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(72) **Inventeurs/Inventors:**
HAZLEHURST, LORI, US;
MCLAUGHLIN, MARK, US;
JAIN, PRIYESH, US;
DALTON, WILLIAM S., US

(73) **Propriétaires/Owners:**
H. LEE MOFFITT CANCER CENTER & RESEARCH INSTITUTE, INC., US;
UNIVERSITY OF SOUTH FLORIDA, US

(74) **Agent:** MBM INTELLECTUAL PROPERTY LAW LLP

(54) **Titre : INHIBITEURS DES INTERACTIONS DE L'INTEGRINE POUR TRAITER LE CANCER**
(54) **Title: INTEGRIN INTERACTION INHIBITORS FOR THE TREATMENT OF CANCER**

(57) **Abrégé/Abstract:**
Integrin interaction inhibitors using a beta-turn promoter are described herein. These peptides are useful in treating cancer, such as multiple myeloma, by administering a therapeutically effective amount of the integrin interaction inhibitor. Data show that integrin interaction inhibitors act synergistically or additively interacts with anti-proliferative agents such as doxorubicin, SAHA, arsenic trioxide, and etopside.

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(71) Applicants *(for all designated States except US)*: **H. LEE MOFFITT CANCER CENTER & RESEARCH INSTITUTE** [US/US]; 12902 Magnolia Drive, Tampa, FL 33612-9497 (US). **UNIVERSITY OF SOUTH FLORIDA** [US/US]; 3802 Spectrum Blvd., Suite 100, Tampa, FL 33612 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **HAZLEHURST, Lori** [US/US]; 818 Bayview Drive, Ruskin, FL 33613 (US). **MCLAUGHLIN, Mark** [US/US]; 3623 W. Tampa Circle, Tampa, FL 33629 (US). **JAIN, Priyesh** [IN/US]; 14308 Wedgewood Circle, Apt #201, Tampa, FL 33613 (US). **DALTON, William, S.** [US/US]; 505 S. Riverhills Drive, Tampa, FL 33617 (US).

(74) Agents: **LADWIG, Glenn, P.** et al.; Saliwanchik, Lloyd & Eisenschenk, P.O. Box 142950, Gainesville, FL 32614-2950 (US).

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(54) Title: INTEGRIN INTERACTION INHIBITORS FOR THE TREATMENT OF CANCER

(57) Abstract: Integrin interaction inhibitors using a beta-turn promoter are described herein. These peptides are useful in treating cancer, such as multiple myeloma, by administering a therapeutically effective amount of the integrin interaction inhibitor. Data show that integrin interaction inhibitors act synergistically or additively interacts with anti-proliferative agents such as doxorubicin, SAHA, arsenic trioxide, and etopside.

INTEGRIN INTERACTION INHIBITORS FOR THE TREATMENT OF CANCER

GOVERNMENT SUPPORT

This invention was made with government support under Grant No. CA122065, awarded by the National Institutes of Health (NIH). The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

This invention relates to treatment of malignancies with peptides. Specifically, the invention provides contacting cancer cells, tumors, or pre-tumorigenic masses with integrin interaction inhibitor proteins to effect treatment.

Multiple myeloma (MM) is a cancer of the plasma cell, which primarily develops in the elderly population. The progression of the tumor is well understood, and it can be diagnosed by the presence of multiple myeloma cells in the bone marrow and monitored by the amount of antibody secretion from the clonal population of plasma cells. A premalignant condition known as monoclonal gammopathy of undetermined significance (MGUS) develops at a certain rates in the US population: 3% at age 50, 5% at age 70, and 7% by age 85; approximately 1% of MGUS patients progress to multiple myeloma on an annual basis (Kyle RA, et. al, Prevalence of monoclonal gammopathy of undetermined significance. *N. Engl. J. Med.* 354, 1362-1369 (2006)). The molecular causes for progression from MGUS to MM are unknown. After the onset of the cancer, multiple myeloma patients suffer from several symptoms, including calcium dysregulation, renal failure, anemia, and bone lesions. A diagnosis of multiple myeloma is established using blood and urine tests. For advanced stage patients, complete skeletal surveys are also used to examine the damage caused by multiple myeloma in the bone marrow. Staging with serum calcium, creatinine, hemoglobin, and most importantly, the concentration of the "monoclonal serum protein" was established in 1975 by Durie and Salmon (Durie BG, Salmon SE, A clinical staging system for multiple

myeloma. Correlation of measured myeloma cell mass with presenting clinical features, response to treatment, and survival. *Cancer* 36, 842-854 (1975)). The International Staging System determined in 2005 uses those markers as well as serum albumin and β-2-microglobulin (Greipp PR, et. al, International staging system for multiple myeloma. *J. Clin. Oncol.* 23, 3412-3420 (2005)). The survival statistics indicate the importance of early detection and proper staging, and show the devastating impact of multiple myeloma. Stage I patients have median survival times of 62 months, stage II 45 months, and stage III patient median survival is reduced to 29 months.

Despite the highly specific and easily detectable biomarkers, many challenges still exist for MM treatment. Several different treatment regimens are under investigation; these strategies have been the subject of numerous recent reviews (Fonseca R, Stewart AK, Targeted therapeutics for multiple myeloma: the arrival of a risk-stratified approach. *Mol. Cancer Ther.* 6, 802-810 (2007); Chanan-Khan AA, Lee K, Pegylated liposomal doxorubicin and immunomodulatory drug combinations in multiple myeloma: rationale and clinical experience. *Clin. Lymph. Myel.* 7, S163-S169 (2007); Thomas S, Alexanian R. Current treatment strategies for multiple myeloma. *Clin. Lymph. Myel.* 7, S139-S144 (2007); Falco P, et al., Melphalan and its role in the management of patients with multiple myeloma. *Expert. Rev. Anticancer Ther.* 7, 945-957(2007)). Novel therapeutic strategies include proteasome inhibition with agents like bortezomib (Voorhees PM, Orlowski RZ, Emerging data on the use of anthracyclines in combination with Bortezomib in multiple myeloma. *Clin. Lymph. Myel.* 7, S156-S162 (2007); Manochakian R, et al., Clinical Impact of Bortezomib in frontline regimens for patients with multiple myeloma. *The Oncologist* 12, 978-990 (2007)) and a combination of cancer cell targeting and immune modulation with thalidomide derivatives like Lenalidomide (Singhal S, Mehta J. Lenalidomide in myeloma. *Curr. Treatment Options in Oncology* 8, 154-163 (2007)). While each of these agents can have some success against multiple myeloma cells, proteasome inhibitors are the only molecularly guided therapy to date: treatment is more effective for patients with myelomas that secrete high levels of monoclonal antibodies (Meister S, et al., Extensive immunoglobulin production sensitizes myeloma cells for proteasome inhibition. *Cancer Res.* 67, 1783-1792 (2007)). The use of the other agents is directed by the expected tolerance for side effects rather than molecular targeting. Regardless, these agents improve the patient outcome when compared to the current standard of care (Ma MH, et al., The proteasome inhibitor PS-341 markedly enhances sensitivity of multiple myeloma tumor cells to

chemotherapeutic agents. *Clin, Cancer Res.* 9, 1136-1144 (2003)), and drug combination strategies are currently in clinical trials (Srikanth M, Davies FE, Morgan GJ, An update on drug combinations for treatment of myeloma. *Expert Opn. Investig. Drugs* 17, 1-12 (2008); Richardson PG, *et al.*, The emerging role of novel therapies for the treatment of relapsed myeloma. *J. Natl. Comp. Cancer Network* 5, 149-162 (2007); Merchionne F, et al., New therapies in multiple myeloma. *Clin. Exp. Med.* 7, 83-97 (2007)). Proteomic research may contribute to guidance of existing and emerging therapies. Identification of novel targets including c-Jun^{v} and the Fanconi anemia pathway (Chen Q, et al., The FA/BRCA pathway is involved in Melphalan-induced DNA interstrand cross-link repair and accounts for Melphalan resistance in multiple myeloma cells. *Blood* 106, 698-705 (2005)) also offers opportunities to examine protein expression, binding partners, and post-translational modification.

Initial treatment is positive, as MM responds to standard chemotherapy treatment. However, relapse of the tumor usually occurs due to unsuccessful elimination of minimal residual disease (MRD). Recurrence of disease is associated with emergence of multi drug resistance (MDR) of tumor cells to standard cytotoxic agents (Hazlehurst, L.A., Alsina, M., Dalton, W.S. *Cancer Research*, **63**, 7900-7906 (2003); Daminao, J.S., Cress, A.E., Hazlehurst, L.A., Shtil, A.A., Dalton, W.S. *Blood*, **93(5)**, 1658-1667 (1999)). MRD is typically found in the bone marrow compartment, suggesting that this particular microenvironment may provide tumor cell survival signals. Multiple myeloma cells adhere to bone marrow, an environment that is rich in extracellular matrices via cell surface receptors. The emergence of drug-resistant cells is an obstacle to treatment of diseases. The bone marrow microenvironment is critical for progression of multiple myeloma and likely contributes to drug resistance; (Li ZW, Dalton WS, Tumor microenvironment and drug resistance in hematologic malignancies. *Blood Rev.* 20(6), 333-342 (2006); Hazlehurst LA, et al., Role of the tumor microenvironment in mediating de novo resistance to drugs and physiological mediators of cell death. *Oncogene* 22, 7396-7402 (2003); Dalton WS. The tumor microenvironment: focus on myeloma. *Cancer Treat Rev.* 29 Suppl 1, 11-19(2003)) this knowledge has led to preclinical models examining multiple myeloma in the context of the bone marrow microenvironment. Plausible targets in the bone marrow microenvironment include cytokine signaling, *e.g.* IL-6, (Chauhan D, et al., Interleukin-6 inhibits Fas-induced apoptosis and stress-activated protein kinase activation in multiple myeloma cells. *Blood* 89, 227-234 (1997); Urashima M, et al., Interleukin-6 overcomes p21WAF1 upregulation and G1 growth arrest induced by dexamethasone and interferon-gamma in multiple myeloma cells.

Blood 90, 279-289 (1997)) and integrin mediated drug resistance (Damiano JS, et al., Cell adhesion mediated drug resistance (CAM-DR): role of integrins and resistance to apoptosis in human myeloma cell lines. *Blood* 93, 1658-1667 (1999)).

In some situations, leukemias have gained resistance through cellular adhesion to extracellular matrix through 1 integrin. (Hazlehurst, et al. *Oncogene.* 2000; 19:4319-4327; Hazlehurst, et al. *Cancer Res.* 2003; 63:7900-7906; Hazlehurst, et al. *Blood.* 2001; 98:1897-1903; Hazlehurst, et al. *Cancer Res.* 2006; 66:2338-2345; Hazlehurst, et al. *Cancer Metastasis Rev.* 2001; 20:43-50; Hazlehurst, et al. *Cancer Res.* 1999; 59:1021-1028; Hazlehurst, et al. *Biochem Pharmacol.* 1995; 50:1087-1094; Hazlehurst, et al. 55 *Oncogene.* 2003; 22:7396-7402). Hazlehurst et. al. have shown that adhesion of leukemia and multiple myeloma cell lines to extracellular matrix component, fibronectin (FN) via integrin influences cell survival and inhibits drug-induced apoptosis (Hazlehurst, L.A., Damiano, J.S., Buyuksalml., Pledger, W.J., Dalton, W.S. *Oncogene*, **38**, 4319-4327 (2000)). Studies have found these findings extend to the clinincal setting, where cell adhesion induced drug resistance (CAMDR) phenotype is operative in clinical samples taken from primary multiple myeloma (Hazlehurst L A, et al. *Cancer Res.* 2003; 63:7900-7906).

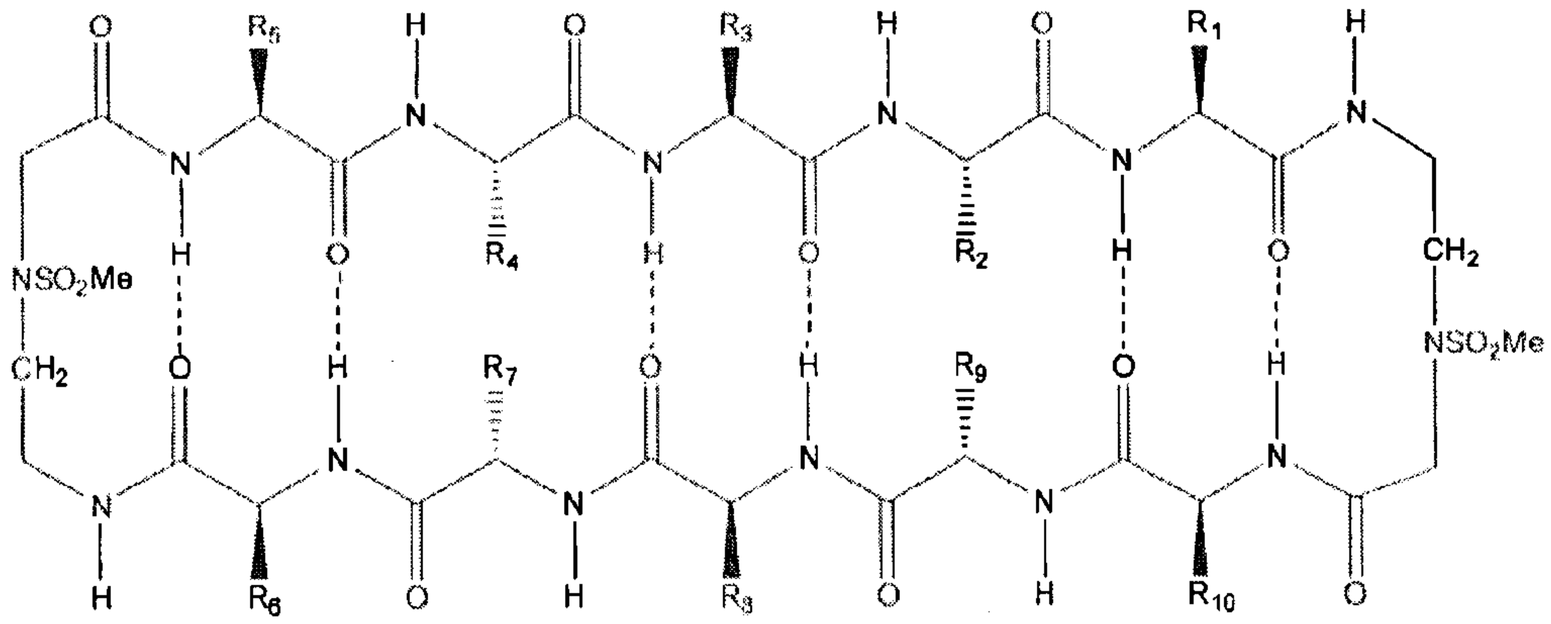
BRIEF SUMMARY OF THE INVENTION

The present invention provides novel designs for integrin interaction inhibitors, compositions comprising these integrin interaction inhibitors, methods of producing these integrin interaction inhibitors, and methods of use. In preferred embodiments, the integrin interaction inhibitors comprise a cyclic compound comprising a recognition sequence and a non-recognition sequence, wherein the recognition sequence comprises at least four amino acids, wherein the non-recognition sequence comprises at least four amino acids, and wherein the recognition sequence is joined to the non-recognition sequence by a first linker and a second linker.

In one aspect of the present invention, there is provided a cyclic compound comprising a recognition sequence and a non-recognition sequence, wherein said recognition sequence comprises at least four amino acids, wherein said non-recognition sequence comprises at least

four amino acids, and wherein said recognition sequence is joined to said non-recognition sequence by a first linker and a second linker.

In certain embodiments, the compound has the following structure:



wherein R_1 is K;

wherein R_2 is L;

wherein R_3 is K;

wherein R_4 is L;

wherein R_5 is K;

wherein R_6 is selected from the group consisting of W, A, and M;

wherein R_7 is selected from the group consisting of S, A, Y, and V;

wherein R_8 is selected from the group consisting of V and A;

wherein R_9 is selected from the group consisting of V, A, and S; and

wherein R_{10} is selected from the group consisting of M, A, W, and nor-Leu.

mediated by cells that exhibit the cell adhesion induced drug resistance (CAM-DR) phenotype.

Another aspect of the invention concerns a method of suppressing the growth of malignant cells, comprising contacting the cells *in vitro* or *in vivo* with an effective amount of at least one integrin interaction inhibitor of the invention. In some embodiments, the malignant cells exhibit the CAM-DR phenotype.

Another aspect of the invention concerns a method of inducing cell death in malignant cells, comprising contacting the cells *in vitro* or *in vivo* with an effective amount of at least one integrin interaction inhibitor of the invention. In some embodiments, the malignant cells are in suspension, *e.g.*, part of a circulating tumor cell (CTC) population, and the peptides kill the CTC. In some embodiments of the *in vivo* method, the integrin interaction inhibitors of the invention prevent or delay onset of metastasis (*e.g.*, to the bone). In some embodiments, the malignant cells exhibit the CAM-DR phenotype. Without being bound by theory, it is proposed that the integrin interaction inhibitors of the invention induce cell death by cell surface binding and activation of a non-caspase dependent cell death mechanism involving autophagy.

Another aspect of the invention concerns a method of inhibiting (*e.g.*, reducing, interfering with, or disrupting) ß1 integrin mediated adhesion, comprising contacting cells *in vitro* or *in vivo* with an effective amount of at least one integrin interaction inhibitor of the invention.

Another aspect of the invention concerns a method for increasing the efficacy of chemotherapy or radiation therapy in a subject, comprising administering at least one integrin interaction inhibitor to the subject. Preferably, the method further comprises administering the chemotherapy and/or radiation treatment to the subject before, during, or after administration of the integrin interaction inhibitor, wherein the effectiveness of the treatment is increased.

In another aspect, the invention pertains to an adhesion trap comprising a substrate (surface) with integrin interaction inhibitor immobilized to the surface, and a method of removing circulating tumor cells (CTC) from blood by contacting a subject's blood with the immobilized integrin interaction inhibitor.

Another aspect of the invention concerns a method of identifying modulators of integrin interaction inhibitor binding (a screen for molecules that displace integrin interaction inhibitor binding), the method comprising providing a candidate agent (such as a chemical

compound, antibody, nucleic acid, peptide, or other substance); and determining whether the candidate agent inhibits (*e.g.*, disrupts, prevents, or interferes with), the ability of an integrin interaction inhibitor of the invention to bind to ß1 integrin on a cancer cell surface and/or inhibit ß1 integrin mediated adhesion, *in vitro* or *in vivo* (*e.g.*, in an animal model). Optionally, the integrin interaction inhibitor is labeled with a detectable moiety (*e.g.*, fluorescently) to facilitate the determining step. The determining step can be carried out by contacting the candidate agent with the cells in the presence of the integrin interaction inhibitor. Optionally, the integrin interaction inhibitor may be immobilized on a surface or in suspension.

In another aspect, the invention concerns a method for detecting circulating tumor cells (CTC). Thus, the invention includes an *in vitro* screening assay for detecting CTC in a biological sample from a subject (such as peripheral blood), comprising obtaining a biological sample from a subject; and determining whether the integrin interaction inhibitor of the invention binds to cells (ß1 integrin on the cell surface) in the sample. Preferably, the integrin interaction inhibitor is labeled with a detectable moiety (*e.g.*, fluorescently) to facilitate the determining step. The integrin interaction inhibitor binding can be carried out using flow cytometry analysis or in tandem with CTC detection machines, for example. Optionally, the integrin interaction inhibitor may be immobilized on a surface, or in suspension. In other embodiments, the integrin interaction inhibitors of the invention can be tested for potency by determining their ability to prevent or interfere with the binding of labeled ligand to target cells. In this case, the ligand is labeled and incubated in the presence of the test cells and unlabelled integrin interaction inhibitor.

Another aspect of the invention concerns an *in vitro* screening test for the presence of malignant cells in a mammalian tissue, the test including: obtaining a sample containing viable cells of the tissue; culturing the sample under conditions promoting growth of the viable cells contained therein; treating the cultured sample with an integrin interaction inhibitor of the invention; and analyzing the treated sample by a method effective to determine percent of cell death as an indicator of presence of malignant cells in the sample.

The invention also concerns a composition comprising an integrin interaction inhibitor and one or more anti-cancer agents (*e.g.*, chemotherapeutic agents). In some embodiments, the anti-cancer agent is selected from among suberoylanilide hydroxamic acid (SAHA) or other histone deacetylase inhibitor, arsenic trioxide, doxorubicin or other anthracycline DNA intercalating agent, and etoposide or other topoisomerase II inhibitor. The

composition is useful for inhibiting the growth of cancer cells (for example, myeloma cells) *in vitro* or *in vivo*, when administered thereto.

The invention also concerns a composition comprising a HYD1 peptide and one or more anti-cancer agents (*e.g.*, chemotherapeutic agents). In some embodiments, the anti-cancer agent is selected from among suberoylanilide hydroxamic acid (SAHA) or other histone deacetylase inhibitor, arsenic trioxide, doxorubicin or other anthracycline DNA intercalating agent, and etoposide or other topoisomerase II inhibitor. The composition is useful for inhibiting the growth of cancer cells (for example, myeloma cells) *in vitro* or *in vivo*, when administered thereto. In some embodiments, the HYD1 peptide comprises the all D-amino acid peptide KIKMVISWKG (HYD1).

As used herein, unless specified, "a HYD1 peptide" is inclusive of the d-amino acid peptide having the sequence: KIKMVISWKG (HYD1), as well as other HYD1-related peptides (which includes d-amino acid containing peptides and non-d-amino acid containing peptides) disclosed in U.S. Patent No. 7,632,814 (Hazelhurst *et al.*, "HYD1 Peptides as Anti-Cancer Agents"). As used herein, reference to c-HYD1, C-HYD1 refers to a cyclized or cyclic peptidomimetic of the invention.

The invention also concerns methods for treating a malignancy in a subject, comprising administering a HYD1 peptide and one or more anti-cancer agents selected from suberoylanilide hydroxamic acid (SAHA) or other histone deacetylase inhibitor, arsenic trioxide, doxorubicin or other anthracycline DNA intercalating agent, and etoposide or other topoisomerase II inhibitor. The HYD1 peptide may be administered before, during, or after the one or more of the aforementioned agents. In some embodiments, the malignancy is multiple myeloma or another hematologic malignancy. In some embodiments, the HYD1 peptide comprises the all D-amino acid peptide KIKMVISWKG (HYD1).

The invention also concerns a method of suppressing the growth of malignant cells, comprising contacting the cells *in vitro* or *in vivo* with an effective amount of a HYD1 peptide, and at least one other anti-cancer agent. In some embodiments, the malignant cells are cells of a multiple myeloma or another hematologic malignancy. In some embodiments, the HYD1 peptide comprises the all D-amino acid peptide KIKMVISWKG (HYD1).

BRIEF DESCRIPTION OF THE DRAWINGS

For a fuller understanding of the invention, reference should be made to the following detailed description, taken in connection with the accompanying drawings, in which:

Figure 1 is a diagram of the cyclic III peptide.

Figure 2 is a diagram of the synthesis scheme for generation of the ß-hairpin turn promoter for cyclic III peptides/ integrin interaction inhibitors.

Figure 3(A) is a graph showing HYD1 is more potent in MM cells (H929) compared to normal hematopoietic cells. CD34 positive cells were isolated from the peripheral blood of a normal donor. HYD1 treatment did not inhibit differentiation or colony formation of CD34 positive cells.

Figure 3(B) is a graph showing HYD1 is more potent in MM cells (H929) compared to normal hematopoietic cells. Peripheral blood mononuclear cells (PBMC) were isolated from a normal donor. Cells were treated with 6.25, 12.5, 25 and 50 ug/ml for six hours and cell death was measured by annexin V positivity and FACS analysis.

Figure 3(C) is a graph showing HYD1 is more potent in MM cells (H929) compared to normal hematopoietic cells. H929 cells are more sensitive to HYD1 induced inhibition of colony growth compared to normal CD34 positive cells. Similar to H929 cells, U226, 8226 and MM1S myeloma cells were all sensitive to HYD1 induced cell death.

Figure 4 is a graph showingHYD1 but not the scrambled control peptide (HYD1S) induces preferential cell death in MM cells. CD138 positive (myeloma) and negative cells were collected, isolated from a BM aspirate, and treated with 50 µg/ml for 24 h; 24 hours after drug treatment, cell death was determined by Annexin V/PI staining and FACS analysis.

Figure 5 is a diagram of the general cyclic III peptide for Table 1.

Figure 6 is a diagram of the general cyclic III peptide for Table 2.

Figure 7 is a graph showing the circular dichroism studies for / integrin interaction inhibitors in 7mM Sodium Acetate buffer at a concentration of 200 µM at pH 7.

Figure 8 shows the structure of an embodiment of the integrin interaction inhibitors of the invention, wherein R_1 is K; R_2 is L; R_3 is K; R_4 is L; R_5 is K; R_6 is selected from the group consisting of W, A, and M; R_7 is selected from the group consisting of S, A, Y, and V; R_8 is selected from the group consisting of V and A; R_9 is selected from the group consisting of V, A, and S; and R_{10} is selected from the group consisting of M, A, W, and nor-Leu.

Figure 9 is Scheme1: Solid-Phase synthesis of cyclic III peptide using solution phase cyclization strategy.

Figure 10 is Scheme2: Solid-Phase synthesis of cyclic III peptide analogs using side chain anchoring strategy.

Figure 11 is Scheme 3: Synthesis of the methylsulfonamido aminoethyl glycine linker T_1.

Figure 12 is Scheme 4: Synthesis of the ether peptidomimetic amino acid linker T_3.

Figure 13 are results of circular dichroism studies for cyclic III peptides 1, 2, 5, 7, 8 and 10 in 7 mM sodium acetate buffer at a concentration of 200 μM at pH 7.

Figures 14A-C. Figure 14A: Labeled positions on the methylsulfonamido aminoethyl glycine turn. Newman projection of the *β*-turn viewed down the d-γ bond: Figure 14B: N-Ms pointing down and away from the *β*-sheet; Figure 14C: N-Ms pointing into the center of the *β*-sheet.

Figure 15 is a Newman Projection of the structurally locked *β*-turn viewed down the d-γ bond.

Figure 16 is a Newman Projection of the T_3 *β*-turn viewed down the O- a' bond. The Pro-R a' proton is labeled as H'.

Figures 17A-17B are peptide **2** NOEs. Figure 17A: same-strand NOEs; Figure 17B: cross-strand NOEs. (Black = strong, Green = Medium, Red = Weak).

Figures 18A-18B are peptide **5** NOEs. Figure 18A: same-strand NOEs; Figure 18B: cross-strand NOEs. (Black = strong, Green = Medium, Red = Weak).

Figures 19A-19B are stereoviews of the 20 lowest energy structures for NOE-constrained calculated structure of peptides **1** (green carbon atoms) (19A) and **5** (gold carbon atoms) (19B). N = blue, O = red, S = yellow; hydrogens omitted for clarity. Thin wire representations prepared with Maestro.

Figures 20A-20H show the N-(2-aminoethyl)-N-methylsulfonamidoglycine linker tert-Butyl N-(2-aminoethyl) glycine 2 (Figure 20A); tert-Butyl N-[2-(N-9-fluorenylmethoxycarbonyl)aminoethyl]glycinate hydrochloride 3 (Figure 20B); tert-Butyl N-[2-(N-9-fluorenylmethoxycarbonyl)aminoethyl N-methylsulfonamido glycinate 4 (Figure 20C); 2-(N-(2-(((9H-fluoren-9-yl)methoxy)carbonylamino)ethyl)methylsulfonamido)acetic acid 5 (Figure 20D); I-tert-butyl 2-((2-tert-butoxy-2-oxoethoxy)methyl)pyrrolidine-1-carboxylate 7 (Figure 20E); 1-tert-butyl 2-(pyrrolidin-2-ylmethoxy) acetate 8 (Figure 20F); I-(9H-fluoren-9-yl)methyl 2-((2-tert-butoxy-2-oxoethoxy)methyl)pyrrolidine-1-carboxylate 9 (Figure 20G); and I-2-((1-(((9H-fluoren-9-yl)methoxy)carbonyl)pyrrolidin-2-yl)methoxy)acetic acid 10 (Figure 20H).

Figures 21-32 are NMR spectra of compounds 2, 3, 5, 7, 9, and 10.

Figures 33A-33C show peptides structural characterization via NOE Peptide 1 NOEs. Figure 33A: Intra-residue NOEs; Figure 33B: Same-strand NOEs; Figure 33C: Cross-strand NOEs.

Figures 34A-34C show peptide **7** NOEs. Figure 34A: Intra-residue NOEs; Figure 34B: Same-strand NOEs; Figure 34C: Cross-strand NOEs.

Figures 35A-35C show peptide **8** NOEs. Figure 35A: Intra-residue NOEs; Figure 35B: Same-strand NOEs; Figure 35C: Cross-strand NOEs.

Figures 36A-36C show peptide **10** NOEs. Figure 36A: Intra-residue NOEs; Figure 36B: Same-strand NOEs; Figure 36C: Cross-strand NOEs.

Figures 37A-37C show peptide **16** NOEs. Figure 37A: Intra-residue NOEs; Figure 37B: Same-strand NOEs; Figure 37C: Cross-strand NOEs.

Figure 38 shows a Ramachandran plot of peptides **1** and **5**. All of the peptide **5** amino acids are in the Beta-sheet region while two amino acids (Val7, L-Pro) of peptide **1** are in the "disallowed regions."[4] The L-Pro phi/psi angles are consistent with the L-Pro phi-psi angles of the cyclic peptide structure published by Fasan et al (PDB 2AXI).[5]

Figure 39 is a graph showing that cyclized HYD1 (represented as C-HYD1 or c-HYD1) is 30-fold more potent compared to the parent linear HYD1 peptide. H929 cells were treated for 24 hours with varying concentrations of peptide. Cell death was measured by Topro-3 staining and FACS analysis. The IC_{50} values were obtained from linear regression analysis and an average value was obtained from 3 independent experiments. In H929 cells, the mean IC_{50} value for HYD1 was 33 μM, while the IC_{50} value for c-HYD1 is 1.2 μM.

Figure 40 is a graph showing IC-50 levels of 24 lung cancer (LC) cell lines treated with cyclized HYD1. The activity of c-HYD1 was screened using a high-throughput CellTiter-Blue cell viability assay. Cell viability was assessed by the ability of the remaining viable cells to bioreduce resazurin to resorufin. Resazurin is dark bluc in color and has little intrinsic fluorescence until it is reduced to resorufin (579nm Ex/584nm Em). The change in fluorescence was measured with a Synergy 4 microplate reader (Bio-Tek Instruments, Inc.). The fluorescence data was transferred to a spreadsheet program to calculate the percent viability relative to the four replicate cell wells that did not receive drug. IC50s were determined as the concentration of drug required for 50% reduction in growth/viability. Shown is the mean IC50 value for each lung cancer cell line tested. Experiments were repeated 2-3 times.

Figure 41 is a graph showing that cyclized HYD1 (represented as C-HYD1 or c-HYD1) treatment significantly reduces tumor growth ($p<0.05$ ANOVA) in a MM SCID-Hu *in vivo* model. Briefly, 50,000 H929 myeloma cells were engrafted into the bone implant for 10 days prior to initiation of peptide treatment. Following tumor engraftment mice were randomized (0 time point) and injected with 8 mg/kg c-HYD1 or vehicle control (VC) daily

(I.P. injections) for 14 days and every other day from day 14-28 at which time treatment stopped. Tumor burden was measured by circulating Kappa levels by ELISA weekly. N=10 mice for vehicle control (VC) and 9 mice for c-HYD1 treated.

Figure 42 is a graph showing the effect of cyclized HYD1 (c-HYD1) injection on the weight of mice. No weight loss or any overt signs of toxicity were observed.

Figure 43 is an embodiment of a synthesis scheme for cyclized peptides. The optimized recognition sequence and the non-recognition sequence are synthesized via standard solution-phase peptide synthesis techniques with a convergent fragment coupling at the beta-turn promoter carboxylate groups which cannot be racemized during fragment coupling or during cyclization. The c-HYD1 peptide is very amenable to convergent solution-phase peptide synthesis methods. The beta-turn promoters in our most active c-HYD1 analog have achiral glycine-like carboxylic acid functional groups that cannot undergo racemization and are therefore excellent sites for peptide fragment coupling, which allows a convergent synthetic approach to making the c-HYD1 analogs. The scheme in Figure 43 is an example of a synthesis approach. Strand A1-A5 and A6-A10 can be the recognition and non-recognition sequences, respectively, or vice versa. The non-recognition sequence position A3 or A8 will have an orthogonal protecting group such as the alloc group which will allow easy derivatization with biotin, FAM1, dimerization, or oligomerization. The inventors have already determined that derivatization of that Lys group does not negatively effect bioactivity.

Figure 44. Biotin-HYD1 interacts with CD44 in H929 cells. Thirty micrograms of membrane extract was incubated with either biotin or biotin-HYD1 bound NeutrAvidin beads. The first lane is 30 μg of membrane extract only. CD44 was detected by western blot analysis using a pan-CD44 antibody.

Figures 45A-45B. Biotin-HYD1 interacts with a4 integrin (Figure 45A) and CD44 (Figure 45B). Biotin-HYD1 or biotin was immobilized to NeutraAvidin beads prior to incubation with 150 μg of membrane extract. The blot was initially probed for a4 integrin and subsequently stripped and re-probed with CD44 antibody.

Figure 46. Biotin-HYD1 binds recombinant CD44 in a direct ELISA. A primary CD44 antibody and HRP conjugated secondary antibody and chemiluminescence detection was used to quantify rCD44 binding to immobilized biotin-HYD1.

DETAILED DISCLOSURE OF THE INVENTION

The present invention concerns integrin interaction inhibitors (also referred to interchangeably herein as "compounds of the invention" and "peptides of the invention"), compounds comprising such inhibitors, and methods of using such inhibitors. Integrin interaction inhibitors, such as those shown in Figure 1, were generated. Generation of the ß-hairpin turn promoter is seen in Figure 2. In some embodiments, the integrin interaction inhibitor is a cyclic peptide disclosed herein.

Since the inventors determined that HYD1 induced an underutilized therapeutic strategy of inducing cell death in tumor cells (programmed necrosis) and binds to a novel target in MM (CD44), it was decided to pursue strategies which would increase the therapeutic potential of this novel agent. To this end, the inventors sought to determine whether cyclization of the peptide was a viable strategy for increasing the potency and *in vivo* efficacy of the peptide. Scanning the sequence of the peptide, it became evident that if a secondary structure was important for binding, that a beta sheet or beta-turn conformation was the most likely candidate. To this end, the inventors initially performed N- and C-terminus truncation studies and identified MVISW as the likely core region of D-HYD1 required for biological activity. Using this information and the finding that V for I replacement gave a more active D-HYD1 analog, the inventors developed a cyclized version of D-HYD1 that is designed to display the core bioactive sequence (MVVSW) in an extended or beta-sheet-like conformation. Side chain-side chain or N- to C-terminus cyclization of linear peptides, to constrain the number of conformations available to the linear peptide, is a well known strategy that increases the affinity of the cyclized peptide for its target when the constraint stabilizes the bound conformation of the peptide. The cyclic beta-hairpin further constrains the recognition portion of the cyclic peptide specifically into an extended or beta-sheet-like conformation.

The inventors first made the all D-amino acid analog of the linear D-HYD1 and found that the cyclized D-HYD1 (c-D-HYD1) was about twice as active as linear D-HYD1. Surprisingly, the inverso (L-HYD1) cyclic compound was 2 fold more potent compared to the cyclic D-HYD1 variant. The inventors have modified the MVVSW recognition strand and found replacing the S for an A makes the compound approximately 10 fold more potent (MVVAW) and replacing the methionine for nor-Leucine (NorLeuVVSW) made the compound 15 fold more potent. Finally, the combination of those modifications (NorLeuVVAW) was 30-fold more potent than HYD1. Going forward herein, the most potent cyclic analog is referred to as c-HYD1. The IC_{50} value of c-HYD1 in H929 cells is 1.2 +/- 0.02 uM while the IC_{50} value of the linear HYD1 is 33 uM in H929 cells (see Figure 39).

As used herein, the terms "treatment" and "treating", and grammatical variations thereof, include therapy and prophylaxis. When used as a therapy, the integrin interaction inhibitors of the invention, by themselves or in conjunction with other agents, alleviate or reduce one or more symptoms associated with a proliferation disorder (*e.g.*, cancer). Thus, the treatment methods may or may not be curative in nature. When used as a prophylactic treatment, the integrin interaction inhibitors of the invention, by themselves or in conjunction with other agents, delay the onset of (and may prevent) one or more symptoms associated with a proliferation disorder (*e.g.*, cancer), or may prevent the genesis of the condition.

In one aspect, the method of the invention is a method for treating a proliferation disorder, such as cancer, comprising administering an effective amount of an integrin interaction inhibitor of the invention to a subject in need thereof.

In another aspect, the method of the invention is a method for inhibiting the growth of cancer cells *in vitro* or *in vivo*, comprising administering an effective amount of an integrin interaction inhibitor of the invention to the cancer cells.

In another aspect, the subject invention provides pharmaceutical compositions comprising at least one integrin interaction inhibitor of the invention; and a pharmaceutically acceptable carrier.

By inhibiting the growth of cells proliferating in an aberrant manner, the methods, integrin interaction inhibitors, and compositions of the present invention can be used to treat a number of cell proliferation disorders, such as cancers, including, but not limited to, leukemias and lymphomas, such as acute lymphocytic leukemia, acute non-lymphocytic leukemias, chronic lymphocytic leukemia, chronic myelogenous leukemia, Hodgkin's Disease, non-Hodgkin's lymphomas, and multiple myeloma, childhood solid tumors such as brain tumors, neuroblastoma, retinoblastoma, Wilms' Tumor, bone tumors, and soft-tissue sarcomas, common solid tumors of adults such as lung cancer, colon and rectum cancer, breast cancer, prostate cancer, urinary cancers, uterine cancers, bladder cancers, oral cancers, pancreatic cancer, melanoma and other skin cancers, stomach cancer, ovarian cancer, brain tumors, liver cancer, laryngeal cancer, thyroid cancer, esophageal cancer, and testicular cancer. The methods of the subject invention can be carried out *in vivo* or *in vitro*, to inhibit the growth of cells (*e.g.*, cancer cells) in humans and non-human mammals. Treatment for a proliferation disorder can proceed by the integrin interaction inhibitor's anti-proliferative activity, or by other mechanisms. Without being bound by theory, in some embodiments, the proliferation disorder is one on which the integrin interaction inhibitor(s) acts by binding to ß1 integrin, and/or inhibits ß1 integrin signaling, and/or ß1 integrin mediated adhesion.

Without being bound by theory, integrin interaction inhibitors of the invention having the capability to modulate (*e.g.*, reduce or eliminate) ß1 integrin signaling *in vitro* and/or *in vivo*, or to inhibit the growth of cancer cells *in vitro* and/or *in vivo* by inhibition of ß1 integrin signaling or a different mechanism, would be considered to have the desired biological activity in accordance with the subject invention. For therapeutic applications, without being bound by theory, integrin interaction inhibitors of the subject invention can have the capability to inhibit ß1 integrin signaling or ß1 integrin mediated adhesion, or to inhibit the growth of cancer cells *in vitro* and/or *in vivo* by inhibition of ß1 integrin signaling or ß1 integrin mediated adhesion or a different mechanism. Treatment for a proliferation disorder can proceed by the integrin interaction inhibitor's anti-proliferative activity, regardless of underlying mechanism.

In some embodiments, the proliferation disorder to be treated is a cancer producing a tumor characterized by ß1 integrin signaling or ß1 integrin mediated adhesion. Examples of susceptible cancer types include, but are not limited to, cancer of the breast, pancreas, prostate, melanoma, myeloma, and lung. In some embodiments, the proliferation disorder to be treated is a cancer producing a tumor characterized by the CAM-DR phenotype. In some embodiments, the proliferation disorder to be treated is a cancer that exhibits elevated levels of the cleaved form of a4 integrin. In some embodiments, the treatment methods further include determining whether the proliferation disorder exhibits the aforementioned characteristics (ß1 integrin signaling or ß1 integrin mediated adhesion; CAM-DR phenotype; elevated a4 integrin level) prior to administration of the one or more integrin interaction inhibitors.

In some embodiments, the proliferation disorder to be treated is characterized by a proliferation of T-cells such as autoimmune disease, *e.g.*, type 1 diabetes, lupus and multiple sclerosis, and pathological states such as graft rejection induced by the presentation of a foreign antigen such as a graft in response to a disease condition (*e.g.*, kidney failure). Other non-malignant diseases characterized by proliferation of cells include cirrhosis of the liver and restenosis.

The methods of the present invention can be advantageously combined with at least one additional treatment method, including but not limited to, chemotherapy, radiation therapy, or any other therapy known to those of skill in the art for the treatment and management of proliferation disorders such as cancer.

While integrin interaction inhibitors of the invention can be administered to cells *in vitro* and *in vivo* as isolated agents, it is preferred to administer these integrin interaction

inhibitors as part of a pharmaceutical composition. The subject invention thus further provides compositions comprising an integrin interaction inhibitor of the invention in association with at least one pharmaceutically acceptable carrier. The pharmaceutical composition can be adapted for various routes of administration, such as enteral, parenteral, intravenous, intramuscular, topical, subcutaneous, and so forth. Administration can be continuous or at distinct intervals, as can be determined by a person of ordinary skill in the art.

The integrin interaction inhibitors of the invention can be formulated according to known methods for preparing pharmaceutically useful compositions. Formulations are described in a number of sources which are well known and readily available to those skilled in the art. For example, *Remington's Pharmaceutical Science* (Martin, E.W., 1995, Easton Pennsylvania, Mack Publishing Company, 19th ed.) describes formulations which can be used in connection with the subject invention. Formulations suitable for administration include, for example, aqueous sterile injection solutions, which may contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient; and aqueous and nonaqueous 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 ampoules and vials, and may be stored in a freeze dried (lyophilized) condition requiring only the condition of the sterile liquid carrier, for example, water for injections, prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powder, granules, tablets, *etc.* It should be understood that in addition to the ingredients particularly mentioned above, the compositions of the subject invention can include other agents conventional in the art having regard to the type of formulation in question.

Examples of pharmaceutically acceptable salts are organic acid addition salts formed with acids that form a physiological acceptable anion, for example, tosylate, methanesulfonate, acetate, citrate, malonate, tartarate, succinate, benzoate, ascorbate, alpha-ketoglutarate, and alpha-glycerophosphate. Suitable inorganic salts may also be formed, including hydrochloride, sulfate, nitrate, bicarbonate, and carbonate salts.

Pharmaceutically acceptable salts of compounds may be obtained using standard procedures well known in the art, for example, by reacting a sufficiently basic compound such as an amine with a suitable acid affording a physiologically acceptable anion. Alkali metal (for example, sodium, potassium or lithium) or alkaline earth metal (for example calcium) salts of carboxylic acids can also be made.

As used herein, the term "analogs" refers to compounds which are substantially the same as another compound but which may have been modified by, for example, adding side groups, oxidation or reduction of the parent structure. Analogs of the integrin interaction inhibitors, and other agents disclosed herein, can be readily prepared using commonly known standard reactions. These standard reactions include, but are not limited to, hydrogenation, alkylation, acetylation, and acidification reactions. Chemical modifications can be accomplished by those skilled in the art by protecting all functional groups present in the molecule and deprotecting them after carrying out the desired reactions using standard procedures known in the scientific literature (Greene, T.W. and Wuts, P.G.M. "Protective Groups in Organic Synthesis" John Wiley & Sons, Inc. New York. 3rd Ed. pg. 819, 1999; Honda, T. *et al. Bioorg. Med. Chem. Lett.*, 1997, 7:1623-1628; Honda, T. *et al. Bioorg. Med. Chem. Lett.*, 1998, 8:2711-2714; Konoike, T. *et al. J. Org. Chem.*, 1997, 62:960-966; Honda, T. *et al. J. Med. Chem.*, 2000, 43:4233-4246).

Analogs, fragments, and variants of the integrin interaction inhibitors exhibiting the desired biological activity (such as induction of cell death, cytotoxicity, cytostaticity, induction of cell cycle arrest, *etc.*) can be identified or confirmed using cellular assays or other *in vitro* or *in vivo* assays. For example, assays that detect ß1 integrin signaling, ß1 integrin mediated adhesion, ERK activation, G_2/M cell cycle arrest, and/or reduction of tumor growth may be utilized. Examples of assays to assess ß1 integrin signaling, ß1 integrin adhesion, and ERK activation are described in Gilcrease, M.S., *Cancer Letters*, 2007, 247(1):1-25; Larsen M. *et al.*, *Current Opinion in Cell Biology*, 2006, 18(5):463-471; Luo B.H. and T.A. Springer, *Current Opinion in Cell Biology*, 2006, 18(5):579-586.

The integrin interaction inhibitors of the invention are useful for various non-therapeutic and therapeutic purposes. The integrin interaction inhibitors may be used for reducing aberrant cell growth in animals and humans. Because of such anti-proliferative properties of the integrin interaction inhibitors, they are useful in reducing unwanted cell growth in a wide variety of settings including *in vitro* and *in vivo*. In addition to their use in treatment methods, the integrin interaction inhibitors of the invention are useful as agents for investigating the role of a4 and ß1 integrin signaling and/or a4 and ß1 integrin mediated adhesion in cellular metabolism, and controlling a4 and/or ß1 integrin mediated malignant or non-malignant cell growth *in vitro* or *in vivo*. They are also useful as standards and for teaching demonstrations.

Therapeutic application of the integrin interaction inhibitors and compositions comprising them can be accomplished by any suitable therapeutic method and technique presently or prospectively known to those skilled in the art. Further, the integrin interaction inhibitors of the invention can be used as starting materials or intermediates for the preparation of other useful compounds and compositions.

Integrin interaction inhibitors of the invention may be locally administered at one or more anatomical sites, such as sites of unwanted cell growth (such as a tumor site, *e.g.*, injected or topically applied to the tumor), optionally in combination with a pharmaceutically acceptable carrier such as an inert diluent. Integrin interaction inhibitors of the invention may be systemically administered, such as intravenously or orally, optionally in combination with a pharmaceutically acceptable carrier such as an inert diluent, or an assimilable edible carrier for oral delivery. They may be enclosed in hard or soft shell gelatin capsules, may be compressed into tablets, or may be incorporated directly with the food of the patient's diet. For oral therapeutic administration, the integrin interaction inhibitors may be combined with one or more excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, aerosol sprays, and the like.

The tablets, troches, pills, capsules, and the like may also contain the following: binders such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, fructose, lactose or aspartame or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring may be added. When the unit dosage form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier, such as a vegetable oil or a polyethylene glycol. Various other materials may be present as coatings or to otherwise modify the physical form of the solid unit dosage form. For instance, tablets, pills, or capsules may be coated with gelatin, wax, shellac, or sugar and the like. A syrup or elixir may contain the active compound, sucrose or fructose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. Of course, any material used in preparing any unit dosage form should be pharmaceutically acceptable and substantially non-toxic in the amounts employed. In addition, the integrin interaction inhibitors may be incorporated into sustained-release preparations and devices.

The active agent (*e.g.*, integrin interaction inhibitors of the invention) may also be administered intravenously or intraperitoneally by infusion or injection. Solutions of the active agent can be prepared in water, optionally mixed with a nontoxic surfactant.

Dispersions can also be prepared in glycerol, liquid polyethylene glycols, triacetin, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations can contain a preservative to prevent the growth of microorganisms.

The pharmaceutical dosage forms suitable for injection or infusion can include sterile aqueous solutions or dispersions or sterile powders comprising the integrin interaction inhibitors of the invention which are adapted for the extemporaneous preparation of sterile injectable or infusible solutions or dispersions, optionally encapsulated in liposomes. The ultimate dosage form should be sterile, fluid and stable under the conditions of manufacture and storage. The liquid carrier or vehicle can be a solvent or liquid dispersion medium comprising, for example, water, ethanol, a polyol (for example, glycerol, propylene glycol, liquid polyethylene glycols, and the like), vegetable oils, nontoxic glyceryl esters, and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the formation of liposomes, by the maintenance of the required particle size in the case of dispersions or by the use of surfactants. Optionally, the prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, buffers or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the inclusion of agents that delay absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the integrin interaction inhibitors of the invention in the required amount in the appropriate solvent with various other ingredients enumerated above, as required, followed by filter sterilization. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze drying techniques, which yield a powder of the active ingredient plus any additional desired ingredient present in the previously sterile-filtered solutions.

For topical administration, the integrin interaction inhibitors may be applied in pure-form, *i.e.*, when they are liquids. However, it will generally be desirable to administer them topically to the skin as compositions, in combination with a dermatologically acceptable carrier, which may be a solid or a liquid.

The integrin interaction inhibitors of the subject invention can be applied topically to a subject's skin to reduce the size (and may include complete removal) of malignant or benign growths. The integrin interaction inhibitors of the invention can be applied directly to the growth. Preferably, the integrin interaction inhibitor is applied to the growth in a

formulation such as an ointment, cream, lotion, solution, tincture, or the like. Drug delivery systems for delivery of pharmacological substances to dermal lesions can also be used, such as that described in U.S. Patent No. 5,167,649 (Zook).

Useful solid carriers include finely divided solids such as talc, clay, microcrystalline cellulose, silica, alumina and the like. Useful liquid carriers include water, alcohols or glycols or water-alcohol/glycol blends, in which the peptide can be dissolved or dispersed at effective levels, optionally with the aid of non-toxic surfactants. Adjuvants such as fragrances and additional antimicrobial agents can be added to optimize the properties for a given use. The resultant liquid compositions can be applied from absorbent pads, used to impregnate bandages and other dressings, or sprayed onto the affected area using pump-type or aerosol sprayers, for example.

Thickeners such as synthetic polymers, fatty acids, fatty acid salts and esters, fatty alcohols, modified celluloses or modified mineral materials can also be employed with liquid carriers to form spreadable pastes, gels, ointments, soaps, and the like, for application directly to the skin of the user. Examples of useful dermatological compositions which can be used to deliver the peptides to the skin are disclosed in Jacquet *et al.* (U.S. Patent No. 4,608,392), Geria (U.S. Patent No. 4,992,478), Smith *et al.* (U.S. Patent No. 4,559,157) and Woltzman (U.S. Patent No. 4,820,508).

Useful dosages of the pharmaceutical compositions of the present invention can be determined by comparing their *in vitro* activity, and *in vivo* activity in animal models. Methods for the extrapolation of effective dosages in mice, and other animals, to humans are known to the art; for example, see U.S. Patent No. 4,938,949.

Accordingly, the present invention includes a pharmaceutical composition comprising an integrin interaction inhibitor of the invention in combination with a pharmaceutically acceptable carrier. Pharmaceutical compositions adapted for oral, topical or parenteral administration, comprising an amount of an integrin interaction inhibitor of the invention constitute a preferred embodiment of the invention. The dose administered to a patient, particularly a human, in the context of the present invention should be sufficient to achieve a therapeutic response in the patient over a reasonable time frame, without lethal toxicity, and preferably causing no more than an acceptable level of side effects or morbidity. One skilled in the art will recognize that dosage will depend upon a variety of factors including the condition (health) of the subject, the body weight of the subject, kind of concurrent treatment, if any, frequency of treatment, therapeutic ratio, as well as the severity and stage of the

pathological condition. Advantageously, in some embodiments, administration of the integrin interaction inhibitors does not induce weight loss or overt signs of toxicity in the subject.

Depending upon the disorder or disease condition to be treated, a suitable dose(s) may be that amount that will reduce proliferation or growth of the target cell(s), or induce cell death. In the context of cancer, a suitable dose(s) is that which will result in a concentration of the active agent (one or more integrin interaction inhibitors of the invention) in cancer tissue, such as a malignant tumor, which is known to achieve the desired response. The preferred dosage is the amount which results in maximum inhibition of cancer cell growth, without unmanageable side effects. Administration of an integrin interaction inhibitor of the invention can be continuous or at distinct intervals, as can be determined by a person of ordinary skill in the art.

To provide for the administration of such dosages for the desired therapeutic treatment, in some embodiments, pharmaceutical compositions of the invention can comprise between about 0.1% and 45%, and especially, 1 and 15%, by weight of the total of one or more of the compounds of the invention based on the weight of the total composition including carrier or diluents. Illustratively, dosage levels of the administered active ingredients can be: intravenous, 0.01 to about 20 mg/kg; intraperitoneal, 0.01 to about 100 mg/kg; subcutaneous, 0.01 to about 100 mg/kg; intramuscular, 0.01 to about 100 mg/kg; orally 0.01 to about 200 mg/kg, and preferably about 1 to 100 mg/kg; intranasal instillation, 0.01 to about 20 mg/kg; and aerosol, 0.01 to about 20 mg/kg of animal (body) weight.

Mammalian species which benefit from the disclosed methods include, but are not limited to, primates, such as apes, chimpanzees, orangutans, humans, monkeys; domesticated animals (*e.g.*, pets) such as dogs, cats, guinea pigs, hamsters, Vietnamese pot-bellied pigs, rabbits, and ferrets; domesticated farm animals such as cows, buffalo, bison, horses, donkey, swine, sheep, and goats; exotic animals typically found in zoos, such as bear, lions, tigers, panthers, elephants, hippopotamus, rhinoceros, giraffes, antelopes, sloth, gazelles, zebras, wildebeests, prairie dogs, koala bears, kangaroo, opossums, raccoons, pandas, hyena, seals, sea lions, elephant seals, otters, porpoises, dolphins, and whales. Other species that may benefit from the disclosed methods include fish, amphibians, avians, and reptiles. As used herein, the terms "patient", "subject", and "individual" are used interchangeably and are intended to include such human and non-human species. Likewise, *in vitro* methods of the present invention can be carried out on cells of such human and non-human species.

Patients in need of treatment using the methods of the present invention can be identified using standard techniques known to those in the medical or veterinary professions, as appropriate.

The terms "cancer" and "malignancy" are used herein interchangeably to refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. The cancer may be multi-drug resistant (MDR) or drug-sensitive. Examples of cancer include but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia. More particular examples of such cancers include breast cancer, prostate cancer, colon cancer, squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, gastrointestinal cancer, pancreatic cancer, cervical cancer, ovarian cancer, peritoneal cancer, liver cancer, *e.g.*, hepatic carcinoma, bladder cancer, colorectal cancer, endometrial carcinoma, kidney cancer, and thyroid cancer. In some embodiments, the cancer is multiple myeloma or another hematologic malignancy.

In some embodiments, the cancer or malignancy is one that expresses CD44. In some embodiments, the methods of the invention further comprise obtaining a sample of the cancer cells and determining whether the cells express CD44 prior to administration of a peptide of the invention. Optionally, the methods may further comprise administering the peptide if the cancer sample expresses CD44.

Other non-limiting examples of cancers are basal cell carcinoma, biliary tract cancer; bone cancer; brain and CNS cancer; choriocarcinoma; connective tissue cancer; esophageal cancer; eye cancer; cancer of the head and neck; gastric cancer; intra-epithelial neoplasm; larynx cancer; lymphoma including Hodgkin's and Non-Hodgkin's lymphoma; melanoma; myeloma; neuroblastoma; oral cavity cancer (*e.g.*, lip, tongue, mouth, and pharynx); retinoblastoma; rhabdomyosarcoma; rectal cancer; cancer of the respiratory system; sarcoma; skin cancer; stomach cancer; testicular cancer; uterine cancer; cancer of the urinary system, as well as other carcinomas and sarcomas. Examples of cancer types that may potentially be treated using the integrin interaction inhibitors of the present invention are also listed in Table 1.

Table 1. Examples of Cancer Types

Acute Lymphoblastic Leukemia, Adult Acute Lymphoblastic Leukemia, Childhood Acute Myeloid Leukemia, Adult Acute Myeloid Leukemia, Childhood	Hairy Cell Leukemia Head and Neck Cancer Hepatocellular (Liver) Cancer, Adult (Primary) Hepatocellular (Liver) Cancer, Childhood (Primary)

Adrenocortical Carcinoma
Adrenocortical Carcinoma, Childhood
AIDS-Related Cancers
AIDS-Related Lymphoma
Anal Cancer
Astrocytoma, Childhood Cerebellar
Astrocytoma, Childhood Cerebral

Basal Cell Carcinoma
Bile Duct Cancer, Extrahepatic
Bladder Cancer
Bladder Cancer, Childhood
Bone Cancer, Osteosarcoma/Malignant Fibrous Histiocytoma
Brain Stem Glioma, Childhood
Brain Tumor, Adult
Brain Tumor, Brain Stem Glioma, Childhood
Brain Tumor, Cerebellar Astrocytoma, Childhood
Brain Tumor, Cerebral Astrocytoma/Malignant Glioma, Childhood
Brain Tumor, Ependymoma, Childhood
Brain Tumor, Medulloblastoma, Childhood
Brain Tumor, Supratentorial Primitive Neuroectodermal Tumors, Childhood
Brain Tumor, Visual Pathway and Hypothalamic Glioma, Childhood
Brain Tumor, Childhood
Breast Cancer
Breast Cancer, Childhood
Breast Cancer, Male
Bronchial Adenomas/Carcinoids, Childhood
Burkitt's Lymphoma

Carcinoid Tumor, Childhood

Carcinoid Tumor, Gastrointestinal

Carcinoma of Unknown Primary

Central Nervous System Lymphoma, Primary

Cerebellar Astrocytoma, Childhood

Cerebral Astrocytoma/Malignant Glioma, Childhood

Hodgkin's Lymphoma, Adult
Hodgkin's Lymphoma, Childhood
Hodgkin's Lymphoma During Pregnancy
Hypopharyngeal Cancer
Hypothalamic and Visual Pathway Glioma, Childhood

Intraocular Melanoma
Islet Cell Carcinoma (Endocrine Pancreas)

Kaposi's Sarcoma
Kidney (Renal Cell) Cancer
Kidney Cancer, Childhood

Laryngeal Cancer
Laryngeal Cancer, Childhood
Leukemia, Acute Lymphoblastic, Adult
Leukemia, Acute Lymphoblastic, Childhood
Leukemia, Acute Myeloid, Adult
Leukemia, Acute Myeloid, Childhood
Leukemia, Chronic Lymphocytic
Leukemia, Chronic Myelogenous
Leukemia, Hairy Cell
Lip and Oral Cavity Cancer
Liver Cancer, Adult (Primary)
Liver Cancer, Childhood (Primary)
Lung Cancer, Non-Small Cell
Lung Cancer, Small Cell
Lymphoma, AIDS-Related
Lymphoma, Burkitt's
Lymphoma, Cutaneous T-Cell, see Mycosis Fungoides and Sézary Syndrome
Lymphoma, Hodgkin's, Adult
Lymphoma, Hodgkin's, Childhood
Lymphoma, Hodgkin's During Pregnancy
Lymphoma, Non-Hodgkin's, Adult
Lymphoma, Non-Hodgkin's, Childhood
Lymphoma, Non-Hodgkin's During Pregnancy
Lymphoma, Primary Central Nervous System

Macroglobulinemia, Waldenström's
Malignant Fibrous Histiocytoma of Bone/Osteosarcoma
Medulloblastoma, Childhood
Melanoma
Melanoma, Intraocular (Eye)
Merkel Cell Carcinoma
Mesothelioma, Adult Malignant
Mesothelioma, Childhood
Metastatic Squamous Neck Cancer with Occult Primary

Cervical Cancer
Childhood Cancers
Chronic Lymphocytic Leukemia
Chronic Myelogenous Leukemia
Chronic Myeloproliferative Disorders
Colon Cancer
Colorectal Cancer, Childhood
Cutaneous T-Cell Lymphoma, see Mycosis Fungoides and Sézary Syndrome

Endometrial Cancer
Ependymoma, Childhood
Esophageal Cancer
Esophageal Cancer, Childhood
Ewing's Family of Tumors
Extracranial Germ Cell Tumor, Childhood
Extragonadal Germ Cell Tumor
Extrahepatic Bile Duct Cancer
Eye Cancer, Intraocular Melanoma
Eye Cancer, Retinoblastoma

Gallbladder Cancer
Gastric (Stomach) Cancer
Gastric (Stomach) Cancer, Childhood
Gastrointestinal Carcinoid Tumor
Germ Cell Tumor, Extracranial, Childhood
Germ Cell Tumor, Extragonadal
Germ Cell Tumor, Ovarian
Gestational Trophoblastic Tumor
Glioma, Adult
Glioma, Childhood Brain Stem
Glioma, Childhood Cerebral Astrocytoma
Glioma, Childhood Visual Pathway and Hypothalamic

Skin Cancer (Melanoma)
Skin Carcinoma, Merkel Cell
Small Cell Lung Cancer
Small Intestine Cancer
Soft Tissue Sarcoma, Adult
Soft Tissue Sarcoma, Childhood
Squamous Cell Carcinoma, see Skin

Multiple Endocrine Neoplasia Syndrome, Childhood
Multiple Myeloma/Plasma Cell Neoplasm
Mycosis Fungoides
Myelodysplastic Syndromes
Myelodysplastic/Myeloproliferative Diseases
Myelogenous Leukemia, Chronic
Myeloid Leukemia, Adult Acute
Myeloid Leukemia, Childhood Acute
Myeloma, Multiple
Myeloproliferative Disorders, Chronic

Nasal Cavity and Paranasal Sinus Cancer
Nasopharyngeal Cancer
Nasopharyngeal Cancer, Childhood
Neuroblastoma
Non-Hodgkin's Lymphoma, Adult
Non-Hodgkin's Lymphoma, Childhood
Non-Hodgkin's Lymphoma During Pregnancy
Non-Small Cell Lung Cancer

Oral Cancer, Childhood
Oral Cavity Cancer, Lip and Oropharyngeal Cancer
Osteosarcoma/Malignant Fibrous Histiocytoma of Bone
Ovarian Cancer, Childhood
Ovarian Epithelial Cancer
Ovarian Germ Cell Tumor
Ovarian Low Malignant Potential Tumor

Pancreatic Cancer
Pancreatic Cancer, Childhood
Pancreatic Cancer, Islet Cell
Paranasal Sinus and Nasal Cavity Cancer
Parathyroid Cancer
Penile Cancer
Pheochromocytoma
Pineoblastoma and Supratentorial Primitive Neuroectodermal Tumors, Childhood
Pituitary Tumor
Plasma Cell Neoplasm/Multiple Myeloma
Pleuropulmonary Blastoma
Pregnancy and Breast Cancer
Pregnancy and Hodgkin's Lymphoma
Pregnancy and Non-Hodgkin's Lymphoma
Primary Central Nervous System Lymphoma
Prostate Cancer

Rectal Cancer
Renal Cell (Kidney) Cancer

Cancer (non-Melanoma) Squamous Neck Cancer with Occult Primary, Metastatic Stomach (Gastric) Cancer Stomach (Gastric) Cancer, Childhood Supratentorial Primitive Neuroectodermal Tumors, Childhood T-Cell Lymphoma, Cutaneous, see Mycosis Fungoides and Sézary Syndrome Testicular Cancer Thymoma, Childhood Thymoma and Thymic Carcinoma Thyroid Cancer Thyroid Cancer, Childhood Transitional Cell Cancer of the Renal Pelvis and Ureter Trophoblastic Tumor, Gestational Unknown Primary Site, Carcinoma of, Adult Unknown Primary Site, Cancer of, Childhood Unusual Cancers of Childhood Ureter and Renal Pelvis, Transitional Cell Cancer Urethral Cancer Uterine Cancer, Endometrial Uterine Sarcoma Vaginal Cancer Visual Pathway and Hypothalamic Glioma, Childhood Vulvar Cancer Waldenström's Macroglobulinemia Wilms' Tumor	Renal Cell (Kidney) Cancer, Childhood Renal Pelvis and Ureter, Transitional Cell Cancer Retinoblastoma Rhabdomyosarcoma, Childhood Salivary Gland Cancer Salivary Gland Cancer, Childhood Sarcoma, Ewing's Family of Tumors Sarcoma, Kaposi's Sarcoma, Soft Tissue, Adult Sarcoma, Soft Tissue, Childhood Sarcoma, Uterine Sezary Syndrome Skin Cancer (non-Melanoma) Skin Cancer, Childhood

As used herein, the term "tumor" refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues. For example, a particular cancer may be characterized by a solid mass tumor or non-solid tumor. The solid tumor mass, if present, may be a primary tumor mass. A primary tumor mass refers to a growth of cancer cells in a tissue resulting from the transformation of a normal cell of that tissue. In most cases, the primary tumor mass is identified by the presence of a cyst, which can be found through visual or palpation methods, or by irregularity in shape, texture

or weight of the tissue. However, some primary tumors are not palpable and can be detected only through medical imaging techniques such as X-rays (*e.g.*, mammography) or magnetic resonance imaging (MRI), or by needle aspirations. The use of these latter techniques is more common in early detection. Molecular and phenotypic analysis of cancer cells within a tissue can usually be used to confirm if the cancer is endogenous to the tissue or if the lesion is due to metastasis from another site. The treatment methods of the invention can be utilized for early, middle, or late stage disease, and acute or chronic disease. In some embodiments, the tumor is characterized as one exhibiting the CAM-DR phenotype.

According to the method of the subject invention, an integrin interaction inhibitor of the invention can be administered to a patient by itself, or co-administered with one or more other agents such as another integrin interaction inhibitor, or a different agent or agents. Co-administration can be carried out simultaneously (in the same or separate formulations) or consecutively. Furthermore, according to the method of the subject invention, integrin interaction inhibitors of the invention can be administered to a patient as adjuvant therapy. For example, integrin interaction inhibitors of the invention can be administered to a patient in conjunction with chemotherapy.

Thus, the integrin interaction inhibitors of the invention, whether administered separately, or as a pharmaceutical composition, can include various other components as additives. Examples of acceptable components or adjuncts which can be employed in relevant circumstances include antioxidants, free radical scavenging agents, peptides, growth factors, antibiotics, bacteriostatic agents, immunosuppressives, anticoagulants, buffering agents, anti-inflammatory agents, anti-angiogenics, anti-pyretics, time-release binders, anesthetics, steroids, and corticosteroids. Such components can provide additional therapeutic benefit, act to affect the therapeutic action of the compounds of the invention, or act towards preventing any potential side effects which may be posed as a result of administration of the compounds. The integrin interaction inhibitors of the subject invention can be conjugated to a therapeutic agent, as well.

Additional agents that can be co-administered to target cells *in vitro* or *in vivo*, such as in a patient, in the same or as a separate formulation, include those that modify a given biological response, such as immunomodulators. For example, proteins such as tumor necrosis factor (TNF), interferon (such as alpha-interferon and beta-interferon), nerve growth factor (NGF), platelet derived growth factor (PDGF), and tissue plasminogen activator can be administered. Biological response modifiers, such as lymphokines, interleukins (such as interleukin-1 (IL-1), interleukin-2 (IL-2), and interleukin-6 (IL-6)), granulocyte macrophage

colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), or other growth factors can be administered. In one embodiment, the methods and compositions of the invention incorporate one or more agents selected from the group consisting of anti-cancer agents, cytotoxic agents, chemotherapeutic agents, anti-signaling agents, and anti-angiogenic agents.

In some embodiments of the methods of the invention, at least one additional anti-cancer agent (*e.g.*, a chemotherapeutic agent) is administered with the integrin interaction inhibitor or HYD1 peptide. In some embodiments, the anti-cancer agent is selected from among suberoylanilide hydroxamic acid (SAHA) or other histone deacetylase inhibitor, arsenic trioxide, doxorubicin or other anthracycline DNA intercalating agent, and etoposide or other topoisomerase II inhibitor.

Integrin interaction inhibitors as described herein may include residues of L-amino acids, D-amino acids, or any combination thereof. In some embodiments, all amino acids of the peptide are D-amino acids. Amino acids may be from natural or non-natural sources. The 20 L-amino acids commonly found in proteins are identified herein by the conventional one-letter abbreviations known in the art, and the corresponding D-amino acids are generally designated by a lower case one letter symbol. Integrin interaction inhibitors may also contain one or more rare amino acids (such as 4-hydroxyproline or hydroxylysine), organic acids or amides and/or derivatives of common amino acids, such as amino acids having the C-terminal carboxylate esterified (*e.g.*, benzyl, methyl or ethyl ester) or amidated and/or having modifications of the N-terminal amino group (*e.g.*, acetylation or alkoxycarbonylamino), with or without any of a wide variety of side chain modifications and/or substitutions (*e.g.*, methylation, benzylation, t-butylation, tosylation, alkoxycarbonylamino, and the like). Such modifications and derivatives of an amino acid sequence, and others known to those of skill in the art, are herein termed "variants". Some derivatives include amino acids having an N-acetyl group (such that the amino group that represents the N-terminus of the linear peptide is acetylated) and/or a C-terminal amide group (*i.e.*, the carboxy terminus of the linear peptide is amidated). Residues other than common amino acids that may be present include, but are not limited to, penicillamine, tetramethylene cysteine, pentamethylene cysteine, mercaptopropionic acid, pentamethylene-mercaptopropionic acid, 2-mercaptobenzene, 2-mercaptoaniline, 2-mercaptoproline, ornithine, diaminobutyric acid, aminoadipic acid, m-aminomethylbenzoic acid, and diaminopropionic acid.

Functional fragments according to the subject invention can comprise a contiguous span of at least 4 consecutive amino acids of a recognition sequence (also referred to as the recognition portion) and/or a non-recognition sequence (also referred to as the non-recognition portion) of the integrin interaction inhibitors disclosed herein. Peptides fragments according to the subject invention can be any integer in length from at least 4 consecutive amino acids to 1 amino acid less than a full length peptide (*e.g.*, 1 amino acid less than the full length peptide). Thus, in some embodiments, functional fragments may be 4, 5, 6, 7, 8, or 9 amino acids in length (*e.g.*, a span of 4, 5, 6, 7, 8, or 9 consecutive amino acids).

Each fragment of the subject invention can also be described in terms of its N-terminal and C-terminal positions. For example, combinations of N-terminal to C-terminal fragments of 6 contiguous amino acids to 1 amino acid less than the full length peptide of are included in the present invention. Thus, a 6 consecutive amino acid fragment could occupy positions selected from the group consisting of 1-6, 2-7, 3-8, 4-9, 5-10, *etc.* It is noted that all ranges used to describe any embodiment of the present invention are inclusive unless specifically set forth otherwise and that fragments of a given peptide can be any integer in length, provided that the length of the peptide fragment is at least one amino acid shorter than the full-length peptide from which the fragment is derived.

Fragments, as described herein, can be obtained by cleaving the peptides of the invention with a proteolytic enzyme (such as trypsin, chymotrypsin, or collagenase) or with a chemical reagent, such as cyanogen bromide (CNBr). Alternatively, peptide fragments can be generated in a highly acidic environment, for example at pH 2.5. Such peptide fragments may be equally well prepared by chemical synthesis or using hosts transformed with an expression vector according to the invention.

In certain preferred embodiments, fragments of the peptides disclosed herein retain at least one property or activity of the full-length peptide from which the fragments are derived. Thus, functional fragments of the invention may have one or more of the following properties or biological activities: 1) specifically bind to antibodies specific for the full-length peptide from which the fragment was derived (such as HYD1); 2) specifically bind ß1 integrin; 3) inhibit ß1 integrin mediated cell adhesion; 4) induce ERK signaling; 5) cause apoptosis in target cells (*e.g.*, malignant cells), by one or more mechanisms of action. Examples of assays to assess ß1 integrin signaling, ß1 integrin adhesion, and ERK activation are described in Gilcrease, M.S., *Cancer Letters*, 2007, 247(1):1-25; Larsen M. *et al.*, *Current Opinion in Cell*

Biology, 2006, 18(5):463-471; Luo B.H. and T.A. Springer, *Current Opinion in Cell Biology*, 2006, 18(5):579-586.

Ligands that may find use with the integrin interaction inhibitors of the present invention can include but not be limited to sugars, lectins, antigens, intercalators, chelators, biotin, digoxygenin and combinations thereof. The particular choice of a dye as a labeling agent or cell uptake facilitator may depend upon physical characteristics such as absorption maxima, emission maxima, quantum yields, chemical stability and solvent solubility. A large number of fluorescent and chemiluminescent compounds have been shown to be useful for labeling proteins and nucleic acids. Examples of compounds that may be used as the dye portion can include but not be limited to xanthene, anthracene, cyanine, porphyrin and coumarin dyes. Examples of xanthene dyes that may be coupled to the peptides of the present invention can include but not be limited to fluorescein, 6-carboxyfluorescein (6-FAM), 5-carboxyfluorescein (5-Fam), 5- or 6-carboxy-4,7,2',7'-tetrachlorofluorescein (TET), 5- or 6-carboxy-4'5'2'4'5'7' hexachlorofluorescein (HEX), 5' or 6'-carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein (JOE), 5-carboxy-2',4',5',7'-tetrachlorofluorescein (ZOE) rhodol, rhodamine, tetramethylrhodamine (TAMRA), 4,7-dichlorotetramethyl rhodamine (DTAMRA), rhodamine X (ROX) and Texas Red. Examples of cyanine dyes that may find use with the peptides of the present invention can include but not be limited to Cy 3, Cy 3.5, Cy 5, Cy 5.5, Cy 7 and Cy 7.5. Other dyes that may find use with the peptides of the present invention can include but not be limited to energy transfer dyes, composite dyes and other aromatic compounds that give fluorescent signals. Chemiluminescent compounds that may be used with the peptides of the present invention can include but not be limited to dioxetane and acridinium esters. It should also be understood that ligands and dyes are not mutually exclusive groups. For instance, fluorescein is a well known example of a moiety that has been used as a fluorescent label and also as an antigen for labeled antibodies.

The integrin interaction inhibitors of the invention may be monomeric or multimeric (*e.g.*, dimers, trimers, tetramers and higher multimers). Accordingly, the present invention relates to monomers and multimers of the integrin interaction inhibitors of the invention, their preparation, and compositions containing them. Multimeric integrin interaction inhibitors of the subject invention can be derived from the same peptide sequence ("homomultimers") or derived from different sequences disclosed herein ("heteromultimers"). A homomultimer may contain peptides having identical or different amino acid sequences; however these sequences are derived from the same original peptide. A heteromultimer refers to a multimeric peptide containing one or more heterologous peptides (*i.e.*, petpides of different

proteins) in addition to the petpides of the invention. Thus, a heteromultimer, in the context of the subject invention can refer to a multimeric peptide that contains any combination of peptides of the invention. Alternatively, a heteromultimeric peptide may comprise any peptide of the invention fused to a peptide or other element that forms a hydrophobic, hydrophilic, ionic and/or covalent association.

Multimeric peptides, as set forth herein, may be formed by hydrophobic, hydrophilic, ionic and/or covalent associations and/or may be indirectly linked, by for example, liposome formation. Thus, in one embodiment, multimers of the invention, such as, for example, homodimers or homotrimers, are formed when peptides of the invention contact one another in solution. In another embodiment, heteromultimers of the invention, such as, for example, heterotrimers or heterotetramers, are formed when peptides of the invention contact antibodies to the peptides of the invention (including antibodies to the heterologous polypeptide sequence in a fusion protein of the invention) in solution. In other embodiments, multimers of the invention are formed by covalent associations with and/or between the peptides of the invention. Examples include those peptide linkers described in U.S. Pat. No. 5,073,627.

Multimeric peptides can also be generated using chemical techniques known in the art. For example, peptides desired to be contained in the multimers of the invention may be chemically cross-linked using linker molecules and linker molecule length optimization techniques known in the art (see, *e.g.*, U.S. Patent Number 5,478,925).

Additionally, multimeric peptides can be generated by introducing disulfide bonds between the cysteine residues located within the sequence of the peptides that are being used to construct the multimeric polypeptide (see, *e.g.*, U.S. Patent Number 5,478,925).

Further, peptides of the invention may be modified by the addition of cysteine or biotin to the C terminus or N-terminus of the polypeptide and techniques known in the art may be applied to generate multimers containing one or more of these modified polypeptides (see, *e.g.*, U.S. Patent Number 5,478,925).

Additionally, other techniques known in the art may be applied to generate liposomes containing the peptides components desired to be contained in the multimer of the invention (see, *e.g.*, U.S. Patent No. 5,478,925).

The peptides expressly provided herein, as well as the fragments thereof, may further comprise linker elements that facilitate the attachment of the fragments to other molecules,

amino acids, or polypeptide sequences. The linkers can also be used to attach the peptides, or fragments thereof, to solid support matrices for use in affinity purification protocols. Non-limiting examples of "linkers" suitable for the practice of the invention include chemical linkers (such as those sold by Pierce, Rockford, IL), or peptides that allow for the connection combinations of peptides (see, for example, linkers such as those disclosed in U.S. Patent Nos. 6,121,424, 5,843,464, 5,750,352, and 5,990,275).

In other embodiments, the linker element can be an amino acid sequence (a peptide linker). In some embodiments, the peptide linker has one or more of the following characteristics: a) it allows for the free rotation of the peptides that it links (relative to each other); b) it is resistant or susceptible to digestion (cleavage) by proteases; and c) it does not interact with the peptides it joins together. In various embodiments, a multimeric construct according to the subject invention includes a peptide linker and the peptide linker is 5 to 60 amino acids in length. More preferably, the peptide linker is 10 to 30, amino acids in length; even more preferably, the peptide linker is 10 to 20 amino acids in length. In some embodiments, the peptide linker is 17 amino acids in length.

Multimeric constructs of the subject invention can also comprise a series of repeating elements, optionally interspersed with other elements. As would be appreciated by one skilled in the art, the order in which the repeating elements occur in the multimeric polypeptide is not critical and any arrangement of the repeating elements as set forth herein can be provided by the subject invention. Thus, a "multimeric construct" according to the subject invention can provide a multimeric peptide comprising a series of peptides, or peptide fragments, that are, optionally, joined together by linker elements (either chemical linker elements or amino acid linker elements).

A "variant" or "variant peptide" (or peptide variant) is to be understood to designate peptides exhibiting, in relation to the peptides disclosed herein, certain modifications. These modifications can include a deletion, addition, or substitution of at least one amino acid (*e.g.*, one, two, three or more amino acids), a truncation, an extension, a chimeric fusion (fusion protein), a mutation, or polypeptides exhibiting post-translational modifications. These modifications can occur anywhere in the peptide, *e.g.*, one or both ends and/or in the middle. Among these homologous variant peptides, are those comprising amino acid sequences exhibiting between at least (or at least about) 20.00% to 99.99% (inclusive) identity to the full length, native, or naturally occurring polypeptide are another aspect of the invention. The aforementioned range of percent identity is to be taken as including, and providing

written description and support for, any fractional percentage, in intervals of 0.01%, between 20.00% and, up to, including 99.99%. These percentages are purely statistical and differences between two polypeptide sequences can be distributed randomly and over the entire sequence length. Thus, variant peptides can have 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 percent identity with the peptide sequences of the instant invention. In a preferred embodiment, a variant or modified peptide exhibits at least 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 percent identity to the reference peptide. The percent identity is calculated with reference to the full-length polypeptide or the length of the fragment of a particular SEQ ID NO: that is identified. Preferably, the variant peptides retain at least one of the biological activities associated with the reference peptide (for example, the ability to: 1) specifically bind to antibodies specific for the full-length peptide from which the fragment was derived (such as HYD1); 2) specifically bind ß1 integrin; 3) to inhibit ß1 integrin mediated cell adhesion; 4) to induce ERK signaling; 5) cause apoptosis in target cells (*e.g.*, malignant cells), regardless of mechanism of action (*e.g.*, caspase-dependent and/or caspase independent)). Examples of assays to assess ß1 integrin signaling, ß1 integrin adhesion, and ERK activation are described in Gilcrease, M.S., *Cancer Letters*, 2007, 247(1):1-25; Larsen M. *et al.*, *Current Opinion in Cell Biology*, 2006, 18(5):463-471; Luo B.H. and T.A. Springer, *Current Opinion in Cell Biology*, 2006, 18(5):579-586.

For example, one or more amino acid residues within the sequence can be substituted by another amino acid of a similar polarity which acts as a functional equivalent, resulting in a silent alteration. In one aspect of the present invention, conservative substitutions for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs (see Table 2). Conservative substitutions also include substitutions by amino acids having chemically modified side chains that do not eliminate the biological function of the resulting variant.

Table 2.

Class of Amino Acid	Examples of Amino Acids

Nonpolar	Ala, Val, Leu, Ile, Pro, Met, Phe, Trp
Uncharged Polar	Gly, Ser, Thr, Cys, Tyr, Asn, Gln
Acidic	Asp, Glu
Basic	Lys, Arg, His

Fusion proteins according to the subject invention comprise one or more heterologous peptide sequences (*e.g.*, tags that facilitate purification of the peptides of the invention (see, for example, U.S. Patent No. 6,342,362;

Altendorf *et al.* [1999-WWW, 2000] "Structure and Function of the F_o Complex of the ATP Synthase from *Escherichia Coli*," *J. of Experimental* Biology 203:19-28, The Co. of Biologists, Ltd., G.B.; Baneyx [1999] "Recombinant Protein Expression in *Escherichia coli*," Biotechnology 10:411-21, Elsevier Science Ltd.; Eihauer *et al.* [2001] "The FLAG™ Peptide, a Versatile Fusion Tag for the Purification of Recombinant Proteins," *J. Biochem Biophys Methods* 49:455-65; Jones *et al.* [1995] *J. Chromatography* 707:3-22; Jones *et al.* [1995] "Current Trends in Molecular Recognition and Bioseparation," *J. of Chromatography A.* 707:3-22, Elsevier Science B.V.; Margolin [2000] "Green Fluorescent Protein as a Reporter for Macromolecular Localization in Bacterial Cells," *Methods* 20:62-72, Academic Press; Puig *et al.* [2001] "The Tandem Affinity Purification (TAP) Method: A General Procedure of Protein Complex Purification," *Methods* 24:218-29, Academic Press; Sassenfeld [1990] "Engineering Proteins for Purification," TibTech 8:88-93; Sheibani [1999] "Prokaryotic Gene Fusion Expression Systems and Their Use in Structural and Functional Studies of Proteins," *Prep. Biochem. & Biotechnol.* 29(1):77-90, Marcel Dekker, Inc.; Skerra *et al.* [1999] "Applications of a Peptide Ligand for Streptavidin: the *Strep*-tag", *Biomolecular Engineering* 16:79-86, Elsevier Science, B.V.; Smith [1998] "Cookbook for Eukaryotic Protein Expression: Yeast, Insect, and Plant Expression Systems," *The* Scientist 12(22):20; Smyth *et al.* [2000] "Eukaryotic Expression and Purification of Recombinant Extracellular Matrix Proteins Carrying the Strep II Tag", *Methods in Molecular Biology*, 139:49-57; Unger [1997] "Show Me the Money: Prokaryotic Expression Vectors and Purification Systems," *The Scientist* 11(17):20

or commercially available tags from vendors such as such as STRATAGENE (La Jolla, CA), NOVAGEN (Madison, WI), QIAGEN, Inc., (Valencia, CA), or InVitrogen (San Diego, CA).

In other embodiments, peptides of the subject invention can be fused to heterologous polypeptide sequences that have adjuvant activity (a polypeptide adjuvant). Non-limiting examples of such polypeptides include heat shock proteins (hsp) (see, for example, U.S. Patent No. 6,524,825).

Peptides as described herein may be synthesized by methods well known in the art, including recombinant DNA methods and chemical synthesis. Chemical synthesis may generally be performed using standard solution phase or solid phase peptide synthesis techniques, in which a peptide linkage occurs through the direct condensation of the amino group of one amino acid with the carboxy group of the other amino acid with the elimination of a water molecule. Peptide bond synthesis by direct condensation, as formulated above, requires suppression of the reactive character of the amino group of the first and of the carboxyl group of the second amino acid. The masking substituents must permit their ready removal, without inducing breakdown of the labile peptide molecule.

In solution phase synthesis, a wide variety of coupling methods and protecting groups may be used (see Gross and Meienhofer, eds., "The Peptides: Analysis, Synthesis, Biology," Vol. 1-4 (Academic Press, 1979); Bodansky and Bodansky, "The Practice of Peptide Synthesis," 2d ed. (Springer Verlag, 1994)). In addition, intermediate purification and linear scale up are possible. Those of ordinary skill in the art will appreciate that solution synthesis requires consideration of main chain and side chain protecting groups and activation method. In addition, careful segment selection is necessary to minimize racemization during segment condensation. Solubility considerations are also a factor..

Solid phase peptide synthesis uses an insoluble polymer for support during organic synthesis. The polymer-supported peptide chain permits the use of simple washing and filtration steps instead of laborious purifications at intermediate steps. Solid-phase peptide synthesis may generally be performed according to the method of Merrifield *et al.*, *J. Am. Chem. Soc.*, 1963, 85:2149, which involves assembling a linear peptide chain on a resin support using protected amino acids. Solid phase peptide synthesis typically utilizes either the Boc or Fmoc strategy, which are well known in the art.

Those of ordinary skill in the art will recognize that, in solid phase synthesis, deprotection and coupling reactions must go to completion and the side-chain blocking groups must be stable throughout the synthesis. In addition, solid phase synthesis is generally most suitable when peptides are to be made on a small scale.

Acetylation of the N-terminal can be accomplished by reacting the final peptide with acetic anhydride before cleavage from the resin. C-amidation is accomplished using an appropriate resin such as methylbenzhydrylamine resin using the Boc technology.

The peptides disclosed here in may be modified by attachment of a second molecule that confers a desired property upon the peptide, such as increased half-life in the body, for example, pegylation. Such modifications also fall within the scope of the term "variant" as used herein.

Covalent attachment of a molecule or solid support may generally be achieved by first reacting the support material with a bifunctional reagent that will also react with a functional group, such as a hydroxyl, thiol, carboxyl, ketone or amino group, on the modulating agent. A preferred method of generating a linkage is via amino groups using glutaraldehyde. A peptide may be linked to cellulose via ester linkages. Similarly, amide linkages may be suitable for linkage to other molecules such as keyhole limpet hemocyanin or other support materials.

Although integrin interaction inhibitors as described herein may preferentially bind to specific tissues or cells, and thus may be sufficient to target a desired site *in vivo*, it may be beneficial for certain applications to include an additional targeting agent. Accordingly, a targeting agent may also, or alternatively, be linked to an integrin interaction inhibitor to facilitate targeting to one or more specific tissues. As used herein, a "targeting agent," may be any substance (such as a compound or cell) that, when linked to a integrin interaction inhibitor, enhances the transport of the inhibitor to a target tissue, thereby increasing the local concentration of the inhibitor. Targeting agents include antibodies or fragments thereof, receptors, ligands and other molecules that bind to cells of, or in the vicinity of, the target tissue. Known targeting agents include serum hormones, antibodies against cell surface antigens, lectins, adhesion molecules, tumor cell surface binding ligands, steroids, cholesterol, lymphokines, fibrinolytic enzymes and those drugs and proteins that bind to a desired target site.

For certain embodiments, it may be beneficial to also, or alternatively, link a drug to an integrin interaction inhibitor. As used herein, the term "drug" refers to any bioactive agent intended for administration to a human or non-human mammal to prevent or treat a disease or other undesirable condition. Drugs include hormones, growth factors, proteins, peptides and other compounds. The use of certain specific drugs within the context of the present invention is discussed below.

Within certain aspects of the present invention, one or more integrin interaction inhibitors as described herein may be present within a pharmaceutical composition. A pharmaceutical composition comprises one or more integrin interaction inhibitors in combination with one or more pharmaceutically or physiologically acceptable carriers, diluents or excipients. Such compositions may comprise buffers (*e.g.*, neutral buffered saline or phosphate buffered saline), carbohydrates (*e.g.*, glucose, mannose, sucrose or dextrans), mannitol, proteins, polypeptides or amino acids such as glycine, antioxidants, chelating agents such as EDTA or glutathione, adjuvants (*e.g.*, aluminum hydroxide) and/or preservatives. Within yet other embodiments, compositions of the present invention may be formulated as a lyophilizate. A integrin interaction inhibitor may, but need not, be encapsulated within liposomes using well known technology. Compositions of the present invention may be formulated for any appropriate manner of administration, including for example, topical, oral, nasal, intravenous, intracranial, intraperitoneal, subcutaneous, or intramuscular administration. For certain topical applications, formulation as a cream or lotion, using well known components, is preferred.

Various techniques may be utilized to facilitate delivery of the integrin interaction inhibitors of the invention to the target cells *in vitro* (including *ex vivo*) and *in vivo* (Cellular Drug Delivery: Principles and Practice, edited by Lu, D.R. and Oie, S., Human Press, Totowa, N.J., 2004). Optionally, it may be desirable to facilitate delivery of the integrin interaction inhibitors of the invention through the outer cell membrane. Various protein carrier molecules may be coupled to the integrin interaction inhibitors of the invention to assist penetration through biological membranes. For example, small regions (*e.g.*, 9-16 amino acids) of proteins called protein transduction domains (PTDs) cell penetrating peptides (CPP) possess the ability to traverse biological membranes through protein transduction (Barnett, E.M. *et al.*, *Invest. Opthalmol. Vis. Sci.*, 2006, 47:2589-2595; Schwarze S.R. *et al.*, *Science*, 1999, 285(5433):1569-1572; Wadia, J.S. and Dowdy, S.F., *Advanced Drug Delivery Reviews*, 2005, 57(4): 579-596; Wadia, J.S. and Dowdy, S.F., *Curr. Opin. Biotechnol.*, 2002, 13(1)52-56; Ho A. *et al.*, *Cancer Research*, 2001, 61:474-477; Futaki *et al.*, *J. Biol. Chem.*, 2001, Feb., 276(8):5836-5840; Cao G. *et al.*, *J. Neurosci.*, 2002, 22(13):5423-5431; Becker-Hapk, M. *et al.*, Methods, 2001, 24:247-256; Snyder, E.L. and Dowdy, S.F., *Curr. Opin. Mol. Ther.*, 2001, 3:147-152; Lewin, M. *et al.*, *Nat. Biotechnol.*, 2000, 18:410-414; Tung, C.H. *et al.*, *Bioorg. Med. Chem.*, 2002, 10:3609-3614; Richard, J.P., *et al.*, *J. Biol. Chem.*, Oct. 30, 2002, epub ahead of print). Transduction can occur in a receptor- and transporter-independent fashion that appears to target the lipid bilayer directly. Proteins (peptides) and

compounds that are linked to PTDs (*e.g.*, covalently) have the capability to traverse outer cell membranes. Preferably, the delivery peptide is a trans-activating transcriptional activator (TAT) peptide or an Antennapedia (ANT) peptide, or a derivative of either. PTDs can be linked to the peptides of the subject invention for transport across the cell membrane. One well characterized PTD is the human immunodeficient virus (HIV)-1 Tat peptide (see, for example, U.S. Patent Nos. 5,804,604; 5,747,641; 5,674,980; 5,670,617; and 5,652,122). Peptides such as the homeodomain of *Drosophila* antennapedia (ANTP) and arginine-rich peptides display similar properties can be employed. VP22, a tegument protein from Herpes simplex virus type 1 (HSV-1), also has the ability to transport proteins across a cell membrane, and may be coupled to the integrin interaction inhibitors of the invention.

Definitions

As used herein, the terms "administering" or "administer" are defined as the introduction of a substance into cells *in vitro* or into the body of an individual *in vivo* by any route (for example, oral, nasal, ocular, rectal, vaginal and parenteral routes). Integrin interaction inhibitors may be administered individually or in combination with other agents via any route of administration, including but not limited to subcutaneous (SQ), intramuscular (IM), intravenous (IV), intraperitoneal (IP), intradermal (ID), via the nasal, ocular or oral mucosa (IN), or orally. For example, the integrin interaction inhibitors can be administered by direct injection into or on a tumor, or systemically (*e.g.*, into the circulatory system), to kill circulating tumor cells (CTC).

In the context of the instant invention, the terms "oligopeptide", "polypeptide", "peptide" and "protein" can be used interchangeably; however, it should be understood that the invention does not relate to the peptides in natural form, that is to say that they are not in their natural environment but that the peptide may have been isolated or obtained by purification from natural sources or obtained from host cells prepared by genetic manipulation (*e.g.*, the peptides, or fragments thereof, are recombinantly produced by host cells, or by chemical synthesis). Integrin interaction inhibitors containing peptides according to the instant invention may also contain non-natural amino acids, as will be described below. The terms "oligopeptide", "polypeptide", "peptide" and "protein" are also used, in the instant specification, to designate a series of residues of any length, typically L-amino acids, connected one to the other, typically by peptide bonds between the α-amino and carboxyl groups of adjacent amino acids. Linker elements can be joined to the peptides of the subject

invention, for example, through peptide bonds or via chemical bonds (*e.g.*, heterobifunctional chemical linker elements) as set forth below. Additionally, the terms "amino acid(s)" and "residue(s)" can be used interchangeably.

As used herein, the terms "treat" or "treatment" refer to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) an undesired physiological change or disorder, such as the development or spread of cancer or other proliferation disorder. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (*i.e.*, not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. For example, treatment with an integrin interaction inhibitor of the invention may include reduction of undesirable cell proliferation, and/or induction of apoptosis and cytotoxicity. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment. Those in need of treatment include those already with the condition or disorder as well as those prone to have the condition or disorder or those in which the condition or disorder is to be prevented or onset delayed. Optionally, the patient may be identified (*e.g.*, diagnosed) as one suffering from the disease or condition (*e.g.*, proliferation disorder) prior to administration of the integrin interaction inhibitor of the invention.

As used herein, the term "(therapeutically) effective amount" refers to an amount of the integrin interaction inhibitor of the invention or other agent (*e.g.*, a drug) effective to treat a disease or disorder in a mammal. In the case of cancer or other proliferation disorder, the therapeutically effective amount of the agent may reduce (*i.e.*, slow to some extent and preferably stop) unwanted cellular proliferation; reduce the number of cancer cells; reduce the tumor size; inhibit (*i.e.*, slow to some extent and preferably stop) cancer cell infiltration into peripheral organs; inhibit (*i.e.*, slow to some extent and preferably stop) tumor metastasis; inhibit, to some extent, tumor growth; reduce ß1 integrin signaling in the target cells, and/or relieve, to some extent, one or more of the symptoms associated with the cancer. To the extent the administered integrin interaction inhibitor prevents growth of and/or kills existing cancer cells, it may be cytostatic and/or cytotoxic. For cancer therapy, efficacy can, for example, be measured by assessing the time to disease progression (TTP) and/or determining the response rate (RR).

As used herein, the term "growth inhibitory amount" of the integrin interaction inhibitor of the invention refers to an amount which inhibits growth or proliferation of a

target cell, such as a tumor cell, either *in vitro* or *in vivo*, irrespective of the mechanism by which cell growth is inhibited (*e.g.*, by cytostatic properties, cytotoxic properties, *etc.*). In a preferred embodiment, the growth inhibitory amount inhibits (*i.e.*, slows to some extent and preferably stops) proliferation or growth of the target cell *in vivo* or in cell culture by greater than about 20%, preferably greater than about 50%, most preferably greater than about 75% (*e.g.*, from about 75% to about 100%).

The terms "cell" and "cells" are used interchangeably herein and are intended to include either a single cell or a plurality of cells, *in vitro* or *in vivo*, unless otherwise specified.

As used herein, the term "anti-cancer agent" refers to a substance or treatment (*e.g.*, radiation therapy) that inhibits the function of cancer cells, inhibits their formation, and/or causes their destruction *in vitro* or *in vivo*. Examples include, but are not limited to, cytotoxic agents (*e.g.*, 5-fluorouracil, TAXOL), chemotherapeutic agents, and anti-signaling agents (*e.g.*, the PI3K inhibitor LY). In one embodiment, the anti-cancer agent administered before, during, after administration of the peptide or encoding polynucleotide of the invention is melphalen. Anti-cancer agents include but are not limited to the chemotherapeutic agents listed Table 3.

As used herein, the term "cytotoxic agent" refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells *in vitro* and/or *in vivo*. The term is intended to include radioactive isotopes (*e.g.*, At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, and radioactive isotopes of Lu), chemotherapeutic agents, toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, and antibodies, including fragments and/or variants thereof.

As used herein, the term "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer, such as, for example, taxanes, *e.g.*, paclitaxel (TAXOL, BRISTOL-MYERS SQUIBB Oncology, Princeton, N.J.) and doxetaxel (TAXOTERE, Rhone-Poulenc Rorer, Antony, France), chlorambucil, vincristine, vinblastine, anti-estrogens including for example tamoxifen, raloxifene, aromatase inhibiting 4(5)-imidazoles, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene (FARESTON, GTx, Memphis, TN), and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin, *etc.* Examples of chemotherapeutic agents that may be used in conjunction with the compounds of the invention are listed in Table 3. In a preferred embodiment, the chemotherapeutic agent is one or more anthracyclines. Anthracyclines are a family of chemotherapy drugs that are also antibiotics. The anthracyclines act to prevent cell division

by disrupting the structure of the DNA and terminate its function by: (1) intercalating into the base pairs in the DNA minor grooves; and (2) causing free radical damage of the ribose in the DNA. The anthracyclines are frequently used in leukemia therapy. Examples of anthracyclines include daunorubicin (CERUBIDINE), doxorubicin (ADRIAMYCIN, RUBEX), epirubicin (ELLENCE, PHARMORUBICIN), and idarubicin (IDAMYCIN).

Table 3. Examples of Chemotherapeutic Agents

- 13-cis-Retinoic Acid	- Mylocel
-2-Amino-6-Mercaptopurine	- Letrozole
	- Neosar
- 2-CdA	- Neulasta
- 2-Chlorodeoxyadenosine	- Neumega
- 5-fluorouracil	- Neupogen
- 5-FU	- Nilandron
- 6 - TG	- Nilutamide
- 6 - Thioguanine	- Nitrogen Mustard
- 6-Mercaptopurine	- Novaldex
- 6-MP	- Novantrone
- Accutane	- Octreotide
- Actinomycin-D	- Octreotide acetate
- Adriamycin	- Oncospar
- Adrucil	- Oncovin
- Agrylin	- Ontak
- Ala-Cort	- Onxal
- Aldesleukin	- Oprevelkin
- Alemtuzumab	- Orapred
- Alitretinoin	- Orasone
- Alkaban-AQ	- Oxaliplatin
- Alkeran	- Paclitaxel
- All-transretinoic acid	- Pamidronate
- Alpha interferon	- Panretin

- Altretamine	- Paraplatin
- Amethopterin	- Pediapred
- Amifostine	- PEG Interferon
- Aminoglutethimide	- Pegaspargase
- Anagrelide	- Pegfilgrastim
- Anandron	- PEG-INTRON
- Anastrozole	- PEG-L-asparaginase
- Arabinosylcytosine	- Phenylalanine Mustard
- Ara-C	- Platinol
- Aranesp	- Platinol-AQ
- Aredia	- Prednisolone
- Arimidex	- Prednisone
- Aromasin	- Prelone
- Arsenic trioxide	- Procarbazine
- Asparaginase	- PROCRIT
- ATRA	- Proleukin
- Avastin	- Prolifeprospan 20 with Carmustine implant
- BCG	- Purinethol
- BCNU	- Raloxifene
- Bevacizumab	- Rheumatrex
- Bexarotene	- Rituxan
- Bicalutamide	- Rituximab
- BiCNU	- Roveron-A (interferon alfa-2a)
- Blenoxane	- Rubex
- Bleomycin	- Rubidomycin hydrochloride
- Bortezomib	- Sandostatin
- Busulfan	- Sandostatin LAR
- Busulfex	- Sargramostim
- C225	- Solu-Cortef
- Calcium Leucovorin	- Solu-Medrol
- Campath	- STI-571
- Camptosar	- Streptozocin

- Camptothecin-11	- Tamoxifen
- Capecitabine	- Targretin
- Carac	- Taxol
- Carboplatin	- Taxotere
- Carmustine	- Temodar
- Carmustine wafer	- Temozolomide
- Casodex	- Teniposide
- CCNU	- TESPA
- CDDP	- Thalidomide
- CeeNU	- Thalomid
- Cerubidine	- TheraCys
- cetuximab	- Thioguanine
- Chlorambucil	- Thioguanine Tabloid
- Cisplatin	- Thiophosphoamide
- Citrovorum Factor	- Thioplex
- Cladribine	- Thiotepa
- Cortisone	- TICE
- Cosmegen	- Toposar
- CPT-11	- Topotecan
- Cyclophosphamide	- Toremifene
- Cytadren	- Trastuzumab
- Cytarabine	- Tretinoin
- Cytarabine liposomal	- Trexall
- Cytosar-U	- Trisenox
- Cytoxan	- TSPA
- Dacarbazine	- VCR
- Dactinomycin	- Velban
- Darbepoetin alfa	- Velcade
- Daunomycin	- VePesid
- Daunorubicin	- Vesanoid
- Daunorubicin hydrochloride	- Viadur
	- Vinblastine

- Daunorubicin liposomal	- Vinblastine Sulfate
- DaunoXome	- Vincasar Pfs
- Decadron	- Vincristine
- Delta-Cortef	- Vinorelbine
- Deltasone	- Vinorelbine tartrate
- Denileukin diftitox	- VLB
- DepoCyt	- VP-16
- Dexamethasone	- Vumon
- Dexamethasone acetate	- Xeloda
- dexamethasone sodium phosphate	- Zanosar
- Dexasone	- Zevalin
- Dexrazoxane	- Zinecard
- DHAD	- Zoladex
- DIC	- Zoledronic acid
- Diodex	- Zometa
- Docetaxel	- Gliadel wafer
- Doxil	- Glivec
- Doxorubicin	- GM-CSF
- Doxorubicin liposomal	- Goserelin
- Droxia	- granulocyte - colony stimulating factor
- DTIC	- Granulocyte macrophage colony stimulating factor
- DTIC-Dome	- Halotestin
- Duralone	- Herceptin
- Efudex	- Hexadrol
- Eligard	- Hexalen
- Ellence	- Hexamethylmelamine
- Eloxatin	- HMM
- Elspar	- Hycamtin
- Emcyt	- Hydrea
- Epirubicin	- Hydrocort Acetate
- Epoetin alfa	- Hydrocortisone

- Erbitux	- Hydrocortisone sodium phosphate
- Erwinia L-asparaginase	- Hydrocortisone sodium succinate
- Estramustine	- Hydrocortone phosphate
- Ethyol	- Hydroxyurea
- Etopophos	- Ibritumomab
- Etoposide	- Ibritumomab Tiuxetan
- Etoposide phosphate	- Idamycin
- Eulexin	- Idarubicin
- Evista	- Ifex
- Exemestane	- IFN-alpha
- Fareston	- Ifosfamide
- Faslodex	- IL - 2
- Femara	- IL-11
- Filgrastim	- Imatinib mesylate
- Floxuridine	- Imidazole Carboxamide
- Fludara	- Interferon alfa
- Fludarabine	- Interferon Alfa-2b (PEG conjugate)
- Fluoroplex	- Interleukin - 2
- Fluorouracil	- Interleukin-11
- Fluorouracil (cream)	- Intron A (interferon alfa-2b)
- Fluoxymesterone	- Leucovorin
- Flutamide	- Leukeran
- Folinic Acid	- Leukine
- FUDR	- Leuprolide
- Fulvestrant	- Leurocristine
- G-CSF	- Leustatin
- Gefitinib	- Liposomal Ara-C
- Gemcitabine	- Liquid Pred
- Gemtuzumab ozogamicin	- Lomustine
- Gemzar	- L-PAM
- Gleevec	- L-Sarcolysin
- Lupron	- Meticorten

- Lupron Depot	- Mitomycin
- Matulane	- Mitomycin-C
- Maxidex	- Mitoxantrone
- Mechlorethamine	- M-Prednisol
-Mechlorethamine Hydrochlorine	- MTC
	- MTX
- Medralone	- Mustargen
- Medrol	- Mustine
- Megace	- Mutamycin
- Megestrol	- Myleran
- Megestrol Acetate	- Iressa
- Melphalan	- Irinotecan
- Mercaptopurine	- Isotretinoin
- Mesna	- Kidrolase
- Mesnex	- Lanacort
- Methotrexate	- L-asparaginase
- Methotrexate Sodium	- LCR
- Methylprednisolone	

As used herein, the term "tumor" refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues. For example, a particular cancer may be characterized by a solid tumor mass. A primary tumor mass refers to a growth of cancer cells in a tissue resulting from the transformation of a normal cell of that tissue. In most cases, the primary tumor mass is identified by the presence of a cyst, which can be found through visual or palpation methods, or by irregularity in shape, texture, or weight of the tissue. However, some primary tumors are not palpable and can be detected only through medical imaging tec;hniques such as X-rays (e.g., mammography), or by needle aspirations. The use of these latter techniques is more common in early detection. Molecular and phenotypic analysis of cancer cells within a tissue will usually confirm if the cancer is endogenous to the tissue or if the lesion is due to metastasis from another site. The peptides of the invention are capable of inducing apoptosis in tumor cells and reducing tumor cell growth. The peptides of the invention (or nucleic acids encoding them) can be administered locally at the site of a tumor (*e.g.*, by direct injection) or remotely. The peptides

of the invention can induce cell death in circulating tumor cells (CTC) in a subject, *e.g.*, by administering the peptides or encoding nucleic acids intravenously. Furthermore, the peptides of the invention can prevent or reduce onset of metastasis to other tissues, *e.g.*, to the bone.

As used herein, the term "signaling" and "signaling transduction" represents the biochemical process involving transmission of extracellular stimuli, via cell surface receptors through a specific and sequential series of molecules, to genes in the nucleus resulting in specific cellular responses to the stimuli.

As used herein, the term "pharmaceutically acceptable salt or prodrug" is intended to describe any pharmaceutically acceptable form (such as an ester, phosphate ester, salt of an ester or a related group) of an integrin interaction inhibitor of the invention or other agent, which, upon administration to a subject, provides the mature or base compound. Pharmaceutically acceptable salts include those derived from pharmaceutically acceptable inorganic or organic bases and acids. Suitable salts include those derived from alkali metals such as potassium and sodium, alkaline earth metals such as calcium and magnesium, among numerous other acids well known in the pharmaceutical art. Pharmaceutically acceptable prodrugs refer to a compound that is metabolized, for example hydrolyzed or oxidized, in the host to form the compound of the present invention. Typical examples of prodrugs include compounds that have biologically labile protecting groups on a functional moiety of the active compound. Prodrugs include compounds that can be oxidized, reduced, aminated, deaminated, hydroxylated, dehydroxylated, hydrolyzed, dehydrolyzed, alkylated, dealkylated, acylated, deacylated, phosphorylated, dephosphorylated to produce the active compound.

The terms "link" or "join" refers to any method known in the art for functionally connecting peptides, including, without limitation, recombinant fusion, covalent bonding, disulfide bonding, ionic bonding, hydrogen bonding, and electrostatic bonding.

The terms "comprising", "consisting of" and "consisting essentially of" are defined according to their standard meaning. The terms may be substituted for one another throughout the instant application in order to attach the specific meaning associated with each term.

The terms "isolated" or "biologically pure" refer to material that is substantially or essentially free from components which normally accompany the material as it is found in its native state. Thus, isolated peptides or integrin interaction inhibitors in accordance with the invention preferably do not contain materials normally associated with the peptides in their *in situ* environment.

As used in this specification, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "a compound" includes more than one such compound. Reference to "an integrin interaction inhibitor" includes more than one such inhibitor. A reference to "a peptide" includes more than one such peptide, and so forth.

The practice of the present invention can employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA technology, electrophysiology, and pharmacology that are within the skill of the art. Such techniques are explained fully in the literature (see, *e.g.*, Sambrook, Fritsch & Maniatis, Molecular Cloning: A Laboratory Manual, Second Edition (1989); DNA Cloning, Vols. I and II (D. N. Glover Ed. 1985); Perbal, B., A Practical Guide to Molecular Cloning (1984); the series, Methods In Enzymology (S. Colowick and N. Kaplan Eds., Academic Press, Inc.); Transcription and Translation (Hames *et al.* Eds. 1984); Gene Transfer Vectors For Mammalian Cells (J. H. Miller *et al.* Eds. (1987) Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.); Scopes, Protein Purification: Principles and Practice (2nd ed., Springer-Verlag); and PCR: A Practical Approach (McPherson *et al.* Eds. (1991) IRL Press)).

Experimental controls are considered fundamental in experiments designed in accordance with the scientific method. It is routine in the art to use experimental controls in scientific experiments to prevent factors other than those being studied from affecting the outcome.

MATERIALS AND METHODS

Reagents. Organic and inorganic reagents (ACS grade) were obtained from commercial sources and used without further purification, unless otherwise noted. Fmoc-protected amino acids and the coupling agent HCTU were obtained from Protein Technologies, Calbiochem-Novabiochem, or Chem-impex International. 2-Chlorotrityl chloride resin was purchased from Anaspec Inc. All linear peptides were synthesized on the Symphony peptide synthesizer, Protein Technologies Instruments. Solvents for peptide synthesis and reverse-phase HPLC were obtained from Applied Biosystems. Other chemicals used were obtained from Aldrich and were of the highest purity commercially available. Thin layer chromatography (TLC) was performed on glass plates (Whatman) coated with 0.25 mm thickness of silica gel 60Å (# 70-230 mesh). High resolution mass spectra were obtained on an Agilent LC-MSD-TOF.

Circular Dichroism Measurement. Circular dichroism experiments were carried out at room temperature on the Aviv (Model # 210) spectropolarimeter flushed with nitrogen. The samples were prepared as stock solutions in sodium acetate buffer and diluted to the desired concentration for measurements. Each spectra was collected from 250 nm to 184 nm using a 0.1 cm path length cylindrical quartz cell. Each spectrum was recorded as an average of three scans taken at a spectral bandwidth of 1 nm. All spectra were corrected for buffer contributions and presented in units of molar ellipticity.

NMR Spectroscopy. All deuterated reagents and solvents were purchased from Cambridge Isotopes. All 1D ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 250 MHz or a Varian INOVA 400 MHz spectrometer in $CDCl_3$ unless otherwise specified and chemical shifts are reported in ppm (δ) relative to internal standard tetramethylsilane (TMS). 2D NMR samples were prepared by dissolving 1-2 mg peptide in 100 µL D_2O and then adjusting the pD to 4.0 (uncorrected) with either 50 mM NaOAc-d_3 or 50 mM AcOH-d_4 to yield a final concentration between 3-7 mM. Chemical shifts are reported in parts per million (ppm) relative to 0.5 mM DSS. NMR experiments were run and processed on a three-channel Varian Inova 500MHz instrument at 298.1 K using a 3-mm I.D. RT probe equipped with Z-axis PFGs running VnmrJ 2.2D. Spectra were then analyzed using ACD labs NMR Manager version 11.0. 1D NMR spectra were collected using 32K data points, between 16 and 64 scans were collected using a 0.5 s delay and 1 s presaturation. 2D TOCSY and NOESY experiments were run with a 5000 Hz window in both dimensions. TOCSY experiments were run with a mixing time of 60 ms, a 0.5 s relaxation delay followed by 1 s of presaturation and 512 increments in the f_1 dimension with 32 transients per increment (collecting 4096 data points per transient in the f_2 dimension). Zero-filling was then applied using 4096 points for each dimension. NOESY experiments were performed using a 500 ms mixing time, 1 s of presaturation and 512 increments of 32 transients each (collecting 4096 data points per transient in the f_2). Zero-filling was then applied using 4096 points for each dimension. Presaturation was used to suppress the water resonance both during the relaxation delay and during the mixing time. All spectra were analyzed using standard window functions (Gaussian without shifting). Assignments were made by using standard methods as described by Wüthrich[1].

Constrained conformation search with MacroModel. Structures were created with Maestro 8.5[2] for peptides 1 and 5. Minimizations of the structures were performed with

MacroModel 9.8[3]. The OPLS 2005[4] force field was used with implicit water and a constant dielectric constant of 1.0 while using the Truncated Newtonian Conjugate Gradient algorithm with a threshold of 0.01 kJ/mol. A Mixed Monte Carlo Multiple Minimum (MCMM)/Low-Mode Conformational Search (LMCS) method was employed with NOEsy data, which were introduced as flat-bottom energetic restraint wells to yield a constrained potential energy. Torsion angles were similarly restrained for all peptide bonds. A 200 kJ/mol energy window of structures were kept during the conformation search where only structures in the lower 100 kJ/mol were outputted. Redundant conformations were eliminated and 20 lowest energy structures were kept for analysis.

Cyclic Peptide Synthesis & Purification.

a. Cyclic III peptide with D-Pro-L-Pro and N-(2-aminoethyl)-N-methylsulfonamido glycine linker.

2-Chlorotrityl chloride resin was treated with Fmoc-Pro-OH and then immediately Fmoc-deprotected using 20% piperidine/2% DBU in DMF. Fmoc quantification of resin indicated a loading of 0.19 mmol/g of resin. For a 25 μmol synthesis, 132 mg of resin was charged to the peptide reaction vessel on a Protein Technologies Symphony Peptide Synthesizer. For each coupling step, 5 equivalents of Fmoc-amino acid and 7.5 equivalents of HCTU are dissolved in 0.4 M NMM in DMF to equal 20 equivalents of NMM, which is added to the reactor. Each coupling reaction was carried out for 10 mins followed by NMP washes. Fmoc deprotection was done using 20% piperidine/2% DBU in DMF for (2 x 2.5 mins). The amino acids used for peptide synthesis were coupled in the following order: Fmoc-D-Pro-OH, Fmoc-Lys(Boc)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-$NHCH_2CH_2N(O_2SCH_3)CH_2COOH$, Fmoc-Trp(Boc)-OH, Fmoc-Ser(t-Bu)-OH, Fmoc-Val-OH, Fmoc-Val-OH, and Fmoc-Met-OH. After synthesis of the protected linear HYD1, the resin was transferred to a manual peptide synthesis vessel and treated with 5 mL of a cleavage solution of 20% trifluoroethanol in DCM for 2 hours. The resin was filtered and washed with 5 mL of cleavage solution. This cleavage cycle was repeated twice. The combined organic filtrates were concentrated to give crude protected linear III peptide. The crude III peptide was dissolved in 15 mL of 1% v/v DIEA in DMF and treated with 4 equivalents of HCTU for one hour. After one hour, the reaction mixture was concentrated to give crude protected cyclized III peptidomimetic. The crude peptidomimetic was then treated with a 10 mL solution of 87.5% TFA/5% H_2O/5% phenol/2.5% triethylsilane for 30 mins. The reaction mixture was concentrated and the thick viscous liquid was triturated twice with 10 mL of cold diethyl ether. The reaction contents were centrifuged

to give crude cyclic III peptidomimetic. The crude peptidomimetic was dissolved in a solution of 0.1% TFA in H_2O and freeze-dried to give a white fluffy powder. All cyclic III peptides and peptidomimetics were purified using semi-preparative reverse phase HPLC (5 μM particle size C_{18} AAPPTEC spirit column, 25 x 2.12 cm) with eluents: A = 0.1% HCO_2H in H_2O, B = 0.1% HCO_2H in H_3CCN. The purification was carried out using a gradient of 5-50% B Buffer over 40 min with a flow rate 20 mL/minute using 222 nm UV detection. All peaks with retention times expected for peptides were collected and lyophilized. The purified peptides were analyzed using similar analytical HPLC conditions and found to have >95% purity and were structurally characterized using a Bruker Autoflex MALDI-TOF instrument with α-cyano hydroxyl cinnamic acid (CHCA) as matrix. We have also characterized the secondary structure of selected cyclic III peptidomimetics and they show concentration independent CD spectra in pH 7.0 sodium acetate buffer at concentrations of 200 μM indicative of beta-sheet-like conformations with a minima around 200 nm for cyclic III and a maxima around 190 nm as expected. This supports the assertion of cyclic beta-hairpin-like structure.

b. Cyclic III peptides with two N-(2-aminoethyl)-N-methylsulfonamidoglycine linkers.

2-Chlorotrityl chloride resin was treated with Fmoc-Met-OH and then immediately Fmoc-deprotected using 20% piperidine/2% DBU in DMF. Fmoc quantification of resin indicated a loading of 0.24 mmol/g of resin. For a 25 μmol synthesis, 104 mg of resin was charged to the peptide reaction vessel on a Protein Technologies Symphony Peptide Synthesizer. Everything else was the same as above except the amino acids were coupled in the following order: Fmoc-$NHCH_2CH_2N(O_2SCH_3)CH_2COOH$, Fmoc-Lys(Boc)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)- OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-$NHCH_2CH_2N(O_2SCH_3)CH_2COOH$, Fmoc-Trp(Boc)-OH, Fmoc-Ser(t-Bu)-OH, Fmoc-Val-OH, and Fmoc-Val-OH. We saw no evidence of Met C-terminal racemization from the C-terminal peptide cyclization stcp, which can be detected by the appearance of diastereomeric peptide side products in the HPLC analysis.

c. Cyclic III Peptide analogs prepared by on resin cyclization using lysine side chain anchoring strategy

p-Nitrophenyl Wang Resin (0.69mmol/g, 0.25g) was swollen in dichloromethane for 15 minutes. N^{α}- Fmoc-Lys-OAllyl. TFA (4equiv.) solution in DCM containing DIEA (8 equiv.) was added to the resin in peptide reaction vessel for 3 hours. The process is repeated twice to ensure maximum loading of the fmoc amino acid on the resin. N^{α}- Fmoc-Lys-OAllyl. TFA salt was prepared by deprotection of N^{α}- Fmoc-Lys(Boc)-OAllyl using 95%

TFA in DCM at 0^0C. Fmoc quantification of resin indicated a loading of 0.59 mmol/g of resin. The linear protected peptide was then synthesized using standard Fmoc solid phase strategy on a Protein Technologies Symphony Peptide Synthesizer. For a 25 μmol synthesis, 42 mg of resin was charged to the peptide reaction vessel. For each coupling step, 5 equivalents of Fmoc-amino acid and 7.5 equivalents of HCTU are dissolved in 0.4 M NMM in DMF to equal 20 equivalents of NMM, which is added to the reactor. Each coupling reaction was carried out for 10 mins followed by NMP washes. Fmoc deprotection was done using 20% piperidine/2% DBU in DMF for (2 x 2.5 mins). The amino acids used for peptide synthesis were coupled in the following order: Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Linker T_3, Fmoc-Trp(Boc)-OH, Fmoc-Ala-OH, Fmoc-Val-OH, Fmoc-Val-OH, Fmoc-Nle-OH and Linker T_1. After synthesis of the protected linear cyclic III peptide, the resin was transferred to a manual peptide synthesis vessel. The Fmoc group from last amino acid was cleaved by 20% piperidine/2% DBU in DMF. The C-terminal allyl group was then removed using $Pd(PPh_3)_4$ dissolved in $CHCl_3$-AcOH-NMM (37:2:1) for two hours. The allyl cleavage procedure was repeated again to ensure complete cleavage. The resulting side chain anchored peptide acid resin was then washed with DCM, NMP, MeOH, DCM and dried. After allyl deprotection, on resin cyclization of linear peptide was carried out by treating peptide side chain anchored peptide acid resin with 4 equivalents of HCTU in 4ml DMF and 8 equivalents of DIEA for one hour.

After on the resin, the peptide was deprotected from the resin using cleavage cocktail of TFA/Triethylsilane/H_2O (95:2.5:5) solution at room temperature for 30 minutes. The reaction mixture was concentrated and the thick viscous liquid was triturated twice with 10 mL of cold diethyl ether. The reaction contents were centrifuged to give crude cyclic III peptidomimetic. The crude peptidomimetic was dissolved in a solution of 0.1% TFA in H_2O and freeze-dried to give a white fluffy powder. All cyclic III peptides and peptidomimetics were purified using semi-preparative reverse phase HPLC (5 μM particle size C_{18} AAPPTEC spirit column, 25 x 2.12 cm) with eluents: A = 0.1% HCO_2H in H_2O, B = 0.1% HCO_2H in H_3CCN. The purification was carried out using a gradient of 5-50% B Buffer over 40 min with a flow rate 20 mL/minute using 222 nm UV detection. All peaks with retention times expected for peptides were collected and lyophilized. The purified peptides were analyzed using similar analytical HPLC conditions and found to have >95% purity and were structurally characterized using a Bruker Autoflex MALDI-TOF instrument with α-cyano hydroxyl cinnamic acid (CHCA) as matrix.

d. Cyclic III Peptide analogs prepared by on resin cyclization using Glutamic acid side chain anchoring strategy

Fmoc protected Rink amide Resin (0.62mmol/g, 0.25g) was swollen in dichloromethane for 15 minutes. The Fmoc group was removed using 20% piperidine/2% DBU in DMF. N^{α}- Fmoc-Glu(OH)-O^{α}Allyl (4equiv.), HCTU (4equiv.) in DMF alongwith DIEA (8 equiv.) was added to the resin in peptide reaction vessel for 2 hours. The process is repeated twice to ensure maximum loading of the fmoc amino acid on the resin. Fmoc quantification of resin indicated a loading of 0.53 mmol/g of resin. The linear protected peptide was then synthesized using standard Fmoc solid phase strategy on a Protein Technologies Symphony Peptide Synthesizer. For a 25 μmol synthesis, 47 mg of resin was charged to the peptide reaction vessel. For each coupling step, 5 equivalents of Fmoc-amino acid and 7.5 equivalents of HCTU are dissolved in 0.4 M NMM in DMF to equal 20 equivalents of NMM, which is added to the reactor. Each coupling reaction was carried out for 10 mins followed by NMP washes. Fmoc deprotection was done using 20% piperidine/2% DBU in DMF for (2 x 2.5 mins). The amino acids used for peptide synthesis were coupled in the following order: Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Linker T_3, Fmoc-Trp(Boc)-OH, Fmoc-Ala-OH, Fmoc-Val-OH, Fmoc-Val-OH, Fmoc-Nle-OH and Linker T_1. After synthesis of the protected linear cyclic III peptide, the resin was transferred to a manual peptide synthesis vessel. The Fmoc group from last amino acid was cleaved by 20% piperidine/2% DBU in DMF. The C-terminal allyl group was then removed using $Pd(PPh_3)_4$ dissolved in $CHCl_3$-AcOH-NMM (37:2:1) for two hours. The allyl cleavage procedure was repeated again to ensure complete cleavage. The resulting side chain anchored peptide acid resin was then washed with DCM, NMP, MeOH, DCM and dried. After allyl deprotection, on resin cyclization of linear peptide was carried out by treating peptide side chain anchored peptide acid resin with 4 equivalents of HCTU in 4ml DMF and 8 equivalents of DIEA for one hour. After on resin, the peptide was deprotected from the resin using cleavage cocktail of TFA/Triethylsilane/H_2O (95:2.5:5) solution at room temperature for 30 minutes. The reaction mixture was concentrated and the thick viscous liquid was triturated twice with 10 mL of cold diethyl ether. The reaction contents were centrifuged to give crude cyclic III peptidomimetic. The crude peptidomimetic was dissolved in a solution of 0.1% TFA in H_2O and freeze-dried to give a white fluffy powder. All cyclic III peptides and peptidomimetics were purified using semi-preparative reverse phase HPLC (5 μM particle size C_{18} AAPPTEC spirit column, 25 x 2.12 cm) with eluents: A = 0.1% HCO_2H in H_2O, B = 0.1% HCO_2H in H_3CCN. The purification was carried out using a

gradient of 5-50% B Buffer over 40 min with a flow rate 20 mL/minute using 222 nm UV detection. All peaks with retention times expected for peptides were collected and lyophilized. The purified peptides were analyzed using similar analytical HPLC conditions and found to have >95% purity and were structurally characterized using a Bruker Autoflex MALDI-TOF instrument with α-cyano hydroxyl cinnamic acid (CHCA) as matrix.

Synthesis of N-(2-aminoethyl)-N-methylsulfonamidoglycine linker.

tert-Butyl N-(2-aminoethyl) glycine 2 (Figure 20A). A solution of tert-butyl bromoacetate (27.6 mL, 0.18 mol) in 150 mL DCM was added dropwise to a solution of ethylenediamine (100 mL, 1.5 mol) in 700 mL DCM at 0°C for a period of 30 mins. The reaction mixture was allowed to warm to room temperature and stirred for 15 hours. The reaction mixture was then washed with (2 x 150 mL) water. The aqueous layer was re-extracted with DCM (3 x 100 mL). The combined organic washes were dried using sodium sulfate and then filtered. The solution was concentrated in vacuo to dryness and was used in next step without further purification (27.4 gm, 85%). ^{1}H NMR (250 MHz, $CDCl_3$) δ 3.30 (s, 2H), 2.83 – 2.76 (m, 2H), 2.72 – 2.64 (m, 2H), 1.60 (b, 3H), 1.47 (s, 9H). ^{13}C NMR (63 MHz, $CDCl_3$) ppm 171.54, 80.59, 51.70, 51.21, 41.34, 27.78.

tert-Butyl N-[2-(N-9-fluorenylmethoxycarbonyl)aminoethyl]glycinate hydrochloride 3 (Figure 20B). Compound 2 (22 gm, 0.13 mol) was dissolved along with DIEA (22 mL, 0.13 mol) in DCM (1000 mL) and N-(9-fluorenylmethoxycarbonyloxy) succinimide (41 gm, 0.12 mol) in 300 mL DCM was added dropwise over one hour. The reaction contents were stirred overnight and washed with (3 x 100 mL) 1M HCl solution and brine solution (100 mL). The organic contents were dried using Na_2SO_4 and filtered. The solution was partially concentrated to 50 mL and cooled in deep freezer (-20°C) for overnight. The white precipitate formed was filtered and washed with DCM. The precipitates were vacuum dried to give compound **3** as the hydrochloride salt (43.1 gm, 90%). ^{1}H NMR (400 MHz, DMSO d_6) δ 9.51 (s, 2H), 7.88 (d, J = 7.5 Hz, 2H), 7.70 (d, J = 7.4 Hz, 2H), 7.65 (d, J = 5.5 Hz, 1H), 7.41 (t, J = 7.4 Hz, 2H), 7.33 (t, J = 7.3 Hz, 2H), 4.31 (d, J = 6.7 Hz, 2H), 4.23 (d, J = 6.6 Hz, 1H), 3.86 (s, 2H), 3.49 – 3.26 (m, 2H), 3.02 (t, J = 5.9 Hz, 2H), 1.45 (s, 9H). ^{13}C NMR (101 MHz, DMSO d_6) ppm 165.52, 156.21, 143.77, 140.70, 127.60, 127.04, 125.17, 120.09, 82.91, 65.64, 54.91, 47.19, 46.63, 46.37, 36.59, 27.59. HRMS *m/z* (ESI): calcd. for $C_{23}H_{29}N_2O_4$ $[M+H]^+$ 397.2122, found 397.2120

tert-Butyl N-[2-(N-9-fluorenylmethoxycarbonyl)aminoethyl N-methylsulfonamido glycinate 4 (Figure 20C). Compound 3 (5.0 gm, 11.5 mmol) was suspended in 50 mL of THF and DIEA (4.0 mL, 23.1 mmol) was added to it at 0°C. Methanesulfonyl chloride (0.9 mL, 11.5 mmol) was added dropwise for period of 10 mins. The reaction mixture was stirred for two hours and allowed to warm to room temperature. The mixture was evaporated to dryness in vacuum and the residue was partitioned between DCM and water. The organic layer was partially concentrated and kept in the refrigerator overnight. The white precipitate that formed was filtered and dried in vacuuo. (4.8 gm, 88%) ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.76 (d, J = 7.1 Hz, 2H), 7.61 (d, J = 7.3 Hz, 2H), 7.44 – 7.27 (m, 5H), 5.48 (s, 1H), 4.38 (d, J = 7.1 Hz, 2H), 4.28 – 4.18 (m, 1H), 4.02 (s, 2H), 3.38 (d, J = 12.6 Hz, 4H), 3.01 (s, 3H), 1.48 (s, 9H).^{13}C NMR (63 MHz, $CDCl_3$) ppm 169.04, 156.58, 143.93, 141.30, 127.69, 127.09, 125.18, 119.96, 82.97, 66.95, 49.45, 47.94, 47.19, 39.71, 39.20, 28.04. HRMS *m/z* (ESI): calcd. for $C_{24}H_{31}N_2O_6SNa$ $[M+Na]^+$ 497.1717, found 497.1712.

2-(N-(2-(((9H-fluoren-9-yl)methoxy)carbonylamino)ethyl)methylsulfonamido)acetic acid 5 (Figure 20D). Compound 4 (4.8 gm, 10 mmol) was dissolved in 1,4-dioxane and 4M HCl was added to it. After completion of the reaction, reaction contents were filtered to give compound **2.5** as white solid in quantitative yield (4.1 gm). ^{1}H NMR (400 MHz, DMSO d_6) δ 12.93 (s, 1H), 7.90 (d, J = 7.5 Hz, 2H), 7.69 (d, J = 7.4 Hz, 2H), 7.43 (t, J = 7.4 Hz, 2H), 7.34 (t, J = 7.3 Hz, 2H), 4.32 (d, J = 6.9 Hz, 2H), 4.23 (t, J = 6.8 Hz, 1H), 4.01 (s, 2H), 3.36 (s, 1H), 3.29 (t, J = 6.3 Hz, 2H), 3.20 (dd, J = 12.0, 6.0 Hz, 2H), 2.98 (s, 3H). ^{13}C NMR (101 MHz, DMSO) ppm 170.99, 156.13, 143.88, 140.73, 127.60, 127.06, 125.13, 120.10, 65.45, 48.25, 46.98, 46.74, 39.02, 38.87. HRMS *m/z* (ESI): calcd. for $C_{20}H_{23}N_2O_6S$ $[M+H]^+$ 419.1271, found 419.1278.

I-tert-butyl 2-((2-tert-butoxy-2-oxoethoxy)methyl)pyrrolidine-1-carboxylate 7 (Figure 20E). To a solution of **6** (0.5 gm, 2.5 mmol) in toluene (10 mL) were added 30% NaOH solution (6 mL), tert-butyl bromoacetate (0.73 mL, 5.0 mmol) and TBAI (0.46 gm, 1.2 mmol) at 0°C. The reaction was carried out for 3 hrs until the TLC showed complete consumption of starting material. The reaction mixture was diluted with water (5 mL) before extracting with ethyl acetate (3 x 20 mL). The combined organic layer was washed with 1M HCl (10 mL), brine (10 mL) and dried over Na_2SO_4. The organic layer was concentrated to leave a residue which was further purified by column chromatography to give compound 7 (0.6 gm) in 77%

yield. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.70 (s, 1H), 3.37 (m, 1H), 3.08 (m, 2H), 1.81-1.67 (m, 4H), 1.22 (s, 9H), 1.21 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 169.3 (1C), 154.2 (1C), 80.9 (1C), 78.9 (1C), 71.7 (1C), 68.7 (1C), 56.1 (1C), 46.4 (1C), 28.4 (1C), 28.2 (3C), 27.8 (3C), 23.0 (1C). HRMS *m/z* (ESI): calcd. for $C_{16}H_{30}NO_5$ $[M+H]^+$ 316.2119, found 316.2164.

1-tert-butyl 2-(pyrrolidin-2-ylmethoxy) acetate 8 (Figure 20F). Compound **7** (0.6 gm, 1.8 mmol) was dissolved in 15 mL DCM and 5 mL trifluoroacetic acid was added to it. The reaction contents were stirred until the starting material was completely consumed. The reaction mixture was concentrated to dryness in vacuo and was used in the next step without further purification.

I-(9H-fluoren-9-yl)methyl 2-((2-tert-butoxy-2-oxoethoxy)methyl)pyrrolidine-1-carboxylate 9 (Figure 20G). Compound 8 (0.5 gm, 1.8 mmol) was dissolved in 20 mL DCM and DIEA (1.0 mL, 5.4 mmol) was added to it. The reaction mixture was cooled to 0°C and FmocOSu (0.6 gm, 1.8 mmol) was added to it. The reaction contents were allowed to warm to room temperature and stirred for two hours. The reaction mixture was concentrated in vacuo and residue was partitioned between DCM (20 mL) and H_2O (15 mL). The organic layer was dried, filtered, concentrated and chromatographed using EtOAc/Hexane (4:1) as eluent to give compound **9** (0.55 gm) in 79%yield. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.74 (d, J = 8 Hz , 2H), 7.58 (d, J = 8 Hz 2H), 7.37 (t, J = 8 Hz, 2H), 7.29 (t, J = 8 Hz, 2H), 4.54 (d, J = 4 Hz, 1H), 4.36 (m, 1H), 4.22 (m, 1H), 3.96 (s, 1H), 3.74-3.55 (m, 3H), 3.44-3.33(m, 2H), 3.24-3.10 (m, 1H), 2.10-1.75 (m, 4H), 1.46 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 169.5 (1C), 154.9 (1C), 144.1 (2C), 141.3 (2C), 127.6 (2C), 127.0 (2C), 125.0 (2C), 119.9 (2C), 81.4 (1C), 71.6 (1C), 68.9 (1C), 66.7 (1C), 56.7 (1C), 47.4 (1C), 46.8 (1C), 28.3 (1C), 28.1 (3C), 23.4 (1C). HRMS *m/z* (ESI): calcd. for $C_{26}H_{32}NO_5$ $[M+H]^+$ 438.2275, found 438.2218.

I-2-((1-(((9H-fluoren-9-yl)methoxy)carbonyl)pyrrolidin-2-yl)methoxy)acetic acid 10 (Figure 20H). Compound **9** (0.6 gm, 1.3 mmol) was dissolved in 10 mL DCM and 10 mL trifluoroacetic acid was added to it. The reaction contents were stirred until the starting material was completely consumed to give compound **10** in quantitative yield. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 10.390 (s, 1H), 7.74 (d, J = 8 Hz , 2H), 7.57 (d, J = 8 Hz 2H), 7.38 (t, J = 8 Hz, 2H), 7.30 (t, J = 8 Hz, 2H), 4.64 (d, J = 16 Hz, 1H), 4.41 (m, 1H), 4.24-4.01 (m, 3H), 3.80 (s, 1H), 3.60 (m, 1H), 3.36 (d, J = 28 Hz, 2H), 3.05(d, J = 44 Hz, 1H), 1.95-1.75 (m, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 174.5 (1C), 155.5 (1C), 143.8 (2C), 141.3 (2C), 127.7

(2C), 127.1 (2C), 124.8 (2C), 119.9 (2C), 71.8 (1C), 68.0 (1C), 67.2 (1C), 56.6 (1C), 47.2 (1C), 46.8 (1C), 28.1 (1C), 23.3 (1C). HRMS *m/z* (ESI): calcd. for $C_{22}H_{24}NO_5$ $[M+H]^+$ 382.1649, found 382.1672.

Following are examples which illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

EXAMPLE 1—Integrin interaction inhibitor Activity in Normal Hematopoietic Progenitor Cells and *In Vivo* Activity.

A colony forming assay was used to compare integrin interaction inhibitors induced cell death in normal hematopoietic cells and MM cells. $CD34^+$ hematopoietic progenitor cells were isolated from peripheral blood and treated for 2 hrs with integrin interaction inhibitors (12.5 and 50 μg/ml) and then plated in a methylcellulose media supplemented with growth factors supporting myeloid and erythroid colonies. Colonies were counted on day 12 post-plating. As shown in Figure 3A, integrin interaction inhibitors did not inhibit colony formation of normal $CD34^+$ cells. In addition, we evaluated the toxicity of integrin interaction inhibitors in normal peripheral blood mononuclear cells (PBMC). As shown in Figure 3B, 6 hours treatment with increasing concentration of integrin interaction inhibitors did not induce cell death up to doses of 50 μg/ml in PBMC. Finally as shown in Figure 3C, and consistent with other endpoints of cell death, integrin interaction inhibitors did inhibit colony formation of H929 cells at doses ranging from 12.5-50 μg/ml integrin interaction inhibitors. The data indicate that integrin interaction inhibitors targets MM cells preferentially when compared to normal hematopoietic cells. Further, recent reports using the SCID-Hu multiple myeloma *in vivo* model show that integrin interaction inhibitor-treated mice demonstrated significant reduction in tumor burden compared to control mice ($P<0.05$, repeated measures test).

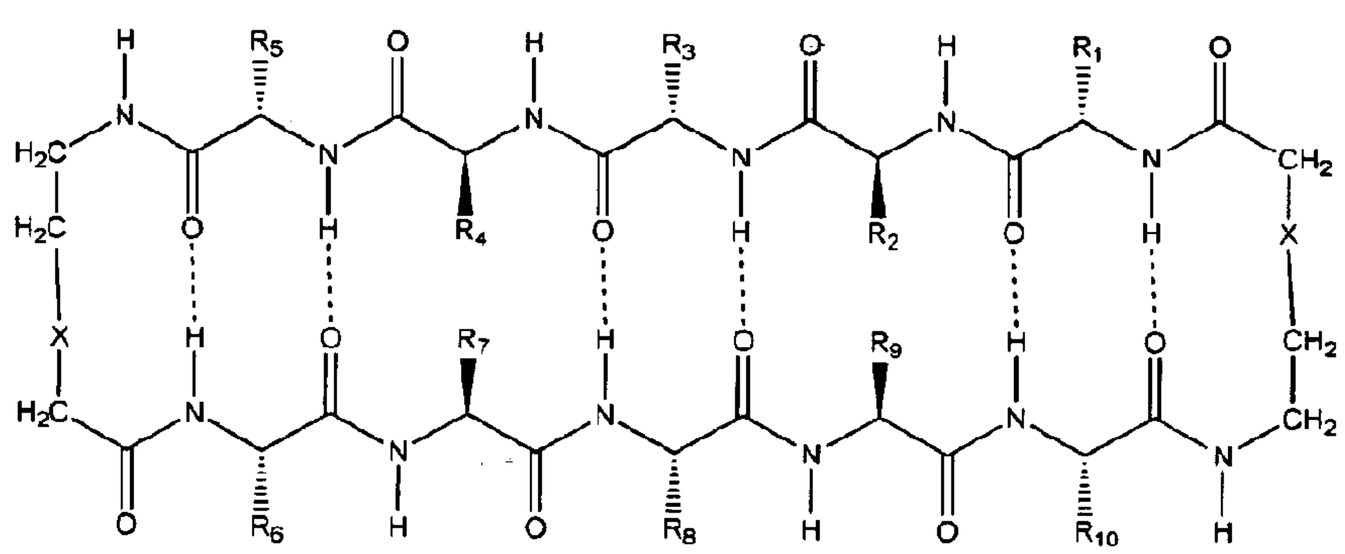
Recent studies demonstrated that integrin interaction inhibitor-induced cell death was necrotic in nature as shown by: (a) decrease in mitochondrial membrane potential ($\Delta\psi_m$); (b) loss of total cellular ATP, and; (c) increase in reactive oxygen species (ROS) production.

Moreover, integrin interaction inhibitor treatment does not result in apoptotic cell death as it did not trigger the activation of caspases or the release of apoptosis-inducing factor (AIF) and Endonuclease G (Endo G) from the mitochondria, nor did it induce double stranded DNA breaks. Integrin interaction inhibitors did initiate autophagy in cells; however, autophagy was found to be an adaptive response contributing to cell survival rather than the cause of cell death. It was further shows that *N*-acetyl-L-cysteine (NAC), a thiol containing free radical scavenger, partially protects MM cells from integrin interaction inhibitor-induced death.

As shown in Figure 4, integrin interaction inhibitors induce cell death in primary myeloma patient specimens. Furthermore, integrin interaction inhibitors showed increased potency in the 138 myeloma population compared to the CD138 negative presumably normal hematopoietic cells obtained from the bone marrow aspirate.

N- and C-terminus truncation studies have been performed, which identified MVISW as the likely core region of the integrin interaction inhibitor required for biological activity. Using this information and the finding that V for I replacement gave a more active integrin interaction inhibitor analog, a cyclized version of the integrin interaction inhibitor was developed that is designed to display the core sequence (MVVSW) in an extended or beta-sheet-like conformation. Side chain-side chain or N- to C-terminus cyclization of linear peptides to constrain the number of conformations available to the linear peptide is a well-known strategy that increases the affinity of the cyclized peptide for its target when the constraint stabilizes the bound conformation of the peptide, but the cyclic beta-hairpin further constrains the recognition portion of the cyclic peptide specifically into a extended or beta-sheet-like conformation.

Table 4: Structure activity relationship in H929 myeloma cells of cyclic integrin interaction inhibitor derivatives.



peptide	R1	R2	R3	R4	R5	T1	R6	R7	R8	R9	R10	T2	IC50

INTEGRIN INTERACTION INHIBITORS													31.8 μM
L-cINTEGRIN INTERACTION INHIBITORS	K	L	K	L	K	X	W	S	V	V	M	X	13.9 μM
L-cINTEGRIN INTERACTION INHIBITORSa6	K	L	K	L	K	X	A	S	V	V	M	X	55.0 μM
L-cINTEGRIN INTERACTION INHIBITORSa7	K	L	K	L	K	X	W	A	V	V	M	X	4.0 μM
L-cINTEGRIN INTERACTION INHIBITORSa8	K	L	K	L	K	X	W	S	A	V	M	X	11.0 μM
L-cINTEGRIN INTERACTION INHIBITORSa9	K	L	K	L	K	X	W	S	V	A	M	X	5.6 μM
L-cINTEGRIN INTERACTION INHIBITORSa10	K	L	K	L	K	X	W	S	V	V	A	X	8.9 μM
L-cINTEGRIN INTERACTION INHIBITORSn10	K	L	K	L	K	X	W	S	V	V	NL	X	2.7 μM
rL-cINTEGRIN INTERACTION INHIBITORS	K	L	K	L	K	X	M	V	V	S	W	X	6.7 μM

T1, T2 = beta-turn 1 or 2; X = H_3CSO_2N; NL = nor-Leu

Table 4 legend: Structure activity relationship in H929 myeloma cells of cyclic INTEGRIN INTERACTION INHIBITORS derivatives. The minimal bioactive core sequence of INTEGRIN INTERACTION INHIBITORS WSVVM was cyclized using 2 novel reduced amide bond beta turn promoters and KLKLK as the non-recognition sequence. H929 cells were treated with varying concentrations of the indicated peptide for 24 h. Cell viability was determined using Topro-3 staining and FACS analysis after drug treatment. IC_{50} values were generated from linear regressions generated from the dose response curves (n = mean of 3 independent experiments). The cyclized analog L-cINTEGRIN INTERACTION INHIBITORS is 2.5-fold more potent than the linear peptide. The alanine scan showed that residues 6, 8, 10 (L-cINTEGRIN INTERACTION INHIBITORSa6, L-cINTEGRIN

INTERACTION INHIBITORSa8, L-cINTEGRIN INTERACTION INHIBITORSa10) decrease in activity and residues 7 and 9 (L-cINTEGRIN INTERACTION INHIBITORSa7, L-cINTEGRIN INTERACTION INHIBITORSa9, L-cINTEGRIN INTERACTION INHIBITORSn10) increase in activity and the retro-inverso peptide (rL-cINTEGRIN INTERACTION INHIBITORS) increases in activity relative to the inverso peptide (L-cINTEGRIN INTERACTION INHIBITORS). Also the nor-leucine replacement of methionine (L-cINTEGRIN INTERACTION INHIBITORSn10) gave the most potent peptide to date.

EXAMPLE 2—Reducing α4 integrin expression confers resistance to integrin interaction inhibitor-induced cell death

Currently, 11 α binding partners for β1 integrin have been identified. An integrin interaction inhibitor -resistant cell line was recently developed by chronically exposing H929 parental MM cells to increasing concentrations of integrin interaction inhibitors. The resistant phenotype correlated with reduced α4 integrin expression and ablated α4 mediated adhesion to the extracellular matrix fibronectin and VCAM1 (data not shown). The cell line was initially tested to determine whether α4 expression is required for integrin interaction inhibitor-mediated cell death. As shown in Figures 3A-3B, reducing α4 levels in H929 cells using shRNA partially blocked integrin interaction inhibitor-induced cell death. The fact that reducing α4 levels did not abrogate integrin interaction inhibitor-induced cell death suggests that additional αXβ1 heterodimers may also contribute to cell death.

EXAMPLE 3—Peptide Design

Using combinatorial peptide libraries and a functional binding assay, several peptides have been identified that inhibited α2β1 and α6β1 integrin mediated adhesion of prostate cancer cells to fibronectin, laminin and collagen IV.[1] They identified an all D-amino acid containing peptide referred as HYD1 (KIKMVISWKG) that blocks binding of epithelial prostate carcinoma cells to extracellular matrix components.[2,3] Hazlehurst and co-workers have truncated the N- and C-termini and alanine scan studies identified MVISW as the likely core region of linear D-HYD1 required for biological activity. Using this information and the finding that Val for Ile replacement gave a more active D-HYD1 analog, we had developed a cyclized version of D-HYD1 that was designed to display the core sequence (MVVSW) in the recognition strand and (KLKLK) as the non-recognition strand. The pentapeptide (KLKLK) was selected as the non-recognition strand to improve the solubility. N- to C-

terminus cyclization of linear peptides was done to restrict the number of conformations available to the linear peptide which increases the affinity of the cyclized peptide for its target when the constraint stabilizes the bound conformation of the peptide. We synthesized cyclic D-HYD1 using a novel methylsulfonamide aminoethyl glycine linker T_1 that connects two anti-parallel strands. As per the cell inhibition assay TOPRO 3, the cyclic D-HYD1 peptide (IC_{50} = 30μM) was found to be twice as active as linear D-HYD1 against H929 cells. The inverso- (peptide **2**, Table 4) and partially modified retro-inverso (PMRI) (peptide **10**) pentapeptide sequences of the recognition strand of cyclic III (Integrin Interaction Inhibitors also known as cyclic L-HYD1) peptide analogs were subsequently investigated for their potential to block beta integrin mediated cell adhesion. The retro-inverso design of biologically active peptides is a well-known strategy to design all D-amino acid peptides from potentially bioactive all L-peptide sequences with increased stability[4-7]. Our retro-inverso peptide analogs have a similar placement of side chain residues as observed for cyclic D-HYD1 and hence similar or greater bioactivity was anticipated for these retro-inverso analogs. It was found that partially modified retro-inverso analogs had better bioactivity than cyclic D-HYD1 analogs whereas cyclic III peptides were twice as active as cyclic D-HYD1 (Table 7).

EXAMPLE 4—Colony Forming Assay

A colony forming assay was used to compare induced cell death in H929 multiple myeloma cells. Cells were treated with integrin interaction inhibitors, shown in Figure 5 and Table 4, and cell death was measured following 24 hour peptide treatment by TOPRO-3 staining and FACS analysis.

An alanine scan of III showed that Tryptophan, Valine and Methionine are critical for the bioactivity of the peptide. Replacement of Methionine with Nor-leucine gave III with enhanced bioactivity. The retro-inverso analog of III has comparable bioactivity for inducing cell death.

Table 5. SAR studies of III in H929 multiple myeloma cells. Variations of the compound III, seen in Figure 5, are shown. Cell death was measured following 24 hour peptide treatment by TOPRO-3 staining and FACS analysis. Shown is the mean IC_{50} value and standard deviation of three independent experiments.

III	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	IC50 (μM)
1	K	L	K	L	K	W	S	V	V	M	15.5 +/-7.7

2	**K**	**L**	**K**	**L**	**K**	**A**	**S**	**V**	**V**	**M**	**57.1 +/- 22**
3	**K**	**L**	**K**	**L**	**K**	**W**	**A**	**V**	**V**	**M**	**4.1 +/-1.9**
4	**K**	**L**	**K**	**L**	**K**	**W**	**S**	**A**	**V**	**M**	**19.0 +/- 6.9**
5	**K**	**L**	**K**	**L**	**K**	**W**	**S**	**V**	**A**	**M**	**6.2 +/- 2.7**
6	**K**	**L**	**K**	**L**	**K**	**W**	**S**	**V**	**V**	**A**	**31.1 +/- 7.6**
7	**K**	**L**	**K**	**L**	**K**	**W**	**S**	**V**	**V**	*****	**2.6 +/- 1.3**
8	**K**	**L**	**K**	**L**	**K**	**M**	**V**	**V**	**S**	**W**	**5.9 +/- 3.4**
9	**K**	**L**	**K**	**L**	**K**	**W**	**Y**	**V**	**V**	*****	**2.9 +/- 1.3**
10	**K**	**L**	**K**	**L**	**K**	**W**	**S**	**V**	**V**	**W**	**5.9**

The structure of the integrin interaction inhibitors showed that integrin interaction inhibitors exhibited secondary β-sheet structure with minima around 200 nm and absorption maxima around 186 nm, as seen in Figure 6 and Table 5.

Table 6. NMR studies of Integrin Interaction Inhibitors

Residue	**H_α (ppm)**	**H_β (ppm)**	**H_γ (ppm)**	**H_δ (ppm)**	**H_ε (ppm)**
Tryptophan	4.692	3.128, 3.226			
Serine	4.599	3.837			
Valine	4.267	1.989	0.802, 0.880		
Valine	4.13	1.94	0.665, 0.875		
Methionine	4.482	2.107	2.434, 2.551		2.121
L-Proline	4.531	2.219, 2.111	2.077, 1.940	3.949, 3.705	
D-Proline	4.731	2.287, 2.155	2.004, 1.896	3.793, 3.539	
Lysine	4.541	1.789, 1.662	1.349, 1.242	1.564	2.864
Leucine	4.985	1.73	1.53	0.851, 0.777	
Lysine	4.291	1.794, 1.667	1.403, 1.261	1.564	2.947
Leucine	4.536	1.569	1.505	0.812, 0.811	
Lysine	4.379	1.784, 1.745	1.403	1.667	2.977
Aeg turn promoter	CH_2	3.162^{E}	3.348^{A}		
	CH_2-CH_2	3.563^{NH}	3.866^{SO}		

	SO_2Me	3.03			

To determine the chirality of the compounds, circular dichroism of different compound III isoforms was detected, as seen in Figure 7.

The integrin interaction inhibitors shown herein have better bioactivity than its parent linear peptides. NMR and circular dichroism studies are consistent with integrin interaction inhibitors adopting a secondary β-sheet structure.

Administration of different integrin interaction inhibitors with traditional chemotherapeutic agents that induce ROS was also found to interact synergistically with and additive with agents that activate the apoptotic pathway. As shown in Table 7, a strong synergism exists with doxorubicin, the HDAC inhibitor SAHA and arsenic trioxide. All three of these compounds are reported to increase ROS levels. Additionally, modest synergy was observed with the topoisomerase II inhibitor etopside. Doxorubicin showed the greatest level of synergy, seen in Table 7.

Table 7. Combination Index (CI) Summary for Combinations in Myeloma Cell Lines.

Treatment	Treatment regimen	U266	Treatment	Treatment regimen	U266
integrin interaction inhibitor & SAHA	Concurrent	0.497±0.155 (n=3) +++	INTEGRIN INTERACTION INHIBITORS&Vel	Concurrent	1.056±0.23 (n=4) ±
integrin interaction inhibitor & Etoposide	Concurrent	0.843±0.439 (n=3) ++	INTEGRIN INTERACTION INHIBITORS&Dox	Concurrent	0.365±0.28 (n=5) +++
integrin interaction inhibitor & Arsenic	Concurrent	0.620±0.328 (n=2) +++	INTEGRIN INTERACTION INHIBITORS&PLAM	Concurrent	5.102±6.365 (n=3) ----

Legend: Values indicate average calculated CI range for 3-5 replicate experiments (----, strong antagonism; ---, antagonism; --, moderate antagonism; -, slight antagonism; +/-, nearly additive; +, slight synergism; ++, moderate synergism; +++, synergism). Synergistic growth inhibition was noted when U226 cells were treated with integrin interaction inhibitor in

combination with SAHA, arsenic trioxide, doxorubicin, and etoposide. U226 cells were treated concurrently with varying concentrations of 2 drugs for 72 hours, and cell viability was measured by the CT-Blue assay (Promega). Data generated were used to calculate a Chou and Talalay Combination Index (CI).

Bioavailability studies and human bone marrow xenograft inhibition of tumor growth studies are conducted in SCID mice using integrin interaction inhibitors with the highest activity. Cyclized derivatives are tested to determine whether the increased specificity towards tumor compared to normal cells and determine whether derivatives induce caspase independent cell death. Testing is also performed to determine whether alpha 4 integrin expression is required for cell death.

EXAMPLE 5—Structure-Activity Relationship (SAR) studies for cyclic III peptides

In an effort to optimize the bioactivity of cyclic III, it was essential to determine the key residues most critical to the bioactivity of the cyclic III peptides. The key residues in the cyclic III peptides that are responsible for the biological activity were identified by performing a sequential Alanine substitution analysis on the recognition strand of the inverso and retro-inverso peptide analogs.

As shown in Table 8, bioactivity data of inverso cyclic III peptide analogs (peptides **3-7**) revealed Tryptophan, Valine and Methionine in peptides **3, 5** and **7** respectively as key residues critical for the binding of cyclic III to integrins. Replacement of the Serine residue with the more hydrophobic Alanine in peptide **4** significantly improved the bioactivity of cyclic III analog. Furthermore, oxidation of Methionine side chain has been observed during peptide isolation for some cyclic III analogs. This problem was overcome by replacing the Methionine side chain with a structurally similar and chemically stable side chain such as Norleucine. Introduction of the hydrophobic Norleucine into the recognition strand of peptide **8** resulted in a further increase of the peptide's bioactivity. Hence, the pentapeptide sequence WAVVN* (N*= Norleucine) was established as the lead recognition strand of inverso cyclic III peptide analog. After the determination of critical residues responsible for the bioactivity of inverso cyclic III peptide, efforts were made to further enhance the bioactivity by making slight changes such as increasing the hydrophobicity or slightly decreasing the hydrophobicity in the recognition strand. Cress and co-workers have previously reported that another peptide RZ-3 (KMVIYWKAG) similar to HYD1 inhibited adhesion of prostate tumor cells to extracellular matrix (ECM) proteins or to human dermal

fibroblasts[8]. To further optimize the recognition strand for the enhancement of bioactivity of cyclic III peptide design, cyclic peptide **9** (N*VVYW) was synthesized with a design similar to the one found in the RZ-3 core sequence of the recognition strand. Peptide **9** had a similar bioactivity as the inverso cyclic III peptide **8**. (Figure 8)

Table 8. Structure-Activity relationship studies of cyclic III peptide analogs

Peptide	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	R_{10}	R_{11}	R_{12}	$IC_{50}(\mu M)$
1	K	L	K	L	K	M	V	V	S	W	T_1	T_2	15
2	K	L	K	L	K	M	V	V	S	W	T_1	T_1	15
3	K	L	K	L	K	M	V	V	S	A	T_1	T_1	57
4	K	L	K	L	K	M	V	V	A	W	T_1	T_1	4
5	K	L	K	L	K	M	V	A	S	W	T_1	T_1	19
6	K	L	K	L	K	M	A	V	S	W	T_1	T_1	6
7	K	L	K	L	K	A	V	V	S	W	T_1	T_1	31
8	K	L	K	L	K	N*	V	V	S	W	T_1	T_1	3
9	K	L	K	L	K	N*	V	V	Y	W	T_1	T_1	3
10	K	L	K	L	K	W	S	V	V	M	T_1	T_1	6
11	K	L	K	L	K	W	A	V	V	N*	T_1	T_1	12
12	K	L	K	L	K	W	A	V	V	A	T_1	T_1	69
13	K	L	K	L	K	W	A	V	A	N*	T_1	T_1	26
14	K	L	K	L	K	W	A	A	V	N*	T_1	T_1	41
15	K	L	K	L	K	A	A	V	V	N*	T_1	T_1	3
16	K	L	K	L	K	N*	V	V	A	W	T_3	T_1	1
17	K	L	Q	L	K	N*	V	V	A	W	T_3	T_1	13
18	Q	L	K	L	K	N*	V	V	A	W	T_3	T_1	ND
19	K	L	K	L	Q	N*	V	V	A	W	T_3	T_1	19
20	K	Q	K	L	K	N*	V	V	A	W	T_3	T_1	18
21	K	L	K	Q	K	N*	V	V	A	W	T_3	T_1	25
22	K	L	K	L	K	W	A	V	V	N*	T_3	T_3	17
23	K	X	K	X	K	W	A	V	V	N*	T_3	T_3	> 150
24	K	L	K	L	K	N*	V	L	A	W	T_3	T_1	10
25	K	L	K	L	K	N*	V	I	A	W	T_3	T_1	10
26	K	L	K	L	K	N*	V	F	A	W	T_3	T_1	9

T_1, T_2, T_3 = Linker 1, 2or 3; T_1= $NH_2CH_2CH_2N(SO_2Me)CH_2COOH$; T_2= LPro-DPro N*= Norleu X=Sarcosine

T_3= $N(CH_2)_3CHCH_2OCH_2COOH$

After determining that the replacement of the Serine and Methionine residues with the Alanine and Norleucine residues respectively yielded inverso cyclic III peptides with improved bioactivity, we attempted to study the structure activity relationship for the retro-inverso cyclic III analog (peptides **12-15**). A sequential Alanine scan was carried out with (WAVVN*) as the core sequence in the recognition strand. It was found that replacement of Norleucine and Valine in peptides **12** & **13** respectively were critical for the bioactivity of the retro-inverso peptides. There was an unexpected improvement in bioactivity for peptide **15** where Tryptophan was substituted for Alanine.

Inverso cyclic peptide design was further improved by bringing additional restraint into the cyclic peptide by introduction of a constrained turn promoter (T_3) at one turn and the methylsulfonamido aminoethyl glycine linker (T_1) as the other turn. The introduction of an ether-peptidomimetic amino acid (proline or 2-piperidine carboxylic acid derivative) as a constrained turn promoter should further reduce the degrees of freedom available to the cyclic peptide and possibly increase its affinity for binding to the target. Conformational search and energy minimization studies suggested that the introduction of the five membered ring D-Proline derivatized ether-peptidomimetic was favorable in stabilizing and sustaining the intramolecular hydrogen-bonding within the cyclic III analog. Based on this information, the inventors synthesized cyclic III peptide analog **16** with bioactivity of 1μM. This peptide provided the best lead scaffold which was further optimized for an improvement in binding affinity towards H929 multiple myeloma cells.

The non-recognition strand of cyclic III peptide was also optimized to determine if it has any effect on the bioactivity. A side chain anchoring strategy was explored for easy preparation of this series of cyclic peptides. Various research groups have applied this solid phase strategy to synthesize monomers[9-17]. This strategy involves side chain anchoring of trifunctional amino acids such as Lysine, Glutamic Acid, Glutamine, Aspartic and Asparagine for peptide elongation and on resin peptide cyclization. A sequential Glutamine substitution analysis on the non-recognition strand of the inverso cyclic III analogs (peptides **17-21**) revealed that these residues did not significantly alter the binding of the peptide to its target. A similar bioactivity observed for the cyclic D-HYD1 and the cyclic III peptide **1** suggests extensive peptide backbone interactions are absent or minimal since these two analogs have opposite backbone sequences. This hypothesis was tested by replacing the amino acid residues that have exo amide hydrogens with N-methylated amino acid residues.

The inventors first replaced all of the Leucine residues in the non-recognition strand with Sarcosine (peptide **23**). The inventors anticipated that N-methylation of the exo amides will not significantly change the beta-hairpin conformation but it should stabilize the cyclic beta-hairpin and eliminate possible peptide aggregation due to beta-sheet like dimerization or oligomerization of one or more cyclic III analogs. The poor bioactivity of peptide **23** led us to believe that the introduction of too many constraints in the molecule might have caused disruption of the internal hydrogen bonding which stabilizes the cyclic peptide. Efforts have been made to further enhance the bioactivity by varying hydrophobicity in the recognition strand of the lead peptide by incorporating hydrophobic residues at position 9 (peptide **24-26**).

EXAMPLE 6— Synthesis of linkers and cyclic III peptides

Cyclic peptides **1-15** were synthesized on 2-chlorotrityl chloride resin as solid support and Fmoc solid phase peptide synthesis strategy was used as shown in Scheme 1 (Figure 9). The linear peptides were synthesized and selectively cleaved from the resin without cleaving the side chain Boc-groups using trifluoroethanol as the cleaving agent. The linear peptide was then cyclized in solution under dilute conditions to afford crude cyclized peptide in modest yields. In order to synthesize a series of cyclic III peptide analogs with better yields, an efficient protocol for peptide cyclization on resin was explored. An alternative peptide cyclization procedure was developed using a side-chain attachment strategy for preparing peptides **16-26**. As shown in Scheme 2 (Figure 10), the N^{ε}-amino group of the Lysine was attached to carbonate Wang resin. The α-carboxyl group was protected with an orthogonal allyl protecting group. After synthesis of the protected linear peptide using our Fmoc-based strategy, the C-terminus α-carboxyl group and Fmoc group from N-terminus were deprotected. The linear peptide was then cyclized on resin and subsequently released from the resin using TFA. For the Glutamine scan of peptides **17-22**, we anchored the γ-side chain carboxyl group of Glutamic Acid to Rink amide resin. The on resin cyclization strategy of synthesizing cyclic peptides enabled us to synthesize and screen a moderate library of cyclic peptides very efficiently and in excellent yields.

Scheme 3 describes the synthesis of the methylsulfonamide aminoethyl glycine linker T_1 (Figure 11). Selective mono-alkylation of excess ethylene diamine with tert-butyl bromoacetate was carried out under dilute conditions to give compound **2** in 85% yield[18]. Compound **2** was used in the next step without further purification and selective Fmoc protection of the primary amine was achieved to give crude Fmoc-protected aminoethyl

glycinate **3**. The crude reaction was then washed with dilute hydrochloric acid and stored overnight in the deep freezer which resulted in the precipitation of pure compound **3** as the hydrochloride salt that can be stored for several months in the refrigerator without decomposing. Mesylation of the secondary amine with methanesulfonyl chloride afforded compound **4** that precipitates from an ethyl acetate solution under cold conditions. Deprotection of the t-butyl group was achieved by employing 4M HCl in dioxane to give the desired compound **5** in excellent yield. The ether peptidomimetic amino acid linker T_3 was prepared from commercially available Boc-D-Prolinol (Scheme 4, Figure 12). First, O-alkylation of **6** with tert-butyl bromoacetate afforded compound **7** in 77% yield[19,20]. Selective removal of the Boc protecting group in compound **7** using trifluoroacetic acid in DCM (1:4) gave compound **8**. Fmoc-group protection of the secondary amine in compound **8** with FmocOSu followed by acidic cleavage of the tert-butyl ester group gave the Proline derived ether-peptidomimetic **10** in 79% yield.

EXAMPLE 7—Structural Determination of Cyclic Peptides: Circular Dichroism Studies

Circular dichroism (CD) is a sensitive measure of the secondary structure of peptides and proteins. Various reports cited in the literature have shown that CD spectra taken from 260-190 nm is analyzed for different secondary structures of peptides and proteins i.e. α-helix, parallel and antiparallel β-sheet, β-turn, etc[21,22]. Peptides with a β-sheet structure usually exhibit an absorption minima around 210 nm and a relatively strong absorption maxima around 190 nm. As seen in Figure 13, CD spectra of peptide 1 shows an absorption minima around 215 nm, which suggests a more stable β-sheet conformation for this peptide whereas peptide **2** displays a negative band around 202-204 nm, which suggests that this peptide deviates from a stable β-sheet conformation and moves towards a more random structure. This can be attributed to the D-Pro-L-Pro turn in peptide **1** which is very structurally rigid thus forcing the peptide into a β-hairpin conformation. In opposite of this rigid turn, the methylsulfonamido linker in peptide **2** is more flexible, allowing the residues to be less structurally rigid and thereby deviate from the β-hairpin conformation. All cyclic III peptides **2-16** display similar CD bands: negative absorption minima around 202 nm and strong positive absorption maxima around 190 nm. Therefore, the inventors can conclude that all of these cyclic peptides have adopted a semi β-sheet structure.

EXAMPLE 8—Structural Determination of Cyclic Peptides: NMR studies for structural determination of cyclic peptides in solution

Complete peak assignments were only done for cyclic peptides **1, 5** and **16**. Assignments for both the recognition sequence and the non-recognition sequence, omitting the turns, were done for the remainder of the peptides. In an effort to de-clutter the 2D spectra sets, the NMR experiments were run in 100% D_2O to remove the exchangeable amide and Lysine ε-NH protons from the spectrum. Even without information from the amide and Lysine ε-NH protons, the results from the NMR experiments clearly show that the peptides have all adopted a β-hairpin structure.

Supporting the CD results, both the chemical shifts of the amino acid α-hydrogen protons (H_α) and the NOE data indicate that the peptides are in a β-hairpin conformation. The NMR results agree with previous empirical analysis which has shown that when β-sheets are formed, there is a downfield shift in the H_α resonances[23,24]. The majority of the amino acid H_α's in our peptides are shifted significantly downfield such that their values indicate a β-hairpin conformation (Table 9).

Table 9. α-Proton Chemical Shifts (ppm) of selective cyclic peptide analogs

	Position											
	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	R_{10}	R_{11}	R_{12}
1	4.38	4.54	4.29	4.99	4.54	4.48	4.13	4.27	4.60	4.69	3.56^S 3.86^R	L-Pro 4.53 D-Pro 4.74
2	4.26	4.35	4.08	4.28	4.57	4.64	4.66	4.32	4.46	4.70	----	----
5	4.31	4.16	4.07	4.04	4.42	4.60	4.58	4.53	4.60	4.61	3.89^S 4.06^R	3.47^S 4.05^R
7	4.44	4.31	4.08	4.28	4.52	4.42	4.63	4.18	4.70	4.67	----	----
8	4.29	4.29	4.09	4.38	4.59	4.45	4.67	4.64	4.45	4.60	----	----
10	4.17	4.53	4.05	4.37	4.35	4.83	4.51	4.52	4.10	4.45	----	----
16	4.55	4.78	4.32	4.63	4.17	4.34	4.44	4.55	4.70	5.05	4.232	3.94^S 4.08^R

R = Pro-R, S = Pro-S

H_α and NOE NMR analysis of peptide **1**, which contains both the Robinson β-hairpin turn promoter template (D-Pro-L-Pro)[25] and the methylsulfonamido aminoethyl glycine turn, confirms the structure of this peptide as a β-sheet. This peptide was then remade using the methylsulfonamido aminoethyl glycine in place of the Robinson template as the β-hairpin turn promoter to give peptide **2**. The resulting H_α chemical shifts for this peptide show it is also a β-sheet.

A comparison between the H_α shifts of these two peptides reveals many similarities. Most of the H_α's on the non-recognition side of peptide **2** have shifted upfield relative to **1**,

suggesting that the structure is less like a β-sheet. While three of these residues have only shifted about 0.2 ppm or less upfield, Leu4's shift of about 0.7 ppm suggests a fair amount of structural change. Interestingly, the only H_α on the non-recognition strand that has an upfield shift is Lys1. More importantly however, all but one of the H_α's on the recognition side of the peptide have shifted downfield indicating a β-sheet conformation. The H_α of Ser9 is the only one that has shifted upfield. Looking at the fact that the H_α's on residues Met6 and Lys5 have shifted downfield after the β-hairpin turn promoter was changed from the Robinson template to our methylsulfonamido aminoethyl glycine turn, it suggests that our turn promoter allows for more β-hairpin-like character at this end of the peptide. Thus, our turn may be a better β-hairpin promoter for certain peptide sequences.

Although most of the H_α shifts were small, about 0.2 ppm or less, there was a large shift in two of the H_α's which appears to be highly structurally significant. While the Leu4's H_α shifted upfield 0.704 ppm, Val7's H_α shifted downfield 0.532 ppm. This suggests that the Leucine is not adopting a predominantly β-sheet conformation. This is most likely due to steric interactions from the γ-protons of Val7 which is directly across from Leu4. Presumably as a direct result of Leu4's structural conformational change, Lys3's H_α is shifted 0.215 ppm (the second largest shift) upfield which removes a small amount of its β-sheet character and thus is further evidence supporting this claim.

An examination of the other peptides reveals a similar phenomenon. The Leu4 H_α is always shifted upfield and the residue in position 7 is always shifted downfield in comparison with peptide **1**. This can be explained by the fact that the D-Pro-L-Pro turn in the Robinson template is very structurally rigid, forcing those residues close to it into a β-hairpin conformation. However, our turn is much more flexible, thus allowing the residues close to the turn to be less rigid in their orientations. It is probably this flexibility that allows the Leu4 H_α to deviate from the β-sheet configuration. NOE analysis supports this view. In peptide **1**, strong NOEs were observed between the H_α's of Leu4 and Val7. However, in peptide **2** the NOE between the H_α's of Leu4 and Val7 was only of low intensity. In its place, there was a semi-strong NOE between the H_α of Leu4 and only 1 γ-CH_3 group of Val7. This significant reduction in the NOE cross-peak intensity between the Leu and Val H_α's combined with the appearance of a new strong cross-peak between the two Leu H_α's is strong evidence in support of the flexibility of our turn.

The inventors also investigated the use of our ether peptidomimetic amino acid linker (T_3) as a β-turn promoter. This promoter is similar to the Robinson template in the fact that they both contain Pyrrolidine rings however; T_3 has a higher degree of flexibility than the D-

Pro-L-Pro turn due to the fact that T_3 contains only 1 ring. An empirical analysis of the H_α's in peptide **16** shows more resonances adopting a β-sheet conformation than any other peptide. This shows that the rigid T_3 linker is quite capable of inducing a β-turn while retaining enough malleable character to allow all of the other residues enough conformational flexibility to adopt a β-sheet. This is opposed to the Robinson template which does not confirm the same flexibility thus fewer residues empirically display β-sheet characteristics.

Comparing the H_α's of peptides **1** and **16** reveals a number of striking similarities and further demonstrates linker T_3's ability to induce a β-turn. Focusing on the non-recognition strands, a comparison of the H_α of the Lysine residues closest to their respective turns (1 Lys5 vs. **16** Lys1) reveals almost no chemical shift difference; **16** Lys1 is shifted downfield by only 0.009 ppm. Moving further away from the respective turns, the chemical shift difference between the two Lys3 H_α's increases slightly to 0.029 ppm implying **16** has slightly more β-sheet character at this residue. Generally, as is the case for **1**, the H_α of the Lysine furthest from the turn is slightly downfield of Lys3's H_α. In peptide **16** however, Lys5's H_α is considerably upfield of Lys3. The difference in chemical shifts between the furthest Lysines (**1** Lys1 vs. **16** Lys5) is greater than 0.2 ppm. Additionally the H_α's of both Lys5 and Nle6 in **16** are significantly upfield of the same protons in peptide **8**. Combined, these facts show that this end of the turn, from Lys5 through the turn to Nle6, adopts slightly less of a β-sheet conformation in **16**. This is most likely caused by trying to relieve a conformational strain generated by either the methylsulfonamido aminoethyl glycine turn or by Lysine's side chain interactions with the other residues pulling the H_α's out of a β-sheet. It should be noted however that all of the protons in the T_1 linker of **16** have been significantly shifted downfield of those in the T_1 linker of **1**, further confirming the superiority of the T_3 linker over the Robinson template.

However, the Leucine residues in the non-recognition strand are in striking contrast from the Lysine residues. In **16** the H_α of Leu2, the one closest to the constrained ring turn, is very upfield (by about 0.205 ppm) from the corresponding H_α of Leu4 in **1** even though they shouldn't be that different. This is especially true given the fact that the chemical shifts of the Lysine H_α's right next to the rigid turns in each peptide are almost exactly identical. This is most likely due to the difference in rigidity between the two linkers as discussed above.

Moving on to the recognition strand, as is expected, there is a downfield shift of Trp's H_α in **16** vs. **1** which is clearly due to its proximity to the rigid β-turn. With regards to the Valine residues, we would expect them to follow a similar trend. Taking into consideration the fact that in peptide **1**, the Valine at residue 7 is very close (two residues away) to the

Proline-Proline turn, it stands to reason that it's H_α should be much more downfield than the same residue on peptide **16** which is much further away (four residues away, twice as far) from its constrained ring turn. However, the H_α's for both Val7 and Val8 in **16** are shifted significantly downfield (0.28 and 0.31 ppm respectively) from those in peptide 1. In a comparison of the H_α's of the Valines at residue 8 between the two peptides, we would expect their H_α chemical shifts to be fairly similar given their central (and identical) location on the recognition strand of the peptide. This however is clearly not the case which indicates that **16**'s recognition strand contains more β-sheet character at this middle point than peptide **1**'s does. Therefore, extrapolation of these findings clearly indicates that the entire recognition strand in peptide **16** has adopted a β-sheet conformation.

The replacement of the Robinson template by our turn T_1 slightly increases the distance between the two sides of the β-sheet. This is due to the fact that this turn isn't rigidly fixed into a certain conformation thereby allowing the chain to expand and contract, much like an accordion. It is this accordion-like action that allows the distance between the two sides of the β-sheet to change. This change in distance can be seen by the decrease in NOE intensity between the Leu and Val residues mentioned previously. NOEs also show the disappearance of the following; a strong NOE between the ε protons of Met6 and the H_α of Lys5, a strong NOE between the γ_1 protons of Val8 and the β and β′ protons of Lys3. Also, the intensity of the NOE interaction between the γ_2 protons of Val8 and the β proton of Lys3 dropped from being semi-strong to being very weak upon replacement of the Robinson turn.

The methylsulfonamido aminoethyl glycine turn itself can be broken down into two parts for discussions sake. The first part is the CH_2 side between the carbonyl and the N-Mesyl group, which we will refer to as the α-protons. The second part is the CH_2-CH_2 side between the N-Mesyl group and the amide NH, which will be referred to as the γ and δ-protons as shown in Figure 14A.

The flexibility of the turn is not just limited to adjusting the distance between the two sides. Rather, NOE analysis shows the existence of 2 distinct conformations. Newman projections of the two different conformations viewed down the δ-γ bond showing specific steric interactions can be seen in Figures 14A-C. The first conformation (Figure 14B) exists as an eclipsed conformer which puts it in the higher energy state of the two. However, the bulky N-Ms group points down and away from the β-sheet, eliminating all steric interactions and placing it in the lowest energy state. The second conformation (Figure 14C) exists as the staggered conformer, thus being the lower energy of the two. This time however, the bulky N-Ms group points directly into the center of the β-sheet causing large amounts of steric

interactions with the sheet's backbone forcing the N-Ms group into a very high energy state. Therefore, it can be assumed that the bulky N-Ms group drives the turn's preference for the (Figure 14B) conformation, picking the lower total energy conformer with the least amount of steric interactions.

Interestingly, conformer (Figure 14B) may also be slightly favored because the Pro-R γ-proton (labeled as H′) is eclipsed with the amide NH and the Pro-R δ-proton is eclipsed with the nitrogen from the N-Ms group. The proton attached to the amide NH is pointed out away from the backbone of the peptide while the lone pair on this nitrogen is pointed upwards towards the inside of the *β*-sheet. Additionally, the lone pair on the nitrogen from the N-Ms group points out towards the middle of the two δ-protons. There is the potential for a favorable attractive interaction between the lone pairs on these nitrogens and their respective eclipsed protons[26,27]. This would forgive some of the strain caused from being in the eclipsed conformation further reducing the total energy of the (Figure 14B) conformation making it the more favorable one.

Indeed, this favorable attractive interaction is supported by the chemical shifts of the respective protons. In peptide **5** for both turns, the Pro-R γ-protons are shifted downfield relative to the Pro-S γ-protons meaning a decrease in shielding as is expected with the interaction of the lone pair of electrons on the amide nitrogen.

For all but one of the peptides, the turn's geminal γ-protons are non-identical giving rise to a Pro-R proton and a Pro-S proton. Peptide **1** is the only one where these protons are identical. This is most likely caused by the Robinson turn locking the methylsulfonamido aminoethyl glycine turn into a more rigid conformation. Observations that support this analysis can be seen by the significant (0.08 ppm and greater) upfield shift of both the γ and δ resonances in **1** vs. those in **5**. The α protons of peptide **1** also experience large (0.18 ppm and greater) upfield shift in comparison to those in peptide **5**, with the exception of the Pro-S α proton in residue 12 of peptide **5**. Thus, this side of the peptide looks less like a *β*-sheet when the Robinson turn is used to induce the *β*-hairpin.

Because only one peak is seen for the γ-protons in peptide **1**'s turn, they must exist in somewhat similar environments and the turn's conformation must be different enough from the two mentioned above. Supported by NOEs, Figure 15 is a Newman projection viewed down the δ-γ bond of the T_1 linker and shows what this altered conformation might look like.

The final turn promoter, T_3, contains characteristics of both turns T_1 and T_2. In T_3, the CH_2 α to the carbonyl (herein referred to as α′) has a Pro-R and Pro-S proton much like the α-position in the T_1 linker. However, the chemical shifts of these two protons are opposite of

those in any of the T_1 linkers such that in T_3, the Pro-S proton is the more downfield one rather than being the more upfield one. As shown in Figure 16, this has to do with the two lone pairs on the ether oxygen which are pointing away from the center of the β-sheet and encompass the Pro-S proton causing its chemical shift to move downfield. Additionally, the difference here in chemical shifts between the Pro-R and Pro-S protons is only 0.044 ppm while the differences between the T_1 α protons are between 0.13 and 0.58 ppm. Therefore, this means that the Pro-R and Pro-S protons are in fairly similar chemical environments. This is clearly the case as the Newman projection shows because one of the lone pairs on the oxygen is also close to the Pro-R proton which would also account for the significant downfield shift of this proton as well.

EXAMPLE 8—Peptide Structural Characterization via NOE

In conjunction with the chemical shifts of the α-protons, the NOE data was used to help determine the 3D structure of peptides. Figures 17A-B and 18A-B show the NOEs found for peptides **2** and **5**, respectively. Analysis of peptide **5** was used as a general model for all the peptides. Cross-strand analysis reveals many NOEs between the Trp10 and Lys1 residues, specifically between Trp4H-LysεH, Trp5H-LysβH, Trp5H-LysεH, Trp6H-LysγH and TrpβH-LysεH to name a few. These suggest that the Tryptophan ring sits between the two strands at an angle with the indole ring facing the rest of the peptide. Additionally, peptide **2** also shows a NOE between TrpβH-LysβH, evidence that the ring spends part of its time in an alternate position between the two strands. Figures 17A and 17B show peptide **2** NOEs: (Figure 17A) Same-strand NOEs; (Figure 17B) Cross-strand NOEs. (Black = strong, Green = Medium, Red = Weak). Figures 18A and 18B show peptide **5**. NOEs: (Figure 18A) Same-strand NOEs; (Figure 18B) Cross-strand NOEs. (Black = strong, Green = Medium, Red = Weak)

Chemical shift analysis supports these two Trp positions, showing that the Tryptophan ring occupies one of the two positions depending on the adjacent residues. With the exception of the H_α's, all of the protons in Lys1 are downfield of their respective ones in either Lys3 or Lys5 even though Lys1 is cross-strand from Trp10 which would suggest an upfield shift due to an interaction with the face of the aromatic rings. The first, and main, position Trp occupies is one that deals with those peptides that contain a Valine at residue 8. Here, the aromatic ring of Trp10 and the hydrophobic γ-methyl groups of Val8 are oriented with each other such that one face of the Tryptophan ring is interacting with the Valine via intra-pair van der Waal's contacts while the other face is interacting slightly with the Lys1

protons via cation-π interaction causing the γ-methyl's to slightly shift upfield[28,29]. This is confirmed by the chemical shifts of the Valine in position 8 because the protons interacting with the Tryptophan ring shift a certain amount upfield relative to their proximity to the Tryptophan ring as is expected due to the increased shielding from the ring. However, peptide **7** does not follow this model. While the Val8 H_α does shift upfield, the β and γ-protons shift downfield which means the face of the Tryptophan ring is not interacting with that Valine to the same extent as the other peptides. This downfield shift is also seen in peptide **16**. In peptide **7**, all of Lys3's protons shift upfield with the exception of the H_α and new prominent NOEs can be seen between Lys3 and Trp10. This combined with the fact that there was very little shift, up or downfield, of Lys1's protons means the faces of the Tryptophan ring are now interacting with Lys 1 and Lys3 rather than with Lys1 and Val8. This is probably due to the replacement of the Methionine with an Alanine at position 6 thus reducing the van der Waal's interactions and eliminating the hydrogen bonding between positions 3 and 6.

The second position Trp occupies is found in peptide **5,** which lacks a Valine at residue 8. Here, the Tryptophan ring sits between the two strands partially over the turn and is at an angle with the indole ring facing the rest of the peptide. In this orientation, there is less interaction between the face of the Tryptophan ring and the protons of Lys1. Therefore, the Lys1 resonances are shifted slightly downfield. Regardless of the presence or absence of a Valine at residue 8, the Lys5 and Lys3 resonances are more upfield due to interactions with each other and the Methionine.

Interestingly, peptide **5** shows only a small NOE between the H_α's of Leu2 and Ser9, much like the Leu4-Val7 interaction mentioned above. There are, however, a few notable NOEs between Leu2 and Ser9 which include SerβH-LeuδH, SerβH-LeuβH and SerβH-Leuβ'H. These imply that the Leucine is oriented such that the β-protons point into the β-sheet while the δ-methyl's are pointing down and away from the β-sheet. It is also important to note the chemical shift of the Serine H_α. There is no difference in the Serine H_α chemical shift between peptides **1** and **5**. This is due to the fact that in 1, the Robinson turn helps to keep everything in a tight β-sheet and so the Serine H_α is most likely artificially changed due to the Tryptophan ring current effects while in **5**, the Tryptophan ring is over the turn. However, in peptides **2** and **8**, the Tryptophan ring is above the Serine H_α shielding it and causing an upfield shift. In peptide 7, the Serine H_α is significantly downfield suggesting that the Tryptophan ring isn't sitting above it; this is supported by the NOE data. NOE data for peptide **16** suggests that while the Tryptophan ring is over the β-sheet, it may be in a more

vertical position over Leu2 and Ala9 interacting with the Lys1 and Lys3 side chains. This possible ring orientation is supported by the downfield shift of Alanine's H_α.

In peptide **5**, the NOEs between the Ala8 and Lys3 residues are of significant intensity. The Alanine β-proton shows an NOE with the β, β′, γ and δ-protons of Lys3. Although the other peptides possess a Valine at position 8, they show the same NOEs with Lys3 and some even show NOEs to Lys1 and Lys5. These cross-strand and diagonal cross-strand NOEs imply that all of the Lysine's are oriented over the β-sheet itself and that when there is a Valine in position 8, it's γ-methyl groups are aligned with the β-sheet and point in opposite directions.

The Valine in position 7 has several cross-strand NOEs, while most are with Leu4 there is one with Leu2 which is quite intense. A few of those with Leu4 include Valγ_2H-LeuγH, Valγ_2H-LeuδH, Valγ_2H-LeuβH, ValβH-LeuαH and ValαH-LeuδH. The NOE with Leu2 is between Valγ_1H and LeuδH. Although the intensity of the NOE between the H_α's of Val7 and Leu4 is quite low, the strengths of the NOEs just mentioned provide compelling evidence that the structure of this part of the peptide is indeed a β-sheet.

NOEs from peptide **5**'s Met6 describe an interesting side-chain shape and a particular orientation with Lys5. Some of these include MetεH-LysαH, Metβ′H-Lysβ′H, Metβ′H-LysεH and MetαH-LysεH. Since these residues are attached to either side of the turn, their cross-strand NOEs are proof that the turn does in fact make a β-sheet rather than a random coil. Additionally, NOEs are also observed between Met6 and Lys3. Some of the most significant ones are MetεH-LysεH, MetγH-LysεH and MetβH-LysεH. These diagonal cross-strand NOEs help to reinforce the fact that these peptides exist as β-sheets despite the lack of a strong NOE between the H_α's of Leu4 and Val7. These NOEs suggest that the Methionine side chain is specifically interacting with these two Lysine side chains. In addition to the standard Van der Waals interactions, weak hydrogen bonding may exist between the Methionine sulfur and the Lysine ε-NH protons holding the chains closer in space thus giving rise to more and stronger NOEs between the chains[30]. This idea is supported by the fact that when Methionine is replaced by Norleucine in **8**, both the amount and the intensity of the cross-strand NOEs decrease significantly. Furthermore, both the ε and γ-methylenes of Lys5 experience a significant downfield shift upon the replacement with Norleucine. Replacing the Methionine residue with a more hydrophobic one removes the hydrogen bonding and causes a change in the side chain conformation, which is evident in peptide **8**.

EXAMPLE 9—Constrained Conformation Search with MacroModel

Structures of peptides **1** and **5** were built with Maestro[31]. NOE-derived distance constraints were applied to optimize the peptide structures with MacroModel[32] to produce 891 and 920 structures within a 100 kJ/mol range for peptides **1** and **5**, respectively. The conformations were reduced in number based on RMSD to 61 and 87 structures of which the 20 lowest energy representative structures shown in Figures 19A and 19B were analyzed. Both sets of calculated structures reveal a twisting of the β-hairpin structure. Further, peptide **1** has a more subtle twist close to its rigid D-Pro-L-Pro linker while the β-hairpin is more contorted near linker T_1. The Ramachandran plot places all phi/psi angles of peptide **5** in the β-sheet region while 2 amino acids, L-Pro and Val 7, of peptide **1** are in the disallowed region (see Figure 38). The averaged energies offer explanation to these structural differences: solvation and electrostatics contribute 80% of the difference in the average energies of the calculated structures (see Table S9). The flexibility of the linker T_1 in comparison to the rigid D-Pro-L-Pro linker allow for a more solvated conformation when in solution, an entropic gain (from less ordered waters) that translates into lower electrostatic and solvation energies. Further, peptide **5** has lower stretch, bend, and torsion energies than peptide **1** accounting for less than 20% of the average energy difference (supporting information, Table S9). Overall, the average energies from the calculated structures indicate more conformational flexibility of peptide **5** over peptide **1**.

Figures 19A and 19B show stereoviews of the 20 lowest energy structures for NOE-constrained calculated structure of peptides **1** (green carbon atoms) and **5** (gold carbon atoms), respectively. N = blue, O = red, S = yellow; hydrogens omitted for clarity. Thin wire representations prepared with Maestro.

EXAMPLE 10—Biotin-HYD1 interacts with CD44.

The inventors used biotin-HYD1 as bait to pulldown binding complexes contained within membrane extracts of H929 MM cells. The pull down assay was directly coupled with an unbiased Mass-Spec analysis to identify HYD1 binding partners. Before performing these studies, the inventors confirmed that biotinylation of HYD1 did not inhibit the bioactivity of the compound, as the IC_{50} value for biotin-HYD1 was slightly decreased in H929 cells. NeutrAvidin beads were used to reduce non-specific binding. The control sample consisted of incubating the membrane extract with biotin and subsequently subjecting the sample to NeutraAvidin beads similar to the biotin-HYD1 sample. In the 30 ug of membrane extract, the only cell surface protein that the inventors identified that was specific for the biotin-HYD1 sample was CD44. The binding experiment was repeated using 300 ug of membrane

extract. In the scaled up reaction, α4 integrin, β1 integrin, NCAM and syndecan-1 were indentified by Mass-Spec analysis. As shown in Figure 44, Western blot analysis was used to confirm that biotin-HYD1 and not biotin interacted with CD44 (antibody used is a pan CD44 antibody). The inventors next determined whether α4 integrin could be detected by western blot analysis. As shown in Figure 45A, the inventors were able to show that α4 was present in the complex; however, stripping the blot and reprobing the membrane revealed that the Biotin-HDY1 complex contains more CD44 compared to α4 integrin (see Figure 45B). To determine whether CD44 was indeed a direct binding partner of CD44 the inventors used recombinant CD44 and an ELISA as a readout of binding. The recombinant CD44 protein (purchased from Abnova) corresponds to Isoform 4 on Swiss Prot. The amino acid sequence is missing 224-266 and 223 is substituted S for T relative to the longest CD44 variant referred to as epican. As shown in Figure 46, the inventors were able to capture CD44 in biotin-HYD1 NeutraAvidin coated 96-well plates. Again, Biotin-coated NetraAvidin showed relatively no binding of CD44. Collectively, these data indicate that CD44 is the likely direct binding target of CD44. Both U226 and H929 cells which are relatively sensitive to HYD1 are reported to express the CD44s (standard form) and the variant forms CD44v3 and CD44v9.

The inventors will verify that biotin conjugated c-HYD1 preferentially binds CD44 in myeloma compared to normal hematopoietic cells. The inventors have synthesized biotin conjugated c-HYD1 derivatives using standard solid-phase peptide synthesis methods and will scale-up synthesis using the solution-phase peptide synthesis methods shown in Figure 43. The inventors will use this reagent to verify that c-HYD1 increases the affinity and specificity for CD44 in MM cell lines compared to normal peripheral blood mononuclear cells. The inventors anticipate that biotin-c-HYD1 will pull down CD44v in MM cell lines but not in membrane extracts obtained from normal mononuclear cells. The c-HYD1 peptide is very amenable to convergent solution-phase peptide synthesis methods. The beta-turn promoters in our most active c-HYD1 analog have achiral glycine-like carboxylic acid functional groups that cannot undergo racemization and are therefore excellent sites for peptide fragment coupling, which allows a convergent synthetic approach to making the c-HYD1 analogs. The scheme in Figure 43 shows our proposed approach.

Strand A1-A5 and A6-A10 can be the recognition and non-recognition sequences, respectively, or vice versa. The non-recognition sequence position A3 or A8 will have an orthogonal protecting group such as the alloc group which will allow easy derivatization with

biotin, FAM1, dimerization, or oligomerization. The inventors have determined that derivatization of that Lys group does not negatively effect bioactivity.

Table S1. Analytical Data for cyclic III peptides

Peptide	Sequence	SEQ ID NO:	Purity %	HPLC Retention Time (min)	MALDI-TOF Data (m/z)
1	KLKLKT$_2$MVVSWT$_1$	1	95	24.71	1585.962
2	KLKLKT$_1$MVVSWT$_1$	2	98	25.4	1569.488
3	KLKLKT$_1$MVVSAT$_1$	3	ND	ND	1455.166
4	KLKLKT$_1$MVVAWT$_1$	4	99	25.29	1544.208
5	KLKLKT$_1$MVASWT$_1$	5	99	30.45	1542.172
6	KLKLKT$_1$MAVSWT$_1$	6	ND	ND	1542.240
7	KLKLKT$_1$AVVSWT$_1$	7	93	23.64	1510.291
8	KLKLKT$_1$N*VVSWT$_1$	8	95	26.93	1552.286
9	KLKLKT$_1$N*VVYWT$_1$	9	99	26.52	1628.341
10	KLKLKT$_1$WSVVMT$_1$	10	93	23.21	1572.091
11	KLKLKT$_1$WAVVN*T$_1$	11	98	25.13	1536.172
12	KLKLKT$_1$WAVVAT$_1$	12	84	24.11	1494.242
13	KLKLKT$_1$WAVAN*T$_1$	13	97	25.93	1508.098
14	KLKLKT$_1$WAAVN*T$_1$	14	87	23.52	1508.200
15	KLKLKT$_1$AAVVN*T$_1$	15	91	25.96	1421.254
16	KLKLKT$_1$N*VVAWT$_3$	16	98	25.68	1499.181
17	KLKQKT$_1$N*VVAWT$_3$	17	97	34.62	1499.313
18	QLKLKT$_1$N*VVAWT$_3$	18	90	30.15	1499.100
19	KLKLQT$_1$N*VVAWT$_3$	19	97	29.74	1615.162
20	KQKLKT$_1$N*VVAWT$_3$	20	87	29.97	1514.242
21	KLKQKT$_1$N*VVAWT$_3$	21	90	29.99	1301.070
22	KLKLKT$_3$WAVVN*T$_3$	22	93	27.54	1462.073
23	KXKXKT$_3$WAVVN*T$_3$	23	86	19.69	1378.046
24	KLKLKT$_1$N*VLAWT$_3$	24	91	35.33	1513.344
25	KLKLKT$_1$N*VIAWT$_3$	25	93	34.98	1513.375
26	KLKLKT$_1$N*VFAWT$_3$	26	97	36.05	1547.507

Table S2. NMR assignments for peptide **1**

	α	β	γ	δ	ε
L-Pro-T$_2$	4.531	2.219, 2.111	2.077, 1.940	3.949, 3.705	
D-Pro-T$_2$	4.741	2.287, 2.155	2.004, 1.896	3.793, 3.539	
Lys-1	4.379	1.784, 1.745	1.403	1.667	2.977
Leu-2	4.536	1.569	1.505	0.812, 0.811	

Lys-3	4.291	1.794, 1.667	1.403, 1.261	1.564	2.947
Leu-4	4.985	1.730	1.530	0.851, 0.777	
Lys-5	4.541	1.789, 1.662	1.349, 1.242	1.564	2.864
Linker T_1	3.563^S, 3.866^R		3.348	3.162	SO_2Me = 3.030
Met-6	4.482	2.107	2.551, 2.434		2.121
Val-7	4.130	1.940	0.875, 0.665		
Val-8	4.267	1.989	0.880, 0.802		
Ser-9	4.599	3.837			
Trp-10	4.692	3.226, 3.128	7.121, 7.146, 7.229, 7.473, 7.561		(5H, 2H, 6H, 7H, 4H)

Table S3. NMR assignments for peptide **2**

	α	β	γ	δ	ε
Lys-1	4.262	1.823, 1.774	1.432, 1.402	1.642	2.976
Leu-2	4.349	1.554	1.427	0.782, 0.767	
Lys-3	4.076	1.759, 1.642	1.373, 1.285	1.588	2.874
Leu-4	4.281	1.945	1.622	0.923, 0.845	
Lys-5	4.574	1.765, 1.657	1.286	1.589	2.879
Met-6	4.643	2.155, 1.989	2.575, 2.522		2.072
Val-7	4.662	1.637	0.874, 0.801		
Val-8	4.320	1.911	0.816, 0.772		
Ser-9	4.462	3.778			
Trp-10	4.701	3.172	7.140, 7.121, 7.238, 7.473, 7.590		(5H, 2H, 6H, 7H, 4H)

Table S4. NMR assignments for peptide **5**

	α	β	γ	δ	ε

Linker T_1	3.891^S, 4.062^R		3.500^S, 3.612^R	3.236	SO_2Me = 3.045
Lys-1	4.311	1.823	1.452	1.691	2.996
Leu-2	4.160	1.525, 1.418	1.359	0.768, 0.645	
Lys-3	4.072	1.740, 1.613	1.281, 1.149	1.506	2.776
Leu-4	4.042	1.994	1.955	0.929, 0.904	
Lys-5	4.423	1.770, 1.657	1.305	1.589	2.879
Linker T_2	3.465^S, 4.047^R		3.490^S, 3.529^R	3.334	SO_2Me = 3.050
Met-6	4.599	2.234, 1.999	2.664, 2.532		2.077
Val-7	4.575	1.618	0.924, 0.797		
Ala-8	4.531	1.183			
Ser-9	4.599	3.822, 3.720			
Trp-10	4.609	3.206, 3.123	7.151, 7.160, 7.239, 7.473, 7.654		(5H, 2H, 6H, 7H, 4H)

Table S5. NMR assignments for peptide 7

	α	β	γ	δ	ε
Lys-1	4.438	1.823, 1.779	1.423	1.662	2.977
Leu-2	4.311	1.946	1.892	0.836, 0.763	
Lys-3	4.081	1.750, 1.638	1.339, 1.261	1.555	2.845
Leu-4	4.282	1.540	1.427	0.787, 0.768	
Lys-5	4.516	1.745, 1.647	1.291	1.564	2.869
Ala-6	4.419	1.383			
Val-7	4.629	1.623	0.865, 0.792		
Val-8	4.184	1.950	0.934, 0.860		
Ser-9	4.697	3.803, 3.754			
Trp-10	4.668	3.172, 3.153	7.146, 7.141, 7.244, 7.478, 7.615		(5H, 2H, 6H, 7H, 4H)

Table S6. NMR assignments for peptide 8

	α	β	γ	δ	ε
Lys-1	4.287	1.799, 1.691	1.437, 1.379	1.623	2.943
Leu-2	4.292	1.579	1.467	0.821, 0.807	
Lys-3	4.086	1.750, 1.682	1.369, 1.266	1.584	2.869
Leu-4	4.375	1.985	1.618	0.914, 0.836	
Lys-5	4.590	1.731, 1.638	1.388, 1.310	1.589	2.943
Nor-6	4.453	1.794	1.711	1.276	0.851
Val-7	4.673	2.038	0.895, 0.880		
Val-8	4.639	1.589	0.861, 0.811		
Ser-9	4.453	3.802			
Trp-10	4.595	3.246, 3.207	7.112, 7.141, 7.239, 7.483, 7.581		(2H, 5H, 6H, 7H, 4H)

Table S7. NMR assignments for peptide 10

	α	β	γ	δ	ε
Lys-1	4.174	1.770, 1.652	1.345	1.379	2.967
Leu-2	4.531	1.657	1.535	0.895, 0.851	
Lys-3	4.052	1.731, 1.608	1.354	1.535	2.943
Leu-4	4.365	1.633	1.525	0.905, 0.875	
Lys-5	4.345	1.819, 1.770	1.437, 1.393	1.687	2.977
Trp-6	4.834	3.436, 3.212	7.141, 7.234, 7.268, 7.493, 7.630		(5H, 6H, 2H, 7H, 4H)
Ser-7	4.512	3.774, 3.754			
Val-8	4.517	1.496	0.816, 0.782		
Val-9	4.096	2.009	0.939, 0.924		
Met-10	4.453	2.048, 1.941	2.562, 2.498		2.083

Table S8. NMR assignments for peptide 16

	α	β	γ	δ	ε
Prolinol-T_1	4.232	3.426	1.544	1.754	α-CH_2-O: 3.704
O-CH_2-CO	4.022^S, 3.978^R				

Lys-1	4.550	1.994, 1.935	1.525, 1.495	1.730	3.010
Leu-2	4.780	1.725, 1.642	1.427	0.889, 0.792	
Lys-3	4.320	1.852, 1.759	1.393, 1.344	1.627	2.918
Leu-4	4.634	1.701	1.564	0.831, 0.816	
Lys-5	4.169	1.828, 1.715	1.422, 1.383	1.661	2.971
Linker T_2	3.944^{S}, 4.076^{R}		3.538^{S}, 3.445^{R}	3.284	SO_2Me = 3.064
Nor-6	4.340	1.720	1.647	1.281	0.860
Val-7	4.442	2.106	0.938, 0.928		
Val-8	4.545	1.950	0.933, 0.875		
Ala-9	4.697	1.285			
Trp-10	5.054	3.309, 2.952	7.223, 7.175, 7.258, 7.497, 7.658		(5H, 6H, 2H, 7H, 4H)

Table S8. NOE-derived constraints and statistics of NMR structure calculations for peptides **1** and **5**.

	Peptide 1	**Peptide 5**
NOE upper-distance limits	40	40
Intraresidue	7	3
Sequential	12	6
Dihedral angle restraints (HN-C=O)	13	12
Mean RMSD values (Å)		
Backbone atoms	0.14±0.08	0.45±0.11
Heavy atoms	0.30±0.18	0.72±0.23

Table S9. Energies for calculated structures of peptides 1 & 5 (kJ/mol)

	Difference	$\%_{Diff.}$[a]	Peptide 1	Peptide 5
Force Field	**-355.91**	*56%*	**-851.684**	**-1207.6**
Stretch	*-8.63*	*1%*	*28.68*	*20.05*
Bend	*-45.21*	*7%*	*127.12*	*81.91*
Torsion	*-61.43*	*10%*	*166.57*	*105.14*
Improper Torsion	*2.08*	*0%*	*4.91*	*7.00*

VDW	*-11.62*	*2%*	*-112.20*	*-123.82*
Electrostatic	*-234.96*	*37%*	*-1066.77*	*-1301.73*
Solvation	**-274.66**	*43%*	**-1337.53**	**-1612.19**
Total	**-633.91**		**-2189.22**	**-2823.13**

[a]Percent difference based on average total energy difference.

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The disclosures of all publications cited above are expressly incorporated herein by reference, each in its entirety, to the same extent as if each were incorporated by reference individually.

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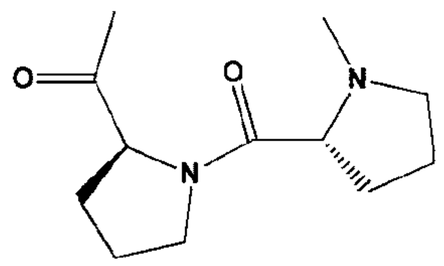
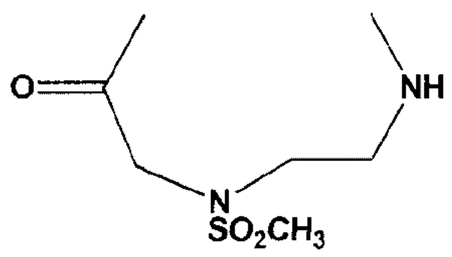
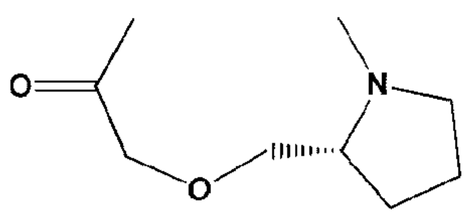
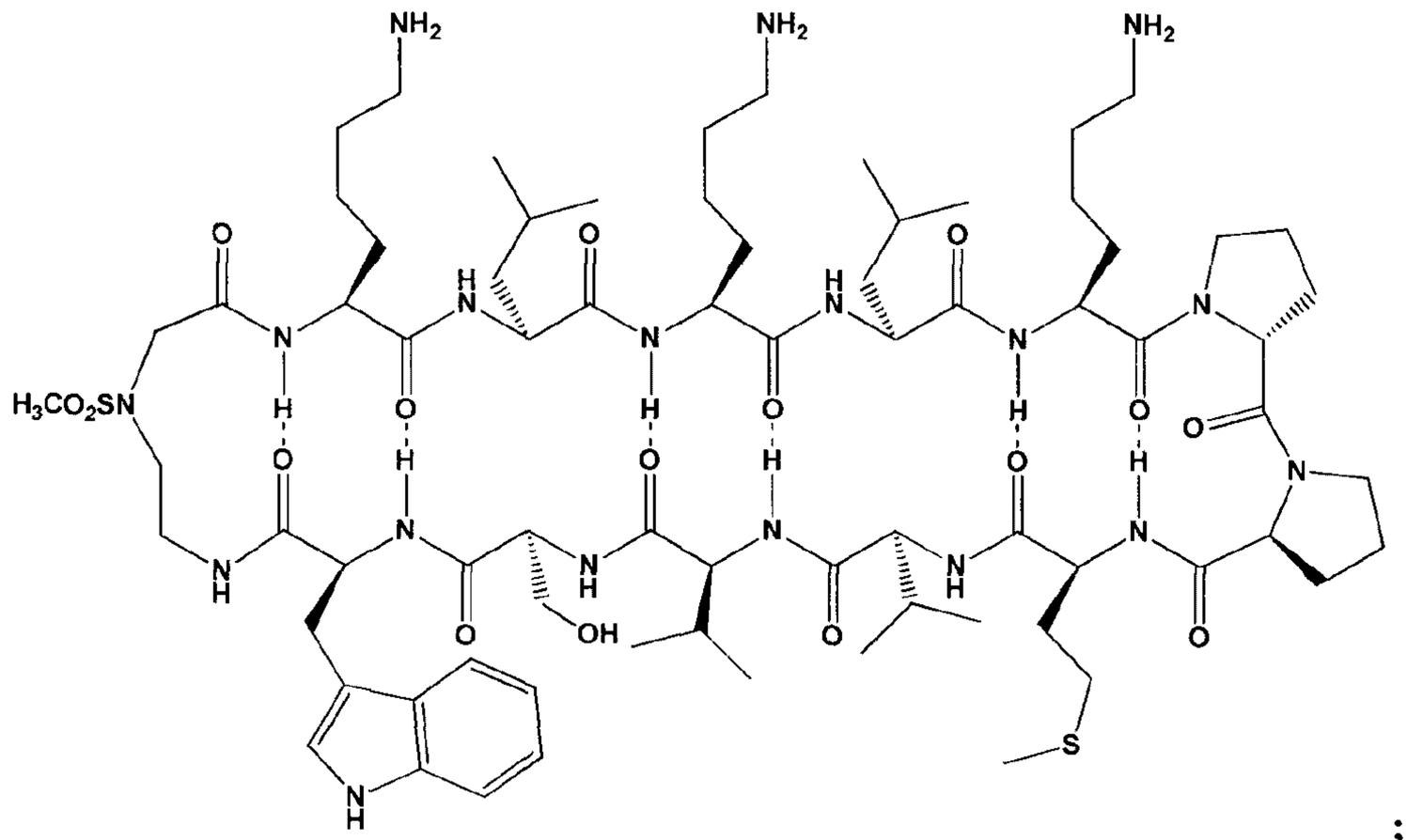
It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims. In addition, any elements or limitations of any invention or embodiment thereof disclosed herein can be combined with any and/or all other elements or limitations (individually or in any combination) or any other invention or embodiment thereof disclosed herein, and all such combinations are contemplated with the scope of the invention without limitation thereto.

THE EMBODIMENTS OF THE INVENTION FOR WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A cyclic compound comprising a recognition sequence and a non-recognition sequence, wherein said recognition sequence is joined to said non-recognition sequence by a first linker and a second linker, wherein said non-recognition sequence is five amino acids selected from KLKLK (SEQ ID NO:27), KLQLK (SEQ ID NO:28), QLKLK (SEQ ID NO:29), KLKLQ (SEQ ID NO:281), KQKLK (SEQ ID NO:30), KLKQK (SEQ ID NO:282), KXKXK (SEQ ID NO:31), or ELKLK (SEQ ID NO:32), where X = sarcosine and said recognition sequence is five amino acids selected from MVVSW (SEQ ID NO:33), MVVSA (SEQ ID NO:34), MVVAW (SEQ ID NO:35), MVASW (SEQ ID NO:36), MAVSW (SEQ ID NO:37), AVVSW (SEQ ID NO:38), N*VVSW (SEQ ID NO:39), N*VVYW (SEQ ID NO:40), N*VVAW (SEQ ID NO:41), AVVAW (SEQ ID NO:42), N*AVAW (SEQ ID NO:43), N*VAAW (SEQ ID NO:44), N*VLAW (SEQ ID NO:45), N*VIAW (SEQ ID NO:46), N*VFAW (SEQ ID NO:47), or WSVVW (SEQ ID NO:48), where N* = norleucine, and where said first linker and said second linker are independently selected from the structures:  (D-Pro-L-Pro);  (methylsulfonamido aminoethylglycine); or  ((pyrrolidin-2-ylmethoxy)acetate, wherein no more than one of said first linker and said second linker is D-Pro-L-Pro.

2. The cyclic compound of claim 1, wherein said cyclic compound has one of the following structures:



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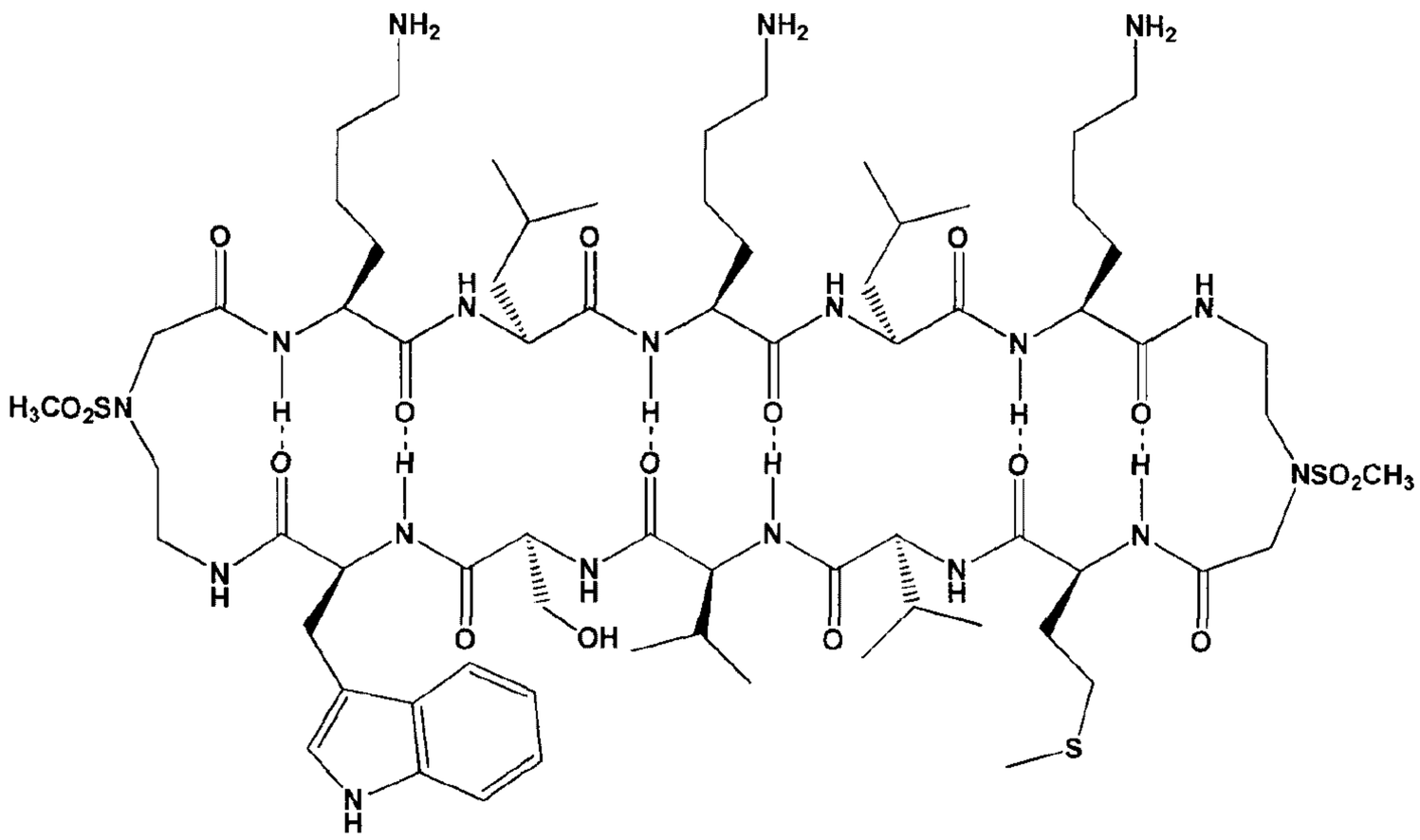

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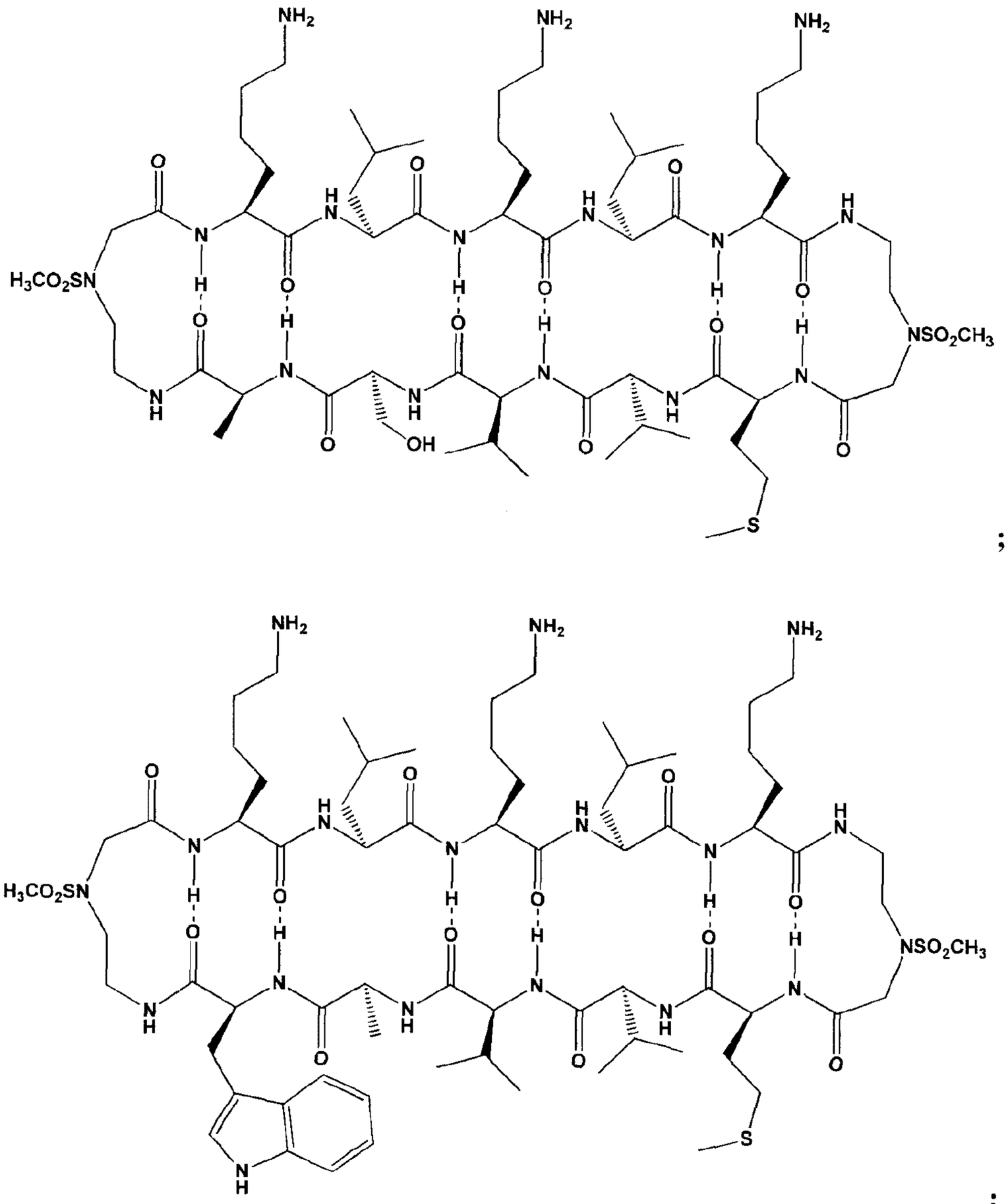

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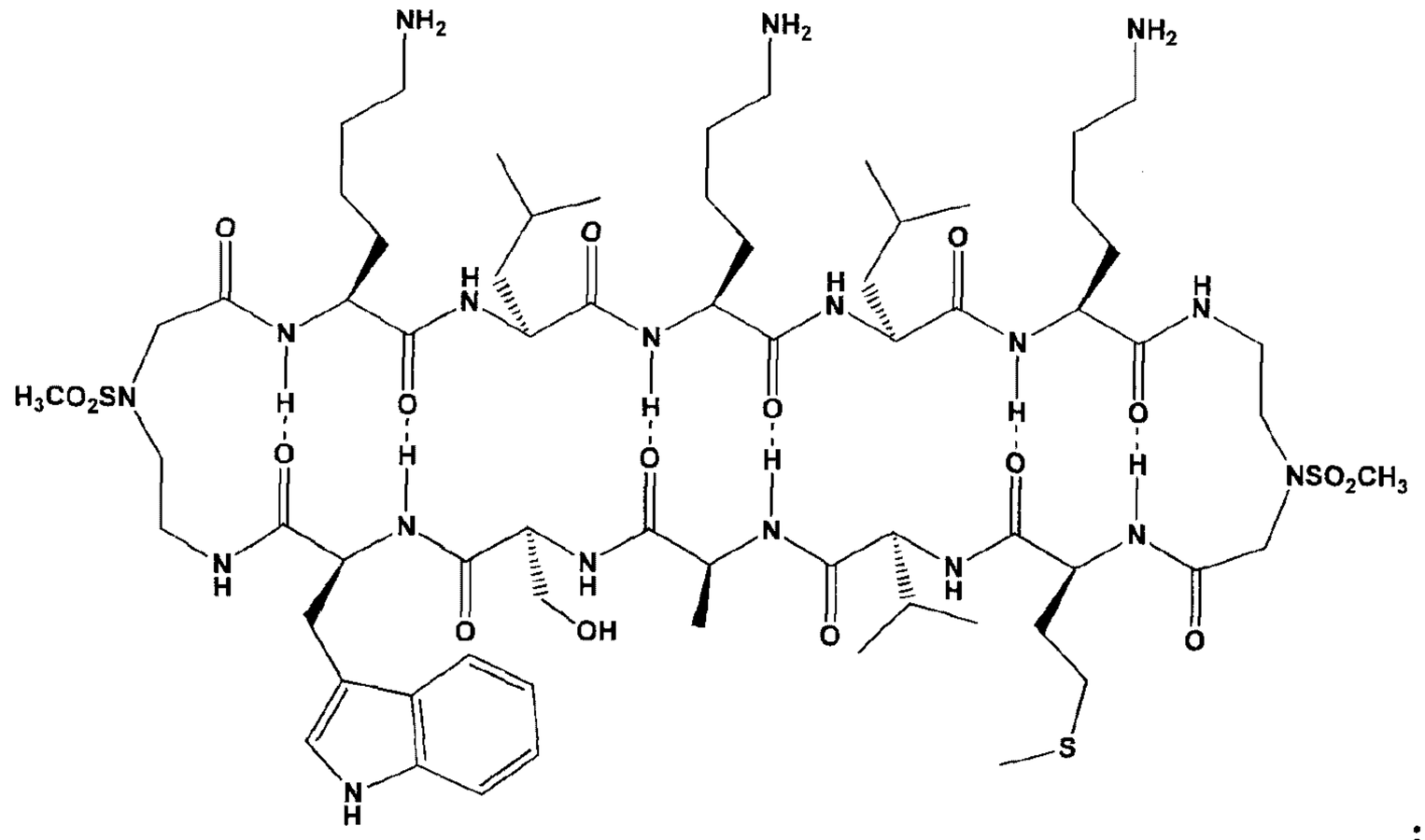

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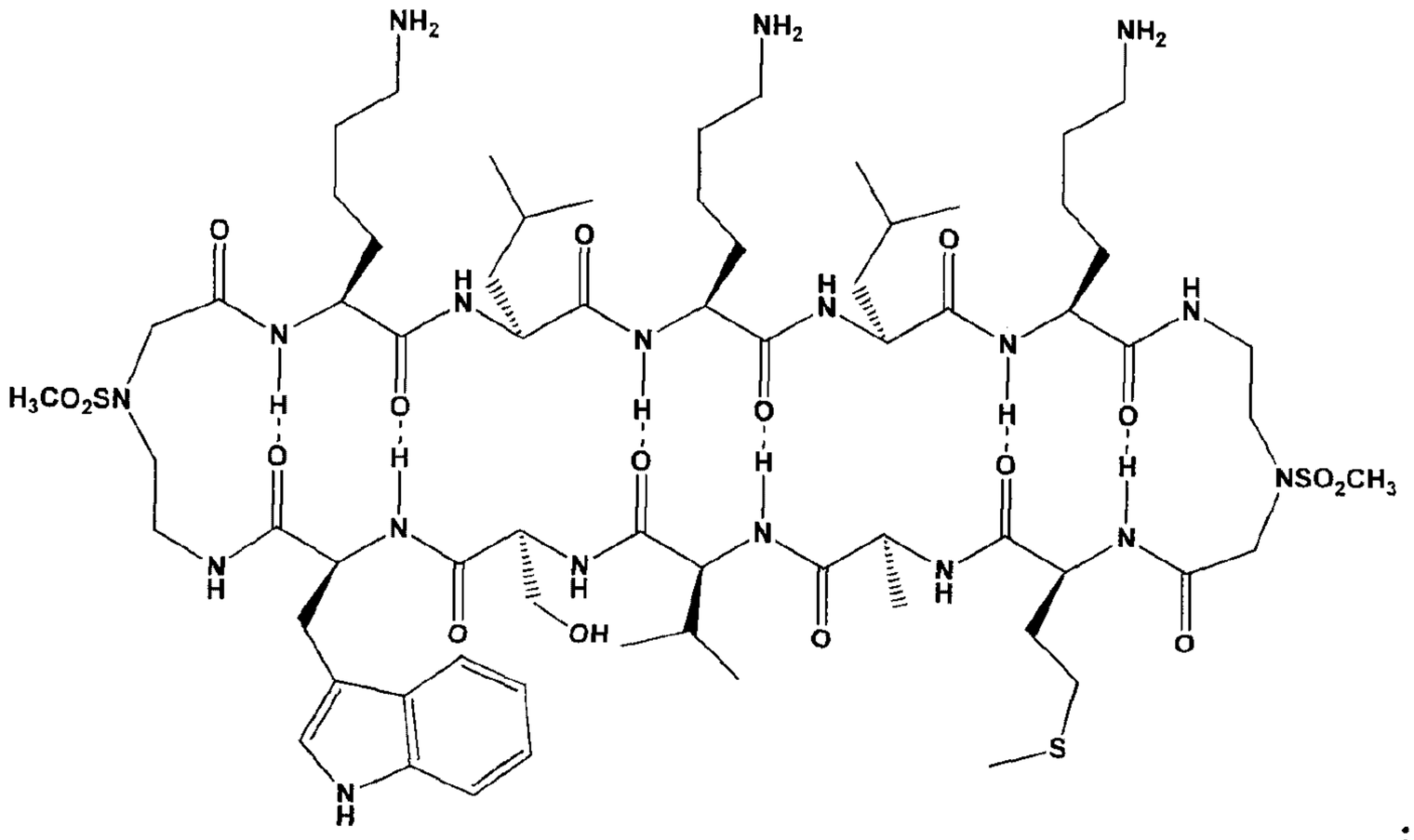

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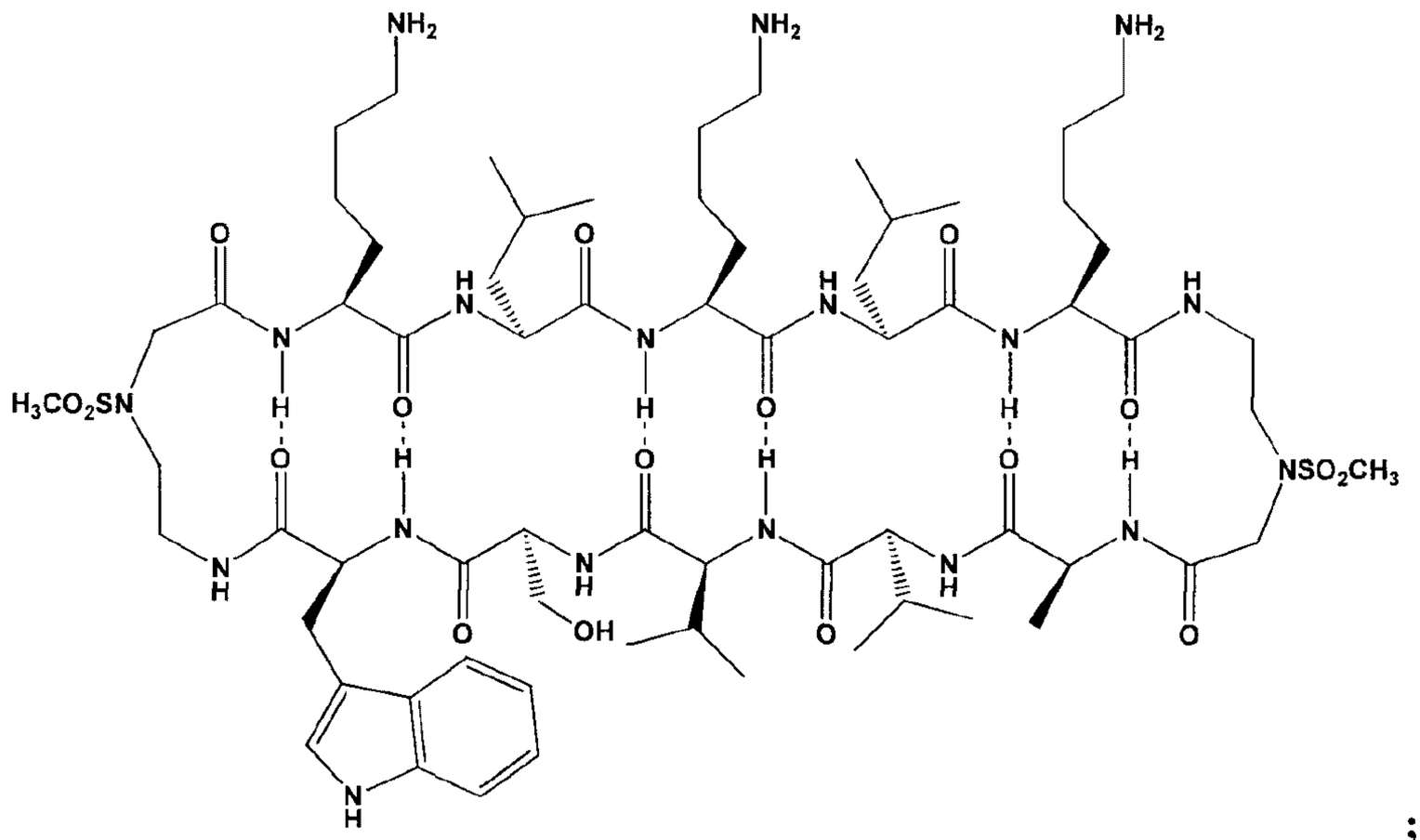

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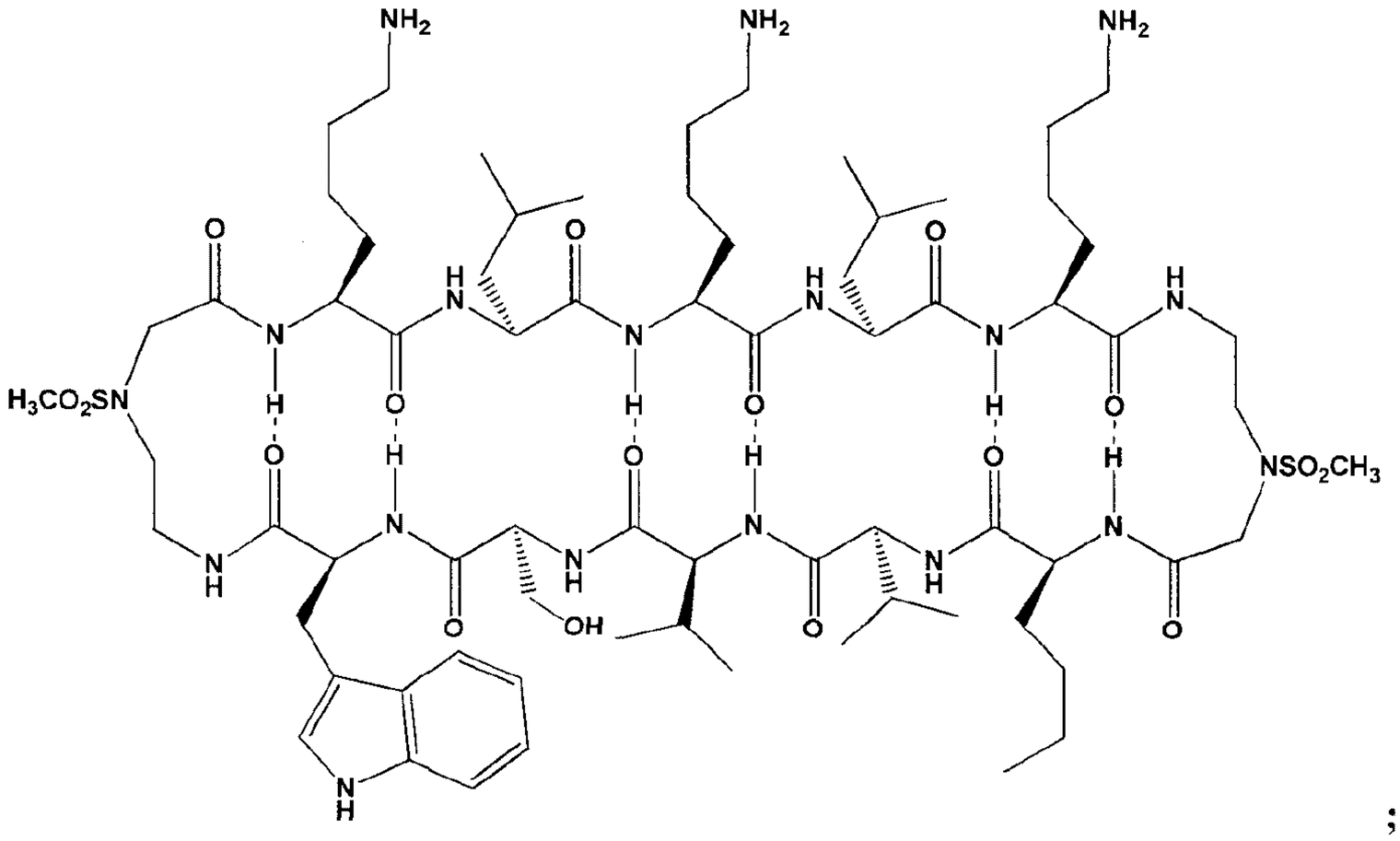

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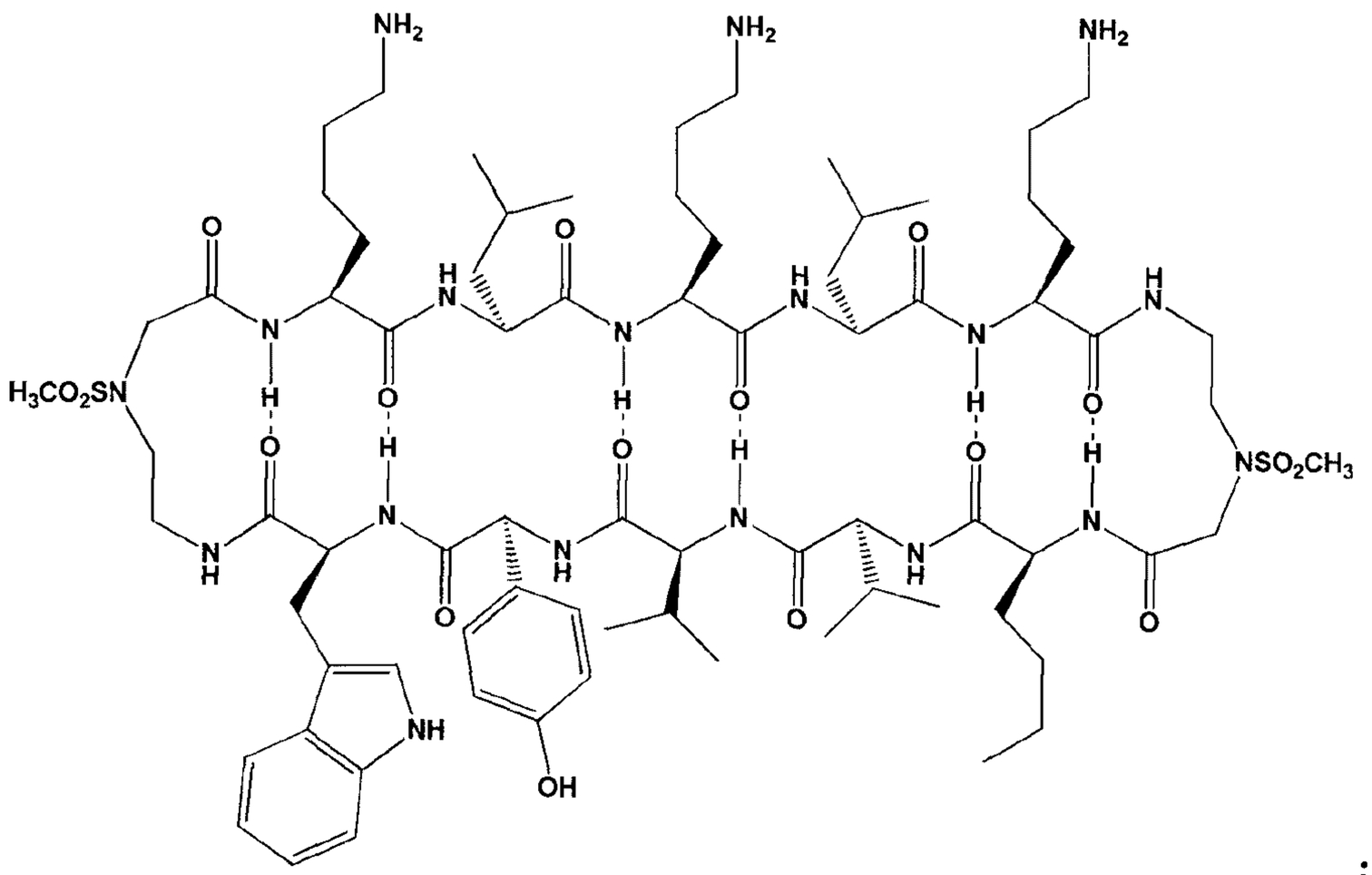

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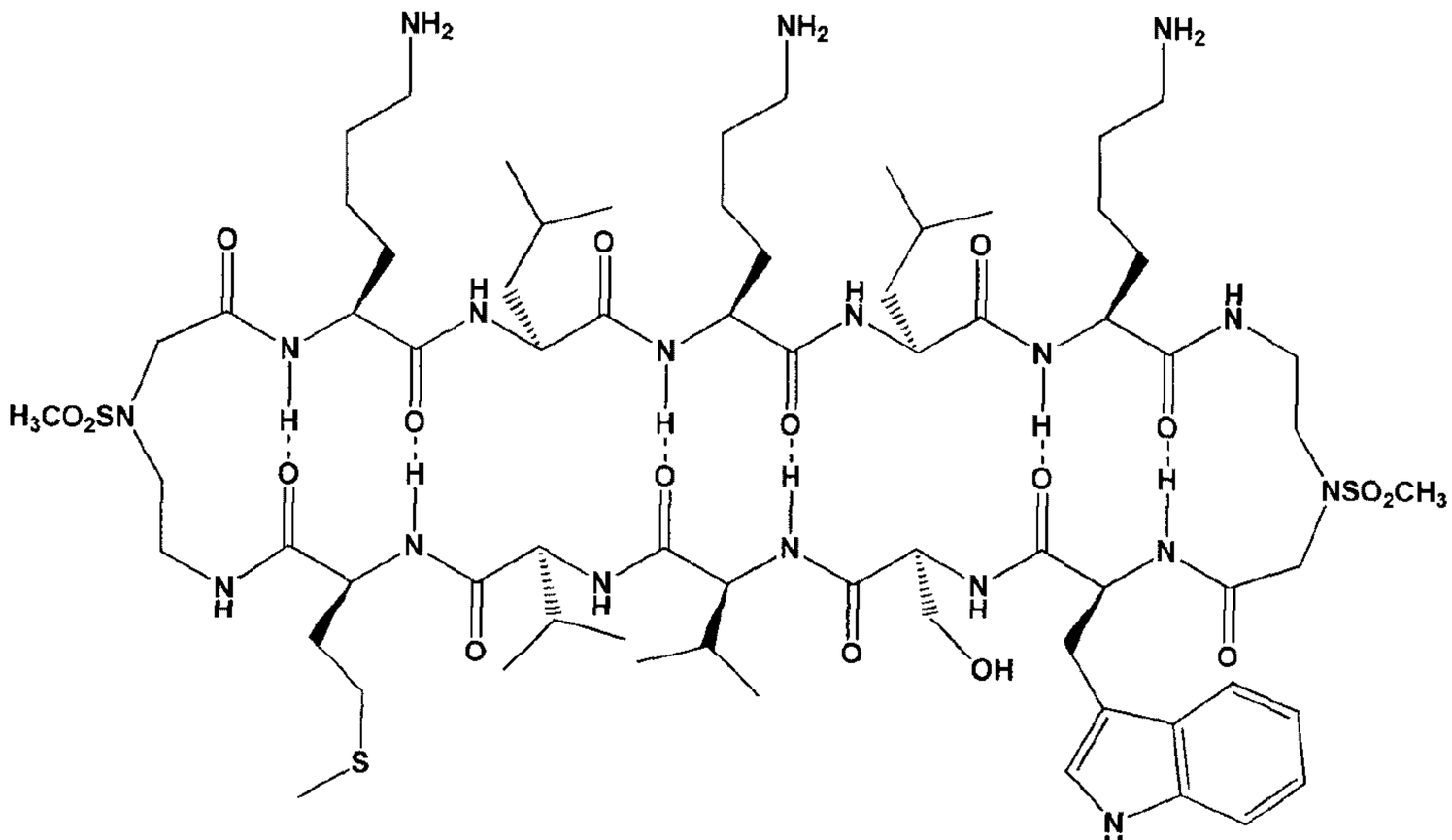

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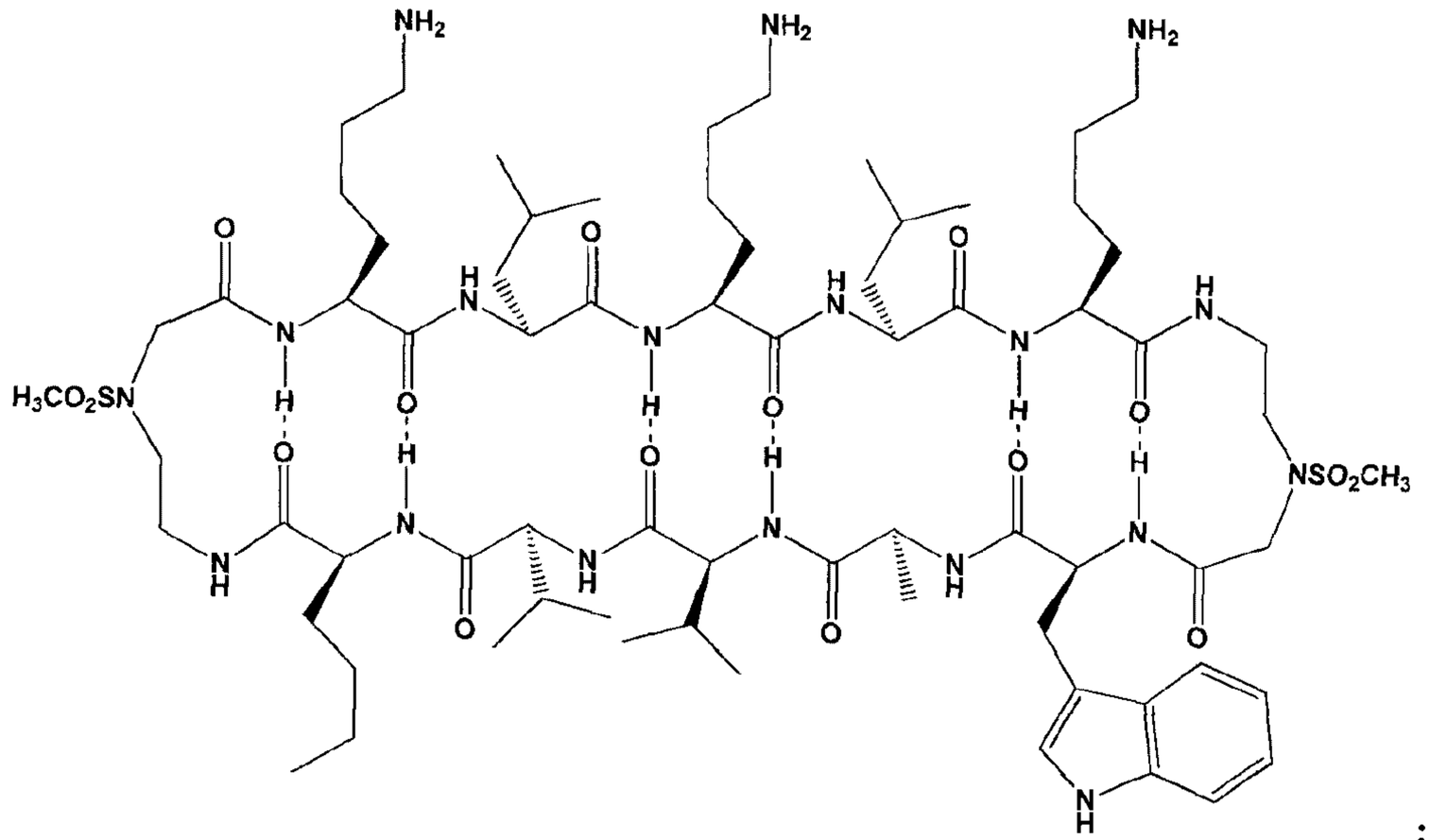

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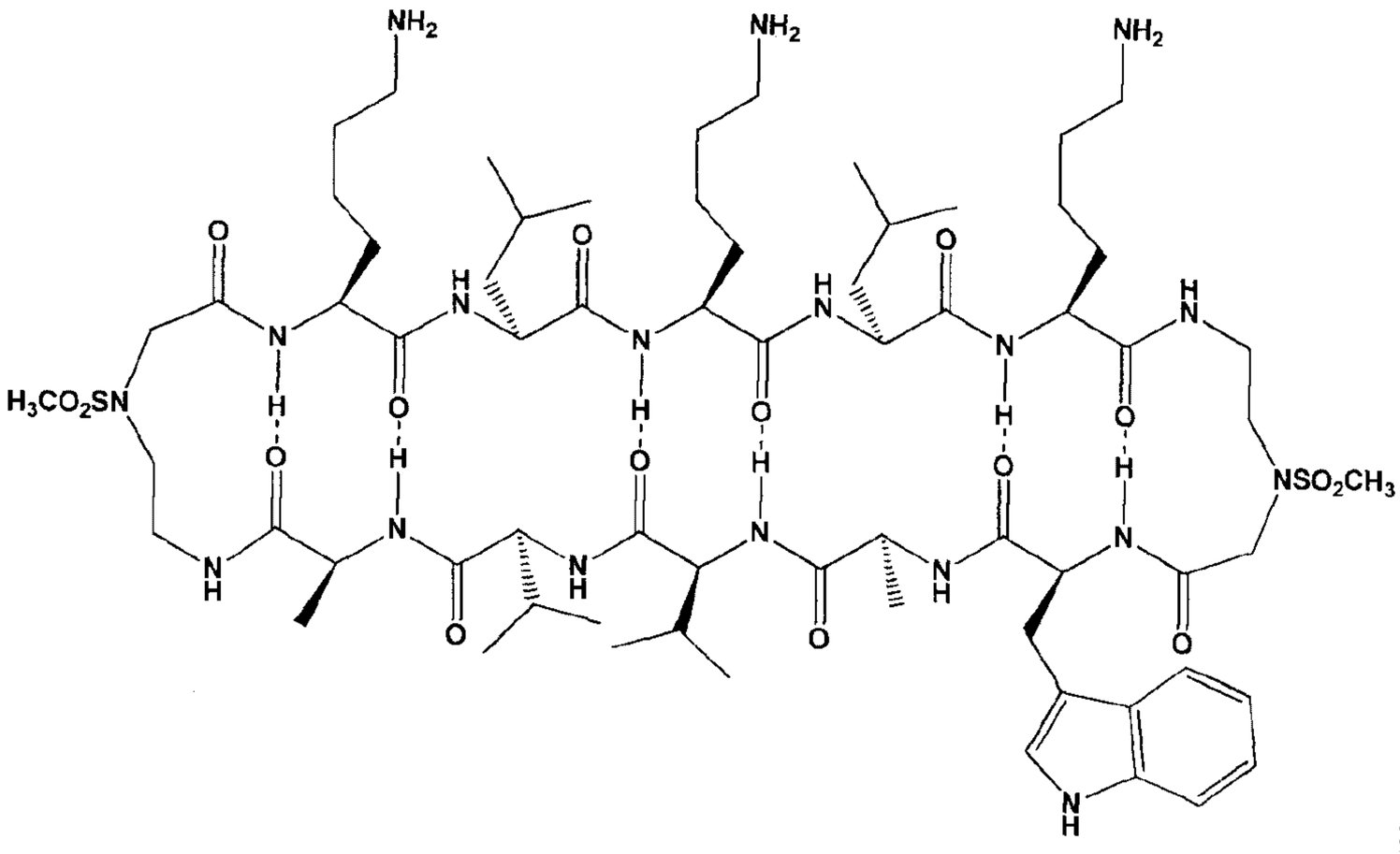

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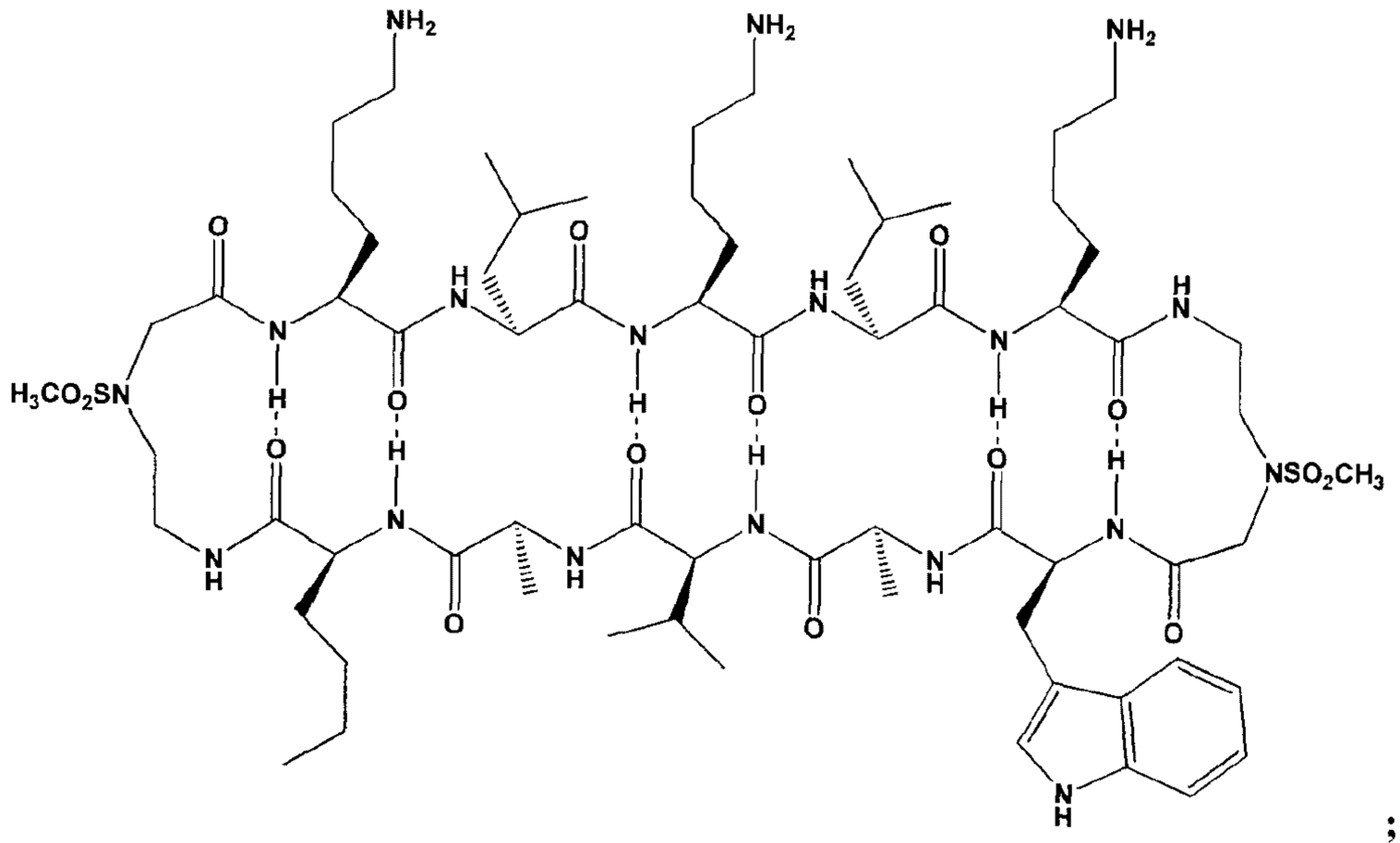

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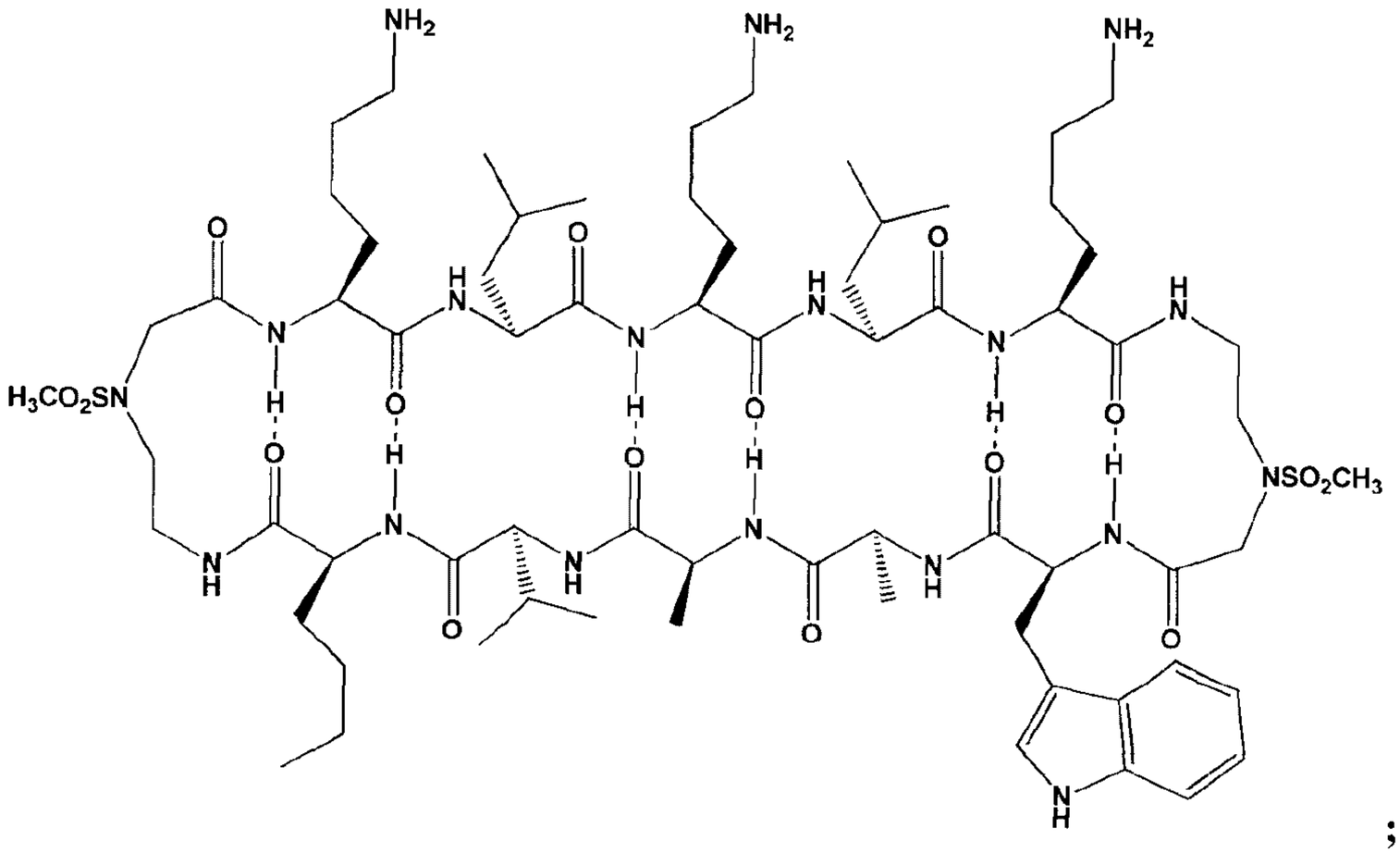

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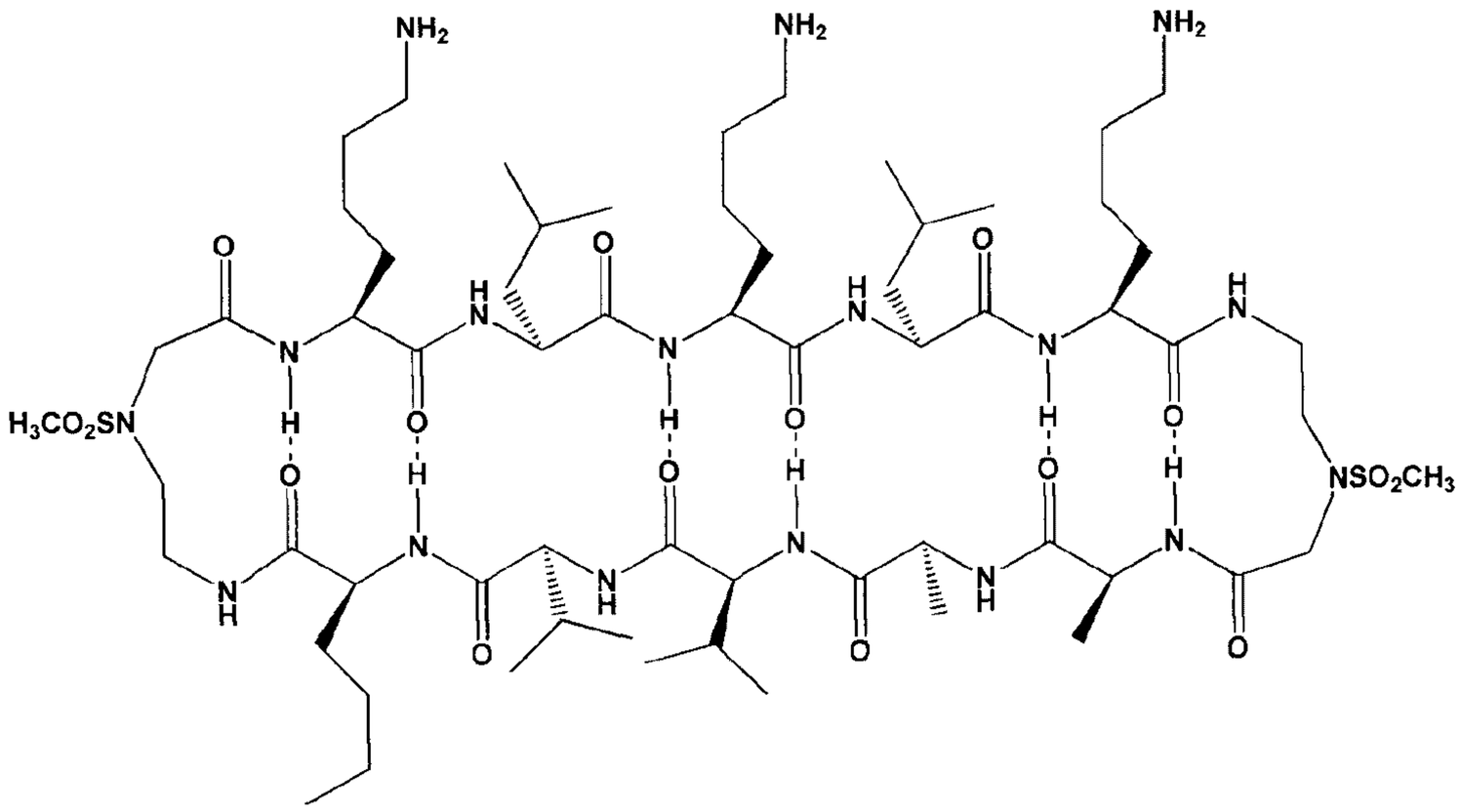

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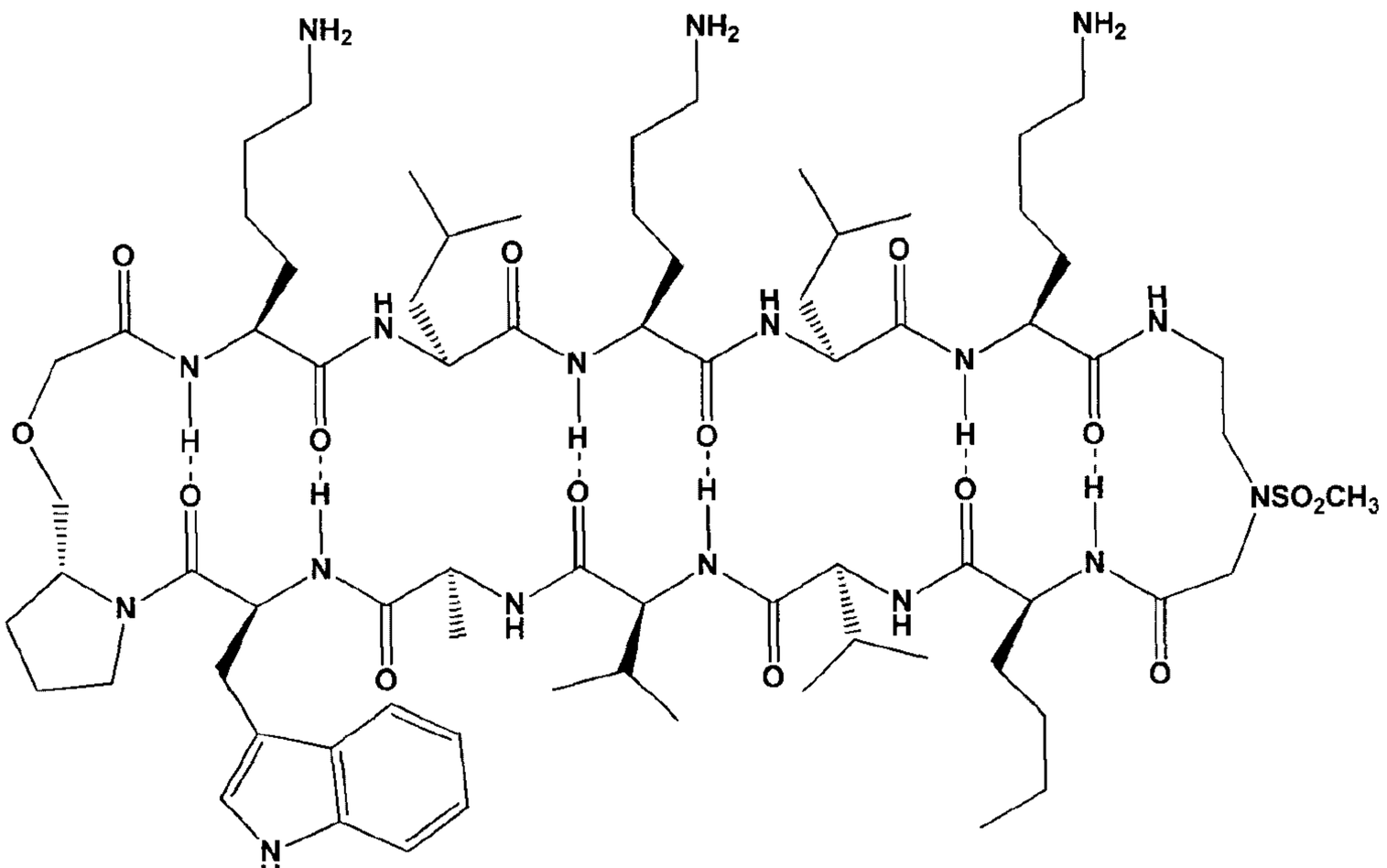

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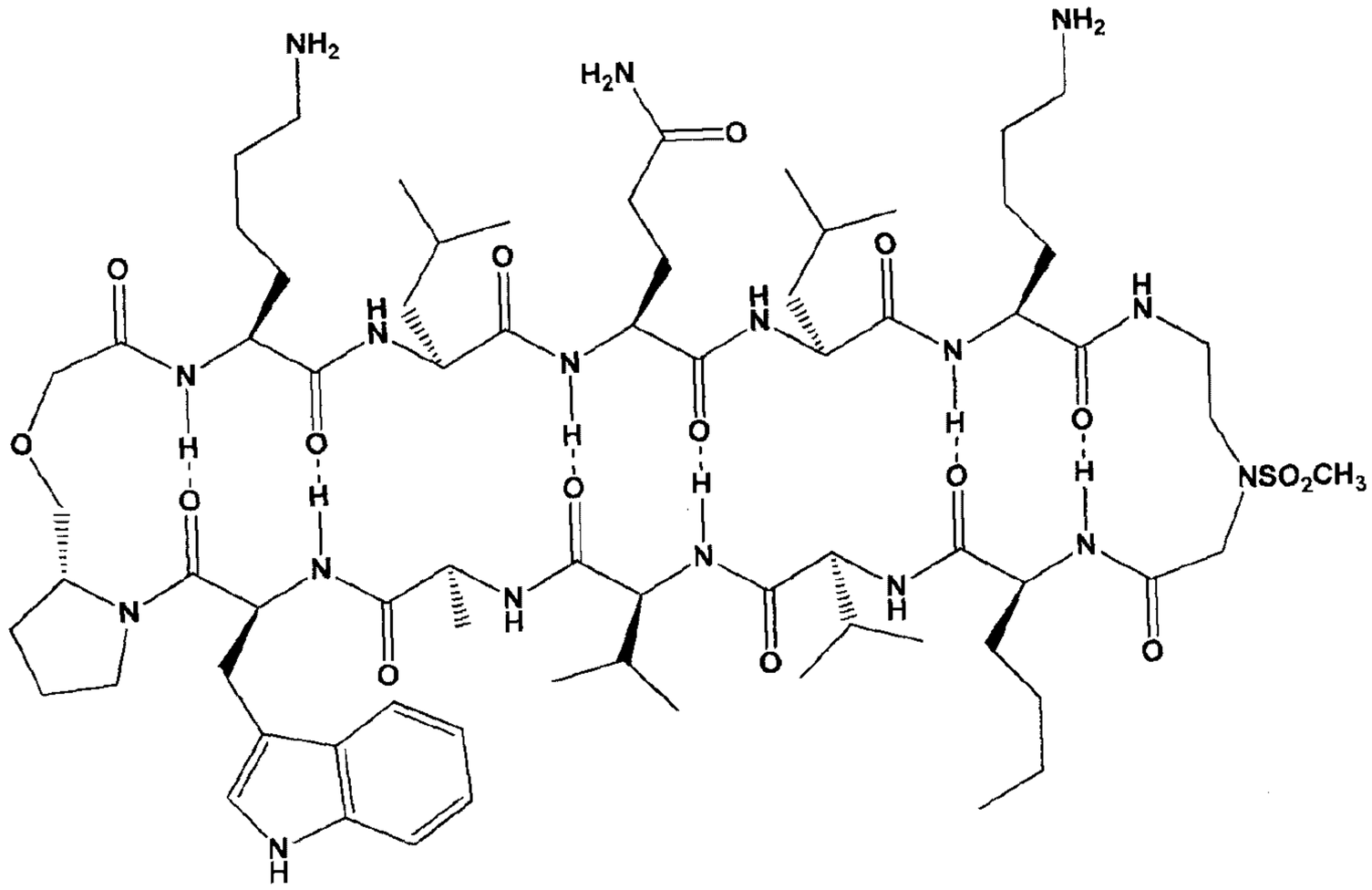

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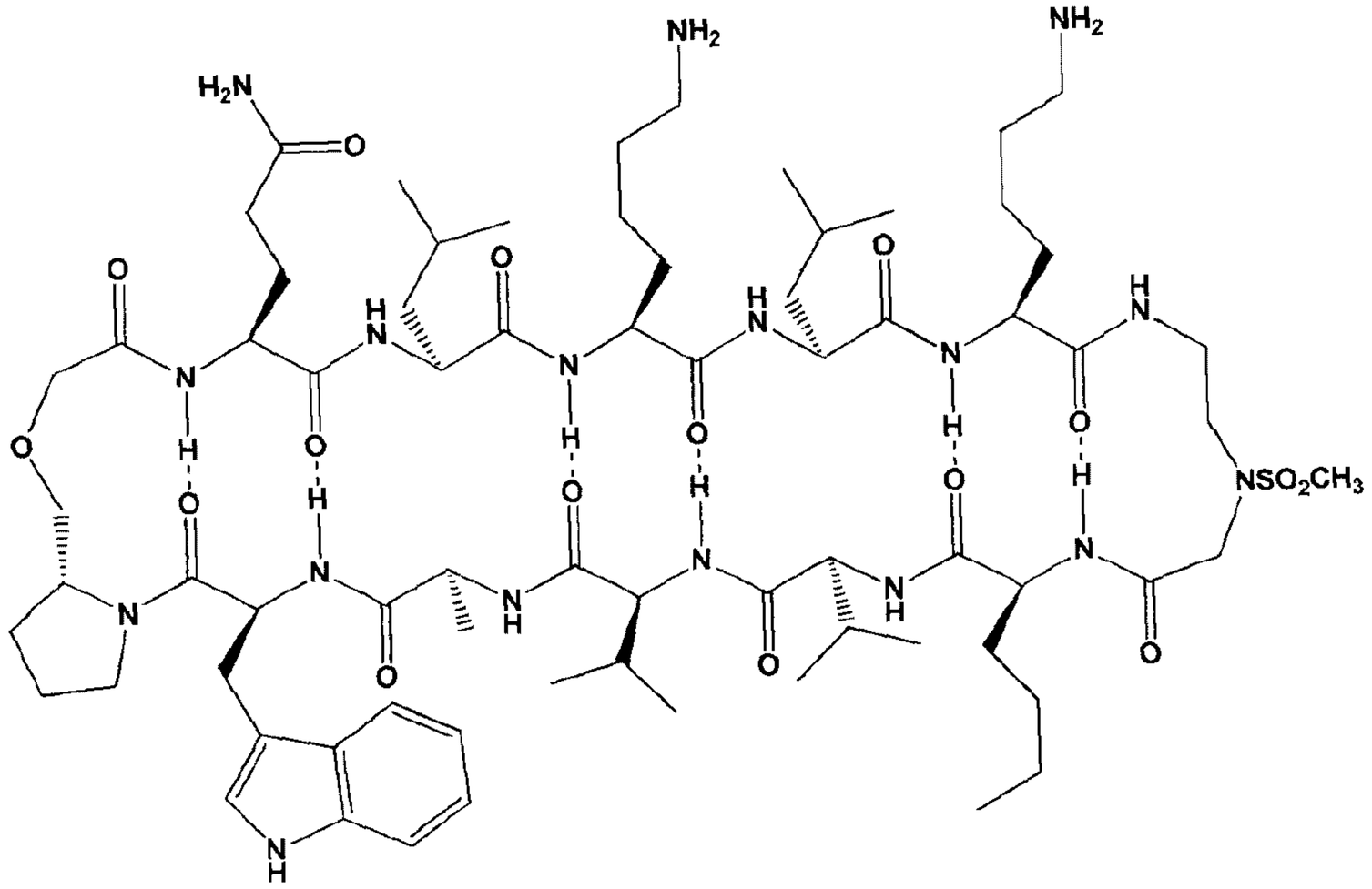

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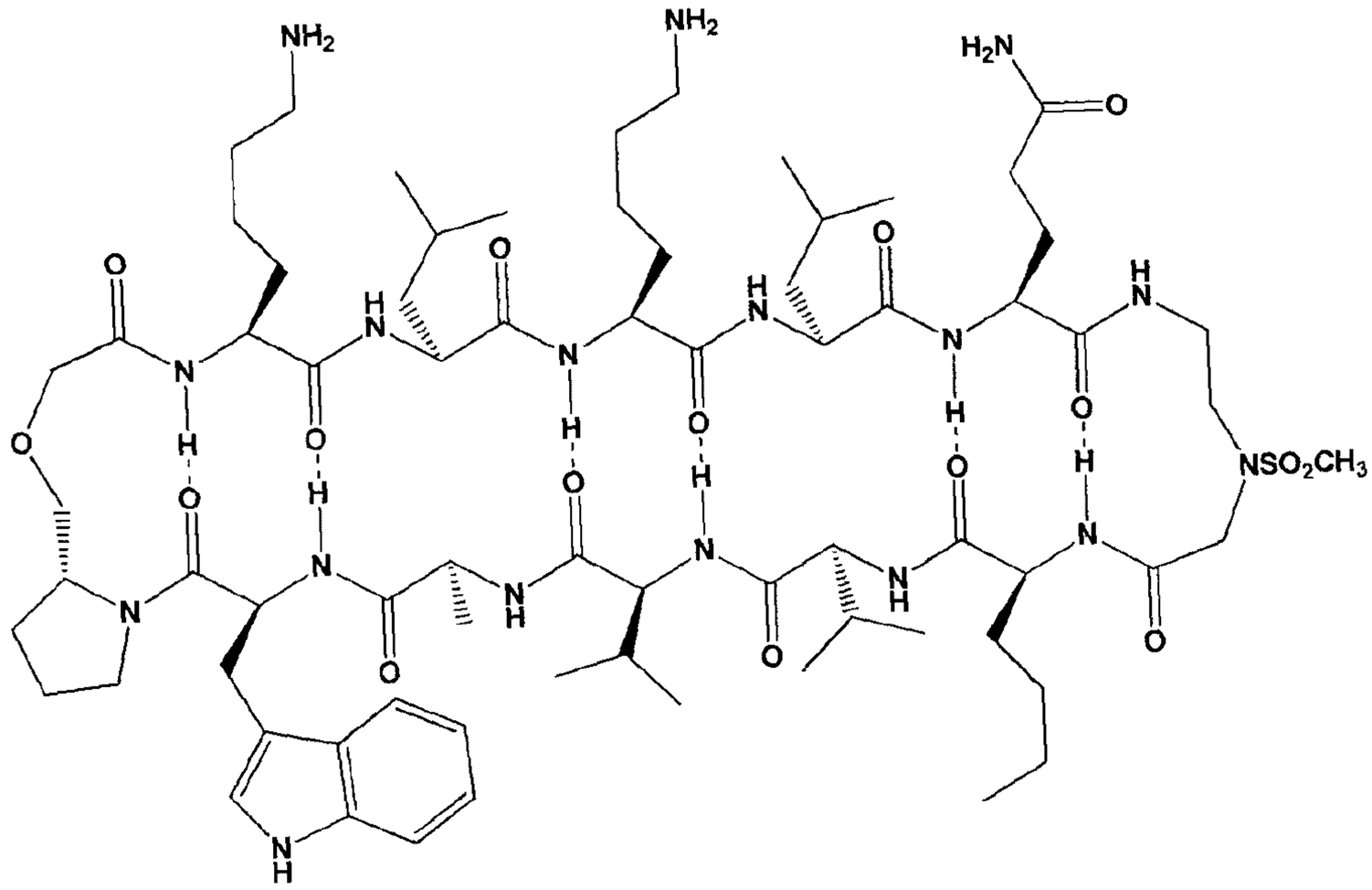

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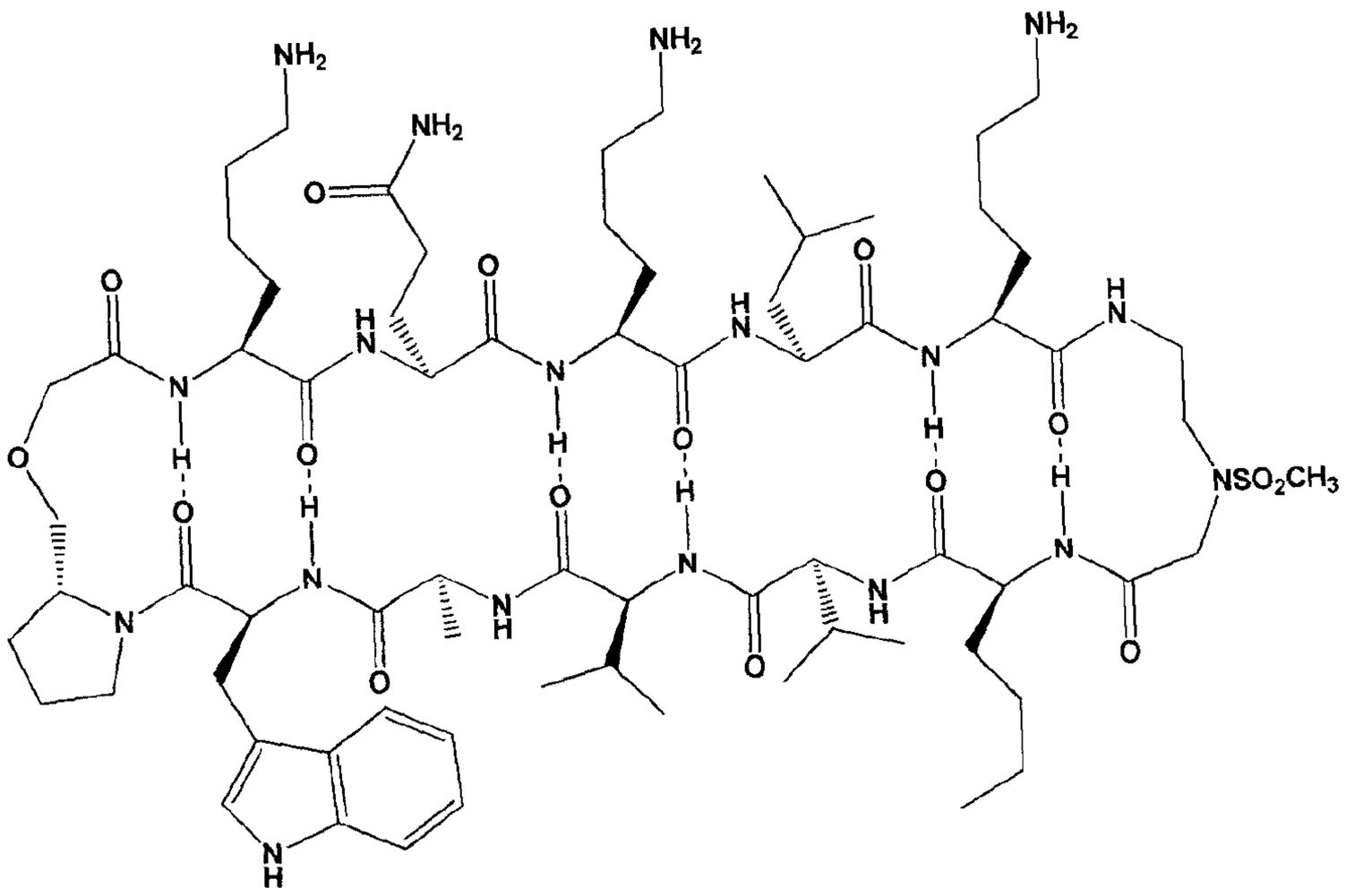

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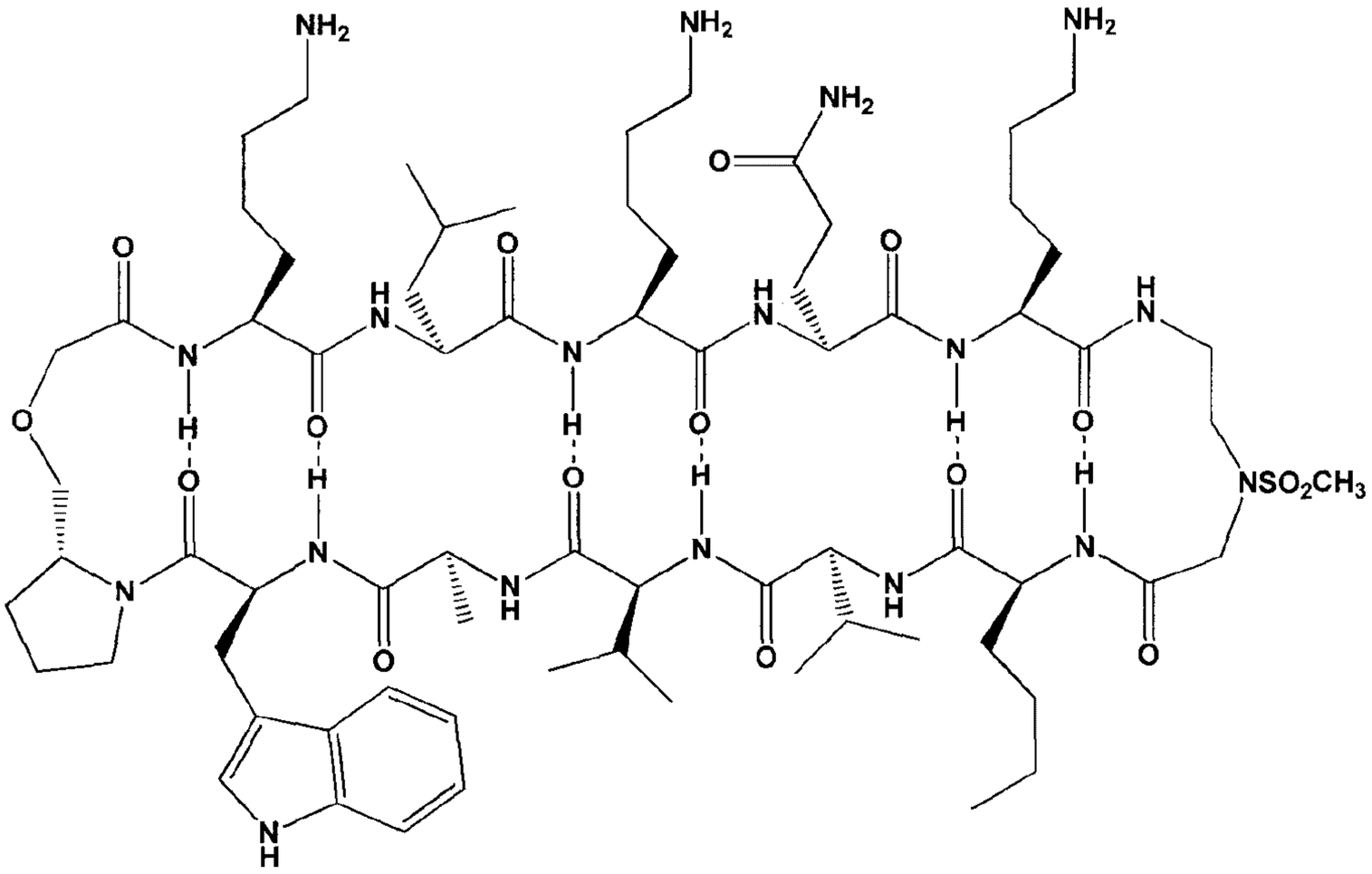

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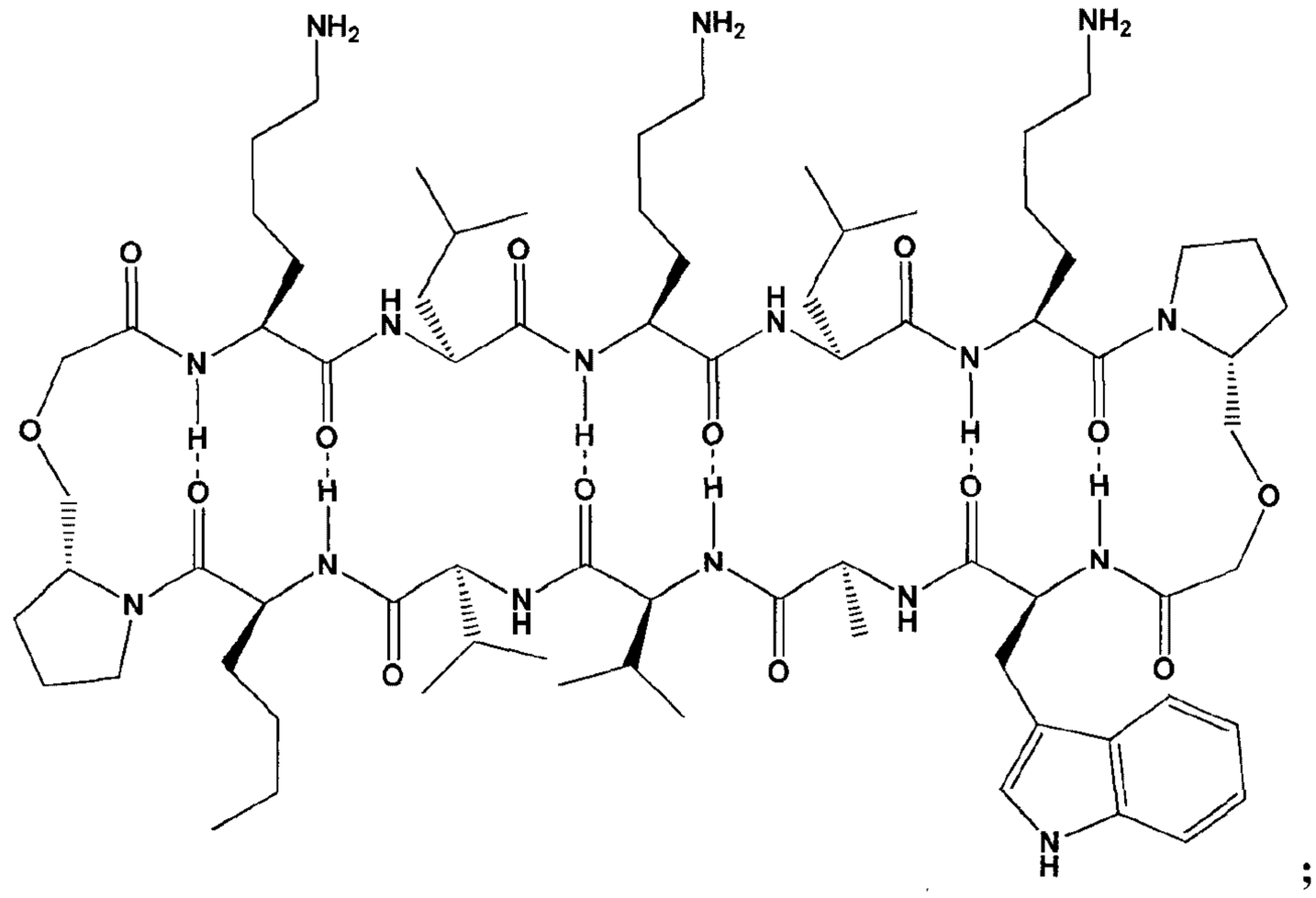

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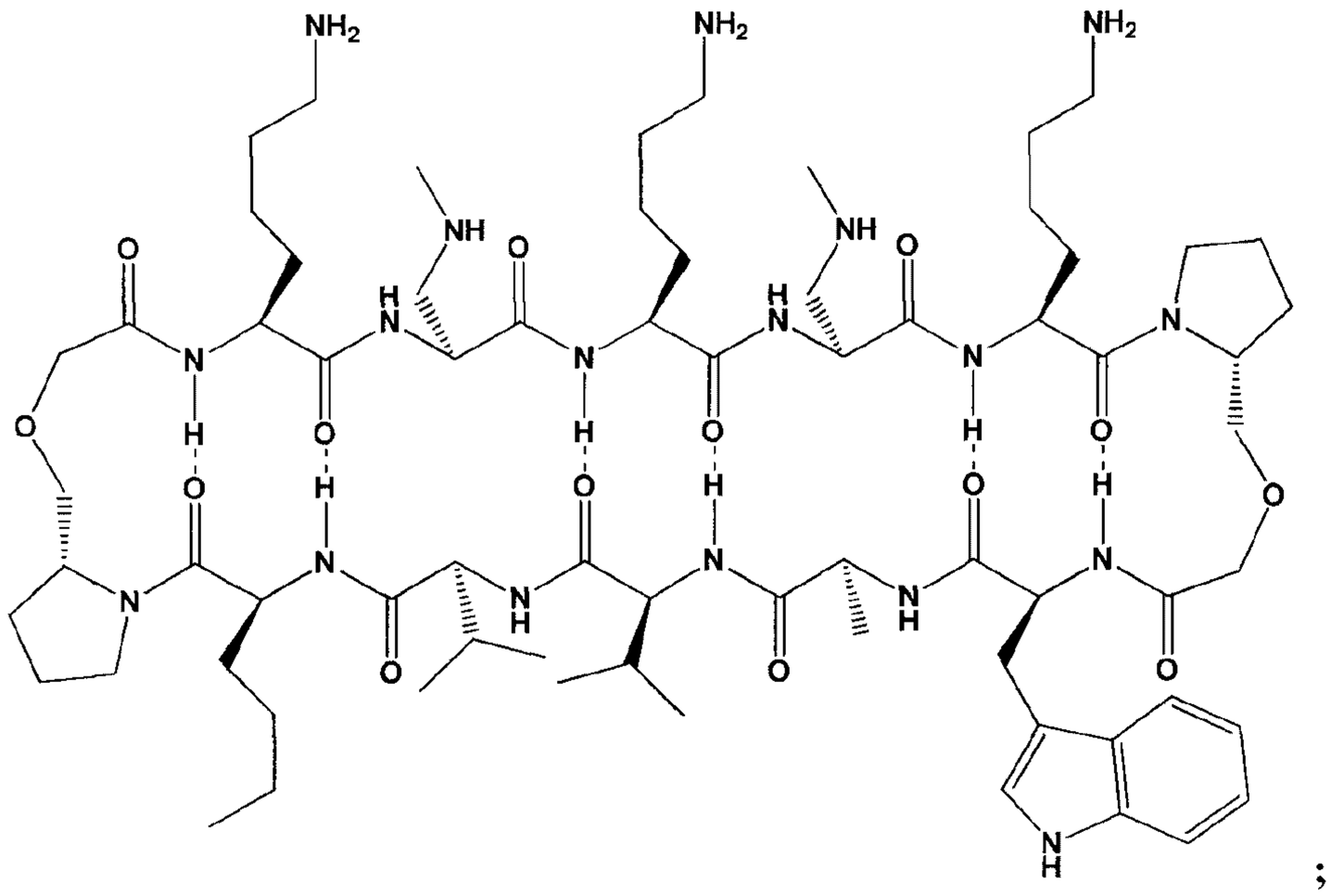

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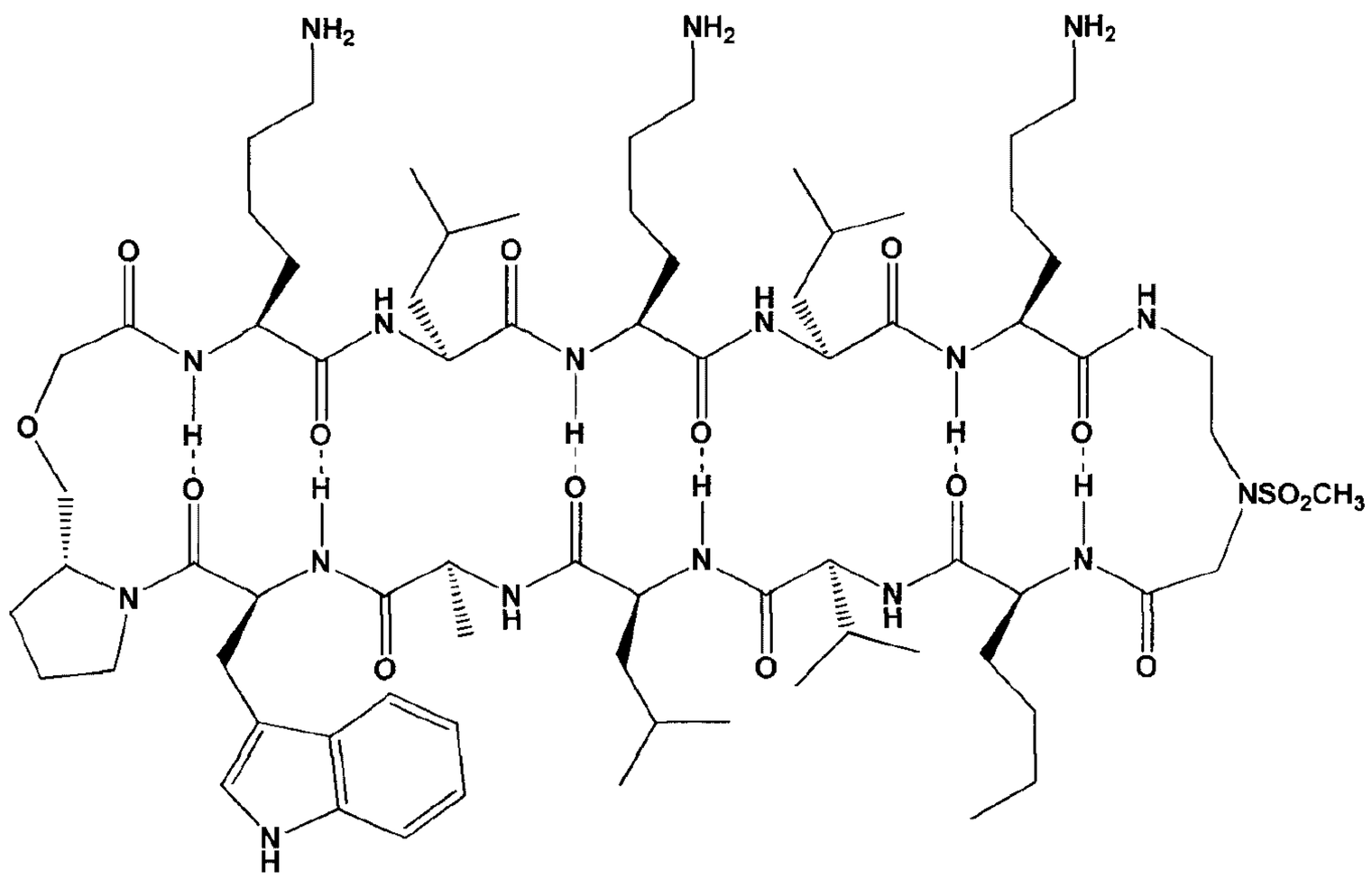

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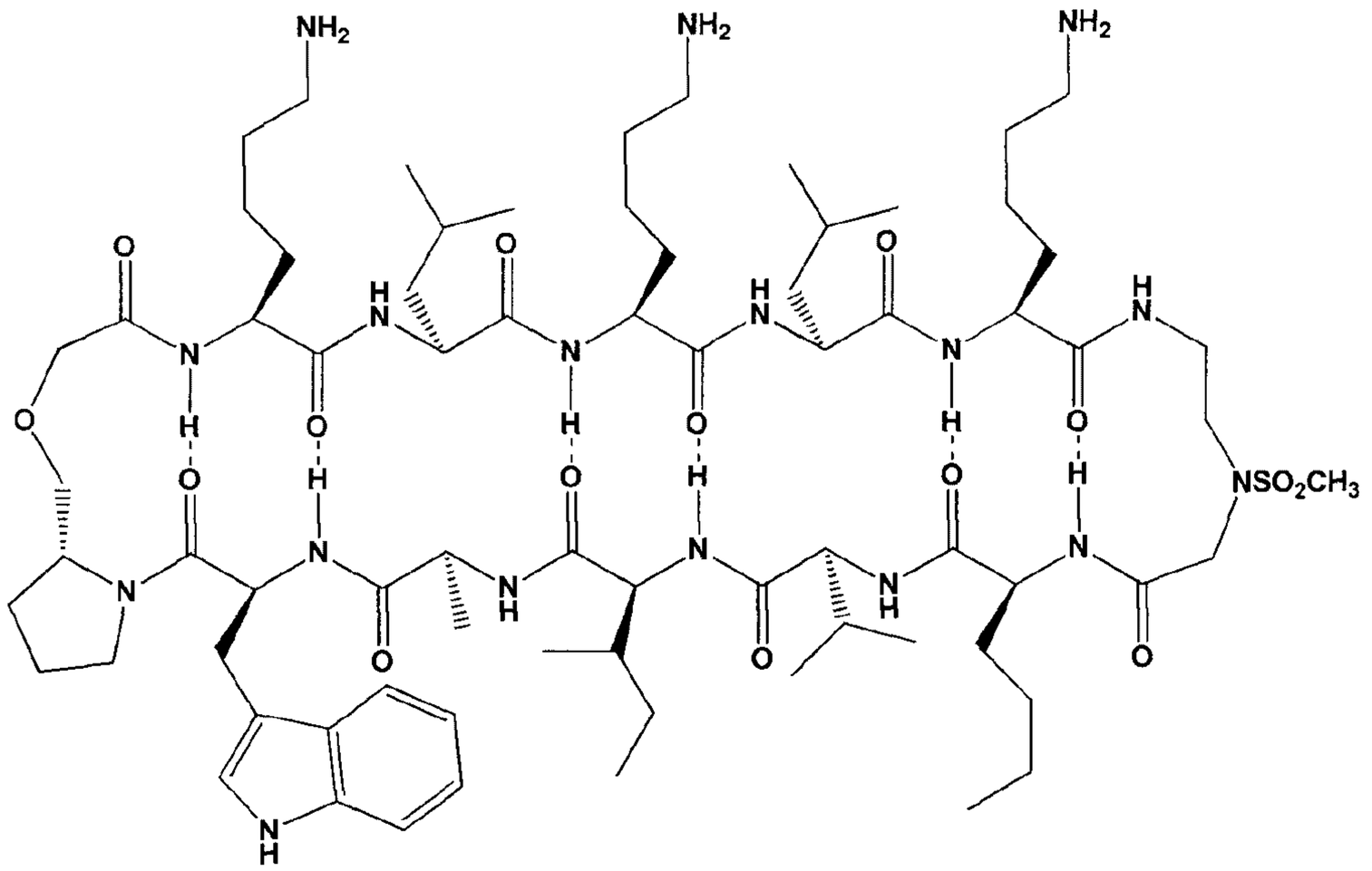

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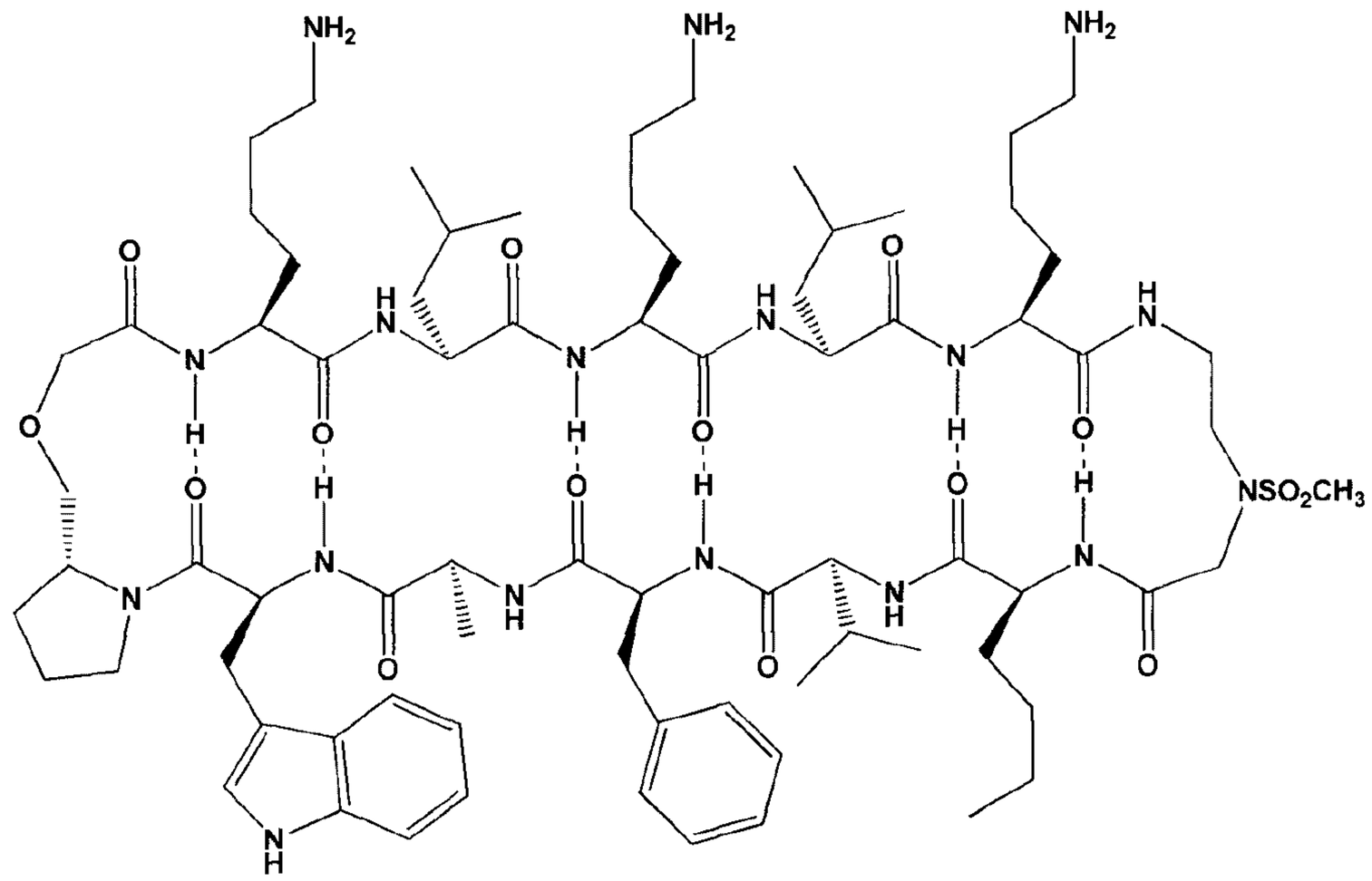
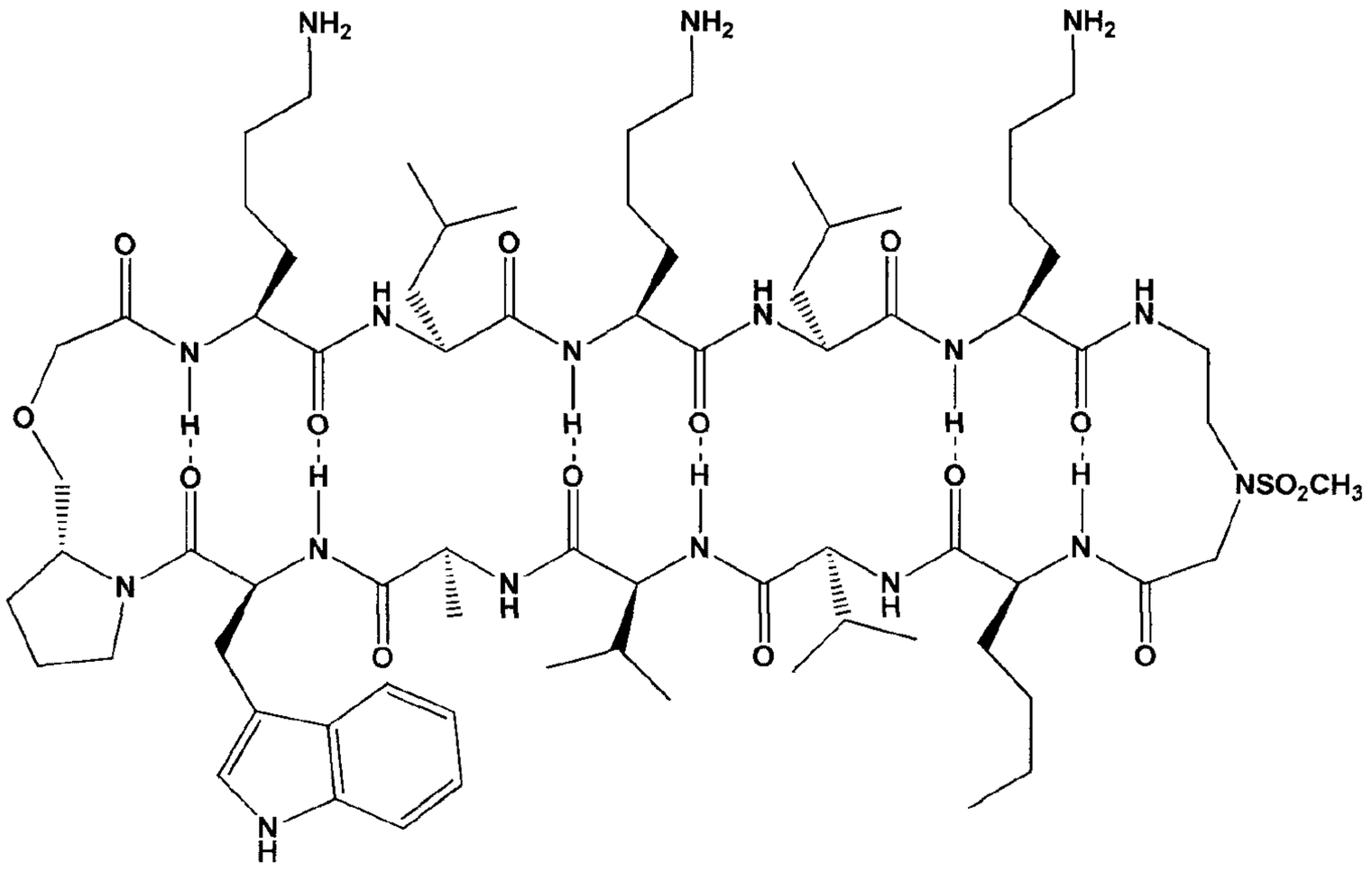


; or a

pharmaceutically acceptable salt of any of the foregoing.

3. The cyclic compound of claim 1, wherein the cyclic compound has the structure:



or a pharmaceutically acceptable salt thereof.

4. Use of an effective amount of the cyclic compound of claim 2 to treat a proliferation disorder in a subject, wherein the proliferation disorder is characterized by proliferation of T-lymphocytes.

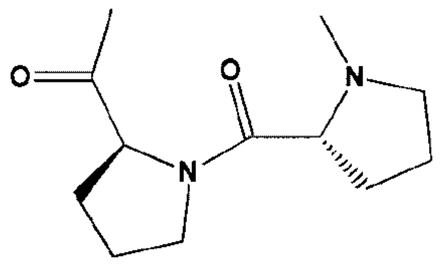
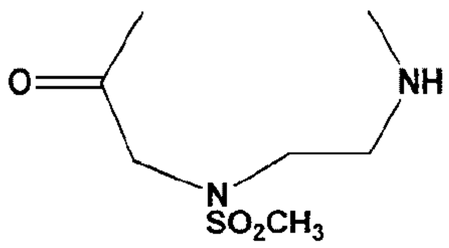
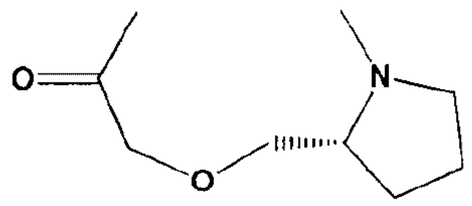
5. Use of an effective amount of the cyclic compound of claim 2 in the manufacture of a medicament to treat a proliferation disorder in a subject, wherein the proliferation disorder is characterized by proliferation of T-lymphocytes.

6. Use of an effective amount of the cyclic compound of claim 2 to treat a malignancy in a subject, wherein the malignancy is selected from among multiple myeloma, lung cancer, acute myeloid leukemia, prostate cancer, and melanoma.

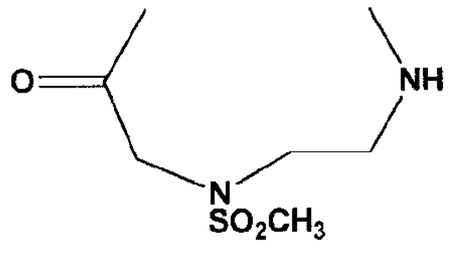
7. Use of the cyclic compound of claim 2 in the manufacture of a medicament to treat a malignancy in a subject, wherein the malignancy is selected from among multiple myeloma, lung cancer, acute myeloid leukemia, prostate cancer, and melanoma.

8. A cyclic compound comprising a recognition sequence and a non-recognition sequence, wherein said recognition sequence is joined to said non-recognition sequence by a first linker and a second linker, wherein said non-recognition sequence is KLKLK (SEQ ID NO:27) and said recognition sequence is five amino acids selected from WAVAW (SEQ ID NO:50), WAVAA (SEQ ID NO:51), WAVAM (SEQ ID NO:52), WAVAN* (SEQ ID NO:53), WAVVN* (SEQ ID NO:54), WAVSN* (SEQ ID NO:55), WAAAW (SEQ ID NO:56), WAAAA (SEQ ID NO:57), WAAAM (SEQ ID NO:58), WAAAN* (SEQ ID NO:59), WAAVW (SEQ ID NO:60), WAAVA (SEQ ID NO:61), WAAVM (SEQ ID NO:62), WAAVN* (SEQ ID NO:63), WAASN* (SEQ ID NO:64), WVVAW (SEQ ID NO:65), WVVAA (SEQ ID NO:66), WVVAM (SEQ ID NO:67), WVVAN* (SEQ ID NO:68), WVVVW (SEQ ID NO:69), WVVVA (SEQ ID NO:70), WVVVM (SEQ ID NO:71), WVVVN* (SEQ ID NO:72), WVVSN* (SEQ ID NO:73), WVAAN* (SEQ ID NO:74), WVAVW (SEQ ID NO:75), WVAVA (SEQ ID NO:76), WVAVM (SEQ ID NO:77), WVAVN* (SEQ ID NO:78), WVASN* (SEQ ID NO:79), WSVAW (SEQ ID NO:80), WSVAA (SEQ ID NO:81), WSVAM (SEQ ID NO:82), WSVAN* (SEQ ID NO:83), WSVVW (SEQ ID NO:48), WSVVA (SEQ ID NO:84), WSVVM (SEQ ID NO:85), WSVVN* (SEQ ID NO:86), WSVSW (SEQ ID NO:87), WSVSA (SEQ ID NO:88), WSVSM (SEQ ID NO:89), WSVSN* (SEQ ID NO:90), WSAAW (SEQ ID NO:91), WSAAA (SEQ ID NO:92), WSAAM (SEQ ID NO:93), WSAAN* (SEQ ID NO:94), WSAVW (SEQ ID NO:95), WSAVA (SEQ ID NO:96), WSAVM (SEQ ID NO:97), WSAVN* (SEQ ID NO:98), WSASW (SEQ ID NO:99), WSASA (SEQ ID NO:100), WSASM (SEQ ID NO:101), WSASN* (SEQ ID NO:102), WYVAW (SEQ ID NO:103), WYVAA (SEQ ID NO:104), WYVAM (SEQ ID NO:105), WYVAN* (SEQ ID NO:106), WYVVW (SEQ ID NO:107), WYVVA (SEQ ID NO:108), WYVVM (SEQ ID NO:109), WYVVN* (SEQ ID NO:110), WYVSW (SEQ ID NO:111), WYVSA (SEQ ID NO:112), WYVSM (SEQ ID NO:113), WYVSN* (SEQ ID NO:114), WYAAW (SEQ ID NO:115), WYAAA (SEQ ID NO:116), WYAAM (SEQ ID NO:117), WYAAN* (SEQ ID NO:118), WYAVW (SEQ ID NO:119), WYAVA (SEQ ID NO:120), WYAVM (SEQ ID NO:121), WYAVN* (SEQ ID NO:122), WYASW (SEQ ID

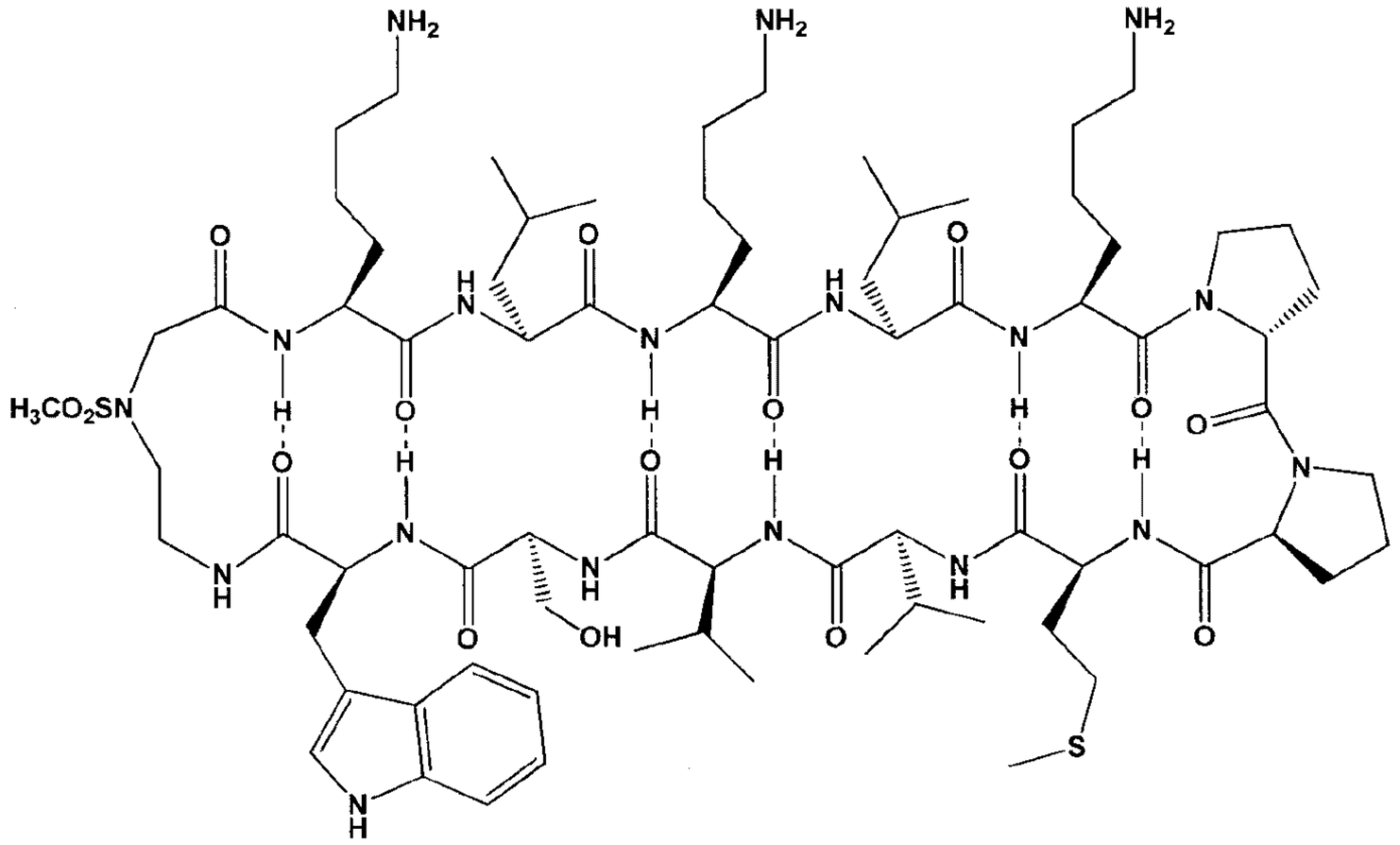
NO:123), WYASA (SEQ ID NO:124), WYASM (SEQ ID NO:125), WYASN* (SEQ ID NO:126), AAVAA (SEQ ID NO:127), AAVAM (SEQ ID NO:128), AAVAN* (SEQ ID NO:129), AAVVN* (SEQ ID NO:130), AAVSN* (SEQ ID NO:131), AAAAA (SEQ ID NO:132), AAAAM (SEQ ID NO:133), AAAAN* (SEQ ID NO:134), AAAVW (SEQ ID NO:135), AAAVA (SEQ ID NO:136), AAAVM (SEQ ID NO:137), AAAVN* (SEQ ID NO:138), AAASM (SEQ ID NO:139), AAASN* (SEQ ID NO:140), AVVAW (SEQ ID NO:141), AVVAA (SEQ ID NO:142), AVVAM (SEQ ID NO:143), AVVAN* (SEQ ID NO:144), AVVVA (SEQ ID NO:145), AVVVM (SEQ ID NO:146), AVVVN* (SEQ ID NO:147), AVVSN* (SEQ ID NO:148), AVAAW (SEQ ID NO:42), AVAAM (SEQ ID NO:149), AVAAN* (SEQ ID NO:150), AVAVA (SEQ ID NO:151), AVAVM (SEQ ID NO:152), AVAVN* (SEQ ID NO:153), AVASN* (SEQ ID NO:154), ASVAW (SEQ ID NO:155), ASVAA (SEQ ID NO:156), ASVAM (SEQ ID NO:157), ASVAN* (SEQ ID NO:158), ASVVW (SEQ ID NO:159), ASVVA (SEQ ID NO:160), ASVVM (SEQ ID NO:161), ASVVN* (SEQ ID NO:162), ASVSA (SEQ ID NO:163), ASVSM (SEQ ID NO:164), ASVSN* (SEQ ID NO:165), ASAAW (SEQ ID NO:166), ASAAA (SEQ ID NO:167), ASAAM (SEQ ID NO:168), ASAAN* (SEQ ID NO:169), ASAVW (SEQ ID NO:170), ASAVA (SEQ ID NO:171), ASAVM (SEQ ID NO:172), ASAVN* (SEQ ID NO:173), ASASA (SEQ ID NO:174), ASASM (SEQ ID NO:175), ASASN* (SEQ ID NO:176), AYVAW (SEQ ID NO:177), AYVAA (SEQ ID NO:178), AYVAM (SEQ ID NO:179), AYVAN* (SEQ ID NO:180), AYVVW (SEQ ID NO:181), AYVVA (SEQ ID NO:182), AYVVM (SEQ ID NO:183), AYVVN* (SEQ ID NO:184), AYVSW (SEQ ID NO:185), AYVSA (SEQ ID NO:186), AYVSM (SEQ ID NO:187), AYVSN* (SEQ ID NO:188), AYAAW (SEQ ID NO:189), AYAAA (SEQ ID NO:190), AYAAM (SEQ ID NO:191), AYAAN* (SEQ ID NO:192), AYAVW (SEQ ID NO:193), AYAVA (SEQ ID NO:194), AYAVM (SEQ ID NO:195), AYAVN* (SEQ ID NO:196), AYASW (SEQ ID NO:197), AYASA (SEQ ID NO:198), AYASM (SEQ ID NO:199), AYASN* (SEQ ID NO:200), MAVAA (SEQ ID NO:201), MAVAM (SEQ ID NO:202), MAVAN* (SEQ ID NO:203), MAVVN* (SEQ ID NO:204), MAVSN* (SEQ ID NO:205), MAAAA (SEQ ID NO:206), MAAAM (SEQ ID NO:207), MAAAN* (SEQ ID NO:208), MAAVW (SEQ ID NO:209), MAAVA (SEQ ID NO:210), MAAVM (SEQ ID NO:211), MAAVN* (SEQ ID NO:212), MAASN* (SEQ ID NO:213), MVVAW (SEQ ID NO:214), MVVAA (SEQ ID NO:215), MVVAM (SEQ ID NO:216), MVVAN* (SEQ ID NO:217), MVVVM (SEQ ID

NO:218), MVVVN* (SEQ ID NO:219), MVVSN* (SEQ ID NO:220), MVAAM (SEQ ID NO:221), MVAAN* (SEQ ID NO:222), MVAVM (SEQ ID NO:223), MVAVN* (SEQ ID NO:224), MVASN* (SEQ ID NO:225), MSVAW (SEQ ID NO:226), MSVAA (SEQ ID NO:227), MSVAM (SEQ ID NO:228), MSVAN* (SEQ ID NO:229), MSVVW (SEQ ID NO:230), MSVVA (SEQ ID NO:231), MSVVM (SEQ ID NO:232), MSVVN* (SEQ ID NO:233), MSVSM (SEQ ID NO:234), MSVSN* (SEQ ID NO:235), MSAAW (SEQ ID NO:236), MSAAA (SEQ ID NO:237), MSAAM (SEQ ID NO:238), MSAAN* (SEQ ID NO:239), MSAVW (SEQ ID NO:240), MSAVA (SEQ ID NO:241), MSAVM (SEQ ID NO:242), MSAVN* (SEQ ID NO:243), MSASM (SEQ ID NO:244), MSASN* (SEQ ID NO:245), MYVAW (SEQ ID NO:246), MYVAA (SEQ ID NO:247), MYVAM (SEQ ID NO:248), MYVAN* (SEQ ID NO:249), MYVVW (SEQ ID NO:250), MYVVA (SEQ ID NO:251), MYVVM (SEQ ID NO:252), MYVVN* (SEQ ID NO:253), MYVSW (SEQ ID NO:254), MYVSA (SEQ ID NO:255), MYVSM (SEQ ID NO:256), MYVSN* (SEQ ID NO:257), MYAAW (SEQ ID NO:258), MYAAA (SEQ ID NO:259), MYAAM (SEQ ID NO:260), MYAAN* (SEQ ID NO:261), MYAVW (SEQ ID NO:262), MYAVA (SEQ ID NO:263), MYAVM (SEQ ID NO:264), MYAVN* (SEQ ID NO:265), MYASW (SEQ ID NO:266), MYASA (SEQ ID NO:267), MYASM (SEQ ID NO:268), or MYASN* (SEQ ID NO:269), where N* = norleucine, and where said first linker and said second linker are independently selected from the structures:  (D-Pro-L-Pro);  (methylsulfonamido aminoethylglycine); or  ((pyrrolidin-2-ylmethoxy)acetate, wherein no more than one of said first linker and said second linker is D-Pro-L-Pro.

9. The cyclic compound of claim 8, wherein said first linker and said second linker are methylsulfonamido aminoethylglycine.

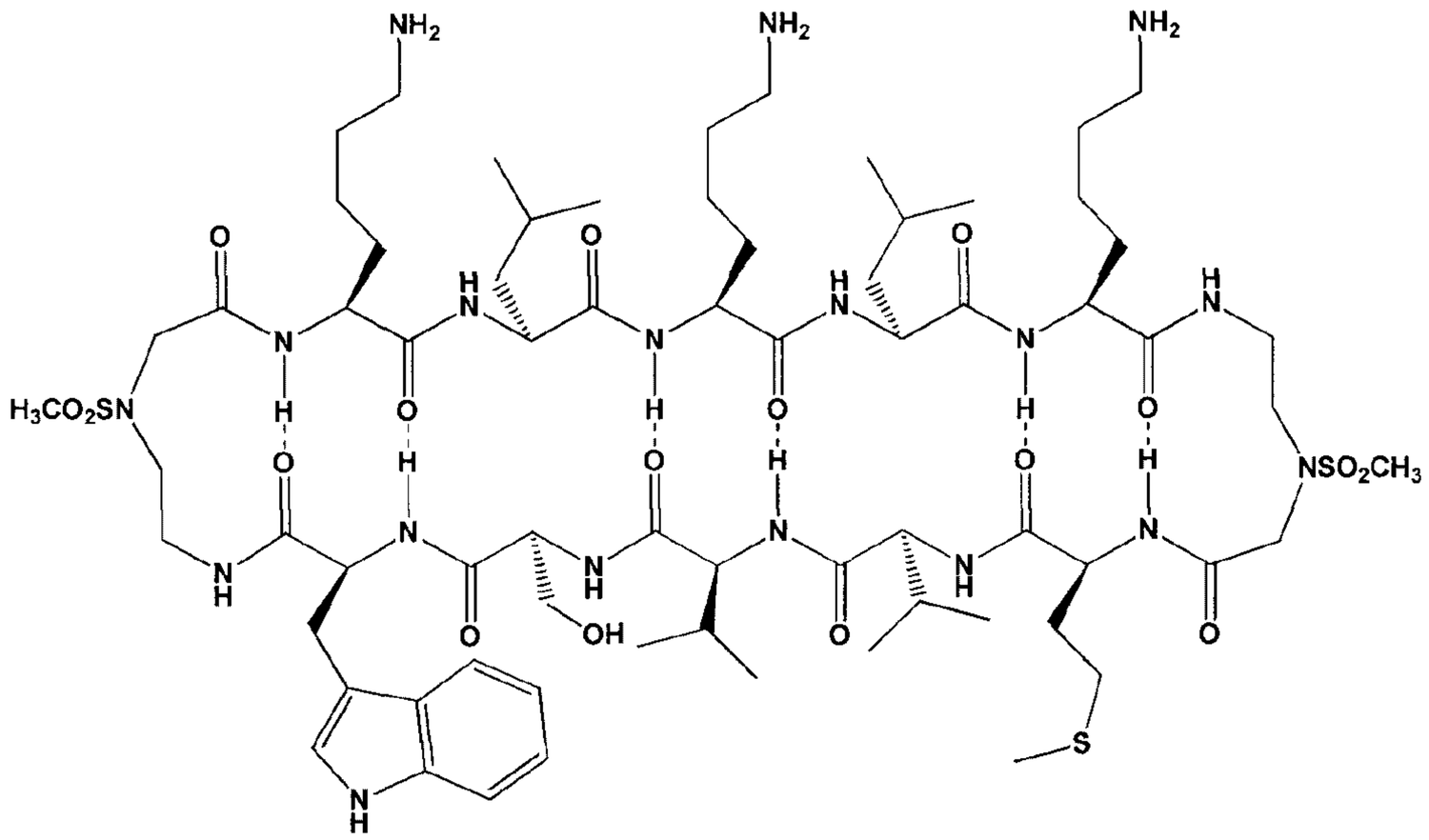
10. The cyclic compound of claim 1, wherein at least one of said first linker and said second linker is  (methylsulfonamido aminoethylglycine).

11. The cyclic compound of claim 1, wherein the cyclic compound has the structure:



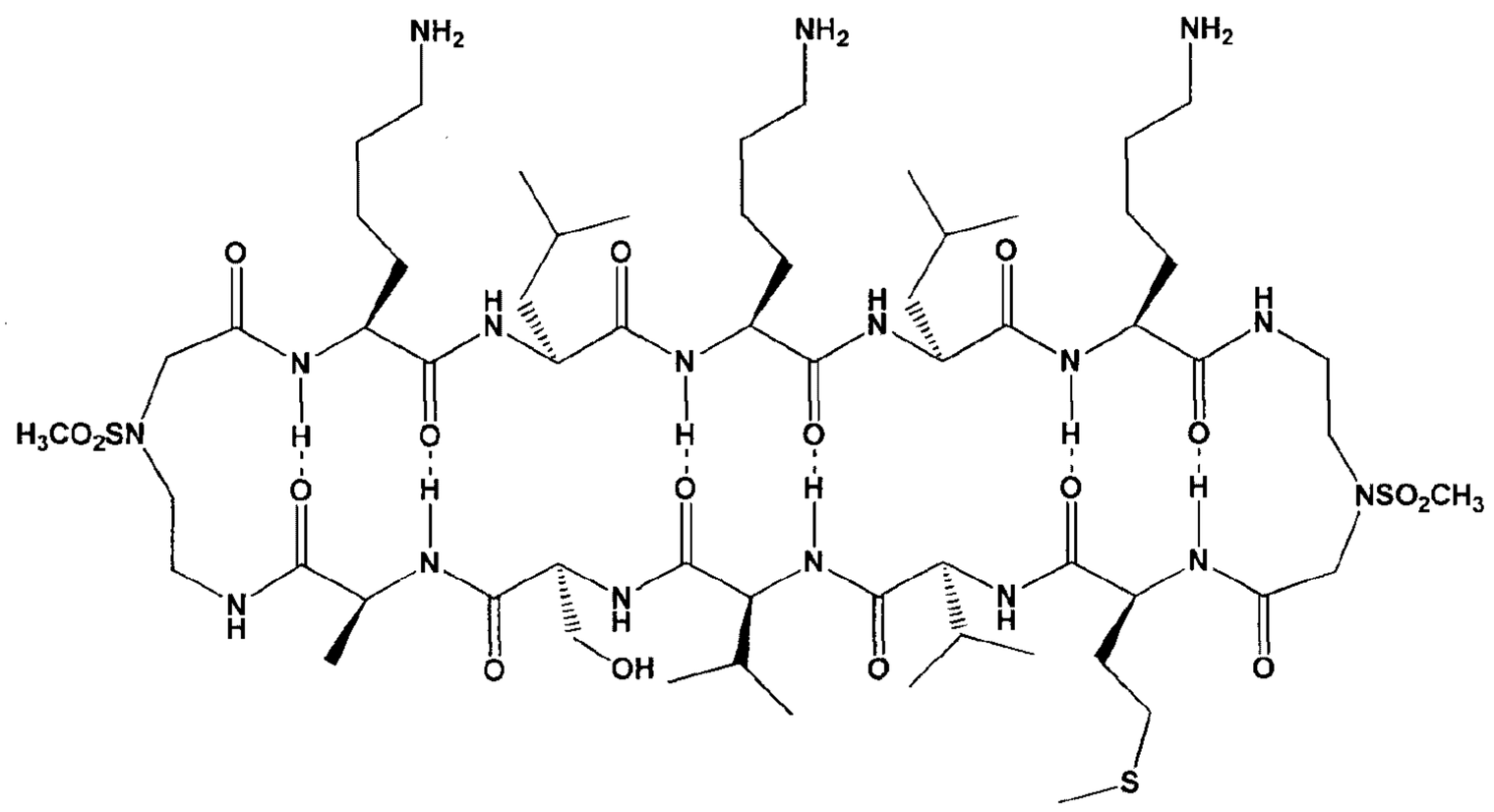
or a pharmaceutically acceptable salt thereof.

12. The cyclic compound of claim 1, wherein the cyclic compound has the structure:



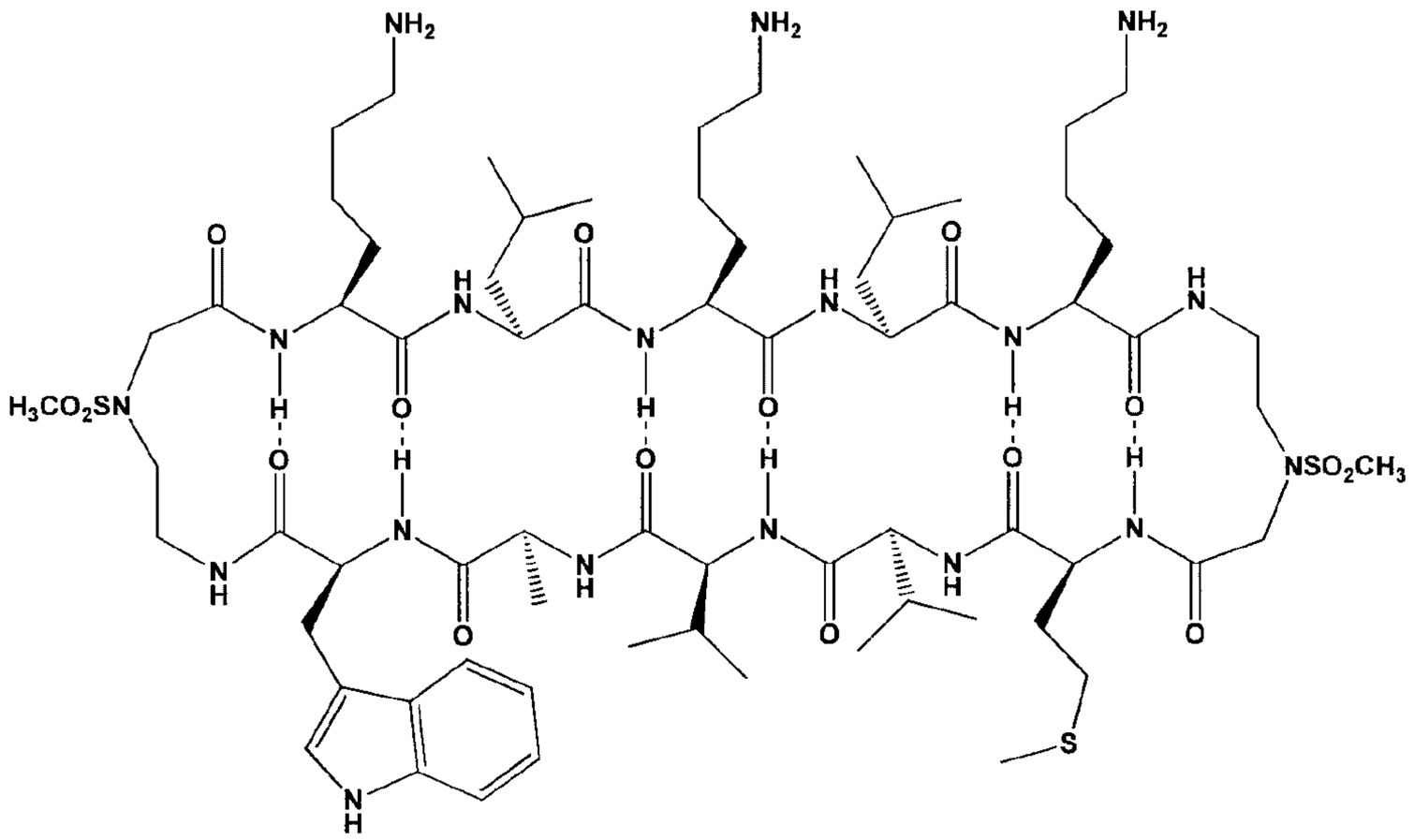
or a pharmaceutically acceptable salt thereof.

13. The cyclic compound of claim 1, wherein the cyclic compound has the structure:



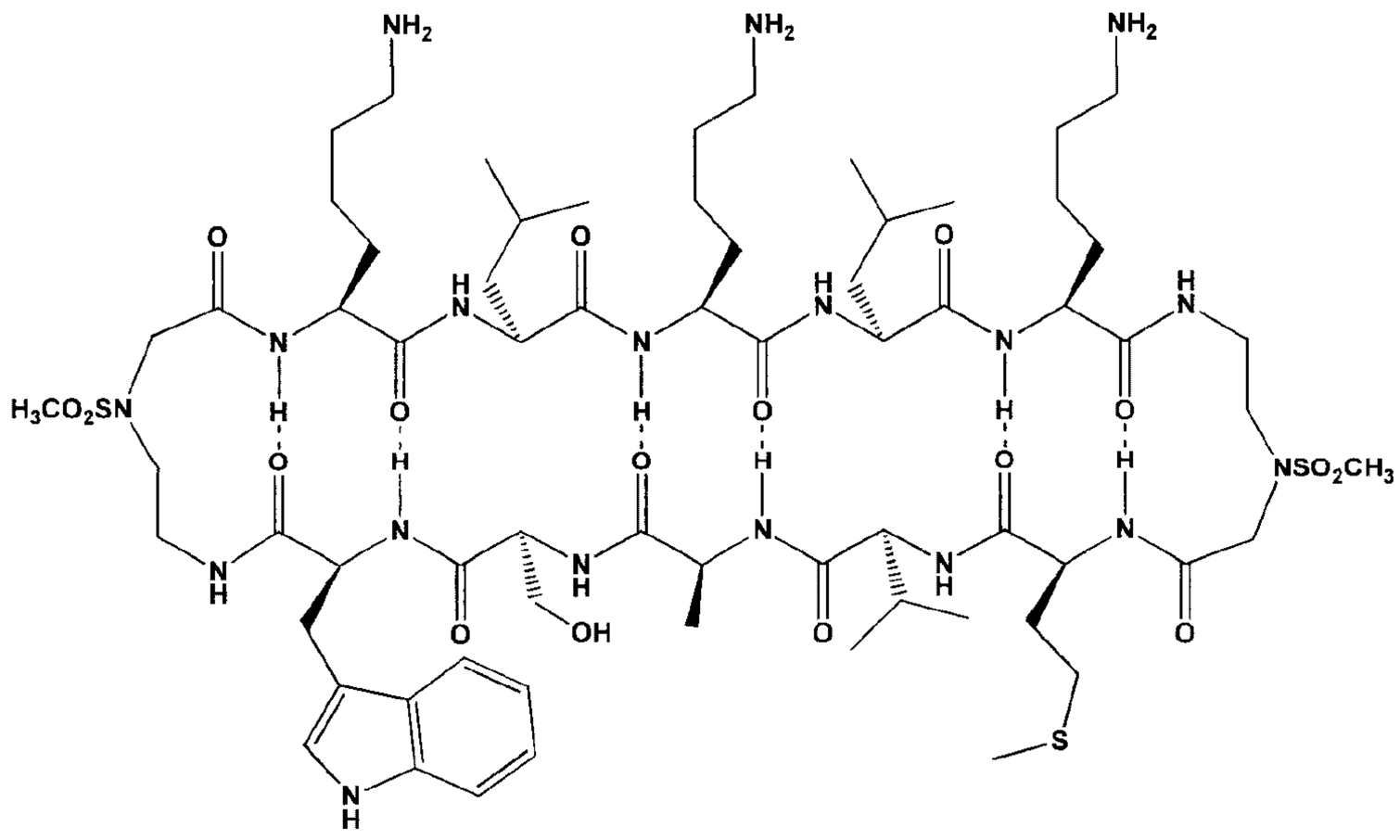
or a pharmaceutically acceptable salt thereof.

14. The cyclic compound of claim 1, wherein the cyclic compound has the structure:



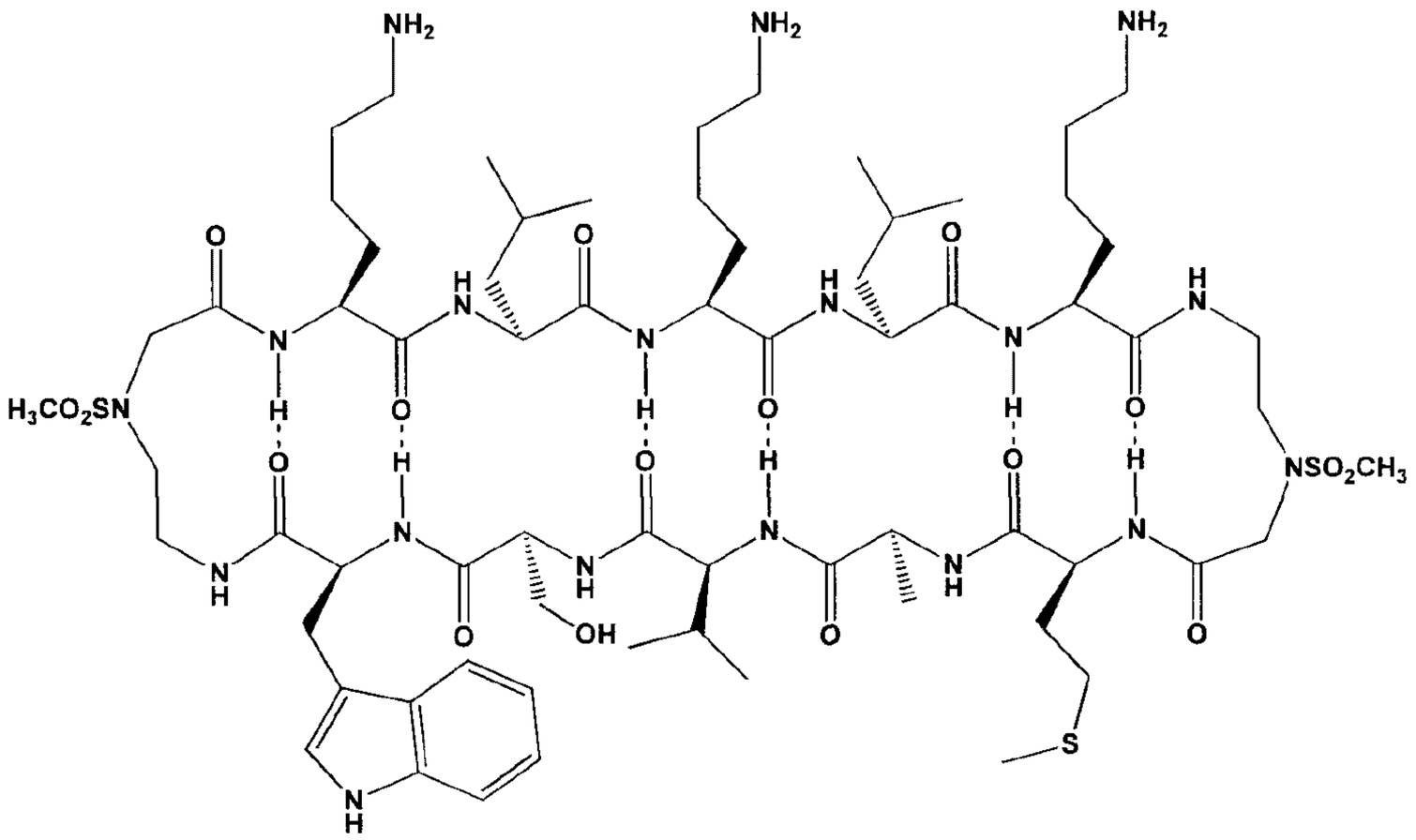
or a pharmaceutically acceptable salt thereof.

15. The cyclic compound of claim 1, wherein the cyclic compound has the structure:



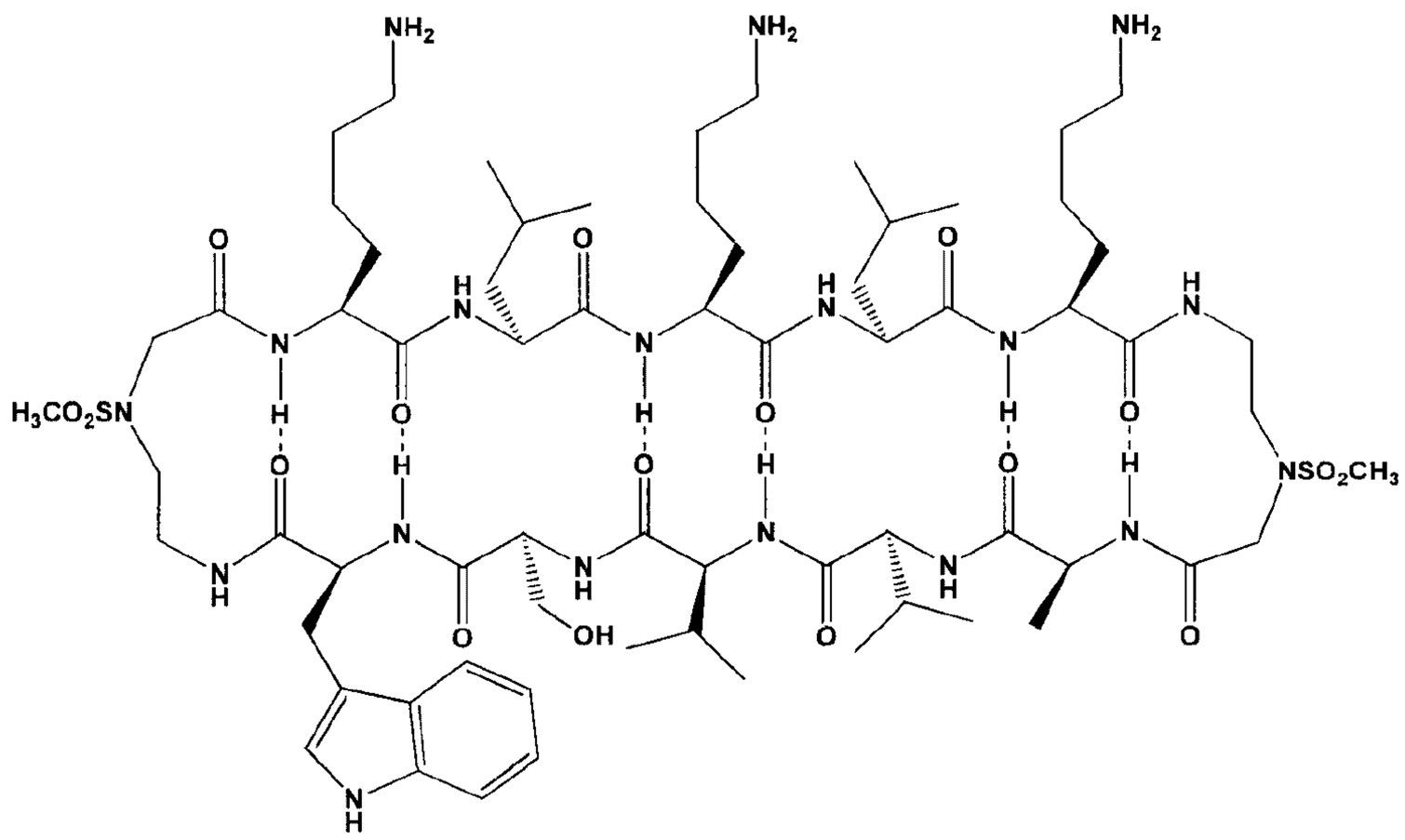
or a pharmaceutically acceptable salt thereof.

16. The cyclic compound of claim 1, wherein the cyclic compound has the structure:



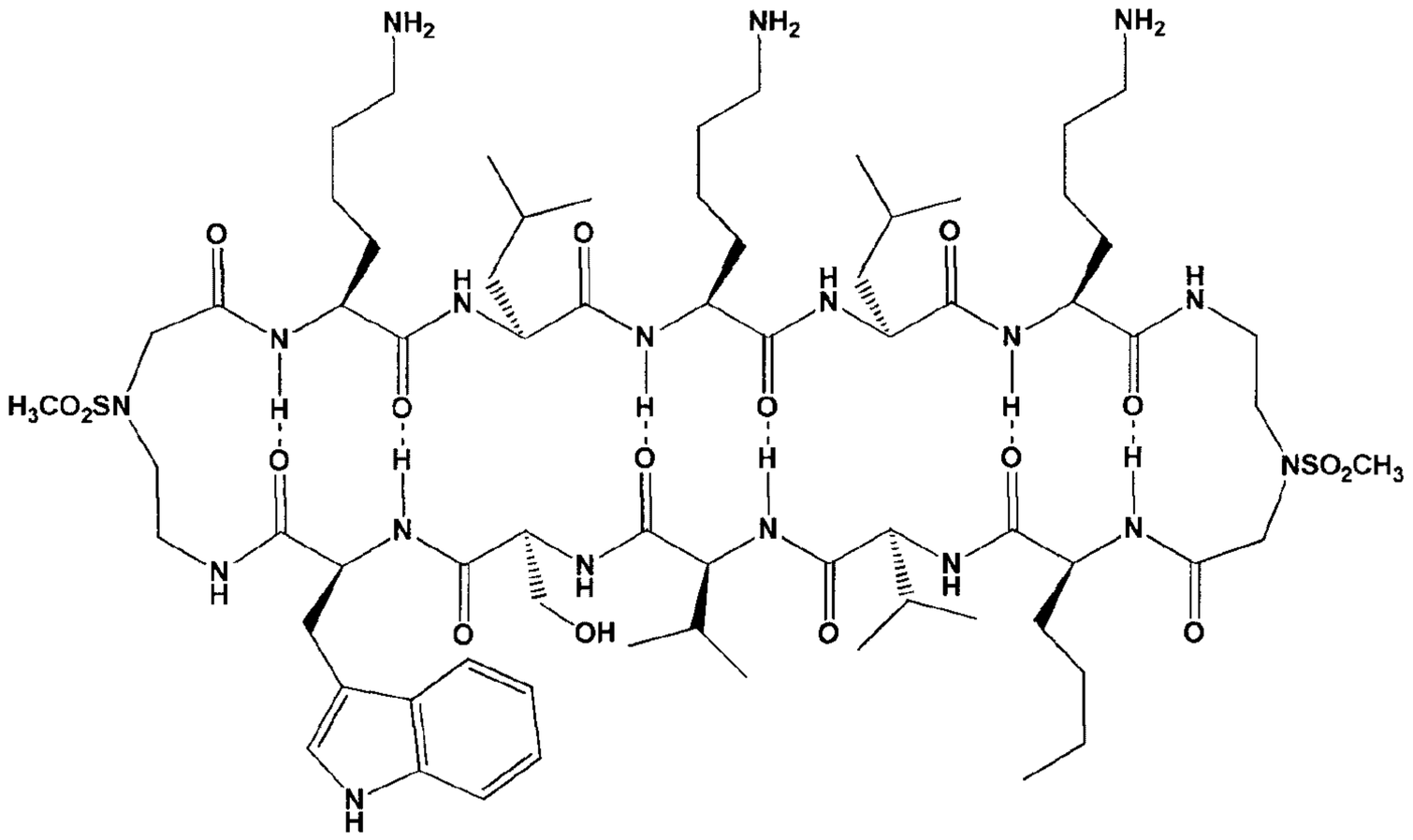
or a pharmaceutically acceptable salt thereof.

17. The cyclic compound of claim 1, wherein the cyclic compound has the structure:



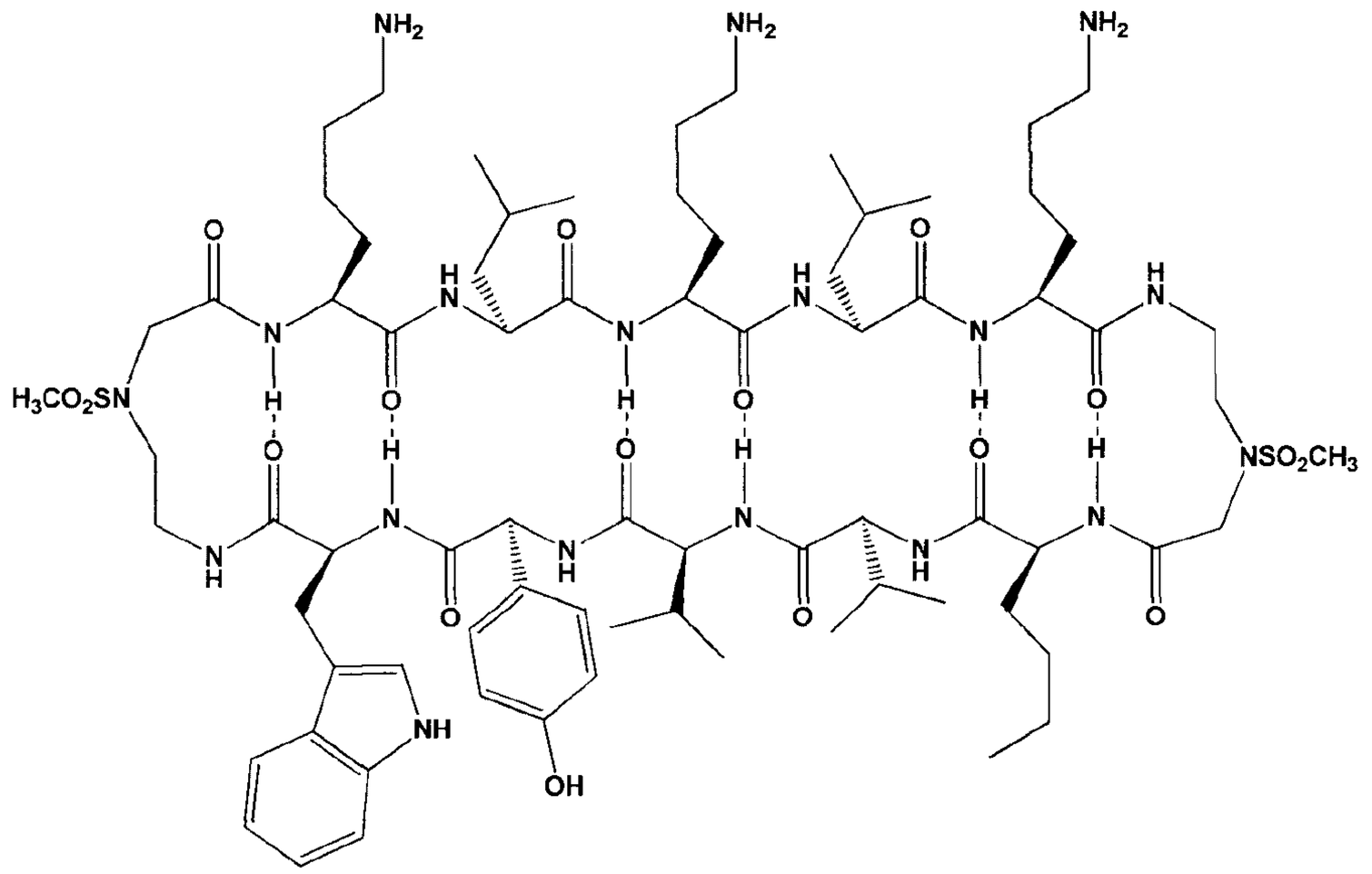
or a pharmaceutically acceptable salt thereof.

18. The cyclic compound of claim 1, wherein the cyclic compound has the structure:



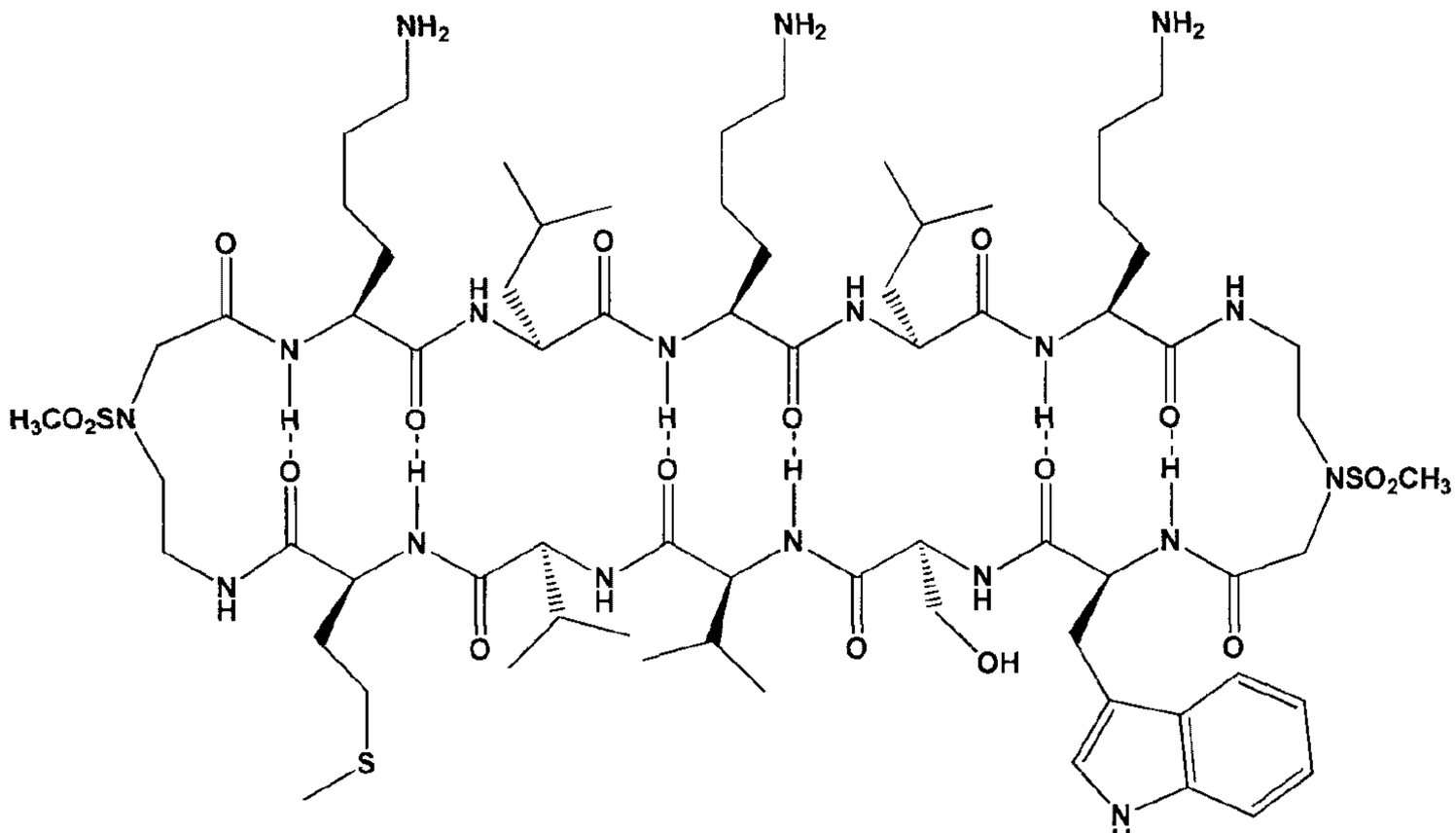
or a pharmaceutically acceptable salt thereof.

19. The cyclic compound of claim 1, wherein the cyclic compound has the structure:



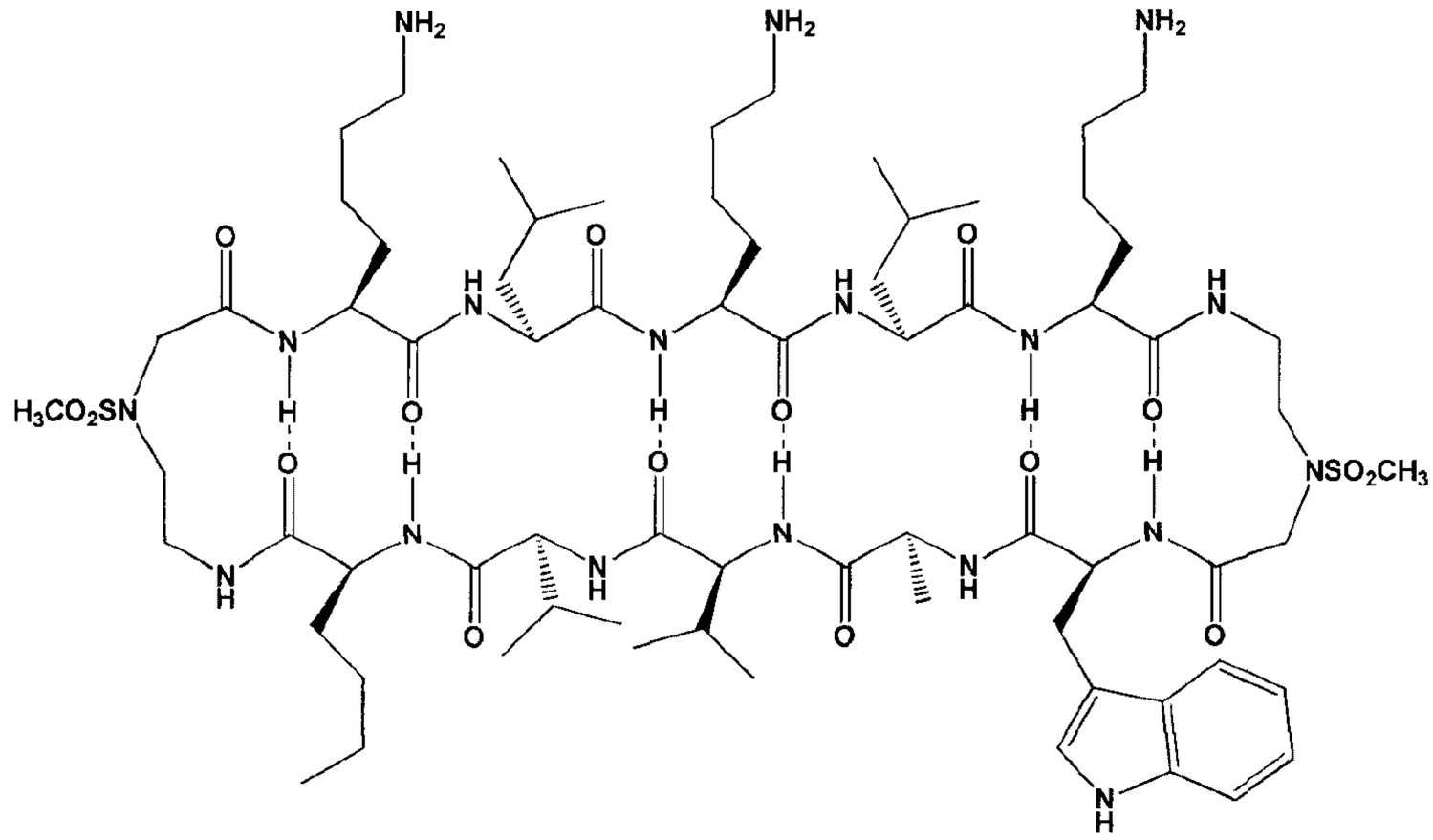
or a pharmaceutically acceptable salt thereof.

20. The cyclic compound of claim 1, wherein the cyclic compound has the structure:



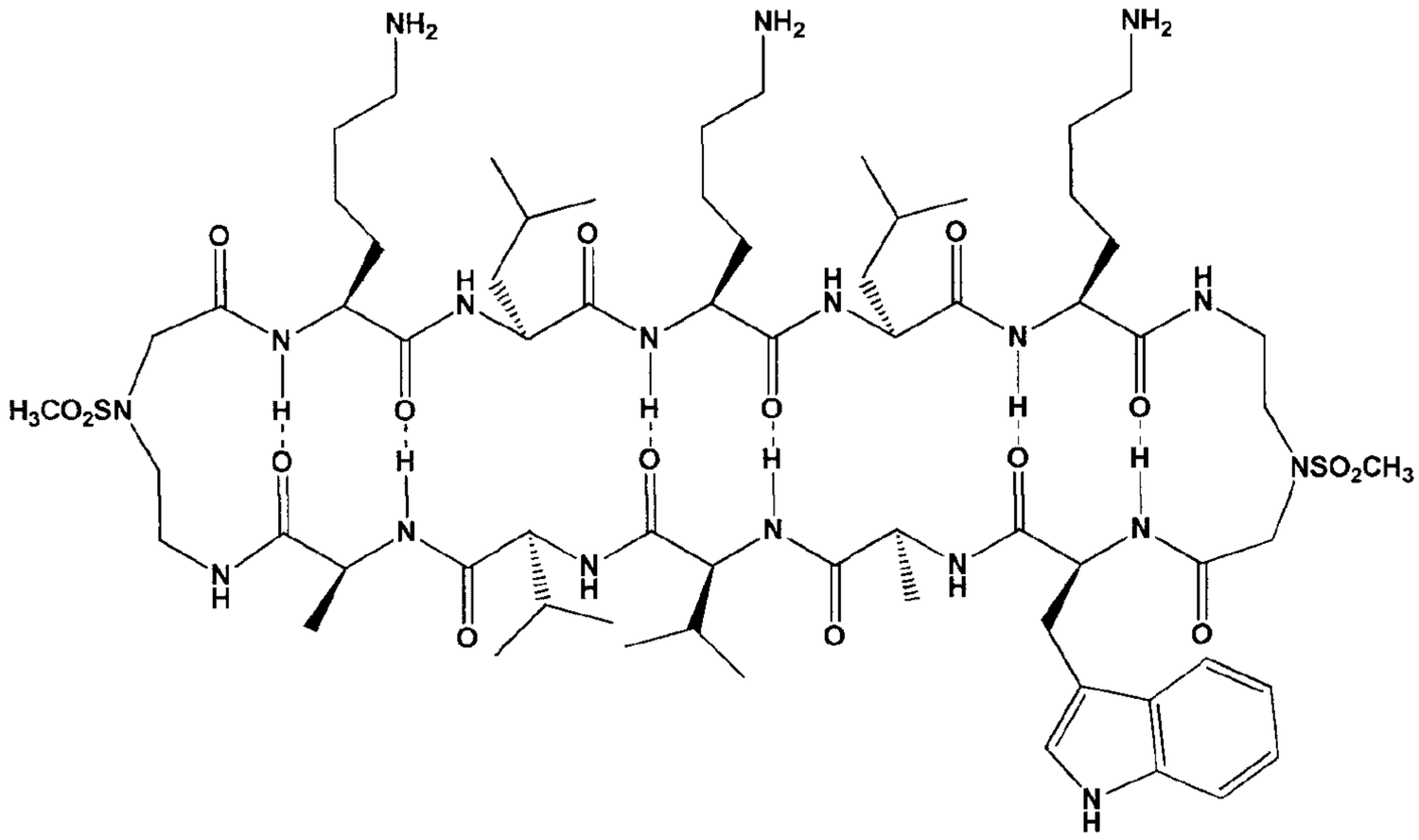
or a pharmaceutically acceptable salt thereof.

21. The cyclic compound of claim 1, wherein the cyclic compound has the structure:



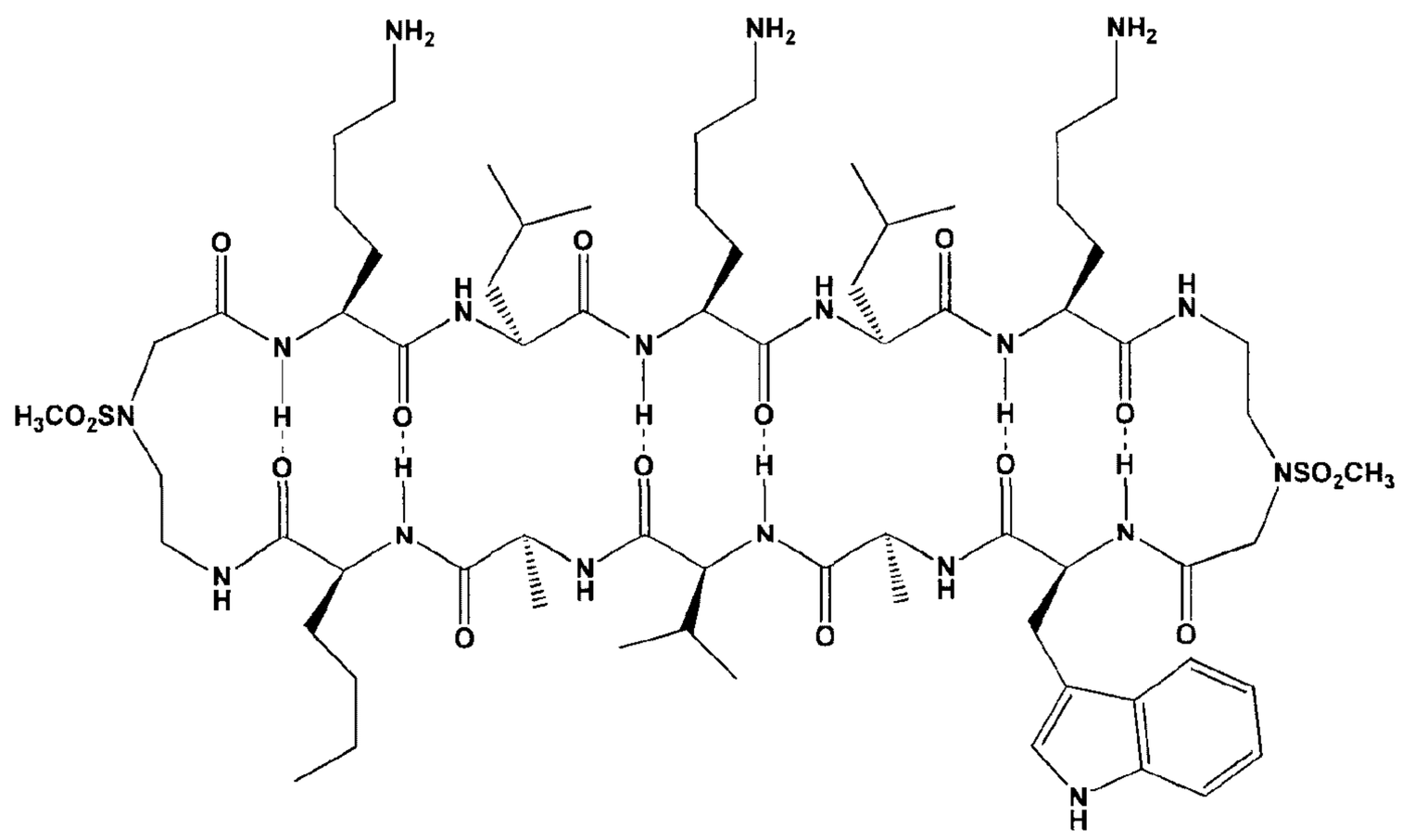
or a pharmaceutically acceptable salt thereof.

22. The cyclic compound of claim 1, wherein the cyclic compound has the structure:



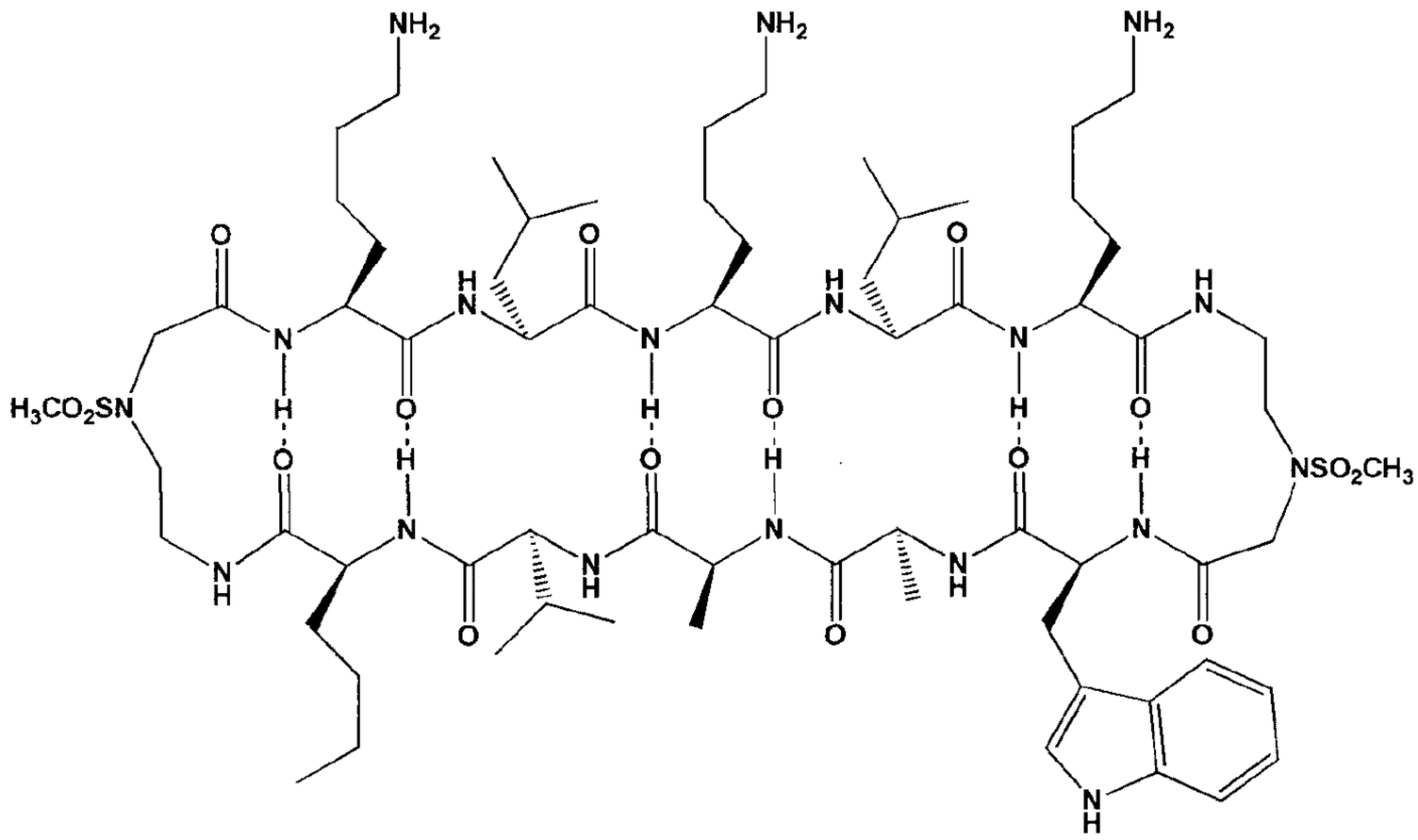
or a pharmaceutically acceptable salt thereof.

23. The cyclic compound of claim 1, wherein the cyclic compound has the structure:



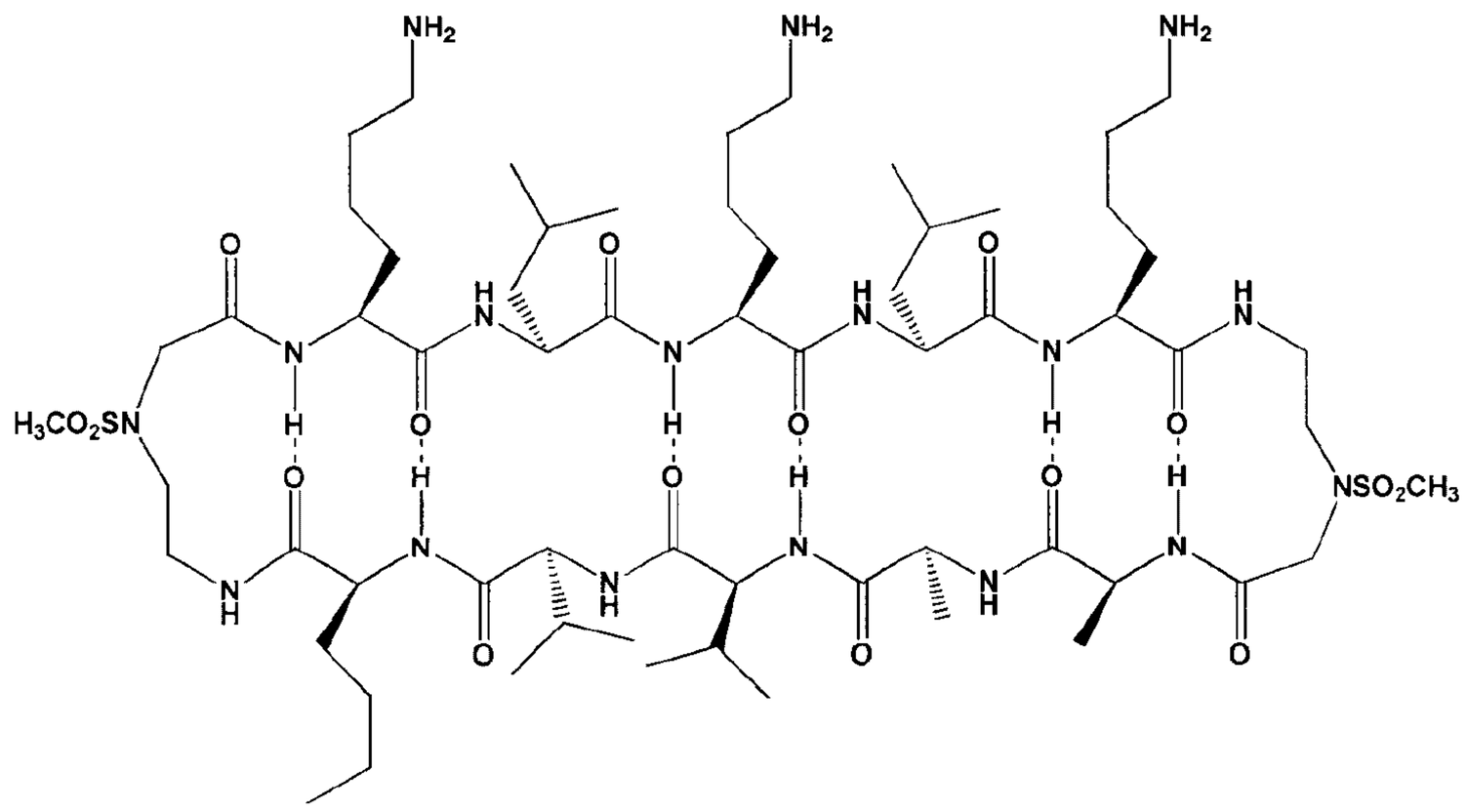
or a pharmaceutically acceptable salt thereof.

24. The cyclic compound of claim 1, wherein the cyclic compound has the structure:



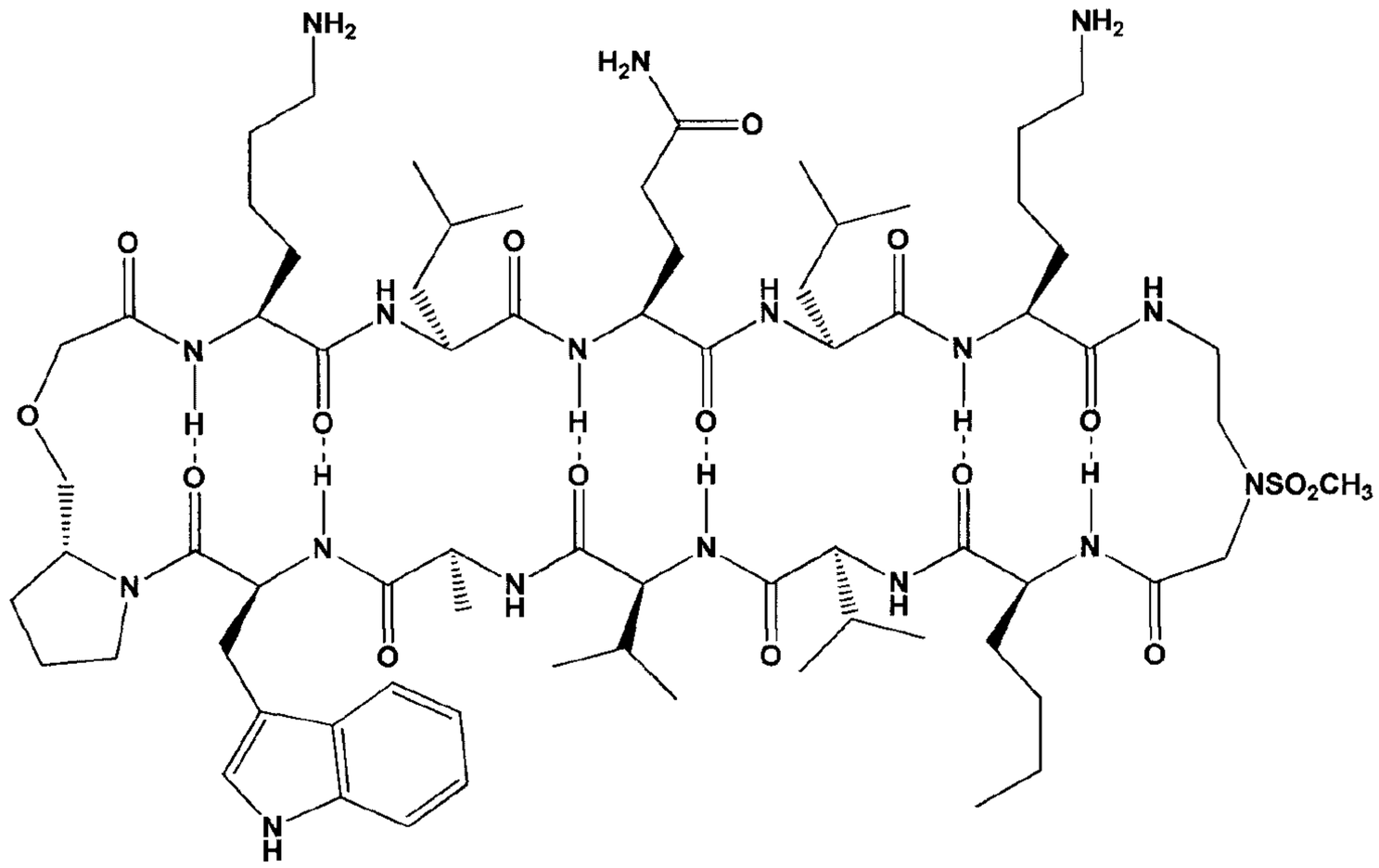
or a pharmaceutically acceptable salt thereof.

25. The cyclic compound of claim 1, wherein the cyclic compound has the structure:



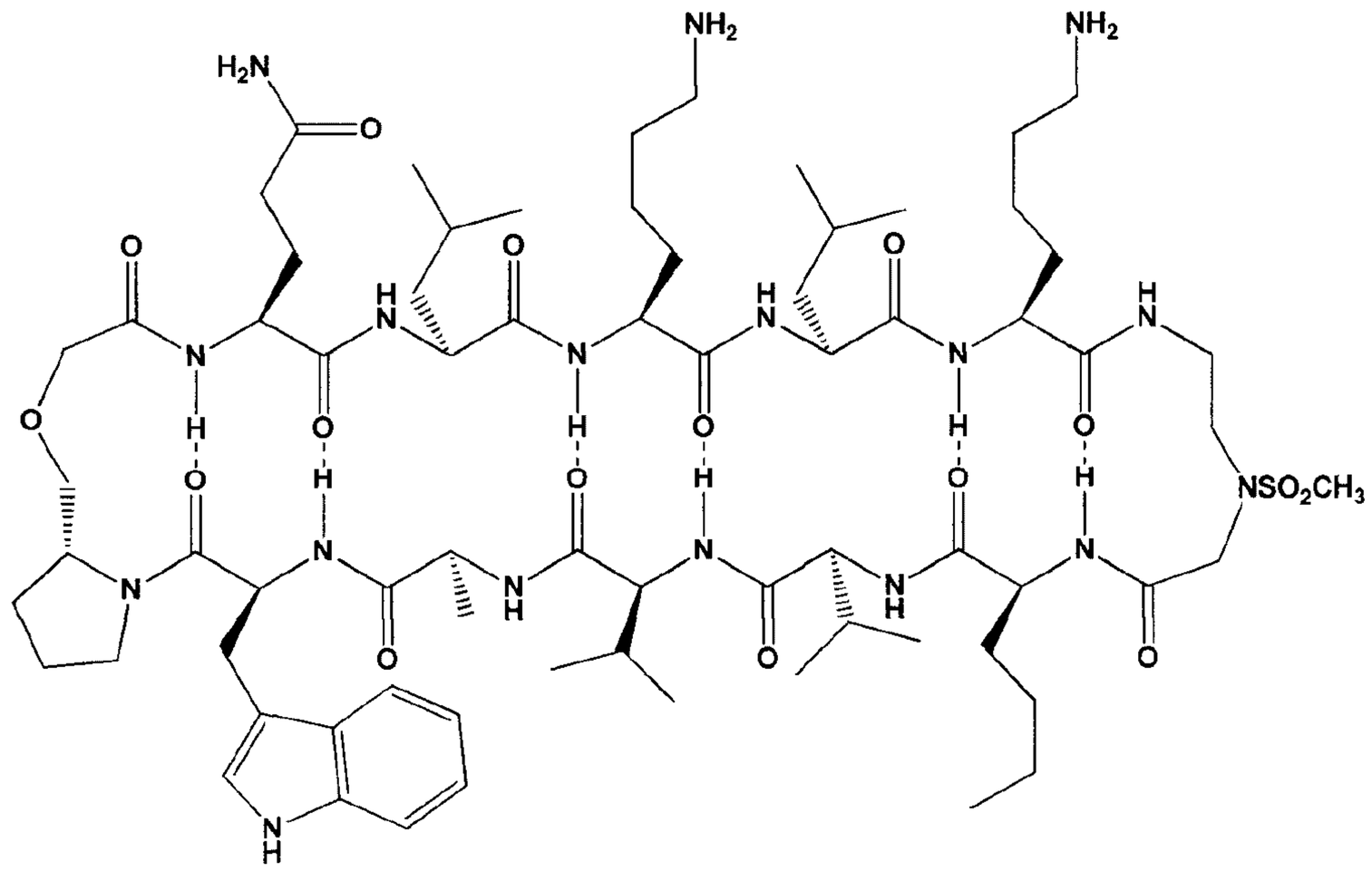
or a pharmaceutically acceptable salt thereof.

26. The cyclic compound of claim 1, wherein the cyclic compound has the structure:



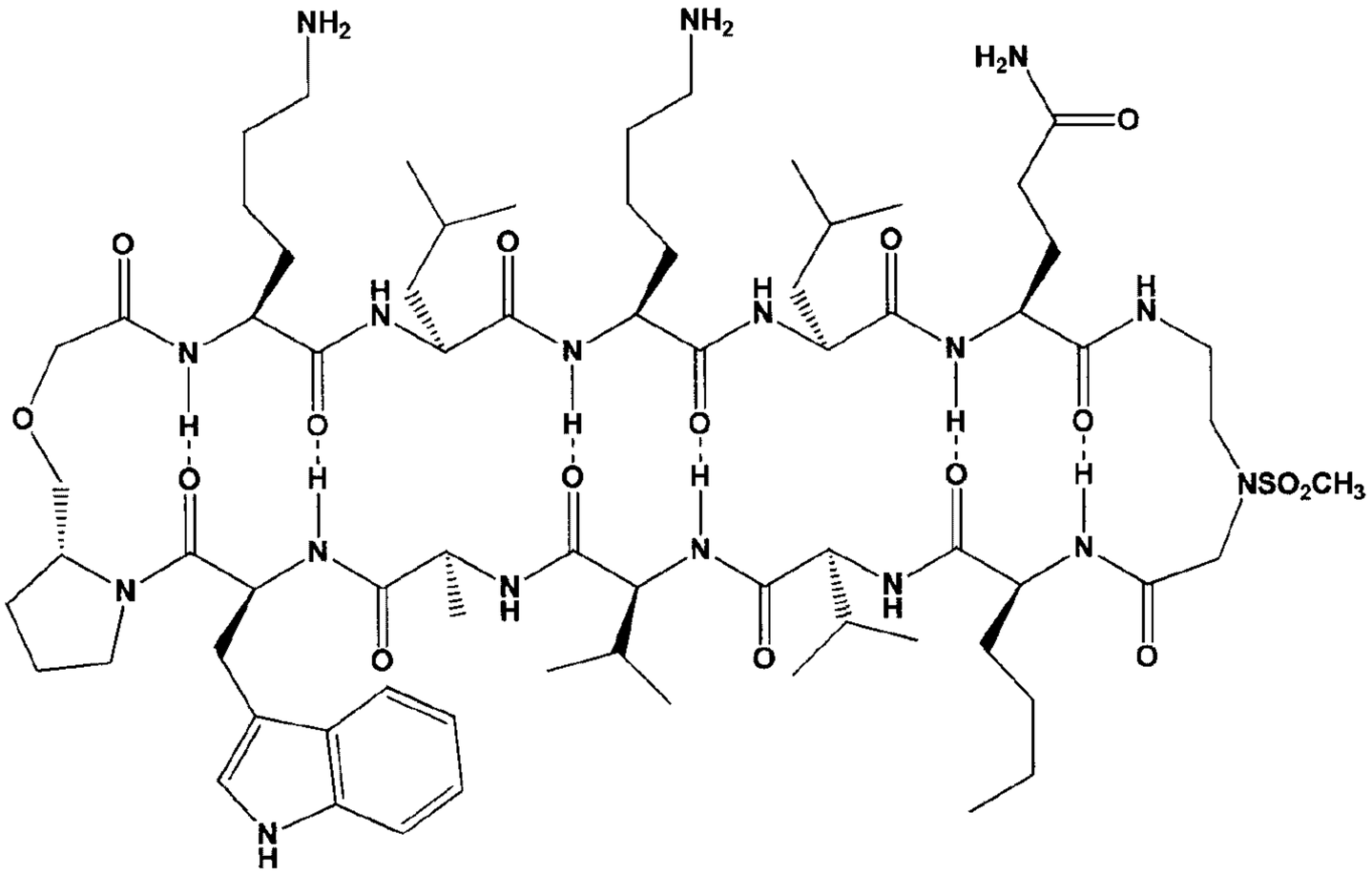
or a pharmaceutically acceptable salt thereof.

27. The cyclic compound of claim 1, wherein the cyclic compound has the structure:



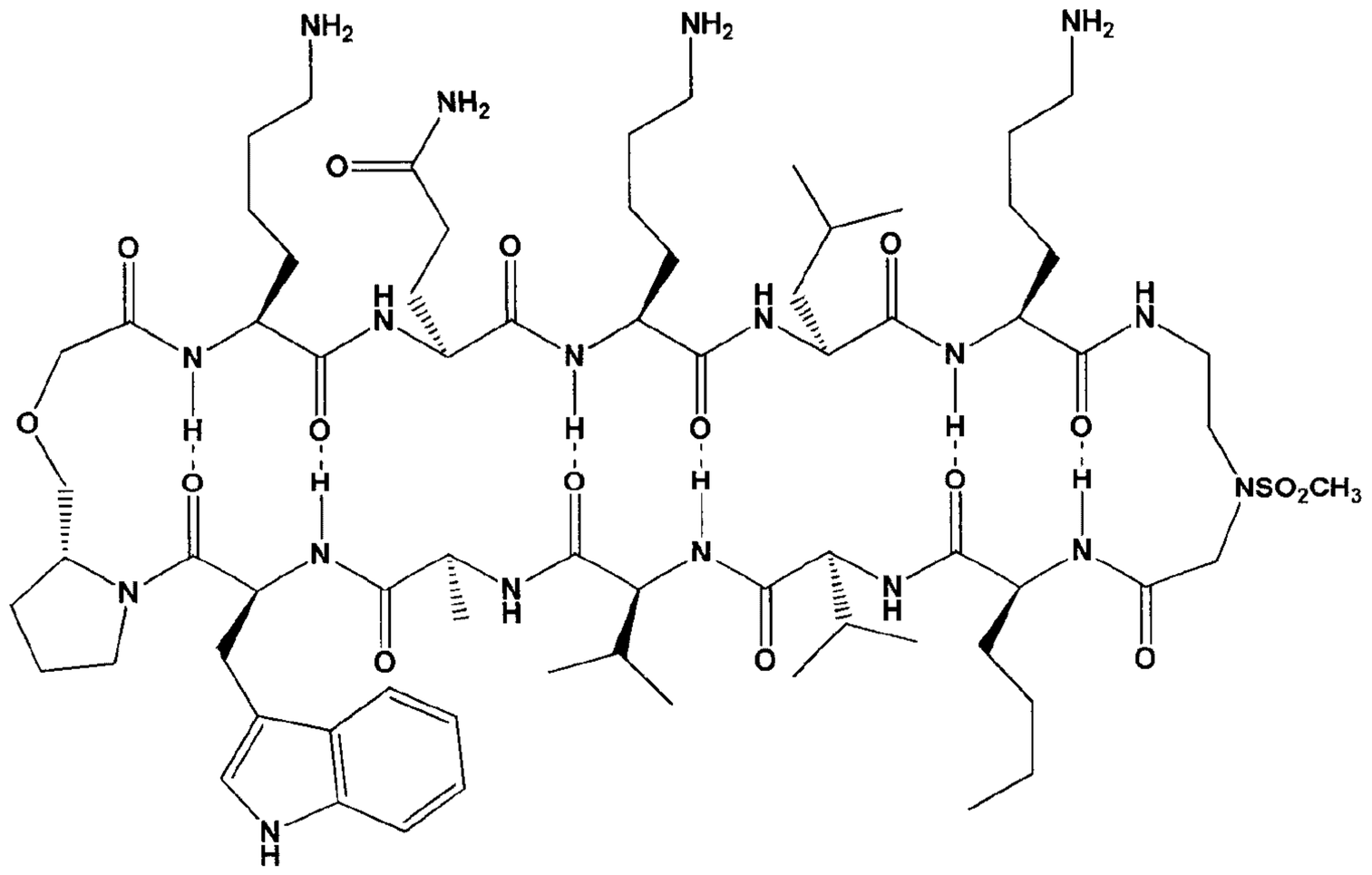
or a pharmaceutically acceptable salt thereof.

28. The cyclic compound of claim 1, wherein the cyclic compound has the structure:



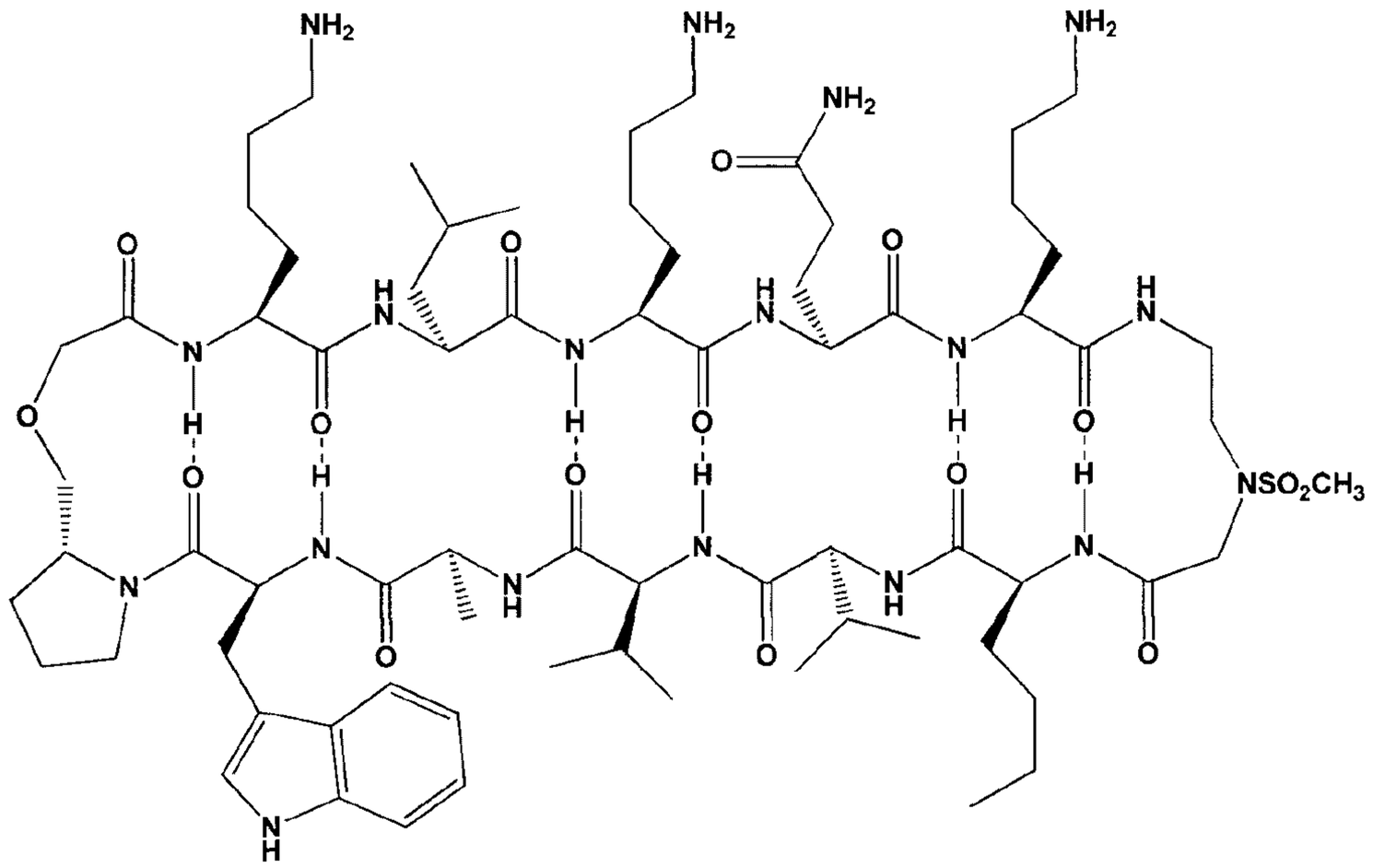
or a pharmaceutically acceptable salt thereof.

29. The cyclic compound of claim 1, wherein the cyclic compound has the structure:



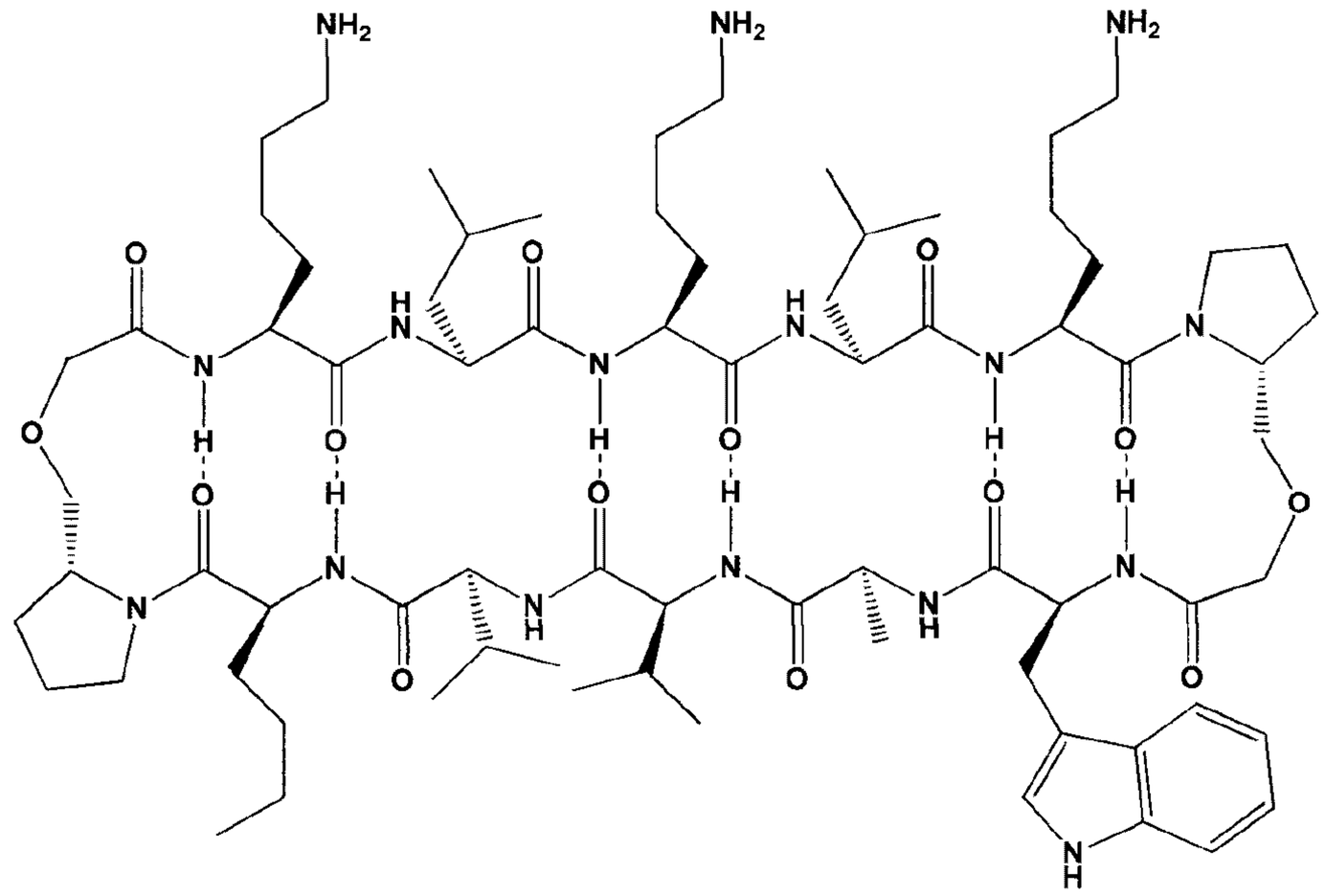
or a pharmaceutically acceptable salt thereof.

30. The cyclic compound of claim 1, wherein the cyclic compound has the structure:



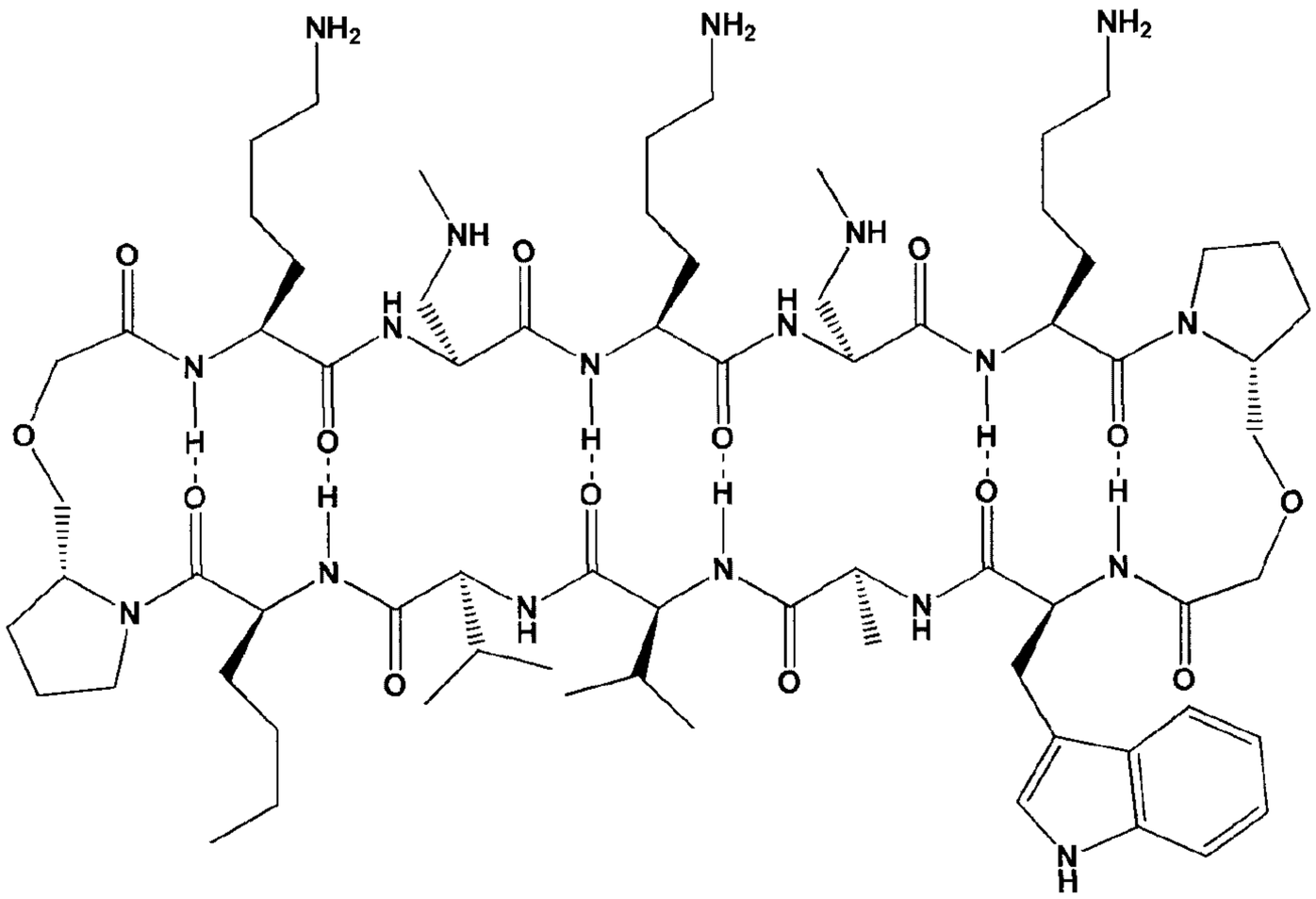
or a pharmaceutically acceptable salt thereof.

31. The cyclic compound of claim 1, wherein the cyclic compound has the structure:



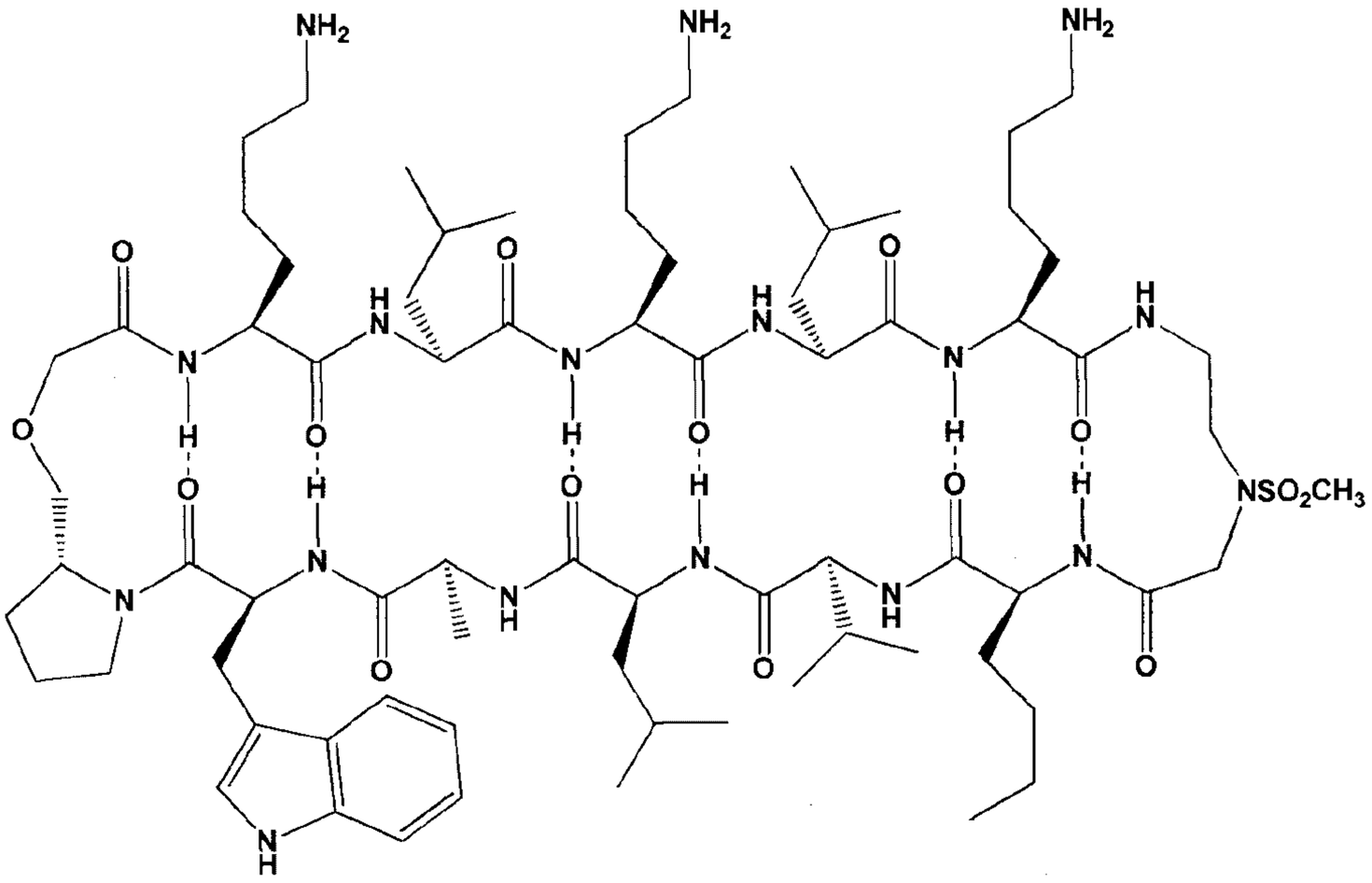
or a pharmaceutically acceptable salt thereof.

32. The cyclic compound of claim 1, wherein the cyclic compound has the structure:



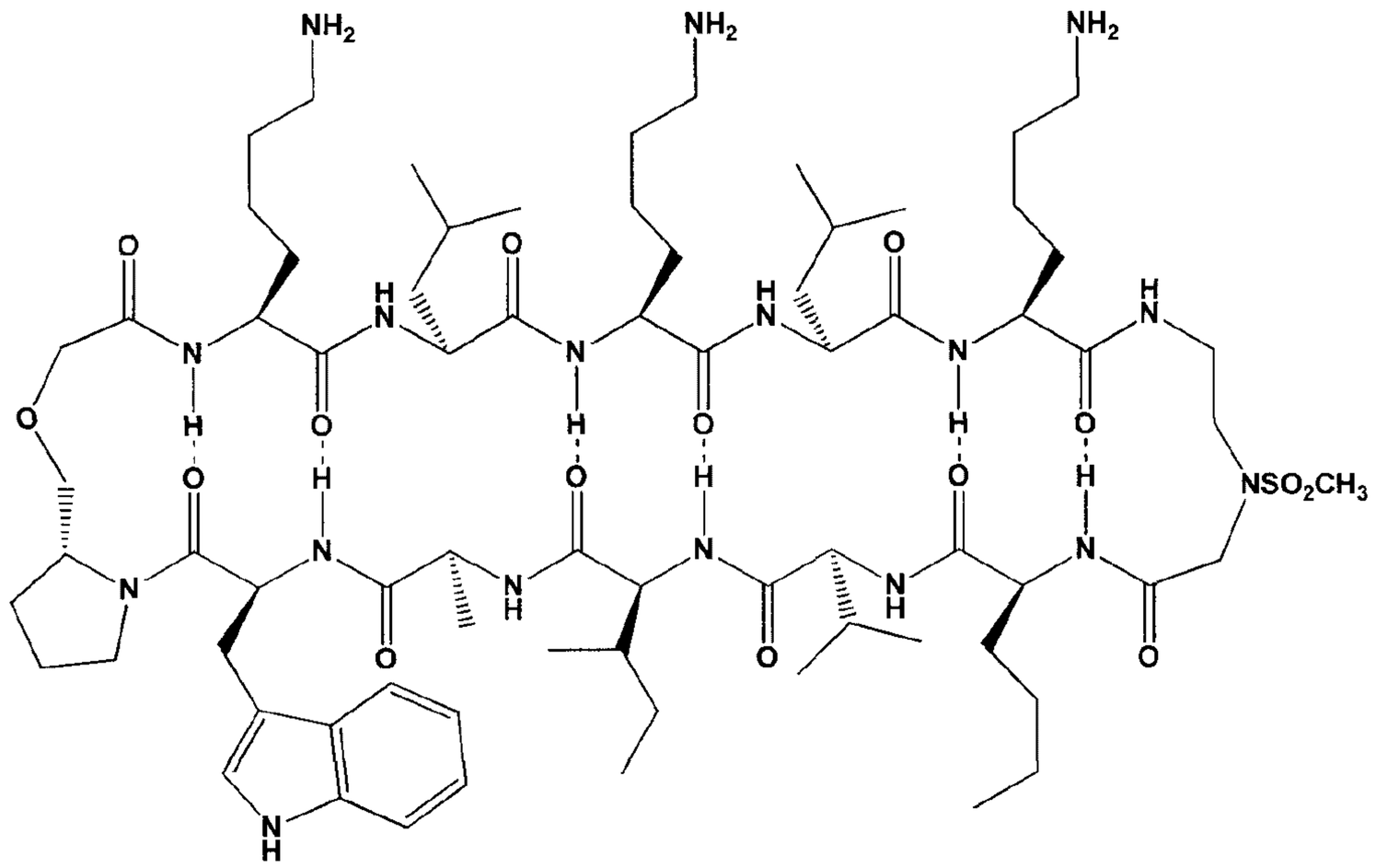
or a pharmaceutically acceptable salt thereof.

33. The cyclic compound of claim 1, wherein the cyclic compound has the structure:



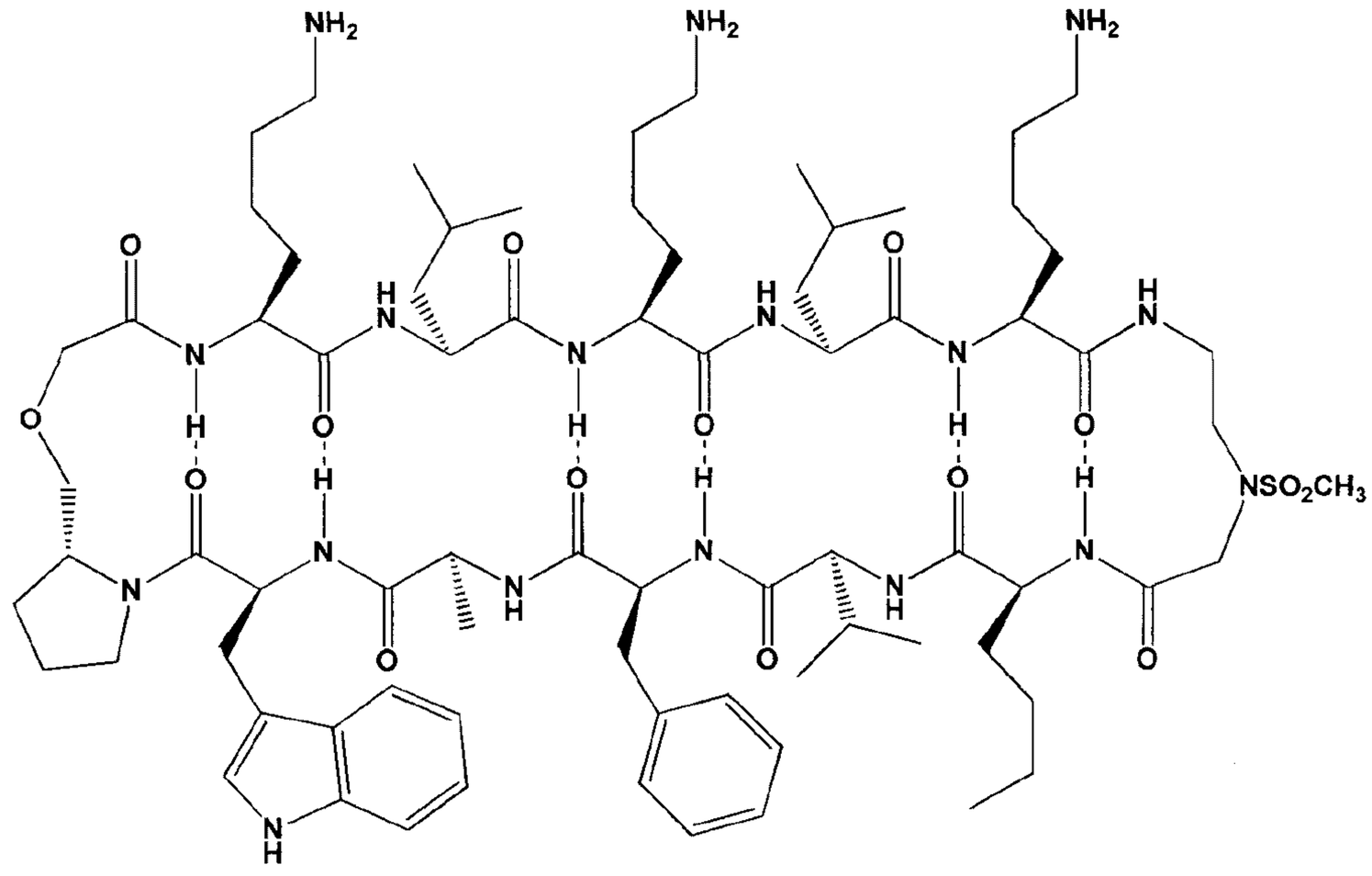
or a pharmaceutically acceptable salt thereof.

34. The cyclic compound of claim 1, wherein the cyclic compound has the structure:



or a pharmaceutically acceptable salt thereof.

35. The cyclic compound of claim 1, wherein the cyclic compound has the structure:



or a pharmaceutically acceptable salt thereof.

36. A composition comprising said cyclic compound of any one of claims 1 to 35; and a pharmaceutically acceptable carrier.

37. The composition of claim 36, further comprising at least one other anti-cancer agent.

38. The use according to claim 4 or 5, wherein the proliferation disorder is an autoimmune disorder.

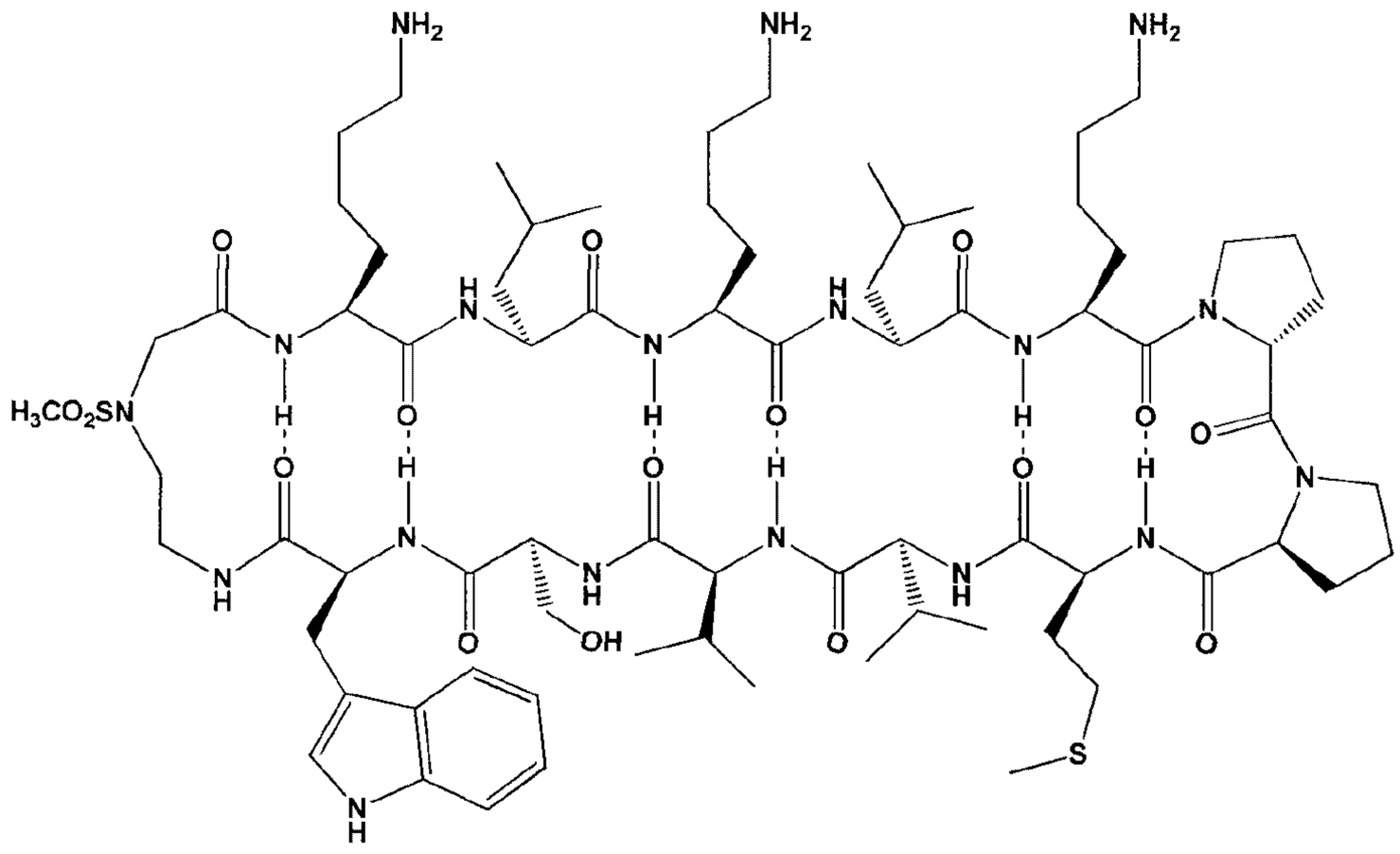
39. The use according to claim 38, wherein the autoimmune disorder is type 1 diabetes.

40. The use according to claim 39, wherein the autoimmune disorder is lupus.

41. The use according to claim 39, wherein the autoimmune disorder is multiple sclerosis.

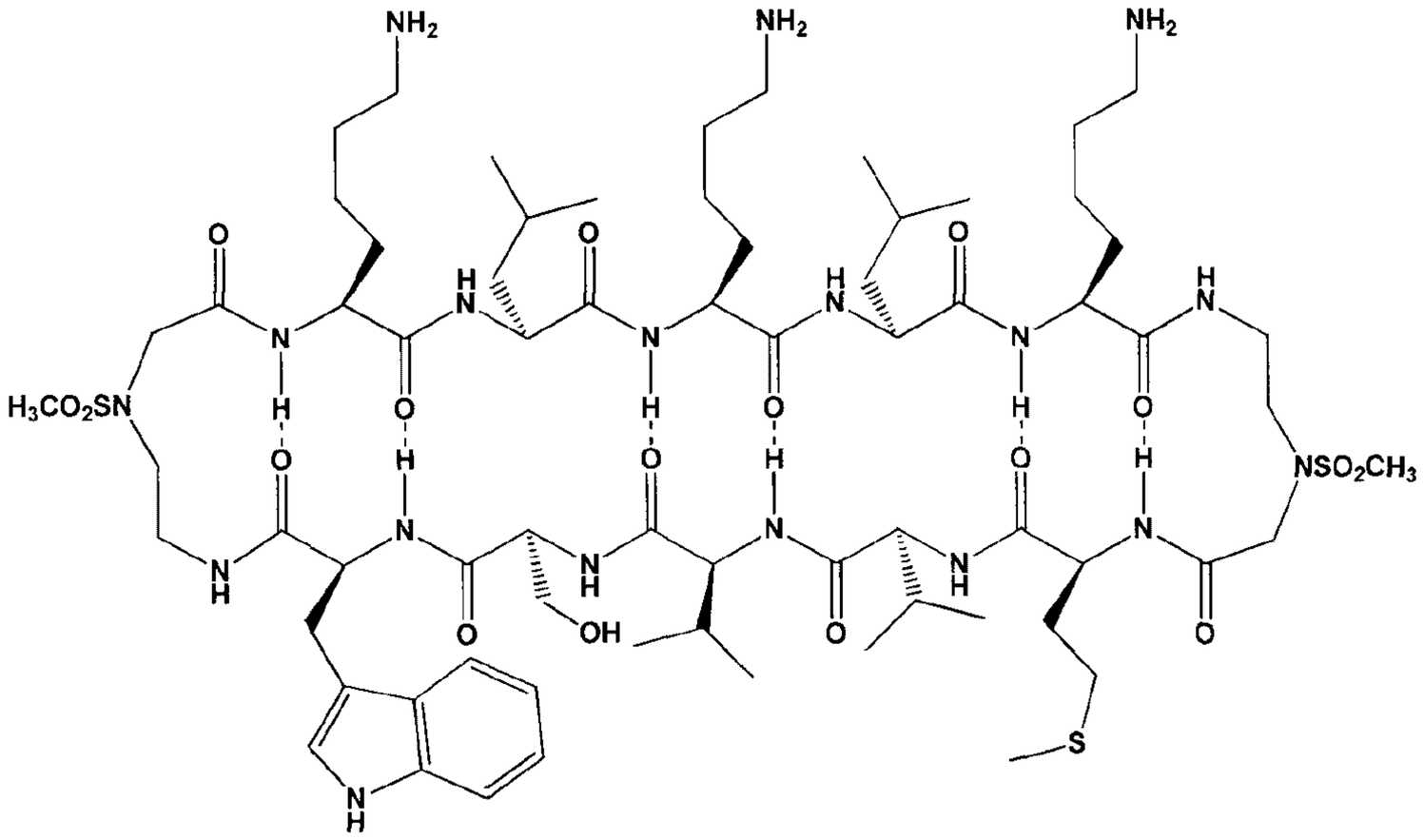
42. The use according to claim 4 or 5, wherein the subject is human.

43. The use according to claim 4 or 5, wherein the cyclic compound has the structure:



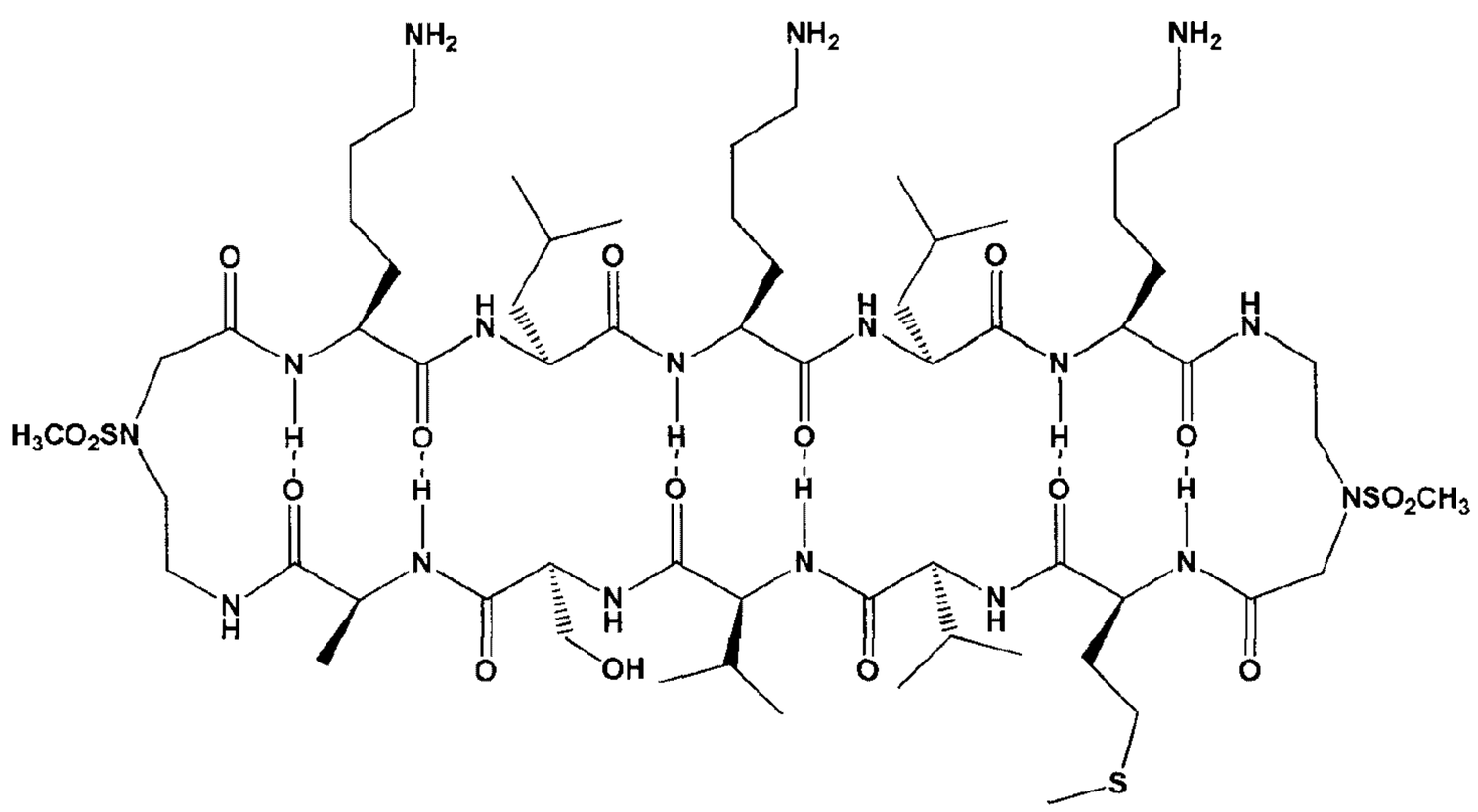
or a pharmaceutically acceptable salt thereof.

44. The use according to claim 4 or 5, wherein the cyclic compound has the structure:



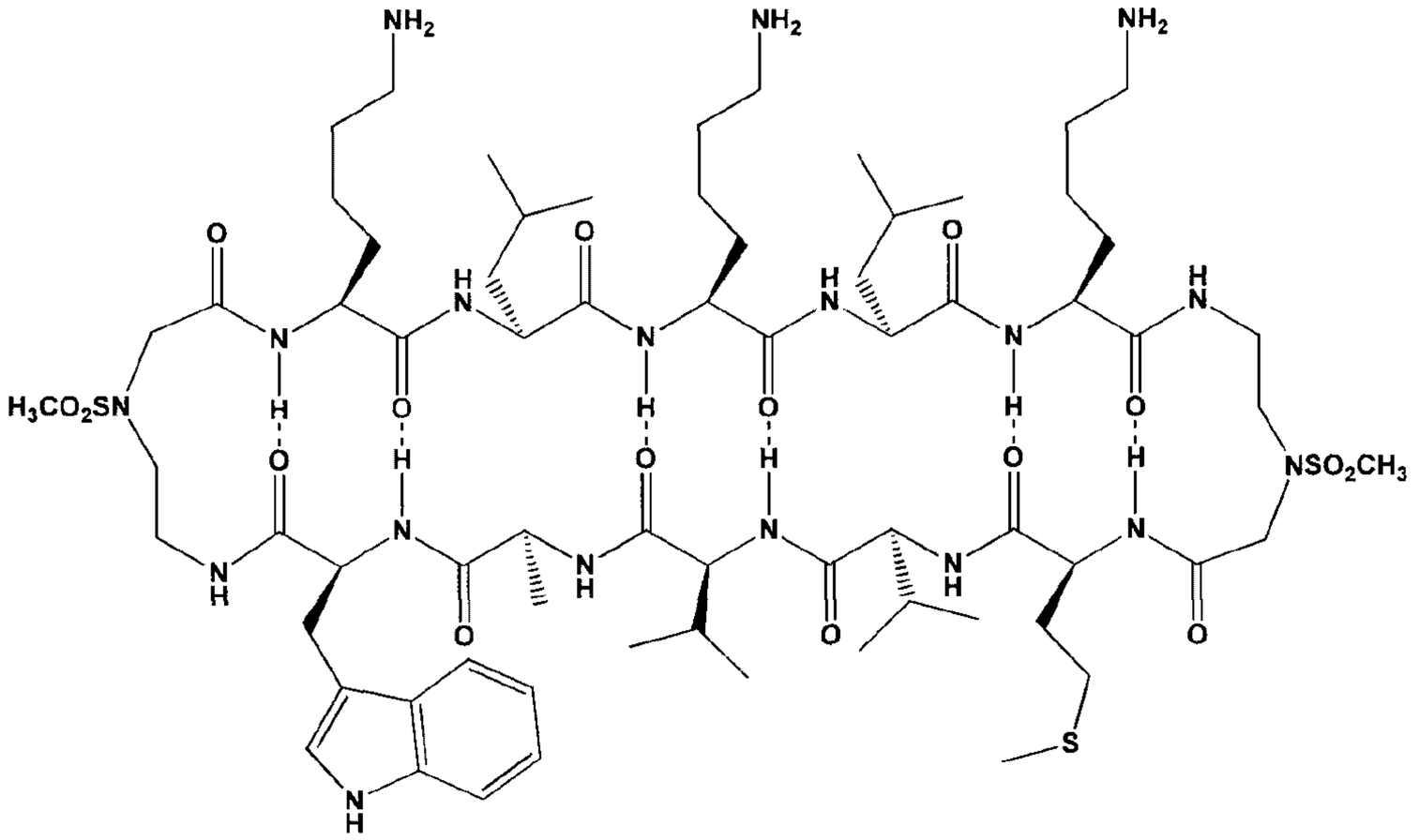
or a pharmaceutically acceptable salt thereof.

45. The use according to claim 4 or 5, wherein the cyclic compound has the structure:



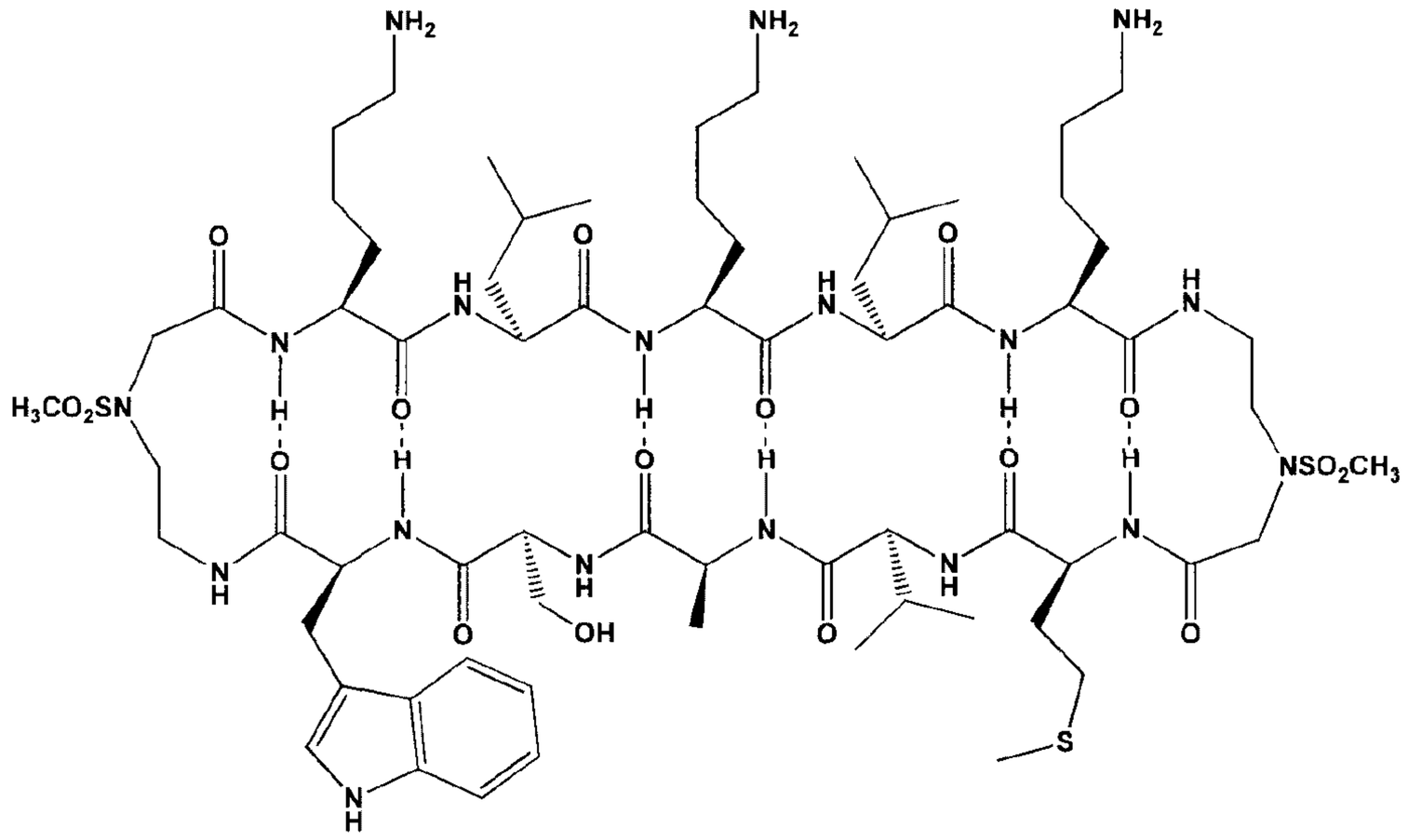
or a pharmaceutically acceptable salt thereof.

46. The use according to claim 4 or 5, wherein the cyclic compound has the structure:



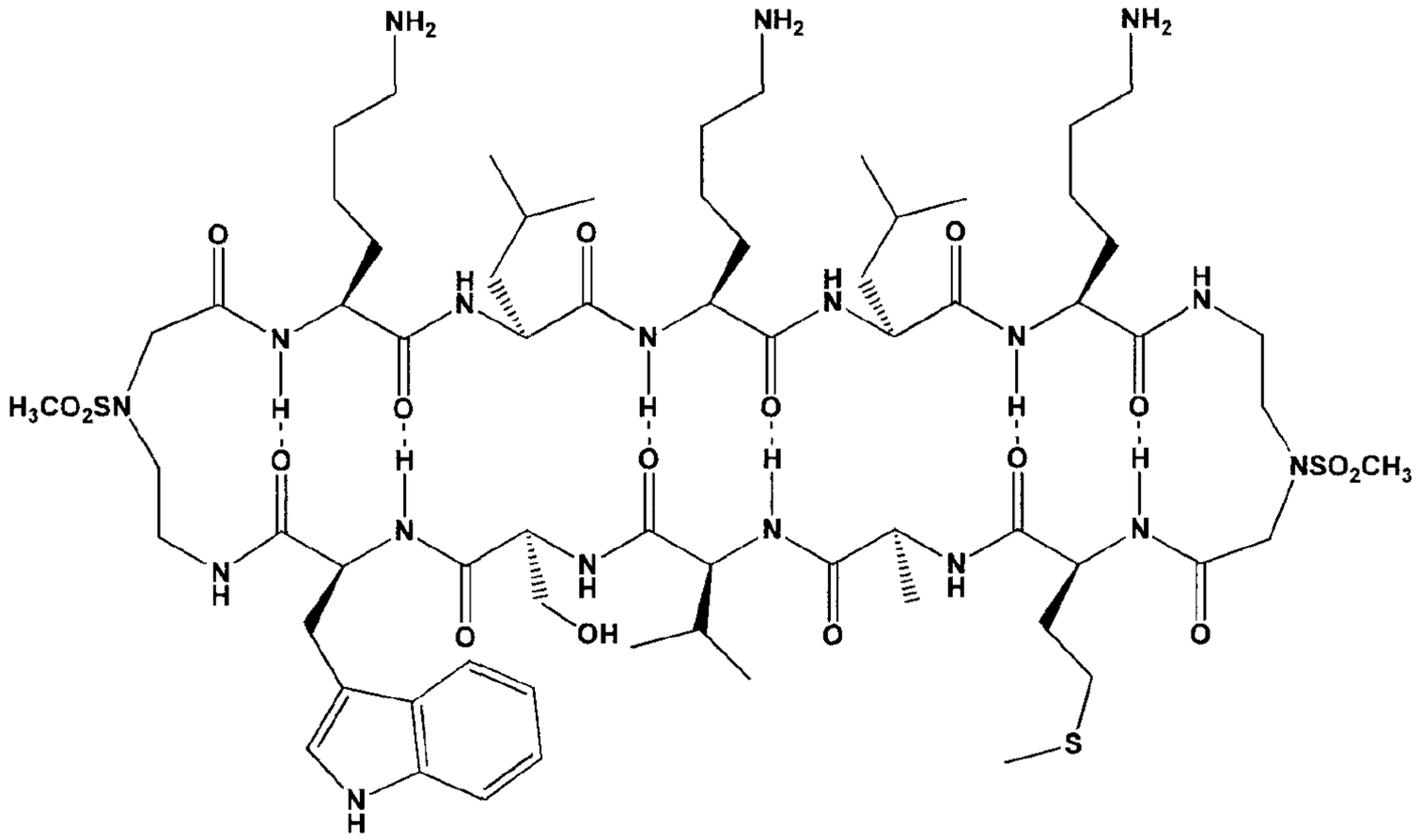
or a pharmaceutically acceptable salt thereof.

47. The use according to claim 4 or 5, wherein the cyclic compound has the structure:



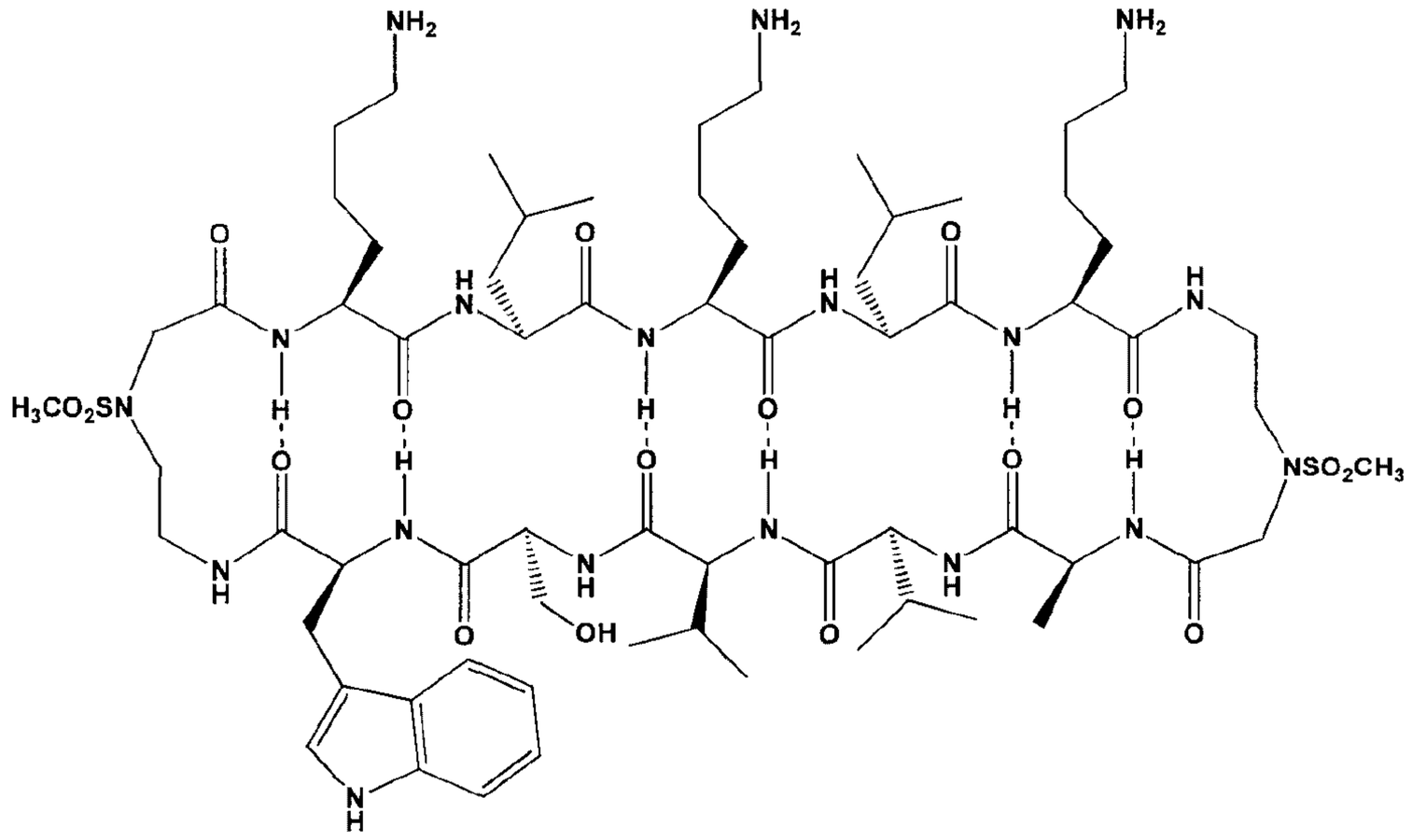
or a pharmaceutically acceptable salt thereof.

48. The use according to claim 4 or 5, wherein the cyclic compound has the structure:



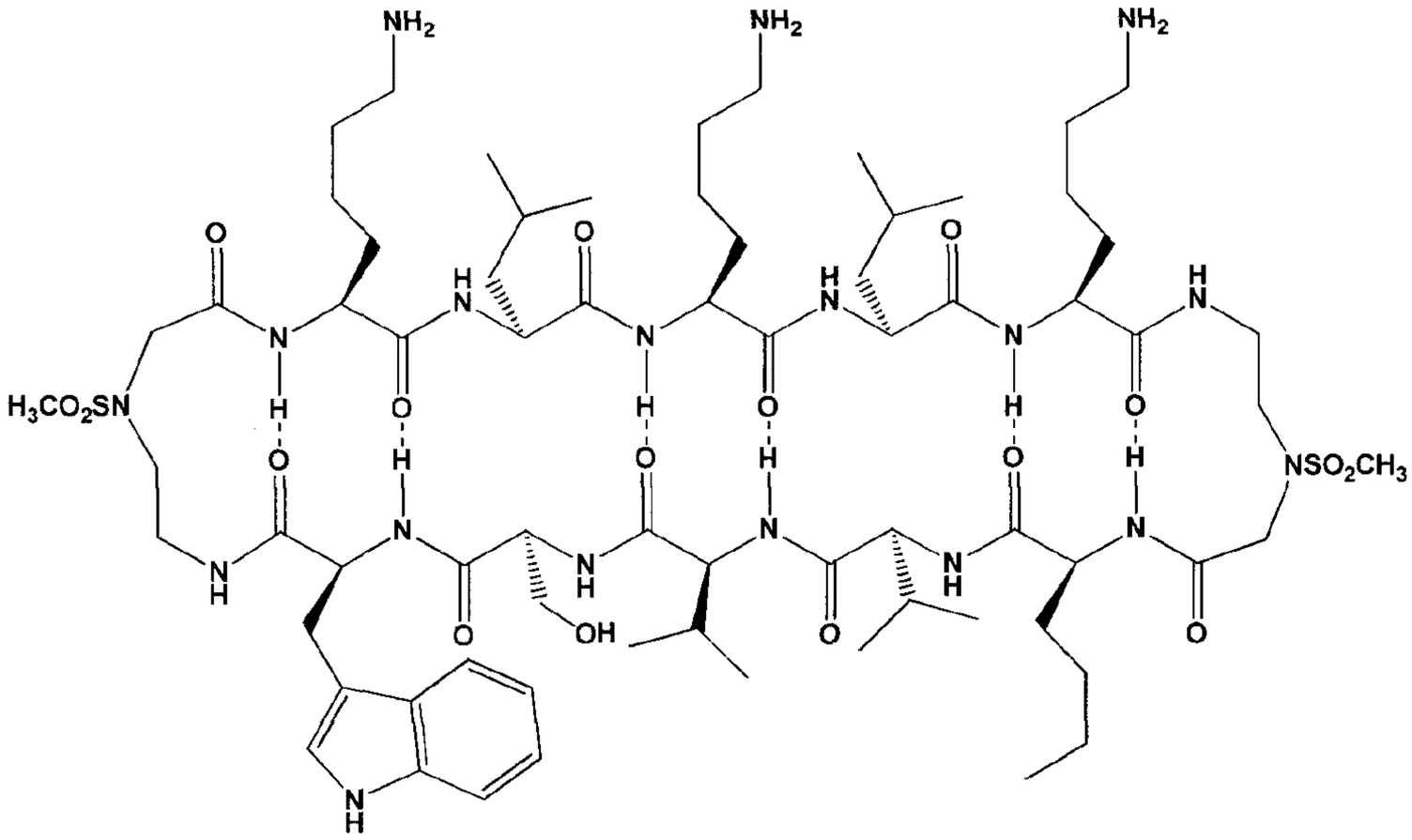
or a pharmaceutically acceptable salt thereof.

49. The use according to claim 4 or 5, wherein the cyclic compound has the structure:



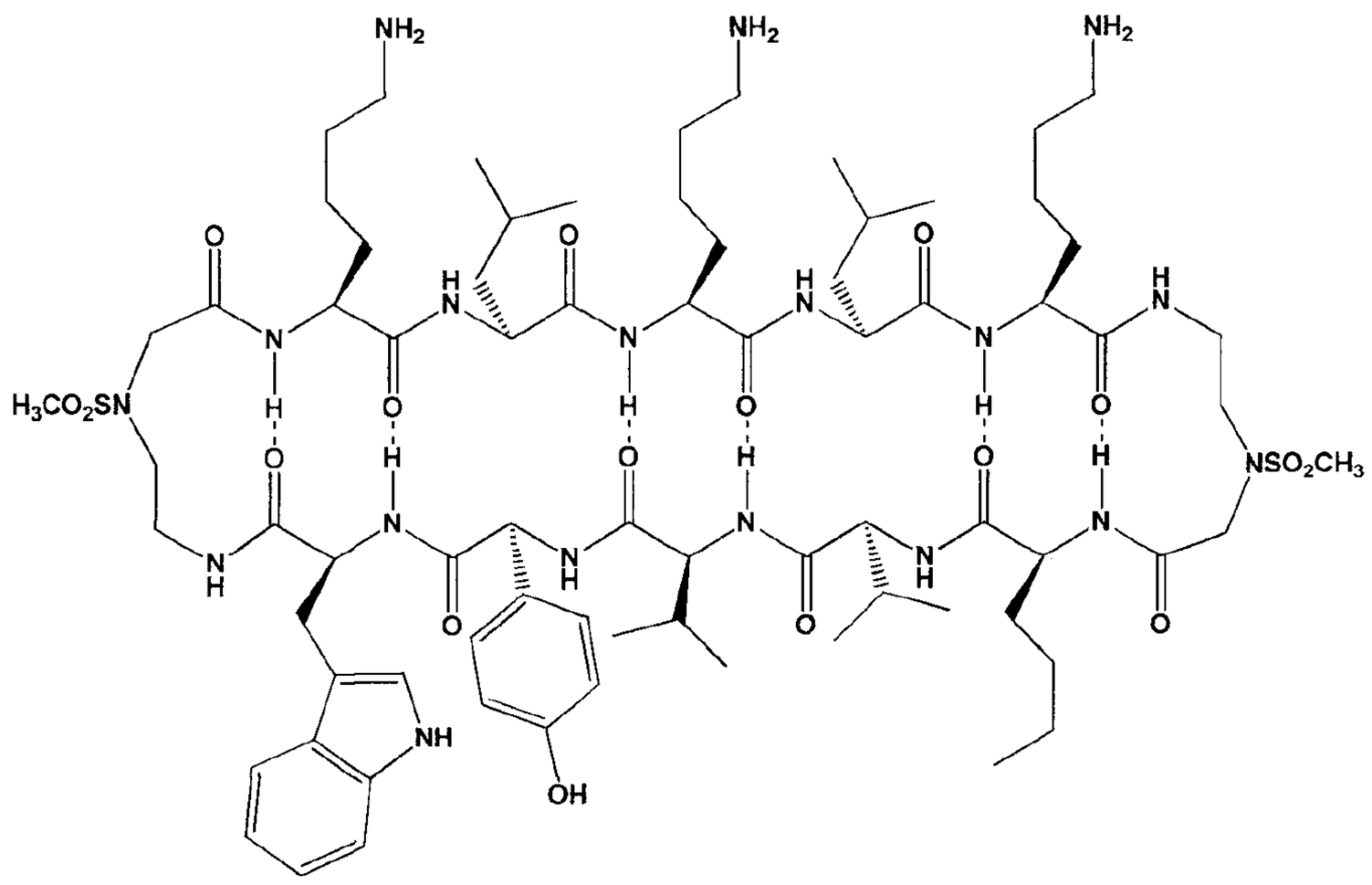
or a pharmaceutically acceptable salt thereof.

50. The use according to claim 4 or 5, wherein the cyclic compound has the structure:



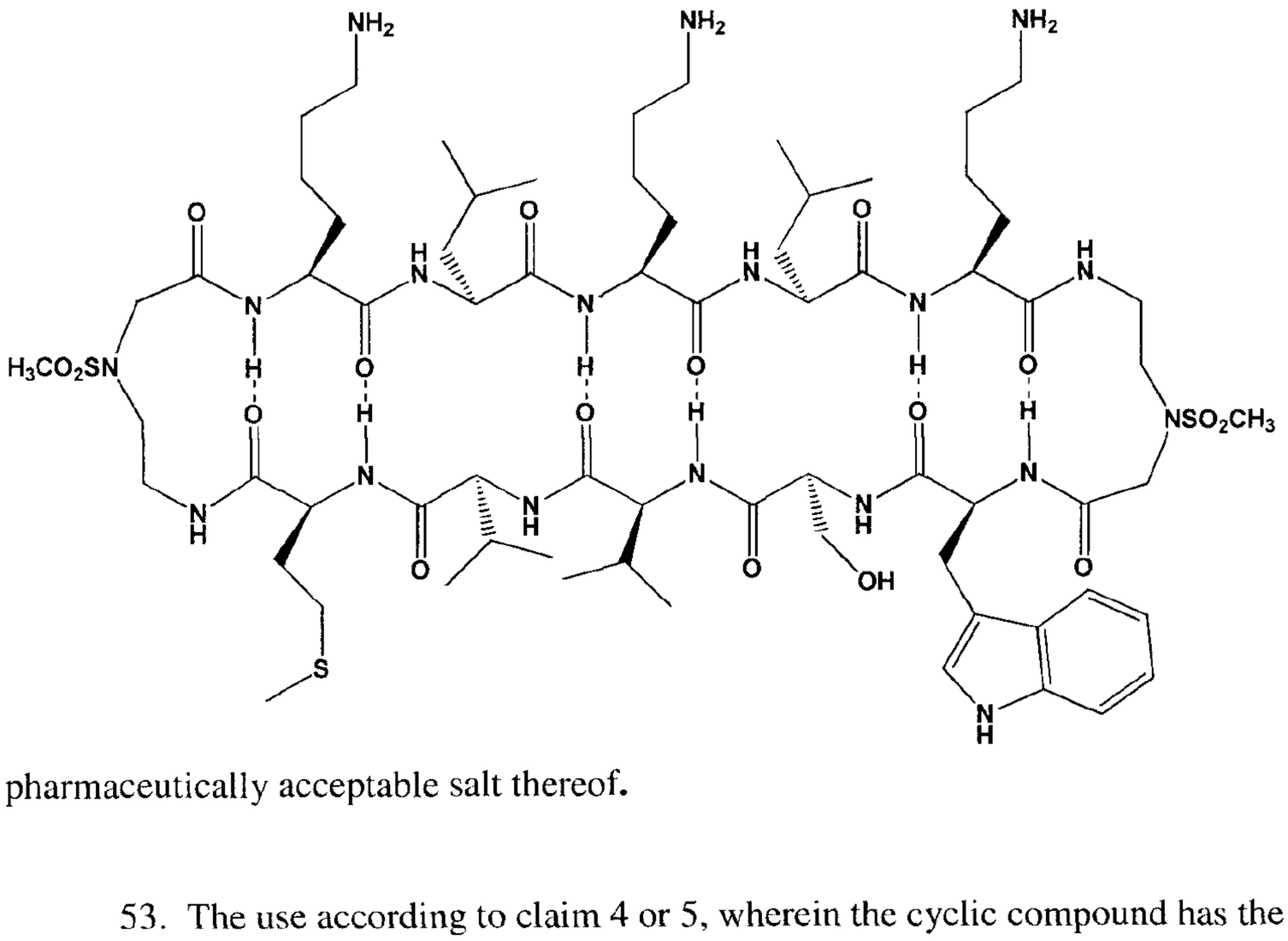
or a pharmaceutically acceptable salt thereof.

51. The use according to claim 4 or 5, wherein the cyclic compound has the structure:



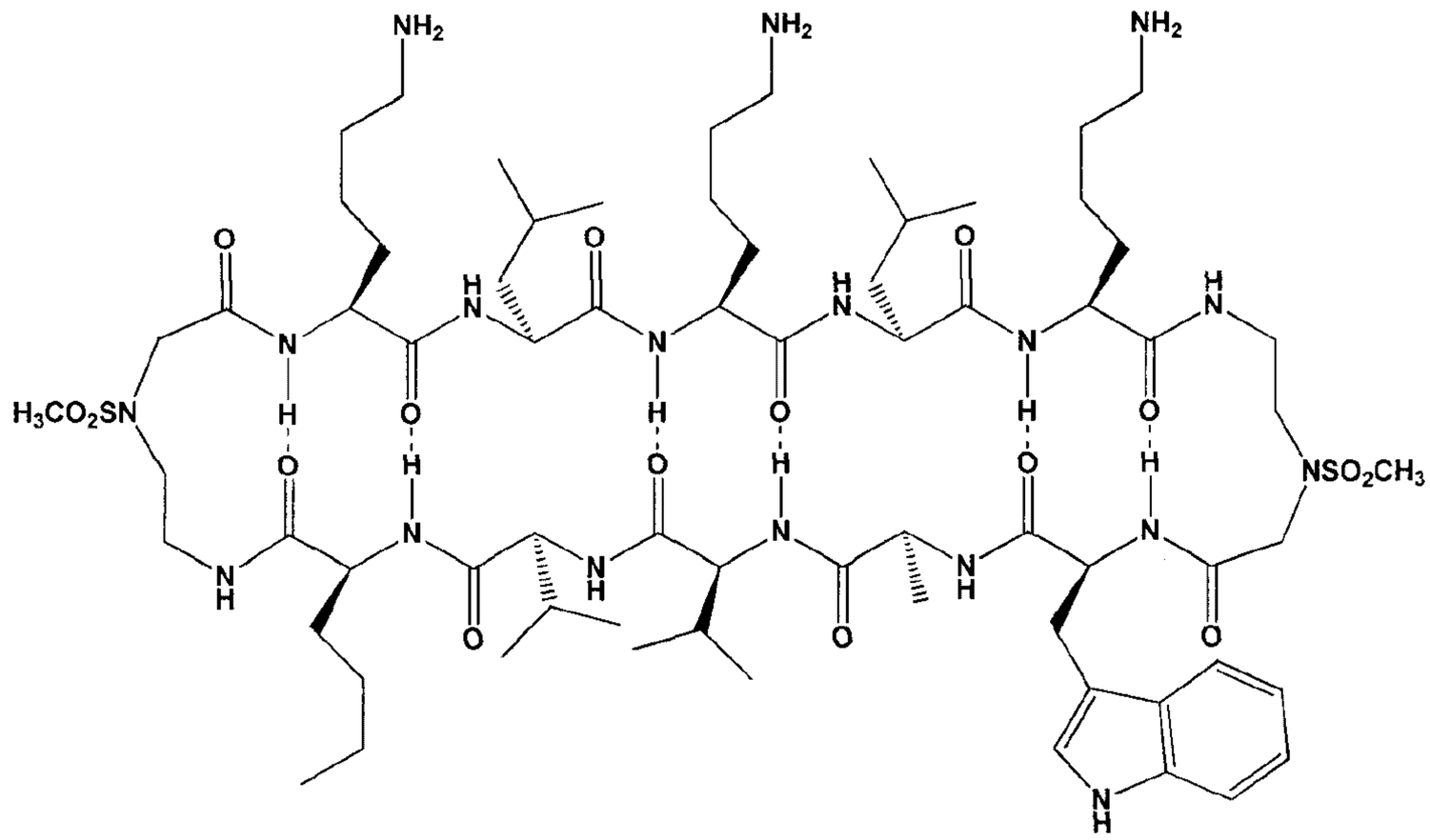
or a pharmaceutically acceptable salt thereof.

52. The use according to claim 4 or 5, wherein the cyclic compound has the structure:



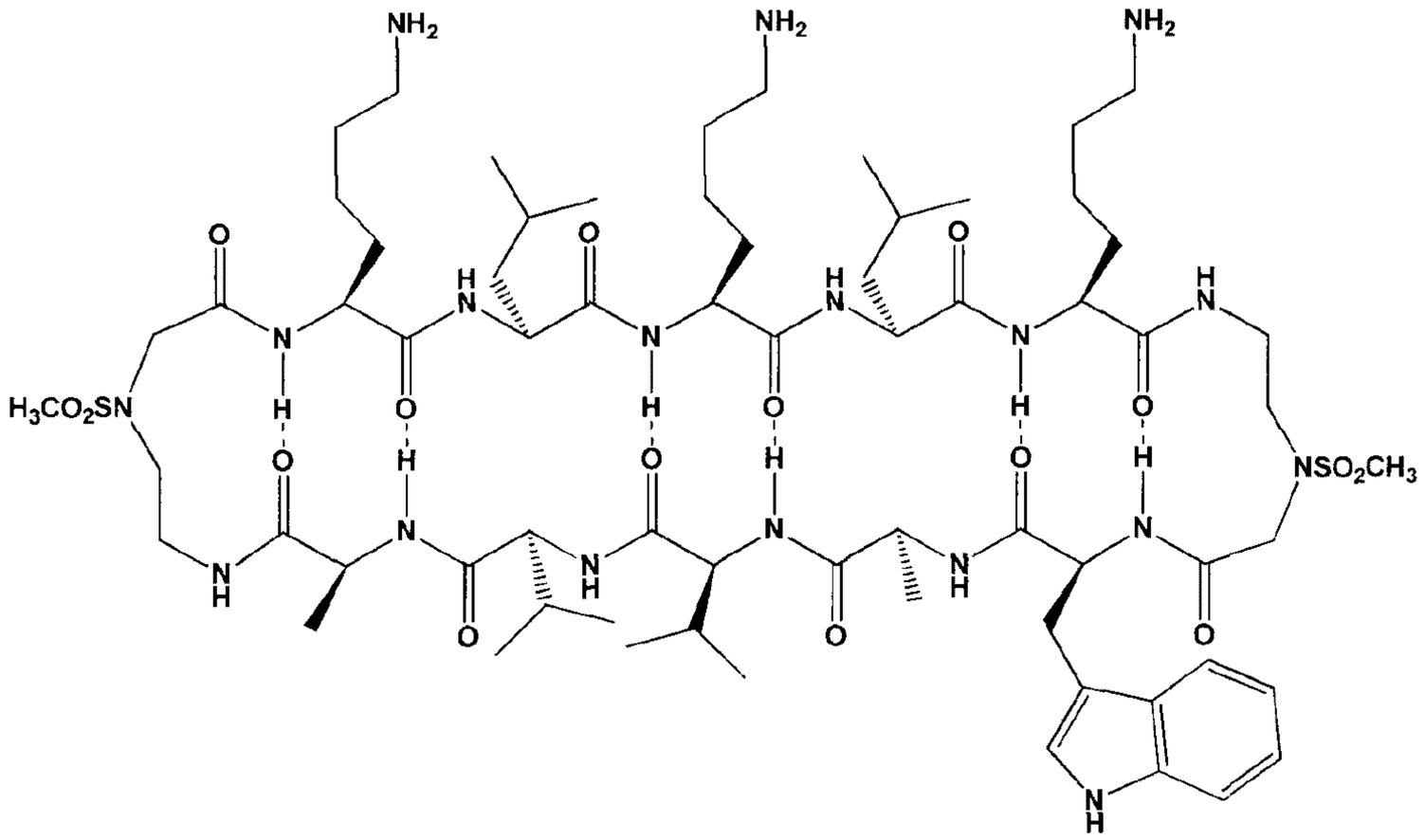
or a pharmaceutically acceptable salt thereof.

53. The use according to claim 4 or 5, wherein the cyclic compound has the structure:



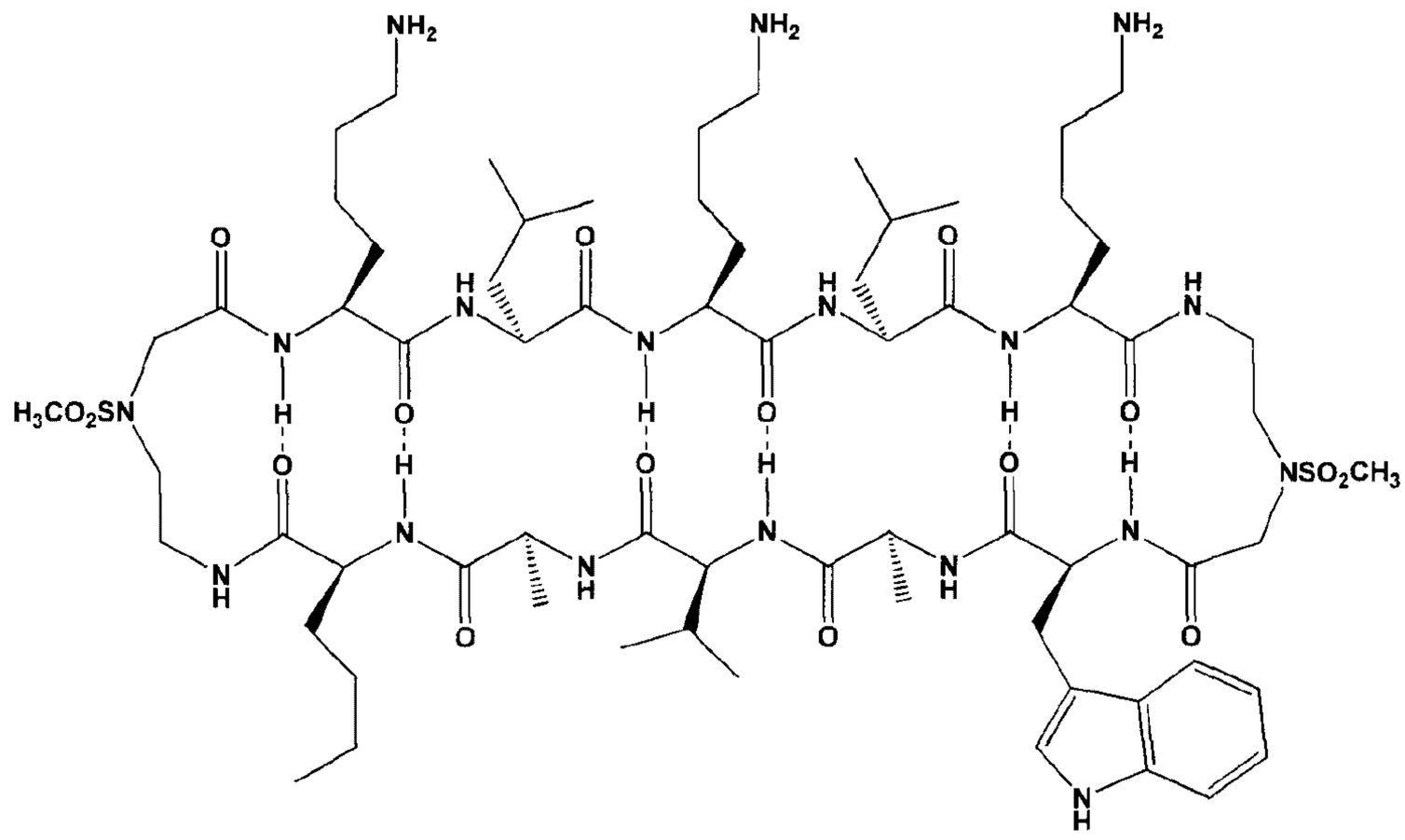
or a pharmaceutically acceptable salt thereof.

54. The use according to claim 4 or 5, wherein the cyclic compound has the structure:



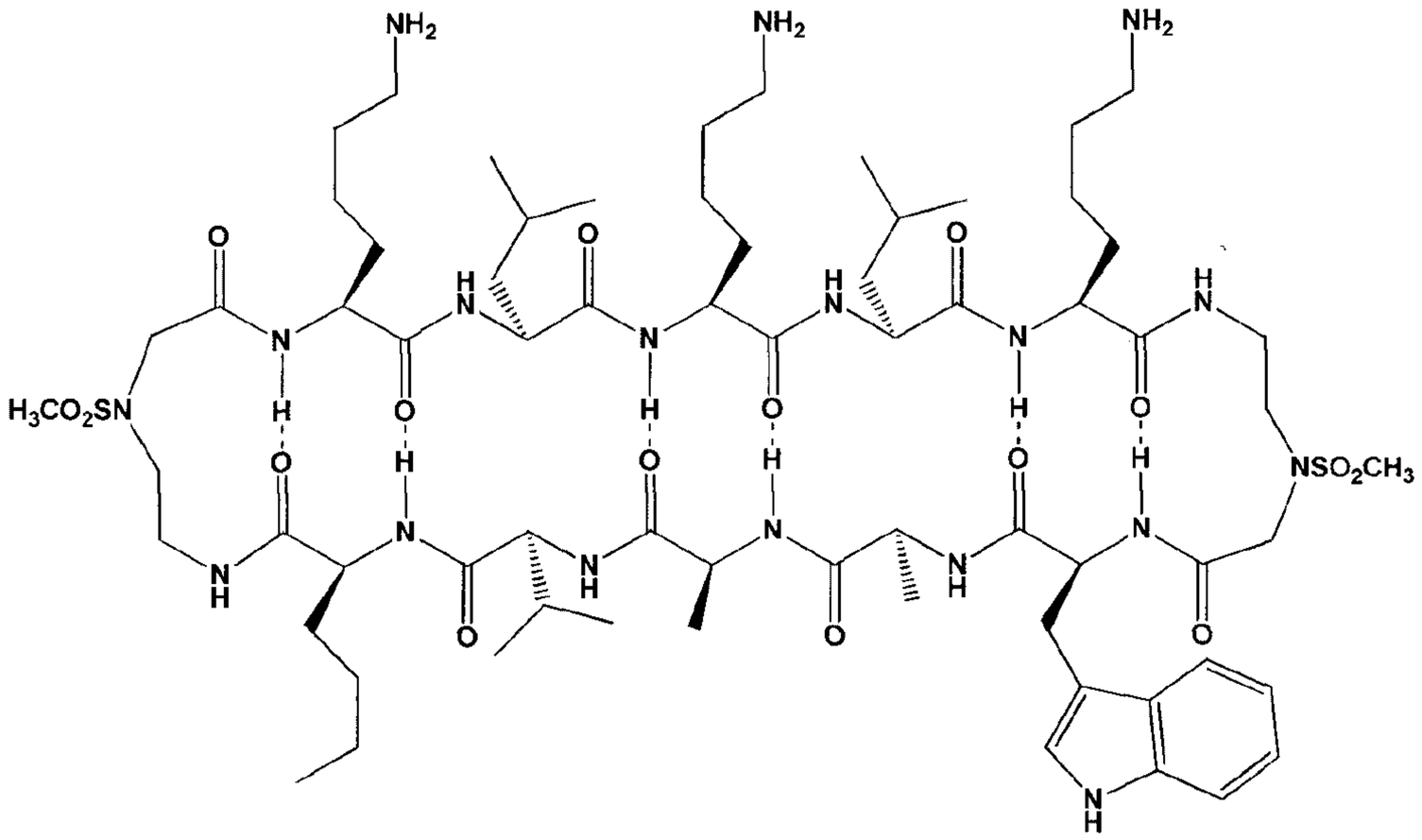
or a pharmaceutically acceptable salt thereof.

55. The use according to claim 4 or 5, wherein the cyclic compound has the structure:



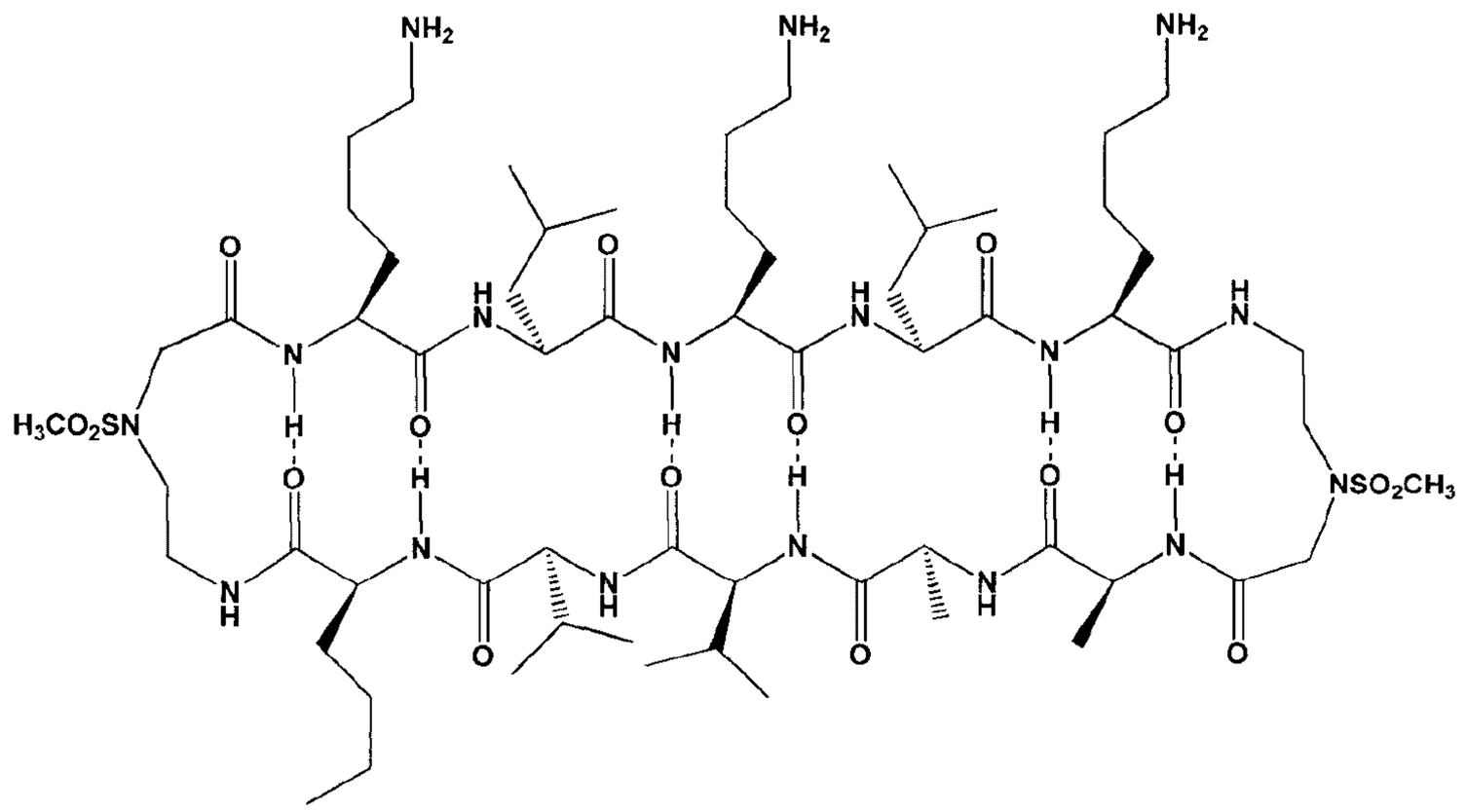
or a pharmaceutically acceptable salt thereof.

56. The use according to claim 4 or 5, wherein the cyclic compound has the structure:



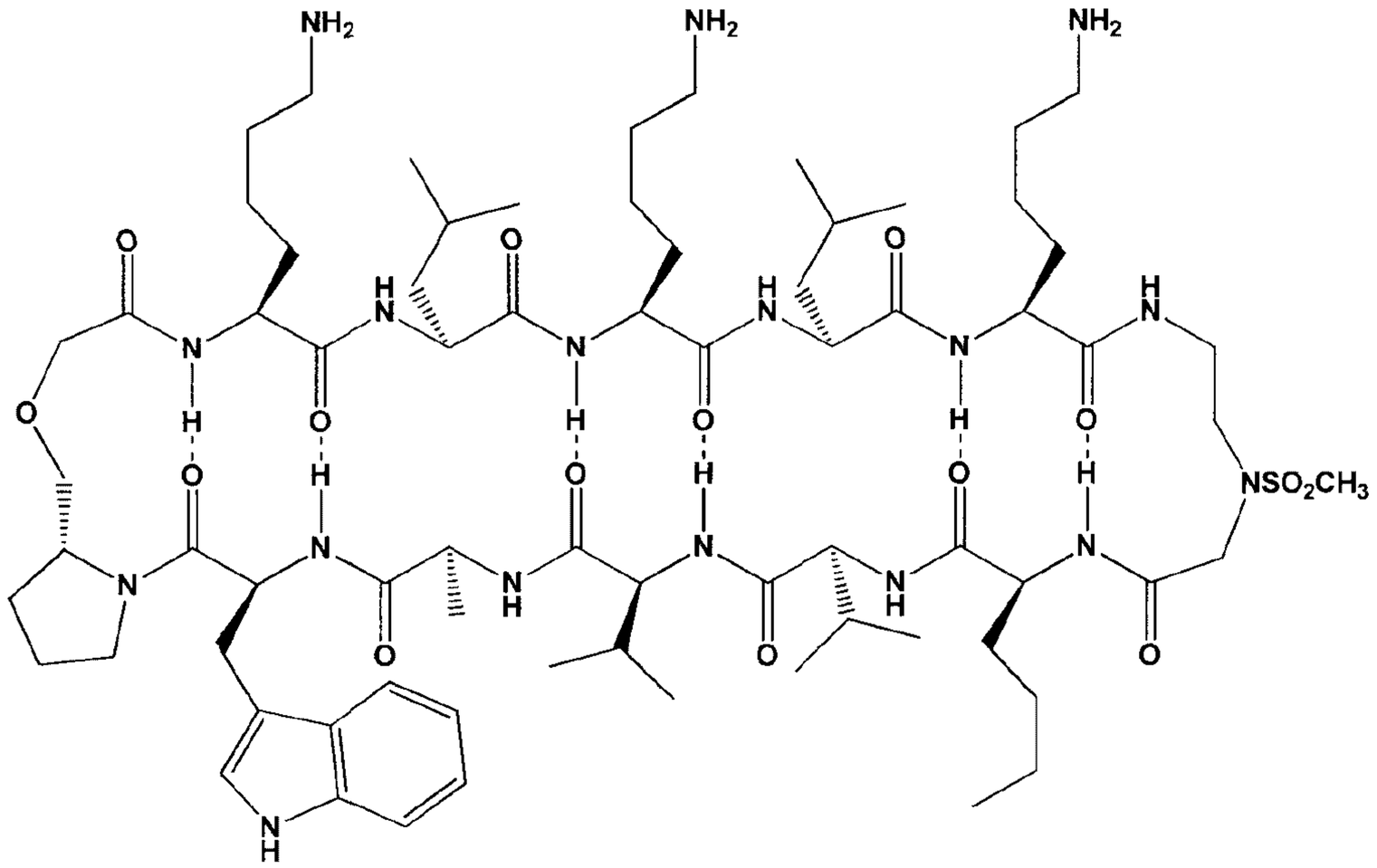
or a pharmaceutically acceptable salt thereof.

57. The use according to claim 4 or 5, wherein the cyclic compound has the structure:



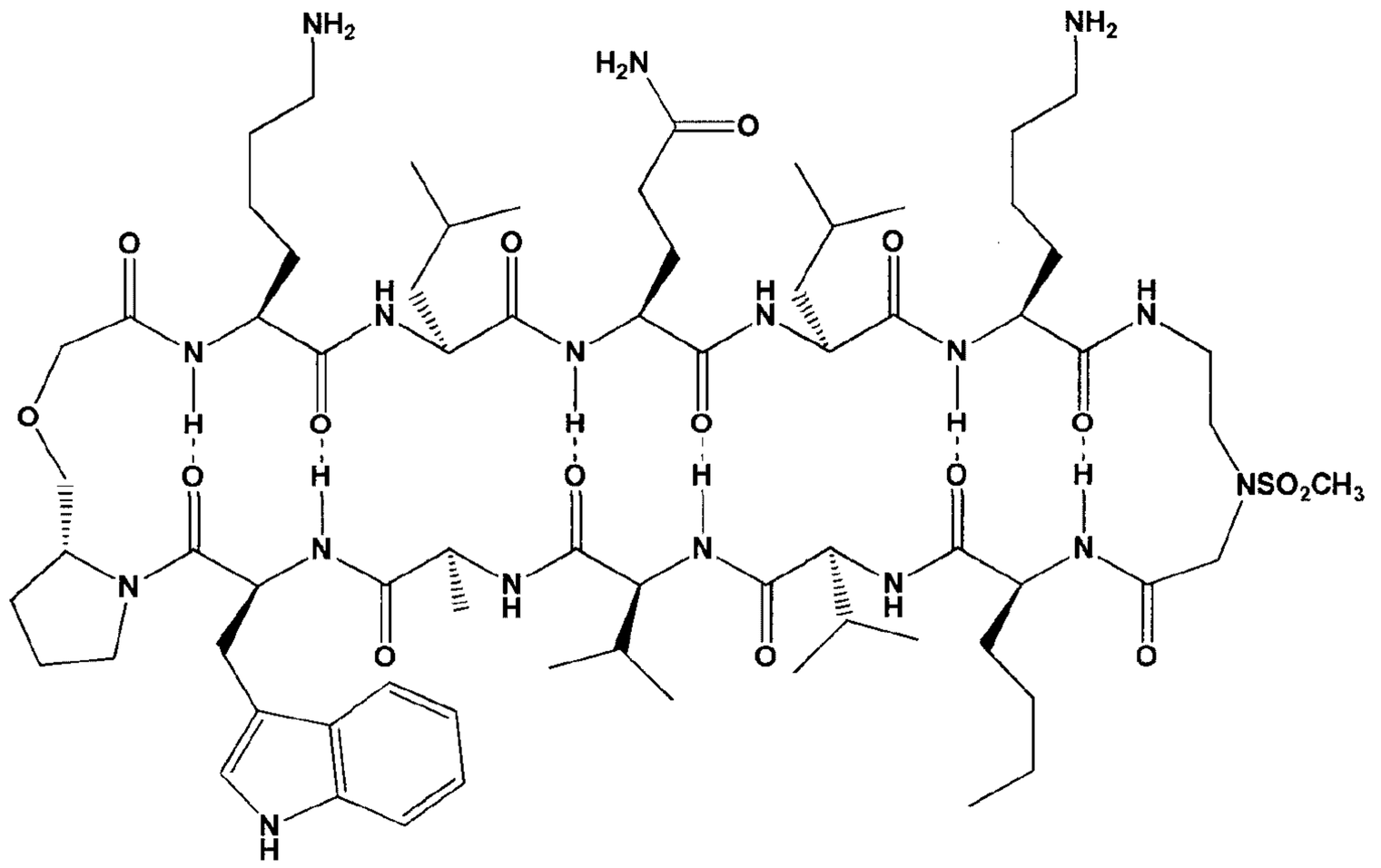
or a pharmaceutically acceptable salt thereof.

58. The use according to claim 4 or 5, wherein the cyclic compound has the structure:



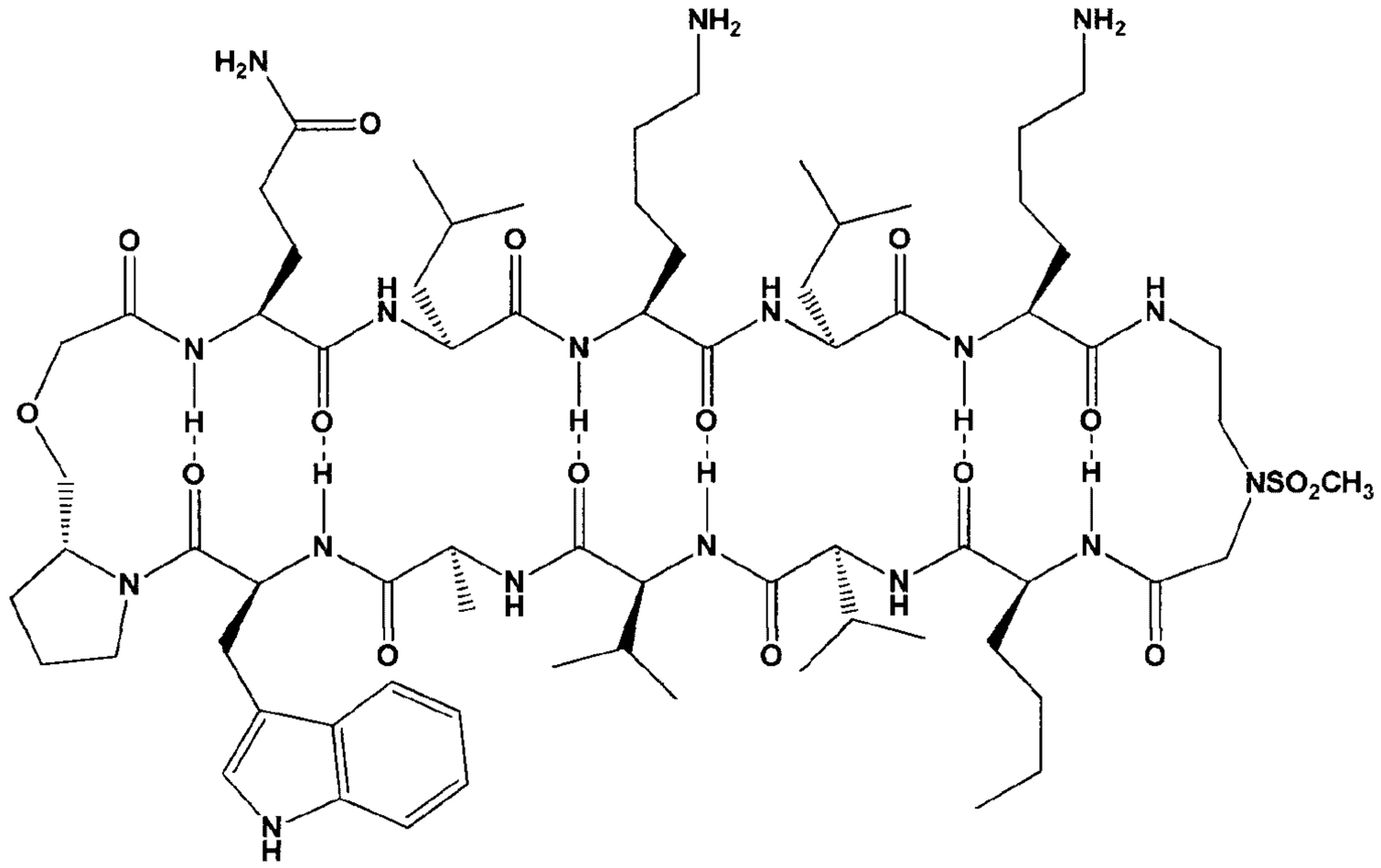
or a pharmaceutically acceptable salt thereof.

59. The use according to claim 4 or 5, wherein the cyclic compound has the structure:



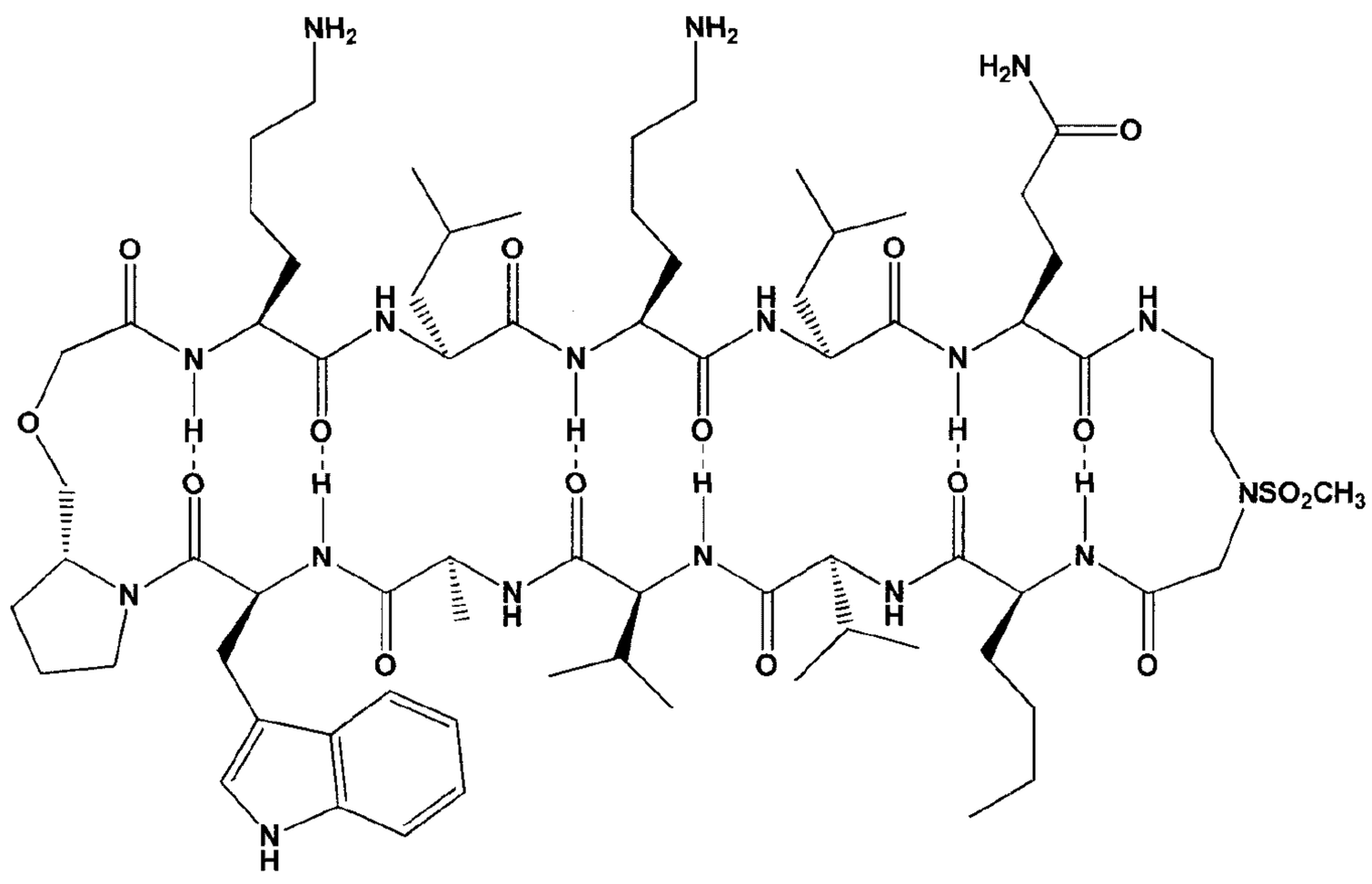
or a pharmaceutically acceptable salt thereof.

60. The use according to claim 4 or 5, wherein the cyclic compound has the structure:



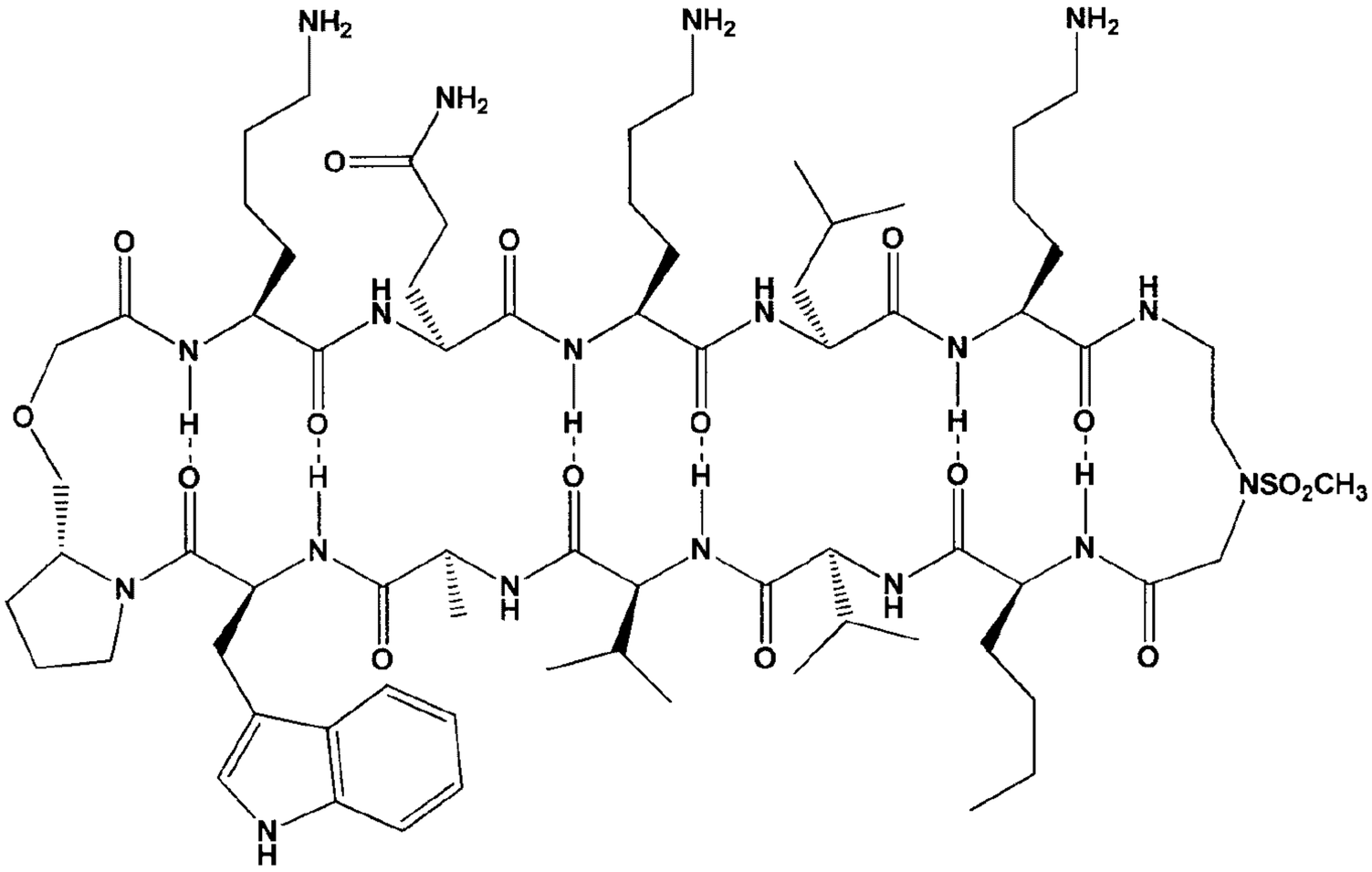
or a pharmaceutically acceptable salt thereof.

61. The use according to claim 4 or 5, wherein the cyclic compound has the structure:



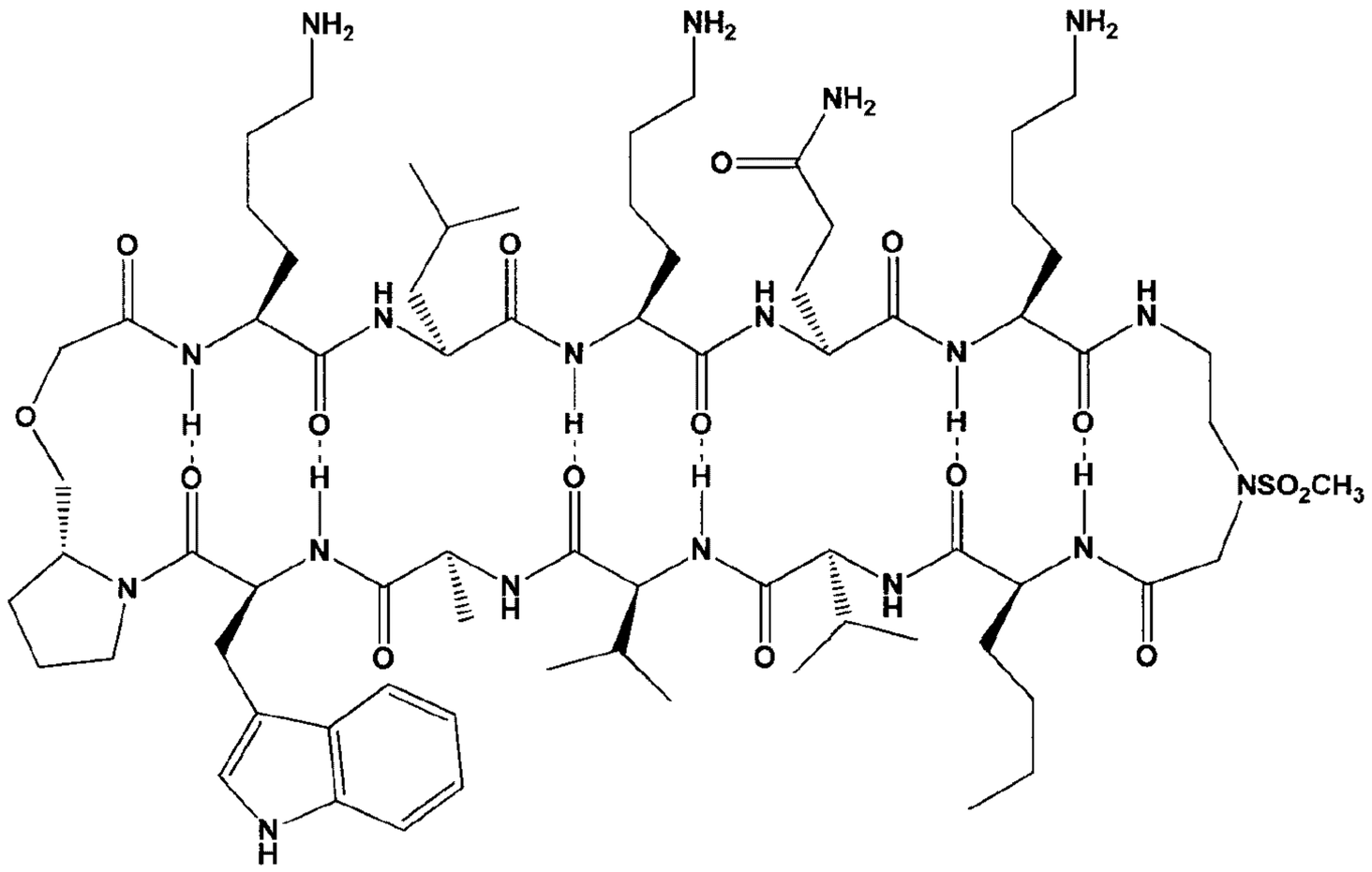
or a pharmaceutically acceptable salt thereof.

62. The use according to claim 4 or 5, wherein the cyclic compound has the structure:



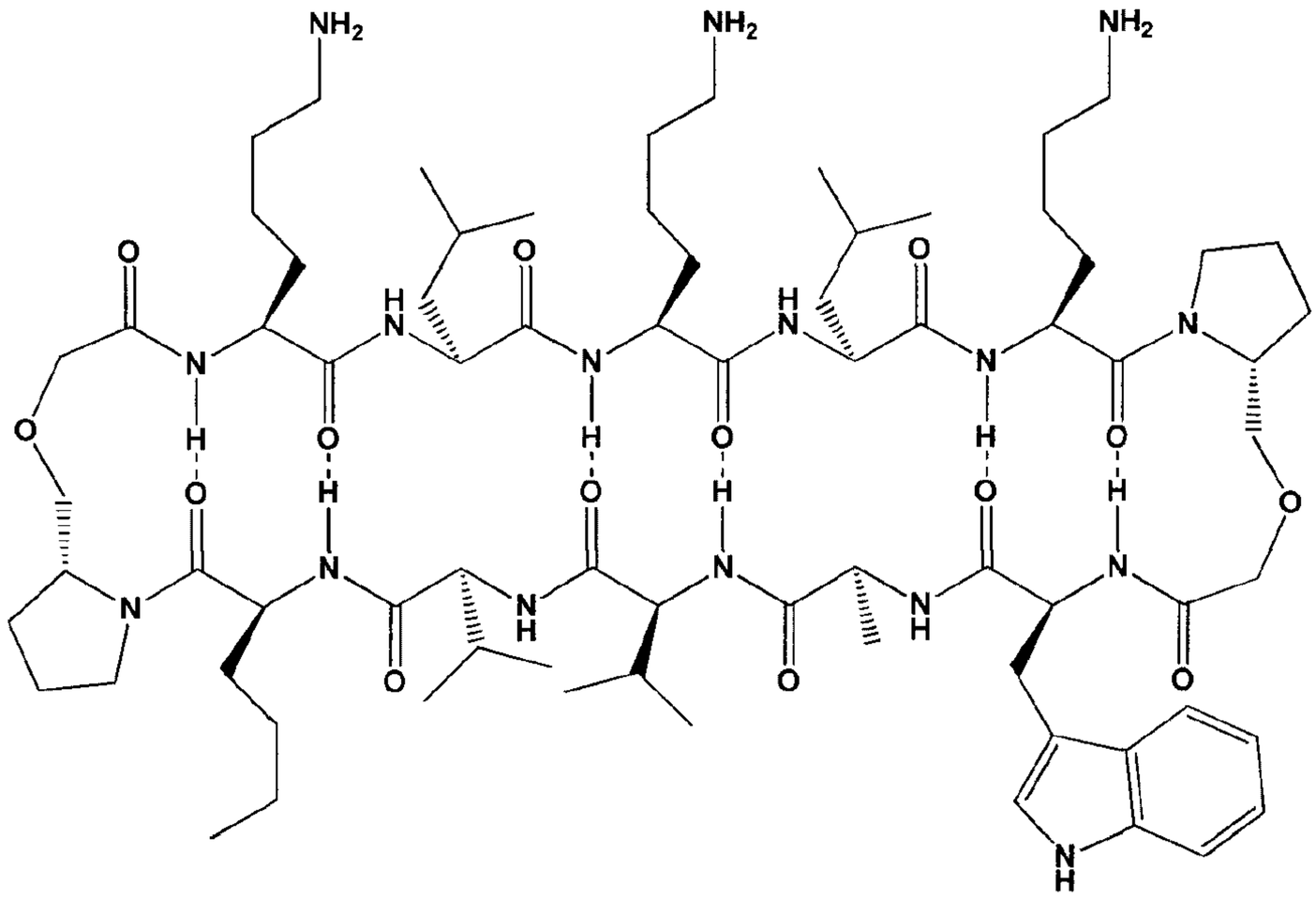
or a pharmaceutically acceptable salt thereof.

63. The use according to claim 4 or 5, wherein the cyclic compound has the structure:



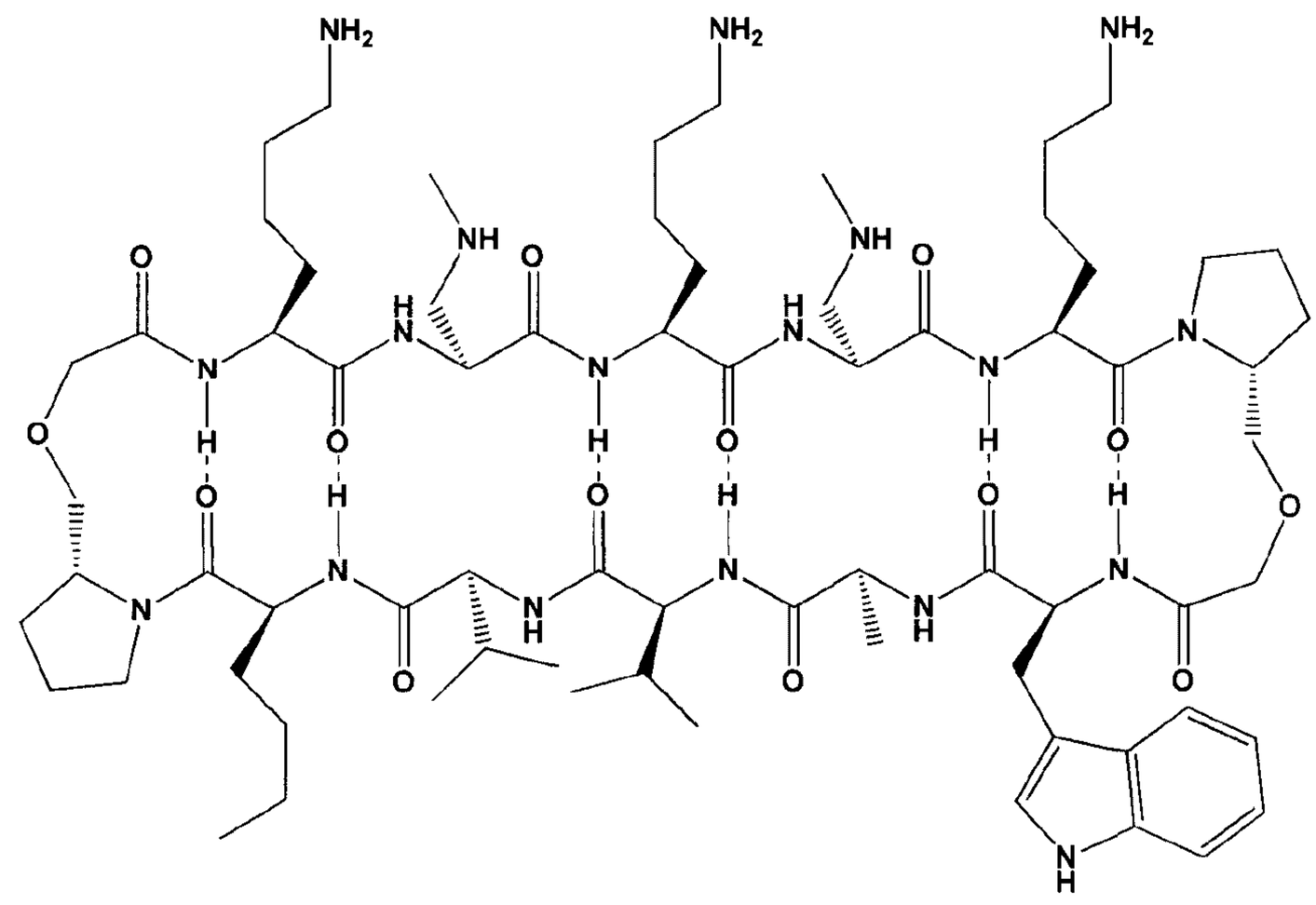
or a pharmaceutically acceptable salt thereof.

64. The use according to claim 4 or 5, wherein the cyclic compound has the structure:



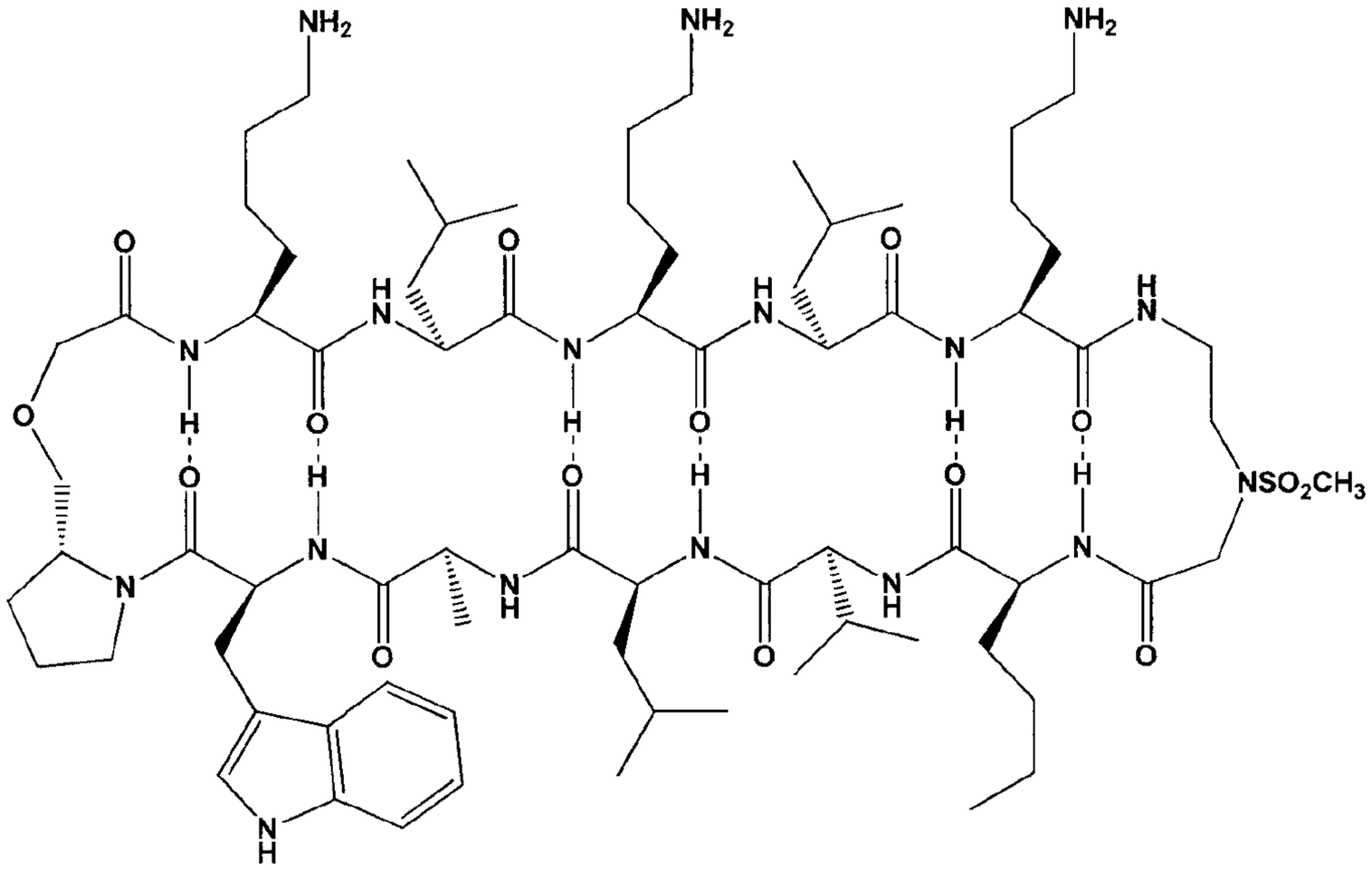
or a pharmaceutically acceptable salt thereof.

65. The use according to claim 4 or 5, wherein the cyclic compound has the structure:



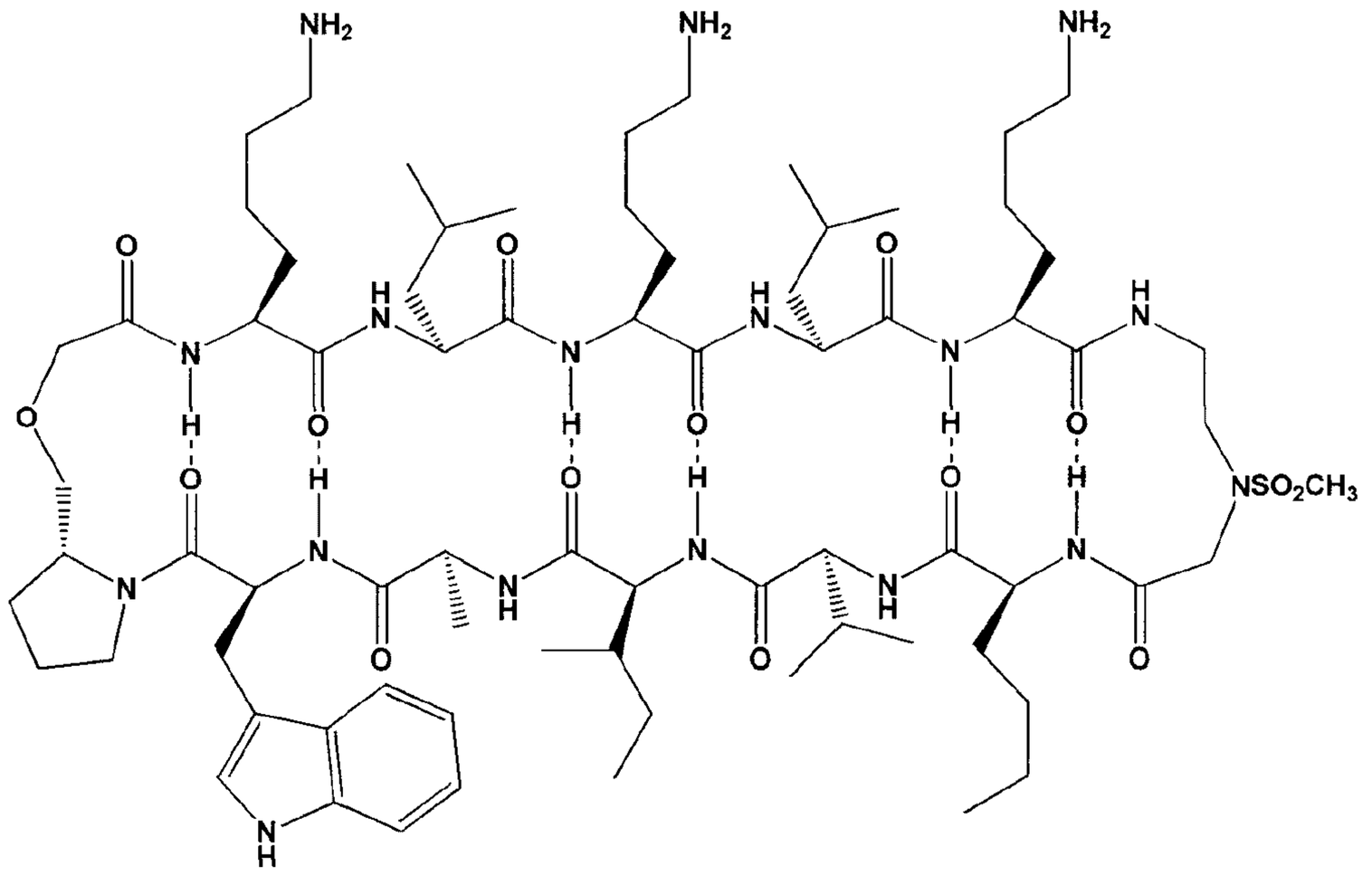
or a pharmaceutically acceptable salt thereof.

66. The use according to claim 4 or 5, wherein the cyclic compound has the structure:



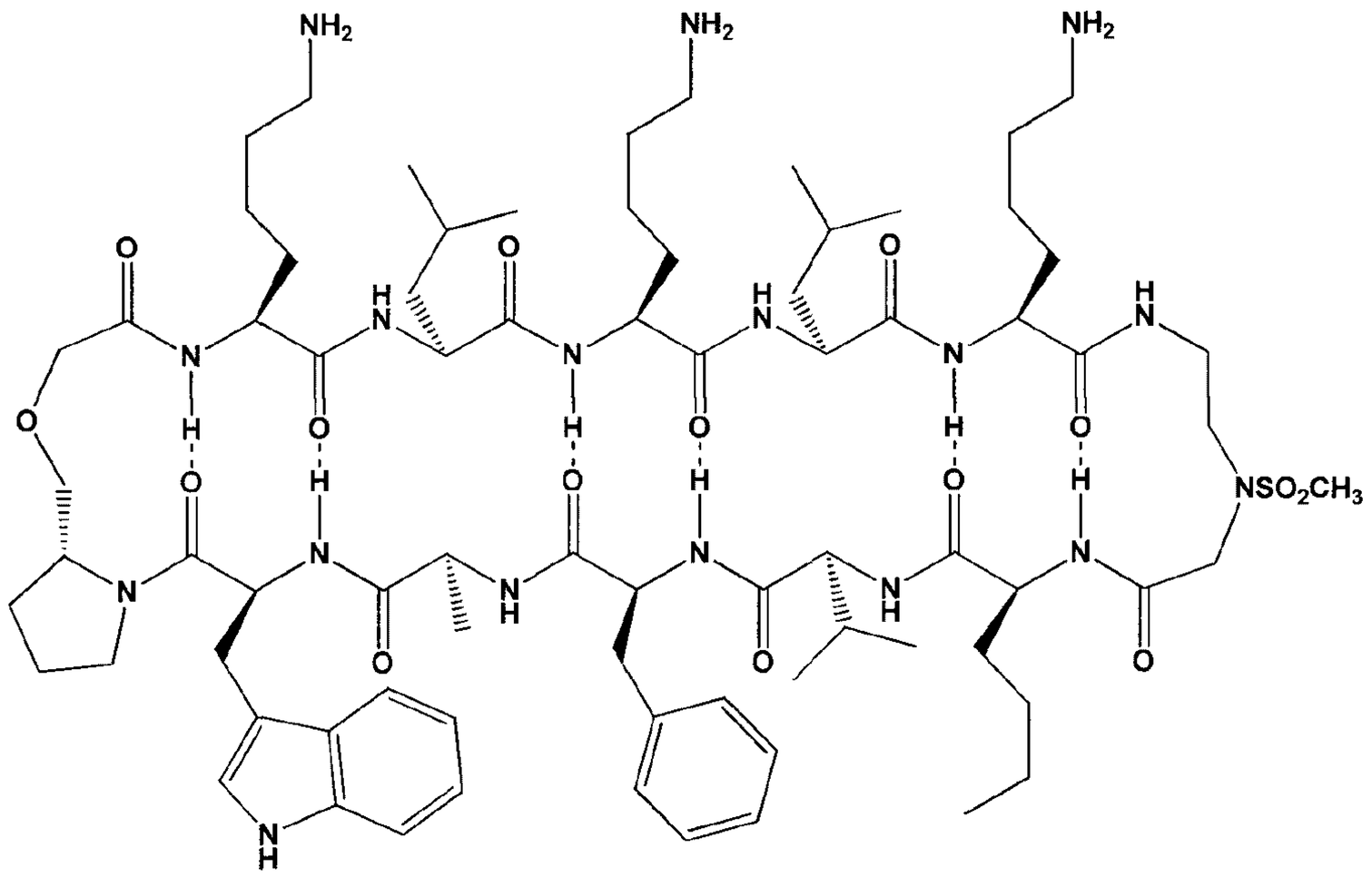
or a pharmaceutically acceptable salt thereof.

67. The use according to claim 4 or 5, wherein the cyclic compound has the structure:



or a pharmaceutically acceptable salt thereof.

68. The use according to claim 4 or 5, wherein the cyclic compound has the structure:



or a pharmaceutically acceptable salt thereof.

69. The use according to claim 6 or 7, wherein the malignancy is multiple myeloma.

70. The use according to claim 6 or 7, wherein the malignancy is lung cancer.

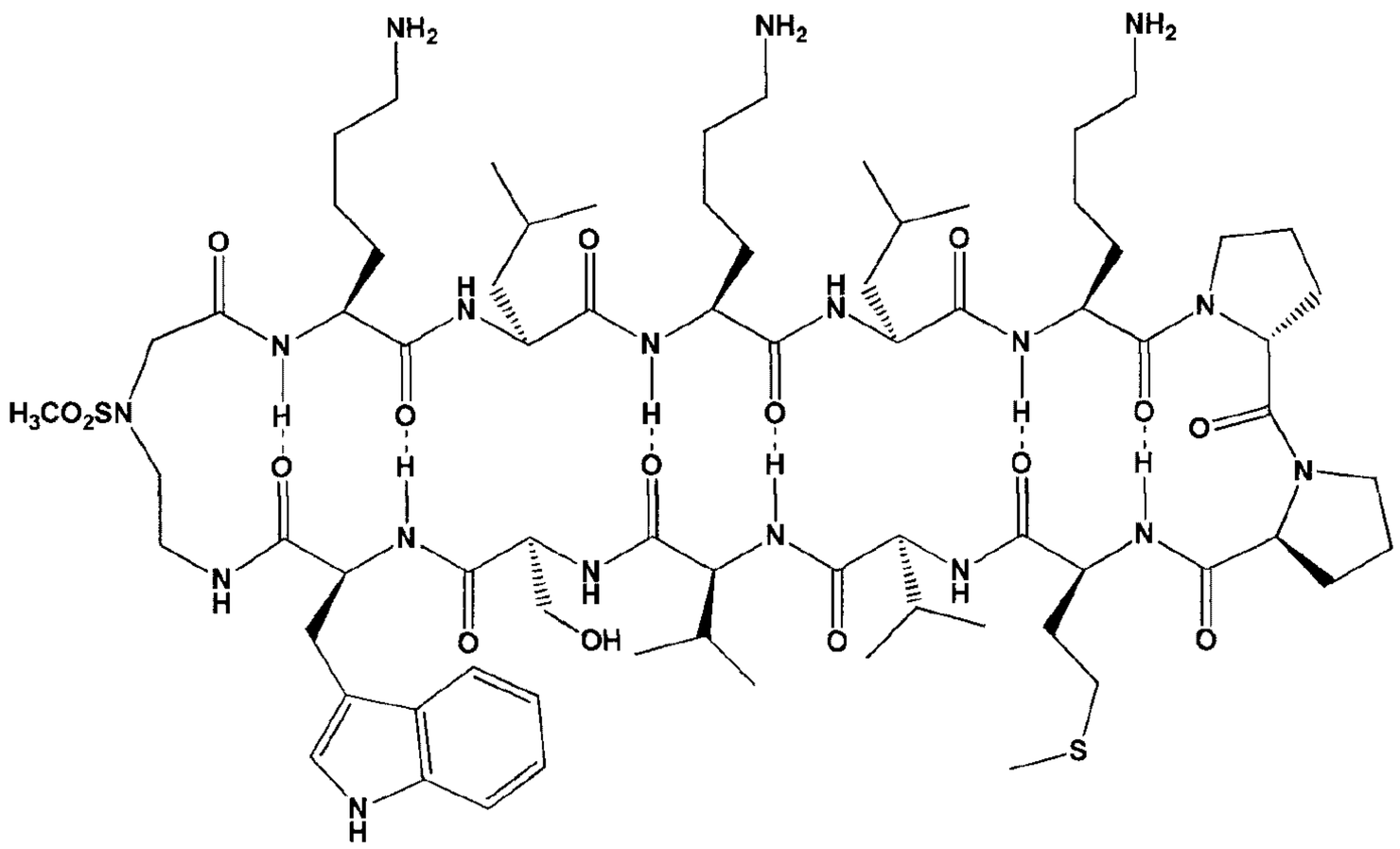
71. The use according to claim 6 or 7, wherein the malignancy is acute myeloid leukemia.

72. The use according to claim 6 or 7, wherein the malignancy is prostate cancer.

73. The use according to claim 6 or 7, wherein the malignancy is melanoma.

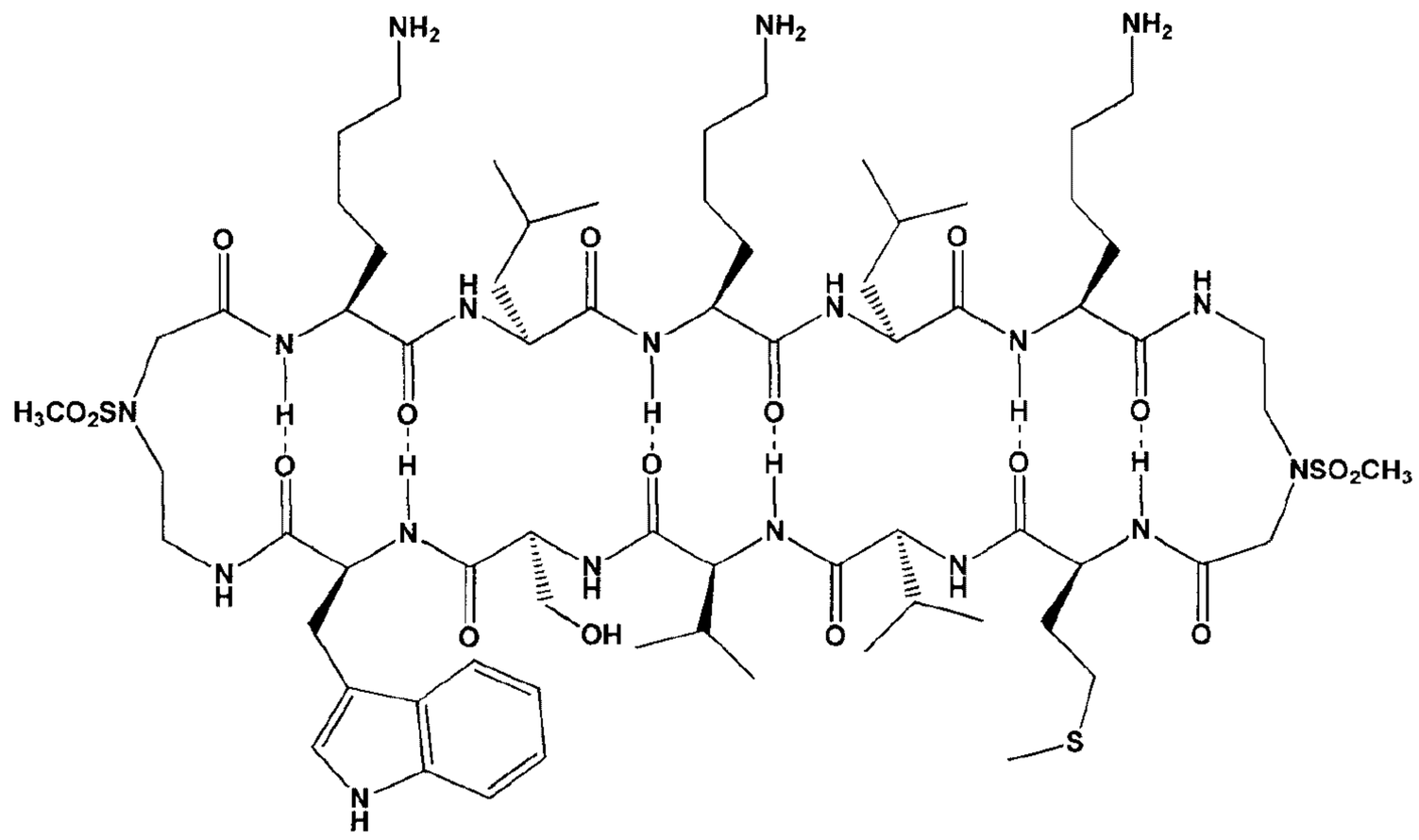
74. The use according to claim 6 or 7, wherein the subject is human.

75. The use according to claim 6 or 7, wherein the cyclic compound has the structure:



or a pharmaceutically acceptable salt thereof.

76. The use according to claim 6 or 7, wherein the cyclic compound has the structure:

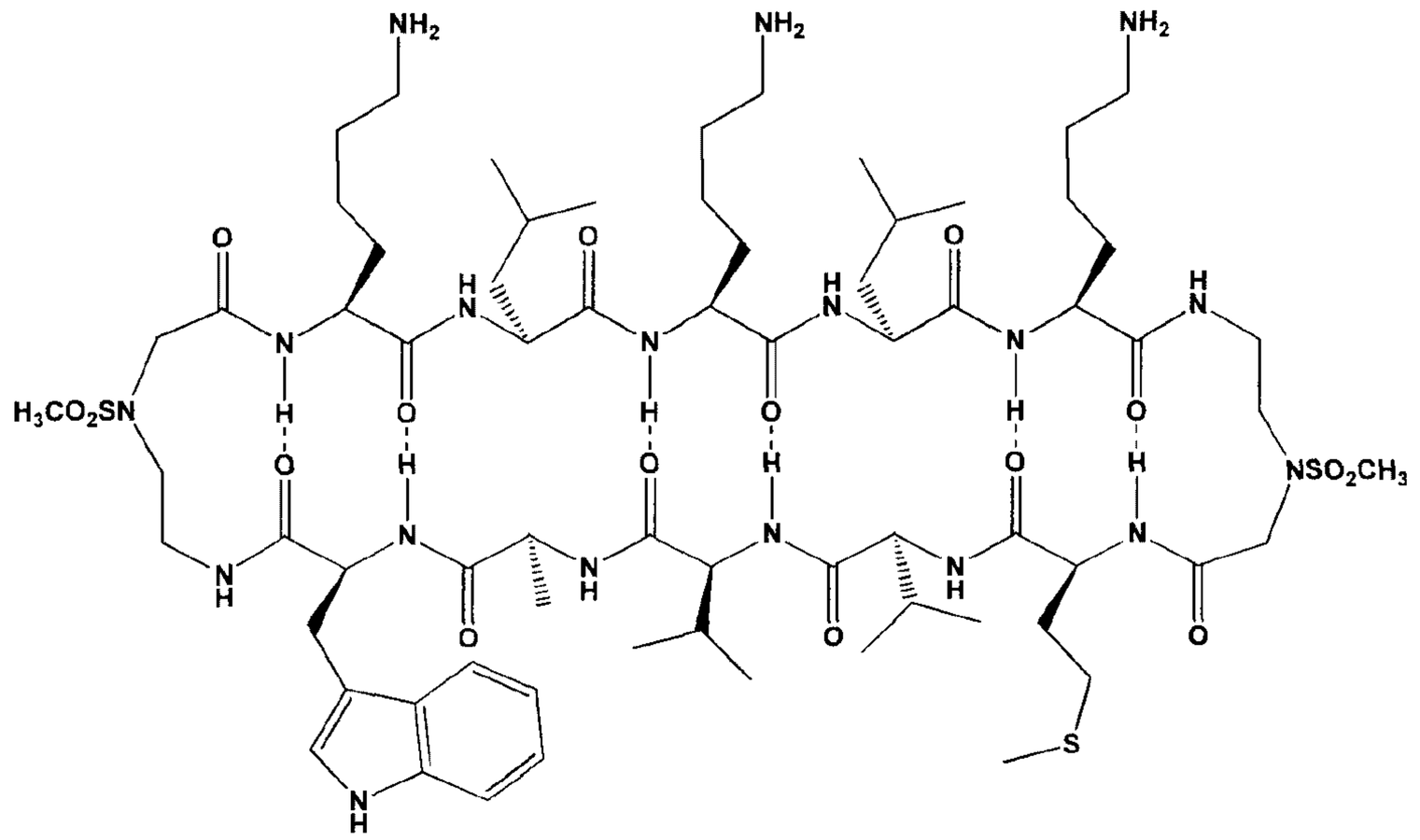


or a pharmaceutically acceptable salt thereof.

77. The use according to claim 6 or 7, wherein the cyclic compound has the structure:

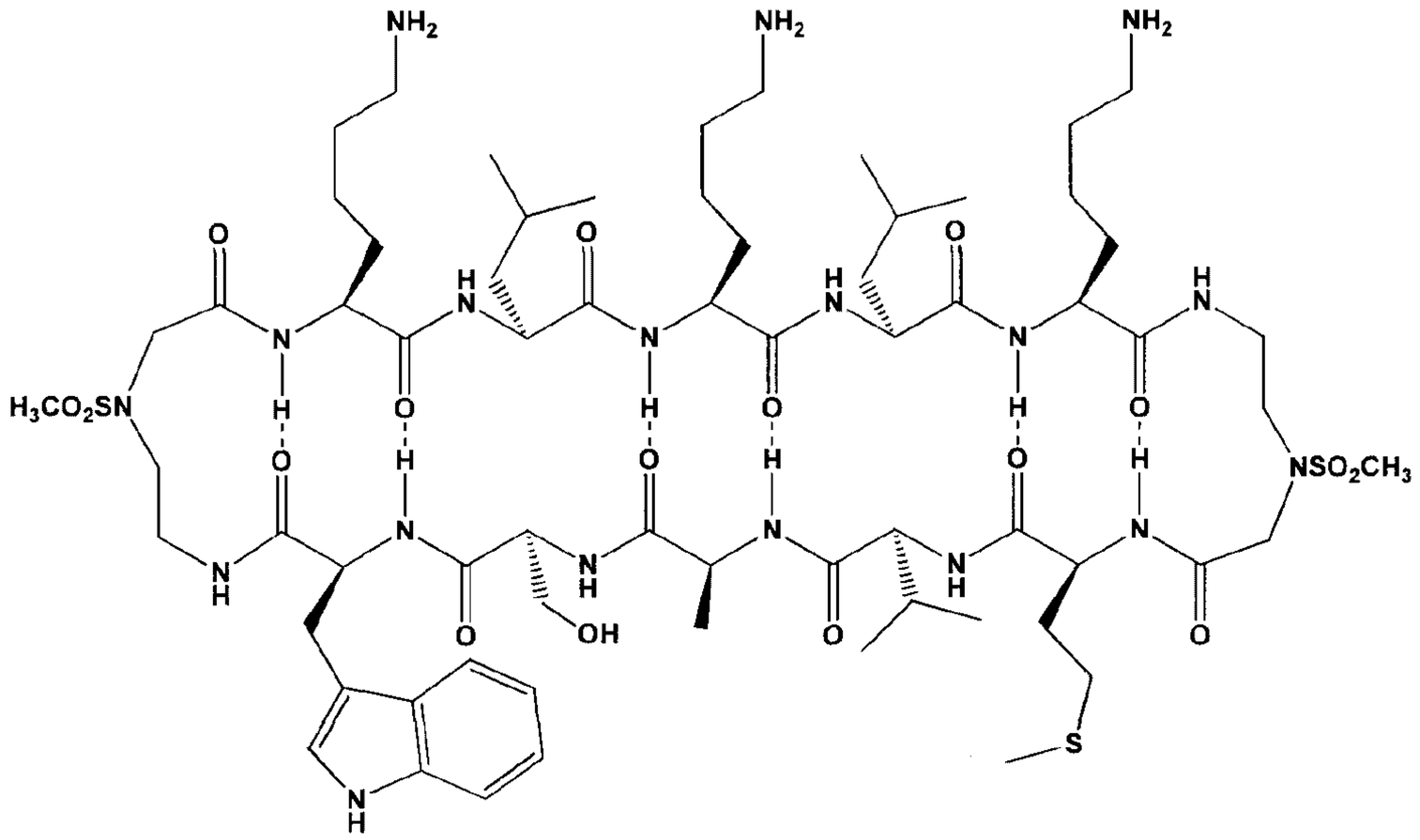
or a pharmaceutically acceptable salt thereof.

78. The use according to claim 6 or 7, wherein the cyclic compound has the structure:



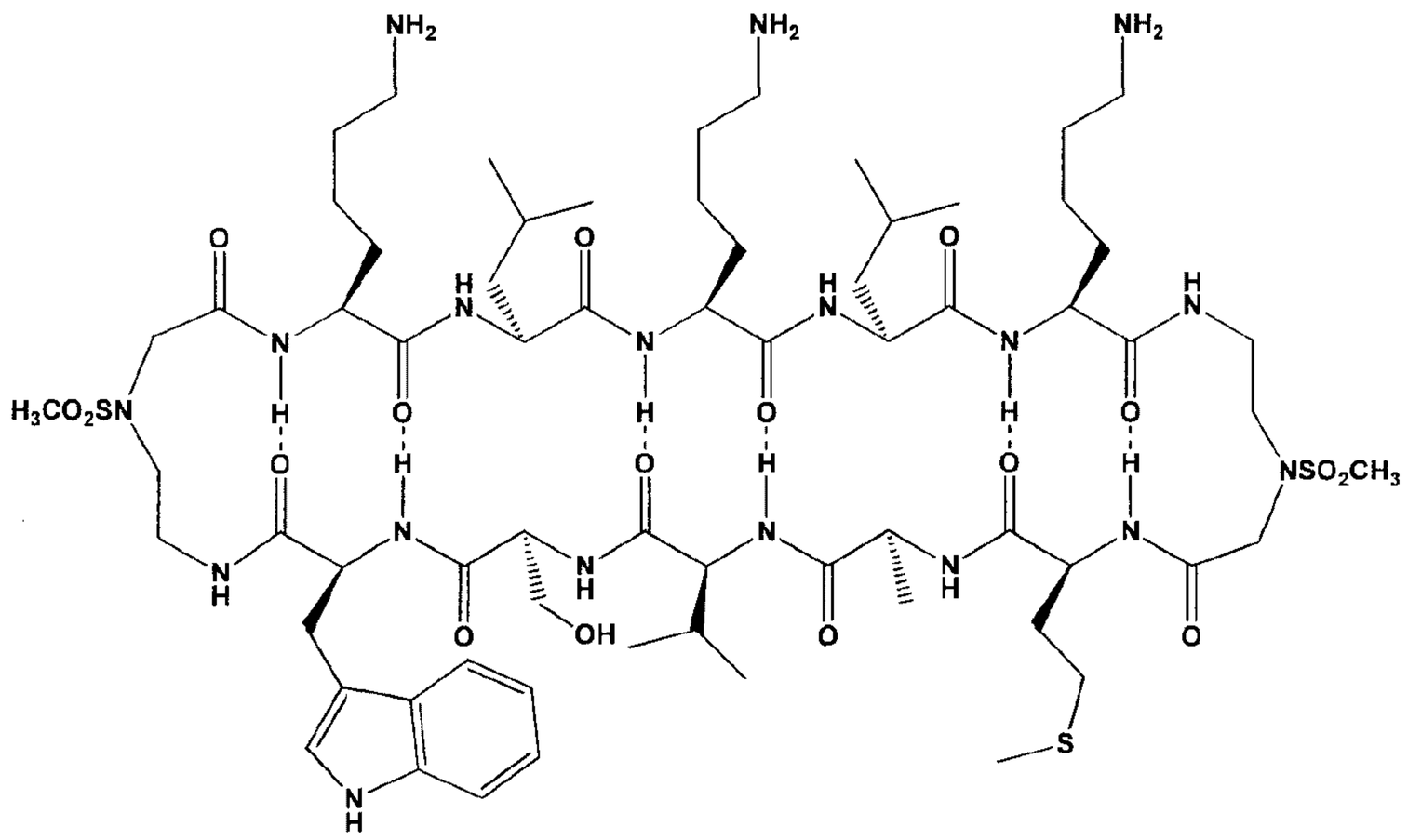
or a pharmaceutically acceptable salt thereof.

79. The use according to claim 6 or 7, wherein the cyclic compound has the structure:



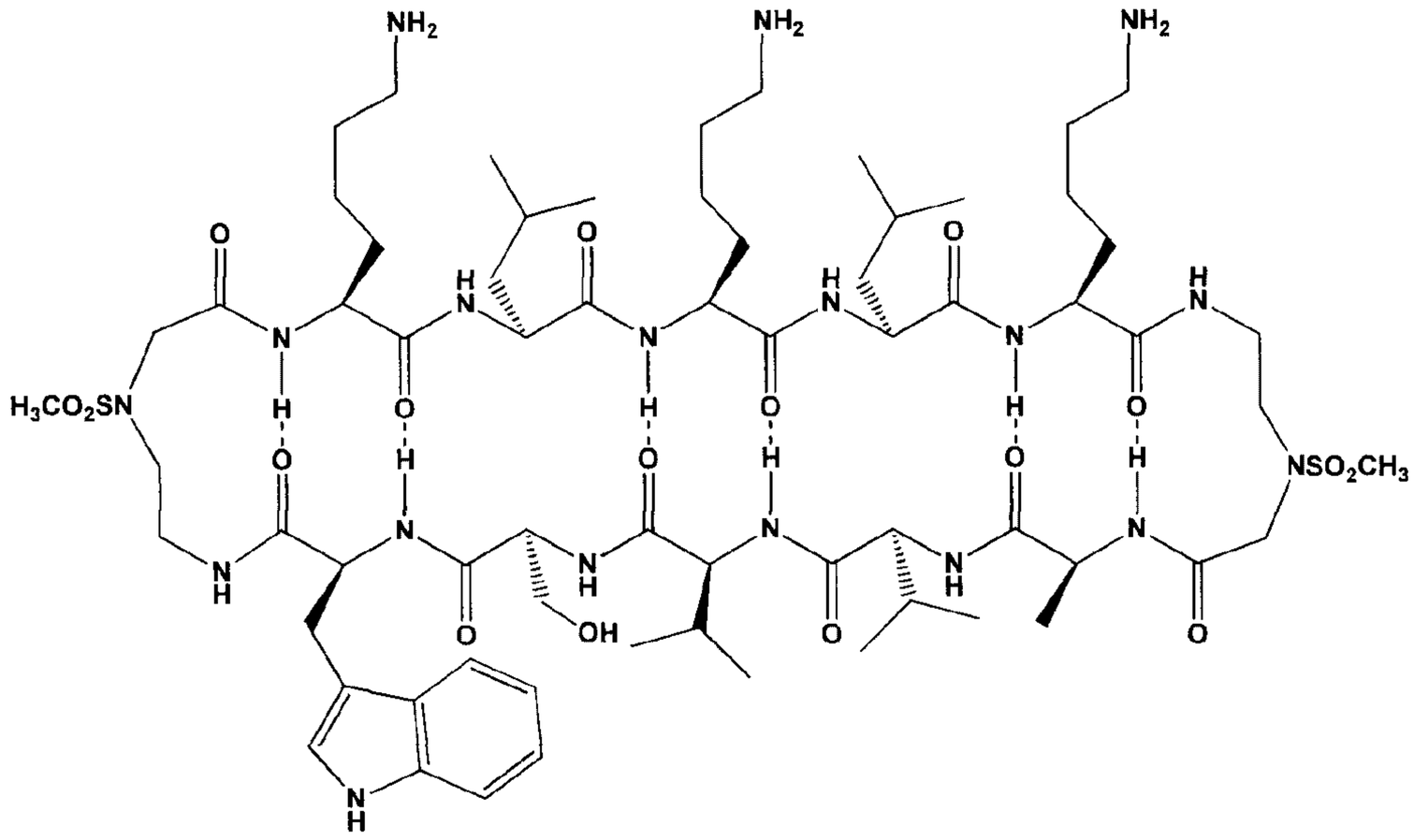
or a pharmaceutically acceptable salt thereof.

80. The use according to claim 6 or 7, wherein the cyclic compound has the structure:



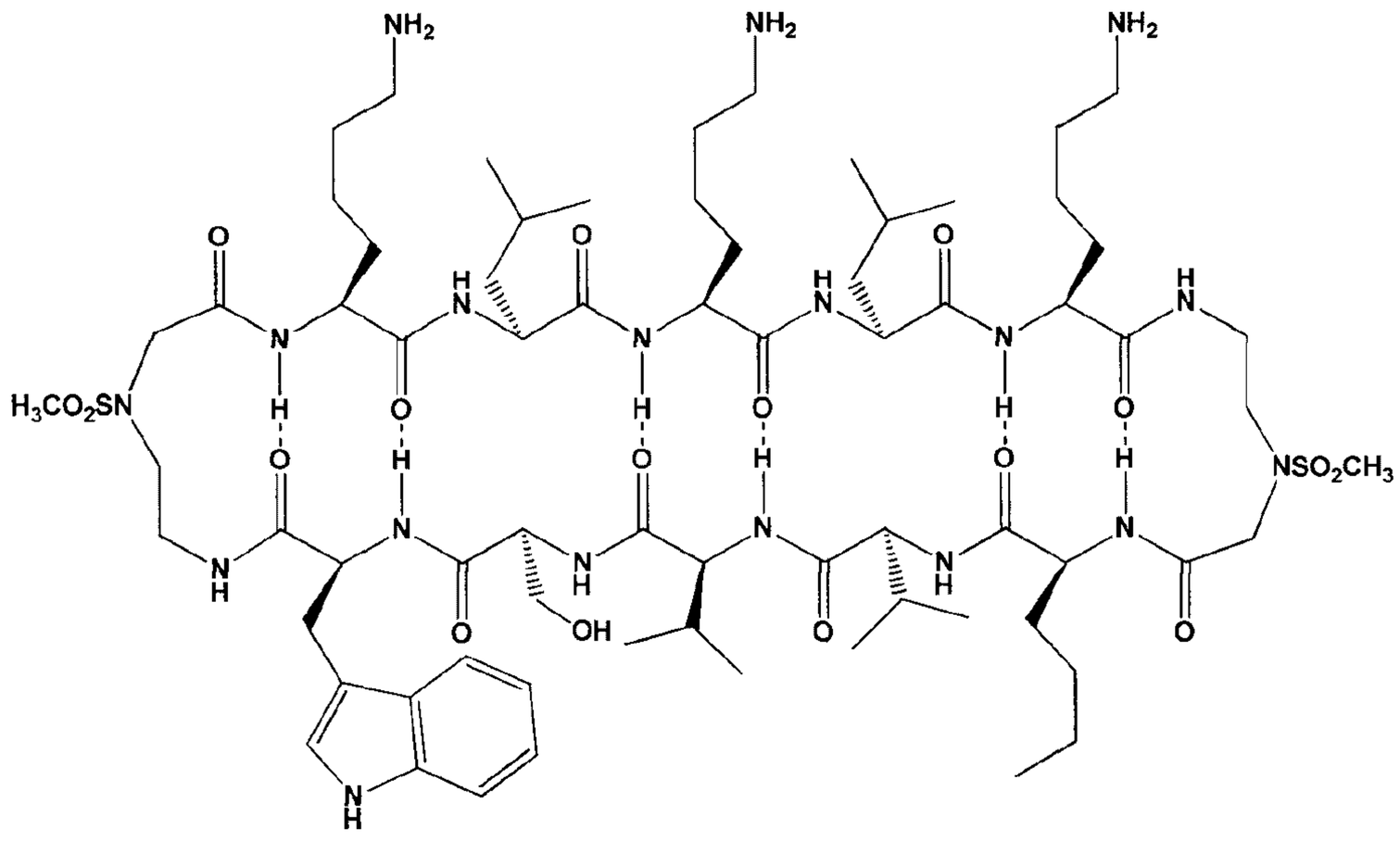
or a pharmaceutically acceptable salt thereof.

81. The use according to claim 6 or 7, wherein the cyclic compound has the structure:



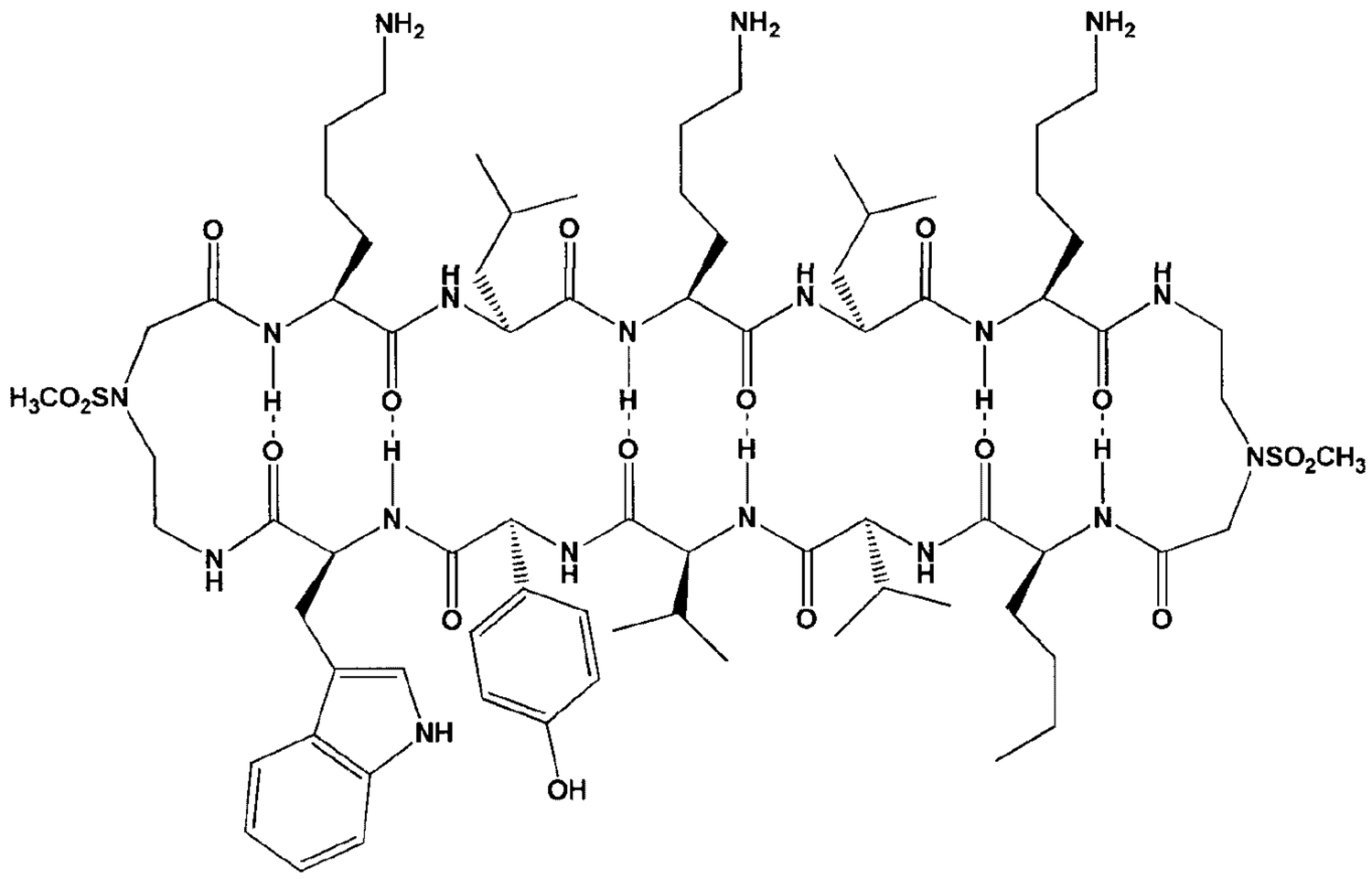
or a pharmaceutically acceptable salt thereof.

82. The use according to claim 6 or 7, wherein the cyclic compound has the structure:



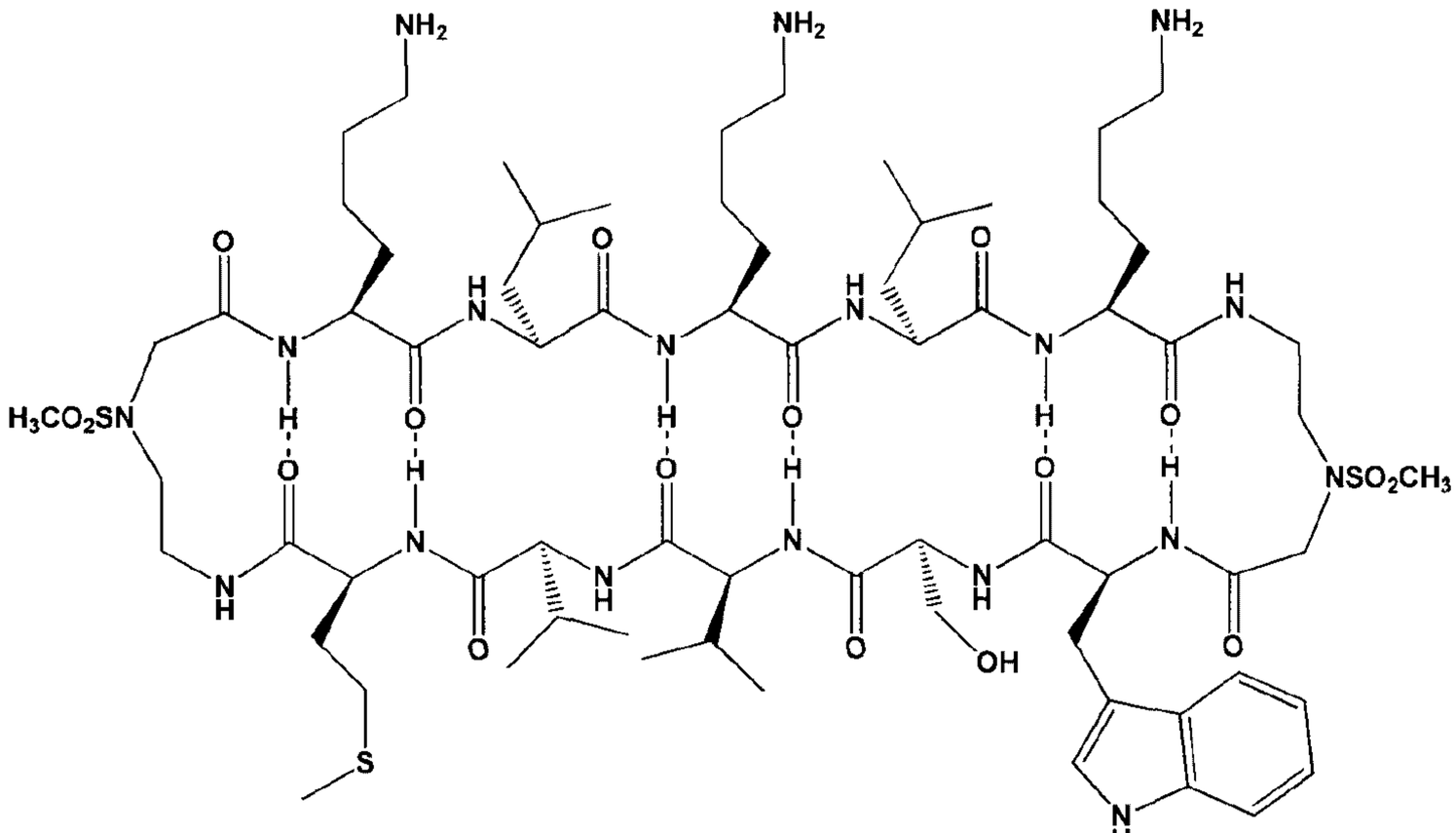
or a pharmaceutically acceptable salt thereof.

83. The use according to claim 6 or 7, wherein the cyclic compound has the structure:



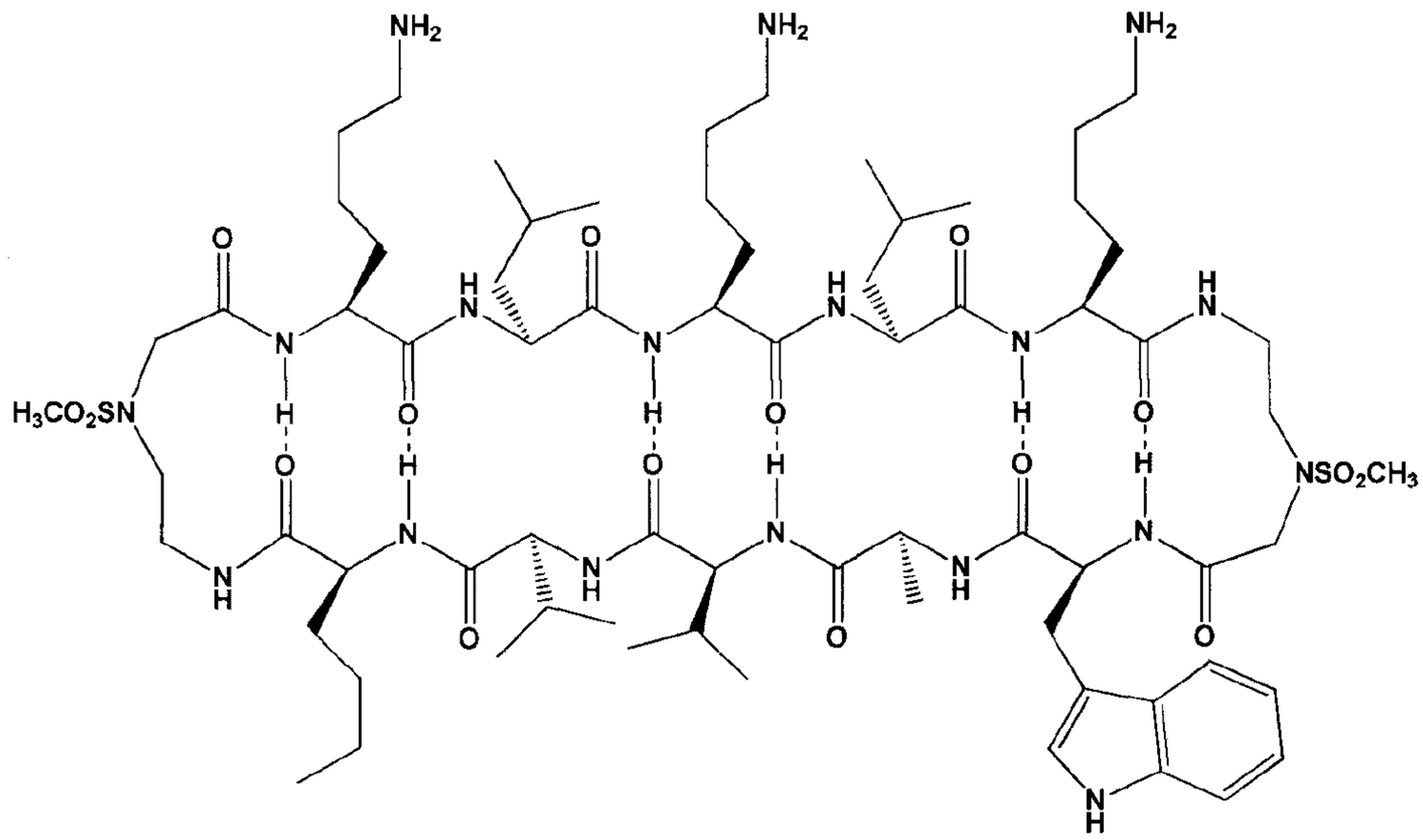
or a pharmaceutically acceptable salt thereof.

84. The use according to claim 6 or 7, wherein the cyclic compound has the structure:



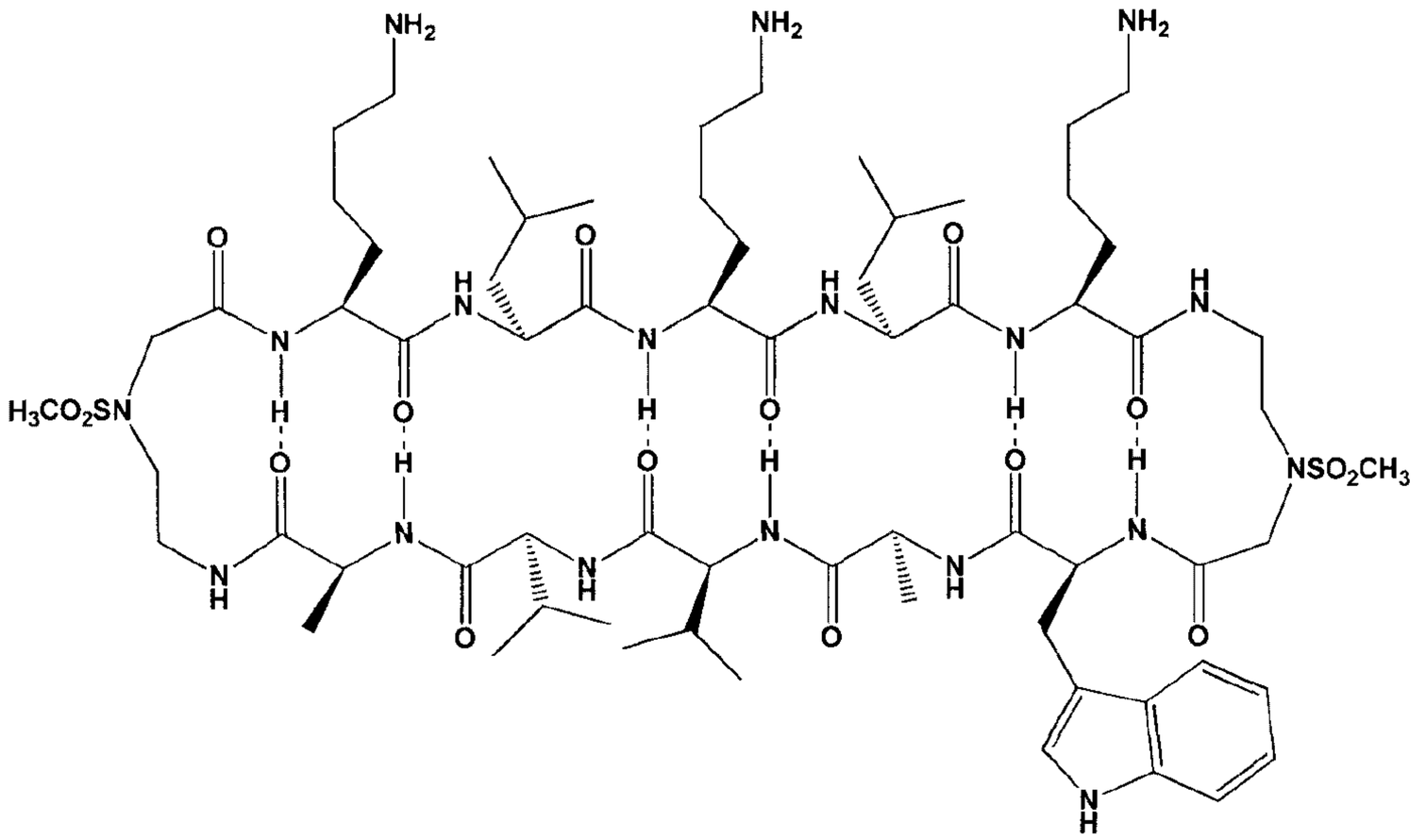
or a pharmaceutically acceptable salt thereof.

85. The use according to claim 6 or 7, wherein the cyclic compound has the structure:



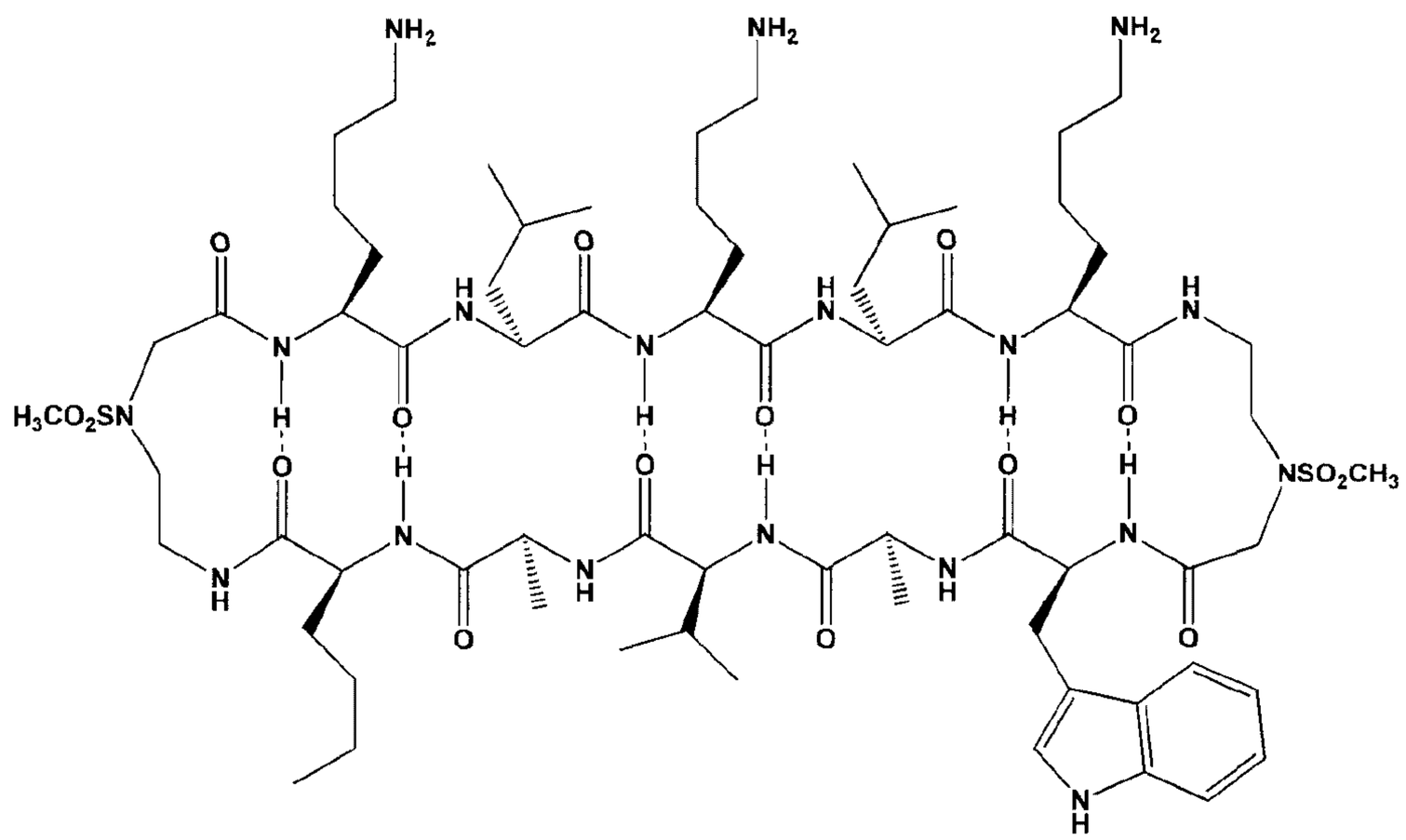
or a pharmaceutically acceptable salt thereof.

86. The use according to claim 6 or 7, wherein the cyclic compound has the structure:



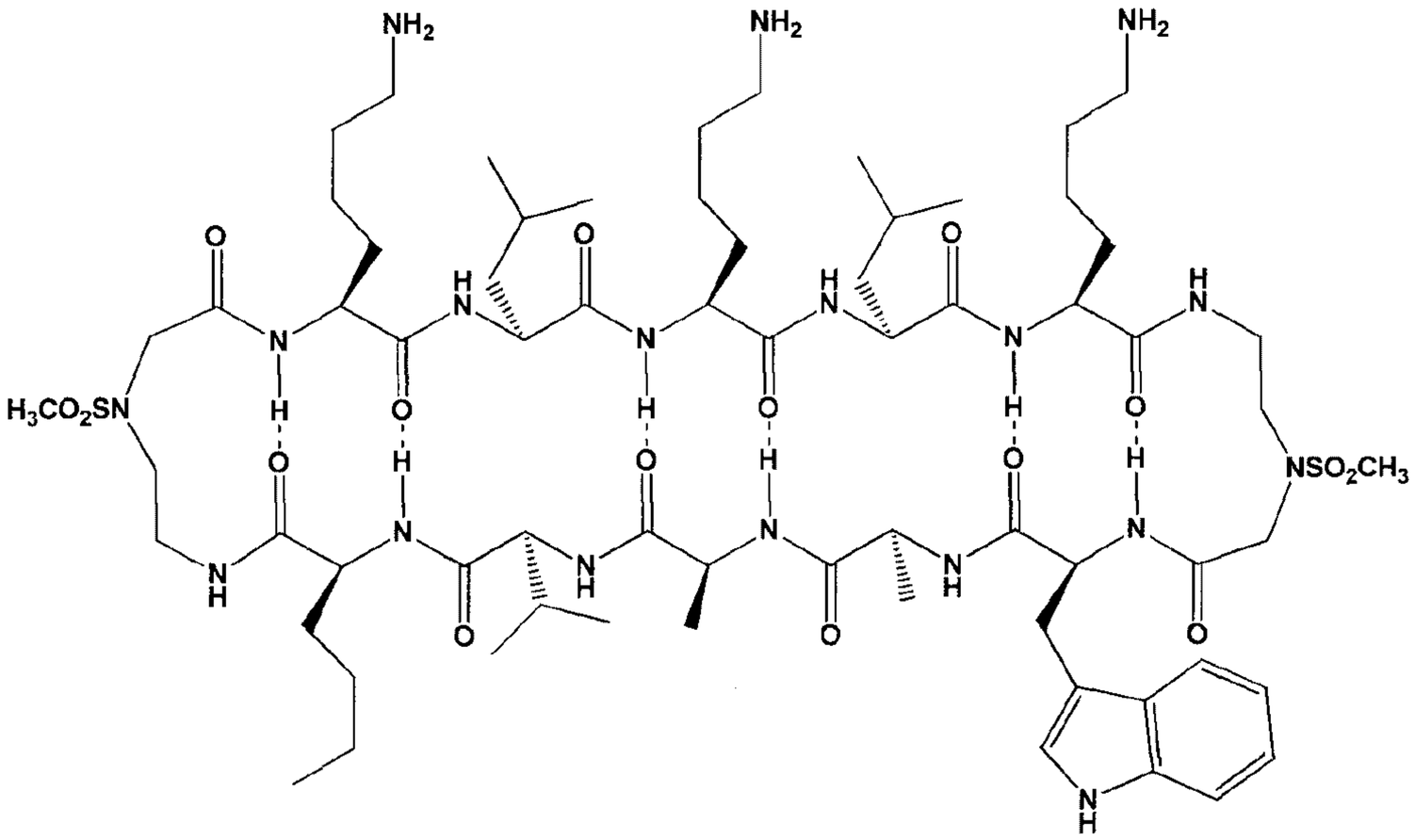
or a pharmaceutically acceptable salt thereof.

87. The use according to claim 6 or 7, wherein the cyclic compound has the structure:



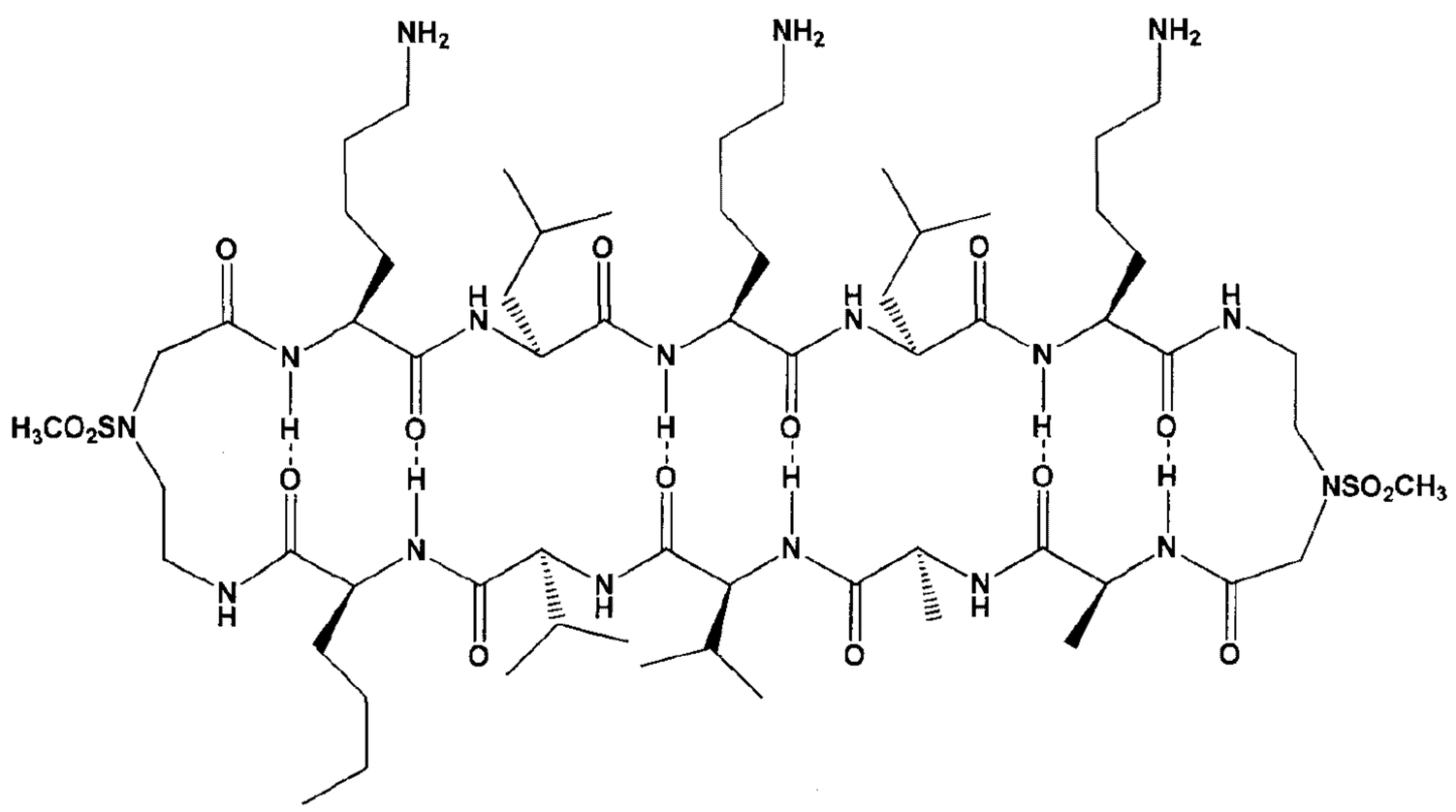
or a pharmaceutically acceptable salt thereof.

88. The use according to claim 6 or 7, wherein the cyclic compound has the structure:



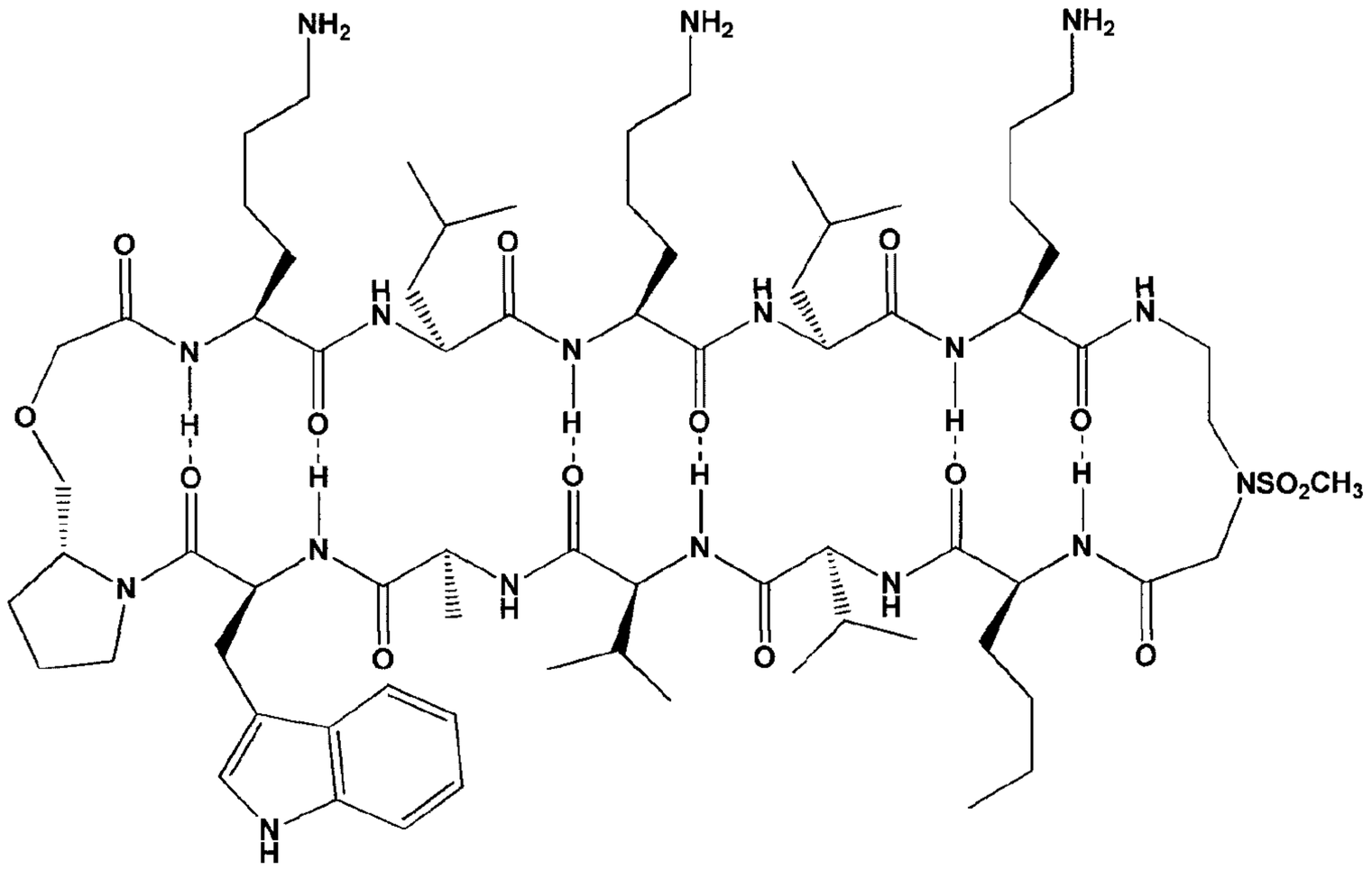
or a pharmaceutically acceptable salt thereof.

89. The use according to claim 6 or 7, wherein the cyclic compound has the structure:



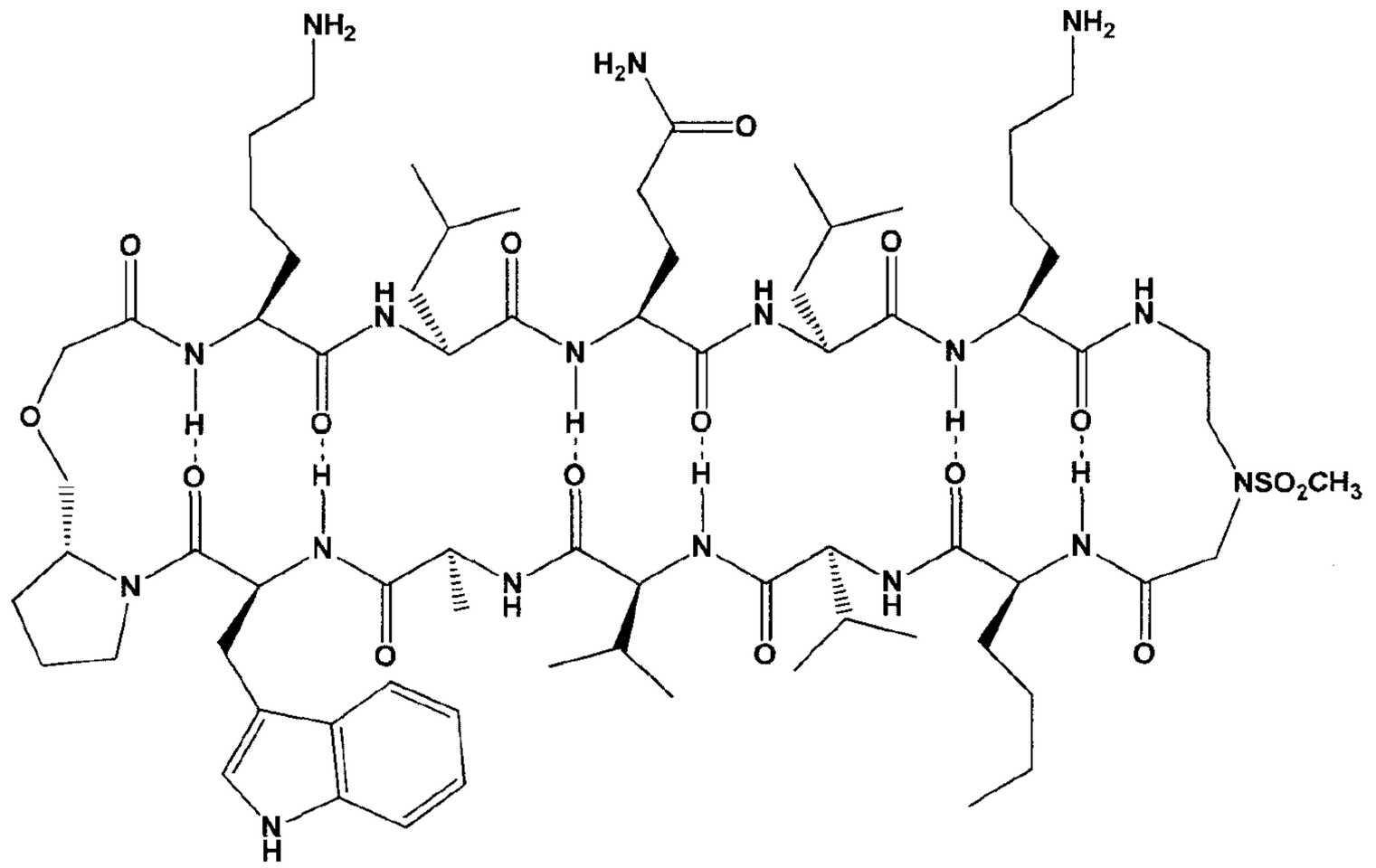
or a pharmaceutically acceptable salt thereof.

90. The use according to claim 6 or 7, wherein the cyclic compound has the structure:



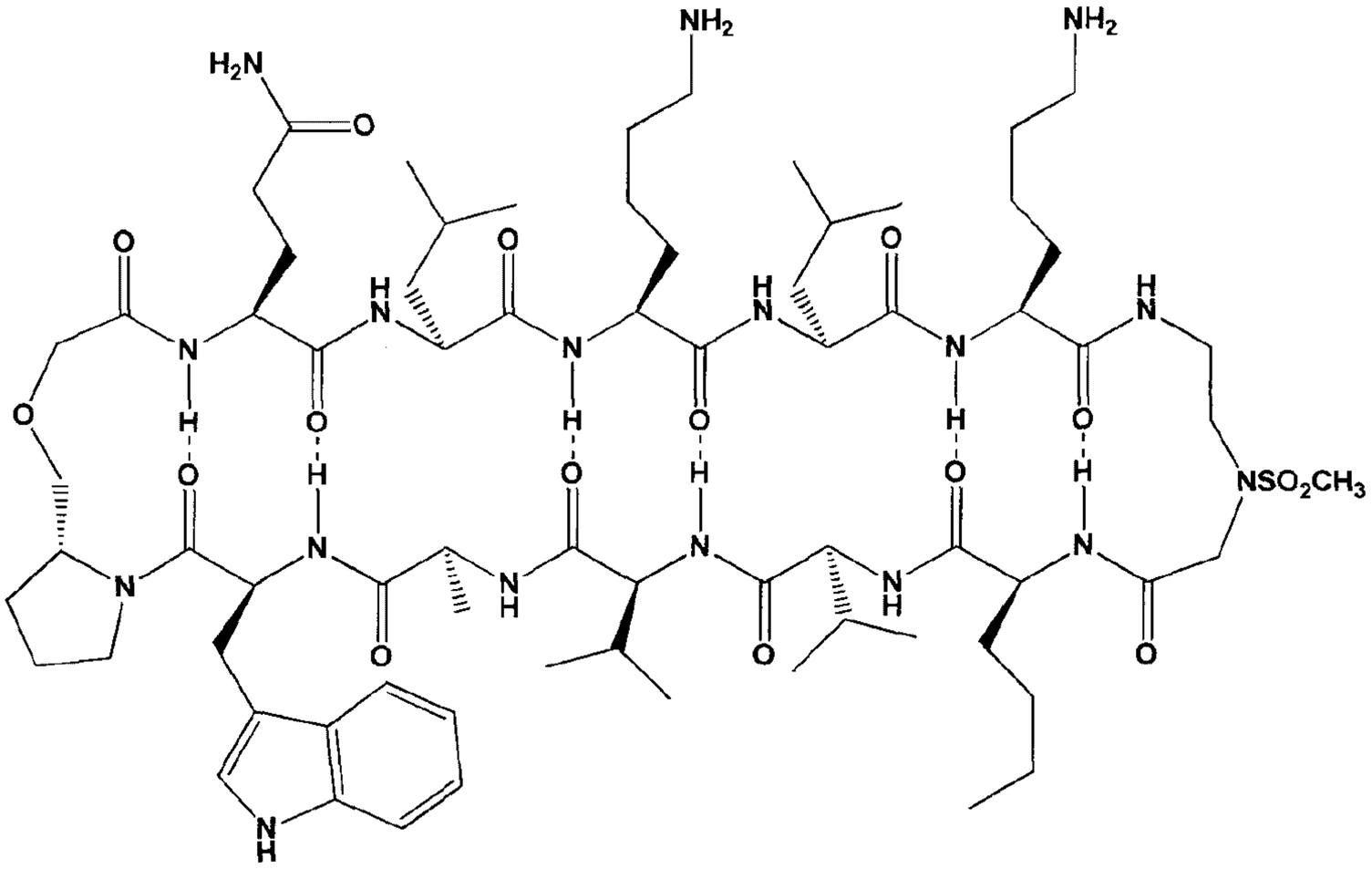
or a pharmaceutically acceptable salt thereof.

91. The use according to claim 6 or 7, wherein the cyclic compound has the structure:



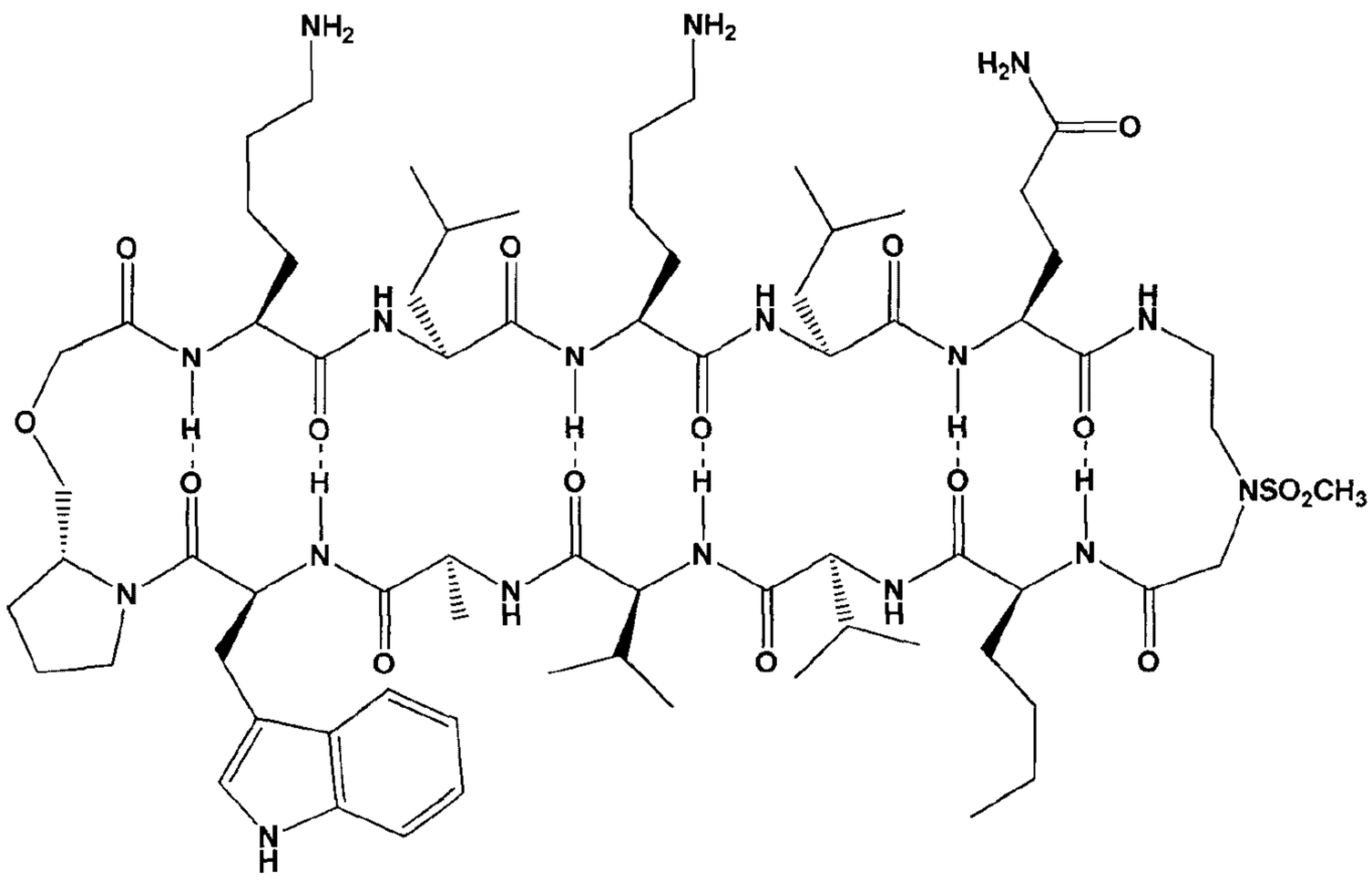
or a pharmaceutically acceptable salt thereof.

92. The use according to claim 6 or 7, wherein the cyclic compound has the structure:



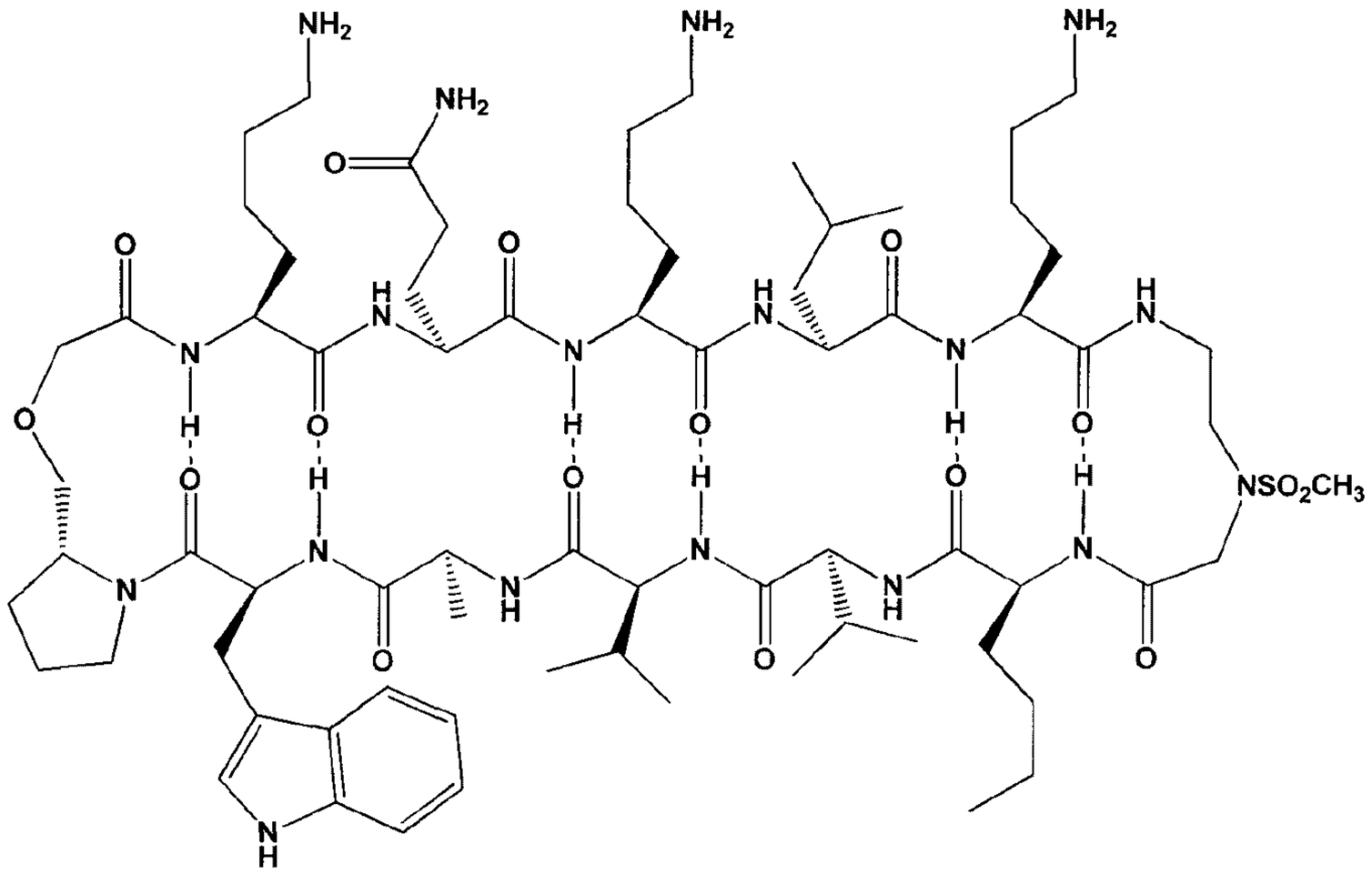
or a pharmaceutically acceptable salt thereof.

93. The use according to claim 6 or 7, wherein the cyclic compound has the structure:



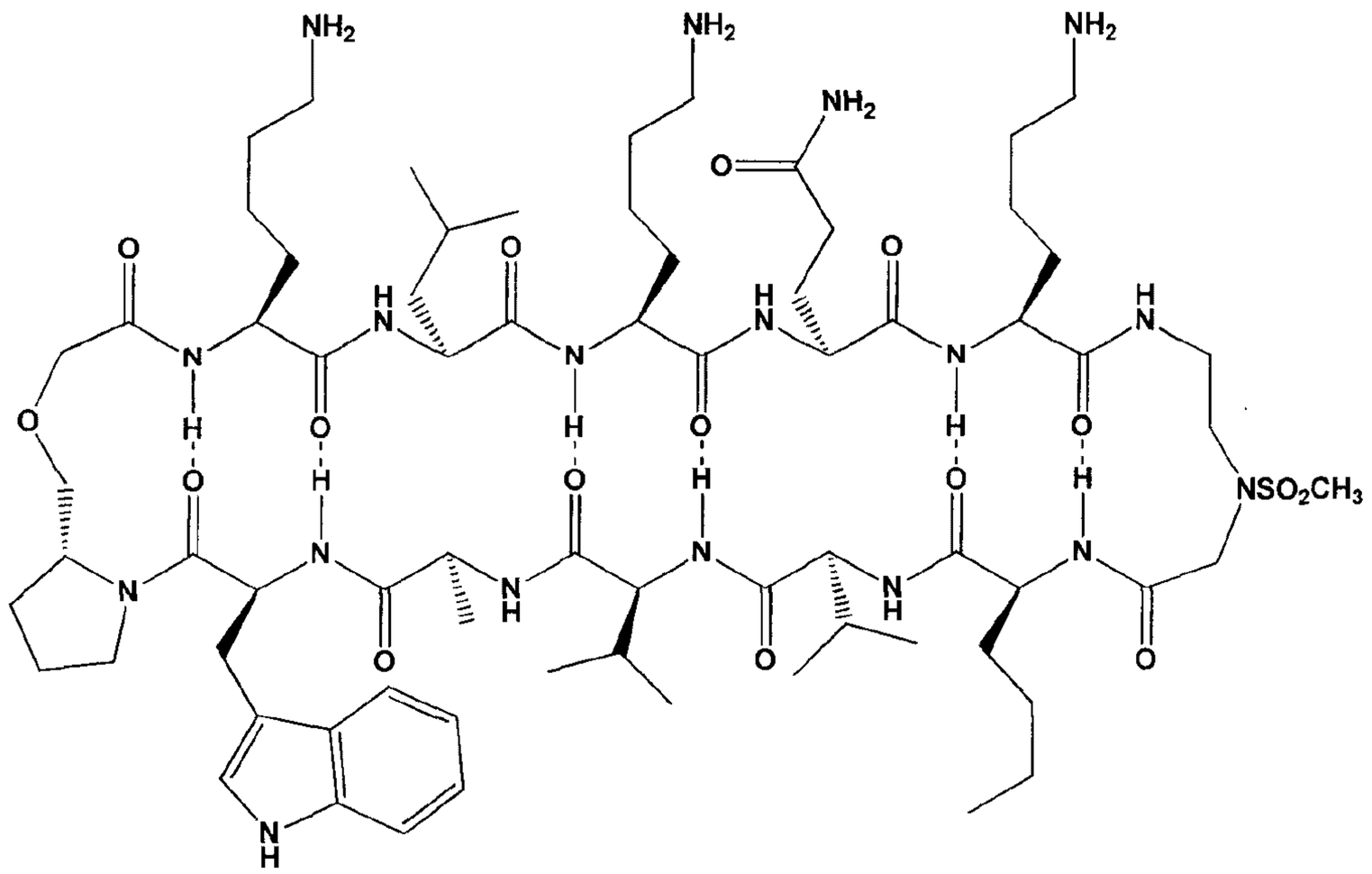
or a pharmaceutically acceptable salt thereof.

94. The use according to claim 6 or 7, wherein the cyclic compound has the structure:



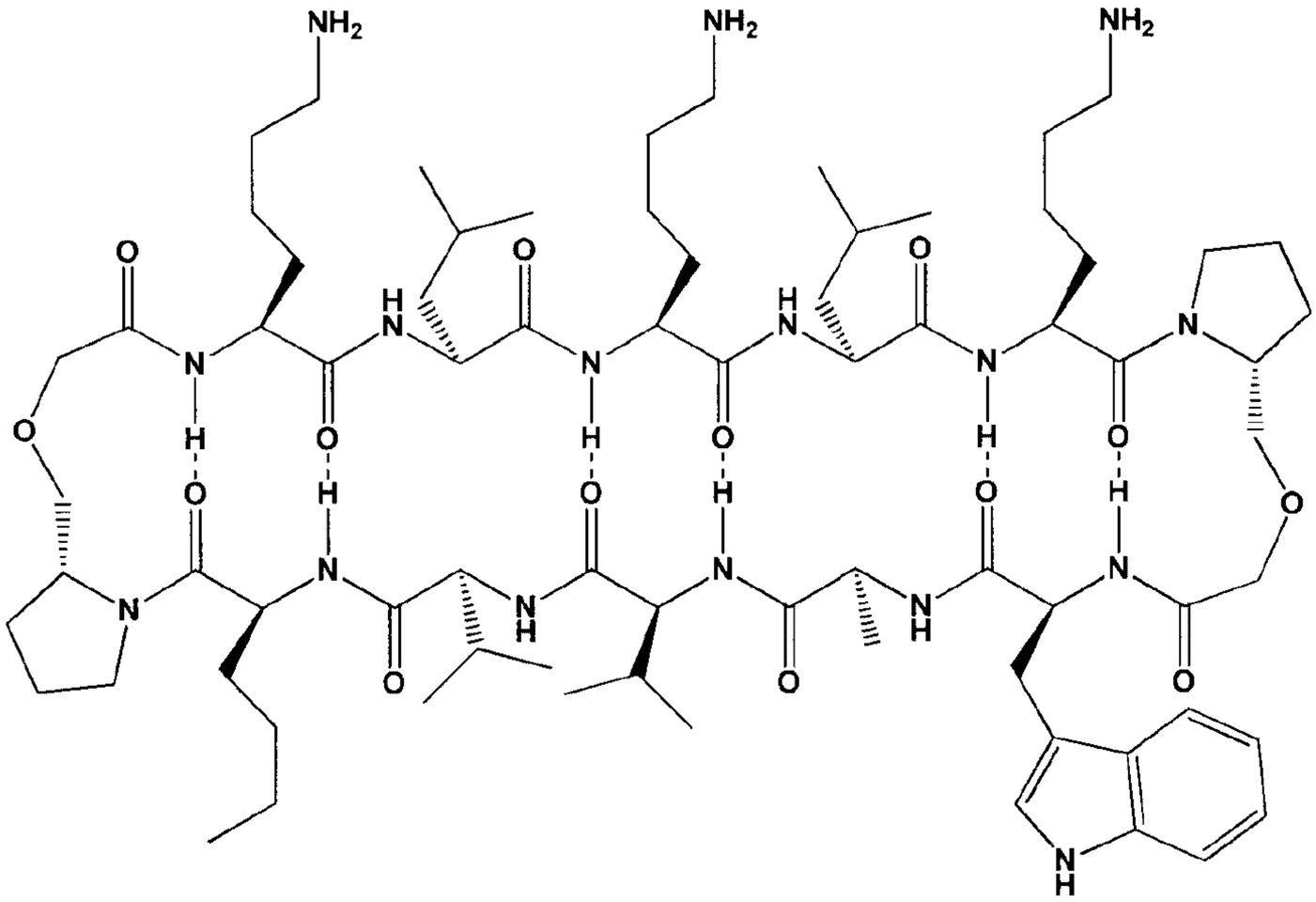
or a pharmaceutically acceptable salt thereof.

95. The use according to claim 6 or 7, wherein the cyclic compound has the structure:



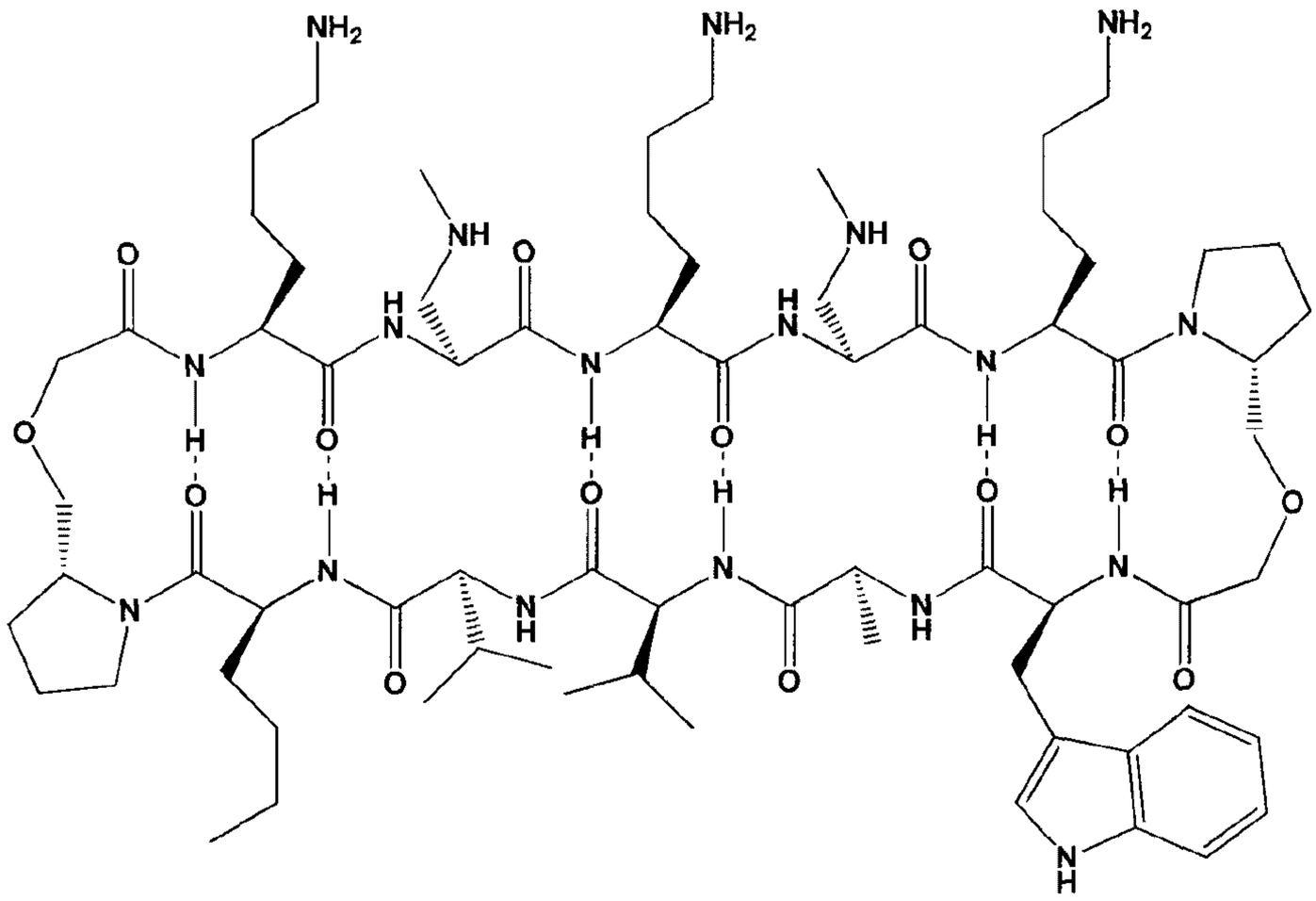
or a pharmaceutically acceptable salt thereof.

96. The use according to claim 6 or 7, wherein the cyclic compound has the structure:



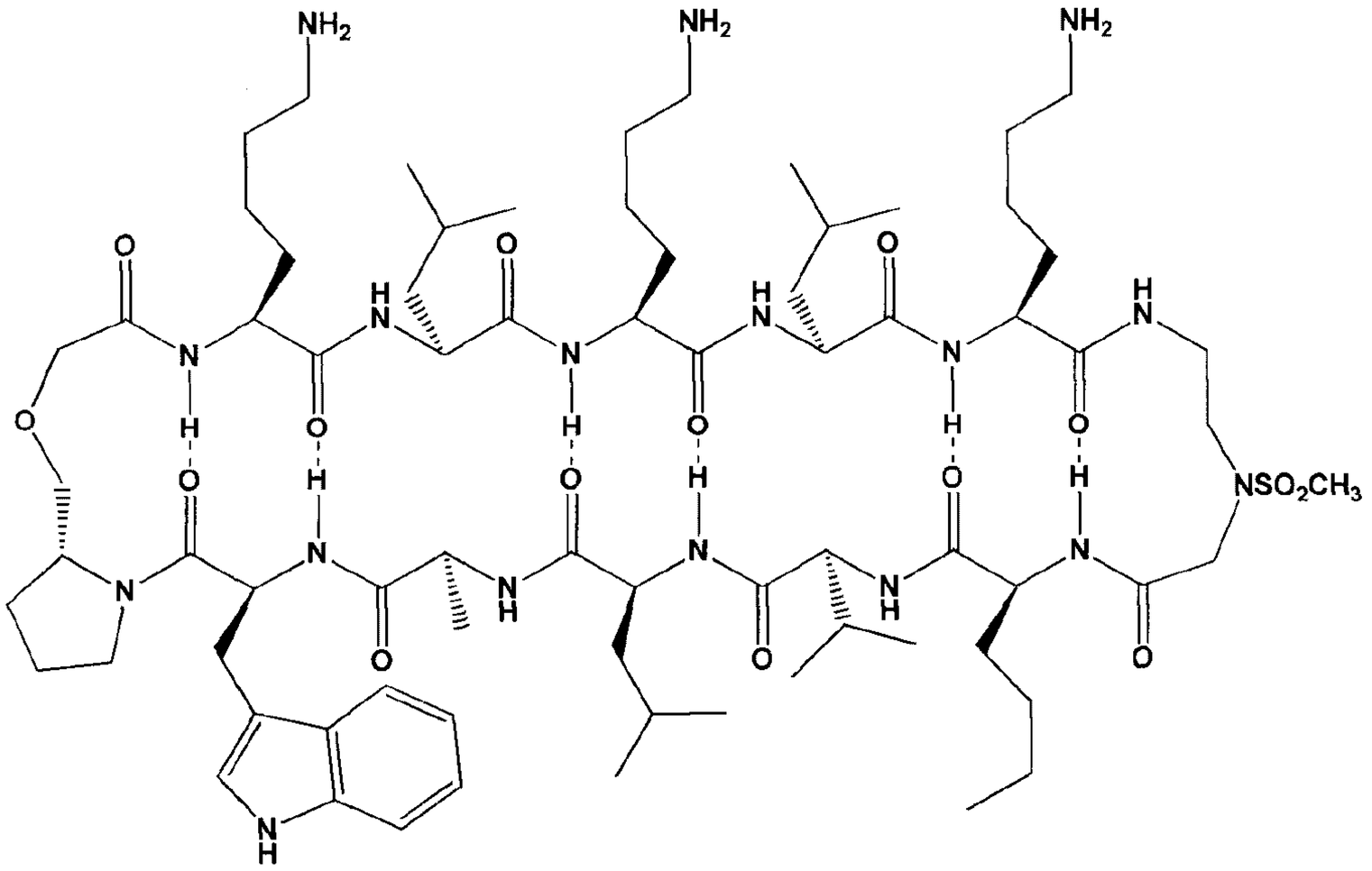
or a pharmaceutically acceptable salt thereof.

97. The use according to claim 6 or 7, wherein the cyclic compound has the structure:



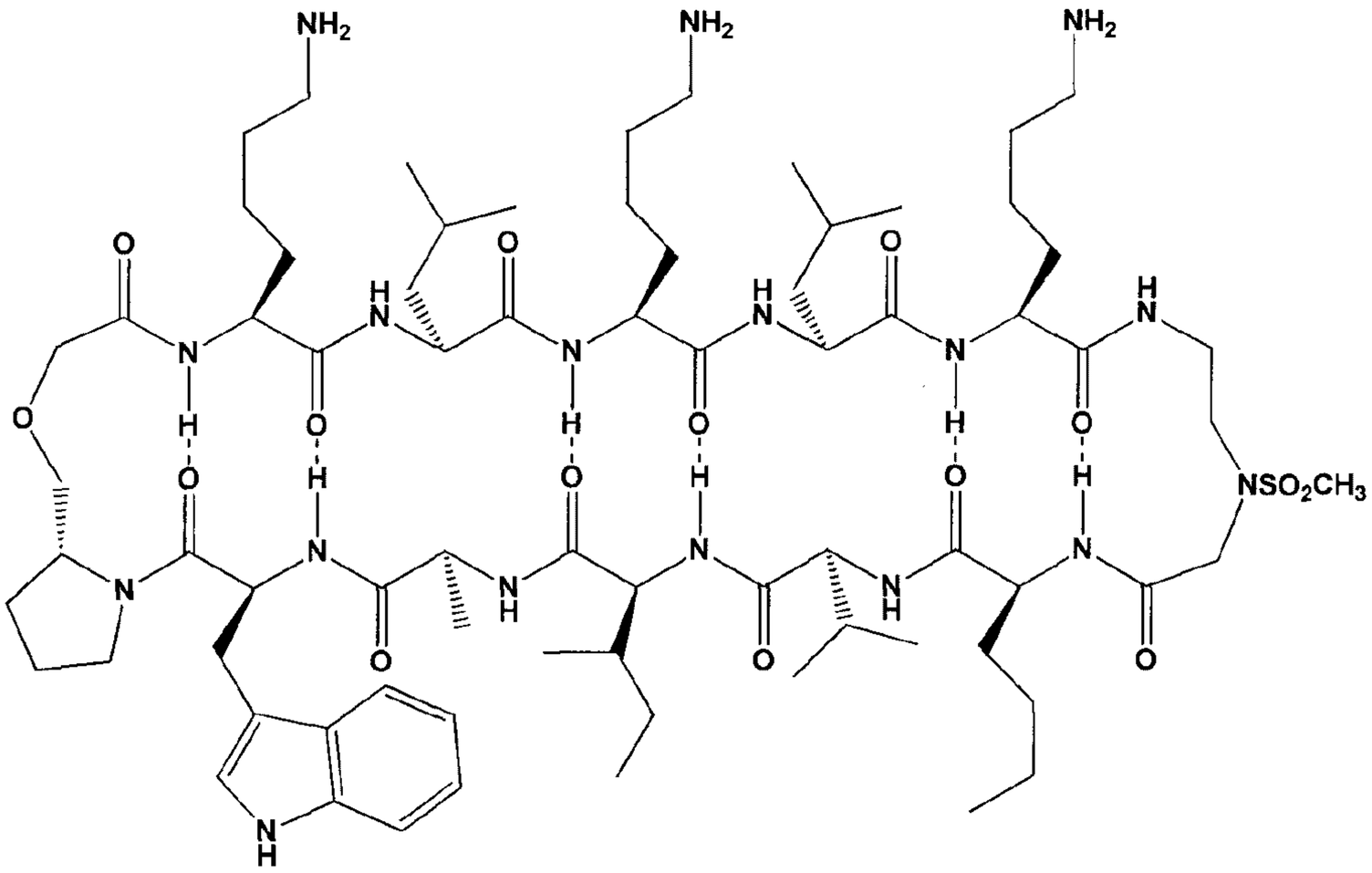
or a pharmaceutically acceptable salt thereof.

98. The use according to claim 6 or 7, wherein the cyclic compound has the structure:



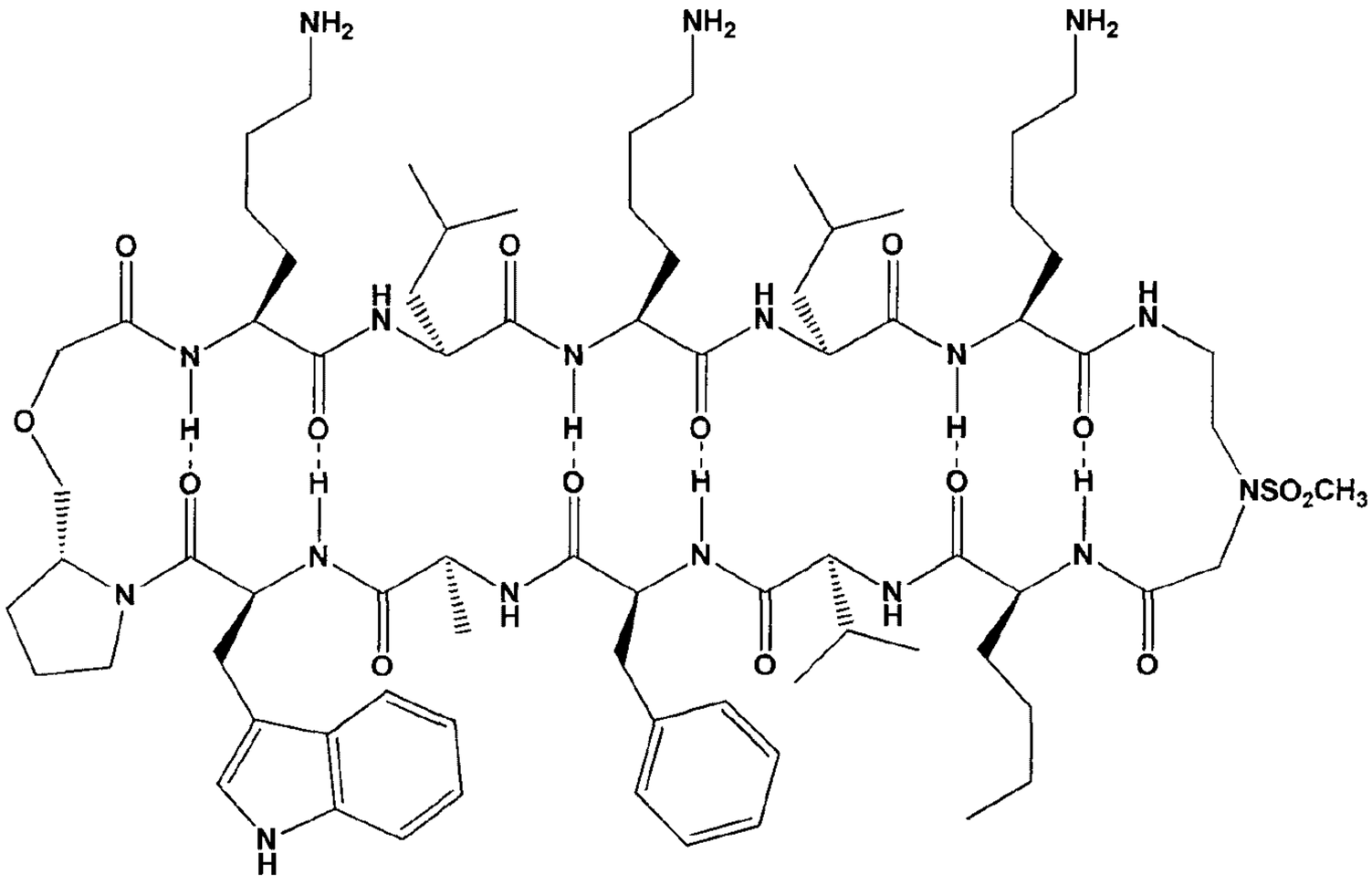
or a pharmaceutically acceptable salt thereof.

99. The use according to claim 6 or 7, wherein the cyclic compound has the structure:



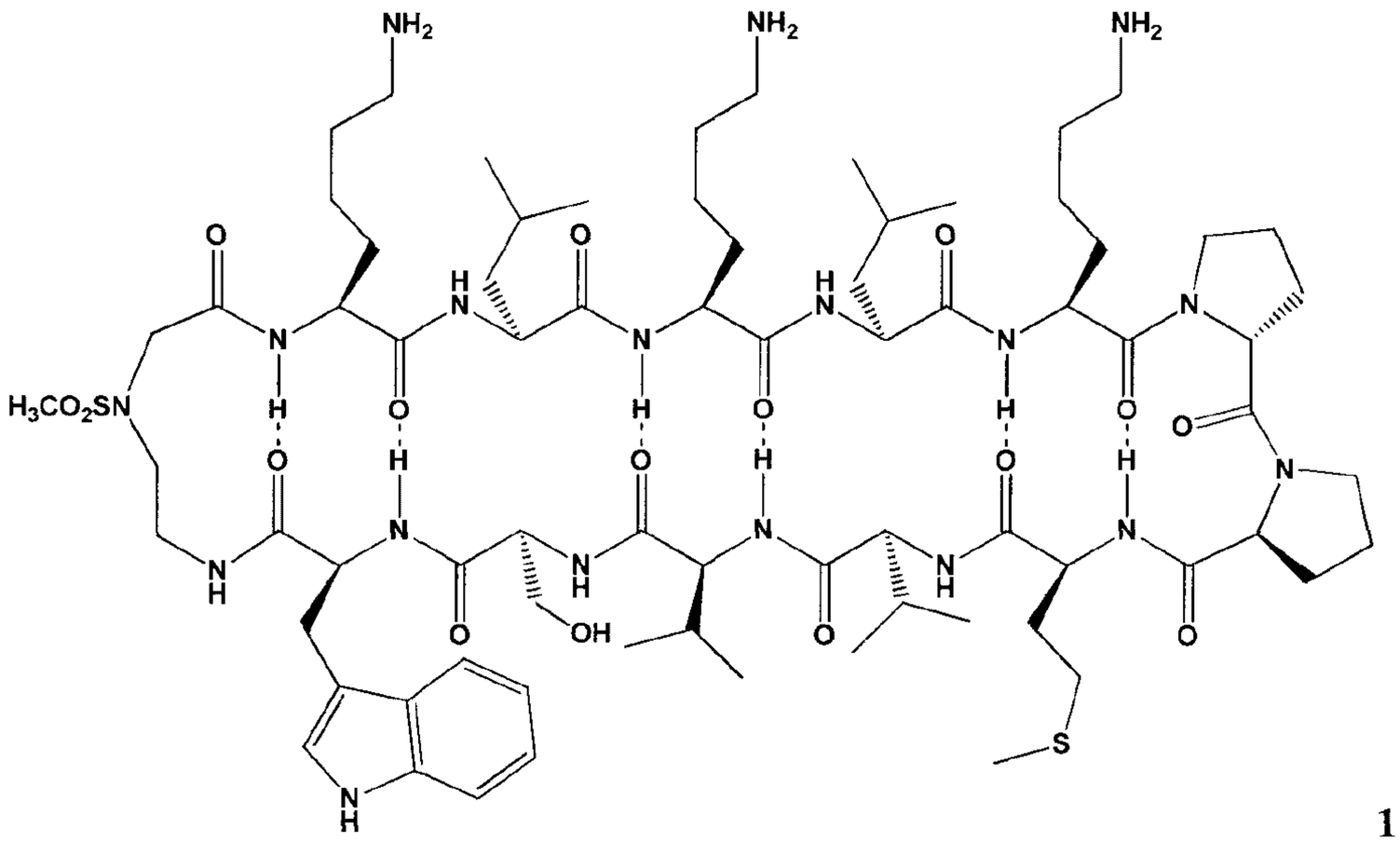
or a pharmaceutically acceptable salt thereof.

100. The use according to claim 6 or 7, wherein the cyclic compound has the structure:

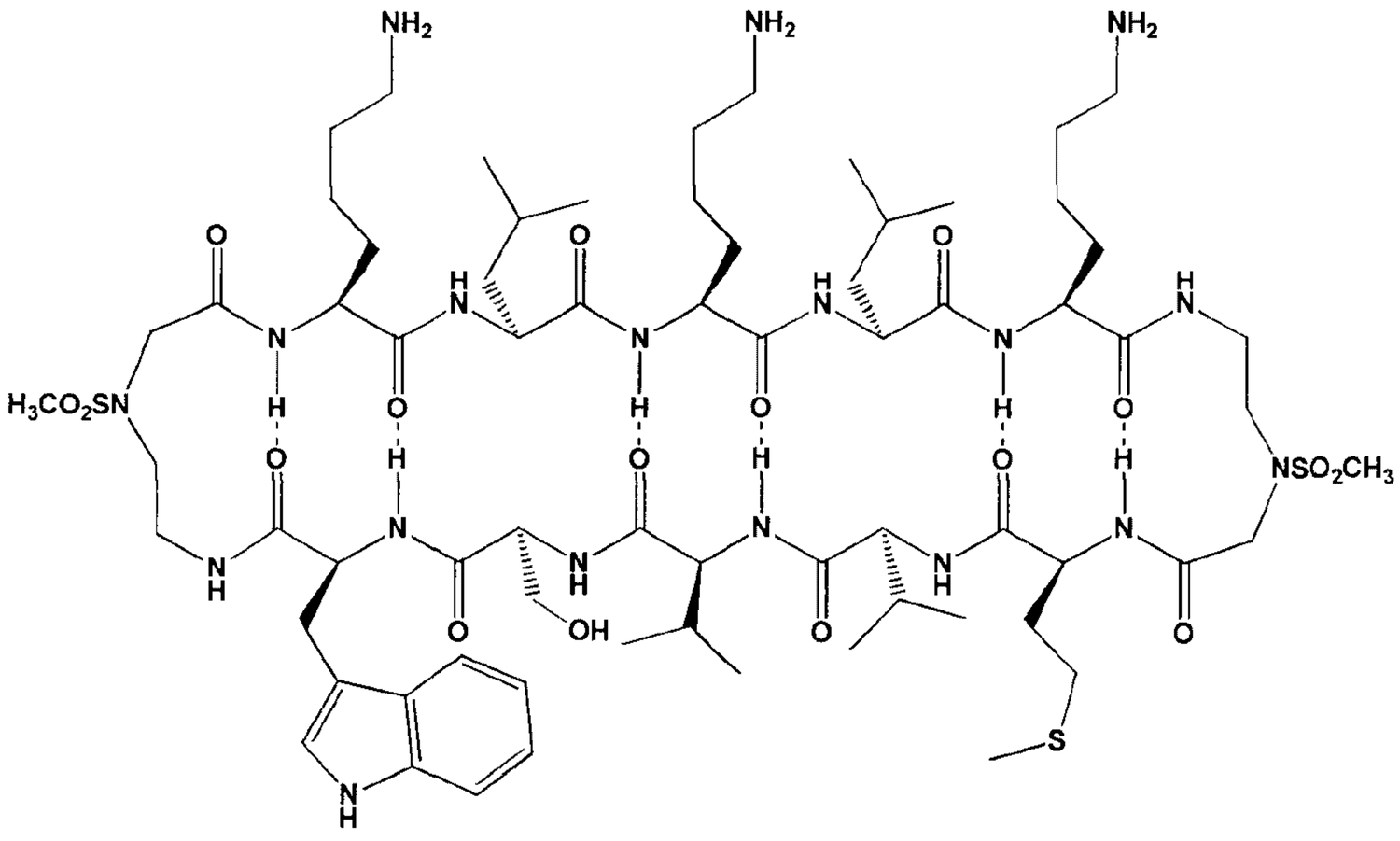


or a pharmaceutically acceptable salt thereof.

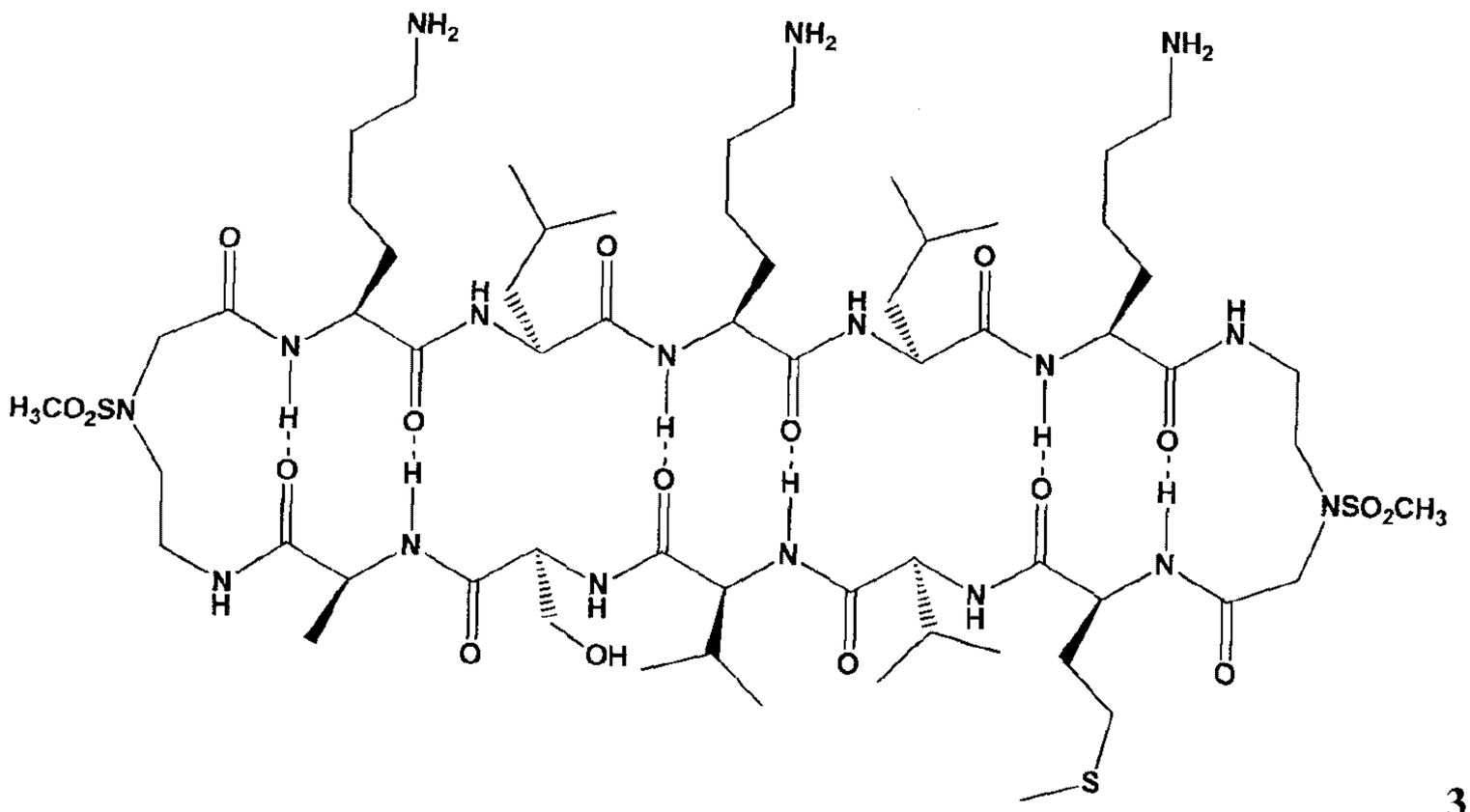
101. A cyclic compound having the following structure, or a pharmaceutically acceptable salt thereof:



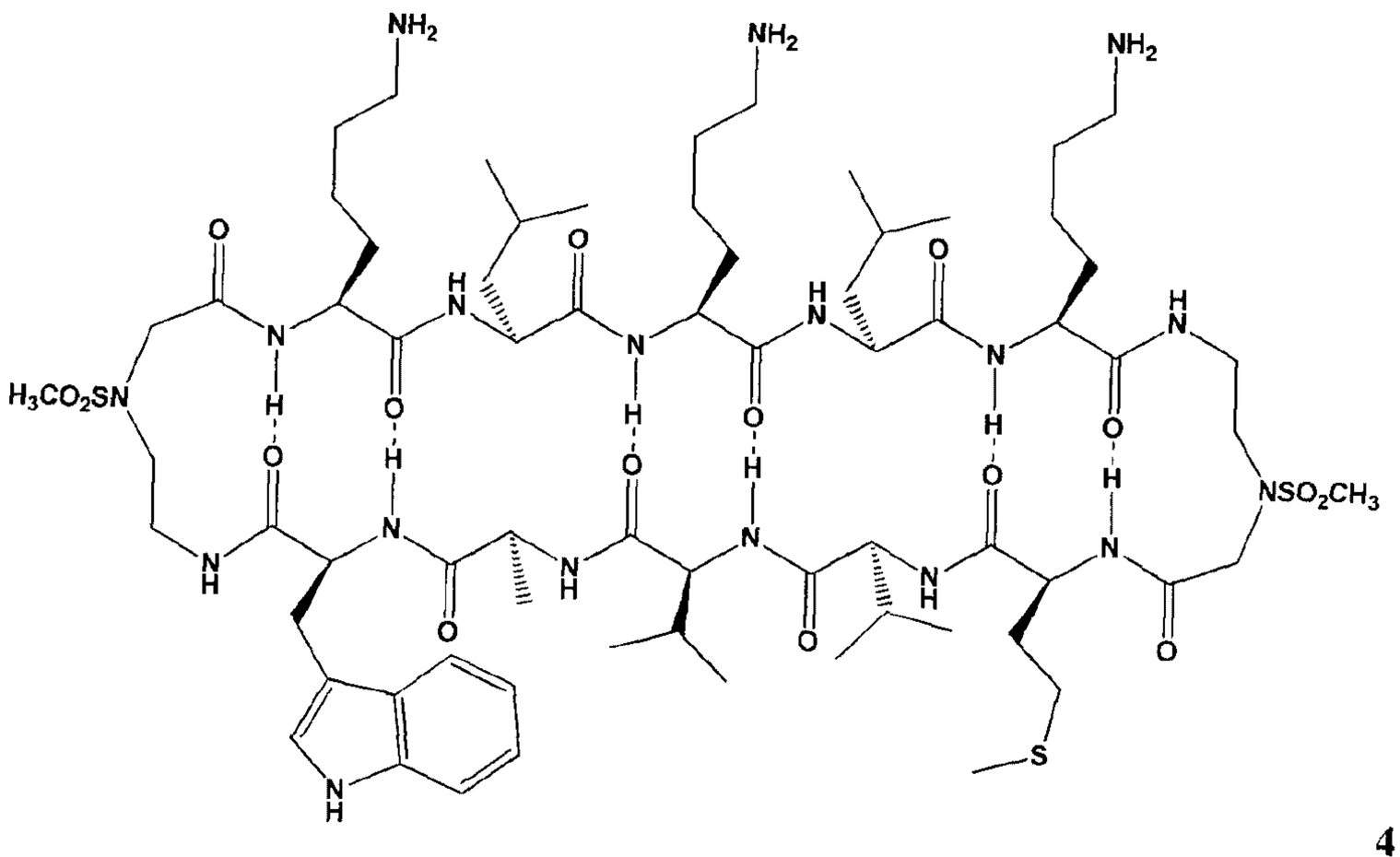
1



2



3



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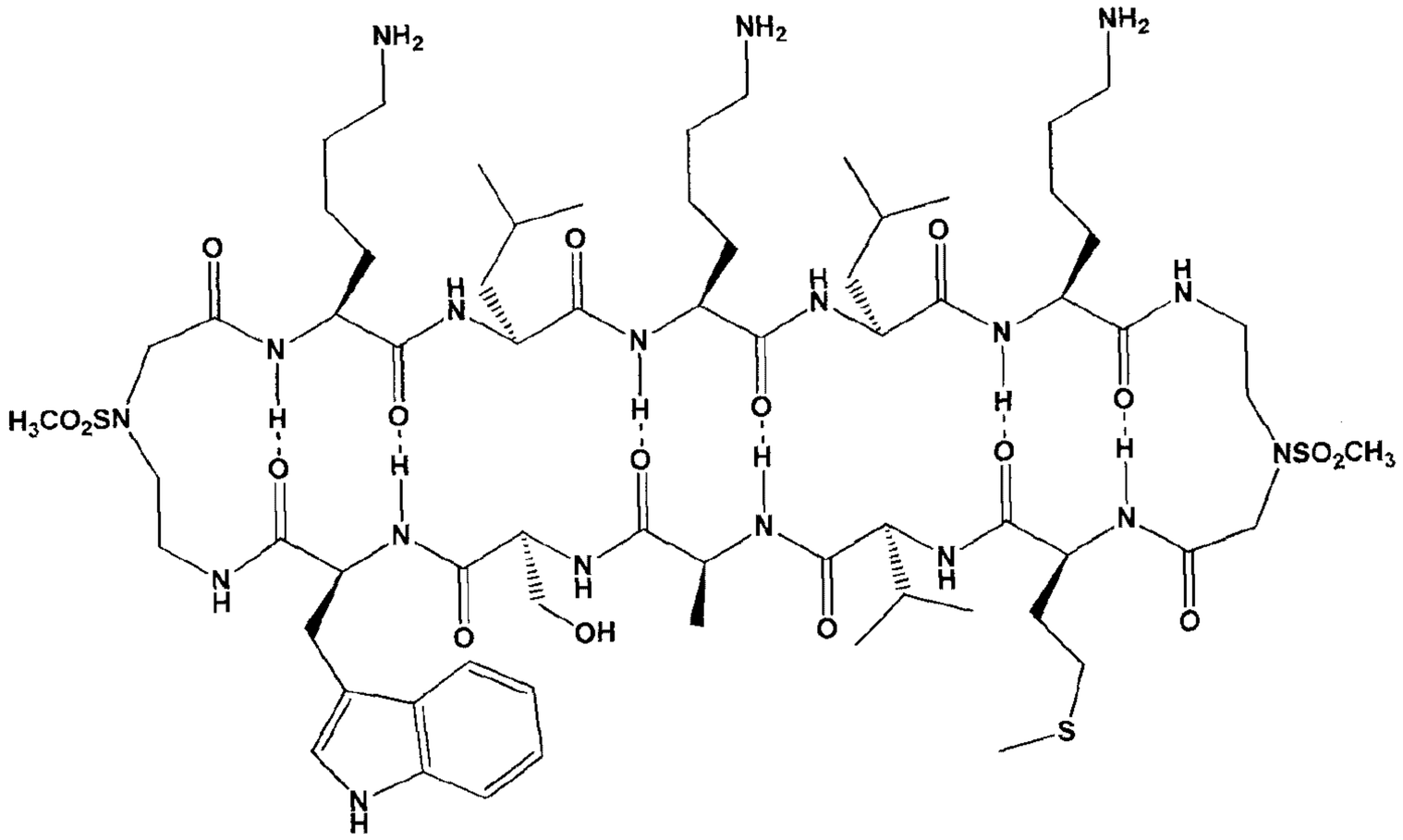

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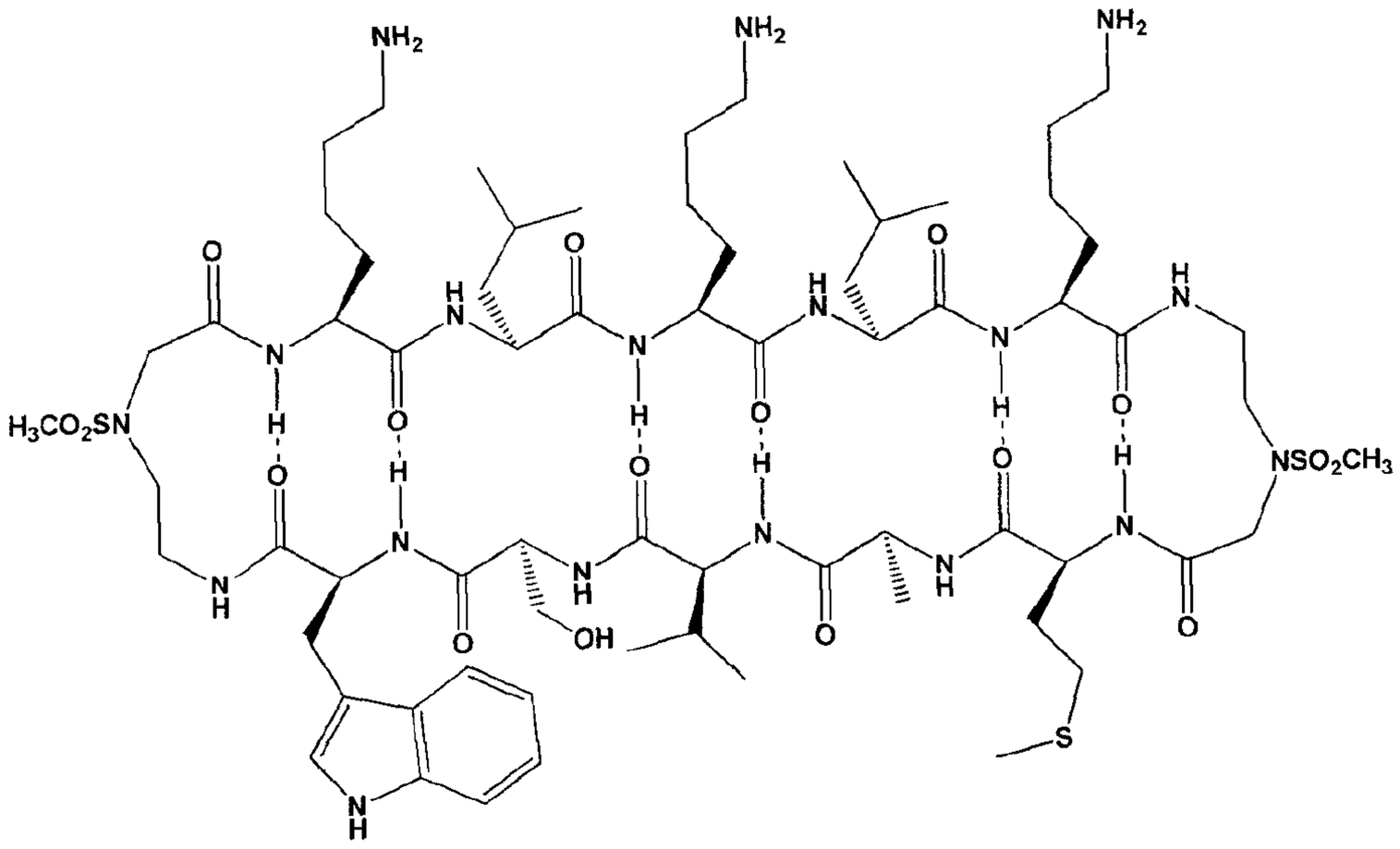

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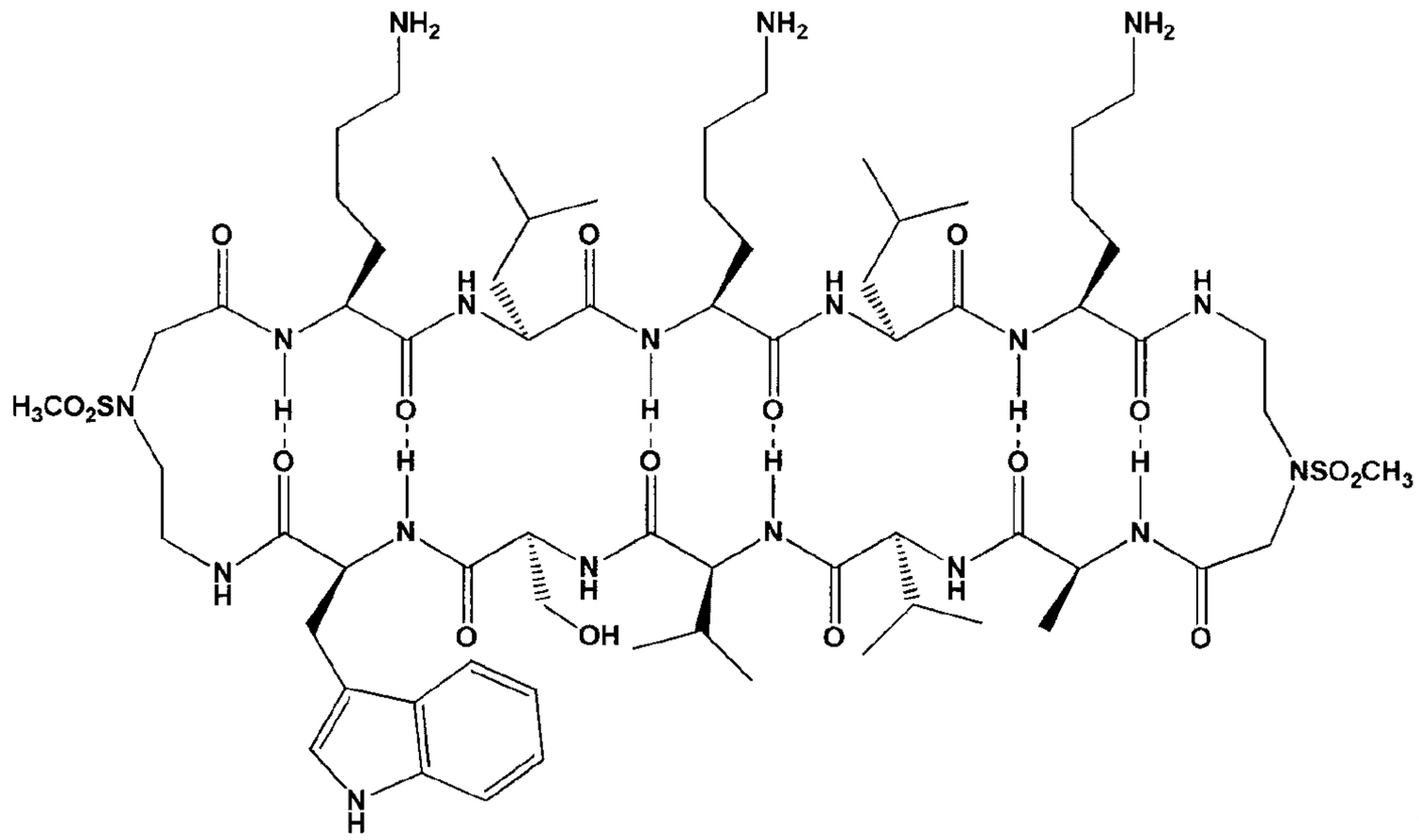

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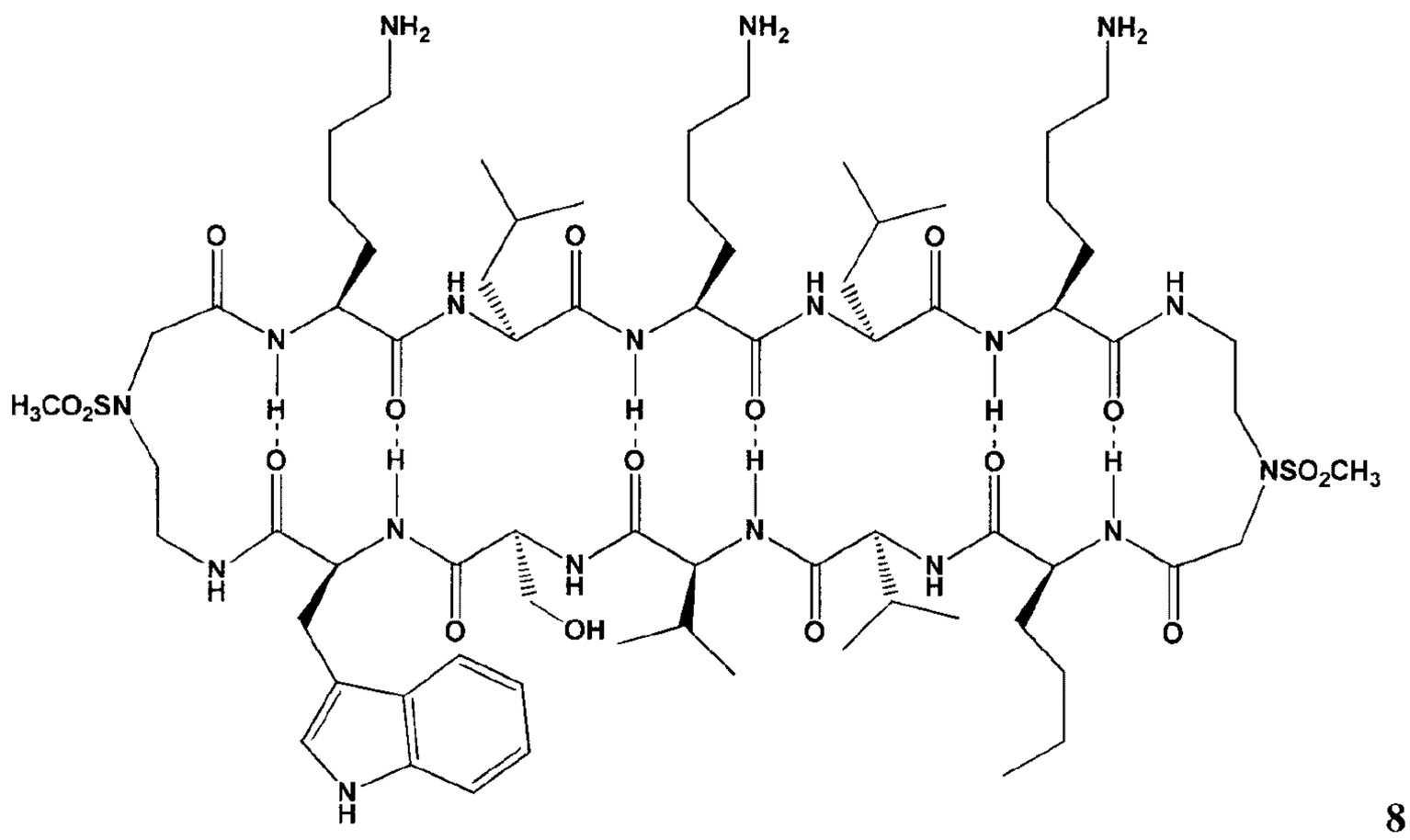
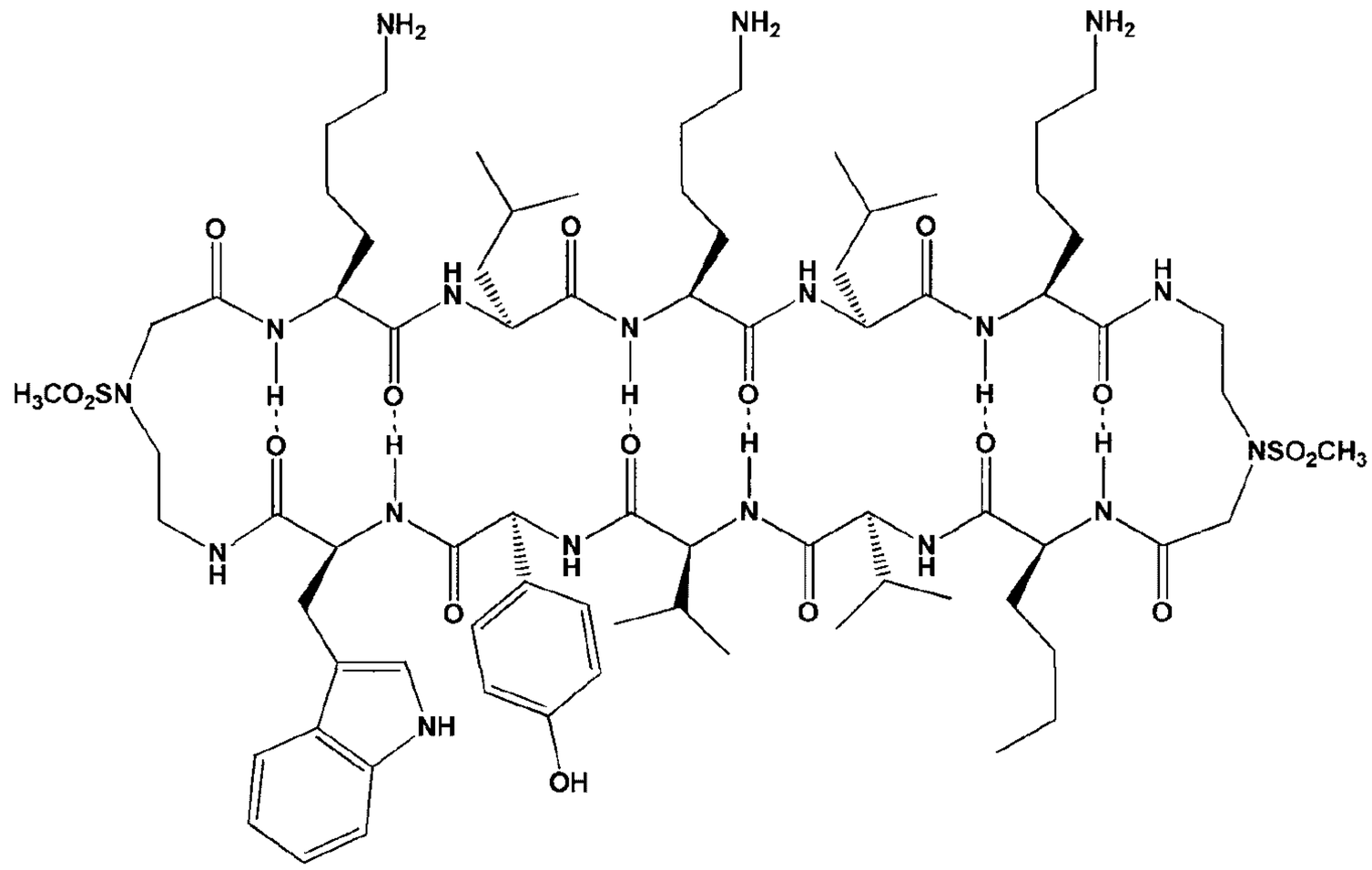


8



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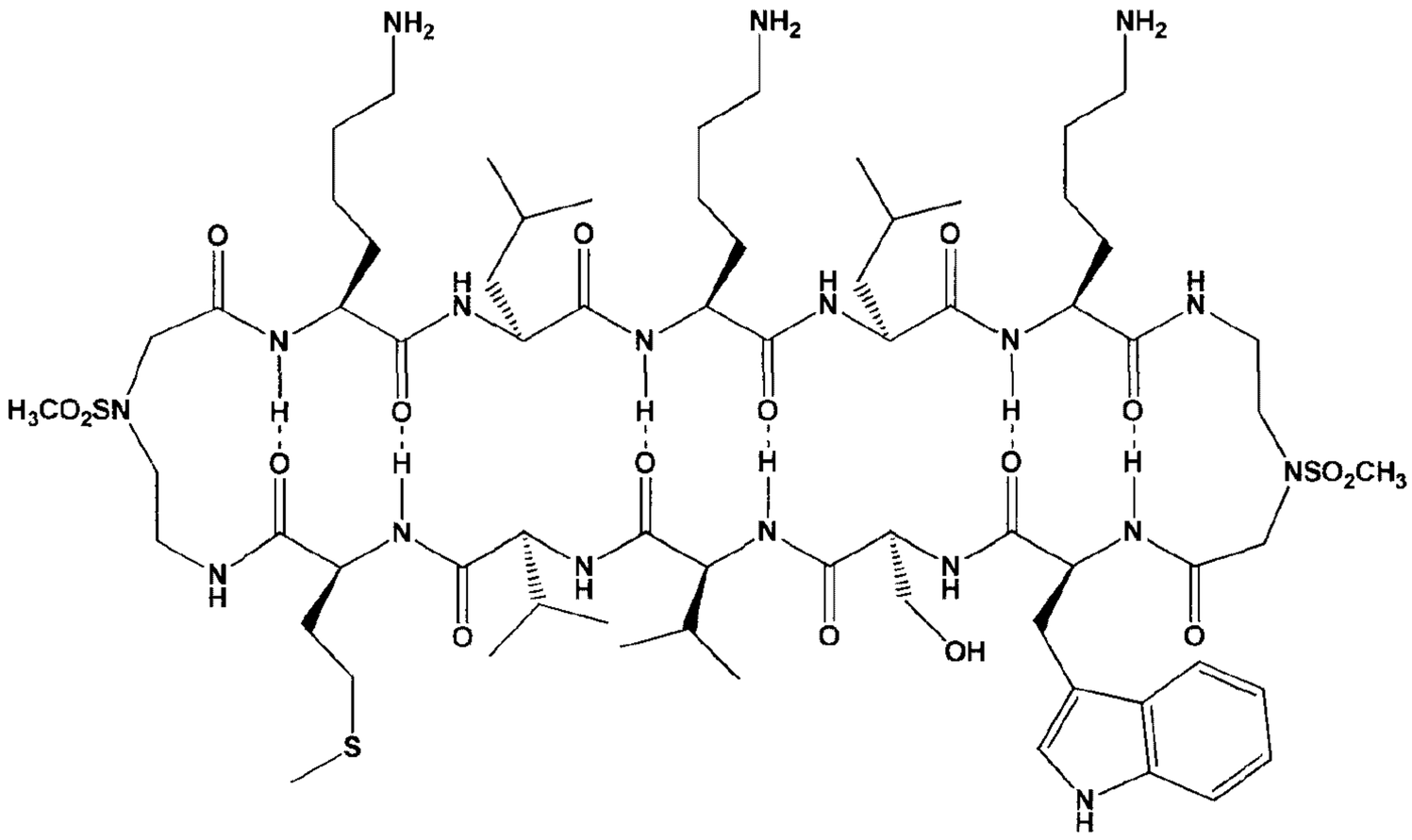

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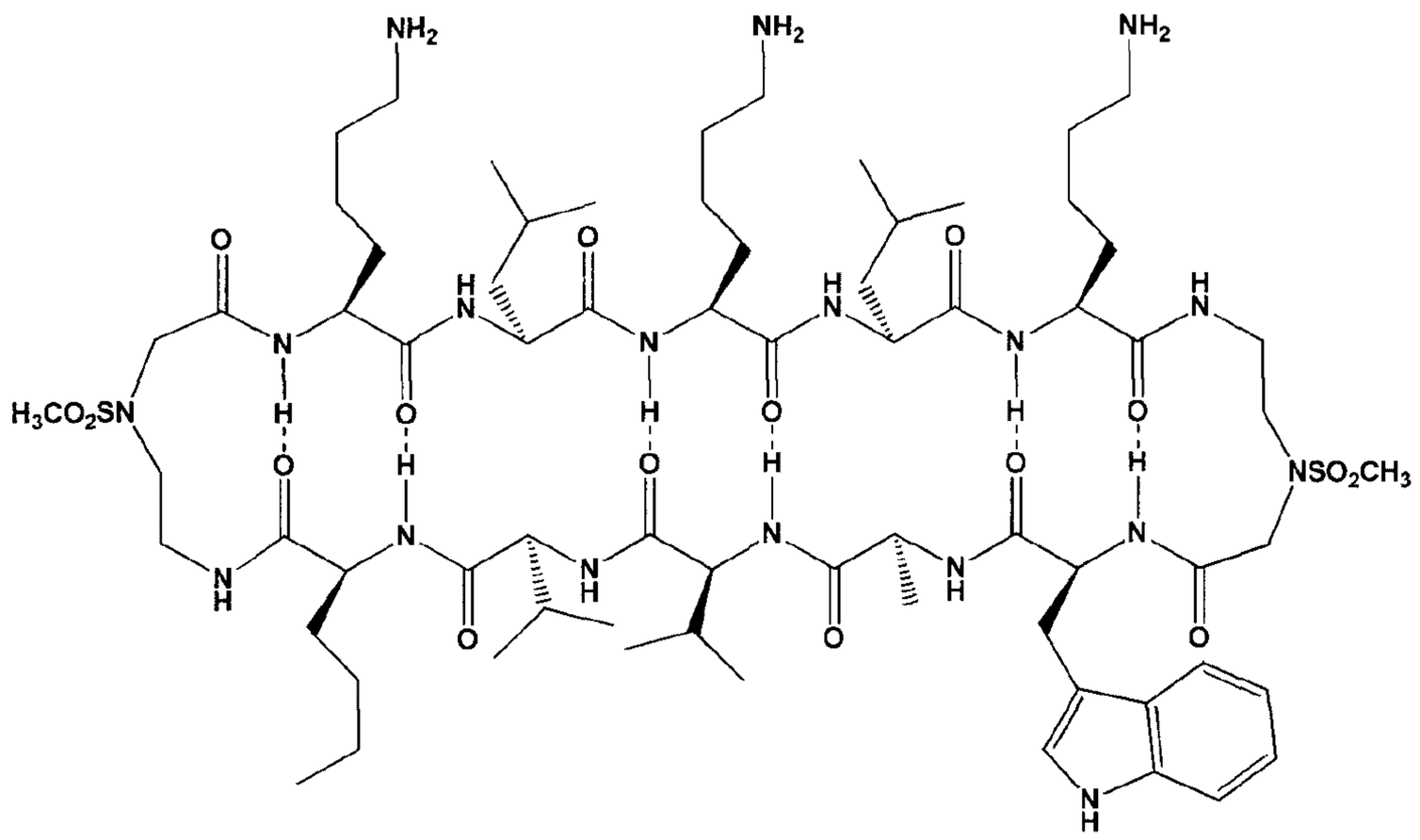

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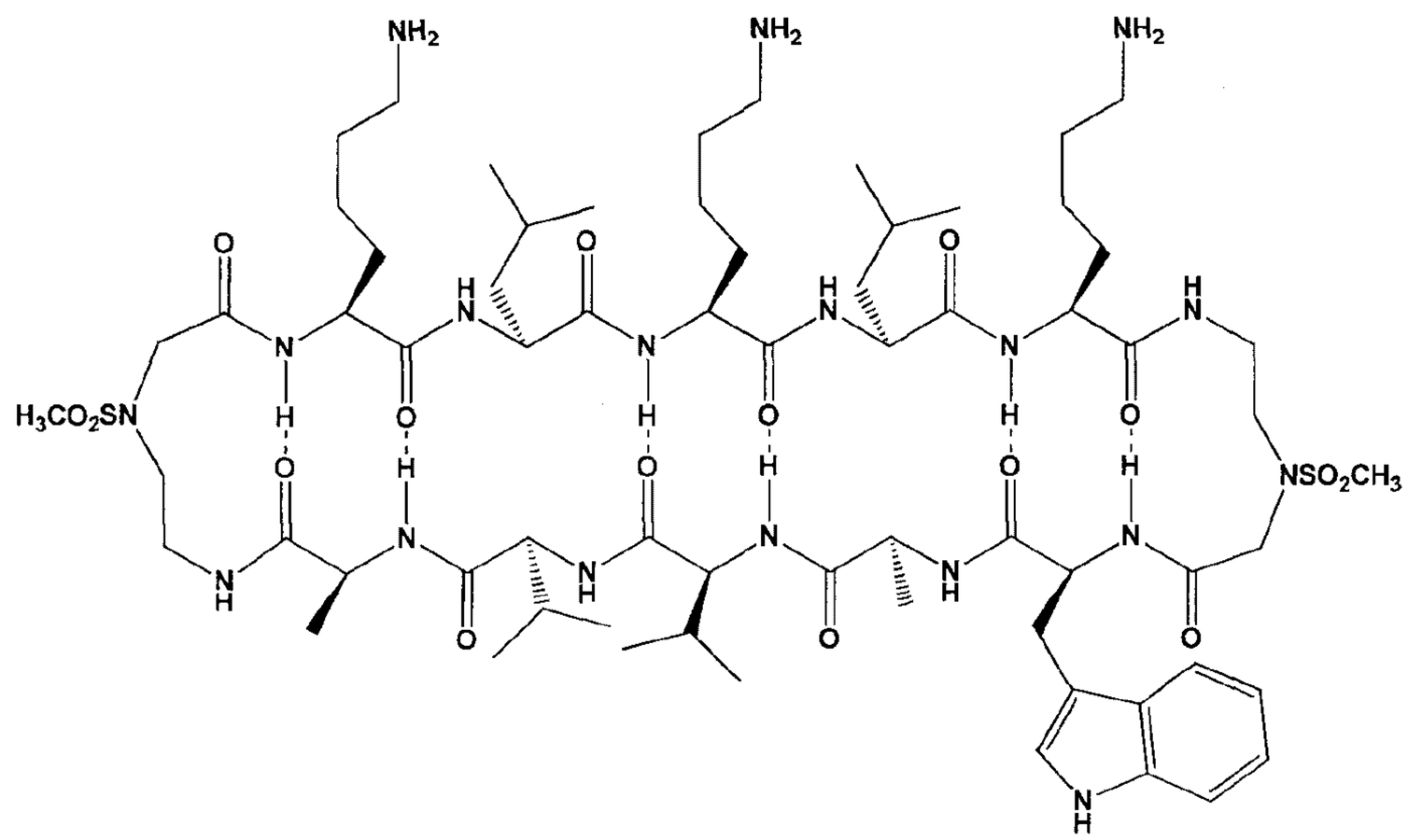

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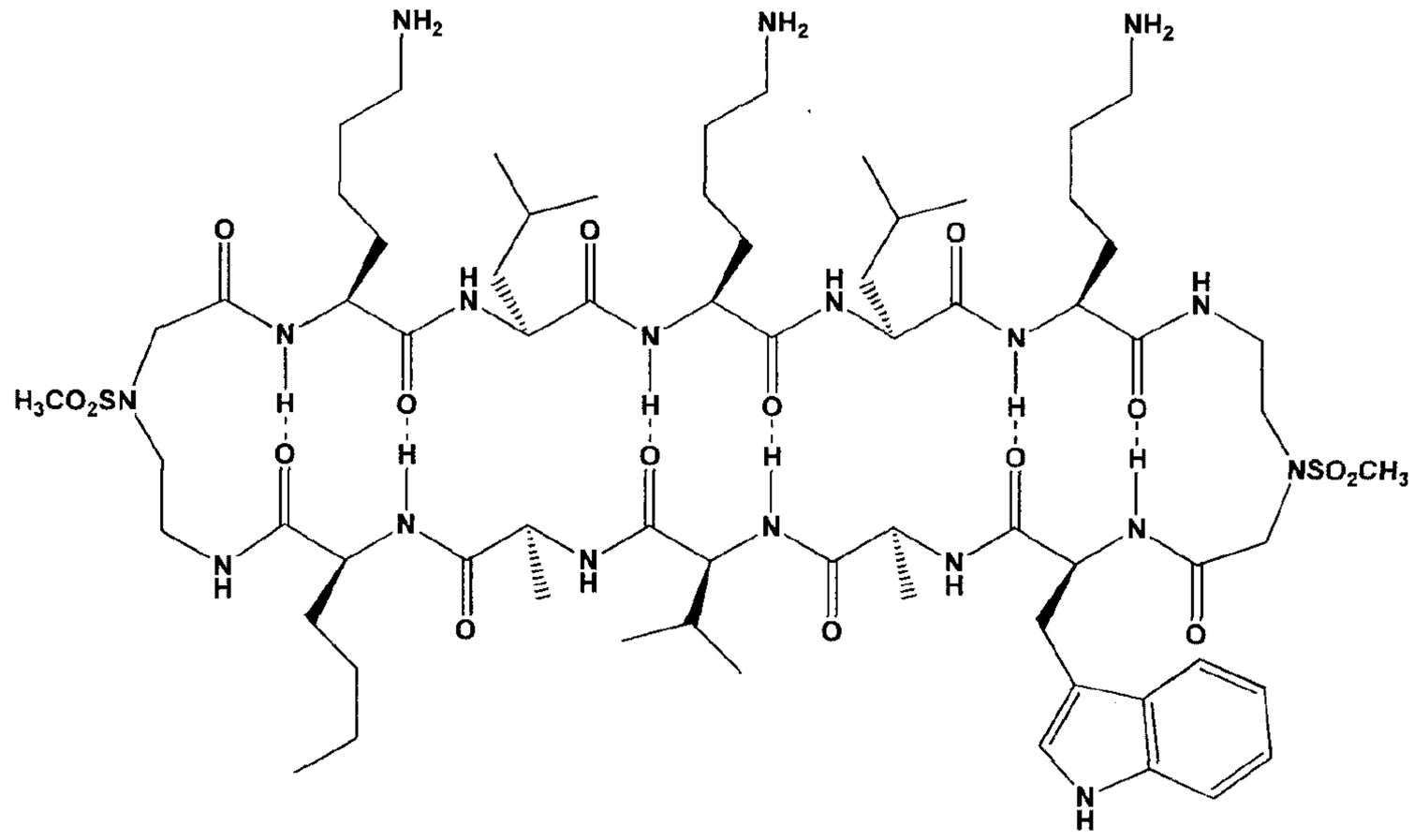

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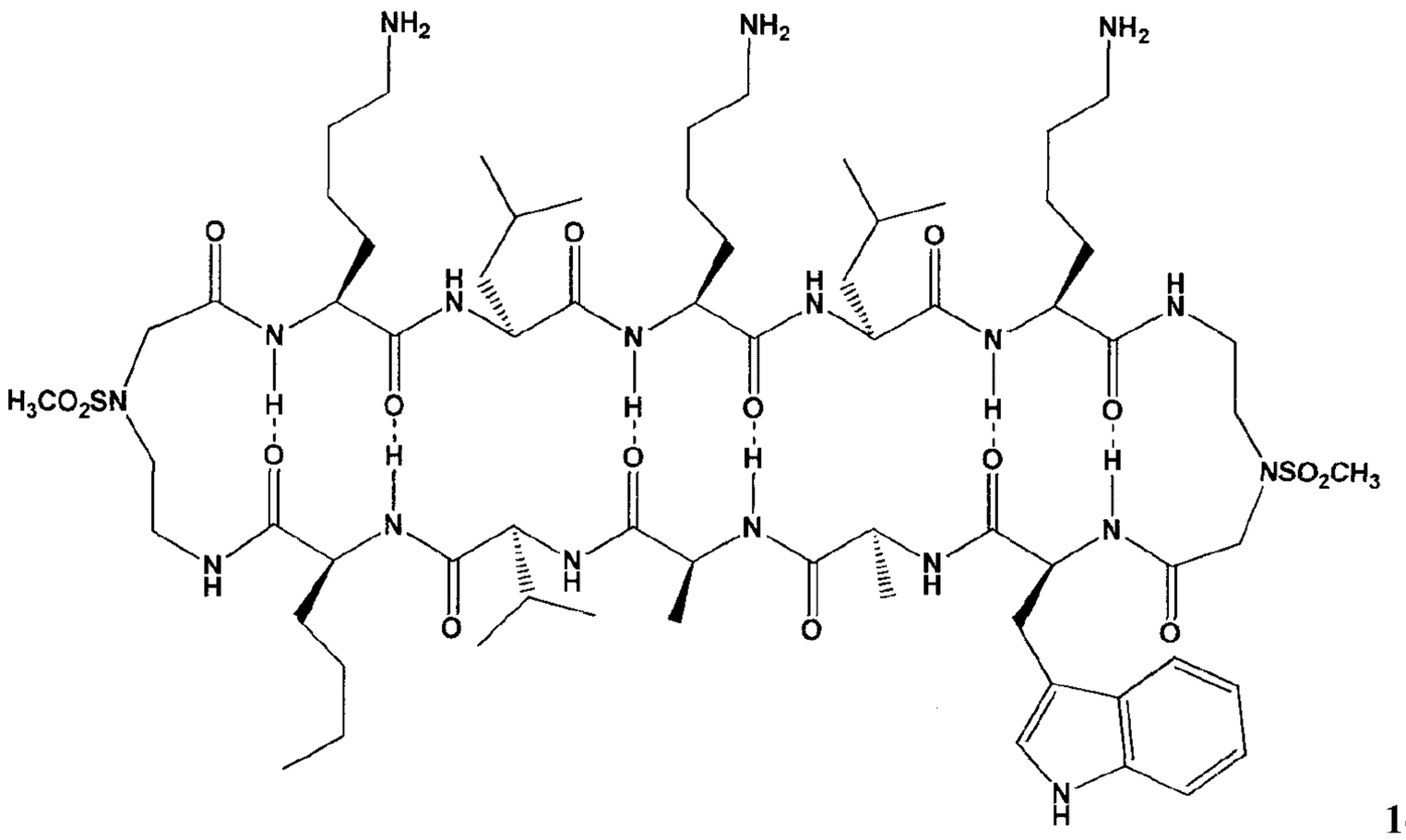

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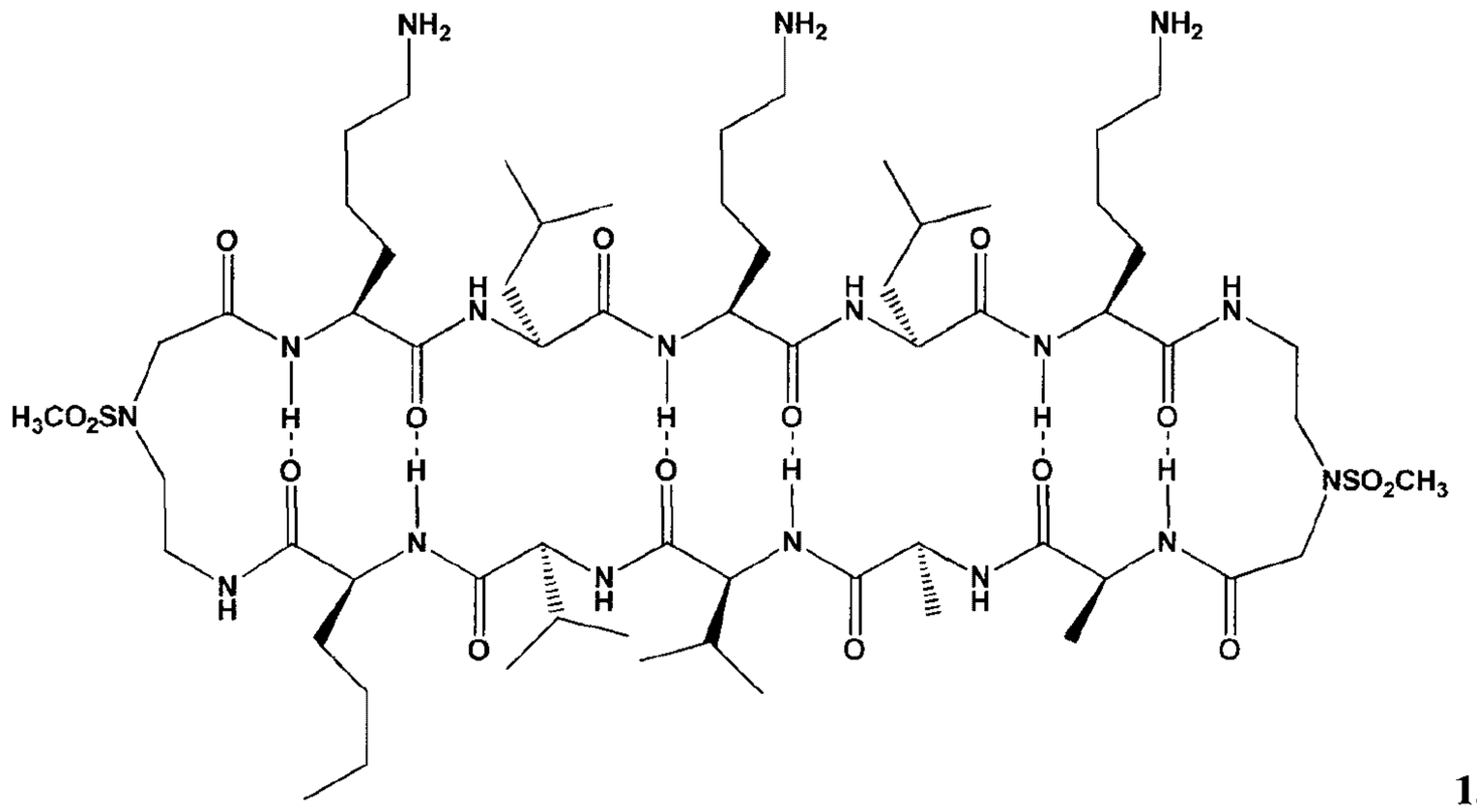

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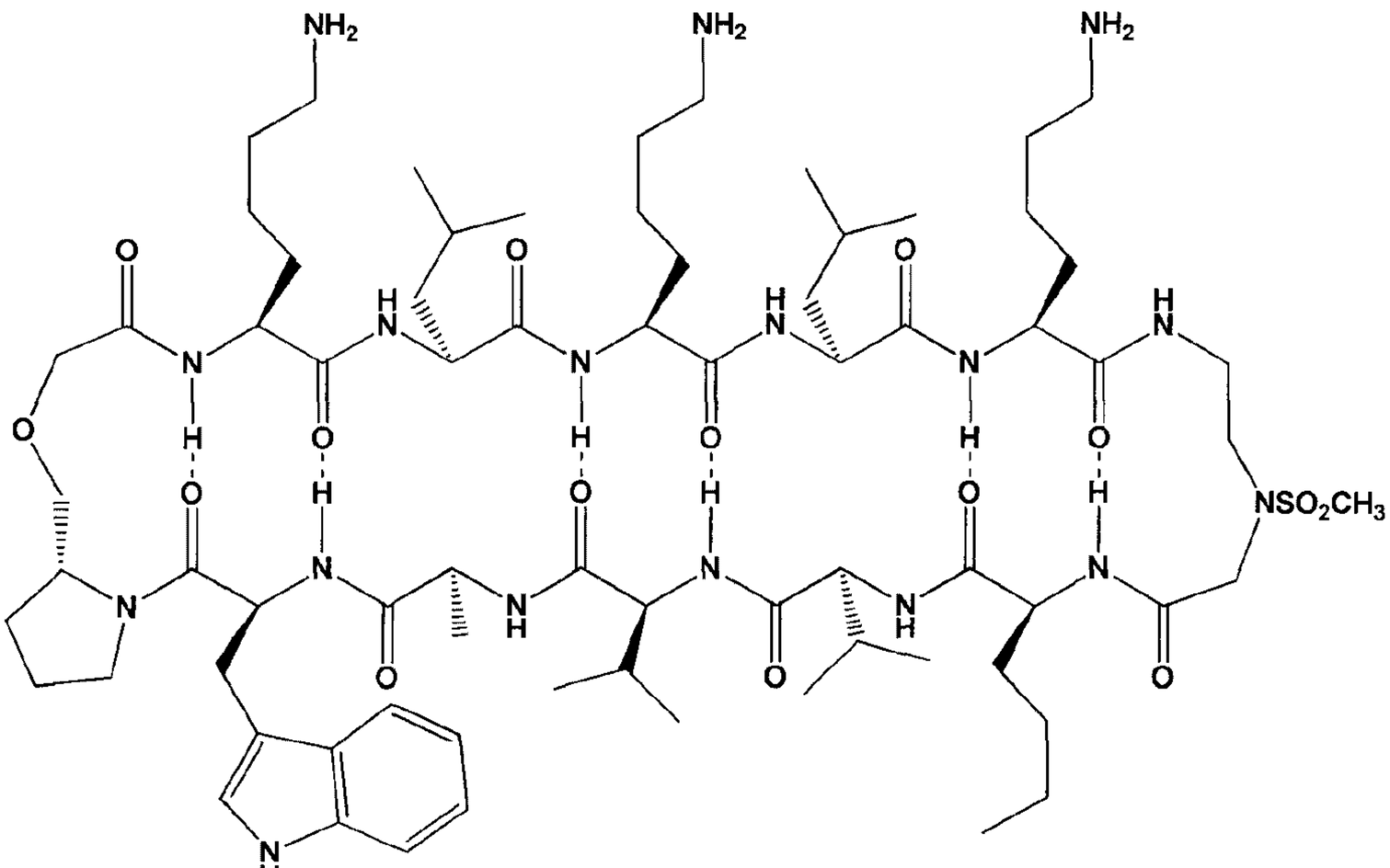

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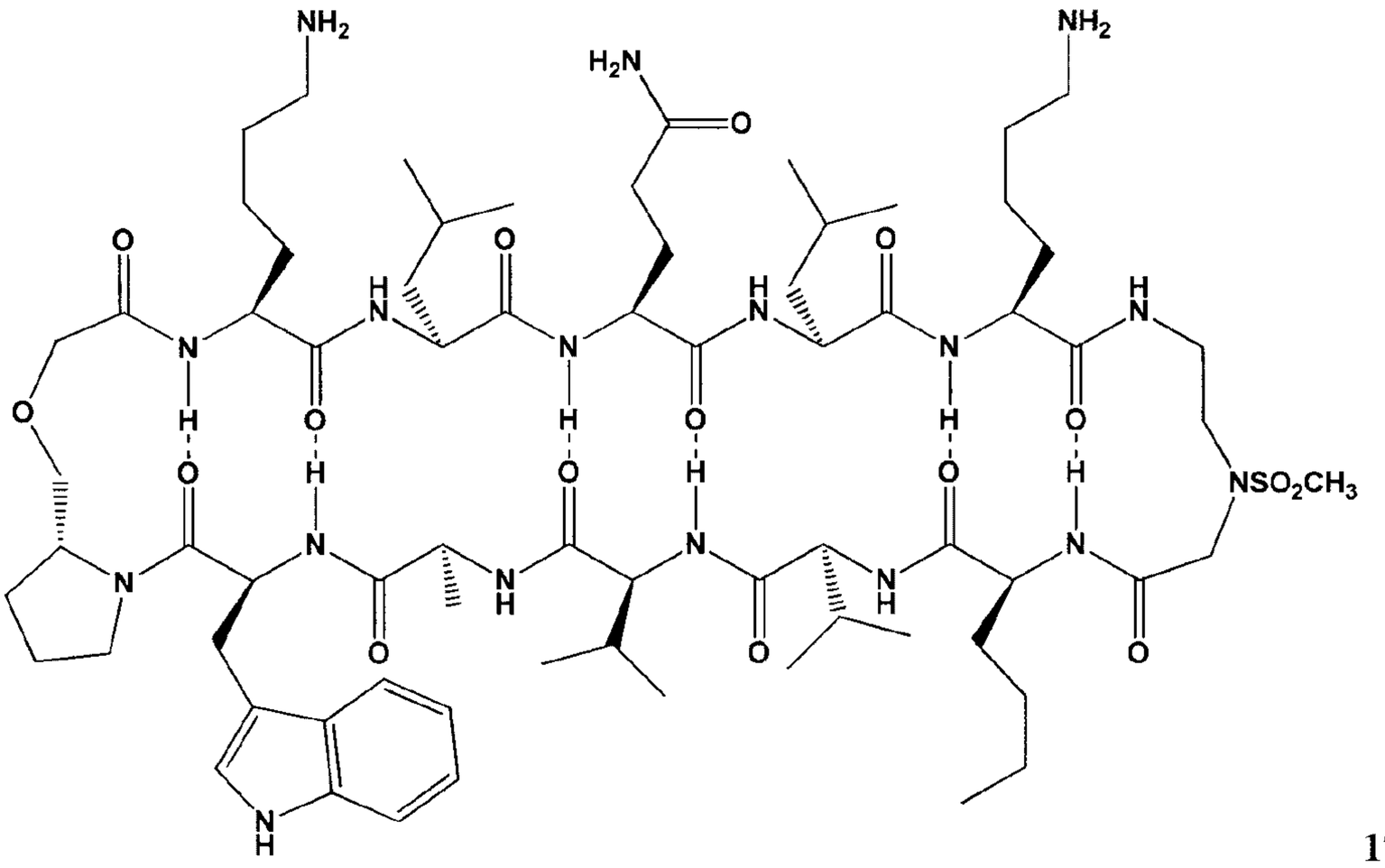
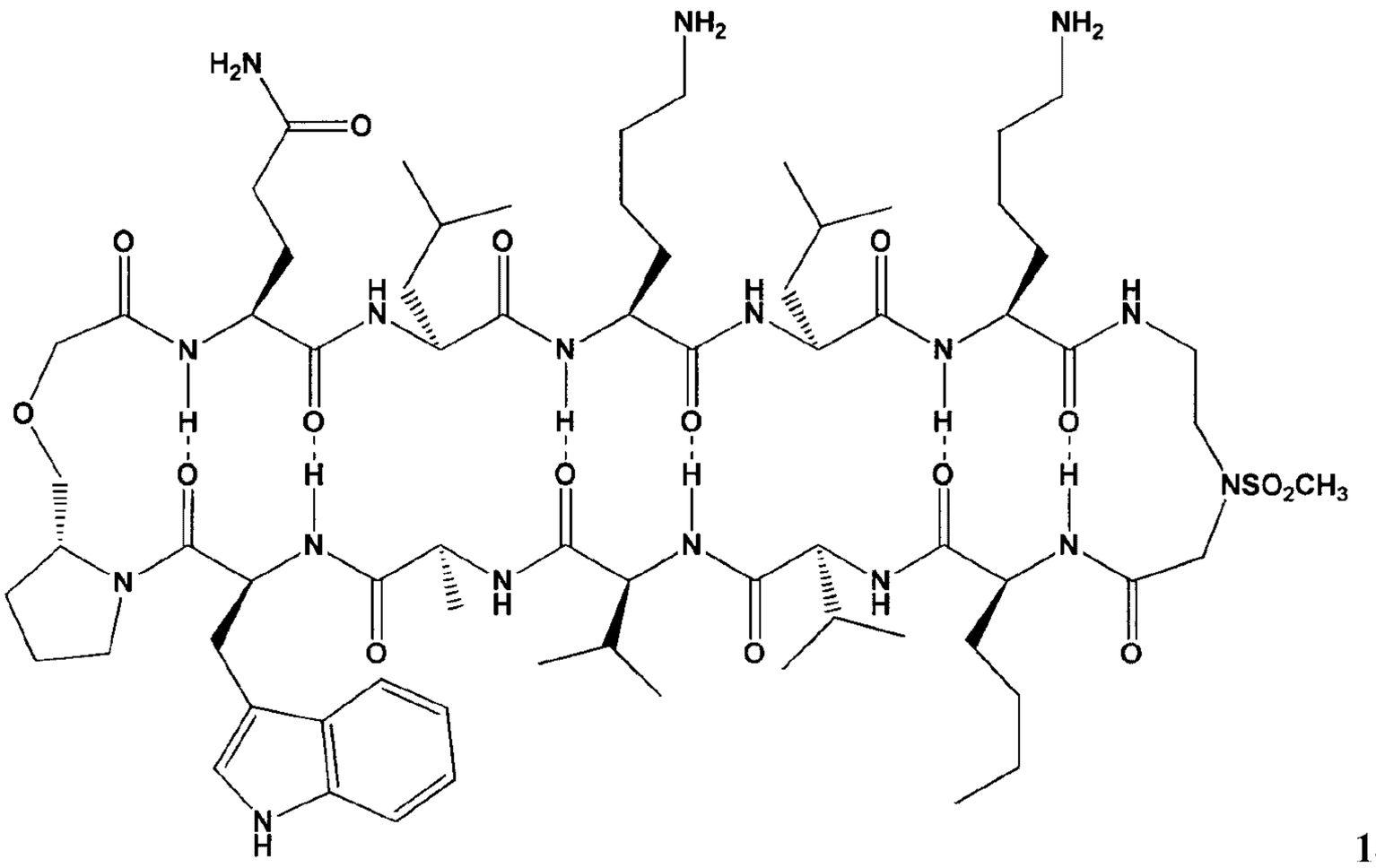
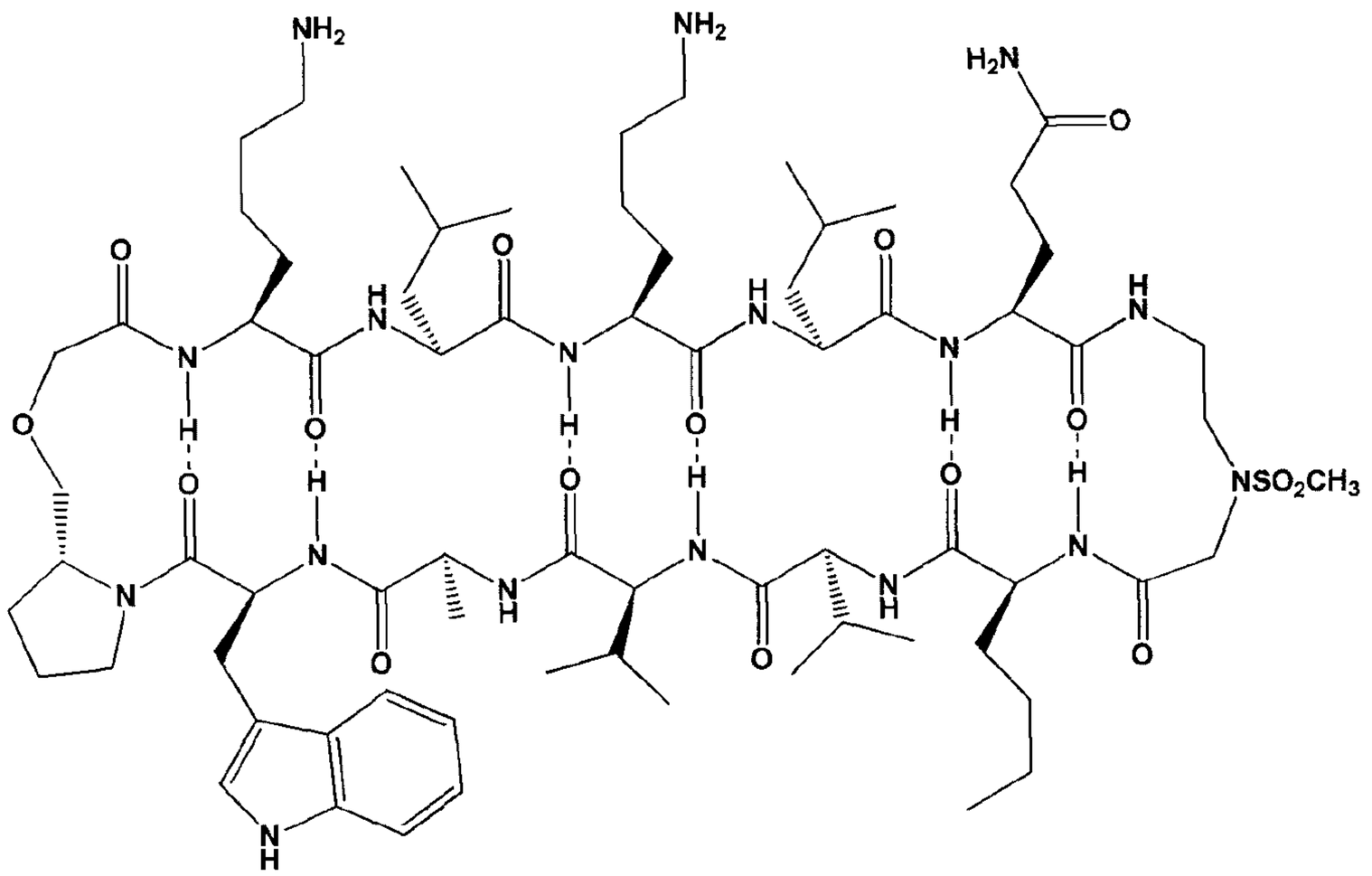


17



18



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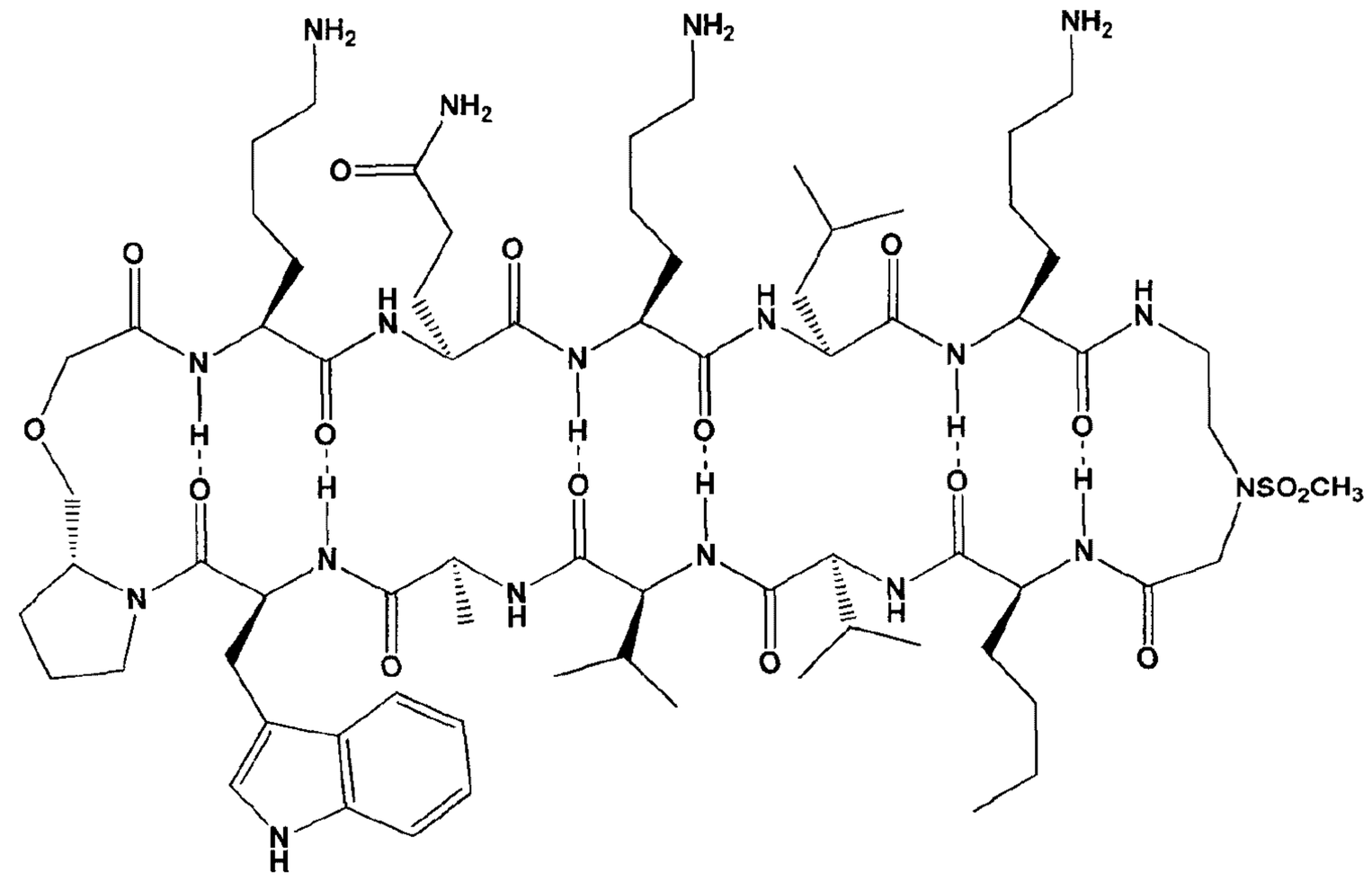
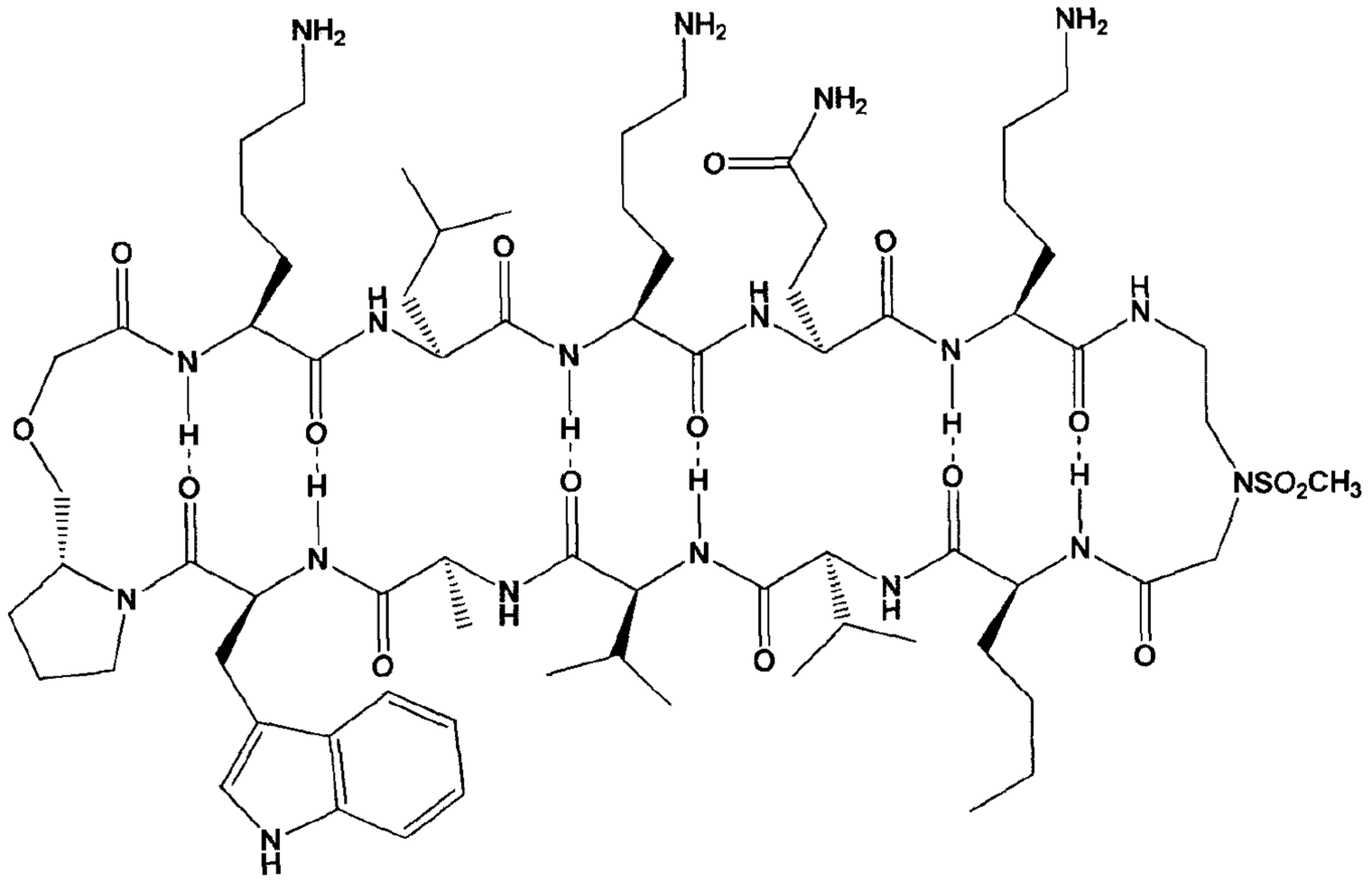
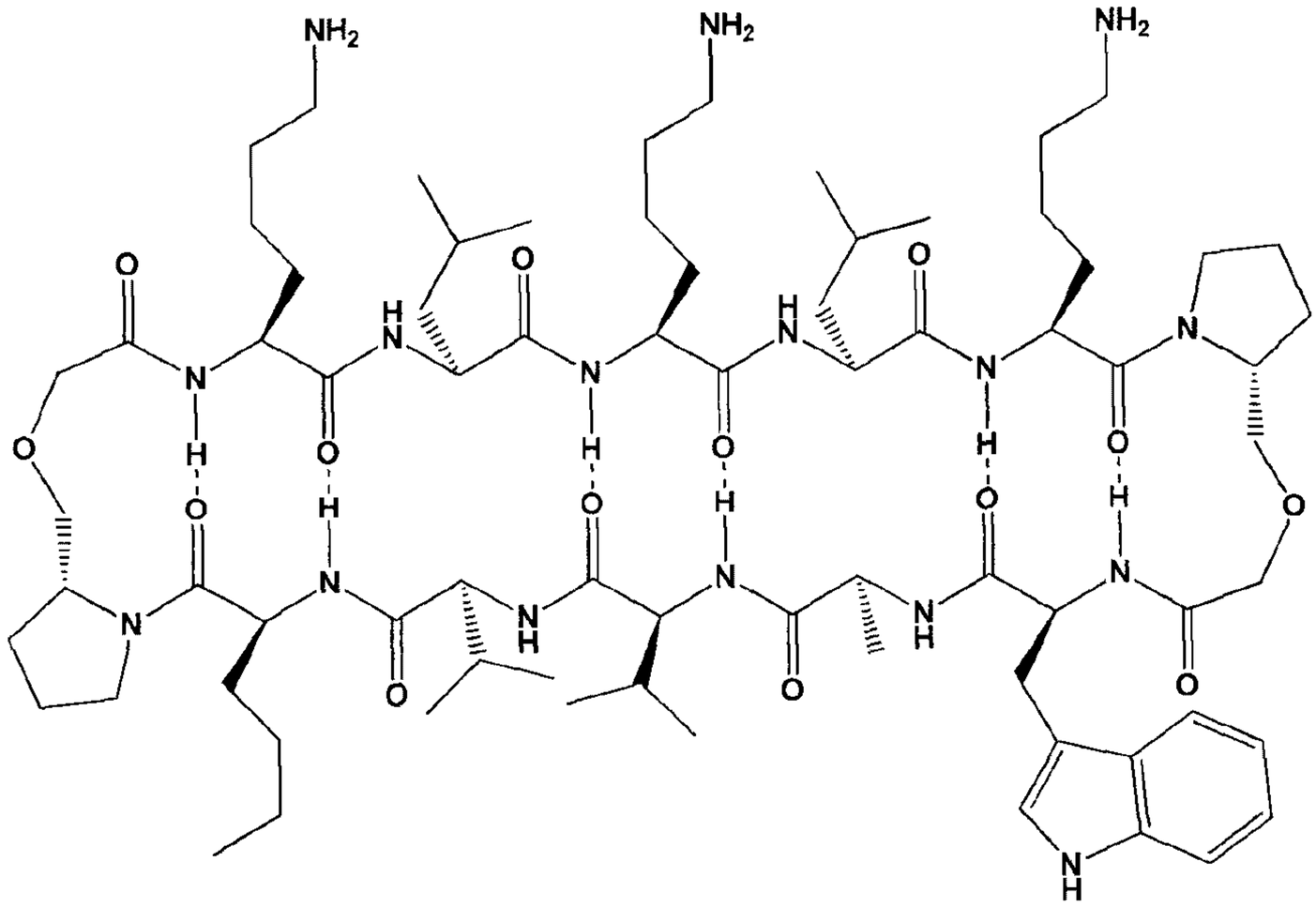


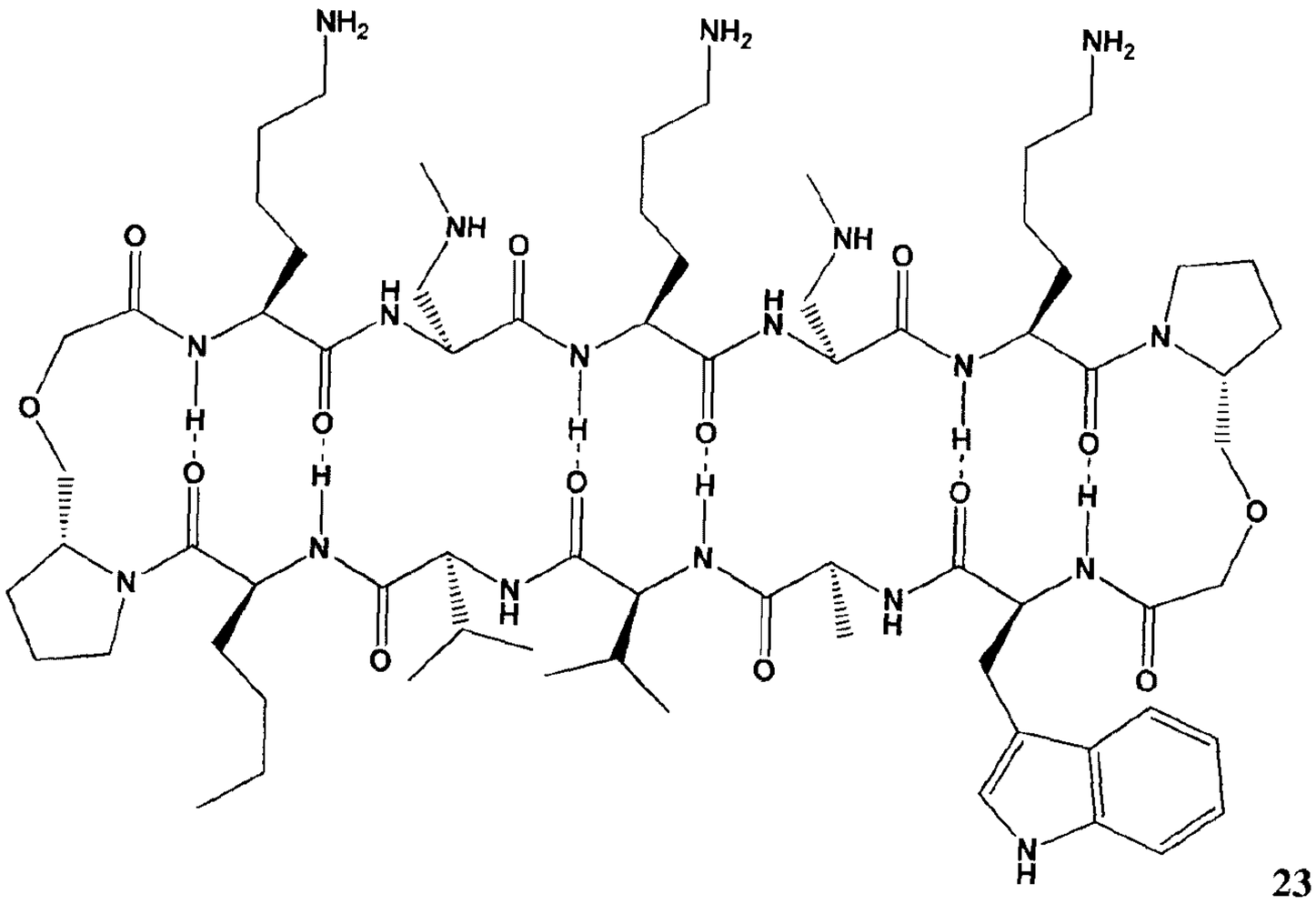
20



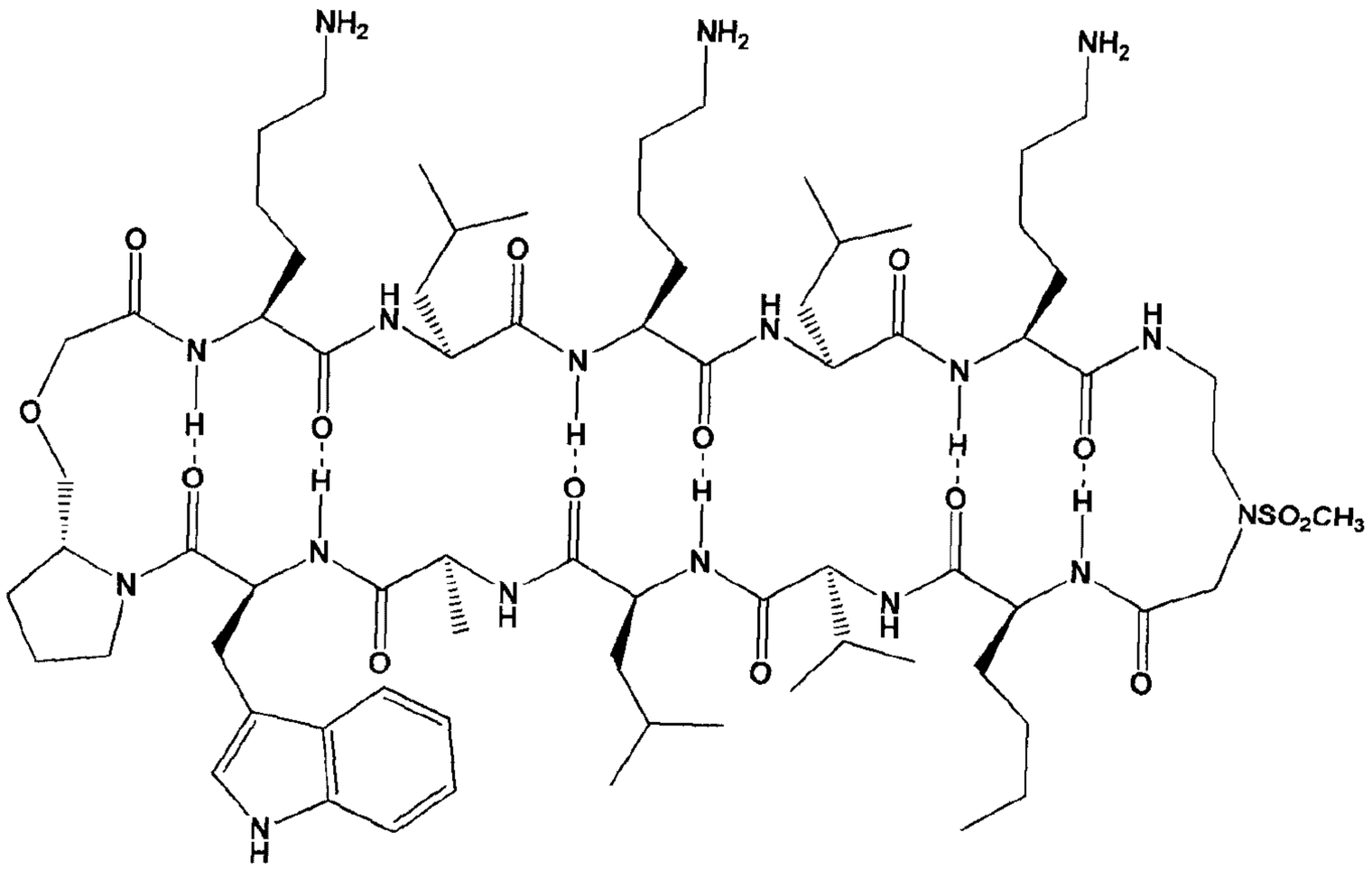
21



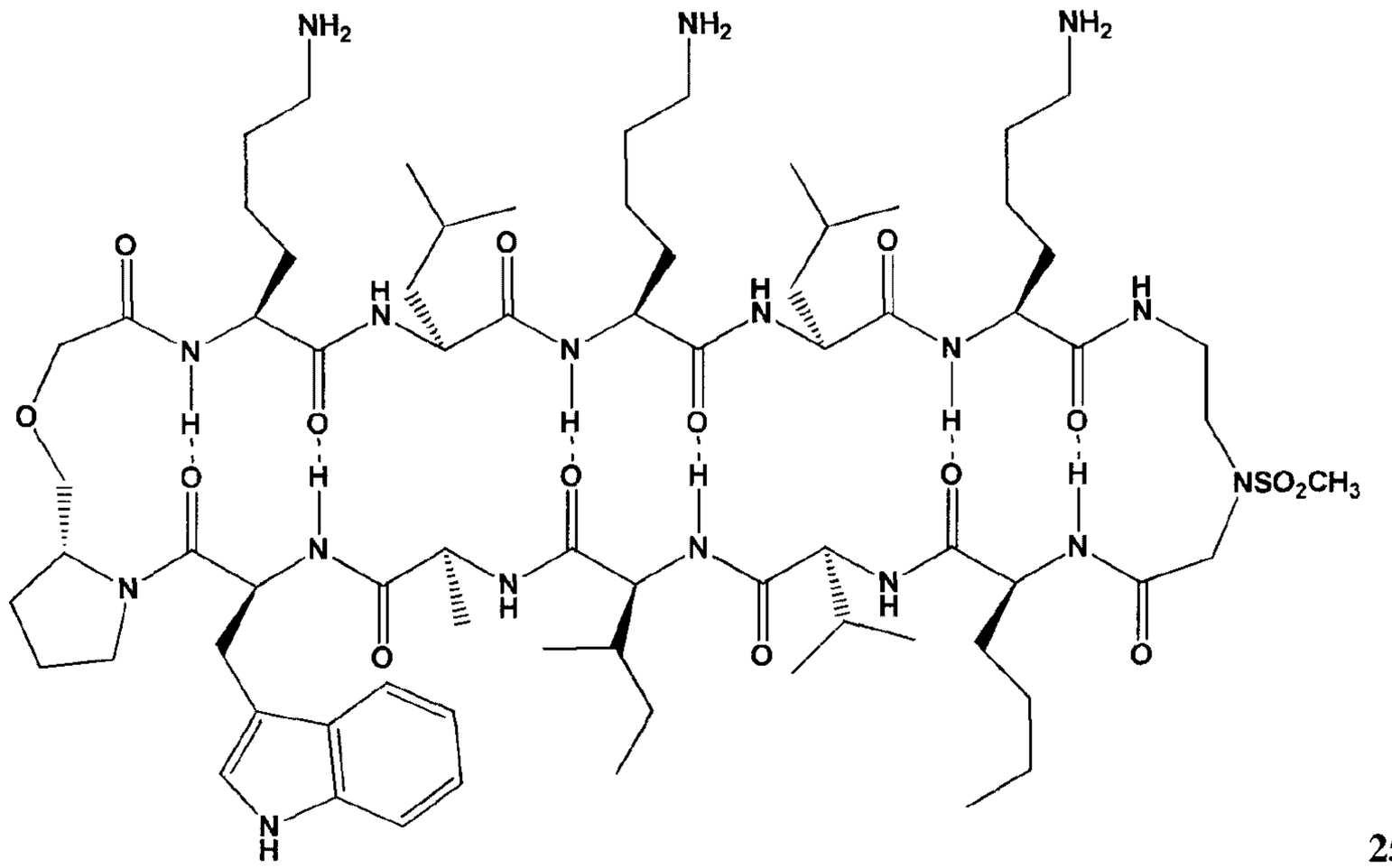
22



23

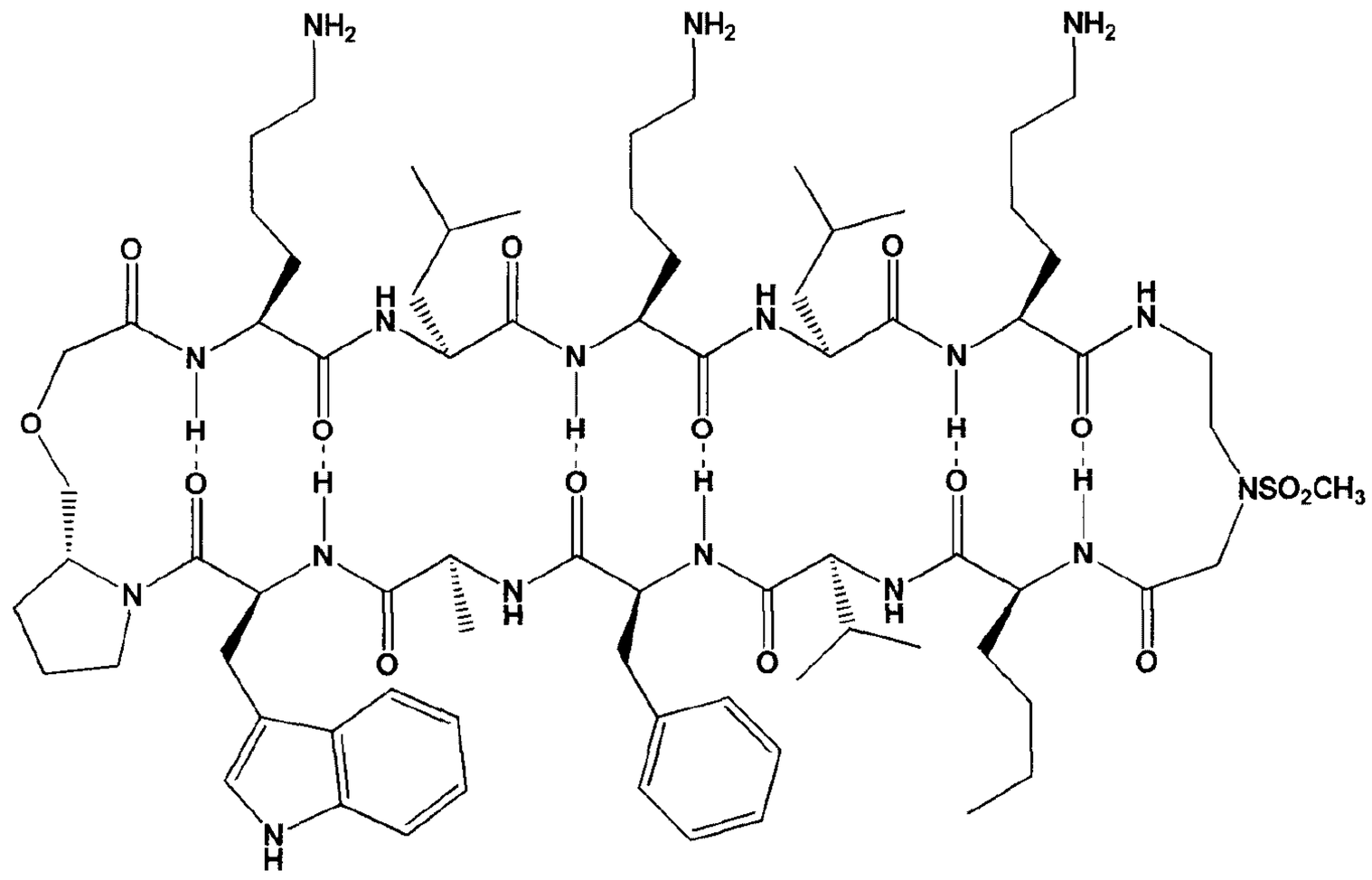


24



25

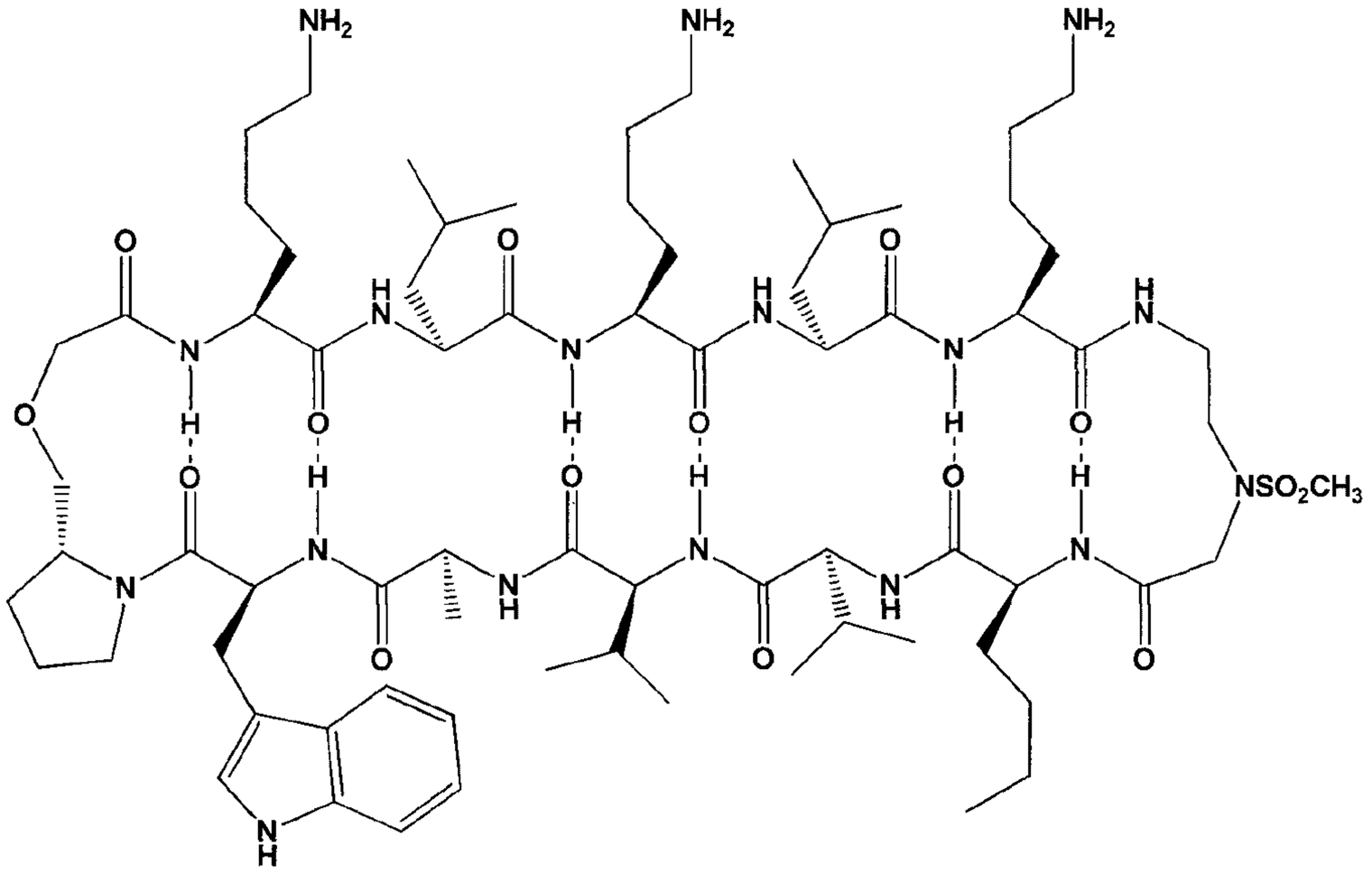
or



26

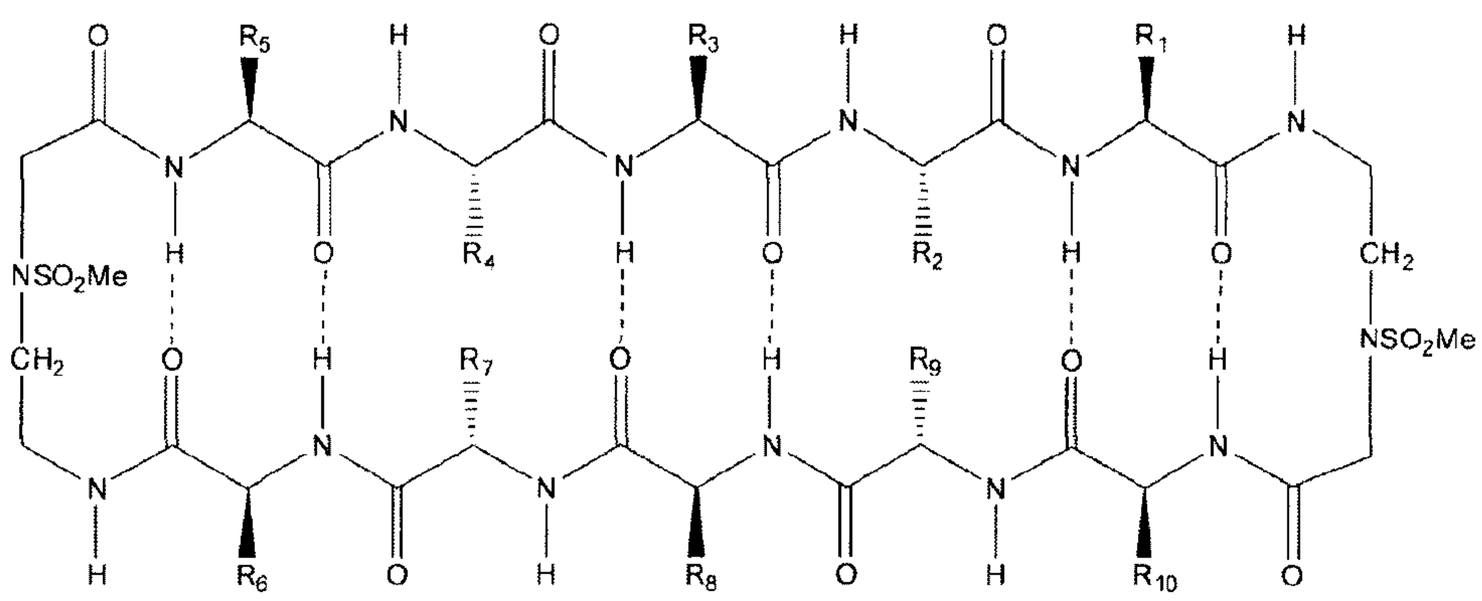
102. A cyclic compound according to claim 101 having the following structure, or a

pharmaceutically acceptable salt thereof:



16

103. A cyclic compound, or a pharmaceutically acceptable salt thereof, having the following structure:



wherein R_1 is K;

wherein R_2 is L;

wherein R_3 is K;

wherein R_4 is L;

wherein R_5 is K;

wherein R_6 is selected from the group consisting of W, A, and M;
wherein R_7 is selected from the group consisting of S, A, Y, and V;
wherein R_8 is selected from the group consisting of V and A;
wherein R_9 is selected from the group consisting of V, A, and S; and
wherein R_{10} is selected from the group consisting of M, A, W, and nor-Leu.

104. A cyclic compound according to claim 103, which is one of compounds 2 to 10 or 12 identified in claim 101.

105. Use of the cyclic compound according to any one claims 101 to 104, for the treatment of a malignancy, wherein the malignancy is selected from among: multiple myeloma, lung cancer, acute myeloid leukemia, prostate cancer, and melanoma.

106. Use of the cyclic compound according to any one claims 101 to 104 in the manufacture of a medicament for treatment of a malignancy, wherein the malignancy is selected from among: multiple myeloma, lung cancer, acute myeloid leukemia, prostate cancer, and melanoma.

107. Use of the cyclic compound according to any one claims 101 to 104 in the treatment of a proliferation disorder characterized by proliferation of T-lymphocytes.

108. Use of the cyclic compound according to any one claims 101 to 104 in the manufacture of a medicament for the treatment of a proliferation disorder characterized by proliferation of T-lymphocytes.

109. A pharmaceutical composition comprising a compound according to any of claims 101 to 104, and a pharmaceutically acceptable carrier.

110. The pharmaceutical composition of claim 109, further comprising at least one anticancer agent selected from among suberoylanilide hydroxamic acid (SAHA) or other histone deacetylase inhibitor, arsenic trioxide, doxorubicin or other anthracycline DNA intercalating

agent, and etoposide or other topoisomerase II inhibitor.

111. The use according to any one of claims 4-7, 43-100, and 105-108, wherein the use comprises use of at least one other anti-cancer agent.

112. The use according to claim 111, wherein the at least one other anti-cancer agent comprises a cytotoxic agent, chemotherapeutic agent, or anti-signaling agent.

113. The use according to claim 111, wherein the at least one other anti-cancer agent comprises a proteasome inhibitor.

114. The use according to claim 111, wherein the at least one other anti-cancer agent comprises bortezomib, or a pharmaceutically acceptable salt thereof.

115. The use according to claim 111, wherein the cyclic compound and the at least one other anti-cancer agent are for administration concurrently.

116. The use according to claim 111, wherein the cyclic compound and the at least one other anti-cancer agent are for administration consecutively.

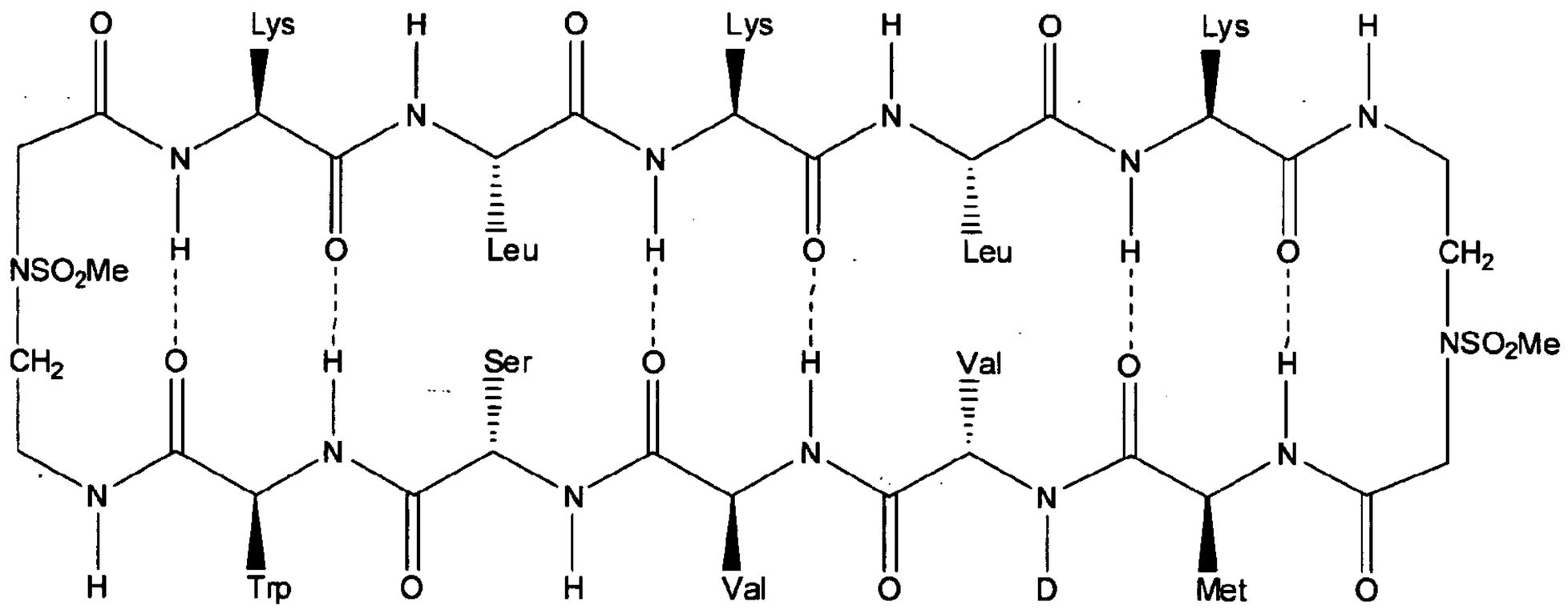
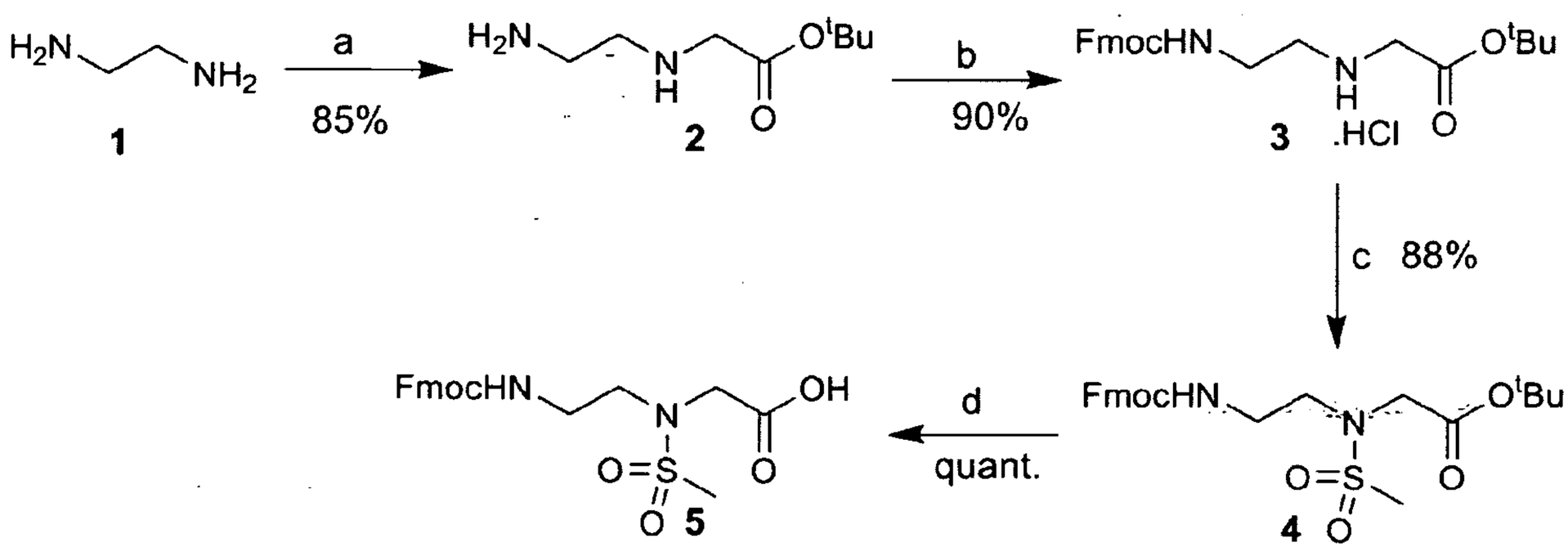


FIG. 1



(a) $BrCH_2CO_2{}^tBu$, THF (b) FmocNOSu, DIEA, DCM (c) $MeSO_2Cl$, DIEA, THF
(d) 1,4-Dioxane,4M HCl

FIG. 2

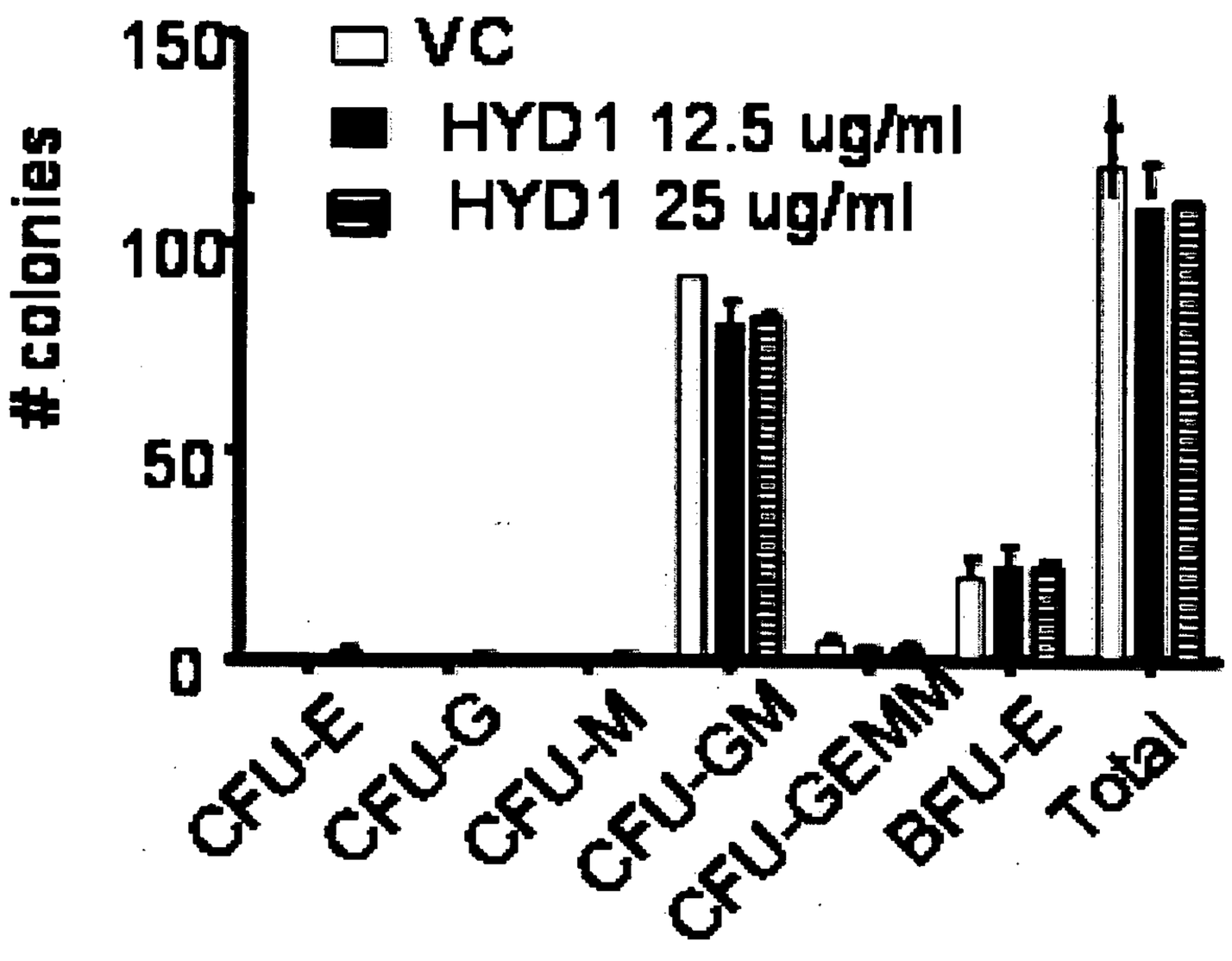

CD34+ Cells
VC
HYD1 12.5 ug/ml
HYD1 25 ug/ml
colonies
150
100
50
0
CFU-E
CFU-G
CFU-M
CFU-GM
CFU-GEMM
BFU-E
Total

FIG. 3A

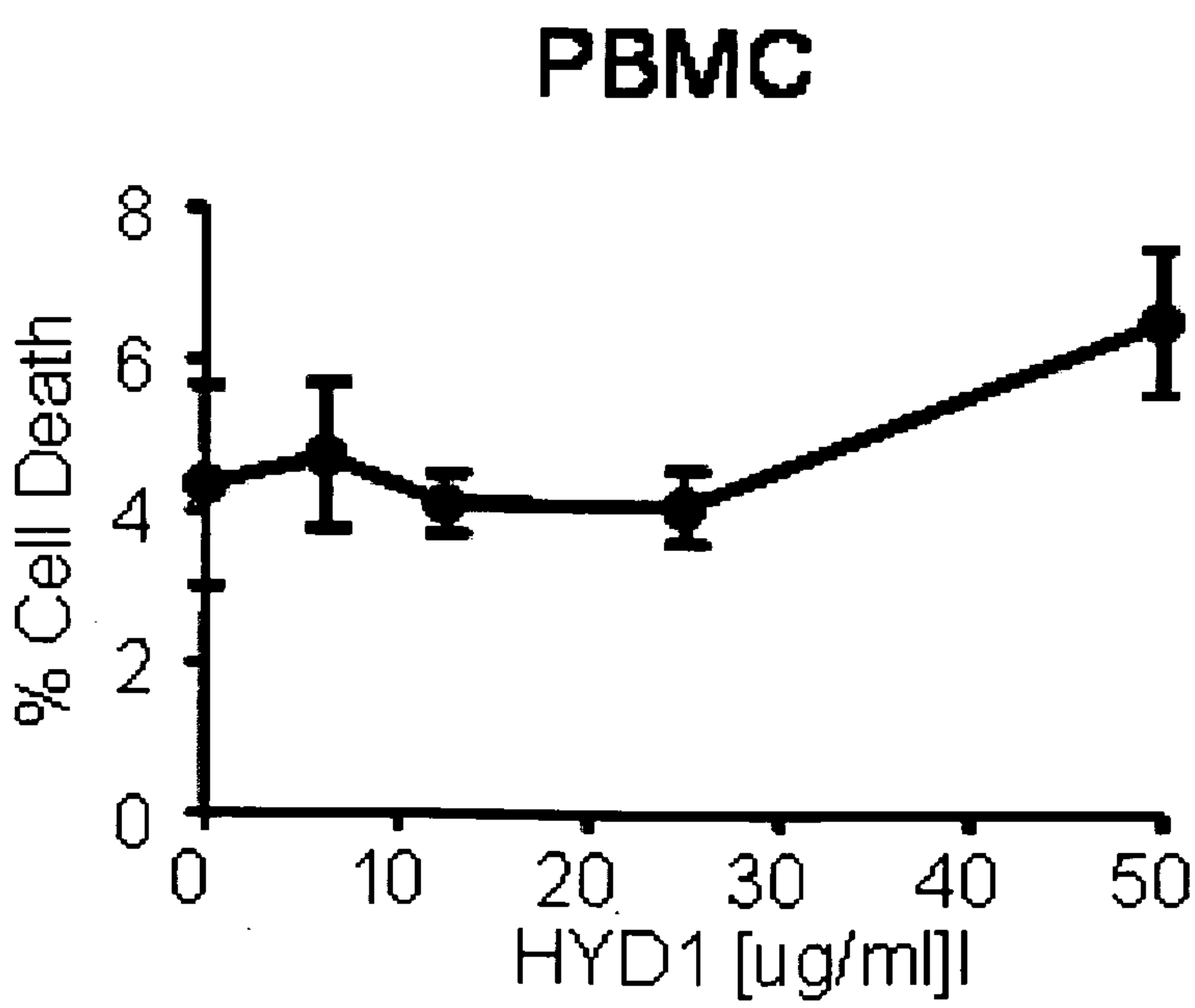

PBMC
% Cell Death
8
6
4
2
0
0
10
20
30
40
50
HYD1 [ug/ml]

FIG. 3B

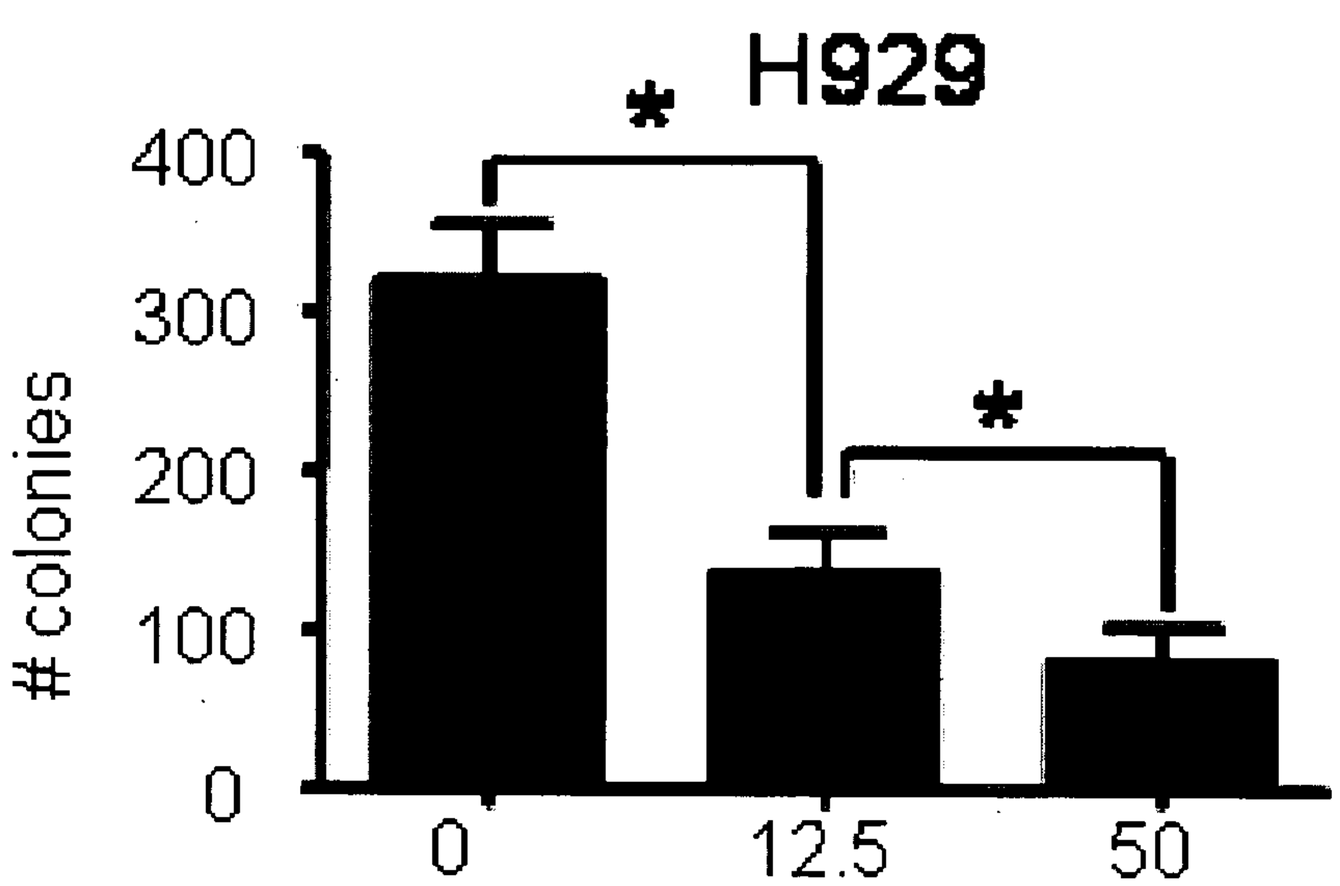


FIG. 3C

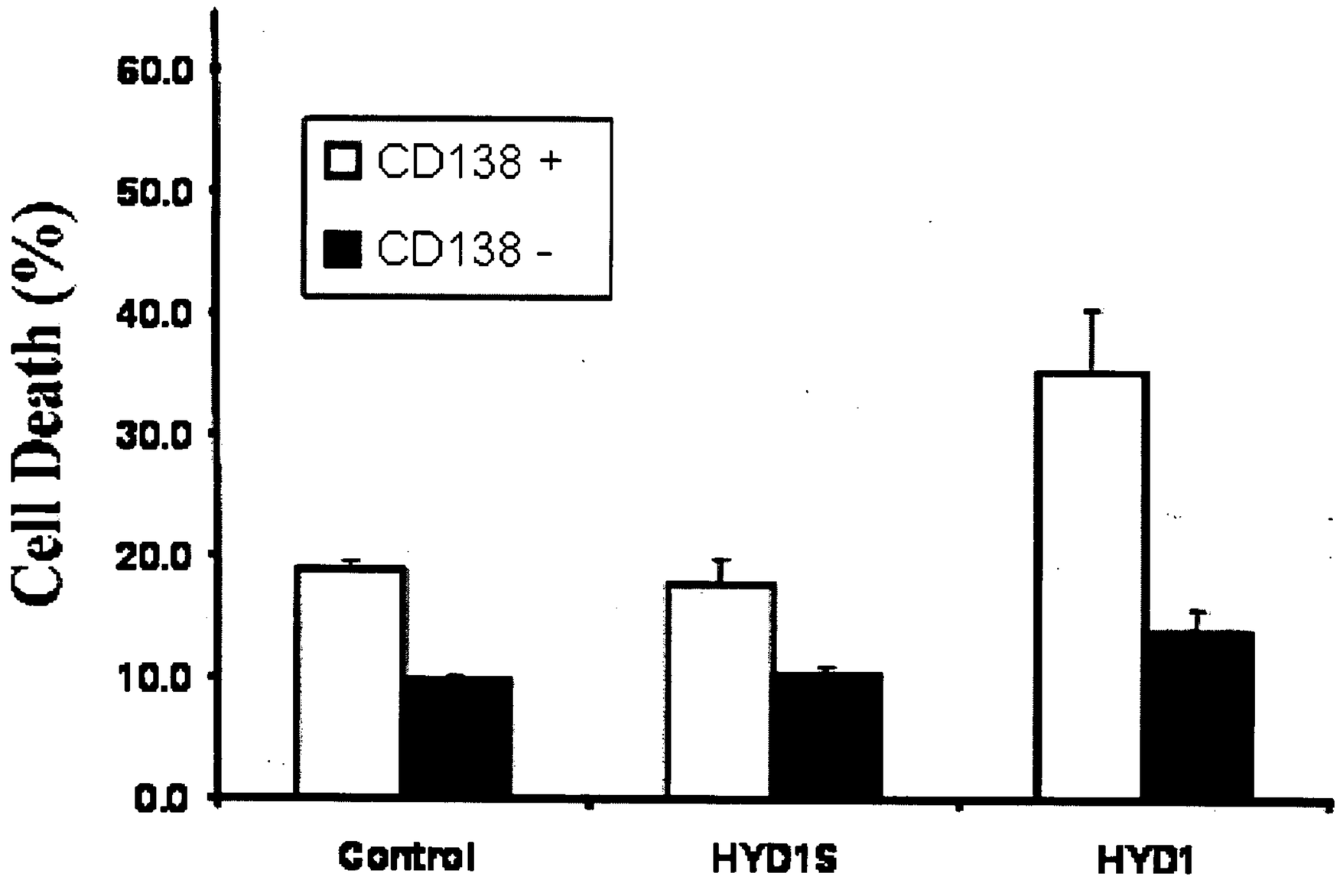

Cell Death (%)
60.0
50.0
40.0
30.0
20.0
10.0
0.0
CD138 +
CD138 -
Control
HYD1S
HYD1

FIG. 4

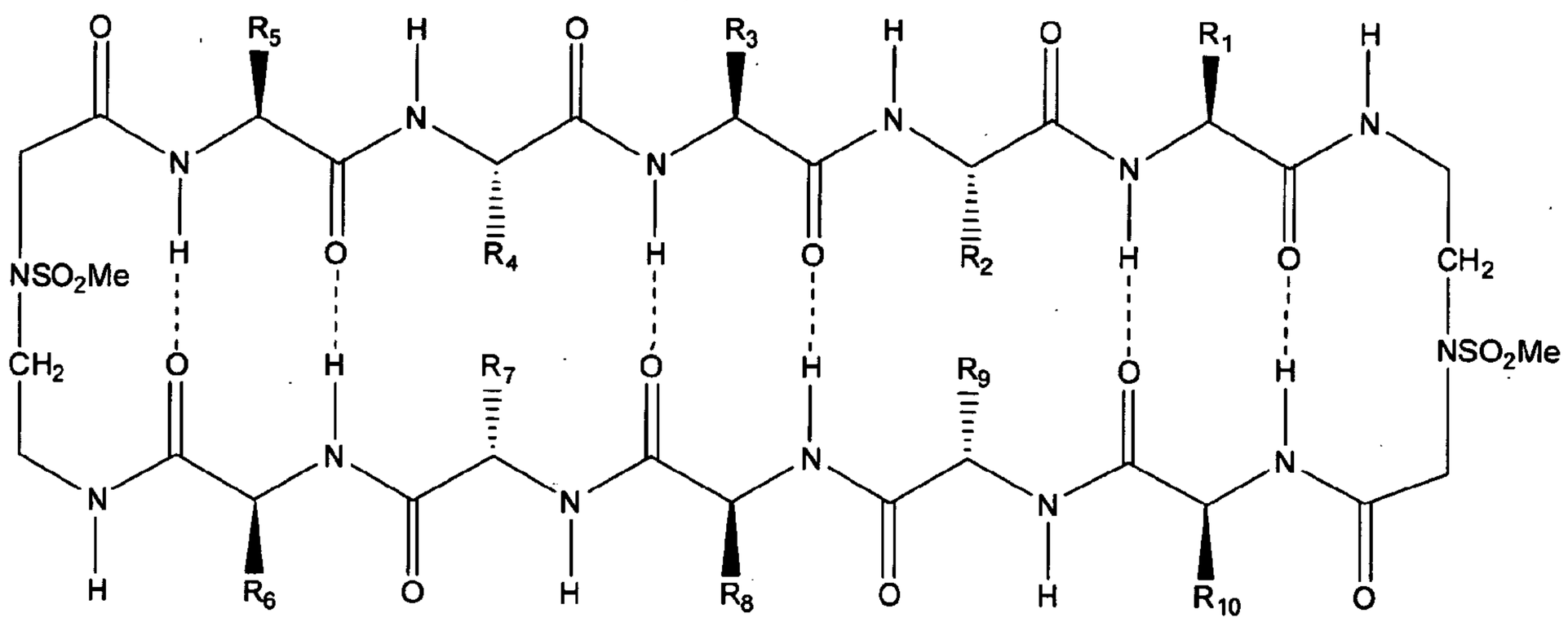

O
R5
H
N
O
R3
H
N
O
R1
H
N
NSO2Me
CH2
N
H
O
R6
H
N
O
R4
H
N
O
R7
H
N
O
R8
H
N
O
R2
H
N
O
R9
H
N
O
R10
H
N
O
CH2
NSO2Me

FIG. 5

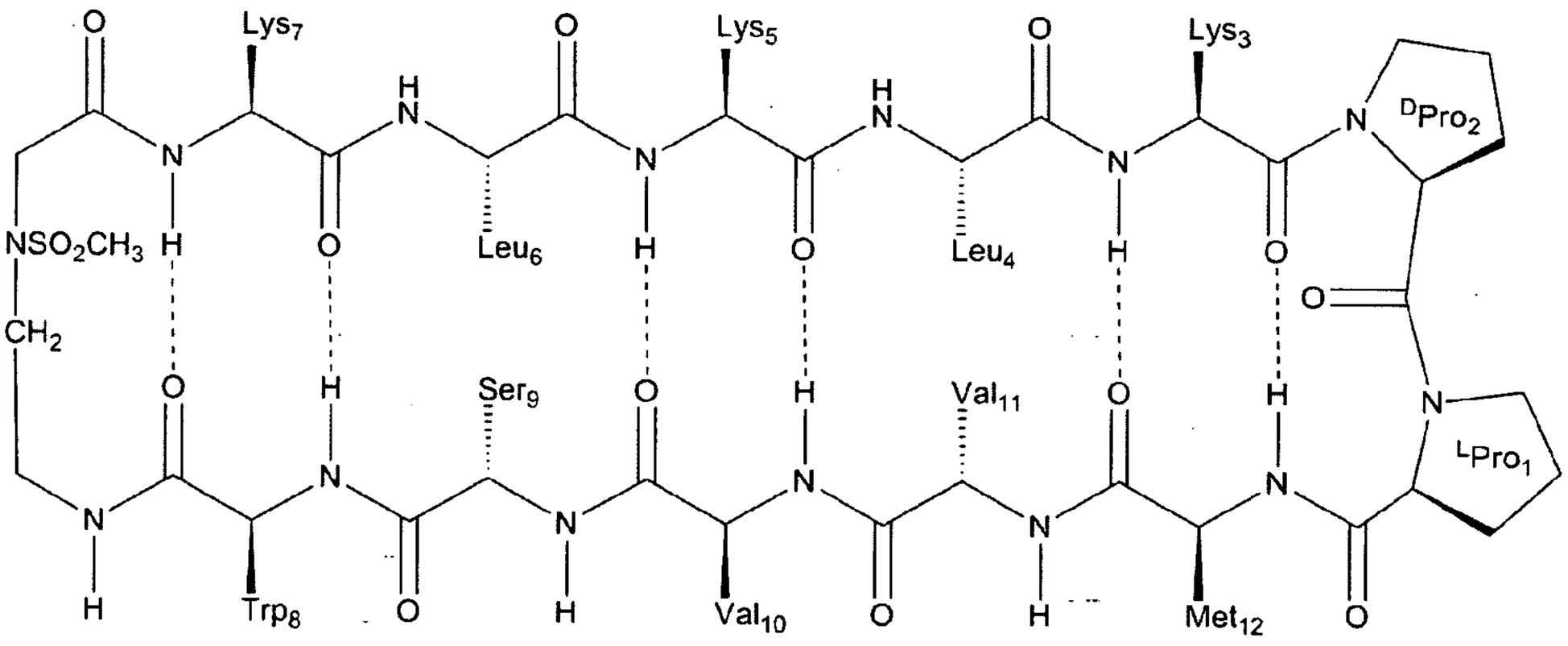

O
Lys7
H
N
O
Lys5
H
N
O
Lys3
N
DPro2
NSO2CH3
H
N
O
Leu6
H
N
O
Leu4
H
N
O
O
CH2
O
H
N
Ser9
O
H
N
Val11
O
H
N
N
LPro1
H
N
Trp8
O
H
N
Val10
O
H
N
Met12
O

FIG. 6

Circular Dichroism for determining secondary structure of Integrin Interaction Inhibitors

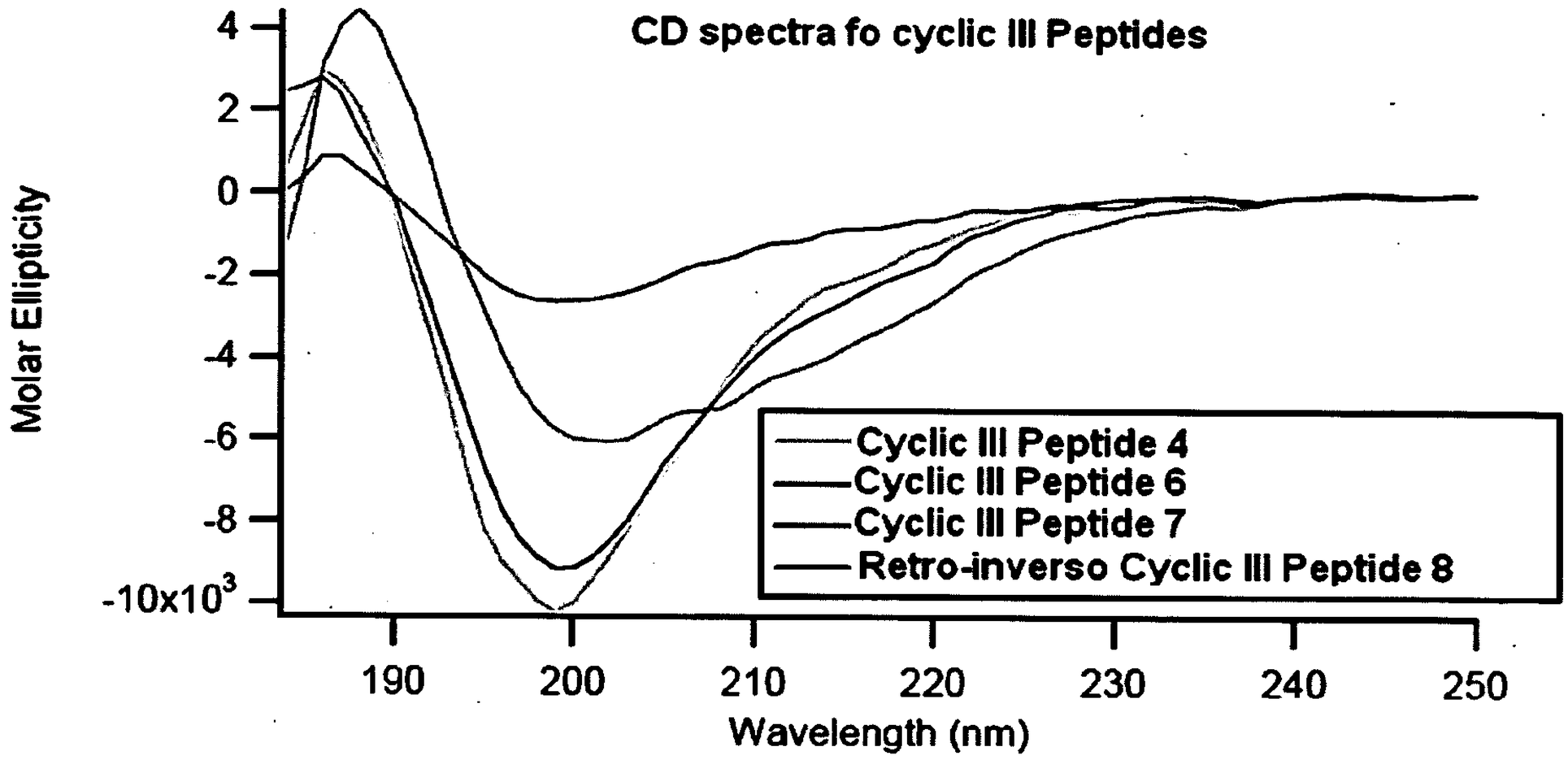


FIG. 7

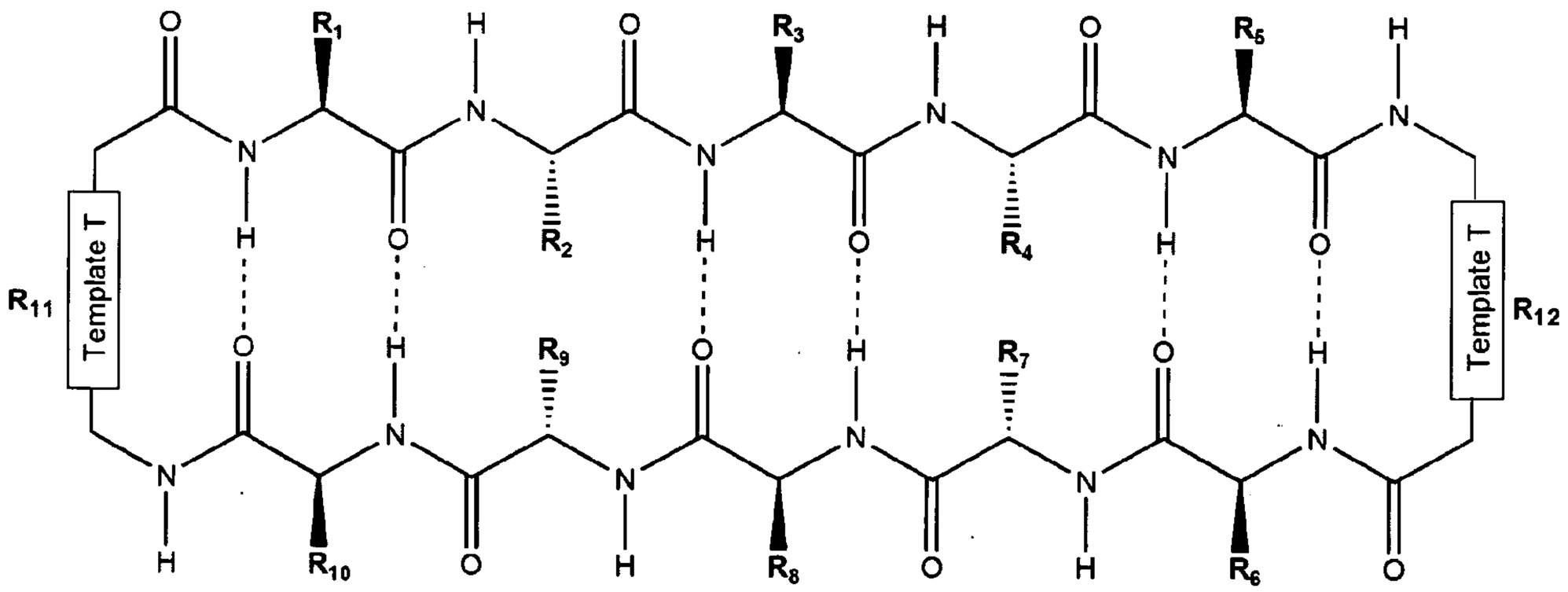
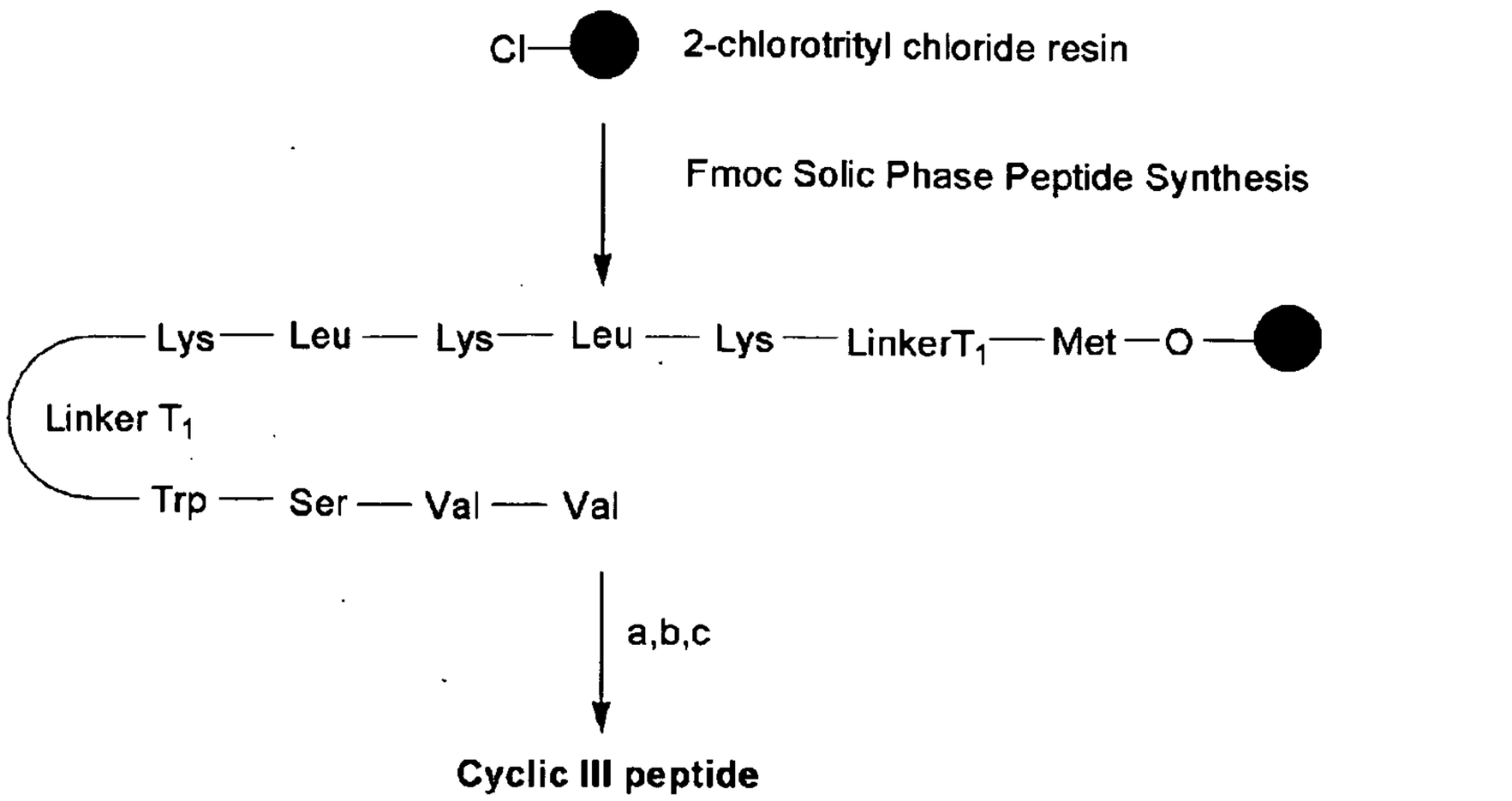
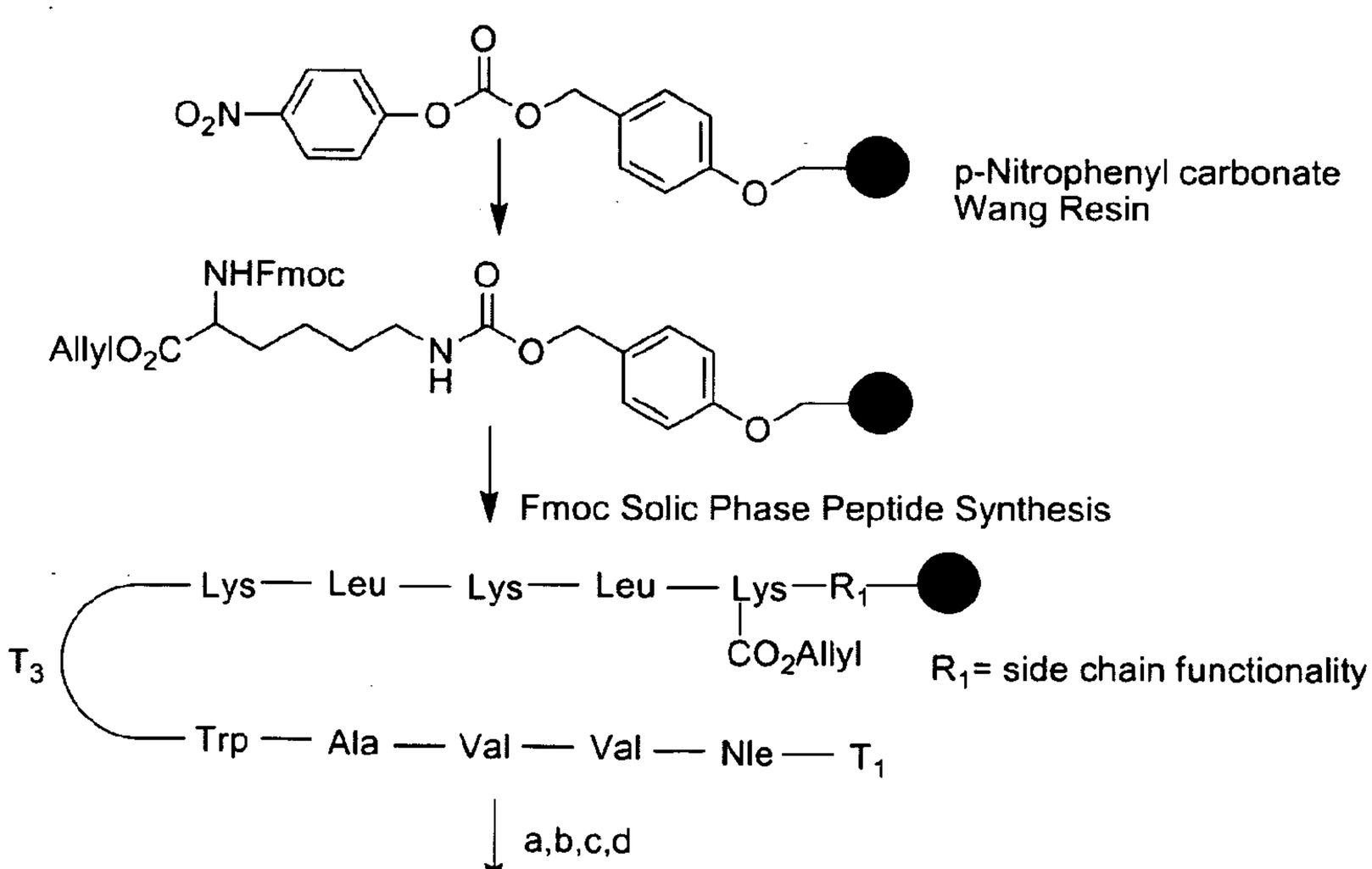


FIG. 8



(a) 20% Trifluoroethanol in DCM, rt (b) HATU, DIEA, DMF (c) 95%TFA, Et_3SiH, H_2O

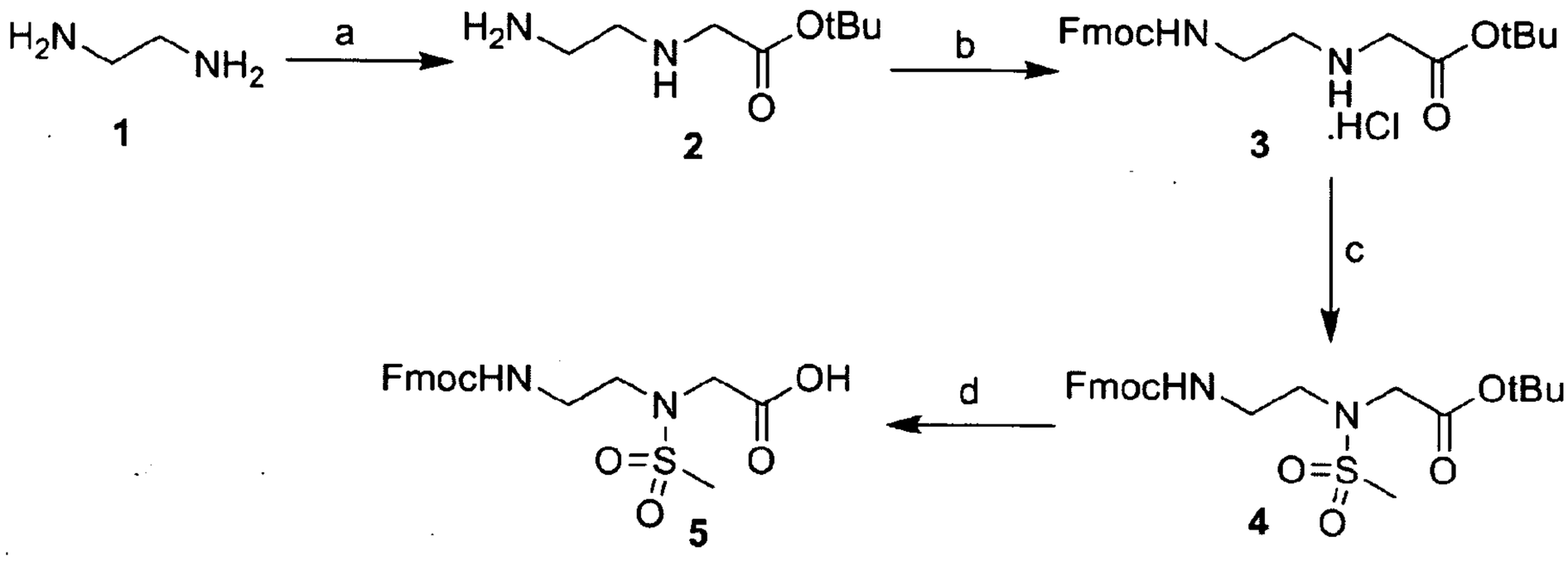
FIG. 9



(a) 20% Piperidine/2%DBU in DMF, rt (b) $Pd(PPh_3)_4$ in $CHCl_3$-AcOH-NMM (37:2:1)

(c)HCTU, DIEA, DMF (d) 95%TFA, Et_3SiH, H_2O

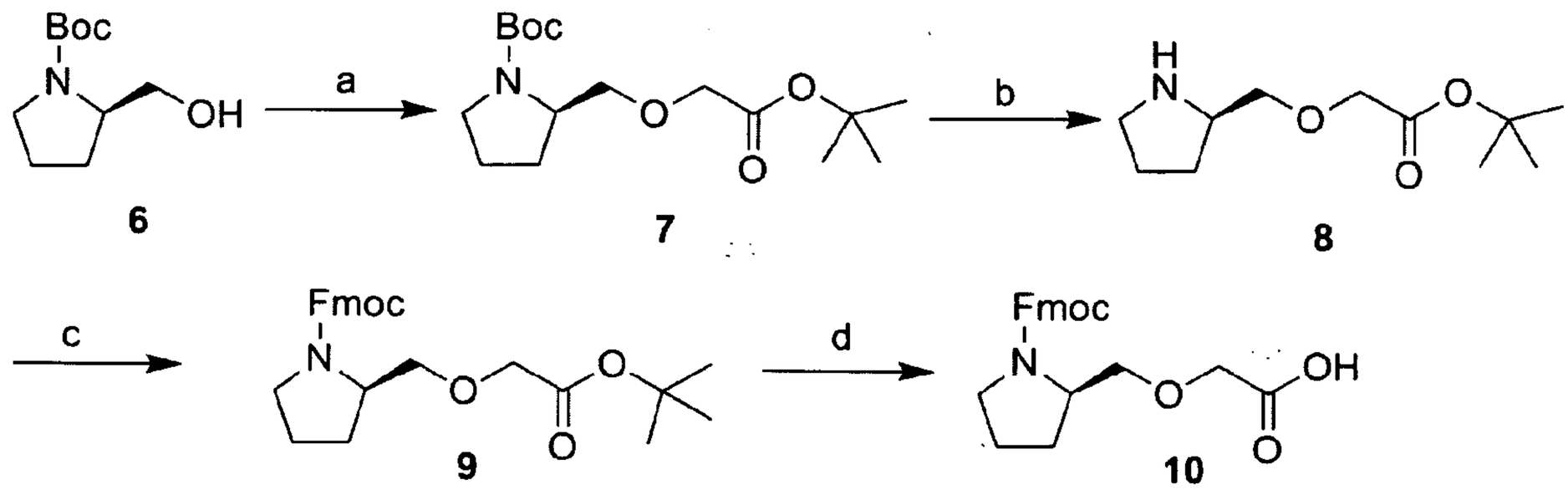
FIG. 10



(a) $BrCH_2CO_2tBu$, THF, 85% (b) FmocNOSu, DIEA, DCM, 90% (c) $MeSO_2Cl$, DIEA, THF, 88%

(d) 1,4-Dioxane,4M HCl, quant.

FIG. 11



(a) NaOH, toluene, $BrCH_2CO_2t$-bu, TBAI, 77% (b) TFA/DCM (4:1) quant. (c) FmocOSu, DIEA, DCM, 79% (d) TFA/DCM,Triethylsilane, quant.

FIG. 12

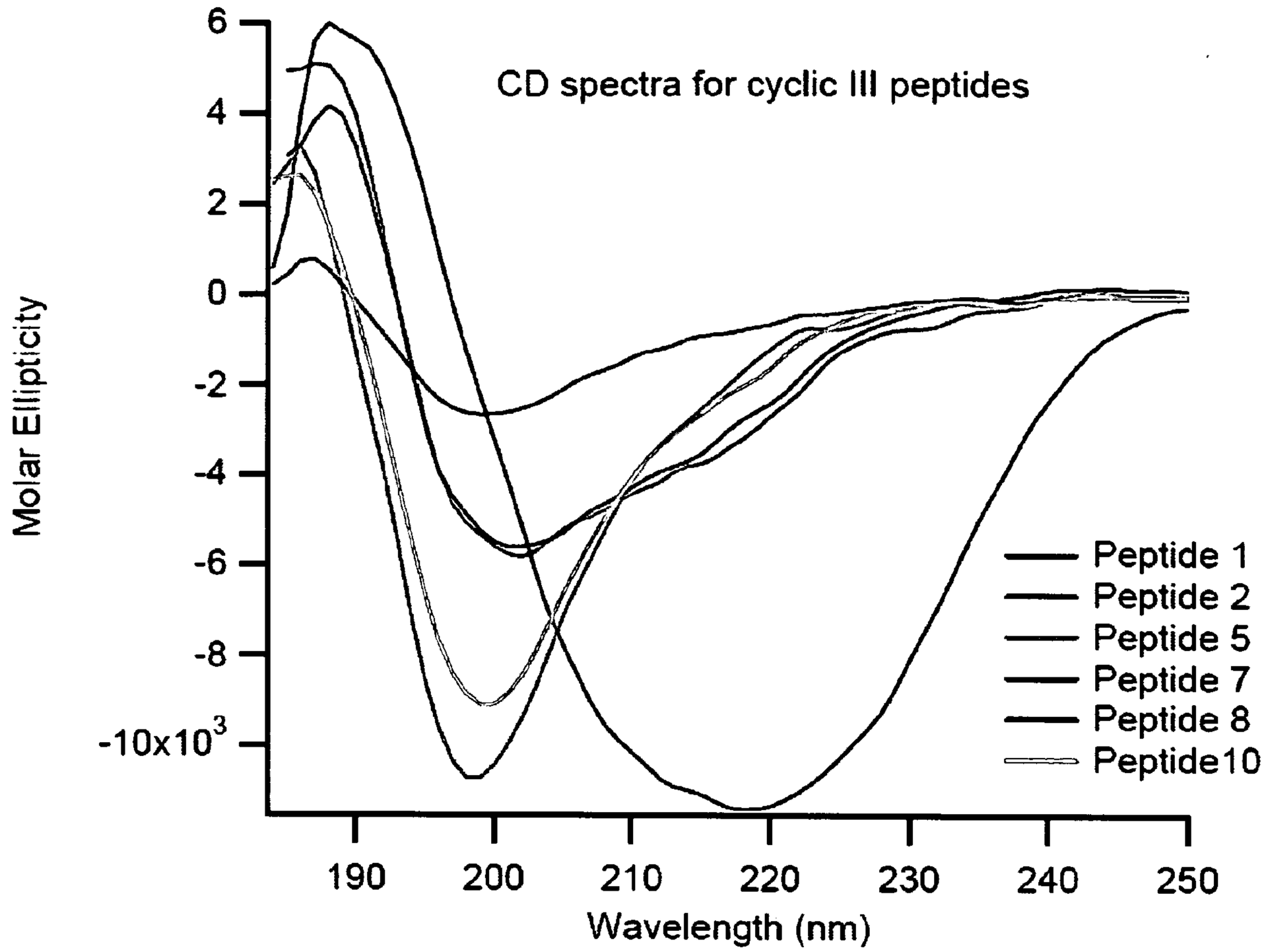

CD spectra for cyclic III peptides
Molar Ellipticity
6
4
2
0
-2
-4
-6
-8
-10x10^3
190
200
210
220
230
240
250
Wavelength (nm)
Peptide 1
Peptide 2
Peptide 5
Peptide 7
Peptide 8
Peptide10

FIG. 13

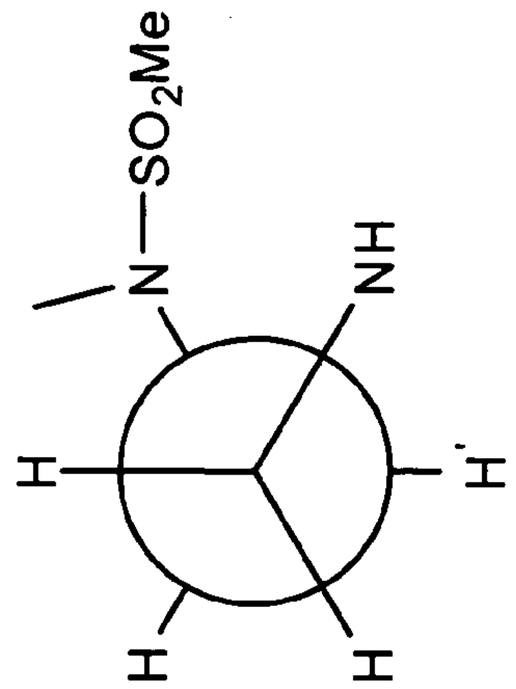


FIG. 14C

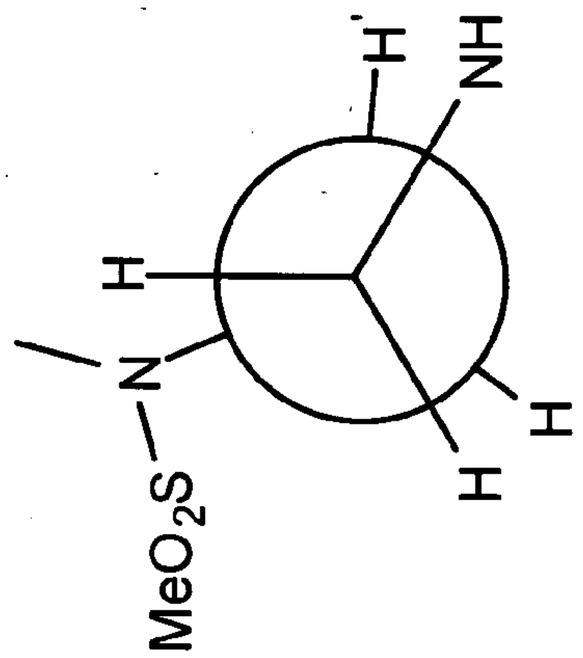


FIG. 14B

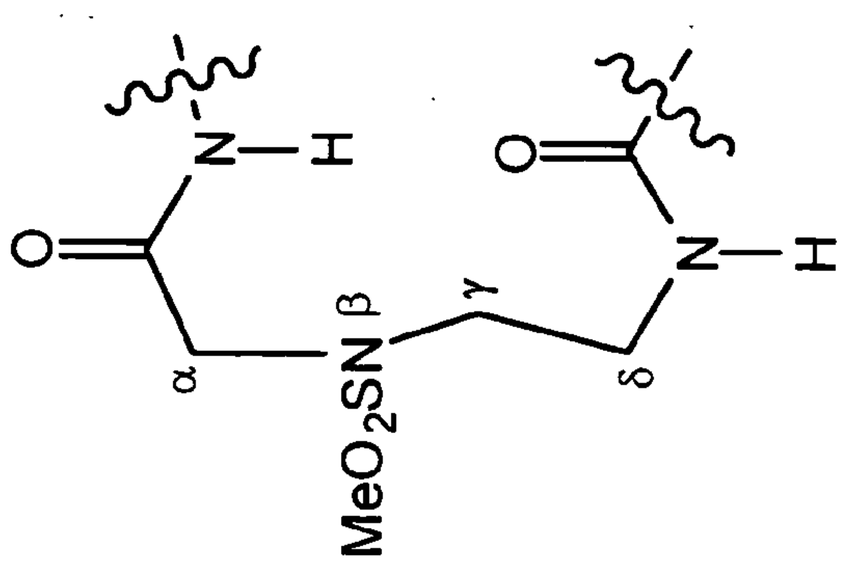


FIG. 14A

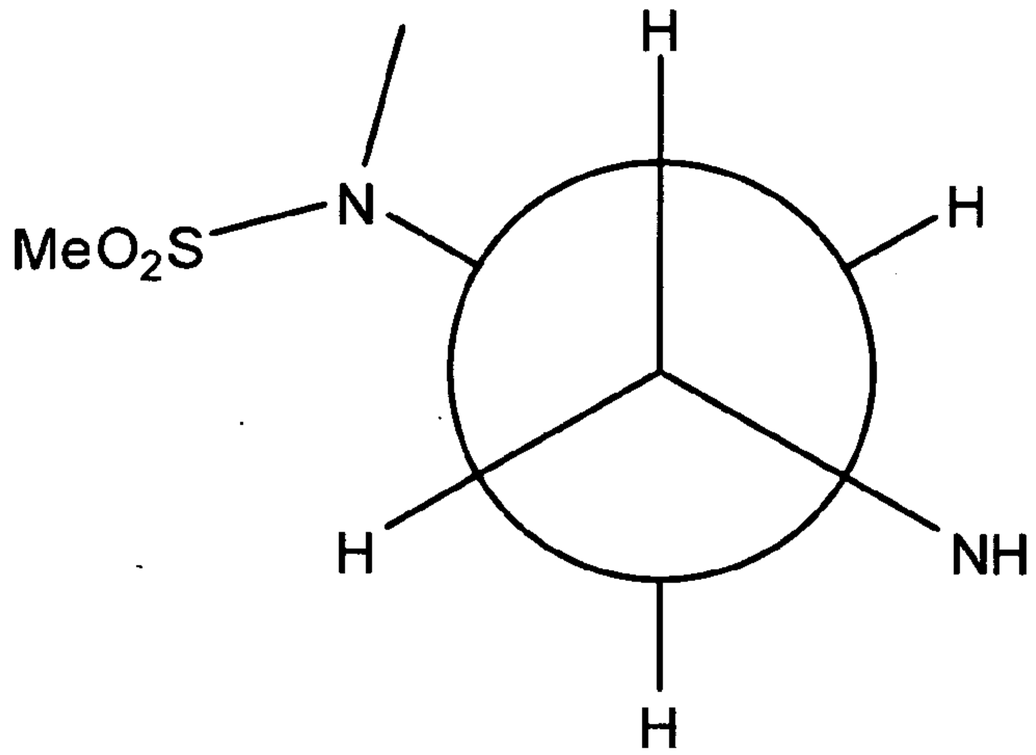


FIG. 15

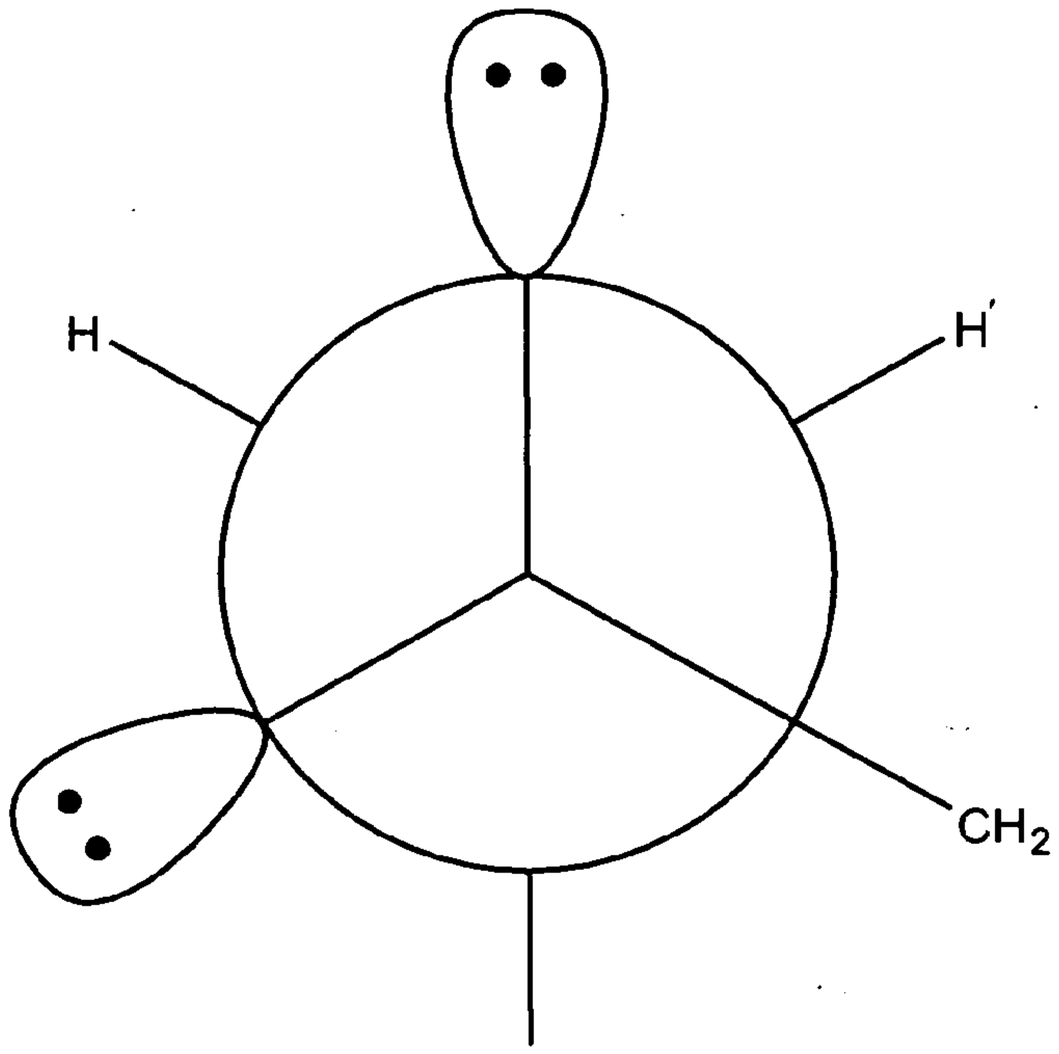
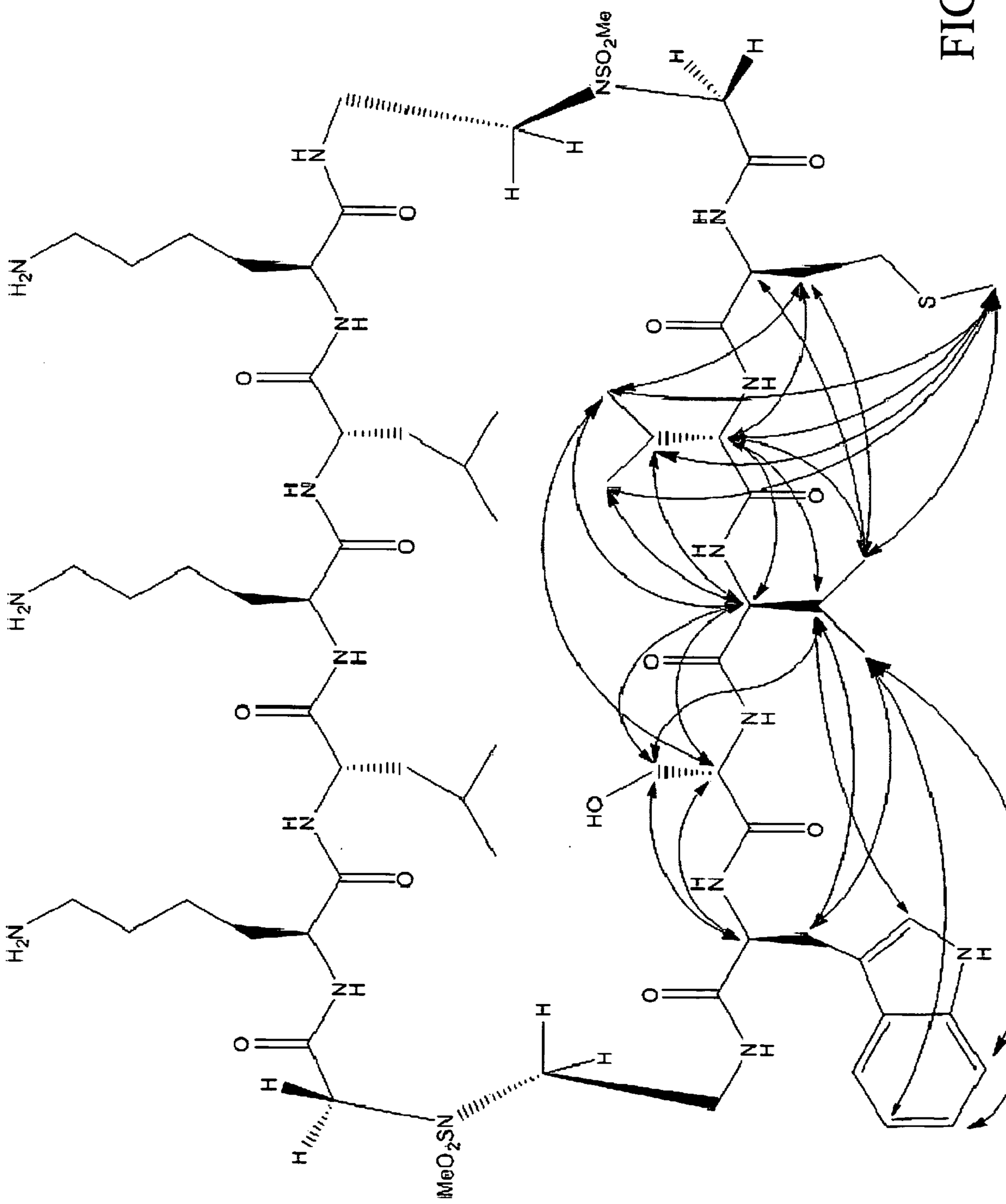


FIG. 16

FIG. 17A



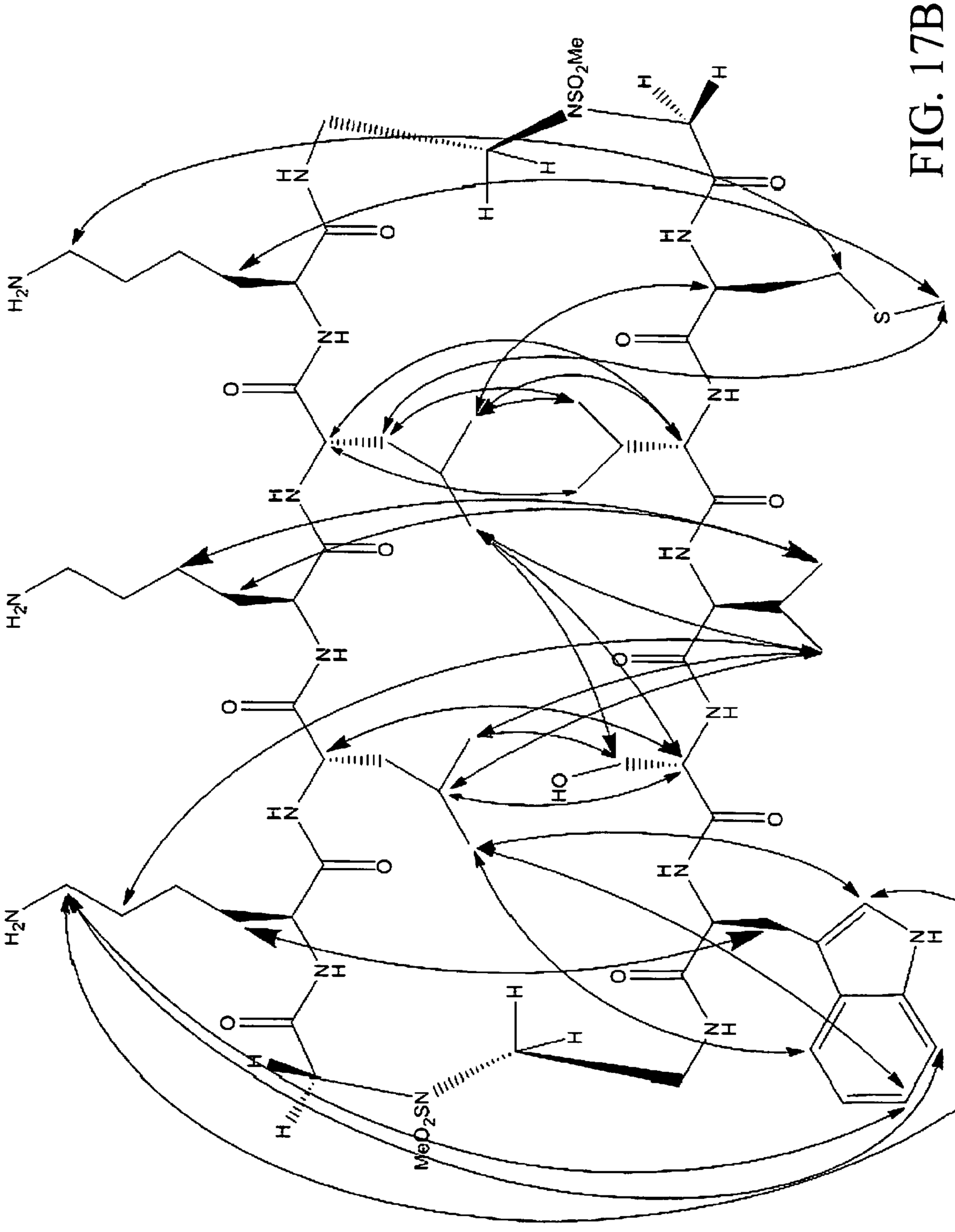
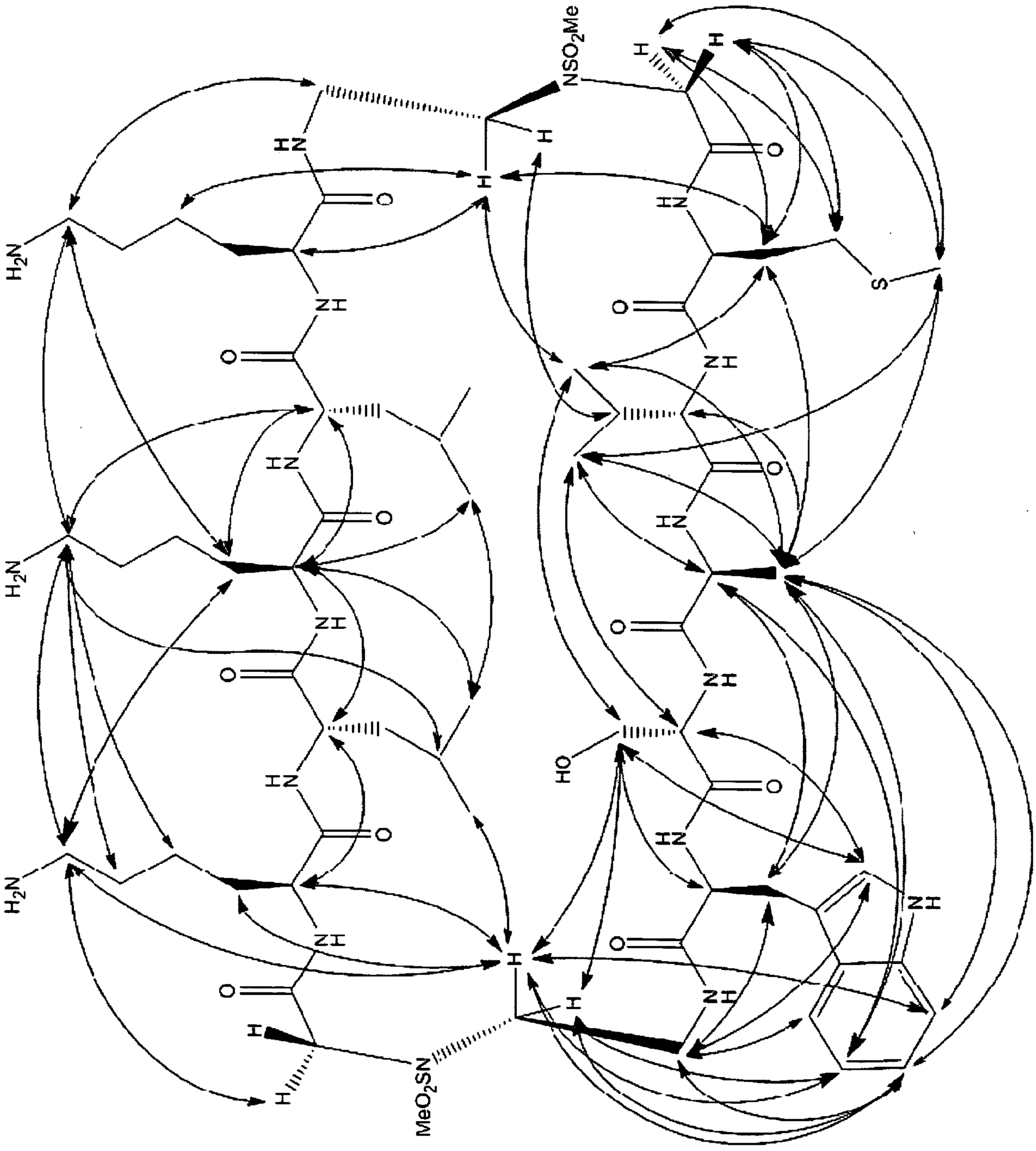


FIG. 17B

FIG. 18A



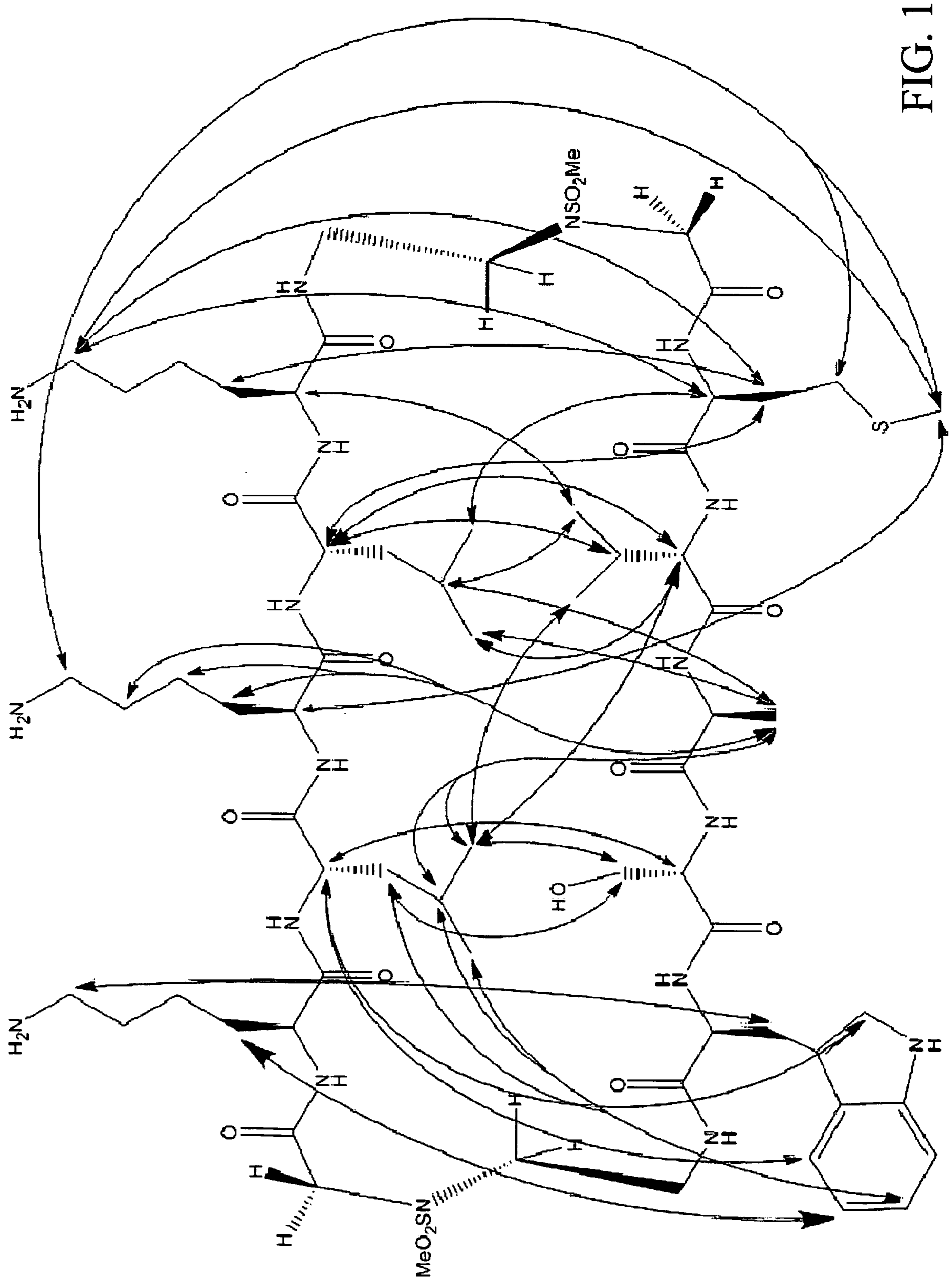


FIG. 18B

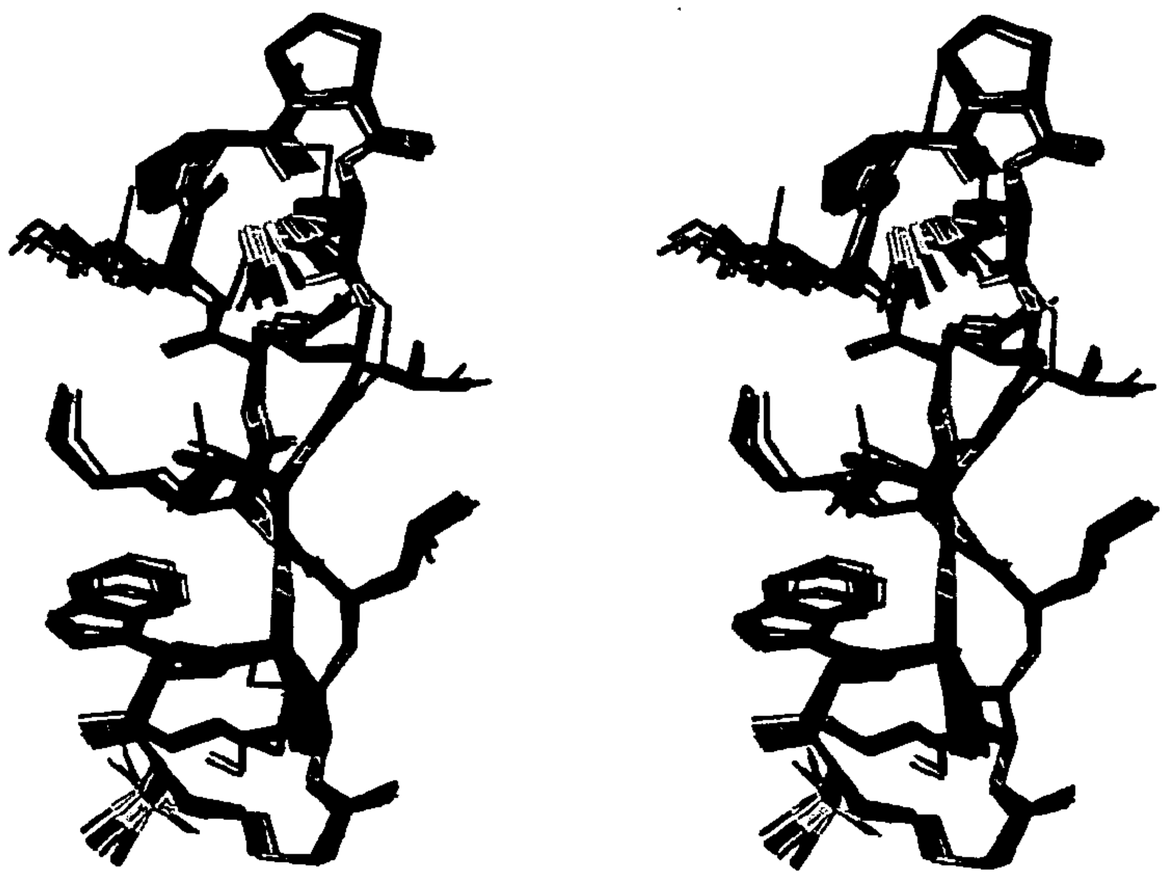


FIG. 19A

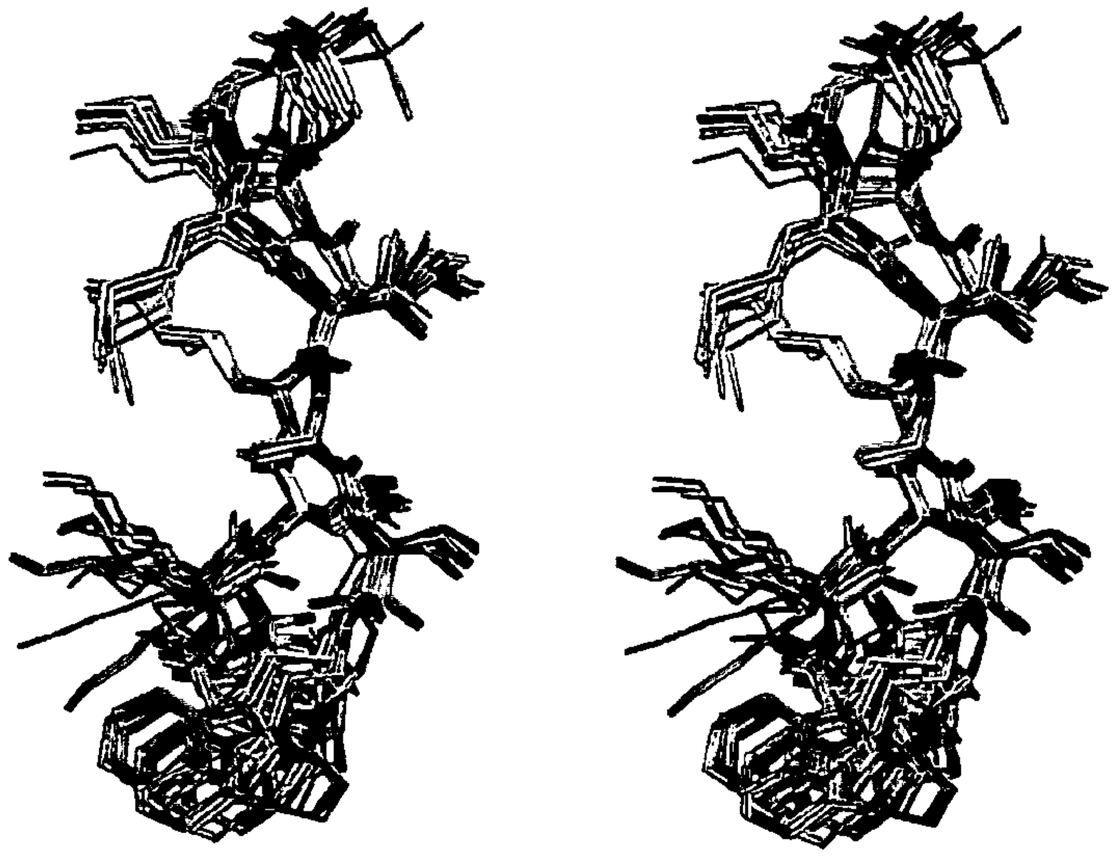


FIG. 19B

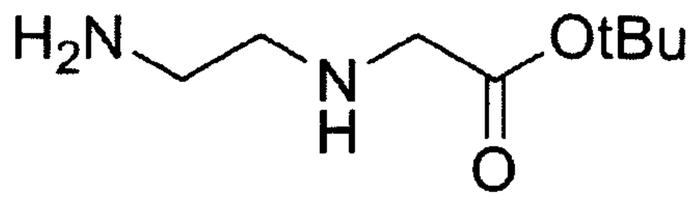


FIG. 20A

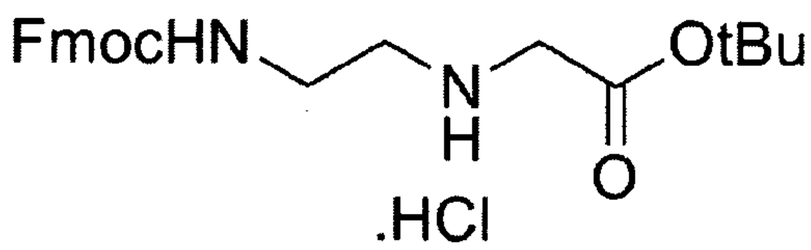


FIG. 20B

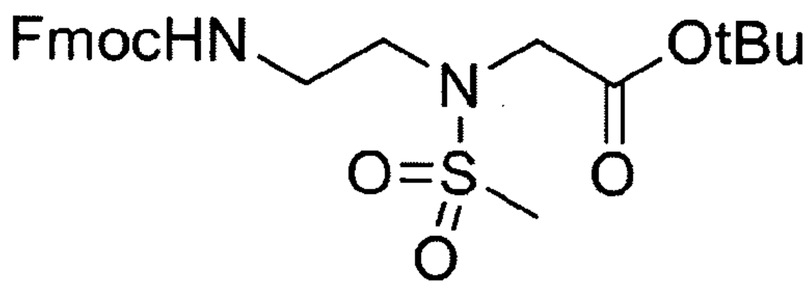


FIG. 20C

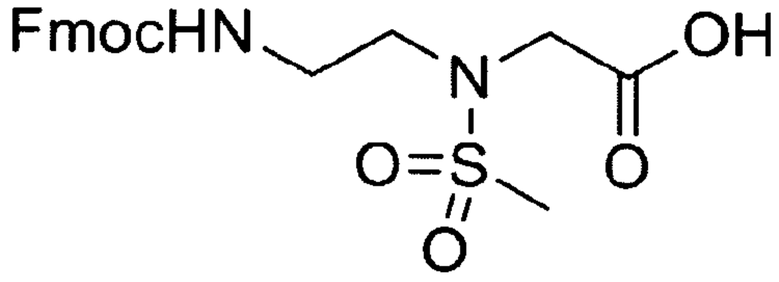


FIG. 20D

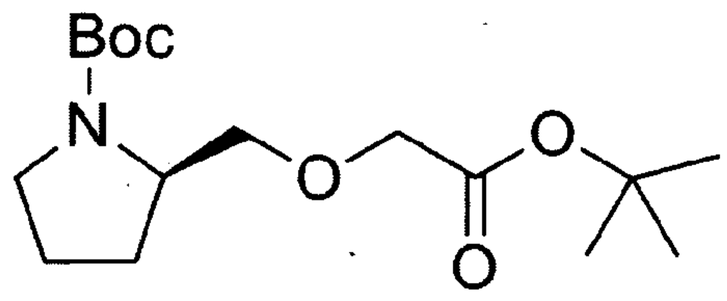


FIG. 20E

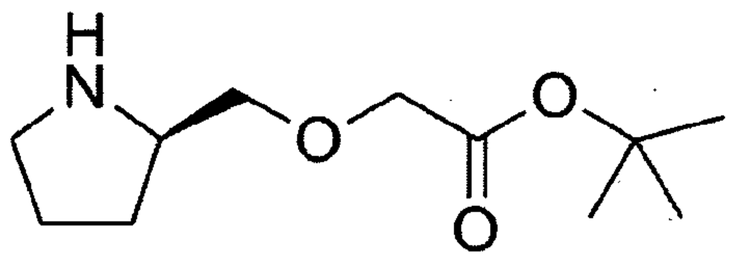


FIG. 20F

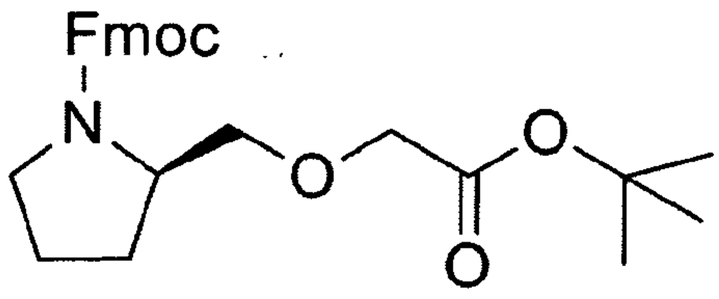


FIG. 20G

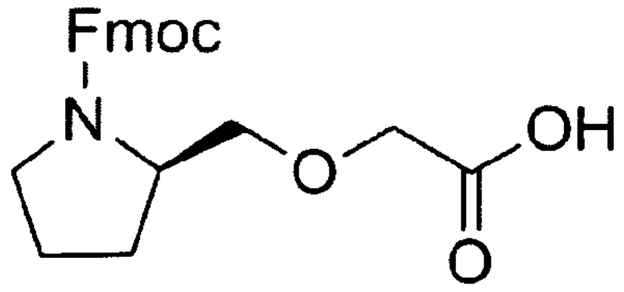


FIG. 20H

FIG. 21

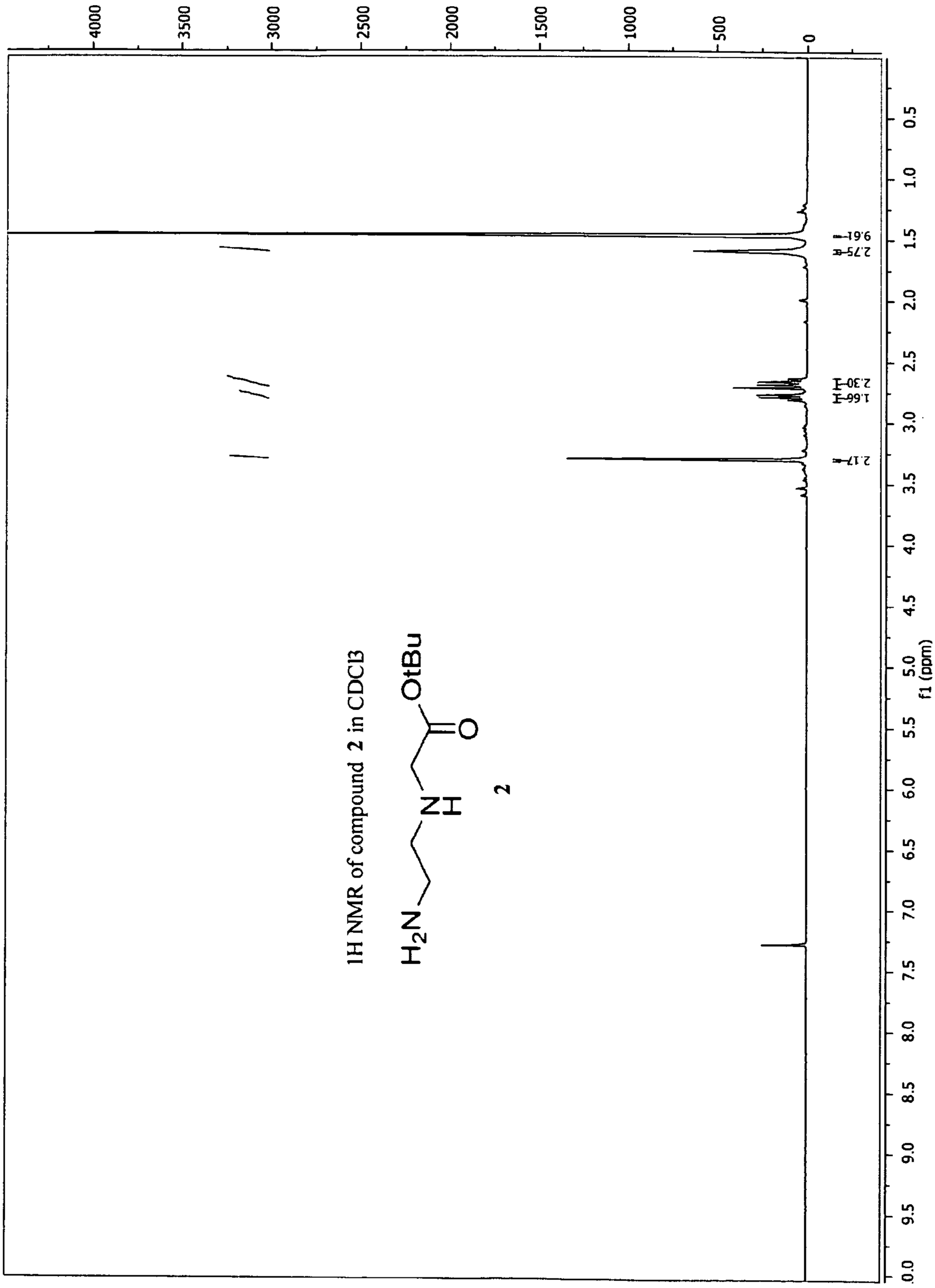

1H NMR of compound 2 in CDCl3
2
H2N
N
H
O
OtBu
9.61
2.75
2.30
1.66
2.17
f1 (ppm)

FIG. 22

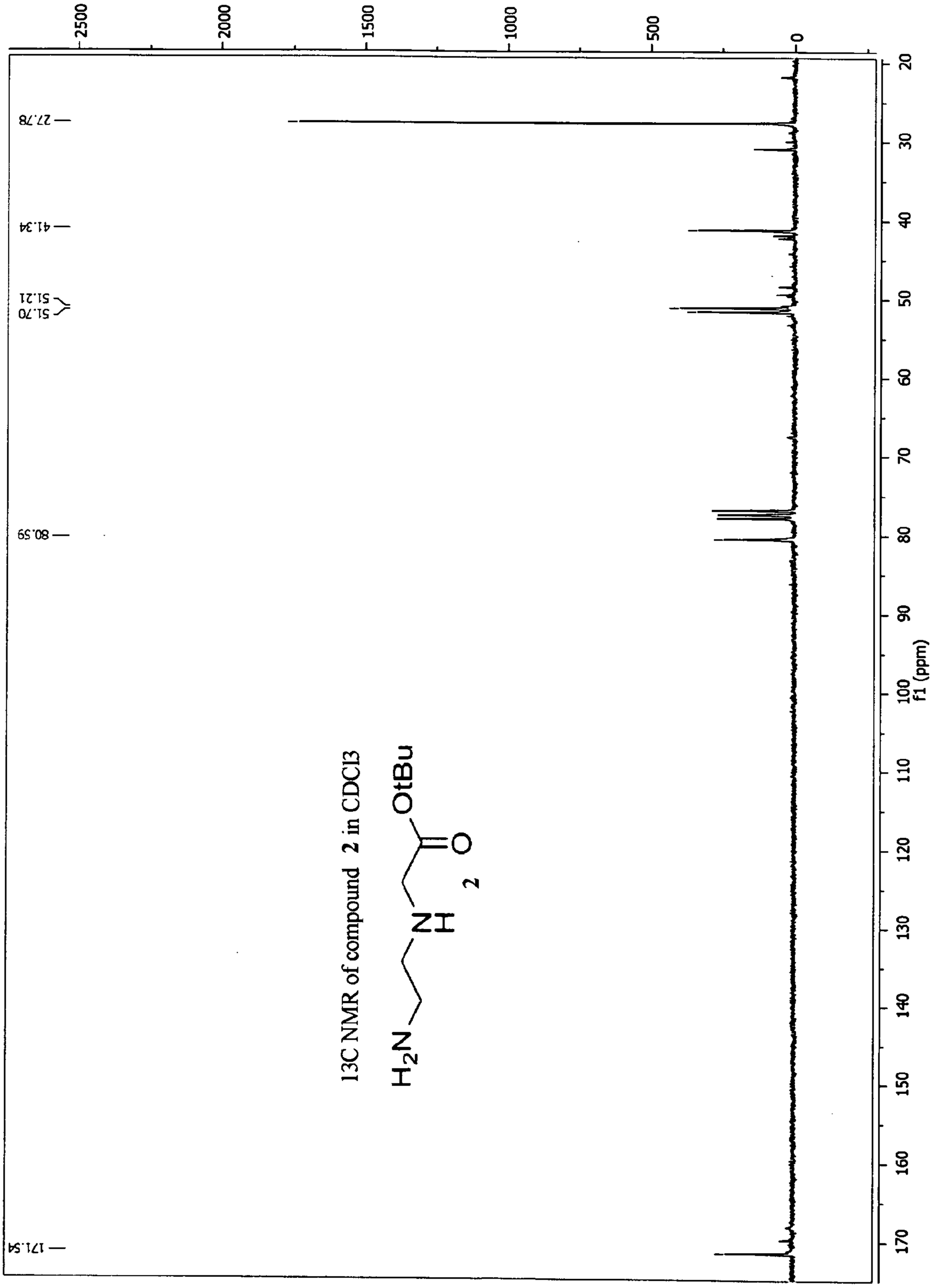
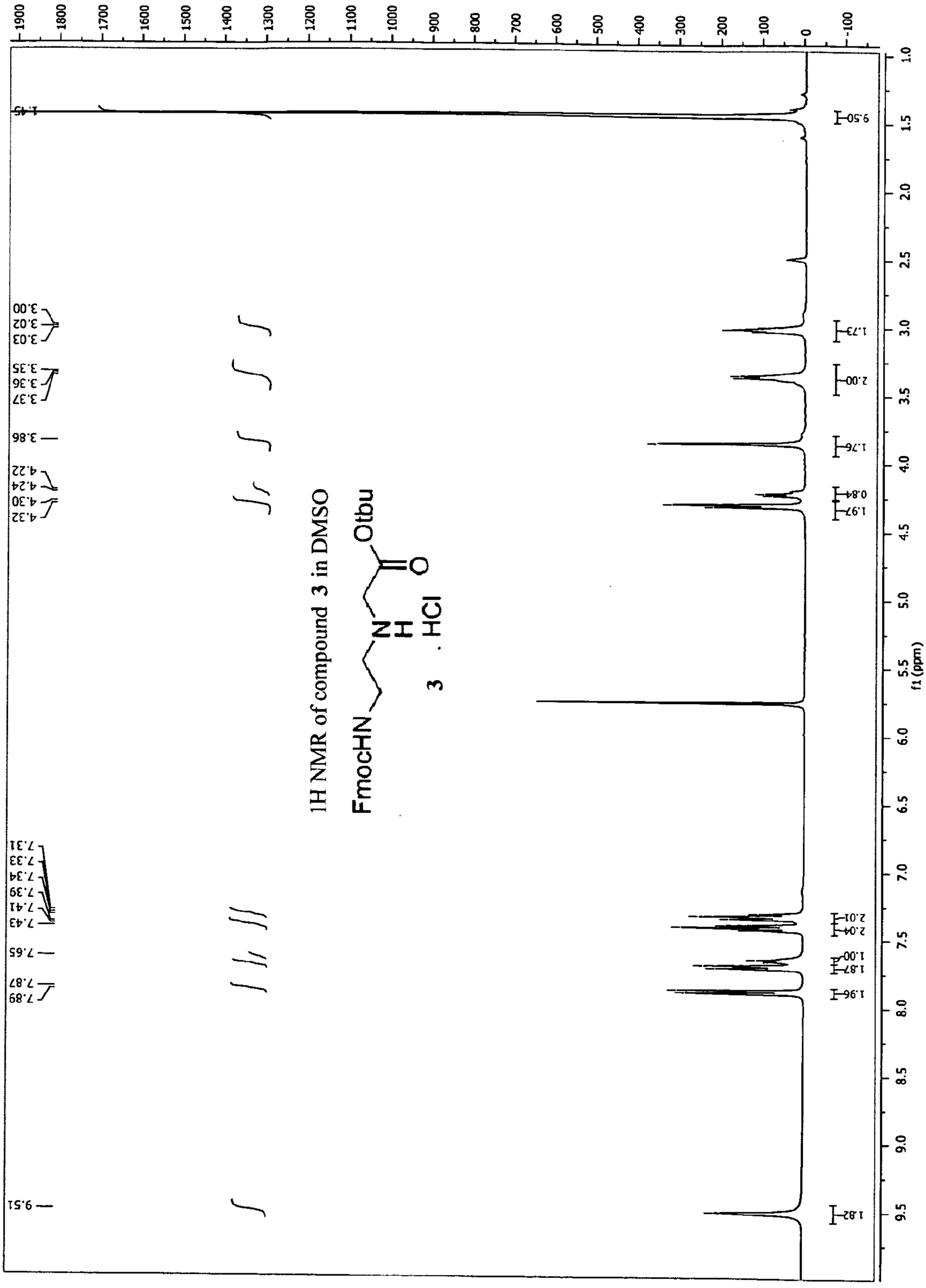

13C NMR of compound 2 in CDCl3
OtBu
O
N
H
2
H2N
2500
2000
1500
1000
500
0
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
f1 (ppm)
27.78
41.34
51.21
51.70
80.59
171.54

FIG. 23



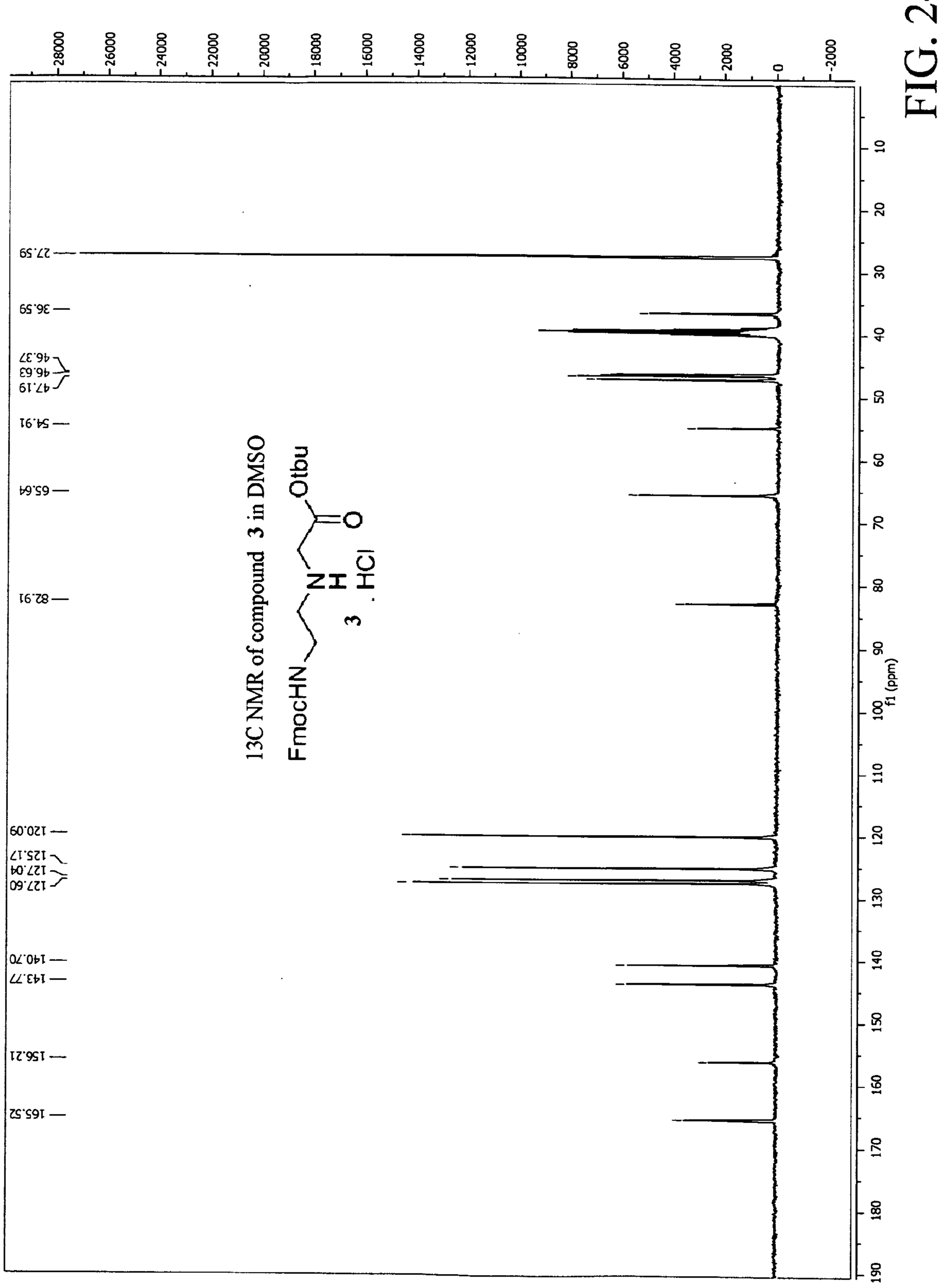

13C NMR of compound 3 in DMSO
FmocHN
N
H
O
O
3 .HCl
27.59
36.59
46.37
46.63
47.19
54.91
65.64
82.91
120.09
125.17
127.04
127.60
140.70
143.77
156.21
165.52
f1 (ppm)

FIG. 24

FIG. 25

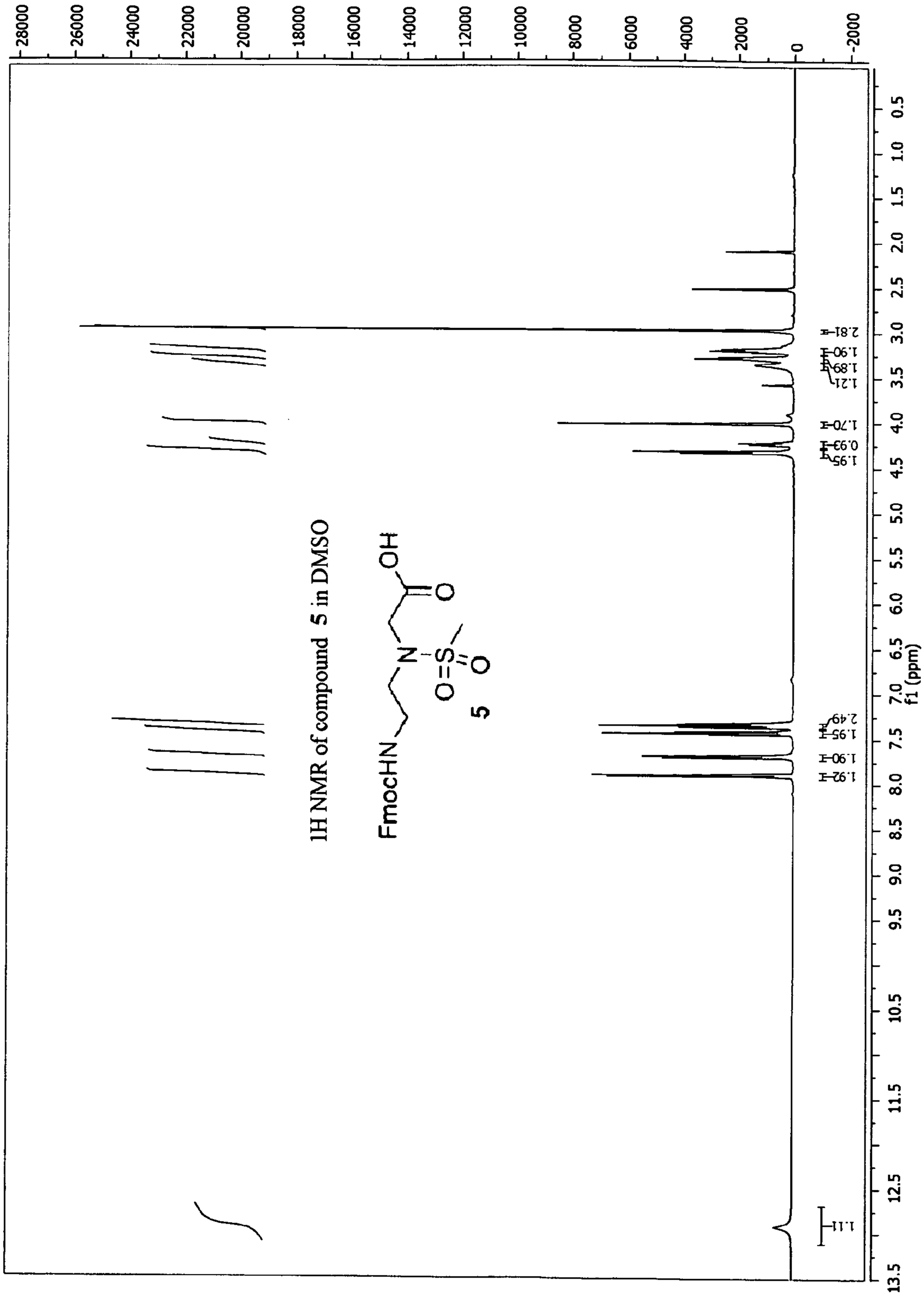
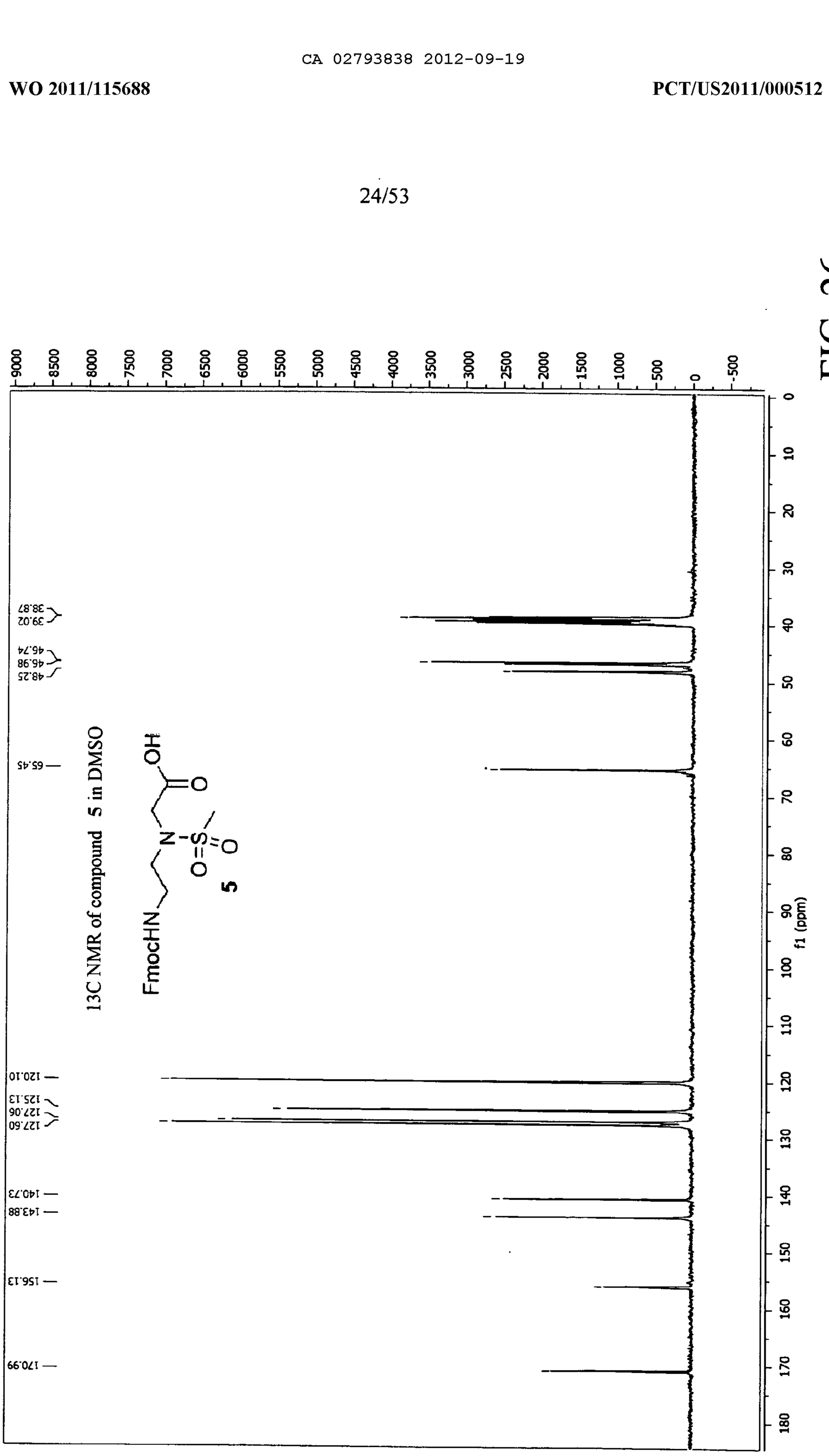

1H NMR of compound 5 in DMSO
FmocHN
OH
O
N
S
O
O
5
28000
26000
24000
22000
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
-2000
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
10.5
11.5
12.5
13.5
f1 (ppm)
2.81
1.90
1.89
1.21
1.70
0.93
1.95
2.49
1.95
1.90
1.92
11.1

FIG. 26


13C NMR of compound 5 in DMSO
FmocHN
OH
O
N
S
O=S=O
5
65.45
38.87
39.02
46.74
46.98
48.25
120.10
125.13
127.06
127.60
140.73
143.88
156.13
170.99
f1 (ppm)

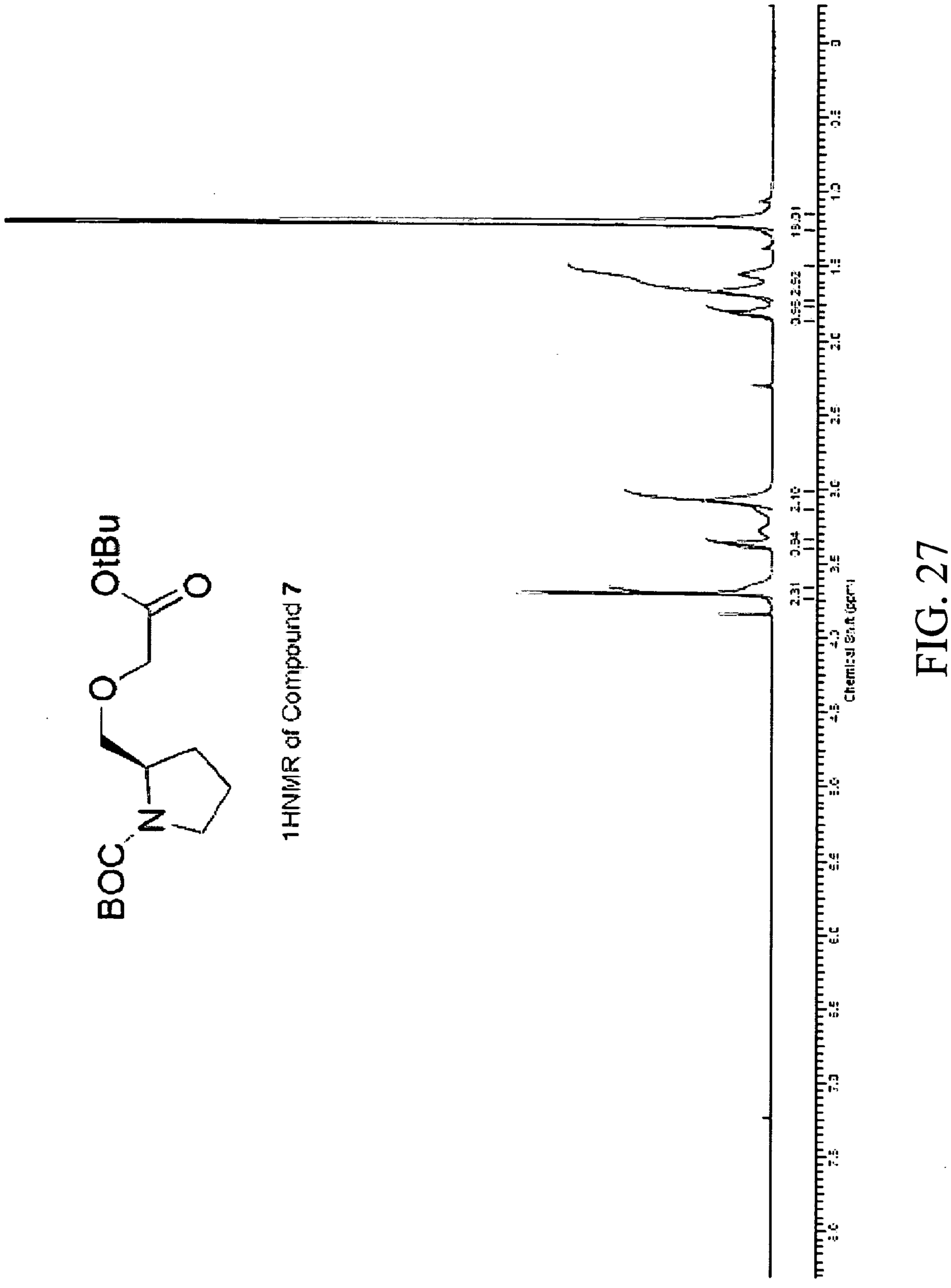


FIG. 27

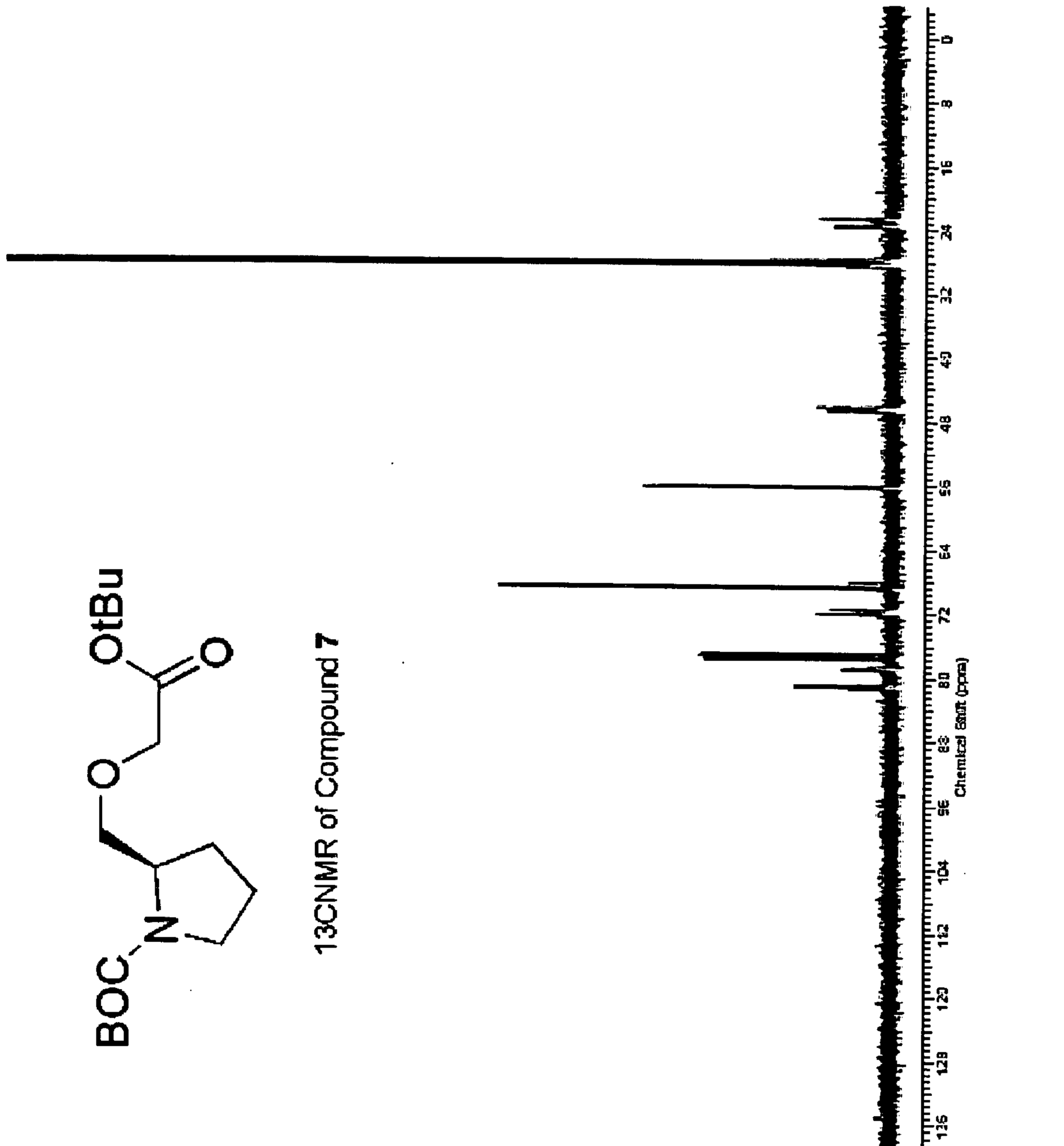


FIG. 28

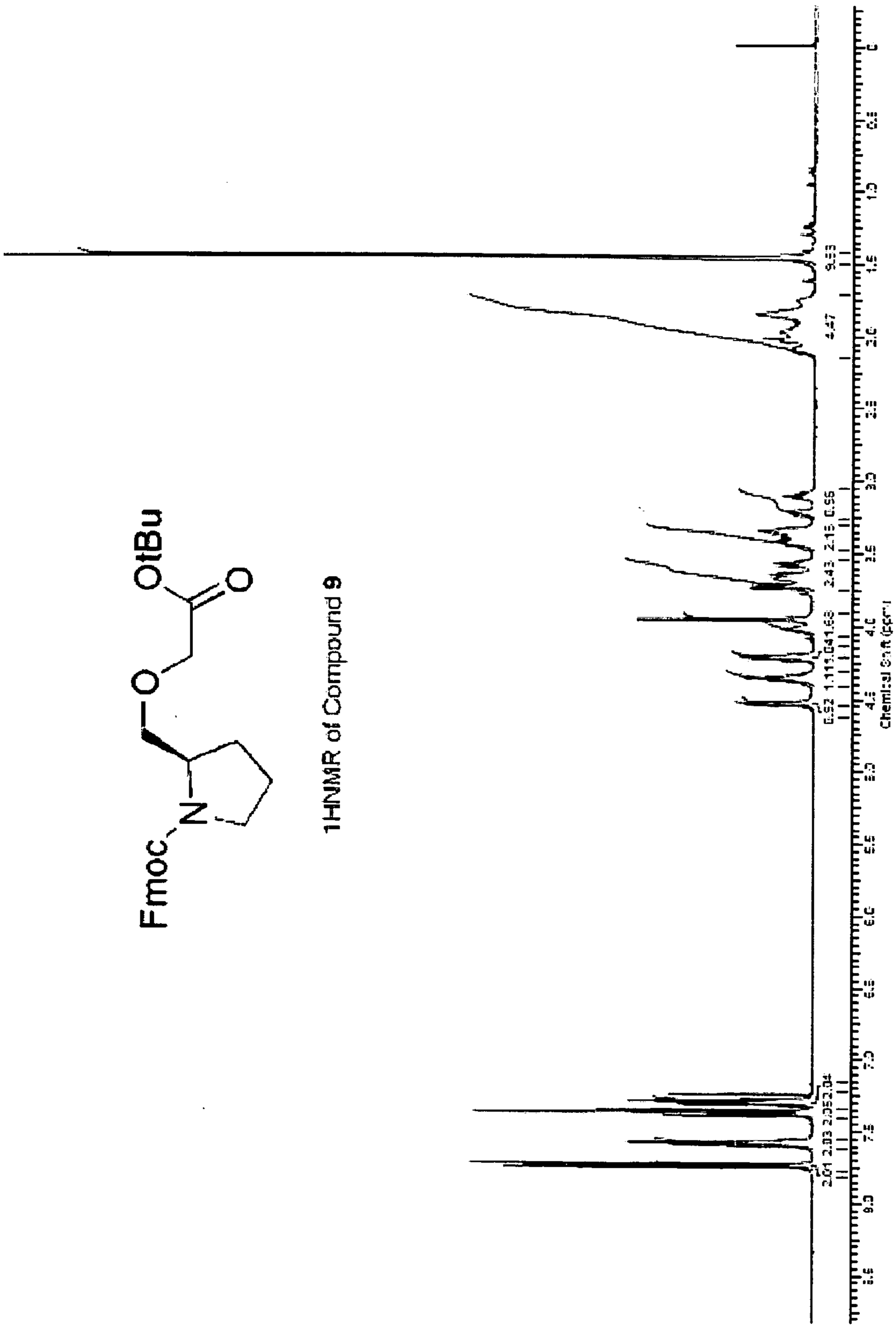


FIG. 29

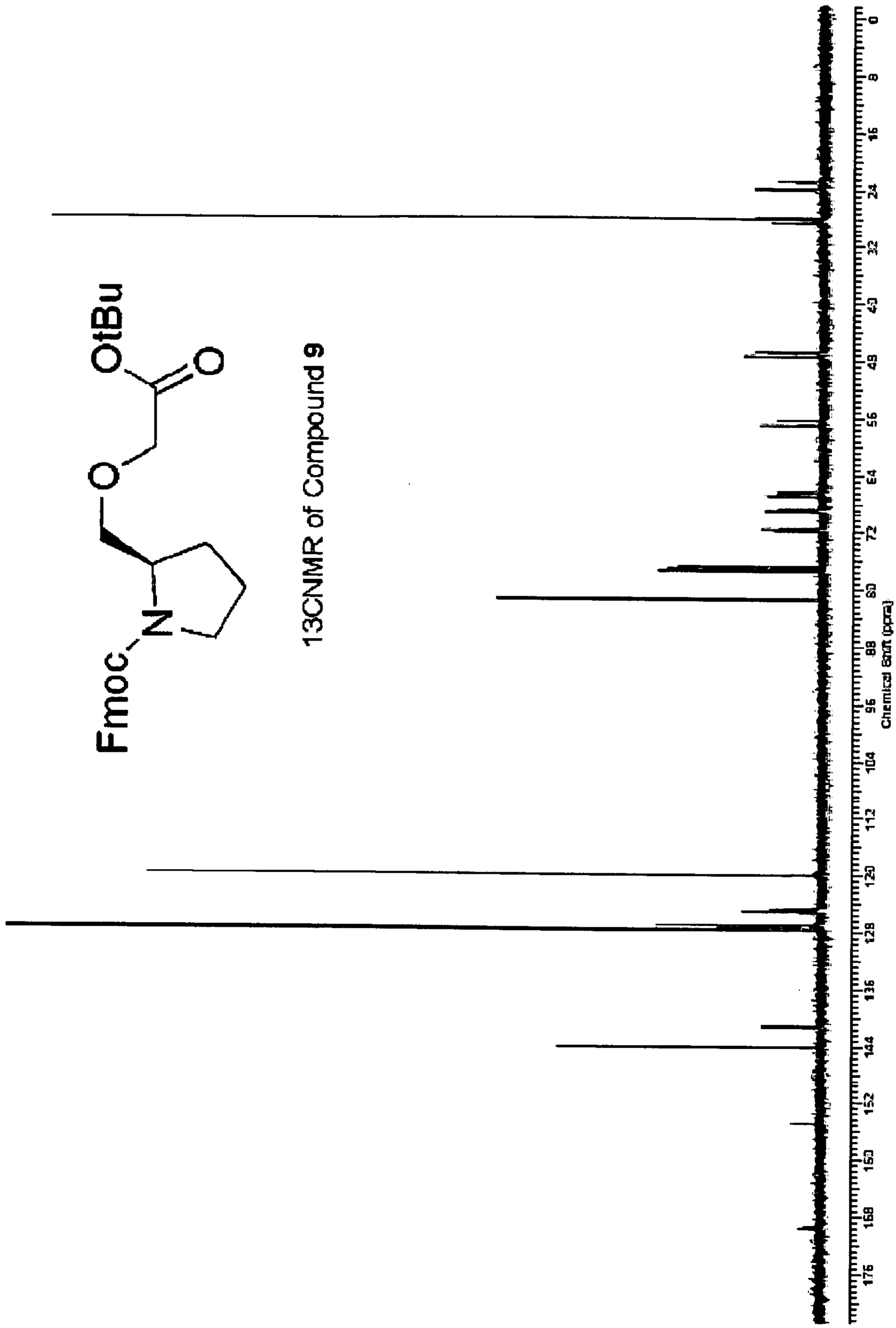


FIG. 30

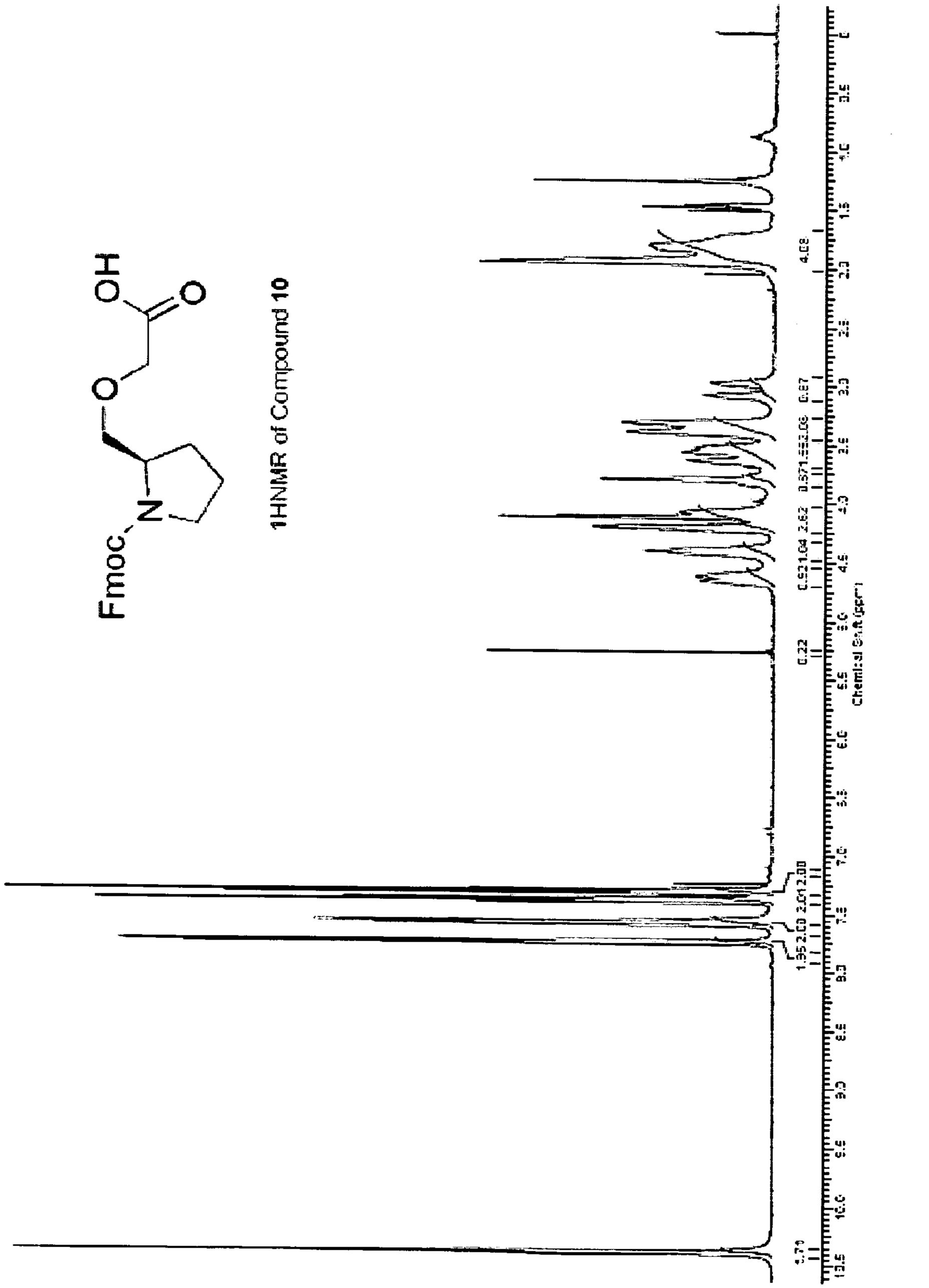


FIG. 31

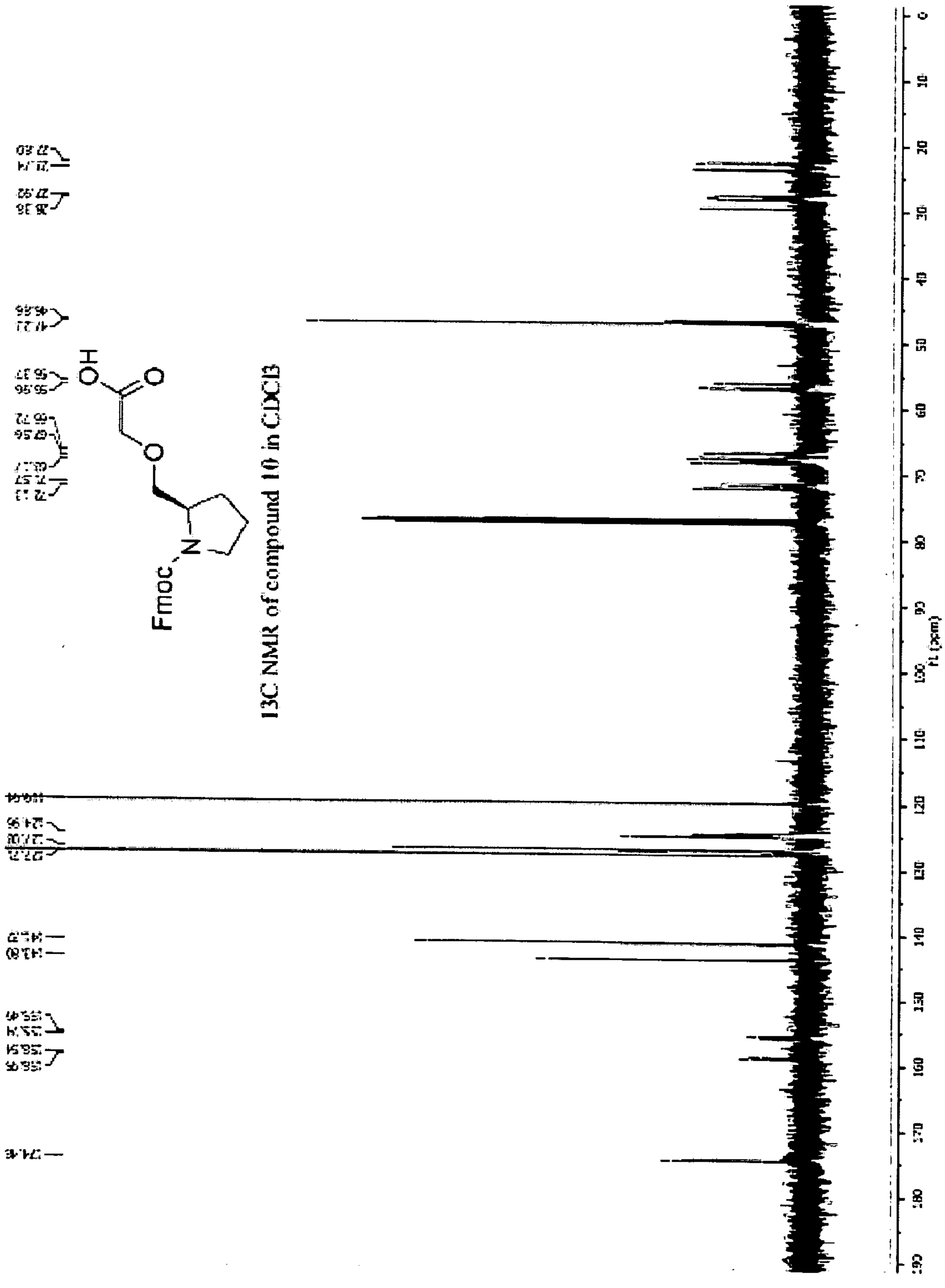


FIG. 32

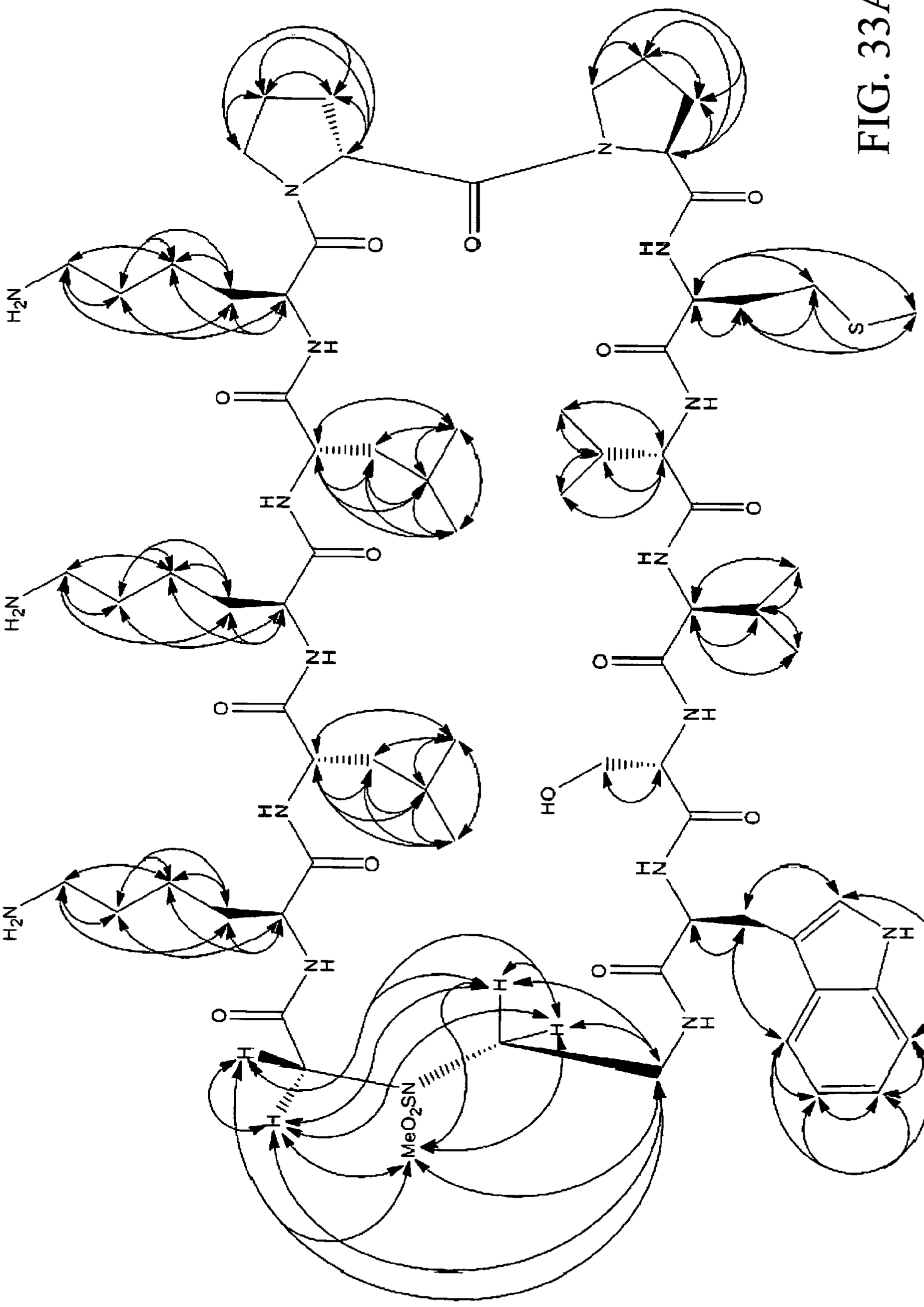


FIG. 33A

FIG. 33B

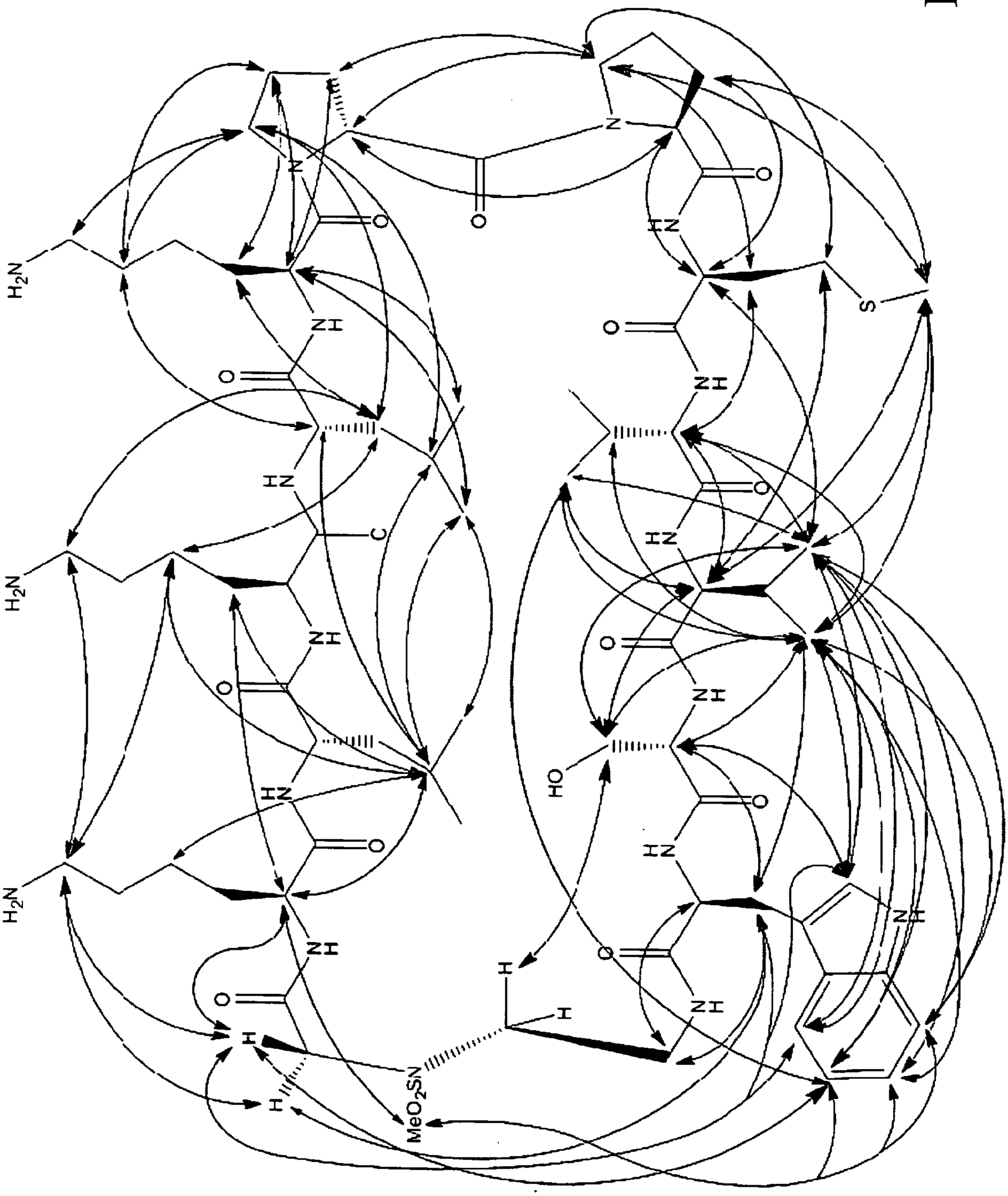
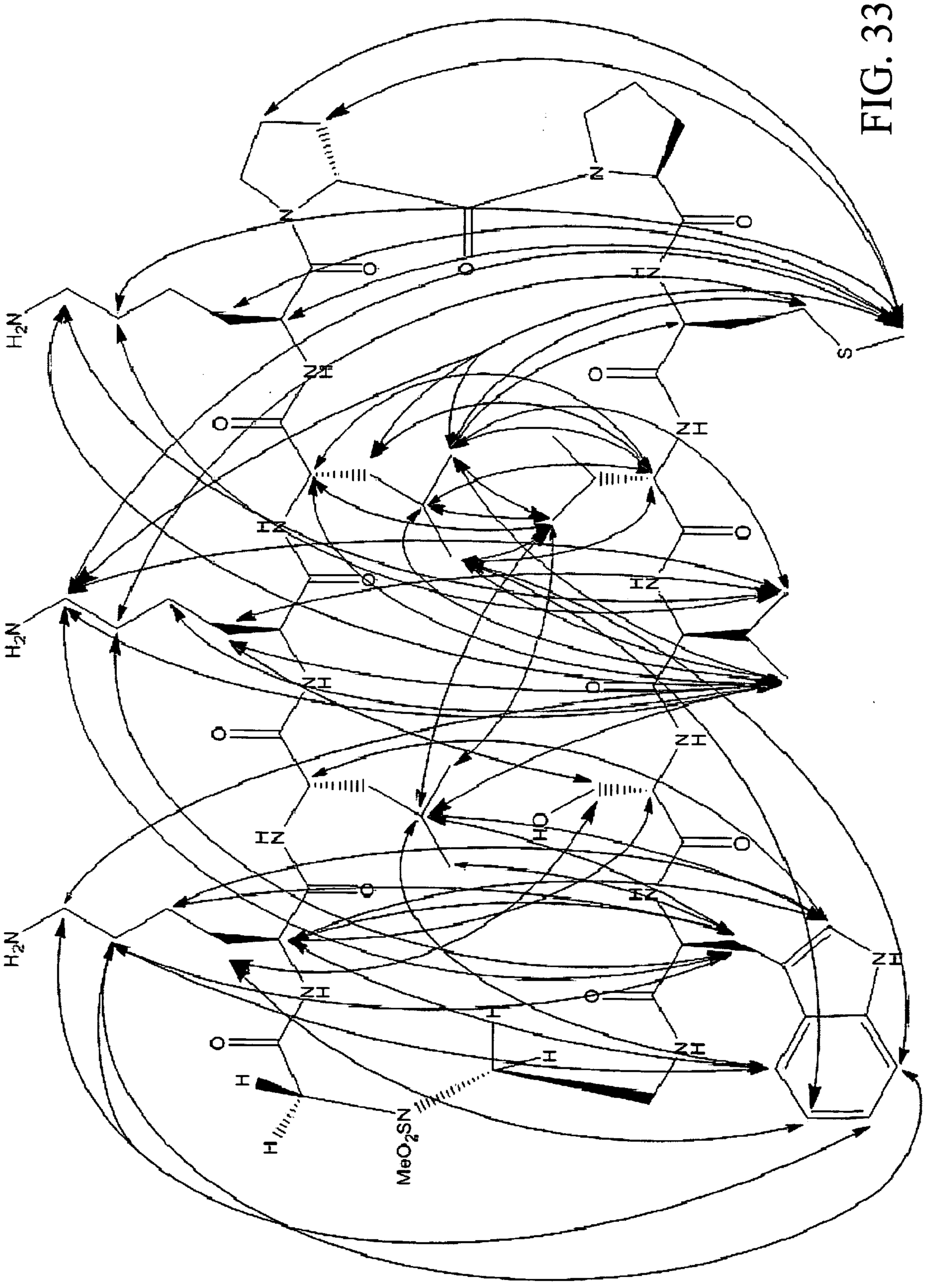


FIG. 33C



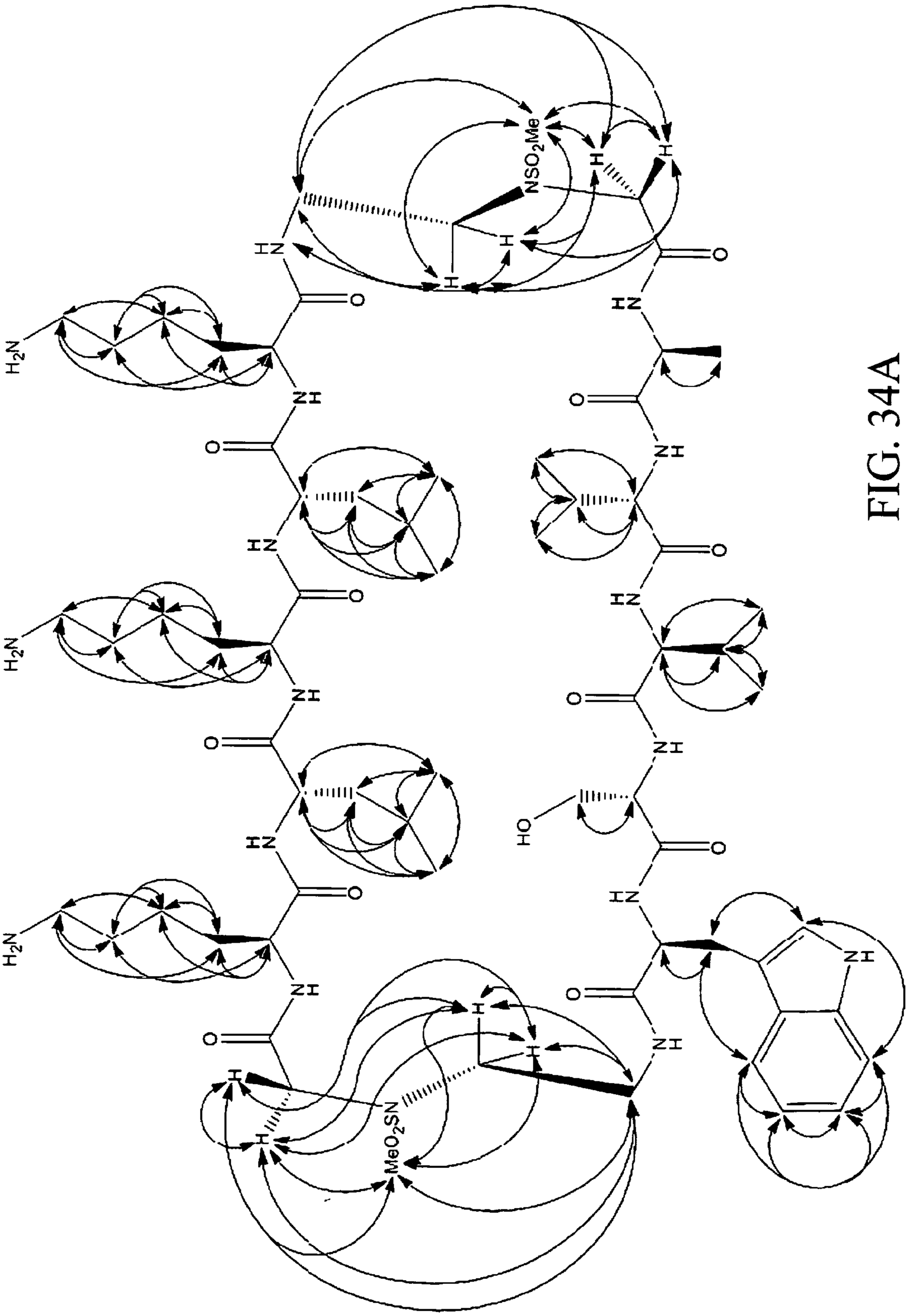

NSO_2Me
H
H
IZ
I
I
O
IZ
H_2N
ZI
O
ZI
O
IZ
O
IZ
H_2N
ZI
O
ZI
O
IZ
H_2N
ZI
O
ZI
HO
O
IZ
ZI
O
I
I
ZI
O
H
MeO_2SN

FIG. 34A

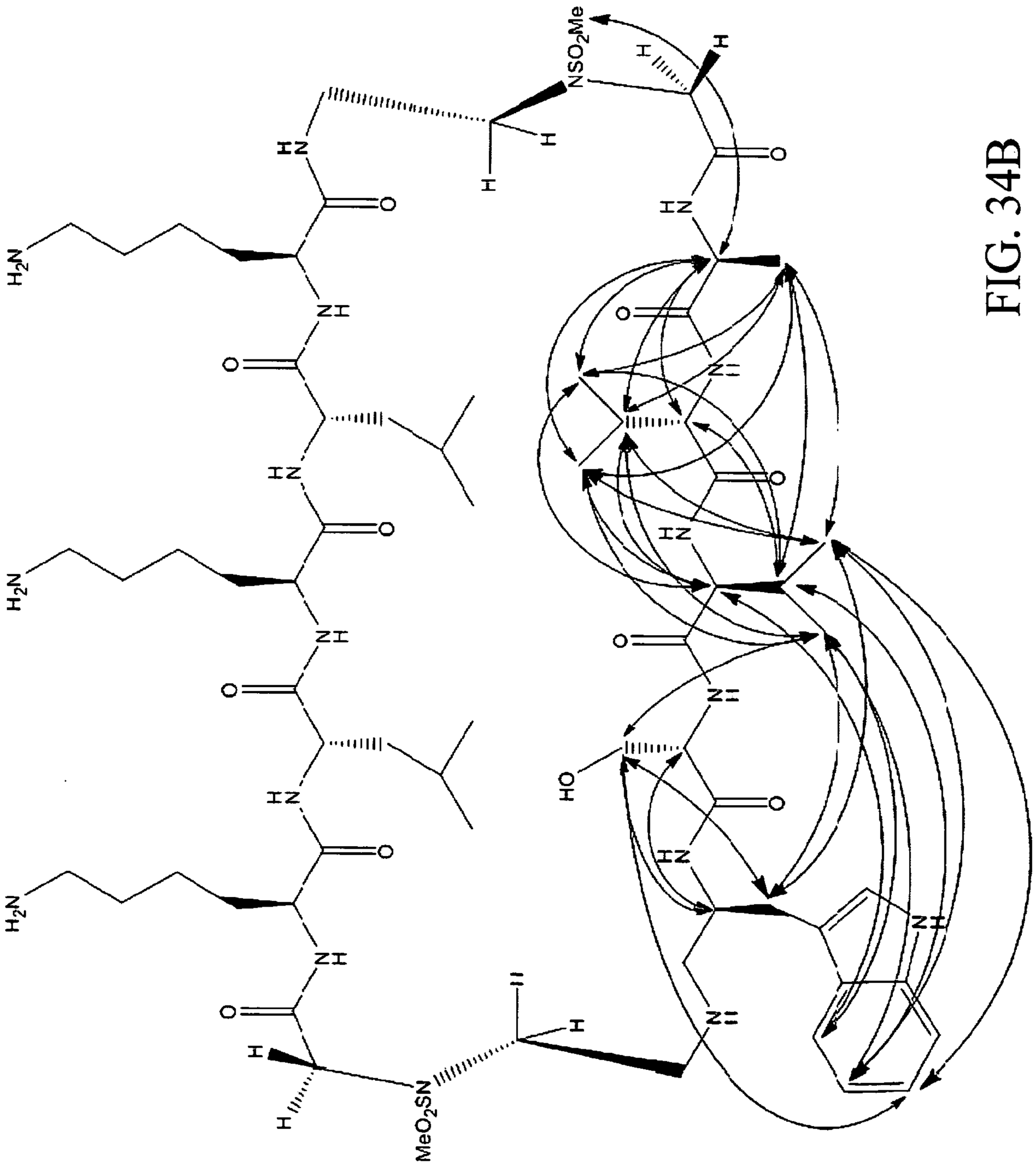


FIG. 34B

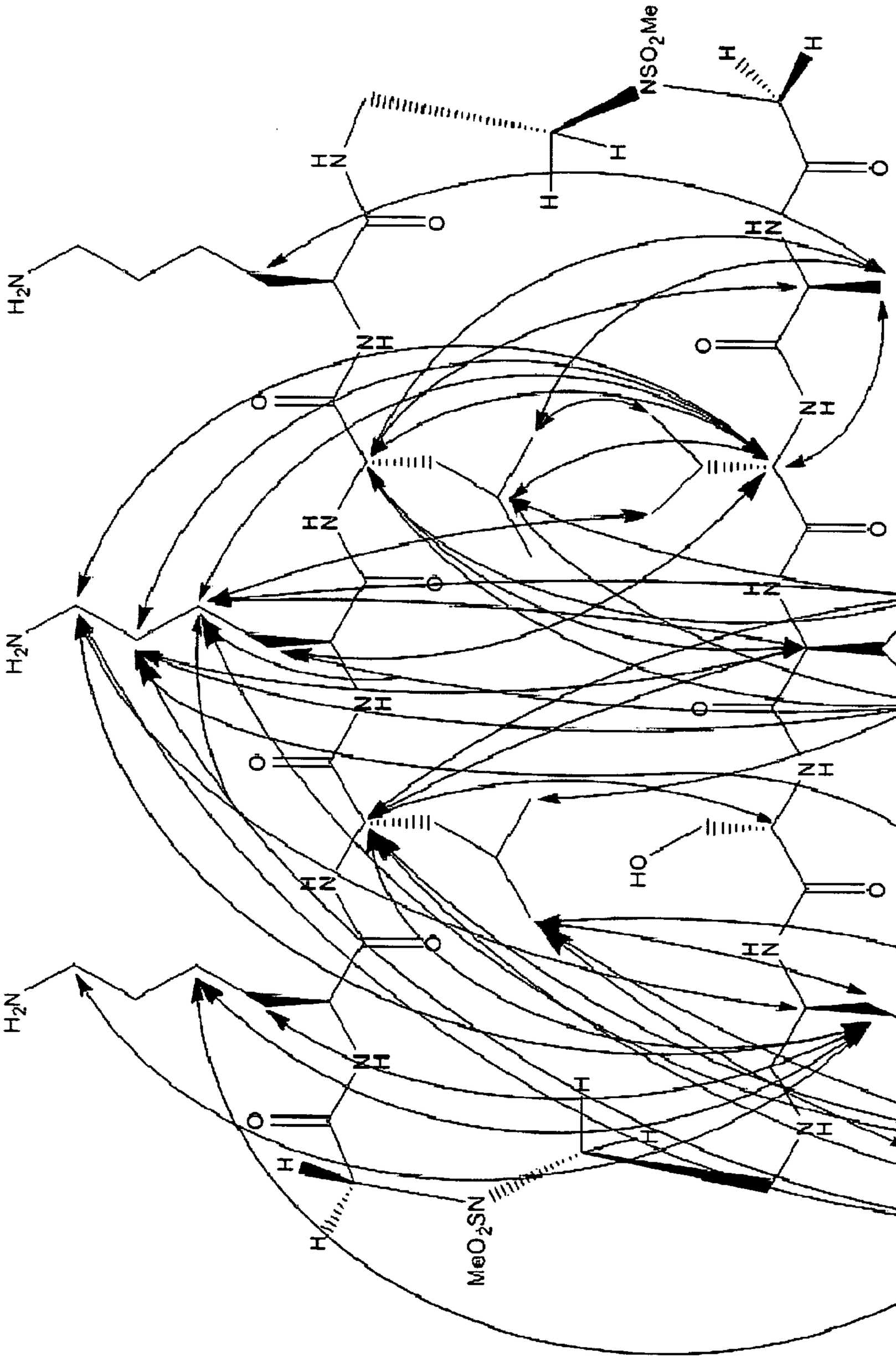

Me
NSO_2
H
H
H
IZ
O
H
O
IZ
H_2N
ZH
O
O
ZH
IZ
IZ
O
O
IZ
H_2N
ZI
O
ZI
O
HO
IZ
IZ
H_2N
ZH
ZI
I
O
I
ZI
H
MeO_2SN

FIG. 34C

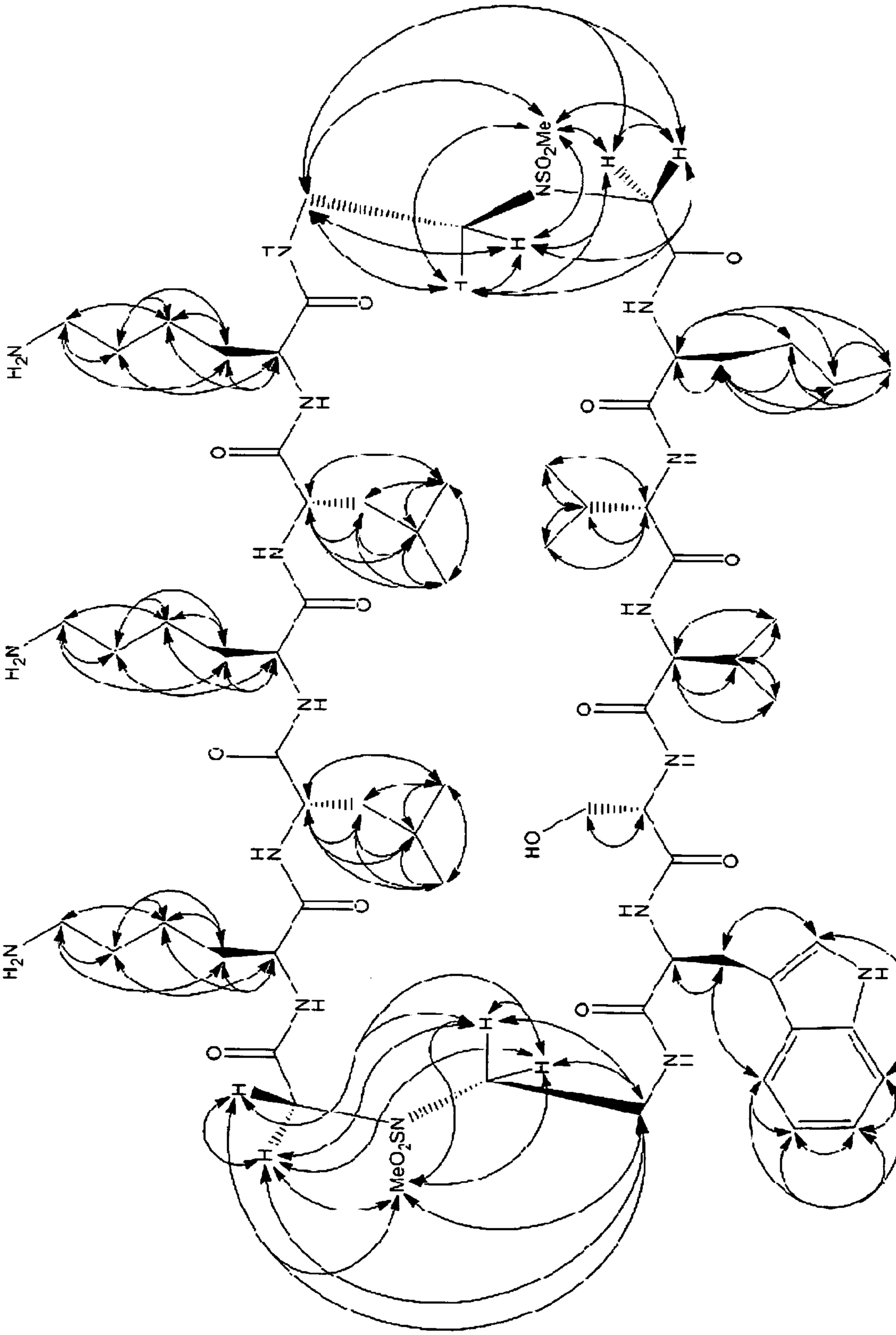
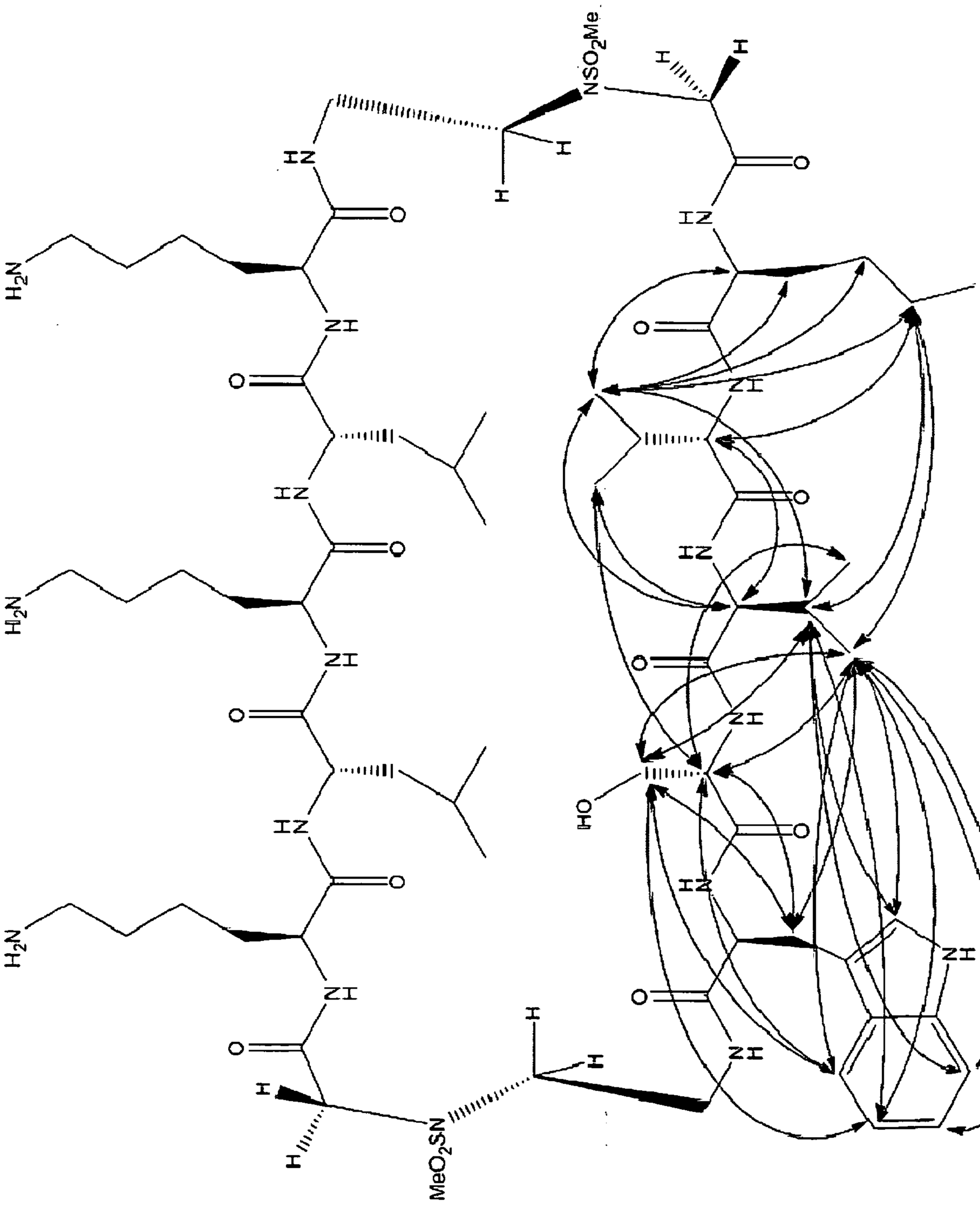


FIG. 35A

FIG. 35B


NSO2Me
H
H
H
H
IZ
O
H2N
ZI
O
IZ
O
ZI
O
IZ
O
H2N
ZI
O
IZ
O
H2N
ZI
O
IZ
O
HO
ZI
O
IZ
O
ZH
MeO2SN

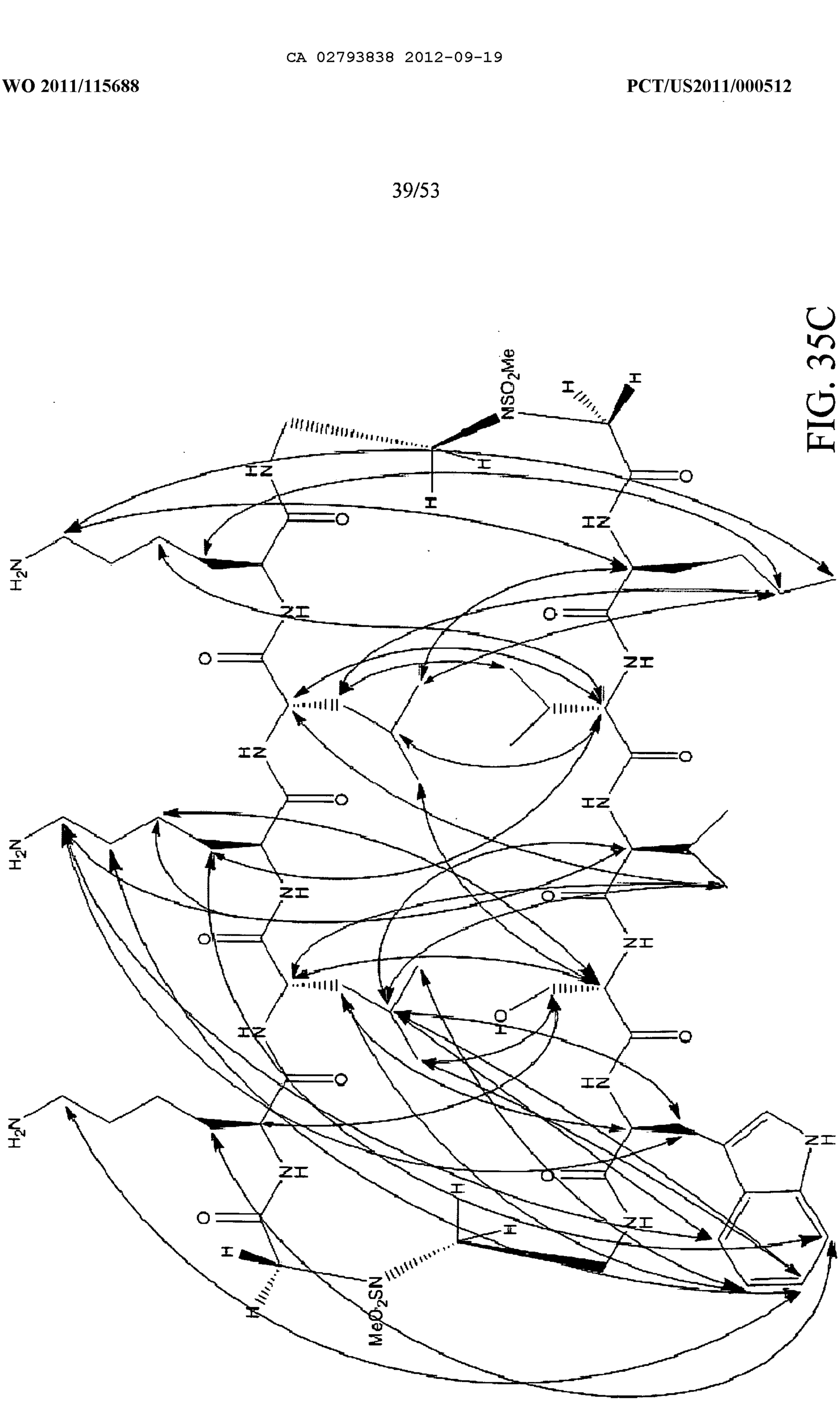


FIG. 35C

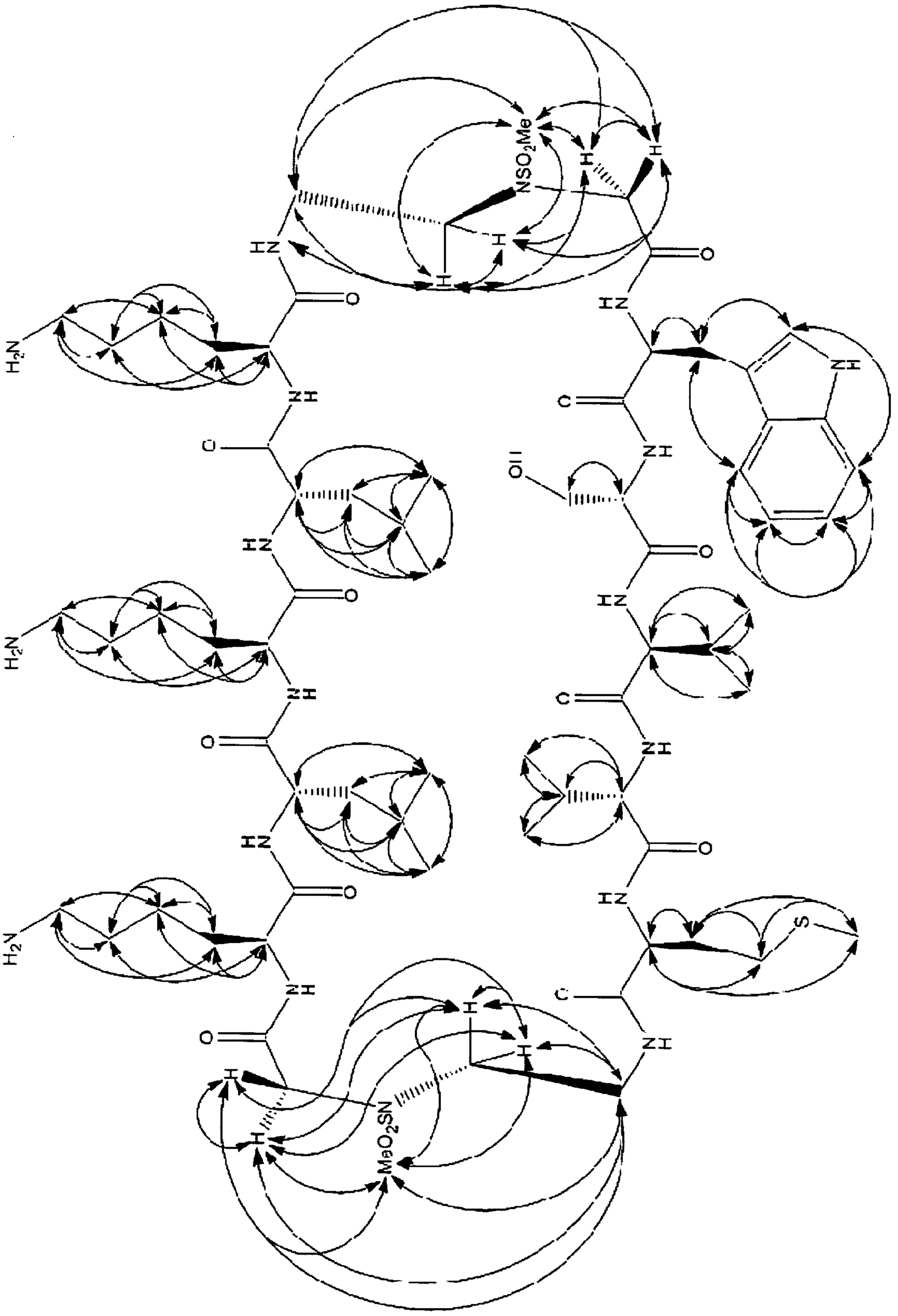


FIG. 36A

FIG. 36B

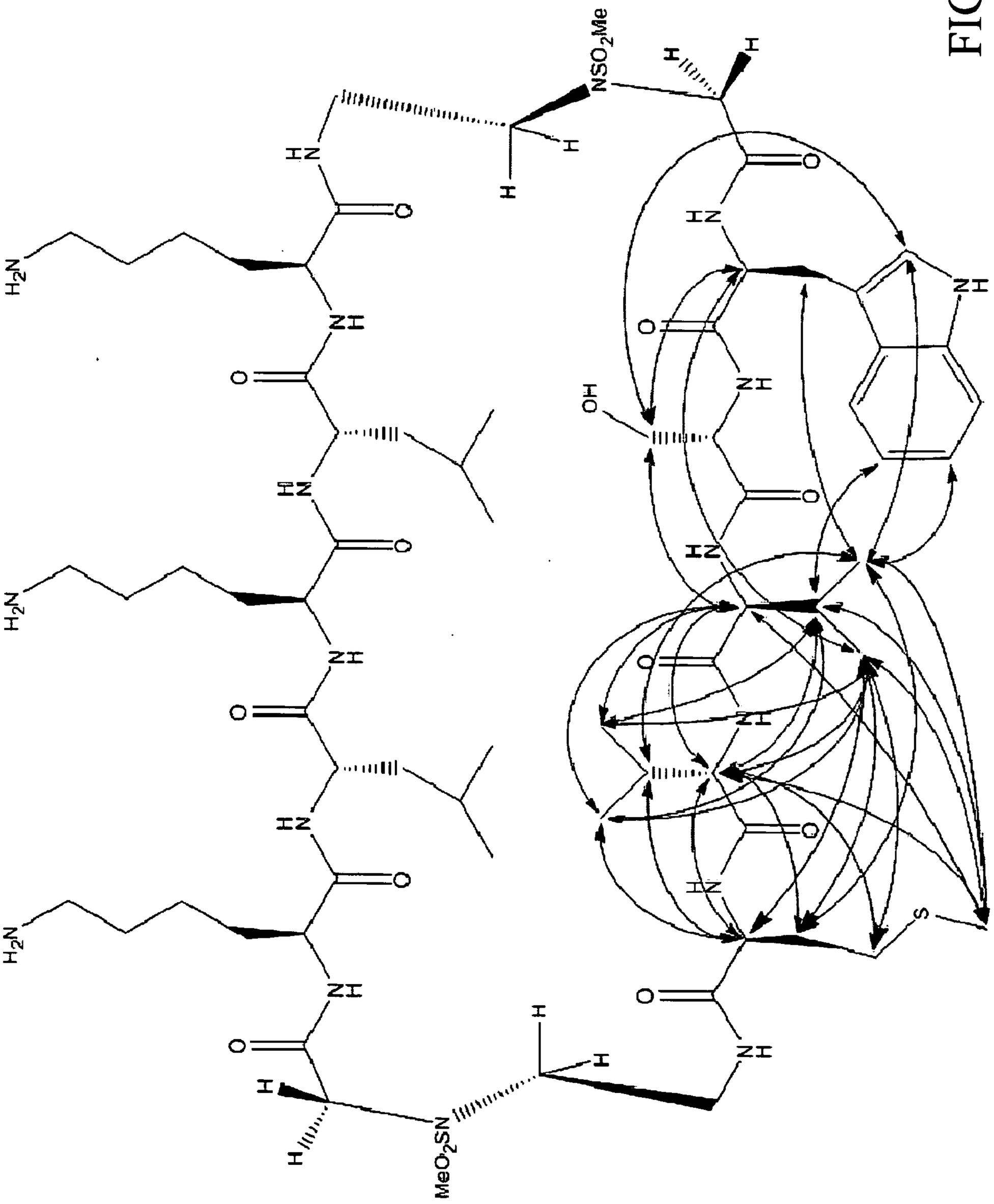
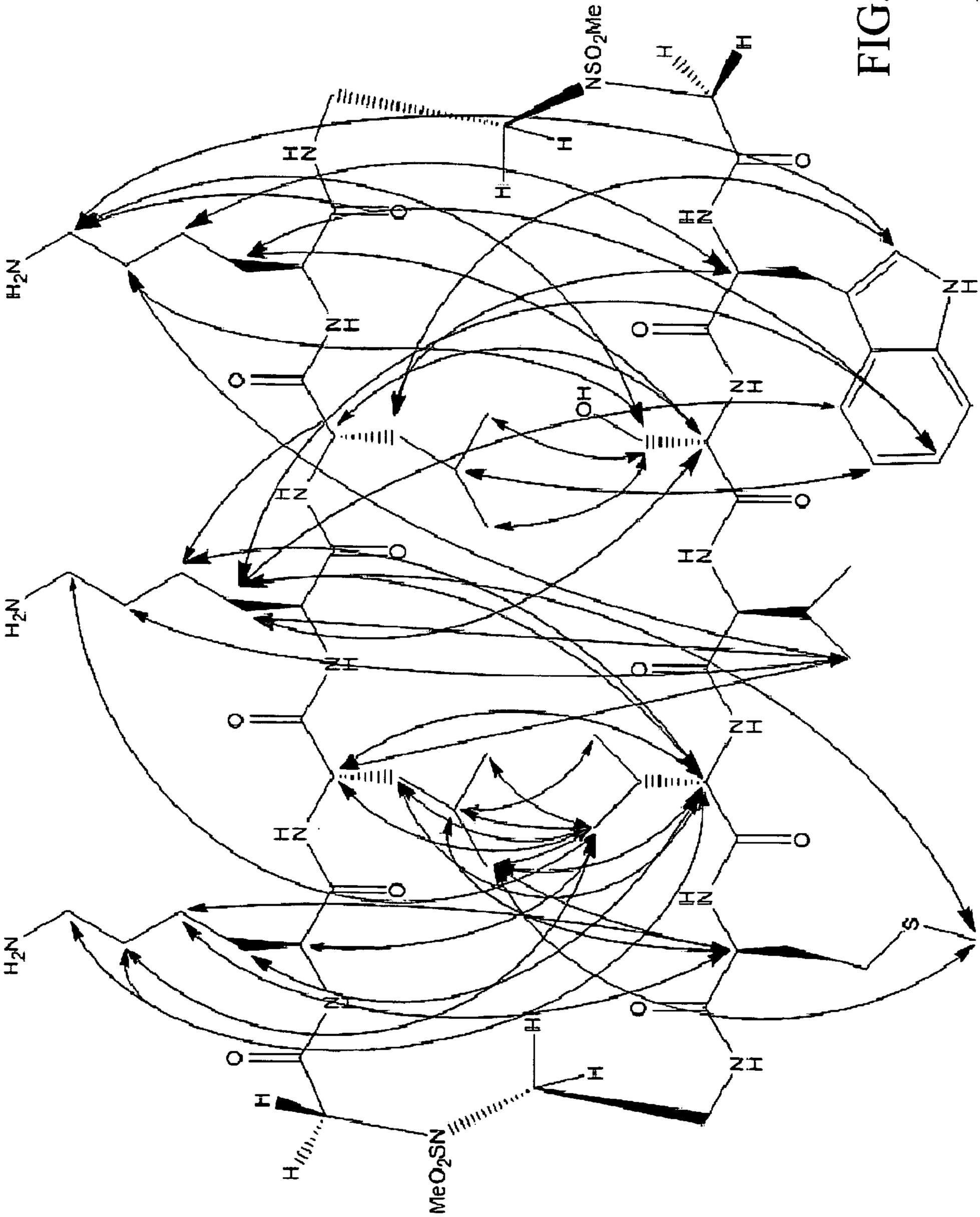

NSO2Me
H
H
H
H
IZ
O
H2N
ZI
O
IZ
O
H2N
ZI
O
IZ
O
H2N
ZI
O
IZ
NH
O
ZI
OH
O
IZ
O
ZI
O
IZ
S
O
ZI
H
H
H
MeO2SN

FIG. 36C



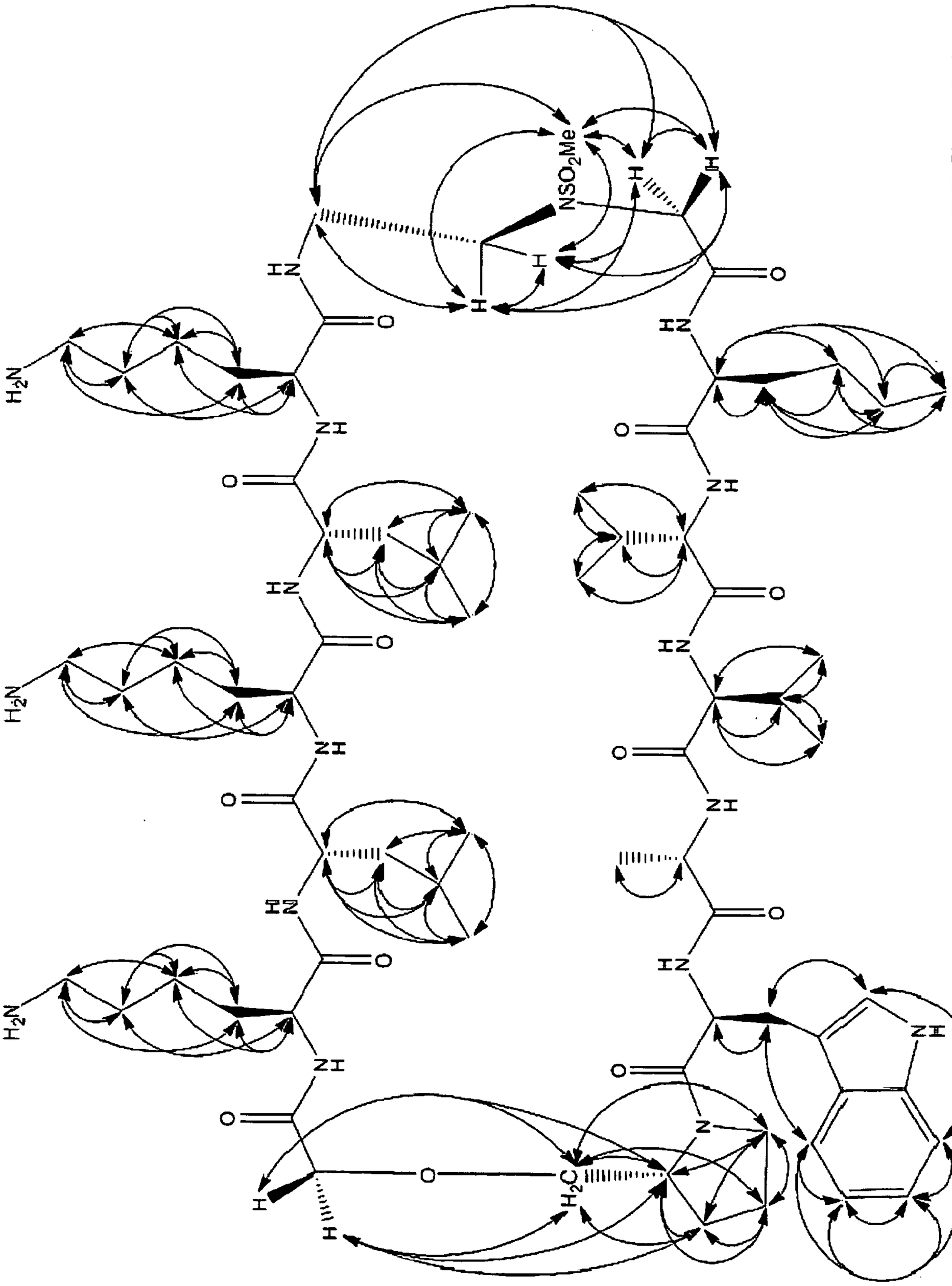
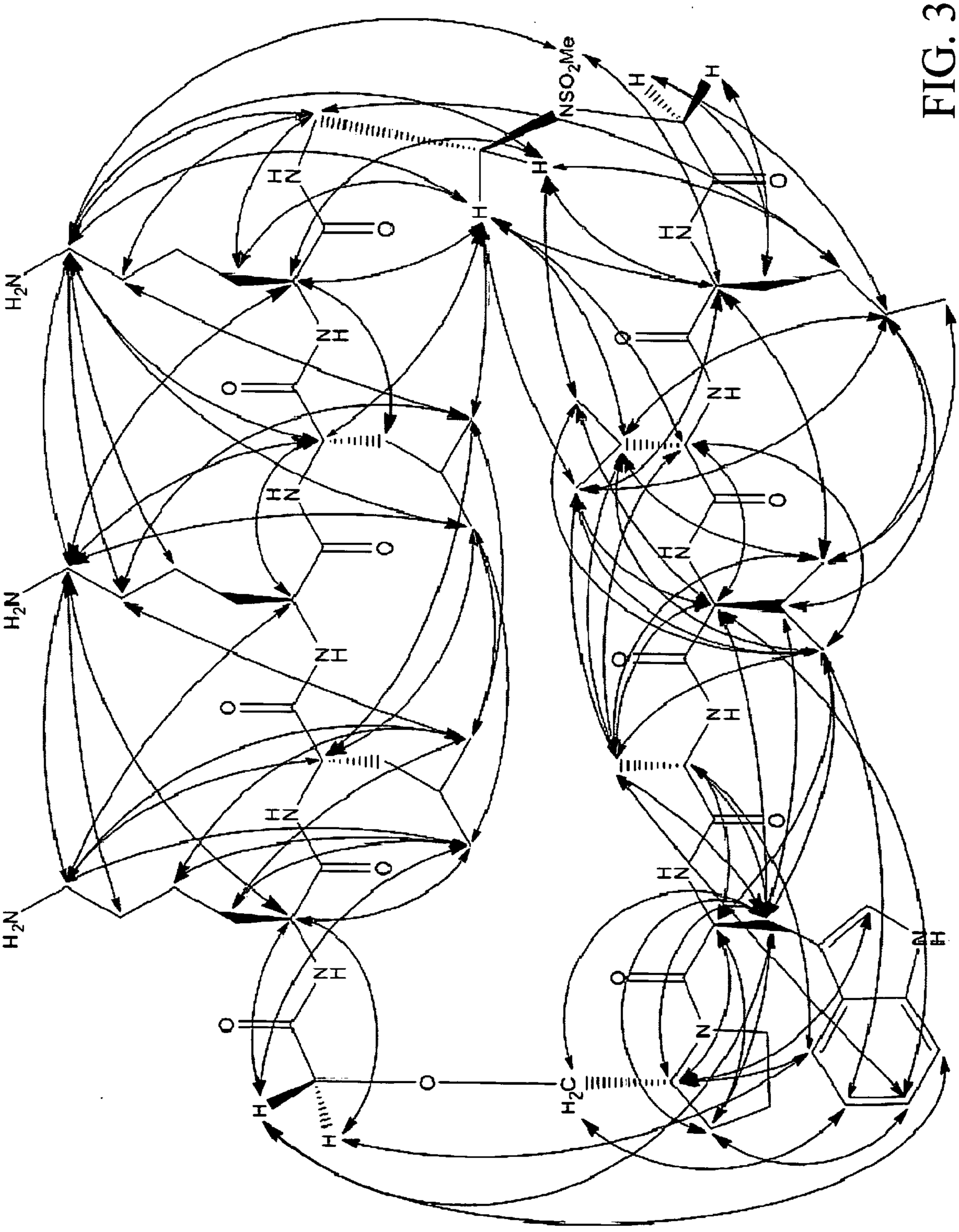

NSO2Me
H
H
I
I
I
IZ
O
H2N
ZI
O
IZ
O
ZI
IZ
O
H2N
ZI
O
IZ
O
H2N
ZI
O
IZ
O
ZI
O
IZ
O
ZI
ZH
O
Z
O
H2C
H
H

FIG. 37A

FIG. 37B



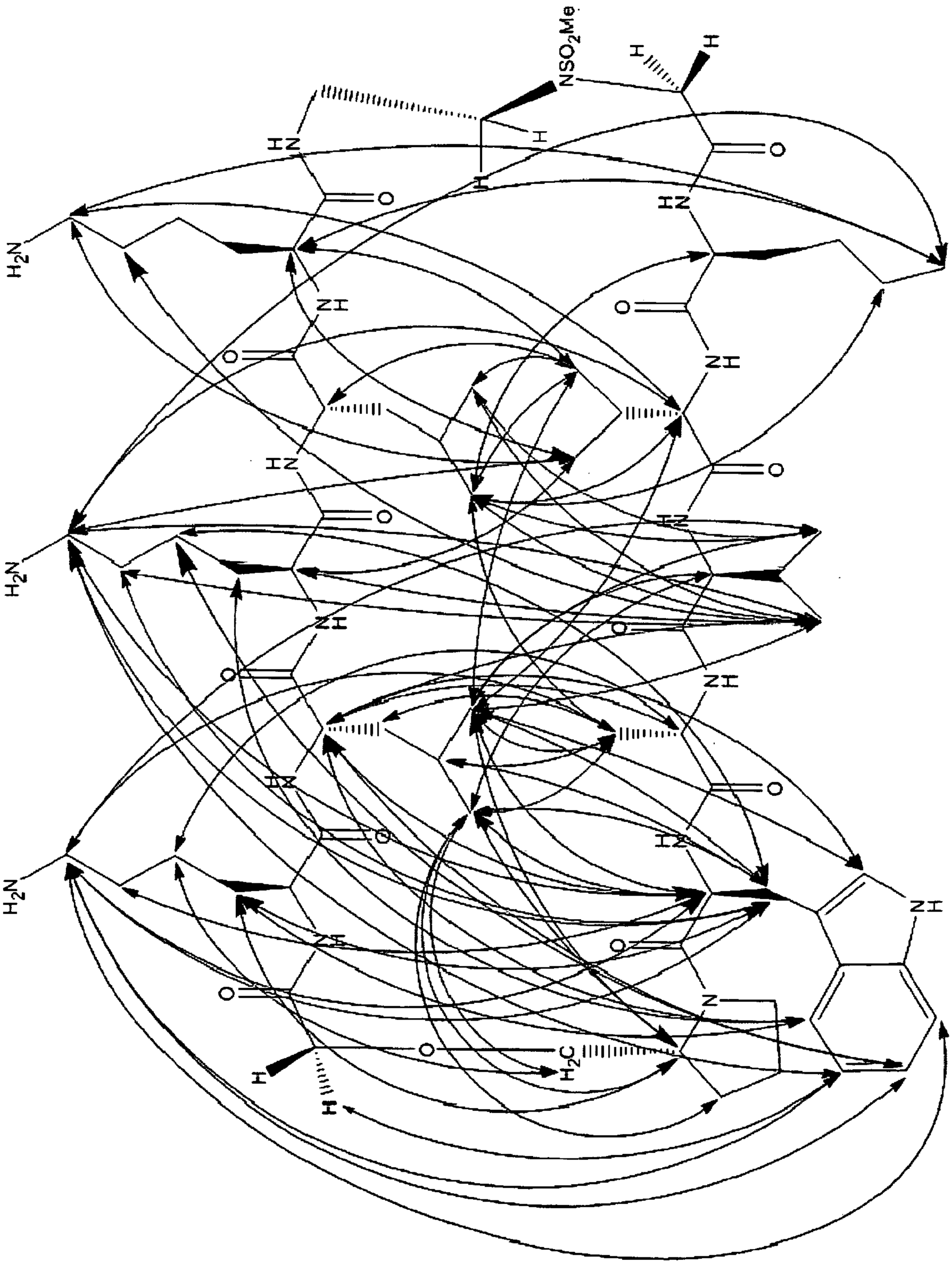


FIG. 37C

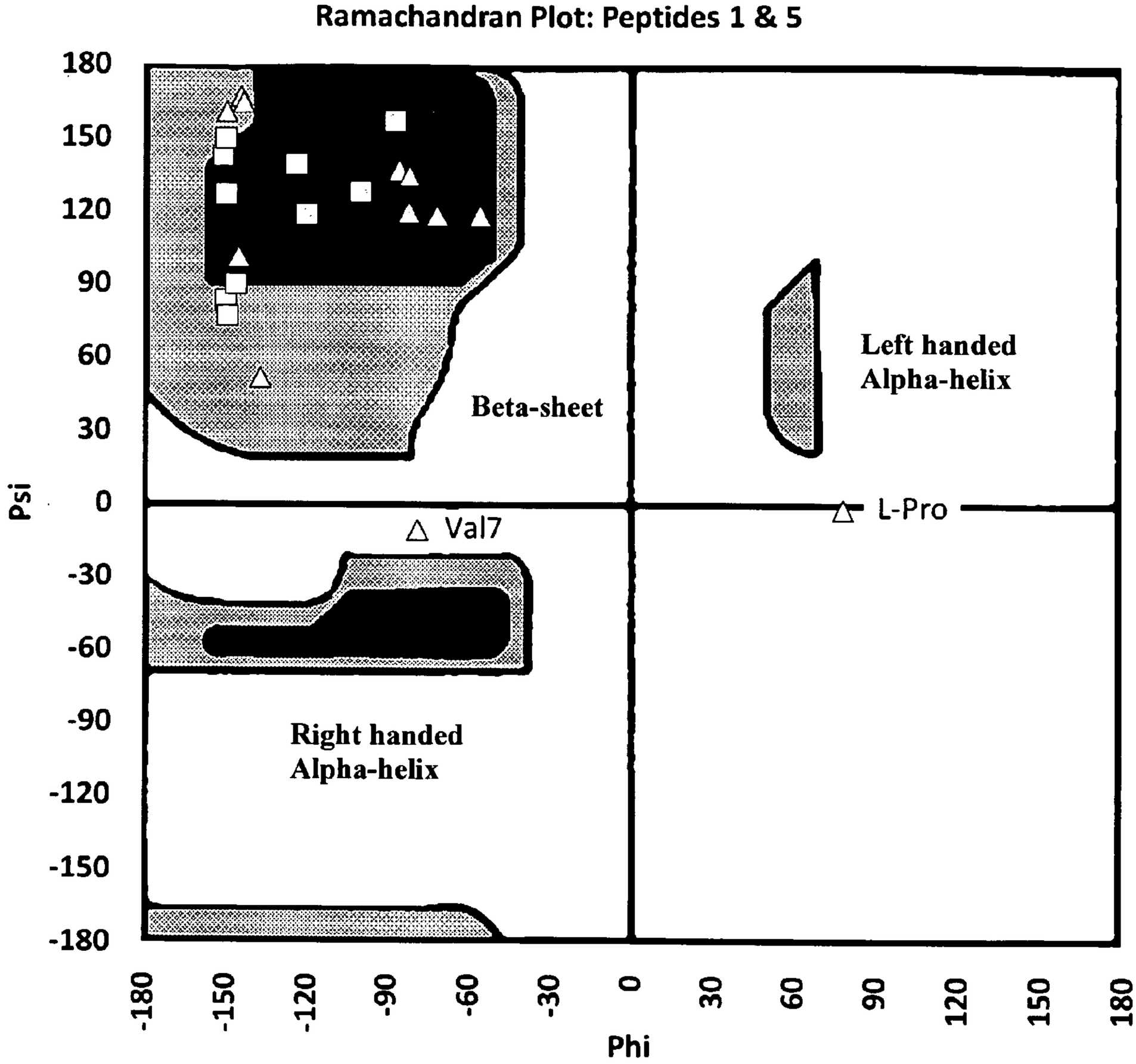

Ramachandran Plot: Peptides 1 & 5
180
150
120
90
60
30
0
-30
-60
-90
-120
-150
-180
Psi
Beta-sheet
Left handed
Alpha-helix
L-Pro
Val7
Right handed
Alpha-helix
-180
-150
-120
-90
-60
-30
0
30
60
90
120
150
180
Phi
Peptide 1
Peptide 5

FIG. 38

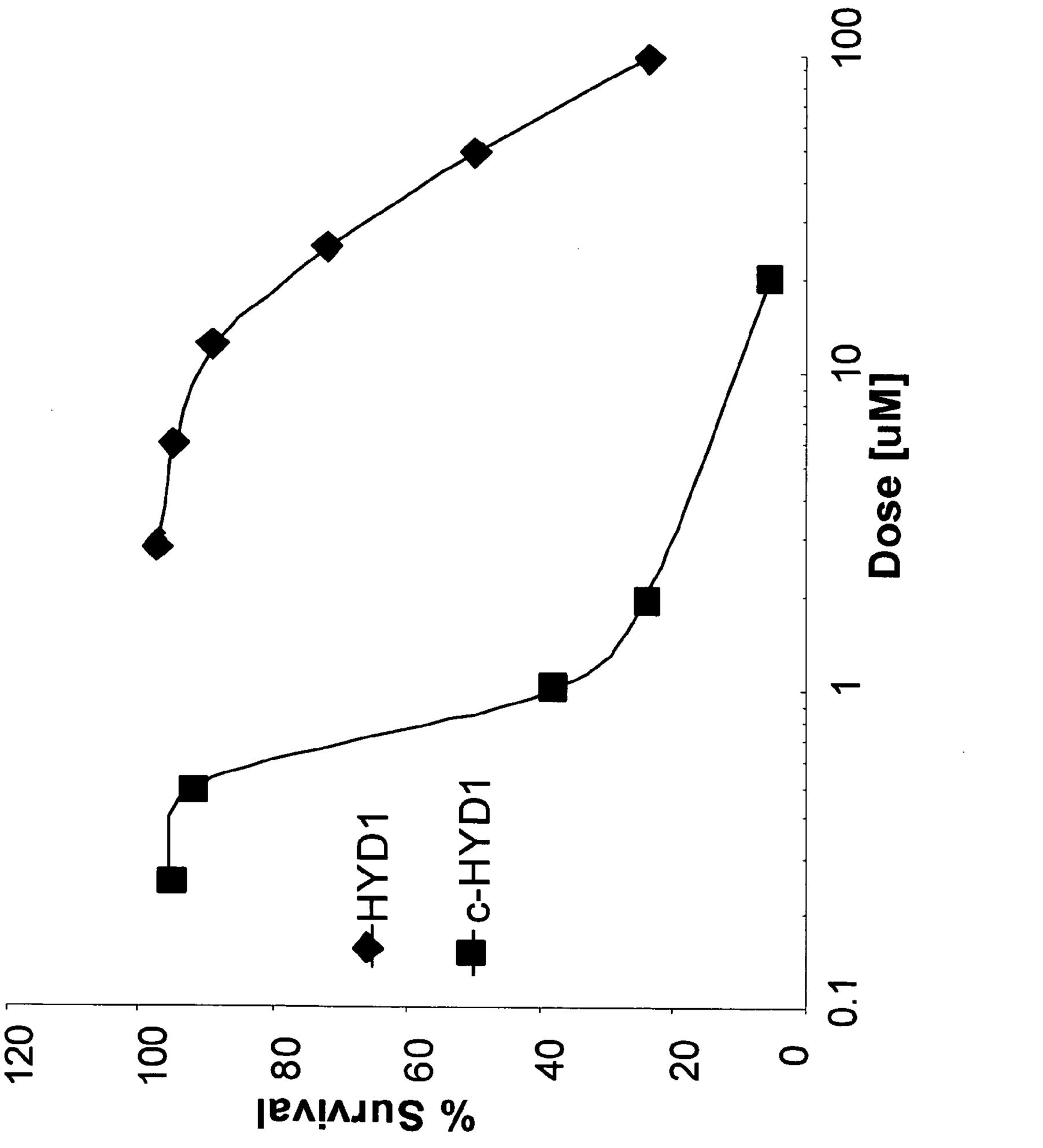


FIG. 39

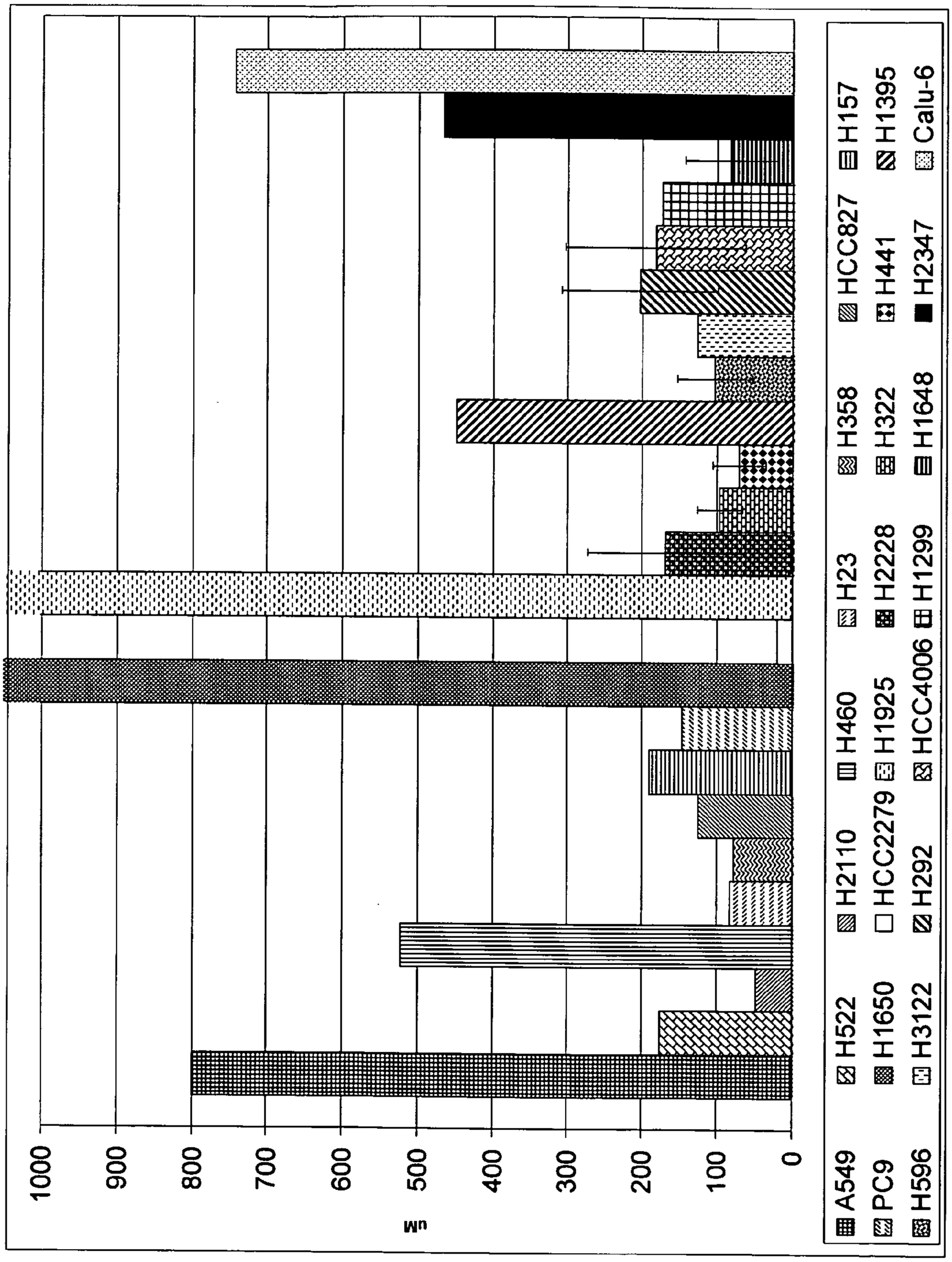

H358
HCC827
H157
H322
H441
H1395
H1648
H2347
Calu-6
H522
H2110
H460
H23
H1650
HCC2279
H1925
H2228
H3122
H292
HCC4006
H1299
A549
PC9
H596
1000
900
800
700
600
500
400
300
200
100
0
uM

FIG. 40

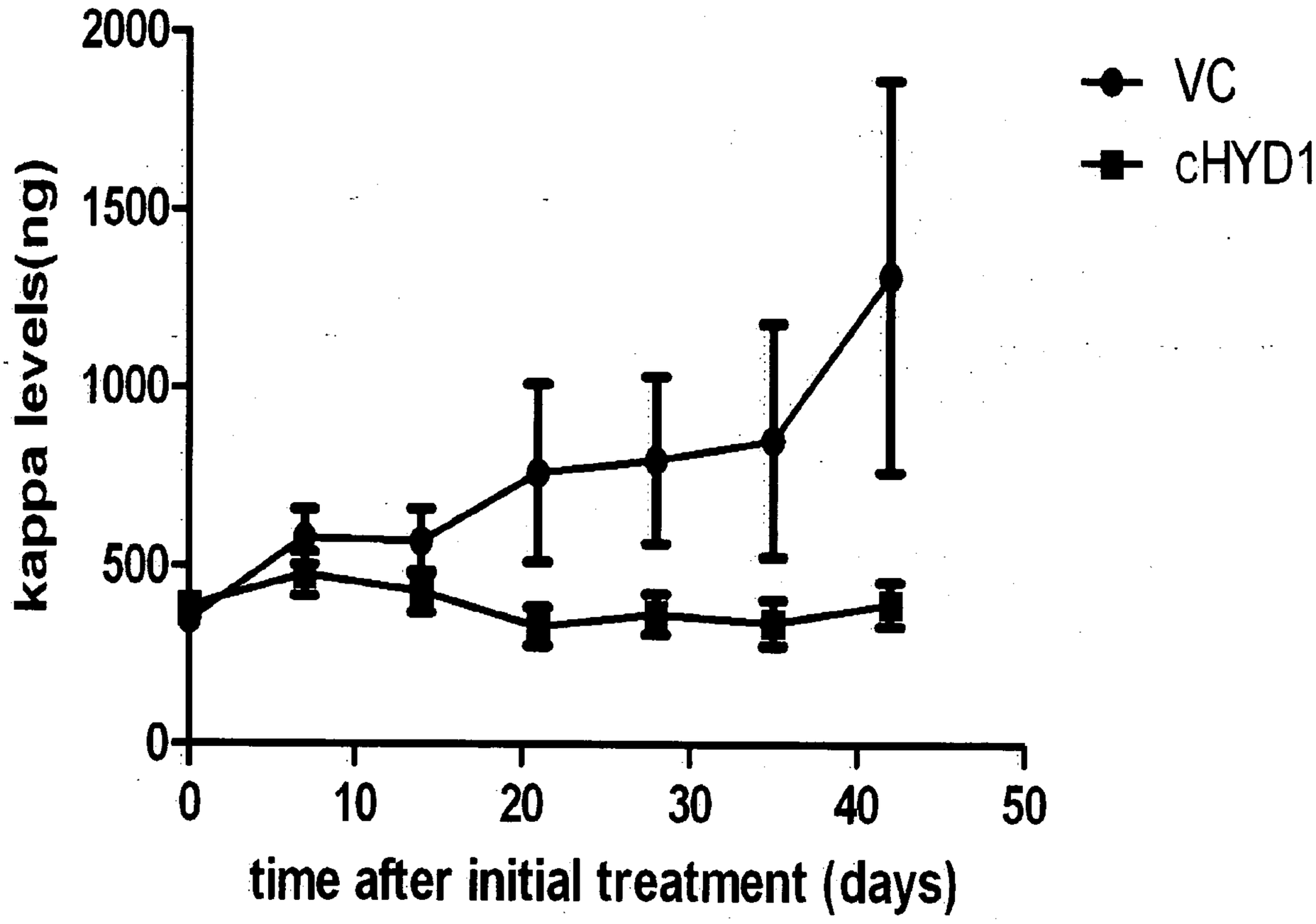

2000
1500
1000
500
0
kappa levels(ng)
0
10
20
30
40
50
time after initial treatment (days)
VC
cHYD1

FIG. 41

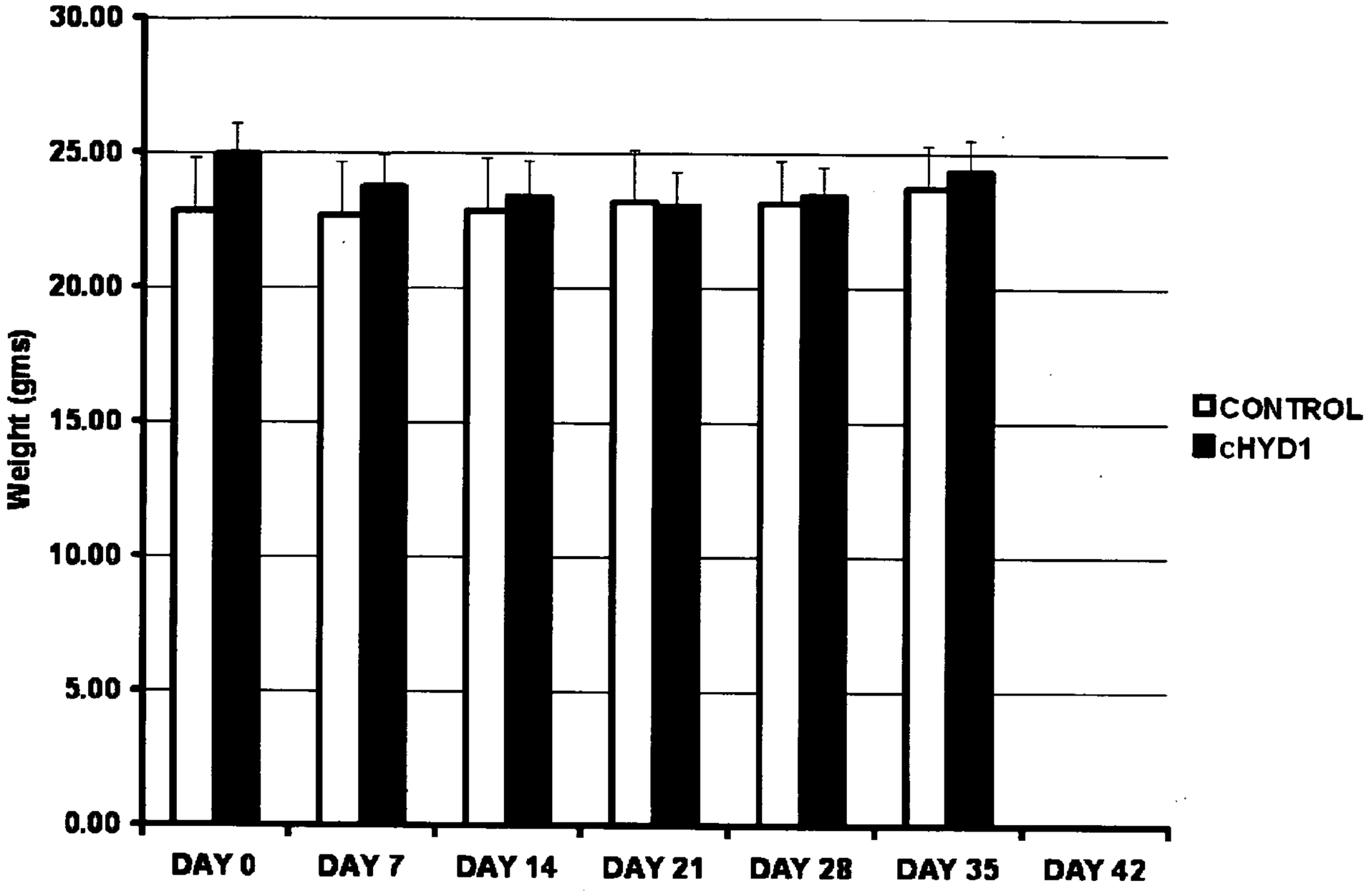

30.00
25.00
20.00
15.00
10.00
5.00
0.00
Weight (gms)
DAY 0
DAY 7
DAY 14
DAY 21
DAY 28
DAY 35
DAY 42
CONTROL
cHYD1

FIG. 42

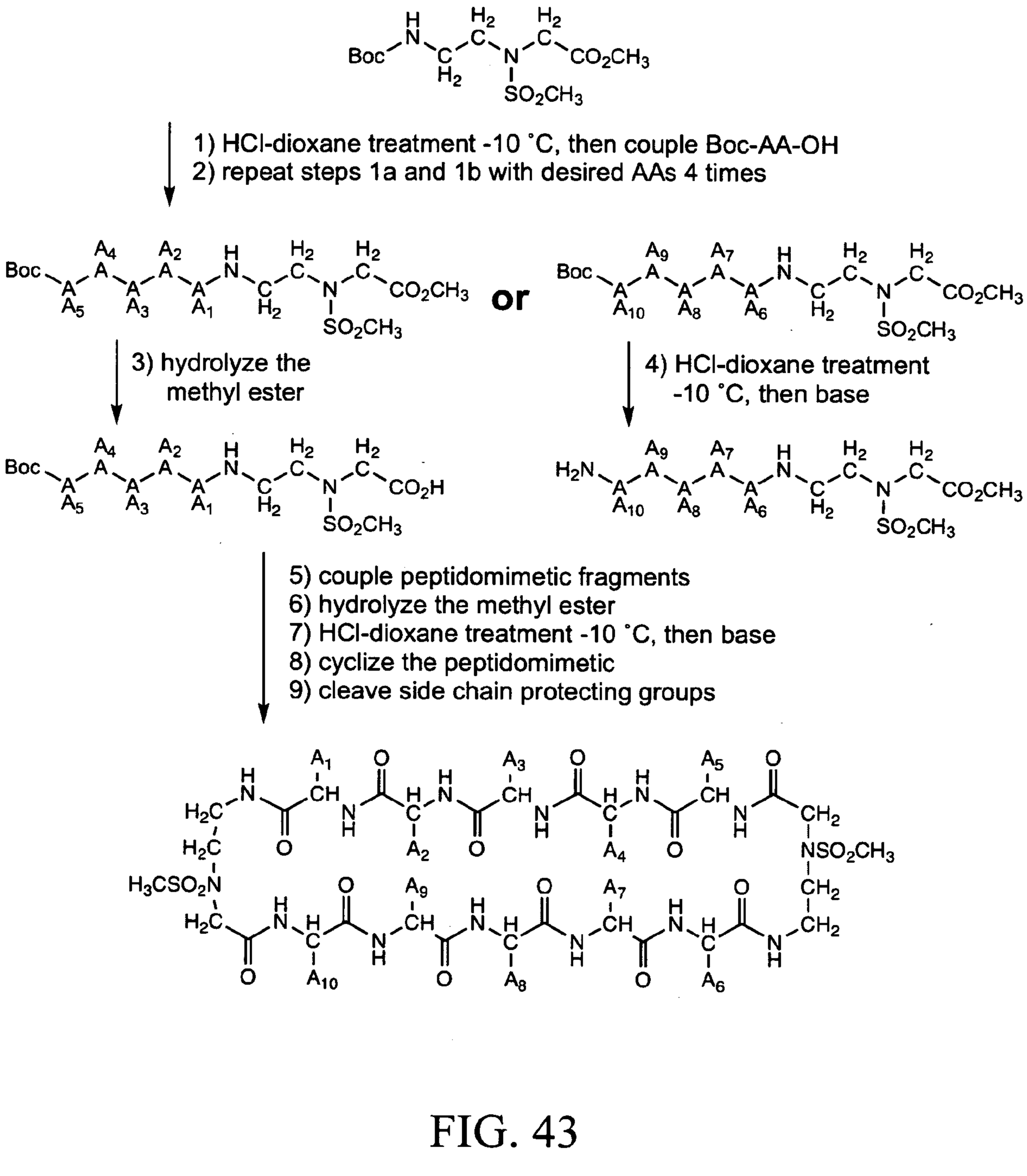

1) HCl-dioxane treatment -10 ˚C, then couple Boc-AA-OH
2) repeat steps 1a and 1b with desired AAs 4 times
or
3) hydrolyze the methyl ester
4) HCl-dioxane treatment -10 ˚C, then base
5) couple peptidomimetic fragments
6) hydrolyze the methyl ester
7) HCl-dioxane treatment -10 ˚C, then base
8) cyclize the peptidomimetic
9) cleave side chain protecting groups

FIG. 43

Membrane Lysate	+	+	+
Biotin	-	+	-
Biotin-HYD1	-	-	+
NeutrAvidin Beads	-	+	+

FIG. 44

Membrane Lysate	+	+	+
Biotin	-	+	-
Biotin-HYD1	-	-	+
NeutrAvidin Beads	-	+	+

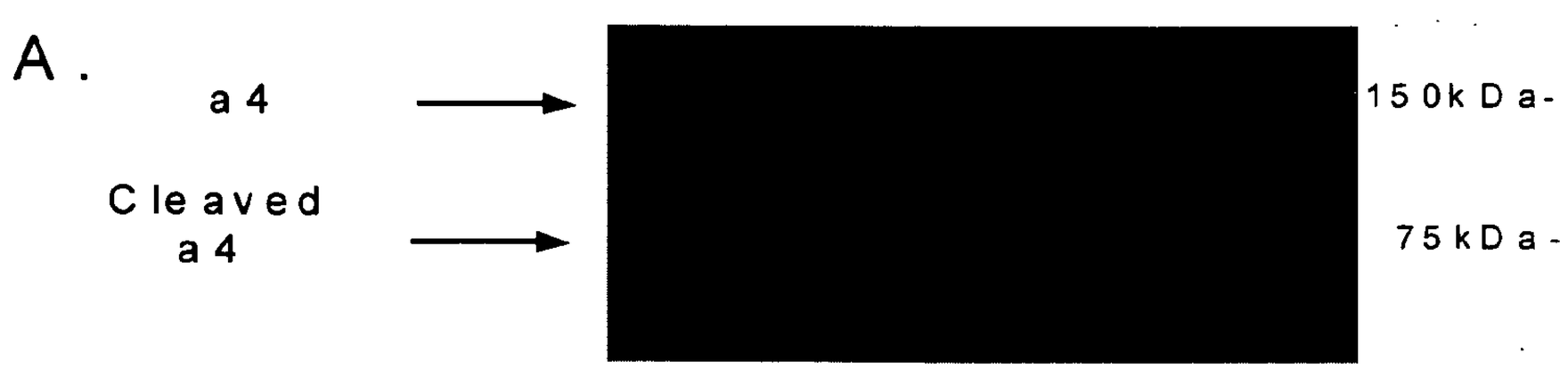


FIG. 45

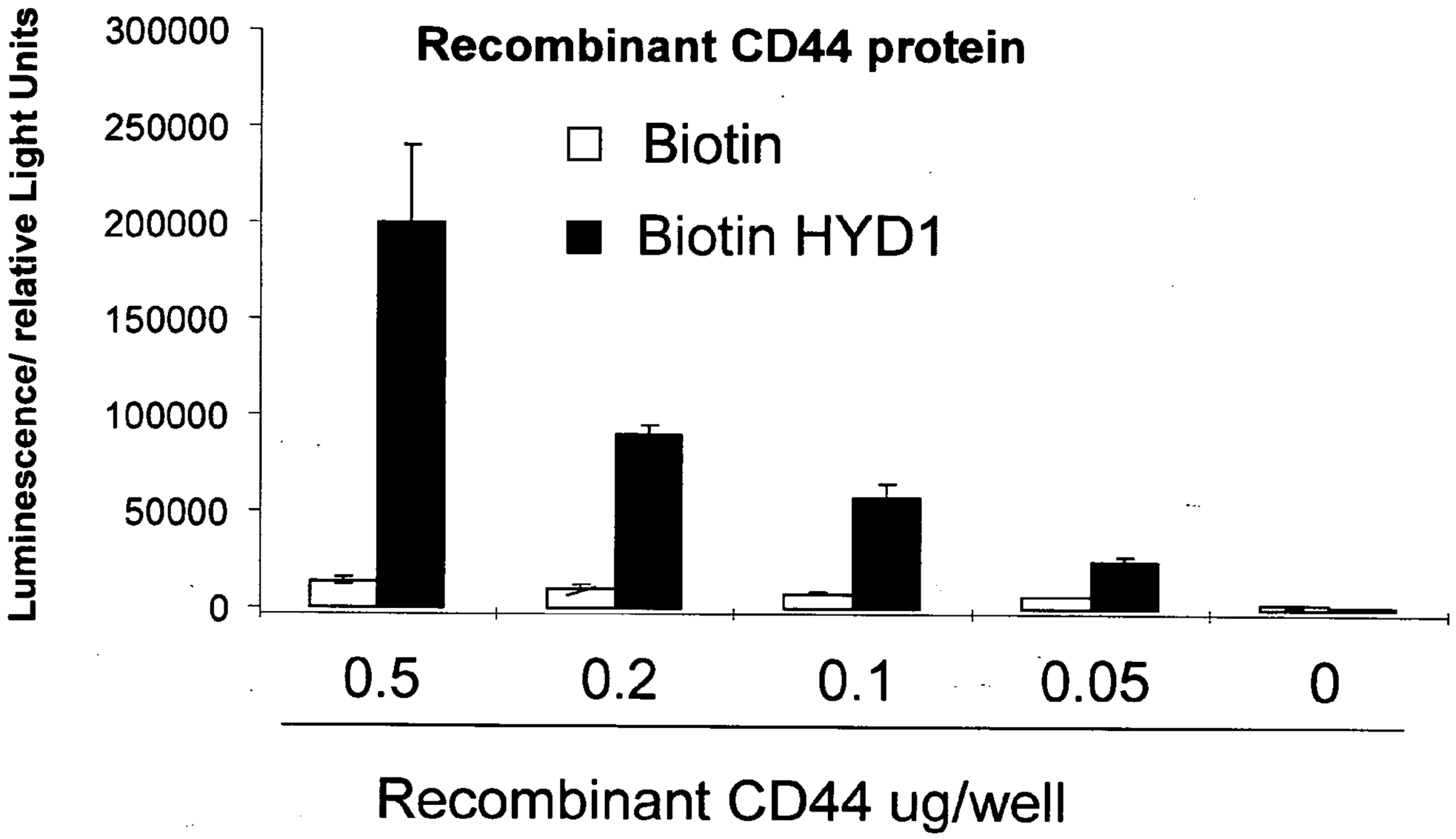

Recombinant CD44 protein
Biotin
Biotin HYD1
Luminescence/ relative Light Units
300000
250000
200000
150000
100000
50000
0
0.5
0.2
0.1
0.05
0
Recombinant CD44 ug/well

FIG. 46