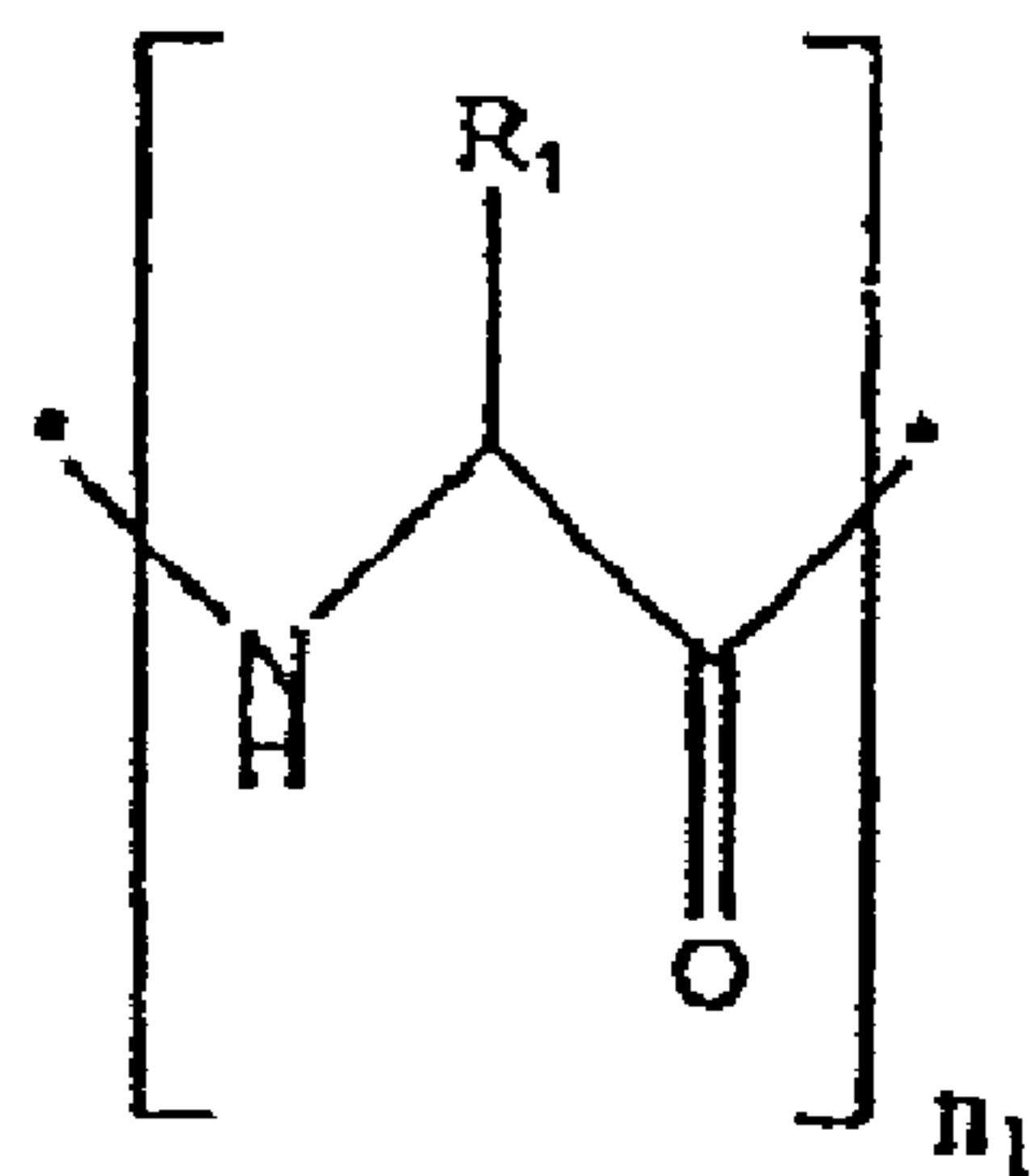




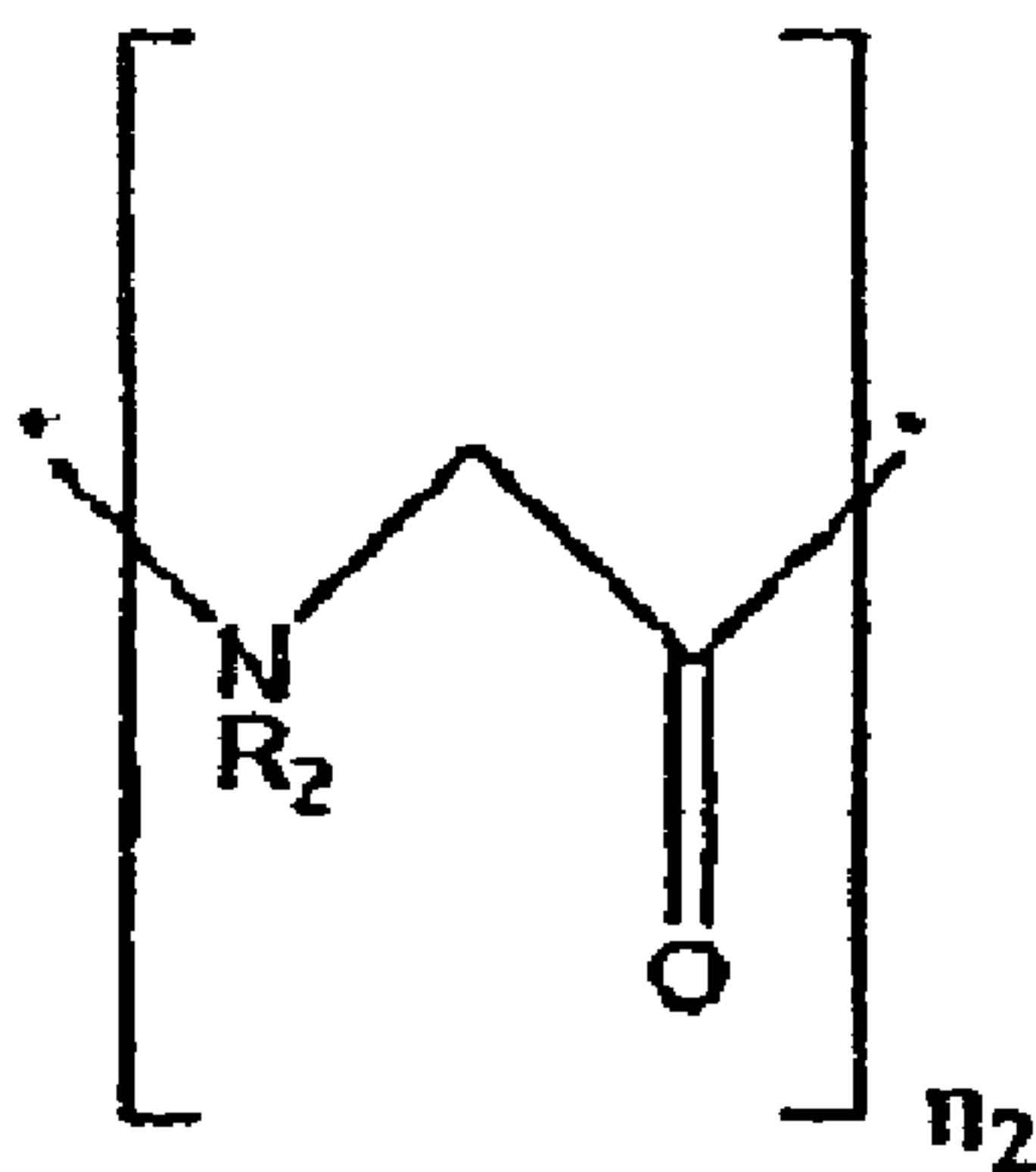
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(54) Titre : POLYMERES PEPTIDOMIMETIQUES POUR SURFACES ANTISALISSURE
(54) Title: PEPTIDOMIMETIC POLYMERS FOR ANTIFOULING SURFACES



I. (Peptide anchor)



II. (Peptoid moiety)

(57) Abrégé/Abstract:

Disclosed is a peptoid moiety that is generally resistant to, or inhibits, protein adsorption or cell fouling of surfaces onto which the composition is coated or attached. Generally speaking the compositions of this invention are referred to as peptidomimetic or

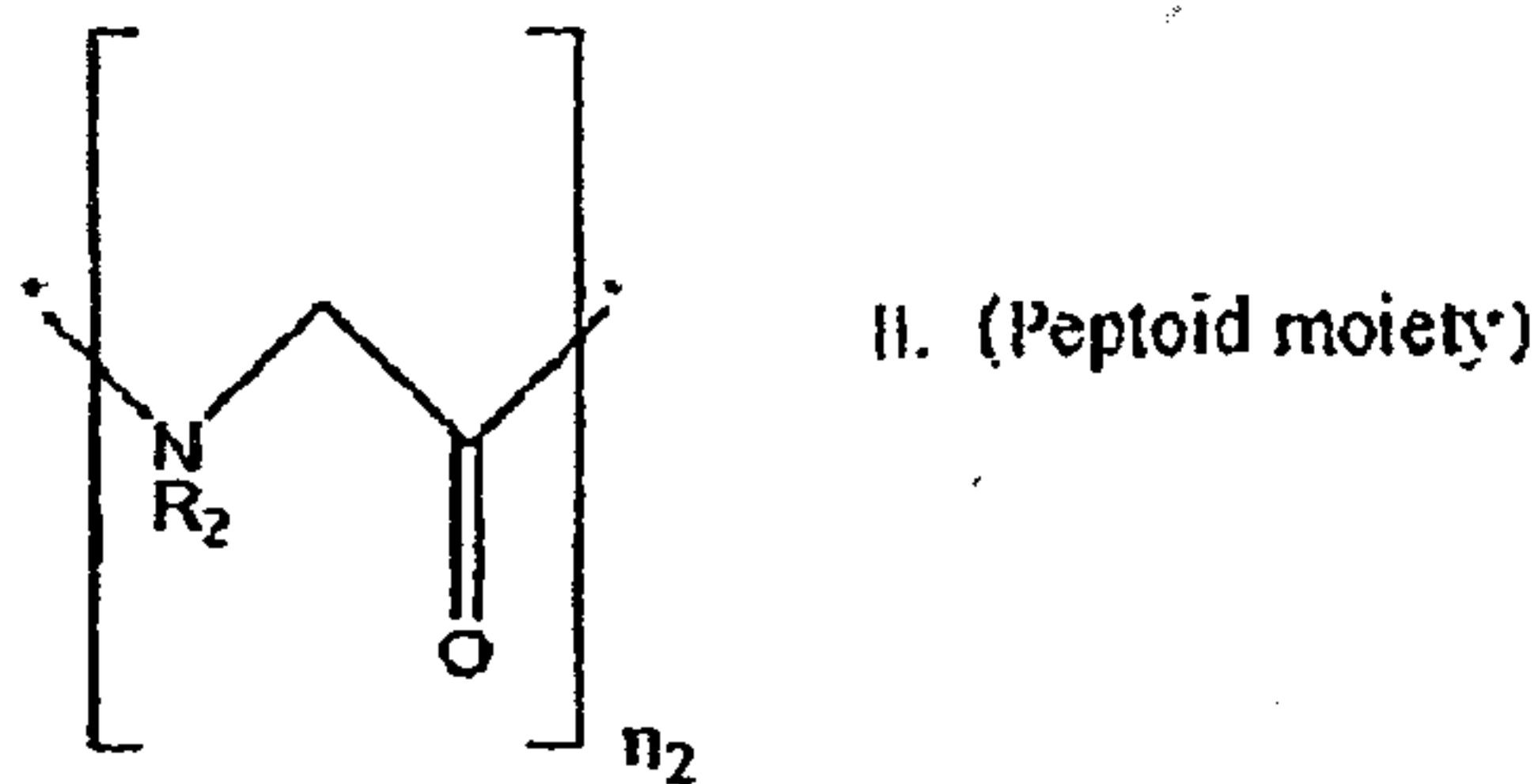
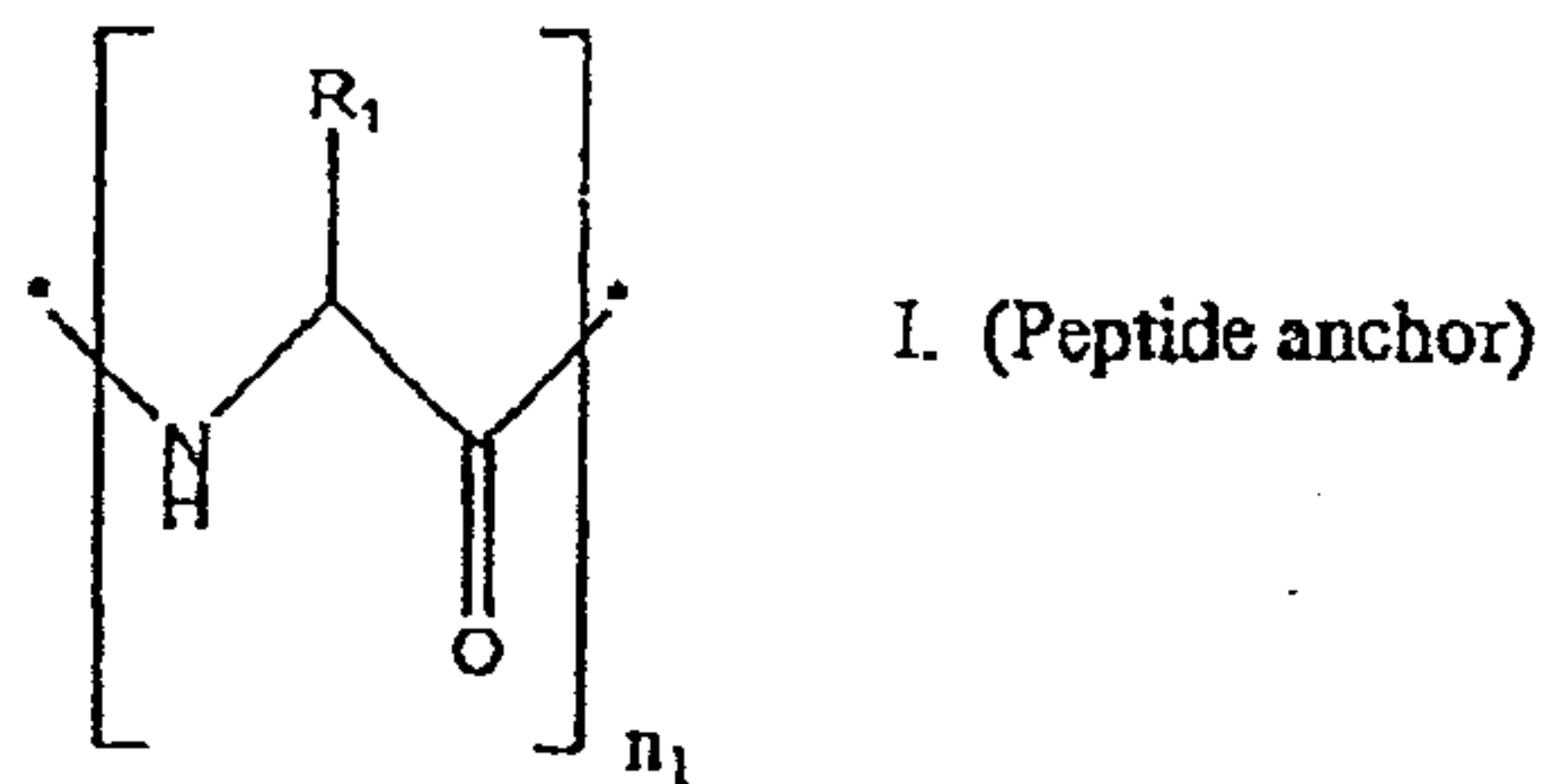


(57) **Abrégé(suite)/Abstract(continued):**

chimeric polymers and comprise coupled anchor and peptoid moieties of the following structure: (see formula I) (Peptide anchor) (see formula II) (Peptoid moiety) wherein R_1 is at least one dihydroxyphenyl alone or in combination with amine-terminated lower alkyl chains having from 1 to about 10 carbon atoms (preferably about 1 to about 7 carbon atoms). The lower alkyl structures of this invention may be branched or unbranched, saturated or unsaturated and contain minor heteroatom (generally O, N, or S,) constituents which do not materially alter the alkyl chain properties. The alkyl structure need not all have the same number of carbon atoms or the same structure. The DHPD's also may be the same or different; R_2 has at least one ether (C-O-C) linkage (multiple ether linkages are contemplated) and comprises ether-linked alkyl groups or chains, each having from 1 (i.e., $\text{CH}_3\text{-O-CH}_2\text{-}$) to about 10 carbon atoms, preferably from 1 to about 7 carbon atoms, and being uncharged, branched, unbranched, saturated or unsaturated; n_1 has a value in the range of about 1 to about 10, preferably 1 to about 8 and most preferably 2 to about 6; and n_2 has a value in the range of about 5 to about 100 or more, preferably about 8 to about 50, and more preferably about 10 to about 30.

ABSTRACT

Disclosed is a peptoid moiety that is generally resistant to, or inhibits, protein adsorption or cell fouling of surfaces onto which the composition is coated or attached. Generally speaking the compositions of this invention are referred to as peptidomimetic or chimeric polymers and comprise coupled anchor and peptoid moieties of the following structure:



wherein R_1 is at least one dihydroxyphenyl alone or in combination with amine-terminated lower alkyl chains having from 1 to about 10 carbon atoms (preferably about 1 to about 7 carbon atoms). The lower alkyl structures of this invention may be branched or unbranched, saturated or unsaturated and contain minor heteroatom (generally O, N, or S,) constituents which do not materially alter the alkyl chain properties. The alkyl structure need not all have the same number of carbon atoms or the same structure. The DHPD's also may be the same or different;

R_2 has at least one ether (C-O-C) linkage (multiple ether linkages are contemplated) and comprises ether-linked alkyl groups or chains, each having from 1 (i.e., $\text{CH}_3\text{-O-CH}_2\text{-}$) to about 10 carbon atoms, preferably from 1 to about 7 carbon atoms, and being uncharged, branched, unbranched, saturated or unsaturated;

n_1 has a value in the range of about 1 to about 10, preferably 1 to about 8 and most preferably 2 to about 6; and

n_2 has a value in the range of about 5 to about 100 or more, preferably about 8 to about 50, and more preferably about 10 to about 30.

PEPTIDOMIMETIC POLYMERS FOR ANTIFOULING SURFACES**BACKGROUND OF THE INVENTION**

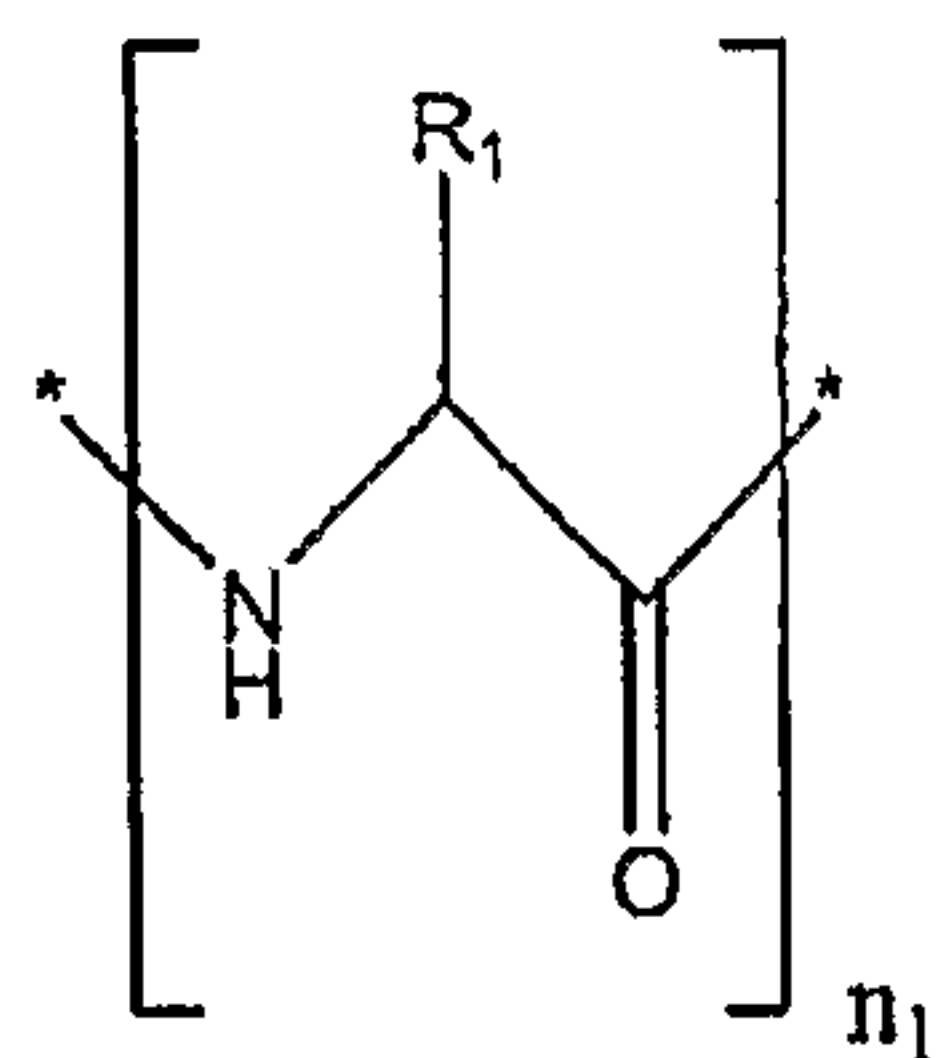
[0003] Protein, cell, and bacterial fouling of surfaces occur spontaneously upon exposure of medical implants and diagnostic devices to physiologic fluids and tissues. In many cases biofouling is an adverse event that can impair function or even cause catastrophic failure of medical devices. Examples of problematic biofouling include occlusion of cardiovascular implants by thrombus, protein accumulation onto biosensor surfaces, and bacterial colonization of indwelling catheters. Complications arising from fouling of medical implants and devices significantly increase the cost of healthcare delivery and can lead to reduction of implant performance, implant failure, and patient infections.

[0004] Strategies in the art for inhibiting biofouling are directed to grafting antifouling polymers or self-assembled monolayers (SAMs) onto surface. Technical issues critical to the longevity and antifouling performance of such organic coatings include the nature of the chemical bond used for anchoring such coatings onto surfaces, as well as the chemical characteristics of the polymer/SAM. Common anchoring chemistries include thiol- and

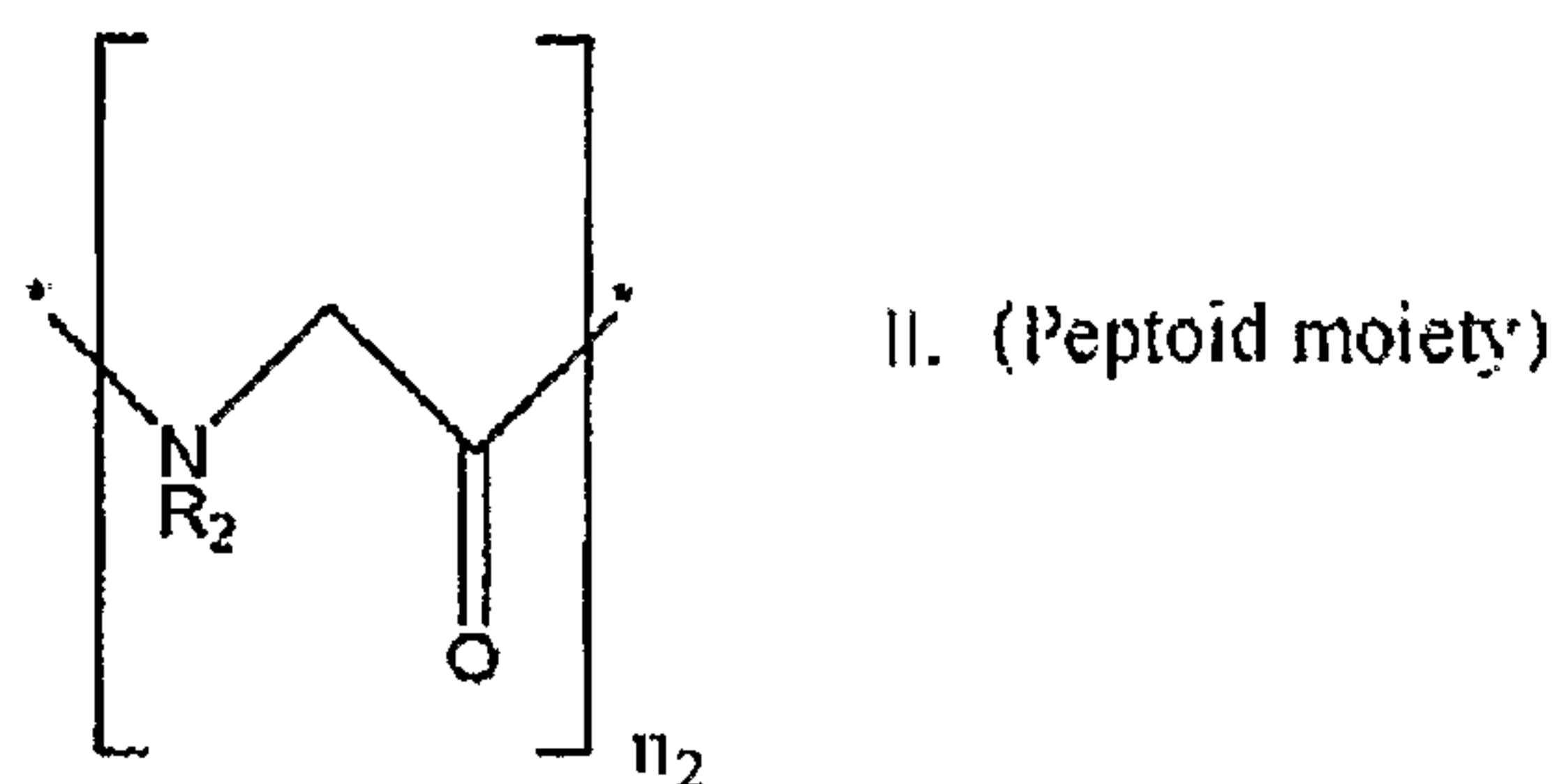
silane-containing molecules on metals and metal oxides, respectively, electrostatic interactions between polyelectrolytes and charged surfaces, and numerous strategies that take advantage of reactive organic functional groups on surfaces and molecules in solution or the vapor phase. While oligoethylene glycol terminated SAMs have shown excellent antifouling properties, their stability under *in-vivo* conditions may be limited in certain applications. A variety of polymers have been investigated as antifouling coatings, including poly(ethylene glycol) (PEG), poly(methoxyethyl acrylate) (PMEA), poly(phosphorylcholine methacrylate), and glycomimetic polymers. Each of these polymers has met with some success in *in-vitro* and *in-vivo* antifouling tests. However, none have yet proven to be ideal for long-term prevention of protein, cell, and bacterial fouling of surfaces.

BRIEF SUMMARY OF THE INVENTION

[0005] Briefly, in one aspect, the present invention comprises macromolecular, antifouling, chimeric compositions or coatings comprising a peptide or polypeptide anchor moiety or component coupled to a peptoid or polypeptoid moiety or component. A peptoid moiety of this invention is generally resistant to, or inhibits, protein adsorption or cell fouling of surfaces onto which the composition is coated or attached. Generally speaking the compositions of this invention are referred to as peptidomimetic or chimeric polymers and comprise coupled anchor and peptoid moieties of the following structure:



I. (Peptide anchor)



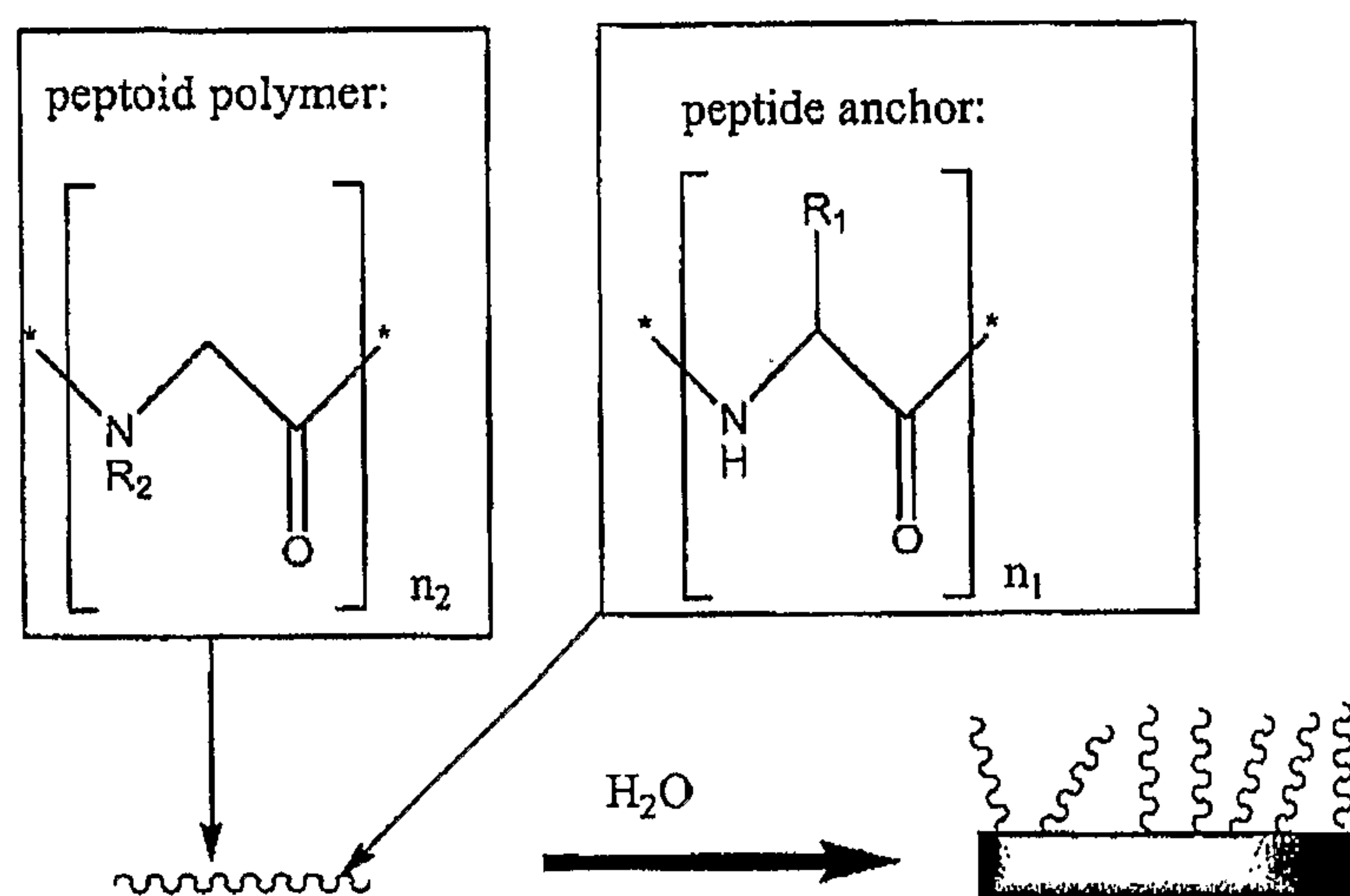
wherein R_1 is at least one dihydroxyphenyl derivative (DHPD) alone or in combination with amine-terminated lower alkyl chains having from 1 to about 10 carbon atoms (preferably about 1 to about 7 carbon atoms). The lower alkyl structures of this invention may be branched or unbranched, saturated or unsaturated and contain minor heteroatom (generally O, N, or S,) constituents which do not materially alter the alkyl chain properties. The alkyl structure need not all have the same number of carbon atoms or the same structure. The DHPD's also may be the same or different;

R_2 has at least one ether (C-O-C) linkage (multiple ether linkages are contemplated) and comprises ether-linked alkyl groups or chains, each having from 1 (i.e., $\text{CH}_3\text{-O-CH}_2\text{-}$) to about 10 carbon atoms, preferably from 1 to about 7 carbon atoms, and being uncharged, branched, unbranched, saturated or unsaturated;

n_1 has a value in the range of about 1 to about 10, preferably 1 to about 8 and most preferably 2 to about 6; and

n_2 has a value in the range of about 5 to about 100 or more, preferably about 8 to about 50, and more preferably about 10 to about 30.

A general preparative route for the coupled moieties of this invention, i.e., the peptidomimetic composition or coating, is shown in Scheme 1, as follows:



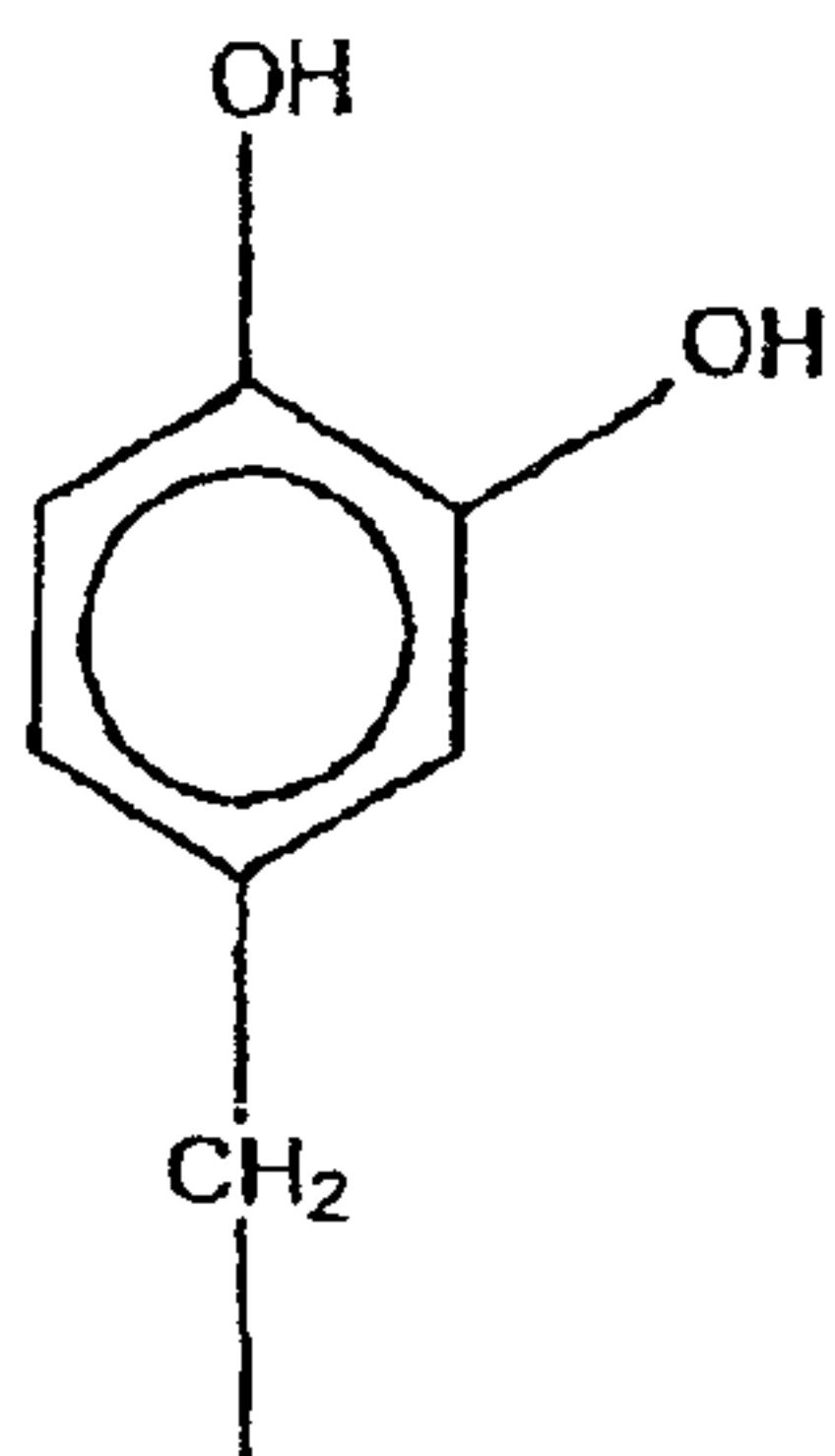
Scheme 1

Scheme 1 shows the general synthetic route for peptidomimetic compositions of the invention as well as a schematic showing of a peptidomimetic coated surface (greatly magnified) of this invention.

[0006] The general design of such chimeric polymers of this invention can be described in a preferred aspect as a functional peptide domain component for robust adsorption deposition, adhesive, absorptive, or other interaction with surfaces, coupled or conjugated to an N-substituted glycine peptoid polymer component that is resistant to protein and cell fouling. The synthetic approach is versatile and allows virtually unlimited variation of composition. Modification of a variety of surfaces can be accomplished with a simple aqueous solution-based adsorption or deposition strategy. In certain embodiments, a peptidomimetic polymer of this invention can be adsorbed to, or deposited on, a metal oxide surface (e.g., titanium oxide), which in turn exhibits significantly reduced serum protein adsorption, and inhibited cell fouling for weeks, months, to several months (or longer) under *in-vitro* conditions.

[0007] The preferred class of anchoring peptide domain or moiety was chosen to mimic the adhesive proteins used by marine mussels to attach to underwater surfaces. Mussels are known for their ability to adhere strongly to a variety of wet surfaces, and for this purpose secrete liquid "glues" containing mussel adhesive proteins (MAPs), which rapidly harden to form a solid adhesive plaque.

[0008] In one aspect, the adhesive or anchor moiety of a composition of this invention comprises dihydroxyphenyl derivatives (DHPD) including, di-(DHPD) wherein the second DHPD is



i.e., a methylene derivative of dihydroxyphenyl. A preferred DHPD is L, 3, 4 dihydroxyphenyl alanine (DOPA) which is more completely described below.

[0009] In a further preferred practice the adhesive moiety comprises DHPD including a pendent chain comprising ethylenic or vinylic unsaturation such as, for example, an alkyl acrylate.

BRIEF DESCRIPTION OF THE FIGURES

[0010] Figure 1 illustrates the molecular structure of a preferred peptidomimetic polymer composition of this invention.

[0011] Figure 2 shows the low mass region of the positive ion ToF-SIMS spectra of Ti modified with a peptidomimetic polymer coating.

[0012] Figure 3 shows mid-range mass region of the negative ion ToF-SIMS spectra of Ti modified with peptidomimetic polymer.

[0013] Figure 4 is a high resolution C(1s) XPS spectra of unmodified and polypeptoid modified TiO₂ substrates.

[0014] Figure 5 is a high-resolution N(1s) XPS spectra of polypeptoid modified TiO₂ substrate.

[0015] Figure 6 is a mass plot of serum protein adsorption on peptoid modified Ti waveguide as measured by OWLS.

[0016] Figure 7 is a total projected cell area of 3T3 fibroblasts on TiO₂ and peptoid modified TiO₂. Cells were reseeded twice weekly throughout the duration of the experiment.

DETAILED DESCRIPTION OF THE INVENTION

[0017] The adhesive properties of the preferred anchoring protein moieties of this invention are believed to be due to the presence of a preferred DHPD, namely L-3,4- dihydroxyphenylalanine (DOPA), an amino acid that is formed by post-translational modification of tyrosine. Of the several blue mussel (*Mytilus edulis*) adhesive pad proteins identified in the art, Mefp-3 and Mefp-5 are of special interest because these proteins have high DOPA content and are structurally located closest to the interface between the adhesive pad and substrate. See, Waite, J.H. and X. Qin, *Polyphosphoprotein from the Adhesive Pads of Mytilus edulis*. Biochemistry, 2001. 40: p. 2887-2893. Papov, V.V., et al., *Hydroxyarginine-containing Polyphenolic Proteins in the Adhesive Plaques of the Marine Mussel Mytilus edulis*. Journal of Biological Chemistry, 1995. 270(34): p. 20183-20192. At approximately 27%, Mefp-5 has the highest DOPA content of any isolated MAP. Furthermore, over 75% of the DOPA residues in Mefp-5 are immediately adjacent to lysine (Lys) residues.

[0018] Accordingly, various non-limiting embodiments can employ a 5-mer peptide mimic of Mefp-5 comprising alternating DOPA and Lys residues (Figure 1) as an anchor for polymer immobilization. Without restriction to any one theory or mode of operation, the catechol side chains of the DOPA residues are hypothesized to form charge transfer complexes to metal oxide surfaces, whereas the cationic nature of the Lys residues should provide electrostatic attraction to a negatively charged oxide surface. Other DOPA-containing peptide anchor components useful in conjunction with the

present inventive polymers will be understood by those skilled in the art made aware of this invention, such components including but not limited to those described in U.S. Patent Application Publication 2003-0087338 published May 8, 2003 particularly paragraphs [0089] through [0092] and Patent Application Publication 2004/026595 published December 30, 2004, each of which may be referred to for further details.

[0019] An antifouling portion of the polymer can comprise a poly-N-substituted glycine oligomer (peptoid) of variable length. Peptoids are non-natural mimics of peptides that have a protein-like backbone, with side chain derivatization at the amide nitrogen instead of the alpha-carbon Formula II. A wide range of N-substituents, corresponding N-substituted glycine residues and related peptoid components will be understood by those skilled in the art made aware of this invention, such residues and peptoid components as can be prepared as described below, in the referenced prior art, U.S. Pat. No. 6,887,845, which may be referred to for details.

[0020] In certain embodiments, the peptoid component can comprise an N-substituted methoxyethyl side chain, according to current understanding in the art of functional groups that provide fouling resistance to surfaces. In an extensive study of protein adsorption onto functionalized SAMs, certain functional group characteristics were identified that render surfaces resistant to protein adsorption. They include hydrophilicity, hydrogen-bond acceptors but not hydrogen-bond donors, and electrical charge neutrality. See, Ostuni, E., et al., *A Survey of Structure-Property Relationships of Surfaces that Resist the Adsorption of Protein*. Langmuir, 2001. 17: p. 5605-5620 which may be referred to for further details. Like PEG and PMEA components, the methoxyethyl side chain of such polypeptoid exhibits all four of these characteristics, including hydrophilicity, hydrogen bond acceptors, no hydrogen bond donors, and no charge. N-substituent identity is limited only by functional effect, meeting one or more of the aforementioned characteristics and/or otherwise demonstrating antifouling properties upon incorporation or one or more such moieties into a corresponding peptoid

polymer component. With respect to the chemical properties of the peptoid backbone, side chain substitution from the nitrogen (instead of the alpha carbon peptides) eliminates the amide hydrogen, removes the capacity for hydrogen bond donation, and significantly decreases incidence of protease degradation.

[0021] The chimeric peptide-peptoid molecule shown in Scheme 1 was synthesized on solid phase resin by first synthesizing the adhesive peptide anchor with standard Fmoc strategy followed by synthesis of a 20-mer N-methoxyethyl glycine peptoid using a known submonomer protocol. See, Zuckerman, R.N., et al., *Efficient Method For the Preparation of Peptoids [Oligo(n-Substituted Blycines)] By Submonomer Solid-Phase Synthesis*. Journal of the American Chemical Society, 1992. 114(26): p. 10646-10647. The amine terminus was acetylated, cleaved from the resin, purified by RP-HPLC and analyzed by mass spectrometry. Silicon wafers coated with 20nm of electron beam evaporated Ti were modified by adsorption of the peptidomimetic polymer from an aqueous solution. Unmodified and modified Ti surfaces were analyzed by time-of-flight secondary ion mass spectrometry (ToF-SIMS) and X-ray photoelectron spectroscopy (XPS). The positive ion ToF-SIMS spectrum of unmodified Ti (not shown) exhibited typical low intensity hydrocarbon contamination peaks ($C_nH_{2n-1}^+$) and ($C_nH_{2n-1}^+$), a Ti peak at $m/z = 47.95$ and a TiO^+ peak at $m/z = 63.95$. The positive ion ToF-SIMS spectrum of peptoid modified Ti revealed numerous fragments representing the presence of adsorbed peptoid polymer (Figure 2). Apparent fragmentation of the methoxyethyl side chain gave rise to peaks for CH_3^+ ($m/z = 15$) $C_2H_5O^+$ ($m/z = 45.06$) and $C_3H_7O^+$ ($m/z = 59.07$) fragments, as well as Lys-derived fragments such as $C_2NH_4^+$ ($m/z = 42.0$). The negative ion spectrum contained peaks for 1-, 2- and 3-mer peptoid fragments as well as other large fragments of the peptoid portion of the polymer (Figure 3).

[0022] XPS analysis of the surfaces modified with the peptidomimetic polymer reveals further evidence of peptidomimetic polymer adsorption onto the Ti surface. XPS spectra showed an increase in the ether (C-O) peak at 286.0 eV when compared to control Ti surfaces (Figure 4). A peak at 284.6 eV is due to the aliphatic and aromatic carbons in the methoxyethyl side chain and the DOPA anchoring group, as well as hydrocarbon contamination. The carbonyl groups of the peptidomimetic polymer backbone are represented by a

peak at 287.5 eV. Furthermore, a strong N(1s) peak was present at 399.7 eV in the spectra of the polypeptoid modified surfaces that was not observed in the unmodified Ti surfaces (Figure 5). While certain embodiments of this invention are demonstrated in conjunction with a titanium oxide substrate, it will be understood by those skilled in the art that various other materials, including but not limited to other metal oxides, can be employed with composites and/or compositions comprising any one or a plurality of the peptidomimetic polymers of this invention. Other such materials include those recognized in the art for implementation of the medical and non-medical applications mentioned herein.

[0023] Optical waveguide lightmode spectroscopy (OWLS) experiments revealed that polypeptoid modification of titanium surfaces resulted in a substantial reduction in protein adsorption (Figure 6). Exposure of unmodified Ti waveguides to whole human serum for 20 minutes resulted in an adsorbed protein layer with a mass between 150 and 230 ng/cm² after rinsing. However, surprisingly and unexpectedly, serum protein adsorption onto representative peptoid modified substrates of this invention under identical conditions was reduced to approximately 4ng/cm². This amount of protein adsorption is similar to that adsorbed onto DOPA-anchored PEG coatings and to oligoethylene glycol terminated SAMs, demonstrating the excellent protein resistance of peptidomimetic polymer compositions of the invention.

[0024] Finally, the ability of polypeptoid modified surfaces to resist cell attachment over a long period of time was determined by culturing 3T3 fibroblast cells on unmodified and modified titanium surfaces in the presence of serum. Fresh cells were seeded twice weekly onto the titanium surfaces for several months, the cell attachment at various time points assayed by fluorescence microscopy and image analysis. Although fibroblasts readily attached to unmodified titanium surfaces and were nearly confluent after several days (Figure 7), the peptoid modified surfaces exhibited low levels of cell attachment throughout the experiment. Since cell attachment to surfaces is typically mediated by adsorbed protein, the results infer that serum protein adsorption remained low throughout the course of the *in vitro* experiment.

[0025] It is interesting to note that fouling resistance persisted for many days in the presence of serum, which typically contains protease enzymes that would be expected gradually to degrade peptide bonds of the polymer backbone. In this respect as well, the design of the peptoid is beneficial in that the placement of the side chain on the amide nitrogen leads to an essentially protease-resistant backbone. Although the adhesive peptide anchor of the molecule may be susceptible to protease degradation, at high polymer density on the surface the peptide anchors are likely to be buried beneath or protected by the peptoid chains and therefore essentially inaccessible to serum proteases.

[0026] As illustrated, above, a new de novo designed peptidomimetic polymers of this invention were synthesized and determined to have excellent and long-lasting antifouling properties when immobilized onto a metal oxide surface. Such chimeric compounds can comprise a mussel adhesive protein mimetic peptide for robust water-resistant anchorage onto substrates, coupled to an oligometric N-substituted glycine peptoid with a side chain (e.g., methoxyethyl) designed for resistance to protein and cell fouling. The modular, solid phase approach known in the art used to synthesize these peptidomimetic polymers offers precise control of molecular weight at high yields, and with virtually unlimited versatility in functionality obtained through variation of N-substituted side chain composition in the form of both natural and non-natural side chains. See, Zuckerman, R.N., et al., *Efficient Method For the Preparation of Peptoids [Oligo(N-Substituted Glycines)] By Submonomer Solid-Phase Synthesis*. Journal of the American Chemical Society, 1992. 114(26): p. 10646-10647. Kirshenbaum, K., et al., Sequence-specific polypeptoids: *A diverse family of heteropolymers with stable secondary structure*. PNAS, 1998. 95(8): p. 4303-4308. The synthetic diversity available through such peptidomimetic polymers can be used to better understand the fundamental relationship between chemical composition of polymers and protein/cell resistance. Enhanced understanding of these relationships may, in turn, lead to improved antifouling strategies for medical and nonmedical applications.

EXAMPLES

Materials

[0027] Bromoacetic acid (BAA) and methoxyethylamine were purchased from Aldrich (Milwaukee, WI).

Polymer Synthesis

[0028] A peptidomimetic polymer composition of the invention was synthesized on 0.25 mmol FmocTM-Rink amide resin (Nova Biochem, San Diego, CA) using an ABI 433A (Applied Biosystems, Foster City, CA) automated peptide synthesizer. Conventional Fmoc strategy of solid phase peptide synthesis with Fmoc-Lys-(N-Boc) and Fmoc-DOPA(acetonid) amino acids (Nova Biochem, San Diego, CA) was used to synthesize the *C-terminal* DOPA-Lys-DOPA-Lys-DOPA peptide anchor, after which the polypeptoid portion was synthesized using submonomer protocol described previously. See Zuckerman, *supra*. Bromoacetylation of the N-terminal amino was accomplished by vortexing 4.15 mL of 1.2M bromoacetic acid in DMF and 1 mL of diisopropylcarbodiimide (DIC) (Aldrich, Milwaukee, WI) with the resin for 60 min. After rinsing 4 times with 7mL of DMF, the resin was vortexed for 60 min. with 4 mL of 1M methoxyethylamine (Aldrich, Milwaukee, WI) in N-methylpyrrolidone (NMP) (Applied Biosystems, Foster City, CA) to introduce the side chain moiety. The liquid was then drained and the resin washed with 7mL of DMF. These two reaction cycles were repeated until the desired number of peptoid monomers was obtained.

[0029] Following completion of the synthesis, the N-terminus of the peptidomimetic polymer was acetylated with acetic anhydride (Applied Biosystems, Foster City, CA). Cleavage of the peptidomimetic polymer from the resin deprotection of the amino acid side chains was accomplished by treating the resin with 95% (v/v) trifluoroacetic acid (Acres Organics, Belgium) with 2.5% H₂O and 2.5% triisopropylsilane (Aldrich, Milwaukee, WI). The cleaved peptidomimetic polymer was isolated by filtration and rinsed several times with acetonitrile and water. The crude product was analyzed by reversed-phase HPLC using a Vydac C18 column and ESI-MS for purity and composition. Purification was performed by preparative HPLC, and purified fractions were frozen at -85°C and lyophilized.

Substrate Preparation

[0030] Silicon wafers were coated with 20 nm of electron beam evaporated Ti and then cut into 8mm by 8mm pieces. The substrates were cleaned ultrasonically for ten minutes in 2-propanol, dried under N₂ and then exposed to O₂ plasma (Harrick Scientific Ossining, USA) at $\leq$ 50 Torr and 100W for three minutes to produce a clean titanium oxide surface. OWLS waveguides were purchased from Microvacuum Ltd. (Budapest, Hungary), consisting of a SiO₂ substrate coated with Si_{6.25}Ti_{6.75}O₂ and a final 10 nm thick coating of TiO₂ produced by a sol-gel process. Voros, J., et al., *Optical grating coupler biosensors*. Biomaterials, 2002. 23: p. 3699-3710. Sensors were cleaned following the same procedure as Ti substrates.

Substrate Modification

[0031] Clean substrates and sensors were immersed in 1mg/ml peptidomimetic polymer in saturated NaCl buffered with 0.1M N-morpholinopropanesulfonic acid (MOPS) at 60°C for 24 hours to form a uniform monolayer. After modification, substrates were exhaustively rinsed with ultrapure H₂O and dried in a stream of filtered N₂.

Surface Characterization

[0032] TM Survey and high resolution XPS spectra were collected on an Omicron FSCALAB (Omicron, Taunusstein, Germany) configured with a monochromated Al K α (1486.8 eV) 300-W X-ray source, 1.5 nm circular spot size, a flood gun counter charging effects, and an ultrahigh vacuum ($<10^{-8}$ Torr). The takeoff angle, defined as the angle between the substrate normal and the detector, was fixed at 45°. Substrates were mounted on standard sample studs by means for double-sided adhesive tape. All binding energies were calibrated using the C(1s) carbon peak (284.6 eV). Analysis included a broad survey scan (50.0 eV pass energy) and a 10-min. high-resolution scan (22.0 eV pass energy) at 270-300 eV for C(1s) and, comparably, for N(1s).

[0033] Secondary ion spectra were recorded on a TRIFT III time-of-flight secondary ion mass spectrometer (Physical Electronics, Eden Prairie,

MN) in the mass range 0-2000 m/z . A Ga -source was used at a beam energy of 15 keV with a 100 μm raster size. Positive and negative spectra were collected and calibrated with a set of low mass ions using the PHI software Cadence.

Cell Culture

[0034] 3T3-Swiss albino fibroblasts (ATCC, Manassas, VA) were maintained at 37°C and 5% CO₂ in Dulbecco's modified Eagle's medium (DMEM, Cellgro, Herndon, VA) containing 10% fetal bovine serum (FBS) and 100 $\mu\text{g}/\text{ml}$ of penicillin and 100 U/ml of streptomycin. Immediately before use, fibroblasts of passage 12-16 were harvested using 0.25% trypsin-EDTA, resuspended in DMEM with 10% FBS and counted using a hemacytometer.

Quantification of Cell Adhesion

[0035] Modified and unmodified TiO₂ substrates were pretreated in a 12-well TCPS plate with 1.0 ml of DMEM containing FBS for 30 minutes at 37°C and 5% CO₂. Fibroblasts were seeded onto the test substrates at a density of 2.9×10^3 cell/cm². For short-term studies, the substrates were maintained in DMEM with 10% FBS at 37°C and 5% CO₂ for 4 hours, after which adherent cells were fixed in 3.7% paraformaldehyde for 5 minutes and stained with 5 M 1,1'-dioctadecyl-3,3,3',3' tetramethylindocarbocyanine perchlorate (DiI; Molecular Probes, Eugene, OR) for inflorescent microscope counting. For long-term adhesion experiments substrates were reseeded with 3T3 fibroblasts at a density of 2.9×10^3 cells/cm² twice per week. The medium was aspirated from each well to remove any non-adherent cells and PBS was used to rinse the substrates and wells. Fibroblasts were stained with 2.5 μM calcein-AM (Molecular Probes) in complete PBS for 1 hour at 37°C twice per week initially and then once per week after 2 weeks.

[0036] Quantitative cell attachment data was obtained by acquiring nine images from random locations on each substrate using an Olympus BX-40 ($\lambda_{\text{Ex}} = 549\text{nm}$, $\lambda_{\text{Em}} = 565\text{nm}$) and a Coolsnap CCD camera (Roper Scientific, Trenton, NJ). The experiments were performed in triplicate for

statistical purposes, resulting in a total of 27 images per time point for each substrate. The resulting images were quantified using thresholding in Metamorph (Universal Imaging, Downingtown, PA).

Protein Adsorption

For *in situ* protein adsorption experiments, TiO₂ coated waveguides were modified *ex situ* with polypeptoid. The waveguides were inserted in the OWLS flow-through cell and equilibrated by exposing to HEPES-2 buffer (10 mM HEPES, 150 mM NaCl, pH 7.4) for at least 6 hours to allow for complete exchange for ions at the TiO₂ surface. The measurement head was mounted in the sample chamber and heated to 37°C; the signal was recorded to ensure a stable baseline and thus adequate equilibrium time. Whole human serum (Control Serum N, Roche Diagnostics, Switzerland, reconstituted in ultrapure water) was injected into the flow-through cell. The waveguide was exposed to serum for 40 minutes and subsequently rinsed with HEPES-2 buffer for another.

[0037] The refractive index of solutions was measured in a refractometer (J157 Automatic Refractometer, Rudolph Research) under identical experimental conditions. A refractive index value of 1.33119 was used for the HEPES-2 buffer and a standard value of 0.182 cm³/g was used for the protein-adsorption calculations. The residual increase in signal intensity versus baseline measured by OWLS can be directly correlated to adsorbed mass of protein.

[0038] One skilled in this art will be prompted to think of many applications for its present invention in light of this disclosure. The compounds, compositions coatings, and/or composites of this invention can be applied, without limitation, to:

- 1) Medical Diagnostics and Therapies, including but not limited to
 - (a) Preparation of Nonfouling Surfaces for
 - Biosensors
 - Cardiovascular implants

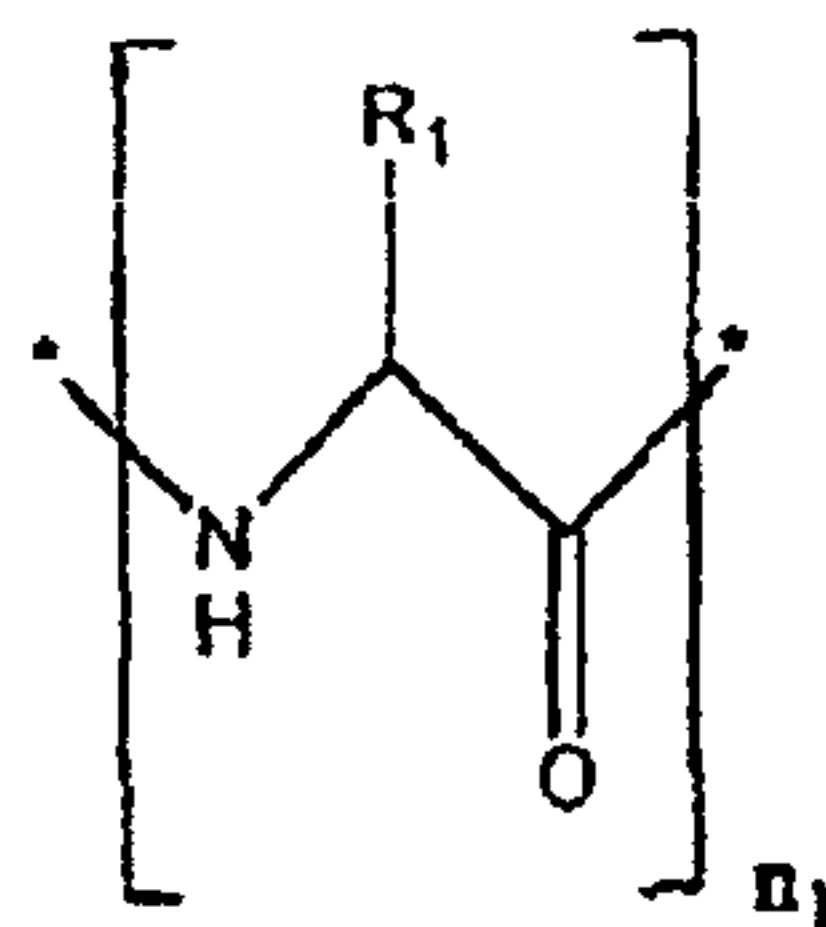
- Catheters
 - Lubricious coatings on catheters, needles, and other percutaneous devices
 - Medical tubing (dialysis)
 - Implantable electronic devices (MEMS)
 - Corrosion resistant coatings on medical grade metal alloys (surface adsorbed catechols are unknown to enhance corrosion resistance of metals); and
- (b) Stabilization of Particles of Diagnostics and Therapy, such as
- Stabilization of proteins, peptides and other therapeutics under in-vivo conditions
 - Nanoparticle-based ex-vivo diagnostics (gold or quantum dot based technologies)
 - Nanoparticle-based in-vivo diagnostics
 - Paramagnetic nanoparticle contrast agents for MRI
 - Nanoparticles for optical imaging
 - Nanoparticle-Based Therapies
 - Superparamagnetic magnetite nanoparticles for hyperthermia, and
- 2) Nonmedical Applications, including but not limited to
- Corrosion resistant coatings (surface adsorbed catechols and polyphenols are known to enhance corrosion resistance of metals, and polyphenol polymers are currently used as corrosion resistant coatings)
 - Antifouling coatings on consumer goods (sunglasses, etc.)
 - Antifouling coatings on electronic devices (MEMS, etc.)
 - Antifouling/anti-icing coatings on aircraft
 - Stabilization of quantum dot suspensions
 - Stabilization of magnetorheological fluids (ferrofluids)
 - Stabilization of inorganic particles (TiO₂, etc.) in paints

Many other such applications will occur to one skilled in the art, in view of the present disclosure.

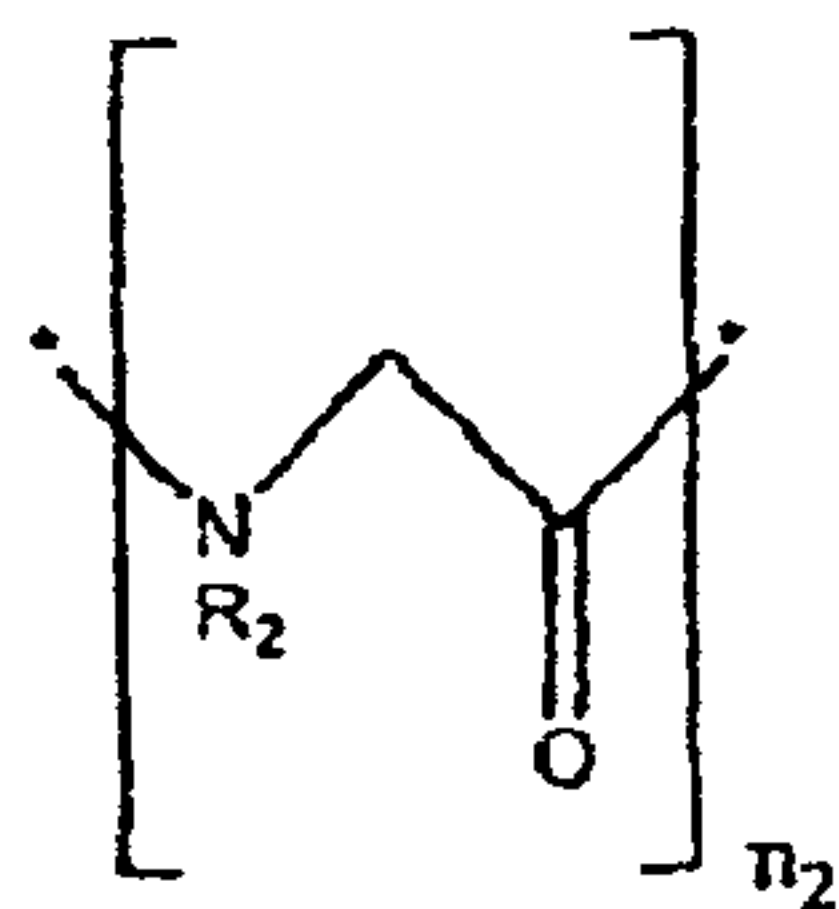
CLAIMS

What is claimed is as follows:

1. A peptidomimetic polymer comprising coupled polypeptide moiety and a polypeptoid moiety, the polypeptide moiety having the structure:



the polypeptoid moiety having the structure:



wherein

R_1 comprises at least one dihydroxyphenyl

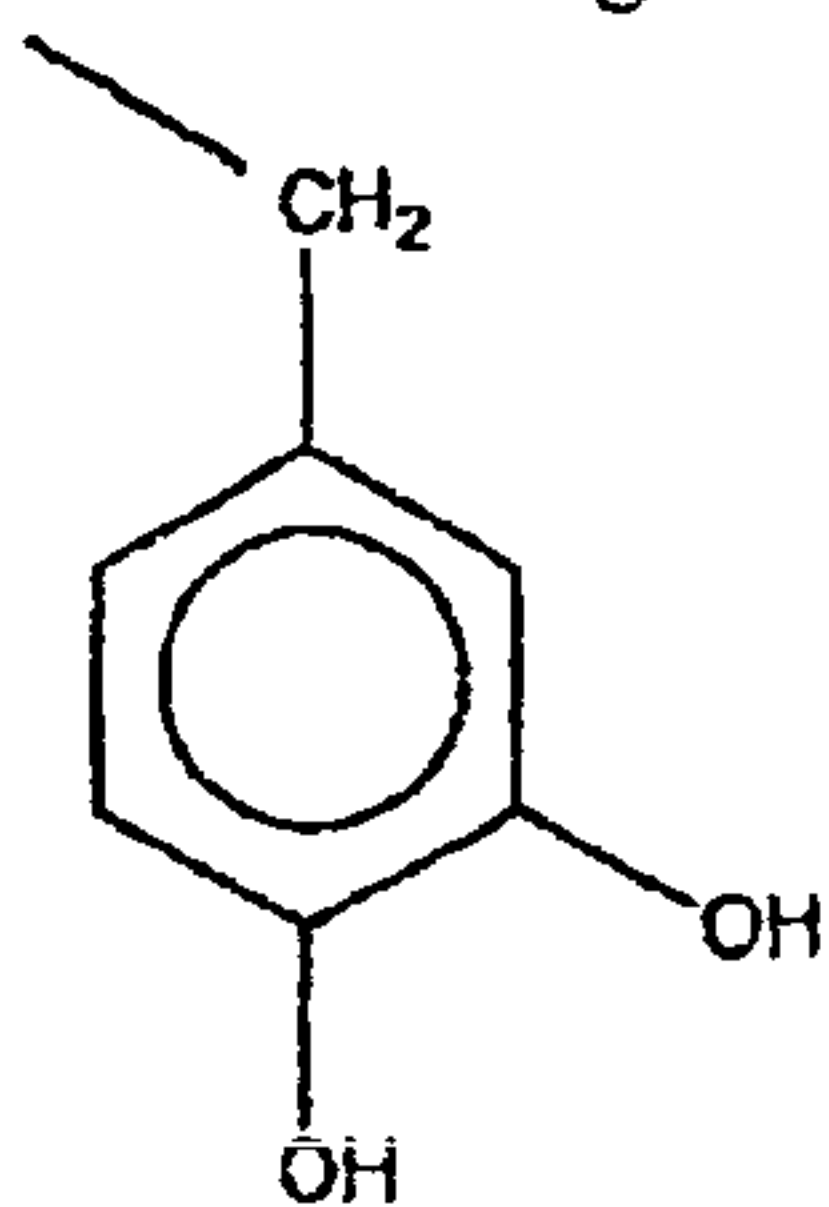
alone or in combination with an amine-terminated lower alkyl chain having from 1 to about 10 carbon atoms;

R_2 comprises at least one ether linkage, which couples alkyl groups or chains, each having from 1 to about 10 carbon atoms;

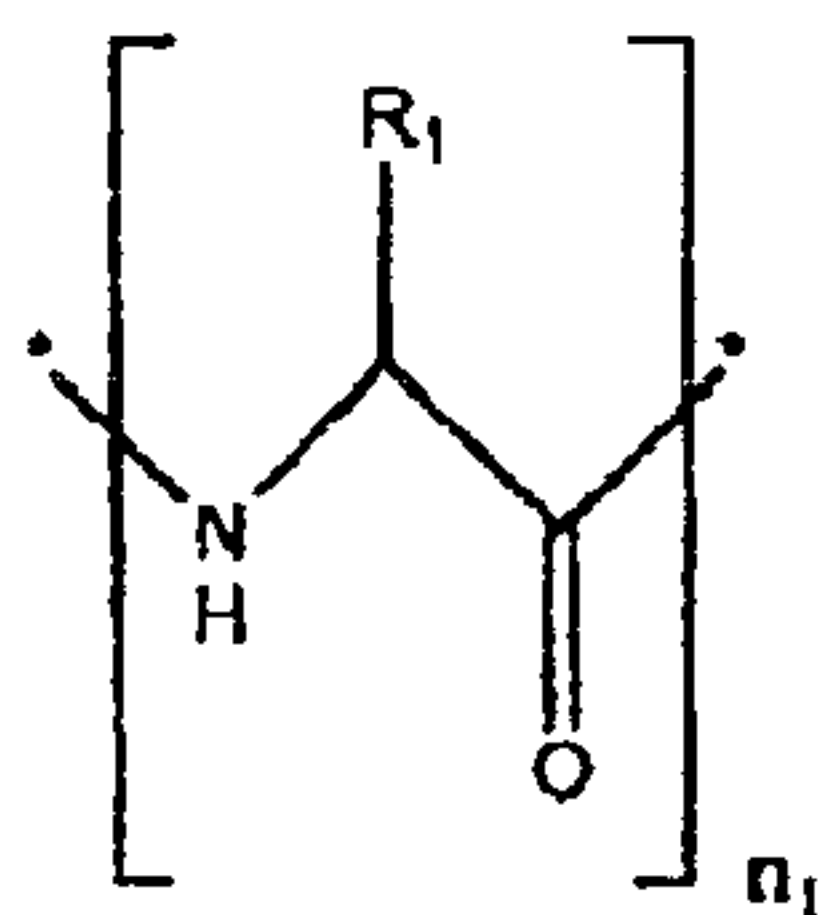
n_1 has a value in the range of about 1 to about 10; and

n_2 has a value in the range of about 5 to about 100.

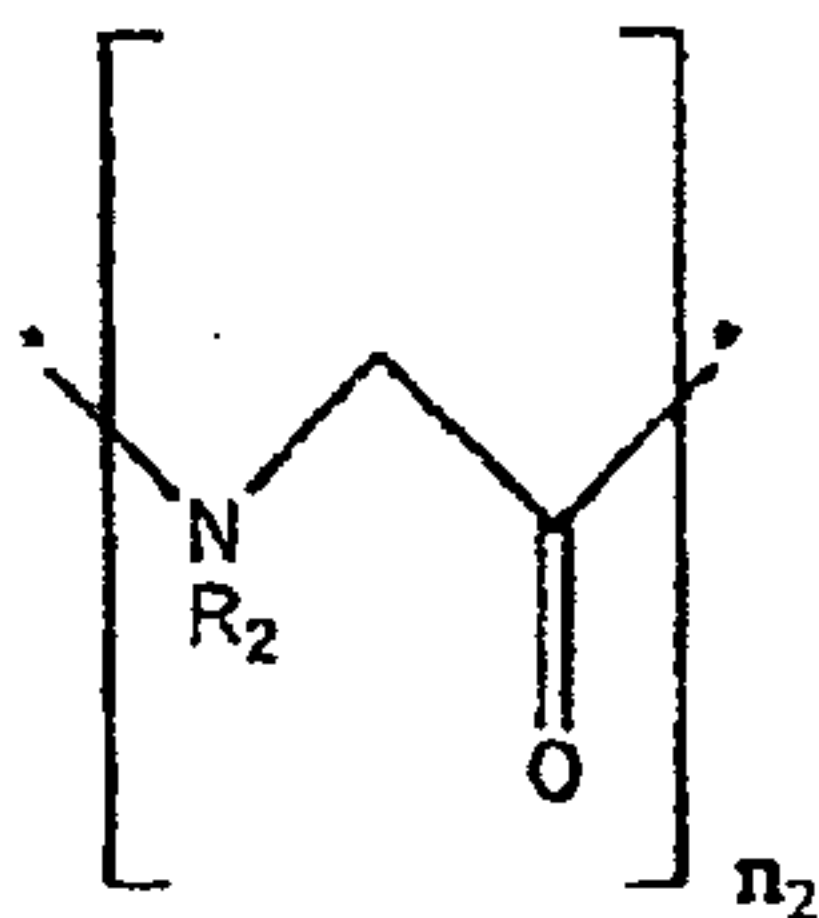
2. A peptidomimetic polymer according to claim 1 wherein R_1 comprises



3. A peptidomimetic polymer according to claim 1 wherein R_2 is $-\text{CH}_2-\text{CH}_2-\text{O}-\text{CH}_3$
4. A peptidomimetic polymer according to claim 1 wherein n_1 is 3.
5. A peptidomimetic polymer according to claim 1 wherein n_2 is 20.
6. A coating comprising a peptidomimetic polymer comprising coupled polypeptide and polypeptoid moieties, the polypeptide moiety having the structure:



the polypeptoid moiety having this structure:



wherein

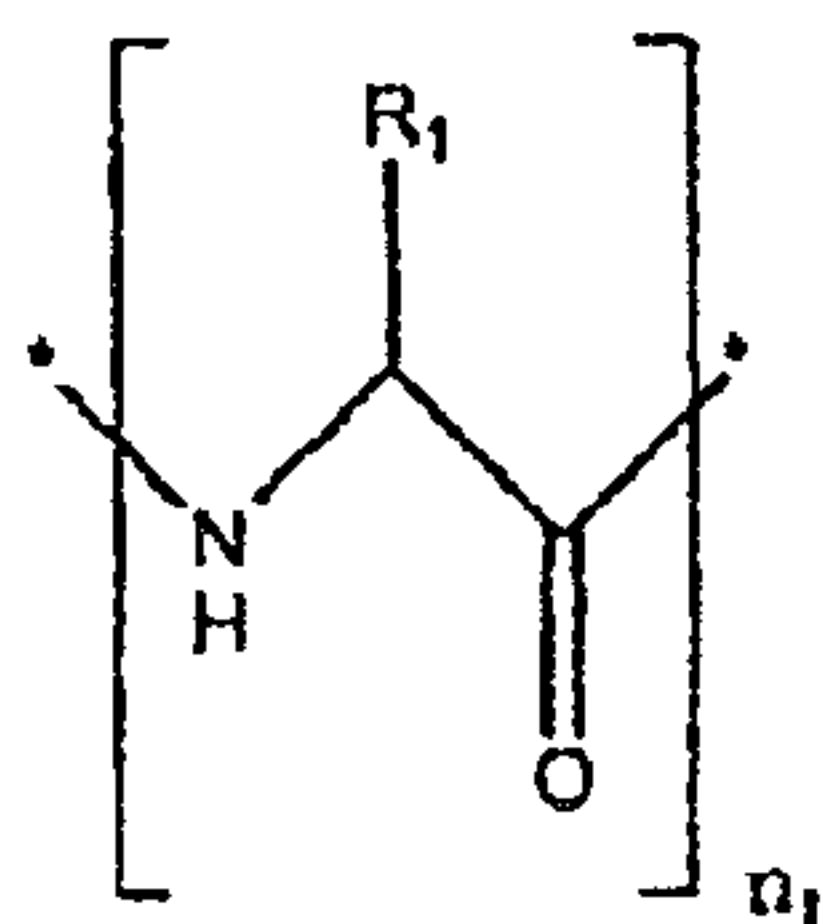
R_1 comprises at least one dihydroxyphenyl alone or in combination with an amine-terminated lower alkyl chain having from 1 to about 10 carbon atoms;

R_2 comprises at least one ether linkage, which couples alkyl groups or chains, each having from 1 to about 10 carbon atoms;

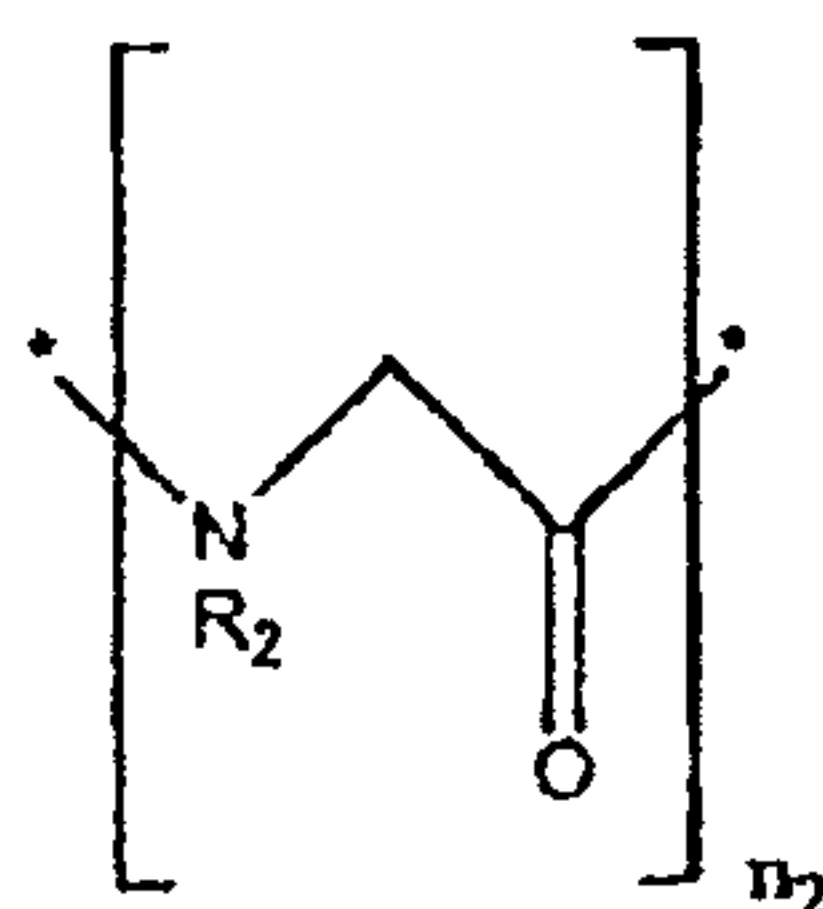
n_1 has a value in the range of about 1 to about 10; and

n_2 has a value in the range of about 5 to about 100.

7. A coated metal work piece comprising a metal substrate having adhered thereto a polypeptidomimetic coating of claim 6.
8. A work piece according to claim 7 wherein the metal substrate is a working surface of a medical device.
9. A work piece according to claim 7 wherein the metal substrate comprises titanium oxide.
10. A antifouling resistant coating comprising a polypeptidomimetic polymer comprising coupled anchoring polypeptide moiety and an antifouling polypeptoid moiety, the anchoring moiety having the structure:



the antifouling moiety having the structure:



wherein

R_1 comprises at least one dihydroxyphenyl alone or in combination with an amine-terminated lower alkyl chain having from 1 to about 10 carbon atoms;

R_2 comprises at least one ether linkage, which couples ether-linked alkyl groups or chains, each having from 1 to about 10 carbon atoms;

n_1 ranges from about 1 to about 10; and

n_2 ranges from about 5 to about 100.

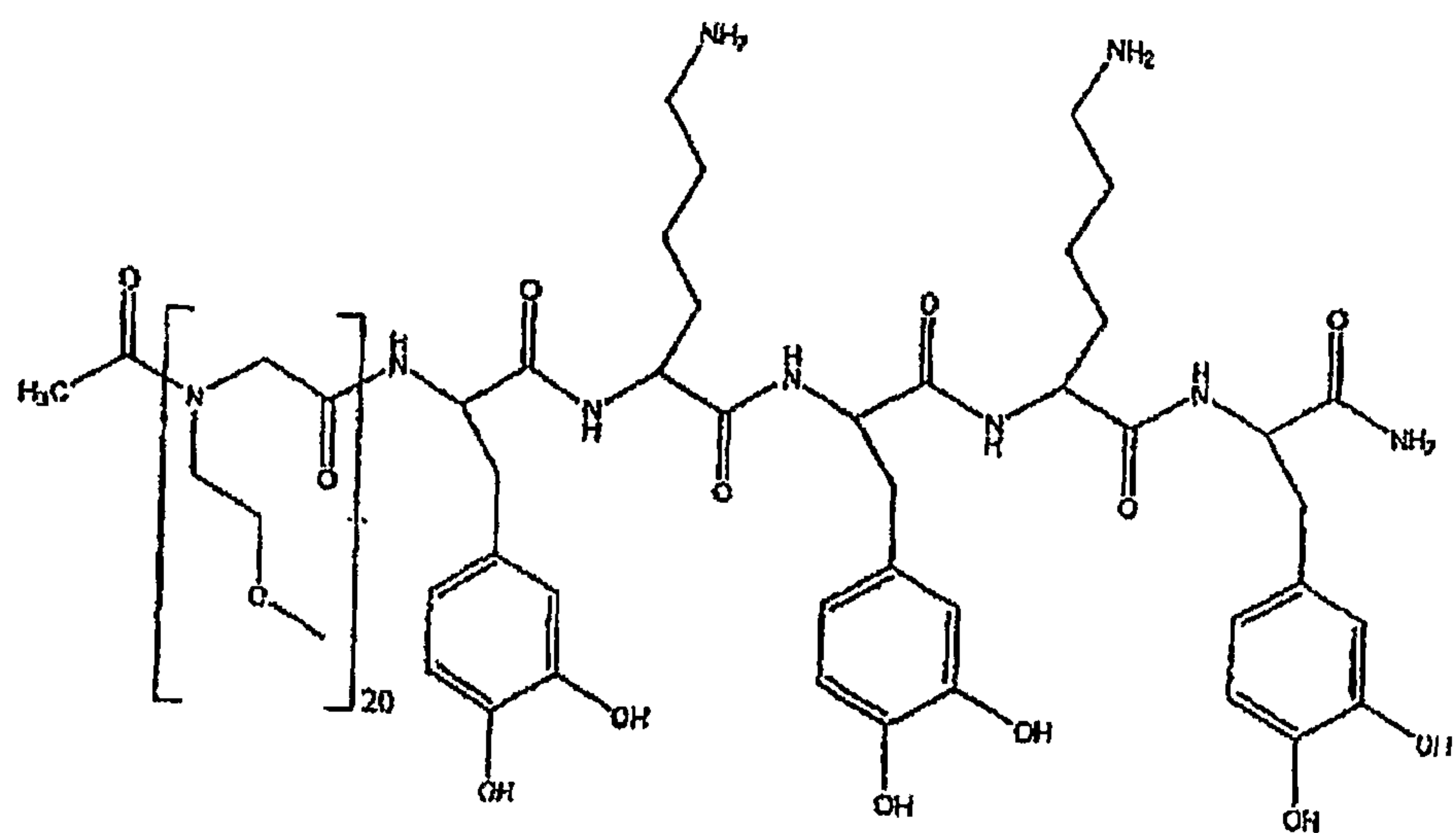


Figure 1

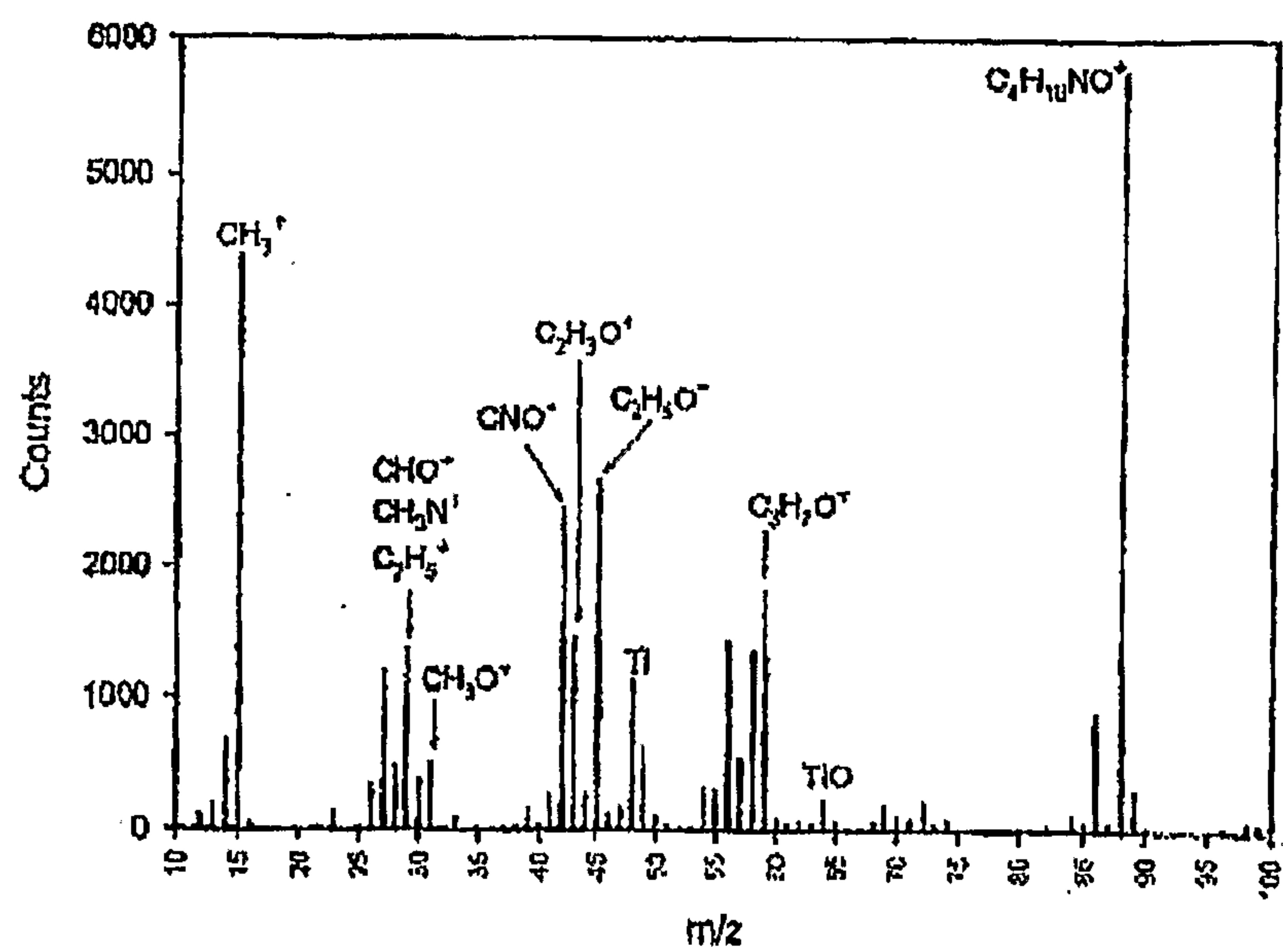


Figure 2

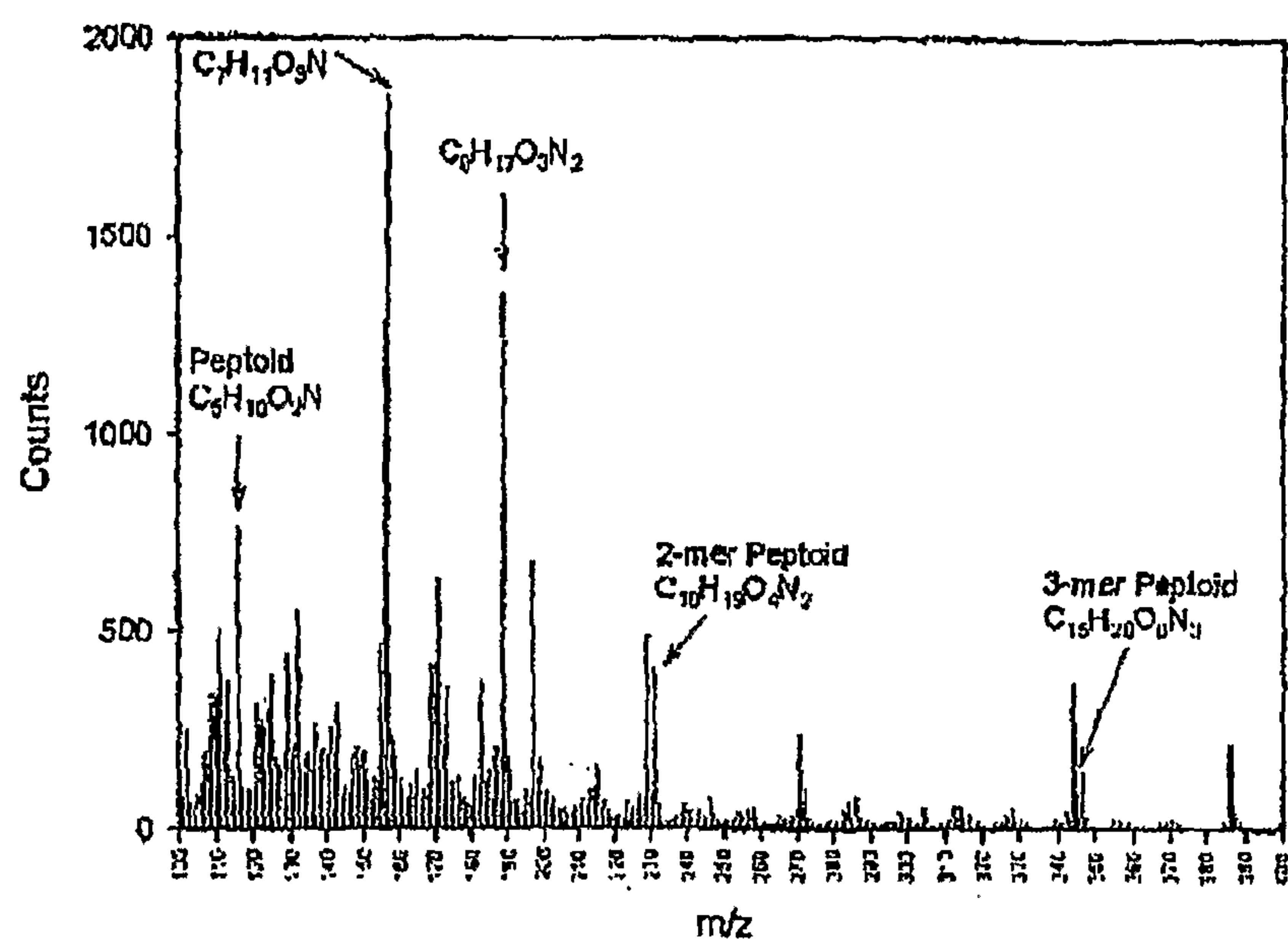


Figure 3

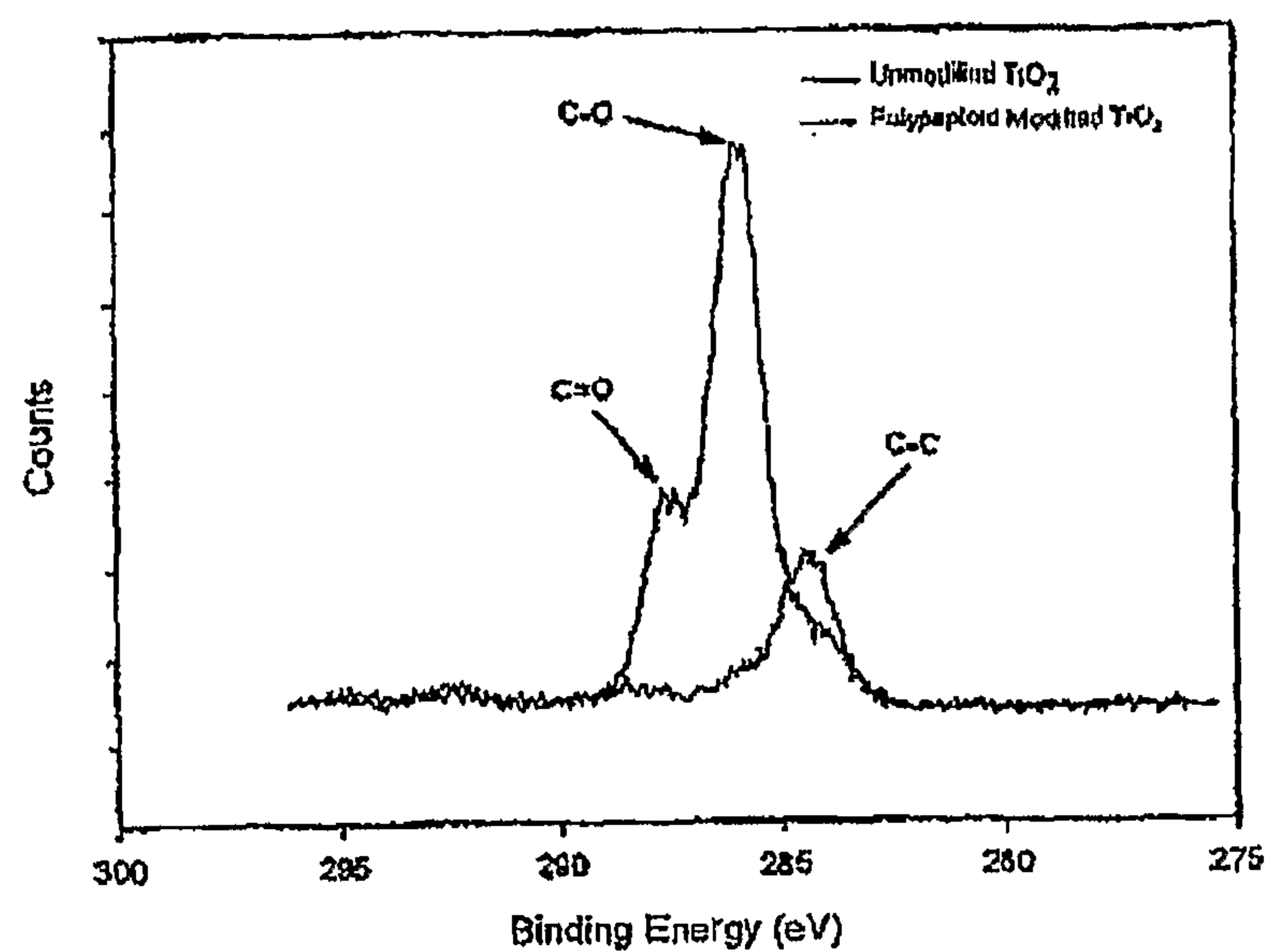


Figure 4

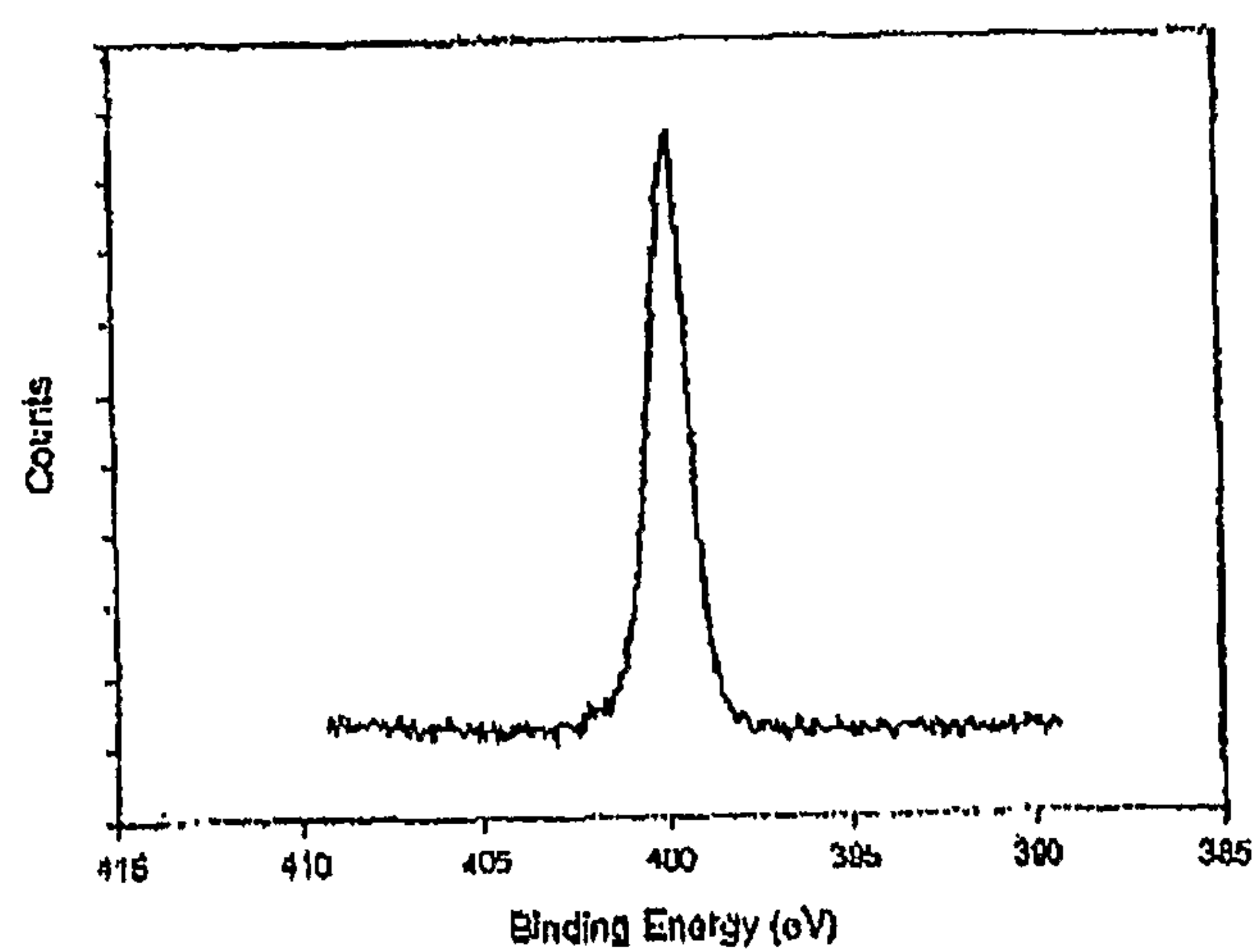


Figure 5

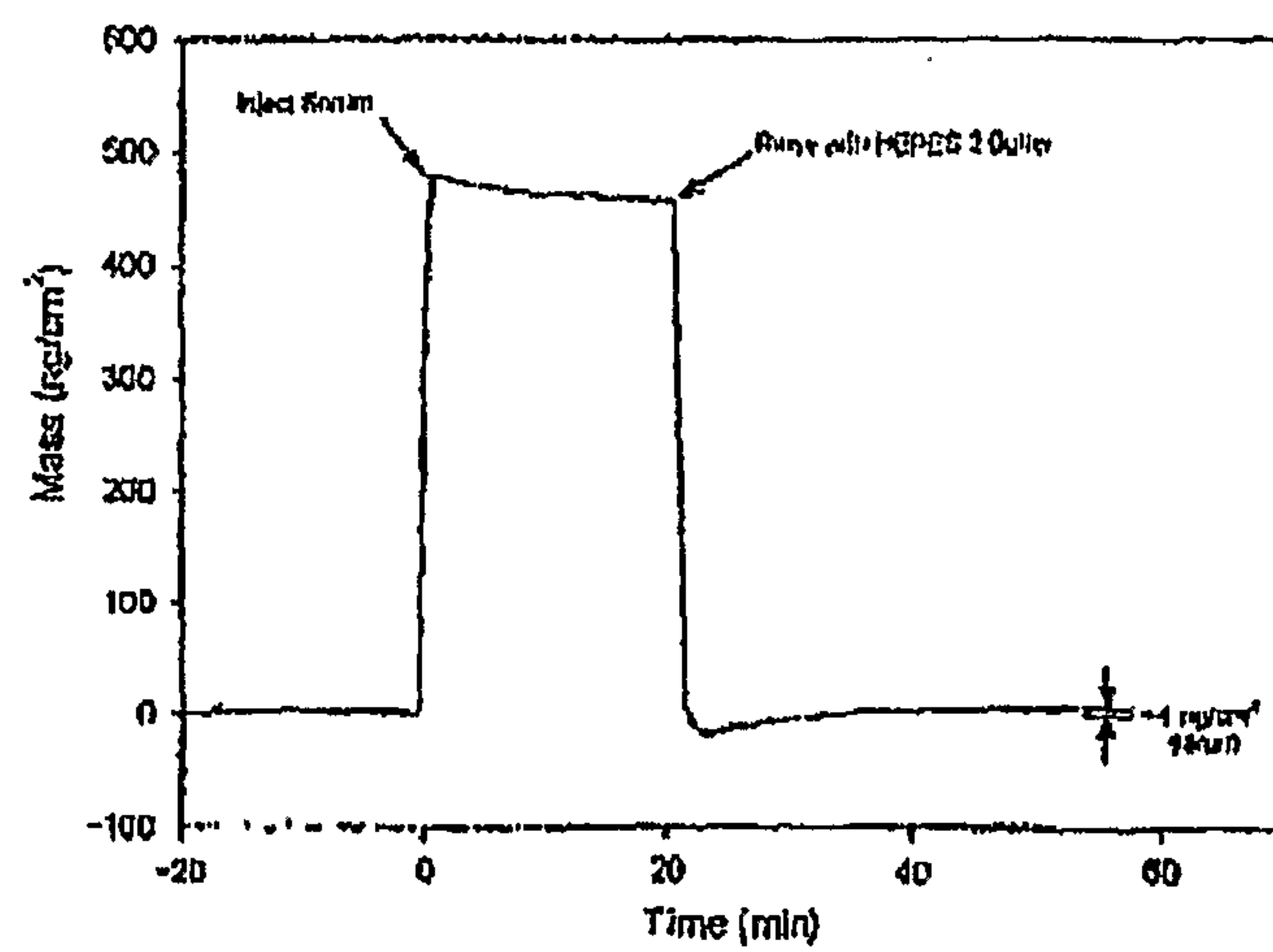
In Situ Determination of Protein Adsorption Using QWLS

Figure 6

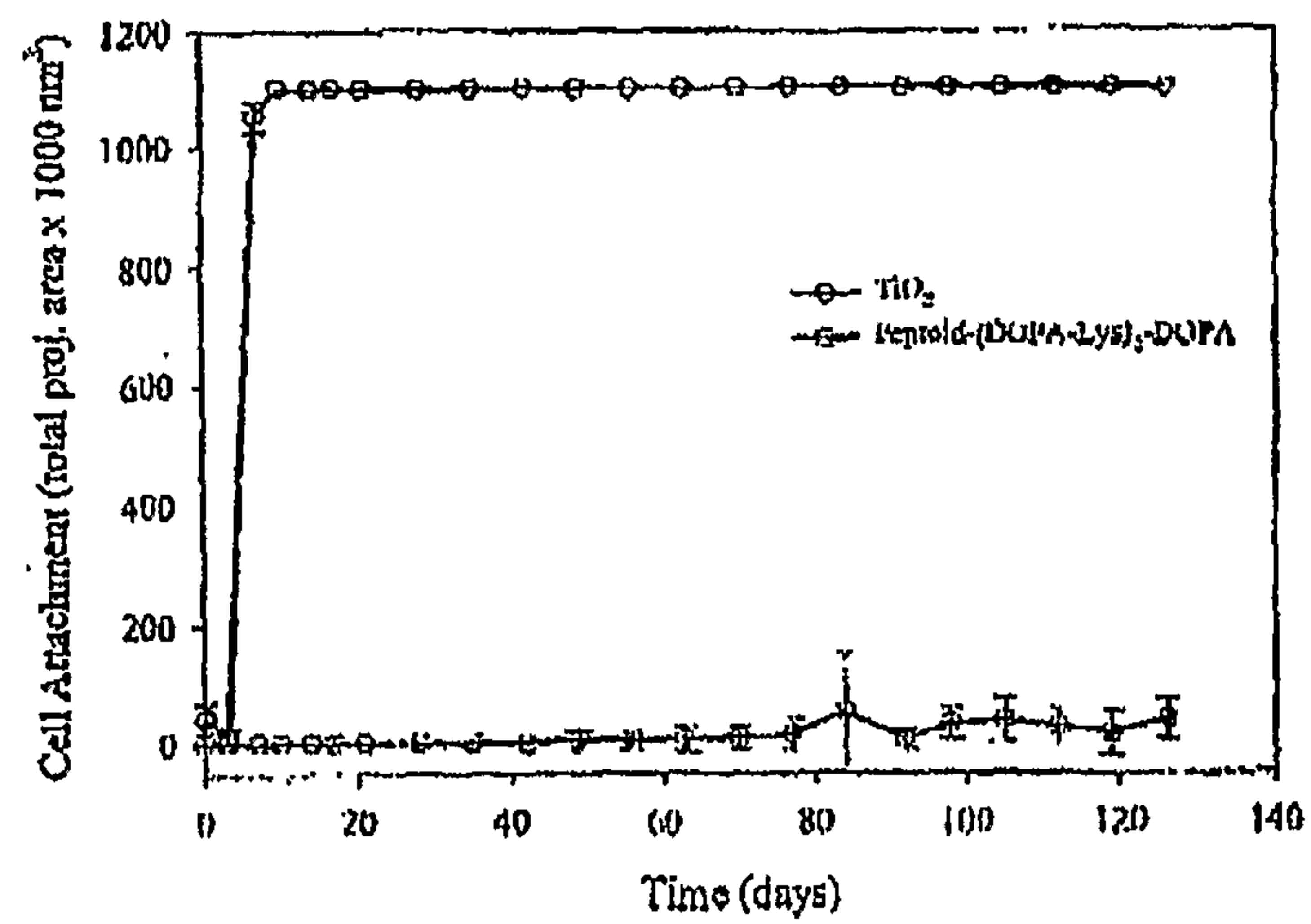
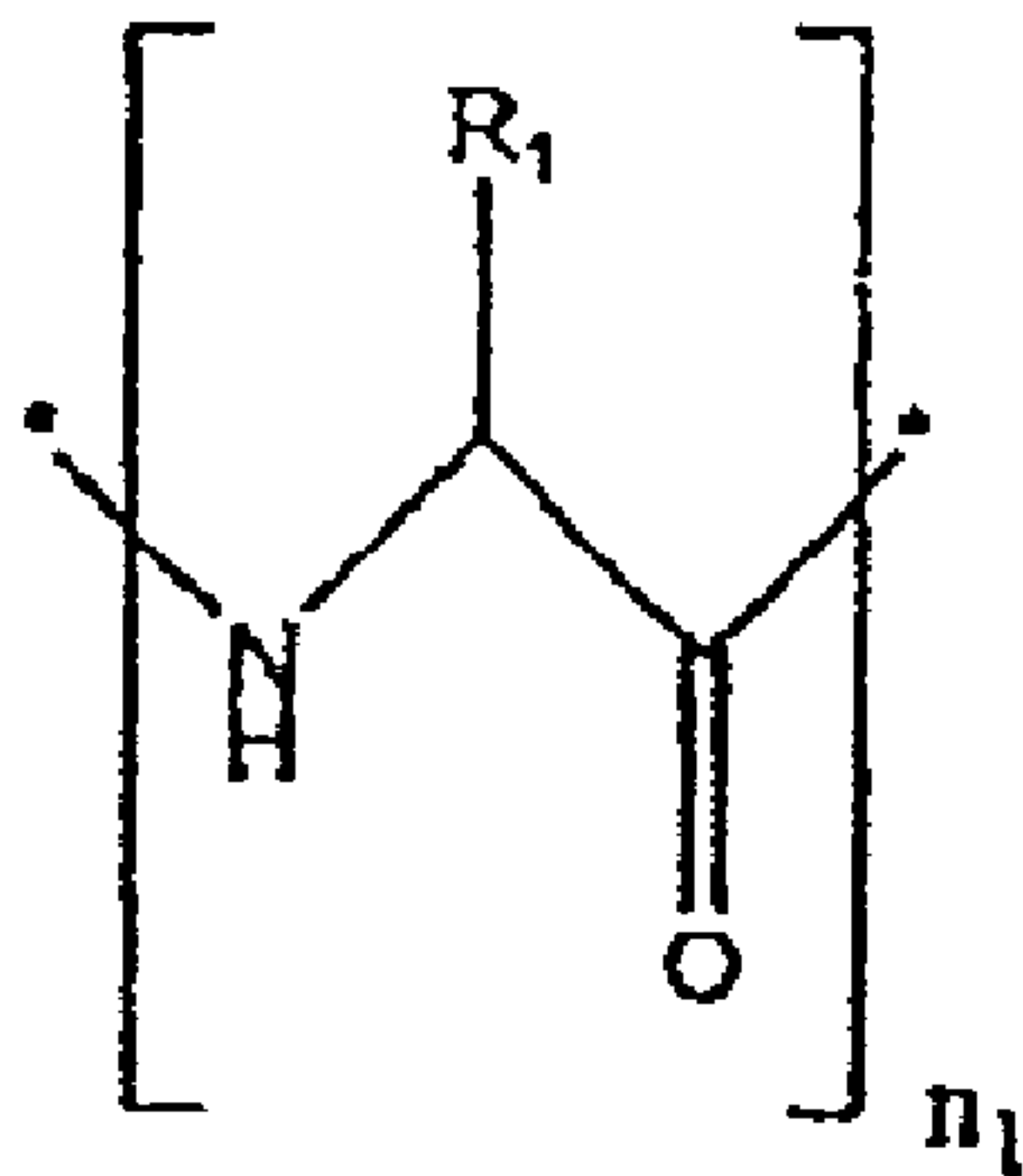
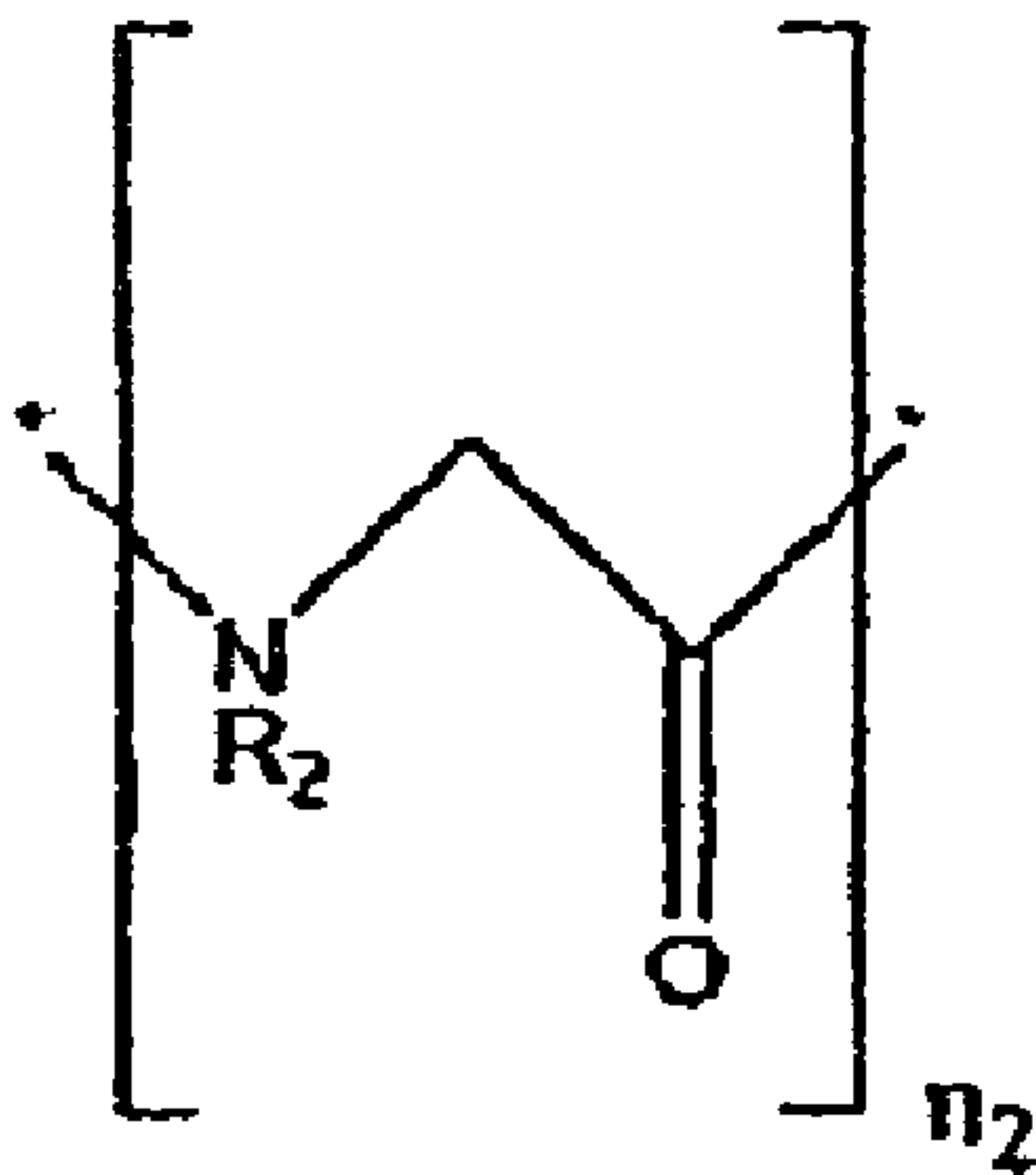


Figure 7



I. (Peptide anchor)



II. (Peptoid moiety)