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(54) Title: POLYOXYALKYLENE COMPOUND AND METHOD FOR MAKING

(57) Abstract: A process for forming a conjugate of a polyoxyalkylene polymer (such as polyethylene glycol) with a compound containing an amine group(s) and/or a sulfide group(s) by reacting the compound with an acrylate terminated polyoxyalkylene (such as polyethylene glycol terminate at one end with acrylate or methacrylate and terminated at the other end with a methoxy group). The reaction is believed to be a Michael addition. When the compound contains primary amine groups (such as the surface primary amine groups of a PAMAM dendrimer), it is usually desirable to convert the primary amine groups to secondary amine groups before the reaction with the acrylate terminated polyoxyalkylene.

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POLYOXYALKYLENE COMPOUND AND METHOD FOR MAKING

This application claims priority from US Application No. 10/770,224 filed February 3, 2004.

BACKGROUND

The instant invention is in the field of chemical compounds comprising polyoxyalkylene sub-structures such as polyethylene glycol sub-structures. The instant invention also relates to methods for producing chemical compounds comprising polyoxyalkylene sub-structures.

Biologically active compounds comprising polyoxyalkylene sub-structures can provide enhanced biocompatibility for the compound, See, for example, USP 5,366,735 and USP 6,280,745. A review of this subject by Zalipsky, in Bioconjugate Chem., 1995, 6, p150-165, identified polyethylene glycol as one of the best biocompatible polymers to conjugate with a biologically active compound (such as a drug, a protein, a peptide or an enzyme) to produce a conjugate having improved properties such as compatible solubility characteristics, reduced toxicity and reduced immunogenicity.

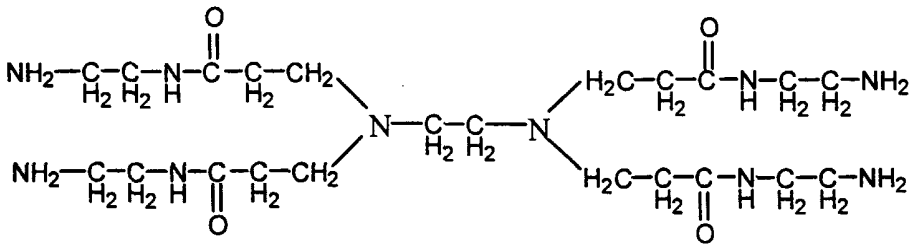
Polyethylene glycol (PEG) is a linear or branched polyoxyalkylene terminated at the ends thereof with hydroxyl groups and generally represented by the formula: $HO-(CH_2CH_2O)_n-CH_2CH_2-OH$. As discussed by Henmanson in Chapter 15 of Bioconjugate Techniques (1966), monomethoxy polyethylene glycol (mPEG) generally represented by the formula: $CH_3O-(CH_2CH_2O)_n-CH_2CH_2-OH$, is usually used to prepare a polyethylene glycol conjugate with a biologically active compound typically by way of a coupling reaction between an amine group of the biologically active compound and an amine receptive derivative (such as trichloro-s-triazine activated mPEG) formed via the remaining terminal hydroxyl group of the monomethoxy polyethylene glycol. An acrylate terminated PEG is offered commercially by Shearwater Corporation (Huntsville, AL) for vinyl polymerization or co-polymerization to produce graft

polymers or cross-linked materials with excellent properties for biomaterial applications.

More recently, so called "second generation" PEGylation chemistry has been developed to, for example, minimize problems of diol impurity contamination of mPEG, to increase the molecular weight of the polyoxyalkylene sub-structure and to increase stability of the conjugate, see Roberts et al., Advanced Drug Delivery Reviews 54 (2002) p459-476.

Dendrimers are hyperbranched, uniformLy distributed structures, having (at least ideally) definite molecular weight, shape and nanometer size characteristics. Dendrimers were discovered by Tomalia and co-workers at the Dow Chemical Company, see Polym. J. 17 (1985) p117-132. Dendrimers have been widely studied as a drug delivery means, see for example Knusli et al., J. Haematology, 82, 654 (1992). Dendrimers *carrying the anti cancer drug* 5-*fluorouracil have been* PEGylated to reduce hemolytic toxicity, drug leakage and macrophageal uptake while improving stability and efficacy, see Bhadra et al., International Journal of Pharmaceutics 257 (2003) p111-124.

PAMAM dendrimers are the most common type of dendrimer and are commercially available from Aldrich (Milwaukee, WI) in the form of various "generations". PAMAM dendrimers are made by a successive Michael addition synthesis scheme involving the reaction of an acrylate group with an amine group. The so called "Generation 0" PAMAM dendrimer has the following formula:



The above Generation 0 PAMAM dendrimer has a molecular weight of about 517 grams per mole. A Generation 1 PAMAM dendrimer has a molecular weight of about 1,430 grams per mole and has eight terminal primary amine groups. A Generation 2 PAMAM dendrimer has a molecular weight of about 3,256 grams per mole and has sixteen terminal primary amine groups. A Generation 10 PAMAM dendrimer has a theoretical molecular weight of almost 935 kilograms per mole and in theory has 4096 primary amine groups on the surface of the dendrimer.

Despite the significant advances that have been made in the field of methods for the PEGylation of biologically active compounds (and more generally in the field of methods for the conjugation of polyoxyalkylene sub-structures with biologically active compounds), the existing methods generally require multiple reactions and extensive purification of the product. It would be an advance in this art if a process were discovered that required only one reaction step and produced no by-products.

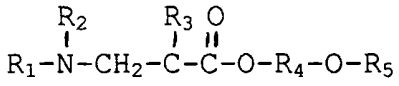
SUMMARY OF THE INVENTION

The method of the instant invention is a solution, at least in part, to the above described problems of the prior art. The instant invention provides a one step pegylation method that ideally produces no by-products. In addition, the method of the instant invention can be practiced at room temperature and under conditions such as solvent compatibility that are mild relative to maintenance of biological activity. In one embodiment, the instant invention is applicable to biologically active compounds containing an amine group. In another embodiment, the instant invention is applicable to biologically active compounds containing a sulfide group. The biologically active compound is reacted with an acrylate

terminated polyoxyalkylene (such as $H_2C=CH-CO-O-PEG-O-CH_3$) in a one step process to produce novel conjugates having many if not all of the benefits of the prior art conjugates.

More specifically, the instant invention is a method for preparing a compound corresponding to the formula:

formula 1



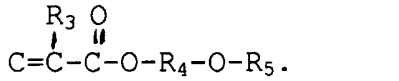
where R_1 is an organic radical

where R_2 is H or an organic radical

where R_3 is H or an organic radical

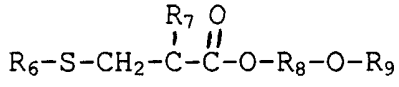
where R_4 is a polyoxyalkylene radical

and where R_5 is an organic radical or H, comprising the step of: reacting A with B, wherein A is R_1-N-R_2 and wherein

B is  .

In another embodiment, the instant invention is a method for preparing a compound corresponding to the formula:

formula 2

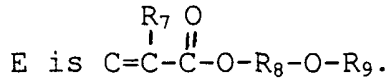


where R_6 is an organic radical

where R_7 is H or an organic radical

where R_8 is a polyoxyalkylene radical

and where R_9 is an organic radical or H, comprising the step of: reacting D with E, wherein D is R_6-S and wherein

E is  .

In addition, the invention is the compound of formula 1 and the compound of formula 2 as described above.

DETAILED DESCRIPTION OF THE INVENTION

In general, the process of the instant invention can be conducted at room temperature. Infrared (IR) spectra are obtained using a thin film on a sodium chloride plate. Spectra are recorded using a Nicolet 20DXB Fourier Transform (FT-IR) Spectrometer and absorption is reported in wave number (cm^{-1}). IR spectra cover the range 1000-4000 cm^{-1}.

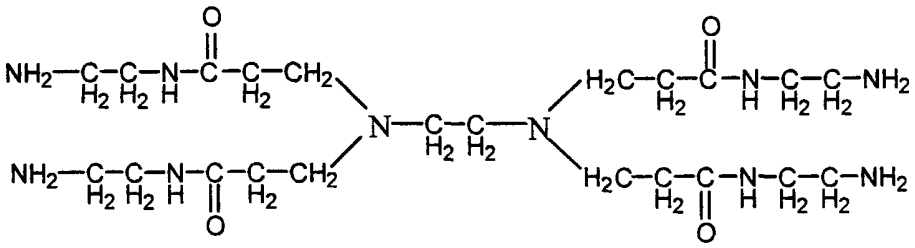
Proton nuclear magnetic resonance spectra and carbon-13 nuclear magnetic resonance spectra are recorded for solutions in appropriate solvents containing tetramethylsilane in case of chloroform and methanol and 3(trimethylsilyl) propane Sulfonic acid sodium salt (DSS) in case of Deuterium Oxide as internal standard using a General Electric QE-300 NMR spectrometer. The NMR shifts are reported in parts per million (ð, PPM). The following standard abbreviations were used in describing NMR data: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet.

Mass Spectra are obtained by using a Hewlett-Packard Model 5995A gas Chromatograph/Mass Spectrometer with an ionizing potential of 70 electron volts.

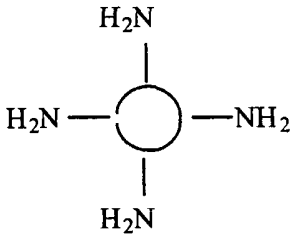
Starting Materials

1. PAMAM Dendrimer (Generation = 0)

Source: Aldrich Chemical; Structure:



The structure above may also be represented

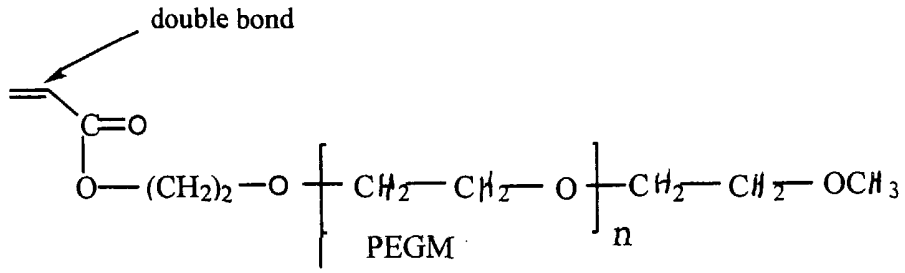


Note: PAMAM Dendrimer is purchased in 20% solution in methanol and used without further purification.

2. Poly Ethylene glycol methyl ether acrylate

Source: Aldrich Chemical Company

Structure:



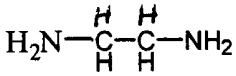
Used without further purification.

A PEG chain may be represented:

3. Ethylene diamine

Source: Aldrich Chemical Company

Structure:

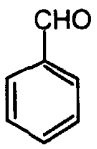


Purification procedure: Ethylene diamine is distilled over CaH_2.

4. Benzaldehyde

Source: Fisher Scientific

Structure:



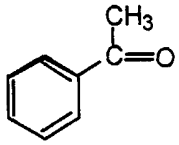
Purification procedure: Benzaldehyde (about 75 mL) is placed in a separatory funnel. It is washed with 10% $NaCO_3$ until no

more CO_2 evolved. Saturated NaCl solution is added to the solution. The solution is then washed with a saturated solution of Na_2SO_3 followed by washing with water. The organic layer is collected and dried with $MgSO_4$. The organic layer is filtered and then distilled under vacuum.

5. Acetophenone

Source: Fisher Scientific

Structure:

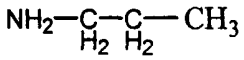


used without further purification.

6. Propylamine

Source: Aldrich Chemical Company

Structure:

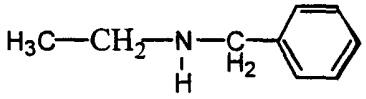


It is distilled over CaH_2 before use.

7. N-ethyl N-benzyl amine

Source: Aldrich Chemical Company

Structure:



used without further purification.

8. Methanol

Source: Burdick and Jackson

Structure : CH_3OH

used without further purification.

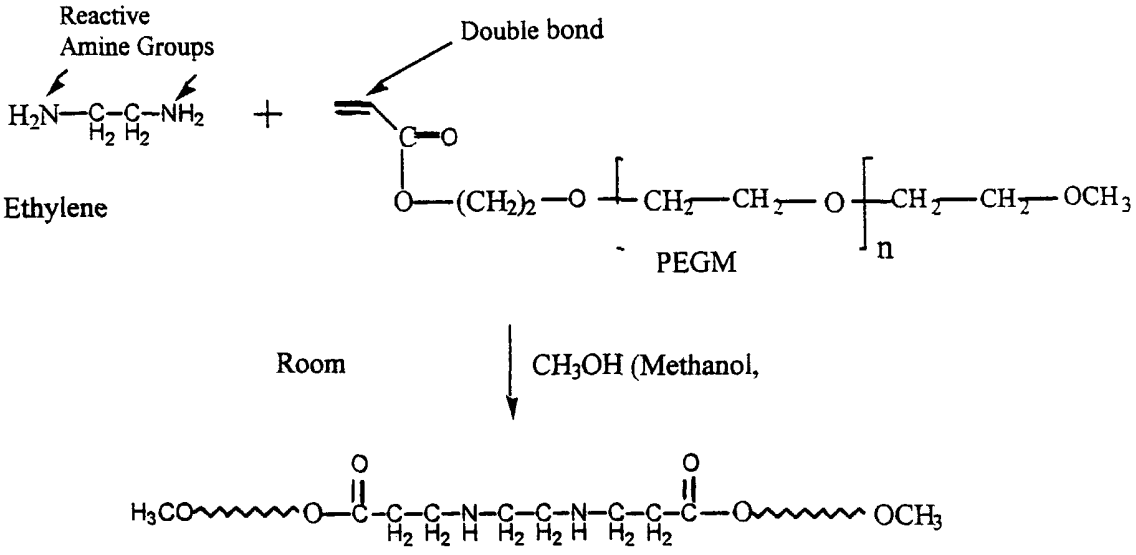
9. Molecular Sieve (Size 3Å)

Source: EM Science

1. Model Reaction

Molecular Weight: 1108 g/mole

Reaction scheme:



1

Procedure: Ethylene diamine (0.5714 g, $0.9525*10^{-3}$ moles) and PEGMA (1 gm, $1.905*10^{-3}$) and 3 mL of methanol are added to a clean and dry vial. The vial is capped and allowed to shake for 2 hours. Methanol is removed under vacuum at room temperature. The product is a sticky solid.

Spectral Data :

IR: cm^{-1}

3514.3, 2873.2, 1723.8, 1656.3, 1462.3, 1449.6, 1407.5, 1344.2, 1293.6 1276.7, 1243.0, 1192.4, 1108.0

^{1}H NMR: (ð ppm) in CD_3OD

2.52(m), 2.69(s), 2.84(t), 3.35(s), 3.53(m), 3.62(d), 3.66(m), 4.70(t)

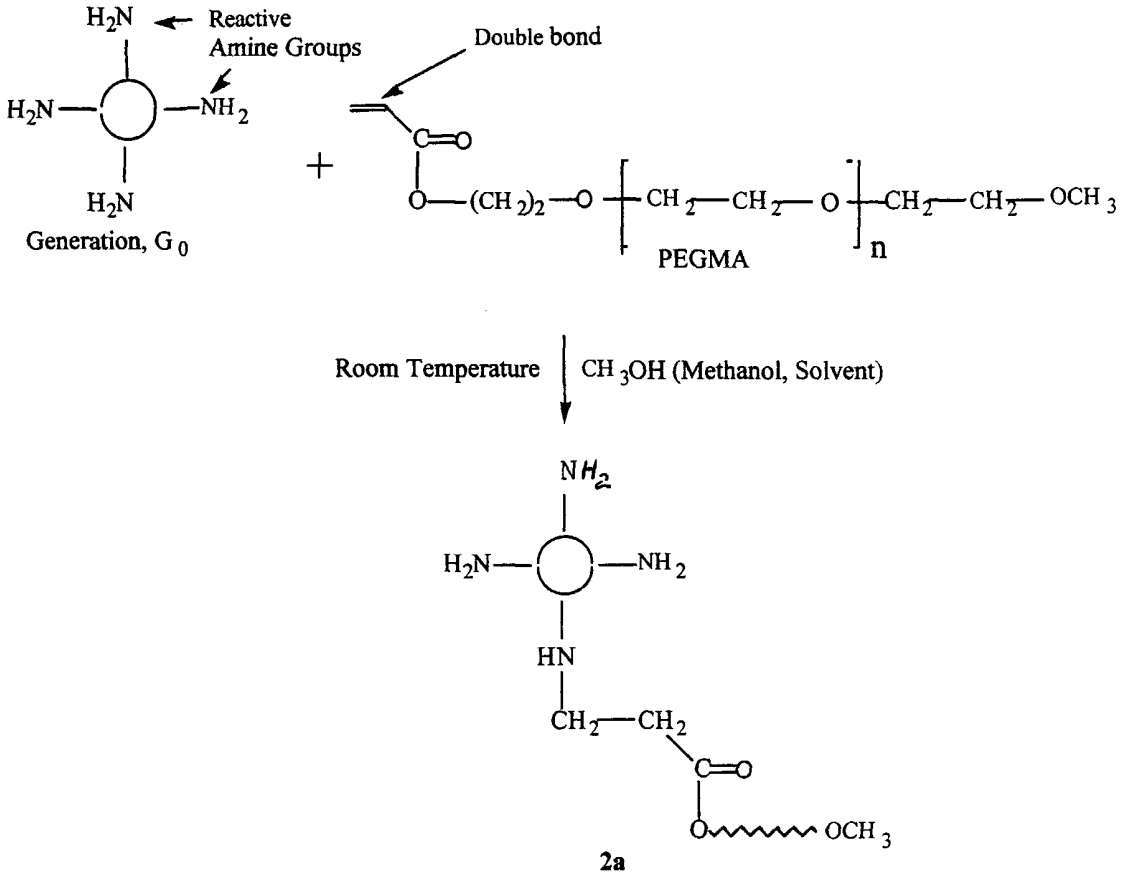
^{13}C NMR: (ð ppm)

33.33, 34.76, 45.67, 52.10, 53.92, 59.08, 62.17, 64.68, 70.05, 71.31, 72.91, 73.64

2. PAMAM Dendrimer (Generation = 0)-PEGMA (1/4 equivalent) conjugate

Molecular weight: 1041 g/mole

Reaction scheme:



Procedure: 1 mL of G_0 solution (20 % solution in methanol) is taken in a clean dry vial. PEGMA (0.0254g, 0.00019 moles) is added to the vial along with the 2 mL methanol. The vial is capped tightly and allowed to shake for about 3 hours. The methanol is removed after the reaction is over. The product is a sticky solid.

Spectral data

^{1}H NMR: (ð ppm) in D_2O

2.42(t), 2.60(s), 2.65(t), 2.70(t), 3.24(t), 3.28(s), 3.40(s), 3.62(m), 3.65(s), 3.80(m), 4.24(t).

^{13}C NMR: (ð ppm)

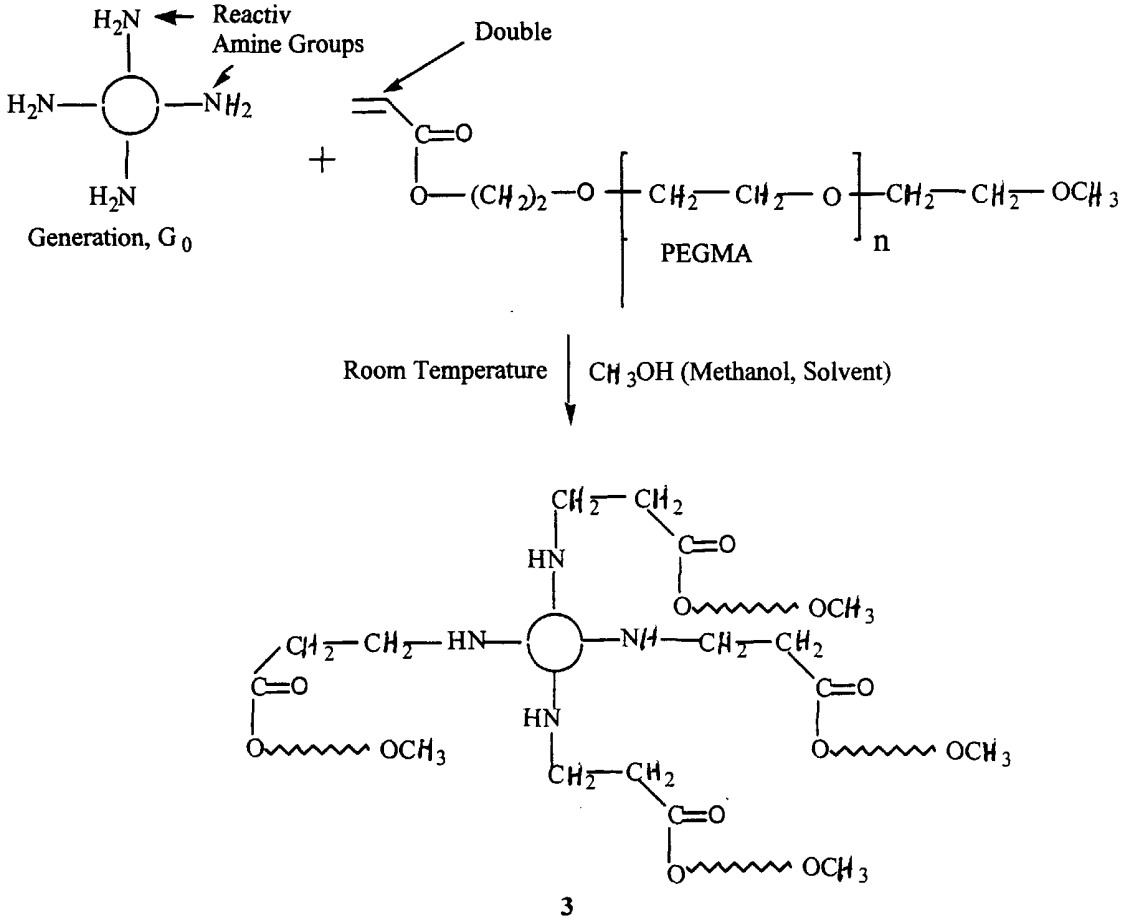
32.59, 36.41, 36.65, 40.96, 41.13, 45.07, 46.97, 48.80, 49.06, 49.96, 58.01, 60.30, 69.41, 69.54, 69.63, 70.94, 71.69, 174.92, 175.03, 175.21, 175.30, 175.97, 180.62, 180.70

.

3. PAMAM Dendrimer (Generation = 0) - PEGMA (4 equivalents) conjugate

Molecular weight : 2613 g/mole

Reaction Scheme:



3

Procedure: 1 mL of G_0 solution (20 % solution in methanol) is taken in a clean dry vial. PEGMA (0.1016g, 0.00076 moles) is added to the vial along with the 2 mL methanol. The vial is capped tightly and allowed to shake for about 3 hours. The methanol is removed after the reaction is over. The product is a sticky solid.

Spectral Data :

^{1}H NMR: (ð ppm) in D_2O

2.42(t), 2.59(m), 2.70(t), 2.82(m), 3.29(t), 3.37(s), 3.62 (m), 3.69(s), 3.80(m), 4.30(d)

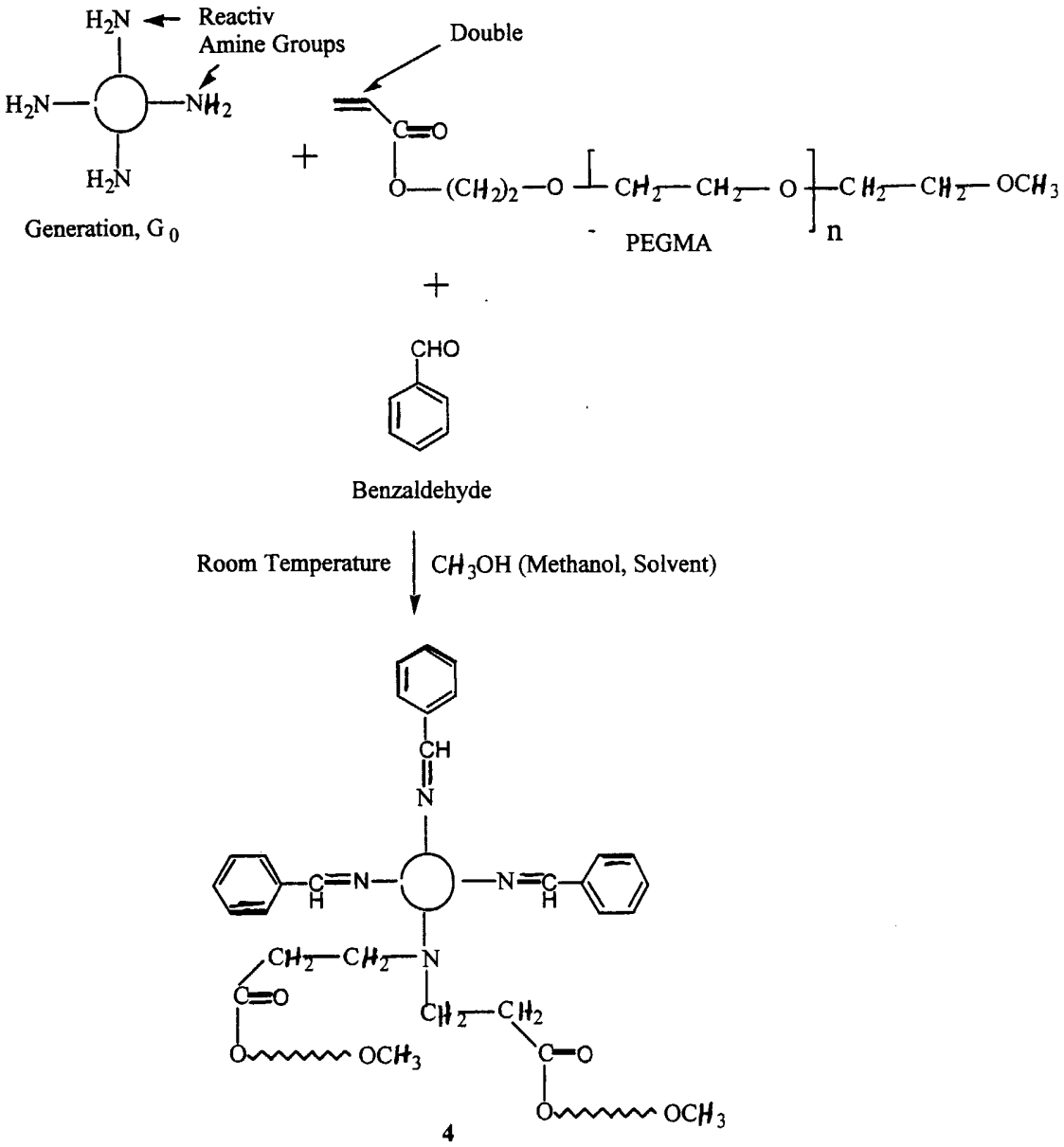
^{13}C NMR: (ð ppm)

21.65, 33.90, 34.52, 34.90, 38.52,.39.21, 45.70, 47.05, 49.29, 49.29, 49.68, 50.82, 51.19, 51.35, 52.28, 53.26, 53.88, 54.01, 54.72, 54.90, 56.92, 60.55, 62.84, 66.43, 70.93, 71.95, 72.08, 72.17, 73.49, 74.23, 176.60, 176.70, 177.06, 177.51, 177.83, 180.58.

4. PAMAM Dendrimer (Generation=0) - PEGMA (2 equivalents) - Benzaldehyde (3 equivalents)

Molecular weight: 1305 g/mol

Reaction scheme:



Procedure: 2mL of G_0 and Benzaldehyde (0.24603 g, 0.00232107 moles) are placed in a clean dry vial, capped tightly and allowed to shake for about 24 hours. PEGMA (0.40612 g, 0.000774 moles) is added thereafter. The vial is allowed to shake for

about 100 hours. The methanol is removed under vacuum at room temperature. The product is a sticky solid.

Spectral data

IR: cm^{-1}

1107.4, 1196.6, 1273.6, 1293.9, 1350.6, 1407.3, 1451.9, 1723.5, 2865.6

^{1}H NMR: (ð ppm) in CD_3OD

2.30 (m), 2.45 (m), 2.60 (m), 2.70 (m), 2.95 (m), 3.34 (s), 3.62 (s), 4.20 (d), 7.40 (m), 7.75 (m), 7.795 (m), 8.30 (d)

^{13}C NMR: (ð ppm)

33.50, 33.63, 33.83, 38.47, 39.11, 40.74, 41.02, 45.14, 50.41, 50.85, 51.76, 52.27, 53.65, 59.07, 60.99, 62.14, 64.76, 71.27, 71.30, 71.45, 72.87, 73.61, 128.78, 129.40, 129.78, 130.26, 131.40, 132.21, 137.01, 165.20, 173.86, 174.09, 174.63, 174.95

5. PAMAM Dendrimer (Generation=0) - PEGMA (2 equivalents)-Acetophenone (3 equivalents)

Molecular weight: 1347 g/mole

Procedure: 1mL of G_0 and Acetophenone (0.1395 g, 0.0021161 moles) are placed in a clean dry vial. 2 scoops of molecular sieve were added to the vial. It was capped tightly and allowed to shake for about 24 hours. PEGMA (0.4062 g, 0.000774

moles) was added thereafter and allowed to shake for about 100 hours. The methanol was removed under pump at room temperature. The product is a sticky solid.

Spectral data

IR: cm^{-1}

1036.2, 1107.4, 1139.8, 1196.6, 1251.3, 1273.6, 1295.9, 1348.6, 1407.3, 1447.3, 1597.8, 1634.3, 1658.6, 1723.5, 2865.6, 3514.1.

^{1}H NMR: (ð ppm) in D_2O

2.22(s), 2.43(t), 2.57(m), 2.65(s), 2.70(m), 2.80(d), 3.28(t), 3.30(m), 3.35(s), 3.38(s), 3.70(s), 3.63(m), 7.46(q), 7.55(t), 7.69(m), 7.98(d).

^{13}C NMR: (ð ppm)

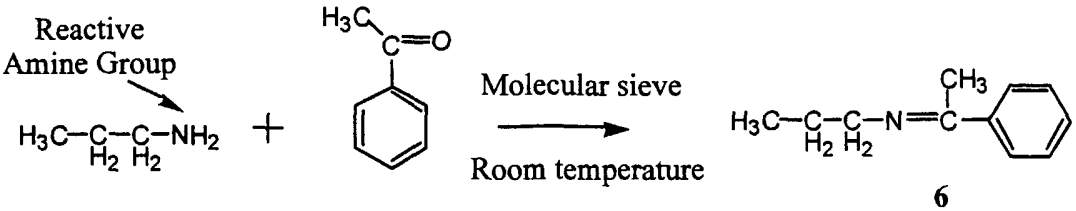
17.65, 21.76, 28.42, 28.92, 32.90, 34.05, 35.21, 36.11, 41.12, 42.21, 42.62, 42.98,46.12, 47.60, 49.61, 51.51, 52.61, 54.87, 57.03, 60.70, 63.00, 72.10, 72.23, 73.63, 74.37, 131.18, 131.47, 136.81, 139.00, 165.00, 177.50, 177.89, 177.80, 182.64.

6. Model Reaction

a. Schiff base formation by the reaction between propylamine and Acetophenone;

Molecular weight: 161g/mol

Structure scheme:



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Procedure: Acetophenone (1.006 g, 0.00779 moles) and Propylamine (2.510 g, 0.0425 moles) are added in a clean dry vial. About 4 scoops of warm molecular sieve are added to the vial and capped tightly. The vial is allowed to shake for about 96 hours. The solution is collected using the solvent CH_2Cl_2 and dried over $MgSO_4$. The solution is filtered through celite and dried under vacuum at room temperature.

Spectral data

IR: cm^{-1} (intensity)

1027.04, 1074.89, 1180.02, 1376.10, 1446.25, 1492.57, 1578.19, 1634.03, 2872.57, 2930.89, 2958.92, 3024.00, 3058.14, 3078.24.

MS: m/e (% of base)

162, 16, 160, 146, 132, 104, 91 (100%), 77

^1H NMR: (ð ppm) in $CDCl_3$

1.01 (t), 1.78 (q), 2.20 (s), 3.42 (t), 7.34 (m), 7.76 (m).

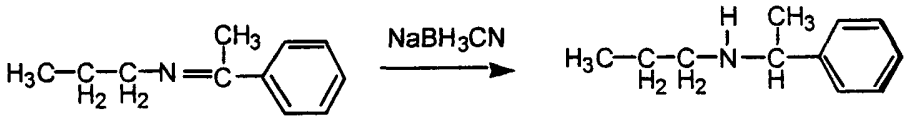
^{13}C NMR (CD_3OD): (ð ppm)

12.07, 15.31, 24.05, 29.05, 53.85, 54.99, 77.00, 125.61, 128.02, 129.10, 141.34, 164.67, 165.41, 168.22.

b. Reduction of imine:

Molecular weight : 163 g/mole

Reaction scheme :



6 7

Procedure: Product (1.348g, 0.008373 moles) of the above reaction is placed in a clean dry vial. NaBH3CN (0.350g, 0.00557moles) was taken in a clean dry vial and about 1.5 mL of methanol is added to that in order to make a clear solution. The solution is added to the reaction vial and capped tightly. The vial is allowed to shake for about 72 hours at room temperature. After 72 hours about 6(N) HCl is added drop by drop until the pH is <2. Then the solution is brought to a pH of >10 with 10% NaOH solution. The opaque solution is then dried over $MgSO_4$, filtered through celite and dried over vacuum at room temperature.

Spectral data

IR: cm^{-1} (intensity)

1027.53, 1071.61, 1286.90, 1370.64, 1450.87, 1492.02, 1601.13, 2871.74, 2931.94, 2959.54, 3025.48, 3062.21.

MS: m/e (% of base)

163, 162,148, 134, 105 (100%), 77

^1H NMR: (δ ppm) in CD_3OD

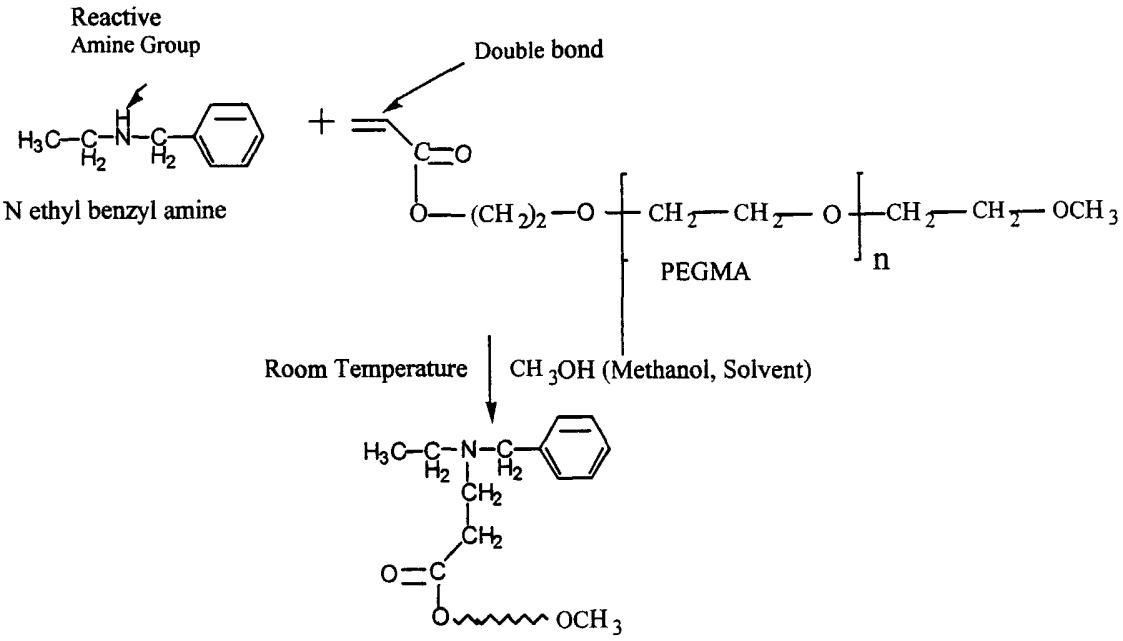
0.94 (t), 3.66 (d), 2.69 (m), 2.88 (m), 3.34 (s), 4.35 (q), 4.93 (s), 7.47 (m).

^{13}C NMR: (ð ppm) in CD_3OD

9.87, 18.30, 19.36, 58.14, 127.15, 129.05, 129.16, 136.54.

c. N-EthylBenzyl amine-PEGMEA conjugates:

Structure:



8

Procedure: PEGMA (1g, 0.00191 moles), N-ethyl benzylamine(0.258 g, 0.00191 moles) and methanol are placed in a clean dry vial. The vial is capped tightly and allowed to shake for about 72 hours. The methanol is evaporated off under vacuum at room temperature.

Spectral data

^{1}H NMR: (ð ppm) in CD_3OD

1.03 (t), 2.48 (q), 2.77 (t), 3.34 (s), 3.57 (m), 3.82 (m), 4.19 (t), 4.28 (t), 7.23 (m), 7.29 (d).

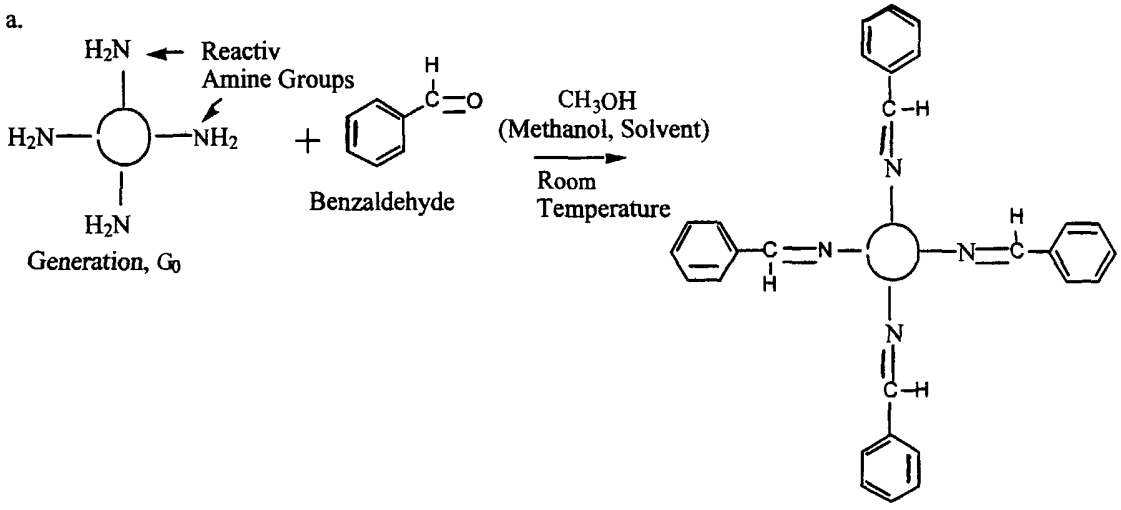
^{13}C NMR: (ð ppm) in CD_3OD

11.96, 32.91, 33.05, 48.06, 49.57, 52.02, 58.80, 59.08, 62.18, 64.66, 64.81, 71.32, 71.51, 72.92, 73.64, 128.06, 129.21, 130.12, 140.15, 167.44, 174.10, 174.63.

7. Pegylation of reduced PAMAM Dendrimer (Generation=0) - Benzaldehyde (4 equivalents) conjugates:

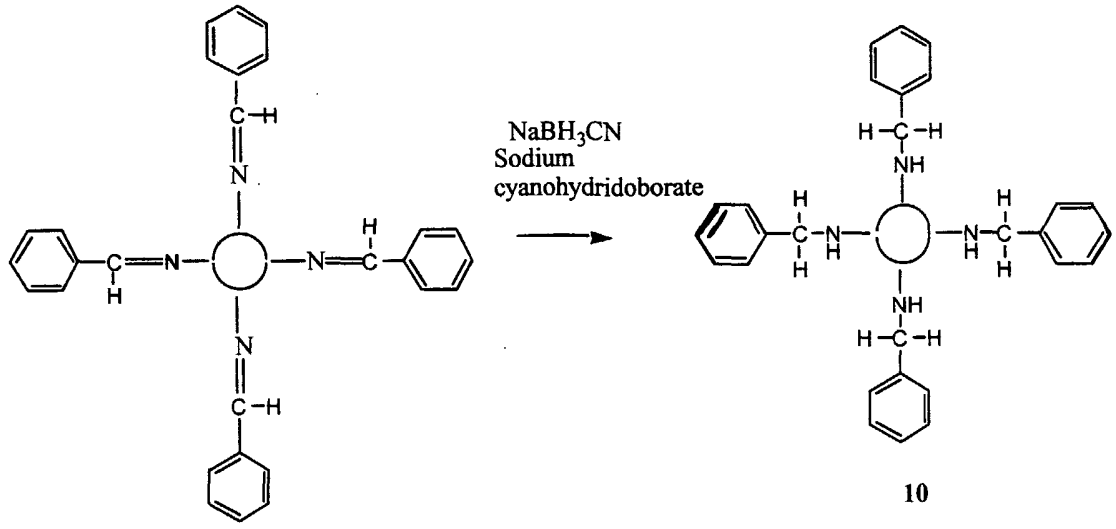
Molecular weight: 2977 g/mole

Reaction scheme:



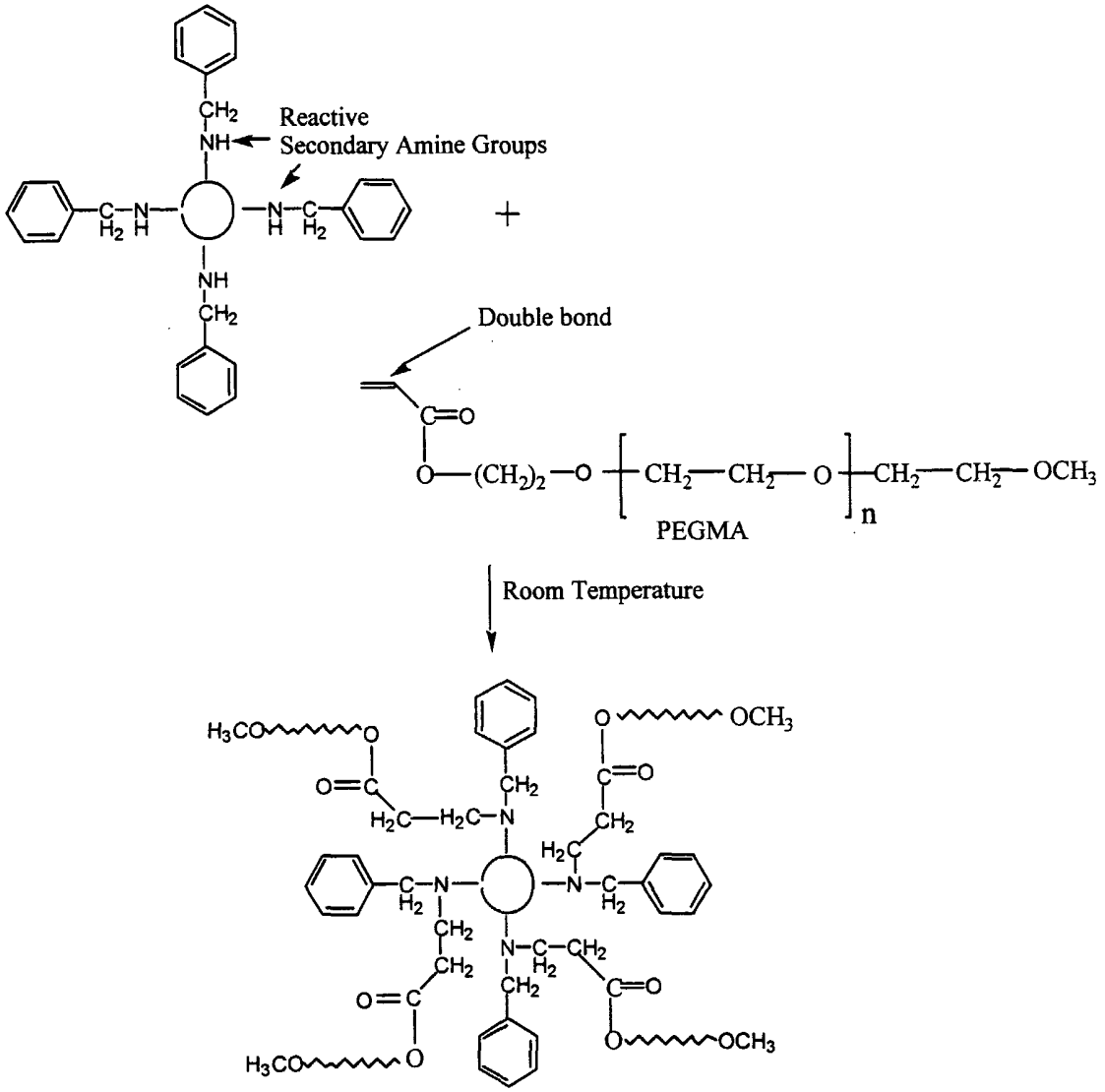
9

b.



9 10

c.



11

Procedure: 2mL ($7.737X\ 10^{-4}$ moles) of G_0 and benzaldehyde (0.328 g, $3.095X10^{-3}$ moles) are placed in a clean dry vial. About 2.5 mL methanol is added to the reaction vial, capped tightly and allowed to shake for about 24 hours. Sodium cyanohydridoborate (0.129 g, $2.058X10^{-3}$ moles) is taken in a clean dry vial and a clean solution with minimum volume (about

1mL) of methanol is made. The solution is added to the reaction vial and capped tightly. The vial is allowed to shake for 24 hours more. Thereafter PEGMA (0.8124 g, $1.547X10^{-3}$ moles) is added and the vial is shaken for another 60 hours. Then concentrated HCl (about 6 N) is added drop by drop until the pH is <2. The acidic solution is kept for about 2 hours and then is brought up to a pH of >10 by the drop by drop addition of 10% NaOH solution and then dried over $MgSO_4$ and filtered through celite. The methanol is then evaporated under vacuum at room temperature.

Spectral data:

A. G_0+ 4Benzaldehyde

^1H NMR: (ð ppm) in CD_3OD

2.23 (t), 2.35 (s), 2.59 (t), 3.34 (s), 3.48 (t), 3.66 (t), 4.92 (s), 5.46 (s), 7.40 (m), 7.53 (t), 7.60 (t), 7.63 (t), 7.65 (t), 7.73 (m), 7.84 (m), 7.86 (t), 8.25 (s), 10.00 (s).

^{13}C NMR: (ð ppm) in CD_3OD

32.56, 34.24, 35.93, 39.10, 40.94, 42.80, 50.93, 51.784, 52.65, 53.58, 59.06, 59.11, 60.86, 61.05, 62.67, 62.73, 128.28, 128.62, 128.92, 129.02, 129.50, 129.59, 130.39, 130.72, 130.73, 130.95, 131.05, 131.15, 131.55, 131.63, 131.71, 133.08, 133.18, 133.28, 134.35, 134.45, 136.83, 136.93, 137.07, 163.94, 166.04, 174.66, 192.77, 192.83, 192.90, 195.09, 195.15, 195.21.

B. G_0+ 4Benzaldehyde+ $NaBH_3CN$

^1H NMR: (ð ppm) in CD_3OD

2.54 (m), 2.53 (m), 2.73 (m), 2.86 (m), 3.34 (s), 3.38 (s), 3.59 (s), 3.76 (s), 4.622 (s), 4.89 (s), 5.01 (t), 5.11 (m), 7.34 (m), 7.54 (m), 7.76 (m), 8.35 (m).

^{13}C NMR: (ð ppm) in CD_3OD

34.67, 38.41, 39.85, 42.01, 42.96, 47.43, 51.23, 52.33, 53.71, 54.15, 57.10, 59.42, 65.19, 116.96, 120.39, 127.92, 128.13, 128.51, 128.76, 128.89, 129.26, 129.43, 129.66, 129.87, 135.37, 136.62, 139.00, 140.63, 142.63, 174.51, 174.60, 175.02, 175.09, 175.16.

C. G_0+ 4Benzaldehyde+ $NaBH_3CN$+PEGMEA

IR: cm^{-1} (intensity)

11091, 1198.77, 1249.93, 1293.76, 1350.91, 1452.86, 1542.64, 1564.72, 1630.09, 1658.20, 1736.78, 2871.20.

^1H NMR: (ð ppm) in DMSO-d_6

2.15 (s), 2.42 (t), 2.49 (m), 2.58 (s), 2.60 (d), 3.08 (d), 3.15 (s), 3.22 (s), 3.41 (t), 3.49 (s), 3.53 (s), 4.47 (d), 4.66 (t), 7.21 (m), 7.29 (m), 7.41 (m), 7.54 (m), 7.88 (m).

^{13}C NMR: (ð ppm) in DMSO-d_6

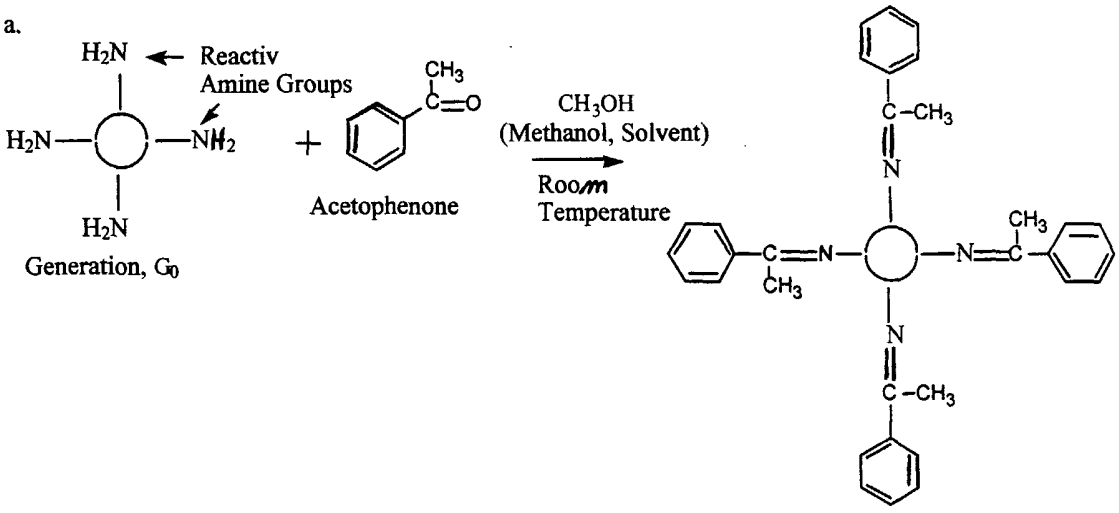
31.86, 32.04, 33.21, 36.48, 48,61, 48.86, 49.79, 51.25, 52.30, 57.57, 58.08, 60.20, 62.87, 69.60, 69.80, 71.30, 72.35, 126.43, 126.63, 126.81, 127.31, 127.51, 128.70, 128.17, 128.51, 128.71, 139.32, 142.57, 171.21, 172.52.

8. Pegylation of reduced PAMAM Dendrimer (Generation=0) - Acetophenone (4 equivalents) conjugates:

Molecular weight: 3033 g/mole

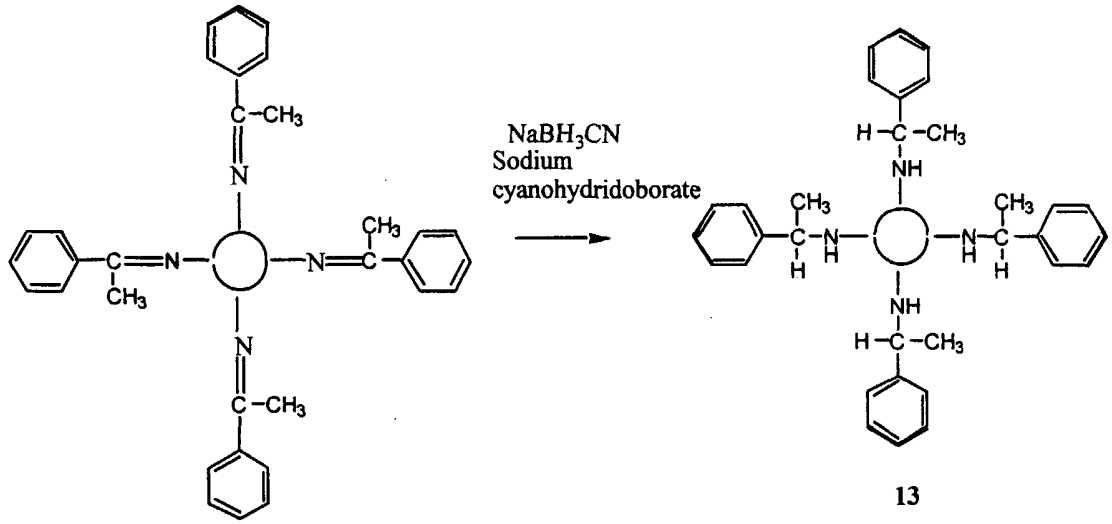
Reaction scheme:

a.



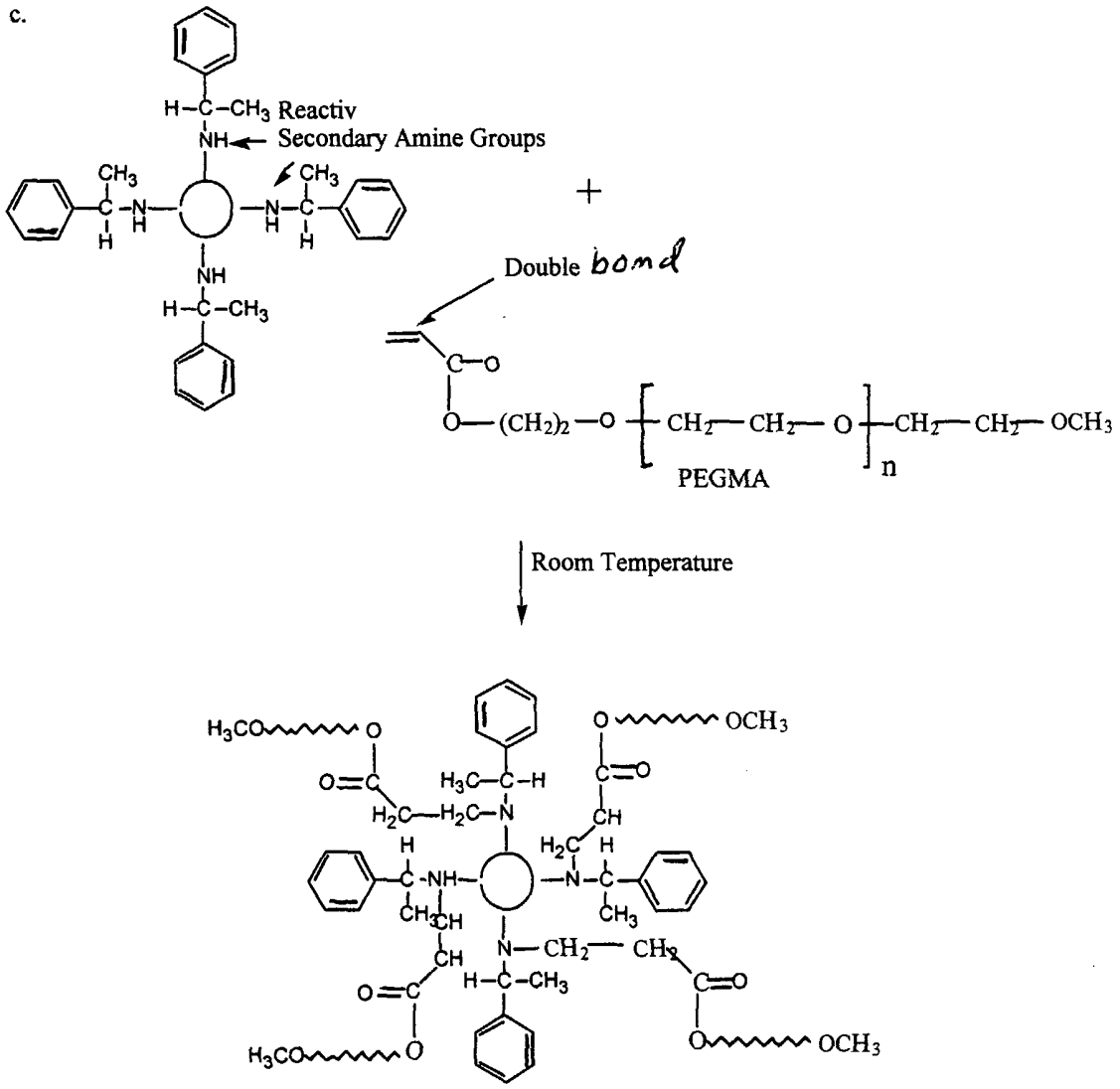
12

b.



12 13

c.



14

Procedure: 2mL (7.737X 10^{-4} moles) of G_0 and acetophenone (0.372 g, 3.095X10^{-3} moles) are placed in a clean dry vial. About 2.5 mL methanol is added to the reaction vial along with 3 scoops of molecular sieve (3A), capped tightly and allowed to shake for about 24 hours. Sodium cyanohydridoborate (0.129 g,

$2.058X10^{-3}$ moles) is taken in a clean dry vial and a clean solution with minimum volume (about 1mL) of methanol is made. The solution is added to the reaction vial and capped tightly. The vial is allowed shake for 72 hours more. Thereafter PEGMA (0.8124 g, $1.547X10^{-3}$ moles) is added and the vial is allowed to shake for another 120 hours. The solution turns to a pink color. Then concentrated HCl (about 6 N) is added drop by drop until the pH is <2. The acidic solution is kept for about 2 hours and then is brought up to a pH of >10 by the drop by drop addition of 10% NaOH solution, dried over $MgSO_4$ and filtered through celite. The methanol is then evaporated under vacuum at room temperature.

Spectral data:

A. G_0+4 Acetophenone:

^1H NMR: (ð ppm) in CDCl3

2.20 (m), 2.65 (d), 2.57 (t), 2.62 (S), 2.72 (t), 3.22 (m), 3.34 (m), 3.49 (d), 3.55 (t), 7.07 (m), 7.27 (s), 7.35 (m), 7.47 (t), 7.59 (t), 7.72 (m), 7.98 (d).

^{13}C NMR: (ð ppm) in $CDCl_3$

16.19, 16.27, 34.03, 34.13, 34.31, 50.36, 50.68, 50.80, 51.38, 51.54, 51.69, 76.58, 77.42, 125.84, 126.56, 128.17, 129.62, 133.10, 140.83, 167.14, 167.32, 172.68, 172.77, 173.01.

B. G_0+4 Acetophenone+ $NaBH_3CN$

^1H NMR: (ð ppm) in CD_3OD

1.37 (m), 1.43 (d), 2.36 (m), 2.51 (t), 2.60 (s), 2.72 (m), 2.85 (t), 3.27 (m), 3.30(m), 3.37 (s), 3.78 (m), 7.22 (m), 7.31(m), 7.47 (m), 7.49 (d), 7.52 (d), 7.58 (d), 7.98 (d), 8.01 (t).

^{13}C NMR: (ð ppm) in CD_3OD

23.99, 25.62, 34.57, 39.96, 41.10, 42.918, 47.66, 49.85, 51.19, 52.29, 59.12, 70.81, 126.42, 127.77, 128.05, 128.15, 129.24, 129.56, 145.93, 147.80, 174.99, 175.21.

C. G_0+4 Acetophenone+ $NaBH_3CN$ +PEGMEA

IR: cm^{-1} (intensity)

1101.38, 1199.40, 1249.53, 1288.77, 1350.85, 1452.80, 1580.67, 1630.78, 1657.81, 1736.70, 2873.04.

^1H NMR: (ð ppm) in DMSO-d_6

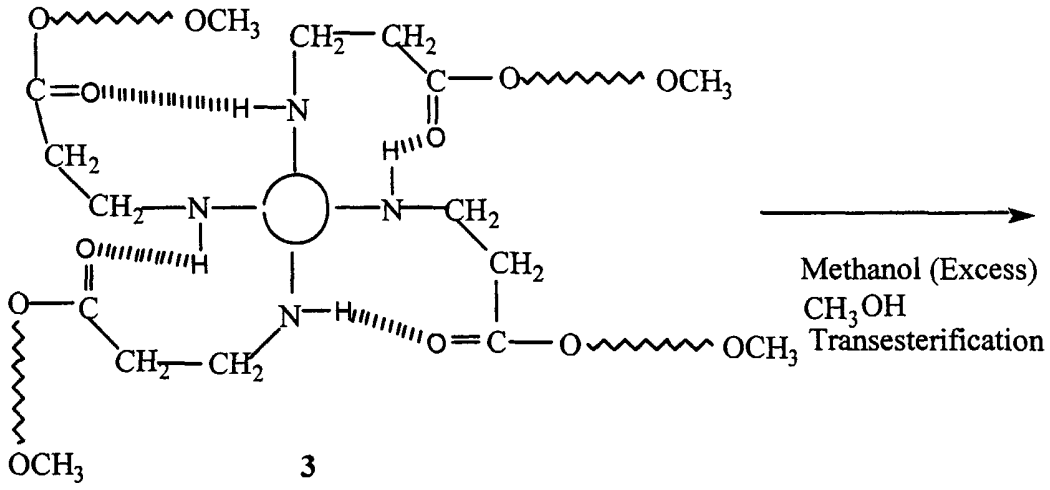
1.18 (d), 1.28 (d), 2.15 (s), 2.36 (t), 2.49 (t), 2.56 (s), 2.65 (t), 3.03 (t), 3.14 (d), 3.21 (s), 3.40 (t), 3.48 (s), 3.54 (m), 4.22 (q), 4.68 (t), 7.64 (d), 7.27 (m), 7.87 (m), 8.07 (m).

^{13}C NMR: (ð ppm) in DMSO-d_6

24.64, 32.11, 33.33, 36.72, 46.76, 48.62, 48.98, 49.98, 49.98, 51.29, 52.34, 57.42, 58.12, 60.22, 69.64, 69.84, 71.34, 72.38, 125.35, 126.54, 127.48, 128.03, 128.23, 146.24, 171.35, 171.43, 172.55.

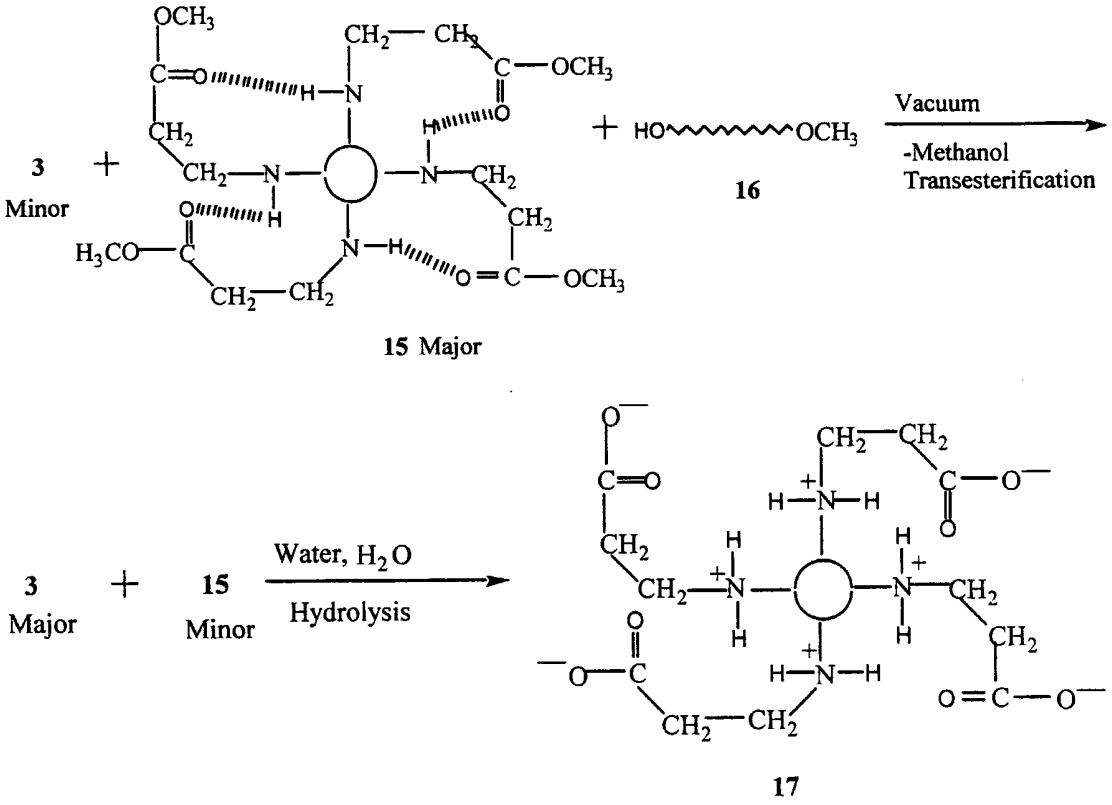
Analysis of spectral data especially the ^1H NMR analysis of the pegylated PAMAM dendrimer revealed the fact that the

double bond of PEGMA is allowed to react with the terminal amine groups of PAMAM dendrimer qualitatively. However, it was observed that the desired product, **3**, is not apparently formed. Detailed analysis of NMR spectra suggested that although **3** forms first, it is rapidly converted into **15** in the presence of solvent methanol. A considerable amount of compound **15** gets converted back to **3** during the removal of methanol at the completion of the reaction. Moreover, both **3** and **15** are unstable in presence of water and are hydrolyzed rapidly to **16**. The mechanism is believed to be a series of substitution reactions described below. The intra molecular hydrogen bond appears to be playing an important role to facilitate above described reactions.



3

Denotes H-bonding



An examination of the above scheme clearly demonstrates the proposition that the hydrogen atom on the nitrogen atom plays a pivotal role in the production of **15** and **17**, instead of the desired product **3**. Furthermore, it is suspected that the dendrimer molecule under investigation can speed up trans-esterification and hydrolysis processes readily. It is also suspected that trans-esterification and hydrolysis processes occurs for quarter as well as per pegylation. It is observed from the NMR spectrum that the intensity ratio of the peaks corresponding to the methoxy groups (OCH_3) of methanol and compound **16** was changed with the time upon the addition of the water. When water is added to the pegylated PAMAM dendrimer,

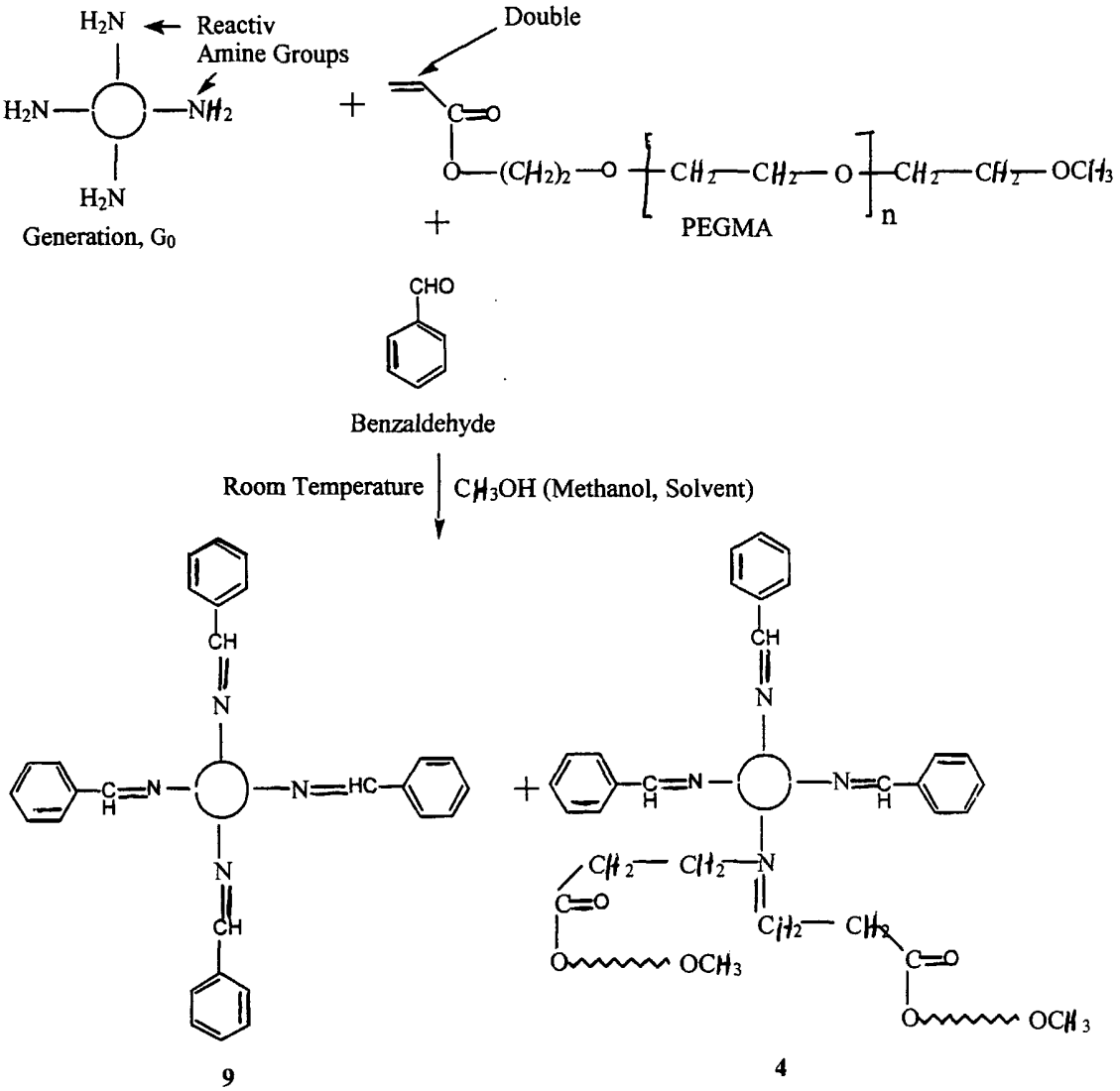
the changes of the intensity ratio of the absorbance correspond to methoxy groups of methanol and compound **16** can be monitored by ^{1}H NMR spectrum. The ratio Vs time is noted as illustrated in Table 1 below. It is suspected from the data that rates of hydrolysis follow the same trend.

Table 1

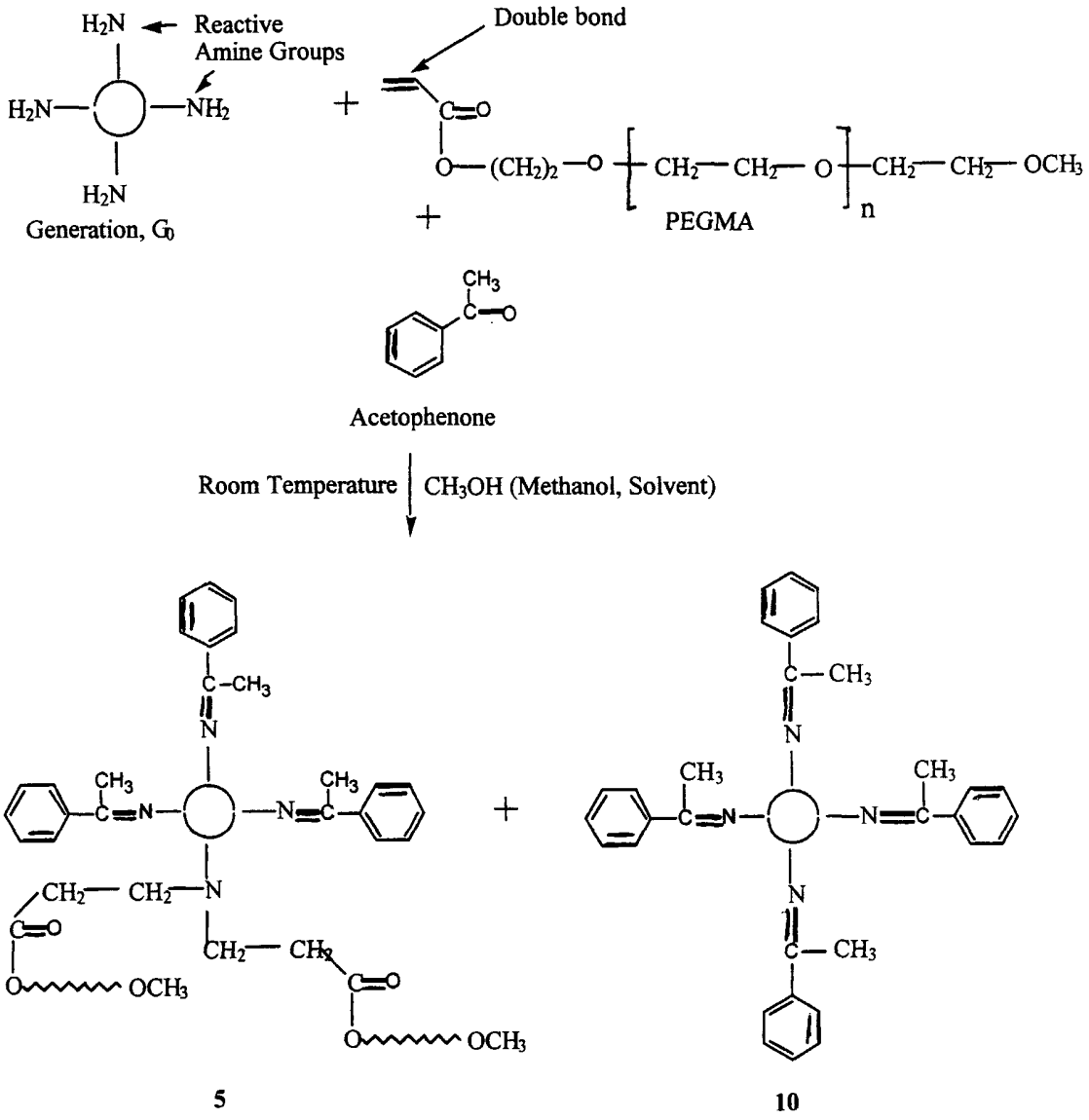
Time(min)	Ratio	1/Rato
7	0.48	2.1
19	0.47	2.13
22	0.51	1.97
27	0.65	1.54
31	0.41	2.44
35	0.57	1.74
39	0.66	1.51
44	0.53	1.87
48	0.67	1.5
52	0.76	1.3
56	0.69	1.44
61	0.71	1.41
67	0.71	1.4
6870	1.49	0.67

In order to circumvent the undesired reactions after pegylation, it was decided to block three of the four amine groups of PAMAM Dendrimer (Generation =0) to remove all the hydrogen attached to three primary residual nitrogen atoms. The remaining primary residual nitrogen atom can then be pegylated without any unwanted product. Benzaldehyde and acetophenone were used for above purpose (and almost any

aldehyde or ketone can be used for this purpose and an aldehyde substituted cyclodextrin is believed to be especially useful in this respect). As carbonyl group of benzaldehyde and acetophenone reacts with primary residual amine groups of dendrimer (Generation = 0) to form an imine. Reactions were carried out using little excess of such reagents. Analysis of the product by NMR indicated that the expected product 5 was formed along with the other side products. Blocking only three residual amine groups out of four was not achieved successfully and was complicated due to distribution of benzaldehyde and acetophenone molecules as well, since side products 9, 10 and others formed along with the expected product 5.


Reactiv
Amine Groups
Double
Generation, G0
PEGMA
n
Benzaldehyde
Room Temperature
CH3OH (Methanol, Solvent)
9
4

5



+

5

Model reactions **6.a** and **6.b** show that formation of Schiff base followed by reduction of imine functional group can be

achieved. The presence of absorbance at 1642 cm^{-1} confirm the formation of imine. On the other hand the absence of that absorbance in IR spectrum after reduction proved that imine functional group can be reduced. Furthermore, the secondary amine can be pegylated and form the desired product. All the four primary amine groups of dendrimer molecules (Generation = 0) can be converted to imine groups by reacting with carbonyl groups of, for example, benzaldehyde as well as acetophenone.

The synthesis routes are shown in scheme **7** and **8** to remove the hydrogen atoms attached to residual primary amine groups. An examination of the spectra reveals the presence of absorbance corresponding to imine functional groups. Analysis of the products by IR and NMR indicates that all amine groups were converted to imines. The imine groups of products **9** and **12** can be reduced by selective reducing agent as dendrimer molecule contains acid amide groups which are very sensitive to reducing agents. Sodium cyanohydridoborate hydride is used for this specific purpose.

An examination of ^{1}H and ^{13}C NMR spectra as well as IR spectra showed that the absence of the absorbance due to imine group. The absorbance of the reduced products **10** and **13** are identified. The hydrogen atoms of compounds **10** and **13** associated with four nitrogen atoms are pegylated in the following step. NMR analysis of the products indicates that

compounds **10** as well as **13** were pegylated. The examination of spectral data showed the formation of alcohol of the corresponding aldehyde as side product as excess aldehyde was used for those reactions. In addition, Boron complex compound was formed during the reduction step. Most of the absorbance of the final product in ^{1}H and ^{13}C NMR spectra is identified with the help of ^{1}H-^{1}H and ^{1}H-^{13}C correlation spectra.

It is noticed that reaction schemes **7** as well as **8** can not apparently be accomplished step by step. It is thought necessary that reduced compounds **10** and **13** to be worked up in order to remove unwanted Boron complex compounds after reduction of the imine.

It is observed that reduced product can not be dissolved further in low boiling point solvent such as methanol once solvent was removed after working up. This is apparently because of the formation of intra-molecular H-bonding after the solvent is removed at room temperature under vacuum. This kind of intra-molecular H-bond makes reduced compounds **10** and **13** reluctant to form further inter-molecular H-bonding with solvent. For the sake of simplicity in isolating the product, a low boiling solvent is probably the best choice for this reaction.

Therefore, a modified procedure is developed in order to avoid such solubility problem. The problem associated with

solubility is avoided by pegylating compounds **10** and **13** before work up. The analysis of ^{1}H NMR indicated that double bonds of PEGMEA are reacted with secondary amine groups of compounds **10** and **13**.

The reactions are carried and dendrimers are pegylated first without working it up. Spectral data showed that dendrimer is successfully pegylated. It can be problematic to work up the pegylated products **11** and **14** as cleavage or hydrolysis could have been possible during work up. However, examination of the ^{1}H, ^{13}C NMR spectra showed that the PEG molecules remain unchanged after treatment with strong acid and base. It is observed that delicate ether and carbonyl moieties of pegylated products **11** and **14** are neither cleaved nor hydrolyzed.

Gel electrophoresis of compound **11** and **14** along with different generation of dendrimer (ladder) are carried out to confirm the formation of compounds **11** and **14**. The migration of **10** and **11** is slower than that of **13** and **14** and is explained on the basis of steric effects.

Aldehyde substituted beta cyclodextrins are especially useful in the instant invention to block primary amines. For example, an amine terminated polyethylene glycol can be reacted with an aldehyde substituted beta cyclodextrin at room temperature in aqueous sodium cyanohydridoborate to couple the

cyclodextrine to the polyethylene glycol via a nitrogen atom to form a pegylated cyclodextrine adduct. Then, the pegylated cyclodextrine adduct can be reacted with the polyoxyalkylene acrylate to form a cyclodextrin adduct that is further pegylated.

Chitosan can be reacted with an aldehyde substituted beta cyclodextrin at room temperature in aqueous sodium cyanohydridoborate to block the primary amines of the chitosan followed by reaction with the polyoxyalkylene acrylate to form a pegylated and beta cyclodextrin substituted chitosan. Peptides, polypeptides and proteins containing primary amines can be reacted with an aldehyde substituted beta cyclodextrin at room temperature in aqueous sodium cyanohydridoborate to block the primary amines of the peptide, polypeptide or protein followed by reaction with the polyoxyalkylene acrylate to form a pegylated and beta cyclodextrin substituted peptide, polypeptide or protein.

As a specific example, the following scheme can be used in the instant invention to first convert the primary amines of poly-L-arginine to secondary amines by the addition of a cyclodextrin to the amine group and then pegylation with a polyethylene glycol acrylate. A 3 necked, 25-mL, round-bottomed, flask is fitted with nitrogen inlet, a condenser with drying tube, a rubber septum, and a magnetic stir bar. The following ingredients are added to the flask: poly-L-arginine

hydrochloride (5mg, 0.663μmol), beta cyclodextrin monoaldehyde (58.0mg, 51.2μmol), 2-mL of deionized water and 1-mL sodium hydroxide solution (0.2586M). Immediately after addition of the base, sodium cyanoborohydride (8.6mg, 0.137mmol) is added. The reaction mixture is stirred for 72 hours at room temperature. Half the reaction mixture (1.5-mL) is then removed and precipitated in 5-mL acetone for analysis to confirm the desired reaction. The remainder of the reaction mixture is mixed with 1-mL of poly(ethylene glycol) methyl ether acrylate aqueous solution (0.0286M). The reaction mixture is then stirred for an additional 72 hours at room temperature and then precipitated in 10-mL of acetone and centrifuged to yield a white solid that is dried under vacuum overnight. Analysis of the white solid confirms the desired formation of a pegylated beta cyclodextrin-poly-L-arginine conjugate.

As a specific further example, the following scheme can be used in the instant invention to first convert the primary amines of poly-L-lysine to secondary amines by the addition of a cyclodextrin to the amine group and then pegylation with a polyethylene glycol acrylate. A 3 necked, 25-mL, round - bottomed, flask is fitted with a nitrogen inlet, a condenser with drying tube, a rubber septum and a magnetic stir bar. The following ingredients are added to the flask: poly-$_L$-lysine

hydrochloride (8mg, 0.5μmol), beta cyclodextrin monoaldehyde (55.0mg, 48.5μmol), 2-mL of deionized water and 1-mL sodium hydroxide solution (0.0485M). Immediately after addition of the base, sodium cyanoborohydride (8.1mg, 0.129mmol) is added. The reaction mixture is then stirred for 72 hours at room temperature. Half the reaction mixture (1.5-mL) is then removed and precipitated in 5-mL acetone for analysis to confirm the production of the desired product. To the remainder of the reaction mixture, 0.5-mL poly(ethylene glycol) methyl ether acrylate aqueous solution (0.0502M) is added. The reaction mixture is then stirred for an additional 72 hours at room temperature and then precipitated in 10-mL of acetone and centrifuged to yield a white sold that was dried under vacuum overnight. Analysis of the white solid confirms the desired formation of a pegylated beta cyclodextrin-poly-L-lysine conjugate.

As a specific additional example, the following scheme can be used in the instant invention to first convert the primary amines of Chitosan to secondary amines by the addition of a cyclodextrin to the amine group and then pegylation with a polyethylene glycol acrylate. A 3 necked, 25-mL, round-bottomed, flask is fitted with a nitrogen inlet, a condenser with drying tube, a rubber septum and a magnetic stir bar. The following ingredients are added to the flask: low molecular

weight chitosan (50mg, 0.185mmol) dissolved in 15-mL of 0.1M hydrochloric acid, 2.00g of beta-glucero-phosphate dissolved in 4-mL of deionized water, beta cyclodextrin monoaldehyde (421mg, 0.370mmol) and sodium cyanoborohydride (33 mg, 0.525mmol). The reaction mixture is then stirred for 72 hours at room temperature. 14-mL of the reaction mixture is removed for analysis to confirm the production of the desired product. To the remainder of the reaction mixture, 4.50-mL poly(ethylene glycol) methyl ether acrylate aqueous solution (0.0220M) is added. The reaction mixture is then stirred for and additional 72 hours at room temperature. The resulting solution is then lyophilized to yield a white fibrous solid. The solid is then washed with acetone using a soxhlet and dried under vacuum overnight. Analysis of the solid confirms the formation of the desired pegylated beta cyclodextrin-chitosan conjugate.

As a yet further specific additional example, the following scheme can be used in the instant invention to pegylate glutathione. Glutathione (0.153 g, 0.0005 mole), poly (ethylene glycol) methyl ether acrylate (0.225 g, 0.0005 mole) are dissolved in 4 mL of a buffer solution (pH= 5.8) in a screw cap vial. The clear aqueous solution is allowed to mix on a tabletop shaker for 3h at room temperature. The entire reaction mixture is lypholized to produce the desired pegylated glutathione.

The following scheme can be used in the instant invention for the pegylation of proteins: (a) the protein is dissolved or dispersed in 0.1M bicarbonate buffer, pH 9.1 (the concentration of proteins as a rule can be measured from their extinction coefficients at 280 nm); (b) mPEG acrylate solutions are prepared at various concentrations in 0.1M bicarbonate buffer, pH 9.1; (c) a known volume of the protein solution is mixed with the mPEG solution in various vials to yield various amino / mPEG ratios; (d) samples are incubated under defined temperatures and times with appropriate control tubes; and (e) after reaction, the reaction mixture is subjected to native gel electrophoresis in 10% polyacrylamide gels (protein staining, as a rule, is performed with Coomassie Blue).

As a final additional specific example, the following scheme can be used in the instant invention to first convert the primary amines of a PAMAM dendrimer to secondary amines by the addition of a cyclodextrin to the amine group and then pegylation with a polyethylene glycol acrylate. A 3 necked, 25-mL, round- bottomed, flask is fitted with a nitrogen inlet, a condenser with drying tube, a rubber septum and a magnetic stir bar. The following ingredients are added to the flask: PAMAM generation 0 dendrimer (160mg, 0.310mmol) dissolved in 1-mL deionized water, beta cyclodextrin monoaldehyde (1.5010g, 1.32mmol) dissolved in 15-mL of deionized water and sodium

cyanoborohydride (229.6mg, 3.65mmol). The reaction mixture is then stirred for 72 hours at room temperature. 6-mL of the reaction mixture is then removed and precipitated in methanol to confirm the production of the desired product. To the remainder of the reaction mixture, 9-mL poly(ethylene glycol) methyl ether acrylate aqueous solution (0.0880M) are added. The reaction mixture is then stirred for an additional 72 hours at room temperature and then precipitated in 10-mL of acetone and centrifuged to yield a white sold that was dried under vacuum overnight. Analysis confirms the desired production of pegylated beta cyclodextrin PAMAM dendrimer conjugate.

Thus, it should be appreciated that in the instant invention any compound containing an amine group can be reacted with the polyoxyalkylene acrylate to form a conjugate comprising a polyoxyalkylene sub-structure. Furthermore, when the amine group is a primary amine, then it may be necessary (such as in the case of a PAMAM dendrimer) as a preliminary step to "block" the primary amine(s), as discussed above in detail, by reaction of such primary amine(s) with an aldehyde or ketone followed by conversion of the resulting imine to a secondary amine. Many drug compounds contain amine group(s) and it should be understood that the instant invention is an excellent means of converting such drugs to a polyoxyalkylene conjugate of the drug.

The term "polyoxyalkylene" is defined in the above referenced US Patent 6,280,745, herein fully incorporated by reference, and includes polyethylene glycol, polypropylene glycol, as well as block and random polyethylene glycol/polypropylene glycol co-polymers. Although acrylate terminated polyethylene glycols are commercially available, acrylate terminated polyethylene glycol can be prepared, for example, by reacting a monomethoxy polyethylene glycol with acryloyl chloride or, for example, with methacroloyl chloride.

The molecular weight of the polyoxyalkylene sub-structure of the instant invention can be tailored so that the conjugate has desired properties such as solubility characteristics that are more compatible with the biologic system. In many cases, the preferred molecular weight of the polyoxyalkylene sub-structure of the instant invention will be in the range of from about 500 to about 5000 grams per mole.

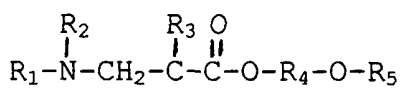
In addition to reactions with amines, the acrylate terminated polyoxylakylene of the instant invention also can be reacted with a terminal sulfur (sulfide) group(s) of a biologically active compound to produce novel compounds. For example an aqueous buffered (pH=5.8) solution of glutathione can be pegylated at room temperature by a two hour reaction with the acrylate terminated polyethylene glycol of the instant invention. Polycysteine can be similarly pegylated.

The process of the instant invention produces novel compounds that, as expected, maintain their biological activity. For example, bovine erythrocyte carbonic anhydrase (CAB) pegylated with mPEG acrylate at room temperature in a pH 9.1 aqueous buffer (mole ratio of CAB to mPEG acrylate of 1:8; 1:2 and 8:1) maintains its biological activity. As a further example, hen egg white lysozyme (HEWL) pegylated with mPEG acrylate at room temperature in a pH 9.1 aqueous buffer (mole ratio of HEWL to mPEG acrylate of 1:2 and 8:1) also maintains its biological activity.

WHAT IS CLAIMED IS:

1. A compound corresponding to the formula:

formula 1



where R_1 is an organic radical

where R_2 is H or an organic radical

where R_3 is H or an organic radical

where R_4 is a polyoxyalkylene radical

and where R_5 is an organic radical or H.

2. The compound of Claim 1, wherein R_1 is derived from a dendrimer.

3. The compound of Claim 1, wherein R_1 is selected from the group consisting of a group derived from a peptide or a protein.

4. The compound of Claim 1, wherein R_4 is derived from polyethylene glycol.

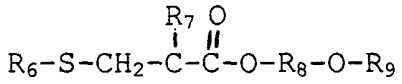
5. The compound of Claim 2, wherein R_2 is derived from a cyclodextrin, wherein R_3 is H, wherein R_4 is derived from polyethylene glycol and wherein R_5 is CH_3.

6. The compound of Claim 3, wherein R_2 is derived from a cyclodextrin, wherein R_3 is H, wherein R_4 is derived from polyethylene glycol and wherein R_5 is CH_3.

7. The compound of Claim 3, wherein R_2 is H, wherein R_3 is H, wherein R_4 is derived from polyethylene glycol and wherein R_5 is CH_3.

8. A compound corresponding to the formula:

formula 2



where R_6 is an organic radical

where R_7 is H or an organic radical

where R_8 is a polyoxyalkylene radical

and where R_9 is an organic radical or H.

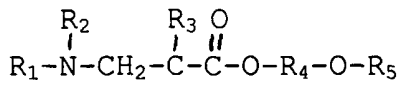
9. The compound of Claim 8, wherein R_6 is selected from the group consisting of a group derived from a peptide or a protein.

10. The compound of Claim 8, wherein R_8 is derived from polyethylene glycol.

11. The compound of Claim 9, wherein R_7 is H, wherein R_8 is derived from polyethylene glycol and wherein R_9 is CH_3.

12. A method for preparing a compound corresponding to the formula:

formula 1



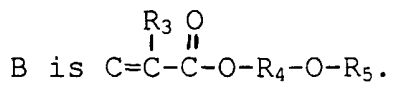
where R_1 is an organic radical

where R_2 is H or an organic radical

where R_3 is H or an organic radical

where R_4 is a polyoxyalkylene radical

and where R_5 is an organic radical or H, comprising the step of: reacting A with B, wherein A is R_1-N-R_2 and wherein



B is $C=C-C-O-R_4-O-R_5$.

13. The process of Claim 12, wherein R_1 is derived from a dendrimer.

14. The process of Claim 12, wherein R_1 is selected from the group consisting of a group derived from a peptide or a protein.

15. The process of Claim 12, wherein R_4 is derived from polyethylene glycol.

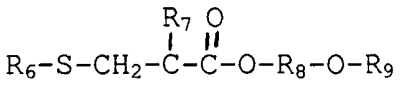
16. The process of Claim 13, wherein R_2 is derived from a cyclodextrin, wherein R_3 is H, wherein R_4 is derived from polyethylene glycol and wherein R_5 is CH_3.

17. The process of Claim 14, wherein R_2 is derived from a cyclodextrin, wherein R_3 is H, wherein R_4 is derived from polyethylene glycol and wherein R_5 is CH_3.

18. The process of Claim 14, wherein R_2 is H, wherein R_3 is H, wherein R_4 is derived from polyethylene glycol and wherein R_5 is CH_3.

19. A method for preparing a compound corresponding to the formula:

formula 2

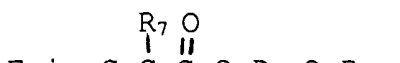


where R_6 is an organic radical

where R_7 is H or an organic radical

where R_8 is a polyoxyalkylene radical

and where R_9 is an organic radical or H, comprising the step of: reacting D with E, wherein D is R_6-S and wherein



E is $C=C-C-O-R_8-O-R_9$.

20. The compound of Claim 19, wherein R_6 is selected from the group consisting of a group derived from a peptide or a protein.

21. The compound of Claim 19, wherein R_8 is derived from polyethylene glycol.

22. The compound of Claim 20, wherein R_7 is H, wherein R_8 is derived from polyethylene glycol and wherein R_9 is CH_3.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US05/03201

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : C07K 2/00; A61K 38/00, 38/02, 31/724
US CL : 530/300; 514/2, 58
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S : 530/300; 514/2, 58

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
X	WO 03/046038 A1 (AVECIA LIMITED) 5 June 2003 (05 06.2003), page 18, example C	1,4,7,12.15,18
X	US 2003/0144222 A1 (WANG et al) 31 July 2003 (31.07 2003), claim 10.	1,2,4-6
X	US 2003/0171285 A1 (FINN et al) 11 September 2003 (11 09 2003), Claim 44, structure at top of right column page 20.	1-4,7
X	YEH et al. Microwave-enhanced liquid-phase synthesis of thiohydantoins and thiotetrahydropyrimidinones. Mol. Divers. 2003, Vol. 7, pages 186-198, especially Scheme 2 and Figure 3, part G and H.	1,4,12,15

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
01 July 2005 (01.07.2005)

Date of mailing of the international search report
22 JUL 2005

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Form PCT/ISA/210 (second sheet) (January 2004)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US05/03201

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2 ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3 ☐ Claims Nos :
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows.
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee

3 ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-7 and 12-18

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ *No protest accompanied the payment of additional search fees.*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US05/03201

BOX III. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group 1, claims 1-7 and 12-18, drawn to a compound corresponding to formula (I) and a method for making.
Group 2, claims 8-11, drawn to a compound corresponding to formula (II).
Group 3, claims 19-22, drawn to a method of preparing a compound correspondi ng to formula (II).

1. This International Searching Authority considers that the international application does not comply with the requirements of unity of invention (Rules 13.1, 13 2 and 13.3) for the reasons indicated below.

The inventions listed as Groups 1-3 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

According to PCT Rule 13.2, unity of invention exists only when the shared or corresponding technical feature is a contribution over the prior art. The technical feature is the structure of formula (I). Compounds embraced by generic formula (I) are taught by PADGET (US Patent 5,043,098; August 27, 1991), e.g.- the Abstract, Claims, compound of Example 14- MeO-$(C_2H_4O)_{22}C(O)C_2H_4NHC_2H_4OH$, and therefore lack novelty, because they do not make a contribution over the prior art.

This application contains claims directed to more than one species of the generic invention. These species are deem ed to lack unity of invention because they are not so linked as to form a single general inventive concept under PCT Rule 13.1.

In order for more than one species to be examined, the appropriate additional examination fees must be paid. The species are as follows:

R1 is derived from a dendrimer, claims 2, 5, 13, and 16
R1 is derived from a peptide or protein, claims 3, 6, 7, 14, 17, and 18.

The following claim(s) are generic: Claims 1, 4, 12, and 15

The species listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: The species are structurally unrelated, not sharing a common core. Furthermore, the species of R1 taught by Example 14 of PADGET is considered to be 'derived from a dendrimer, and thus the species lack novelty over the prior art, and the species lack unity.

An international and a national stage application shall relate to one invention only or to a group of inventions so linked as to form a single general inventive concept ("requirement of unity of invention"). Where a group of inventions is claimed in an application, the requirement of unity of invention shall be fulfilled only when there is a technical relationship among those inventions involving one or more of the same or corresponding special technical features. The expression "special technical features" shall mean those technical features that define a contribution which each of the claimed inventions, considered as a whole, makes over the prior art. An international or a national stage application containing claims to different categories of invention will be considered to have unity of invention if the claims are drawn only to one of the following combinations of categories: (1) a product and a process specially adapted for the manufacture of said product; or (2) a product and a process of use of said product; or (3) a product, a process specially adapted

for the manufacture of the said product, and a use of the said product; or (4) a process and an apparatus or means specifically designed for carrying out the said process; or (5) a product, a process specially adapted for the manufacture of the said product, and an apparatus or means specifically designed for carrying out the said process
If multiple products, processes of manufacture or uses are claimed, the first invention of the category first mentioned in the claims of the application and the first recited invention of each of the other categories related thereto will be considered as the main invention in the claims, see PCT Article 17(3)(a) and 1.476(c)
In the instant case, the first named invention of Group I is the compound of formula I where R2 and R3 are H, R1 and R5 are organic radical of undefined structure, and R4 is polyethylene glycol

Continuation of B. FIELDS SEARCHED Item 3:
EPO, JPO, DERWENT, USPAT, US PGPUB, CAPLUS· PEG, mPEG, polyethylene glycol. cojugate, polymer, dextrin, cyclodextrin, peptide.
STN registry. structure claim 1