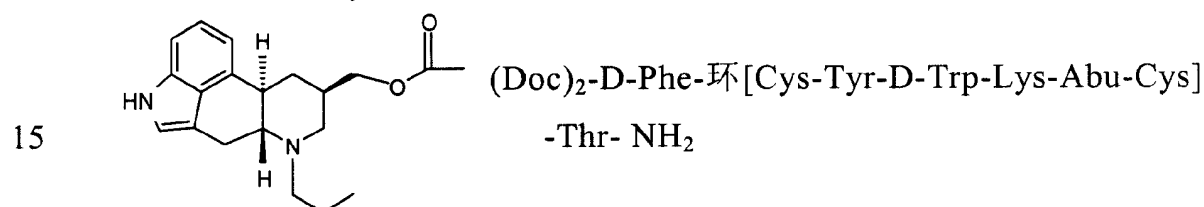
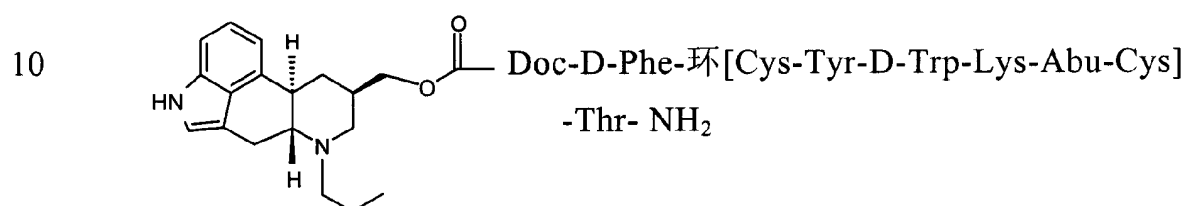
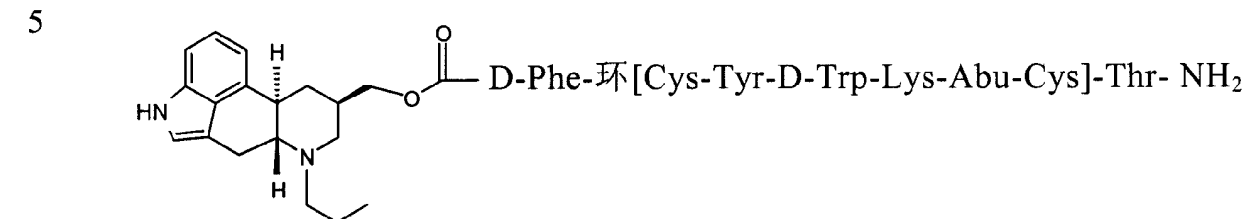
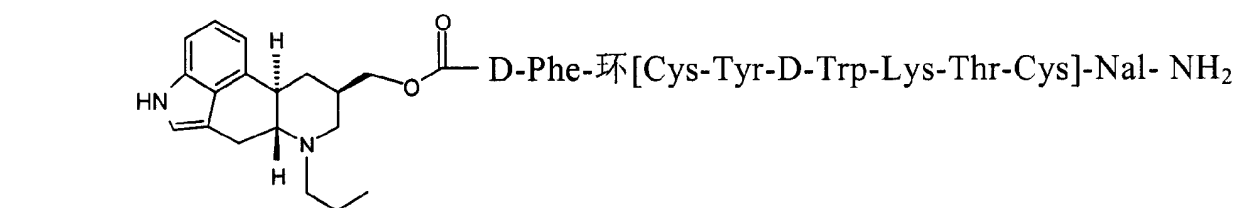
30

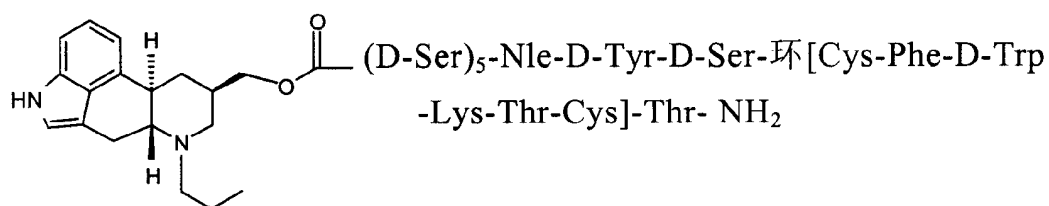




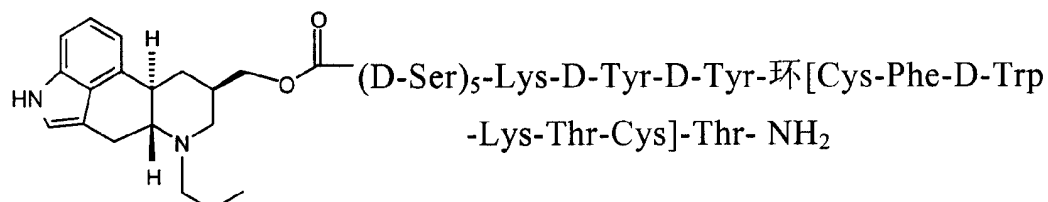


- 
 (Doc)₂-Lys-D-Tyr-D-Tyr-环[Cys-Phe
-D-Trp-Lys-Thr-Cys]-Thr- NH₂
- 5
- 
 (Doc)₃-Lys-D-Tyr-D-Tyr-环[Cys-Phe
-D-Trp-Lys-Thr-Cys]-Thr- NH₂
- 10
- 
 (D-Ser)₅-Nle-D-Tyr-D-Ser-环[Cys-Phe
-D-Trp-Lys-Thr-Cys]-Thr- NH₂
- 15
- 
 (D-Ser)₁₀-Lys-D-Tyr-D-Tyr-环[Cys-Phe
-D-Trp-Lys-Thr-Cys]-Thr- NH₂
- 20
- 
 (D-Ser)₅-Lys-D-Tyr-D-Tyr-环[Cys-Phe-D-Trp-Lys
-Thr-Cys]-Thr- NH₂
- 25
- 
 D-Nal-环[Cys-Tyr-D-Trp-Lys-Val-Cys]-Thr- NH₂
- 30
- 
 D-Phe-环[Cys-(3-溴-Tyr)-D-Trp-Lys
-Thr-Cys]-Thr- NH₂

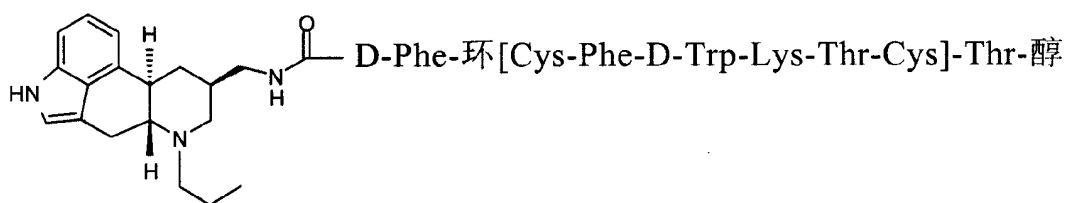




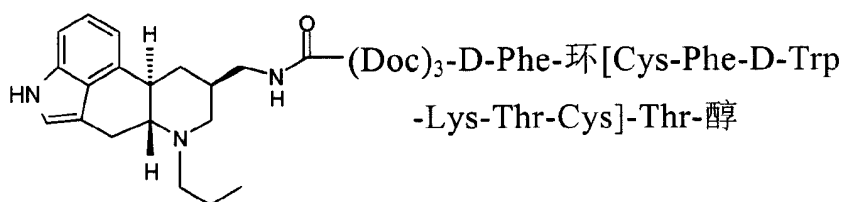
5



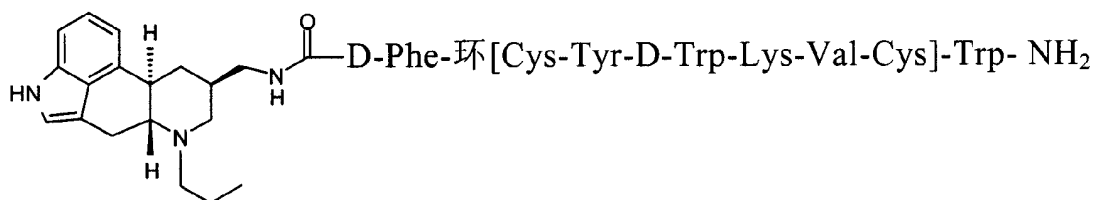
10



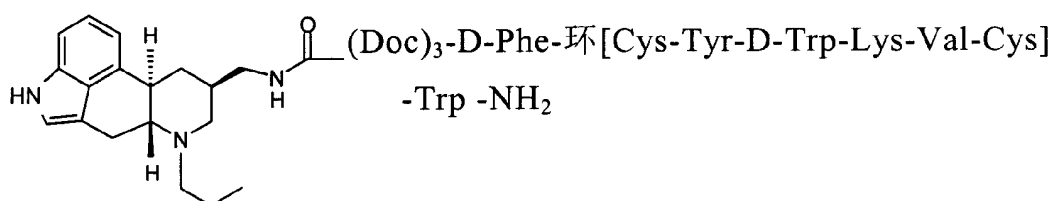
15



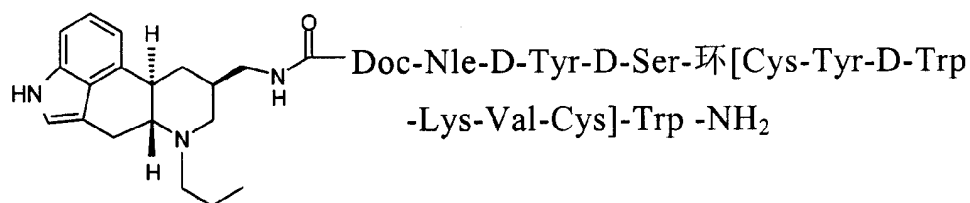
20

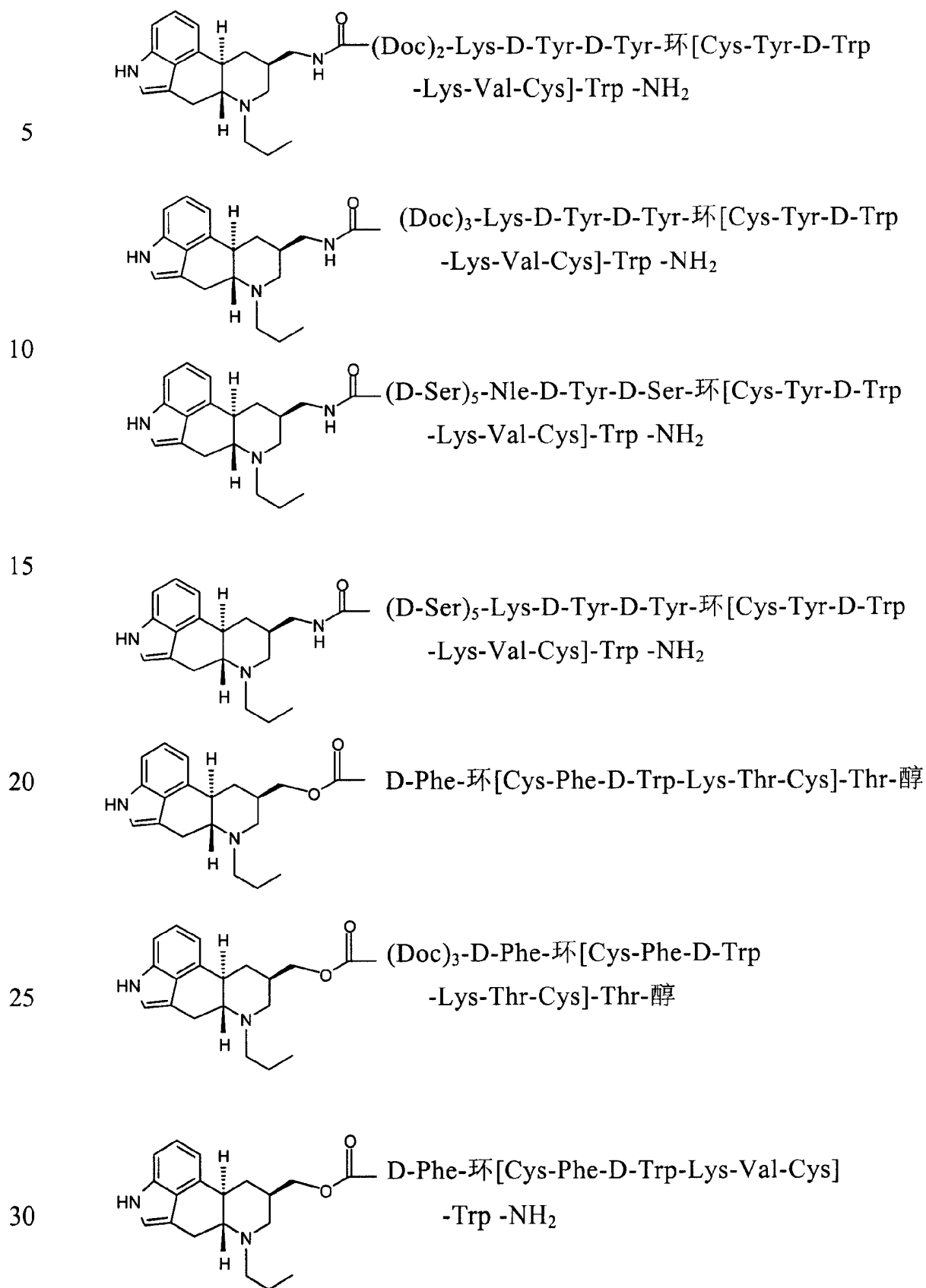


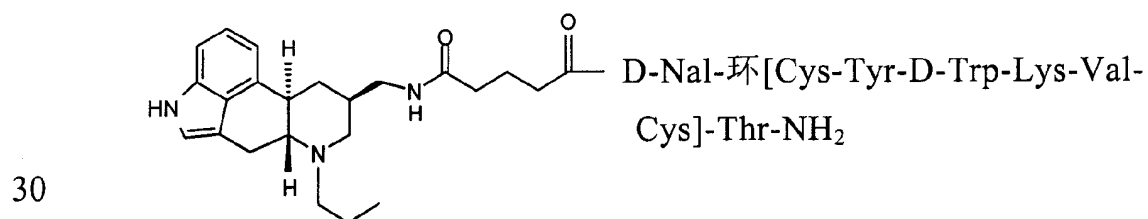
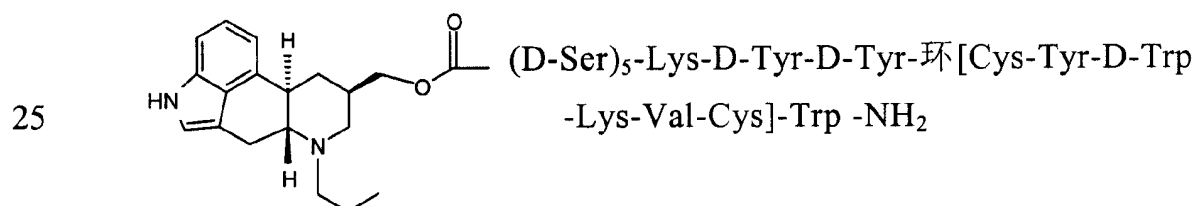
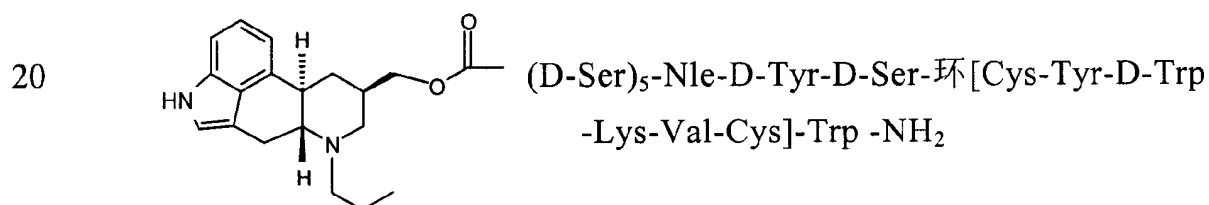
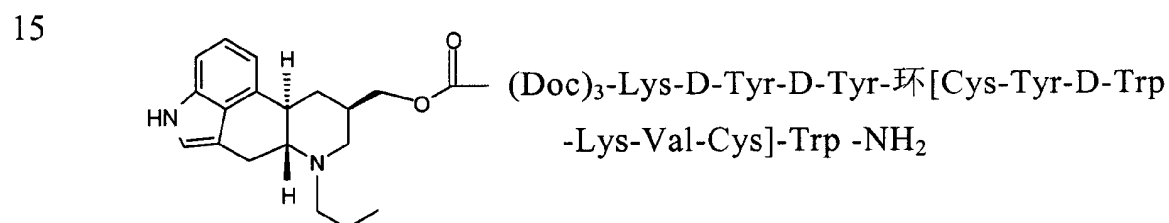
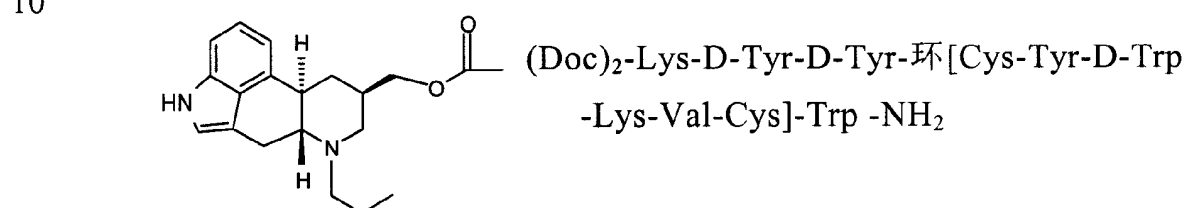
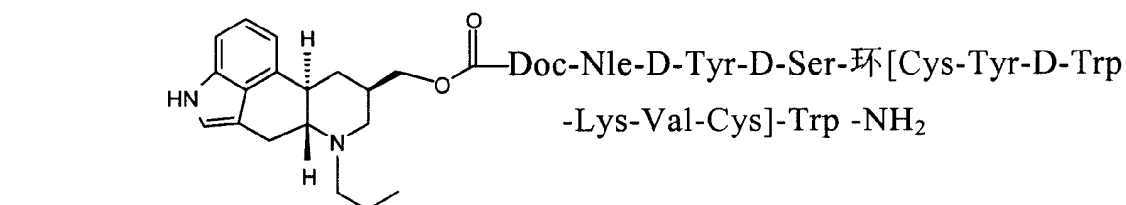
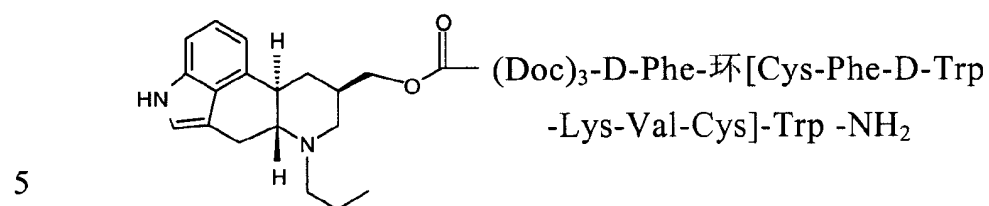
25

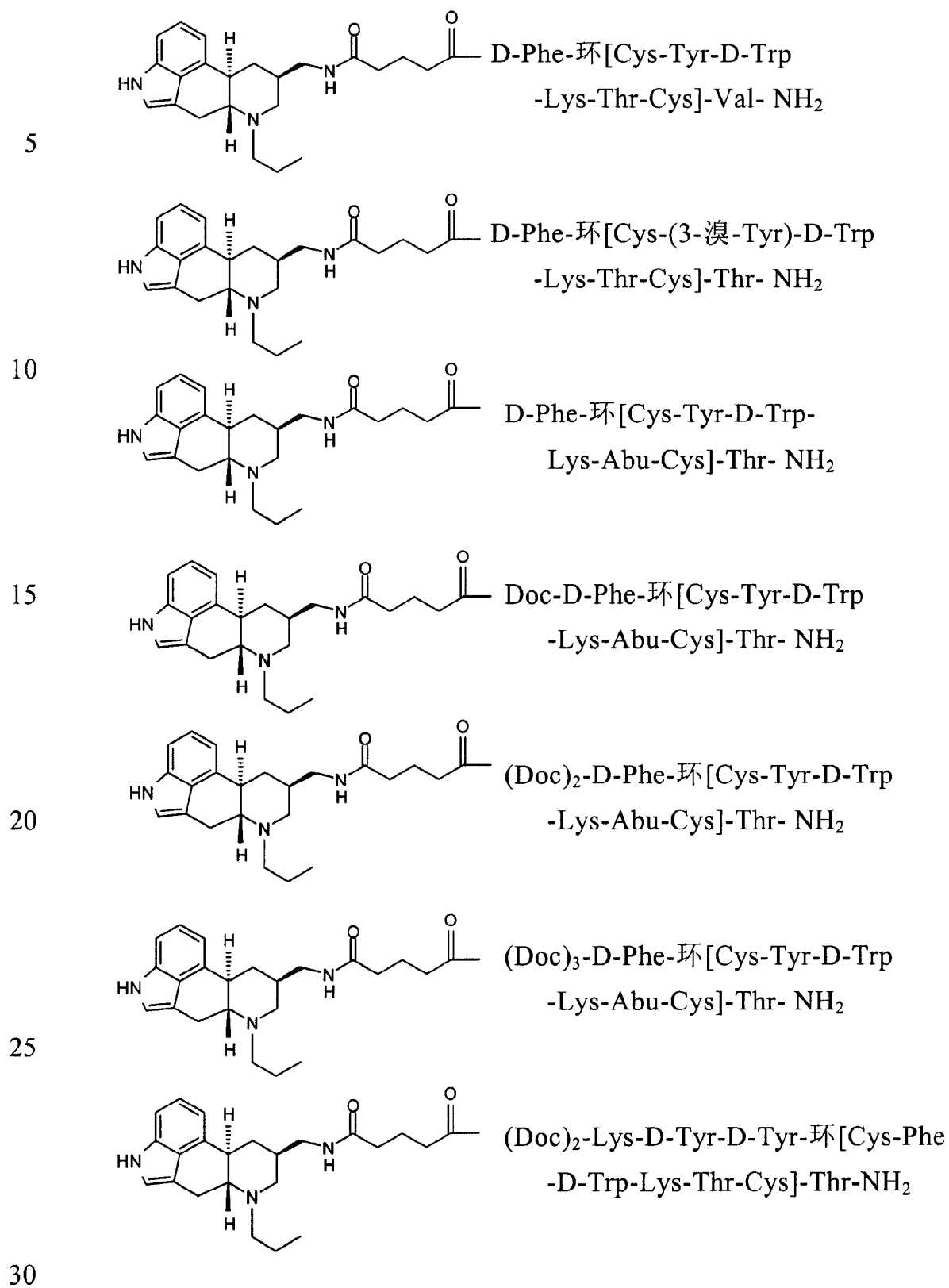


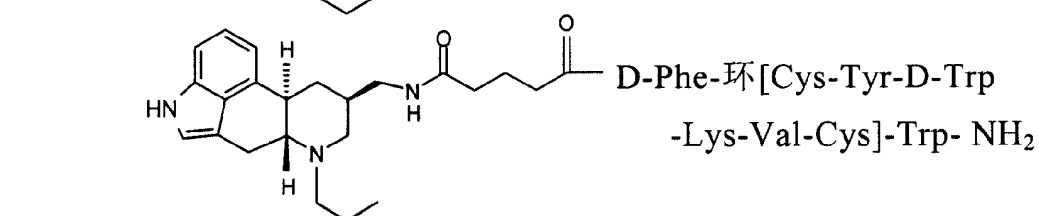
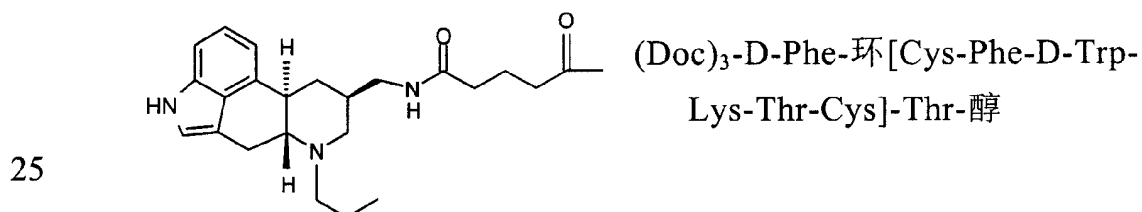
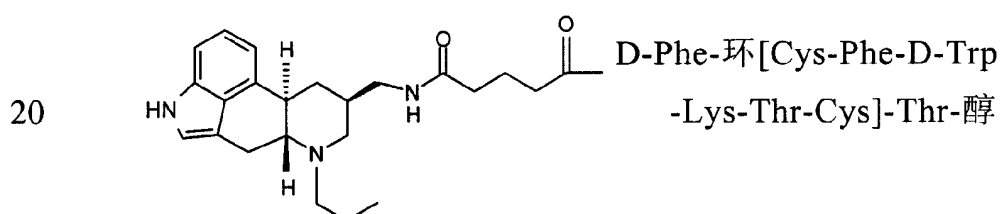
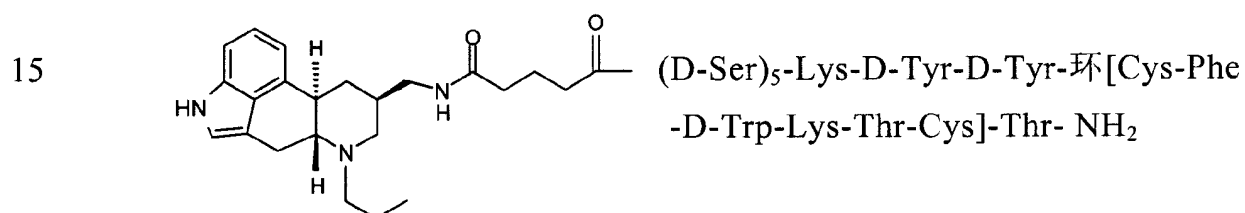
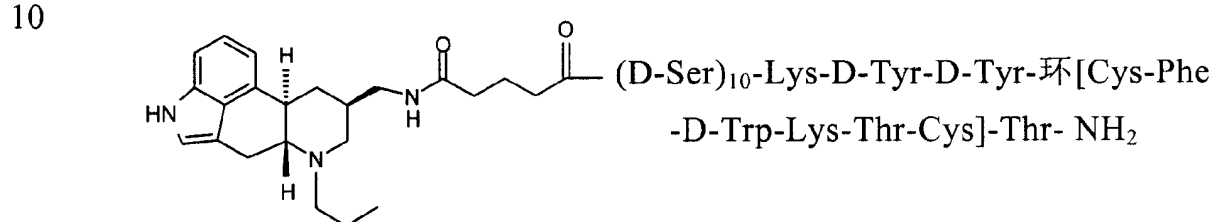
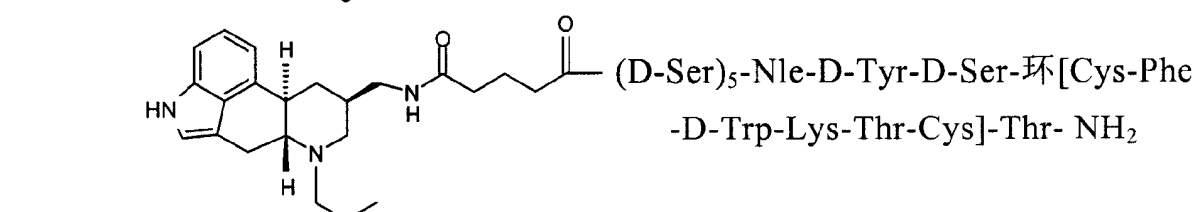
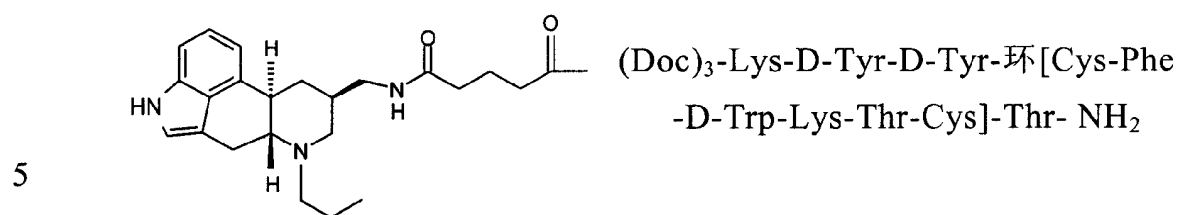
30

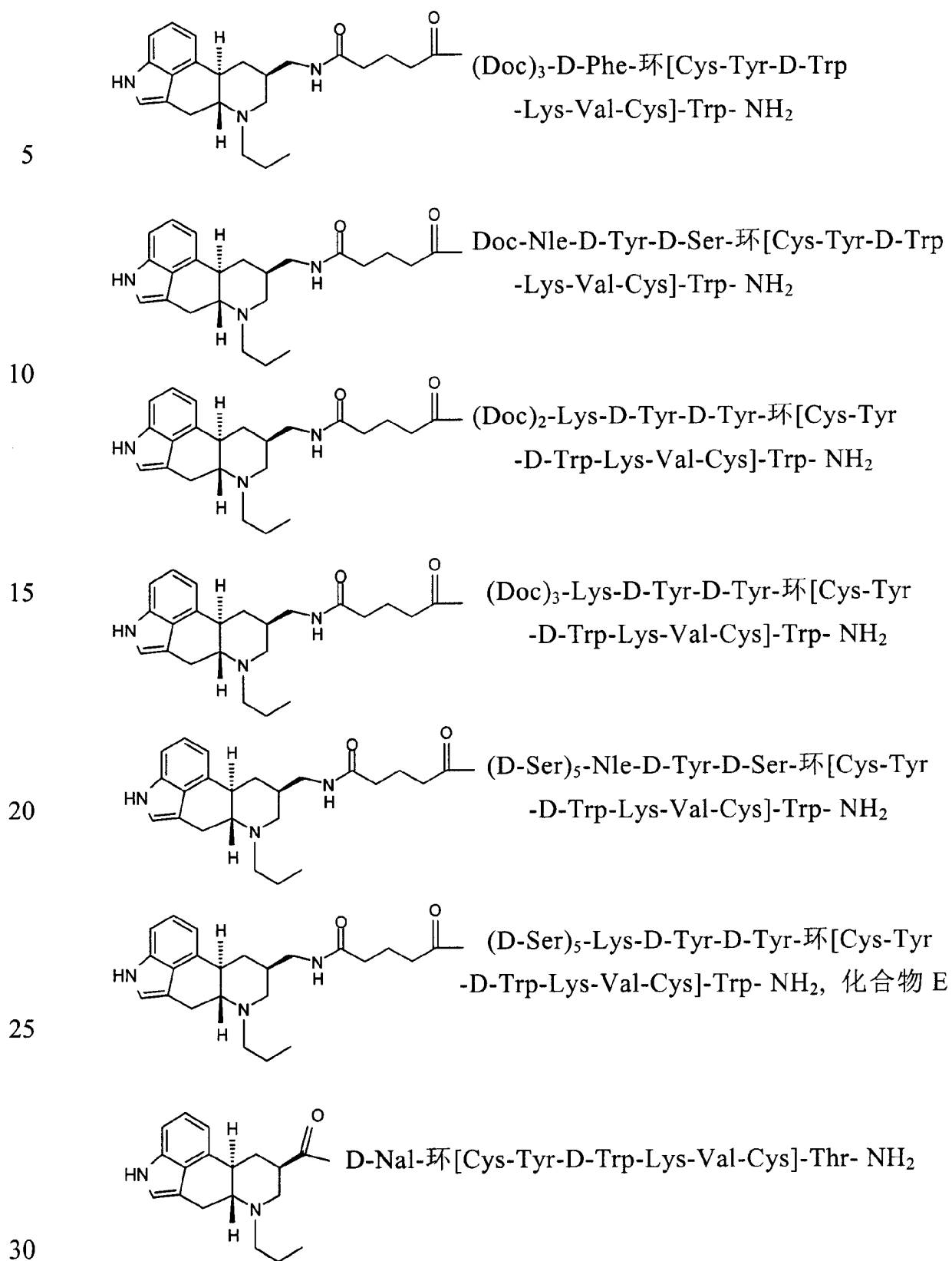


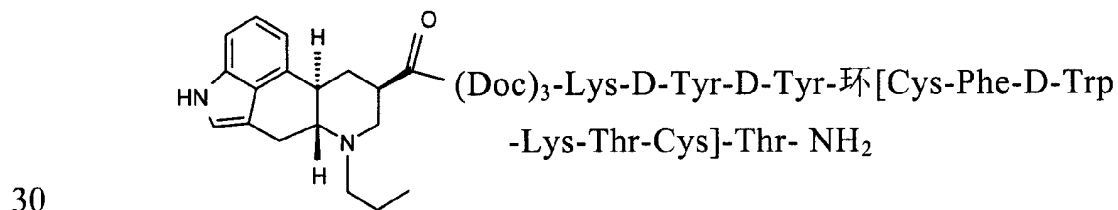
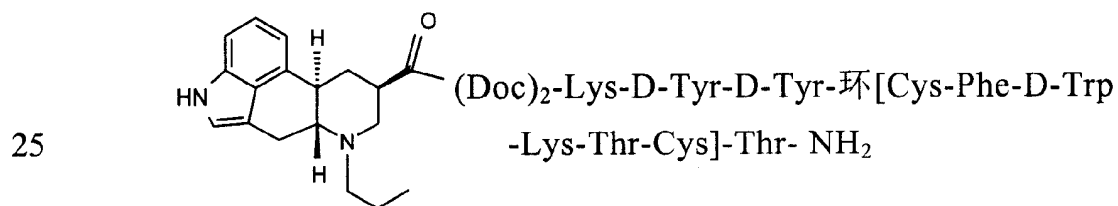
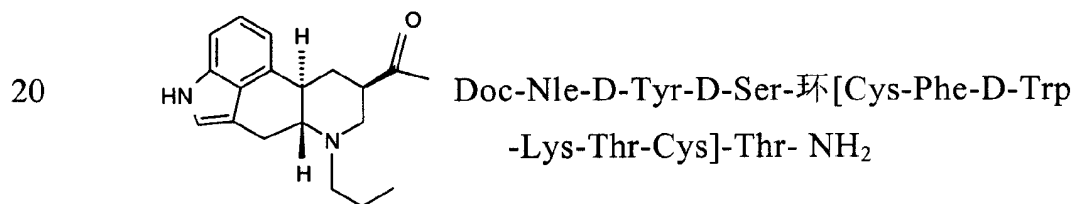
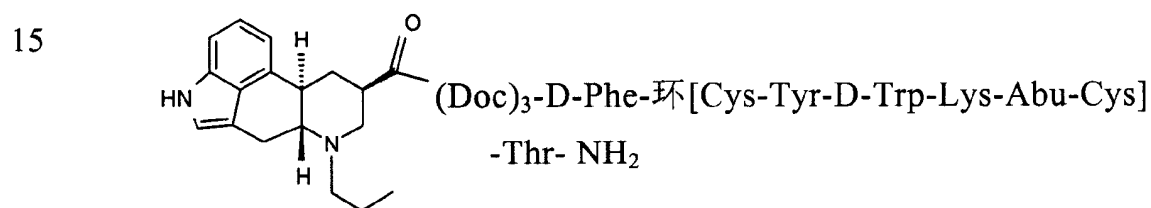
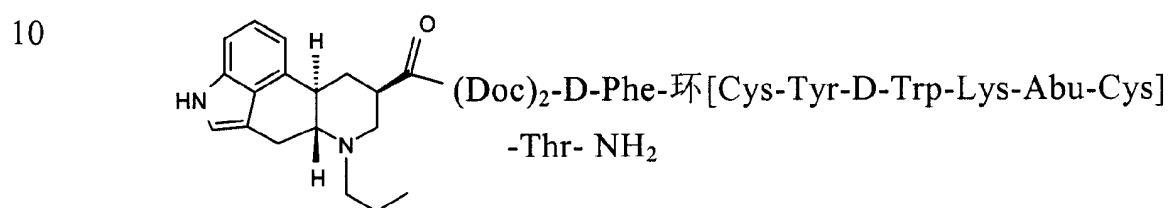
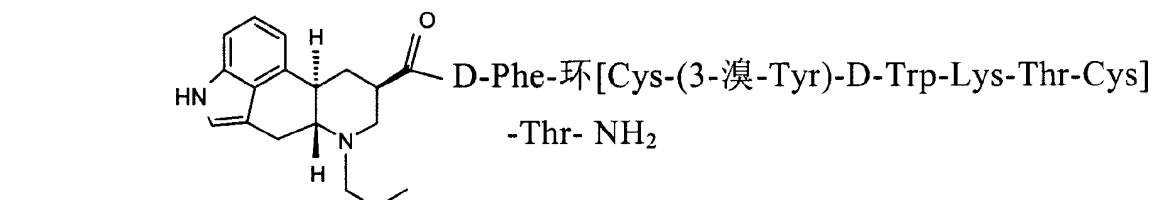
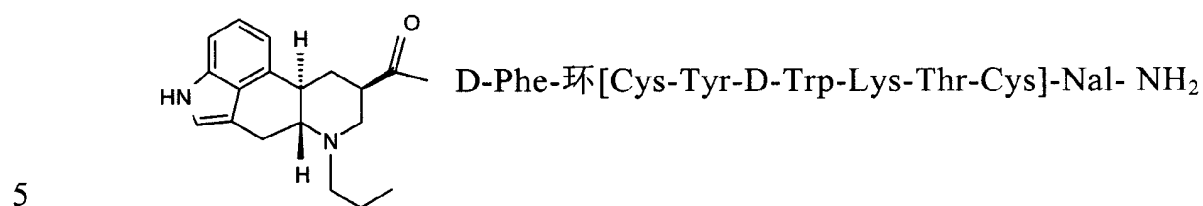


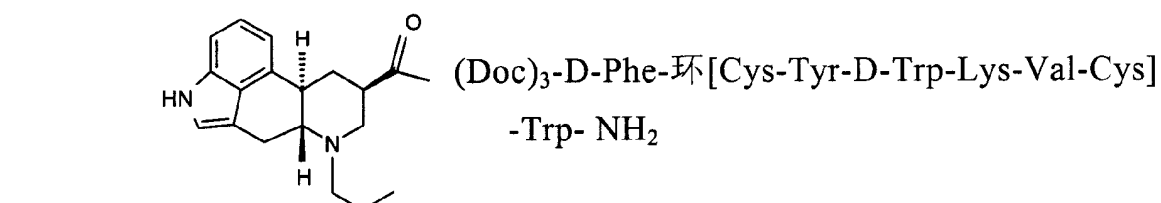
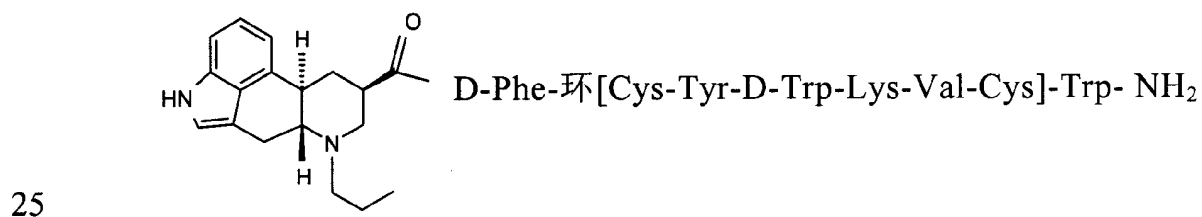
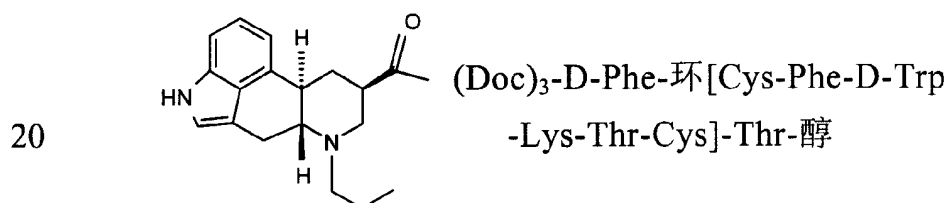
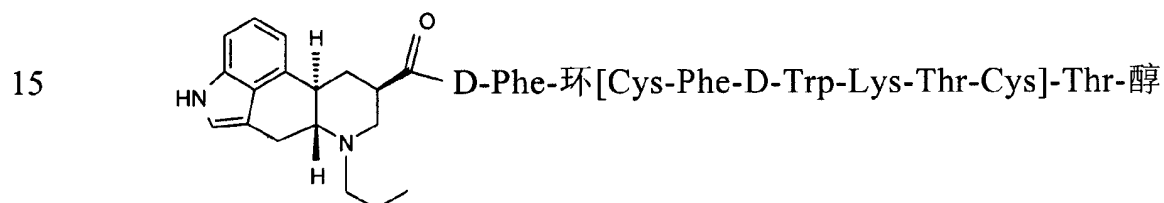
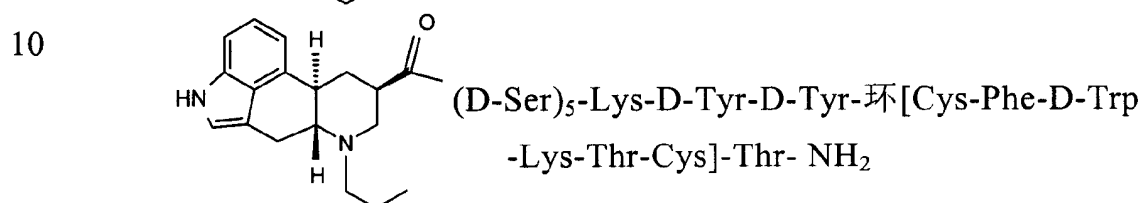
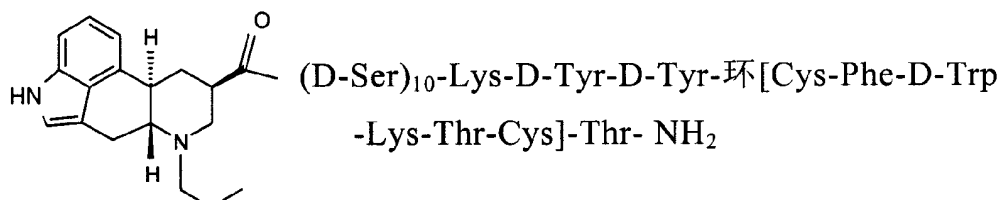
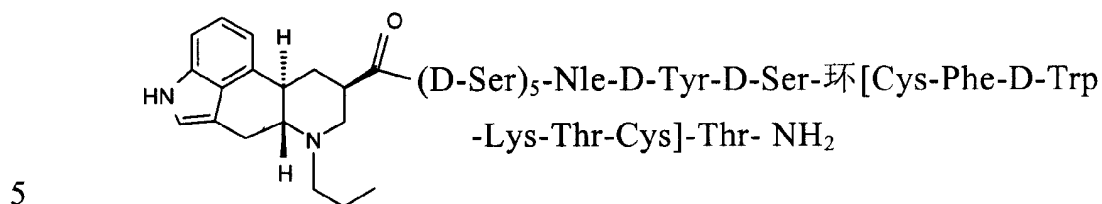


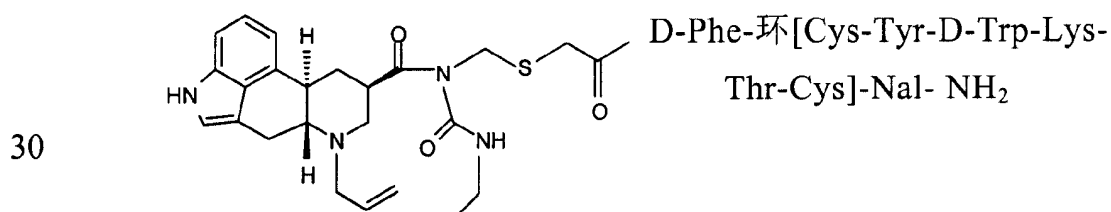
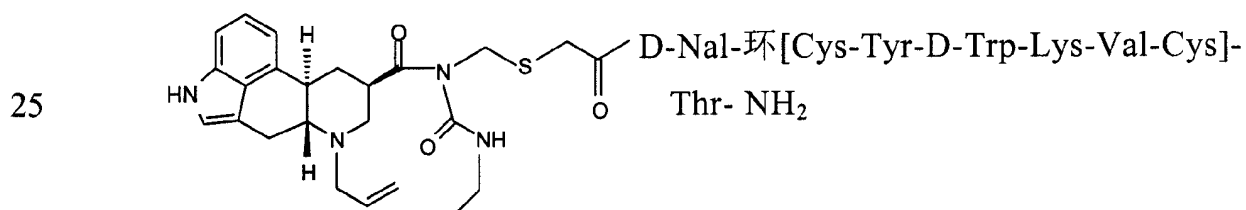
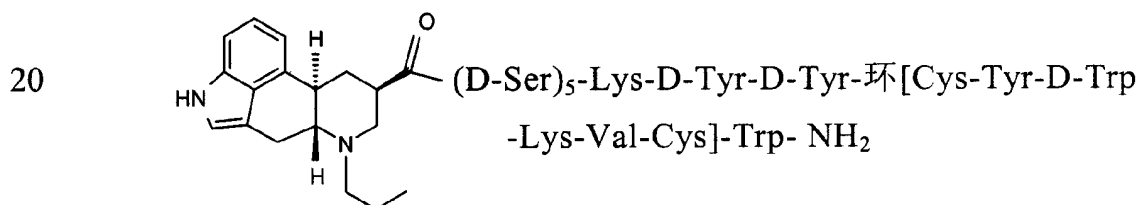
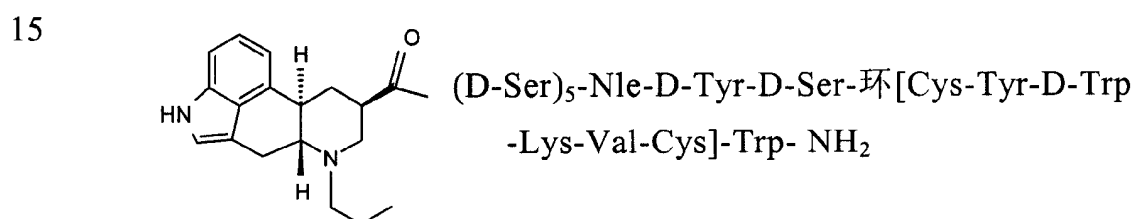
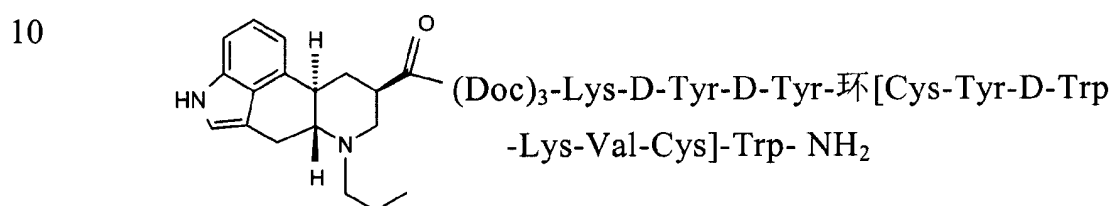
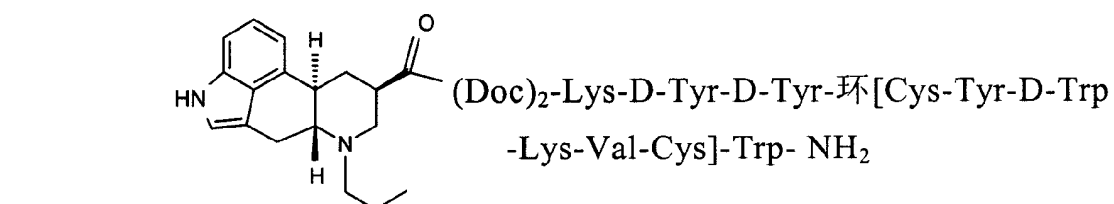
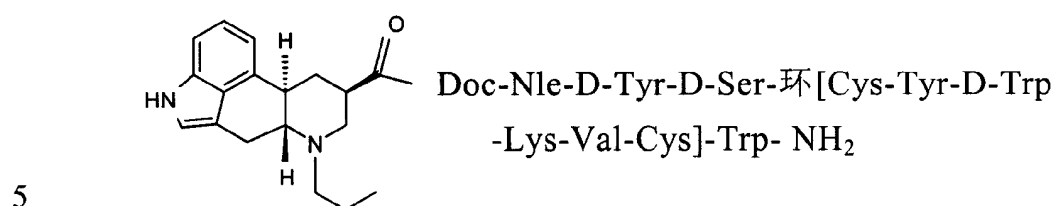




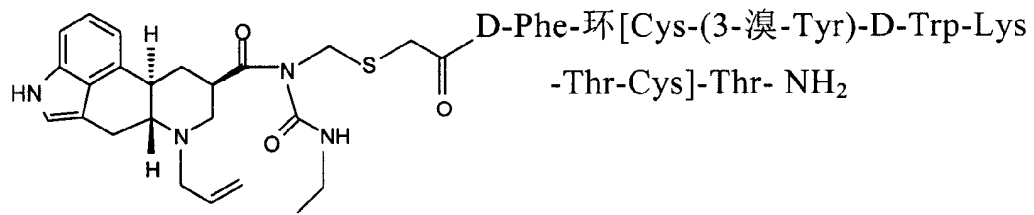




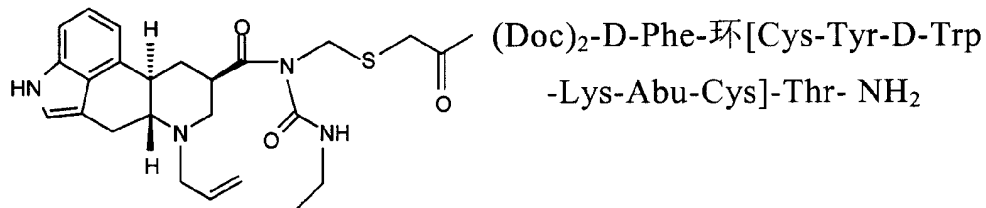




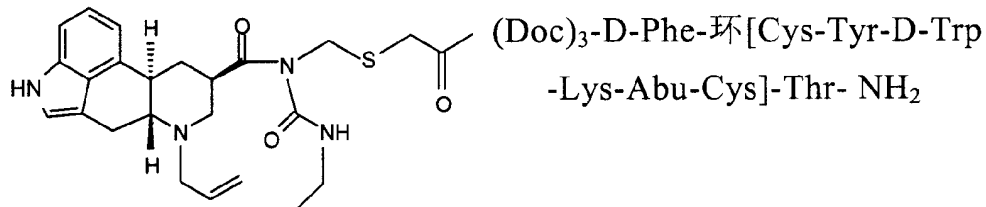
5



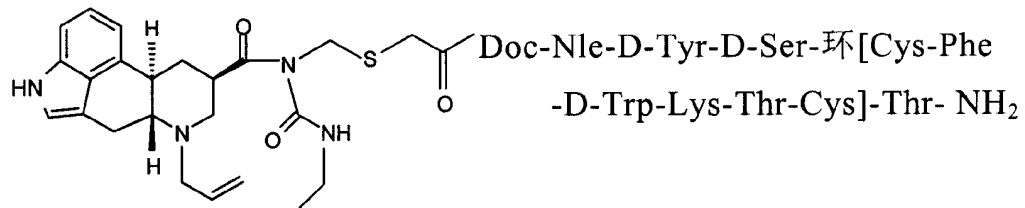
10



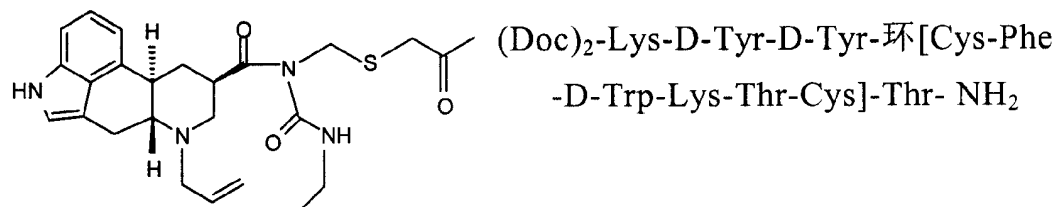
15



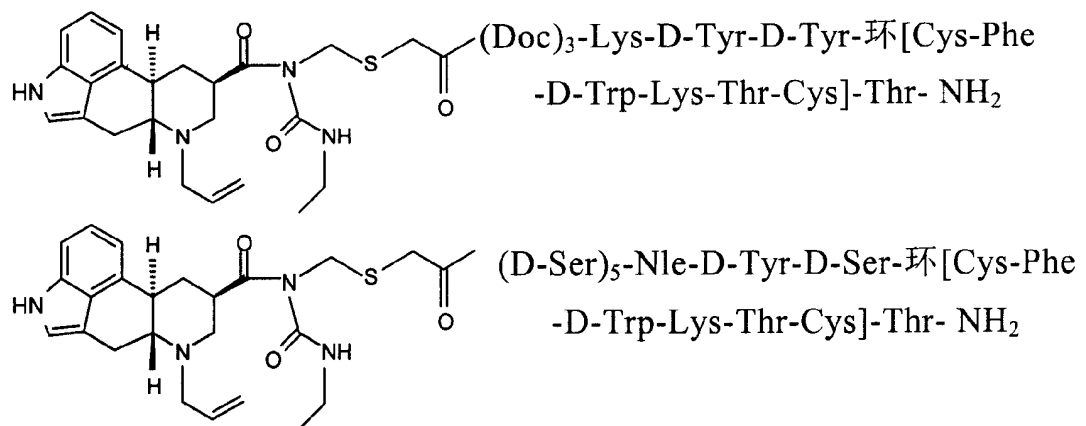
20

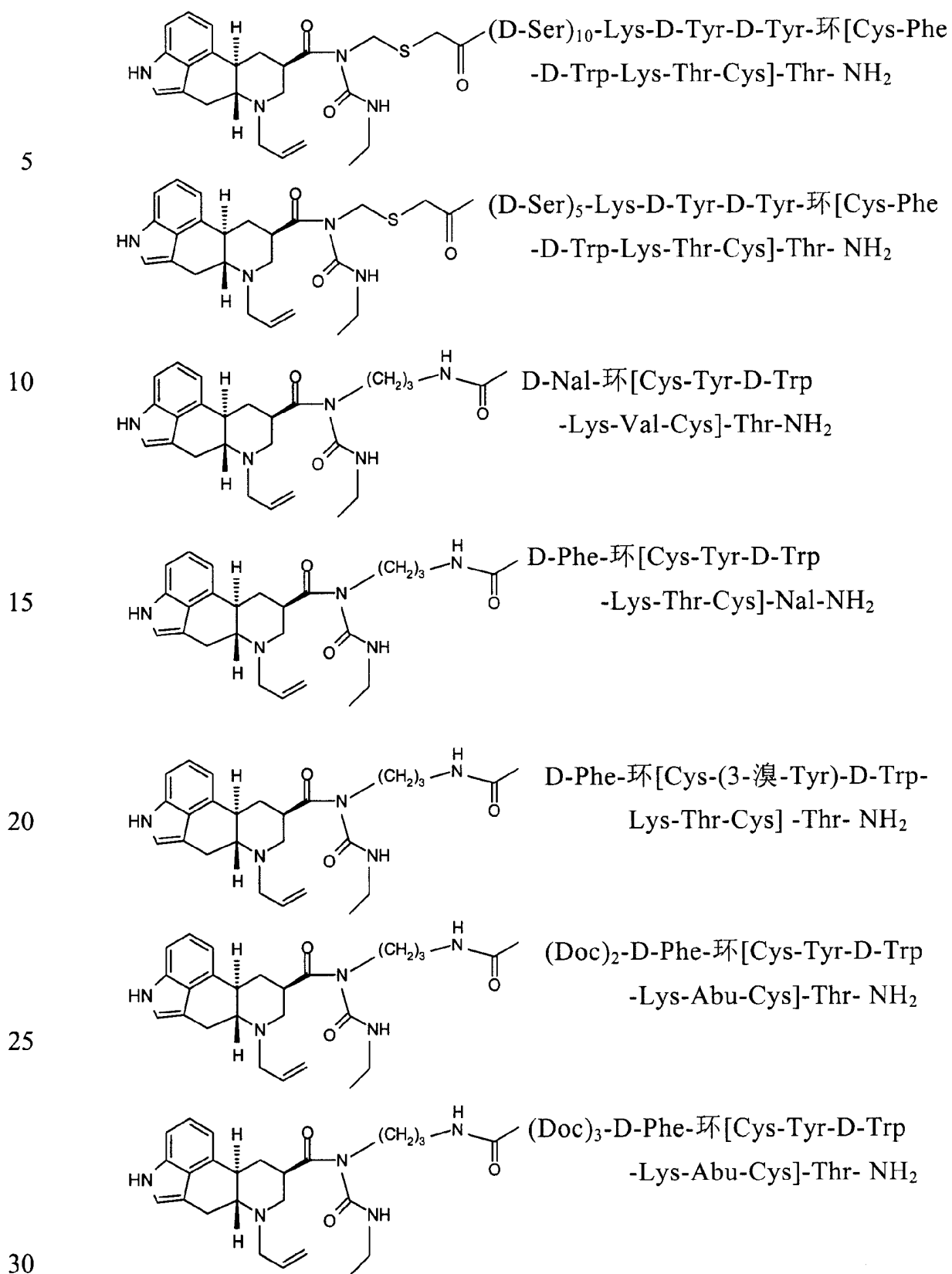


25

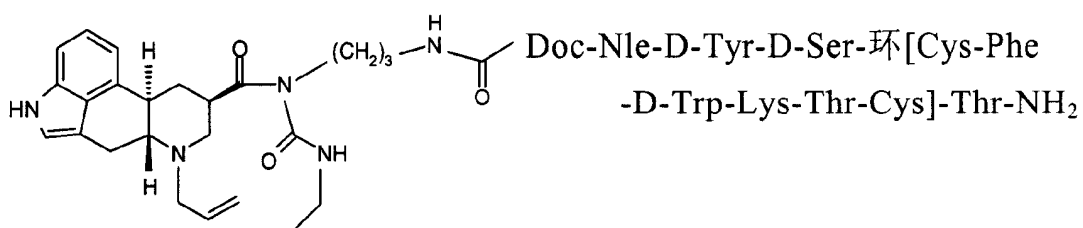


30

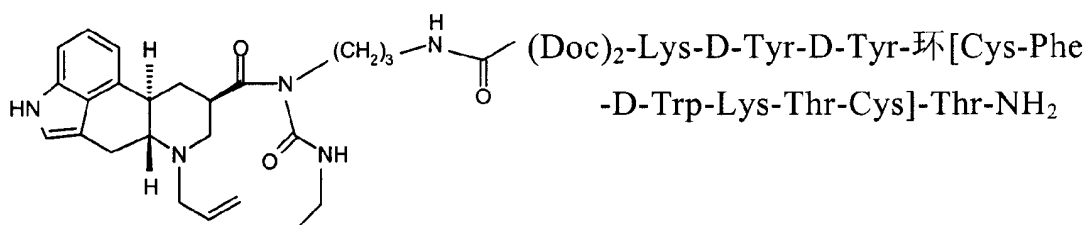




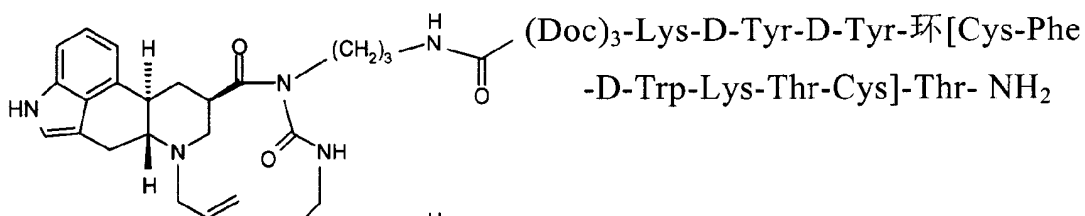
5



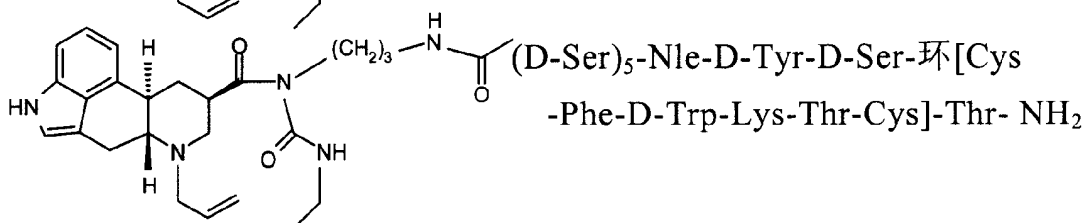
10



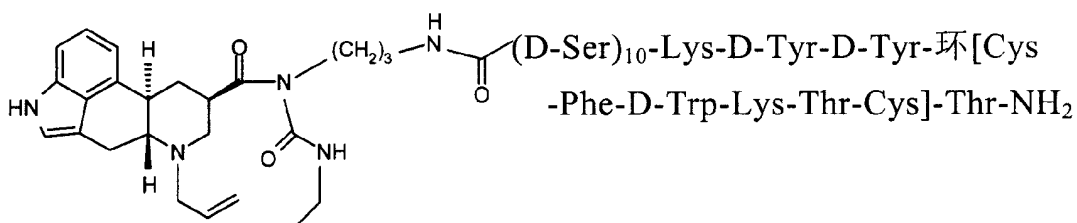
15



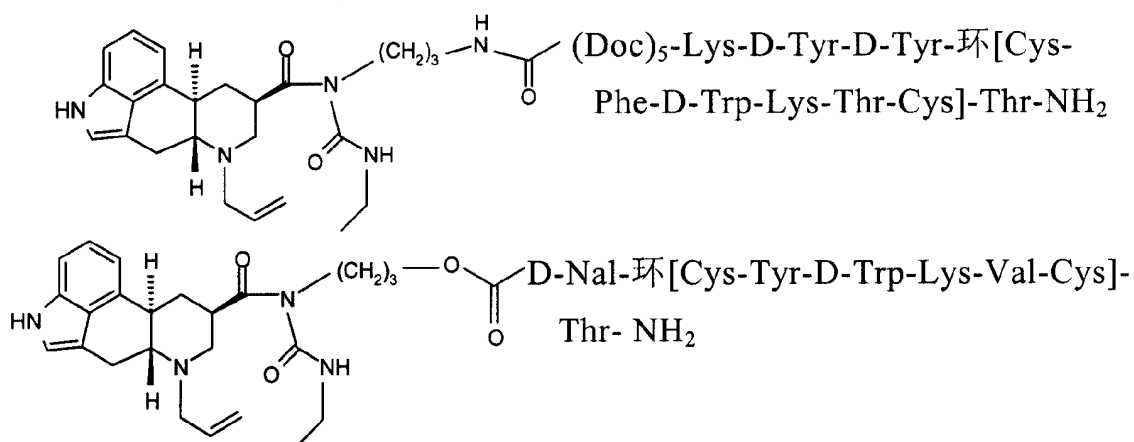
20



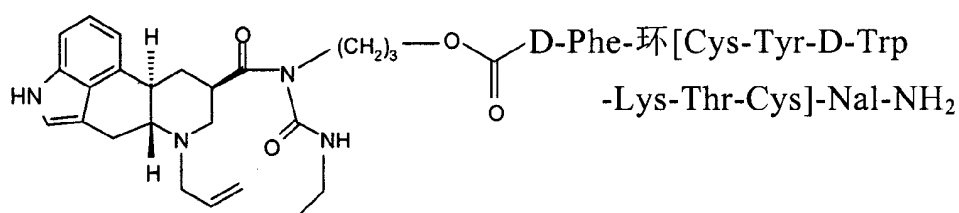
25



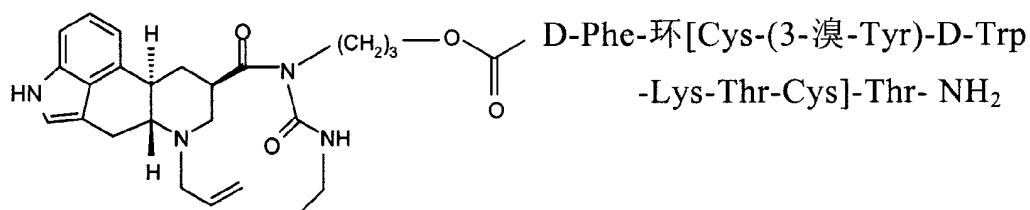
30



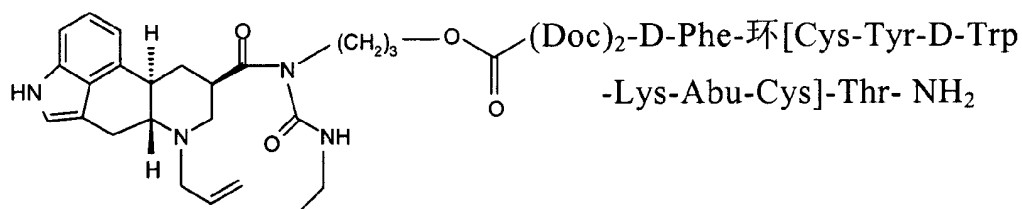
5



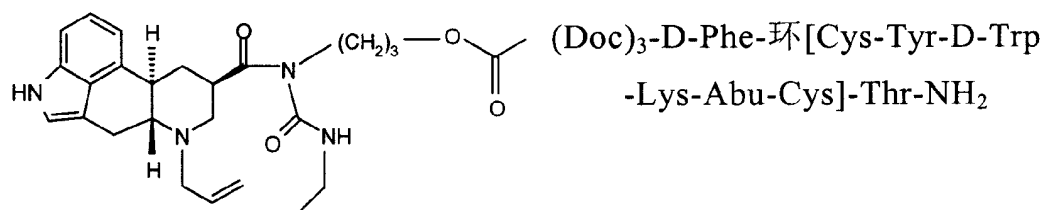
10



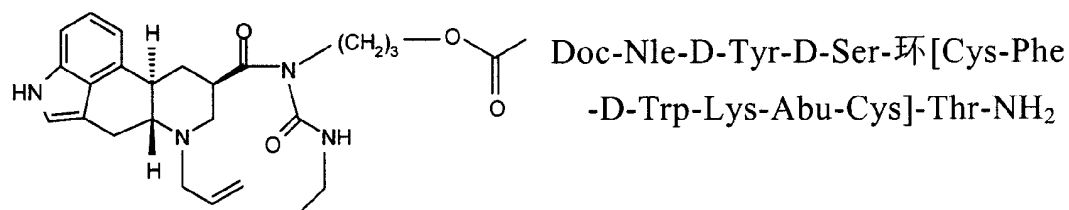
15



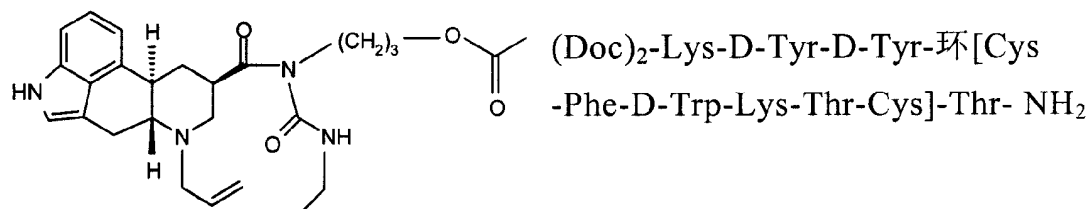
20



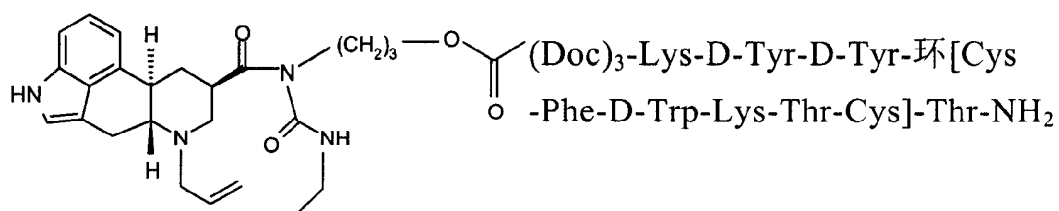
25



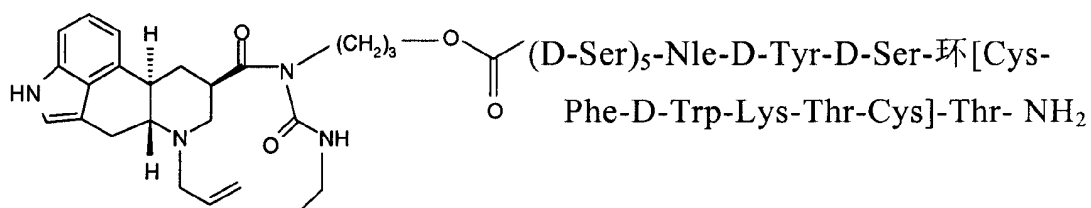
30



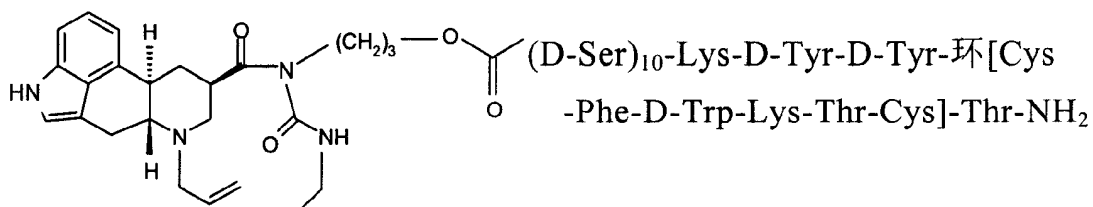
5



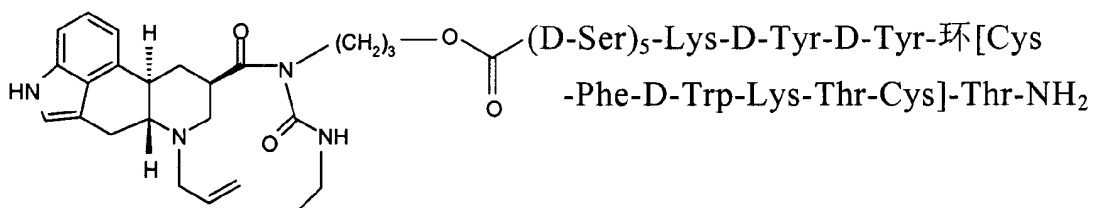
10



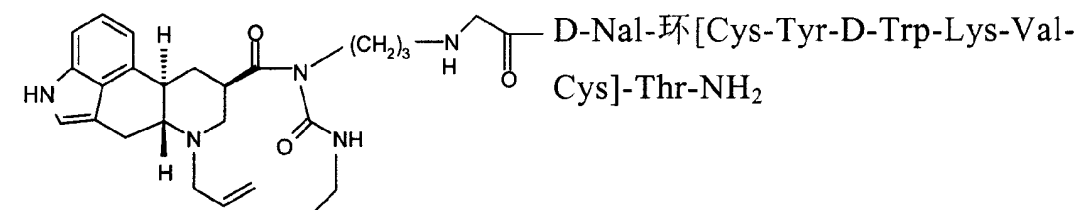
15



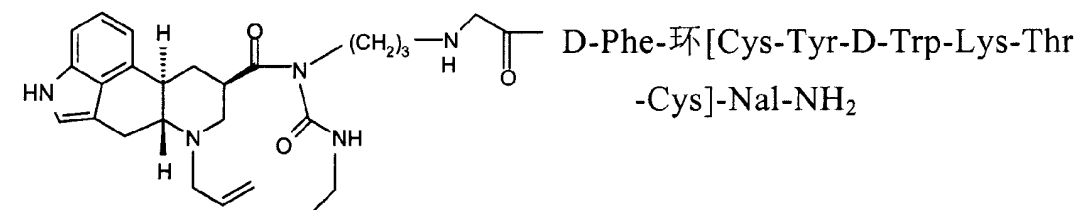
20

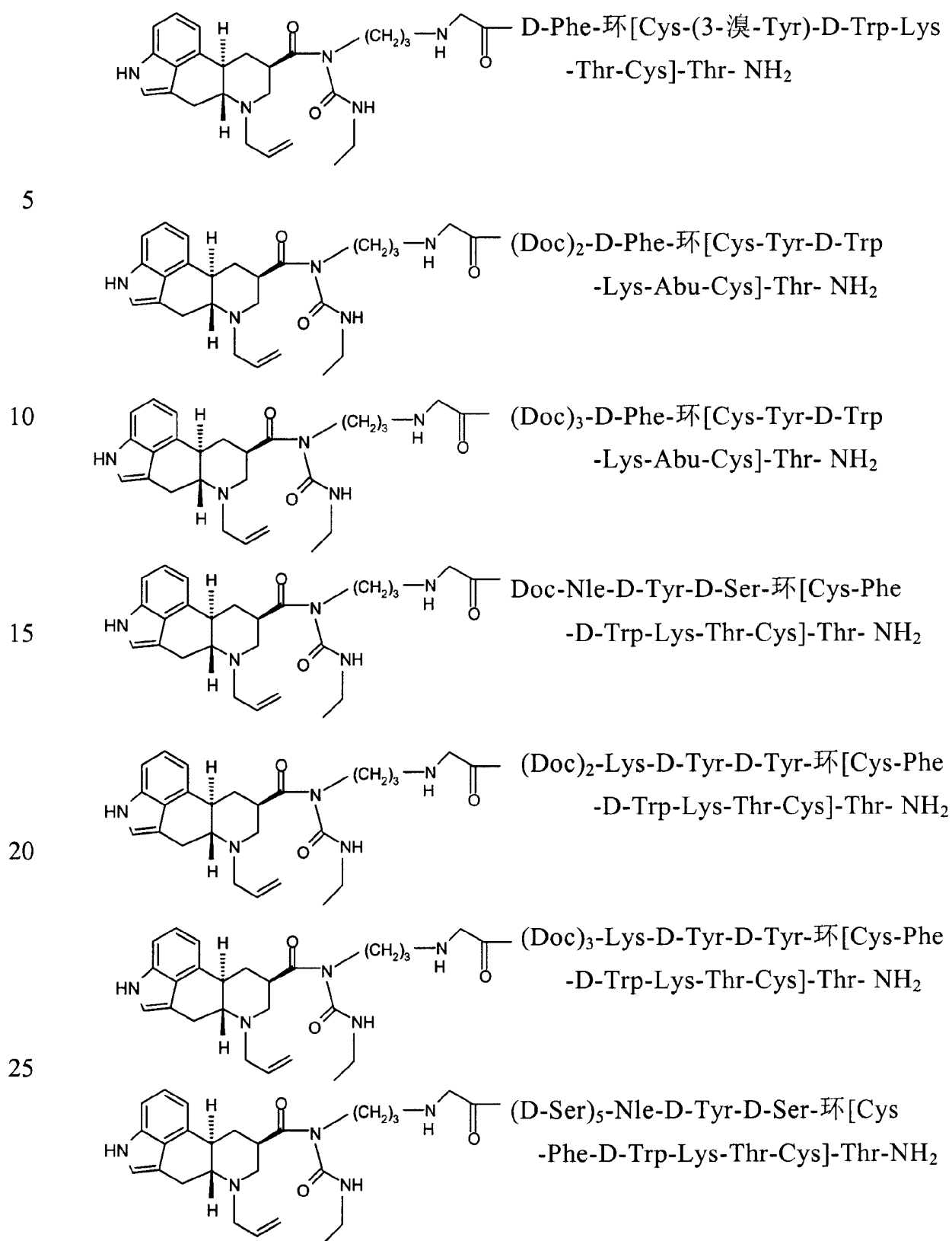


25

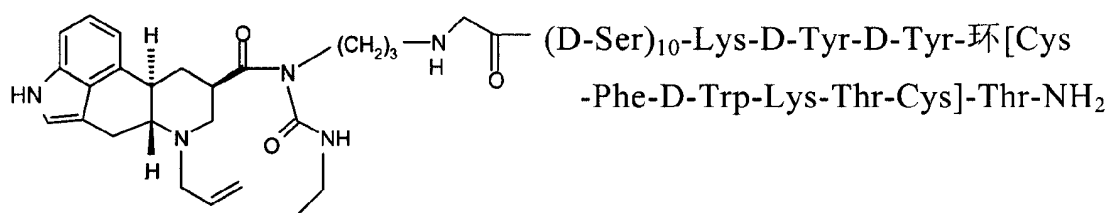


30

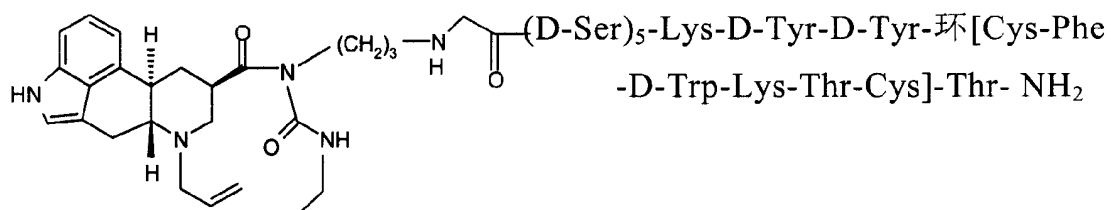




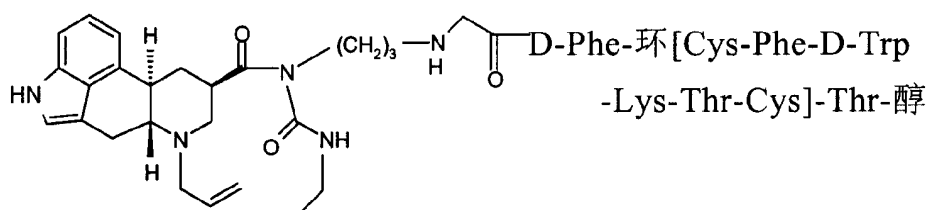
5



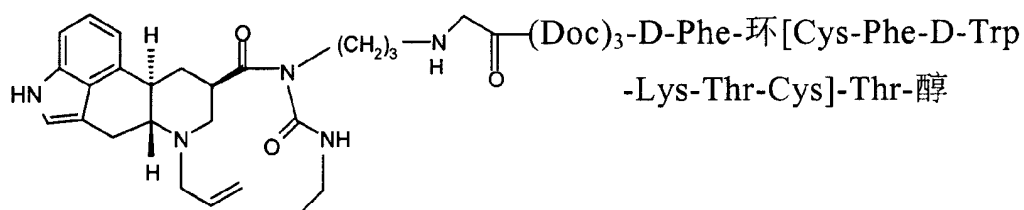
10



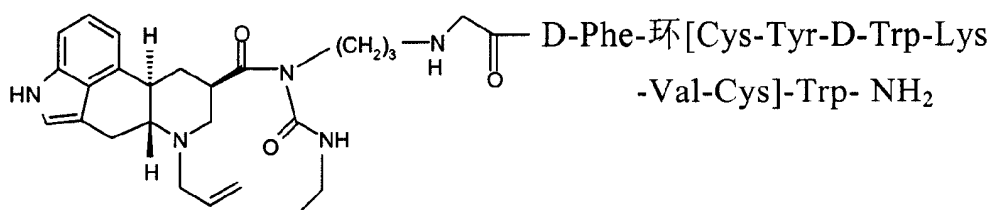
15



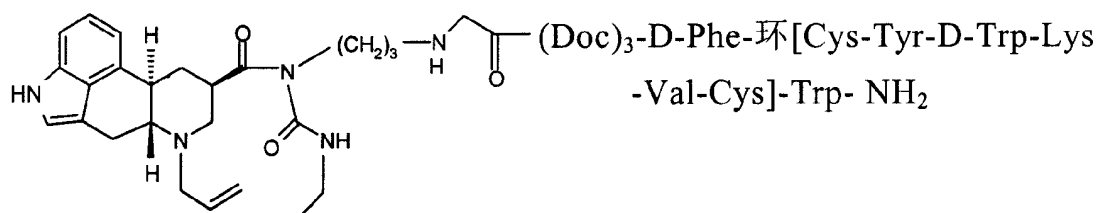
20



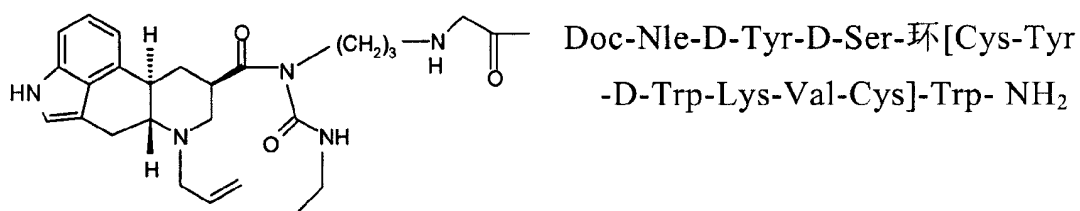
25



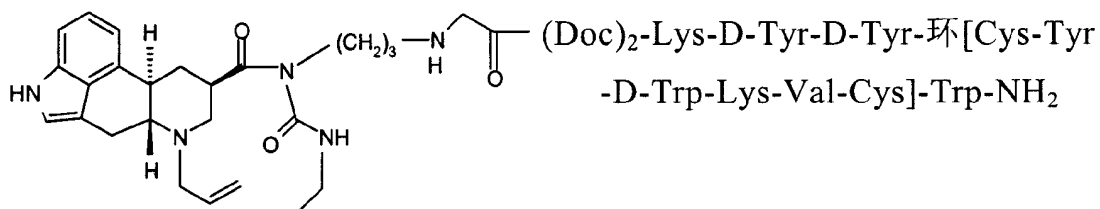
30



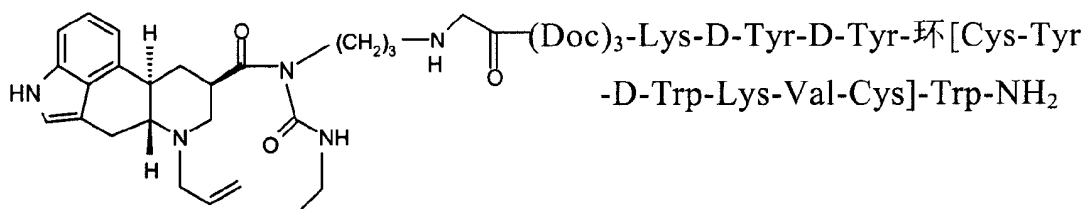
5



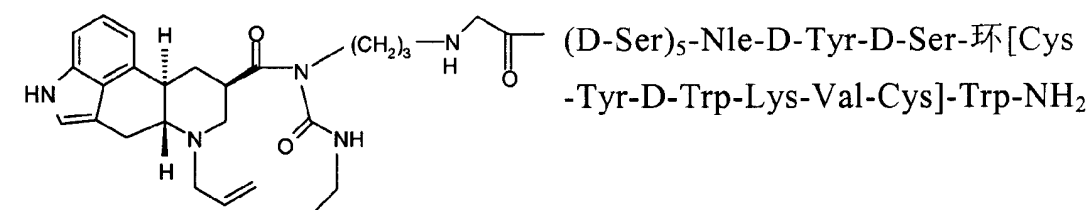
10



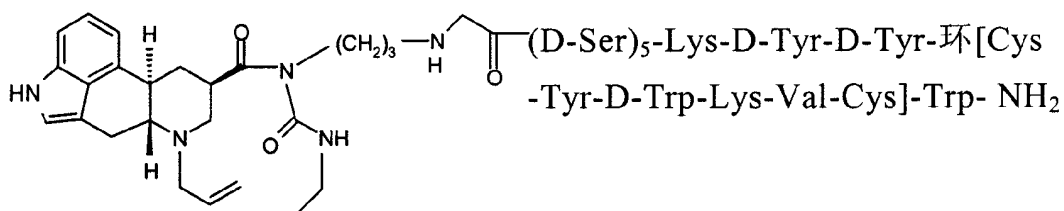
15



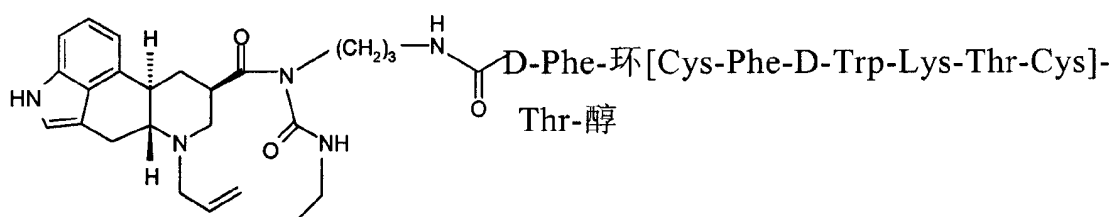
20



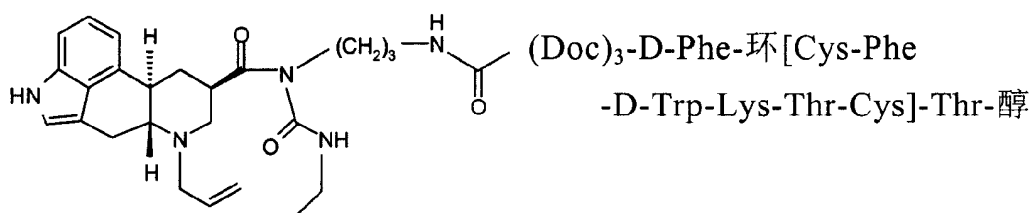
25



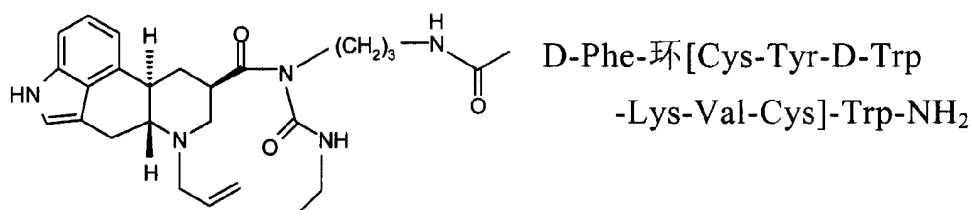
30



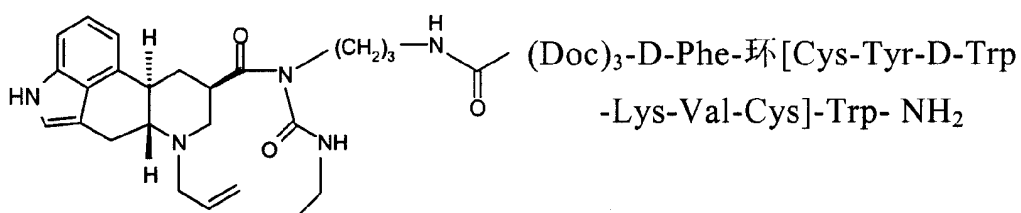
5



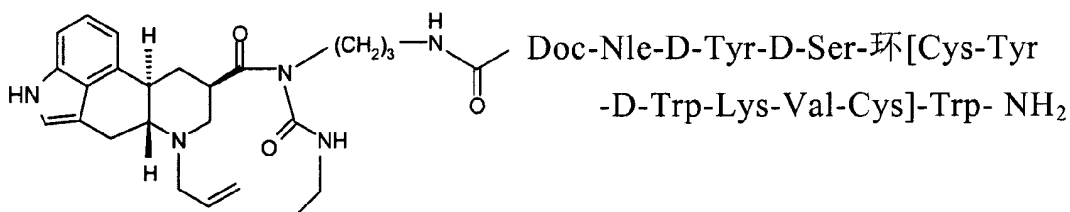
10



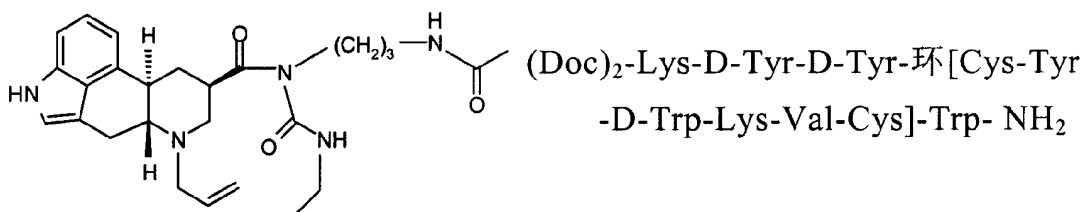
15



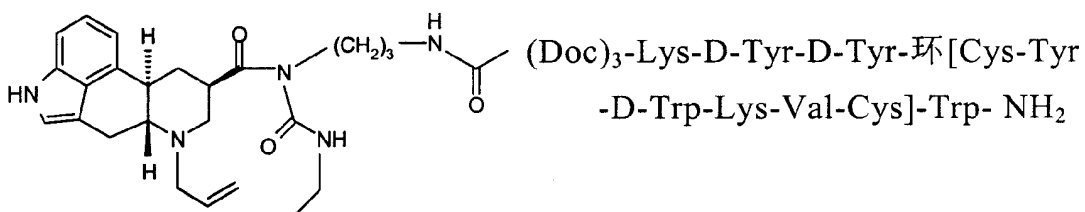
20



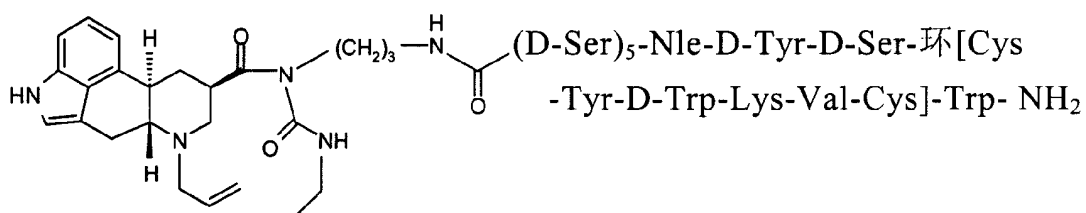
25



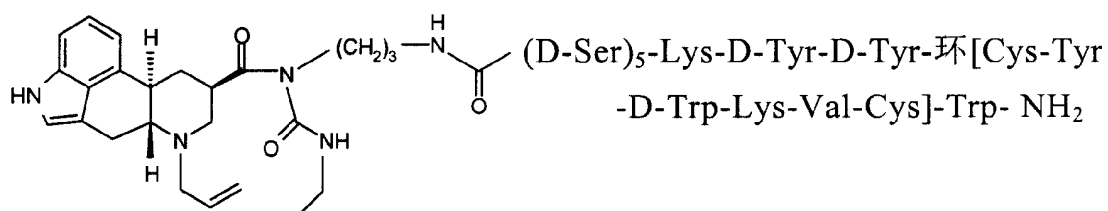
30



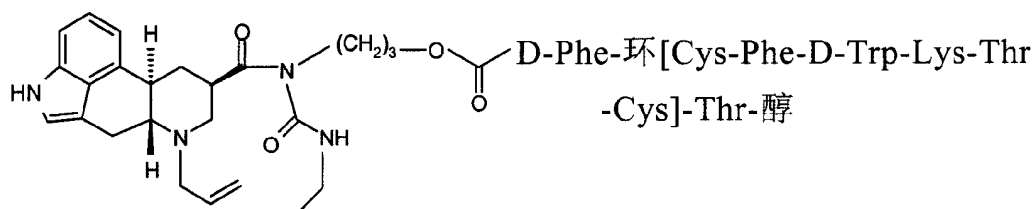
5



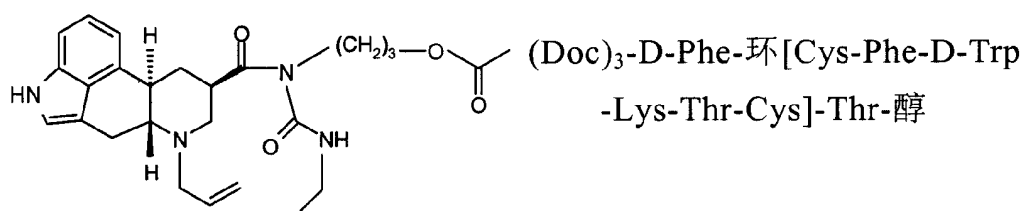
10



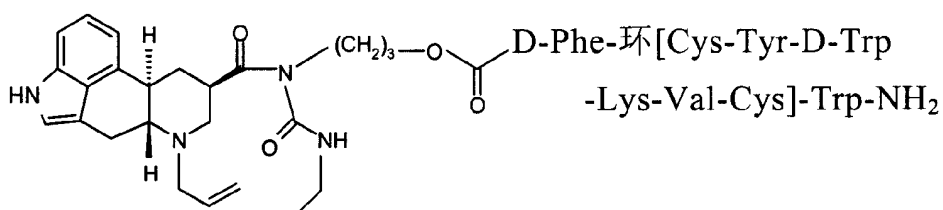
15



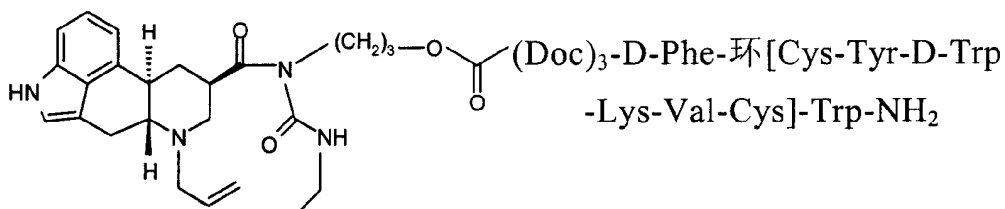
20

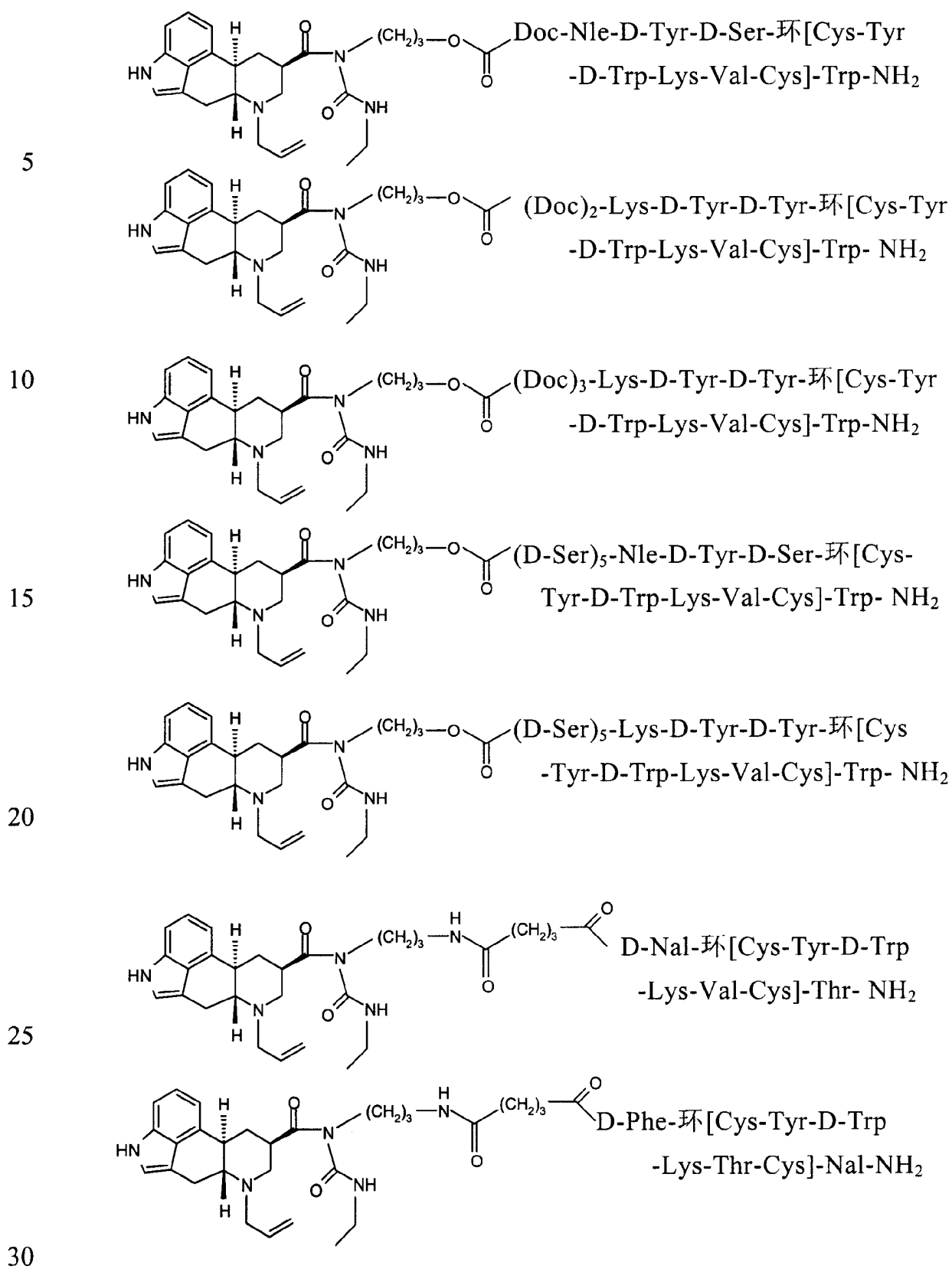


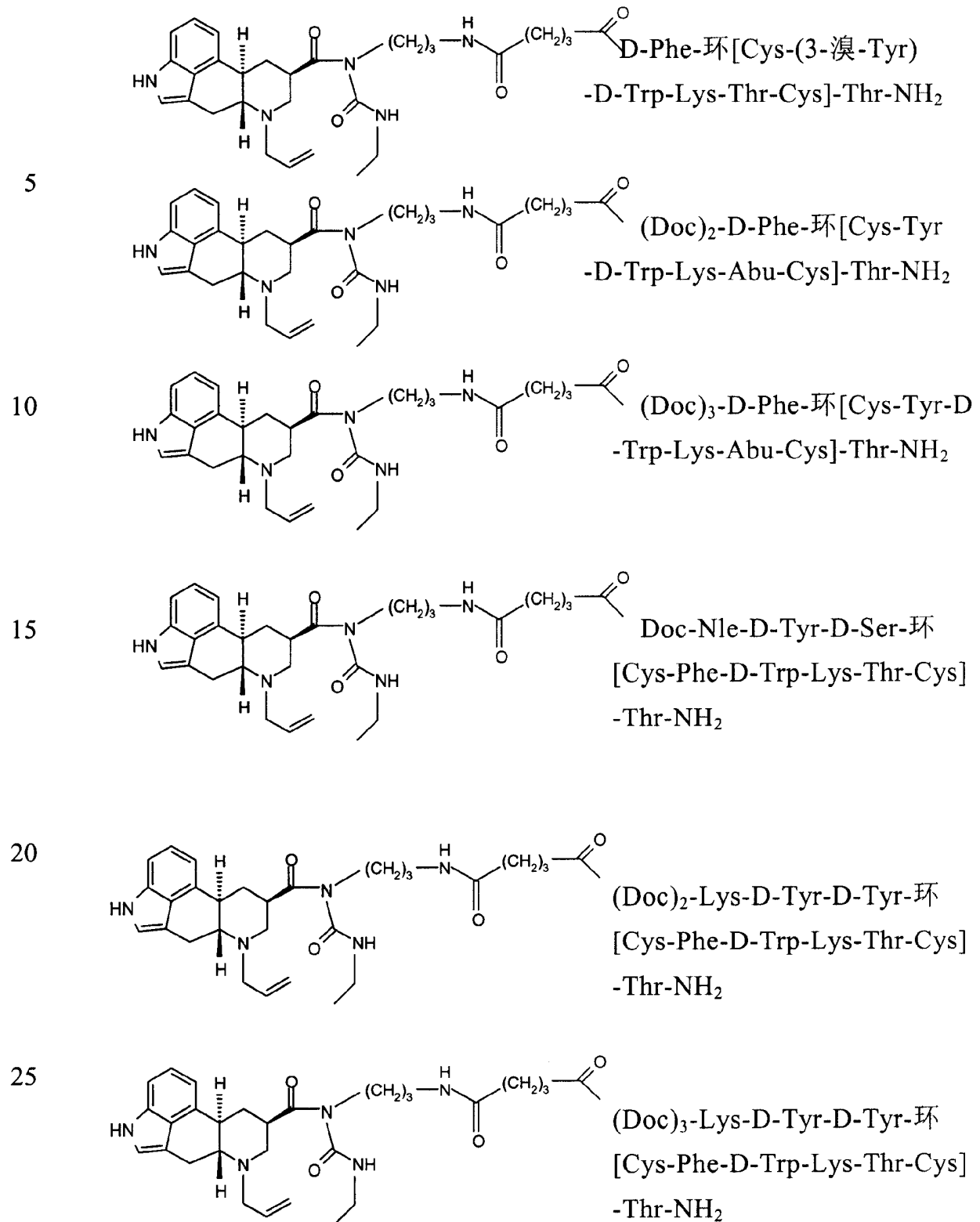
25

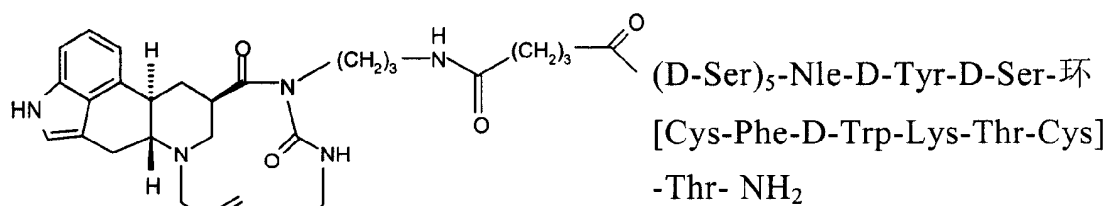


30

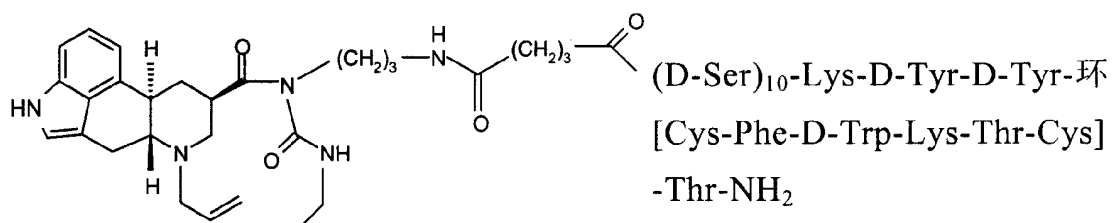




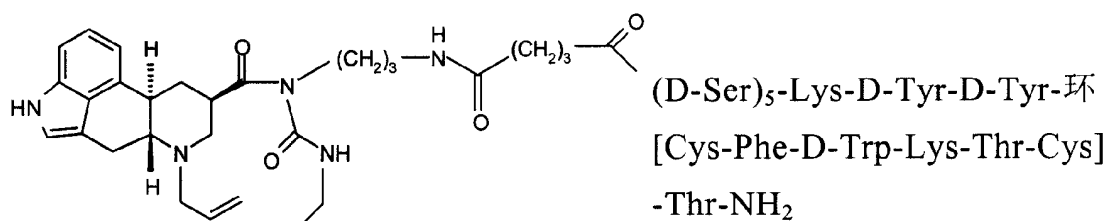




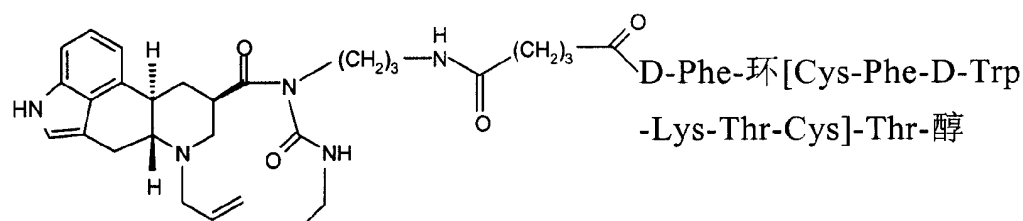
5



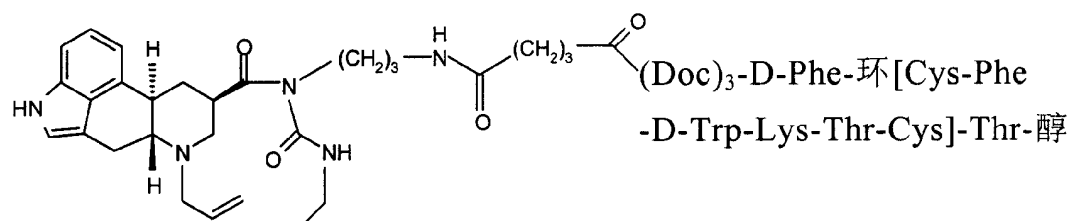
10



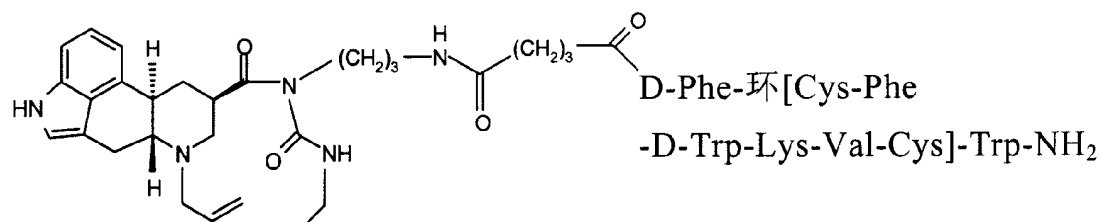
15



20

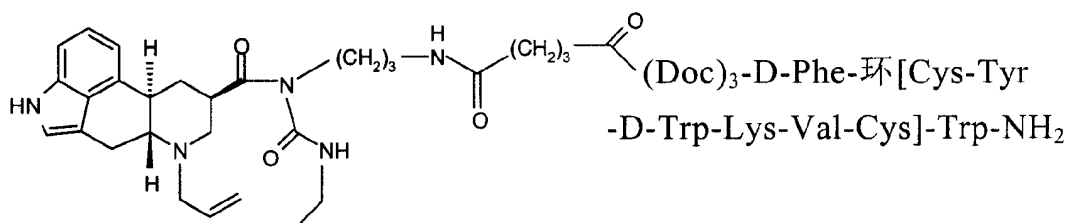


25

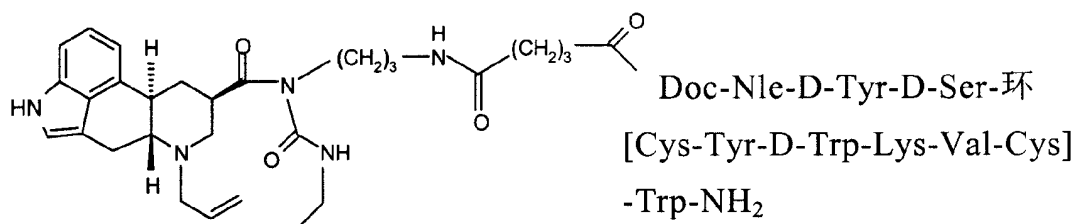


30

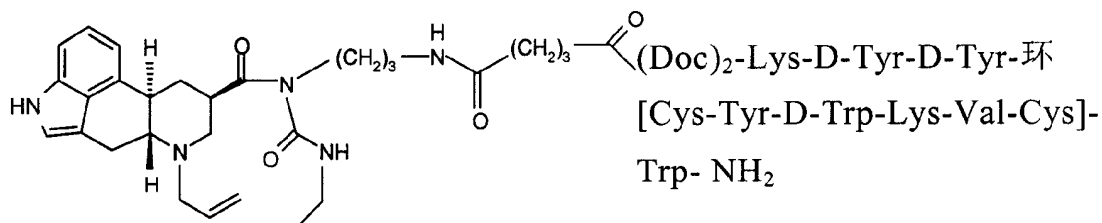
5



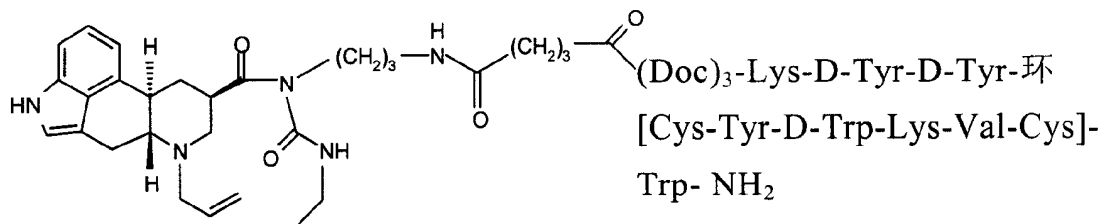
10



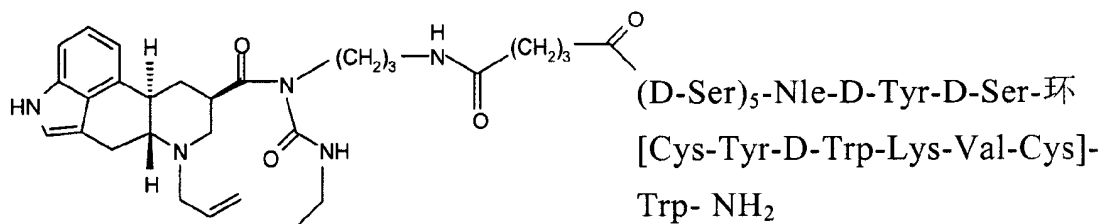
15



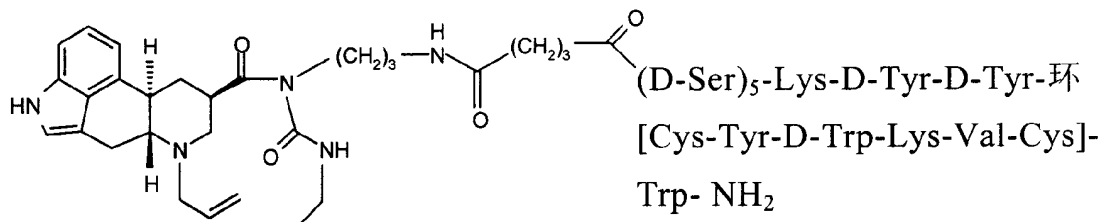
20

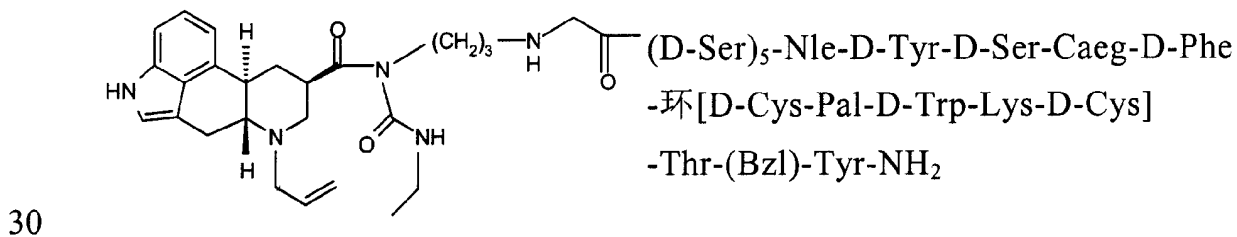
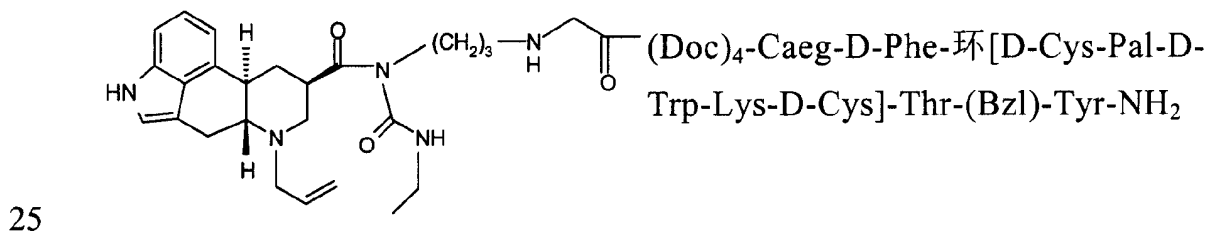
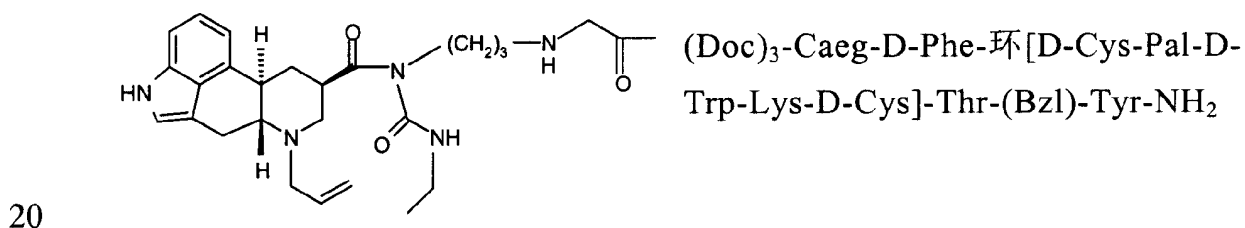
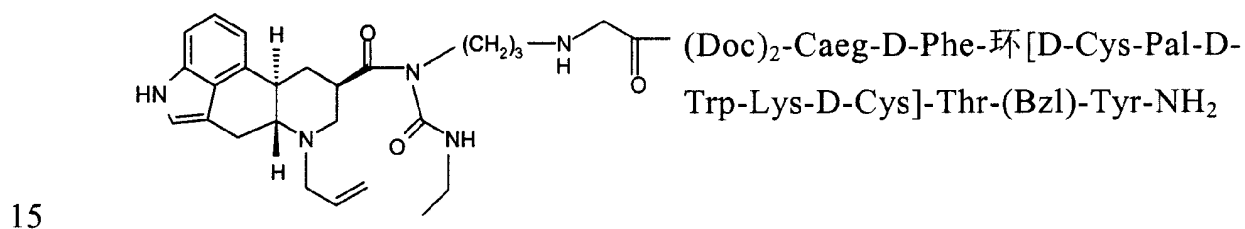
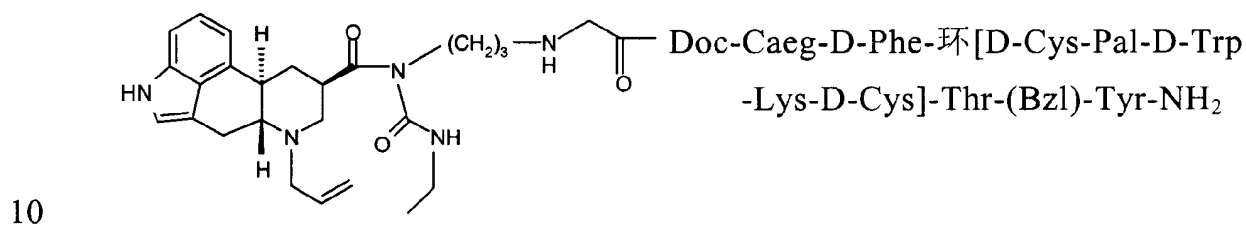
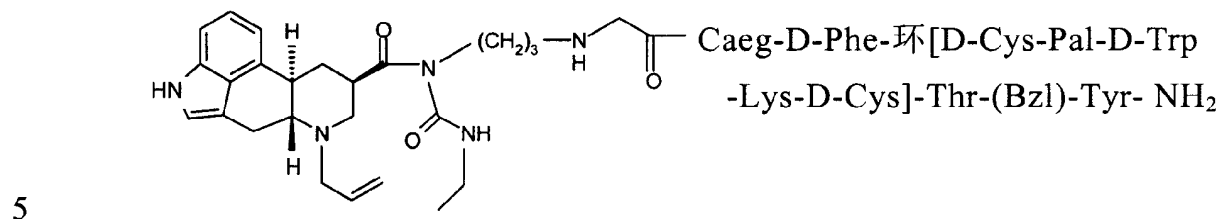


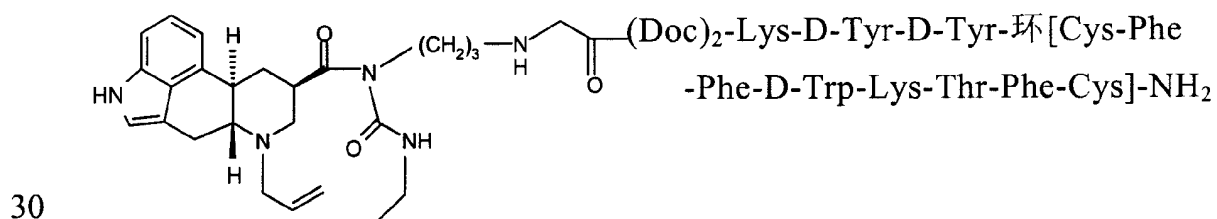
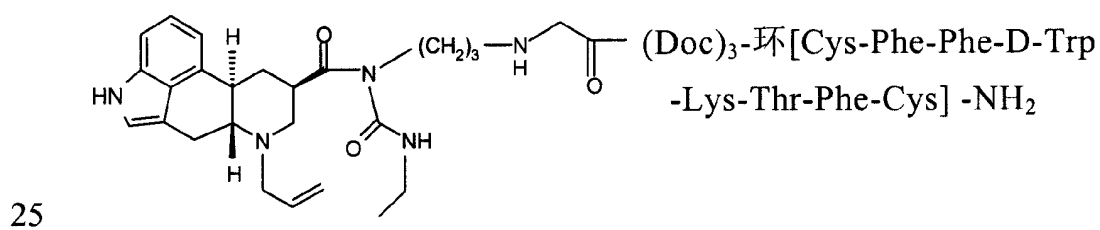
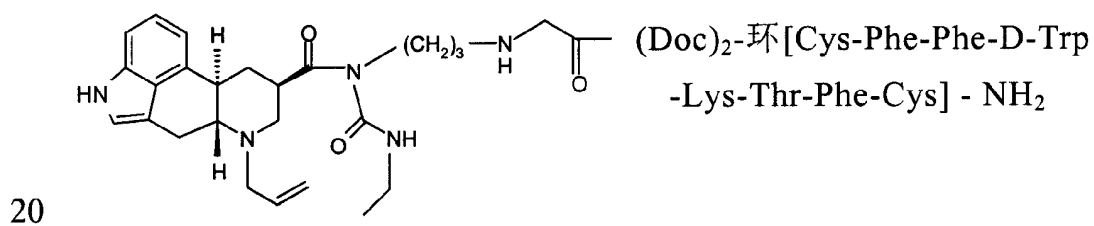
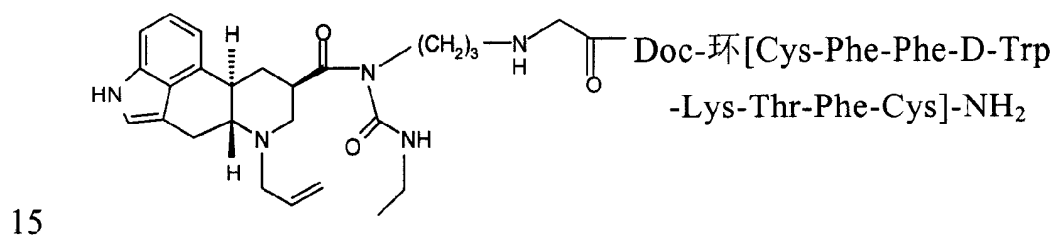
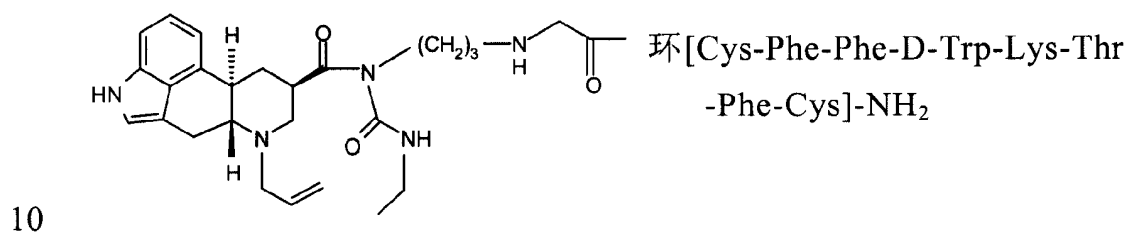
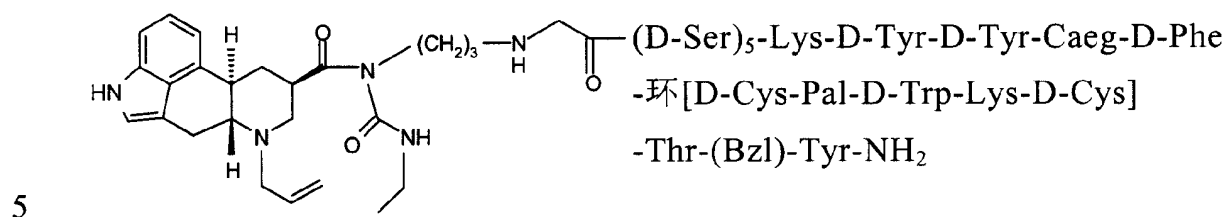
25

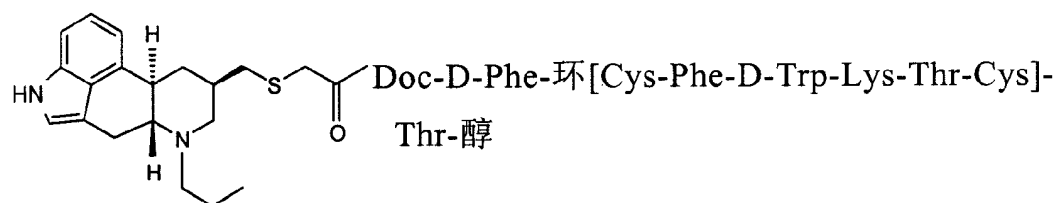
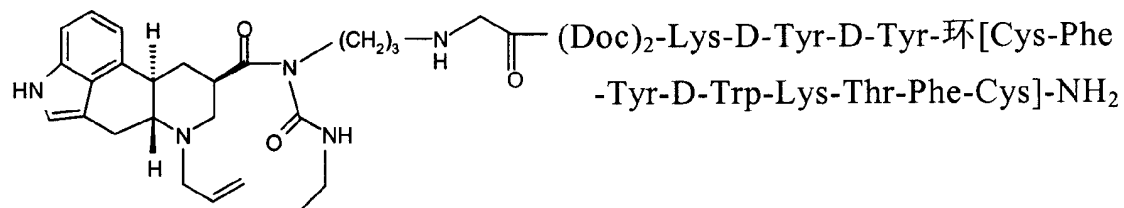
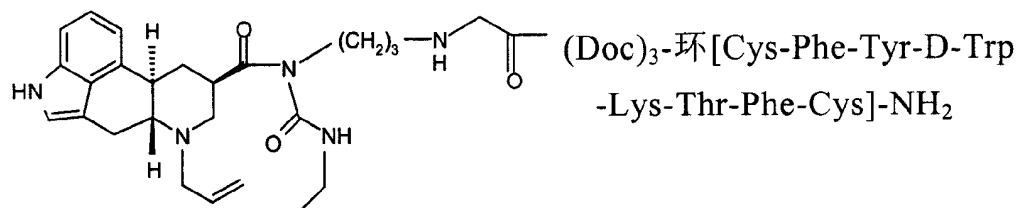
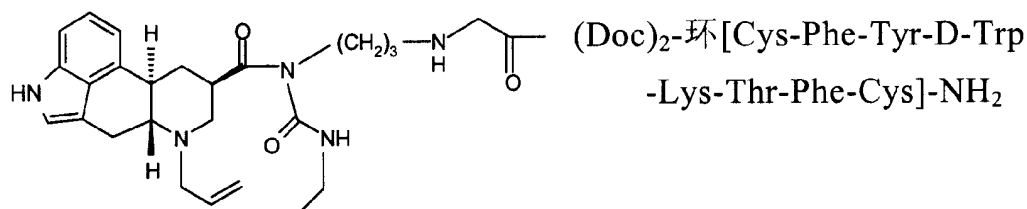
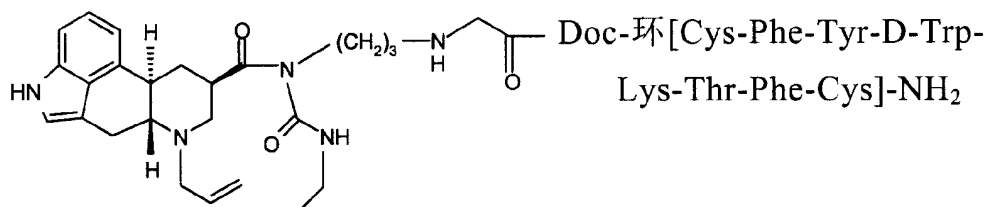
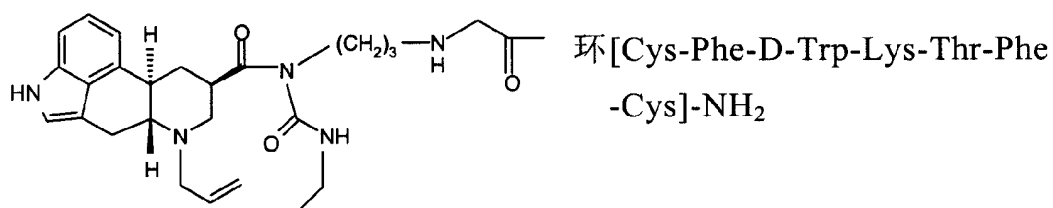


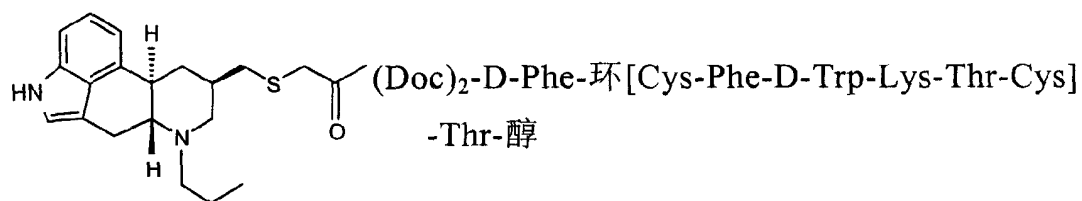
30



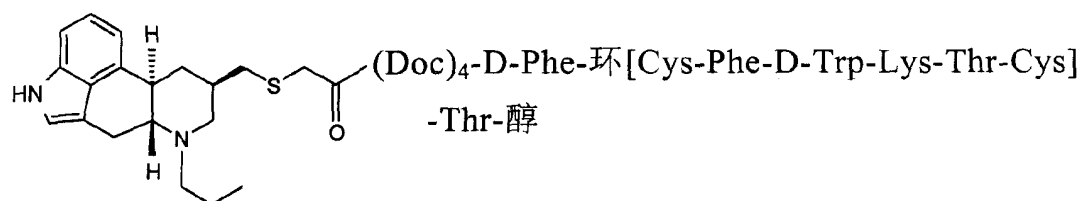




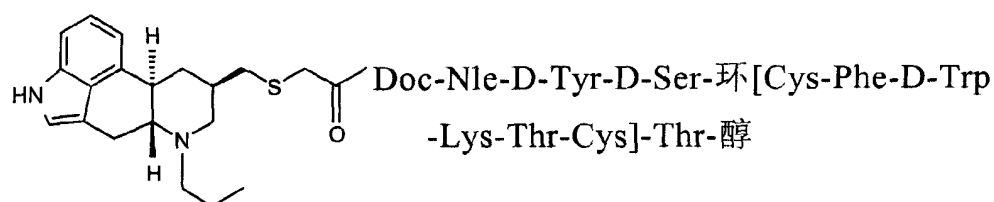




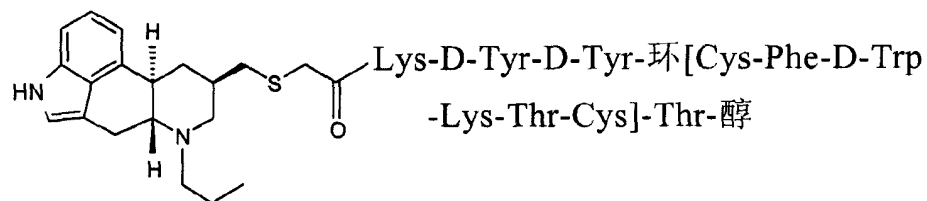
5



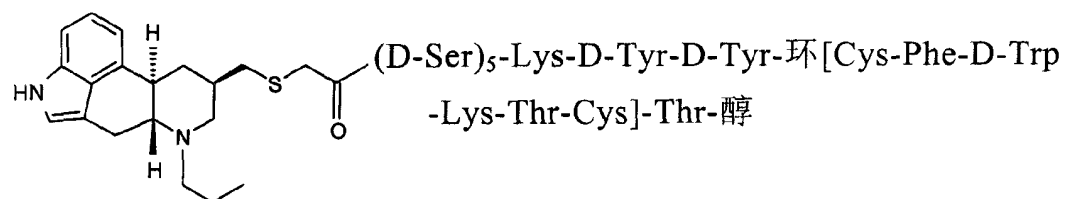
10



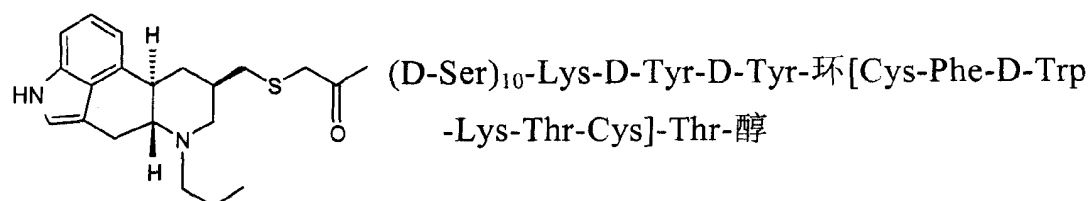
15



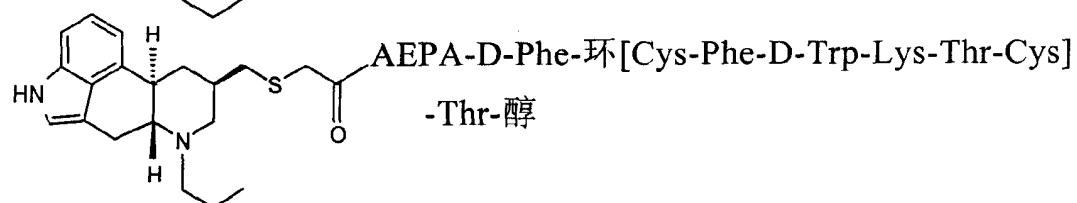
20

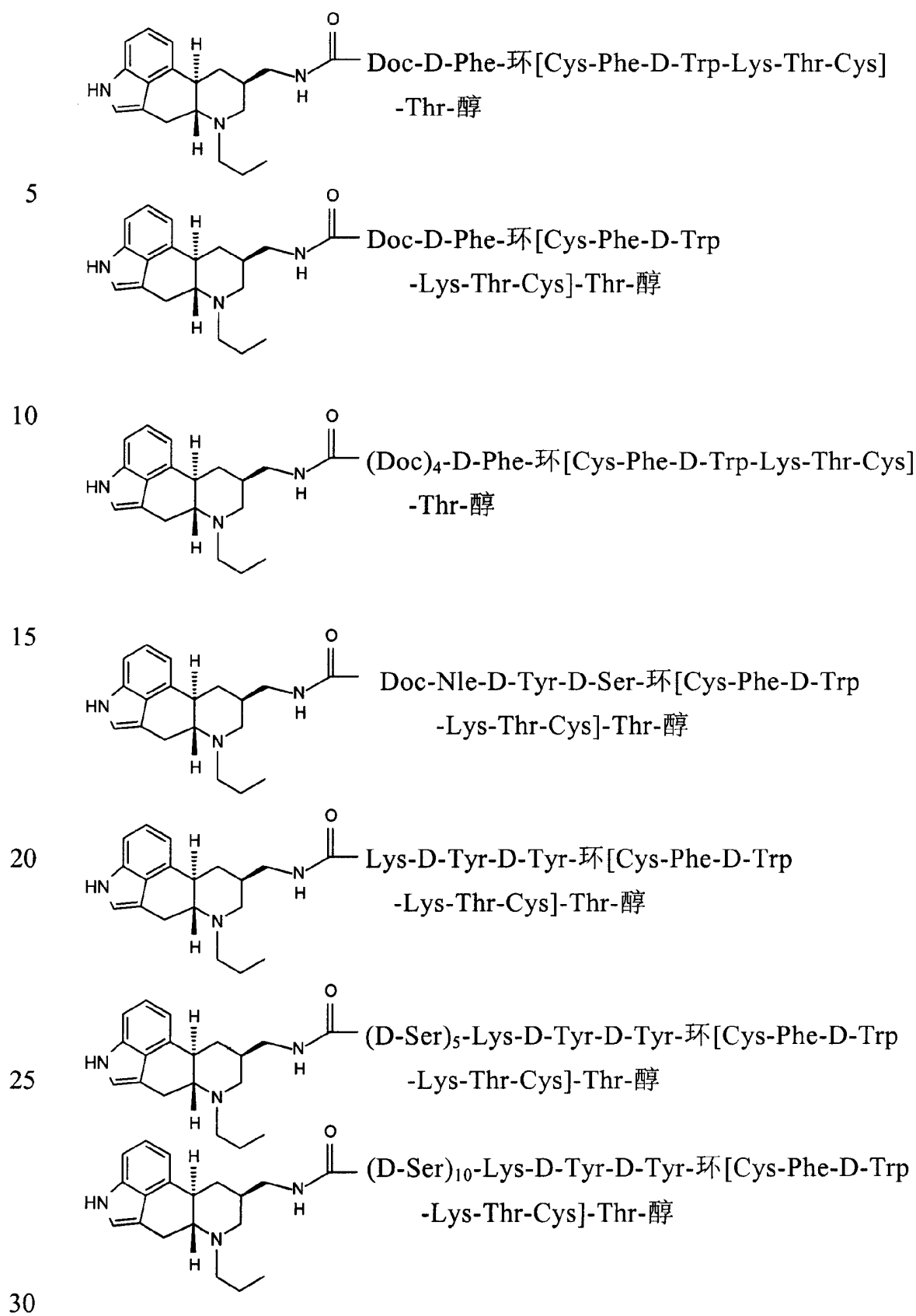


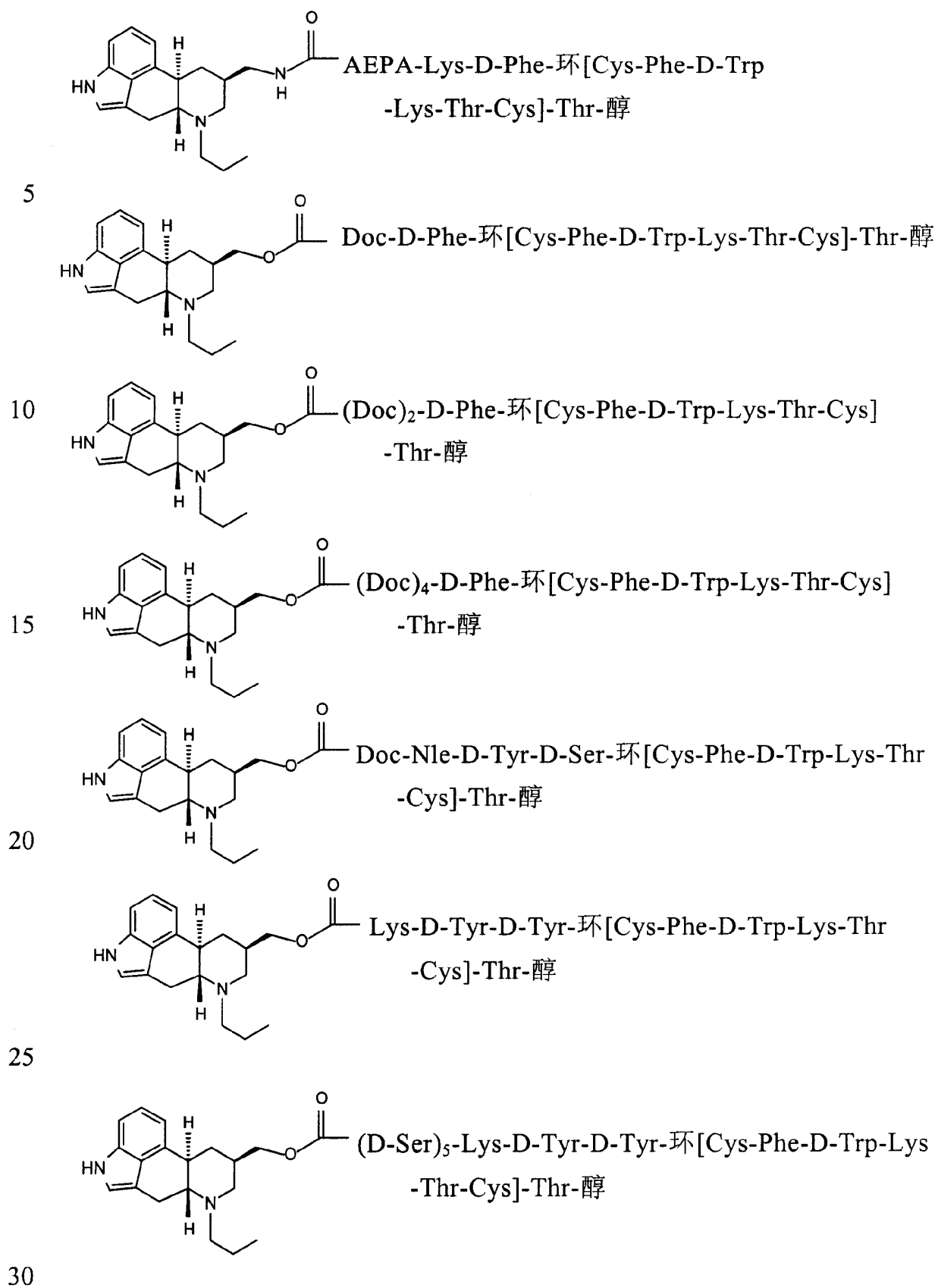
25

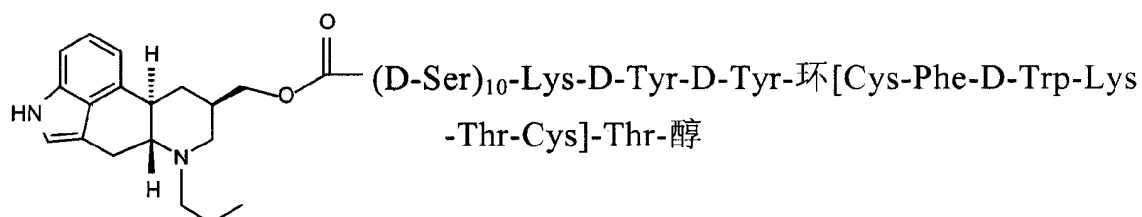


30

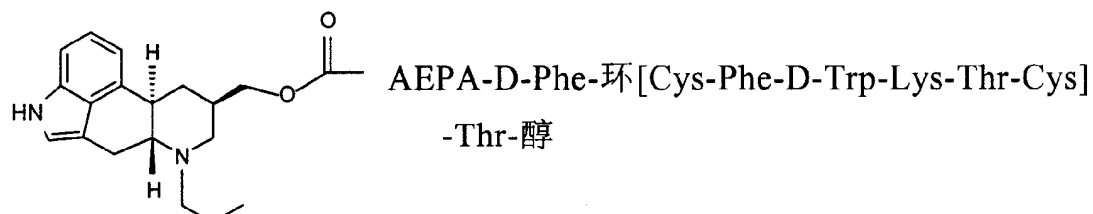




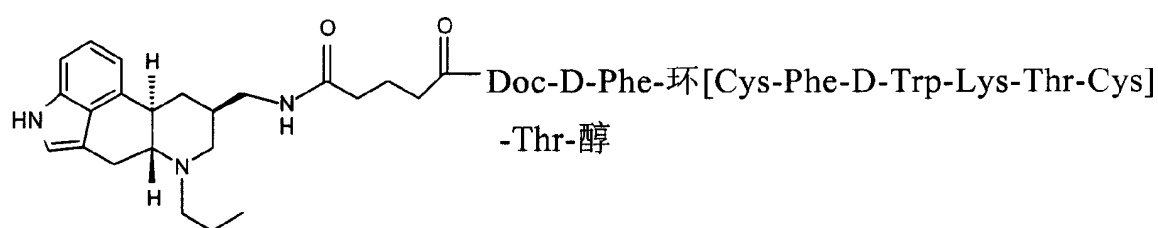




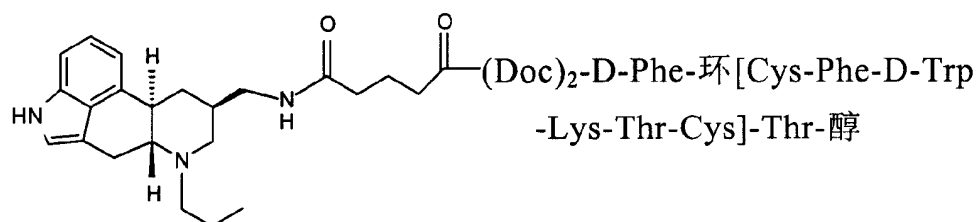
5



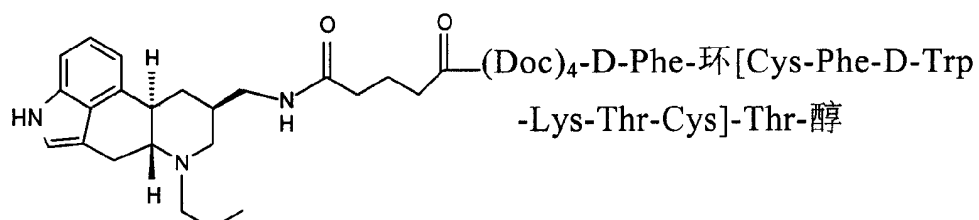
10



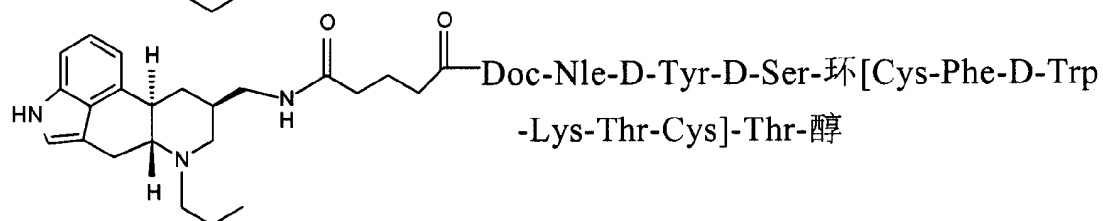
15



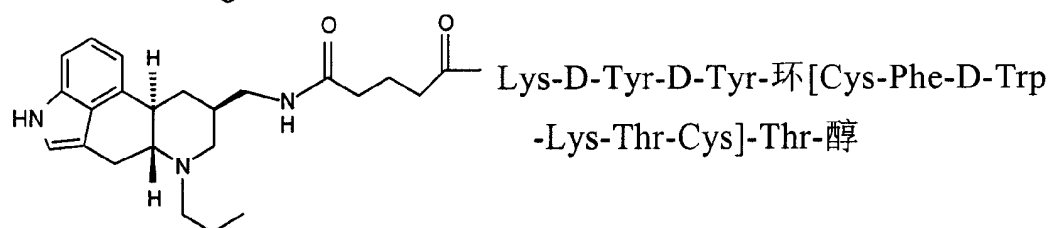
20

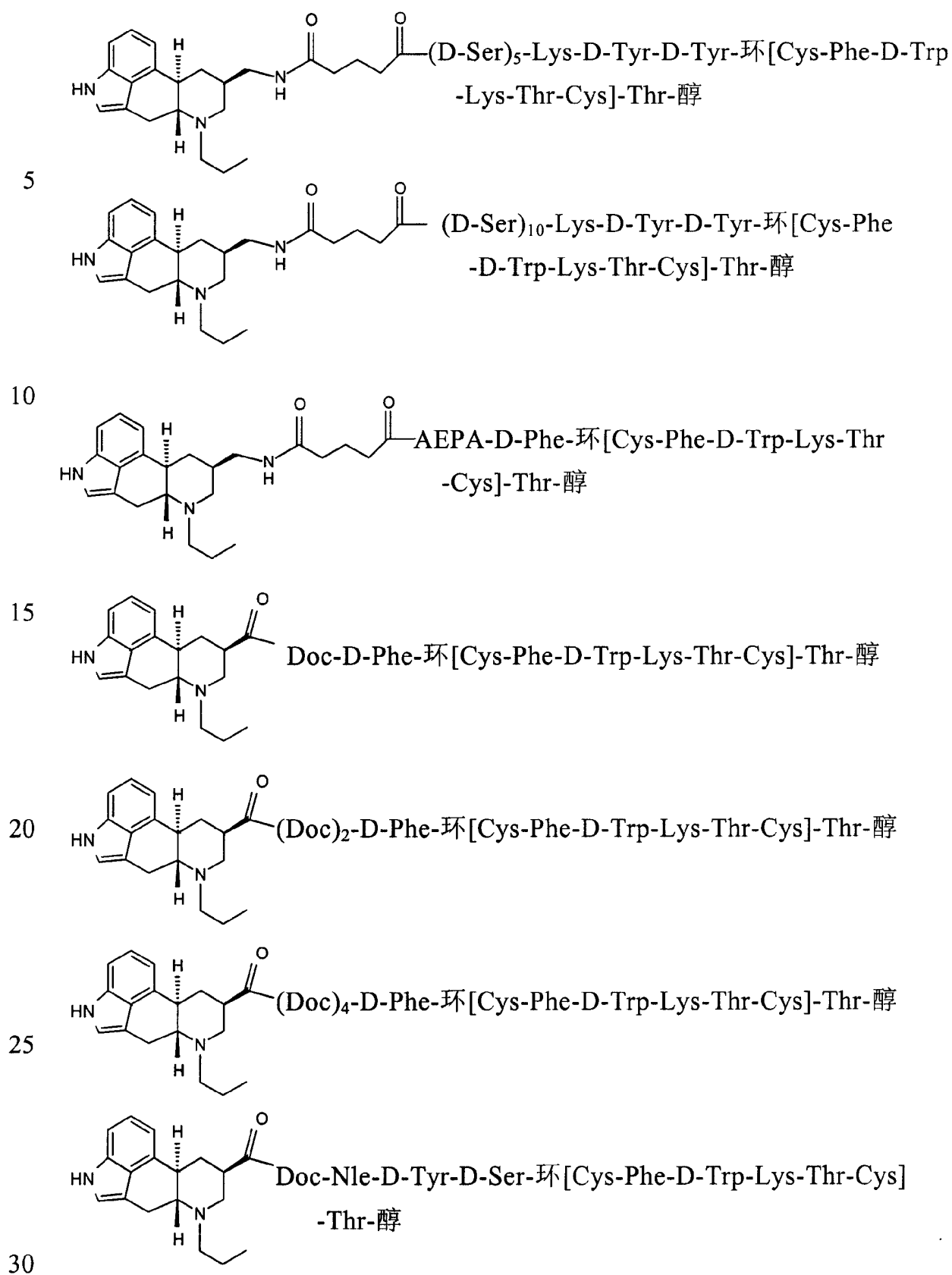


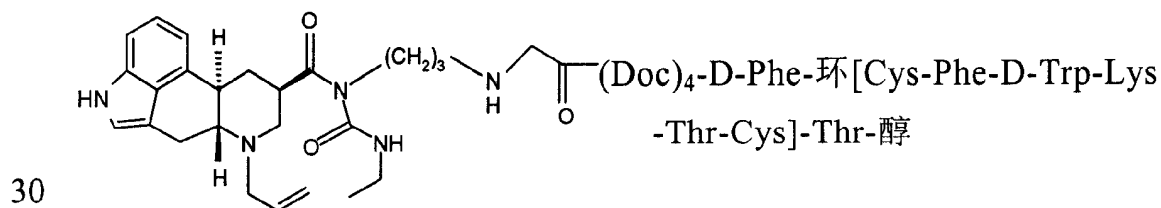
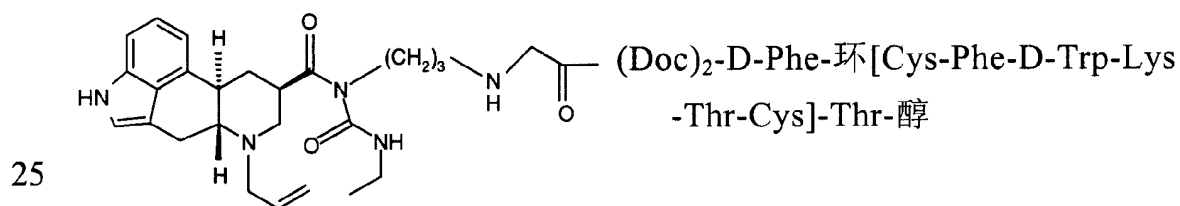
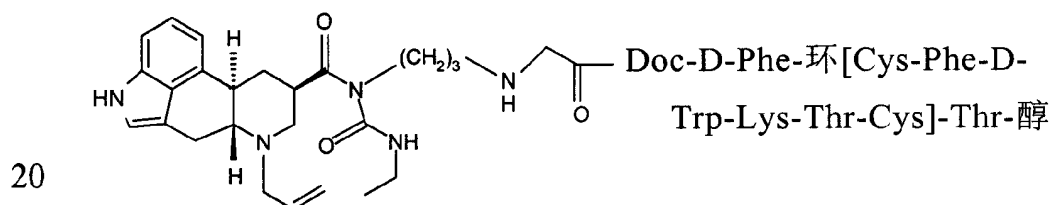
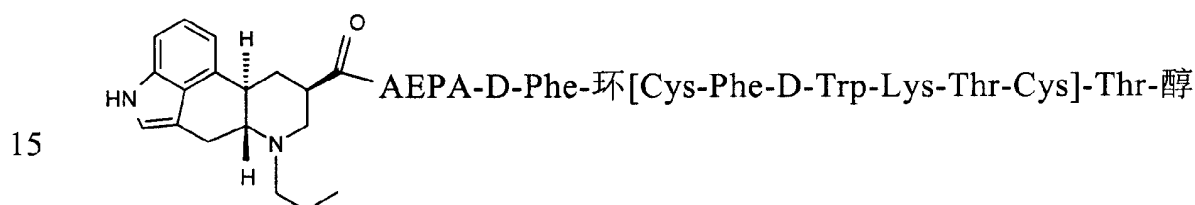
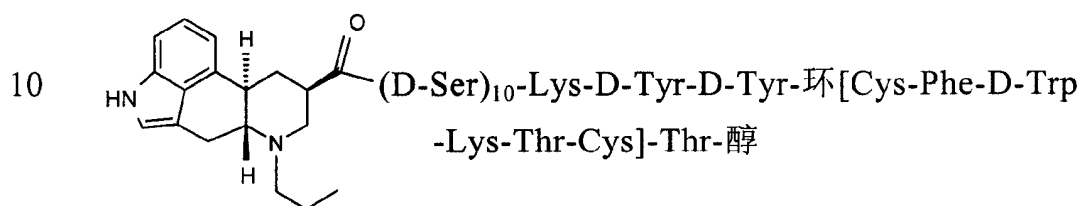
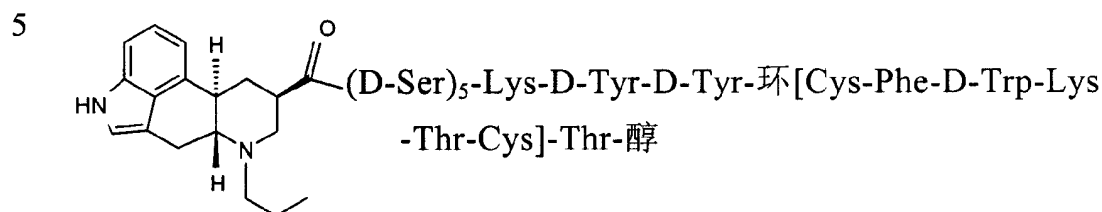
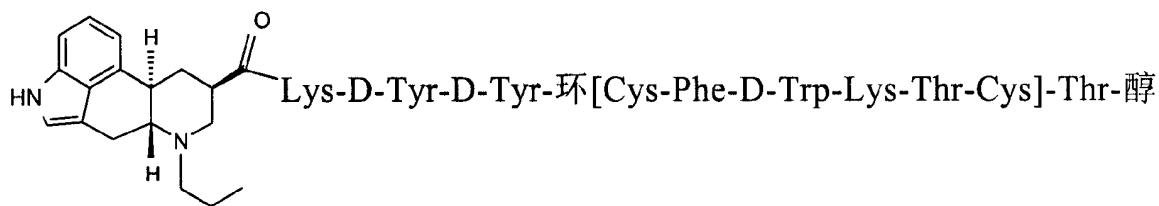
25

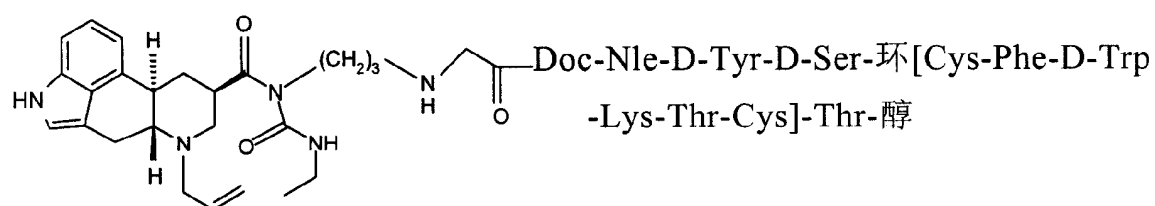


30

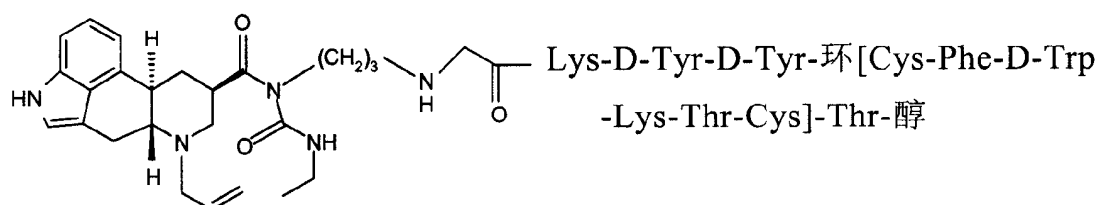




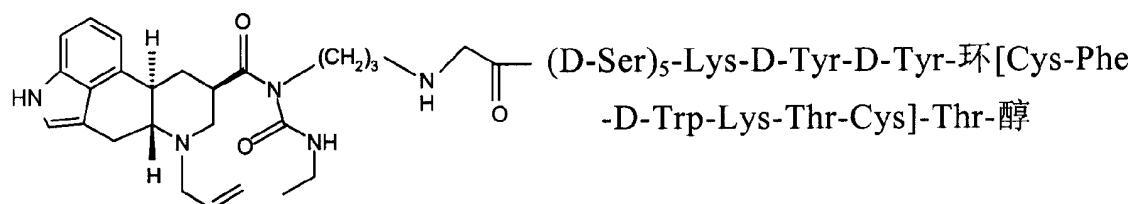




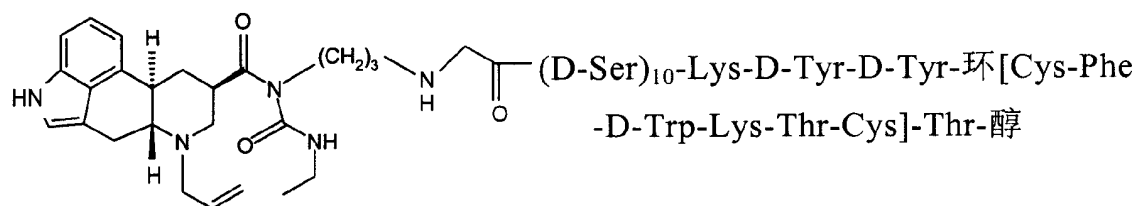
5



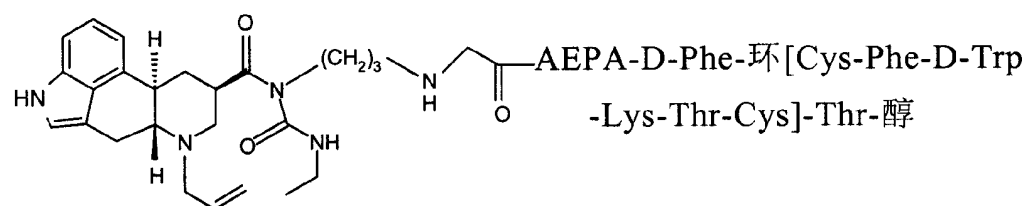
10



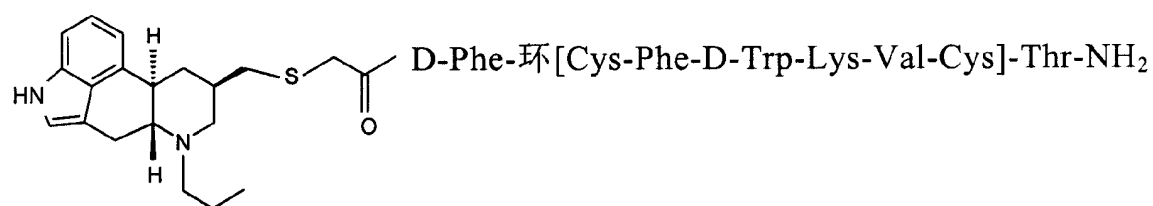
15



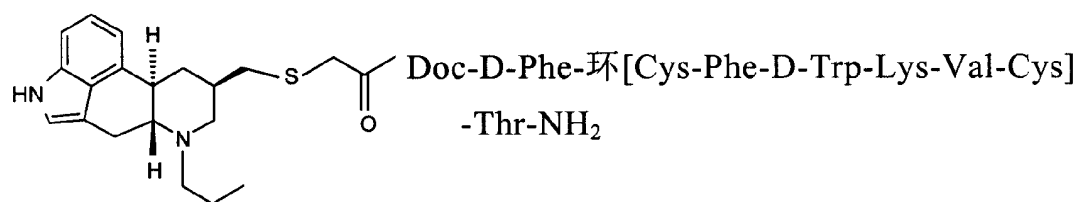
20



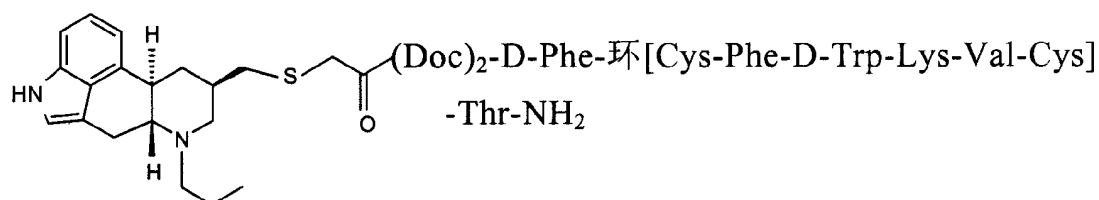
25



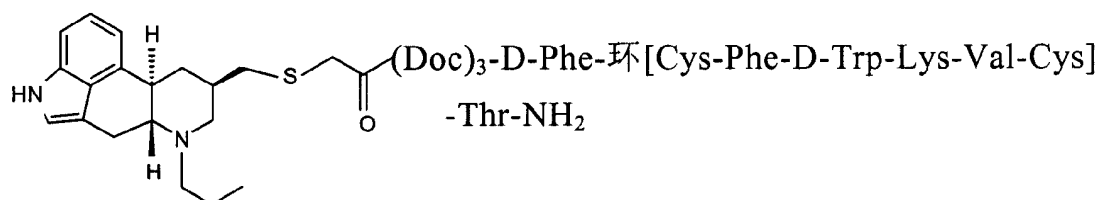
30



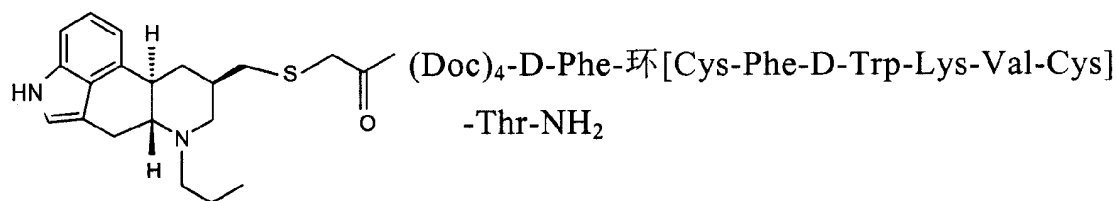
5



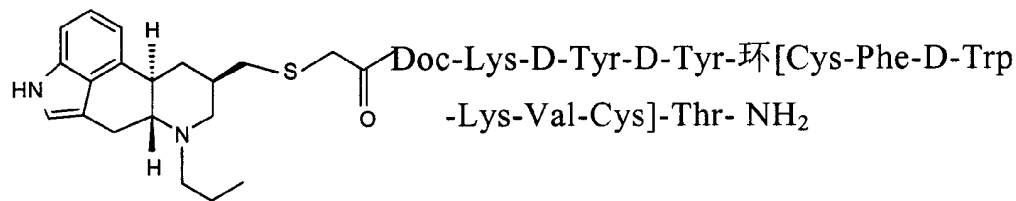
10



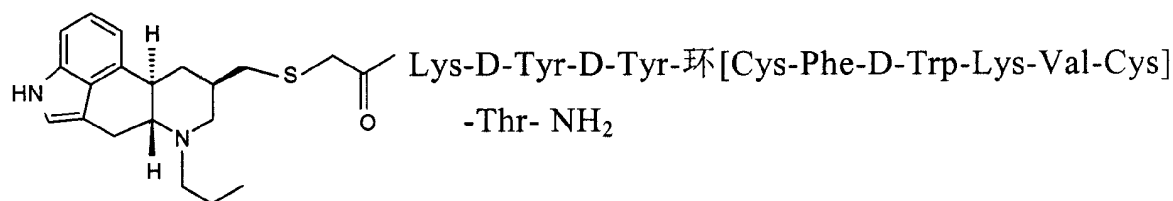
15



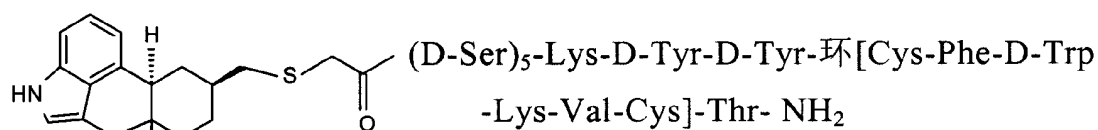
20



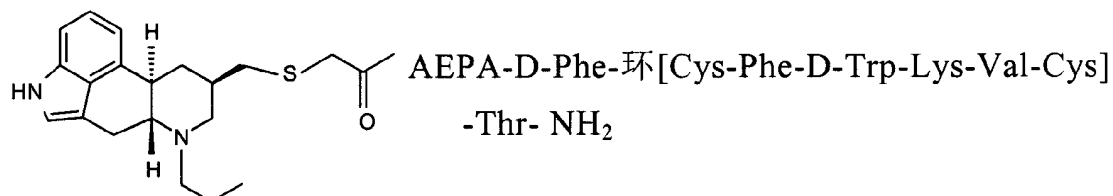
25



30

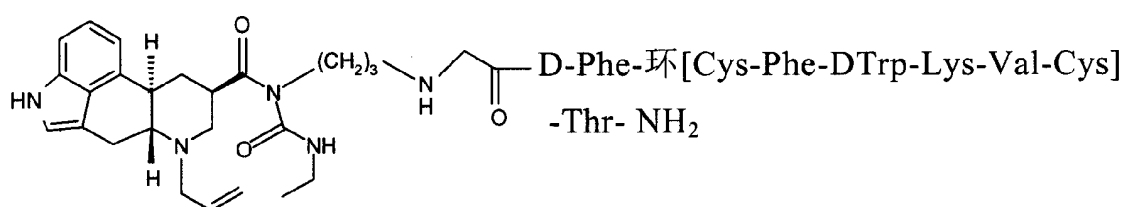


5

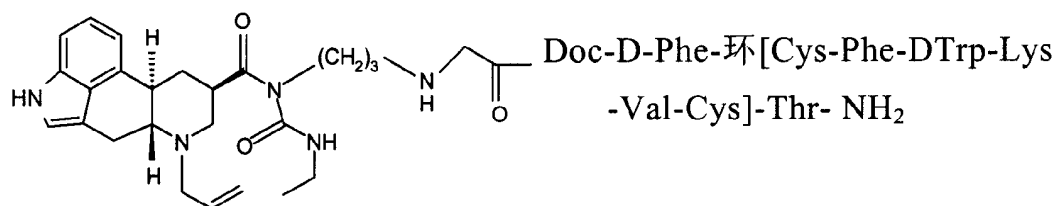


AEPA=4-(2-氨基乙基)-1-羧甲基-哌嗪

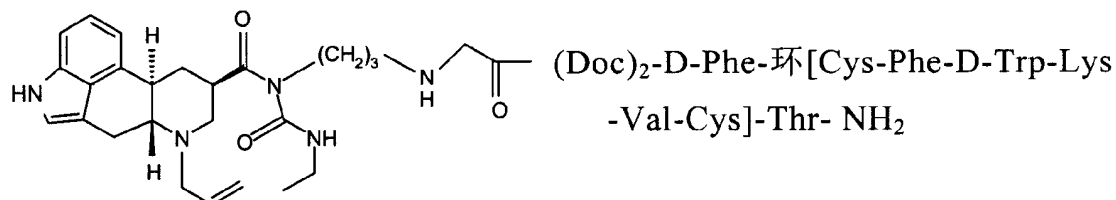
10



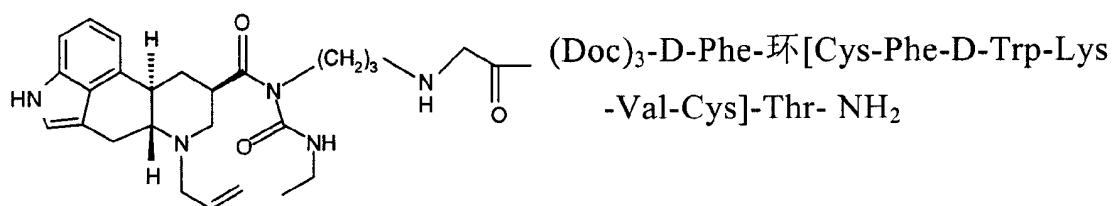
15



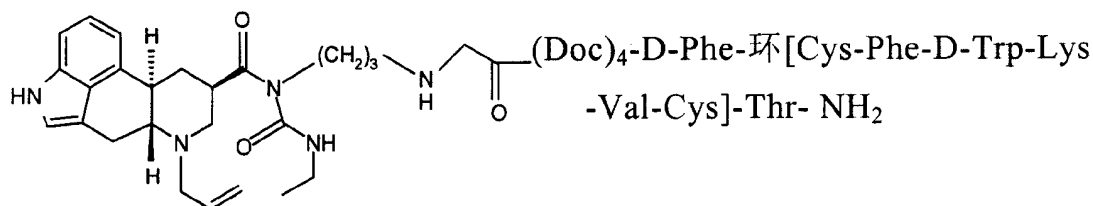
20

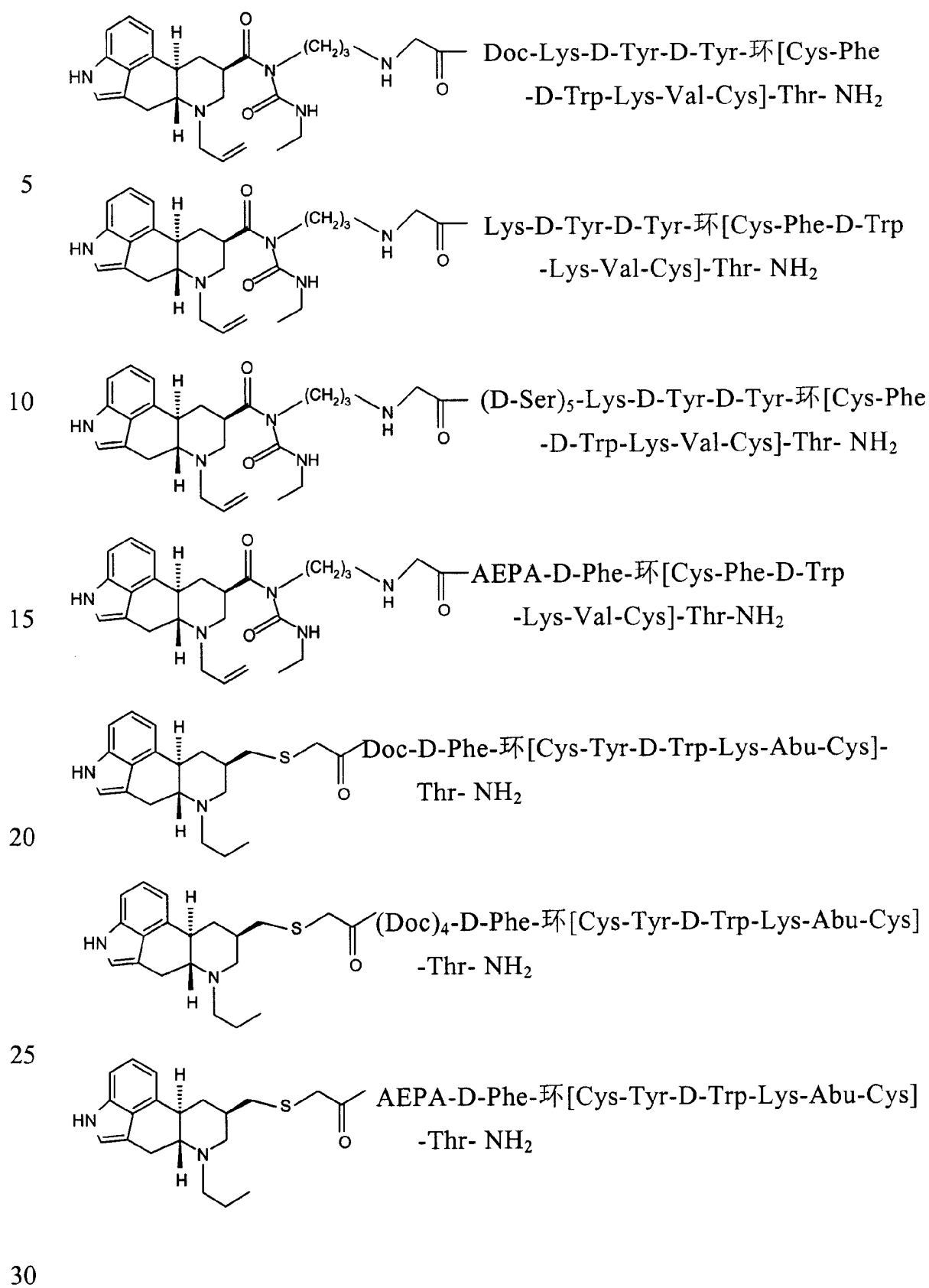


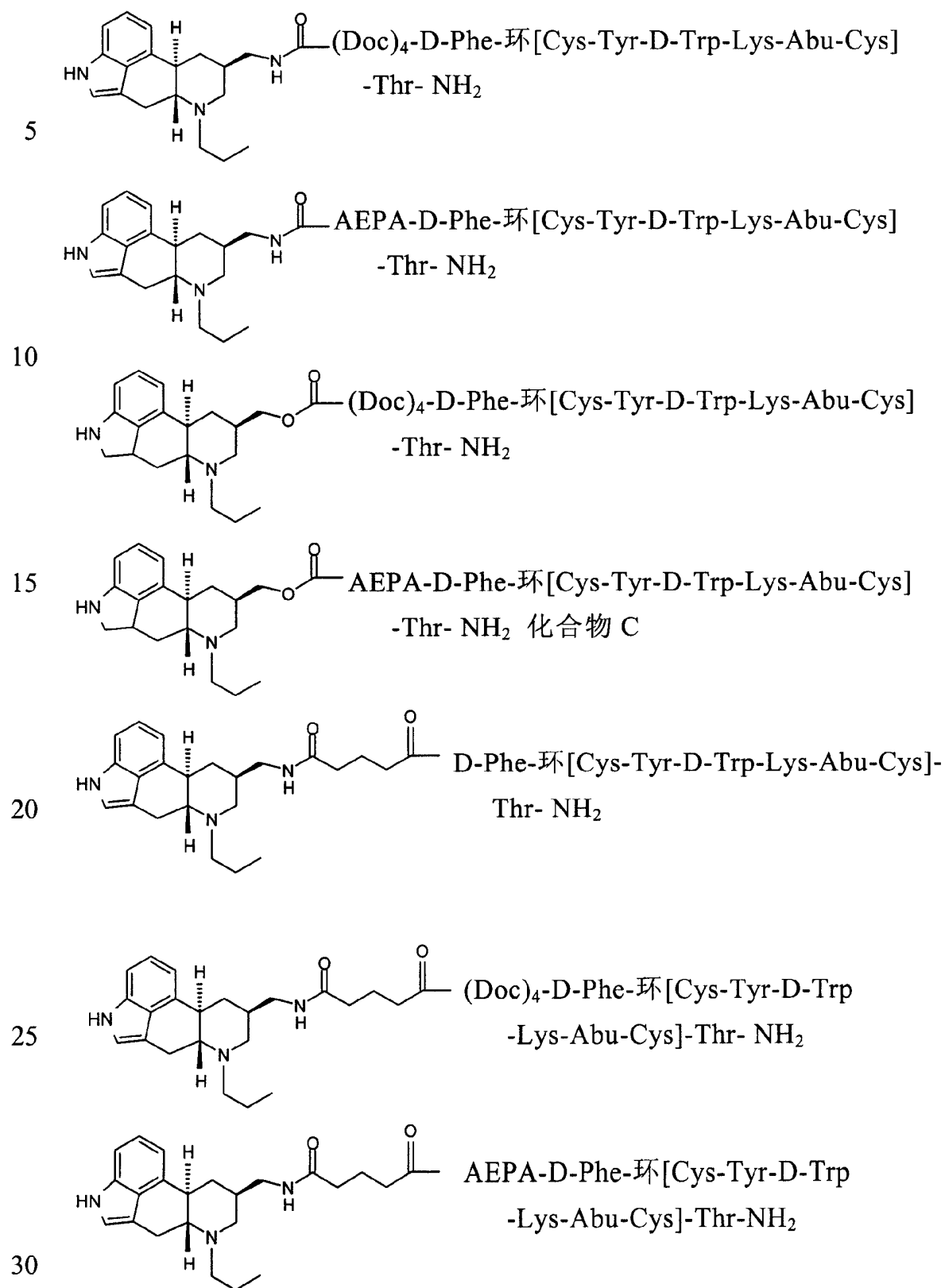
25

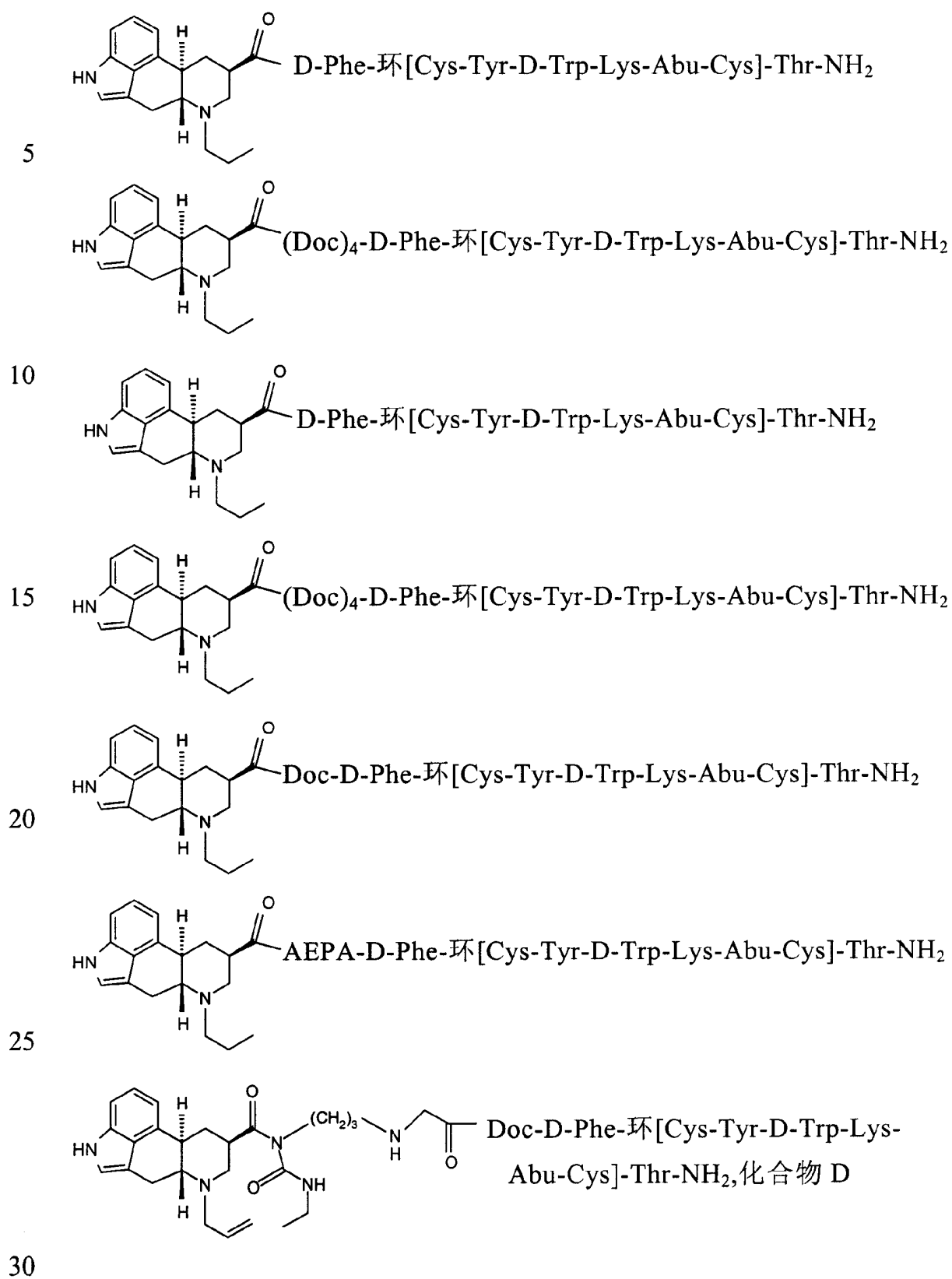


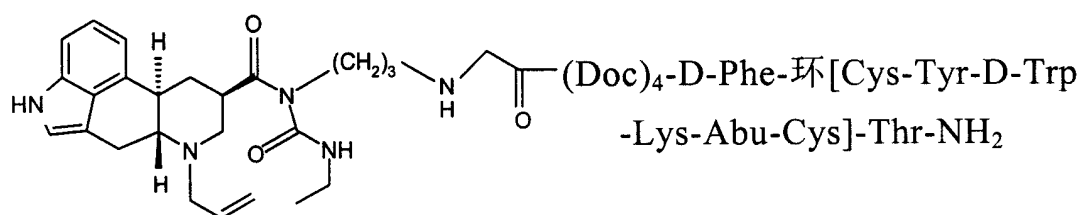
30



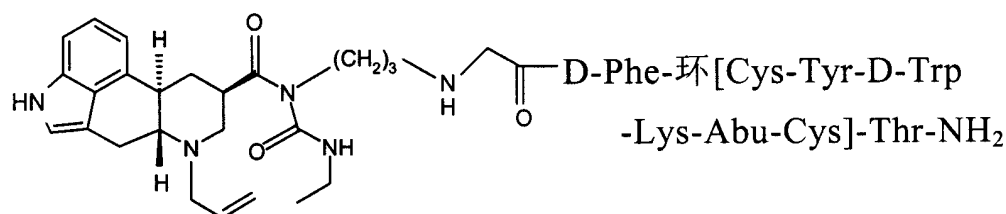




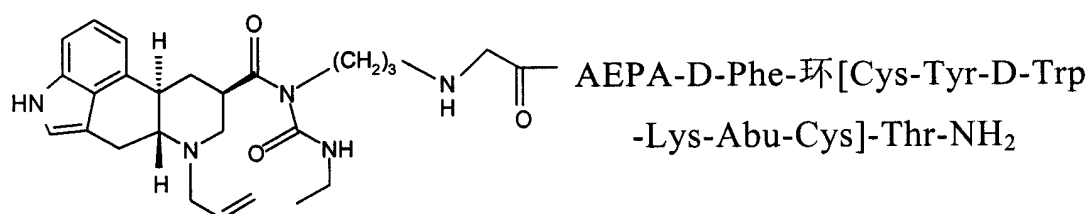




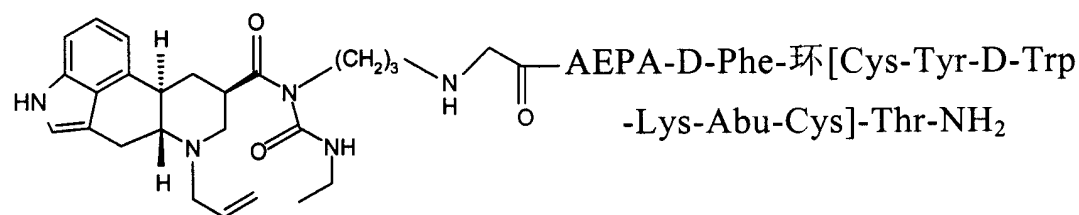
5



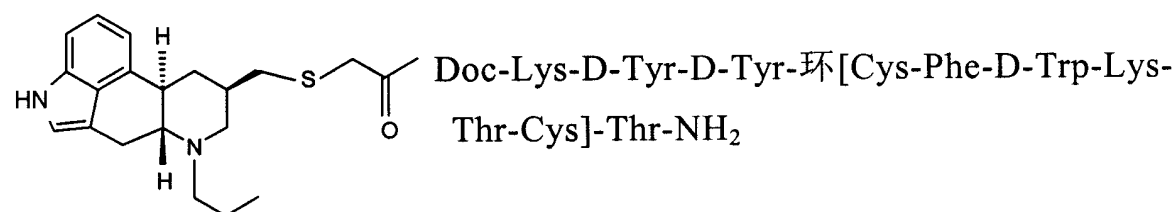
10



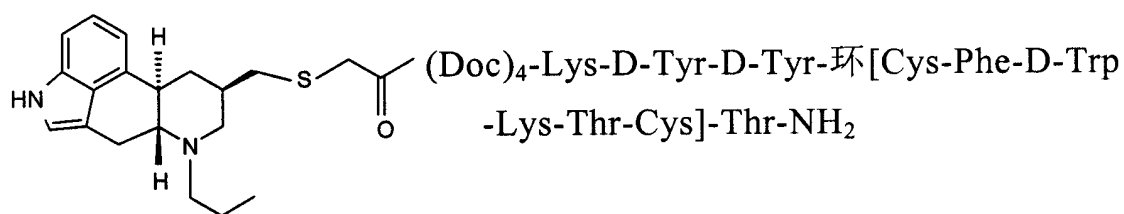
15



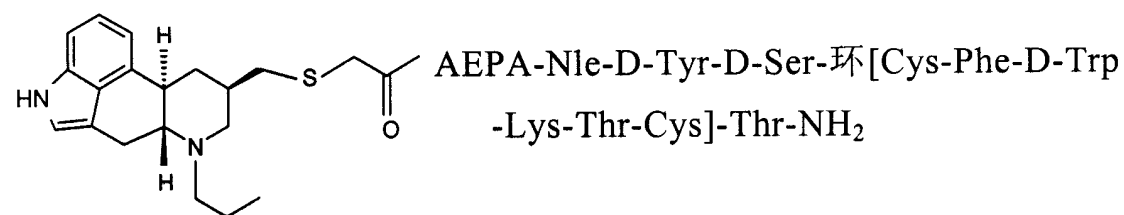
20

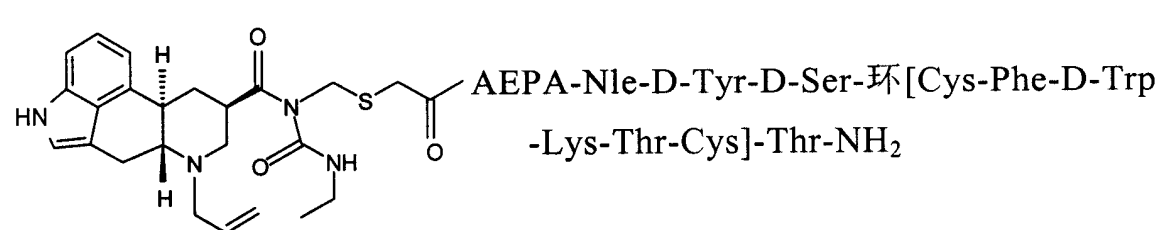
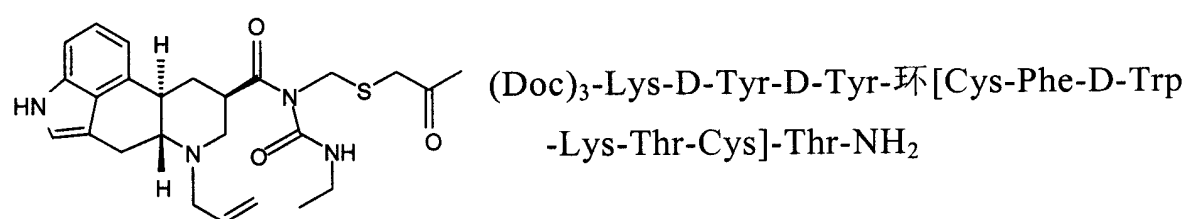
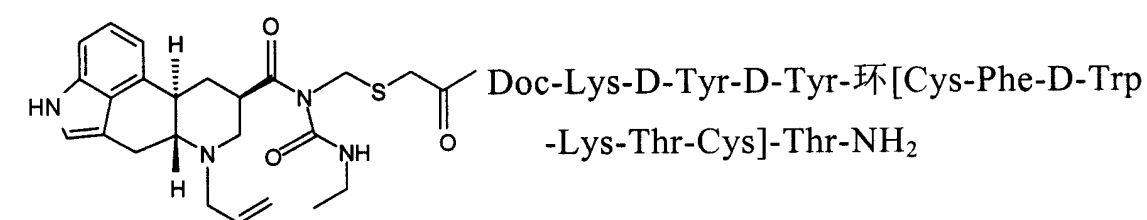
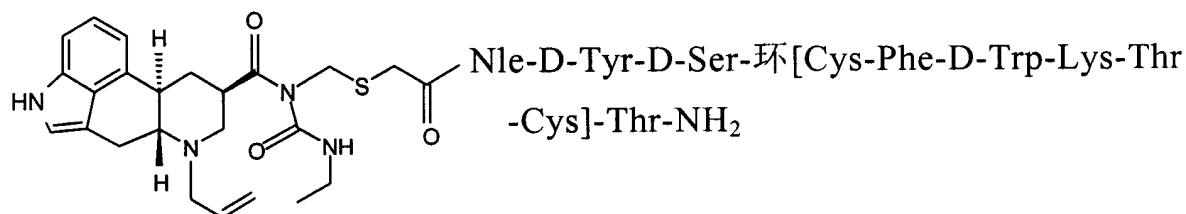
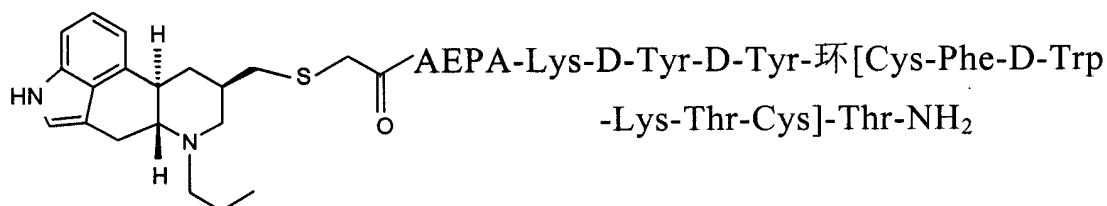


25

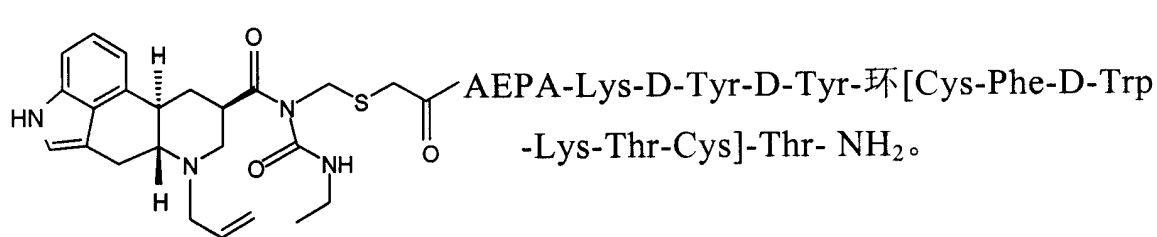


30

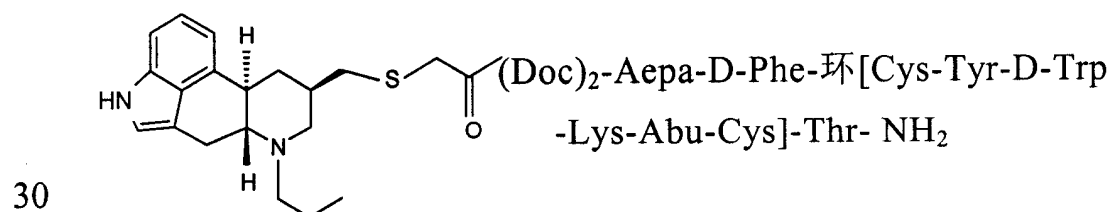
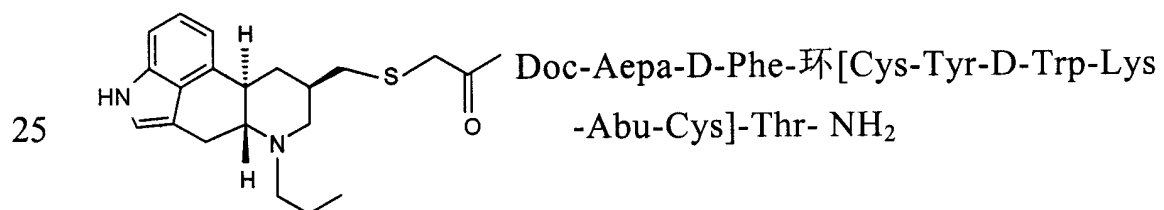
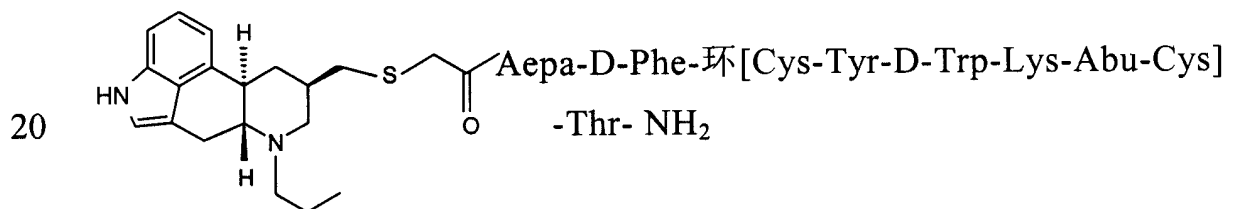
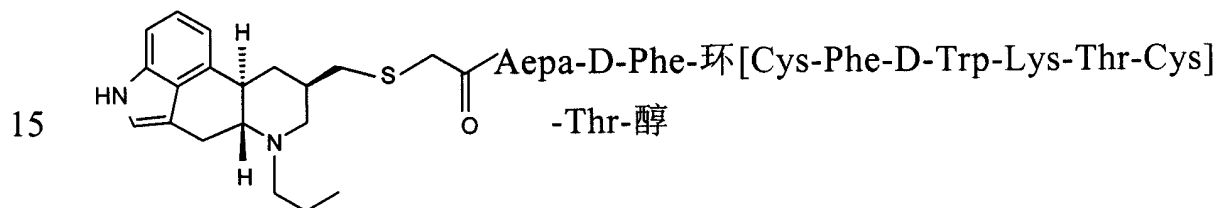
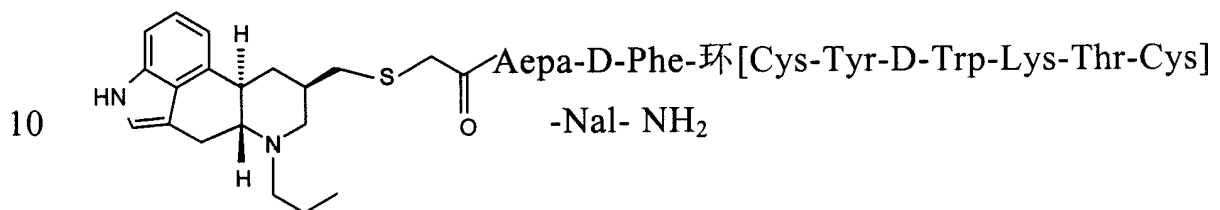
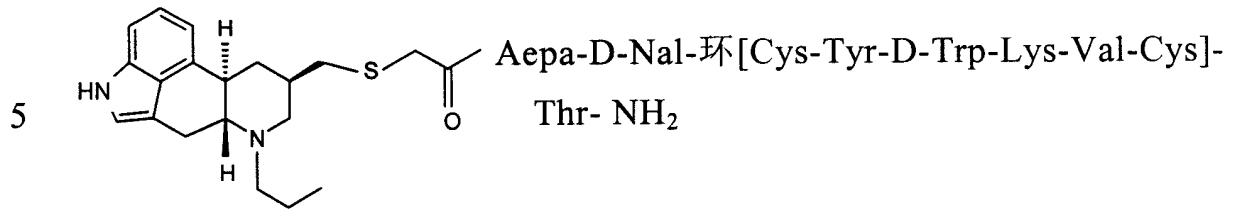


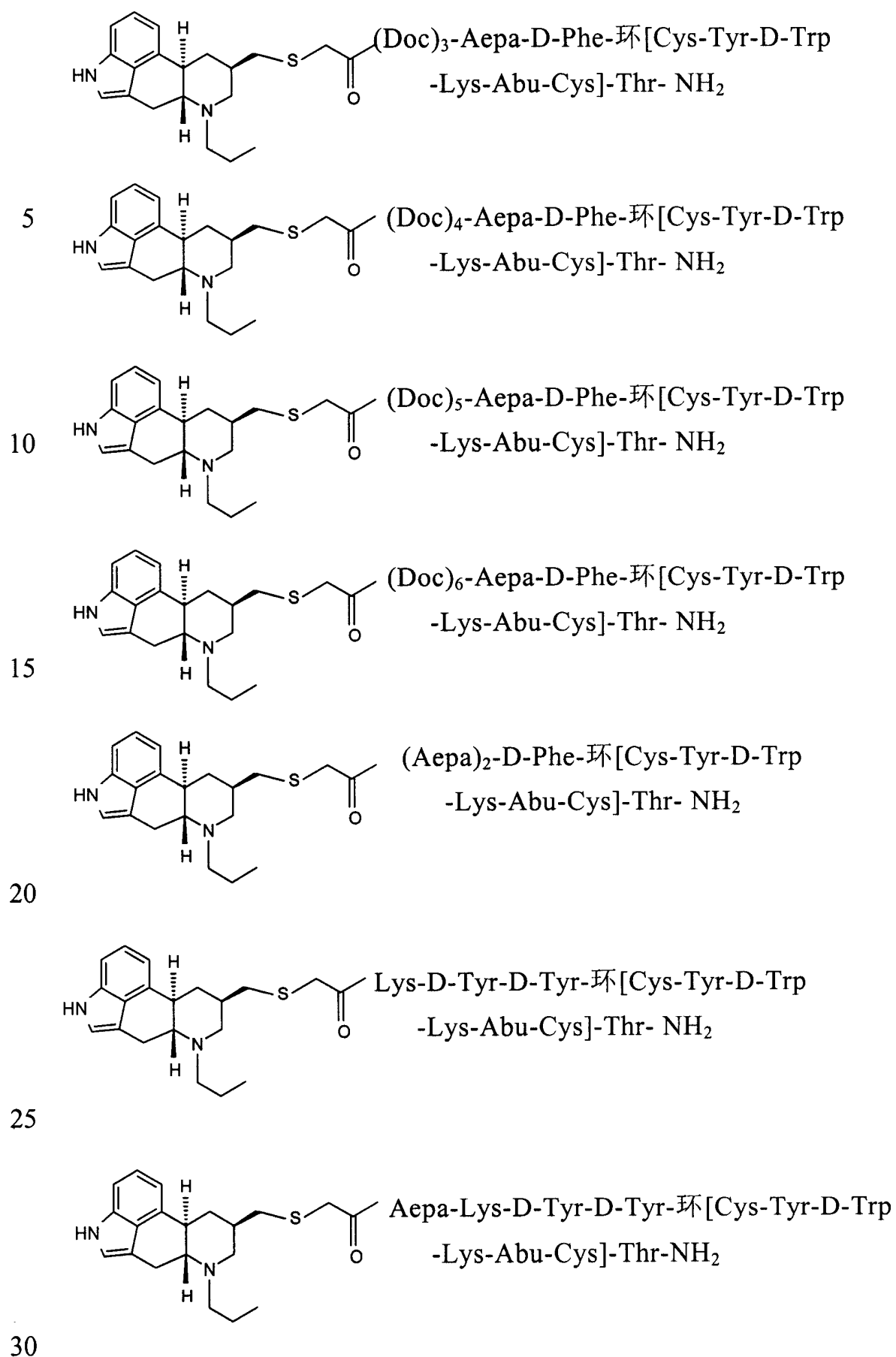


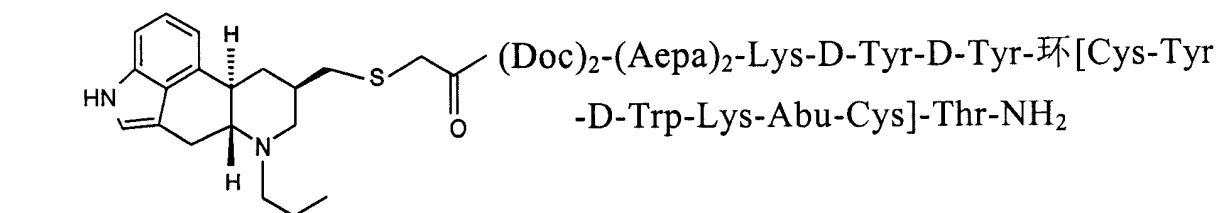
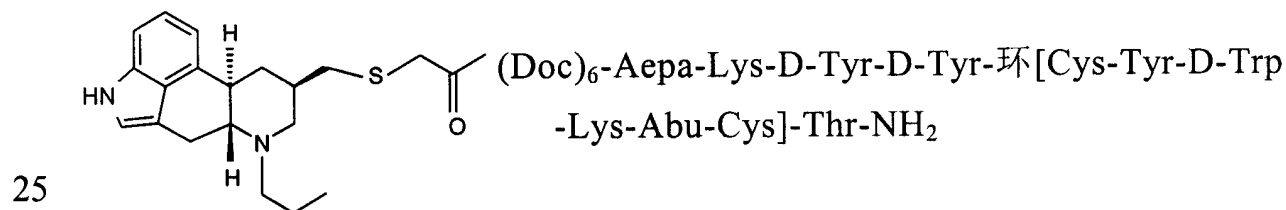
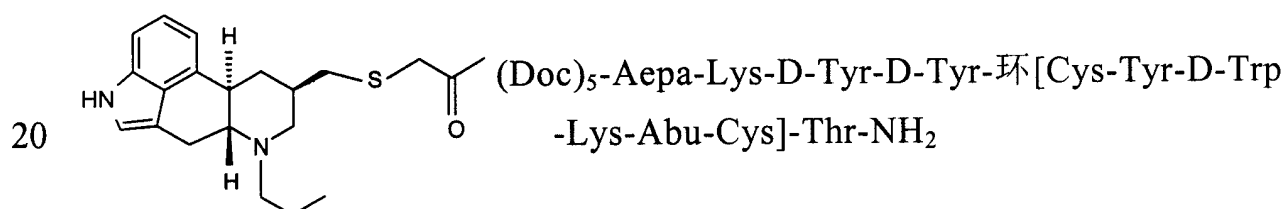
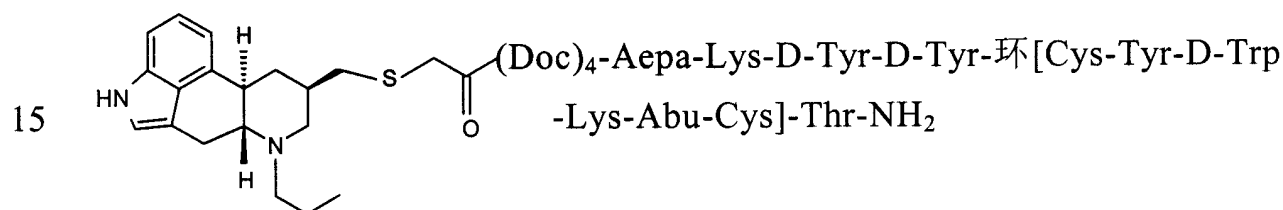
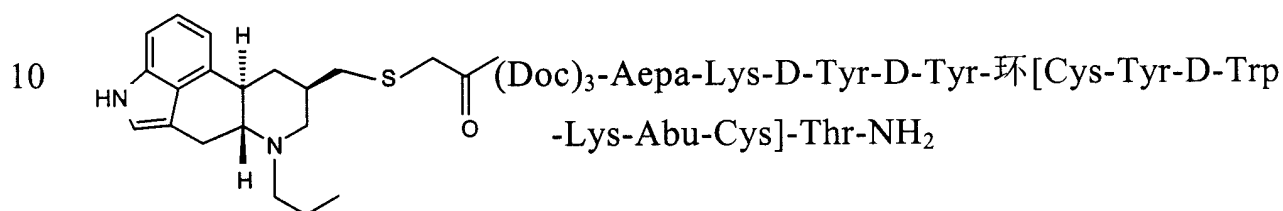
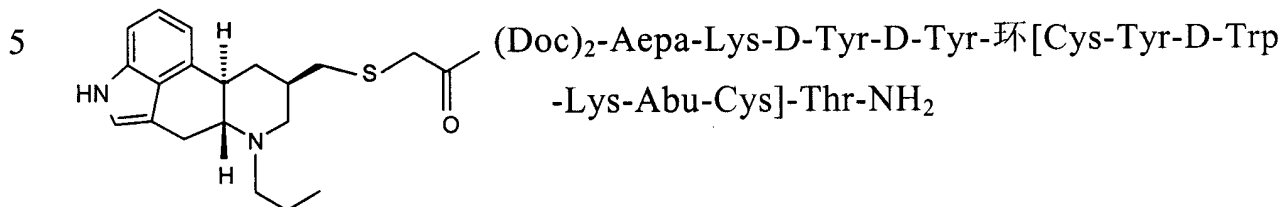
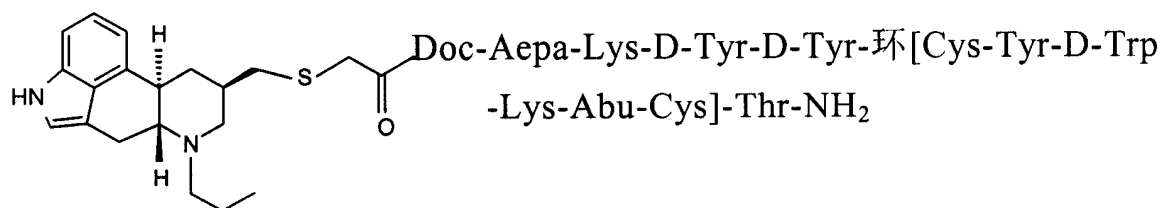
以及

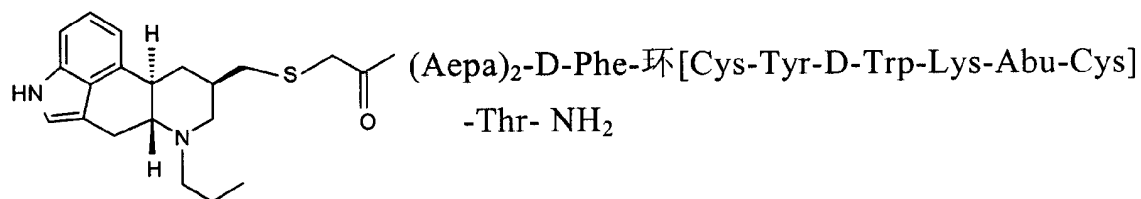


4、下式化合物或其药学上可接受的盐：

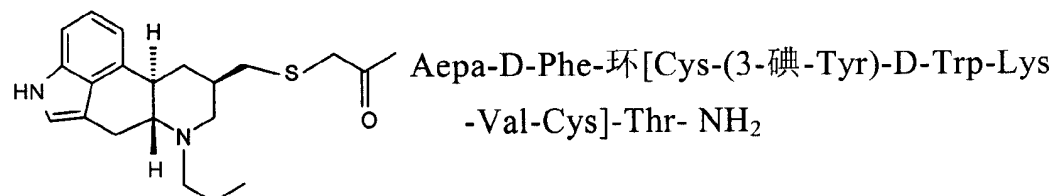




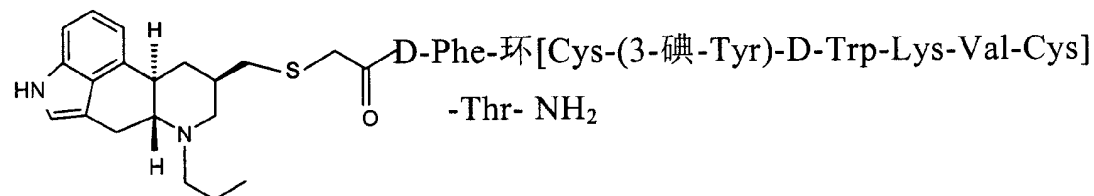




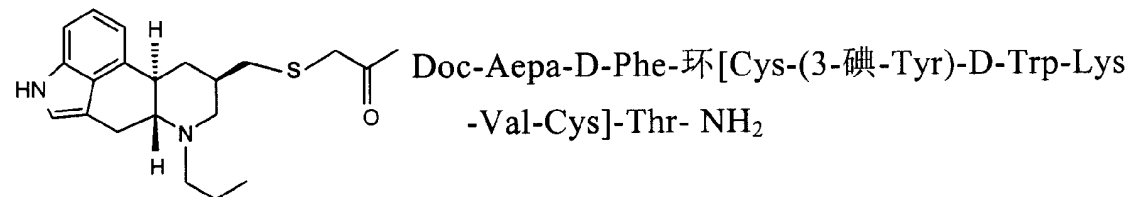
5



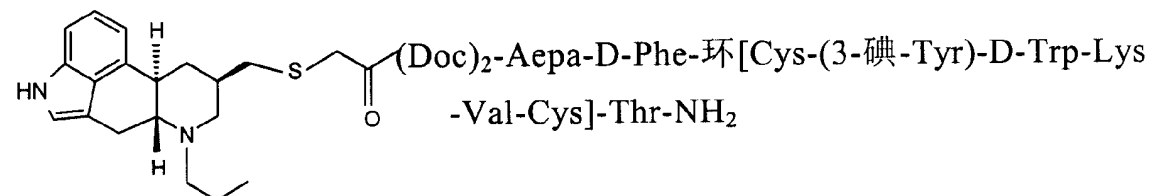
10



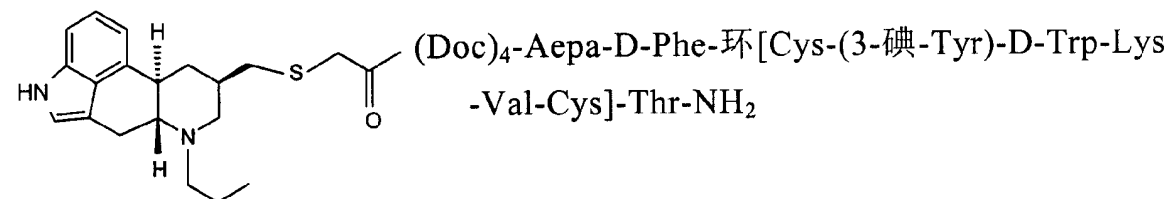
15



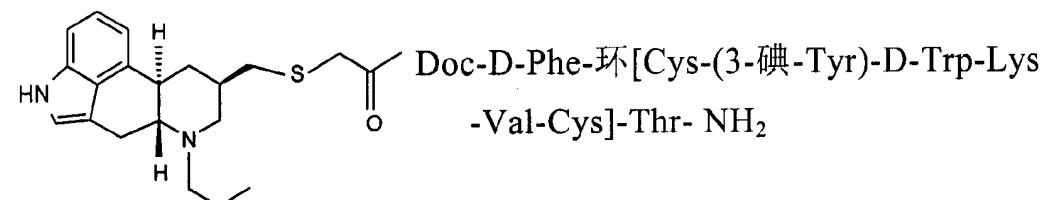
20

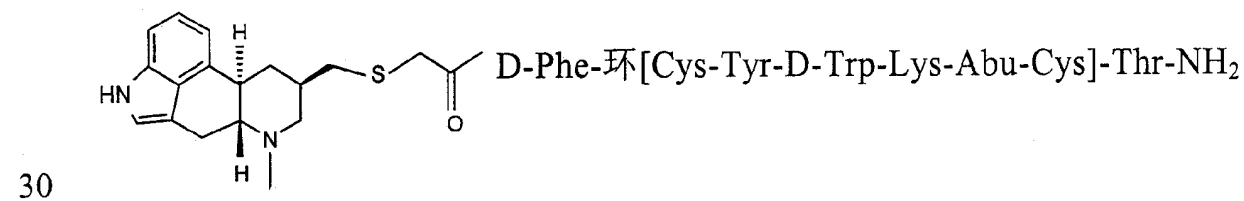
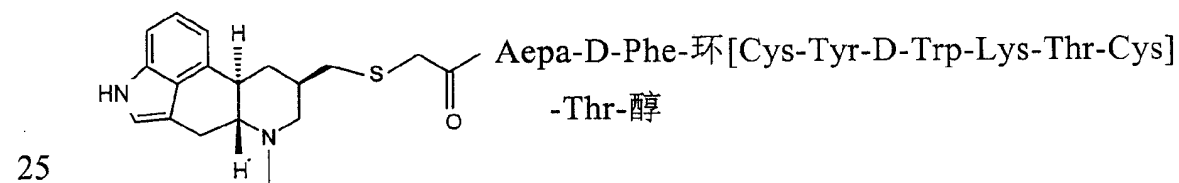
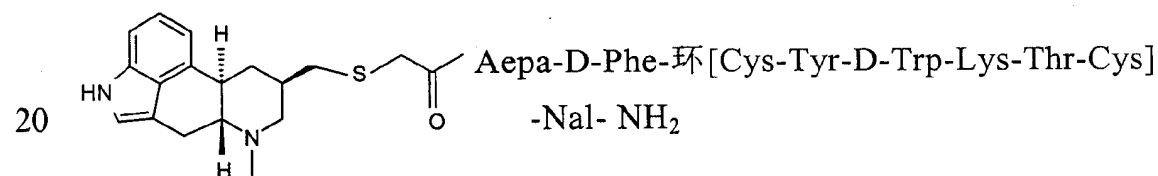
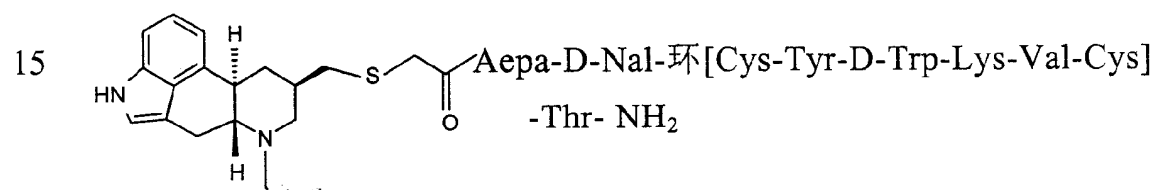
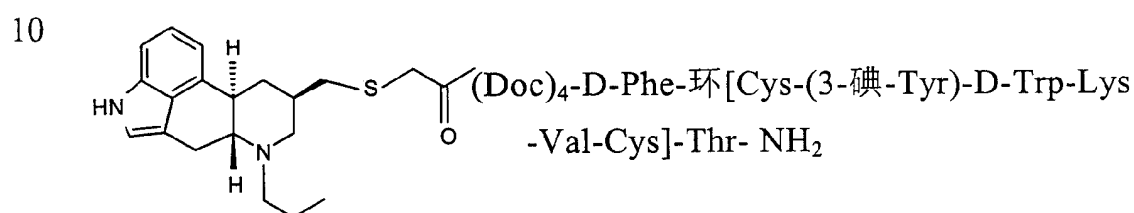
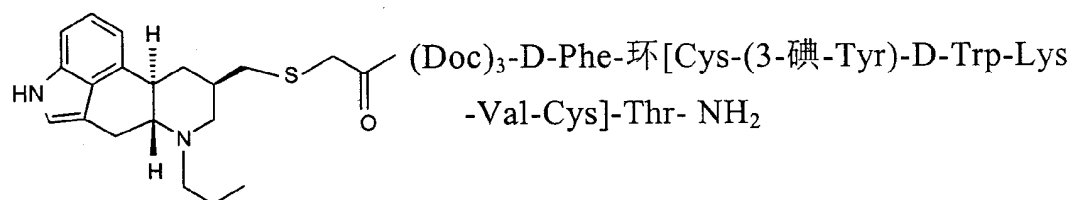
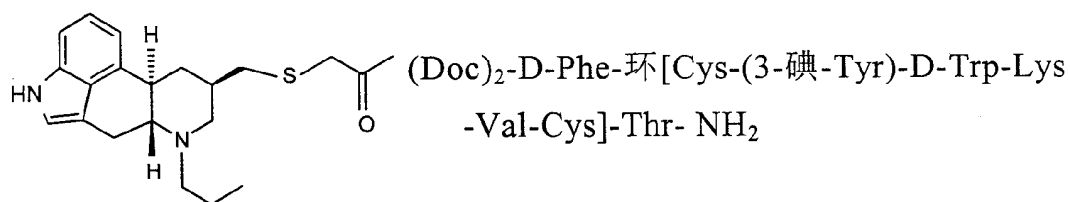


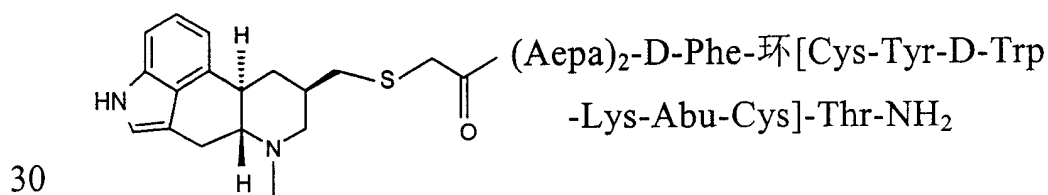
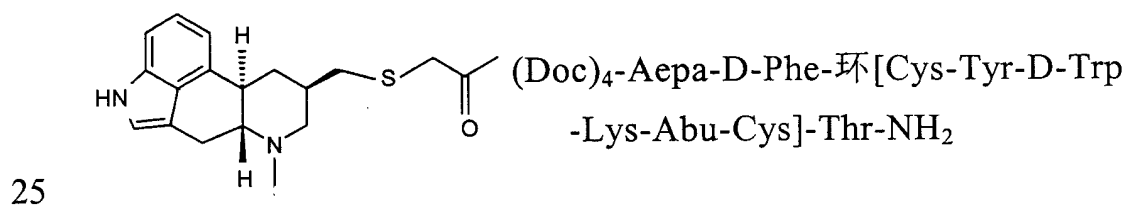
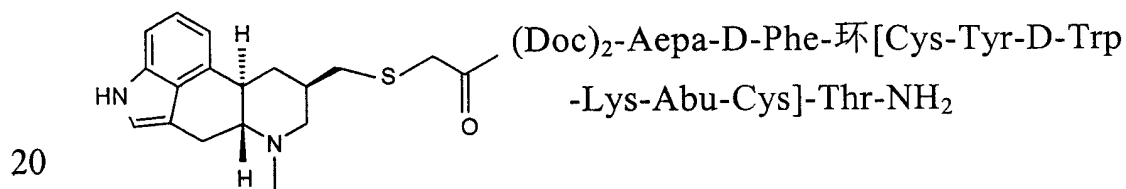
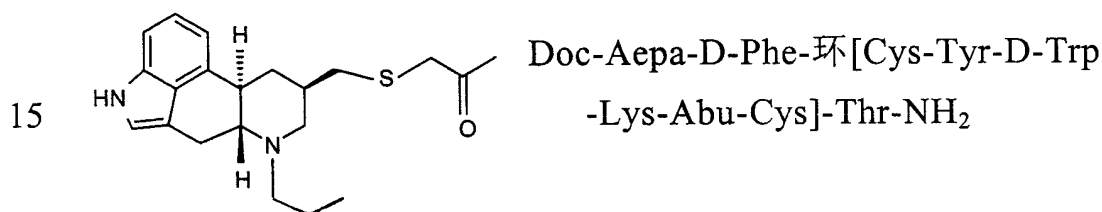
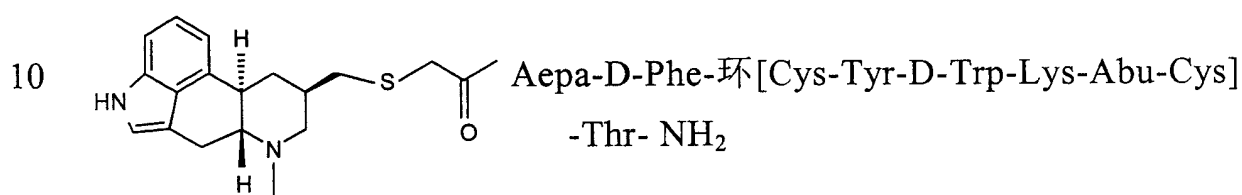
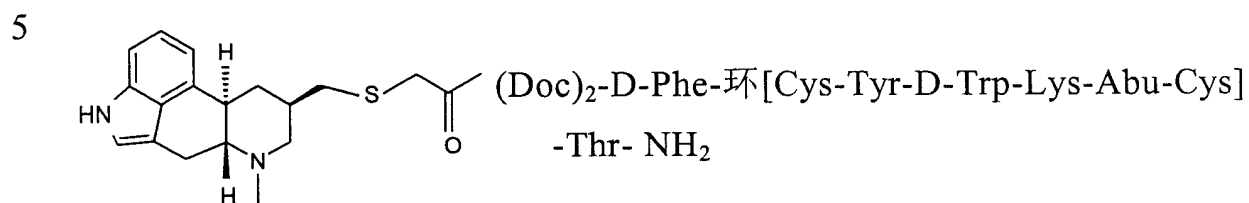
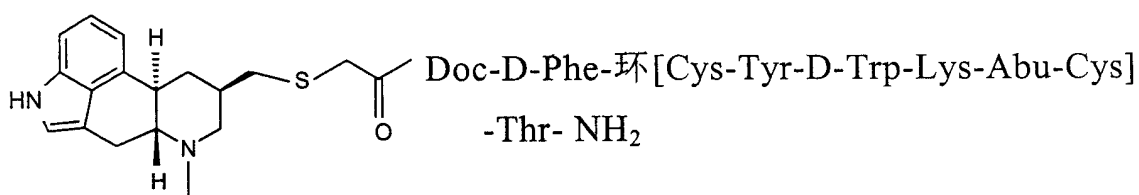
25



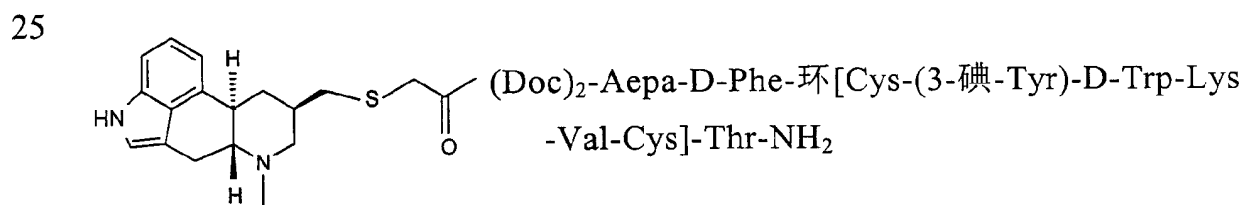
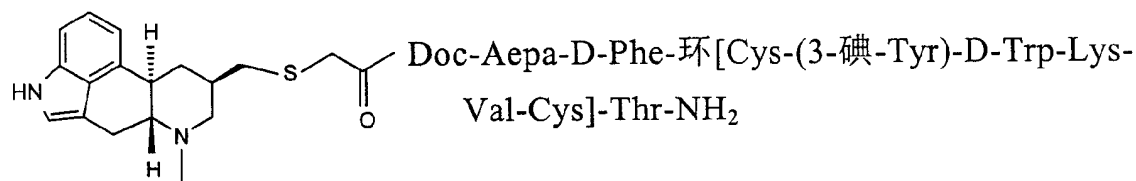
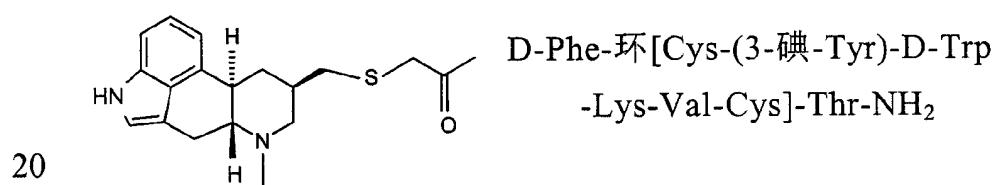
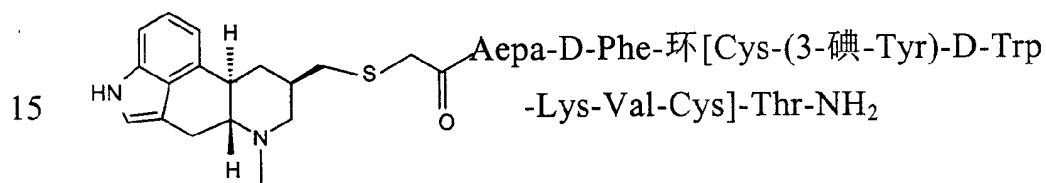
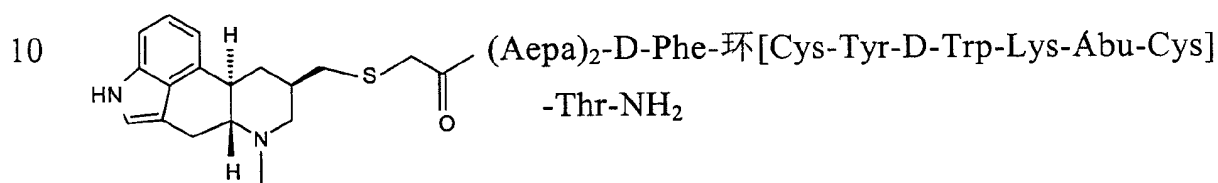
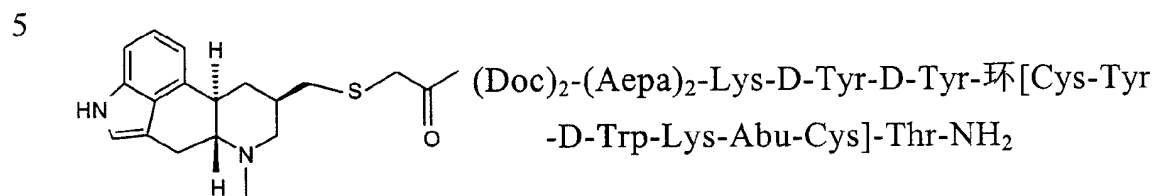
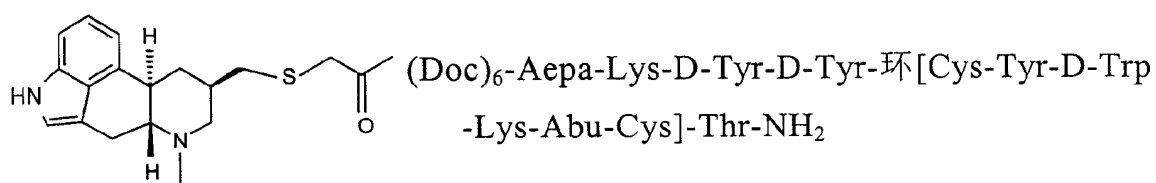
30



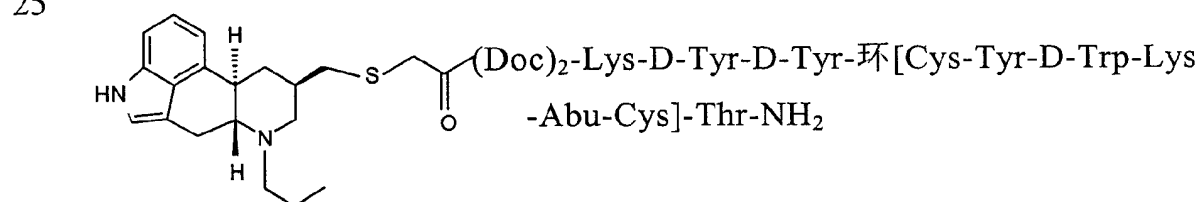
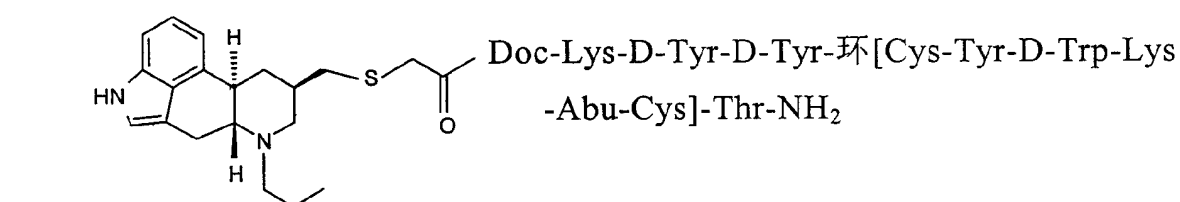
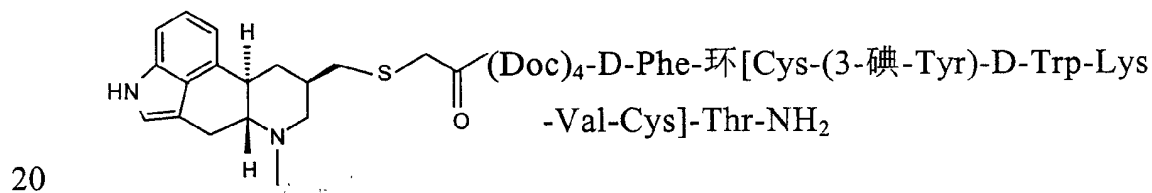
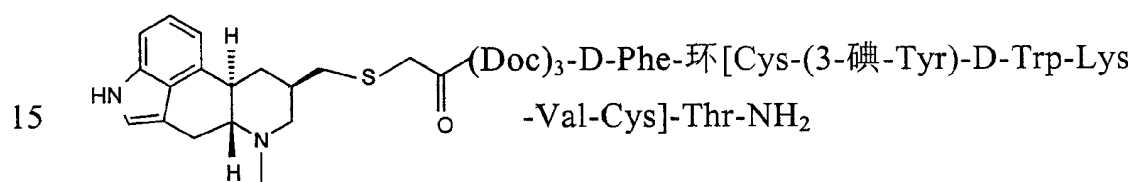
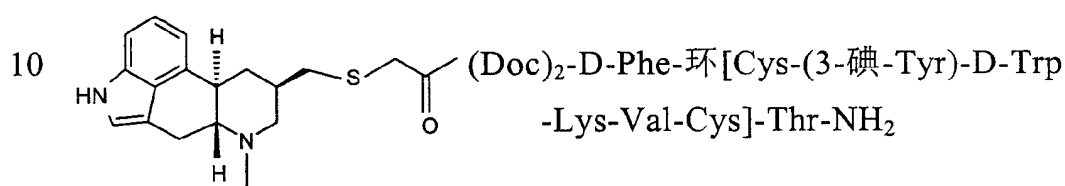
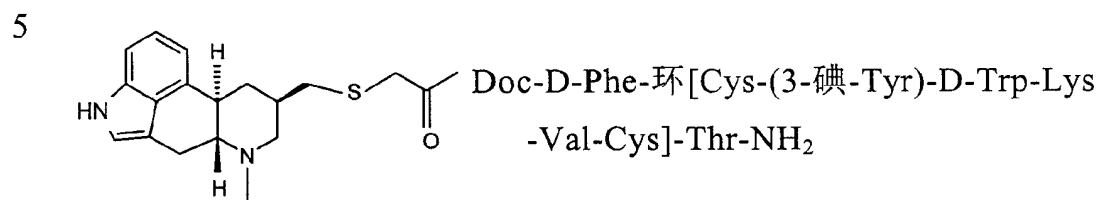
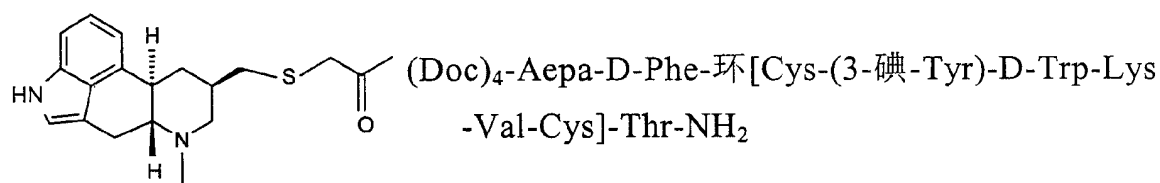


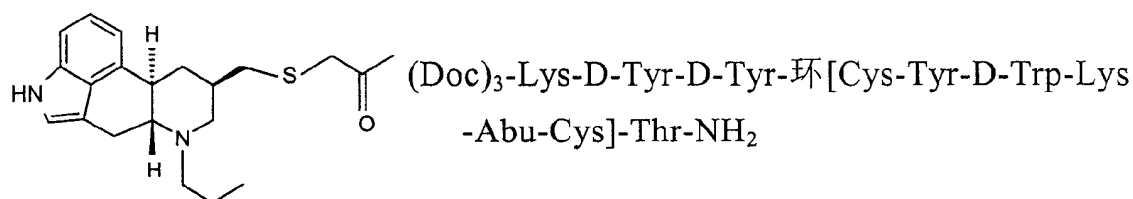




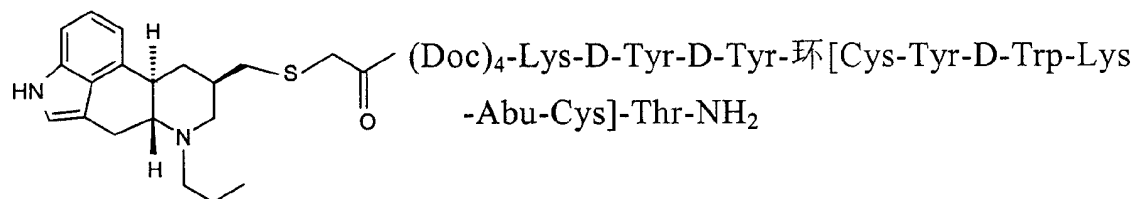


30

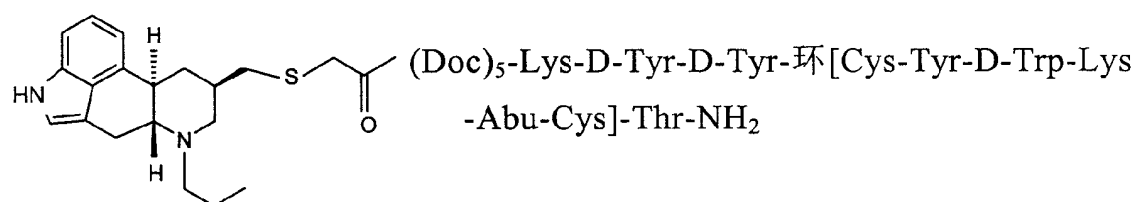




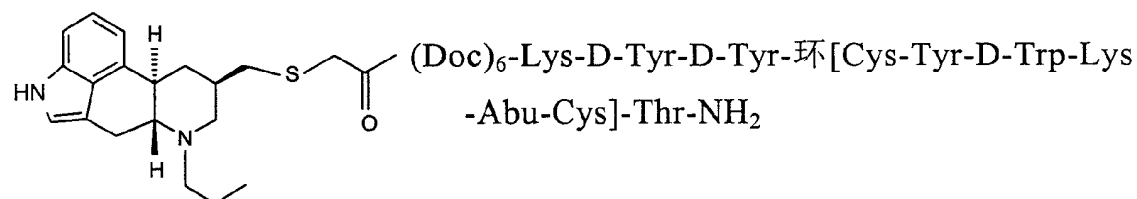
5



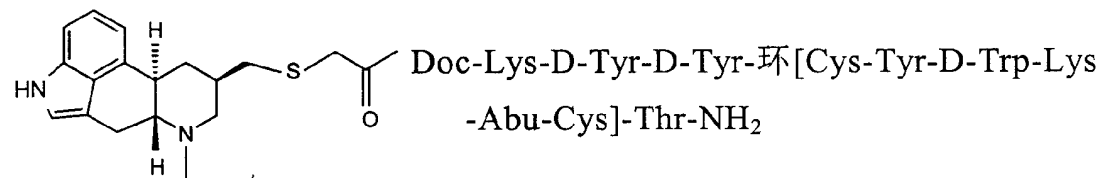
10



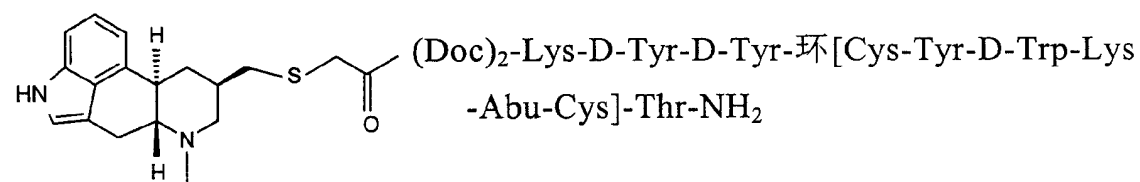
15



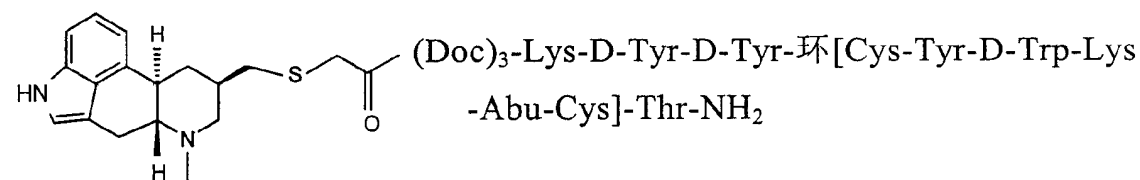
20

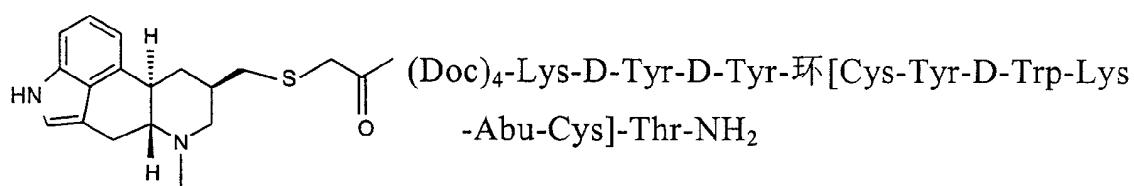


25

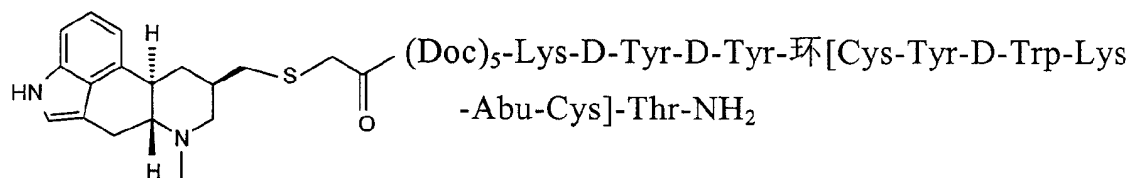


30

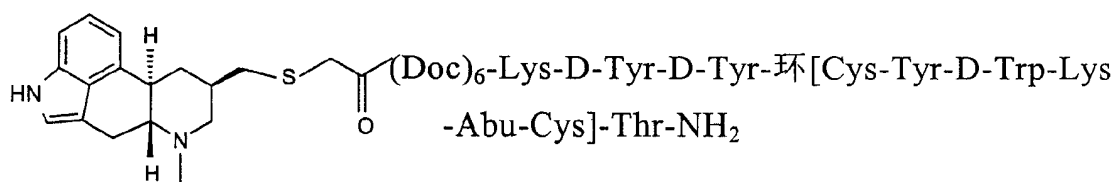




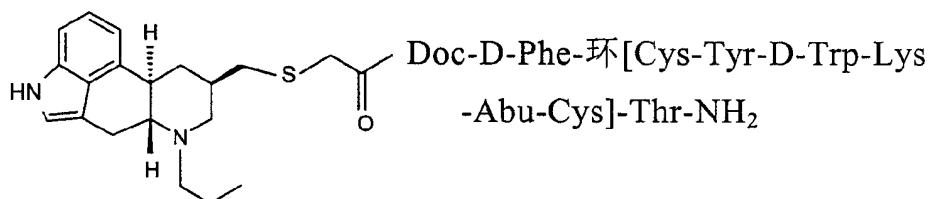
5



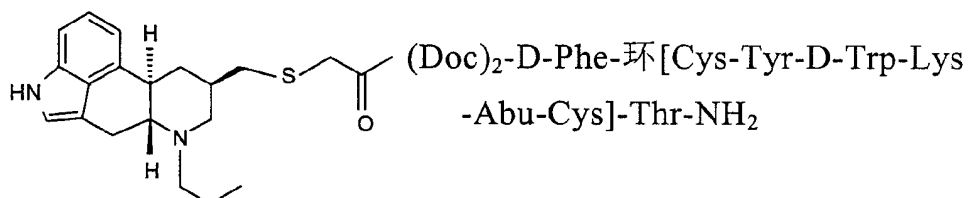
10



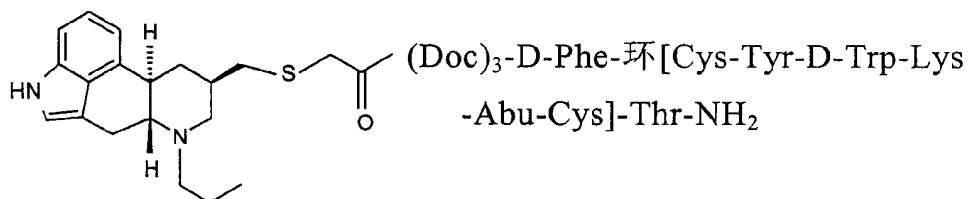
15



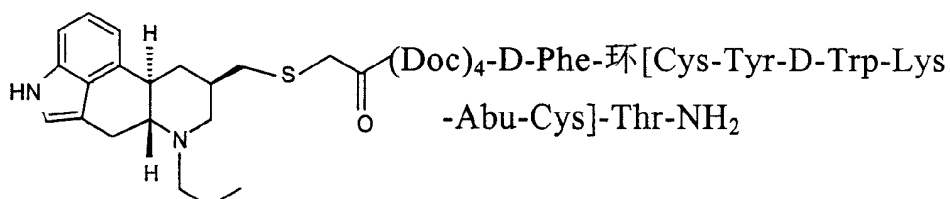
20

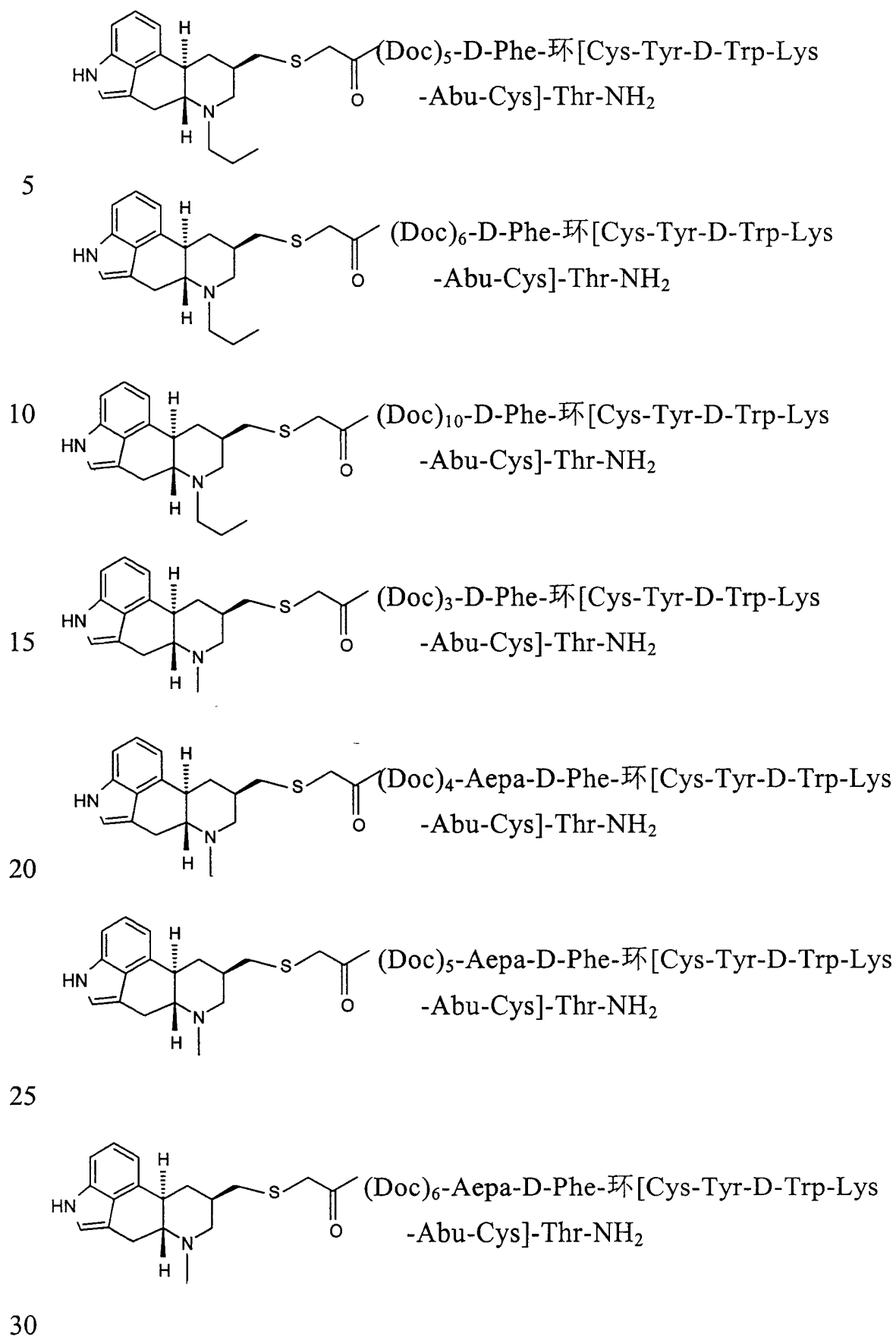


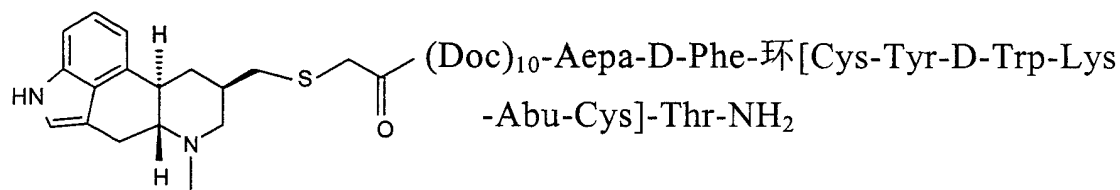
25



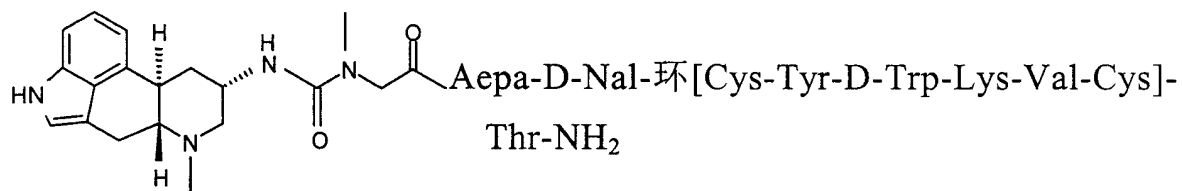
30



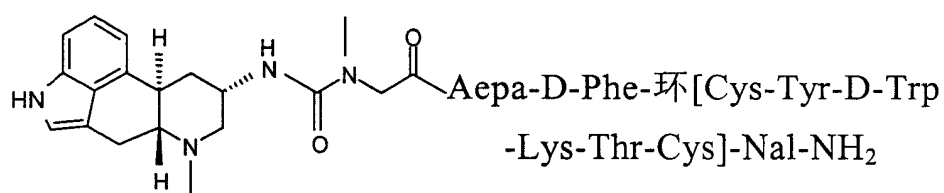




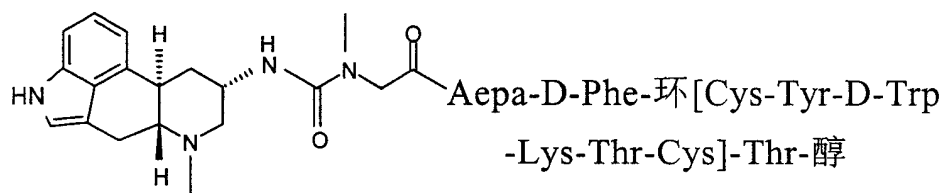
5



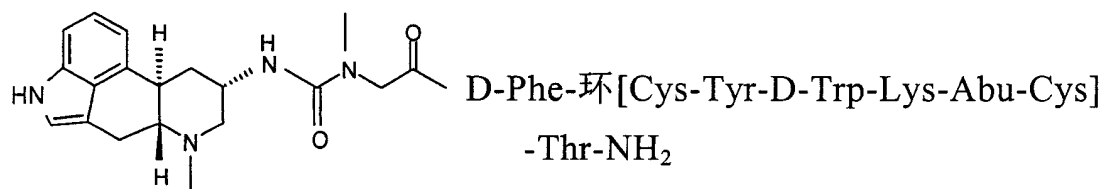
10



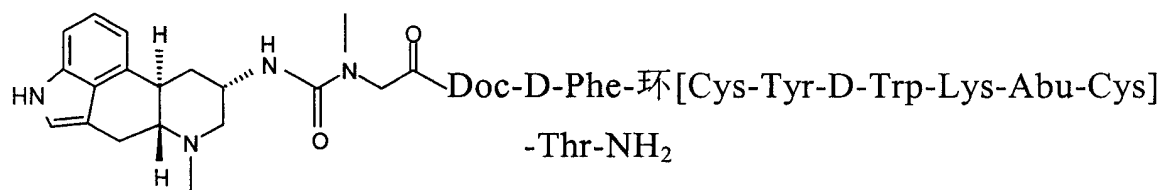
15



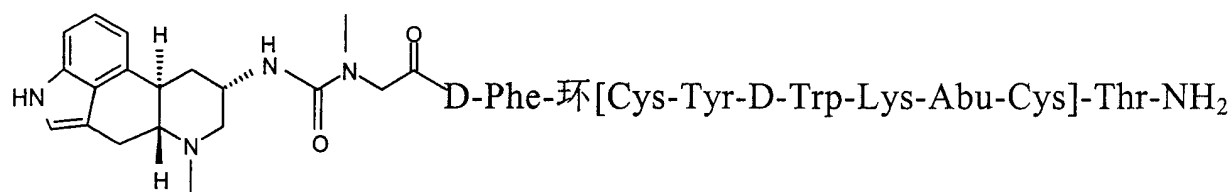
20

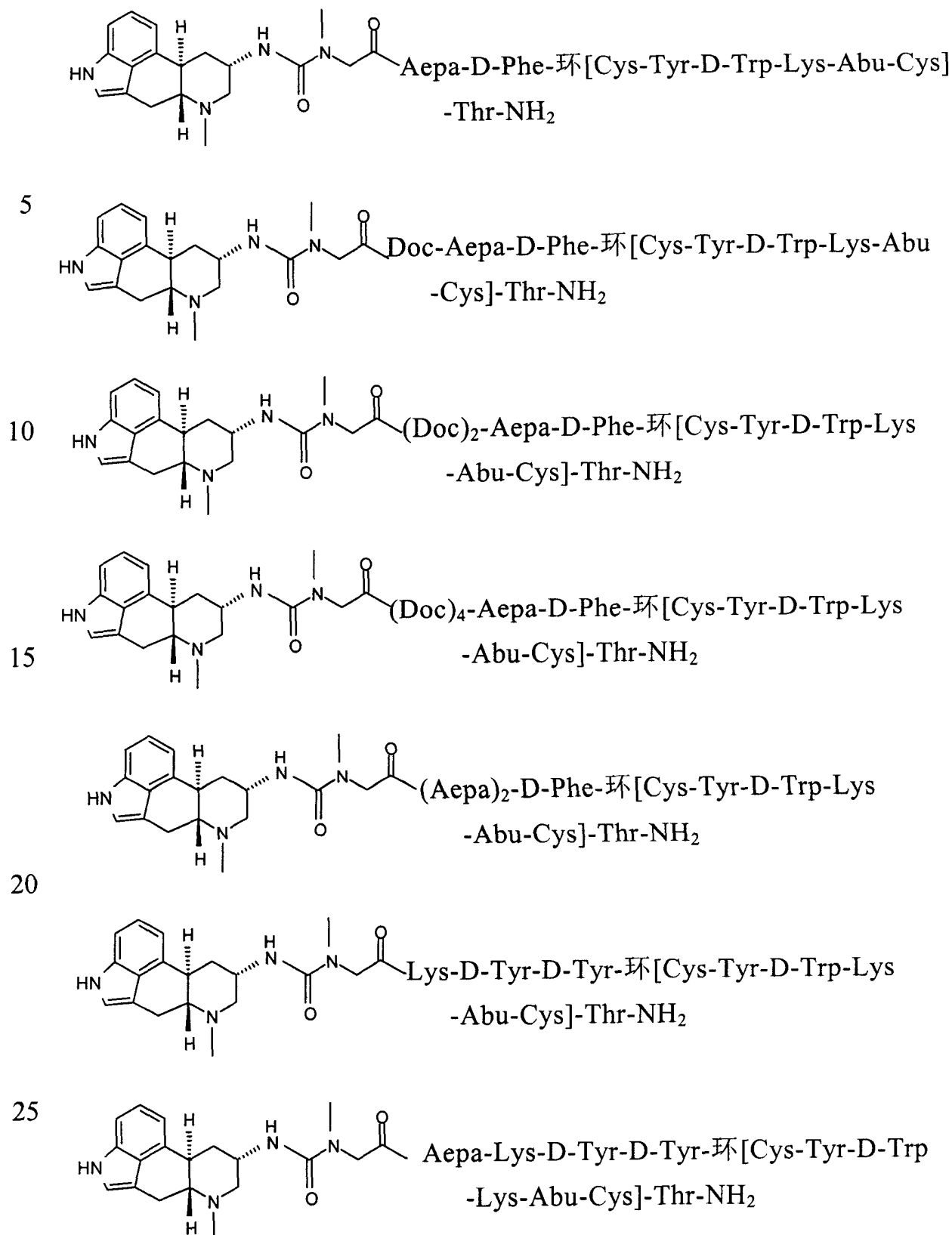


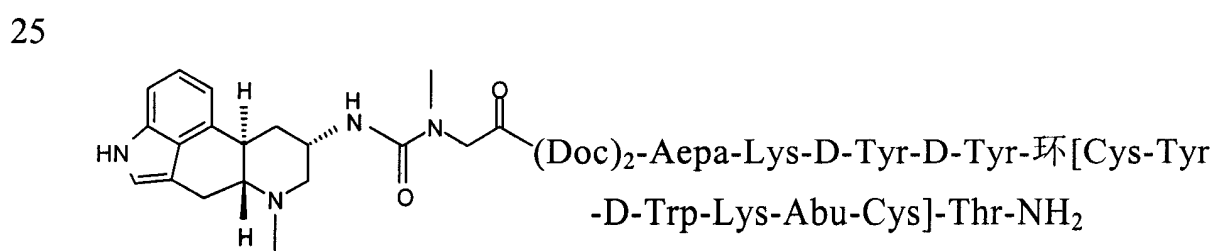
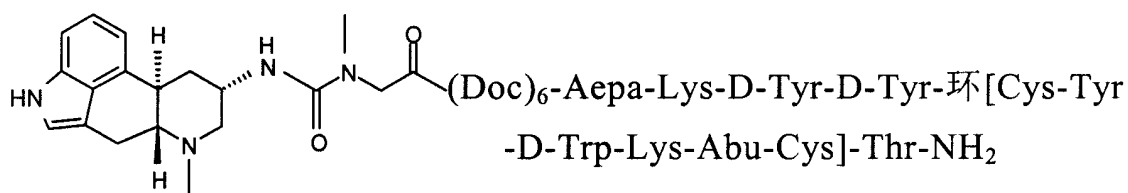
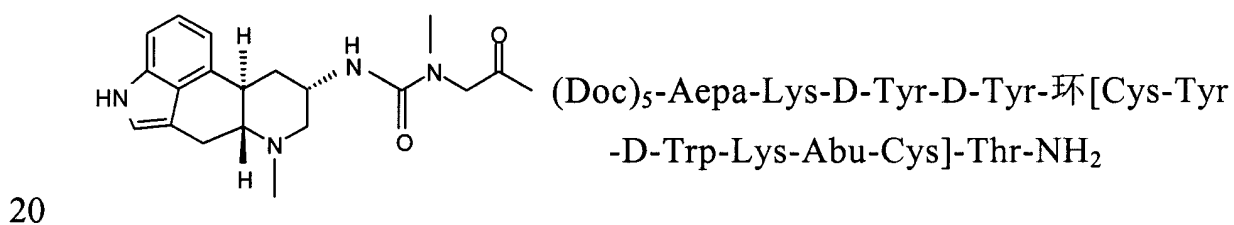
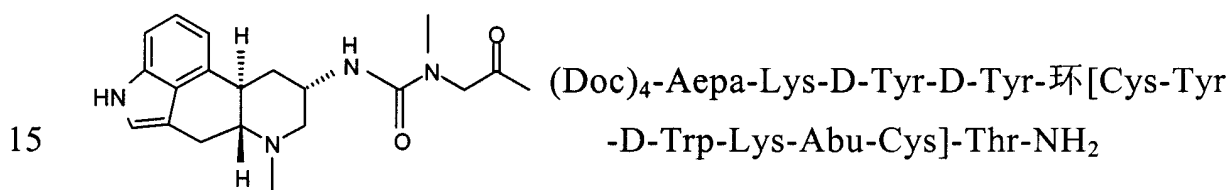
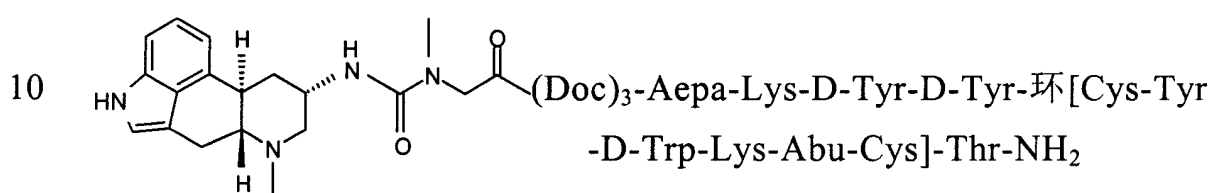
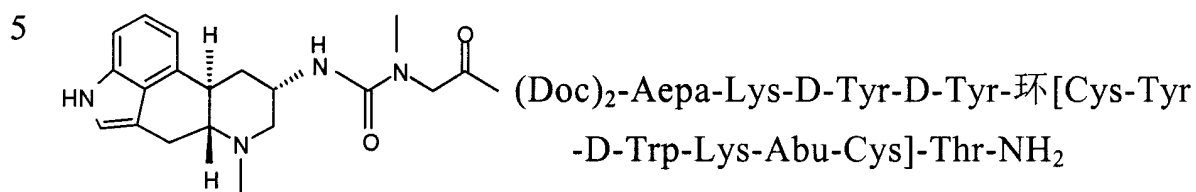
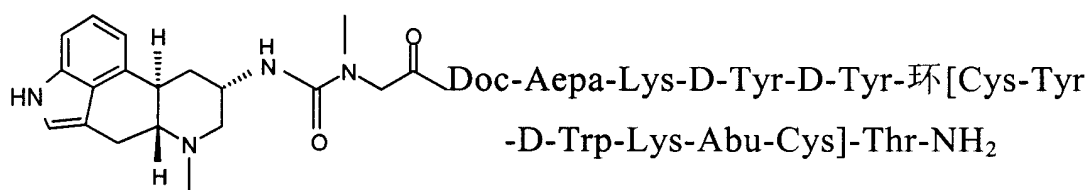
25

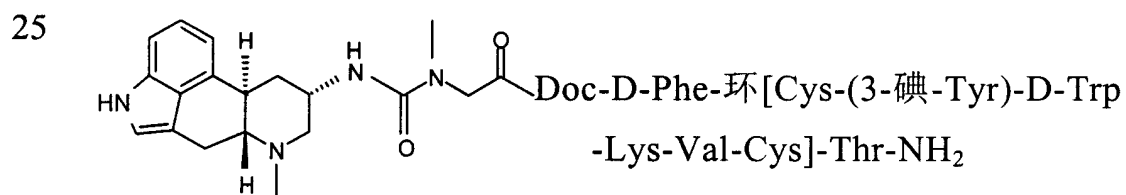
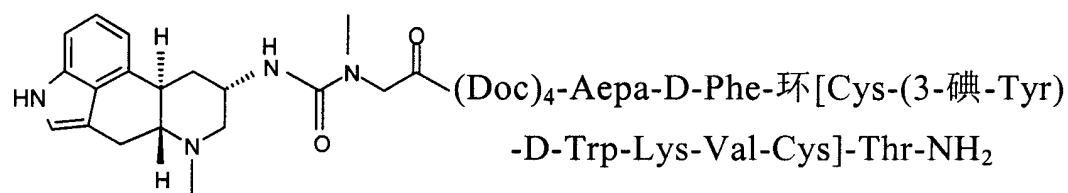
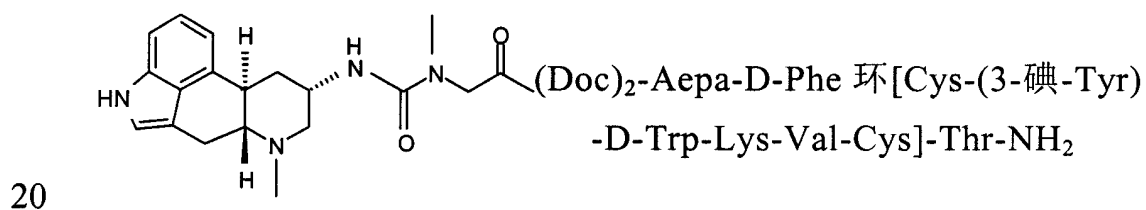
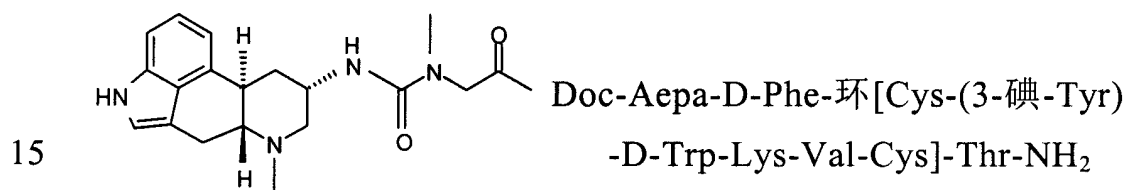
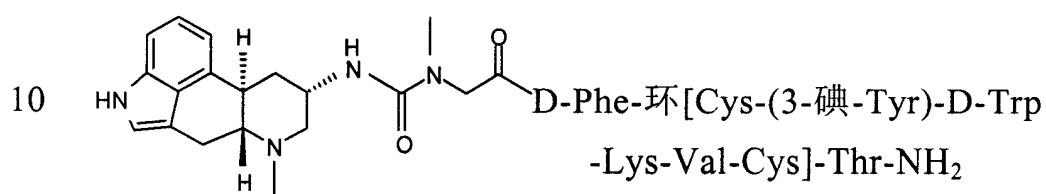
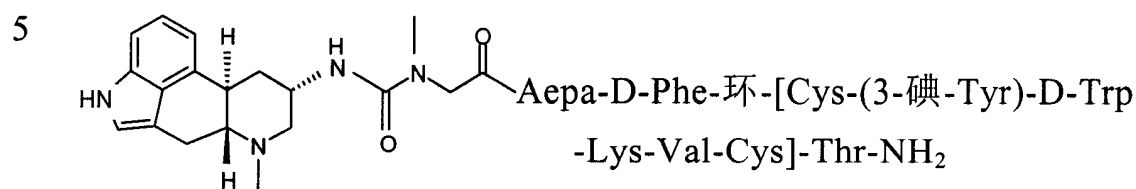
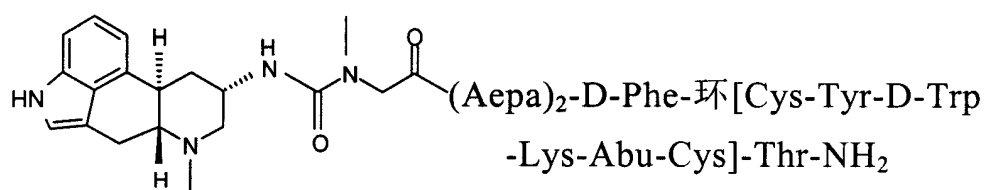


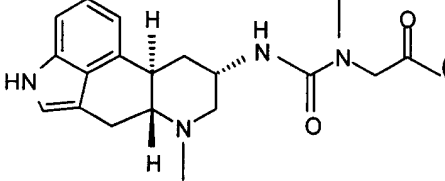
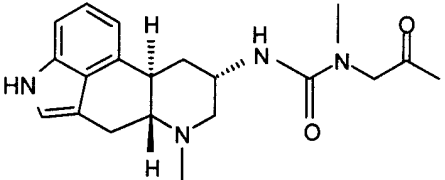
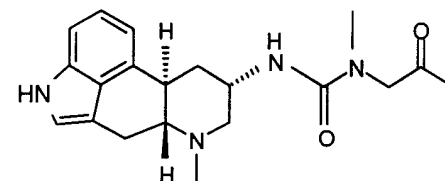
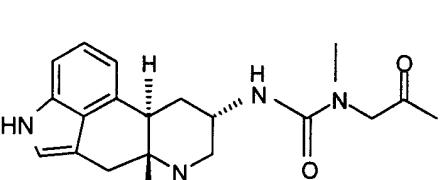
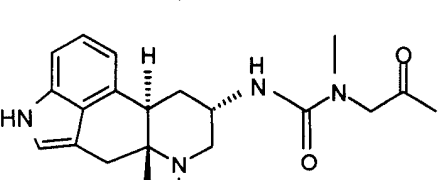
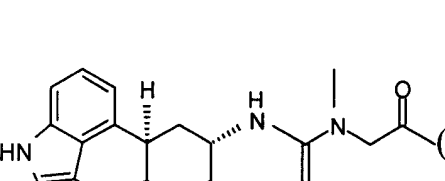
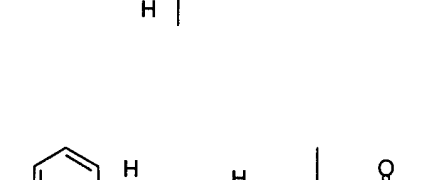
30

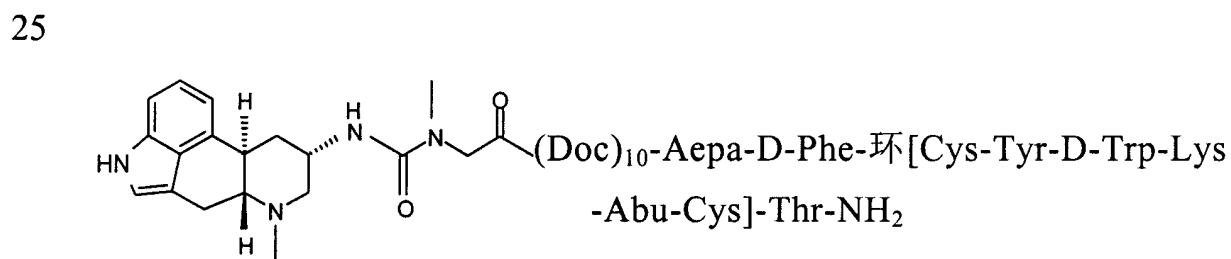
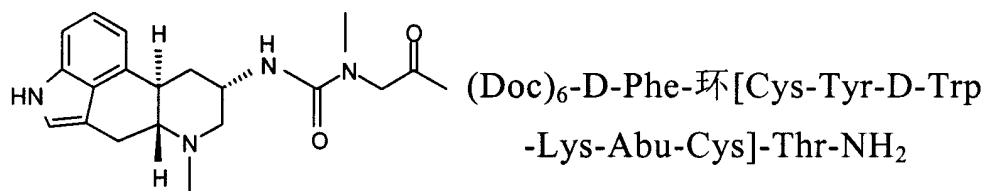
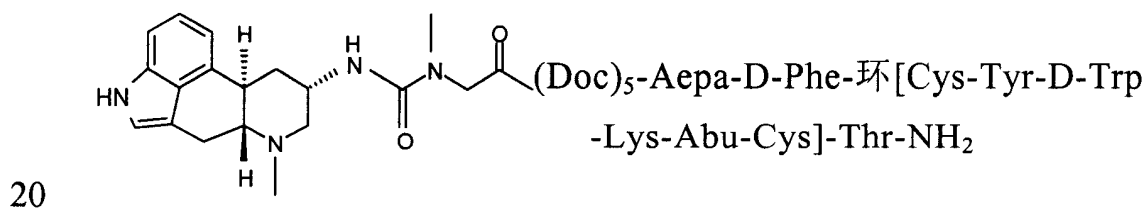
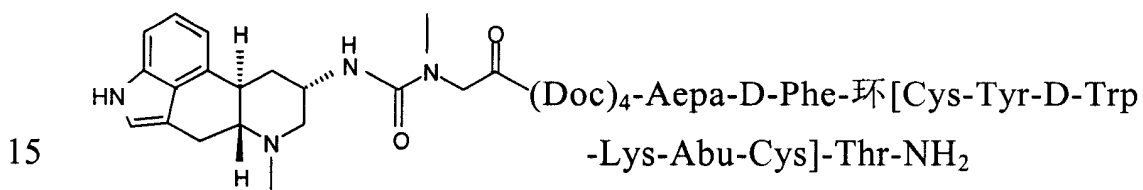
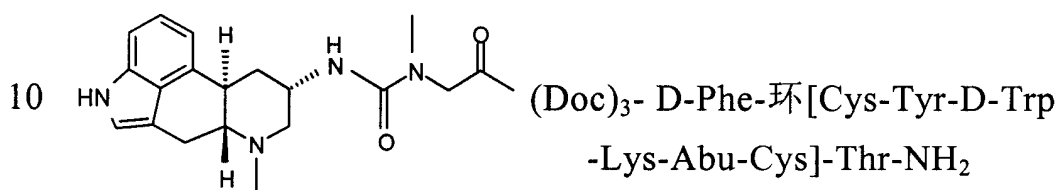
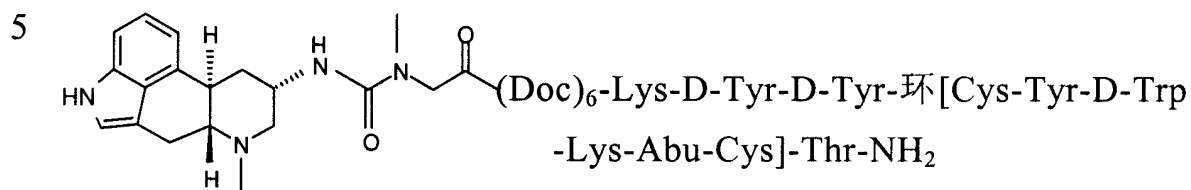
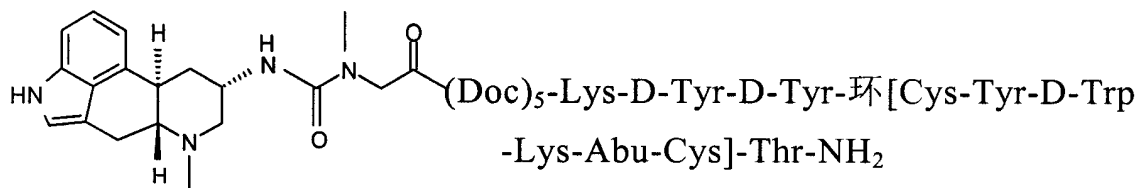


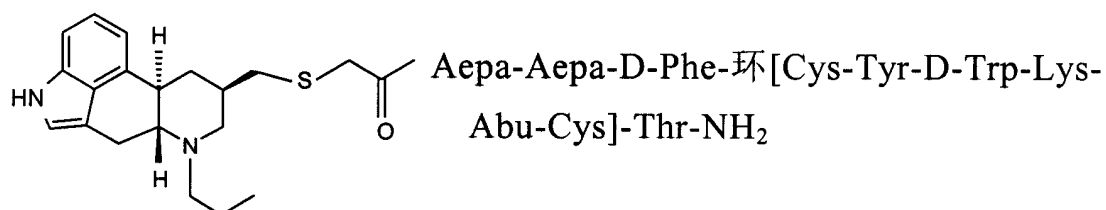




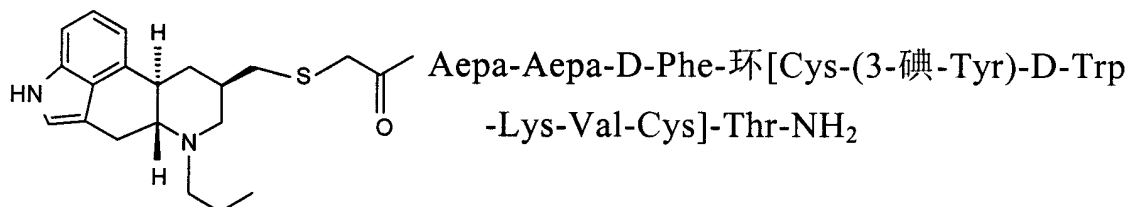


- 
 (Doc)₂-D-Phe-环[Cys-(3-碘-Tyr)-D-Trp
-Lys-Val-Cys]-Thr-NH₂
- 5
 
 (Doc)₃-D-Phe-环[Cys-(3-碘-Tyr)-D-Trp
-Lys-Val-Cys]-Thr-NH₂
- 10
 
 (Doc)₄-D-Phe 环[Cys-(3-碘-Tyr)-D-Trp
-Lys-Val-Cys]-Thr-NH₂
- 15
 
 Doc-Lys-D-Tyr-D-Tyr-环-[Cys-Tyr-D-Trp
-Lys-Abu-Cys]-Thr-NH₂
- 20
 
 (Doc)₂-Lys-D-Tyr-D-Tyr-环[Cys-Tyr-D-Trp
-Lys-Abu-Cys]-Thr-NH₂
- 25
 
 (Doc)₃-Lys-D-Tyr-D-Tyr-环[Cys-Tyr-D-Trp
-Lys-Abu-Cys]-Thr-NH₂
- 25
 
 (Doc)₄-Lys-D-Tyr-D-Tyr-环[Cys-Tyr-D-Trp
-Lys-Abu-Cys]-Thr-NH₂

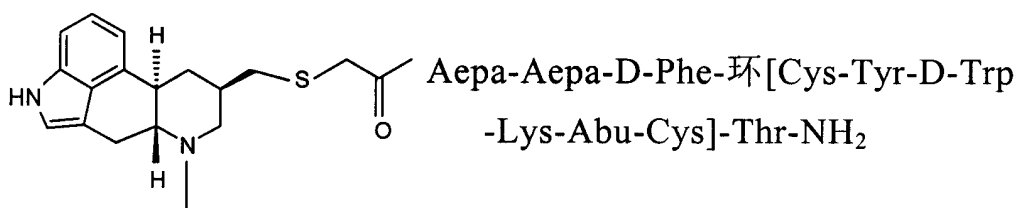




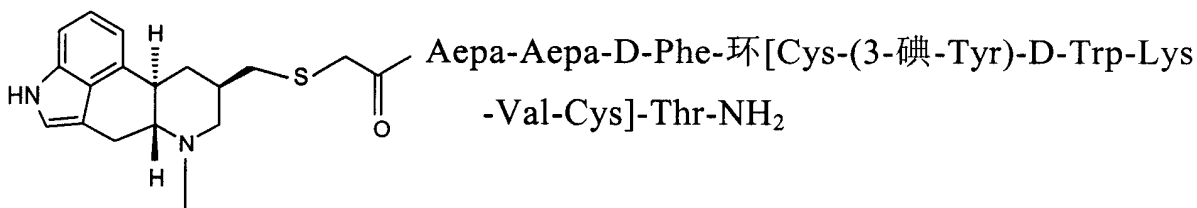
5



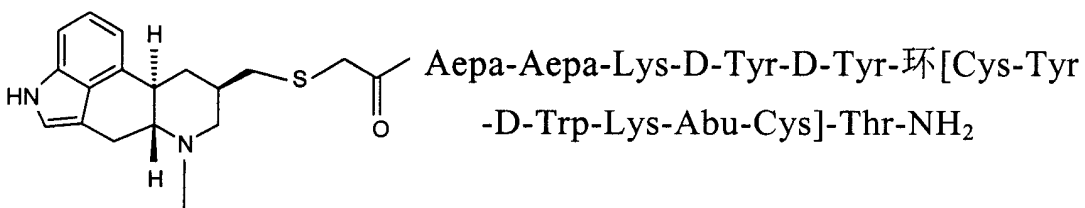
10



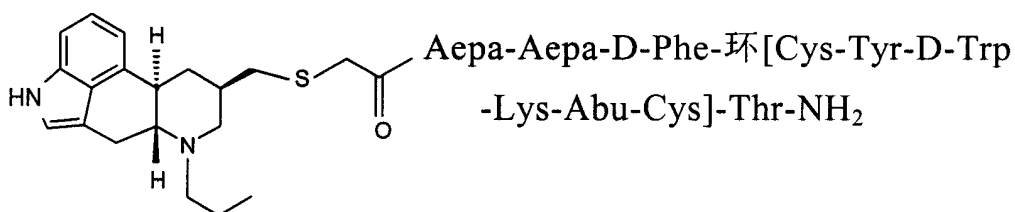
15



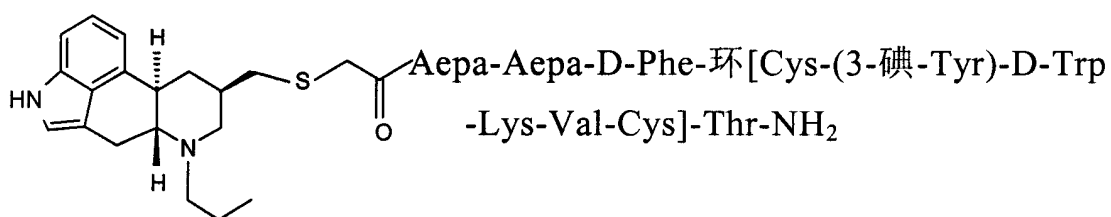
20

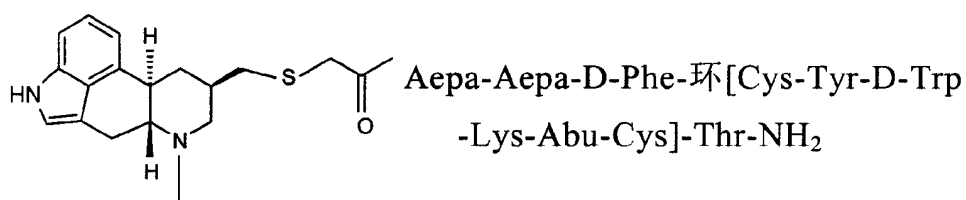


25

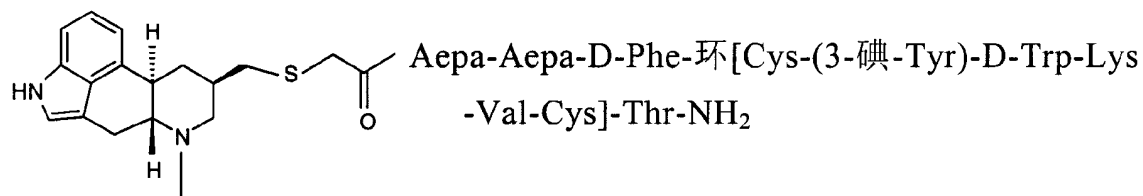


30



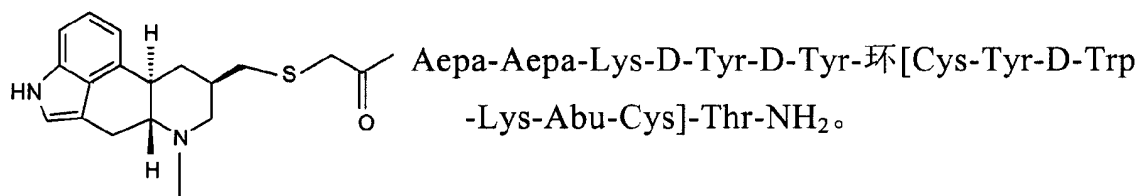


5



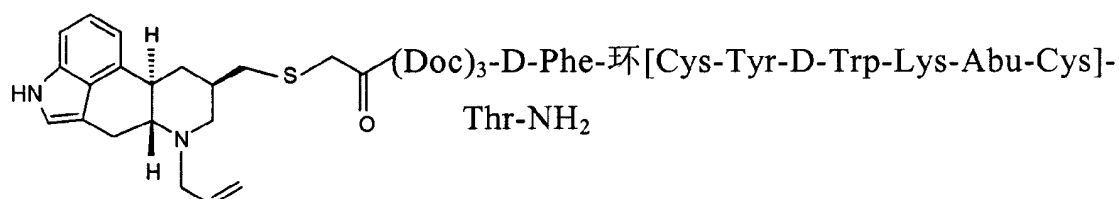
或

10

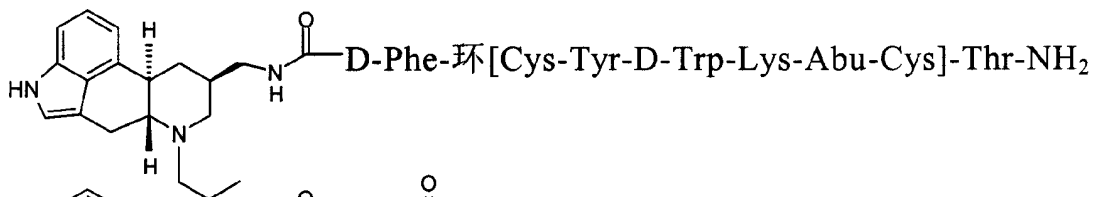


15

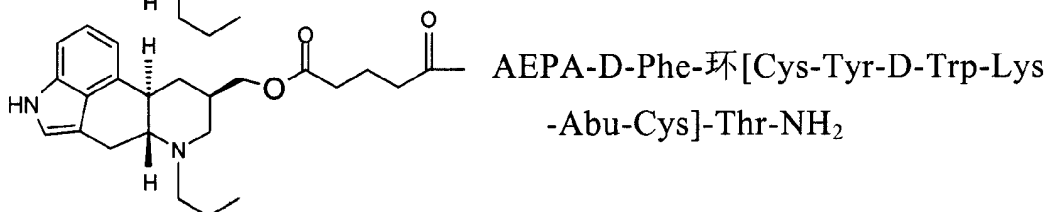
5、下式化合物或其药学上可接受的盐：



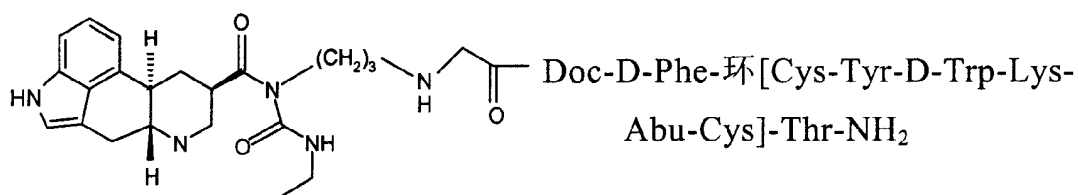
20

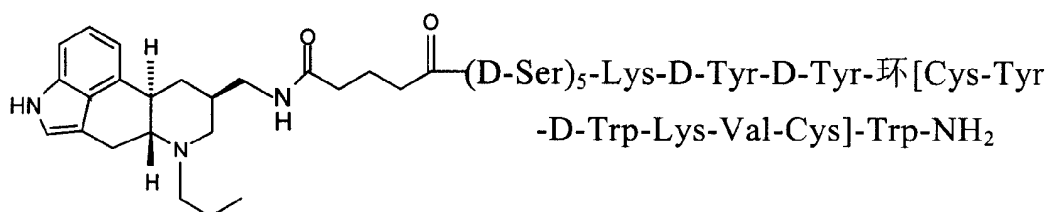


25



30





乙基-[6-甲基-8β-麦角灵基甲基]硫代乙酸酯;

6- 甲 基 -8β- 麦 角 灵 基 甲 基 硫 代 乙 酰 基 -D-Phe- 环
(Cys-Tyr-D-Trp-Lys-Abu-Cys)-Thr-NH₂;

10

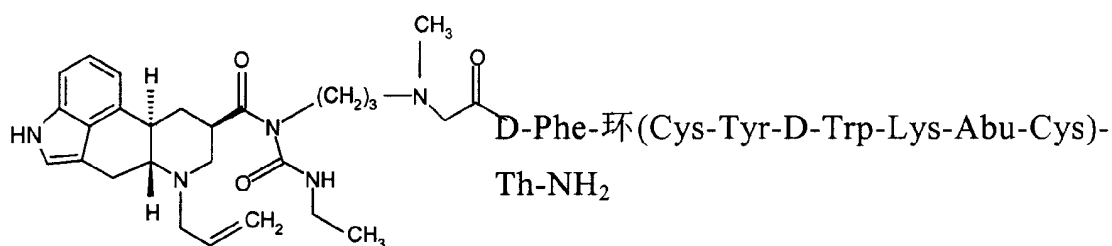
乙基-(6-正丙基)-8β-麦角灵基)甲基硫代乙酸酯;

6- 正 丙 基 -8β- 麦 角 灵 基 甲 基 硫 代 乙 酰 基 -D-Phe- 环
[Cys-Tyr-D-Trp-Lys-Abu-Cys]-Thr-NH₂;

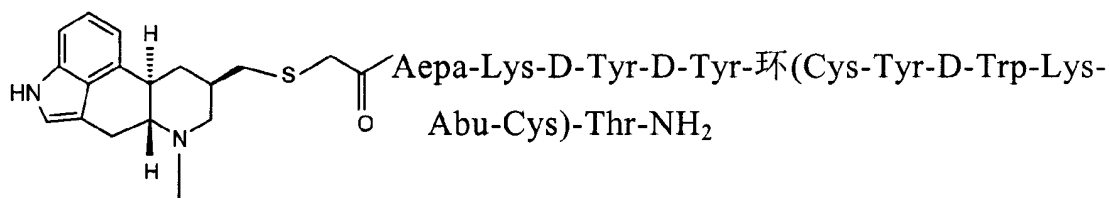
15

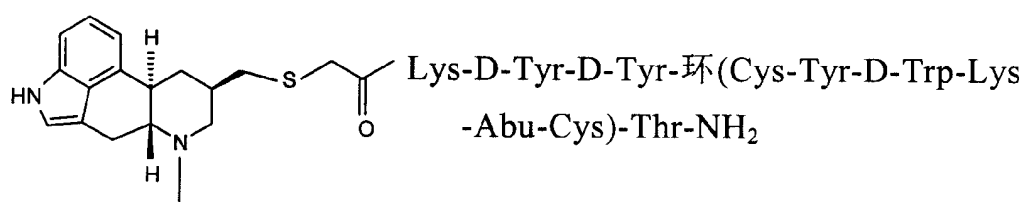
6-D- 甲 基 -8β- 麦 角 灵 基 甲 基 硫 代 氨 基 琥 珀 酰 基
(ergolinylmethylthlaminosuccinoyl)-D-Phe-环[Cys-Tyr-D-Trp
-Lys-Abu-Cys]-Thr-NH₂;

20

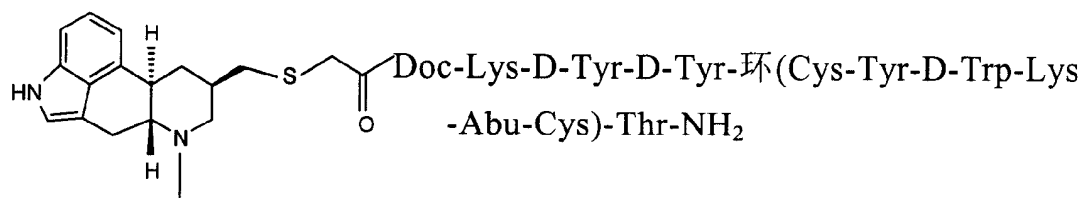


25

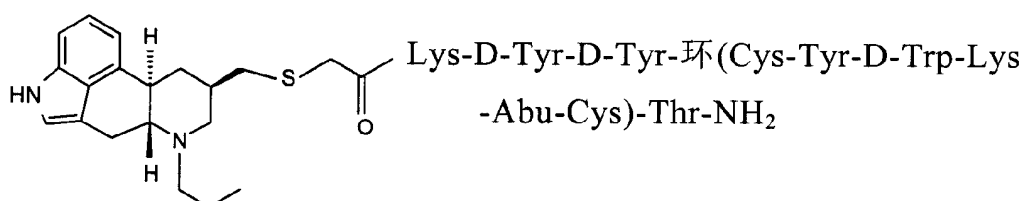




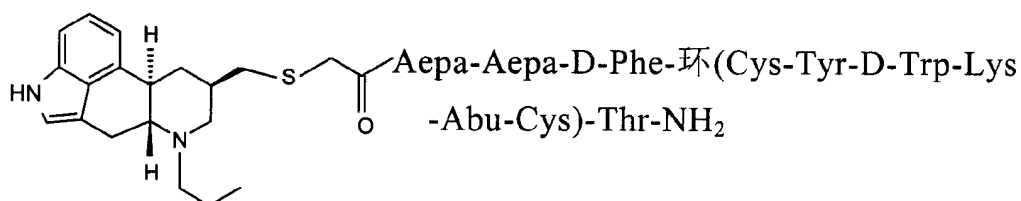
5



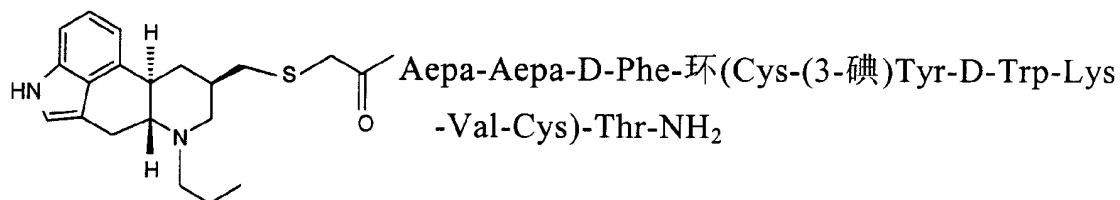
10



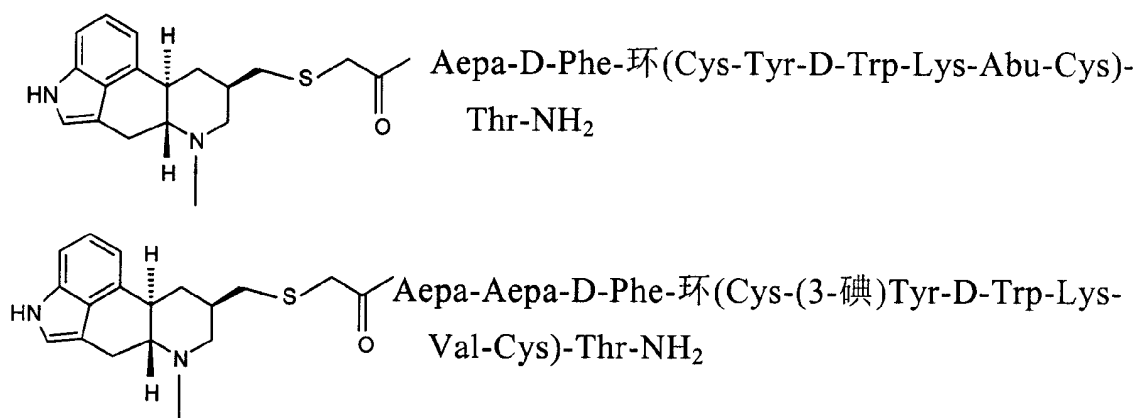
15

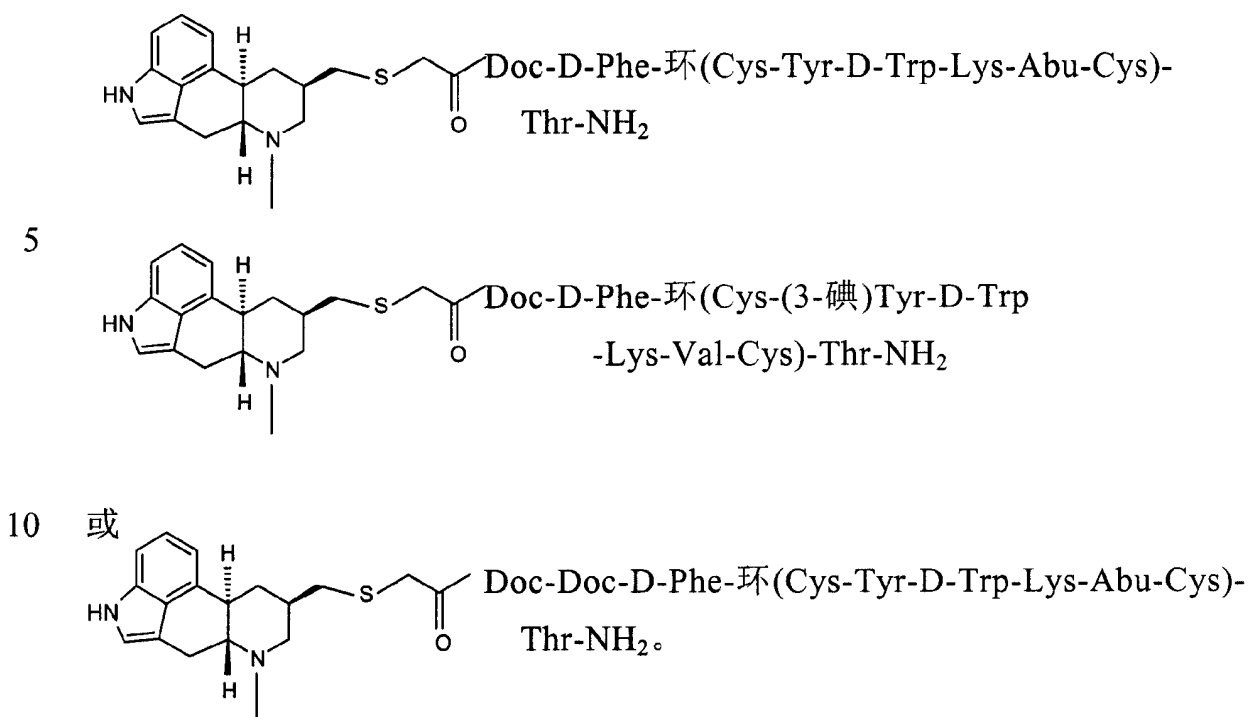


20

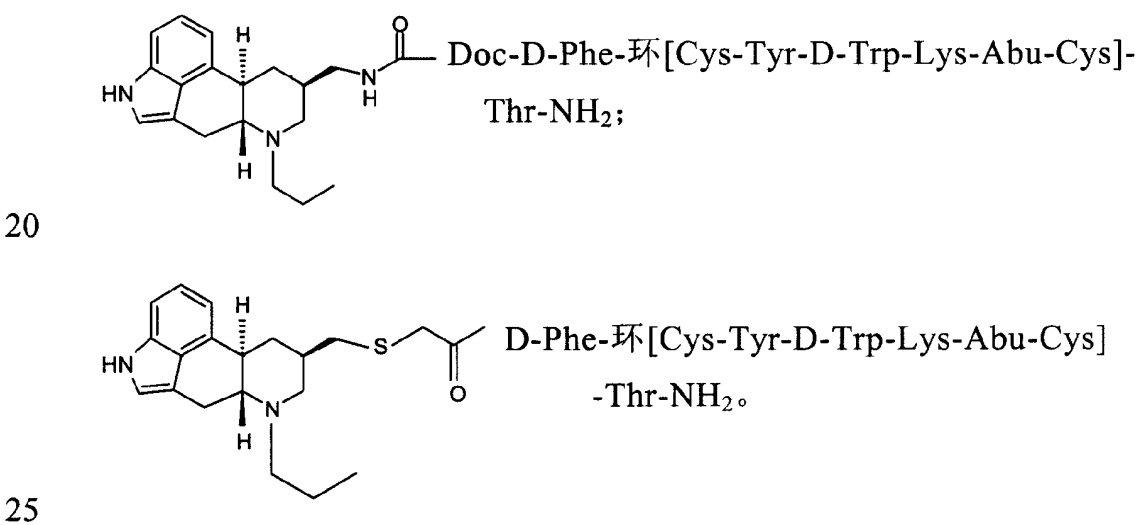


25





15 6、下式化合物或其药学上可接受的盐：



7、一种在需要的患者体内引起多巴胺受体激动剂作用的方法，其中，所述方法包括以有效量的权利要求 1-6 中任一项所述的化合物或其药学上可接受的盐对所述患者给药。

8、一种在需要的患者体内引起生长激素释放抑制因子受体激动剂作用的方法，其中，所述方法包括以有效量的权利要求 1-6 中任一项所述的

化合物或其药学上可接受的盐对所述患者给药。

- 9、一种在需要的患者体内同时引起多巴胺受体激动剂作用和生长激素释放抑制因子受体激动剂作用的方法，其中，所述方法包括以有效量的权利要求 1-6 中任一项所述的化合物或其药学上可接受的盐对所述患者给药。

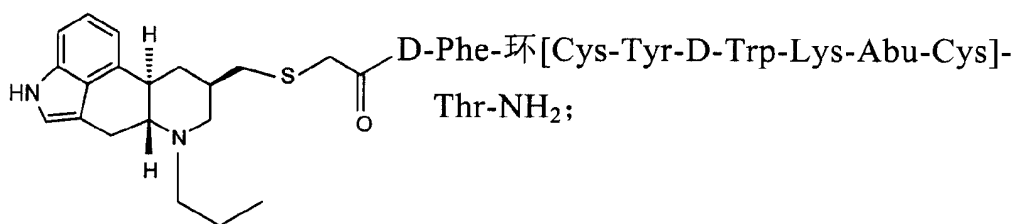
10、一种药物组合物，包括有效量的权利要求 1-6 中任一项所述的化合物或其药学上可接受的盐。

- 11、一种为患者治疗疾病的方法，所述方法包括以治疗有效量的权利要求 1-6 任一项所述的化合物对所述患者给药，其中所述疾病选自肺癌、神经胶质瘤、厌食症、甲状腺机能减退、醛固酮过多症、幽门螺旋杆菌增生、肢端肥大症、再狭窄、克朗氏病、系统性硬化症、外部和内部的胰腺假囊肿和腹水、舒血管肠肽瘤、胰岛细胞弥漫性增生、胰岛素分泌过多、胃泌素瘤、卓-艾二氏综合症、腹泻、与 AIDS 相关的腹泻、与化疗相关的腹泻、硬皮病、过敏性肠综合症、胰腺炎、小肠梗阻、胃食管回流、十二指肠胃回流、库兴氏综合症、促性腺素瘤、甲状旁腺机能亢进、格雷夫斯氏病、糖尿病性神经病、Paget 氏病、多囊卵巢病、甲状腺癌、肝细胞瘤、白血病、脑膜炎、癌性恶病质、直立低血压、餐后低血压、焦虑发作、GH 分泌腺瘤、肢端肥大症、TSH 分泌腺瘤、催乳素分泌腺瘤、胰岛腺瘤、高血糖素瘤、糖尿病、高脂血症、胰岛素麻木、X 综合症、血管病、增生性视网膜炎、黎明现象、肾病、胃酸分泌、消化器官溃疡、肠皮肤瘻管、胰腺皮肤瘻管、倾倒综合症、含水腹泻综合症、胰腺炎、胃肠激素分泌肿瘤；血管生成、关节炎；同种异体抑制排斥、移植脉管出血、入口高血压、胃肠出血、肥胖、以及鸦片样物质过量。

- 12、根据权利要求 11 所述的方法，其中，所述的疾病或症状是肢端肥大症。

13、根据权利要求 7, 8, 9 或 12 中任一项所述的方法，其中，所述的化合物为

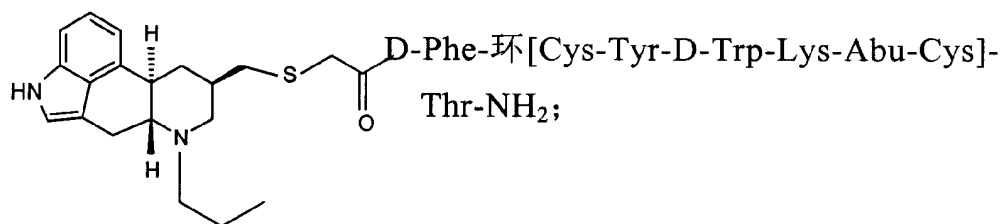
5



或其药学上可接受的盐。

14、根据权利要求 10 所述的药物组合物，其中，所述化合物是

10



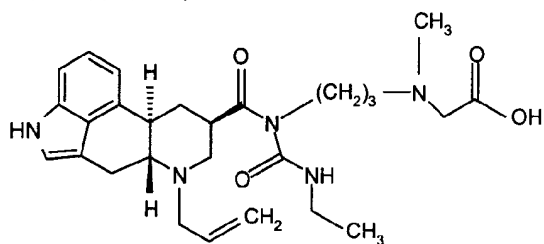
或其药学上可接受的盐。

15、根据权利要求 1 或 2 所述的化合物或其药学接受的盐，其中，Z 是含有-H, -OH, (C₁-C₆)烷氧基，芳氧基，-NH₂或-NR⁹R¹⁰的基团。

16、根据权利要求 15 的化合物或其药学接受的盐，其中，Z 是含有-H, -OH, (C₁-C₆)烷氧基或苄基的基团。

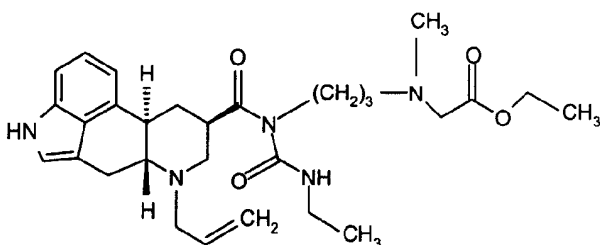
17、根据权利要求 15 的化合物或其药学上可接受的盐，其中，所述化合物具有下式

20



或

25



或其药学可接受的盐。

生长激素释放抑制因子-多巴胺嵌合类似物

发明背景

5 本发明描述了生长激素释放抑制因子-多巴胺嵌合类似物。

多巴胺是一种牵涉在帕金森病和精神分裂症的发病机制中的儿茶酚胺神经递质。Gradybiel 等人., *Adv. Neurol.* 53, 17-29 (1990); Goldstein 等人., *FASEB J.* 6, 2413-2421(1992); Olanow 等人., *Annu. Rev. Neurosci.* 22, 123-144(1999). Egan 等人., *Curr. Opin. Neurobiol.* 7, 701-707(1997)。多巴胺及相关分子在大鼠
10 中显示出可以抑制一些类型的恶性肿瘤的生长,这种活性被不同地归因于对肿瘤细胞增生的抑制,对肿瘤免疫的刺激或在恶性黑色素瘤中的黑色素代谢的结果。Wick, M.M., *J. Invest. Dermatol.* 71,163-164(1978); Wick, M.M., *J. Natl. Cancer Inst.* 63, 1465-1467(1979); Wick, M.M., *Cancer Treat. Rep.* 63, 991-997 (1979); Wick, M.M., *Cancer Res.* 40, 1414-1418 (1980); Wick, M.M.,*Cancer*
15 *Treat. Rep.* 65; 861-867 (1981); Wick, M.M.& Mui, *J. Natl. Cancer Inst.* 66, 351-354 (1981); Dasgupta 等人., *J. Cancer Res. Clin. Oncol.* 113, 363-368 (1987); Basu 等人., *Endocrine* 12, 237-241(2000); Basu 等人., *J. Neuroimmunol.* 102, 113-124 (2000)。最近的研究表明在内皮细胞的 D2 多巴胺受体的存在。Ricci 等人., *J. Auton. Pharmacol.*, 14, 61-68 (1994);Bacic 等人., *J. Neurochem.* 57,
20 1774-1780 (1991)。最近报道多巴胺在非毒性水平强烈并选择性地抑制 VPF/VEGF 的血管渗透性和血管原性活性。Basu 等人., *Nat. Med.* 7(5), 569-574 (2001)。

生长激素释放抑制因子(SS),即一种由 Brazeau 等人发现的十四肽,显示在组织中如脑下垂体、胰和胃肠道中对不同的分泌过程具有有效的抑制作用。
25 SS 在中枢神经系统中也可作为神经调节物。SS 的这些在本质上都具有抑制作用的生物学效果是通过一系列 G 蛋白偶联受体引起,其中的五种不同亚型已经被表征(SSTR1-SSTR5) (Reubi JC 等人, *Cancer Res* 47:551-558, Reisine T 等人, *Endocrine Review* 16:427-442, Lamberts SW 等人, *Edocr Rev* 12:450-482, 4 Patel YC, 1999 *Front Neuroendocrinology* 20: 157-198)。这五种亚型对内生的

SS 配体具有相似的免疫亲和力但在不同的组织中具有不同的分布。生长激素释放抑制因子与五个受体亚型以每个亚型相对较高和相等的免疫亲和力进行结合。

有证据表明, 通过由 SSTR1, 2, 4 和 5 亚型阻止细胞生长, SS 调节细胞
5 增生(Buscail L.等人., 1995 *Proc Natl Acad Sci USA* 92: 1580-1584; Buscail L.等人, 1994 *Proc Natl Acad Sci USA* 91: 2315-2319; Florio T,等人, 1999 *Mol Endocrinol* 13:24-37; Sharma K,等人, 1999 *Mol Endocrinol* 13:82-90), 或通过由 SSTR3 亚型诱导凋亡作用, SS 可调节细胞增生(Sharma K 等人, 1996 *Mol Endocrinol* 10: 1688-1696)。SS 和不同的类似物已经显示出在活体内和体外通
10 过特定的 SS 受体(SSTR's)(Patel YC, 1999 *Front Neuroendocrinology* 20:157-198)以及可能不同的受体后反应 (Weckbecker G.等人, *Pharmacol Ther* 60:245-264; Bell GI, Reisine T 1993 *Trends Neurosci* 16:34-38; Patel YC 等人, *Biochem Biophys Res Commun* 198:605-612; Law SF 等人, *Cell SigNal* 7:1-8)抑制正常细胞和瘤细胞的增生。另外, 有证据表明, 不同的 SSTR 亚型在正常人体组织和
15 瘤组织(9)中进行表达, 由于不同 SS 类似物和对起治疗效果的不稳定的临床反应, 从而产生了不同的组织免疫亲和力。

不同类型的生长激素释放抑制因子受体亚型的结合与多种病症和/或疾病的治疗相关。(SSTR2) (Raynor 等人, *Molecular Pharmacol.* 43:838(1993); Lloyd 等人, *Am. J. Physiol.* 268: G 102 (1995))。胰岛素的抑制则归因于生长激素释放抑制因子 5-型受体(SSTR5) (Coy 等人, 197: 366-371(1993))。2 型和 5 型的活化与生长激素的抑制有关, 尤其与 GH 分泌腺瘤(肢端肥大症)和 TSH 分泌腺瘤有关。2 型而并非是 5 型的活化与治疗催乳素分泌腺瘤相关。其他与生长激素释放因子受体亚型相关的适应症包括对用于治疗糖尿病、血管病、增生性视网膜炎、, 黎明现象(dawn Phenomenon)和肾病的抑制胰岛素和/或胰增血
20 糖素进行抑制; 抑制胃酸分泌并尤其是抑制消化器官溃疡, 肠皮肤(enterocutaneous)和胰腺皮肤(pancreaticocutaneous)瘻管、过敏性肠综合症、倾倒综合症、含水腹泻综合症、与 AIDS 相关的腹泻、化疗引发的腹泻、急性和慢性胰腺炎和胃肠激素分泌肿瘤; 治疗癌症如肝细胞瘤; 抑制血管生成; 治疗炎症性疾病如关节炎、视网膜炎、慢性同种异体抑制排斥、血管成形术; 防止脉

管移植和胃肠出血。优选的类似物对特定的生长激素释放因子受体亚型具有选择性,或对能得到所希望的生物学反应的亚型具有选择性,因此减少了与其他会导致不希望的副作用的受体亚型进行反应。

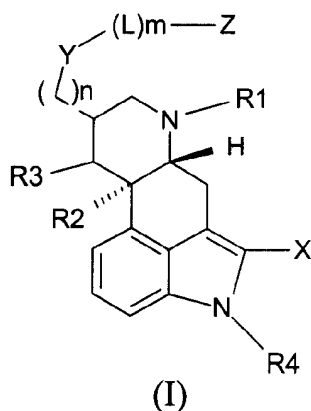
生长激素释放因子(SS)和其受体(SSTR1-SSTR5)在正常的人体滤泡旁
 5 (parafollicular)C 细胞和髓状甲状腺癌(MTC)中进行表达。MTC 是一种由产生降钙素(CT)、生长激素抑制因子以及一些其他的肽的甲状腺滤泡旁 C 细胞引起的肿瘤(Moreau JP 等人, Metabolism 45(8 Suppl 1):24-26)。最近, Mato 等人证明 SS 和 SSTR'S 在人类 MTC 中进行表达。(Mato E 等人, J Clin
 Endocrinol Metab 83:2417-2420)。文献中还记载了 SS 和其类似物在血浆 CT
 10 水平引起下降和在 MTC 病人中得到了症状改善。然而,直至现在, SS 类似物对肿瘤细胞的抗增生活性还没有得到明确的证明(Mahler C 等人, Clin
 Endocrinol 33:261-9; Lupoli G 等人, Cancer 78:1114-8; Smid WM 等人, Neth J. Med. 40: 240-243)。因此, SSTR 亚型类似物选择性地对 MTC 细胞生长的发展和评估为临床研究提供了有用的工具。直至现在,没有报道过关于 SSTR 亚型
 15 与 MTC 细胞生长调节有关的数据。

发明概述

本发明涉及一系列生长激素释放抑制因子-多巴胺嵌合类似物,其在活体内既保留了生长激素释放因子的活性和又保留了多巴胺的活性,包括,其中的一些对单独的天然生长激素释放因子和多巴胺类似物显示出提高的生物学活性及其治疗用途。

一方面,本发明的特征在于式(I)的多巴胺-生长激素释放因子嵌合体,

25



其中,

X 是 H, Cl, Br, I, F, -CN, 或 C_{1-5} 烷基;

R1 是 H, C_{1-4} 烷基, 烯丙基, 链烯基或 -CN;

R2 和 R3 分别独立地为 H 或不存在, 条件是当 R2 和 R3 不存在时, 它们所连

5 接的碳原子之间有双键;

R4 是 H 或 $-CH_3$;

Y 是 $-O-$, $-C(O)-$, $-S-$, $-S-(CH_2)S-C(O)-$, $-S(O)-$, $-S(O)_2-$, $-SC(O)-$, $-OC(O)-$, $-N(R5)-C(O)-$, 或 $-N(R6)-$;

R5, R6, R7 和 R8 分别独立地是 H 或 C_{1-5} 烷基;

10 R6 是 H 或 C_{1-5} 烷基;

m 是 0 或 1;

n 是 0-10;

当 Y 是 $-S-$, $-S(O)-$, $-S(O)_2-$, $-O-$ 或 $-N(R6)-$ 时; L 是 $-(CH_2)_p-C(O)-$,

当 Y 是 $-N(R6)-$, $-O-$, 或 $-S-$ 时; L 是 $-C(O)-(CR7R8)_q-C(O)-$,

15 p 是 1-10;

q 是 2-4;

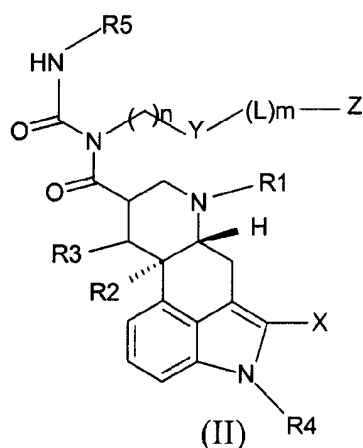
s 是 1-10;

t 是 1-10; 和

z 是生长激素释放因子类似物,

20 或其药学上可接受的盐。

另一方面, 本发明的特征在于式 (II) 的多巴胺-生长激素释放因子嵌合体,



其中:

X 是 H, Cl, Br, I, F, CN, 或 C₁₋₅ 烷基;

R₁ 是 C₁₋₄ 烷基, H, 烯丙基, 链烯基或-CN;

5 R₂ 和 R₃ 分别独立地为 H 或不存在, 条件是当 R₂ 和 R₃ 不存在时, 它们所连接的碳原子之间有双键;

R₄ 是 H 或-CH₃;

R₅ 是 C₁₋₅ 烷基基团, 或式-CH₂rN(CH₃)_q 的基团;

Y 是 -O-, -C(O)-, -S-, -SC(O)-, -OC(O)-, -N(R₆)-C(O), -N(R₇)-, 或 -N(R₈)-(CH₂)_s-C(O)-;

10 R₆, R₇, R₈, R₉ 和 R₁₀ 分别独立为 H 或 C₁₋₅ 烷基;

当 Y 是-S-, -O-或 -N(R₇)-时, L 是-(CH₂)_p-C(O)-;

当 Y 是-N(R₇)-, -O-或-S-时, L 是-(CO)-(CR₉R₁₀)_q-C(O)-;

当 Y 是-C(O)-, -SC(O)-, -OC(O)-, -N(R₈)-(CH₂)_s-C(O)-,或-N(R₆)-C(O)-时, L 是-(Doc)_t-;

15 m 是 0 或 1;

n 是 2-10;

q 是 2-4;

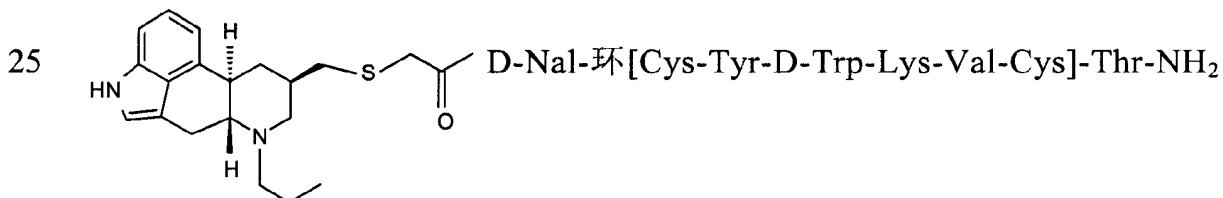
p 是 1-10;

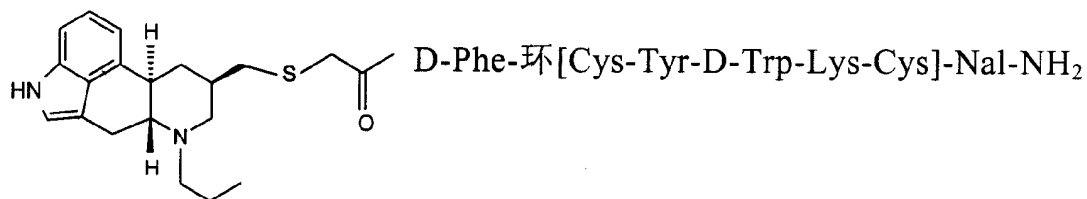
s 是 1-10;

20 t 是 1-10; 和

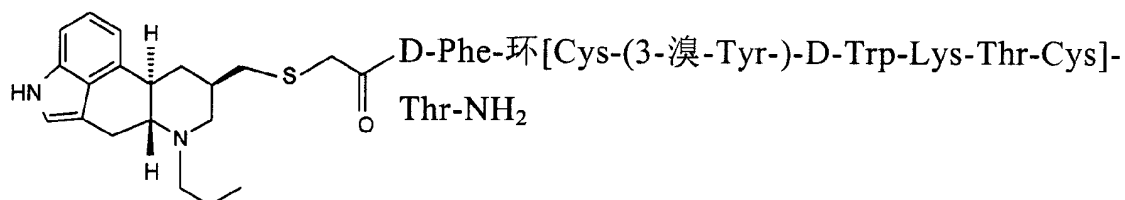
z 是生长激素释放因子类似物,
或其药学上可接受的盐。

本发明的一个实施方式在于下式化合物或其药学上可接受的盐:

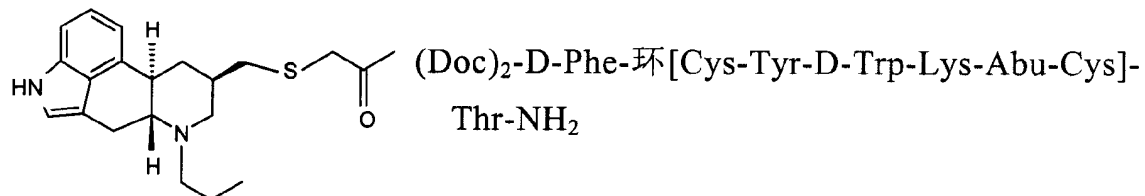




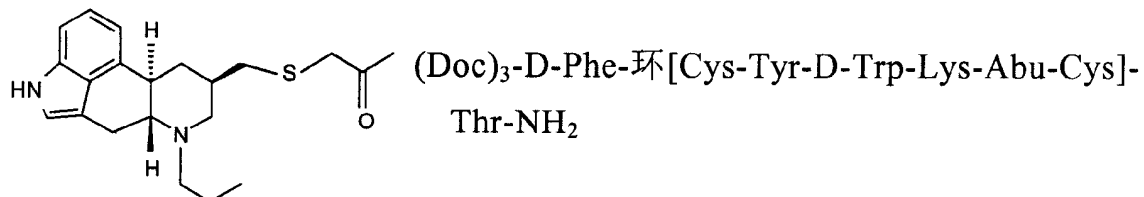
5



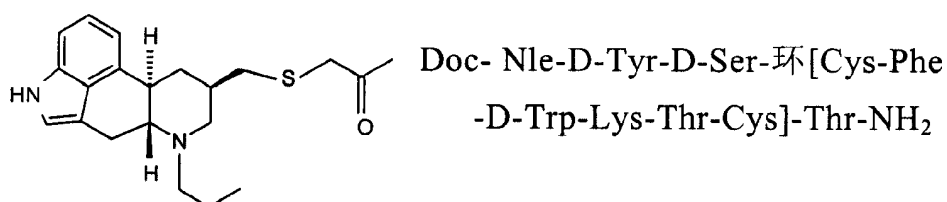
10



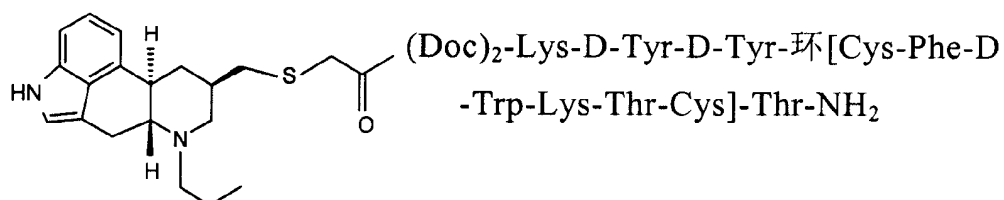
15



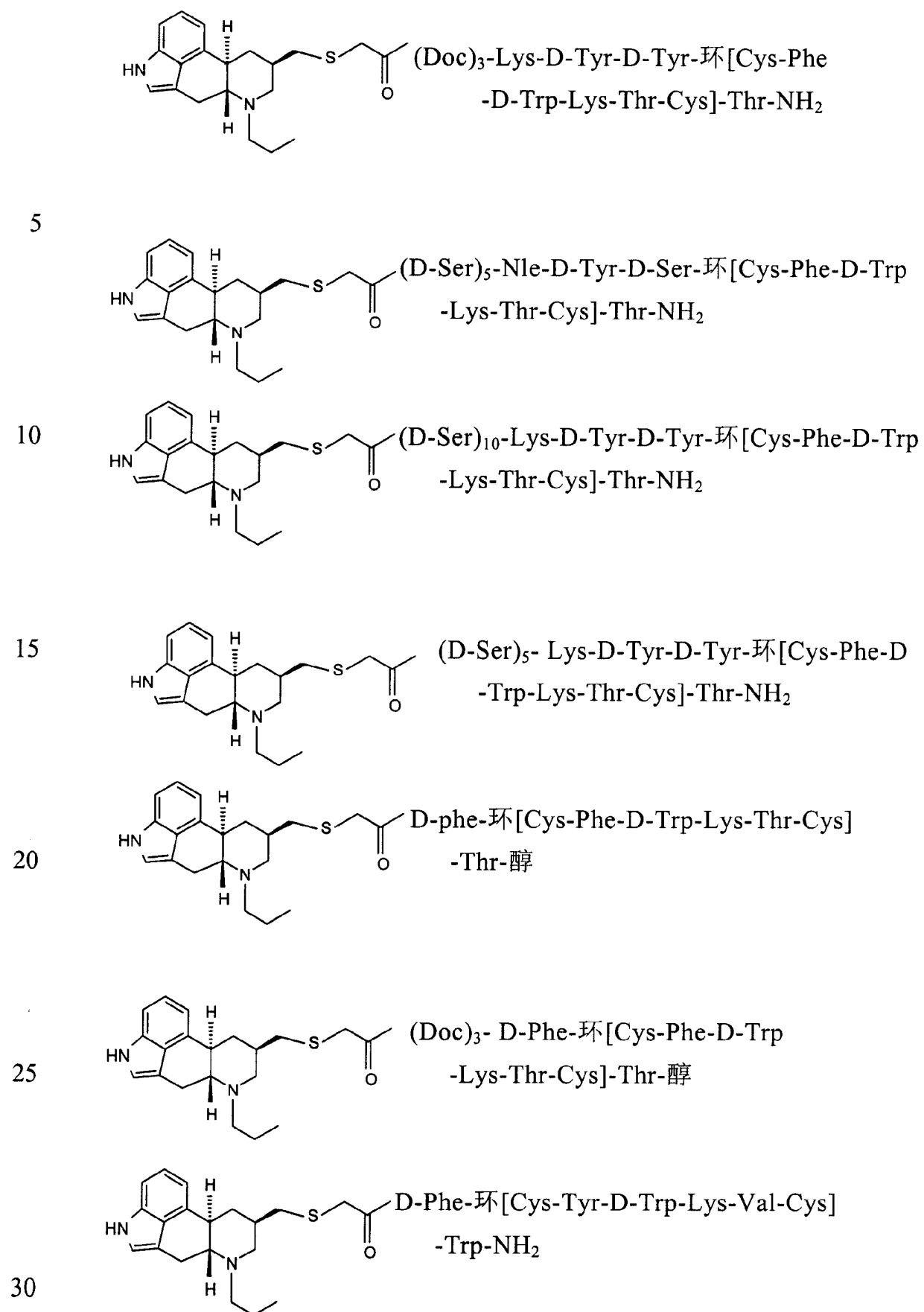
20

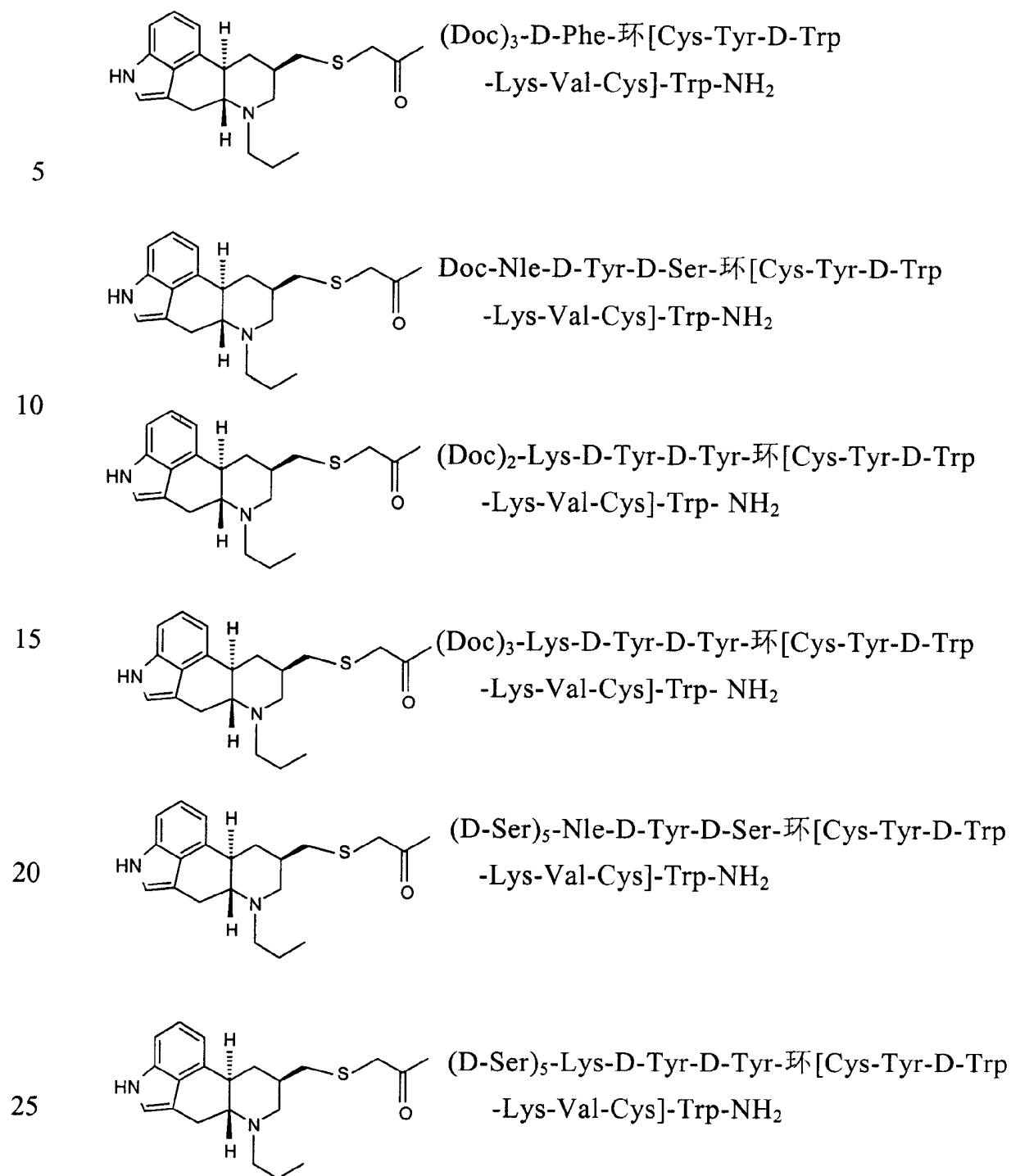


25

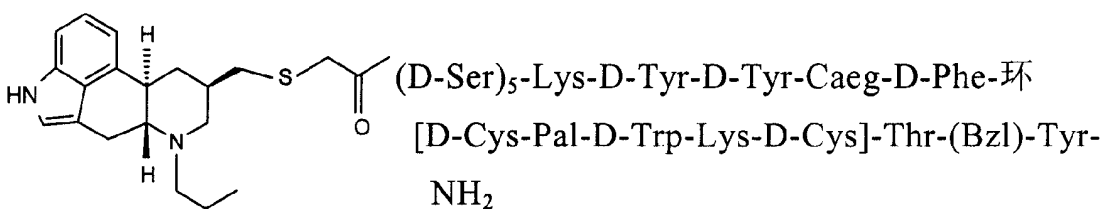
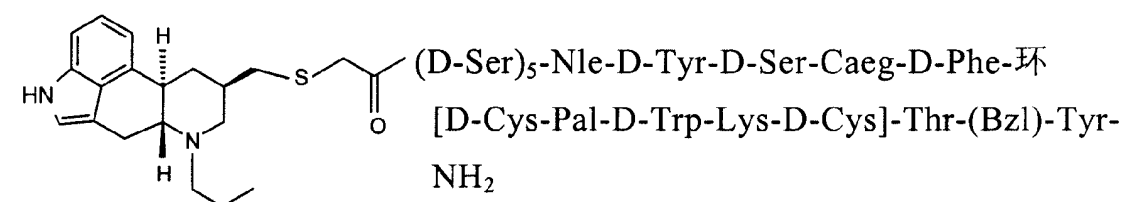
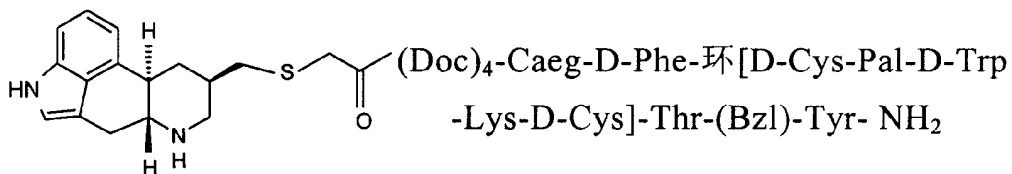
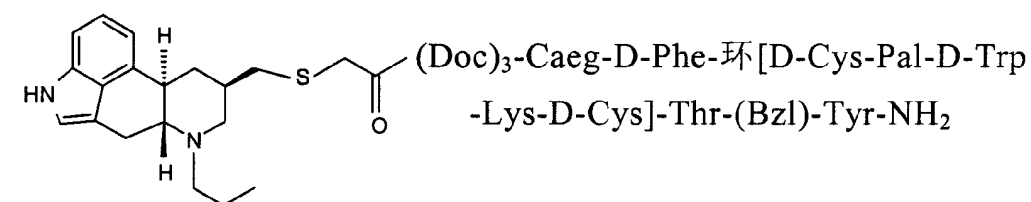
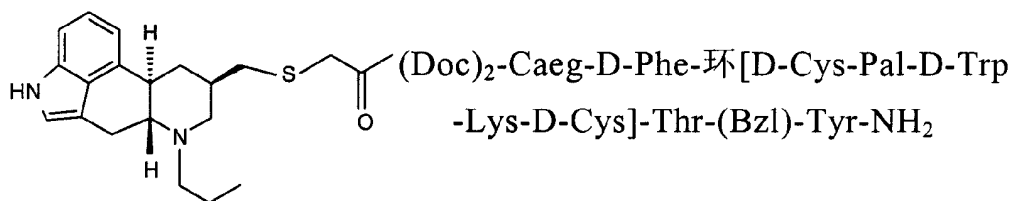
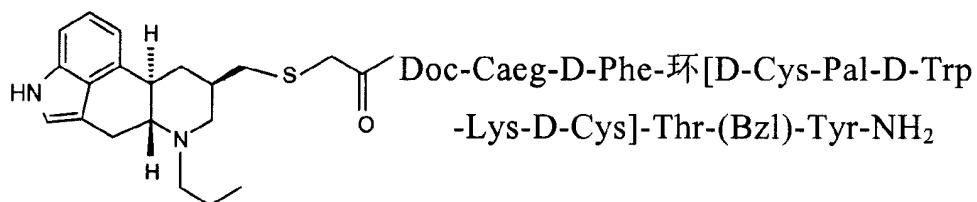
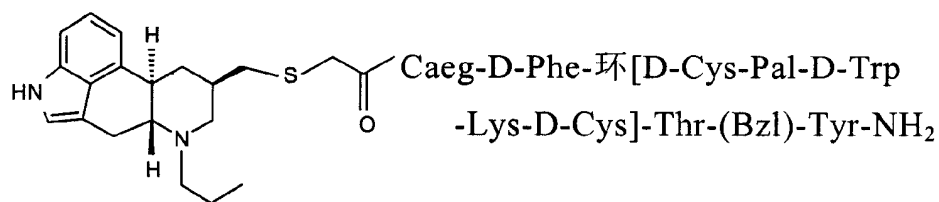


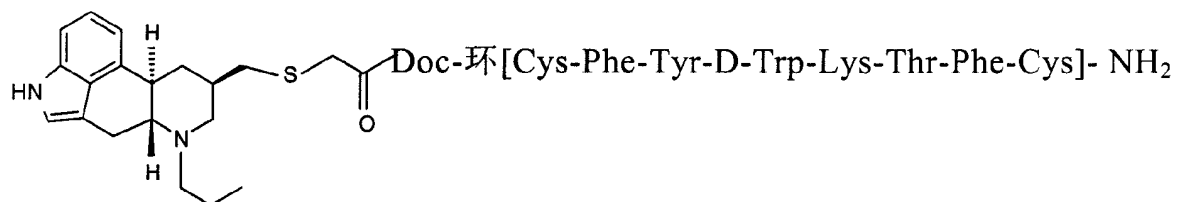
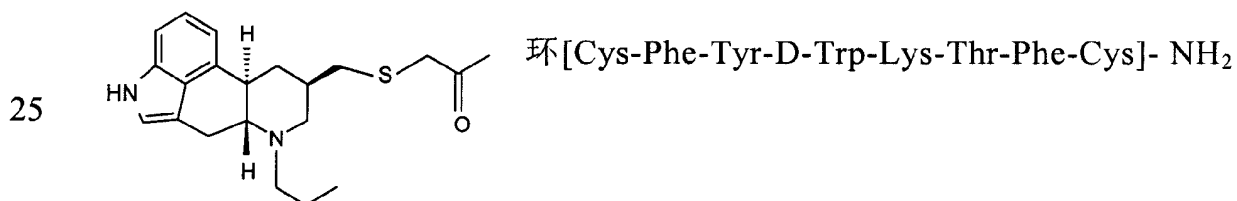
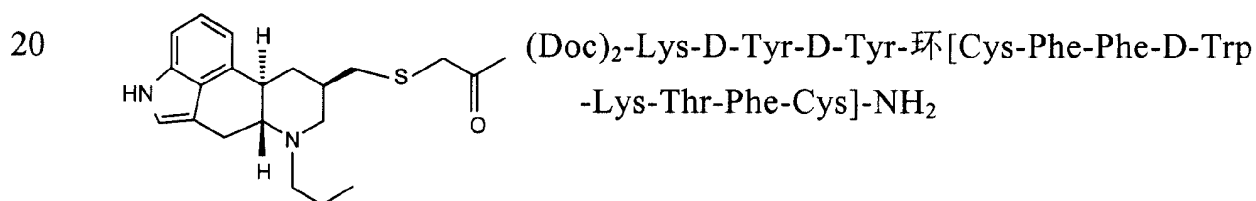
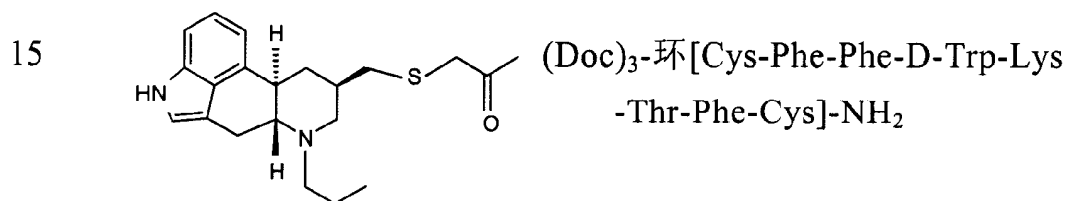
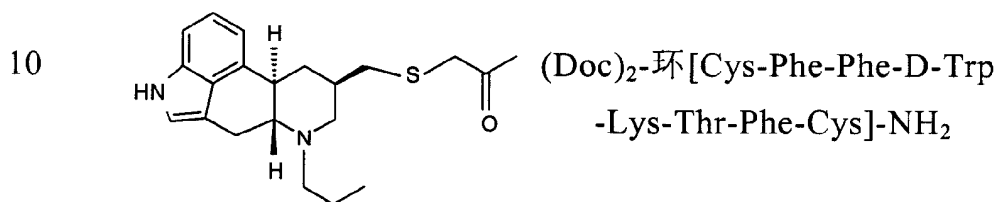
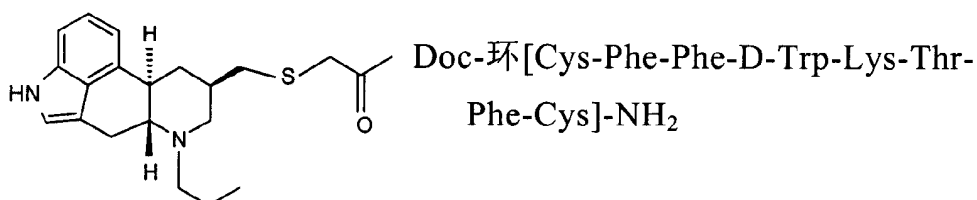
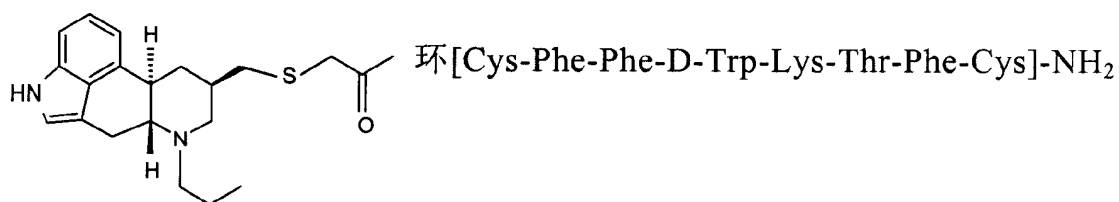
30

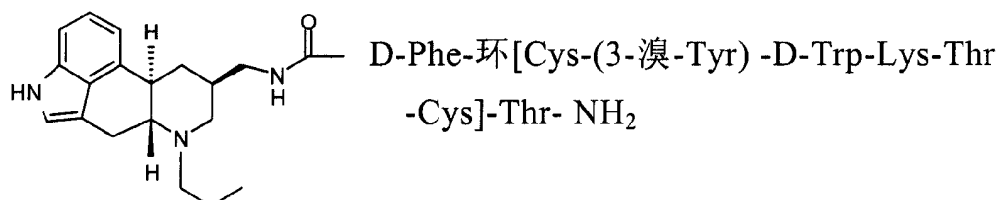
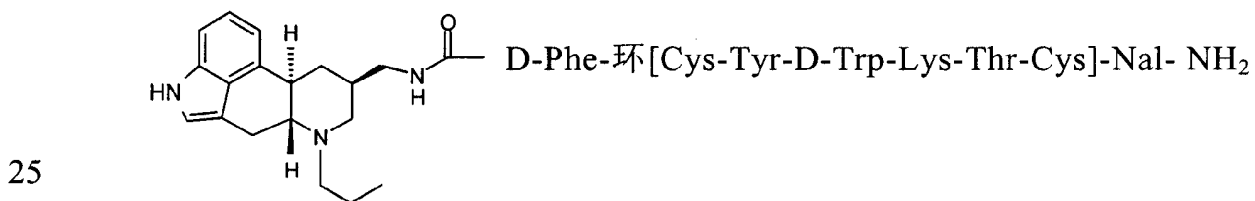
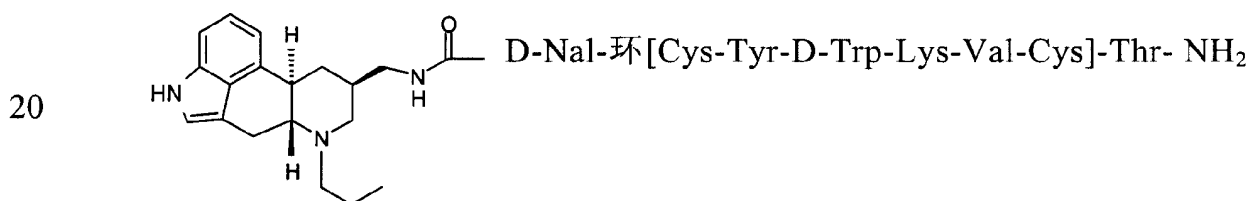
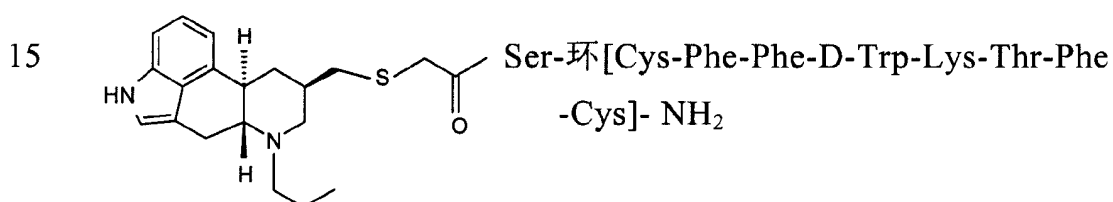
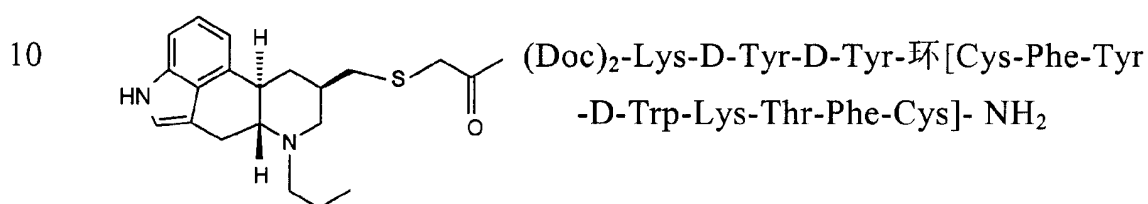
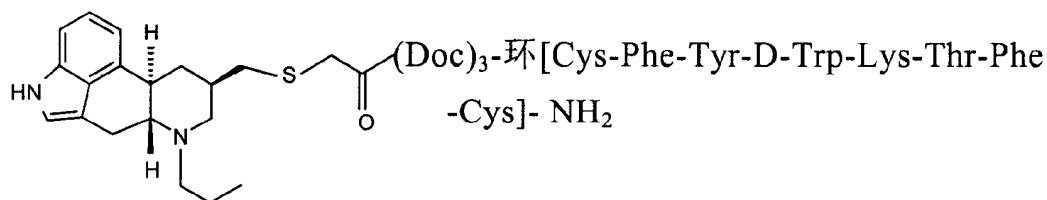
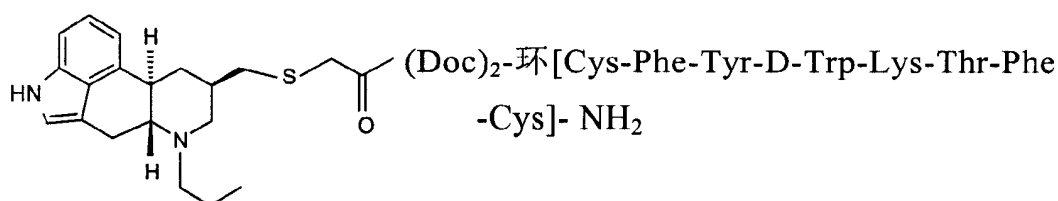


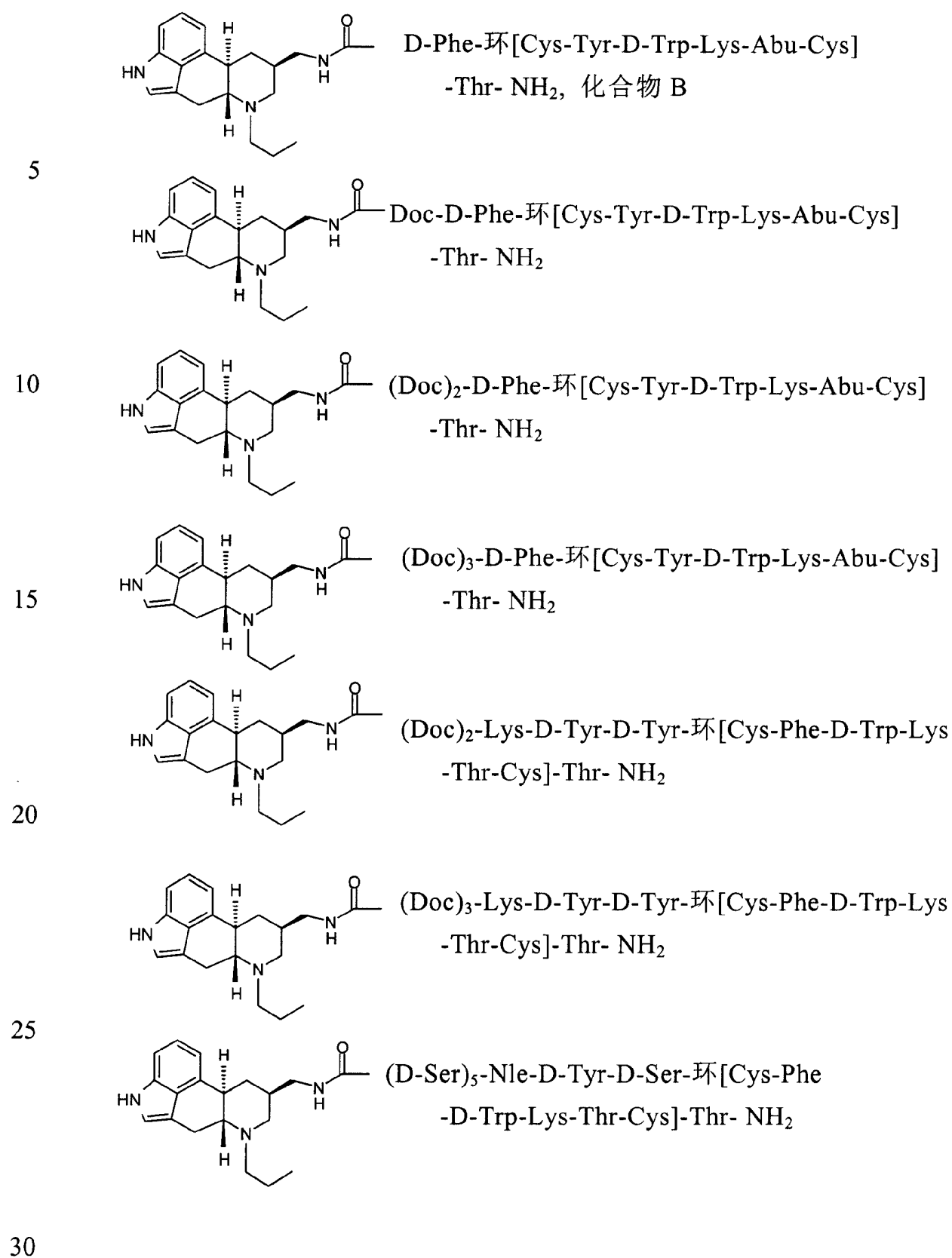


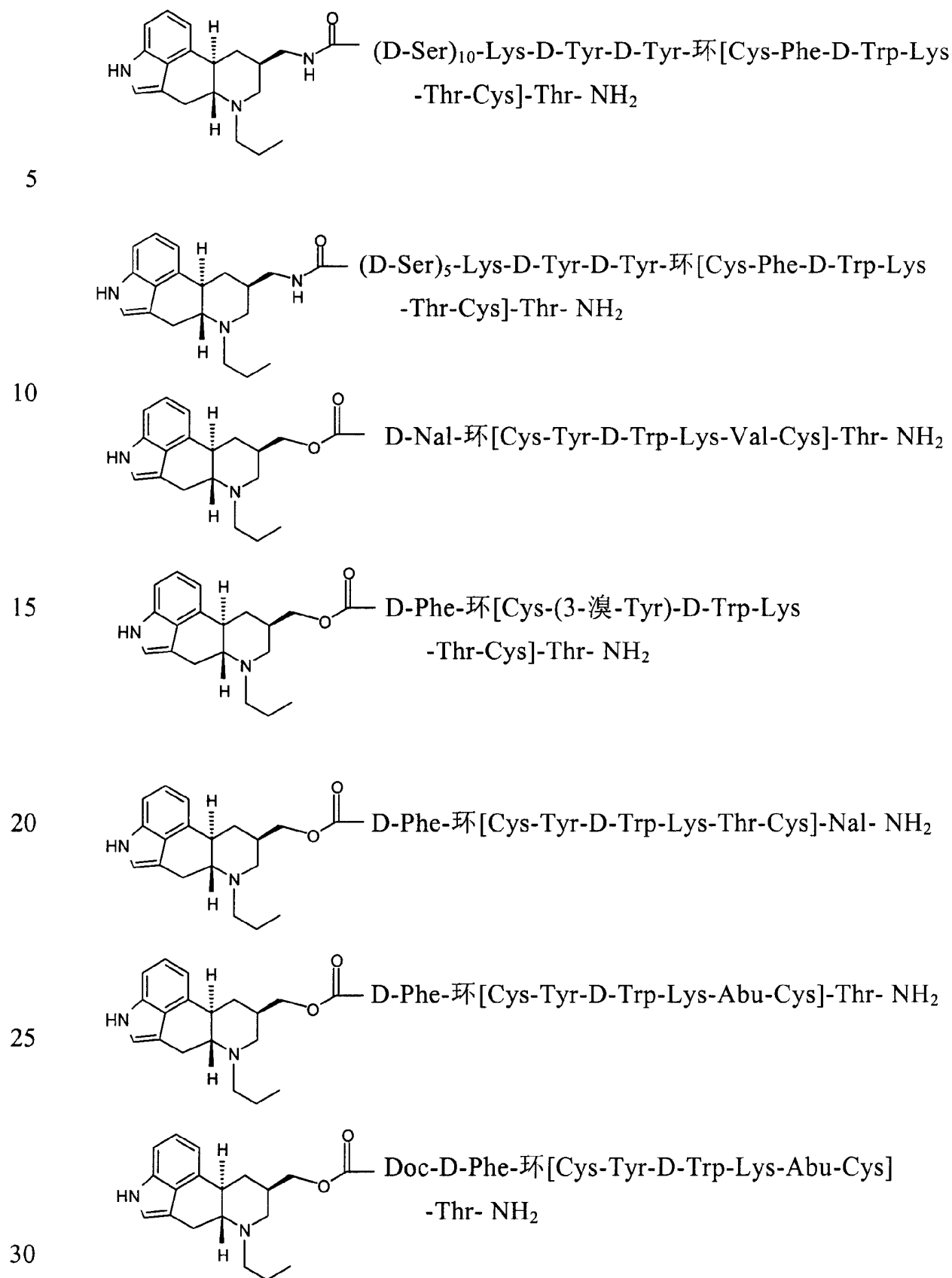
Caeg= N-(2-氨基乙基)-N-(2-胞嘧啶基-1-氧代-乙基)-甘氨酸

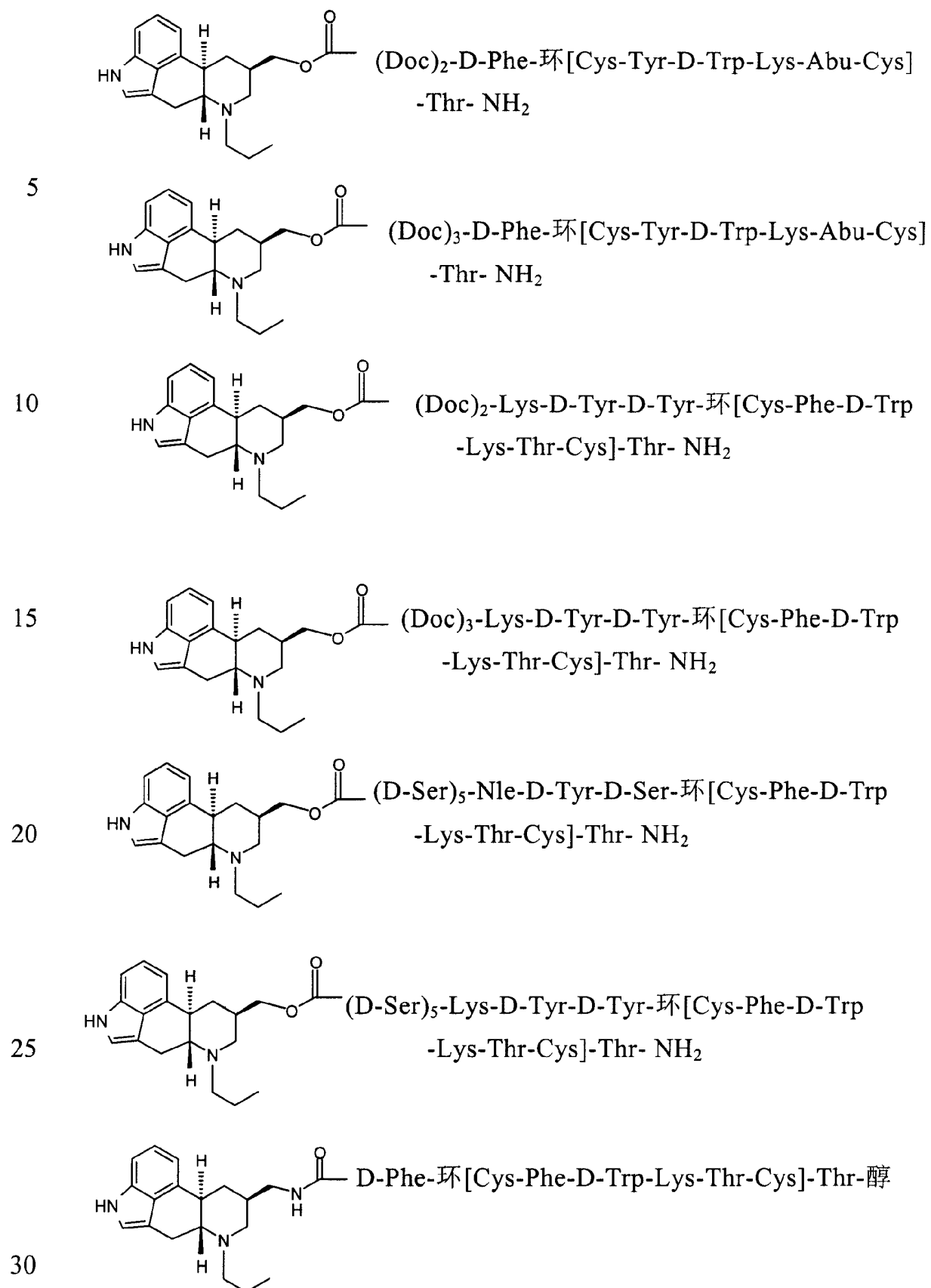


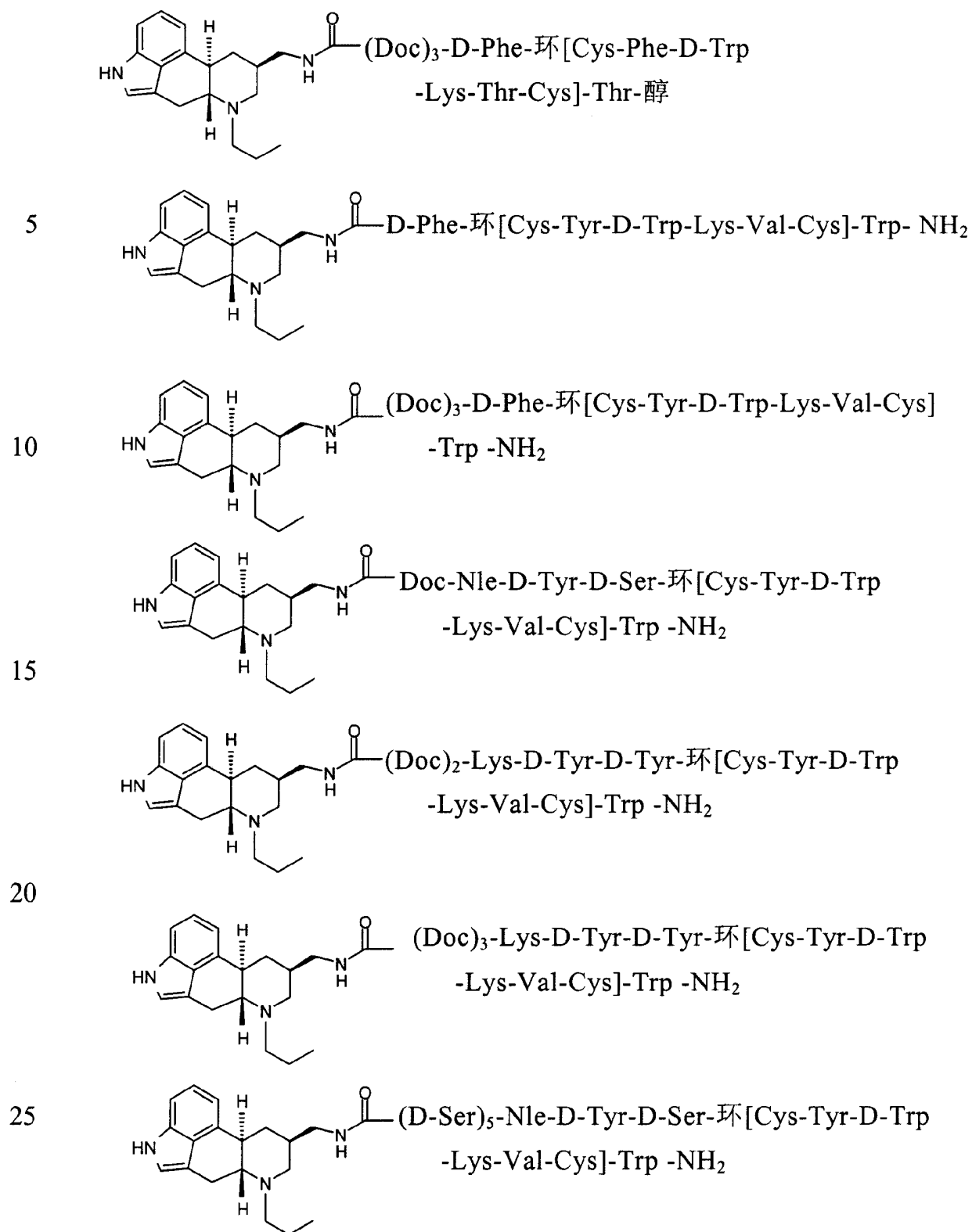


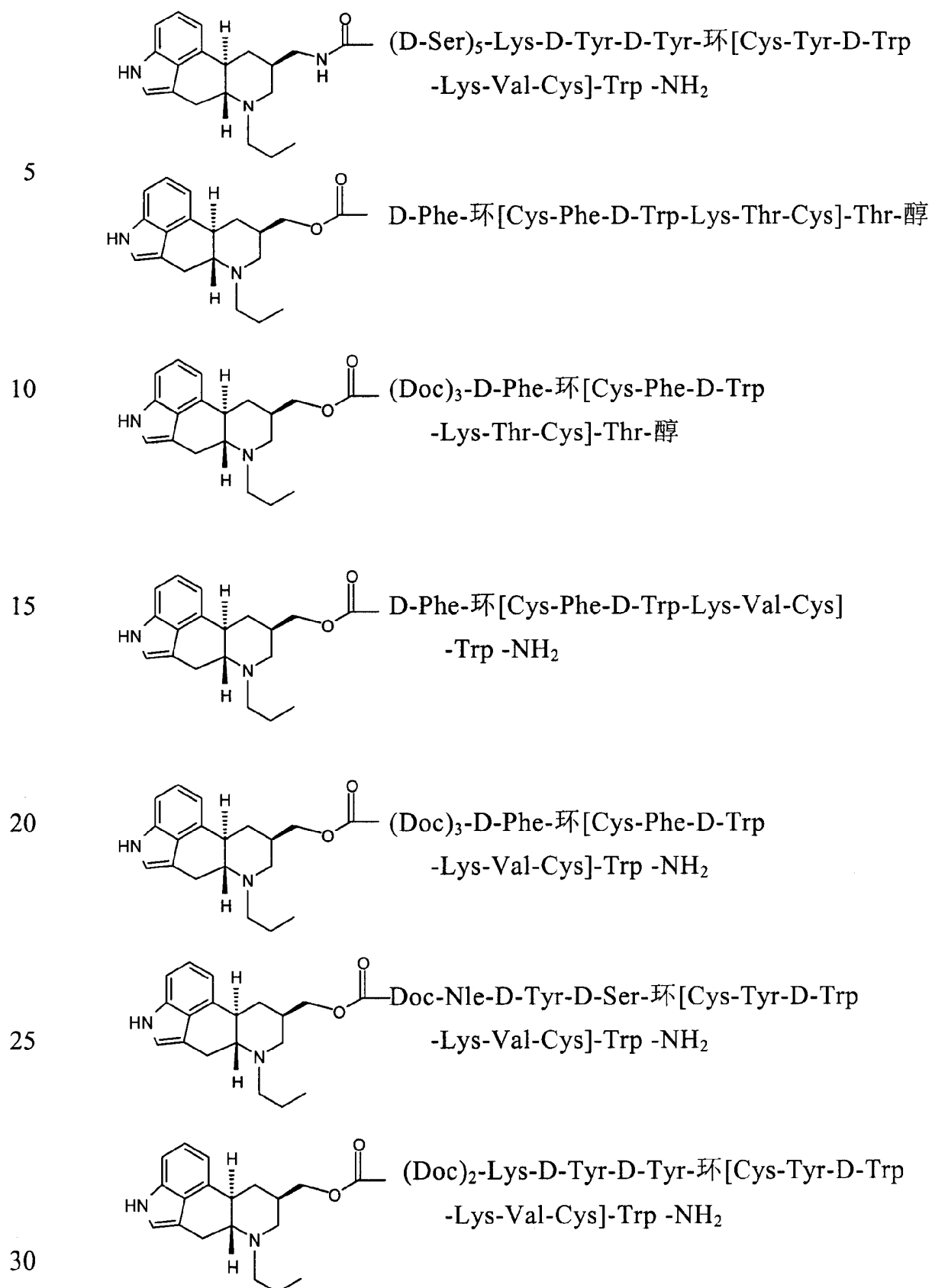


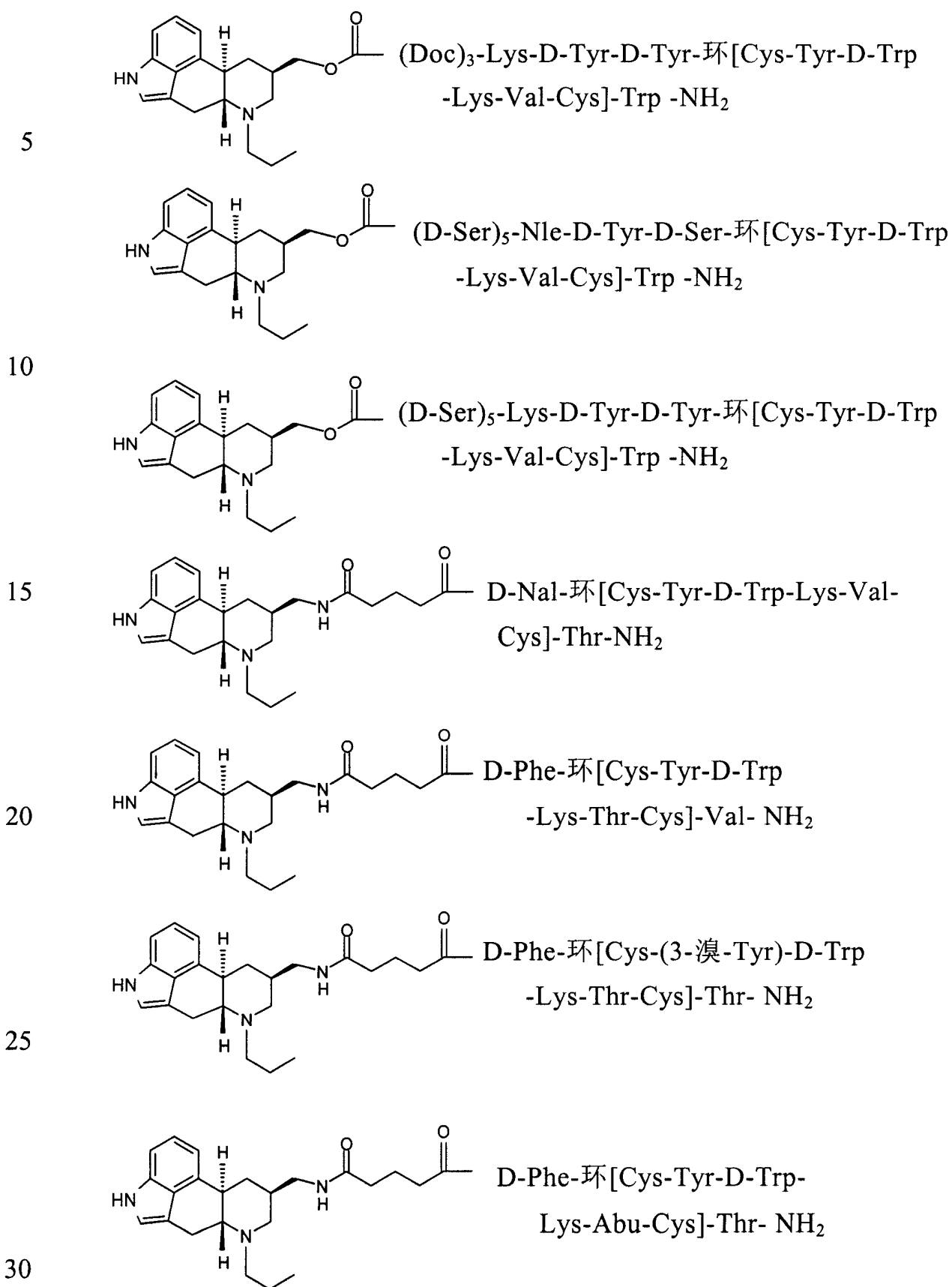




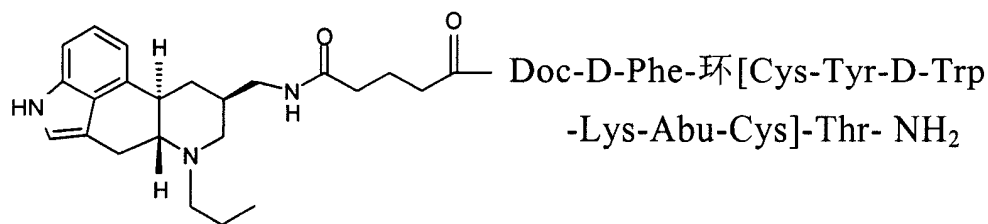




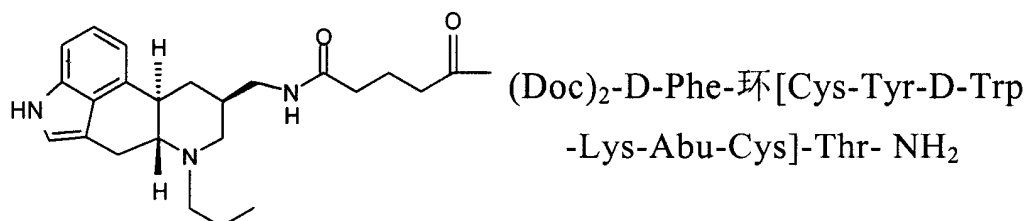




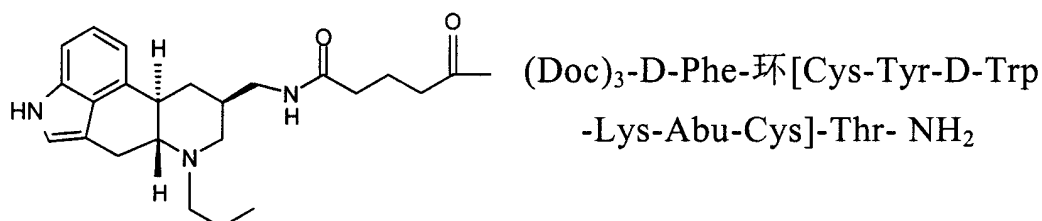
5



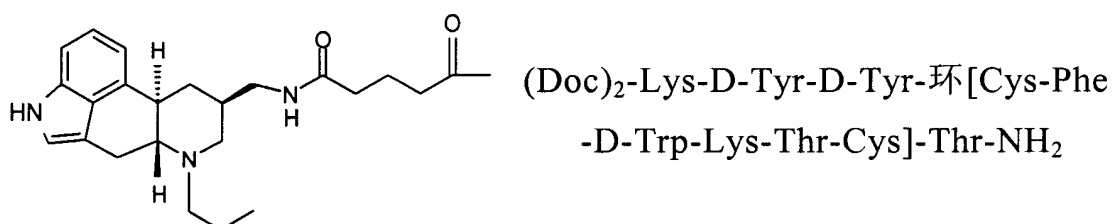
10



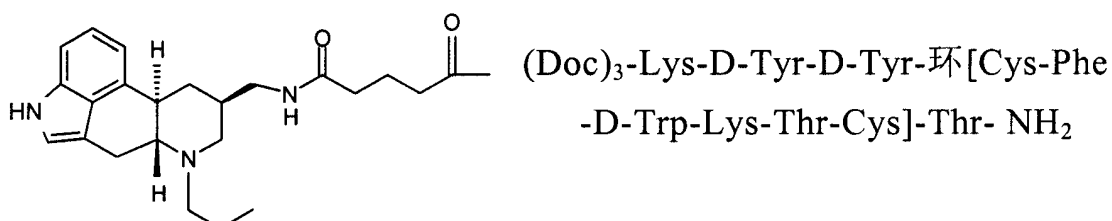
15



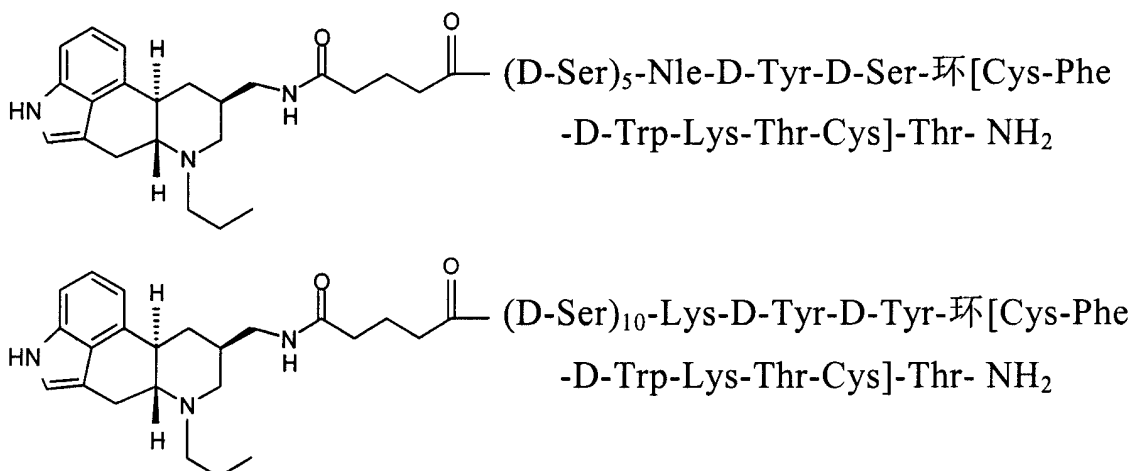
20

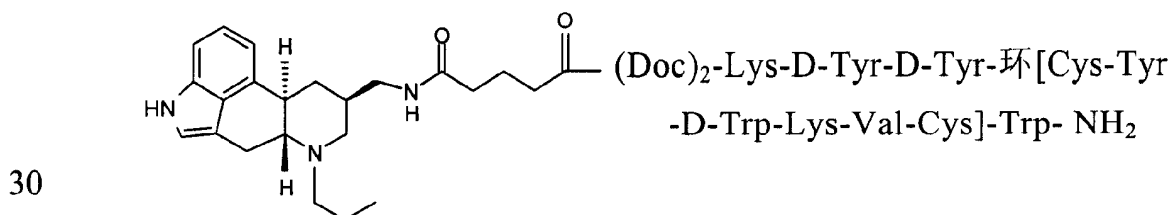
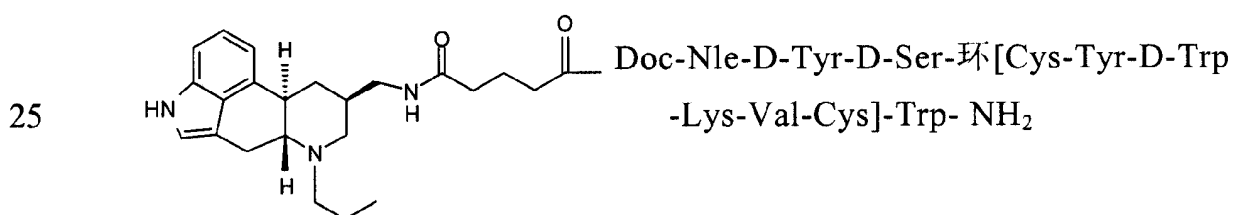
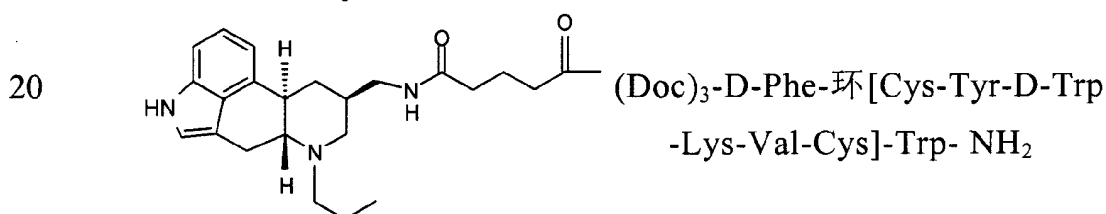
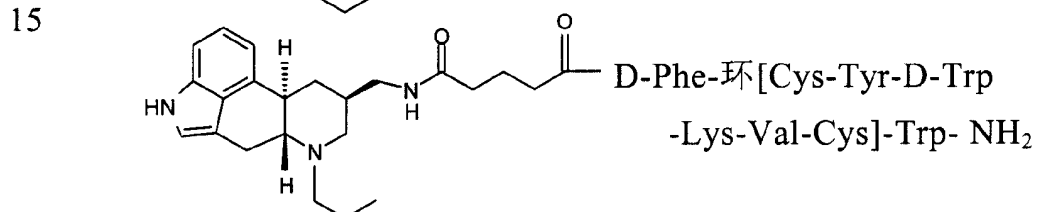
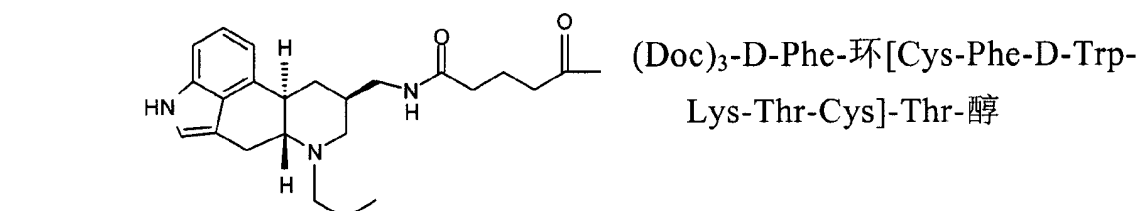
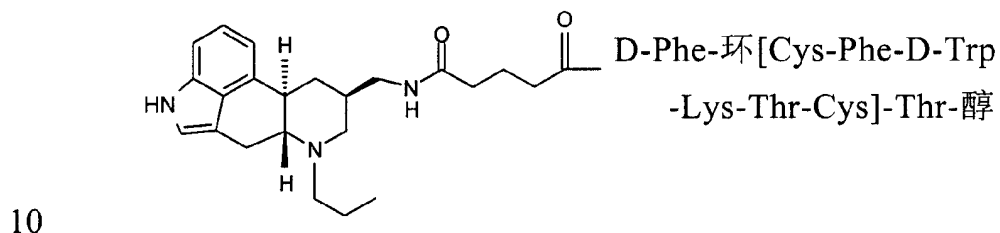
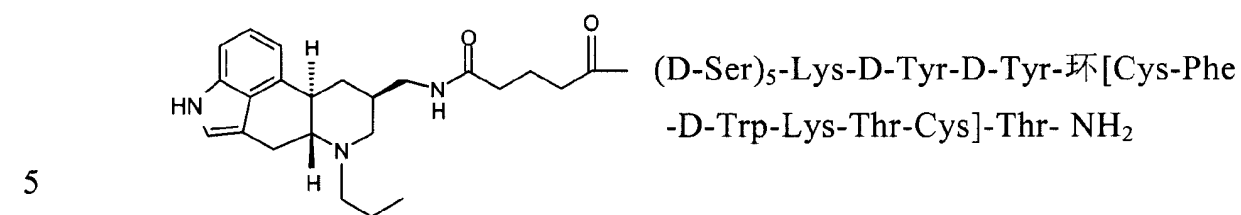


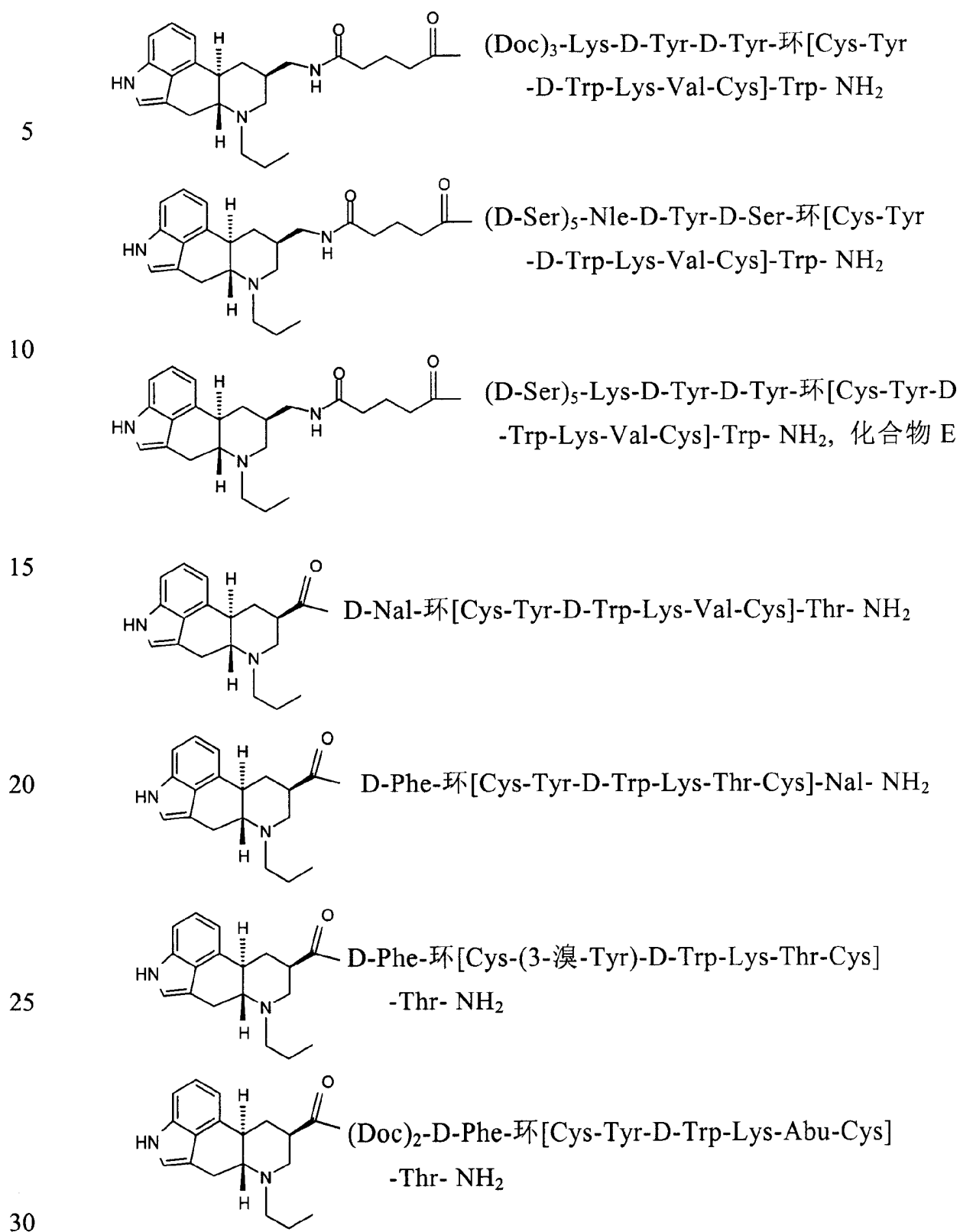
25

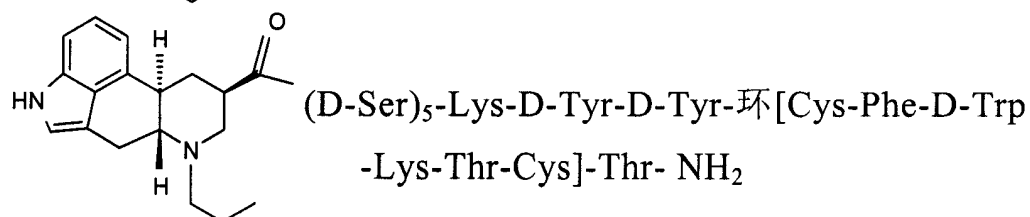
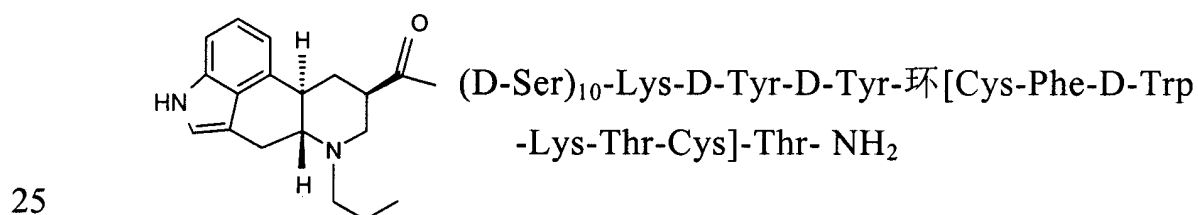
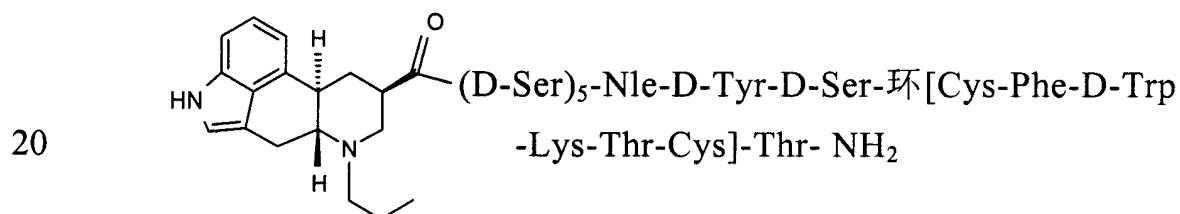
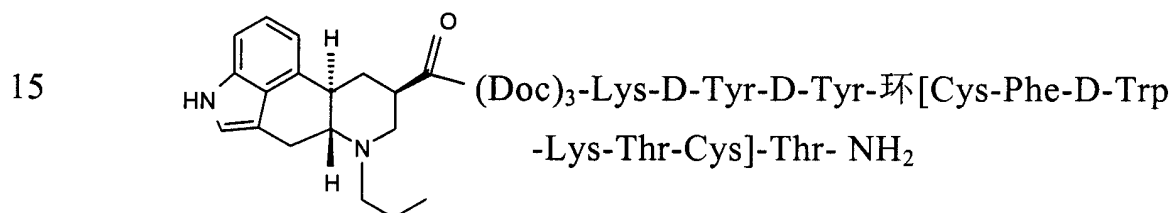
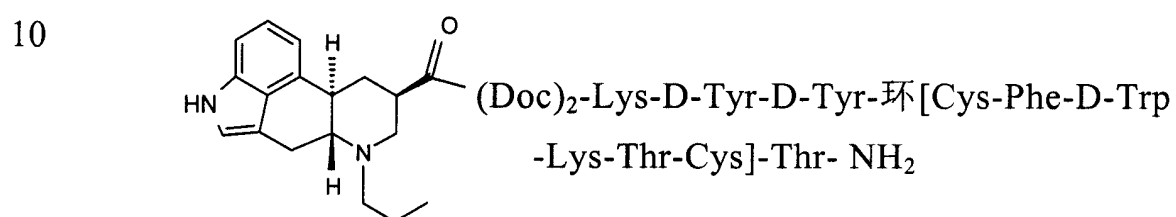
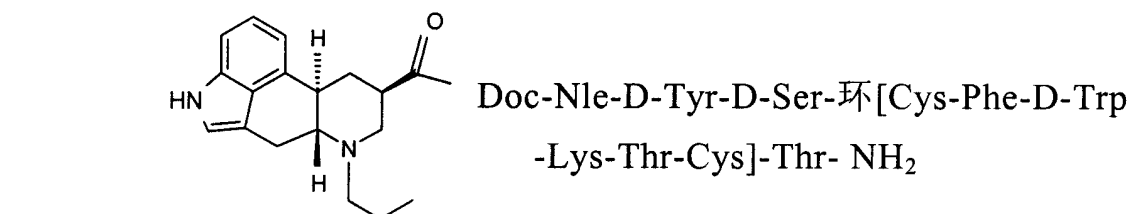
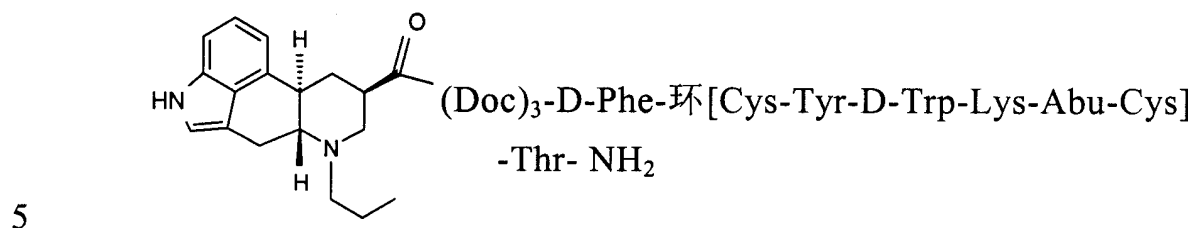


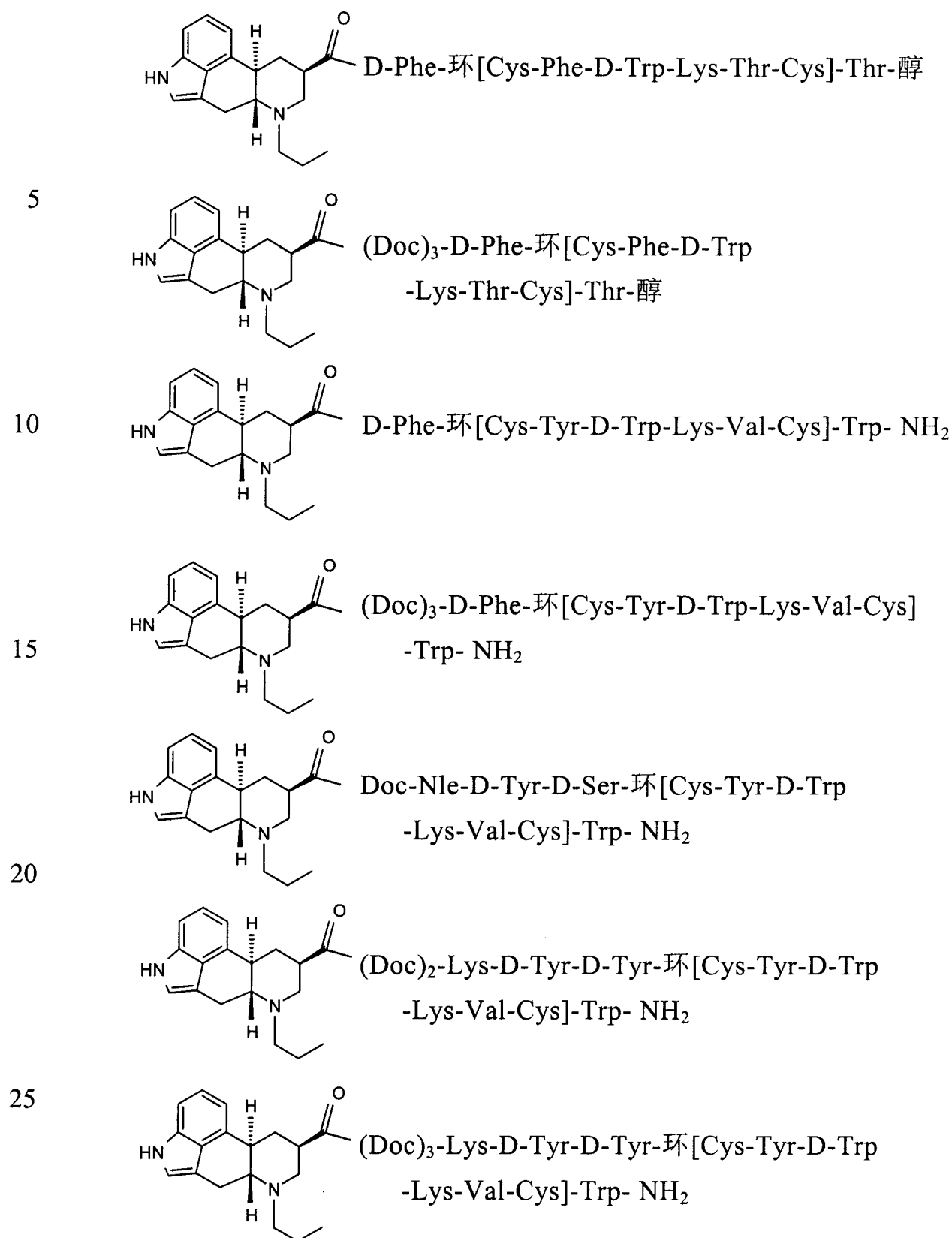
30

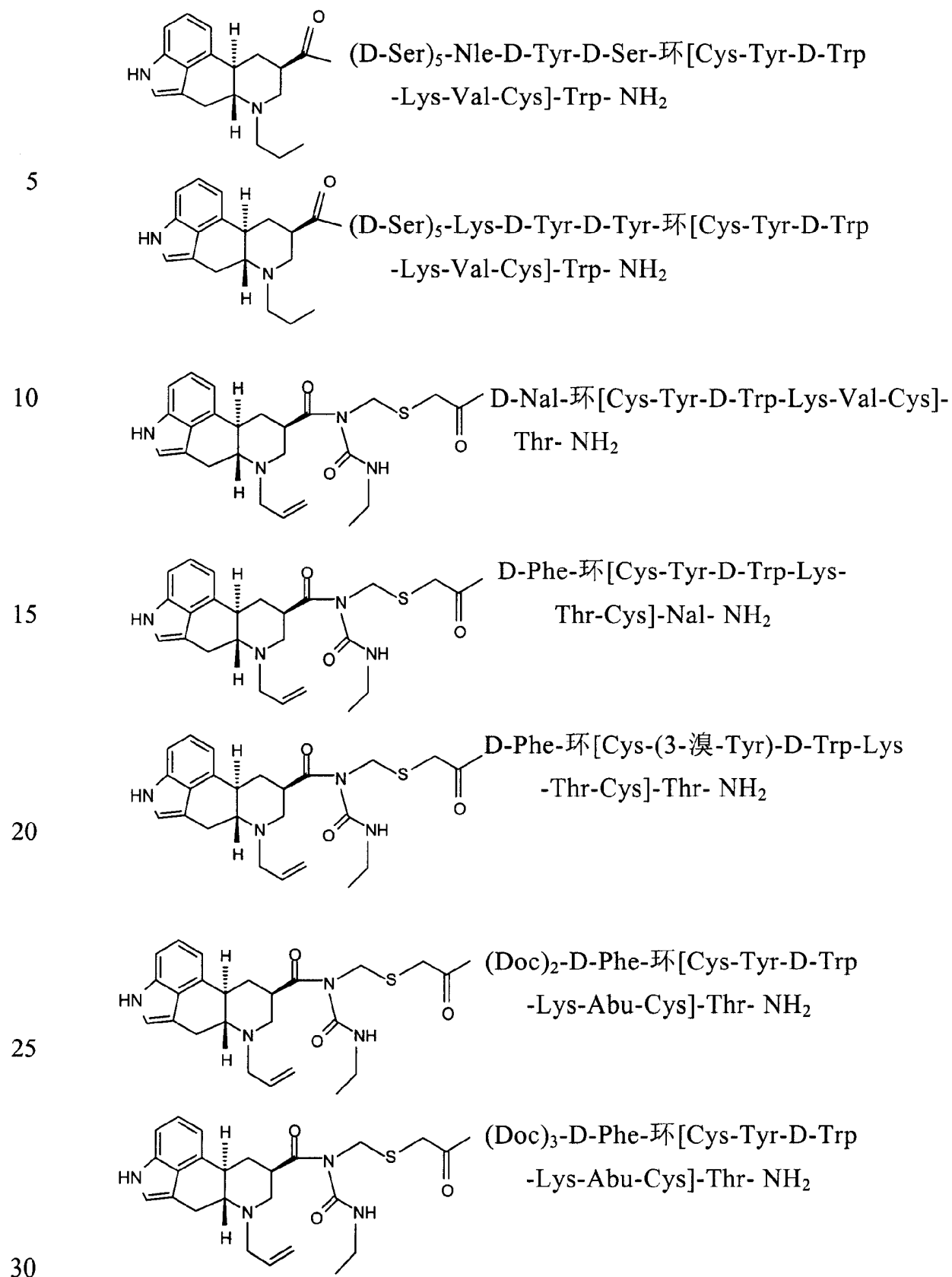




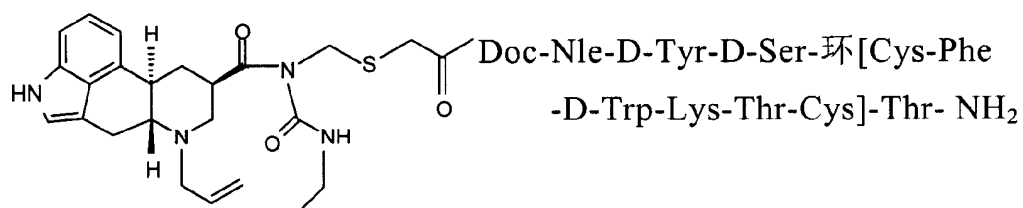




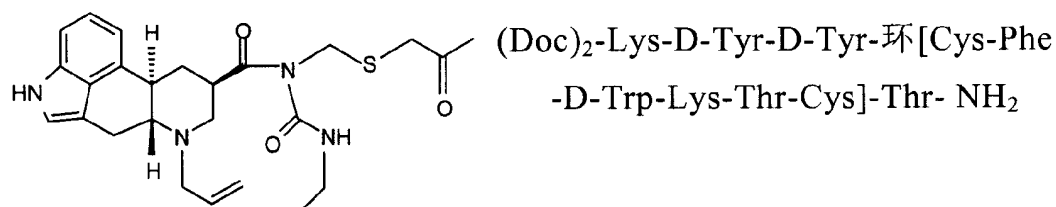




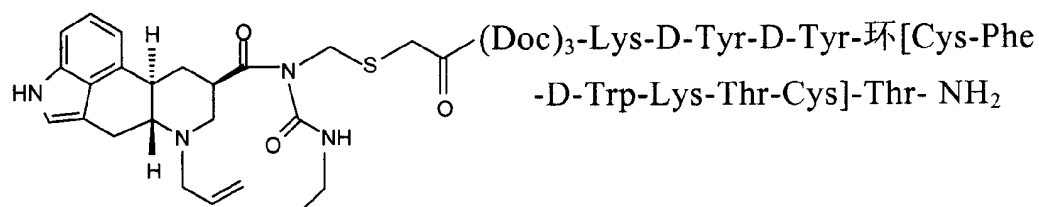
5



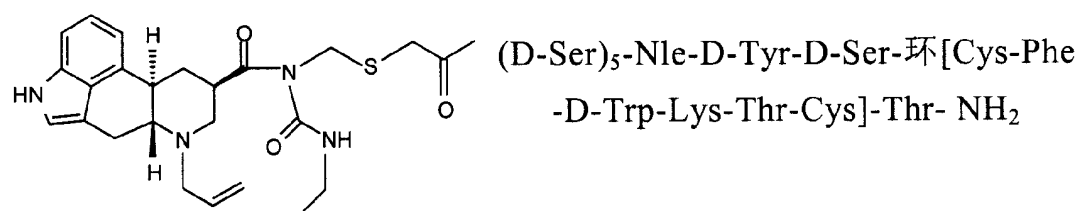
10



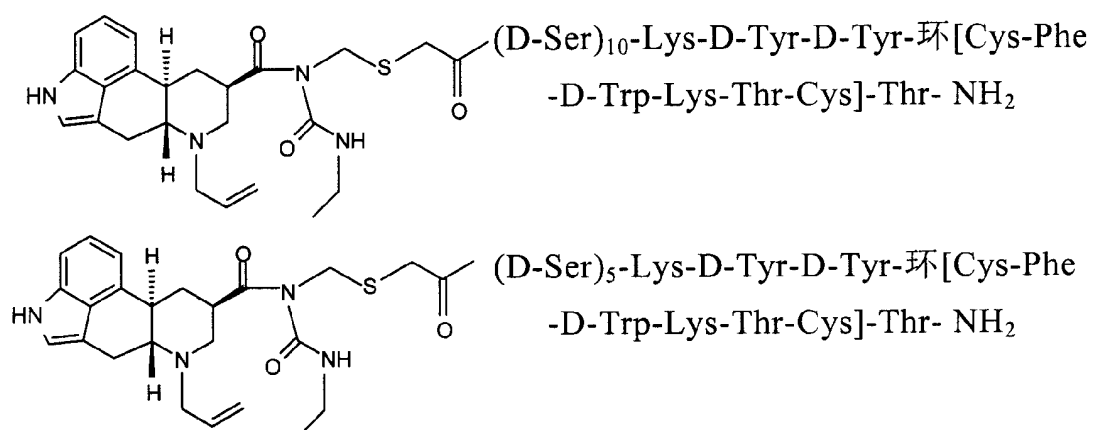
15

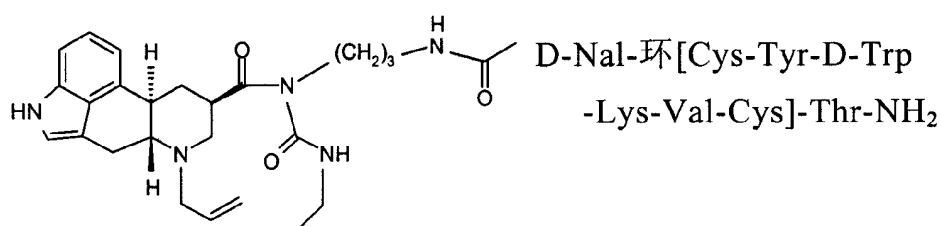


20

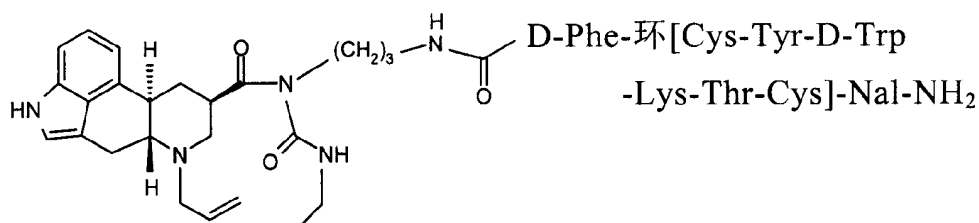


25

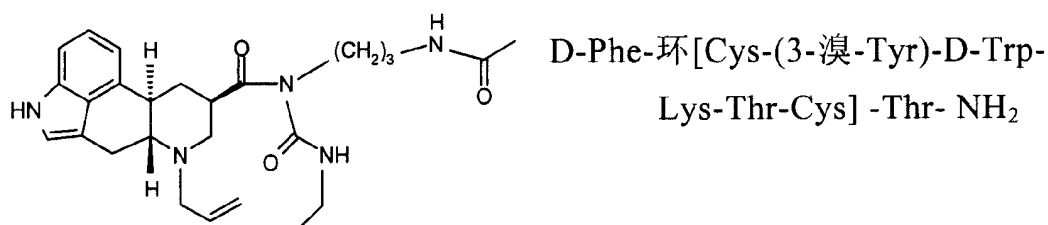




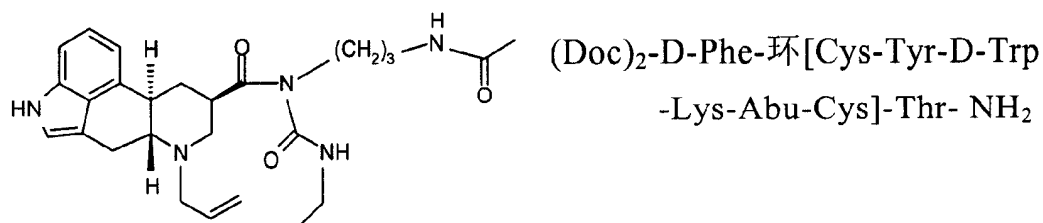
5



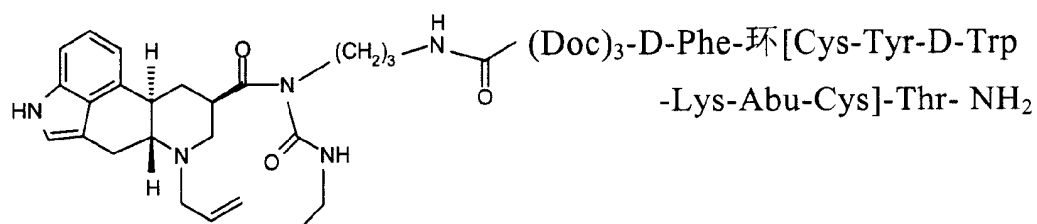
10



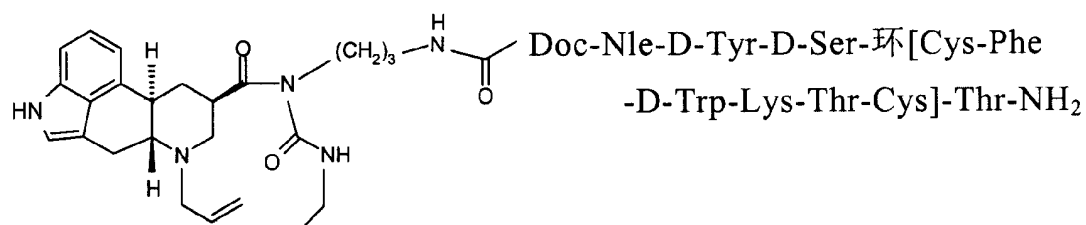
15



20

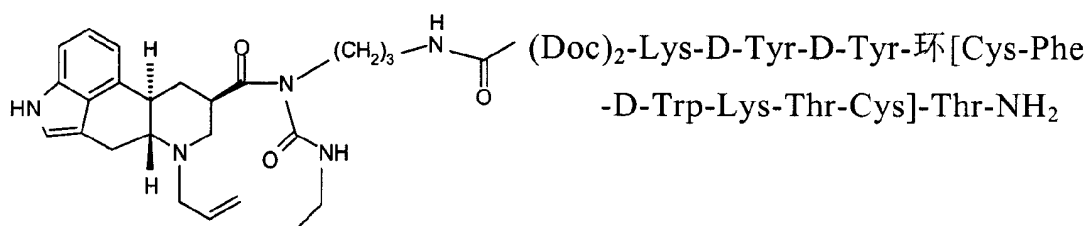


25

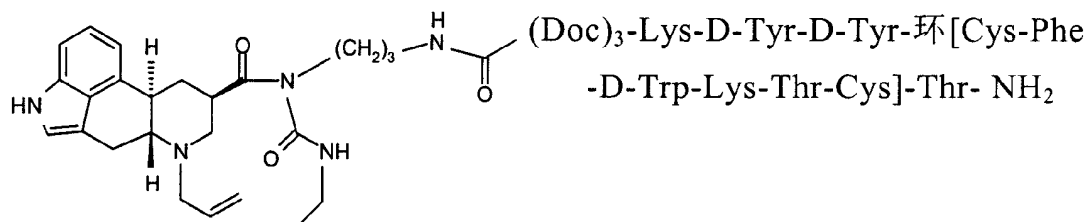


30

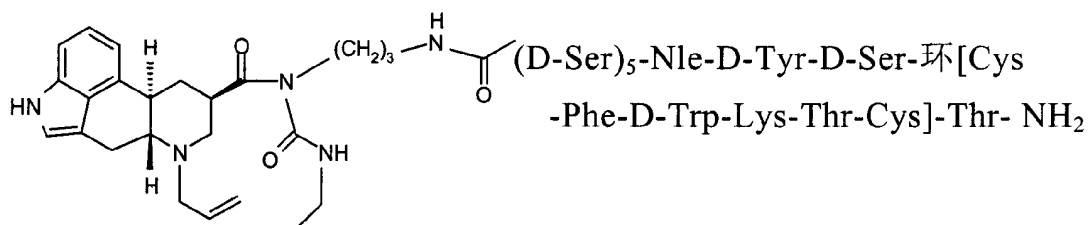
5



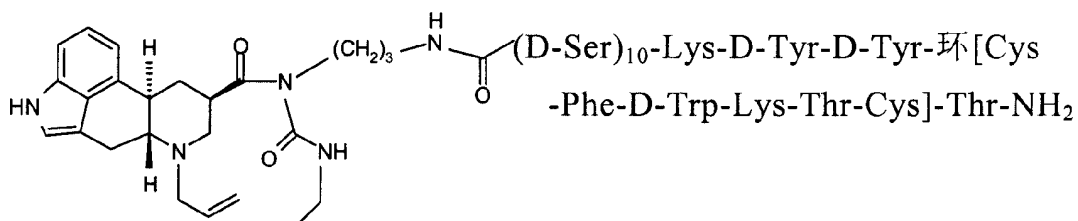
10



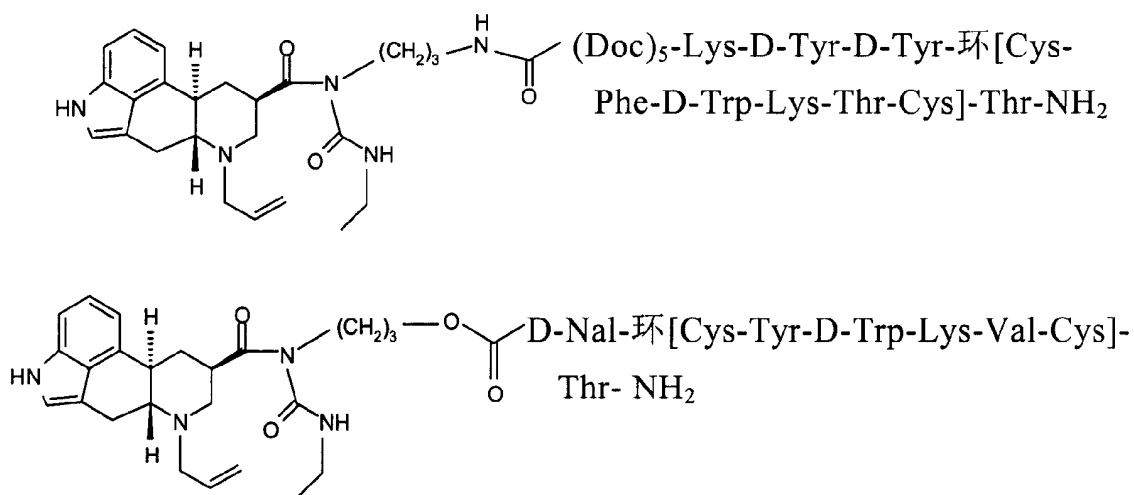
15



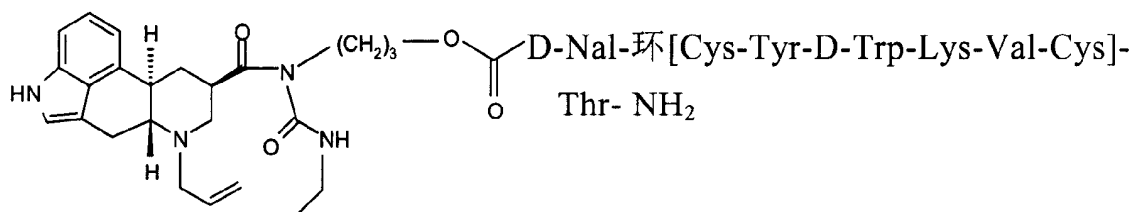
20



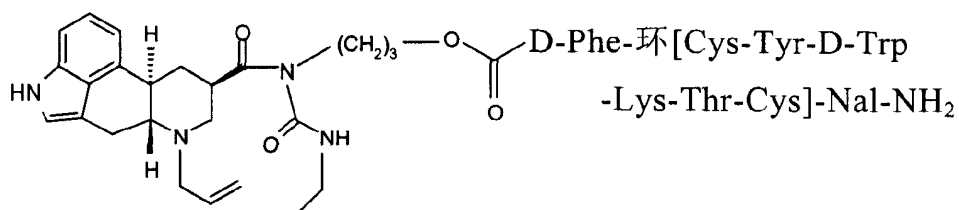
25



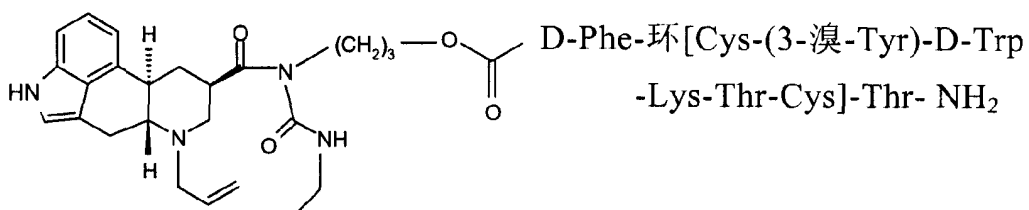
30



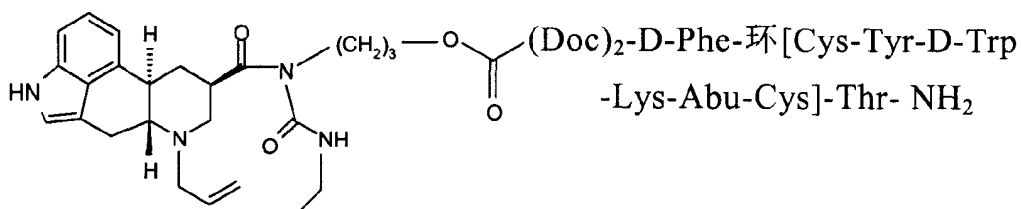
5



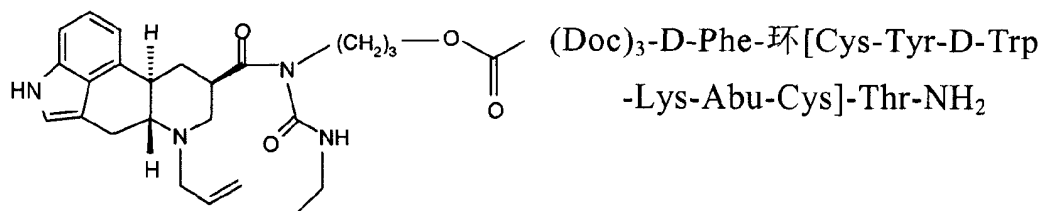
10



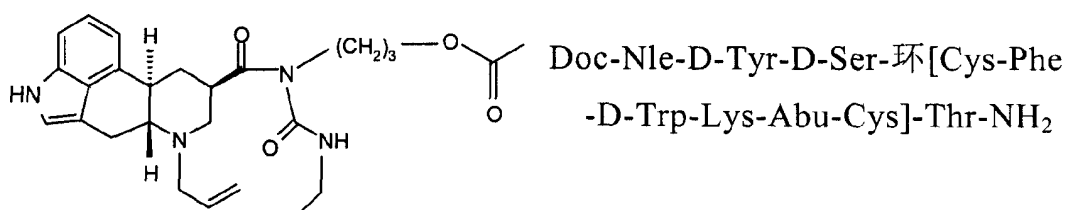
15



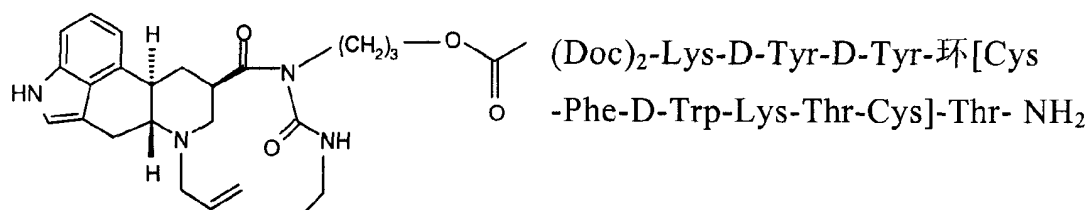
20



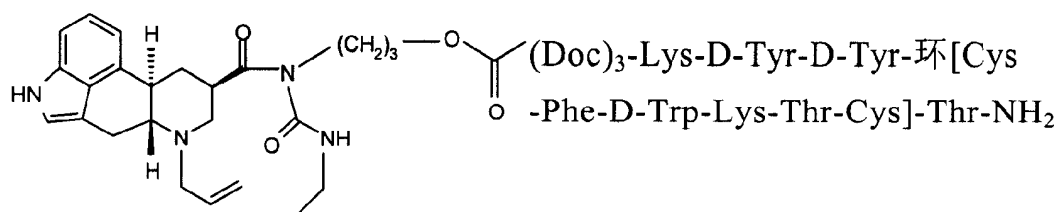
25



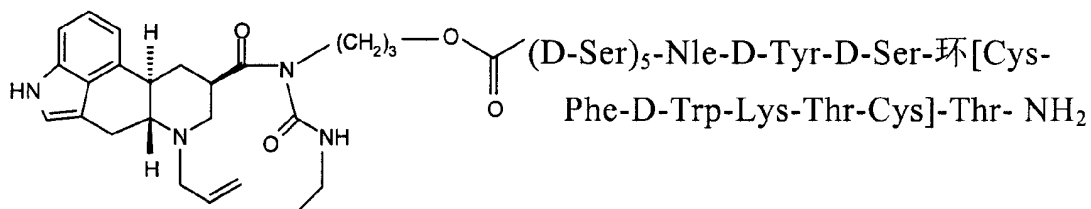
30



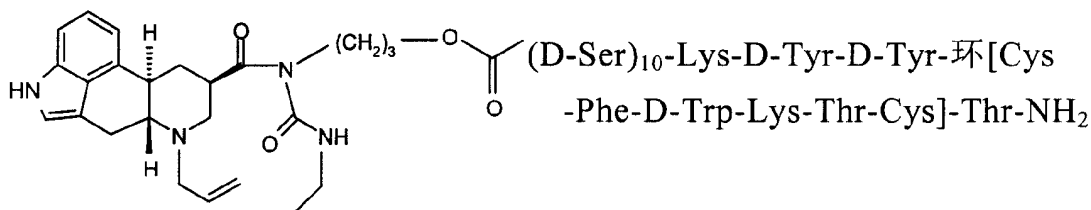
5



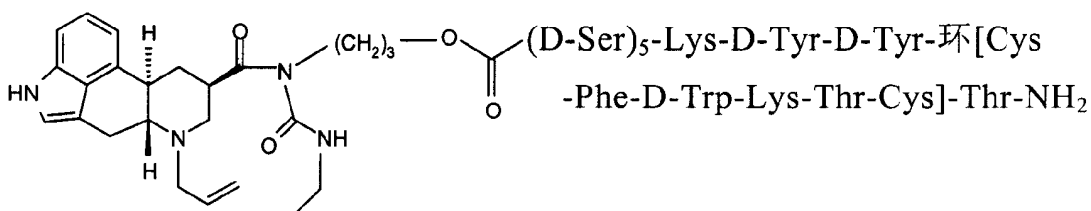
10



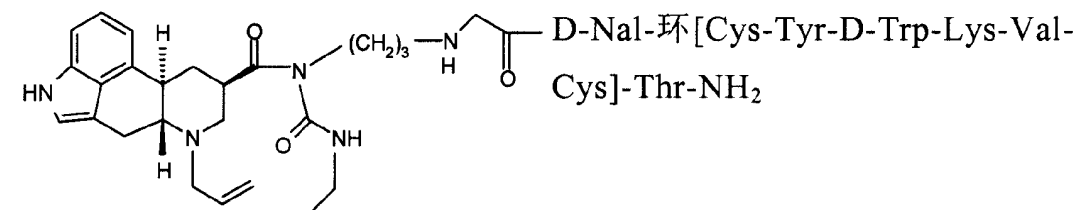
15



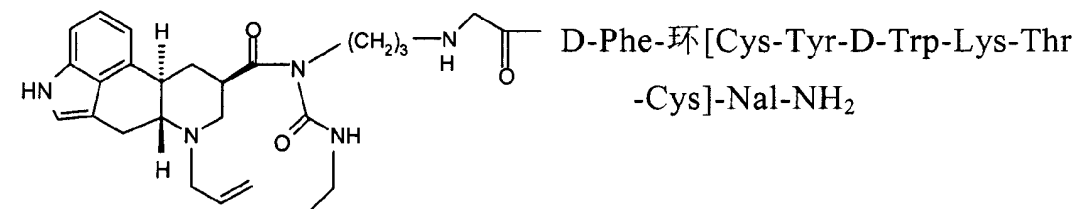
20

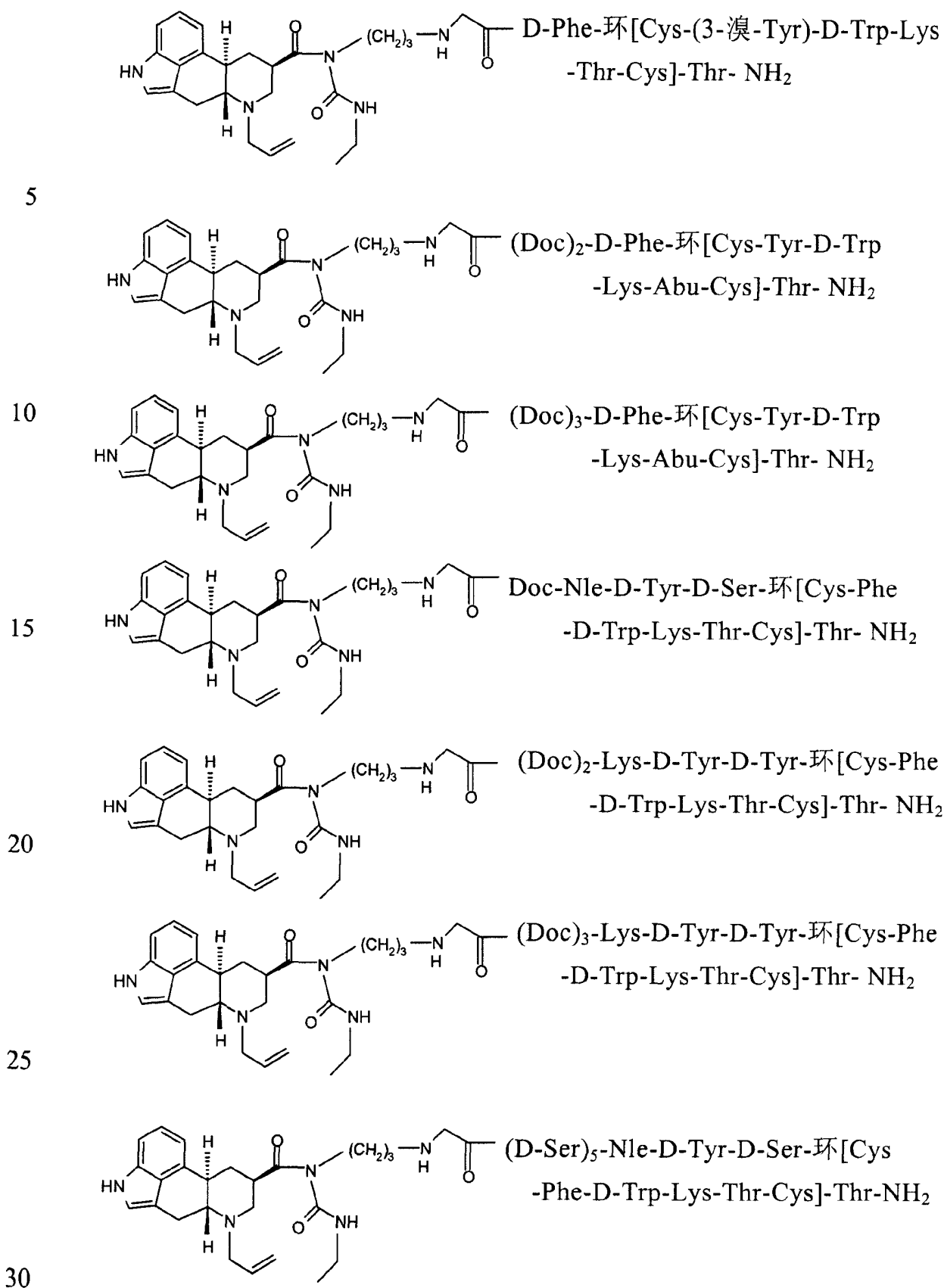


25

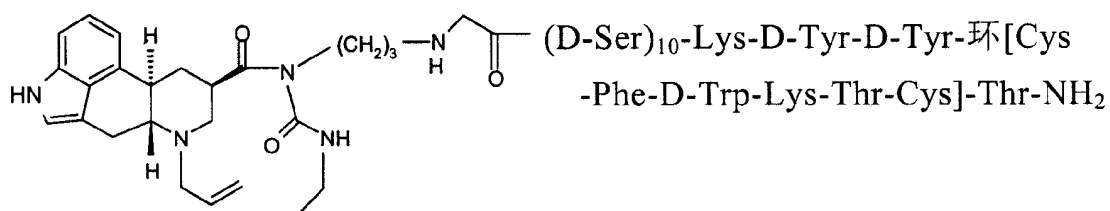


30

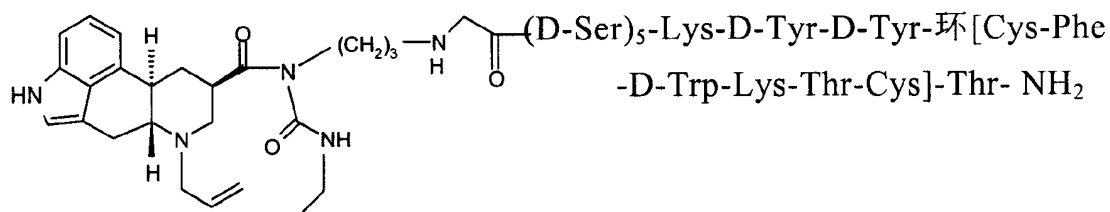




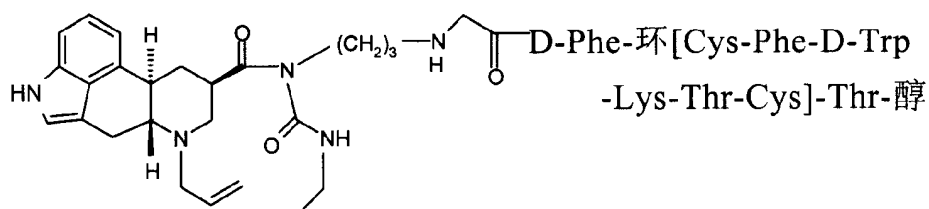
5



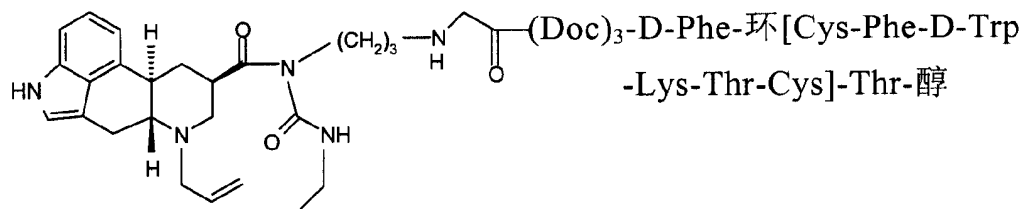
10



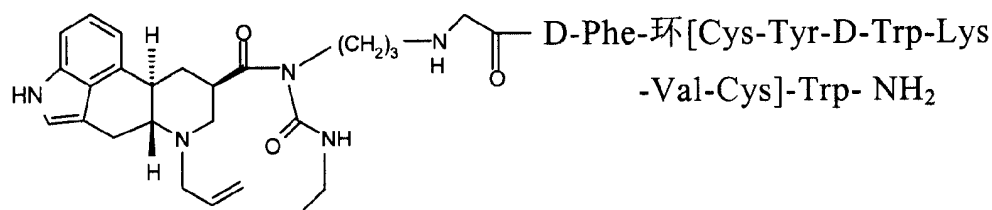
15



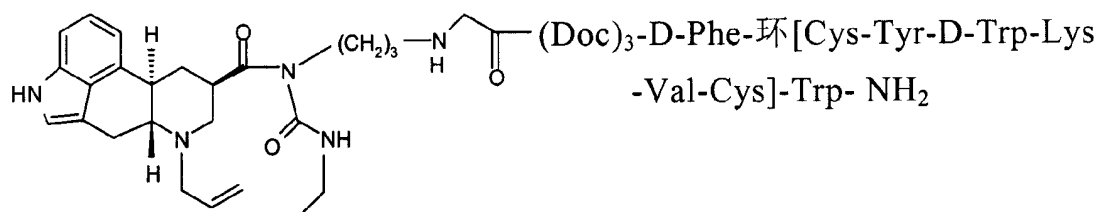
20

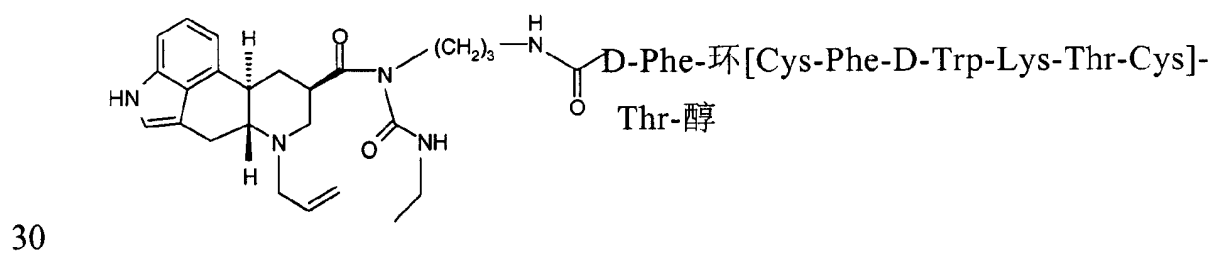
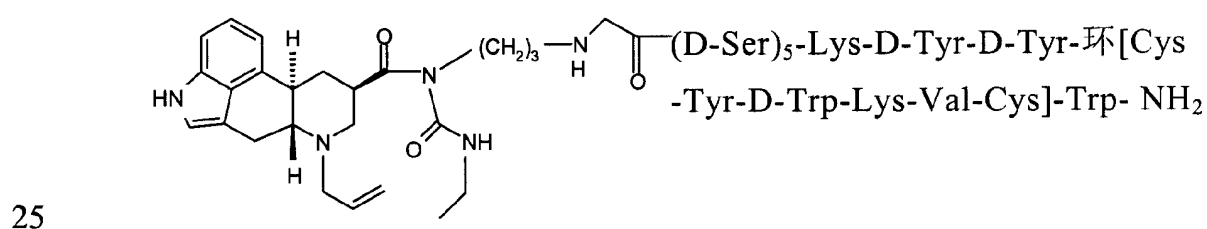
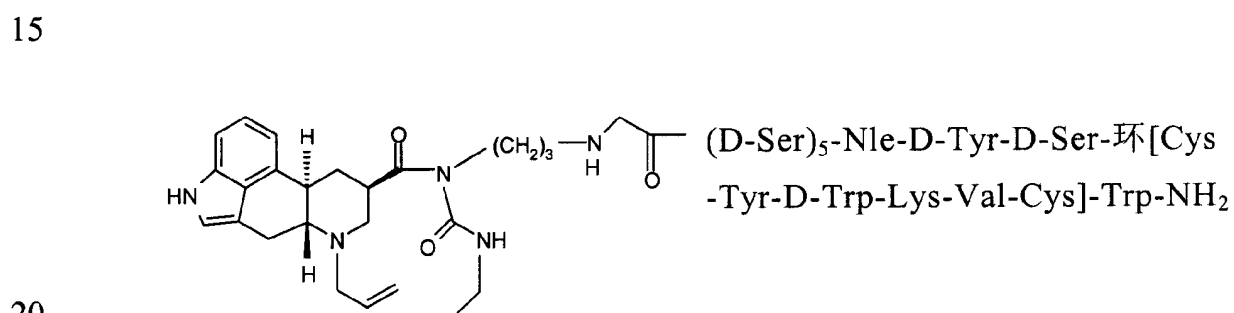
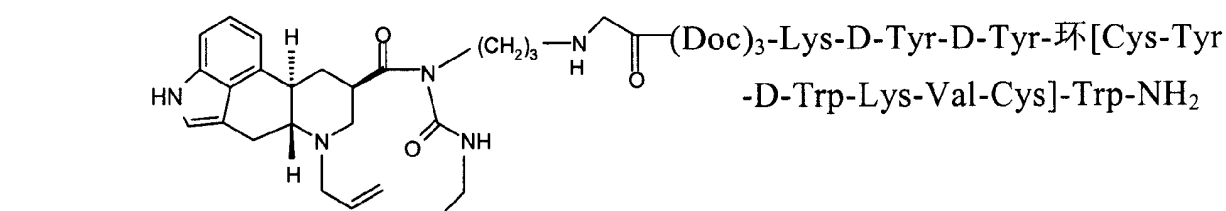
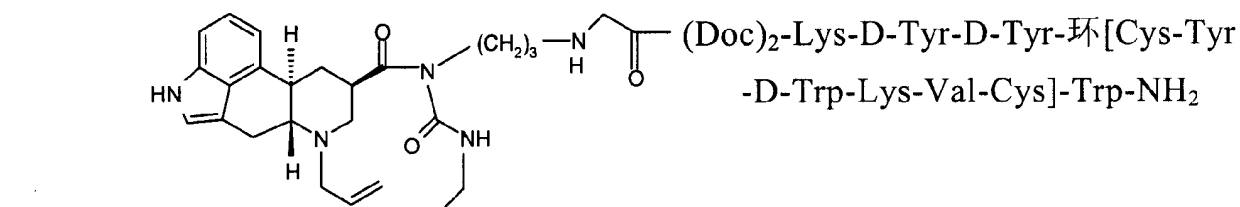
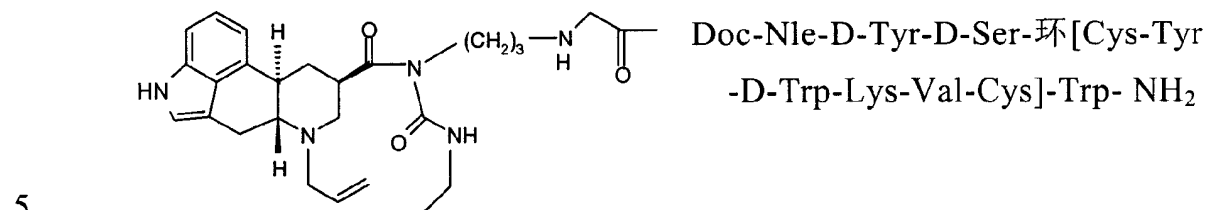


25

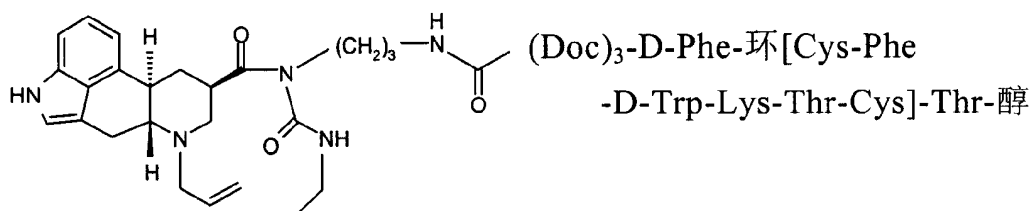


30

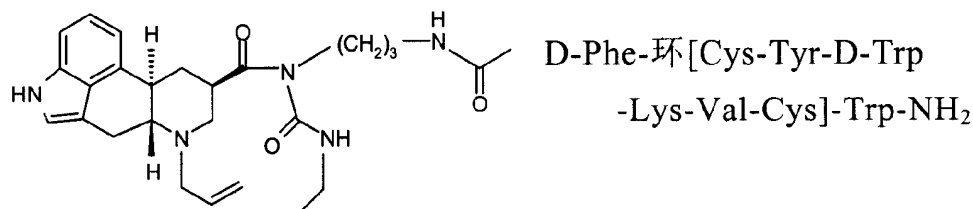




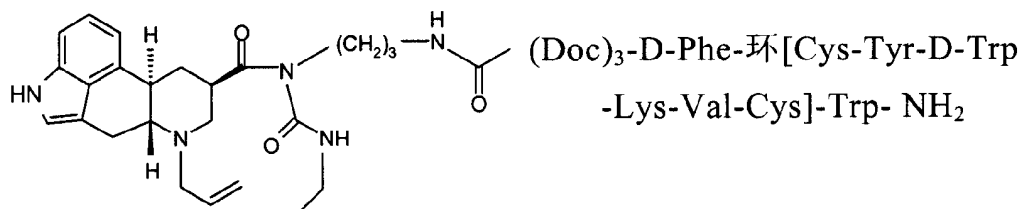
5



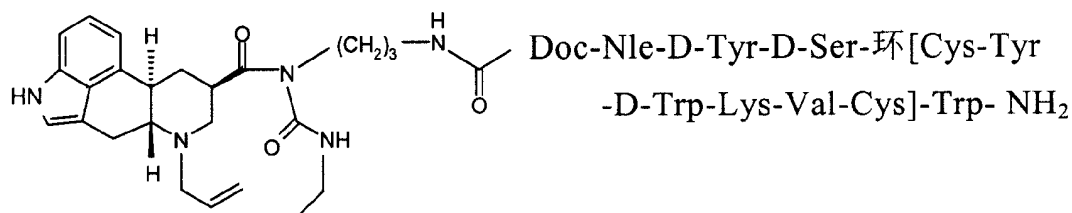
10



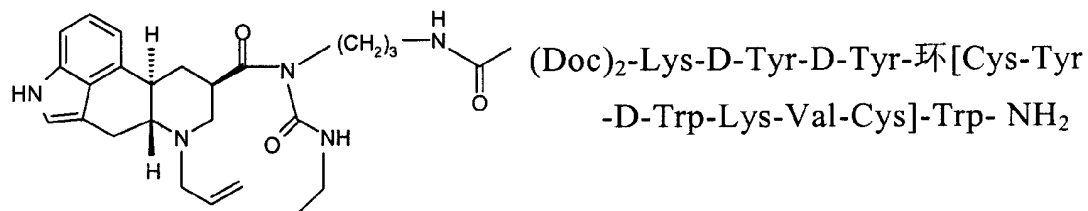
15



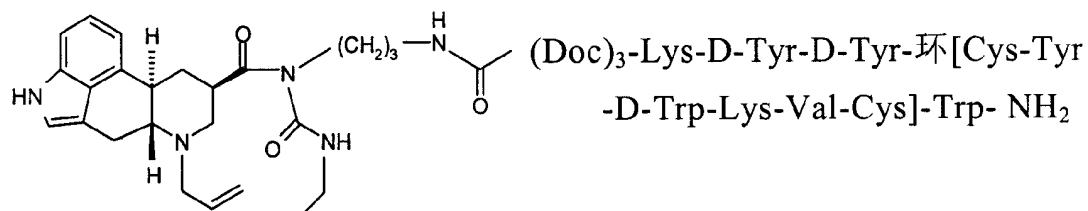
20

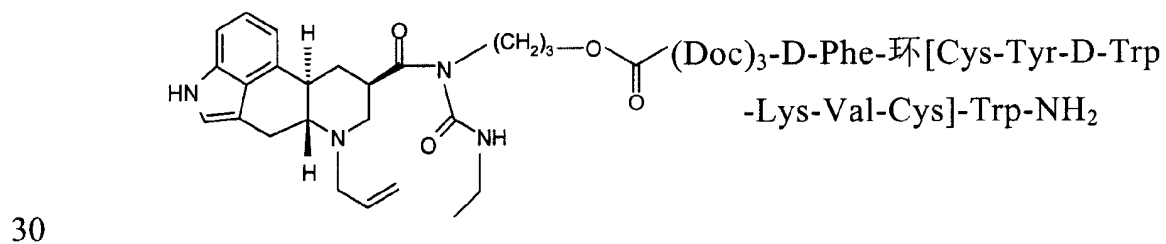
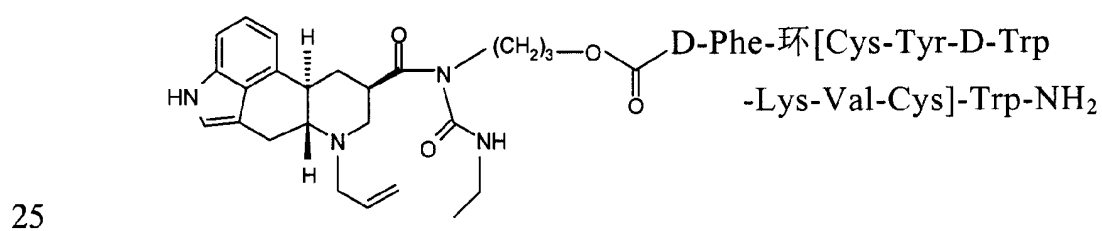
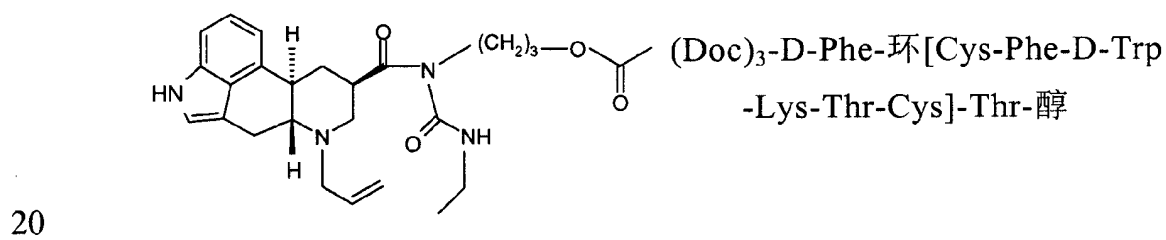
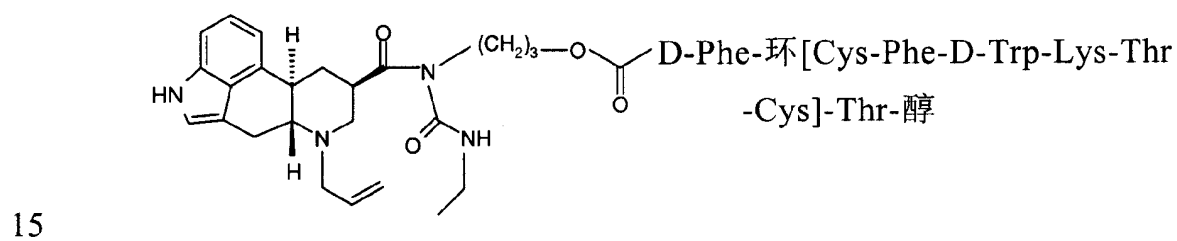
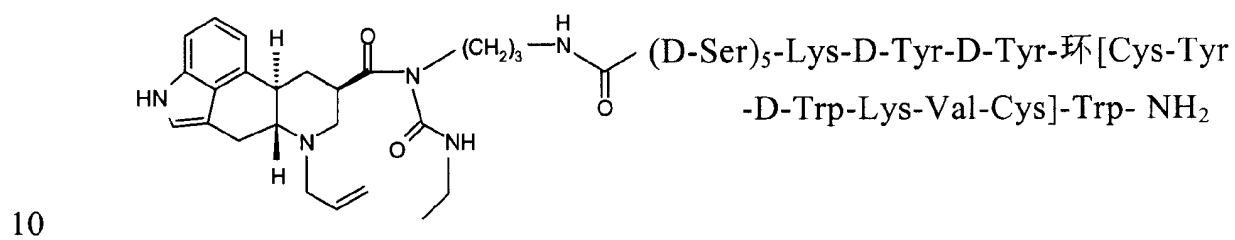
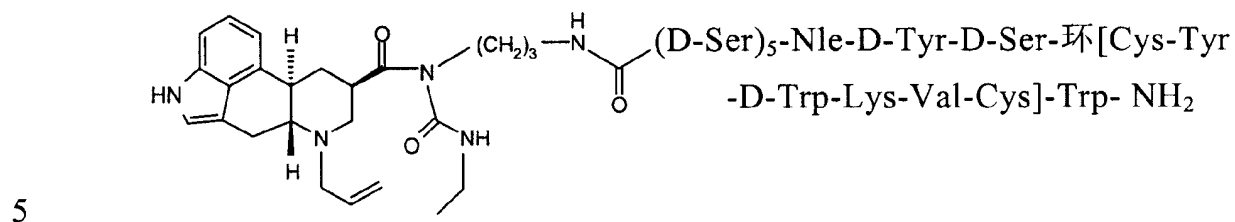


25

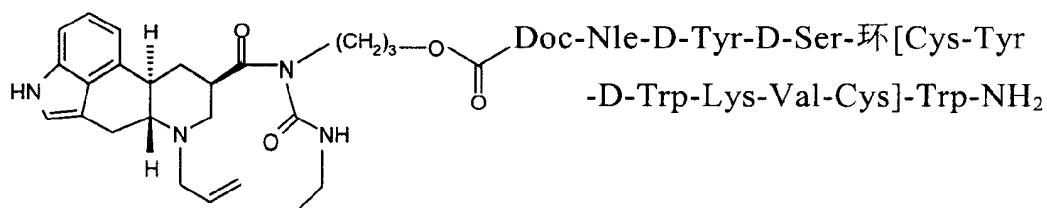


30

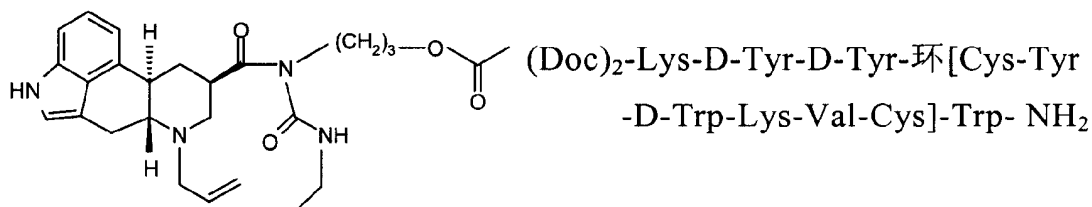




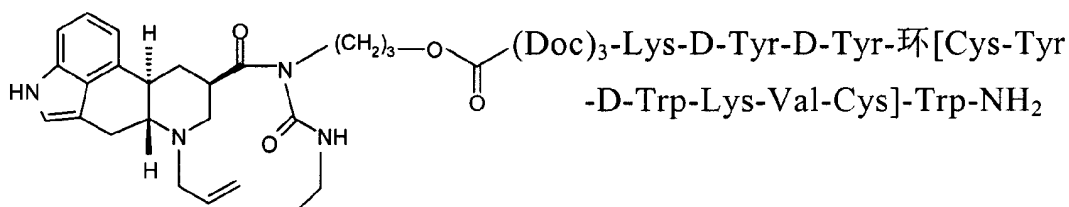
5



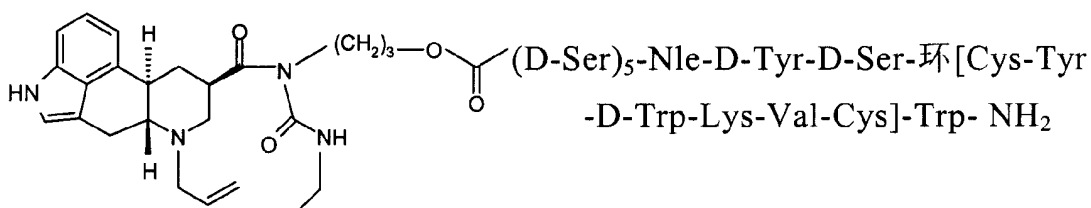
10



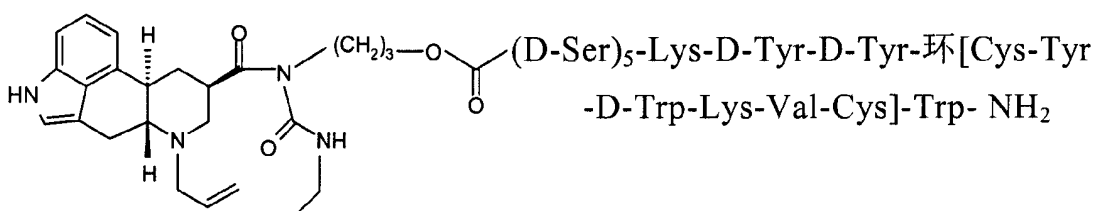
15



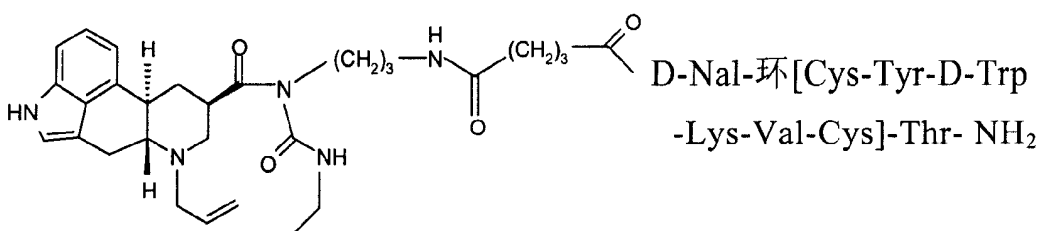
20



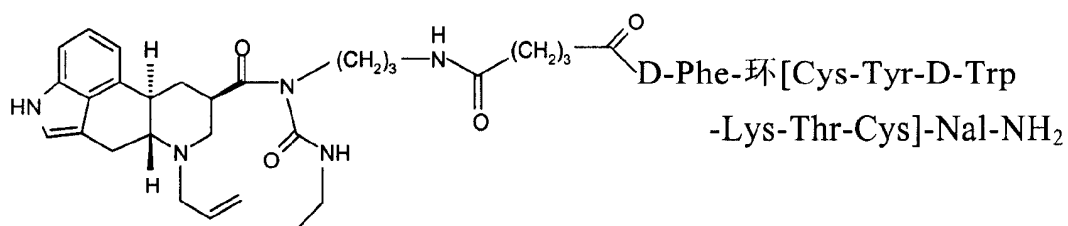
25



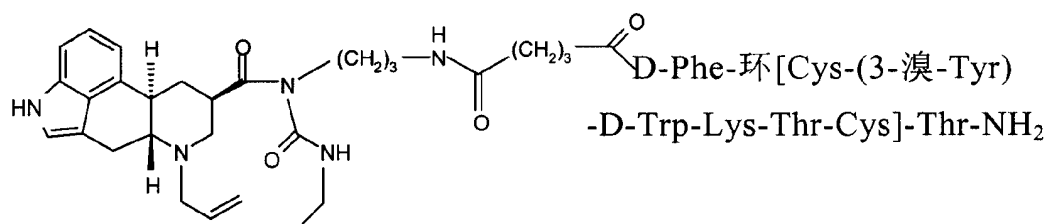
30



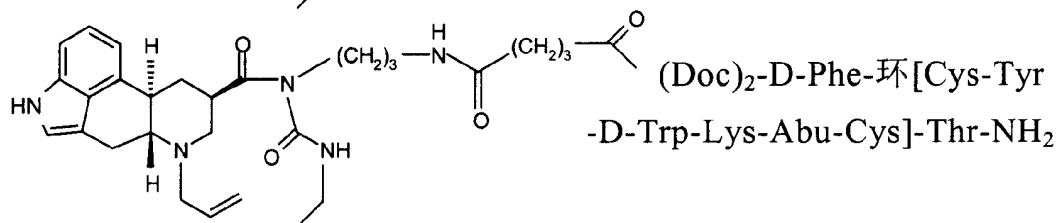
5



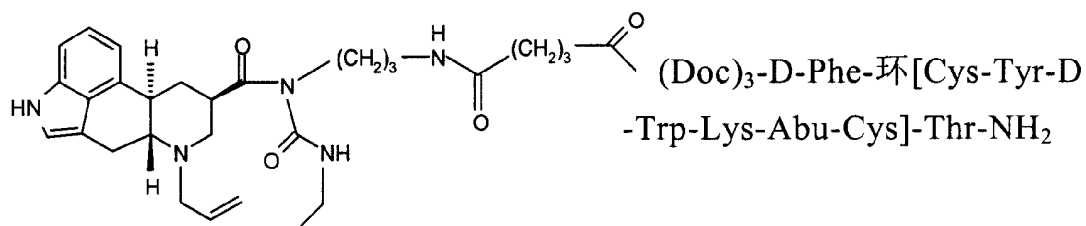
10



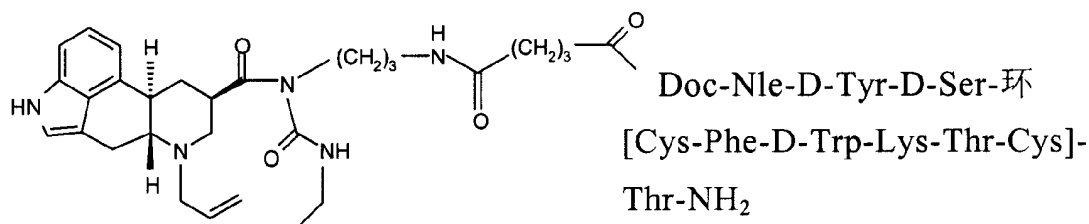
15



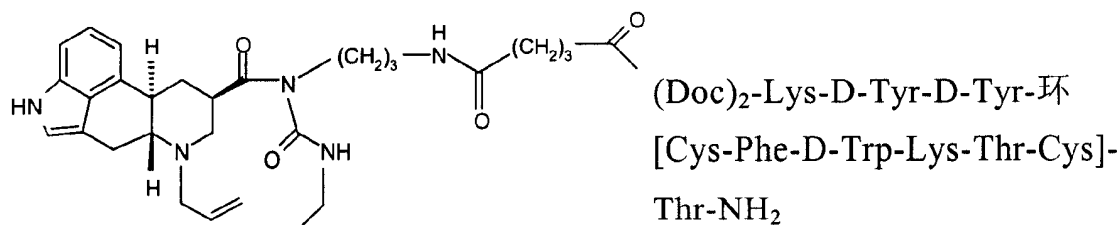
20

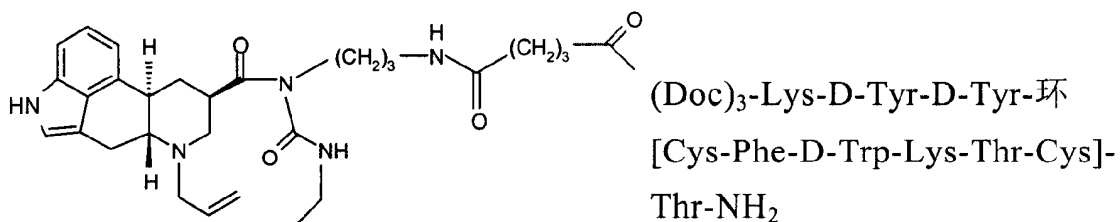


25

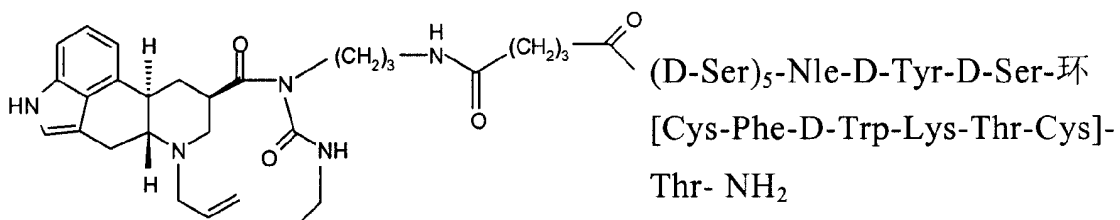


30

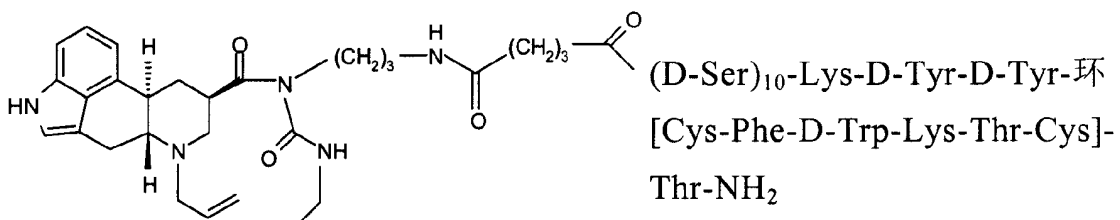




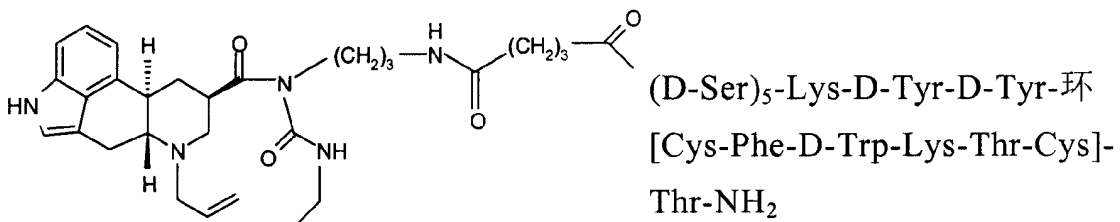
5



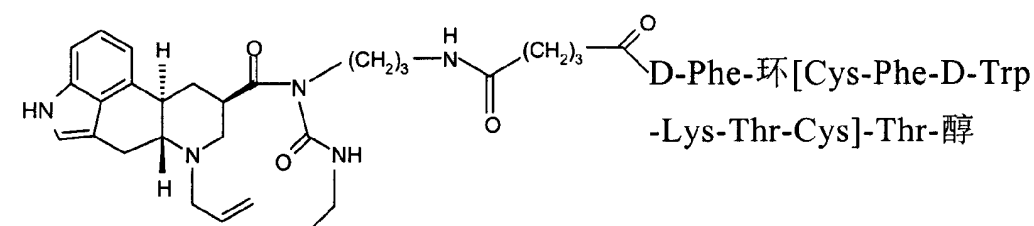
10



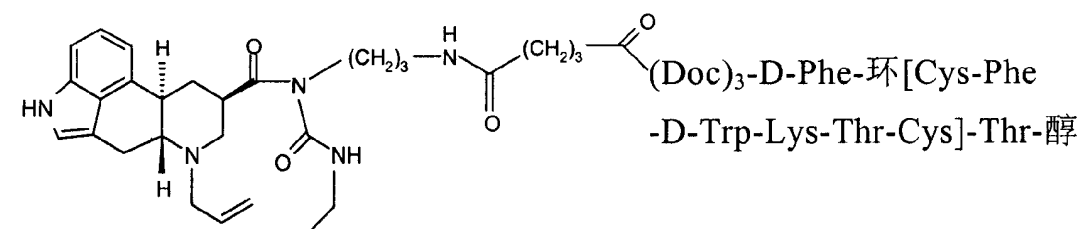
15



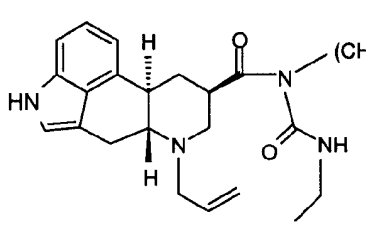
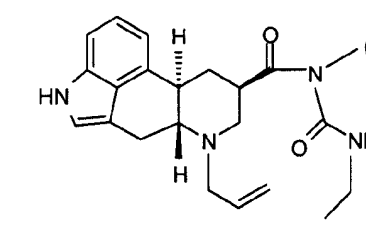
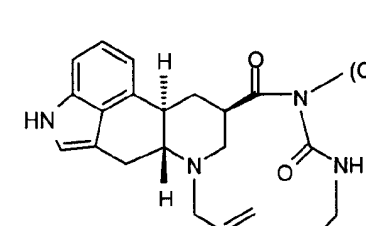
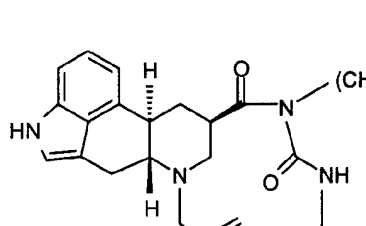
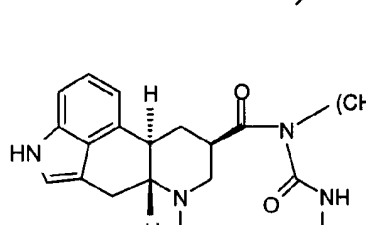
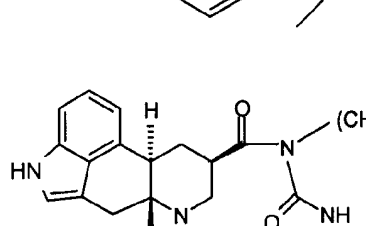
20



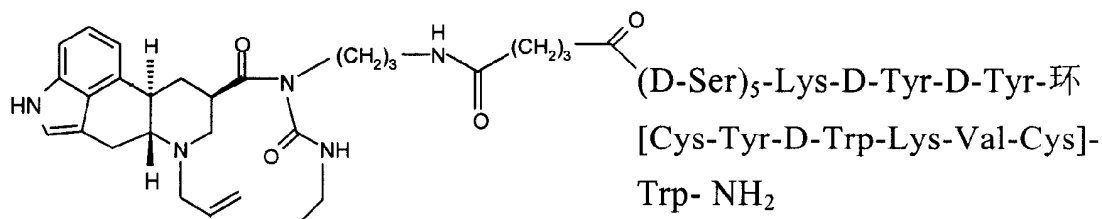
25



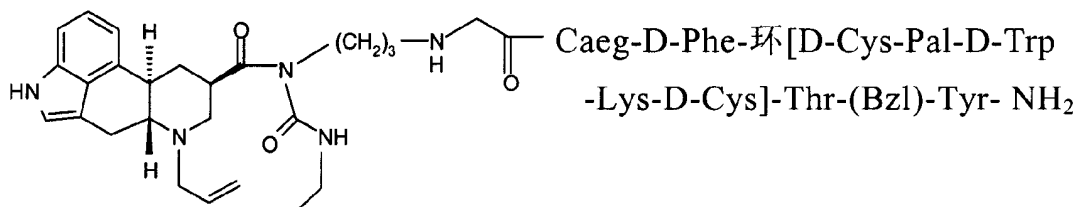
30

- 5
- 
- D-Phe-环[Cys-Phe
-D-Trp-Lys-Val-Cys]-Trp-NH₂
- 10
- 
- (Doc)₃-D-Phe-环[Cys-Tyr
-D-Trp-Lys-Val-Cys]-Trp-NH₂
- 15
- 
- Doc-Nle-D-Tyr-D-Ser-环
[Cys-Tyr-D-Trp-Lys-Val-Cys]
-Trp-NH₂
- 20
- 
- (Doc)₂-Lys-D-Tyr-D-Tyr-环
[Cys-Tyr-D-Trp-Lys-Val-Cys]-
Trp- NH₂
- 25
- 
- (Doc)₃-Lys-D-Tyr-D-Tyr-环
[Cys-Tyr-D-Trp-Lys-Val-Cys]-
Trp- NH₂
- 30
- 
- (D-Ser)₅-Nle-D-Tyr-D-Ser-环
[Cys-Tyr-D-Trp-Lys-Val-Cys]-
Trp- NH₂

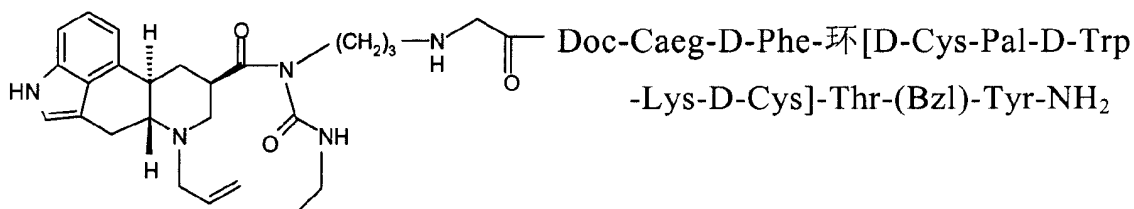
5



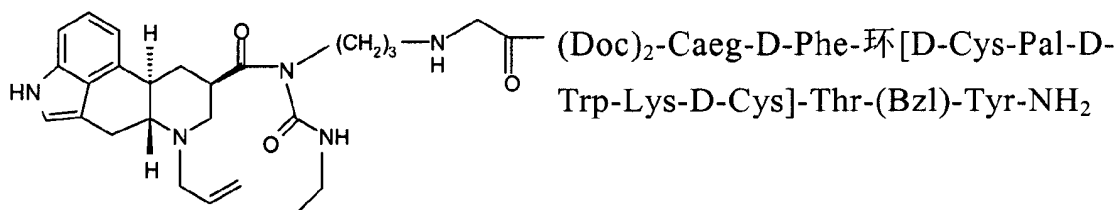
10



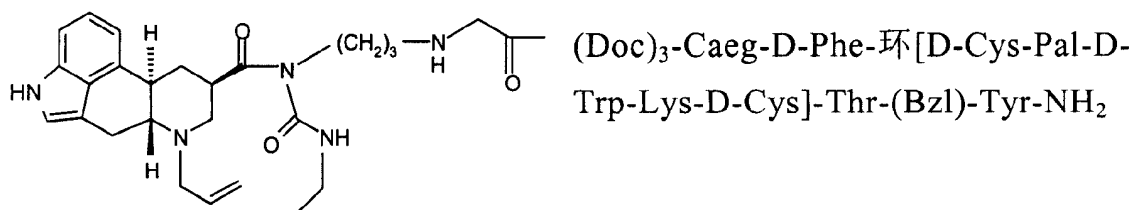
15



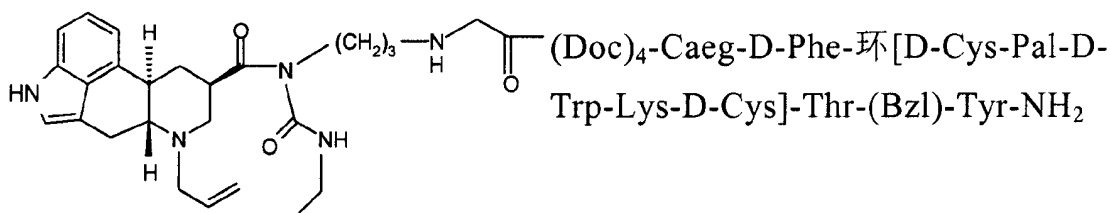
20



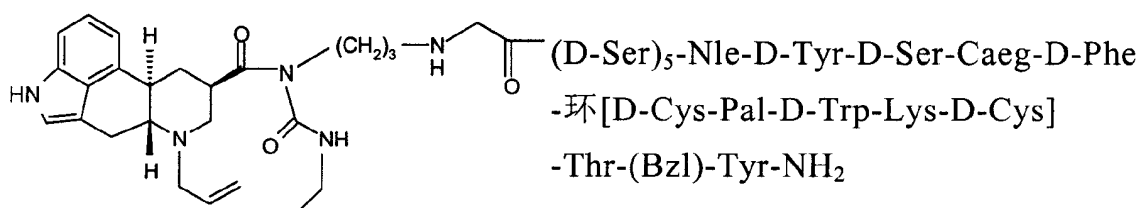
25



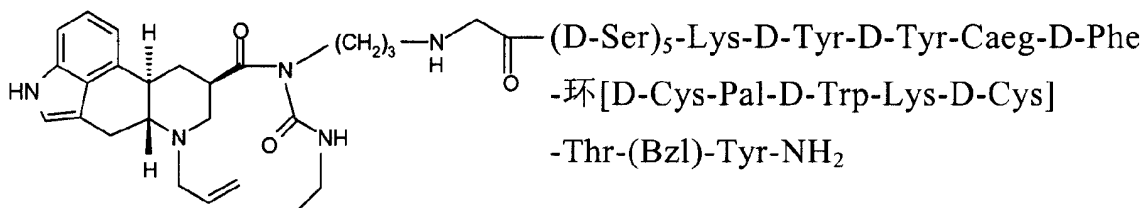
30



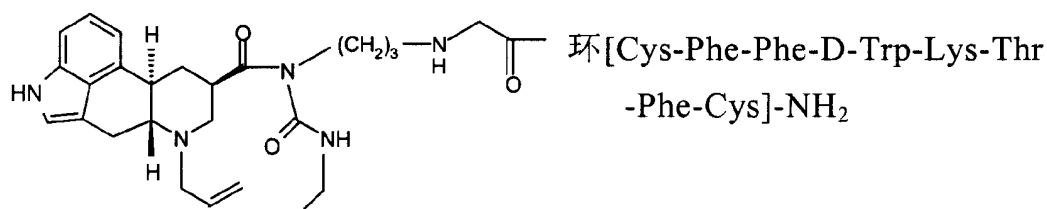
5



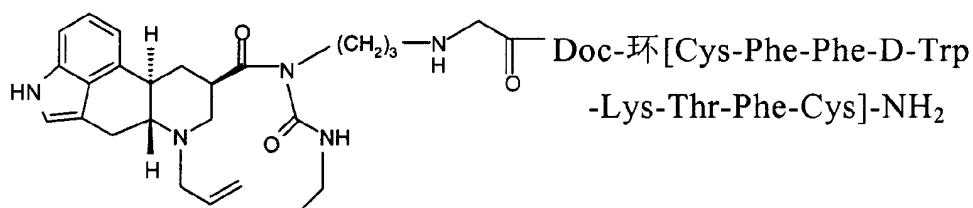
10



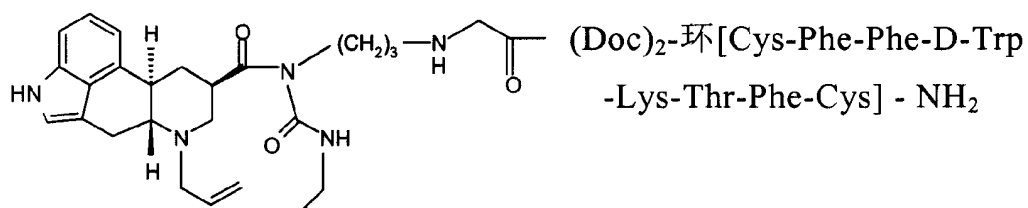
15



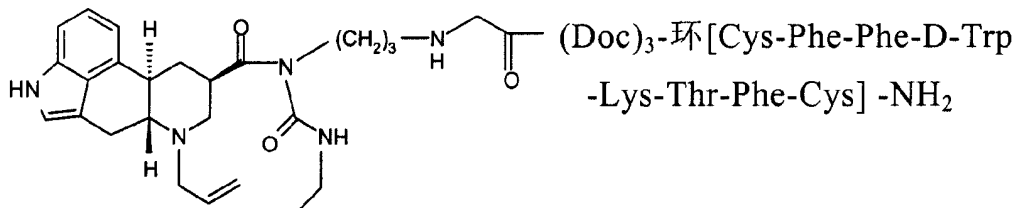
20

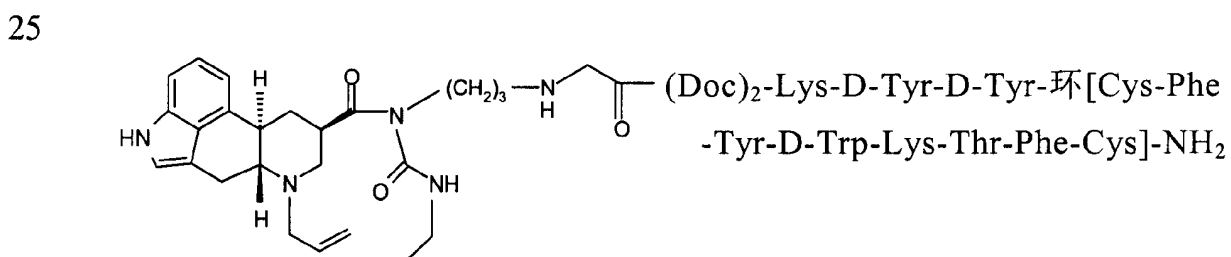
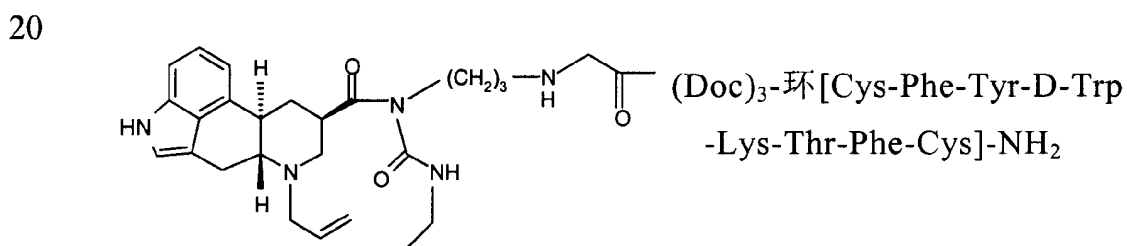
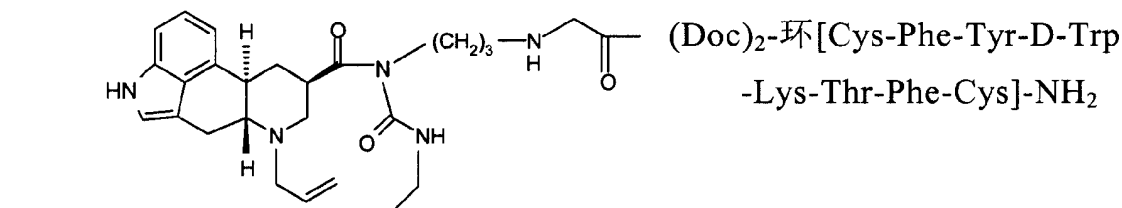
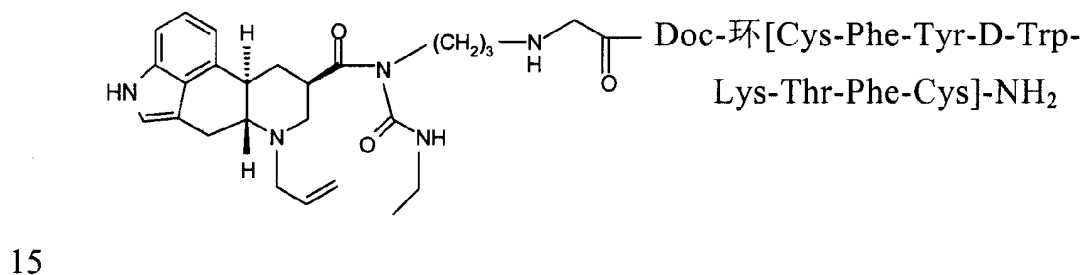
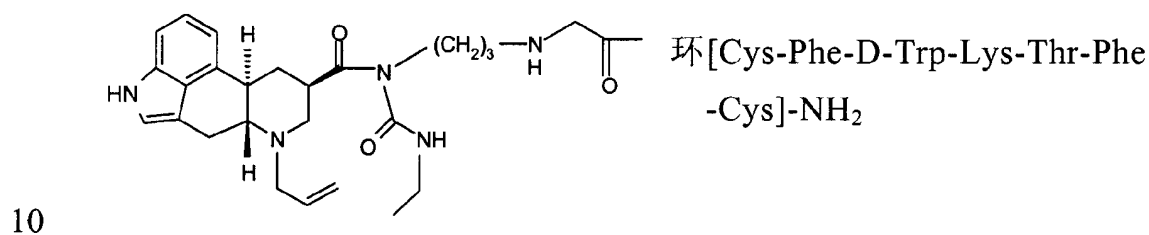
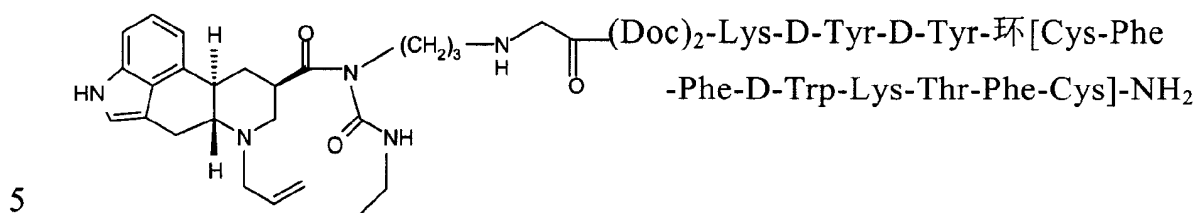


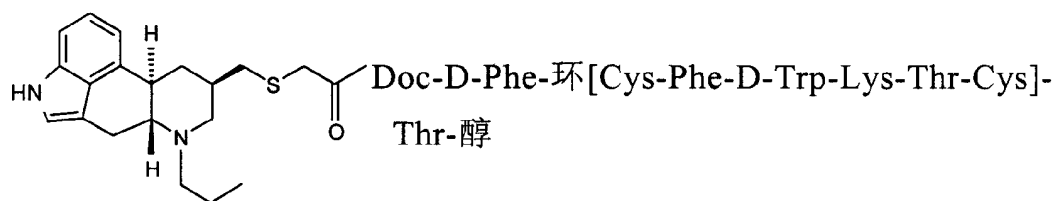
25



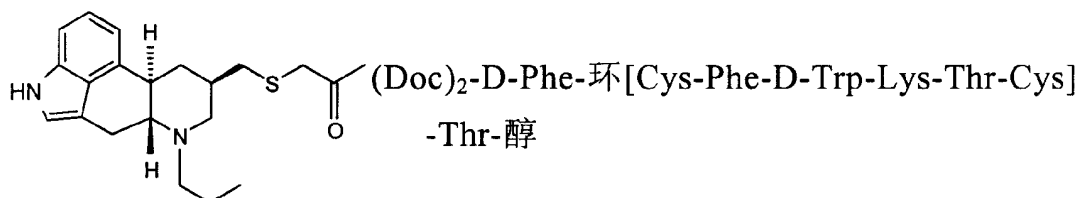
30



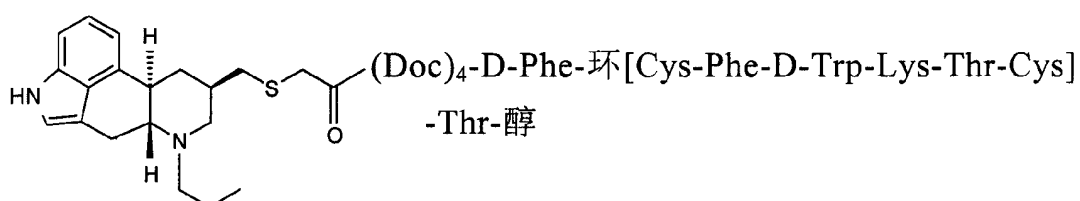




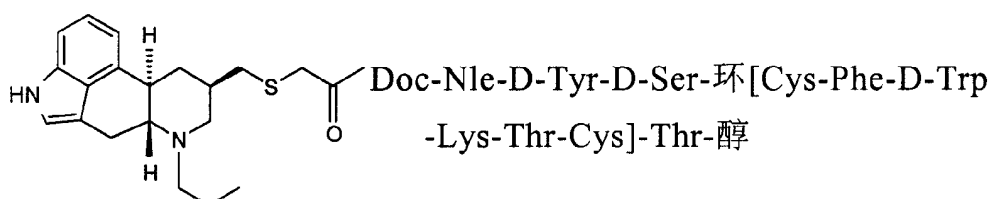
5



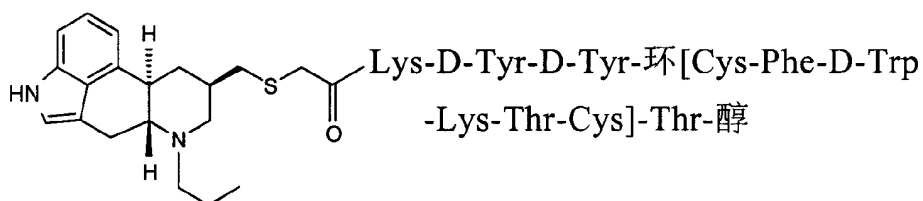
10



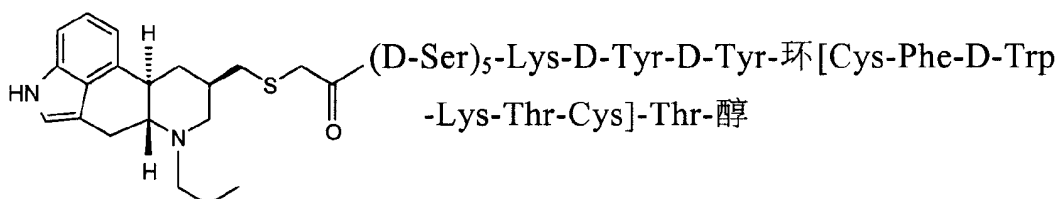
15



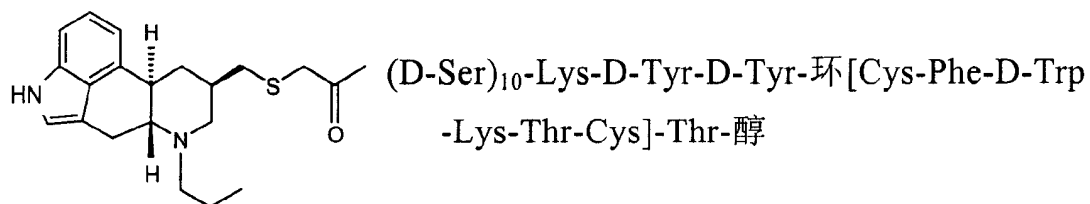
20

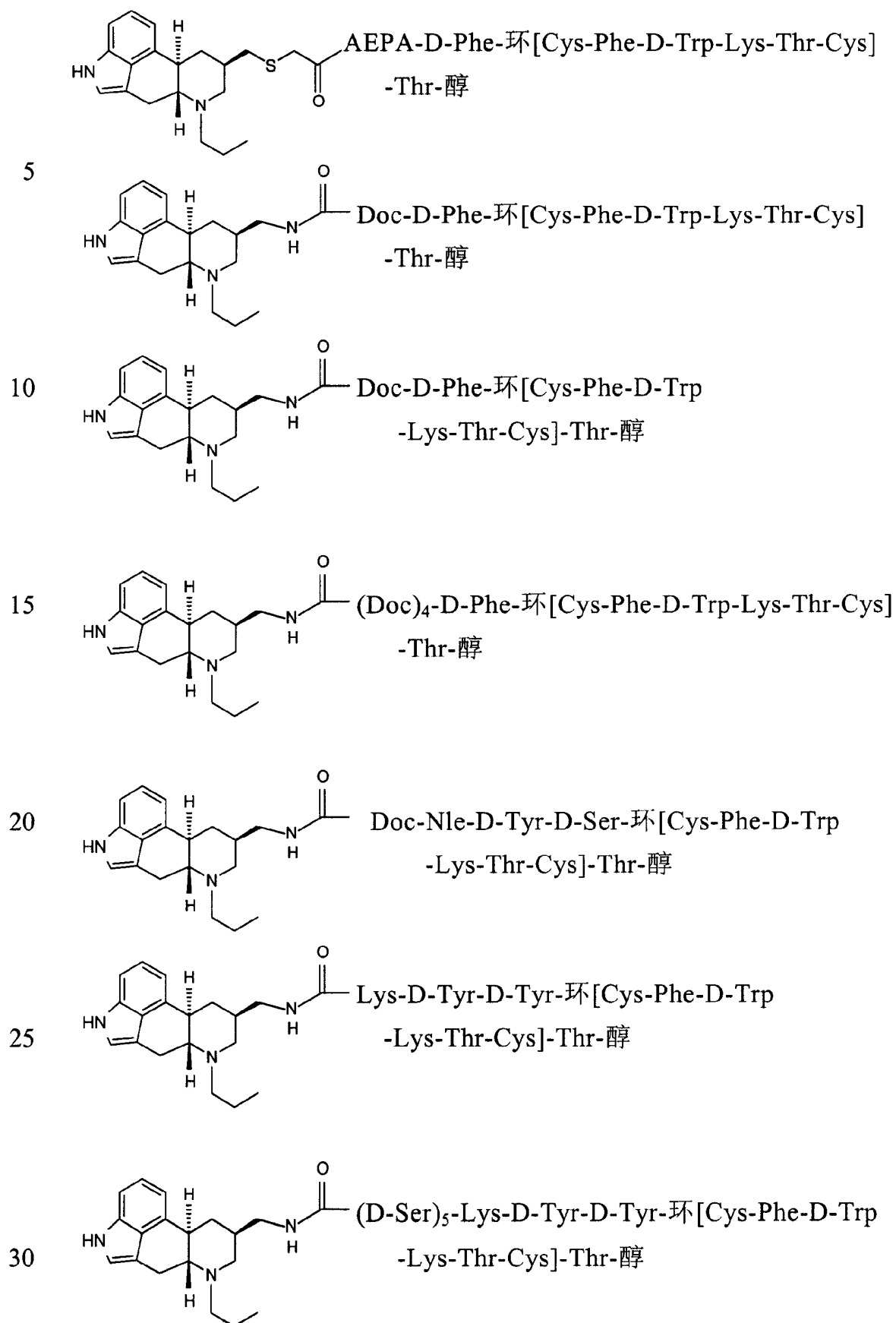


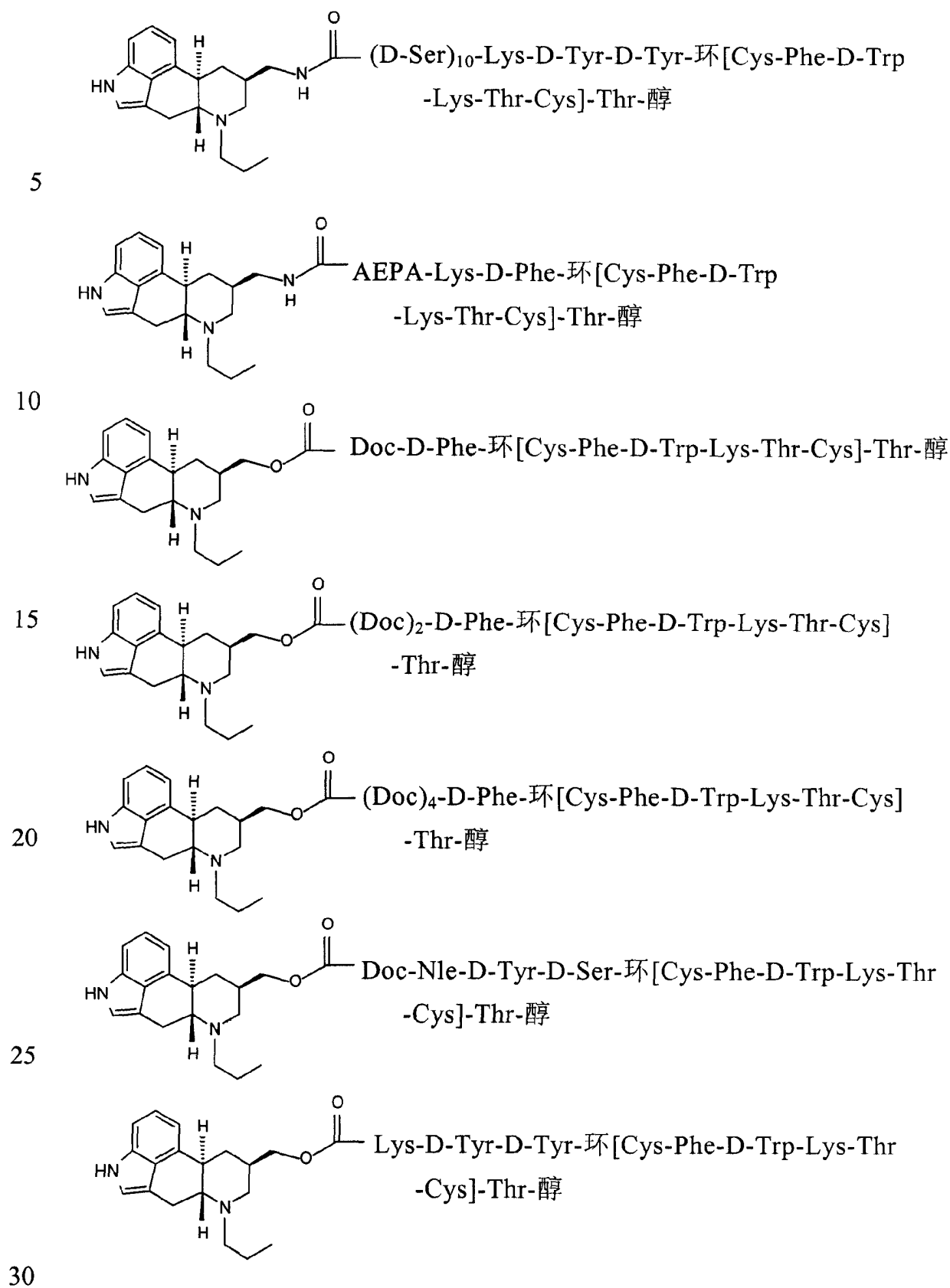
25

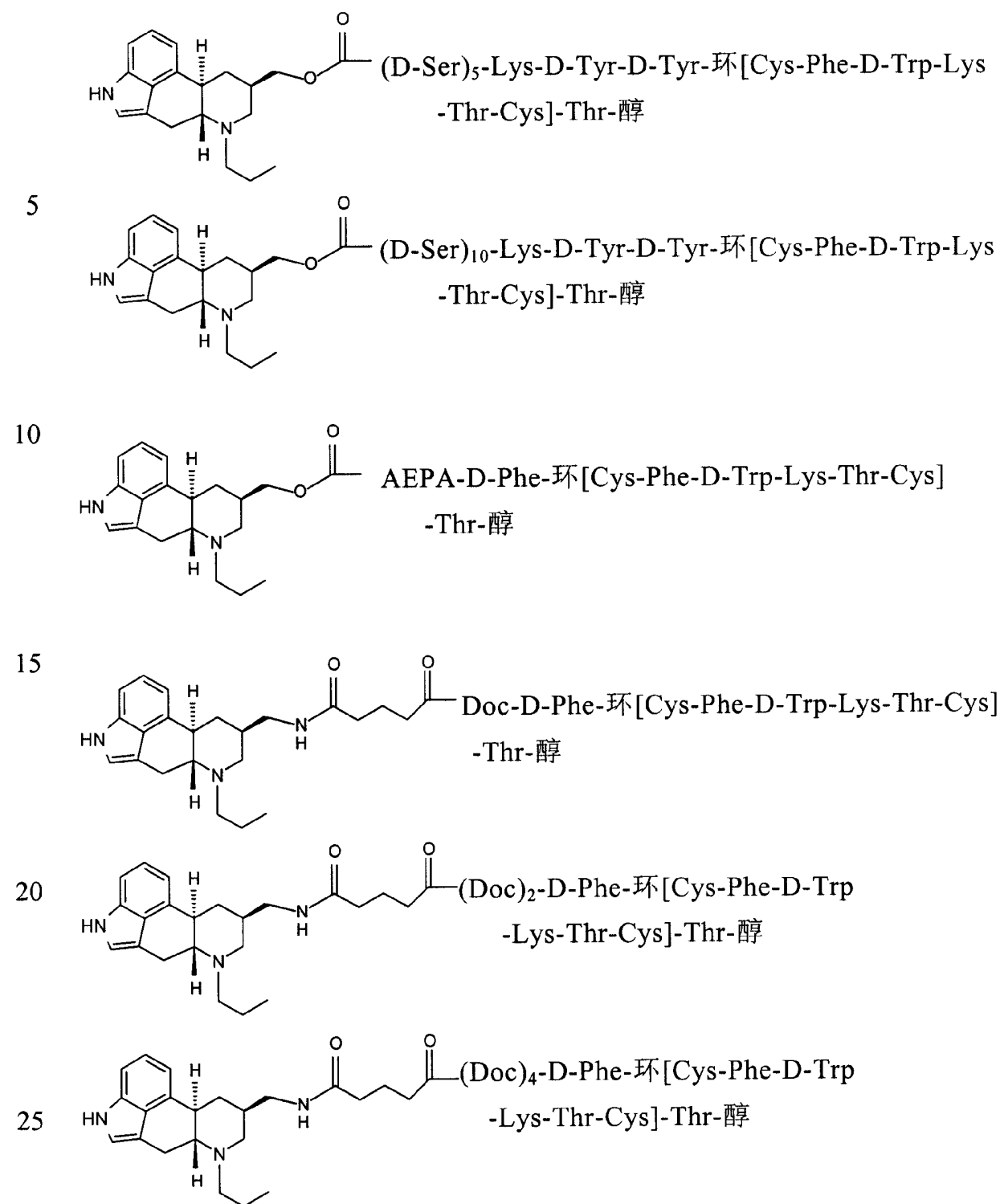


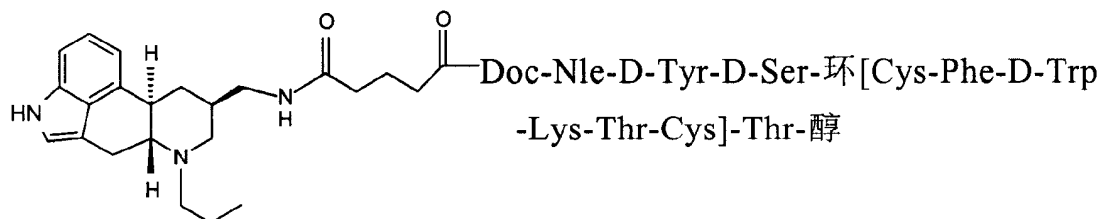
30



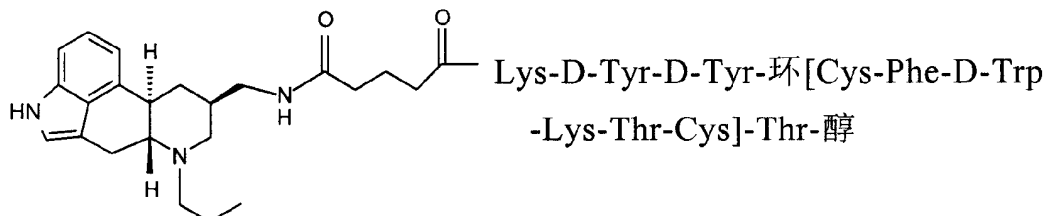




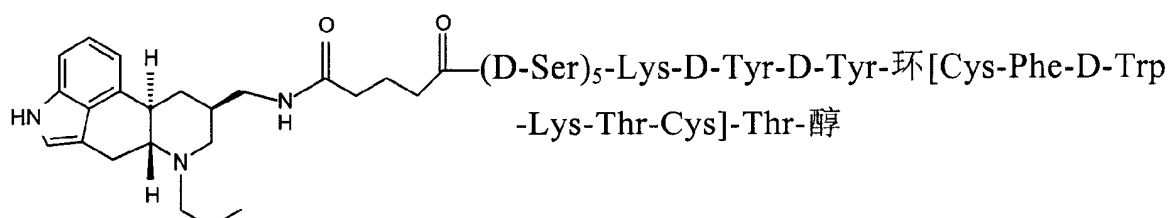




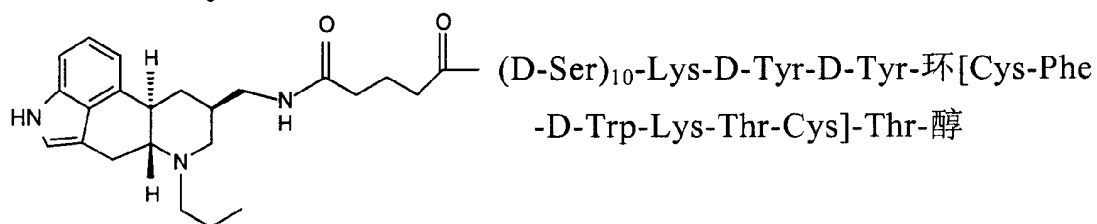
5



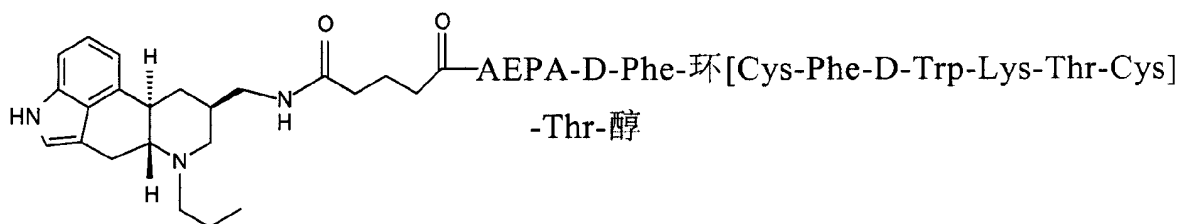
10



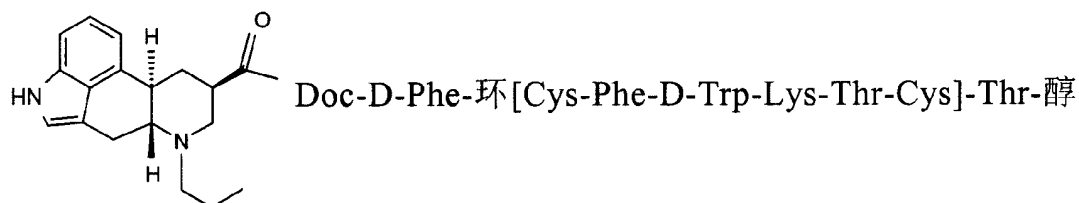
15



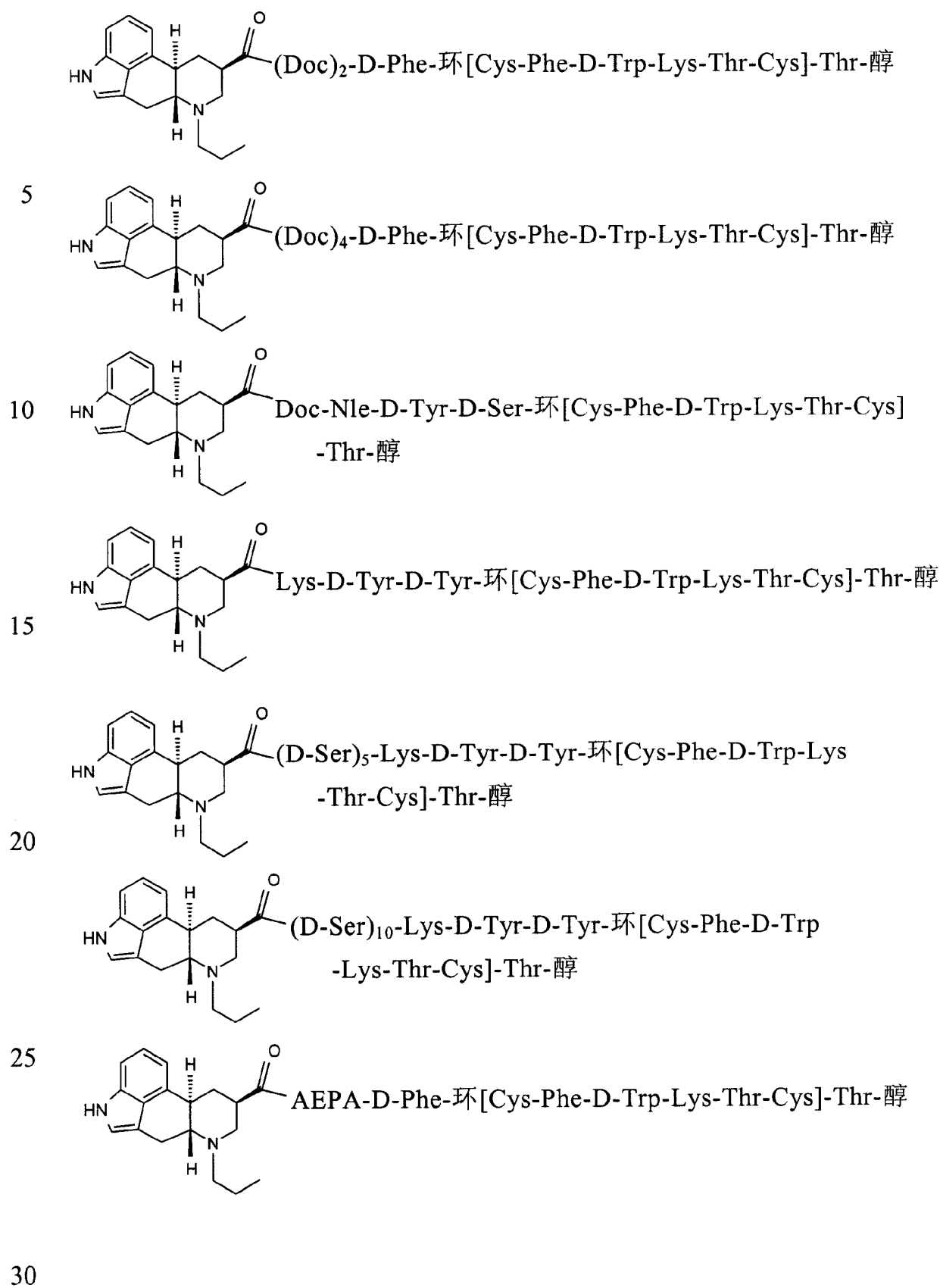
20

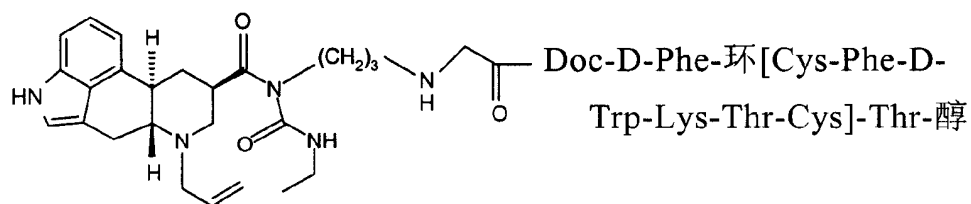


25

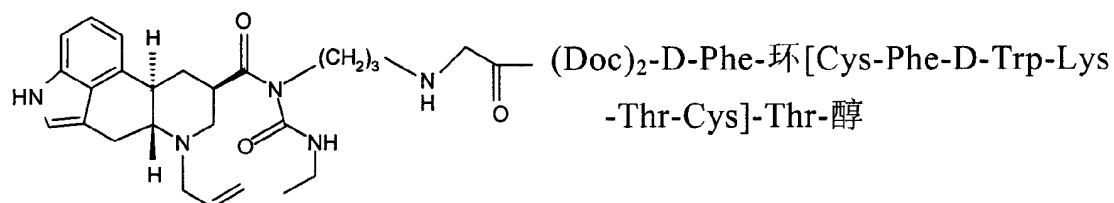


30

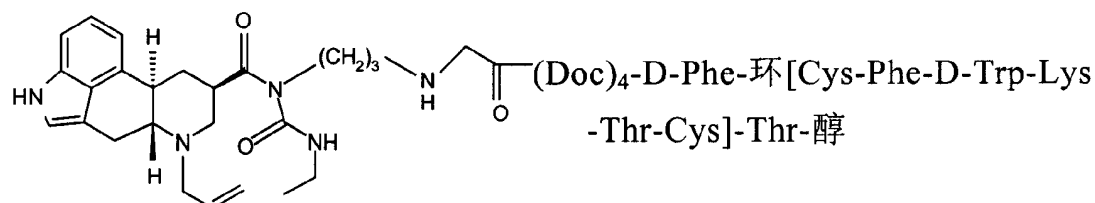




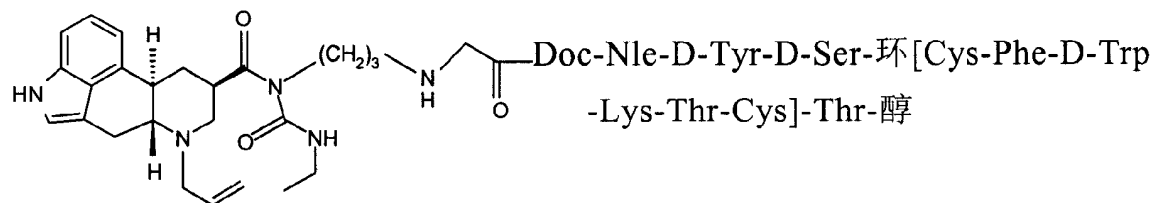
5



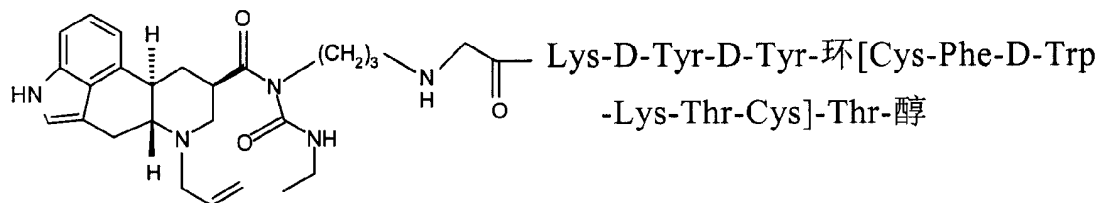
10



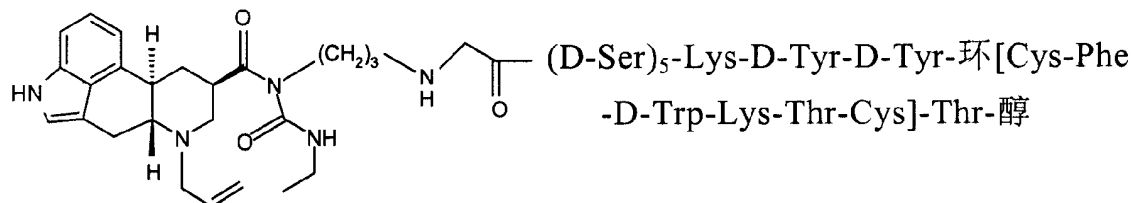
15



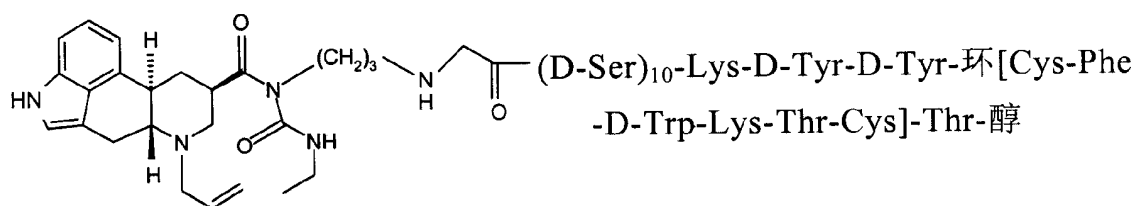
20



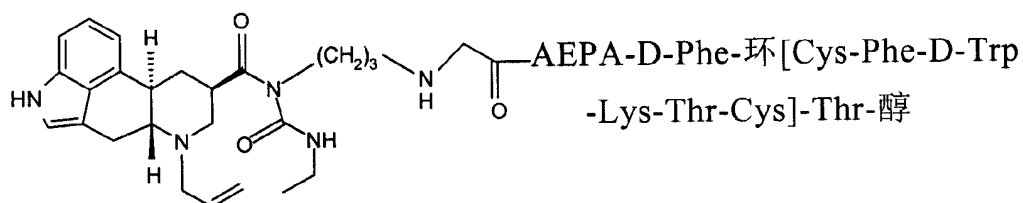
25



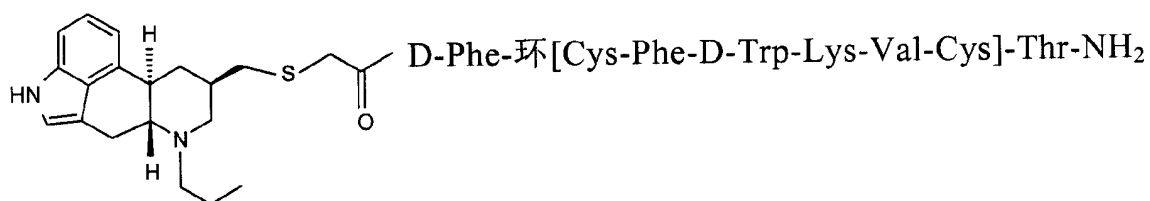
30



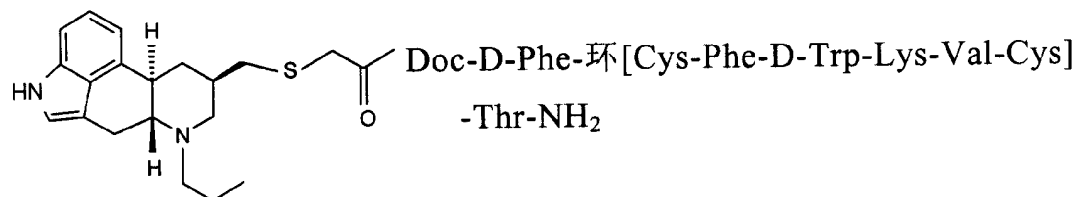
5



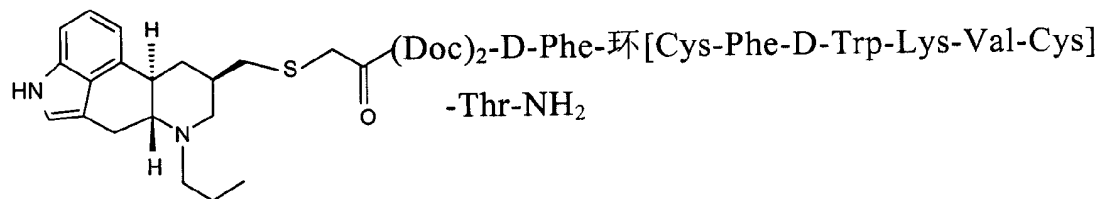
10



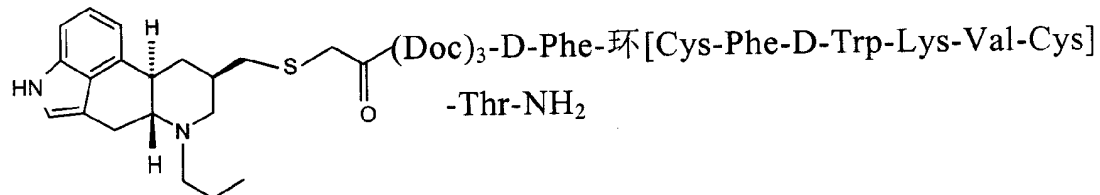
15



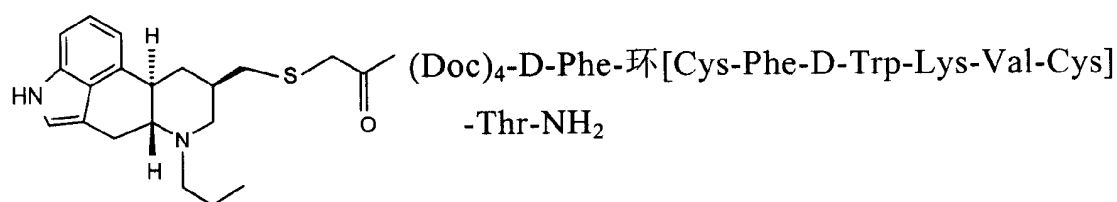
20



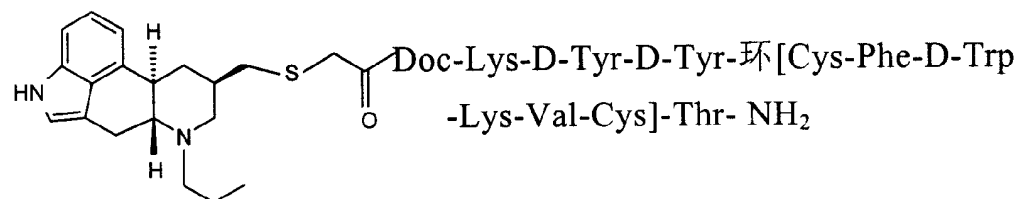
25



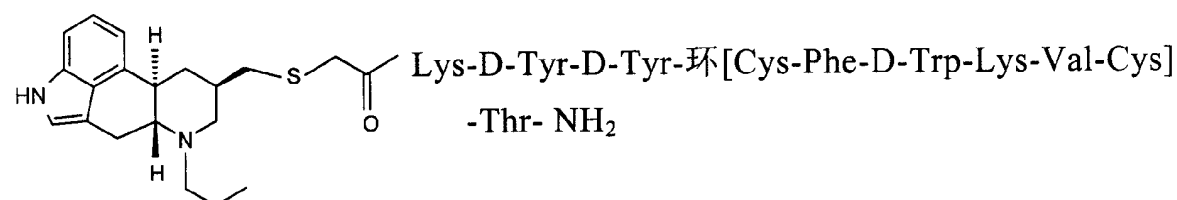
30



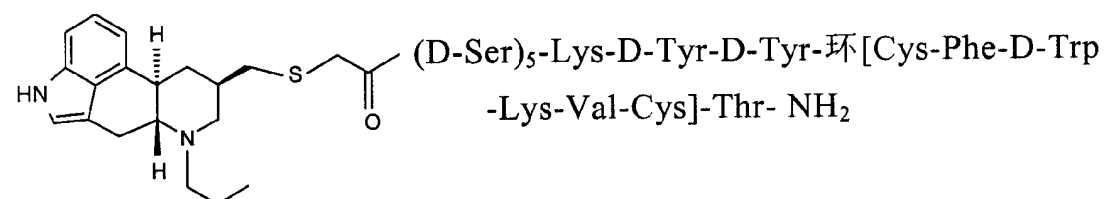
5



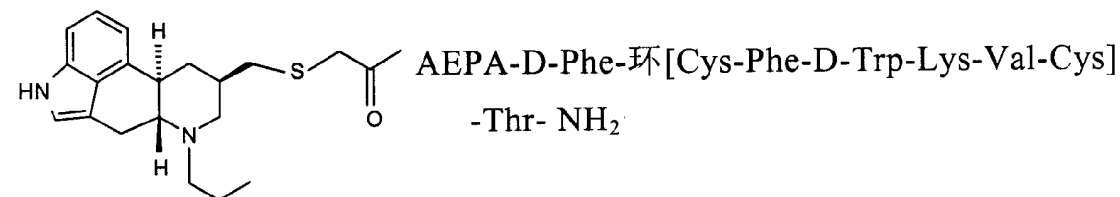
10



15

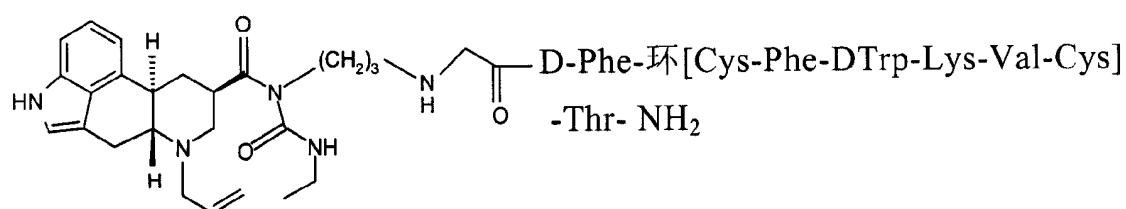


20

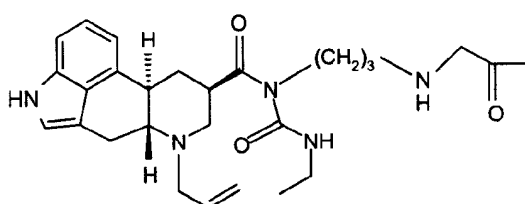
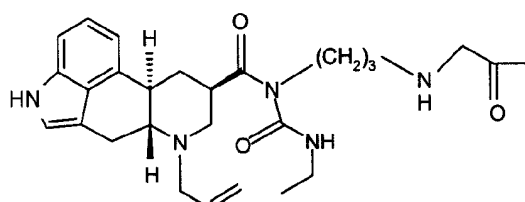
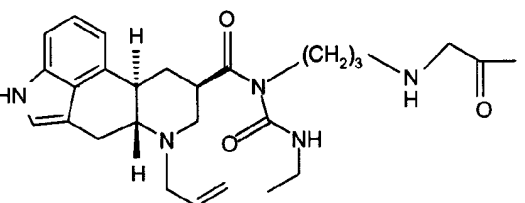
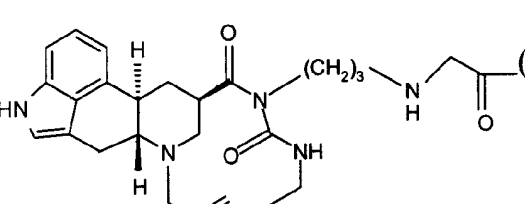
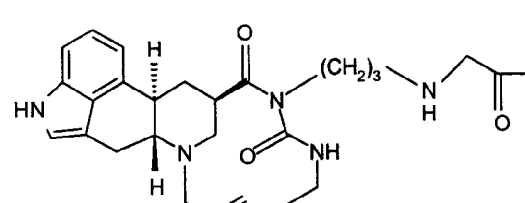
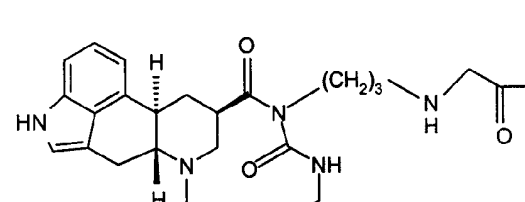


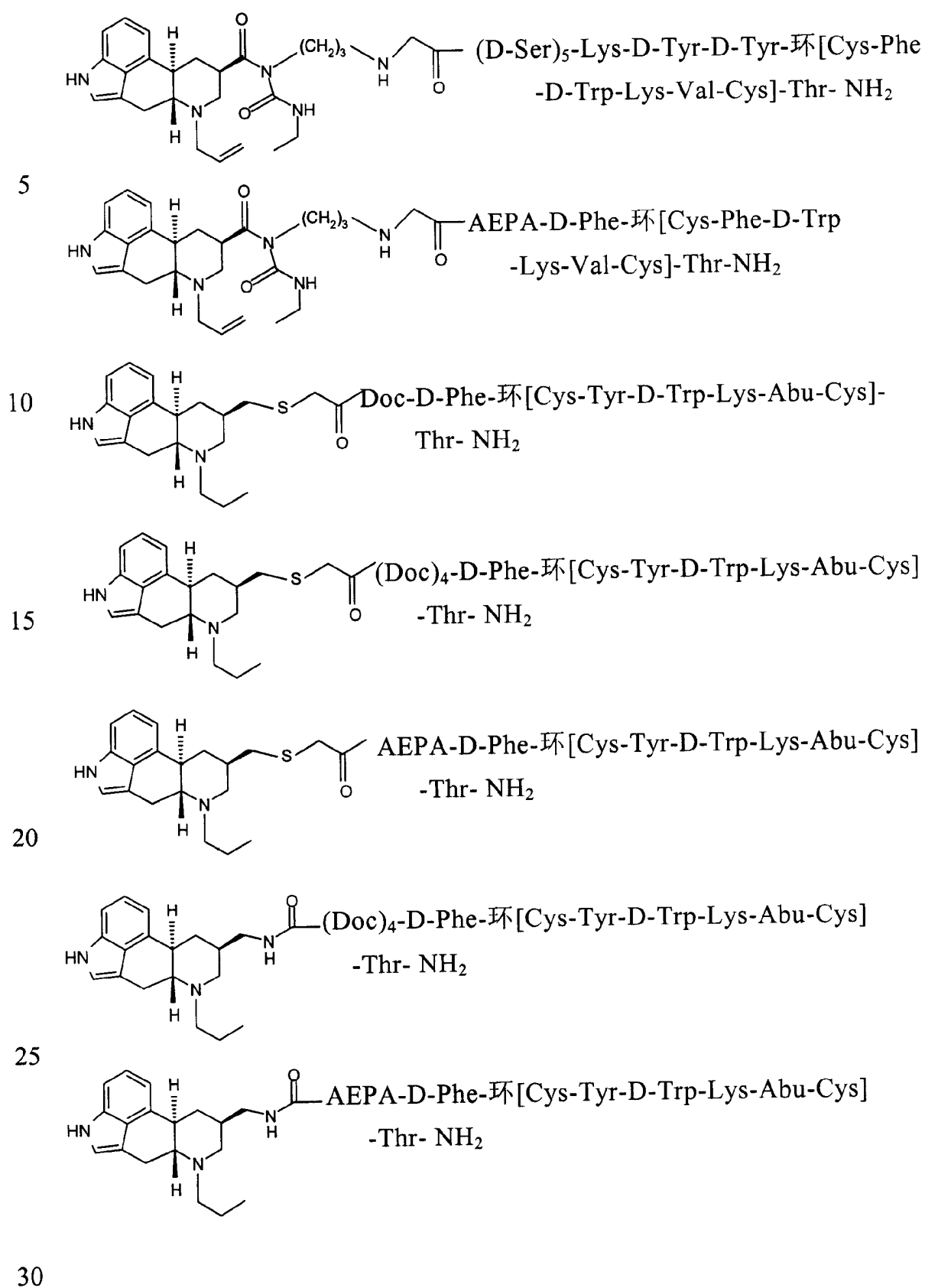
AEPA=4-(2-氨基乙基)-1-羧甲基-哌嗪

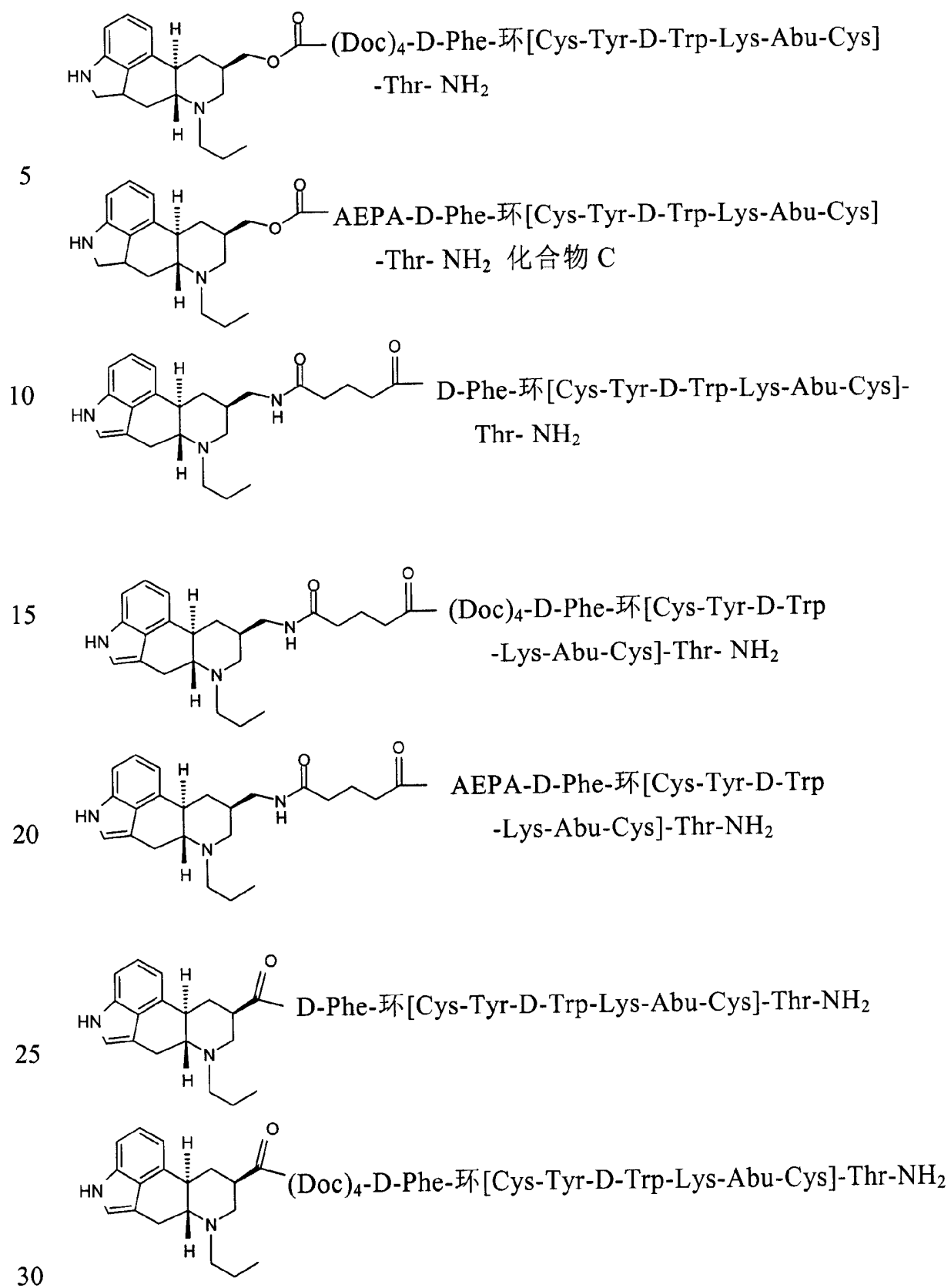
25

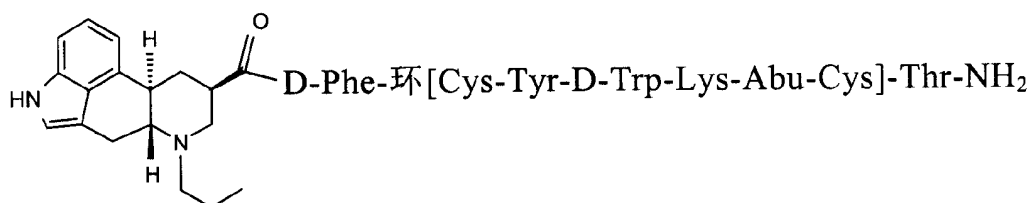


30

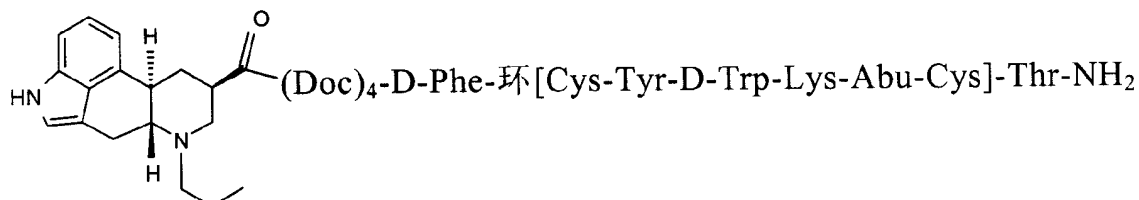
- 5  Doc-D-Phe-环[Cys-Phe-D-Trp-Lys-Val-Cys]-Thr- NH₂
- 10  (Doc)₂-D-Phe-环[Cys-Phe-D-Trp-Lys-Val-Cys]-Thr- NH₂
- 15  (Doc)₃-D-Phe-环[Cys-Phe-D-Trp-Lys-Val-Cys]-Thr- NH₂
- 20  (Doc)₄-D-Phe-环[Cys-Phe-D-Trp-Lys-Val-Cys]-Thr- NH₂
- 25  Doc-Lys-D-Tyr-D-Tyr-环[Cys-Phe-D-Trp-Lys-Val-Cys]-Thr- NH₂
- 30  Lys-D-Tyr-D-Tyr-环[Cys-Phe-D-Trp-Lys-Val-Cys]-Thr- NH₂



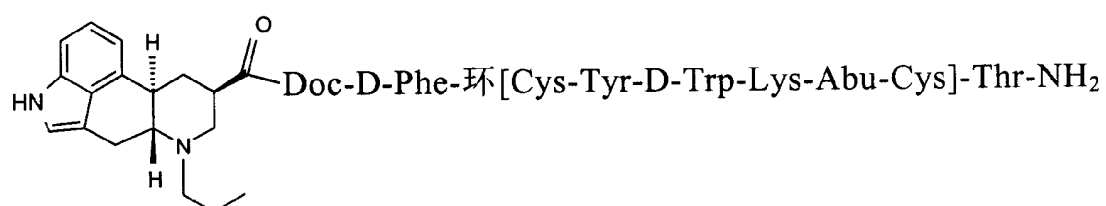




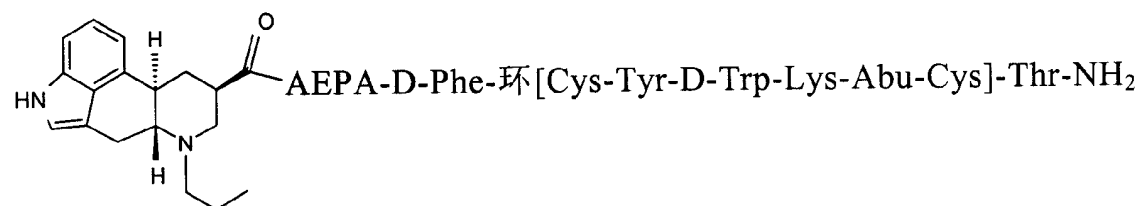
5



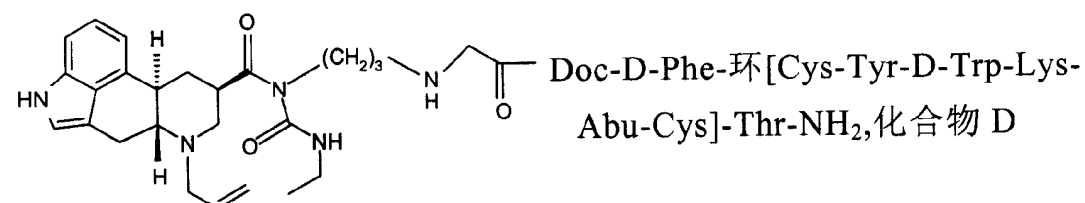
10



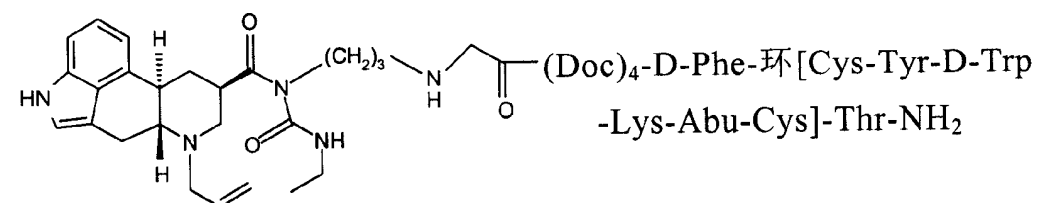
15



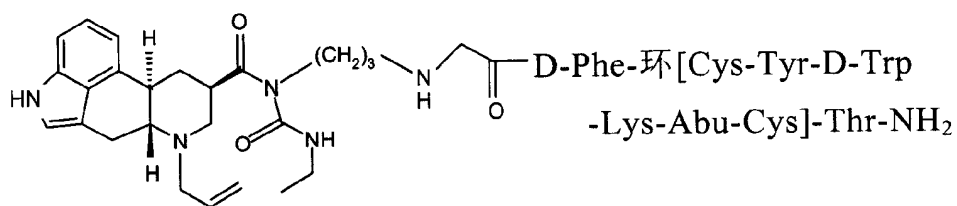
20



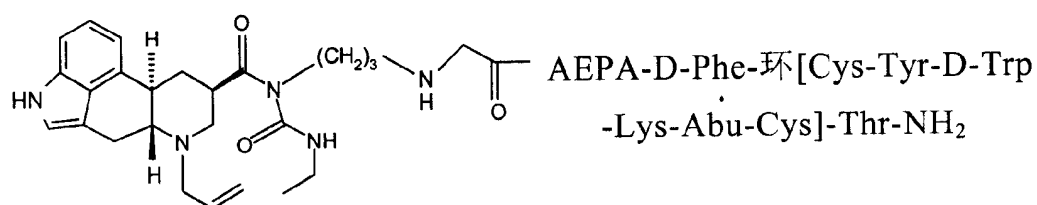
25



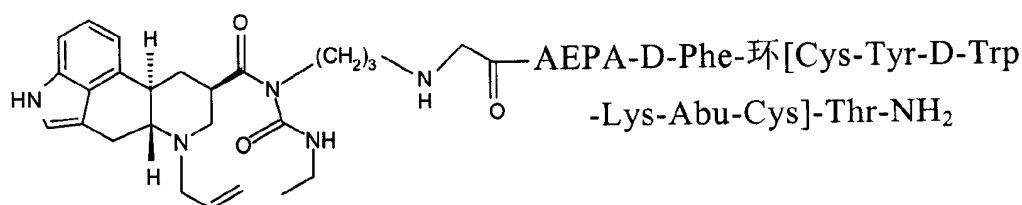
30



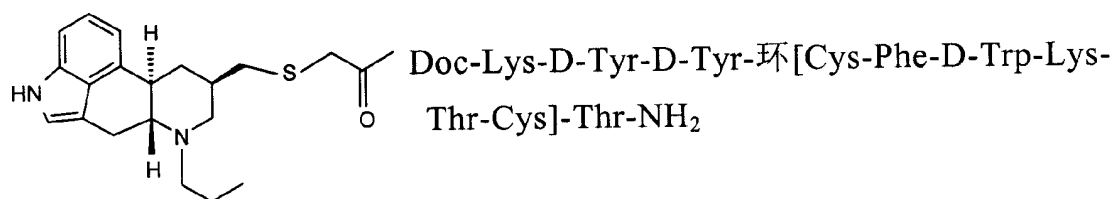
5



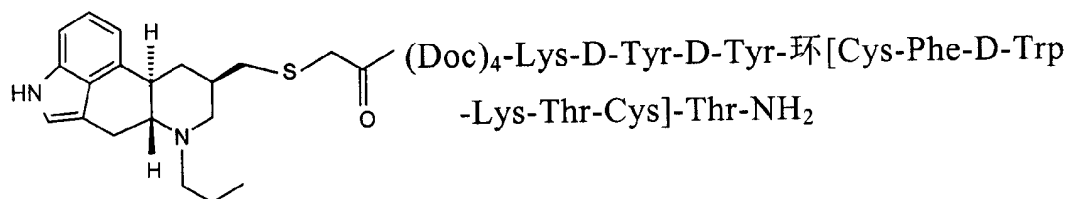
10



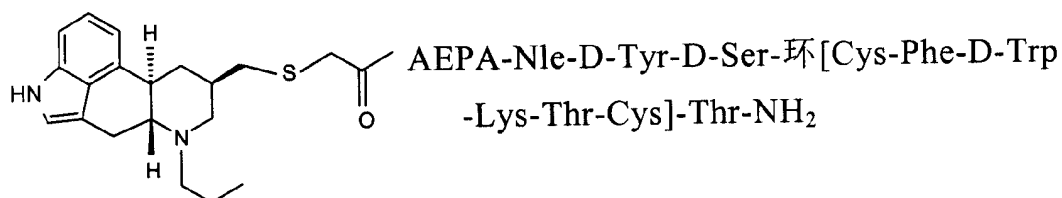
15



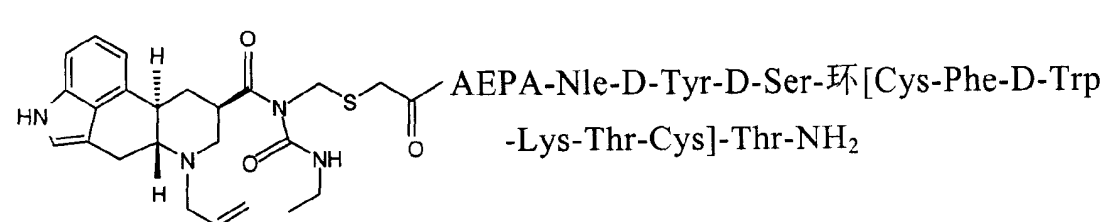
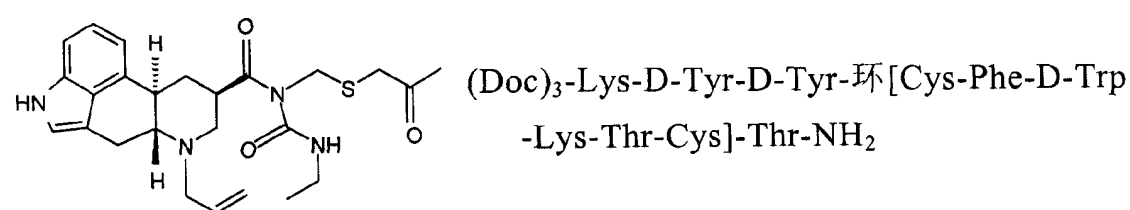
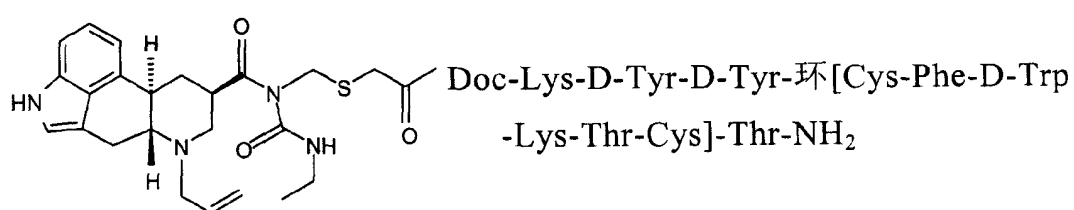
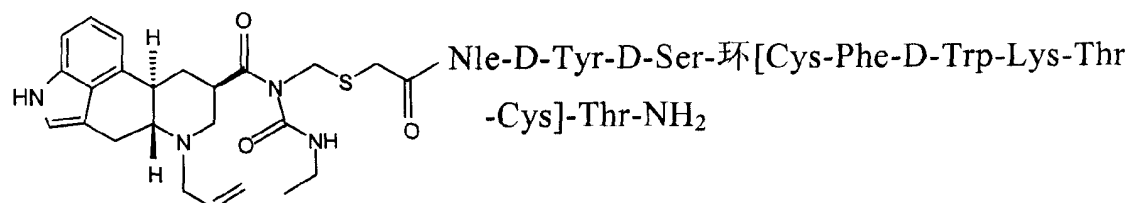
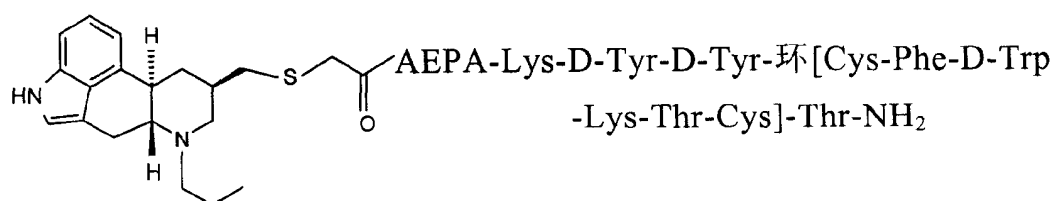
20



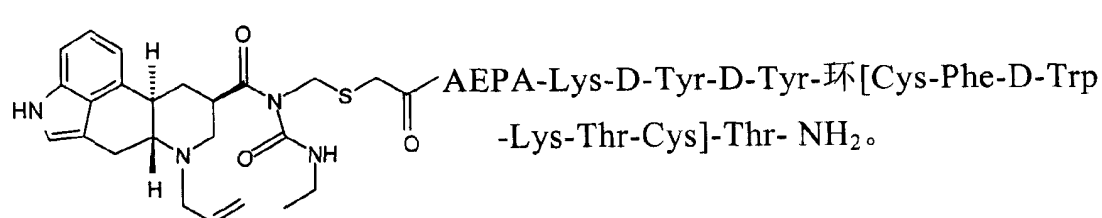
25



30

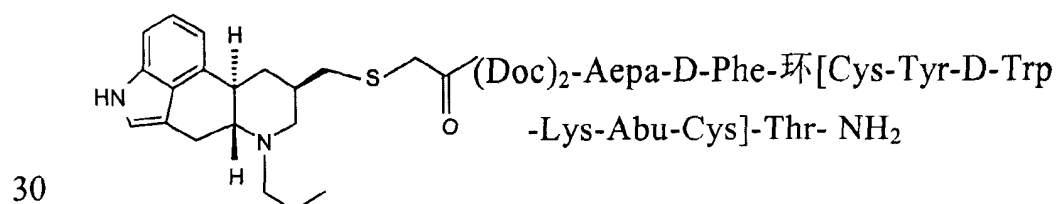
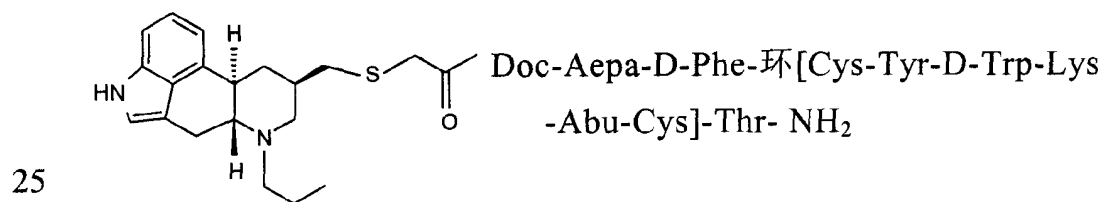
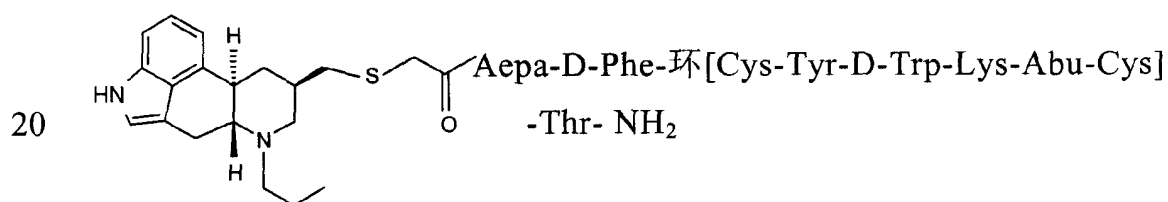
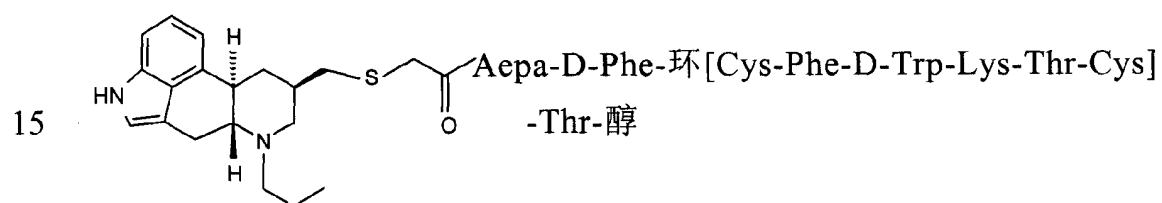
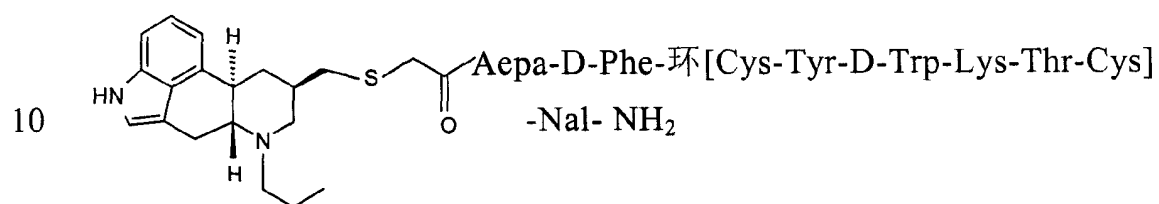
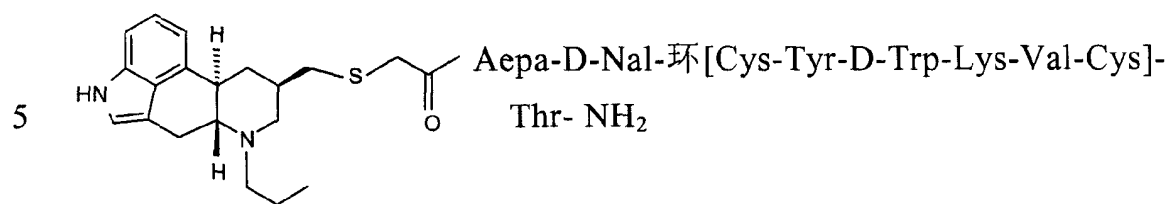


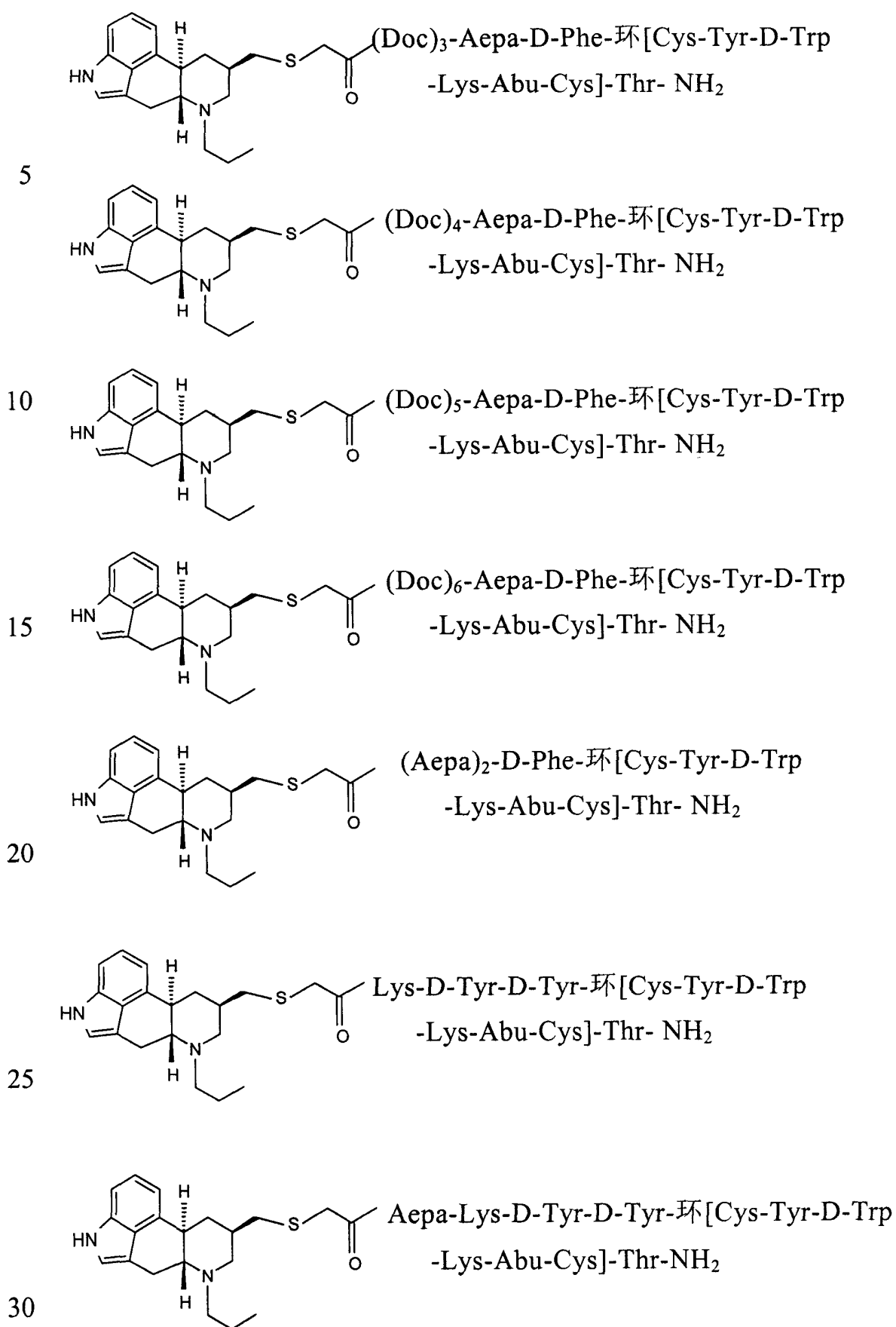
以及

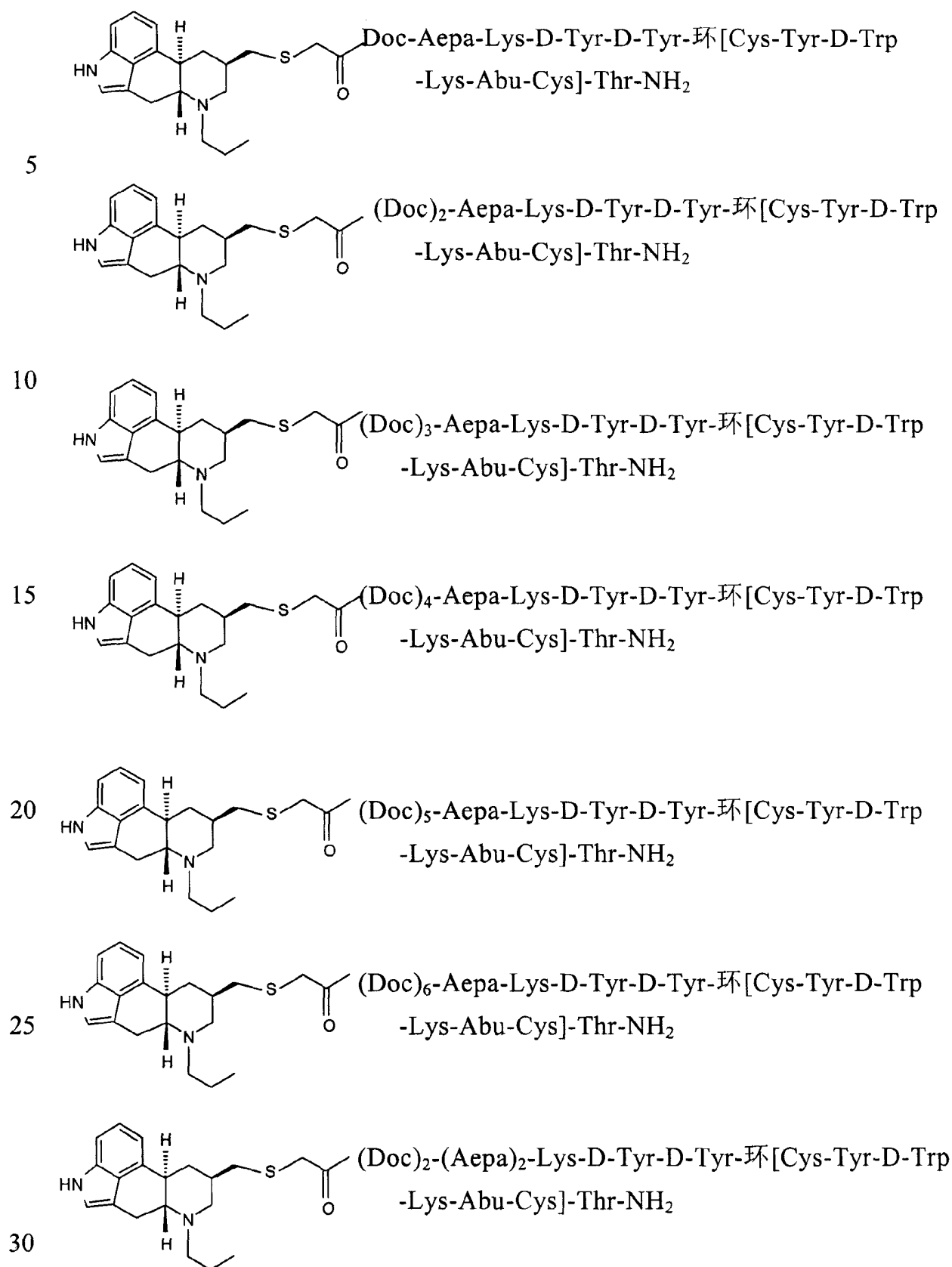


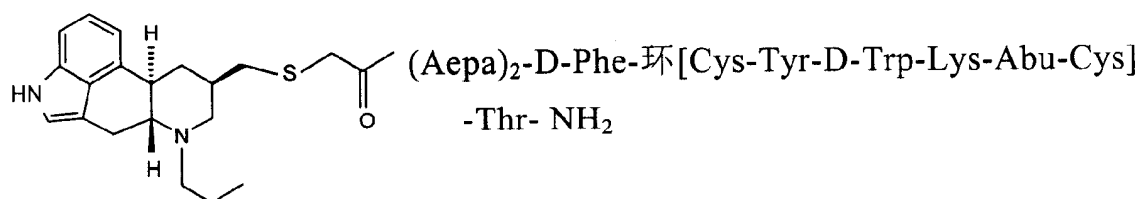
在另一种实施方式中，本发明的特征在于下式的化合物或其药学上可

接受的盐:

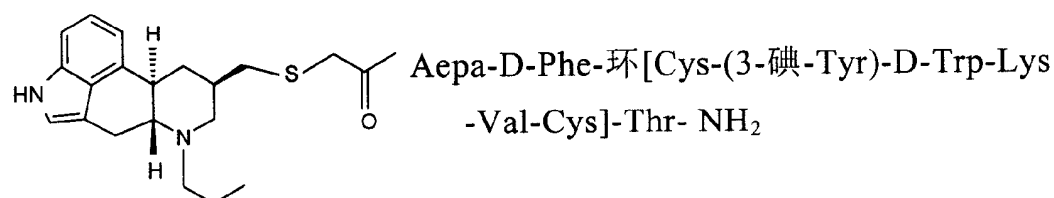




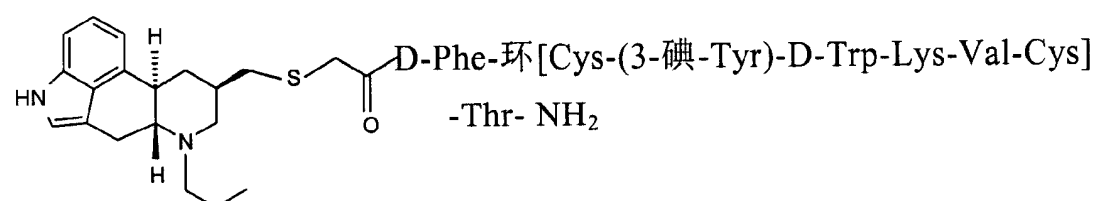




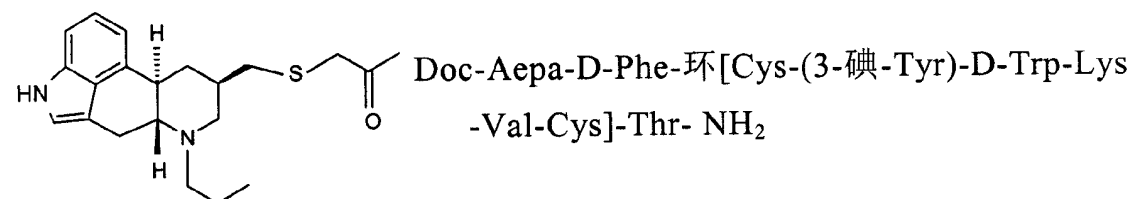
5



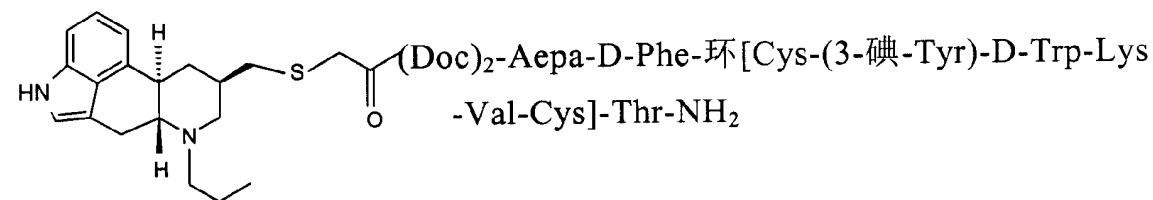
10



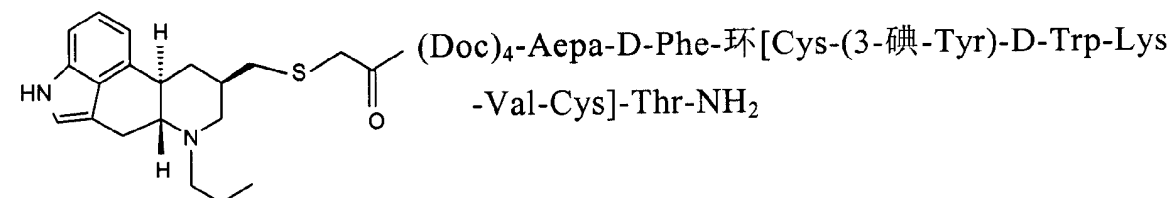
15



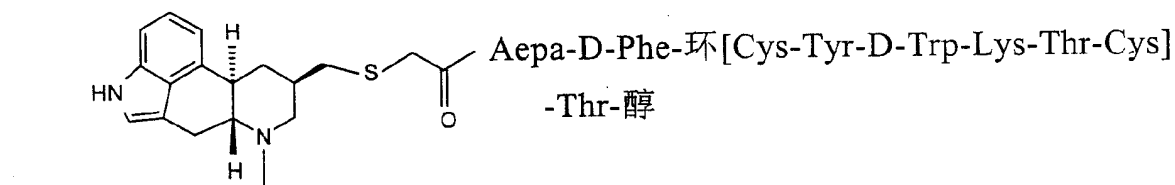
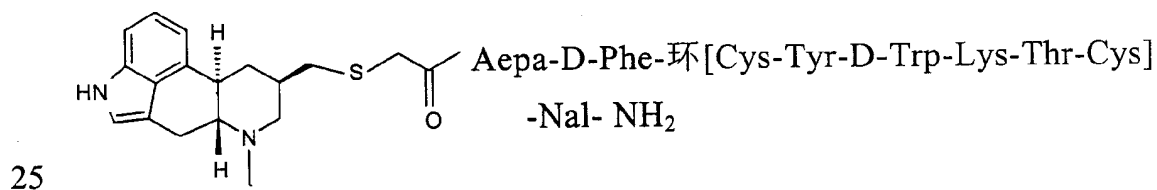
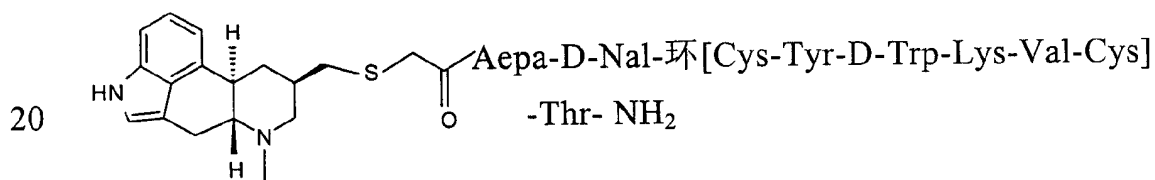
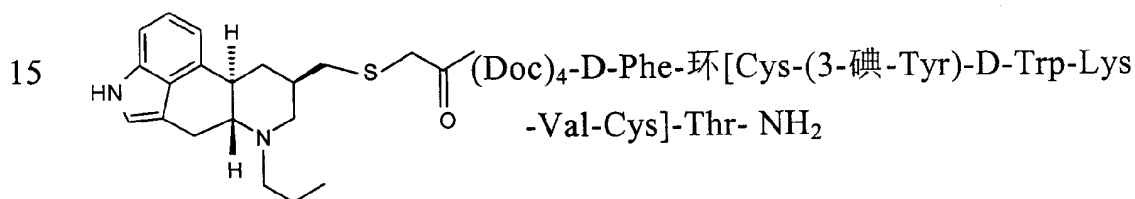
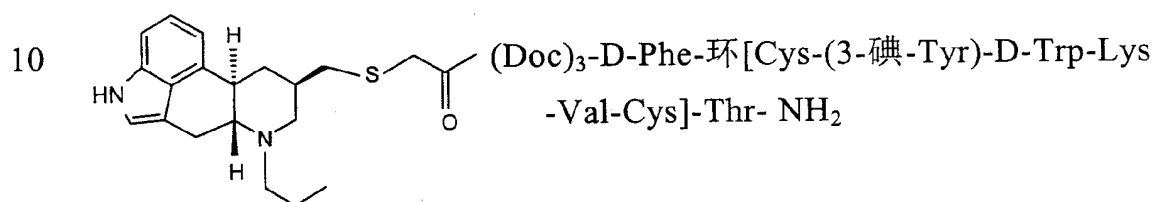
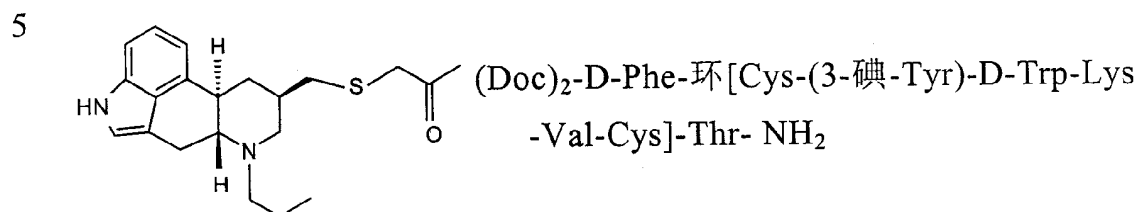
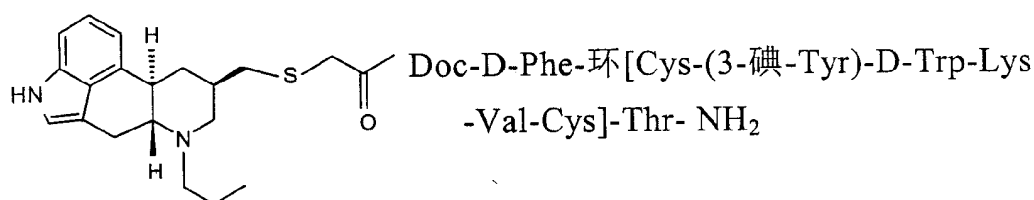
20

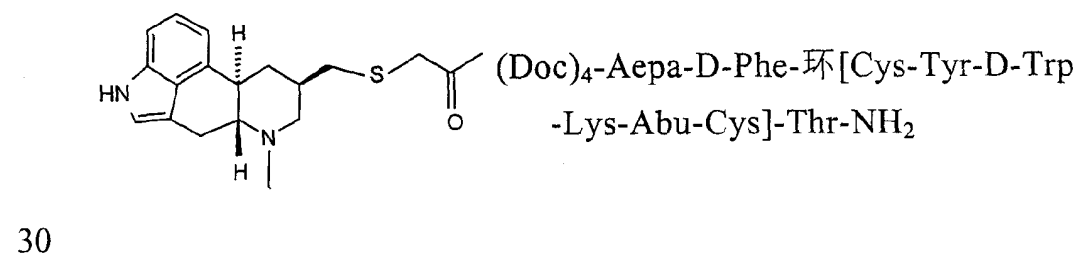
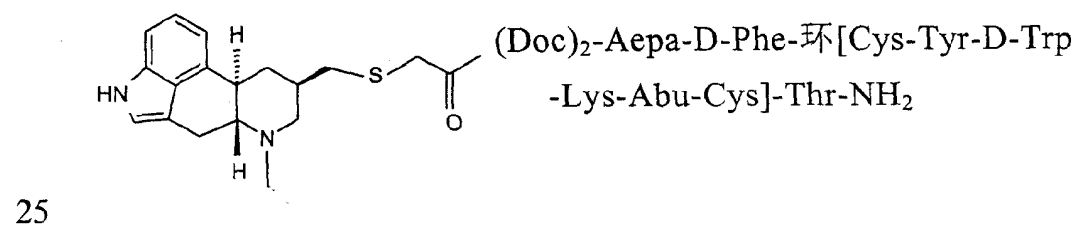
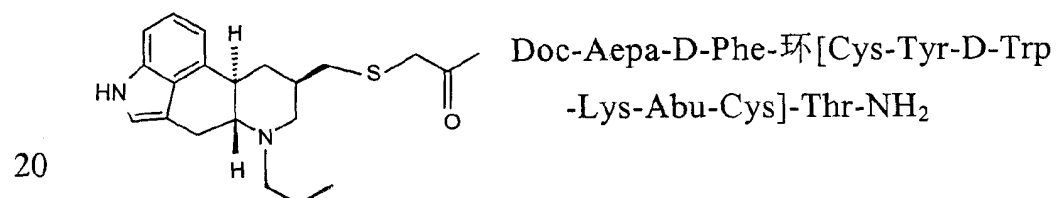
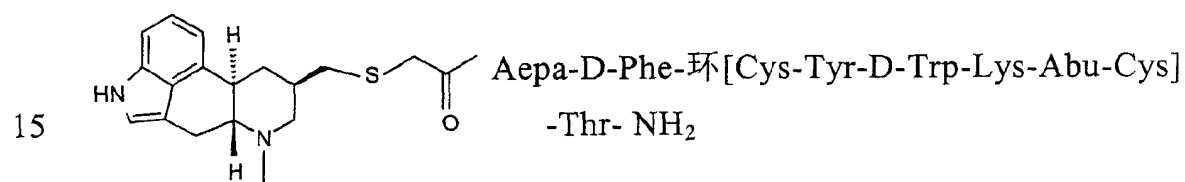
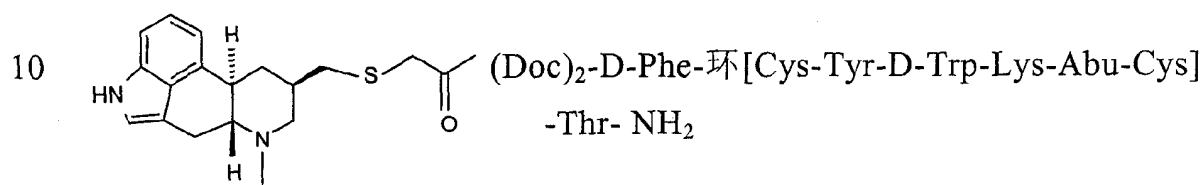
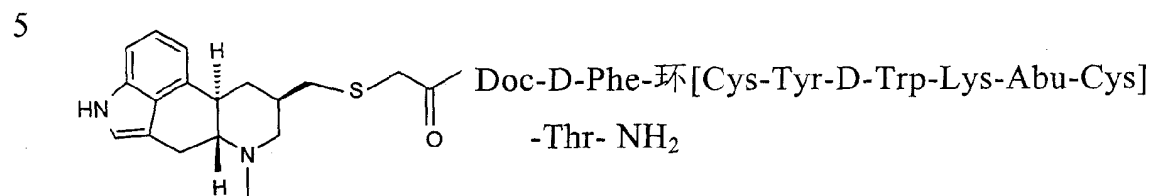
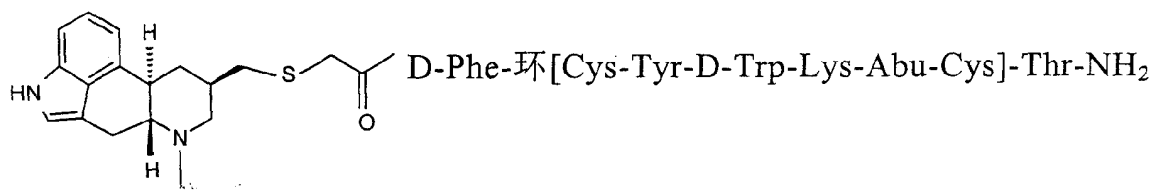


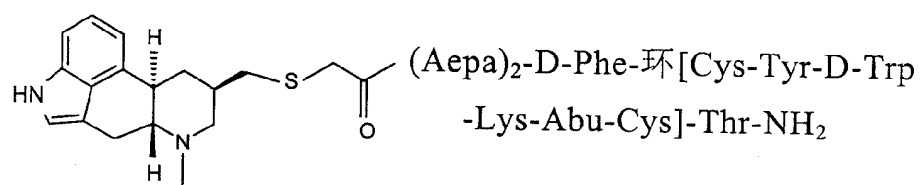
25



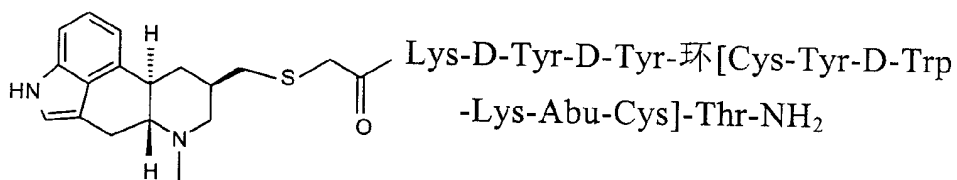
30



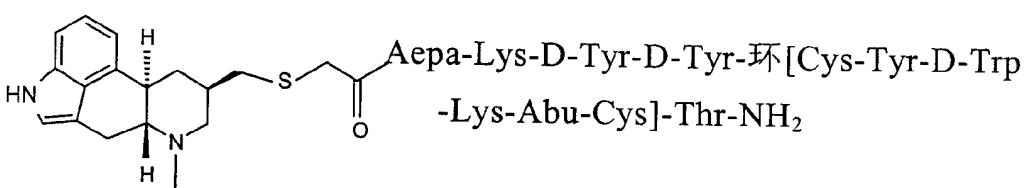




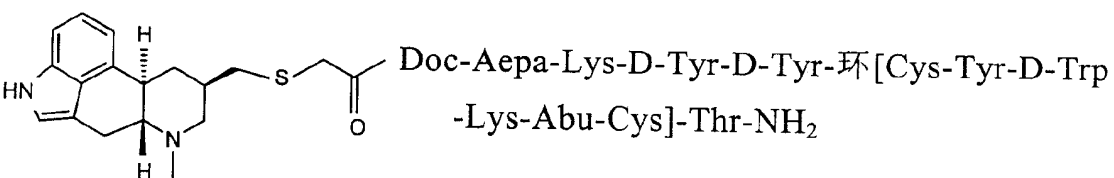
5



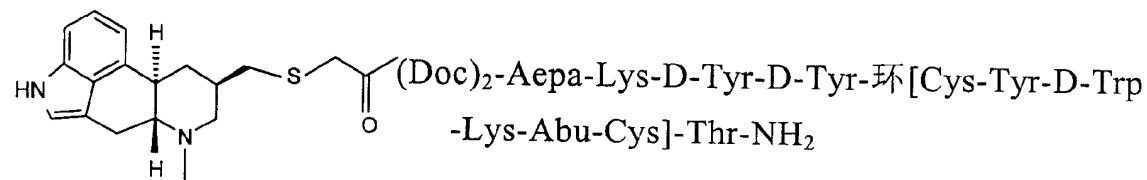
10



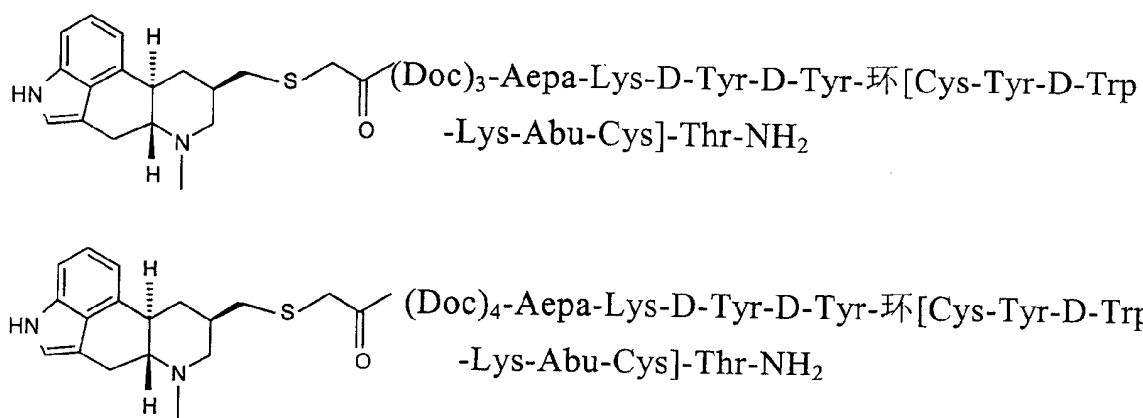
15



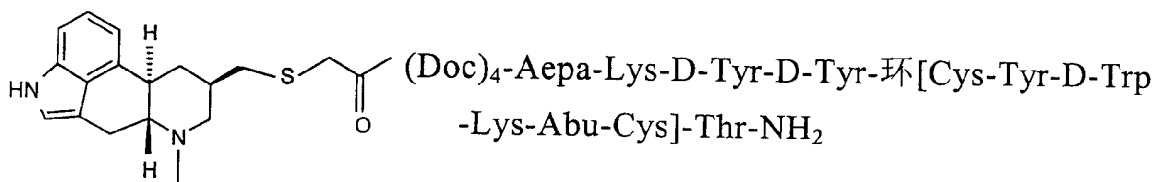
20

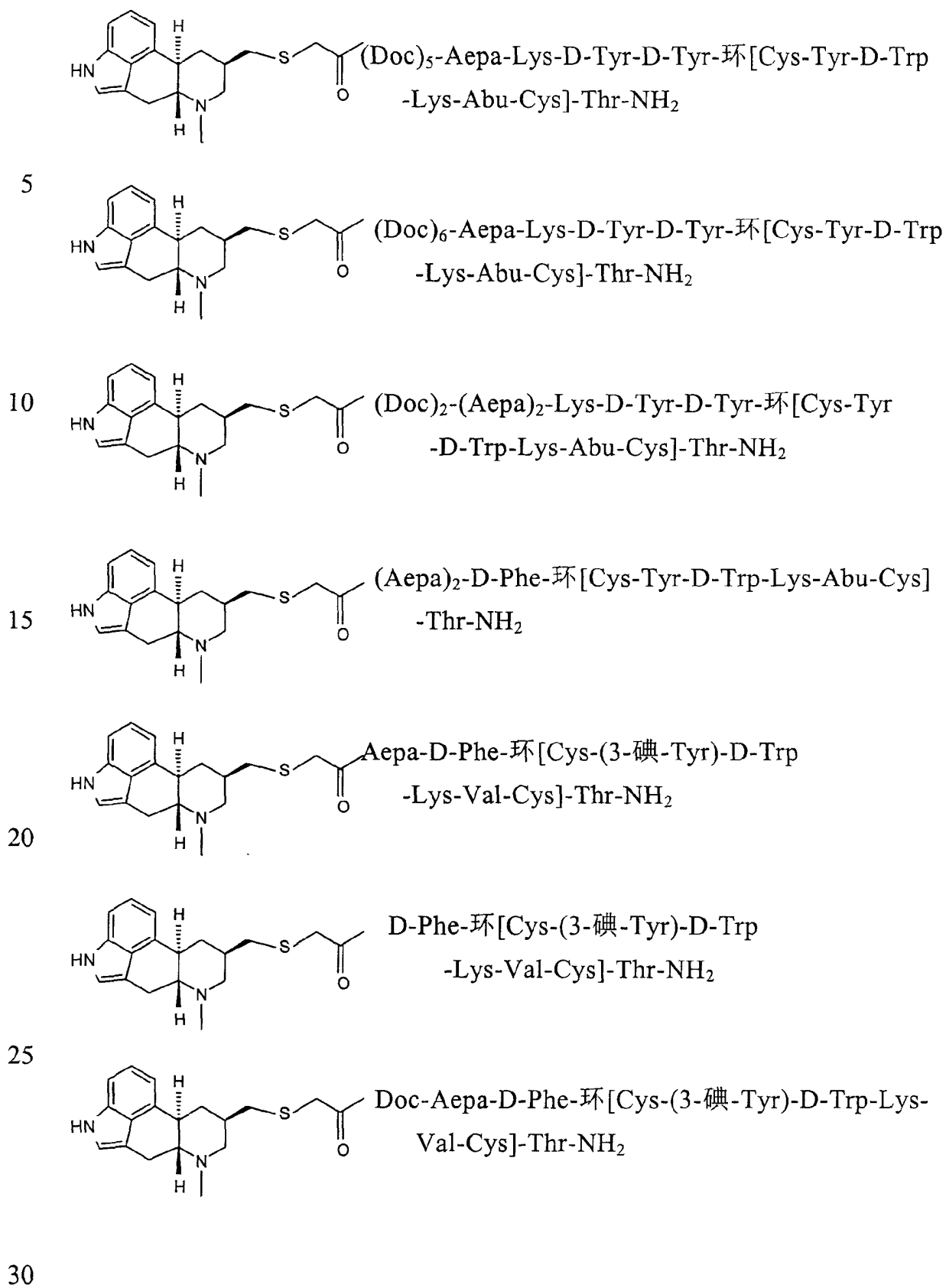


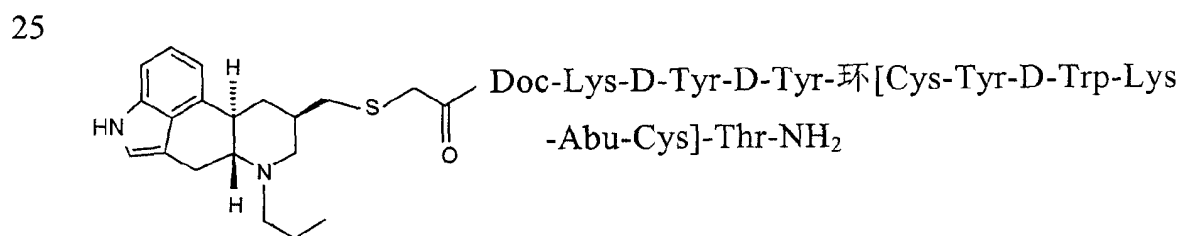
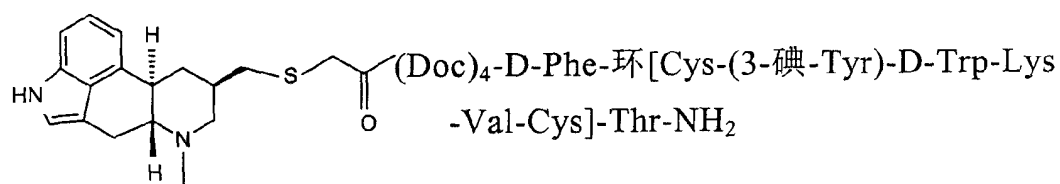
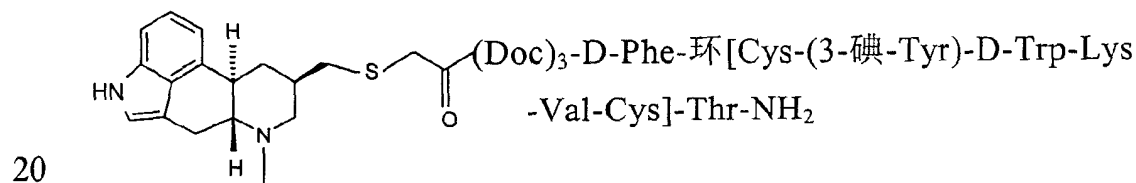
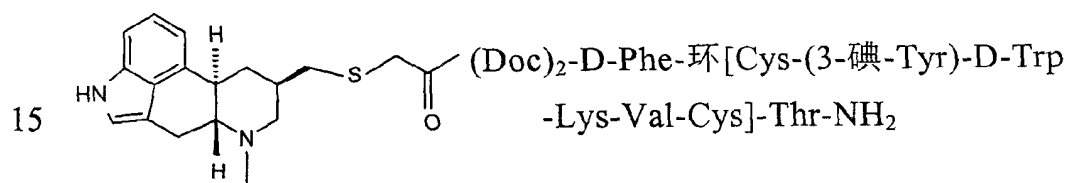
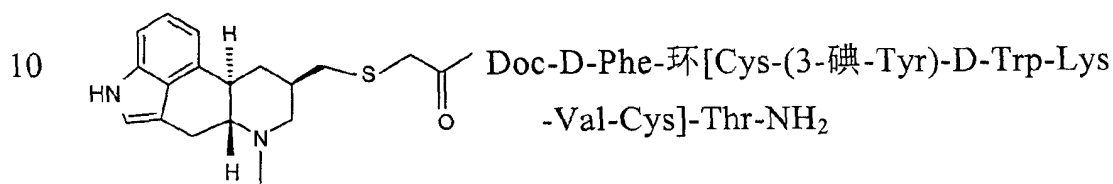
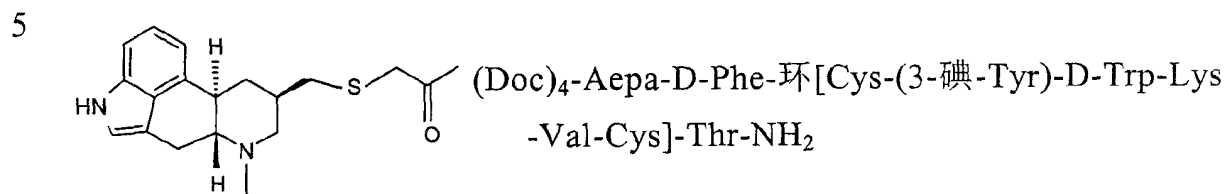
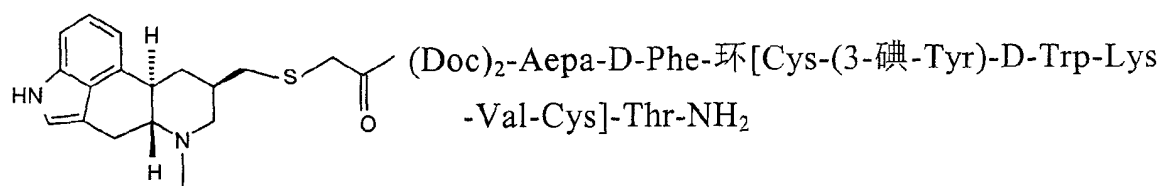
25



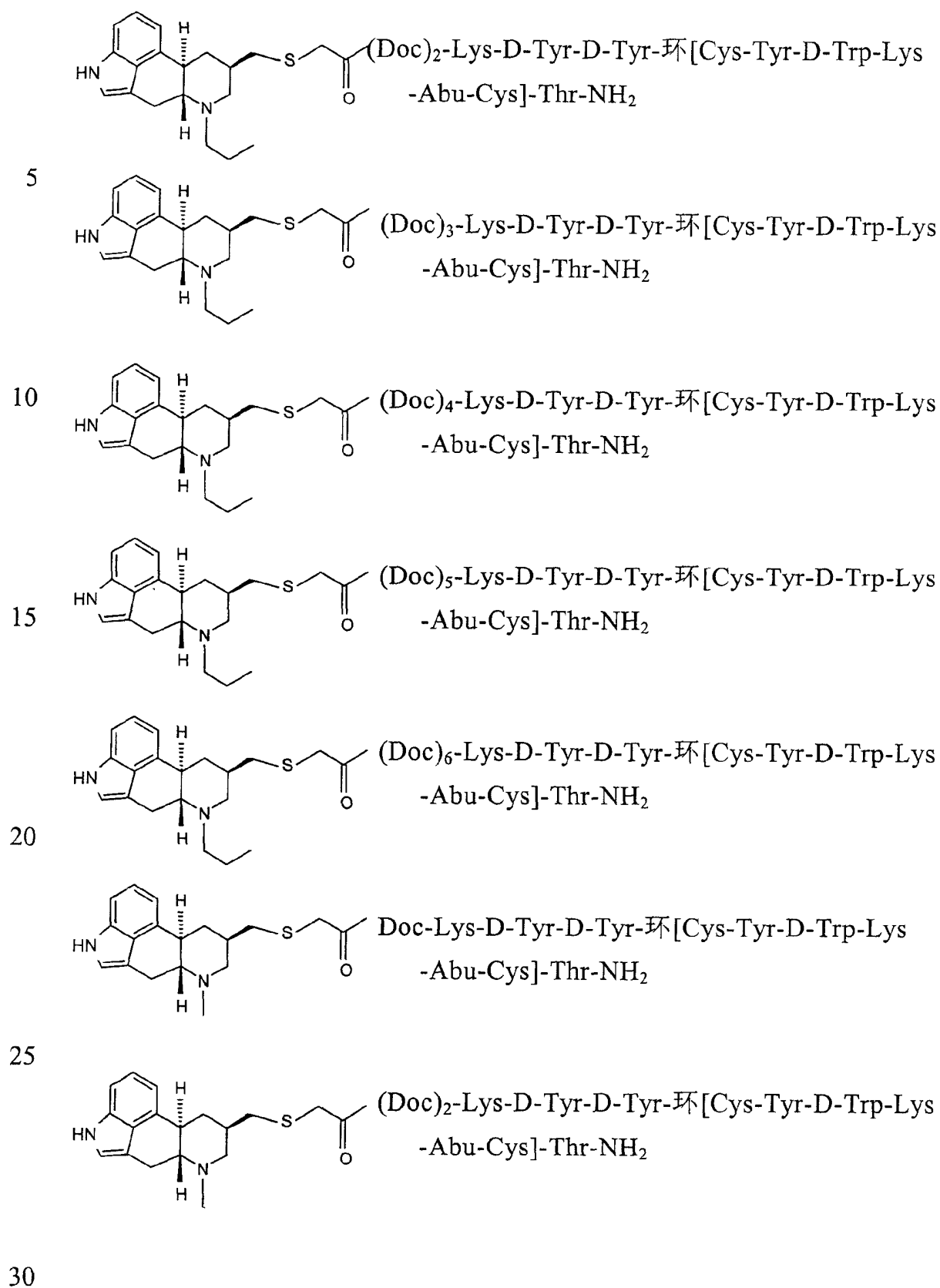
30

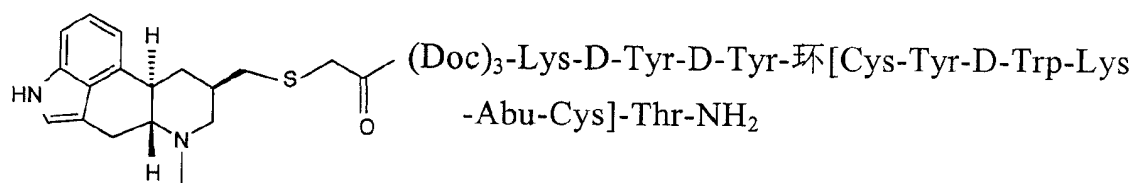




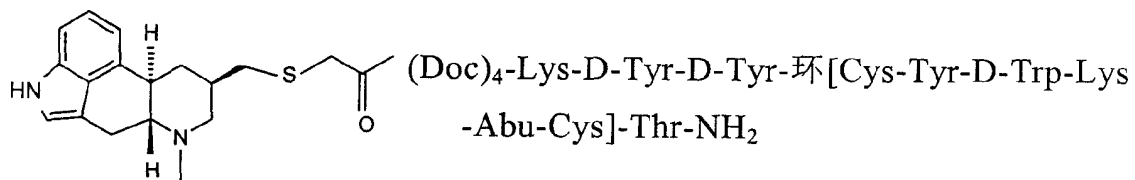


30

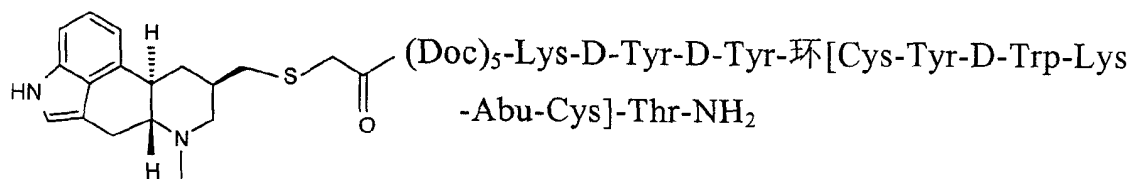




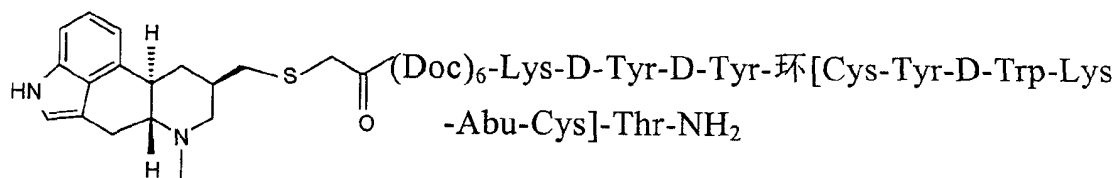
5



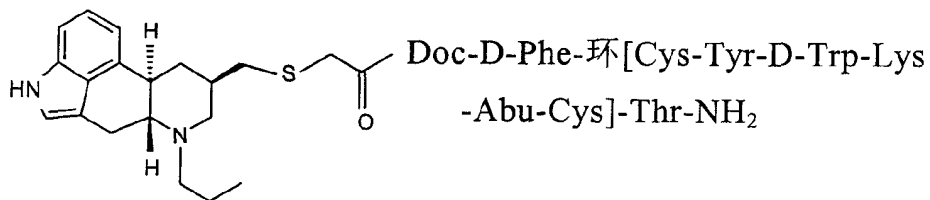
10



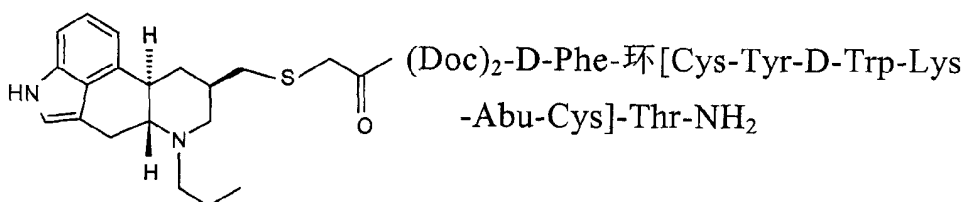
15



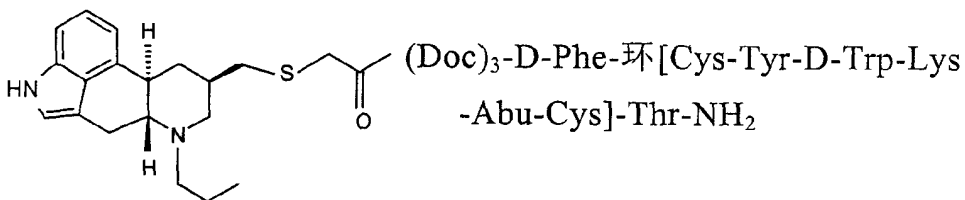
20

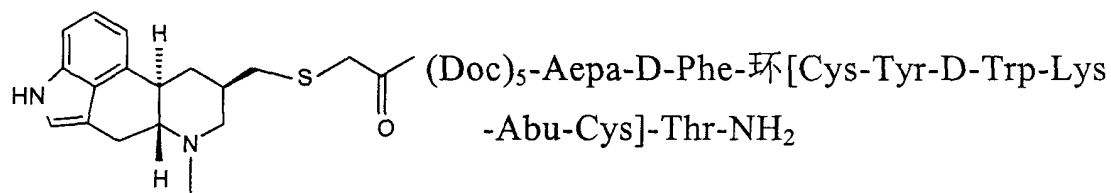
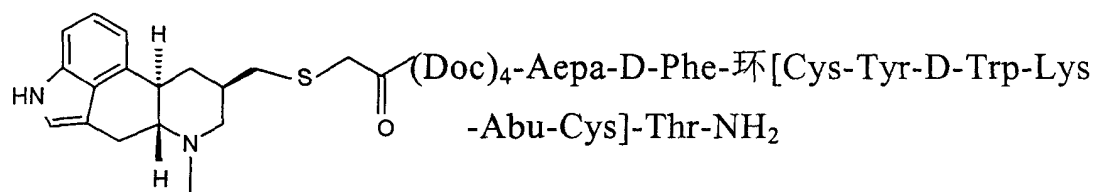
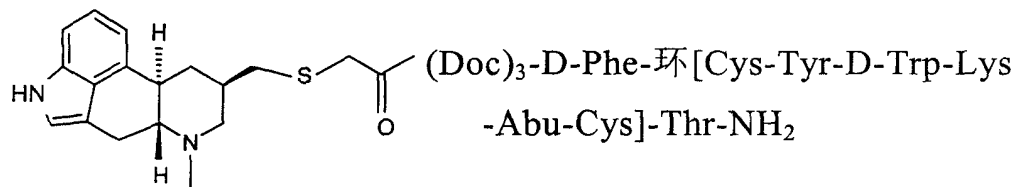
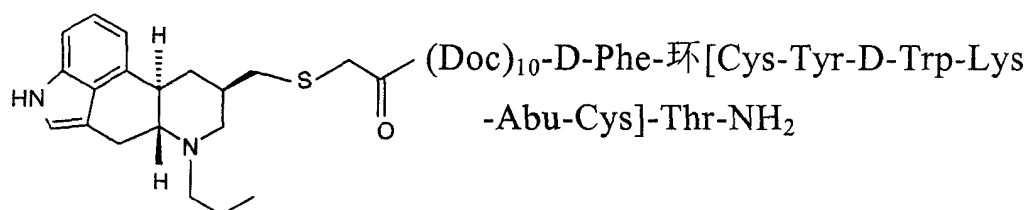
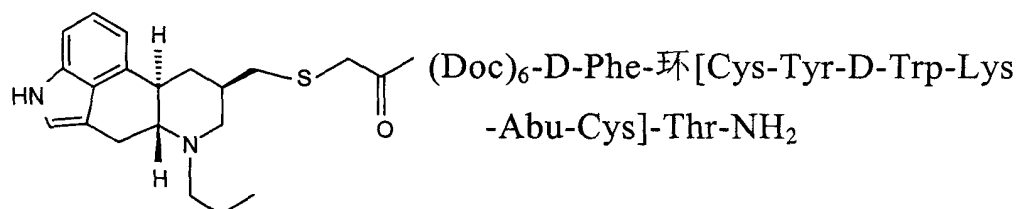
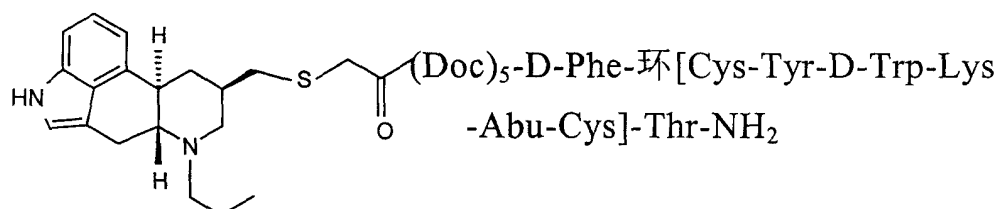
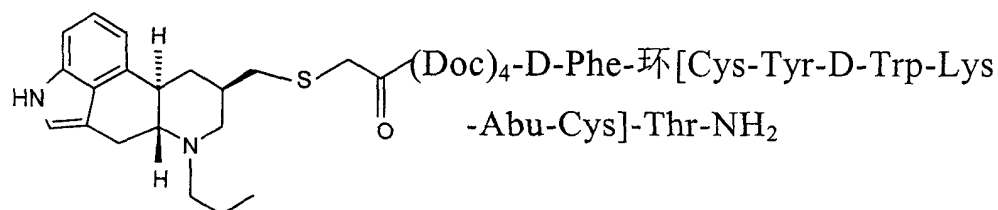


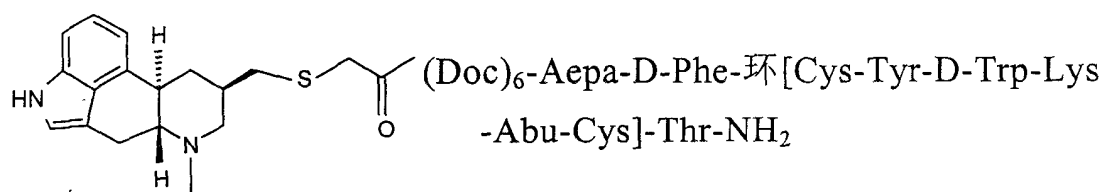
25



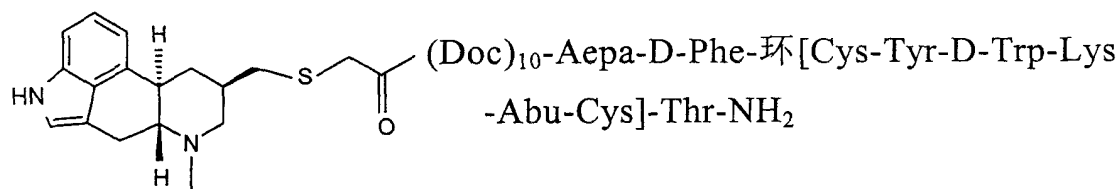
30



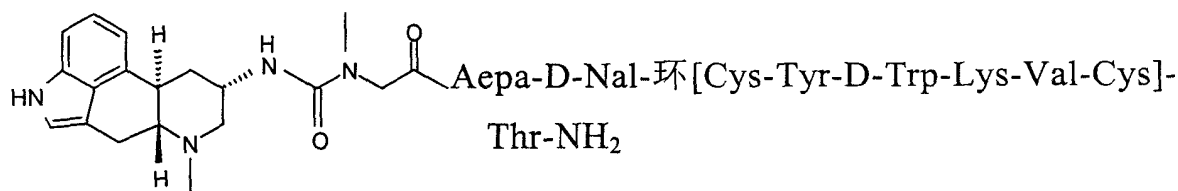




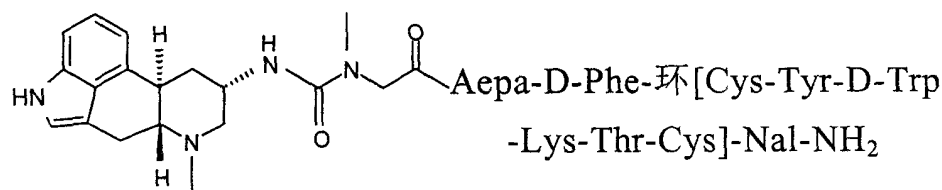
5



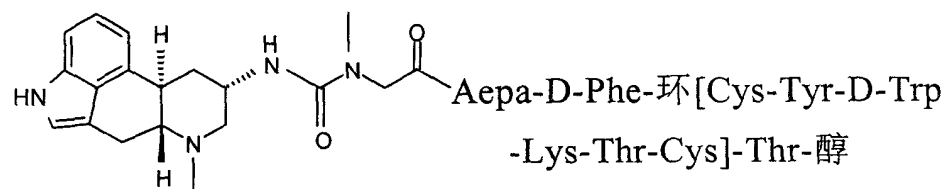
10



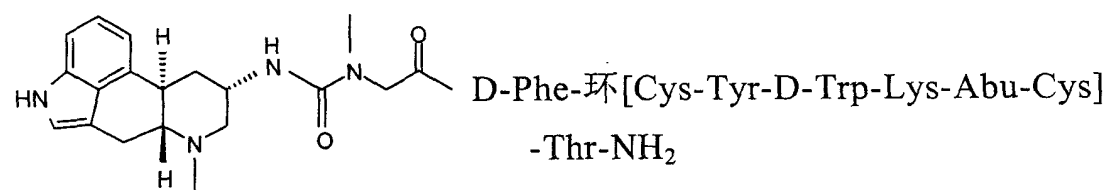
15



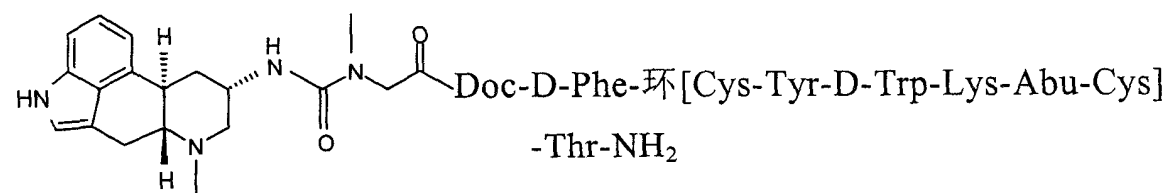
20

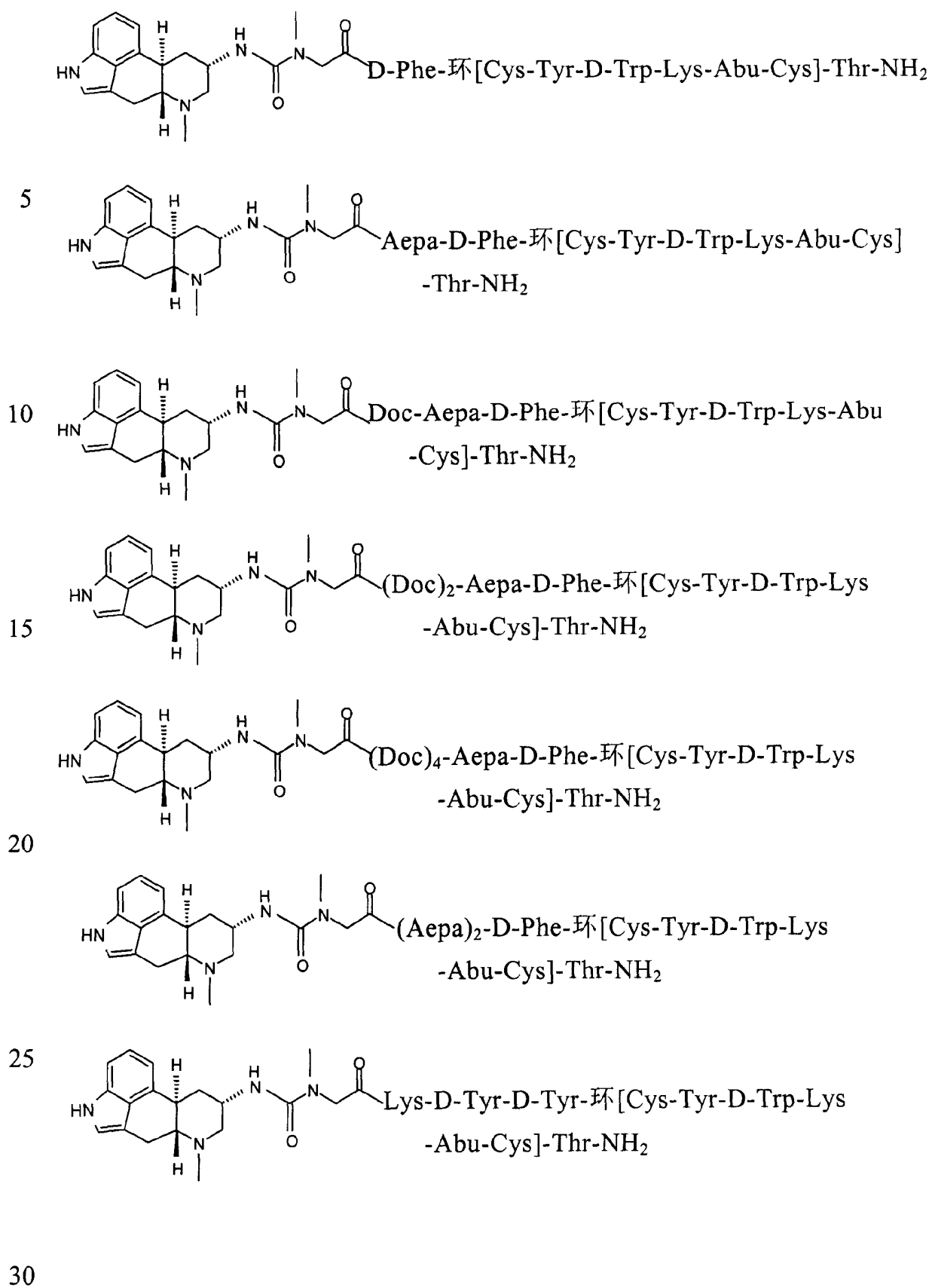


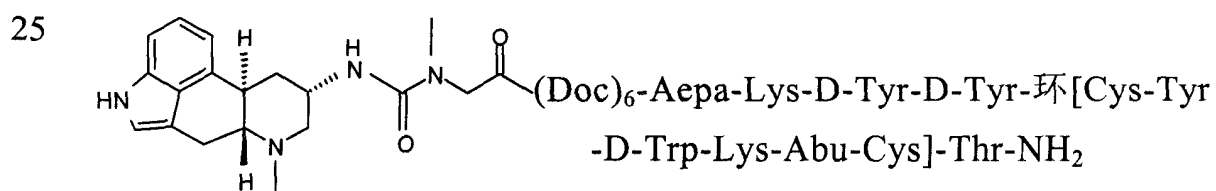
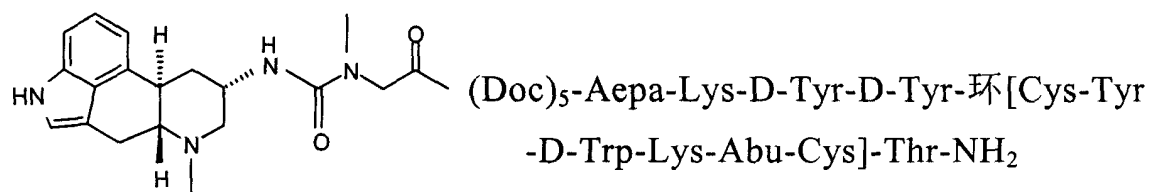
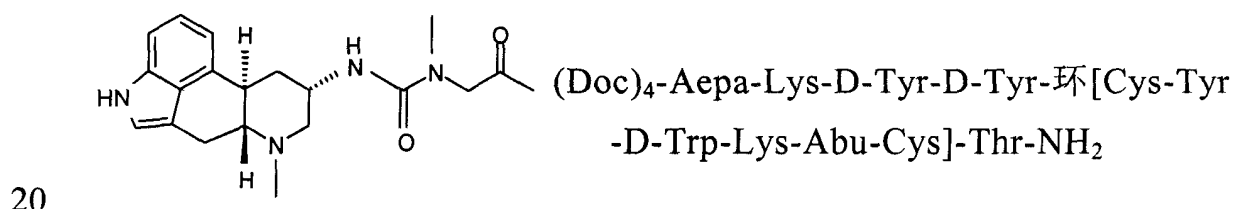
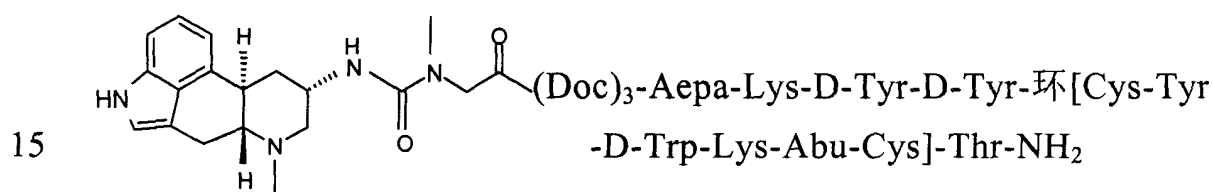
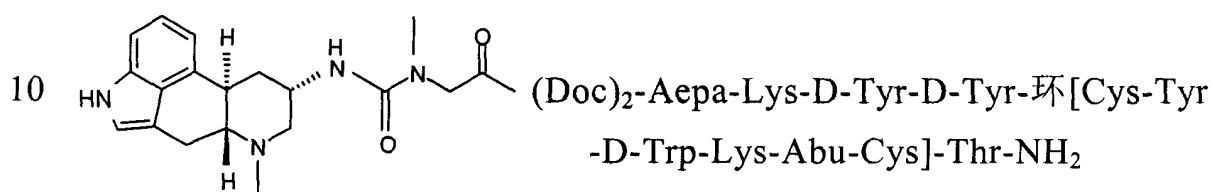
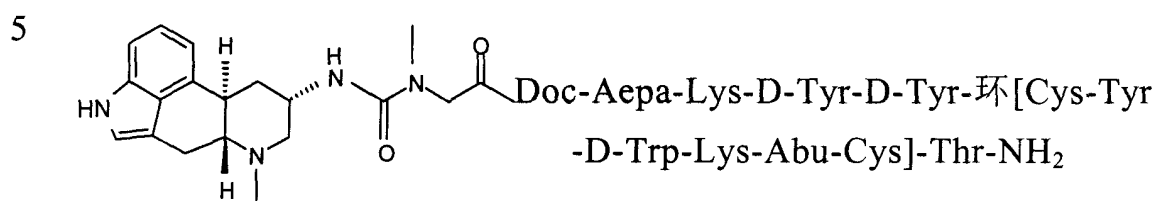
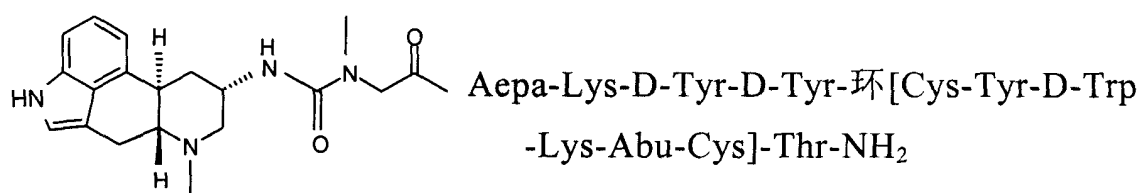
25

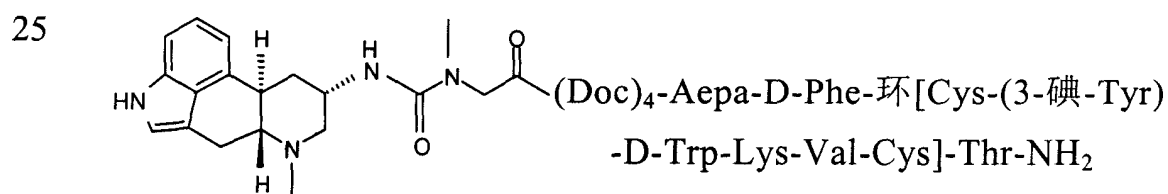
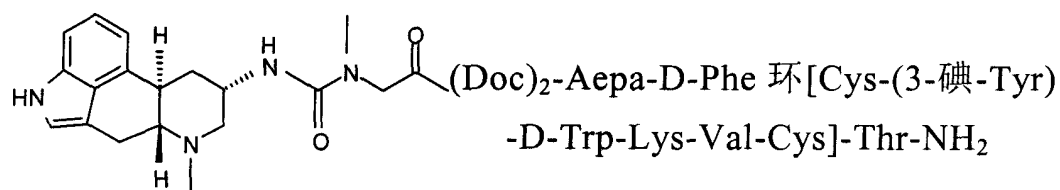
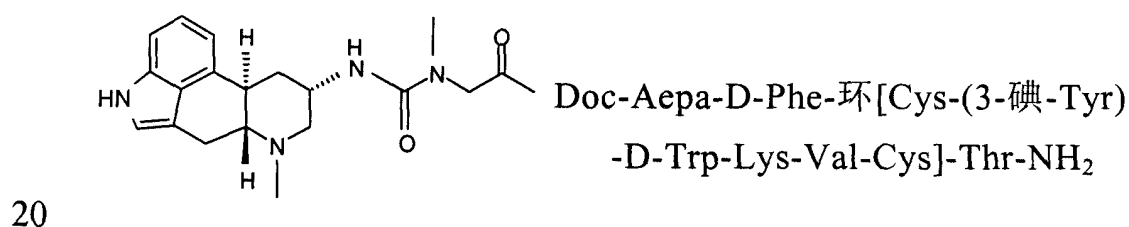
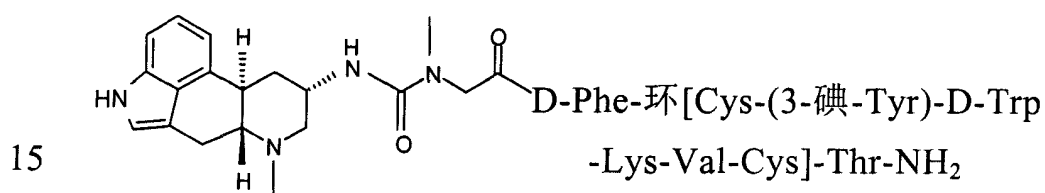
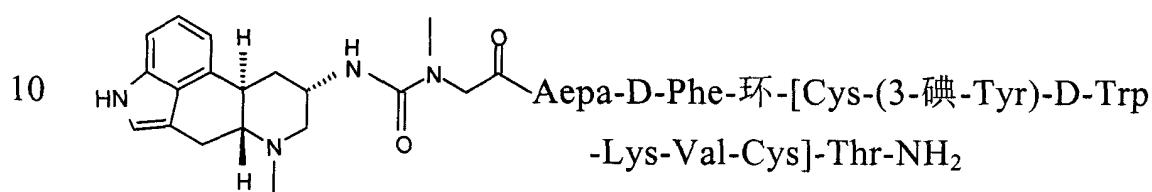
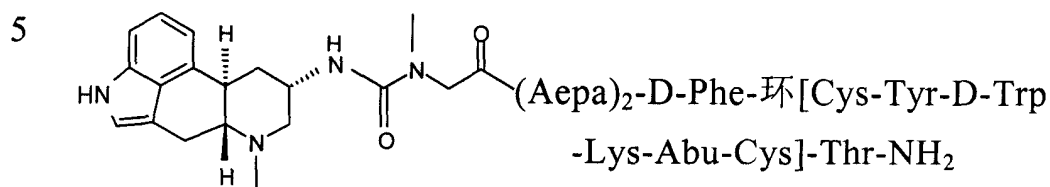
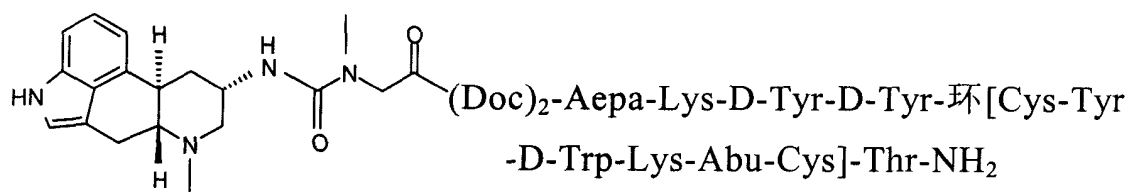


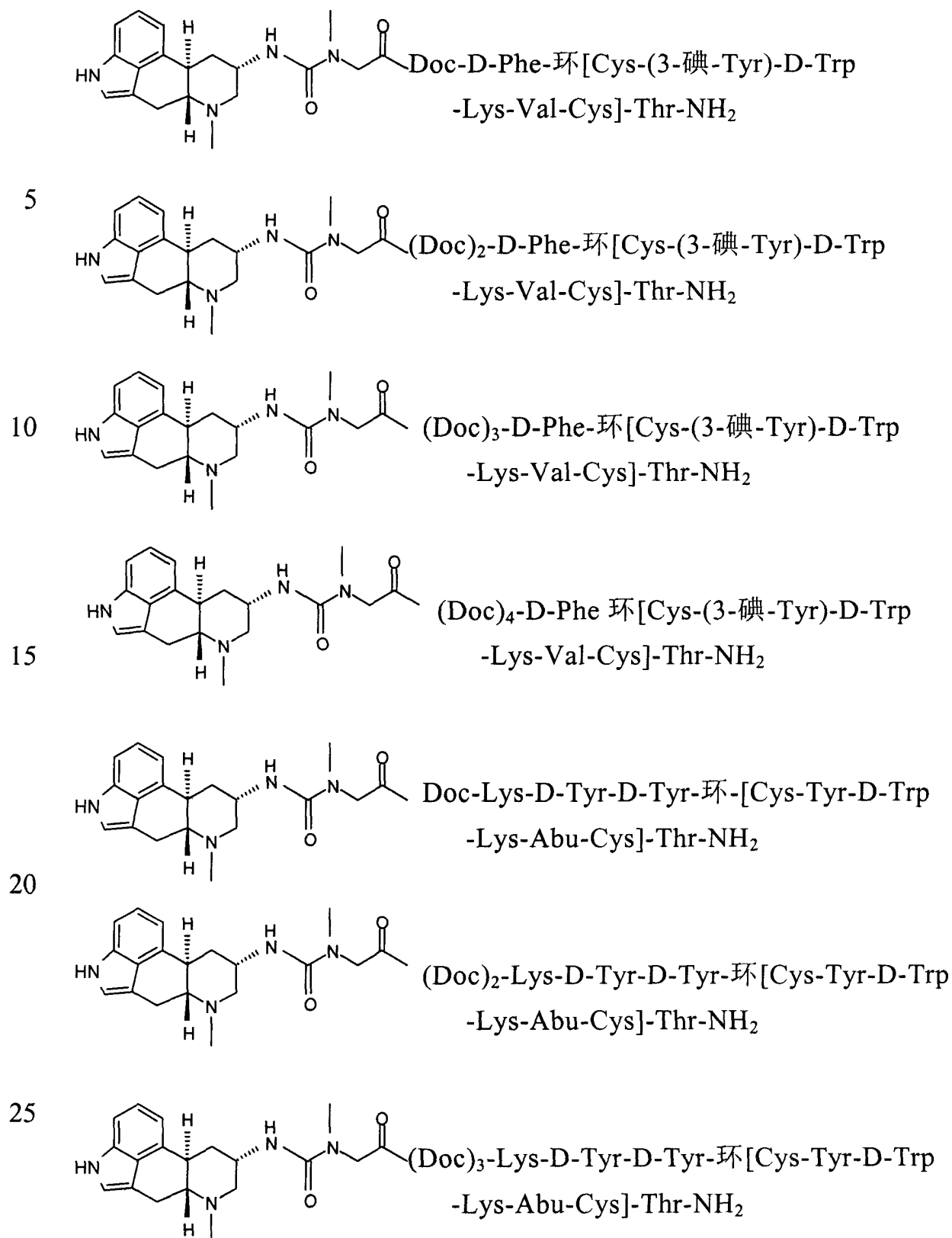
30

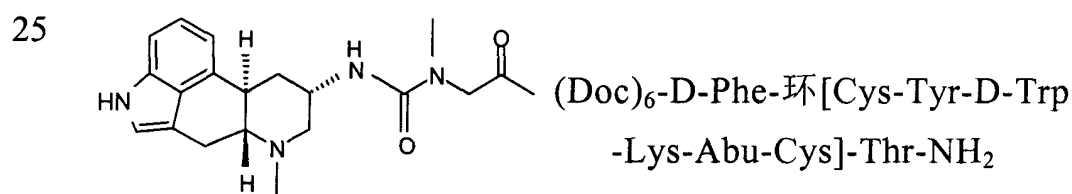
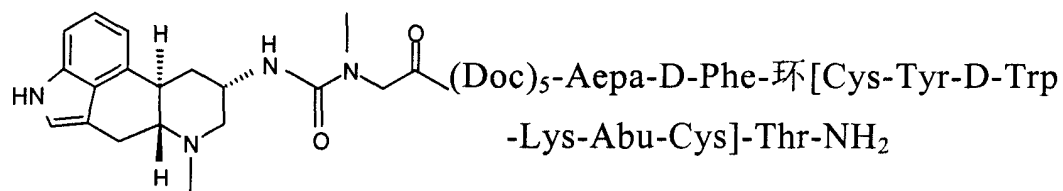
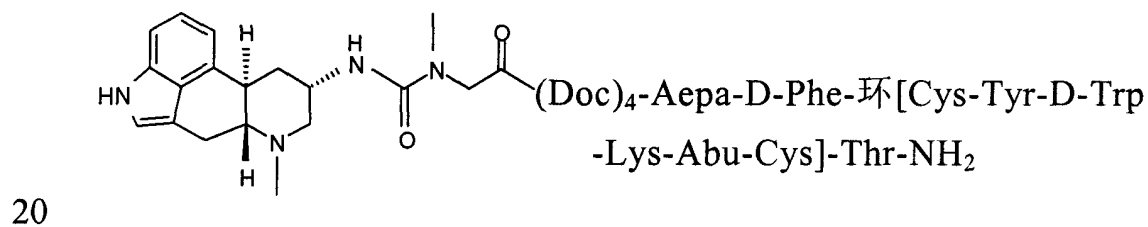
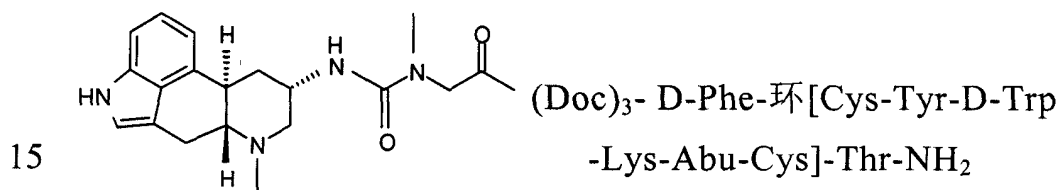
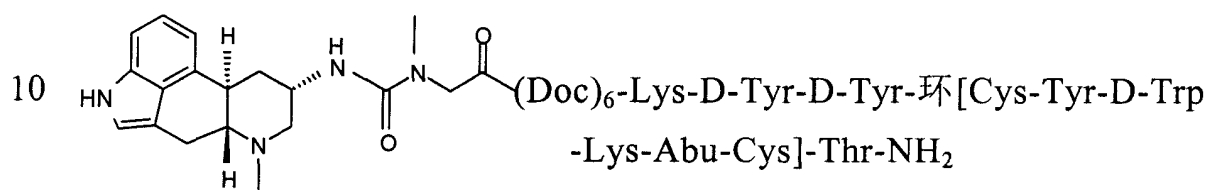
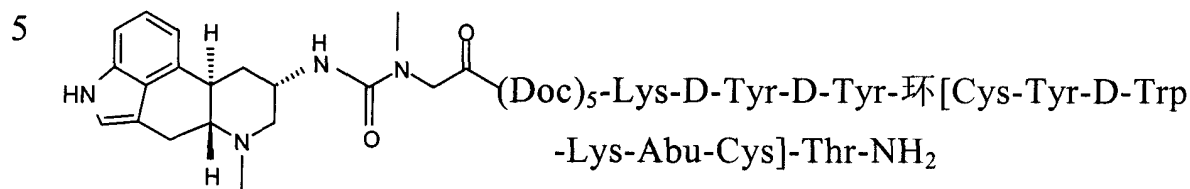
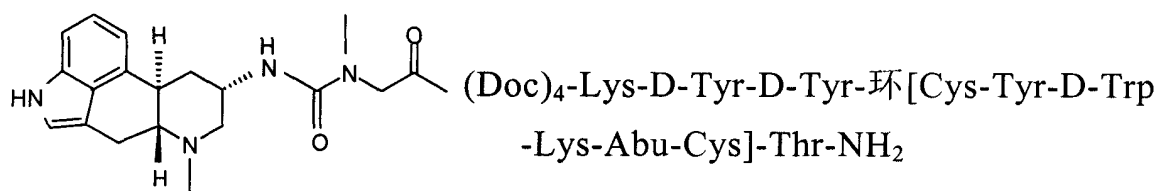


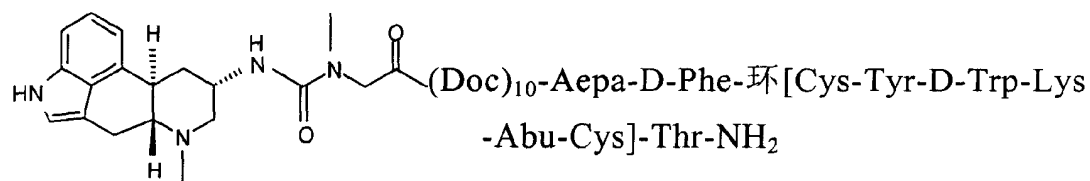




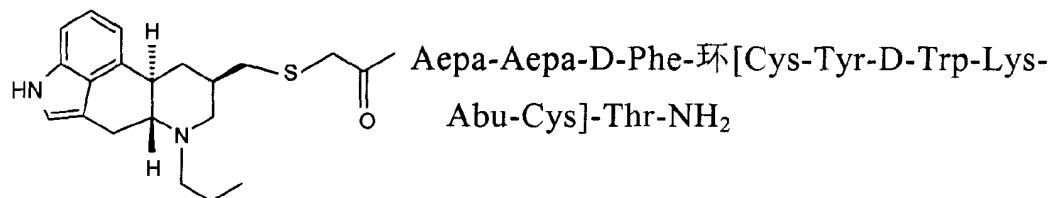




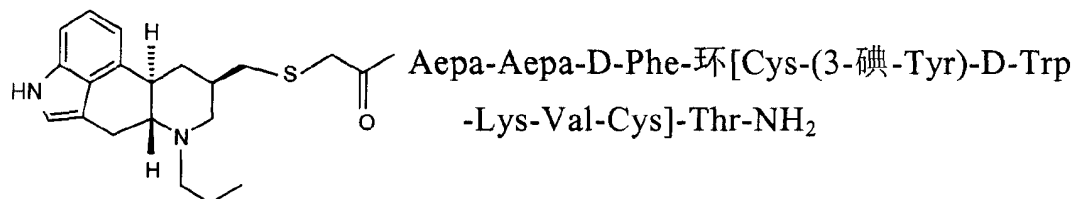




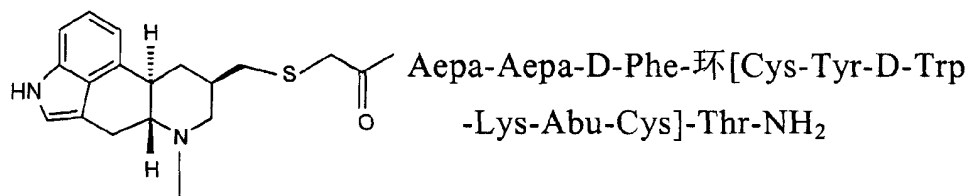
5



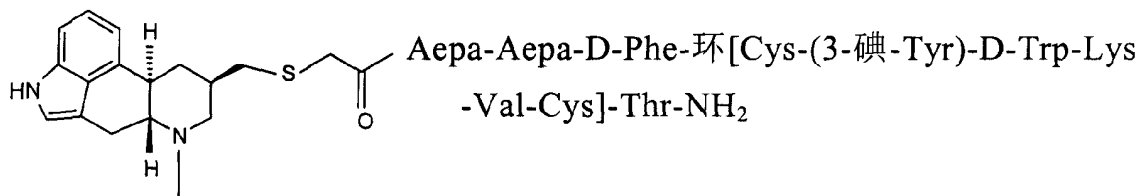
10



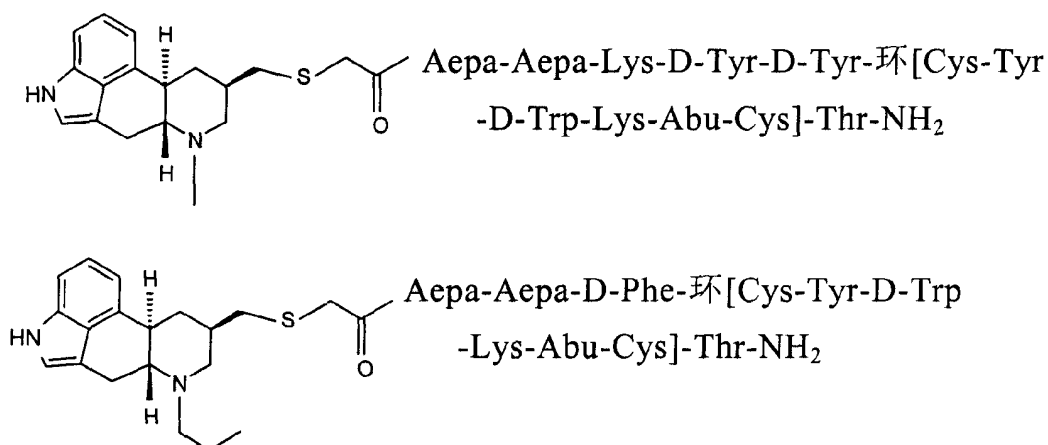
15



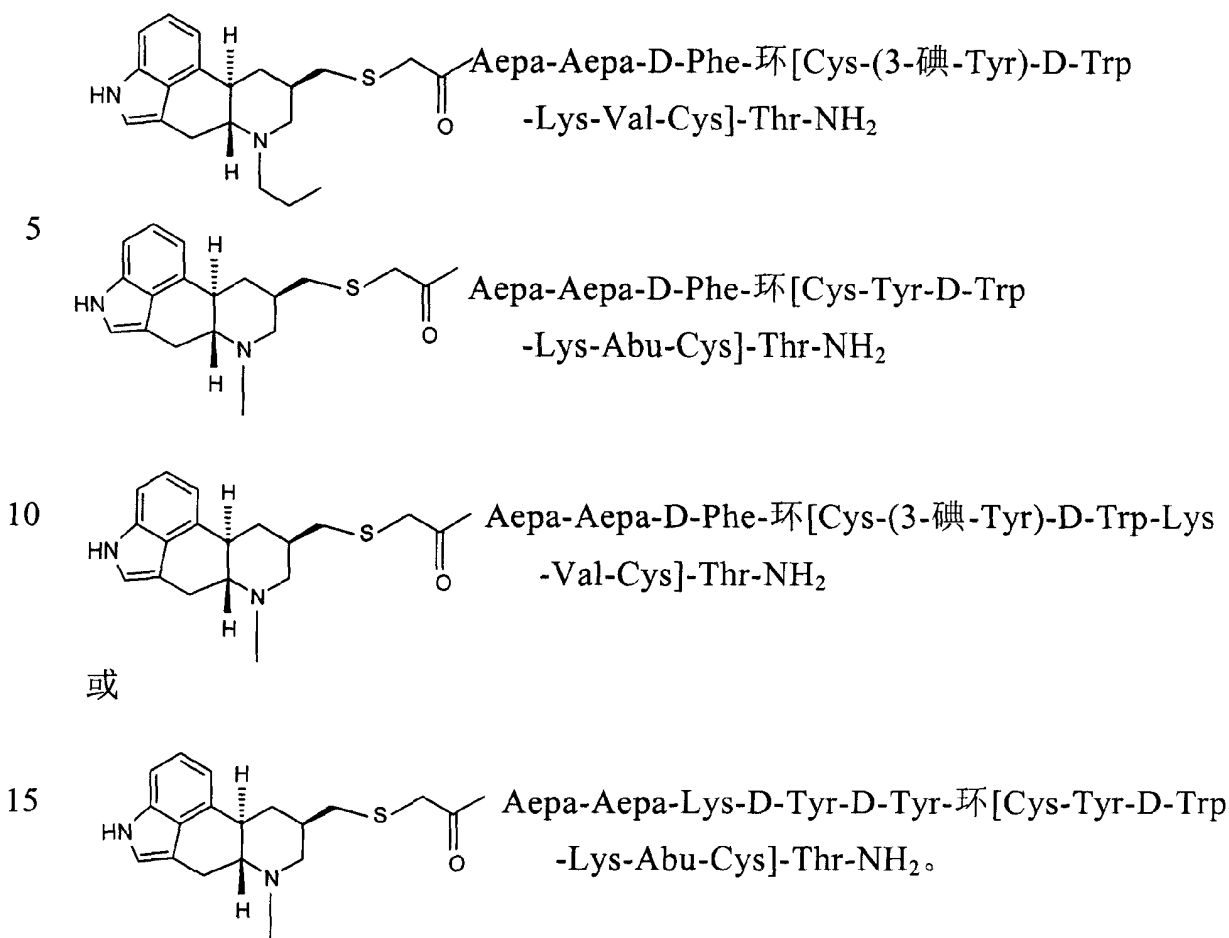
20



25



30



一方面本发明的特征在于一种在需要的患者体内引起多巴胺受体激动剂作用的方法，其中所述方法包括以有效量的式(I)或式(II)化合物或其药学上可接受的盐对所述患者给药。在这一方面的一个优选的实施方式是所述化合物选自本发明在这里所公开的化合物。

本发明的另一方面的特征在于一种在需要的患者体内引起生长激素释放抑制因子受体激动剂作用的方法，其中所述方法包括以有效量的式(I)或式(II)化合物或其药学上可接受的盐对所述患者给药。在这一方面的一个优选的实施方式是所述化合物选自本发明在这里所公开的化合物。

本发明的另一方面的特征在于一种在需要的患者体内同时引起多巴胺受体激动剂作用和生长激素释放抑制因子受体激动剂作用的方法，其中所述方法包括以有效量的式(I)或式(II)化合物或其药学上可接受的盐对所述患者给药。在这一方面的一个优选的实施方式是所述化合物选自本发明在这里所公开的

化合物。

本发明的另一方面的特征在于一种药物组合物，其包括有效量的式(I)或式(II)化合物，或其药学上可接受的盐。在这一方面的一个优选的实施方式是所述化合物选自本发明在这里所公开的化合物。

- 5 本发明的另一方面的特征在于一种为患者治疗疾病或症状的方法，所述方法包括以治疗有效量的式(I)或式(II)化合物或其药学上可接受的盐对患者给药，其中所述疾病选自肺癌、神经胶质瘤、厌食症、甲状腺机能减退、醛固酮过多症、幽门螺旋杆菌(*H. pylori*)增生、肢端肥大症、再狭窄、克朗氏病、系统性硬化症、外部和内部胰腺假囊肿和腹水、舒血管肠肽瘤(VIPoma)、胰岛细胞弥漫性增生、胰岛素分泌过多、胃泌素瘤、卓-艾二氏综合症、腹泻、与 AIDS 相关的腹泻、与化疗相关的腹泻、硬皮病、过敏性肠综合症、胰腺炎、小肠梗阻、胃食管回流、十二指肠胃回流、库兴氏综合症、促性腺素瘤、甲状旁腺机能亢进、格雷夫斯氏病、糖尿病性神经病、Paget 氏病、多囊卵巢病、甲状腺癌、肝细胞瘤(hepatome)、白血病、脑膜炎、癌性恶病质、直立低血压、餐后低血压、焦虑发作、GH 分泌腺瘤、肢端肥大症、TSH 分泌腺瘤、催乳素分泌腺瘤、胰岛腺瘤、高血糖素瘤、糖尿病、高脂血症、胰岛素麻木、X 综合症、血管病、增生性视网膜炎、黎明现象(dawn phenomenon)、肾病、胃酸分泌、消化器官溃疡、肠皮肤瘻管、胰腺皮肤瘻管、倾倒综合症、含水腹泻综合症、胰腺炎、胃肠激素分泌肿瘤；血管生成、关节炎；同种异体抑制排斥、移植脉管出血、入口高血压(portal hypertension)、胃肠出血、肥胖、以及鸦片样物质过量。在优选的实施方式中化合物选自本文公开的化合物。在本发明这一方面的一个更优选的实施方式中所述疾病或症状是肢端肥大症。
- 10
15
20

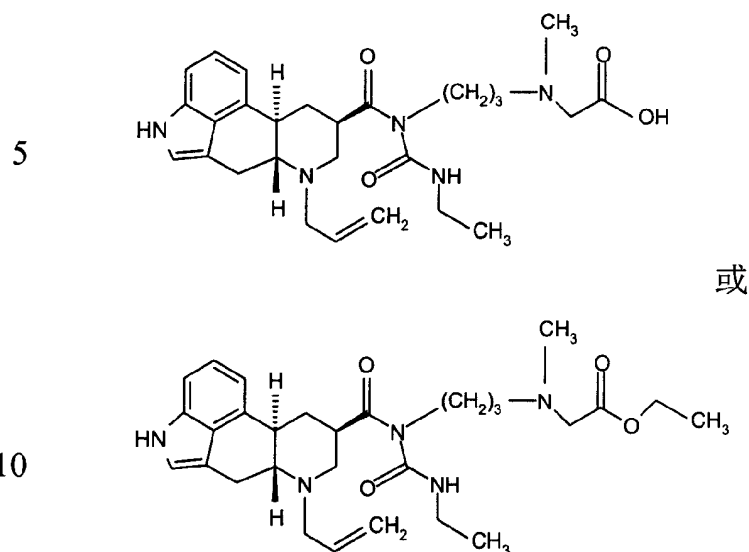
在前述方法的具体优选实施方式中，化合物选自所列的化合物 A 到 K，或选自所列的实施例 L 到 V，其题目为“生长激素释放抑制因子-多巴胺嵌合体的合成”，如下文所公开。

25

本发明另一方面的特征在于如上式(I)和式(II)化合物的多巴胺激动剂，其中生长激素释放抑制因子类似物“z”被包括下列的部分取代：-H, -OH, (C₁-C₆) 烷氧基、芳基烷氧基(如苄基、取代苄基等)、-NH₂、-NR₉R₁₀，其中 R₉ 和 R₁₀ 如式(II)中定义。在这一方面的优选实施方式中，所述多巴胺激动剂选自如本文所公开的生长激素释放抑制因子-多巴胺嵌合体的多巴胺部分，或其药

30

学上可接受的盐。在最优选的实施方式中，多巴胺激动剂是：



或其药学可接受的盐。

15 发明详述

我们相信本领域的普通技术人员根据本说明书可以完全理解本发明。因此下面的实施方式只是示例性的说明，而无论如何不是对本发明公开的剩余部分的限制。

除非特别定义，本文所用的所有的科技术语与本发明所属领域的技术人员通常理解的意思相同。所有的出版物、专利申请、专利和其他参考文献均全文选入作为参考。

已经分离出不同的生长激素释放抑制因子受体(SSTR's)，如 SSTR-1, SSTR-2, SSTR-3, SSTR-4 和 SSTR-5。因此生长激素释放抑制因子激动剂可以是一个或多个 SSTR-1 激动剂, SSTR-2 激动剂, SSTR-3 激动剂, SSTR-4 激动剂和 SSTR-5 激动剂。例如生长激素释放抑制因子 2-型受体激动剂 (即 SSTR-2 激动剂) 的意思是一种具有对 SSTR-2 具有高结合免疫亲和力 (如 K_i 小于 100nM, 或优选小于 10nm, 或更优选小于 1nM) 的化合物 (如由下面描述的受体结合测定来定义)。生长激素释放抑制因子 2-型受体选择性激动剂的意思是一种对 SSTR-2 比对其他生长激素释放抑制因子受体具有更高的结合免疫亲

和力的生长激素释放抑制因子 2-型受体激动剂。

在一个实施方式中, SSTR-2 激动剂也是一种 SSTR-2 选择性激动剂。可以用来实践本发明的 SSTR-2 激动剂的实例包括但不限于:

D-Nal-环[Cys-Tyr-D-Trp-Lys-Val-Cys]-Thr-NH₂;

5 环-[Tic-Tyr-D-Trp-Lys-Abu-Phe];

4-(2- 羟 乙 基)-1- 哌 嗪 乙 酰 基 -D-Phe- 环
(Cys-Tyr-D-Trp-Lys-Abu-Cys)-Thr-NH₂; 和

4-(2- 羟 乙 基)-1- 哌 嗪 -2- 乙 磺 酰 基 -D-Phe- 环
-(Cys-Tyr-D-Trp-Lys-Abu-Cys)-Thr-NH₂。

10 生长激素释放抑制因子激动剂的更多实例是由下列出版物特别提到的化学式所覆盖的化合物, 每篇文献均全文引入作为参考。

欧洲专利申请号 P5 164 EU(发明人: G. Keri);

Van Binst, G 等人. 肽研究 5: 8 (1992);

15 Horvath, A 等人, 摘要 “具有抗肿瘤活性的生长激素释放抑制因子类似物的确认”, 22nd European peptide Symposium, 1992 年 9 月 13-19, Interlaken, 瑞士。

PCT 申请号 WO 91/09056(1991)

欧洲专利申请号 0 363 589 A2 (1990)

美国专利号 4,904,642 (1990);

美国专利号 4,871,717 (1989);

20 美国专利号 4,853,371 (1989);

美国专利号 4,725,577 (1988);

美国专利号 4,684,620 (1987);

美国专利号 4,650,787 (1987);

美国专利号 4,603,120 (1986);

25 美国专利号 4,585,755 (1986);

欧洲专利申请号 0 203 031 A2(1986);

美国专利号 4,522,813 (1985);

美国专利号 4,486,415 (1984);

美国专利号 4,485,101 (1984);

30 美国专利号 4,435,385 (1984);

-
- 美国专利号 4,395,403 (1983);
美国专利号 4,369,179 (1983);
美国专利号 4,360,516 (1982);
美国专利号 4,358,439 (1982);
5 美国专利号 4,328,214 (1982);
美国专利号 4,316,890 (1982);
美国专利号 4,310,518 (1982);
美国专利号 4,291,022 (1981);
美国专利号 4,238,481 (1980);
10 美国专利号 4,235,866 (1980);
美国专利号 4,224,199 (1980);
美国专利号 4,211,693 (1980);
美国专利号 4,190,648 (1980);
美国专利号 4,146,612 (1979);
15 美国专利号 4,133,782 (1979);
美国专利号 5,506,339 (1996);
美国专利号 4,261,885 (1981);
美国专利号 4,728,638 (1988);
美国专利号 4,282,143 (1981);
20 美国专利号 4,215,039 (1980);
美国专利号 4,209,426 (1980);
美国专利号 4,190,575 (1980);
欧洲专利号 0 389 180 (1990);
欧洲专利申请号 0 505 680 (1982);
25 欧洲专利申请号 0 030 920 (1980);
PCT 申请号 WO 88/05052 (1988);
PCT 申请号 WO 90/12811 (1990);
PCT 申请号 WO 97/01579 (1997);
PCT 申请号 WO 91/18016 (1991);
30 英国申请号 GB 2,095,261 (1981)和

法国申请号 FR 2,522,655 (1983)。

注意，对于本文描述的所有生长激素释放抑制因子激动剂，每个氨基酸基团代表-NH-C(R)H-CO-的结构，其中 R 是侧链(例如丙氨酸上的 CH₃)。氨基酸基团之间的线代表连接氨基酸的肽键。当氨基酸基团具有光学活性时，除非明确指定 D-构型，其意思是 L-型构型。为清楚起见，在 Cys 基团的两个游离硫醇间的二硫键没有表示出来。常规氨基酸的缩写与 IUPAC-IUB 的建议相符。

生长激素释放抑制因子激动剂的合成

文献中已报道过合成肽生长激素释放抑制因子激动剂的方法，因此该类方法属于本领域的普通技术人员的能力范围之内。例如，肽是在 Rink 酰胺 MBHA 树脂(4-(2'4'-二甲氧基苯基-Fmoc-氨基甲基)-苯氧基乙酰胺基-正亮氨酸基-MBHA 树脂)上使用 Fmoc 化学的标准固相法合成的。在 N-端带有自由氨基官能团的肽-树脂用含有多巴胺部分的相应化合物处理。最终产品可以从树脂和三氟乙酸(TFA)/水/三异丙基硅烷(TIS)混合物中分离出来。

例如，H-D-Phe-Phe-Phe-D-Trp-Lys-Thr-Phe-Thr-NH₂ 可以通过欧洲专利申请 0 395 417A1 的实施例 1 所述的方法合成。带有取代的 N-端的生长激素释放抑制因子激动剂的合成可以通过下列申请所描述的方法实现：PCT 公开号 WO 88/02756, PCT 公开号 WO 94/04752 和/或欧洲专利申请号 0 329 295。

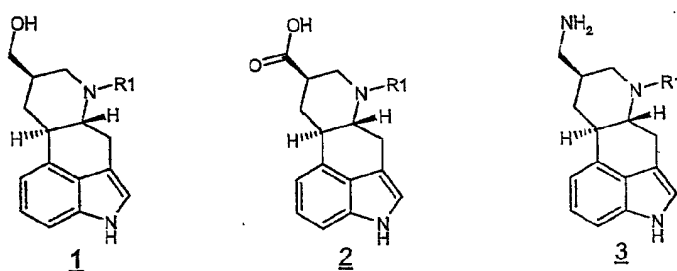
用碘的甲醇/水溶液可以使肽环化，使用乙腈-0.1%三氟乙酸/水-0.1%三氟乙酸缓冲剂使肽在 C18 反向制备高效液相色谱(HPLC)上纯化。用分析高效液相色谱和质谱来测定均一性，结果表明每种肽的均一性都大于 95%。

从下述的零售商可购买一些非常规的氨基酸：从 Chem-Impex International Inc. (Wood Dale, IL, USA) 购买 Fmoc-Doc-OH 和 Fmoc-AEPA。从 PerpSeptive Biosystems (Framingham MA, USA) 购买 Fmoc-Caeg (Bhoc)-OH。Bhoc 表示二苯甲氧羰基。

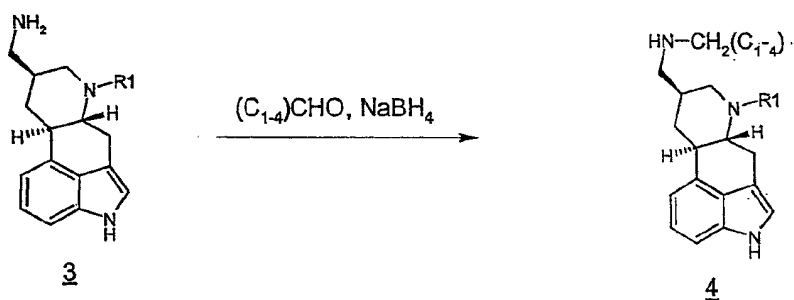
多巴胺激动剂的合成

已有文献报道过合成多种多巴胺激动剂的方法,因此合成方法属于本领域的技术人员可以实现的能力之内。如下的反应路线和实施例中公开了进一步的合成方法。

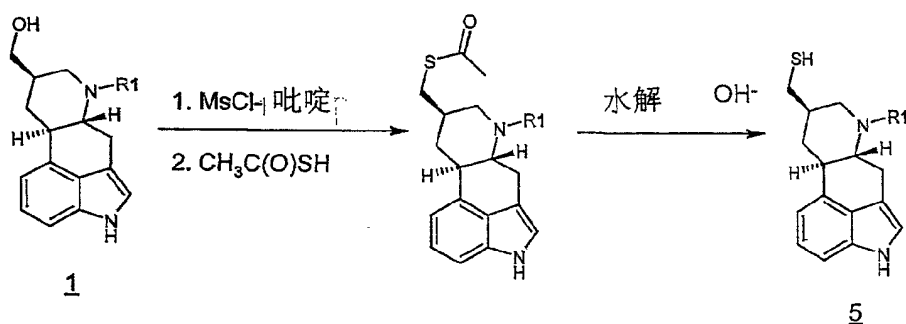
5



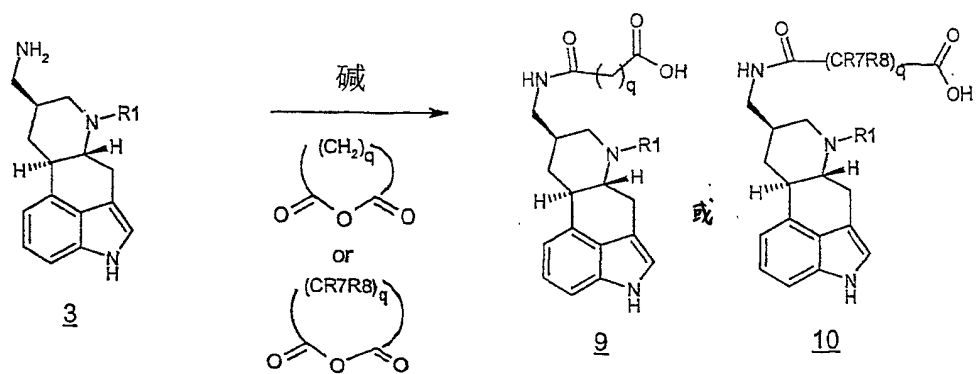
反应路线 1



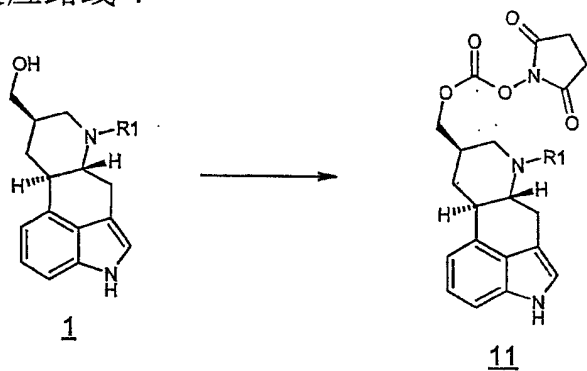
反应路线 2



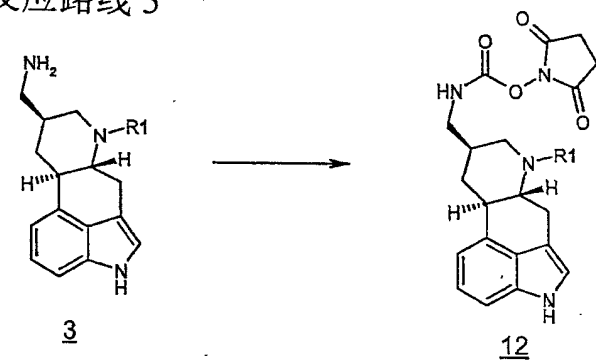
反应路线 3



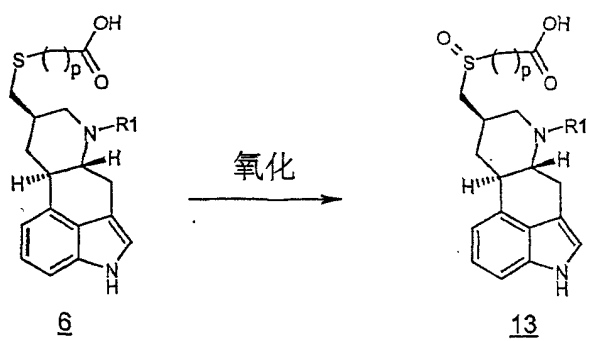
反应路线 4



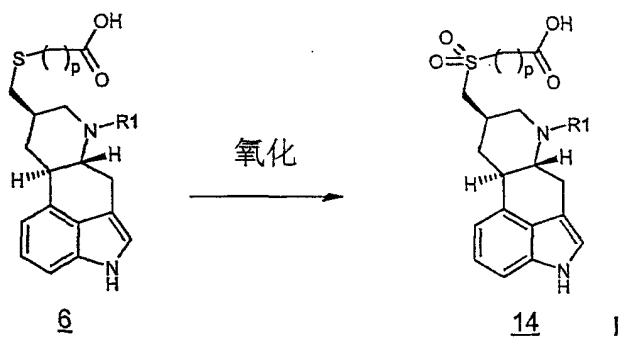
反应路线 5



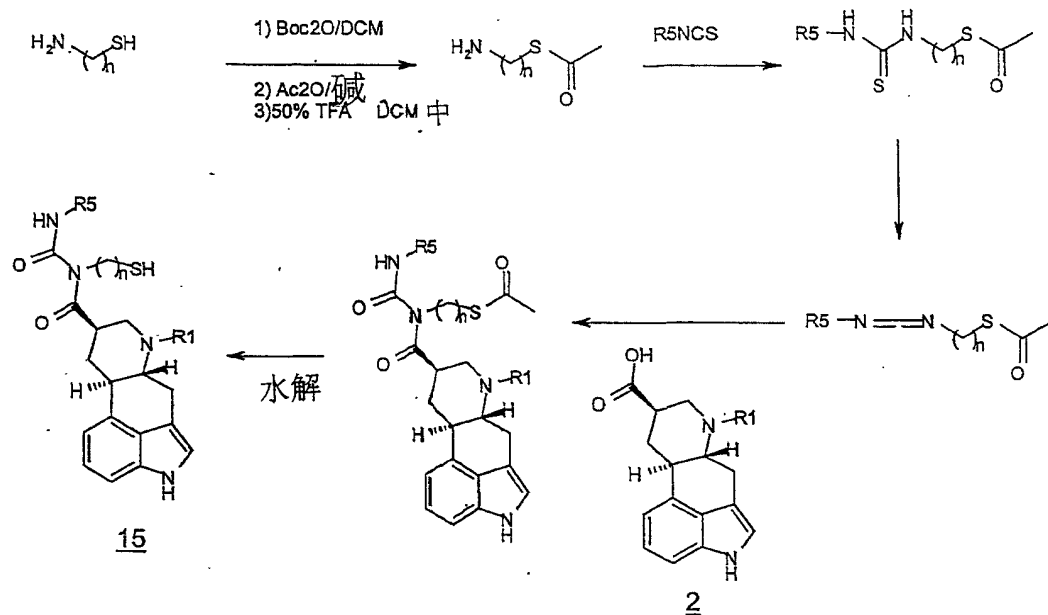
反应路线 6



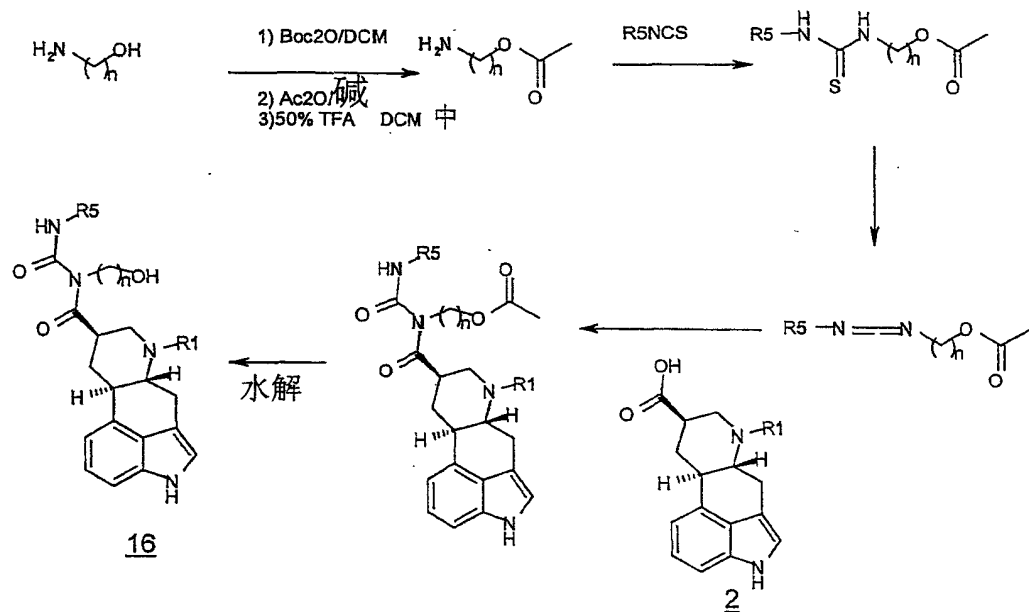
反应路线 7



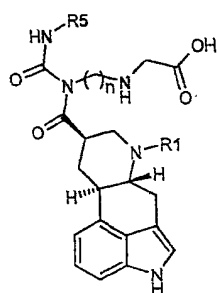
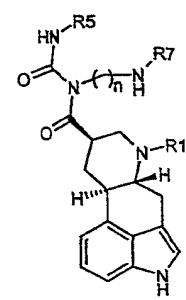
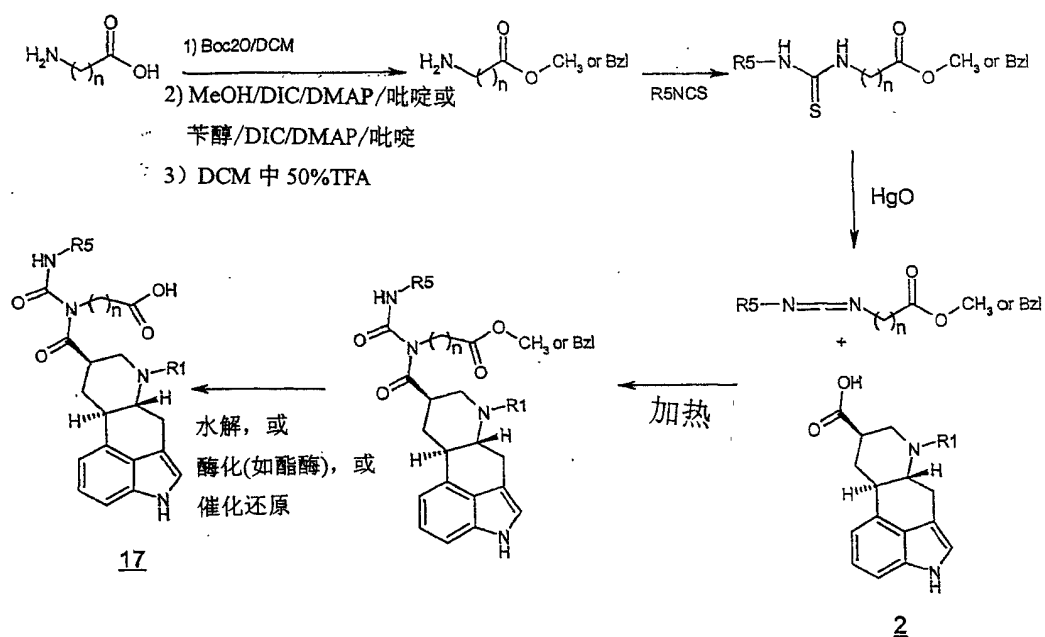
反应路线 8



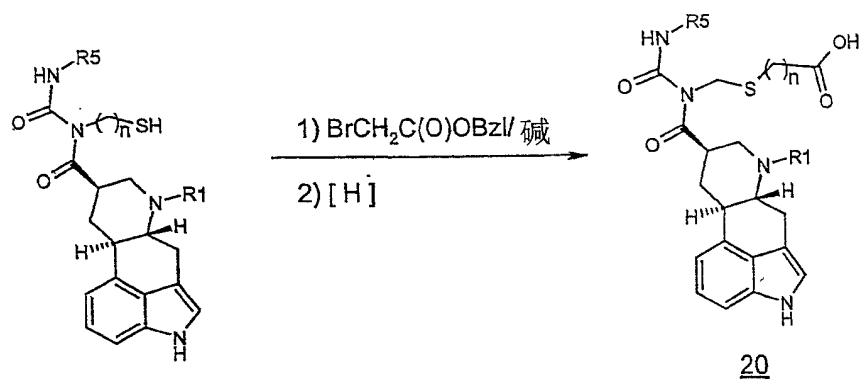
反应路线 9



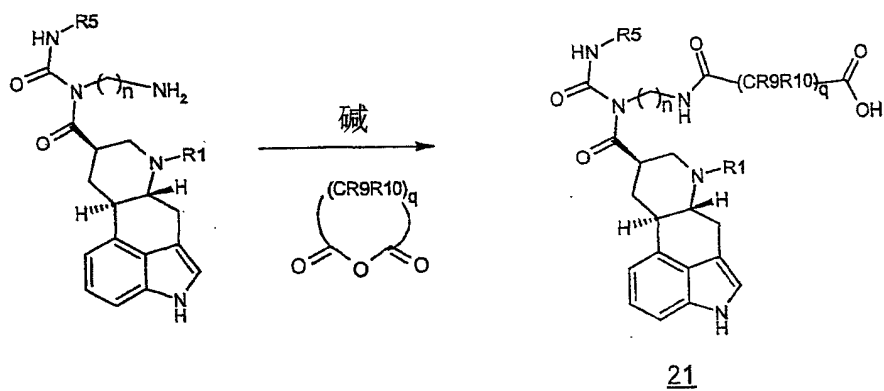
反应路线 10:



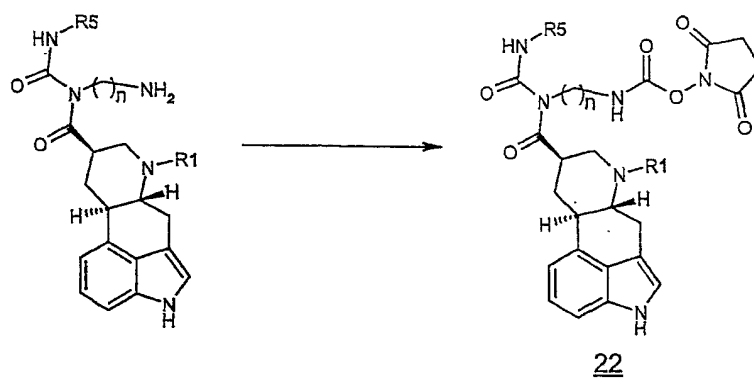
反应路线 11:



反应路线 12:

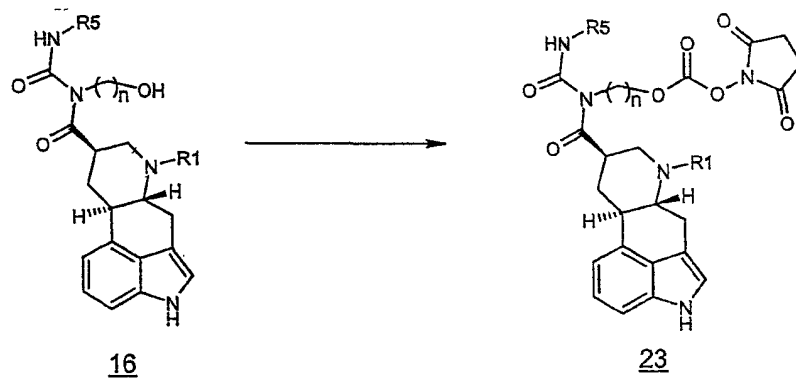


反应路线 13:

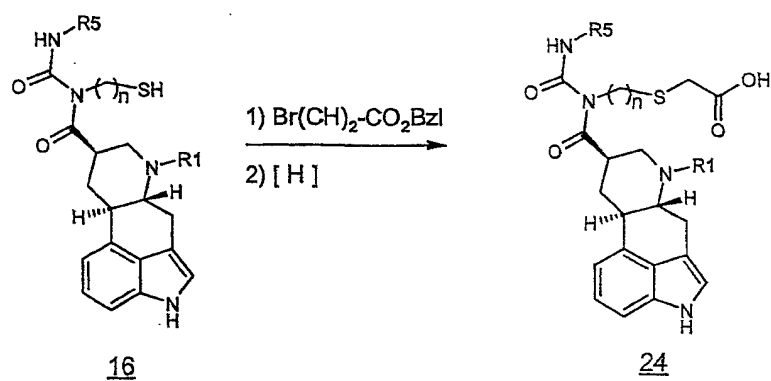


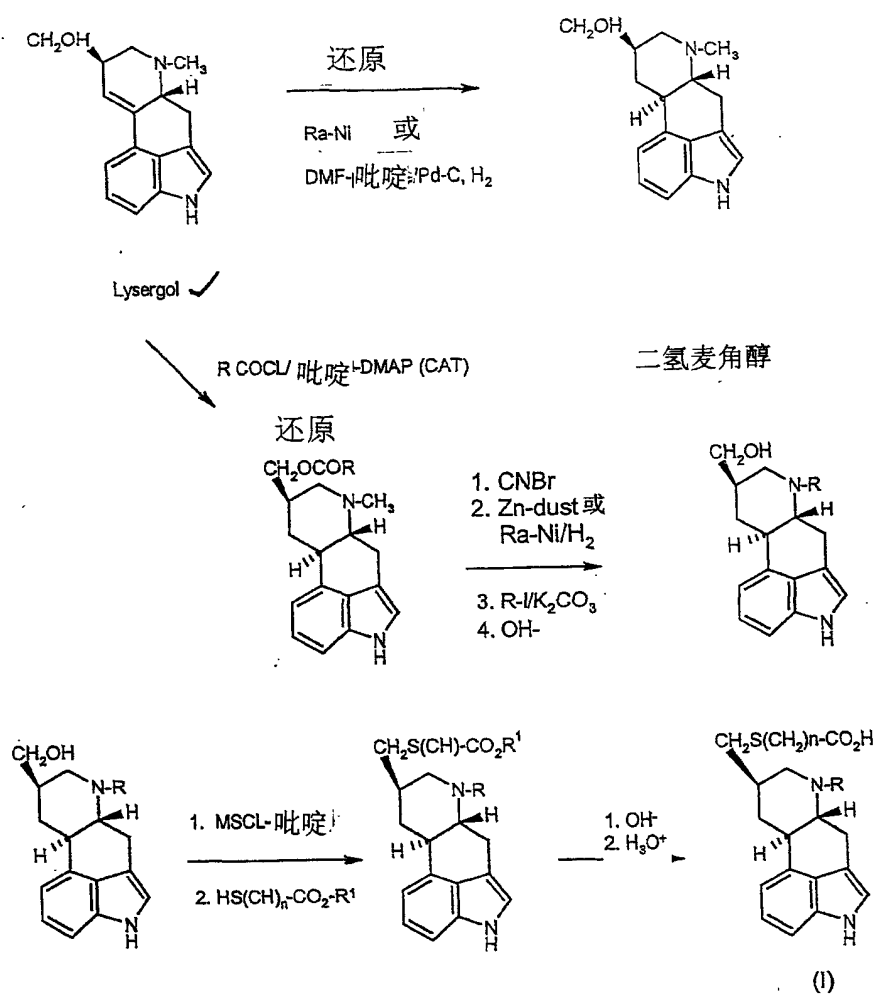
10

反应路线 14:

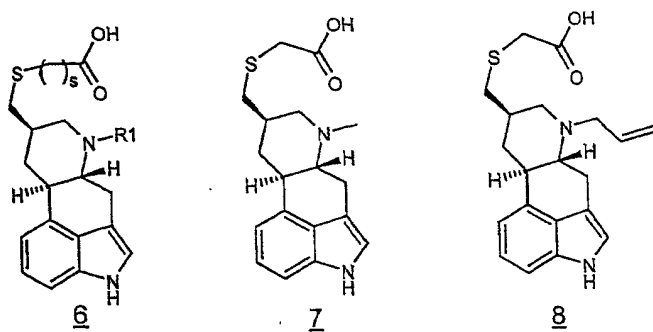


反应路线 15:

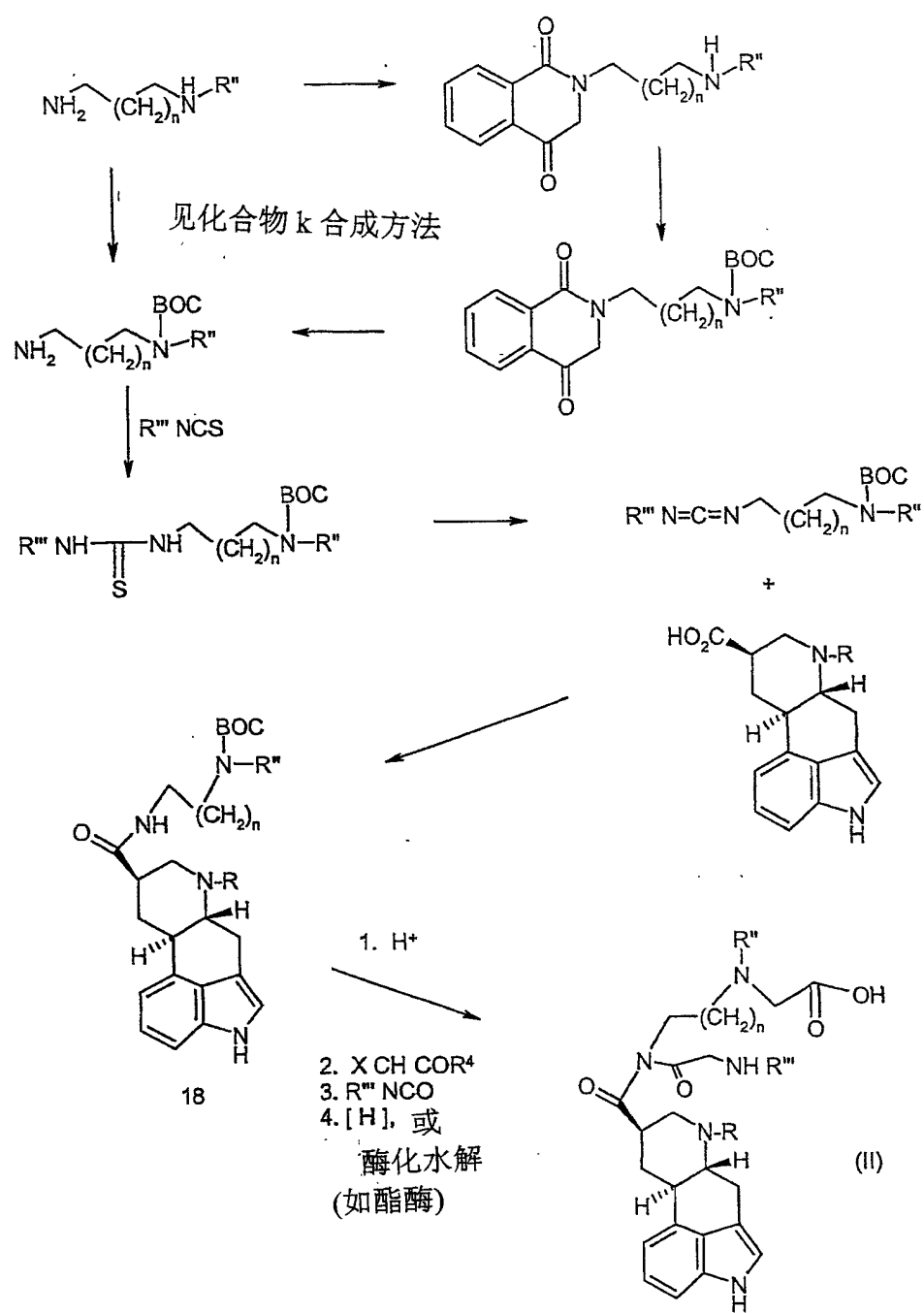




与化合物 6, 7, 8 相似 :

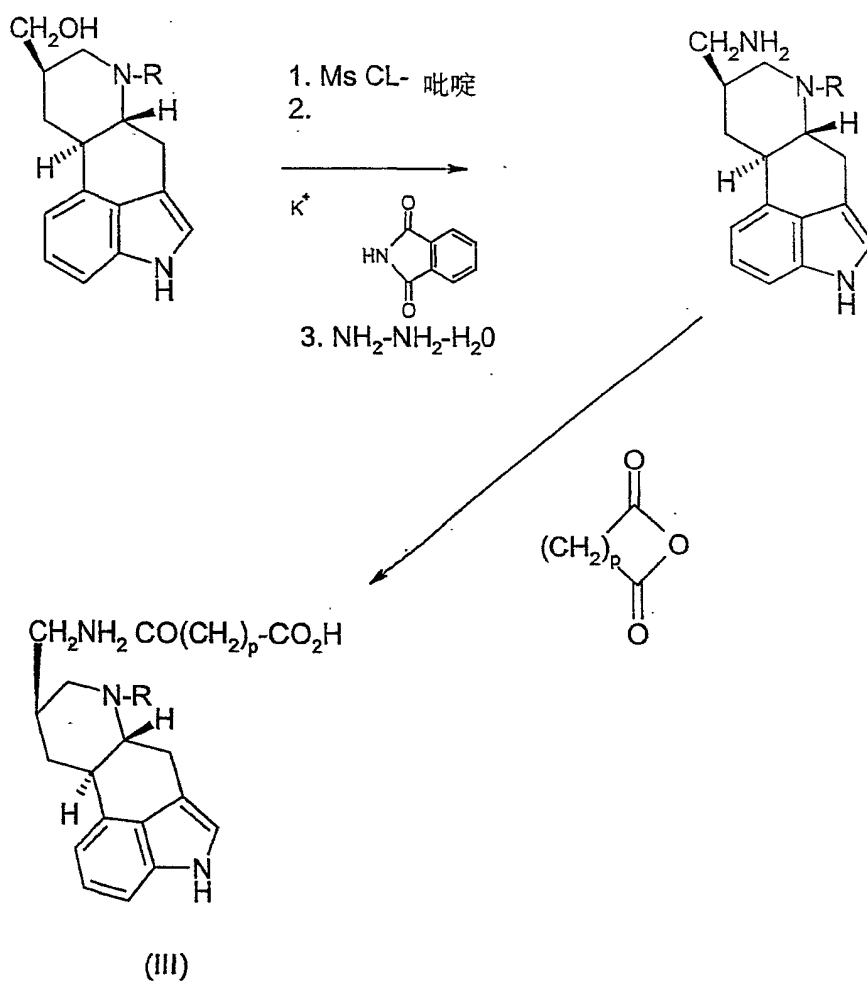


反应路线 I

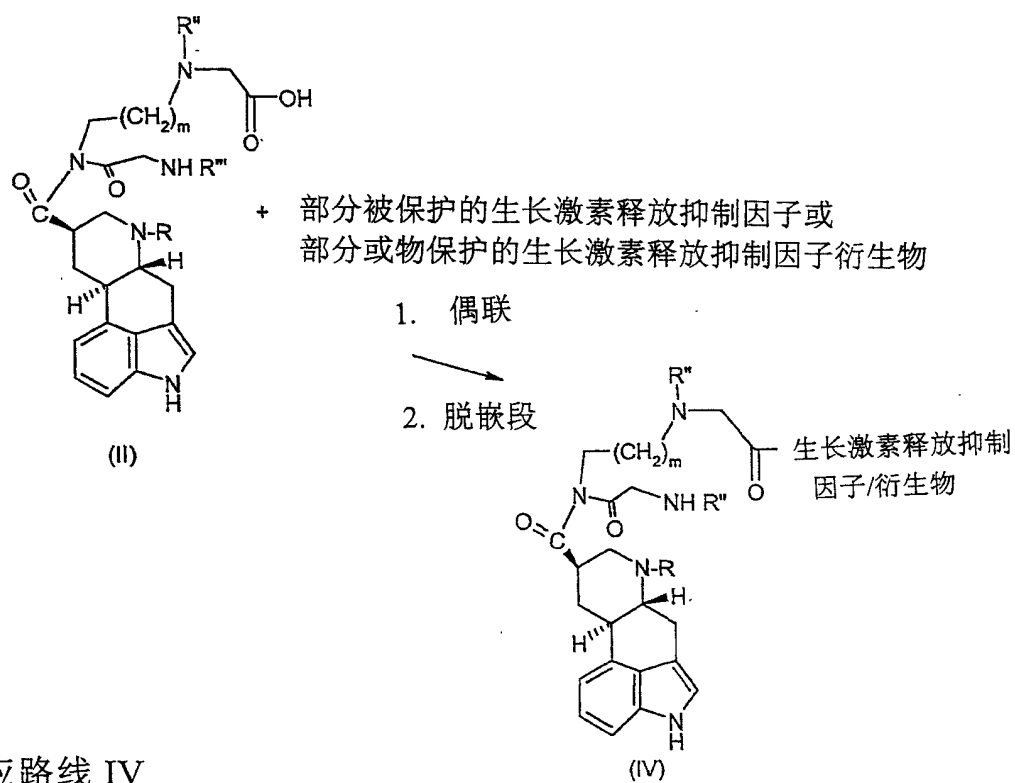
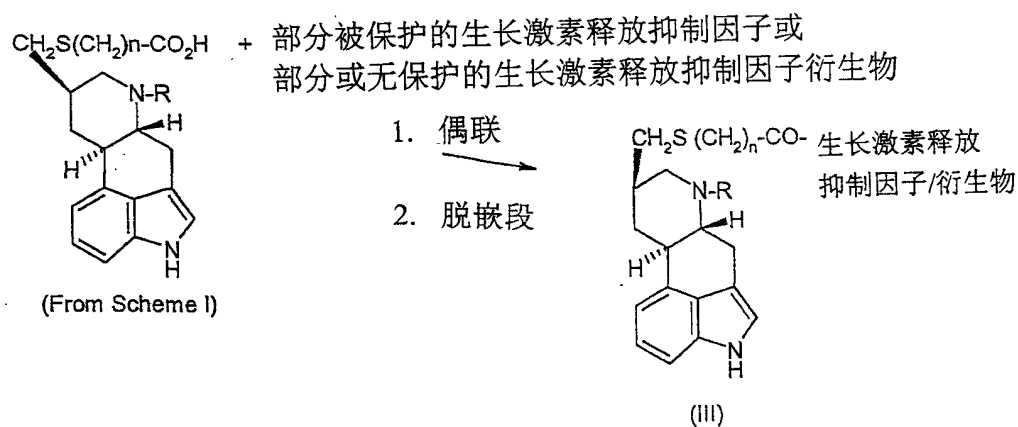


其中 R'' 和 R''' 独立地是 H 或 C_1-C_4 烷基。

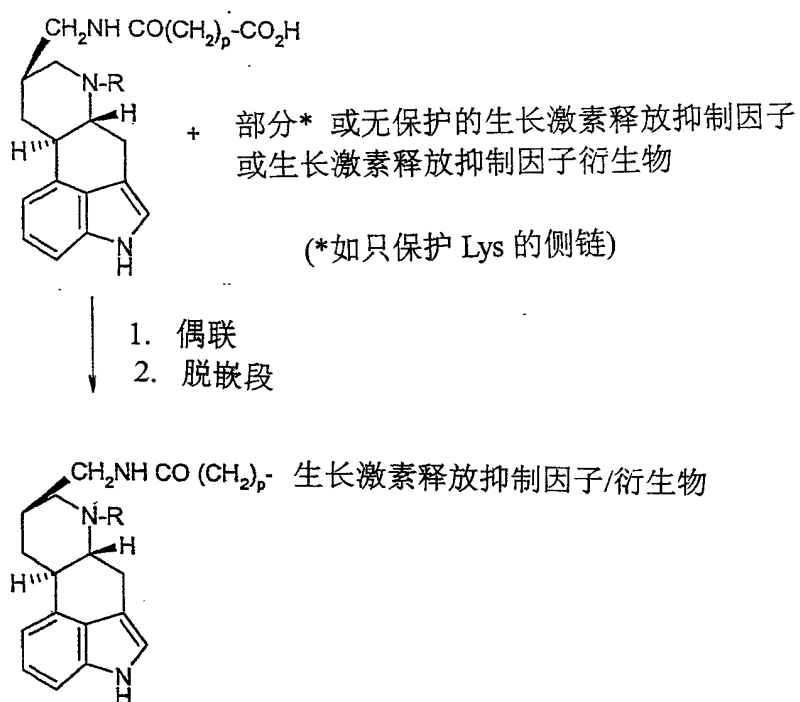
反应路线 II



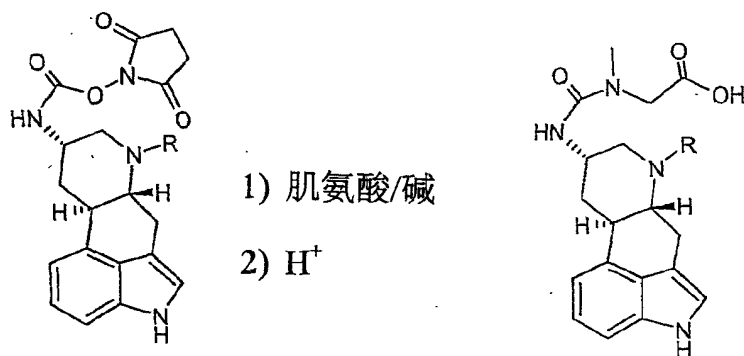
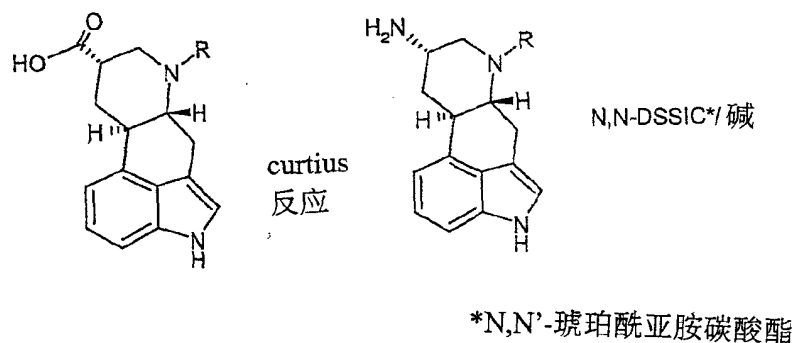
反应路线 III



反应路线 IV



反应路线 V



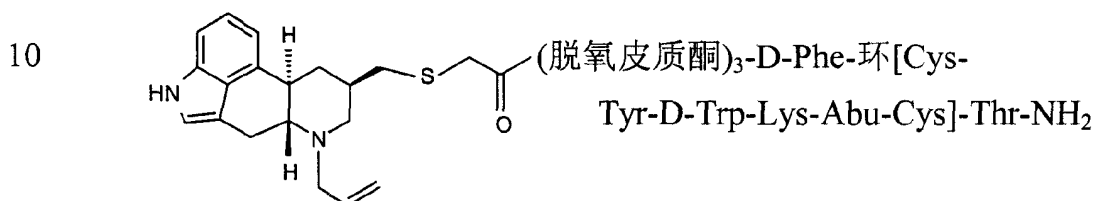
反应式 VI

25 生长激素释放抑制因子-多巴胺嵌合体的合成

生长激素释放抑制因子-多巴胺嵌合体可以根据下列反应路线和实施例来合成。在反应式 I, II 和 III 中表示的为制造化合物(I), (II)和(III)的起始原料和中间体分别可商购或由下列文献中的记载来制备: Pharmazie 39,537 (1984);

Collect Czech. Chem. Commun. 33, 577 (1966); Helv. Chim. Acta 32, 1947, (1949); USP. 5,097,031; USP 3,901,894; EP 0003667; USP 4,526,892。肽的合成是本领域的技术人员公知的,但也可以从下列文献得到,见例如 Stewart 等人,固相合成, Pierce Chemical, 2nd Ed. 1984; G.A. Grant; 合成肽. WH., Freenand Co., New York, 1992; M. Bodenszky A. Bodanszky, 肽合成的实践. Spring Venlag. N. Y. 1984.

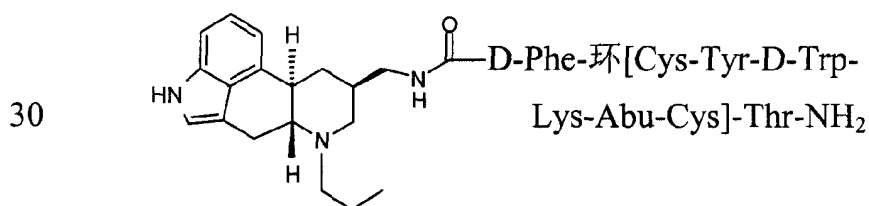
化合物 A 的制备:



将化合物 8 (3 eq.)与 H-(脱氧皮质酮)₃-D-Phe-Cys(丙烯酰胺)-Tyr(叔丁基)-D-Trp(Boc)-Lys(Boc)-Abu-Cys(丙烯酰胺)-Thr(叔丁基)-Rink 酰胺 MBHA 树脂(1 eq.), HBTU(2.9 eq.), HOBt(3.0 eq)和 DIEA (6 eq.)在二甲基甲酰胺(DMF)中混合。将混合物在室温下震荡 4 小时。用二甲基甲酰胺(DMF)和二氯甲烷(DCM)冲洗树脂,并在减压下干燥。将干燥的树脂用 TFA/TIS/水 (92/5/3, v/v)在室温下处理 1 小时。将溶液过滤并浓缩。在浓缩液中加入冷的乙醚,收集沉淀物将其溶解在水-甲醇溶剂体系中。向溶液中加入溶于甲醇的碘溶液直至出现棕色。然后将溶液在室温下静置 1 小时。再向溶液中加入含水 Na₂S₂O₃ 溶液直至棕色消失。用 C18 反相制备性高效液相色谱纯化所得到的溶液,以线性梯度的缓冲液 A (1%TFA 水溶液)/缓冲液 B(1%TFA 的 CH₃CN 溶液)进行洗脱。用分析高效液相色谱检测馏分。合并含有所需要的纯化合物,并冷冻至干燥。

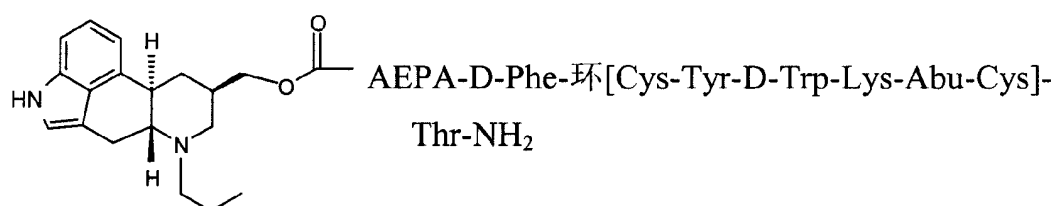
25 化合物的分子量用配有电雾化源的 MS 测定。

化合物 B 的制备:



- 将化合物 12(其中 R1 是正丙基(1.5eq))与 H-D-Phe-Cys(丙烯酰胺)-Tyr(叔丁基)-D-Trp(Boc)-Lys(Boc)-Abu-Cys(丙烯酰胺)-Thr(叔丁基)-Rink 酰胺 MBHA 树脂(1 eq)和 DIEA (2 eq)在二甲基甲酰胺(DMF)中混合。将混合物在室温下震荡 5 小时。用二甲基甲酰胺(DMF)和二氯甲烷(DCM)冲洗树脂并在减压下干燥。用 TFA/TIS/水 (92/5/3, v/v) 在室温下处理干燥的树脂达 1 小时。将溶液过滤和浓缩。在浓缩液中加入冷的乙醚, 收集沉淀物将其溶解在水-甲醇溶剂体系中。向溶液中加入溶于甲醇中的碘溶液直至出现棕色。然后将溶液在室温下静置 1 小时。再向溶液中加入 $\text{Na}_2\text{S}_2\text{O}_3$ 溶液直至棕色消失。用 C18 反相制备性高效液相色谱纯化所得到的溶液, 以线性梯度的缓冲液 A (1%TFA 水溶液)/缓冲液 B(1%TFA 的 CH_3CN 溶液)进行洗脱。用分析高效液相色谱检测馏分。合并含有所需要的纯化合物并冷冻至干燥。化合物的分子量用配有电雾化源的 MS 测定。

15 化合物 C 的制备:

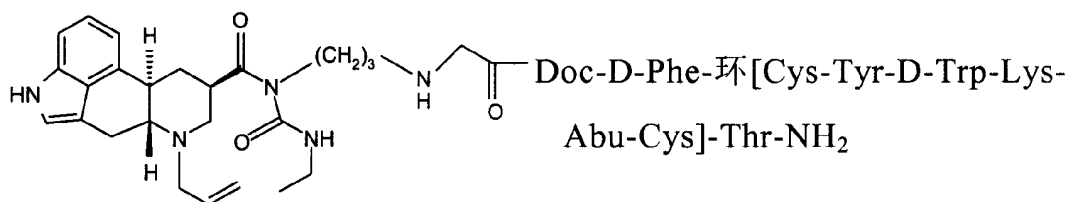


- 20 将其中 R1 是正丙基(1.5 eq)的化合物 11 与 H-AEPA-D-Phe-Cys(丙烯酰胺)-Tyr(叔丁基)-D-Trp(Boc)-Lys(Boc)-Abu-Cys(丙烯酰胺)-Thr(叔丁基)Rink 酰胺 MBHA 树脂(1 eq)和 DIEA (2 eq)在二甲基甲酰胺(DMF)中混合。将混合物在室温下震荡 5 小时。用二甲基甲酰胺(DMF)和二氯甲烷(DCM)冲洗树脂并在减压下干燥。用 TFA/TIS/水 (92/5/3, v/v) 在室温下处理干燥的树脂达 1 小时。将溶液过滤并浓缩。在浓缩液中加入冷的乙醚, 收集沉淀物将其溶解在水-甲醇溶剂体系中。向溶液中加入溶于甲醇的碘溶液直至出现棕色。然后将溶液在室温下静置 1 小时。再向溶液中加入 $\text{Na}_2\text{S}_2\text{O}_3$ 溶液直至棕色消失。用 C18 反相制备性高效液相色谱纯化所得到的溶液, 以线性梯度的缓冲液 A (1%TFA 水溶液)/缓冲液 B(1%TFA 的 CH_3CN 溶液)进行洗脱。用分析高效液相色谱检

测馏分。合并含有所需要的纯化合物并冷冻至干燥。化合物的分子量用配有电雾化源的 MS 测定。

5

化合物 D 的制备:



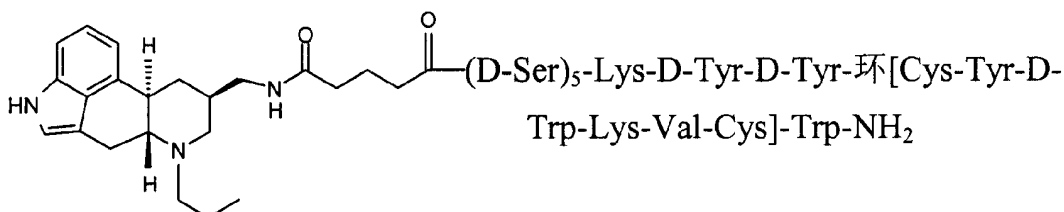
10

将化合物 25 (3 eq) 与 H-Doc-D-Phe-Cys(丙烯酰胺)-Tyr(叔丁基)-D-Trp(Boc)-Lys(Boc)-Abu-Cys(丙烯酰胺)-Thr(叔丁基)Rink 酰胺 MBHA 树脂(1 eq)、HBTU(2.9 eq), HOBT(3.0 eq)和 DIEA (6 eq)在二甲基甲酰胺中混合。将混合物在室温下震荡 4 小时。用二甲基甲酰胺(DMF)和二氯甲烷(DCM)冲洗树脂并在减压下干燥。用 TFA/TIS/水 (92/5/3, v/v) 在室温下处理干燥的树脂达 1 小时。将溶液过滤并浓缩。在浓缩液中加入冷的乙醚, 收集沉淀物将其溶解在水-甲醇溶剂体系中。向溶液中加入溶于甲醇的碘溶液直至出现棕色。然后将溶液在室温下静置 1 小时。再向溶液中加入 Na₂S₂O₃ 溶液直至棕色消失。用 C18 反相制备性高效液相色谱纯化所得到的溶液, 以线性梯度的缓冲液 A (1%TFA 水溶液)/缓冲液 B(1%TFA 的 CH₃CN 溶液)进行洗脱。用分析高效液相色谱检查馏分。合并含有所需要的纯化合物并冷冻至干燥。化合物的分子量用配有电雾化源的 MS 测定。

15

20

化合物 E 的制备:



25

将化合物 26(3 eq) 与 H-(D-Ser(叔丁基))₅-Lys(Boc)-D-Tyr(叔丁基)

- 基)-D-Tyr(叔丁基)-Tyr(叔丁基)-D-Trp(Boc)-Lys(Boc)-Val-Cys(丙烯酰胺)-Trp(Boc)-Rink 酰胺 MBHA 树脂(1 eq)和 HBTU(2.9 eq), HOBT(3.0 eq)和 DIEA (6 eq)在二甲基甲酰胺(DMF)中混合。将混合物在室温下震荡 4 小时。用二甲基甲酰胺(DMF)和二氯甲烷(DMC)冲洗树脂并在减压下干燥。用 TFA/TIS/
- 5 水 (92/5/3, v/v) 在室温下处理干燥的树脂达 1 小时。将溶液过滤并浓缩。在浓缩液中加入冷的乙醚, 收集沉淀物将其溶解在水-甲醇溶剂体系中。向溶液中加入溶于甲醇的碘溶液直至出现棕色。然后将溶液在室温下静置 1 小时。再向溶液中加入 $\text{Na}_2\text{S}_2\text{O}_3$ 溶液直至棕色消失。用 C18 反相制备性高效液相色谱纯化所得到的溶液, 以线性梯度的缓冲液 A (1%TFA 水溶液)/缓冲液 B(1%TFA 的 CH_3CN 溶液)进行洗脱。用分析高效液相色谱检测馏分。合并含有所需要的纯化合物并冷冻至干燥。化合物的分子量用配有电雾化源的 MS 测定。

化合物 F 的制备:

乙基-[6-甲基-8 β -麦角灵基甲基(ergolinylmethyl)]硫代乙酸酯

- 15 在溶有 240mg 二氢麦角醇(dihydroLySergol)的 10ml 吡啶溶液中加入 250 μl 甲磺酰氯。在室温下搅拌 2 小时后, 将反应混合物倒入 100ml 水中, 用氯仿($2 \times 20\text{ml}$)抽提, 用水洗涤有机层, 然后用 MgSO_4 干燥, 在真空下除去溶剂至干燥, 得到 140mg 浅棕色固体。用 NaHCO_3 碱性化后再一次从溶液中抽提, 得到另外的 100mg 产品。共 240mg。质谱(电雾化)335.2。
- 20 向上述 D-6-甲基-8 β -甲磺酰基羟甲基-麦角灵 (140mg)在 3ml 二甲基甲酰胺所形成的溶液中加入粉末 K_2CO_3 (150mg), 然后加入 150 μl 乙基-2-巯基乙酸酯, 将混合物在氮气下在 40 $^\circ\text{C}$ 下加热 2 小时。在真空下除去溶剂至干燥, 剩余物在氯仿和水之间进行分配, 用 MgSO_4 干燥有机层, 蒸发掉溶剂后, 剩余物用氯仿/甲醇(9:1)作为展开剂流过制备性硅胶薄层色谱, 分离出相应的部分,
- 25 用氯仿-甲醇抽提后, 在真空下除去溶剂至干燥。得到浅棕色固体 100mg。质谱(电雾化)359.2。

化合物 G 的制备:

- 6-甲基-8 β -麦角灵基甲基硫代乙酰基-D-Phe-环
- 30 (Cys-Tyr-D-Trp-Lys-Abu-Cys)-Thr- NH_2

向 6-甲基-8 β -麦角灵基甲基硫代乙酸(反应路线 I, 化合物 7)和用 Fmoc-化学法由固相合成制备的 D-Phe-环(Cys-Tyr(OBT)-D-Trp-Lys(BOC)-Abu-Cys)-Thr-NH₂(100mg)在 100ml 二甲基甲酰胺的溶液中加入 200mg EDC (1-[3-(二甲氨基)-丙基]-3-乙基碳二亚胺-HCL), 100mg HOAT (1-羟基-7-氮杂苯并三唑(azabenzotriazole)), 然后加入 200 μ l 二异丙基乙胺, 将混合物在室温下搅拌过夜。在真空下除去挥发性物质至干燥, 剩余物在氯代甲醇和溴之间进行分配。用 NaHCO₃ 水溶液洗涤有机物层, 用 MgSO₄ 干燥。溶剂蒸发后, 剩余物用氯仿-甲醇(85:15)做为展开剂流过制备性薄层色谱。分离出相应的部分, 用氯仿-甲醇抽提, 在真空下除去溶剂, 得到 40mg 被保护的产品。质谱(电雾化)1500.7。

将被保护的产品用 30%的三氟乙酸在含有几滴三异丙基硅烷的二氯甲烷(10ml)中处理 30 分钟, 在真空下除去挥发性组分至干燥, 剩余物用 vydac C18 高效液相色谱和 CH₃CN/0.1%TFA 水溶液纯化, 得到 17mg 白色固体。质谱(电雾化). 1344.8, 673.2。

15

化合物 H 的制备:

乙基-(6-正丙基-8 β -麦角灵基)甲基硫代乙酸酯

从 D-正丙基-8 β -羟甲基麦角灵开始(其制备方法见 EP 000,667), 用类似于化合物 F 的制备方法制备得到浅黄色固体。质谱(电雾化)387.2。

20

化合物 I 的制备

6-正丙基-8 β -麦角灵基甲基硫代乙酰基-D-Phe-环(Cys-Tyr-D-Trp-Lys-Abu-Cys)-Thr-NH₂

从 6-正丙基-8 β -麦角灵基甲基硫代乙酸 (反应路线 I, 化合物 6, 其中 R1 是丙基且 s=1)和 D-Phe-环[Cys-Tyr (OBT)-D-Trp-Lys(BOC)- Abu-Cys]-Thr-NH₂ 开始, 用与化合物 G 的制备方法类似的方法制备得到白色固体。质谱(电雾化)1372.5, 687.3。

化合物 J 的制备:

6-D-甲基-8 β -麦角灵基甲基硫基氨基琥珀酰基

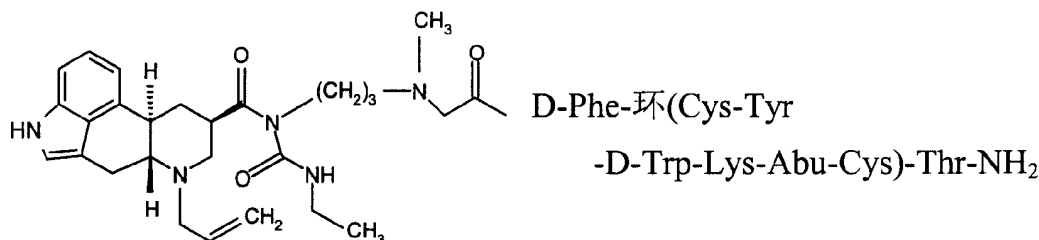
(ergolinylmethylthlaminosuccinoyl)-D-Phe-环[Cys-Tyr-D-Trp-Lys
-Abu-Cys]-Thr-NH₂。

从 6-D- 甲基 -8 β - 琥珀酰氨基麦角灵和 D-Phe- 环
[Cys-Tyr(OBT)-D-Trp-Lys(BOC)-Abu-Cys]-Thr-NH₂ 开始, 以化合物 G 的制备
5 方法相类似的方法制备得到白色固体。质谱(电雾化)1344.8, 673.2。

化合物 K 的制备:

6-烯丙基-8 β -(1-乙基-(3-N-甲基-3-羰甲基)氨基丙基-脲基羰基-麦角灵
-D-Phe-环(Cys-Tyr-D-Trp-Lys-Abu-Cys)-Thr-NH₂, 即下列结构式的化合物

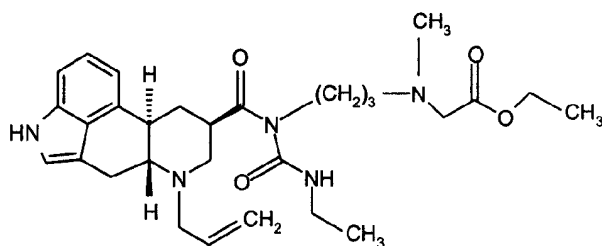
10



15

A. 1-[[6-烯丙基麦角灵-8 β -基]羰基]-1-[3-(N-(乙氧基羰基)甲基,N-甲基)氨基-丙基]-3-乙脲, 即下式化合物

20



(1) 3,3-BOC, N-甲基丙二胺

向 3N-甲基丙二胺(1.8g)的二氯甲烷 (30ml)溶液加入无水 MgSO₄(5.5gm),
25 然后加入苯甲醛(2.3g), 将混合物在室温下搅拌过夜。过滤后, 滤出物用
(BOC)₂(4.3g)和 DMAP (0.35g) 处理并搅拌约 1 小时。然后用 5%柠檬酸水溶液
洗涤混合物, 然后用 MgSO₄ 干燥。

溶剂蒸发后, 将剩余物溶解在乙醇中(50ml), 然后加入 Pd(OH)₂ (600mg),
乙酸(1ml)和环己烯(3ml), 整夜进行加氢反应。将混合物用硅藻土衬片过滤,
30 滤出物在真空下蒸发至干燥, 得到 2.3g 3,3-BOC, N-甲基丙二胺无色液体。质

谱(电雾化)=189.1

(2) 6-烯丙基-8 β -(3,3-BOC,N-甲基-氨基丙基-氨基甲酰基-麦角灵

向 6-烯丙基-二氢麦角酸(dihydroLySeric acid)(150mg)(由 EP 0 003667 公开的方法制备)和 3,3-BOC,N-甲基-丙二胺(150MG)在二甲基甲酰胺(DMF)(5ml)中形成的溶液中加入二异丙基乙胺(175 μ l), 然后加入二乙基氰基膦酸酯(150 μ l), 将混合物在室温下搅拌过夜。在真空下除去挥发性物质至干燥, 剩余物在 CH₃Cl 和水之间进行分配。然后用 NaHCO₃ 水溶液洗涤有机层, 用 MgSO₄ 干燥, 在真空下除去溶剂, 得到 6-烯丙基-8 β -(3,3-BOC,N-甲基-氨基丙基-氨基甲酰基)-麦角灵。

10 (3) 6-烯丙基-8 β -(3-N-甲基-氨基丙基-氨基甲酰基-麦角灵, TFA 盐

将上一步骤得到的 6-烯丙基-8 β -(3,3-BOC,N-甲基-氨基丙基-氨基甲酰基)-麦角灵用 30%TFA 的二氯甲烷溶液洗涤 30 分钟, 在真空下除去挥发性物质至干燥, 得到 250mg 6-烯丙基-8 β -(3-N-甲基-氨基丙基-氨基甲酰基)-麦角灵, TFA 盐。质谱(电雾化)=367.2.

15 (4) 6-烯丙基-8 β -(3-N-甲基,3-乙酯基甲基)-氨基丙基-氨基甲酰基-麦角灵

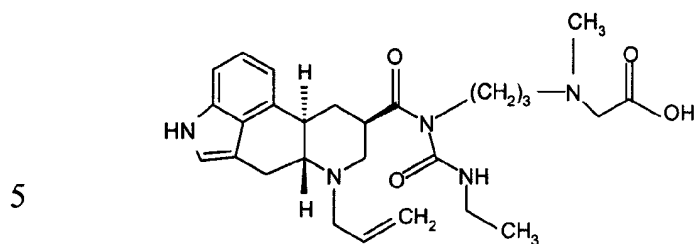
向 6-烯丙基-8 β -(3-N-甲基-氨基丙基-氨基甲酰基)-麦角灵 TFA 盐(250mg)和 K₂CO₃(140mg)在二甲基甲酰胺(DMF)(5ml)的溶液中加入溴代乙酸乙酯(70 μ l), 将得到的混合物在室温下搅拌过夜。蒸发掉溶剂后, 剩余物在氯仿和水之间进行分配。用 MgSO₄ 干燥有机层, 然后在真空下除去溶剂, 得到粗 6-烯丙基-8 β -(3-N-甲基,3-乙酯基甲基)-氨基丙基-氨基甲酰基-麦角灵(240mg)。质谱(电雾化)=453.2

(5) 6-烯丙基-8 β -(1-乙基-(3-N-甲基-3-乙酯基甲基)-氨基丙基-脲基羰基-麦角灵

将上一步骤得到的 6-烯丙基-8 β -(3-N-甲基,3-乙酯基甲基)-氨基丙基-氨基甲酰基-麦角灵溶解在甲苯(10 ml)中, 然后加入异氰酸乙酯(3 ml)。将混合物在氮气下回流 3 天, 蒸发掉挥发性物质后, 剩余物以氯仿/甲醇为展开剂通过制备性硅胶色谱。用氯仿/甲醇抽提适当的组分, 然后在真空下除去溶剂, 得到 6-烯丙基-8 β -(1-乙基-(3-N-甲基 3-乙酯基甲基)-氨基丙基-脲基羰基-麦角灵, 其是浅黄色粘性物质(30mg)。质谱(电雾化)=524.3.

30 B. 6-烯丙基-8 β -(1-乙基-(3-N-甲基-3-羧甲基)-氨基丙基-脲基羰基-麦角

灵，即下式化合物：

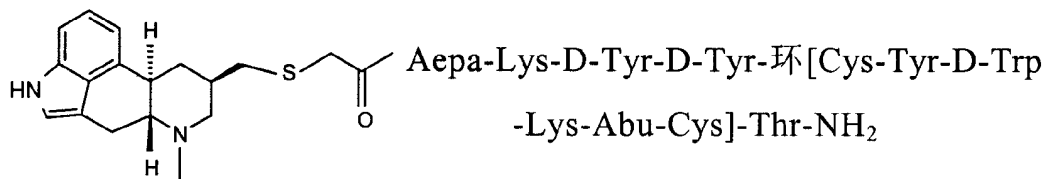


向 6-烯丙基-8β-(1-乙基-(3-N-甲基-3-羧甲基)-氨基丙基-脲基羰基-麦角灵(520mg)在 10ml 丙酮中的混合物中加入 15ml 0.2M 磷酸盐缓冲液(pH=约 7)和 0.6ml ChiroCLEC-BL (Altus Biologics, Cambridge, MA)。将混合物在约 40
10 °C下在旋转式混合器中温育过夜。用 5%柠檬酸水溶液酸化混合物，然后用 CHCl₃-甲醇抽提。干燥有机抽提物，在真空下除去溶剂，得到 6-烯丙基-8β-(1-乙基-(3-N-甲基-3-羧甲基)-氨基丙基-脲基羰基-麦角灵。

C. 6-烯丙基-8β-(1-乙基-(3-N-甲基-3-羧甲基)-氨基丙基-脲基羰基-麦角灵
15 -D-Phe-环(Cys-Tyr-Trp-Lys-Abu-Cys)-Thr-NH₂，即化合物 K

向 6-烯丙基-8β-(1-乙基-(3-N-甲基-3-羧甲基)-氨基丙基-脲基羰基-麦角灵(50mg)和 D-Phe-环(Cys-Tyr-D-Trp-Lys(FMOC)-Abu-Cys)-Thr-NH₂(100mg,固相合成制备)在二甲基甲酰胺(10ml)的溶液中加入 200mg EDC (1-[3-(二甲氨基)-丙基]-3-乙基碳二亚胺-HCl)，100mg HOAT (1-羟基-7-氮杂苯并三唑)，然后加入
20 200 μl 二异丙基乙胺，将混合物在室温下搅拌过夜，在真空下除去挥发性物质至干燥。剩余物在氯仿甲醇和溴之间进行分配。用 NaHCO₃ 水溶液洗涤有机层，然后用 MgSO₄ 干燥。溶剂蒸发后，将被保护的产品用溶于二甲基甲酰胺(10 ml)中的 5%哌啶溶液处理 30 分钟。在真空下除去挥发性物质直至很小的体积(约 2 ml)。用 VYDAC C₁₈ HPLC 和 CH₃CN /0.1% TFA 水溶液纯化，得到
25 纯的，脱保护的产品。

实施例 L

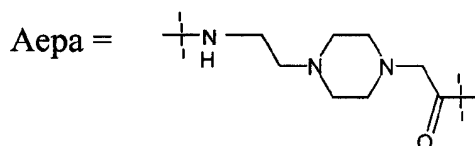


5

A. H-Aepa-Lys(Boc)-D-Tyr(叔丁基)-Cys(trt)-Tyr(叔丁基)-D-Trp(Boc)-Lys(Boc)-Abu-Cys(trt)-Thr(叔丁基)-Rink 酰胺 MBHA 树脂

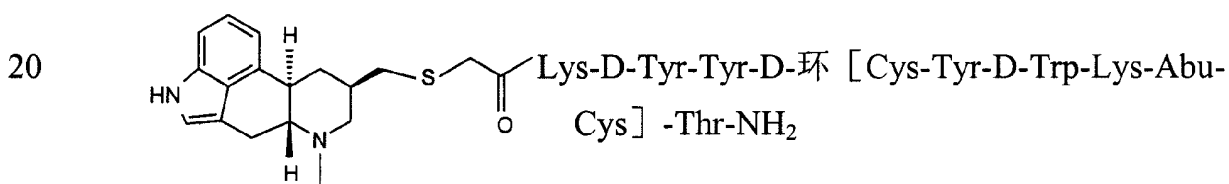
采茆基甲氧基羰基(Fmoc)化学, 在应用生物系统(Applied Biosystems) (Foster City, CA)模型 433A 肽合成器上可以自动合成被保护的肽-树脂。使用
 10 取代度为 0.72 mmol/g 的 Rink 酰胺 MBHA 树脂 (Novabiochem., San Diego, CA)。Fmoc 氨基酸(AnaSpec, San Jose, CA)用于下列的侧链保护: Fmoc-Thr(叔丁基)-OH, Fmoc-Cys(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-D-Trp(Boc)-OH, Fmoc-Tyr(叔丁基)-OH, Fmoc-D-Tyr(叔丁基)-OH, Fmoc-Phe-OH, Fmoc-Cys(Trt)-OH, Fmoc-Thr(叔丁基)-OH 和 Fmoc-Abu-OH。Fmoc-Aepa-OH
 15 从 Chem-Impex InternatioNal, Inc. (Wood Dale, IL)购买。合成在 0.25mmol 规模上进行。通过用浓度为 20%的哌啶的 N-甲基吡咯烷酮(NMP)溶液处理 30 分钟除去 Fmoc 基团。在每个连接步骤中, Fmoc 氨基酸 (4 eq, 1mmol)在 2ml 0.45M 的六氟磷酸化 2-(1-H-苯并三唑-1-基)-1,1,2,3-四甲基脲盐 (tetramethyluronium)/1-羟基-苯并三唑(HBTU/UOBT)在 N,N-二甲基甲酰胺 (DMF)溶液中预活化。将该活化的氨基酸酯, 1ml 二异丙基乙胺(DIEA)和 1ml
 20 NMP 加入到树脂中。AB1 433A 肽合成器被编程以实现下列反应循环: (1) 用 NMP 洗涤, (2)用溶于 NMP 中的 20%哌啶除去 Fmoc 保护基团达 30 分钟, (3) 用 NMP 洗涤, (4)与预活化的 Fmoc 氨基酸偶联达 1 小时。树脂被依次偶联。肽链组装后, 除去 Fmoc, 用 DMF 和二氯甲烷(DCM)彻底洗涤。

25 MBHA=4-甲基二苯甲胺(Methylbenzylhydramine)

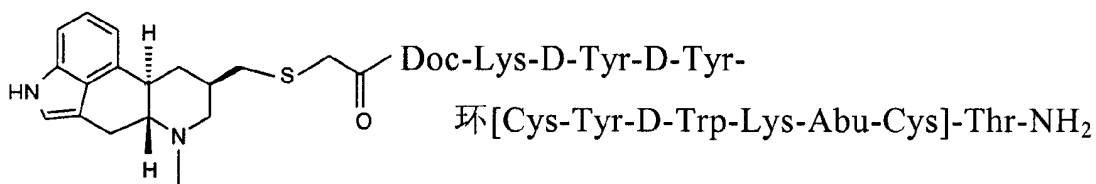


- B. 将得到的 H-Aepa-Lys(Boc)-D-Tyr(叔丁基)-Cys(Trt)- Tyr(叔丁基)-D-Trp(Boc)-Lys(Boc)-Abu-Cys(Trt)-Thr(叔丁基)-Rink 酰胺 MBHA 树脂 (0.188 mmol)与化合物 7(92mg, 0.28mmol, 1.5 eq), [7-氮杂苯并三唑-1-基-三(吡咯烷酮)磷(PyAOP) (146mg, 0.28mmol, 1.5eq)和 1-羟基-7-氮杂苯并三唑(HOAT) (38mg, 0.28mmol, 1.5 eq)在 5mL DCM 中混合。将混合物震荡过夜。树脂干燥后, 依次用 DMF, 甲醇和 DCM 洗涤。在空气中干燥后, 用 TFA, 水和三异丙基硅烷(TIS)的混合物(9.5ml/0.85ml/0.8ml)处理该树脂达 2 小时。过滤该树脂, 然后将滤出物倒入 50ml 冷乙醚中。离心分离后, 收集沉淀物。将粗产品溶解在 100ml 5%AcOH 水溶液中, 滴加碘的甲醇溶液直至保留有黄色。将反应溶液再搅拌 1 小时。加入 10%Na₂S₂O₃ 水溶液猝灭多余的碘。用有 C18 DYNAMAX-100 Å 柱的制备性 HPLC 系统 (Varian, Walnut Creek, CA)纯化溶液中的粗产品。以从 80%A 和 20%B 至 55%A 和 45%B 的线性梯度在 50 分钟内洗脱该柱, 其中 A 是 0.1%TFA 水溶液, B 是 0.1%TFA 的乙腈溶液。用分析 HPLC 检测馏分。合并含有纯产品的那些物质并冷冻至干燥。收率 40%。
- 根据分析性 HPLC 的分析, 纯度为 96.8%。MS (电雾化): 1820.8 (与计算的分子量 1821.3 一致)。

实施例 M



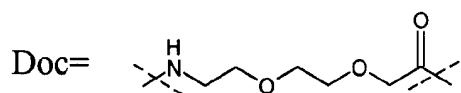
- 实施例 M 基本上用 H-Lys(Boc)-D-Tyr(叔丁基)- Tyr(叔丁基)-Cys(Trt)-Tyr(叔丁基)-D-Trp(Boc)-Lys(Boc)-Abu-Cys(Trt)-Thr(叔丁基)-Rink 酰胺 MBHA 树脂按照实施例 L 的方法合成。根据 HPLC 的分析, 最终产物的纯度为 97.9%。MS (电雾化): 1652.1 (与计算的分子量 1652.03 一致)。

实施例 N

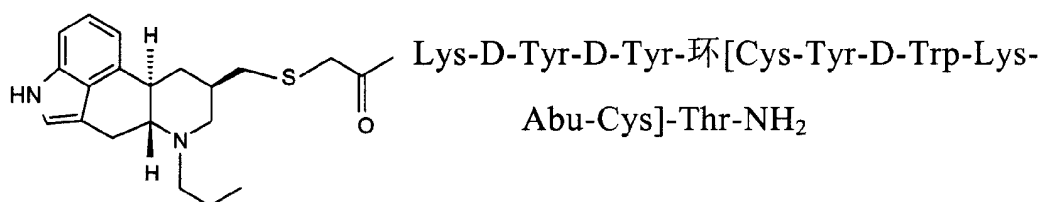
5

实施例 N 基本上是使用 H-Doc-Lys(Boc)-D-Tyr(叔丁基)-D-Tyr(叔丁基)-Lys(Trt)-Tyr(叔丁基)-D-Trp(Boc)-Lys(Boc)-Abu-Cys(Trt)-Thr(叔丁基)-Rink 酰胺 MBHA 树脂按照实施例 L 所描述的方法合成。根据 HPLC 的分析, 最终产物的纯度为 99.2%。MS (电雾化): 1797.1 (与计算的分子量 1797.19 一致)。

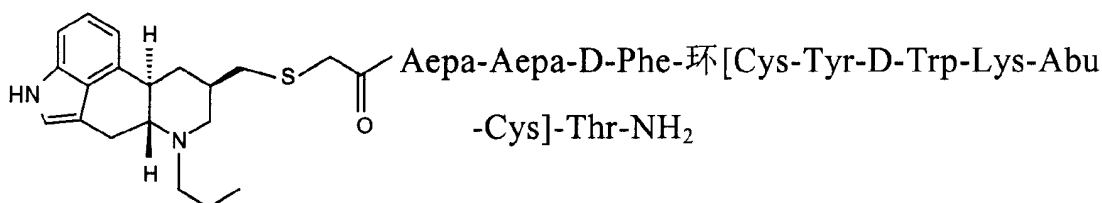
10 Fmoc-Doc-OH 可从 Chem-Impex InternatioNal, Inc. (Wood Dale, IL)商购。

实施例 O

15

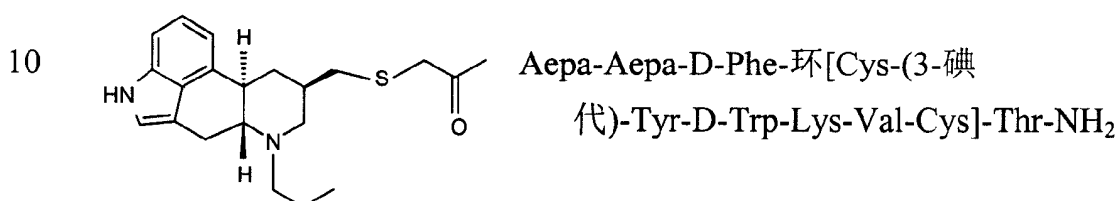


实施例 O 基本上是使用(6-N-丙基-8β-麦角灵基)甲基硫代乙酸和
20 H-Lys(Boc)-D-Tyr(叔丁基)-D-Tyr(叔丁基)-Cys(Trt)-Tyr(叔丁基)-D-Trp(Boc)-Lys(Boc)-Abu-Cys(Trt)-Thr(叔丁基)-Rink 酰胺 MBHA 树脂按照实施例 L 所描述的方法合成。根据 HPLC 的分析, 最终产物的纯度为 97.4%。MS (电雾化): 1680.6 (与计算的分子量 1680.1 一致)。

25 实施例 P

实施例 P 基本上是使用(6-N-丙基-8 β -麦角灵基)甲基硫代乙酸和 H-Aepa-Aepa-D-Phe-Cys(Trt)-Tyr(叔丁基)-D-Trp(Boc)-Lys(Boc)-Abu-Cys(Trt)-Thr(叔丁基)-Rink 酰胺 MBHA 树脂按照实施例 L 所描述的方法合成。根据 HPLC 的分析,最终产物的纯度为 99.9%。MS (电雾化): 1710.7 (与计算的分子量 1711.2 一致)。

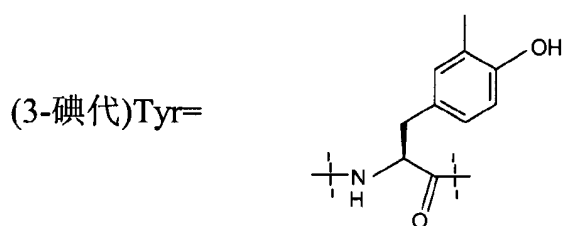
实施例 Q



实施例 Q 基本上是使用(6-N-丙基-8 β -麦角灵基)甲基硫代乙酸和 H-Aepa-Aepa-D-Phe-Cys(Trt)-(3-碘代)-Tyr-D-Trp(Boc)-Lys(Boc)-Val-Cys(Trt)-Thr(叔丁基)-Rink 酰胺 MBHA 树脂按照实施例 L 所描述的方法合成。根据 HPLC 的分析,最终产物的纯度为 99%。MS (电雾化): 1851.1 (与计算的分子量 1851.1 一致)。

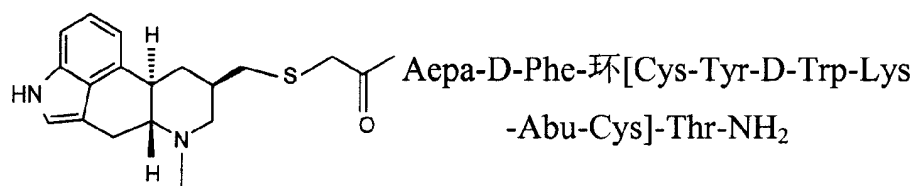
Fmoc-(3-碘代)-Tyr-OH 可从 Advanced ChemTech (Louisville, KY)商购。

20



25

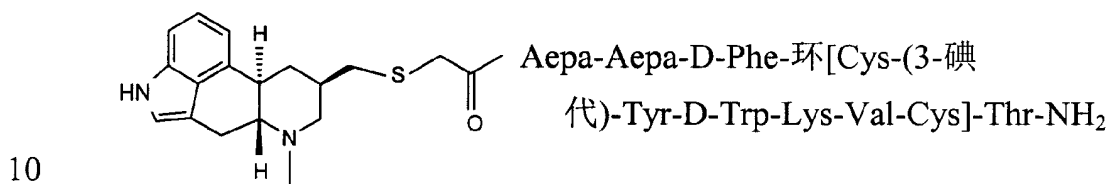
实施例 R



实施例 R 基本上是使用 H-Aepa-Aepa-D-Phe-Cys(Trt)-Tyr(叔丁基)-D-Trp(Boc)-Lys(Boc)-Abu-Cys(Trt)-Thr(叔丁基)-Rink 酰胺 MBHA 树脂按照实施例 L 所描述的方法合成。根据 HPLC 的分析, 最终产物的纯度为 98.3%。MS (电雾化): 1513.8 (与计算的分子量 1513.9 一致)。

5

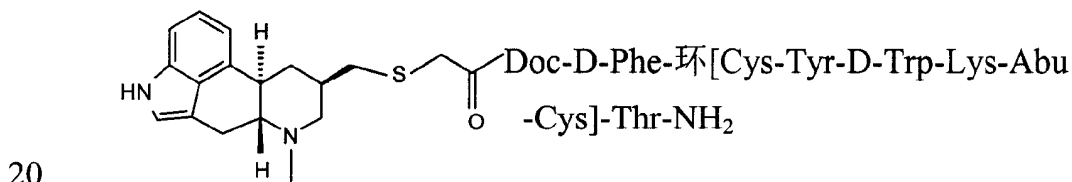
实施例 S



实施例 S 基本上是使用 H-Aepa-Aepa-D-Phe-Cys(Trt)-(3-碘代)-Tyr-D-Trp(Boc)-Lys(Boc)-Val-Cys(Trt)-Thr(叔丁基)-Rink 酰胺 MBHA 树脂按照实施例 L 所描述的方法合成。根据 HPLC 的分析, 最终产物的纯度为 85.7%。MS (电雾化): 1822.9 (与计算的分子量 1823.06 一致)。

15

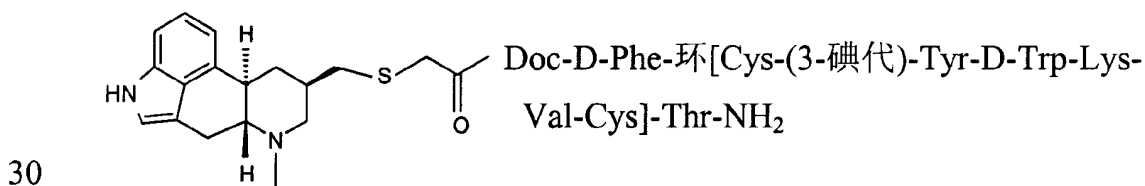
实施例 T



实施例 T 基本上是使用 H-Doc-D-Phe-Cys(Trt)-Tyr(叔丁基)-D-Trp(Boc)-Lys(Boc)-Abu-Cys(Trt)-Thr(叔丁基)-Rink 酰胺 MBHA 树脂根据实施例 L 所描述的方法合成。根据 HPLC 的分析, 最终产物的纯度为 98.9%。MS (电雾化): 1489.6 (与计算的分子量 1489.84 一致)。

25

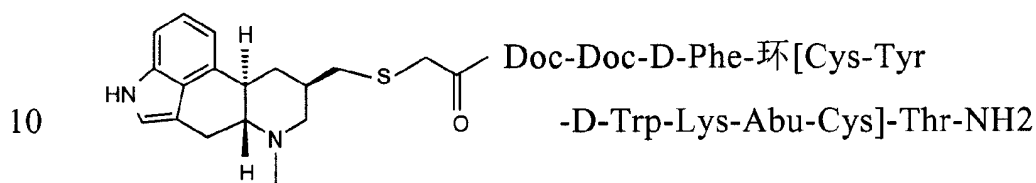
实施例 U



实施例 U 基本上是使用 H-Doc-D-Phe-Cys(Trt)-(3-碘代)Tyr-D-Trp(Boc)-Lys(Boc)-Val-Cys(Trt)-Thr(叔丁基)-Rink 酰胺 MBHA 树脂按照实施例 L 所描述的方法合成。MS (电雾化): 1629.8 (与计算的分子量 1629.7 一致)

5

实施例 V



该标题化合物基本上是使用 H-Doc-Doc-D-Phe-Cys(Trt)-Tyr(叔丁基)-D-Trp(Boc)-Lys(Boc)-Abu-Cys(Trt)-Thr(叔丁基)-Rink 酰胺 MBHA 树脂按照实施例 L 所描述的方法合成。根据 HPLC 的分析, 最终产物的纯度为 99%。

15 MS (电雾化): 1635.0 (与计算的分子量 1633 一致)。

本发明的某些化合物可具有至少一个不对称中心。分子中也可以有另外的不对称中心, 这取决于分子上不同取代基的性质。每个这样的不对称中心产生两个旋光异构体, 所有这些旋光异构体如分离的, 纯的或部分纯的旋光异构体、外消旋混合物或其非对映混合物均包括在本发明的范围内。

20 本发明的化合物通常可以其药学上可接受的酸加成盐的形式分离出来, 如使用无机酸或有机酸得到的盐。所述酸的实例包括盐酸、硝酸、硫酸、磷酸、甲酸、乙酸、三氟乙酸、丙酸、马来酸、琥珀酸、D-酒石酸、L-酒石酸、丙二酸、甲磺酸等。另外, 某些含有酸官能团如羧基的化合物可以其无机盐的形式分离出来, 其中的抗衡离子选自 Na, K, Li, Ca, Mg 等, 也可从有机碱中分离出来。

25

所述药学上可接受的盐可通过下述方法得到: 取约 1 当量的本发明的化合物(如下述化合物 C), 使其与约 1 当量或更多的所希望得到的相应盐的酸接触, 合成方法和分离方法是本领域的技术人员所公知的。

本发明的化合物可以通过下列方式给药: 口服、胃肠外(如肌内、腹膜内、

静脉内或皮下注射，或移植)、鼻腔给药、阴道、直肠、舌下、或局部给药，本发明的化合物可与药学上可接受的载体一起制剂以提供与各种给药途径相适应的药剂形式。因此，本发明包括含有下列的药物组合物：作为活性组分的至少一种本发明的化合物，其与药学上可接受的载体相联合。

5 用于口服给药的固体剂型包括胶囊、片剂、丸剂、粉末剂和粒剂。在这些固体剂型中，活性成分化合物与至少一种惰性的药学上可接受的载体混合，如蔗糖、乳糖、或淀粉。这些剂型也可包括除惰性稀释剂以外的物质，如润滑剂(如硬酯酸镁)。胶囊、片剂和丸剂还可含有缓冲剂。片剂和丸剂还可包有肠溶衣。

10 用于口服给药的液体剂型包括药学上可接受的乳液、溶液、悬浮液、糖浆、含有本领域常用的惰性稀释剂如水的酏剂。除上述惰性稀释剂以外，组合物也可包括辅剂，如润湿剂、乳化剂、悬浮剂和甜味剂、调味剂和芳香剂。

 本发明用于胃肠外给药的制剂包括无毒水溶液或非水溶液、悬浮液或乳液。非水溶剂或载体的实例包括丙二醇、聚乙二醇、植物油如橄榄油和玉米油，
15 明胶和可注射的有机酯类如油酸酯。该剂型还可包括辅剂如防腐剂、润湿剂、乳化剂和分散剂。它们可通过下列方式消毒：通过细菌保留过滤器(bacteria-retaining filter)进行过滤，在组合物中加入消毒剂，照射组合物或加热组合物。在使用前，可以制成溶解在无菌水或一些其他无菌可注射介质中的无菌固体组合物的形式。

20 用于直肠或阴道给药的组合物优选是栓剂，除活性物质外，其可含有赋型剂如可可乳脂或栓剂蜡。

 用于鼻腔或舌下给药的组合物可与本领域公知的标准赋型剂一起制备。

 通常，本发明的组合物中的活性组分的有效剂量可以不同，但活性组分的量必须是获得适宜剂型的量。选择的剂量取决于所希望达到的治疗效果、给药
25 途径、和治疗持续的时间，这些都是本领域的公知常识。通常，对人和其他动物如哺乳动物的给药剂量每天在 0.0001-100mg/kg 体重。

 优选的剂量范围在每天 0.01-10.0 mg/kg 体重，其可以是一次给药的剂量或分为多次给药的剂量。

30 生长激素释放抑制因子特性和选择性分析

用来合成生长激素释放抑制因子-多巴胺嵌合体的生长激素释放抑制因子类似物的特性和选择性取决于对被每种 SSTR 亚型转染的 CHO-K1 细胞所进行的放射性配体结合试验, 如下所述。

SSTR 1, 2, 3 和 4 基因的完全编码序列和 SSTR 5 的 cDNA 无性系被亚克隆到哺乳纲表达载体 pCMV 上(Life Technologies, Lilano, Italy)。稳定表达 SSTR's 1-5 的克隆细胞系由使用磷酸钙共沉淀法(Davis L 等人, 1994, 分子生物学的基本方法, 第二版, Appleton & Lange, Norwalk, CT, USA: 611-646)转染至 CHO-K1 细胞中(ATCC, Manassas, Va, USA)而得到。pRSV-neo(ATCC)质粒作为可选择的标记包含于其中。在含有 0.5 mg/ml G418 (Life Technologies, Milano, Italy)的 RPMI 1640 介质中选择克隆细胞系, 进行环克隆, 并在培养物中扩展。

在冰冷的 50nM Tris-HCl 中匀化表达 SSTR's 亚型的 CHO-K1 细胞, 在 39000g(10 分钟)离心分离两次, 并立刻在新鲜的缓冲剂中进行再悬浮, 得到用于体外受体结合分析的薄膜。最终的小颗粒在 10nM Tris-HCl 中进行再悬浮, 以用于分析。为了分析 SSTR 1, 3, 4 和 5, 使薄膜制剂的等分试样在 25°C 下与 0.05nM [¹²⁵I-Tyr11]SS-14 在 50mM HEPES (pH7.4)中温育 90 分钟, 该 HEPES 含有 10mg/ml BSA, 5mM MgCl₂, 200 KIU/ml 抑肽酶(Trasylol), 0.02 mg/ml USP 杆菌肽, 和 0.02mg/ml 苯甲磺酰氟。最后的分析体积是 0.3ml。为分析 SSTR 2, 采用 0.05nM [¹²⁵I]MK-678 为放射性配体, 温育时间在 25°C 下为 90 分钟。使用 Brandel 过滤歧管通过 GF/B 过滤器将其快速过滤, 从而终止温育。然后用 5ml 冰冷缓冲剂的等分试样分别冲洗每个管和过滤器 3 次。特异性结合定义为放射性配体的结合总数减去出现在 1000 nM SS-14 中的结合(对于 SSTR 1, 3, 4 和 5)或 1000 nM MK-678 中的结合(对于 SSTR2)。

25 多巴胺受体特性和选择性分析

用于合成生长激素释放抑制因子-多巴胺嵌合体的多巴胺类似物的多巴胺-2 受体的特性和选择性, 取决于放射性配体结合试验, 如下所述。

采用 Brinkman Polytron (设定为 6, 15 秒), 在 20ml 冰冷的 50 nM Tris-HCl 中匀化冷冻的鼠体纹状体(Zivic Laboratories, Pittsburgh, PA), 得到粗膜。加入缓冲剂使终体积为 40 ml, 匀浆在 SorVal SS-34 转子以 39000g 在 0-4°C 下离心

分离 10 分钟。倒掉所得上层清液,再将小颗粒在冰冷的缓冲剂中匀化,在 37°C 下预先温育 10 分钟,如前述进行稀释和离心分离。在缓冲剂中再悬浮最终小颗粒,并保存在冰上,用于受体结合分析试验。

5 为了进行分析,将洗涤过的薄膜制剂的等分试样和测试化合物与 0.25nM [³H] 螺环哌啶酮(16.5Ci. mmol, New England Nuclear, Boston, MA)一起在 50 mM Tris HCl, 120mM NaCl, 5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂ 中温育 15 分钟。最终分析体积为 1.0ml。使用 Brandel 过滤歧管通过 GF/B 过滤器进行快速过滤,从而终止温育。然后用 5ml 冰冷缓冲剂的等分试样分别冲洗每个管和过滤器 3 次。特异性结合定义为放射性配体的结合总数减去出现在 1000 nM (+) 10 丁克吗(butaclamol)中的结合。

其他实施方式

应该理解,当本发明结合详细说明来描述时,前述说明只是用于举例说明而并非是为了限制由后面的权利要求数所定义的本发明的范围。其他的方面、15 优点和改进也属于本发明的权利要求的范围。而且,本文所提到的所有文献均全文引入作为参考。