



(86) Date de dépôt PCT/PCT Filing Date: 2014/01/21
(87) Date publication PCT/PCT Publication Date: 2014/07/24
(85) Entrée phase nationale/National Entry: 2015/07/15
(86) N° demande PCT/PCT Application No.: US 2014/012337
(87) N° publication PCT/PCT Publication No.: 2014/113794
(30) Priorité/Priority: 2013/01/19 (US61/754,575)

(51) Cl.Int./Int.Cl. *C07D 403/06* (2006.01),
A61K 31/496 (2006.01), *A61K 45/06* (2006.01)

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(54) Titre : OLIGOOXOPIPERAZINES POUR LA REACTIVATION DU P53
(54) Title: OLIGOOXOPIPERAZINES FOR P53 REACTIVATION

(57) Abrégé/Abstract:

The present invention relates to oligooxopiperazines for reactivating p53. Methods of using the oligooxopiperazines are also disclosed.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
24 July 2014 (24.07.2014)

(10) International Publication Number
WO 2014/113794 A3

(51) International Patent Classification:

A61K 31/497 (2006.01)

(21) International Application Number:

PCT/US2014/012337

(22) International Filing Date:

21 January 2014 (21.01.2014)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

61/754,575 19 January 2013 (19.01.2013) US

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(81) Designated States (unless otherwise indicated, for every

kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every

kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

(88) Date of publication of the international search report:

12 September 2014

(54) Title: OLIGOOXOPIPERAZINES FOR P53 REACTIVATION

(57) Abstract: The present invention relates to oligooxopiperazines for reactivating p53. Methods of using the oligooxopiperazines are also disclosed.



WO 2014/113794 A3

OLIGOOXOPIPERAZINES FOR p53 REACTIVATION

[0001] This application claims the priority benefit of U.S. Provisional Patent Application Serial No. 61/754,575, filed January 19, 2013, which is hereby incorporated by reference in its entirety.

5 [0002] This invention was made with government support under grant numbers R01CA135096 and R01GM073943 awarded by the National Institutes of Health. The government has certain rights in this invention.

FIELD OF THE INVENTION

10 [0003] This invention is directed generally to oligooxopiperazines for targeting the E6-p300 interaction.

BACKGROUND OF THE INVENTION

[0004] Human papillomaviruses (HPV) are small, double-stranded DNA viruses that infect the epithelium. More than 100 HPV types have been identified. They are differentiated by the genetic sequence of the outer capsid protein L1. Most
15 HPV types infect the cutaneous epithelium and cause common skin warts. About 40 types infect the mucosal epithelium; these are categorized according to their epidemiologic association with cervical cancer. Infection with low-risk, or nononcogenic types, such as types 6 and 11, can cause benign or low-grade cervical cell abnormalities, genital warts and laryngeal papillomas. High-risk, or oncogenic,
20 HPV types act as carcinogens in the development of cervical cancer and other anogenital cancers. High-risk types (currently including types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 69, 73, 82) can cause low-grade cervical cell abnormalities, high-grade cervical cell abnormalities that are precursors to cancer, and anogenital cancers. High-risk HPV types are detected in 99% of cervical cancers. Type 16 is the
25 cause of approximately 50% of cervical cancers worldwide, and types 16 and 18 together account for about 70% of cervical cancers.

[0005] Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer with approximately 600,000 new cases worldwide (Kamangar et al., "Patterns of Cancer Incidence, Mortality, and Prevalence Across Five Continents:
30 Defining Priorities to Reduce Cancer Disparities in Different Geographic Regions of

- 2 -

the World,” *J. Clin. Oncol.* 24(14):2137–50 (2006)). HPV infection is recognized as a major risk factor for the development of a subset of HNSCC, oropharyngeal SCC. HPV16 is the most prevalent subtype and accounts for ~90% of HPV-positive HNSCC (Gillison et al., “Evidence for a Causal Association Between Human Papillomavirus and a Subset of Head and Neck Cancers,” *J. Nat’l Cancer Inst.* 92(9):709–20 (2000); Klusmann et al., “Expression of p16 Protein Identifies a Distinct Entity of Tonsillar Carcinomas Associated With Human Papillomavirus,” *Am. J. Pathol.* 162(3):747–53 (2003)). Epidemiological data indicate that the prevalence of HPV-positive HNSCC has increased by ~3-fold in the past three decades in the United States and Europe (Licitra et al., “Advances in the Changing Patterns of Aetiology of Head and Neck Cancers,” *Curr. Opin. Otolaryngol. Head Neck Surg.* 14(2):95–99 (2006); Shiboski et al., “Tongue and Tonsil Carcinoma: Increasing Trends in the U.S. Population Ages 20–44 Years,” *Cancer* 103(9):1843–49 (2005); Sturgis & Cinciripini, “Trends in Head and Neck Cancer Incidence in Relation to Smoking Prevalence: An Emerging Epidemic of Human Papillomavirus-Associated Cancers?” *Cancer* 110(7):1429–35 (2007)). Data obtained from the Swedish Cancer Registry showed a 2.8-fold increase in the incidence of oropharyngeal SCC in the Stockholm area between 1970 and 2002. Interestingly, over the same time period, the incidence of HPV-positive oropharyngeal SCC increased by ~3-fold from 23% in the 1970s to 68% in the 2000s (Hammarstedt et al., “Human Papillomavirus as a Risk Factor for the Increase in Incidence of Tonsillar Cancer,” *Int’l J. Cancer* 119(11):2620–23 (2006). Based on these alarming numbers, it has been suggested that an epidemic of HPV-positive HNSCC will emerge in the near future (Sturgis & Cinciripini, “Trends in Head and Neck Cancer Incidence in Relation to Smoking Prevalence: An Emerging Epidemic of Human Papillomavirus-Associated Cancers?” *Cancer* 110(7):1429–35 (2007); Hammarstedt et al., “Human Papillomavirus as a Risk Factor for the Increase in Incidence of Tonsillar Cancer,” *Int’l J. Cancer* 119(11):2620–23 (2006)).

[0006] There is concrete clinical data that the HPV vaccine, Gardasil, protects against HPV-positive cervical, vaginal, and vulvar carcinomas (Group FIS, “Quadrivalent Vaccine Against Human Papillomavirus to Prevent High-Grade Cervical Lesions,” *N. Engl. J. Med.* 356(19):1915–27 (2007)). It is assumed that the HPV vaccine will protect against HPV-positive HNSCC; however, there is no clinical

- 3 -

evidence to support this expectation. The HPV vaccine uptake in females has been modest even though the Centers for Disease Control and Prevention issued a recommendation to vaccinate females, between the ages of 9 to 26, for high-risk HPV in 2006. A study using the 2010 National Health Interview Survey showed that only about 30% and 15% of eligible females received one dose and the full three-dose series of the HPV vaccine, respectively (Laz et al., “An Update on Human Papillomavirus Vaccine Uptake Among 11–17 Year Old Girls in the United States: National Health Interview Survey, 2010,” *Vaccine* 30(24):3534–40 (2012)). Gardasil was approved for males, 9 to 26 years old, in 2009; however, vaccine uptake was reported to be extremely poor at 2% (Reiter et al., “HPV Vaccine and Adolescent Males,” *Vaccine* 29(34):5595–602 (2012)). It is clear that a significant number of age eligible females and males are not vaccinated and may remain unprotected against HPV-positive carcinomas, including HNSCC, over their lifetime. Gardasil was shown to be highly effective to protect against cervical carcinoma for HPV-infection naïve individuals but provided much more limited benefit to individuals already exposed to high-risk HPV, including HPV16 (Munoz et al., “Impact of Human Papillomavirus (HPV)-6/11/16/18 Vaccine on All HPV-Associated Genital Diseases in Young Women,” *J. Nat’l Cancer Inst.* 102(5):325–39 (2010); Sigurdsson et al., “The Efficacy of HPV 16/18 Vaccines on Sexually Active 18–23 Year Old Women and the Impact of HPV Vaccination on Organized Cervical Cancer Screening,” *Acta Obstet. Gynecol. Scand.* 88(1):27–35 (2009)). HPV vaccination is not recommended for adults >26 years old since these individuals are likely to be exposed to high-risk HPV already. Therefore, several generations of individuals already exposed to high-risk HPV or are >26 years old will not be vaccinated routinely or even if vaccinated will have minimal protection against HPV-positive carcinomas, including HNSCC. In light of these points, there is a clinical need to develop alternative therapeutic strategies to manage an anticipated growing number of HPV-positive HNSCC patients.

[0007] In contrast to HPV-negative HNSCC, p53 is predominantly wildtype in HPV-positive HNSCC (Balz et al., “Is the p53 Inactivation Frequency in Squamous Cell Carcinomas of the Head and Neck Underestimated? Analysis of p53 Exons 2–11 and Human Papillomavirus 16/18 E6 Transcripts in 123 Unselected Tumor Specimens,” *Cancer Res.* 63(6):1188–91 (2003); Agrawal et al., “Exome Sequencing

- 4 -

of Head and Neck Squamous Cell Carcinoma Reveals Inactivating Mutations in NOTCH1,” *Science* 333(6046):1154–57 (2011); Stransky et al., “The Mutational Landscape of Head and Neck Squamous Cell Carcinoma,” *Science* 333(6046):1157–60 (2011)). However, high-risk HPV E6 inactivates p53 through two distinct mechanisms. E6 associates with E6AP to degrade p53 through the proteasome pathway and associates with p300 to block p300-mediated p53 acetylation (Huibregtse et al., “A Cellular Protein Mediates Association of p53 With the E6 Oncoprotein of Human Papillomavirus Types 16 or 18,” *EMBO J.* (13):4129–35 (1991); Scheffner et al., “The HPV-16 E6 and E6-AP Complex Functions as a Ubiquitin-Protein Ligase in the Ubiquitination of p53,” *Cell* 75(3):495–505 (1993); Talis et al., “The Role of E6AP in the Regulation of p53 Protein Levels in Human Papillomavirus (HPV)-Positive and HPV-Negative Cells,” *J. Biol. Chem.* 273(11):6439–45 (1998); Zimmermann et al., “The Human Papillomavirus Type 16 E6 Oncoprotein Can Down-Regulate p53 Activity by Targeting the Transcriptional Coactivator CBP/p300,” *J. Virol.* 73(8):6209–19 (1999); Patel et al., “The E6 Protein of Human Papillomavirus Type 16 Binds to and Inhibits Co-Activation by CBP and p300,” *EMBO J.* 18(18):5061–72 (1999); Thomas & Chiang, “E6 Oncoprotein Represses p53-Dependent Gene Activation Via Inhibition of Protein Acetylation Independently of Inducing p53 Degradation,” *Mol. Cell* 17(2):251–64 (2005)). Acetylation of p53 enhances p53 stability, and transcriptional activity (Zimmermann et al., “The Human Papillomavirus Type 16 E6 Oncoprotein Can Down-Regulate p53 Activity by Targeting the Transcriptional Coactivator CBP/p300,” *J. Virol.* 73(8):6209–19 (1999); Patel et al., “The E6 Protein of Human Papillomavirus Type 16 Binds to and Inhibits Co-Activation by CBP and p300,” *EMBO J.* 18(18):5061–72 (1999); Thomas & Chiang, “E6 Oncoprotein Represses p53-Dependent Gene Activation Via Inhibition of Protein Acetylation Independently of Inducing p53 Degradation,” *Mol. Cell* 17(2):251–64 (2005); Ito et al., “MDM2-HDAC1-Mediated Deacetylation of p53 Is Required for Its Degradation,” *EMBO J.* 21(22):6236–45 (2002); Li et al., “Acetylation of p53 Inhibits Its Ubiquitination by Mdm2,” *J. Biol. Chem.* 277(52):50607–11 (2002)). Inactivation of p53 by E6 is indispensable for HPV-mediated tumorigenesis suggesting that reactivation of p53 may be a strategy to ablate HPV-positive carcinoma cells. Several genetic and chemical strategies to reactivate p53 have been demonstrated in HPV-positive cervical carcinomas. Most of

- 5 -

these approaches focused on targeting E6 levels, E6AP levels, or E6-E6AP association to increase p53 stability and accumulation (Beerheide et al., "Potential Drugs Against Cervical Cancer: Zinc-Ejecting Inhibitors of the Human Papillomavirus Type 16 E6 Oncoprotein," *J. Nat'l Cancer Inst.* 91(14):1211–20 (1999); Beerheide et al., "Inactivation of the Human Papillomavirus-16 E6 Oncoprotein by Organic Disulfides," *Bioorg. Med. Chem.* 8(11):2549–60 (2000); Courtete et al., "Suppression of Cervical Carcinoma Cell Growth by Intracytoplasmic Codelivery of Anti-Oncoprotein E6 Antibody and Small Interfering RNA," *Mol. Cancer Ther.* 6(6):1728–35 (2007); Beer-Romero et al., "Antisense Targeting of E6AP Elevates p53 in HPV-Infected Cells but Not in Normal Cells," *Oncogene* 14(5):595–602 (1997); Koivusalo et al., "Activation of p53 in Cervical Cancer Cells by Human Papillomavirus E6 RNA Interference Is Transient, but Can Be Sustained by Inhibiting Endogenous Nuclear Export-Dependent p53 Antagonists," *Cancer Res.* 66(24):11817–24 (2006); Zhao et al., "Rescue of p53 Function by Small-Molecule RITA in Cervical Carcinoma by Blocking E6-Mediated Degradation," *Cancer Res.* 70(8):3372–81 (2010).

[0008] There is a clinical need to develop alternate therapeutic strategies to manage the growing number of HPV-positive HNSCC patients (and those with other HPV-associated cancers). The present invention is directed to overcoming these and other deficiencies in the art.

SUMMARY OF THE INVENTION

[0009] A first aspect of the present invention relates to a method of treating or preventing in a subject a disorder mediated by interaction of E6 with CREB-binding protein and/or p300, the method comprising administering to the subject an oligooxopiperazine that substantially mimics helix α B of the C-terminal transactivation domain of Hypoxia-Inducible Factor 1 α under conditions effective to treat or prevent the disorder.

[0010] A second aspect of the present invention relates to a method of inducing apoptosis of a cell, the method comprising contacting the cell with an oligooxopiperazine that substantially mimics helix α B of the C-terminal transactivation domain of HIF1 α under conditions effective to induce apoptosis of the cell.

- 6 -

[0011] A third aspect of the present invention relates to a method of decreasing survival and/or proliferation of a cell, the method comprising contacting the cell with an oligooxopiperazine that substantially mimics helix α B of the C-terminal transactivation domain of HIF1 α under conditions effective to decrease survival and/or proliferation of the cell.

[0012] A fourth aspect of the present invention relates to a method of preventing or reversing inactivation of p53 in a cell, the method comprising contacting the cell with an oligooxopiperazine that substantially mimics helix α B of the C-terminal transactivation domain of HIF1 α under conditions effective to prevent or reverse inactivation of p53 in a cell.

[0013] A fifth aspect of the present invention relates to a method of inhibiting p300-mediated acetylation of a transcription factor in a cell, the method comprising contacting the cell with an oligooxopiperazine that substantially mimics helix α B of the C-terminal transactivation domain of HIF1 α under conditions effective to inhibit p300-mediated acetylation of the transcription factor in the cell.

[0014] In this study, we took a novel approach and functionally reactivated p53 in HPV-positive HNSCC by blocking the interaction between E6 and p300. Ectopic expression of the CH1 domain of p300 squelched E6 to disrupt E6-p300 association resulting in an increase in p53 acetylation, accumulation, and activity. Exogenous CH1 promoted a pleiotropic, anti-cancer effect in HPV-positive HNSCC partly due to a reduction in the cancer initiating cell (CIC) population. CH1 domain inhibitors reactivated p53 and dramatically potentiated the efficacy of cis-platinum in HPV-positive HNSCC. Taken together, our work revealed a novel druggable approach to reactivate p53 in HPV-positive HNSCC that is expected to translate to other HPV-positive carcinomas.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] Figures 1A–C are analytical HPLC traces of the CH1 inhibitors CH1iA (Figure 1A), CH1iB (Figure 1B), and CH1iB-mut (Figure 1C).

[0016] Figures 2A–E demonstrate that exogenous CH1 reactivates p53 by blocking the association between HPV16 E6 and p300. Stable polyclonal UMSCC47/empty, UMSCC47/CH1, SCC90/empty, and SCC90/CH1 cells were generated by transfection and antibiotic selection. Figure 2A is a pair of western blots

- 7 -

showing HPV16 E6-p300 association. Cell lysates were extracted, immunoprecipitated with anti-E6 (left panel) or anti-p300 (right panel) antibody, and immunoblotted with anti-V5, anti-E6, or anti-p300 antibody. Cell lysates were immunoblotted with anti-V5, anti-E6, or anti-p300 antibody for input control. Figure 2B is a western blot of total and acetylated p53 levels. Cell lysates were immunoblotted with anti-p53 or anti-acetylated[K382]-p53 antibody. Figure 2C is a graph of p53 transcriptional activity. Cells were co-transfected with a p53 Firefly luciferase reporter plasmid and a control Renilla luciferase plasmid. Firefly luciferase activity was normalized to Renilla luciferase activity to control for transfection efficiency. Data is normalized to empty vector cells and presented as mean $\pm$ SEM. *P<0.01, n=4. Figure 2D is a pair of graphs of p300 and p53 expression in UMSCC47 (top panel) and SCC90 (bottom panel) cells. Figure 2E is a pair of graphs of p21, miR-34a, and miR-200c expression in UMSCC47 (top panel) and SCC90 (bottom panel) cells. mRNA expression was determined using qPCR with validated TaqMan assays. Data is normalized to empty vector control cells and presented as mean $\pm$ SEM. *P<0.01, n=3.

[0017] Figures 3A–H demonstrate that exogenous CH1 has a pleiotropic anti-tumor effect in HPV16-positive HNSCC cells. Figure 3A is a pair of graphs of cell proliferation. UMSCC47 (left panel) and SCC90 (right panel) cells were treated with control (vehicle) or cis-platinum (10 μ M) for 24, 48, and 72 hours. Data is normalized to Day 0 and presented as mean $\pm$ SEM. *P<0.01, control vs. CH1, cis-platinum, or CH1 + cis-platinum, **P<0.01 CH1 or cis-platinum vs. CH1 + cis-platinum, n=6. Figure 3B is a graph of clonogenic survival. UMSCC47 (left panel) and SCC90 (right panel) were treated with control (vehicle) or cis-platinum (10 μ M). Colonies were stained with crystal violet. Data is normalized to empty/control cells and presented as mean $\pm$ SEM. *P<0.01, control vs. CH1, cis-platinum, or CH1 + cis-platinum, **P<0.01 CH1 or cis-platinum vs. CH1 + cis-platinum, n=3. Figure 3C is a graph of apoptosis. UMSCC47 (left panel) and SCC90 (right panel) were treated with control (vehicle) or cis-platinum (10 μ M). FACS was used to quantitate Annexin V-positive apoptotic cells. Data is presented as mean $\pm$ SEM. *P<0.01, control vs. CH1, cis-platinum, or CH1 + cis-platinum, **P<0.01 CH1 or cis-platinum vs. CH1 + cis-platinum, n=3. Figure 3D is a table of *in vivo* tumor incidence. Two different dilutions, 3×10^5 or 3×10^4 , of UMSCC47/empty and UMSCC47/CH1 cells were

- 8 -

implanted in the flanks of NOD/SCID mice. Tumor incidence was monitored for 49 days following tumor cell implantation. *P<0.02, n=8. Figure 3E is a graph of *in vivo* tumor growth. Tumors were measured weekly using a digital caliper and tumor volumes were calculated. Data is presented as mean $\pm$ SEM. *P<0.01, n=6. Figure 3F is a pair of graphs of ALDH (left panel) and CD44 (right panel). ALDH^{high} cells were quantitated using the ALDEFLUOR assay. Data is presented as mean $\pm$ SEM. *P<0.01, n=3. CD44 intensity was determined using FACS with an anti-PE-CD44 antibody and presented as a histogram. Figure 3G is a pair of graphs of tumorsphere formation efficiency (left panel) and diameter (right panel). Tumorsphere formation efficiency was calculated as the number of tumorspheres ($\geq 50 \mu\text{m}$ in diameter) formed divided by the original number of cells seeded. Tumorsphere diameter was measured using NIS-Elements software. Data is presented as mean $\pm$ SEM. *P<0.01, n=6. Figure 3H is a pair of images representative of *in vivo* tumor incidence of a single tumorsphere. NOD/SCID mice were implanted with a single UMSCC47 tumorsphere (mean diameter of 60–80 μm with ~ 100 cells) or 1×10^3 UMSCC47 cells. A representative UMSCC47 tumorsphere (left image) and the resulting tumor grown in NOD/SCID mice (right image) are shown. Tumor incidence was monitored over a 6 month period. *P<0.005, n=11 for single UMSCC47 tumorsphere and n=10 for 1×10^3 UMSCC47 cells.

20 [0018] Figures 4A–F demonstrate that exogenous CH1 has a pleiotropic anti-tumor effect in HPV-negative HNSCC. Stable polyclonal UMSCC74A/empty and UMSCC74A/CH1 cells were generated by transfection and antibiotic selection. Figure 4A is a western blot showing total and acetylated p53 levels. Cell lysates were immunoblotted with anti-V5, anti-p53, or anti-acetylated[K382]-p53 antibody. Figure 25 4B is a graph of p53 transcriptional activity. Cells were co-transfected with a p53 Firefly luciferase reporter plasmid and a control Renilla luciferase plasmid. Firefly luciferase activity was normalized to Renilla luciferase activity to control for transfection efficiency. Data is normalized to empty vector cells and presented as mean $\pm$ SEM. *P<0.01, n=4. Figure 4C is a western blot showing MDM2–p300 30 association. Cell lysates were extracted, immunoprecipitated with anti-p300 antibody, and immunoblotted with anti-MDM2. Cell lysates were immunoblotted with anti-MDM2 or anti-p300 antibody for input control. Figure 4D is a graph of cell proliferation. Cells were treated with control (vehicle) or cis-platinum (10 μM) for 24

- 9 -

and 48 hours. Data is normalized to Day 0 and presented as mean $\pm$ SEM. *P<0.01, control vs. CH1 or cis-platinum, **P<0.01 CH1 or cis-platinum vs. CH1 + cis-platinum, n=4. Figure 4E is a graph of clonogenic survival. Cells were treated with control (vehicle) or cis-platinum (10 μ M). Colonies were stained with crystal violet.

5 Data is normalized to empty/control cells and presented as mean $\pm$ SEM. *P<0.01, control vs. CH1, cis-platinum, or CH1 + cis-platinum, **P<0.01 CH1 or cis-platinum vs. CH1 + cis-platinum. Figure 4F is graph of apoptosis. Cells were treated with control (vehicle) or cis-platinum (10 μ M). FACS was used to quantitate Annexin V-positive apoptotic cells. Data is presented as mean $\pm$ SEM. *P<0.05, control vs.

10 CH1, cis-platinum, or CH1 + cis-platinum, **P<0.05 CH1 or cis-platinum vs. CH1 + cis-platinum.

[0019] Figures 5A–G demonstrate that CH1iB preferentially reactivates p53 in HPV16-positive HNSCC. Figure 5A is an schematic illustration showing that CH1 has two distinct target sites. HIF1- α /p300 structures were used as guides to design

15 helix mimetics that target site A and site B on the CH1 domain of p300. Figure 5B shows the structures of the synthetic helices. CH1iA and CH1iB were designed to mimic two helices from the C-terminal domain of HIF-1 α . The peptides were locked into the helical conformation by the hydrogen bond surrogate method. Figure 5C is a graph of p53 activity. UMSCC47 (left) and UMSCC74A (right) cells were co-

20 transfected with a p53 Firefly luciferase reporter plasmid and a control Renilla luciferase plasmid. After 24 hours, cells were treated with control (vehicle), CH1iA (10 μ M), or CH1iB (10 μ M) for 24 hours. Firefly luciferase activity was normalized to Renilla luciferase activity to control for transfection efficiency. Data is normalized to control and presented as mean $\pm$ SEM. *P<0.05, n=3. Figure 5D is a western blot

25 showing total and acetylated p53 levels. UMSCC47 (left) and UMSCC74A (right) cells were treated with control (vehicle), CH1iA (10 μ M), or CH1iB (10 μ M) for 24 hours. Cell lysates were immunoblotted with anti-p53 or anti-acetylated[K382]-p53 antibody. Figure 5E is a pair of graphs of p300, p53, p21, miR-34a, and miR-200c expression. UMSCC47 (top panel) and UMSCC74A (bottom panel) cells were

30 treated with control (vehicle), CH1iA (10 μ M), or CH1iB (10 μ M) for 24 hours. mRNA expression was determined using qPCR with validated TaqMan assays. Data is normalized to control cells and presented as mean $\pm$ SEM. *P<0.01, control vs. CH1iB, n=3. Figure 5F is a western blot showing HPV16 E6-p300 association.

- 10 -

UMSCC47 cells were treated with control (vehicle), CH1iA (10 μ M), or CH1iB (10 μ M) for 24 hours. Cell lysates were extracted, immunoprecipitated with anti-p300 antibody, and immunoblotted with anti-E6 antibody. Cell lysates were immunoblotted with anti-E6 or anti-p300 antibody for input control. Figure 5G is a set of graphs of cell proliferation. UMSCC47 (top left panel), UMSCC74A (top right panel), and IMR90 (human normal fibroblasts) (bottom panel) cells were treated with control (vehicle), CH1iA (10 μ M), CH1iB (10 μ M), cis-platinum (10 μ M), CH1iA (10 μ M) and cis-platinum (10 μ M), or CH1iB (10 μ M) and cis-platinum (10 μ M) for 24, 48, or 72 hours. Data is normalized to Day 0 and presented as mean $\pm$ SEM.

*P<0.01, control vs. CH1iB or cis-platinum, **P<0.01 CH1iB or cis-platinum vs. CH1iB and cis-platinum, n=6.

[0020] Figures 6A–F demonstrate that CH1iB potentiates the efficacy of cis-platinum in HPV16-positive HNSCC. Figure 6A is a western blot showing total and acetylated p53 levels. UMSCC47 cells were treated with control (vehicle), CH1iB (10 μ M), cis-platinum (10 μ M), or CH1iB (10 μ M) and cis-platinum (10 μ M) for 24 hours. Cell lysates were immunoblotted with anti-p53 or anti-acetylated[K382]-p53 antibody. Figure 6B is a graph of p53 transcriptional activity. UMSCC47 cells were co-transfected with a p53 Firefly luciferase reporter plasmid and a control Renilla luciferase plasmid. After 24 hours, cells were treated with control (vehicle), CH1iB (10 μ M), cis-platinum (10 μ M), or CH1iB (10 μ M) and cis-platinum (10 μ M) for 24 hours. Firefly luciferase activity was normalized to Renilla luciferase activity to control for transfection efficiency. Data is normalized to control and presented as mean $\pm$ SEM. *P<0.01, control vs. CH1iB, cis-platinum or CH1iB + cis-platinum, **P<0.01 CH1iB or cis-platinum vs. CH1iB + cis-platinum, n=5. Figure 6C is a graph of p300, p53, p21, miR-34a, and miR-200c expression. UMSCC47 cells were treated with control (vehicle), CH1iB (10 μ M), cis-platinum (10 μ M), or CH1iB (10 μ M) and cis-platinum (10 μ M) for 24 hours. mRNA expression was determined using qPCR with validated TaqMan assays. Data is normalized to control cells and presented as mean $\pm$ SEM. *P<0.01, control vs. CH1iB, cis-platinum or CH1iB + cis-platinum, **P<0.01 CH1iB or cis-platinum vs. CH1iB + cis-platinum, n=3. Figure 6D is a pair of graphs of apoptosis. UMSCC47 cells were treated with control (vehicle), CH1iB (10 μ M), cis-platinum (10 μ M), or CH1iB (10 μ M) and cis-platinum (10 μ M) for 24 hours. FACS was used to quantitate Annexin V-positive apoptotic

- 11 -

cells (left panel). Data is presented as mean $\pm$ SEM. * $P < 0.01$, control vs. CH1iB, cis-platinum or CH1iB + cis-platinum, ** $P < 0.01$ CH1iB or cis-platinum vs. CH1iB + cis-platinum, $n = 3$. Figure 6E is a series of images and a graph of clonogenic survival. UMSCC47 cells were treated with control (vehicle), CH1iB (10 μ M), cis-platinum (10 μ M), or CH1iB (10 μ M) and cis-platinum (10 μ M) at day 0 and colonies were stained with crystal violet at 14 days. Data is normalized to control and presented as mean $\pm$ SEM. * $P < 0.01$, control vs. CH1iB, cis-platinum or CH1iB + cis-platinum, ** $P < 0.01$ CH1iB or cis-platinum vs. CH1iB + cis-platinum, $n = 3$. Figure 6F is a pair of graphs of tumorsphere formation efficiency (top panel) and diameter (bottom panel). UMSCC47 cells were seeded on low-attachment plates and treated with control (vehicle), CH1iB (10 μ M), cis-platinum (3 μ M), or CH1iB (10 μ M) and cis-platinum (3 μ M). Tumorsphere formation efficiency was calculated as the number of tumorspheres (≥ 50 μ m in diameter) formed in 7 days divided by the original number of cells seeded. Tumorsphere diameter was measured using NIS-Elements software. Data is normalized to control and presented as mean $\pm$ SEM. * $P < 0.01$, control vs. CH1iB, cis-platinum or CH1iB + cis-platinum, ** $P < 0.01$ CH1iB or cis-platinum vs. CH1iB + cis-platinum, $n = 8$.

[0021] Figures 7A–B relate to the effect of CH1iB-mut, an inactive analog of CH1iB, on p53 transcriptional activity and cell proliferation. Figure 7A is a graph of p53 transcriptional activity. UMSCC47 cells were co-transfected with a p53 Firefly luciferase reporter plasmid and a control Renilla luciferase plasmid. After 24 hours, cells were treated with control (vehicle) or CH1iB-mut (10 μ M) for 24 hours. Firefly luciferase activity was normalized to Renilla luciferase activity to control for transfection efficiency. Data is presented as mean $\pm$ SEM. * $P < 0.05$, $n = 5$. Figure 7B is a graph of cell proliferation. UMSCC47 cells were treated with control (vehicle), CH1iB-mut (10 μ M), cis-platinum (10 μ M), or CH1iB-mut (10 μ M) and cis-platinum (10 μ M) for 24 or 48 hours. Data is normalized to Day 0 and presented as mean $\pm$ SEM. * $P < 0.01$, control or CH1iB-mut vs. cis-platinum or cis-platinum + CH1iB-mut, $n = 6$.

[0022] Figure 8 is an analytical HPLC trace of BB2-125. Gradient: 5 to 95% acetonitrile/water in 30 minutes.

[0023] Figure 9 is an analytical HPLC trace of BB2-162. Gradient: 5 to 95% acetonitrile/water in 30 minutes.

- 12 -

[0024] Figure 10 is an analytical HPLC trace of BB2-164. Gradient: 5 to 95% acetonitrile/water in 30 minutes.

[0025] Figure 11 is a graph of p53 transcriptional activity. UMSCC47 cells were co-transfected with a p53 Firefly luciferase reporter plasmid and a control Renilla luciferase plasmid. After 24 hours, cells were treated with control (vehicle), CH1iB (10 μ M), or BB2-162 (“OOP1”) (10 μ M) for 24 hours.

[0026] Figure 12 illustrates the design of amino acid-derived oligooxopiperazines (Tošovska & Arora, *Org. Lett.* 12:1588 (2010)). The oligooxopiperazines are obtained by linking neighboring amide nitrogen atoms in peptides with ethylene bridges, as depicted.

[0027] Figure 13 is a general synthesis scheme for making oligooxopiperazines using microwave-assisted solid phase synthesis.

[0028] Figure 14 shows the design and structure of model oligooxopiperazine dimers A–C and a model oligooxopiperazine trimer. An overlay of the predicted structure of each model oligooxopiperazine and its target α -helix is also shown.

[0029] Figure 15 shows the design and structure of a model oligooxopiperazine dimer B that mimics the α B helix of HIF-1 α .

[0030] Figure 16 shows the design and structure of a model oligooxopiperazine dimer C that mimics the α B helix of HIF-1 α .

[0031] Figure 17 shows oligooxopiperazines BB2-164, BB2-162, BB2-125, and BB2-282.

[0032] Figure 18 illustrates the design of oligooxopiperazine BB2-162. The sequence of helix α B (SEQ ID NO: 2) is also shown with a box around each hotspot residue.

[0033] Figure 19 is a graph of cellular viability upon exposure to varying concentrations of oligooxopiperazines BB2-125 or BB2-162.

DETAILED DESCRIPTION OF THE INVENTION

[0034] The incidence of human papillomavirus (HPV)-positive head and neck squamous cell carcinoma (HNSCC) has rapidly increased over the past 30 years prompting the suggestion that an epidemic may be on the horizon. Therefore, there is a clinical need to develop alternate therapeutic strategies to manage the growing

number of HPV-positive HNSCC patients, as well as other HPV-associated cancers. E6, the oncogenic protein of high-risk HPV serotypes, inactivates p53 through two distinct mechanisms: association with E6AP to degrade p53 and association with p300 to block p300-mediated p53 acetylation and activation. As described herein

5 targeting the E6-p300 interaction is an effective approach to reactivate p53 in HPV-positive cancers. Ectopic expression of the CH1 domain of p300 in HPV-positive HNSCC blocks the association between E6 and p300, increases total and acetylated p53 levels, and enhances p53 transcriptional activity. Moreover, expression of p21, miR-34a, and miR-200c are increased, demonstrating functional p53 reactivation.

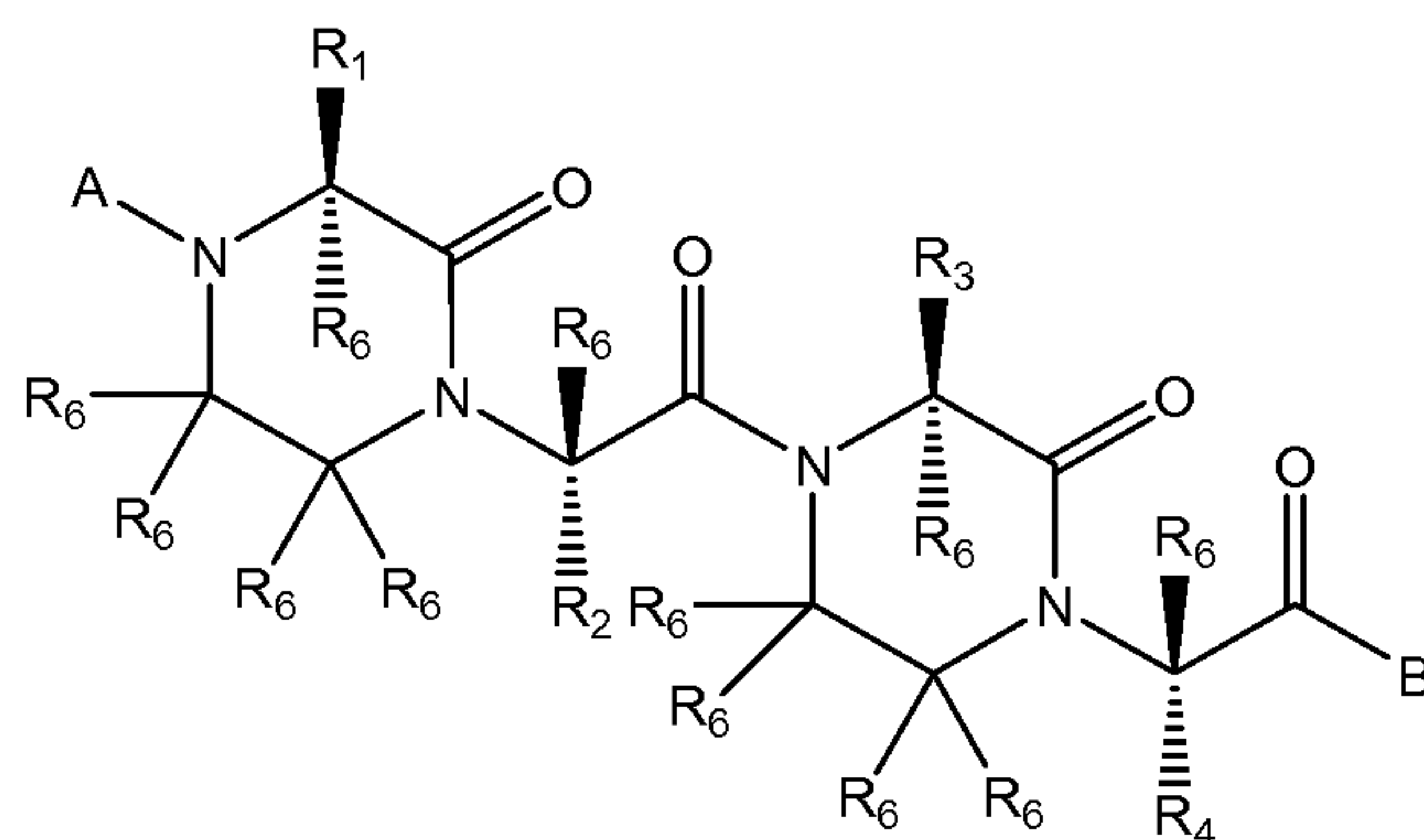
10 CH1 overexpression in HPV-positive HNSCC has a global anti-cancer effect resulting in a decrease in cell proliferation and clonogenic survival and an increase in apoptosis. The *in vivo* tumor initiating ability of HPV-positive HNSCC is severely compromised with CH1 overexpression, in part through a reduction in the cancer initiating cell population. Oligooxopiperazines that target the CH1 domain of p300

15 disrupt the E6-p300 interaction, reactivating p53, and potentiate the anti-cancer activity of *cis*-platinum in HPV-positive cancer cells. The oligooxopiperazines described herein represent a class of p53 reactivation therapeutics for managing HPV-positive cancer patients.

[0035] One aspect of the present invention relates to a method of treating or

20 preventing in a subject a disorder mediated by interaction of E6 with CREB-binding protein and/or p300, the method comprising administering to the subject an oligooxopiperazine that substantially mimics helix α B of the C-terminal transactivation domain of Hypoxia-Inducible Factor 1 α under conditions effective to treat or prevent the disorder.

25 [0036] Suitable oligooxopiperazines according to this and all aspects of the present invention include those of Formula I:



I, wherein:

- 14 -

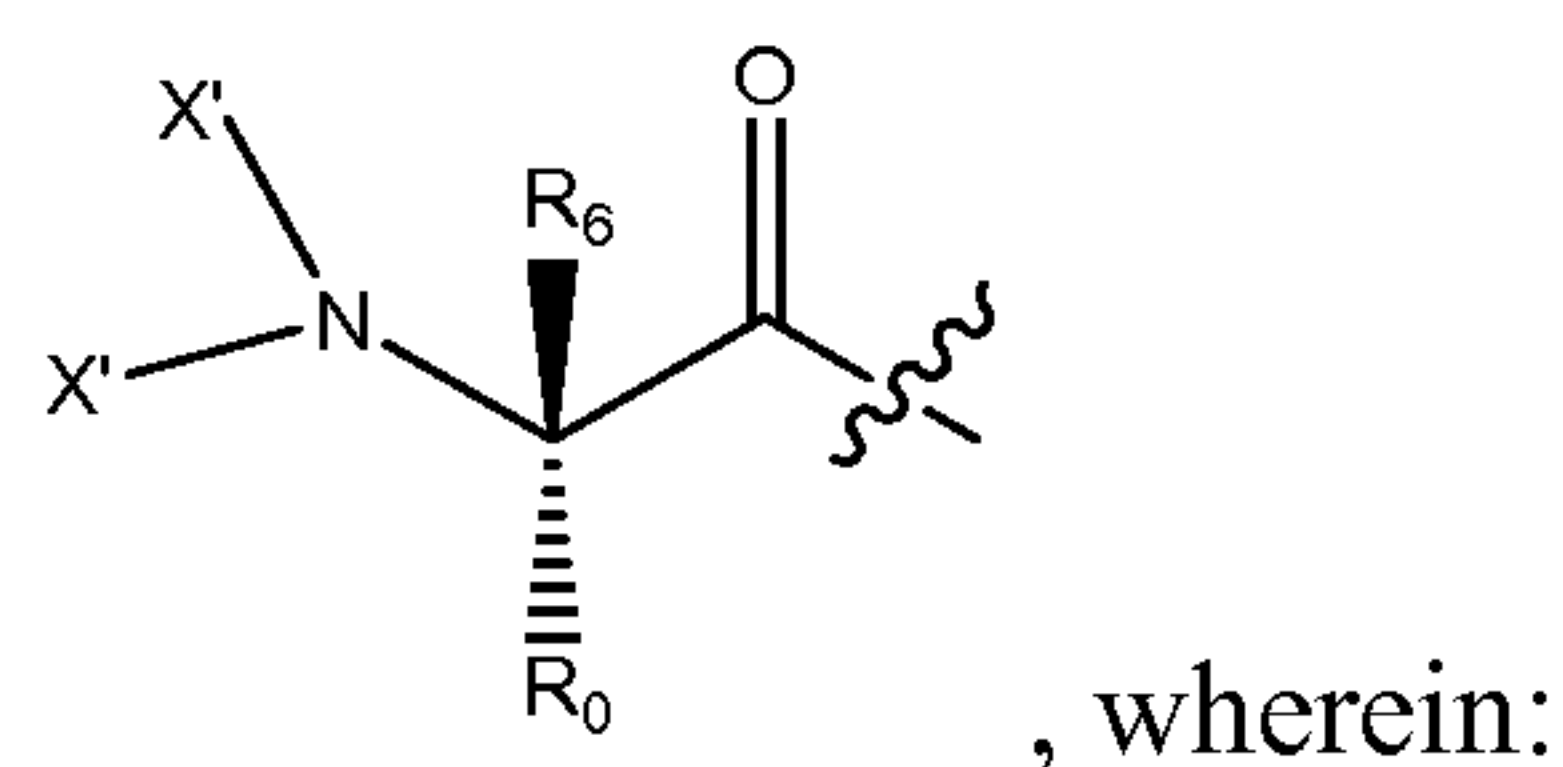
each of R_1 , R_2 , R_3 , and R_4 is independently an amino acid side chain, H, $N(R)_2$, OR, halogen, an alkyl, or an aryl; wherein each R is independently H, an alkyl, or an aryl;

each R_6 is independently H, $N(R)_2$, OR, halogen, an alkyl, or an aryl; wherein each R is independently H, an alkyl, or an aryl;

A is X_1 or C, wherein:

X_1 is H, COR' , CO_2R' , $CONR'$, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a protecting group for protection of an amine, a targeting moiety, or a tag; wherein R' is H, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; and

C is a moiety of the formula



each X' is independently H, COR' , CO_2R' , $CONR'$, $N(R'')_2$, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; wherein:

R' is H, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; and

each R'' is independently H, CO_2R' , $CONR'$, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag;

R_0 is an amino acid side chain, H, $N(R)_2$, OR, halogen, an alkyl, or an aryl; wherein each R is independently H, an alkyl, or an aryl; and

R_6 is H, $N(R)_2$, OR, halogen, an alkyl, or an aryl; wherein each R is independently H, an alkyl, or an aryl; and

B is Y or D, wherein:

Y is OR' , COR' , $N(R''')_2$, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a protecting group for protection of a carboxylic acid, a targeting moiety, or a tag; wherein:

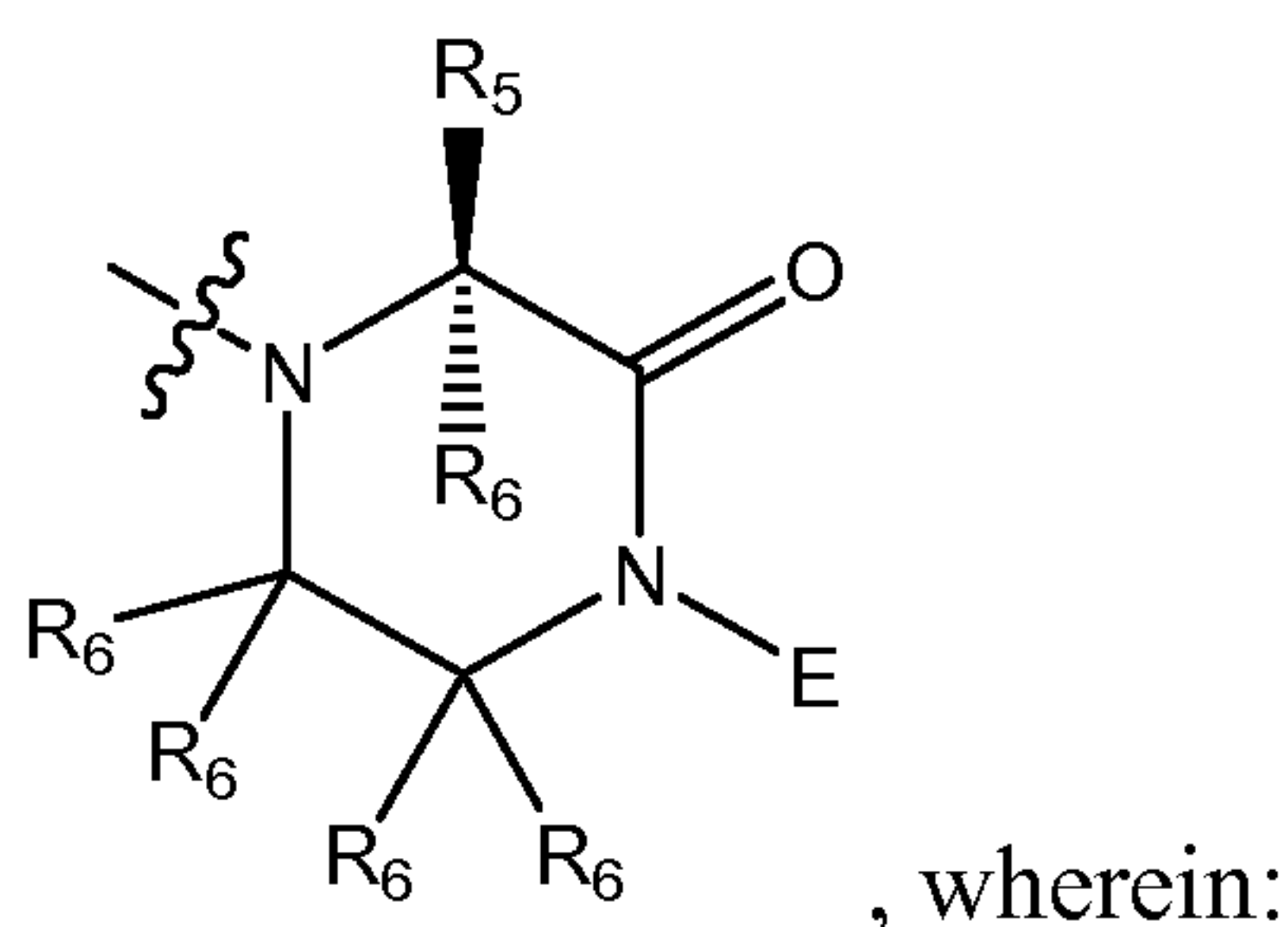
- 15 -

R' is H, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; and

each R''' is independently H, CO₂R', CONR', an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; and

5

D is a moiety of the formula



R₅ is an amino acid side chain, H, N(R)₂, OR, halogen, an alkyl, or an aryl; wherein each R is independently H, an alkyl, or an aryl;

10

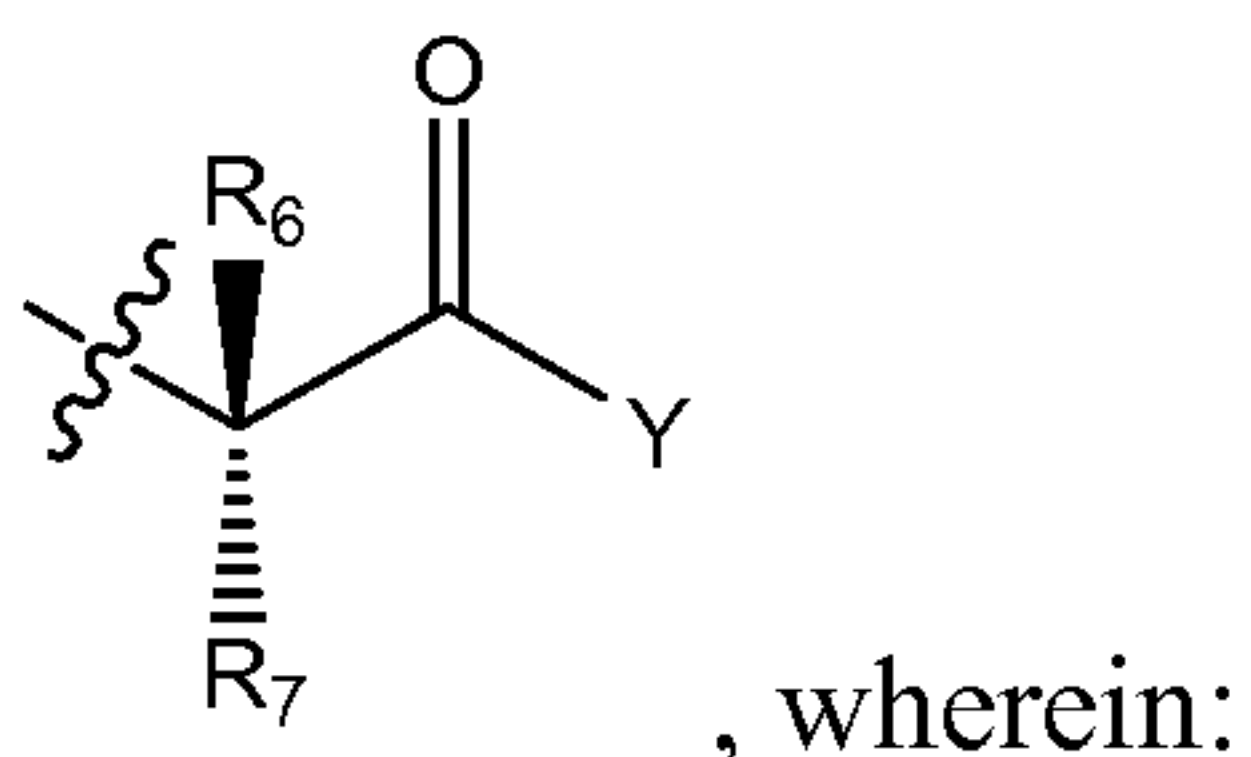
R₆ is H, N(R)₂, OR, halogen, an alkyl, or an aryl; wherein each R is independently H, an alkyl, or an aryl; and

E is X₂ or F, wherein:

X₂ is H, COR', CO₂R', CONR', an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a protecting group for protection of an amine, a targeting moiety, or a tag; wherein R' is H, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; and

15

F is a moiety of the formula



20

R₆ is H, N(R)₂, OR, halogen, an alkyl, or an aryl;

wherein each R is independently H, an alkyl, or an aryl;

R₇ is an amino acid side chain; and

25

Y is OR', COR', N(R''')₂, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a protecting group for

- 16 -

protection of a carboxylic acid, a targeting moiety, or a tag; wherein:

R' is H, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; and

each R''' is independently H, CO₂R', CONR', an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag.

[0037] Amino acid side chains according to this and all aspects of the present invention can be any amino acid side chain from natural or nonnatural amino acids, including from alpha amino acids, beta amino acids, gamma amino acids, L-amino acids, and D-amino acids.

[0038] As used herein, the term "alkyl" means an aliphatic hydrocarbon group which may be straight or branched having about 1 to about 6 carbon atoms in the chain. Branched means that one or more lower alkyl groups such as methyl, ethyl, or propyl are attached to a linear alkyl chain. Exemplary alkyl groups include methyl, ethyl, n-propyl, i-propyl, n-butyl, t-butyl, n-pentyl, and 3-pentyl.

[0039] As used herein, the term "cycloalkyl" refers to a non-aromatic saturated or unsaturated mono- or polycyclic ring system which may contain 3 to 6 carbon atoms, and which may include at least one double bond. Exemplary cycloalkyl groups include, without limitation, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cyclopropenyl, cyclobutenyl, cyclopentenyl, cyclohexenyl, *anti*-bicyclopropane, or *syn*-bicyclopropane.

[0040] As used herein, the term "aryl" refers to an aromatic monocyclic or polycyclic ring system containing from 6 to 19 carbon atoms, where the ring system may be optionally substituted. Aryl groups of the present invention include, but are not limited to, groups such as phenyl, naphthyl, azulenyl, phenanthrenyl, anthracenyl, fluorenyl, pyrenyl, triphenylenyl, chrysenyl, and naphthacenyl.

[0041] The term "arylalkyl" refers to a radical of the formula -R^aR^b where R^a is an alkyl radical as defined above and R^b is an aryl radical as defined above. The alkyl radical and the cycloalkyl radical may be optionally substituted as defined above.

- 17 -

[0042] As used herein, "heteroaryl" refers to an aromatic ring radical which consists of carbon atoms and from one to five heteroatoms selected from the group consisting of nitrogen, oxygen, and sulfur. Examples of heteroaryl groups include, without limitation, pyrrolyl, pyrazolyl, imidazolyl, triazolyl, furyl, thiophenyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, oxadiazolyl, thiadiazolyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, triazinyl, thienopyrrolyl, furopyrrolyl, indolyl, azaindolyl, isoindolyl, indolinyl, indoliziny, indazolyl, benzimidazolyl, imidazopyridinyl, benzotriazolyl, benzoxazolyl, benzoxadiazolyl, benzothiazolyl, pyrazolopyridinyl, triazolopyridinyl, thienopyridinyl, benzothiadiaazolyl, benzofuyl, benzothiophenyl, quinolinyl, isoquinolinyl, tetrahydroquinolyl, tetrahydroisoquinolyl, cinnolinyl, quinazolinyl, quinoliziliny, phthalazinyl, benzotriazinyl, chromenyl, naphthyridinyl, acrydinyl, phenanzinyl, phenothiazinyl, phenoxazinyl, pteridinyl, and purinyl. Additional heteroaryls are described in COMPREHENSIVE HETEROCYCLIC CHEMISTRY: THE STRUCTURE, REACTIONS, SYNTHESIS AND USE OF HETEROCYCLIC COMPOUNDS (Katritzky et al. eds., 1984), which is hereby incorporated by reference in its entirety.

[0043] The oligooxopiperazines of Formula I may comprise a protecting group that is suitable for the protection of an amine or a carboxylic acid. Such protecting groups function primarily to protect or mask the reactivity of functional groups. Protecting groups that are suitable for the protection of an amine group are well known in the art, including without limitation, carbamates, amides, *N*-alkyl and *N*-aryl amines, imine derivatives, enamine derivatives, and *N*-hetero atom derivatives as described by THEODORA W. GREENE & PETER G.M. WUTS, PROTECTIVE GROUPS IN ORGANIC SYNTHESIS 494–615 (1999), which is hereby incorporated by reference in its entirety. Protecting groups that are suitable for the protection of a carboxylic acid are also well known in the art. Suitable carboxylic acid protecting groups include, without limitation, esters (*e.g.*, substituted methyl esters, 2-substituted ethyl esters, 2,6-dialkylphenyl esters, substituted benzyl esters, silyl esters, and stannyl esters), amides, and hydrazides as described by THEODORA W. GREENE & PETER G.M. WUTS, PROTECTIVE GROUPS IN ORGANIC SYNTHESIS 372–450 (1999), which is hereby incorporated by reference in its entirety. Methods of protecting and deprotecting amine and carboxylic acids vary depending on the chosen protecting group; however, these methods are well known in the art and described in

- 18 -

THEODORA W. GREENE & PETER G.M. WUTS, PROTECTIVE GROUPS IN ORGANIC SYNTHESIS 372–450 and 494–615 (1999), which is hereby incorporated by reference in its entirety.

[0044] A “tag” as used herein includes any labeling moiety that facilitates the detection, quantitation, separation, and/or purification of the compounds of the present invention. Suitable tags include purification tags, radioactive or fluorescent labels, and enzymatic tags.

[0045] Purification tags, such as poly-histidine (His₆–), a glutathione-S-transferase (GST–), or maltose-binding protein (MBP–), can assist in compound purification or separation but can later be removed, *i.e.*, cleaved from the compound following recovery. Protease-specific cleavage sites can be used to facilitate the removal of the purification tag. The desired product can be purified further to remove the cleaved purification tags.

[0046] Other suitable tags include radioactive labels, such as, ¹²⁵I, ¹³¹I, ¹¹¹In, or ⁹⁹Tc. Methods of radiolabeling compounds are known in the art and described in U.S. Patent No. 5,830,431 to Srinivasan et al., which is hereby incorporated by reference in its entirety. Radioactivity is detected and quantified using a scintillation counter or autoradiography. Alternatively, the compound can be conjugated to a fluorescent tag. Suitable fluorescent tags include, without limitation, chelates (europium chelates), fluorescein and its derivatives, rhodamine and its derivatives, dansyl, Lissamine, phycoerythrin, and Texas Red. The fluorescent labels can be conjugated to the compounds using techniques disclosed in CURRENT PROTOCOLS IN IMMUNOLOGY (Coligen et al. eds., 1991), which is hereby incorporated by reference in its entirety. Fluorescence can be detected and quantified using a fluorometer.

[0047] Enzymatic tags generally catalyze a chemical alteration of a chromogenic substrate which can be measured using various techniques. For example, the enzyme may catalyze a color change in a substrate, which can be measured spectrophotometrically. Alternatively, the enzyme may alter the fluorescence or chemiluminescence of the substrate. Examples of suitable enzymatic tags include luciferases (*e.g.*, firefly luciferase and bacterial luciferase; *see e.g.*, U.S. Patent No. 4,737,456 to Weng et al., which is hereby incorporated by reference in its entirety), luciferin, 2,3-dihydrophthalazinediones, malate dehydrogenase, urease, peroxidases (*e.g.*, horseradish peroxidase), alkaline phosphatase, β -galactosidase,

- 19 -

glucoamylase, lysozyme, saccharide oxidases (*e.g.*, glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase), heterocyclic oxidases (*e.g.*, uricase and xanthine oxidase), lactoperoxidase, microperoxidase, and the like. Techniques for conjugating enzymes to proteins and peptides are described in O'Sullivan et al.,
5 *Methods for the Preparation of Enzyme—Antibody Conjugates for Use in Enzyme Immunoassay*, in METHODS IN ENZYMOLOGY 147–66 (Langone et al. eds., 1981), which is hereby incorporated by reference in its entirety.

[0048] A targeting moiety according to the present invention functions to (i) promote the cellular uptake of the oligooxopiperazine, (ii) target the
10 oligooxopiperazine to a particular cell or tissue type (*e.g.*, signaling peptide sequence), or (iii) target the oligooxopiperazine to a specific sub-cellular localization after cellular uptake (*e.g.*, transport peptide sequence).

[0049] To promote the cellular uptake of an oligooxopiperazine of the present invention, the targeting moiety may be a cell penetrating peptide (CPP). CPPs
15 translocate across the plasma membrane of eukaryotic cells by a seemingly energy-independent pathway and have been used successfully for intracellular delivery of macromolecules, including antibodies, peptides, proteins, and nucleic acids, with molecular weights several times greater than their own. Several commonly used CPPs, including polyarginines, transportant, protamine, maurocalcine, and M918, are
20 suitable targeting moieties for use in the present invention and are well known in the art (*see* Stewart et al., “Cell-Penetrating Peptides as Delivery Vehicles for Biology and Medicine,” *Organic Biomolecular Chem* 6:2242–2255 (2008), which is hereby incorporated by reference in its entirety). Additionally, methods of making CPP are described in U.S. Patent Application Publication No. 20080234183 to Hallbrink et al.,
25 which is hereby incorporated by reference in its entirety.

[0050] Another suitable targeting moiety useful for enhancing the cellular uptake of a compound is an “importation competent” signal peptide as disclosed by U.S. Patent No. 6,043,339 to Lin et al., which is hereby incorporated by reference in its entirety. An importation competent signal peptide is generally about 10 to about
30 50 amino acid residues in length—typically hydrophobic residues—that render the compound capable of penetrating through the cell membrane from outside the cell to the interior of the cell. An exemplary importation competent signal peptide includes the signal peptide from Kaposi fibroblast growth factor (*see* U.S. Patent No.

- 20 -

6,043,339 to Lin et al., which is hereby incorporated by reference in its entirety).

Other suitable peptide sequences can be selected from the SIGPEP database (*see* von Heijne G., "SIGPEP: A Sequence Database for Secretory Signal Peptides," *Protein Seq. Data Anal.* 1(1):41–42 (1987), which is hereby incorporated by reference in its entirety).

5 [0051] Another suitable targeting moiety is a signal peptide sequence capable of targeting the oligooxopiperazine to a particular tissue or cell type. The signaling peptide can include at least a portion of a ligand binding protein. Suitable ligand binding proteins include high-affinity antibody fragments (*e.g.*, Fab, Fab' and F(ab')₂),
10 single-chain Fv antibody fragments), nanobodies or nanobody fragments, fluorobodies, or aptamers. Other ligand binding proteins include biotin-binding proteins, lipid-binding proteins, periplasmic binding proteins, lectins, serum albumins, enzymes, phosphate and sulfate binding proteins, immunophilins, metallothionein, or various other receptor proteins. For cell specific targeting, the signaling peptide is
15 preferably a ligand binding domain of a cell specific membrane receptor. Thus, when the modified oligooxopiperazine is delivered intravenously or otherwise introduced into blood or lymph, the oligooxopiperazine will adsorb to the targeted cell, and the targeted cell will internalize the oligooxopiperazine. For example, if the target cell is a cancer cell, the oligooxopiperazine may be conjugated to an anti-C3B(I) antibody as
20 disclosed by U.S. Patent No. 6,572,856 to Taylor et al., which is hereby incorporated by reference in its entirety. Alternatively, the oligooxopiperazine may be conjugated to an alphafeto protein receptor as disclosed by U.S. Patent No. 6,514,685 to Moro, or to a monoclonal GAH antibody as disclosed by U.S. Patent No. 5,837,845 to Hosokawa, which are hereby incorporated by reference in their entirety. For targeting
25 an oligooxopiperazine to a cardiac cell, the oligooxopiperazine may be conjugated to an antibody recognizing elastin microfibril interfacer (EMILIN2) (Van Hoof et al., "Identification of Cell Surface for Antibody-Based Selection of Human Embryonic Stem Cell-Derived Cardiomyocytes," *J Proteom Res* 9:1610–18 (2010), which is hereby incorporated by reference in its entirety), cardiac troponin I, connexin-43, or
30 any cardiac cell-surface membrane receptor that is known in the art. For targeting an oligooxopiperazine to a hepatic cell, the signaling peptide may include a ligand domain specific to the hepatocyte-specific asialoglycoprotein receptor. Methods of

- 21 -

preparing such chimeric proteins and peptides are described in U.S. Patent No. 5,817,789 to Heartlein et al., which is hereby incorporated by reference in its entirety.

[0052] Another suitable targeting moiety is a transport peptide that directs intracellular compartmentalization of the compound once it is internalized by a target cell or tissue. For transport to the endoplasmic reticulum (ER), for example, the compound can be conjugated to an ER transport peptide sequence. A number of such signal peptides are known in the art, including the signal peptide MMSFVSLLLVGILFYATEAEQLTKCEVFQ (SEQ ID NO: 1). Other suitable ER signal peptides include the N-terminus endoplasmic reticulum targeting sequence of the enzyme 17 β -hydroxysteroid dehydrogenase type 11 (Horiguchi et al., “Identification and Characterization of the ER/Lipid Droplet-Targeting Sequence in 17 β -hydroxysteroid Dehydrogenase Type 11,” *Arch. Biochem. Biophys.* 479(2):121–30 (2008), which is hereby incorporated by reference in its entirety), or any of the ER signaling peptides (including the nucleic acid sequences encoding the ER signal peptides) disclosed in U.S. Patent Application Publication No. 20080250515 to Reed et al., which is hereby incorporated by reference in its entirety. Additionally, the compound of the present invention can contain an ER retention signal, such as the retention signal KEDL (SEQ ID NO: 2). Methods of modifying the compounds of the present invention to incorporate transport peptides for localization of the compounds to the ER can be carried out as described in U.S. Patent Application Publication No. 20080250515 to Reed et al., which is hereby incorporated by reference in its entirety.

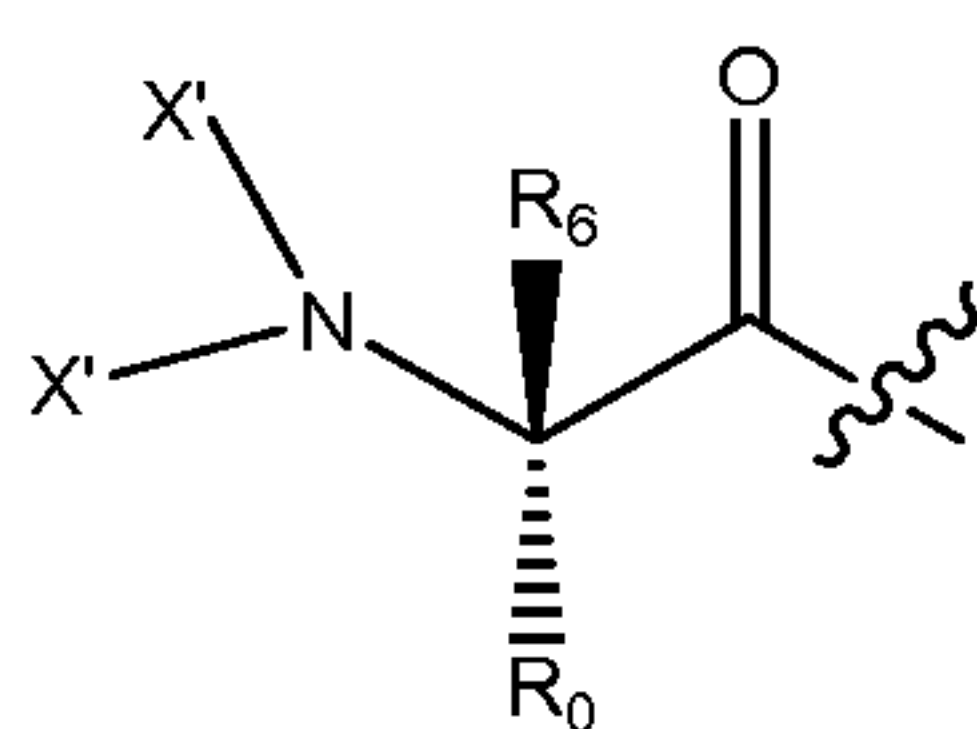
[0053] For transport to the nucleus, the compounds of the present invention can include a nuclear localization transport signal. Suitable nuclear transport peptide sequences are known in the art, including the nuclear transport peptide PPKKKRKV (SEQ ID NO:3). Other nuclear localization transport signals include, for example, the nuclear localization sequence of acidic fibroblast growth factor and the nuclear localization sequence of the transcription factor NF-KB p50 as disclosed by U.S. Patent No. 6,043,339 to Lin et al., which is hereby incorporated by reference in its entirety. Other nuclear localization peptide sequences known in the art are also suitable for use in the compounds of the present invention.

[0054] Suitable transport peptide sequences for targeting to the mitochondria include MLSLRQSIRFFKPATRTLCSRYLL (SEQ ID NO: 4). Other suitable transport peptide sequences suitable for selectively targeting the compounds of the

- 22 -

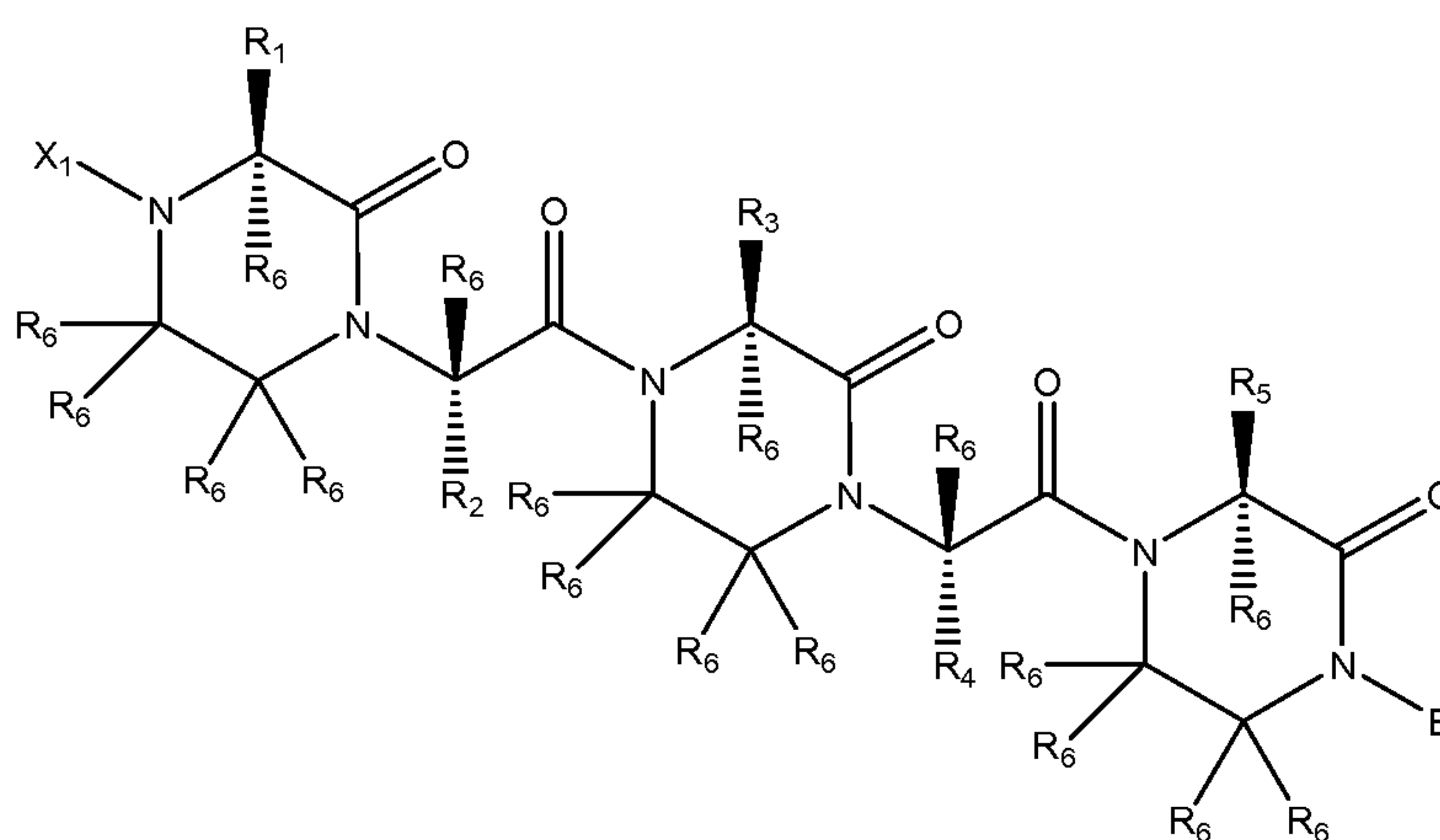
present invention to the mitochondria are disclosed in U.S. Patent Application Publication No. 20070161544 to Wipf, which is hereby incorporated by reference in its entirety.

- [0055] In at least one embodiment, the oligooxopiperazine is an
 5 oligooxopiperazine of Formula I in which (i) R_1 and R_2 are hydrophobic and R_4 is a hydrogen bond acceptor or hydrogen bond donor or A is a moiety of formula



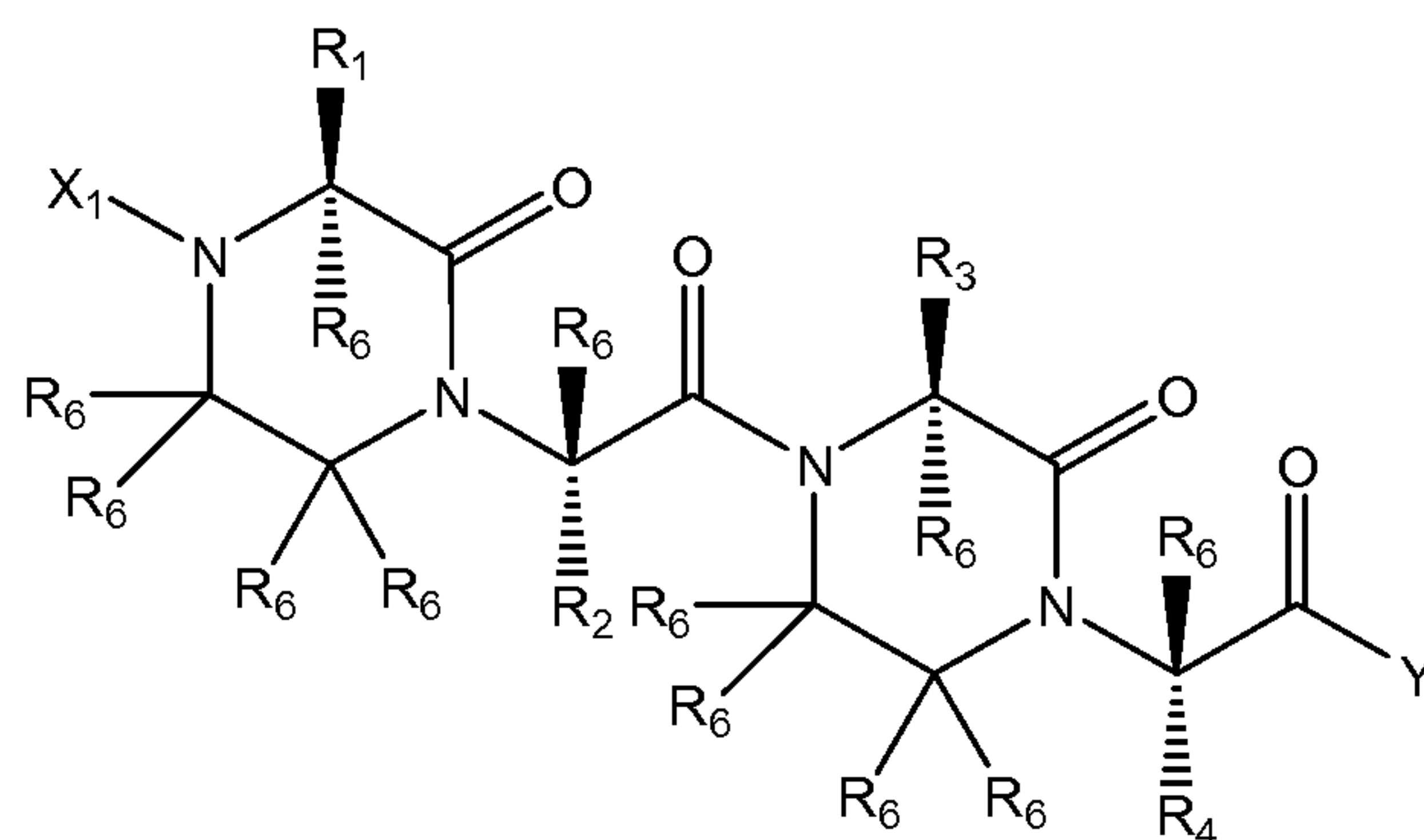
- , R_0 and R_3 are hydrophobic, and R_4 is a hydrogen bond acceptor or hydrogen bond donor; (ii) each R'' is independently H, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; and (iii) B is not D.
 10 Hydrogen bond acceptors contain an atom with an electron lone-pair to interact with a proton on an electronegative atom. Suitable examples include, without limitation, carbonyl groups and aromatic amines such as pyridine and imidazole. Hydrogen bond donors contain an electronegative atom with at least one proton to share. Suitable examples include, without limitation, amine, amide, carboxylic acids,
 15 hydroxyl, and thiol functional groups.

[0056] In one embodiment of the present invention, the oligooxopiperazine of Formula I has a formula of Formula IA:



- IA. In one
 embodiment, R_1 , R_2 , R_4 , and R_5 of the oligooxopiperazine of Formula IA mimic the
 20 amino acid side chain of, respectively, residues i , $i+4$, $i+6$, and $i+7$ of the α -helix.

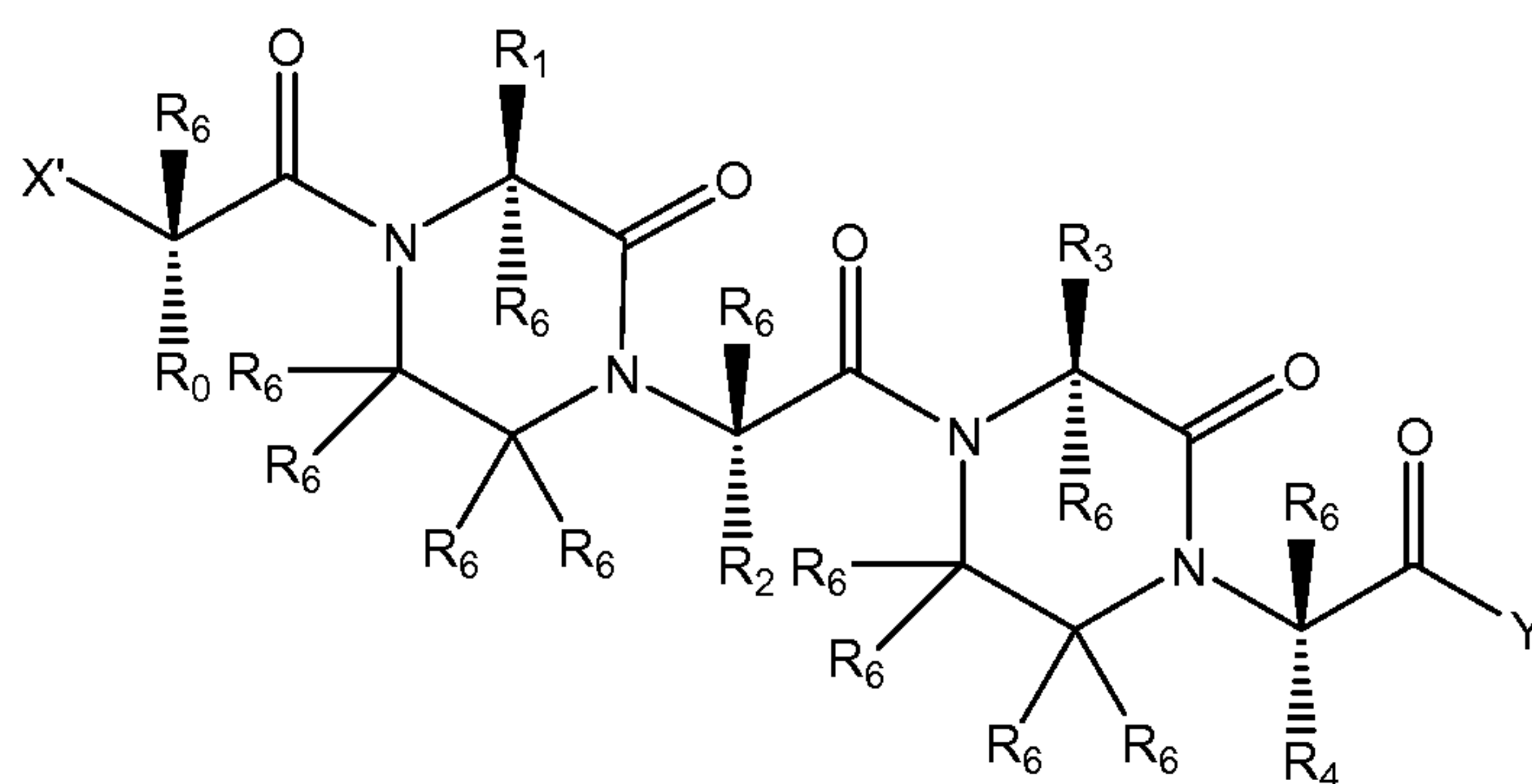
[0057] In another embodiment of the present invention, the oligooxopiperazine of Formula I has a formula of Formula IB:



IB. In one embodiment, R_1 ,

R_2 , and R_4 of the oligooxopiperazine of Formula IB mimic the amino acid side chain
5 of, respectively, residues i , $i+4$, and $i+7$, of the α -helix.

[0058] In another embodiment of the present invention, the oligooxopiperazine of Formula I has a formula of Formula IC:



IC. In one embodiment,

R_0 , R_1 , R_2 , R_3 , and R_4 of the oligooxopiperazine of Formula IC mimic the amino acid
10 side chain of, respectively, residues i , $i+2$, $i+3$, $i+4$, and $i+7$ of the α -helix.

[0059] Exemplary oligooxopiperazine compounds of Formula I include, without limitation BB2-125, BB2-162, and BB2-164.

[0060] Oligooxopiperazine for use in the present invention may be made using methods in the art. Suitable methods include those described in U.S. Patent
15 Application No. 12/917,176, which is hereby incorporated by reference in its entirety.

[0061] Disorders mediated by the interaction of E6 with CREB-binding protein and/or p300 include, for example, HPV-associated cancers. HPV-associated cancers are those that are caused (at least in part) by high-risk, or oncogenic, HPV types, *e.g.*, HPV-16, HPV-18, HPV-31, HPV-33, HPV-35, HPV-39, HPV-45, HPV-
20 51, HPV-52, HPV-56, HPV-58, HPV-59, HPV-68, HPV-69, HPV-73, and HPV-82.

- 24 -

These cancers include anogenital cancers, including cervical cancer, vulvar cancer, vaginal cancer, penile cancer, anal cancer, as well as cancers of the head and neck, including HNSCC and oropharyngeal cancer.

5 [0062] The subject according to this aspect of the present invention is preferably a human subject.

[0063] The compounds of the present invention can be administered orally, parenterally, for example, subcutaneously, intravenously, intramuscularly, intraperitoneally, by intranasal instillation, or by application to mucous membranes, such as, that of the nose, throat, and bronchial tubes. They may be administered alone
10 or with suitable pharmaceutical carriers, and can be in solid or liquid form such as, tablets, capsules, powders, solutions, suspensions, or emulsions.

[0064] The active compounds of the present invention may be orally administered, for example, with an inert diluent, or with an assimilable edible carrier, or they may be enclosed in hard or soft shell capsules, or they may be compressed
15 into tablets, or they may be incorporated directly with the food of the diet. For oral therapeutic administration, these active compounds may be incorporated with excipients and used in the form of tablets, capsules, elixirs, suspensions, syrups, and the like. Such compositions and preparations should contain at least 0.1% of active compound. The percentage of the compound in these compositions may, of course,
20 be varied and may conveniently be between about 2% to about 60% of the weight of the unit. The amount of active compound in such therapeutically useful compositions is such that a suitable dosage will be obtained. Preferred compositions according to the present invention are prepared so that an oral dosage unit contains between about 1 and 250 mg of active compound.

25 [0065] The tablets, capsules, and the like may also contain a binder 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; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose, or saccharin. When the dosage unit form is a capsule, it may contain, in
30 addition to materials of the above type, a liquid carrier, such as a fatty oil.

[0066] Various other materials may be present as coatings or to modify the physical form of the dosage unit. For instance, tablets may be coated with shellac, sugar, or both. A syrup may contain, in addition to active ingredient, sucrose as a

- 25 -

sweetening agent, methyl and propylparabens as preservatives, a dye, and flavoring such as cherry or orange flavor.

[0067] These active compounds may also be administered parenterally.

Solutions or suspensions of these active compounds can be prepared in water suitably
5 mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof in oils.

Illustrative oils are those of petroleum, animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, or mineral oil. In general, water, saline, aqueous dextrose and related sugar solution, and glycols such as, propylene glycol or
10 polyethylene glycol, are preferred liquid carriers, particularly for injectable solutions. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

[0068] The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous
15 preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol
20 (e.g., glycerol, propylene glycol, and liquid polyethylene glycol), suitable mixtures thereof, and vegetable oils.

[0069] The compounds of the present invention may also be administered directly to the airways in the form of an aerosol. For use as aerosols, the compounds of the present invention in solution or suspension may be packaged in a pressurized
25 aerosol container together with suitable propellants, for example, hydrocarbon propellants like propane, butane, or isobutane with conventional adjuvants. The materials of the present invention also may be administered in a non-pressurized form such as in a nebulizer or atomizer.

[0070] As will be apparent to the skilled artisan, the oligooxopiperazine may
30 be administered in the form of a pharmaceutical formulation comprising any of the above described oligooxopiperazines and a pharmaceutically acceptable carrier. Acceptable pharmaceutical carriers include solutions, suspensions, emulsions,

- 26 -

excipients, powders, or stabilizers. The carrier should be suitable for the desired mode of delivery.

[0071] In addition, the pharmaceutical formulations may further comprise one or more pharmaceutically acceptable diluents, adjuvants, excipients, or vehicles, such as preserving agents, fillers, disintegrating agents, wetting agents, emulsifying agents, suspending agents, sweetening agents, flavoring agents, perfuming agents, antibacterial agents, antifungal agents, lubricating agents and dispensing agents, depending on the nature of the mode of administration and dosage forms. Examples of suspending agents include ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar—agar and tragacanth, or mixtures of these substances. Prevention of the action of microorganisms can be ensured by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, and the like. It may also be desirable to include isotonic agents, for example sugars, sodium chloride, and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for example, aluminum monostearate and gelatin. Examples of suitable carriers, diluents, solvents, or vehicles include water, ethanol, polyols, suitable mixtures thereof, vegetable oils (such as olive oil), and injectable organic esters such as ethyl oleate. Examples of excipients include lactose, milk sugar, sodium citrate, calcium carbonate, and dicalcium phosphate. Examples of disintegrating agents include starch, alginic acids, and certain complex silicates. Examples of lubricants include magnesium stearate, sodium lauryl sulphate, talc, as well as high molecular weight polyethylene glycols.

[0072] As indicated by Example 18 below, agents that disrupt the E6-p300 interaction, like the oligo oxopiperazines for use in the present invention, can potentiate the anti-cancer effect of other anti-cancer agents. Thus, in some embodiments, the oligo oxopiperazines are used together with one or more other anti-cancer agents. Suitable agents include, without limitation, 13-cis-Retinoic Acid, 2-CdA, 2-Chlorodeoxyadenosine, 5-Azacitidine, 5-Fluorouracil, 5-FU, 6-Mercaptopurine, 6-MP, 6-TG, 6-Thioguanine, Abraxane, Accutane, Actinomycin-D, Adcetris, Adriamycin, Adrucil, Afinitor, Agrylin, Ala-Cort, Aldesleukin, Alemtuzumab, ALIMTA, Alitretinoin, Alkaban-AQ, Alkeran, All-transretinoic Acid, Alpha Interferon, Altretamine, Amethopterin, Amifostine, Aminoglutethimide,

- 27 -

Anagrelide, Anandron ®, Anastrozole, Arabinosylcytosine, Ara-C, Aranesp ®, Aredia
 ®, Arimidex ®, Aromasin ®, Arranon ®, Arsenic Trioxide, Arzerra™, Asparaginase,
 ATRA, Avastin ®, Axitinib, Azacitidine, BCG, Bendamustine, Bevacizumab,
 Bexarotene, BEXXAR ®, Bicalutamide, BiCNU, Blenoxane ®, Bleomycin,
 5 Bortezomib, Bosulif, Bosutinib, Brentuximab Vedotin, Busulfan, Busulfex ®, C225,
 Cabazitaxel, Cabozantinib, Calcium Leucovorin, Campath ®, Camptosar ®,
 Camptothecin-11, Capecitabine, Caprelsa, Carac™, Carboplatin, Carfilzomib,
 Carmustine, Carmustine Wafer, Casodex ®, CC-5013, CCI-779, CCNU, CDDP,
 CeeNU, Cerubidine ®, Cetuximab, Chlorambucil, Cisplatin, Cisplatinum, Citrovorium
 10 Factor, Cladribine, Cometriq, Cortisone, Cosmegen ®, CPT-11, Crizotinib,
 Cyclophosphamide, Cytadren ®, Cytarabine, Cytarabine Liposomal, Cytosar-U ®,
 Cytoxan ®, Dacarbazine, Dacogen, Dactinomycin, Darbepoetin Alfa, Dasatinib,
 Daunomycin, Daunorubicin, daunorubicin-hydrochloride, Daunorubicin Liposomal,
 DaunoXome ®, Decadron, Decitabine, Delta-Cortef ®, Deltasone ®, Denileukin
 15 Difitox, Denosumab, DepoCyt™, Dexamethasone, Dexamethasone Acetate,
 Dexamethasone Sodium Phosphate, Dexasone, Dexrazoxane, DHAD, DIC, Diodex,
 Docetaxel, Doxorubicin, Doxorubicin Liposomal, Droxia™, DTIC, DTIC-Dome ®,
 Duralone ®, Eculizumab, Efudex ®, Ellence™, Eloxatin™, Elspar ®, Emcynt ®,
 Epirubicin, Epoetin Alfa, Erbitux, Eribulin, Erivedge, Erlotinib, Erwinia L-
 20 asparaginase, Estramustine, Ethyol, Etopophos, Etoposide, Etoposide Phosphate,
 Eulexin ®, Everolimus, Evista ®, Exemestane, Fareston ®, Faslodex ®, Femara ®,
 Filgrastim, Floxuridine, Fludara ®, Fludarabine, Fluoroplex ®, Fluorouracil,
 Fluorouracil (cream), Fluoxymesterone, Flutamide, Folinic Acid, Folutyn, FUDR ®,
 Fulvestrant, G-CSF, Gefitinib, Gemcitabine, Gemtuzumab ozogamicin, Gemzar,
 25 Gleevec™, Gliadel Wafer ®, GM-CSF, Goserelin, Granulocyte - Colony Stimulating
 Factor, Granulocyte Macrophage Colony Stimulating Factor, Halaven ®, Halotestin
 ®, Herceptin ®, Hexadrol, Hexalen ®, Hexamethylmelamine, HMM, Hycamtin ®,
 Hydrea ®, Hydrocort Acetate ®, Hydrocortisone, Hydrocortisone Sodium Phosphate,
 Hydrocortisone Sodium Succinate, Hydrocortone Phosphate, Hydroxyurea,
 30 Ibritumomab, Ibritumomab Tiuxetan, Idamycin, ICLUSIG®, Ifex ®, IFN-alpha,
 Ifosfamide, IL-11, IL-2, Imatinib Mesylate, Imidazole Carboxamide, Inlyta ®,
 Interferon-Alfa, Interferon Alfa-2b (PEG Conjugate), Interleukin-2, Interleukin-11,
 Intron A® (interferon alfa-2b), Ipilimumab, Irinotecan, Isotretinoin, Istodax,

- 28 -

Ixabepilone, Jevtana, Kidrolase, Kyprolis, Lanacort, Lapatinib, L-asparaginase, LCR,
 Lenalidomide, Letrozole, Leucovorin, Leukeran, Leukine, Leuprolide, Leurocristine,
 Leustatin, Liposomal Ara-C, Liquid Pred, Lomustine, L-PAM, L-Sarcolysin, Lupron,
 Lupron Depot, Marqibo, Matulane, Maxidex, Mechlorethamine, Mechlorethamine
 5 Hydrochloride, Medralone, Medrol, Megace, Megestrol, Megestrol Acetate,
 Melphalan, Mercaptopurine, Mesna, Mesnex, Methotrexate, Methotrexate Sodium,
 Methylprednisolone, Meticorten, Mitomycin, Mitomycin-C, Mitoxantrone, M-
 Prednisol, MTC, MTX, Mustargen, Mustine, Mutamycin, Myleran, Mylocel,
 Mylotarg, Navelbine, Nelarabine, Neosar, Neulasta, Neumega, Neupogen, Nexavar,
 10 Nilandron, Nilotinib, Nilutamide, Nipent, Nitrogen Mustard, Novaldex, Novantrone,
 Nplate, Octreotide, Octreotide Acetate, Ofatumumab, Oncospar, Oncovin, Ontak,
 Onxal, Oprelvekin, Orapred, Orasone, Oxaliplatin, Paclitaxel, Paclitaxel Protein-
 bound, Pamidronate, Panitumumab, Panretin, Paraplatin, Pazopanib, Pediapred, PEG
 Interferon, Pegaspargase, Pegfilgrastim, PEG-INTRON, PEG-L-asparaginase,
 15 PEMETREXED, Pentostatin, Perjeta, Pertuzumab, Phenylalanine Mustard, Platinol,
 Platinol-AQ, Ponatinib, Pralatrexate, Prednisolone, Prednisone, Prelone,
 Procarbazine, PROCRIT, Proleukin, Prolia, Prolifeprospan 20 with Carmustine
 Implant, Provenge, Purinethol, Raloxifene, Regorafenib, Revlimid, Rheumatrex,
 Rituxan, Rituximab, Roferon-A (Interferon Alfa-2a), Romidepsin, Romiplostim,
 20 Rubex, Rubidomycin Hydrochloride, Sandostatin, Sandostatin LAR, Sargramostim,
 Sipuleucel-T, Soliris, Solu-Cortef, Solu-Medrol, Sorafenib, SPRYCEL, STI-571,
 Stivarga, Streptozocin, SU11248, Sunitinib, Sutent, Tamoxifen, Tarceva, Targretin,
 Tasigna, Taxol, Taxotere, Temodar, Temozolomide, Temsirolimus, Teniposide,
 TESPA, Thalidomide, Thalomid, TheraCys, Thioguanine, Thioguanine Tabloid,
 25 Thiophosphoamide, Thioplex, Thiotepa, TICE, Toposar, Topotecan, Toremifene,
 Torisel, Tositumomab, Trastuzumab, Treanda, Tretinoin, Trexall, Trisenox, TSPA,
 TYKERB, Valrubicin, Valstar, Vandetanib, VCR, Vectibix, Velban, Velcade,
 VePesid, Vesanoid, Viadur, Vidaza, Vinblastine, Vinblastine Sulfate, Vincasar Pfs,
 Vincristine, Vincristine Liposomal, Vinorelbine, Vinorelbine Tartrate, Vismodegib,
 30 VLB, VM-26, Vorinostat, Votrient, VP-16, Vumon, Xalkori Capsules, Xeloda,
 Xgeva, Yervoy, Zaltrap, Zanosar, Zelboraf, Zevalin, Zinecard, Ziv-aflibercept,
 Zoladex, Zoledronic Acid, Zolinza, and Zometa.

- 29 -

[0073] Another aspect of the present invention relates to a method of inducing apoptosis of a cell, the method comprising contacting the cell with an oligooxopiperazine that substantially mimics helix α B of the C-terminal transactivation domain of HIF1 α under conditions effective to induce apoptosis of the cell.

[0074] Suitable oligooxopiperazines include those noted above.

[0075] Suitable cells according to this and all aspects of the present invention include, without limitation, mammalian cells. Preferably, the cells are human cells. In at least one embodiment, the cells are cancer cells. Suitable cancer cells include, *e.g.*, anogenital cancer cells, including cervical cancer cells, vulvar cancer cells, vaginal cancer cells, penile cancer cells, anal cancer cells, as well as cancer cells of the head and neck, including HNSCC cells and oropharyngeal cancer cells. In at least one embodiment, the cells are infected with a high-risk HPV, as described above.

[0076] Another aspect of the present invention relates to a method of decreasing survival and/or proliferation of a cell, the method comprising contacting the cell with an oligooxopiperazine that substantially mimics helix α B of the C-terminal transactivation domain of HIF1 α under conditions effective to decrease survival and/or proliferation of the cell.

[0077] Suitable oligooxopiperazines and suitable cells include those noted above.

[0078] Another aspect of the present invention relates to a method of preventing or reversing inactivation of p53 in a cell, the method comprising contacting the cell with an oligooxopiperazine that substantially mimics helix α B of the C-terminal transactivation domain of HIF1 α under conditions effective to prevent or reverse inactivation of p53 in a cell.

[0079] Suitable oligooxopiperazines and suitable cells include those noted above.

[0080] Yet another aspect of the present invention relates to a method of inhibiting p300-mediated acetylation of a transcription factor in a cell, the method comprising contacting the cell with an oligooxopiperazine that substantially mimics helix α B of the C-terminal transactivation domain of HIF1 α under conditions effective to inhibit p300-mediated acetylation of the transcription factor in the cell.

- 30 -

[0081] Suitable transcription factors according to this aspect of the present invention include any transcription factor whose acetylation is mediated by p300. In at least one embodiment, the transcription factor is p53.

5 [0082] Suitable oligooxopiperazines and suitable cells include those noted above.

[0083] In all aspects of the present invention directed to methods involving contacting a cell with one or more oligooxopiperazines, contacting can be carried out using methods that will be apparent to the skilled artisan, and can be done *in vitro* or *in vivo*.

10 [0084] One approach for delivering agents into cells involves the use of liposomes. Basically, this involves providing a liposome which includes agent(s) to be delivered, and then contacting the target cell, tissue, or organ with the liposomes under conditions effective for delivery of the agent into the cell, tissue, or organ.

15 [0085] This liposome delivery system can also be made to accumulate at a target organ, tissue, or cell via active targeting (*e.g.*, by incorporating an antibody or hormone on the surface of the liposomal vehicle). This can be achieved according to known methods.

[0086] An alternative approach for delivery of protein- or polypeptide-containing agents (*e.g.*, oligooxopiperazines for use in the present invention containing one or more protein or polypeptide side chains) involves the conjugation of the desired agent to a polymer that is stabilized to avoid enzymatic degradation of the conjugated protein or polypeptide. Conjugated proteins or polypeptides of this type are described in U.S. Patent No. 5,681,811 to Ekwuribe, which is hereby incorporated by reference in its entirety.

25 [0087] Yet another approach for delivery of agents involves preparation of chimeric agents according to U.S. Patent No. 5,817,789 to Heartlein et al., which is hereby incorporated by reference in its entirety. The chimeric agent can include a ligand domain and the agent (*e.g.*, a oligooxopiperazine of the invention). The ligand domain is specific for receptors located on a target cell. Thus, when the chimeric agent is delivered intravenously or otherwise introduced into blood or lymph, the
30 chimeric agent will adsorb to the targeted cell, and the targeted cell will internalize the chimeric agent.

- 31 -

[0088] Oligooxopiperazines for use in the present invention may be delivered directly to the targeted cell/tissue/organ.

[0089] Additionally and/or alternatively, the oligooxopiperazines may be administered to a non-targeted area along with one or more agents that facilitate migration of the oligooxopiperazines to (and/or uptake by) a targeted tissue, organ, or cell. As will be apparent to one of ordinary skill in the art, the oligooxopiperazine itself can be modified to facilitate its transport to a target tissue, organ, or cell, including its transport across the blood-brain barrier; and/or to facilitate its uptake by a target cell (*e.g.*, its transport across cell membranes).

10 [0090] *In vivo* administration can be accomplished either via systemic administration to the subject or via targeted administration to affected tissues, organs, and/or cells, as described above. Typically, the therapeutic agent (*i.e.*, a oligooxopiperazine of the present invention) will be administered to a patient in a vehicle that delivers the therapeutic agent(s) to the target cell, tissue, or organ.

15 Typically, the therapeutic agent will be administered as a pharmaceutical formulation, such as those described above.

[0091] Exemplary routes of administration include, without limitation, orally, topically, transdermally, parenterally, subcutaneously, intravenously, intramuscularly, intraperitoneally, intraventricularly, and intralesionally; by intratracheal inoculation, aspiration, airway instillation, aerosolization, nebulization, intranasal instillation, oral or nasogastric instillation, intraperitoneal injection, intravascular injection, intravenous injection, intra-arterial injection (such as via the pulmonary artery), intramuscular injection, and intrapleural instillation; by application to mucous membranes (such as that of the nose, throat, bronchial tubes, genitals, and/or anus);

20 and by implantation of a sustained release vehicle.

[0092] For use as aerosols, a oligooxopiperazine of the present invention in solution or suspension may be packaged in a pressurized aerosol container together with suitable propellants, for example, hydrocarbon propellants like propane, butane, or isobutane with conventional adjuvants. The oligooxopiperazines for use in the present invention also may be administered in a non-pressurized form.

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[0093] Exemplary delivery devices include, without limitation, nebulizers, atomizers, liposomes (including both active and passive drug delivery techniques) (Wang & Huang, "pH-Sensitive Immunoliposomes Mediate Target-cell-specific

- 32 -

Delivery and Controlled Expression of a Foreign Gene in Mouse,” *Proc. Nat’l Acad. Sci. USA* 84:7851–5 (1987); Bangham et al., “Diffusion of Univalent Ions Across the Lamellae of Swollen Phospholipids,” *J. Mol. Biol.* 13:238–52 (1965); U.S. Patent No. 5,653,996 to Hsu; U.S. Patent No. 5,643,599 to Lee et al.; U.S. Patent No. 5,885,613 to Holland et al.; U.S. Patent No. 5,631,237 to Dzau & Kaneda; and U.S. Patent No. 5,059,421 to Loughrey et al.; Wolff et al., “The Use of Monoclonal Anti-Thy1 IgG1 for the Targeting of Liposomes to AKR-A Cells *in Vitro* and *in Vivo*,” *Biochim. Biophys. Acta* 802:259–73 (1984), each of which is hereby incorporated by reference in its entirety), transdermal patches, implants, implantable or injectable protein depot compositions, and syringes. Other delivery systems which are known to those of skill in the art can also be employed to achieve the desired delivery of the oligooxopiperazine to the desired organ, tissue, or cells *in vivo* to effect this aspect of the present invention.

[0094] Contacting (including *in vivo* administration) can be carried out as frequently as required and for a duration that is suitable to provide the desired effect. For example, contacting can be carried out once or multiple times, and *in vivo* administration can be carried out with a single sustained-release dosage formulation or with multiple (*e.g.*, daily) doses.

[0095] The amount to be administered will, of course, vary depending upon the particular conditions and treatment regimen. The amount/dose required to obtain the desired effect may vary depending on the agent, formulation, cell type, culture conditions (for *ex vivo* embodiments), the duration for which treatment is desired, and, for *in vivo* embodiments, the individual to whom the agent is administered.

[0096] Effective amounts can be determined empirically by those of skill in the art. For example, this may involve assays in which varying amounts of the oligooxopiperazine of the invention are administered to cells in culture and the concentration effective for obtaining the desired result is calculated. Determination of effective amounts for *in vivo* administration may also involve *in vitro* assays in which varying doses of agent are administered to cells in culture and the concentration of agent effective for achieving the desired result is determined in order to calculate the concentration required *in vivo*. Effective amounts may also be based on *in vivo* animal studies.

- 33 -

[0097] The present invention may be further illustrated by reference to the following examples.

EXAMPLES

Example 1 — Materials and Methods: Cell Lines

5 [0098] UMSCC47 and UMSCC74A were obtained from Dr. Thomas Carey at the University of Michigan. UPCI:SCC090 was provided by Dr. Susanne Gollin at the University of Pittsburgh (White et al., “The Influence of Clinical and Demographic Risk Factors on the Establishment of Head and Neck Squamous Cell Carcinoma Cell Lines,” *Oral Oncol.* 43(7):701–12 (2007), which is hereby
10 incorporated by reference in its entirety). UMSCC47, UMSCC74A, and UPCI:SCC090 cells were grown in DMEM containing 10% FBS, 2 mM glutamine, 100 mg/mL streptomycin and 100 U/mL penicillin and maintained in a humidified atmosphere of 5% CO₂ at 37°C.

Example 2 — Materials and Methods: Plasmid Construction and Transfection

15 [0099] The CH1 domain of p300 (nucleotides 332-417) was amplified by PCR (forward primer: 5'-GGATCCATGCCAGAGAAGCGCAAGCTCATCCAGC-3'; reverse primer: 5'-CTCGAGATCACCAGCATTTTTGAGGGGGAGACAC-3') and inserted into pcDNA3.1 between the BamHI and XhoI restriction enzyme sites. UMSCC47, UMUPCI:SCC090, and UMSCC74A cells were transfected with
20 pcDNA3.1/empty or pcDNA3.1/CH1 using Lipofectamine2000 (Invitrogen, Carlsbad, CA). Stable polyclonal populations were selected and maintained in the presence of G418 (Invitrogen).

Example 3 — Materials and Methods: Western Blot

[0100] Whole cell lysates were mixed with Laemmli loading buffer, boiled,
25 separated by SDS-PAGE, and transferred to a nitrocellulose membrane. Subsequently, immunoblot analyses were performed using antibodies specific to V5 (Invitrogen), p53 (sc-126, Santa Cruz Biotechnology, Santa Cruz, CA), or acetylated[K382]-p53 (2525, Cell Signaling Technology). The signal was developed using the SuperSignal Western Blotting Kit (Pierce, Rockford, IL).

- 34 -

Example 4 — Materials and Methods: p53 Transcriptional Activity

[0101] Cells were transfected with 100 ng of Cignal p53 reporter (SABiosciences, Valencia, CA) using Lipofectamine 2000. Cignal p53 reporter contains tandem repeats of the p53 consensus transcriptional response element. After 5 48 hours, cells were washed with cold PBS, lysed in passive lysis buffer (Promega), and measured for Firefly/Renilla dual luciferase activities in a luminometer using the Dual-Light System (Applied Biosystems, Foster City, CA). Renilla luciferase activity was normalized to Firefly luciferase activity to control for transfection efficiency. A modification to the protocol was used for compound treatment. UMSCC47 cells were 10 transfected with 100 ng of Cignal p53 reporter. After 24 hours, cells were treated with vehicle, CH1iB (10 μ M), cis-platinum (10 μ M), or combination of CH1iB (10 μ M) and cis-platinum (10 μ M) and measured for Firefly/Renilla dual luciferase activities 24 hours post-treatment.

Example 5 — Materials and Methods: Quantitative Real-Time PCR

15 [0102] Cells were extracted for total RNA using the TRIzol® reagent (Invitrogen) or TaqMan PreAmp Cells-to-CT kit (Applied Biosystems). Expression of p300, p53, p21, miR-34a and miR-200c were determined using the Applied Biosystems 7900HT Fast Real-Time PCR System with validated TaqMan gene expression assays (Applied Biosystems). p53, p300, and p21 expression were 20 normalized to GAPDH and miR-34a and miR-200c expression were normalized to RNU44 using the $\Delta\Delta C_t$ method.

Example 6 — Materials and Methods: Immunoprecipitation

[0103] Cells were lysed with NP buffer [50 mM Tris (pH 8.0), 150 mM NaCl, 0.5% deoxycholate, 0.1% SDS, and 1.0% NP-40] containing 1 $\times$ Protease Inhibitor 25 Cocktail (Roche, Switzerland) at 4°C with gentle rocking for 15 min. The supernatant was pre-cleared to block nonspecific binding with 50 μ L protein A/G Agarose beads (Pierce Biotechnology) that had been pre-washed with NP buffer before use. Equal amounts of anti-E6 antibody (Abcam), anti-p300 antibody (Millipore), or IgG antibody (Cell Signaling) were added to the respective samples. After 4 hours 30 incubation at 4°C, 50 μ L pre-washed protein A/G-agarose beads were added to each tube and immunoprecipitation was performed by rocking overnight at 4°C. The immunoprecipitated complexes were washed with NP buffer and then eluted using

- 35 -

2XSDS sample buffer. Eluted sample and 10% of input were resolved by SDS-PAGE for Western blot analysis with anti-E6, anti-p300, or anti-V5 antibodies.

Example 7 — Materials and Methods: Cell Proliferation, Clonogenic Survival, and Apoptosis

5 [0104] Cell proliferation was assessed using the MTT reagent (Roche Molecular Biochemicals, Nutley, NJ) to detect metabolic active cells. Absorbance was measured at 570nm in the Spectra Max 190 ELISA reader (Molecular Devices, Sunnyvale, CA) after overnight incubation. For clonogenic survival, 200 cells per well were plated in complete growth media and allowed to grow until visible colonies
10 were formed (14 days). Cell colonies were fixed with cold methanol, stained with 0.25% crystal violet in 25% methanol, washed, and air dried. For apoptosis, cells were harvested, washed with cold PBS, and co-stained with Annexin V and propidium iodide according to the manufacturer's protocol (ApoAlert Annexin V-FITC Apoptosis Kit; Clontech). Apoptotic cells were analyzed using BD FACS Calibur
15 (BD Biosciences Corporation, Franklin Lakes, NJ) at The Ohio State University Comprehensive Cancer Center Analytical Cytometry Core.

Example 8 — Materials and Methods: Tumor Incidence and Growth in Athymic Nude Mice

[0105] UMSCC47/empty and UMSCC47/CH1 cells were suspended in 50:50
20 DMEM:Matrigel and implanted subcutaneously into the left and right flanks of 6-week old athymic nude mice (8 mice/group), respectively. After 3 weeks, tumors were measured once a week using a digital caliper and tumor volumes were calculated using the formula $d1 \times d2 \times d3 \times 0.5236$, where "d" represents the three orthogonal diameters. Tumor growth and incidence were monitored for 49 days following tumor
25 cell implantation.

Example 9 — Materials and Methods: ALDH and CD44

[0106] Cells were assessed for ALDH activity using the ALDEFLUOR kit according to the manufacturer's protocol (Stem Cell Technologies, British Columbia, Canada). Cells were suspended in ALDEFLUOR assay buffer containing ALDH
30 substrate (bidipy-aminoacetaldehyde, 1 μ M per 1×10^6 cells) and incubated for 40 minutes at 37°C. For each experiment, a sample of cells was incubated with 50 mM of diethylaminobenzaldehyde (DEAB), a specific ALDH inhibitor, to serve as the negative control. For CD44 expression, cells were harvested and resuspended in

- 36 -

incubation buffer with PE-CD44 antibody (Abcam) or mouse PE-IgG (Abcam) for 50 minutes on ice. Suspensions were centrifuged at 300x g for 5 minutes at 4°C and resuspend in 0.5 mL of 1% paraformaldehyde solution for analysis. Fluorescence activated cell sorting (FACS) analyses were performed using BD FACS Calibur at
5 The Ohio State University Comprehensive Cancer Center Analytical Cytometry Core.

Example 10 — Materials and Methods: Tumorsphere Formation

[0107] Cells were harvested and seeded in a serum-free defined medium consisting of KSF medium supplemented with epidermal growth factor, basic fibroblast growth factor, insulin, and hydrocortisone in low-attachment plates
10 (Corning Incorporated, Corning, NY) for tumorspheres. Tumorsphere formation efficiency was calculated as the number of tumorspheres ($\geq 50 \mu\text{m}$ in diameter) formed in 7 days divided by the original number of cells seeded. Tumorsphere diameter was measured using NIS-Elements software.

Example 11 — Materials and Methods: Tumor Incidence with a Single Tumorsphere
15

[0108] Tumorsphere derived from UMSCC47 cells were generated and measured using NIS-Elements software. A single tumorsphere (60-80 μm in diameter) was suspended in 50:50 KSF:Matrigel and implanted subcutaneously into the flank of 6-week old NOD/SCID mice (n=11). In a separate set of animals,
20 parental UMSCC47 cells (1×10^3) were suspended in 50:50 DMEM:Matrigel and implanted subcutaneously into the flank of 6-week old NOD/SCID mice (n=10). Tumor incidence was monitored for 180 days following tumorsphere or tumor cell implantation.

Example 12 — Materials and Methods: Synthesis of Inhibitors

25 [0109] Synthetic helices were synthesized as previously described (Henchey et al., "Inhibition of Hypoxia Inducible Factor 1-Transcription Coactivator Interaction by a Hydrogen Bond Surrogate Alpha-Helix," *J. Am. Chem. Soc.* 132(3):941–43 (2010); Patgiri et al., "Solid-Phase Synthesis of Short Alpha-Helices Stabilized by the Hydrogen Bond Surrogate Approach," *Nat. Protoc.* 5(11):1857–65 (2010), which are
30 hereby incorporated by reference in their entirety). Compounds were purified by reverse-phase HPLC (see Figures 1A–C) and characterized by ESI-MS, as shown in Table 1 below.

Table 1. Mass Spectroscopic Characterization of CH1 Inhibitors

COMPOUND	SEQUENCE ^A	CALCULATED [M+H] ⁺	OBSERVED [M+H] ⁺
CH1IA	XTAA*DCEYNAR	1206.5	1206.4
CH1IB	XELA*RALDQ-NH ₂	1008.5	1008.5
CH1IB-MUT	XELA*RAADQ-NH ₂	966.5	966.5

X denotes 4-pentenoic acid; A* = N-allylalanine.

Example 13 — Materials and Methods: Statistical Analysis

5 [0110] Data were analyzed by two-tailed Student's *t*-test. P-values < 0.05 were considered significant.

Example 14 — Exogenous CH1 Reactivates p53 by Blocking the Association Between HPV16 E6 and p300

[0111] High-risk HPV E6 was reported to associate with p300 to inhibit p300-
10 mediated p53 acetylation (Zimmermann et al., “The Human Papillomavirus Type 16 E6 Oncoprotein Can Down-Regulate p53 Activity by Targeting the Transcriptional Coactivator CBP/p300,” *J. Virol.* 73(8):6209–19 (1999); Patel et al., “The E6 Protein of Human Papillomavirus Type 16 Binds to and Inhibits Co-Activation by CBP and p300,” *EMBO J.* 18(18):5061–72 (1999); Thomas & Chiang, “E6 Oncoprotein
15 Represses p53-Dependent Gene Activation Via Inhibition of Protein Acetylation Independently of Inducing p53 Degradation,” *Mol. Cell* 17(2):251–64 (2005), which are hereby incorporated by reference in their entirety). Acetylation is a critical regulatory mechanism to control p53 stability and transcriptional activity (Zimmermann et al., “The Human Papillomavirus Type 16 E6 Oncoprotein Can
20 Down-Regulate p53 Activity by Targeting the Transcriptional Coactivator CBP/p300,” *J. Virol.* 73(8):6209–19 (1999); Patel et al., “The E6 Protein of Human Papillomavirus Type 16 Binds to and Inhibits Co-Activation by CBP and p300,” *EMBO J.* 18(18):5061–72 (1999); Thomas & Chiang, “E6 Oncoprotein Represses p53-Dependent Gene Activation Via Inhibition of Protein Acetylation Independently
25 of Inducing p53 Degradation,” *Mol. Cell* 17(2):251–64 (2005); Ito et al., “MDM2-HDAC1-Mediated Deacetylation of p53 Is Required for Its Degradation,” *EMBO J.* 21(22):6236–45 (2002); Li et al., “Acetylation of p53 Inhibits Its Ubiquitination by

- 38 -

Mdm2,” *J. Biol. Chem.* 277(52):50607–11 (2002), which are hereby incorporated by reference in their entirety). Therefore, targeting the E6-p300 interaction may be a novel approach to reactivate p53 in HPV-positive HNSCC. Published work showed that high-risk HPV E6 binds to the CH1, CH3, and C-terminal domain of p300 (Patel et al., “The E6 Protein of Human Papillomavirus Type 16 Binds to and Inhibits Co-Activation by CBP and p300,” *EMBO J.* 18(18):5061–72 (1999), which is hereby incorporated by reference in its entirety). Thus, we determined if targeting one of the contact sites, the CH1 domain, is a tractable approach to block the association between E6 and p300 and reactivate p53. As shown in Figure 2A, exogenous CH1

10 squelched E6 to reduce the association between E6 and p300 in UMSCC47 and UPCI:SCC090, two HPV16-positive HNSCC cell lines. An accumulation of total p53 and an increase in acetylated p53 was revealed in CH1 overexpressing UMSCC47 (UMSCC47/CH1) and UPCI:SCC090 (SCC090/CH1) cells (Figure 2B). p53 transcription activity was elevated by 85% ($P<0.01$) and 50% ($P<0.01$) in

15 UMSCC47/CH1 and UPCI:SCC090/CH1 cells, respectively (Figure 2C). Overexpression of CH1 had no effect on *p53* and *p300* expression but enhanced the expression of three well-recognized p53 targets. *p21*, miR-34a, and miR-200c expression were increased by 114%, 323%, and 80% in UMSCC47/CH1 cells ($P<0.01$) and 39%, 134%, and 49% in UPCI:SCC090/CH1 cells ($P<0.01$),

20 respectively (Figure 2E). These results demonstrate that blocking the E6-p300 interaction is an efficient approach to reactive p53, through p53 accumulation and acetylation, in HPV-positive HNSCC.

Example 15 — Exogenous CH1 Has a Pleiotropic Anti-Tumor Effect in HPV16-Positive HNSCC

25 [0112] We determined if reactivation of p53 is sufficient to promote an anti-tumor response in HPV-positive HNSCC cells. Cell proliferation and clonogenic survival were reduced by 20% and 55% in UMSCC47/CH1 cells and 11% and 58% in UMUPCI:SCC090/CH1 cells, respectively (Figures 3A and 3B). Moreover, CH1 overexpression increased apoptosis by 60% in UMSCC47 cells and 27% in

30 UPCI:SCC090 cells (Figure 3C). A similar response in cell proliferation, clonogenic survival, and apoptosis was observed for empty vector-transfected UMSCC47 and UPCI:SCC090 cells treated with single agent cis-platinum (10 μ M). UMSCC47/CH1 and UPCI:SCC090/CH1 cells were dramatically more responsive to cis-platinum

- 39 -

(10 μ M) than UMSCC47/empty and UPCI:SCC090/empty cells. The combination of CH1 overexpression and cis-platinum treatment reduced cell proliferation by 46% and 23%, reduced clonogenic survival by 85% and 77%, and enhanced apoptosis by 194% and 157% in UMSCC47 and UPCI:SCC090, respectively ($P < 0.01$). Our results indicate that reactivation of p53 was sufficient to promote a broad anti-tumor response and furthermore, enhanced the efficacy of cis-platinum in HPV-positive HNSCC cells.

[0113] Next, we determined if CH1 overexpression modulates the *in vivo* tumorigenicity of HPV-positive HNSCC cells. Two different dilutions, 3×10^5 or 3×10^4 , of UMSCC47/empty and UMSCC47/CH1 cells were implanted in the flanks of athymic nude mice (Figure 3D). At a dilution of 3×10^5 cells, tumor incidence was the same between UMSCC47/empty and UMSCC47/CH1 cells however a difference ($P < 0.01$, $n = 6$) in tumor volume was observed. Mean tumor volume was 142 mm^3 for UMSCC47/empty and 67 mm^3 for UMSCC47/CH1 (Figure 3E). Interestingly, at a dilution of 3×10^4 cells, tumor incidence was 50% (4/8) for UMSCC47/empty but 0% (0/8) for UMSCC47/CH1 ($P < 0.02$). This observation suggests that the CIC population may be compromised in HPV16-positive HNSCC following p53 reactivation. CICs are a sub-set of cancer cells within the tumor with the exclusive capacity to divide and expand the CIC pool or to differentiate into heterogeneous non-tumorigenic cells that constitute the bulk of the tumor. CICs are postulated to be the unique cells responsible for disease recurrence and/or metastasis. Therefore, elimination of CICs may be essential to optimally manage cancer patients. ALDH and CD44 are two markers used to identify the CIC population in HNSCC (Prince et al., "Identification of a Subpopulation of Cells With Cancer Stem Cell Properties in Head and Neck Squamous Cell Carcinoma," *Proc. Nat'l Acad. Sci. U.S.A.* 104(3):973–78 (2007); Clay et al., "Single-Marker Identification of Head and Neck Squamous Cell Carcinoma Cancer Stem Cells With Aldehyde Dehydrogenase," *Head Neck* 32(9):1195–201 (2010); Chen et al., "Aldehyde Dehydrogenase 1 Is a Putative Marker for Cancer Stem Cells in Head and Neck Squamous Cancer," *Biochem. Biophys. Res. Commun.* 385(3):307–13 (2009), which are hereby incorporated by reference in their entirety). As shown in Figure 3F, CH1 overexpression reduced the ALDH^{high} population by 46% ($P < 0.01$) and CD44^{high} population by 31% in UMSCC47 cells ($P < 0.01$). Moreover, FACS analysis showed that CD44 levels were

- 40 -

reduced by 33% in UMSCC47/CH1 cells compared to UMSCC47/empty cells.

Tumorsphere formation is an *in vitro* assay to assess the CIC population.

Overexpression of CH1 in UMSCC47 cells inhibited tumorsphere formation efficiency by 42% ($P<0.01$) and reduced tumorsphere diameter by 25% ($P<0.01$)

5 (Figure 3G). To confirm the tumor initiating potential of tumorspheres, NOD/SCID mice were implanted with a single tumorsphere (mean diameter of 60-80 μm with ~ 100 cells) and monitored for tumor incidence over a 6 month period (Figure 3H). Mice implanted with a single tumorsphere had a tumor incidence rate of 55% (6/11). In contrast, all the mice implanted with 1×10^3 UMSCC47 cells failed to develop
10 tumors over a 6 month period. Our work demonstrate that reactivation of p53 suppress the *in vivo* tumorigenicity of HPV-positive HNSCC, in part through a reduction in the CIC population.

Example 16 — Exogenous CH1 Has a Pleiotropic Anti-Tumor Effect in HPV-Negative HNSCC

15 [0114] There is evidence that p300 is indispensable for MDM2-mediated p53 degradation (Grossman et al., “p300/MDM2 Complexes Participate in MDM2-Mediated p53 Degradation,” *Mol. Cell* 2(4):405–15 (1998); Kobet et al., “MDM2 Inhibits p300-Mediated p53 Acetylation and Activation by Forming a Ternary Complex With the Two Proteins,” *Proc. Nat’l Acad. Sci. U.S.A.* 97(23):12547–52
20 (2000), which are hereby incorporated by reference in their entirety). MDM2 was shown to bind to the CH1 domain of p300 and overexpression of CH1 was sufficient to enhance p53 stability in p53 wildtype human osteosarcoma U2OS cells (Grossman et al., “p300/MDM2 Complexes Participate in MDM2-Mediated p53 Degradation,” *Mol. Cell* 2(4):405–15 (1998); Kobet et al., “MDM2 Inhibits p300-Mediated p53
25 Acetylation and Activation by Forming a Ternary Complex With the Two Proteins,” *Proc. Nat’l Acad. Sci. U.S.A.* 97(23):12547–52 (2000), which are hereby incorporated by reference in their entirety). In line with these observations, ectopic expression of CH1 increased total and acetylated p53 in p53 wildtype, HPV-negative UMSCC74A HNSCC cells (Figure 4A). p53 transcription activity was elevated by 68% ($P<0.05$)
30 in UMSCC74A/CH1 compared to UMSCC74A/empty cells (Figure 4B). As shown in Figure 4C, the interaction between p300 and MDM2 in UMSCC74A cells was disrupted with the introduction of CH1. Overexpression of CH1 inhibited cell proliferation (48%, $P<0.01$) and clonogenic survival (70%, $P<0.01$), and increased

- 41 -

apoptosis (95%, $P < 0.05$) in UMSCC74A cells. In addition, UMSCC74A/CH1 cells were more responsive to the anti-tumor effects of cis-platinum (10 μ M) than UMSCC74A/empty cells. Our work demonstrates that exogenous CH1 blocked p300-MDM2 interaction, enhanced p53 activity, and promoted a broad anti-tumor response in HPV-negative HNSCC cells.

Example 17 — CH1iB, a Small Molecule CH1 Inhibitor, Preferentially Reactivates p53 in HPV16-Positive HNSCC

[0115] Our results showed that exogenous CH1 reactivated p53 in HPV-positive and HPV-negative HNSCC. We determined if small molecule CH1 ligands can function as competitive inhibitors to mask the E6 and MDM2 binding sites on p300 and block the E6-p300 and MDM2-p300 association. HIF-1 α recruits and binds to the CH1 domain of p300 to facilitate HIF-1 α -mediated transcription of target genes (Dames et al., “Structural Basis for Hif-1 Alpha /CBP Recognition in the Cellular Hypoxic Response,” *Proc. Nat’l Acad. Sci. U.S.A.* 99(8):5271–76 (2002); Freedman et al., “Structural Basis for Recruitment of CBP/p300 by Hypoxia-Inducible Factor-1 Alpha,” *Proc. Nat’l Acad. Sci. U.S.A.* 99(8):5367–72 (2002), which are hereby incorporated by reference in their entirety). A stabilized α -helical mimic, constrained by the hydrogen bond surrogate methodology (Patgiri et al., “A Hydrogen Bond Surrogate Approach for Stabilization of Short Peptide Sequences in Alpha-Helical Conformation,” *Acc. Chem. Res.* 41(10):1289–300 (2008), which is hereby incorporated by reference in its entirety), of HIF-1 α (CH1iA) was reported to function as a CH1 inhibitor and compete with endogenous HIF-1 α for p300 resulting in a reduction in HIF-1 α -mediated transcription of vascular endothelial growth factor (Henchey et al., “Inhibition of Hypoxia Inducible Factor 1-Transcription Coactivator Interaction by a Hydrogen Bond Surrogate Alpha-Helix,” *J. Am. Chem. Soc.* 132(3):941–43 (2010), which is hereby incorporated by reference in its entirety). The CH1 domain of p300 features multiple binding sites for individual α -helices (Dames et al., “Structural Basis for Hif-1 Alpha /CBP Recognition in the Cellular Hypoxic Response,” *Proc. Nat’l Acad. Sci. U.S.A.* 99(8):5271–76 (2002); Freedman et al., “Structural Basis for Recruitment of CBP/p300 by Hypoxia-Inducible Factor-1 Alpha,” *Proc. Nat’l Acad. Sci. U.S.A.* 99(8):5367–72 (2002), which are hereby incorporated by reference in their entirety) suggesting that targeting a distinct CH1-

- 42 -

binding partner may be a possibility. We tested the ability of synthetic helices that target binding site A and site B in CH1 in HPV-negative and HPV-positive HNSCC (Figure 5B). CH1iA and CH1iB did not modulate p53 activity and levels in UMSCC74A, a HPV-negative, p53 wildtype HNSCC cell line (Figures 5C and 5D).
 5 Expression of *p300*, *p53*, and p53-regulated genes were unchanged following CH1iA or CH1iB treatment in UMSCC74A cells (Figure 5E). In HPV-positive UMSCC47 cells, CH1iA had no effect whereas CH1iB enhanced p53 activity (71% increase, $P<0.01$), p53 accumulation, and acetylated p53 levels. A modest but significant increase in *p21*, miR-34a, and miR-200c expression was shown following CH1iB
 10 treatment (Figure 5E). Furthermore, the association between E6 and p300 was reduced with CH1iB but not with CH1iA treatment in HPV-positive HNSCC cells (Figure 5F). These results reveal that the critical binding contacts between E6 and CH1 are located within or in proximity to binding site B of the CH1 domain. In Figure 5G, CH1iA was inactive but CH1iB inhibited the proliferation of HPV-
 15 positive UMSCC47 cells as single-agent and potentiated the anti-proliferative efficacy of cis-platinum. CH1iA and CH1iB had no effect and did not augment the efficacy of cis-platinum on the proliferation of HPV-negative UMSCC74A cells and human normal IMR90 fibroblasts. Taken together, our work demonstrates that targeting binding site B in CH1 with CH1iB preferentially reactivates p53 in HPV-positive
 20 HNSCC cells by disrupting the association between E6 and p300.

Example 18 — CH1iB Potentiates the Efficacy of *cis*-Platinum in HPV16-Positive HNSCC

[0116] Our results showed that introduction of CH1 potentiated the effects of cis-platinum on cell proliferation, clonogenic survival, and apoptosis in HPV-positive
 25 HNSCC. In addition, CH1iB enhanced the anti-proliferative action of cis-platinum in UMSCC47 cells. As shown in Figures 6A–F, CH1iB potentiated the effect of cis-platinum on p53 accumulation, acetylation, and activity. Expression of p21, miR-34a, and miR-200c was dramatically higher ($P<0.01$) with the combination treatment than with either single-agent treatment (Figure 6C). Compared to vehicle-treated
 30 UMSCC47 cells, CH1iB reduced clonogenic survival by 35% and tumorsphere formation by 20%, and enhanced apoptosis by 353% ($P<0.01$). Furthermore, the combination regimen was highly active and almost completely ablated the clonogenic survival (91% inhibition, $P<0.01$) of HPV16-positive HNSCC cells. Apoptosis

- 43 -

induced by the combination treatment was increased by 984% ($P < 0.01$) and 443% ($P < 0.01$) compared to CH1iB and cis-platinum, respectively (Figure 6D). In addition, tumorsphere formation was suppressed by a greater extent with the combination regimen than single-agent CH1iB or cis-platinum (Figure 6F). CH1iB-mut, a
5 designed specificity control for CH1iB in which one energetically important leucine residue is mutated to alanine, showed a minimal but significant increase (14% increase, $P < 0.05$) in p53 activity but, importantly, had no effect as single-agent or in combination with cis-platinum to inhibit cell proliferation in UMSCC47 cells (Figures 7A–B). These results indicate that CH1iB, a CH1 inhibitor, potentiates the anti-tumor
10 activity of cis-platinum in HPV-positive HNSCC.

Discussion of Examples 1–18

[0117] High-risk HPV is recognized as an etiological agent for the pathogenesis of anogenital and head and neck squamous cell carcinomas. HPV E6 inactivates p53 through two distinct and independent pathways. It is well recognized
15 that E6 complexes with E6AP to form an active E3-ubiquitin ligase to target p53 for proteasome-dependent proteolysis (Talis et al., “The Role of E6AP in the Regulation of p53 Protein Levels in Human Papillomavirus (HPV)-Positive and HPV-Negative Cells,” *J. Biol. Chem.* 273(11):6439–45 (1998), which is hereby incorporated by reference in its entirety). A second but much more under-appreciated mechanism is
20 that E6 associates with the p300–p53 complex to block p300-mediated acetylation and activation of p53 (Thomas & Chiang, “E6 Oncoprotein Represses p53-Dependent Gene Activation Via Inhibition of Protein Acetylation Independently of Inducing p53 Degradation,” *Mol. Cell* 17(2):251–64 (2005), which is hereby incorporated by reference in its entirety). p300 acetylates p53 at multiple lysine residues, including
25 K370, 372, 381, and 382 (Gu & Roeder, “Activation of p53 Sequence-Specific DNA Binding by Acetylation of the p53 C-Terminal Domain,” *Cell* 90(4):595–606 (1997), which is hereby incorporated by reference in its entirety). Acetylation was shown to control p53 function through multiple mechanisms, including an increase in protein stability, tetramerization, DNA binding, and co-activator recruitment (Thomas &
30 Chiang, “E6 Oncoprotein Represses p53-Dependent Gene Activation Via Inhibition of Protein Acetylation Independently of Inducing p53 Degradation,” *Mol. Cell* 17(2):251–64 (2005); Li et al., “Acetylation of p53 Inhibits Its Ubiquitination by

- 44 -

Mdm2,” *J. Biol. Chem.* 277(52):50607–11 (2002); Gu & Roeder, “Activation of p53 Sequence-Specific DNA Binding by Acetylation of the p53 C-Terminal Domain,” *Cell* 90(4):595–606 (1997), which are hereby incorporated by reference in their entirety).

5 [0118] Several groups reported that reactivation of p53 is achievable in HPV-positive cervical carcinomas using different strategies to reduce E6 or E6AP levels (Beerheide et al., “Potential Drugs Against Cervical Cancer: Zinc-Ejecting Inhibitors of the Human Papillomavirus Type 16 E6 Oncoprotein,” *J. Nat’l Cancer Inst.* 91(14):1211–20 (1999); Beerheide et al., “Inactivation of the Human Papillomavirus-
10 16 E6 Oncoprotein by Organic Disulfides,” *Bioorg. Med. Chem.* 8(11):2549–60 (2000); Courtete et al., “Suppression of Cervical Carcinoma Cell Growth by Intracytoplasmic Codelivery of Anti-Oncoprotein E6 Antibody and Small Interfering RNA,” *Mol. Cancer Ther.* 6(6):1728–35 (2007); Beer-Romero et al., “Antisense Targeting of E6AP Elevates p53 in HPV-Infected Cells but Not in Normal Cells,”
15 *Oncogene* 14(5):595–602 (1997); Koivusalo et al., “Activation of p53 in Cervical Cancer Cells by Human Papillomavirus E6 RNA Interference Is Transient, but Can Be Sustained by Inhibiting Endogenous Nuclear Export-Dependent p53 Antagonists,” *Cancer Res.* 66(24):11817–24 (2006); Zhao et al., “Rescue of p53 Function by Small-Molecule RITA in Cervical Carcinoma by Blocking E6-Mediated Degradation,”
20 *Cancer Res.* 70(8):3372–81 (2010), which are hereby incorporated by reference in their entirety). Treatment with E6AP anti-sense oligonucleotides accumulated p53 but did not promote apoptosis (Beer-Romero et al., “Antisense Targeting of E6AP Elevates p53 in HPV-Infected Cells but Not in Normal Cells,” *Oncogene* 14(5):595–602 (1997), which is hereby incorporated by reference in its entirety). These authors
25 suggest that a threshold level of p53 levels may be required for p53-mediated apoptosis. An alternate explanation is the ablation of E6AP may be inefficient to reactivate p53 since E6 is still available to suppress p300-mediated acetylation and activation of p53. Co-delivery of a HPV16 E6 antibody and E6 siRNA enhanced p53 levels and decreased clonogenic survival; however, an apoptotic response was not
30 detected (Courtete et al., “Suppression of Cervical Carcinoma Cell Growth by Intracytoplasmic Codelivery of Anti-Oncoprotein E6 Antibody and Small Interfering RNA,” *Mol. Cancer Ther.* 6(6):1728–35 (2007), which is hereby incorporated by reference in its entirety). An interesting study showed that siRNA-mediated ablation

- 45 -

of E6 results in a transient increase in p53 protein and activity despite a sustained E6 knockdown suggesting that a compensatory p53 degradation and/or inactivation mechanism is quickly triggered in HPV-positive cervical carcinomas cells under these experimental conditions (Koivusalo et al., “Activation of p53 in Cervical Cancer Cells by Human Papillomavirus E6 RNA Interference Is Transient, but Can Be Sustained by Inhibiting Endogenous Nuclear Export-Dependent p53 Antagonists,” *Cancer Res.* 66(24):11817–24 (2006), which is hereby incorporated by reference in its entirety).

[0119] Disruption of E6-p300 association is an approach that has not been utilized to reactivate p53 in HPV-positive carcinomas. Restoration of p300-mediated acetylation of p53 may be an ideal strategy since acetylation controls p53 function through multiple mechanisms, including stability and transcriptional activation. Our results with stable CH1 overexpressing HNSCC cells indicate that targeting the E6-p300 interaction is sufficient to maintain elevated p53 accumulation, acetylation, and activity *ad infinitum*. Exogenous CH1 inhibits cell proliferation and clonogenic survival and enhances apoptosis in HPV-positive HNSCC. Importantly, the *in vivo* tumorigenicity of UMSCC47/CH1 cells is severely compromised in part through a reduction in the CIC population. Thus, our data showed that restoration of p300-mediated p53 acetylation induces a sustained p53 reactivation and anti-tumor response in HPV-positive HNSCC.

[0120] It was reported that p300 functions as a scaffold for MDM2 and p53 to facilitate MDM2-mediated degradation of p53 (Grossman et al., “p300/MDM2 Complexes Participate in MDM2-Mediated p53 Degradation,” *Mol. Cell* 2(4):405–15 (1998), which is hereby incorporated by reference in its entirety). Overexpression of the CH1 domain of p300 enhanced p53 accumulation in human osteosarcoma U2OS cells presumably by blocking the physical interaction between p300 and MDM2 (Grossman et al., “p300/MDM2 Complexes Participate in MDM2-Mediated p53 Degradation,” *Mol. Cell* 2(4):405–15 (1998), which is hereby incorporated by reference in its entirety). Also, binding of MDM2 to the p300-p53 complex blocked p300-mediated acetylation and activation of p53 (Kobet et al., “MDM2 Inhibits p300-Mediated p53 Acetylation and Activation by Forming a Ternary Complex With the Two Proteins,” *Proc. Nat’l Acad. Sci. U.S.A.* 97(23):12547–52 (2000), which is hereby incorporated by reference in its entirety). These results indicate that the MDM2-p300-p53 complex is intimately involved in p53 turnover, acetylation, and

- 46 -

activation. In direct support, our results showed that exogenous CH1 disrupts MDM2-p300 association and increases p53 levels and activity in p53 wildtype, HPV-negative UMSCC74A HNSCC cells. In addition, CH1 overexpression sensitized UMSCC74A cells to the anti-tumor efficacy of cis-platinum. These results

5 demonstrate that targeting the CH1 domain of p300 may be a tractable approach to enhance p53 activity in HNSCC cells with wildtype p53, regardless of HPV status, albeit through distinct mechanisms. CH1iB, but not CH1iA, blocked E6-p300 association and reactivated p53 in HPV-positive HNSCC indicating that binding site B in the CH1 domain contains the critical contacts for E6 and p300 interaction.

10 Interestingly, selective targeting of the CH1 domain with CH1iA and CH1iB did not enhance p53 accumulation and activity in UMSCC74A cells. The preferential activity of CH1iB for HPV-positive HNSCC over HPV-negative HNSCC suggests that the CH1-binding interface for E6 may be distinct from the CH1-binding interface for MDM2. Another possibility is that MDM2 may have a tighter binding association for

15 p300 than E6 and thus, CH1iB and CH1iA were unable to successfully compete against MDM2 for p300 binding. In any event, our work reveals that CH1iB preferentially reactivates p53 activity in HPV-positive HNSCC cells providing initial evidence that discrete chemical targeting of the CH1 domain of p300 can be realized.

[0121] The role of p53 in normal stem cell regulation is established and is

20 beginning to emerge for CICs. Inhibition of p53 dramatically enhanced the transformation efficiency of differentiated cells into induced pluripotent stem cells (Hong et al., "Suppression of Induced Pluripotent Stem Cell Generation by the p53-p21 Pathway," *Nature* 460(7259):1132–35 (2009); Kawamura et al., "Linking the p53 Tumour Suppressor Pathway to Somatic Cell Reprogramming," *Nature*

25 460(7259):1140–44 (2009); Utikal et al., "Immortalization Eliminates a Roadblock During Cellular Reprogramming Into iPS Cells," *Nature* 460(7259):1145–48 (2009), which are hereby incorporated by reference in their entirety). Loss of p53 favored self-renewal, symmetric cell division, of mammary stem cells resulting in an expansion of the stem cell population (Cicalese et al., "The Tumor Suppressor p53

30 Regulates Polarity of Self-Renewing Divisions in Mammary Stem Cells," *Cell* 138(6):1083–95 (2009), which is hereby incorporated by reference in its entirety). Two p53 targets, miR-34a and p21, were shown to contribute to p53 repression of induced pluripotent stem cells (Hong et al., "Suppression of Induced Pluripotent Stem

- 47 -

Cell Generation by the p53-p21 Pathway,” *Nature* 460(7259):1132–35 (2009) ; Kawamura et al., “Linking the p53 Tumour Suppressor Pathway to Somatic Cell Reprogramming,” *Nature* 460(7259):1140–44 (2009); Choi et al., “miR-34 miRNAs Provide a Barrier for Somatic Cell Reprogramming,” *Nat. Cell Biol.* 13(11):1353–60 (2011), which are hereby incorporated by reference in their entirety). Additionally, miR-34a blocked prostate CIC expansion (Liu et al., “The MicroRNA miR-34a Inhibits Prostate Cancer Stem Cells and Metastasis by Directly Repressing CD44,” *Nat. Med.* 17(2):211–15 (2011), which is hereby incorporated by reference in its entirety). Loss of p53 in mammary epithelial cells led to reduced miR-200c expression resulting in an increase in EMT-associated CIC population (Chang et al., “p53 Regulates Epithelial-Mesenchymal Transition and Stem Cell Properties Through Modulating miRNAs,” *Nat. Cell Biol.* 13(3):317–23 (2011), which is hereby incorporated by reference in its entirety). Our results are in line with these studies and further support the link between p53 and CICs. We show that reactivation of p53 in HPV-positive HNSCC increase the expression of *p21*, miR-34a, and miR-200c and reduce the CIC population. These observations suggest that the p53-p21/miR-34a/miR-200c circuitry to limit normal stem cell expansion, either through reprogramming or self-renewal, can be triggered in HPV-positive HNSCC to block CIC expansion through p53 reactivation. It is unclear at this time whether the reduction in the CIC population is due to a shift in favor of asymmetric CIC division and/or differentiation of CICs. Additional work will be necessary to address this question and to dissect the contributions of p21, miR-34a, and miR-200c in controlling the CIC population.

[0122] High-dose cis-platinum-based therapy is the standard of care for definitive treatment of HPV-positive cancers, but is associated with high toxicities and difficult for patients to tolerate (Pan et al., “Pharmacotherapy of Head and Neck Squamous Cell Carcinoma,” *Expert Opin. Pharmacother.* 10(14):2291–302 (2009), which is hereby incorporated by reference in its entirety). Treatment-associated toxicities from high-dose cis-platinum-based therapy are a major concern and have prompted considerable discussion whether alternate treatment or de-intensification of treatment should be offered for the HPV-positive HNSCC population. Considering there are limited clinical options for HPV-positive HNSCC at this time, alternative treatment strategies are critically needed. We showed that CH1iB, a CH1 inhibitor,

- 48 -

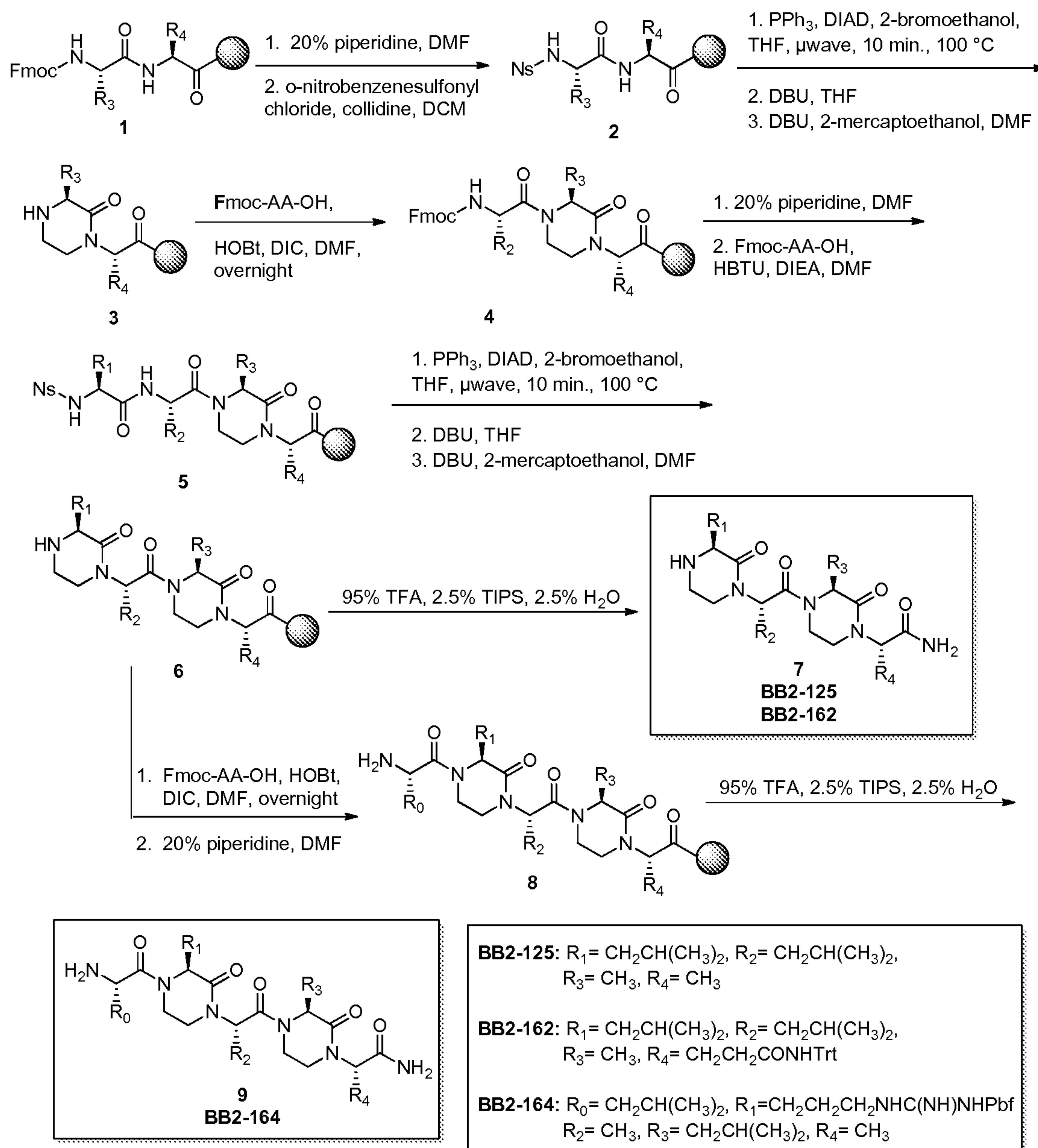
reactivates p53 in HPV-positive HNSCC. Single-agent CH1iB exhibits broad anti-cancer activity to suppress cell proliferation and clonogenic survival and enhance apoptosis in UMSCC47 and UPCI:SCC090 cells. Interestingly, CH1iB potentiates cis-platinum-mediated p53 activity and anti-tumor efficacy. HPV-positive HNSCC cells are almost completely eliminated following treatment with the combination of CH1iB and cis-platinum. Based on these results, we speculate that fewer cycles or a tapered dose of cis-platinum may be sufficient in the presence of a CH1 inhibitor to effectively manage HPV-positive HNSCC patients with a better toxicity profile. Our data strongly supports further development of CH1 inhibitors as p53 reactivation therapeutics for HPV-positive HNSCC.

[0123] The E6 viral protein is conserved across HPV serotypes (*E.g.*, Scheffner et al., “The E6 Oncoprotein Encoded by Human Papillomavirus Types 16 and 18 Promotes the Degradation of p53,” *Cell* 63(6):1129–36 (1990), which is hereby incorporated by reference in its entirety). Thus, our results reveal that targeting the E6-p300 association is a novel approach to reactivate p53 in HPV-positive cancer cells, such as HNSCC. CH1iB, a small molecule CH1 inhibitor, reactivates p53 and potentiates the anti-tumor activity of cis-platinum in HPV-positive HNSCC cells. Our work indicates that CH1 domain inhibitors, like those described herein, represent a novel class of p53 reactivation therapeutics for HPV-positive cancers.

Example 19 — Synthesis of Oligooxopiperazines

[0124] The oligooxopiperazines were synthesized via solid phase synthesis as described in U.S. Patent Application No. 12/917,176 to Arora et al., which is hereby incorporated by reference in its entirety, as shown in Scheme 1 below.

- 49 -



Scheme 1

[0125] Dipeptide **1** was synthesized by standard Fmoc solid phase peptide synthesis on Knorr resin in a solid phase reaction vessel. The Fmoc group was removed by treatment with 20% piperidine in dimethylformamide (DMF) and the resin was washed sequentially with DMF, dichloromethane (DCM), methanol (MeOH), and diethyl ether and dried under vacuum. *o*-Nitrobenzenesulfonyl chloride (Ns-Cl, 10 eq) and collidine (10 eq) were dissolved in dry DCM and added to the reaction vessel. The mixture was shaken for 2 hours at 23°C to obtain **2**. The resin

- 50 -

containing **2** was then washed sequentially with DMF, DCM, MeOH, and diethyl ether and dried for 12 hours under vacuum.

[0126] The resin containing **2** was transferred to a glass microwave tube (CEM). Triphenylphosphine (PPh₃, 5 eq) was added and the tube was flushed with
5 nitrogen gas for 30 minutes. Tetrahydrofuran (THF), diisopropylazodicarboxylate (DIAD, 10 eq), and 2-bromoethanol (10 eq) were added and the reaction mixture was subjected to microwave irradiation (200 watts, 250 psi) for 10 minutes at 100°C. Resin was washed sequentially with THF, DMF, and DCM. Next, the resin was transferred to a solid phase vessel, treated with 1,8-diazabicyclo[5.4.0]undec-7-ene
10 (DBU) in THF for 2 hours, then washed with THF, DMF, DCM, and diethyl ether and dried for 30 minutes followed by treatment with DBU and 2-mercaptoethanol in DMF for 2 hours. The resin, now containing **3**, was washed with DMF, DCM, MeOH, and diethyl ether and dried.

[0127] The desired pre-activated Fmoc-amino acid (Fmoc-AA-OH) was
15 added to the resin containing **3** and the mixture was shaken at 23°C for 12 hours. Nosyl protection and the ring formation steps were repeated to obtain oxopiperazine dimers BB2-125 and BB2-162 after cleavage from the resin with 95% trifluoroacetic acid (TFA), 2.5% water, and 2.5% triisopropylsilane (TIPS). For the formation of BB2-164, resin bound oxopiperazine dimer **6** was treated with a preactivated Fmoc-
20 amino acid in DMF followed by cleavage from resin. Analytical HPLC traces for BB2-125, BB2-162, and BB2-164 are shown in Figure 8, Figure 9, and Figure 10, respectively.

Example 20 — MTT Cellular Viability

[0128] Human breast carcinoma (MCF7) or human lung carcinoma (A549)
25 cells were seeded into 96-well plates (Greiner) at a density of 5,000–10,000 cells in 200 µL of the fresh media per well; MCF7 cells in RPMI media (Gibco) with 10% FBS (Irvine Scientific), A549 cells (ATCC) in F-12K media (ATCC) with 2% FBS (Irvine Scientific). The plates were then placed into the incubator (37°C, 5-10% CO₂) until the desired confluency (ca. 70%) was reached (ca. 24–72 hours). After that, the
30 media in all the wells was replaced with a solution of BB2-125 or BB2-162 (150 µL for 48 hour study, 200 µL for 72 hour study, respectively) in the appropriate media. The plates were then maintained for 48 or 72 hours at 37°C, 5–10% CO₂, in the

- 51 -

incubator. After 48 (or 72) hours of incubation with the compound, MTT (5mg/ml in PBS, Sigma-Aldrich) was added into every well (10% v/v) and mixed carefully and thoroughly. The plates were further incubated for 3–4 hours at 37°C and 5–10% CO₂, and then the media was removed carefully and completely. Resulting purple precipitate was dissolved in DMSO (200 µL/well) and the absorption of each well was measured at 562 nm using microplate reader (Synergy II, BioTek, Inc).

Example 21 — BB2-162 Preferentially Reactivates p53 in HPV16-Positive HNSCC

[0129] We tested the ability of BB2-162, an oligooxopiperazine that targets binding site B in CH1, to modulate p53 transcriptional activity in HPV-positive HNSCC (Figure 11). BB2-162 enhanced p53 activity in UMSCC74 to an even greater extent than did CH1iB.

Discussion of Examples 19–21

[0130] Oligooxopiperazines are non-peptidic helix mimetics that feature a chiral backbone (see Figure 12). These compounds are easily synthesized from α-amino acids allowing rapid diversification of the scaffold. Conformational analysis of oxopiperazine dimers and trimers composed of α-amino acids suggests that these compounds span the length of 7–10mer α-helices. Crystal structures of related compounds, quantum mechanical calculations, and molecular mechanics simulations have been used to better understand the conformation of the oxopiperazine scaffold. Results support the hypothesis that oligooxopiperazines provide a stable and geometrically appropriate helix mimetic.

[0131] As shown in Figure 13, an efficient microwave-assisted solid phase synthesis of oxopiperazine oligomers has been developed (U.S. Patent Application No. 12/917,176 to Arora et al., which is hereby incorporated by reference in its entirety). The key step in the synthesis involves ring closure, and Mitsunobu conditions with nosyl-protected amino acid residues was found to offer the highest yields.

[0132] As shown in Figure 14, four potential options—based on the predicted lowest energy conformation—for the design of oligooxopiperazines to mimic functionality on one face of a two-turn helix were contemplated. These configurations are termed “Dimers A–C” and “Trimer” in Figure 14. The Dimer configurations differ from each other in the overlay of oxopiperazine residues on to

- 52 -

the canonical helix; Dimer C contains an extra N-terminal residue. In Dimer A and B, oxopiperazine positions R_1 and R_2 overlay onto the i and $i+4$ residues whereas in Dimer C positions R_1 and R_3 overlay with these positions. Dimers A and B differ in the relative positioning of the axis of the oxopiperazine scaffold; their overlays suggest that R_4 may align with the $i+6$ or the $i+7$ residues. Dimer C design uses a different alignment, with an N-terminal amino acid residue providing the first contact.

[0133] As described herein and illustrated in Figure 15 and Figure 16, HIF-1 α mimetics for dimer B (Figure 10) and dimer C (Figure 11) helical overlays have been designed. As shown in Figure 17, four analogs were designed based on

10 computational analysis. BB2-164 oligooxopiperazine is based on the dimer C design. BB2-162 is based on the dimer B design. BB2-125 is a negative control of BB2-162, where the glutamine residue is replaced with an alanine group. BB2-282 is also a negative control and displays no critical residues. In designing mimetics, the natural amino acid sequence of helix α B (PDB code 1L8C, residues 139–147) of HIF-1 α was
15 utilized, as illustrated in Figure 18. Figure 18 shows an overlay of oligooxopiperazine BB2-162 and helix α B.

[0134] Cellular viability MTT assays were performed to evaluate the toxicity of the oligooxopiperazines. As shown in Figure 19, oligooxopiperazines exert no toxic effect on HeLa cells up to concentrations of 20 μ M, with GI₅₀ values of 30 μ M.

20 [0135] BB2-162 was evaluated for its ability to reactivate p53 in HPV-positive HNSCC cells. BB2-162 was found to be even more effective than CH1iB. This indicates that oligooxopiperazines that mimic the helix α B are also CH1 domain inhibitors suitable for restoring p53 activity. Thus, it is expected that oligooxopiperazines that mimic the helix α B of HIF1 α , like those described herein,
25 can also be used as p53 reactivation therapeutics for HPV-positive cancers.

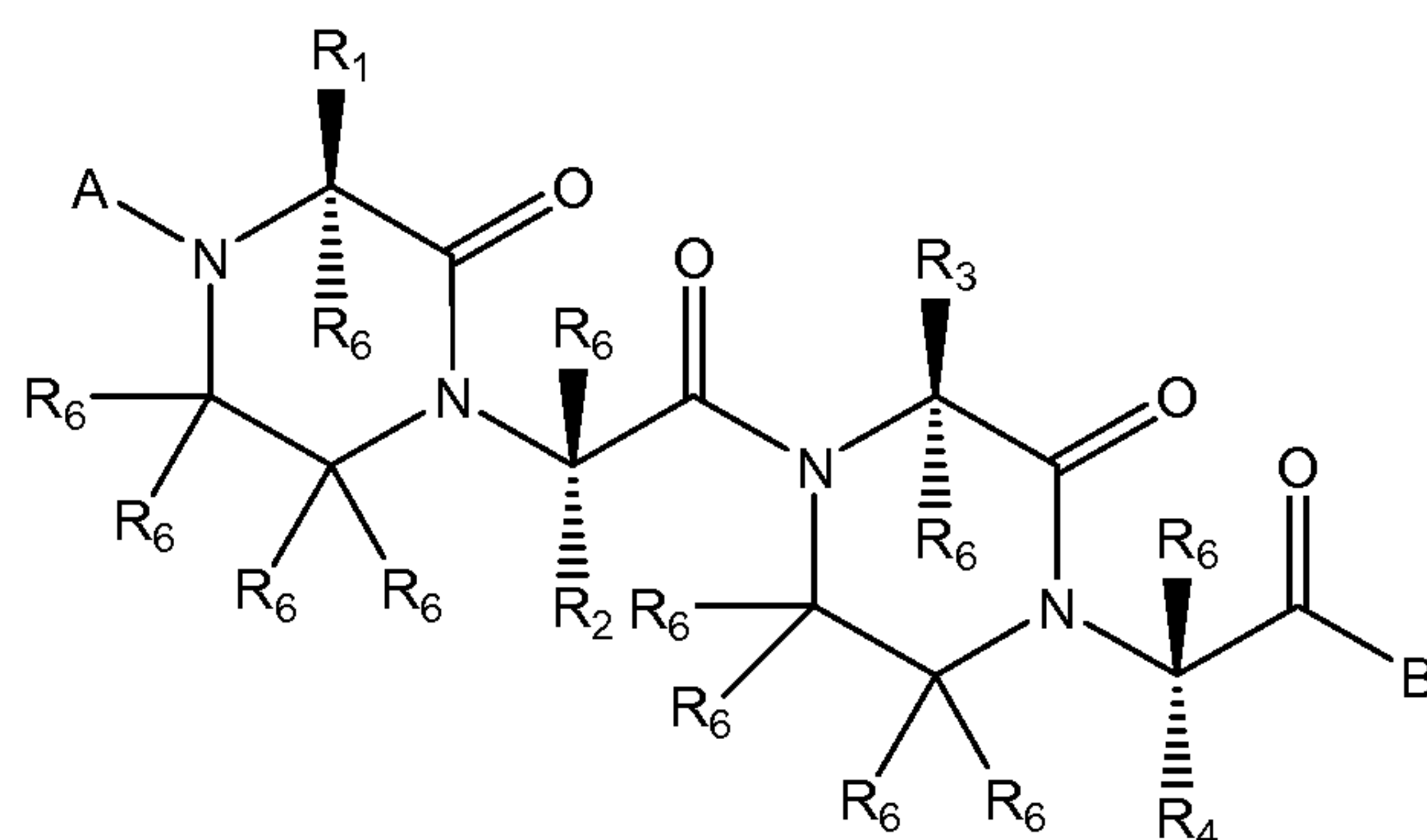
[0136] Although preferred embodiments have been depicted and described in detail herein, it will be apparent to those skilled in the relevant art that various modifications, additions, substitutions, and the like can be made without departing from the spirit of the invention and these are therefore considered to be within the
30 scope of the invention as defined in the claims which follow.

WHAT IS CLAIMED:

1. A method of treating or preventing in a subject a disorder mediated by interaction of E6 with CREB-binding protein and/or p300, said method comprising:

5 administering to the subject an oligooxopiperazine that substantially mimics helix α B of the C-terminal transactivation domain of Hypoxia-Inducible Factor 1 α under conditions effective to treat or prevent the disorder.

2. The method according to claim 1, wherein the oligooxopiperazine is an oligooxopiperazine of Formula I:



I, wherein:

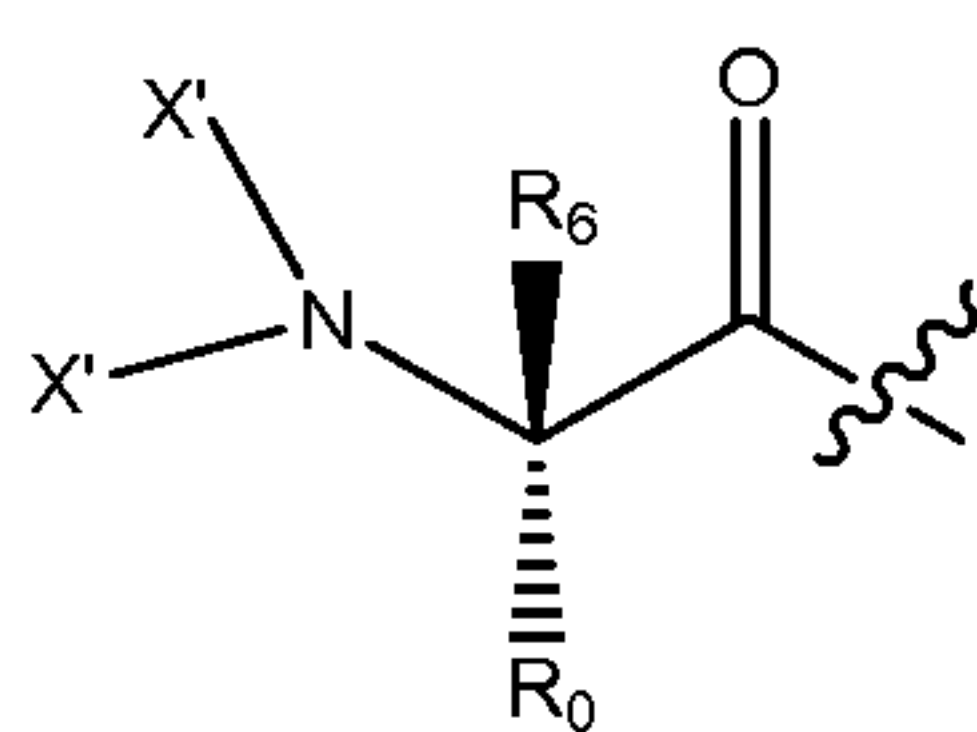
each of R_1 , R_2 , R_3 , and R_4 is independently an amino acid side chain, H, $N(R)_2$, OR, halogen, an alkyl, or an aryl; wherein each R is independently H, an alkyl, or an aryl;

each R_6 is independently H, $N(R)_2$, OR, halogen, an alkyl, or an aryl; wherein each R is independently H, an alkyl, or an aryl;

A is X_1 or C, wherein:

X_1 is H, COR' , CO_2R' , $CONR'$, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a protecting group for protection of an amine, a targeting moiety, or a tag; wherein R' is H, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; and

C is a moiety of the formula



, wherein:

- 54 -

each X' is independently H, COR' , CO_2R' , $CONR'$, $N(R'')_2$, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; wherein:

5 R' is H, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; and
each R'' is independently H, CO_2R' , $CONR'$, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag;

10 R_0 is an amino acid side chain, H, $N(R)_2$, OR, halogen, an alkyl, or an aryl; wherein each R is independently H, an alkyl, or an aryl; and

R_6 is H, $N(R)_2$, OR, halogen, an alkyl, or an aryl; wherein each R is independently H, an alkyl, or an aryl; and

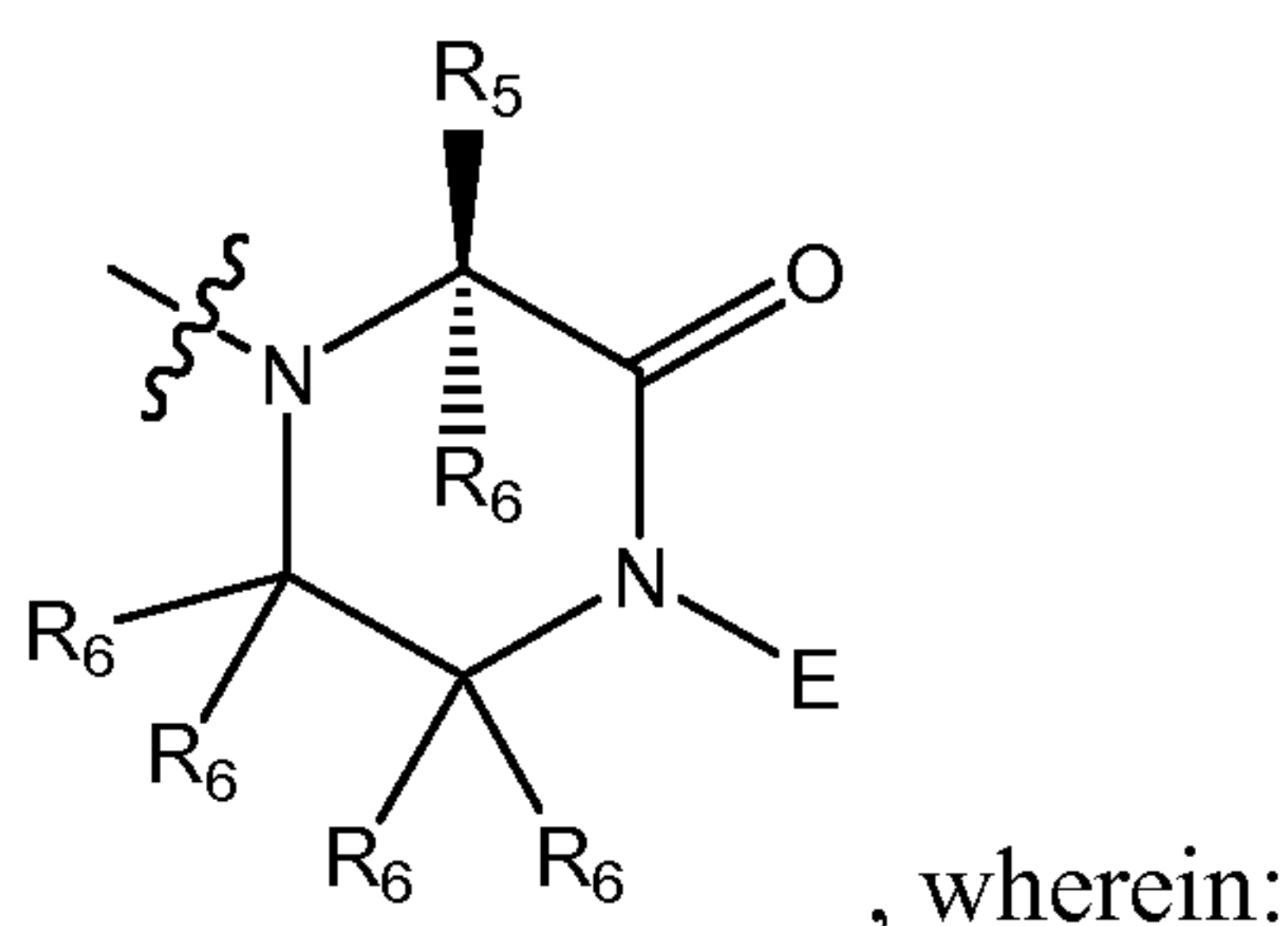
B is Y or D, wherein:

15 Y is OR' , COR' , $N(R''')_2$, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a protecting group for protection of a carboxylic acid, a targeting moiety, or a tag; wherein:

R' is H, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; and

20 each R''' is independently H, CO_2R' , $CONR'$, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; and

D is a moiety of the formula



25 R_5 is an amino acid side chain, H, $N(R)_2$, OR, halogen, an alkyl, or an aryl; wherein each R is independently H, an alkyl, or an aryl;

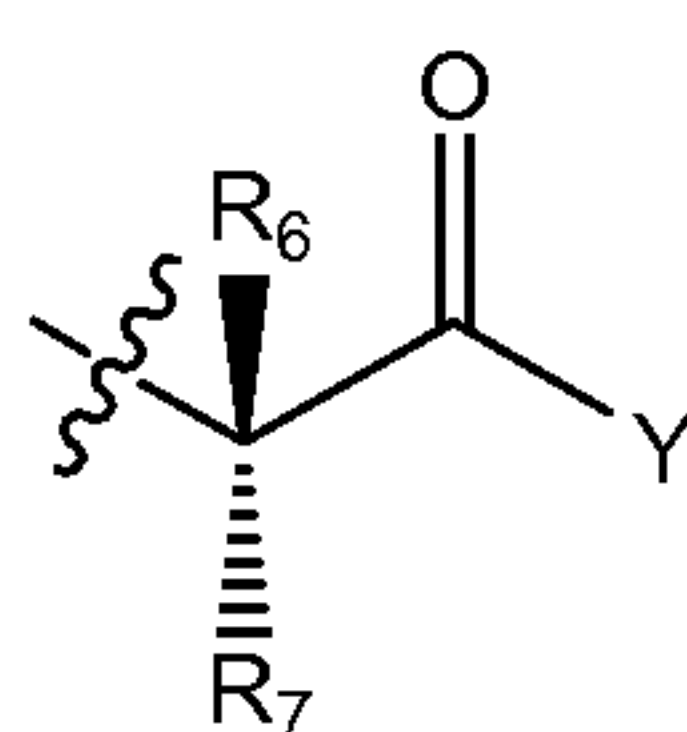
R_6 is H, $N(R)_2$, OR, halogen, an alkyl, or an aryl; wherein each R is independently H, an alkyl, or an aryl; and

- 55 -

E is X_2 or F, wherein:

X_2 is H, COR' , CO_2R' , $CONR'$, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a protecting group for protection of an amine, a targeting moiety, or a tag; wherein R' is H, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; and

F is a moiety of the formula



, wherein:

R_6 is H, $N(R)_2$, OR, halogen, an alkyl, or an aryl;

wherein each R is independently H, an alkyl, or an aryl;

R_7 is an amino acid side chain; and

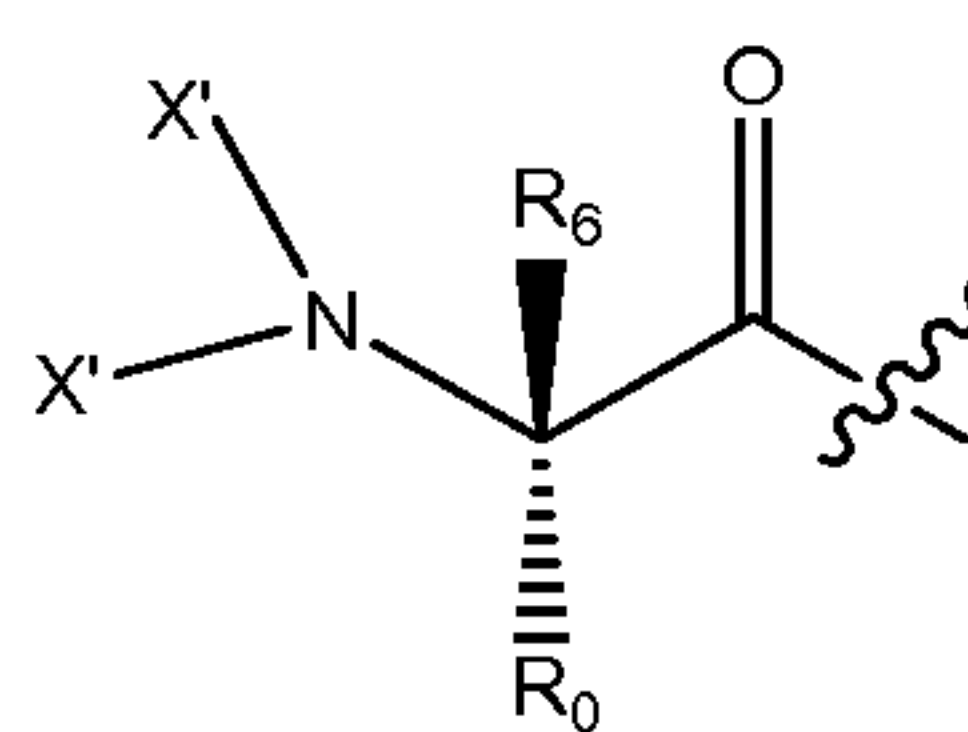
Y is OR' , COR' , $N(R''')_2$, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a protecting group for protection of a carboxylic acid, a targeting moiety, or a tag; wherein:

R' is H, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; and

each R''' is independently H, CO_2R' , $CONR'$, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag.

3. The method according to claim 2, wherein:

(i) R_1 and R_2 are hydrophobic and R_4 is a hydrogen bond acceptor or hydrogen bond



donor or A is a moiety of formula

, R_0 and R_3 are

hydrophobic, and R_4 is a hydrogen bond acceptor or hydrogen bond donor;

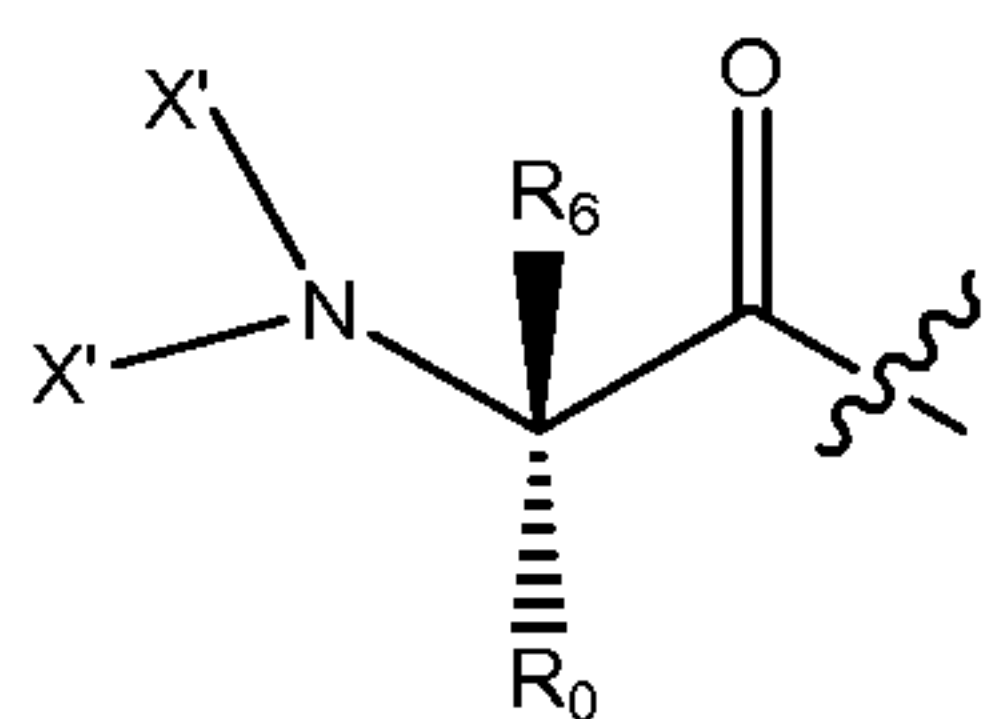
- 56 -

- (ii) each R'' is independently H, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; and
- (iii) B is not D.

4. The method according to claim 3, wherein R₁ and R₂ are hydrophobic and R₄ is a hydrogen bond acceptor or hydrogen bond donor.

5. The method according to claim 4, wherein the oligooxopiperazine is BB2-125 or BB2-162.

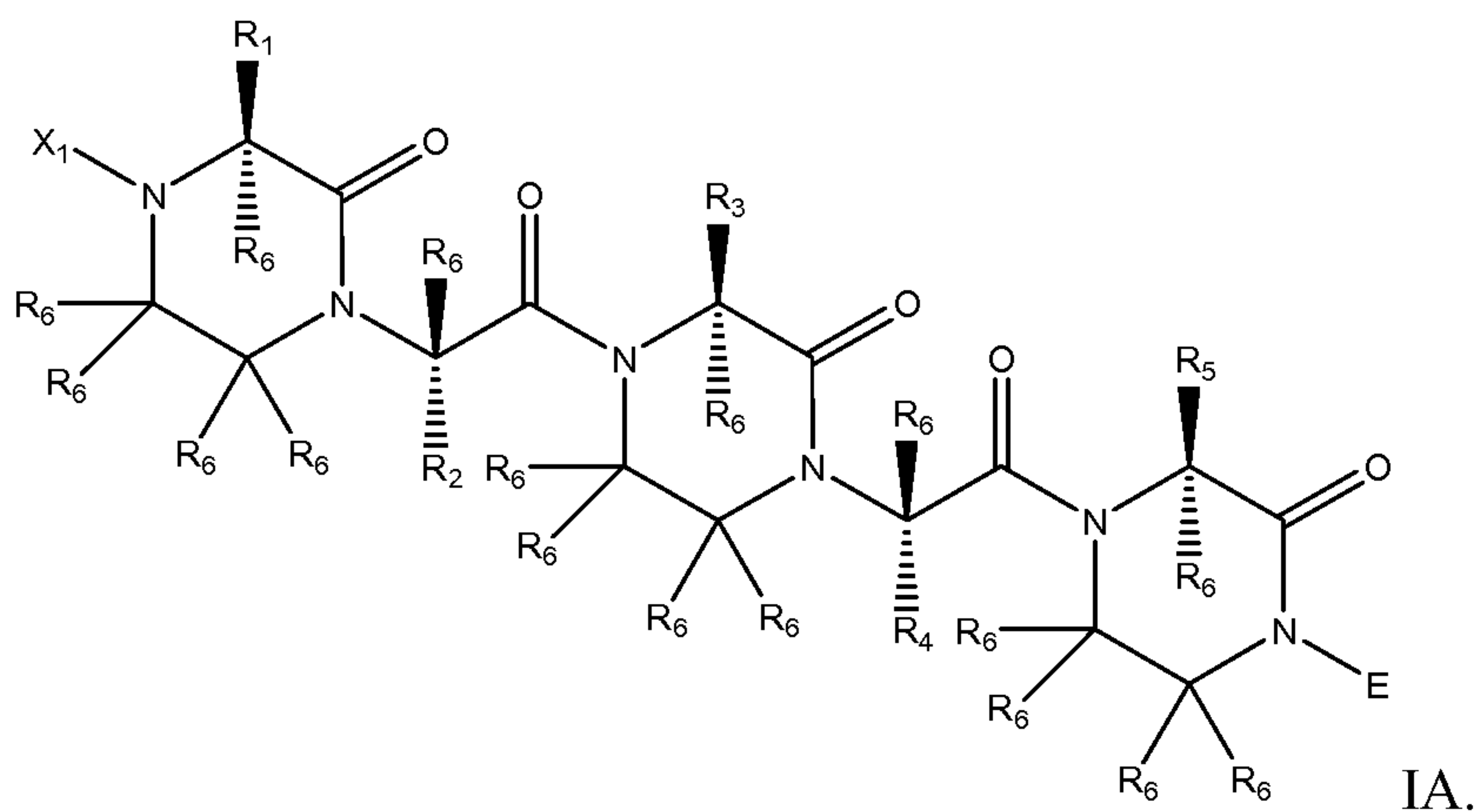
6. The method according to claim 3, wherein A is a moiety of



formula R_0 and R_3 are hydrophobic, and R_4 is a hydrogen bond acceptor or hydrogen bond donor.

7. The method according to claim 6, wherein the oligooxopiperazine is BB2-164.

8. The method according to claim 2, wherein the oligooxopiperazine has a formula of Formula IA:

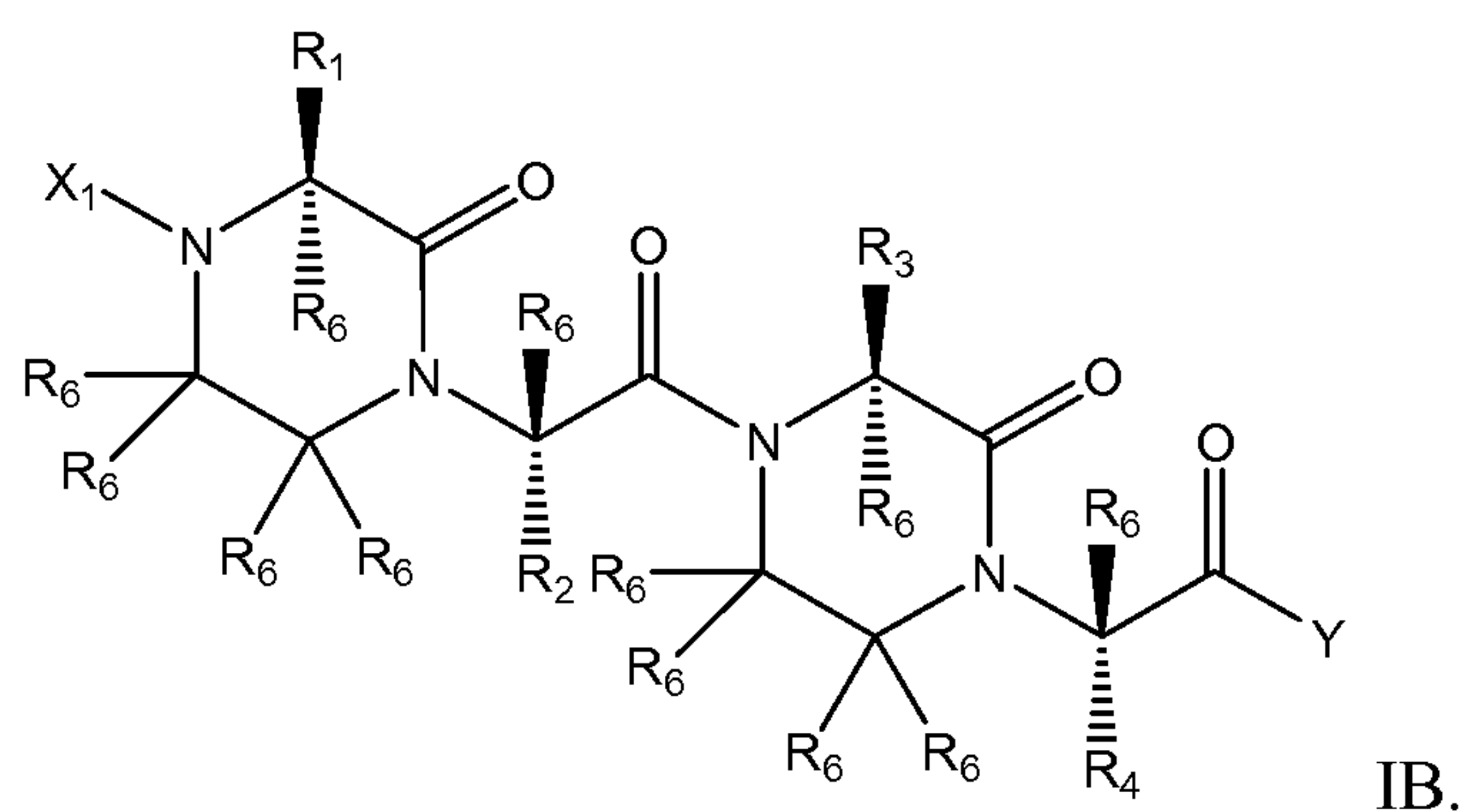


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9. The method according to claim 8, wherein R₁, R₂, R₄, and R₅ of the oligooxopiperazine of Formula IA mimic the amino acid side chain of, respectively, residues *i*, *i*+4, *i*+6, and *i*+7 of the α -helix.

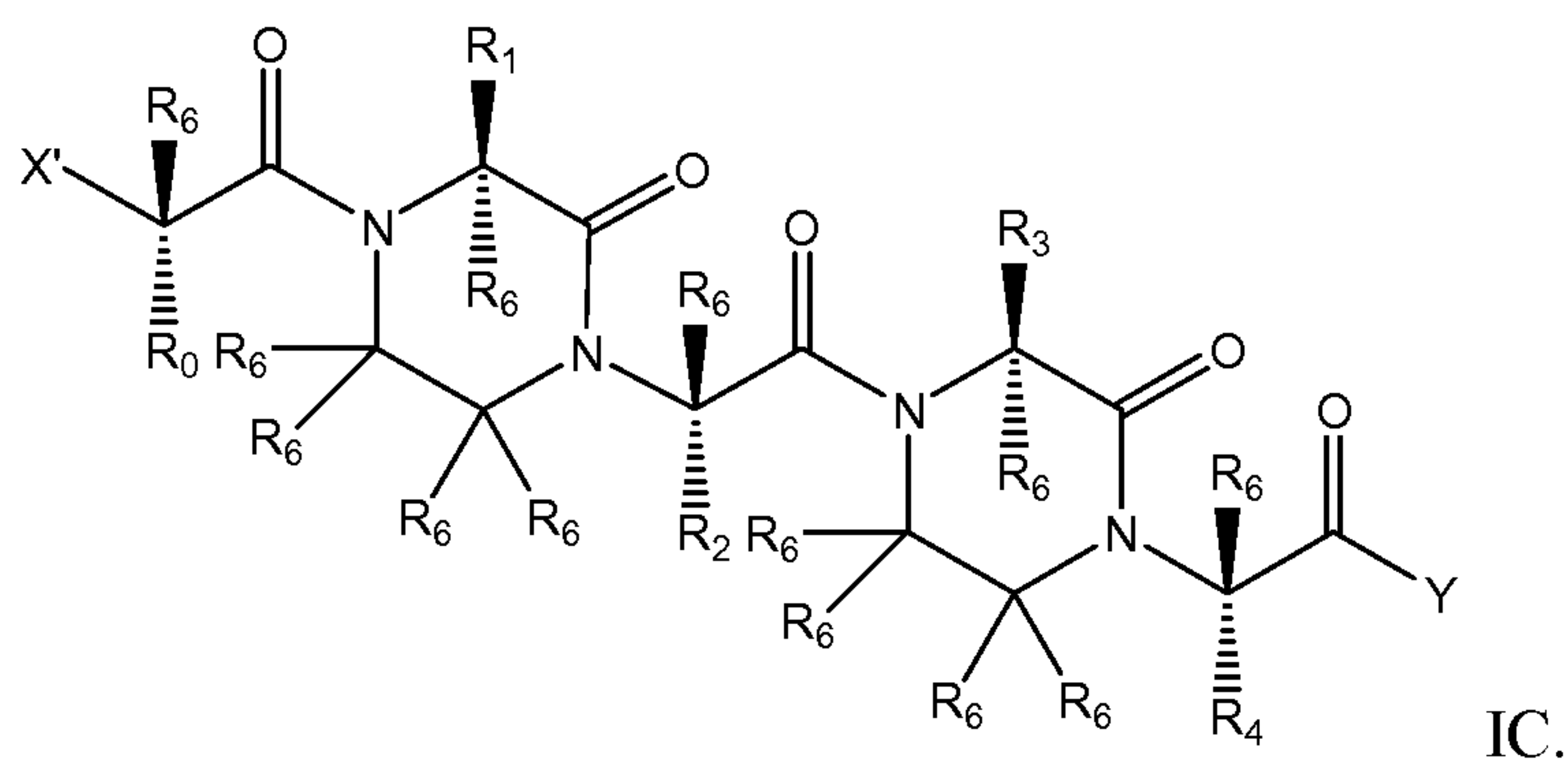
- 57 -

10. The method according to claim 2, wherein the oligooxopiperazine has a formula of Formula IB:



11. The method according to claim 10, wherein R_1 , R_2 , and R_4 of the oligooxopiperazine of Formula IB mimic the amino acid side chain of, respectively, residues i , $i+4$, and $i+7$, of the α -helix.

12. The method according to claim 2, wherein the oligooxopiperazine has a formula of Formula IC:



13. The method according to claim 12, wherein R_0 , R_1 , R_2 , R_3 , and R_4 of the oligooxopiperazine of Formula IC mimic the amino acid side chain of, respectively, residues i , $i+2$, $i+3$, $i+4$, and $i+7$ of the α -helix.

14. The method according to claim 1, wherein the disorder is an HPV-associated cancer.

15. The method according to claim 1, wherein the HPV is selected from the group consisting of HPV-16, HPV-18, HPV-31, HPV-33, HPV-35, HPV-39,

- 58 -

HPV-45, HPV-51, HPV-52, HPV-56, HPV-58, HPV-59, HPV-68, HPV-69, HPV-73, and HPV-82.

16. The method according to claim 1, wherein the HPV-associated cancer is selected from the group of anogenital cancer, cervical cancer, vulvar cancer, vaginal cancer, penile cancer, anal cancer, oropharyngeal cancer, and head and neck squamous cell carcinoma.

17. The method according to claim 1 further comprising:
administering to the subject one or more anti-cancer agents.

18. The method according to claim 17, wherein the one or more anti-cancer agents are selected from the group consisting of 13-cis-Retinoic Acid, 2-CdA, 2-Chlorodeoxyadenosine, 5-Azacitidine, 5-Fluorouracil, 5-FU, 6-Mercaptopurine, 6-MP, 6-TG, 6-Thioguanine, Abraxane, Accutane, Actinomycin-D, Adcetris, Adriamycin, Adrucil, Afinitor, Agrylin, Ala-Cort, Aldesleukin, Alemtuzumab, ALIMTA, Alitretinoin, Alkaban-AQ, Alkeran, All-transretinoic Acid, Alpha Interferon, Altretamine, Amethopterin, Amifostine, Aminoglutethimide, Anagrelide, Anandron®, Anastrozole, Arabinosylecytosine, Ara-C, Aranesp®, Aredia®, Arimidex®, Aromasin®, Arranon®, Arsenic Trioxide, Arzerra™, Asparaginase, ATRA, Avastin®, Axitinib, Azacitidine, BCG, Bendamustine, Bevacizumab, Bexarotene, BEXXAR®, Bicalutamide, BiCNU, Blenoxane®, Bleomycin, Bortezomib, Bosulif, Bosutinib, Brentuximab Vedotin, Busulfan, Busulfex®, C225, Cabazitaxel, Cabozantinib, Calcium Leucovorin, Campath®, Camptosar®, Camptothecin-11, Capecitabine, Caprelsa, Carac™, Carboplatin, Carfilzomib, Carmustine, Carmustine Wafer, Casodex®, CC-5013, CCI-779, CCNU, CDDP, CeeNU, Cerubidine®, Cetuximab, Chlorambucil, Cisplatin, Cisplatinum, Citrovorium Factor, Cladribine, Cometriq, Cortisone, Cosmegen®, CPT-11, Crizotinib, Cyclophosphamide, Cytradren®, Cytarabine, Cytarabine Liposomal, Cytosar-U®, Cytoxan®, Dacarbazine, Dacogen, Dactinomycin, Darbepoetin Alfa, Dasatinib, Daunomycin, Daunorubicin, daunorubicin-hydrochloride, Daunorubicin Liposomal, DaunoXome®, Decadron, Decitabine, Delta-Cortef®, Deltasone®, Denileukin Diftitox, Denosumab, DepoCyt™, Dexamethasone, Dexamethasone Acetate, Dexamethasone Sodium Phosphate, Dexasone, Dexrazoxane, DHAD, DIC, Diodex,

- 59 -

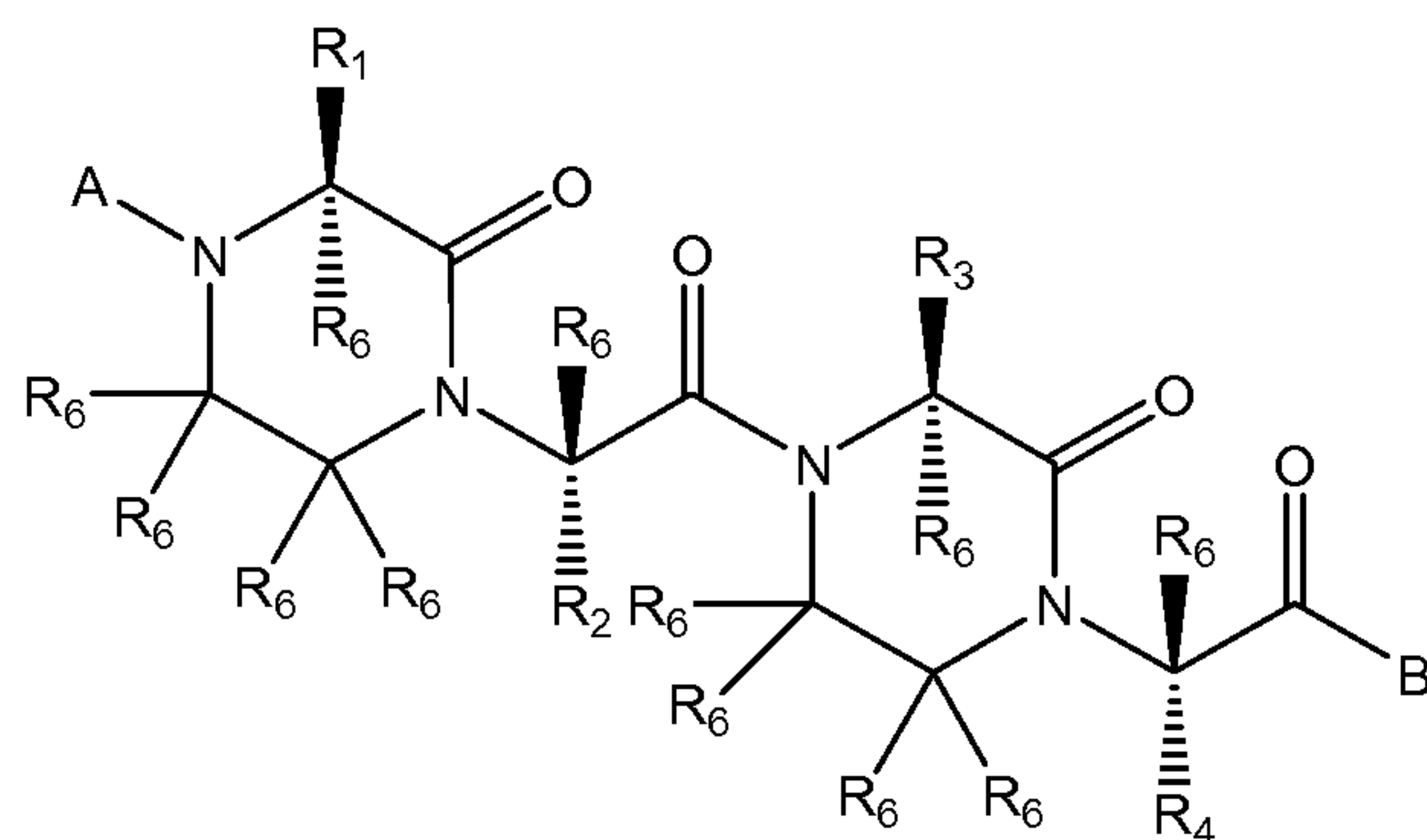
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 Epirubicin, Epoetin Alfa, Erbitux, Eribulin, Erivedge, Erlotinib, Erwinia L-
 asparaginase, Estramustine, Ethyol, Etopophos, Etoposide, Etoposide Phosphate,
 5 Eulexin [®], Everolimus, Evista [®], Exemestane, Fareston [®], Faslodex [®], Femara [®],
 Filgrastim, Floxuridine, Fludara [®], Fludarabine, Fluoroplex [®], Fluorouracil,
 Fluorouracil (cream), Fluoxymesterone, Flutamide, Folinic Acid, Folotyn, FUDR [®],
 Fulvestrant, G-CSF, Gefitinib, Gemcitabine, Gemtuzumab ozogamicin, Gemzar,
 Gleevec TM, Gliadel Wafer [®], GM-CSF, Goserelin, Granulocyte - Colony Stimulating
 10 Factor, Granulocyte Macrophage Colony Stimulating Factor, Halaven [®], Halotestin
[®], Herceptin [®], Hexadrol, Hexalen [®], Hexamethylmelamine, HMM, Hycamtin [®],
 Hydrea [®], Hydrocort Acetate [®], Hydrocortisone, Hydrocortisone Sodium Phosphate,
 Hydrocortisone Sodium Succinate, Hydrocortone Phosphate, Hydroxyurea,
 Ibritumomab, Ibritumomab Tiuxetan, Idamycin, ICLUSIG[®], Ifex [®], IFN-alpha,
 15 Ifosfamide, IL-11, IL-2, Imatinib Mesylate, Imidazole Carboxamide, Inlyta [®],
 Interferon-Alfa, Interferon Alfa-2b (PEG Conjugate), Interleukin-2, Interleukin-11,
 Intron A[®] (interferon alfa-2b), Ipilimumab, Irinotecan, Isotretinoin, Istodax,
 Ixabepilone, Jevtana, Kidrolase, Kyprolis, Lanacort, Lapatinib, L-asparaginase, LCR,
 Lenalidomide, Letrozole, Leucovorin, Leukeran, Leukine, Leuprolide, Leurocristine,
 20 Leustatin, Liposomal Ara-C, Liquid Pred, Lomustine, L-PAM, L-Sarcolysin, Lupron,
 Lupron Depot, Marqibo, Matulane, Maxidex, Mechlorethamine, Mechlorethamine
 Hydrochloride, Medralone, Medrol, Megace, Megestrol, Megestrol Acetate,
 Melphalan, Mercaptopurine, Mesna, Mesnex, Methotrexate, Methotrexate Sodium,
 Methylprednisolone, Meticorten, Mitomycin, Mitomycin-C, Mitoxantrone, M-
 25 Prednisol, MTC, MTX, Mustargen, Mustine, Mutamycin, Myleran, Mylocel,
 Mylotarg, Navelbine, Nelarabine, Neosar, Neulasta, Neumega, Neupogen, Nexavar,
 Nilandron, Nilotinib, Nilutamide, Nipent, Nitrogen Mustard, Novaldex, Novantrone,
 Nplate, Octreotide, Octreotide Acetate, Ofatumumab, Oncospar, Oncovin, Ontak,
 Onxal, Oprelvekin, Orapred, Orasone, Oxaliplatin, Paclitaxel, Paclitaxel Protein-
 30 bound, Pamidronate, Panitumumab, Panretin, Paraplatin, Pazopanib, Pediapred, PEG
 Interferon, Pegaspargase, Pegfilgrastim, PEG-INTRON, PEG-L-asparaginase,
 PEMETREXED, Pentostatin, Perjeta, Pertuzumab, Phenylalanine Mustard, Platinol,
 Platinol-AQ, Ponatinib, Pralatrexate, Prednisolone, Prednisone, Prelone,

- 60 -

Procarbazine, PROCRIT, Proleukin, Prolia, Prolifeprospan 20 with Carmustine
 Implant, Provenge, Purinethol, Raloxifene, Regorafenib, Revlimid, Rheumatrex,
 Rituxan, Rituximab, Roferon-A (Interferon Alfa-2a), Romidepsin, Romiplostim,
 Rubex, Rubidomycin Hydrochloride, Sandostatin, Sandostatin LAR, Sargramostim,
 5 Sipuleucel-T, Soliris, Solu-Cortef, Solu-Medrol, Sorafenib, SPRYCEL, STI-571,
 Stivarga, Streptozocin, SU11248, Sunitinib, Sutent, Tamoxifen, Tarceva, Targretin,
 Tassigna, Taxol, Taxotere, Temodar, Temozolomide, Temsirolimus, Teniposide,
 TESPAs, Thalidomide, Thalomid, TheraCys, Thioguanine, Thioguanine Tabloid,
 Thiophosphoamide, Thioplex, Thiotepa, TICE, Toposar, Topotecan, Toremifene,
 10 Torisel, Tositumomab, Trastuzumab, Treanda, Tretinoin, Trexall, Trisenox, TSPA,
 TYKERB, Valrubicin, Valstar, Vandetanib, VCR, Vectibix, Velban, Velcade,
 VePesid, Vesanoid, Viadur, Vidaza, Vinblastine, Vinblastine Sulfate, Vincasar Pfs,
 Vincristine, Vincristine Liposomal, Vinorelbine, Vinorelbine Tartrate, Vismodegib,
 VLB, VM-26, Vorinostat, Votrient, VP-16, Vumon, Xalkori Capsules, Xeloda,
 15 Xgeva, Yervoy, Zaltrap, Zanosar, Zelboraf, Zevalin, Zinecard, Ziv-aflibercept,
 Zoladex, Zoledronic Acid, Zolinza, and Zometa.

19. A method of inducing apoptosis of a cell, said method
 comprising:
 contacting the cell with an oligooxopiperazine that substantially
 20 mimics helix α B of the C-terminal transactivation domain of HIF1 α under conditions
 effective to induce apoptosis of the cell.

20. The method according to claim 19, wherein the
 oligooxopiperazine is an oligooxopiperazine of Formula I:



I, wherein:

- 61 -

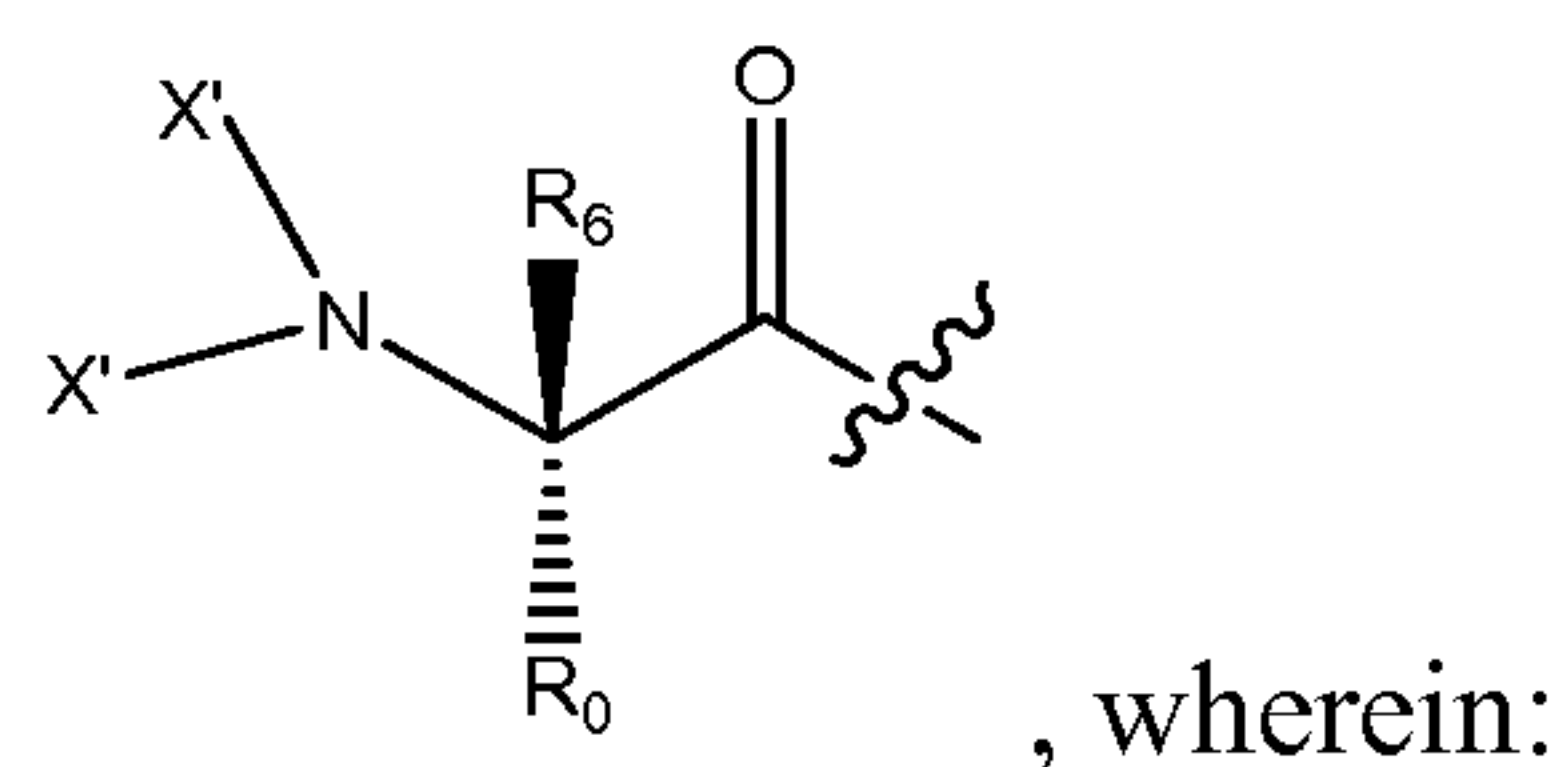
each of R_1 , R_2 , R_3 , and R_4 is independently an amino acid side chain, H, $N(R)_2$, OR, halogen, an alkyl, or an aryl; wherein each R is independently H, an alkyl, or an aryl;

each R_6 is independently H, $N(R)_2$, OR, halogen, an alkyl, or an aryl; wherein each R is independently H, an alkyl, or an aryl;

A is X_1 or C, wherein:

X_1 is H, COR' , CO_2R' , $CONR'$, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a protecting group for protection of an amine, a targeting moiety, or a tag; wherein R' is H, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; and

C is a moiety of the formula



each X' is independently H, COR' , CO_2R' , $CONR'$, $N(R'')_2$, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; wherein:

R' is H, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; and

each R'' is independently H, CO_2R' , $CONR'$, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag;

R_0 is an amino acid side chain, H, $N(R)_2$, OR, halogen, an alkyl, or an aryl; wherein each R is independently H, an alkyl, or an aryl; and

R_6 is H, $N(R)_2$, OR, halogen, an alkyl, or an aryl; wherein each R is independently H, an alkyl, or an aryl; and

B is Y or D, wherein:

Y is OR' , COR' , $N(R''')_2$, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a protecting group for protection of a carboxylic acid, a targeting moiety, or a tag; wherein:

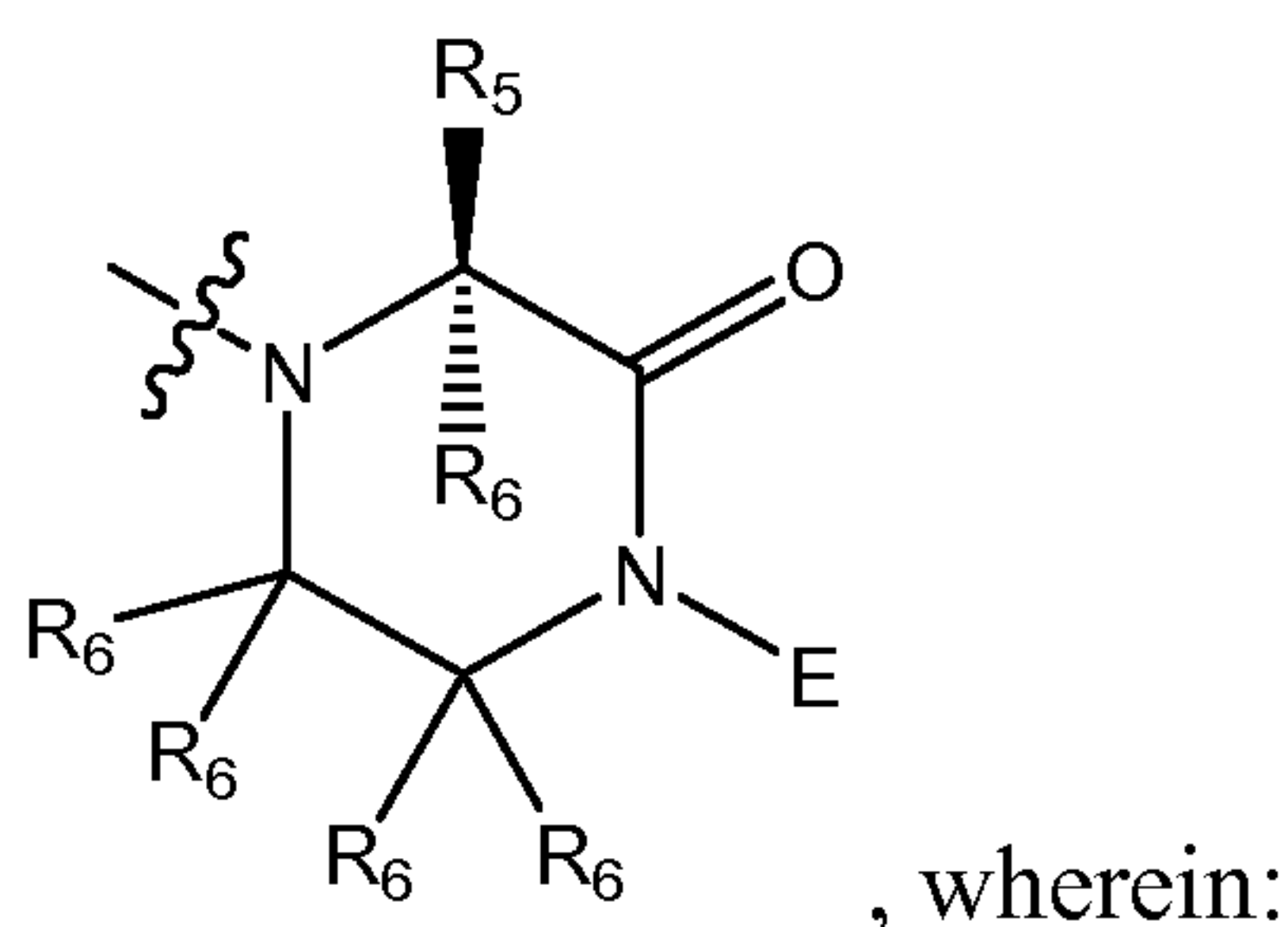
- 62 -

R' is H, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; and

each R''' is independently H, CO₂R', CONR', an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; and

5

D is a moiety of the formula



R₅ is an amino acid side chain, H, N(R)₂, OR, halogen, an alkyl, or an aryl; wherein each R is independently H, an alkyl, or an aryl;

10

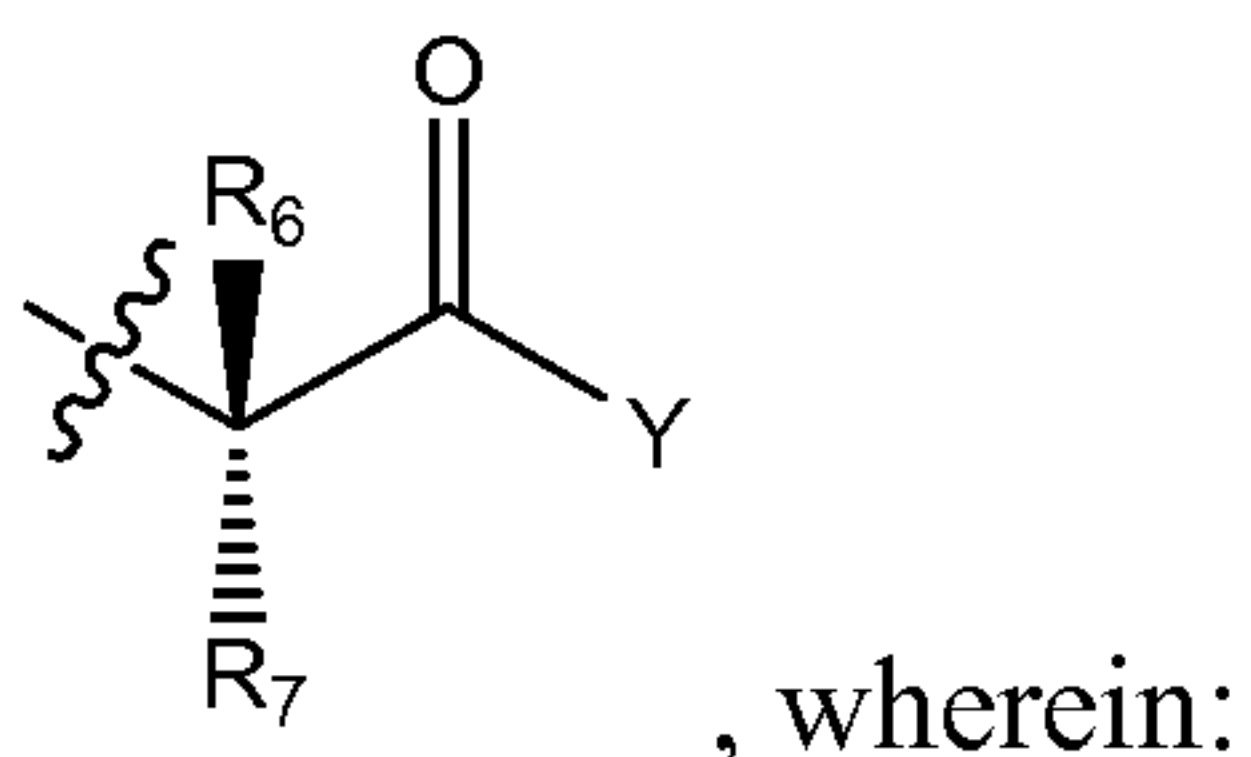
R₆ is H, N(R)₂, OR, halogen, an alkyl, or an aryl; wherein each R is independently H, an alkyl, or an aryl; and

E is X₂ or F, wherein:

X₂ is H, COR', CO₂R', CONR', an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a protecting group for protection of an amine, a targeting moiety, or a tag; wherein R' is H, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; and

15

F is a moiety of the formula



20

R₆ is H, N(R)₂, OR, halogen, an alkyl, or an aryl;

wherein each R is independently H, an alkyl, or an aryl;

R₇ is an amino acid side chain; and

25

Y is OR', COR', N(R''')₂, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a protecting group for

- 63 -

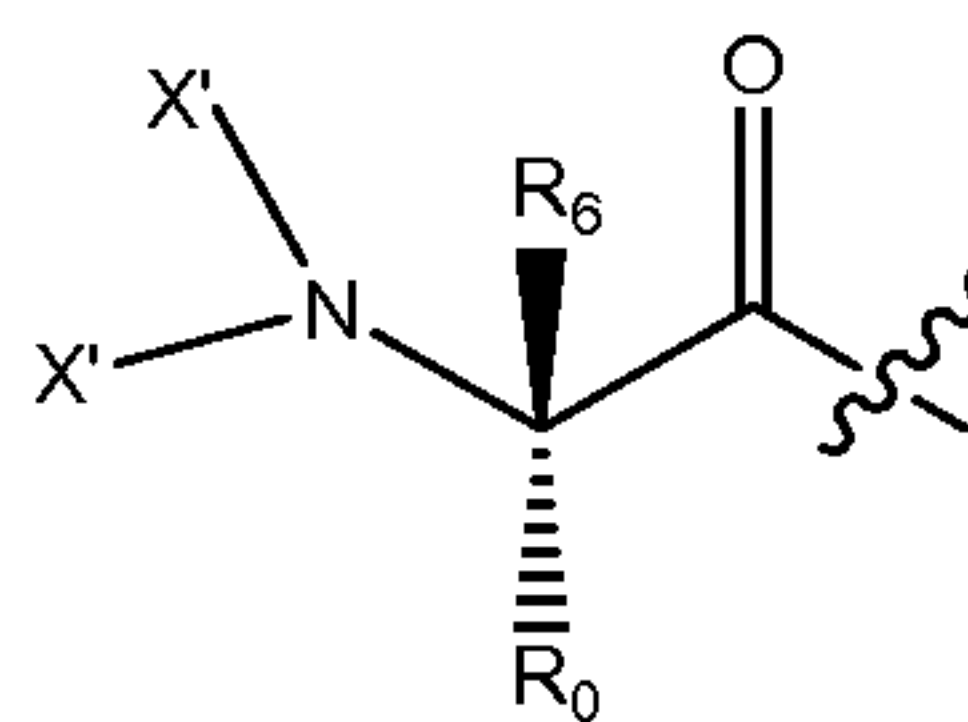
protection of a carboxylic acid, a targeting moiety, or a tag; wherein:

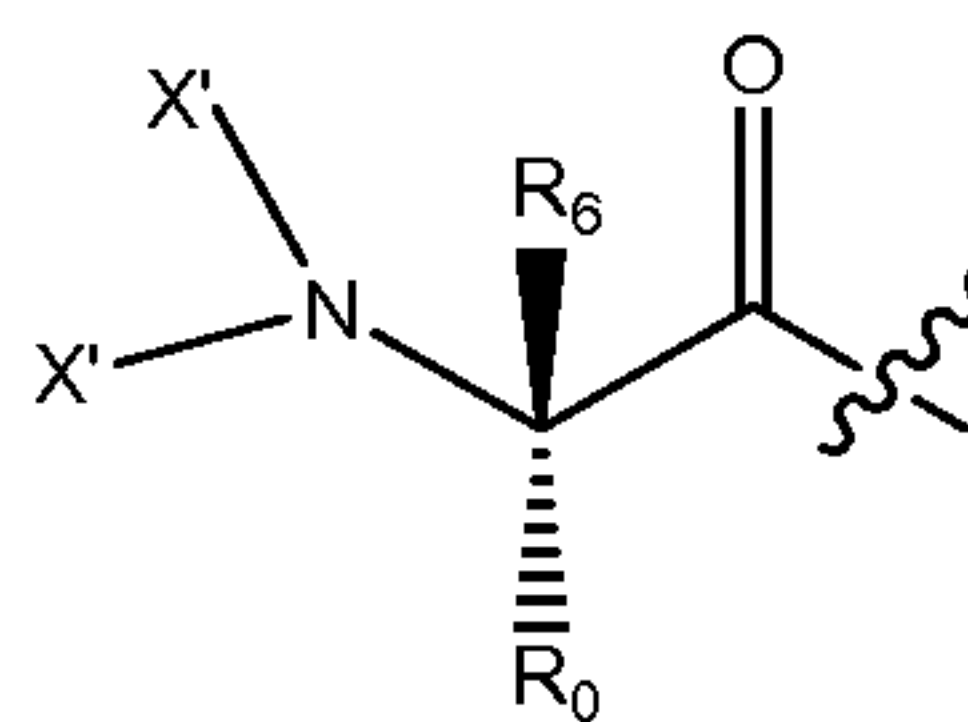
R' is H, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; and

each R''' is independently H, CO₂R', CONR', an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag.

21. The method according to claim 20, wherein:

10 (i) R₁ and R₂ are hydrophobic and R₄ is a hydrogen bond acceptor or hydrogen bond



donor or A is a moiety of formula , R₀ and R₃ are hydrophobic, and R₄ is a hydrogen bond acceptor or hydrogen bond donor;

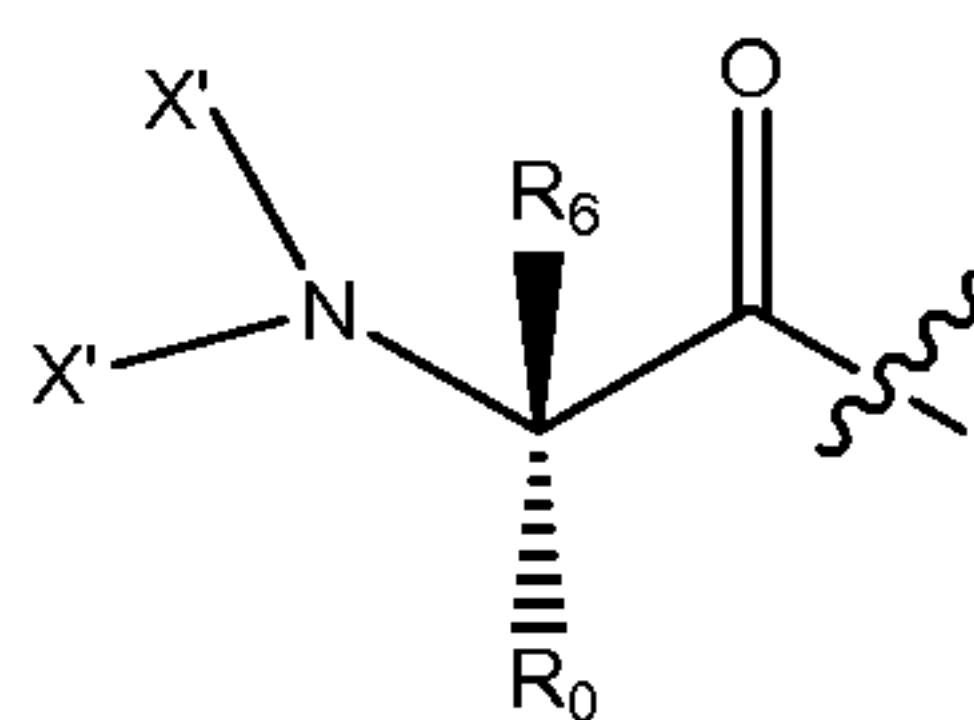
(ii) each R'' is independently H, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; and

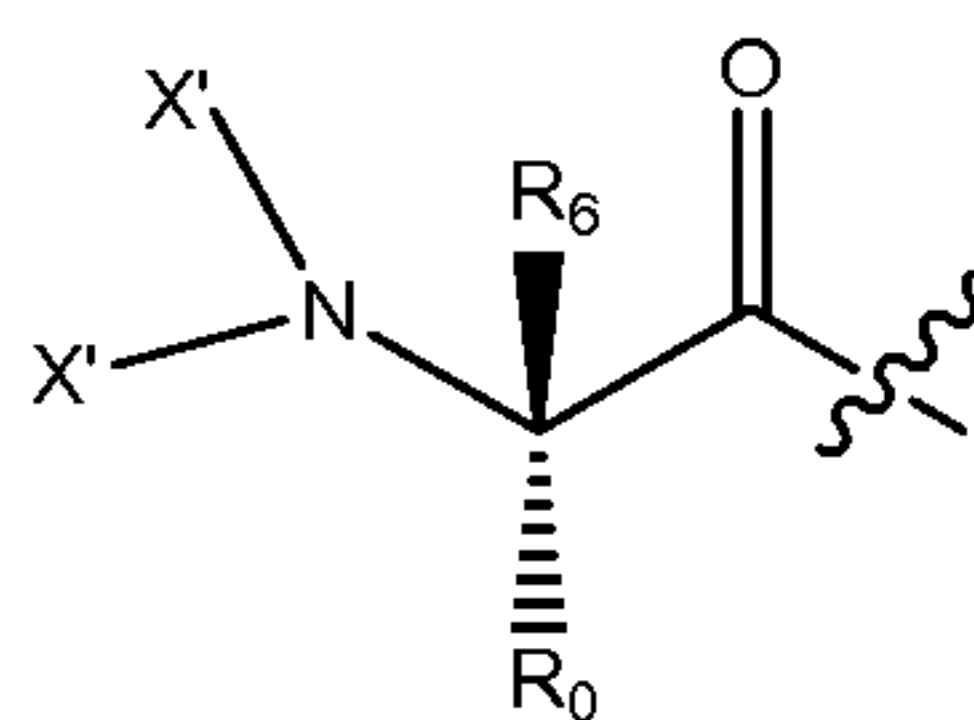
15 (iii) B is not D.

22. The method according to claim 21, wherein R₁ and R₂ are hydrophobic and R₄ is a hydrogen bond acceptor or hydrogen bond donor.

23. The method according to claim 22, wherein the oligooxopiperazine is BB2-125 or BB2-162.

20 24. The method according to claim 21, wherein A is a moiety of

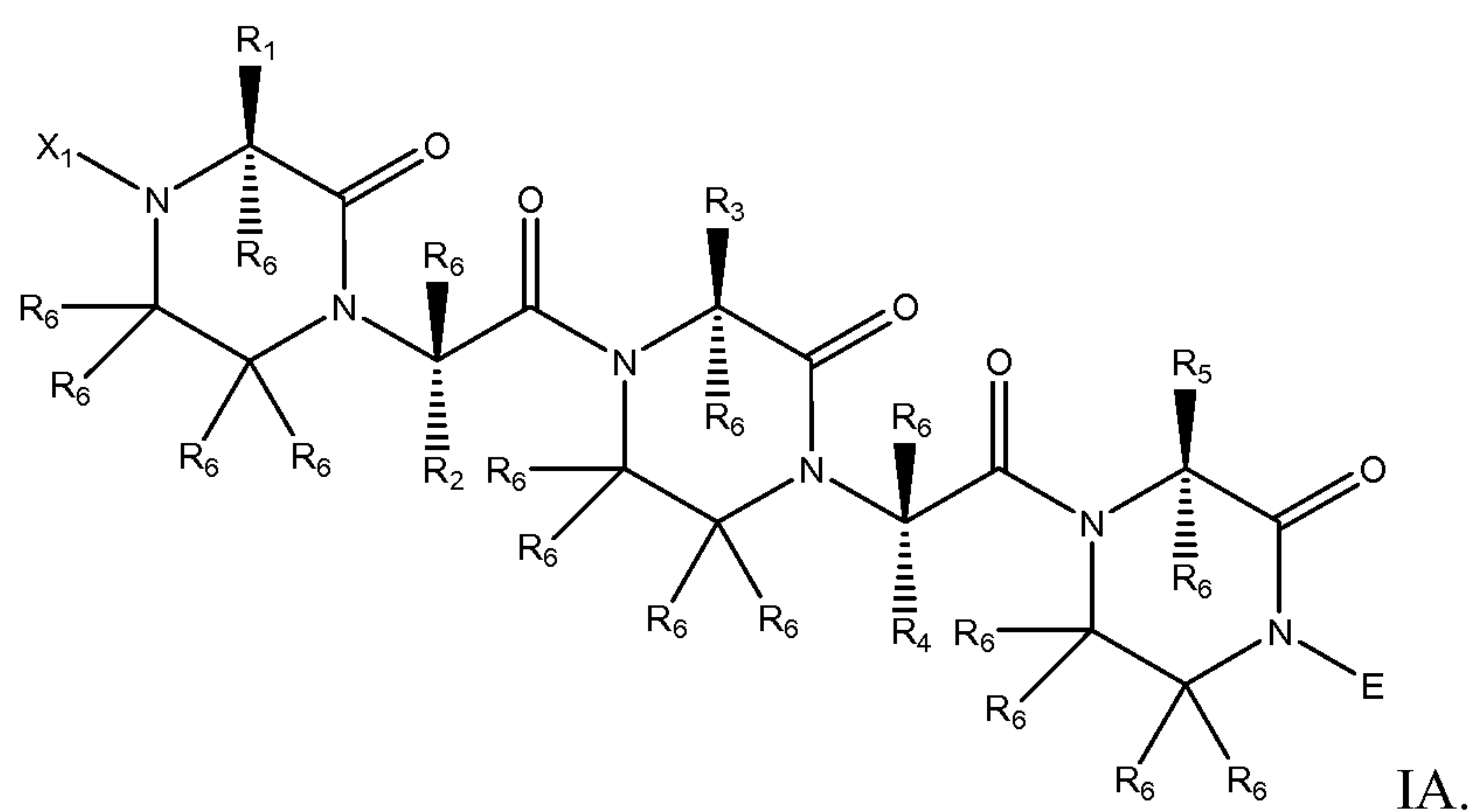


formula , R₀ and R₃ are hydrophobic, and R₄ is a hydrogen bond acceptor or hydrogen bond donor.

25. The method according to claim 24, wherein the oligooxopiperazine is BB2-164.

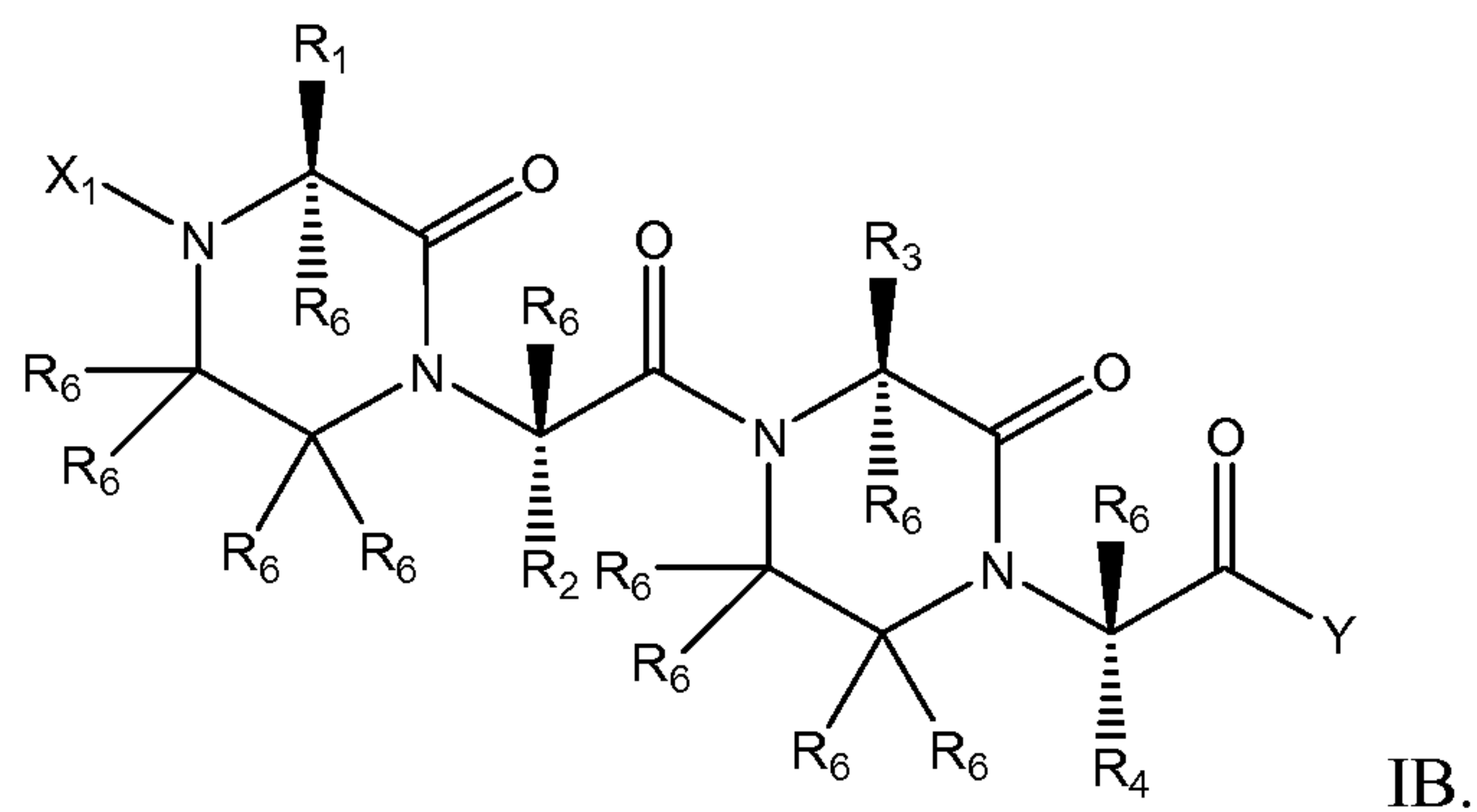
- 64 -

26. The method according to claim 20, wherein the oligooxopiperazine has a formula of Formula IA:



27. The method according to claim 26, wherein R_1 , R_2 , R_4 , and R_5 of the oligooxopiperazine of Formula IA mimic the amino acid side chain of, respectively, residues i , $i+4$, $i+6$, and $i+7$ of the α -helix.

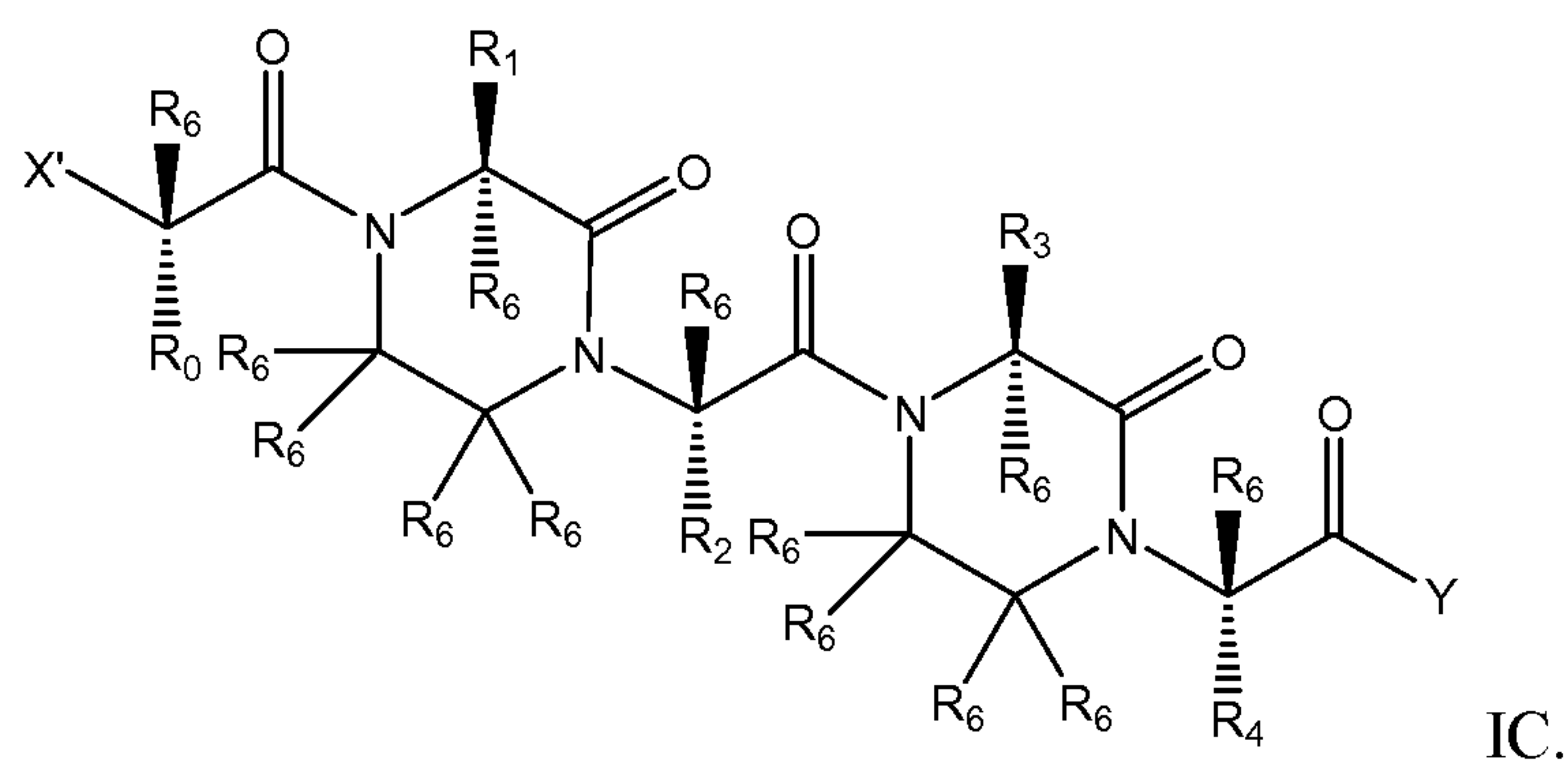
28. The method according to claim 20, wherein the oligooxopiperazine has a formula of Formula IB:



29. The method according to claim 28, wherein R_1 , R_2 , and R_4 of the oligooxopiperazine of Formula IB mimic the amino acid side chain of, respectively, residues i , $i+4$, and $i+7$, of the α -helix.

- 65 -

30. The method according to claim 20, wherein the oligooxopiperazine has a formula of Formula IC:



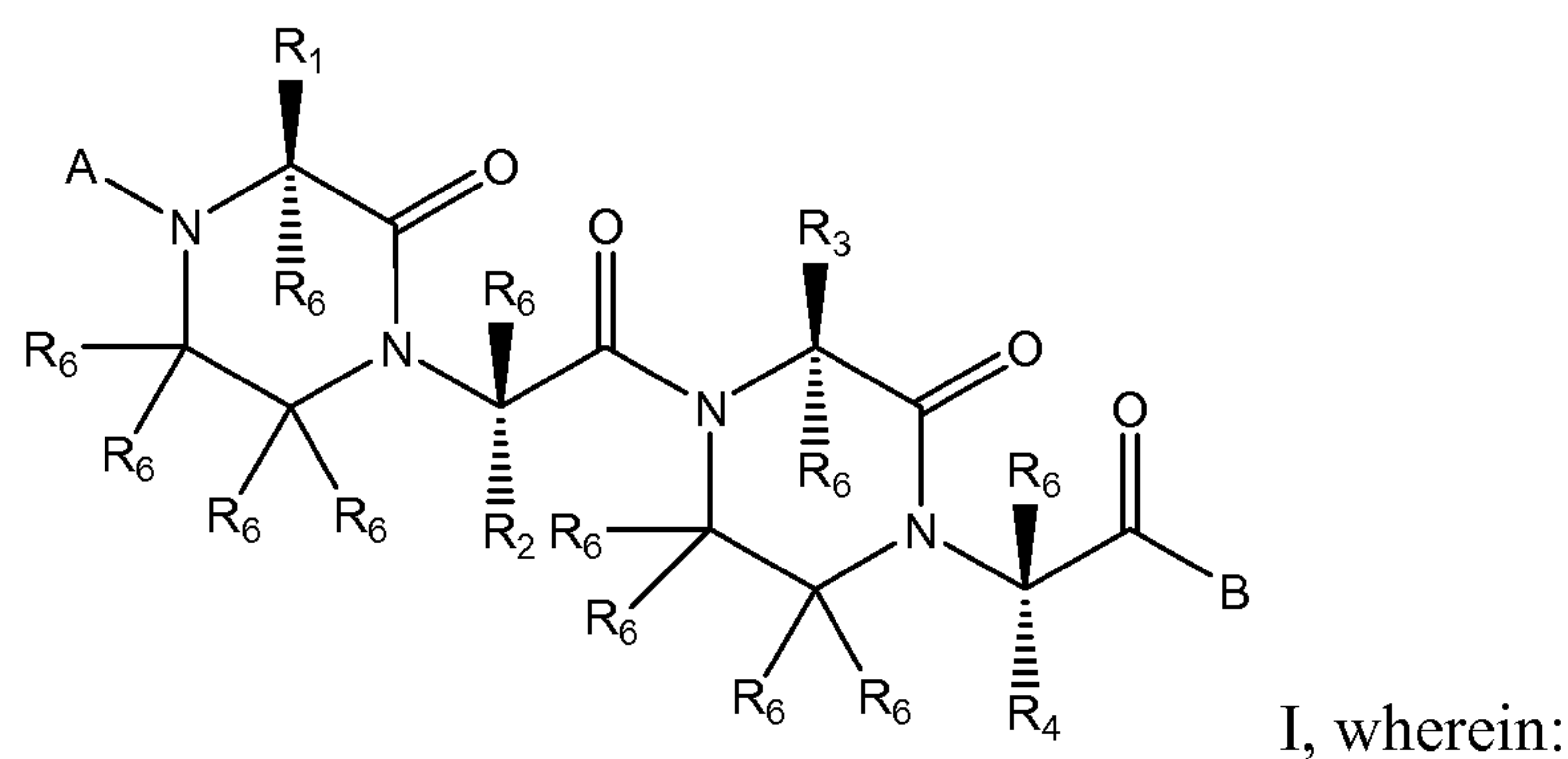
31. The method according to claim 30, wherein R_0 , R_1 , R_2 , R_3 , and R_4 of the oligooxopiperazine of Formula IC mimic the amino acid side chain of, respectively, residues i , $i+2$, $i+3$, $i+4$, and $i+7$ of the α -helix.

32. A method of decreasing survival and/or proliferation of a cell, said method comprising:

contacting the cell with an oligooxopiperazine that substantially mimics helix αB of the C-terminal transactivation domain of HIF1 α under conditions effective to decrease survival and/or proliferation of the cell.

33. The method according to claim 32, wherein the cell is cancerous or is contained in the endothelial vasculature of a tissue that contains cancerous cells.

34. The method according to claim 32, wherein the oligooxopiperazine is an oligooxopiperazine of Formula I:



- 66 -

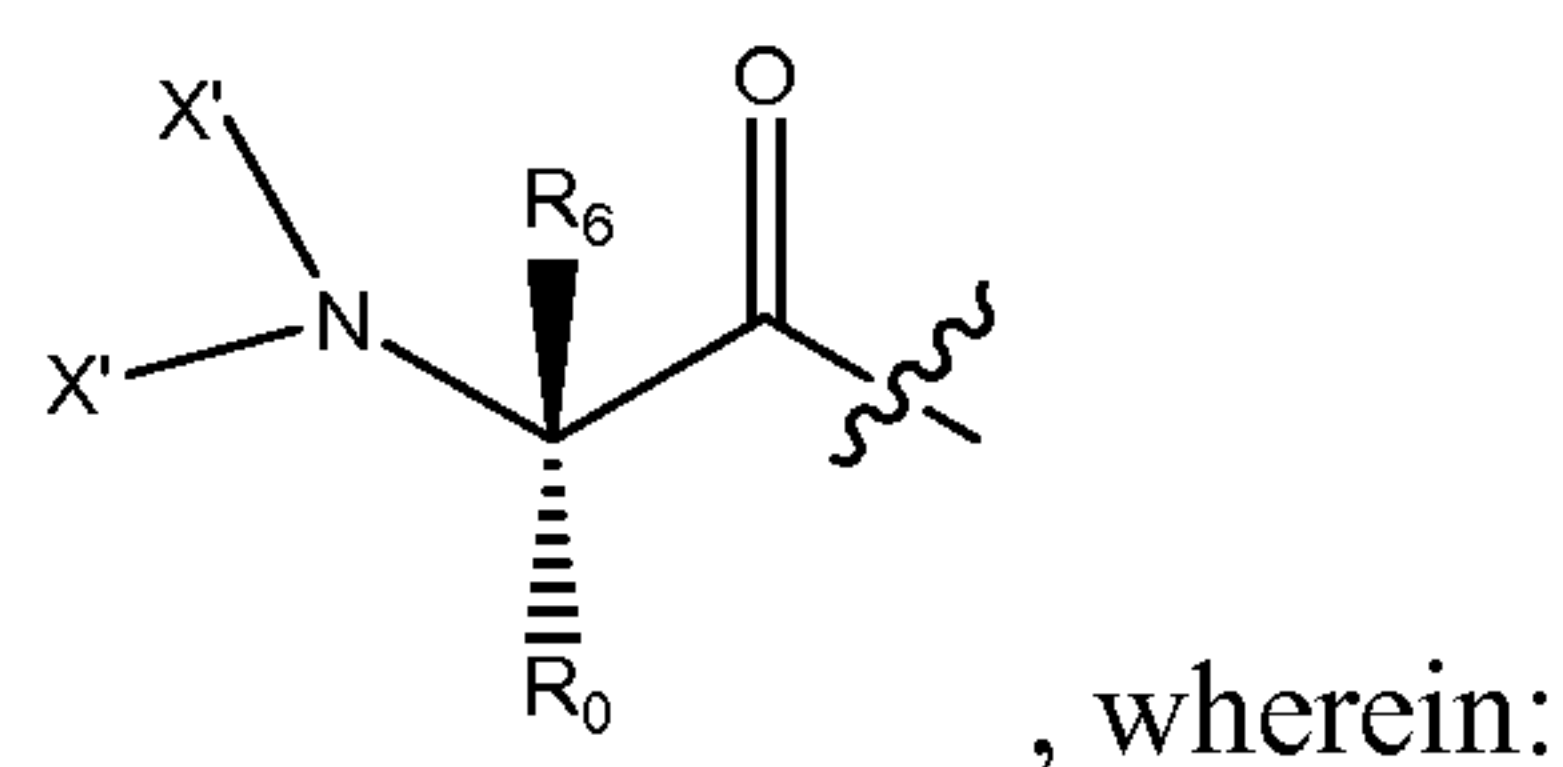
each of R_1 , R_2 , R_3 , and R_4 is independently an amino acid side chain, H, $N(R)_2$, OR, halogen, an alkyl, or an aryl; wherein each R is independently H, an alkyl, or an aryl;

each R_6 is independently H, $N(R)_2$, OR, halogen, an alkyl, or an aryl; wherein each R is independently H, an alkyl, or an aryl;

A is X_1 or C, wherein:

X_1 is H, COR' , CO_2R' , $CONR'$, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a protecting group for protection of an amine, a targeting moiety, or a tag; wherein R' is H, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; and

C is a moiety of the formula



each X' is independently H, COR' , CO_2R' , $CONR'$, $N(R'')_2$, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; wherein:

R' is H, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; and

each R'' is independently H, CO_2R' , $CONR'$, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag;

R_0 is an amino acid side chain, H, $N(R)_2$, OR, halogen, an alkyl, or an aryl; wherein each R is independently H, an alkyl, or an aryl; and

R_6 is H, $N(R)_2$, OR, halogen, an alkyl, or an aryl; wherein each R is independently H, an alkyl, or an aryl; and

B is Y or D, wherein:

Y is OR' , COR' , $N(R''')_2$, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a protecting group for protection of a carboxylic acid, a targeting moiety, or a tag; wherein:

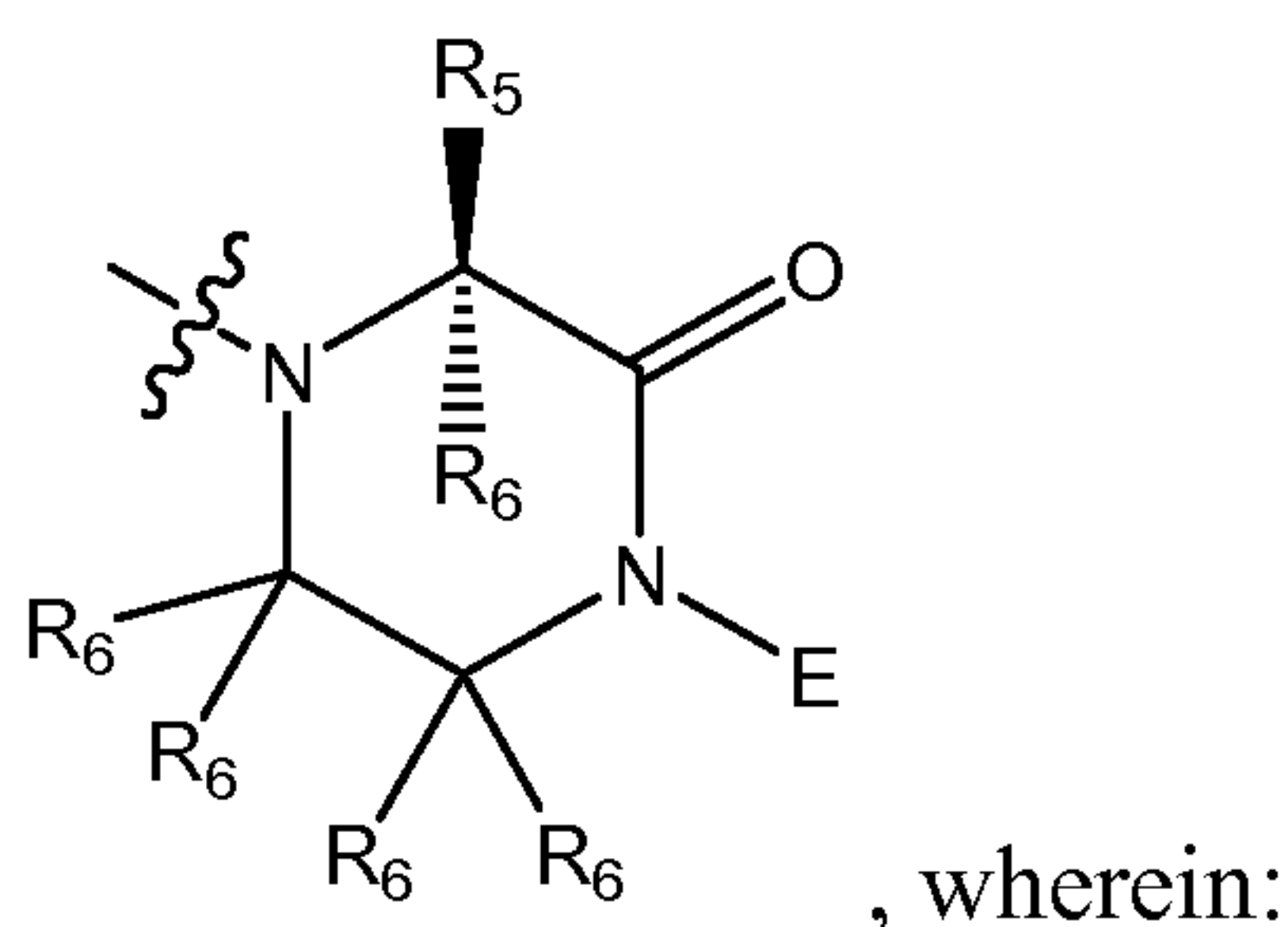
- 67 -

R' is H, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; and

each R''' is independently H, CO₂R', CONR', an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; and

5

D is a moiety of the formula



R₅ is an amino acid side chain, H, N(R)₂, OR, halogen, an alkyl, or an aryl; wherein each R is independently H, an alkyl, or an aryl;

10

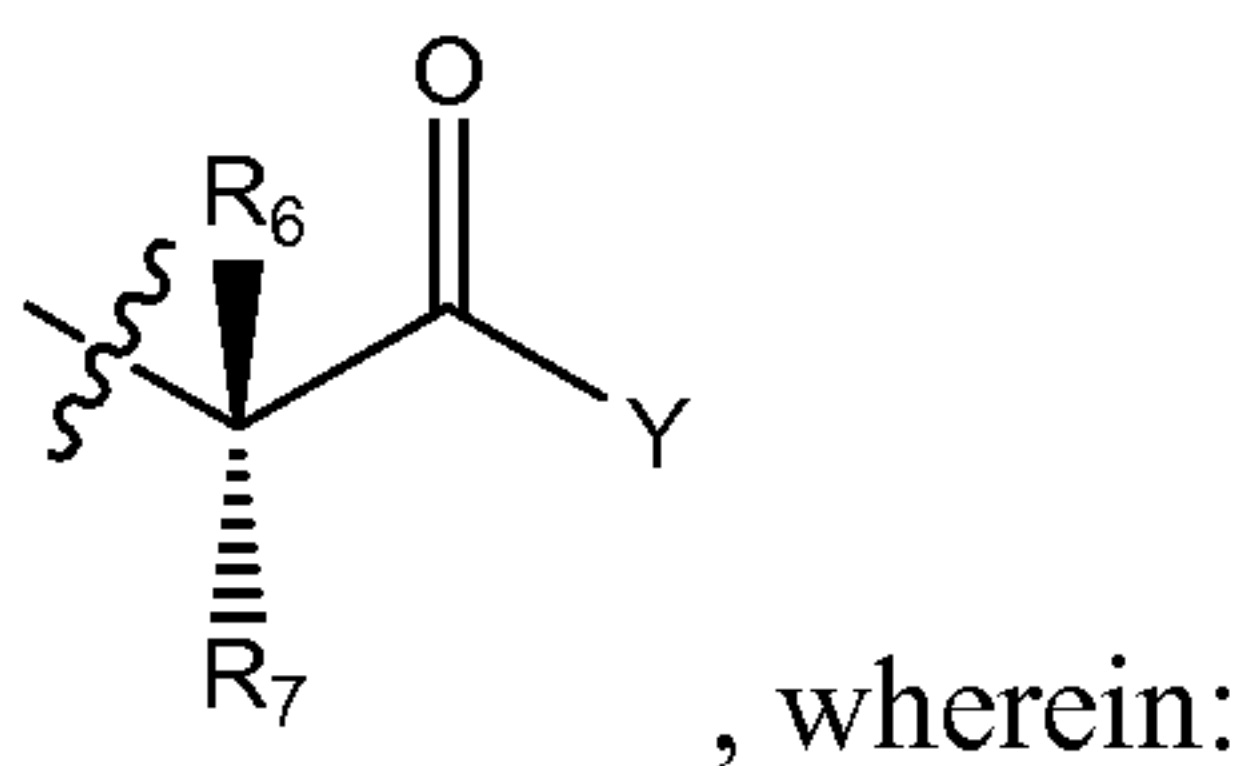
R₆ is H, N(R)₂, OR, halogen, an alkyl, or an aryl; wherein each R is independently H, an alkyl, or an aryl; and

E is X₂ or F, wherein:

X₂ is H, COR', CO₂R', CONR', an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a protecting group for protection of an amine, a targeting moiety, or a tag; wherein R' is H, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; and

15

F is a moiety of the formula



20

R₆ is H, N(R)₂, OR, halogen, an alkyl, or an aryl; wherein each R is independently H, an alkyl, or an aryl;

R₇ is an amino acid side chain; and

25

Y is OR', COR', N(R''')₂, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a protecting group for

- 68 -

protection of a carboxylic acid, a targeting moiety, or a tag; wherein:

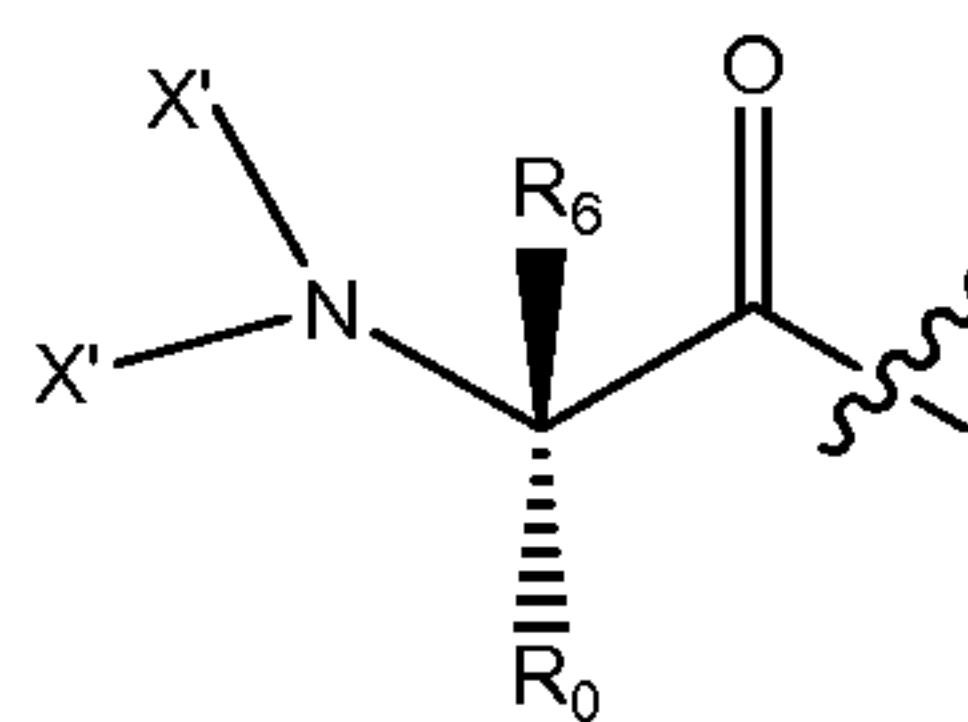
R' is H, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; and

each R''' is independently H, CO₂R', CONR', an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag.

5

35. The method according to claim 34, wherein:

10 (i) R₁ and R₂ are hydrophobic and R₄ is a hydrogen bond acceptor or hydrogen bond



donor or A is a moiety of formula , R₀ and R₃ are hydrophobic, and R₄ is a hydrogen bond acceptor or hydrogen bond donor;

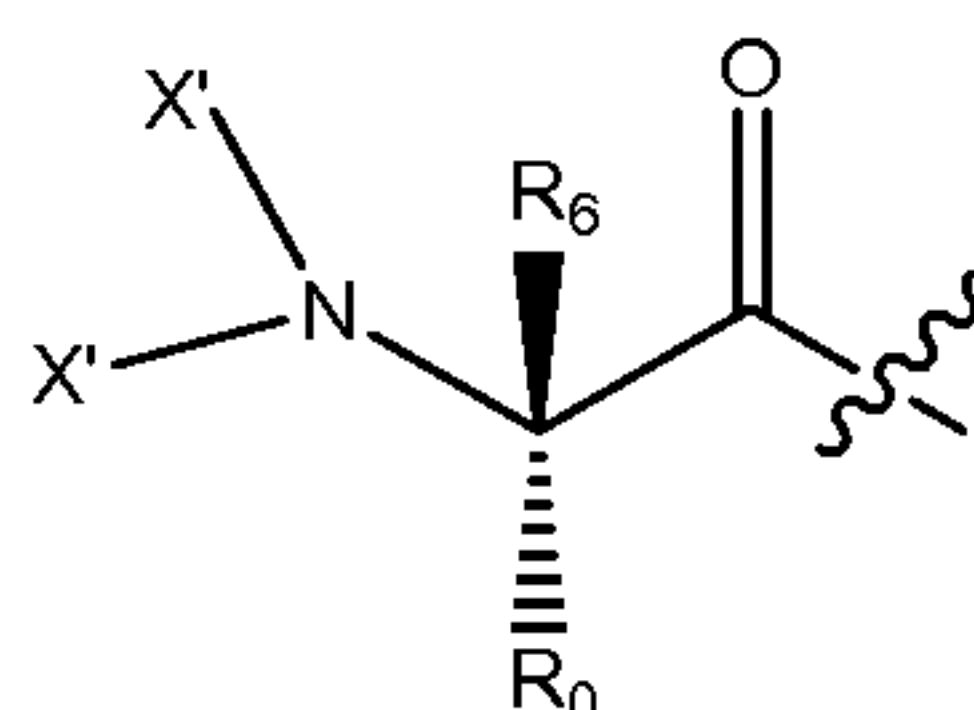
(ii) each R'' is independently H, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; and

15 (iii) B is not D.

36. The method according to claim 35, wherein R₁ and R₂ are hydrophobic and R₄ is a hydrogen bond acceptor or hydrogen bond donor.

37. The method according to claim 36, wherein the oligooxopiperazine is BB2-125 or BB2-162.

20 38. The method according to claim 35, wherein A is a moiety of

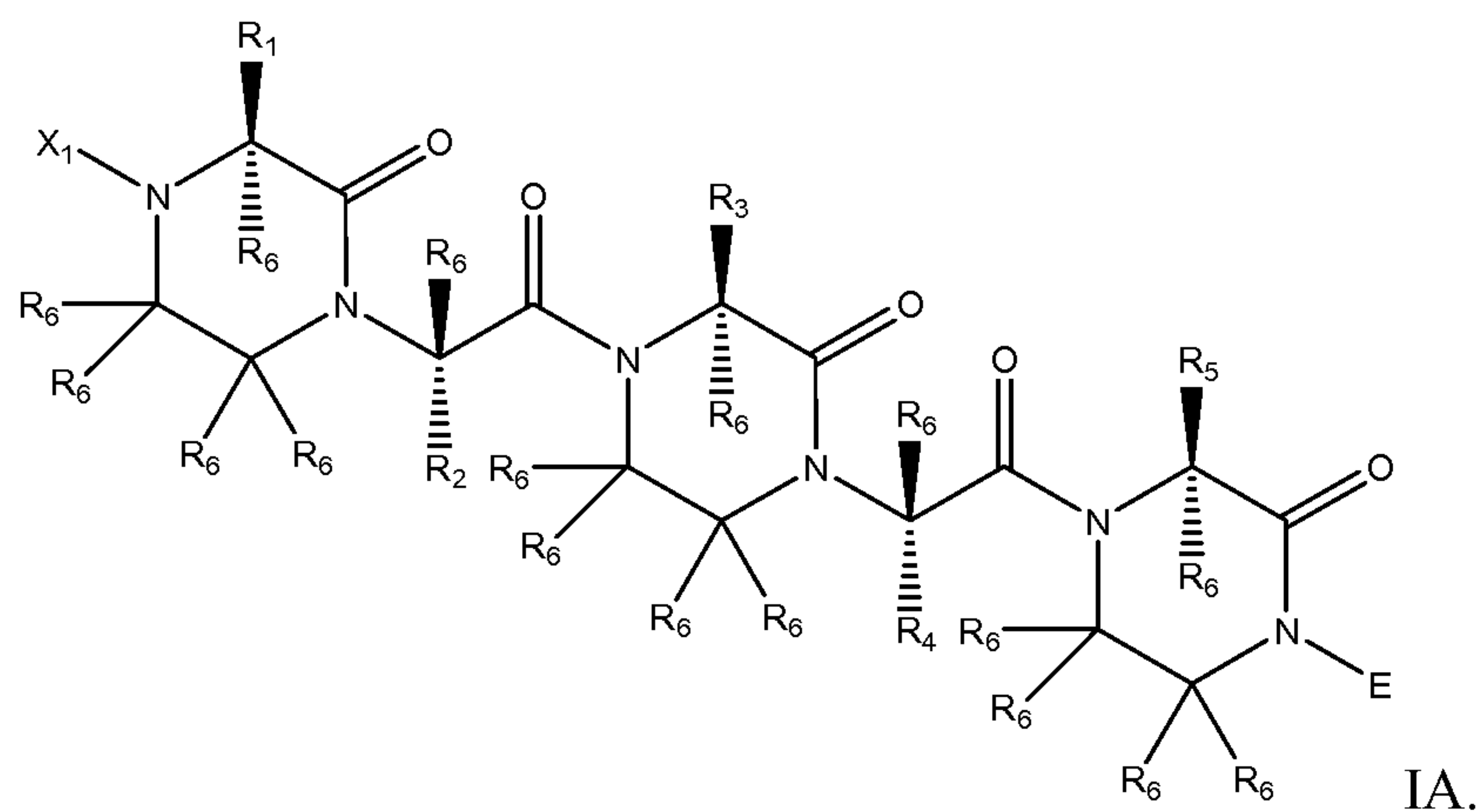


formula , R₀ and R₃ are hydrophobic, and R₄ is a hydrogen bond acceptor or hydrogen bond donor.

39. The method according to claim 38, wherein the oligooxopiperazine is BB2-164.

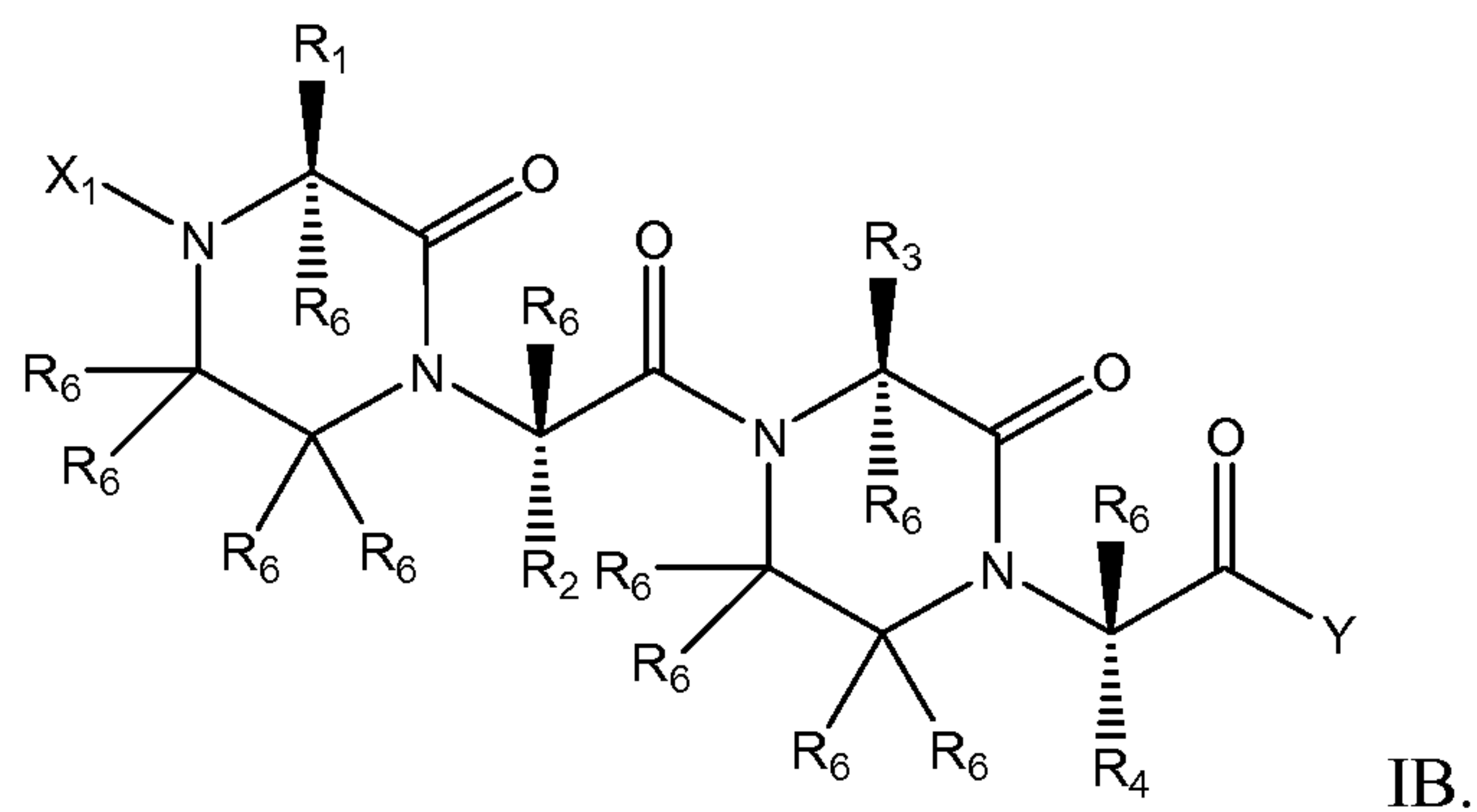
- 69 -

40. The method according to claim 34, wherein the oligooxopiperazine has a formula of Formula IA:



41. The method according to claim 40, wherein R_1 , R_2 , R_4 , and R_5 of the oligooxopiperazine of Formula IA mimic the amino acid side chain of, respectively, residues i , $i+4$, $i+6$, and $i+7$ of the α -helix.

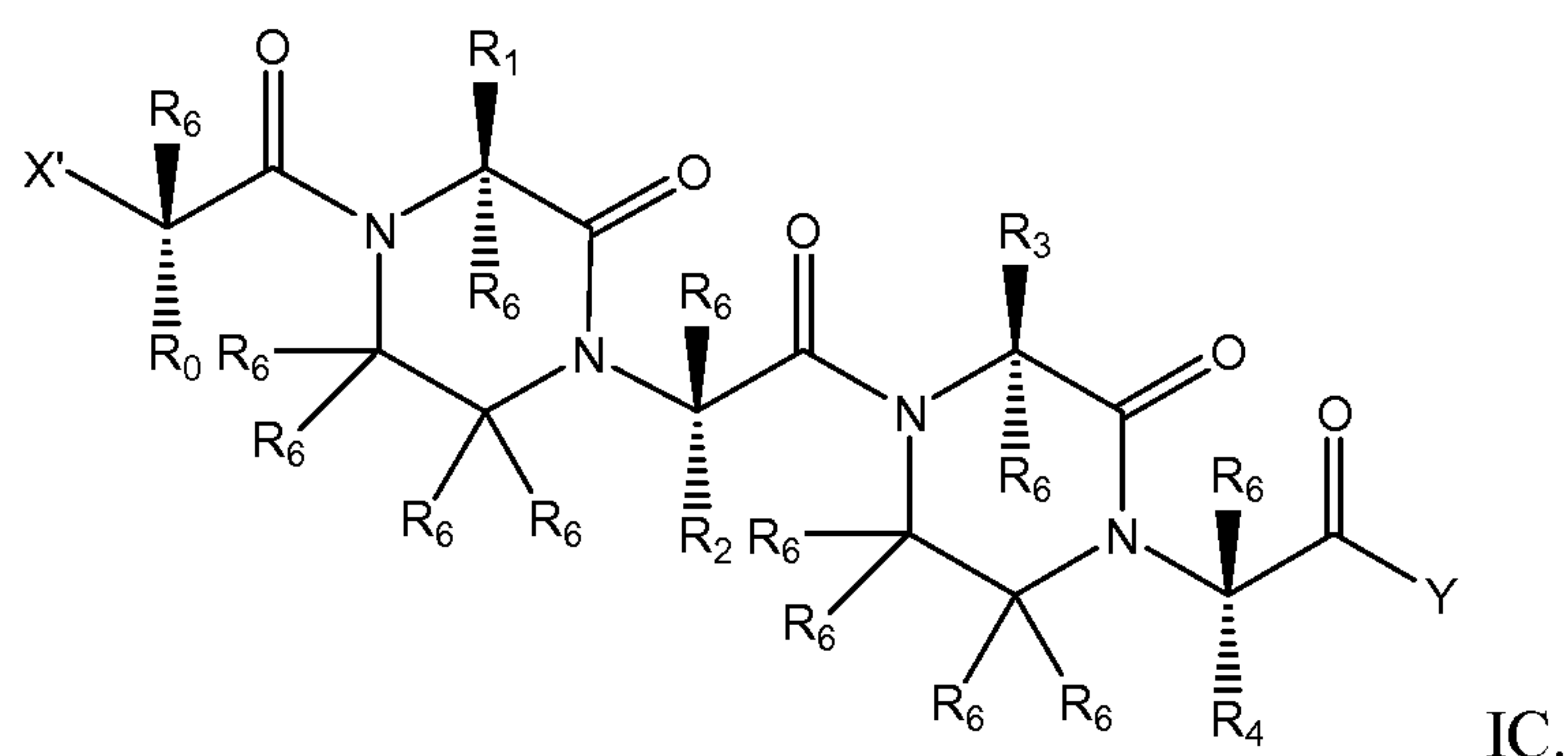
42. The method according to claim 34, wherein the oligooxopiperazine has a formula of Formula IB:



43. The method according to claim 42, wherein R_1 , R_2 , and R_4 of the oligooxopiperazine of Formula IB mimic the amino acid side chain of, respectively, residues i , $i+4$, and $i+7$, of the α -helix.

- 70 -

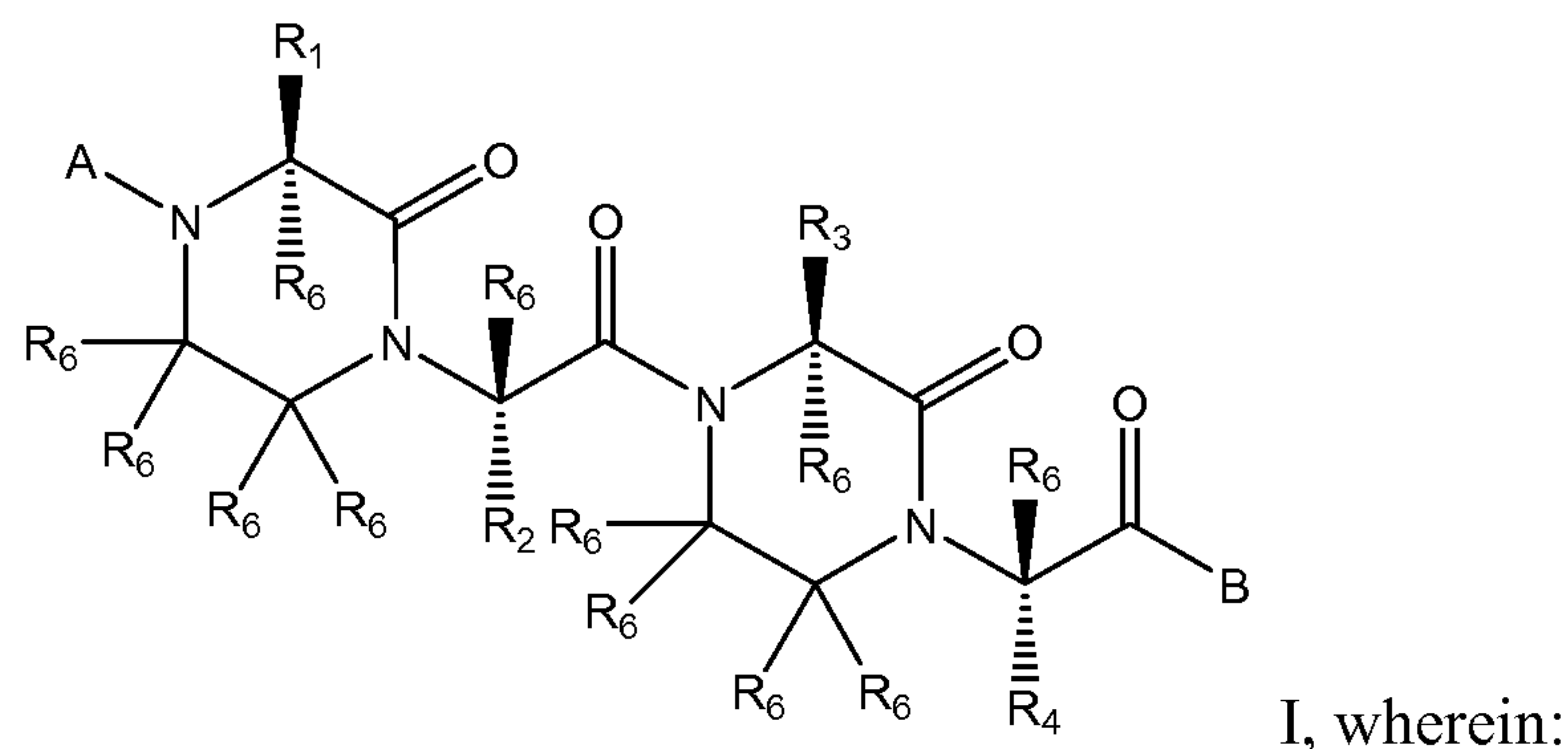
44. The method according to claim 34, wherein the oligooxopiperazine has a formula of Formula IC:



45. The method according to claim 44, wherein R_0 , R_1 , R_2 , R_3 , and R_4 of the oligooxopiperazine of Formula IC mimic the amino acid side chain of, respectively, residues i , $i+2$, $i+3$, $i+4$, and $i+7$ of the α -helix.

46. A method of preventing or reversing inactivation of p53 in a cell, said method comprising:
 contacting the cell with an oligooxopiperazine that substantially mimics helix αB of the C-terminal transactivation domain of HIF1 α under conditions effective to prevent or reverse inactivation of p53 in a cell.

47. The method according to claim 46, wherein the oligooxopiperazine is an oligooxopiperazine of Formula I:



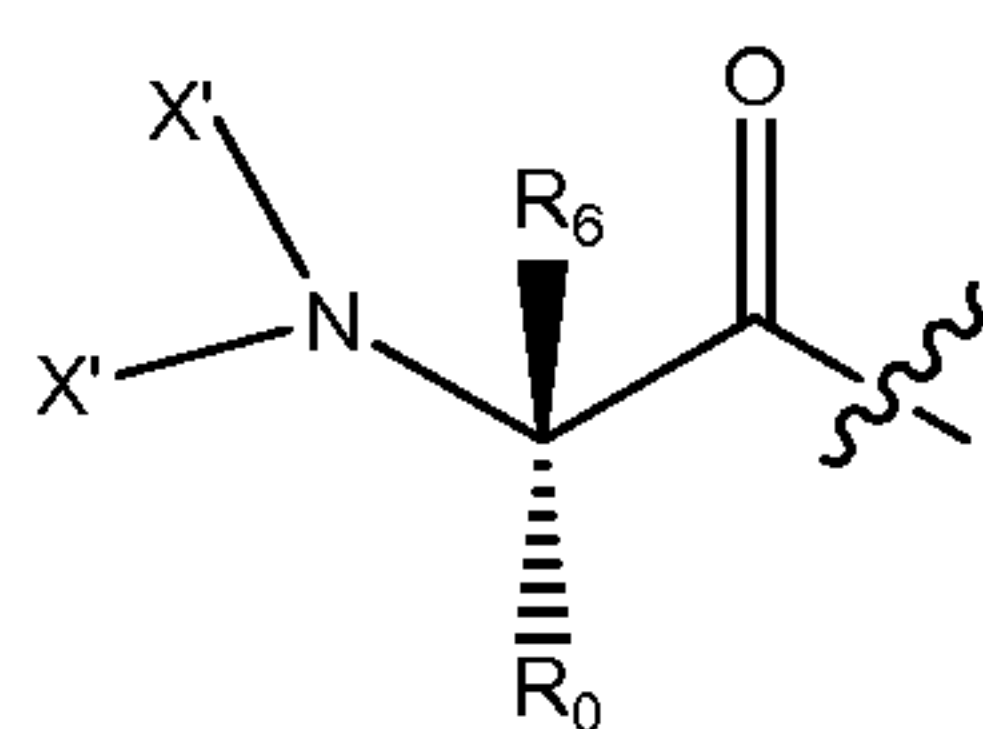
each of R_1 , R_2 , R_3 , and R_4 is independently an amino acid side chain, H, $N(R)_2$, OR, halogen, an alkyl, or an aryl; wherein each R is independently H, an alkyl, or an aryl;
 each R_6 is independently H, $N(R)_2$, OR, halogen, an alkyl, or an aryl; wherein each R is independently H, an alkyl, or an aryl;

- 71 -

A is X_1 or C, wherein:

X_1 is H, COR' , CO_2R' , $CONR'$, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a protecting group for protection of an amine, a targeting moiety, or a tag; wherein R' is H, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; and

C is a moiety of the formula



, wherein:

each X' is independently H, COR' , CO_2R' , $CONR'$, $N(R'')_2$, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; wherein:

R' is H, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; and

each R'' is independently H, CO_2R' , $CONR'$, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag;

R_0 is an amino acid side chain, H, $N(R)_2$, OR, halogen, an alkyl, or an aryl; wherein each R is independently H, an alkyl, or an aryl; and

R_6 is H, $N(R)_2$, OR, halogen, an alkyl, or an aryl; wherein each R is independently H, an alkyl, or an aryl; and

B is Y or D, wherein:

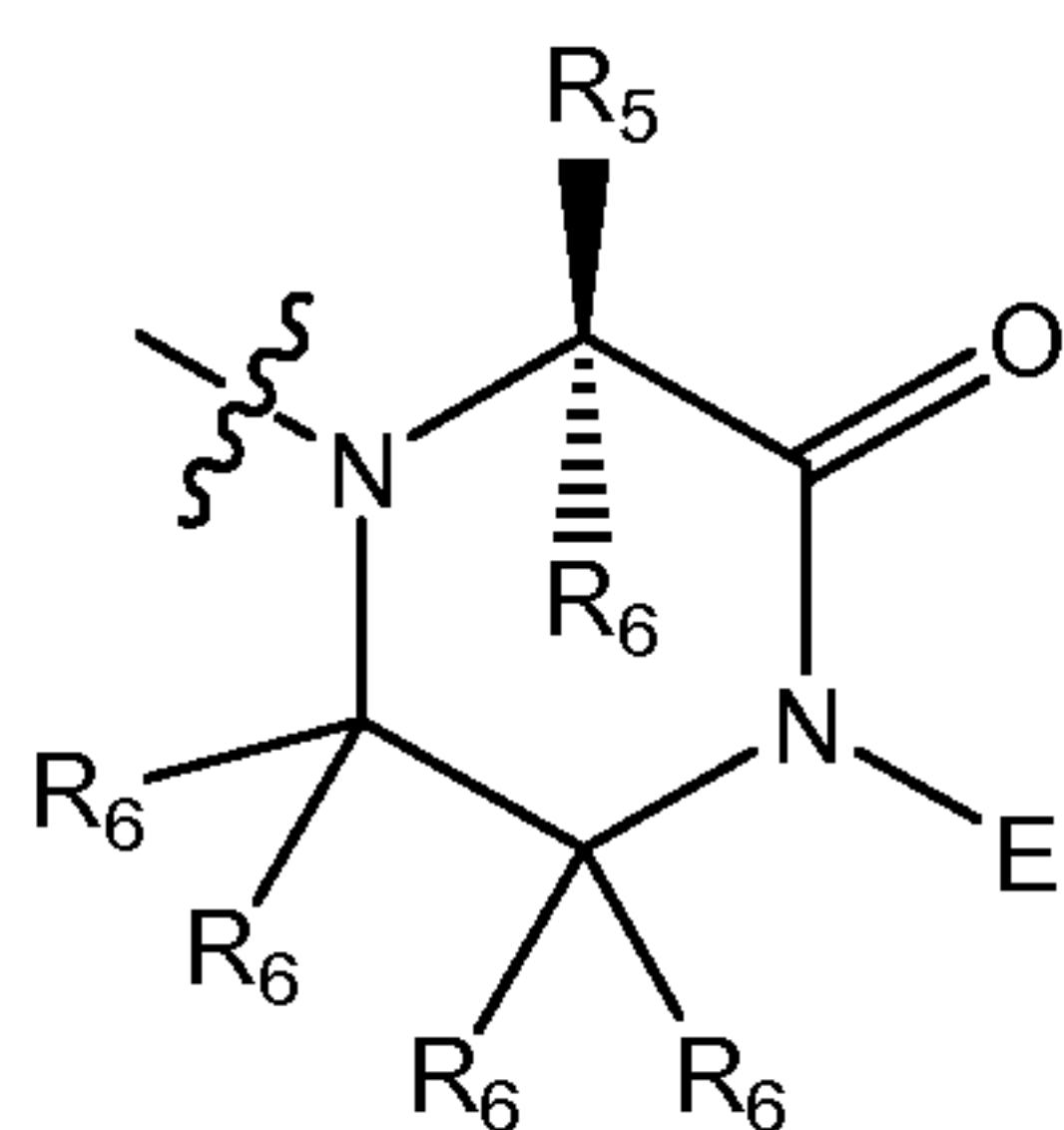
Y is OR' , COR' , $N(R''')_2$, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a protecting group for protection of a carboxylic acid, a targeting moiety, or a tag; wherein:

R' is H, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; and

each R''' is independently H, CO_2R' , $CONR'$, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; and

- 72 -

D is a moiety of the formula



, wherein:

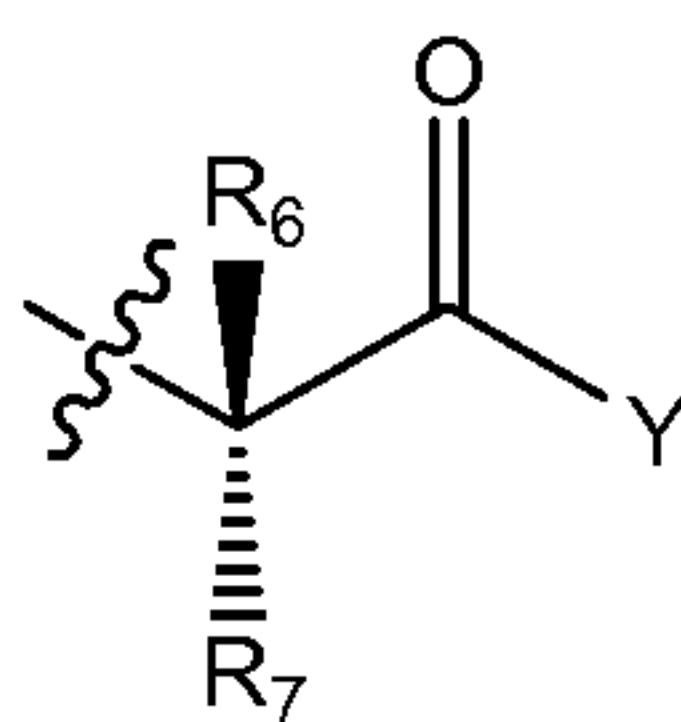
R₅ is an amino acid side chain, H, N(R)₂, OR, halogen, an alkyl, or an aryl; wherein each R is independently H, an alkyl, or an aryl;

5 R₆ is H, N(R)₂, OR, halogen, an alkyl, or an aryl; wherein each R is independently H, an alkyl, or an aryl; and

E is X₂ or F, wherein:

10 X₂ is H, COR', CO₂R', CONR', an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a protecting group for protection of an amine, a targeting moiety, or a tag; wherein R' is H, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; and

F is a moiety of the formula



15 , wherein:

R₆ is H, N(R)₂, OR, halogen, an alkyl, or an aryl;

wherein each R is independently H, an alkyl, or an aryl;

R₇ is an amino acid side chain; and

20 Y is OR', COR', N(R'')₂, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a protecting group for protection of a carboxylic acid, a targeting moiety, or a tag; wherein:

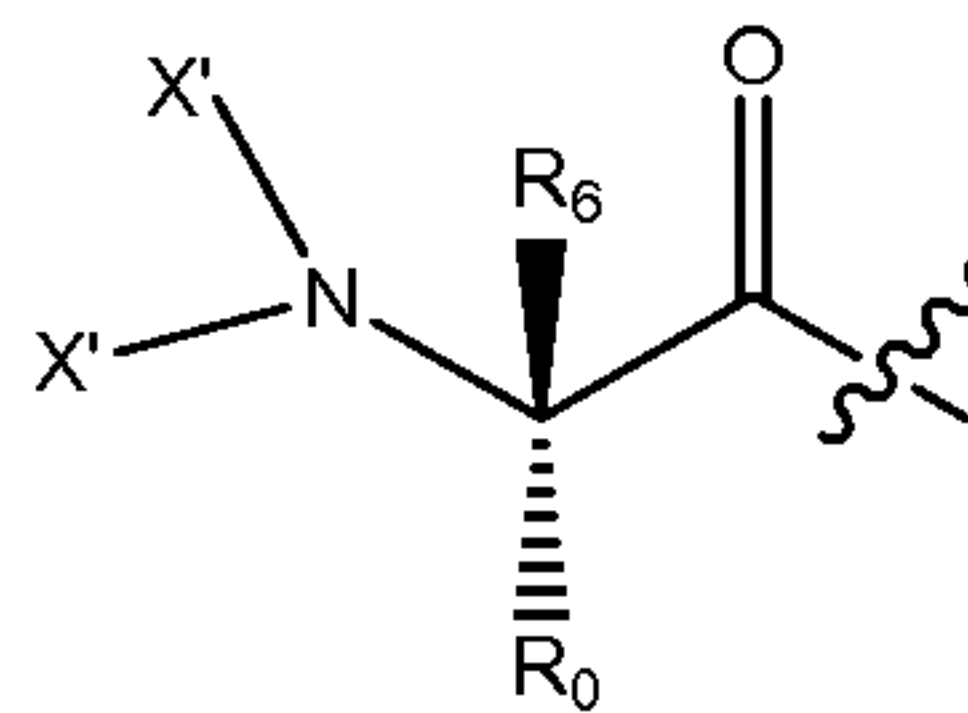
25 R' is H, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; and

- 73 -

each R''' is independently H, CO₂R', CONR', an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag.

48. The method according to claim 47, wherein:

5 (i) R₁ and R₂ are hydrophobic and R₄ is a hydrogen bond acceptor or hydrogen bond



donor or A is a moiety of formula

, R₀ and R₃ are

hydrophobic, and R₄ is a hydrogen bond acceptor or hydrogen bond donor;

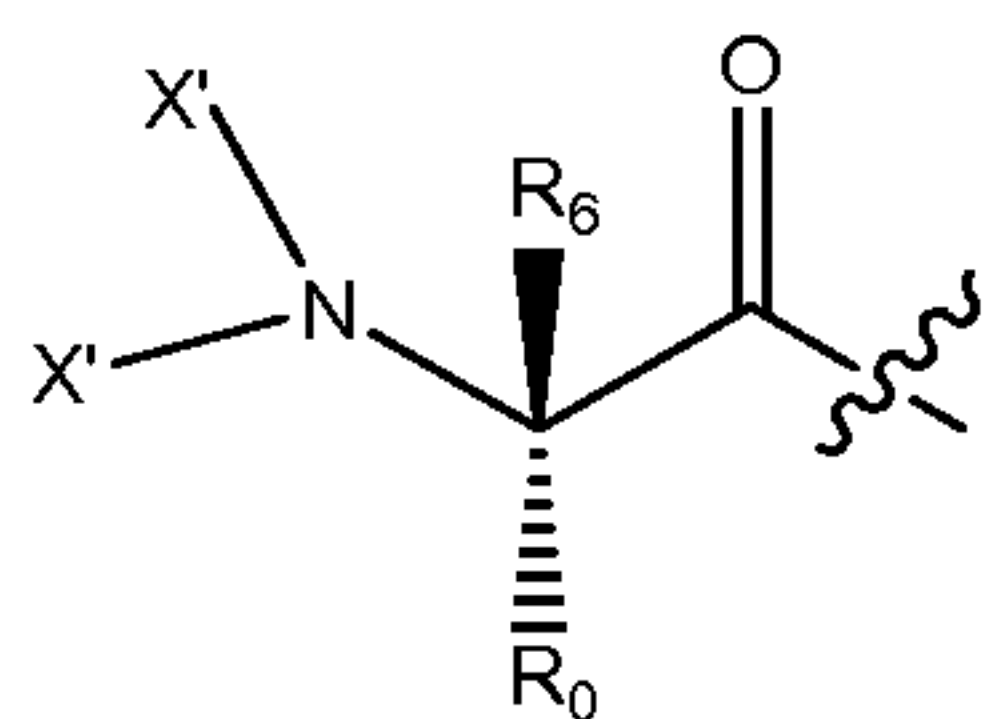
(ii) each R'' is independently H, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; and

10 (iii) B is not D.

49. The method according to claim 48, wherein R₁ and R₂ are hydrophobic and R₄ is a hydrogen bond acceptor or hydrogen bond donor.

50. The method according to claim 49, wherein the oligooxopiperazine is BB2-125 or BB2-162.

15 51. The method according to claim 48, wherein A is a moiety of

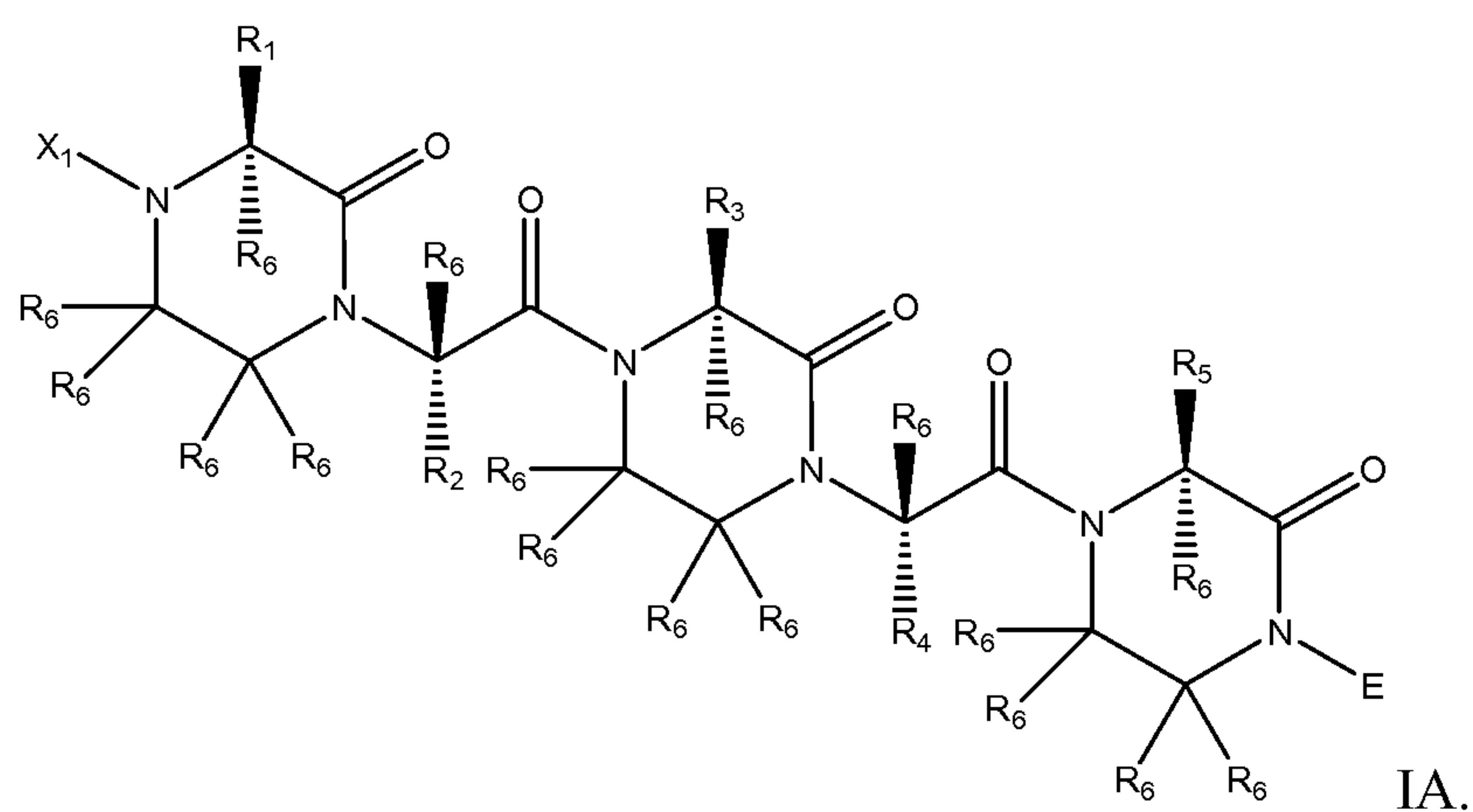


formula

, R₀ and R₃ are hydrophobic, and R₄ is a hydrogen bond acceptor or hydrogen bond donor.

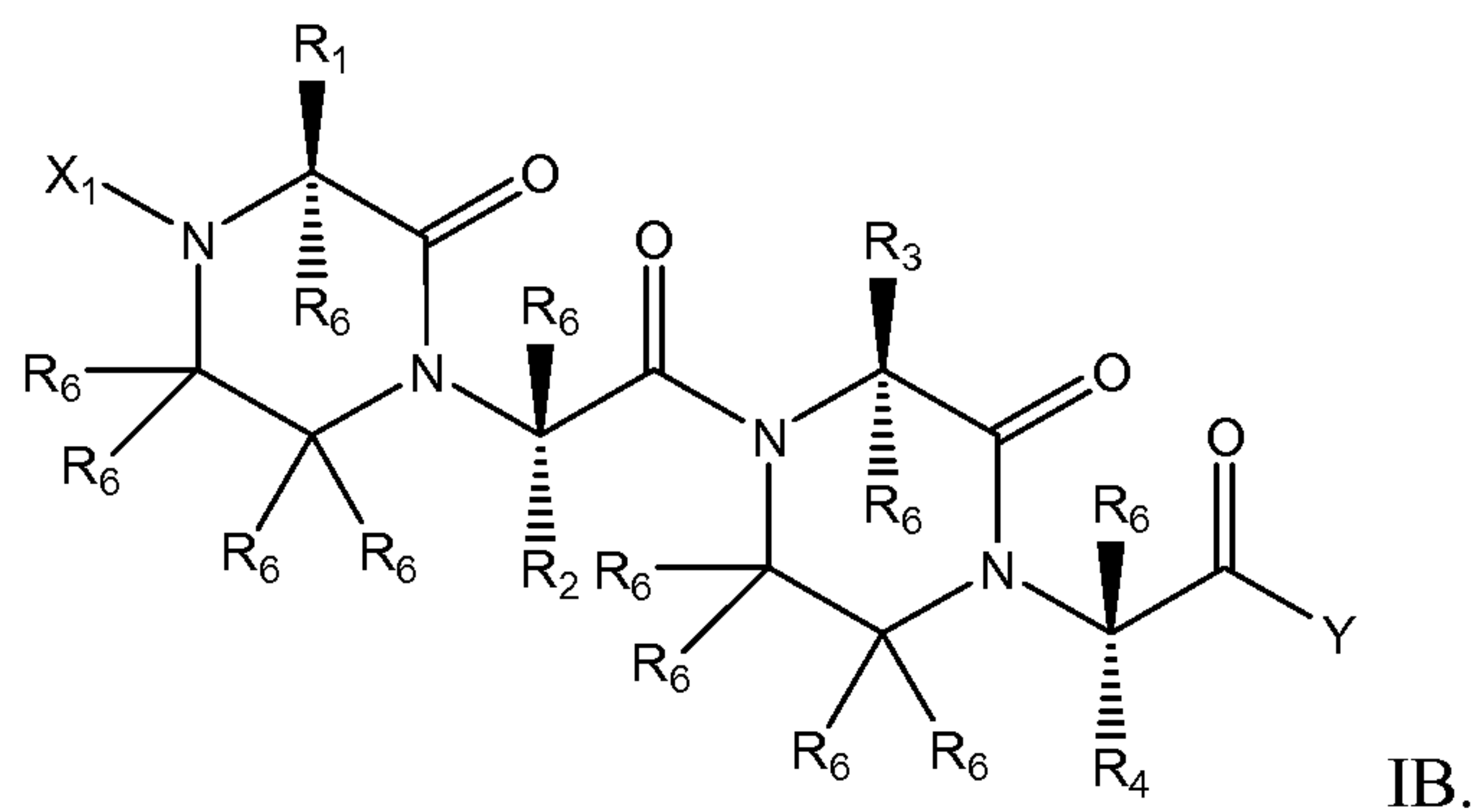
52. The method according to claim 51, wherein the oligooxopiperazine is BB2-164.

53. The method according to claim 47, wherein the oligooxopiperazine has a formula of Formula IA:



54. The method according to claim 53, wherein R_1 , R_2 , R_4 , and R_5 of the oligooxopiperazine of Formula IA mimic the amino acid side chain of, respectively, residues i , $i+4$, $i+6$, and $i+7$ of the α -helix.

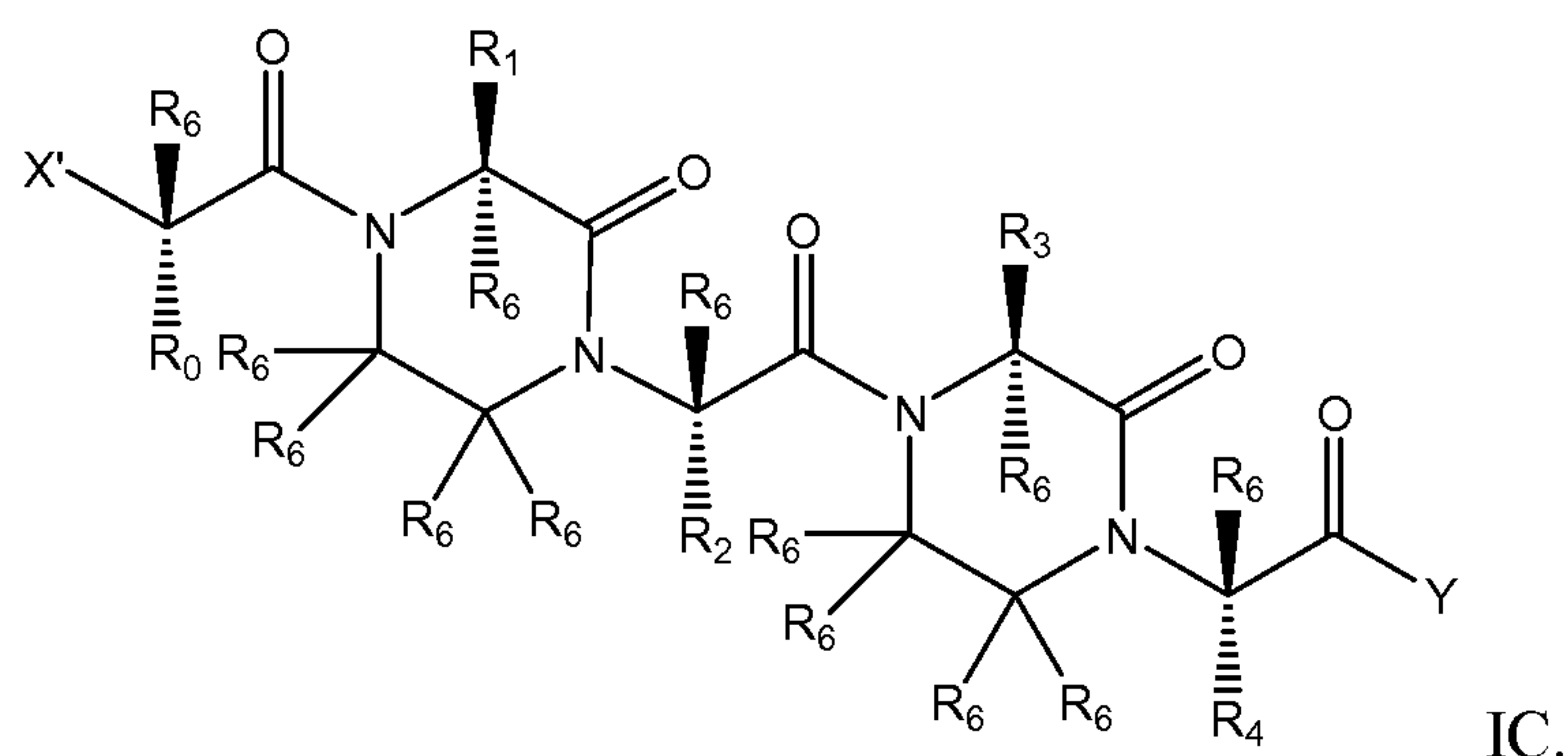
55. The method according to claim 47, wherein the oligooxopiperazine has a formula of Formula IB:



56. The method according to claim 55, wherein R_1 , R_2 , and R_4 of the oligooxopiperazine of Formula IB mimic the amino acid side chain of, respectively, residues i , $i+4$, and $i+7$, of the α -helix.

- 75 -

57. The method according to claim 47, wherein the oligooxopiperazine has a formula of Formula IC:

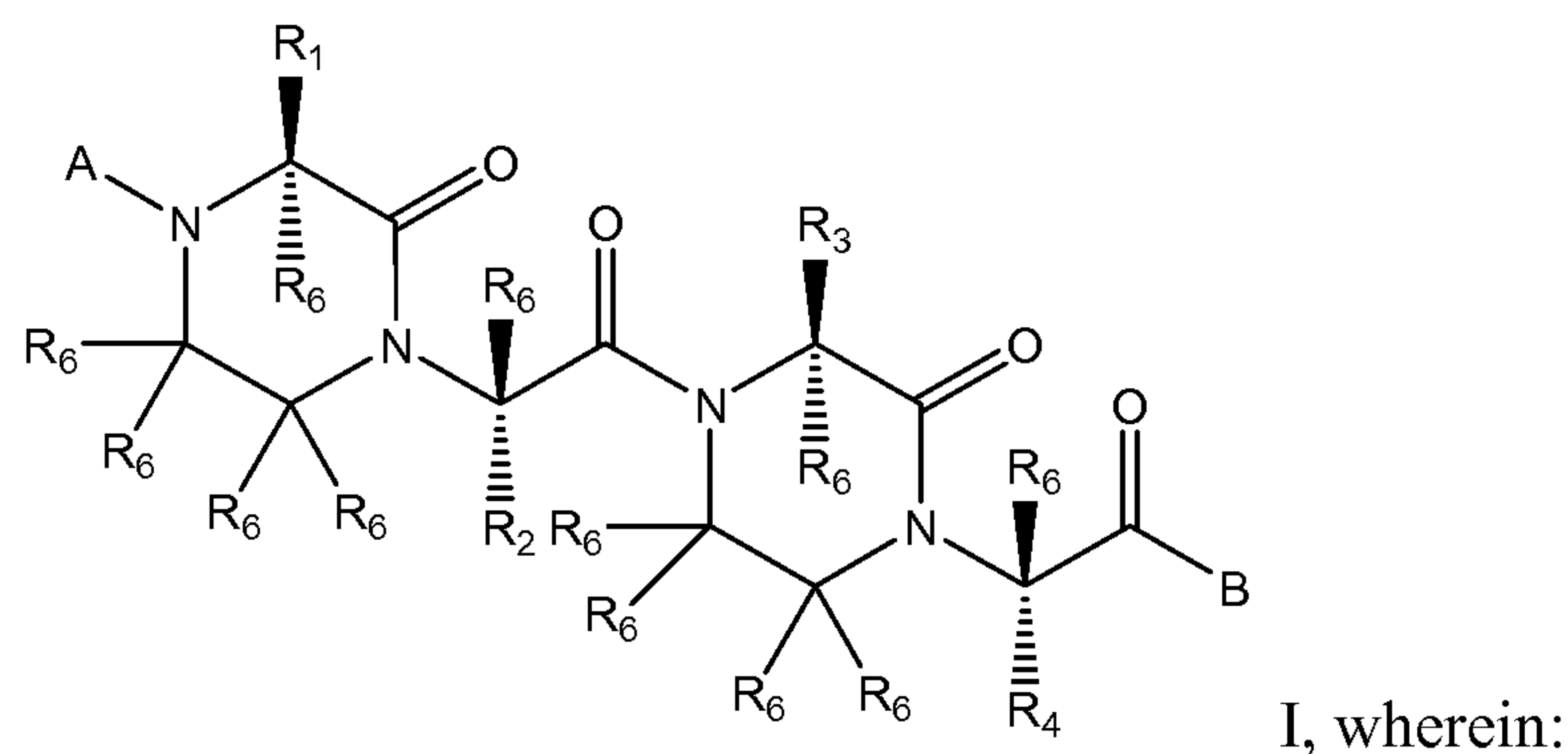


58. The method according to claim 57, wherein R_0 , R_1 , R_2 , R_3 , and R_4 of the oligooxopiperazine of Formula IC mimic the amino acid side chain of, respectively, residues i , $i+2$, $i+3$, $i+4$, and $i+7$ of the α -helix.

59. A method of inhibiting p300-mediated acetylation of a transcription factor in a cell, said method comprising:
 contacting the cell with an oligooxopiperazine that substantially
 mimics helix α B of the C-terminal transactivation domain of HIF1 α under conditions
 effective to inhibit p300-mediated acetylation of the transcription factor in the cell.

60. The method according to claim 59, wherein the transcription factor is p53.

61. The method according to claim 59, wherein the oligooxopiperazine is an oligooxopiperazine of Formula I:



- 76 -

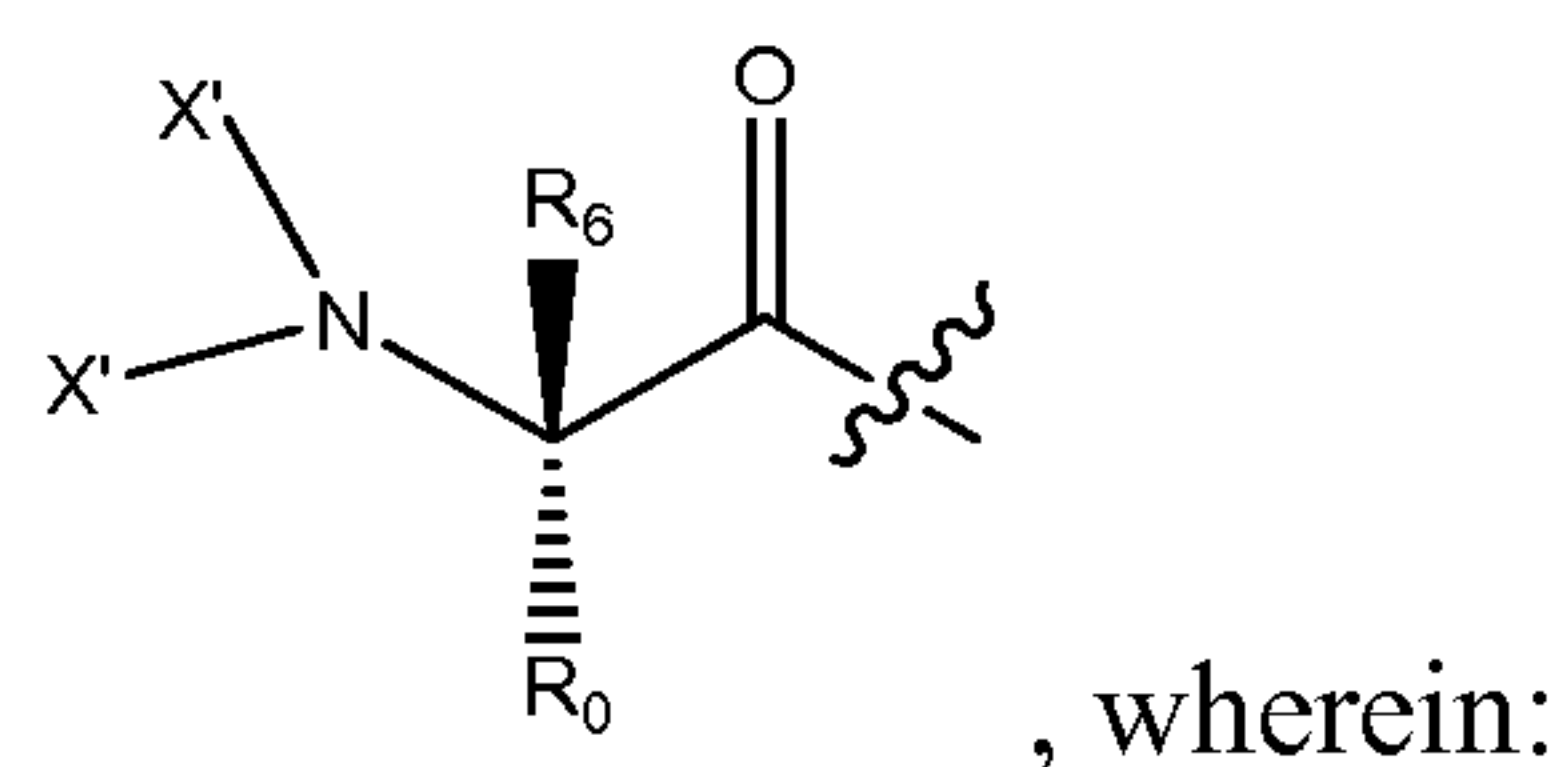
each of R_1 , R_2 , R_3 , and R_4 is independently an amino acid side chain, H, $N(R)_2$, OR, halogen, an alkyl, or an aryl; wherein each R is independently H, an alkyl, or an aryl;

each R_6 is independently H, $N(R)_2$, OR, halogen, an alkyl, or an aryl; wherein each R is independently H, an alkyl, or an aryl;

A is X_1 or C, wherein:

X_1 is H, COR' , CO_2R' , $CONR'$, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a protecting group for protection of an amine, a targeting moiety, or a tag; wherein R' is H, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; and

C is a moiety of the formula



each X' is independently H, COR' , CO_2R' , $CONR'$, $N(R'')_2$, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; wherein:

R' is H, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; and

each R'' is independently H, CO_2R' , $CONR'$, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag;

R_0 is an amino acid side chain, H, $N(R)_2$, OR, halogen, an alkyl, or an aryl; wherein each R is independently H, an alkyl, or an aryl; and

R_6 is H, $N(R)_2$, OR, halogen, an alkyl, or an aryl; wherein each R is independently H, an alkyl, or an aryl; and

B is Y or D, wherein:

Y is OR' , COR' , $N(R''')_2$, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a protecting group for protection of a carboxylic acid, a targeting moiety, or a tag; wherein:

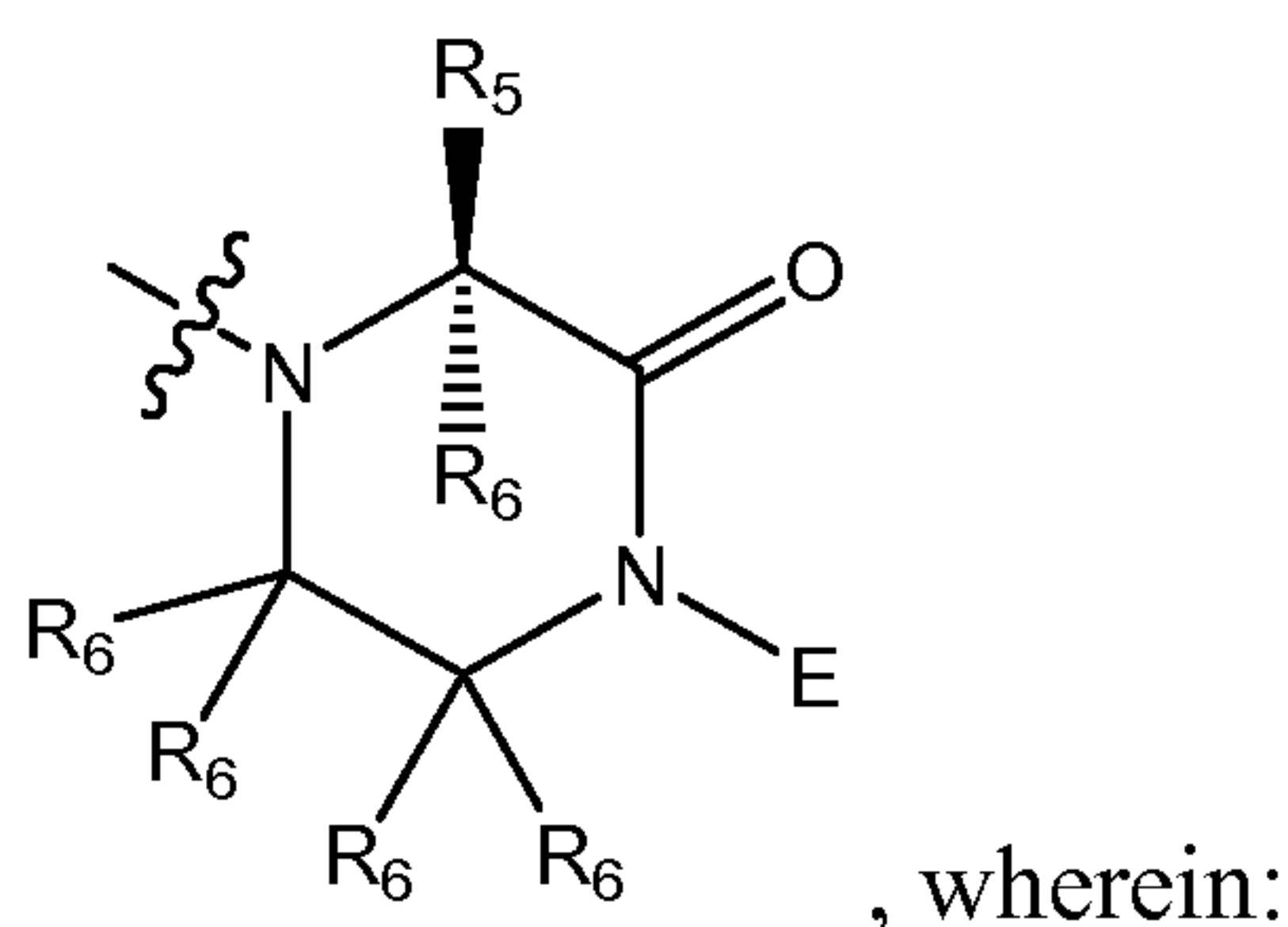
- 77 -

R' is H, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; and

each R''' is independently H, CO₂R', CONR', an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; and

5

D is a moiety of the formula



R₅ is an amino acid side chain, H, N(R)₂, OR, halogen, an alkyl, or an aryl; wherein each R is independently H, an alkyl, or an aryl;

10

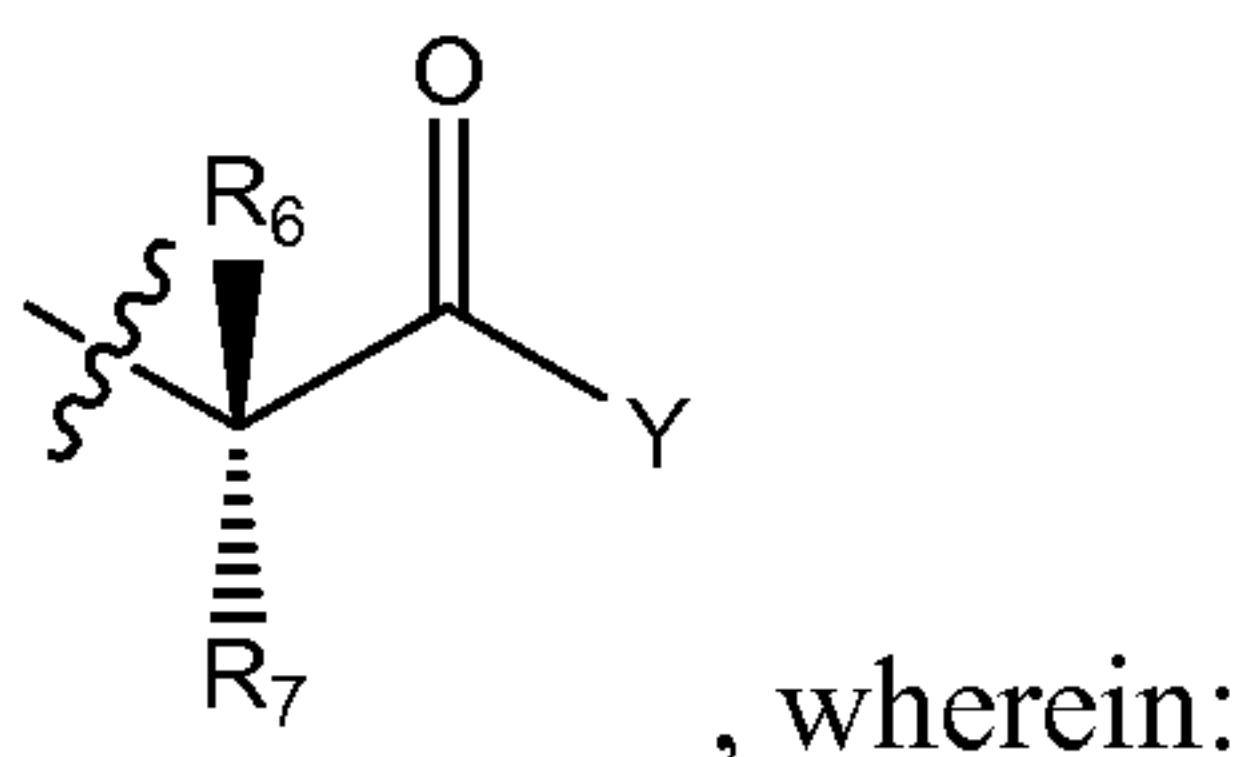
R₆ is H, N(R)₂, OR, halogen, an alkyl, or an aryl; wherein each R is independently H, an alkyl, or an aryl; and

E is X₂ or F, wherein:

X₂ is H, COR', CO₂R', CONR', an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a protecting group for protection of an amine, a targeting moiety, or a tag; wherein R' is H, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; and

15

F is a moiety of the formula



20

R₆ is H, N(R)₂, OR, halogen, an alkyl, or an aryl;

wherein each R is independently H, an alkyl, or an aryl;

R₇ is an amino acid side chain; and

25

Y is OR', COR', N(R''')₂, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a protecting group for

- 78 -

protection of a carboxylic acid, a targeting moiety, or a tag; wherein:

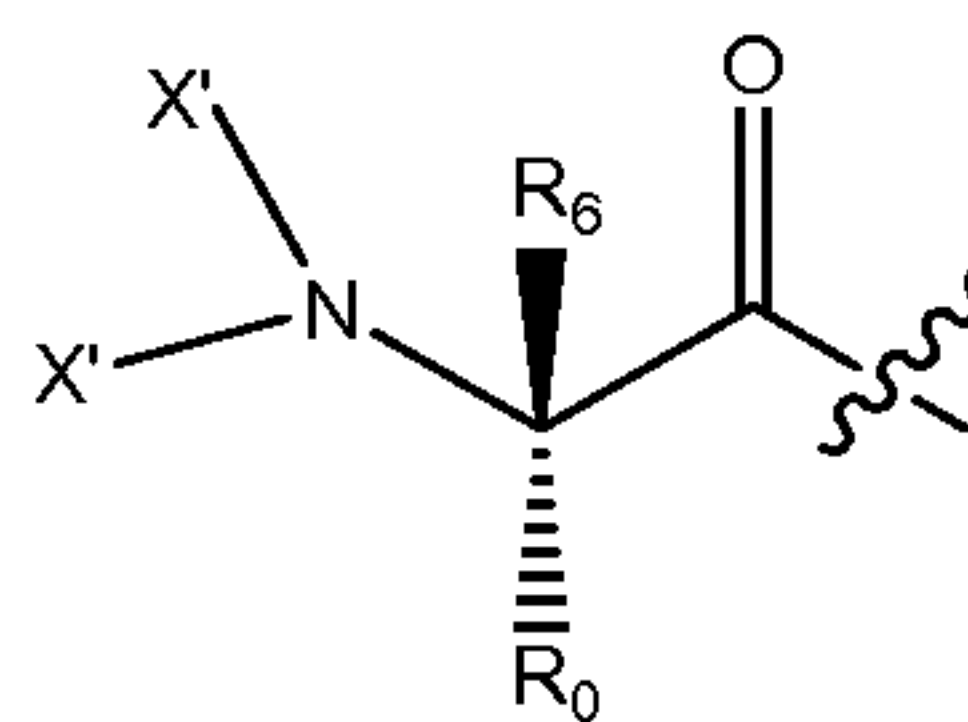
R' is H, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; and

each R''' is independently H, CO₂R', CONR', an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag.

5

62. The method according to claim 61, wherein:

10 (i) R₁ and R₂ are hydrophobic and R₄ is a hydrogen bond acceptor or hydrogen bond



donor or A is a moiety of formula

, R₀ and R₃ are

hydrophobic, and R₄ is a hydrogen bond acceptor or hydrogen bond donor;

(ii) each R'' is independently H, an alkyl, an aryl, an arylalkyl, a cycloalkyl, a heteroaryl, a targeting moiety, or a tag; and

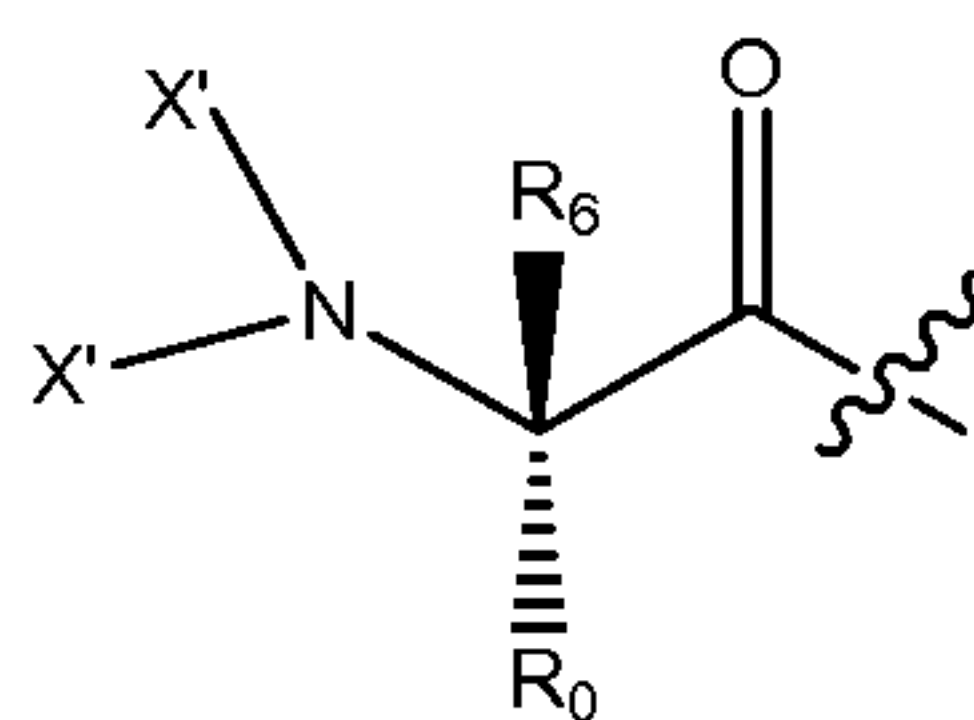
15 (iii) B is not D.

63. The method according to claim 62, wherein R₁ and R₂ are hydrophobic and R₄ is a hydrogen bond acceptor or hydrogen bond donor.

64. The method according to claim 63, wherein the oligooxopiperazine is BB2-125 or BB2-162.

20

65. The method according to claim 62, wherein A is a moiety of



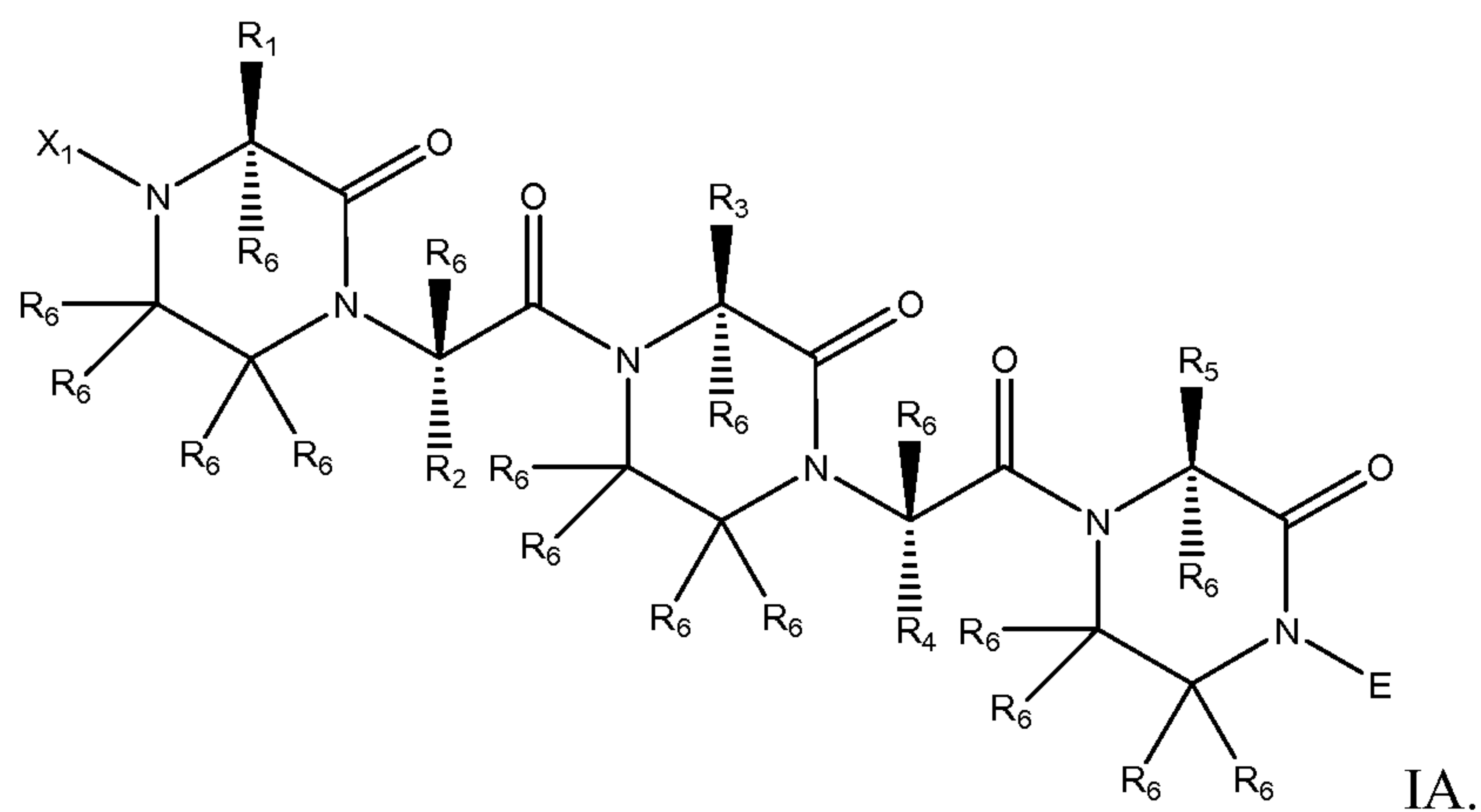
formula

, R₀ and R₃ are hydrophobic, and R₄ is a hydrogen bond acceptor or hydrogen bond donor.

66. The method according to claim 65, wherein the oligooxopiperazine is BB2-164.

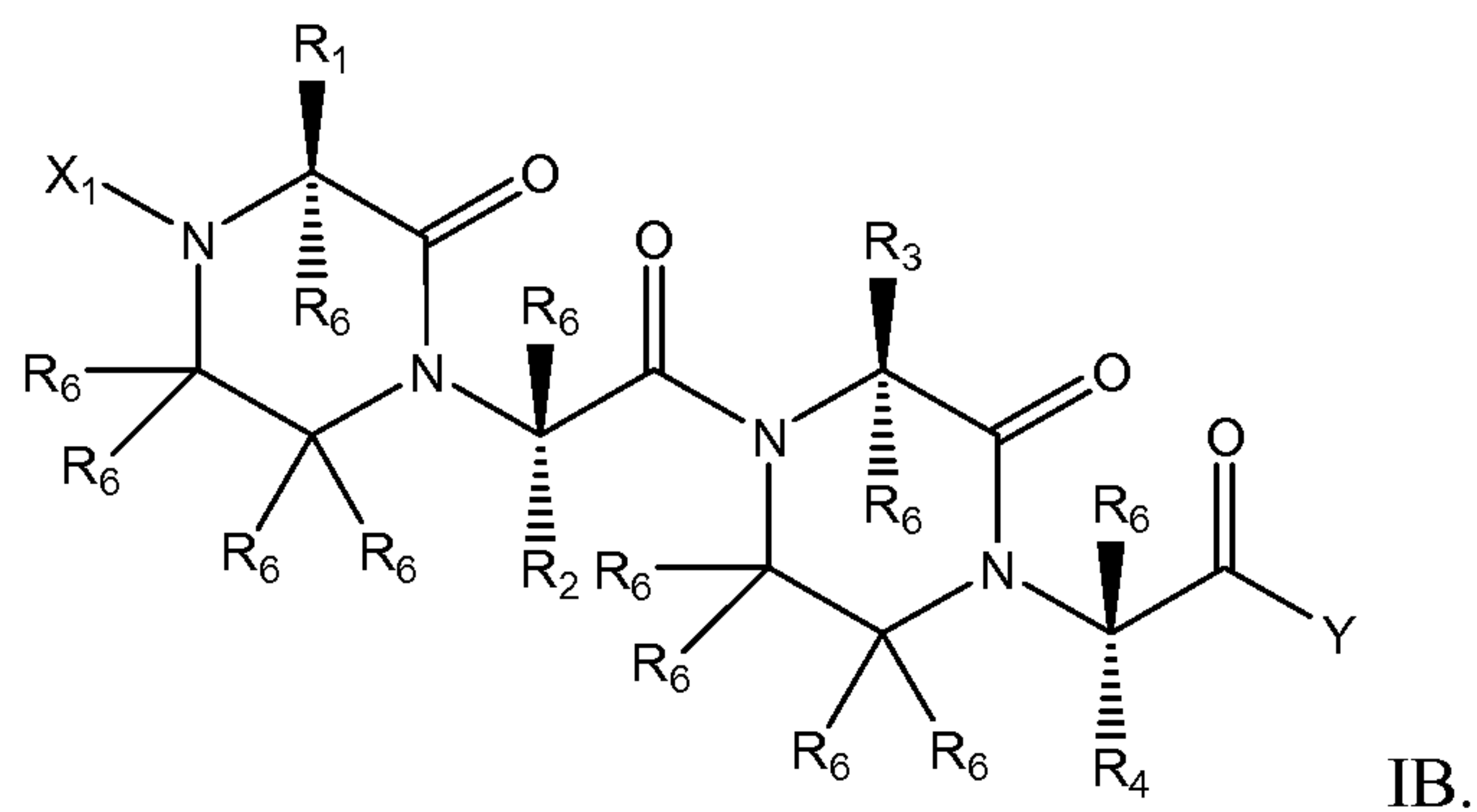
- 79 -

67. The method according to claim 61, wherein the oligooxopiperazine has a formula of Formula IA:



68. The method according to claim 67, wherein R_1 , R_2 , R_4 , and R_5 of the oligooxopiperazine of Formula IA mimic the amino acid side chain of, respectively, residues i , $i+4$, $i+6$, and $i+7$ of the α -helix.

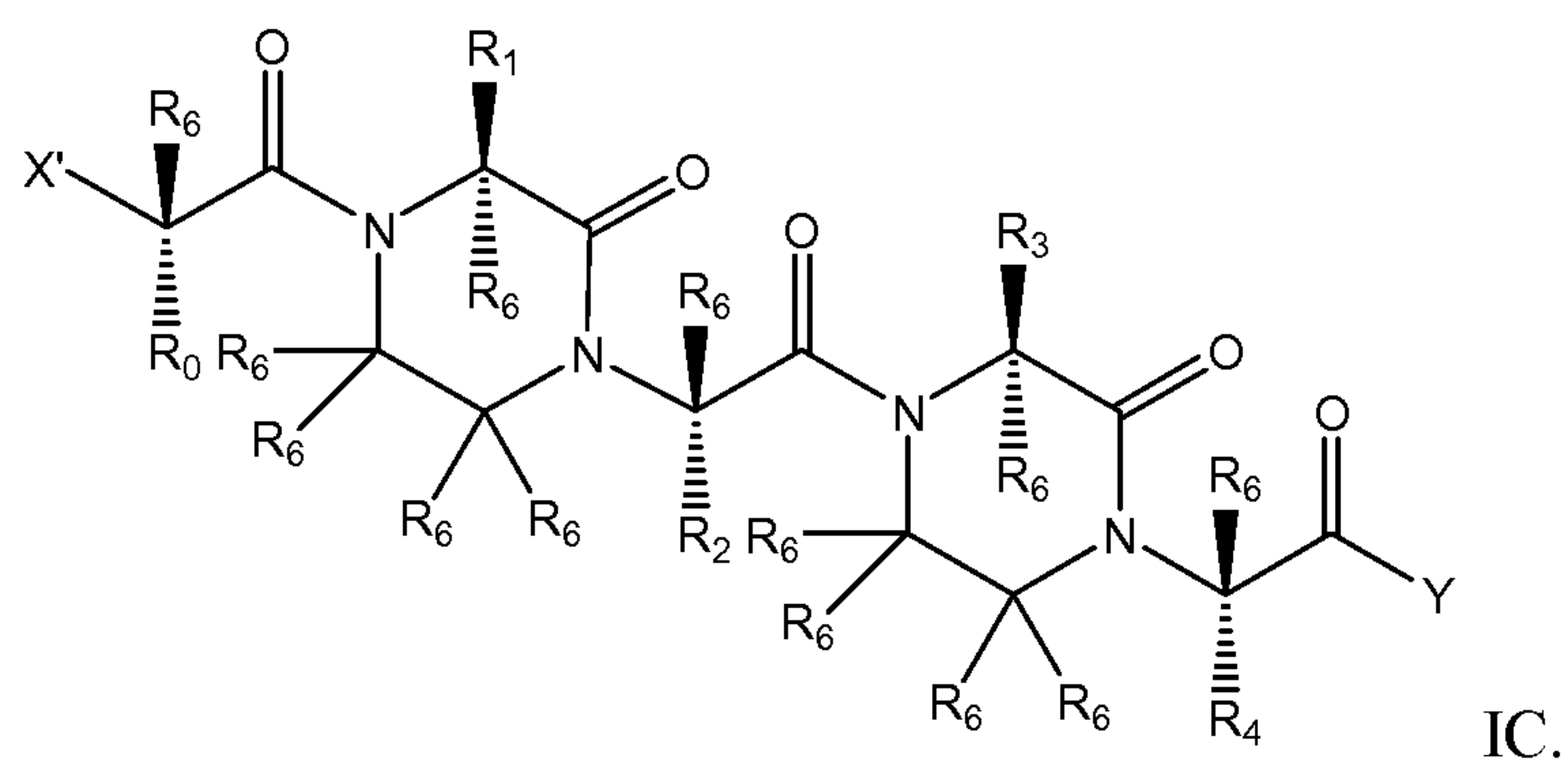
69. The method according to claim 61, wherein the oligooxopiperazine has a formula of Formula IB:



70. The method according to claim 69, wherein R_1 , R_2 , and R_4 of the oligooxopiperazine of Formula IB mimic the amino acid side chain of, respectively, residues i , $i+4$, and $i+7$, of the α -helix.

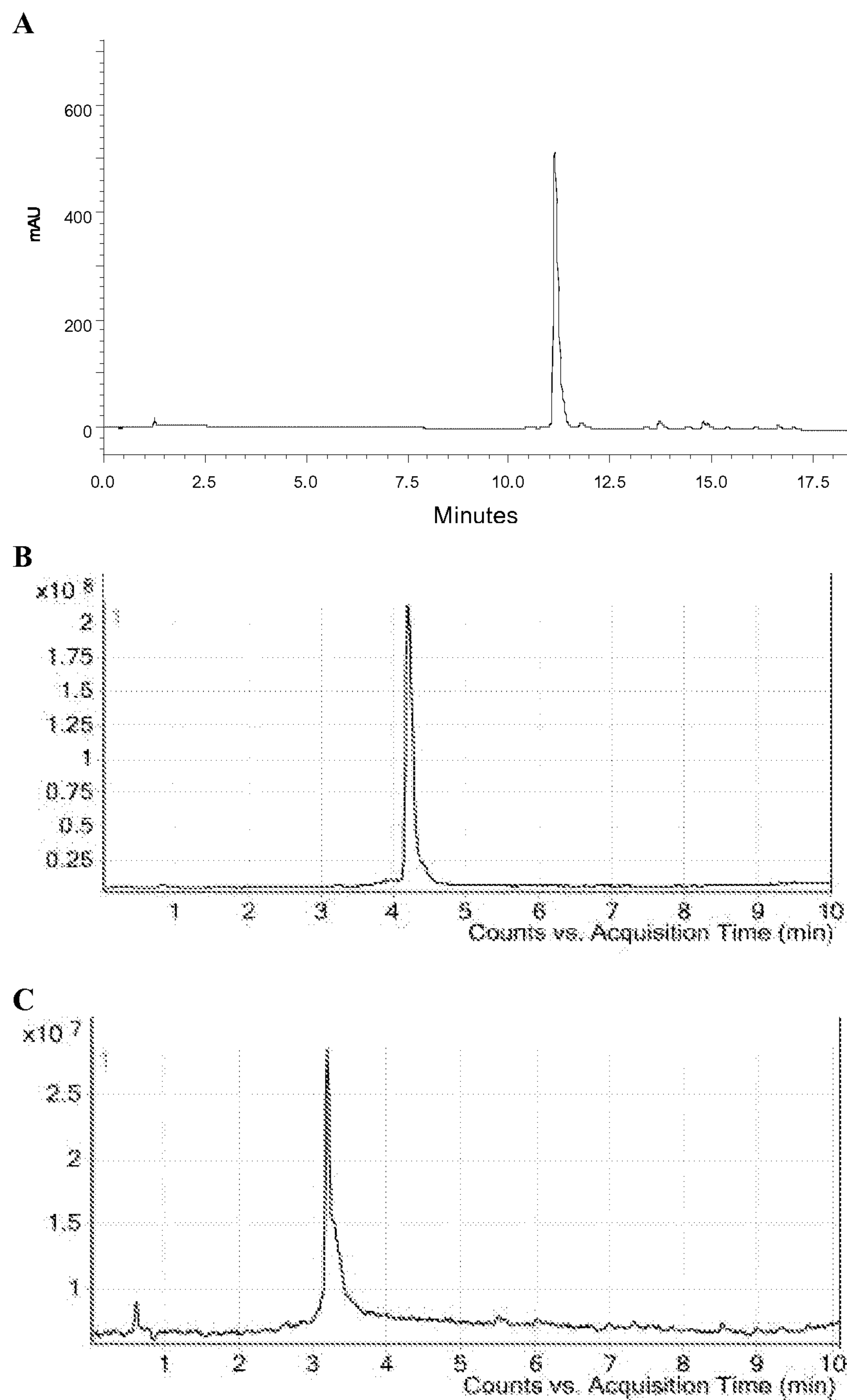
- 80 -

71. The method according to claim 61, wherein the oligooxopiperazine has a formula of Formula IC:

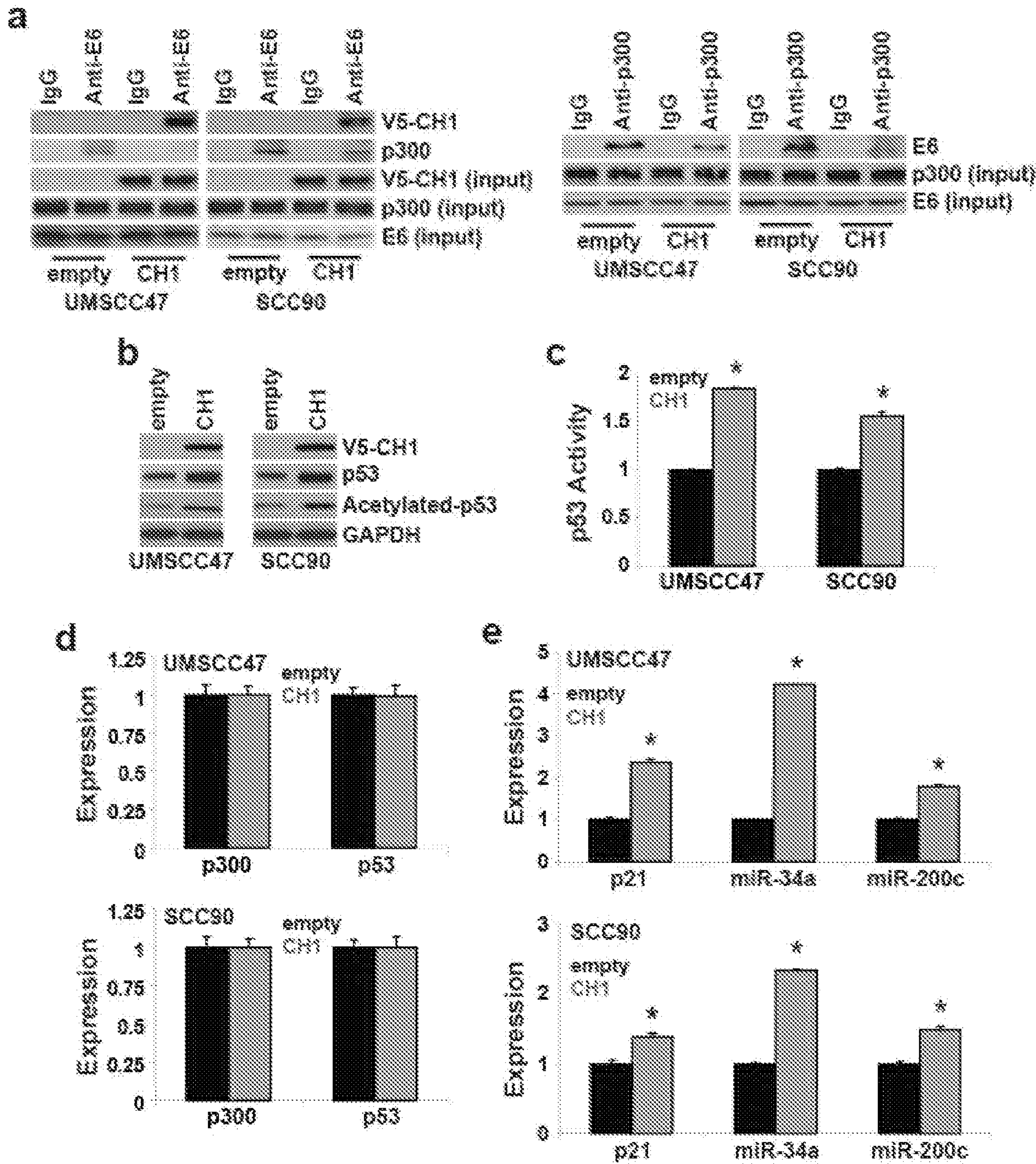


72. The method according to claim 71, wherein R_0 , R_1 , R_2 , R_3 , and R_4 of the oligooxopiperazine of Formula IC mimic the amino acid side chain of, respectively, residues i , $i+2$, $i+3$, $i+4$, and $i+7$ of the α -helix.

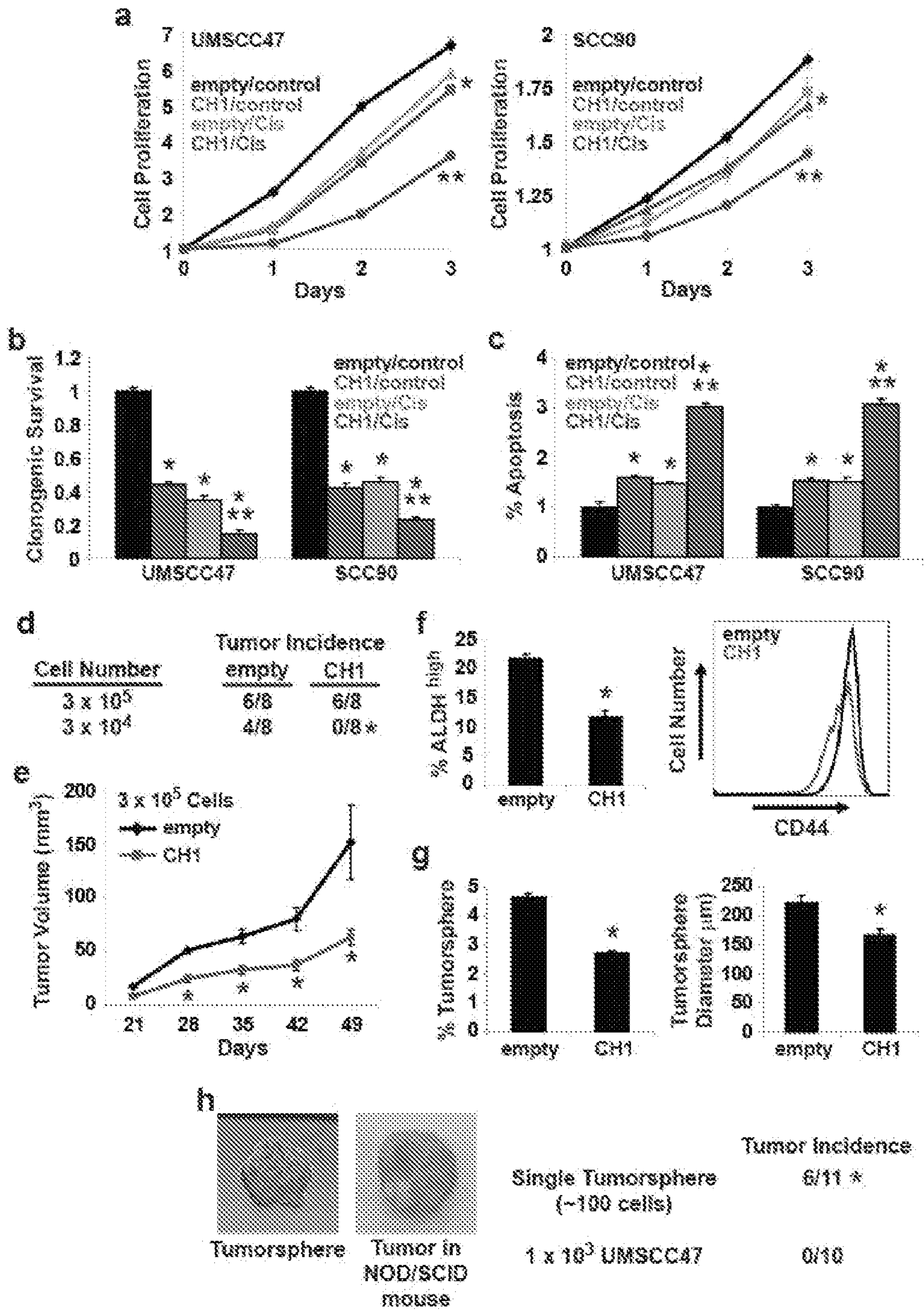
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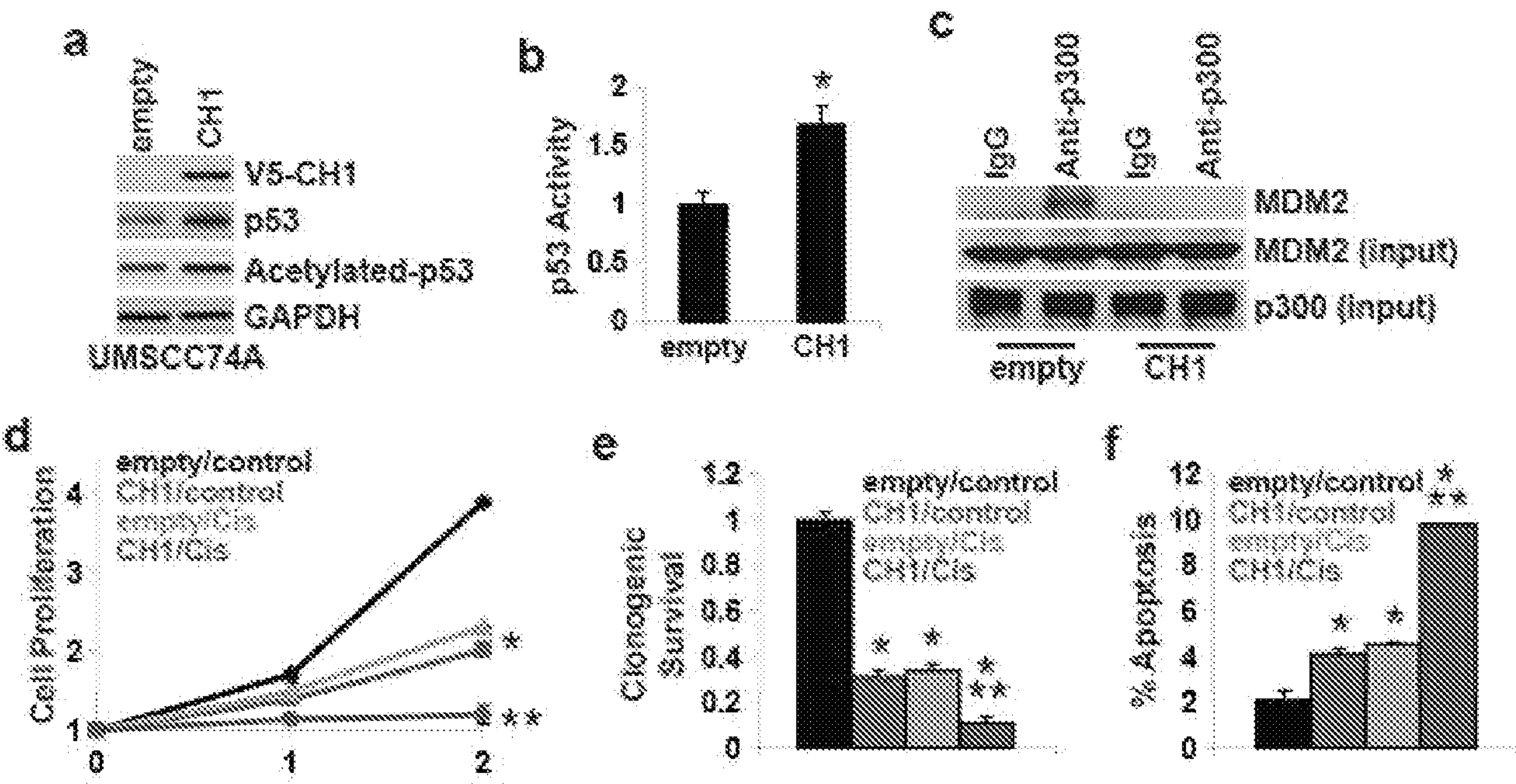
Figures 1A–C



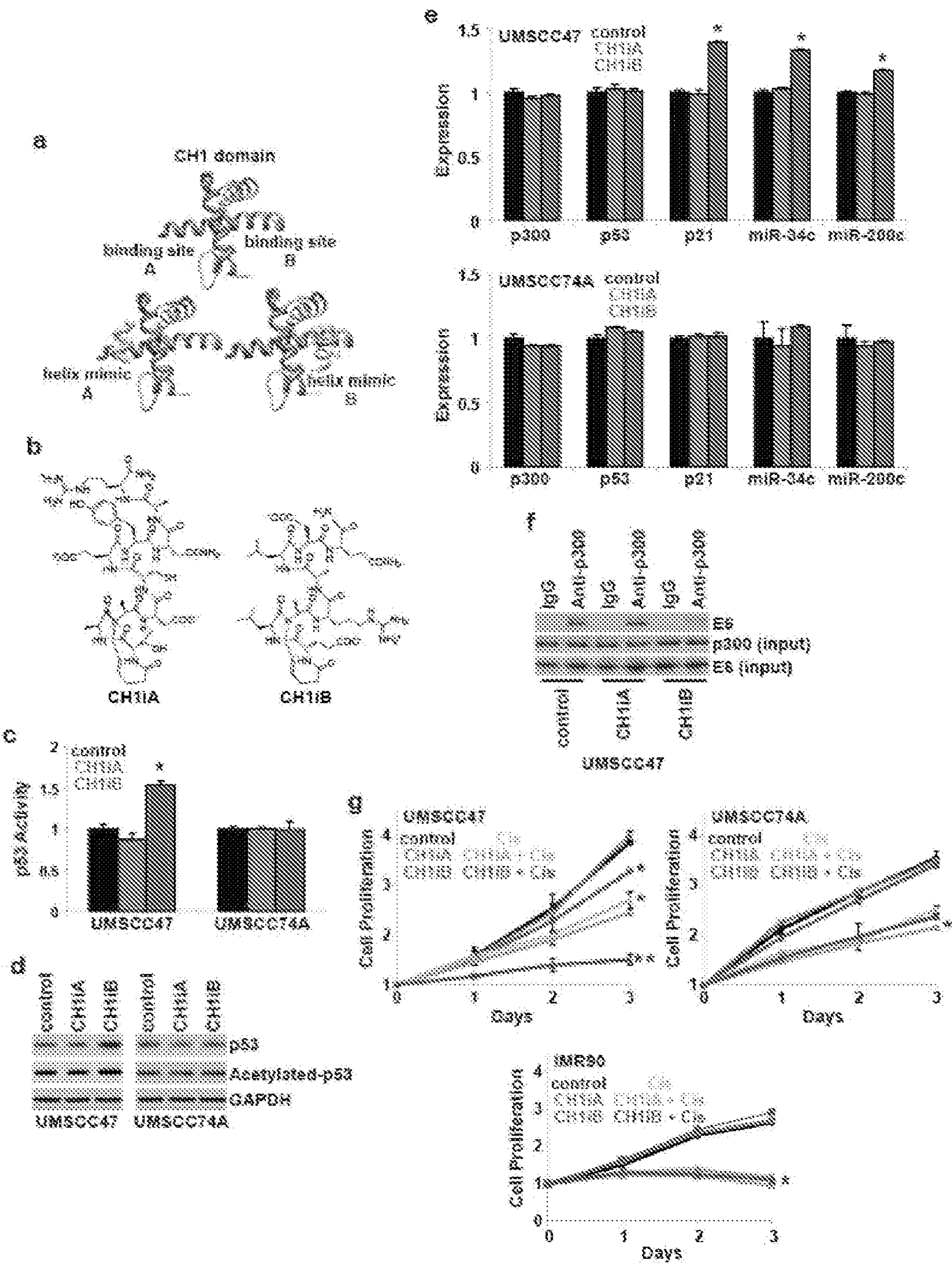
Figures 2A–E



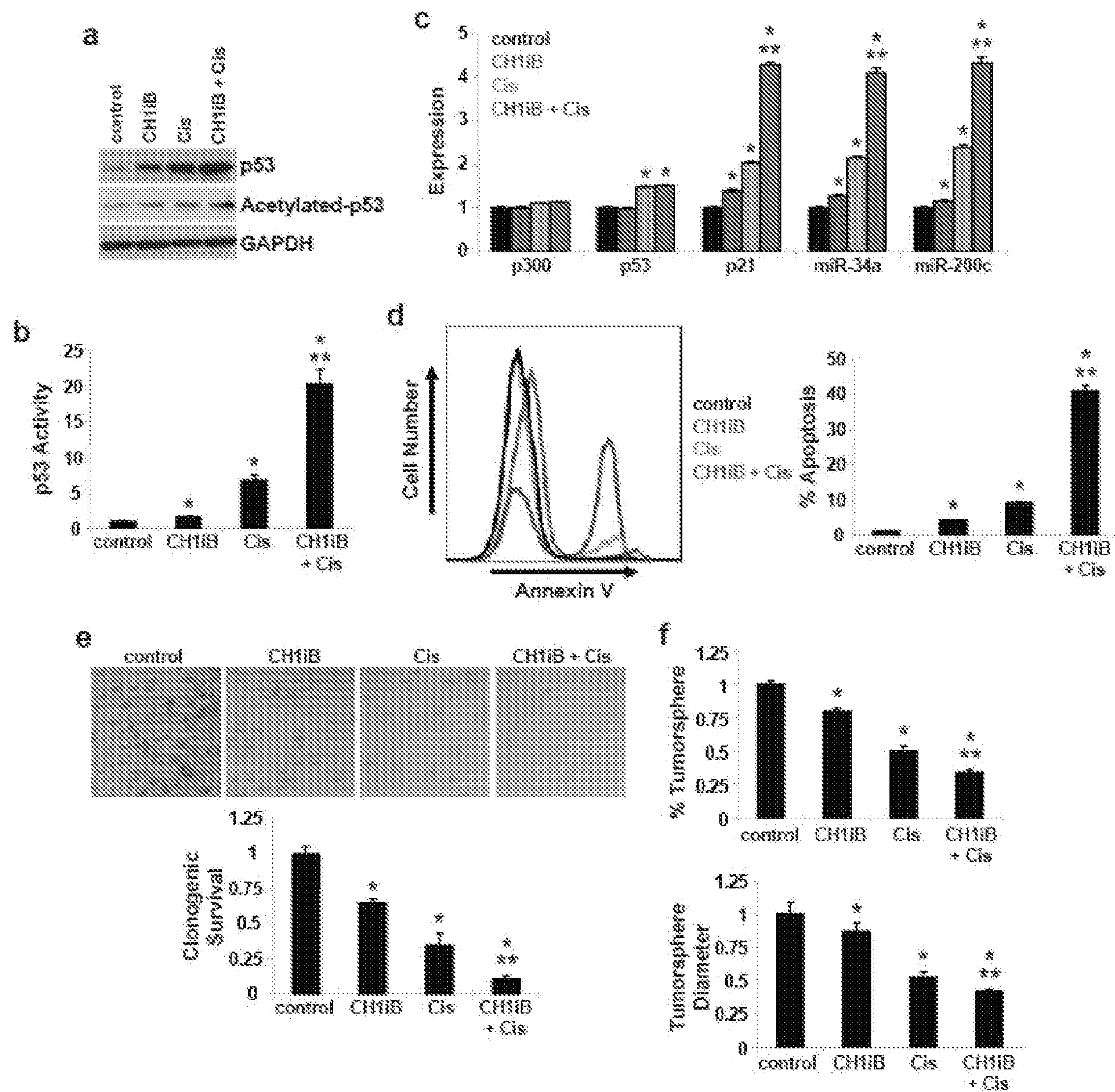
Figures 3A–H



Figures 4A–F

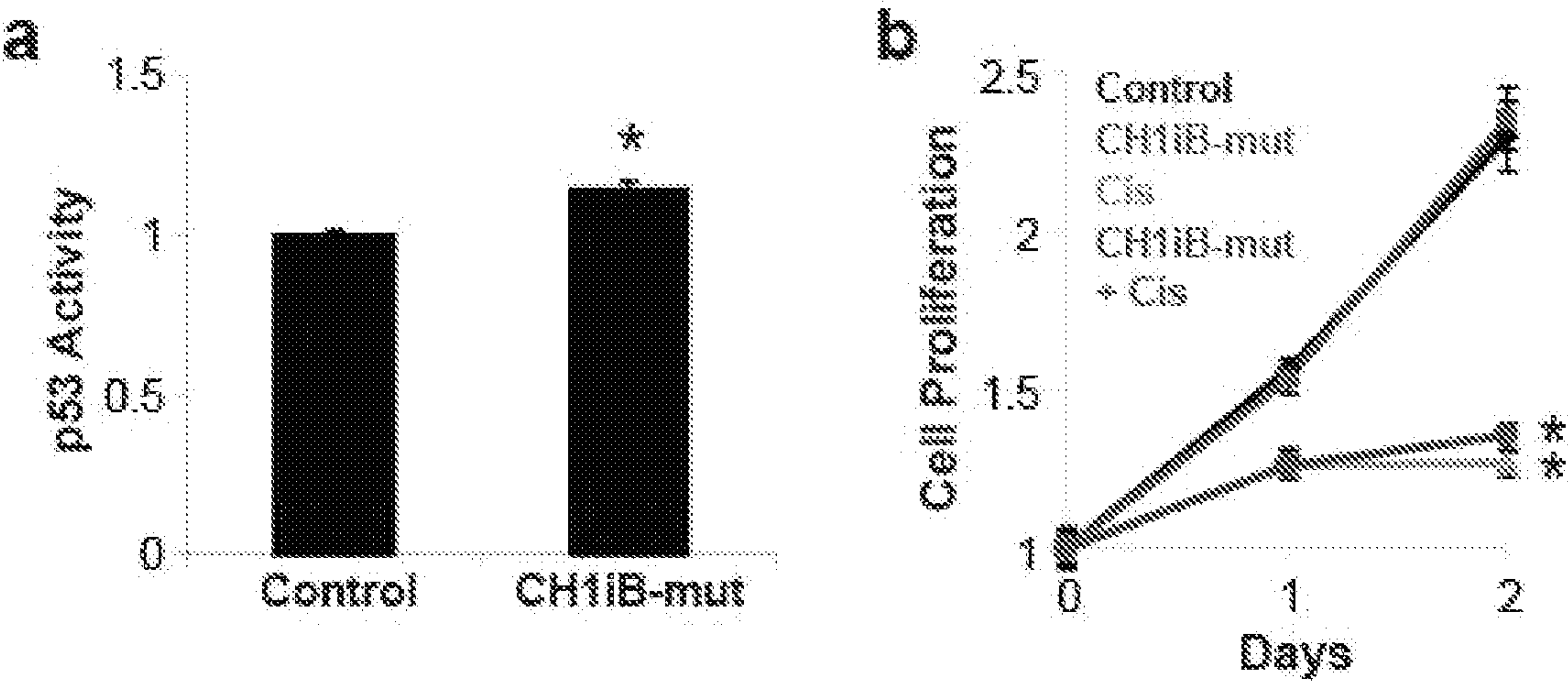


Figures 5A–G



Figures 6A–F

7/14



Figures 7A-B

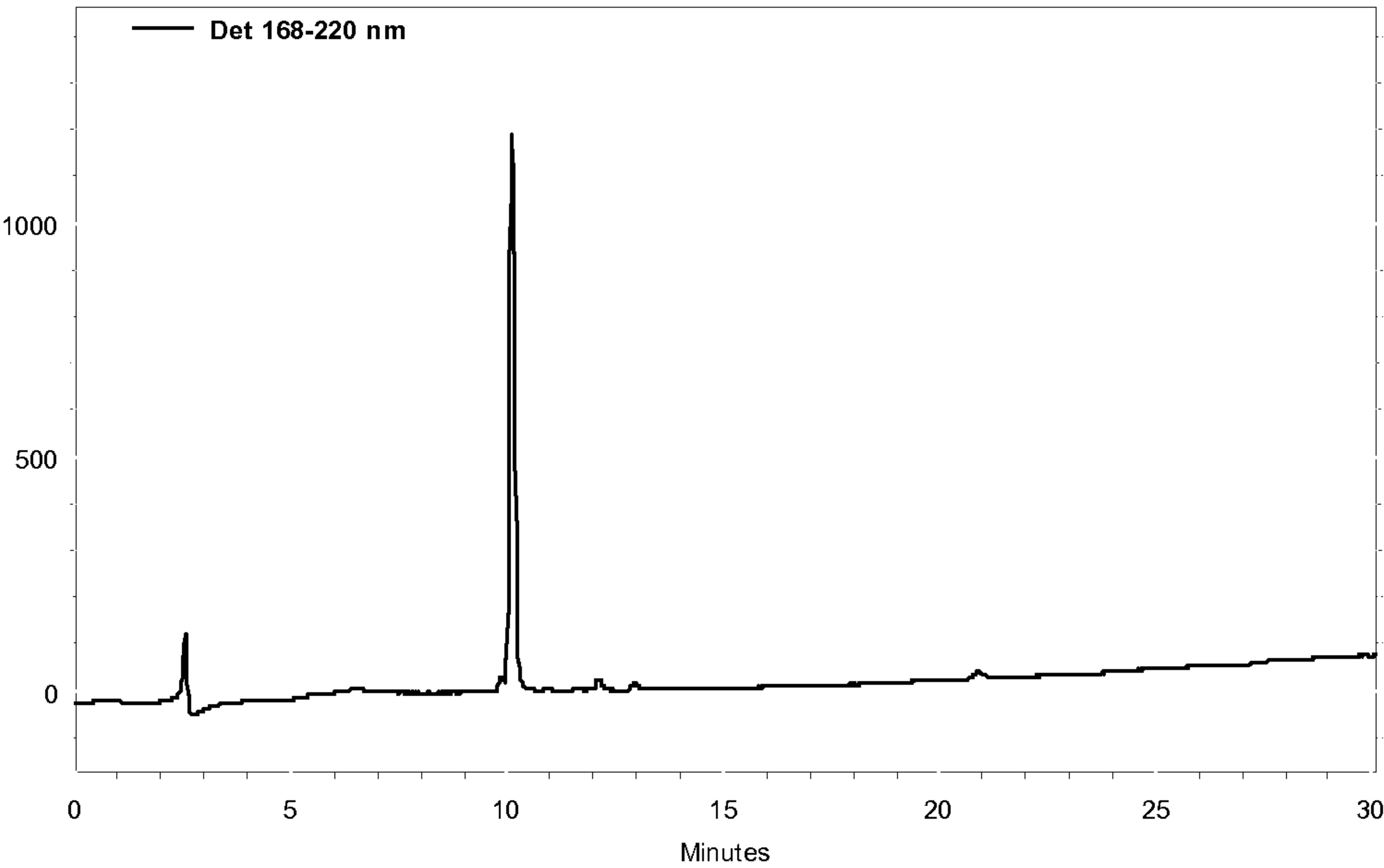


Figure 8

8/14

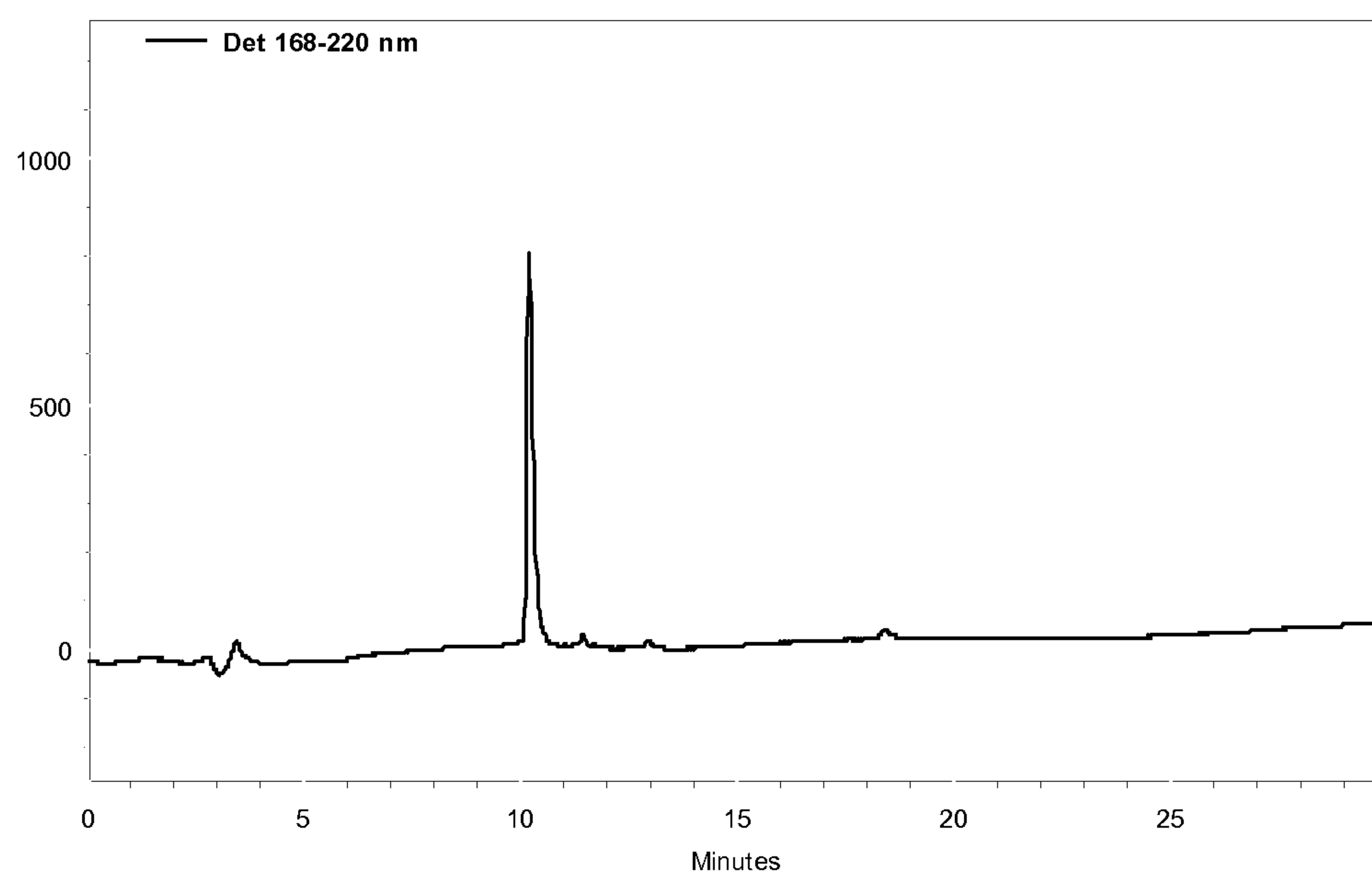


Figure 9

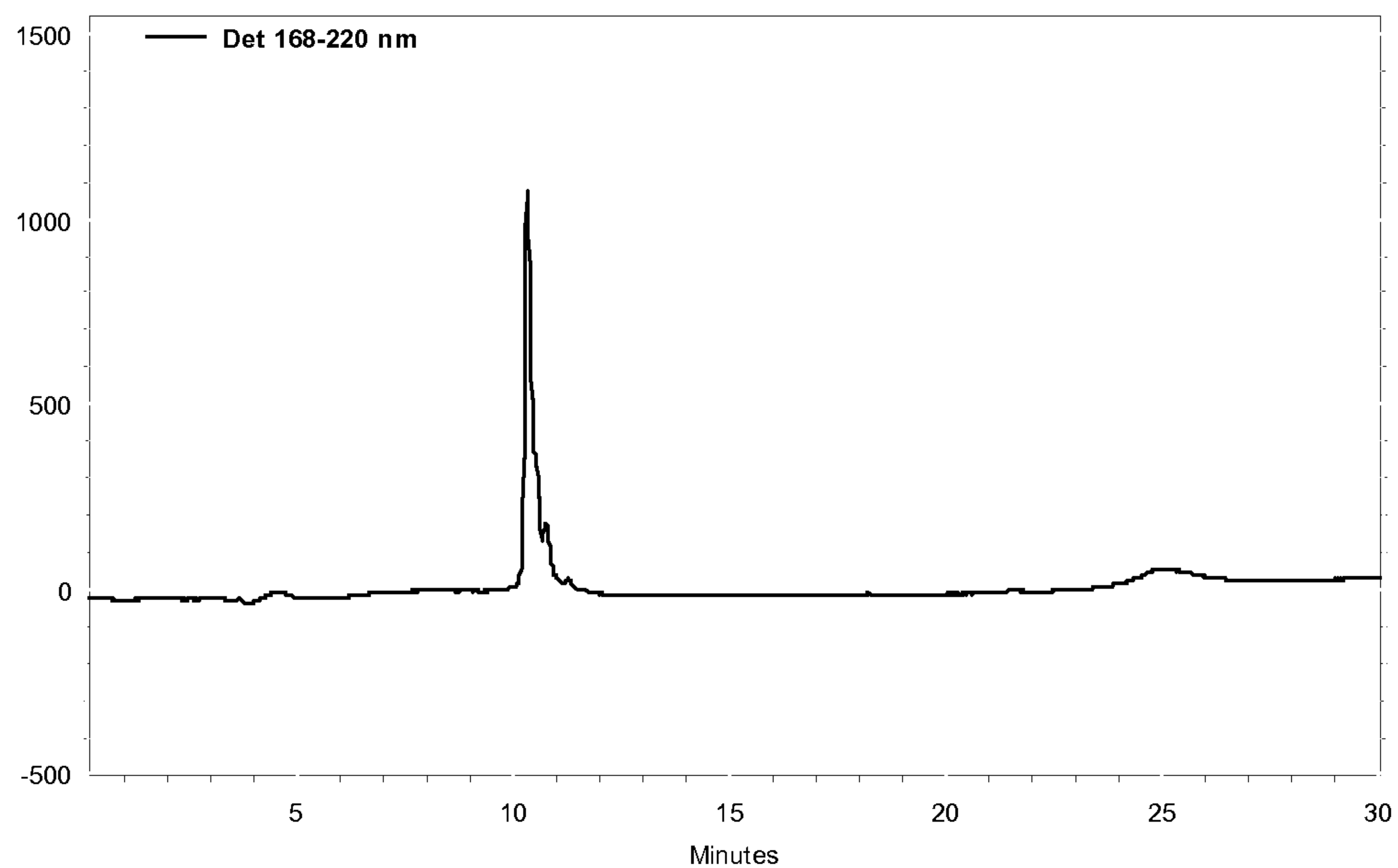


Figure 10

9/14

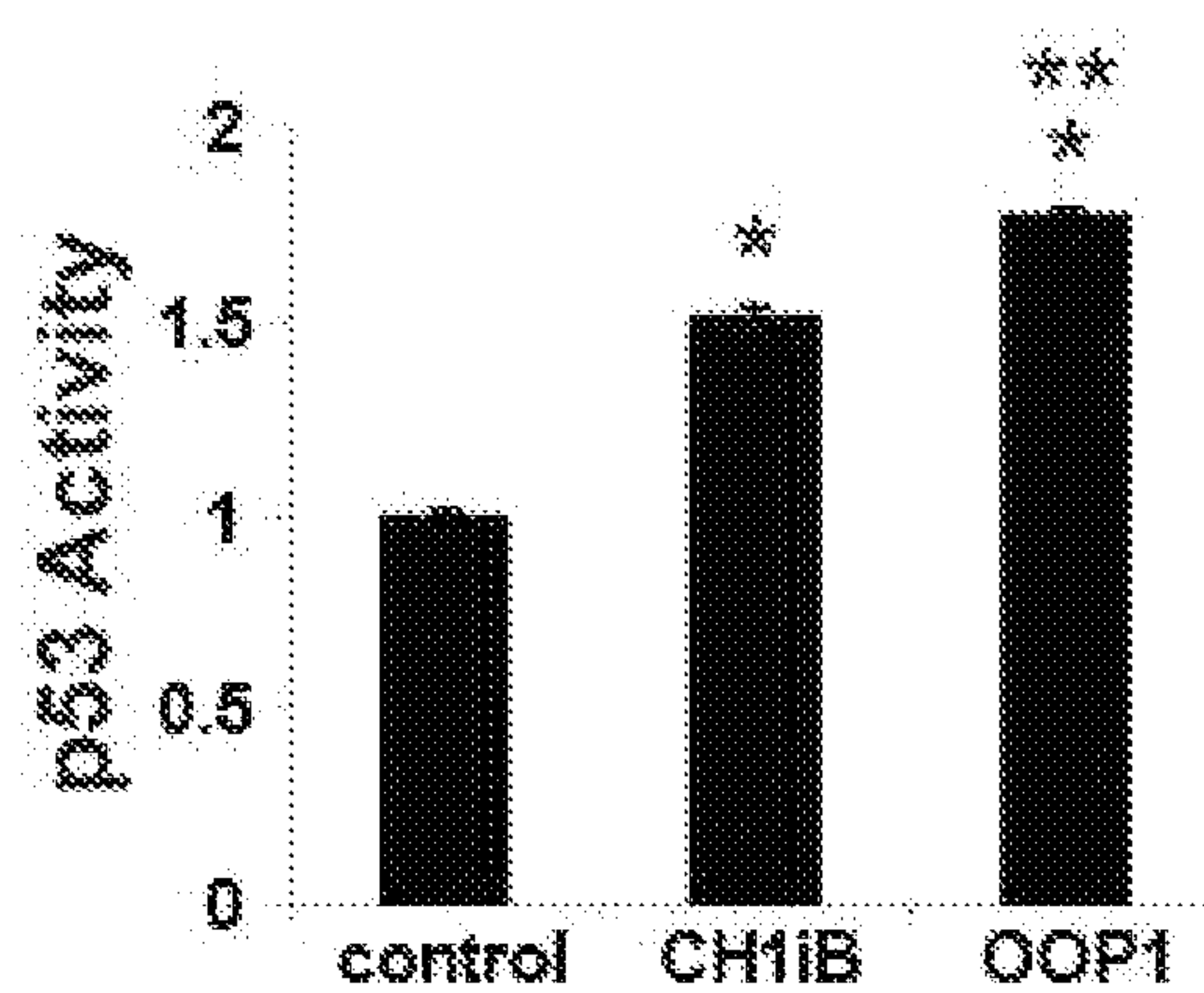


Figure 11

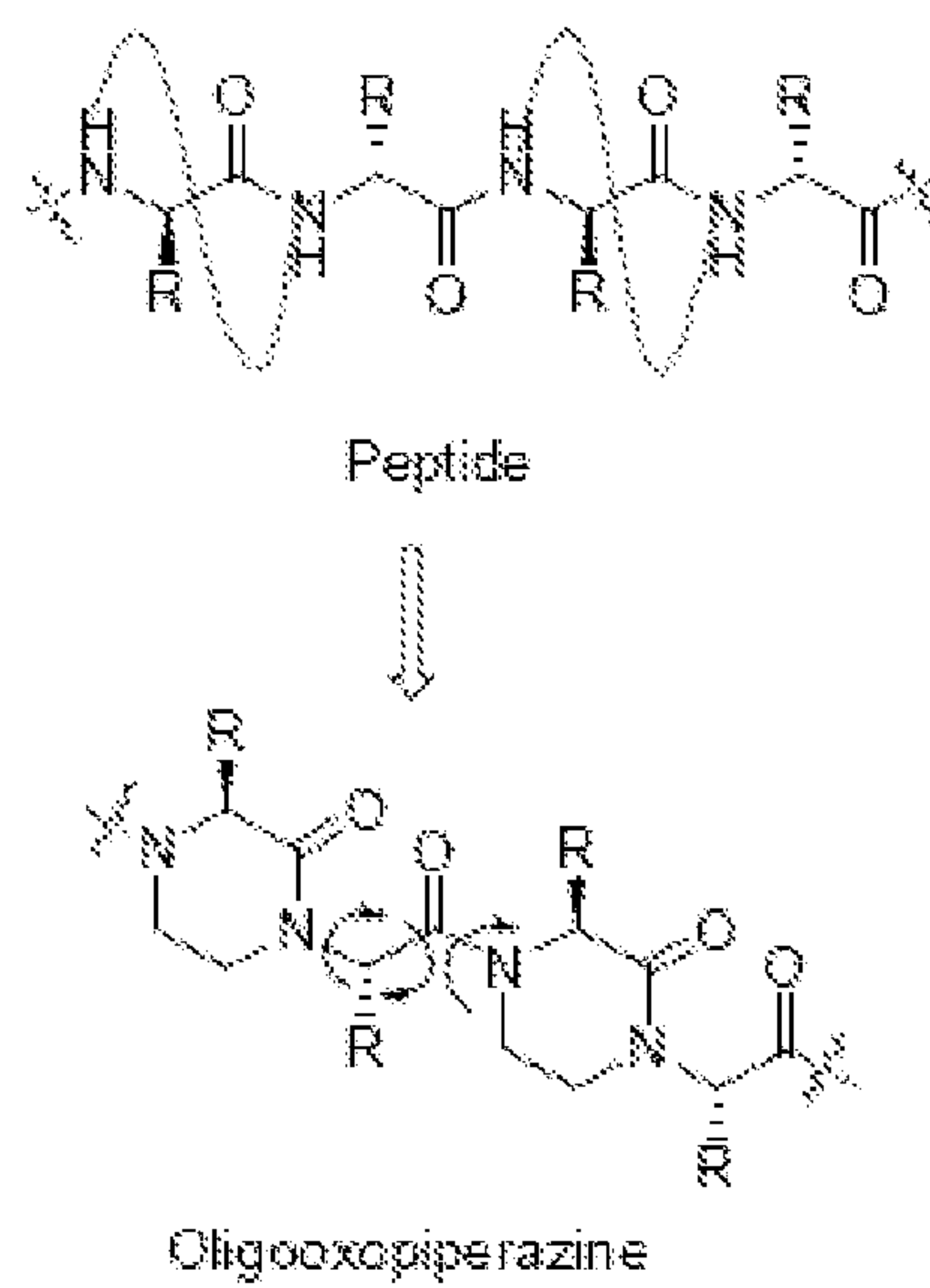


Figure 12

10/14

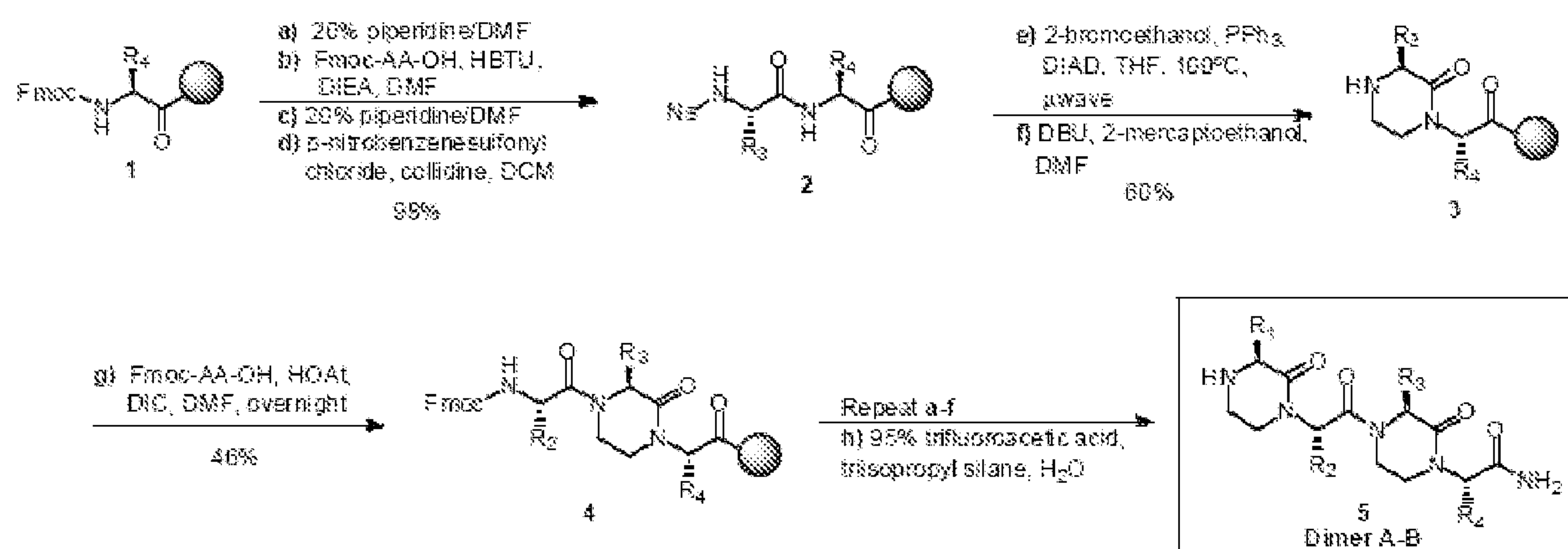


Figure 13

11/14

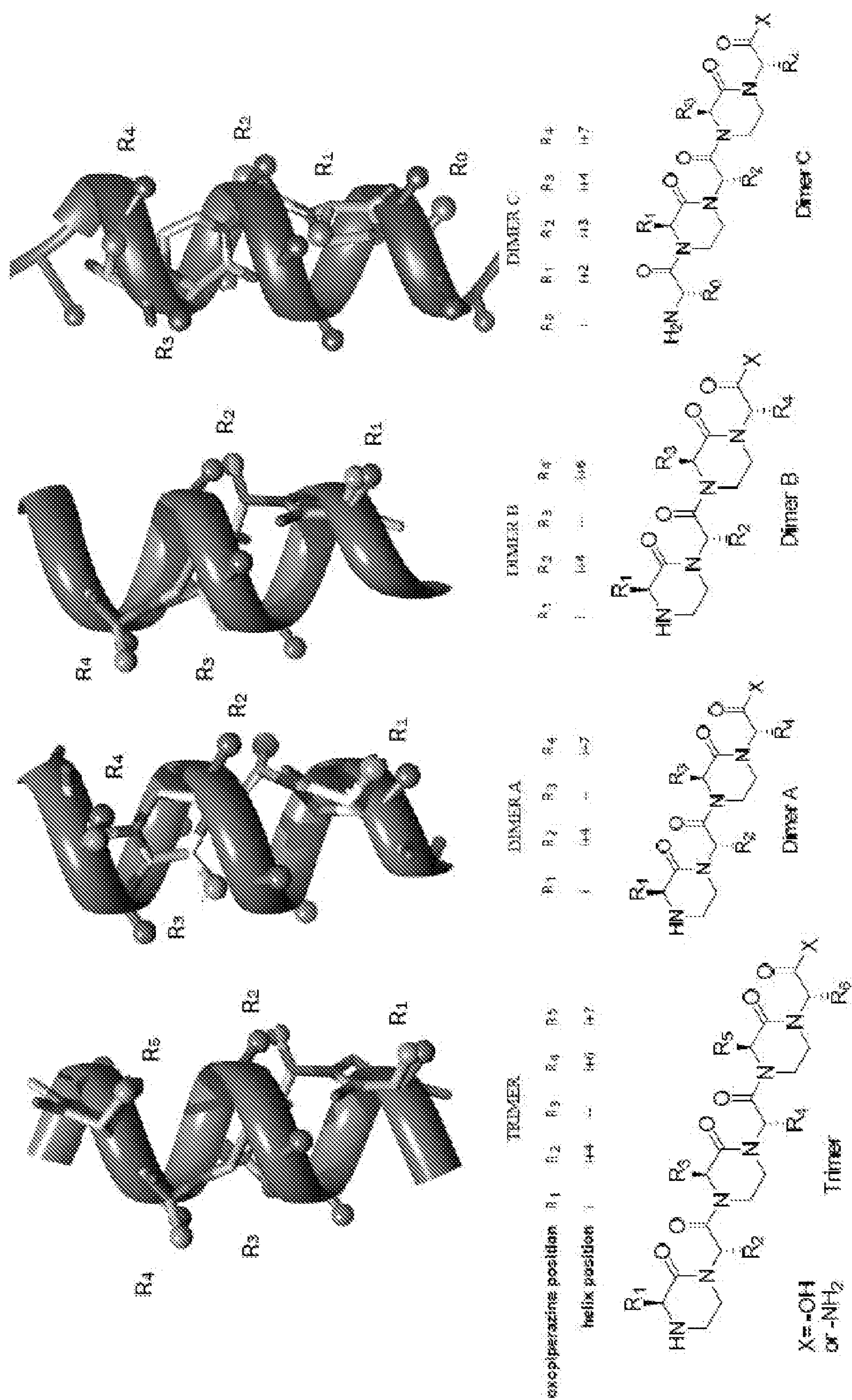


Figure 14

12/14

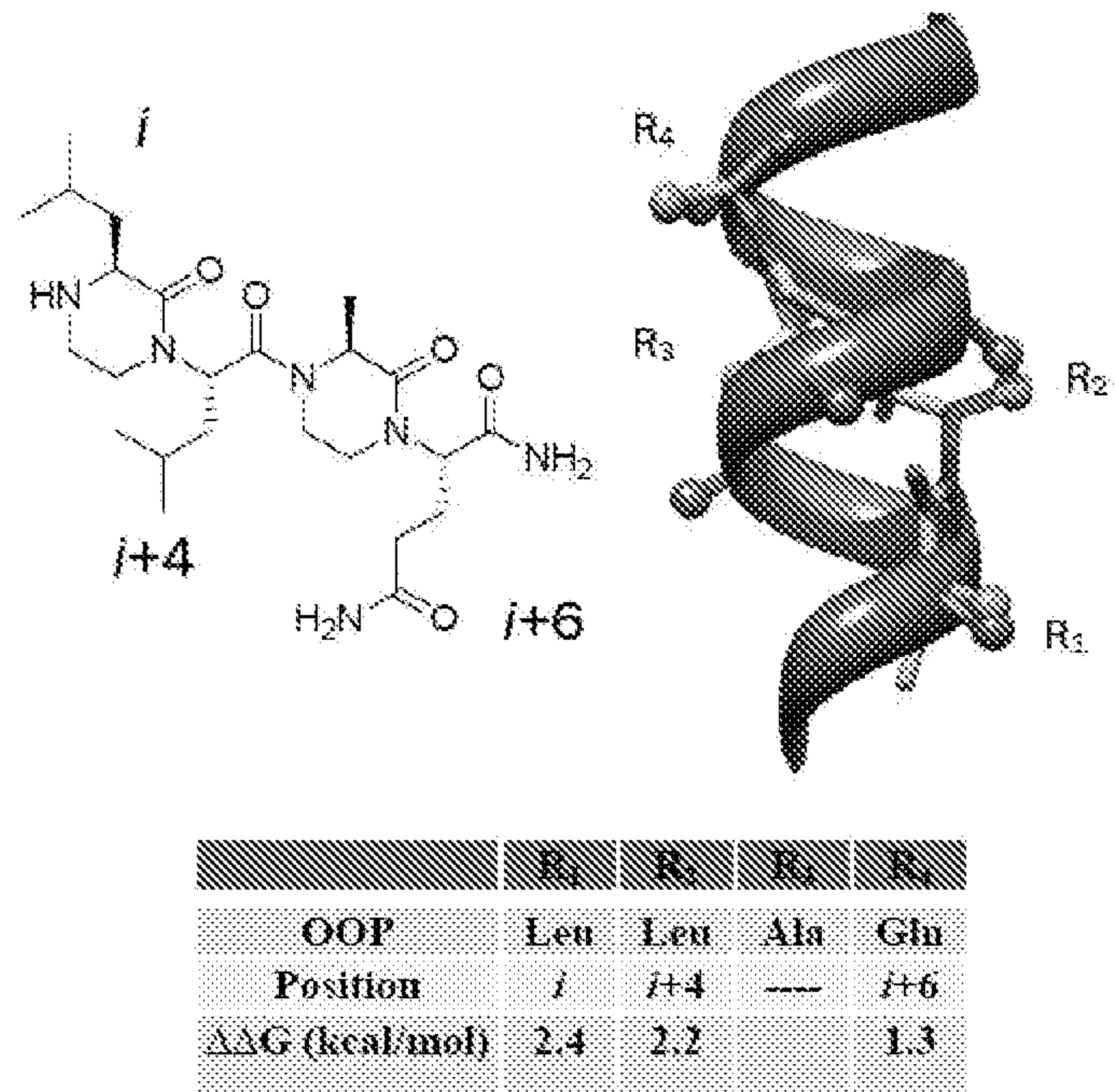


Figure 15

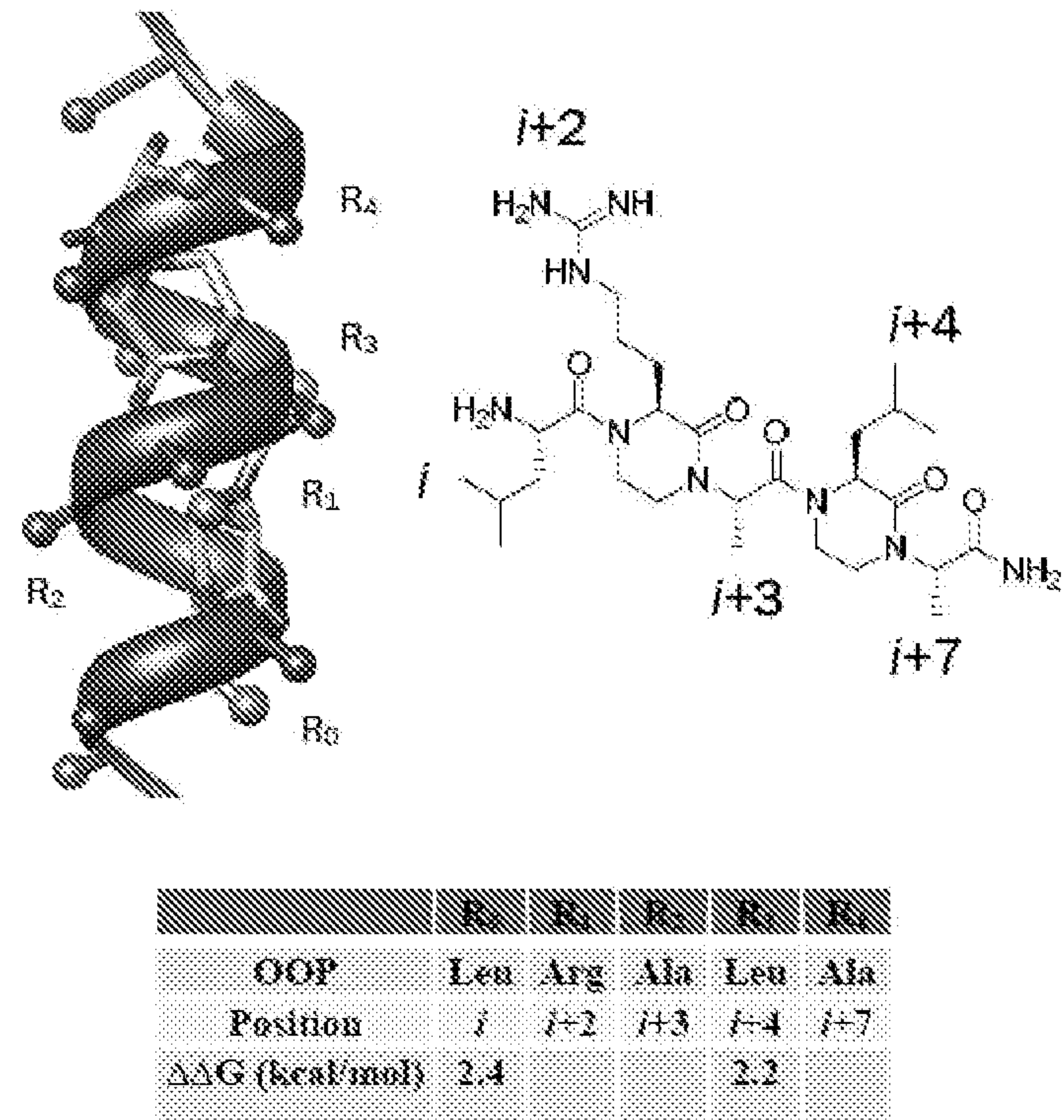


Figure 16

13/14

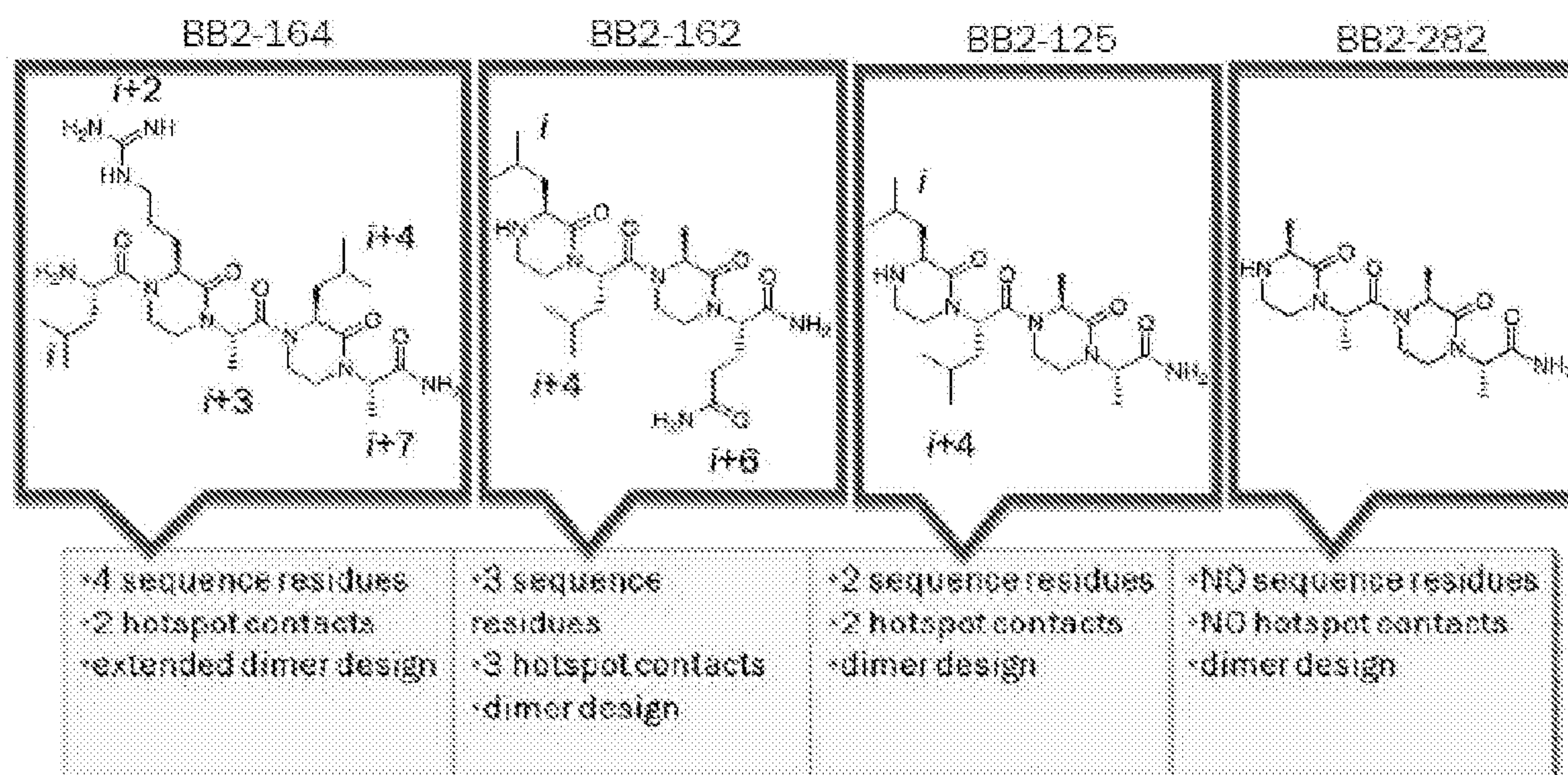


Figure 17

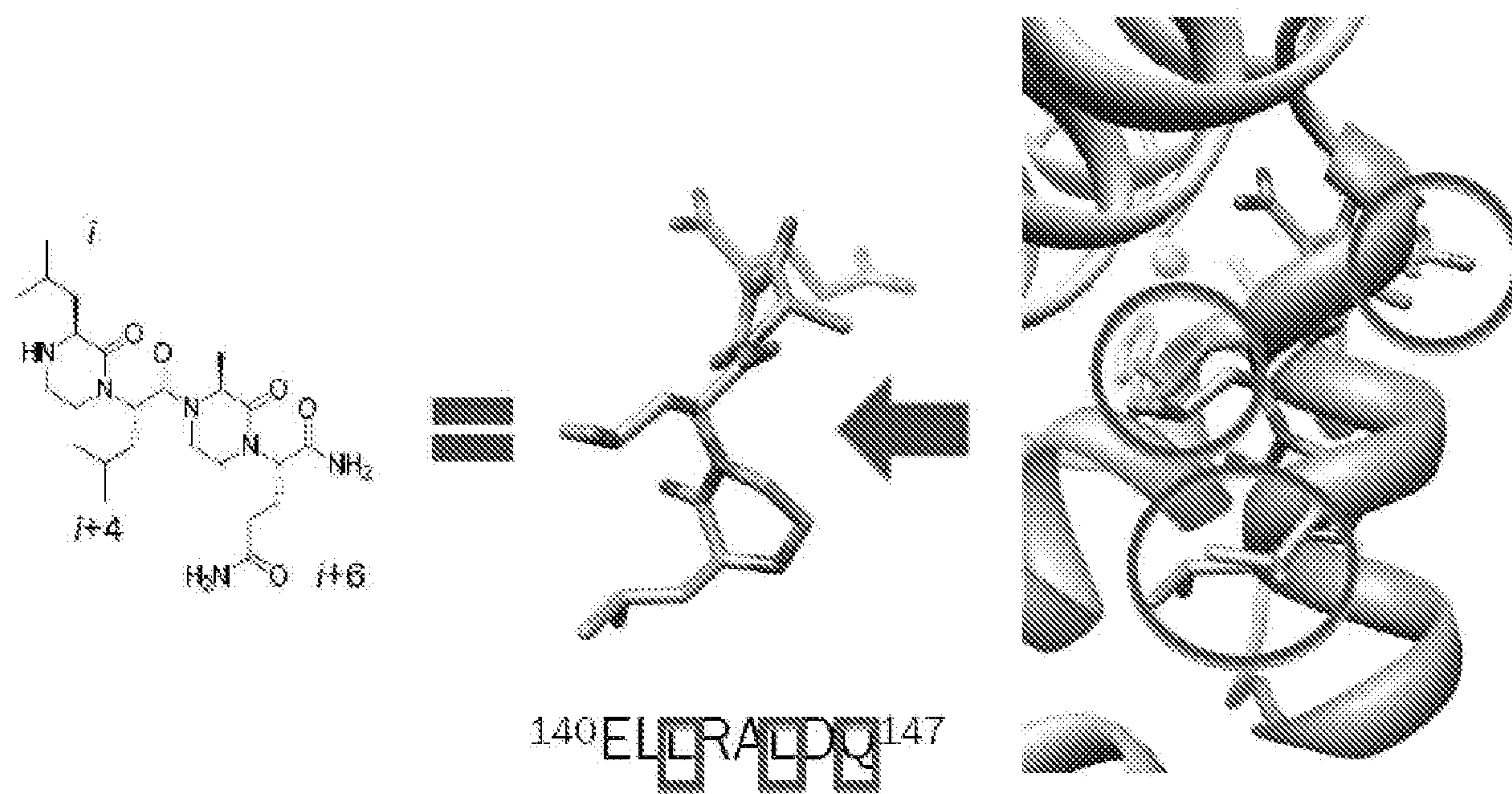


Figure 18

14/14

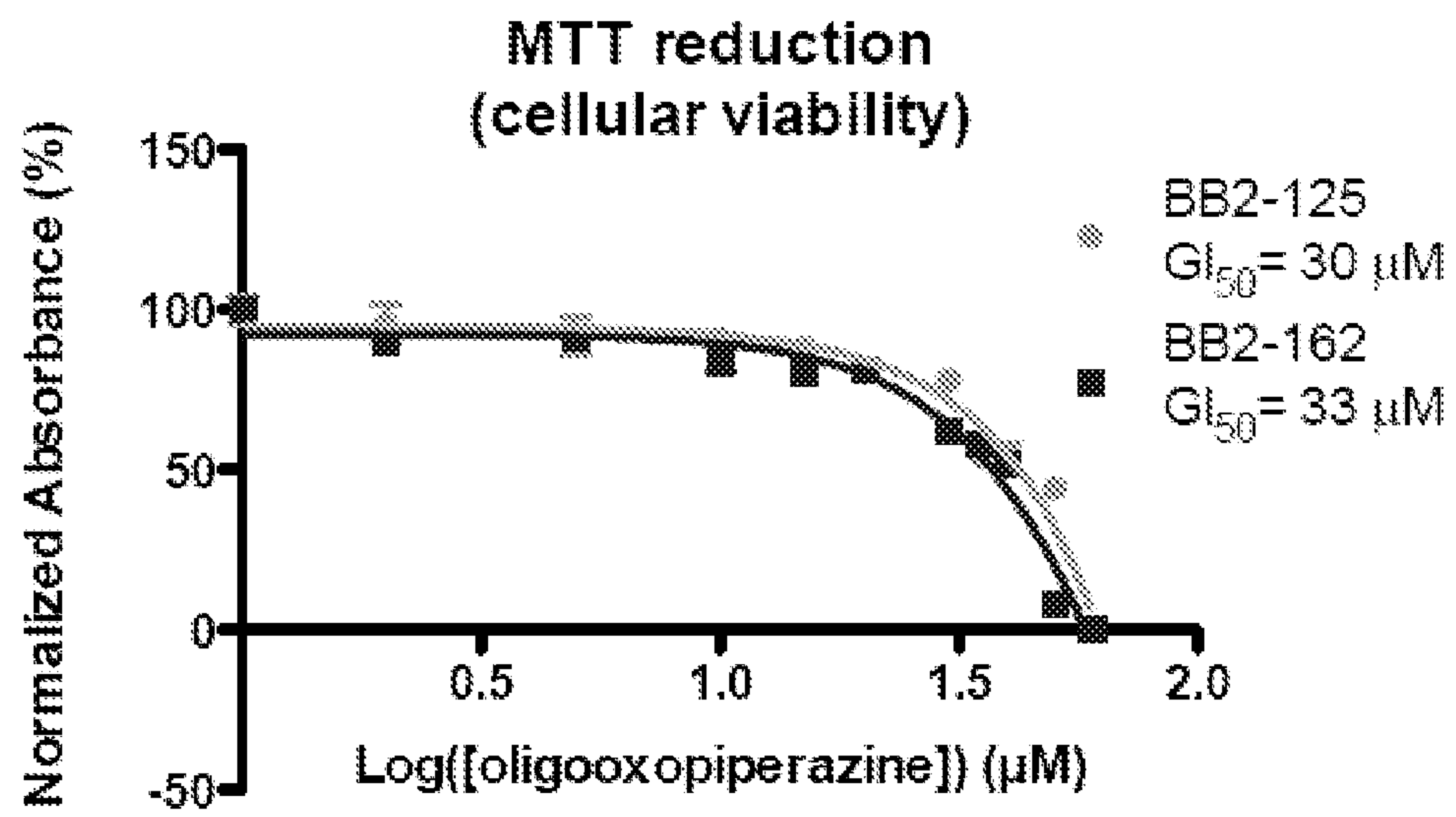


Figure 19