α-Helical Polypeptide Films Grown From Sulfide or Thiol Linkers on Gold Surfaces

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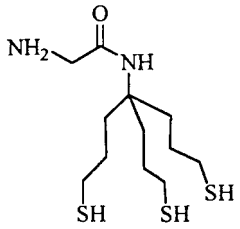
Dedicated to Professor *André M. Braun* on the occasion of his 60th birthday

We prepared two new linkers, S-functionalized adamantane derivatives **2** and **3**, which bind as monolayers on polycrystalline gold. From these surface anchors, both L- and D-isomers of alanine can be grown as thin films of α-helical polypeptides directed from the gold surface by using the appropriate *N*-carboxyalanine anhydride. FT-IR Studies show that these layers are roughly 1000-Å thick and that, under the same growth conditions, the L-polypeptide layers grow at a rate *ca.* 30% greater than that of the non-natural D-amino acid. X-Ray photoelectron spectroscopy studies show that, upon equilibration, all three S-atoms of the sulfide moieties of **2** are bound to the gold surface, and that, on average, three of the four thiols of **3** are chemoadsorbed. The essential role of H_2O on the surface of these films as a necessary component in these gas-phase polymerization reactions is demonstrated.

1. Introduction. – Previously [1], we reported the controlled growth of α-helical peptides from the surface of gold and of indium-tin oxide (ITO) modified by a thiol linking agent. The resulting layers are oriented nearly vertically to the metal surface and are relatively thick, exceeding 1000 Å. Furthermore, we have shown that the layers can be smoothed to a fairly uniform length by enzymatically grooming longer peptide chains with retention complete surface coverage and α-helicity of the layers [2]. These highly oriented supramolecular materials may serve as optical switches based on presumed nonlinear optical properties resulting from unidirectional alignment of polarization [3][4].



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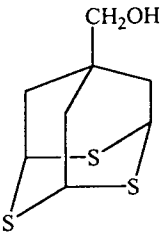

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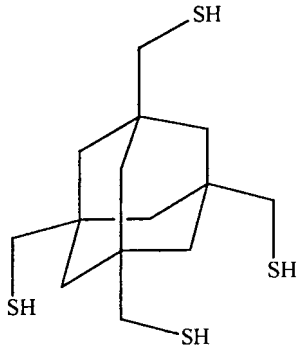

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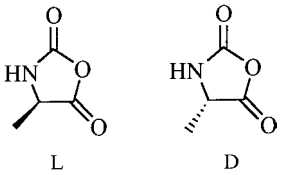

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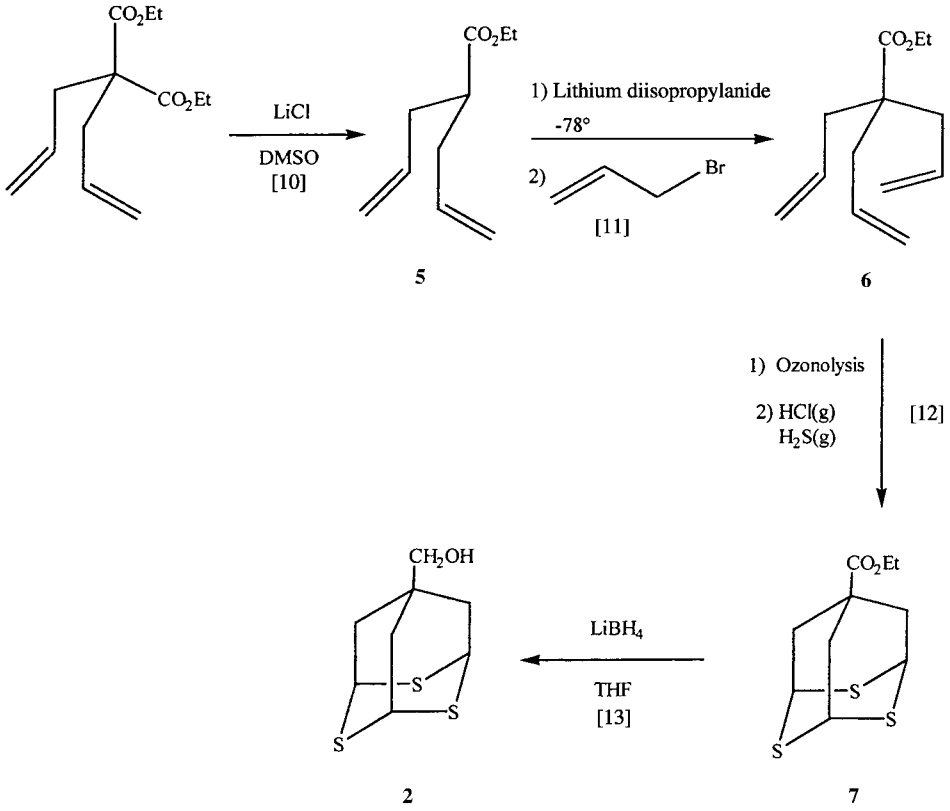
Aminotrimercaptoamide **1** has been shown to be a versatile linker, having been used both as a seed site for the growth of oriented helical peptides and as an attachment site for anchoring preformed polymers of defined composition [5]. In addition, the amino functional group in **1** participates in *in situ* coupling with active esters, *e.g.*, for the attachment of the emissive dye fluorescamine [6]. Aminotrimercaptoamide **1** has been used as a linker for antibodies on colloidal metals (Au, Ag, and Ni) for the quantitative monocloning of CD4 and CD8 lymphocytes and anti-red-blood-cell KC16 in whole blood by forward light scatter using flow cytometry; these results compared well with standard analysis utilizing fluorescent markers [7][8].

Although **1** is an effective linking agent for binding surface probes, monolayers of aminotrimercaptoamide **1** are substantially disordered, with an average 30% of the mercapto groups not bound to the surface [6]. Seeking more-ordered surface layers, we now synthesized two new surface linkers, the trithiaadamantane derivative **2** from **5** *via* **6** and **7** and the adamantane-tetrathiol derivative **3** from **8** *via* **9**–**11** (*Schemes 1* and *2*). We report here the binding properties of these linkers, and the utility of both the hydroxy group of **2** and the unbound thiol appendage of **3** as sites for the initiation of surface polymerization of *N*-carboxyalanine anhydride **4** to form surface-bound polypeptides.

2. Experimental. – 2.1. *General*. All reagents were purchased from *Aldrich* and used without further purification unless otherwise specified. Et_2O and THF were freshly distilled from a deep blue soln. resulting from Na and benzophenone. Ozone was generated using a *Welsbach* generator, and the flow rate was determined prior to and after use by titration. All glassware was flame-dried before use, and reactions were carried out under a positive pressure of Ar. Chromatography or *Chromatotron*^{TM} (*Harrison Research*): according to *Still* [9], silica gel. HPLC: *Waters Prep-500* system with two *Prep-Pak* cartridges; prior to HPLC, products were run through a short column of silica gel. M.p.: *Mel-Temp* apparatus; uncorrected. Transmission FT-IR spectra: *Nicolet 510P* spectrometer; KBr pellet; in cm^{-1}. Grazing angle reflectance (GAR) FT-IR spectra: *Nicolet 550-FTIR* instrument, p-polarized light at an incidence angle $\theta = 80°$, equipped with a liq.-N_2-cooled *MCT/A* detector; typically, 512 scans at a resolution of 4 cm^{-1} were collected. NMR Spectra: *General Electric QE-300-MHz* spectrometer; in $CDCl_3$; chemical shifts δ in ppm, coupling constants J in Hz. High resolution (HR) mass spectra were obtained at the NC State University Mass Spectrometry Laboratory for Biotechnology.

2.2. *2,4,9-Trithiaadamantane-7-methanol* (=*2,4,9-Trithiatricyclo[3.3.1.1*^{3,7}*]decane-7-methanol*; **2**). *Ethyl Diallylacetate* (= *2-(Prop-2-enyl)pent-4-enoic Acid Ethyl Ester*; **5**). According to the procedure of *Krapcho et al.* [10].

Scheme 1. *Synthesis of Trithiaadamantanemethanol* **2**

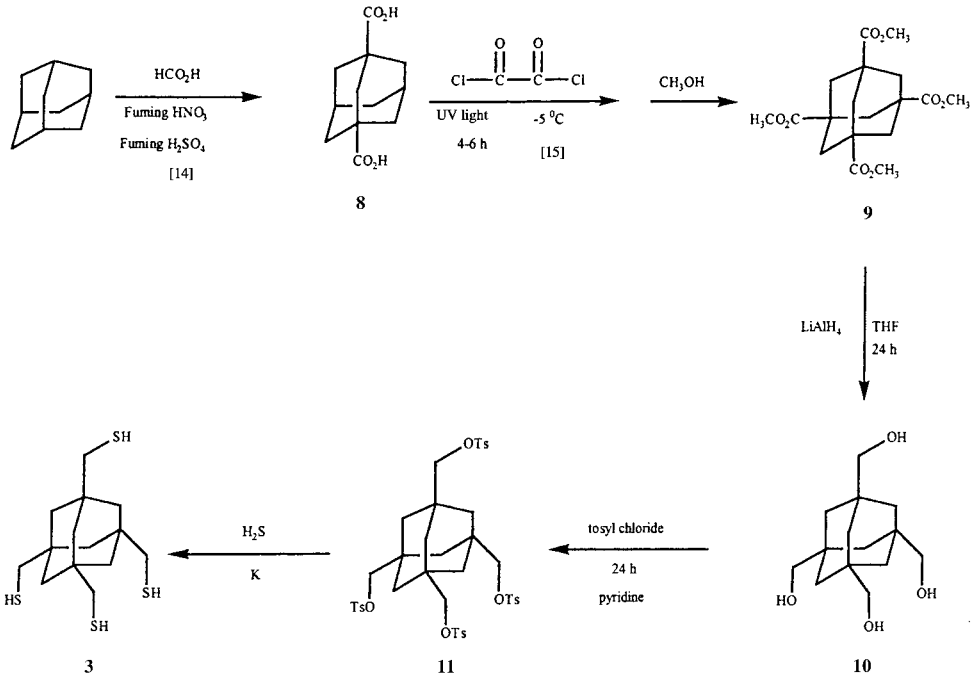


Ethyl Triallylacetate (= *2,2-Di(prop-2-enyl)pent-4-enoic Acid Ethyl Ester*; **6**). According to the procedure of *Beaulieu* and *Deslongchamps* [11].

Ethyl 2,4,9-Trithiaadamantane-7-carboxylate (= *2,4,9-Trithiatricyclo[3.3.1.1^{3,7}]decane-7-carboxylic Acid Ethyl Ester*; **7**). According to a modified procedure of *Lindgren* [12]. A stirred soln. of **6** (3.0 g, 14 mmol) in abs. EtOH (60 ml) was cooled under N_2 to $-78°$ and then ozonized until the blue color of excess ozone persisted (average 2.5 h). Ar was bubbled through the soln. to purge excess ozone, and when the blue color had dissipated, a soln. of Me_2S (1.4 ml, 19 mmol, 1.3 equiv.) in abs. EtOH (9 ml) was added rapidly dropwise. The cooling bath was removed and the soln. allowed to warm to r.t. The soln. was concentrated to *ca.* 30 ml *in vacuo* at r.t. and then added dropwise with cooling in an ice bath to a stirred sat. soln. of HCl gas in abs. EtOH (60 ml) that had H_2S gas bubbling through it. Almost immediately, a yellow precipitate of polymeric sulfur began to appear. After addition was complete, the cooling bath was removed, and H_2S bubbling was continued for another 15 min. The mixture was poured into H_2O and extracted with CH_2Cl_2 (3×50 ml). The combined org. layer was washed with H_2O (2×50 ml), filtered through cotton, and evaporated. The residue was filtered through silica gel with hexanes/AcOEt 4:1 and then purified by semi-prep. HPLC (hexanes/AcOEt 6:1). The main product was further purified by recrystallization from hexanes/AcOEt: 0.95 g (25%) of **7**. White crystals. M.p. 156°. TLC (silica gel, hexanes/AcOEt 6:1): R_f 0.41. FT-IR (KBr): 2972*m*, 2954*m*, 2923*m*, 1727*s*, 1441*m*, 1419*m*, 1363*m*, 1284*m*, 1265*s*, 1210*s*, 1193*m*, 1096*m*, 1048*s*, 1009*m*, 929*m*, 853*m*. ^{1}H-NMR: 4.33 (t, $J = 3.2$, 3 H); 4.19 (q, $J = 7.1$, 2 H); 2.90 (d, $J = 3.8$, 6 H); 1.28 (t, $J = 3.2$, 3 H). ^{13}C-NMR (75 MHz): 174.9; 61.1; 41.2; 40.0; 38.4; 14.1. HR-CI-MS: 263.02437 ($C_{10}H_{15}O_2S_3^+$; calc. 263.02342).

2,4,9-Trithiaadamantane-7-methanol (**2**). According to a modified procedure of *Brown* and *Narasimhan* [13]. To a soln. of **7** (0.44 g, 1.7 mmol) in anh. THF (20 ml) was added $LiBH_4$ (0.15 g, 6.7 mmol, 4.0 equiv.). The

Scheme 2. *Synthesis of Adamantanetetramethanethiol* **3**



suspension was left stirring at r.t. under Ar overnight. After being heated under reflux for 1 h, the mixture was cooled in ice. Dry MeOH (from 3 Å molecular sieves) was added carefully (with foaming). The soln. was warmed to r.t. and made weakly acidic with conc. HCl soln. Solvents were evaporated, Et_2O was added, and the resulting white solid was distributed between H_2O and Et_2O. The aq. layer was extracted with Et_2O (3×25 ml), the combined Et_2O extract washed with brine, dried (4 Å molecular sieves), and evaporated, and the residue dried under high vacuum for 24 h: 0.38 g (100%) of crude **2**. The solid was purified by filtration through silica gel with 5% MeOH/CH_2Cl_2 followed by semi-prep. HPLC (2% MeOH/CH_2Cl_2): **2**. White solid. M.p. 199–200°. TLC (silica gel, 2% MeOH/CH_2Cl_2): R_f 0.34. ^{1}H-NMR: 4.33 (*t*, *J* = 3.2, 3 H); 3.38 (*s*, 2 H); 2.58 (*d*, *J* = 3.6, 6 H); 1.53 (*s*, 1 H). ^{13}C-NMR (75 MHz): 73.5; 42.0; 40.5; 31.9. HR-MS: 220.0047 ($C_8H_{12}OS_3^+$; calc. 220.0050).

2.3. *Adamantane-1,3,5,7-tetramethanethiol* (= *Tricyclo[3.3.1.1^{3,7}]decane-1,3,5,7-tetramethanethiol*; **3**). *Adamantane-1,3-dicarboxylic Acid* (= *Tricyclo[3.3.1.1^{3,7}]decane-1,3-dicarboxylic Acid*; **8**). According to the procedure of *Grimme et al.* [14].

Tetramethyl Adamantane-1,3,5,7-tetracarboxylate (= *Tricyclo[3.3.1.1^{3,7}]decane-1,3,5,7-tetracarboxylic Acid Tetramethyl Ester*; **9**). According to the procedure of *Bashir-Hashemi* and *Li* [15] or to this procedure but with 4.5 g (25 mmol) of adamantane-1-carboxylic acid.

Adamantane-1,3,5,7-tetramethanol (= *Tricyclo[3.3.1.1^{3,7}]decane-1,3,5,7-tetramethanol*; **10**). According to the procedure of *Landa* and *Kamycek* [16]. ^{1}H-NMR (300 MHz, $CD_3OD/CDCl_3$ 1:1): 4.49 (*s*); 3.25 (*s*, 8 H); 1.16 (*s*, 12 H). ^{13}C-NMR (75 MHz, $CD_3OD/CDCl_3$ 1:1): 72.5; 40.4; 36.3.

Adamantane-1,3,5,7-tetramethanol Tetratosylate (= *Tricyclo[3.3.1.1^{3,7}]decane-1,3,5,7-tetramethanol Tetrakis(4-methylbenzenesulfonate)*; **11**). According to the procedure of *Nakazaki* and *Naemura* [17]. ^{1}H-NMR (300 MHz, $CDCl_3$): 7.72 (*d*, *J* = 8.3, 8 H); 7.36 (*d*, *J* = 8.2, 8 H); 3.53 (*s*, 8 H); 2.47 (*s*, 12 H); 1.10 (*s*, 12 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 145.2; 132.5; 130.0; 127.9; 77.4; 38.7; 34.7; 21.7.

Adamantane-1,3,5,7-tetramethanethiol (= *Tricyclo[3.3.1.1^{3,7}]decane-1,3,5,7-tetramethanethiol*, **3**). According to a modified procedure by *Grimme et al.* [14]. Dry 2-ethoxyethanol (sequentially dried over 4 Å molecular sieves; 15 ml) was deoxygenated by sparging with Ar in an ultrasonic bath for 30 min and cooled in ice with stirring. Then 0.58 g (15 mmol) of K metal was added in small pieces. H_2S Gas was bubbled through the ice-

cooled soln. for 30 min, and then **11** (1.3 g, 1.5 mmol) was added. The soln. was warmed to r.t. and then heated under reflux for 12 h. The mixture was cooled, diluted with H_2O and CH_2Cl_2, and acidified with 2N HCl. The org. layer was extracted twice more with CH_2Cl_2, the combined org. layer washed with H_2O (2×25 ml), filtered through cotton, and evaporated, and the residue dried under high vacuum. The product was purified by semi-prep. HPLC (silica gel, hexanes/AcOEt 10:1): 0.20 g (40%) of **3**. White solid. M.p. 144–145°. FT-IR (KBr): 2916*s*, 2891*m*, 2841*m*, 2571*w* (SH), 1447*m*, 1412*w*, 1348*s*, 1280*m*, 1244*w*, 1212*w*, 1152*w*, 1038*w*, 978*w*, 696*m*. ^{1}H-NMR (300 MHz, $CDCl_3$): 2.43 (*d*, $J = 8.8$, 8 H); 1.22 (*s*, 12 H); 1.19 (*t*, $J = 8.8$, 4 H). ^{13}C-NMR (75 MHz, $CDCl_3$): 43.4; 37.2; 35.7. HR-MS: 320.0755 ($C_{14}H_{24}S_4^+$; calc. 320.0761).

Monolayer Formation. Gold surfaces for monolayer formation were prepared on a Si wafer by evaporation of Cr (80 Å), followed by Au (2000 Å) (*Biomedical Microsensor Laboratory*, NCSU). The freshly prepared gold surfaces were washed sequentially with H_2SO_4 soln./40% aq. H_2O_2 soln. 3:1, copious amounts of *Millipore*-filtered H_2O (which had a resistance of 18 MΩcm), and abs. EtOH, before being dried under a stream of Ar. Monolayers of the S-compound **2** or **3** were deposited by allowing a freshly cleaned gold wafer to soak for 24 h at r.t. in the dark in 10 ml of mM **2** or **3** in toluene that had been degassed by bubbling with Ar for 15 min. After the gold wafer had been added, the solns. were heated gently until they were fully transparent. The solns. were then allowed to cool and to stand under Ar for 24 h at r.t. in the dark. The self-assembled monolayers (SAMs) were rinsed sequentially with toluene, abs. EtOH, H_2O, and then again with EtOH, before being dried under Ar just before use.

Peptide Polymerization. Growth of the polypeptides was accomplished by the gas-phase polymerization of the *N*-carboxyalanine anhydride (=4-methyloxazolidine-2,5-dione; **4**) prepared by the reaction of L- or D-alanine with carbonic dichloride [18]. A small glassy vacuum chamber fitted with two horizontal glass rods and an outlet with stopcock was used for growth of polyalanine films in vacuum. The silicon wafer with a gold layer and a monolayer of either **2** or **3** was placed in the chamber with the gold surface up. A small quantity (*ca*. 50 mg) of *N*-carboxyalanine anhydride (**4**) was placed underneath the wafer. The chamber was wrapped with heating tape and evacuated (<0.1 Torr), and the temp. raised to and held at 70°.

Film thicknesses were determined by grazing angle reflectance (GAF) FT-IR spectroscopy, where it has been previously shown that each 0.1 absorbance represents *ca*. 300 Å [2]. We were able to grow layers approximately 1000 Å thick from both linkers **2** and **3**.

3. Results and Discussion. – 3.1. *X-Ray Photoelectron Spectroscopy.* The *Table* lists XPS results that define the extent of S-binding to the gold surface. For an S-atom bound to a highly electropositive atom such as Au, a band appears at *ca*. 160 eV, whereas for a free thiol a band appears at *ca*. 162.9 eV [19]. For trithiaadamantanemethanol **2**, the rigid adamantane-like structural constraint of the sulfide moieties apparently forces all three S-atoms to be bound to the gold surface. Indeed, the data in the *Table* (for **2**) shows that all three sulfide atoms are bound to the gold surface as soon as the monolayer forms (within 120 min). For the tetramethanethiol **3**, reasonable geometric constraints dictate that there should be at least one S-atom directed away from the

Table. *X-Ray Photoelectron Spectroscopy for Compounds* **2** *and* **3** *as SAMs on Gold*

	% Peak area Bound sulfide moiety or thiol[a])[b])	% Peak area Free sulfide moiety or thiol[a])[c])
2 at 2 h	100	0
3 at 1 h	15	85
3 at 2 h	35	65
3 at 4 h	55	45
3 at 6 h	73	27
3 at 24 h	74	26

[a]) Peak areas are reported as a percentage of the total areas from the intensity of the peaks at 160 and 162.9 V. [b]) Band for the S-atom bound to Au at 160 eV. [c]) Band for a free thiol at 162.9 eV.

metal surface, *i.e.*, not bound to the surface. The data in the *Table* shows that, initially, a monolayer of **3** on gold binds, on average, one S-atom after 1 h. As time elapses, further thiol binding takes place as the monolayer comes to full equilibration. With tight surface packing in *ca.* 6 h, three of the four S-atoms are attached to the gold surface.

3.2. *Grazing Angle Reflectance* (GAR) *FT-IR*. *Figs. 1* and *2* are IR spectra for monolayers of **2** and **3**, respectively. Both spectra exhibit the characteristic frequencies

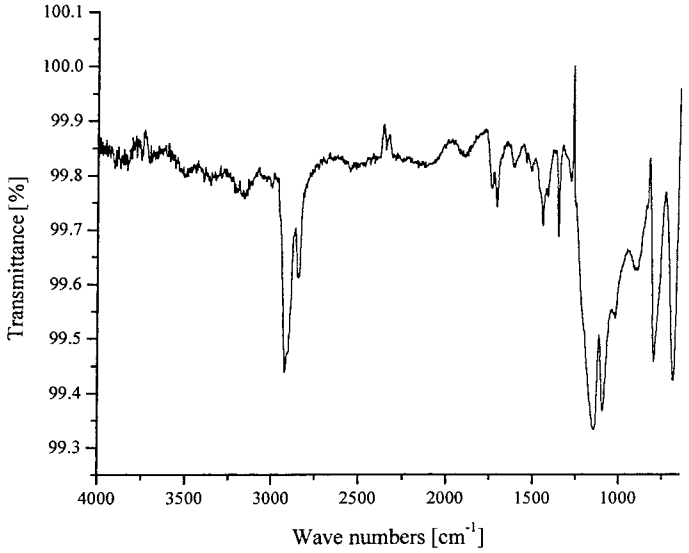


Fig. 1. *GAR-FT-IR Spectrum of* **2** *as SAM on gold*

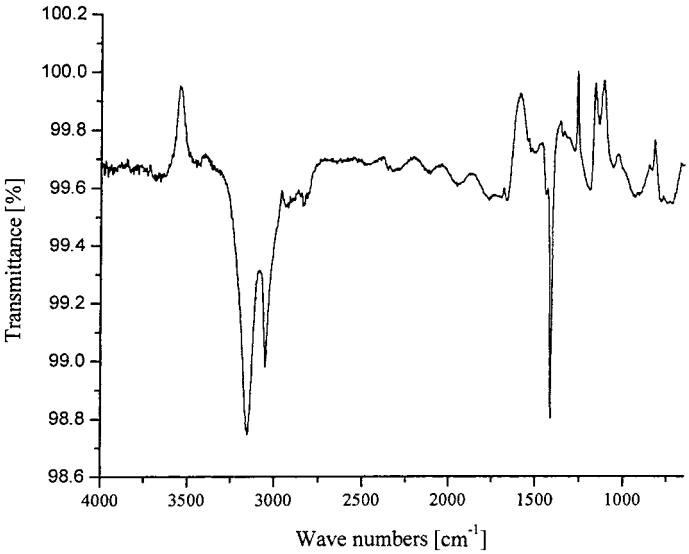


Fig. 2. *GAR-FT-IR Spectrum of* **3** *as SAM on gold*

for the alkyl C–H out-of-plane bending (960–1080 cm^{-1}) and for alkyl C–H asymmetric (2925–2935 cm^{-1}) and symmetric (2850–2865 cm^{-1}) stretching. The spectrum for **2** also includes a band assigned as an OH stretch (3650–3575 cm^{-1}) and that for **3** a weak SH stretch (2600–2550 cm^{-1}). *Figs. 3* and *4* are the spectra of the polyalanine layers obtained from the D- and L-monomers grown on **2**, and *Figs. 5* and *6* show spectra for the same polypeptides on **3**. Each spectrum for the helical peptide

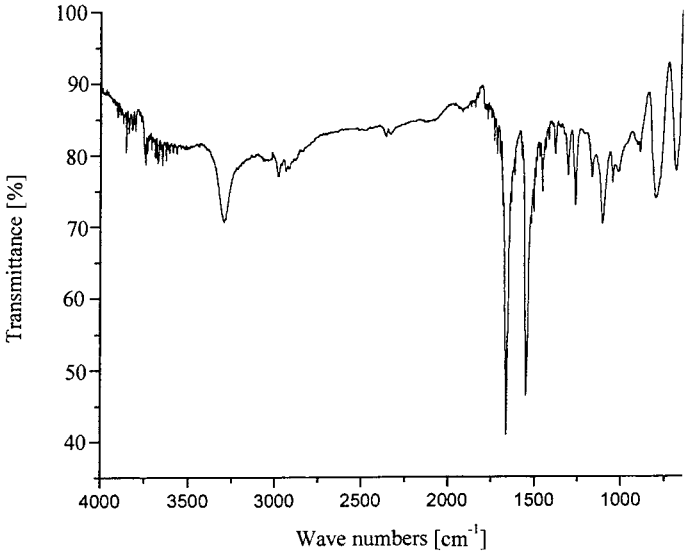


Fig. 3. *GAR-FT-IR Spectrum of poly*(L-*alanine) grown from* **2** *on gold*

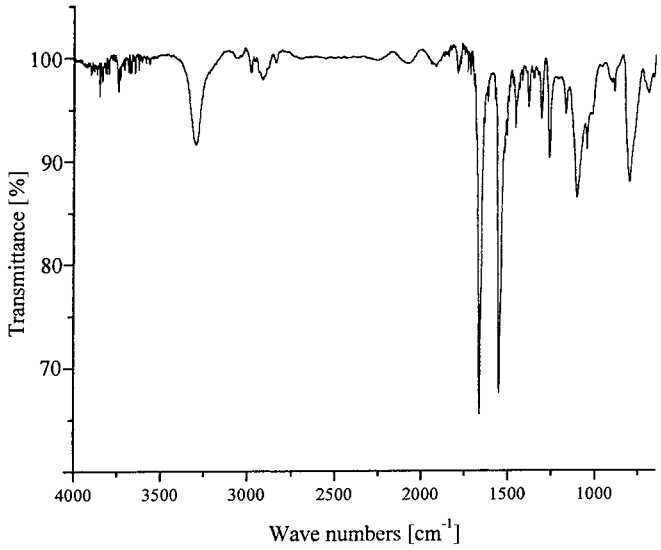


Fig. 4. *GAR-FT-IR Spectrum of poly*(D-*alanine) grown from* **2** *on gold*

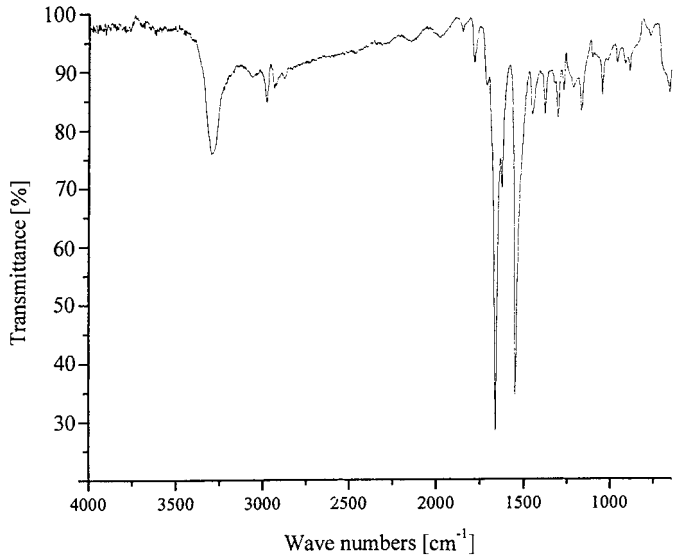


Fig. 5. *GAR-FT-IR Spectrum of poly*(L-*alanine) grown from* **3** *on gold*

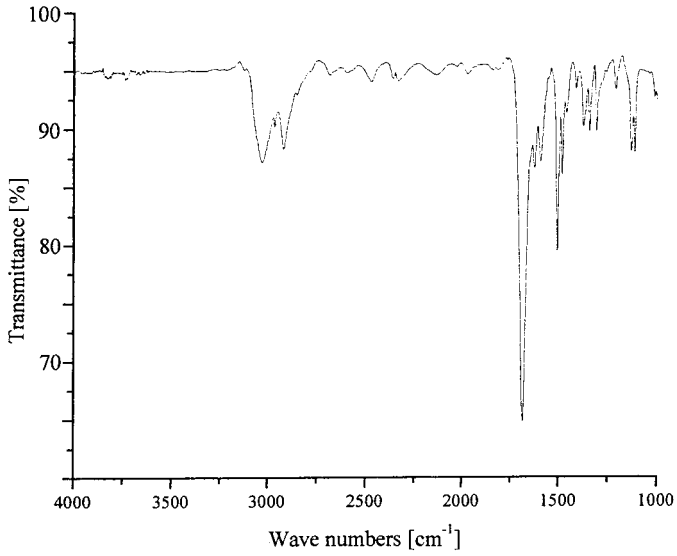


Fig. 6. *GAR-FT-IR Spectrum of poly*(D-*alanine) grown from* **3** *on gold*

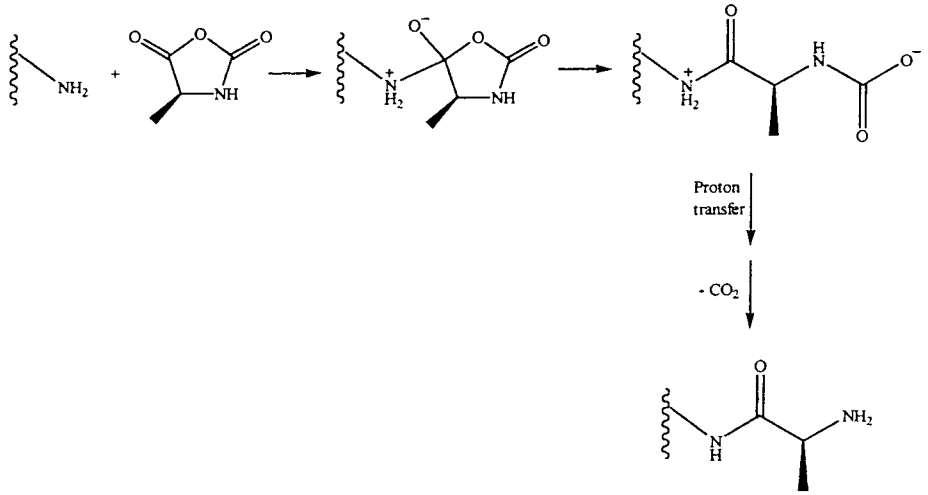
polymerized on gold exhibits extremely narrow absorptions for the amide-I and -II bands (*ca.* 1660 and *ca.* 1545 cm^{-1}) [1][20]. It has been demonstrated before that the axis of the helix is aligned normally to the gold surface from IR spectra of helical polyalanine on ITO glass taken with vertically and horizontally polarized light and from comparison of the intensities of the amide-I and -II bands, these being greatly attenuated in the latter case [1]. Thus, we conclude that the helices grown in this study

are aligned mainly normal to the surface. From the amide-I IR absorptions of the peptides, we estimate that our films contain 800–1000 alanine units. Knowing that each amino acid residue contributes *ca.* 1.5 Å to the length of the helix, the polymer layers are 1200–1500 Å thick. Of note is the fact that, irrespective of the initiator, once polymerization begins, the results are the same for both **2** and **3**.

The films grown with L- and D-amino acids are of different thicknesses: for the naturally occurring L-amino acid, the films are generally 30–50% thicker under the same experimental conditions. The D-amino acid likely contains the L-isomer, and this small amount of the naturally occurring isomer inhibits peptide growth. We base our conclusion on the reported amine-initiated polymerization of the γ-benzyl *N*-carboxyglutamate anhydride in dimethylformamide [21]. A mixture of D- and L-isomers showed a 35–50% decrease in the rate of polymerization compared to the pure L- or D-isomers. This inhibition and/or termination of polymerization arises from the nonselectivity of polypeptide propagation that occurs before helical configuration is reached, *i.e.*, at around 12 residues. When a 50 : 50 mixture of L- and D-isomers was used to grow peptides on **2** and **3**, very little growth was found ($\leq$25 Å) with no further polymerization after 72 h.

3.3. *Role of Water in Polypeptide Polymerization*. In our efforts to grow longer polypeptides, we sought to understand the mechanism of polymerization. It has long been known that high molecular mass polypeptides from (carboxyamino) acid anhydrides are prepared in solution in the presence of aqueous acid or base [22]. Under these conditions, polypeptides are routinely synthesized with a degree of polymerization over 5000. The corresponding reaction in the gas phase in the absence of aqueous components has a degree of polymerization of 32 [23]. In an amine-initiated polymerization, a zwitterionic intermediate is likely (*Scheme 3*), but should be disfavored in the gas phase [24]. However, the presence of H_2O will not only stabilize the transition state leading to the zwitterion but may also facilitate proton transfer in a similar fashion to enzymatic catalysis [25].

Scheme 3. *Mechanism for Amine-Initiated Polypeptide Polymerization*



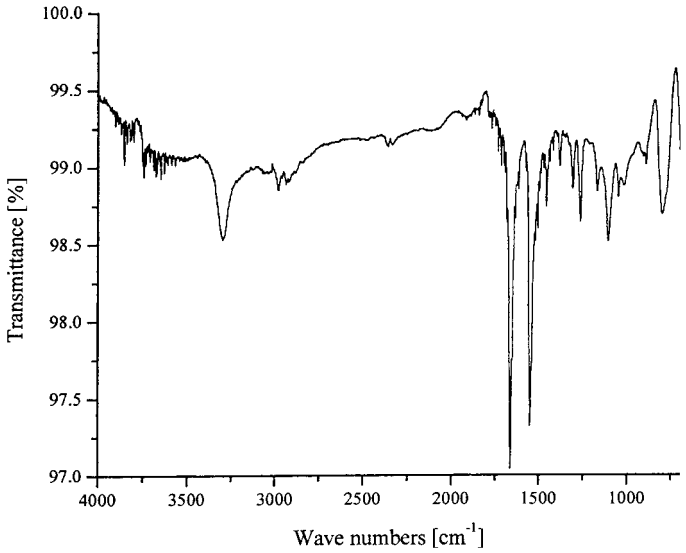


Fig. 7. *GAR-FT-IR Spectrum of a 50:50 mixture of poly* (L- *and* D-*alanine) grown from* **3** *on gold*

To understand the role that H_2O may play in gas-phase polypeptide polymerizations, the following experiments were conducted: first, as a control, the polymerization was run under static vacuum for five days which resulted in formation of a 45 Å thick film. Second, the same experimental conditions were used but with injections of 1 µl of H_2O every 24 h, which produced a 140 Å thick film. When the frequency of the injections was increased to every 12, 8, then finally 4 h, the thickness of the films correspondingly increased from 150 to 180, and finally to 240 Å. There were not any further increases in film thickness with increasing the frequency of the H_2O injections, *i.e.*, every 2 h or every hour. Third, the same experiment was run but with exposure of the slide to the atmosphere for 10 min, re-evacuation of the chamber, a rest period of 45 min, then repeating this cycle every hour[2]). This procedure assumed that the amount of H_2O from the atmosphere would be sufficient to cover the surface of the film, which, in turn, would facilitate polymerization. Indeed, this proved to be the case, as a 220 Å thick film was grown. These experiments clearly demonstrate the necessity of the presence of H_2O for gas-phase polypeptide polymerization.

4. Conclusions. – We prepared two new S-containing adamantane derivatives, which act as surface-bound linkers on which to initiate peptide growth on polycrystalline gold. Both the L and D-isomers of the amino acid alanine grew as α-helical polypeptides perpendicular to the gold surface. FT-IR Studies show that peptides can be routinely grown to thicknesses of more than 1000 Å while retaining nearly perfect α-helicity. The L-amino acid grows layers 30% faster than the non-natural D-amino acid. X-Ray photoelectron spectroscopy studies show that all three S-atoms of the sulfide moieties

[2]) This experiment was also conducted by substituting dry Ar for air to fill the reaction chamber and produced a 55 Å thick film after 5 days.

of **2** are bound to the gold surface and that three of the four thiol groups of **3** are attached, with appreciable equilibration required for the expected orientation of **3**. The presence of H_2O on the surface of the films was shown to be necessary for gas-phase polymerization by stabilizing the zwitterionic intermediate and possibly facilitating proton transfer.

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