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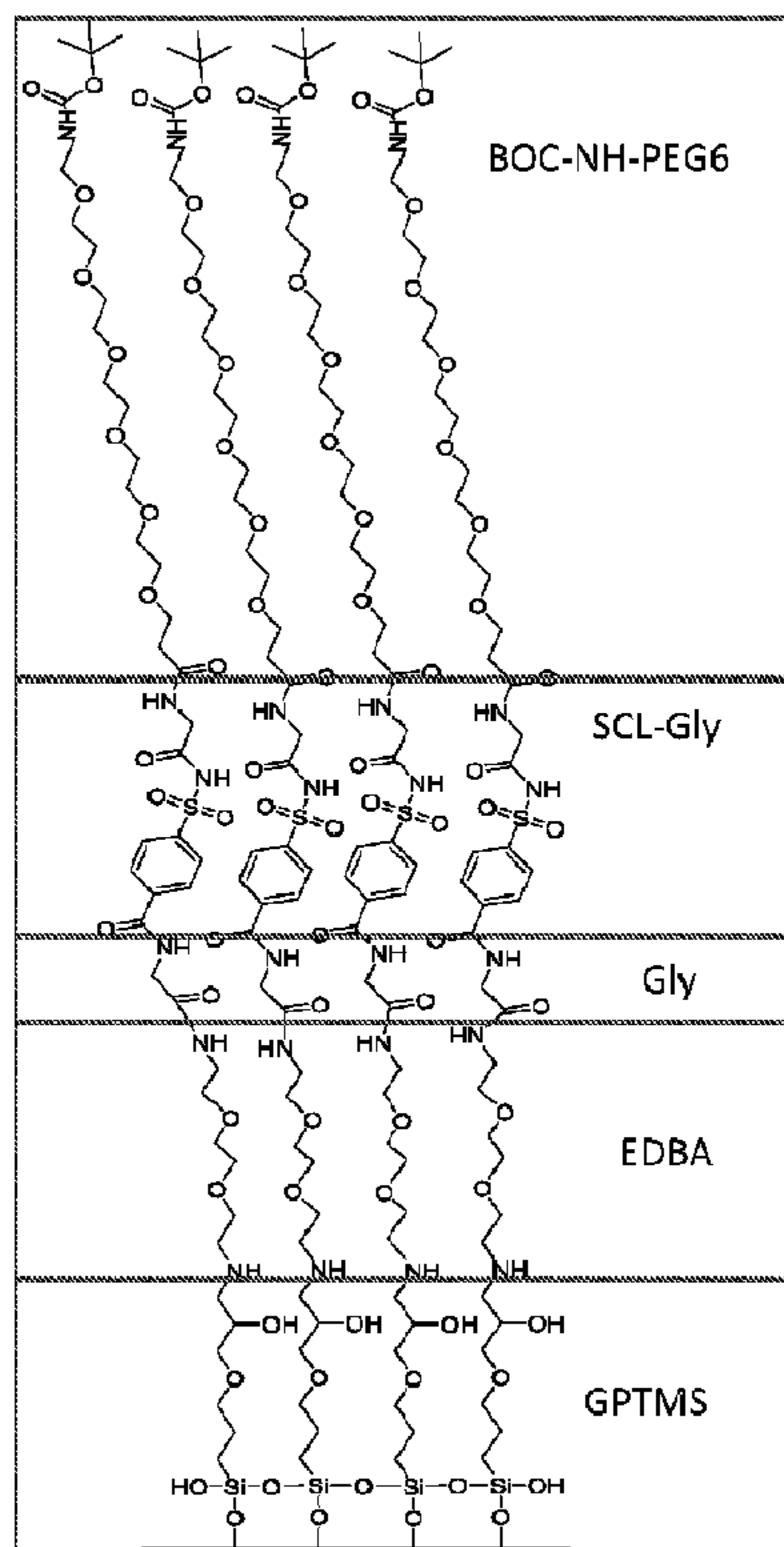
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(54) Titre : LIEURS CHIMIOSELECTIFS PRE-ASSEMBLES, PROTEGES, CHIMIQUEMENT STABLES
(54) Title: PRE-ASSEMBLED, PROTECTED, CHEMICALLY STABLE, CHEMOSELECTIVE LINKERS

FIG. 1

PL8 Molecule (HT960)



(57) Abrégé/Abstract:

Molecules compounds are provided having the structure in Formula I, or a salt thereof, wherein n^1 is independently 0, 1, 2, or 3; n^2 is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20; n^3 is from 0, 1, 2, or 3; n^4 is 0 or 1; and n^5 is 0, 1, 2, or 3;

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

and wherein X is O, N, or S; Y, Z, XX, and YY are the same or different and are independently O or S; ZZ comprises nitrogen, oxygen, sulfur, or selenium; and wherein R¹, R², R³, R⁴, R⁵, R⁶, R⁷, R⁸, R⁹, R¹⁰, R¹¹, R¹² are as described herein. Methods are also provided for the synthesis of and use of the provided molecules in applications for diagnostic testing.

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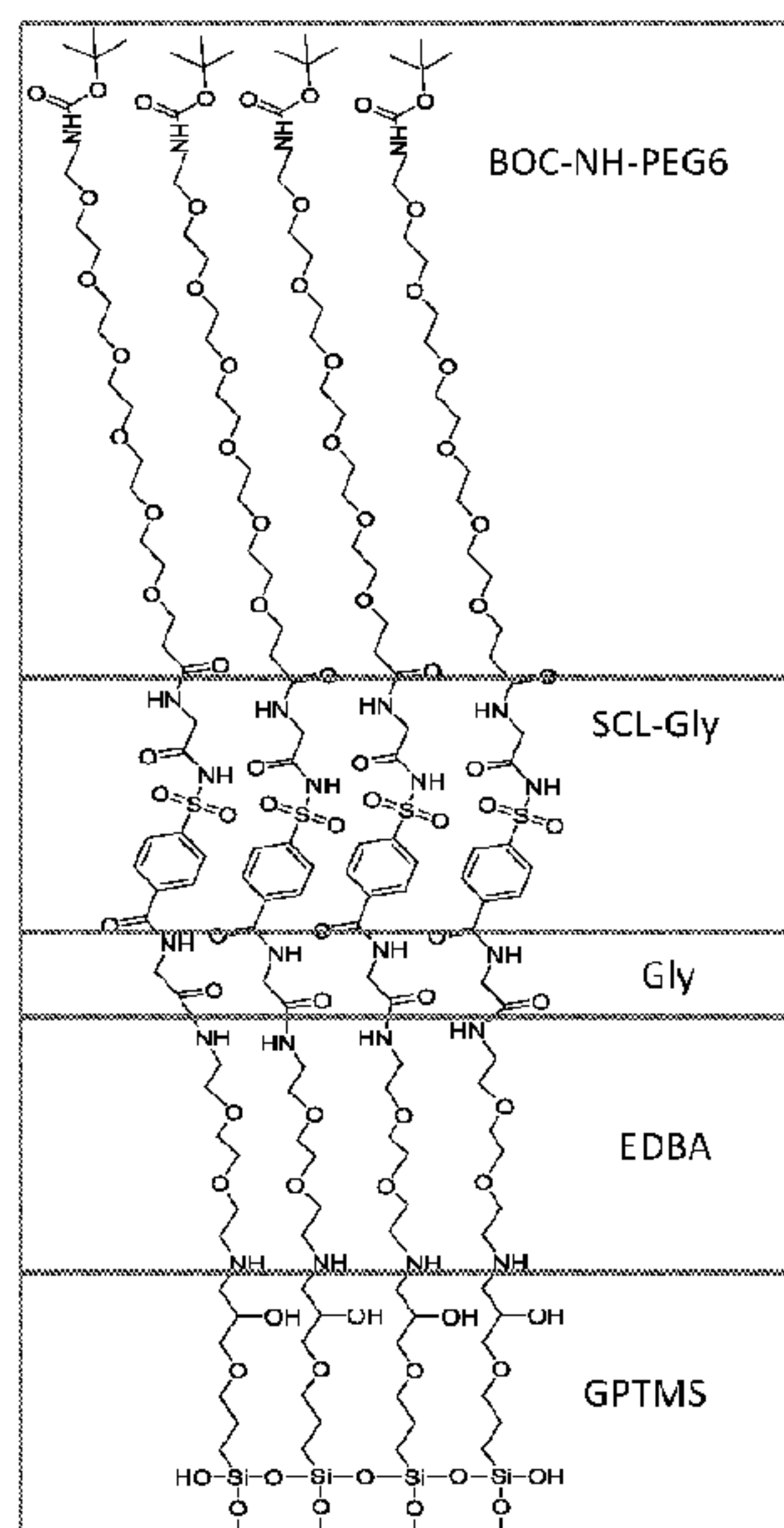
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(54) Title: PRE-ASSEMBLED, PROTECTED, CHEMICALLY STABLE, CHEMOSELECTIVE LINKERS

(57) Abstract: Molecules compounds are provided having the structure in Formula I, or a salt thereof, wherein n_1 is independently 0, 1, 2, or 3; n_2 is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20; n_3 is from 0, 1, 2, or 3; n_4 is 0 or 1; and n_5 is 0, 1, 2, or 3; and wherein X is O, N, or S; Y, Z, XX, and YY are the same or different and are independently O or S; ZZ comprises nitrogen, oxygen, sulfur, or selenium; and wherein $R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}$ are as described herein. Methods are also provided for the synthesis of and use of the provided molecules in applications for diagnostic testing.

FIG. 1

PL8 Molecule (HT960)



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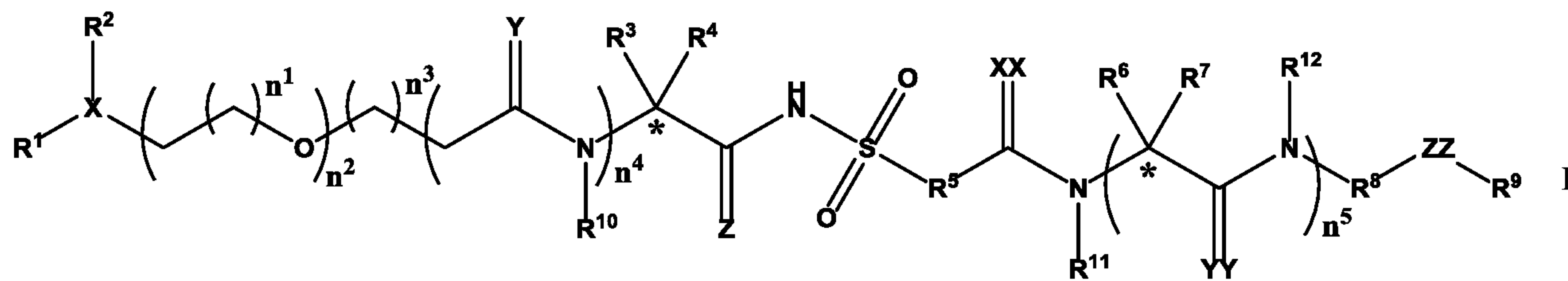
PRE-ASSEMBLED, PROTECTED, CHEMICALLY STABLE, CHEMOSELECTIVE LINKERS

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 62/419,861, filed November 9, 2016, which is incorporated herein by reference in its entirety.

SUMMARY OF THE INVENTION

[0002] Disclosed herein are molecules having the structure:



or a salt thereof,

wherein n^1 can be independently 0, 1, 2, or 3;

n^2 can be 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20;

n^3 can be from 0, 1, 2, or 3;

n^4 can be 0 or 1; and

n^5 can be 0, 1, 2, or 3; and wherein

X can be O, N, or S;

Y, Z, XX, and YY can be the same or different and are independently O or S;

R^1 and R^2 can be the same or different and can be independently hydrogen, alkyl, alkenyl, aryl,

heteroaryl, alkynyl, arylalkyl, arylalkenyl, haloalkyl, cycloalkyl, sulfonamidyl, acyl, electron lone pair, or $-\text{CO}_2\text{R}^{13}$, wherein R^{13} can be alkyl, alkenyl, aryl, arylalkyl, arylalkenyl, haloalkyl, cycloalkyl, cycloalkylalkyl, or arylcycloalkylalkyl; or wherein R^1 and R^2 and the N to which they can be bound form a ring; or wherein R^1 or R^2 can comprise a nucleoside, nucleotide, polynucleotide, peptide, peptoid, saccharide, aptamer, or antibody or fragment thereof;

R^3 , R^4 , R^6 , and R^7 are the same or different and can be independently hydrogen, halo, alkyl, alkenyl, aryl, heteroalkyl, arylalkyl, hydroxyarylalkyl, heteroarylalkyl, cycloalkyl, thioalkyl, selenoalkyl, hydroxyalkyl, or amino-substituted alkyl;

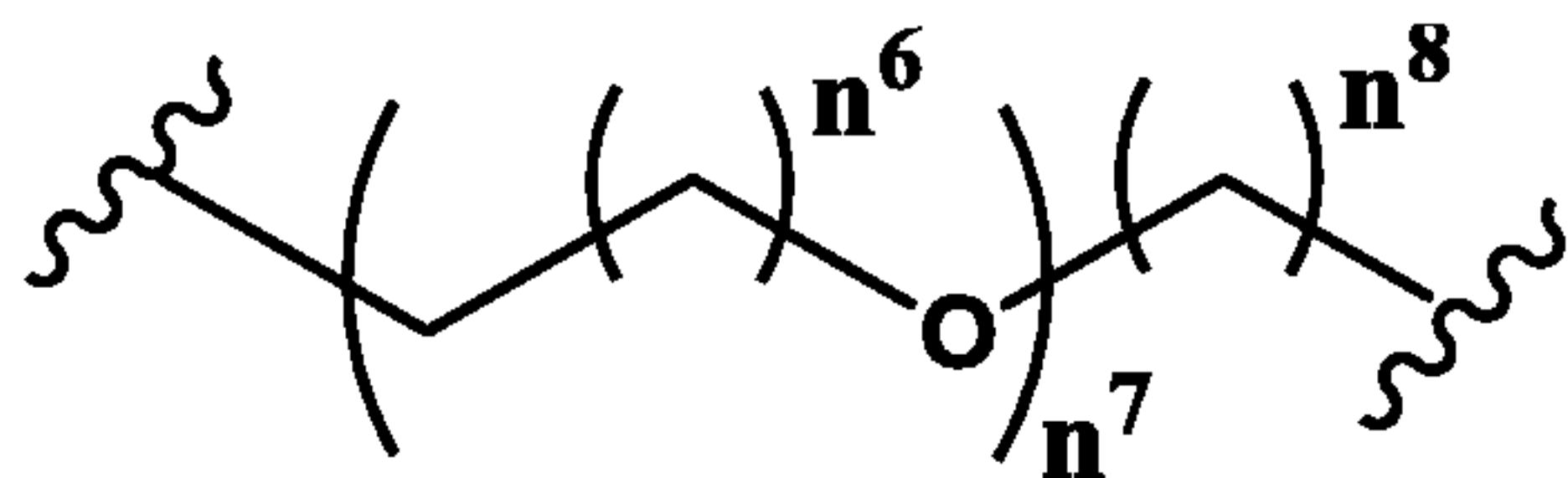
R^5 can be alkyl, alkenyl, alkynyl or aryl, all optionally substituted with hydrogen, halo, alkyl, polyhaloalkyl, alkoxy, haloalkoxy, polyhaloalkoxy, alkoxycarbonyl, alkenyl, alkynyl, cycloalkyl, cycloalkylalkyl, hydroxyl, hydroxyalkyl, nitro, cyano, amino, substituted amino, alkylamino, dialkylamino, thiol, alkylthio, alkylcarbonyl, acyl, alkoxycarbonyl, aminocarbonyl, alkylsulfinyl, sulfonamide, or sulfonyl;

R^8 , oriented from N to ZZ, can be alkyl, heteroalkyl, amino-substituted alkyl, amino-substituted heteroalkyl, amidoalkyl, amidoheteroalkyl, amino-substituted amidoheteroalkyl, each optionally substituted with an alkyl, heteroalkyl, amino-substituted alkyl, amino-substituted heteroalkyl,

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amidoalkyl, amidoheteroalkyl, or amino-substituted amidoheteroalkyl; or wherein R^8 is ξ - $(CR^{15}R^{16}CR^{17}R^{18})_m$ - ξ wherein m is 1 to 100, and R^{15} , R^{16} , R^{17} , and R^{18} can be the same or different and are independently hydrogen, halo, alkyl, heteroalkyl, amino-substituted alkyl, amino-substituted heteroalkyl, amidoalkyl, amidoheteroalkyl, amino-substituted amidoheteroalkyl; or wherein R^8 can be



wherein n^6 and n^8 can be the same or different and can be independently 1, 2, or 3, and n^8 can be 1 to 20; R^9 can be an alkyl, heteroalkyl, hydroxyheteroalkyl, silylalkyl, siloxyalkyl siloxyheteroalkyl,

siloxyhydroxyheteroalkyl, or wherein R^9 can be $R^{19}-(CH_2)_3-Si(Z)_p$, wherein p can be from 1 to 3, wherein Z can be selected from halo or alkoxy, and wherein R^{19} can be selected from a hydroxyalkyl or carbamate;

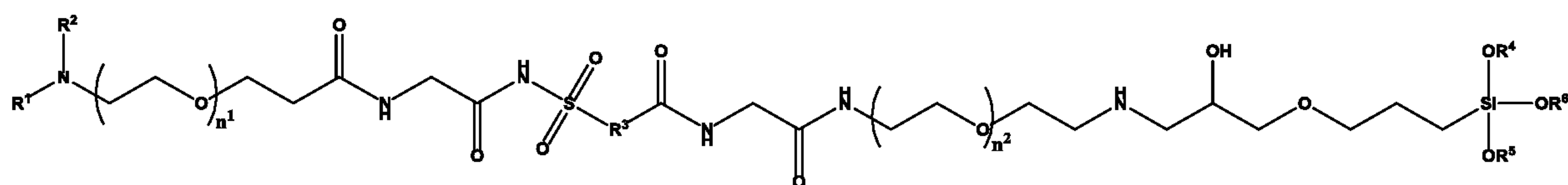
R^{10} , R^{11} , and R^{12} are the same or different and can be independently selected from hydrogen or alkyl; and wherein R^9 can be optionally linked to a solid phase;

ZZ is NR^{20} , O, S, or Se, wherein R^{20} can be hydrogen or alkyl; and

(*) is a carbon center, wherein said carbon center can be independently a stereogenic center or a non-stereogenic center.

[0003] In some aspects are molecules or salts of structure I, wherein X can be N, and wherein R^1 and R^2 can be the same or different and can be independently selected from hydrogen, acyl, acyloxy, benzyl, triphenylmethyl, benzylidenyl, or *p*-toluenesulfonyl. In some aspects are molecules or salts of structure I, wherein the solid phase can comprise a substrate, a bead, or a chromatographic packing material. In some aspects are molecules or salts of structure I, wherein either of R^3 and R^4 can comprise a C_1 - C_{10} alkyl, cycloalkyl, aryl, or heteroaryl side chain. In one aspect are molecules or salts of structure I, wherein either of R^3 and R^4 can comprise a functional group that is at least partially protonated at a pH of about 7.3. In some aspects are molecules or salts of structure I, wherein either of R^3 and R^4 can comprise a functional group that is at least partially deprotonated at a pH of about 7.3. In some aspects are salts or molecules of structure I, wherein said side chain can comprise an amide, alcohol, or thiol. In one aspect are molecules or salts of structure I, wherein R^3 and R^4 can be hydrogen. In some aspects are molecules or salts of structure I, wherein either of R^6 and R^7 can comprise a C_1 - C_{10} alkyl, cycloalkyl, aryl, or heteroaryl side chain. In some aspects are molecules or salts of structure I, wherein either of R^6 and R^7 can comprise a functional group that is at least partially protonated at a pH of about 7.3. In some aspects are molecules or salts of structure I, wherein either of R^6 and R^7 can comprise a functional group that is at least partially deprotonated at a pH of about 7.3. In some aspects are molecules or salts of structure I, wherein a side chain can comprise an amide, alcohol, or thiol. In some aspects are molecules or salts of structure I, wherein R^6 and R^7 can be hydrogen.

[0004] Molecules of formula I will include the molecule having the structure:



IA

or a salt thereof,

wherein $n^1=1-15$ and $n^2=1-6$; and

wherein R^1 and R^2 can be the same or different and are independently hydrogen or acyloxy;

R^3 can be alkyl, alkenyl, alkynyl or aryl, all optionally substituted with 1, 2, 3, or 4 groups selected from hydrogen, halo, alkyl, polyhaloalkyl, alkoxy, haloalkoxy, polyhaloalkoxy, alkoxycarbonyl, alkenyl, alkynyl, cycloalkyl, cycloalkylalkyl, hydroxyl, hydroxyalkyl, nitro, cyano, amino, substituted amino, alkylamino, dialkylamino, thiol, alkylthio, alkylcarbonyl, acyl, alkoxycarbonyl, aminocarbonyl, alkylsulfinyl, sulfonamide, or sulfonyl; and

R^4 , R^5 , and R^6 can be the same or different and are independently selected from hydrogen, alkyl, silyl, or siloxy.

[0005] In some aspects are molecules or salts of structure IA, wherein R^3 can be *p*-phenyl. In some aspects are molecules or salts of structure IA, wherein wherein R^3 can be *n*-propyl. In some aspects are molecules or salts of structure I or IA, wherein R^1 or R^2 can comprise a peptide. In some aspects, the molecule or salt of structure I or IA can comprise a peptide. In one aspect, a peptide can comprise from 2 to 100 amino acids.

[0006] Also disclosed herein are arrays comprising the molecules or salts of structure I or IA. In one aspect, an array can comprise a peptide. In one aspect, an array can comprise at least 10,000 peptides per square centimeter. In one aspect, an array can comprise at least 300,000 peptides per square centimeter. In one aspect, an array can comprise at least 1 million peptides per square centimeter. In some aspects, an array can comprise a binding moiety. A binding moiety can comprise a nucleoside, nucleotide, polynucleotide, peptide, peptoid, saccharaide, polysaccharide, aptamer, or antibody or fragment thereof. In some aspects, an array can comprise a fatty acid. A fatty acid can be fluorinated. Also disclosed herein are methods for the synthesis of arrays comprising the molecules or salts of structure I or IA. In one aspect, making an array comprises associating the molecules or salts of structure I or IA with a substrate. A substrate can be a silicon or silicon oxide wafer. In one aspect, an array can comprise at least 10,000 peptides per square centimeter. In one aspect, an array can comprise at least 300,000 peptides per square centimeter. In one aspect, an array can comprise at least 1 million peptides per square centimeter.

[0007] Also disclosed herein is a method. In some aspects, the method can comprise forming a solid support. Forming a solid support can comprise associating the solid support with molecules or salts of structures I or IA. A solid support can be a substrate, bead, polymer, or a chromatographic packing material.

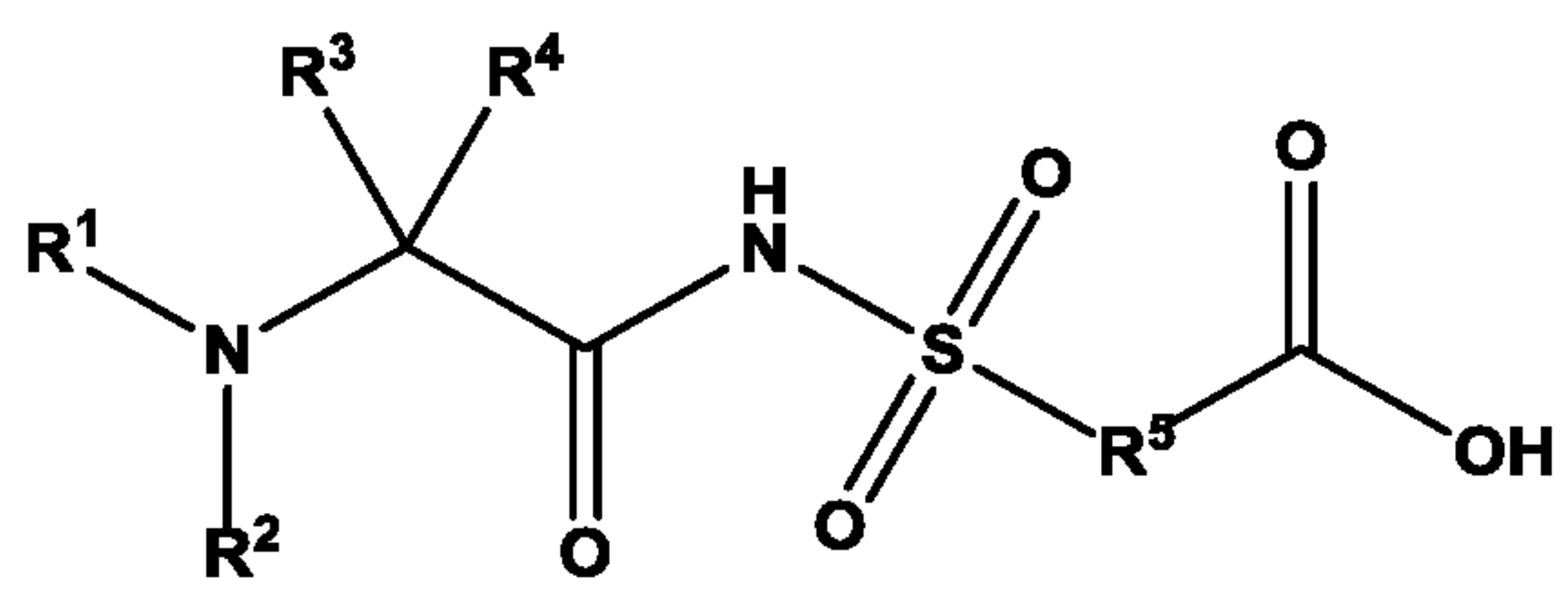
[0008] Also disclosed herein is a use of the molecules or salts of structures I or IA for binding a nucleotide, polynucleotide, polypeptide, aptamer, or antibody or fragment thereof. Also disclosed herein

is a use of the molecules or salts of structures I or IA for binding proteins or antibodies. Also disclosed herein is a method comprising binding a binding moiety to the molecules or salts of structures I or IA. In some aspects, the method can comprise identifying a binding moiety. Identifying can comprise an immunofluorescence assay. In some aspects, the binding moiety can comprise a nucleotide, polynucleotide, polypeptide, aptamer, or antibody or fragment thereof. In some aspects, the binding moiety can be obtained from a subject. In some aspects, the method can comprise determining the likelihood that the subject has a disease or a condition. A subject can be a human. In some aspects, a disease or condition can be an autoimmune disease, an infection, or cancer. In some aspects an autoimmune disease can be lupus, ulcerative colitis, Crohn's disease, or rheumatoid arthritis. In some aspects, an infection can be bacterial, viral, fungal, or parasitic. In some cases, the method further can comprise communicating a result via a communication medium.

[0009] Also disclosed herein is a method comprising cleaving a bond in the molecules or salts of structures I or IA. In some aspects, a bond can be the C-N bond of an *N*-acylsulfonamide. In some aspects, cleaving can comprise a first activating step and a second cleavage step. In some aspects, a first activating step can comprise alkylating an *N*-acylsulfonamide. In some aspects, alkylating can comprise use of an alkylating agent. In some aspects, an alkylating agent can be a diazo compound or alkyl halide. In some aspects, an alkylating agent can be diazomethane, methyl iodide, or iodoacetonitrile. In some aspects, the cleavage can comprise a vapor phase cleavage reaction. In some aspects, the cleavage can comprise a vapor-phase ammonia cleavage reaction.

[0010] Also disclosed herein are kits. In some aspects, the kits can comprise the molecules or salts of structures I or IA. In some cases a kit can comprise instructions for use. Also disclosed herein are methods of making a kit disclosed herein.

[0011] Also disclosed herein is a molecule having the structure:



II

or a salt thereof, wherein:

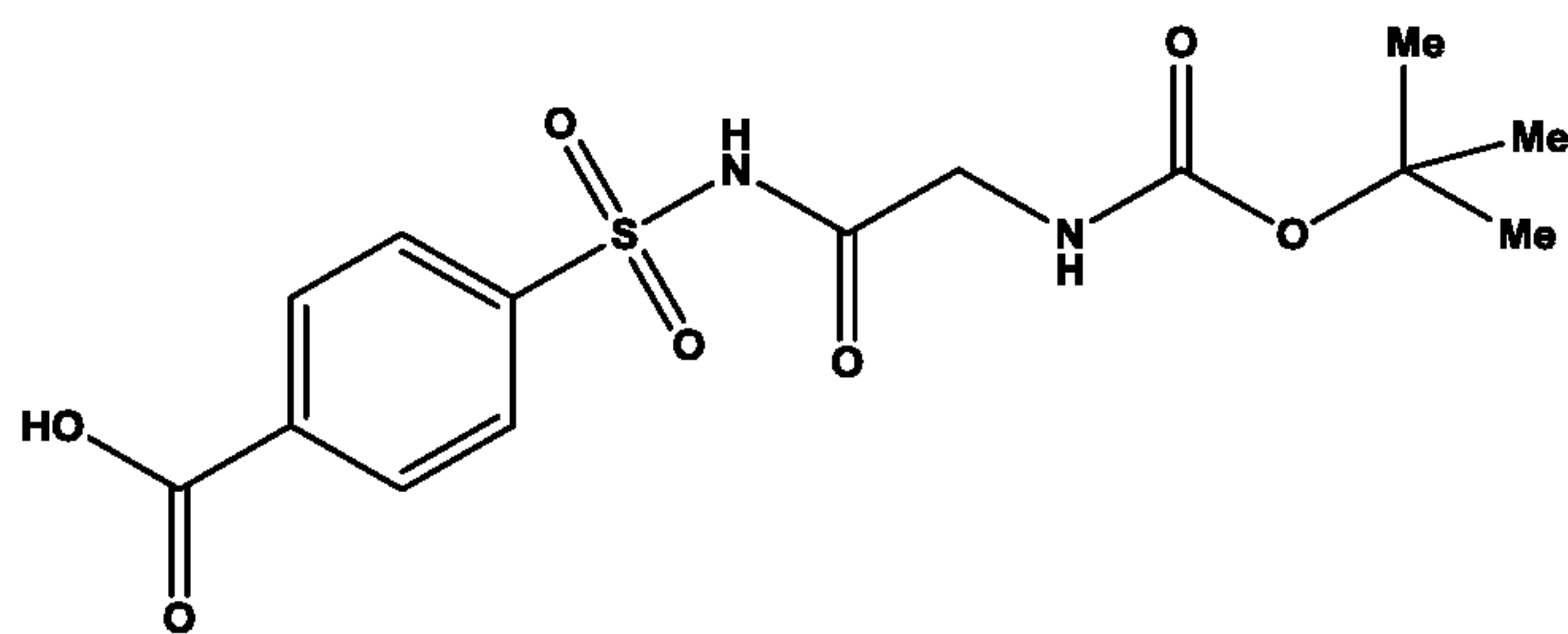
R^1 and R^2 can be the same or different and are independently hydrogen, alkyl, alkenyl, aryl, heteroaryl, alkynyl, arylalkyl, arylalkenyl, haloalkyl, cycloalkyl, sulfonamidyl, acyl, electron lone pair, or $-CO_2R^6$, wherein R^6 can be alkyl, alkenyl, aryl, arylalkyl, arylalkenyl, haloalkyl, cycloalkyl, cycloalkylalkyl, or arylcycloalkylalkyl; or wherein R^1 and R^2 and the N to which they can be bound form a ring;

R^3 , R^4 can be the same or different and are independently hydrogen, halo, alkyl, alkenyl, aryl, heteroalkyl, arylalkyl, hydroxyarylalkyl, heteroarylalkyl, cycloalkyl, thioalkyl, selenoalkyl, hydroxyalkyl, or amino-substituted alkyl; and

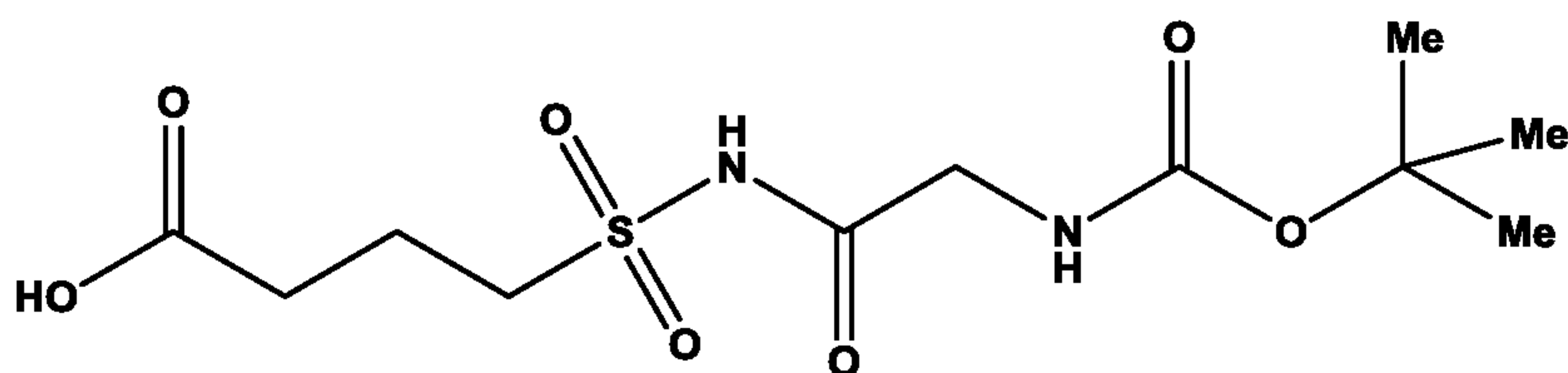
R⁵ can be alkyl, alkenyl, alkynyl or aryl, each optionally substituted with hydrogen, halo, alkyl, polyhaloalkyl, alkoxy, haloalkoxy, polyhaloalkoxy, alkoxycarbonyl, alkenyl, alkynyl, cycloalkyl, cycloalkylalkyl, hydroxyl, hydroxyalkyl, nitro, cyano, amino, substituted amino, alkylamino, dialkylamino, thiol, alkylthio, alkylcarbonyl, acyl, alkoxycarbonyl, aminocarbonyl, alkylsulfinyl, sulfonamide, or sulfonyl.

[0012] In some aspects are molecules or salts of structure II, wherein R¹ and R² can be the same or different and are independently hydrogen, acyl, acyloxy, phthalimidyl, benzyl, triphenylmethyl, benzylidenyl, or *p*-toluenesulfonyl. In some aspects are molecules or salts of structure II, wherein either of R³ and R⁴ can comprise a C₁-C₁₀ alkyl, cycloalkyl, aryl, or heteroaryl side chain. In some aspects are molecules or salts of structure II, wherein either of R³ and R⁴ can comprise a functional group that is at least partially protonated at a pH of about 7.3. In some aspects are molecules or salts of structure II, wherein either of R³ and R⁴ can comprise a functional group that is at least partially deprotonated at a pH of about 7.3. In some aspects, a side chain can comprise an amide, alcohol, or thiol. In some aspects are molecules or salts of structure II, wherein R³ and R⁴ can be hydrogen.

[0013] The compound of structure II includes the structures:



IIA

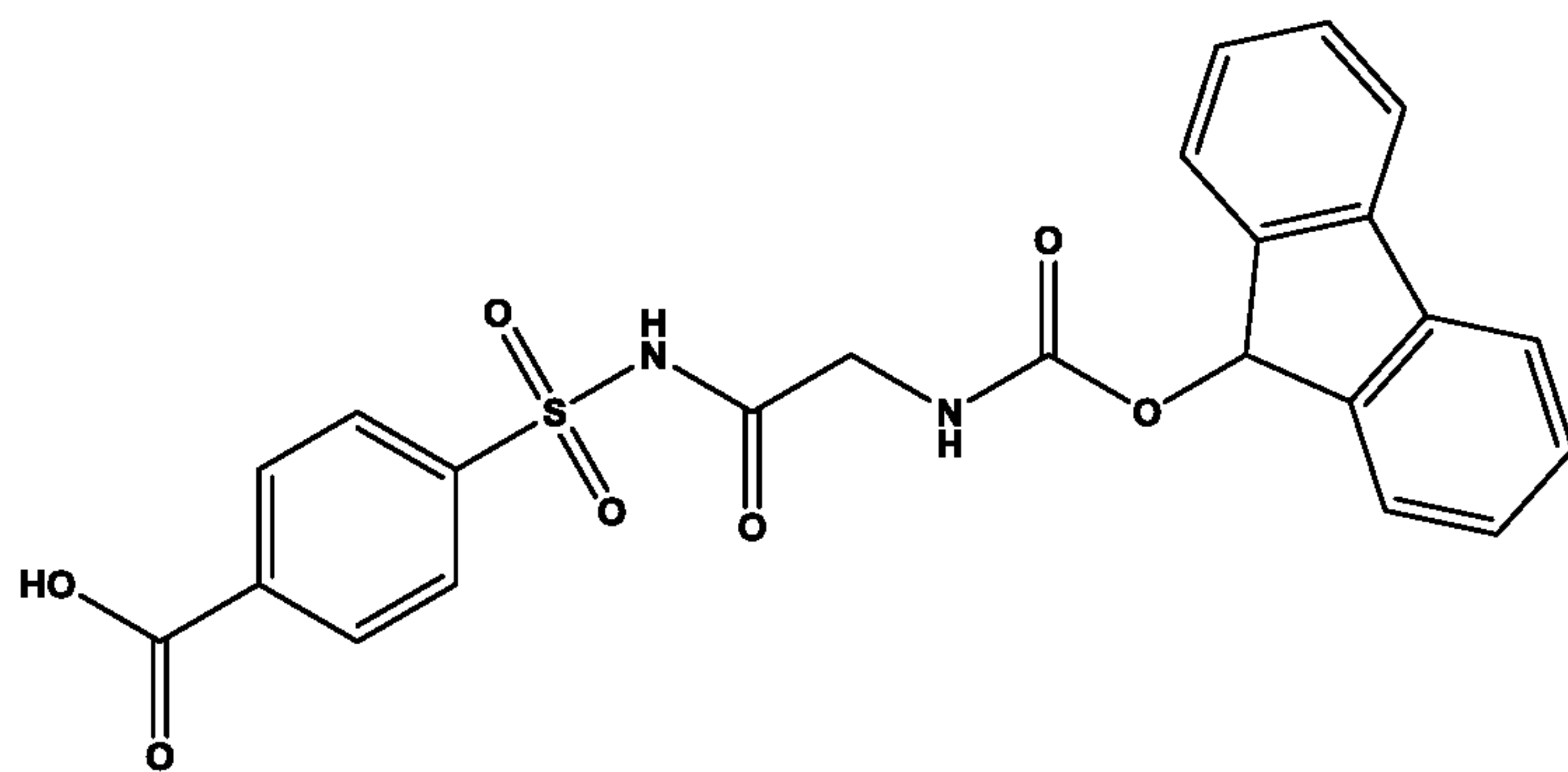


IIB

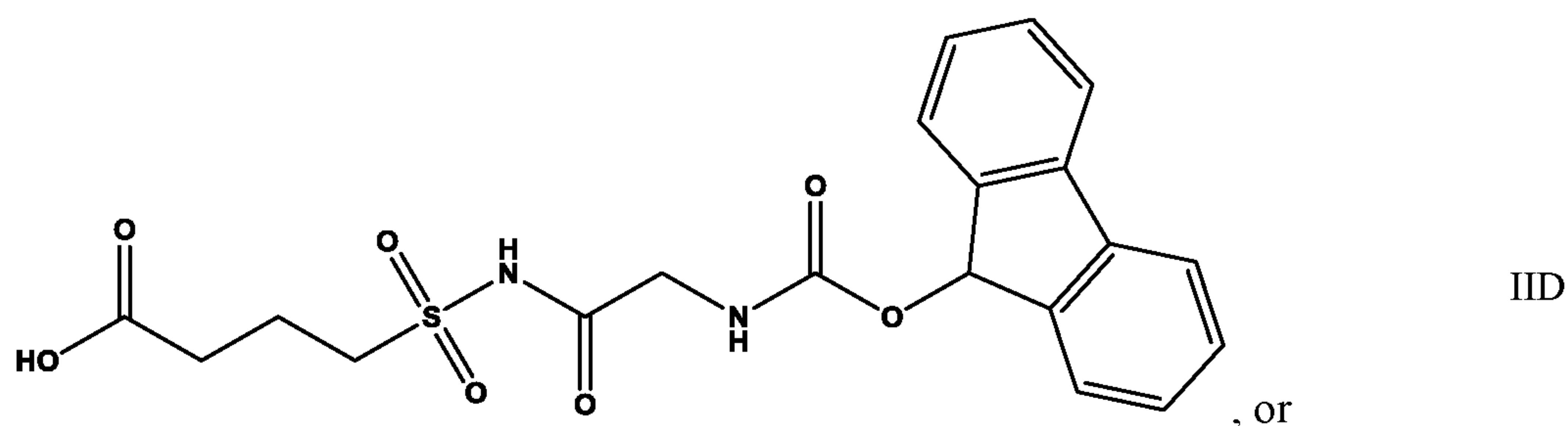
, or

salts thereof.

[0014] The compound of structure II includes the structures:



IIC



salts thereof.

[0015] Also disclosed herein is a method for synthesizing molecules. In some aspects, the method can comprise forming molecules with the compounds of structures IIA, IIB, IIC, or IID. In some aspects, the method can comprise coupling the compounds of structures IIA, IIB, IIC, or IID to an amino group on a solid phase. In some aspects, the method can further comprise deprotecting the compounds of structures IIA, IIB, IIC, or IID to form a second amino group. In some aspects, a solid phase can be a substrate, a bead, a polymer, or a chromatographic packing material. In some aspects, a deprotection can comprise a photoacid or a photoacid generator. In some aspects, a deprotection can comprise a photobase or a photobase generator. In some aspects, the method further can comprise an amine capping step. In some aspects, the amine capping step can be performed after the coupling step, and before the deprotection step. In some aspects, the amine capping step can comprise reacting the first amino group with a capping agent. In some aspects, the capping step can form an alkylamine, arylamine, acetamide, carbamate, phthalimide, enamine, sulfonamide, or *N*-protected amino acid. In some aspects, the protected amino acid can be an *N*-acyl-protected amino acid. In some aspects, the protected amino acid can be acetylglycine. In some aspects, the capping agent can be acetic anhydride, acetyl chloride, acetyl fluoride, or an acylglycine. Also disclosed herein are molecules made by the process of the any of the disclosed methods.

INCORPORATION BY REFERENCE

[0016] All publications, patents, and patent applications herein are incorporated by reference in their entireties. In the event of a conflict between a term herein and a term in an incorporated reference, the term herein controls.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] The novel features described herein are set forth with particularity in the appended claims. A better understanding of the features and advantages of the features described herein will be obtained by reference to the following detailed description that sets forth illustrative examples, in which the principles of the features described herein are utilized, and the accompanying drawings of which:

[0018] FIG. 1. depicts a molecule structure according to structure I. SCL-Gly is safety-catch-glycine. Gly is glycine. EDDBA is (ethylenedioxy)bis(ethylamine). GPTMS is 3-glycidioxypropyltrimethoxysilane.

[0019] FIG. 2. depicts schematic molecule structures of molecules according to structure I.

[0020] FIG. 3. depicts MALDI analysis and coupling yields for step-wise coupling of tetrapeptides to a PL8 Molecule: HT960.

[0021] FIG. 4. depicts MALDI analysis and coupling yields for step-wise coupling of tetrapeptides to a PL8 Molecule: HT963.

- [0022] FIG. 5 depicts a molecule. n can be 1-20.
- [0023] FIG. 6 depicts a molecule. n can be 1-20.
- [0024] FIG. 7 depicts a molecule. n can be 1-20.
- [0025] FIG. 8 depicts a molecule.
- [0026] FIG. 9 depicts the use of a molecular array for immunosignaturing.
- [0027] FIG. 10 depicts the arrangement of features on a molecular array.
- [0028] FIG. 11 depicts substrate characteristics, coating compositions, deposition methods, and analytical characterization methods
- [0029] FIG. 12 depicts a scheme for peptide synthesis.
- [0030] FIG. 13 depicts protected amino acids.
- [0031] FIG. 14 depicts aminosilane coating structures.
- [0032] FIG. 15 depicts why surface properties control important.
- [0033] FIG. 16 depicts thickness and water contact angle analysis of amino coatings.
- [0034] FIG. 17 depicts a scheme for a reactive amine density assay.
- [0035] FIG. 18 depicts an amine density analysis of amino coatings.
- [0036] FIG. 19 depicts an AFM analysis of the smoothness of deposition of amino coatings.
- [0037] FIG. 20 depicts an experimental process for side chain deprotection.
- [0038] FIG. 21 depicts an outline of a peptide synthesis process.
- [0039] FIG. 22 depicts a thickness analysis of peptide-functionalized amino coatings.
- [0040] FIG. 23 depicts thickness and XPS analysis of amino coatings.
- [0041] FIG. 24 depicts a scheme for peptide synthesis, amine capping, and MALDI-MS analysis.
- [0042] FIG. 25 depicts a scheme for peptide synthesis, amine capping, and MALDI-MS analysis.
- [0043] FIG. 26 depicts a thickness analysis of amino coatings.
- [0044] FIG. 27 depicts a thickness analysis of amino coatings.
- [0045] FIG. 28 depicts procedures for surface preparation for MALDI-MS analysis.
- [0046] FIG. 29 depicts a MALDI-MS analysis of array coatings.
- [0047] FIG. 30 depicts a purity analysis of peptides synthesized on amino coatings.
- [0048] FIG. 31 depicts a MALDI-MS analysis of array coatings.
- [0049] FIG. 32 depicts a purity analysis of peptides synthesized on amino coatings.
- [0050] FIG. 33 depicts a MALDI-MS analysis of array coatings.
- [0051] FIG. 34 depicts a purity analysis of peptides synthesized on amino coatings.
- [0052] FIG. 35 depicts a MALDI-MS analysis of array coatings.
- [0053] FIG. 36 depicts a purity analysis of peptides synthesized on amino coatings.
- [0054] FIG. 37 depicts a summary of amino coating properties.

DETAILED DESCRIPTION

[0055] Several aspects are described below with reference to example applications for illustration. It should be understood that numerous specific details, relationships, and methods are set forth to provide a full understanding of the features described herein. One having ordinary skill in the relevant art, however,

will readily recognize that the features described herein can be practiced without one or more of the specific details or with other methods. The features described herein are not limited by the illustrated ordering of acts or events, as some acts can occur in different orders and/or concurrently with other acts or events. Furthermore, not all illustrated acts or events are required to implement a methodology in accordance with the features described herein.

[0056] The terminology used herein is for the purpose of describing particular cases only and is not intended to be limiting. As used herein, the singular forms “a”, “an” and “the” are intended to include the plural forms as well, unless the context clearly indicates otherwise. Furthermore, to the extent that the terms “including”, “includes”, “having”, “has”, “with”, or variants thereof are used in either the detailed description and/or the claims, such terms are intended to be inclusive in a manner similar to the term “comprising”.

[0057] The term “about” or “approximately” can mean within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, i.e. the limitations of the measurement system. For example, “about” can mean within 1 or more than 1 standard deviation. Alternatively, “about” can mean a range of up to 20%, up to 10%, up to 5%, or up to 1% of a given value. Alternatively, particularly with respect to biological systems or processes, the term can mean within an order of magnitude, within 5-fold, and more preferably within 2-fold, of a value. Where particular values are described in the application and claims, unless otherwise stated the term “about” meaning within an acceptable error range for the particular value should be assumed. The term “about” has the meaning as commonly understood by one of ordinary skill in the art. In some embodiments, the term “about” refers to $\pm 10\%$. In some embodiments, the term “about” refers to $\pm 5\%$.

Overview

[0058] Detecting and diagnosing immune-mediated disorders, including autoimmune disorders, infections, and cancer, is challenging, with patients having a difficult time receiving an accurate or correct diagnosis. In many instances, patients are often misdiagnosed with other autoimmune conditions because of the closely related nature of these diseases. The disclosure, in one aspect, relates to compounds, methods, and devices that identify differential patterns of peripheral-blood antibody binding to a array-bound molecular library. Differential binding of patient samples to the array results in specific binding patterns or signatures indicative of the disease state of the patient. These binding signatures can accurately determine or diagnose a disease activity, including but not limited to autoimmune disease activity, infectious disease activity, cancer activity, and diabetes disease activity. The identification of such differential binding activity, or signature, is referred to as “immunosignaturing.” Synthesized peptide libraries have been commonly used for antibody binding characterization. However, protein and robotically printed peptide arrays have been cost-prohibitive and *in situ* synthesized peptide arrays have suffered from lack of scalability, poor reproducibility and low production quality. The technologies herein, in one aspect, will enable reliable, low cost, and scalable methods for construction and use of arrays for immunosignaturing assays.

[0059] In some embodiments, arrays with chemical libraries produced by the technologies disclosed herein are used for immune-based diagnostic assays, for example, immunosignature assays. In one aspect, using a patient's antibody repertoire from a drop of blood bound to the arrays, a fluorescence binding profile image of the bound array provide sufficient information to identify and classify a disease state. The arrays disclosed herein incorporate analytical measurements capability within each synthesized array using orthogonal analytical methods including ellipsometry, mass spectrometry, and fluorescence. These measurements enable longitudinal qualitative and quantitative assessment of array synthesis performance.

[0060] In some embodiments, detection of antibody binding on a peptide array poses some challenges that can be addressed by the technologies disclosed herein. The technologies disclosed herein address two potential shortcomings of using molecular arrays to profile antibody binding. First, non-specific antibody binding on a array is minimized by coating the solid support with a moderately hydrophilic monolayer comprising, in some embodiments, polyethylene glycol. In some embodiments, the hydrophilic monolayer is homogeneous. Second, synthesized library are linked to the surface using a linker that moves the peptide away from the surface of the solid support so that the peptide may be presented to an antibody in an unhindered orientation. The technologies disclosed herein include such linkers, which, in one aspect, connect chemical libraries to solid supports, including, in some aspects, arrays for immunosignaturing.

[0061] The synthesis of linkers by iterative coupling of linker subunits has limitations when certain functional groups are incorporated into the linker. As an example, sulfonamides exhibit poor reactivity in peptide coupling reactions. When linkers contain sulfonamide-terminated subunits, the reaction between the nitrogen of the sulfonamide and the carboxylic acid of the next subunit of the linker is slow, requiring elevated reaction times and temperatures to achieve even mediocre reaction of the sulfonamide. Residual, unreacted sulfonamides present the problem that, while less reactive than primary amines, they will still exhibit reactivity as peptide synthesis progresses. In subsequent coupling steps, undesired coupling of initially unreacted sulfonamides leads ultimately to truncation and deletion products in the final synthesized linker products. Furthermore, poor reactivity lowers the overall yield of final synthesized linker products. Furthermore, the poor reactivity of sulfonamides in coupling reactions is exacerbated in coupling performed on solid phase systems, because the chemical sensitivity of the solid phase prevents aggressive reaction conditions required to force coupling reactions to completion. In one aspect, disclosed herein, the problem of poor sulfonamide reactivity under standard peptide coupling conditions is overcome by the coupling of pre-assembled sulfonamide-amino building blocks. In one aspect, the pre-assembled sulfonamide-amino building blocks comprise a first moiety comprising a sulfonamide functional group at a first end, and a carboxylic acid functional group at a second end. In one aspect, the pre-assembled sulfonamide-amino building block comprises a second moiety comprising a C=O functional group at a first end, covalently bound at the carbon to the nitrogen of the sulfonamide functional group, and an amino group at a second end. In one aspect, the amino group is protected. In one

aspect the amino group is protected as a tert-butyl carbamate (Boc)-protected amine or as a 9-fluorenylmethyl carbamate (Fmoc)-protected amine.

[0062] Further disclosed herein are arrays comprising the molecules disclosed herein. In some aspects, the arrays comprise a nucleoside, nucleotide, polynucleotide, peptide, peptoid, saccharide, aptamer, lipid, lipo-peptide, or antibody or fragment thereof chemically bound to the linker. In one aspect, the nucleoside, nucleotide, polynucleotide, peptide, peptoid, saccharide, aptamer, or antibody or fragment thereof comprise a chemical library. In some embodiments, the array is a peptide array. In some aspects, the peptide array is synthesized *in situ*.

[0063] One of the major deficiencies of *in situ* synthesized peptide arrays has been the inability to directly measure purity of the synthesized peptide features. In some embodiments, the technologies include qualitative *in situ* mass spectrometry of synthesized peptides directly from solid support. Mass spectrometry is performed by incorporating a gas-phase cleavable linker between the solid support and the synthesized peptides so that cleavage of the peptide is done without diffusion from the array feature. Following peptide cleavage, Matrix-Assisted Laser Desorption Ionization (MALDI) mass spectrometry is performed directly on the solid support by applying a thin aerosol matrix layer and subsequently focusing the MALDI laser on individual peptide features to acquire a mass spectrum for each synthesized peptide. Qualitative *in situ* MALDI mass spectrum from a peptide array feature produced using the photolithographic synthesis approach are also included in the methods and devices described herein. Other analyses known to those of skill in the art may also be used to quantify and/or qualify the fidelity of the *in situ* synthesis process disclosed herein.

[0064] In one aspect, the use of a pre-assembled sulfonamide-amino building block reduces background autofluorescence of the synthesized linker associated with heating the surface of a solid phase in the presence of solvent, base, and peptide coupling reagents. In one aspect, the use of a pre-assembled sulfonamide-amino building block increases fidelity of the overall peptide coupling-based linker synthesis. In one aspect, the synthesized linkers further comprise a nucleoside, nucleotide, polynucleotide, peptide, peptoid, saccharide, aptamer, or antibody or fragment thereof. In one aspect, the incorporation of a sulfonamide into linkers allows for cleavage of the linker. In one aspect, cleavage of the linker further comprises analysis of the nucleoside, nucleotide, polynucleotide, peptide, peptoid, saccharide, aptamer, or antibody or fragment thereof. In some aspects, analysis of the peptide can comprise liquid chromatography or gas chromatography. In some aspects, the analysis comprises spectroscopic or spectrometric analysis. In some embodiments, spectrometric analysis is Matrix-assisted laser desorption/ionization spectrometry (MALDI). In one aspect, the incorporation of a sulfonamide linkers provides a site for cleavage that is chemically stable to the conditions for cleaving protected functional groups on the nucleoside, nucleotide, polynucleotide, peptide, peptoid, saccharide, aptamer, or antibody or fragment thereof. In one aspect, linkers are analyzed by ellipsometry.

Definitions

[0065] The terms “attach”, “bind”, “couple”, and “link” are used interchangeably and refer to covalent interactions (*e.g.*, by chemically coupling), or non-covalent interactions (*e.g.*, ionic interactions,

hydrophobic interactions, hydrogen bonds, hybridization, etc.). The terms “specific”, “specifically”, or “specificity” refer to the preferential recognition, contact, and formation of a stable complex between a first molecule and a second molecule compared to that of the first molecule with any one of a plurality of other molecules (*e.g.*, substantially less to no recognition, contact, or formation of a stable complex between the first molecule and any one of the plurality of other molecules). For example, two molecules may be specifically attached, specifically bound, specifically coupled, or specifically linked.

Furthermore, “binding” may refer to either a specific interaction, such as the interaction of an antibody with an epitope, or it may refer to a non-specific interaction.

Nomenclature

[0066] Unless otherwise indicated, the term “alkyl” as employed herein alone or as part of another group can include both straight and branched chain hydrocarbons, containing, for instance, 1 to 20 carbons, 1 to 10 carbons, or 1 to 8 carbons, in the normal chain, such as methyl, ethyl, propyl, isopropyl, butyl, t-butyl, isobutyl, pentyl, hexyl, isohexyl, heptyl, 4,4-dimethylpentyl, octyl, 2,2,4-trimethyl-pentyl, nonyl, decyl, undecyl, dodecyl, the various branched chain isomers thereof, and the like as well as such groups including 1 to 4 substituents such as halo, for example F, Br, Cl or I or CF₃, alkyl, alkoxy, aryl, aryloxy, aryl(aryl) or diaryl, arylalkyl, arylalkyloxy, alkenyl, cycloalkyl, cycloalkylalkyl, cycloalkylalkyloxy, amino, hydroxyl, hydroxyalkyl, acyl, heteroaryl, heteroaryloxy, heteroarylalkyl, heteroarylalkoxy, aryloxyalkyl, alkylthio, arylalkylthio, aryloxyaryl, alkylamido, alkanoylamino, arylcarbonylamino, nitro, cyano, thiol, haloalkyl, trihaloalkyl and/or alkylthio.

[0067] Unless otherwise indicated, the term “cycloalkyl” as employed herein alone or as part of another group can include saturated or partially unsaturated (containing 1 or 2 double bonds) cyclic hydrocarbon groups containing 1 to 3 rings, including monocyclic alkyl, bicyclic alkyl (or bicycloalkyl) and tricyclic alkyl (tricycloalkyl), containing a total of 3 to 20 carbons forming the ring and which may be fused to 1 or 2 aromatic rings as described for aryl, which includes cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclodecyl and cyclododecyl, cyclohexenyl, adamantyl, and the like, any of which may be optionally substituted with 1 to 4 substituents such as halogen, alkyl, alkoxy, hydroxyl, aryl, aryloxy, arylalkyl, cycloalkyl, hydroxyalkyl, alkylamido, alkanoylamino, oxo, acyl, arylcarbonylamino, amino, nitro, cyano, thiol and/or alkylthio and/or any of the substituents for alkyl.

[0068] The term “alkanoyl” as used herein alone or as part of another group can refer to alkyl linked to a carbonyl group.

[0069] Unless otherwise indicated, the term “alkenyl” as used herein by itself or as part of another group can refer to straight or branched chain radicals of, for instance, 2 to 20 carbons in the normal chain, which include one to six double bonds in the normal chain, such as vinyl, 2-propenyl, 3-butenyl, 4-pentenyl, 3-pentenyl, 2-hexenyl, 3-hexenyl, 2-heptenyl, 3-heptenyl, 4-heptenyl, 3-octenyl, 3-nonenyl, 4-decenyl, 3-undecenyl, 4-dodecenyl, 4, 8, 12-tetradecatrienyl, and the like, and which may be optionally substituted with 1 to 4 substituents, namely, halogen, haloalkyl, alkyl, alkoxy, alkenyl, alkynyl, aryl, arylalkyl, cycloalkyl, amino, hydroxyl, heteroaryl, cycloheteroalkyl, alkanoylamino, alkylamido, arylcarbonyl-amino, nitro, cyano, thiol, alkylthio and/or any of the alkyl substituents set out herein.

[0070] Unless otherwise indicated, the term “alkynyl” as used herein by itself or as part of another group can refer to straight or branched chain radicals of 2 to 20 carbons in the normal chain, which include one triple bond in the normal chain, such as 2-propynyl, 3-butynyl, 2-butynyl, 4-pentynyl, 2-hexynyl, 3-hexynyl, 2-heptynyl, 3-heptynyl, 4-heptynyl, 3-octenyl, 3-nonenyl, 4-decenyl, 3-undecenyl, 4-dodecenyl and the like, and which may be optionally substituted with 1 to 4 substituents, namely, halogen, haloalkyl, alkyl, alkoxy, alkenyl, alkynyl, aryl, arylalkyl, cycloalkyl, amino, heteroaryl, cycloheteroalkyl, hydroxyl, alkanoylamino, alkylamido, arylcarbonylamino, nitro, cyano, thiol, and/or any of the alkyl substituents set out herein.

[0071] The term “halogen” or “halo” as used herein alone or as part of another group can refer to chlorine, bromine, fluorine, and iodine, as well as CF₃.

[0072] Unless otherwise indicated, the term “aryl” as employed herein alone or as part of another group can refer to monocyclic and bicyclic aromatic groups containing 6 to 10 carbons in the ring portion (such as phenyl or naphthyl including 1-naphthyl and 2-naphthyl) and may optionally include one to three additional rings fused to a carbocyclic ring or a heterocyclic ring (such as aryl, cycloalkyl, heteroaryl or cycloheteroalkyl rings) and may be optionally substituted through available carbon atoms with 1, 2, or 3 groups selected from hydrogen, halo, haloalkyl, alkyl, alkoxy, haloalkoxy, alkenyl, trifluoromethyl, trifluoromethoxy, alkynyl, cycloalkylalkyl, cycloheteroalkyl, cycloheteroalkylalkyl, aryl, heteroaryl, arylalkyl, aryloxy, aryloxyalkyl, arylalkoxy, arylthio, arylazo, heteroarylalkyl, heteroarylalkenyl, heteroarylheteroaryl, heteroaryloxy, hydroxyl, nitro, cyano, amino, substituted amino wherein the amino can include 1 or 2 substituents (which are alkyl, aryl or any of the other aryl compounds mentioned in the definitions), thiol, alkylthio, arylthio, heteroarylthio, arylthioalkyl, alkoxyarylthio, alkylcarbonyl, arylcarbonyl, alkylaminocarbonyl, arylaminocarbonyl, alkoxycarbonyl, aminocarbonyl, alkylcarbonyloxy, arylcarbonyloxy, alkylcarbonyloxy, arylcarbonylamino, arylsulfinyl, arylsulfinylalkyl, arylsulfonylamino or arylsulfonaminocarbonyl and/or any of the alkyl substituents set out herein.

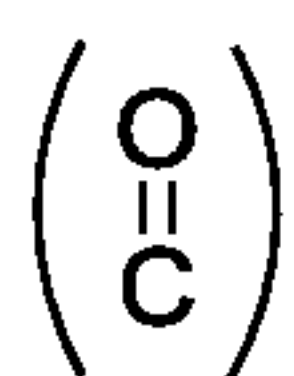
[0073] Unless otherwise indicated, the term “amino-substituted” as employed herein alone or as part of another group can refer to a chemical group having from 1 to 10 amino groups substituted thereon.

[0074] Unless otherwise indicated, the term “alkylthio” (also known as “thioalkyl”) or “arylthio” (also known as “thioaryl”) as employed herein alone or as part of another group can include any of the above alkyl or aryl groups linked to a sulfur atom.

[0075] Unless otherwise indicated, the term “selenoalkyl” as employed herein alone or as part of another group can include any of the above alkyl groups linked to a selenium atom.

[0076] Unless otherwise indicated, the term “alkylamino” or “arylamino” as employed herein alone or as part of another group can include any of the above alkyl or aryl groups linked to a nitrogen atom.

[0077] Unless otherwise indicated, the term “acyl” as employed herein by itself as part of another group, as defined herein, can refer to an organic radical linked to a carbonyl



group; examples of acyl groups include any of the R groups attached to a carbonyl, such as alkanoyl, alkenoyl, aroyl, aralkanoyl, heteroaroyl, cycloalkanoyl, cycloheteroalkanoyl and the like.

[0078] Unless otherwise indicated, the term “cycloheteroalkyl” as used herein alone or as part of another group can refer to a 5-, 6-, or 7-membered saturated or partially unsaturated ring which can include 1 to 2 heteroatoms such as nitrogen, oxygen and/or sulfur, linked through a carbon atom or a heteroatom, where possible, optionally via the linker (CH₂)_r (where r is 1, 2, or 3).

[0079] Unless otherwise indicated, the term “heteroaryl” as used herein alone or as part of another group can refer to a 5- or 6-membered aromatic ring which can include 1, 2, 3 or 4 heteroatoms such as nitrogen, oxygen or sulfur, and such rings fused to an aryl, cycloalkyl, heteroaryl or cycloheteroalkyl ring, and includes possible N-oxides. The heteroaryl group may optionally include 1 to 4 substituents such as any of the substituents set out above for alkyl.

[0080] Unless otherwise indicated, the term “heteroalkyl” as used herein alone or as part of another group can refer to an alkyl group, as defined herein, which can include 1, 2, 3, or 4 heteroatoms such as nitrogen, oxygen or sulfur. The heteroalkyl group may optionally include 1 to 4 substituents such as any of the substituents set out above for alkyl.

[0081] All stereoisomers of compounds are contemplated, either in admixture or in pure or substantially pure form. Compounds can have asymmetric carbon centers at any of the carbon atoms including any one of the R substituents. Compounds can be either optically active or optically inactive. Asymmetric carbon centers can be independently in an *R*- or *S*-configuration. As defined herein asymmetric carbons are carbons that are a stereogenic center. Consequently, compounds of structures I, IA, or II can exist in enantiomeric or diastereomeric forms or in mixtures thereof. Enantiomeric mixtures can exist with an enantiomeric excess of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5% or 100%. Diastereomeric mixtures can exist with a diastereomeric ratio of 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 20:1, 50:1, 100:1, or 500:1. The processes for preparation of the molecules disclosed herein can utilize racemates, enantiomers or diastereomers as starting materials. When diastereomeric or enantiomeric products are prepared, they can be separated by conventional methods, for example, by chromatographic or fractional crystallization.

[0082] A polynucleotide, as used herein, can be any type of nucleic acid molecule, including DNA, RNA, a hybridization thereof, or any combination thereof. For example, a polynucleotide can be cDNA, genomic DNA, mRNA, tRNA, rRNA, or microRNA.

[0083] A peptide, polypeptide, or protein can be contemplated to include any fragments thereof, in particular, immunologically detectable fragments. A peptide can be contemplated to include an α -peptide, a β -peptide, or a γ -peptide.

Supports/Substrates/Solid Phases

[0084] The present disclosure provides solid supports (also known as solid phases, substrates, or supports). The nature and geometry of a support or substrate can depend upon a variety of factors, including the type of array (e.g., one-dimensional, two-dimensional or three-dimensional). Generally, a substrate can be composed of any material which will permit coupling of a nucleoside, nucleotide,

polynucleotide, peptide, peptoid, saccharide, aptamer, or antibody or fragment thereof, which will not melt or degrade under the conditions used to couple said nucleoside, nucleotide, polynucleotide, peptide, peptoid, saccharide, aptamer, or antibody or fragment thereof to said solid support. A solid support can be composed of any material which will permit coupling of a target analyte, and/or other moiety at one or more discrete regions and/or discrete locations within the discrete regions. A solid support can be composed of any material which will permit washing or physical or chemical manipulation without dislodging a target analyte or binding moiety from the solid support.

[0085] A substrate may take a variety of configurations ranging from simple to complex, depending on the intended use of the array. Thus, a substrate can have an overall slide or plate configuration, such as a rectangular or disc configuration. A standard microplate configuration can be used. In some embodiments, the surface may be smooth or substantially planar, or have irregularities, such as depressions or elevations. For example, the substrates of the presently disclosed subject matter can include at least one surface on which a pattern of recombinant virion microspots can be coupled or deposited. In some instances, a substrate may have a rectangular cross-sectional shape, having a length of from about 10-200 mm, 40-150 mm, or 75-125 mm; a width of from about 10-200 mm, 20-120 mm, or 25-80 mm, and a thickness of from about 0.01-5.0 mm, 0.1-2 mm, or 0.2 to 1 mm.

[0086] A support may be organic or inorganic; may be metal (*e.g.*, copper or silver) or non-metal; may be a polymer or nonpolymer; may be conducting, semiconducting or nonconducting (insulating); may be reflecting or nonreflecting; may be porous or nonporous; etc. A solid support as described above can be formed of any suitable material, including metals, metal oxides, semiconductors, polymers (particularly organic polymers in any suitable form including woven, nonwoven, molded, extruded, cast, etc.), silicon, silicon oxide, and composites thereof.

[0087] Suitable materials for use as substrates include, but are not limited to, polycarbonate, gold, silicon, silicon oxide, silicon oxynitride, indium, tantalum oxide, niobium oxide, titanium, titanium oxide, platinum, iridium, indium tin oxide, diamond or diamond-like film, acrylic, styrene-methyl methacrylate copolymers, ethylene/acrylic acid, acrylonitrile-butadiene-styrene (ABS), ABS/polycarbonate, ABS/polysulfone, ABS/polyvinyl chloride, ethylene propylene, ethylene vinyl acetate (EVA), nitrocellulose, nylons (including nylon 6, nylon 6/6, nylon 6/6-6, nylon 6/9, nylon 6/10, nylon 6/12, nylon 11 and nylon 12), polyacrylonitrile (PAN), polyacrylate, polycarbonate, polybutylene terephthalate (PBT), poly(ethylene) (PE) (including low density, linear low density, high density, cross-linked and ultra-high molecular weight grades), poly(propylene) (PP), cis and trans isomers of poly(butadiene) (PB), cis and trans isomers of poly(isoprene), polyethylene terephthalate (PET), polypropylene homopolymer, polypropylene copolymers, polystyrene (PS) (including general purpose and high impact grades), polycarbonate (PC), poly(epsilon-caprolactone) (PECL or PCL), poly(methyl methacrylate) (PMMA) and its homologs, poly(methyl acrylate) and its homologs, poly(lactic acid) (PLA), poly(glycolic acid), polyorthoesters, poly(anhydrides), nylon, polyimides, polydimethylsiloxane (PDMS), polybutadiene (PB), polyvinylalcohol (PVA), polyacrylamide and its homologs such as poly(N-isopropyl acrylamide), fluorinated polyacrylate (PFOA), poly(ethylene-butylene) (PEB), poly(styrene-acrylonitrile) (SAN),

polytetrafluoroethylene (PTFE) and its derivatives, polyolefin plastomers, fluorinated ethylene-propylene (FEP), ethylene-tetrafluoroethylene (ETFE), perfluoroalkoxyethylene (PFA), polyvinyl fluoride (PVF), polyvinylidene fluoride (PVDF), polychlorotrifluoroethylene (PCTFE), polyethylene-chlorotrifluoroethylene (ECTFE), styrene maleic anhydride (SMA), metal oxides, glass, silicon oxide or other inorganic or semiconductor material (*e.g.*, silicon nitride), compound semiconductors (*e.g.*, gallium arsenide, and indium gallium arsenide), and combinations thereof.

[0088] Examples of well-known solid supports include polypropylene, polystyrene, polyethylene, dextran, nylon, amylases, glass, natural and modified celluloses (*e.g.*, nitrocellulose), polyacrylamides, agaroses and magnetite. In some instances, the solid support can be silica or glass because of its great chemical resistance against solvents, its mechanical stability, its low intrinsic fluorescence properties, and its flexibility of being readily functionalized. In one embodiment, the substrate can be glass, particularly glass coated with nitrocellulose, more particularly a nitrocellulose-coated slide (*e.g.*, FAST slides).

[0089] In some embodiments, the support can be planar. In some instances, the support can be spherical. In some instances, the support can be a bead. In some instances, a support can be magnetic. In some instances, a magnetic solid support can comprise magnetite, maghemite, FePt, SrFe, iron, cobalt, nickel, chromium dioxide, ferrites, or mixtures thereof. In some instances, a support can be nonmagnetic. In some embodiments, the nonmagnetic solid support can comprise a polymer, metal, glass, alloy, mineral, or mixture thereof. In some instances a nonmagnetic material can be a coating around a magnetic solid support. In some instances, a magnetic material may be distributed in the continuous phase of a magnetic material. In some embodiments, the solid support comprises magnetic and nonmagnetic materials. In some instances, a solid support can comprise a combination of a magnetic material and a nonmagnetic material. In some embodiments, the magnetic material is at least about 5, 10, 20, 30, 40, 50, 60, 70, or about 80 % by weight of the total composition of the solid support. In some embodiments, the bead size can be quite large, on the order of 100-900 microns or in some cases even up to a diameter of 3 mm. In other embodiments, the bead size can be on the order of 1-150 microns. The average particle diameters of beads can be in the range of about 2 μm to several millimeters, *e.g.*, diameters in ranges having lower limits of 2 μm , 4 μm , 6 μm , 8 μm , 10 μm , 20 μm , 30 μm , 40 μm , 50 μm , 60 μm , 70 μm , 80 μm , 90 μm , 100 μm , 150 μm , 200 μm , 300 μm , or 500 μm , and upper limits of 20 μm , 30 μm , 40 μm , 50 μm , 60 μm , 70 μm , 80 μm , 90 μm , 100 μm , 150 μm , 200 μm , 300 μm , 500 μm , 750 μm , 1 mm, 2 mm, or 3 mm.

[0090] In some embodiments, the support can comprise an array. In some embodiments, the array comprises a target analyte. In some embodiments, the target analyte comprises a nucleoside, a nucleotide, a polynucleotide, a peptide, a peptoid, a saccharide, a polysaccharide, an aptamer, or an antibody or fragment thereof. In some embodiments, the target analyte comprises a library of target analytes.

[0091] In some embodiments, an array comprises a library of molecules. In some embodiments, the array can comprise at least about 100, 1000, 10,000, 100,000, 10^6 , 10^7 , 10^8 , 10^9 , 10^{10} , 10^{11} , 10^{12} , 10^{13} , or

10^{14} , 10^{15} molecules per 1 cm^2 . In some embodiments, a molecule can comprise a sequence of monomers. In some embodiments, the sequence of monomers can comprise a sequence of amino acids. In some embodiments, a feature can be a region on a substrate from about 0.5 microns to about 200 microns in diameter. In some embodiments, the array can have a plurality of features. In some embodiments, the center-to-center distance between features can be from about 1 micron to about 300 microns. In some embodiments, the array can comprise at least about 1,000, 10,000, 100,000, 200,000, 300,000, 400,000, 500,000, 600,000, 700,000, 800,000, 900,000, 1 million, 1.5 million, 2 million, 2.5 million, 3 million, 3.5 million, or 4 million features per 1 cm^2 . In some embodiments, at least about 40% of the molecules in the library are distinct. In some embodiments, at least about 50% of the molecules in the library are distinct. In some embodiments, at least about 60% of the molecules in the library are distinct. In some embodiments, at least about 70% of the molecules in the library are distinct. In some embodiments, at least about 80% of the molecules in the library are distinct. In some embodiments, at least about 90% of the molecules in the library are distinct. In some embodiments, at least 50% of the molecules in the library are at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, 40, 50, 60, 70, 80, 90, or 100 monomers in length. In some embodiments, at least 50% of the molecules in the library are at most 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, 40, 50, 60, 70, 80, 90, or 100 monomers in length. In some embodiments, the library comprises a median monomer length of about 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, 40, 50, 60, 70, 80, 90, or 100 monomers. In some embodiments, the array can comprise at least 10,000, 50,000, 100,000, 200,000, 300,000, 400,000, 500,000, 600,000, 700,000, 800,000, 900,000, 10^6 , or 10^7 sequentially distinct library molecules. In some embodiments, the array substrate can be selected from wafers, slides, and beads. In some embodiments, the library can be an *in-situ* synthesized chemical library. In some embodiments, the molecules can be polynucleotides, peptides, peptoids, or polysaccharides.

Binding Moiety

[0092] An analyte binding moiety, also referred to as a binding moiety (or domain) can be the region, molecule, domain, portion, fragment, or moiety that binds to a target analyte. Thus, a binding moiety confers the ability to bind or specifically bind to given target. A binding moiety can be a nucleic acid molecule or can be proteinaceous. Binding moieties include, but are not limited to, RNAs DNAs, RNA-DNA hybrids, small molecules (e.g., drugs or metabolites), aptamers, polypeptides, proteins, antibodies, viruses, virus particles, cells, fragments thereof, and combinations thereof.

[0093] In some embodiments, a binding moiety can be a polypeptide, a protein, or any fragment thereof. In some embodiments, a polypeptide or protein can be an engineered or recombinant polypeptide or protein. In some embodiments, a binding moiety is an antibody or fragment thereof. An antibody can be of any isotype (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (IgG₁, IgG₂, IgG₃, IgG₄, IgA₁, IgA₂), subclass or modified version thereof. Antibodies may include complete immunoglobulins or fragments thereof. An antibody fragment can refer to one or more fragments of an antibody that retains the ability to specifically bind to a target molecule, such as an antigen.

[0094] In some embodiments, a binding moiety can be an aptamer. An aptamer is an isolated nucleic acid molecule that binds with high specificity and affinity to a target analyte, such as a protein. An aptamer comprises a three dimensional structure held in certain conformation(s) that provide chemical contacts to specifically bind a given target. In some embodiments, a binding moiety is small molecule. For example, a small molecule can be a macrocyclic molecule, an inhibitor, a drug, or chemical compound. In some embodiments, a binding moiety is a cell. For example, a binding moiety can be an intact cell, a cell treated with a compound (e.g. a drug), a fixed cell, a lysed cell, or any combination thereof.

Detection Methods

[0095] Detection methods for detecting bound binding moieties can include photometric and non-photometric means. In some embodiments, such methods process includes a method to detect and measure absorbance, fluorescence, refractive index, polarization or light scattering. These include direct and/or indirect means to measure such parameters. Methods involving fluorescence include fluorescent tagging in immunological methods such as ELISA or sandwich assay. Methods involving refractive index include surface Plasmon resonance (SPR), grating coupled methods (e.g. sensors uniform grating couplers, wavelength-interrogated optical sensors (WIOS) and chirped grating couplers), resonant mirror and interferometric techniques. Methods involving polarization include ellipsometry. Light scattering methods may also be used. Other means for tagging and/or separating and/or detecting can also include magnetic means. Magnetic resonance imaging (MRI), or gas phase ion spectrometry, may all be used.

[0096] Non-photometric methods of detection include, without limitation, magnetic resonance imaging, gas phase ion spectrometry, atomic force microscopy and multipolar coupled resonance spectroscopy. Magnetic resonance imaging (MRI) is based on the principles of nuclear magnetic resonance (NMR), a spectroscopic technique used by scientists to obtain microscopic chemical and physical information about molecules. Gas phase ion spectrometers include mass spectrometers, ion mobility spectrometers and total ion current measuring devices.

[0097] Binding assays can also be useful, e.g., for identifying disease related antibodies (binding moieties) that interact with the target analytes described herein. For example, antibodies or other molecules that bind target analytes can be identified in binding assays. Binding assays can involve, but are not limited to, use of isolated polypeptides, crude extracts, or cell-based assays. In some embodiments the assays described herein can be used to a) identify subjects who have a first disease or a second disease; (b) assess the impact of a disease therapy; and (c) monitor disease progression.

[0098] Binding assays can involve contacting a target analyte with a sample comprising a binding moiety (antibody) and allowing sufficient time for the molecule and test agents to form a binding complex. Any binding complexes formed can be detected using any of a number of established analytical techniques. Binding assays include, but are not limited to, methods that measure co-precipitation or co-migration on non-denaturing SDS- polyacrylamide gels, co-migration on Western blots, enzyme linked immunosorbent assay (ELISA), radioimmunoassay (RIA), immunoradiometric assay,

fluoroimmunoassay, chemiluminescent assay, bioluminescent assay, fluorescence activated cell sorting (FACS), or fluorescence resonance energy transfer (FRET).

Diagnostics

[0099] The methods and apparatus disclosed herein can be used to screen for various diseases or conditions, including an alteration in the state of the body or of some of the organs, interrupting or disturbing the performance of the functions and/or causing symptoms such as discomfort, dysfunction, distress, or even death to the person afflicted or those in contact with the person. A disease or condition can also include a distemper, ailment, malady, disorder, sickness, illness, complain, interdisposition and/or affectation.

[00100] For example, samples containing binding moieties from a diseased animal can be simultaneously screened for the binding moieties' ability to interact with an array. These interactions can be compared to those of samples from individuals that are not in a disease state, not presenting symptoms of persons in the disease state, or presenting symptoms of the disease state. For example, the levels of binding moieties in samples from a diseased animal can be simultaneously determined. These levels can be compared to those of samples from individuals that are not in a disease state, not presenting symptoms of persons in the disease state, or presenting symptoms of the disease state.

[00101] The methods, kits, and compositions described herein can be used in medical diagnostics, drug discovery, molecular biology, immunology and toxicology. Arrays can be used for large scale binding assays in numerous diagnostic and screening applications. The multiplexed measurement of quantitative variation in levels of large numbers of target analytes (*e.g.* proteins) allows the recognition of patterns defined by several to many different target analytes. The multiplexed identification of large numbers of interactions between target analytes and binding moieties allows for the recognition of binding and interaction patterns defined by several to many different interactions between target analytes and binding moieties. Many physiological parameters and disease-specific patterns can be simultaneously assessed. One embodiment involves the separation, identification and characterization of proteins present in a biological sample. For example, by comparison of disease and control samples, it is possible to identify disease specific target analytes. These target analytes can be used as targets for drug development or as molecular markers of disease.

[00102] In some embodiments, methods can be methods for diagnosing or detecting a disease or condition such as a cancer, inflammatory disease, immune disease, autoimmune disease, cardiovascular disease, neurological disease, infectious disease, metabolic disease, or a perinatal condition. For example, the disease or condition can be a tumor, neoplasm, or cancer. The cancer can be, but is not limited to, breast cancer, ovarian cancer, lung cancer, colon cancer, hyperplastic polyp, adenoma, colorectal cancer, high grade dysplasia, low grade dysplasia, prostatic hyperplasia, prostate cancer, melanoma, pancreatic cancer, brain cancer (such as a glioblastoma), hematological malignancy, hepatocellular carcinoma, cervical cancer, endometrial cancer, head and neck cancer, esophageal cancer, gastrointestinal stromal tumor (GIST), renal cell carcinoma (RCC) or gastric cancer. The colorectal cancer can be CRC Dukes B or Dukes C-D. The hematological malignancy can be B-Cell Chronic Lymphocytic Leukemia, B-Cell

Lymphoma-DLBCL, B-Cell Lymphoma-DLBCL-germinal center-like, B-Cell Lymphoma-DLBCL-activated B-cell-like, or Burkitt's lymphoma. The disease or condition can also be a premalignant condition, such as Barrett's Esophagus. The disease or condition can also be an inflammatory disease, immune disease, or autoimmune disease. For example, the disease may be inflammatory bowel disease (IBD), Crohn's disease (CD), ulcerative colitis (UC), pelvic inflammation, vasculitis, psoriasis, diabetes, autoimmune hepatitis, Multiple Sclerosis, Myasthenia Gravis, Type I diabetes, Rheumatoid Arthritis, Psoriasis, Systemic Lupus Erythematosus (SLE), Hashimoto's Thyroiditis, Grave's disease, Ankylosing Spondylitis Sjogrens Disease, CREST syndrome, Scleroderma, Rheumatic Disease, organ rejection, Primary Sclerosing Cholangitis, or sepsis. The disease or condition can also be a cardiovascular disease, such as atherosclerosis, congestive heart failure, vulnerable plaque, stroke, or ischemia. The cardiovascular disease or condition can be high blood pressure, stenosis, vessel occlusion or a thrombotic event. The disease or condition can also be a neurological disease, such as Multiple Sclerosis (MS), Parkinson's Disease (PD), Alzheimer's Disease (AD), schizophrenia, bipolar disorder, depression, autism, Prion Disease, Pick's disease, dementia, Huntington disease (HD), Down's syndrome, cerebrovascular disease, Rasmussen's encephalitis, viral meningitis, neuropsychiatric systemic lupus erythematosus (NPSLE), amyotrophic lateral sclerosis, Creutzfeldt-Jacob disease, Gerstmann-Straussler-Scheinker disease, transmissible spongiform encephalopathy, ischemic reperfusion damage (*e.g.* stroke), brain trauma, microbial infection, or chronic fatigue syndrome. The condition may also be fibromyalgia, chronic neuropathic pain, or peripheral neuropathic pain. The disease or condition may also be an infectious disease, such as a bacterial, viral or yeast infection. For example, the disease or condition may be Whipple's Disease, Prion Disease, cirrhosis, methicillin-resistant staphylococcus aureus, HIV, hepatitis, syphilis, meningitis, malaria, tuberculosis, or influenza. The disease or condition can also be a perinatal or pregnancy related condition (*e.g.* preeclampsia or preterm birth), zika virus, dengue fever, flavivirus, or a metabolic disease or condition, such as a metabolic disease or condition associated with iron metabolism.

[00103] In some embodiments, methods are methods for diagnosing or detecting an autoimmune disorder. In some embodiments, methods can be methods for determining a disease or condition or the progression of a disease or condition. Non-limiting examples of disorder associated with the immune system can include: autoimmune disorders, inflammatory diseases, HIV, rheumatoid arthritis, diabetes mellitus type 1, systemic lupus erythematosus, scleroderma, multiple sclerosis, severe combined immunodeficiency (SCID), DiGeorge syndrome, ataxia-telangiectasia, seasonal allergies, perennial allergies, food allergies, anaphylaxis, mastocytosis, allergic rhinitis, atopic dermatitis, Parkinson's, Alzheimer's, hypersplenism, leukocyte adhesion deficiency, X-linked lymphoproliferative disease, X-linked agammaglobulinemia, selective immunoglobulin A deficiency, hyper IgM syndrome, autoimmune lymphoproliferative syndrome, Wiskott-Aldrich syndrome, chronic granulomatous disease, common variable immunodeficiency (CVID), hyperimmunoglobulin E syndrome, Hashimoto's thyroiditis.

Kits

[00104] Also provided are kits that find use in practicing the subject methods, as mentioned above. A kit can include one or more of the compositions described herein. A kit can include at least one nucleoside, nucleotide, polynucleotide, peptide, peptoid, saccharide, aptamer, or antibody or fragment thereof. A kit can include at least one binding moiety.

[00105] A kit can include a solid support. In some embodiments, the solid support is already functionalized with at least one molecule of structure I. In some embodiments, the solid support is already functionalized with at least one nucleoside, nucleotide, polynucleotide, peptide, peptoid, saccharide, aptamer, or antibody or fragment thereof. A kit can include a reagent for coupling at least one nucleoside, nucleotide, polynucleotide, peptide, peptoid, saccharide, aptamer, or antibody or fragment thereof to the solid support.

[00106] The kit components may be present in separate containers, or one or more of the components may be present in the same container, where the containers may be storage containers and/or containers that are employed during the assay for which the kit is designed.

[00107] In addition to the above components, the subject kits may further include instructions for practicing the subject methods. These instructions may be present in the subject kits in a variety of forms, such as printed information on a suitable medium or substrate (e.g., a piece or pieces of paper on which the information is printed), in the packaging of the kit, in a package insert, etc. Yet another means would be a computer readable medium (e.g., diskette, CD, etc.), on which the information has been recorded. Yet another means that may be present is a website address which may be used via the internet to access the information at a removed site.

Communicating a Result

[00108] Additional embodiments relate to the communication of assay results or diagnoses or both to technicians, physicians or subjects, for example. In certain embodiments, computers will be used to communicate results of the assessing or diagnoses or both to interested parties, e.g., physicians and their subjects. In some embodiments, the assessing can be performed or results analyzed in a country or jurisdiction which differs from the country or jurisdiction to which the results or diagnoses are communicated. In some embodiments, a diagnosis based on the presence or absence in a test subject of a binding moiety or a binding signature, or signal identified may be communicated to the subject as soon as possible after the diagnosis is obtained. The diagnosis may be communicated to the subject by the subject's treating physician. Alternatively, the diagnosis may be sent to a test subject by email or communicated to the subject by phone. A computer may be used to communicate the diagnosis by email or phone. In certain embodiments, the message containing results of a diagnostic test may be generated and delivered automatically to the subject using a combination of computer hardware and software which will be familiar to artisans skilled in telecommunications. One example of a healthcare-oriented communications system is described in U.S. Patent No. 6,283,761; however, the present disclosure is not limited to methods which utilize this particular communication system. In certain embodiments, all or

some of the method steps, including the assaying of samples, diagnosing of diseases, and communicating of method results or diagnoses, may be carried out in diverse (e.g., foreign) jurisdictions.

Other Embodiments

[00109] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

[00110] It is to be understood that the methods and compositions described herein are not limited to the particular methodology, protocols, constructs, and reagents described herein and as such may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the methods and compositions described herein, which will be limited only by the appended claims. While preferred embodiments of the present disclosure have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the disclosure. It should be understood that various alternatives to the embodiments of the disclosure described herein may be employed in practicing the disclosure. It is intended that the following claims define the scope of the disclosure and that methods and structures within the scope of these claims and their equivalents be covered thereby.

[00111] Several aspects are described with reference to example applications for illustration. Unless otherwise indicated, any embodiment can be combined with any other embodiment. It should be understood that numerous specific details, relationships, and methods are set forth to provide a full understanding of the features described herein. A skilled artisan, however, will readily recognize that the features described herein can be practiced without one or more of the specific details or with other methods. The features described herein are not limited by the illustrated ordering of acts or events, as some acts can occur in different orders and/or concurrently with other acts or events. Furthermore, not all illustrated acts or events are required to implement a methodology in accordance with the features described herein.

[00112] Some inventive embodiments herein contemplate numerical ranges. When ranges are present, the ranges include the range endpoints. Additionally, every sub range and value within the range is present as if explicitly written out. The term “about” or “approximately” can mean within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, e.g., the limitations of the measurement system. For example, “about” can mean within 1 or more than 1 standard deviation, per the practice in the art. Alternatively, “about” can mean a range of up to 20%, up to 10%, up to 5%, or up to 1% of a given value. Alternatively, particularly with respect to biological systems or processes, the term can mean within an order of magnitude, within 5-fold, or within 2-fold, of a value. Where particular values are described in the application and claims, unless otherwise stated the term “about” meaning within an acceptable error range for the particular value can be assumed.

EXAMPLES

Example 1 – PL8 Linker (HT960-963)

[00113] FIG. 1 illustrates a molecule (HT960).

[00114] FIG. 2 illustrates schematically four molecules (HT960-HT963).

Example 2 – Stepwise Coupling of tetrapeptides to PL8 Linker

[00115] The molecule of Example 1 was coupled sequentially to amino acids to form 16 linker-bound tetrapeptides. Vapor phase ammonia cleavage and MALDI analysis were performed after each coupling step to assess the identity and yield for each coupling step.

[00116] FIG. 3 shows the results of the coupling sequence for HT960. Average yield after first coupling step was 64%. Average yield after second coupling step was 100%. Average yield after third coupling step was 100%. Average yield after fourth coupling step was 100%.

[00117] FIG. 4 shows the results of the coupling sequence for HT962. Average yield after first coupling step was 62%. Average yield after second coupling step was 100%. Average yield after third coupling step was 100%. Average yield after fourth coupling step was 100%.

Example 3 (Prophetic)

[00118] FIG. 5 illustrates a molecule.

Example 4 (Prophetic)

[00119] FIG. 6 illustrates a molecule.

Example 5 (Prophetic)

[00120] FIG. 7 illustrates a molecule

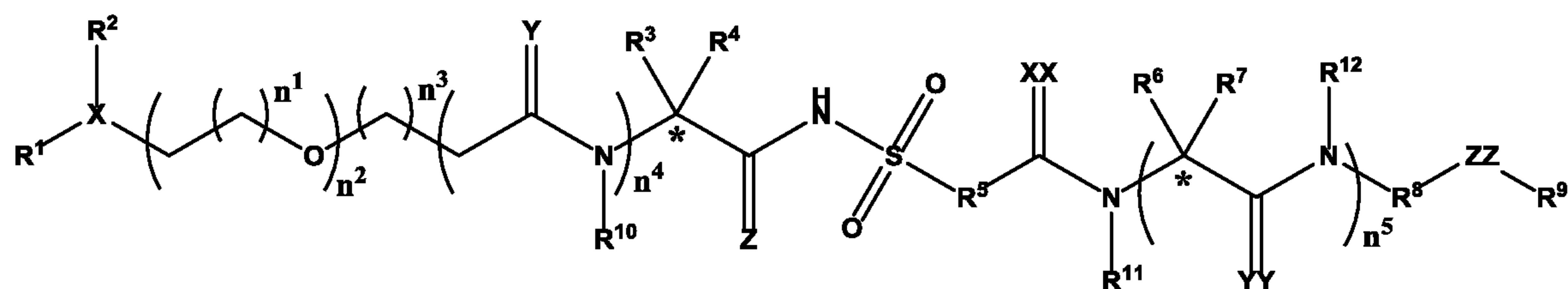
Example 6 (Prophetic)

[00121] FIG. 8 illustrates a molecule

CLAIMS

WHAT IS CLAIMED IS

1. A molecule having the structure:



or a salt thereof,

wherein n^1 is independently 0, 1, 2, or 3;

n^2 is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20;

n^3 is from 0, 1, 2, or 3;

n^4 is 0 or 1; and

n^5 is 0, 1, 2, or 3; and wherein

X is O, N, or S;

Y , Z , XX , and YY are the same or different and are independently O or S;

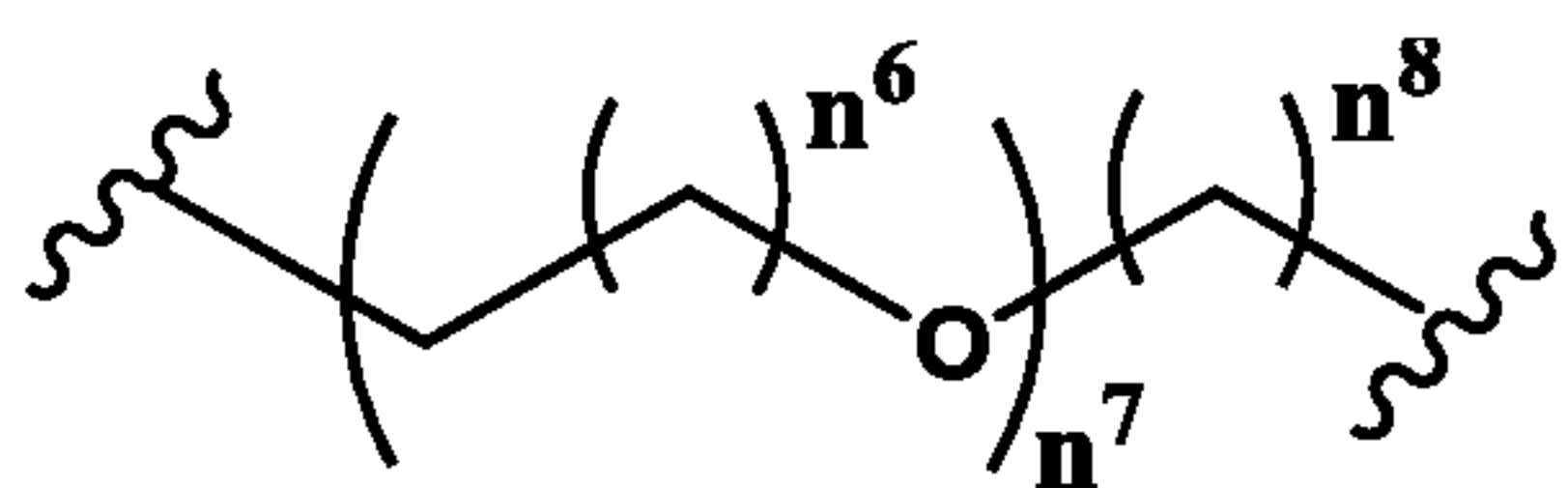
R^1 and R^2 are the same or different and are independently hydrogen, alkyl, alkenyl, aryl, heteroaryl, alkynyl, arylalkyl, arylalkenyl, haloalkyl, cycloalkyl, sulfonamidyl, acyl, electron lone pair, or $-CO_2R^{13}$, wherein R^{13} is alkyl, alkenyl, aryl, arylalkyl, arylalkenyl, haloalkyl, cycloalkyl, cycloalkylalkyl, or arylcycloalkylalkyl; or wherein R^1 and R^2 and the N to which they are bound form a ring; or wherein R^1 or R^2 comprise a nucleoside, nucleotide, polynucleotide, peptide, peptoid, saccharide, aptamer, or antibody or fragment thereof;

R^3 , R^4 , R^6 , and R^7 are the same or different and are independently hydrogen, halo, alkyl, alkenyl, aryl, heteroalkyl, arylalkyl, hydroxyarylalkyl, heteroarylalkyl, cycloalkyl, thioalkyl, selenoalkyl, hydroxyalkyl, or amino-substituted alkyl;

R^5 is alkyl, alkenyl, alkynyl or aryl, all optionally substituted with hydrogen, halo, alkyl, polyhaloalkyl, alkoxy, haloalkoxy, polyhaloalkoxy, alkoxycarbonyl, alkenyl, alkynyl, cycloalkyl, cycloalkylalkyl, hydroxyl, hydroxyalkyl, nitro, cyano, amino, substituted amino, alkylamino, dialkylamino, thiol, alkylthio, alkylcarbonyl, acyl, alkoxycarbonyl, aminocarbonyl, alkylsulfinyl, sulfonamide, or sulfonyl;

R^8 , oriented from N to ZZ, is alkyl, heteroalkyl, amino-substituted alkyl, amino-substituted heteroalkyl, amidoalkyl, amidoheteroalkyl, amino-substituted amidoheteroalkyl, each optionally substituted with an alkyl, heteroalkyl, amino-substituted alkyl, amino-substituted heteroalkyl, amidoalkyl, amidoheteroalkyl, or amino-substituted amidoheteroalkyl; or wherein R^8 is $\xi-(CR^{15}R^{16}CR^{17}R^{18})_m-\xi$, wherein m is 1 to 100, and R^{15} , R^{16} , R^{17} , and R^{18} are the same or different and are independently hydrogen, halo, alkyl, heteroalkyl, amino-substituted alkyl, amino-

substituted heteroalkyl, amidoalkyl, amidoheteroalkyl, amino-substituted amidoheteroalkyl; or wherein R⁸ is

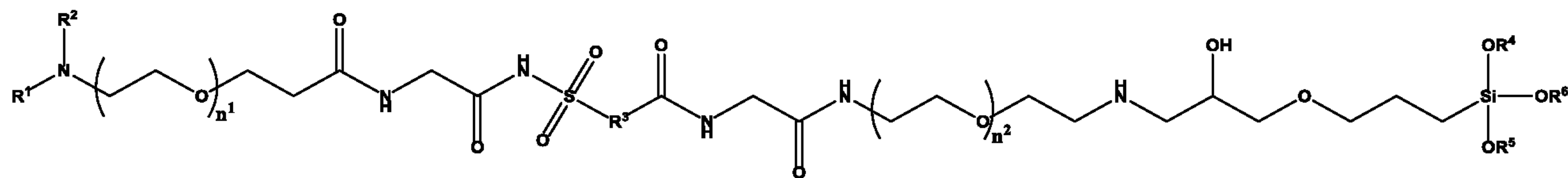


wherein n⁶ and n⁸ are the same or different and are independently 1, 2, or 3, and n⁸ is 1 to 20; R⁹ is an alkyl, heteroalkyl, hydroxyheteroalkyl, silylalkyl, siloxyalkyl siloxyheteroalkyl, siloxyhydroxyheteroalkyl, or wherein R⁹ may be R¹⁹-(CH₂)₃-Si(Z)_p, wherein p is from 1 to 3, wherein Z is selected from halo or alkoxy, and wherein R¹⁹ is selected from a hydroxyalkyl or carbamate; R¹⁰, R¹¹, and R¹² are the same or different and are independently selected from hydrogen or alkyl; and wherein R⁹ is optionally linked to a solid phase; ZZ is NR²⁰, O, S, or Se, wherein R²⁰ is hydrogen or alkyl; and (*) is a carbon center, wherein said carbon center can be independently a stereogenic center or a non-stereogenic center.

2. The molecule or salt of claim 1, wherein X is N, and wherein R¹ and R² are the same or different and are independently selected from hydrogen, acyl, acyloxy, benzyl, triphenylmethyl, benzylidenyl, or *p*-toluenesulfonyl.
3. The molecule or salt of claim 1 or 2, wherein said solid phase comprises a substrate, a bead, or a chromatographic packing material.
4. The molecule or salt of any one of claims 1 to 3, wherein either of R³ and R⁴ comprises a C₁-C₁₀ alkyl, cycloalkyl, aryl, or heteroaryl side chain.
5. The molecule or salt of any one of claims 1 to 4, wherein either of R³ and R⁴ comprises a functional group that is at least partially protonated at a pH of about 7.3.
6. The molecule or salt of any one of claims 1 to 4, wherein either of R³ and R⁴ comprises a functional group that is at least partially deprotonated at a pH of about 7.3.
7. The molecule or salt of claim 4, wherein said side chain comprises an amide, alcohol, or thiol.
8. The molecule or salt of any one of claims 1 to 3, wherein R³ and R⁴ are hydrogen.
9. The molecule or salt of any one of claims 1 to 8, wherein either of R⁶ and R⁷ comprises a C₁-C₁₀ alkyl, cycloalkyl, aryl, or heteroaryl side chain.
10. The molecule or salt of any one of claims 1 to 9, wherein either of R⁶ and R⁷ comprises a functional group that is at least partially protonated at a pH of about 7.3.
11. The molecule or salt of any one of claims 1 to 9, wherein either of R⁶ and R⁷ comprises a functional group that is at least partially deprotonated at a pH of about 7.3.
12. The molecule or salt of claim 9, wherein said side chain comprises an amide, alcohol, or thiol.

13. The molecule or salt of any one of claims 1 to 8, wherein R^6 and R^7 are hydrogen.

14. A molecule having the structure:



, or a salt thereof,

wherein $n^1=1-15$ and $n^2=1-6$; and

wherein R^1 and R^2 are the same or different and are independently hydrogen or acyloxy;

R^3 is alkyl, alkenyl, alkynyl or aryl, all optionally substituted with 1, 2, 3, or 4 groups selected from hydrogen, halo, alkyl, polyhaloalkyl, alkyoxy, haloalkoxy, polyhaloalkoxy, alkoxycarbonyl, alkenyl, alkynyl, cycloalkyl, cycloalkylalkyl, hydroxyl, hydroxyalkyl, nitro, cyano, amino, substituted amino, alkylamino, dialkylamino, thiol, alkylthio, alkylcarbonyl, acyl, alkoxycarbonyl, aminocarbonyl, alkylsulfinyl, sulfonamide, or sulfonyl; and

R^4 , R^5 , and R^6 are the same or different and are independently selected from hydrogen, alkyl, silyl, or siloxy.

15. The molecule or salt of claim 14, wherein R^3 is *p*-phenyl.

16. The molecule or salt of claim 14, wherein R^3 is *n*-propyl.

17. The molecule or salt of any one of claims 1 to 16, wherein R^1 or R^2 comprise a peptide.

18. The molecule or salt of claim 17, wherein said peptide comprises from 2 to 100 amino acids.

19. An array comprising the molecule or salt of any one of claims 1 to 18.

20. The array of claim 19, wherein said array comprises at least about 10,000, 300,000, or 1 million peptide features per 1 cm^2 .

21. The array of claim 19 or 20, further comprising a binding moiety.

22. The array of claim 21, wherein said binding moiety comprises a nucleoside, nucleotide, polynucleotide, peptide, peptoid, saccharide, polysaccharide, aptamer, or antibody or fragment thereof.

23. The array of any one of claims 19 to 22, further comprising a fatty acid.

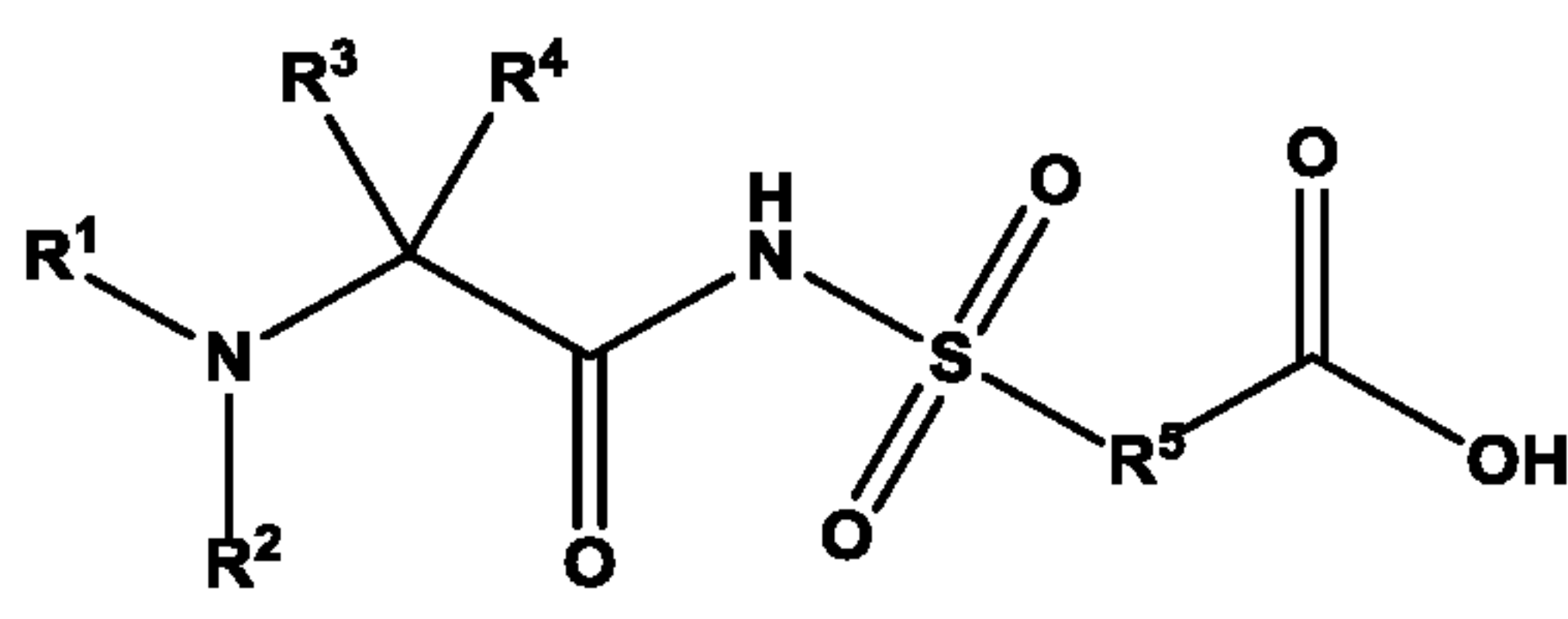
24. The array of claim 23, wherein said fatty acid is a fluorinated fatty acid.

25. A method of making an array, comprising associating the molecule or salt of any one of claims 1 to 18 with a substrate.

26. The method of claim 25, wherein said substrate is a Si/SiO₂ wafer.

27. The method of claim 25 or 26, wherein said array comprises at least about 10,000, 300,000, or 1 million peptide features per 1 cm².
28. A method of forming a solid support comprising associating a solid support with the molecule of any one of claims 1 to 18, wherein said solid support is a substrate, bead, polymer, or chromatographic packing material.
29. The use of the molecule or salt of any one of claims 1 to 18 for binding a nucleotide, polynucleotide, polypeptide, aptamer, or antibody or fragment thereof.
30. The use of the molecule or salt of any one of claims 1 to 18 for binding antibodies or proteins.
31. A method, comprising binding a binding moiety to the molecule or salt of any one of claims 1 to 18.
32. The method of claim 31, further comprising identifying said binding moiety.
33. The method of claim 32, wherein said identifying comprises an immunofluorescence assay.
34. The method of claim 32, wherein said binding moiety comprises a nucleotide, polynucleotide, polypeptide, aptamer, or antibody or fragment thereof.
35. The method of any one of claims 31 to 34, wherein said binding moiety is obtained from a subject.
36. The method of any one of claims 31 to 35, further comprising determining the likelihood that said subject has a disease or condition.
37. The method of claim 36, wherein said subject is a human.
38. The method of claim 37, wherein said disease or condition is an autoimmune disease.
39. The method of claim 38, wherein said autoimmune disease is lupus, ulcerative colitis, Crohn's disease, or rheumatoid arthritis.
40. The method of claim 37, wherein said disease or condition is an infection.
41. The method of claim 40, wherein said infection is bacterial, viral, fungal, or parasitic.
42. The method of claim 37, wherein said disease or condition is cancer.
43. The method of any one of claims 31 to 42, further comprising communicating a result via a communication medium.
44. A method comprising cleaving a bond in the molecule of any one of claims 1 to 18.
45. The method of claim 44, wherein said bond is the C-N bond of an *N*-acylsulfonamide.
46. The method of claim 45, wherein said cleaving comprises a first activating step and a second cleavage step.
47. The method of claim 46, wherein said first activating step comprises alkylating said *N*-acylsulfonamide with an alkylating agent.

48. The method of claim 47, wherein said alkylating agent is a diazo compound or an alkyl halide.
49. The method of claim 48, wherein said alkylating agent is diazomethane, methyl iodide, or iodoacetonitrile.
50. The method of claim 45, wherein said cleavage comprises a vapor-phase cleavage reaction.
51. The method of claim 50, wherein said cleavage comprises a vapor-phase ammonia cleavage reaction.
52. A kit, comprising the molecule of any one of claims 1 to 18 and instructions for use.
53. A method of making a kit, comprising forming the kit with the molecule of any one of claims 1 to 18.
54. A compound having the structure:

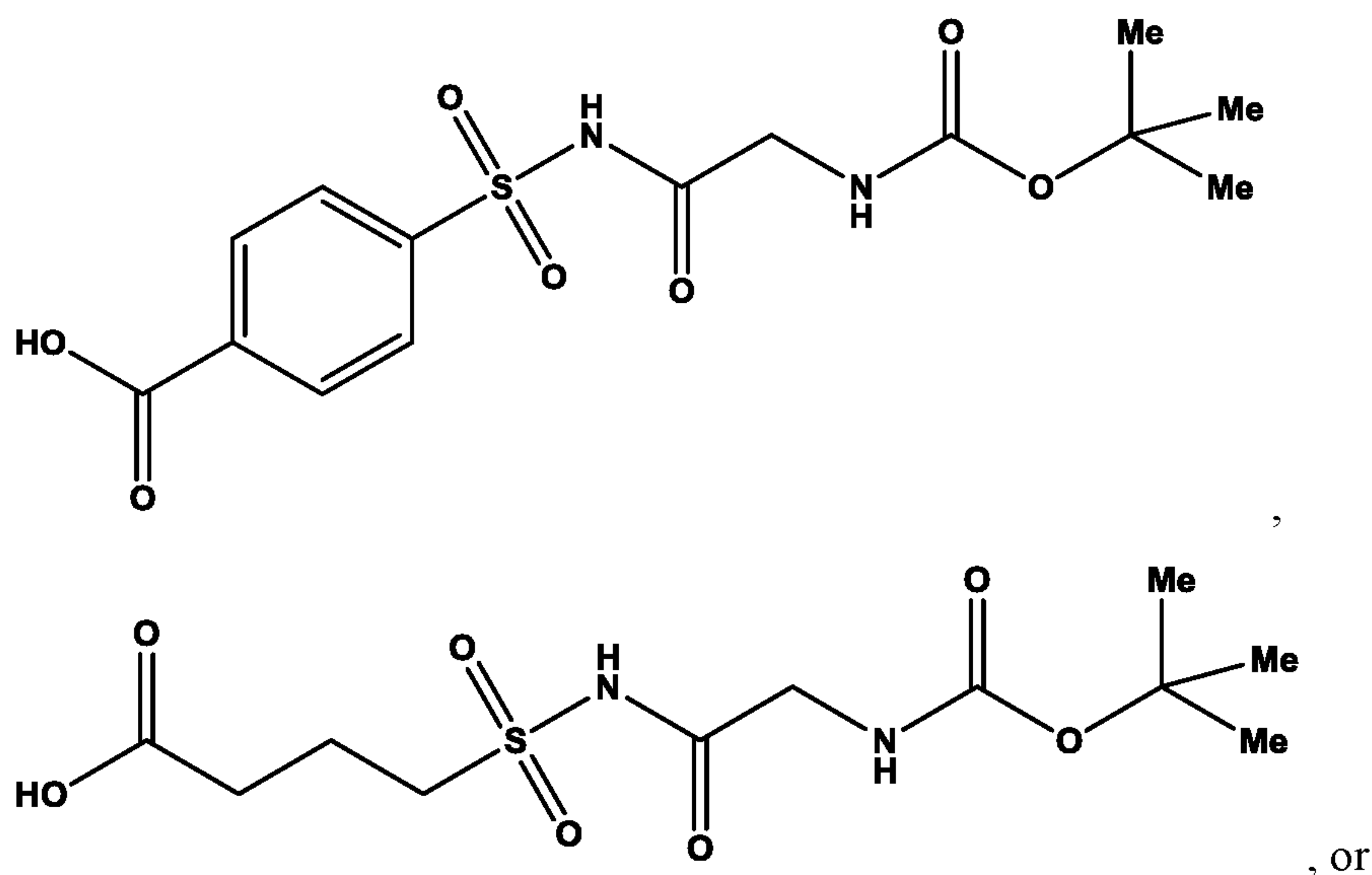


or salt thereof, wherein:

- R^1 and R^2 are the same or different and are independently hydrogen, alkyl, alkenyl, aryl, heteroaryl, alkynyl, arylalkyl, arylalkenyl, haloalkyl, cycloalkyl, sulfonamidyl, acyl, electron lone pair, or $-CO_2R^6$, wherein R^6 is alkyl, alkenyl, aryl, arylalkyl, arylalkenyl, haloalkyl, cycloalkyl, cycloalkylalkyl, or arylcycloalkylalkyl; or wherein R^1 and R^2 and the N to which they are bound form a ring;
- R^3 , R^4 are the same or different and are independently hydrogen, halo, alkyl, alkenyl, aryl, heteroalkyl, arylalkyl, hydroxyarylalkyl, heteroarylalkyl, cycloalkyl, thioalkyl, selenoalkyl, hydroxyalkyl, or amino-substituted alkyl; and
- R^5 is alkyl, alkenyl, alkynyl or aryl, each optionally substituted with hydrogen, halo, alkyl, polyhaloalkyl, alkoxy, haloalkoxy, polyhaloalkoxy, alkoxycarbonyl, alkenyl, alkynyl, cycloalkyl, cycloalkylalkyl, hydroxyl, hydroxyalkyl, nitro, cyano, amino, substituted amino, alkylamino, dialkylamino, thiol, alkylthio, alkylcarbonyl, acyl, alkoxycarbonyl, aminocarbonyl, alkylsulfinyl, sulfonamide, or sulfonyl.
55. The compound or salt of claim 54, wherein R^1 and R^2 are the same or different and are independently hydrogen, acyl, acyloxy, phthalimidyl, benzyl, triphenylmethyl, benzylidenyl, or *p*-toluenesulfonyl.
56. The compound or salt of claim 54 or 55, wherein either of R^3 and R^4 comprises a C_1 - C_{10} alkyl, cycloalkyl, aryl, or heteroaryl side chain.
57. The compound or salt of claim 56, wherein either of R^3 and R^4 comprises a functional group that is at least partially protonated at a pH of about 7.3.
58. The compound or salt of claim 56, wherein either of R^3 and R^4 comprises a functional group that is at least partially deprotonated at a pH of about 7.3.
59. The compound or salt of claim 56, wherein said side chain comprises an amide, alcohol, or thiol.

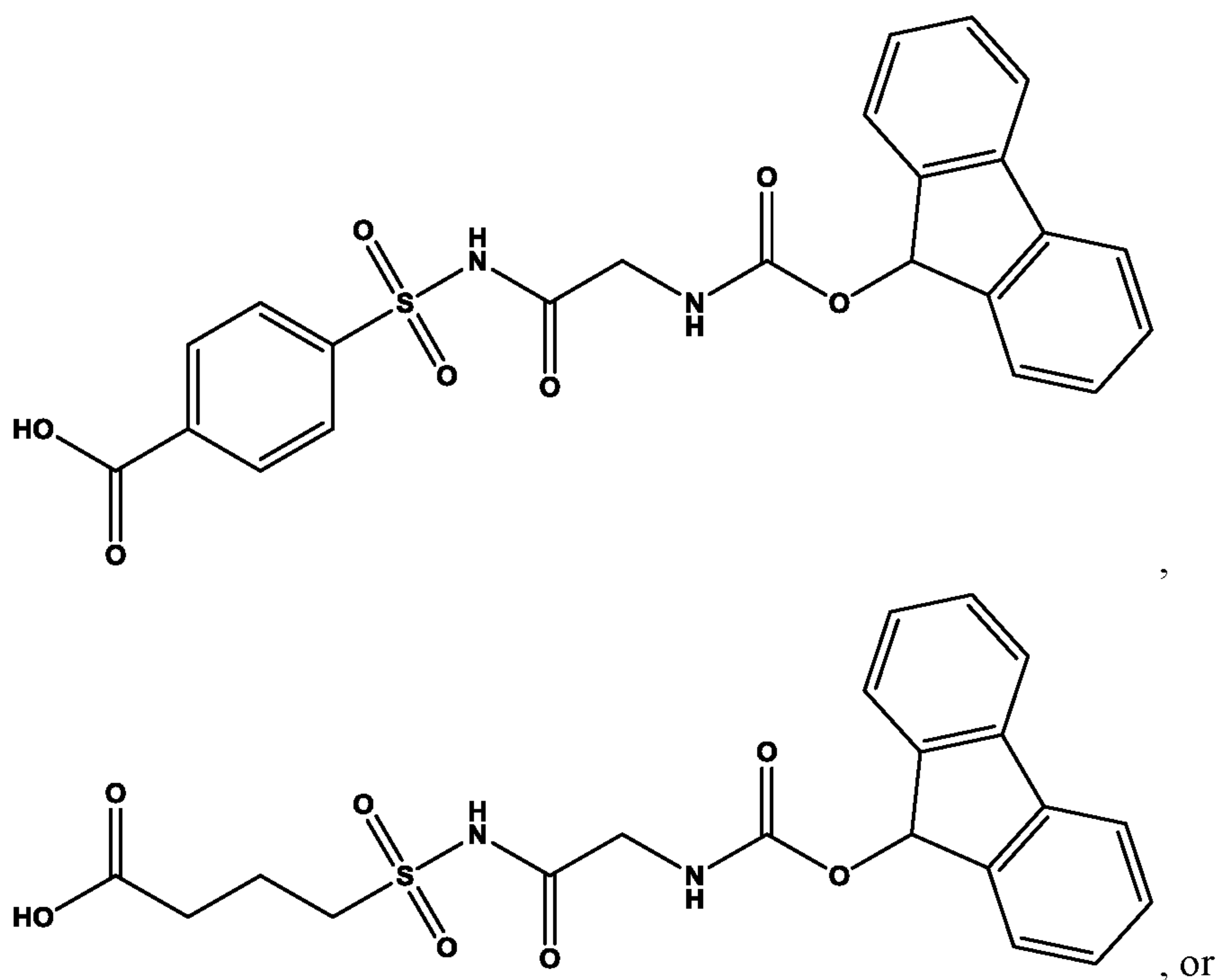
60. The compound or salt of claim 54 or 55, wherein R³ and R⁴ are hydrogen.

61. The compound of claim 54, having the structure:



a salt thereof.

62. The compound of claim 54, having the structure:



a salt thereof.

63. A method of synthesizing a molecule, comprising forming a molecule with the compound or salt of any one of claims 54 to 62, comprising:

- a. coupling said compound to a first amino group on a solid phase; and
- b. deprotecting said compound to form a second amino group.

64. The method of claim 63, wherein said solid phase comprises a substrate, a bead, a polymer, or a chromatographic packing material.

65. The method of claim 63 or 64, wherein said deprotection comprises a photoacid or a photoacid generator.
66. The method of claim 63 or 64, wherein said deprotection comprises a photobase or a photobase generator.
67. The method of any one of claims 63 to 65, further comprising an amine capping step after step (a) and before step (b), comprising reacting said first amino group with a capping agent.
68. The method of claim 67, wherein said amine capping step forms an alkylamine, arylamine, acetamide, carbamate, phthalimide, enamine, sulfonamide, or protected amino acid.
69. The method of claim 67, wherein said capping agent is acetic anhydride, acetyl chloride, acetyl fluoride, or an acylglycine.
70. A molecule made by the process of any one of claims 63 to 69.

FIG. 1

PL8 Molecule (HT960)

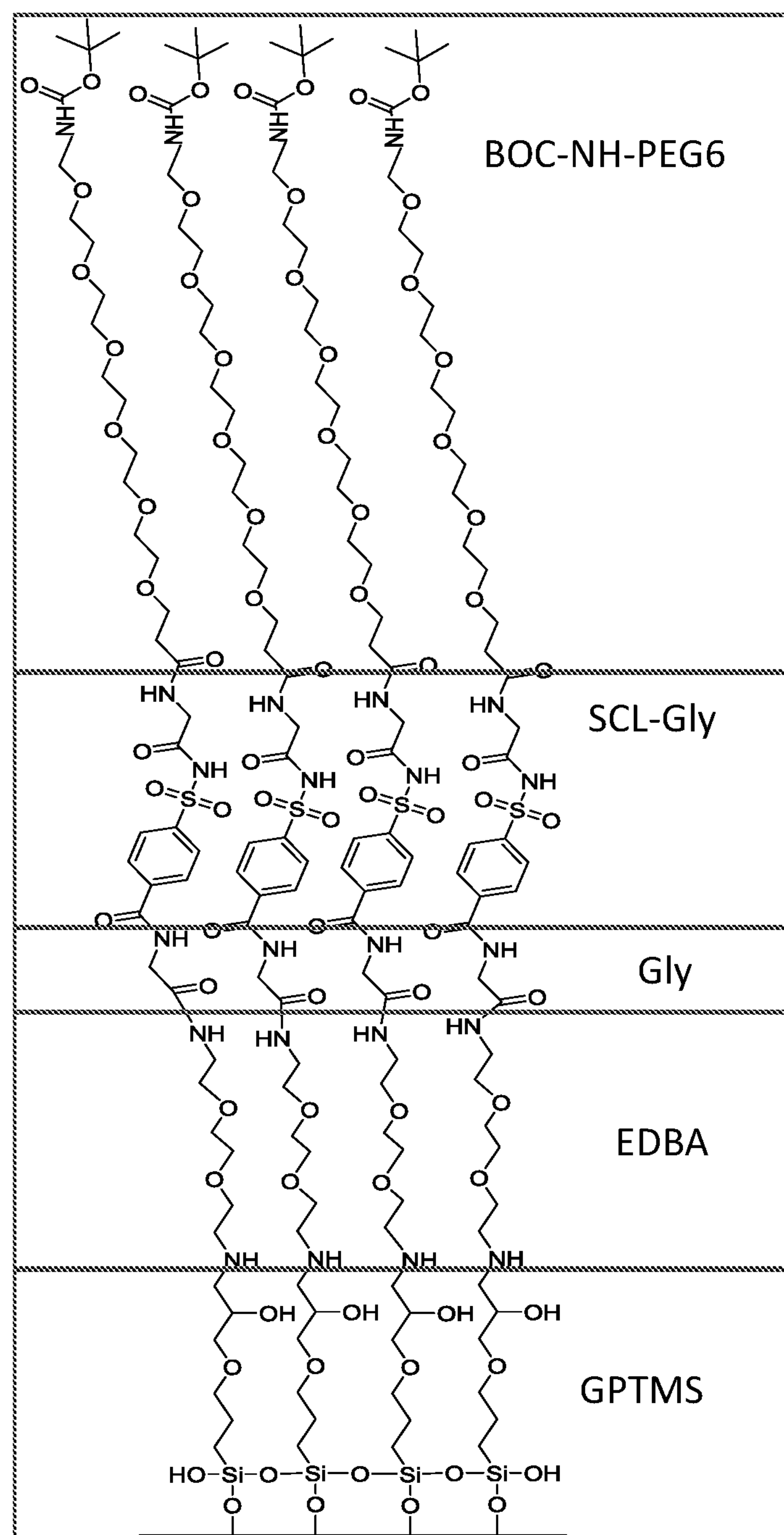


FIG. 2

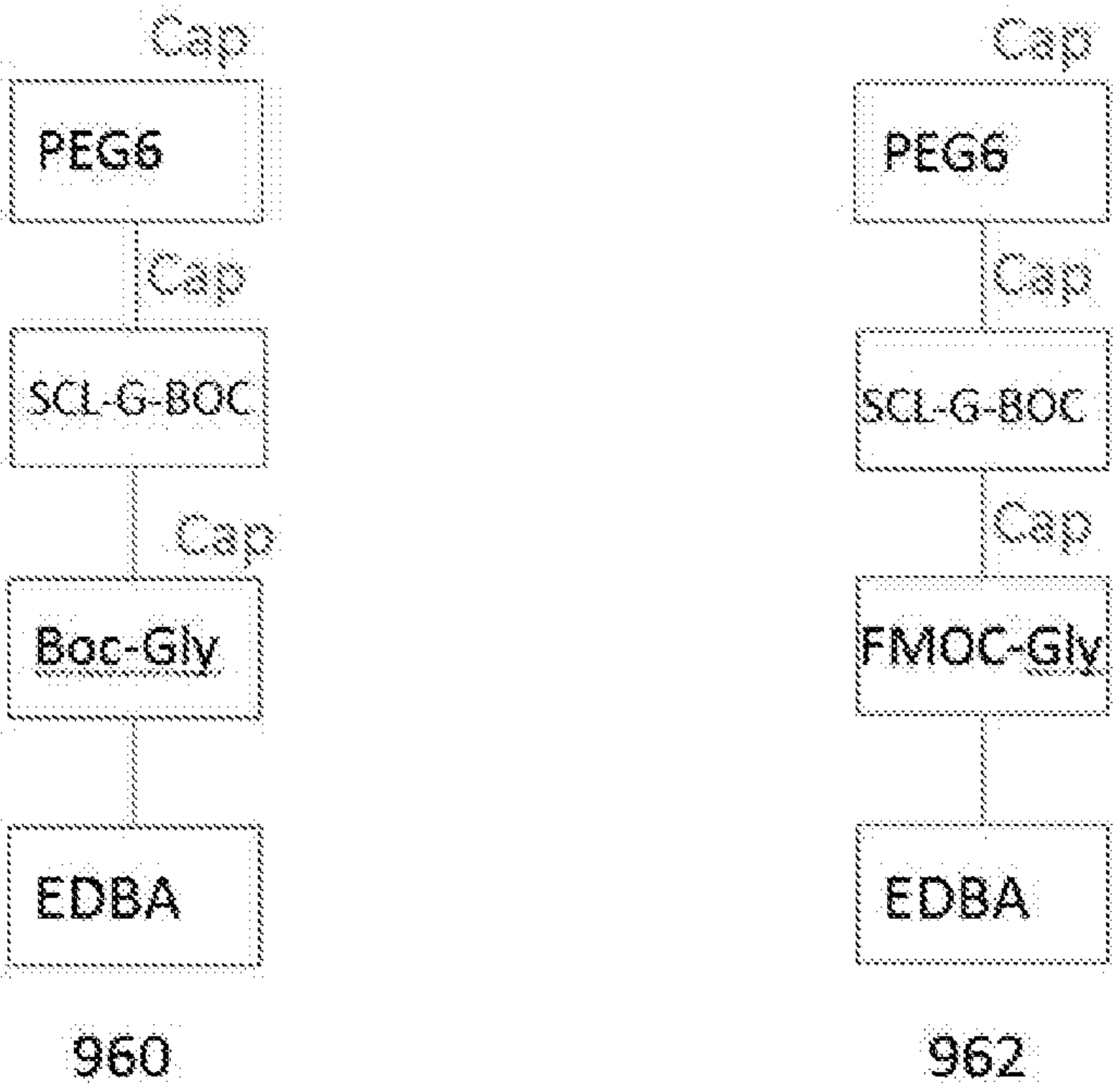


FIG. 3

MALDI-MS (SSA) Measurement: Fraction Desired Yield

HT960_S4_modifiedwith W oxidation, H-to-N conversion, E protective group and R manual integration

LAYER 1			LAYER 2			LAYER 3			LAYER 4		
ANIONIC ACID	AVG YIELD (n=2)	REMARKS %CV	ANIONIC ACID	AVG YIELD (n=2)	REMARKS %CV	ANIONIC ACID	AVG YIELD (n=2)	REMARKS %CV	ANIONIC ACID	AVG YIELD (n=2)	REMARKS %CV
C	0.70	0.57	D	1.00	0.00	D	1.00	0.00	D	1.00	0.00
F	0.64	0.11	D	1.00	0.00	D	1.00	0.00	D	1.00	0.00
S	0.64	0.10	E	1.00	0.00	E	1.00	0.00	E	1.00	0.00
Q	0.64	0.08	Q	1.00	0.00	Q	1.00	0.00	Q	1.00	0.00
H	0.60	0.01	H	1.00	0.00	H	1.00	0.00	H	1.00	0.00
A	0.78	0.70	L	0.99	0.00	L	1.00	0.00	L	1.00	0.00
D	0.78	1.04	V	1.00	0.00	V	1.00	0.00	V	1.00	0.00
X	0.78	0.01	H	1.00	0.00	H	1.00	0.00	H	1.00	0.00
G	0.73	0.08	H	1.00	0.00	H	1.00	0.00	H	1.00	0.00
Y	0.83	0.02	K	1.00	0.00	K	1.00	0.00	K	1.00	0.00
N	0.64	0.08	D	1.00	0.00	D	1.00	0.00	D	1.00	0.00
D	0.55	0.03	Q	1.00	0.00	Q	1.00	0.00	Q	1.00	0.00
A	0.61	0.10	W	1.00	0.00	W	1.00	0.00	W	1.00	0.00
K	0.40	0.14	A	1.00	0.00	A	1.00	0.00	A	1.00	0.00
Q	0.38	0.03	I	1.00	0.00	I	1.00	0.00	I	1.00	0.00
H	0.01	0.11	P	1.00	0.00	P	1.00	0.00	P	1.00	0.00

Layer 1
Average Yield: 0.64

Layer 2
Average Yield: 1.00

Layer 3
Average Yield: 1.00

Layer 4
Average Yield: 1.00

FIG. 4

MALDI-MS (SSA) Measurement: Fraction Desired Yield

HT962_54_modifiedwith W oxidation, H-to-N conversion, E protective group and R manual integration

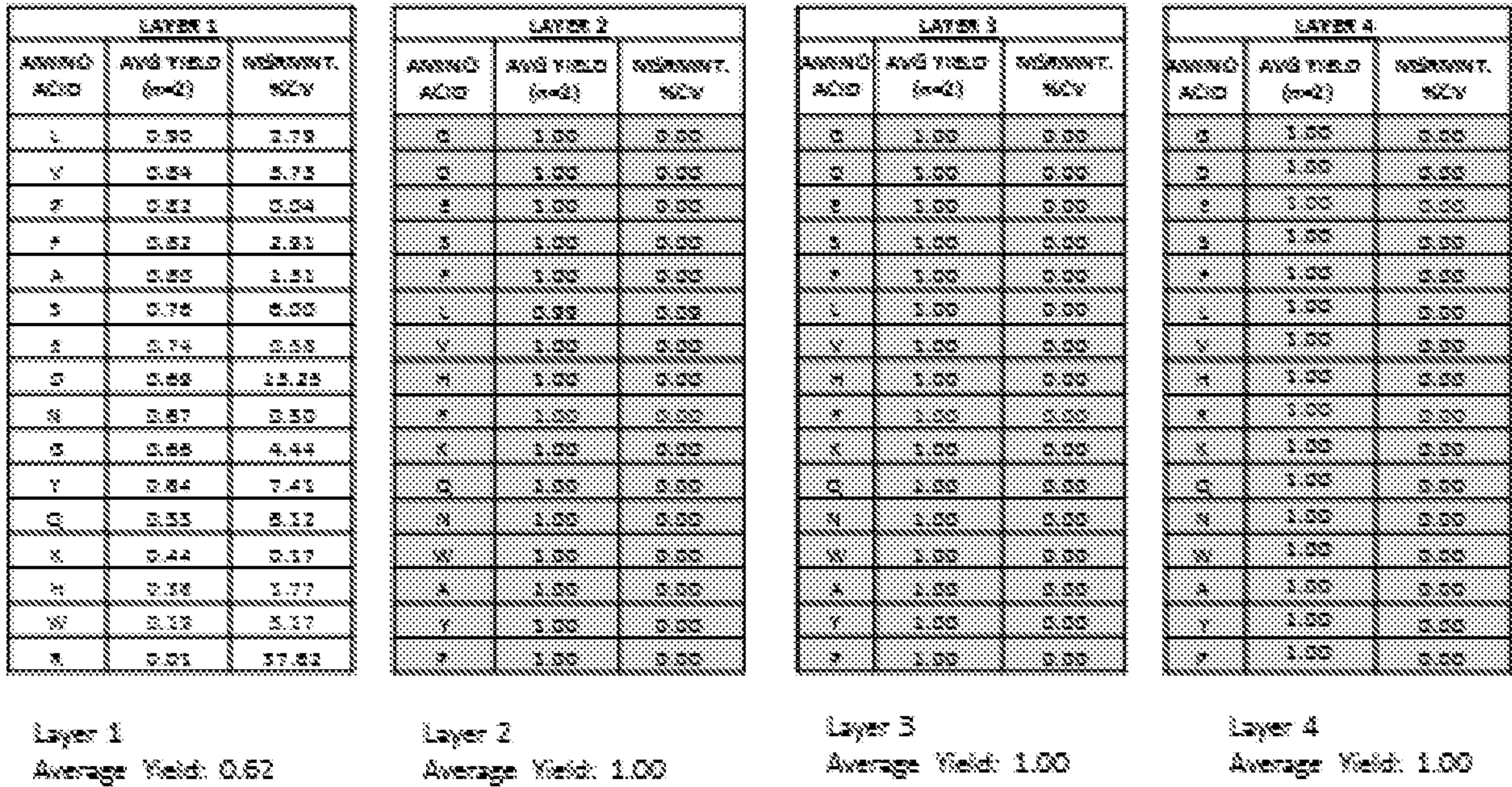


FIG. 5

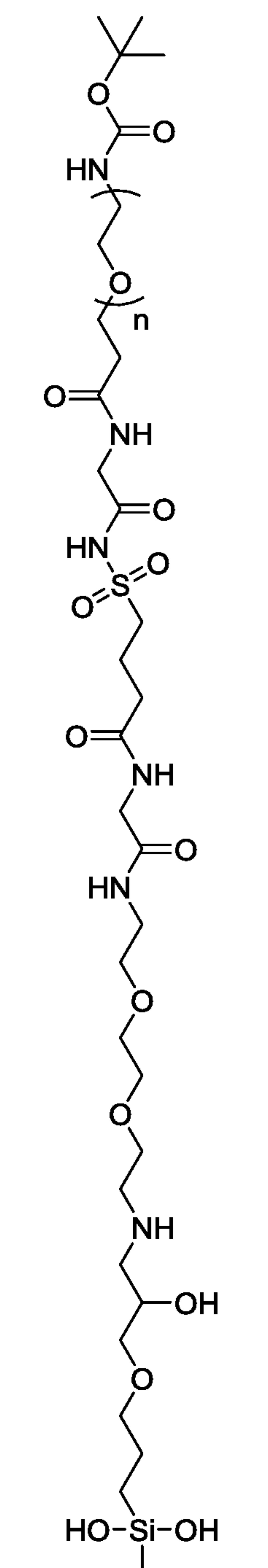


FIG. 6

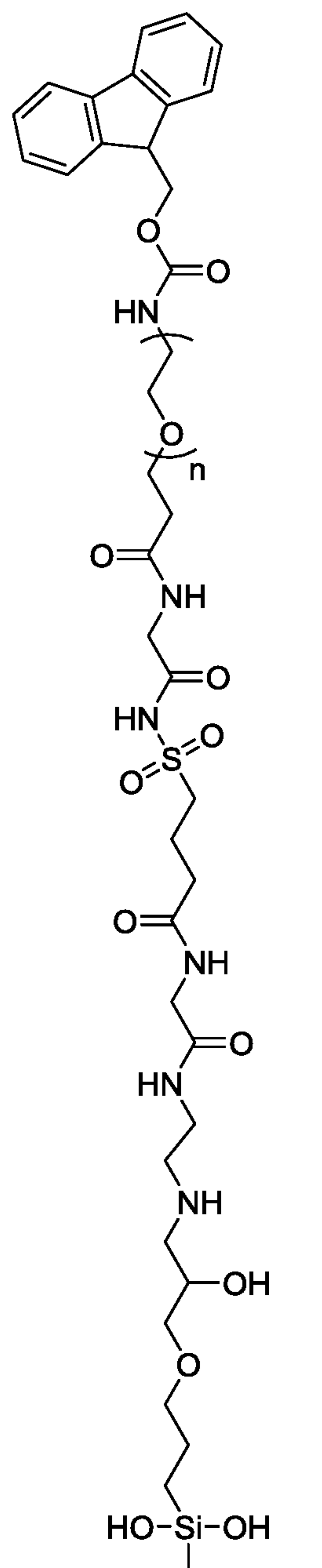


FIG. 7

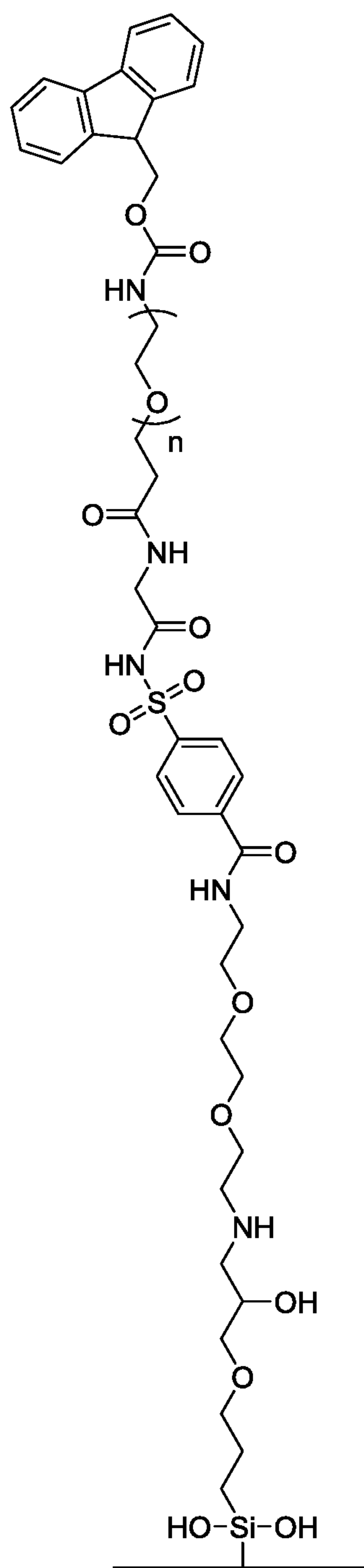


FIG. 8

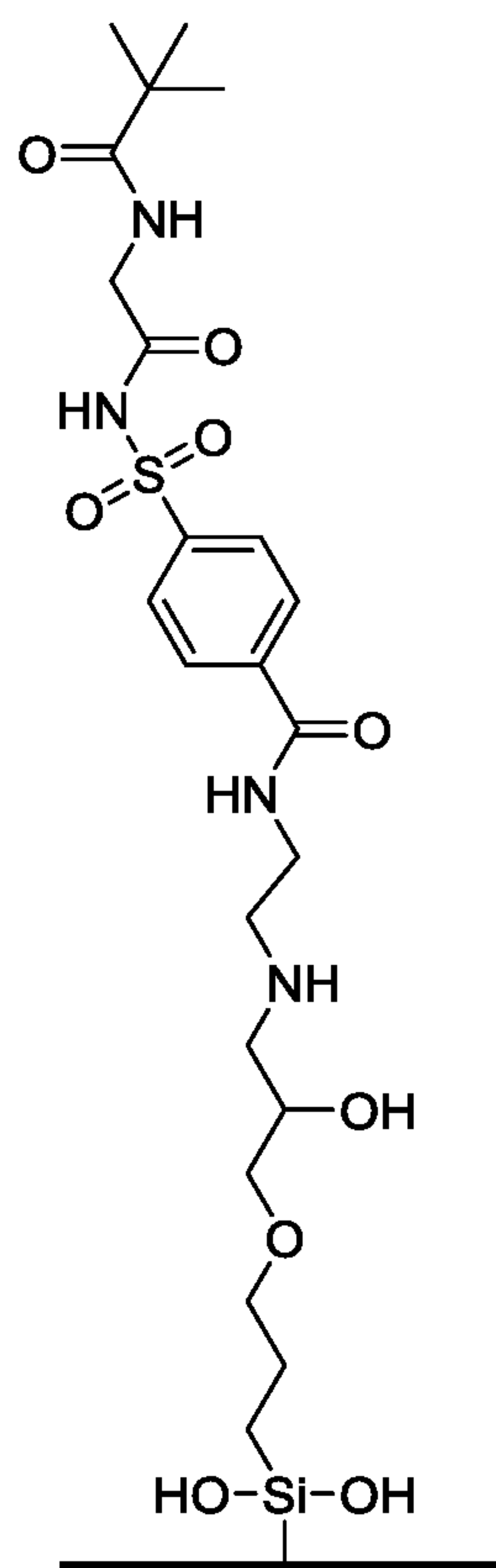
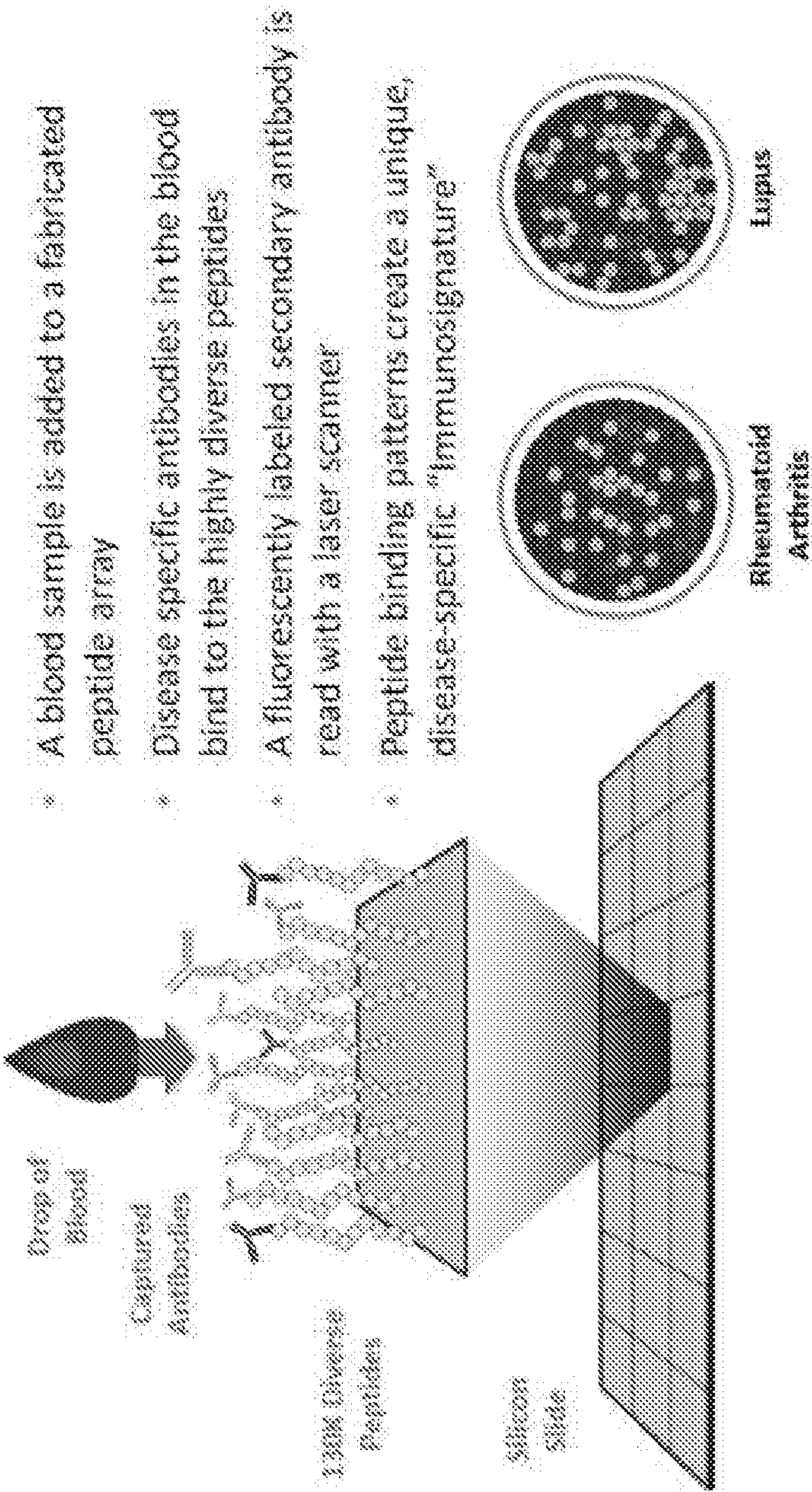


FIG. 9

HealthTell's Approach Provides a Snapshot of a Person's Immune Status from Small Amount of Blood



Any disease that generates a B-Cell response can be measured with the same array

FIG. 10

HealthTell's platform generates peptide arrays via in situ synthesis

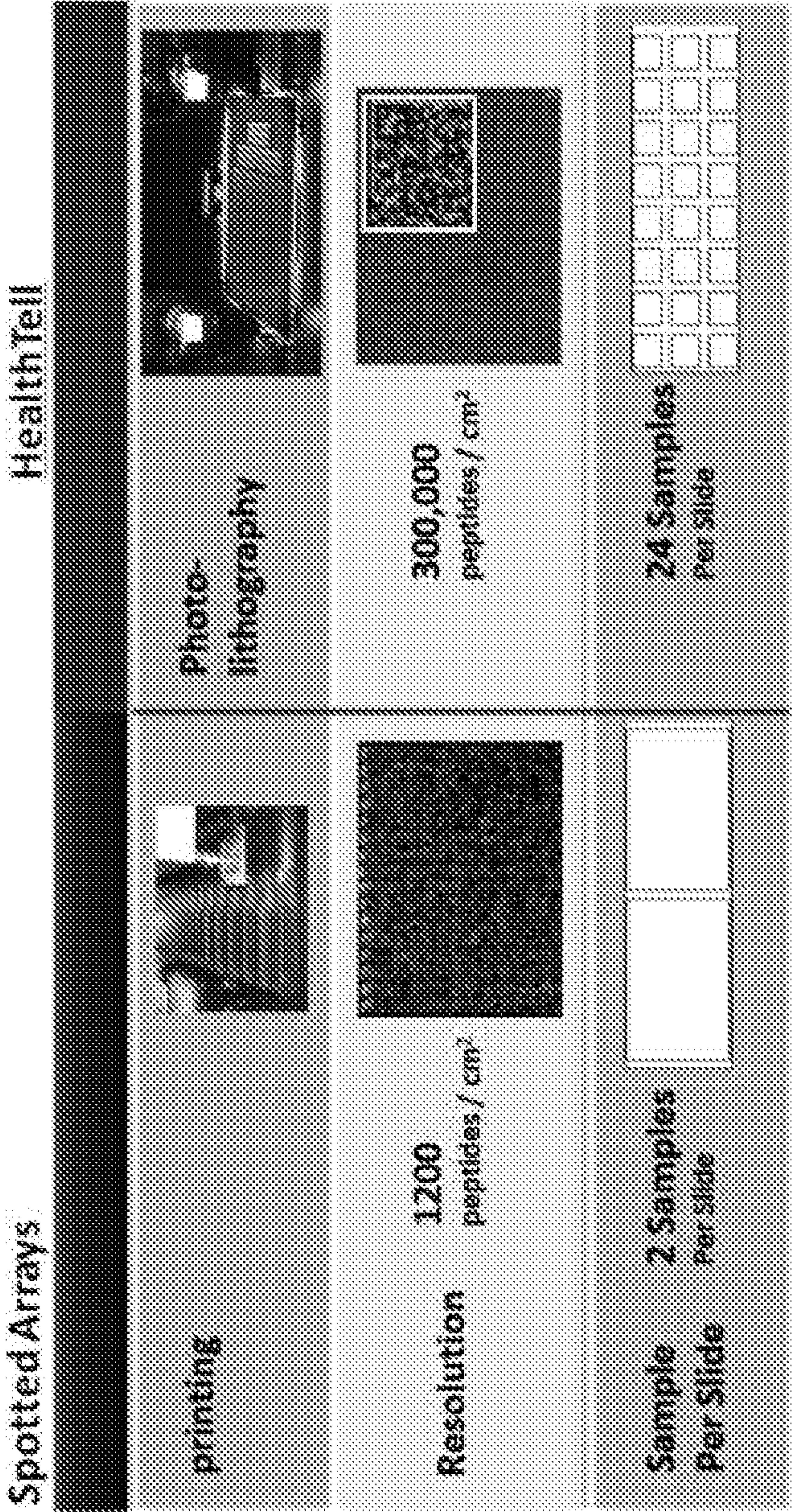


FIG. 11

Experimental

- Substrate: Si (<100> orientation) substrate containing ~250 nm thick thermal SiO₂
- Aminosilane coatings studied:
 - 3-aminopropyltriethoxysilane (APTES)
 - 3-aminopropylmethyldiethoxysilane (APDEMS)
 - 3-aminopropyl-diisopropylethoxysilane (APDIPES)
- Deposition method: Chemical vapor deposition using commercial CVD system
- Analytical characterization: Ellipsometry, WCA, XPS, AFM, Colorimetry, and MADLL-MS

FIG. 12

Peptide Synthesis – General Scheme

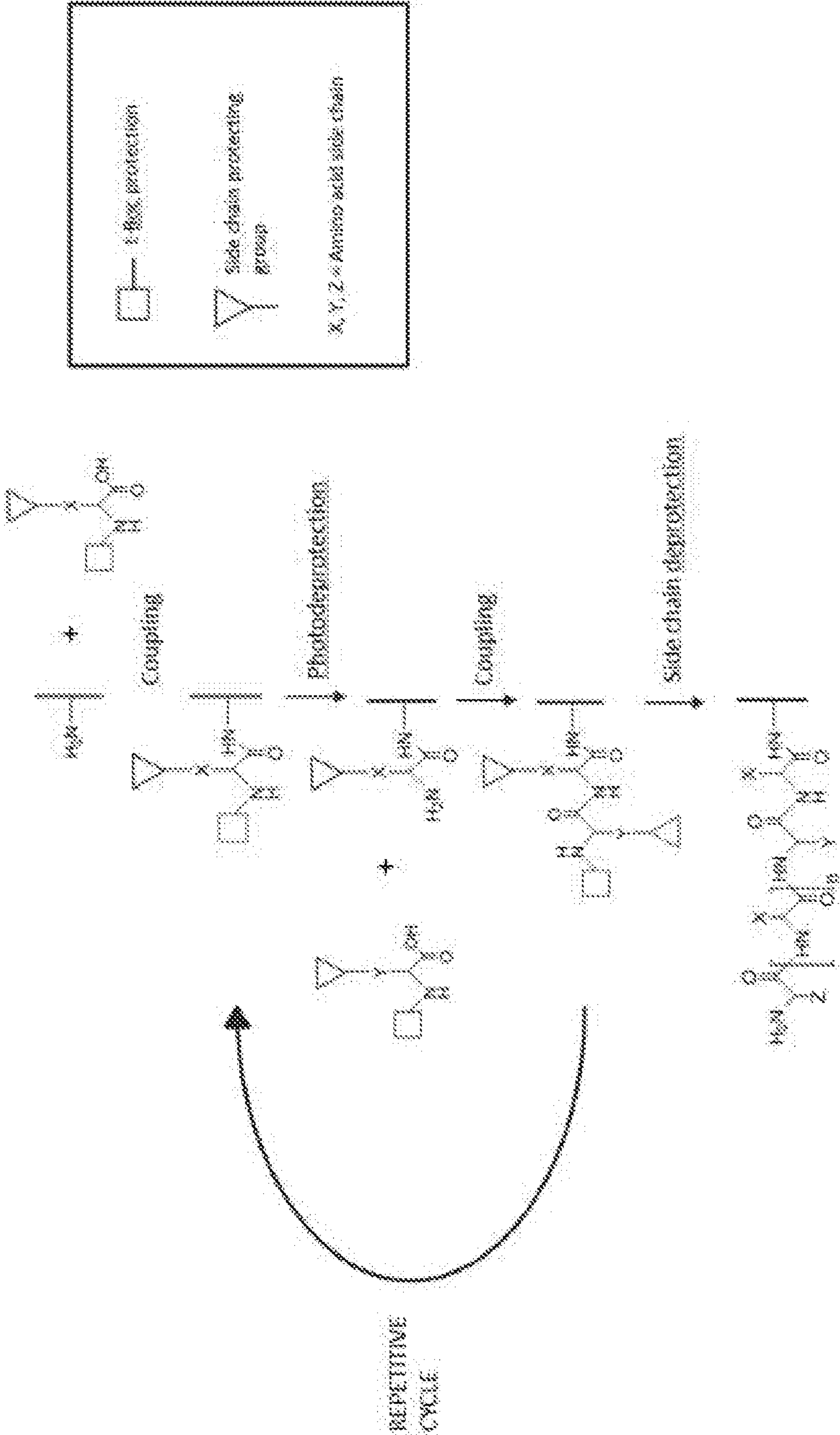


FIG. 13

t-Boc-Protected Amino Acids Used in the Study

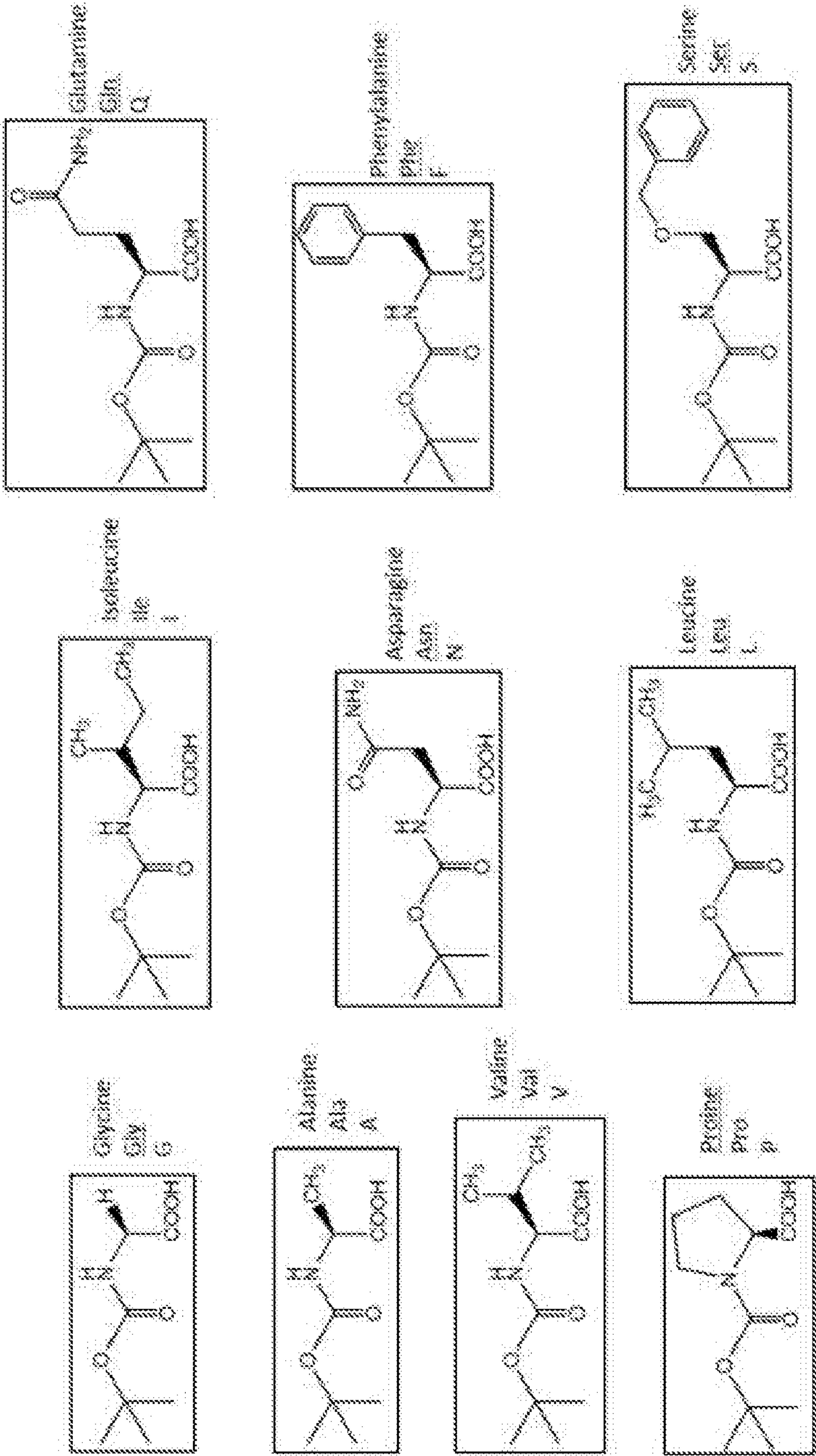


FIG. 14

Aminosilanes Coatings have Different Molecular Structures

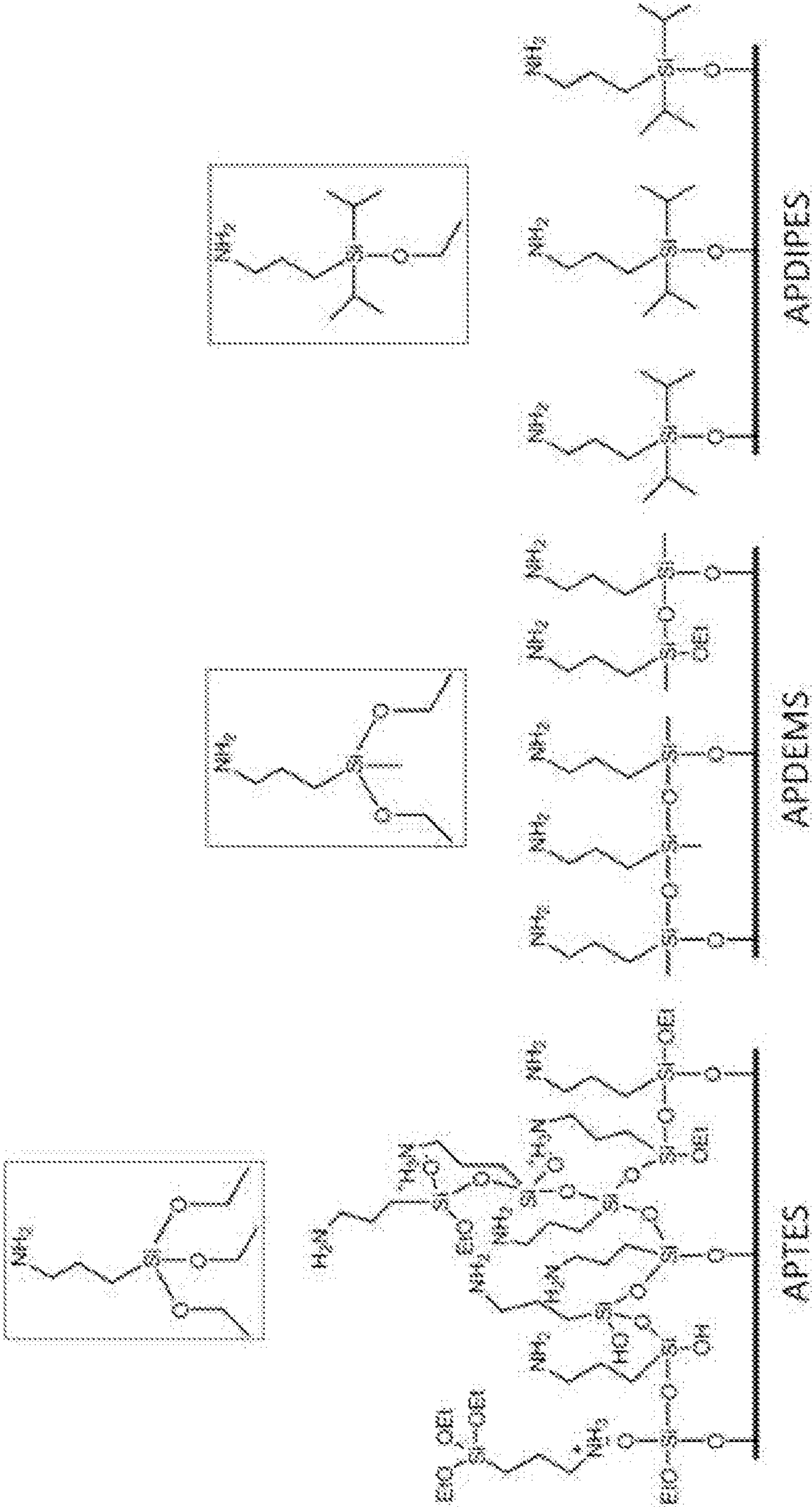


FIG. 15

Why is Surface Properties Control Important?

- Surface properties impact
 - Purity and number density of probes
 - Non-specific interaction of biological molecules with the substrate
 - Presentation of immobilized probes to biological molecules

FIG. 16

APTES and APDIPES Coatings are Most and Least Hydrophilic, Respectively

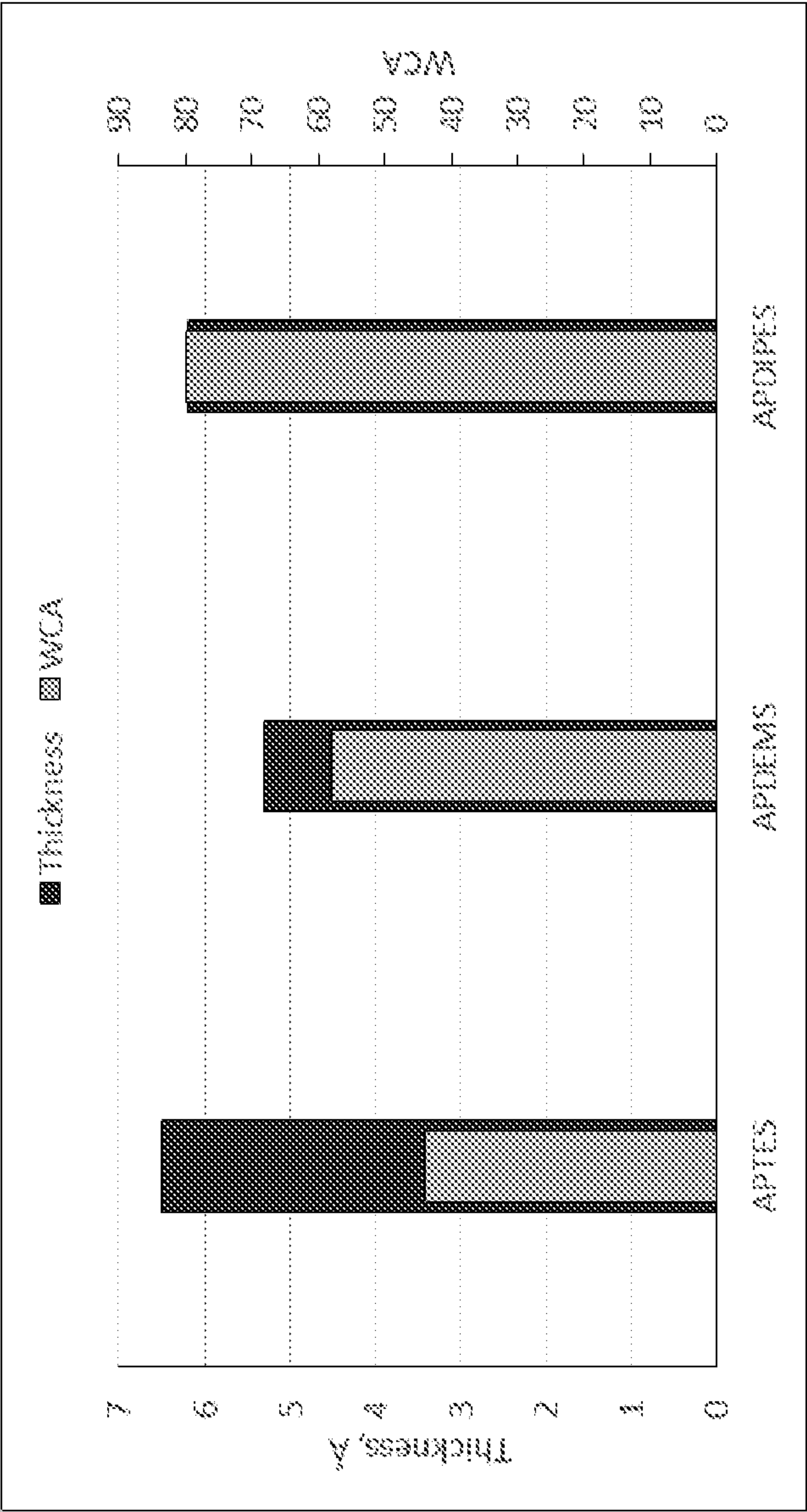


FIG. 17

Reactive Amine Density Assay

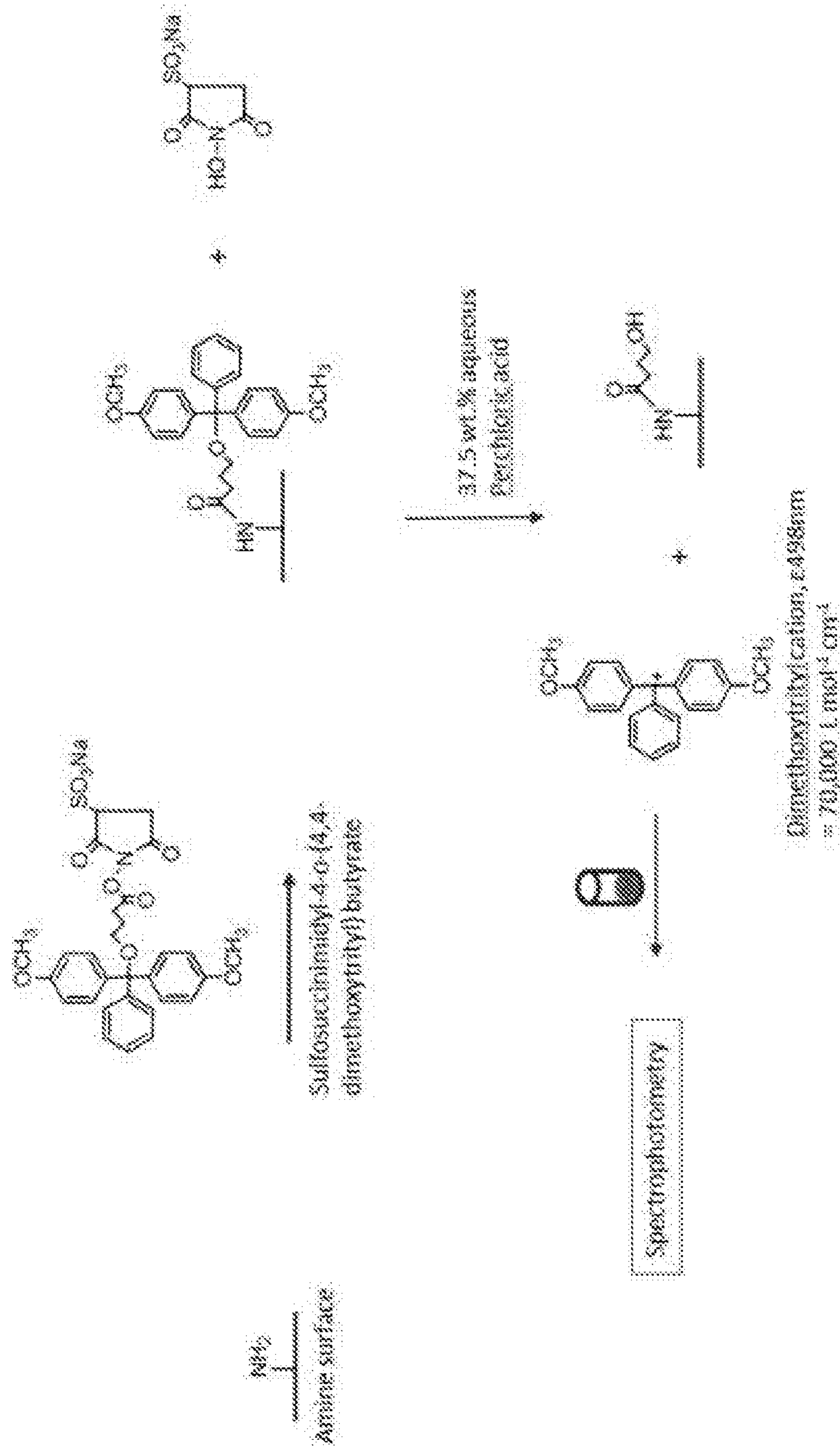


FIG. 18

APTES Coating is Densest Among Three Coatings

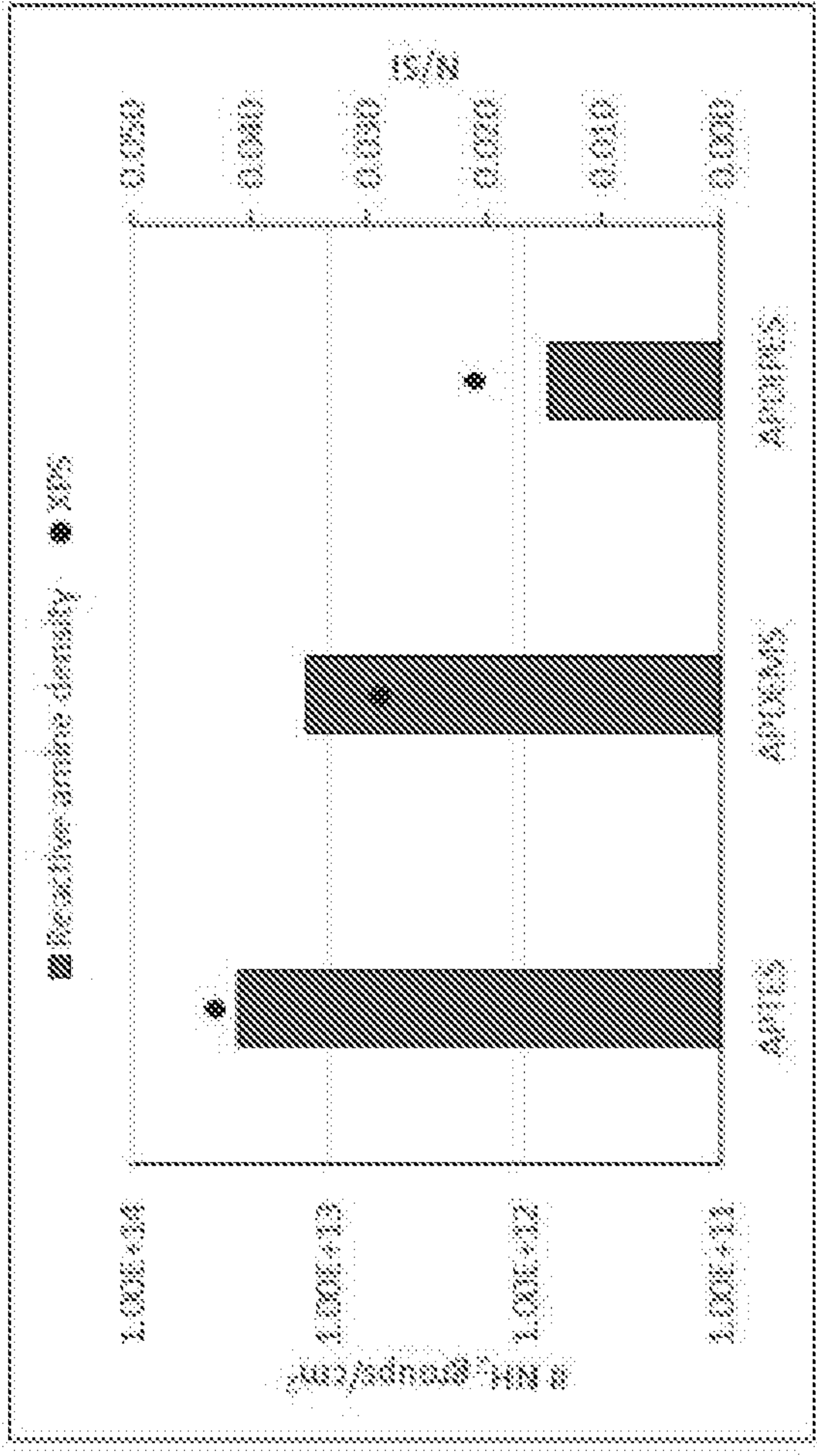
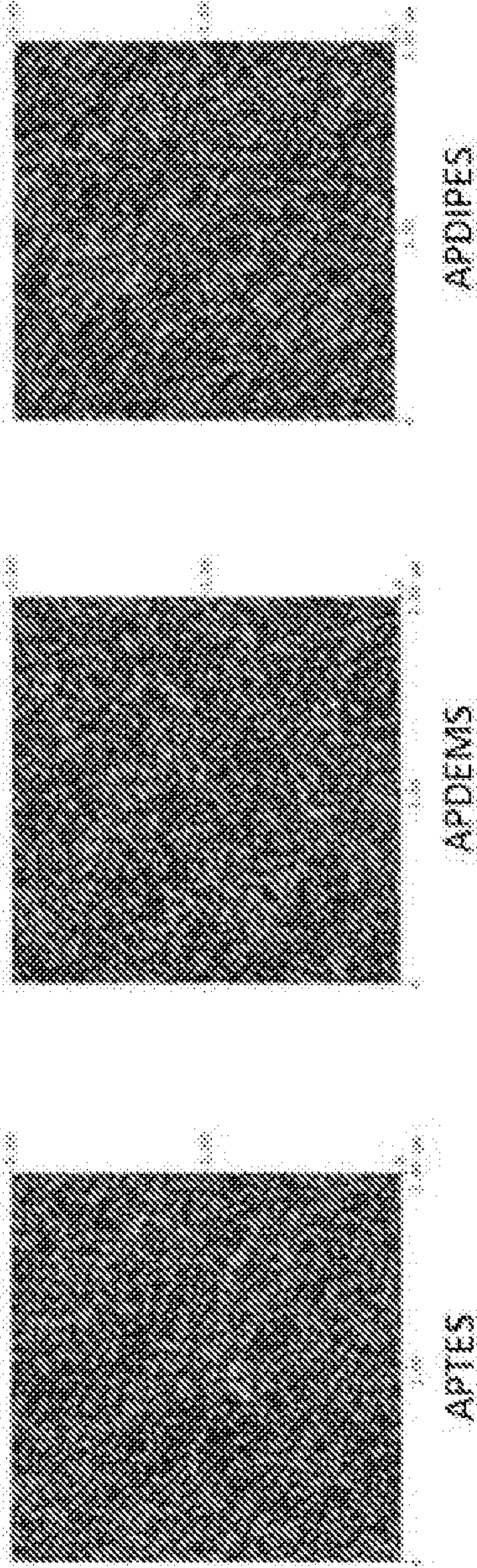


FIG. 19

All Three Aminosilane Coatings are Deposited Smoothly Over the Substrate



Coating type	RMS (Rq), nm
APTES	0.239
APDEMS	0.239
APDIPES	0.248

FIG. 20

Side Chain Deprotection (SCD) Process

- 1st treatment
 - TFA : DMS : m-cresol : DODT : TIOH (0.49 : 0.29 : 0.10 : 0.02 : 0.10) at 0°C for 3 hrs.
- 2nd treatment
 - TFA : thioanisole : DODT : TIOH (0.83 : 0.04 : 0.03 : 0.10) at RT for 1.5 hrs.

TFA: Trifluoroacetic acid
DMS: Dimethyl sulfide
TIOH: Trifluoromethanesulfonic acid
DODT: 2,2'-(Ethylenedioxy)diethanethiol

FIG. 21

Peptide Synthesis for Chemical Stability Study

- Preparatory synthesis
 - Aminosilane-coated surface was functionalized with tetramer peptide linker (GSGS).
- Main synthesis
 - A mixture of 9 amino acids (A, F, G, L, I, N, P, Q, and V) with no side chain protecting groups was coupled 12 times to create a diverse library of peptides.

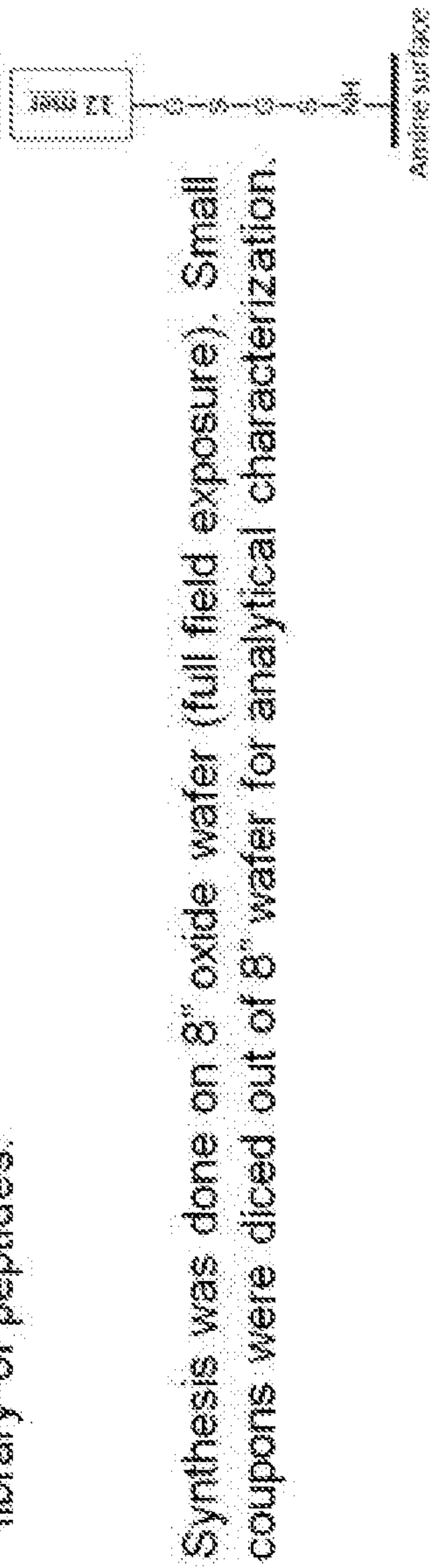
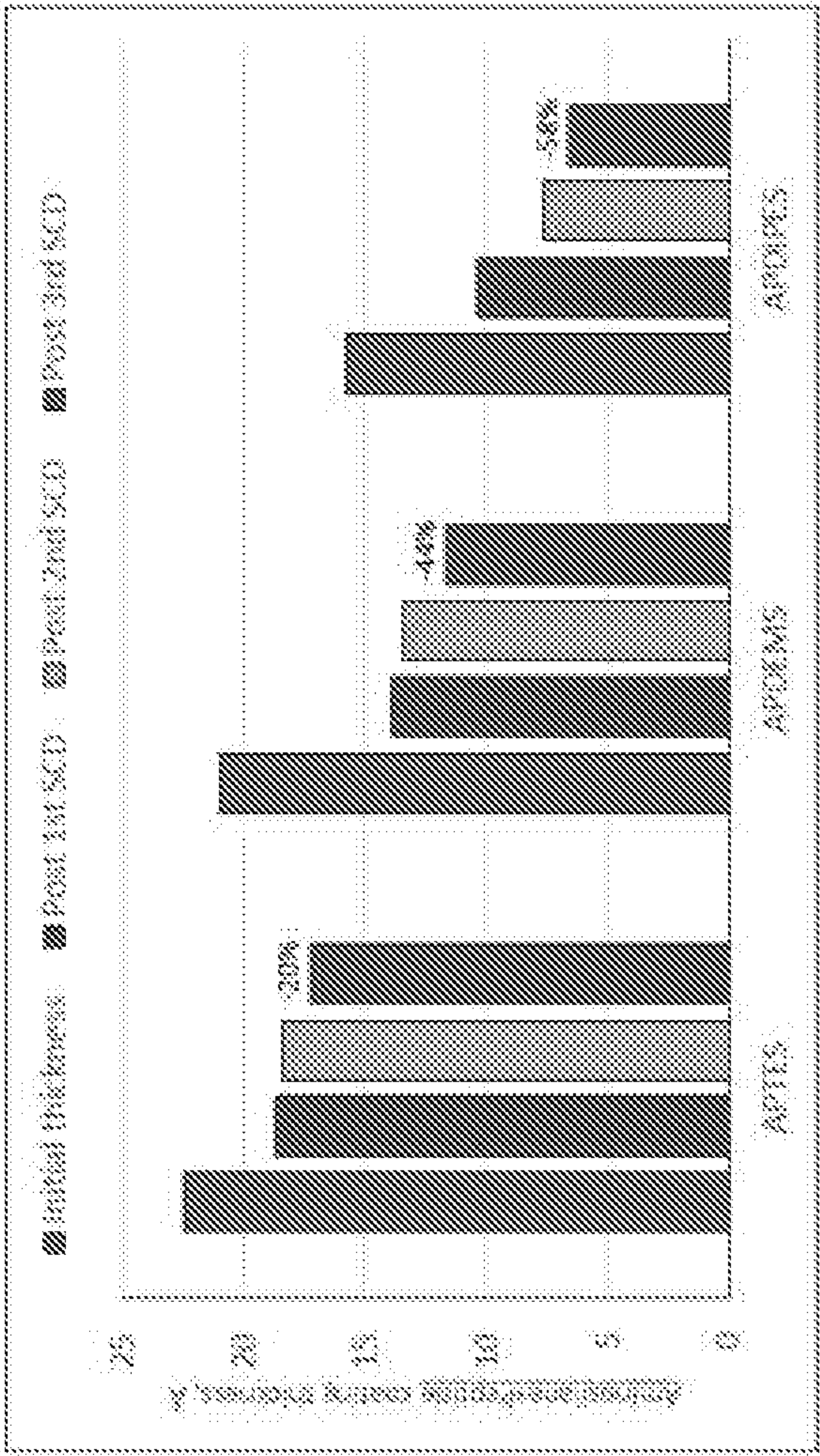


FIG. 22

Chemical Stability of Peptide-Functionalized Aminosilane Surface: APTES > APDEMS > APDIPES



- SCD mixture contains fluorinated acids i.e., Trifluoroacetic acid and Trifluoromethanesulfonic acid.
- Cumulative percent thickness loss after multiple SCDs is reported for three surfaces.

FIG. 23

Decrease in Thickness is Corroborated by XPS Data

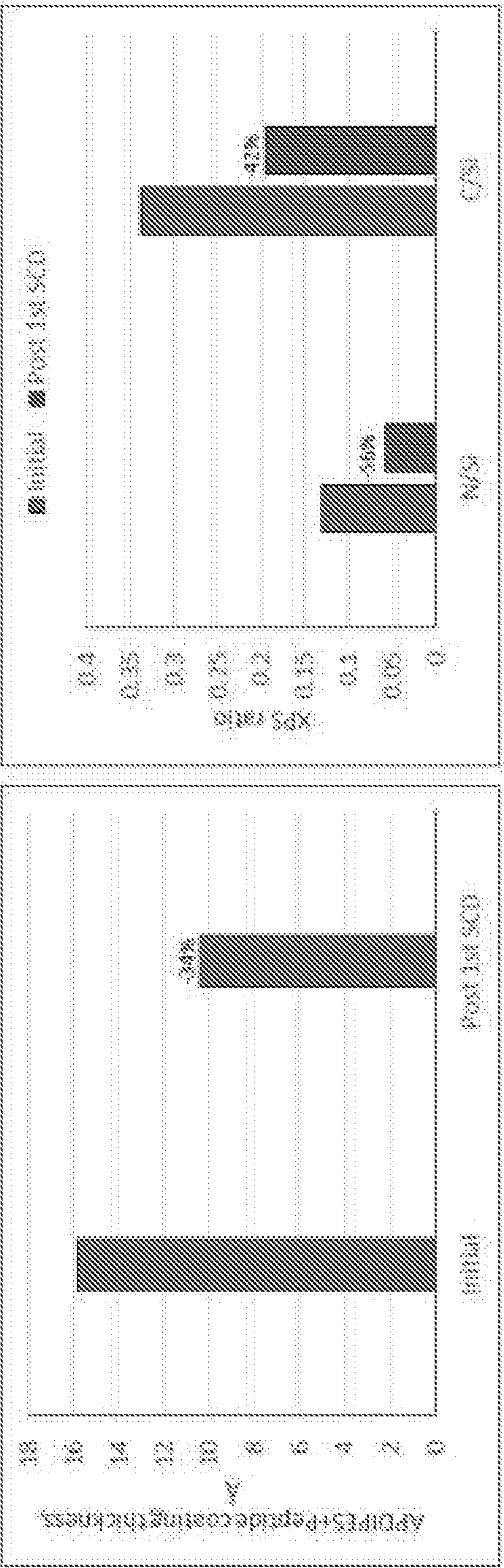
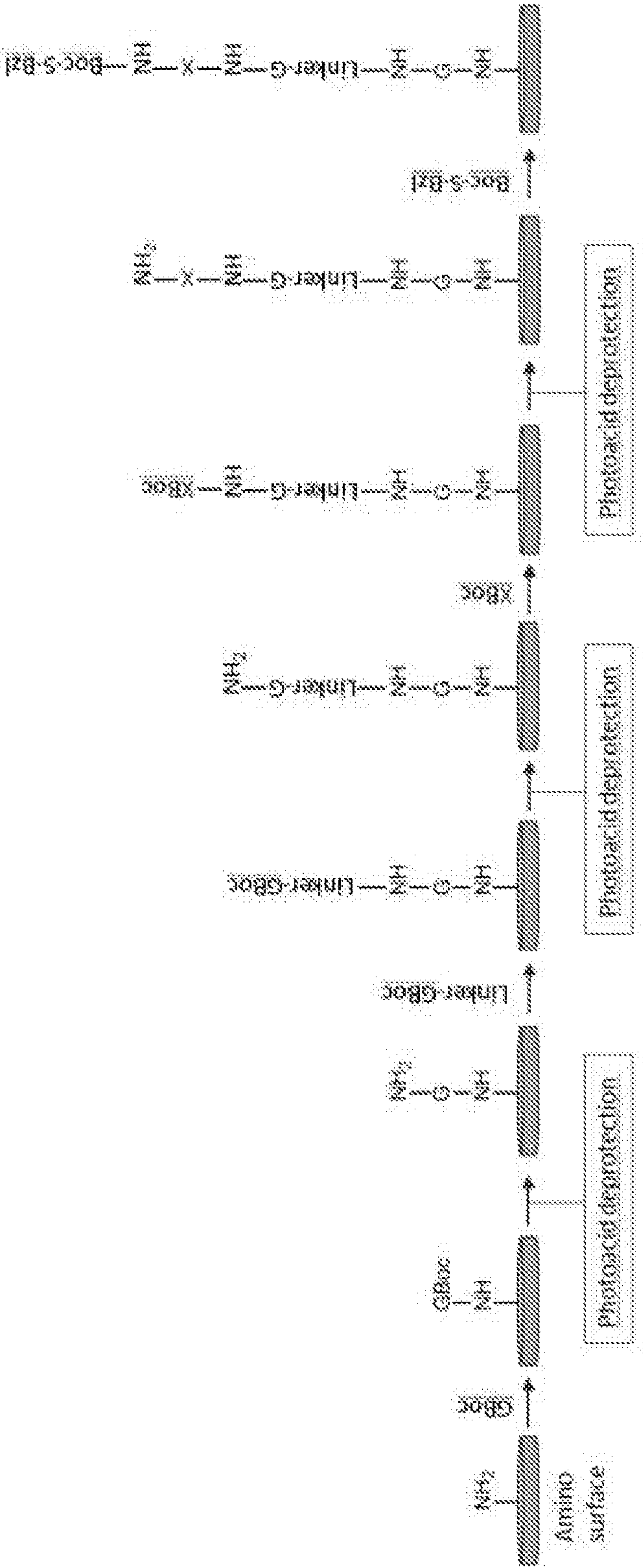


FIG. 24

Peptide Synthesis for MALDI-MS Analysis –
Capping



X = P, L, V, and F

FIG. 25

Peptide Synthesis for MALDI-MS Analysis – With Capping

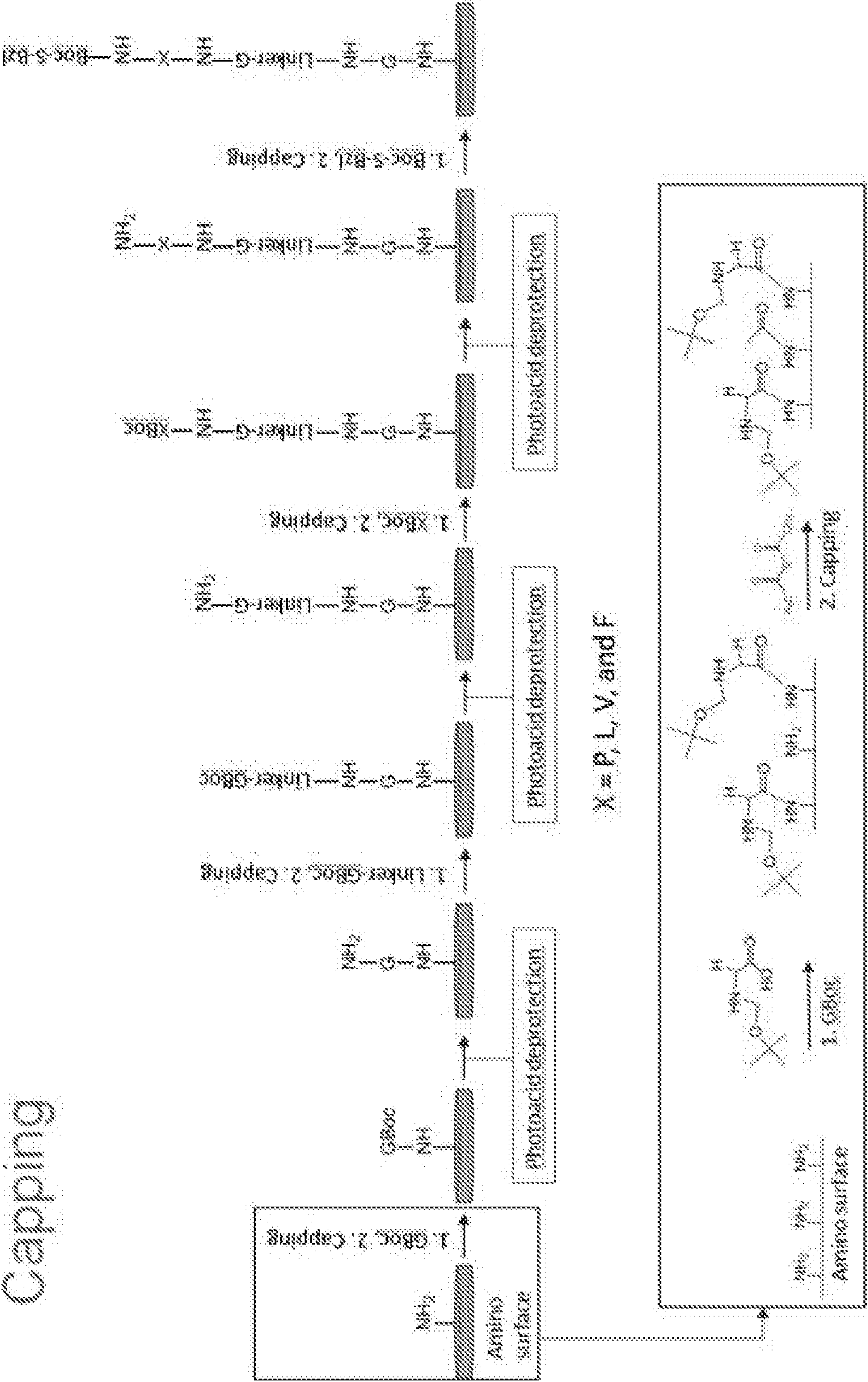


FIG. 26

APTES Surface has Highest Number of Uncoupled Amines; APDIPES has Least

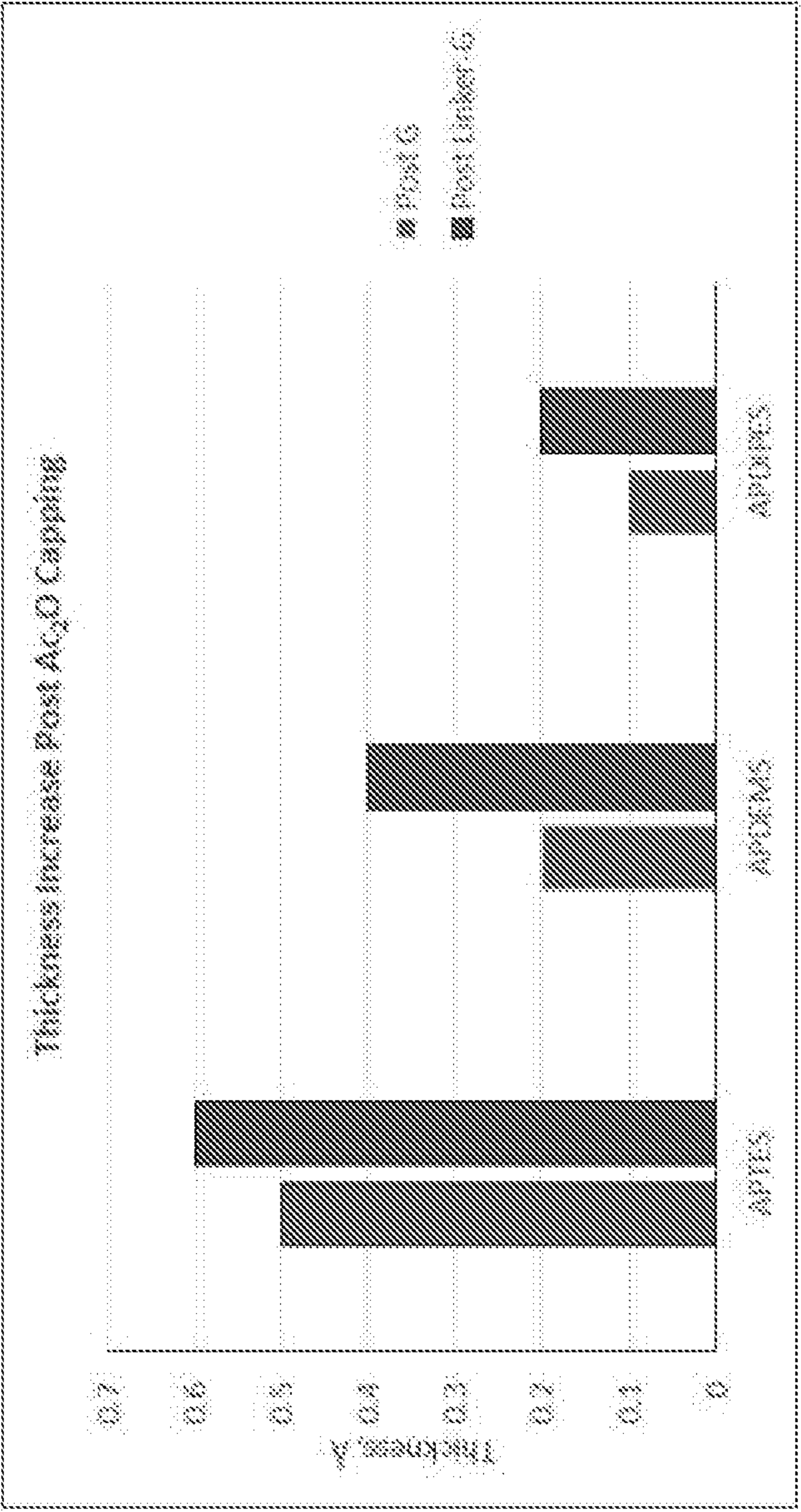
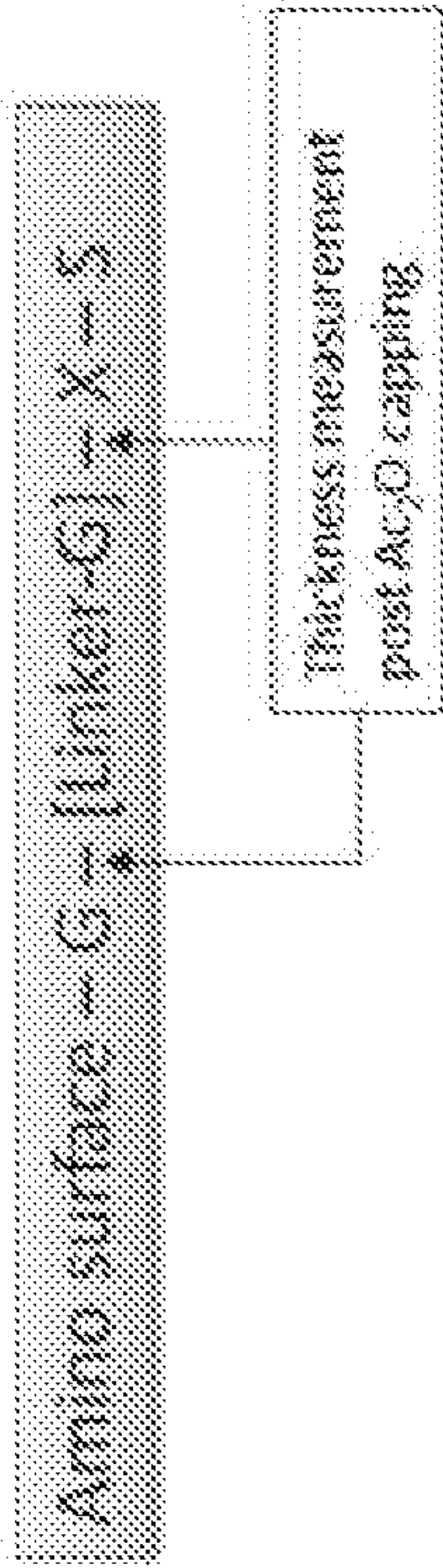
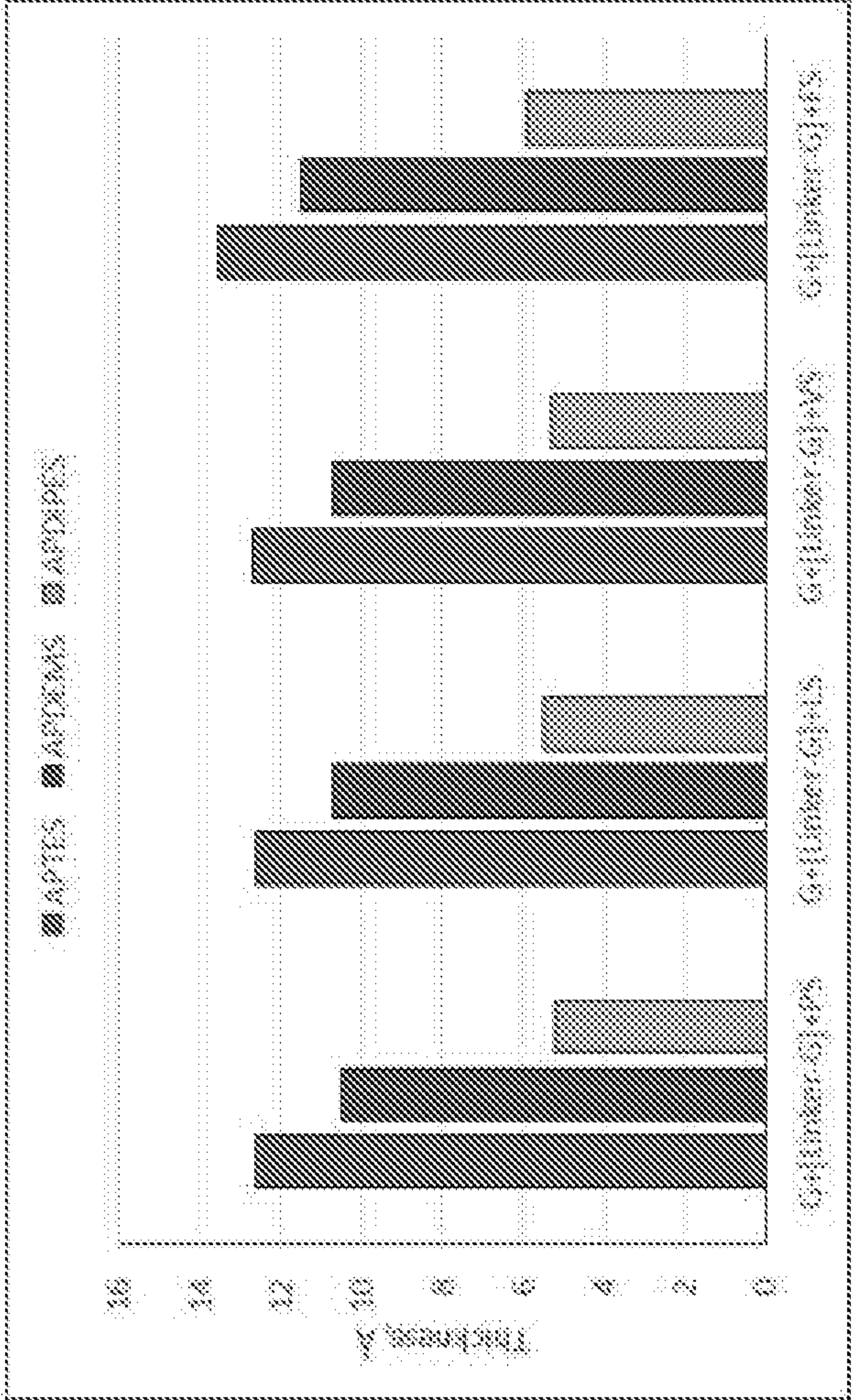


FIG. 27

Peptide Coating Thickness is Consistent with XPS
and Reactive Amine Density Data



Amino surface ~ G ~ [Linker-G] ~ X ~ S, where X = P,L,V,F

Reactive amine density and N/Si by XPS: APTES > APDMS > APDIPES

FIG. 28

Surface Preparation for MALDI-MS Analysis

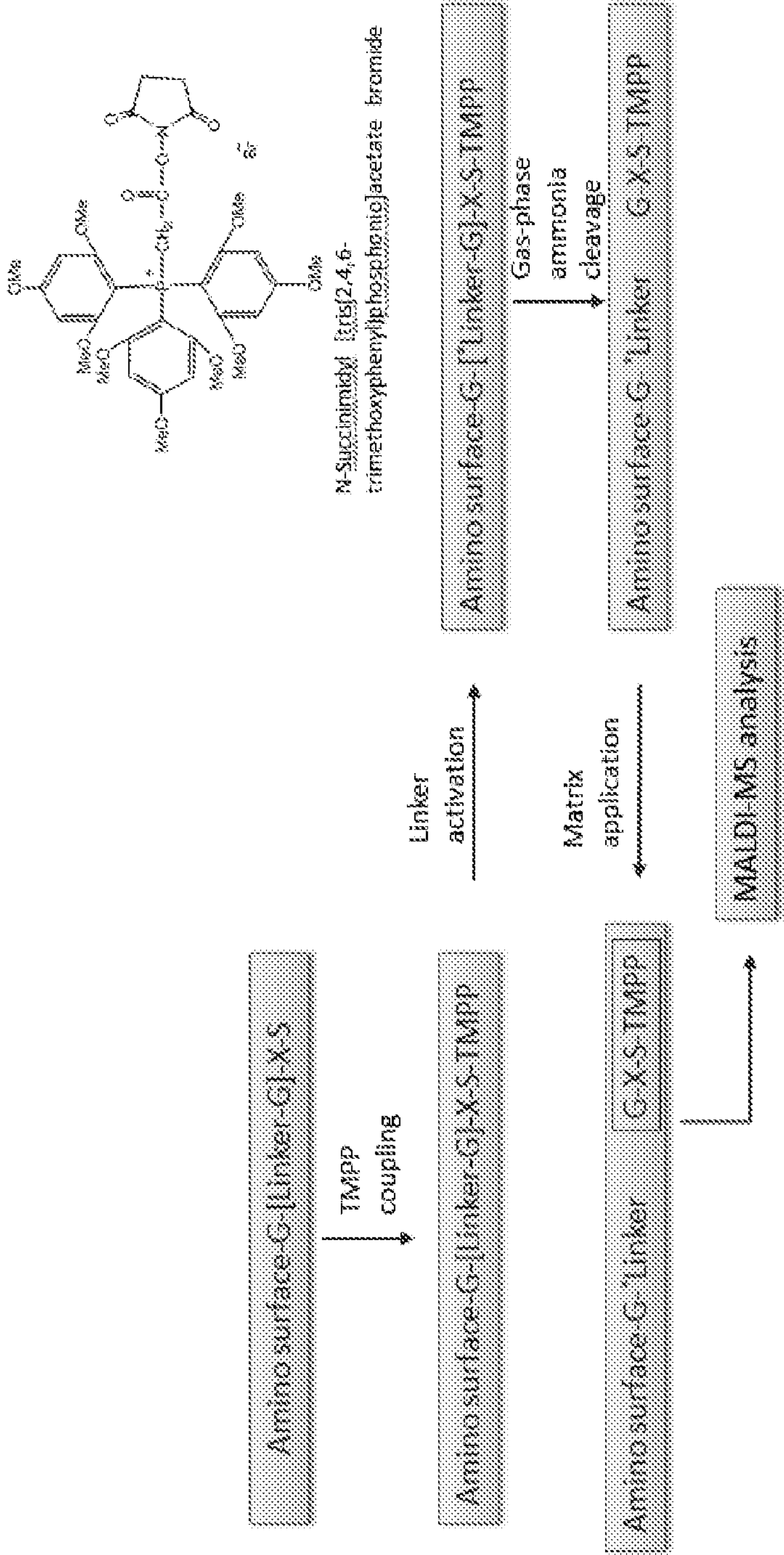


FIG. 29

MALDI-MS Spectra – SPG Synthesis

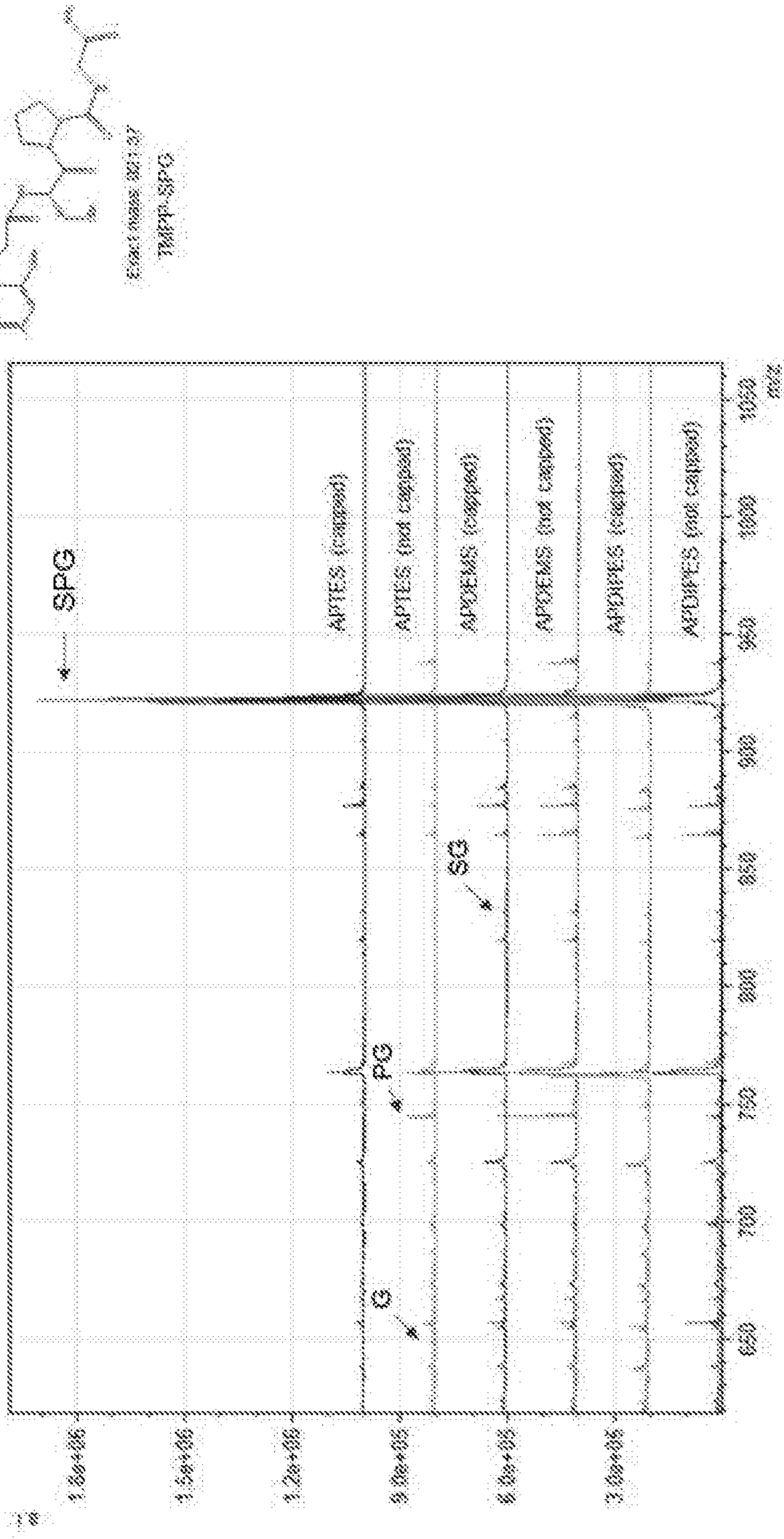
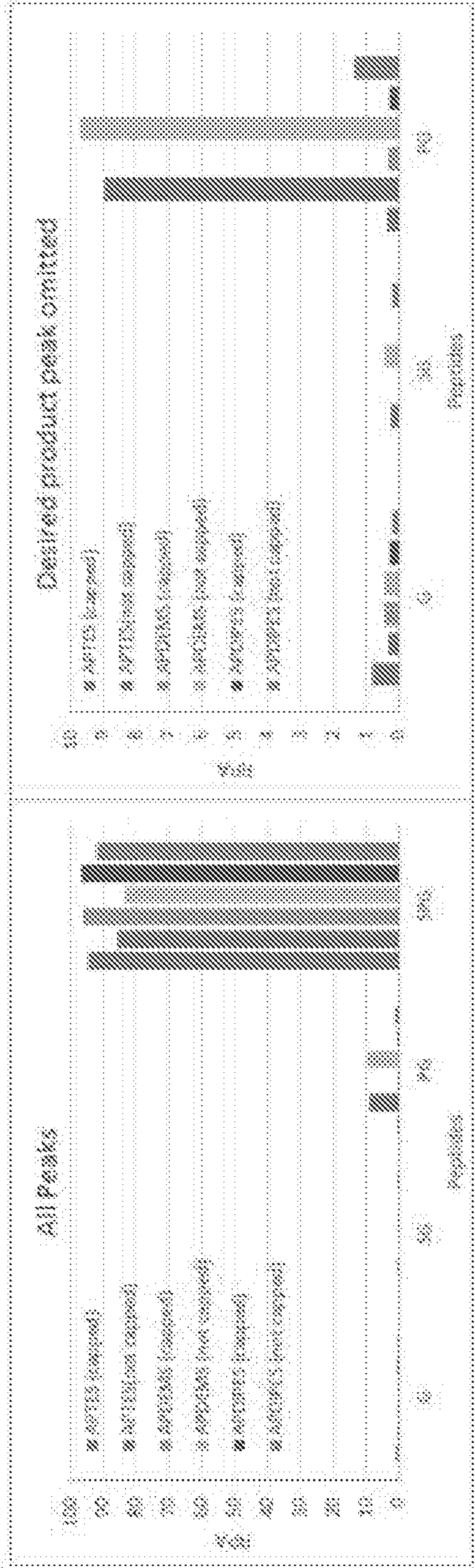


FIG. 30

SPG Synthesis – Capping Improves Probe Purity;
APDIPES Surface Produces Highest Percentage of Pure
Probes

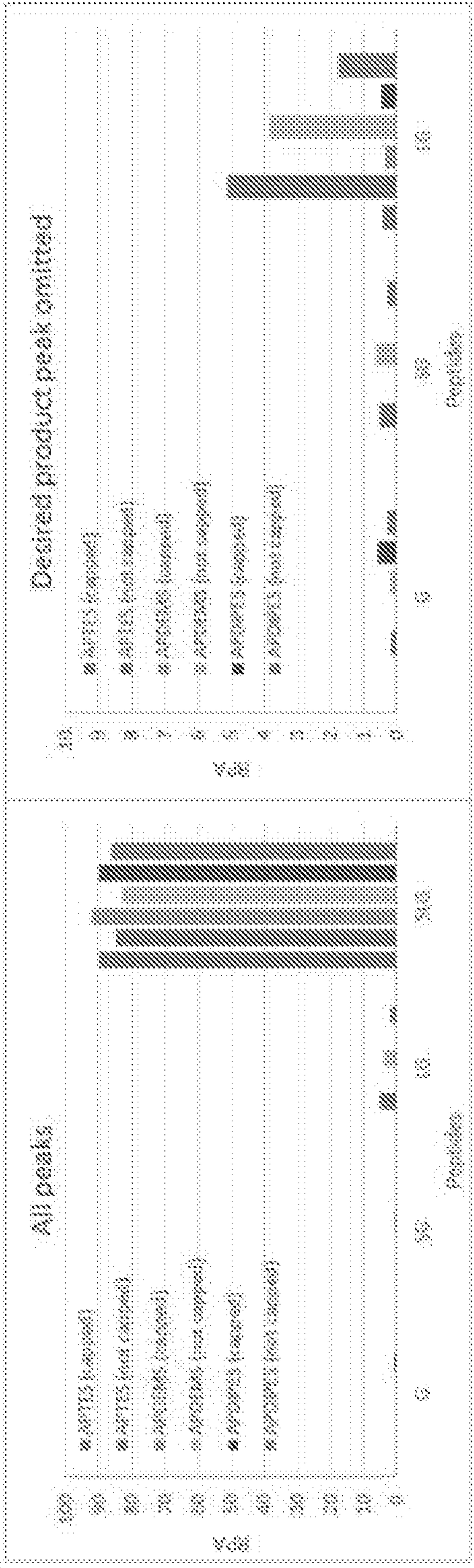


Surface type	RPA desired/ Sum (RPA undesired peaks)
APTES (capped)	82
APTES (not capped)	9
APDIPES (capped)	190
APDIPES (not capped)	176
APDIPES (not capped)	52

RPA: Relative percent abundance

FIG. 32

SLC Synthesis – Capping Improves Probe Purity; Capped APDEMS Surface Produces Highest Percentage of Pure Probes



Surface type	RPA desired/ Sum (RPA undesired peak)
APTES (capped)	214
APTES (not capped)	255
APDEMS (capped)	18
APDEMS (not capped)	86
APDEMS (not capped)	37

RPA: Relative percent abundance

FIG. 33

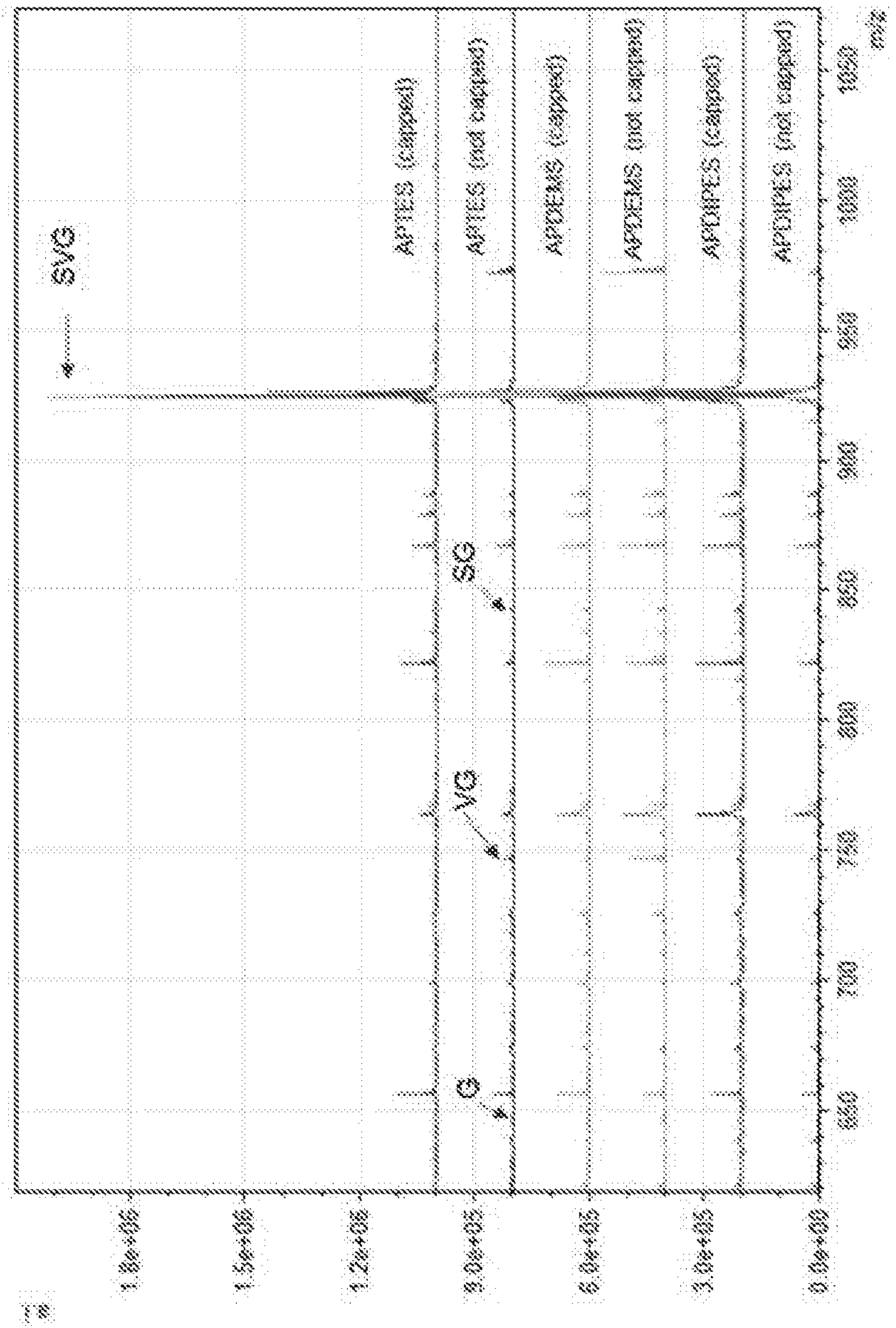
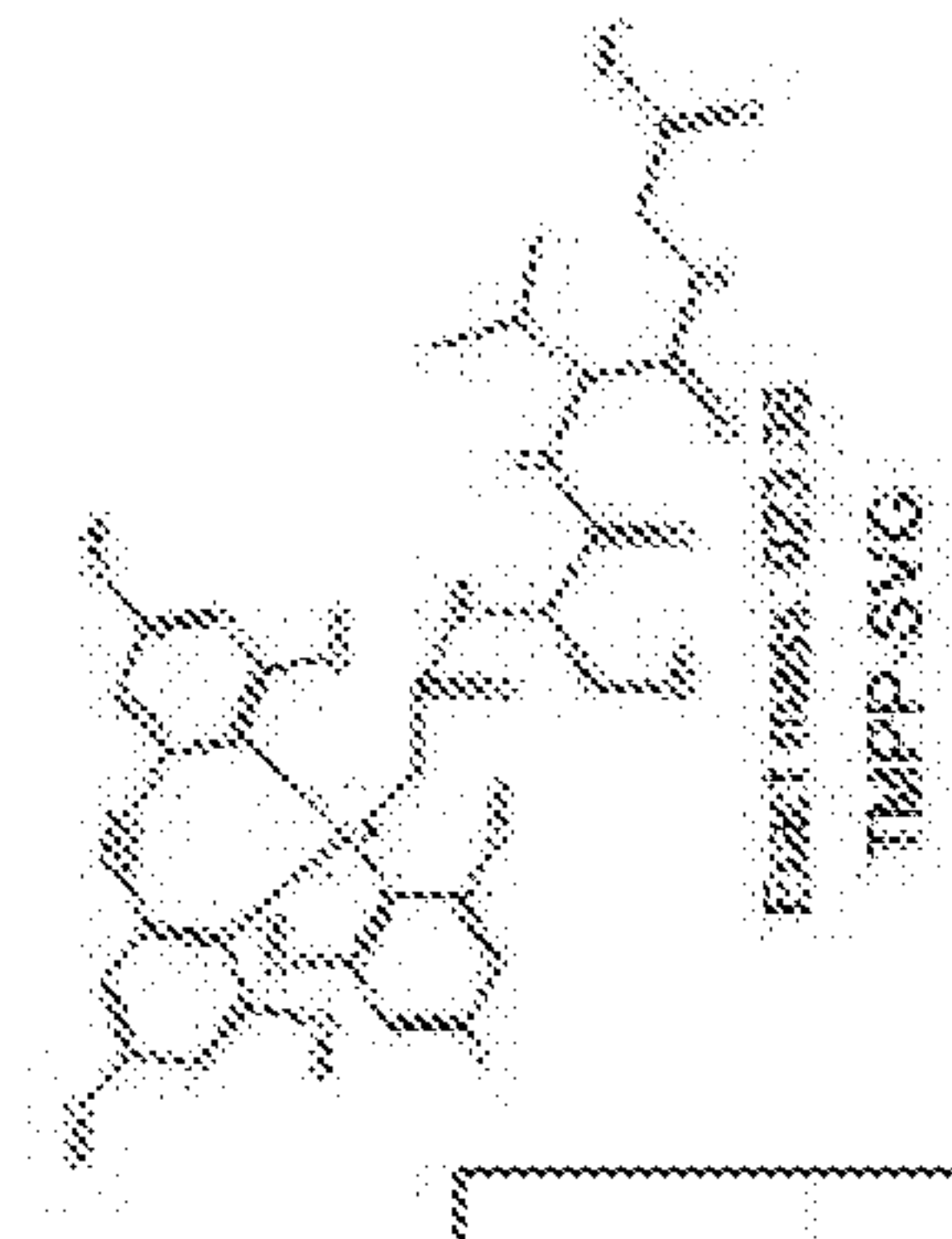
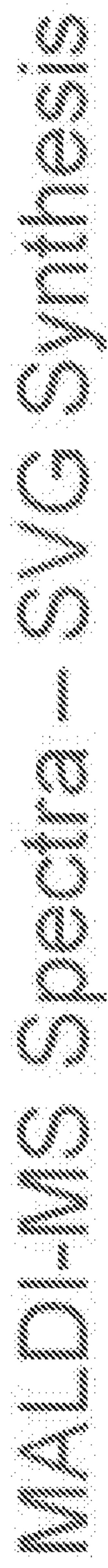


FIG. 34

SVG Synthesis – Capped APDIPES Surface
Produces Highest Percentage of Pure Probes

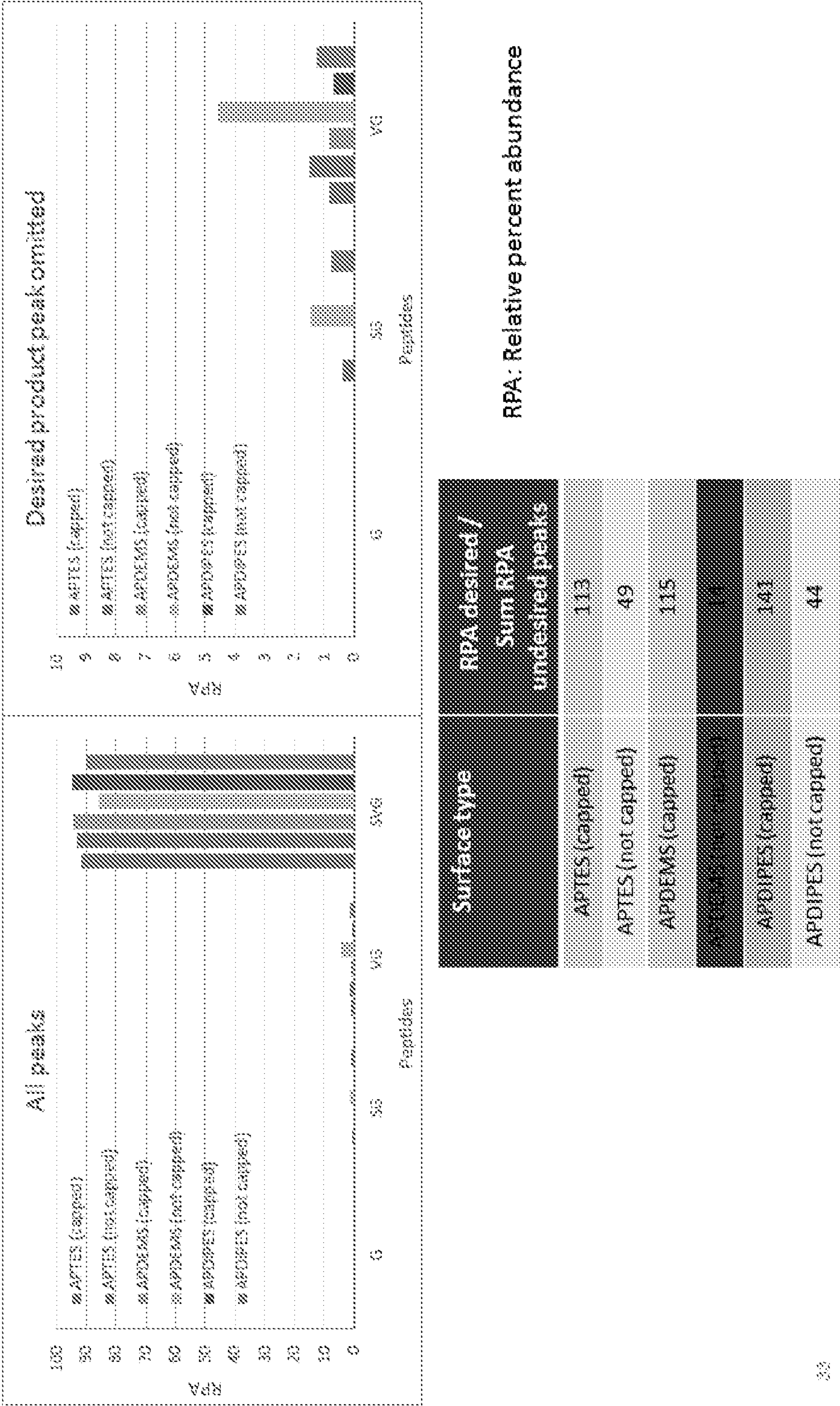


FIG. 35

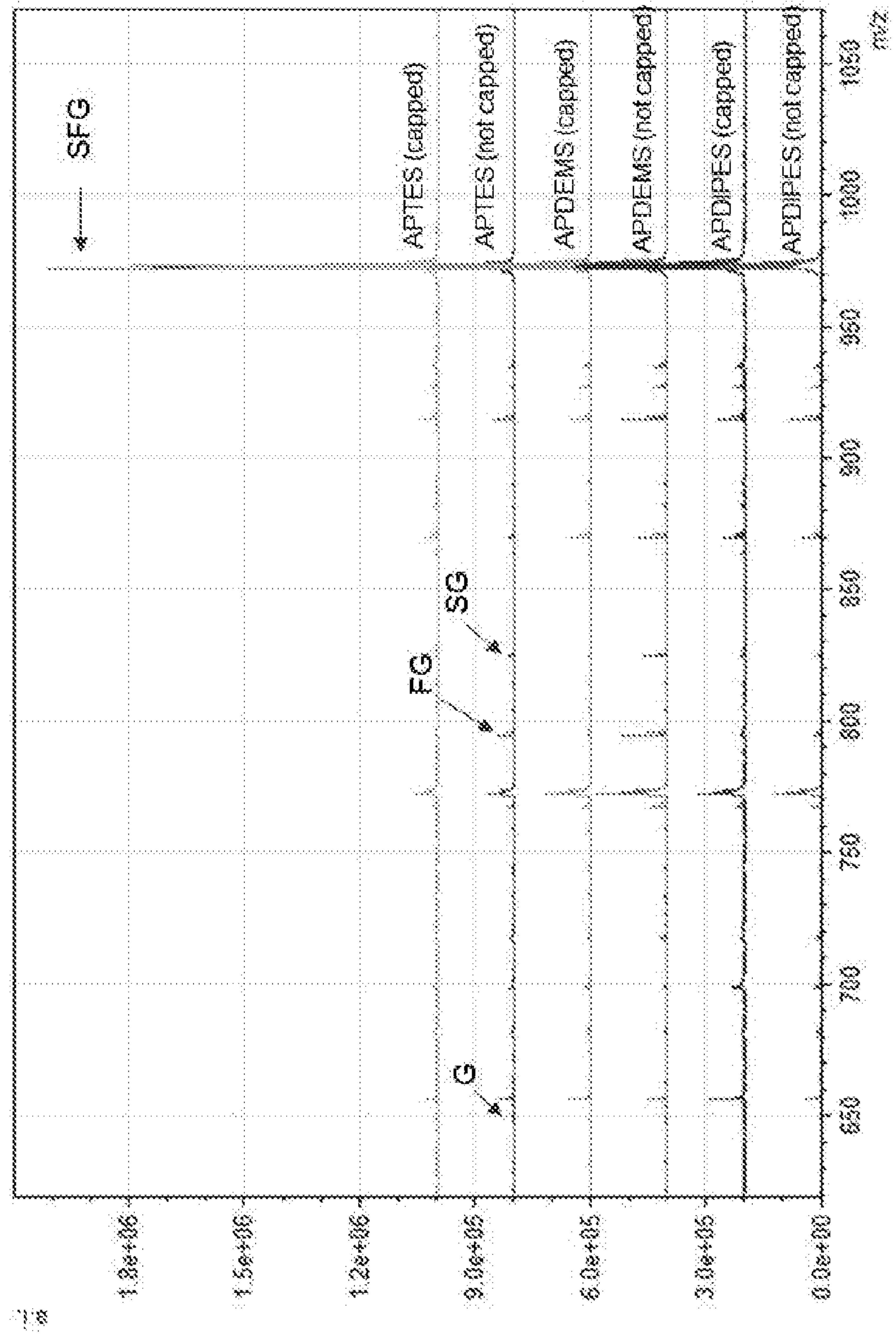
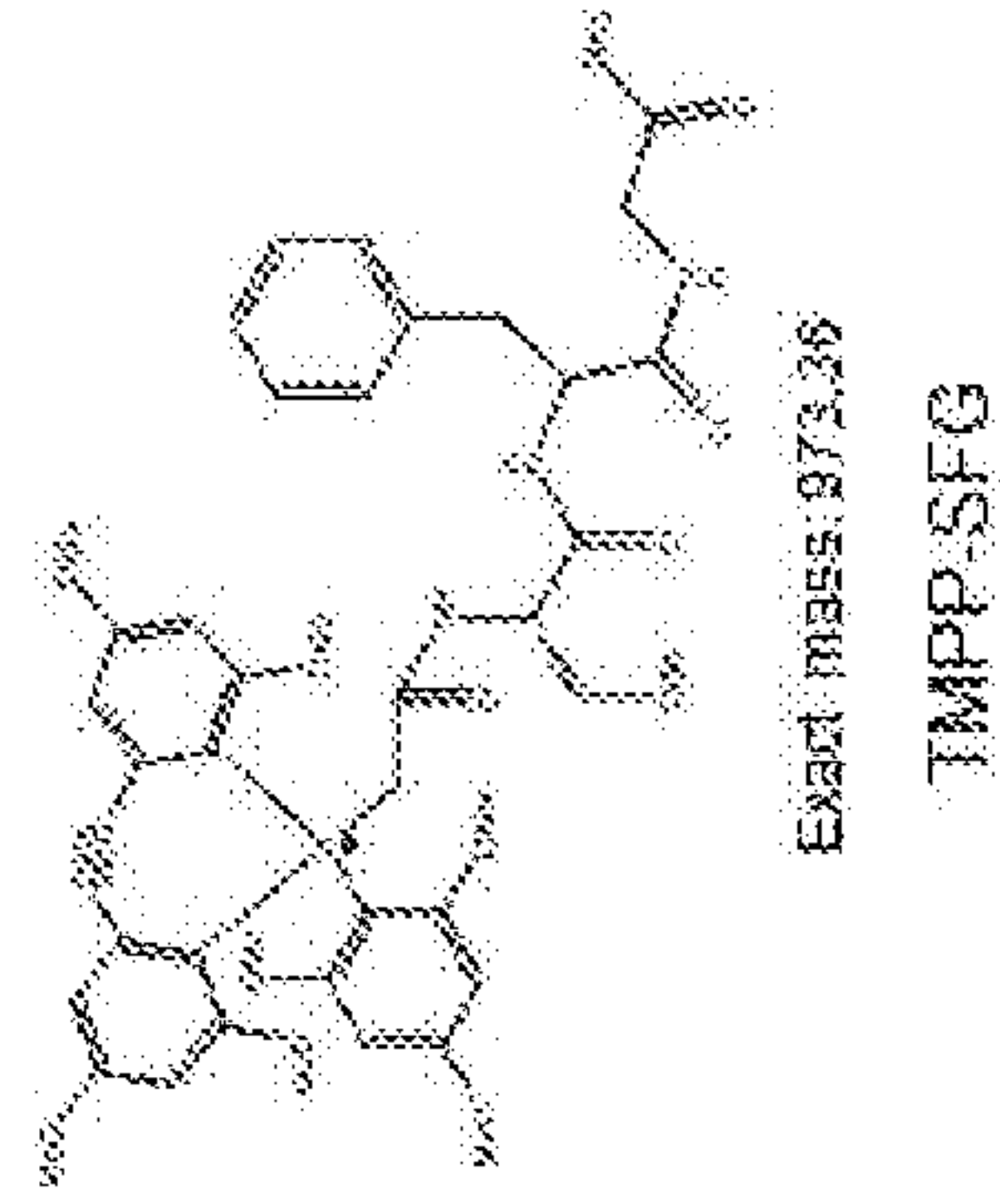
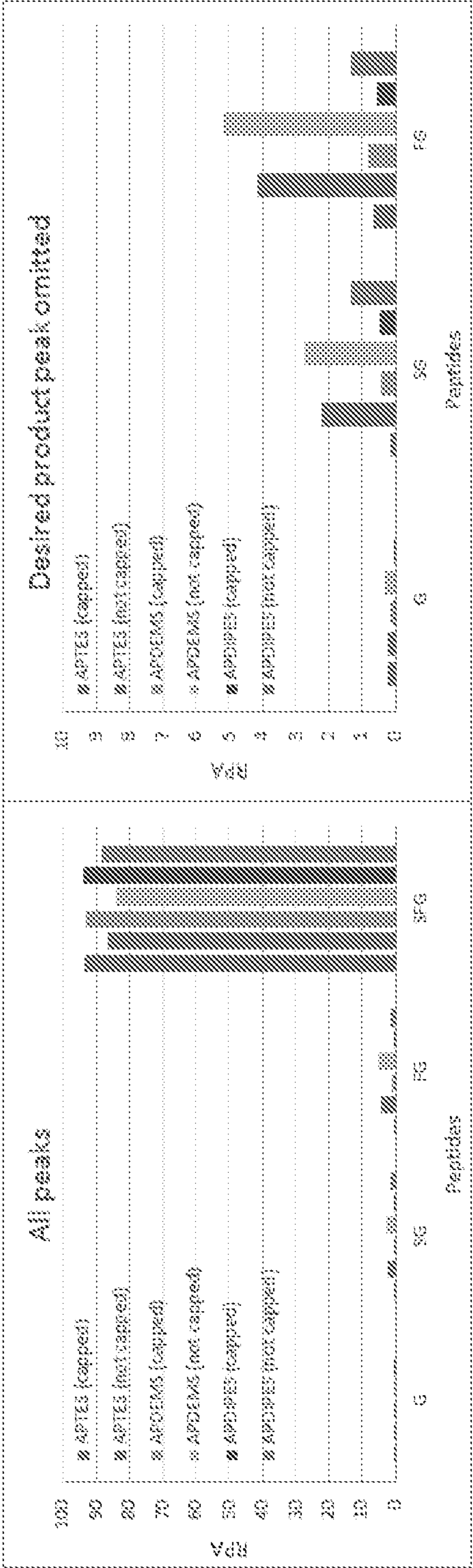


FIG. 36

SFG Synthesis – Capping Improves Probe Purity;
Capped Surface Produces Highest Percentage of Pure
Probes



Surface type	RPA desired/ Sum (RPA undesired peaks)
APTES (capped)	95
APTES (not capped)	13
APDEMS (capped)	67
APDEMS (not capped)	1
APDIPES (capped)	82
APDIPES (not capped)	33

RPA: Relative percent abundance

FIG. 37

Summary

- Three aminosilanes yield coatings with comparable thickness but different hydrophilicity.
- Hydrophilicity of the surface: APTES > APDEMS > APDIPES
- Nitrogen content of the coating is a function of number of ethoxy groups in aminosilane.
- N content of aminosilane coating: APTES > APDEMS > APDIPES
- All aminosilanes generate smooth coatings.
- Chemical stability of peptide-functionalized aminosilane surface to SCD: APTES > APDEMS > APDIPES
- Acetic anhydride capping helps improve probe purity.
- APTES produces lowest percentage of pure probes. In general, APDIPES surface produces highest percentage of pure probes.

FIG. 1

PL8 Molecule (HT960)

