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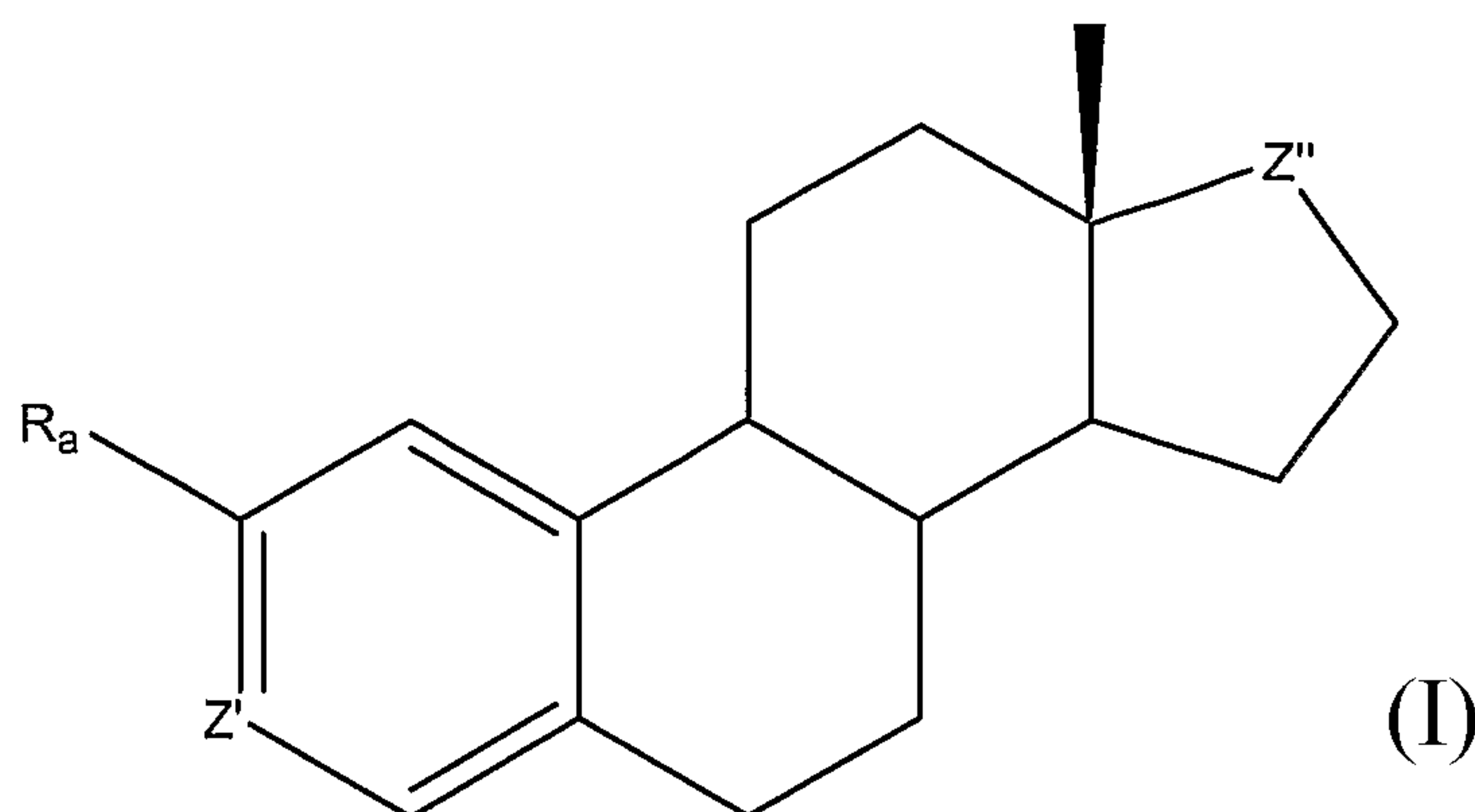
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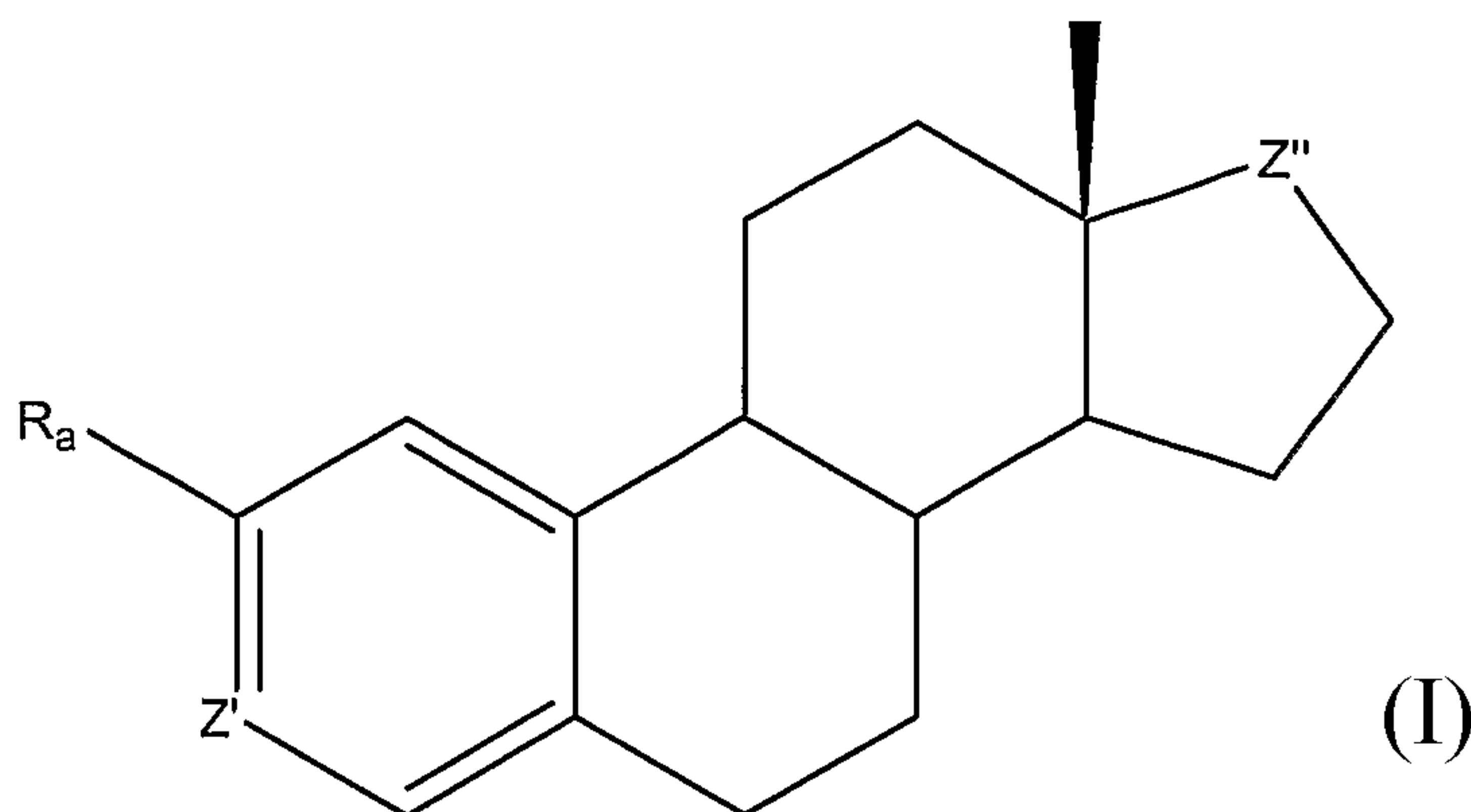
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(I)

(57) Abstract: Compositions and methods for treating mammalian disease characterized by undesirable angiogenesis by administering compounds of the general formula (I) wherein the variables are defined in the specification.

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ANTIANGIOGENIC AGENTS

FIELD OF THE INVENTION

The present invention relates to treating disease states characterized by abnormal
5 cell mitosis and to treating disease states characterized by abnormal angiogenesis and to
treating disease states characterized by a combination of these events. More particularly,
the present invention relates to analogs of 2-methoxyestradiol (2ME₂) and their effect on
diseases characterized by abnormal cell mitosis and/or abnormal angiogenesis.

10 BACKGROUND OF THE INVENTION

Angiogenesis is the generation of new blood vessels into a tissue or organ. Under
normal physiological conditions, humans and animals undergo angiogenesis only in very
specific, restricted situations. For example, angiogenesis is normally observed in wound
healing, fetal and embryonal development, and formation of the corpus luteum,
15 endometrium and placenta.

Angiogenesis is controlled through a highly regulated system of angiogenic
stimulators and inhibitors. The control of angiogenesis has been found to be altered in
certain disease states and, in many cases, pathological damage associated with the diseases
is related to uncontrolled angiogenesis. Both controlled and uncontrolled angiogenesis are
20 thought to proceed in a similar manner. Endothelial cells and pericytes, surrounded by a
basement membrane, form capillary blood vessels. Angiogenesis begins with the erosion
of the basement membrane by enzymes released by endothelial cells and leukocytes.
Endothelial cells, lining the lumen of blood vessels, then protrude through the basement
membrane. Angiogenic stimulants induce the endothelial cells to migrate through the
25 eroded basement membrane. The migrating cells form a "sprout" off the parent blood
vessel where the endothelial cells undergo mitosis and proliferate. The endothelial sprouts
merge with each other to form capillary loops, creating a new blood vessel.

Persistent, unregulated angiogenesis occurs in many disease states, tumor
metastases, and abnormal growth by endothelial cells. The diverse pathological disease
30 states in which unregulated angiogenesis is present have been grouped together as
angiogenic-dependent or angiogenic-associated diseases.

One example of a disease dependent on angiogenesis is ocular neovascular disease.
This disease is characterized by invasion of new blood vessels into the structures of the

eye, such as the retina or cornea. It is the most common cause of blindness and is involved in approximately twenty eye diseases. In age-related macular degeneration, the associated visual problems are caused by an ingrowth of choroidal capillaries through defects in Bruch's membrane with proliferation of fibrovascular tissue beneath the retinal pigment epithelium. Angiogenic damage is also associated with diabetic retinopathy, retinopathy of prematurity, corneal graft rejection, neovascular glaucoma, and retrolental fibroplasia. Other diseases associated with corneal neovascularization include, but are not limited to, epidemic keratoconjunctivitis, Vitamin A deficiency, contact lens overwear, atopic keratitis, superior limbic keratitis, and pterygium keratitis sicca. Other diseases associated with undesirable angiogenesis include Sjögren's syndrome, acne rosacea, phlyctenulosis, syphilis, *Mycobacteria* infections, lipid degeneration, chemical burns, bacterial ulcers, fungal ulcers, *Herpes simplex* infection, *Herpes zoster* infections, protozoan infections, Kaposi's sarcoma, Mooren's ulcer, Terrien's marginal degeneration, marginal keratolysis, rheumatoid arthritis, systemic lupus, polyarteritis, trauma, Wegener's sarcoidosis, scleritis, Stevens-Johnson's disease, pemphigoid, and radial keratotomy.

Diseases associated with neovascularization include, but are not limited to, retinal/choroidal neovascularization, diabetic retinopathy, macular degeneration, sickle cell anemia, sarcoidosis, syphilis, pseudoxanthoma elasticum, Paget's disease, vein occlusion, artery occlusion, carotid obstructive disease, chronic uveitis/vitritis, *Mycobacteria* infections, Lyme's disease, systemic lupus erythematosus, retinopathy of prematurity, Eales' disease, Behcet's disease, infections causing retinitis or choroiditis, presumed ocular histoplasmosis, Best's disease, myopia, optic pits, Stargardt's disease, pars planitis, chronic retinal detachment, hyperviscosity syndromes, toxoplasmosis, trauma and post-laser complications. Other eye-related diseases include, but are not limited to, diseases associated with rubeosis (neovascularization of the angle) and diseases caused by the abnormal proliferation of fibrovascular or fibrous tissue, including all forms of proliferative vitreoretinopathy.

Another angiogenesis associated disease is rheumatoid arthritis. The blood vessels in the synovial lining of the joints undergo angiogenesis. In addition to forming new vascular networks, the endothelial cells release factors and reactive oxygen species that lead to pannus growth and cartilage destruction. Angiogenesis may also play a role in osteoarthritis. The activation of the chondrocytes by angiogenic-related factors contributes to the destruction of the joint. At a later stage, the angiogenic factors promote new bone

growth. Therapeutic intervention that prevents the cartilage destruction could halt the progress of the disease and provide relief for persons suffering with arthritis.

Chronic inflammation may also involve pathological angiogenesis. Such diseases as ulcerative colitis and Crohn's disease show histological changes with the ingrowth of new blood vessels into inflamed tissues. Bartonellosis, a bacterial infection found in South America, can result in a chronic stage that is characterized by proliferation of vascular endothelial cells. Another pathological role associated with angiogenesis is found in atherosclerosis. The plaques formed within the lumen of blood vessels have been shown to have angiogenic stimulatory activity.

The hypothesis that tumor growth is angiogenesis-dependent was first proposed in 1971. (Folkman, *New Eng. J. Med.*, 285:1182-86 (1971)). In its simplest terms, this hypothesis states: "Once tumor 'take' has occurred, every increase in tumor cell population must be preceded by an increase in new capillaries converging on the tumor." Tumor 'take' is currently understood to indicate a prevascular phase of tumor growth in which a population of tumor cells occupying a few cubic millimeters volume, and not exceeding a few million cells, can survive on existing host microvessels. Expansion of tumor volume beyond this phase requires the induction of new capillary blood vessels. For example, pulmonary micrometastases in the early prevascular phase in mice would be undetectable except by high power microscopy on histological sections.

Examples of the indirect evidence which support this concept include:

(1) The growth rate of tumors implanted in subcutaneous transparent chambers in mice is slow and linear before neovascularization, and rapid and nearly exponential after neovascularization. (Algire, *et al.*, *J. Nat. Cancer Inst.*, 6:73-85 (1945)).

(2) Tumors grown in isolated perfused organs where blood vessels do not proliferate are limited to 1-2 mm³ but expand rapidly to >1000 times this volume when they are transplanted to mice and become neovascularized. (Folkman, *et al.*, *Annals of Surgery*, 164:491-502 (1966)).

(3) Tumor growth in the avascular cornea proceeds slowly and at a linear rate, but switches to exponential growth after neovascularization. (Gimbrone, Jr., *et al.*, *J. Nat. Cancer Inst.*, 52:421-27 (1974)).

(4) Tumors suspended in the aqueous fluid of the anterior chamber of a rabbit eye remain viable, avascular, and limited in size to < 1 mm³. Once they are implanted on the iris vascular bed, they become neovascularized and grow rapidly, reaching 16,000

times their original volume within 2 weeks. (Gimbrone, Jr., *et al.*, *J. Exp. Med.*, 136:261-76).

(5) When tumors are implanted on a chick embryo chorioallantoic membrane, they grow slowly during an avascular phase of >72 hours, but do not exceed a mean diameter of 0.93 ± 0.29 mm. Rapid tumor expansion occurs within 24 hours after the onset of neovascularization, and by day 7 these vascularized tumors reach a mean diameter of 8.0 ± 2.5 mm. (Knighton, *British J. Cancer*, 35:347-56 (1977)).

(6) Vascular casts of metastases in a rabbit liver reveal heterogeneity in size of the metastases, but show a relatively uniform cut-off point for the size at which vascularization is present. Tumors are generally avascular up to 1 mm in diameter, but are neovascularized beyond that diameter. (Lien, *et al.*, *Surgery*, 68:334-40 (1970)).

(7) In transgenic mice that develop carcinomas in the beta cells of the pancreatic islets, pre-vascular hyperplastic islets are limited in size to < 1 mm. At 6-7 weeks of age, 4-10% of the islets become neovascularized, and from these islets arise large vascularized tumors of more than 1000 times the volume of the pre-vascular islets. (Folkman, *et al.*, *Nature*, 339:58-61 (1989)).

(8) A specific antibody against VEGF (vascular endothelial growth factor) reduces microvessel density and causes "significant or dramatic" inhibition of growth of three human tumors which rely on VEGF as their sole mediator of angiogenesis (in nude mice). The antibody does not inhibit growth of the tumor cells *in vitro*. (Kim, *et al.*, *Nature*, 362:841-44 (1993)).

(9) Anti-bFGF monoclonal antibody causes 70% inhibition of growth of a mouse tumor which is dependent upon secretion of bFGF as its only mediator of angiogenesis. The antibody does not inhibit growth of the tumor cells *in vitro*. (Hori, *et al.*, *Cancer Res.*, 51:6180-84 (1991)).

(10) Intraperitoneal injection of bFGF enhances growth of a primary tumor and its metastases by stimulating growth of capillary endothelial cells in the tumor. The tumor cells themselves lack receptors for bFGF, and bFGF is not a mitogen for the tumor cells *in vitro*. (Gross, *et al.*, *Proc. Am. Assoc. Cancer Res.*, 31:79 (1990)).

(11) A specific angiogenesis inhibitor (AGM-1470) inhibits tumor growth and metastases *in vivo*, but is much less active in inhibiting tumor cell proliferation *in vitro*. It inhibits vascular endothelial cell proliferation half-maximally at 4 logs lower concentration

than it inhibits tumor cell proliferation. (Ingber, *et al.*, *Nature*, 48:555-57 (1990)). There is also indirect clinical evidence that tumor growth is angiogenesis dependent.

5 (12) Human retinoblastomas that are metastatic to the vitreous develop into avascular spheroids that are restricted to less than 1 mm³ despite the fact that they are viable and incorporate ³H-thymidine (when removed from an enucleated eye and analyzed *in vitro*).

(13) Carcinoma of the ovary metastasizes to the peritoneal membrane as tiny avascular white seeds (1-3 mm³). These implants rarely grow larger until one or more of them becomes neovascularized.

10 (14) Intensity of neovascularization in breast cancer (Weidner, *et al.*, *New Eng. J. Med.*, 324:1-8 (1991); Weidner, *et al.*, *J Nat. Cancer Inst.*, 84:1875-87 (1992)) and in prostate cancer (Weidner, *et al.*, *Am. J. Pathol.*, 143(2):401-09 (1993)) correlates highly with risk of future metastasis.

15 (15) Metastasis from human cutaneous melanoma is rare prior to neovascularization. The onset of neovascularization leads to increased thickness of the lesion and an increased risk of metastasis. (Srivastava, *et al.*, *Am. J. Pathol.*, 133:419-23 (1988)).

20 (16) In bladder cancer, the urinary level of an angiogenic protein, bFGF, is a more sensitive indicator of status and extent of disease than is cytology. (Nguyen, *et al.*, *J. Nat. Cancer Inst.*, 85:241-42 (1993)).

25 Thus, it is clear that angiogenesis plays a major role in the metastasis of cancer. If this angiogenic activity could be repressed or eliminated, then the tumor, although present, would not grow. In the disease state, prevention of angiogenesis could avert the damage caused by the invasion of the new microvascular system. Therapies directed at control of the angiogenic processes could lead to the abrogation or mitigation of these diseases.

30 Angiogenesis has been associated with a number of different types of cancer, including solid tumors and blood-borne tumors. Solid tumors with which angiogenesis has been associated include, but are not limited to, rhabdomyosarcomas, retinoblastoma, Ewing's sarcoma, neuroblastoma, and osteosarcoma. Angiogenesis is also associated with blood-borne tumors, such as leukemias, any of various acute or chronic neoplastic diseases of the bone marrow in which unrestrained proliferation of white blood cells occurs, usually accompanied by anemia, impaired blood clotting, and enlargement of the lymph nodes,

liver and spleen. It is believed that angiogenesis plays a role in the abnormalities in the bone marrow that give rise to leukemia tumors and multiple myeloma diseases.

One of the most frequent angiogenic diseases of childhood is the hemangioma. A hemangioma is a tumor composed of newly formed blood vessels. In most cases the tumors are benign and regress without intervention. In more severe cases, the tumors progress to large cavernous and infiltrative forms and create clinical complications. Systemic forms of hemangiomas, hemangiomatoses, have a high mortality rate. Therapy-resistant hemangiomas exist that cannot be treated with therapeutics currently in use.

Angiogenesis is also responsible for damage found in heredity diseases such as Osler-Weber-Rendu disease, or heredity hemorrhagic telangiectasia. This is an inherited disease characterized by multiple small angiomas, tumors of blood or lymph vessels. The angiomas are found in the skin and mucous membranes, often accompanied by epistaxis (nose bleeds) or gastrointestinal bleeding and sometimes with pulmonary or hepatic arteriovenous fistula.

What is needed, therefore, is a composition and method that can inhibit angiogenesis. What is also needed is a composition and method that can inhibit the unwanted growth of blood vessels, especially in tumors.

Angiogenesis is also involved in normal physiological processes, such as reproduction and wound healing. Angiogenesis is an important step in ovulation and also in implantation of the blastula after fertilization. Prevention of angiogenesis could be used to induce amenorrhea, to block ovulation, or to prevent implantation by the blastula.

In wound healing, excessive repair or fibroplasia can be a detrimental side effect of surgical procedures and may be caused or exacerbated by angiogenesis. Adhesions are a frequent complication of surgery and lead to problems such as small bowel obstruction.

Several compounds have been used to inhibit angiogenesis. Taylor, *et al.* (*Nature*, 297:307 (1982)) have used protamine to inhibit angiogenesis. The toxicity of protamine limits its practical use as a therapeutic. Folkman, *et al.* (*Science*, 221:719 (1983)), and U.S. Pat. Nos. 5,001,116 and 4,994,443 have disclosed the use of heparin and steroids to control angiogenesis. Steroids, such as tetrahydrocortisol, which lack glucocorticoid and mineralocorticoid activity, have been found to be angiogenic inhibitors.

Other factors found endogenously in animals, such as a 4 kDa glycoprotein from bovine vitreous humor and a cartilage derived factor, have been used to inhibit angiogenesis. Cellular factors, such as interferon, inhibit angiogenesis. For example,

interferon alpha or human interferon beta have been shown to inhibit tumor-induced angiogenesis in mouse dermis stimulated by human neoplastic cells. Interferon beta is also a potent inhibitor of angiogenesis induced by allogeneic spleen cells. (Sidky, *et al.*, *Cancer Res.*, 47:5155-61(1987)). Human recombinant interferon (alpha/A) was reported to
5 be successfully used in the treatment of pulmonary hemangiomas, an angiogenesis-induced disease. (White, *et al.*, *New Eng. J. Med.*, 320:1197-1200 (1989)).

Other agents that have been used to inhibit angiogenesis include ascorbic acid ethers and related compounds. (Japanese Kokai Tokkyo Koho No.58-13 (1978)). Sulfated polysaccharide DS 4152 also inhibits angiogenesis. (Japanese Kokai Tokkyo Koho No.
10 63-119500). Additional anti-angiogenic compounds include Angiostatin® (U.S. Patent Nos. 5,639,725; 5,792,845; 5,885,795; 5,733,876; 5,776,704; 5,837,682; 5,861,372, and 5,854,221) and Endostatin (U.S. Patent No. 5,854,205).

Another compound which has been shown to inhibit angiogenesis is thalidomide. (D'Amato, *et al.*, *Proc. Natl. Acad. Sci.*, 90:4082-85 (1994)). Thalidomide is a
15 hypnosedative that has been successfully used to treat a number of diseases, such as rheumatoid arthritis (Gutierrez-Rodriguez, *Arthritis Rheum.*, 27 (10):1118-21 (1984); Gutierrez-Rodriguez, *et al.*, *J. Rheumatol.*, 16(2):158-63 (1989)), Behcet's disease (Handley, *et al.*, *Br. J. Dermatol.*, 127 Suppl, 40:67-8 (1992); Gunzler, *Med. Hypotheses*, 30(2):105-9 (1989)).

20 Although thalidomide has minimal side effects in adults, it is a potent teratogen. Thus, there are concerns regarding its use in women of child-bearing age. Although minimal, there are a number of side effects that limit the desirability of thalidomide as a treatment. One such side effect is drowsiness. In a number of therapeutic studies, the initial dosage of thalidomide had to be reduced because patients became lethargic and had
25 difficulty functioning normally. Another side effect limiting the use of thalidomide is peripheral neuropathy, in which individuals suffer from numbness and dysfunction in their extremities.

Thus, improved methods and compositions are needed that are easily administered and capable of inhibiting angiogenesis. What is needed are safe and effective treatments
30 that do not create unwanted side effects.

2-Methoxyestradiol is an endogenous metabolite of estradiol (E2). When administered orally, it exhibits anti-tumor and anti-proliferative activity with little toxicity. *In vitro* data suggests that 2-methoxyestradiol does not engage the estrogen receptor for its

anti-proliferative activity and is not estrogenic over a wide range of concentrations, as assayed by estrogen dependant MCF-7 cell proliferation. However, the presence of metabolizing enzymes, such as demethylases, *in vivo* and *in vitro* may metabolize this compound to products, such as 2-hydroxyestradiol, which has been shown to be estrogenic by several approaches. What is needed is a means to improve the bioavailability of estradiol derivatives or 2-methoxyestradiol and to reduce the formation of estrogenic 2-methoxyestradiol metabolites. What is also needed is a means to modify estradiol derivatives or 2-methoxyestradiol in such a way as to prevent conversion into an estrogenic derivative, metabolic conjugation and/or conversion to estrones.

SUMMARY OF THE INVENTION

The present invention provides certain analogs of 2-methoxyestradiol that are effective in treating diseases characterized by abnormal mitosis and/or abnormal angiogenesis. Specifically the present invention relates to analogs of 2-methoxyestradiol that have been modified at the 2, 3 and 17 positions thereof. Compounds within the general formulae that inhibit cell proliferation are preferred. Compounds within Formula I that inhibit angiogenesis are also preferred. Preferred compositions may also exhibit a change (increase or decrease) in estrogen receptor binding, improved absorption, transport (*e.g.*, through blood-brain barrier and cellular membranes), biological stability, or decreased toxicity. The invention also provides compounds useful in the method, as described by the general formulae of the claims.

A mammalian disease characterized by undesirable cell mitosis, as defined herein, includes but is not limited to excessive or abnormal stimulation of endothelial cells (*e.g.*, atherosclerosis), solid tumors and tumor metastasis, benign tumors, for example, hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas, vascular malfunctions, abnormal wound healing, inflammatory and immune disorders, Bechet's disease, gout or gouty arthritis, abnormal angiogenesis accompanying: rheumatoid arthritis, skin diseases, such as psoriasis, diabetic retinopathy and other ocular angiogenic diseases such as retinopathy of prematurity (retrolental fibroplastic), macular degeneration, corneal graft rejection, neovascular glaucoma and Osler Weber syndrome (Osler-Weber-Rendu disease). Other undesired angiogenesis involves normal processes including ovulation and implantation of a blastula. Accordingly, the compositions described above

can be used to block ovulation and implantation of a blastula or to block menstruation (induce amenorrhea).

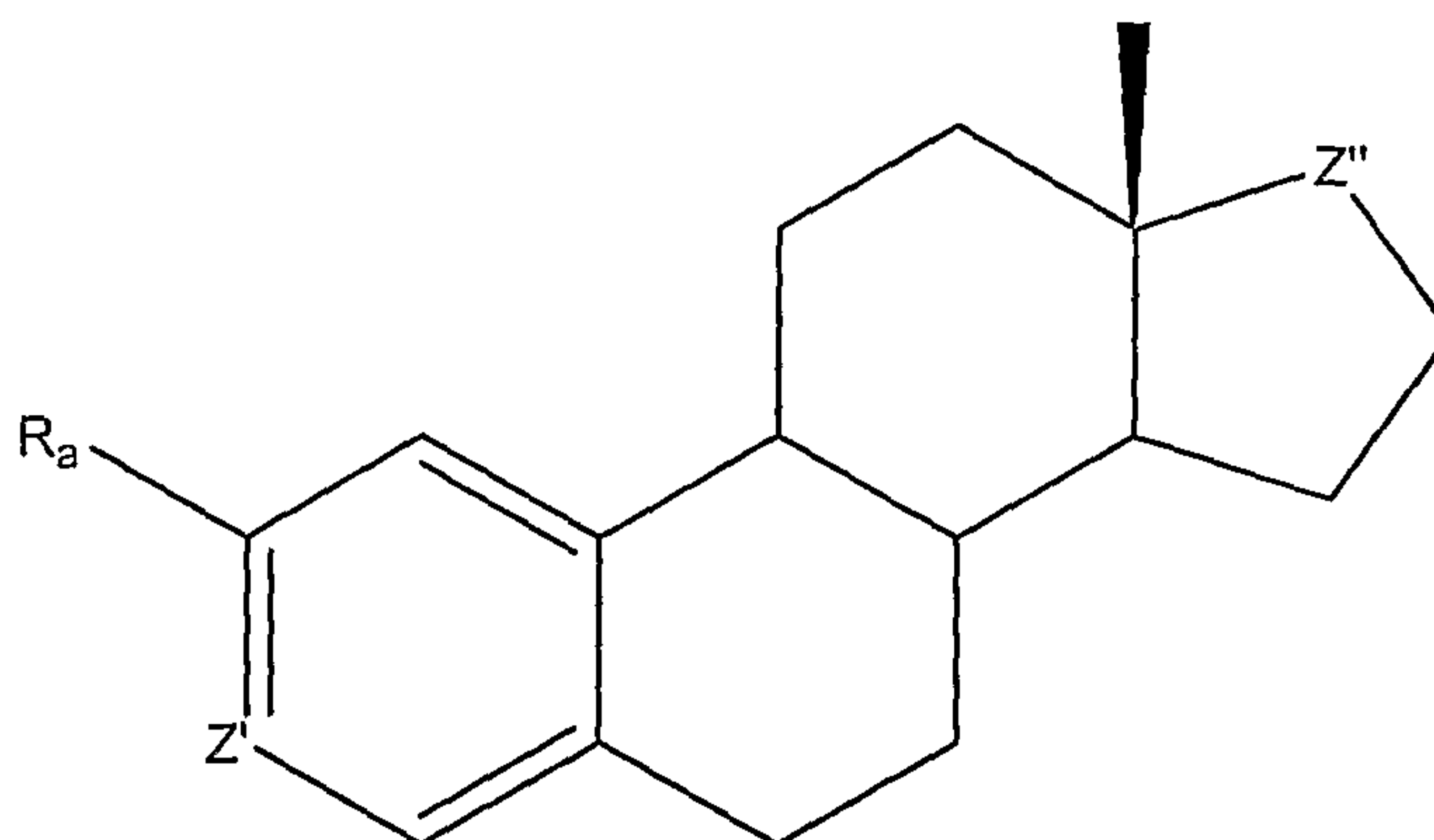
A novel series of compounds have been prepared that retain the biological activities of 2ME₂ but are believed to have reduced metabolism. 17-Position alkylated analogs lack the hydroxyl moiety and cannot be metabolized to estrones or conjugated at that position but retain antiproliferative activity in HUVEC and tumor cells. Replacement of the 2-methoxy group by other moieties, such as a propynyl group, retains antiproliferative activity, but these groups cannot be de-methylated to yield the estrogenic 2-hydroxyl derivatives. Contrary to what is observed with 2ME₂, several of these new analogs have selective *in vitro* antiproliferative activity for the endothelial cells over the tumor cell line assessed.

Also disclosed are compounds and methods for altering the chemical nature of positions 3 and 17 of 2-methoxyestradiol for preventing conversion to 2-methoxyestrone and/or the conjugation of 2-methoxyestradiol (or metabolites) with other molecules and subsequent loss during excretion of the resulting compounds.

Other features and advantages of the invention will be apparent from the following description of preferred embodiments thereof.

DETAILED DESCRIPTION OF THE INVENTION

As described below, compounds that are useful in accordance with the invention include novel 2-methoxyestradiol derivatives that exhibit anti-mitotic, anti-angiogenic and/or anti-tumor properties. Preferred compounds of the invention are 2-methoxyestradiol derivatives modified at the 2, 3 and 17 positions. Preferred compounds are those of the general Formula (I):



wherein, R_a is selected from $-OCH_3$, $-OCH_2CH_3$, or $-CCCH_3$; Z' is selected from $>C-F$, $>C-NH_2$, $>CCONH_2$, $>C-NHCOH$, $>C-OSO_2NH_2$, or $>C-CHCH_2$; and Z'' is selected from $>C(H_2)$, $>C(H)-CH_3$, $>C=CH_2$, $>C=CHCH_3$, or $>C=O$; provided that when Z' is $>C-OSO_2NH_2$ and Z'' is $>C(H_2)$ or $>C=O$, R_a is neither $-OCH_3$ nor $-OCH_2CH_3$. Specific compounds according to the invention are described below.

In an alternate disclosed embodiment of the present invention, compounds according to the present invention are those of Formula I, wherein R_a is $-OCH_3$; Z' is selected from $>C-NH_2$, $>CCONH_2$, $>C-NHCOH$, $>C-OSO_2NH_2$, or $>C-CHCH_2$; and Z'' is selected from $>C(H_2)$, $>C(H)-CH_3$, $>C=CH_2$, $>C=CHCH_3$, or $>C=O$.

In another alternate disclosed embodiment of the present invention, compounds according to the present invention are those of Formula I, wherein R_a is $-OCH_2CH_3$; Z' is selected from $>C-NH_2$, $>CCONH_2$, $>C-NHCOH$, $>C-OSO_2NH_2$, or $>C-CHCH_2$; and Z'' is selected from $>C(H_2)$, $>C(H)-CH_3$, $>C=CH_2$, $>C=CHCH_3$, or $>C=O$.

In a further alternate disclosed embodiment of the present invention, compounds according to the present invention are those of Formula I, wherein R_a is $-CCCH_3$; Z' is selected from $>C-NH_2$, $>CCONH_2$, $>C-NHCOH$, $>C-OSO_2NH_2$, or $>C-CHCH_2$; and Z'' is selected from $>C(H_2)$, $>C(H)-CH_3$, $>C=CH_2$, $>C=CHCH_3$, or $>C=O$.

In another alternate disclosed embodiment of the present invention, compounds according to the present invention are those of Formula I, wherein Z' is selected from $>C-F$; R_a is selected from $-OCH_3$, $-OCH_2CH_3$, or $-CCCH_3$; and Z'' is selected from $>C(H_2)$, $>C(H)-CH_3$, $>C=CH_2$, $>C=CHCH_3$, or $>C=O$.

In another alternate disclosed embodiment of the present invention, compounds according to the present invention are those of Formula I, wherein Z' is $>C-NH_2$; R_a is selected from $-OCH_3$, $-OCH_2CH_3$, or $-CCCH_3$; and Z'' is selected from $>C(H_2)$, $>C(H)-CH_3$, $>C=CH_2$, $>C=CHCH_3$, or $>C=O$.

In still another alternate disclosed embodiment of the present invention, compounds according to the present invention are those of Formula I, wherein Z' is selected from $>CCONH_2$; R_a is $-OCH_3$, $-OCH_2CH_3$, or $-CCCH_3$; and Z'' is selected from $>C(H_2)$, $>C(H)-CH_3$, $>C=CH_2$, $>C=CHCH_3$, or $>C=O$.

In a further alternate disclosed embodiment of the present invention, compounds according to the present invention are those of Formula I, wherein Z' is selected from $>C-OSO_2NH_2$; R_a is $-OCH_3$, $-OCH_2CH_3$, or $-CCCH_3$; and Z'' is selected from $>C(H_2)$, $>C(H)-CH_3$, $>C=CH_2$, $>C=CHCH_3$, or $>C=O$.

In another alternate disclosed embodiment of the present invention, compounds according to the present invention are those of Formula I, wherein Z' is selected from >C-CHCH₂; R_a is -OCH₃, -OCH₂CH₃, or -CCCH₃; and Z'' is selected from >C(H₂), >C(H)-CH₃, >C=CH₂, >C=CHCH₃, or >C=O.

5 In yet alternate disclosed embodiment of the present invention, compounds according to the present invention are those of Formula I, wherein Z'' is selected from >C(H₂); R_a is selected from -OCH₃, -OCH₂CH₃, or -CCCH₃; and Z' is selected from >C-NH₂, >CCONH₂, >C-NHCOH, >C-OSO₂NH₂, or >C-CHCH₂.

10 In another alternate disclosed embodiment of the present invention, compounds according to the present invention are those of Formula I, wherein Z'' is selected from >C(H)-CH₃; R_a is selected from -OCH₃, -OCH₂CH₃, or -CCCH₃; and Z' is selected from >C-NH₂, >CCONH₂, >C-NHCOH, >C-OSO₂NH₂, or >C-CHCH₂.

15 In a further alternate disclosed embodiment of the present invention, compounds according to the present invention are those of Formula I, wherein Z'' is selected from >C=CH₂; R_a is selected from -OCH₃, -OCH₂CH₃, or -CCCH₃; and Z' is selected from >C-NH₂, >CCONH₂, >C-NHCOH, >C-OSO₂NH₂, or >C-CHCH₂.

20 In another alternate disclosed embodiment of the present invention, compounds according to the present invention are those of Formula I, wherein Z'' is selected from >C=CHCH₃; R_a is selected from -OCH₃, -OCH₂CH₃, or -CCCH₃; and Z' is selected from >C-NH₂, >CCONH₂, >C-NHCOH, >C-OSO₂NH₂, or >C-CHCH₂.

In still another alternate disclosed embodiment of the present invention, compounds according to the present invention are those of Formula I, wherein Z'' is selected from >C=O; R_a is selected from -OCH₃, -OCH₂CH₃, or -CCCH₃; and Z' is selected from >C-NH₂, >CCONH₂, >C-NHCOH, >C-OSO₂NH₂, or >C-CHCH₂.

25 In a further alternate disclosed embodiment of the present invention, compounds according to the present invention are those of Formula I, wherein R_a is selected from -OCH₃, -OCH₂CH₃, or -CCCH₃; Z' is selected from >C-NH₂, >CCONH₂, >C-NHCOH, or >C-CHCH₂; and Z'' is selected from >C(H₂), >C(H)-CH₃, >C=CH₂, >C=CHCH₃, or >C=O.

30 In another alternate disclosed embodiment of the present invention, compounds according to the present invention are those of Formula I, wherein R_a is selected from -OCH₃, -OCH₂CH₃, or -CCCH₃; Z' is selected from >C-NH₂, >CCONH₂, >C-NHCOH, >C-OSO₂NH₂, or >C-CHCH₂; and Z'' is selected from >C(H₂), >C(H)-CH₃, >C=CH₂,

$>C=CHCH_3$, or $>C=O$; provided that when Z' is $>C-OSO_2NH_2$, Z'' is neither $>C(H_2)$ nor $>C=O$.

Those skilled in the art will appreciate that the invention extends to other compounds within the formulae given in the claims below, having the described characteristics. These characteristics can be determined for each test compound using the assays detailed below and elsewhere in the literature.

Although not wishing to be bound by theory, it is believed that 2-methoxyestrone (2ME₁) is formed through the same enzymatic pathway as estrone is formed from estradiol. Although not wishing to be bound by theory, it is further believed that the enzymes responsible for this reaction on estradiol are the 17 β -hydroxysteroid dehydrogenases (17 β -HSD), which utilize NADP⁺ as a co-factor (Han *et al.*, *J. Biol. Chem.* 275:2, 1105-1111 (Jan. 12, 2000) and other references cited earlier). Each of the four members of this enzyme family, types 1, 2, 3, and 4 have distinct activity. It appears that 17 β -HSD type 1 catalyzes the reductive reaction (estrone to estradiol), while 17 β -HSD type 2 catalyzes the oxidation reaction (estradiol to estrone), and type 3 catalyzes 4-androstenedione to testosterone. It is also believed that an additional metabolic deactivation pathway results in conjugation of 2-methoxyestradiol or 2-methoxyestrone with molecules such as sulfate or glucuronic acid and subsequent loss via excretion. In this invention, positions 3 and 17 of 2-methoxyestradiol, and derivatives thereof, may be modified to prevent these metabolic pathways from occurring.

Since 2-methoxyestradiol is metabolized to a much less active metabolite, the present invention adds steric bulk and/or modification of chemical or electrostatic characteristics at positions 3 and 17 of 2-methoxyestradiol for retarding or preventing interaction of the family of 17 β -hydroxysteroid dehydrogenases and co-factor NADP⁺ on this substrate. Addition of steric bulk and/or modification of chemical or electrostatic characteristics at positions 3 and 17 of 2-methoxyestradiol may also retard or prevent conjugation, such as glucuronidation or sulfation. It is believed that retardation or prevention of these two metabolic deactivation pathways prolongs the serum lifetime of 2-methoxyestradiol and other estradiol derivatives while retaining the desired anti-angiogenic and anti-tumor activity.

Aside from preventing the possible metabolism of 2ME₂ to 2ME₁, which may occur by making these steroids poor substrates for 17 β -HSD (by either steric and/or electronic effects), it is not possible for some of these analogs modified at the 2 position to undergo

the demethylation known to occur with 2ME₂ since there is no methyl ether group at that position. This is desirable since it has been demonstrated that 2-hydroxyestradiol (the product of demethylation of 2ME₂) has estrogenic activity.

It is well known that orally-delivered steroids, such as estradiol (E₂) and ethynyl-E₂, are extensively metabolized during passage through the gastrointestinal tract and by first-pass metabolism in the liver. Two major metabolic pathways that lead to rapid deactivation and excretion are well studied (Fotsis, T.; Zhang, Y.; Pepper, M. S.; Adlercrutz, H.; Montesano, R.; Nawroth, P. P.; Schweigerer, L., The Endogenous Estrogen Metabolite 2-Methoxyestradiol Inhibits Angiogenesis and Suppresses Tumor. *Nature*, 1994, 368, 237-239; Wang, Z.; Yang, D.; Mohanakrishnan, A. K.; Fanwick, P. E.; Nampoothiri, P.; Hamel, E.; Cushman, M. "Synthesis of B-Ring Homologated Estradiol Analogs that Modulate Tubulin Polymerization and Microtubule Stability." *J. Med. Chem.*, 2000, 43, 2419-2429) e.g. oxidation at the D-ring's 17-hydroxy group of E₂ to form estrone and conjugation with sulfate and/or glucuronate at the hydroxyls of position-3 on the A-ring and position-17 on the D-ring.

Several studies have been conducted to determine SAR of 2ME₂ analogs (D'Amato, R. J.; Lin, C. M.; Flynn, E.; Folkman, J.; Hamel, E. Inhibition of Angiogenesis and Breast Cancer in Mice by the Microtubule Inhibitors 2-Methoxyestradiol and Taxol", *Cancer Res.*, 1997, 57, 81-86; Cushman, M.; He, M.-H.; Katzenellenbogen, J. A.; Lin, C. M.; Hamel, E. "Synthesis, Antitubulin and Antimitotic Activity, and Cytotoxicity of Analogs of 2-Methoxyestradiol, an Endogenous Mammalian Metabolite of Estradiol that inhibits Tubulin Polymerization by Binding to the Colchicine Binding Site." *J. Med. Chem.* 1995, 38, 2041-2049) but none to reduce or stop its metabolic pathway.

In the preferred embodiment of the invention, 2-methoxyestradiol, and derivatives thereof, are modified at the 3 and 17 positions.

Anti-Proliferative Activity *In Situ*

Anti-proliferative activity can be evaluated *in situ* by testing the ability of an improved estradiol derivative to inhibit the proliferation of new blood vessel cells (angiogenesis). A suitable assay is the chick embryo chorioallantoic membrane (CAM) assay described by Crum et al. *Science* 230:1375 (1985). See also, U.S. Patent 5,001,116, hereby incorporated by reference, which describes the CAM assay. Briefly, fertilized chick embryos are removed from their shell on day 3 or 4, and a methylcellulose disc

containing the drug is implanted on the chorioallantoic membrane. The embryos are examined 48 hours later and, if a clear avascular zone appears around the methylcellulose disc, the diameter of that zone is measured. Using this assay, a 100 µg disk of the estradiol derivative 2-methoxyestradiol was found to inhibit cell mitosis and the growth of new blood vessels after 48 hours. This result indicates that the anti-mitotic action of 2-methoxyestradiol can inhibit cell mitosis and/or angiogenesis.

Anti-Proliferative Activity *In Vitro*

The process by which 2ME₂ affects cell growth remains unclear, however, a number of studies have implicated various mechanisms of action and cellular targets. 2ME₂ induced changes in the levels and activities of various proteins involved in the progression of the cell cycle. These include cofactors of DNA replication and repair, e.g., proliferating cell nuclear antigen (PCNA) (Klauber, N., Parangi, S., Flynn, E., Hamel, E. and D'Amato, R.J. (1997), Inhibition of angiogenesis and breast cancer in mice by the microtubule inhibitors 2-methoxyestradiol and Taxol., *Cancer Research* 57, 81-86; Lottering, M-L., de Kock, M., Viljoen, T.C., Grobler, C.J.S. and Seegers, J.C. (1996) 17β-Estradiol metabolites affect some regulators of the MCF-7 cell cycle. *Cancer Letters* 110, 181-186); cell division cycle kinases and regulators, e.g., p34^{cdc2} and cyclin B (Lottering *et al.* (1996); Attalla, H., Mäkelä, T.P., Adlercreutz, H. and Andersson, L.C. (1996) 2-Methoxyestradiol arrests cells in mitosis without depolymerizing tubulin. *Biochemical and Biophysical Research Communications* 228, 467-473; Zoubine, M.N., Weston, A.P., Johnson, D.C., Campbell, D.R. and Banerjee, S.K. (1999) 2-Methoxyestradiol-induced growth suppression and lethality in estrogen- responsive MCF-7 cells may be mediated by down regulation of p34cdc2 and cyclin B1 expression. *Int J Oncol* 15, 639-646); transcription factor modulators, e.g., SAPK/JNK (Yue, T-L., Wang, X., Loudon, C.S., Gupta, L.S., Pillarisetti, K., Gu, J-L., Hart, T.K., Lysko, P.G. and Feuerstein, G.Z. (1997) 2-Methoxyestradiol, an endogenous estrogen metabolite induces apoptosis in endothelial cells and inhibits angiogenesis: Possible role for stress-activated protein kinase signaling pathway and fas expression. *Molecular Pharmacology* 51, 951-962; Attalla, H., Westberg, J.A., Andersson, L.C., Aldercreutz, H. and Makela, T.P. (1998) 2-Methoxyestradiol-induced phosphorylation of bcl-2: uncoupling from JNK/SAPK activation. *Biochem and Biophys Res Commun* 247, 616-619); and regulators of cell arrest and apoptosis, e.g., tubulin (D'Amato, R.J., Lin, C.M., Flynn, E., Folkman, J. and Hamel, E. (1994) 2-

Methoxyestradiol, and endogenous mammalian metabolite, inhibits tubulin polymerization by interacting at the colchicine site. Proc. Natl. Acad. Sci. USA 91, 3964-3968; Hamel, E., Lin, C.M., Flynn, E. and D'Amato, R.J. (1996) Interactions of 2-methoxyestradiol, and endogenous mammalian metabolite, with unpolymerized tubulin and with tubulin polymers. Biochemistry 35, 1304-1310), p21^{WAF1/CIP1} (Mukhopadhyay, T. and Roth, J.A. (1997) Induction of apoptosis in human lung cancer cells after wild-type p53 activation by methoxyestradiol. Oncogene 14, 379-384), bcl-2 and FAS (Yue *et al.* (1997); Attalla *et al.* (1998)), and p53 (Kataoka, M., Schumacher, G., Cristiano, R.J., Atkinson, E.N., Roth, J.A. and Mukhopadhyay, T. (1998) An agent that increases tumor suppressor transgene product coupled with systemic transgene delivery inhibits growth of metastatic lung cancer *in vivo*. Cancer Res 58, 4761-4765; Mukhopadhyay *et al.* (1997); Seegers, J.C., Lottering, M-L., Grobler C.J.S., van Papendorp, D.H., Habbersett, R.C., Shou, Y. and Lehnert B.E. (1997) The mammalian metabolite, 2-methoxyestradiol, affects p53 levels and apoptosis induction in transformed cells but not in normal cells. J. Steroid Biochem. Molec.Biol. 62, 253-267).

The effects on the level of cAMP, calmodulin activity and protein phosphorylation may also be related to each other. More recently, 2ME₂ was shown to upregulate Death Receptor 5 and caspase 8 in human endothelial and tumor cell lines (LaVallee TM, Zhan XH, Johnson MS, Herbstritt CJ, Swartz G, Williams MS, Hembrough WA, Green SJ, Pribluda VS. 2-methoxyestradiol up-regulates death receptor 5 and induces apoptosis through activation of the extrinsic pathway. Cancer Res. (2003) 63#2:468-75). Additionally, 2ME₂ has been shown to interact with superoxide dismutase (SOD) 1 and SOD 2 and to inhibit their enzymatic activities (Huang, P., Feng, L., Oldham, E. A., Keating, M. J., and Plunkett, W. 2000. Superoxide dismutase as a target for the selective killing of cancer cells, Nature. 407:390-5.). All cellular targets described above are not necessarily mutually exclusive to the inhibitory effects of 2ME₂ in actively dividing cells.

The high affinity binding of 2ME₂ to SHBG has been mechanistically associated to its efficacy in a canine model of prostate cancer, in which signaling by estradiol and 5 α -androstan-3 α ,17 β -diol were inhibited by 2ME₂ (Ding, V.D., Moller, D.E., Feeney, W.P., Didolkar, V., Nakhla, A.M., Rhodes, L., Rosner, W. and Smith, R.G., *Sex hormone-binding globulin mediates prostate androgen receptor action via a novel signaling pathway*, Endocrinology 139, 213-218 (1998)).

The more relevant mechanisms described above have been extensively discussed in Victor S. Pribluda, Theresa M. LaVallee and Shawn J. Green, *2-Methoxyestradiol: A novel*

endogenous chemotherapeutic and antiangiogenic in The New Angiotherapy, Tai-Ping Fan and Robert Auerbach eds., Human Press Publisher.

Assays relevant to these mechanisms of action and inhibition of cell proliferation are well-known in the art. For example, anti-mitotic activity mediated by effects on tubulin polymerization activity can be evaluated by testing the ability of an estradiol derivative to inhibit tubulin polymerization and microtubule assembly *in vitro*. Microtubule assembly can be followed in a Gilford recording spectrophotometer (model 250 or 2400S) equipped with electronic temperature controllers. A reaction mixture typically contains 1.0 M monosodium glutamate (pH 6.6), 1.0 mg/ml (10 μ M) tubulin, 1.0 mM MgCl₂, 4% (v/v) dimethylsulfoxide and 20-75 μ M of a composition to be tested. The reaction mixtures are incubated for 15 min. at 37 $^{\circ}$ C and then chilled on ice. After addition of 10 μ l 2.5 mM GTP, the reaction mixture is transferred to a cuvette at 0 $^{\circ}$ C, and a baseline established. At time zero, the temperature controller of the spectrophotometer is set at 37 $^{\circ}$ C. Microtubule assembly is evaluated by increased turbidity at 350 nm. Alternatively, inhibition of microtubule assembly can be followed by transmission electron microscopy as described in Example 2 of U.S. Patent Nos. 5,504,074, 5,661,143, and 5,892,069, the disclosures of which are incorporated herein by reference.

Other such assays include counting of cells in tissue culture plates or assessment of cell number through metabolic assays or incorporation into DNA of labeled (radiochemically, for example ³H-thymidine, or fluorescently labeled) or immuno-reactive (BrdU) nucleotides. In addition, antiangiogenic activity may be evaluated through endothelial cell migration, endothelial cell tubule formation, or vessel outgrowth in *ex-vivo* models such as rat aortic rings.

Indications

The invention can be used to treat any disease characterized by abnormal cell mitosis and/or abnormal angiogenesis. Such diseases include, but are not limited to: abnormal stimulation of endothelial cells (*e.g.*, atherosclerosis), solid tumors and tumor metastasis, benign tumors, for example, hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas, vascular malfunctions, abnormal wound healing, inflammatory and immune disorders, Bechet's disease, gout or gouty arthritis, abnormal angiogenesis accompanying: rheumatoid arthritis, skin diseases, such as psoriasis, diabetic

retinopathy, and other ocular angiogenic diseases such as retinopathy of prematurity (retrolental fibroplastic), macular degeneration, corneal graft rejection, neurovascular glaucoma, liver diseases and Osler-Weber Rendu syndrome (Osler-Weber Rendu disease).

Diseases associated with neovascularization can be treated according to the present invention. Such diseases include, but are not limited to, diabetic retinopathy, retinopathy of prematurity, corneal graft rejection, neovascular glaucoma and retrolental fibroplasias, epidemic keratoconjunctivitis, Vitamin A deficiency, contact lens overwear, atopic keratitis, superior limbic keratitis, pterygium keratitis sicca, Sjögren's, acne rosacea, phlyctenulosis, syphilis, *Mycobacteria* infections, lipid degeneration, chemical burns, bacterial ulcers, fungal ulcers, *Herpes simplex* infections, *Herpes zoster* infections, protozoan infections, Kaposi's sarcoma, Mooren's ulcer, Terrien's marginal degeneration, marginal keratolysis, trauma, rheumatoid arthritis, systemic lupus, polyarteritis, Wegener's sarcoidosis, Scleritis, Steven-Johnson disease, pemphigoid, radial keratotomy, and corneal graft rejection.

Other diseases associated with neovascularization can be treated according to the present invention. Such diseases include, but are not limited to, diabetic retinopathy, macular degeneration, sickle cell anemia, sarcoid, syphilis, pseudoxanthoma elasticum, Paget's disease, vein occlusion, artery occlusion, carotid obstructive disease, chronic uveitis/vitritis, mycobacterial infections, Lyme's disease, systemic lupus erythematosus, retinopathy of prematurity, Eales' disease, Bechet's disease, infections causing a retinitis or choroiditis, presumed ocular histoplasmosis, Best's disease, myopia, optic pits, Stargart's disease, pars planitis, chronic retinal detachment, hyperviscosity syndromes, toxoplasmosis, trauma and post-laser complications. Other diseases include, but are not limited to, diseases associated with rubeosis (neovascularization of the angle) and diseases caused by the abnormal proliferation of fibrovascular or fibrous tissue including all forms of proliferative vitreoretinopathy, whether or not associated with diabetes.

The present invention may also be used to treat cancerous diseases. Cancerous diseases include, but are not limited to, rhabdomyosarcoma, retinoblastoma, Ewing sarcoma, neuroblastoma, osteosarcoma, acoustic neuromas, neurofibromas, hemangiomas, breast cancer, prostate cancer, renal cell cancer, brain tumors, ovarian cancer, colon cancer, bladder cancer, cutaneous melanoma, liver cancer, and lung cancer.

Another disease that can be treated according to the present invention is rheumatoid arthritis. It is believed that the blood vessels in the synovial lining of the joints undergo

angiogenesis. In addition to forming new vascular networks, the endothelial cells release factors and reactive oxygen species that lead to pannus growth and cartilage destruction. The factors involved in angiogenesis may actively contribute to, and help maintain, the chronically inflamed state of rheumatoid arthritis.

5 Other diseases that can be treated according to the present invention are hemangiomas, Osler-Weber-Rendu disease, or hereditary hemorrhagic telangiectasia, solid or blood borne tumors and acquired immune deficiency syndrome.

10 In addition, the invention can be used to treat a variety of post-menopausal symptoms, osteoporosis, cardiovascular disease, Alzheimer's disease, to reduce the incidence of strokes, and as an alternative to prior estrogen replacement therapies. The compounds of the present invention can work by estrogenic and non-estrogenic biochemical pathways.

15 Also contemplated by the present invention are implants or other devices comprised of the compounds or drugs of Formula I or prodrugs thereof where the drug or prodrug is formulated in a bio-degradable or non-biodegradable polymer for sustained release. Non-biodegradable polymers release the drug in a controlled fashion through physical or mechanical processes without the polymer itself being degraded. Biodegradable polymers are designed to gradually be hydrolyzed or solubilized by natural processes in the body, allowing gradual release of the admixed drug or prodrug. Both bio-degradable and non-20 biodegradable polymers and the process by which drugs are incorporated into the polymers for controlled release are well known to those skilled in the art. Examples of such polymers can be found in many references such as Brem et al, J. Neurosurg 74: pp. 441-446 (1991). These implants or devices can be implanted in the vicinity where delivery is desired, for example, at the site of a tumor or a stenosis.

25 Because anything not formed in the body as a natural component may elicit extreme and unexpected responses, such as blood vessel closure due to thrombus formation or spasm, and because damage to blood vessels by the act of insertion of a vascular stent may be extreme and unduly injurious to the blood vessel surface, it is prudent to protect against such events. Restenosis is a re-narrowing or blockage of an artery at the same site where 30 treatment, such as an angioplasty or stent procedure, has already taken place. If restenosis occurs within a stent that has been placed in an artery, it is technically called "in-stent restenosis," the end result being a narrowing in the artery caused by a build-up of substances that may eventually block the flow of blood. The compounds that are part of

the present invention are especially useful to coat vascular stents to prevent restenosis. The coating should preferably be a biodegradable or non-biodegradable polymer that allows for a slow release of a compound of the present invention thereby preventing the restenosis event.

5 Prodrug

The present invention also relates to conjugated prodrugs and uses thereof. More particularly, the invention relates to conjugates of estradiol compounds such as compounds of Formula I, and the use of such conjugates in the prophylaxis or treatment of conditions associated with enhanced angiogenesis or accelerated cell division, such as cancer, and
10 inflammatory conditions such as asthma and rheumatoid arthritis and hyperproliferative skin disorders including psoriasis. The invention also relates to compositions including the prodrugs of the present invention and methods of synthesizing the prodrugs.

In one aspect, the present invention provides a conjugated prodrug of an estradiol compound, preferably compounds of Formula I, conjugated to a biological activity
15 modifying agent.

Alternatively, the conjugated prodrug according to the present invention includes the compounds of Formula I conjugated to a peptide moiety.

The incorporation of an estradiol compound, such as the compounds of Formula I, into a disease-dependently activated pro-drug enables significant improvement of potency
20 and selectivity of this anti-cancer and anti-inflammatory agent.

In addition to the compounds of the present invention, the pharmaceutical composition of this invention may also contain, or be co-administered (simultaneously or sequentially) with, one or more pharmacological agents of value in treating one or more disease conditions referred to hereinabove.

25 In addition, the compounds of Formula I or prodrug thereof may be incorporated into biodegradable or non-biodegradable polymers allowing for sustained release, the polymers being implanted in the vicinity of where delivery is desired, for example, at the site of a tumor. Polymers and their use are described in detail in Brem et al., J. Neurosurg 74:441-446 (1991).

30 A person skilled in the art will be able by reference to standard texts, such as Remington's Pharmaceutical Sciences 17th edition, to determine how the formulations are to be made and how these may be administered.

In a further aspect of the present invention there is provided use of compounds of Formula I or prodrugs thereof according to the present invention for the preparation of a medicament for the prophylaxis or treatment of conditions associated with angiogenesis or accelerated cell division or inflammation.

5 In a further aspect of the present invention there is provided a pharmaceutical composition comprising compounds of Formula I or prodrugs thereof according to the present invention, together with a pharmaceutically acceptable carrier, diluent or excipient.

The pharmaceutical composition may be used for the prophylaxis or treatment of conditions associated with angiogenesis or accelerated cell division or inflammation.

10 In a still further aspect of the present invention there is provided a method of prophylaxis or treatment of a condition associated with angiogenesis or accelerated or increased amounts of cell division hypertrophic growth or inflammation, said method including administering to a patient in need of such prophylaxis or treatment an effective amount of compounds of Formula I or prodrugs thereof according to the present invention,
15 as described above.

It should be understood that prophylaxis or treatment of said condition includes amelioration of said condition.

By "an effective amount" is meant a therapeutically or prophylactically effective amount. Such amounts can be readily determined by an appropriately skilled person,
20 taking into account the condition to be treated, the route of administration and other relevant factors. Such a person will readily be able to determine a suitable dose, mode and frequency of administration.

Pharmaceutically acceptable salts of the compound of the formula may be prepared in any conventional manner for example from the free base and acid. *In vivo* hydrolysable
25 esters, amides and carbamates may be prepared in any conventional manner.

Improved Estradiol Derivative Synthesis

Known compounds that are used in accordance with the invention and precursors to novel compounds according to the invention can be purchased, *e.g.*, from Sigma Chemical
30 Co., St. Louis, Steraloids or Research Plus. Other compounds according to the invention can be synthesized according to known methods from publicly available precursors.

The chemical synthesis of estradiol has been described (Eder, V. et al., *Ber* 109, 2948 (1976); Oppolzer, D.A. and Roberts, D.A. *Helv. Chim. Acta.* 63, 1703, (1980)). The

synthetic pathways used to prepare some of the derivatives of the present invention are based on modified published literature procedures for estradiol derivatives (Trembley et al., Bioorganic & Med. Chem. 1995 3, 505-523; Fevig et al., J. Org. Chem., 1987 52, 247-251; Gonzalez et al., Steroids 1982, 40, 171-187; Trembley et al., Synthetic
5 Communications 1995, 25, 2483-2495; Newkome et al., J. Org. Chem. 1966, 31, 677-681; Corey et al Tetrahedron Lett 1976, 3-6; Corey et al., Tetrahedron Lett, 1976, 3667-3668) and German Patent No. 2757157 (1977).

Administration

10 The compositions described above can be provided as physiologically acceptable formulations using known techniques, and these formulations can be administered by standard routes. In general, the combinations may be administered by the topical, oral, rectal or parenteral (e.g., intravenous, subcutaneous or intramuscular) route. In addition, the combinations may be incorporated into polymers allowing for sustained release, the
15 polymers being implanted in the vicinity of where delivery is desired, for example, at the site of a tumor or within or near the eye. The dosage of the composition will depend on the condition being treated, the particular derivative used, and other clinical factors such as weight and condition of the patient and the route of administration of the compound. However, for oral administration to humans, a dosage of 0.01 to 100 mg/kg/day, especially
20 0.01-20 mg/kg/day, is generally preferred.

The formulations include those suitable for oral, rectal, nasal, inhalation, topical (including dermal, transdermal, buccal and sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intraocular, intratracheal, and
25 epidural) and inhalation administration. The formulations may conveniently be presented in unit dosage form and may be prepared by conventional pharmaceutical techniques. Such techniques include the step of bringing into association the active ingredient and a pharmaceutical carrier(s) or excipient(s). In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

30 Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a

suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil emulsion, etc.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing, in a suitable machine, the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, preservative, surface-active or dispersing agent. Molded tablets may be made by molding, in a suitable machine, a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide a slow or controlled release of the active ingredient therein.

Formulations suitable for topical administration in the mouth include lozenges comprising the ingredients in a flavored basis, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the ingredient to be administered in a suitable liquid carrier.

Formulations suitable for topical administration to the skin may be presented as ointments, creams, gels and pastes comprising the ingredient to be administered in a pharmaceutical acceptable carrier. A preferred topical delivery system is a transdermal patch containing the ingredient to be administered.

Formulations for rectal administration may be presented as a suppository with a suitable base comprising, for example, cocoa butter or a salicylate.

Formulations suitable for nasal administration, wherein the carrier is a solid, include a coarse powder having a particle size, for example, in the range of 20 to 500 microns which is administered in the manner in which snuff is taken, *i.e.*, by rapid inhalation through the nasal passage from a container of the powder held close up to the nose. Suitable formulations, wherein the carrier is a liquid, for administration, as for example, a nasal spray or as nasal drops, include aqueous or oily solutions of the active ingredient.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing, in addition to the active ingredient, ingredients such as carriers as are known in the art to be appropriate.

Formulation suitable for inhalation may be presented as mists, dusts, powders or spray formulations containing, in addition to the active ingredient, ingredients such as carriers as are known in the art to be appropriate.

Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampules and vials, and may be stored in a freeze-dried (lyophilized) conditions requiring only the addition of the sterile liquid carrier, for example, water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kinds previously described.

Preferred unit dosage formulations are those containing a daily dose or unit, daily sub-dose, as herein above recited, or an appropriate fraction thereof, of the administered ingredient.

It should be understood that in addition to the ingredients, particularly mentioned above, the formulations of the present invention may include other agents conventional in the art having regard to the type of formulation in question, for example, those suitable for oral administration may include flavoring agents.

The present invention includes compositions and methods for treating mammalian disease characterized by pathogenic angiogenesis by administering compounds of Formula I. The 2-methoxyestradiol, and derivatives thereof, are modified at the 3 and 17 positions. Combinations which are physically impossible are not contemplated by this invention, such as a carbon atom containing 5 bonds.

100% pure isomers are contemplated by this invention, however a stereochemical isomer labeled as α or β may be a mixture of both in any ratio, where it is chemically possible by one skilled in the art. Also contemplated by this invention are both classical and non-classical bioisosteric atom and substituent replacements, such as are described by Patani and Lavoie (Bio-isosterism: a rational approach in drug design Chem. Rev. (1996) p. 3147-3176) and are well known to one skilled in the art. Such bioisosteric replacements include, for example, substitution of =S or =NH for =O.

The synthetic routes for this series of analogs are summarized in Schemes 1-7. Schemes 1 and 2 present the preparation of the singly and doubly substituted templates that are required as precursors for the triply substituted analogs. Schemes 3-7 present on the

modification of position 3 of the templates derived from Schemes 1 and 2. These synthetic routes present one potential way to prepare this series of analogs, and other synthetic routes (including modifying the order of synthetic steps or reagents) are possible to someone skilled in the art.

5 In Scheme 1, the 2-methoxy, 2-ethoxy or 2-propynyl derivatives, which are either commercially available or can be readily prepared by literature methods (Wang *et al*, *Synthetic Comm* 1998, 28, 4431, Cushman et al *J. Med. Chem.* 1995, 38, 2041, Cushman et al *J. Med. Chem.* 1997, 40, 2323, Cushman et al *J. Med. Chem.* 2002, 45, 4748), are oxidized using the Oppenauer oxidation. The resulting ketone can be deoxygenated using
10 the Wolf-Kishner reduction (Shapiro R, J. et al *J. Org. Chem.* 1964, 86, 2825-2832.) or olefinated (Schow et al *J. Org. Chem.* 1979, 44, 22. Krubner et al *J. Org. Chem.* 1968, 33, 1715.) using the Wittig reaction. Both the 17-methylene and 17-ethylene estrane analogs can be reduced to the alkane using catalytic reduction.

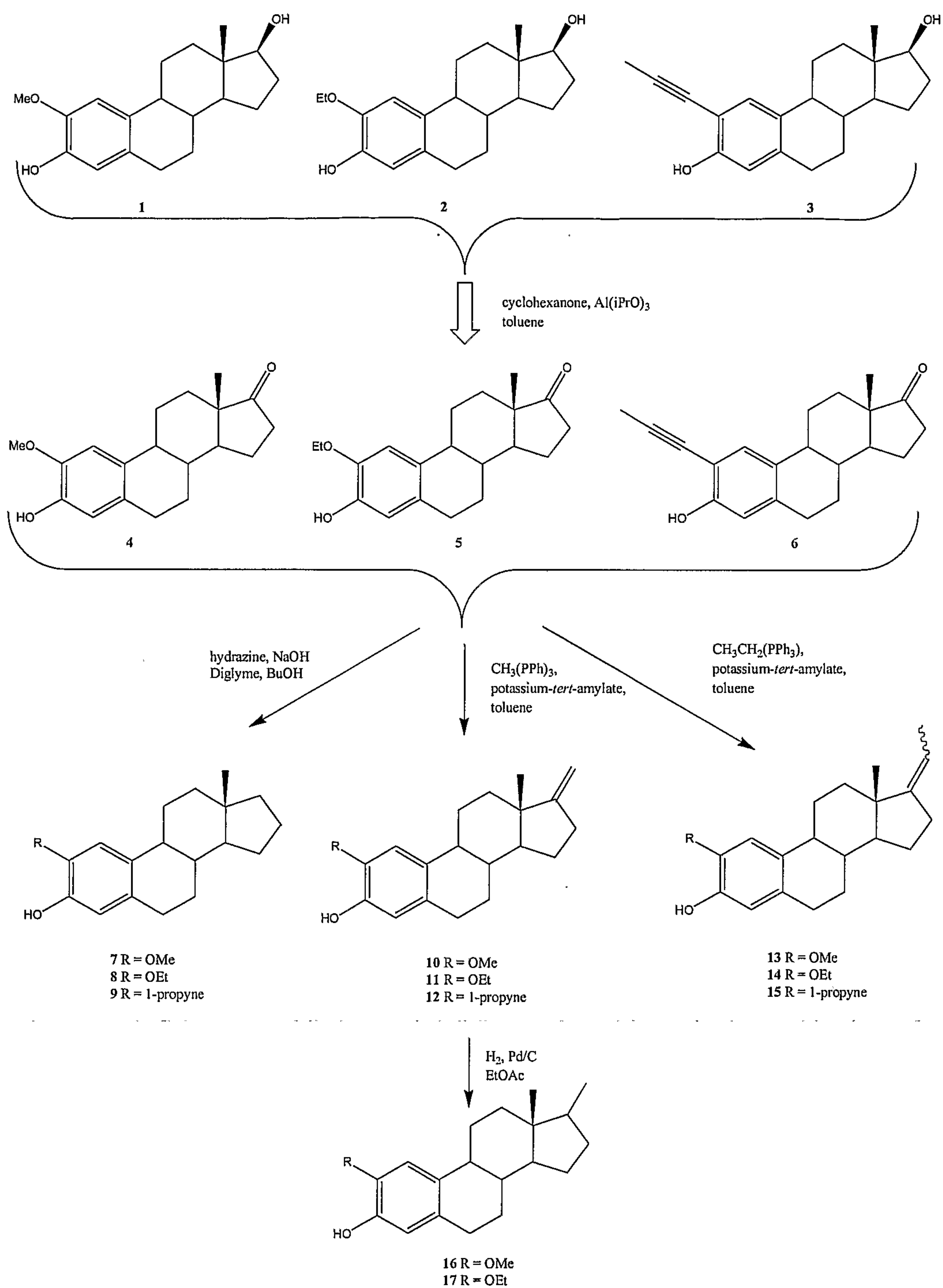
In specific cases, the nature of protecting groups or the order of reactions may have
15 to be altered to reach the desired products. These changes to the general synthetic schemes would be well understood to one skilled in the art. For instance, in the case where the desired 2 functionality is a propyne and the desired 17-functionality is an alkyl group, catalytic hydrogenation could not be carried out on the 17-olefin since the 2-alkyne would also be reduced. Scheme 2 presents a synthetic route to prepare analog 18. In this
20 example, the 17 methyl is introduced as in Scheme 1 starting with estrone, and subsequently the 2-propyne is incorporated using a literature method (Cushman et al *J. Med. Chem.* 2002, 45, 4748).

Schemes 3-7 feature the further modification of the templates generated in Schemes 1 and 2. In Scheme 3, templates 4, 5 or 6 can be converted to the 3-sulfamate derivative
25 (19, 20 or 21) with sulfamoyl chloride and either sodium hydride (Howarth et al *J. Med. Chem.* 1994, 37, 219) or 2,6-di-*tert*-butyl-4-methylpyridine (Coibanu et al *J. Med. Chem.* 1999, 42, 2280). To prepare analogs 22-24, triflic anhydride was added to a solution of 4, 5 or 6 in dichloromethane and pyridine at 0°C using Echavarren's procedure (Echvarren et al *J. Am. Chem.* 1987, 109, 5478). The triflate is a versatile synthetic intermediate and was
30 used to incorporate a wide range of functional groups at position 3. One utility of the triflate is its conversion to the vinyl analog via palladium catalyzed substitution with a vinyl functionality (analog 25-27) (Shi et al *J. Med Chem.* 2002, 124, 6921). The

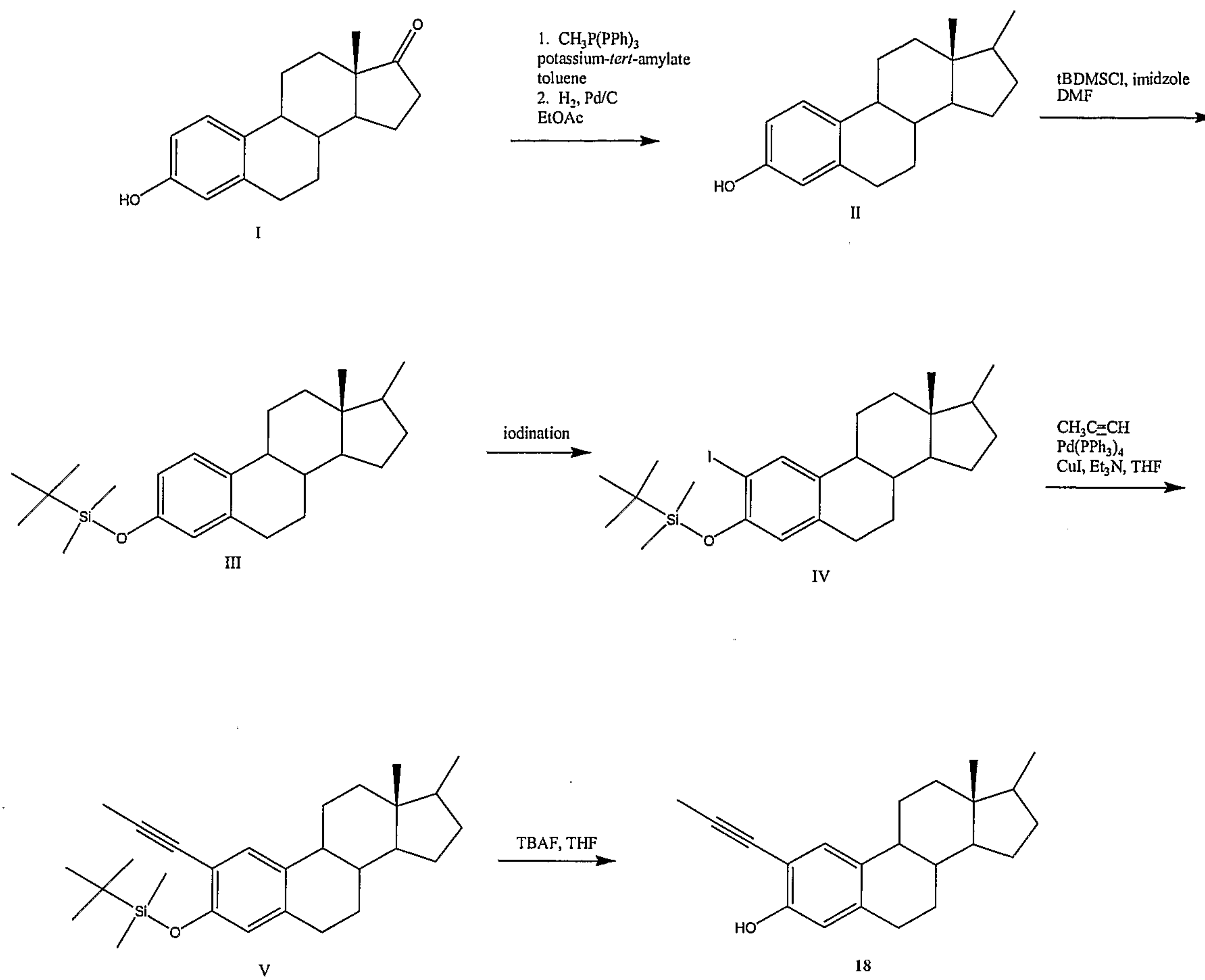
carboxylic acid derivatives (28-30) were also prepared by palladium catalysis as well (Shi et al *Chem. & Biol.* 2001, 8, 501). The carboxylic acid could also be converted to the corresponding amide using thionyl chloride and ammonia gas (Tomas de, Paulis, et. al. *J. Med. Chem.* 1986, 29, 61). The triflate could be converted directly to a carboxamide using
5 a procedure described by Morera (*Tetrahedron Letters*, 1998, 39, 2835-2838). Alternatively, the triflate could be converted to an amine via palladium catalyzed substitution of the triflate with benzophenone imine, subsequent hydrolysis yielded the corresponding amine (analogs 34-36) (Wolfe et al *Tetrahedron Lett* 1997, 38, 6367). The 3-amine could be converted to 3-fluoro analogs using the diazonium salt intermediate
10 (Morrow *J. Med. Chem.* 1966, 9, 249). Additionally, ketones 34-36 can be reduced to the corresponding alcohols 37-39 with lithium aluminum hydride at -78°C then converted to amides 43-45 with trimethylacetic formyl anhydride (Vleitstra et al *Recueil* 1982, 101, 460). Schemes 4-7 use analogous chemistry to prepare other triply modified steroid analogs.

15 Compounds according to the present invention may be prepared using the reaction schemes shown below:

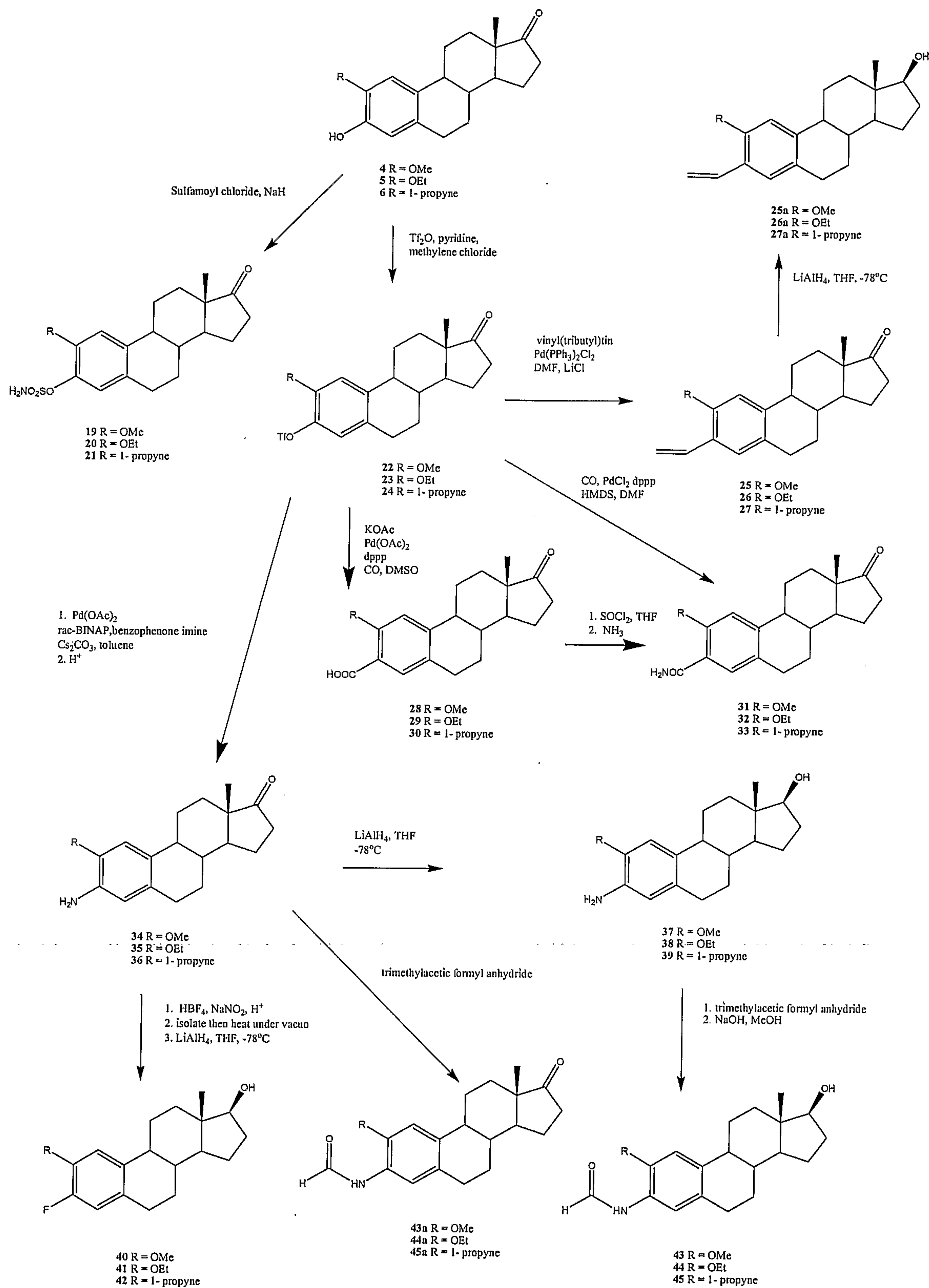
Scheme 1



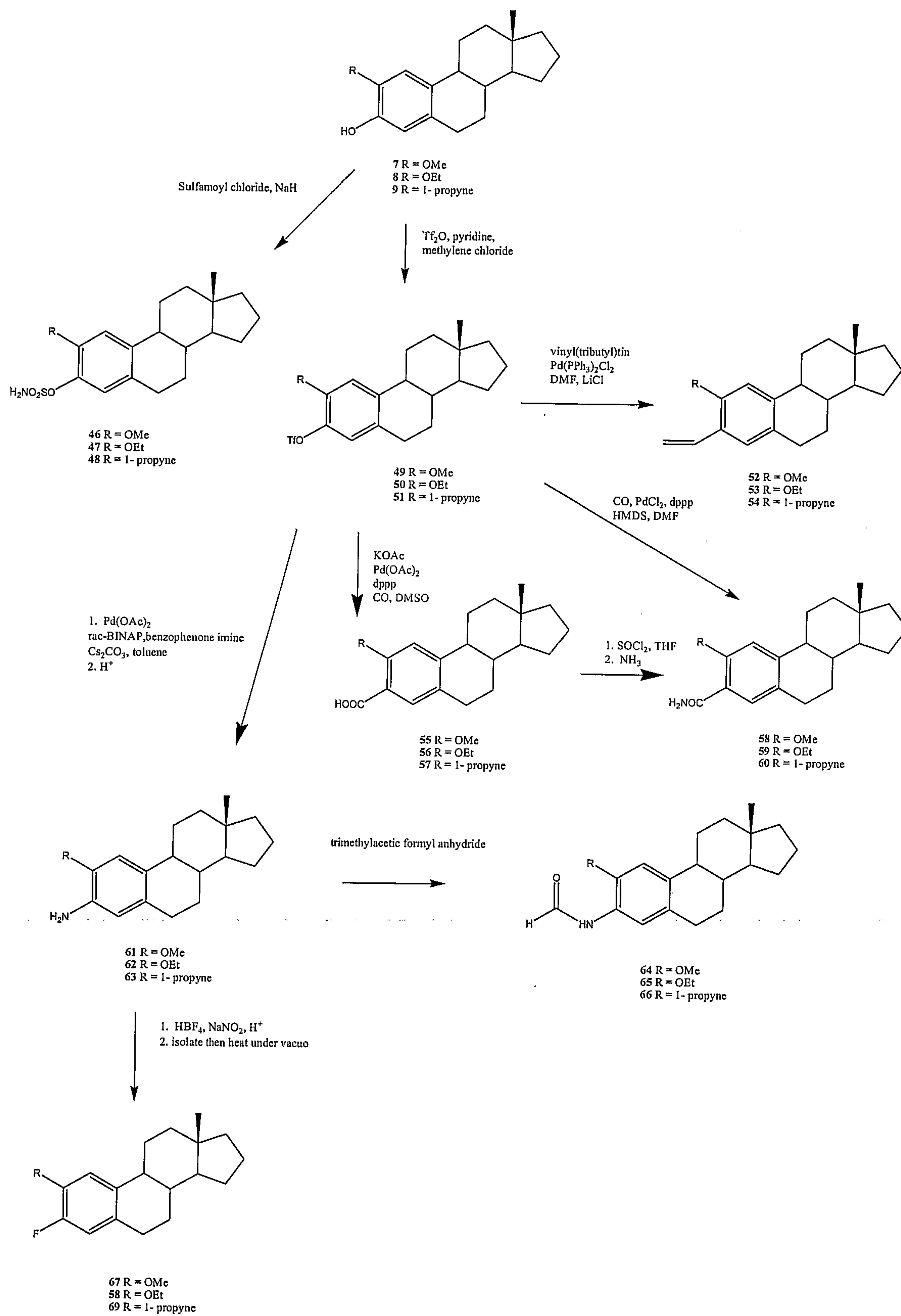
Scheme 2



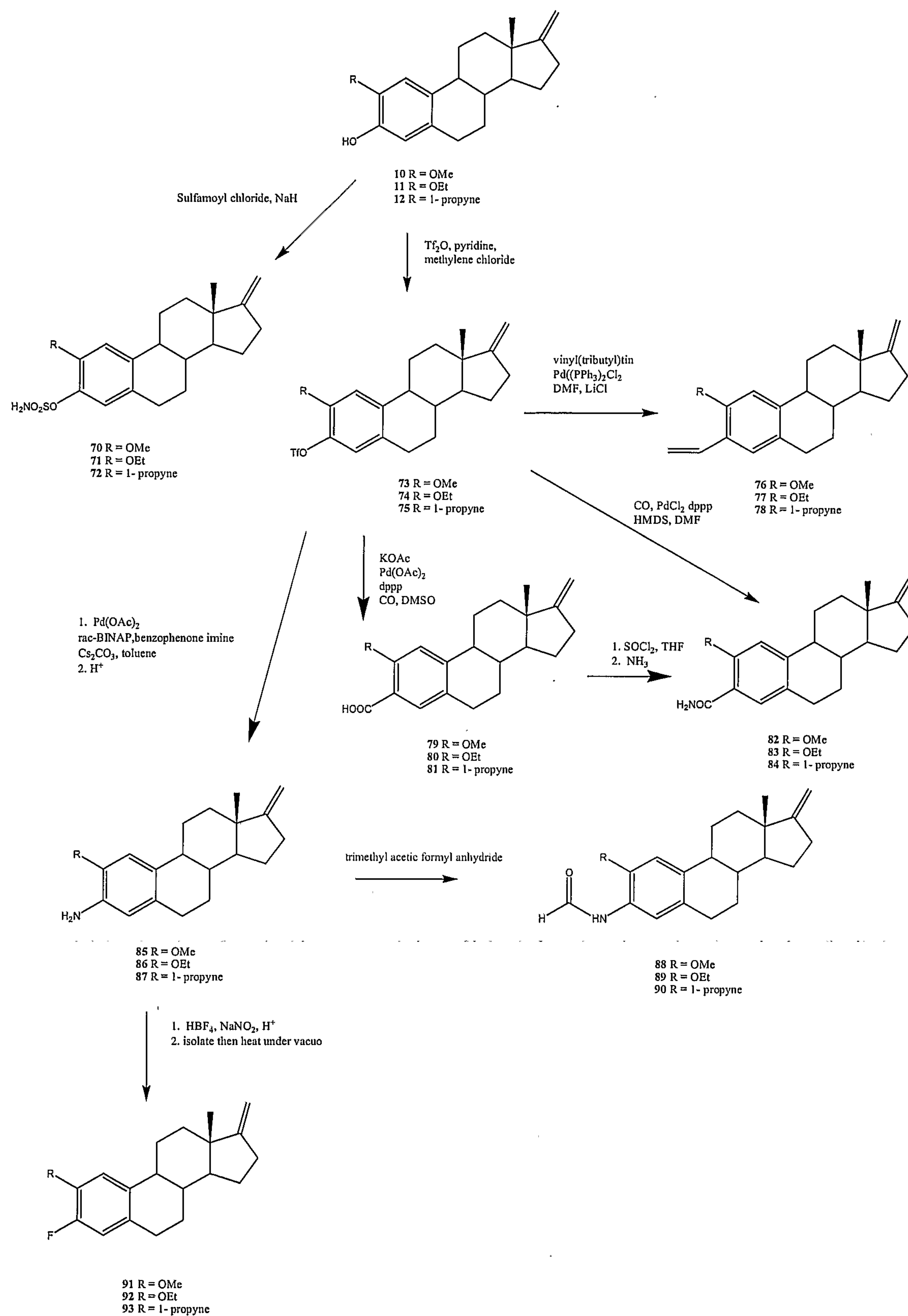
Scheme 3



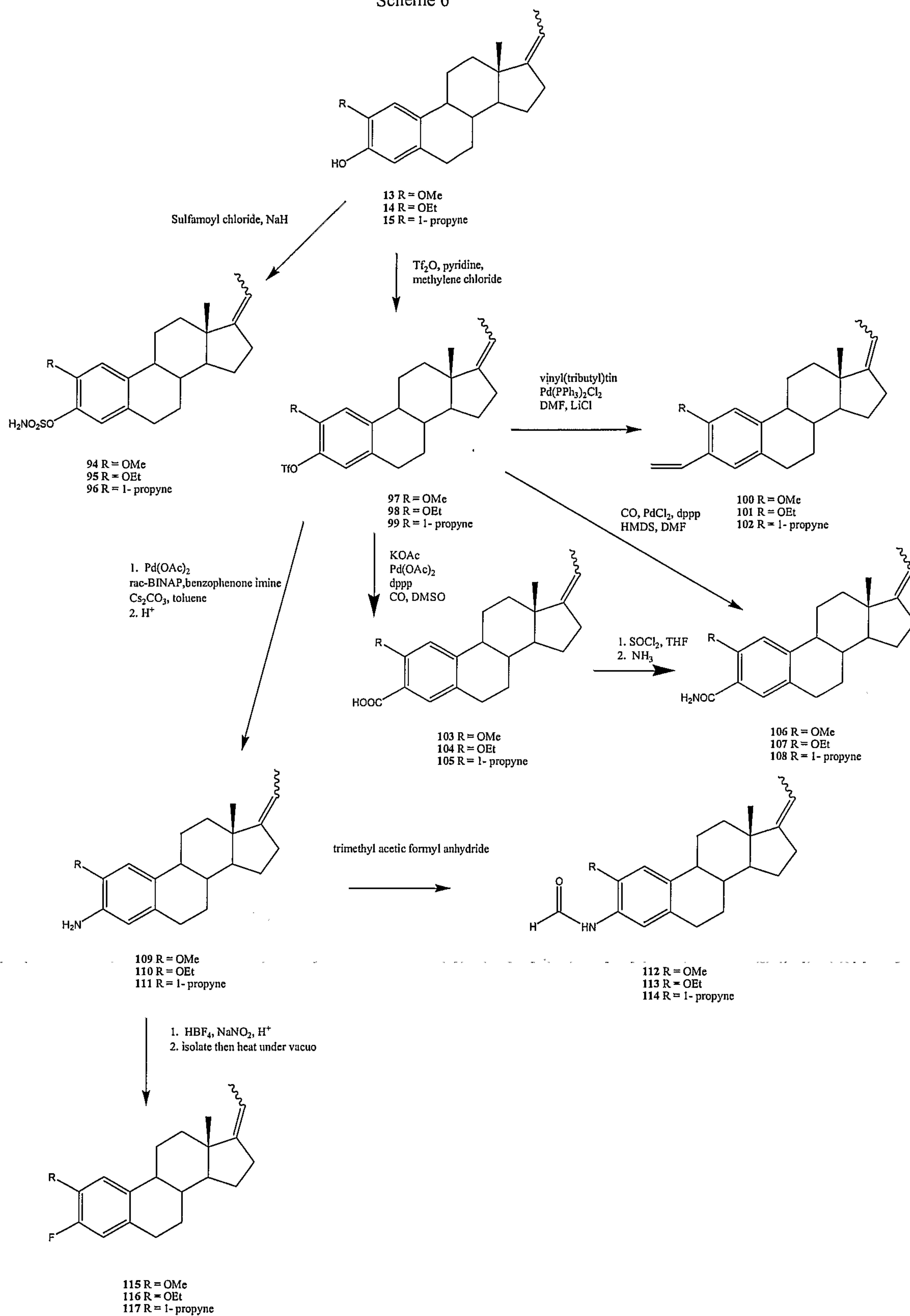
Scheme 4



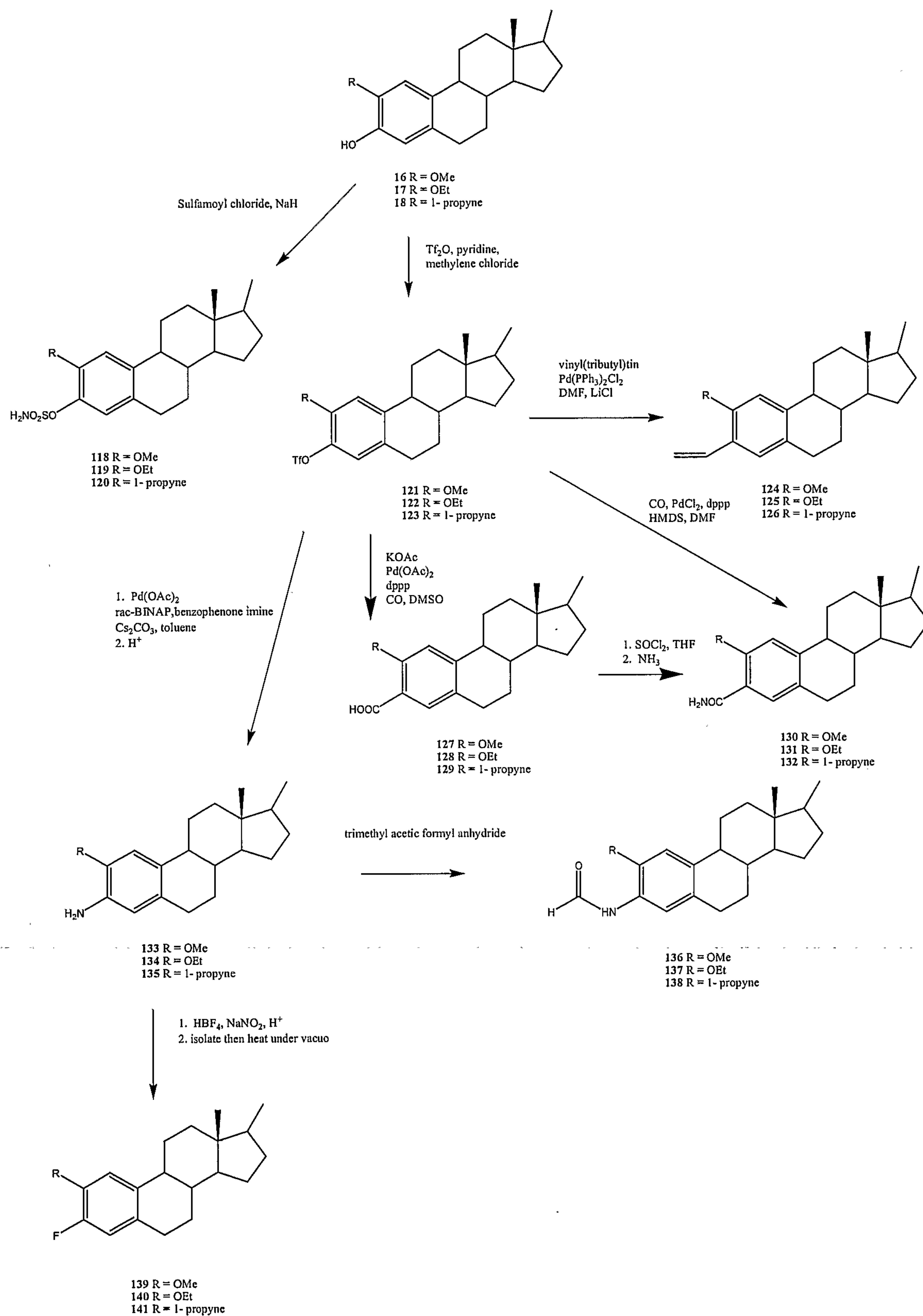
Scheme 5



Scheme 6



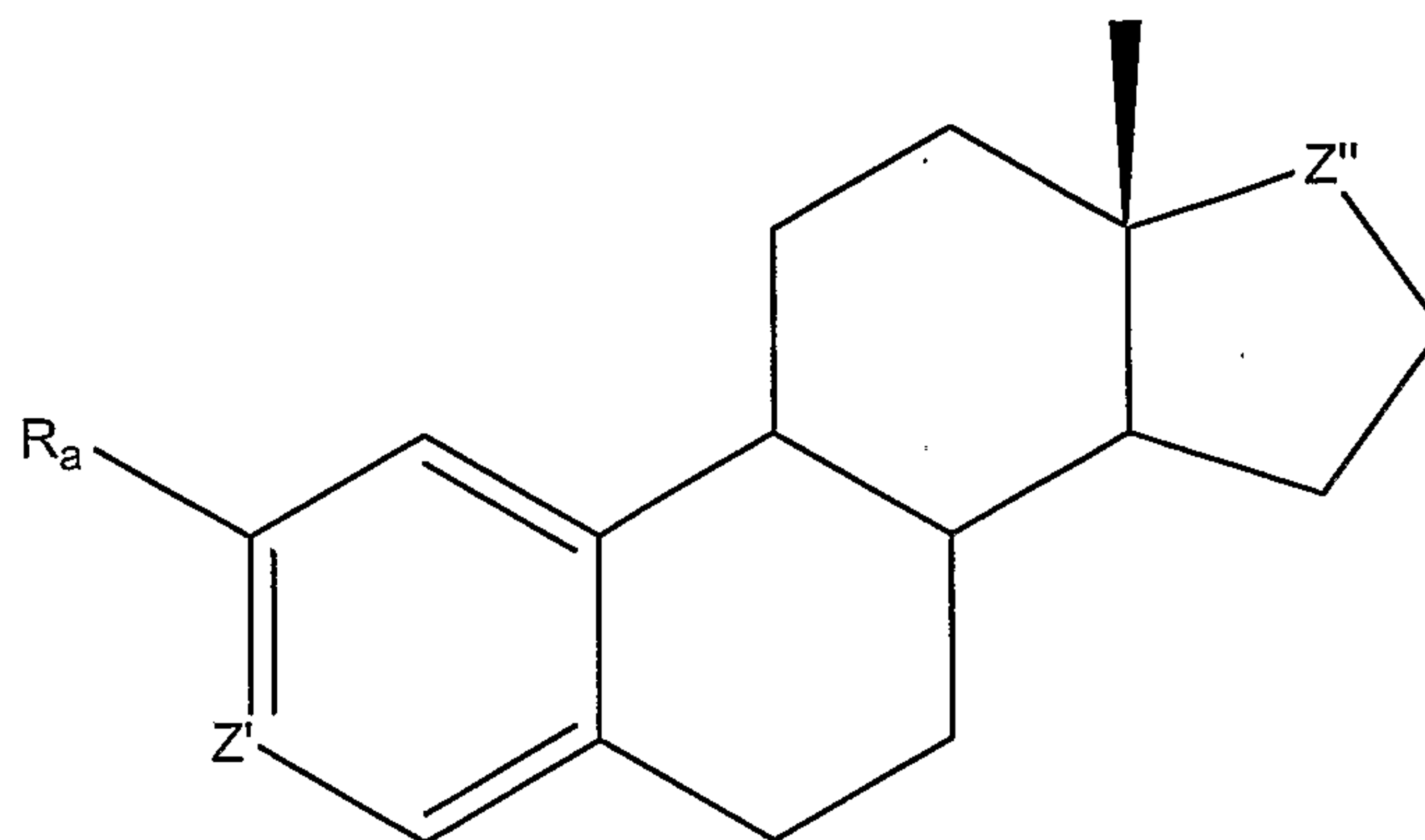
Scheme 7



5

EXPERIMENTAL DATA

The following Examples refer to compounds of the following general Formula I:



10

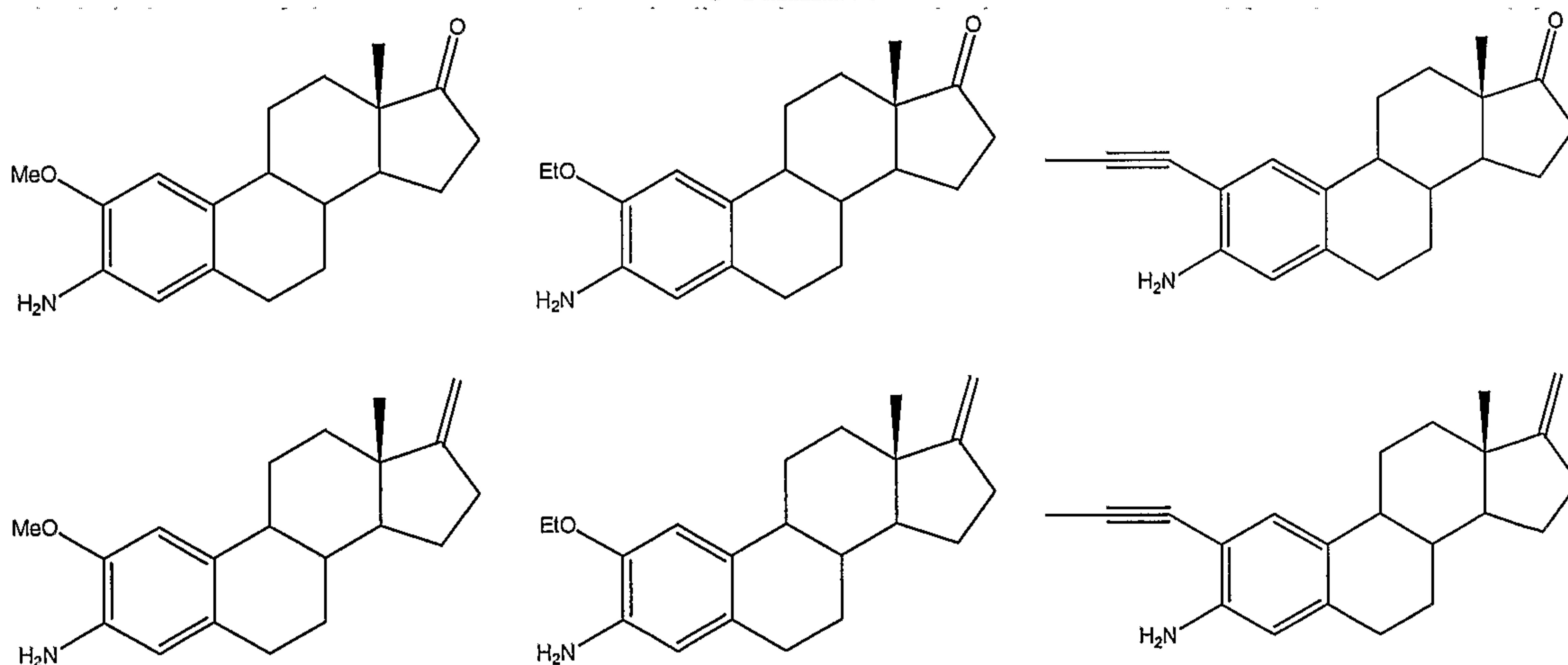
(I)

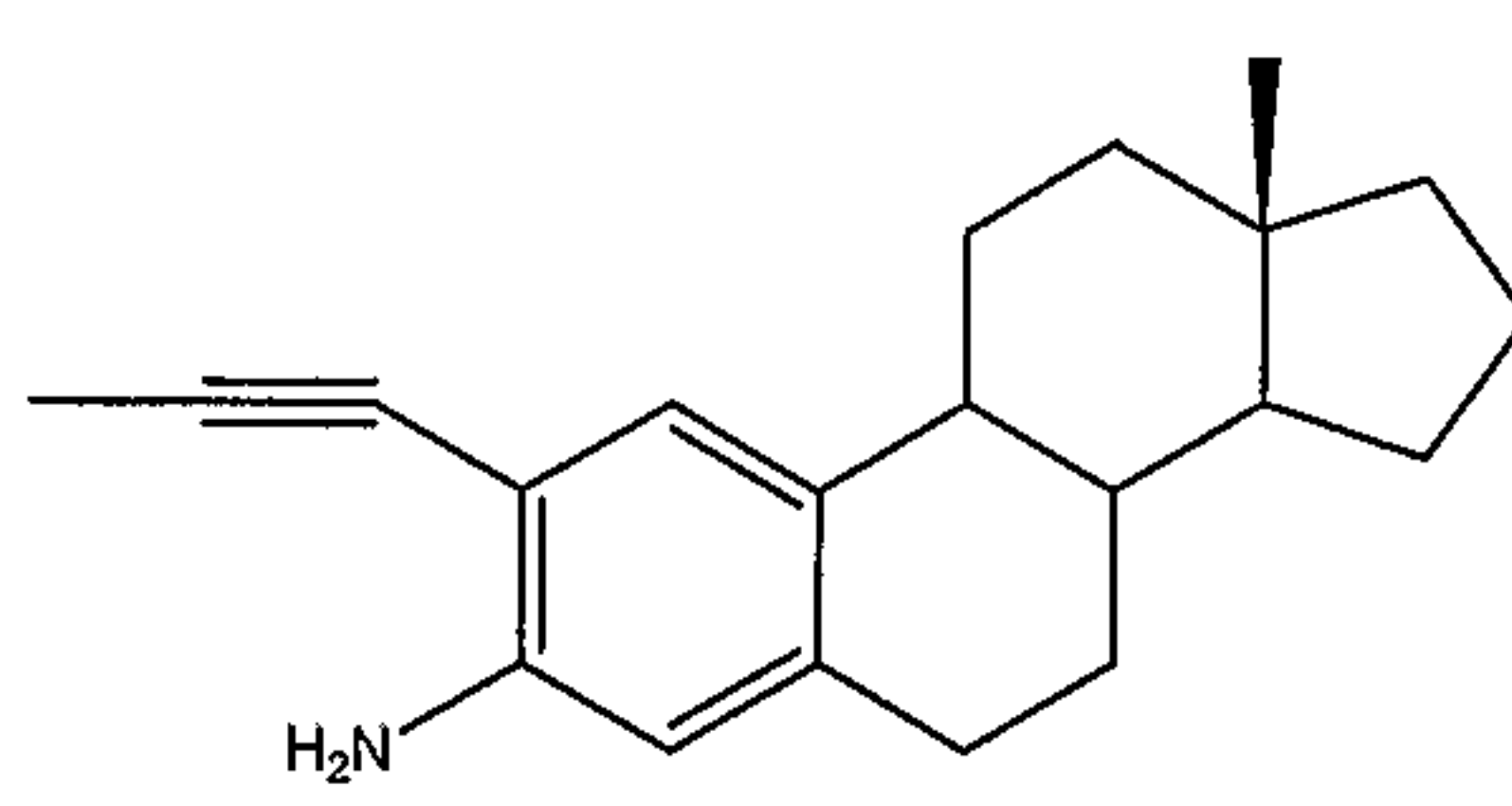
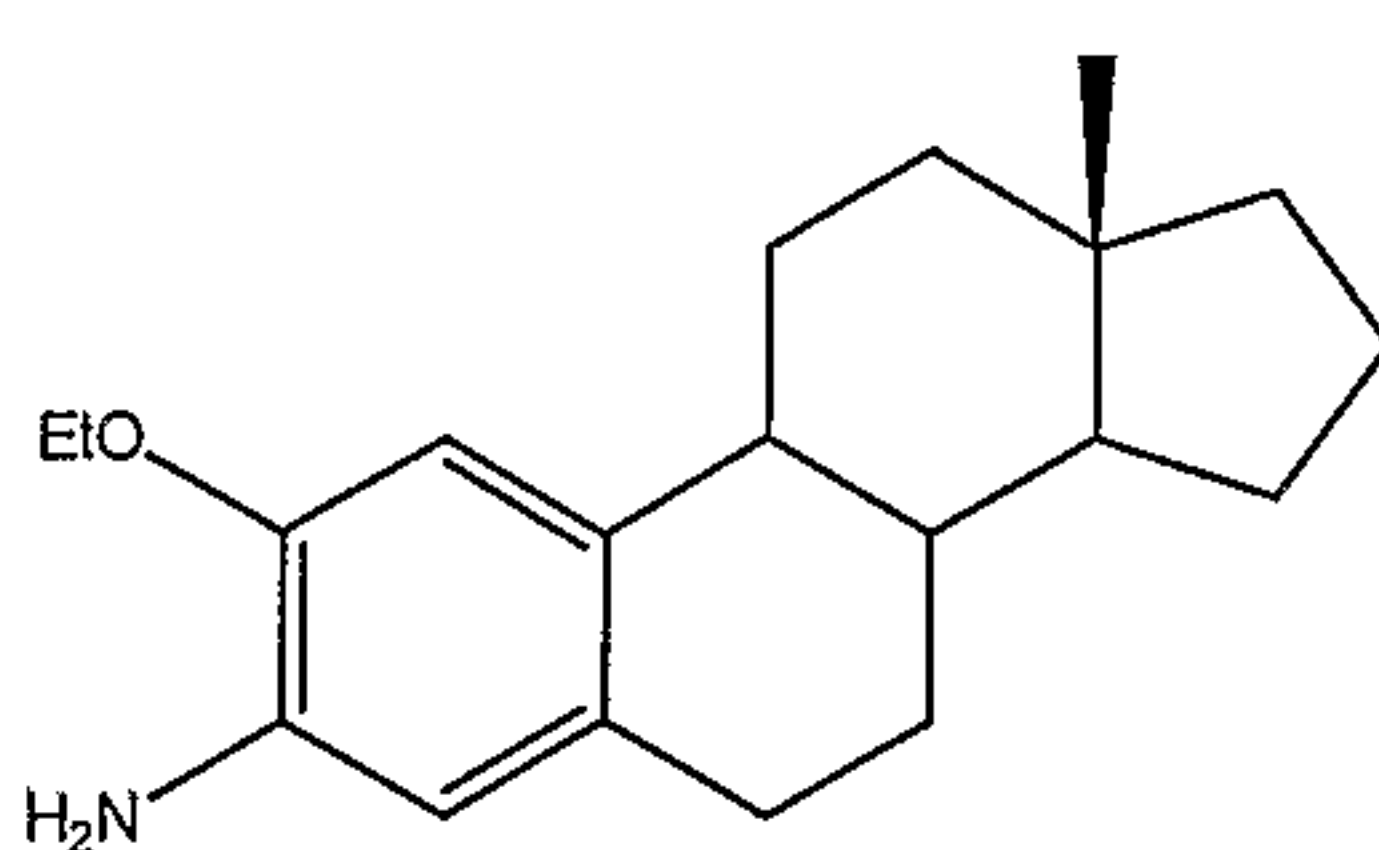
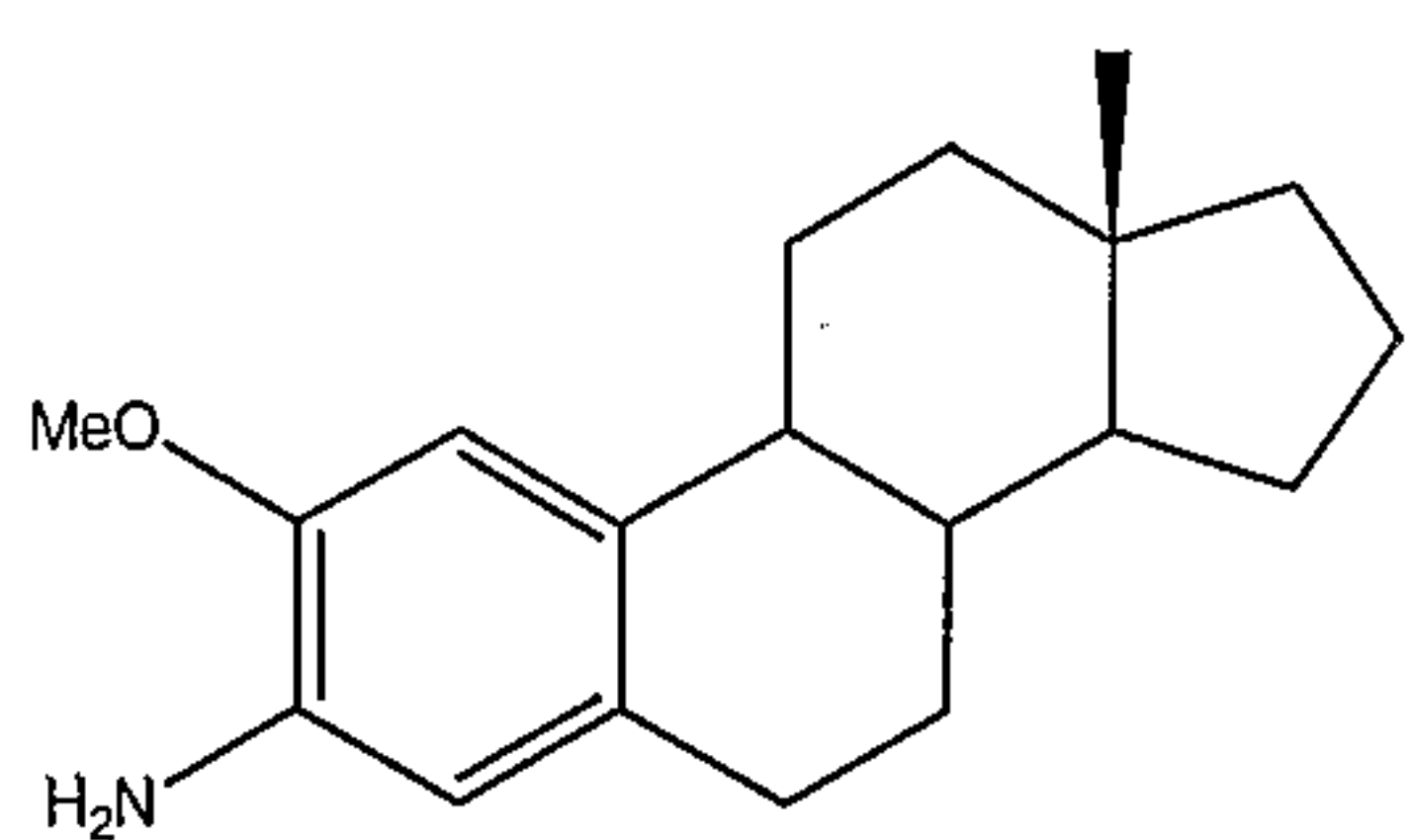
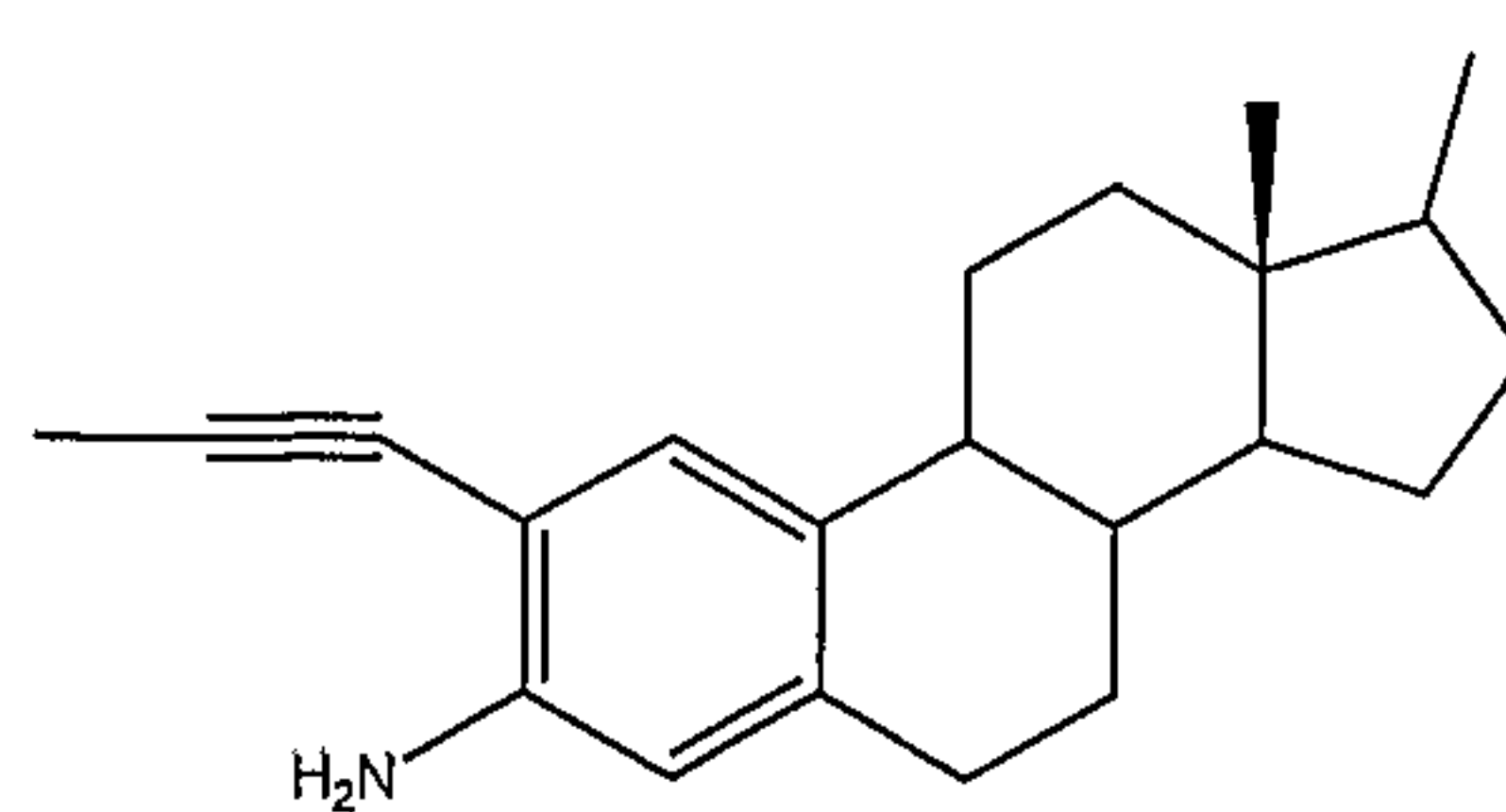
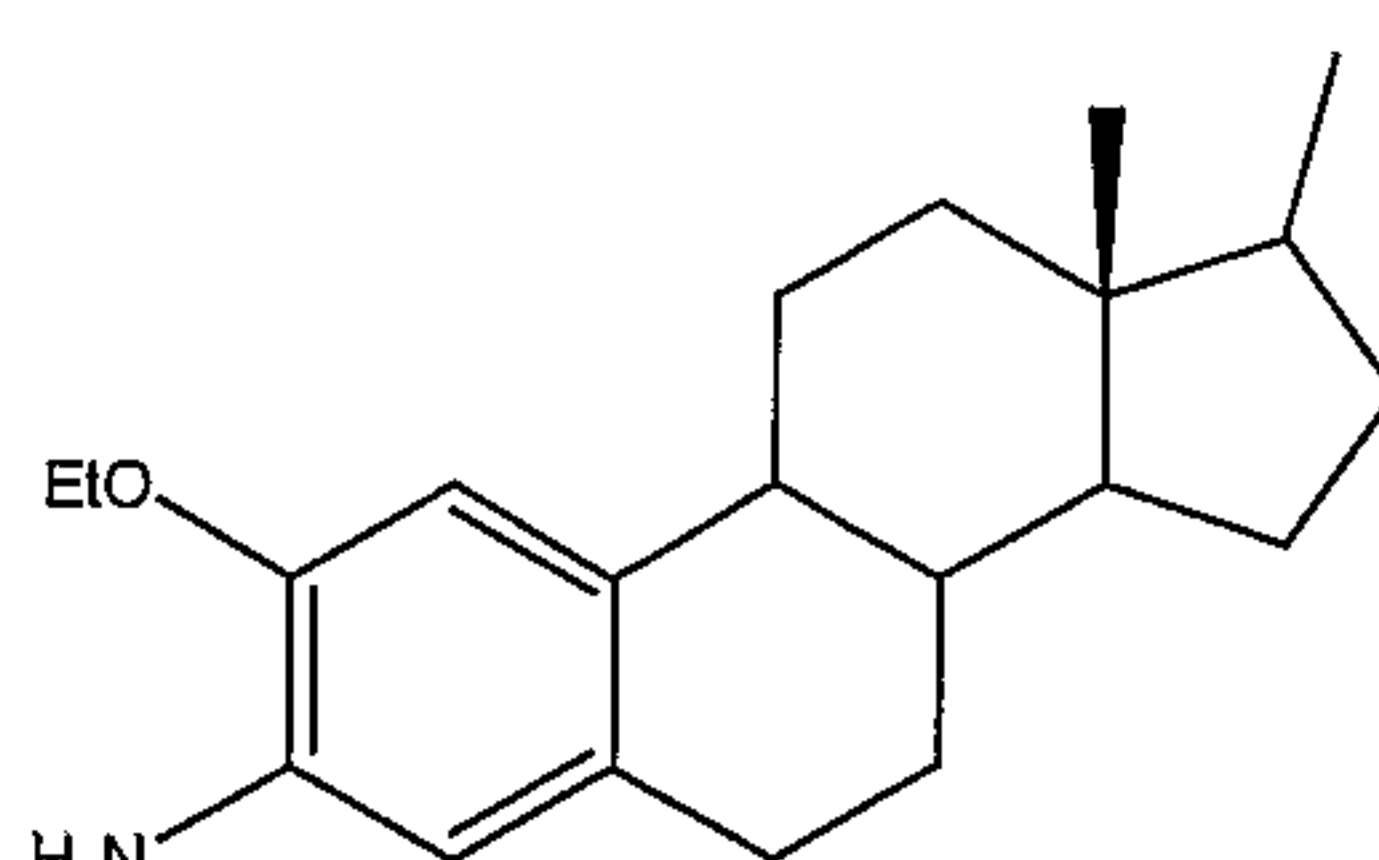
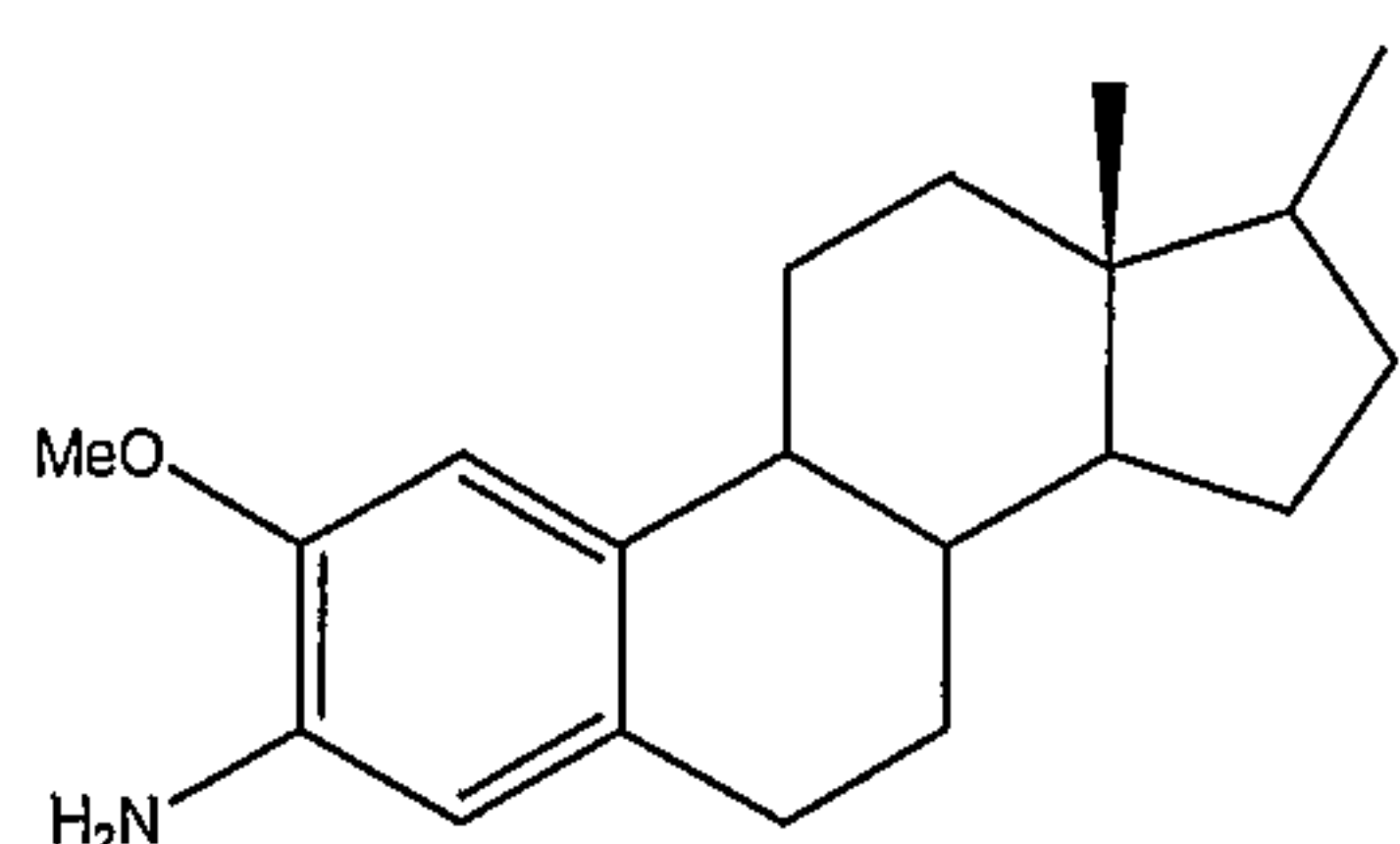
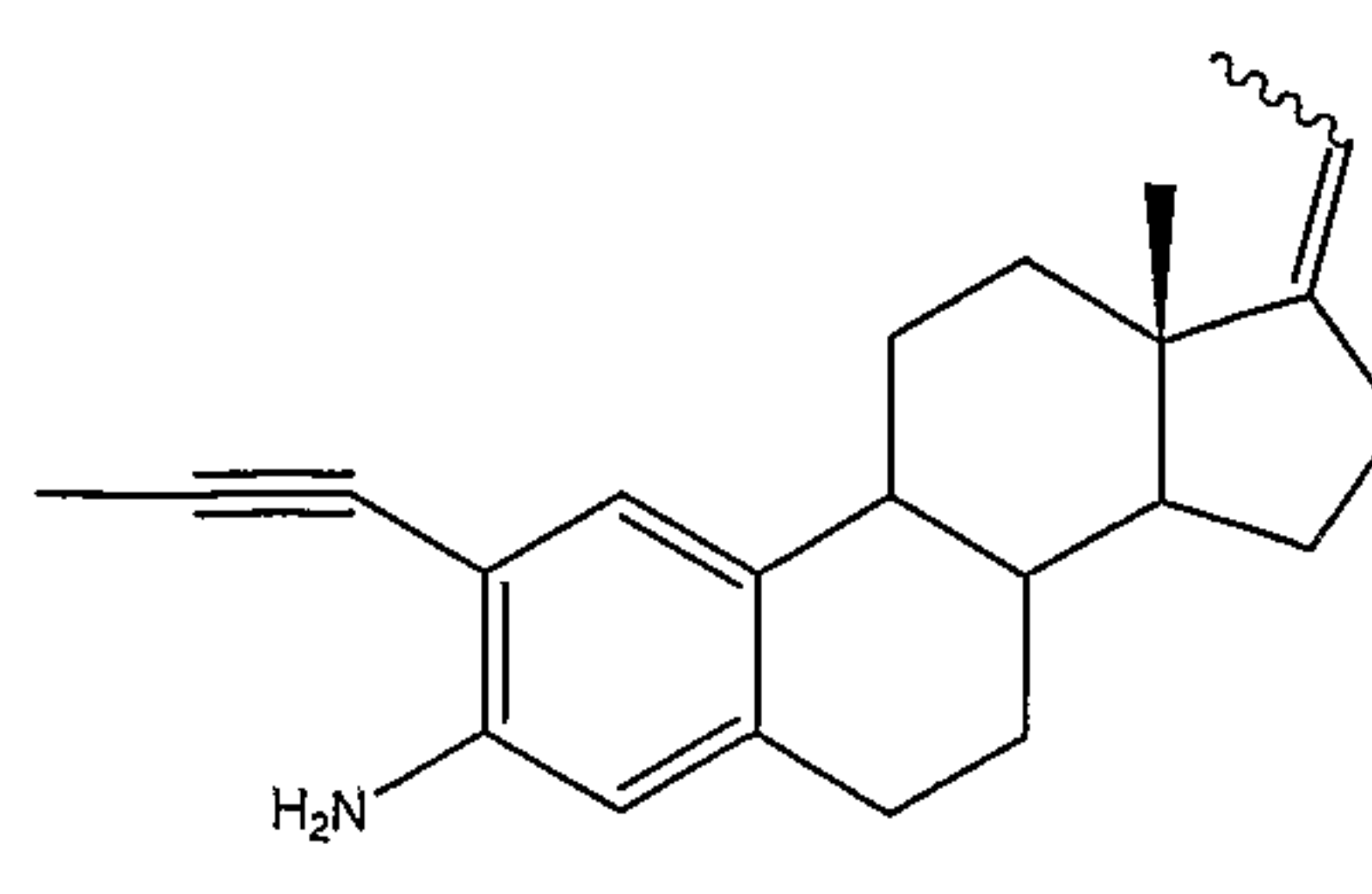
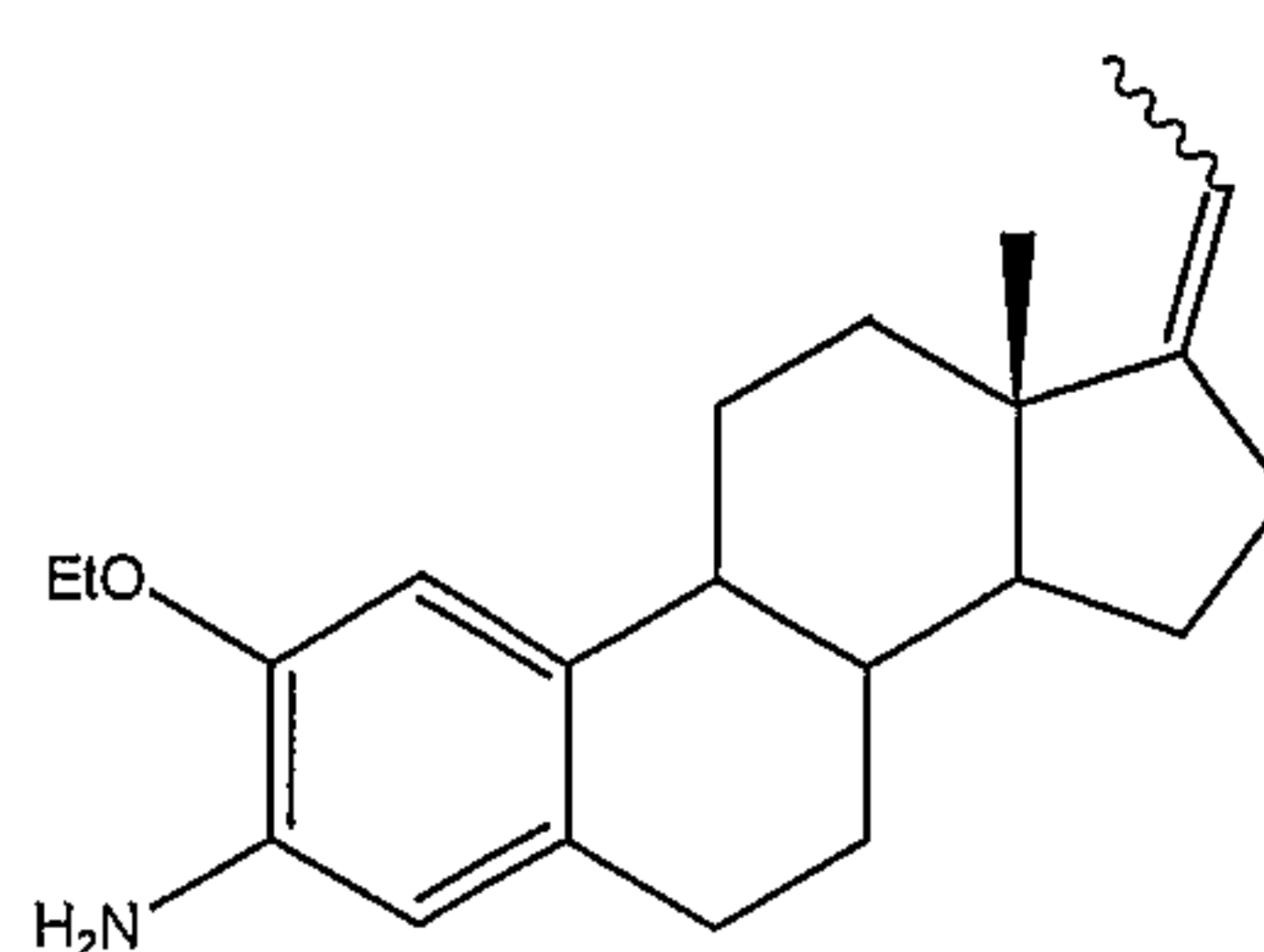
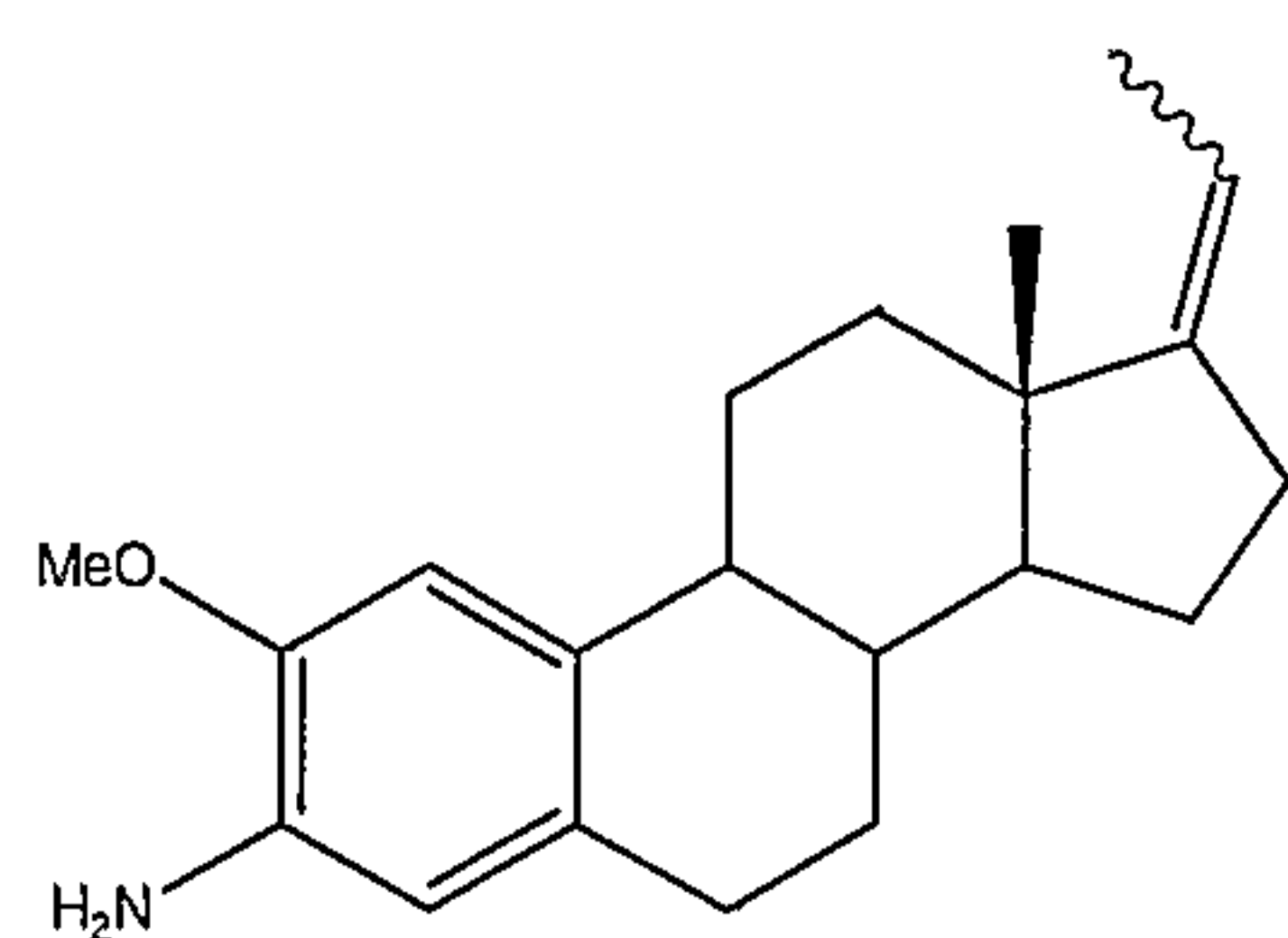
wherein R_a is selected from $-\text{OCH}_3$, $-\text{OCH}_2\text{CH}_3$ or $-\text{CCCH}_3$; Z' is selected from $>\text{C}-\text{F}$, $>\text{C}-\text{NH}_2$, $>\text{CCONH}_2$, $>\text{C}-\text{NHCOH}$, $>\text{C}-\text{OSO}_2\text{NH}_2$ or $>\text{C}-\text{CHCH}_2$, and Z'' is selected from $>\text{C}(\text{H}_2)$, $>\text{C}(\text{H})-\text{CH}_3$, $>\text{C}=\text{CH}_2$, $>\text{C}=\text{CHCH}_3$ or $>\text{C}=\text{O}$; provided that when Z' is $>\text{C}-\text{OSO}_2\text{NH}_2$ and Z'' is $>\text{C}(\text{H}_2)$ or $>\text{C}=\text{O}$, R_a is neither $-\text{OCH}_3$ nor $-\text{OCH}_2\text{CH}_3$. Preferred species from the foregoing genus that are useful in the present invention include, but are not limited to, the compounds shown in Table I.

15

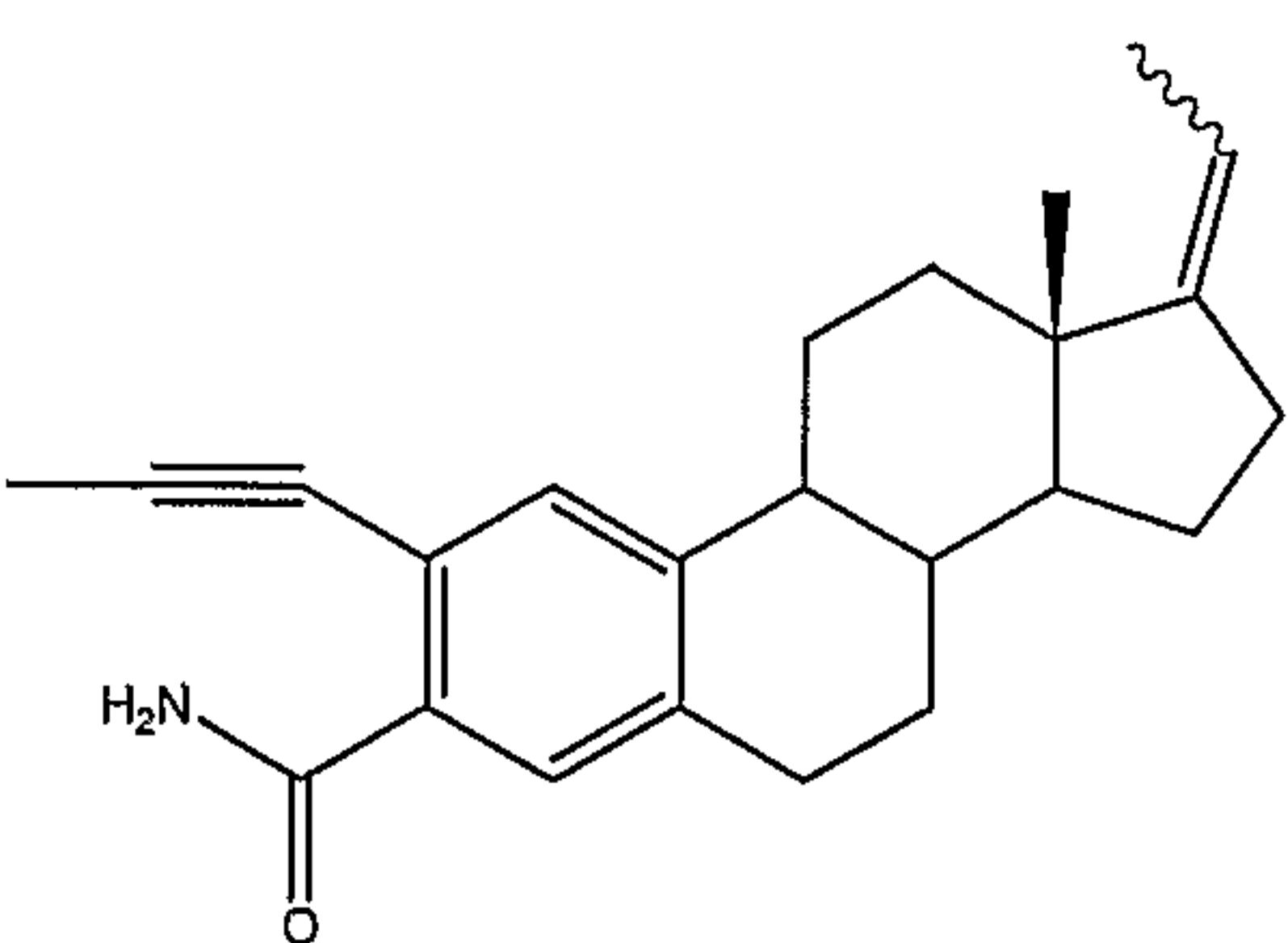
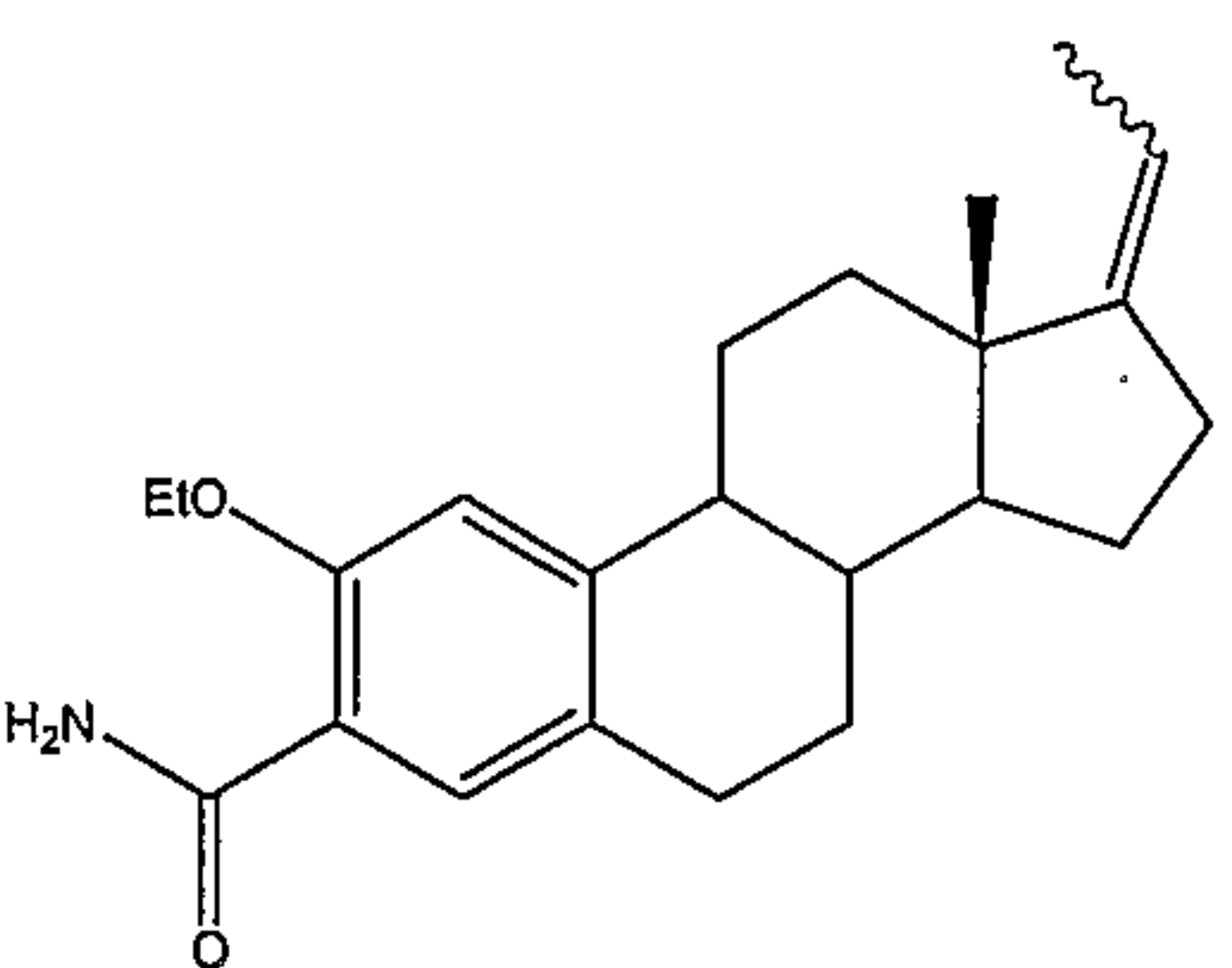
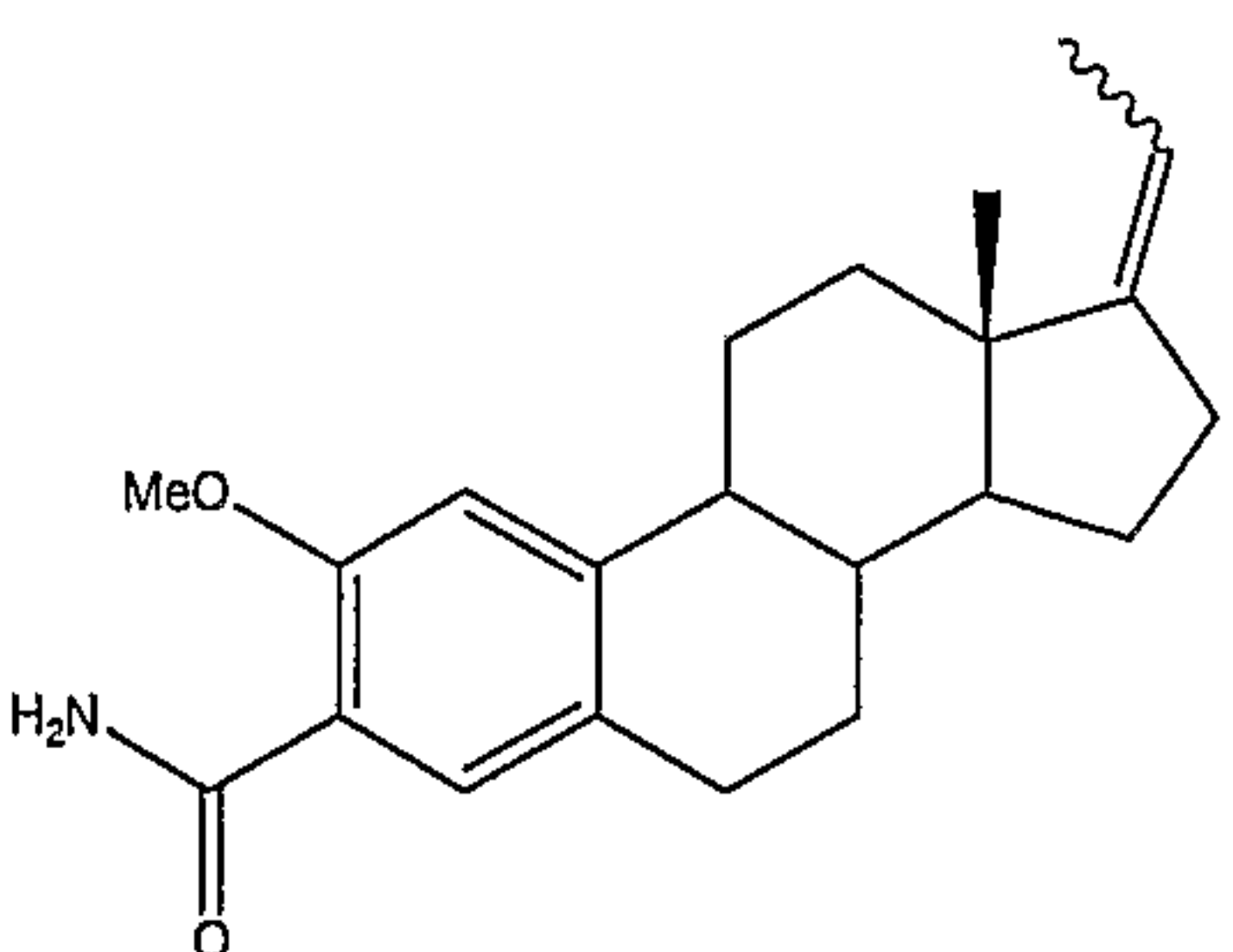
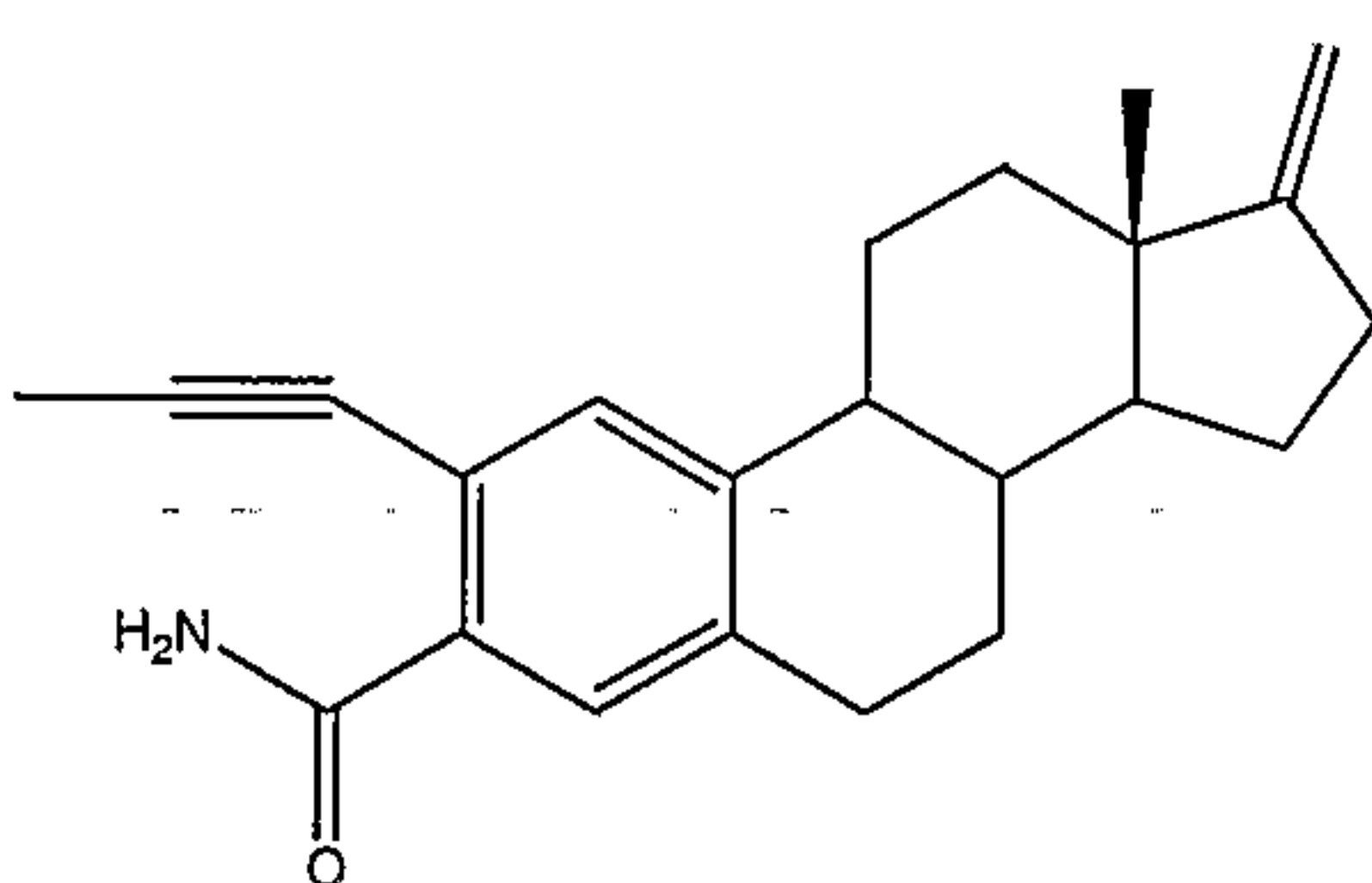
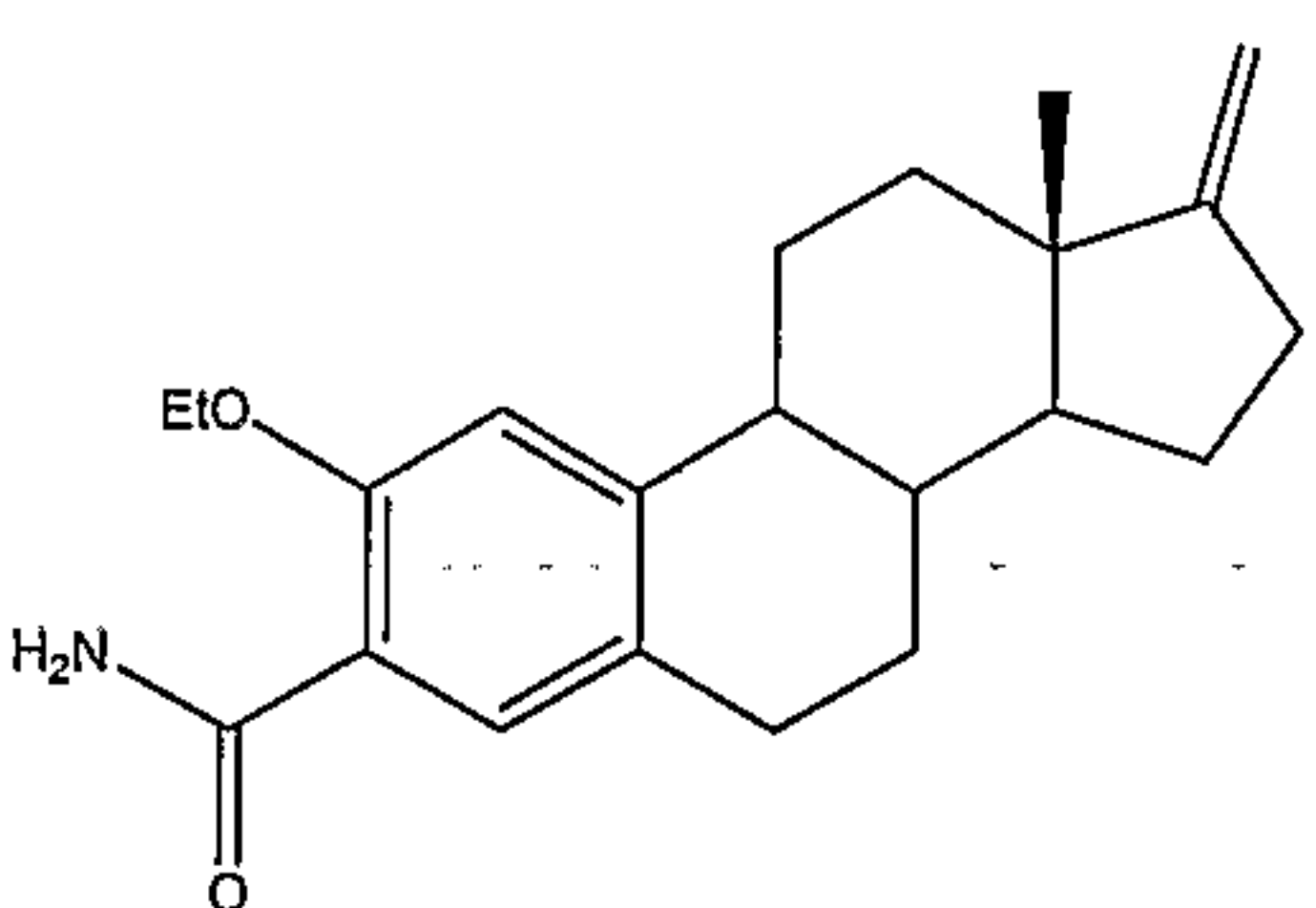
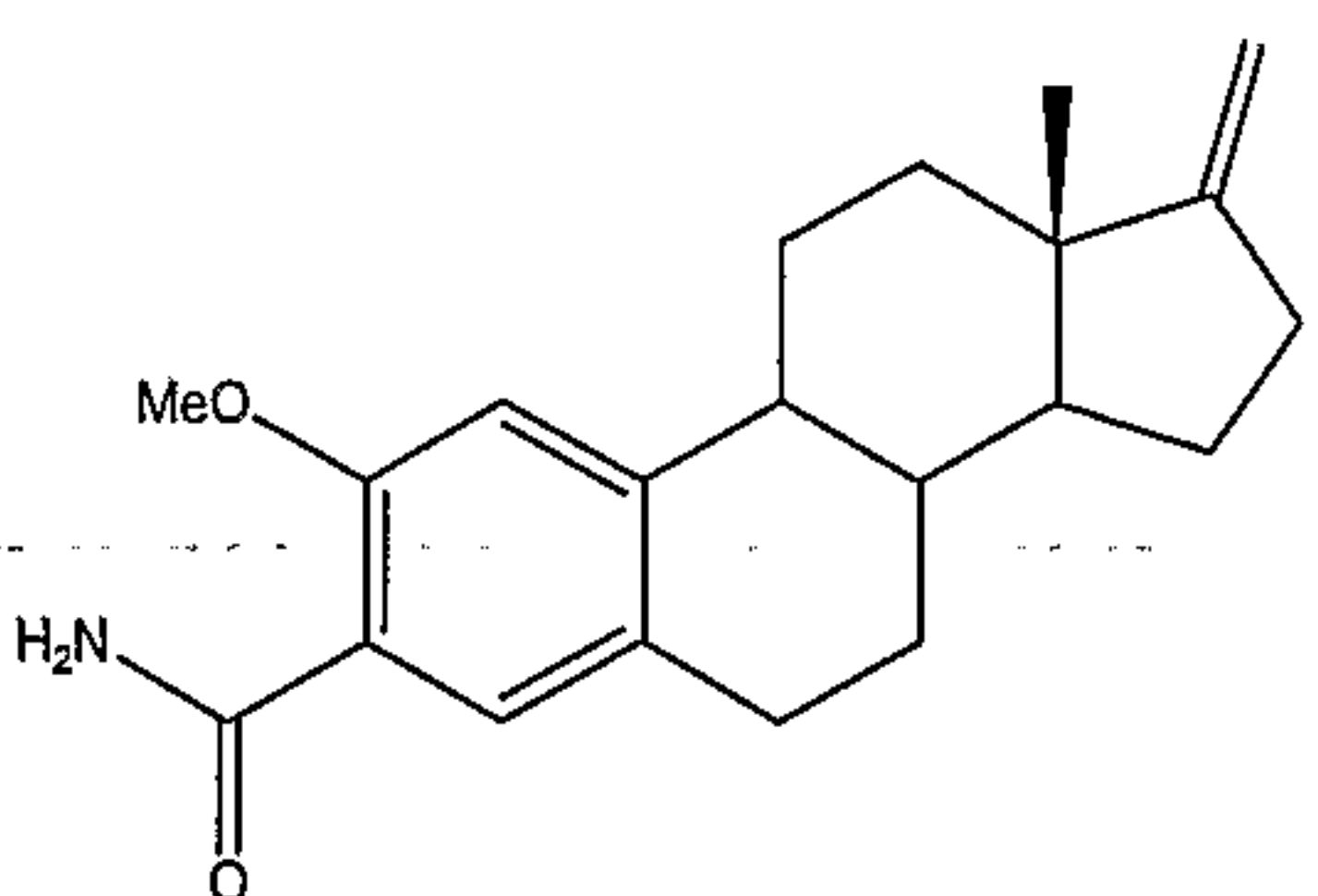
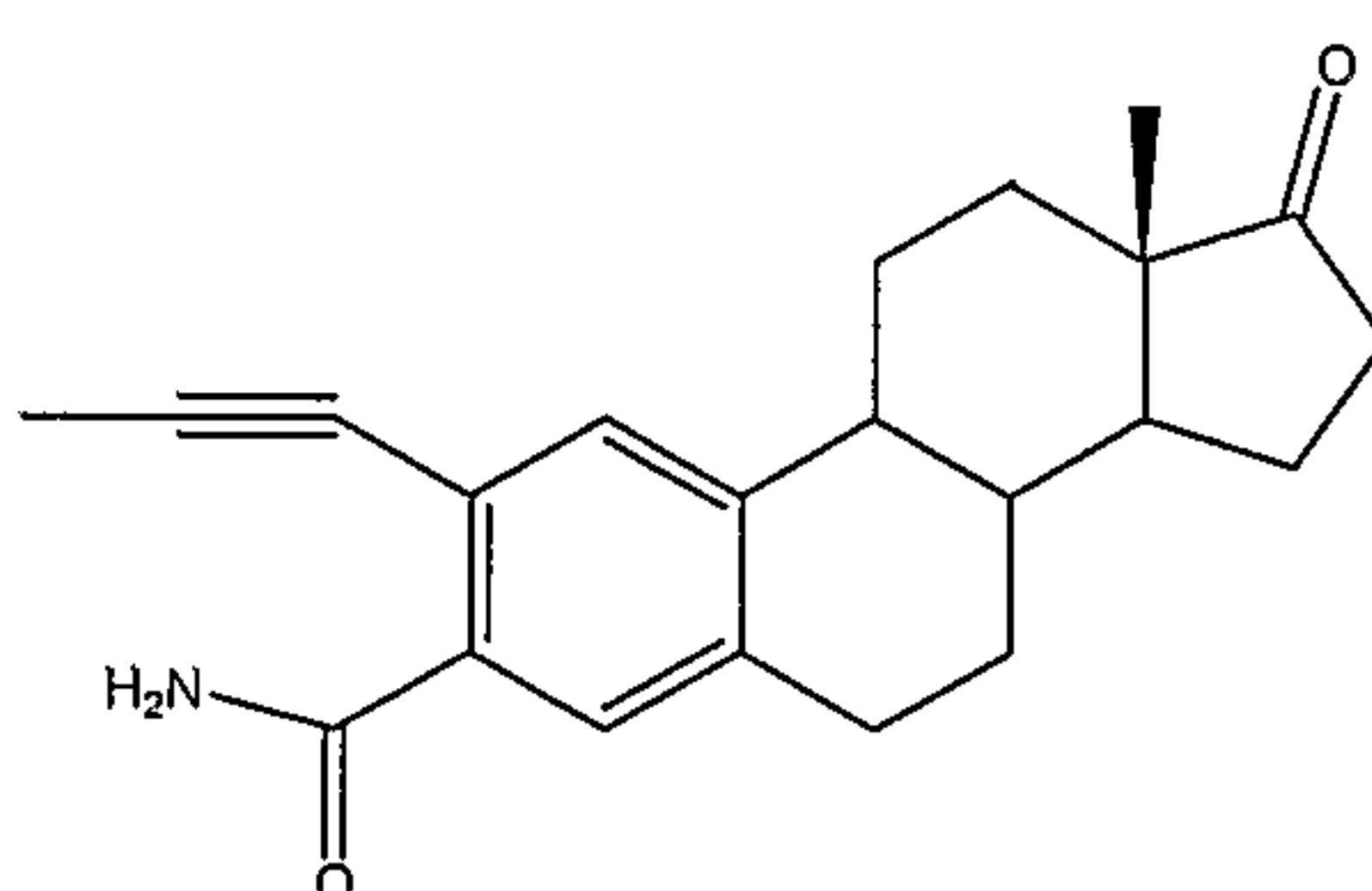
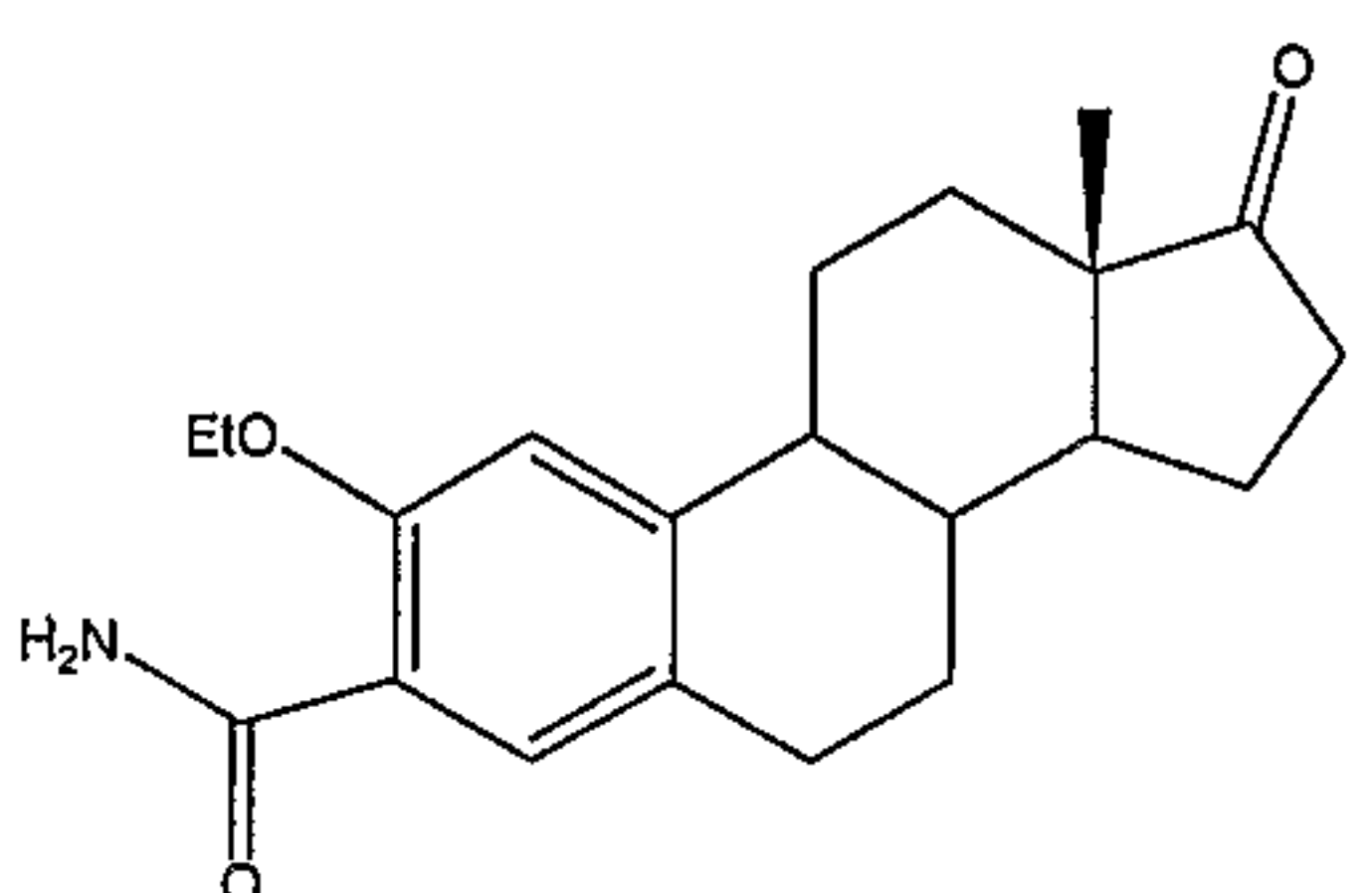
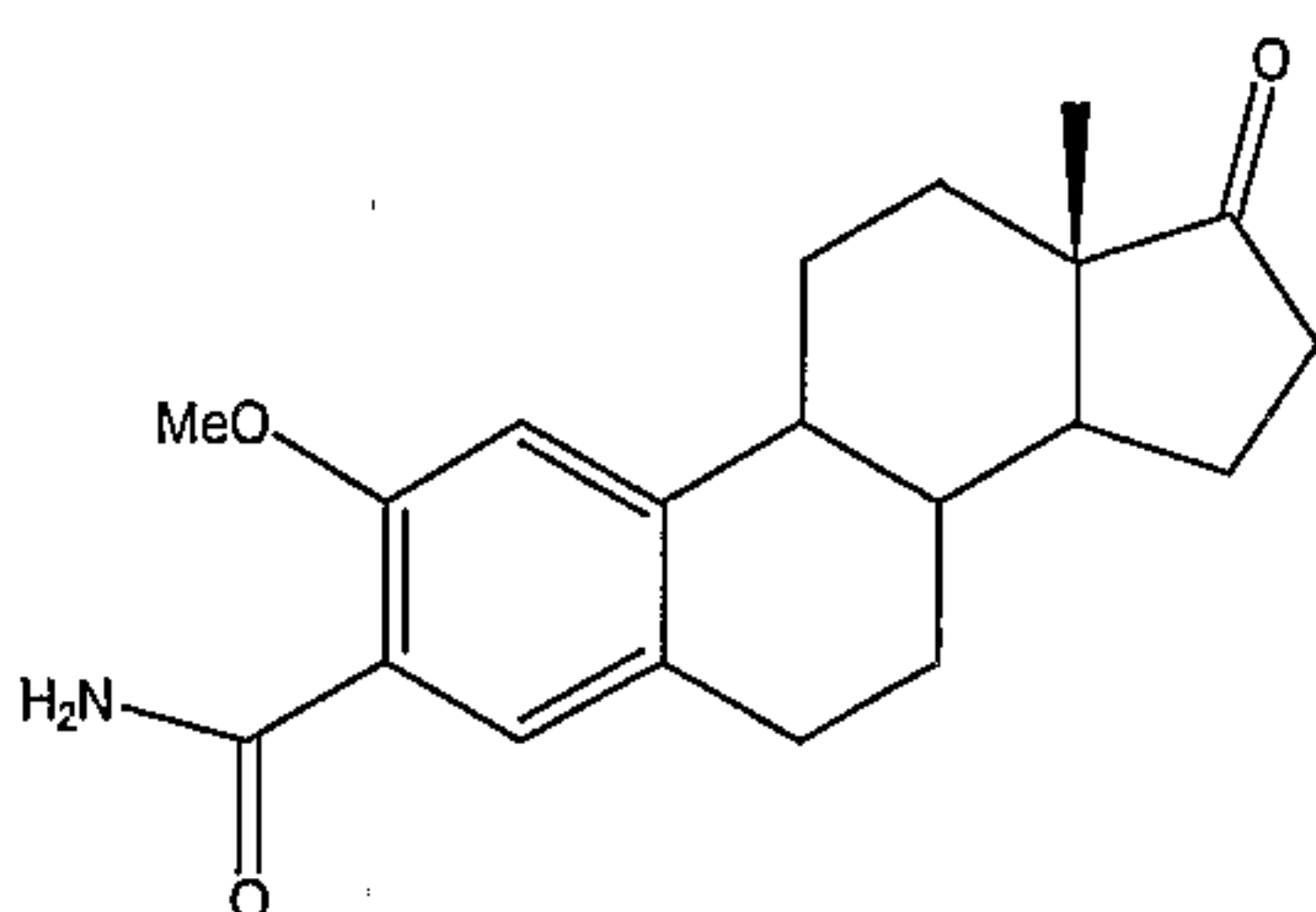
TABLE I

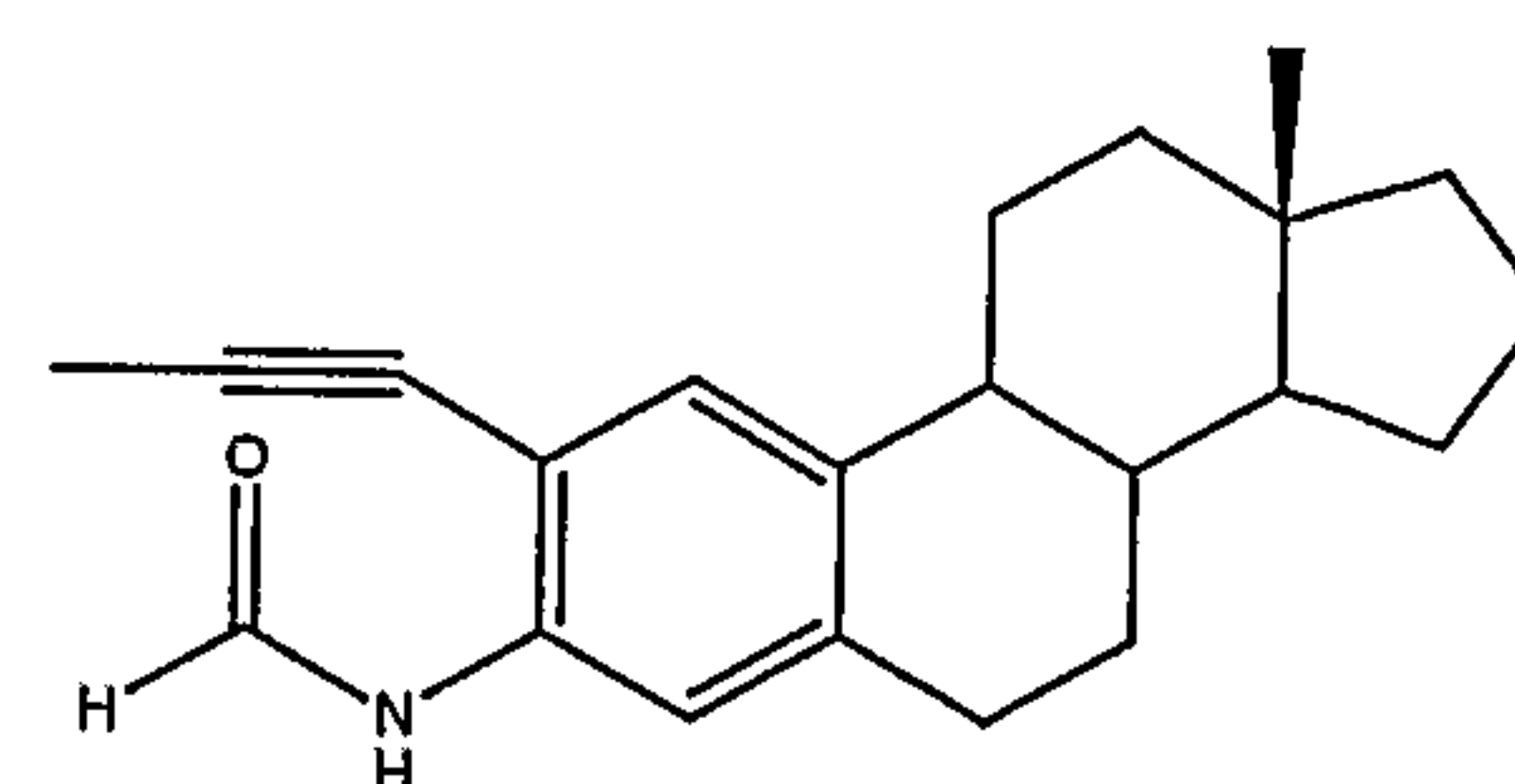
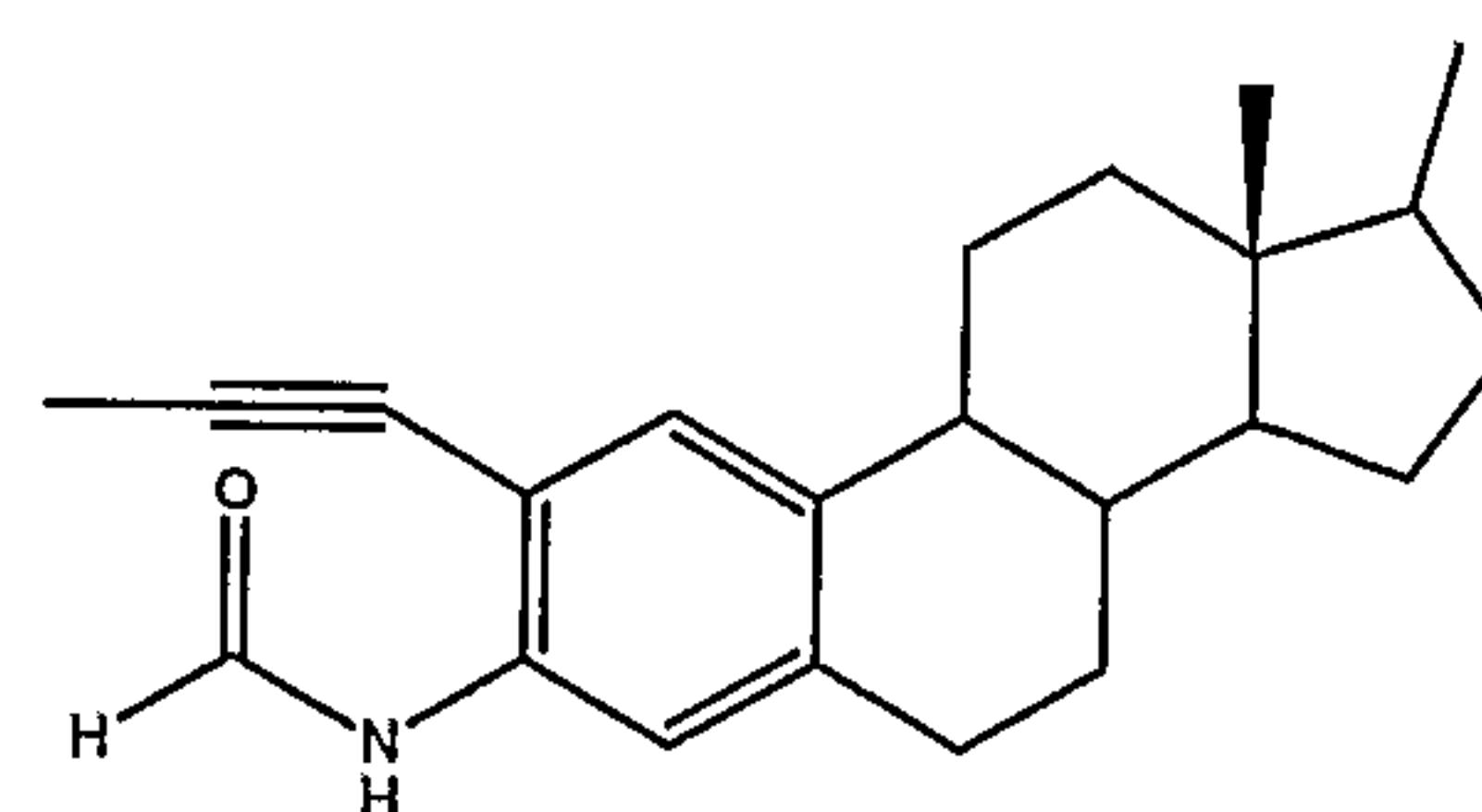
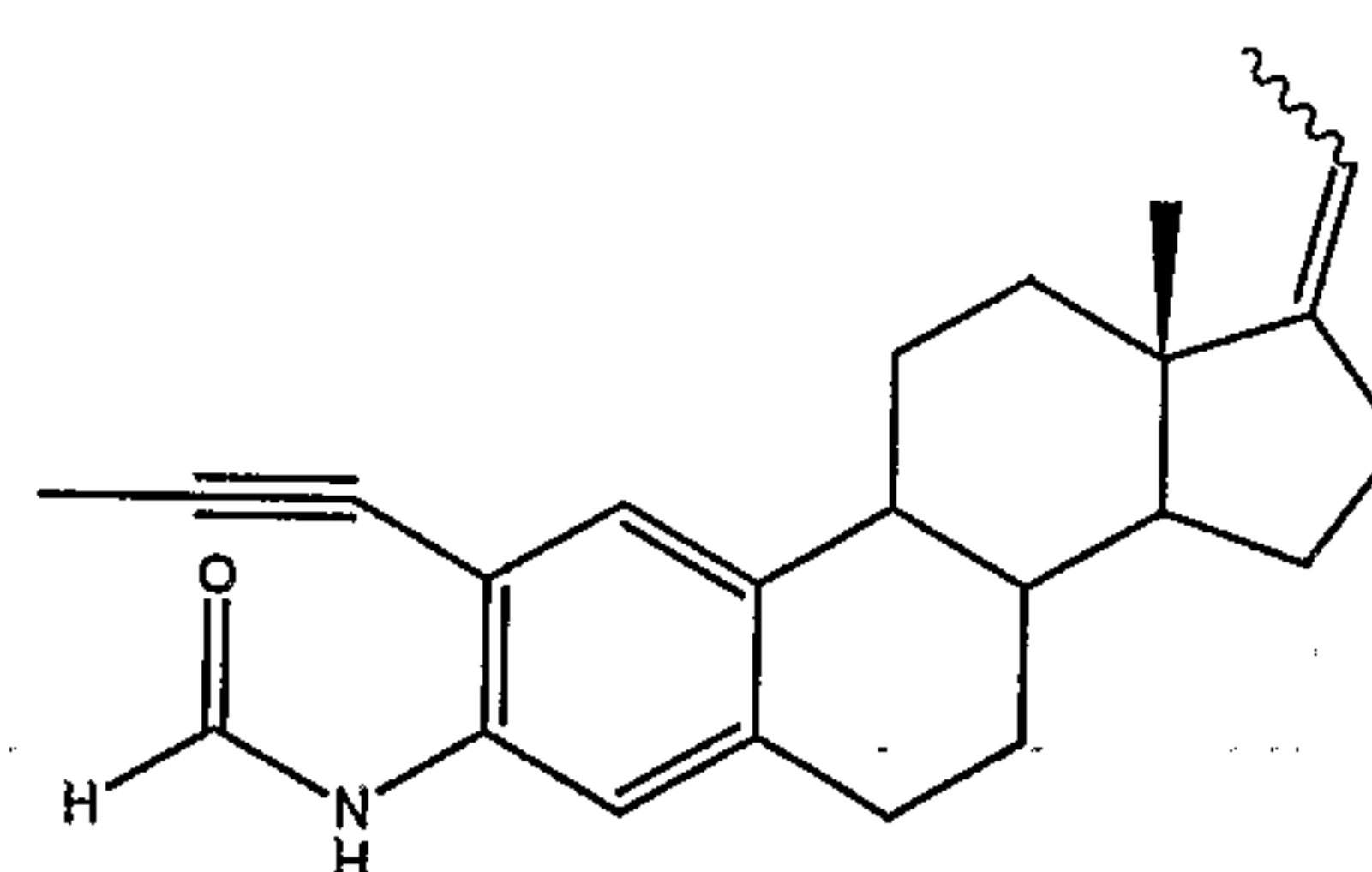
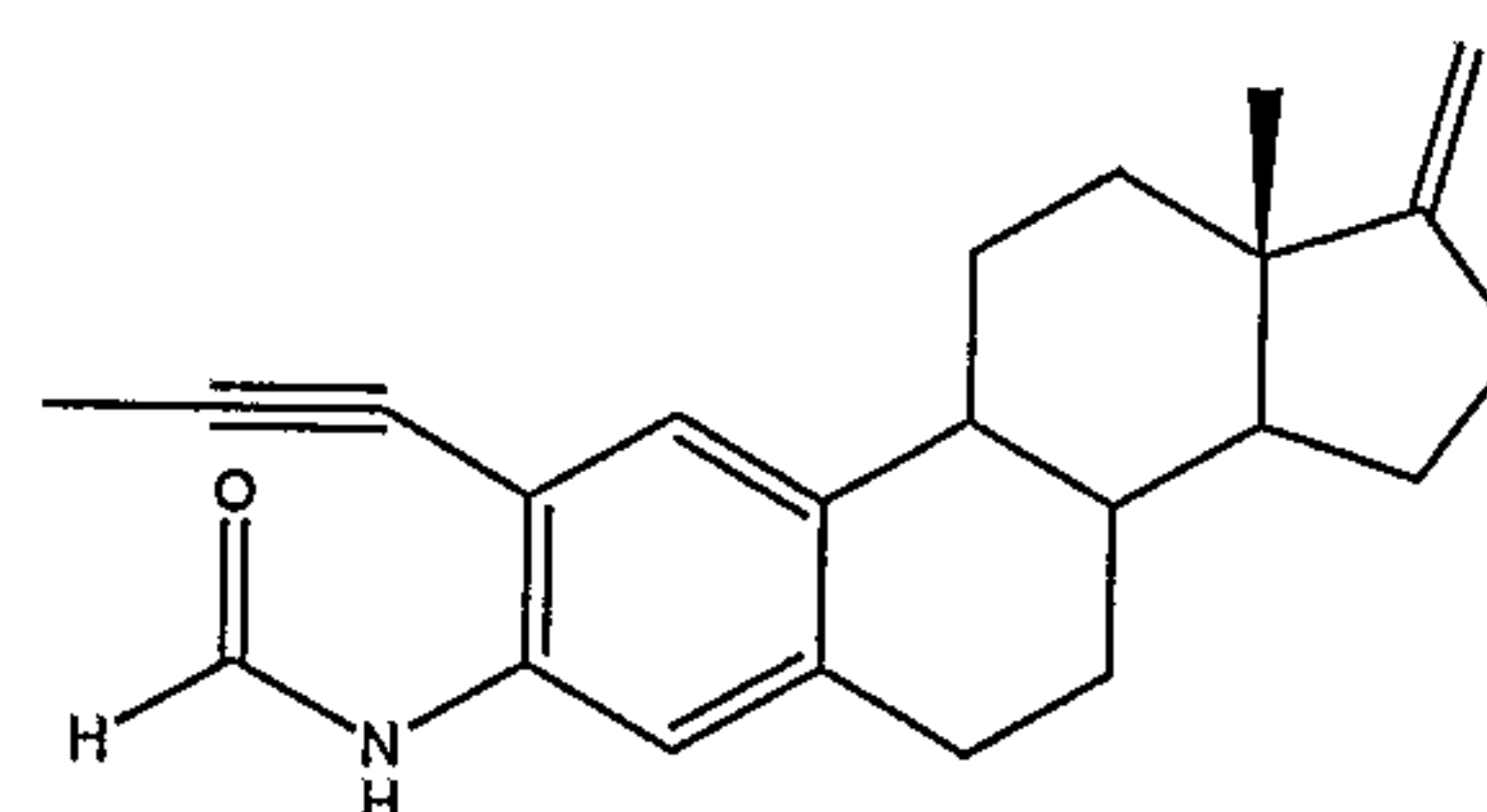
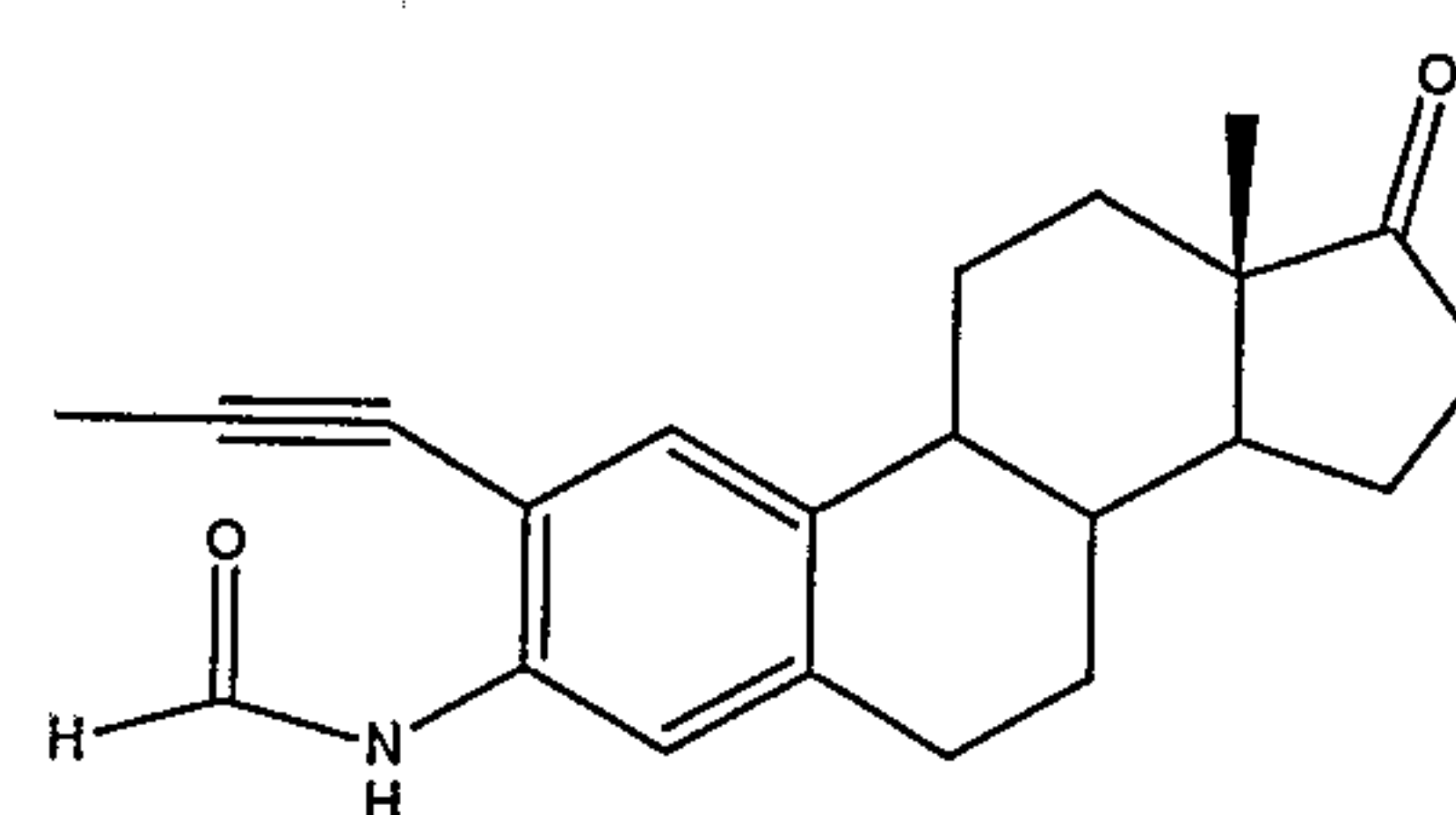
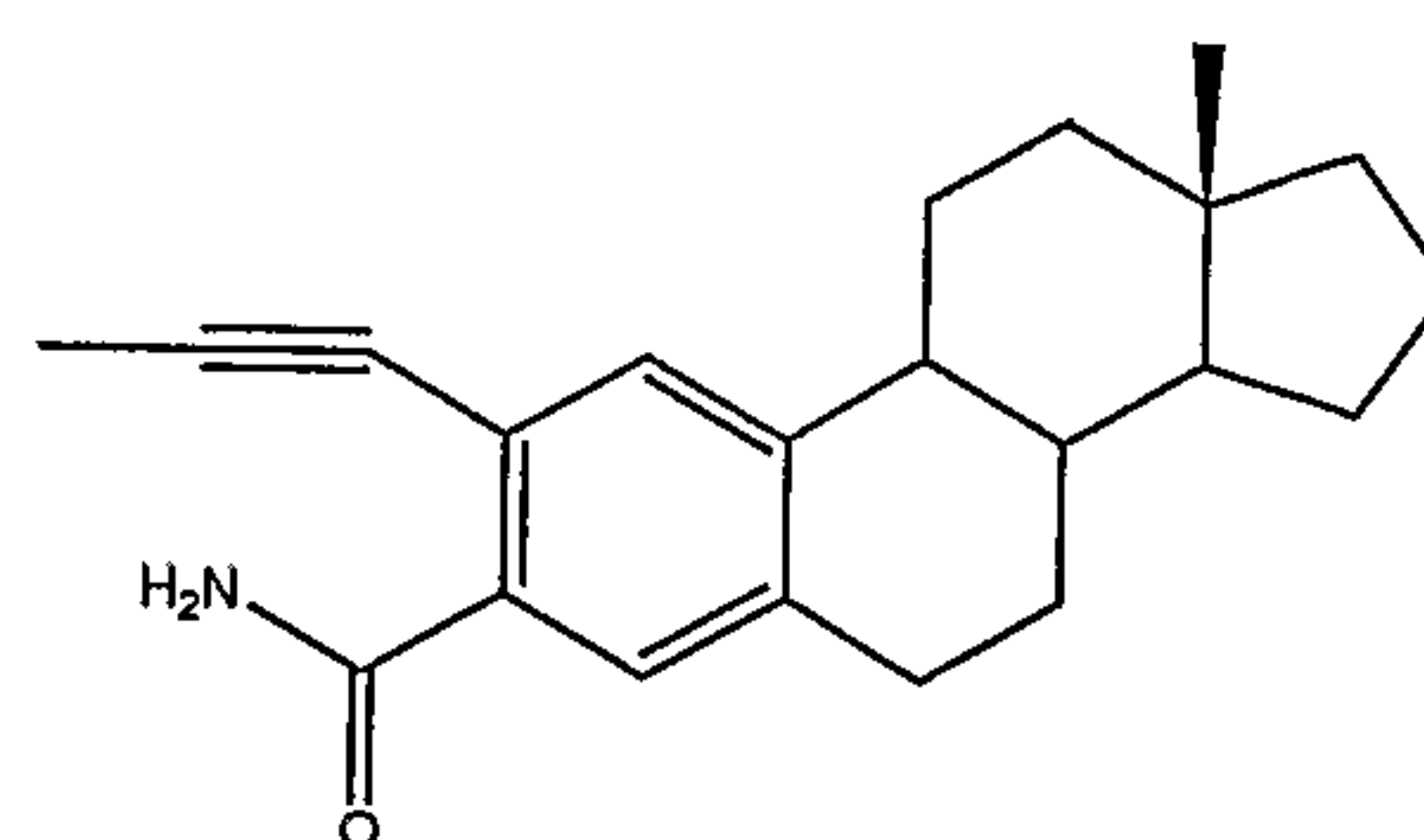
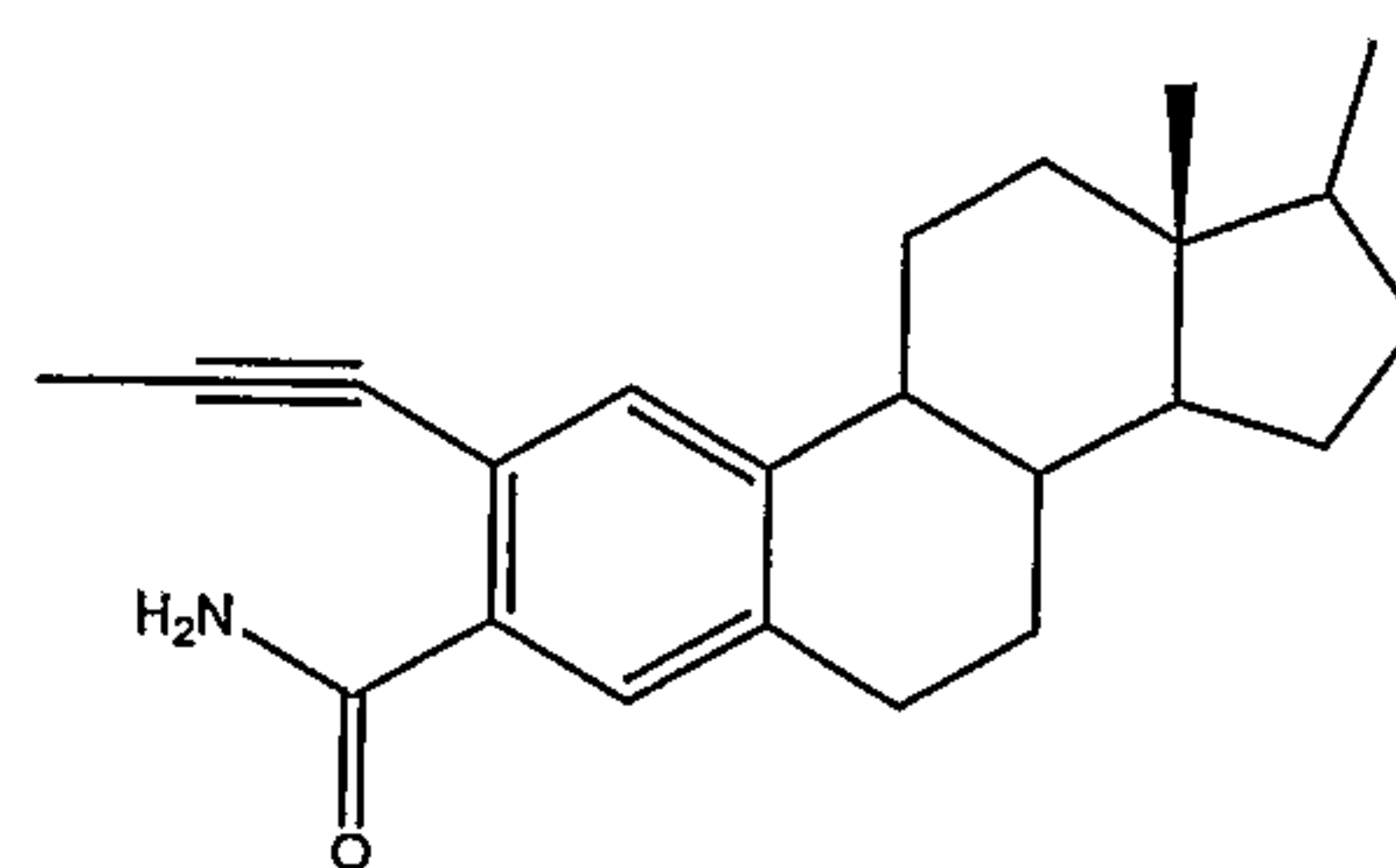
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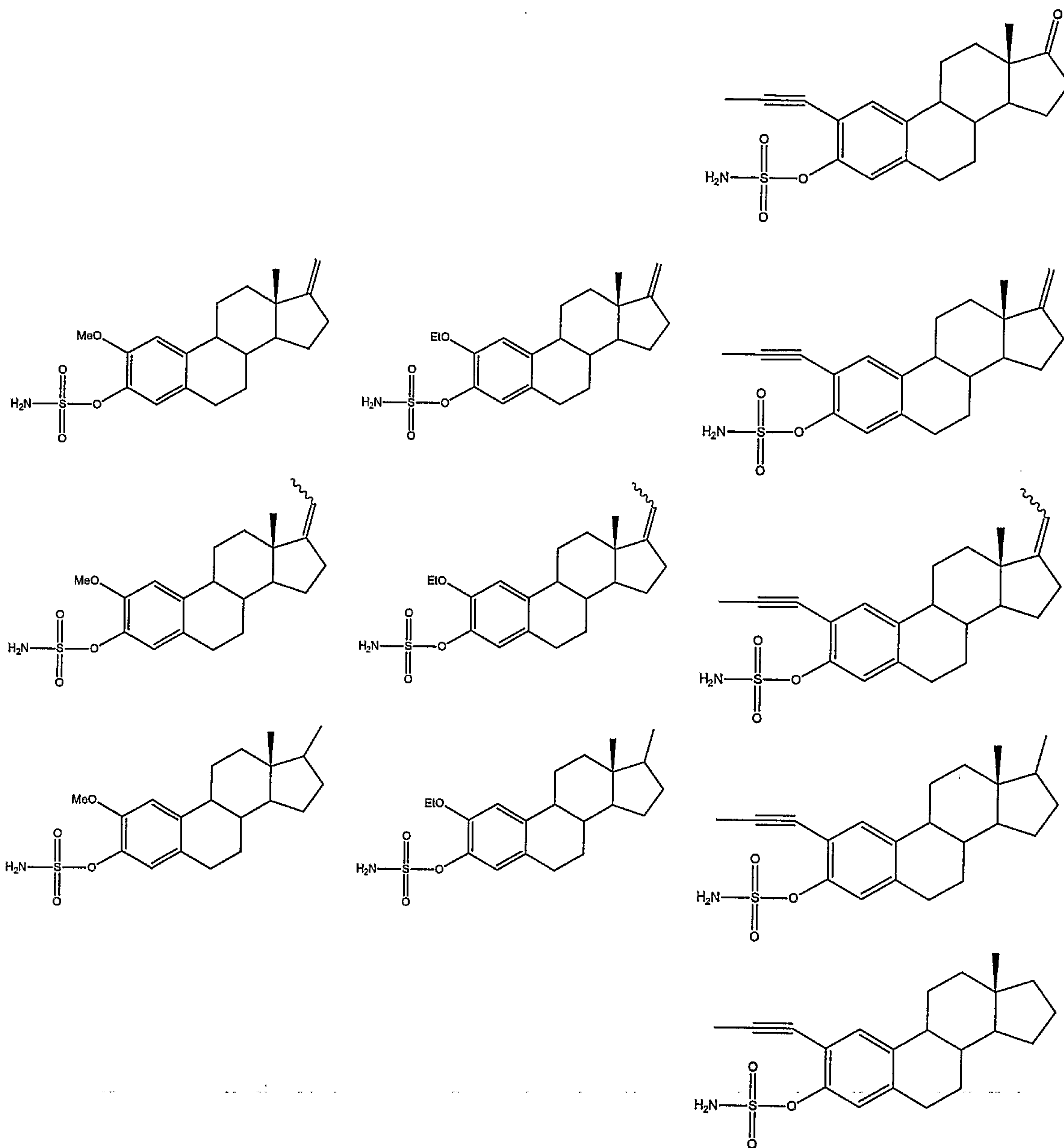


3-Carbamides

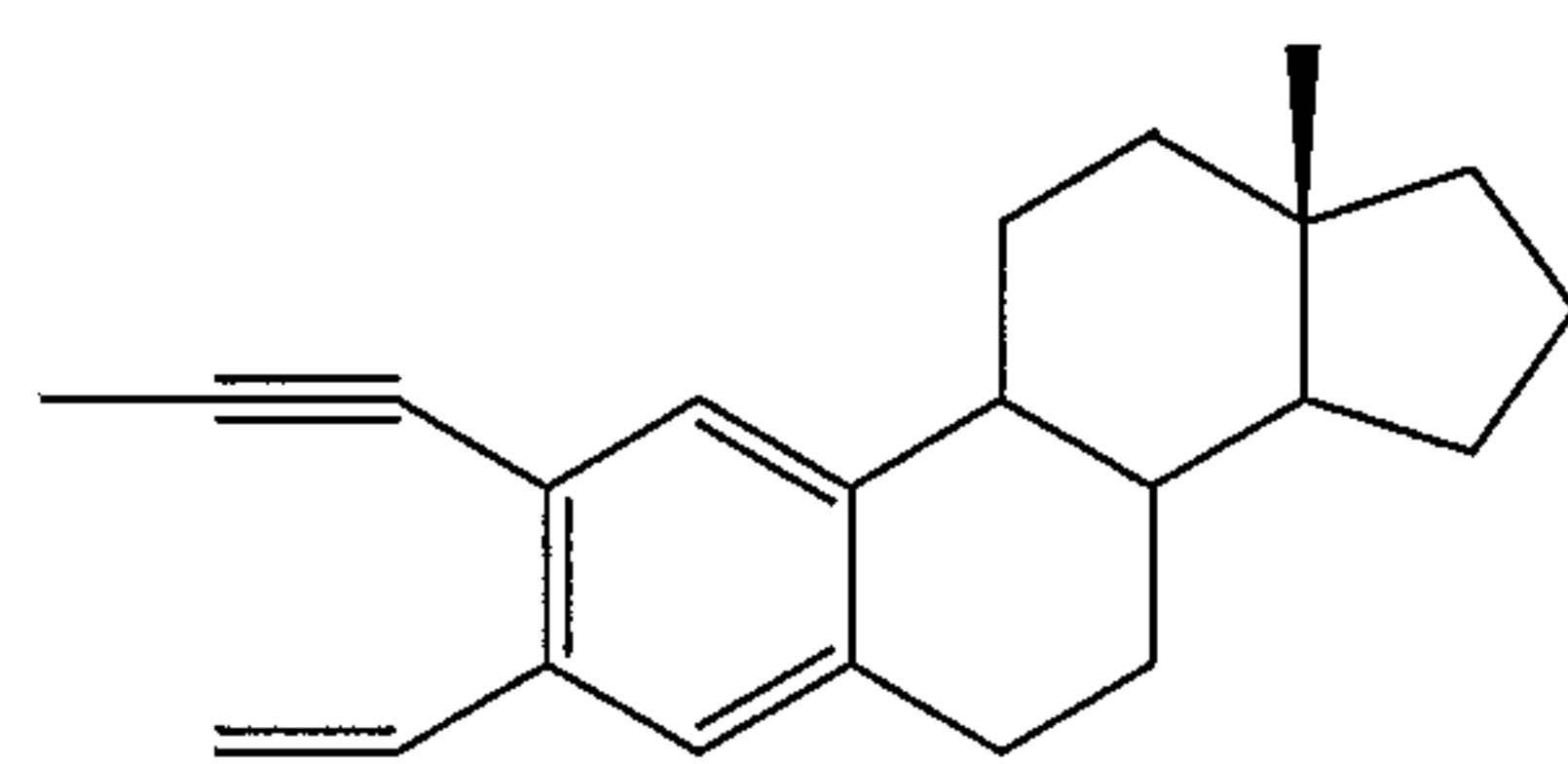
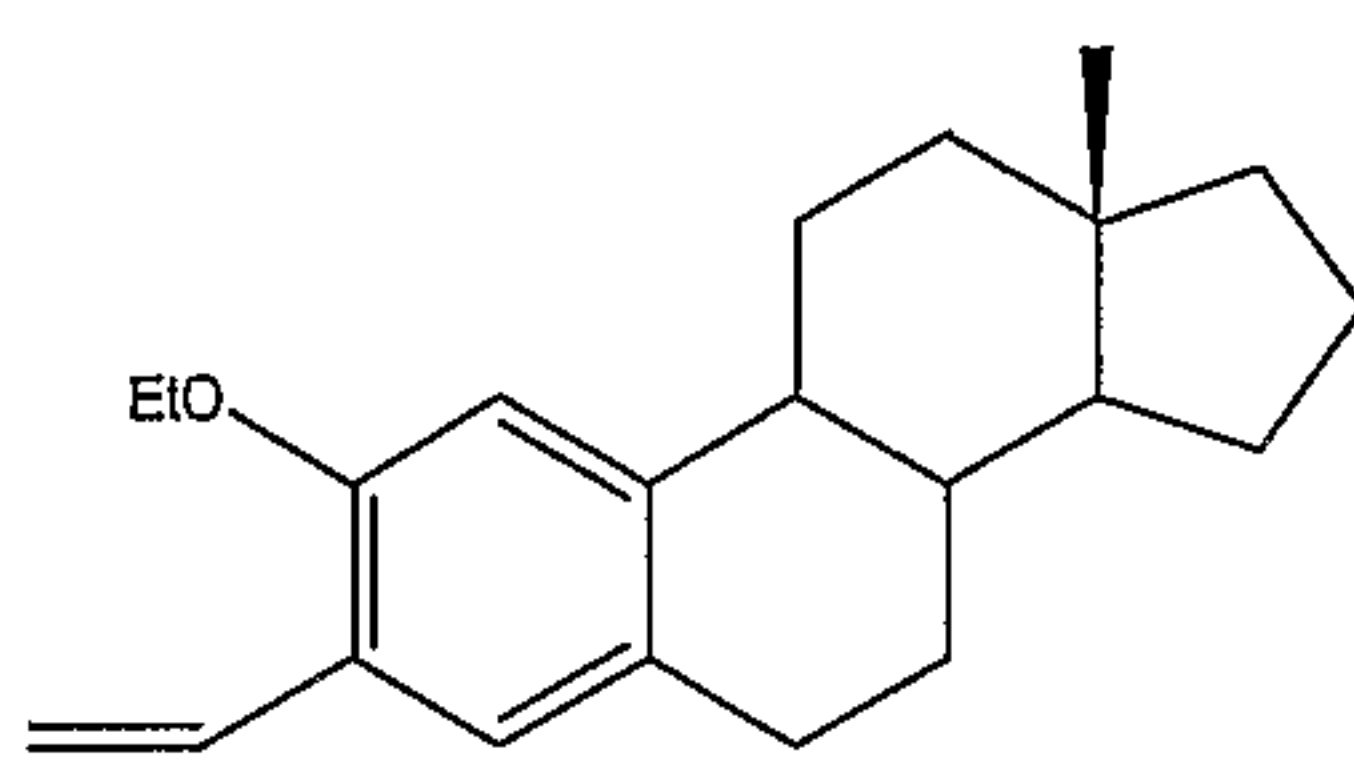
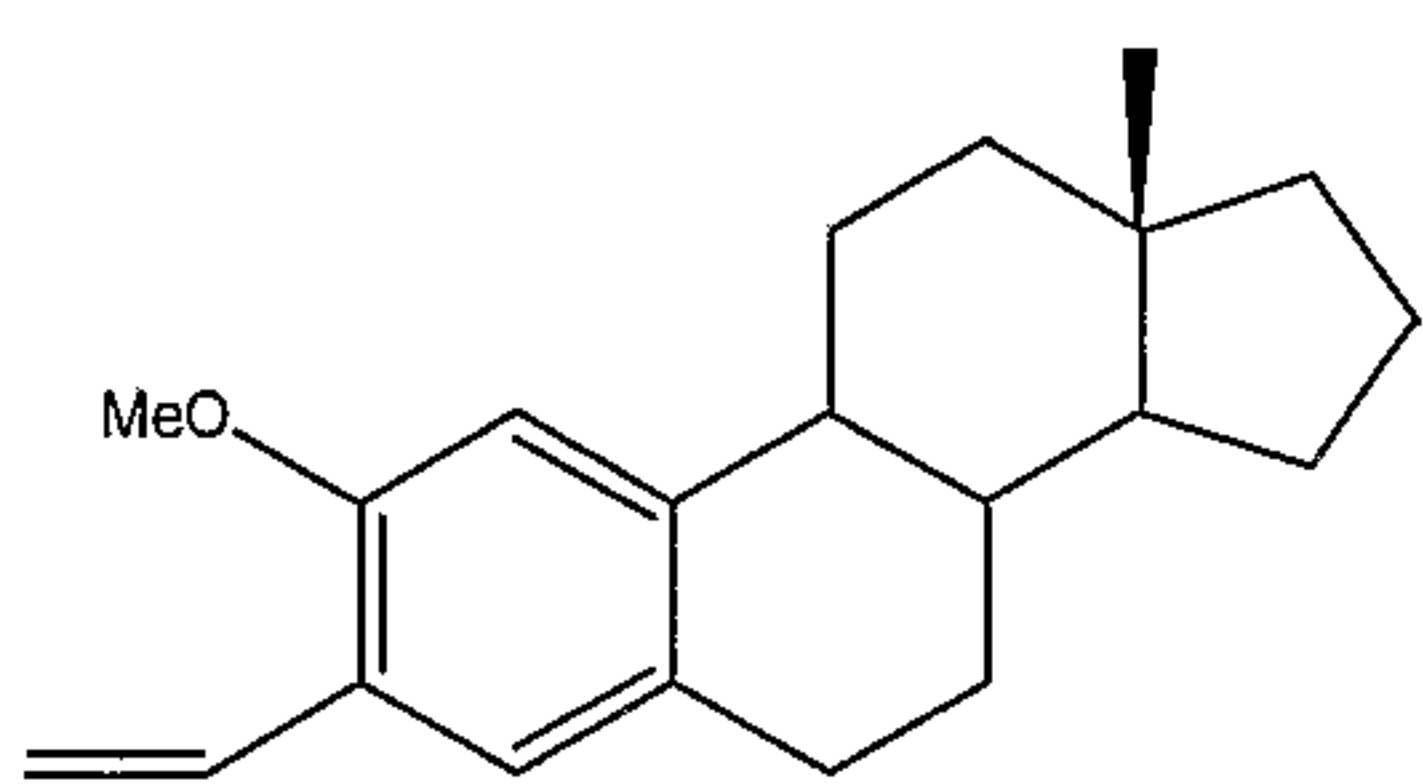
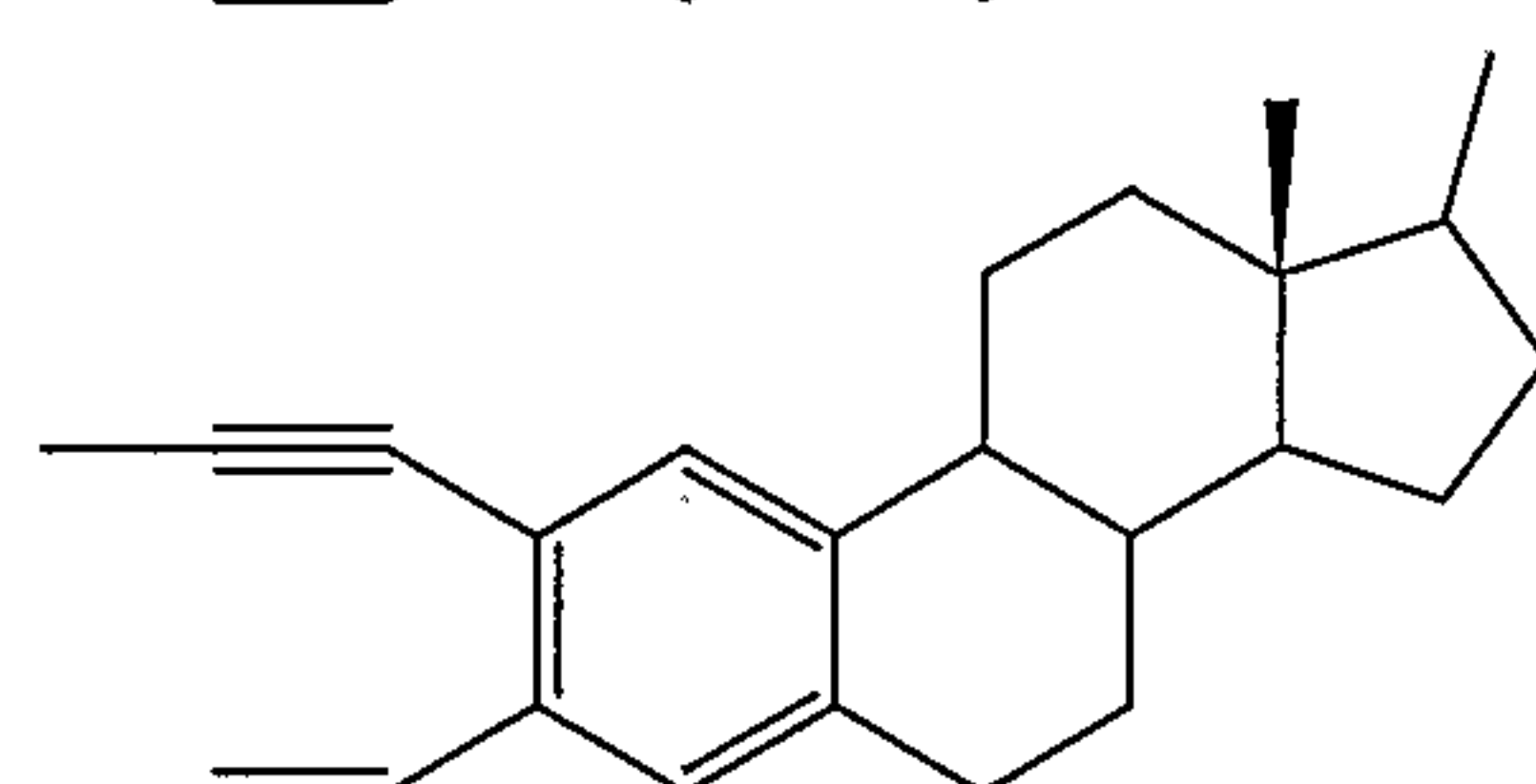
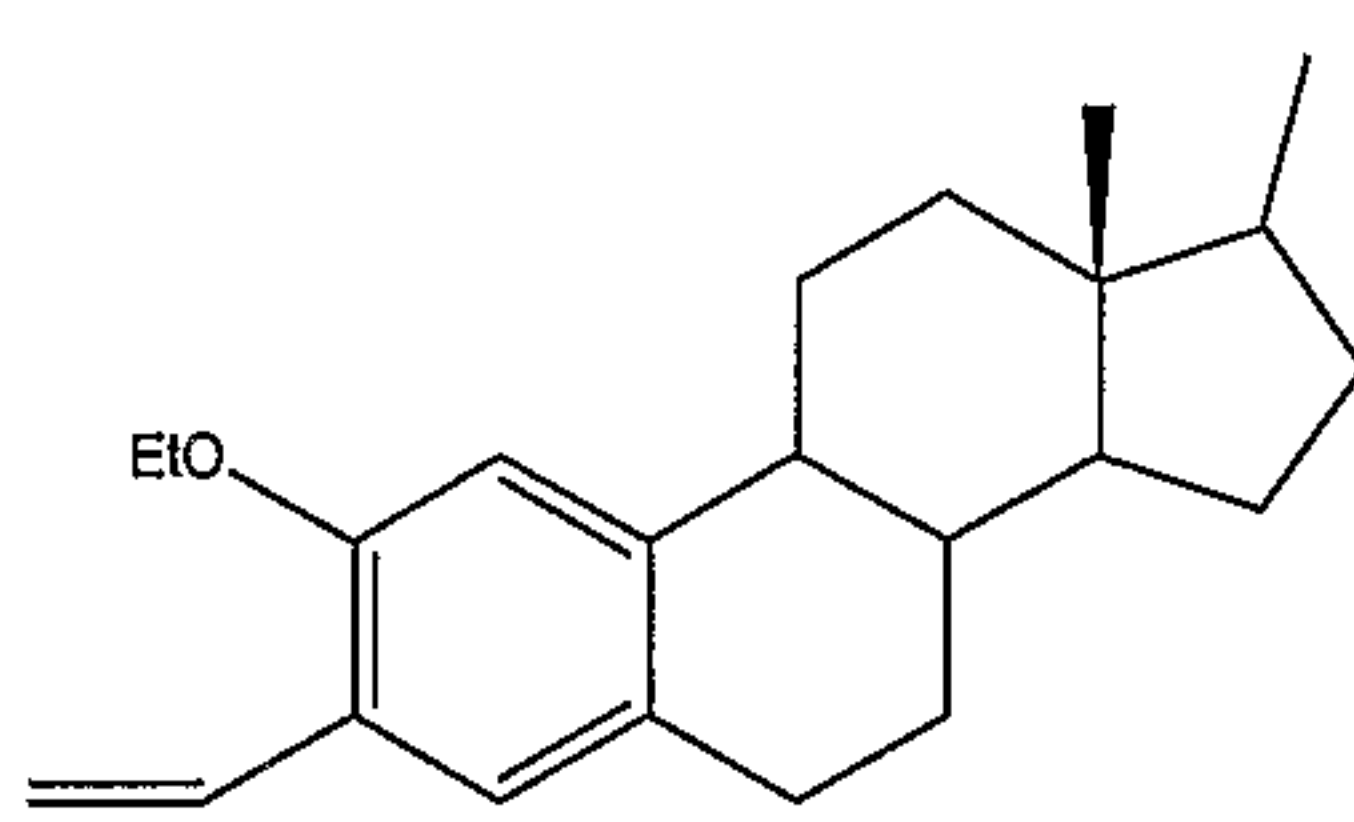
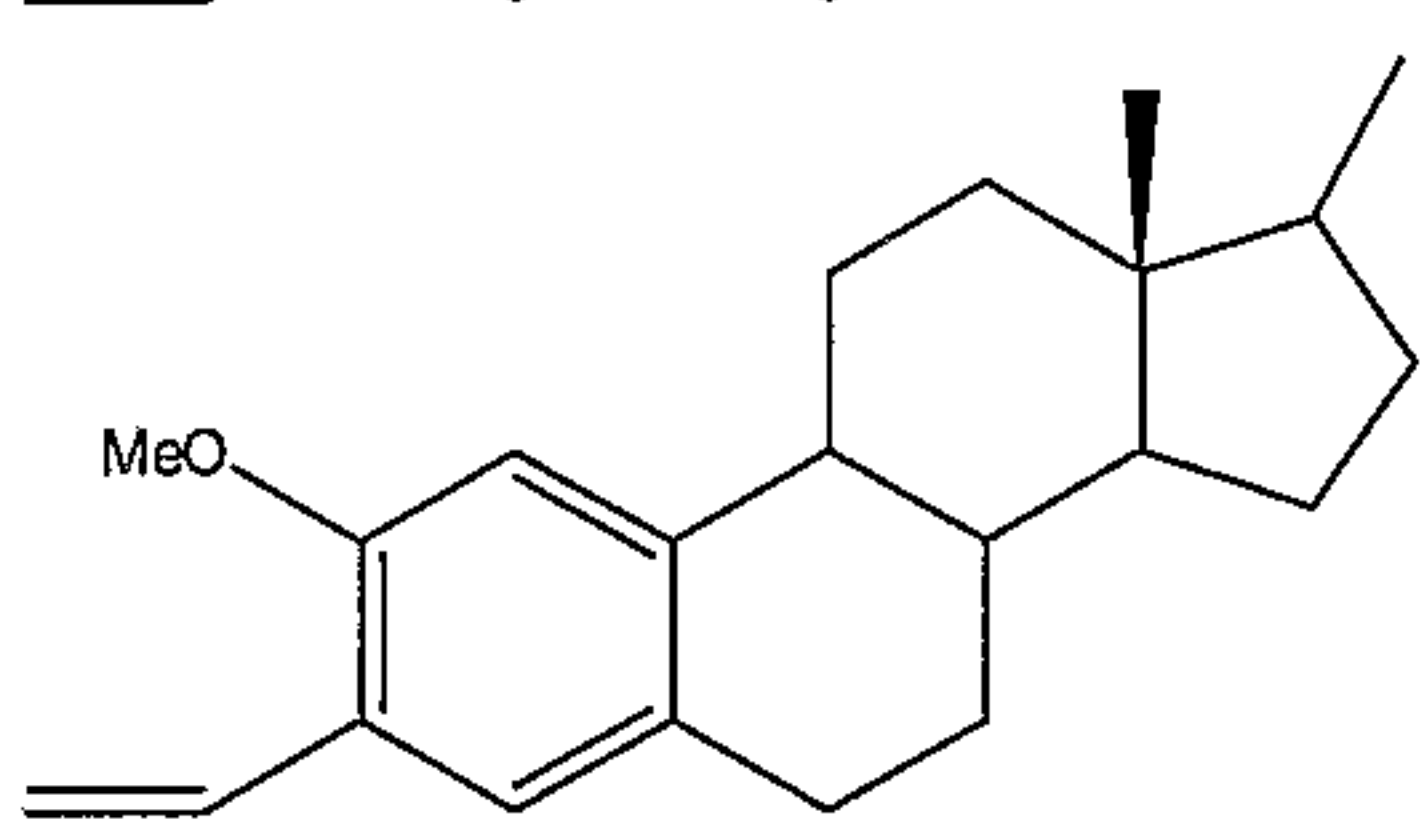
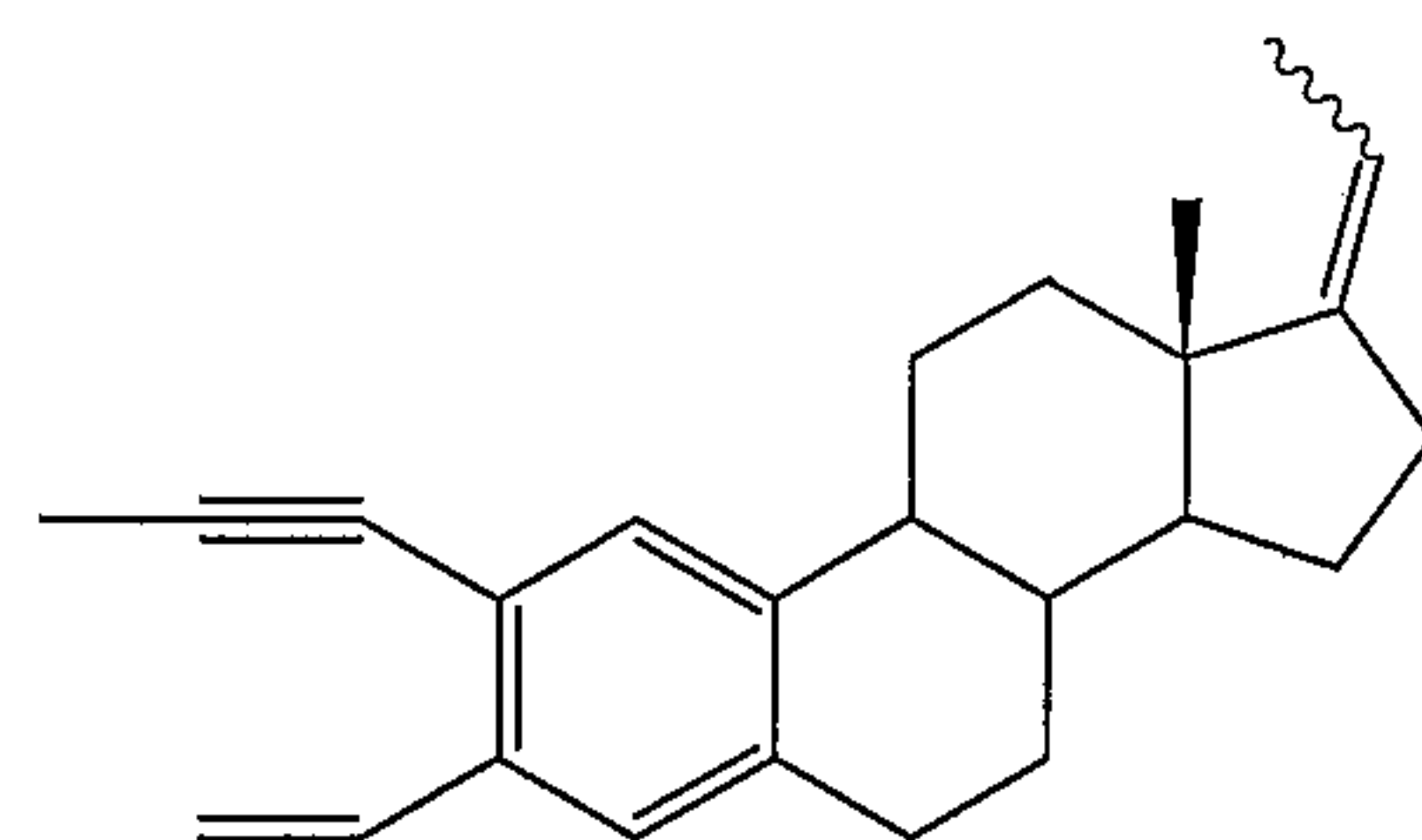
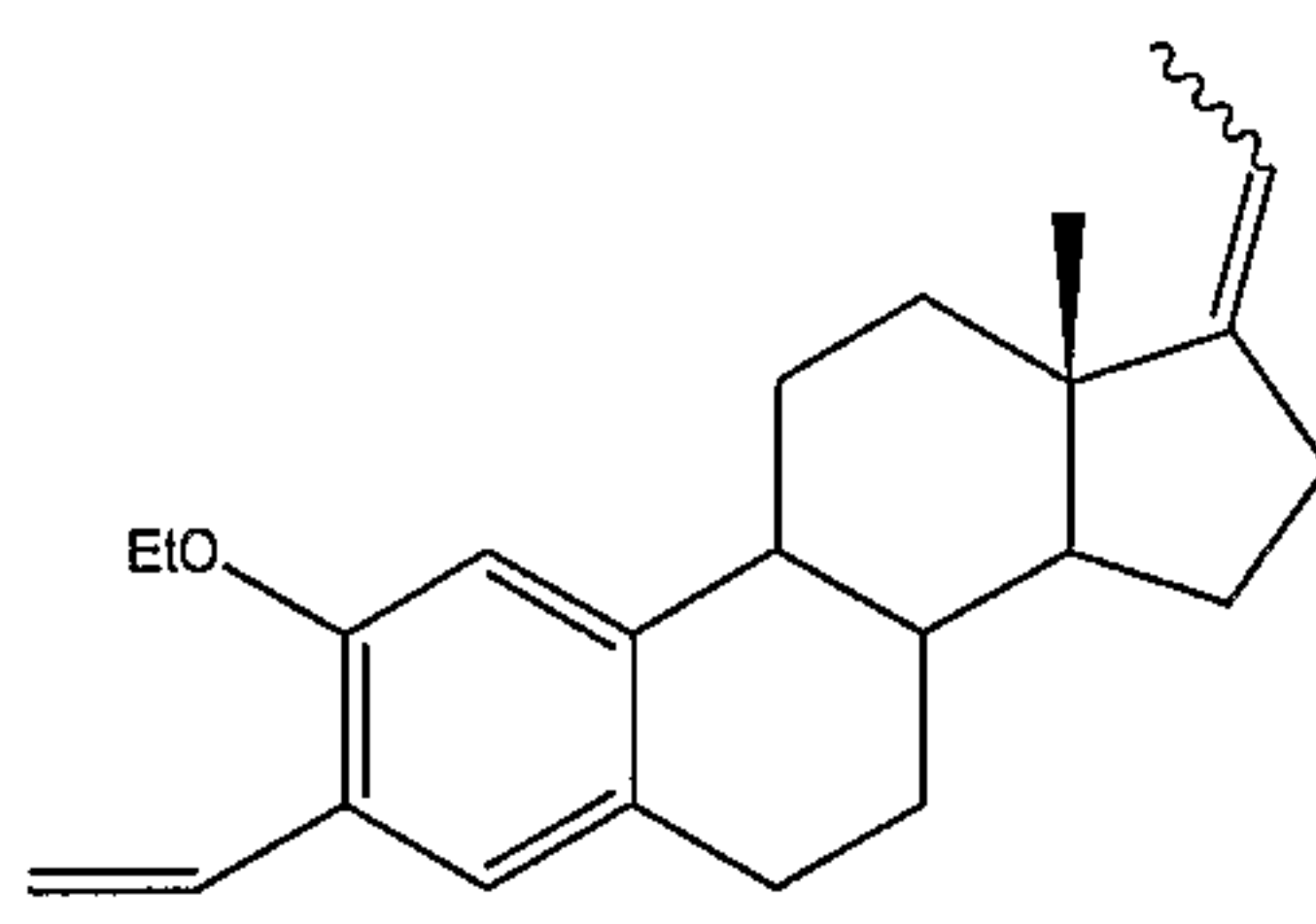
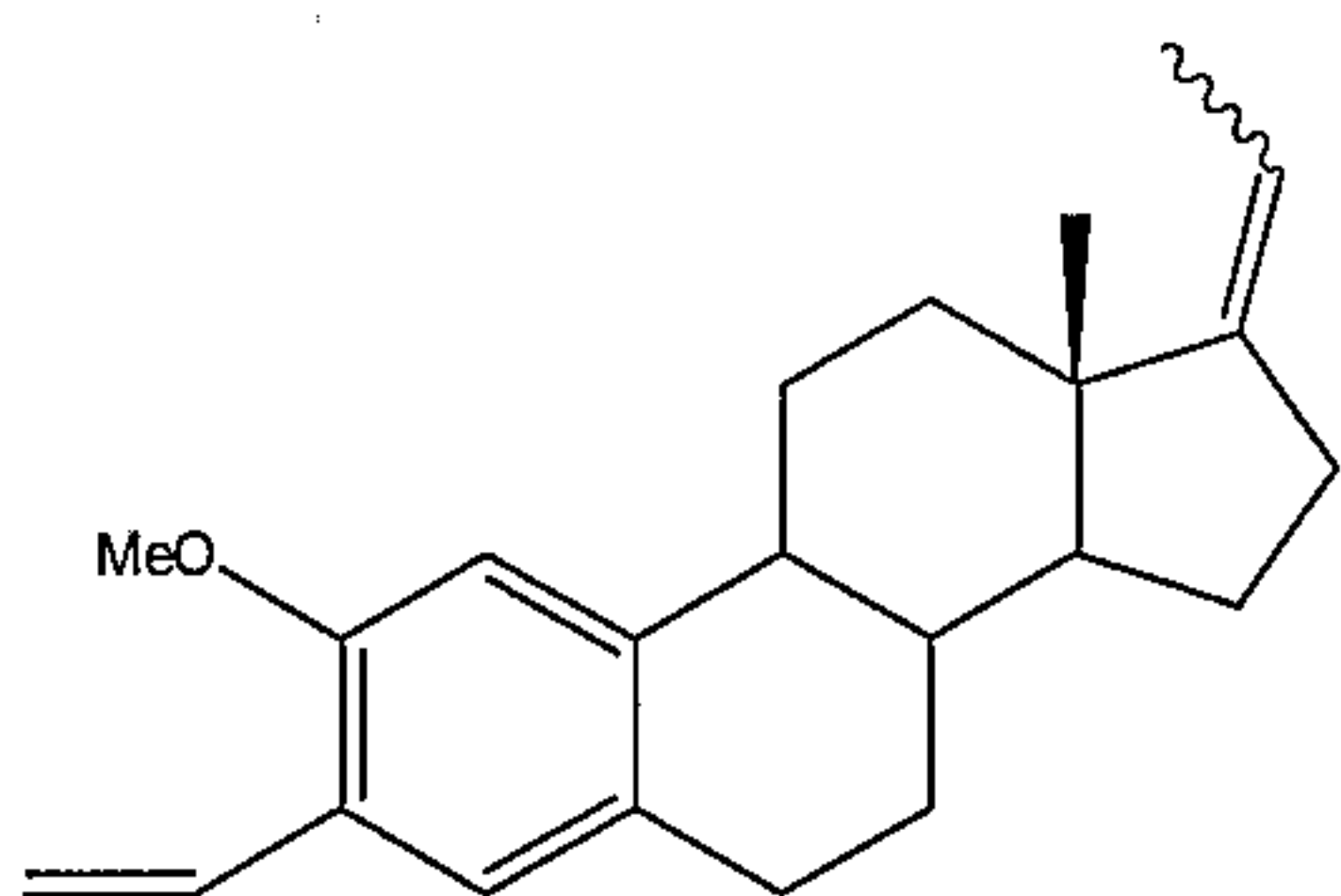
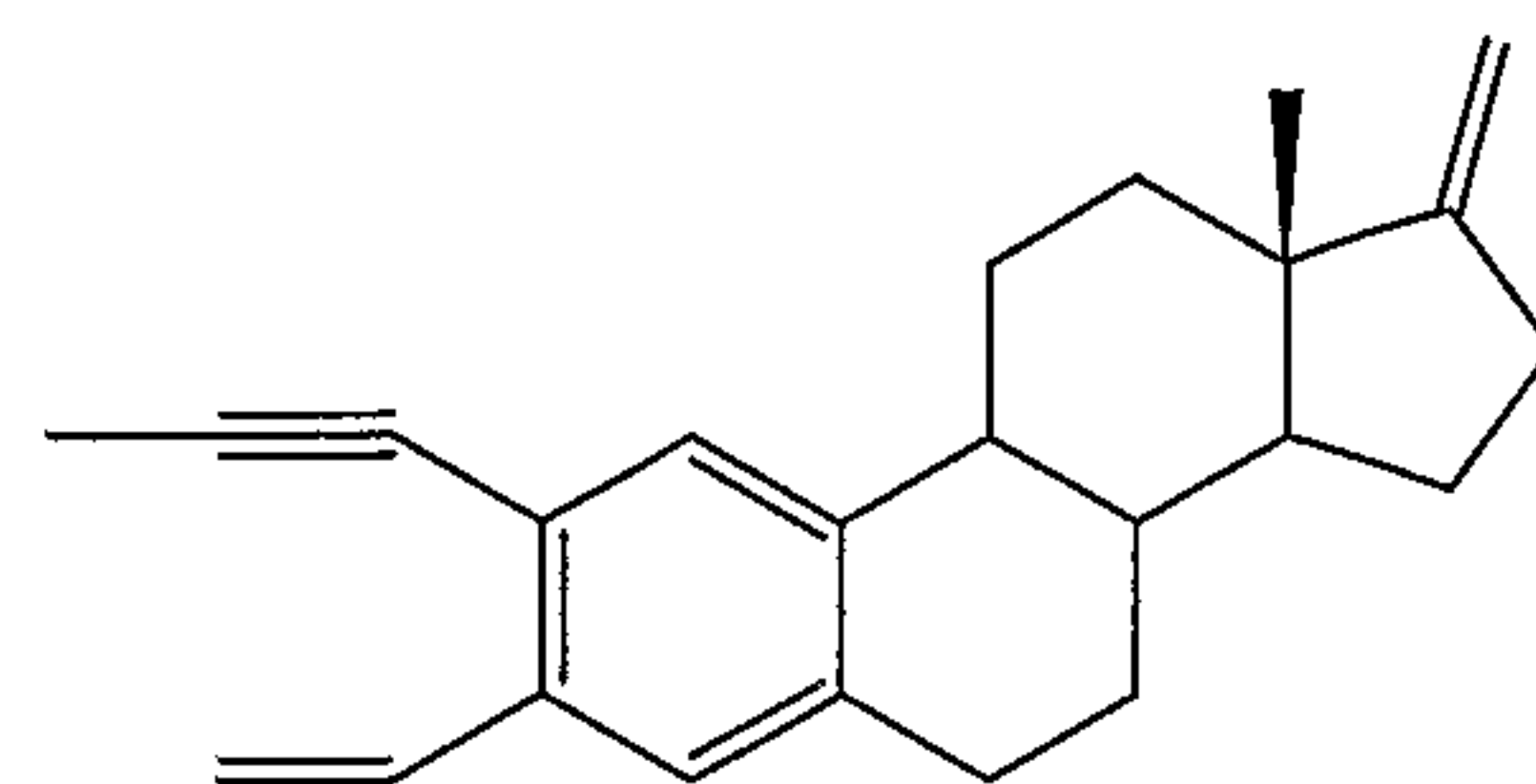
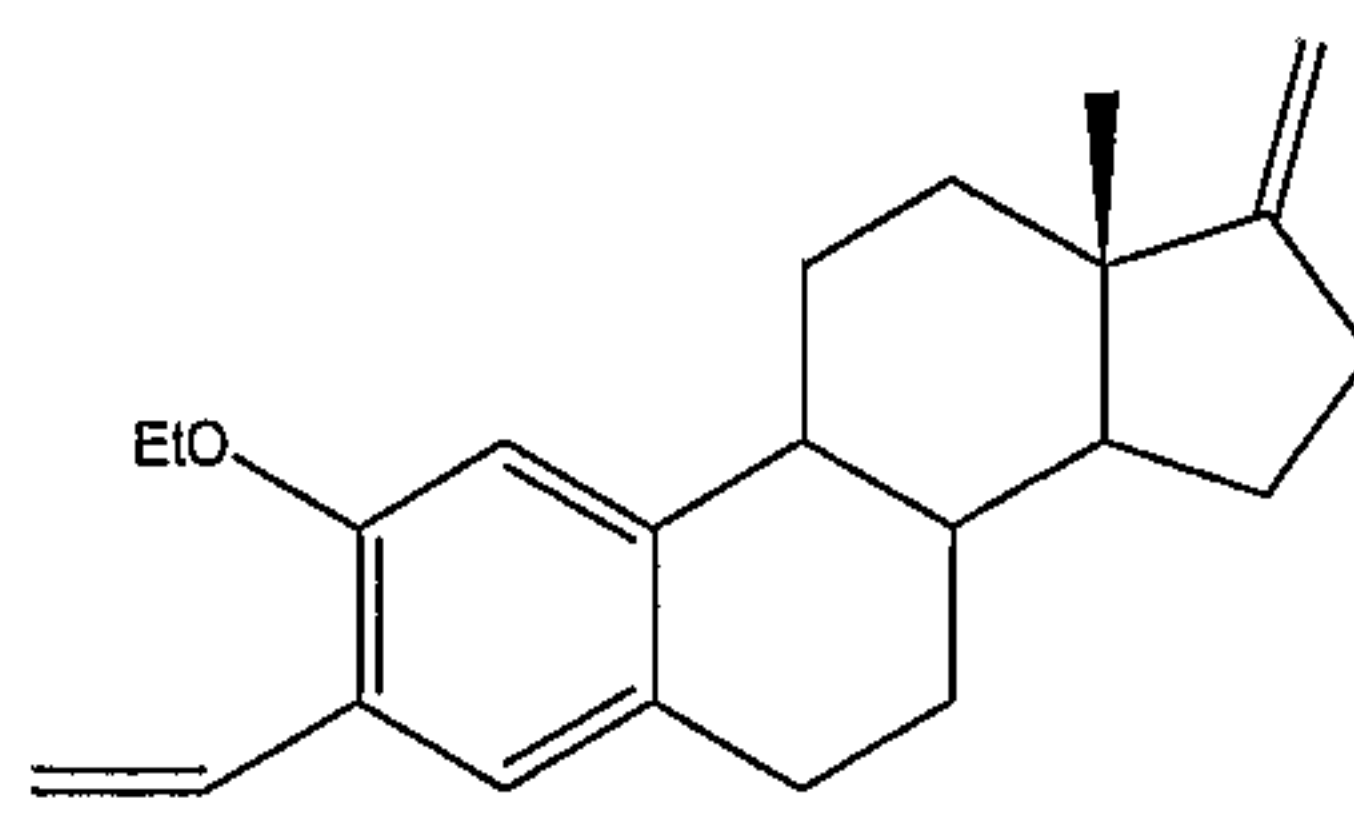
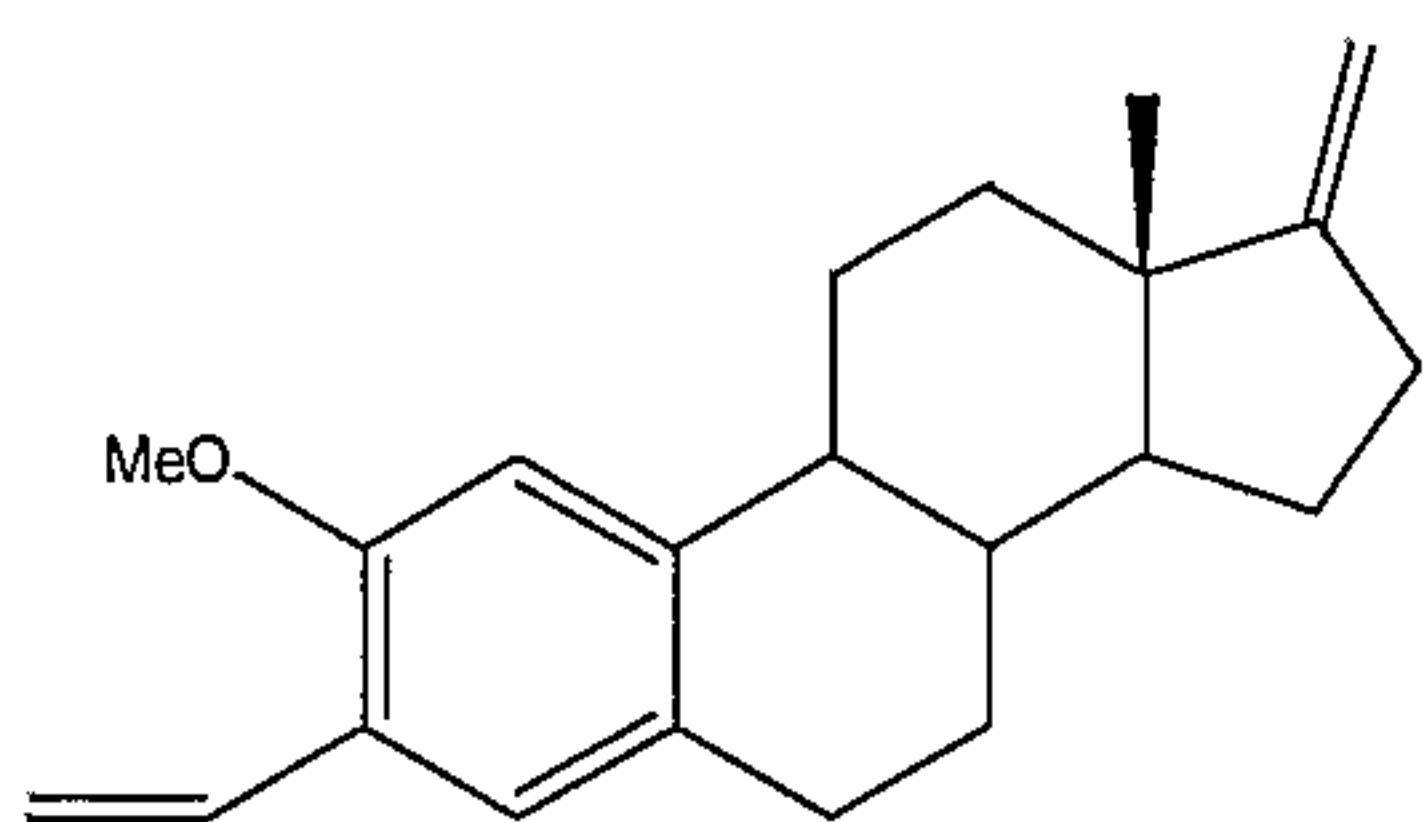
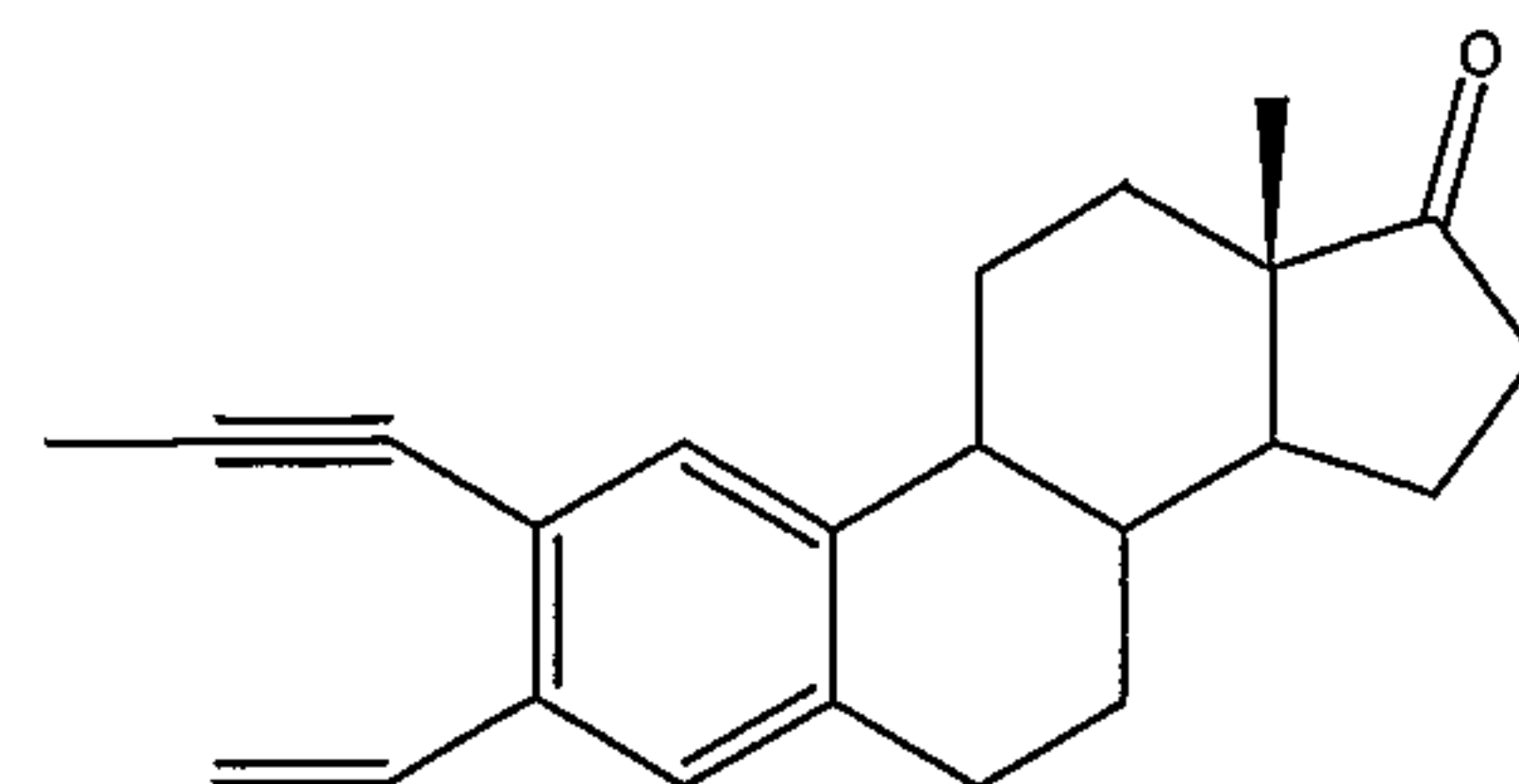
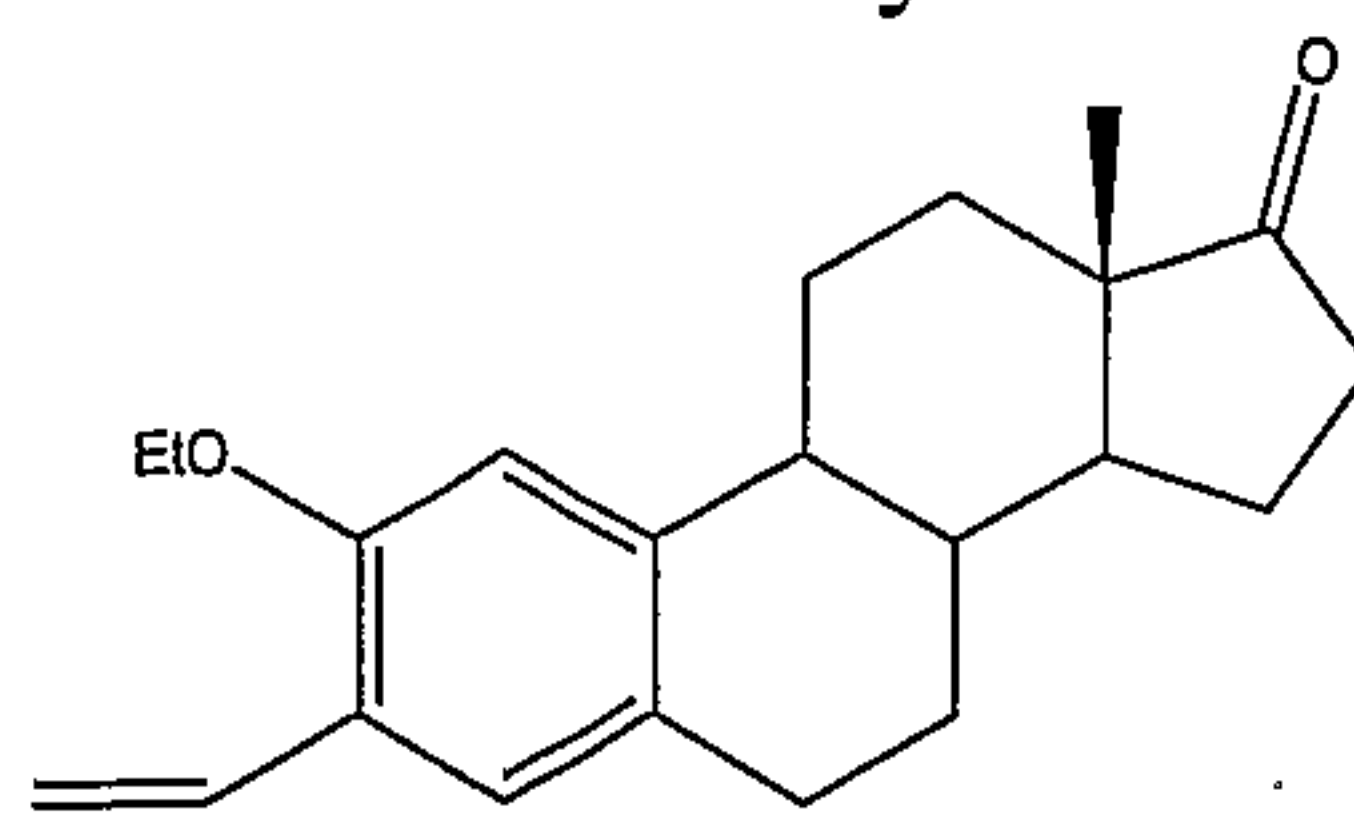
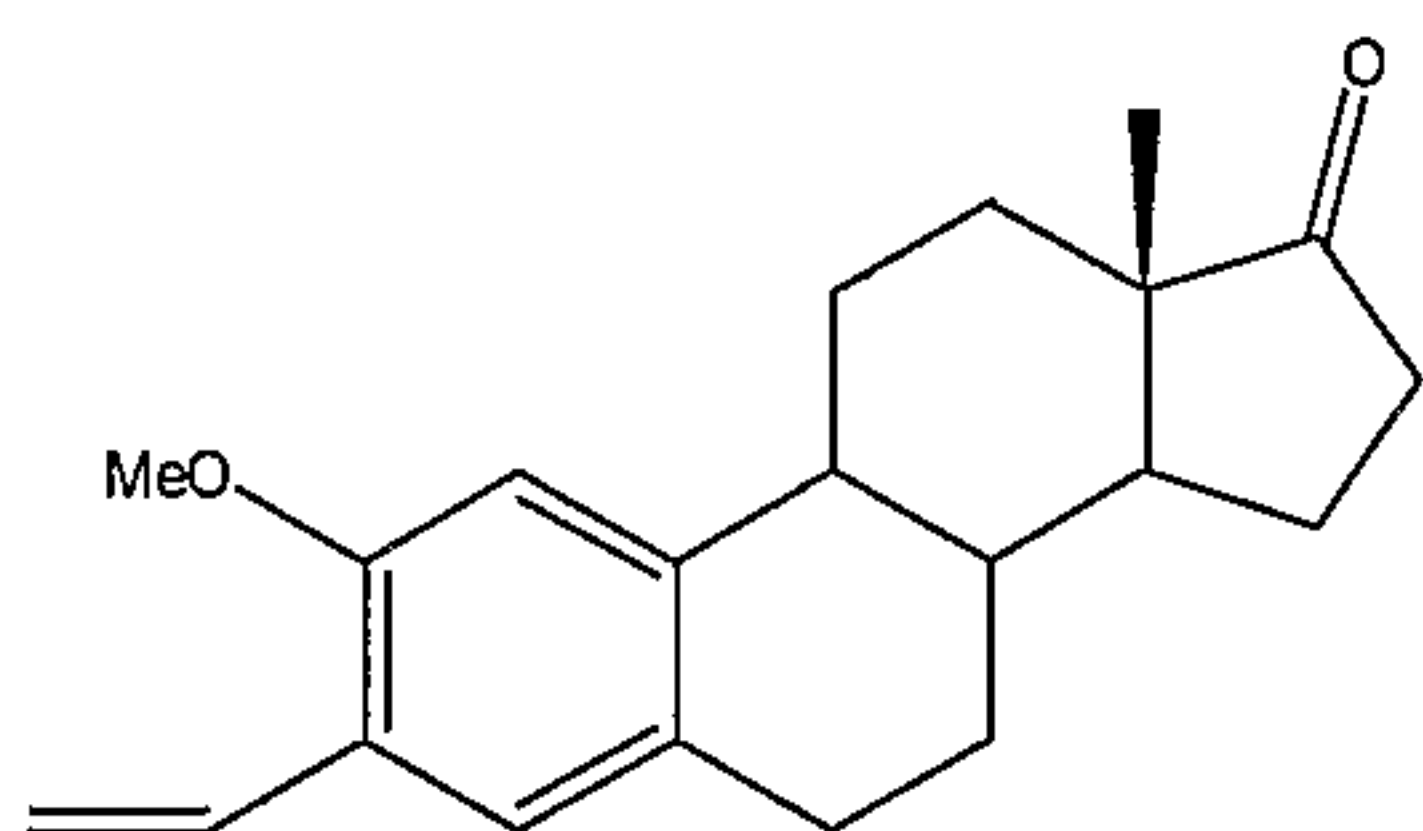




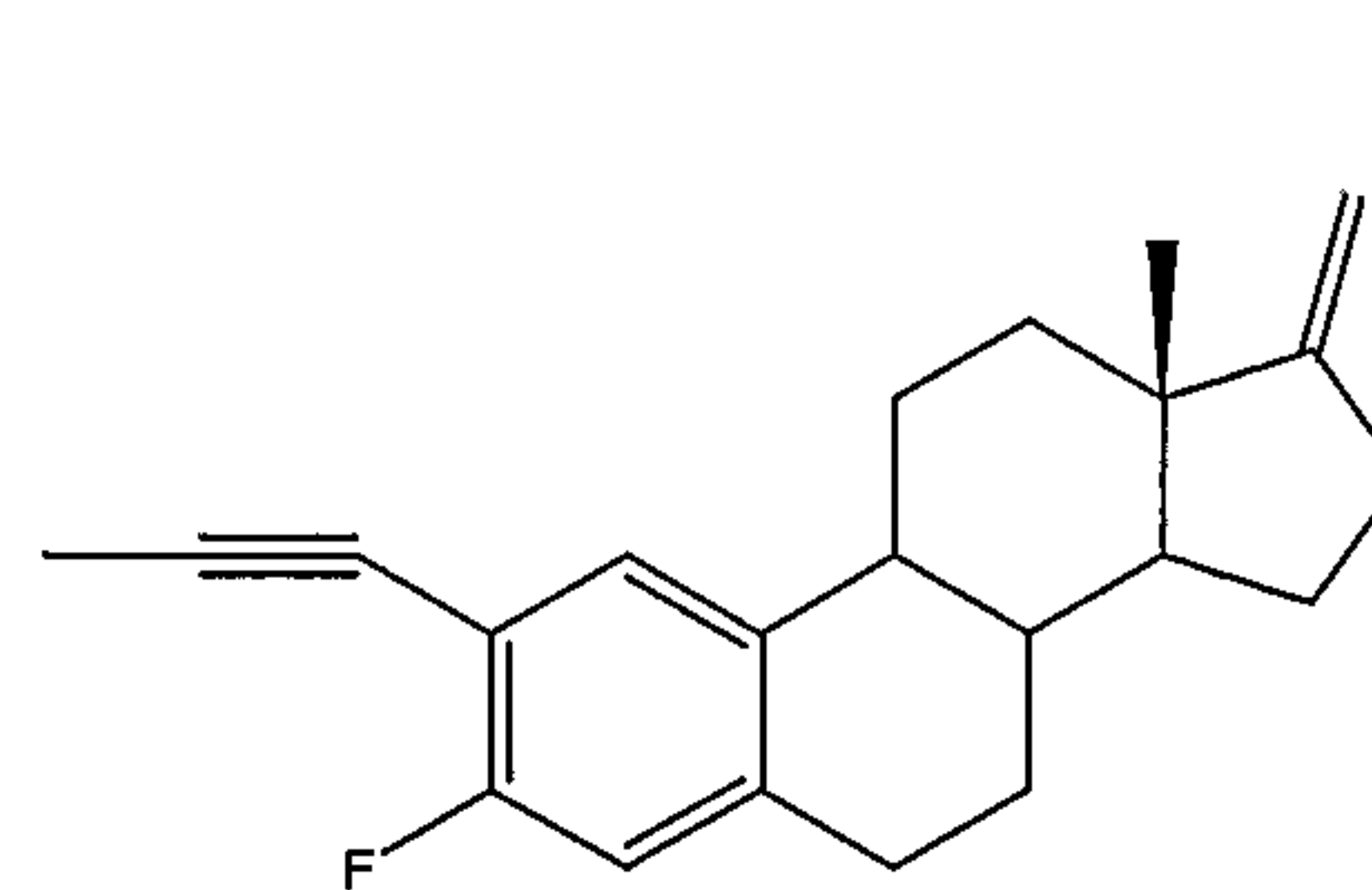
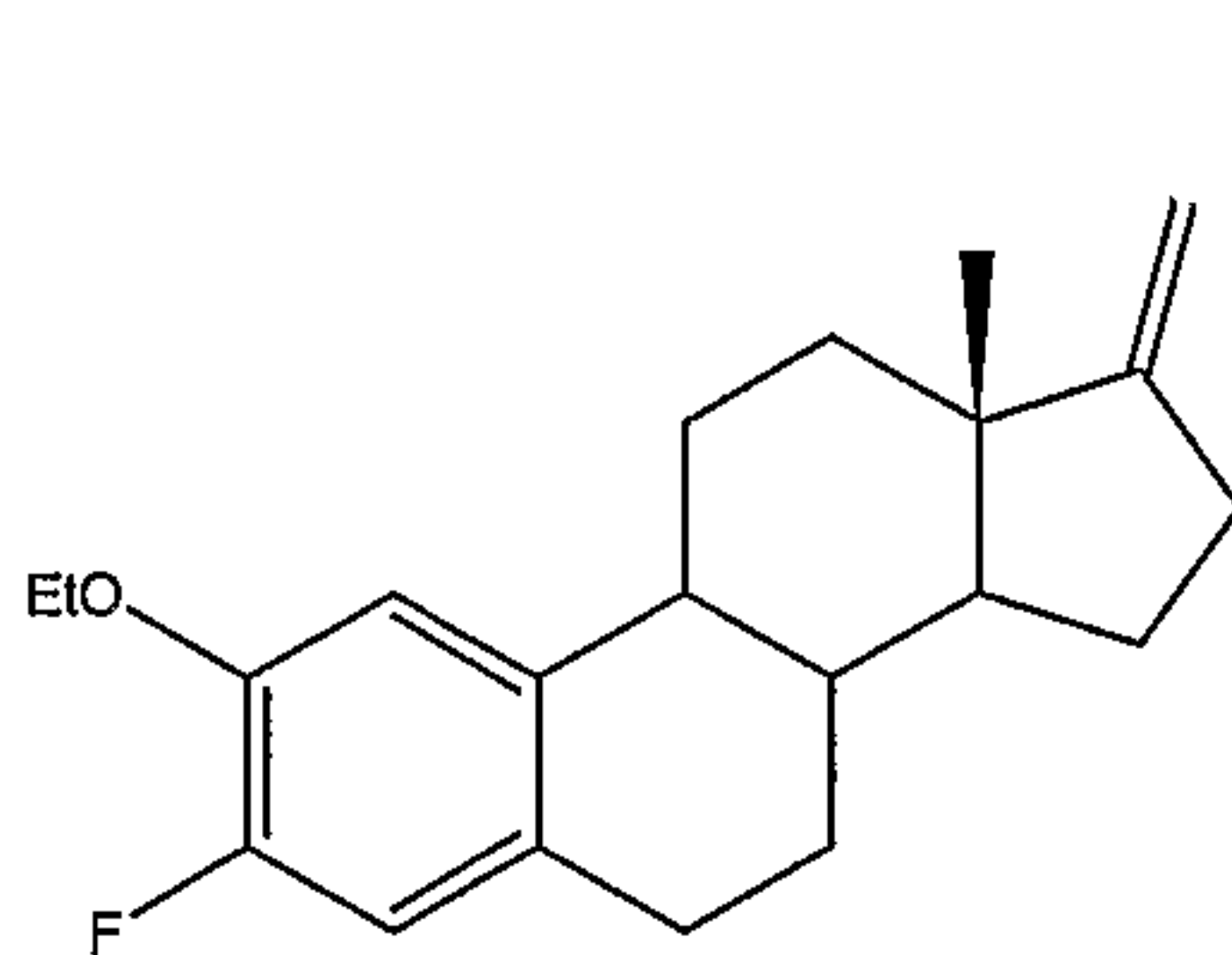
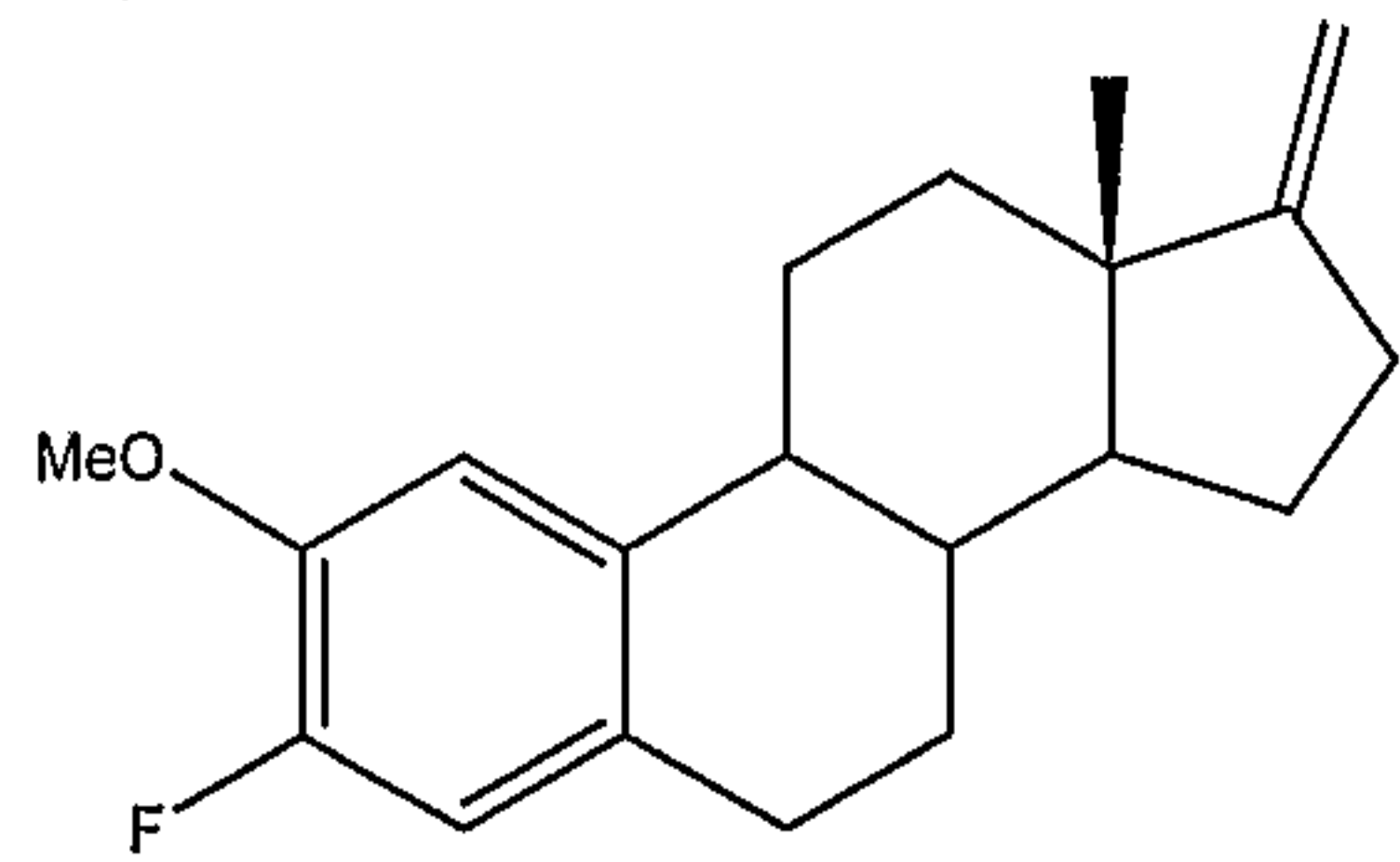
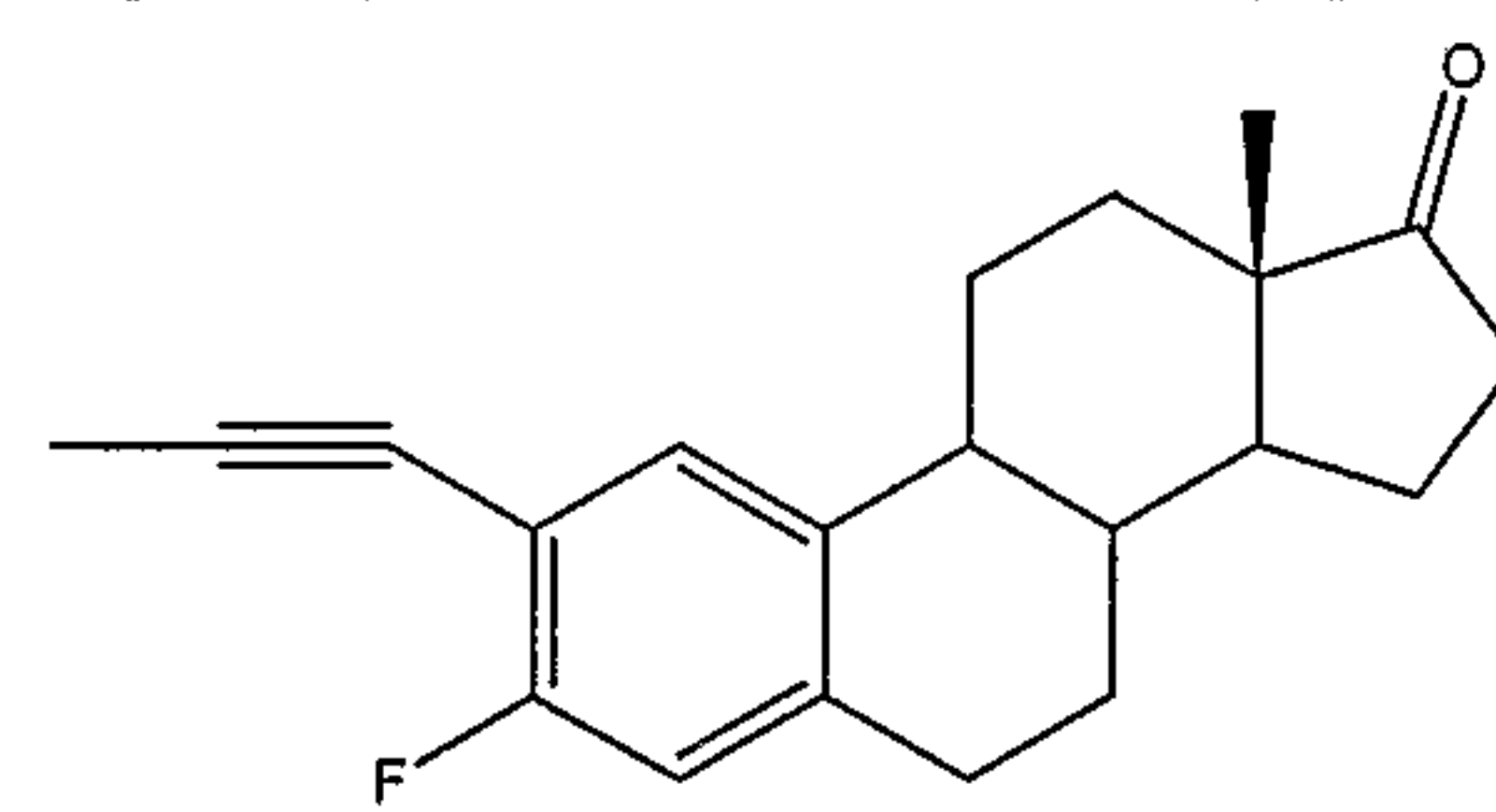
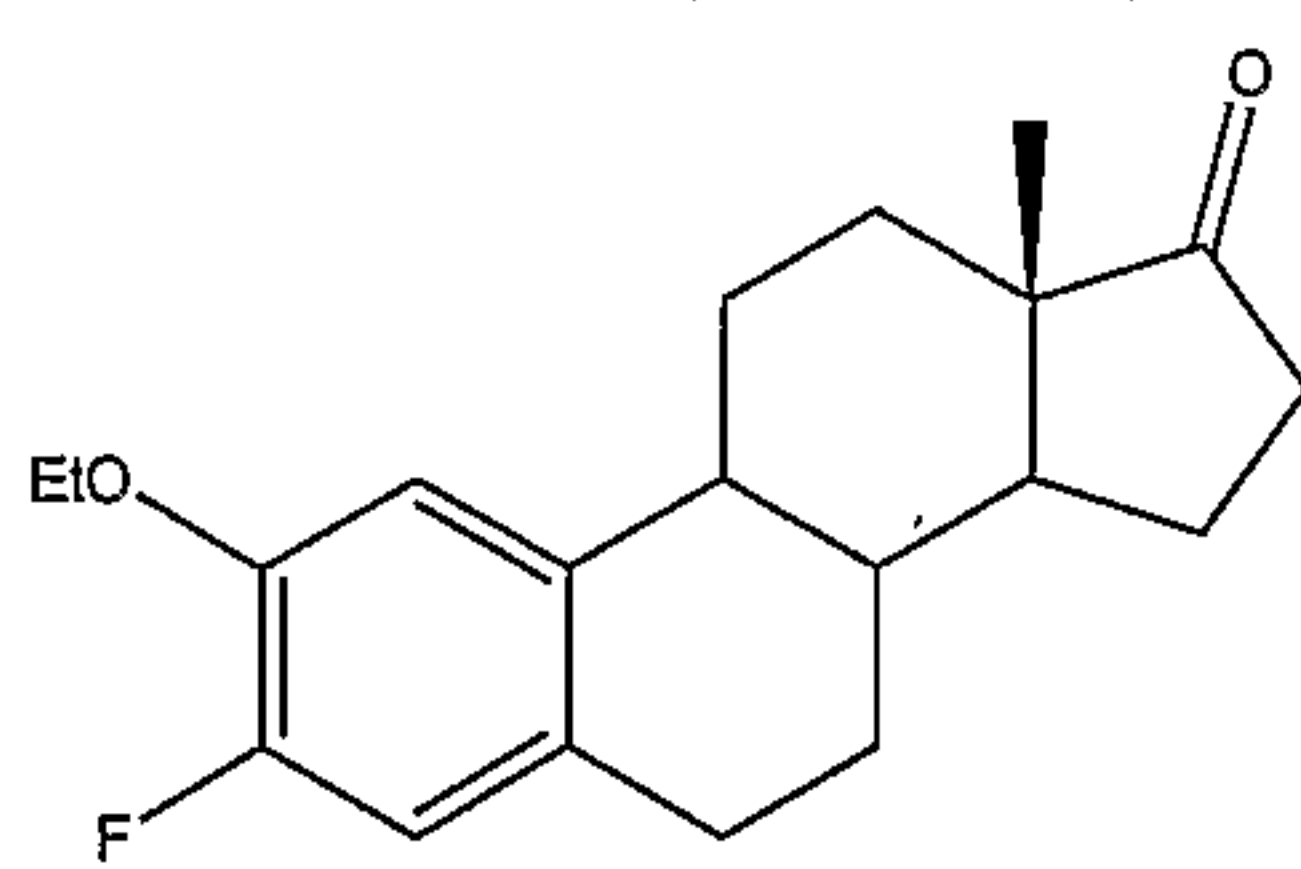
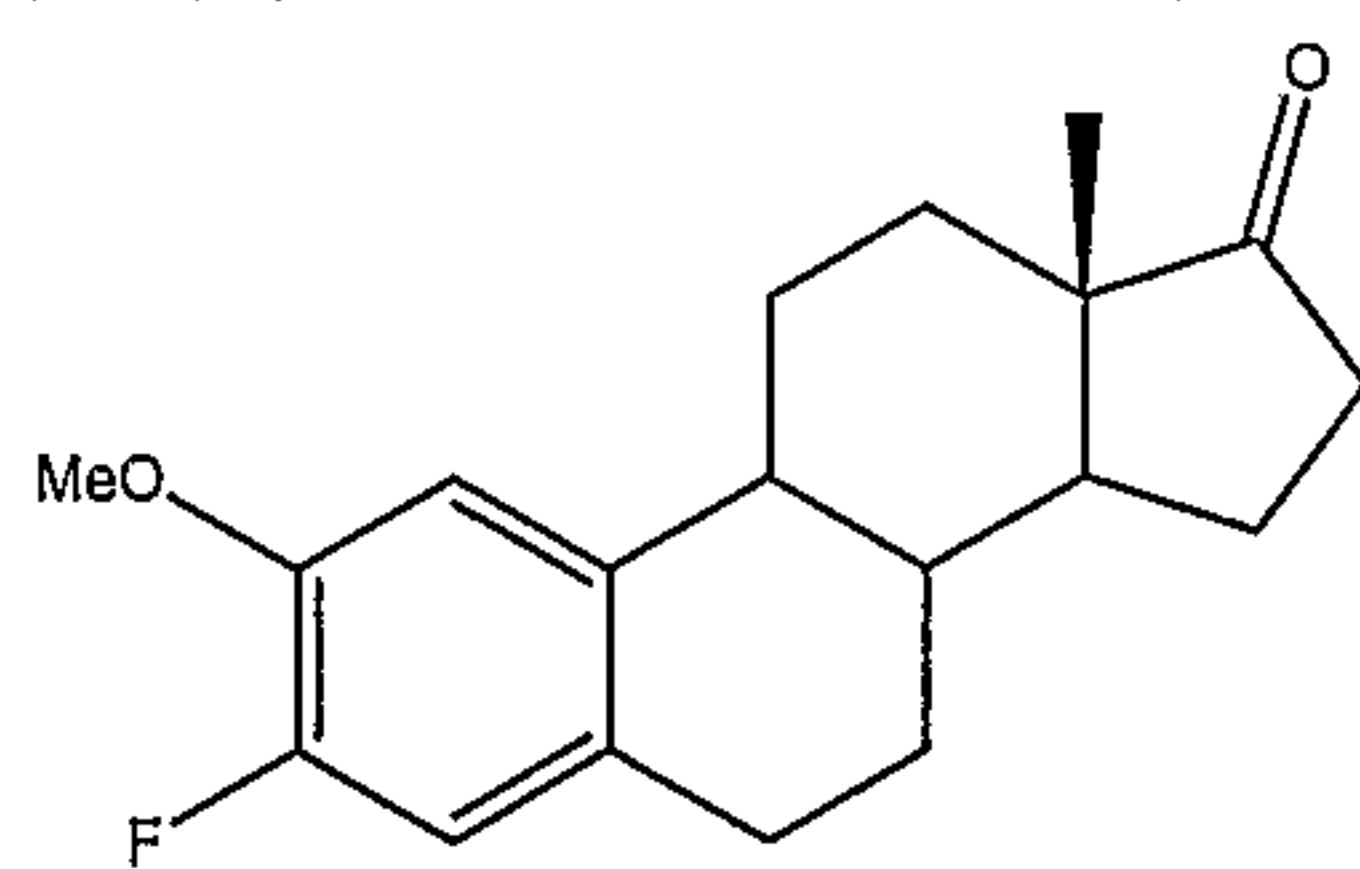
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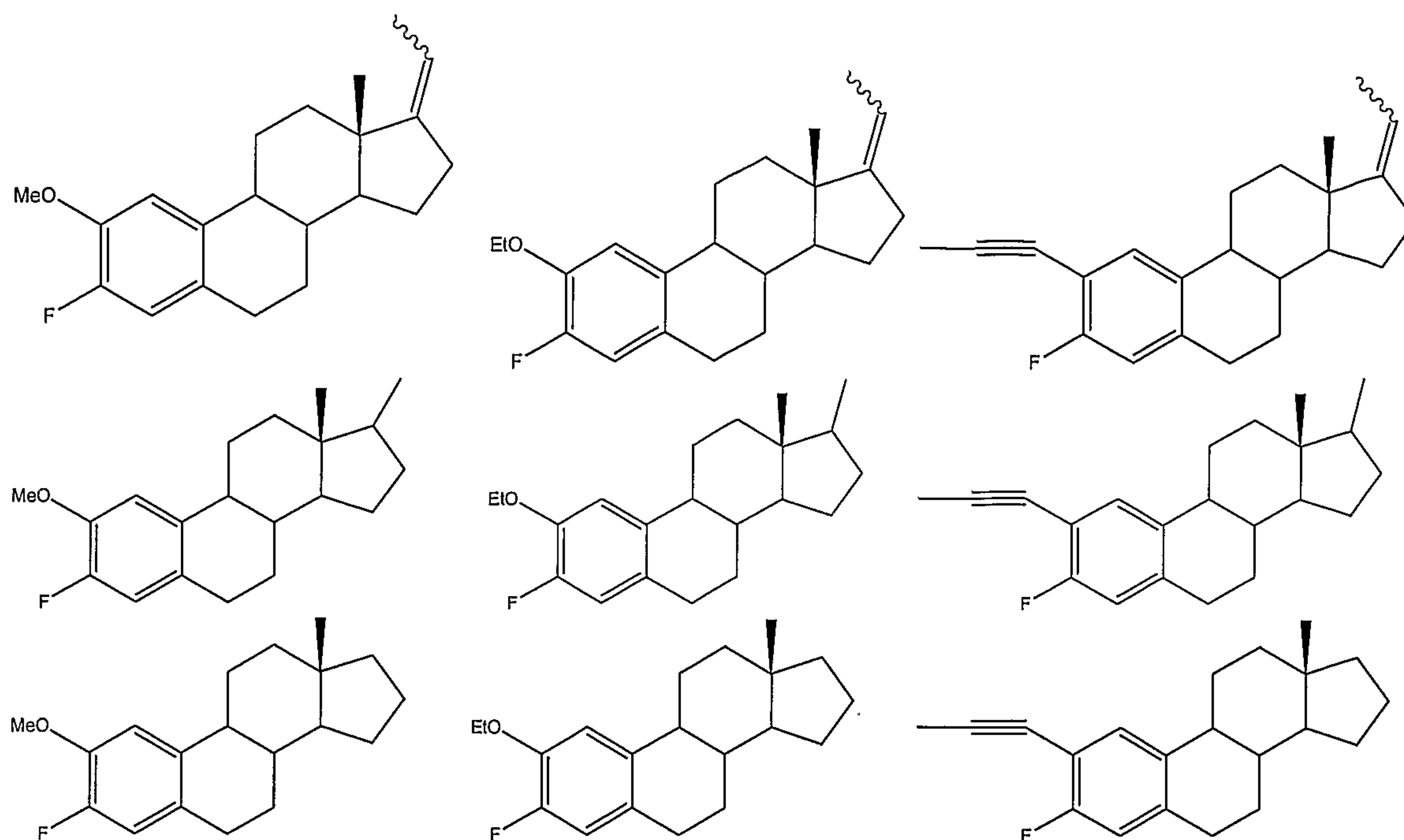


3-Vinyls



3-Fluoro

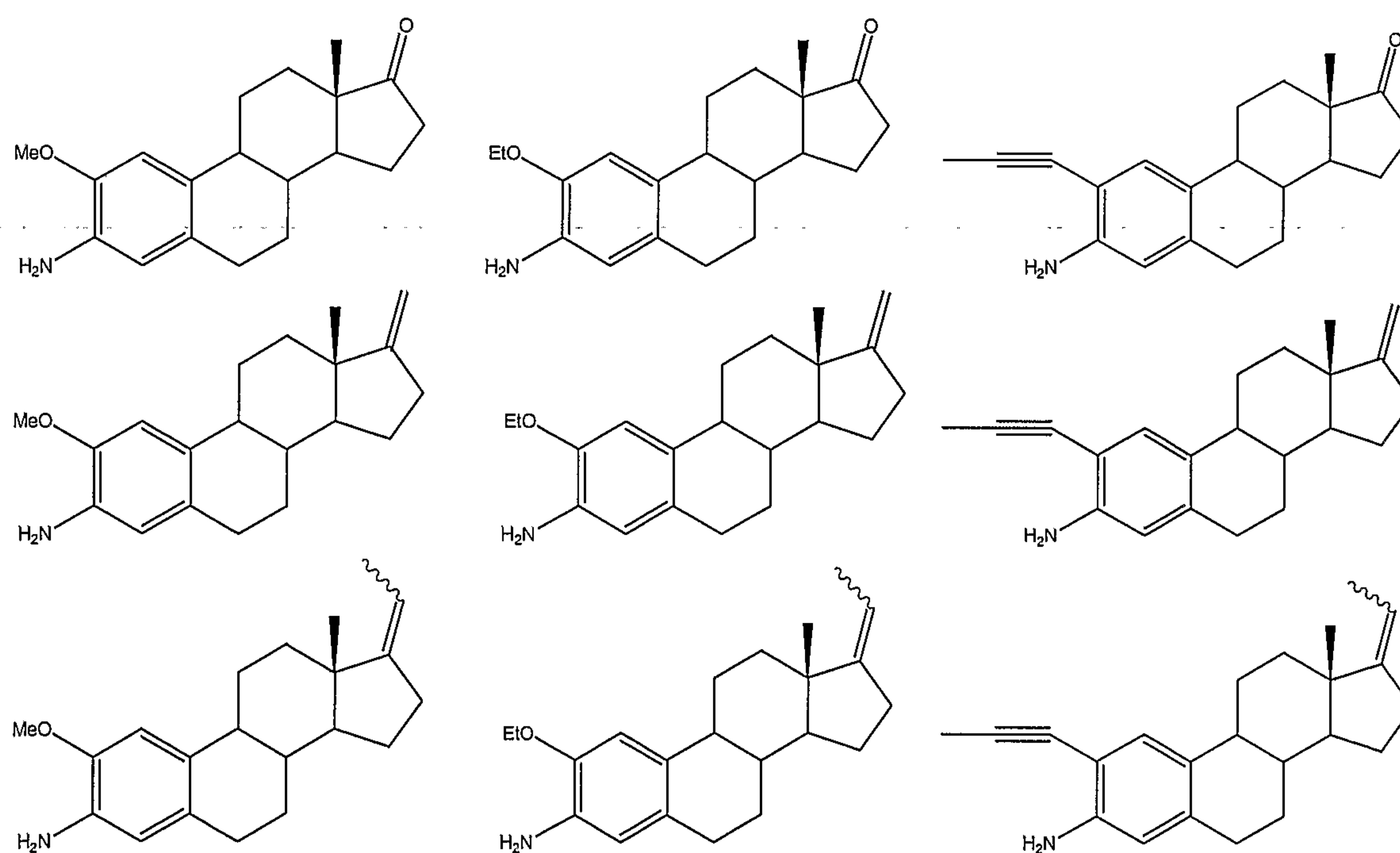


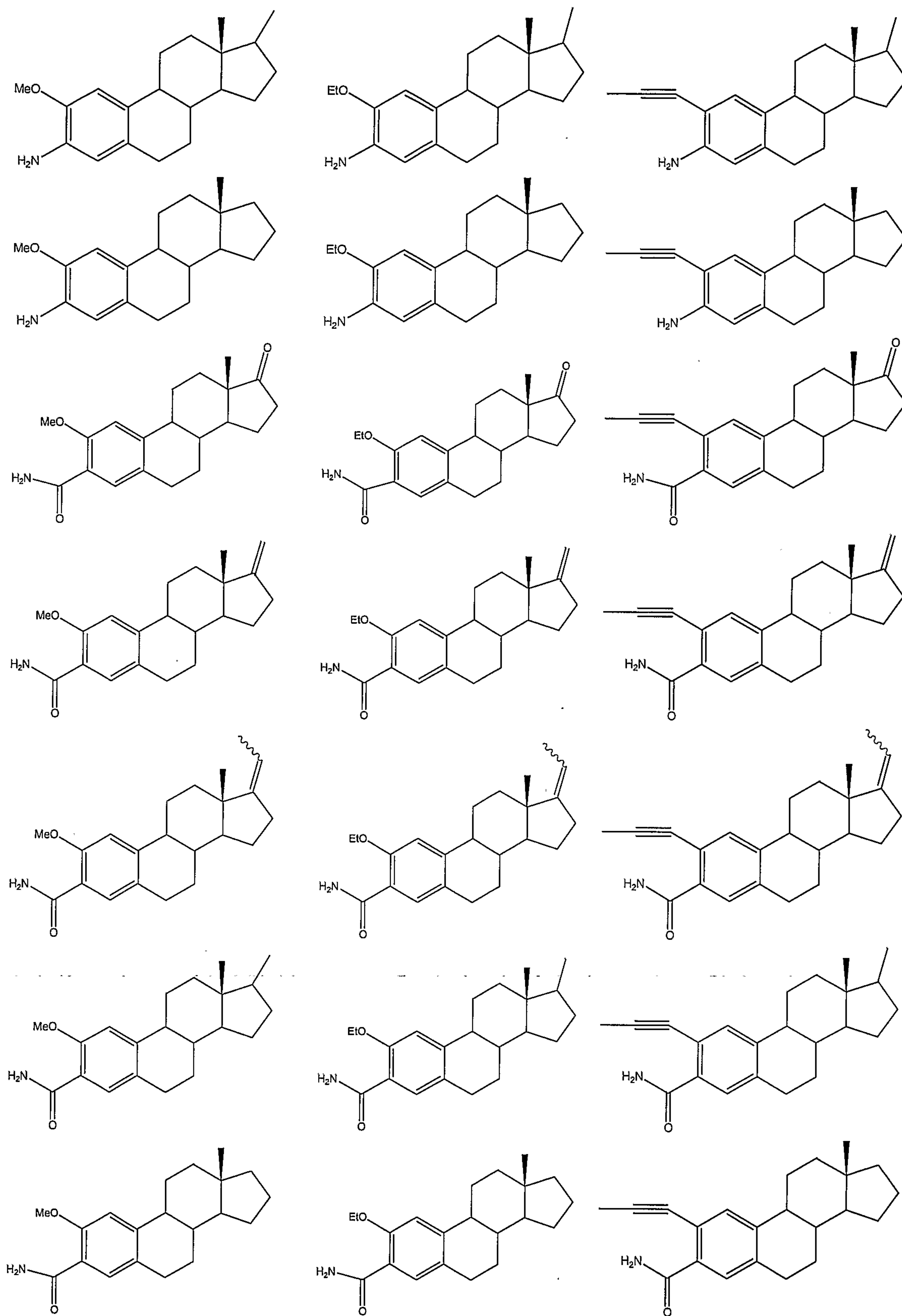


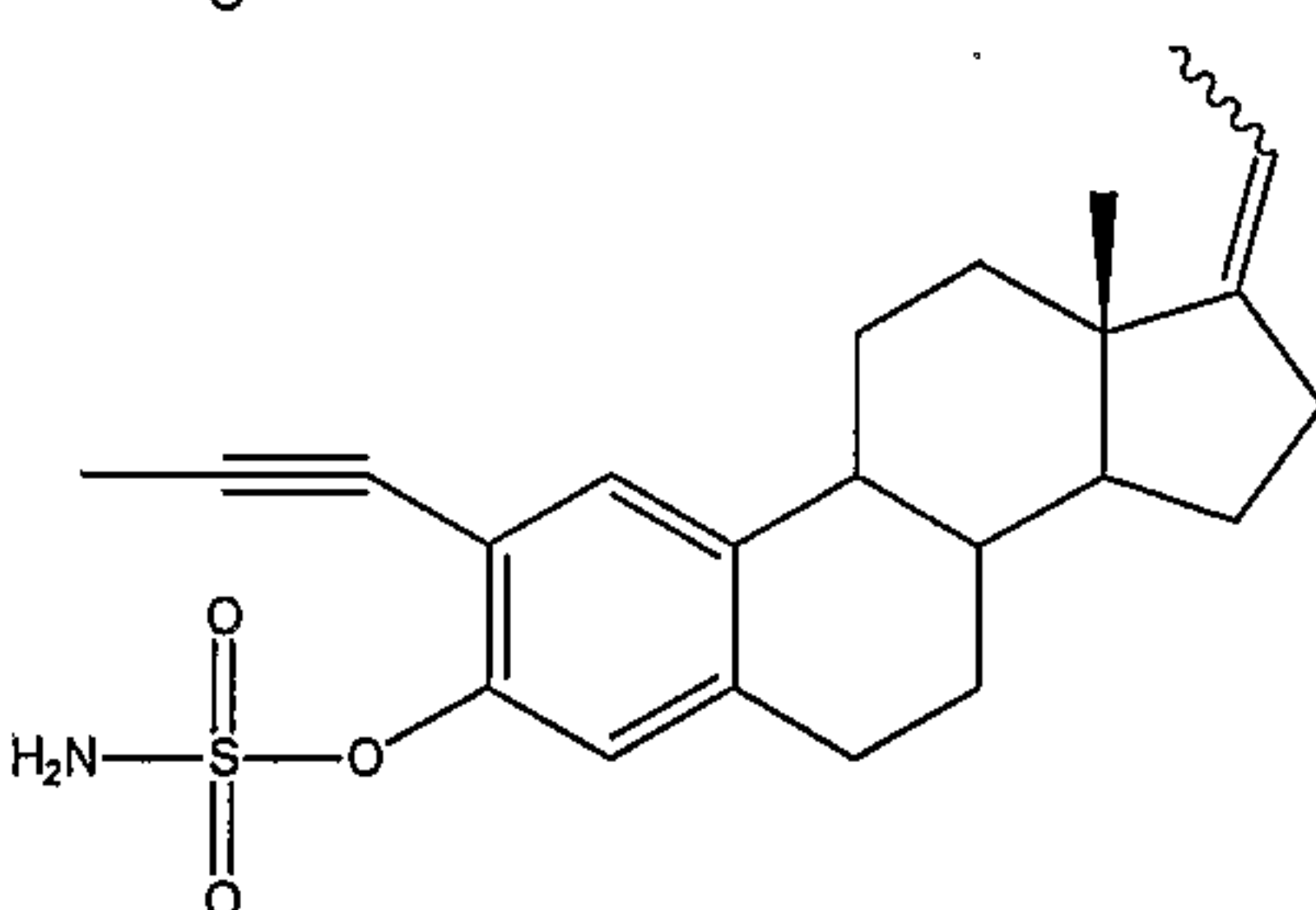
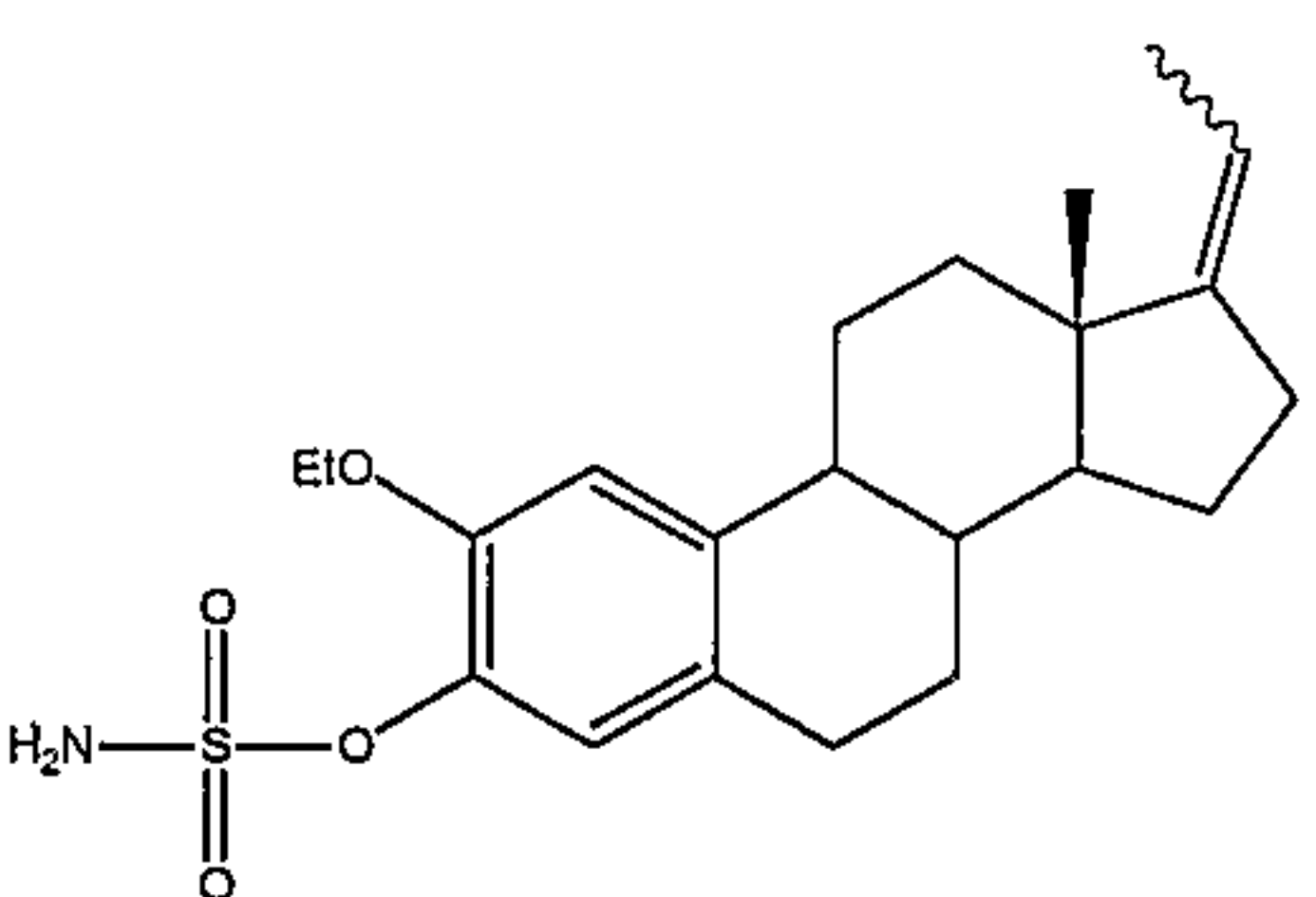
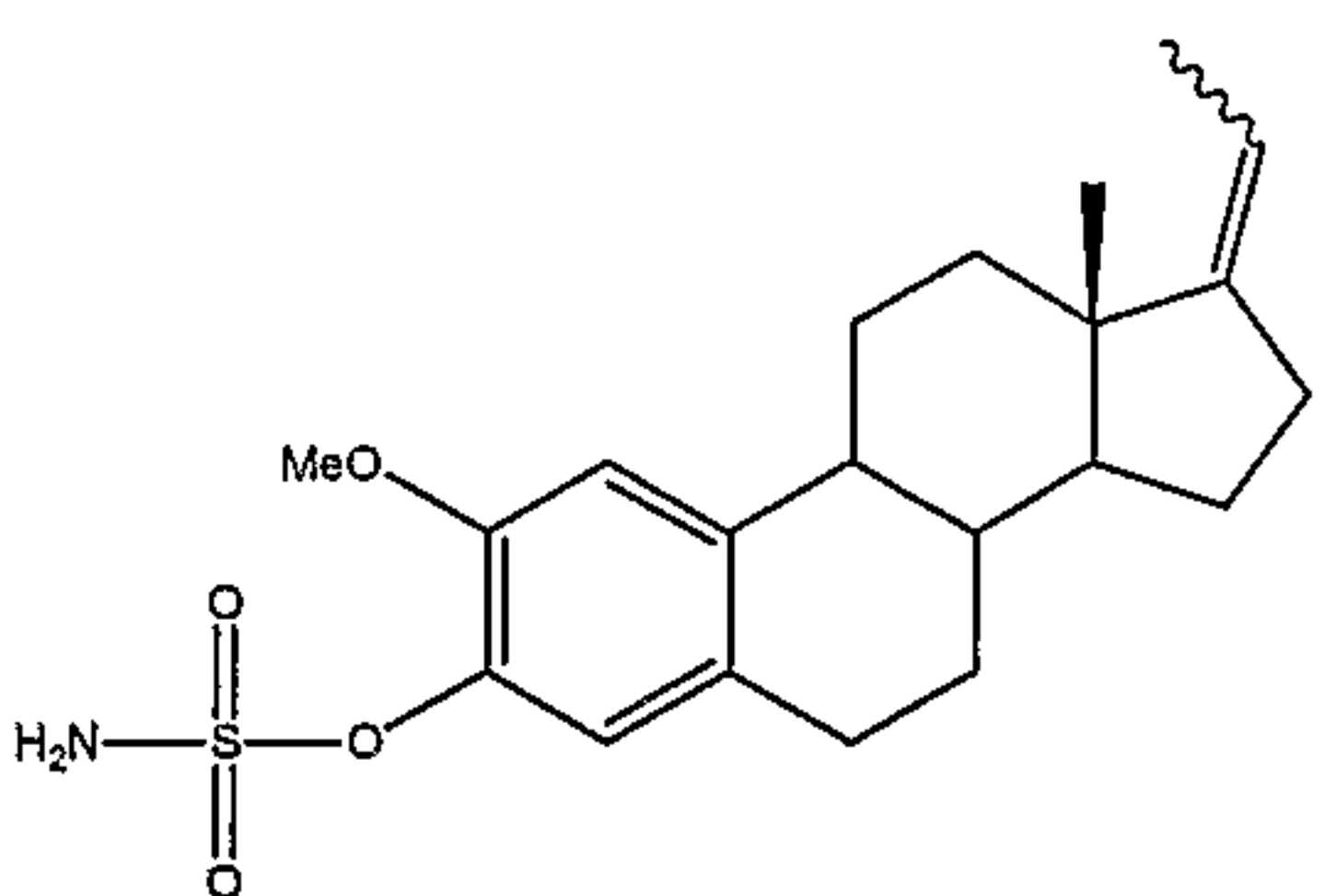
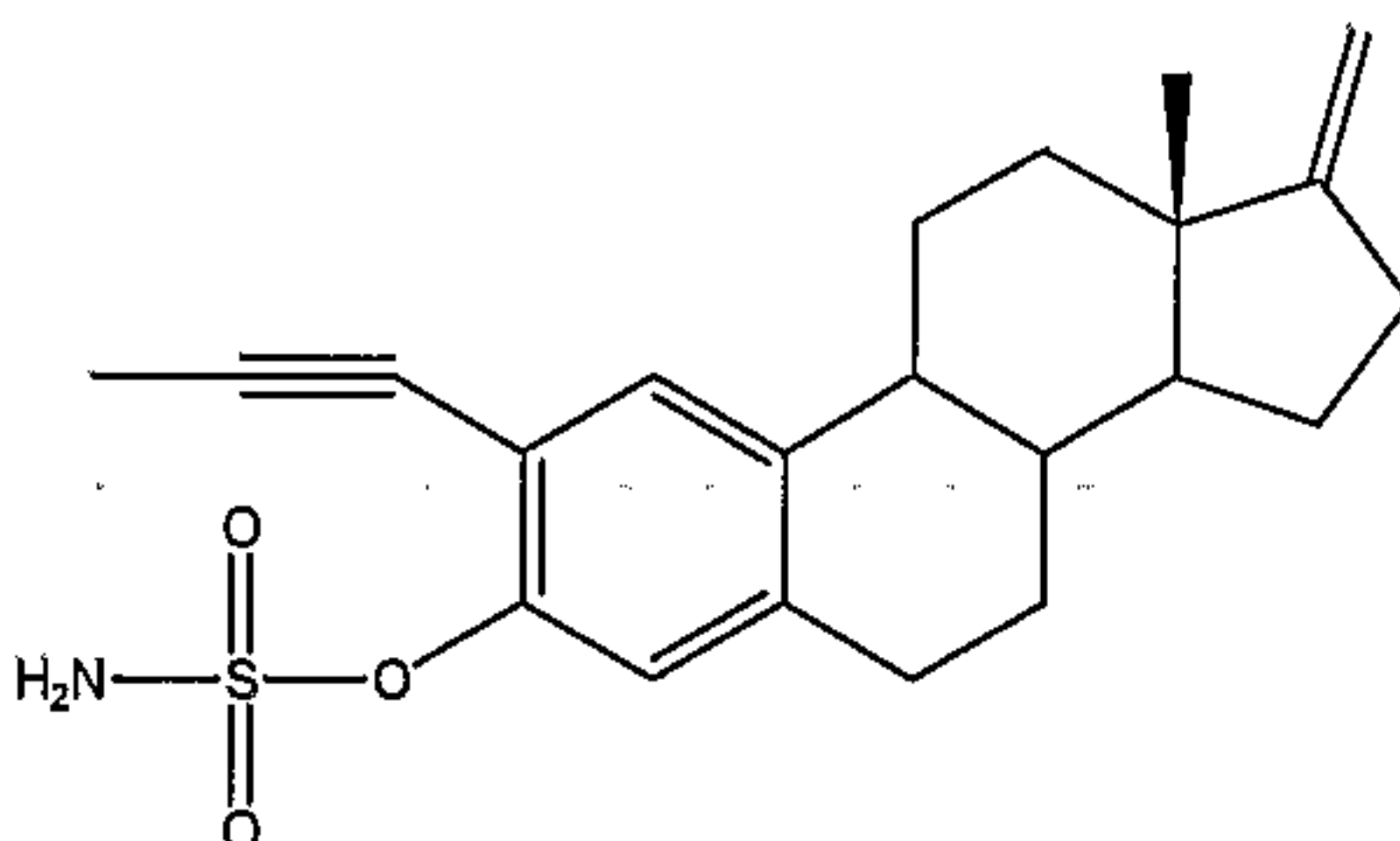
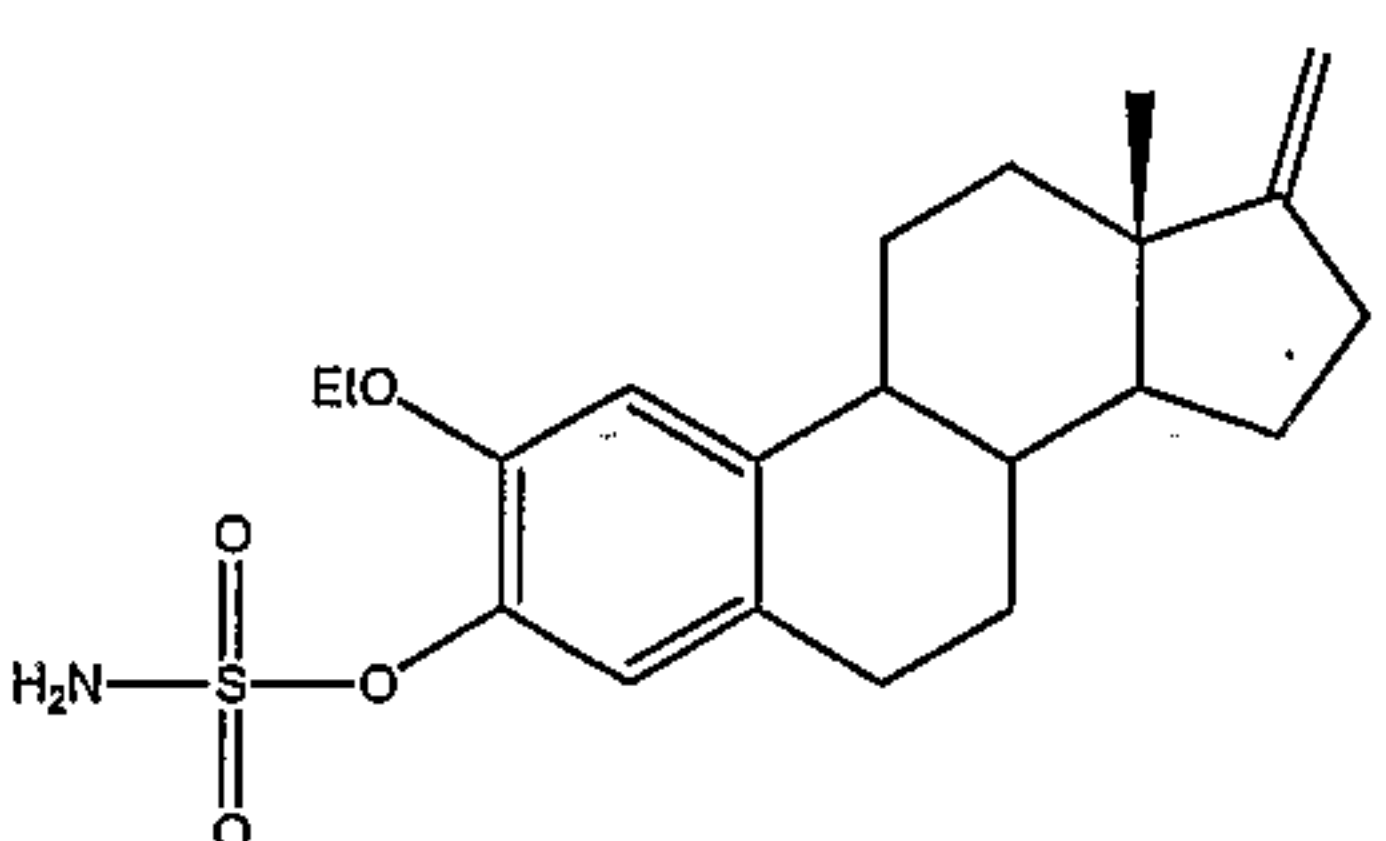
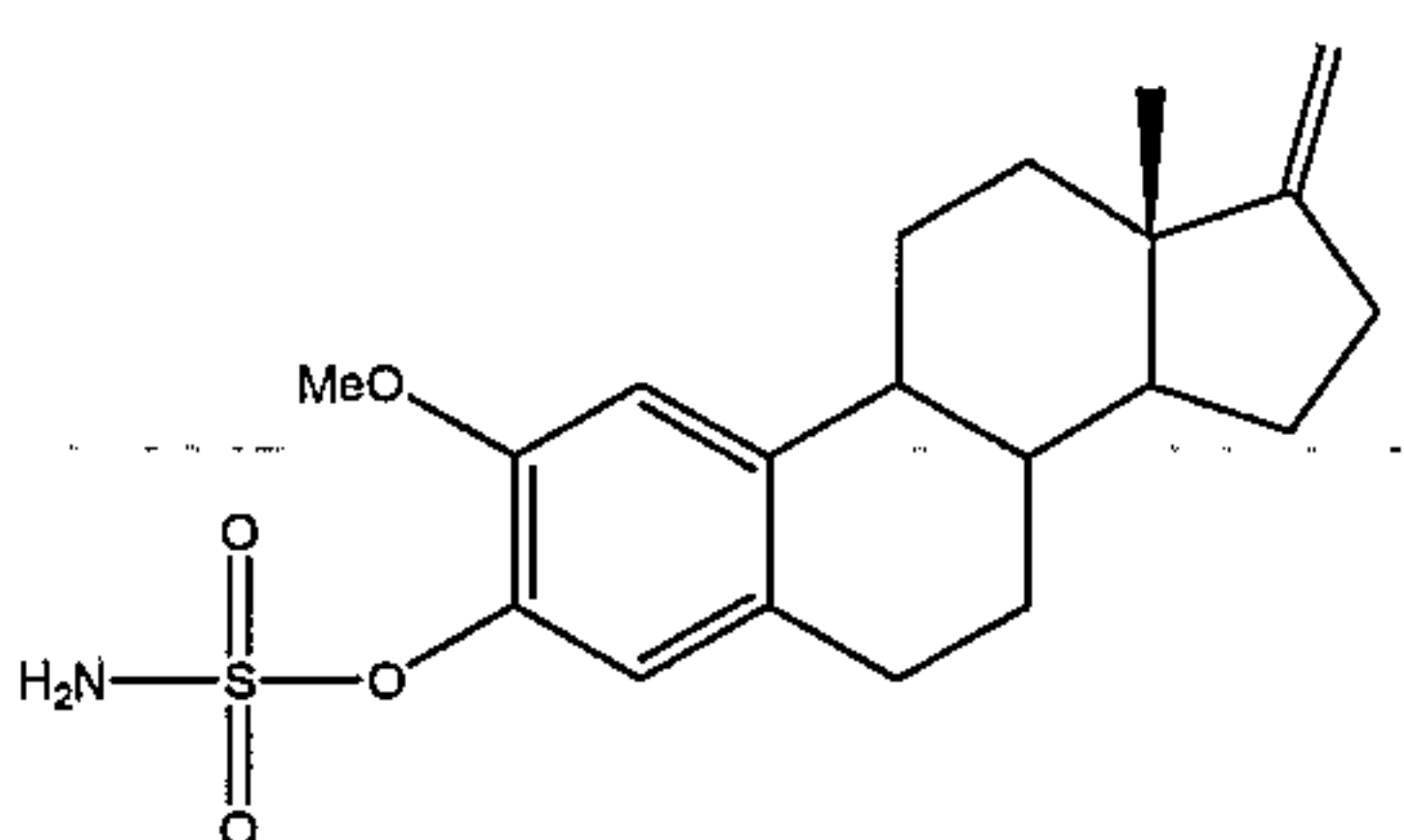
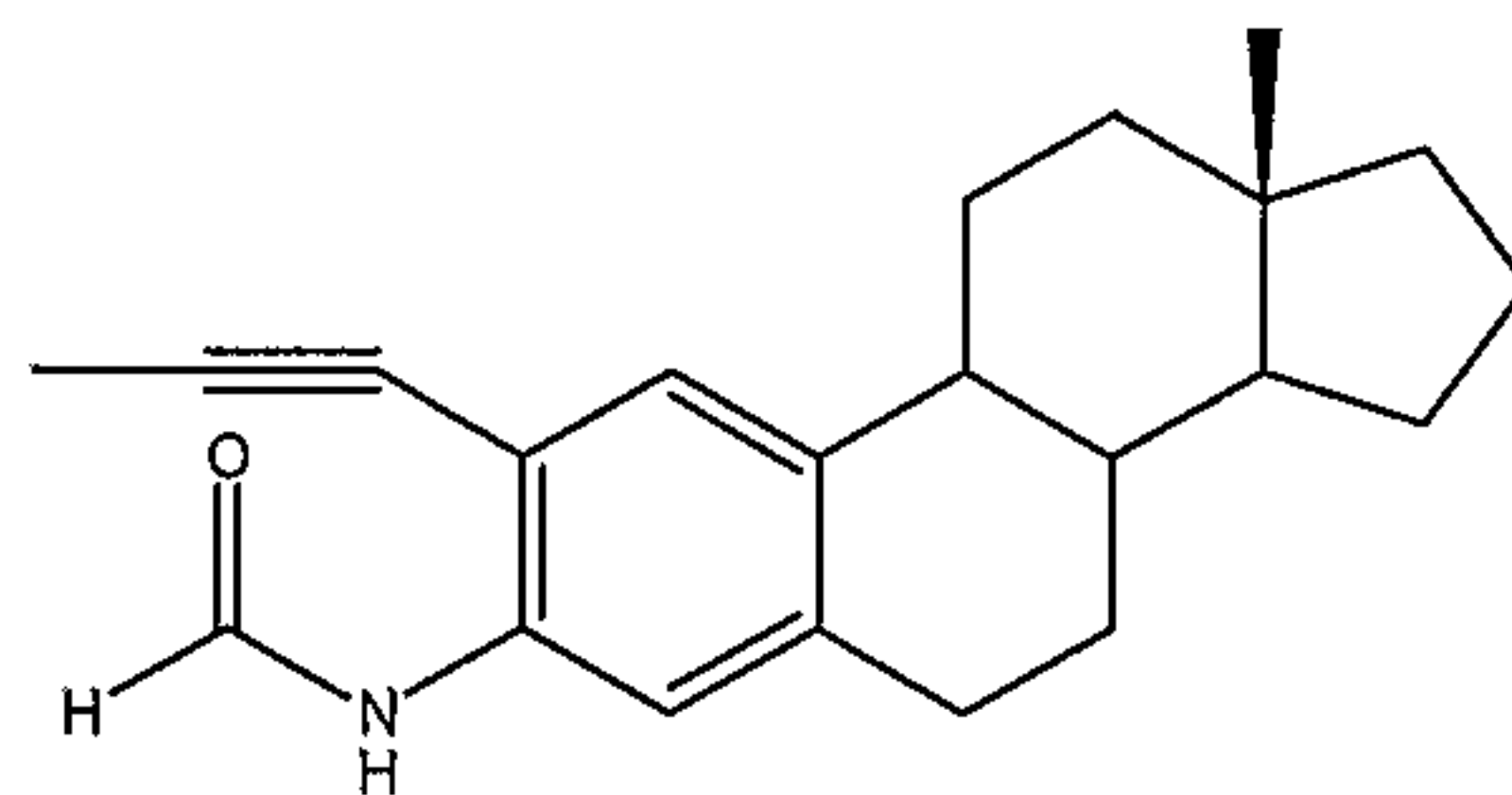
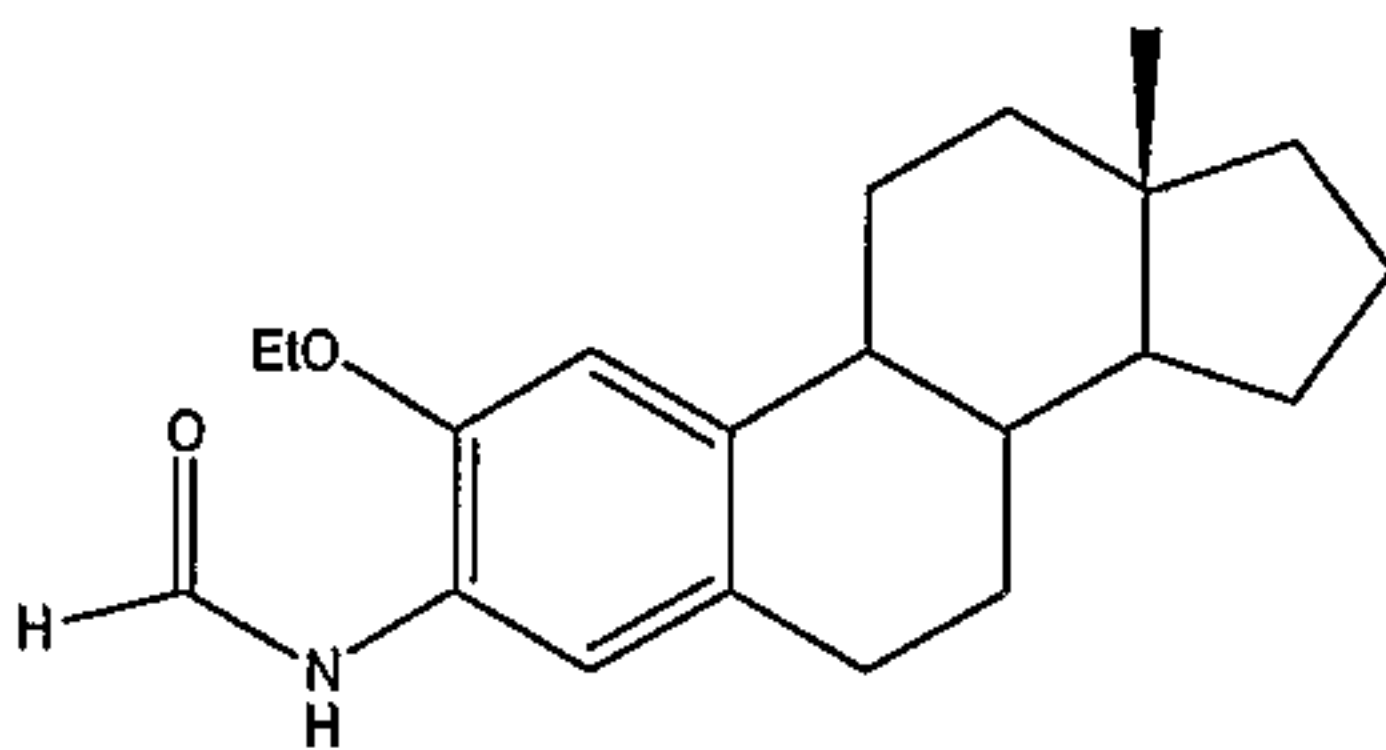
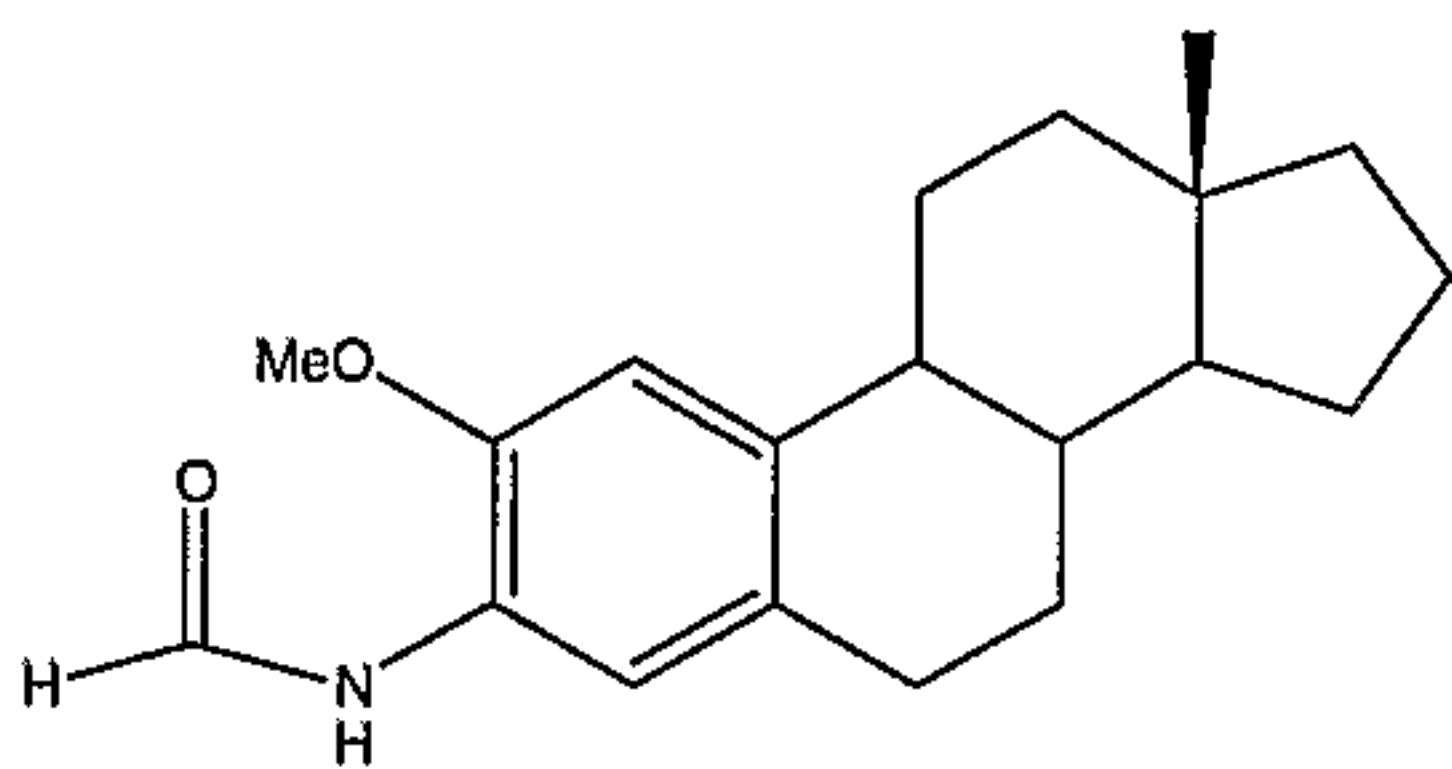
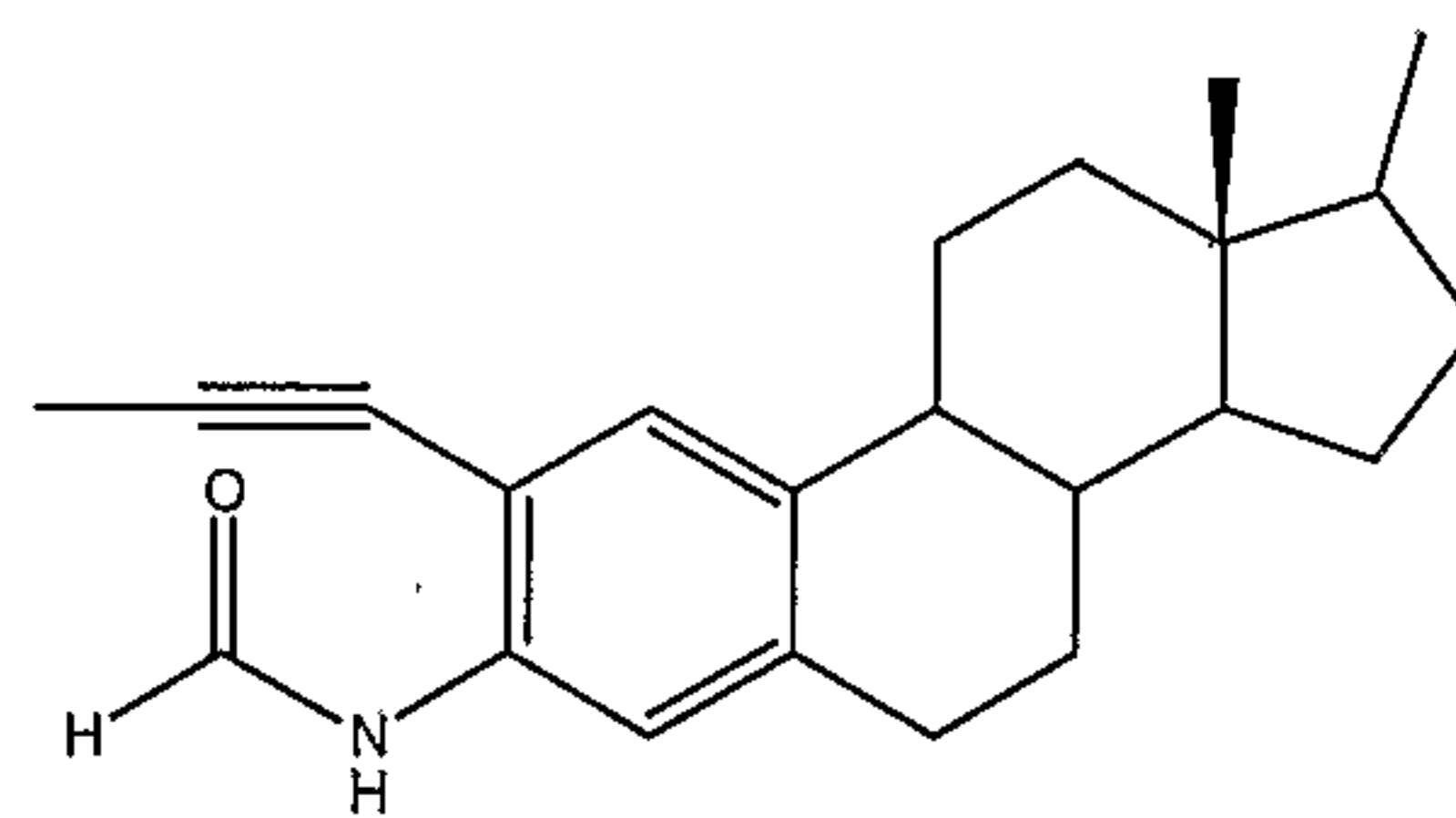
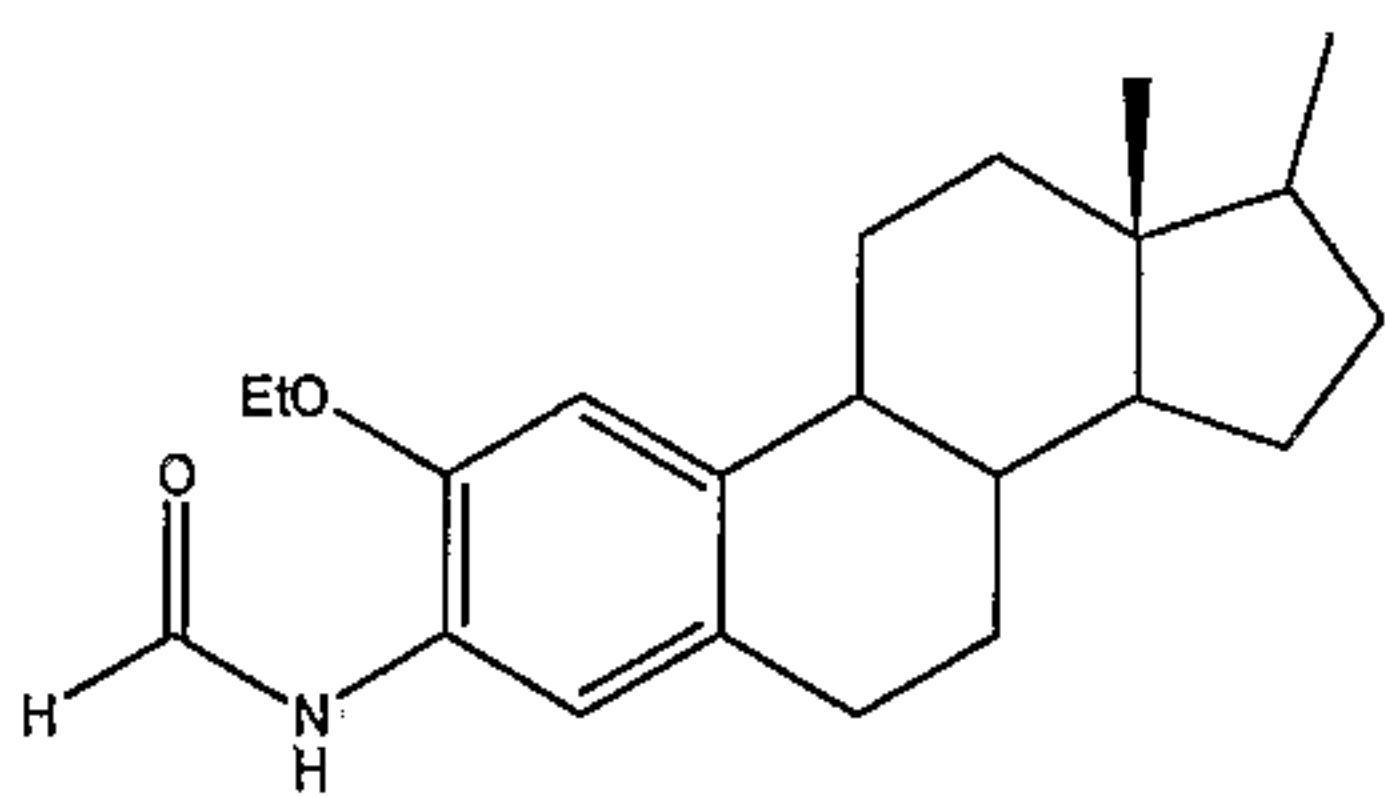
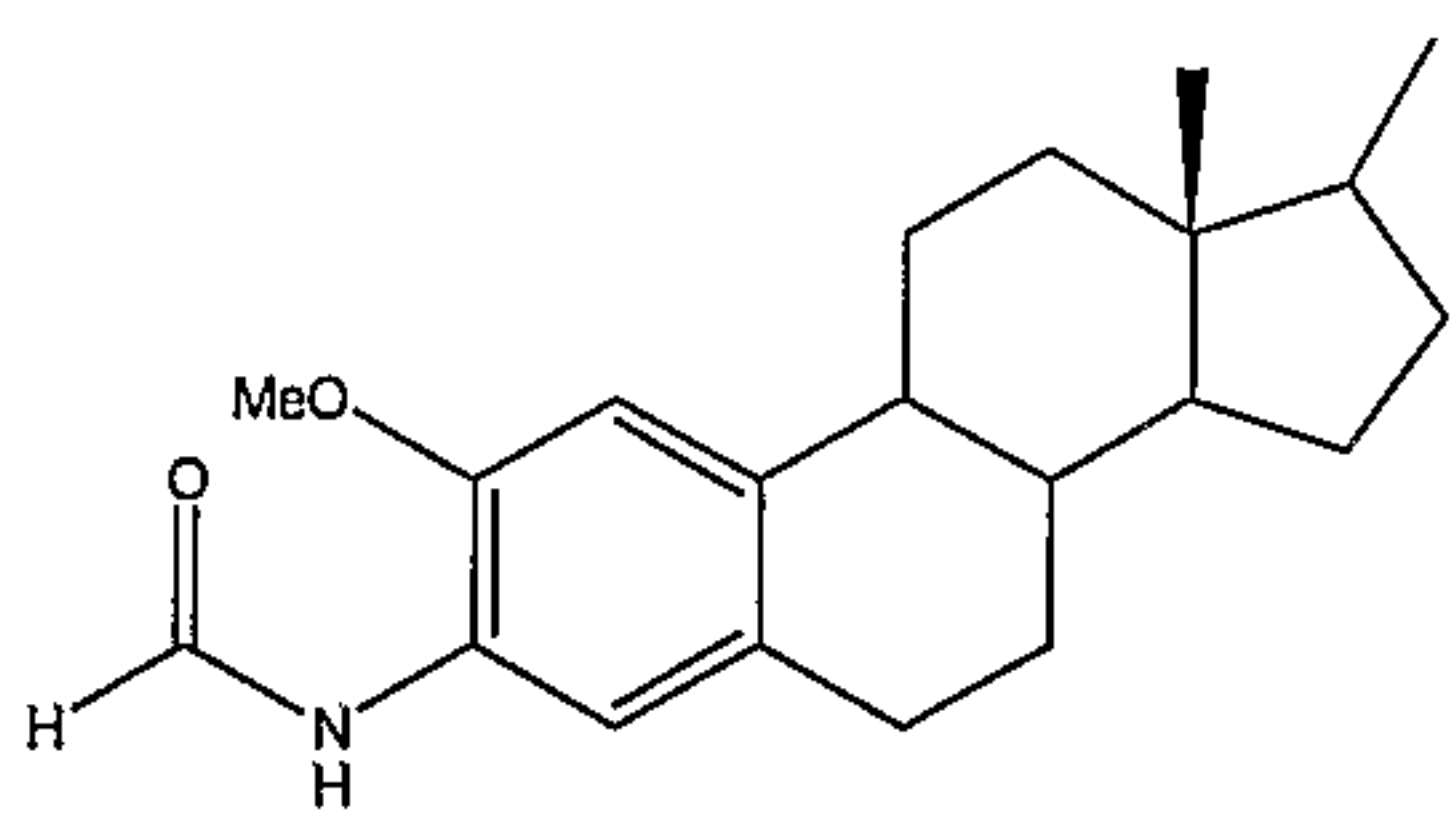
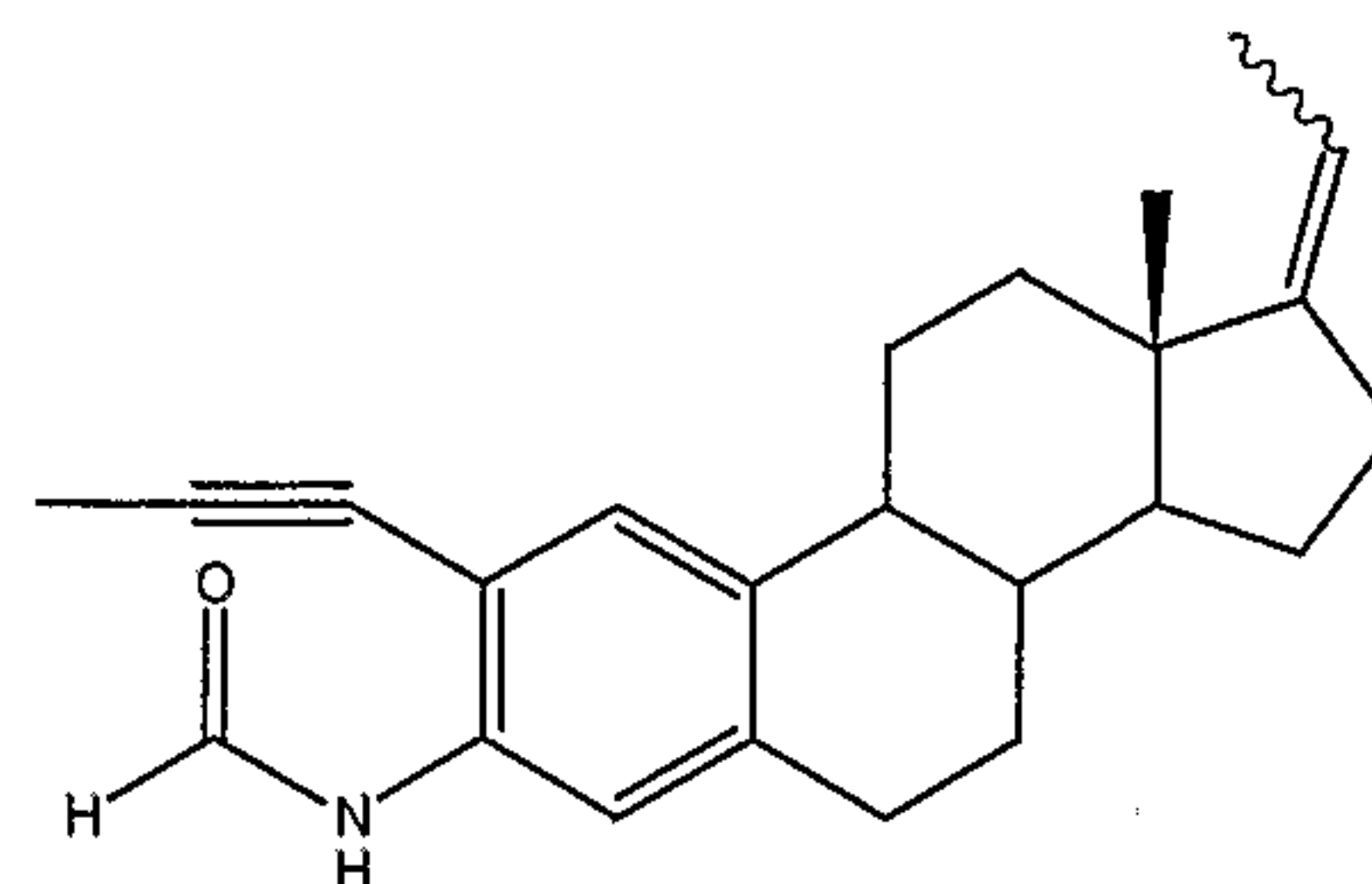
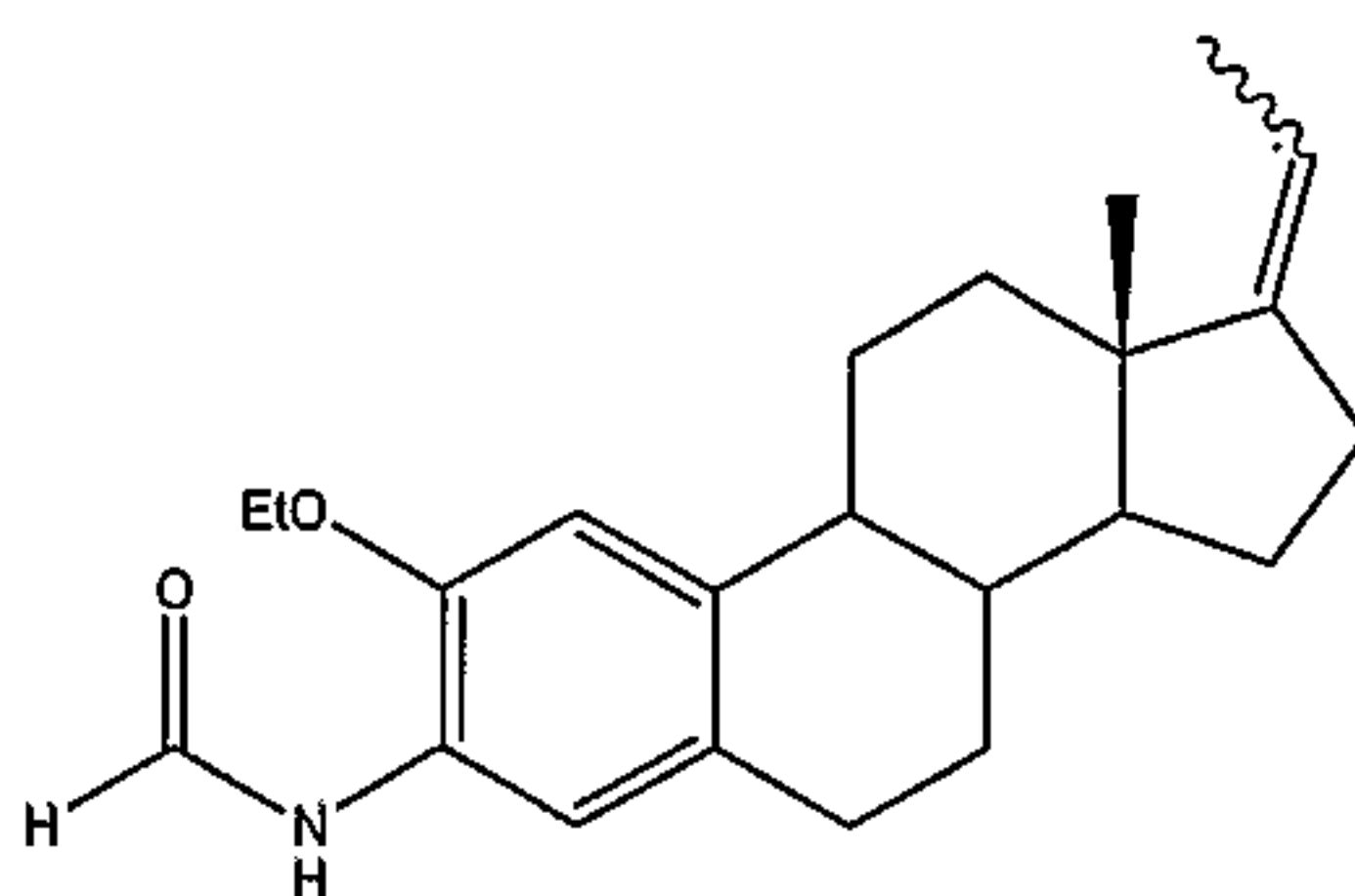
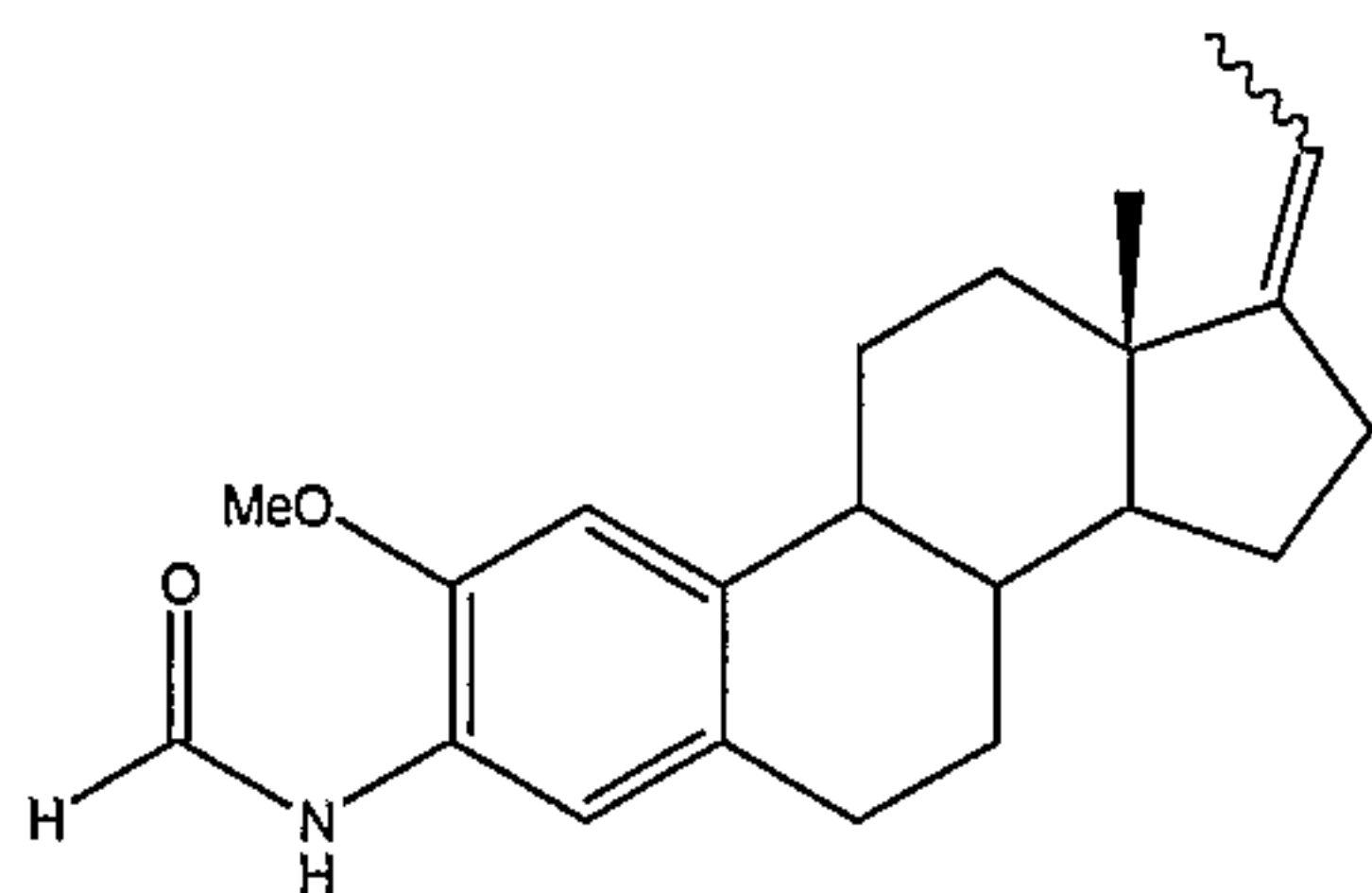
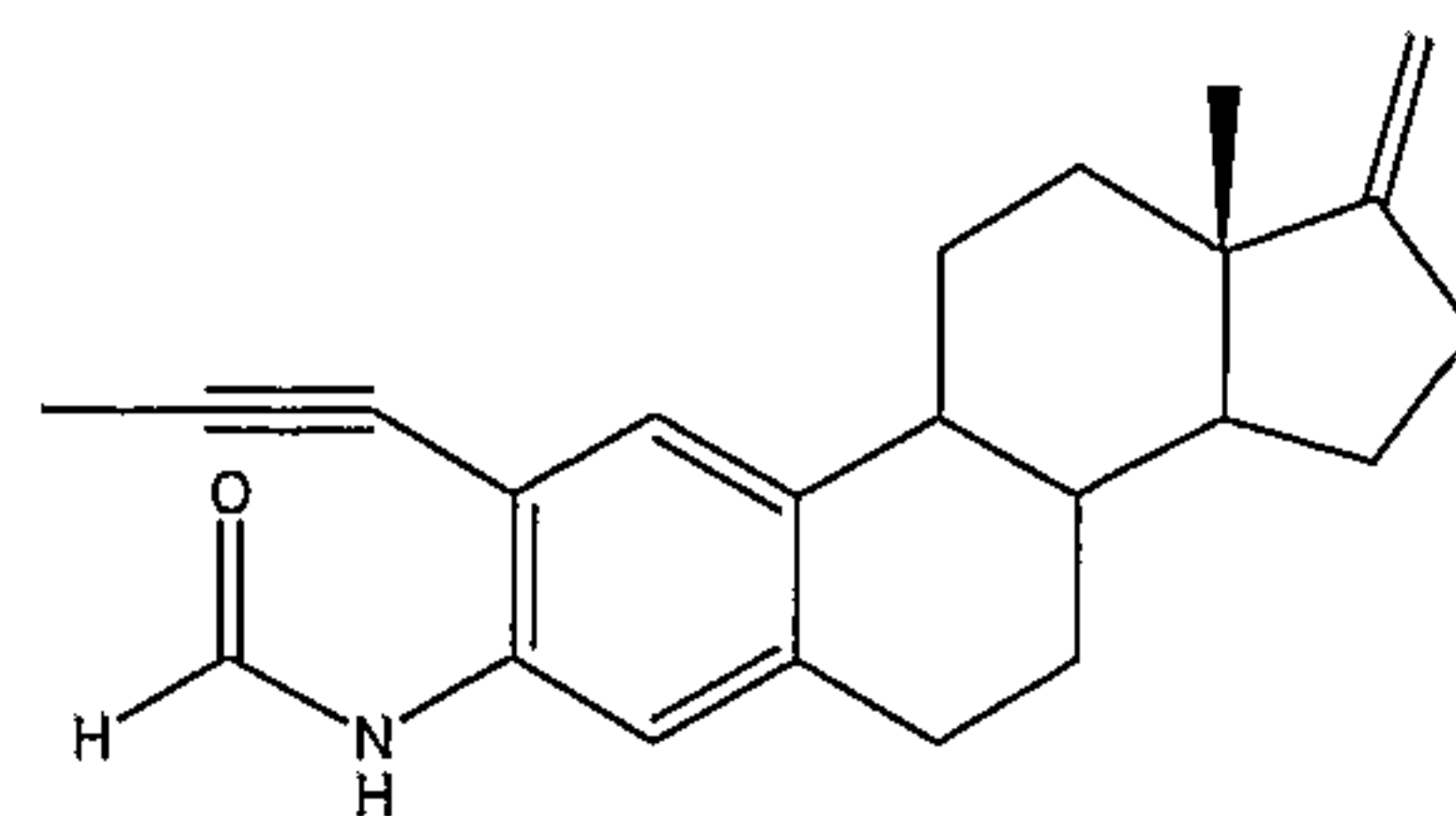
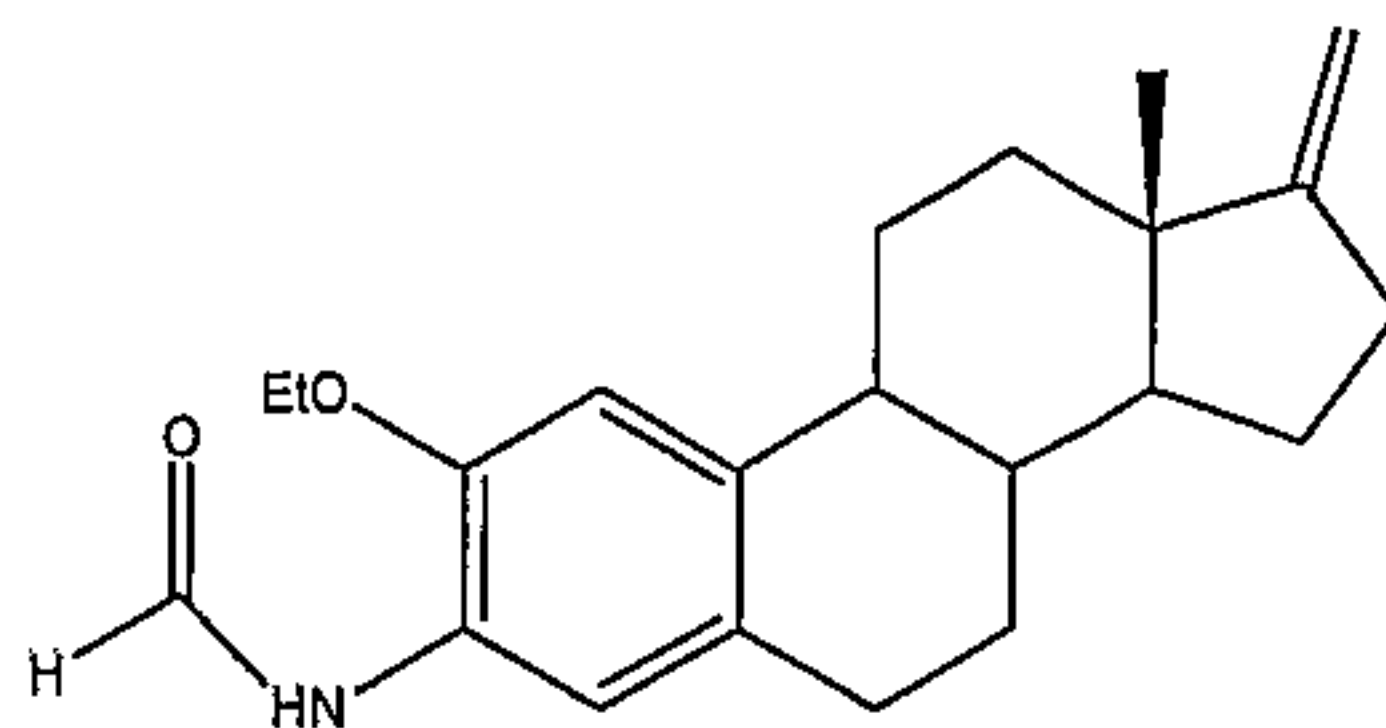
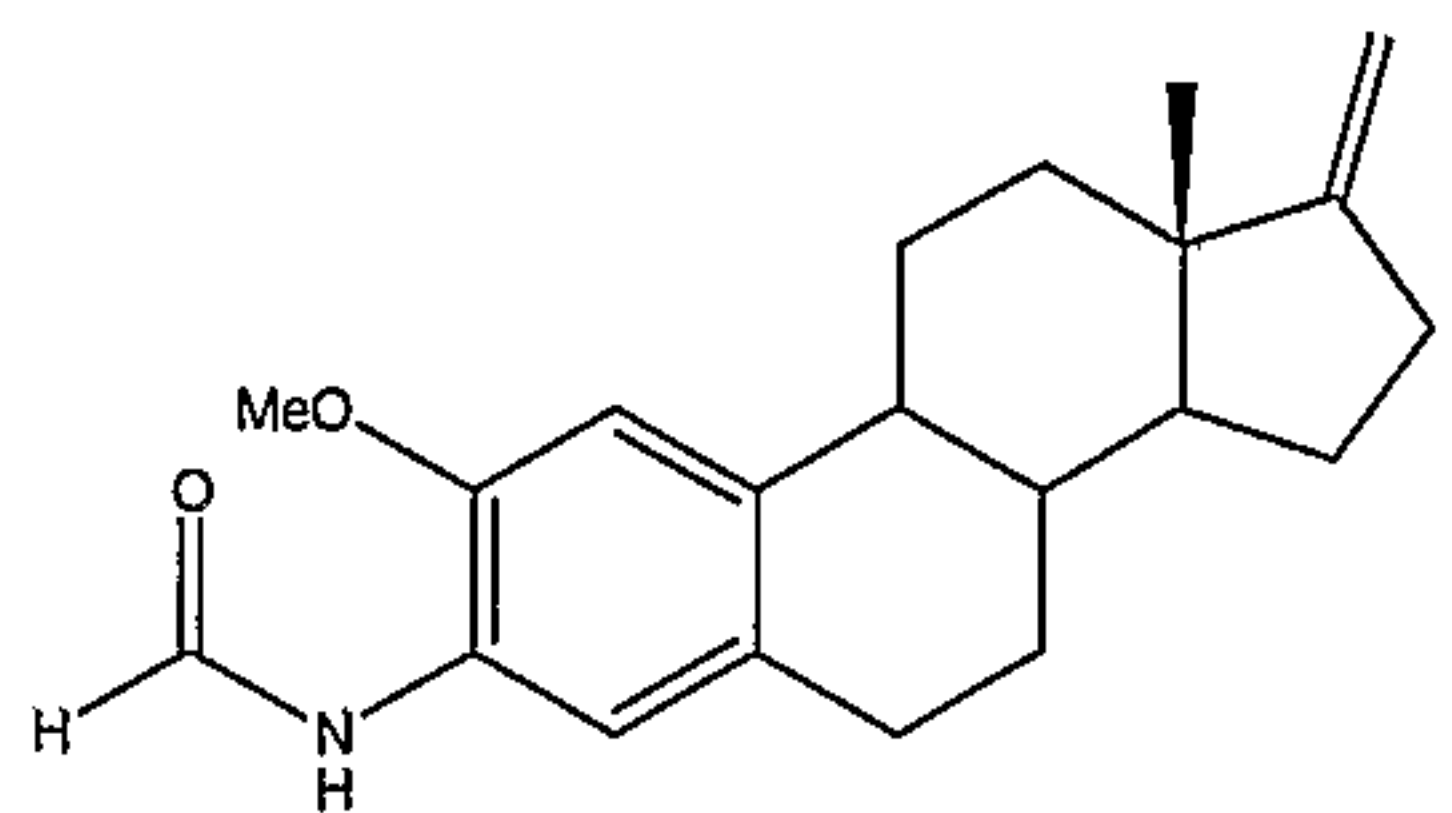
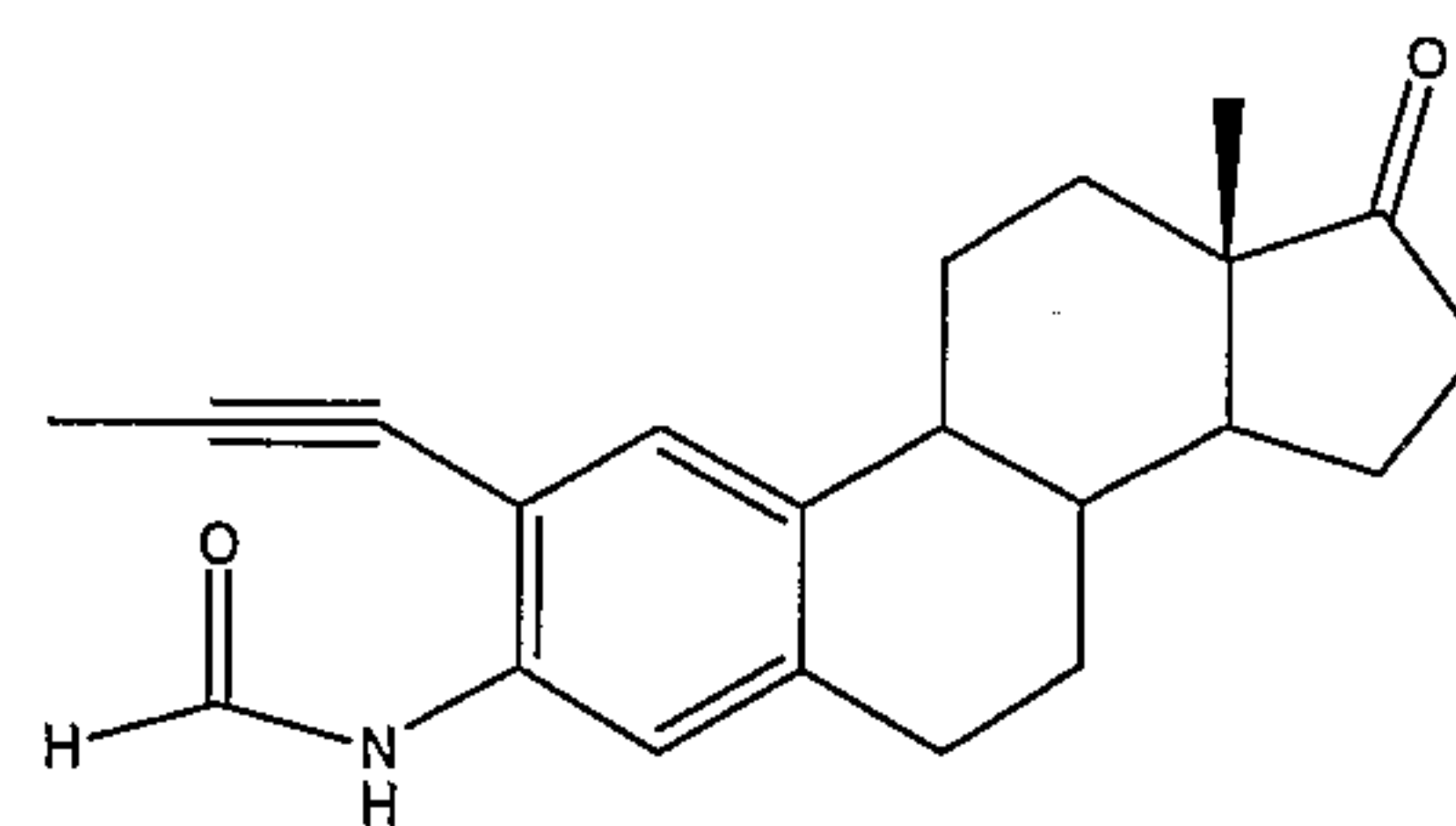
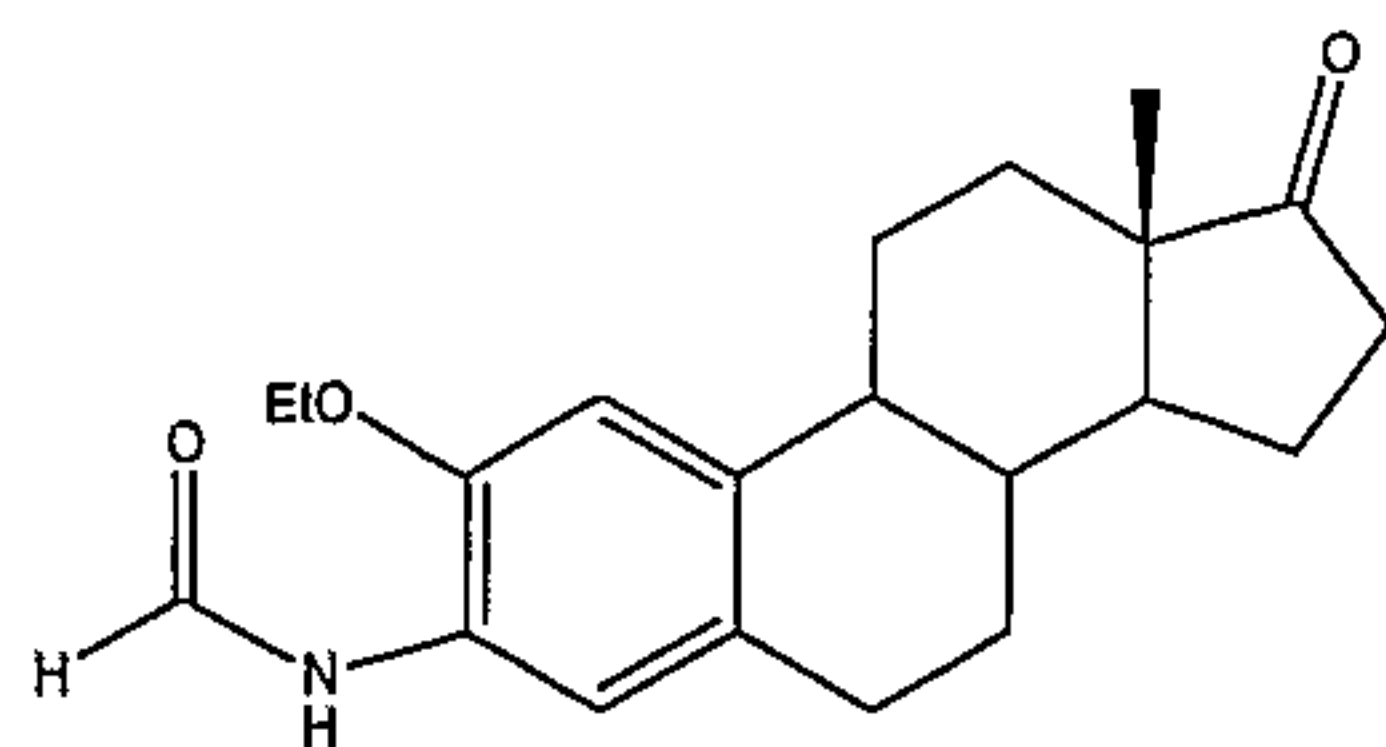
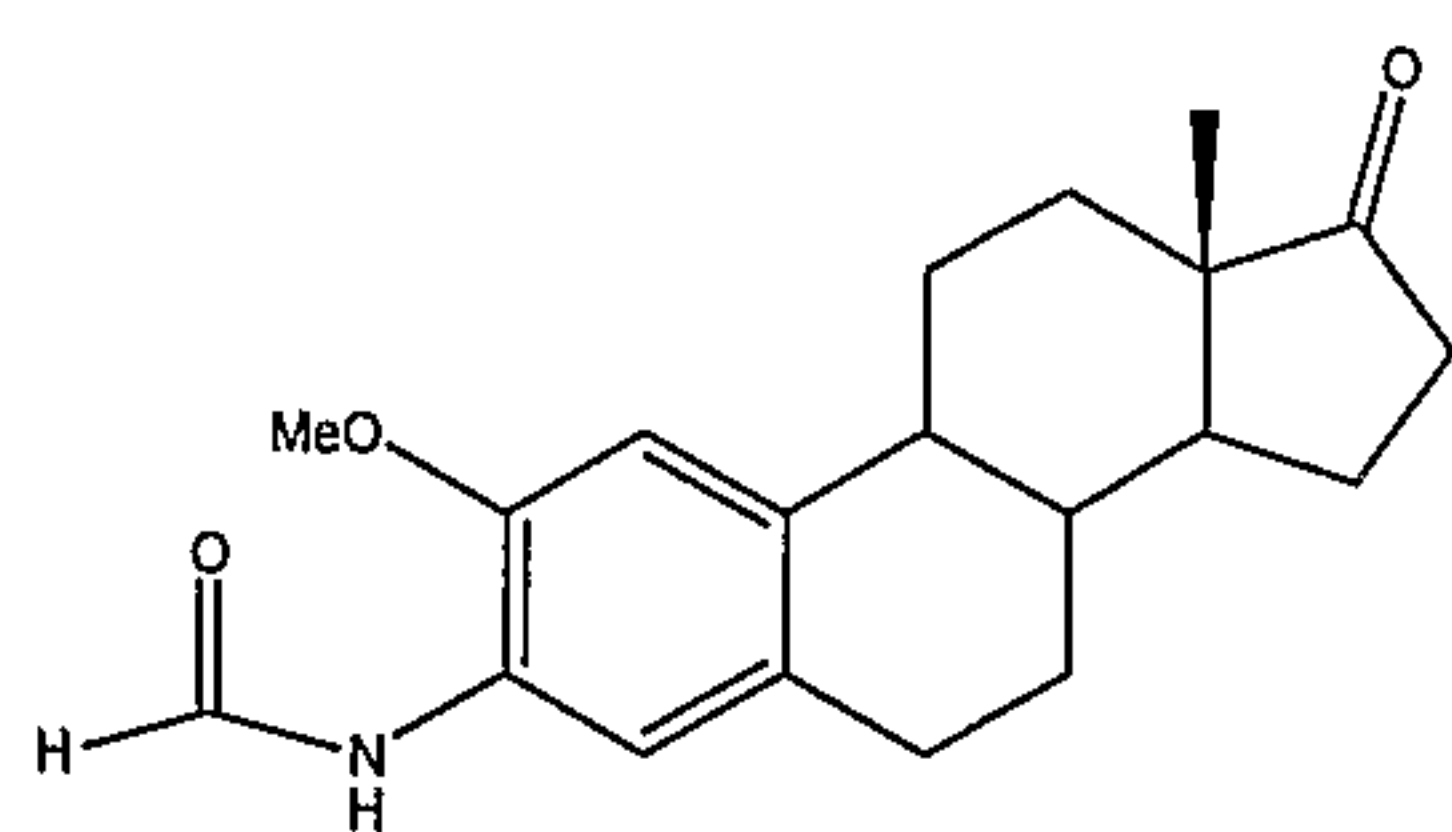
Each of the foregoing compounds from Table I are found to have anti-mitotic, anti-angiogenic and/or anti-tumor properties.

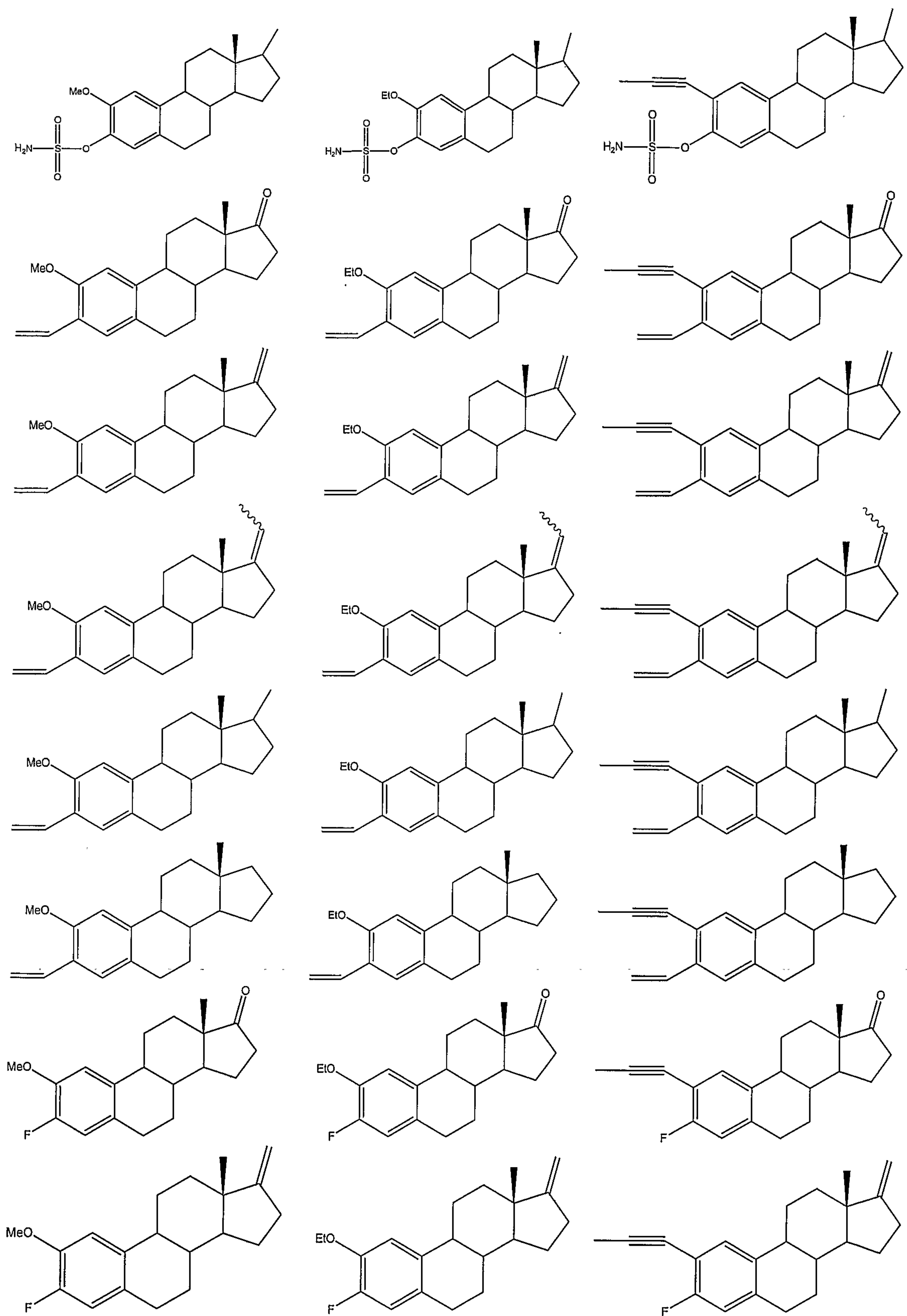
5 In an alternate disclosed embodiment of the present invention, preferred species from the foregoing genus that are useful in the present invention include, but are not limited to, the compounds shown in Table II.

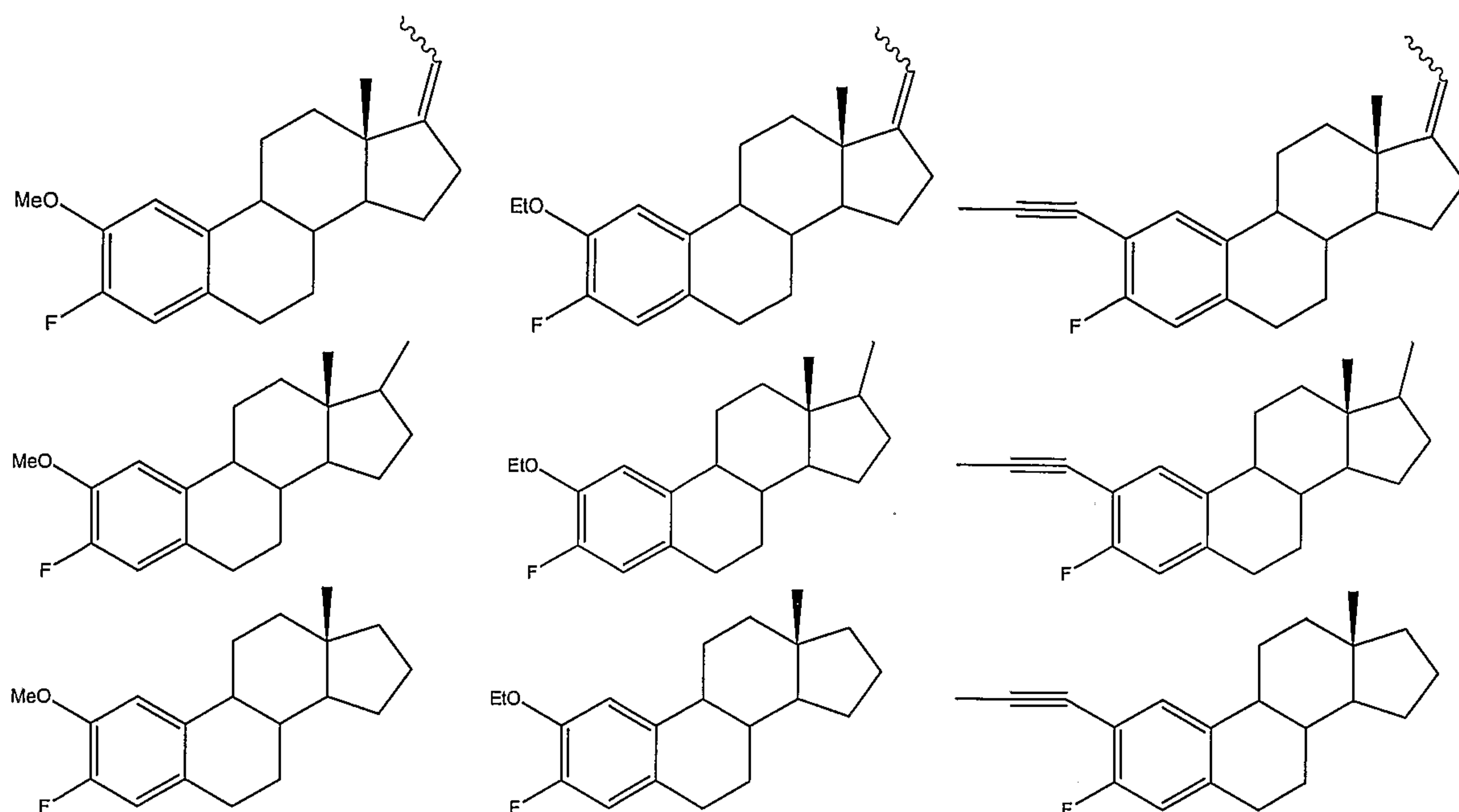
Table II







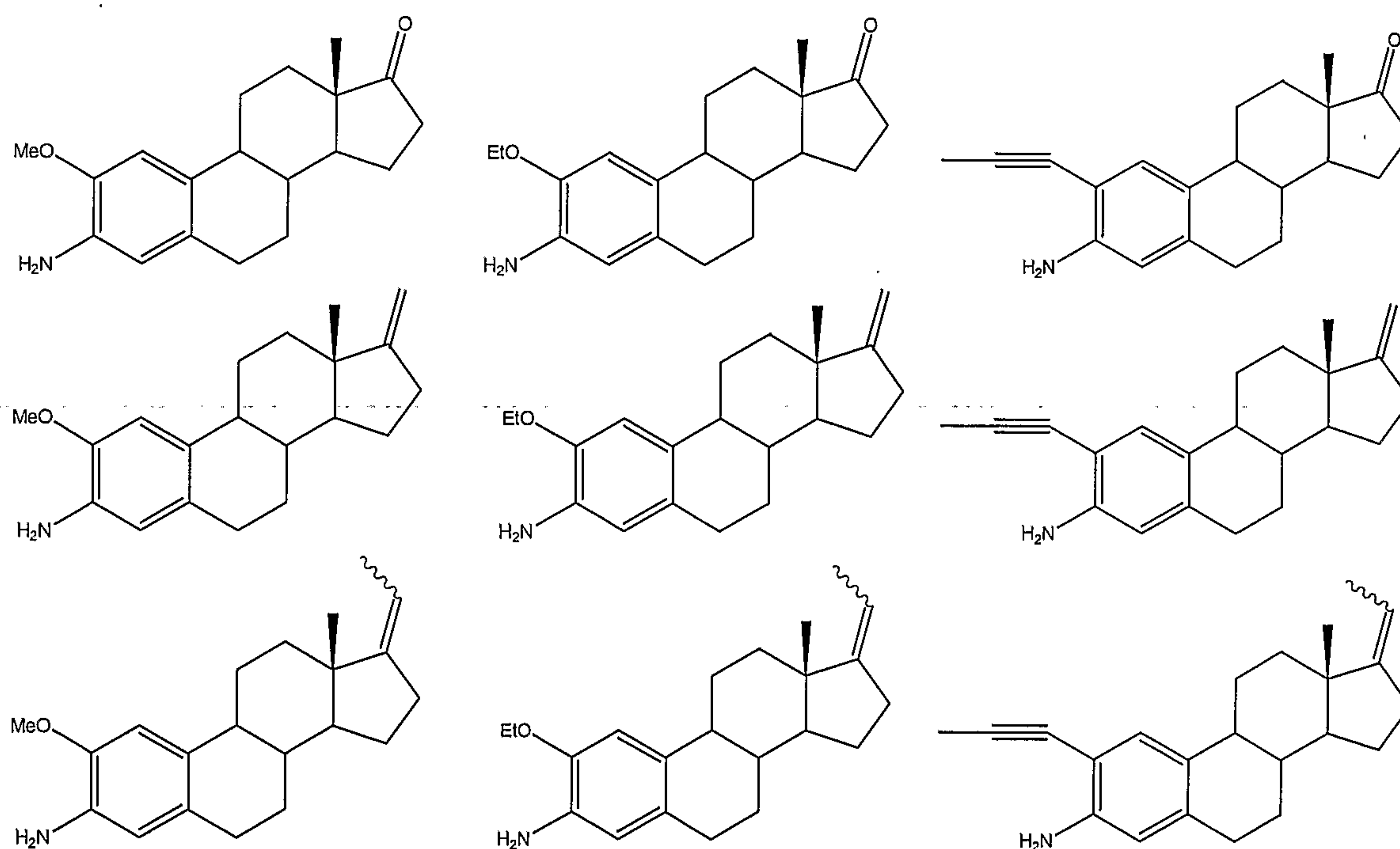


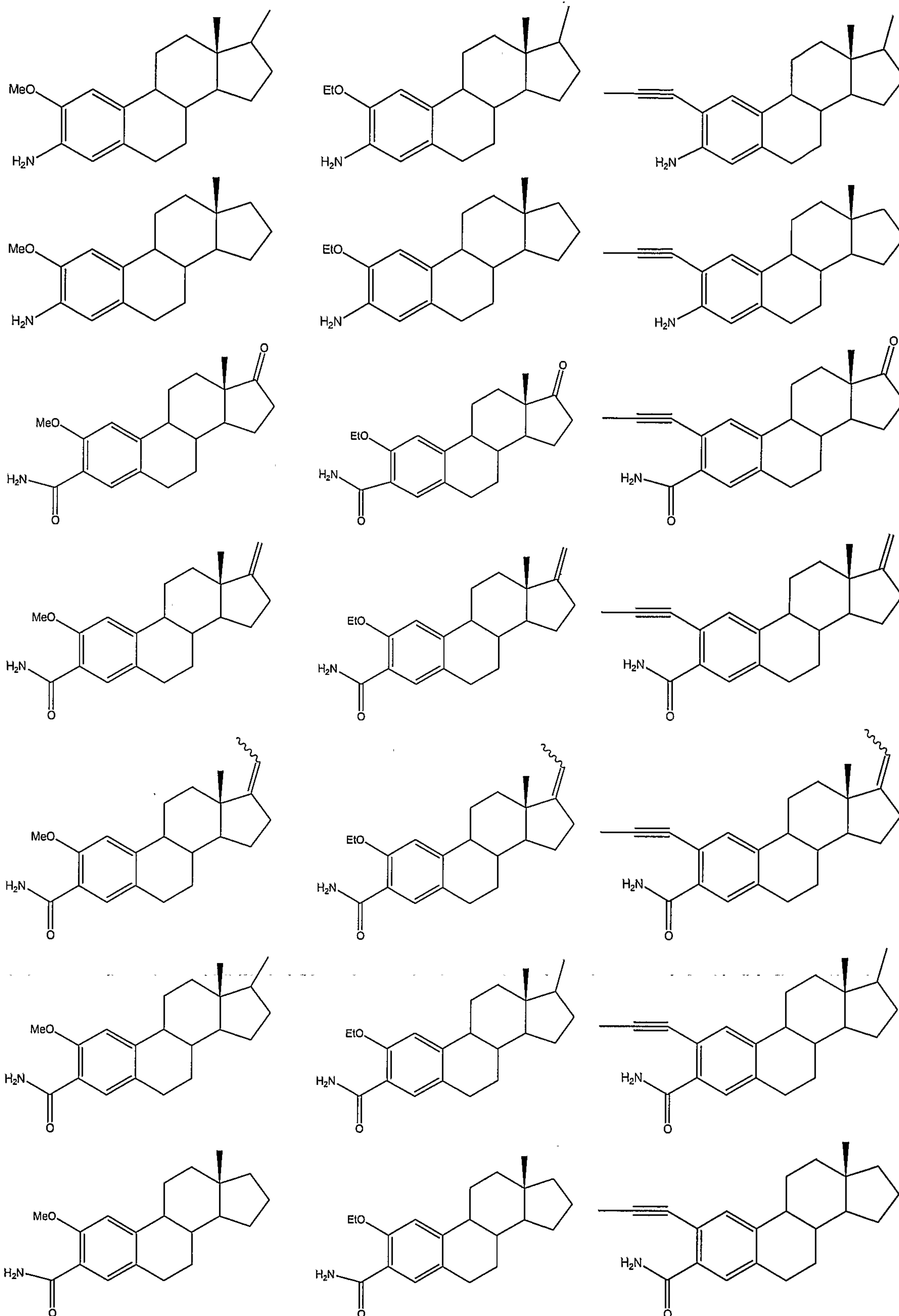


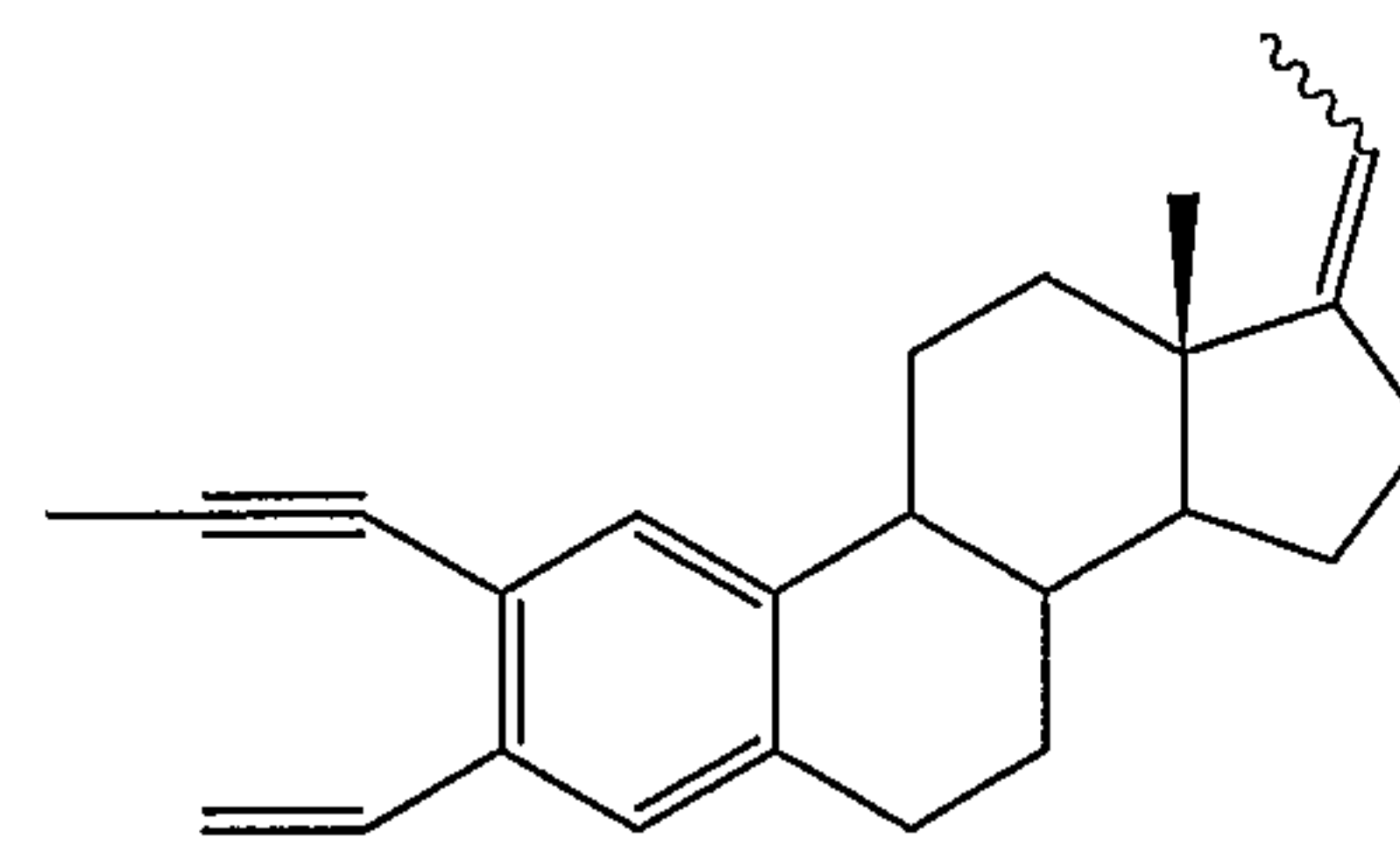
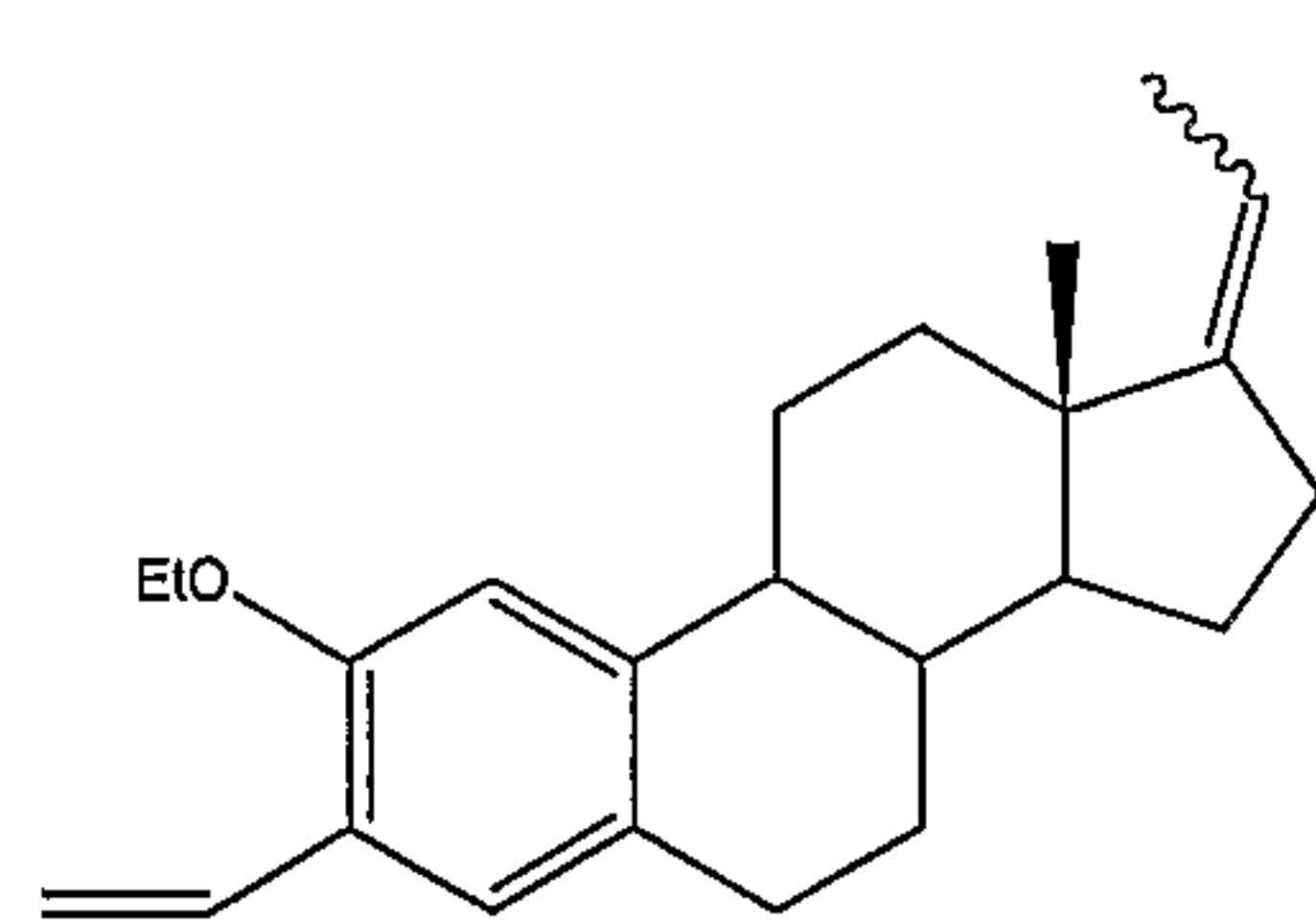
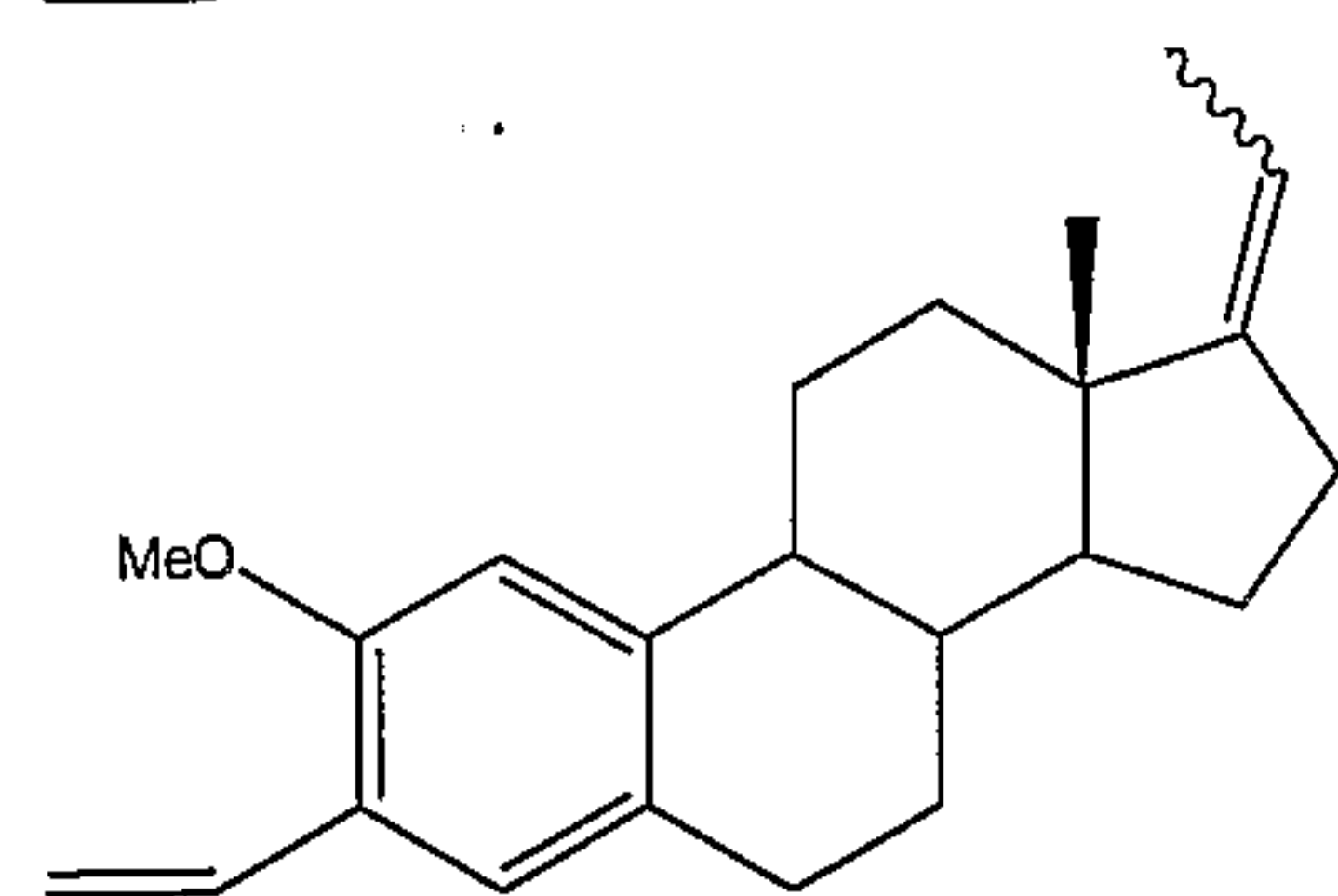
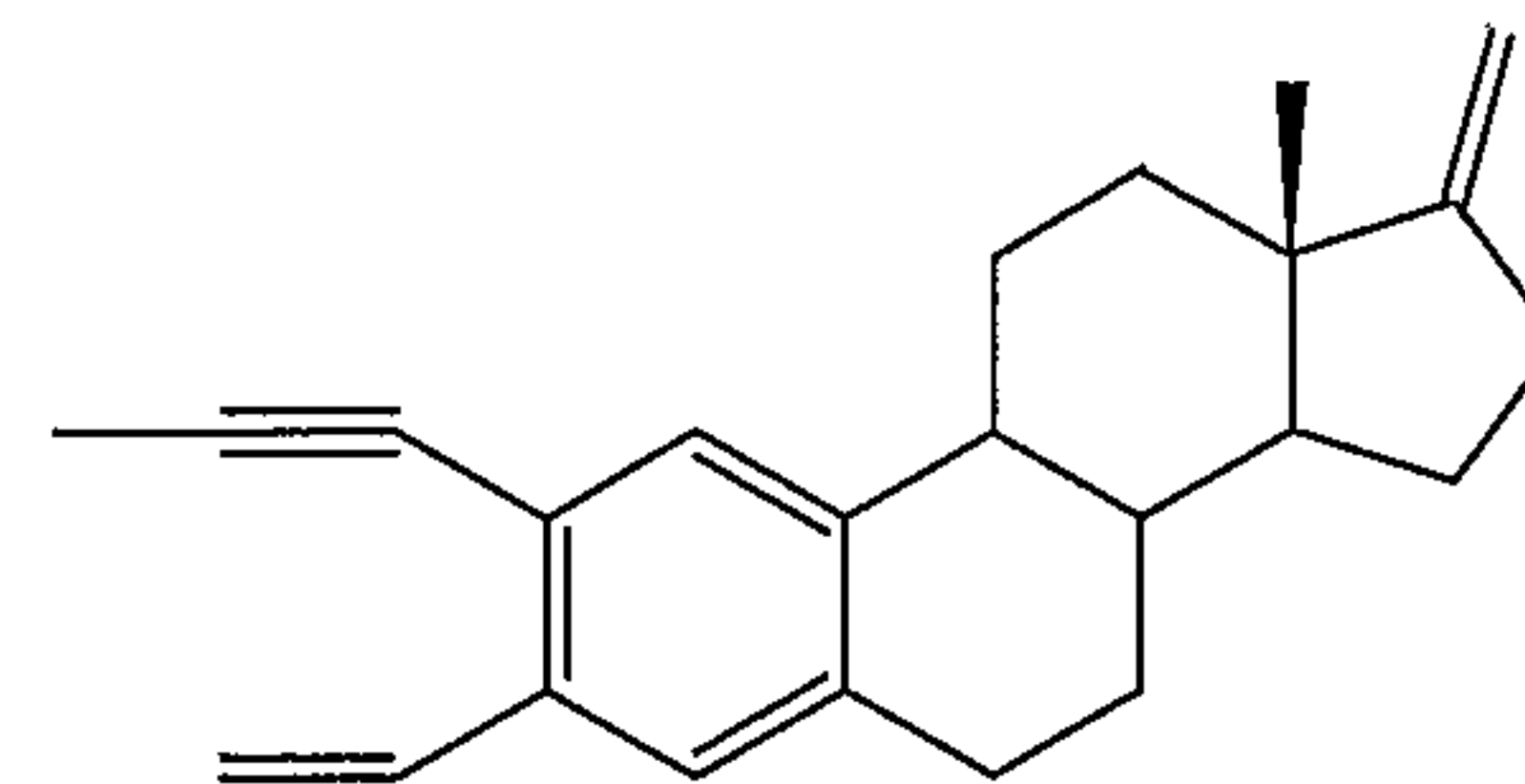
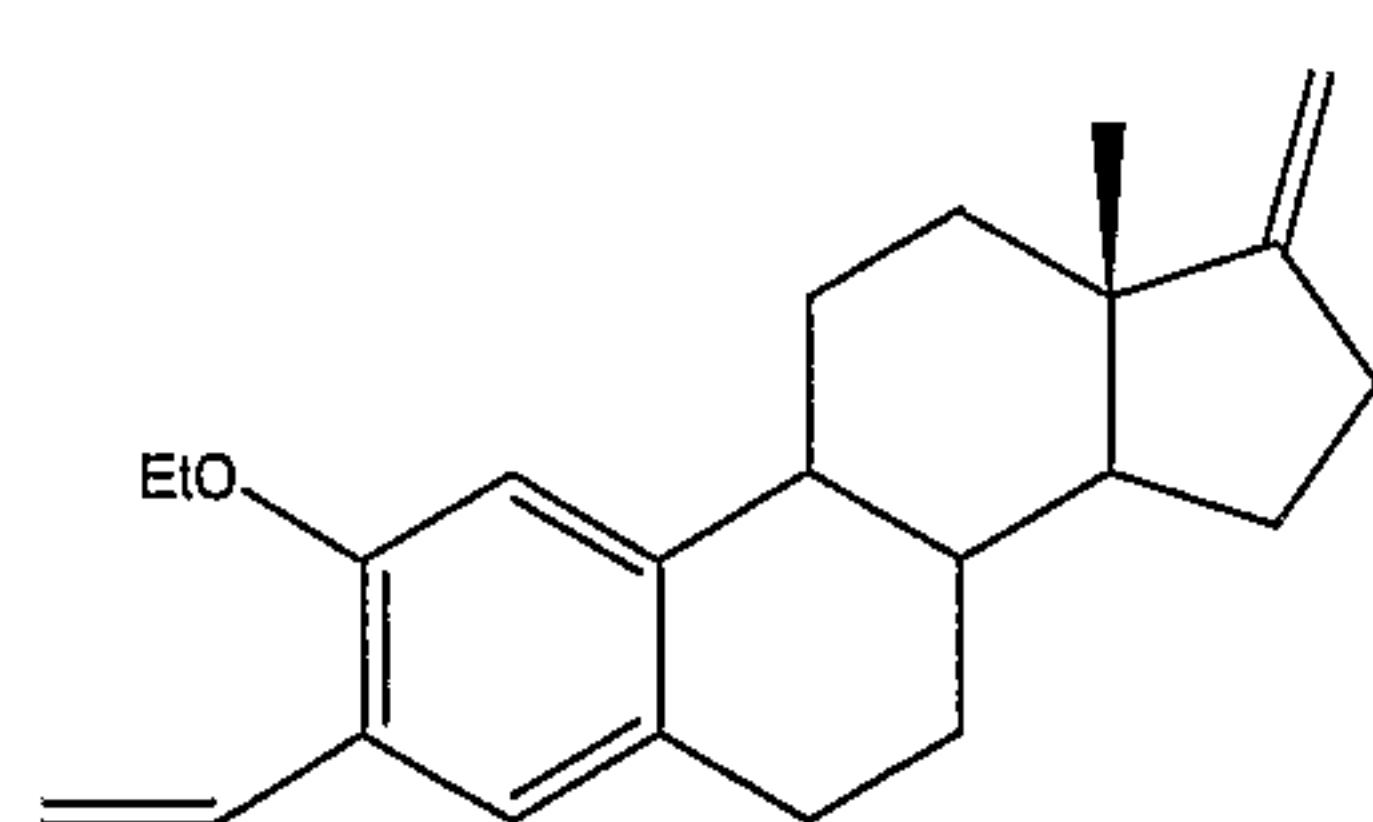
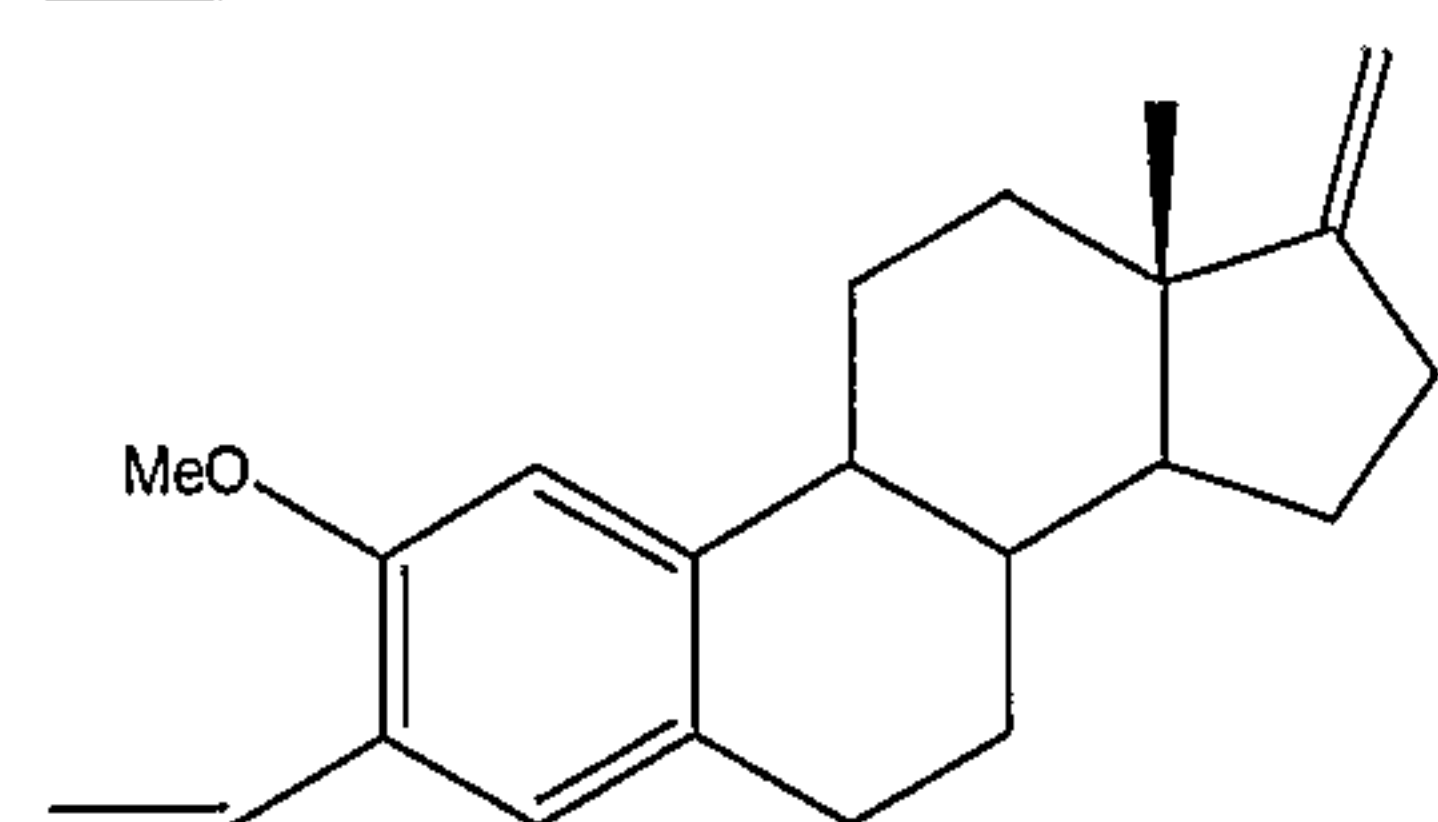
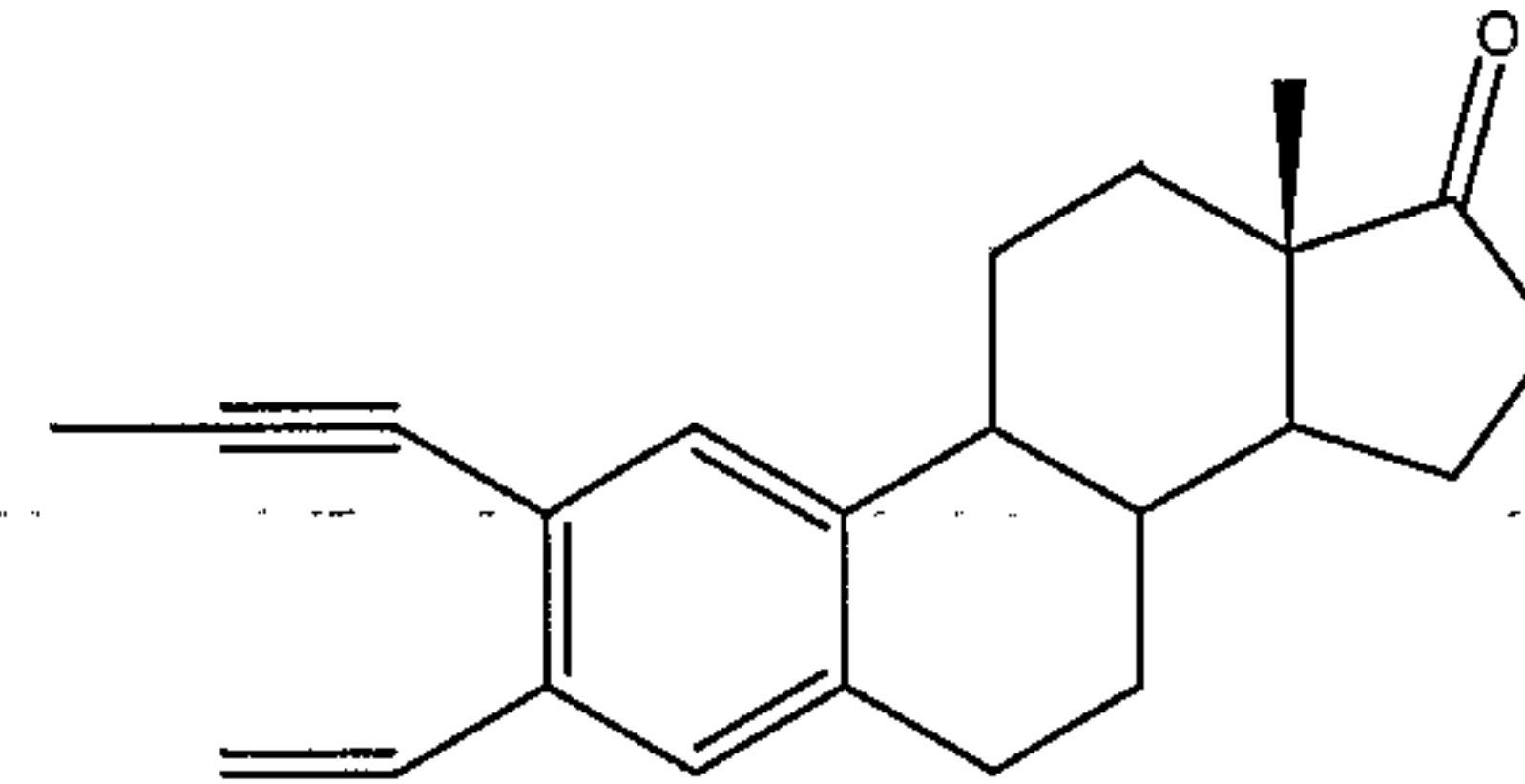
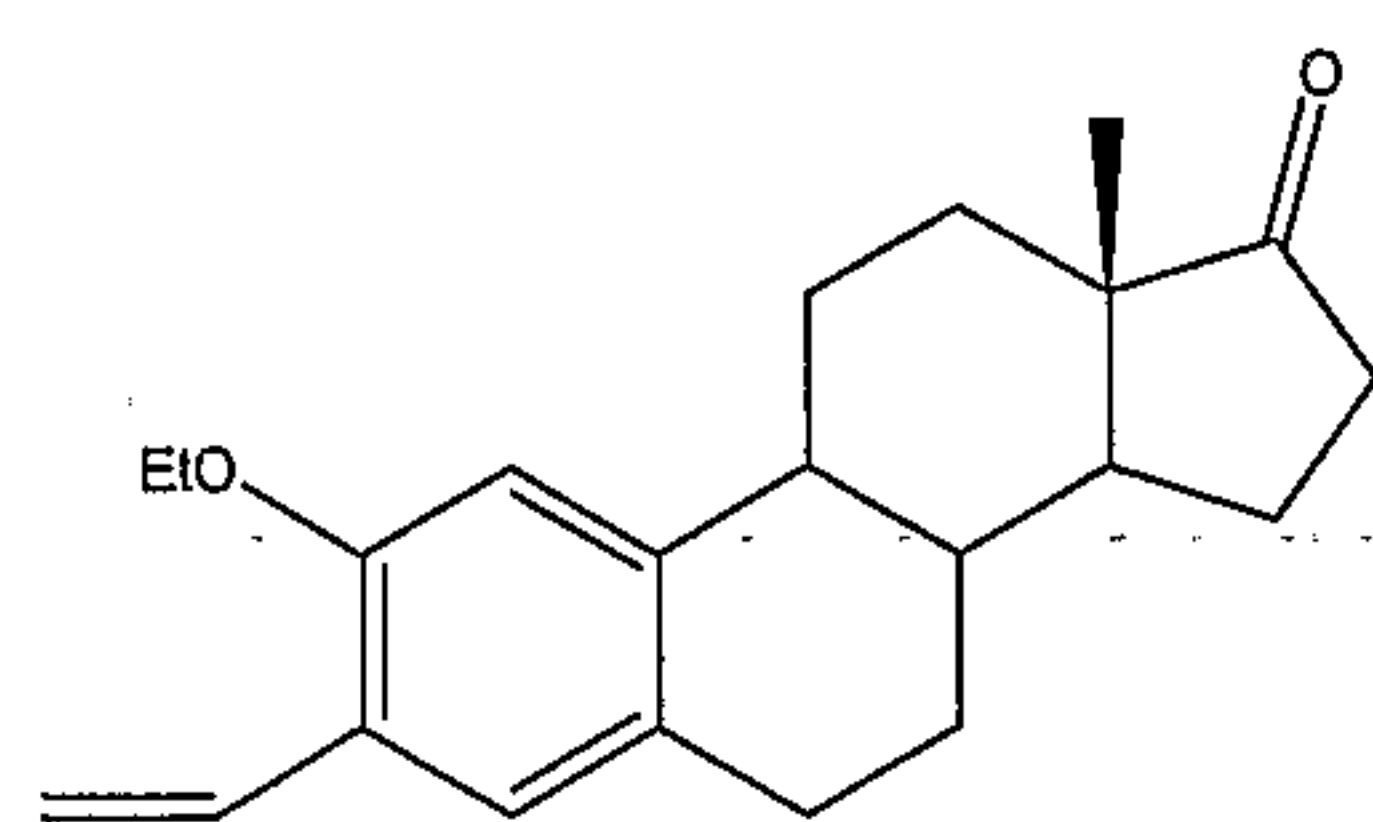
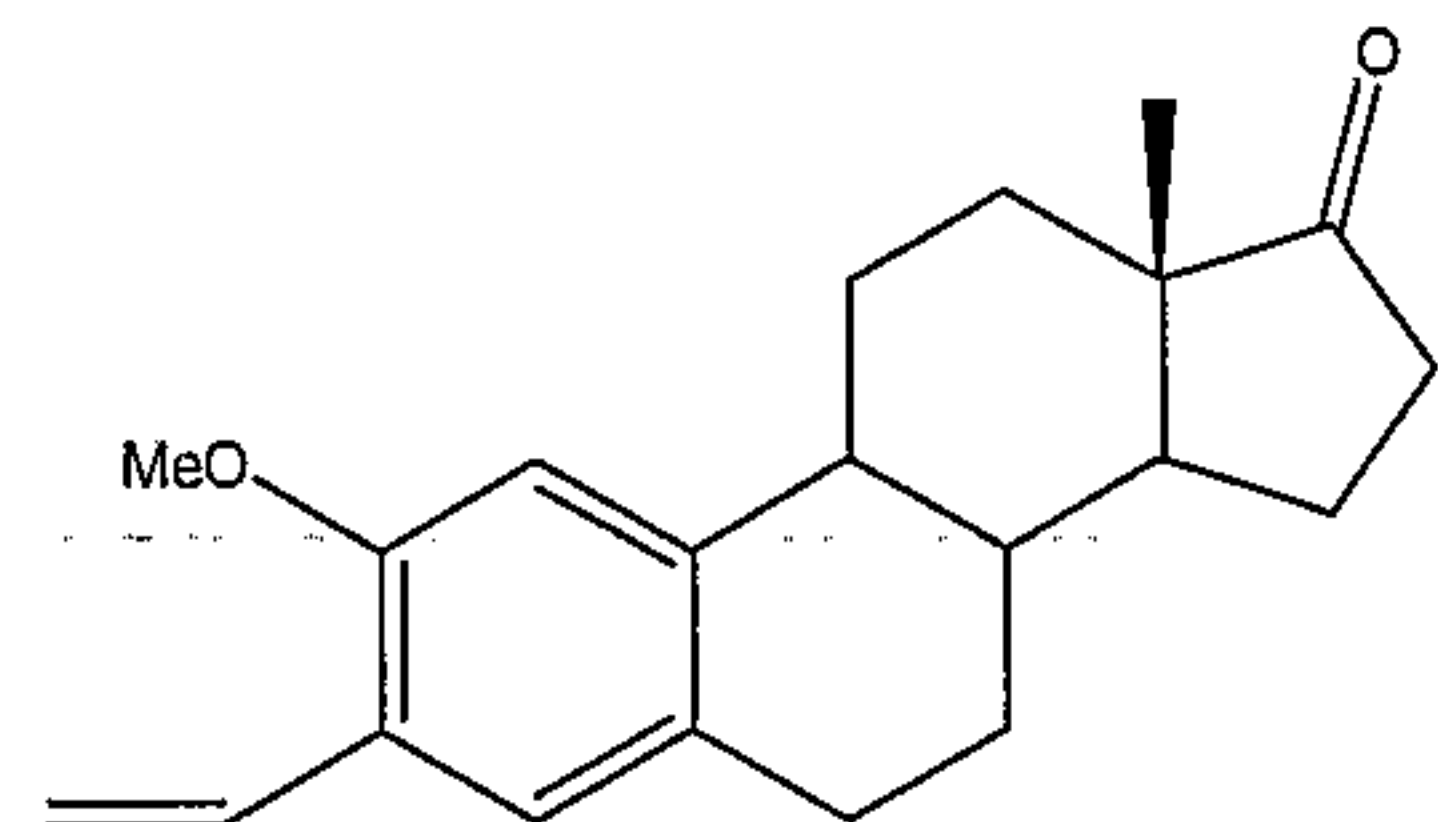
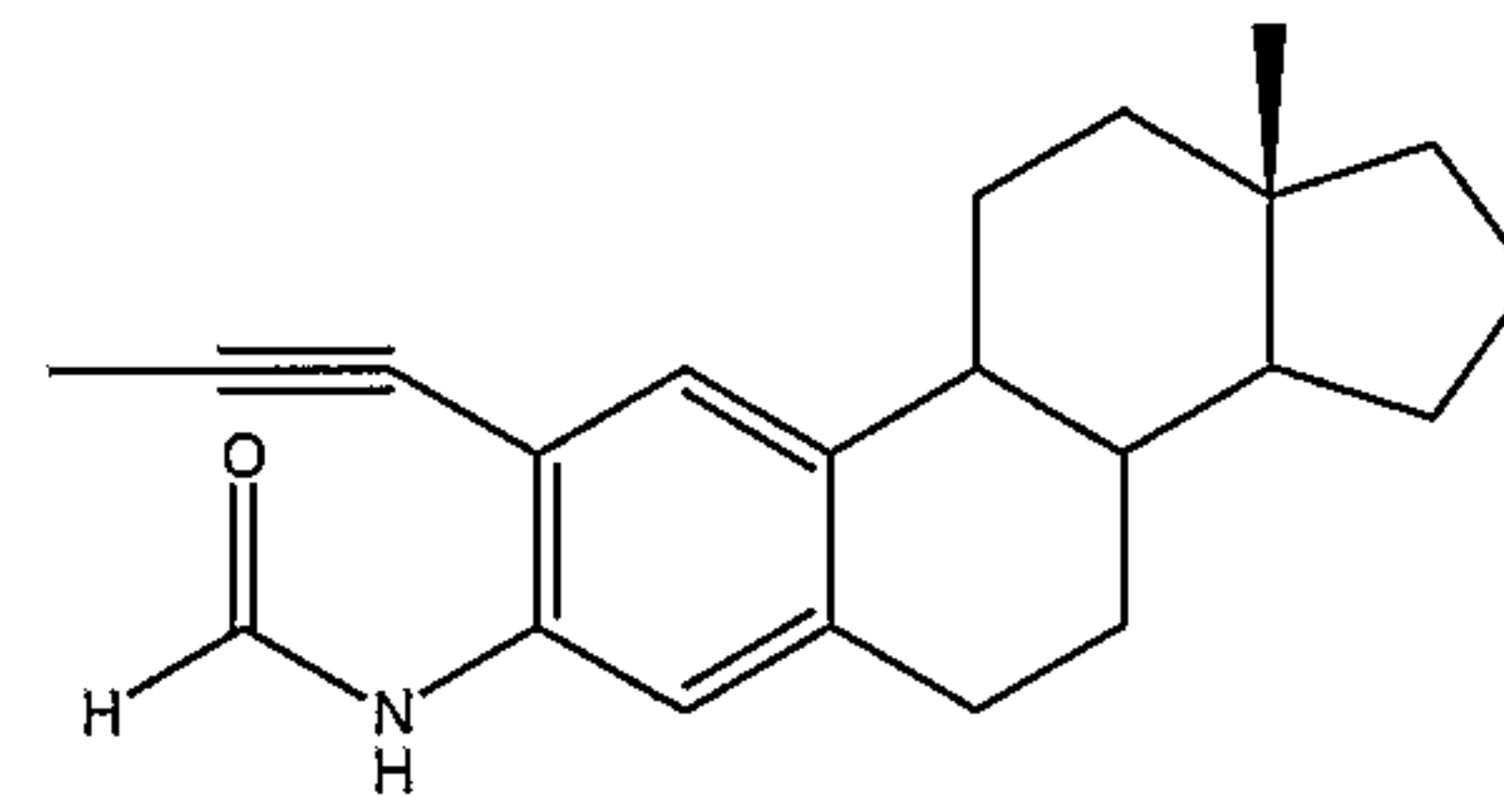
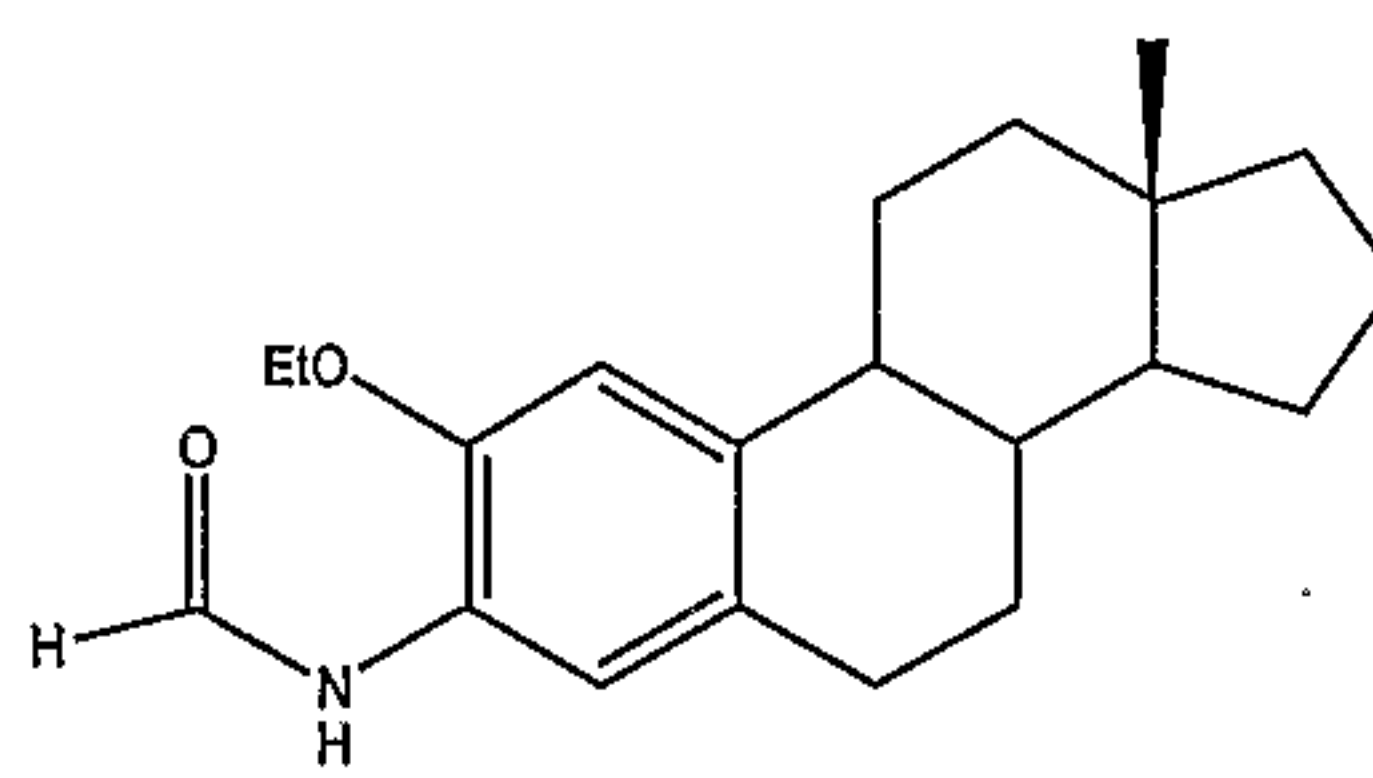
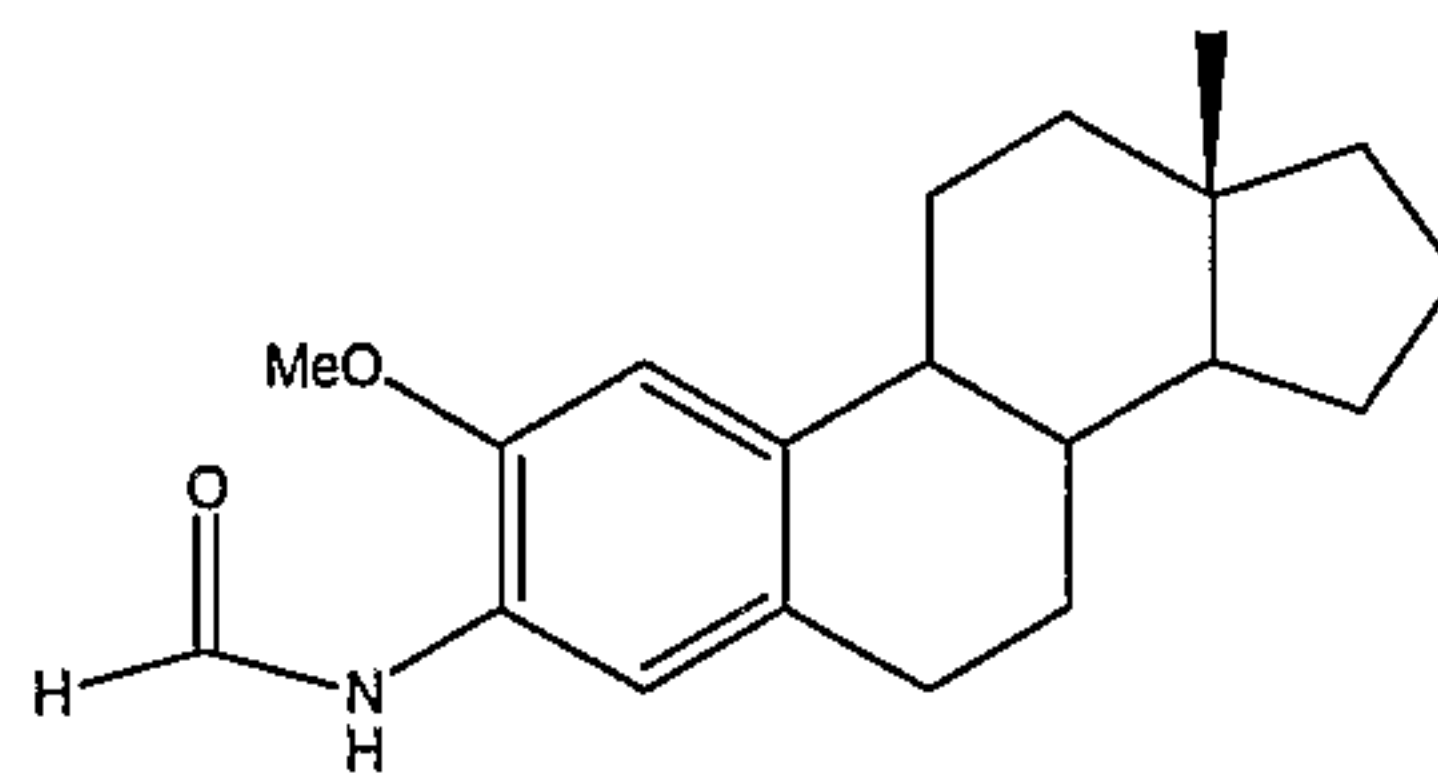
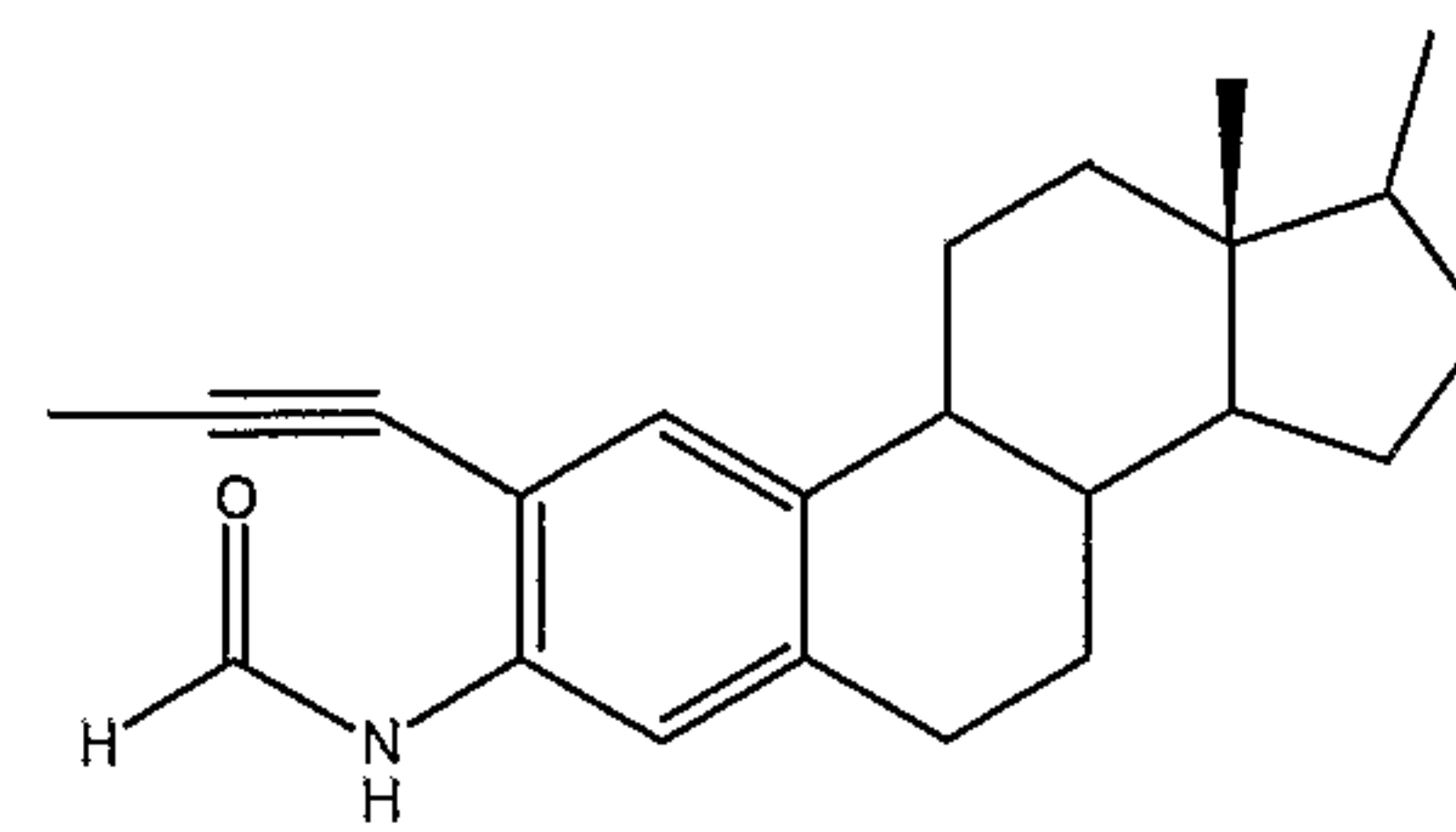
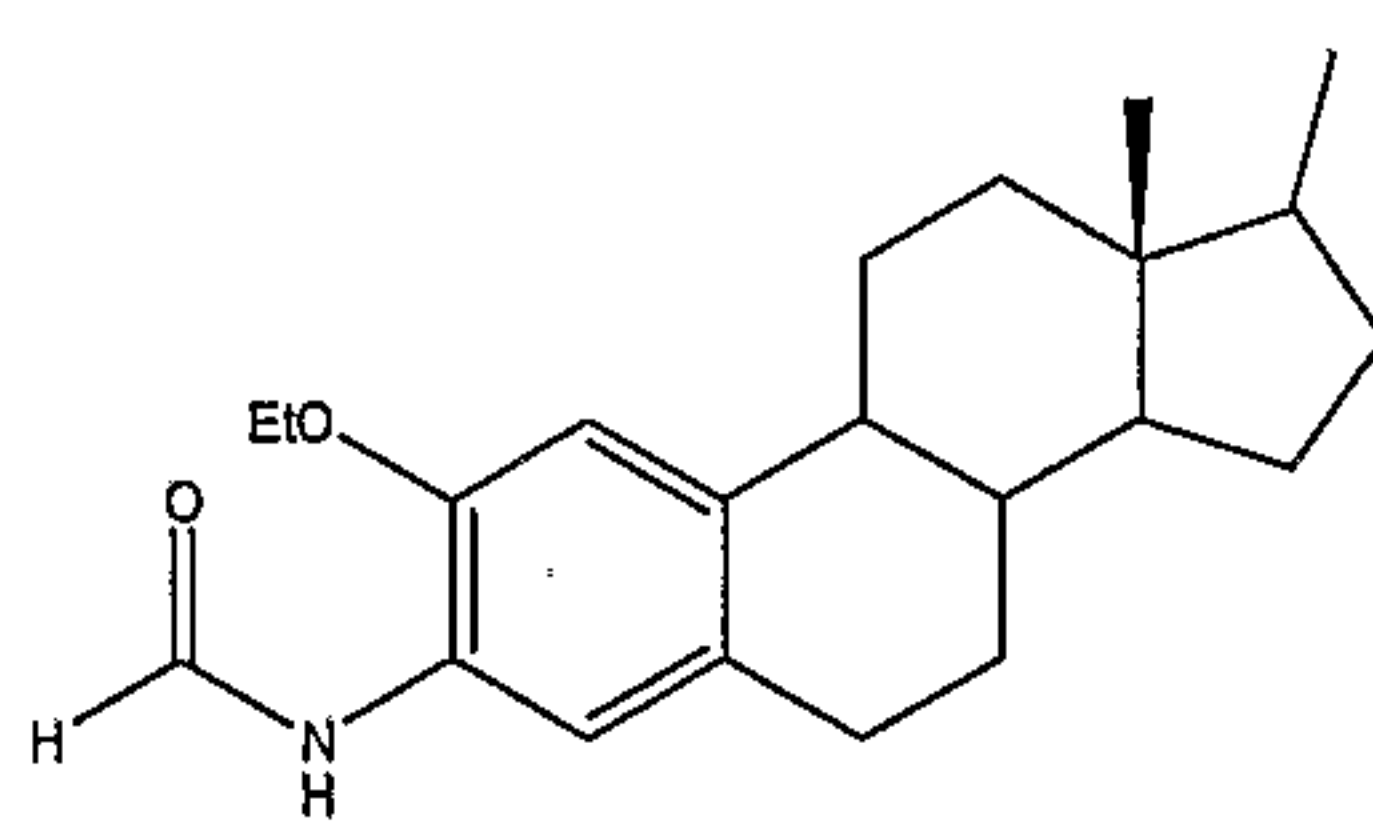
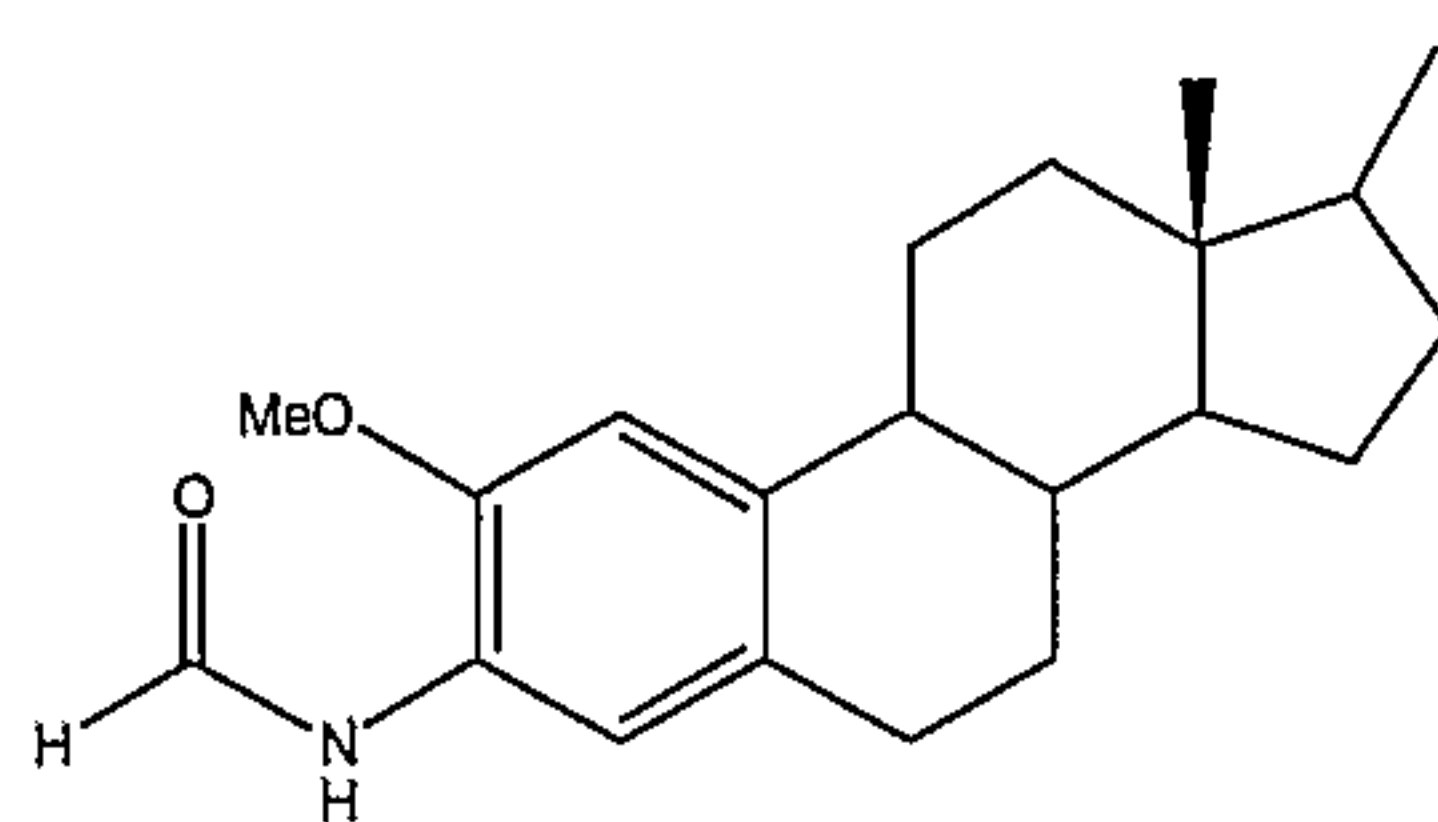
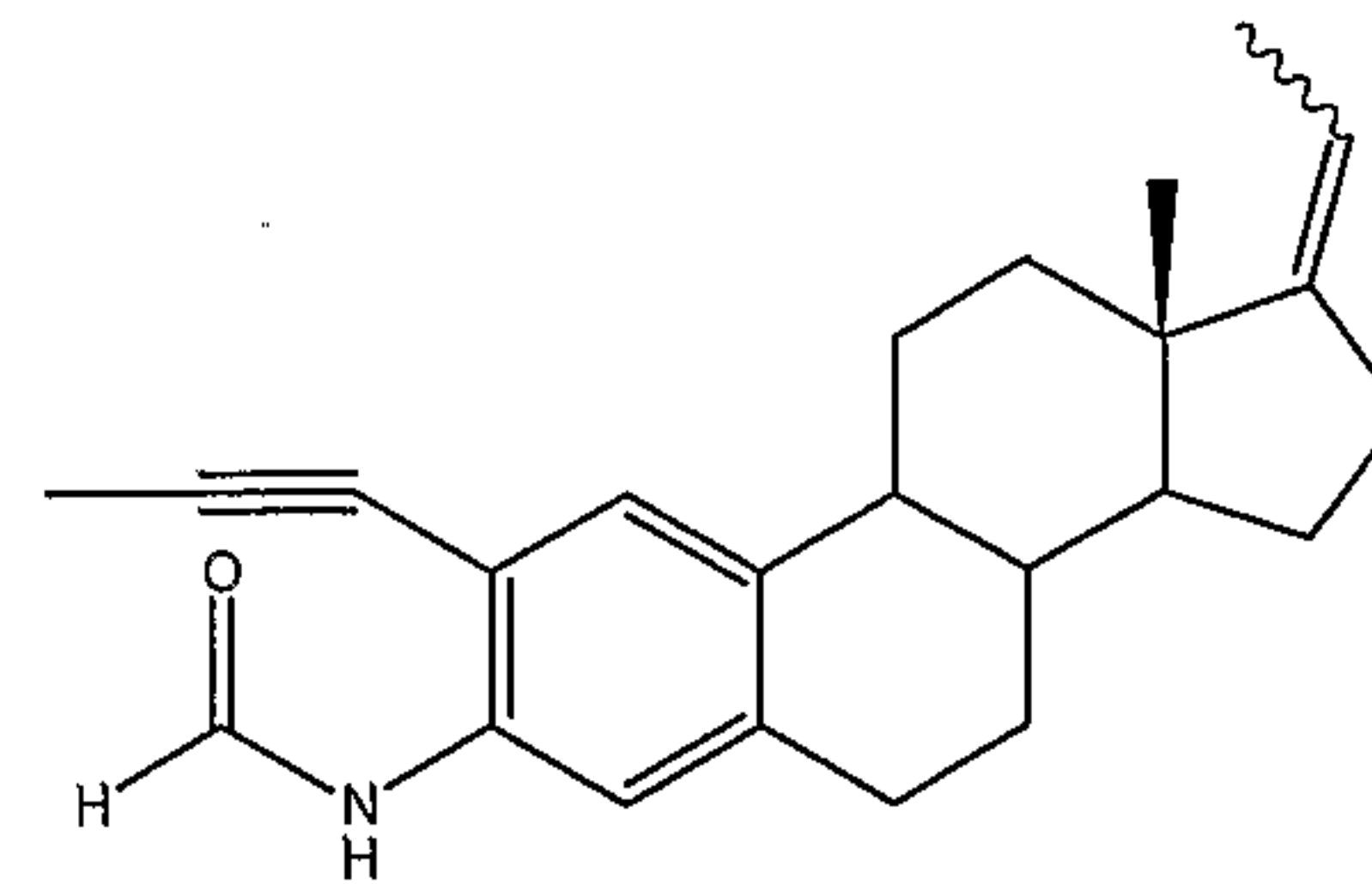
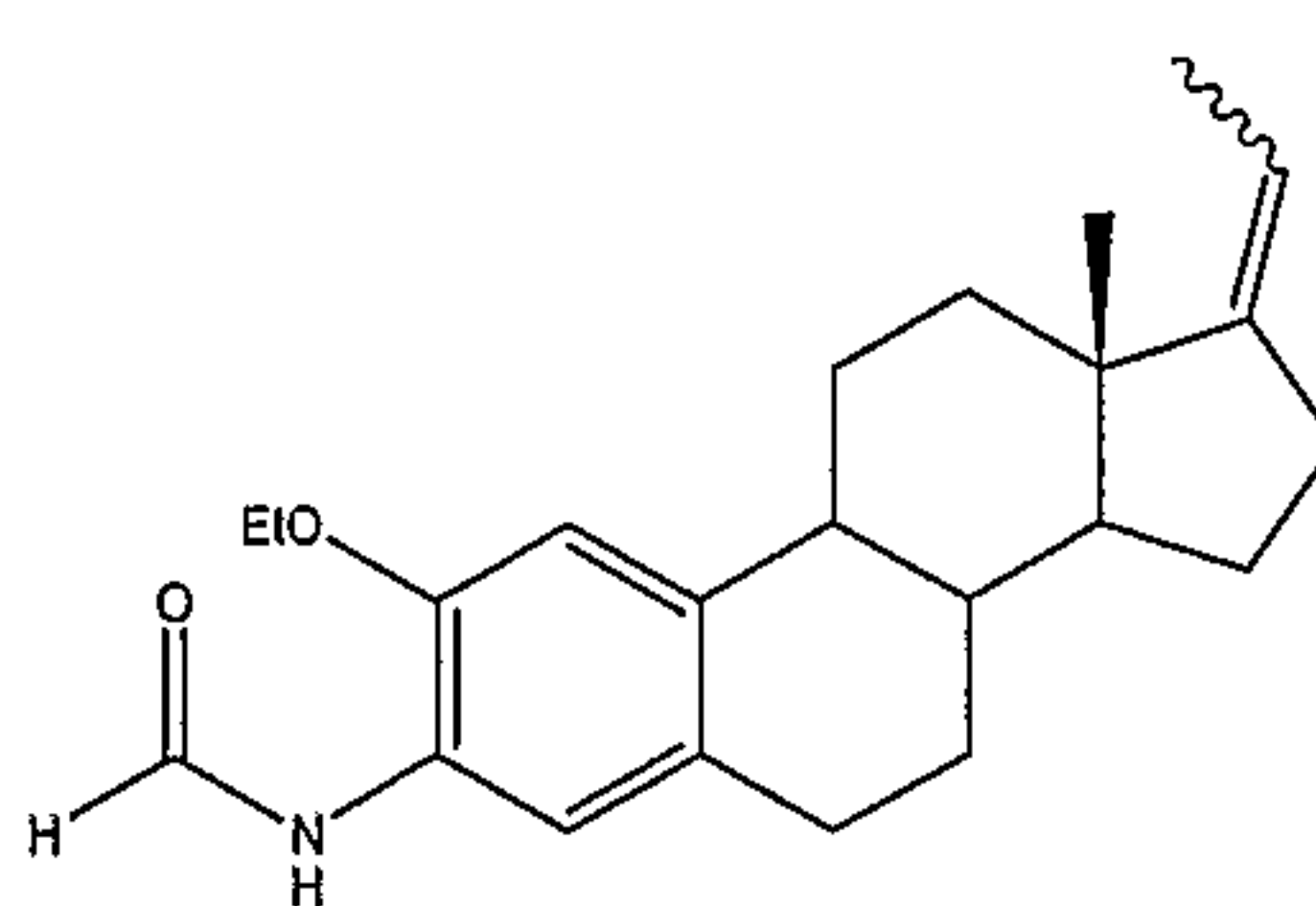
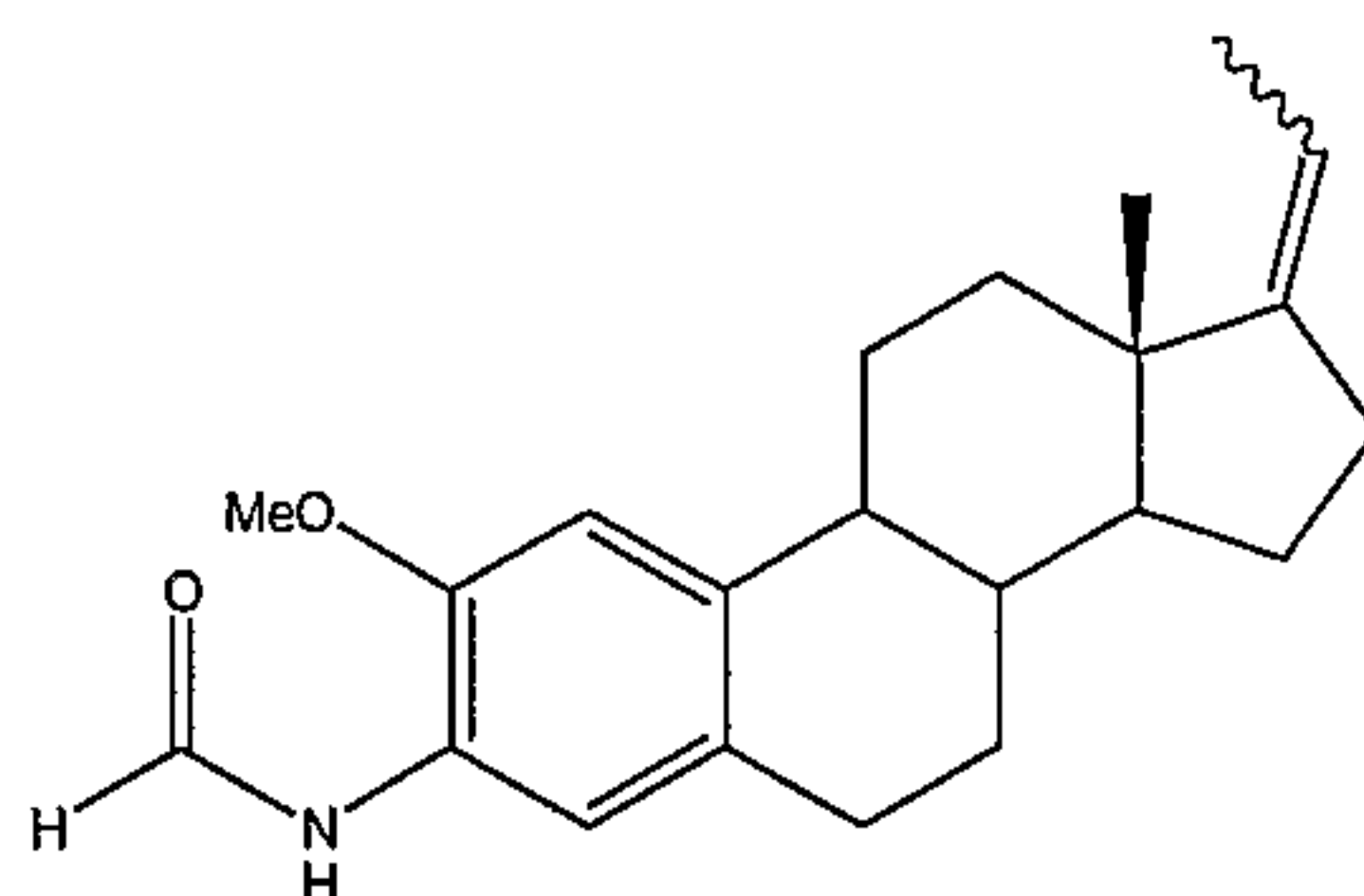
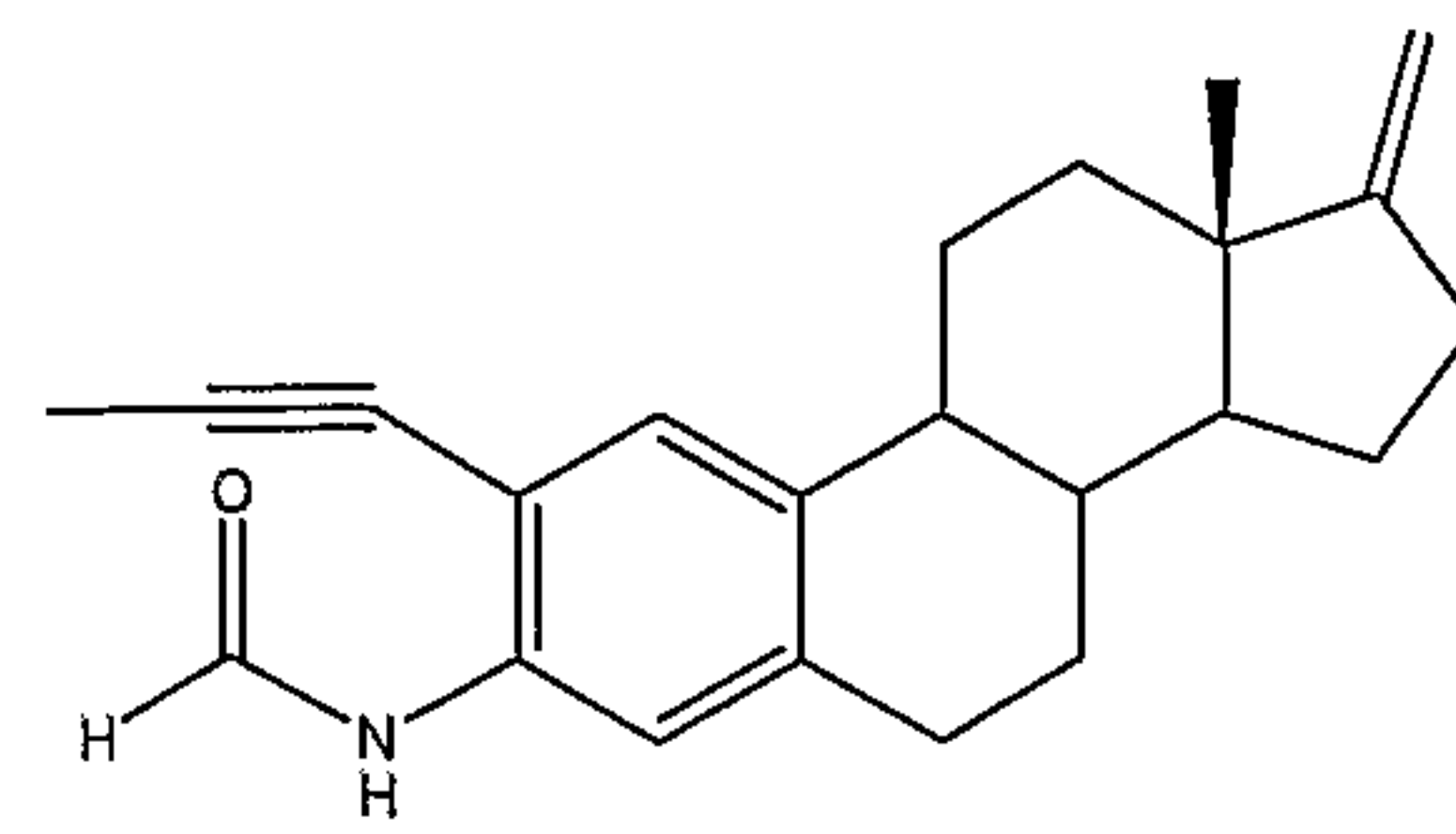
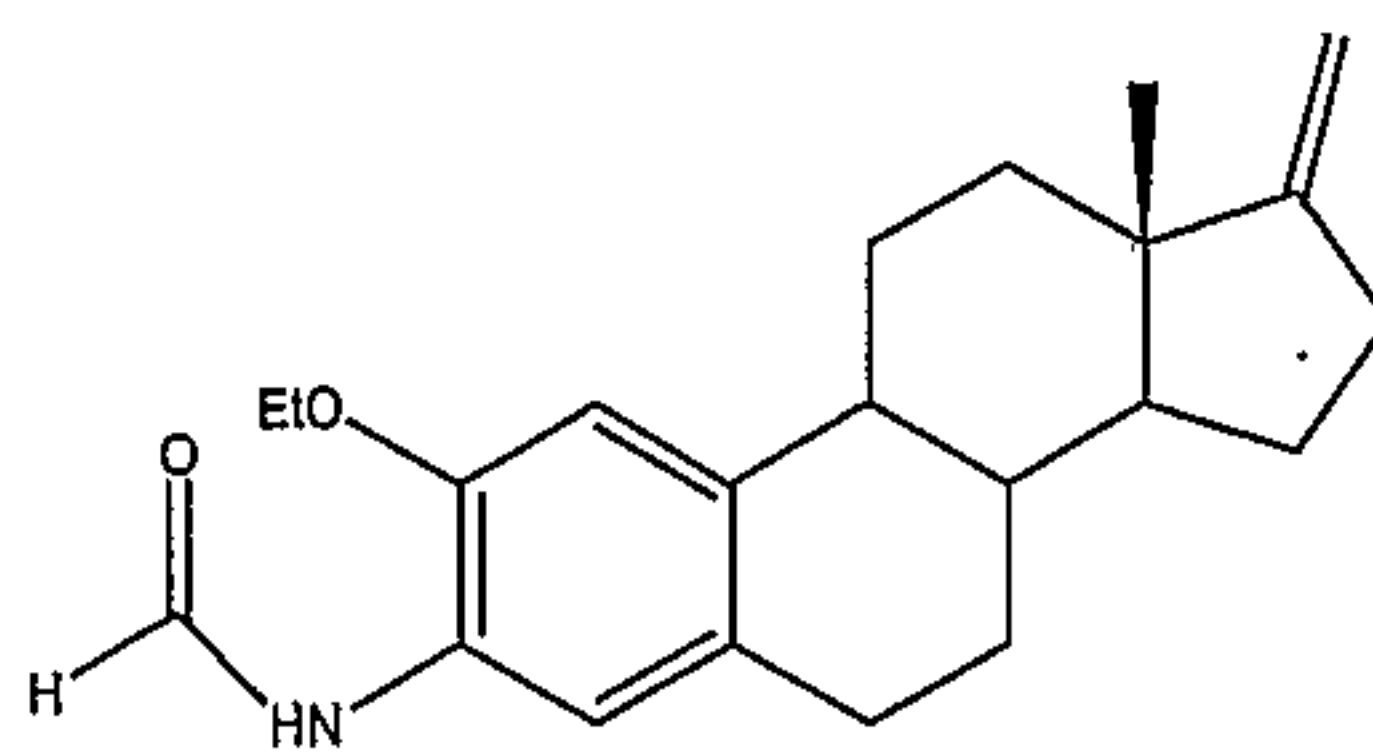
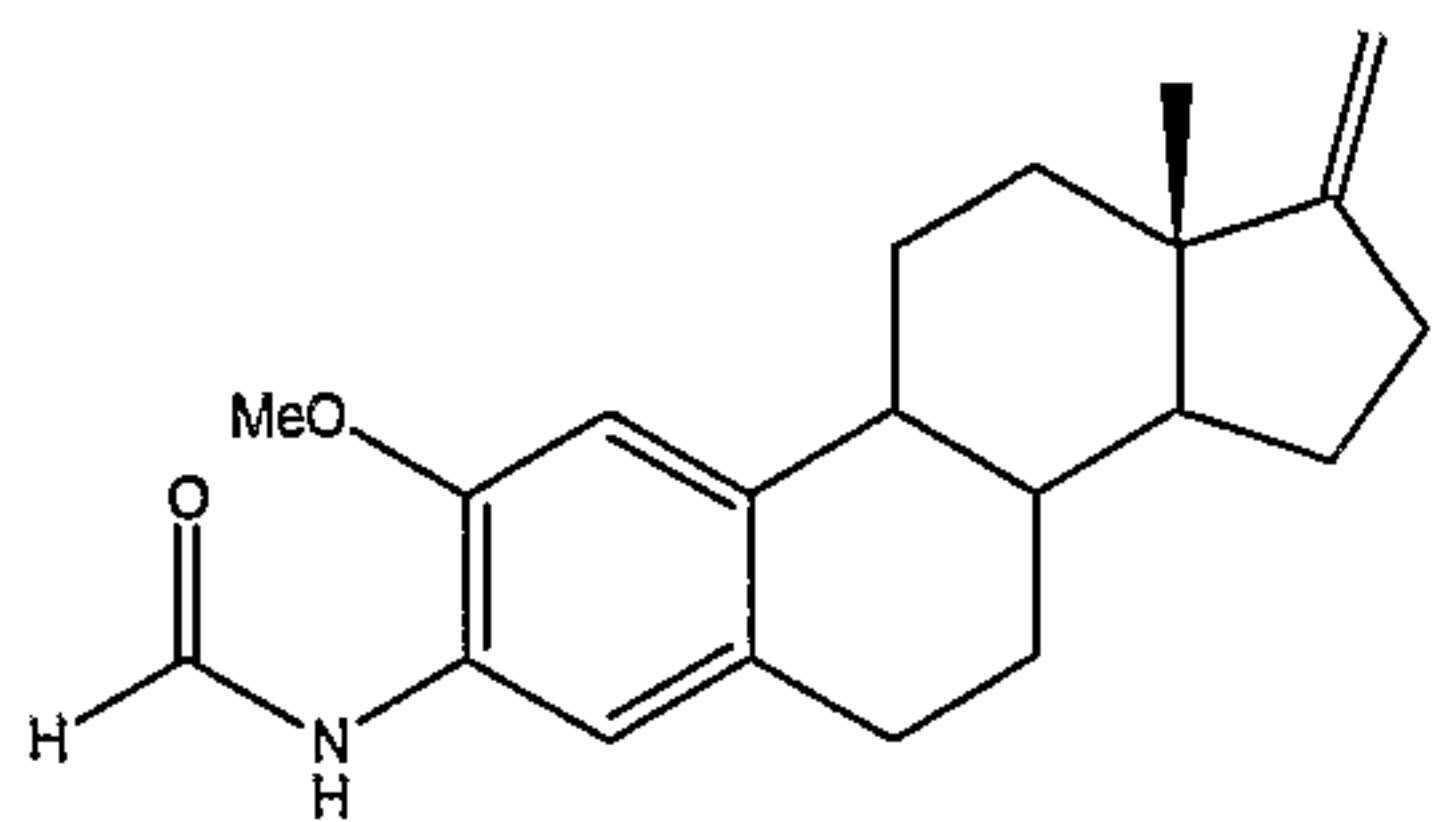
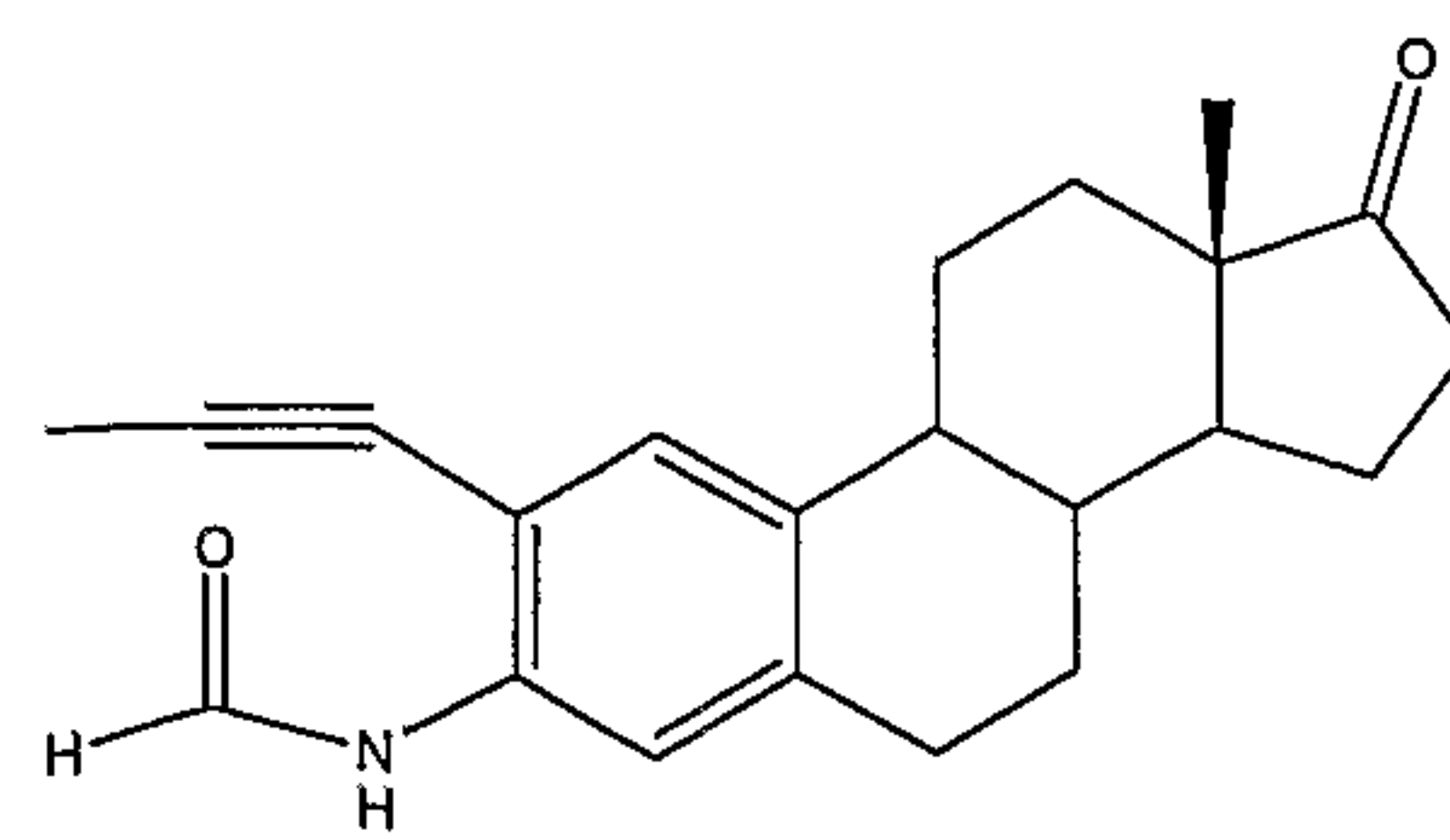
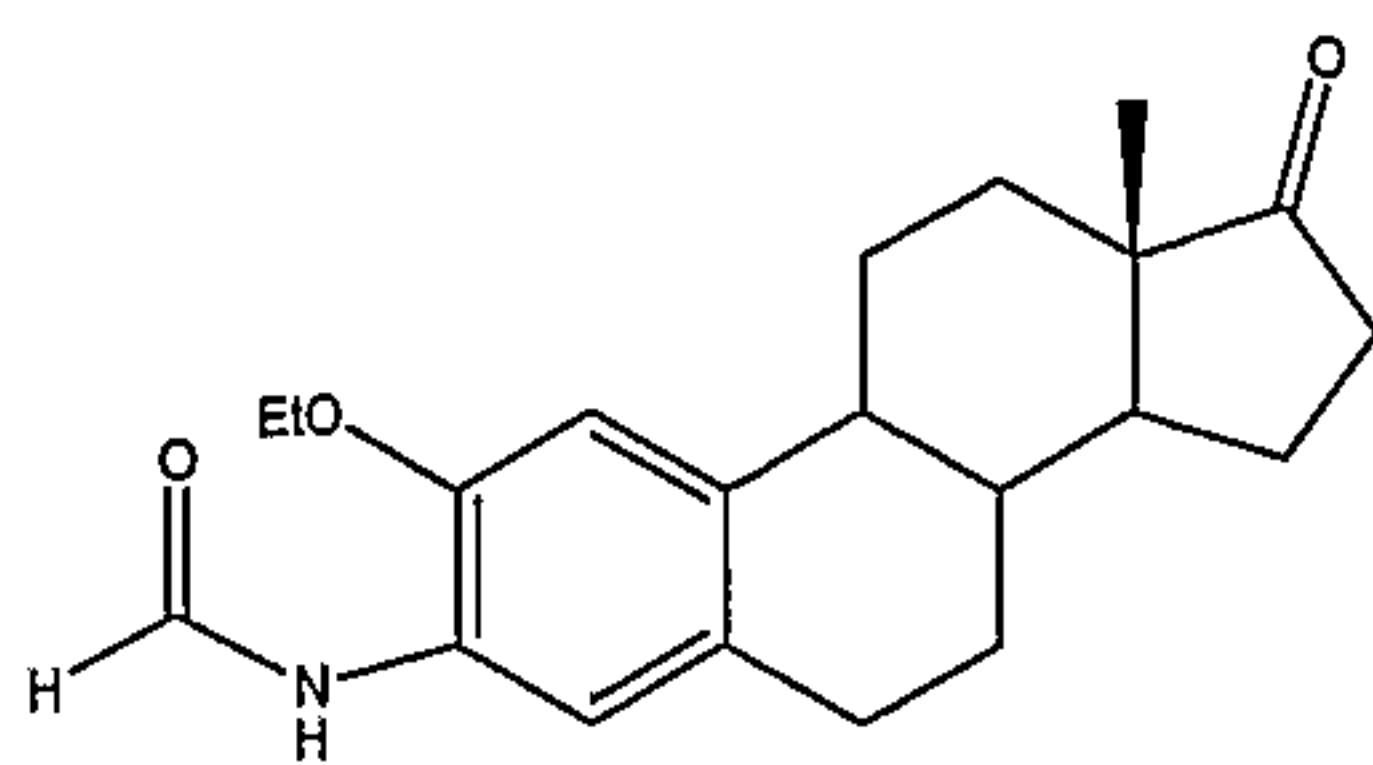
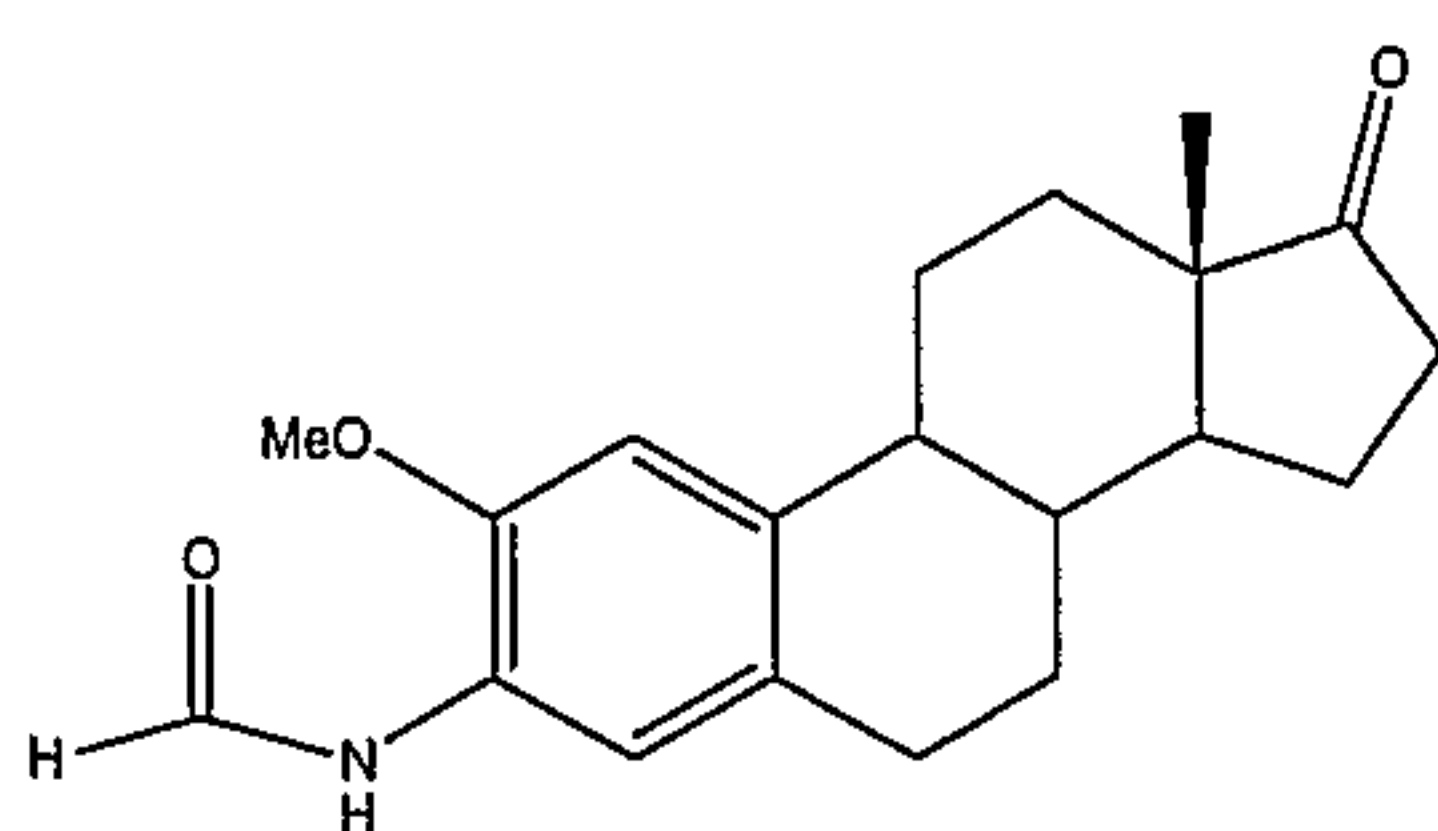
In another alternate disclosed embodiment of the present invention, preferred species from the foregoing genus that are useful in the present invention include, but are not limited to, the compounds shown in Table III.

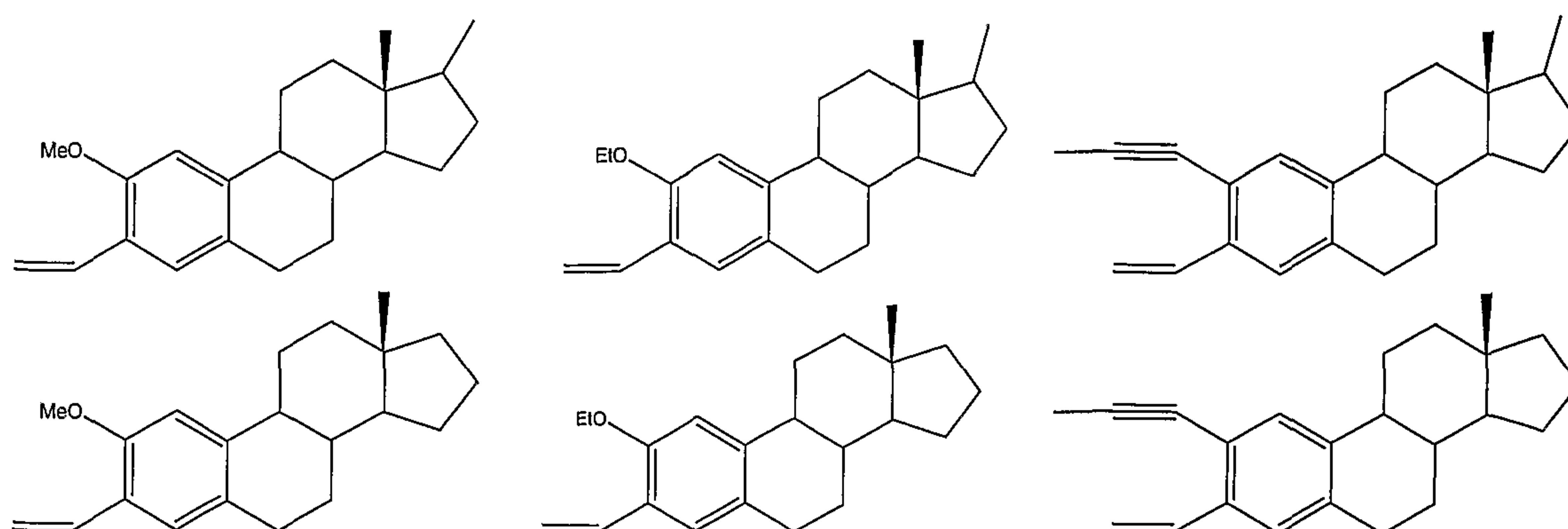
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Table III









EXAMPLE 1

The procedures described below are for specific compounds. However, these reactions can be applied to all examples in this patent by one skilled in the art. References to compound #s below correspond to the numbers assigned to the compounds shown in the synthesis Schemes 1-7 above.

Representative oxidation of estradiol analogs to estrone analogs: 2-ethoxyestra-1,3,5(10)-trien-3-ol-17-one (Compound #5): 2-Ethoxyestra-1,3,5(10)-trien-3,17-diol (#2, 1.88 g, 5.67 mmol) was placed in a 250 mL round bottom flask that was equipped with a 25 mL Dean-Stark trap and a reflux condenser. The entire apparatus had been flame dried under an argon atmosphere. Toluene (50 mL) was added to dissolve the starting material. Aluminum isopropoxide (5.7 g, 28.4 mmol) and cyclohexanone (23.5 mL, 2.26.8 mmol) were added and the entire reaction mixture was heated at reflux (145-150°C) for 20h. Saturated aqueous sodium bicarbonate solution (100 mL) was added after the reaction mixture was allowed to cool to room temperature. The organic material was extracted with dichloromethane (3 x 150 mL). The aqueous emulsion was acidified with 3 N HCl (~20 mL) until the emulsion separated and again, the aqueous layer was extracted with ethyl acetate (2 x 75 mL). The combined organic extracts were dried over magnesium sulfate and condensed in vacuo. The crude product was purified via flash chromatography (silica gel, hexanes ethyl acetate 8 : 1 by volume) to produce #5 as a white solid (1.7 g, 94 %). ¹H NMR (300 MHz, CDCl₃) δ 6.78 (s, 1H), 6.66 (s, 1H), 5.49 (s, 1H), 4.07 (dq, J = 6.9 and 1.6 Hz, 2H), 2.83 (m, 2H), 2.52 (m, 2H), 2.36-1.37 (m, 11H), 1.42 (t, J = 7 Hz, 3H), 0.92 (s, 3H).

Synthesis of 2-methoxyestra-1,3,5(10)-triene-3-ol (Compound #7): Into a stirring suspension of 2-methoxyestra-1,3,5(10)-trien-3-ol-17-one (#4, 8.1 g, 30 mmol) in diethylene glycol (60mL), 1-butanol (20 mL) and hydrazine anhydrous (2 mL, 60mmol)

were added. The reaction mixture was heated under reflux for 1 hour and the solution cleared. After cooling reaction mixture to 50 °C, KOH pellets (5.04 g, 90 mmol) were added and the butanol was distilled. The reaction mixture was heated at 50 °C for 2 hours and then cooled to room temperature. After the mixture was poured onto ice (50 g), 6N HCl (20 mL) was added with stirring to give white solid product. The product was separated by filtration, washed with cold water and dried under vacuum to give 7.5 g (90 %) product. The product was purified on silica gel column eluted with CHCl₃/MeOH 99:1. ¹H NMR in CDCl₃ confirmed the product as 2-methoxyestra-1,3,5(10)-triene-3-ol (7).

Representative procedure for preparation of 17-olefin-2-alkoxyestrane or 17-olefin-2-alkylestrane analogs -- 2-methoxy-17(20)-methyleneestra-1,3,5(10)-triene-3-ol (Compound #10): Potassium-*tert*-amylate (1.54 M, toluene, 4.35 mL 6.69 mmol, prepared as in Schow et al *J. Org. Chem.* 1979, 44, 3760) was added to a suspension of methyl triphenylphosphonium bromide (2.39 g, 6.69 mmol) in anhydrous benzene and refluxed for 30 min. 2-Methoxyestrone (#4, 300 mg, 1 mmol) in warm benzene (5 mL) was added and the mixture was refluxed for 3 h. The reaction was cooled to room temperature, poured into 100 mL water, washed with ether (2 x 100 mL). The combined organics were washed with 6 M HCl (1 x 100 mL), NaHCO₃ (saturated, 1 x 100 mL), water (1 x 100 mL), and brine (1 x 100 mL). Dry with sodium sulfate, filter and rotoevap to give a semi solid-yellowish oil. Purify by silica gel column chromatography using 95:5 chloroform:methanol as an eluent. Obtain 220 mg 2-methoxy-17(20)-methyleneestra-1,3,5(10)-triene-3-ol (#10, 0.738 mmol, 73 % yield). Selected spectral data: ¹H NMR (300 MHz, CDCl₃) δ 6.83 (s, 1H), 6.67 (s, 1H), 5.44 (br s, 1H), 4.70 (t, J=2.26 Hz, 2H), 3.89 (s, 3H), 2.86-2.74 (m, 2H), 2.64-2.49 (m, 1H), 2.39-2.17 (m, 3H), 2.02-1.78 (m, 3H), 1.65-1.19 (m, 6H), 0.85 (s, 3H). ¹³C NMR (75 MHz, CDCl₃) δ 162.2, 144.9, 143.8, 132.3, 130.0, 115.0, 108.5, 101.2, 77.6, 56.5, 53.9, 44.7, 39.2, 36.2, 29.9, 29.5, 28.1, 27.3, 24.3, 19.0. Analytical (C₂₀H₂₆O₂); calculated C=80.48, H=8.79; found C=80.60, H=8.77.

2-Methoxy-19-norpregna-1,3,5(10)17(20)-tetraene-3-ol (Compound #13): Reaction conditions in general same as in preparation of #10 except reaction scale was doubled and ethyl triphenylphosphonium bromide was used, from 2-methoxyestrone (613 mg, 2.04 mmol) obtain 540 mg (1.73 mmol, 84 % yield) of final product. Selected spectral data: ¹H NMR (300 MHz, CDCl₃) δ 6.82 (s, 1 H), 6.67 (s, 1 H), 5.44 (s, 1 H), 5.23-5.07 (m, 1 H), 3.88 (s, 3 H), 2.86-2.72 (m, 2 H), 2.51-2.38 (m, 2 H), 2.38-2.17 (m, 3 H), 1.99-1.88 (m, 1

H), 1.83-1.68 (m, 4 H), 1.49-1.20 (m, 6 H), 0.94 (s, Z isomer) and 0.80 (s, E isomer, total 3H, ratio 5:1 respectively). ¹³C NMR (75 MHz, CDCl₃) δ 153.0 (E isomer) and 150.7 (Z isomer), 145.0, 143.8, 132.4, 130.0, 115.0, 113.8, 110.6 (Z isomer) and 108.4 (E isomer), 56.5, 55.6, 54.1, 45.0 (Z isomer) and 44.5 (E isomer), 39.0 (E isomer) and 38.7 (Z isomer), 37.7 (Z isomer) and 36.6 (E isomer), 31.9, 29.5, 28.1 (E isomer) and 28.0 (Z isomer), 27.7 (Z isomer) and 27.4 (E isomer), 24.5 (Z isomer) and 24.4 (E isomer), 19.5 (E isomer) and 17.4 (Z isomer), 14.0 (E isomer) and 13.6 (Z isomer). Analytical (C₂₁H₂₈O₂); calculated C=80.73 H=8.79; found C=80.60 H=8.77.

Representative procedure for preparation of 17-alkyl-2-methoxyestradiol analogs:
 2-methoxy-17β-methylestra-1,3,5(10)-triene-3-ol (Compound #16): 17-Methylene-2-methoxyestra-1,3,5(10)triene-3-ol (471.9 mg, 1.58 mmol) was dissolved ethyl acetate (20 ml) in a Parr bottle which was then flushed with Ar. Pd/C 10% (47.5 mg) was added and reaction mixture was then subjected to hydrogenation in Parr hydrogenater for an hour under 30 psi of hydrogen. Reaction mixture was then filtered through Celite and solvent was removed via rotary evaporation to yield 472.5 mg white crystals (1.57 mmol, 99% yield) of the final product 2-methoxy-17β-methylestra-1,3,5(10)-triene-3-ol. Selected spectral data: ¹H NMR (300 MHz, CDCl₃) δ 6.82 (s, 1 H), 6.66 (s, 1 H), 5.43 (s, 1 H), 3.88 (s, 3 H), 2.85-2.70 (m, 2 H), 2.32-2.15 (m, 2 H), 1.94-1.68 (m, 4 H), 1.52-1.12 (m, 8 H), 0.90 (d, J=6.9 Hz, 3 H), 0.61 (s, 3 H). ¹³C NMR (75 MHz, CDCl₃) δ 144.90, 143.75, 132.65, 130.11, 114.99, 108.51, 56.46, 55.21, 45.58, 44.85, 42.74, 39.39, 37.97, 30.65, 29.52, 28.38, 27.21, 24.83, 14.34, 12.44. Analytical (C₂₀H₂₈O₂); calculated C=79.96 H=9.39; found C=79.98 H=9.49.

Preparation of 3-sulfamate-2-methoxyestra-1,3,5(10)-trien-17-one (Compound #19): Prepared as depicted in Scheme 3 using representative procedures. ¹H NMR (300 MHz, CDCl₃) δ 7.06 (s, 1H), 6.93 (s, 1H), 4.93 (s, 2H), 3.88 (s, 3H), 2.86 (m, 2H), 2.58-1.26 (m, 313 H), 0.92 (s, 1H).

Preparation of 3-sulfamate-2-ethoxyestra-1,3,5(10)-trien-17-one (Compound #20): Prepared as depicted in Scheme 3 using representative procedures. ¹H NMR (300 MHz, CDCl₃) δ 7.02 (s, 1H), 6.93 (s, 1H), 5.04 (br s, 2H), 4.12 (m, 2H), 3.73 (t, J = 8.33 Hz, 1H), 2.78 (m, 2H), 2.28-1.17 (m, 13H), 1.43 (t, J = 6.98 Hz, 3H), 0.78 (s, 3H).

Representative procedure for the preparation of triflic esters: Preparation of 2-methoxy-3-trifluoromethanesulfonylestra-1,3,5(10)-trien-17-one (Compound #22): 2-

Methoxyestra-1,3,5(10)-trien-17-one-3-ol (2.4 g, 8.0 mmol) was dissolved in anhydrous dichloromethane (80 mL), anhydrous pyridine (20 mL) was added and the mixture was cooled to 0°C. Triflic anhydride (2 mL, 11.89 mmol, 1.5 eq) was added drop wise, then allowed to warm to room temperature with stirring for 18 h. The reaction mixture was poured into water (300 mL) and additional dichloromethane (150 mL) was added. The layers were separated, and the organic was washed with water (200 mL), 10 % HCl (2 x 100 mL) and brine (100 mL). The organic layer was dried with MgSO₄, filtered and solvent was removed under reduced pressure. The product was purified using a SiO₂ Biotage FLASH apparatus using 5 : 1 hexanes : ethyl acetate as eluent, obtain 2.3801 g (5.51 mmol, 68 % yield) as final product. ¹H NMR (300 MHz, CDCl₃) δ 6.96 (s, 1H), 6.95 (s, 1H), 3.90 (s, 3H), 2.87 (dd, J = 4.2, 6.4 Hz, 2H), 2.61-2.95 (m, 8H), 1.74-1.36 (m, 5H), 0.94 (s, 3H).

Synthesis of 2-methoxy-3-(vinyl)estra-1,3,5(10)-triene-17-one (Compound #25): Tributyl(vinyl)tin(I) (200 μL, 0.6 mmol), lithium chloride (85 mg, 2 mmol), dichlorobis(triphenylphosphine)palladium (II) (10 mg, 0.05 mmol), 2,6-di-tert-butyl-4-methylphenol (2 mg) was added to a solution of 2-methoxy-3-trifluoromethanesulfoneestra-1,3,5(10)-triene-17-one (216 mg, 0.5 mmol) in anhydrous DMF (4 mL) at room temperature. The reaction was heated at 90°C for 18 h and then cooled to room temperature. HF-pyridine (4 mL) was added and the reaction mixture was stirred for an additional 6 h. The mixture was diluted with EtOAc (20 mL) and filtered through Celite filter agent. The combined organics were washed with 1 N HCl (1 X 100 mL), water (1 X 100 mL), and brine (1 X 100 mL), dried with sodium sulfate, filtered, and concentrated via rotary evaporation to give a semi solid viscous oil. The product was purified on a flash silica gel column eluted with a hexane/EtOAc 5:1 mixture to give a white solid product (75 mg, 30 %); ¹H NMR (CDCl₃) δ 7.20 (s, 1 H), 7.00 (dd, J = 14, 11 Hz, vinyl, 1H), 6.82 (s, 1 H), 5.70 (d, J = 18, vinyl, 1H), 5.22 (d, J = 11, vinyl, 1H) 3.88 (s, 3 H), 2.85 (m, 2 H), 2.52 (dd, J = 3 Hz, 1H), 2.44-1.88 (m, 6 H), 1.72-1.35 (m, 6 H), 0.95 (s, 3 H).

Alternate synthesis of 2-methoxy-3-(vinyl)estra-1,3,5(10)-triene-17β-ol (Compound #25a): Into a solution of 3-triflate-2-methoxyestra-1,3,5(10)-trien-17β-ol (218 mg, 0.5 mmol, prepared as in Shi *J. Am. Chem. Soc.* 2002, 124, 6921) in 4 mL DMF (anhydrous), was added tributyl(vinyl)Tin(I) (200 μL, 0.6 mmol), lithium chloride (85 mg, 2 mmol), dichlorobis(triphenylphosphine)palladium (II) (10 mg, 0.05 mmol), 2,6-di-tert-butyl-4-

methylphenol (2 mg) at room temperature. The reaction was heated at 90° C for 24 h and then cooled to room temperature. After adding HF-pyridine (4 mL) the reaction mixture was stirred for 16 h. After diluting with EtOAc (20 mL) the reaction mixture was filtered through Celite filter agent and the combined organics were washed with 1 N HCl (1 X 100 mL), water (1 X 100 mL), and brine (1 X 100 mL), then dried with sodium sulfate, filtered, and concentrated via rotary evaporation to give a semi solid viscous oil. The product was purified on a flash silica gel column eluted with a hexane/EtOAc 2:1 mixture to give a white solid product (140 mg, 60 %). ¹H NMR (CDCl₃) δ 7.20 (s, 1 H), 7.00 (dd, J = 14, 11 Hz, vinyl, 1H) 6.80 (s, 1 H), 5.70 (d, J = 18 Hz, vinyl, 1H), 5.22 (d, J = 11 Hz, vinyl, 1H), 3.85 (s, 3 H), 2.72 (t, J = 4 Hz, 1H), 2.85 (dd, J = 5 Hz, 2H), 2.44-1.88 (m, 6 H), 1.80-1.15 (m, 8 H), 0.80 (s, 3 H).

Synthesis of 2-methoxy-3-(carboxylic acid)estra-1,3,5(10)-triene-17-one (Compound #28): Potassium acetate (786 mg, 8 mmol), palladium (II) acetate (40 mg, 0.1 mmol), 1,1-bis(diphenylphosphine)-ferrocene (dppp) (200 mg, 0.4 mmol) were added to a solution of #22 (175 mg, 0.4 mmol) in 4 mL DMSO (anhydrous) at room temperature. The mixture was purged with CO gas for five minutes at room temperature then heated at 50° C for 18 h under CO (balloon) and cooled to room temperature. After diluting with water, the reaction mixture was extracted with CH₂Cl₂ (1 X 20 mL) washed with 1 N HCl (1 X 10 mL), water (1 X 10 mL), and brine (1 X 10 mL), then dried with sodium sulfate, filtered, and concentrated via rotary evaporation to give a dark viscous oil. The product was purified on a flash silica gel column eluted with a hexane/EtOAc 1:2 mixture to give a white solid product (40 mg, 30 %). ¹H NMR (CDCl₃) δ 10.92 (br s, 1H), 7.91 (s, 1H), 6.95 (s, 1 H), 4.05 (s, 3 H), 2.85 (dd, J = 5 Hz, 2H), 2.42-1.85 (m, 6 H), 1.65-1.15 (m, 8 H), 0.80 (s, 3 H).

Representative procedure for conversion of 3-(carboxyl acid)estrane derivative to 3-(carboxamide)estrane analog: Synthesis of 2-methoxy-3-carboxamideestra-1,3,5(10)-triene-17-one (Compound #31): A 150 mg portion of #28 (0.5 mmol) was dissolved in SOCl₂ at room temperature and then reaction mixture was heated at 50-60 °C for 2h until gas evolution ceased. The remaining SOCl₂ was removed under vacuum to give a dark viscous oil. The product was diluted with THF anhydrous (5 mL) and NH₃ gas was bubbled for 5 minutes. Soon a white precipitate of NH₄Cl formed. The reaction mixture was diluted with THF, filtered and evaporated to give viscous product. The product was purified on a flash silica gel column eluted with a hexane/EtOAc 1:2 mixture to give a

white solid product (60 g, 30 %). ^1H NMR (CDCl_3) δ 7.95 (s, 1H), 7.82 (br s, 1H), 6.95 (s, 1 H), 5.72 (br s, 1H), 3.95 (s, 3 H), 2.95 (dd, $J = 5$ Hz, 2H), 2.42-1.85 (m, 6 H), 1.65-1.15 (m, 8 H), 0.80 (s, 3 H).

Representative preparation of 3-(benzophenone imine)estrane derivatives –
 5 Preparation of 3-dibenzylimine-2-methoxyestra-1,3,5(10)-trien-17-one: 3-Trifluoromethanesulfonyl-2-methoxyestra-1,3,5(10)-trien-17-one (908 mg, 2.367 mmol) was dissolved in anhydrous toluene (6 mL) and $\text{Pd}(\text{OAc})_2$ (30 mg, 5.7 mol %), rac BINAP (135 mg, 9.2 mol %), Cs_2CO_3 (1.0729 g, 3.29 mmol, dried overnight in vacuum oven) and benzophenone imine (436 μL , 2.6 mmol) were added. The mixture was refluxed for 60 h,
 10 cooled to room temperature, and diethyl ether (200 mL) was then added. The resulting mixture was filtered through celite, and solvent was removed under reduced pressure. The mixture was purified using SiO_2 with a Biotage FLASH apparatus with 5:1 hexanes : ethyl acetate as eluent. Obtain 906 mg (1.96 mmol, 58 % yield) as final product. ^1H NMR (300 MHz, CDCl_3) δ 7.86-7.14 (m, 10H), 6.67 (s, 1H), 6.37 (s, 1H), 3.65 (s, 3H), 2.80-2.59 (m,
 15 2H), 2.58-1.85 (m, 8H), 1.69-1.23 (m, 5H), 0.93 (s, 3H).

Representative procedure for hydrolysis of 3-(benzophenone imine)estrane derivatives to 3-aminoestrane derivatives – Preparation of 3-amino-2-methoxyestra-1,3,5(10)-trien-17-one (Compound #34): 3-Dibenzylimine-2-methoxyestra-1,3,5(10)-trien-17-one (906 mg, 1.96 mmol) was dissolved in THF (10 mL) and 2M HCl (2 mL) was
 20 added with stirring for 4 h. The reaction mixture was partitioned between 2:1 hexanes : ethyl acetate and 0.5M HCl (100 mL each). The aqueous layer was adjusted to pH=10 with 10M NaOH and then washed with dichloromethane (2 x 150 mL). The organic layer was washed with brine (100 mL), dried with Na_2SO_4 , filtered and solvent was removed under reduced pressure. Obtain 438 mg (1.46 mmol, 75 % yield) as final product. ^1H
 25 NMR (300 MHz, CDCl_3) δ 6.74 (s, 1H), 6.49 (s, 1H), 3.95 (br s, 2H), 3.84 (s, 3H), 2.89-2.69 (m, 2H), 2.60-1.88 (m, 8H), 1.74-1.34 (m, 5H), 0.93 (s, 3H).

3-Amino-2-ethoxyestra-1,3,5(10)-trien-17-one (Compound #35): Prepared as depicted in Scheme 3 using the representative procedures: ^1H NMR (300 MHz, CDCl_3) δ 6.74 (s, 1H), 6.50 (s, 1H), 4.05 (q, $J = 6.8$ Hz, 2H), 3.70 (br s, 2H), 2.86-2.73 (m, 2H),
 30 2.60-1.91 (m, 13H), 1.44 (t, $J = 6.8$ Hz, 3H), 0.93 (s, 3H).

Representative procedure to prepare 3-fluoroestrane analogs: Preparation of 3-fluoro-2-methoxyestra-1,3,5(10)-trien-17-one (Compound #40): 3-Amino-2-methoxyestra-1,3,5(10)-trien-17-one (#34, 439 mg, 1.468 mmol) was dissolved in ethanol (10 mL) and

HBF_4 (48 % aqueous, 5 mL) and cooled to 0°C . NaNO_2 (124 mg, 1.797 mmol in water (0.5 mL) was added and stirred for 2.5 h. Diethyl ether (500 mL) was added, caused an oil to form. The supernatant was decanted off, and the flask was dried under vacuo causing the oil to foam. This foam was stored overnight under vacuo at 80°C and was subsequently

 5 purified using SiO_2 Biotage FLASH apparatus with 2 : 1 hexanes : ethyl acetate as eluent. Obtain 92 mg (0.305 mmol, 21 % yield). ^1H NMR (300 MHz, CDCl_3) δ 6.91 (d, $J = 8.3$ Hz, 1H), 6.82 (d, $J = 12.1$ Hz, 1H), 3.88 (s, 3H), 2.95-2.77 (m, 2H), 2.61-1.92 (m, 8H), 1.73-1.36 (m, 5H), 0.94 (s, 3H). This compound was reduced with NaBH_4 at room temperature or LiAlH_4 at -78°C to give the corresponding 17 alcohol.

10 Preparation of 3-Formamide-2-methoxyestra-1,3-5(10)-trien-17-ol (Compound #34): Same reaction procedure as the preparation of #88, except the 17-ester was cleaved with methanolic NaOH . ^1H NMR (300 MHz, CDCl_3) δ 8.7 (d, $J=11.7$ Hz, rotamer 2), 8.44 (s, rotamer 1) and 8.10 (s, rotamer 2, total 1H), 7.72 (br s) and 7.58 (m, exchangeable amide H), 6.90 (d, $J=16.2$ Hz, rotamer 1), 6.84 (s, rotamer 1, 1H) (total of all H from 8.7-

 15 6.84 (m, 4H), 3.88 (s, rotamer 1) and 3.86 (s, rotamer 2, total 3H), 3.76 (m, 1H), 2.88-2.77 (m, 2H), 2.40-1.13 (m, 14H), 0.81 (s, 3H).

Preparation of 3-formamide-2-methoxyestra-1,3,5(10)-triene-17-one (Compound 43a): Prepared as depicted in Scheme 3 using representative procedures. ^1H NMR (300 MHz, CDCl_3) δ 8.73 (d, $J = 11.5$ Hz, rotamer 1), 8.46 (d, $J = 1.9$ Hz, rotamer 1) and 8.13

 20 (s, rotamer 2, total 1H), 7.76 (br s, rotamer 1) and 7.68-7.61 (m, rotamer 2, total 1H), 6.94 (s, rotamer 1) and 6.85 (s, rotamer 2), 6.82 (s, 1H) (all H between 8.73 and 6.82 total 4H), 4.16-4.01 (m, 2H), 2.94-2.76 (m, 2H), 2.62-1.15 (m, 15 H), 1.47 (app t, $J = 7.0$ and 7.7 Hz, 3H), 0.94 (s, 3H).

25 Preparation of 3-Formamide-2-ethoxyestra-1,3-5(10)-trien-17-one (Compound #44a): Prepared as depicted in Scheme 3 using representative procedure for #88. ^1H NMR (300 MHz, CDCl_3) δ 8.74 (d, $J = 11.7$ Hz, rotamer 2), 8.46 (d, $J = 2.08$ Hz, rotamer 1) and 8.13 (s, rotamer 2, total 1H), 7.76 (br s, rotamer 1) and 7.69-7.61 (m, rotamer 2, total 1H), 6.88 (d, $J = 15$ Hz, rotamer 1), 6.28 (s, 1H) (all protons from 8.74-6.28 total 4H), 4.16-4.01

 30 (m, 2H), 2.95-2.77 (m, 2H), 2.63-1.18 (m, 16H), 0.94 (s, 3H).

Preparation of 3-sulfamate-2-methoxyestra-1,3,5(10)-triene (Compound #46): Prepared as depicted in Scheme 4 using representative procedures. ^1H NMR (300 MHz,

CDCl₃) δ 7.06 (s, 1H), 6.97 (s, 1H), 4.96 (br s, 2H), 3.89 (s, 3H), 2.85-2.78 (m, 2H), 2.33-2.19 (m, 2H), 2.00-1.11 (m, 13H), 0.77 (s, 3H).

Preparation of 3-Triflic-2-methoxyestra-1,3,5(10)-triene (Compound #49): Same general procedure as the preparation of #22. ¹H NMR (300 MHz, CDCl₃) δ 6.97 (s, 1H), 6.92 (s, 1H), 3.89 (s, 3H), 2.93-2.75 (m, 2H), 2.35-2.19 (m, 2H), 2.03-1.06 (m, 15H), 0.77 (s, 3H).

Preparation of 3-Vinyl-2-methoxyestra-1,3,5(10)-triene (Compound #52): Prepared as depicted in Scheme 4 using the representative procedures: ¹H NMR (300 MHz, CDCl₃) δ 7.20 (s, 1H), 7.00 (dd, *J* = 14.5, 11.2 Hz, 1H), 6.85 (s, 1H), 5.71 (d, *J* = 17.7 Hz, 1H), 5.22 (d, *J* = 11.3 Hz, 1H), 3.85 (s, 3H), 2.88-2.78 (m, 2H), 2.35-2.22 (m, 2H), 2.01-1.10 (m, 11H), 0.95 (t, *J* = 7.54, 2H), 0.77 (s, 3H).

Preparation of (2-Methoxyestra-1,3,5(10)-trien-3-yl)-3-carboxamide (Compound #58): A 500 mL round-bottomed flask fitted with an overhead stirrer, a Claisen adaptor with a thermocouple probe and carbon monoxide inlet, and a vacuum inlet, was charged with 2-methoxyestra-1,3,5(10)-trien-3-yl-(trifluoromethyl)sulfonate (#49, 24 g, 57.35 mmol), anhydrous dimethylformamide (185 mL), palladium (II) chloride (0.500 g, 2.87 mmol), 1,3-bis(diphenylphosphino)propane (2.37 g, 5.74 mmol), and 1,1,1,3,3,3-hexamethyldisilazane (48 mL, 229 mmol). The resulting yellow solution was stirred and evacuated, flushed with carbon monoxide (balloon) several times, then heated to 102°C for 12 h. Additional palladium (II) chloride (0.500 g), 1,3-bis(diphenylphosphino)propane (2.40 g), and hexamethyldisilazane (30 mL) were added and the mixture was re-evacuated, charged with carbon monoxide, and heated at 102°C for an additional 12 h. Methanol (50 mL) was added and, after several minutes, the dark solution was partitioned with ethyl acetate (1000 mL) and 2 N sulfuric acid (1000 mL). The aqueous phase was extracted with ethyl acetate (2 x 250 mL), the combined organic extracts were washed with additional sulfuric acid (500 mL), and the aqueous phase was back-extracted with ethyl acetate (2 x 250 mL), and the total combined dark organic layers were washed with saturated aqueous sodium bicarbonate (500 mL) and dried over sodium sulfate (400 g). Suction-filtration through a plug of silica gel 60 (136 g) and concentration afforded 19 g of crude product as a red paste. This was purified by flash chromatography using 430 g of silica gel, and eluting with 10% ethyl acetate-dichloromethane. The product-containing fractions were concentrated, then taken up in acetone and re-concentrated (2 x 2000 mL). The yellow solid was then slurried in *n*-heptane (200 mL) overnight and isolated by suction-

filtration. Removal of residual solvent, and drying to a constant weight over 3 h in a vacuum oven at 70°C and 0.5 torr, afforded 5.07 g (28% overall) of #58 as an off-white powder. ¹H NMR (300 MHz, CDCl₃) δ 10.82 (br s, 1H), 7.99 (s, 1H), 6.95 (s, 1 H), 4.05 (s, 3 H), 2.95 (dd, 2 H, J = 5), 2.15 (m 2H,) 2.02-1.65 (m, 6 H), 1.55-1.15 (m, 8 H), 0.80 (s, 3 H). C NMR (125 MHz, CDCl₃) δ 167.6, 156.1, 147.1, 132.9, 130.0, 116.0, 108.7, 56.2, 54.0, 45.2, 41.2, 40.7, 39.0, 38.6, 28.8, 28.2, 26.7, 25.5, 20.8, 17.8.

Preparation of 3-amino-2-methoxyestra-1,3,5(10)-triene (Compound #61): Prepared as depicted in Scheme 4 using representative procedures. ¹H NMR, (CDCl₃,) δ 6.80 (1H, s, aromatic), 6.48 (1H, s, aromatic), 3.87 (3H, s), 3.65 (2H, broad, NH₂), 2.75 (2H, dd, J= 5.0, 3.0 Hz), 2.25 (2H, m), 1.97 (2H, m), 1.80-1.05 (11H, m), 0.80 (3H, s).

Preparation of 3-formamide-2-methoxyestra-1,3,5(10)-triene (Compound #64): Prepared as depicted in Scheme 4 using representative procedures. ¹H NMR (300 MHz, CDCl₃) δ 8.70 (d, J = 11.7 Hz, rotamer 2), 8.44 (d, J = 1.7 Hz, rotamer 2) and 8.09 (s, rotamer 1, total 1H), 7.71 (br s, rotamer 1) and 7.62-7.53 (m, rotamer 2, total 1H), 6.90 (d, J = 10.4 Hz, rotamer 1), 6.85 (s, 1H) (all protons from 8.70-6.85 total 4H), 3.88 (s, rotamer 1) and 3.86 (s, rotamer 2, total 3H), 2.91-2.77 (m, 2H), 2.33-2.18 (m, 2H), 2.00-1.08 (m, 13H), 0.77 (s, 3H).

Representative procedure for preparation of sulfamate derivatives: 17-methylene-3-sulfamate-2-methoxyestra-1,3,5(10)-triene (Compound #70): Sodium hydride (268 mg, 11.2 mmol) was added to anhydrous DMF (40 mL) and cooled to 0°C, then 17-methylene-2-methoxyestra-1,3,5(10)-triene-3-ol in anhydrous DMF (8 mL) was added drop wise. The mixture was stirred for 2 h at room temperature. Sulfamoyl chloride (prepared freshly as in Peterson *et al J. Med. Chem.* 1992, 35, 3991) was added in portions at 0°C and stirred overnight at room temperature. The mixture was poured into water (100 mL) and washed with ethyl acetate (3 x 100 mL). The combined organic layers were washed with water (4 x 100 mL) and brine (100 mL). The organics were dried with MgSO₄, filtered and solvent was removed under reduced pressure to obtain a white solid. After drying under vacuo for 24 h, obtain 441 mg product (70 % yield, 1.17 mmol). ¹H NMR (300 MHz, CDCl₃) δ 7.07 (s, 1H), 6.98 (s, 1H), 4.96 (s, 2H), 4.71 (s, 2H), 3.91 (s, 3H), 2.93-2.73 (m, 2H), 2.65-1.15 (m, 13 H), 0.85 (s, 3H).

Preparation of 3-triflic-17-methylene-2-methoxyestra-1,3,5(10)-triene (Compound #73): Prepared as depicted in Scheme 5 using representative procedures. ¹H NMR (300

MHz, CDCl₃) δ 6.98 (s, 1H), 6.93 (s, 1H), 4.73-4.68 (m, 2H), 3.90 (s, 3H), 2.88-2.78 (m, 2H), 2.64-2.50 (m, 1H), 2.41-1.18 (m, 13H), 0.85 (s, 3H).

Alternate preparation of 3-carboxamide estrane derivatives directly from triflate –
Preparation of 2-Methoxy-17-methyleneestra-1,3,5(10)-triene-3-carboxamide (Compound
#82): General procedure based on *Tetrahedron Letters*, 1998, 39, 2835-2838. A 250 mL
three-necked flask equipped with an overhead stirrer, thermocouple, and nitrogen inlet, was
charged with 2-Methoxy-17-methyleneestra-1,3,5(10)-trien-3-yl (trifluoromethyl)-sulfonate
#73 (20.0 g, 46.5 mmol), palladium (II) chloride (0.41 g, 2.3 mmol), 1,3-
bis(diphenylphosphino) propane (1.9 g, 4.6 mmol), 1,1,1,3,3,3-hexamethyldisilazane (38.8
mL, 186 mmol) and anhydrous dimethylformamide (150 mL). The resulting orange solution
was evacuated and back-filled with nitrogen three times, then evacuated and back-filled with
carbon monoxide three times. The reaction was warmed to 100°C and stirred under a carbon
monoxide atmosphere (balloon) for 18 hours, during which time the solution became dark red.
The heat was removed, methanol (40 mL) was added, and the solution allowed to stir for 10
minutes. The solution was poured into ethyl acetate (1 L) and extracted with 2 N H₂SO₄ (1 L).
The aqueous layer was extracted with ethyl acetate (3 x 500 mL). Each of the ethyl acetate
extracts was washed with 2 N H₂SO₄ (500 mL). The combined organic phases were washed
with saturated aqueous sodium bicarbonate (1 L) and dried over sodium sulfate (500 g). This
suspension was suction-filtered through a bed of silica gel 60 (102 g), and the filtrate
concentrated to dryness affording 16.02 g (106% recovery) of crude #82. The crude product
was purified on silica gel 60 (500 g, flash column) eluting with methylene chloride (2 L) then
2% methanol-methylene chloride (4 L) then 4% methanol-methylene chloride (4 L).
Chromatography failed to remove all impurities so fractions containing product were
combined, concentrated to dryness, and repurified on silica gel 60 (500 g, flash column)
eluting with methylene chloride (2 L) then 1:5 ethyl acetate-methylene chloride.
Concentration of the pure fractions (TLC, 1:5 ethyl acetate-methylene chloride, R_f=0.3, UV
detection) and drying in a vacuum oven at 80°C, afforded 12.3 g (81% overall) of compound
#82 as a light-yellow solid with residual methylene chloride by NMR. The material was
dissolved in 10:1 acetone/methanol (1.35 L), concentrated to dryness to remove residual
solvents, and dried *in vacuo* to yield 11.36 g of compound #16 (75% overall yield) as a light-
yellow solid. ¹H-NMR (showed 0.09 wt% acetone present), ¹³C-NMR and Mass Spectral
analysis were consistent with desired structure. ¹H NMR (300 MHz, CDCl₃) δ 7.94 (s, 1H),
7.63 (br s, 1H), 6.95 (s, 1H), 5.70 (br s, 1H), 4.71 (s, 2H), 3.97 (s, 3H), 2.97-2.82 (m, 2H),

2.65-1.19 (m, 13H), 0.86 (s, 3H). ^{13}C NMR (CDCl_3 , 125 MHz) δ 167.2, 161.4, 155.8, 146.3, 132.6, 129.6, 118.0, 108.4, 100.9, 55.9, 53.5, 44.9, 44.2, 38.2, 35.6, 29.4, 28.4, 27.4, 26.4, 23.9, 18.5.

Preparation of 3-amino-17-methylene-2-methoxyestra-1,3,5(10)-triene (Compound #85): Prepared as depicted in Scheme 5 using representative procedures. ^1H NMR (300 MHz, CDCl_3) δ 6.78 (s, 1H), 6.49 (s, 1H), 4.72 -4.67 (m, 2H), 3.85 (s, 2H), 3.67 (br s, 2H), 2.83-2.71 (m, 2H), 2.63-2.48 (m, 1H), 2.41-1.14 (m, 13H), 0.85 (s, 3H).

Preparation of 3-amino-17-methylene-2-ethoxyestra-1,3,5(10)-triene (Compound #86): Prepared as depicted in Scheme 5 using representative procedures. ^1H NMR (300 MHz, CDCl_3) δ 6.77 (s, 1H), 6.48 (s, 1H), 4.69 (s, 2H), 4.06 (q, $J = 7.0$ Hz, 2H), 3.70 (br s, 2H), 2.87-2.65 (m, 2H), 2.63-2.48 (m, 1H), 2.40-2.11 (m, 2H), 2.03-1.17 (m, 10H), 1.43 (t, $J = 6.8$ Hz, 3H), 0.84 (s, 3H).

Representative preparation of 3-formamide estrane derivatives: Preparation of 3-formamide-17-methylene-2-methoxyestra-1,3,5(10)-triene (Compound #88): 3-Amino-17-methylene-2-methoxyestra-1,3,5(10)-triene (293 mg, 0.99 mmol) was dissolved in chloroform (1mL) and trimethylacetyl formic anhydride (170 mg, 1.30 mmol, prepared as in Vlietstra *et al Recueil*, 1982, 101, 460) was added. The mixture was stirred 40 min, after which, the solvent was removed under reduced pressure. The product was purified by titrating with hexanes and isolating the precipitate by decanting after the precipitate was centrifuged. Obtain 150 mg white powder (0.417 mmol, 42 % yield). ^1H NMR (300 MHz, CD_3OD) δ 8.27 (s, 1H), 7.88 (s, 1H), 6.94 (s, 1H), 4.72-4.67 (m, 2H), 3.88 (s, rotamer 1) and 3.86 (s, rotamer 2, total 3H), 2.87-2.76 (m, 2H), 2.66-1.19 (m, 13H), 0.87 (s, 3H).

Preparation of 3-formamide-17-methylene-2-ethoxyestra-1,3,5(10)-triene (Compound #89): Prepared as depicted in Scheme 5 using representative procedures. ^1H NMR (300 MHz, CDCl_3) δ 8.73 (d, $J = 11.7$ Hz, rotamer 2), 8.46 (d, $J = 1.88$ Hz, rotamer 2) and 8.11 (s, total 1H), 7.75 (br s, rotamer 1) and 7.62 (br s, rotamer 2, exchangeable amide H), 6.90 (d, $J = 18$ Hz, rotamer 1), 6.84 (s, all protons from 8.73-6.84 total 4H), 4.70 (s, 2H), 4.16-4.02 (m, 2H), 2.93-2.79 (m, 2H), 2.66-1.16 (m, 16H), 0.85 (s, 3H).

Preparation of 3-sulfamate-17-ethylene-2-methoxyestra-1,3,5(10)-triene (Compound #94): Prepared as depicted in Scheme 6 using representative procedures. ^1H NMR (300 MHz, CDCl_3) δ 7.06 (s, 1H), 6.96 (s, 1H), 5.24-5.13 (m, 1H), 4.96 (s, 2H),

3.90 (s, 3H), 2.90-2.78 (m, 2H), 2.51-1.20 (m, 16H), 0.94 (s, Z isomer) and 0.80 (s, E isomer, total 3H, ratio 5:1 respectively).

Preparation of 3-amino-17-ethylene-2-methoxyestra-1,3,5(10)-triene (Compound #109): Prepared as depicted in Scheme 6 using representative procedures. ¹H NMR (300 MHz, CDCl₃) δ 6.76 (s, 1H), 6.48 (s, 1H), 5.22-5.12 (m, 1H), 3.85 (s, 3H), 3.66 (br s, 2H), 2.86-2.66 (m, 2H), 2.53-2.16 (m, 4H), 2.00-1.20 (m, 12H), 0.93 (s, Z isomer) and 0.80 (s, E isomer, total 3H, ratio 5:1 respectively).

Preparation of 3-formamide-17-ethylene-2-methoxyestra-1,3,5(10)-triene (Compound #112): Prepared as depicted in Scheme 6 using representative procedures. ¹H NMR (300 MHz, CDCl₃) δ 8.70 (d, J = 11.7 Hz, rotamer 2), 8.44 (d, J = 1.89 Hz, rotamer 1) and 8.09 (s, rotamer 2, total 1H), 7.72 (br s, rotamer 1) and 7.63-7.52 (m, rotamer 2, exchangeable amide H), 6.90 (d, J = 14.7 Hz, rotamer 1), 6.84 (s, 1H) (all protons from 8.70-6.84 total 4H), 5.24-5.14 (m, 1H), 3.89 (s, rotamer 1) and 3.86 (s, rotamer 2, total 3H), 2.90-2.78 (m, 2H), 2.53-2.18 (m, 5H), 2.03-1.23 (m, 11H), 0.93 (s, Z isomer) and 0.80 (s, E isomer, total 3H, ratio 5:1 respectively).

Preparation of 3-sulfamate-17-methyl-2-methoxyestra-1,3,5(10)-triene (Compound #118): Prepared as depicted in Scheme 7 using representative procedures. ¹H NMR (300 MHz, CDCl₃) δ 7.06 (s, 1H), 6.97 (s, 1H), 4.95 (s, 2H), 3.90 (s, 3H), 2.89-2.74 (m, 2H), 2.35-2.15 (m, 2H), 1.99-1.12 (m, 12H), 0.91 (d, J = 6.8 Hz, 3H), 0.62 (s, 3H).

Preparation of 3-sulfamate-17-methyl-2-ethoxyestra-1,3,5(10)-triene (Compound #119): Prepared as depicted in Scheme 7 using representative procedures. ¹H NMR (300 MHz, CDCl₃) δ 7.042 (s, 1H), 6.96 (s, 1H), 4.99 (s, 2H), 4.21-4.06 (m, 2H), 2.87-2.74 (m, 2H), 2.32-1.12 (m, 17H), 0.90 (d, J = 5.30 Hz, β isomer) and 0.79 (d, J = 3.77 Hz, α isomer, total 3H, ratio 4:1 respectively), 0.61 (s, 3H).

Preparation of 3-amino-17-methyl-2-methoxyestra-1,3,5(10)-triene (Compound #133): Prepared as depicted in Scheme 7 using representative procedures. ¹H NMR (300 MHz, CDCl₃) δ 6.77 (s, 1H), 6.48 (s, 1H), 3.85 (s, 3H), 2.87-2.66 (m, 2H), 2.33-2.14 (m, 2H), 1.95-1.11 (m, 14H), 0.89 (d, J = 6.78 Hz, β isomer) and 0.79 (d, J = 3.77 Hz, α isomer, total 3H, ratio 4:1 respectively), 0.61 (s, 3H).

Preparation of 3-formamide-17-methyl-2-methoxyestra-1,3,5(10)-triene (Compound #136): Prepared as depicted in Scheme 7 using representative procedures. ¹H NMR (300 MHz, CDCl₃) δ 8.70 (d, J = 11.7 Hz, rotamer 2), 8.43 (d, J = 1.89 Hz, rotamer

1) and 8.09 (s, rotamer 2, total 1H), 7.72 (br s) and 7.61-7.52 (m, exchangeable H), 6.90 (d, J = 10.9 Hz, rotamer 1), 6.85 (s, 1H) (all protons from 8.70-6.85 total 4H), 3.88 (s, rotamer 1) and 3.86(s, rotamer 2, total 3H), 2.93-2.73 (μ , 2H), 2.34-1.10 (μ , 14H), 0.90 (d, J = 6.78 Hz, β isomer) and 0.79 (d, J = 3.7 Hz, α isomer, total 3H, ratio 4:1 respectively), 0.61 (s, 3H).

EXAMPLE 2

Determination of *in vitro* anti-proliferative activity of substituted estradiol analogs: *In vitro* anti-proliferative or anti-mitogenic activity was determined using a commercially available cell-based assay in 96-well tissue culture plates with assessment of proliferation by evaluating DNA synthesis through incorporation into DNA of immuno-reactive (BrdU) nucleotides. The cell types used are commercially available (MDA-MB-231: breast cancer; U87-MG: glioblastoma; PC3: prostate cancer; HUVEC: non-transformed early passage human umbilical vein endothelial cells). These assays, and the many assay variations possible to determine *in vitro* anti-proliferative or anti-mitogenic activity, are well known to those skilled in the art. The concentration which causes 50% inhibition of proliferation (IC_{50}) was estimated from a dose-response curve generally carried out with a range of concentrations from ≥ 100 microg/mL to ≤ 0.01 microg/mL. The results of the tests are shown below in Table IV.

TABLE IV

Compound #	IC_{50} for MDA-MB-231 (μ M)	IC_{50} for U87-MG (μ M)	IC_{50} for PC3 (μ M)	IC_{50} for HUVEC (μ M)
1	0.69 ± 0.14	1.48 ± 0.62	1.08 ± 0.50	0.68 ± 0.15
2	0.650 ± 0.23			0.14 ± 0.07
3	3.99 ± 1.12	2.68 ± 0.22	2.22 ± 0.13	0.52 ± 0.14
4	11.7	10.5	13.7	12.6
7	2.20 ± 0.52	7.35 ± 0.43	8.49 ± 2.49	1.32 ± 0.56
10	0.58 ± 0.31			0.26 ± 0.23
13	2.63 ± 0.20	2.37 ± 0.34	1.15 ± 0.40	0.37 ± 0.08
16	3.33 ± 0.23	9.39 ± 0.43	6.46 ± 2.31	2.40 ± 0.45
19	0.60 ± 0.43	0.80 ± 0.23	0.51 ± 0.24	0.18 ± 0.04
20	7.52 ± 2.04	19.82 ± 2.32	8.93 ± 0.61	2.59 ± 0.25
25	2.57 ± 0.29	2.20 ± 0.06	1.43 ± 0.40	0.58 ± 0.01
25a	8.93 ± 0.61	7.81 ± 1.30	6.34 ± 0.08	1.96 ± 0.05
28	$>100 \pm 0$	$>100 \pm 0$	$>100 \pm 0$	$>100 \pm 0$
31	0.7 ± 0.12	1.07 ± 0.15	0.7 ± 0.03	0.79 ± 0.20
34	46.91 ± 8.15	81.54 ± 20.37	>100	23.42 ± 4.14
35	10.64 ± 2.98	26.05 ± 1.40	9.25 ± 1.58	16.46 ± 2.02
37	2.48 ± 0.50	10.83 ± 1.04	9.79 ± 1.93	2.32 ± 0.38
40	2.94 ± 0.80	25.98 ± 3.38	9.26 ± 0.73	2.10 ± 0.23

43	0.78 ± 0.01	2.70 ± 0.22	1.34 ± 0.02	0.07 ± 0.02
43A	1.22 ± 0.60	0.94 ± 0.09	0.61 ± 0.27	0.53 ± 0.22
44A	24.87 ± 5.63	21.10	27.70 ± 0.62	13.17 ± 2.20
46	0.38 ± 0.15	0.72 ± 0.04	0.38 ± 0.21	0.21 ± 0.15
52	1.86 ± 0.22	3.14 ± 0.52	2.12 ± 0.64	0.58 ± 0.04
58	5.23 ± 1.95	4.52 ± 1.3	2.58 ± 0.36	1.42 ± 0.41
61	7.62 ± 0.43	10.20 ± 3.78	8.63 ± 0.18	6.43 ± 1.32
64	8.29 ± 1.64	23.24 ± 2.0	9.24 ± 0.59	5.80 ± 0.73
70	0.56 ± 0.02	0.70 ± 0.06	0.91 ± 0.32	0.58 ± 0.03
73	>100	>100	>100	>100
82	0.19 ± 0.08	0.25 ± 0.02	0.25 ± 0.01	0.12 ± 0.06
85	2.11 ± 0.57	5.51 ± 0.46	2.40 ± 0.39	1.00 ± 0.19
86	2.13 ± 0.12	7.29 ± 2.26	2.75 ± 0.22	2.31 ± 0.05
88	0.30 ± 0.08	0.53 ± 0.24	0.24 ± 0.06	0.22 ± 0.03
89	2.41 ± 0.13	7.73	1.59 ± 0.19	2.10 ± 0.02
94	0.58 ± 0.02	0.64 ± 0.09	0.66 ± 0.05	0.59 ± 0.00
109	1.94 ± 0.27	2.35 ± 0.41	0.92 ± 0.11	0.72 ± 0.10
112	0.56 ± 0.02	0.77 ± 0.06	0.74 ± 0.01	0.58 ± 0.00
113	11.98	19.13	20.64	7.08
118	0.66 ± 0.10	0.64 ± 0.01	0.82 ± 0.06	0.59 ± 0.1
119	1.55 ± 0.39	6.41	0.83	0.91 ± 0.09
133	7.31 ± 0.58	14.87 ± 0.01	7.16 ± 1.42	2.65 ± 0.77
136	0.61 ± 0.00	0.64 ± 0.01	0.70 ± 0.10	0.59 ± 0.00

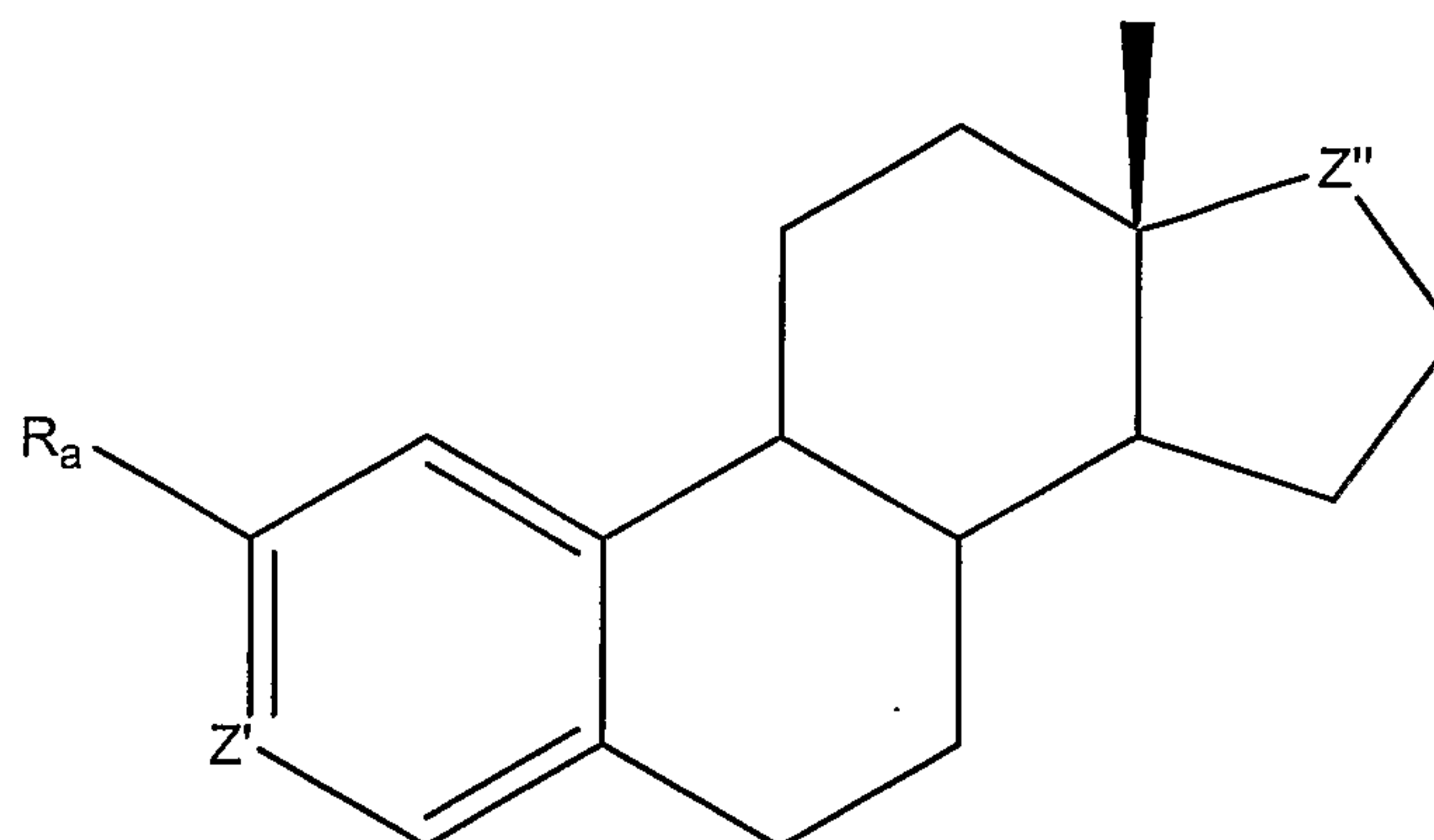
All of the publications mentioned herein are hereby incorporated by reference in their entireties. The above examples are merely demonstrative of the present invention,
5 and are not intended to limit the scope of the appended claims.

CLAIMS

We claim:

5

1. A compound of the general formula:

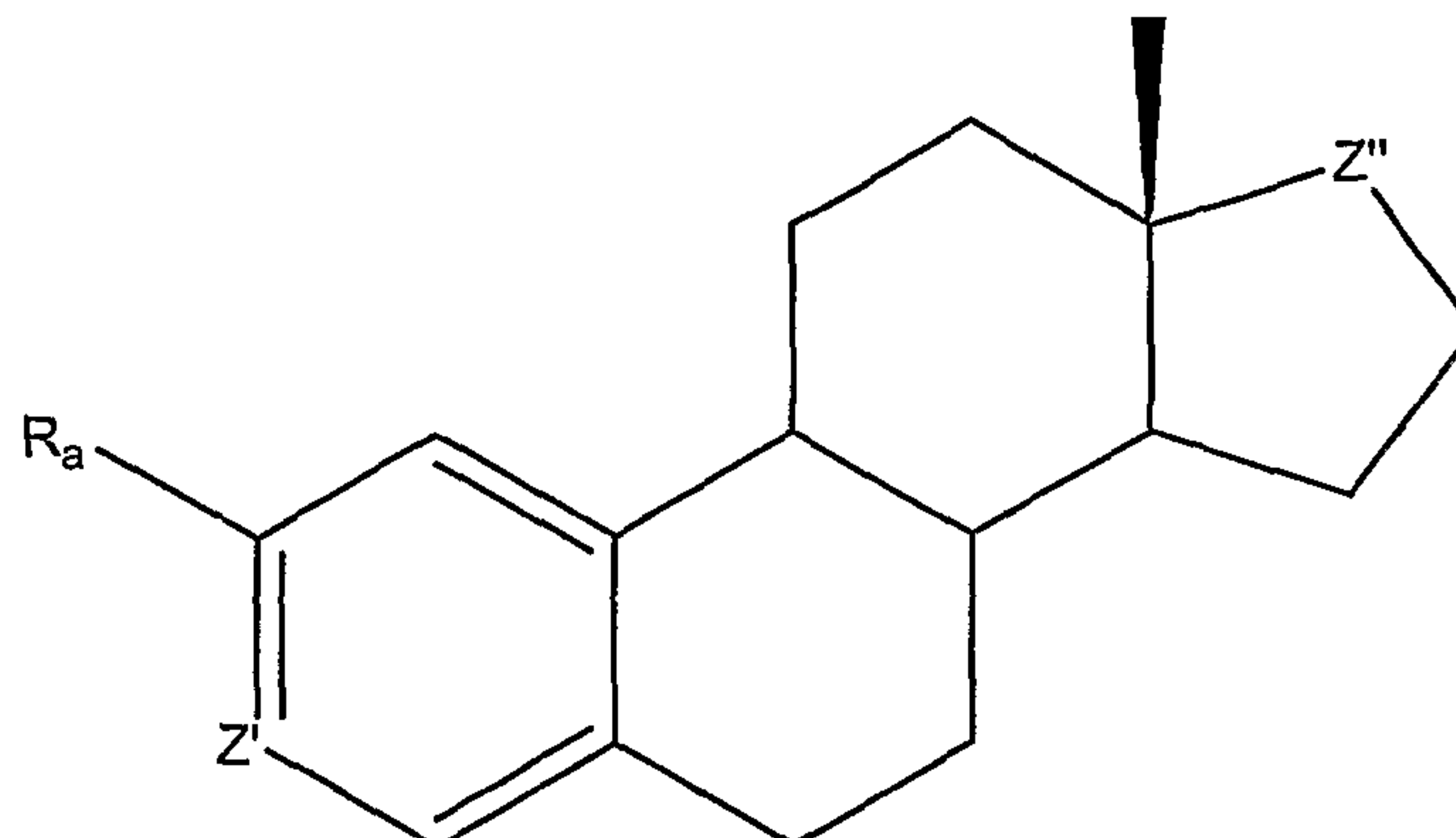


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wherein R_a is selected from $-OCH_3$; $-OCH_2CH_3$; or $-CCCH_3$; Z' is selected from $>C-F$, $>C-NH_2$, $>CCONH_2$, $>C-NHCOH$, $>C-OSO_2NH_2$, or $>C-CHCH_2$, and Z'' is selected from $>C(H_2)$, $>C(H)-CH_3$, $>C=CH_2$, $>C=CHCH_3$, or $>C=O$; provided that when Z' is $>C-OSO_2NH_2$ and Z'' is $>C(H_2)$ or $>C=O$, R_a is neither $-OCH_3$ nor $-OCH_2CH_3$.

15

2. A method of inhibiting angiogenesis in a human or animal comprising administering to the human or animal an angiogenesis-inhibiting amount of a compound having the formula:



- 5 wherein, R_a is selected from $-\text{OCH}_3$; $-\text{OCH}_2\text{CH}_3$; or $-\text{CCCH}_3$; Z' is selected from $>\text{C}-\text{F}$, $>\text{C}-\text{NH}_2$, $>\text{CCONH}_2$, $>\text{C}-\text{NHCOH}$, $>\text{C}-\text{OSO}_2\text{NH}_2$, or $>\text{C}-\text{CHCH}_2$, and Z'' is selected from $>\text{C}(\text{H}_2)$, $>\text{C}(\text{H})-\text{CH}_3$, $>\text{C}=\text{CH}_2$, $>\text{C}=\text{CHCH}_3$, or $>\text{C}=\text{O}$; provided that when Z' is $>\text{C}-\text{OSO}_2\text{NH}_2$ and Z'' is $>\text{C}(\text{H}_2)$ or $>\text{C}=\text{O}$, R_a is neither $-\text{OCH}_3$ nor $-\text{OCH}_2\text{CH}_3$.

10

3. The method of Claim 2, wherein the administration of the angiogenesis-inhibiting amount of the compound is in a daily dose, a daily sub-dose, or any appropriate fraction thereof to the human or animal to inhibit the effects of angiogenesis.

15

4. The method of Claim 2, wherein the amount of the compound administered is approximately 0.1 to approximately 300 mg/kg/day.

20

5. The method of Claim 2, wherein the amount of the compound administered is approximately 0.5 to approximately 50 mg/kg/day.

6. The method of Claim 2, wherein the amount of the compound administered is approximately 1 to approximately 10 mg/kg/day.

25

- 5
7. The method of Claim 2, wherein the administration of the compound is oral, parenteral, transdermal, topical, intravenous, subcutaneous, intramuscular, intradermal, ophthalmic, epidural, intratracheal, sublingual, buccal, rectal, vaginal, nasal or inhalation.
- 10
8. The method of Claim 2, wherein the compound is administered in a composition comprising an additive selected from an anti-oxidant, a buffer, a bacteriostat, a liquid carrier, a solute, a suspending agent, a thickening agent, a flavoring agent, a gelatin, glycerin, a binder, a lubricant, an inert diluent, a preservative, a surface active agent, a dispersing agent, a biodegradable polymer, or any combination thereof.
- 15
9. The method of Claim 2, wherein the compound is administered in the form of a tablet, a capsule, a lozenge, a cachet, a solution, a suspension, an emulsion, a powder, an aerosol, a suppository, a spray, a pastille, an ointment, a cream, a paste, a foam, a gel, a tampon, a pessary, a granule, a bolus, a mouthwash, or a transdermal patch.
- 20
10. The method of Claim 2, wherein the angiogenesis is associated with diabetic retinopathy.
- 25
11. The method of Claim 2, wherein the angiogenesis is associated with retinopathy of prematurity.
- 30
12. The method of Claim 2, wherein the angiogenesis is associated with corneal graft rejection.
13. The method of Claim 2, wherein the angiogenesis is associated with neovascular glaucoma.
14. The method of Claim 2, wherein the angiogenesis is associated with retrolental fibroplasia.

15. The method of Claim 2, wherein the angiogenesis is associated with epidemic keratoconjunctivitis.
- 5 16. The method of Claim 2, wherein the angiogenesis is associated with Vitamin A deficiency.
17. The method of Claim 2, wherein the angiogenesis is associated with contact lens overwear.
- 10 18. The method of Claim 2, wherein the angiogenesis is associated with atopic keratitis.
19. The method of Claim 2, wherein the angiogenesis is associated with superior limbic keratitis.
- 15 20. The method of Claim 2, wherein the angiogenesis is associated with pterygium keratitis sicca.
- 20 21. The method of Claim 2, wherein the angiogenesis is associated with Sjögren's syndrome.
22. The method of Claim 2, wherein the angiogenesis is associated with acne rosacea.
- 25 23. The method of Claim 2, wherein the angiogenesis is associated with phlyctenulosis.
24. The method of Claim 2, wherein the angiogenesis is associated with syphilis.
- 30 25. The method of Claim 2, wherein the angiogenesis is associated with *Mycobacteria* infections.

26. The method of Claim 2, wherein the angiogenesis is associated with lipid degeneration.
- 5 27. The method of Claim 2, wherein the angiogenesis is associated with chemical burns.
28. The method of Claim 2, wherein the angiogenesis is associated with bacterial ulcers.
- 10 29. The method of Claim 2, wherein the angiogenesis is associated with fungal ulcers.
30. The method of Claim 2, wherein the angiogenesis is associated with *Herpes simplex* infections.
- 15 31. The method of Claim 2, wherein the angiogenesis is associated with *Herpes zoster* infections.
32. The method of Claim 2, wherein the angiogenesis is associated with protozoan infections.
- 20 33. The method of Claim 2, wherein the angiogenesis is associated with Kaposi's sarcoma.
- 25 34. The method of Claim 2, wherein the angiogenesis is associated with Mooren's ulcer.
35. The method of Claim 2, wherein the angiogenesis is associated with Terrien's marginal degeneration.
- 30 36. The method of Claim 2, wherein the angiogenesis is associated with marginal keratolysis.

37. The method of Claim 2, wherein the angiogenesis is associated with trauma.
38. The method of Claim 2, wherein the angiogenesis is associated with rheumatoid arthritis.
- 5 39. The method of Claim 2, wherein the angiogenesis is associated with systemic lupus.
- 10 40. The method of Claim 2, wherein the angiogenesis is associated with polyarteritis.
41. The method of Claim 2, wherein the angiogenesis is associated with Wegener's sarcoidosis.
- 15 42. The method of Claim 2, wherein the angiogenesis is associated with scleritis.
43. The method of Claim 2, wherein the angiogenesis is associated with Stevens-Johnson disease.
- 20 44. The method of Claim 2, wherein the angiogenesis is associated with radial keratotomy.
45. The method of Claim 2, wherein the angiogenesis is associated with macular degeneration.
- 25 46. The method of Claim 2, wherein the angiogenesis is associated with sickle cell anemia.
47. The method of Claim 2, wherein the angiogenesis is associated with sarcoid.
- 30 48. The method of Claim 2, wherein the angiogenesis is associated with pseudoxanthoma elasticum.

49. The method of Claim 2, wherein the angiogenesis is associated with Paget's disease.
50. The method of Claim 2, wherein the angiogenesis is associated with vein occlusion.
51. The method of Claim 2, wherein the angiogenesis is associated with artery occlusion.
52. The method of Claim 2, wherein the angiogenesis is associated with carotid obstructive disease.
53. The method of Claim 2, wherein the angiogenesis is associated with chronic uveitis.
54. The method of Claim 2, wherein the angiogenesis is associated with chronic vitritis.
55. The method of Claim 2, wherein the angiogenesis is associated with Lyme's disease.
56. The method of Claim 2, wherein the angiogenesis is associated with Eales' disease.
57. The method of Claim 2, wherein the angiogenesis is associated with Behcet's disease.
58. The method of Claim 2, wherein the angiogenesis is associated with myopia.
59. The method of Claim 2, wherein the angiogenesis is associated with optic pits.

- 5
60. The method of Claim 2, wherein the angiogenesis is associated with Stargardt's disease.
61. The method of Claim 2, wherein the angiogenesis is associated with pars planitis.
62. The method of Claim 2, wherein the angiogenesis is associated with chronic retinal detachment.
- 10 63. The method of Claim 2, wherein the angiogenesis is associated with hyperviscosity syndromes.
64. The method of Claim 2, wherein the angiogenesis is associated with toxoplasmosis.
- 15 65. The method of Claim 2, wherein the angiogenesis is associated with post-laser complications.
- 20 66. The method of Claim 2, wherein the angiogenesis is associated with abnormal proliferation of fibrovascular or fibrous tissue.
67. The method of Claim 2, wherein the angiogenesis is associated with hemangiomas.
- 25 68. The method of Claim 2, wherein the angiogenesis is associated with Osler-Weber-Rendu disease.
69. The method of Claim 2, wherein the angiogenesis is associated with solid tumors.
- 30 70. The method of Claim 2, wherein the angiogenesis is associated with blood-borne tumors.

71. The method of Claim 2, wherein the angiogenesis is associated with acquired immune deficiency syndrome.
- 5 72. The method of Claim 2, wherein the angiogenesis is associated with ocular neovascular disease.
73. The method of Claim 2, wherein the angiogenesis is associated with age-related macular degeneration.
- 10 74. The method of Claim 2, wherein the angiogenesis is associated with osteoarthritis.
75. The method of Claim 2, wherein the angiogenesis is associated with diseases caused by chronic inflammation.
- 15 76. The method of Claim 2, wherein the angiogenesis is associated with Crohn's disease.
77. The method of Claim 2, wherein the angiogenesis is associated with ulcerative colitis.
- 20 78. The method of Claim 2, wherein the angiogenesis is associated with tumors of rhabdomyosarcoma.
- 25 79. The method of Claim 2, wherein the angiogenesis is associated with tumors of retinoblastoma.
80. The method of Claim 2, wherein the angiogenesis is associated with Ewing's sarcoma.
- 30 81. The method of Claim 2, wherein the angiogenesis is associated with neuroblastoma.

82. The method of Claim 2, wherein the angiogenesis is associated with tumors of osteosarcoma.
- 5 83. The method of Claim 2, wherein the angiogenesis is associated with leukemia.
84. The method of Claim 2, wherein the angiogenesis is associated with psoriasis.
- 10 85. The method of Claim 2, wherein the angiogenesis is associated with atherosclerosis.
86. The method of Claim 2, wherein the angiogenesis is associated with pemphigoid.
- 15 87. The method of Claim 2, wherein the angiogenesis is associated with infections causing retinitis or choroiditis.
88. The method of Claim 2, wherein the angiogenesis is associated with presumed ocular histoplasmosis.
- 20 89. The method of Claim 2, wherein the angiogenesis is associated with Best's disease.
- 25 90. The method of Claim 2, wherein the angiogenesis is associated with proliferative vitreoretinopathy.
91. The method of Claim 2, wherein the angiogenesis is associated with Bartonellosis.
- 30 92. The method of Claim 2, wherein the angiogenesis is associated with acoustic neuromas.

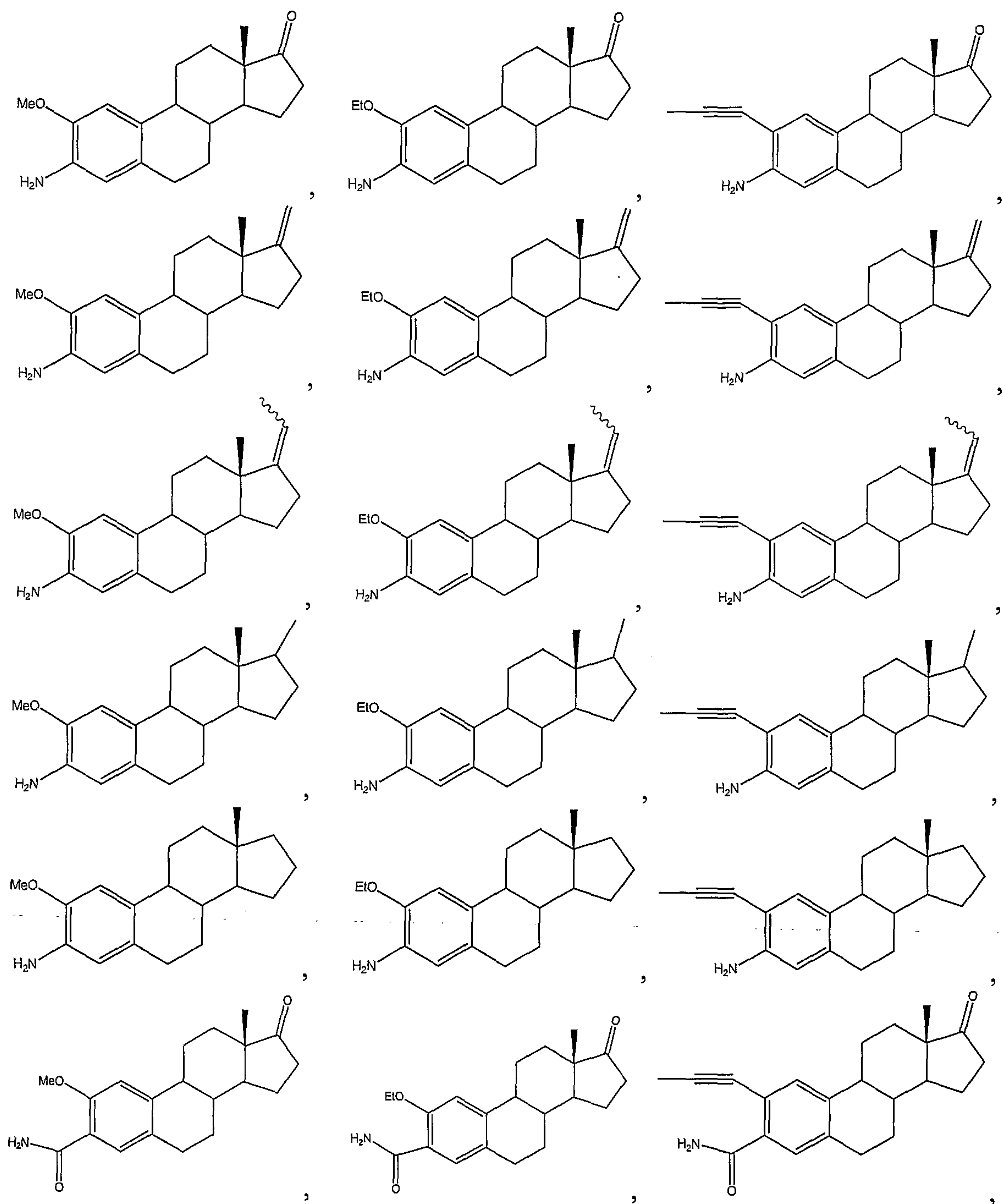
93. The method of Claim 2, wherein the angiogenesis is associated with neurofibroma.

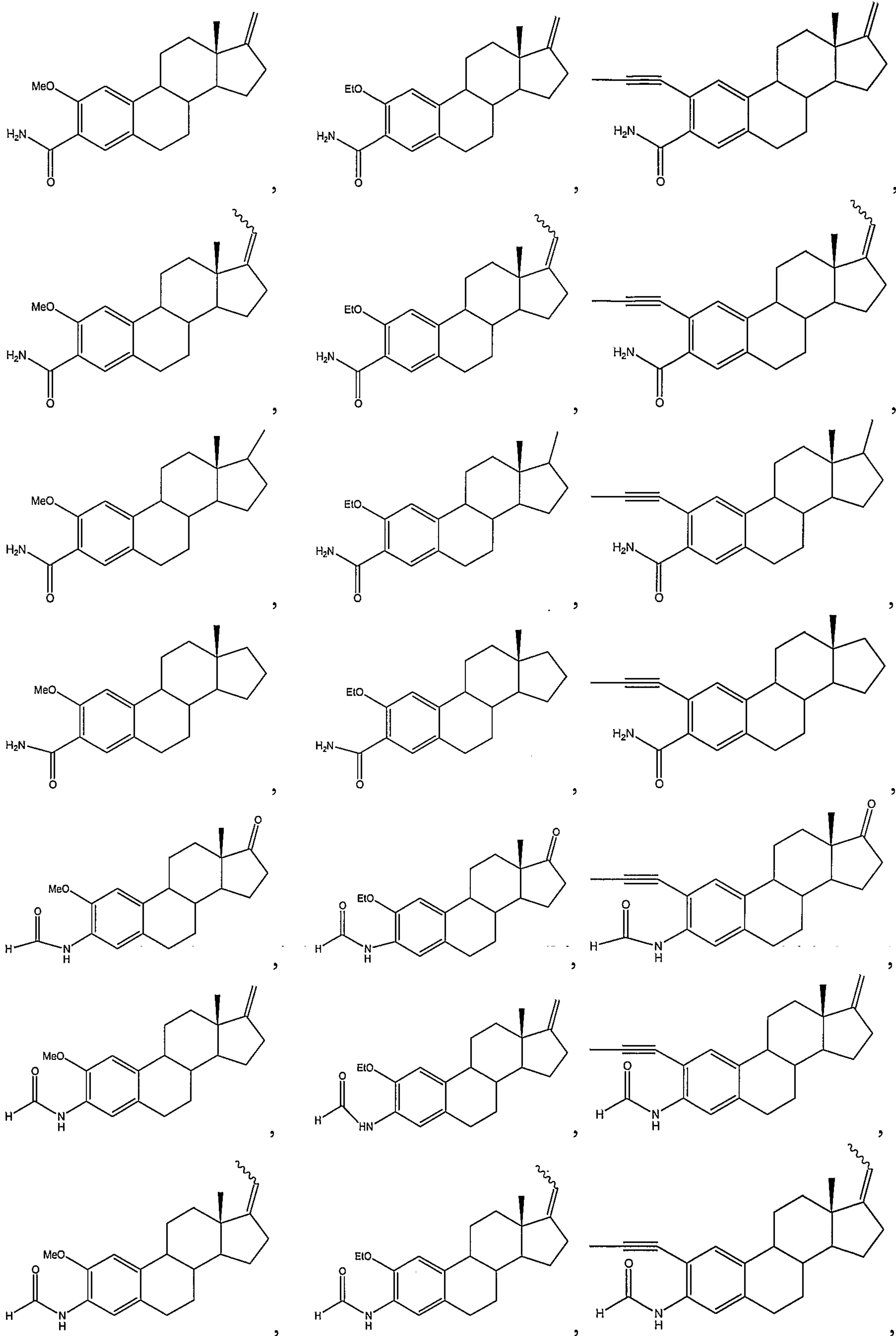
5 94. The method of Claim 2, wherein the angiogenesis is associated with trachoma.

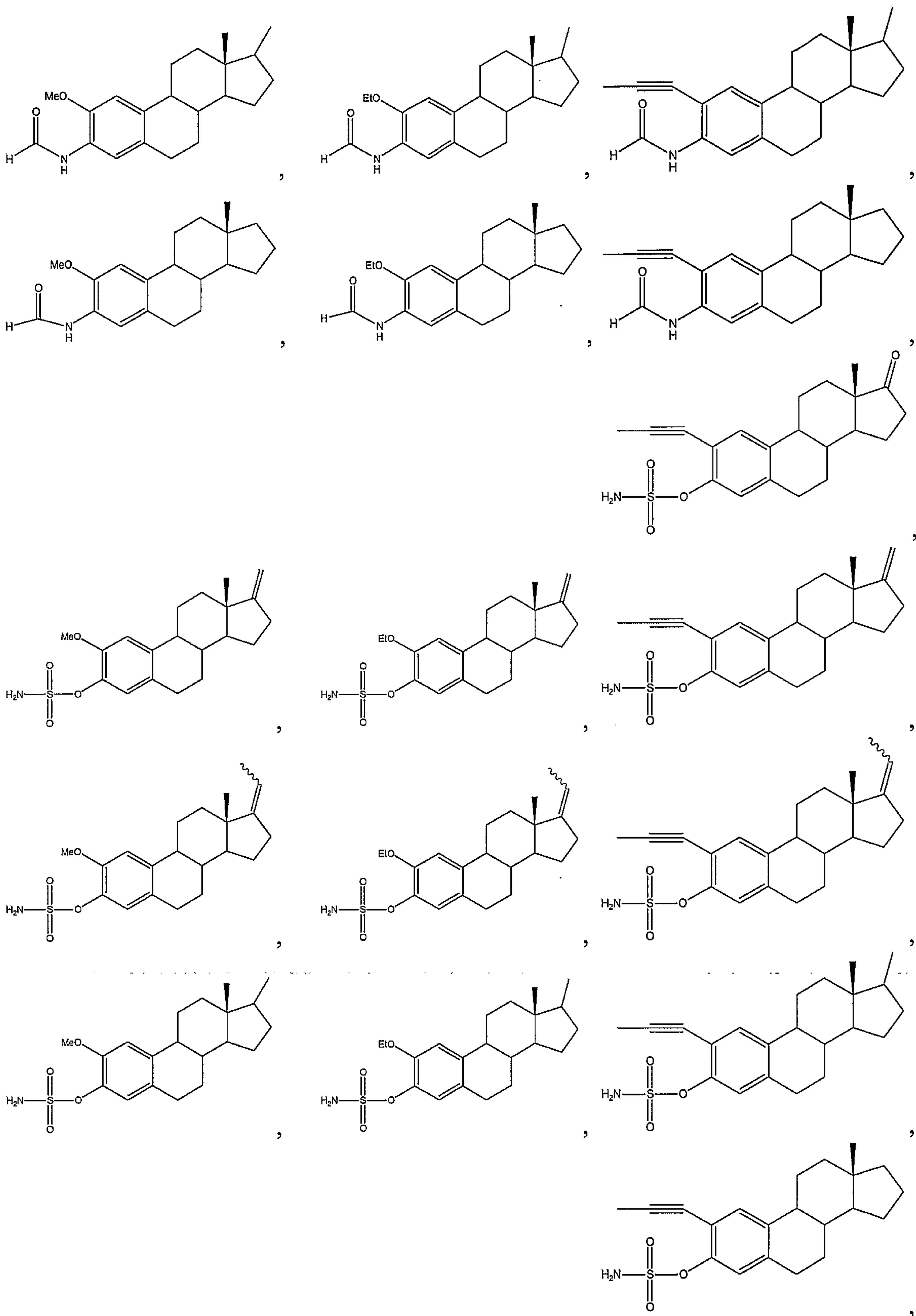
95. The method of Claim 2, wherein the angiogenesis is associated with pyogenic granulomas.

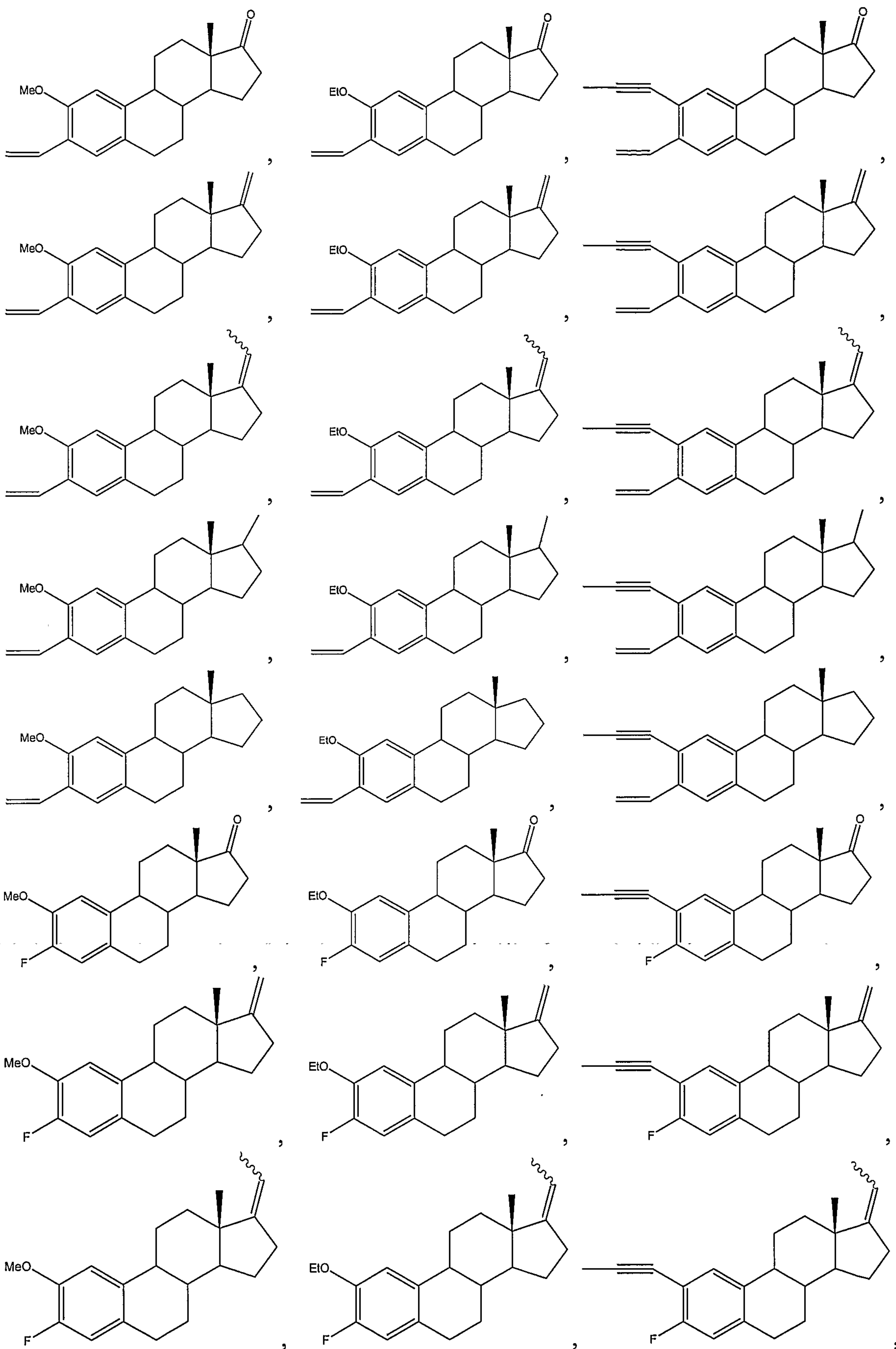
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96. A method of treating an eye condition in a human or an animal comprising administering to the human or animal a compound having the formula



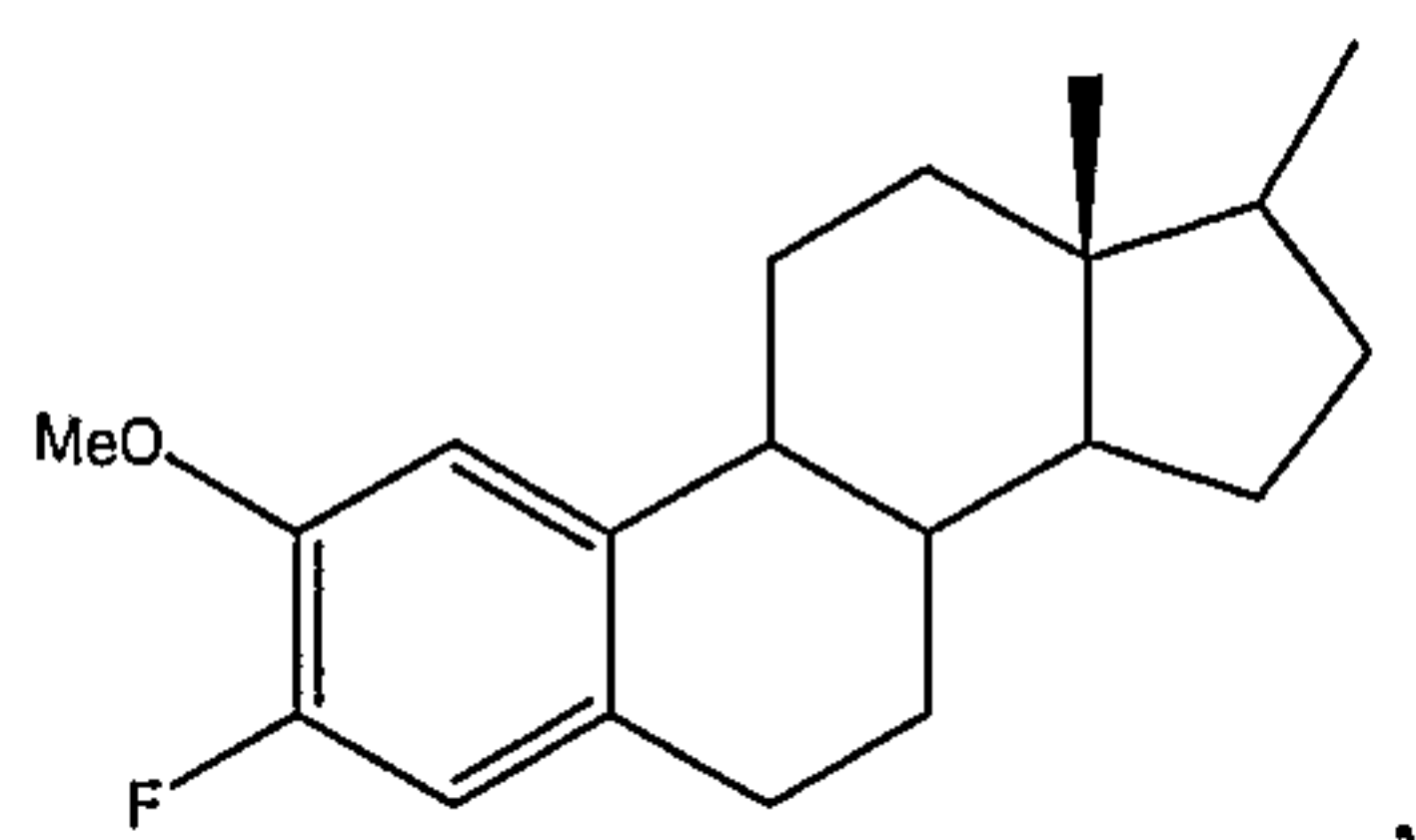




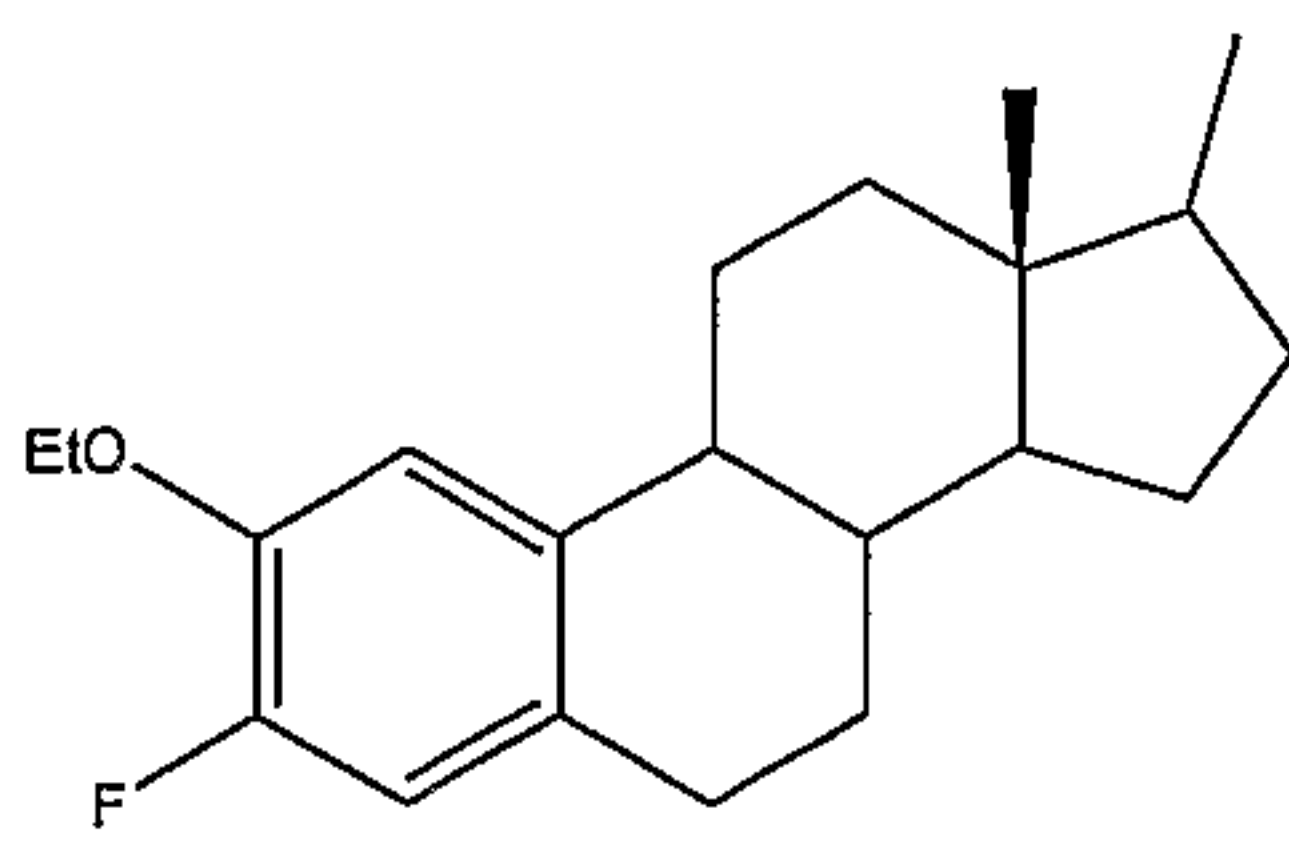


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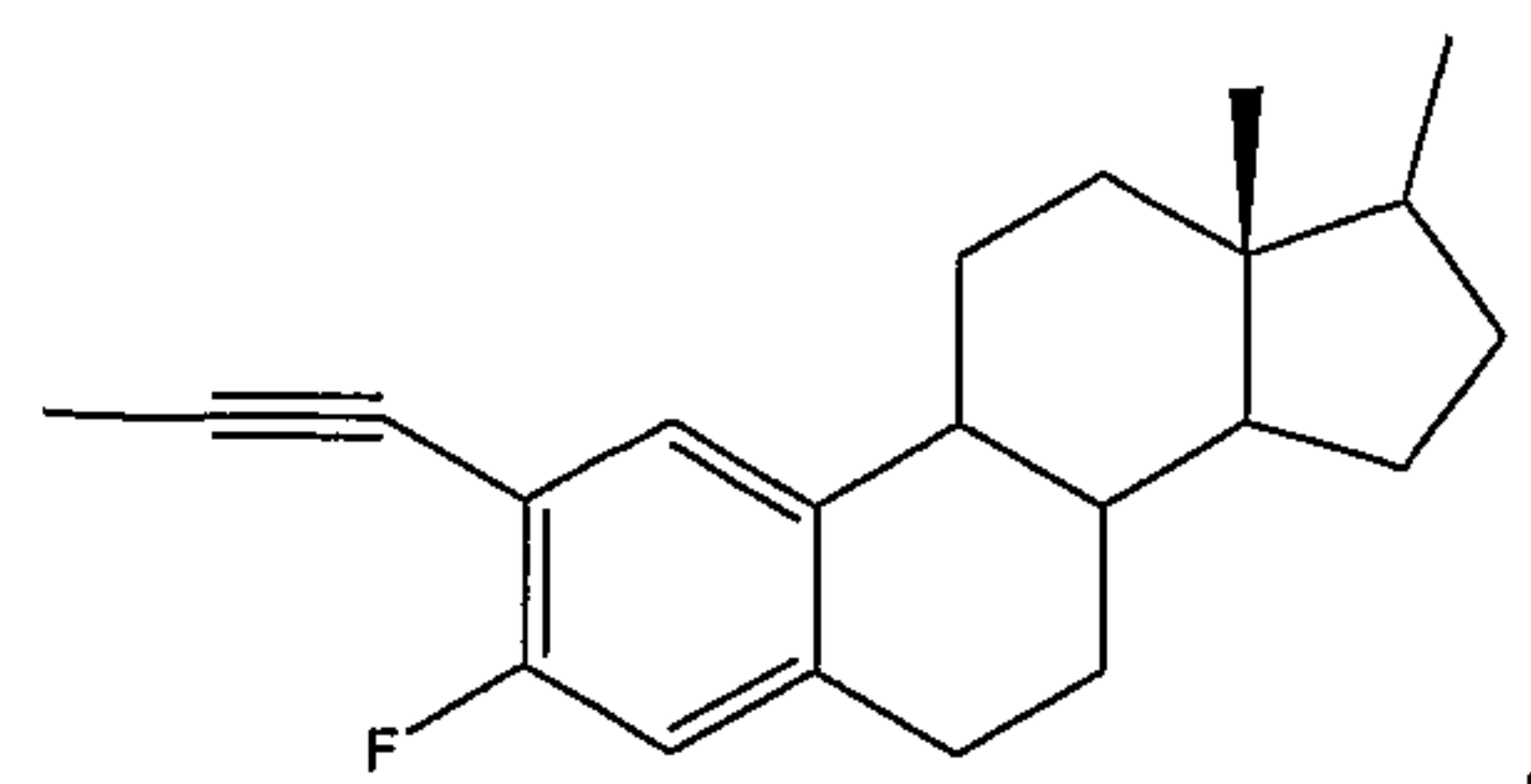
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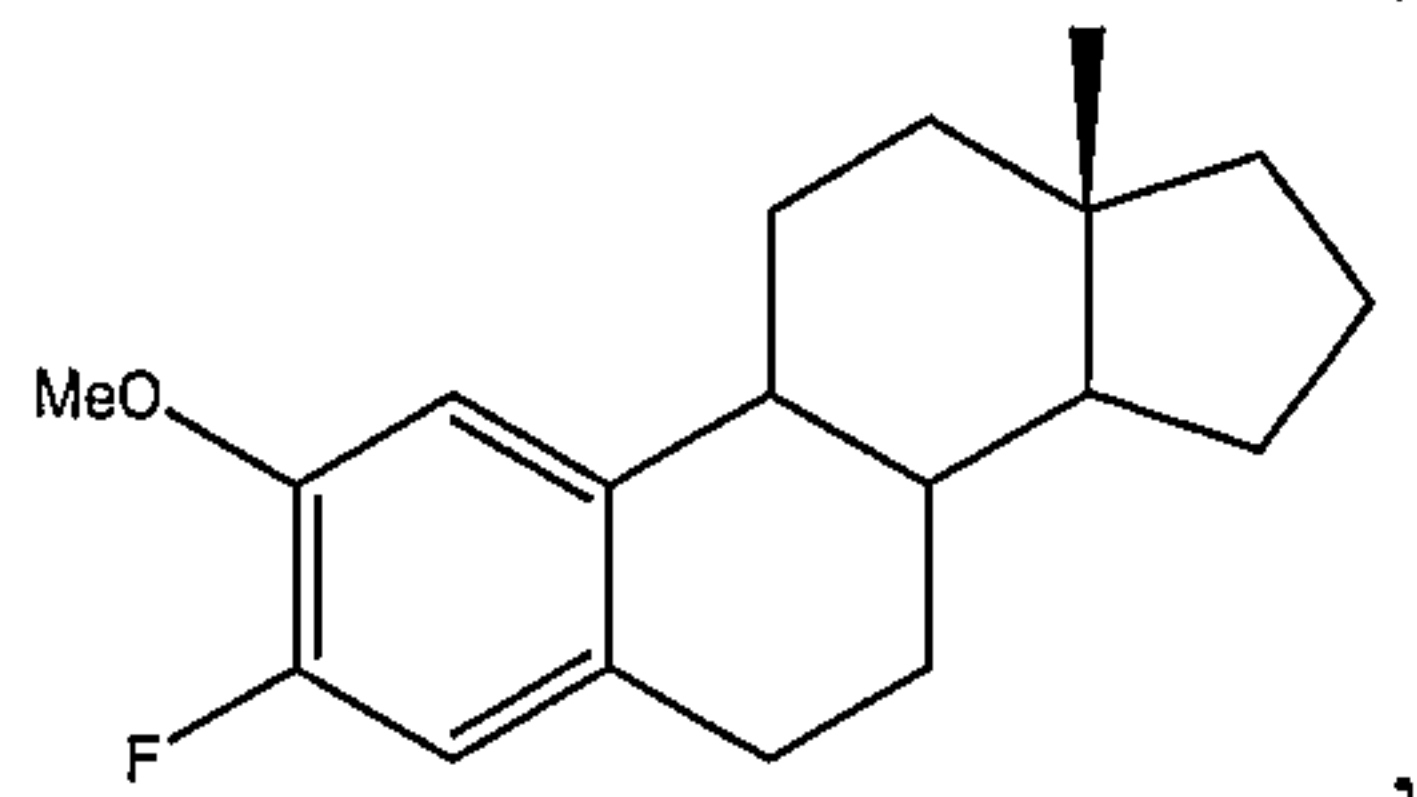
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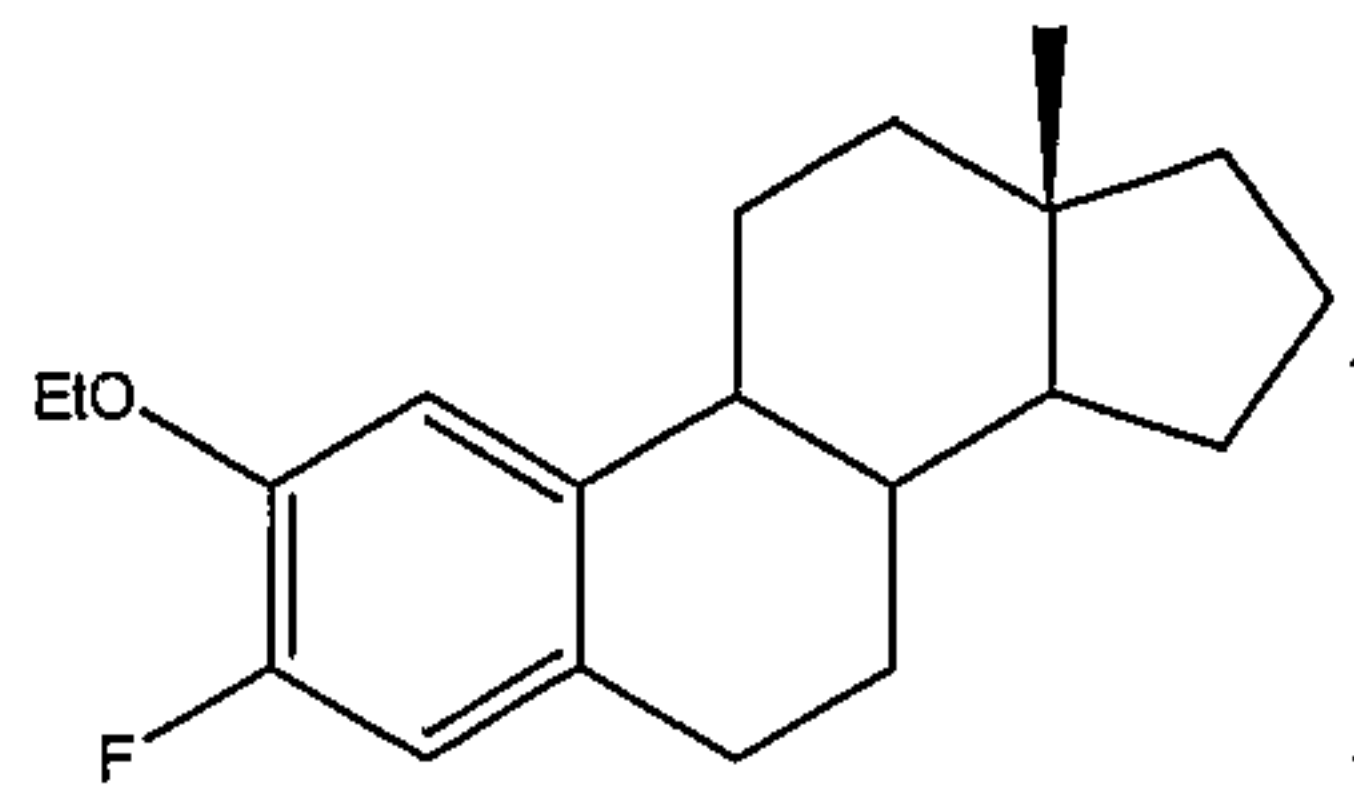
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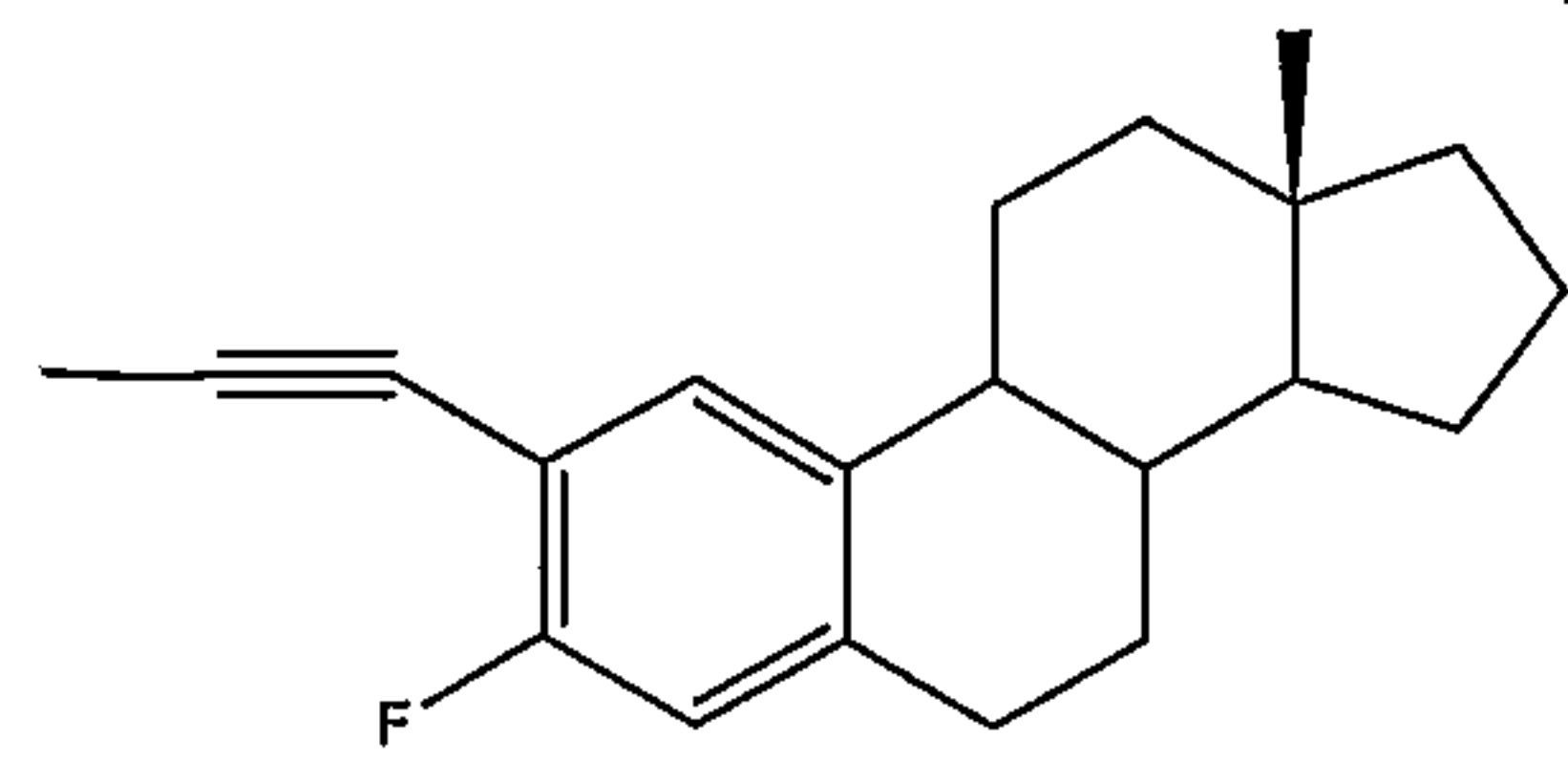
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or



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97. The method of Claim 96, wherein the eye condition is an ocular neovascular disease.
98. The method of Claim 96, wherein the eye condition is diabetic retinopathy.
99. The method of Claim 96, wherein the eye condition is retinopathy of prematurity.
- 10
100. The method of Claim 96, wherein the eye condition is macular degeneration.
101. The method of Claim 96, wherein the eye condition is corneal graft rejection.
- 15
102. The method of Claim 96, wherein the eye condition is neovascular glaucoma.
103. The method of Claim 96, wherein the eye condition is retrolental fibroplasia.
- 20
104. The method of Claim 96, wherein the eye condition is epidemic keratoconjunctivitis.
105. The method of Claim 96, wherein the eye condition is due to contact lens overwear.
- 25
106. The method of Claim 96, wherein the eye condition is atopic keratitis.
107. The method of Claim 96, wherein the eye condition is superior limbic keratitis.
- 30
108. The method of Claim 96, wherein the eye condition is pterygium keratitis sicca.

109. The method of Claim 96, wherein the eye condition is myopia.

110. The method of Claim 96, wherein the eye condition is chronic retinal detachment.

5

111. The method of Claim 96, wherein the eye condition is optic pits.

112. The method of Claim 96, wherein the eye condition is Terrien's marginal degeneration.

10

113. The method of Claim 96, wherein the eye condition is hyperviscosity syndromes.

114. The method of Claim 96, wherein the eye condition is chronic uveitis.

15

115. The method of Claim 96, wherein the eye condition is chronic vitritis.

116. The method of Claim 96, wherein the eye condition is presumed ocular histoplasmosis.

20

117. The method of Claim 96, wherein the eye condition is retinitis.

118. The method of Claim 96, wherein the eye condition is choroiditis.

25

119. The method of Claim 96, wherein the eye condition is proliferative vitreoretinopathy.

120. The method of Claim 96, wherein the eye condition is scleritis.

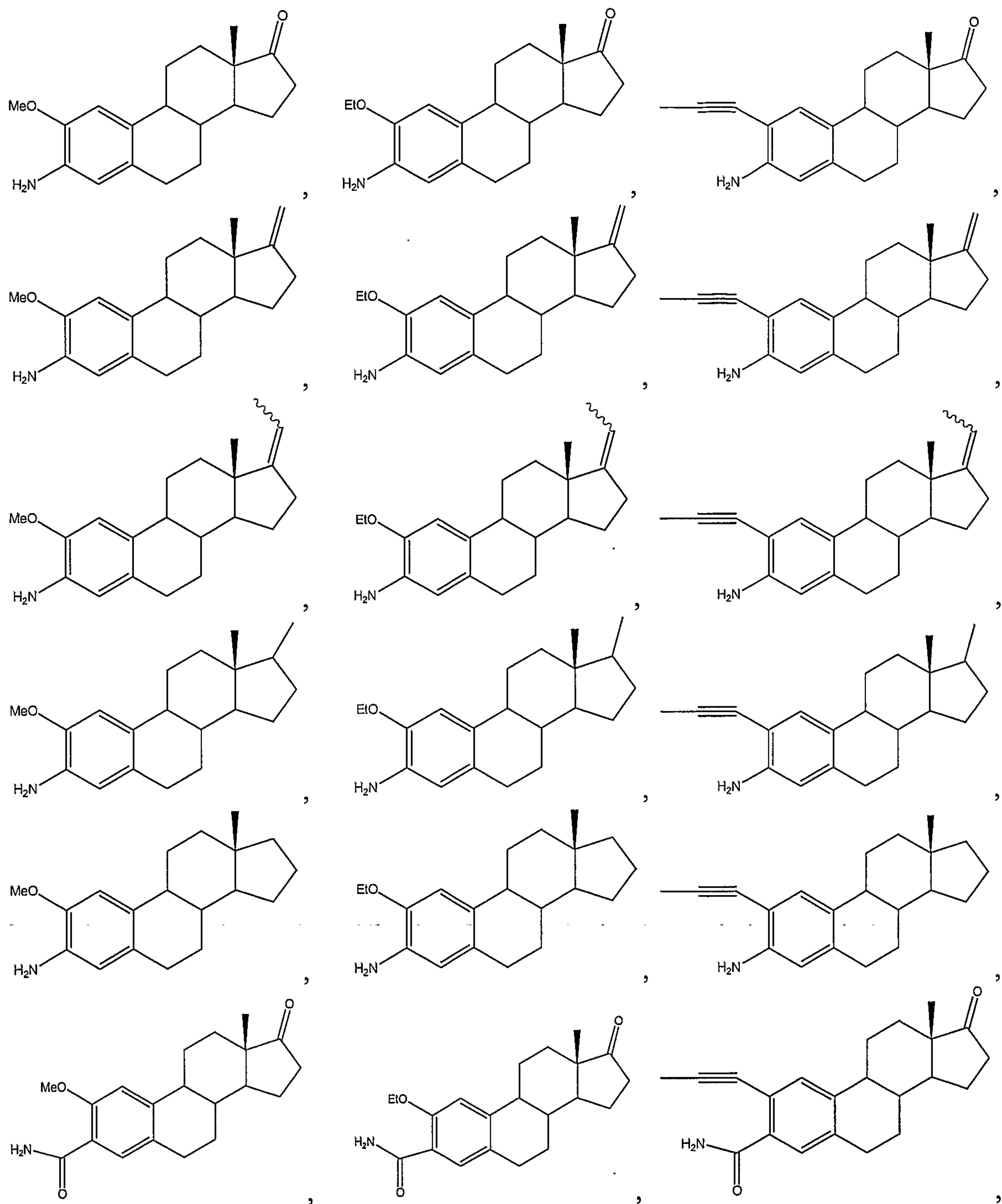
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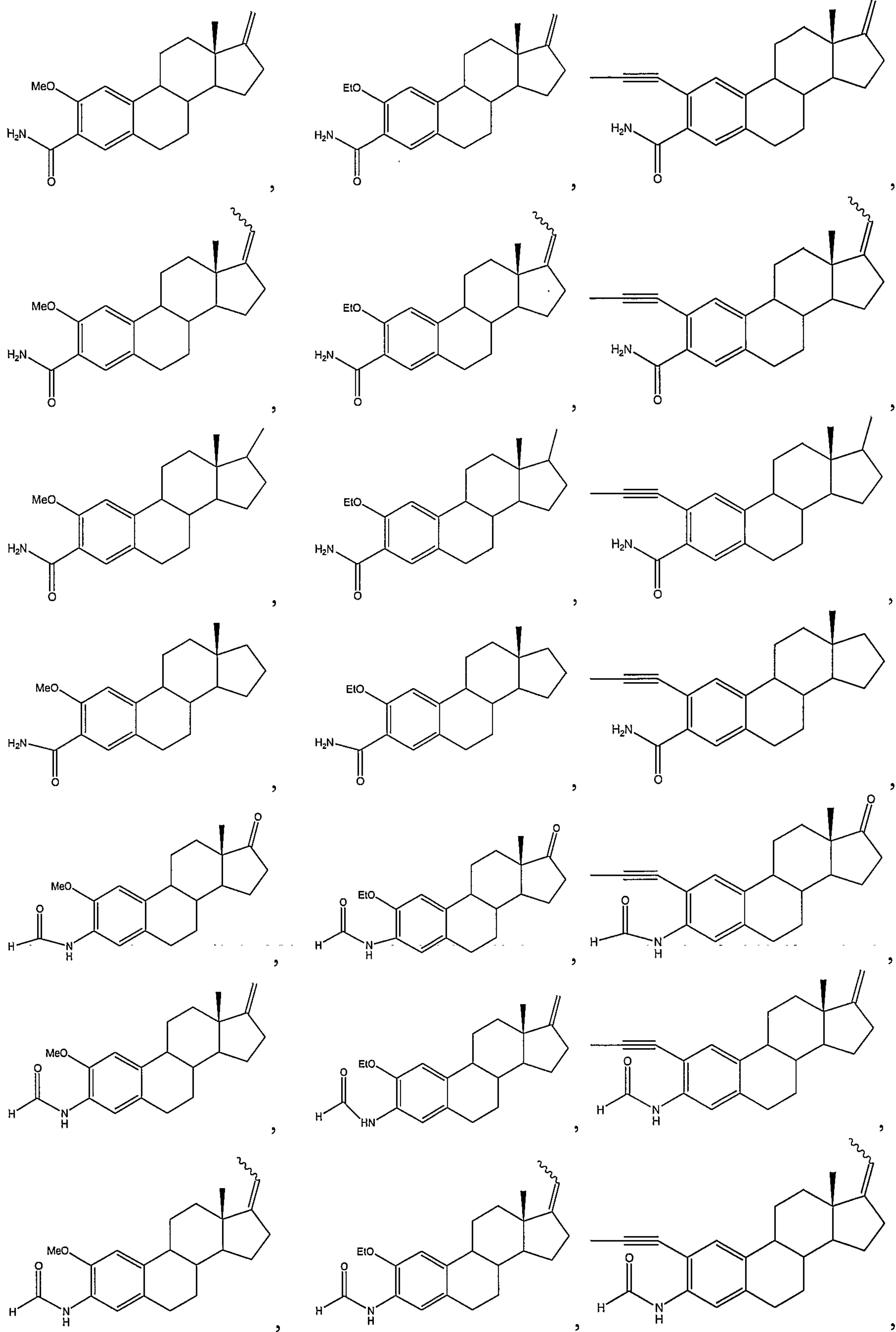
121. The method of Claim 96, wherein the eye condition is Eales' disease.

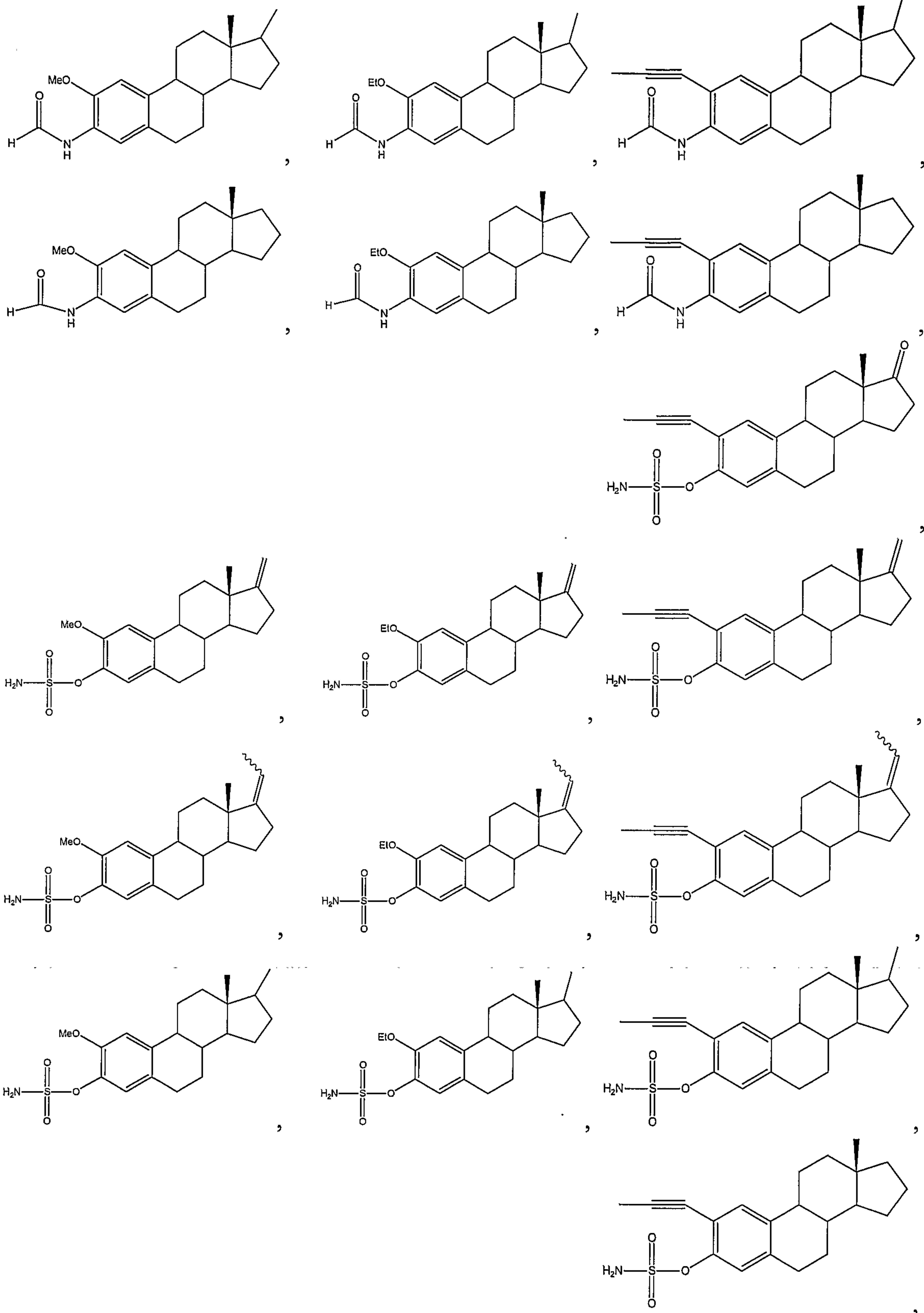
122. The method of Claim 96, wherein the eye condition is Best's disease.

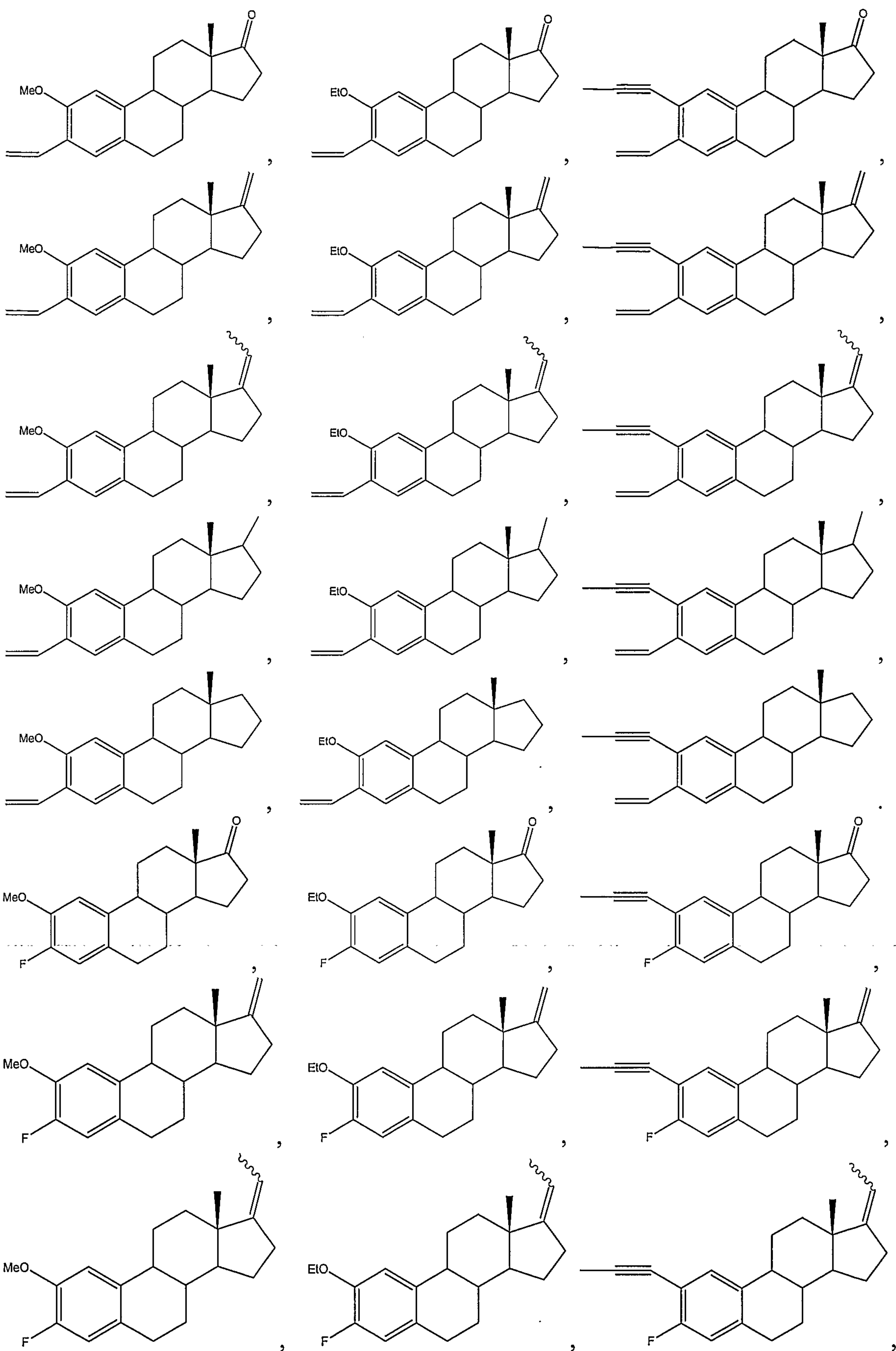
123. The method of Claim 96, wherein the eye condition is trachoma.
124. The method of Claim 96, wherein the eye condition is due to post-laser complications.

125. A method of treating an inflammatory or immune mediated disease in a human or an animal comprising administering to the human or animal a compound selected from



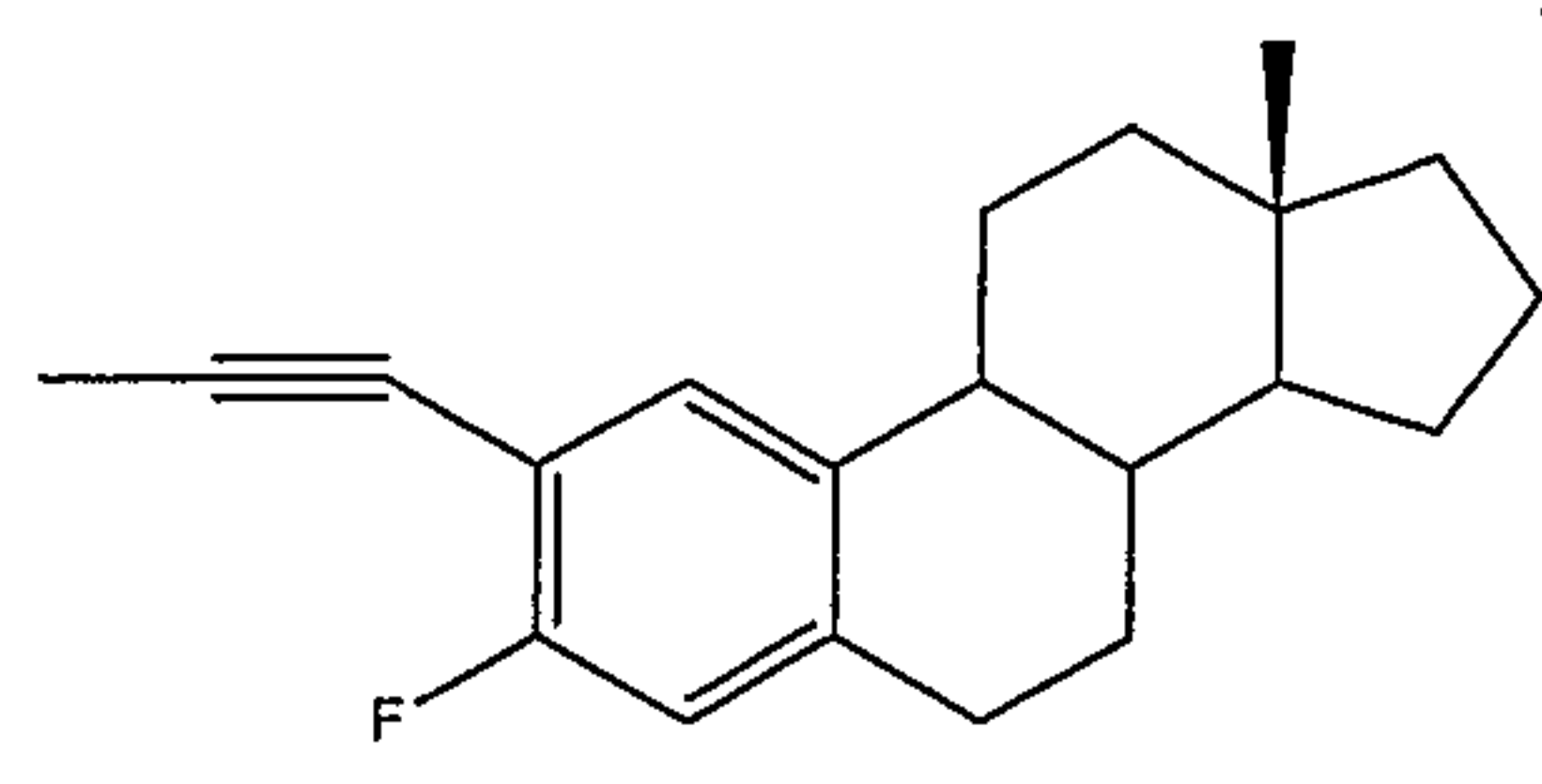
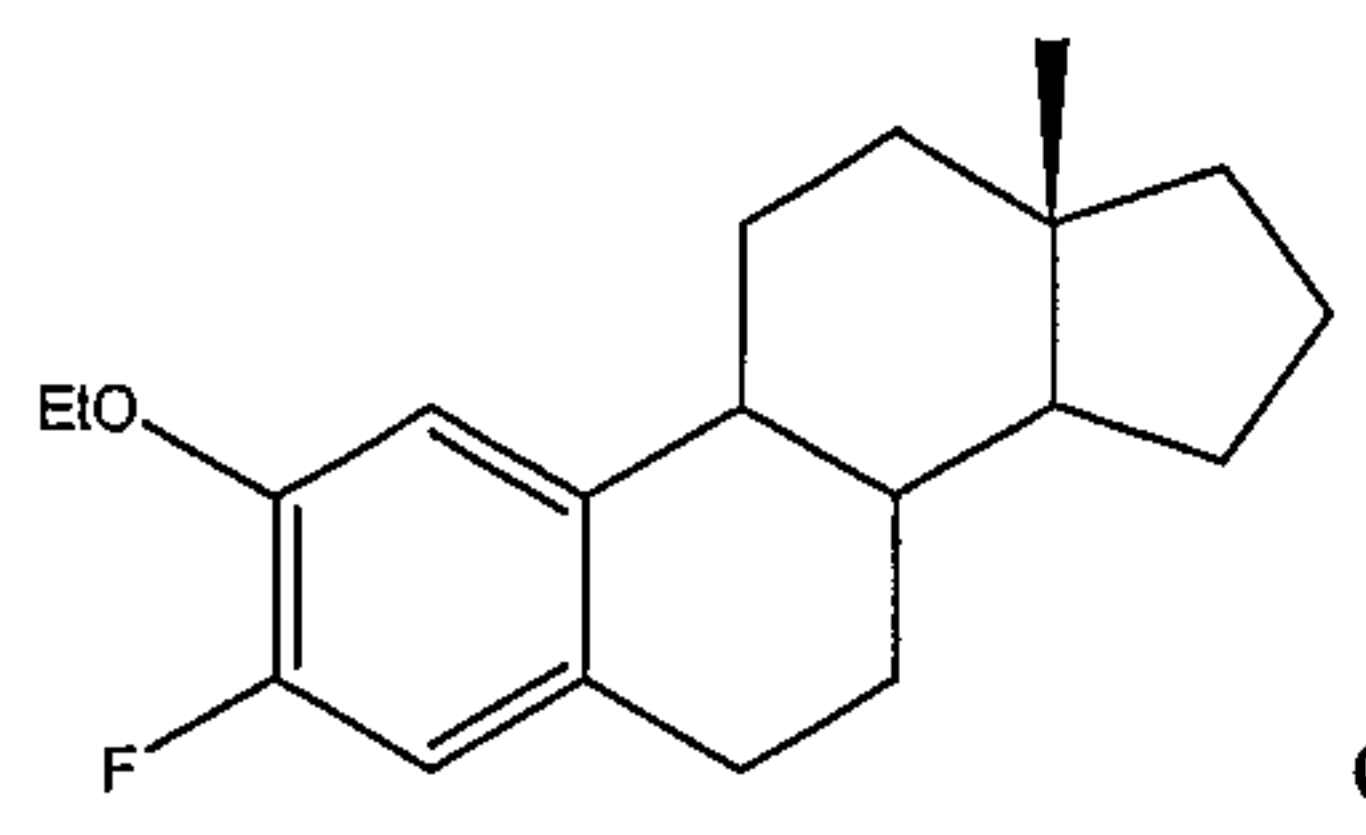
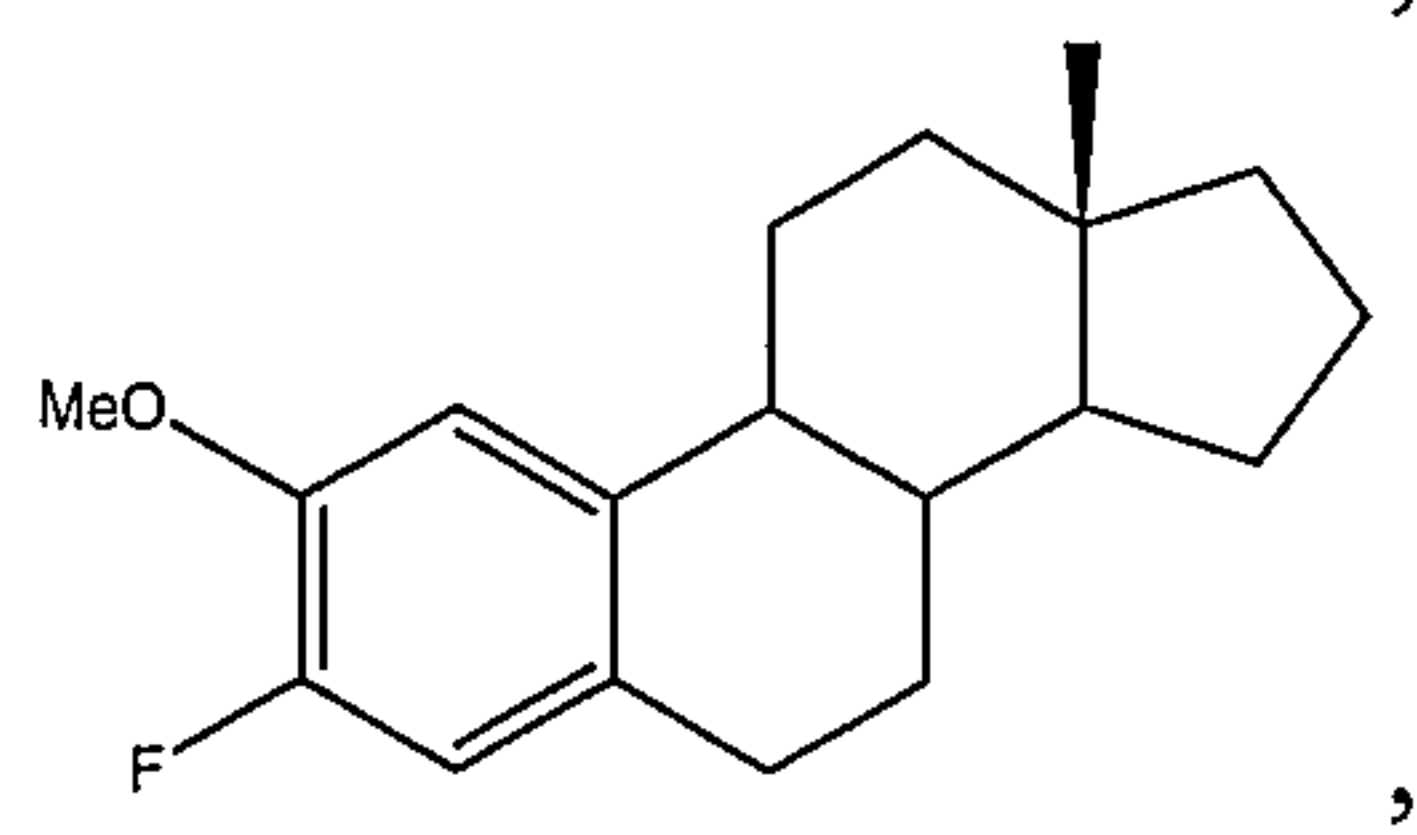
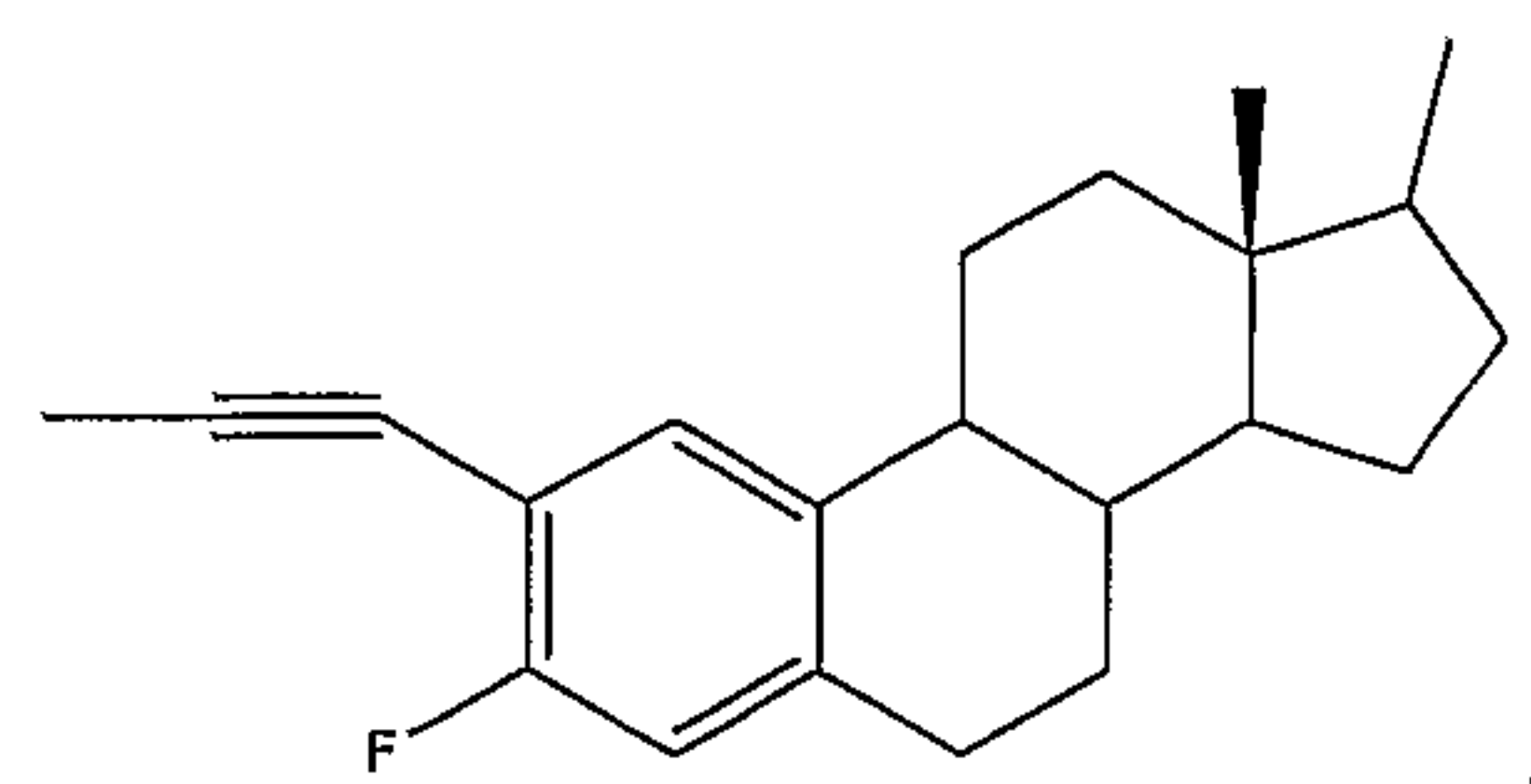
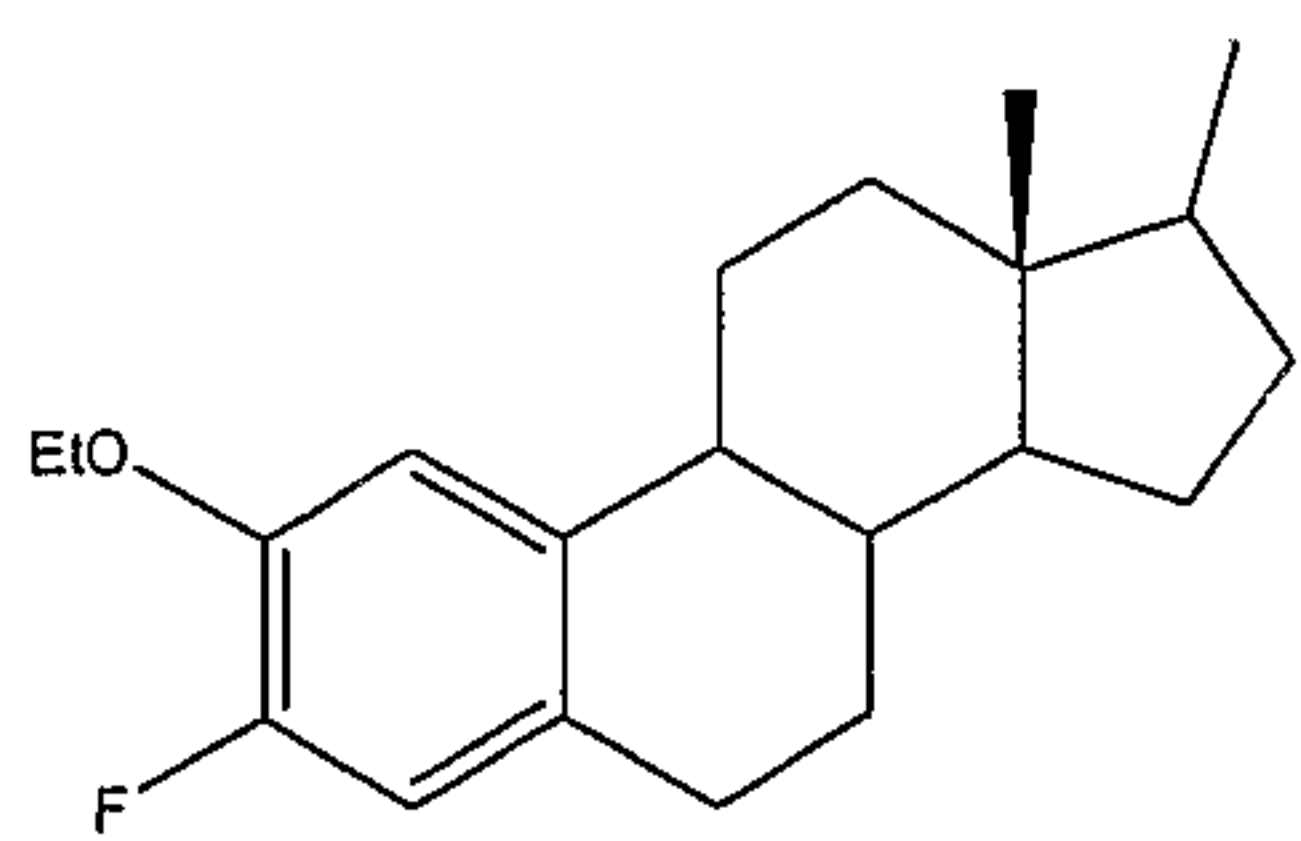
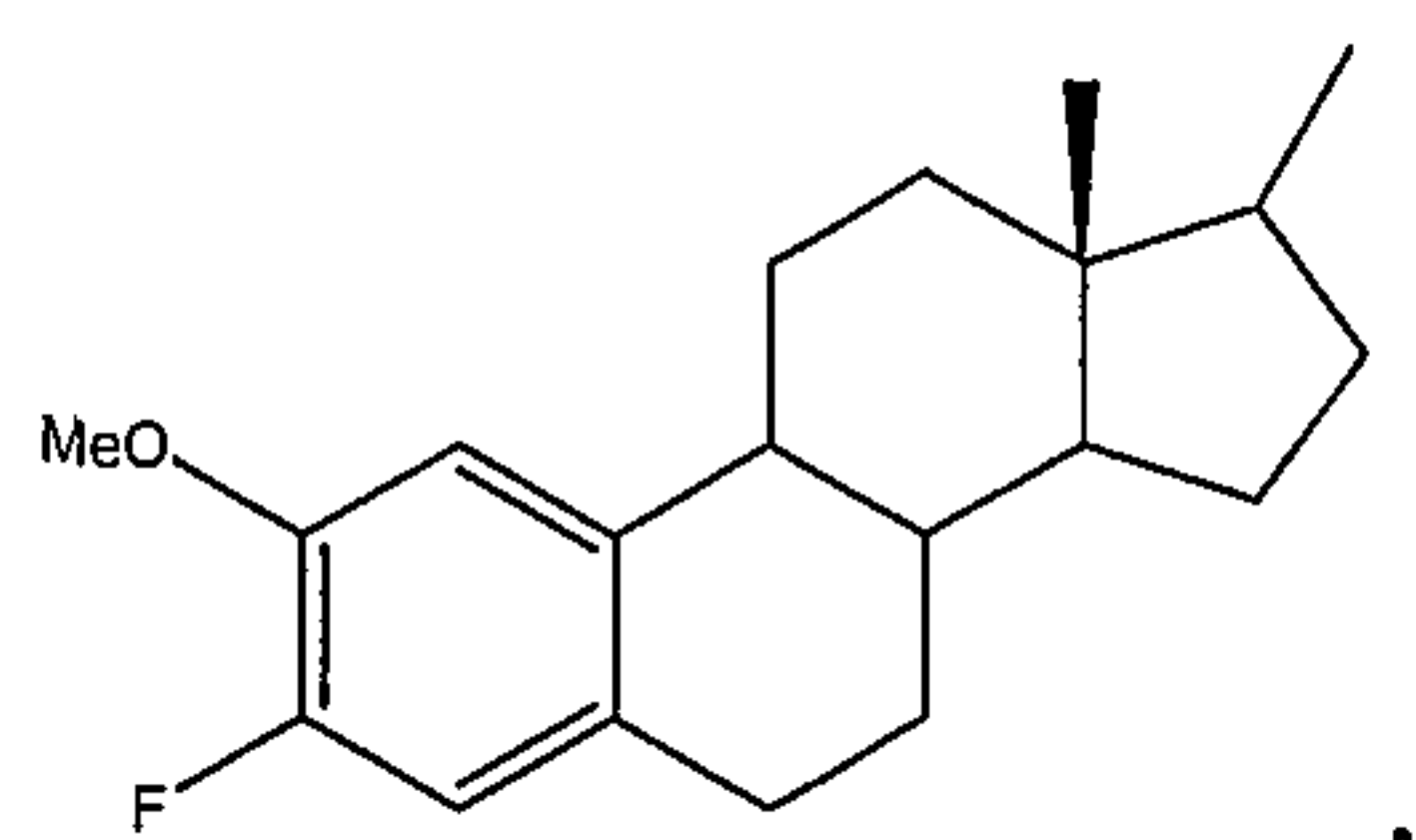






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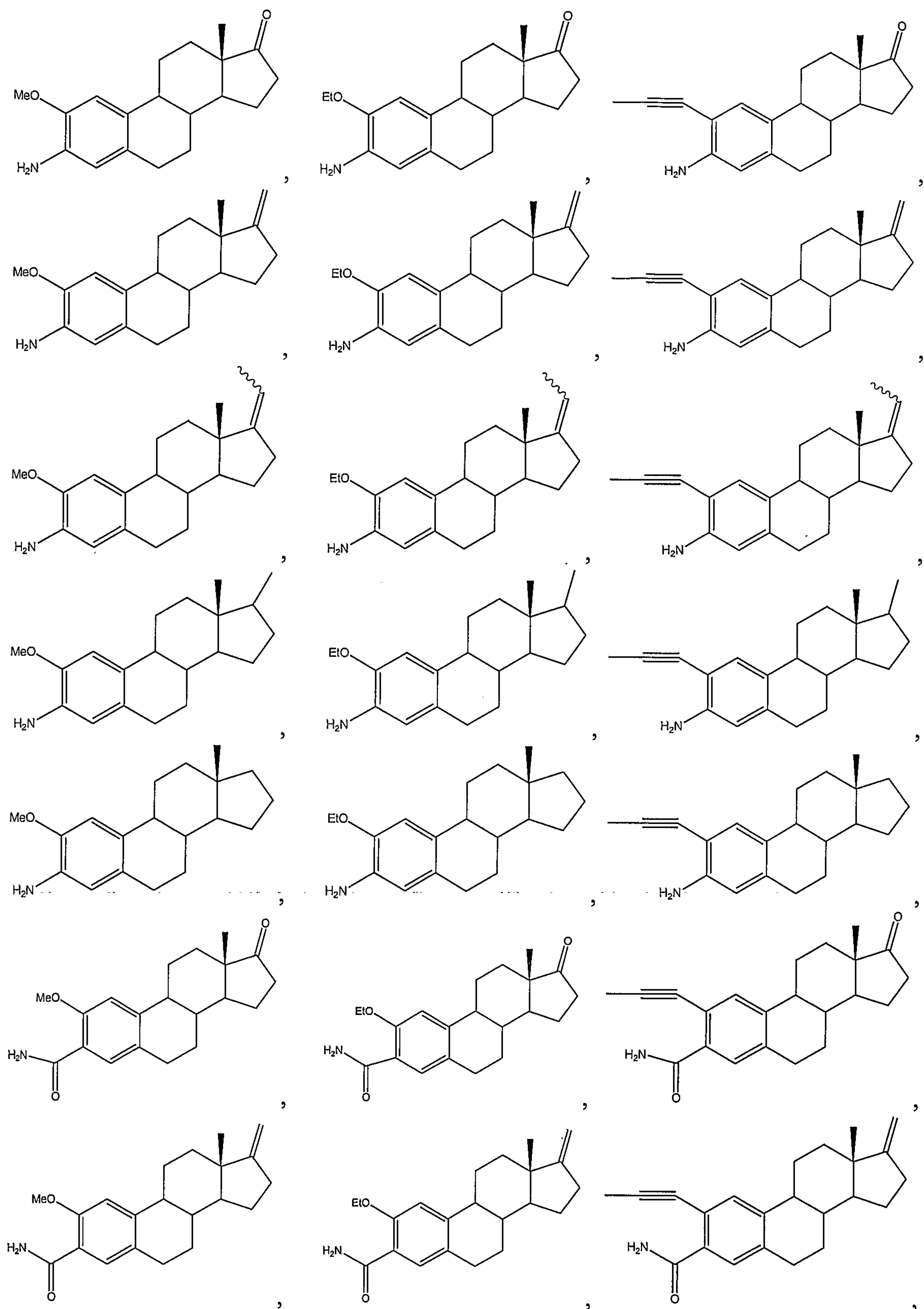
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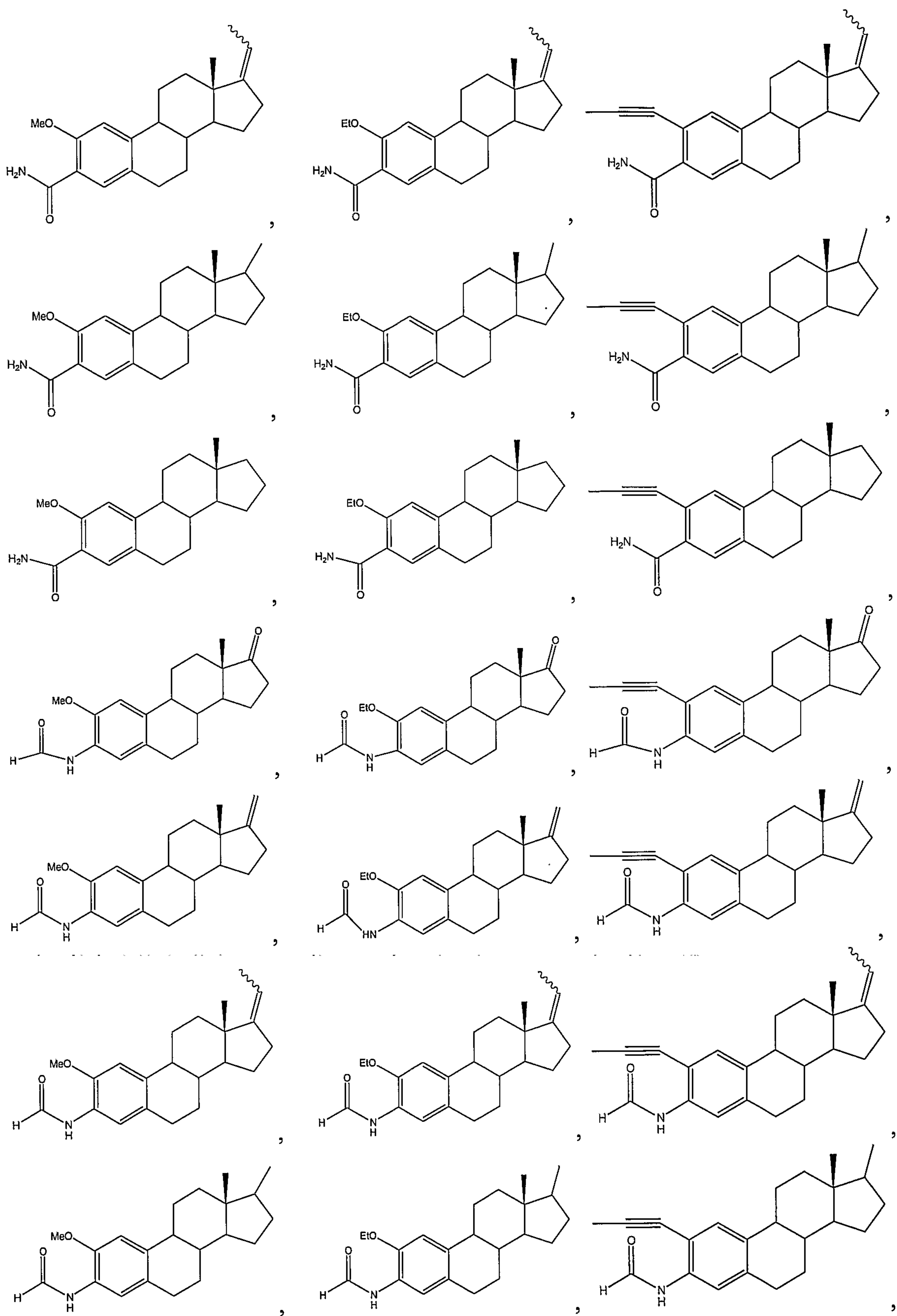


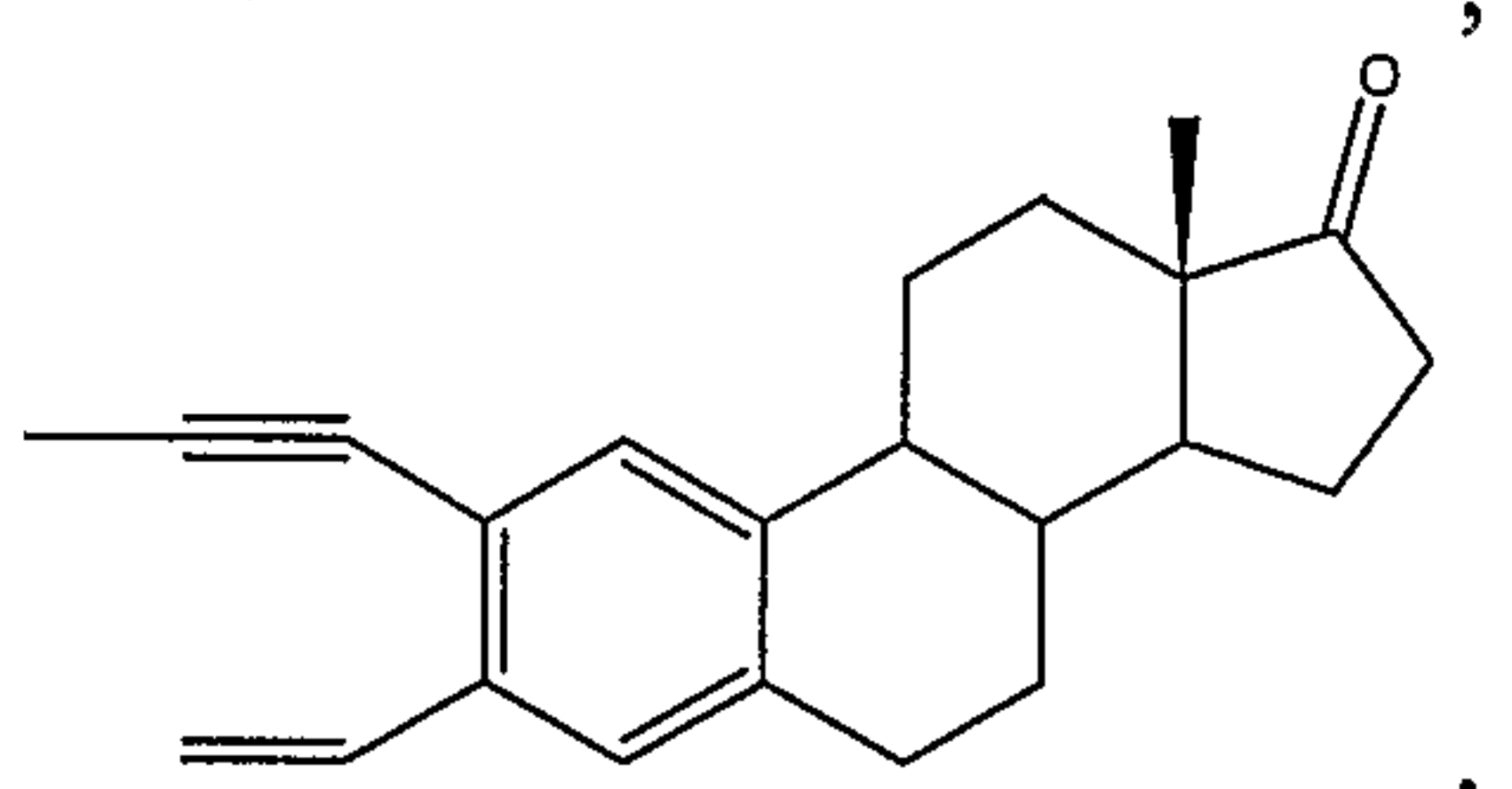
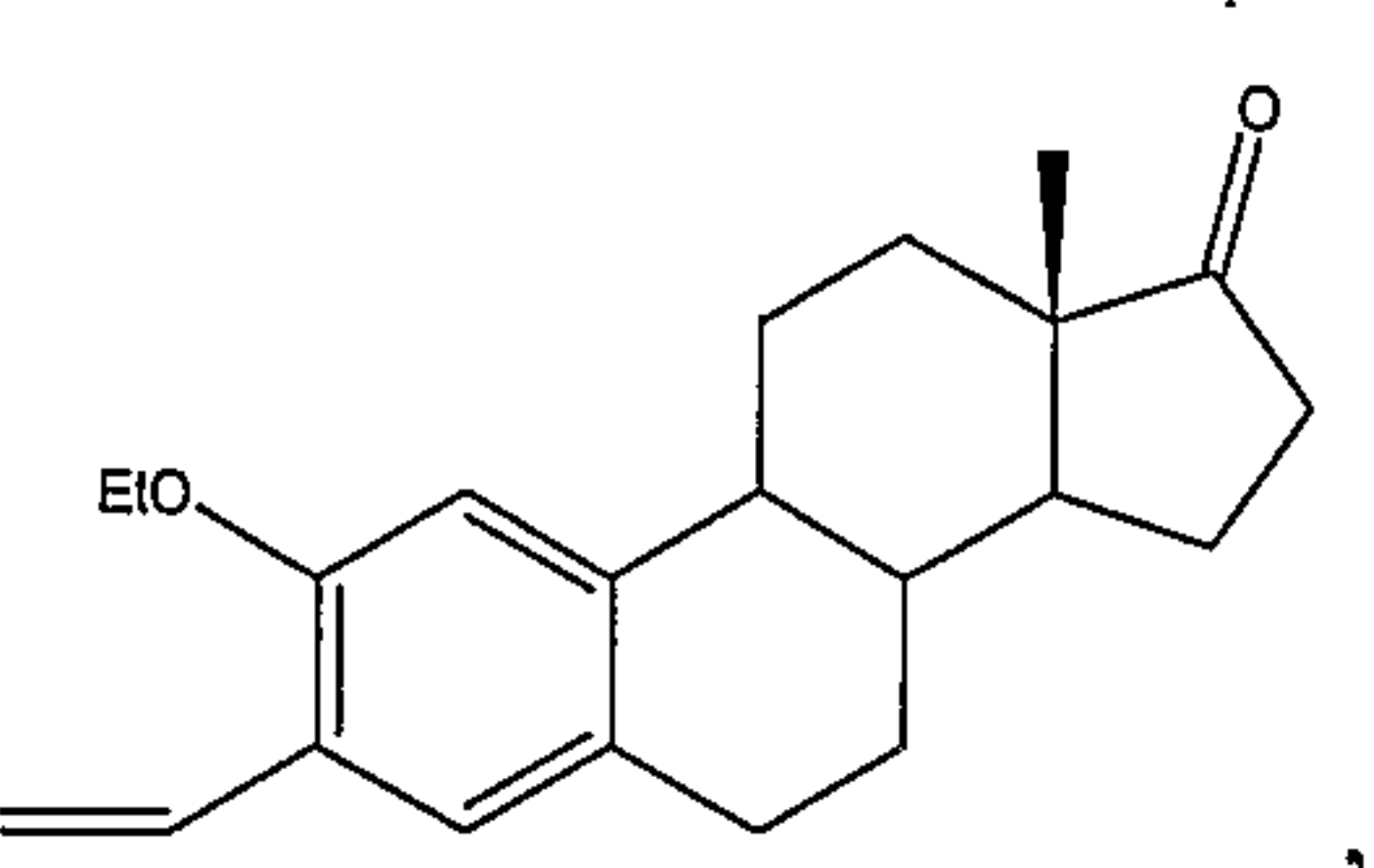
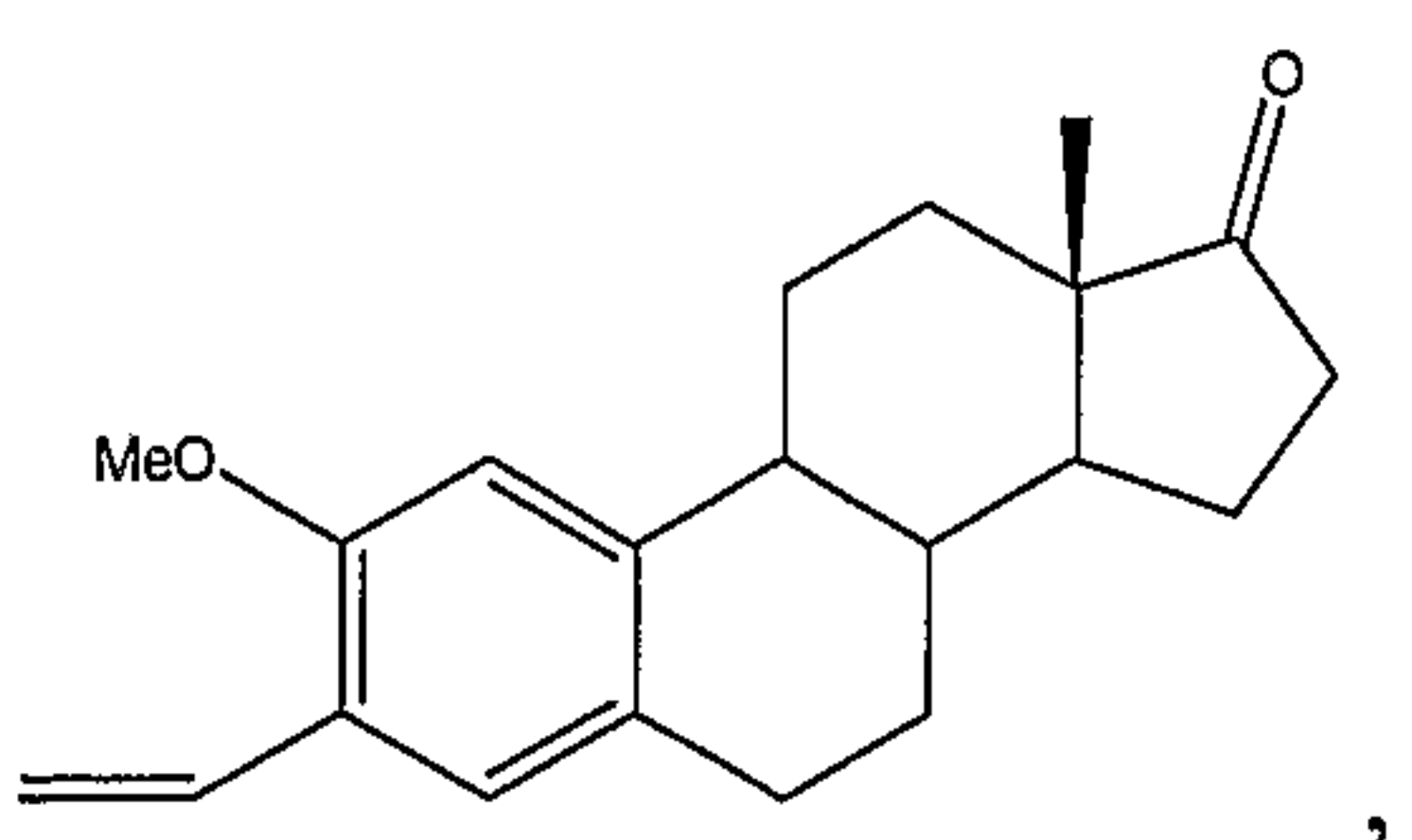
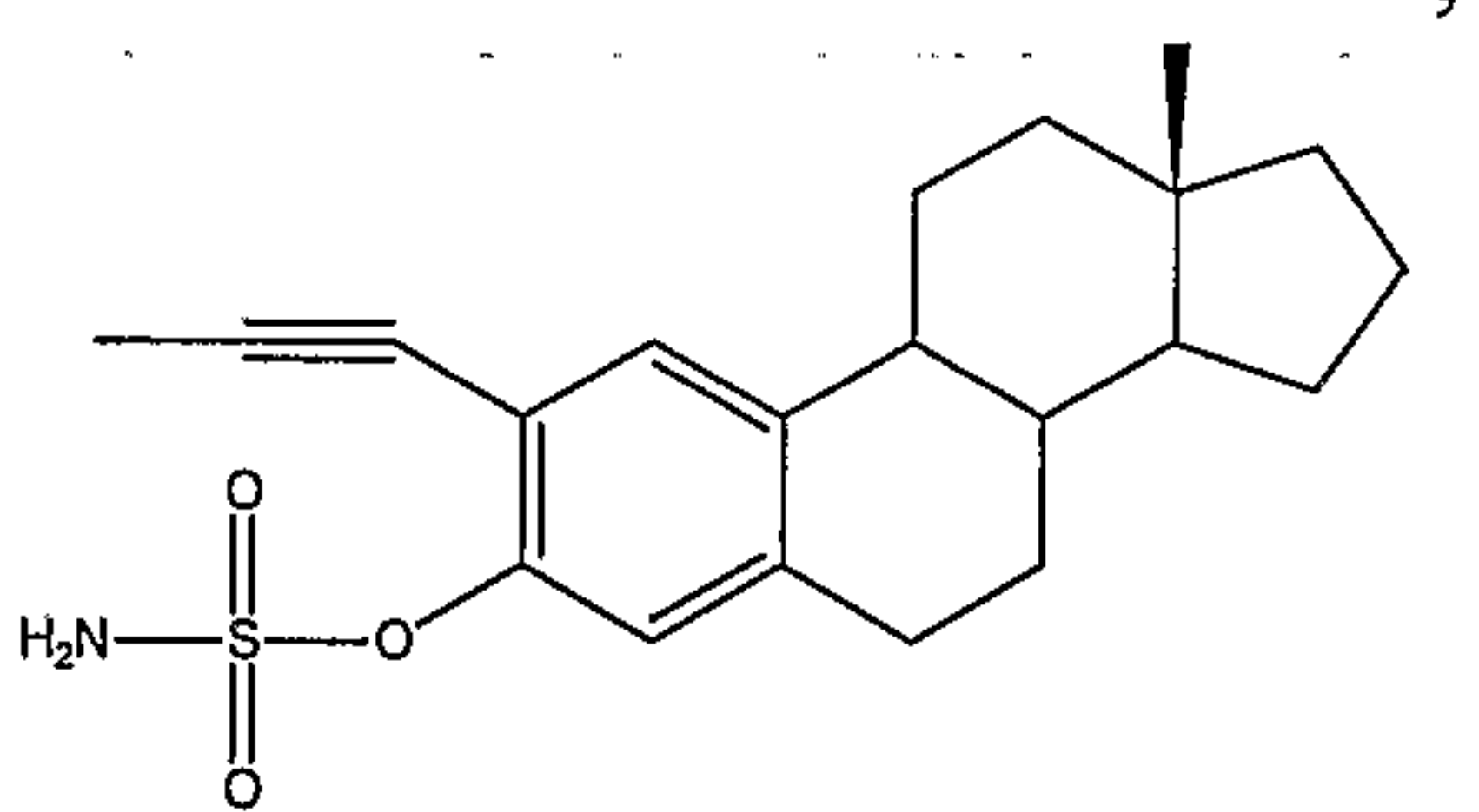
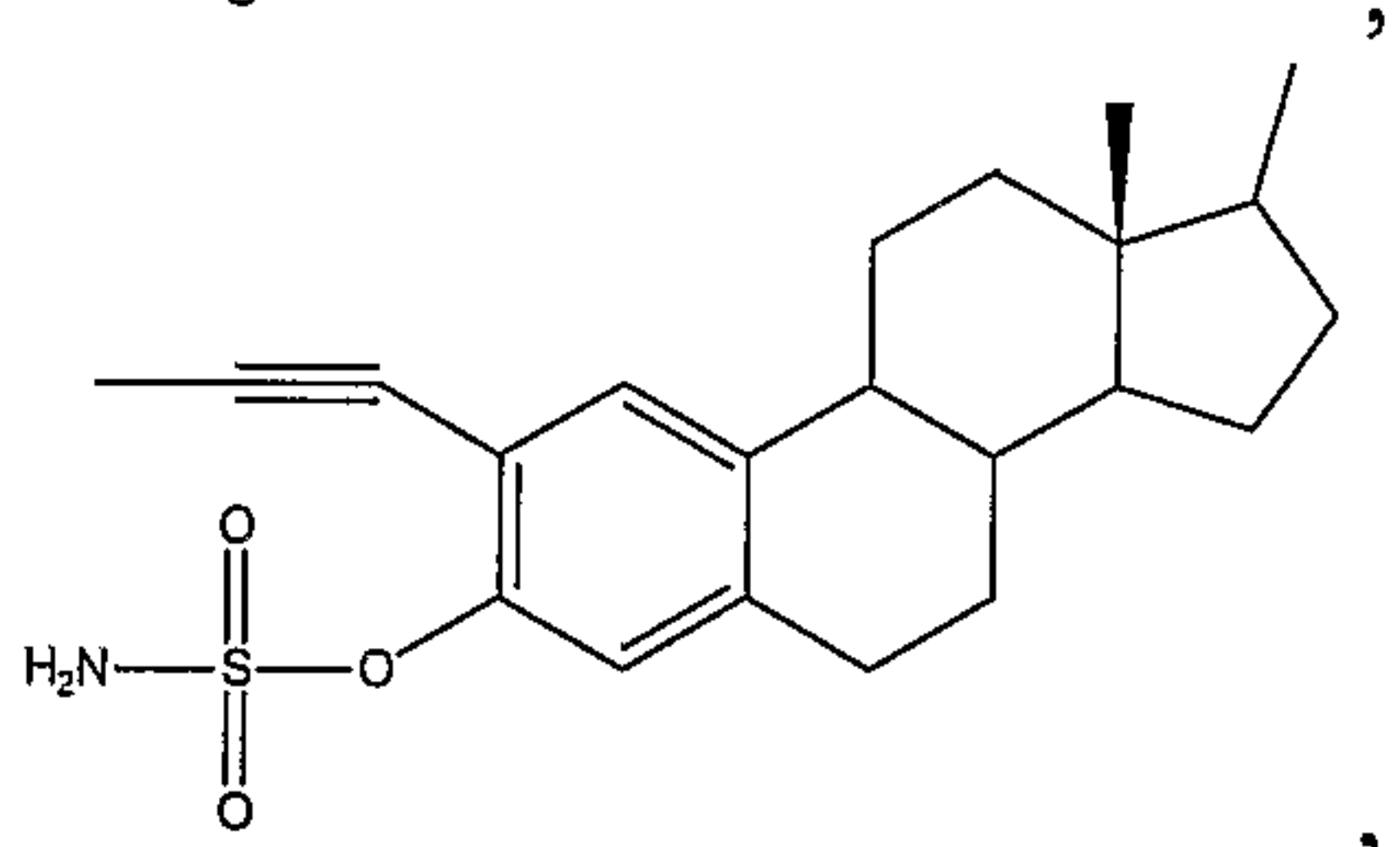
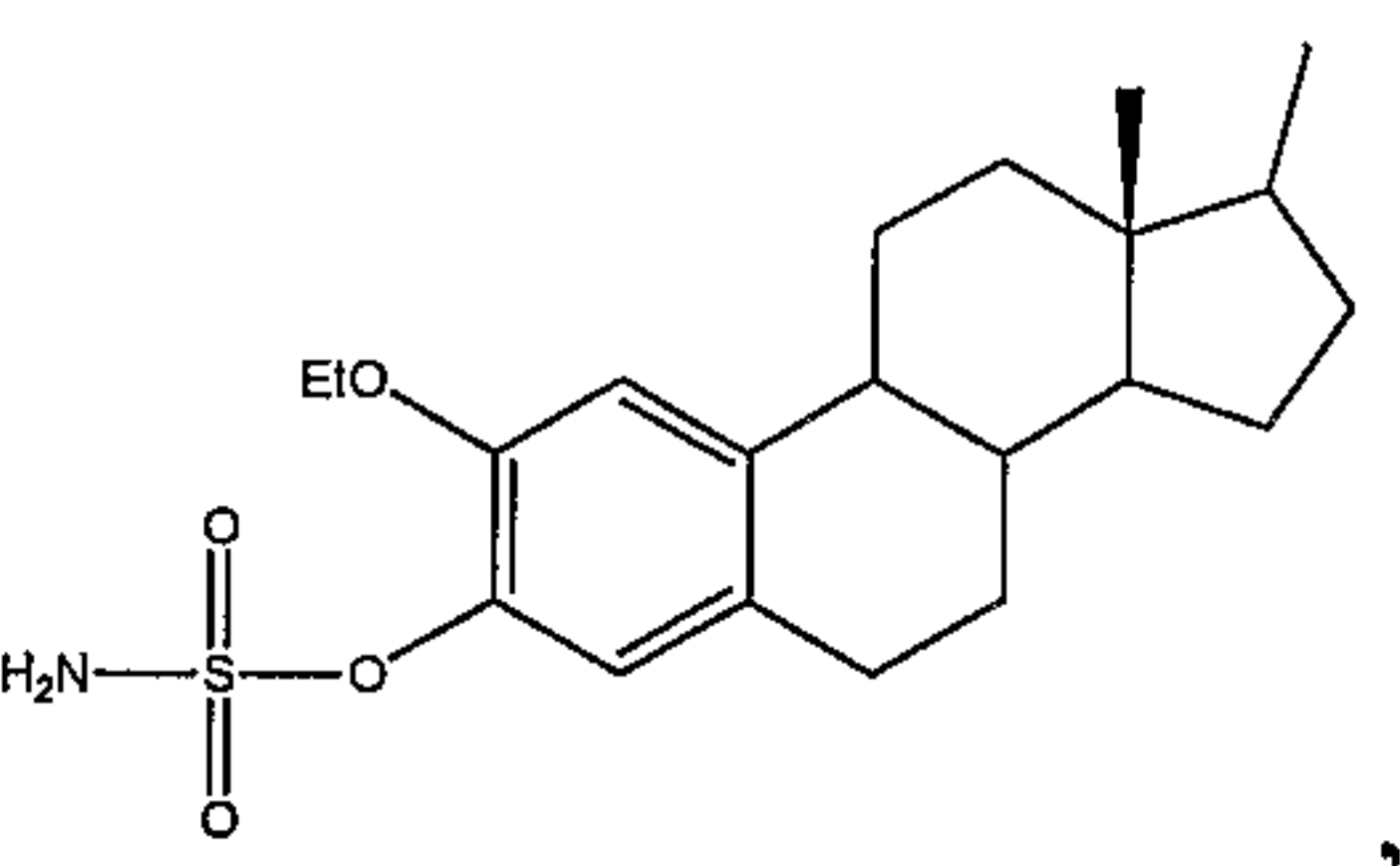
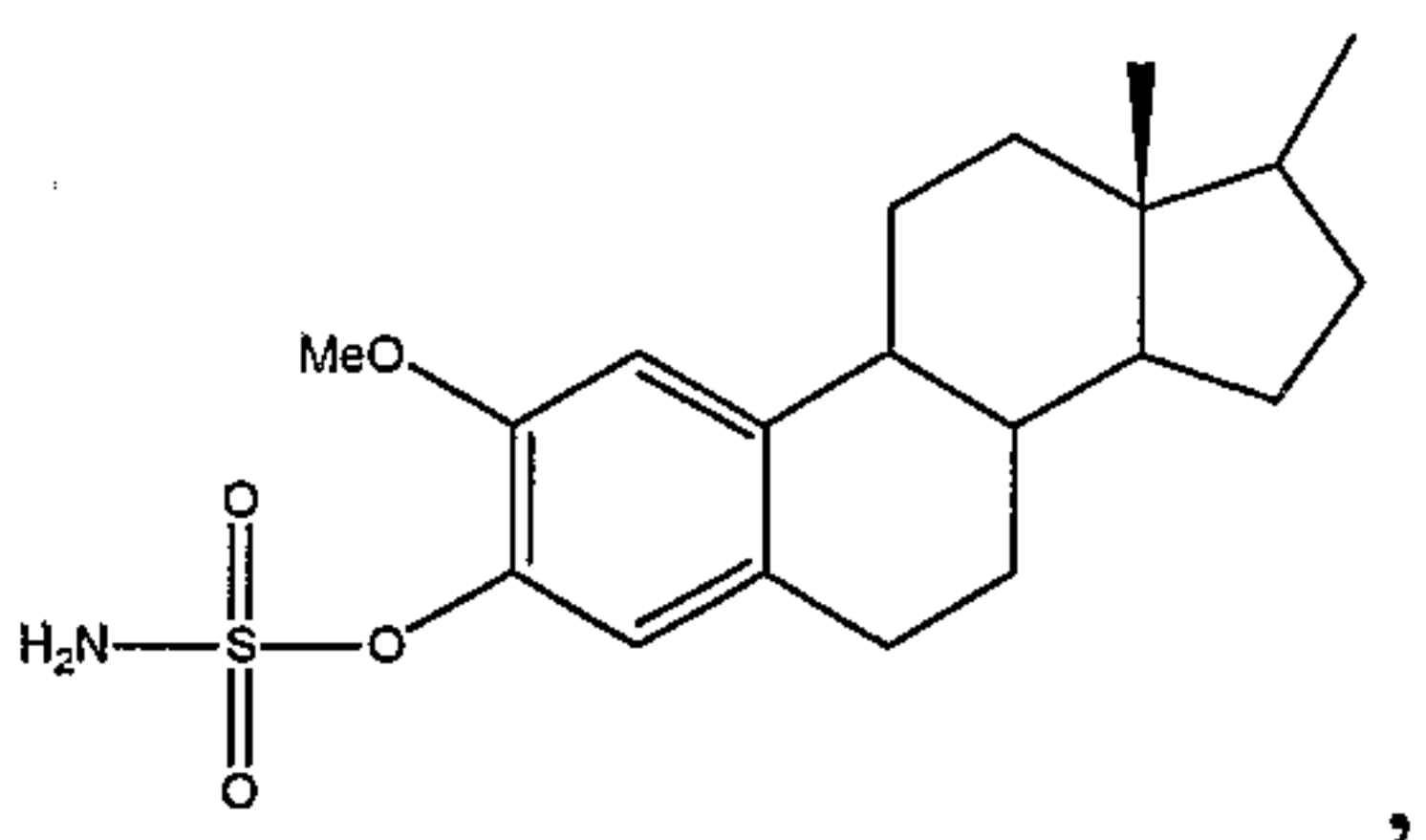
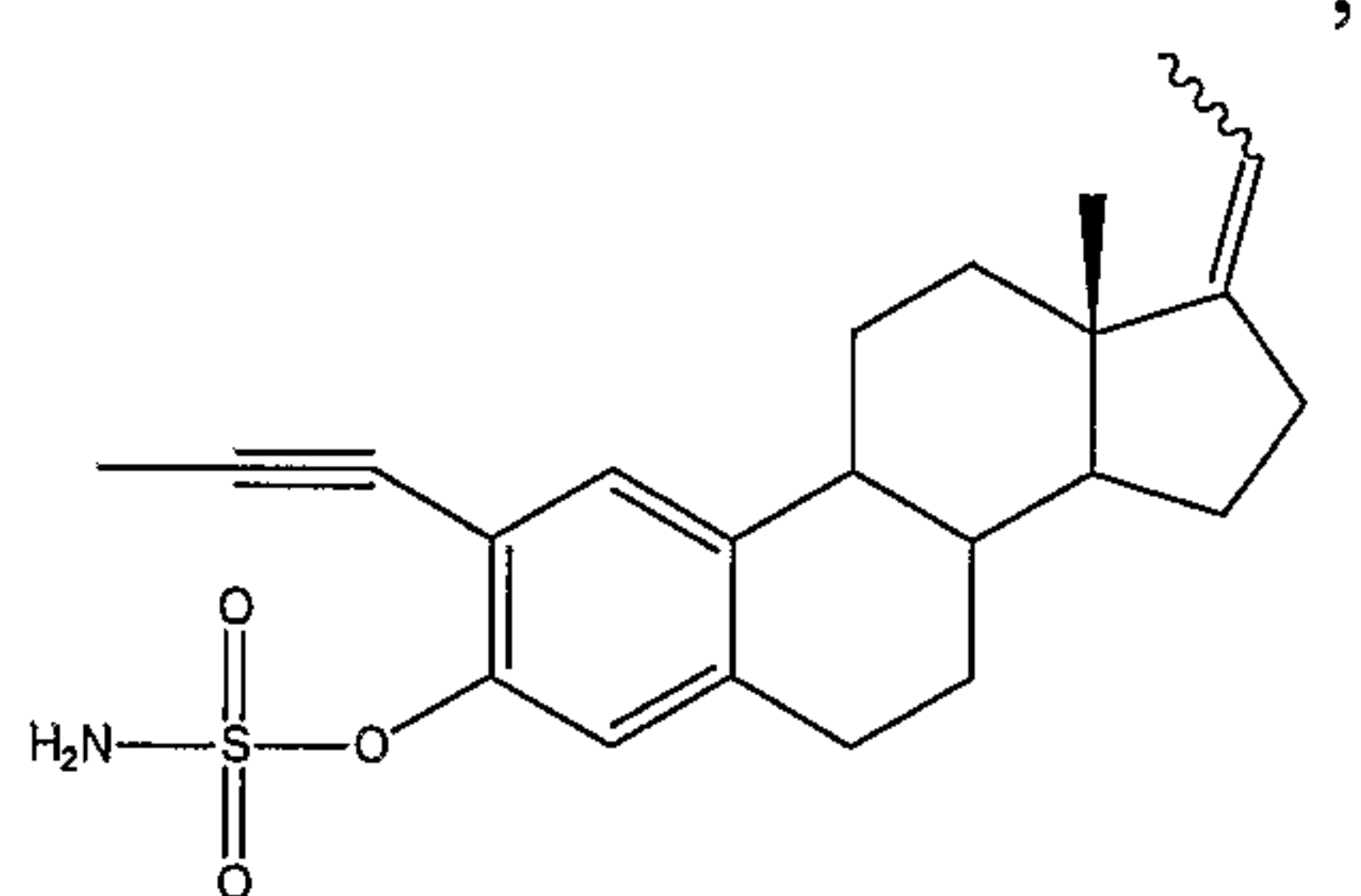
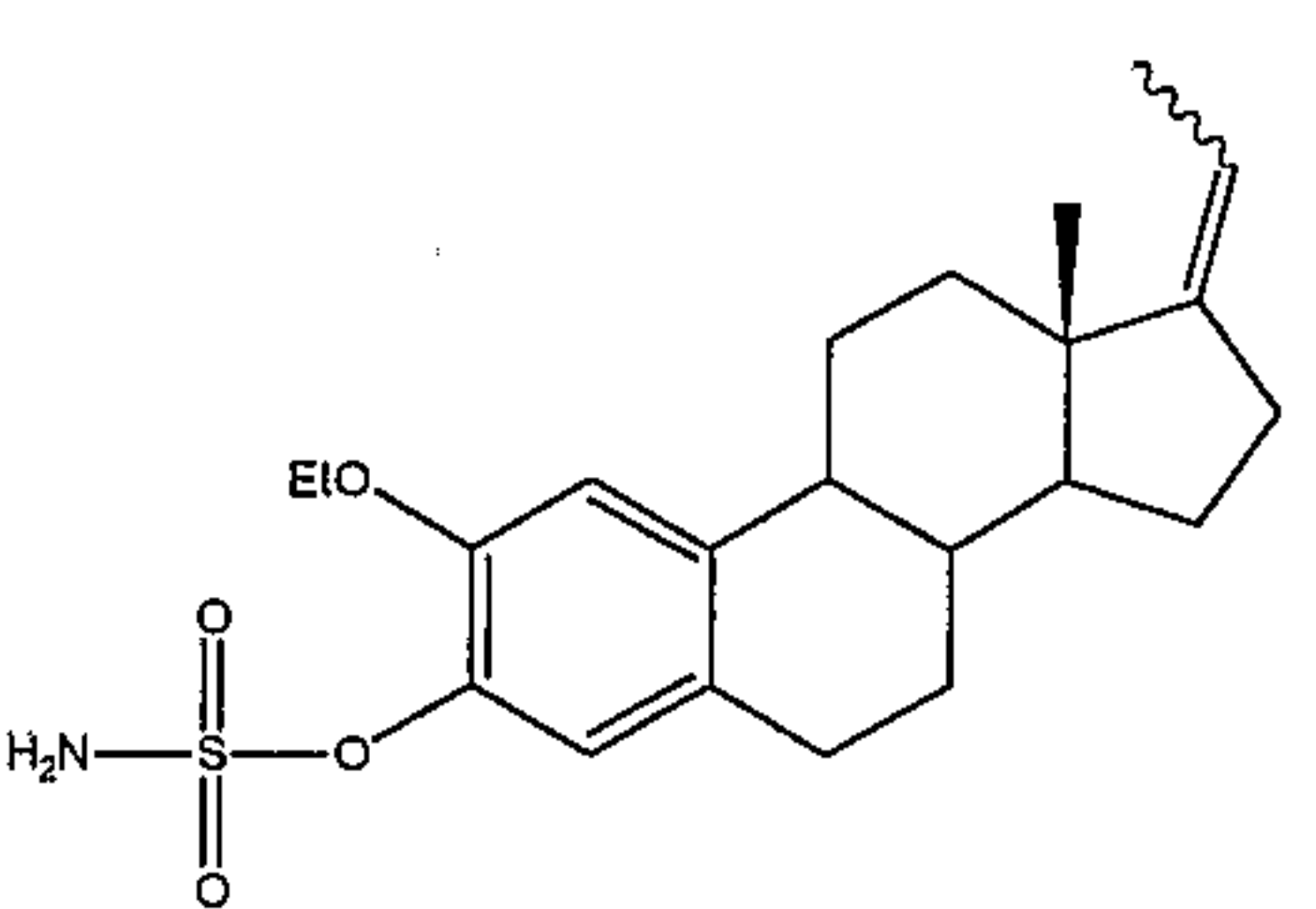
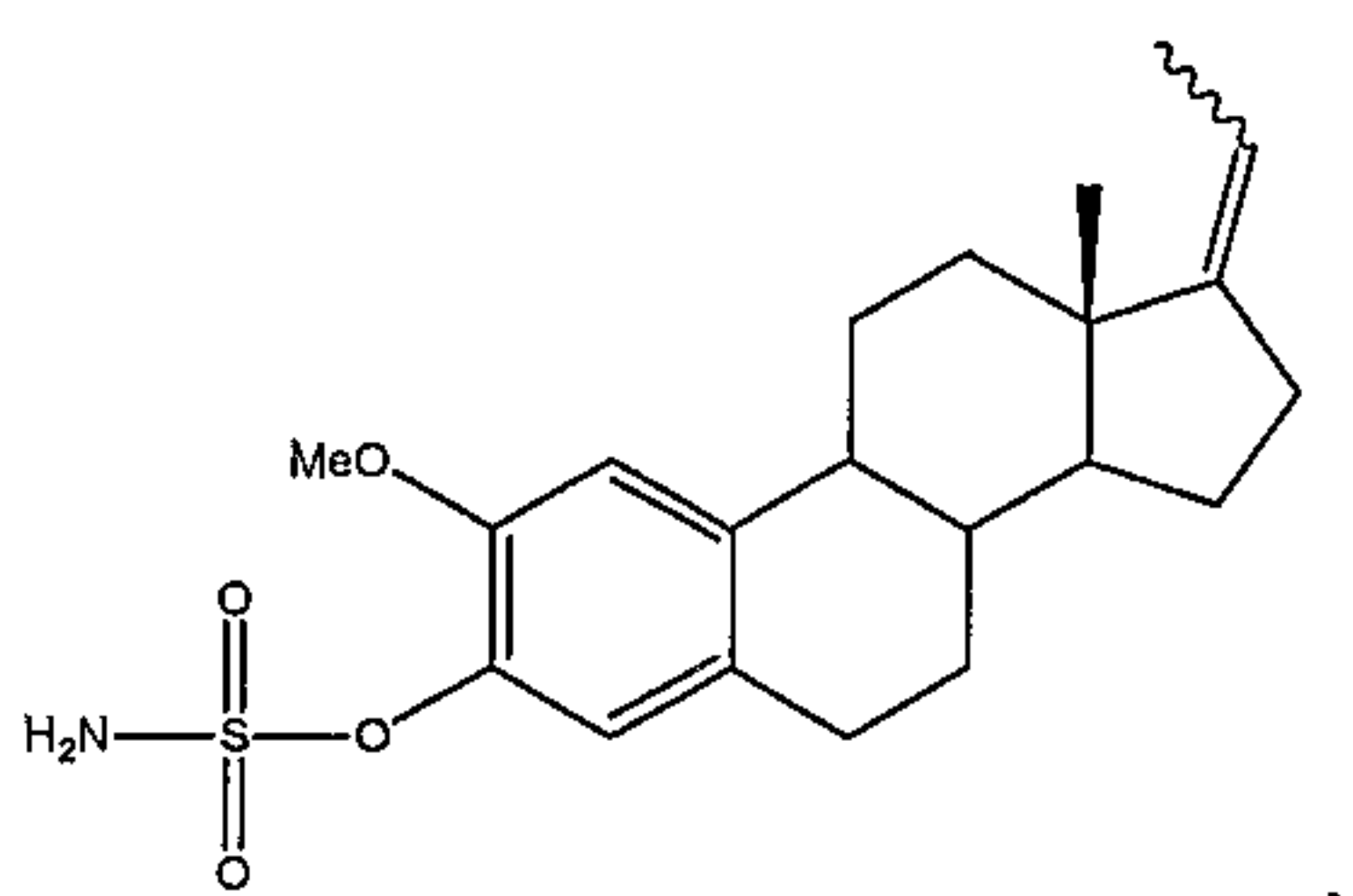
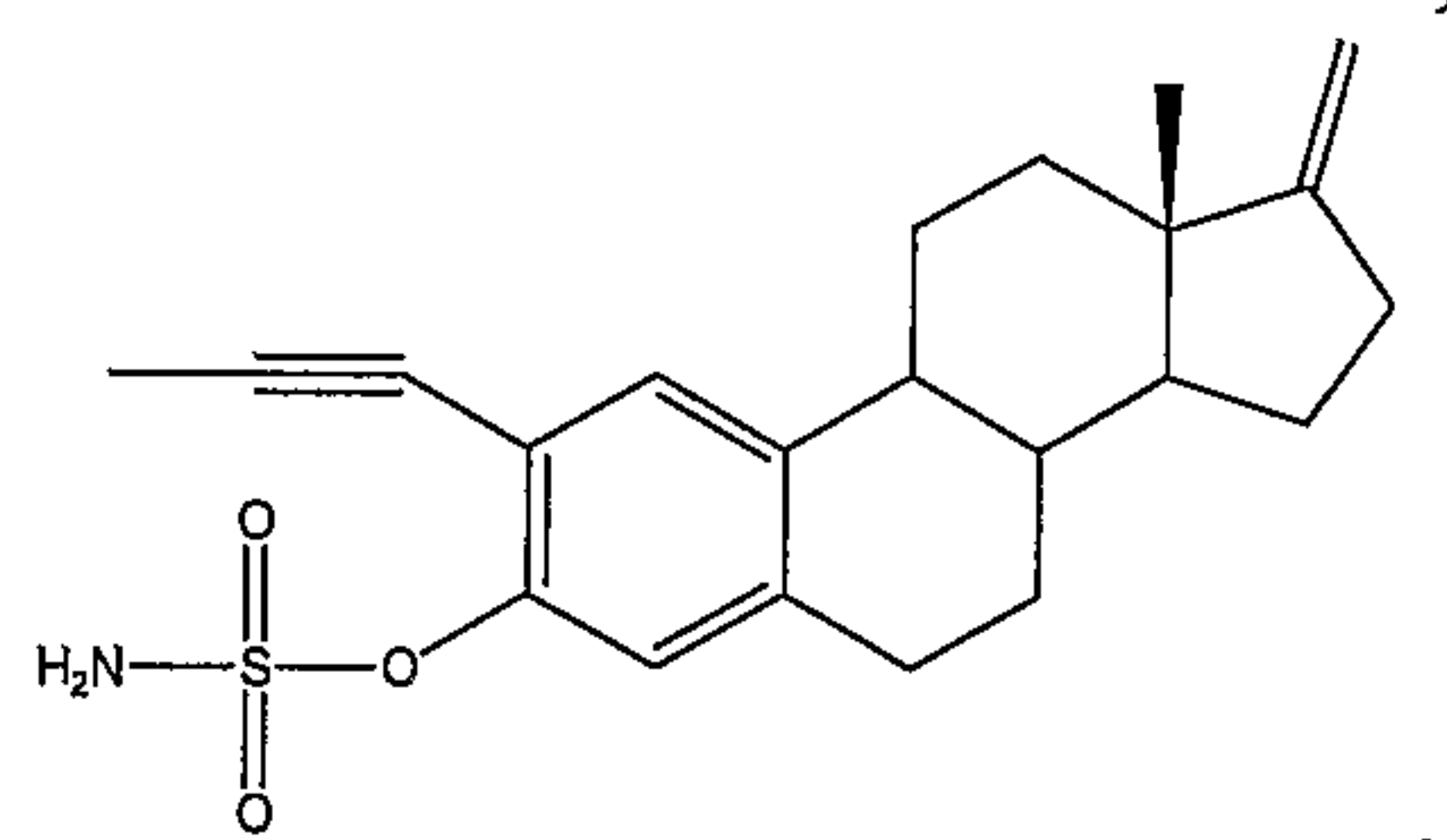
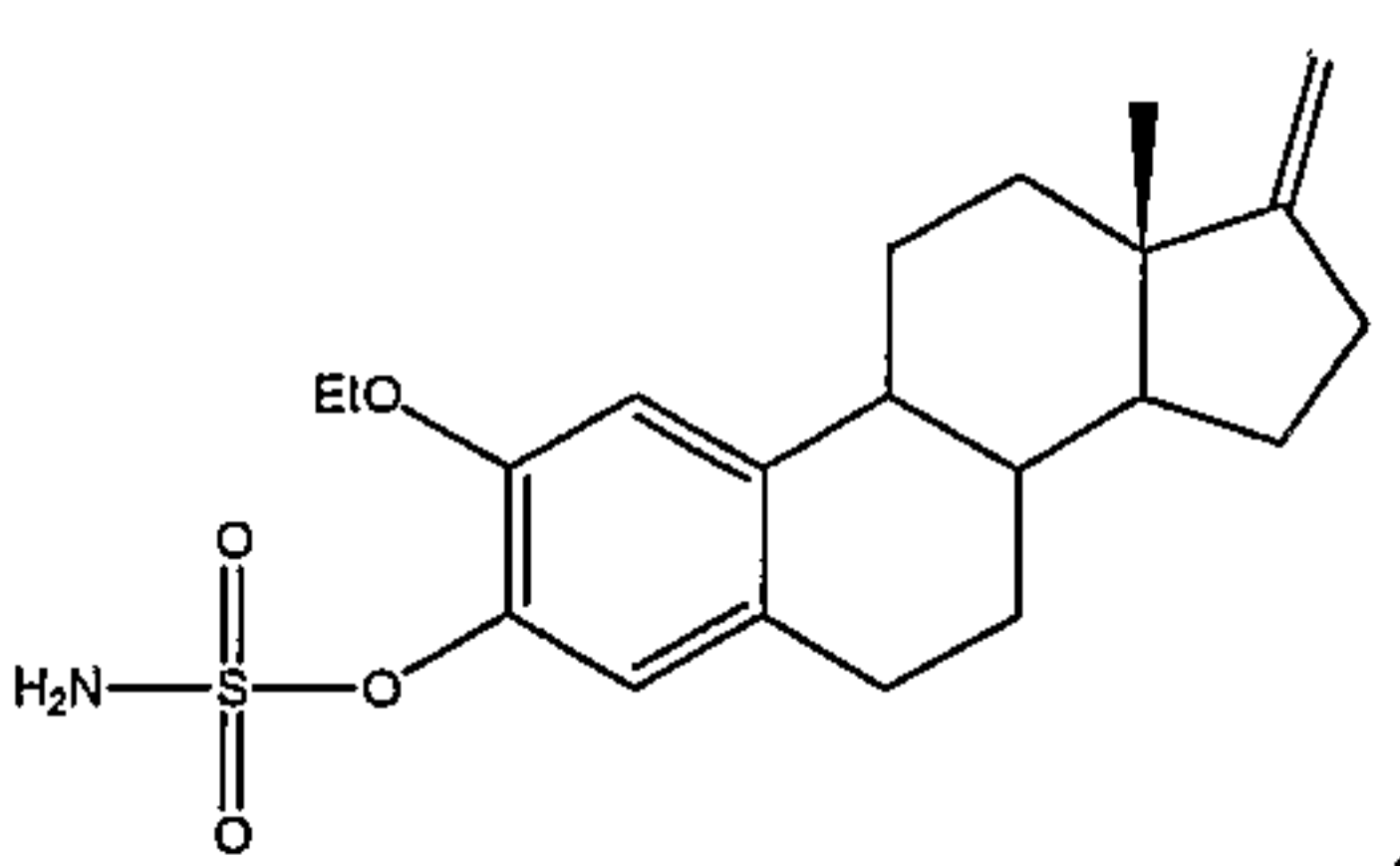
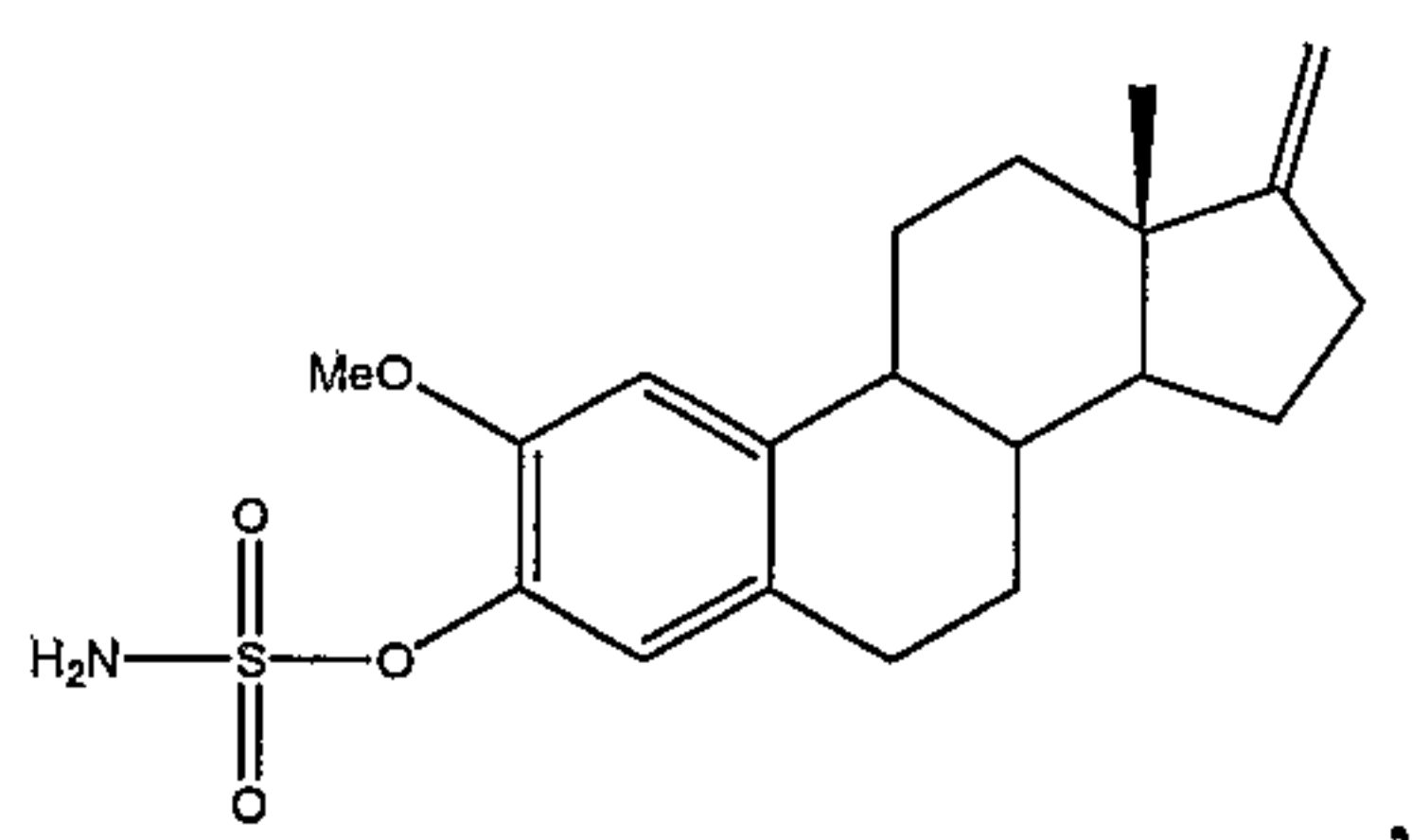
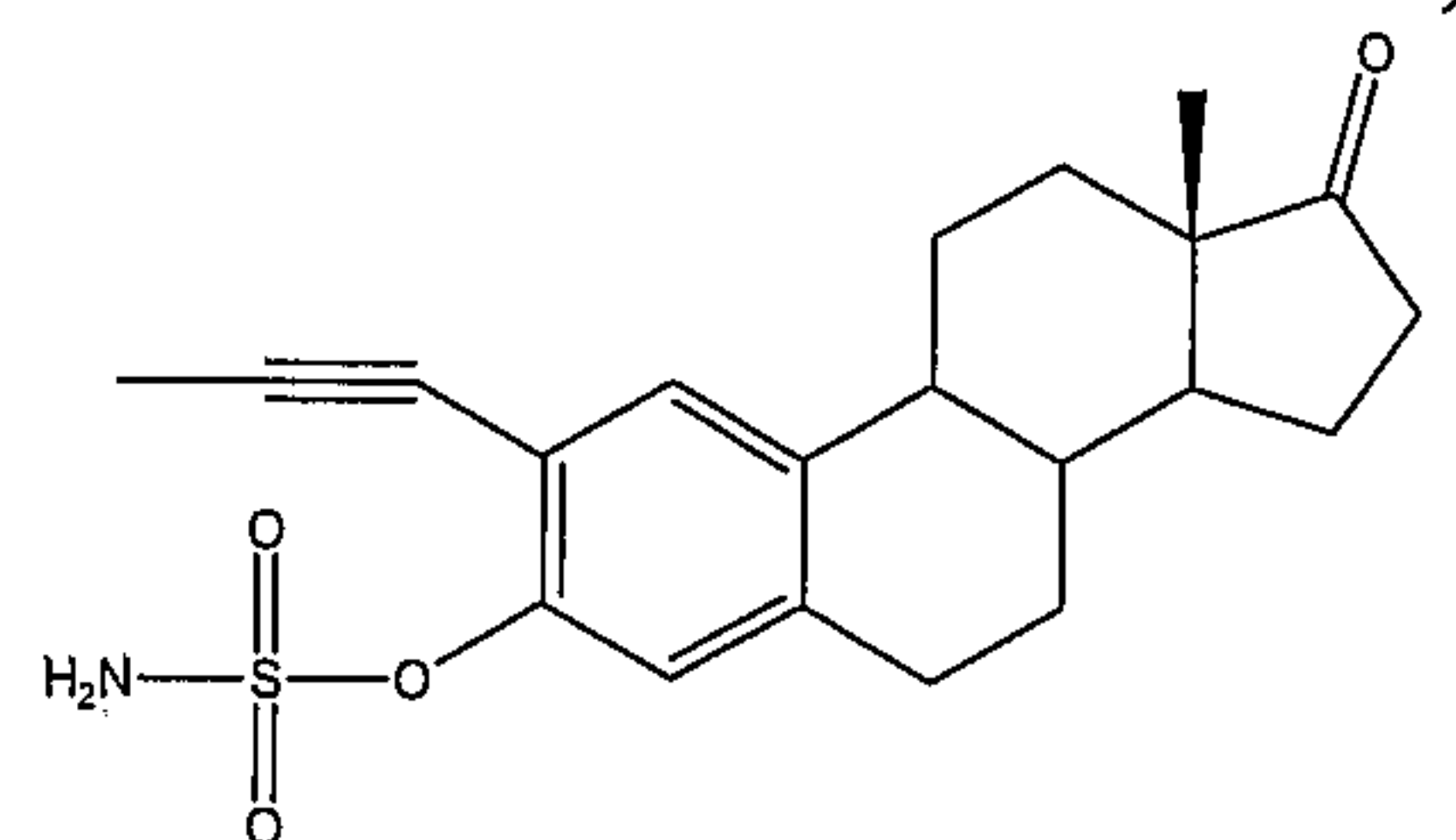
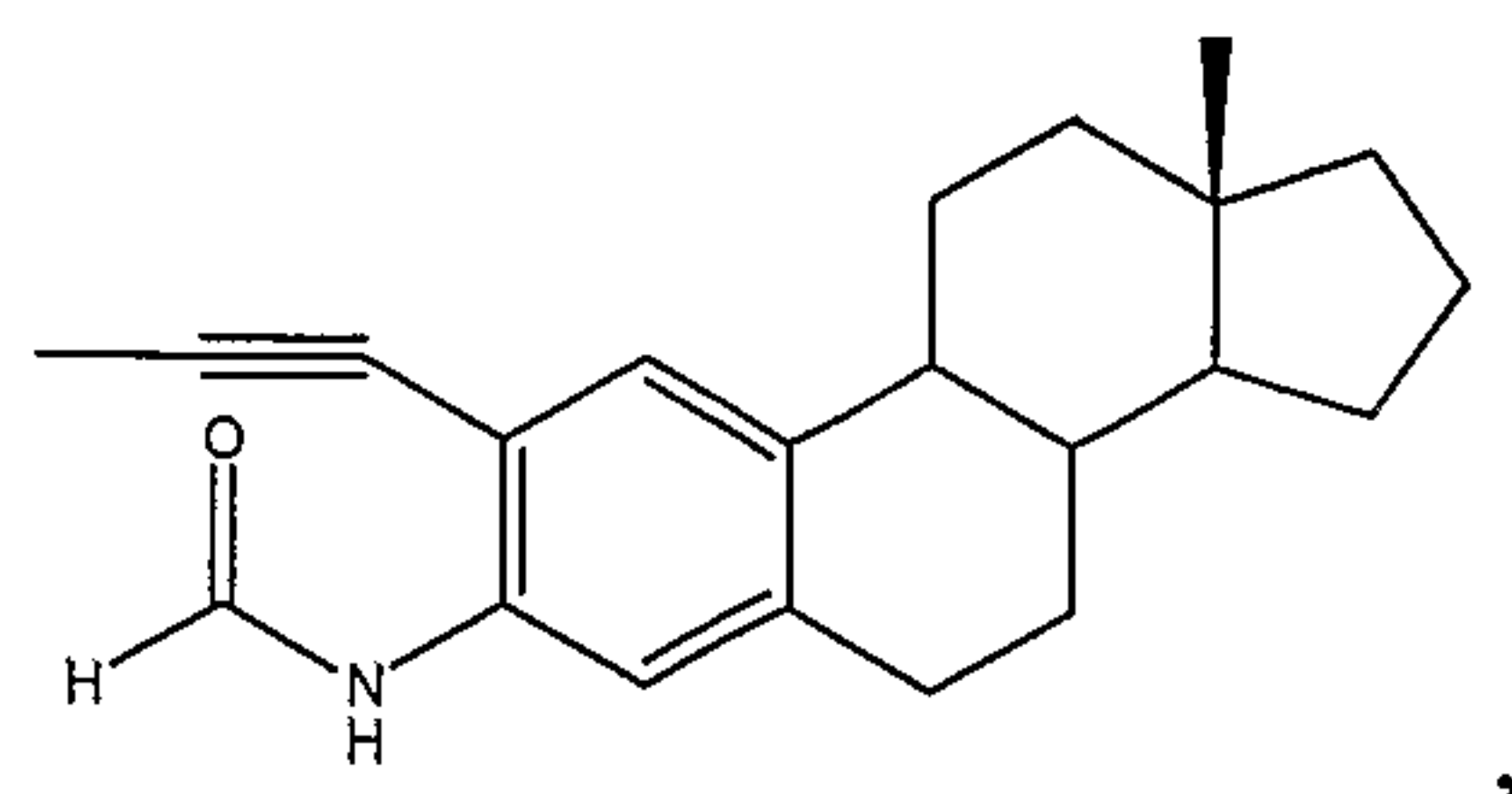
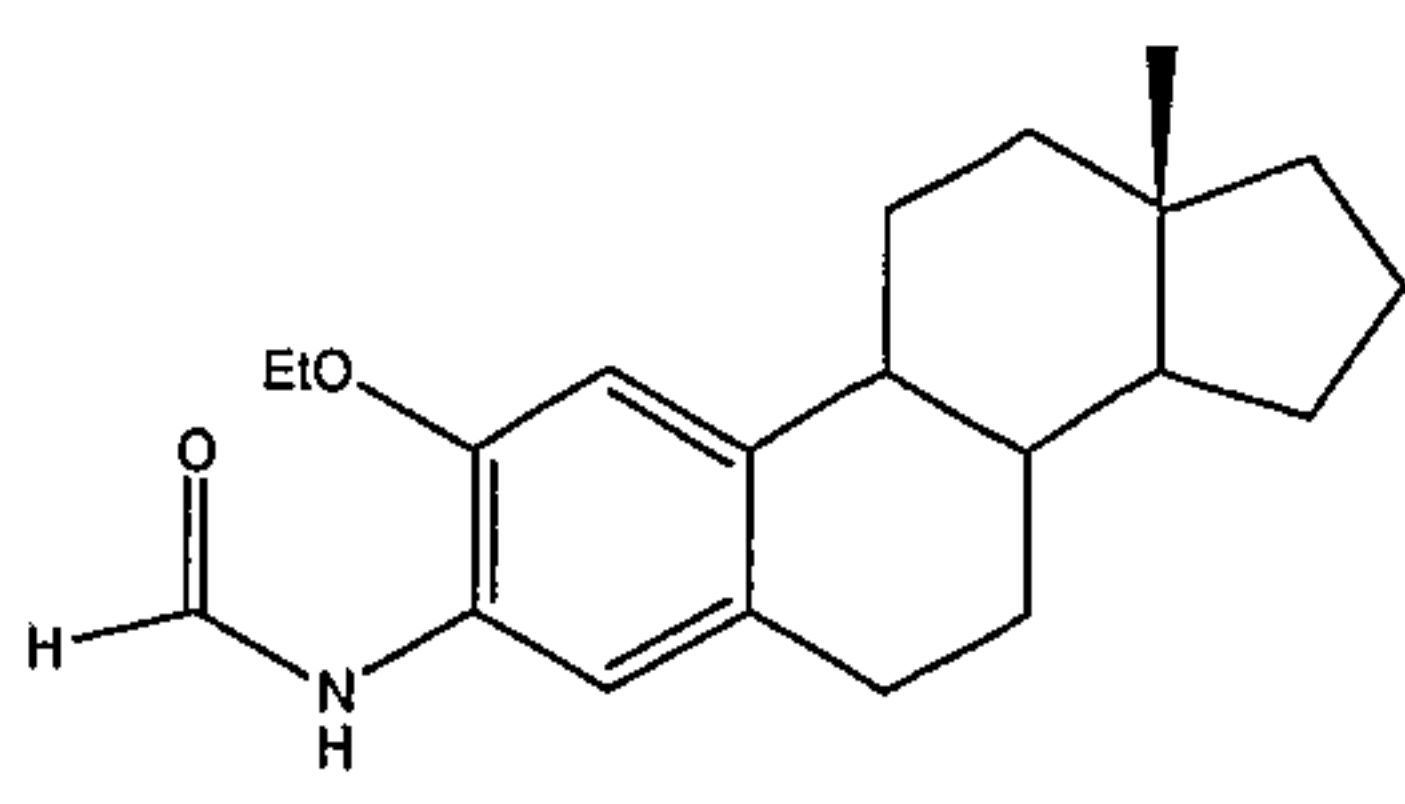
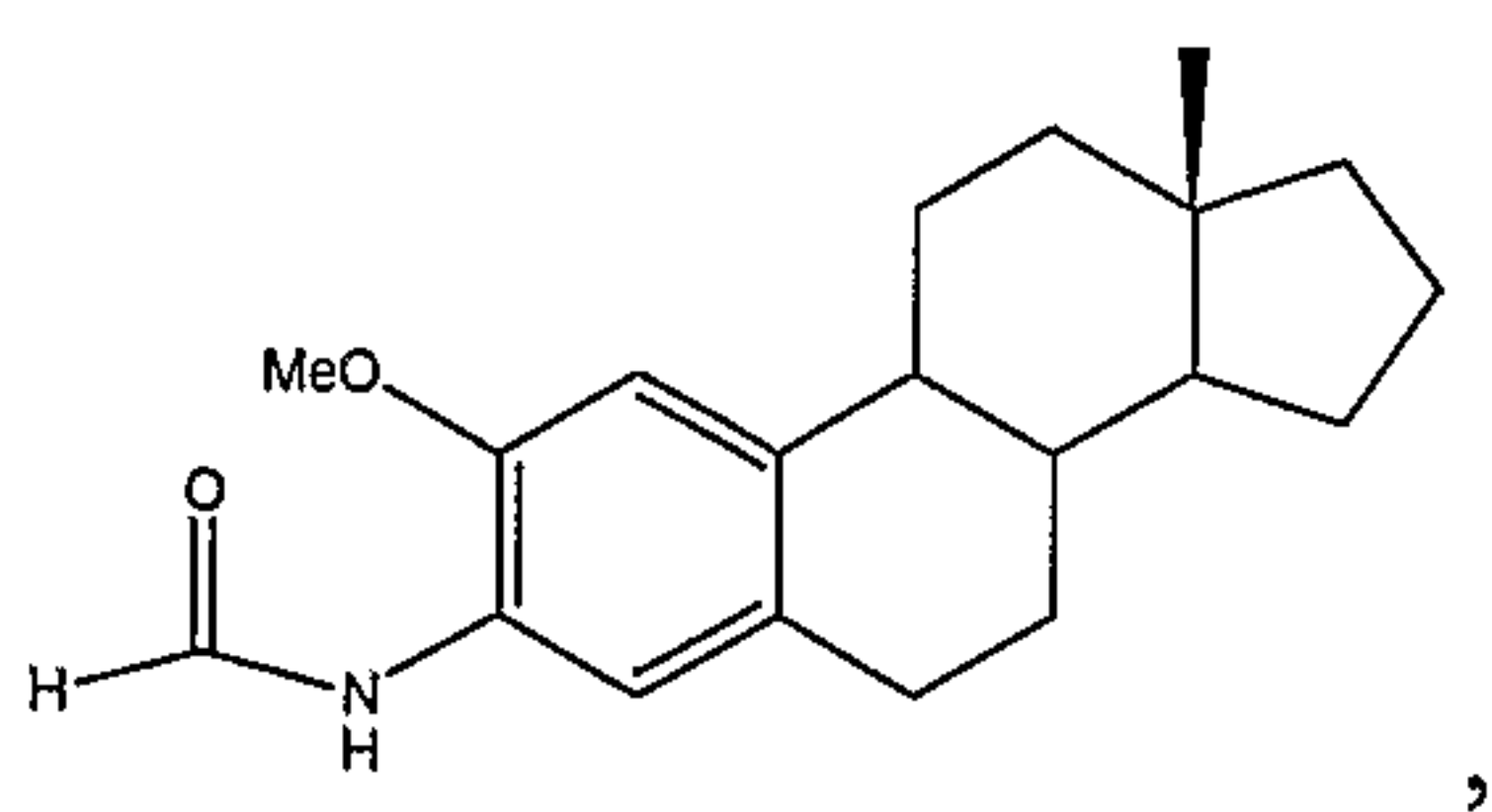
126. The method of Claim 125, wherein the inflammatory or immune mediated disease is rheumatoid arthritis.
- 5 127. The method of Claim 125, wherein the inflammatory or immune mediated disease is osteoarthritis.
128. The method of Claim 125, wherein the inflammatory or immune mediated disease is ulcerative colitis.
- 10 129. The method of Claim 125, wherein the inflammatory or immune mediated disease is Crohn's disease.
130. The method of Claim 125, wherein the inflammatory or immune mediated disease is Mooren's ulcer.
- 15 131. The method of Claim 125, wherein the inflammatory or immune mediated disease is arthritis.
132. The method of Claim 125, wherein the inflammatory or immune mediated disease is sarcoidosis.
- 20 133. The method of Claim 125, wherein the inflammatory or immune mediated disease is an inflammatory or immune mediated bowel disease.
- 25 132. The method of Claim 125, wherein the inflammatory or immune mediated disease is systemic lupus.
133. The method of Claim 125, wherein the inflammatory or immune mediated disease is Wegener's syndrome.
- 30 134. The method of Claim 125, wherein the inflammatory or immune mediated disease is Stevens-Johnson disease.

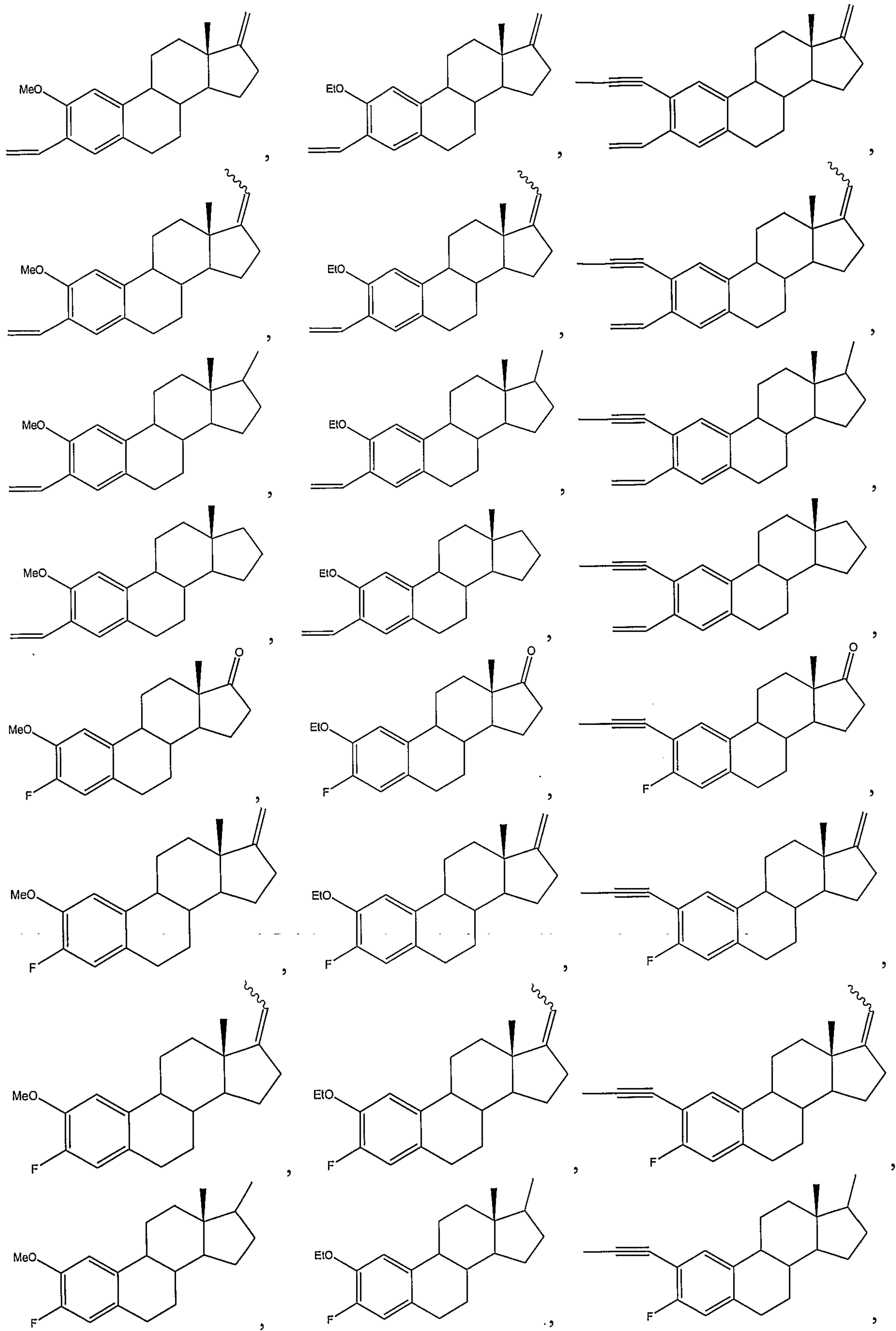
135. The method of Claim 125, wherein the inflammatory or immune mediated disease is Behcet's disease.
- 5 136. The method of Claim 125, wherein the inflammatory or immune mediated disease is pemphigoid.
137. The method of Claim 125, wherein the inflammatory or immune mediated disease is Lyme's disease.
- 10 138. The method of Claim 125, wherein the inflammatory or immune mediated disease is acquired immune deficiency syndrome.

139. A method of treating an infectious disease in a human or an animal comprising administering to the human or animal a compound selected from

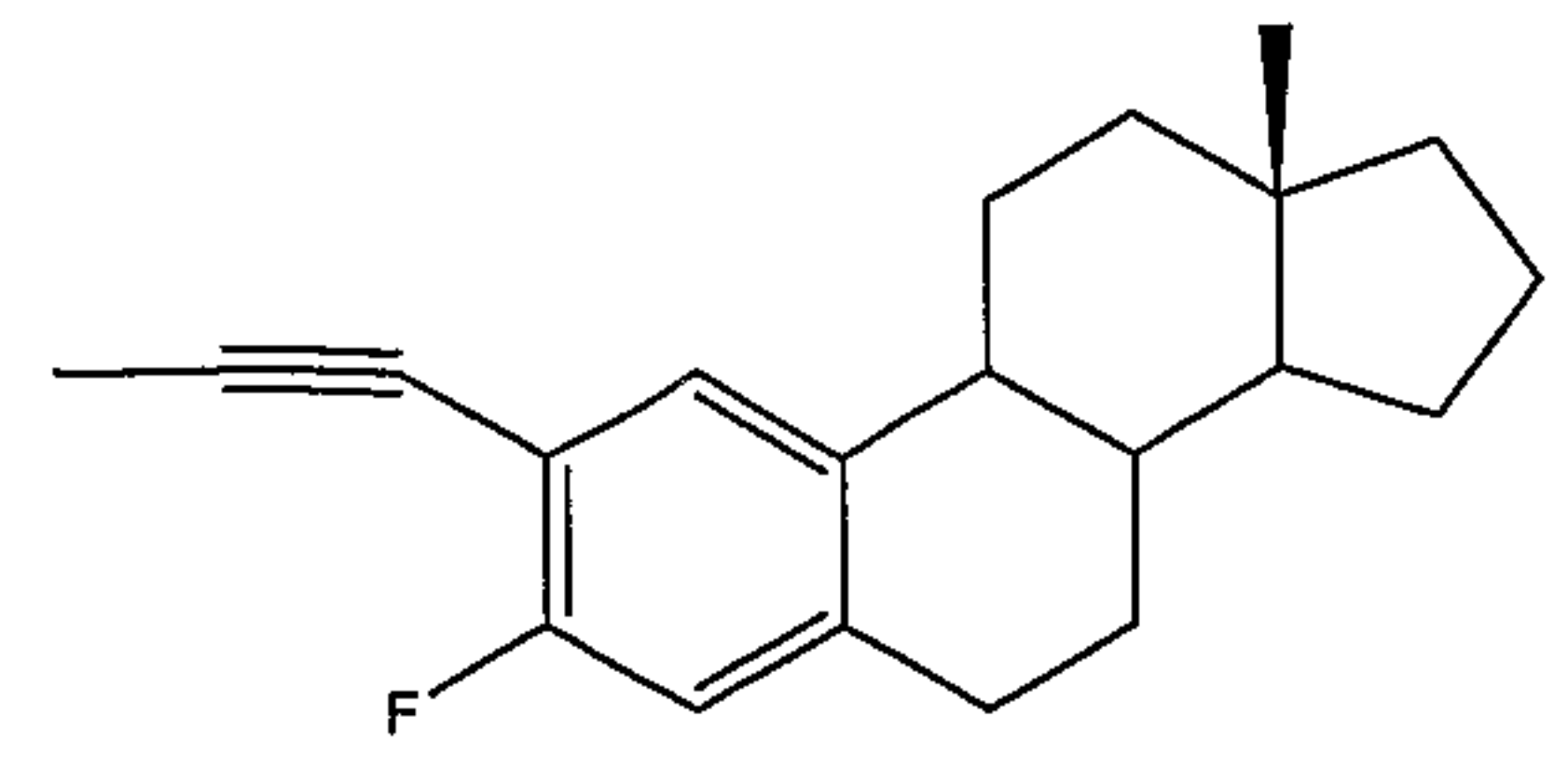
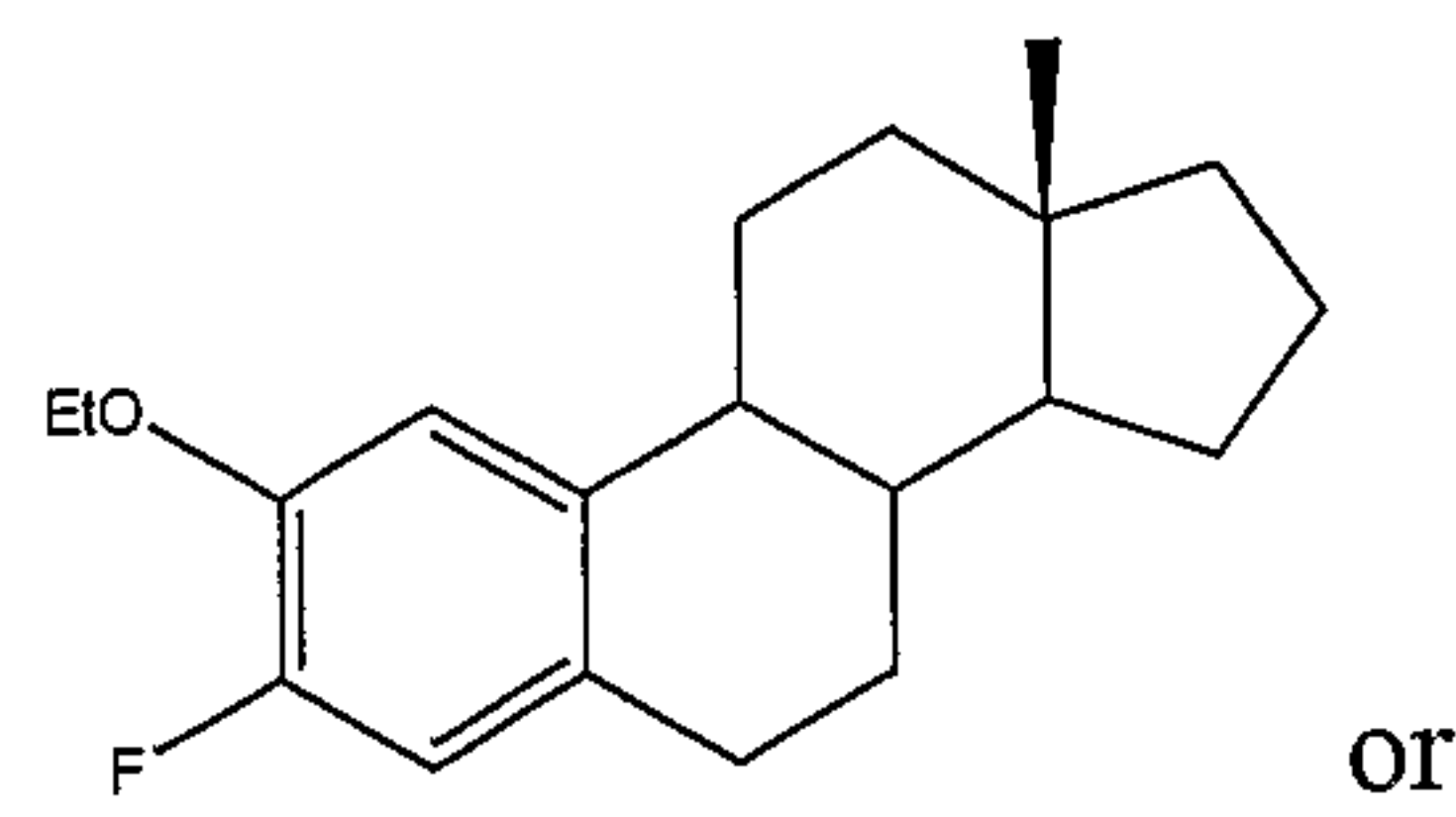
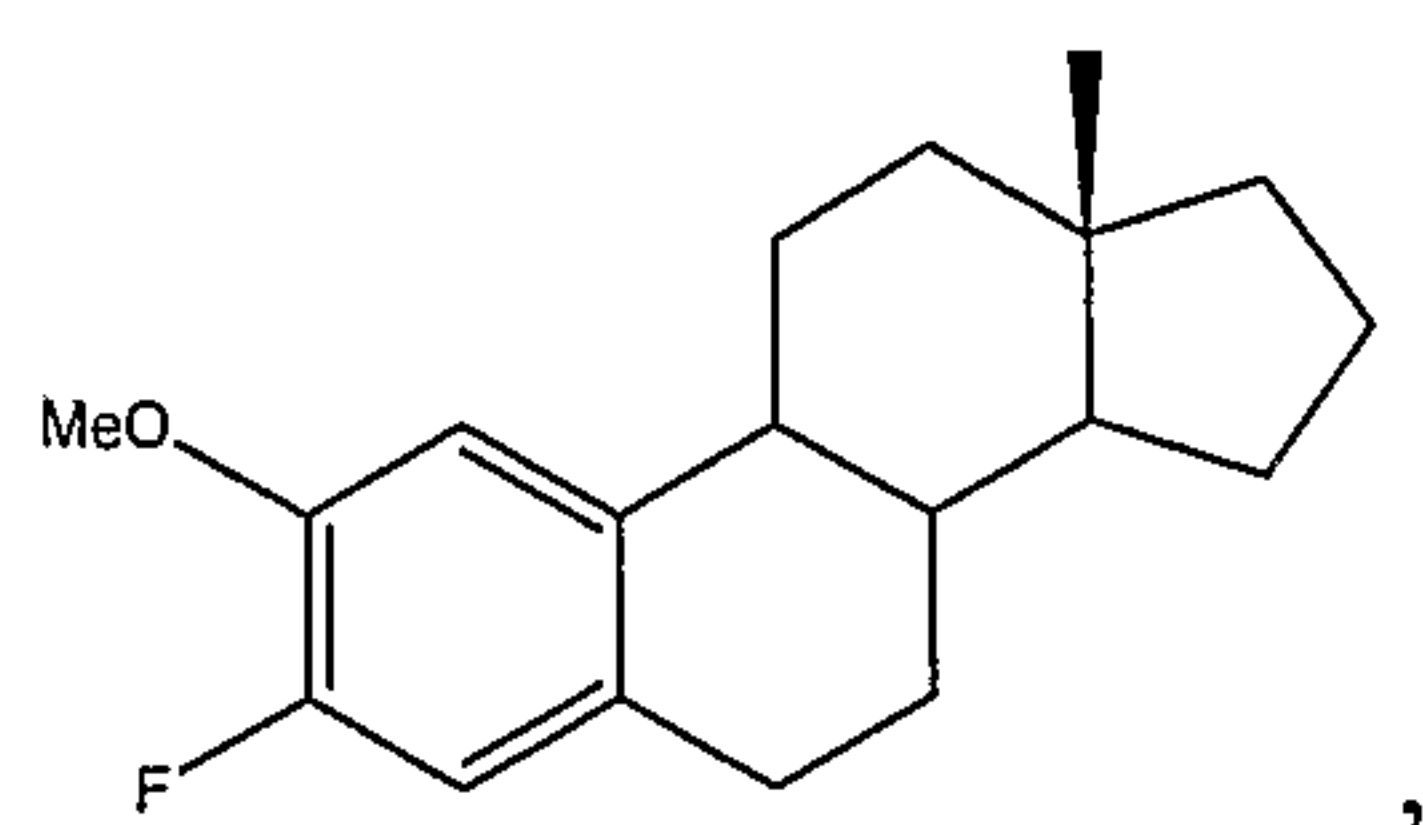








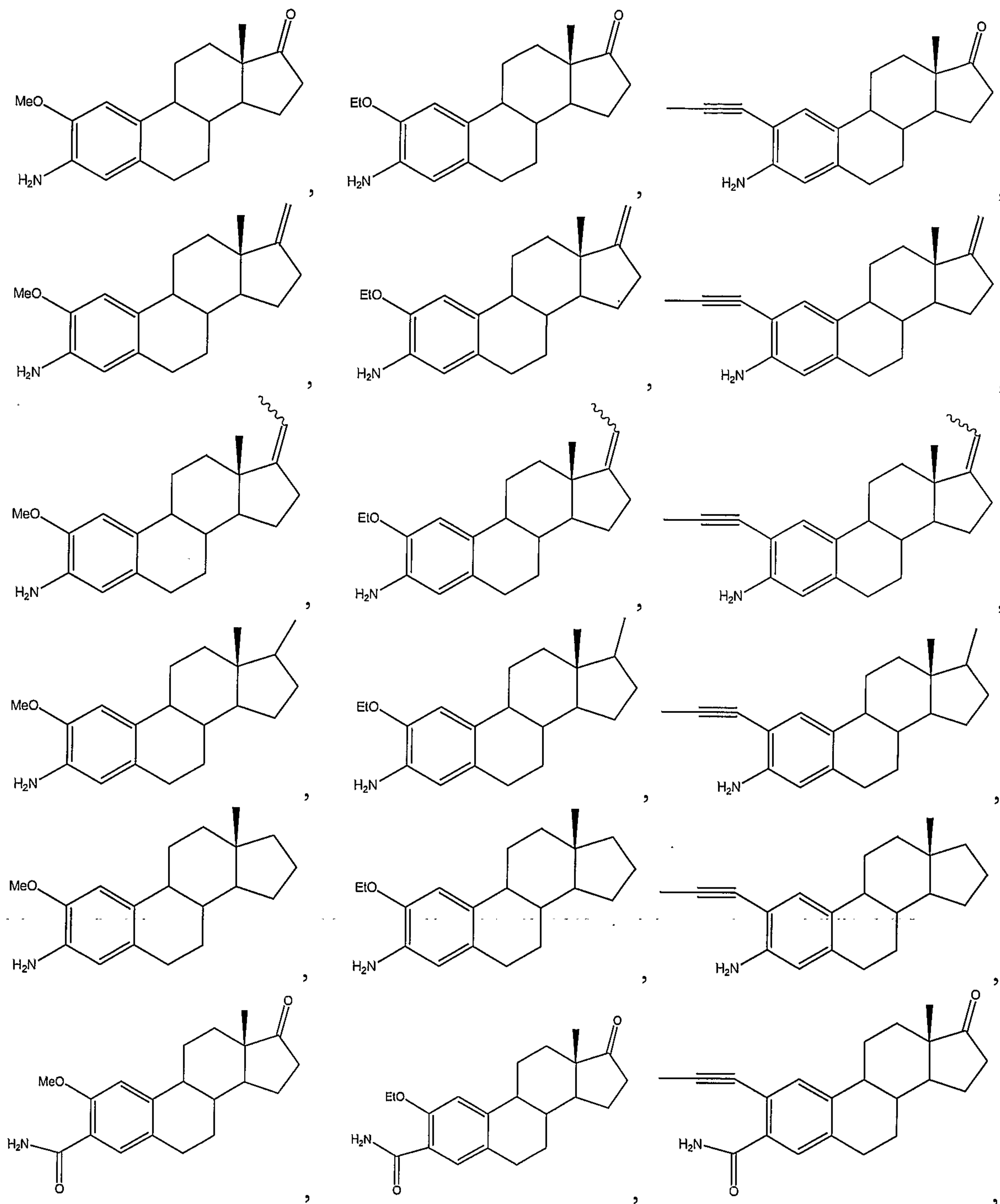
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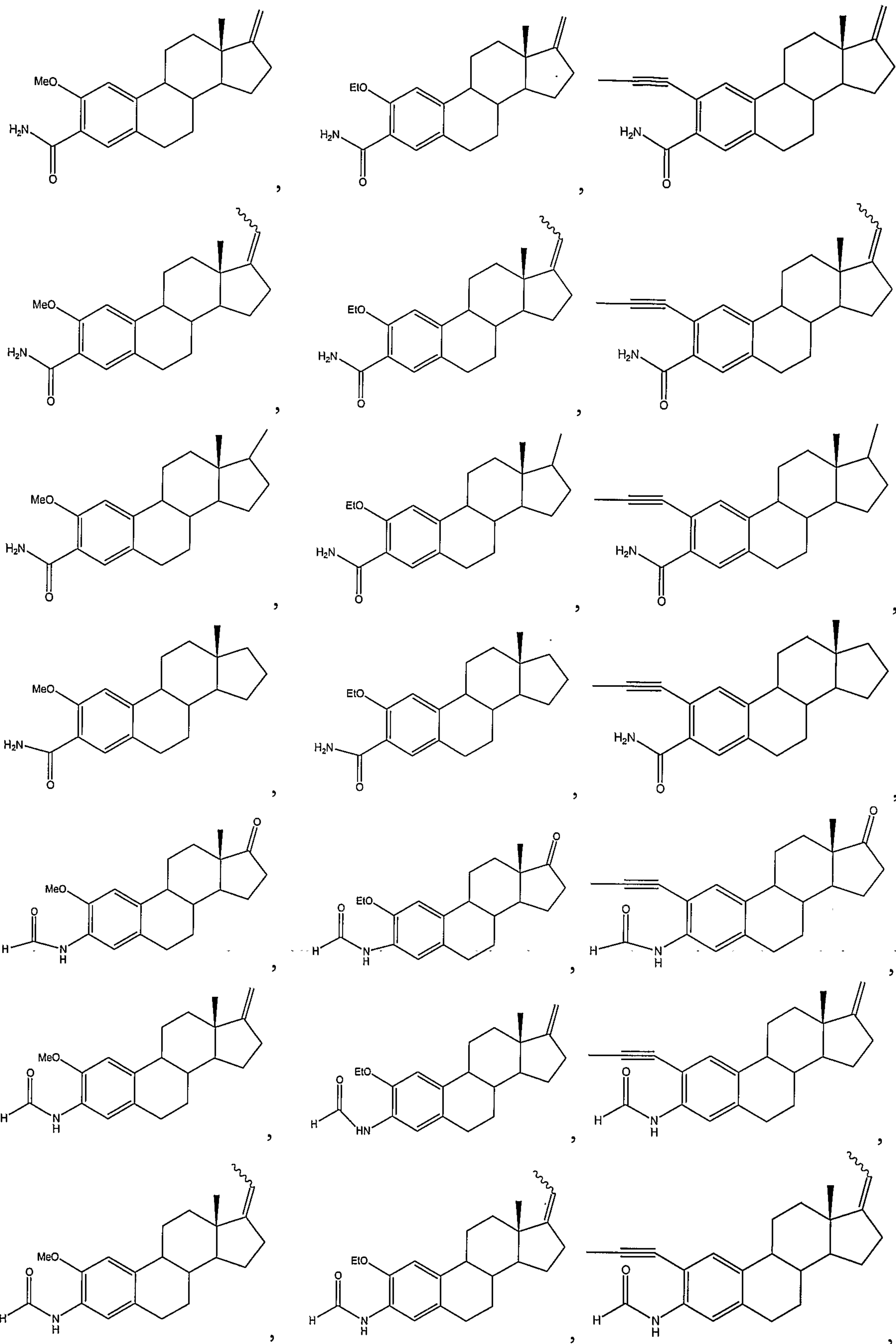


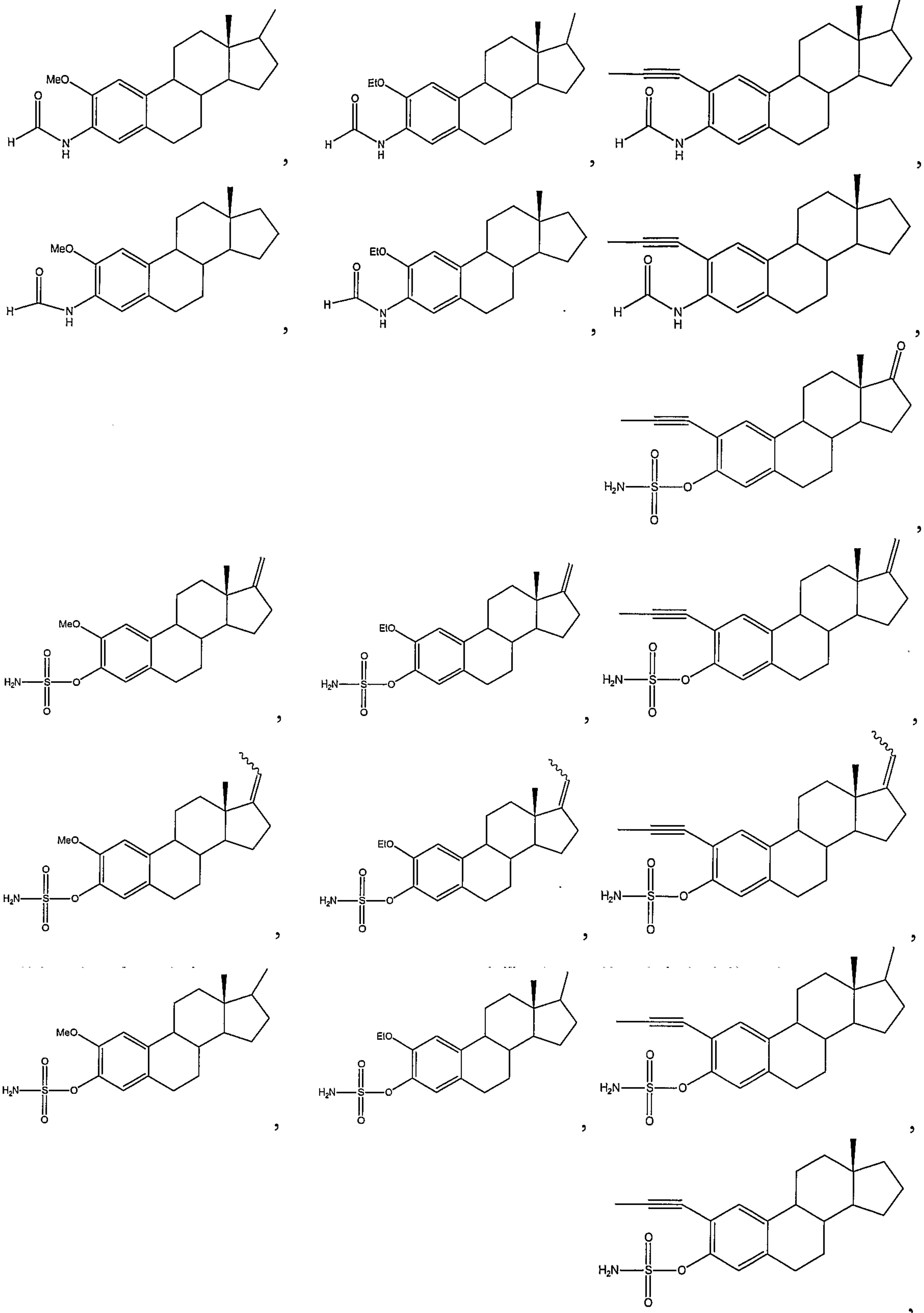
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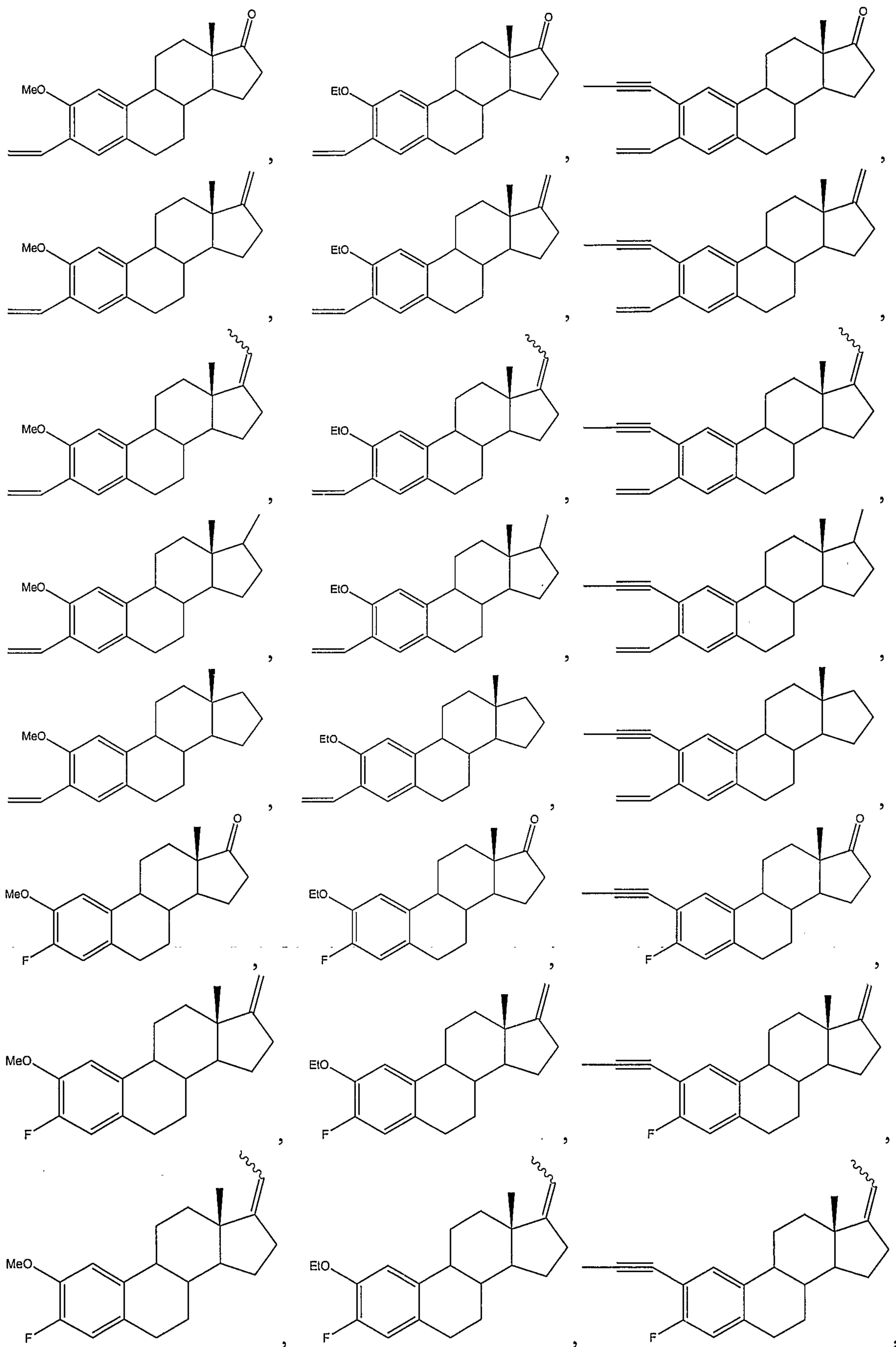
140. The method of Claim 139, wherein the infectious disease is syphilis.
141. The method of Claim 139, wherein the infectious disease is a bacterial infection.
- 5 142. The method of Claim 139, wherein the infectious disease is a mycobacterial infection.
143. The method of Claim 139, wherein the infectious disease is a bacterial ulcer.
- 10 144. The method of Claim 139, wherein the infectious disease is a fungal ulcer.
145. The method of Claim 139, wherein the infectious disease is an Herpes simplex infection.
- 15 146. The method of Claim 139, wherein the infectious disease is an Herpes zoster infection.
147. The method of Claim 139, wherein the infectious disease is a protozoan infection.
- 20 148. The method of Claim 139, wherein the infectious disease is a Bartonellosis infection.
- 25 149. The method of Claim 139, wherein the infectious disease is toxoplasmosis.

150. A method of treating a cancerous disease in a human or an animal comprising administering to the human or animal an effective cancerous disease treating amount of a compound selected from



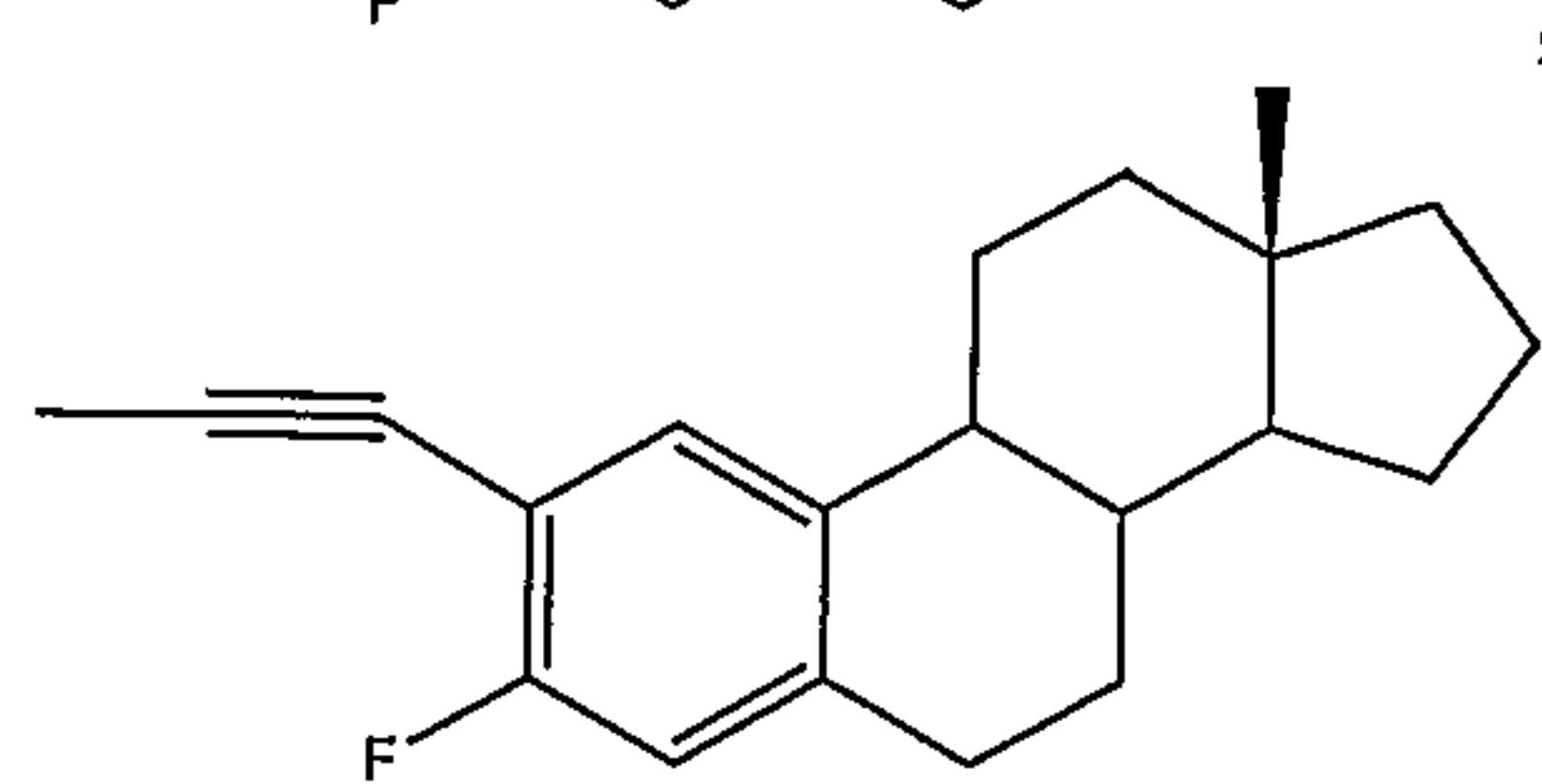
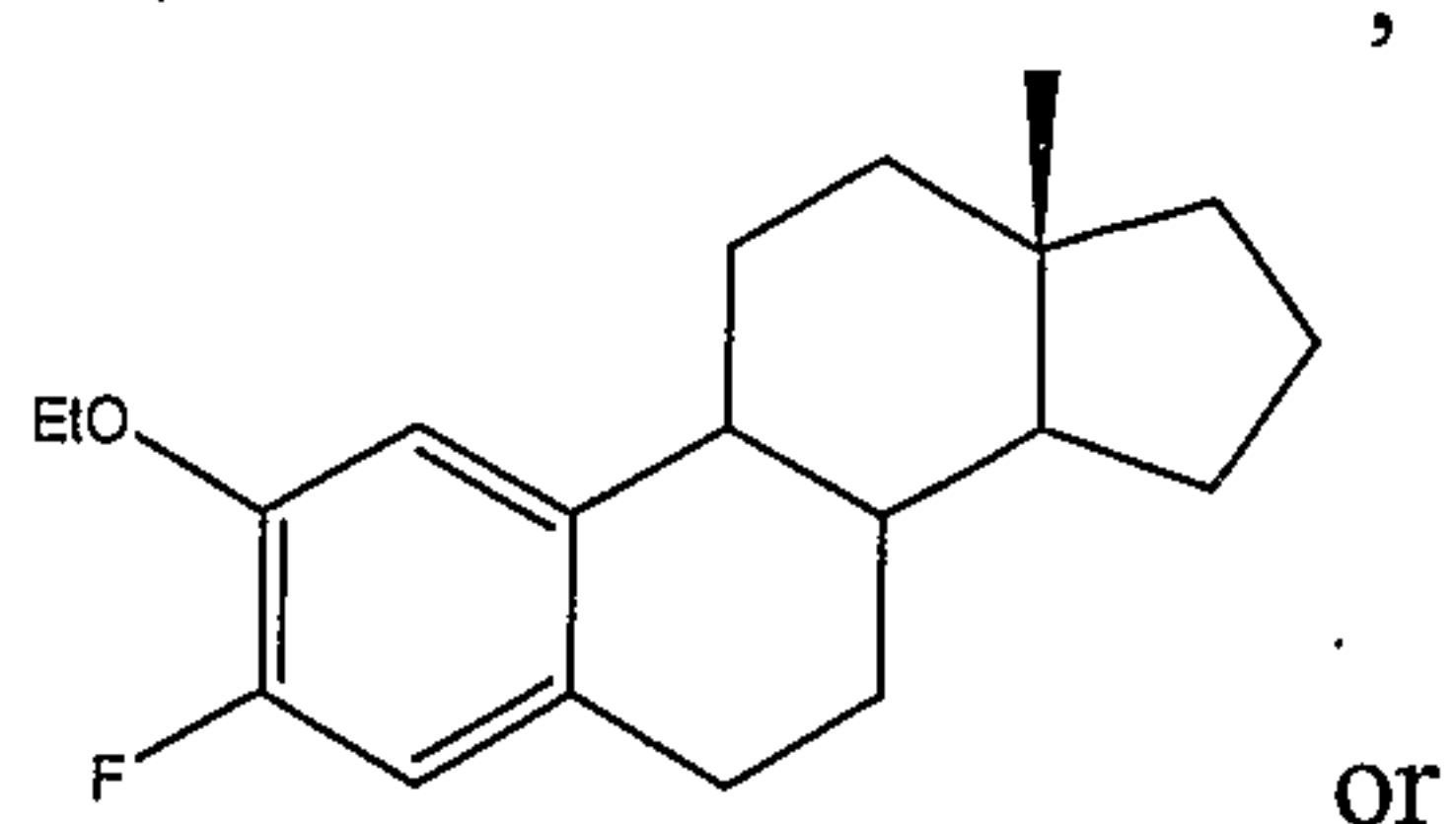
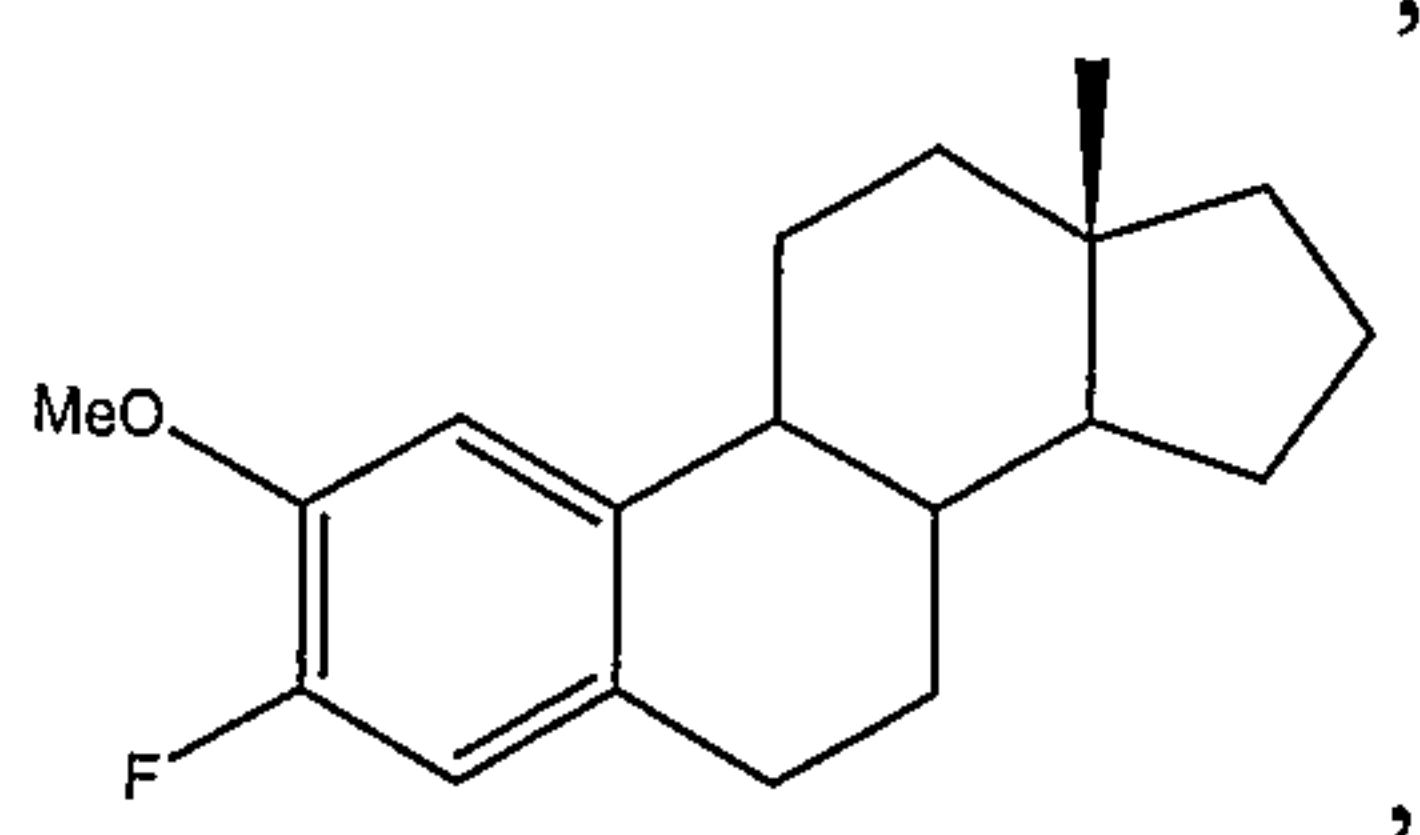
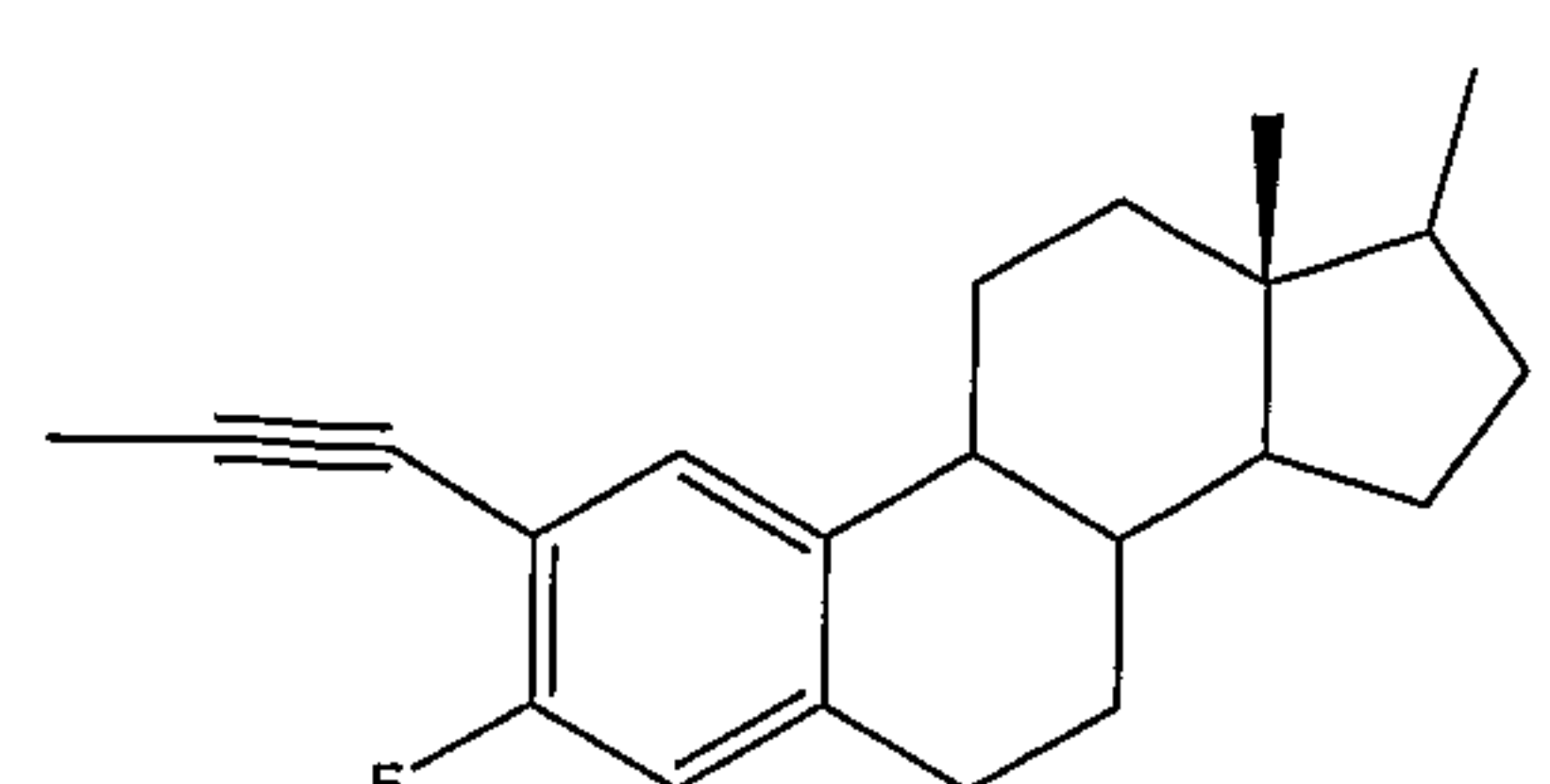
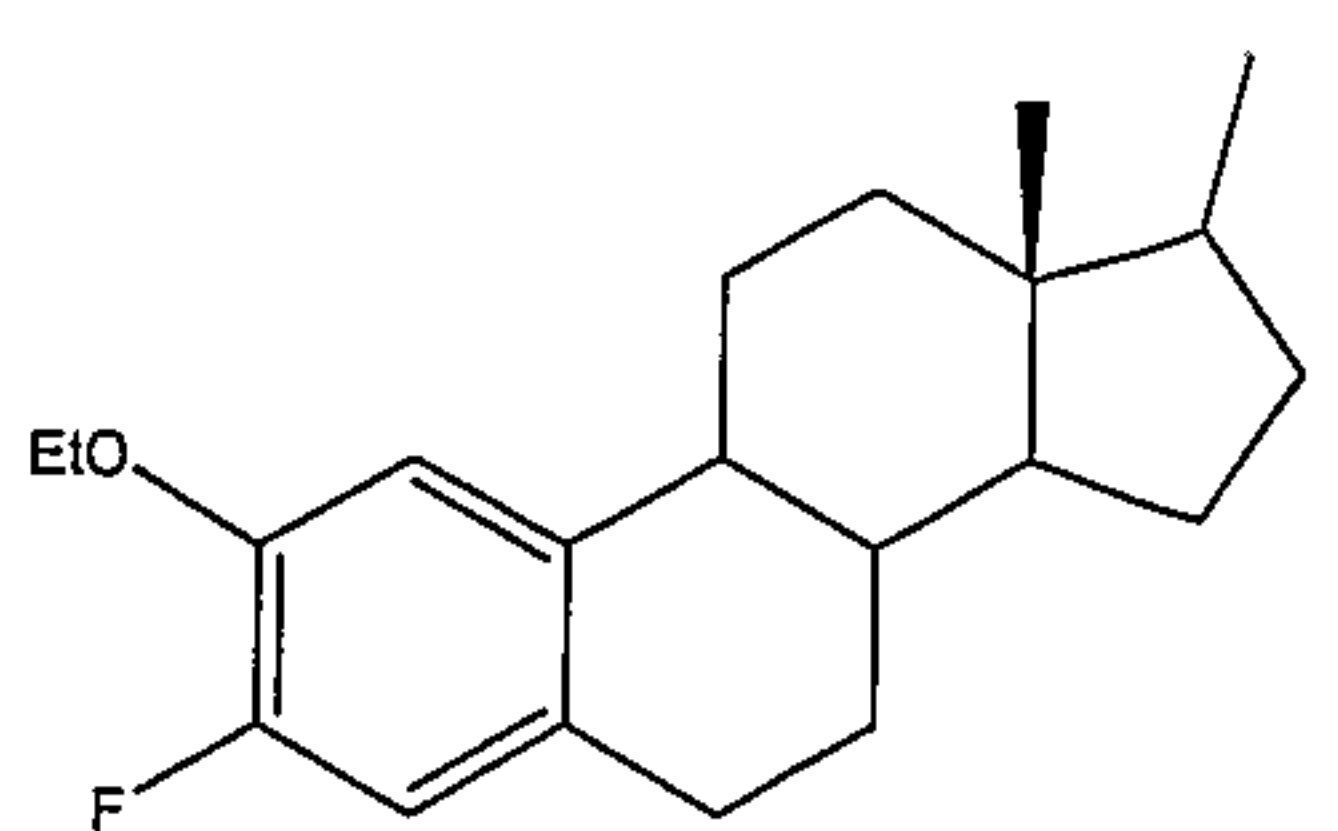
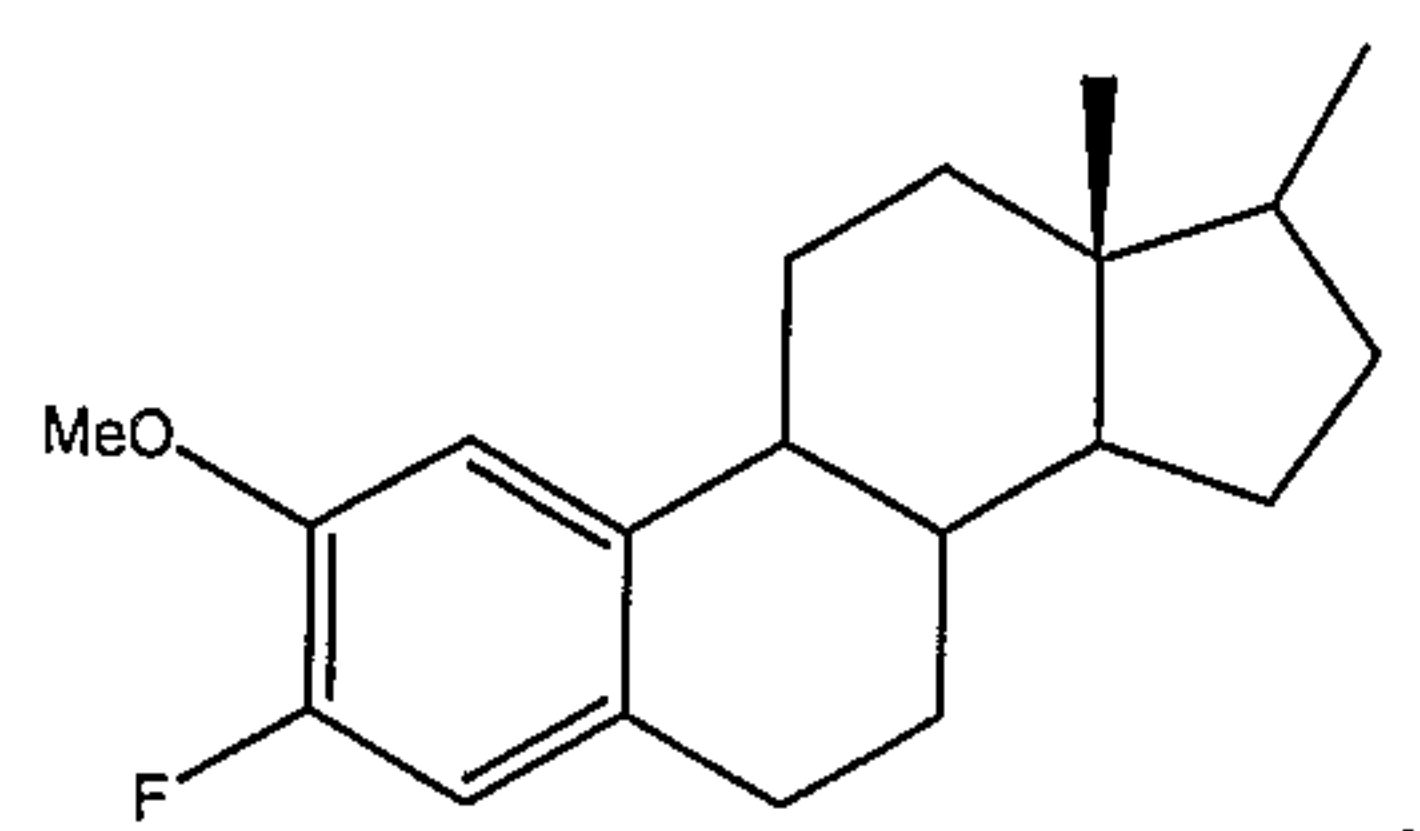






WO 2005/030120

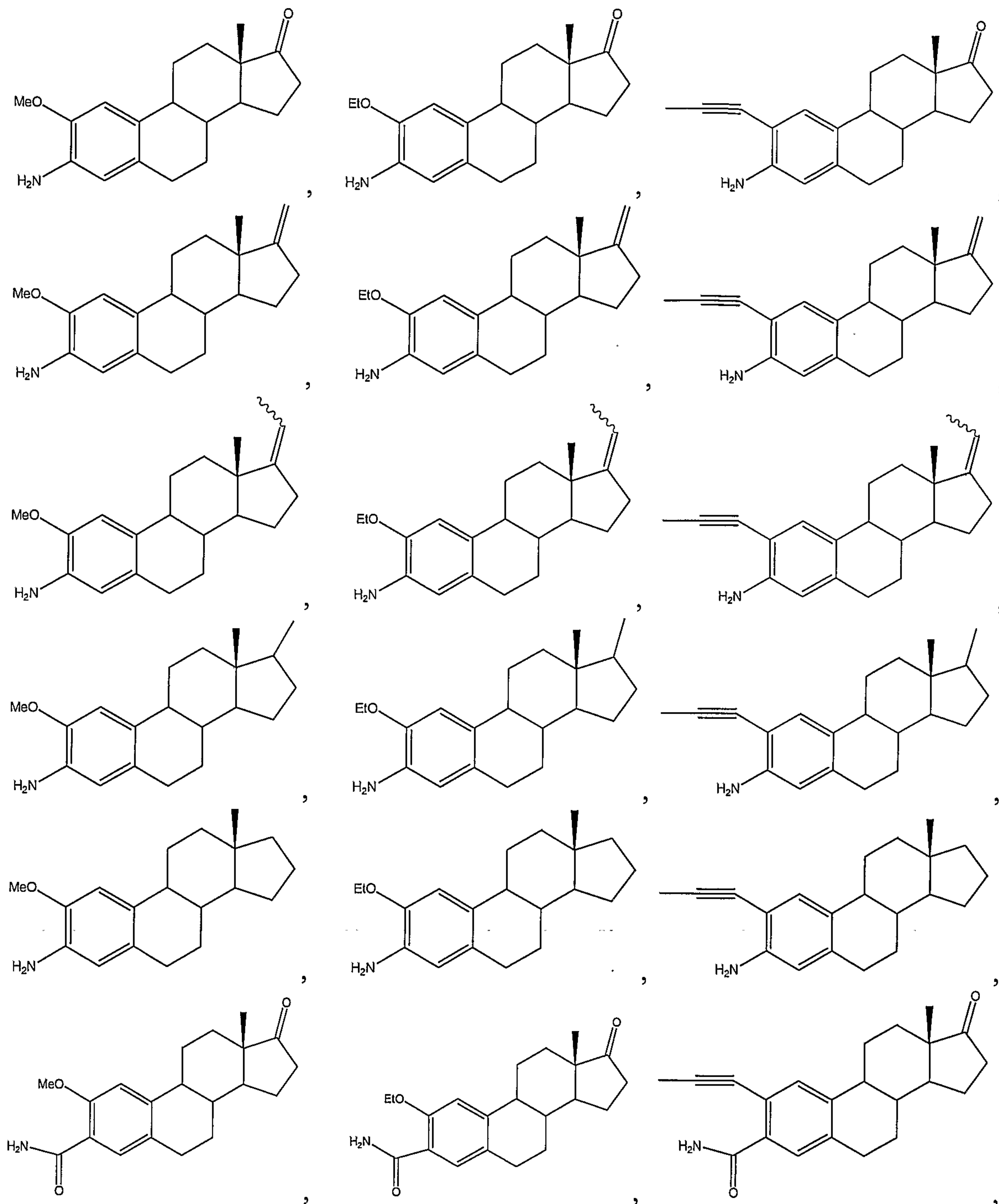
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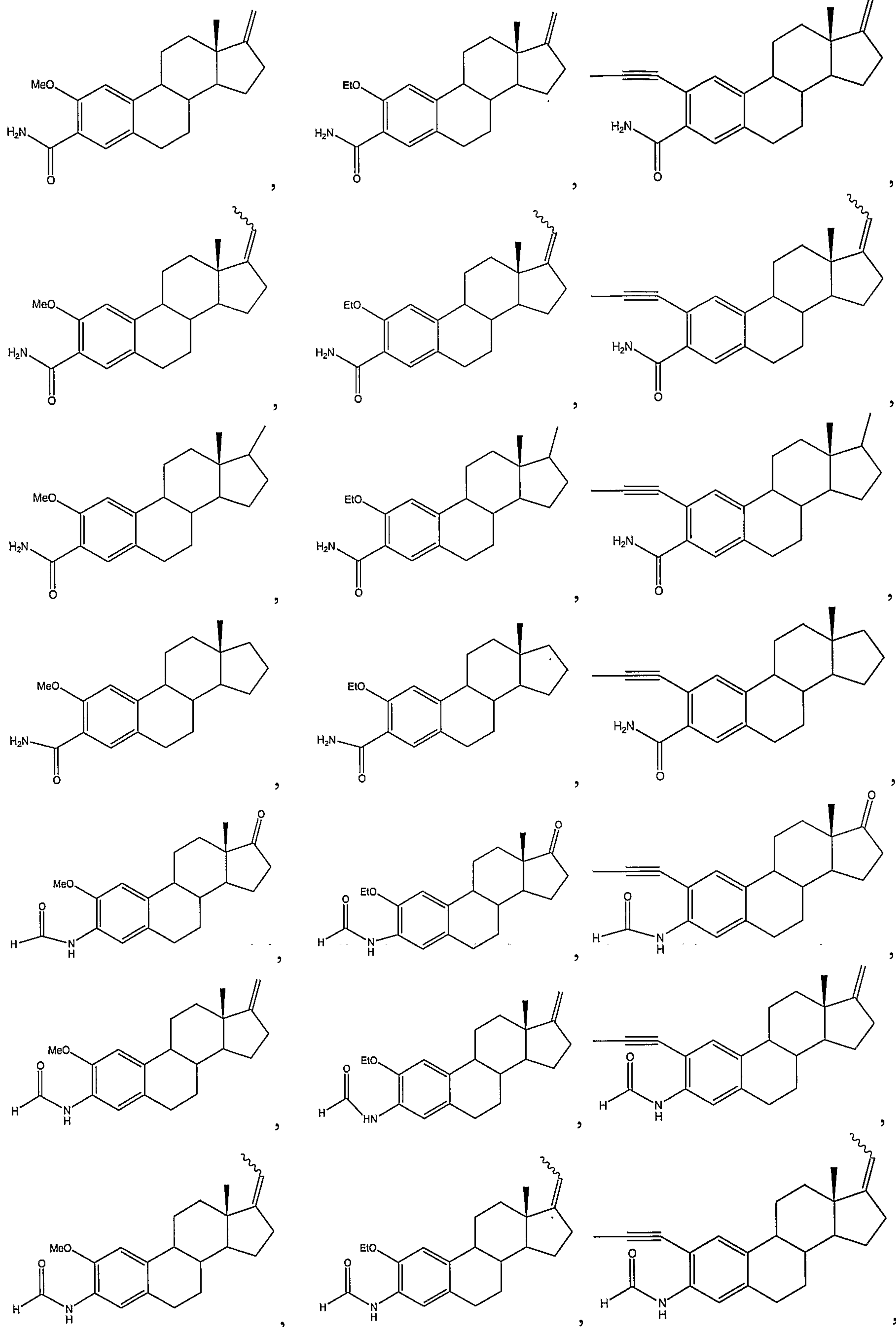


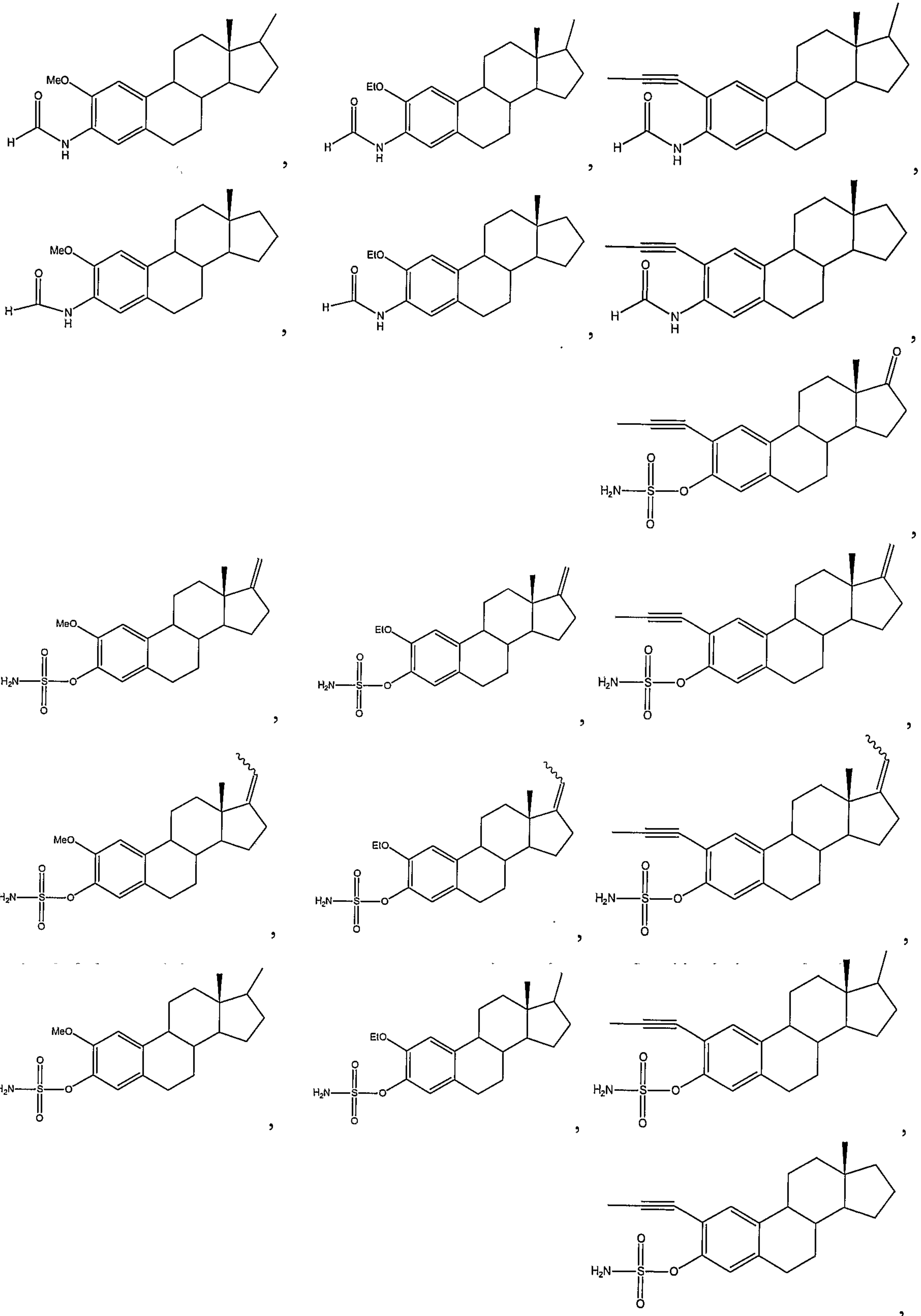
151. The method of Claim 150, wherein the cancerous disease is rhabdomyosarcoma.
152. The method of Claim 150, wherein the cancerous disease is retinoblastoma.
- 5 153. The method of Claim 150, wherein the cancerous disease is Ewing sarcoma.
154. The method of Claim 150, wherein the cancerous disease is neuroblastoma.
- 10 155. The method of Claim 150, wherein the cancerous disease is osteosarcoma.
156. The method of Claim 150, wherein the cancerous disease is acoustic neuroma.
- 15 157. The method of Claim 150, wherein the cancerous disease is neurofibroma.
158. The method of Claim 150, herein the cancerous disease is hemangioma.
159. The method of Claim 150, wherein the cancerous disease is breast cancer.
- 20 160. The method of Claim 150, wherein the cancerous disease is prostate cancer.
161. The method of Claim 150, wherein the cancerous disease is renal cell cancer.
- 25 162. The method of Claim 150, wherein the cancerous disease is a brain tumor.
163. The method of Claim 150, wherein the cancerous disease is ovarian cancer.
164. The method of Claim 150, wherein the cancerous disease is colon cancer.
- 30 165. The method of Claim 150, wherein the cancerous disease is liver cancer.
166. The method of Claim 150, wherein the cancerous disease is lung cancer.

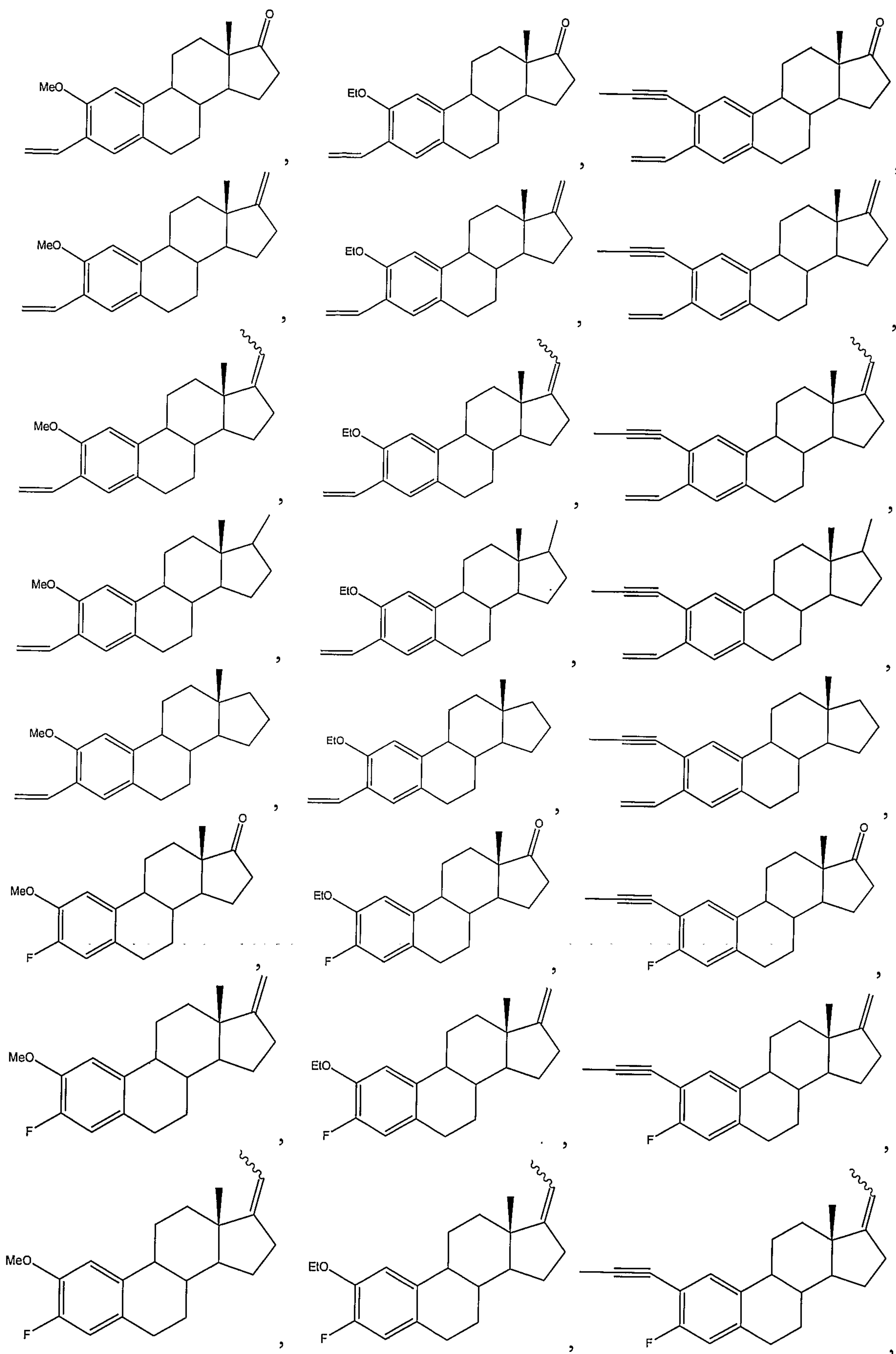
166. The method of Claim 150, wherein the cancerous disease is bladder cancer.

167. A method of treating a blood or blood vessel disease in a human or an animal comprising administering to the human or animal a compound selected from



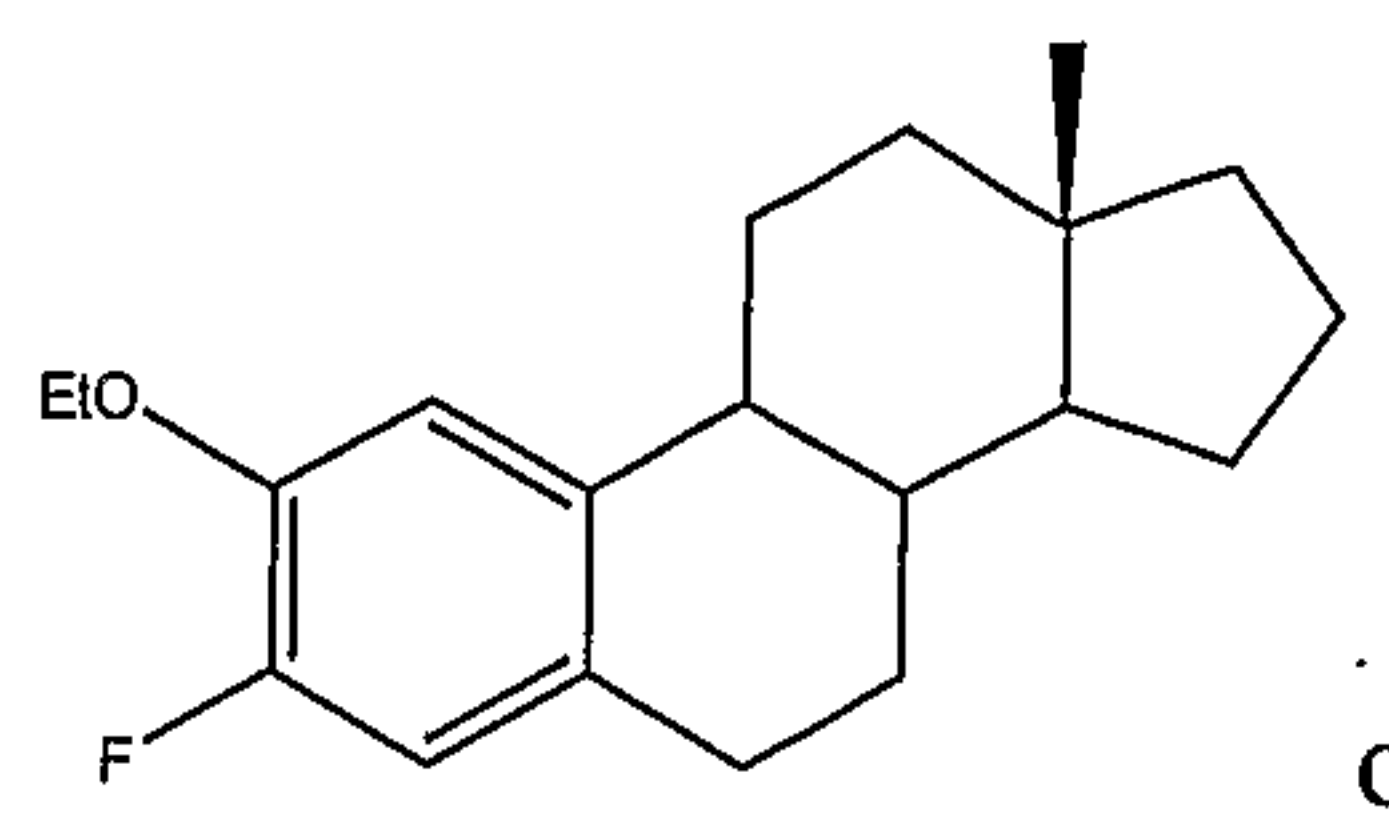
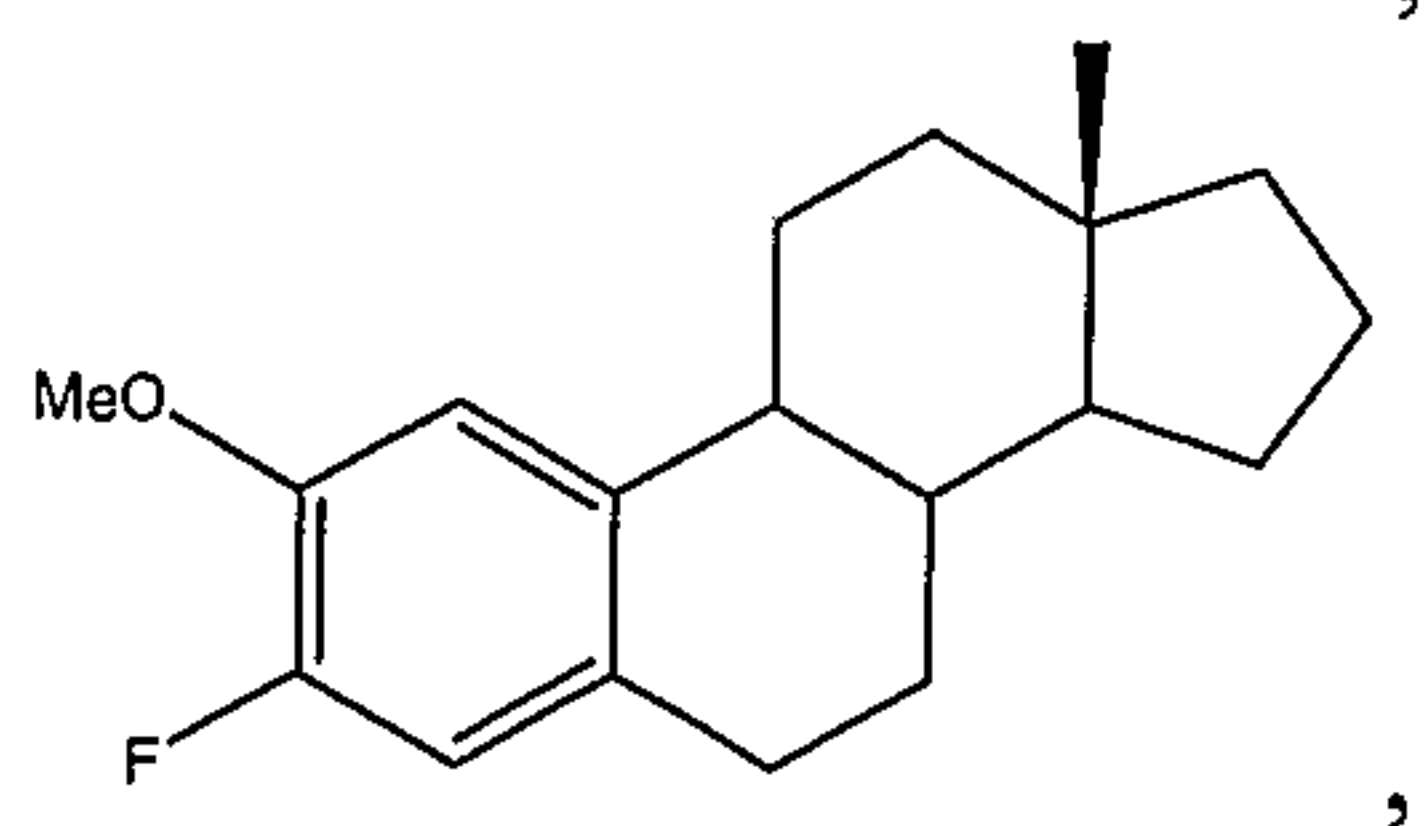
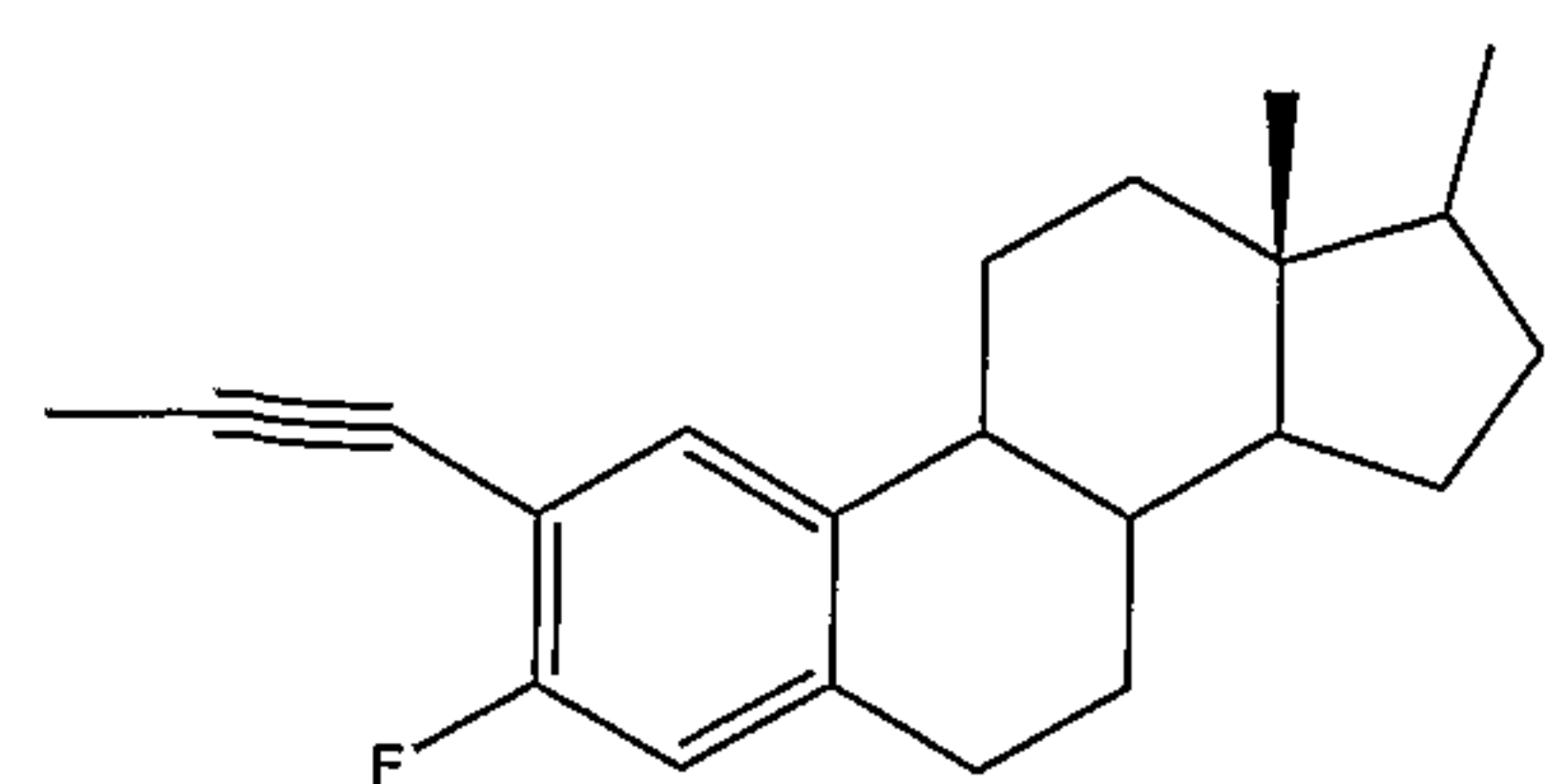
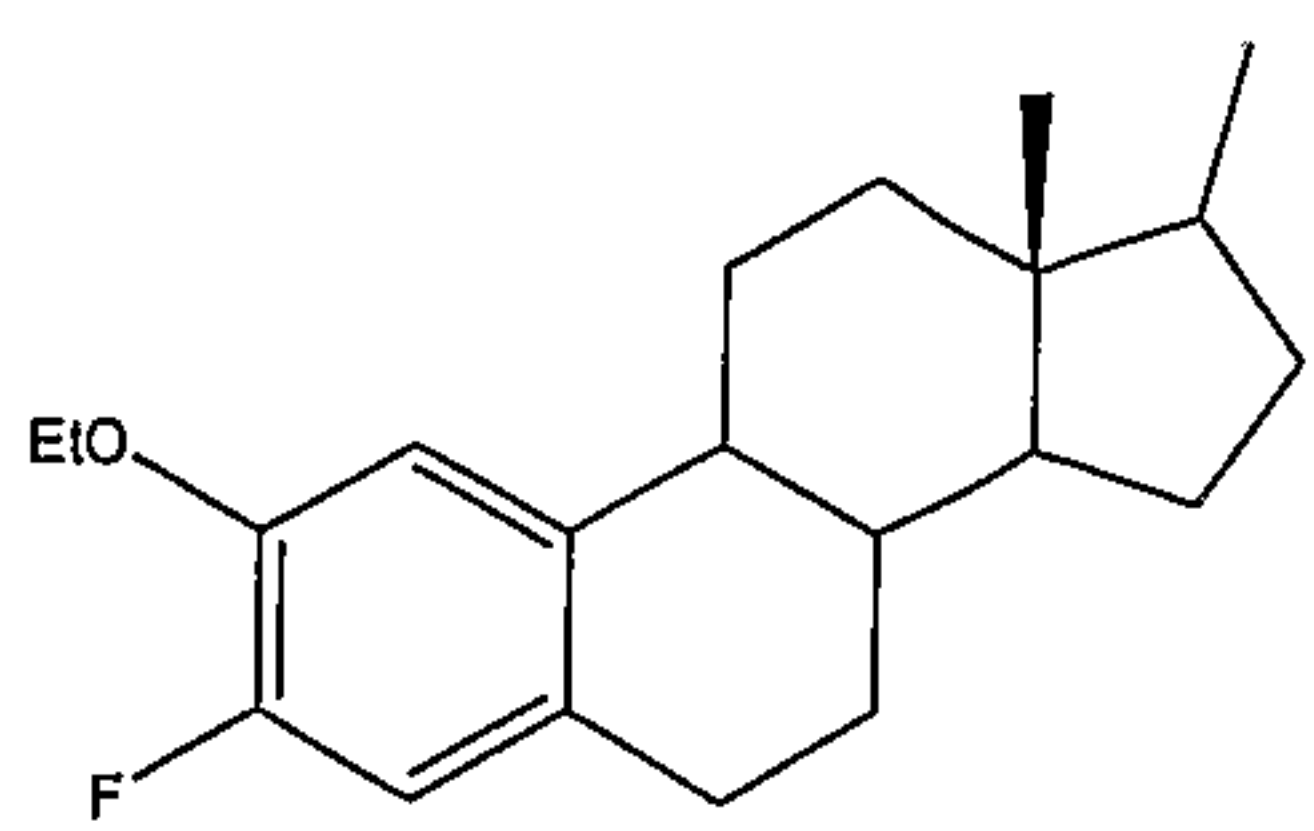
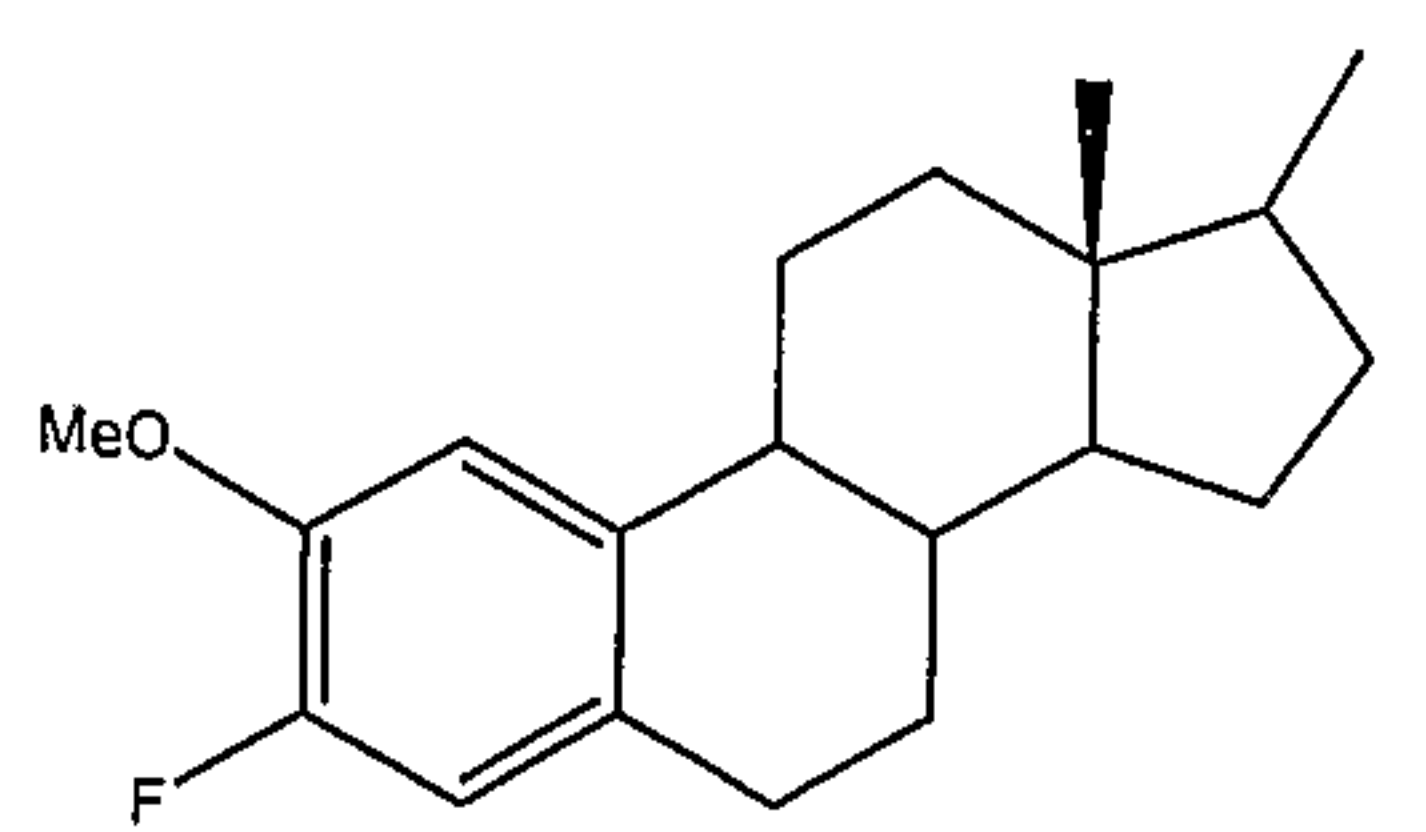




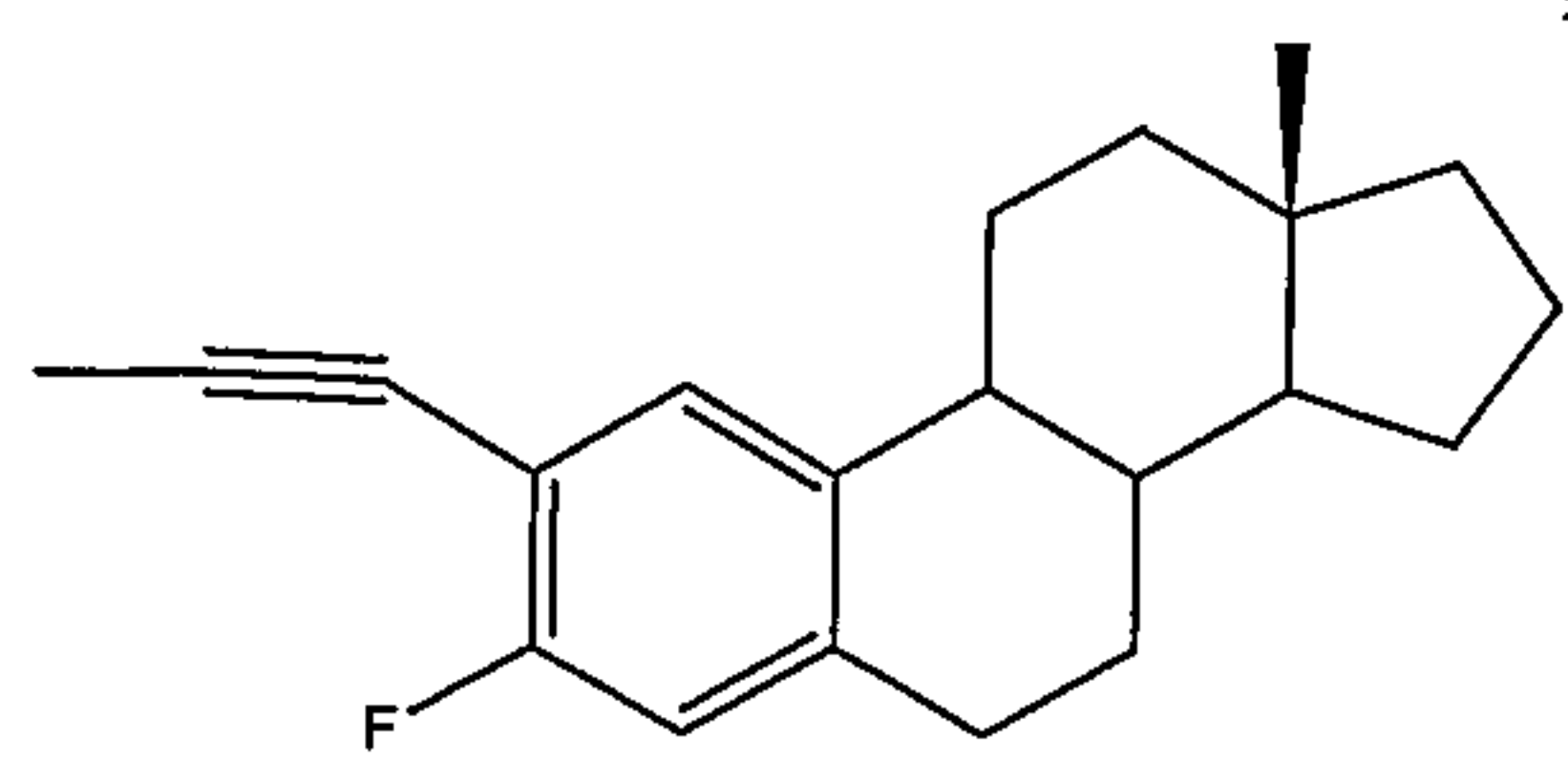


WO 2005/030120

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OR



168. The method of Claim 167, wherein the blood or blood vessel disease is vein occlusion.
- 5 168. The method of Claim 167, wherein the blood or blood vessel disease is artery occlusion.
169. The method of Claim 167, wherein the blood or blood vessel disease is carotid obstructive disease.
- 10 170. The method of Claim 167, wherein the blood or blood vessel disease is polyarteritis.
171. The method of Claim 167, wherein the blood or blood vessel disease is atherosclerosis.
- 15 172. The method of Claim 167, wherein the blood or blood vessel disease is Osler-Weber-Rendu disease.
173. The method of Claim 167, wherein the blood or blood vessel disease is sickle cell anemia.
- 20 174. The method of Claim 167, wherein the blood or blood vessel disease is leukemia.
- 25 175. The method of Claim 167, wherein the blood or blood vessel disease is an acute or chronic neoplastic disease of the bone marrow.
176. The method of Claim 175, wherein the acute or chronic neoplastic disease of the bone marrow is multiple myeloma.
- 30 177. The method of Claim 175, wherein the acute or chronic neoplastic disease of the bone marrow is myelo dysplastic syndrome.

178. The method of Claim 167, wherein the blood or blood vessel disease is a hemangioma.

5 179. The method of Claim 167, wherein the blood or blood vessel disease is a hereditary hemorrhagic telangiectasia.

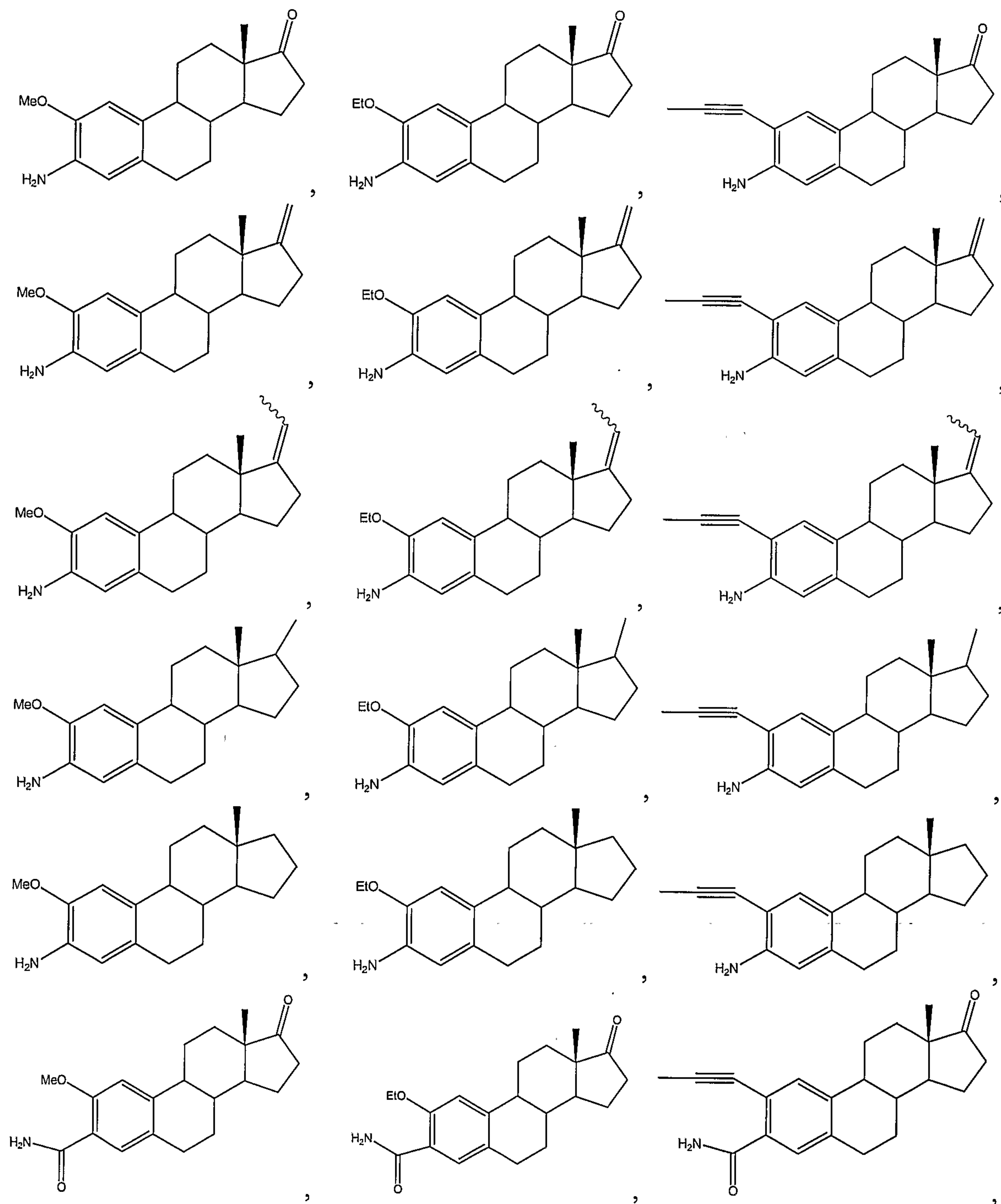
180. The method of Claim 167, wherein the blood or blood vessel disease is a disease of the bone marrow.

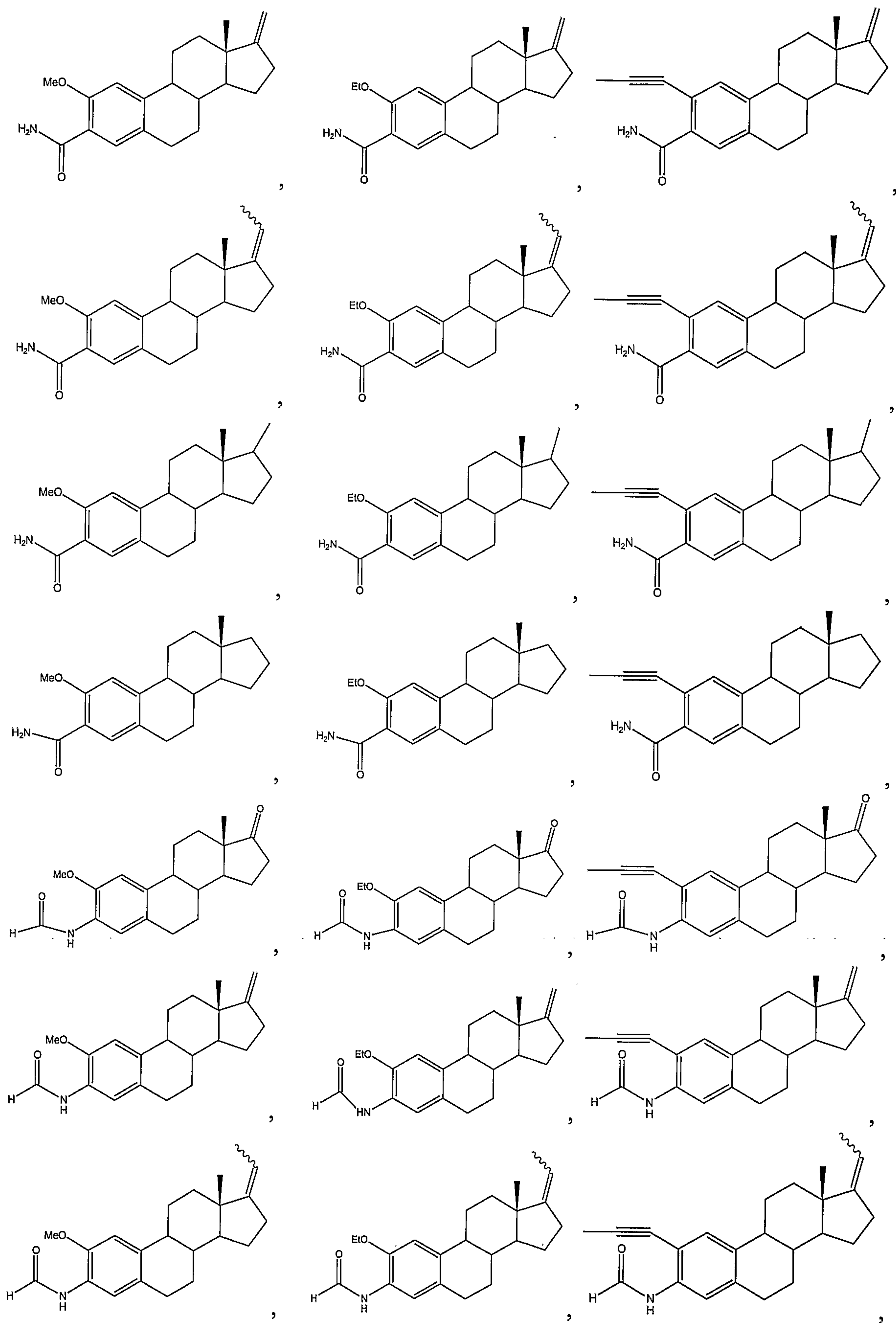
10 181. The method of Claim 167, wherein the blood or blood vessel disease is anemia.

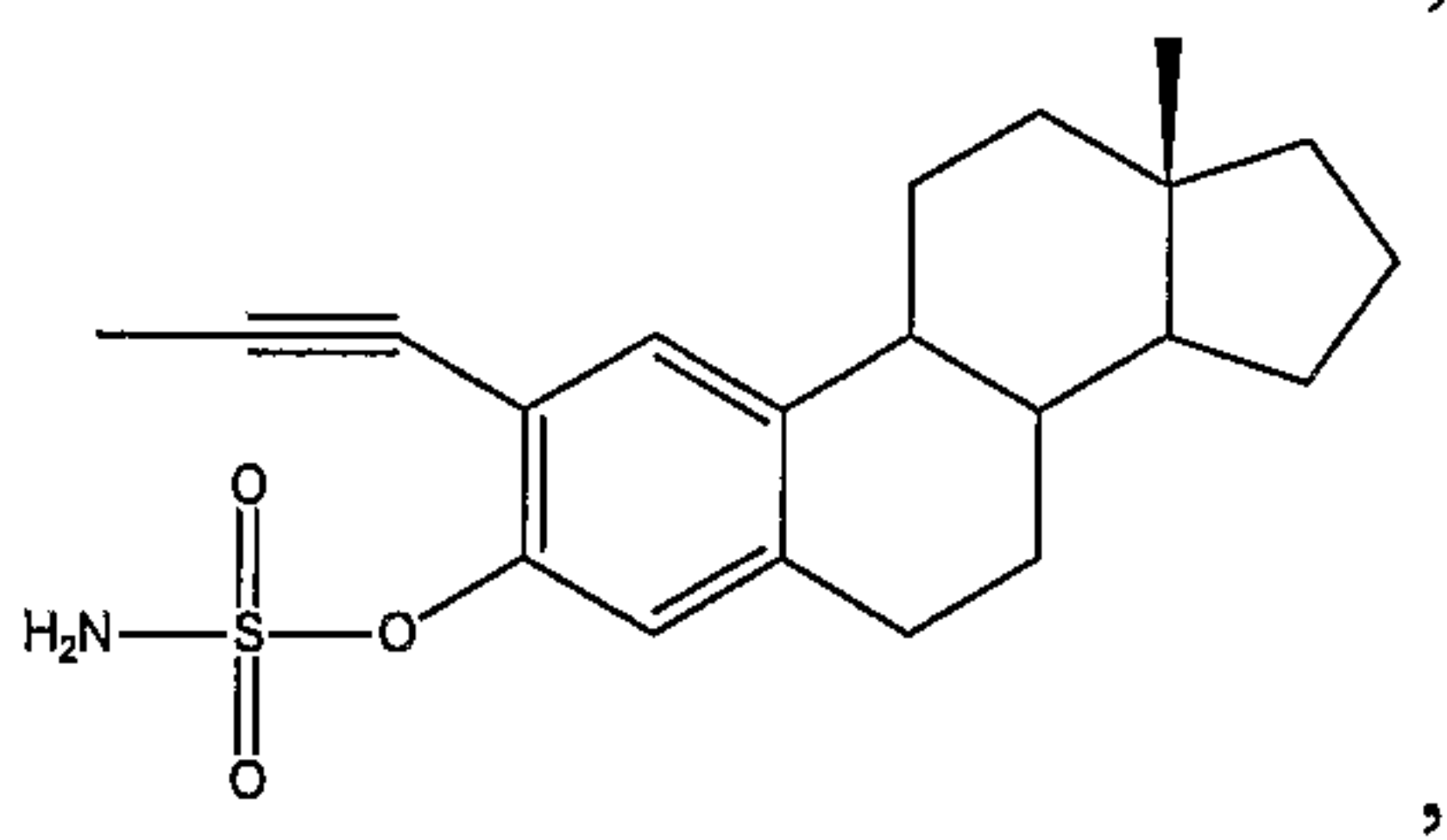
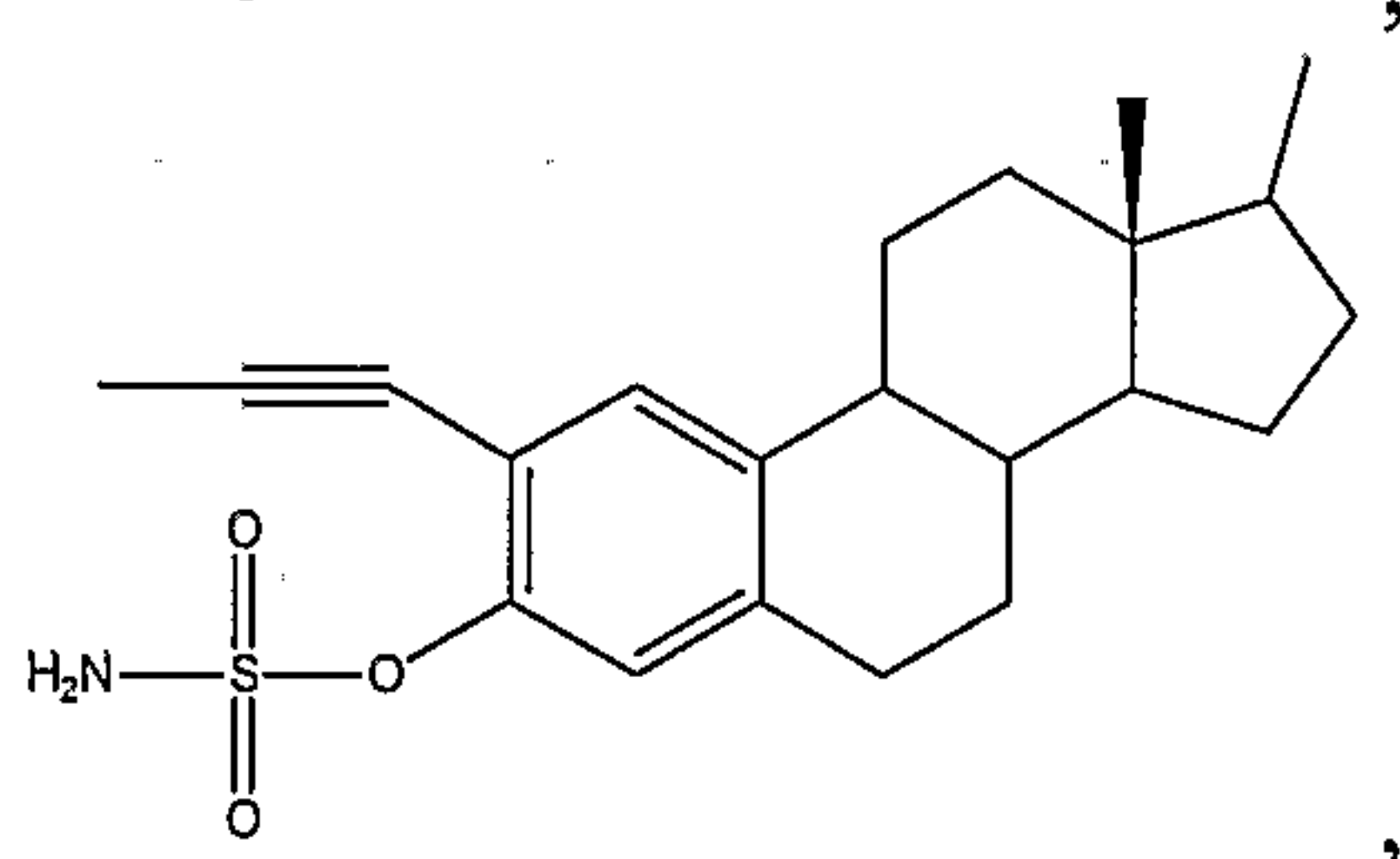
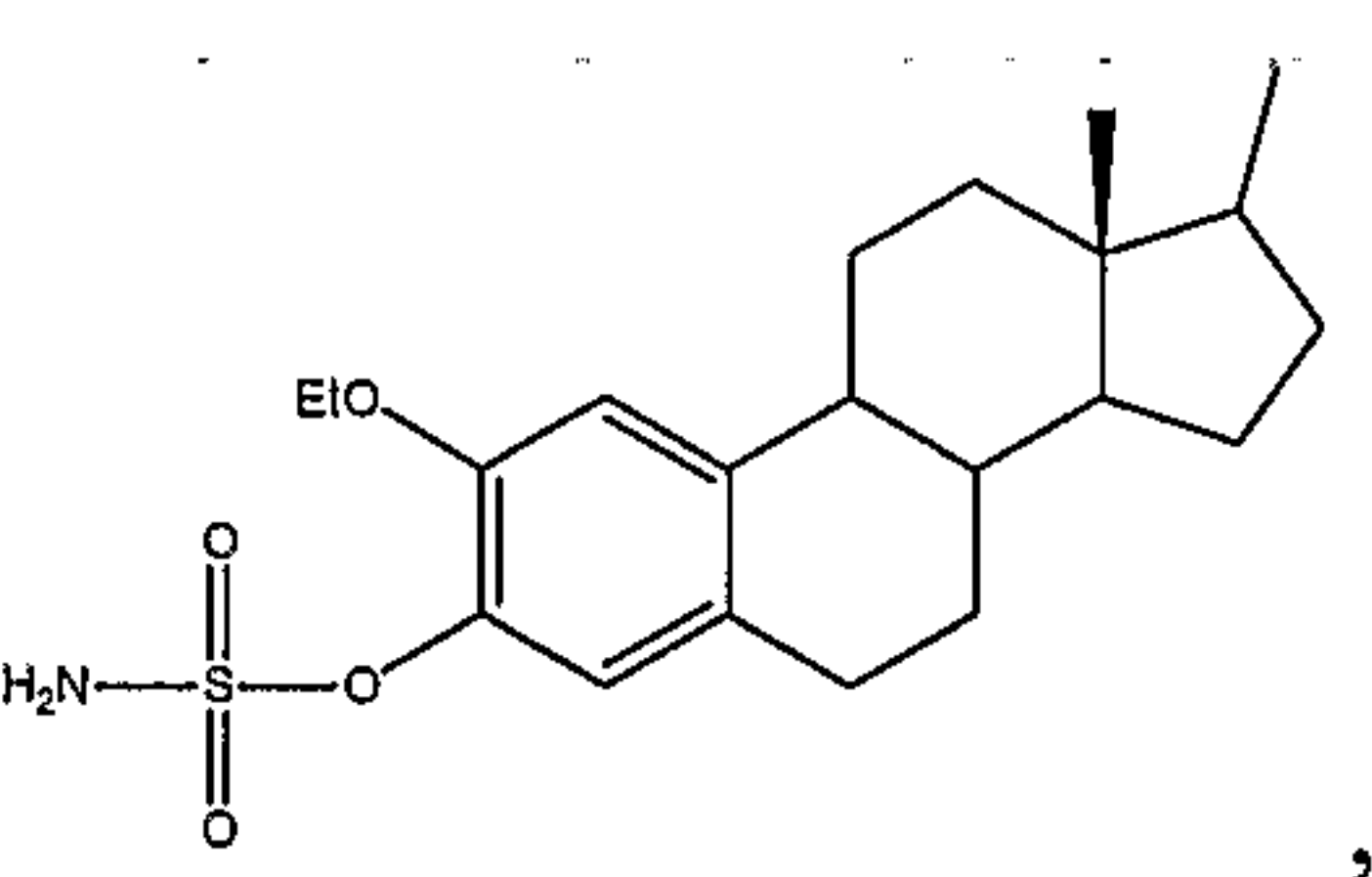
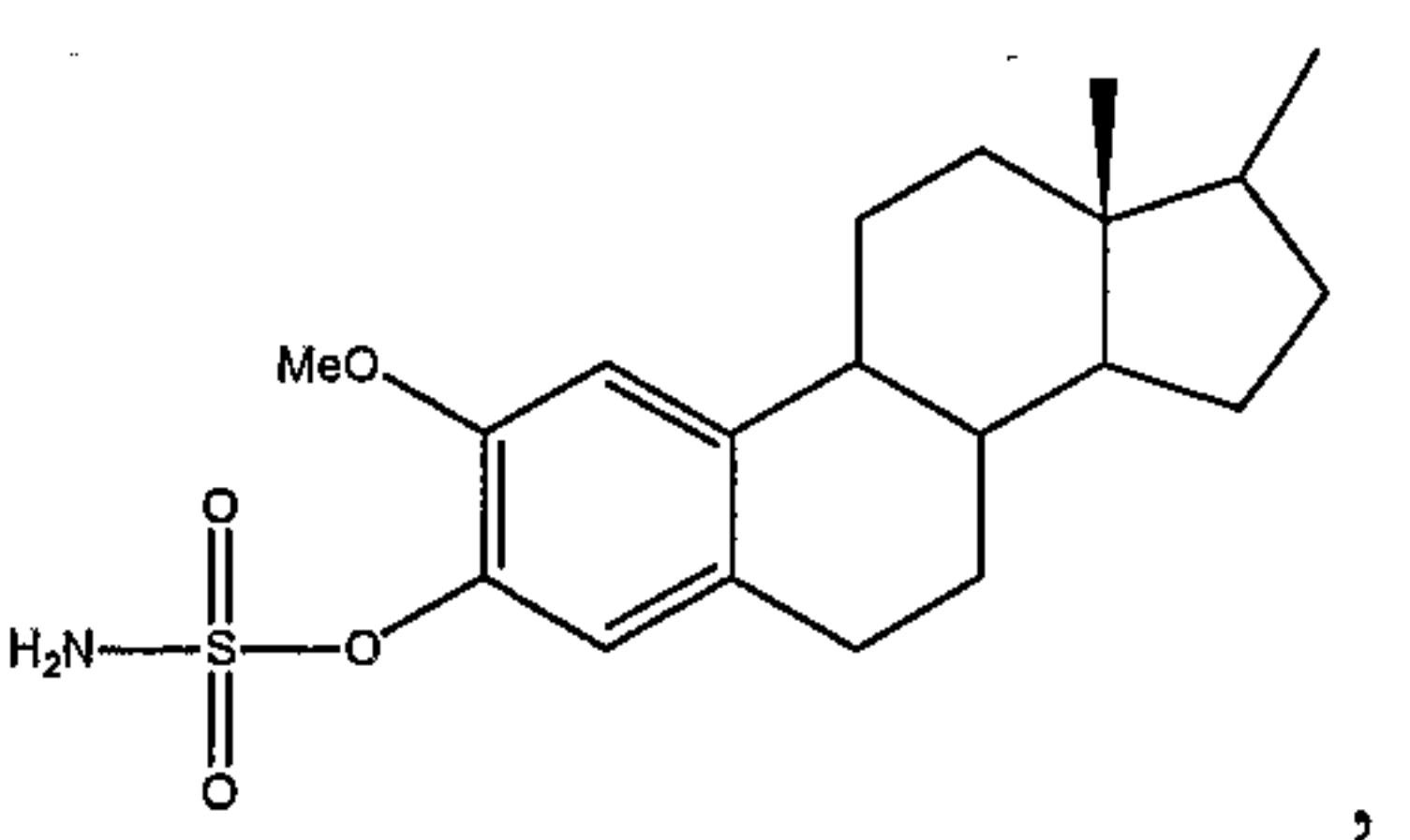
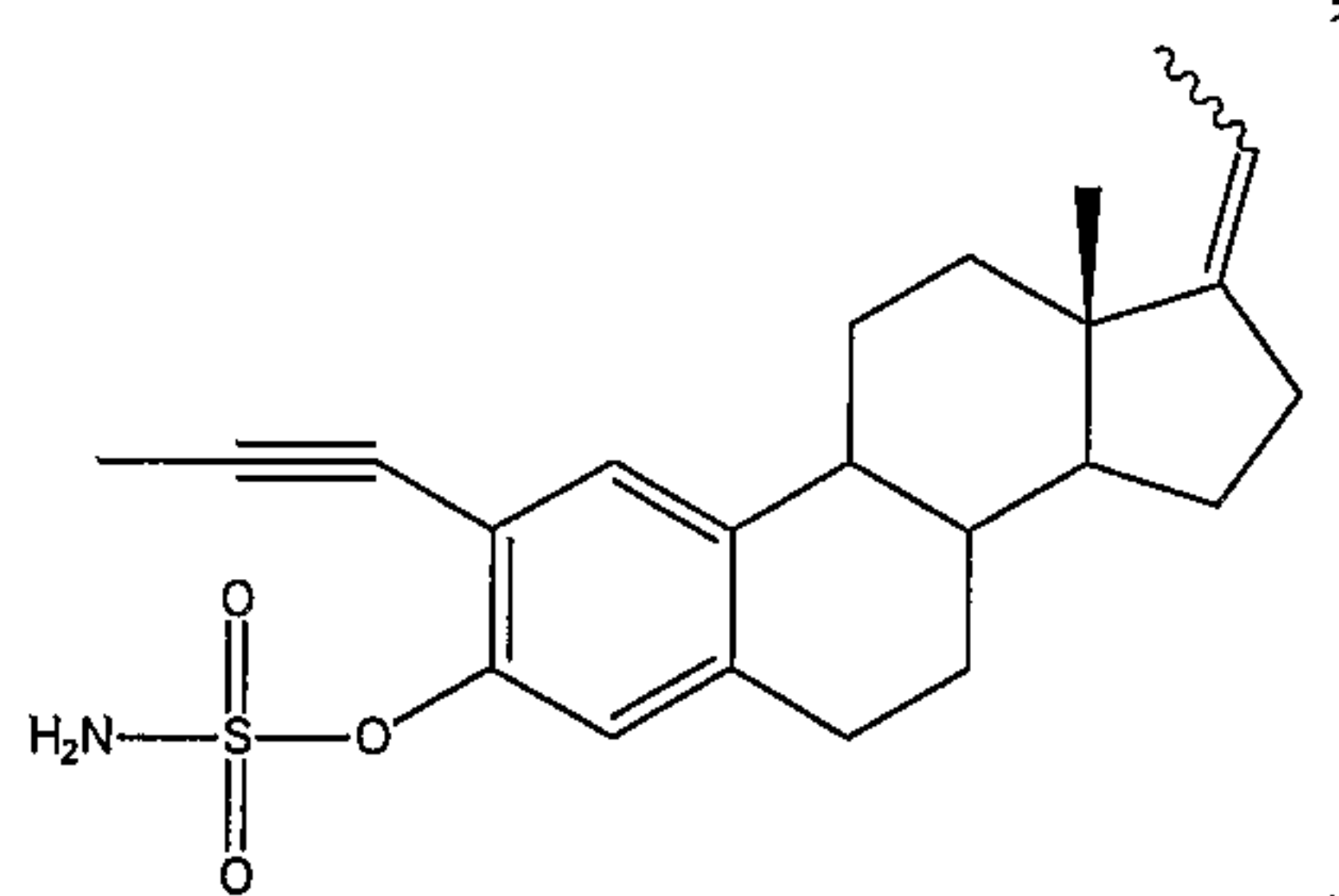
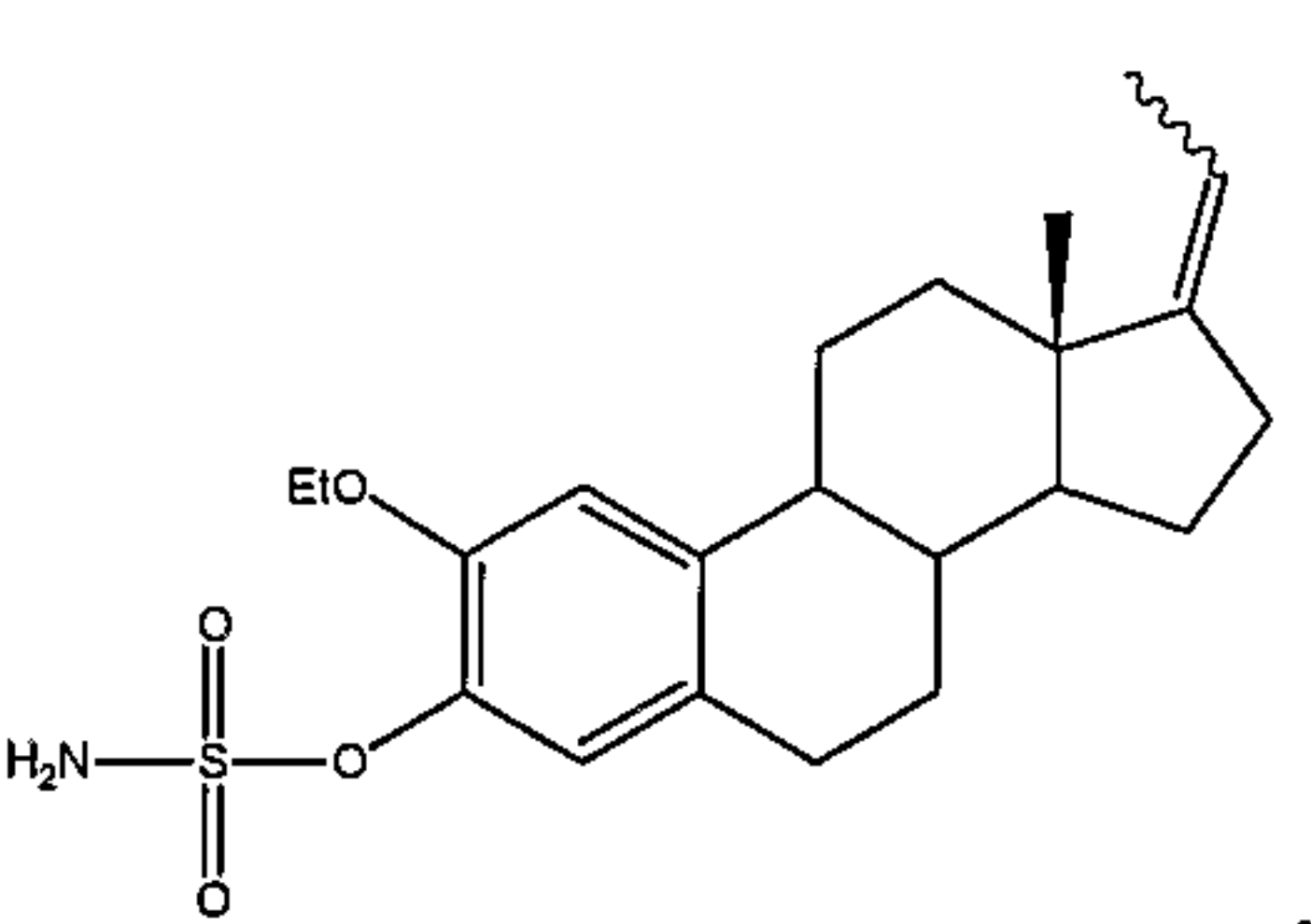
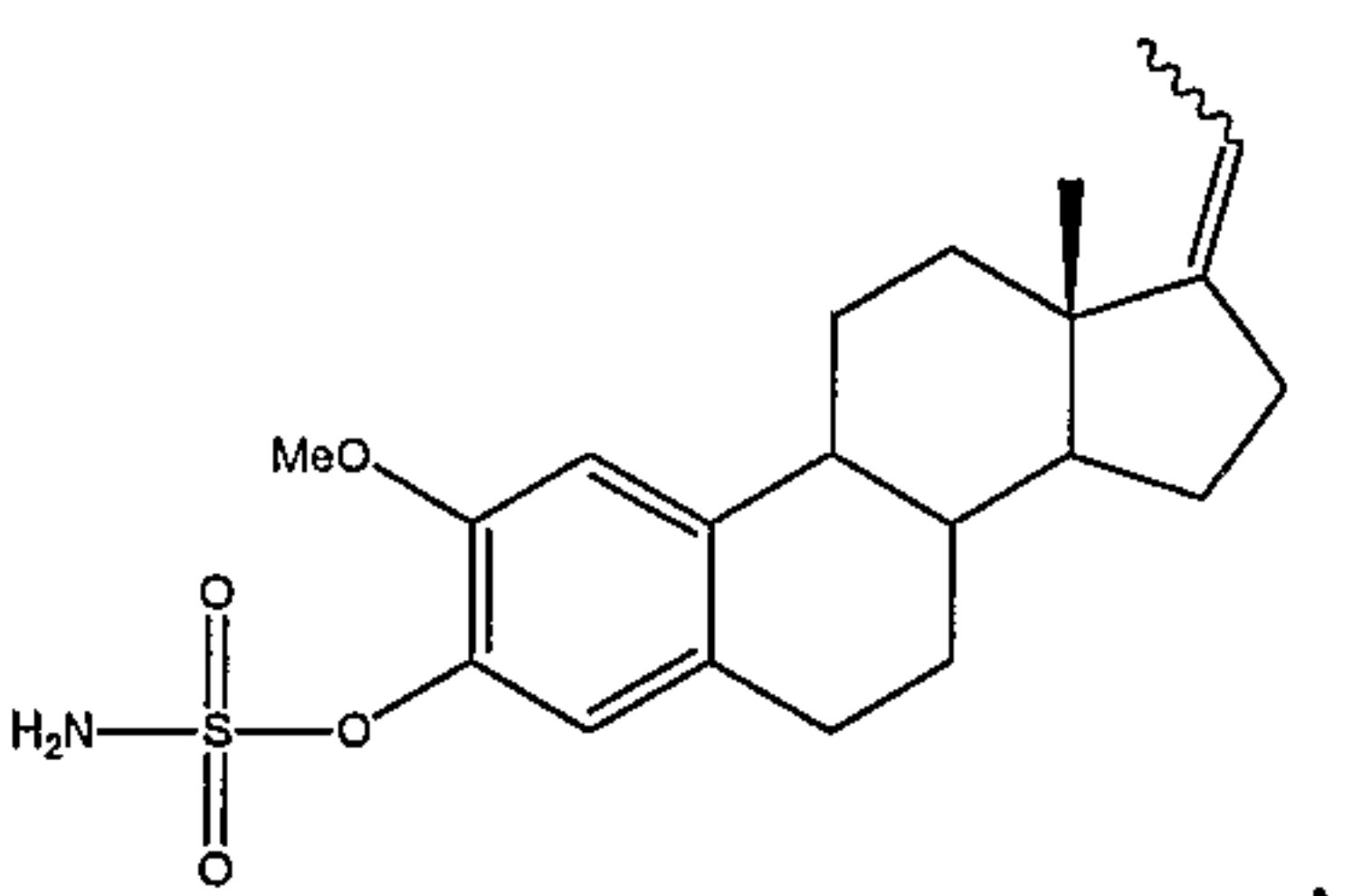
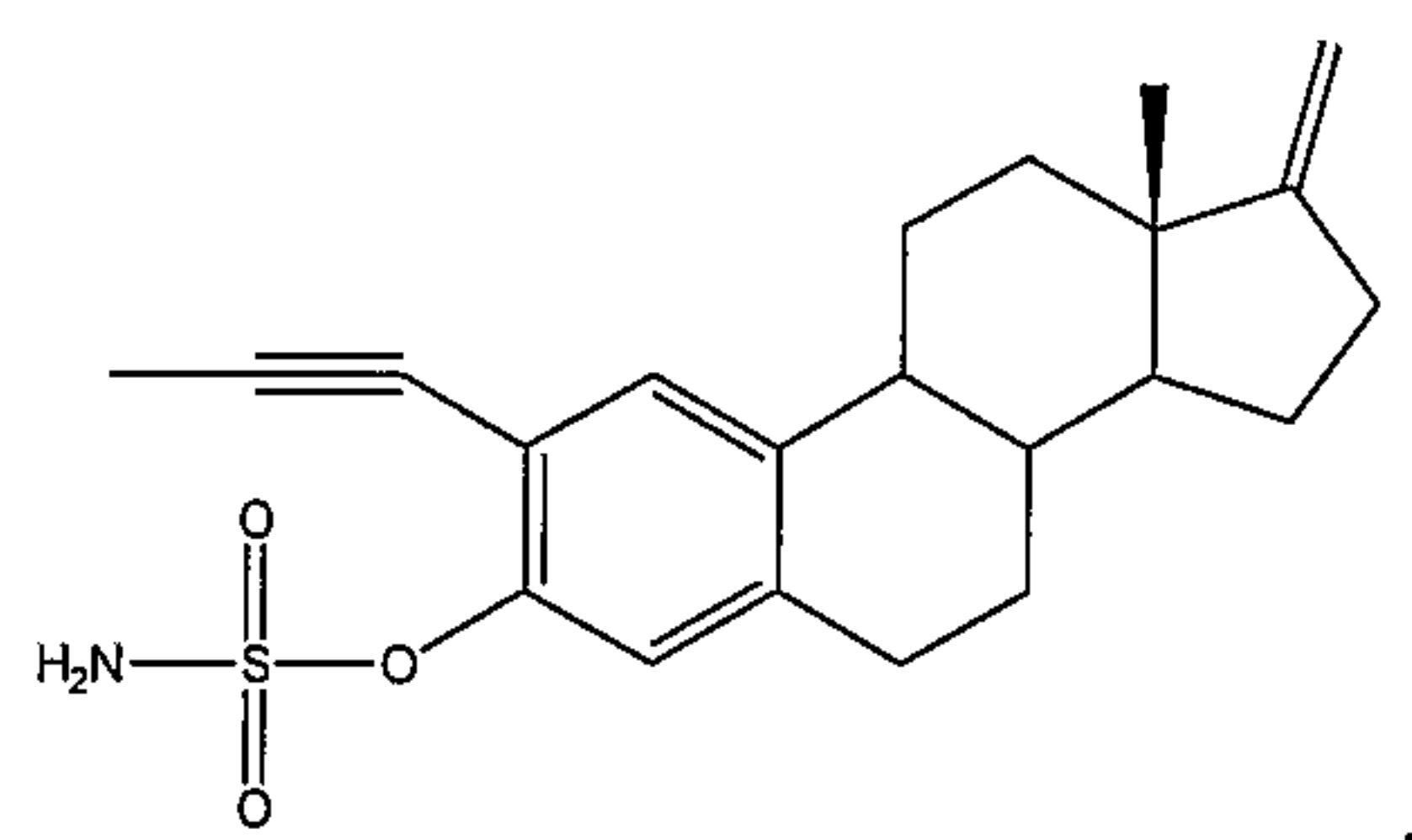
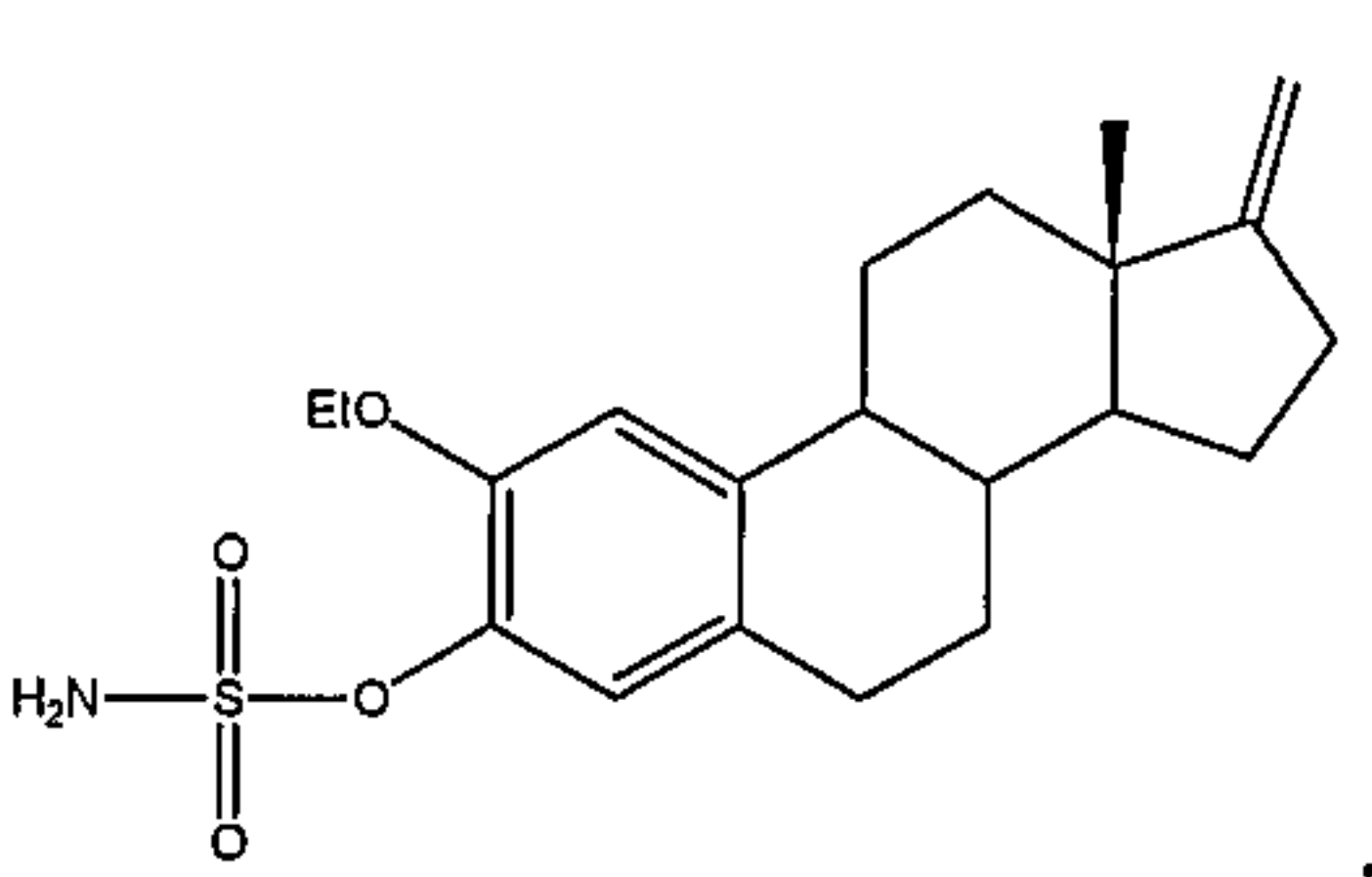
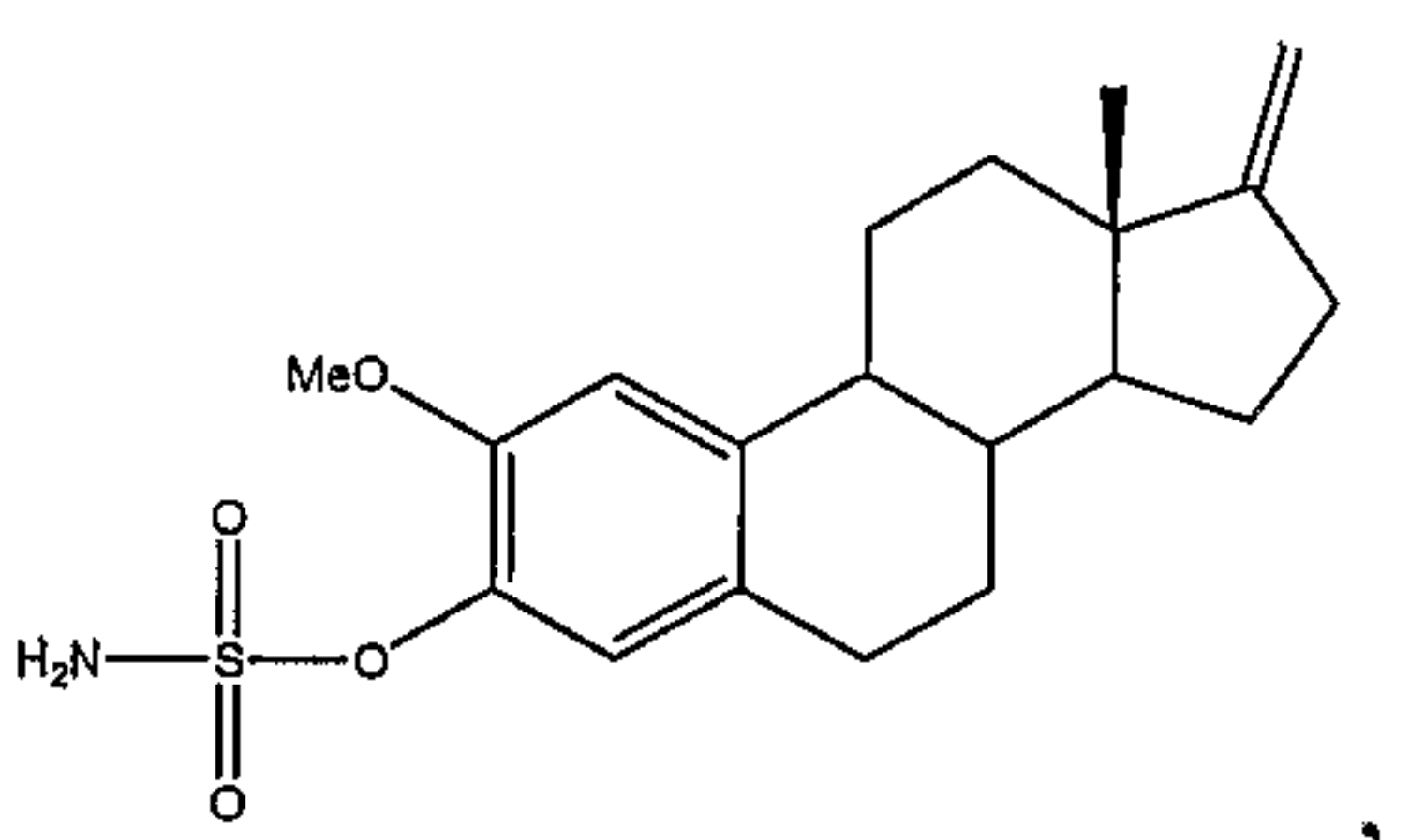
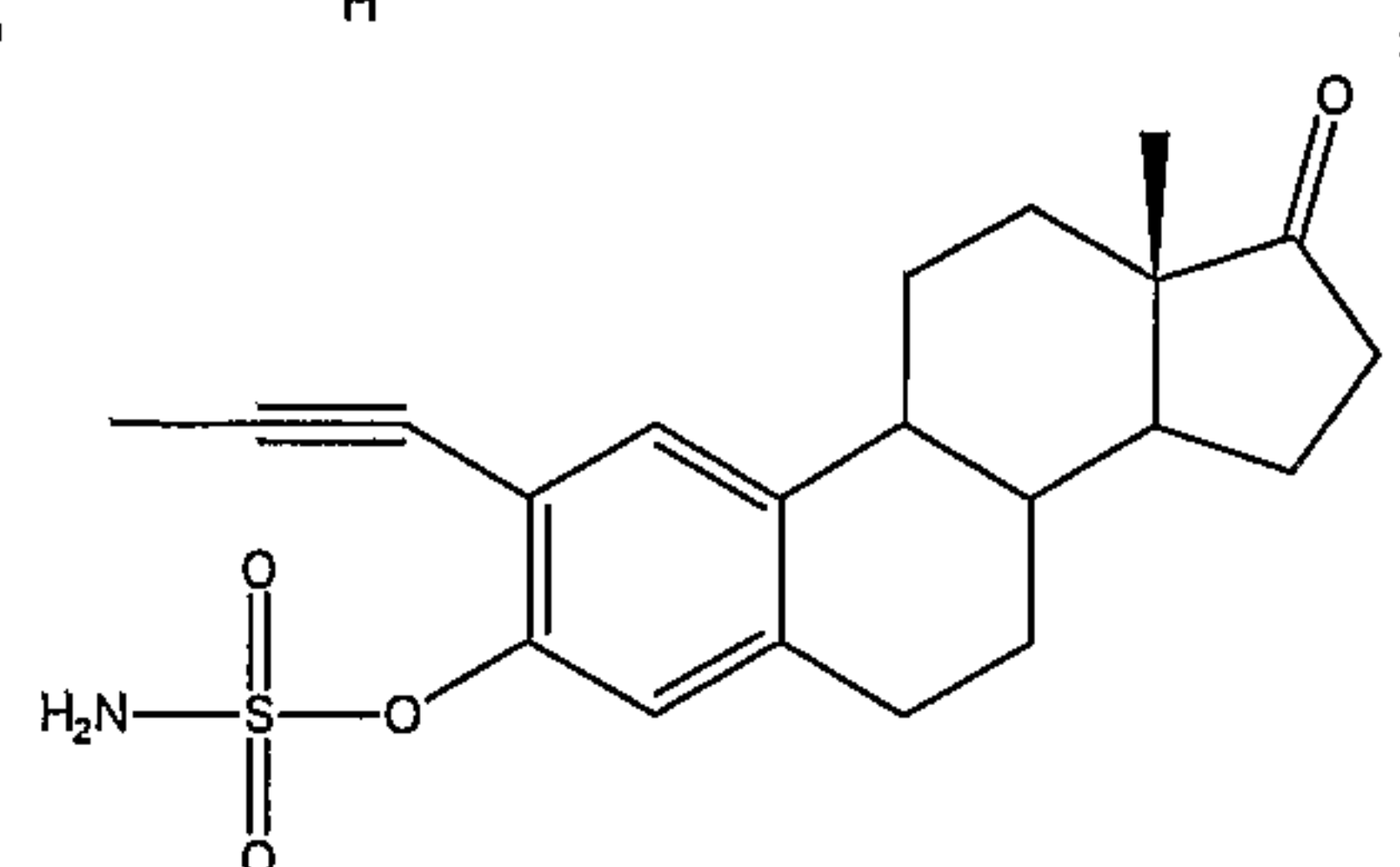
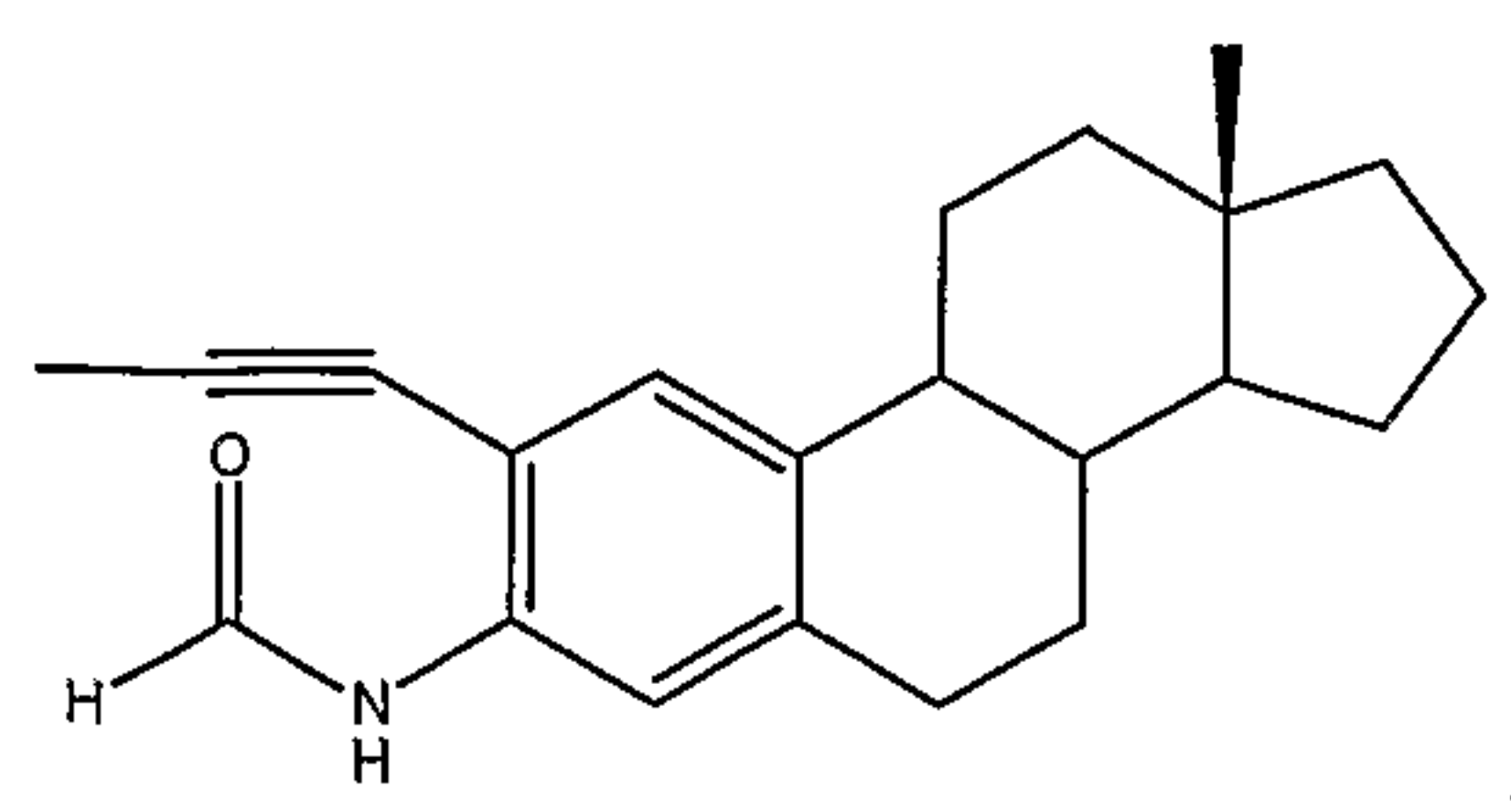
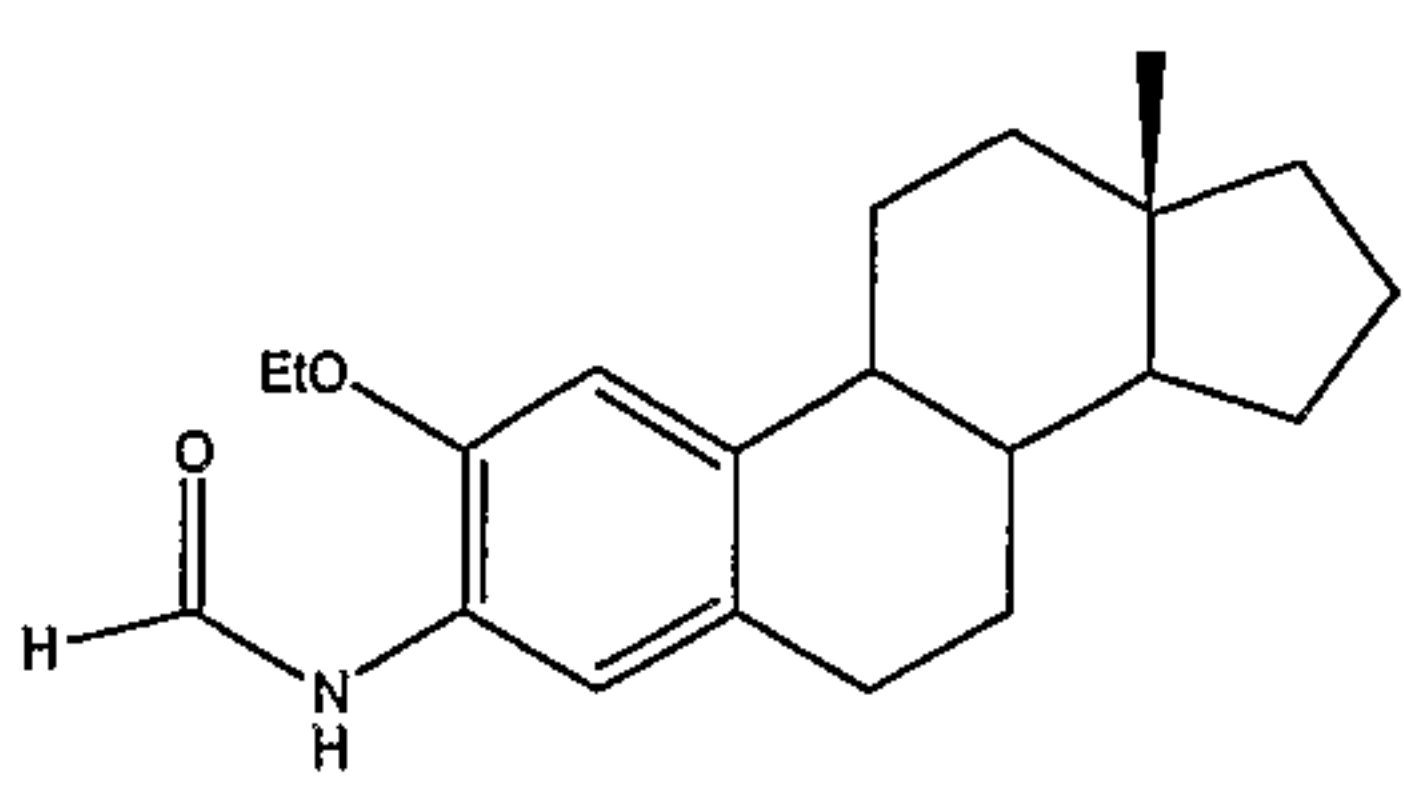
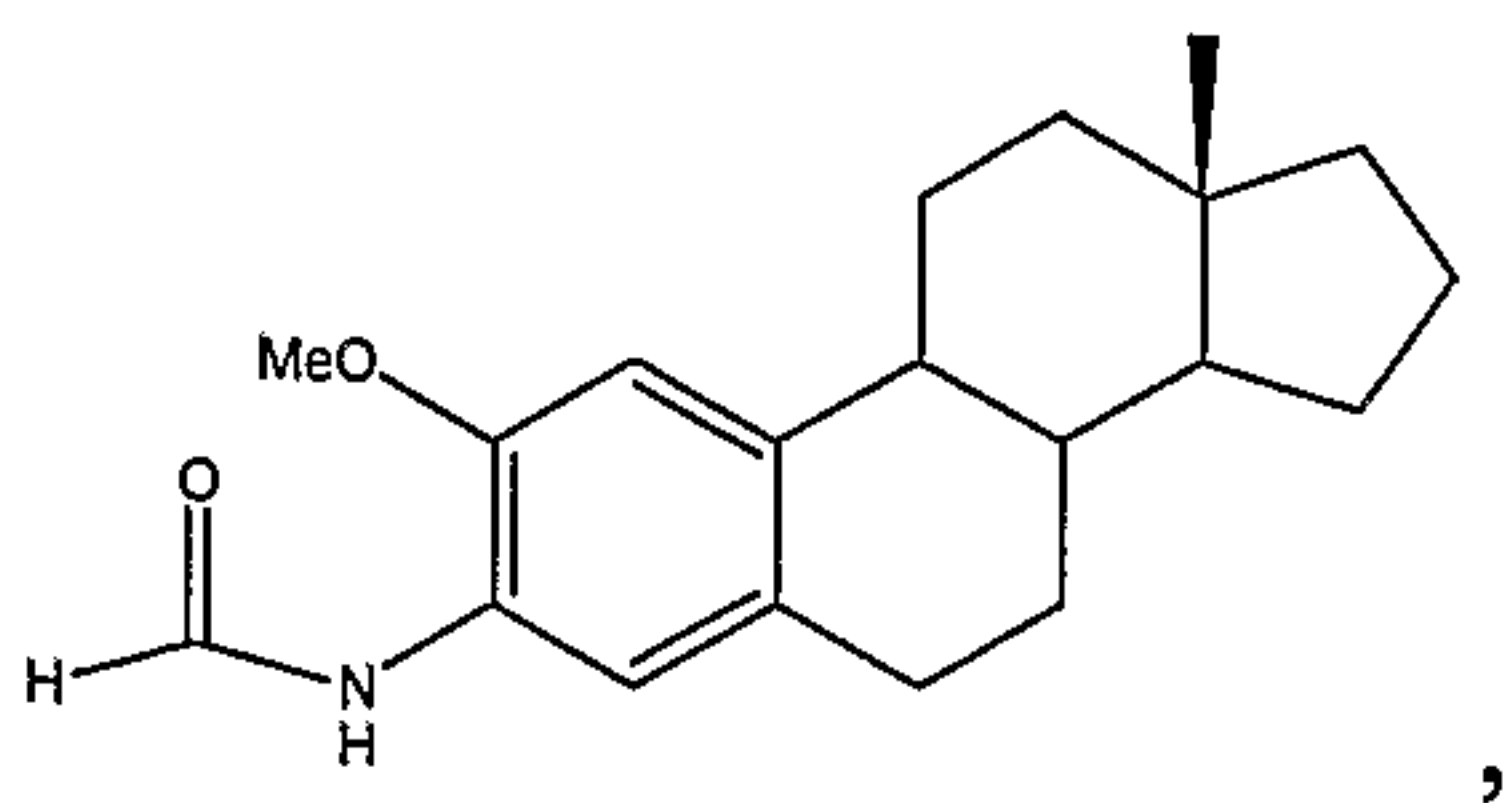
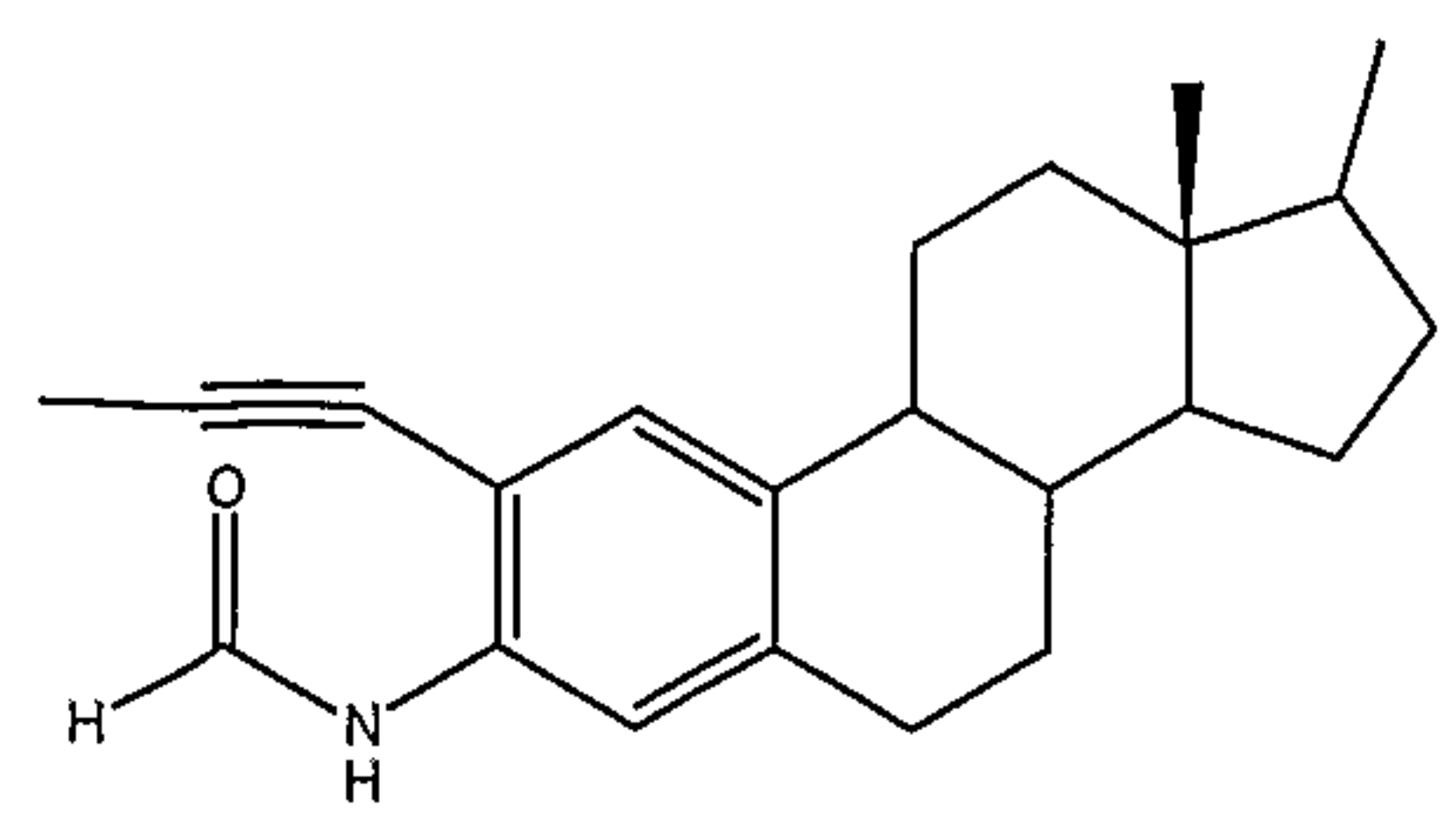
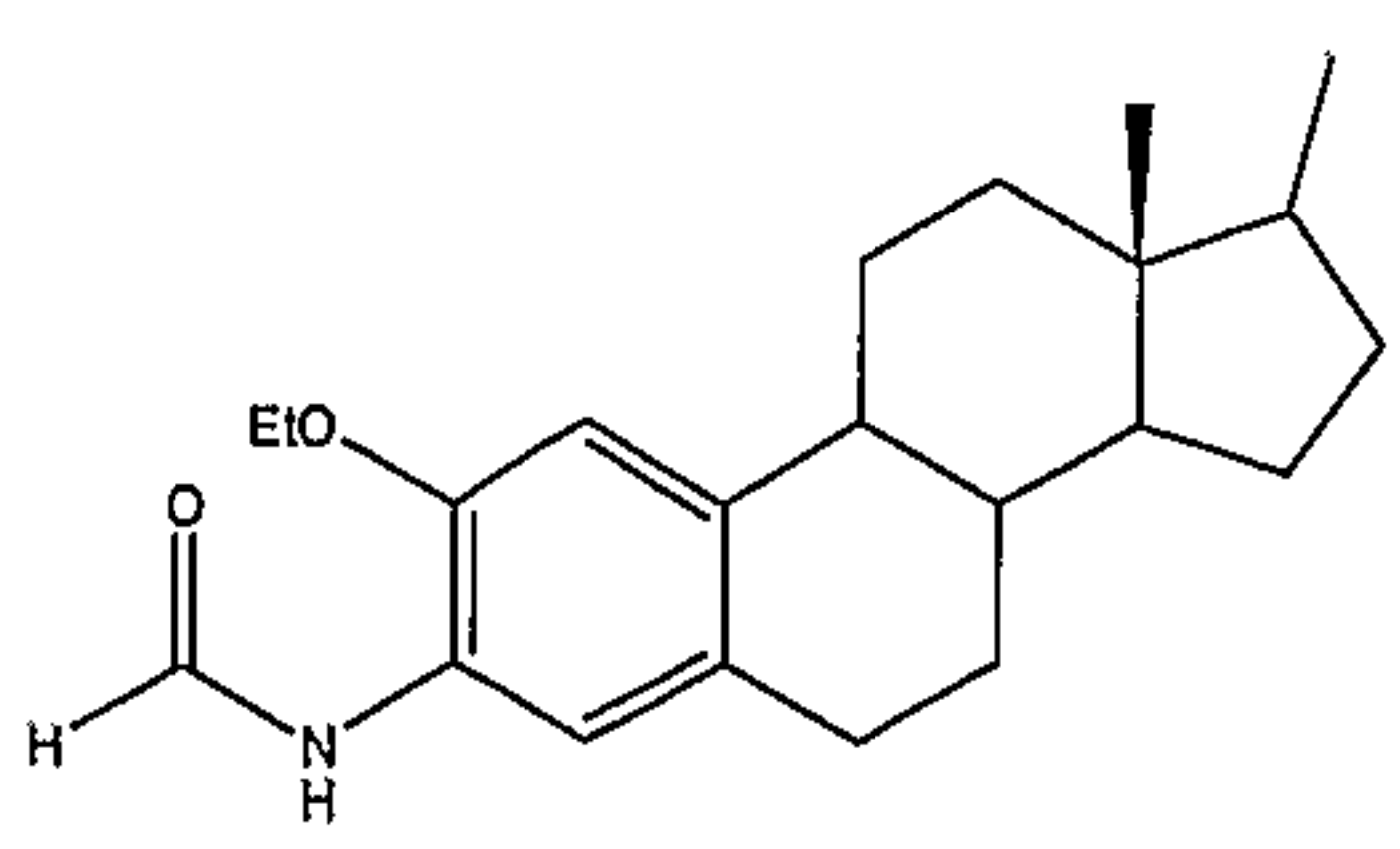
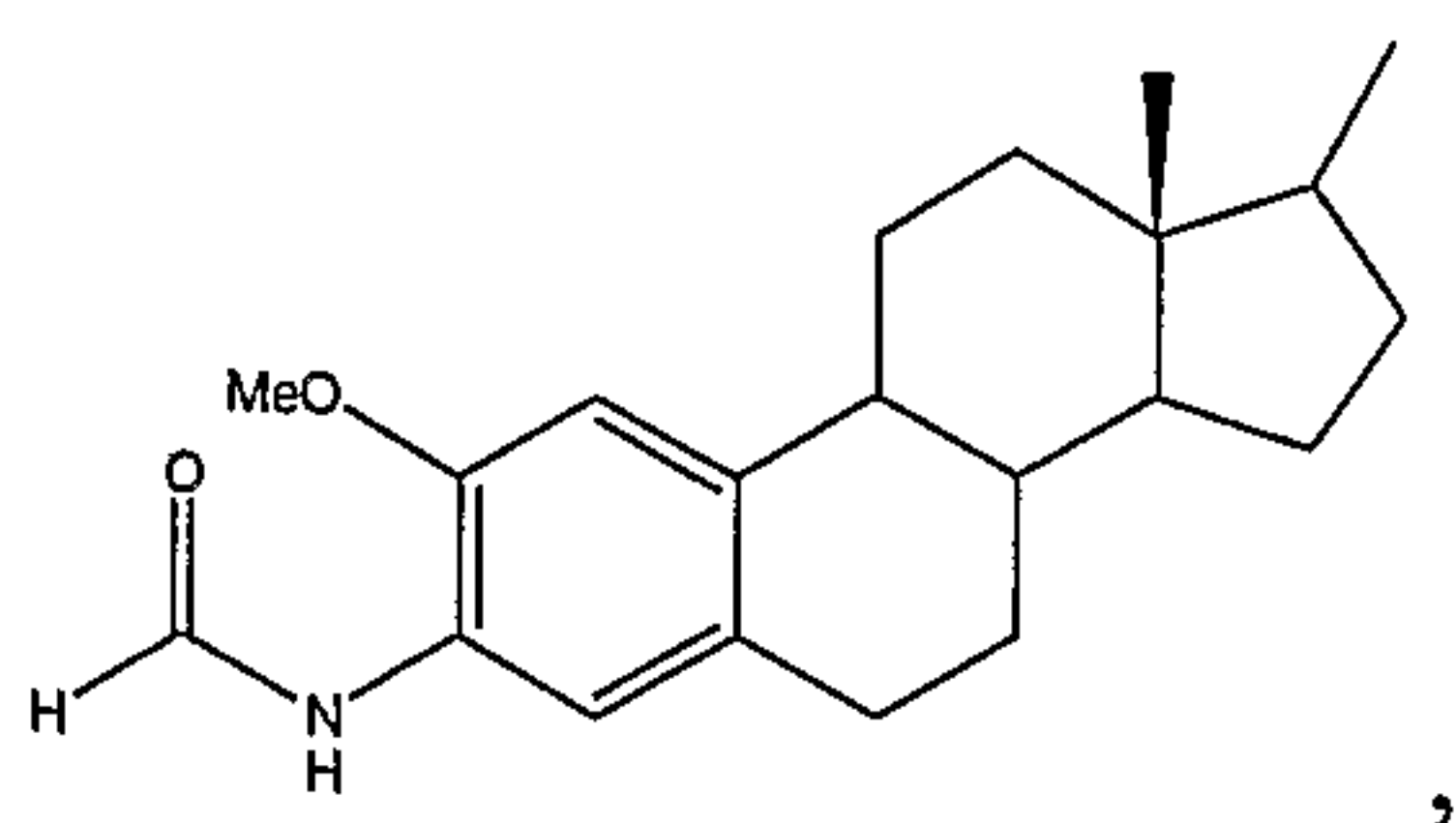
182. The method of Claim 167, wherein the blood or blood vessel disease is impaired blood clotting.

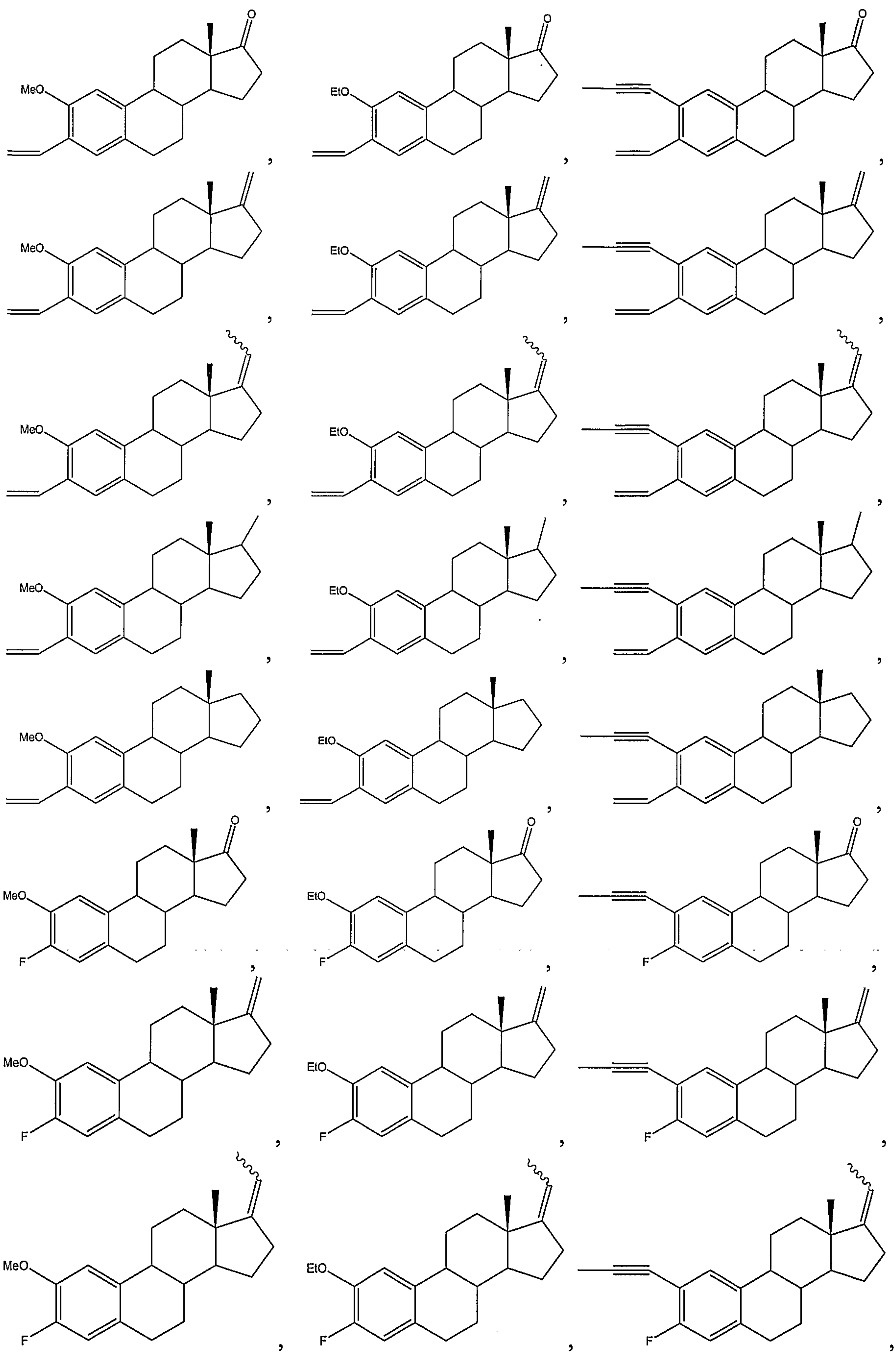
15 183. The method of Claim 167, wherein the blood or blood vessel disease is enlargement of the lymph nodes, liver, or spleen.

184. A method of treating a skin condition in a human or an animal comprising administering to the human or animal a compound selected from



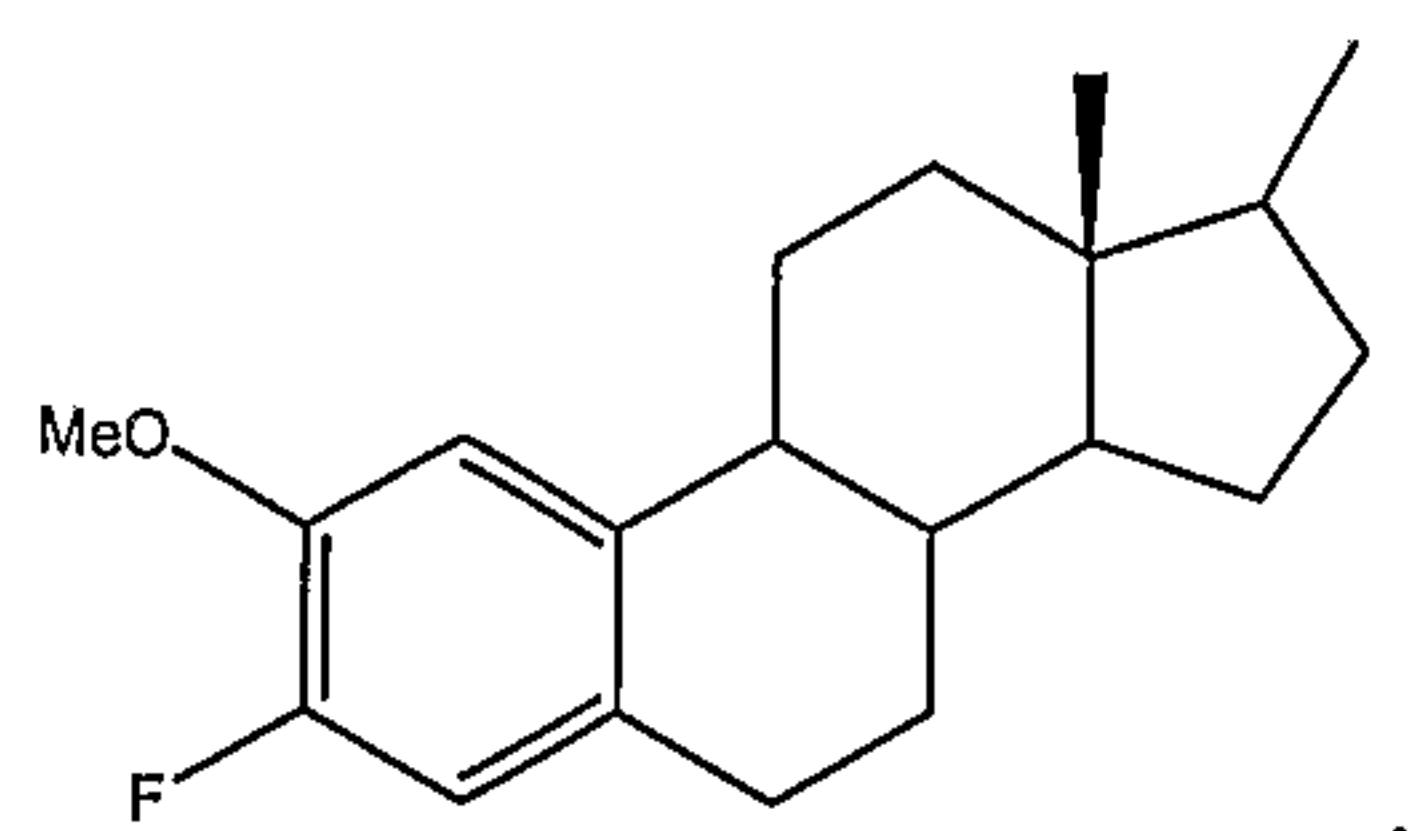




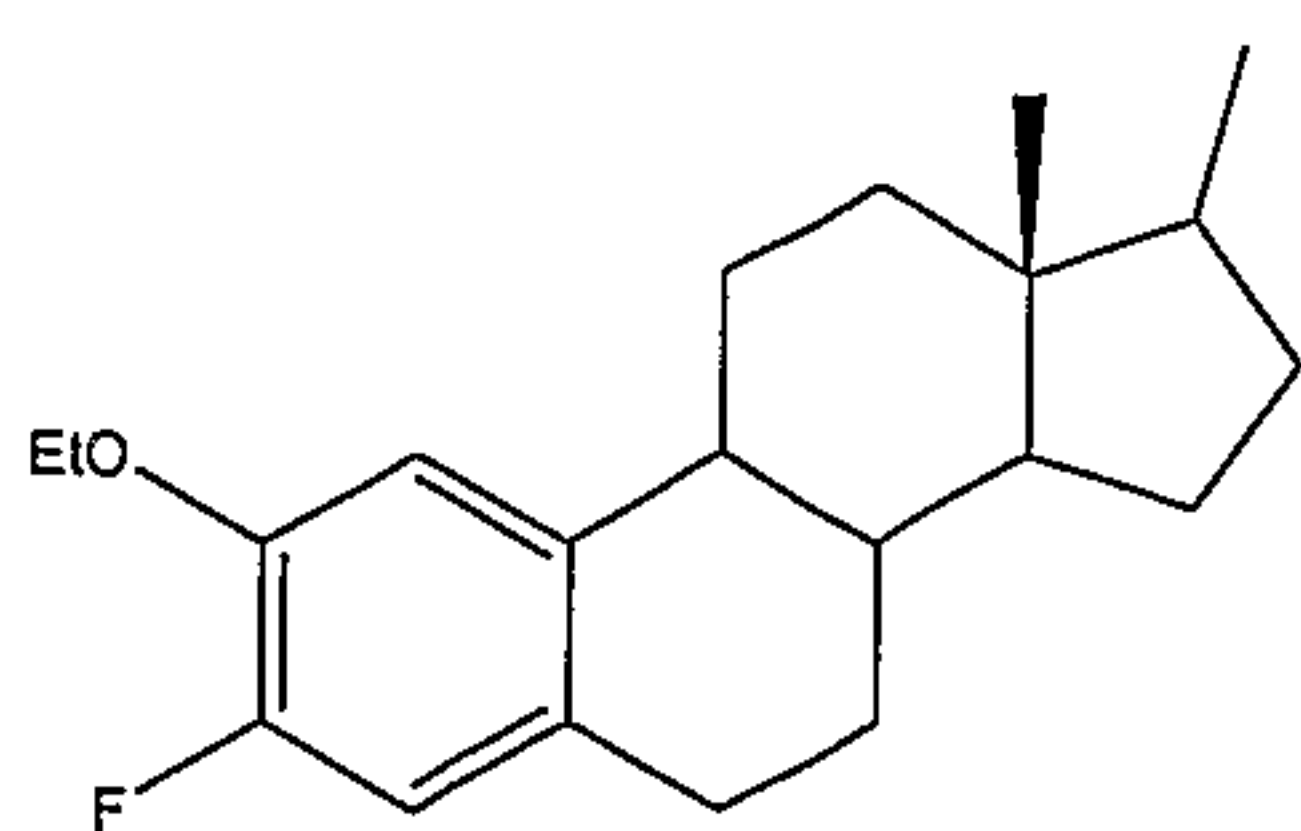


WO 2005/030120

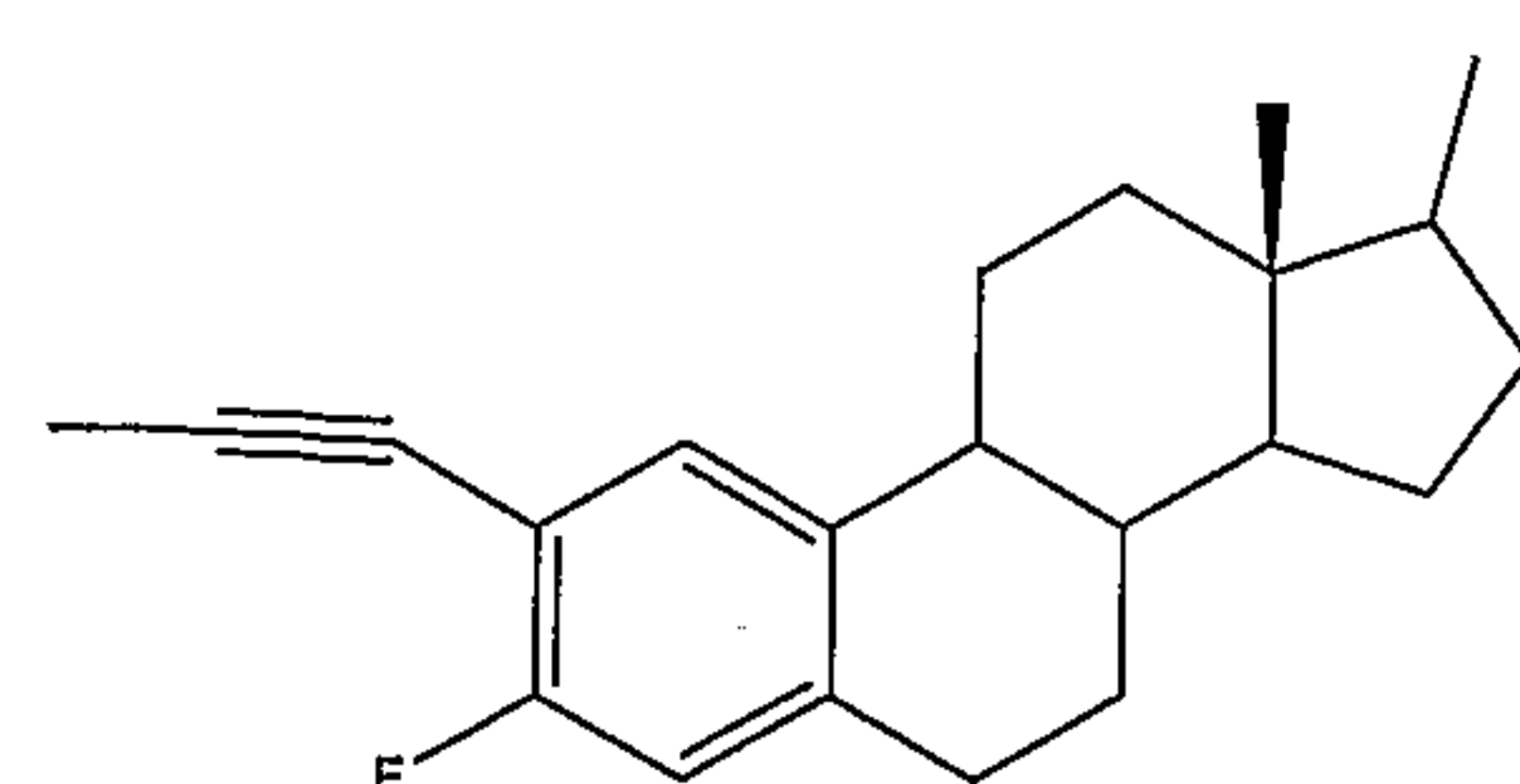
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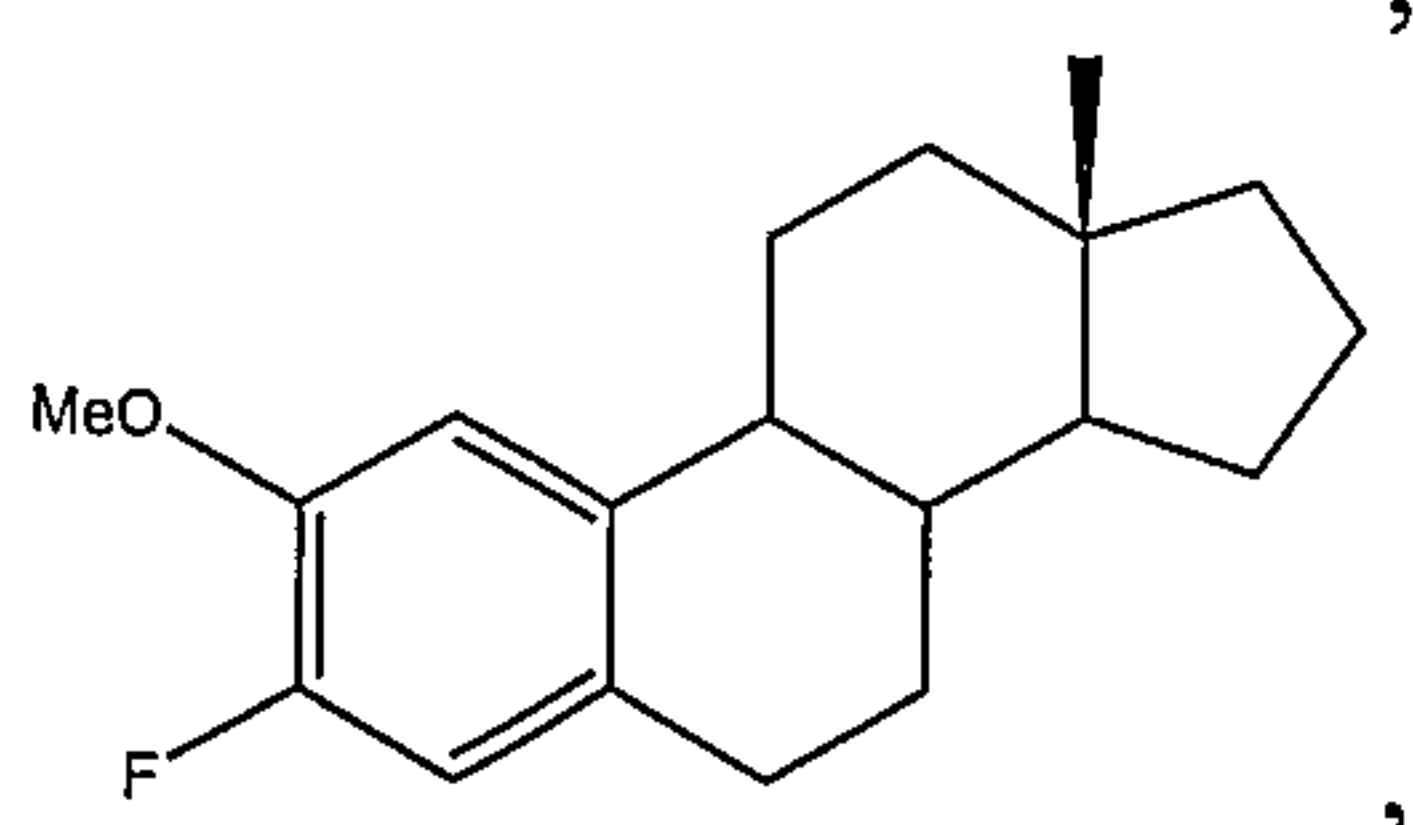
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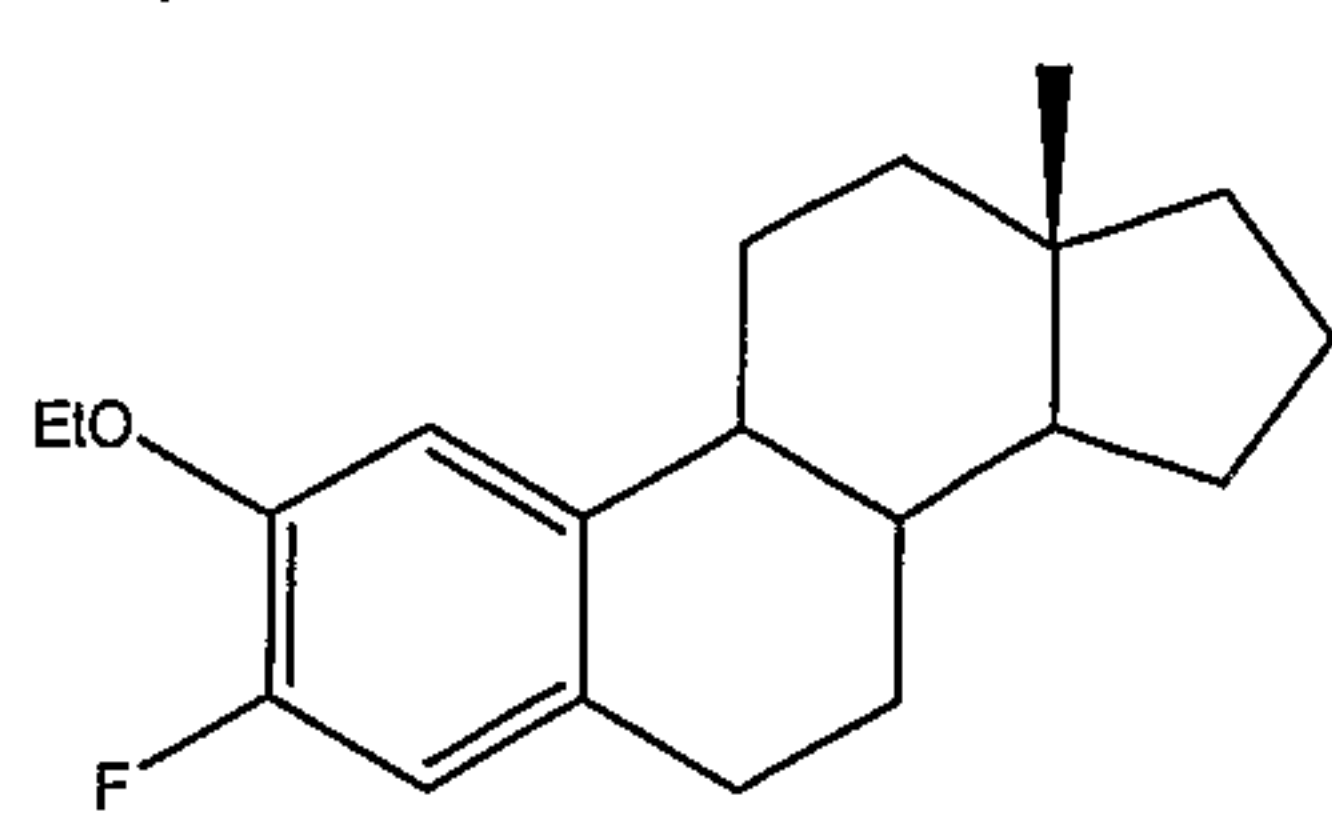
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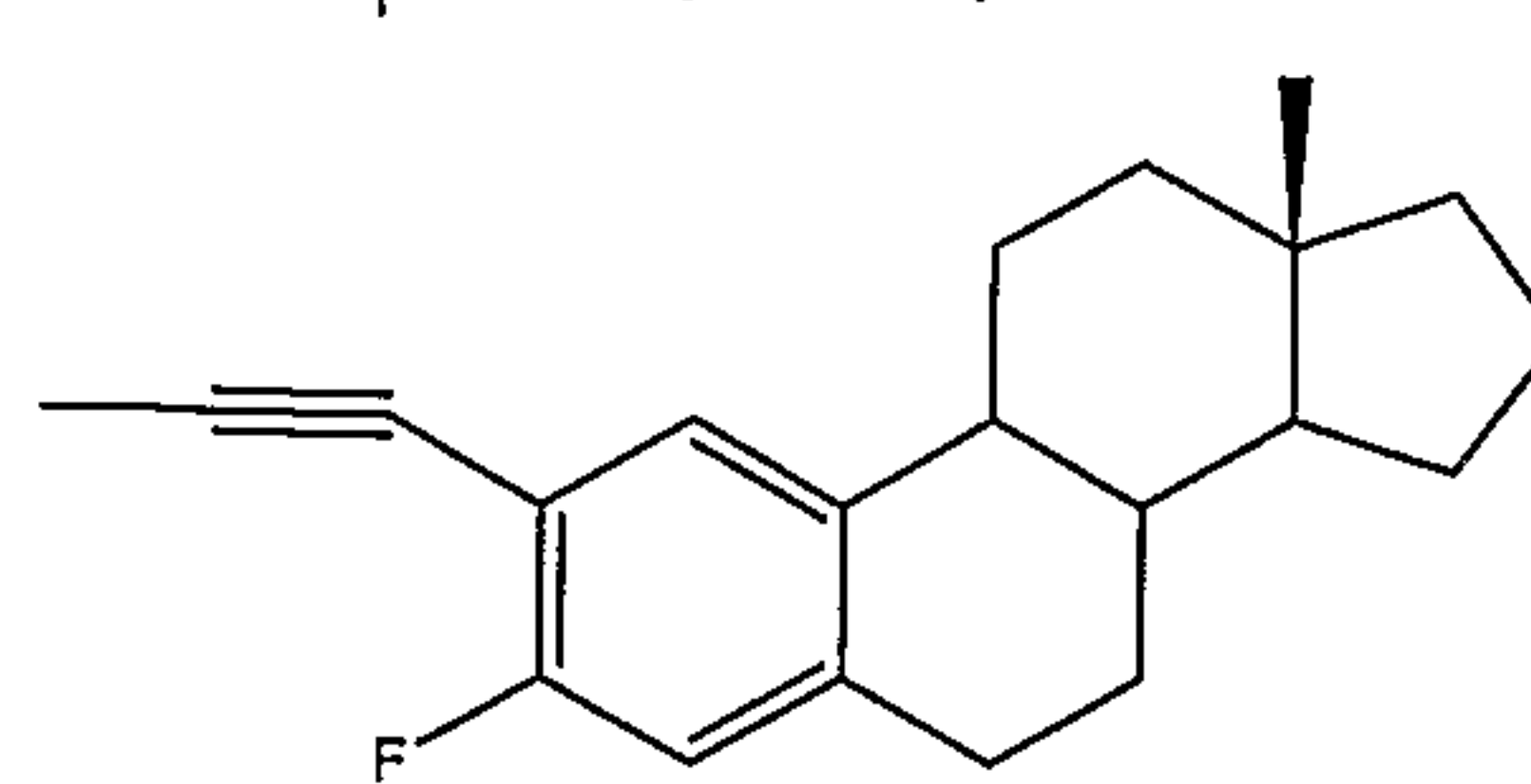
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or



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185. The method of Claim 184, wherein the skin condition is abnormal wound healing.

186. The method of Claim 184, wherein the skin condition is acne rosacea.

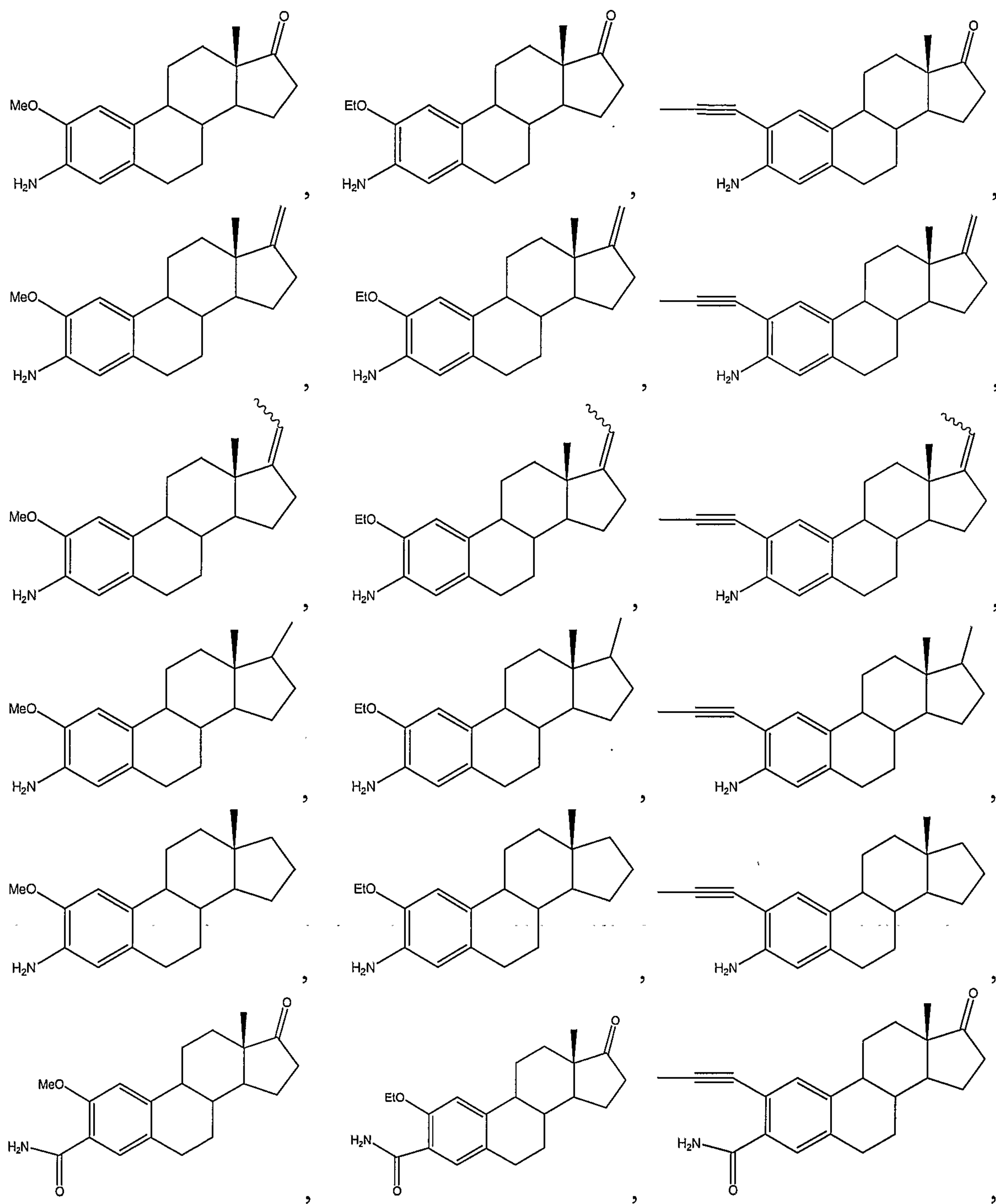
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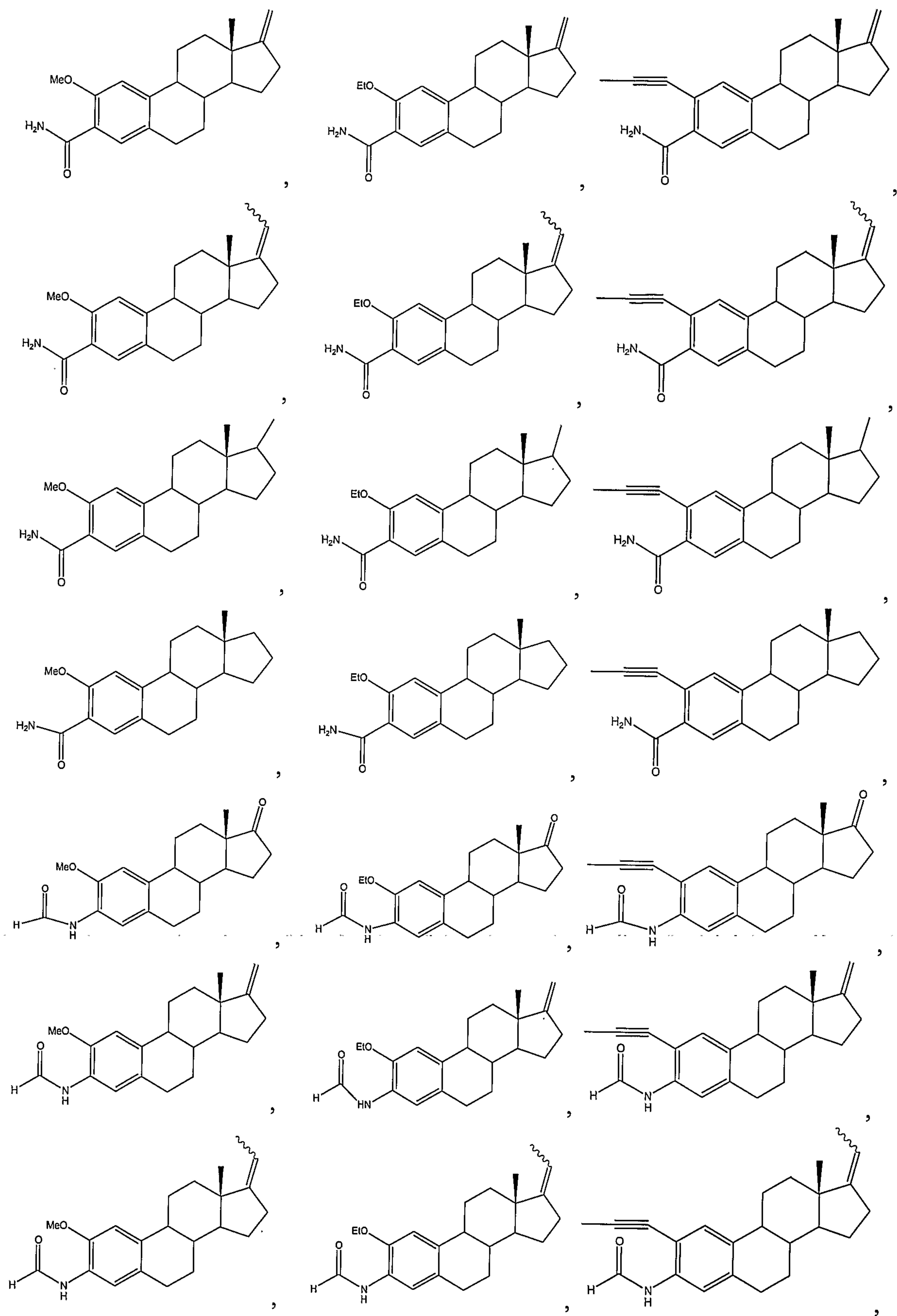
187. The method of Claim 184, wherein the skin condition is due to chemical burns.

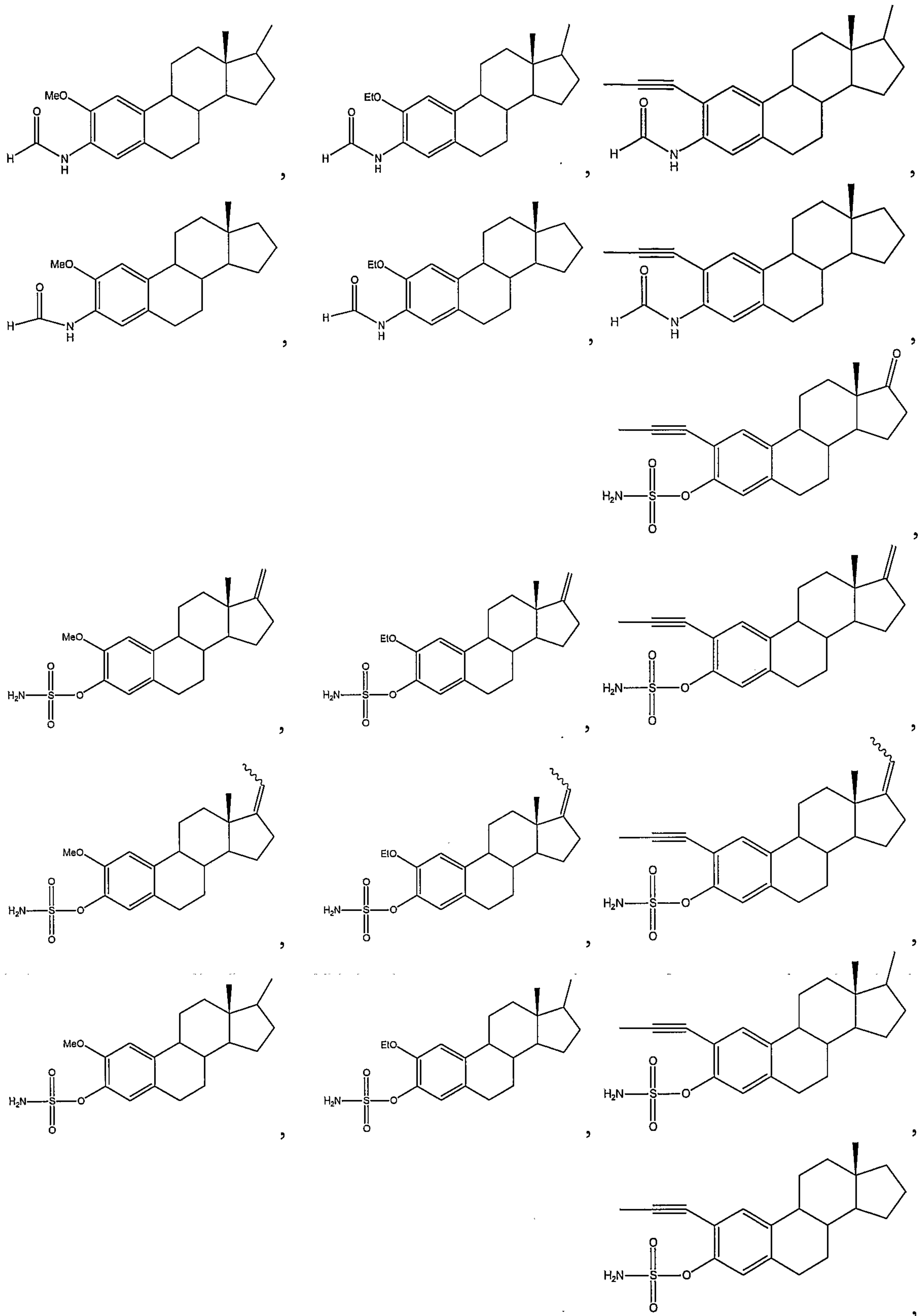
188. The method of Claim 184, wherein the skin condition is psoriasis.

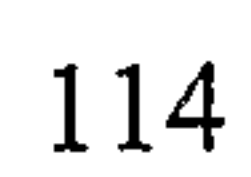
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189. A method of treating a tumor in a human or an animal comprising administering to the human or animal a compound having the formula



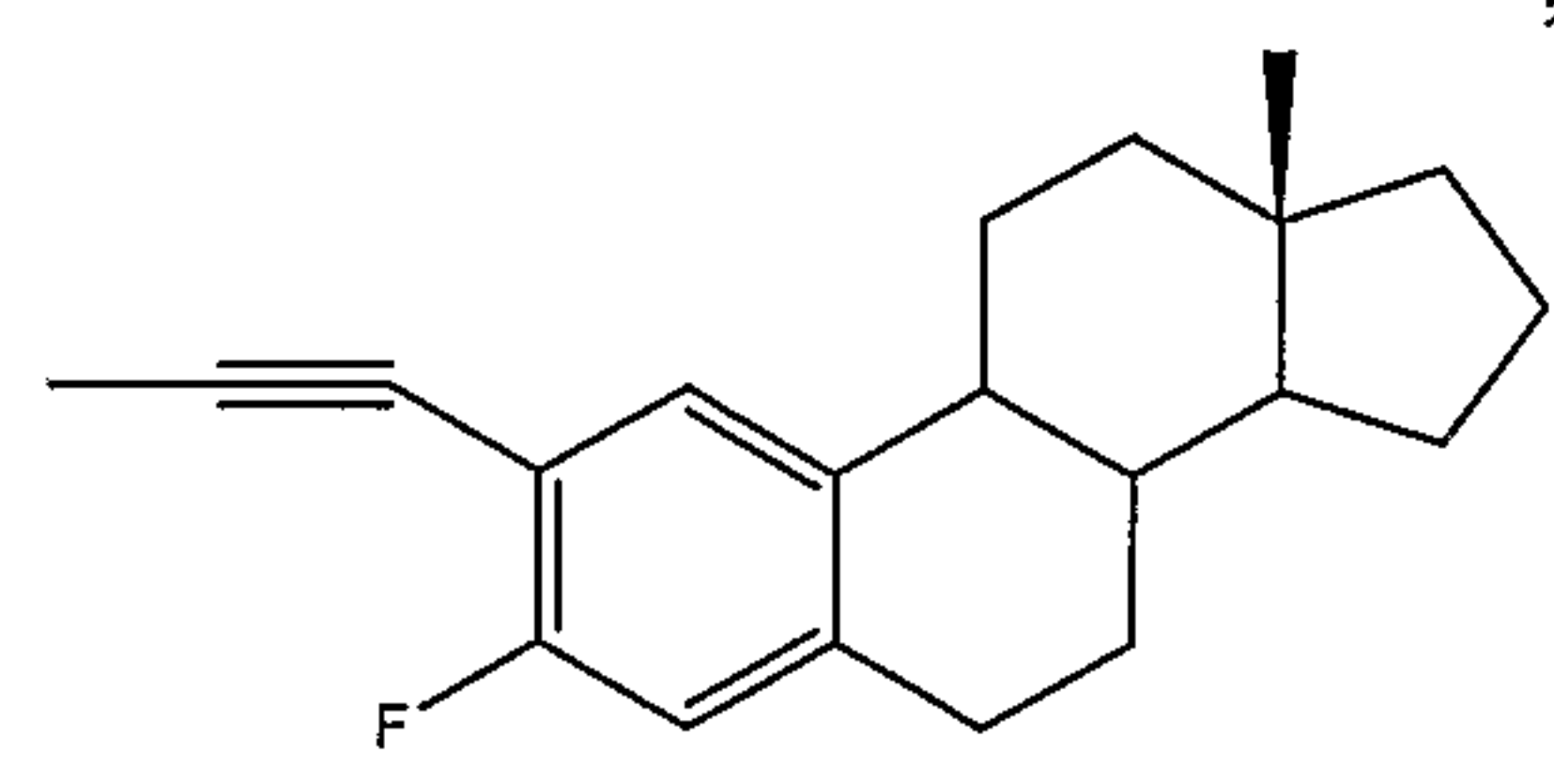
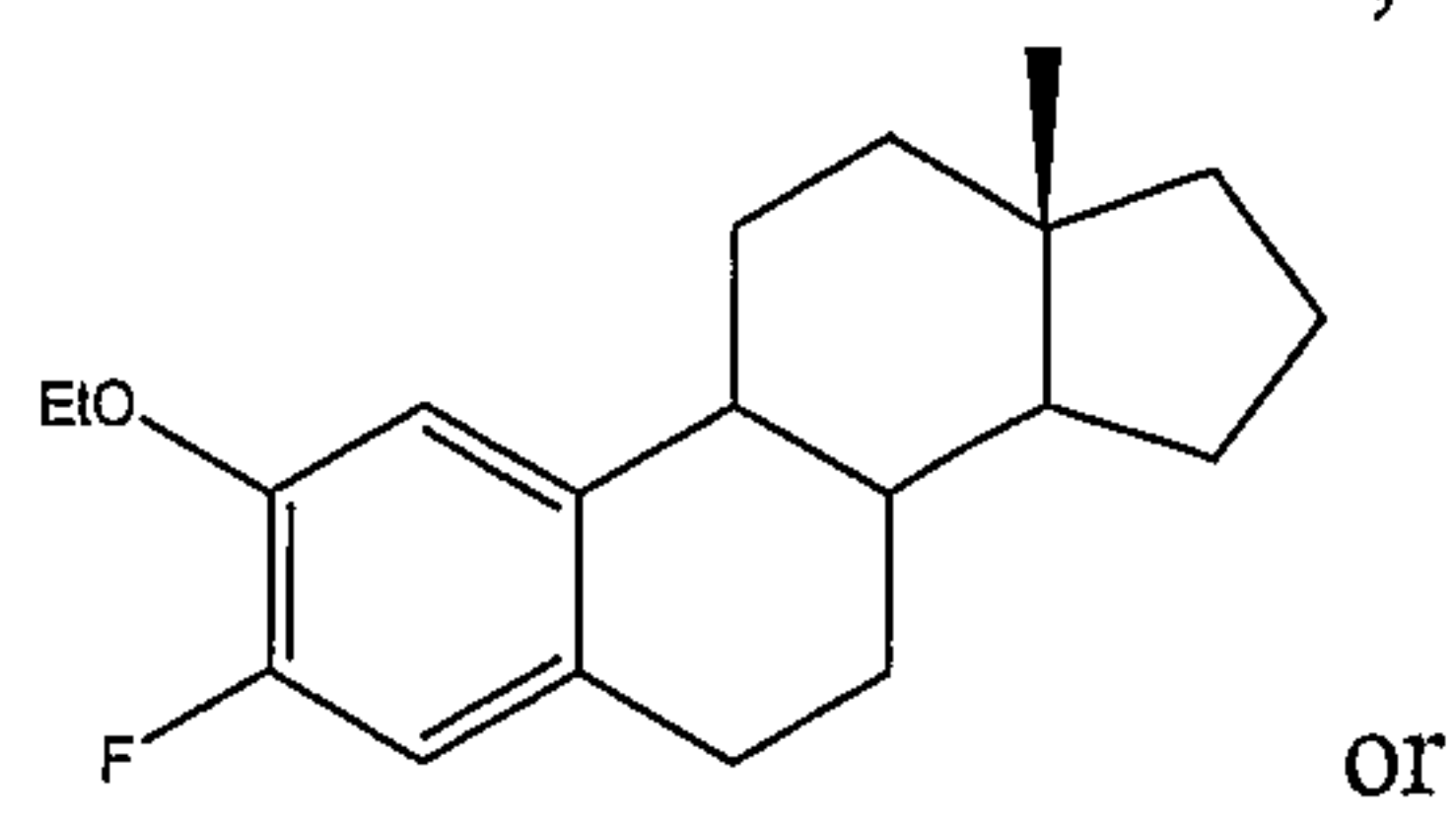
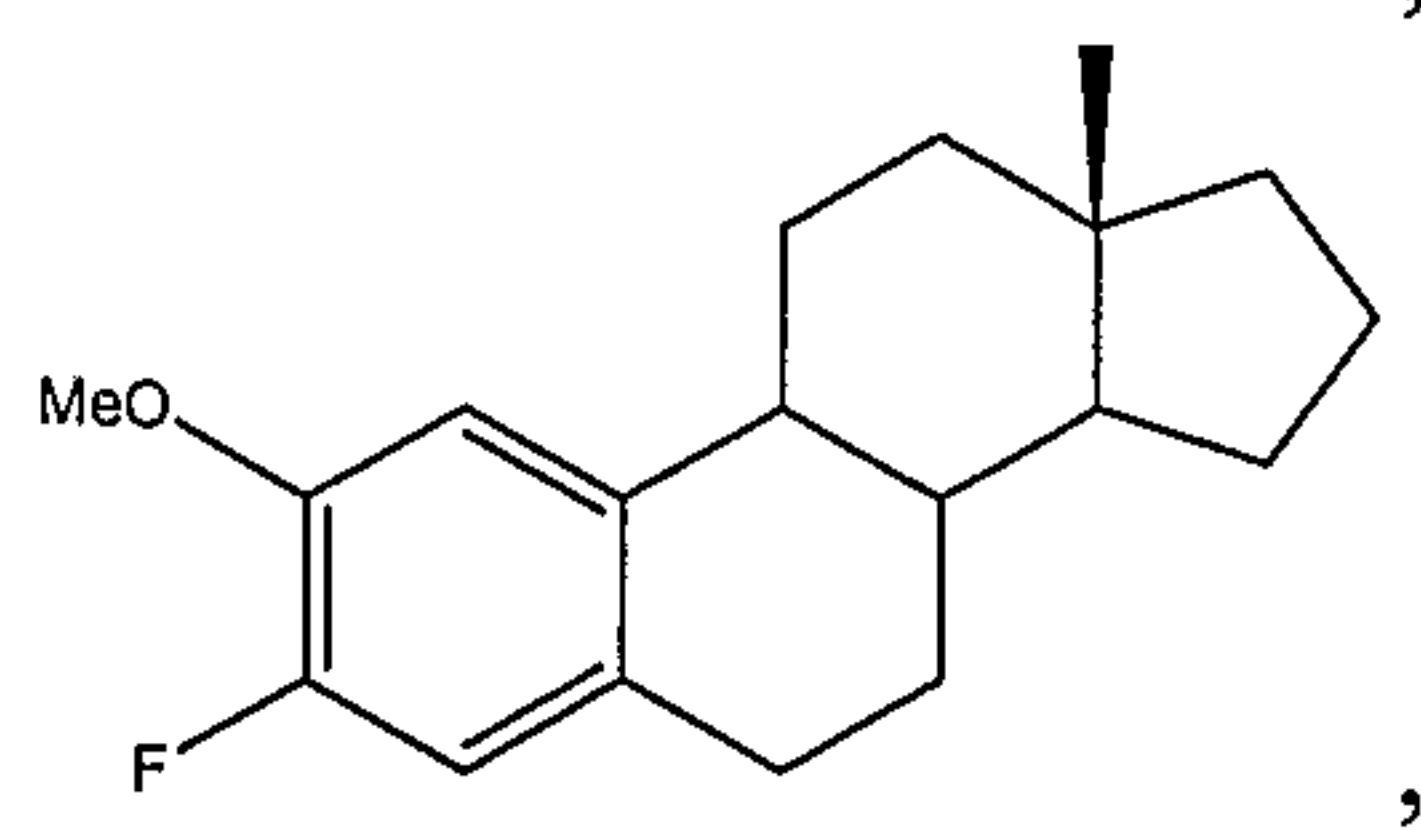
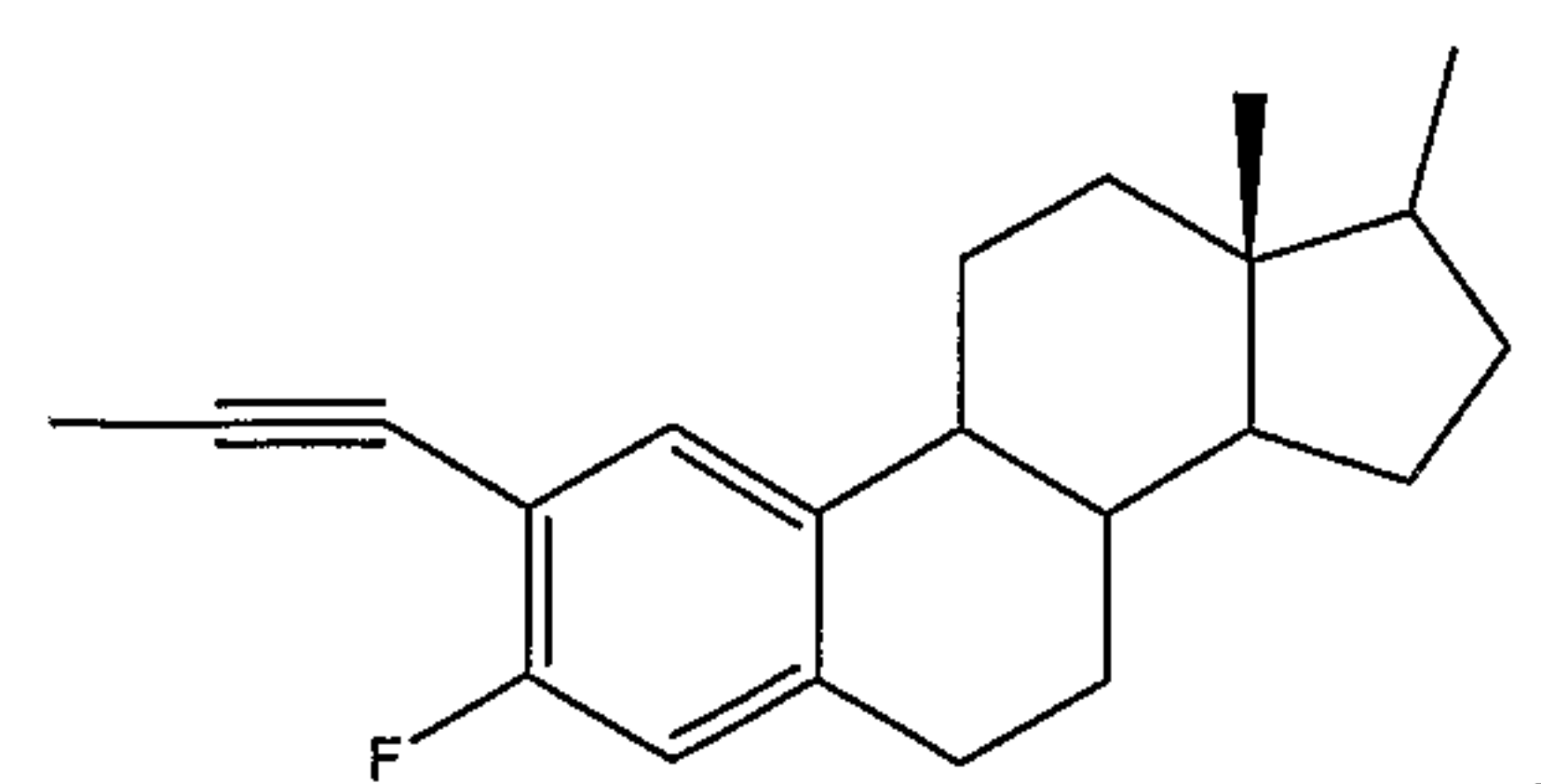
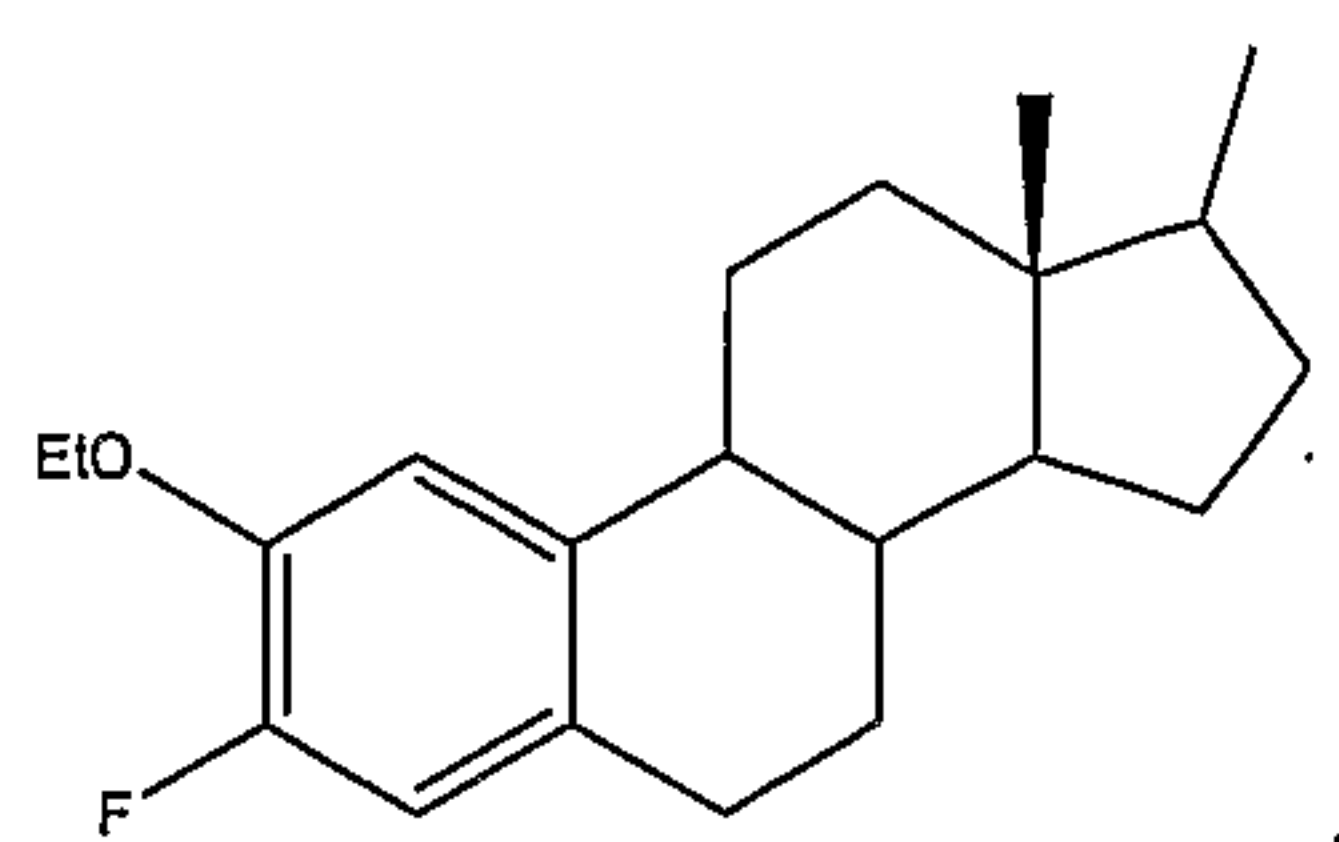
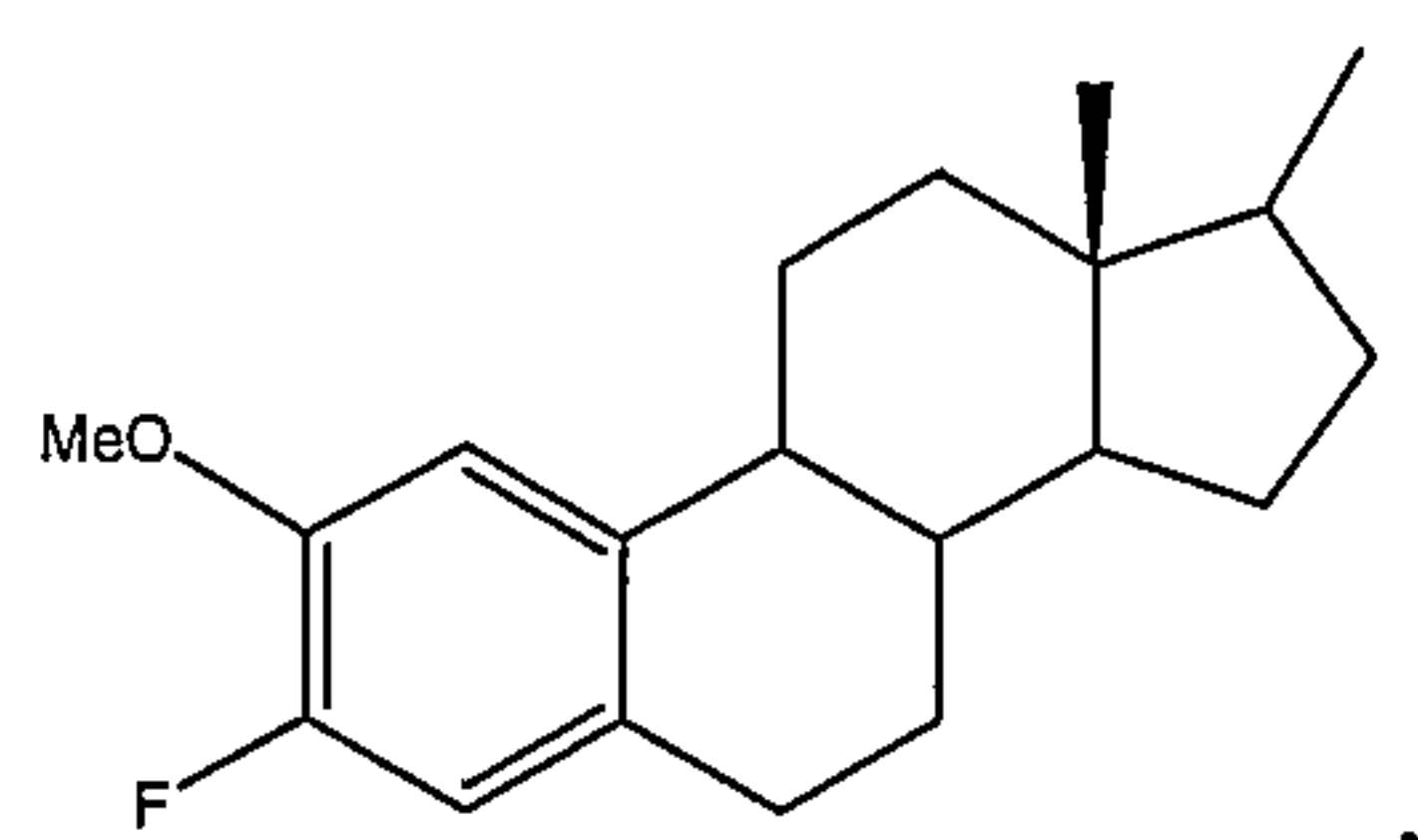






WO 2005/030120

PCT/US2004/016831



190. The method of Claim 189, wherein the tumor is a blood borne tumor.

191. The method of Claim 190, wherein the blood borne tumor is a cancerous blood borne tumor.

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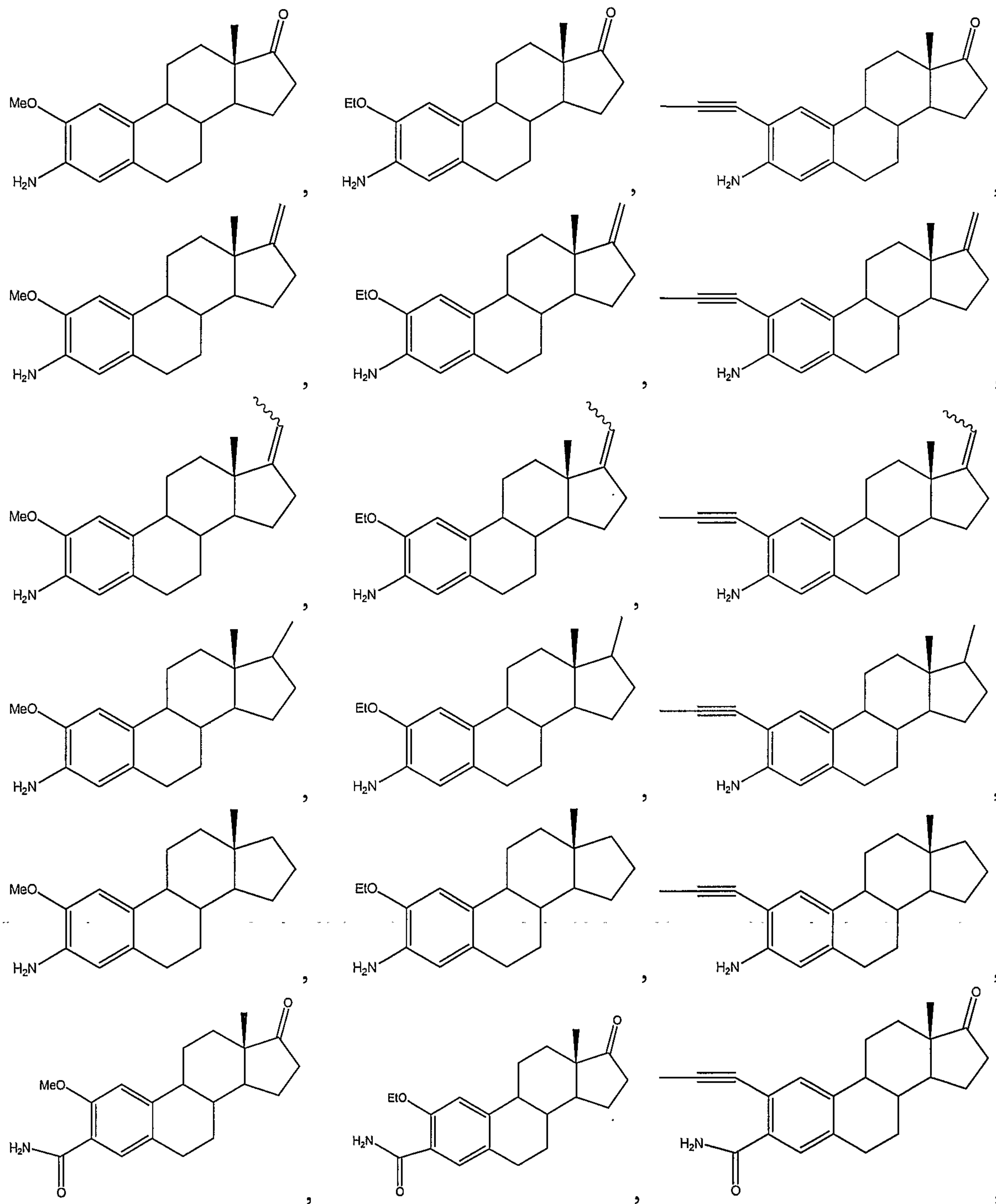
192. The method of Claim 189, wherein the tumor is a solid tumor.

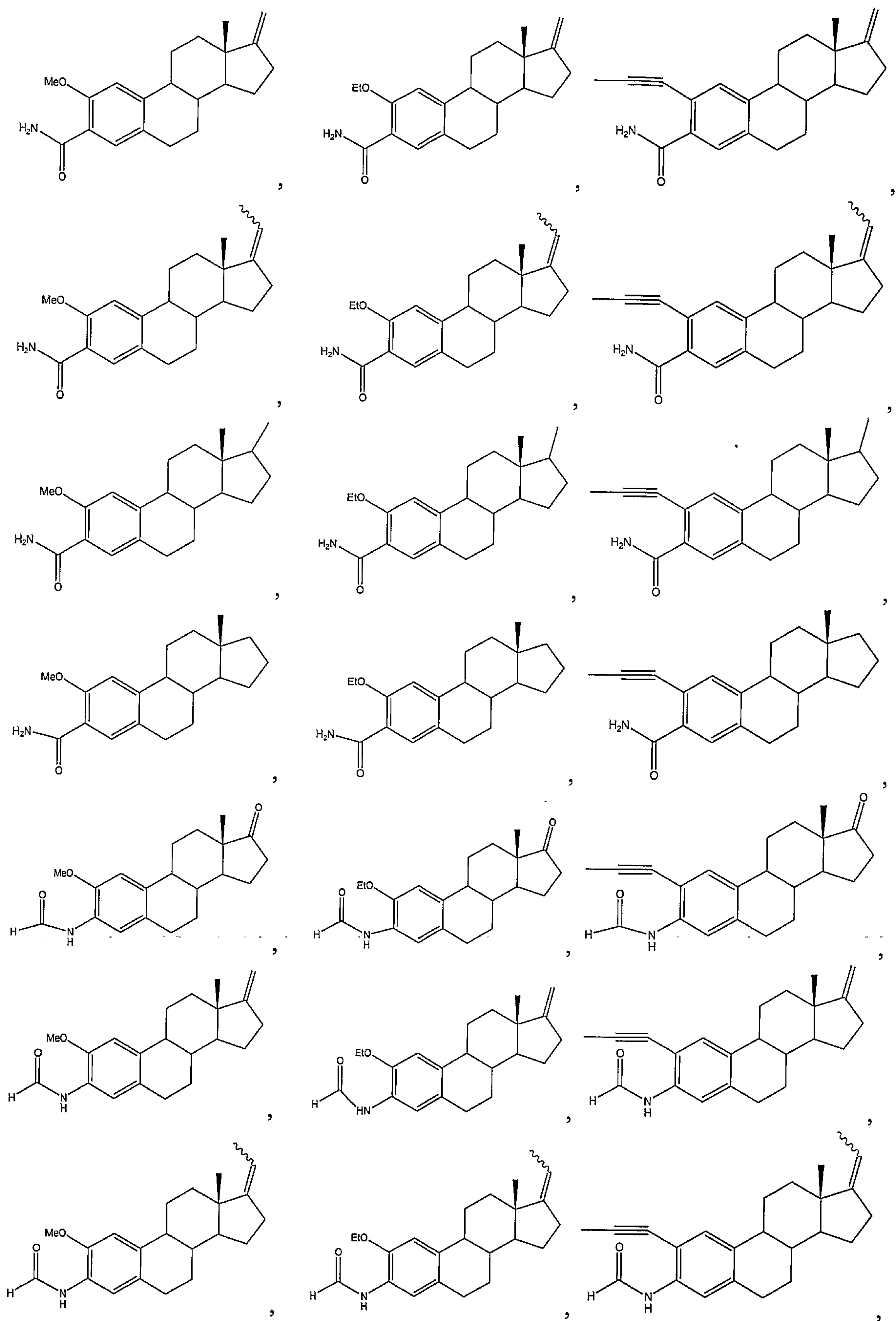
193. The method of Claim 192, wherein the solid tumor is a benign tumor.

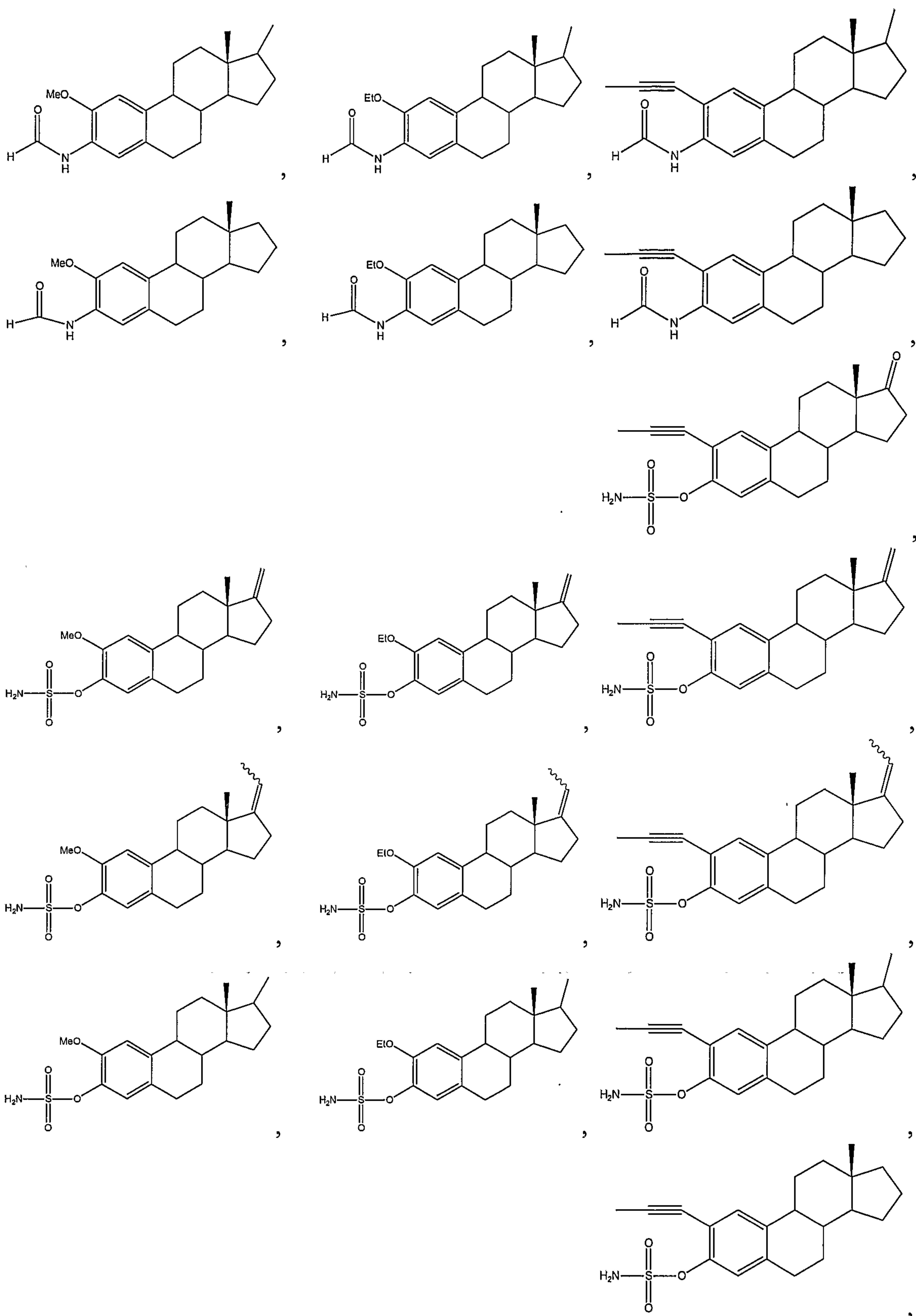
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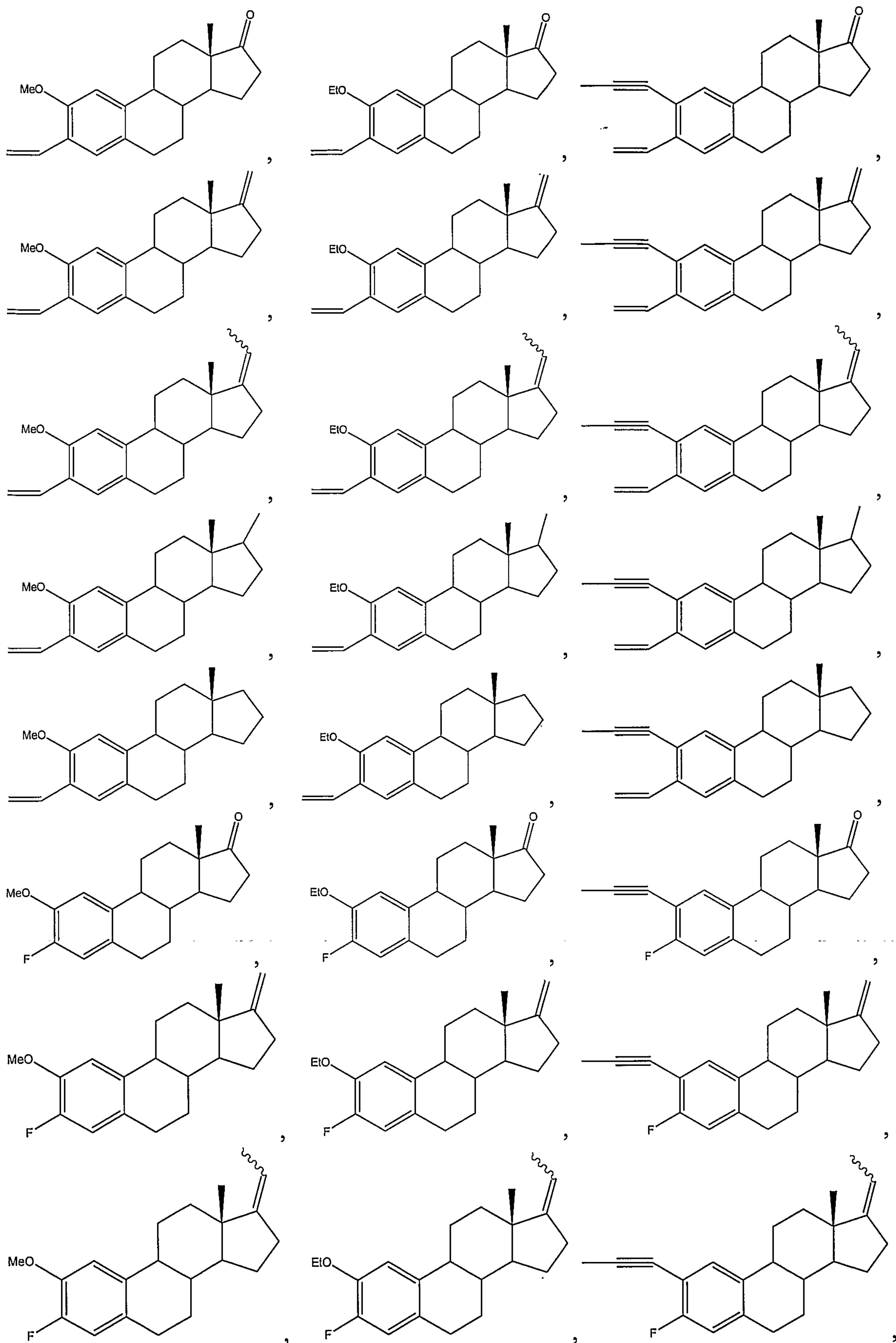
194. The method of Claim 192, wherein the solid tumor is a cancerous tumor.

195. A method of inhibiting angiogenesis in a human or animal comprising administering to the human or animal an angiogenesis-inhibiting amount of a compound selected from



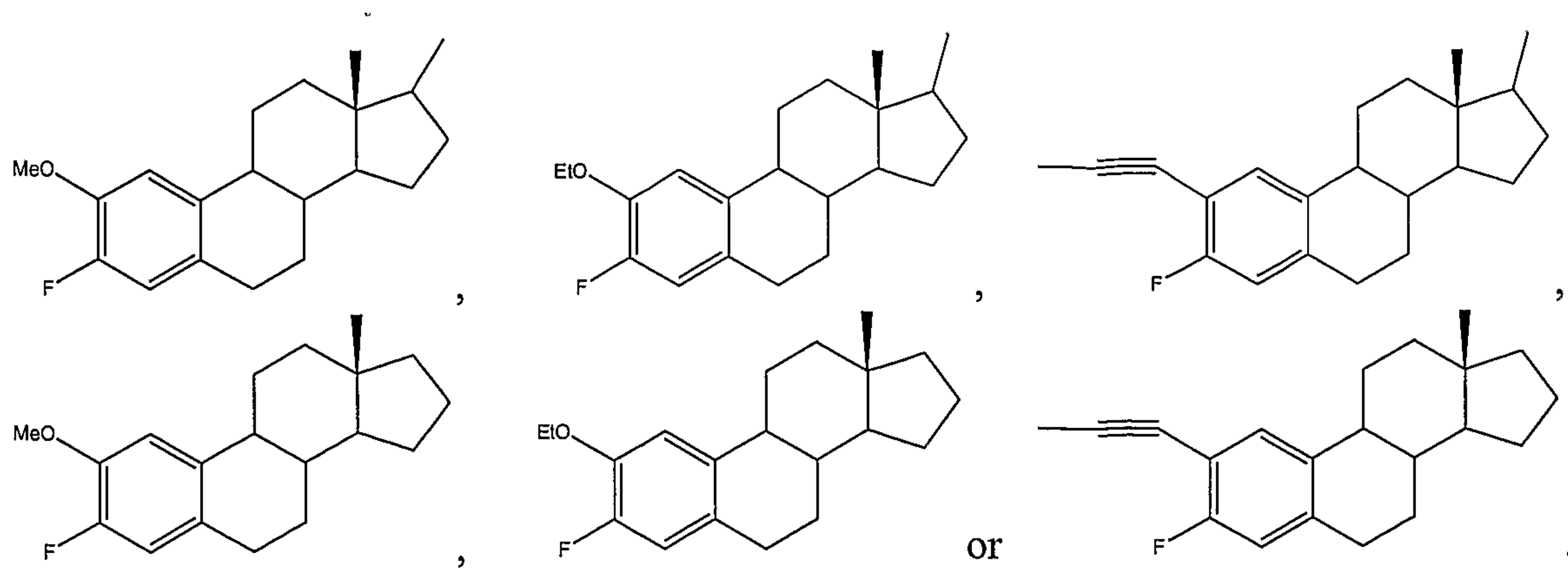




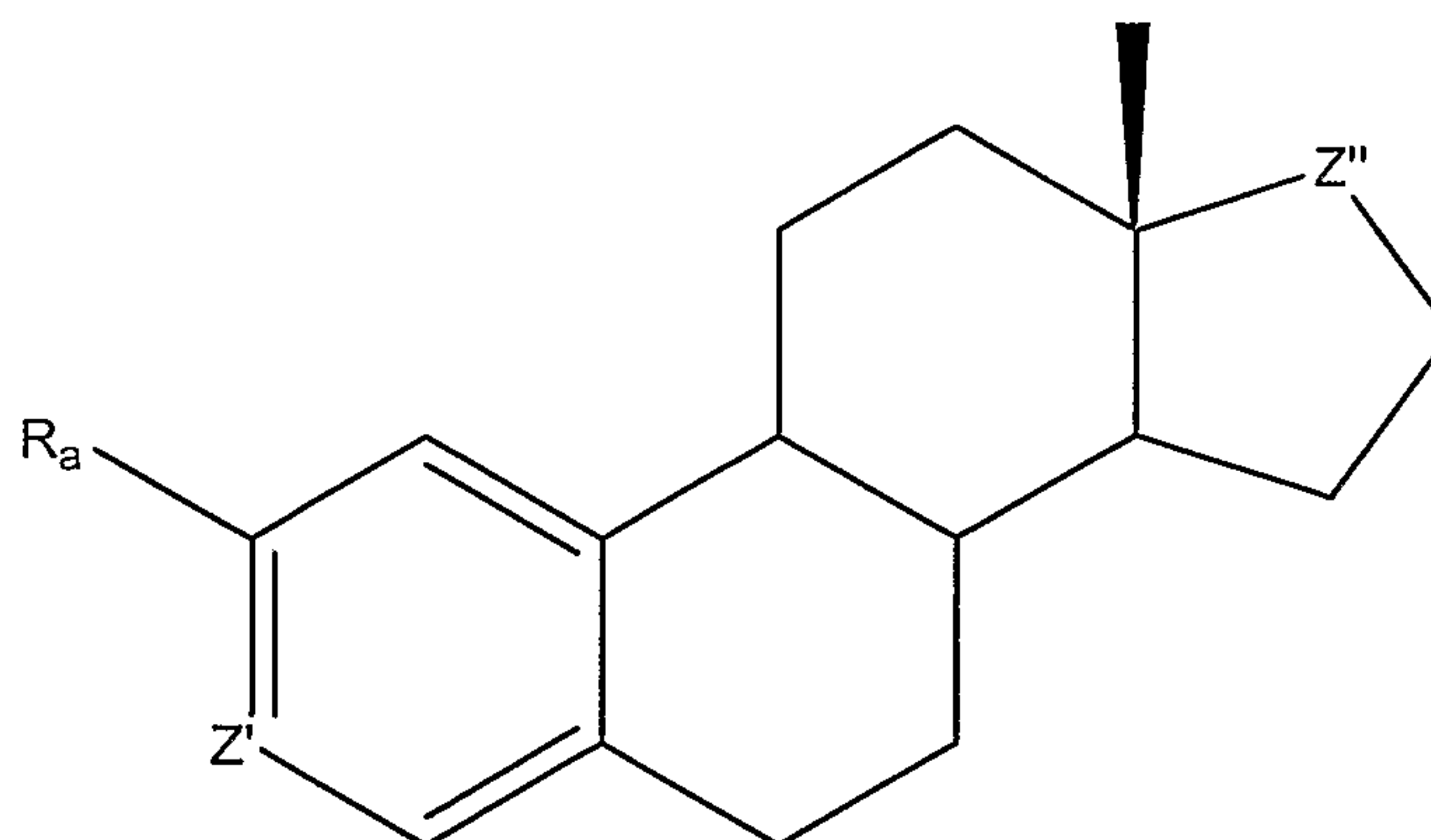


WO 2005/030120

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196. A pharmaceutical composition comprising:
a pharmaceutical carrier or excipient; and
a compound having the formula



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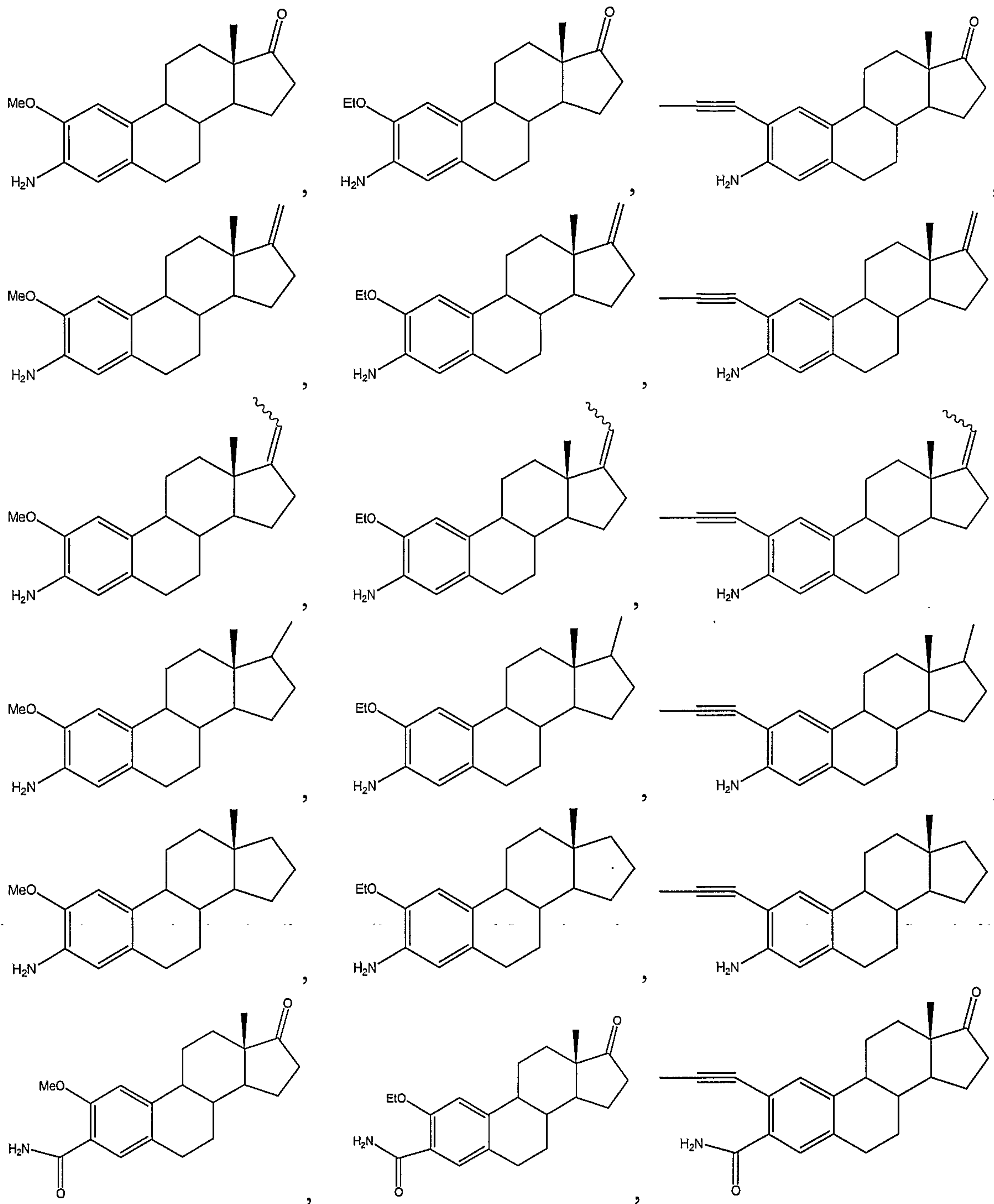
wherein R_a is selected from $-OCH_3$, $-OCH_2CH_3$ or $-CCCH_3$;

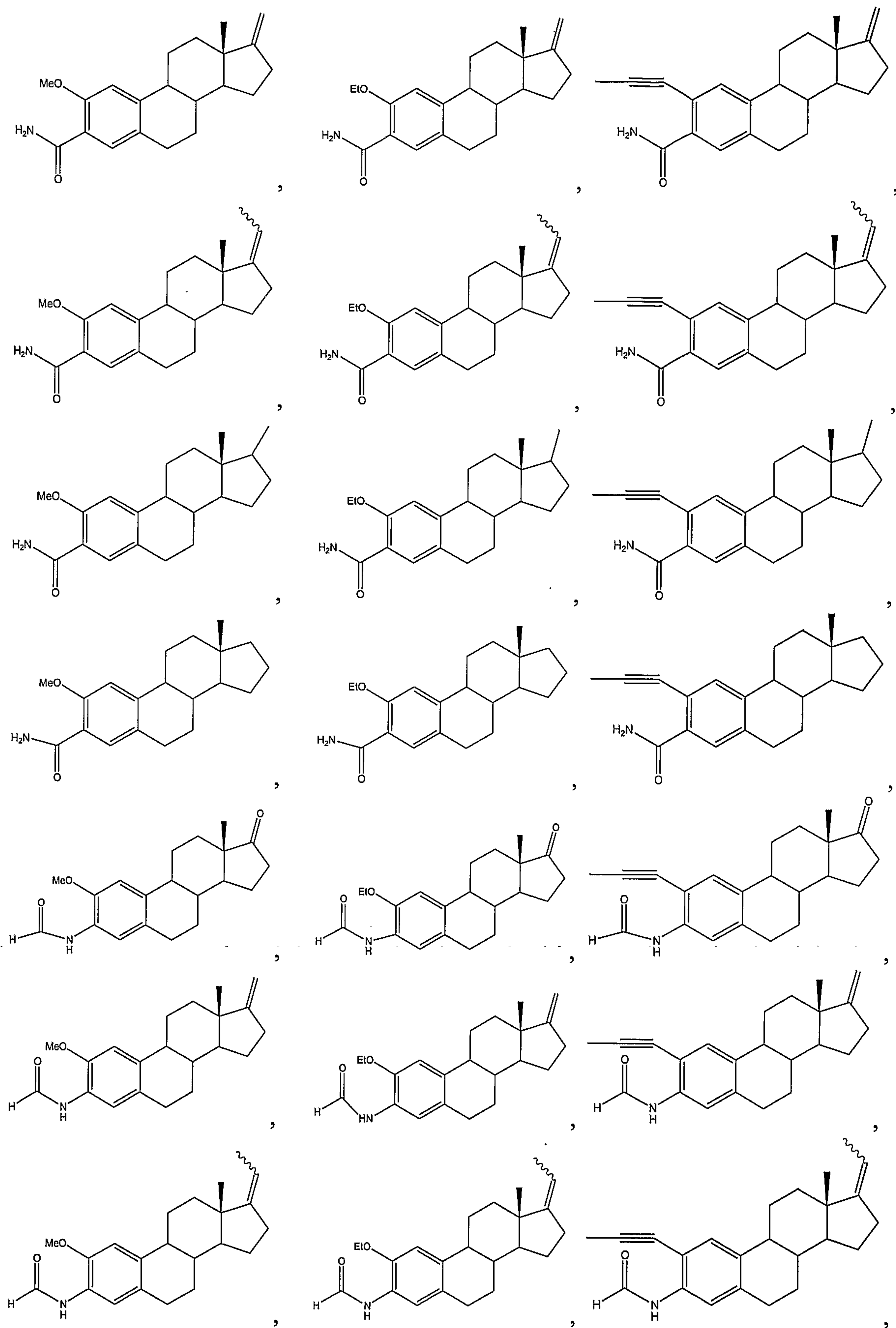
Z' is selected from $>C-F$, $>C-NH_2$, $>CCONH_2$, $>C-NHCOH$, $>C-OSO_2NH_2$ or $>C-CHCH_2$; and

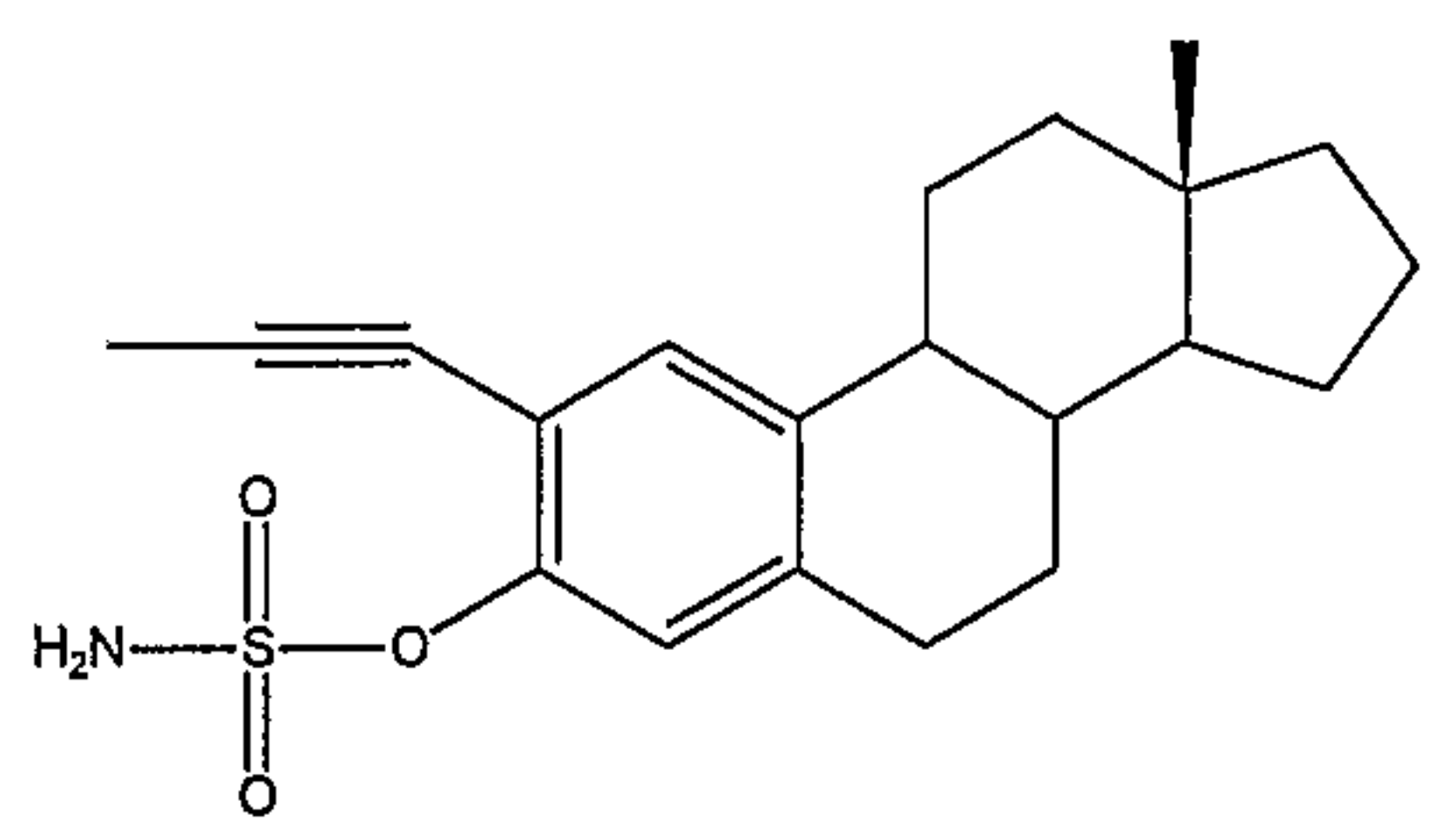
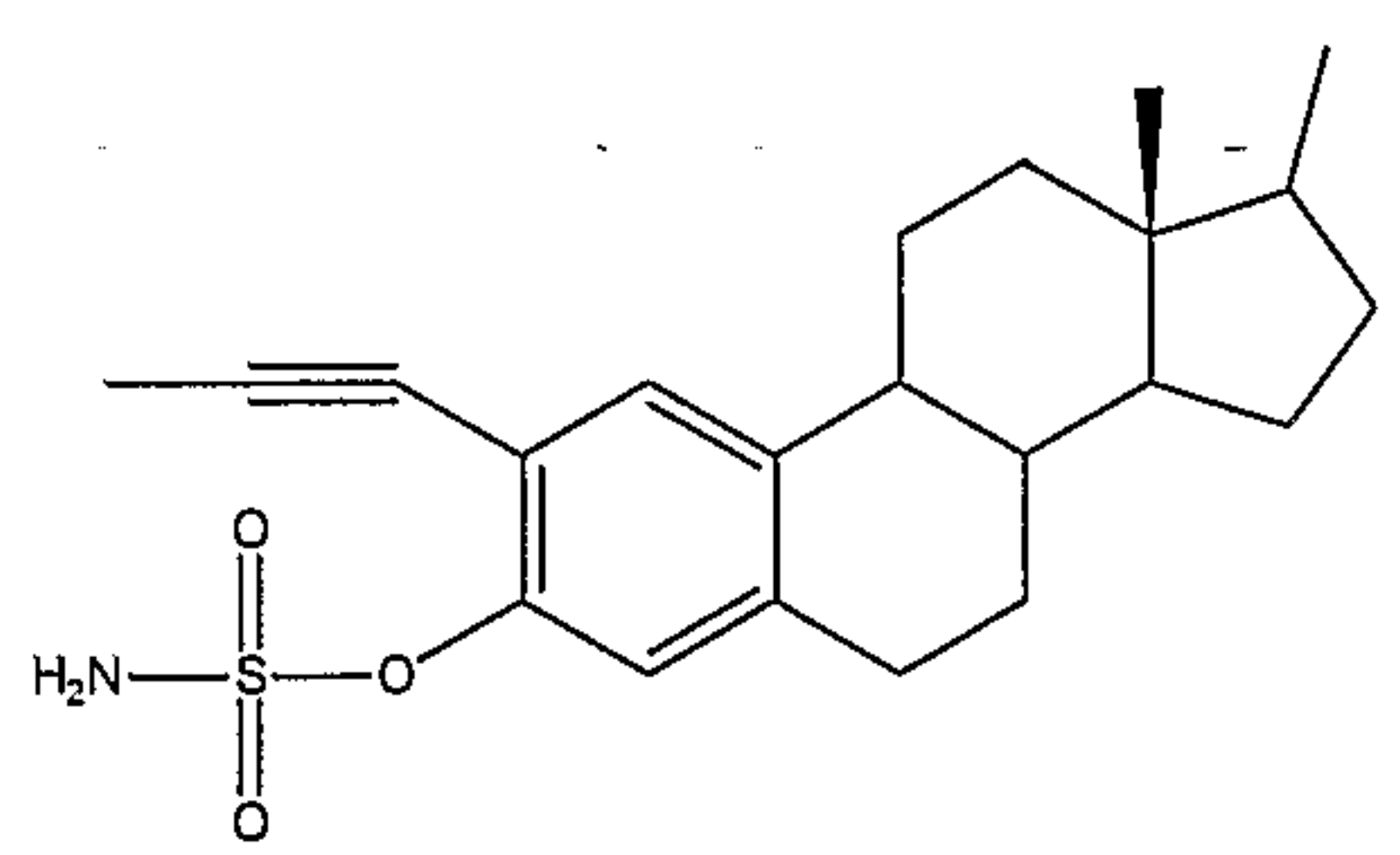
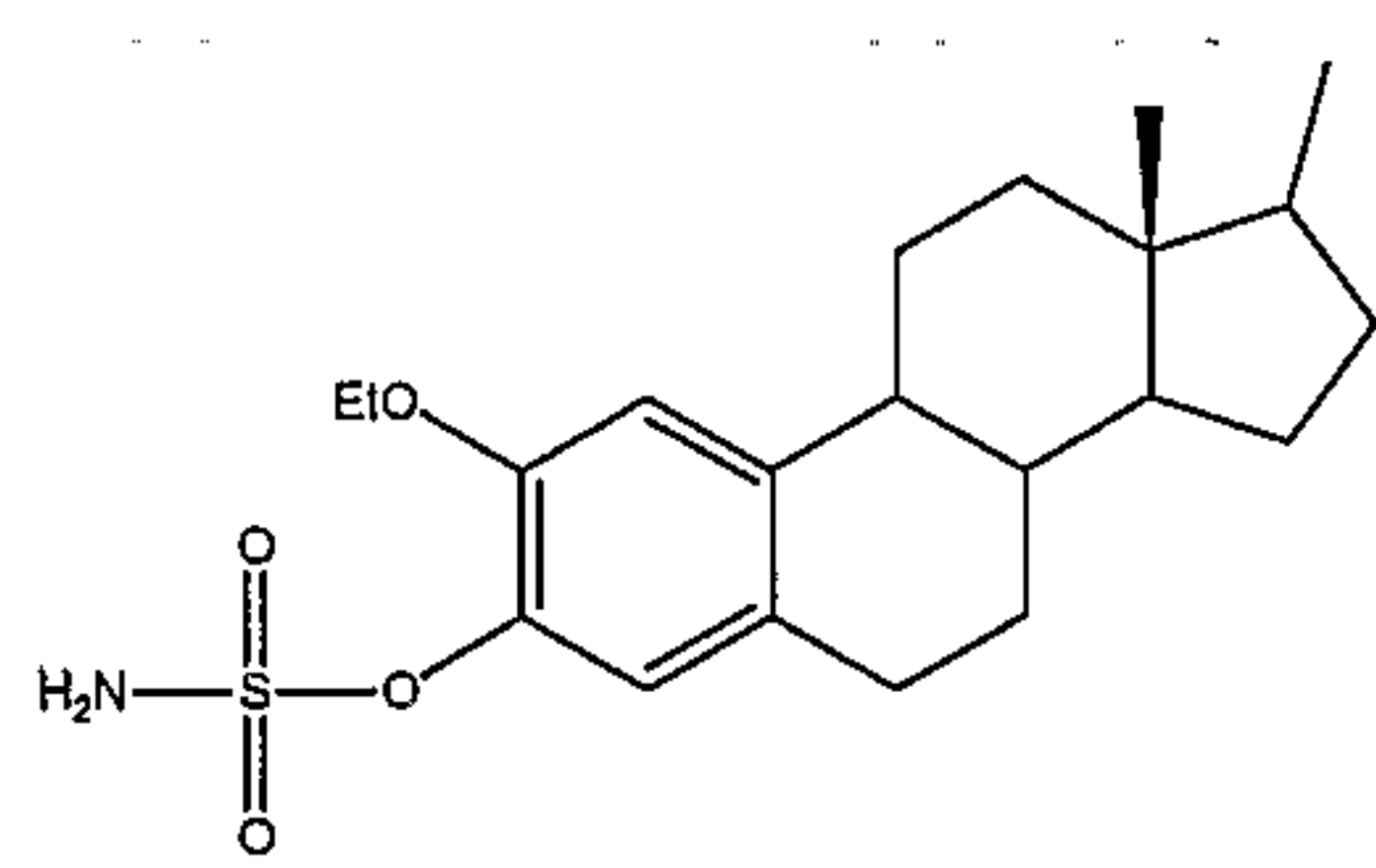
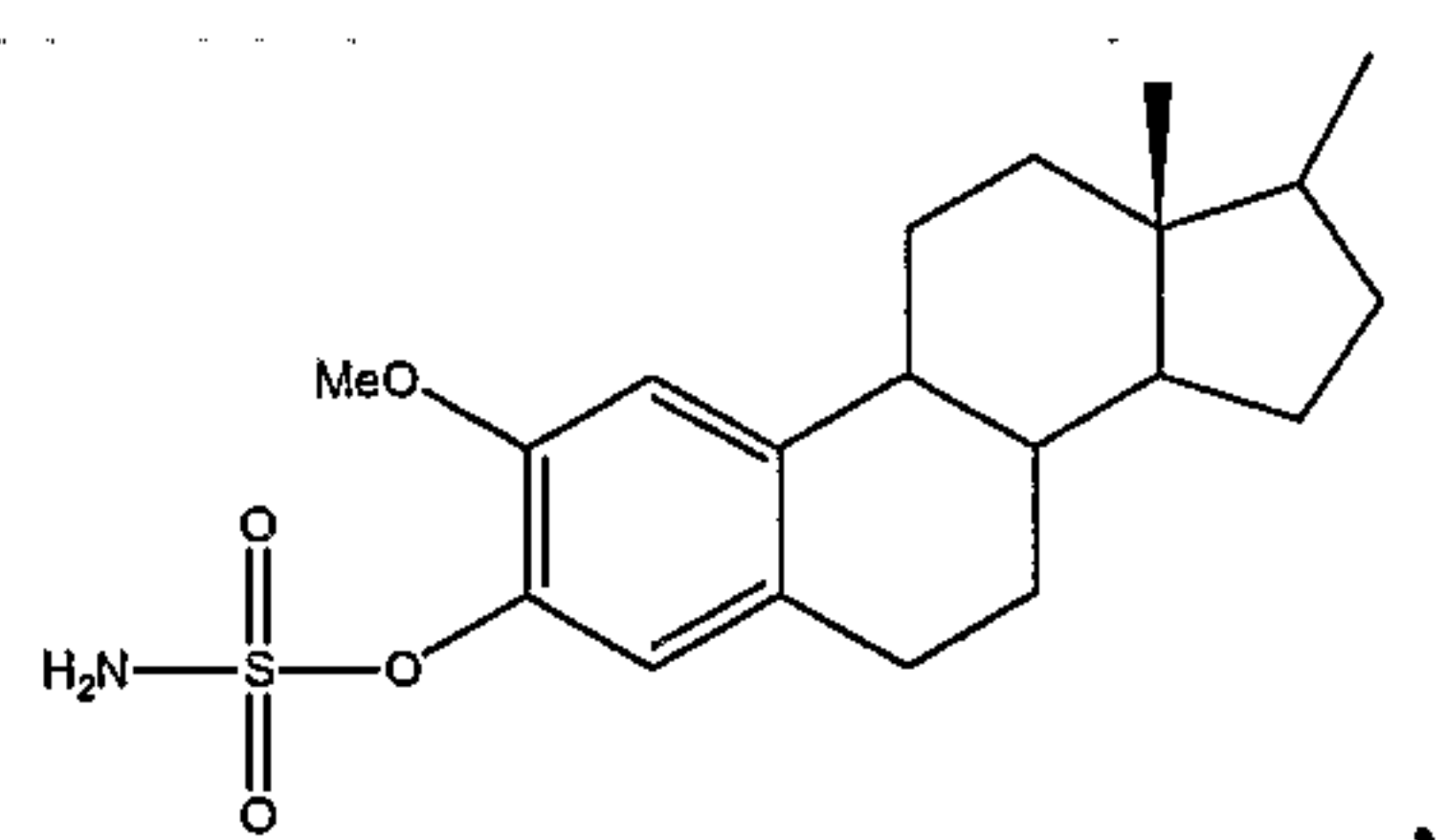
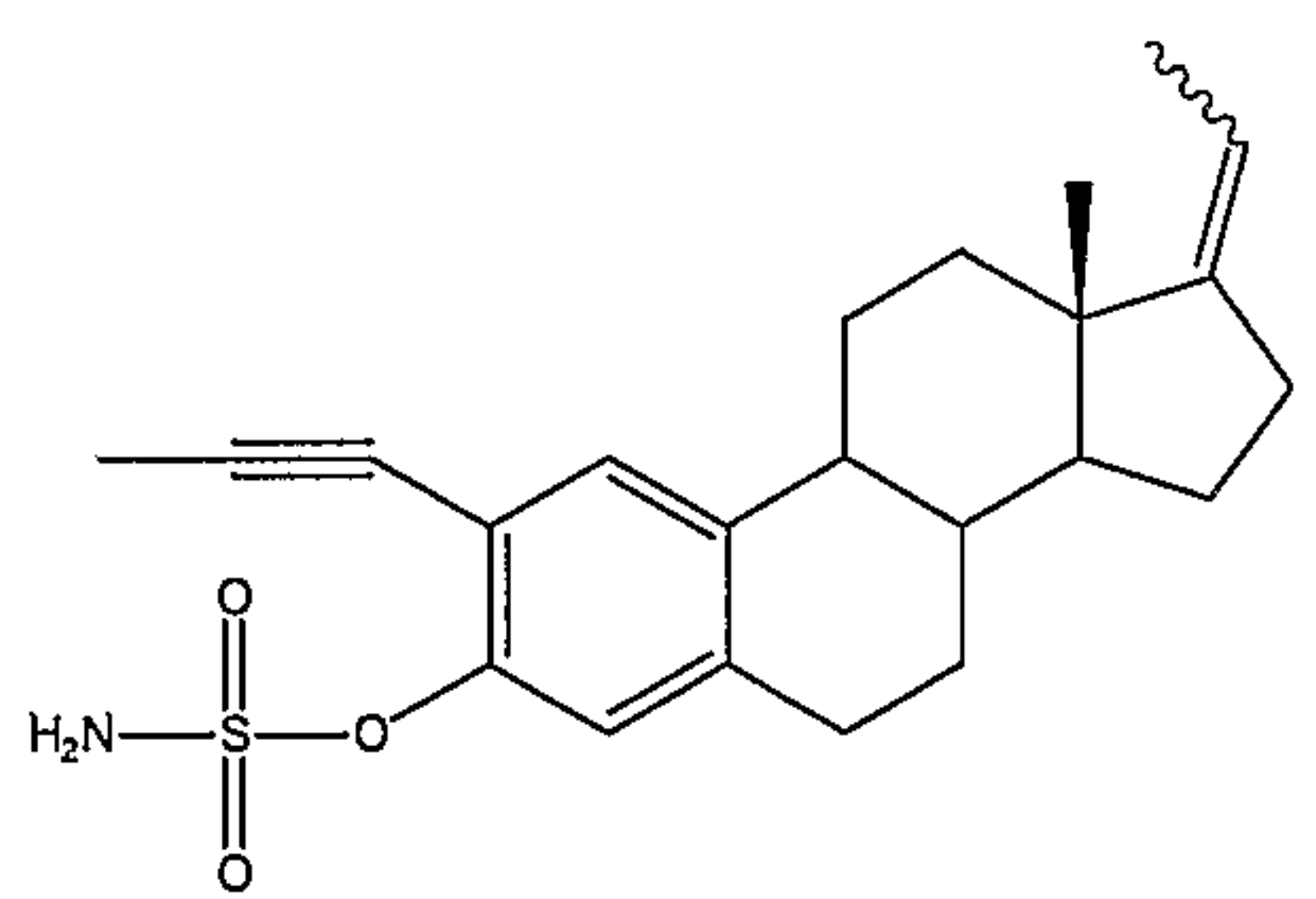
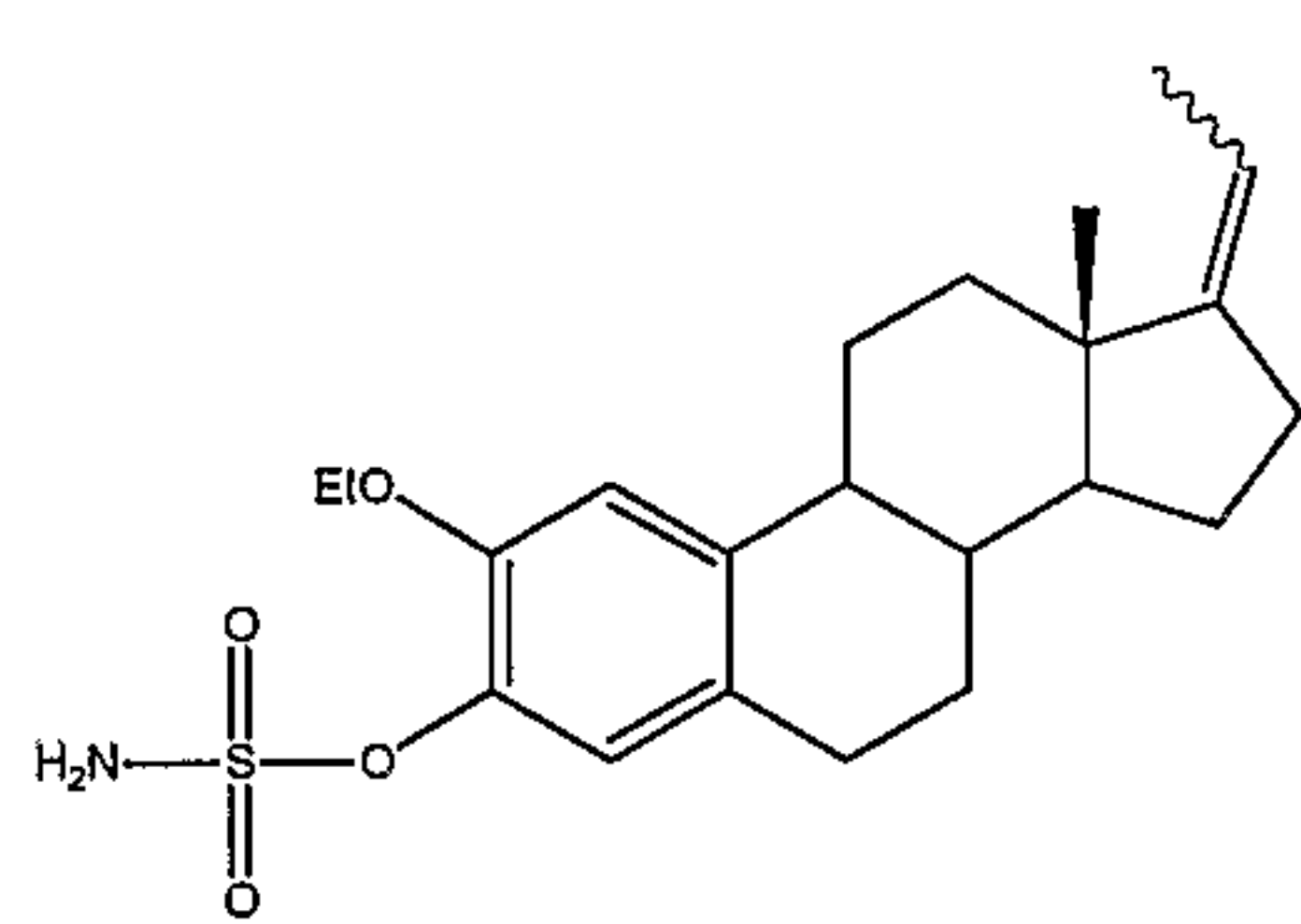
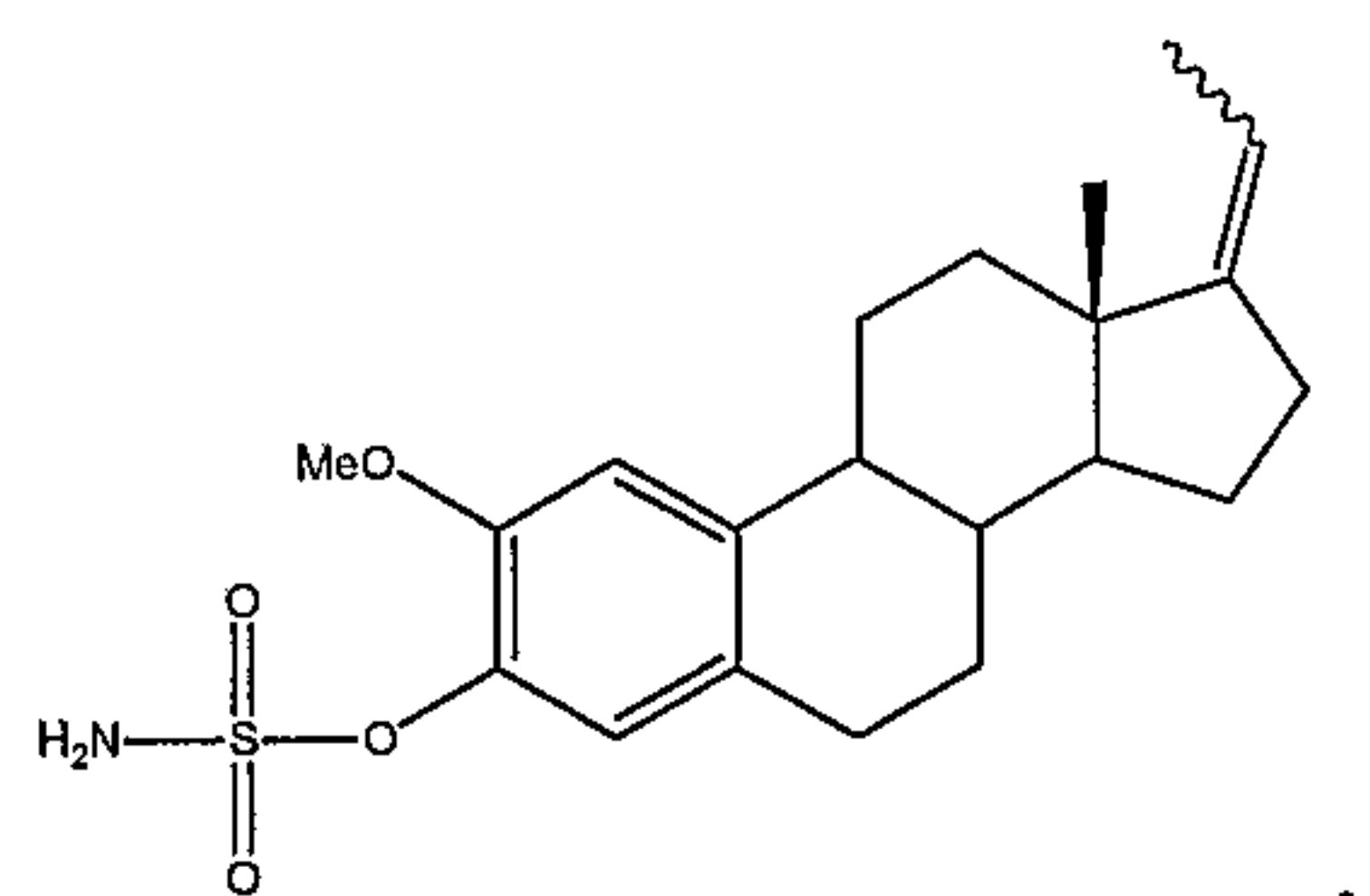
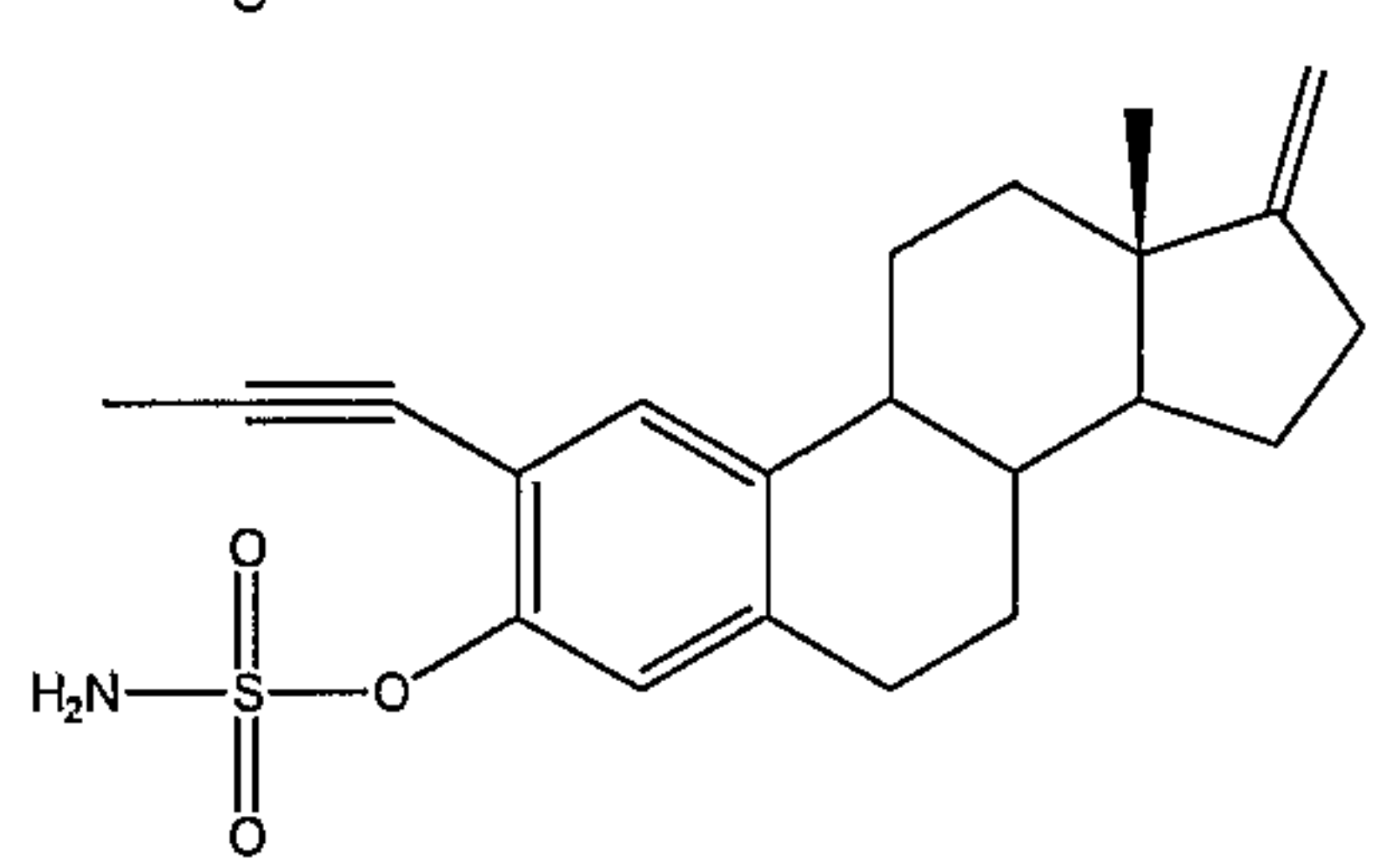
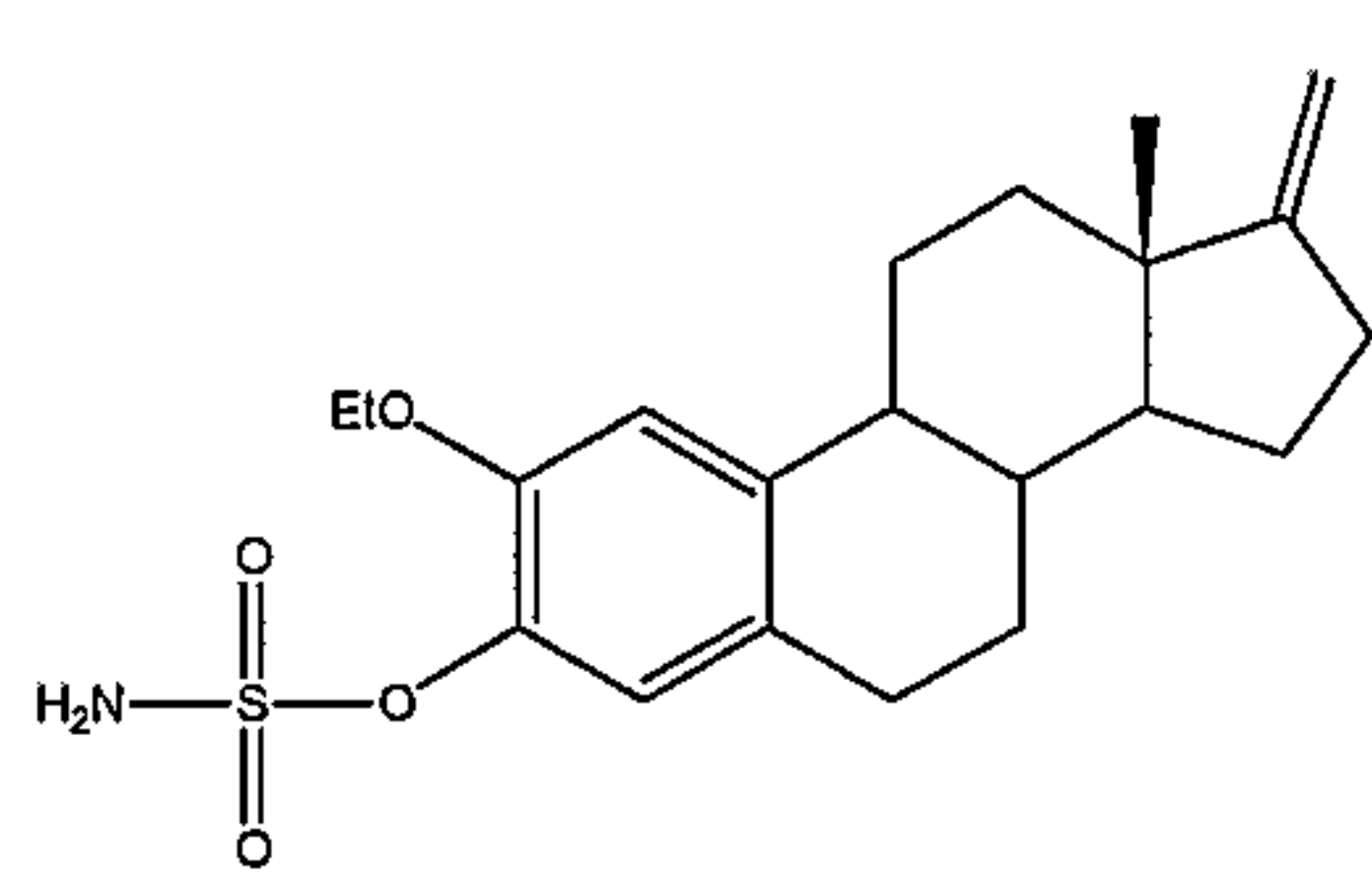
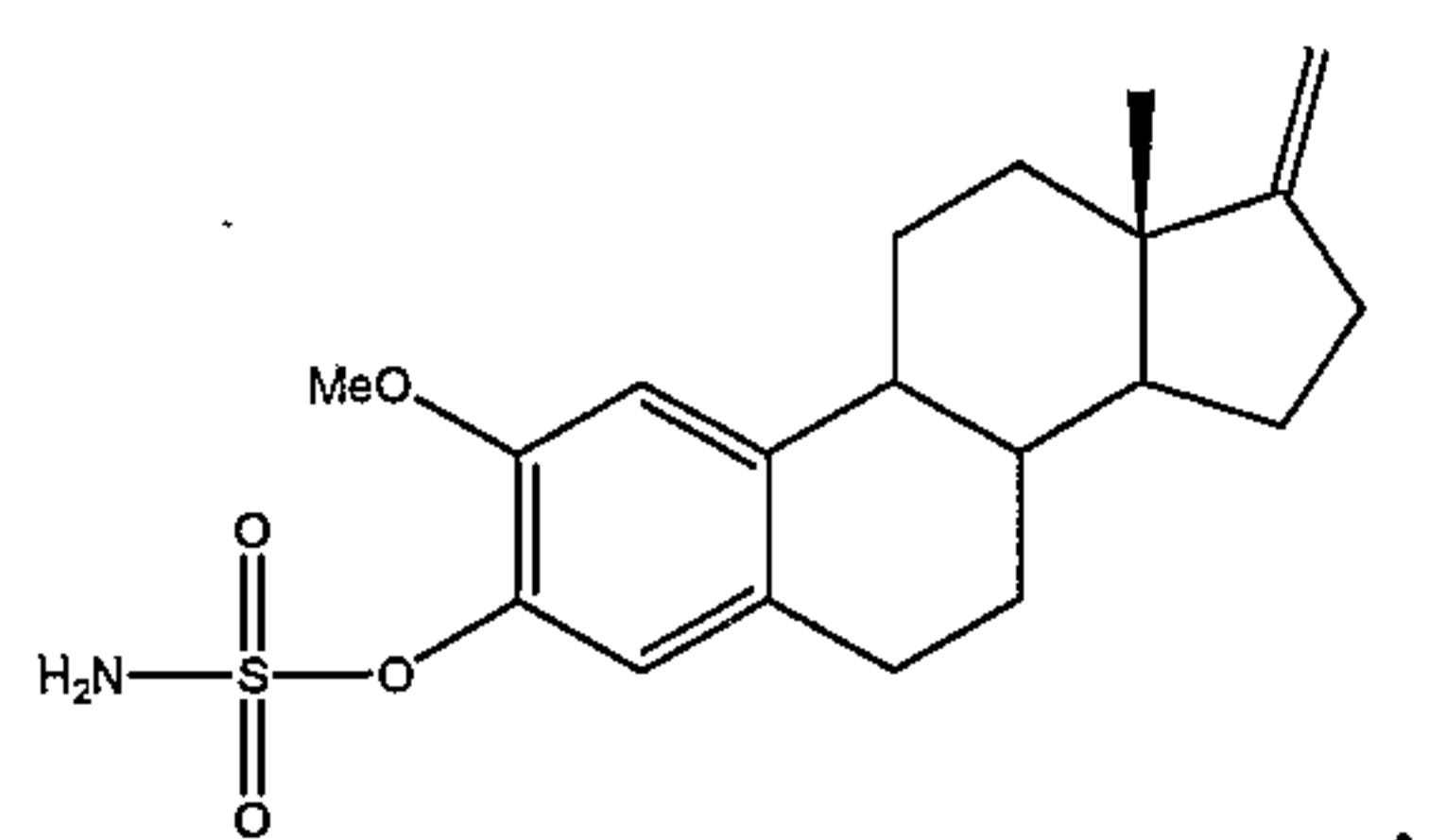
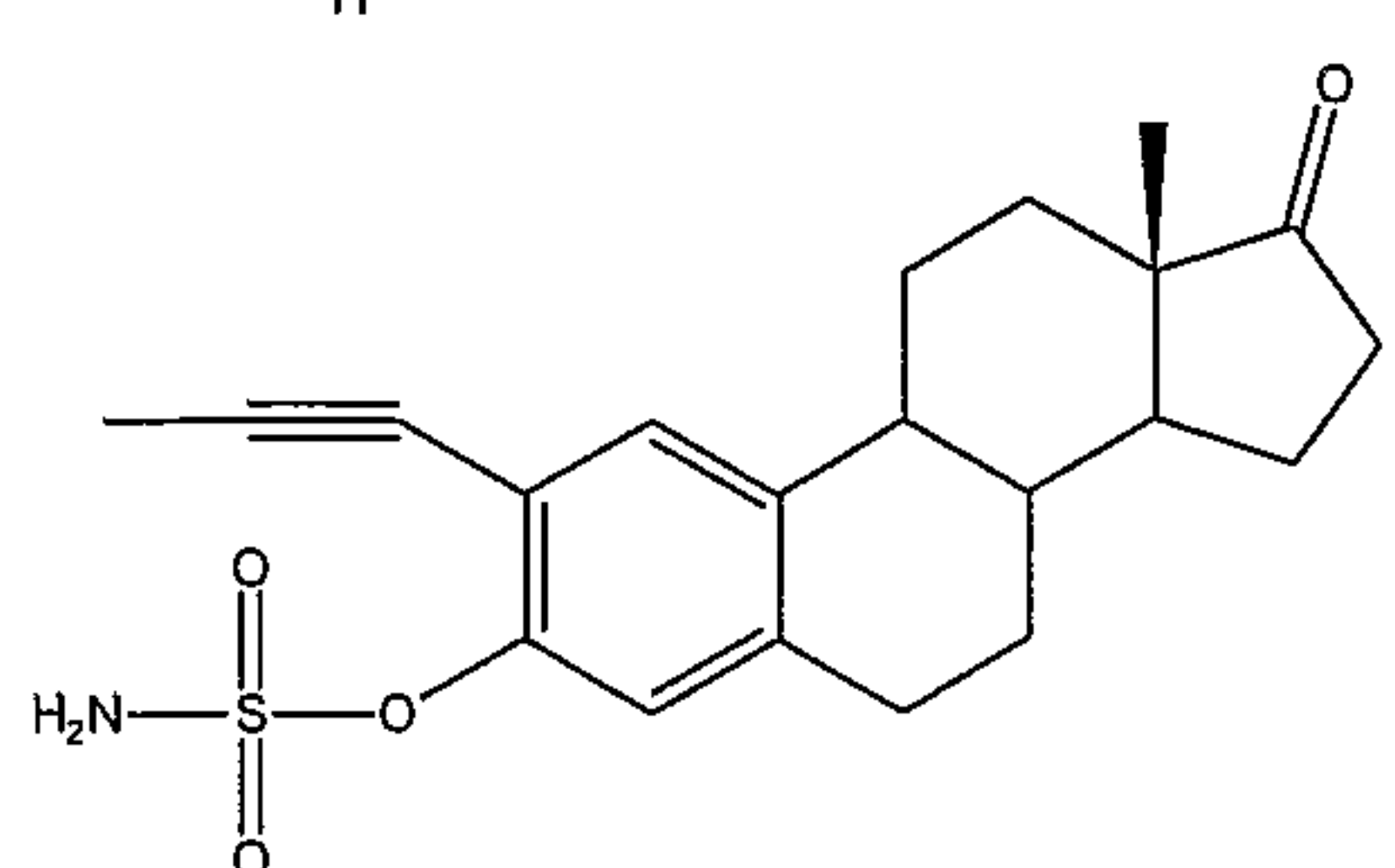
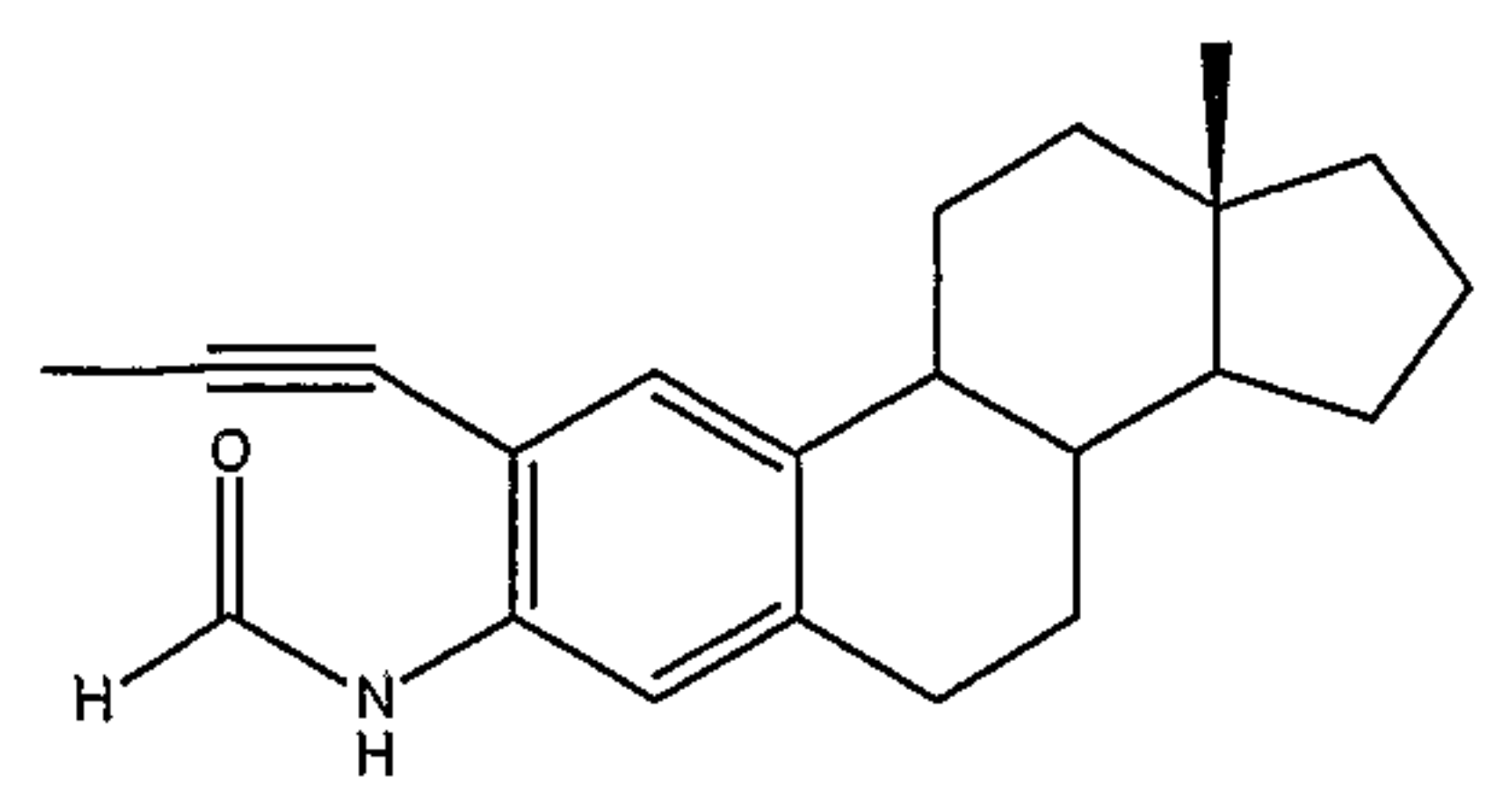
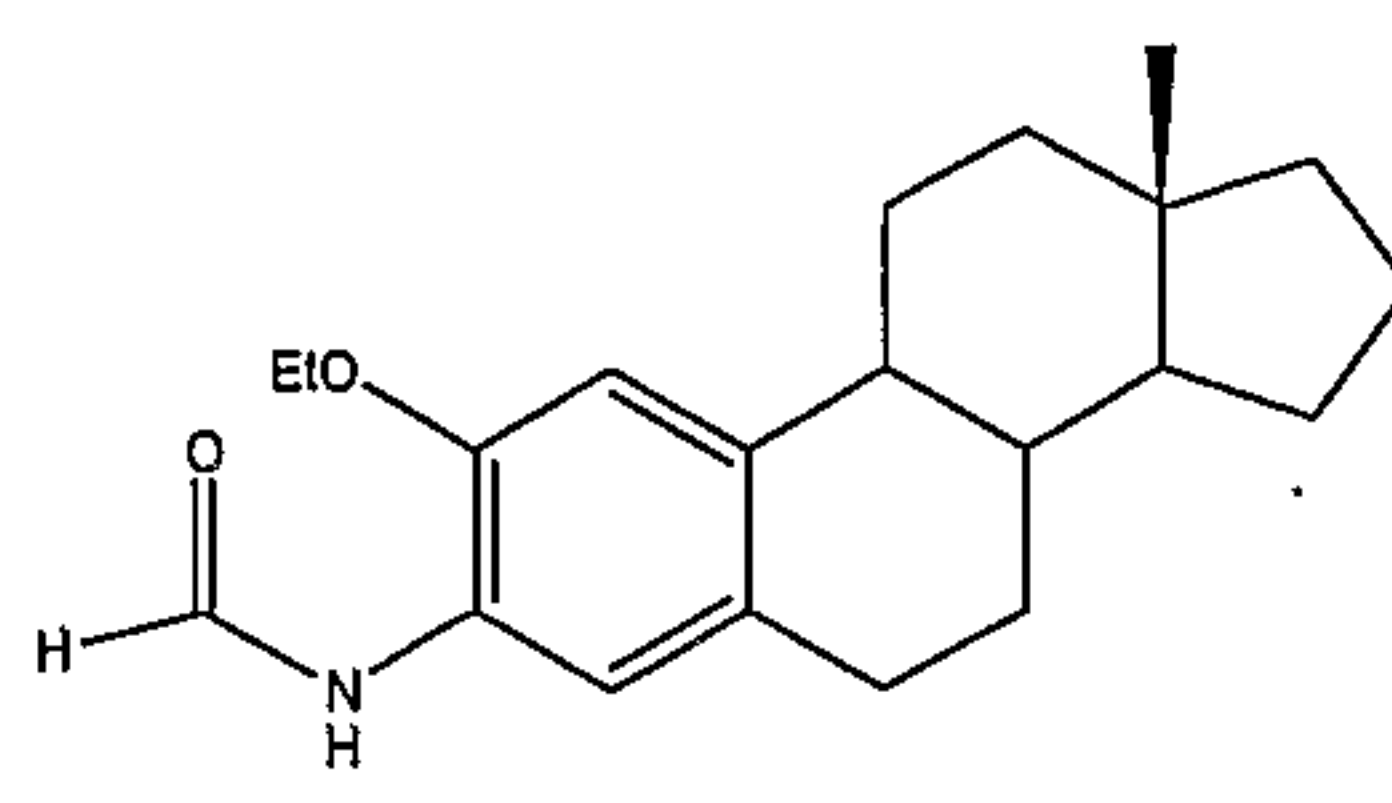
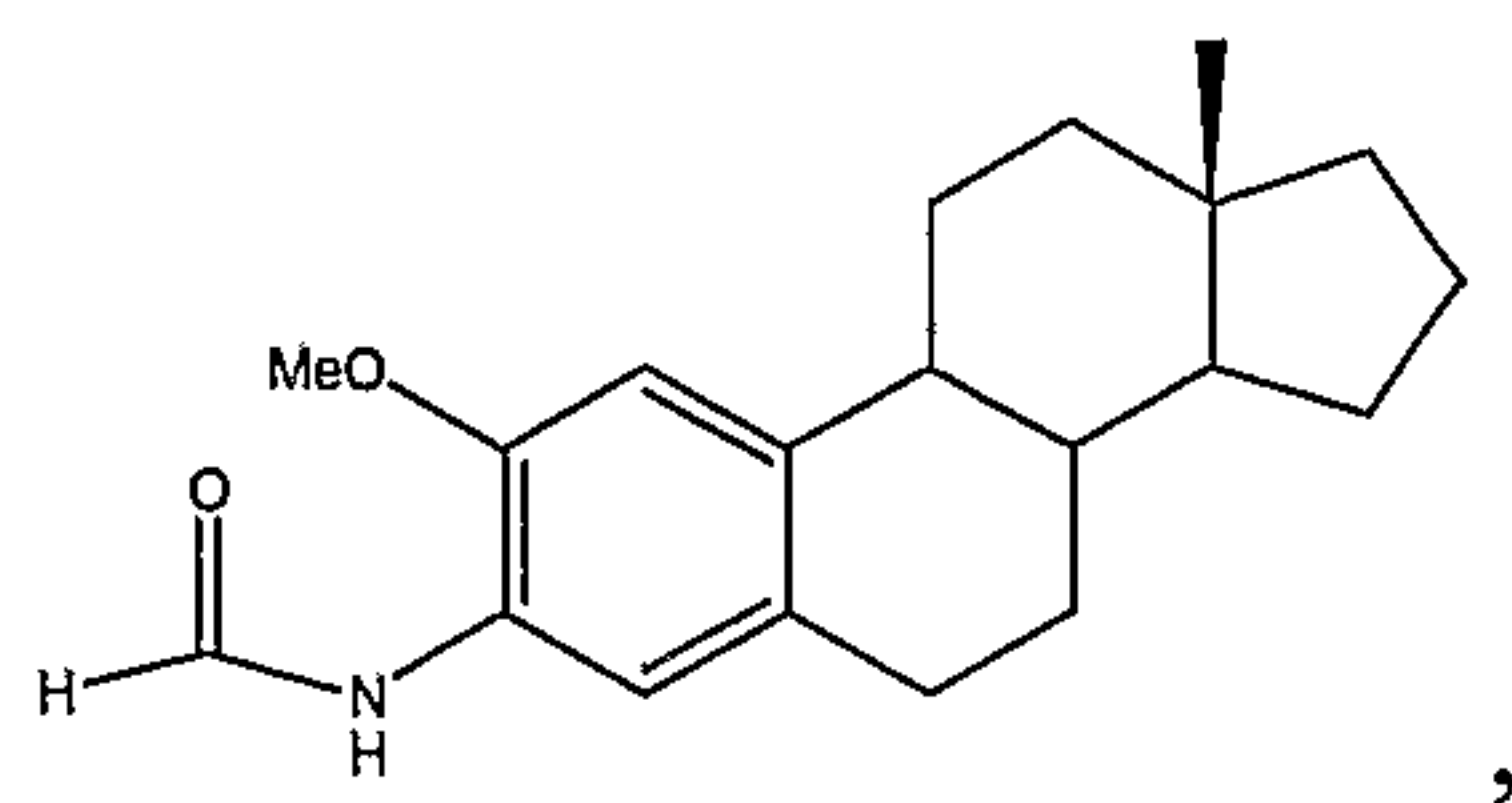
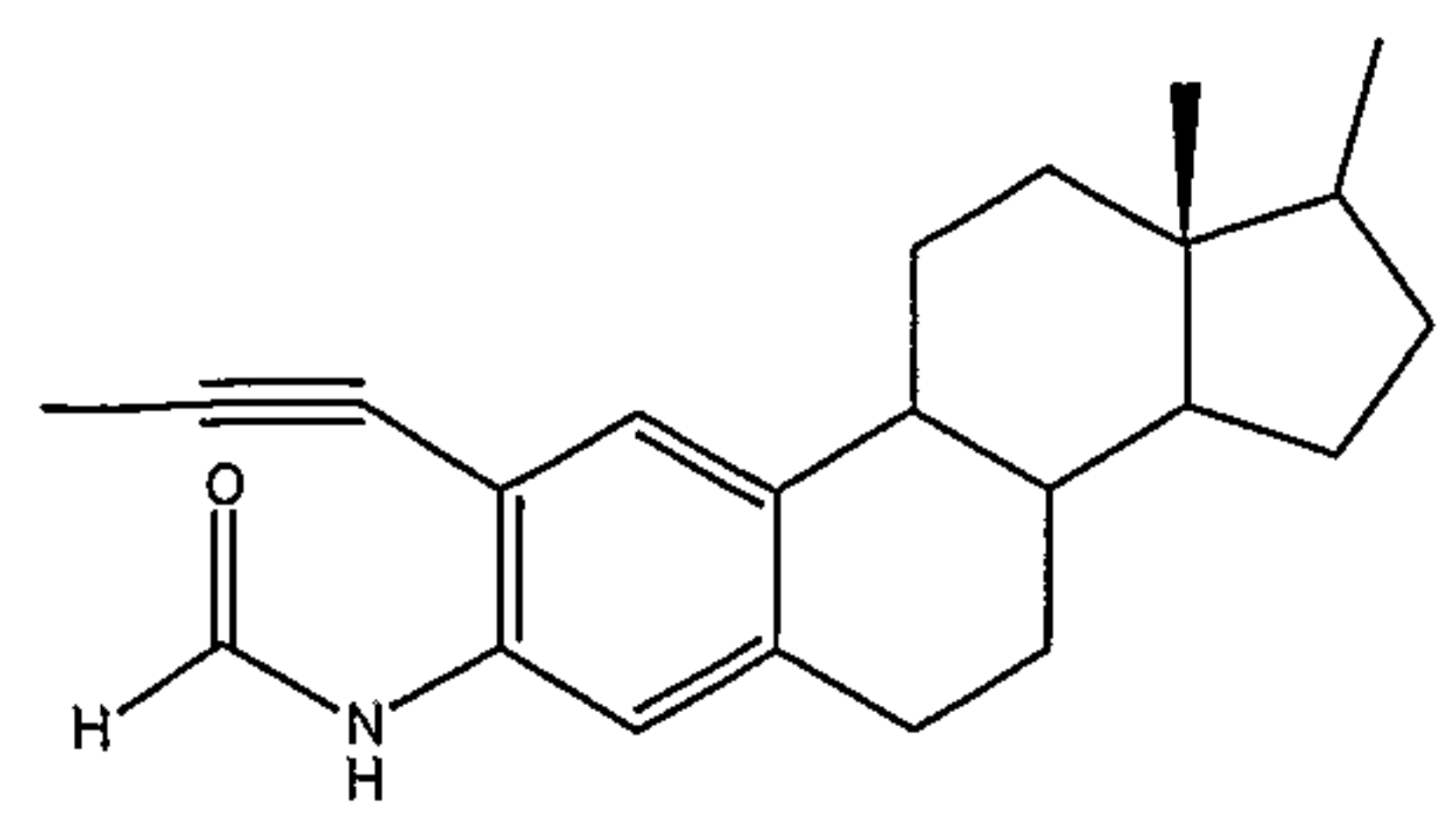
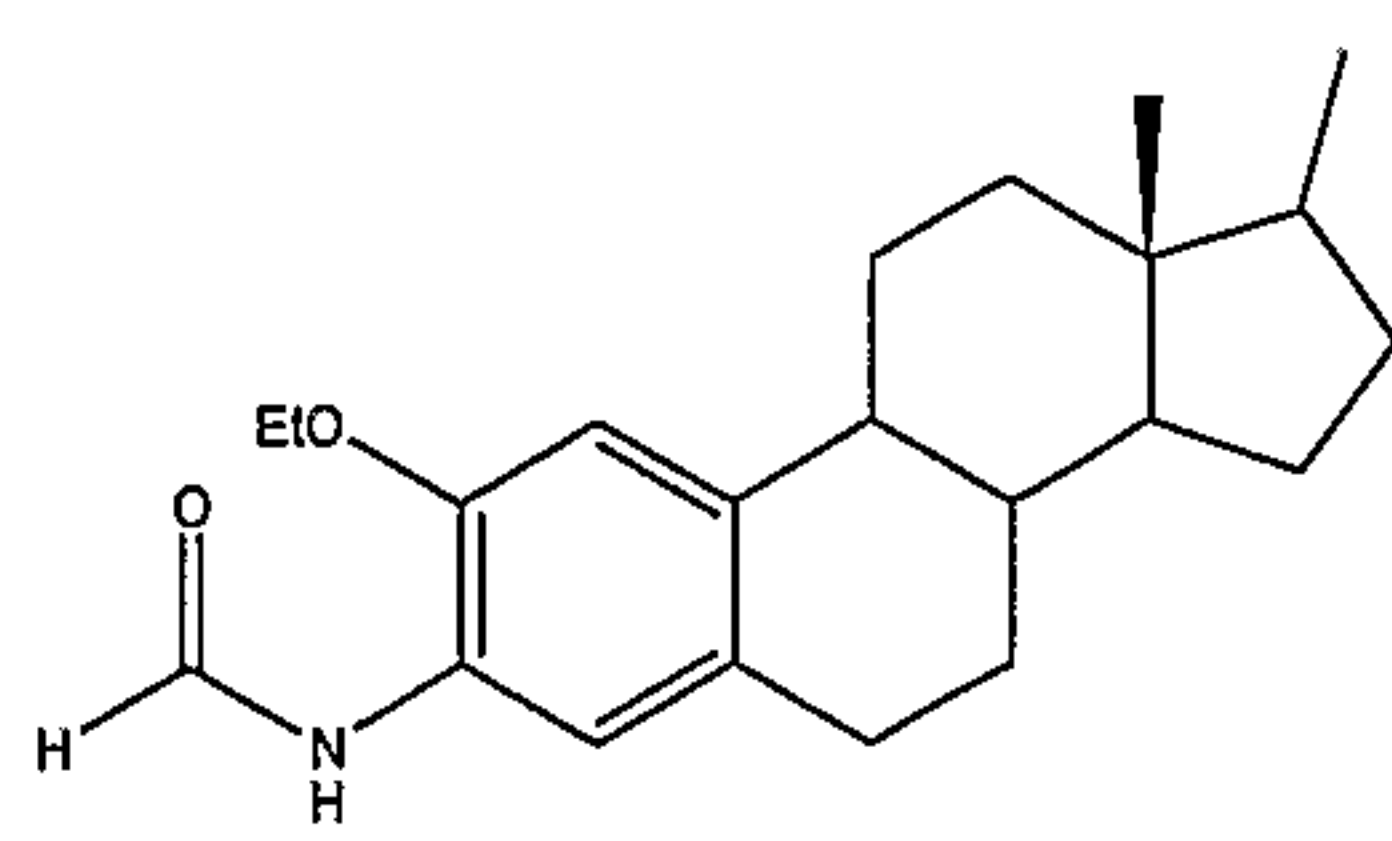
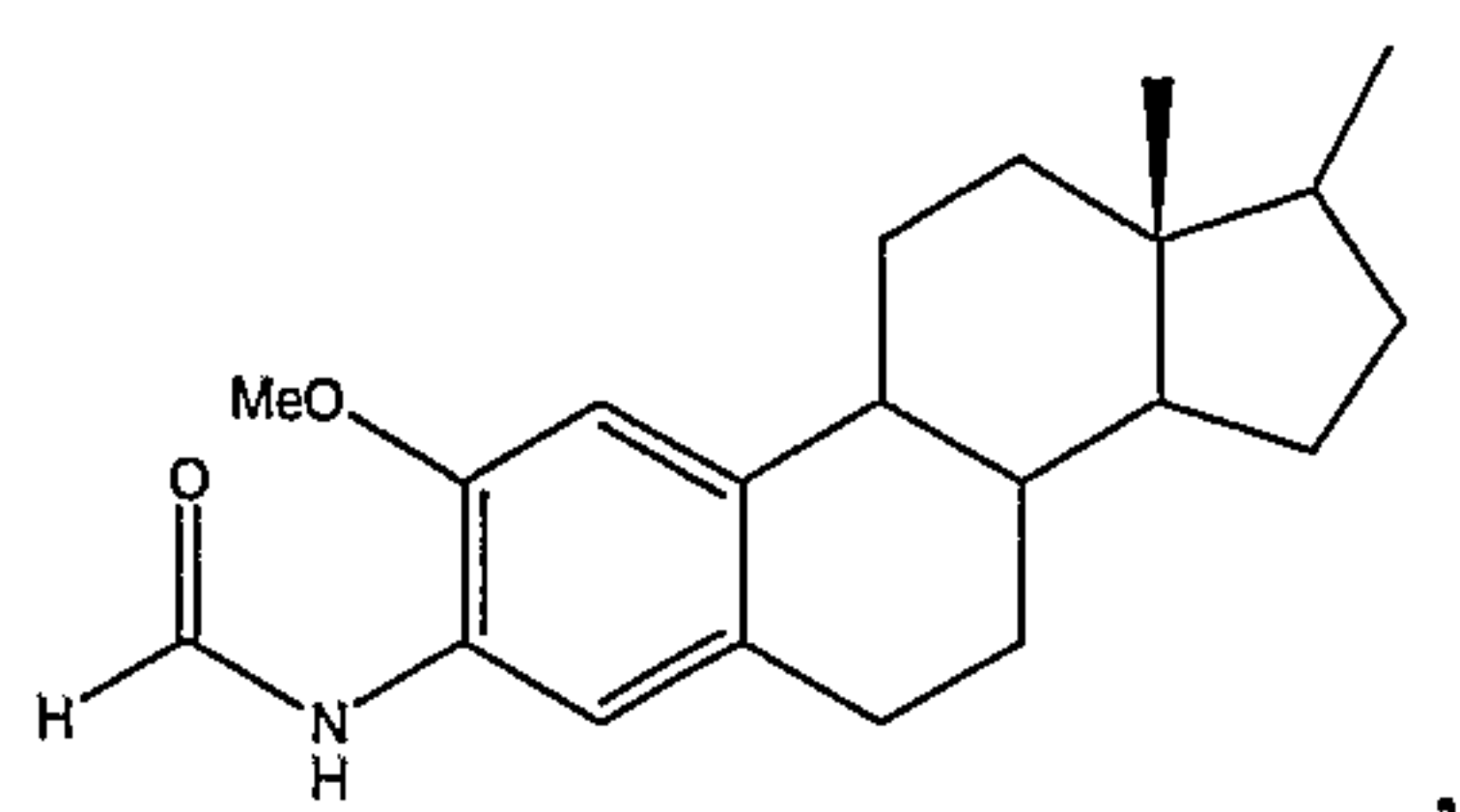
Z'' is selected from $>C(H_2)$, $>C(H)-CH_3$, $>C=CH_2$, $>C=CHCH_3$ or $>C=O$, provided that when Z' is $>C-OSO_2NH_2$ and Z'' is $>C(H_2)$ or $>C=O$, R_a is neither $-OCH_3$ nor $-OCH_2CH_3$, in an effective amount upon administration in a daily dose, a daily sub-dose, or an appropriate fraction thereof to a human or an animal to inhibit angiogenesis.

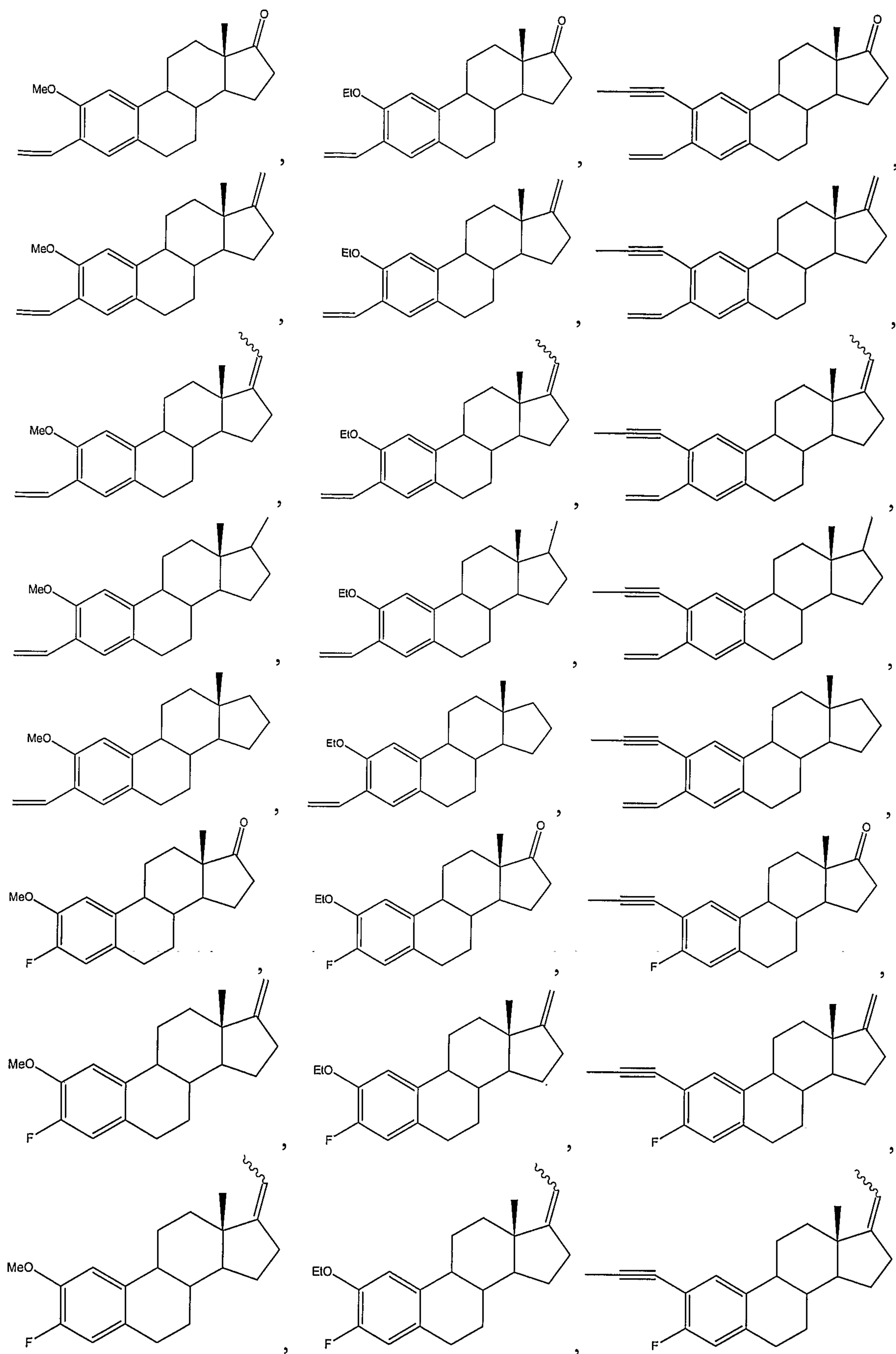
15

197. A pharmaceutical composition comprising:
a pharmaceutical carrier or excipient; and
a compound selected from



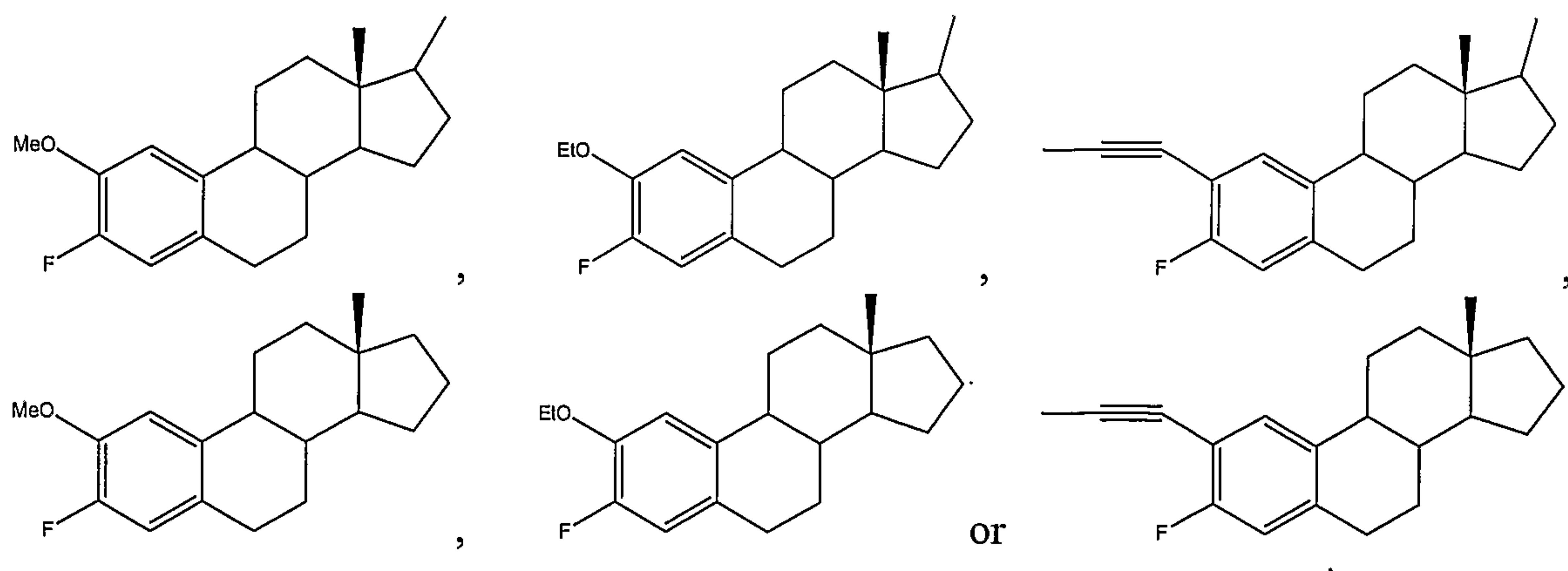






WO 2005/030120

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in an effective amount upon administration in a daily dose, a daily sub-dose, or an appropriate fraction thereof to a human or an animal to inhibit angiogenesis.

