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Facile Synthesis of Polyester Dendrimers as Drug Delivery Carriers

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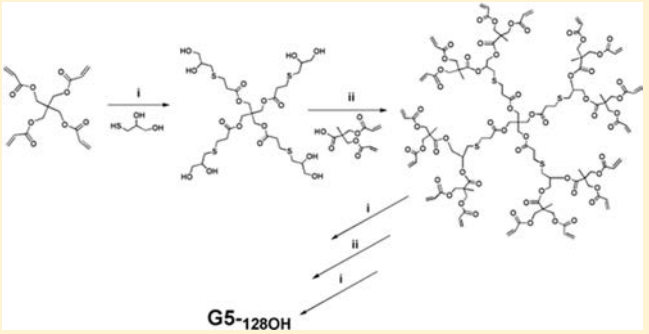
ABSTRACT: Aliphatic polyester dendrimers are attractive carriers for *in vivo* delivery of bioactive molecules due to their biocompatibility and biodegradability, but efficient precision synthesis of these dendrimers without tedious purifications remains challenging. Herein, we report an efficient synthesis approach to polyester dendrimers from two AB_2-type monomers via combining a click reaction of thiol/acrylate Michael addition with esterification. The reaction solution of each generation contains only the targeted dendrimer macromolecules; thus, the only required separation is simple precipitation. The resulting hydroxyl-terminated fifth-generation dendrimer is thermoresponsive with a LCST of 41 °C. The dendrimer could be further pegylated to obtain a water-soluble biocompatible dendrimer capable of encapsulation and controlled release of a hydrophobic anticancer drug, doxorubicin.



INTRODUCTION

Dendrimers are highly branched macromolecules characterized by monodispersity, uniform and controlled sizes, copious surface functionalities,[1] and low intrinsic viscosity in solution.[2] These characteristics make them ideal nanocarriers for biomedical applications,[1a,3] of which polyamidoamine (PAMAM) dendrimers are the most studied.[4] However, PAMAM dendrimers are not biodegradable *in vivo* and carry positive charges on their surface, thus inducing cytotoxicity,[4a,5] hemolytic toxicity,[6] rapid blood clearance,[6] and quick opsonization (RES).[7] These drawbacks hinder their translation to clinical applications. Aliphatic polyester dendrimers, for example the dendrimers from an AB_2-type monomer 2,2-bis(hydroxymethyl)propionic acid (bis-MPA), are biodegradable and biocompatible with very low toxicity and low immunogenicity[8] and thus have been proposed as carriers for *in vivo* biodelivery or imaging.[9]

The traditional polyester dendrimers were synthesized by repeated esterification of bis-MPA with protected either carboxylic acid group[10] or hydroxyl groups[11] followed by deprotection. These synthesis techniques are straightforward, but the protection/deprotection reactions may be incomplete and thus introduce defects amplified in the subsequent generations and also make the synthesis tedious.

The azide/acetylene-based click reaction characteristic of specificity, quantitative yields, and almost perfect fidelity[12] has been used extensively in synthesis of dendrimers with fewer steps, less purification procedures, and higher overall yields.[13] For instance, acetylene or azide groups were separately introduced to bis-MPA to produce asymmetric clickable monomers used for speed synthesis of dendrimers.[14] The thiol/allyl- or thiol/acetylene-based thiol−ene reactions have some characteristics of click reaction and have also been used to synthesize dendrimers.[14,15] For instance, bis-MPA was functionalized with allyl or acetylene groups and was used for dendrimer synthesis via thiol−ene reaction.[16] Very recently, Hawker and Malkoch introduced thiol and azides or acetylene and allyl groups to bis-MPA separately and obtained AB_2 and CD_2 monomers. Alternative azide−acetylene and thiol−ene click reactions produced dendrimers quickly and efficiently.[17] However, the radical nature of thiol−ene reactions caused cross-linking due to radical coupling, particularly in the synthesis of dendrimers higher than fifth generations, causing broader polydispersity (PDI > 1.2).

Aliphatic polyester dendrimers without heterocyclics have better biocompatibility and biodegradability for translational nanocarriers. Taking advantage of highly efficient thiol/acrylate Michael addition reactions, we developed a simple but efficient strategy to synthesize bis-MPA-based dendrimers without any protection/deprotection steps. The monomers were easily obtained and the reactions were fast under mild conditions. A dendrimer with 128 terminal hydroxyl groups was constructed in five steps (Scheme 1) with a high overall yield. We also demonstrated an application of the dendrimer as a drug carrier.

EXPERIMENTAL SECTION

Materials. 2,2-Bis(hydroxymethyl)propionic acid (bis-MPA, 98%), pentaerythritol (≥99%), *N*,*N*′-diisopropylcarbodiimide (DIC, 99%), acryloyl chloride (98%), triethylamine (Et_3N, 99.5%), sodium carbonate (≥99%), 1-thioglycerol (99%), succinic anhydride

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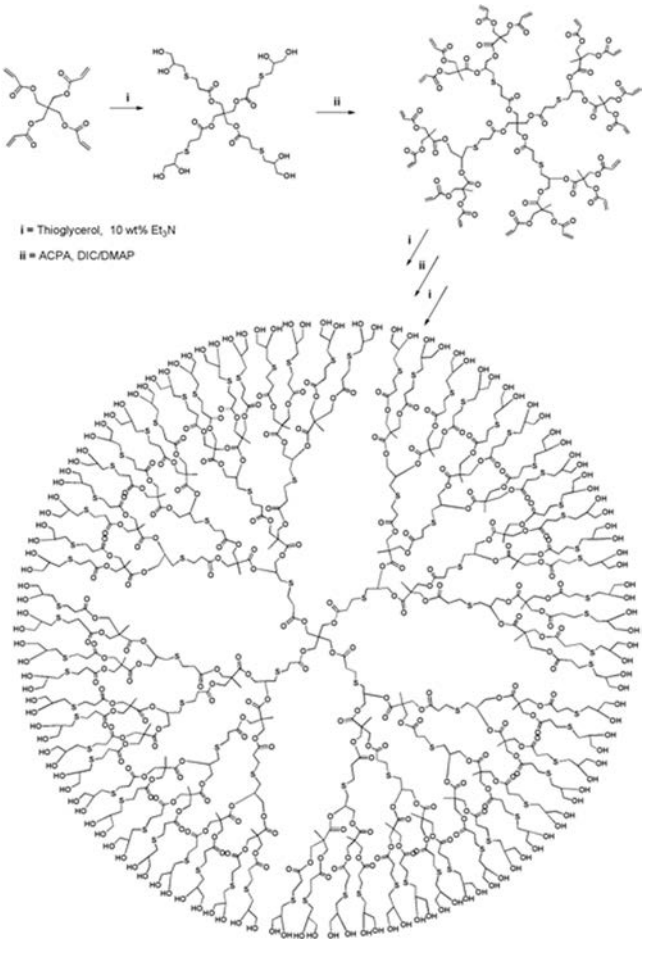
Scheme 1. Dendrimer Synthesis from a AB_2 Monomer Pair 2,2-Bis(acryloyloxymethyl)propionic Acid (ACPA) and 1-Thioglycerol



(≥99%), 4-(*N*,*N*-dimethylamino)pyridine (DMAP, ≥99%), poly(ethylene glycol) monomethyl ether (PEG2k, MW 2000 Da), and doxorubicin hydrochloride (DOX·HCl, ≥98%) were purchased from Sigma-Aldrich (Milwaukee, WI). Hydrochloric acid (37%), anhydrous dichloromethane (99.96%), ethyl ether (99.0%), methanol (99.0%), tetrahydrofuran (THF, ≥99.0%), and hexane (98.5%) were from Fisher Scientific (Pittsburgh, PA). Anhydrous dimethyl sulfoxide (DMSO, 99.9%) was from EMD Chemicals (Gibbstown, NJ). All chemicals were used as received.

Instrumentation. Gel permeation chromatography (GPC) was performed on a Waters SEC equipped with a Waters 2414 refractive index detector, a PD2000 dynamic laser light scattering detector with 15° and 90° scattered light collecting angles, and two 300 mm Solvent-Saving GPC Columns (molecular weight ranges: 5×10^2–3×10^4, 5×10^3–6×10^5) set at 30 °C. THF with 3% v/v Et_3N was used as eluent at a flow rate of 0.30 mL/min. Data were recorded and processed using the Waters software package. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance DRX-400 spectrometer using $CDCl_3$ or DMSO-d_6 as solvent. Chemical shifts were reported downfield from 0.00 ppm using TMS as an internal reference. Matrix-assisted laser-desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS; Applied Biosystems, Voyager DE Pro) was performed in a positive-ion mode with a source temperature of 200 °C at a sample concentration of about 100 μM using 2,5-dihydroxybenzoic acid as the matrix. The size of the G5-$_{PEG}$/DOX was determined using a Nano-ZS Nanosizer (Malvern Instruments, Worcestershire, UK) with a laser wavelength of 632.8 nm and a scattering angle of 173°.

Synthesis of 2,2-Bis(acryloyloxymethyl)propionic Acid (ACPA). Bis-MPA (30 g, 0.22 mol) in 300 mL of dichloromethane, DMAP (1.40 g), and TEA (77 mL) were charged to a 1 L flask and cooled to 0 °C. Acryloyl chloride (38.5 mL, 0.47 mol) was added dropwise to the solution for 3 h with stirring. The mixture was extracted with a Na_2CO_3 (10%) aqueous solution. The aqueous phase was acidified with concentrated hydrochloride acid and then extracted with dichloromethane. The organic phase was dried with sodium sulfate, and then the solvent was evaporated. The crude product was further purified with column chromatography (hexane/ethyl acetate 5:1) to obtain pure ACPA acid as a white powder (21.7 g). ^{1}H NMR ($CDCl_3$, 400 MHz): δ_{ppm}: 6.41 (d, J = 17.6 Hz, 2H), 6.09 (m, 2H), 5.86 (d, J = 11.2 Hz, 2H), 4.37 (s, 4H), 1.33 (s, 3H). ^{13}C NMR ($CDCl_3$, 400 MHz): δ_{ppm}: 178.34, 165.65, 131.63, 127.67, 65.26, 46.14, 17.69.

Synthesis of Pentaerythritol Tetraacrylate (PTA). Pentaerythritol (0.5 g, 3.67 mmol) was suspended in a solution of triethylamine (2.5 mL, 18 mmol) and dichloromethane (10 mL) and was cooled to 0 °C. Acryloyl chloride (1.30 mL, 16.1 mmol) was added dropwise to the solution in 0.5 h with stirring, and the reaction mixture was stirred for 2 h at room temperature. The mixture was then washed with a Na_2CO_3 (10%) aqueous solution. The organic phase was dried with Na_2SO_4, and then the solvent was evaporated to give the crude product. It was further purified with column chromatography (hexane/ethyl acetate 10:1) to give the PTA as a colorless oil with a 96.6% yield (1.25 g). ^{1}H NMR ($CDCl_3$, 400 MHz): δ_{ppm}: 6.44 (d, J = 17.6 Hz, 4H), 6.11 (m, 4H), 5.89 (d, J = 10.4 Hz), 4.29 (s, 8H). ^{13}C NMR ($CDCl_3$, 400 MHz): δ_{ppm}: 165.06, 131.32, 127.76, 62.45, 41.96.

Synthesis of G1-$_{8OH}$. 1-Thioglycerol (1.56 g, 14.42 mmol) was added to the solution of PTA (0.85 g, 2.41 mmol) and Et_3N (0.24 g, 2.39 mmol) in DMSO (4 mL). The solution was stirred at room temperature for 0.5 h and then diluted with methanol (4 mL). The mixture was poured into ether (30 mL). The product was isolated and purified by reprecipitation in ether. The G1-$_{8OH}$ was obtained as a colorless oil (1.81 g) at a yield of 95.9%. ^{1}H NMR (DMSO-d_6, 400 MHz): δ_{ppm}: 4.76 (d, J = 4.8 Hz, 4H), 4.57 (t, J = 4.8 Hz, 4H), 4.09 (s, 8H), 3.54 (m, 4H), 3.32 (m, 8 H), 2.71 (d, J = 6.8 Hz, 8H), 2.62(t, J = 7.2 Hz, 8H), 2.45 (t, J = 7.2 Hz, 8H). ^{13}C NMR (DMSO-d_6, 400 MHz): δ_{ppm}: 171.59, 71.92, 64.91, 62.56, 35.47, 34.80, 22.51. MS (MALDI-TOF, m/z) Calcd for $C_{29}H_{52}O_{16}S_4$: 784.2138; found: 808.4278 (M + Na^+); found: 824.4160 (M + K^+). GPC: M_n, 830 Da; PDI: 1.003.

Synthesis of G2-$_{16acrylate}$. G1-$_{8OH}$ (0.50 g, 0.64 mmol), ACPA (3.67 g, 15.29 mmol), DIC (2.83 mL, 18.35 mmol), and DMAP (0.22 g, 1.81 mmol) were dissolved in 10 mL of dichloromethane. The solution was stirred overnight at room temperature. After evaporation of the solvent, ether was added to the residue and filtered. The filtrate was precipitated in hexane (50 mL × 3), and the precipitation was dried in the vacuum to obtain the product G2-$_{16acrylate}$ as colorless oil (1.54 g) at a yield of 90.1%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ_{ppm}: 6.37 (d, J = 17.2 Hz, 16H), 6.09 (m, 16H), 5.83 (d, J = 8.4 Hz, 16H), 5.13 (m, 4H), 4.43–4.12 (m, 48H), 2.76 (t, J = 6.8 Hz, 8H), 2.67 (t, J = 6.8 Hz, 8H), 2.59 (t, J = 6.8 Hz, 8H), 1.25 (s, 24H). ^{13}C NMR ($CDCl_3$, 400 MHz): δ_{ppm}: 172.13, 171.90, 170.90, 165.72, 165.40, 165.37, 131.65, 131.62, 131.60, 131.57, 130.98, 128.15, 127.77, 127.71, 70.93, 65.24, 64.18, 62.13, 46.51, 46.48, 42.11, 34.24, 31.89, 27.05, 17.76. MS (MALDI-TOF, m/z) Calcd for $C_{117}H_{148}O_{56}S_4$: 2576.7616; found: 2599.6706 (M + Na^+). GPC: M_n, 2600 Da; PDI: 1.031.

Synthesis of G3-$_{32OH}$. Reaction of G2-$_{16acrylate}$ (0.40 g, 0.16 mmol) with 1-thioglycerol (0.66 g, 6.11 mmol) in the presence of Et_3N (0.15 mL, 1.05 mmol) in DMSO (5 mL) following the procedure used in the G1-$_{8OH}$ synthesis produced G3-$_{32OH}$ as a colorless oil (0.61 g) with a 94.9% yield. ^{1}H NMR (DMSO-d_6, 400 MHz): δ_{ppm}: 5.11 (b, 4H), 4.75 (b, 32H), 4.60 (b, 32H), 4.16 (b, 48H), 3.66 (m, 16H), 3.54 (m, 32H), 2.71 (t, J = 6.8 Hz, 48H), 2.59 (t, J = 7.2 Hz, 48H), 2.46 (t, J = 6.8 Hz, 20H), 1.18 (s, 24H). ^{13}C NMR (DMSO-d_6, 400 MHz): δ_{ppm}: 172.37, 172.09, 171.53, 171.41, 71.84, 70.66, 70.64, 65.32, 64.96, 64.89, 55.27, 46.43, 46.40, 43.53, 43.49, 35.46, 34.75, 27.48, 17.77, 17.70. MS (MALDI-TOF, m/z) Calcd for $C_{165}H_{276}O_{88}S_{20}$: 4305.1536; found: 4325.9125 (M + Na^+). GPC: M_n, 4400 Da; PDI: 1.036.

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Synthesis of G4-$_{64acrylate}$. Reaction of G3-$_{32OH}$ (0.09 g, 0.021 mmol) with ACPA (0.80 g, 3.33 mmol) in the presence of DIC (0.62 mL, 3.99 mmol) and DMAP (0.05 g, 0.41 mmol) in CH_2Cl_2 (5 mL) following the procedure used in the G2-$_{16acrylate}$ synthesis produced G4-$_{64Acrylate}$ as a colorless oil (0.22 g) at a 91.6% yield. ^{1}H NMR ($CDCl_3$, 400 MHz): δ_{ppm}: 6.40 (d, J = 17.2 Hz, 64H), 6.09 (m, 64H), 5.83 (d, J = 10.4 Hz, 64H), 5.13 (b, 20H), 4.46–4.17 (m, 208H), 2.78 (t, J = 6.4 Hz, 40H), 2.70 (t, J = 6.4 Hz, 40H), 2.59 (t, J = 6.8 Hz, 40H), 1.25 (s, 120H). ^{13}C NMR ($CDCl_3$, 400 MHz): δ_{ppm}: 172.17, 171.91, 170.94, 165.42, 131.67, 131.64, 128.10, 127.81, 127.74, 70.98, 65.26, 64.26, 46.53, 46.51, 34.26, 31.96, 27.09, 17.79. MS (MALDI-TOF, m/z) Calcd for $C_{517}H_{660}O_{248}S_{20}$: 11475.3448; found: 11 500.5951 (M + Na^+). GPC: M_n, 12 000 Da; PDI: 1.044.

Synthesis of G5-$_{128OH}$. Reaction of G4-$_{64acrylate}$ (0.40 g, 0.035 mmol) with 1-thioglycerol (1.92 g, 17.8 mmol) in the presence of Et_3N (0.32 mL, 2.30 mmol) in DMSO (5 mL) following the procedure used in the G1-$_{8OH}$ synthesis produced G5-$_{128OH}$ as a colorless oil (0.58 g) at a 90.6% yield. ^{1}H NMR (DMSO-d_6, 400 MHz): δ_{ppm}: 5.14 (b, 20H), 4.76 (b, 128H), 4.57 (b, 128H), 4.17 (b, 208H), 3.55 (m, 128H), 3.34 (m, 256 H), 2.72 (d, J = 6.8 Hz, 168H), 2.62 (m, 168H), 2.45 (t, J = 6.8 Hz, 168H), 1.24 (s, 120H). ^{13}C NMR (DMSO-d_6, 400 MHz): δ_{ppm}: 173.56, 172.36, 172.09, 171.52, 171.39, 71.89, 71.21, 65.28, 64.92, 49.07, 48.55, 46.40, 36.27, 35.49, 34.76, 34.42, 31.51, 27.50, 26.87, 17.82, 17.73, 17.38. GPC: M_n, 19 400 Da; PDI: 1.052. The diameter of G5-$_{128OH}$ measured by DLS in water (1 mg/mL) was 5.20 ± 0.10 nm.

Synthesis of PEG2k-COOH. PEG2k (10 g), succinic anhydride (2 g, 20 mmol), and DMAP (1.22 g, 10 mmol) were dissolved in 40 mL of CH_2Cl_2 and stirred at room temperature for 24 h. The solvent was removed under reduced pressure, and then the residue was dissolved in 50 mL of deionized (DI) water. The product was extracted with CH_2Cl_2 (30 mL × 3), and the organic layer was dried with sodium sulfate. After removing the solvent under vacuum, the product was obtained as a white solid (9.3 g). ^{1}H NMR ($CDCl_3$, 400 MHz): δ_{ppm}: 4.26 (m, 2H), 3.74–3.47 (m, 180H), 3.46 (s, 3H), 2.66 (m, 4H). ^{13}C NMR ($CDCl_3$, 400 MHz): δ_{ppm}: 173.18, 171.85, 71.58, 70.22, 68.66, 63.40, 58.59, 58.56, 53.89, 53.85, 28.73, 28.35. GPC: M_n, 2200 Da; PDI: 1.052.

Synthesis of Pegylated G5 (G5-$_{PEG}$). G5-$_{128OH}$ (44 mg, 2.38 μmol), PEG2k-COOH (0.96 g, 0.46 mmol), DIC (0.08 mL, 0.52 mmol), and DMAP (0.02 g) were dissolved in THF (5 mL) and stirred overnight at room temperature. The filtrate was precipitated in a solvent of THF and ether (v/v 1:1). Precipitate was dried under vacuum for 6 h, and the product was obtained as a white powder (130 mg). ^{1}H NMR ($CDCl_3$, 400 MHz): δ_{ppm}: 5.18 (b), 4.22 (m), 3.63 (m), 3.47 (s), 2.81 (m), 2.61 (m), 1.27 (b). ^{13}C NMR ($CDCl_3$, 400 MHz): δ_{ppm}: 172.27, 172.10, 171.79, 171.53, 171.02, 170.55, 170.31, 71.83, 71.76, 71.71, 70.47, 69.15, 68.93, 68.52, 67.10, 66.49, 63.78, 58.95, 49.83, 46.05, 35.71, 34.46, 34.30, 32.40, 31.96, 30.64, 28.94, 28.88, 28.79, 28.73, 27.36, 27.20, 26.27, 25.39, 25.27, 24.67, 17.86, 17.82, 17.74. GPC: M_n, 145 500 Da; PDI: 1.061. The ^{1}H NMR spectrum showed that on average each G5 molecule was conjugated with about 56 PEG2k chains (G5-$_{PEG}$). The average diameter of G5$_{-PEG}$ in water (1 mg/mL) measured by DLS was about 12.2 nm.

Determination of the Lower Critical Solution Temperature of G5-$_{128OH}$ and G3-$_{32OH}$. The lower critical solution temperatures (LCSTs) of the G1-$_{8OH}$, G3-$_{32OH}$, and G5-$_{128OH}$ were detected using a cloud point method. In brief, the dendrimers were dissolved in DI water at different concentrations. The dendrimer solution was equilibrated for 5 min at a set temperature controlled by a RC20 thermostat (Brinkmann, Dallas, TX). Transmittance through the aqueous dendrimer solution at the wavelength of 500 nm was recorded using a UV–vis spectrometer (UV-1201, Shimadzu, Japan). The transmittance was plotted versus temperature, and the LCST was defined as the midpoint of the transition.

Loading DOX to the G5-$_{PEG}$ Dendrimer. Doxorubicin hydrochloride salt (4 mg) was dissolved in 5 mL of DMSO, and two drops of triethylamine were added to the solution. G5-$_{PEG}$ (20 mg) was dissolved in 10 mL of DI water and dropped in the DOX solution. The mixture was stirred at room temperature for 3 h and then loaded into a dialysis bag (Spectra Por-7, molecular weight cutoff, MWCO, 3500) and dialyzed against pH 8.5 buffer for 24 h. The DOX content was analyzed by measuring its UV–vis absorbance at 486 nm in DMSO against a standard curve constructed with DOX solutions of known concentrations. The average diameter of G5-$_{PEG}$/DOX measured by DLS was about 19.5 nm.

Measurement of the Drug Release of G5-$_{PEG}$/DOX. G5-$_{PEG}$ loaded with 15.2 wt % DOX (3.8 mg in 10 mL of buffer solution) was loaded in a dialysis bag (MWCO 3500, Spectrum) and incubated in 100 mL of phosphate buffer saline (PBS, pH 5.0 or 7.4) at 37 °C in a water bath with shaking. At timed intervals, 10 mL of the solution was taken from outside the dialysis bag and 10 mL fresh PBS was added. The DOX concentration was determined by UV–vis absorbance at 486 nm, and the percentage of DOX released was calculated.

***In Vitro* Cytotoxicity Assay.** The cytotoxicity assay was carried out using the MTT cell-proliferation assay kit (ATCC, Manassas, VA) according to the modified manufacturer's protocol. SKOV-3 ovarian cancer cells were cultured in a medium (Invitrogen Corp., Carlsbad, CA) for at least 2 weeks before use. They were then seeded onto 96-well plates at a density of 10 000 cells per well and incubated for 72 h. The original medium (200 mL) was replaced with the G5$_{-PEG}$, G5$_{-PEG}$/DOX, or free DOX·HCl solutions at different concentrations. The cells were incubated for 72 h, and then the medium in each well was replaced with fresh cell culture medium and further incubated for 48 h. MTT reagent (10 mL) was then added to each well and incubated for 6 h. Finally, the detergent reagent (100 mL) was added to each well, and the plates were incubated at 37 °C for 18 h to dissolve the crystals. The absorbance intensity at 570 nm was recorded, and the cytotoxicity was expressed as a percentage of the control.

RESULTS AND DISCUSSION

The hydroxyl groups in bis-MPA must be protected first to avoid self-esterification or convert to other functional groups that cannot cause cross-linking.[11b,18] A monomer pair of thioglycerol (AB_2) and ACPA (CD_2) simplified the reaction requiring no protection/deprotection steps. The Michael addition reaction of thiol–acrylate is almost quantitative without side reactions and considered to be a click reaction in polymer synthesis[19] and functionalization.[20] Different from the radical mechanism of the thiol–ene/yne reactions, the thiol–(meth)acrylate reaction does not involve radicals, avoiding side reactions via radical coupling.[15a,21]

The PTA was first reacted with the thiol group in thioglycerol to produce the first-generation dendrimer with eight hydroxyl groups (Scheme 1, step i). Pendant hydroxyl groups were esterified with ACPA with catalysis of DIC/DMAP (Scheme 1, step ii). Alternating the two steps easily produced the fifth generation of the dendrimers at high overall yields (68%). The synthesis strategy is shown in Scheme 1.

The reaction between PTA and a slight excess of 1-thioglycerol in the presence of a catalytic amount of the triethylamine was carried out at room temperature. Completion of the acrylate groups' reaction was confirmed by ^{1}H NMR and MALDI-TOF mass spectra. The ^{1}H NMR spectrum of the reaction mixture showed that the signals at 5.8–6.4 ppm (Figure 1a) assigned to the acrylate protons disappeared, indicating a quantitative reaction (Figure 1b). The MALDI-TOF MS spectrum of the reaction solution confirmed that the targeted molecules—theoretical MW 784; found 807 (M + Na^+) and 824 (M + K^+)—were the only products in the solution (Figure 2).

Simple precipitation of the solution in ethyl ether removed the unreacted 1-thioglycerol, yielding the pure first generation (G1-$_{8OH}$). G1-$_{8OH}$ was then reacted with ACPA catalyzed by the DIC/DMAP coupling agents. The esterification of G1 was

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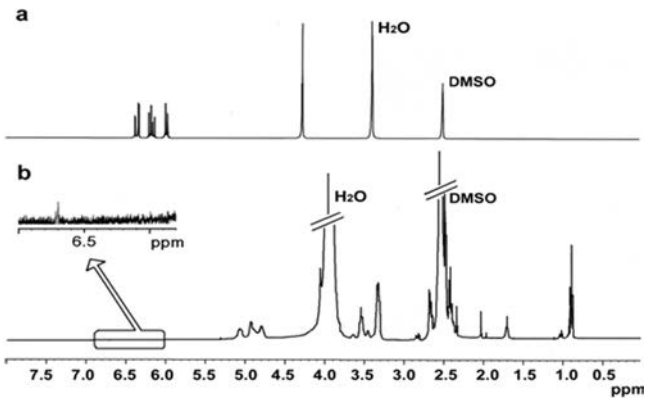

Figure 1. ^{1}H NMR spectra of PTA (a) and its reaction with 1-thioglycerol in DMSO (thiol/acrylate molar ratio of 1.5, room temperature, 0.5 h) (b).

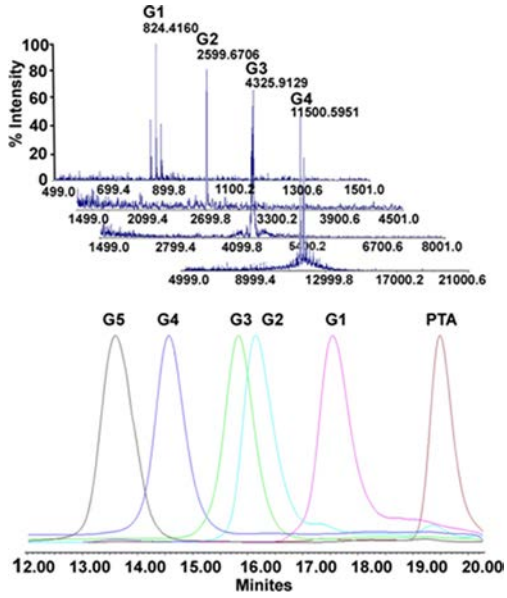

Figure 2. Molecular-weight progress of the dendrimers from the reaction of ACPA and thioglycerol measured by (a) MALDI-TOF MS and (b) GPC. The MALDI-TOF MS spectra were obtained from the reaction solutions without any purification.

monitored using MALDI-TOF analysis to ensure completion. We found that a three-to-one ratio of ACPA relative to each hydroxyl group (COOH/OH = 3) was needed to complete the esterification and produce the target G2-$_{16acrylate}$ (Figure 2a). DCC and DMAP also catalyzed this reaction to completion under the same conditions, but DCC and its byproducts were difficult to remove from the product. DIC and G2-$_{16acrylate}$ are soluble in ether, but the product of *N,N′*-diisopropylurea is not. Therefore, G2-$_{16acrylate}$ was easily isolated by ether extraction and precipitation.

Figure 2a shows the MALDI-TOF MS spectra of the reaction solutions. Clearly, the reaction solution in each generation only contained the targeted dendrimer molecules in agreement with the calculated molecular weight. There were almost no signals of incomplete molecules. For example, the reaction solution of the fourth-generation dendrimer G4-$_{64acrylate}$ had a molecular ion at 11 500.59, which was the sodium ion adduct with the molecule (11 475.34; Figure 2a). The MALDI-TOF spectrum of the fifth generation had a poor resolution due to difficult evaporation as a result of its high molecular weight. However, its GPC trace was as narrow as that of the prior generation (Figure 2b), and DLS showed that it had a diameter of 5.2 nm in water with a low PDI (data not shown), indicating the fifth generation also had similar perfect structure. Thus, the reaction solution contained only the targeted dendrimer macromolecules and a small amount of the unreacted monomers. As a result, the purification required only simple precipitation. The typical ^{1}H NMR spectra of the acrylate- and hydroxyl-terminated dendrimers (G4-$_{64acrylate}$ in $CDCl_3$ and G5-$_{128OH}$ in DMSO-d_6) are shown in Figure 3.

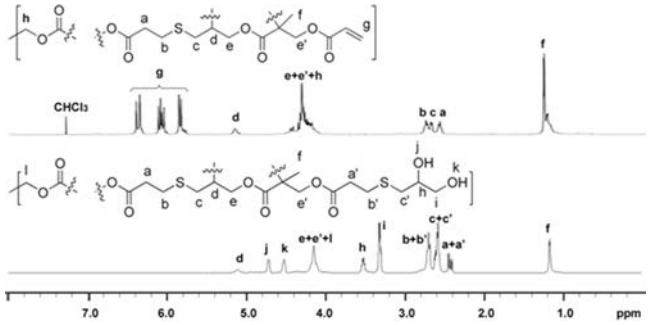

Figure 3. ^{1}H NMR spectra of G4-$_{64acrylate}$ (in $CDCl_3$) and G5-$_{128OH}$ (in DMSO-d_6).

The hydroxyl-terminated dendrimers were water-soluble at room temperature. G1-$_{8OH}$ and G3-$_{32OH}$ remained water-soluble at a high concentration (up to 5 wt %) at high temperatures (up to 80 °C) (Figure 4). Upon increasing the temperature

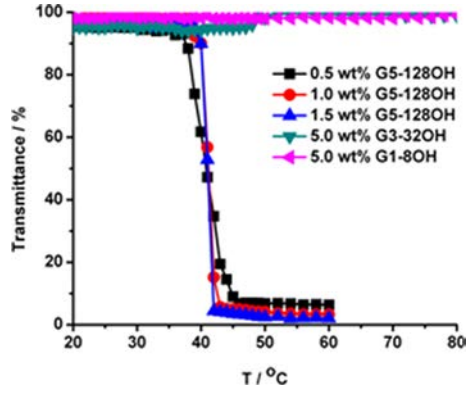

Figure 4. Transmittance of the solutions of G1-$_{8OH}$, G3-$_{32OH}$, and G5-$_{128OH}$ in DI water as a function of temperature at different concentrations.

higher than 41 °C, the clear solution of G5-$_{128OH}$ dendrimer suddenly became cloudy and the dendrimer precipitated (Figure 4). This soluble/insoluble transition at the lower critical solution temperature (LCST) was generally due to the disturbance of hydrophilicity/hydrophobicity balance of the polymer chains.[22] The average hydrophobic segment per hydroxyl group was 126.7 Da for G5-$_{128OH}$ but 98.0 Da for G1-$_{8OH}$ and 117.5 Da for G3-$_{32OH}$. Therefore, G5-$_{128OH}$ was more hydrophobic than G1-$_{8OH}$ and G3-$_{32OH;}$ upon heating some hydroxyl groups were dehydrated, further increasing the overall hydrophobicity of the dendrimer and leading to precipitation. This phenomenon was consistent with our recent results of thermally responsive polyester dendrimers[22b] and

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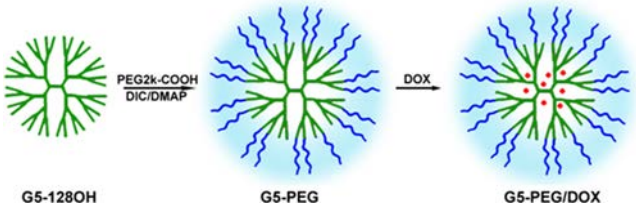
other reports.[23] Interestingly, this phenomenon was opposite to the conclusion reported by Adronov et al. that the bis-MPA polyester dendrimers functionalized with carborane had a LCST, but the parent dendrimers exhibited no phase transition.[24]

An important application of this type of dendrimer is as a drug carrier owing to its hydrophobic interior. To demonstrate this concept, PEG2 kDa chains were first introduced onto the dendrimer surface of G5-$_{128OH}$ to further grant it stealth properties. The esterification of G5-$_{128OH}$ using PEG2k-COOH in the presence of DIC and DMAP (Scheme 2) introduced a

Scheme 2. Synthesis of G5-$_{PEG}$ and Its Loading with DOX



controlled number of PEG chains. One such sample contained about 56 PEG chains (G5-$_{PEG}$) as determined by the integration of the methyl group (1.27 ppm) of dendrimer and PEG (3.6 ppm) in its ^{1}H NMR spectrum. DOX, a hydrophobic anticancer drug, was easily encapsulated into G5-$_{PEG}$ via dialysis. The DOX content was 15.2% with a loading efficiency of 99%, which suggests that the G5-PEG accommodated DOX very well via the hydrophobic−hydrophobic interaction.

As shown in Figure 5, G5-$_{PEG}$/DOX released DOX with a slight burst at pH 7.4 and 37 °C followed by a very slow

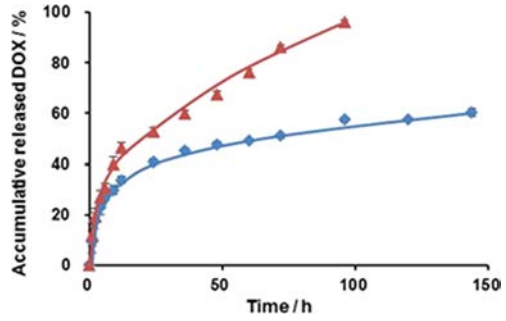


Figure 5. DOX release from G5-$_{PEG}$/DOX at pH 7.4 (▲) or 5 (◆) and 37 °C as a function of time.

release; about 40% of the DOX was released in 24 h and less than 60% in 100 h. This is a great improvement over most micellar drug carriers that generally have a severe burst release.[25] The DOX release was greatly enhanced at acidic pH. At pH 5, more than 90% of the DOX was released in 100 h. The faster degradation of the polyester structure and the protonation of DOX probably were the reason for the fast release of DOX at pH 5.

The cytotoxicity of free DOX, G5-$_{PEG/DOX}$ (15.2 wt % DOX), and G5-$_{PEG}$ to SKOV-3 ovarian cancer cells was evaluated using the (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) (MTT) assay (see Figure 6). G5-$_{PEG}$ was not toxic even at high doses. The IC$_{50}$ of the DOX in the G5-$_{PEG}$/DOX

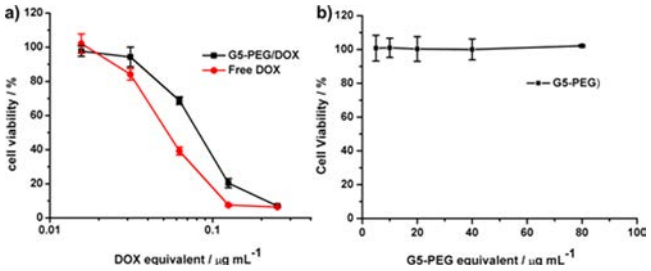


Figure 6. Cytotoxicity of free DOX, G5-$_{PEG}$/DOX (15.2 wt % DOX) (a) and G5-$_{PEG}$ (b) to SKOV-3 ovarian cancer cells estimated by MTT assay. Cells were exposed to the indicated drug or polymer for 72 h in medium. Data represent mean ± sd, n = 5.

to SKOV-3 ovarian cancer cells was 0.085 μg mL^{-1}, no significant difference from that of free DOX (0.056 μg mL^{-1}).

CONCLUSION

In summary, we successfully developed an efficient synthesis of monodispersed bis-MPA polyester dendrimers using thiol−acrylate reaction and the traditional esterification reaction under mild conditions. The 64-acrylate-terminated dendrimer was obtained in four steps, and the 128-hydroxyl-terminated dendrimer was produced in five steps. The simple synthesis and purification make the dendrimer synthesis straightforward for large-scale production. The hydroxyl-terminated dendrimers were thermoresponsive, and the LCST was 41 °C, which is near the physiological temperature. The biocompatible dendrimer G5-$_{PEG}$ showed an excellent capacity for the encapsulation and controlled release of a hydrophobic anticancer drug such as DOX. Further applications of the dendrimers as drug carriers are under exploration.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) (a) Tekade, R. K.; Kumar, P. V.; Jain, N. K. *Chem. Rev.* **2009**, *109*, 49−87. (b) Astruc, D.; Boisselier, E.; Ornelas, C. *Chem. Rev.* **2010**, *110*, 1857−1959. (c) Buhleier, E.; Wehner, W.; Vögtle, F. *Synthesis* **1978**, 155−158. (d) Zeng, F.; Zimmerman, S. C. *J. Am. Chem. Soc.* **1996**, *118*, 5326−5327. (d) Brauge, L.; Magro, G.; Caminade, A.-M.; Majoral, J.-P. *J. Am. Chem. Soc.* **2001**, *123*, 6698−6699. (e) Maraval., V.; Caminade., A. M.; Majoral., J. P.; Blais., J. C. *Angew. Chem., Int. Ed.* **2003**, *42*, 1822−1826.

(2) Mourey, T. H.; Turner, S. R.; Rubinstein, M.; Fréchet, J. M. J.; Hawker, C. J.; Wooley, K. L. *Macromolecules* **1992**, *25*, 2401−2406.

(3) (a) Jang, W.-D.; Kamruzzaman Selim, K. M.; Lee, C.-H.; Kang, I.-K. *Prog. Polym. Sci.* **2009**, *34*, 1−23. (b) Wolinsky, J. B.; Grinstaff, M. W. *Adv. Drug Delivery Rev.* **2008**, *60*, 1037−1055. (c) Cheng, Y. Y.; Zhao, L. B.; Li, Y. W.; Xu, T. W. *Chem. Soc. Rev.* **2011**, *40*, 2673−2703. (d) Wu, H. M.; Pan, S. R.; Chen, M. W.; Wu, Y.; Wang, C.; Wen, Y.

T.; Zeng, X.; Wu, C. B. *Biomaterials* **2011**, *32*, 1619−1634. (e) Mintzer, M. A.; Dane, E. L.; O'Toole, G. A.; Grinstaff, M. W. *Mol. Pharmaceutics* **2012**, *9*, 342−354. (f) Yan, H. H.; Wang, L.; Wang, J. Y.; Weng, X. F.; Lei, H.; Wang, X. X.; Jiang, L.; Zhu, J. H.; Lu, W. Y.; Wei, X. B.; Li, C. *ACS Nano* **2012**, *6*, 410−420. (g) van der Poll, D. G.; Kieler-Ferguson, H. M.; Floyd, W. C.; Guillaudeu, S. J.; Jerger, K.; Szoka, F. C.; Fréchet, J. M. *Bioconjugate Chem.* **2010**, *21*, 764−773.

(4) (a) Sadekar, S.; Ghandehari, H. *Adv. Drug Delivery Rev.* **2012**, *64*, 571−588. (b) Shen, Y.; Zhou, Z.; Sui, M.; Tang, J.; Xu, P.; Van Kirk, E. A.; Murdoch, W. J.; Fan, M.; Radosz, M. *Nanomedicine* **2010**, *5*, 1205−1217. (c) Wang, Y.; Guo, R.; Cao, X.; Shen, M.; Shi, X. *Biomaterials* **2011**, *32*, 3322−3329.

(5) Mishra, V.; Gupta, U.; Jain, N. K. *J. Biomater. Sci., Polym. Ed.* **2009**, *20*, 141−166.

(6) Malik, N.; Wiwattanapatapee, R.; Klopsch, R.; Lorenz, K.; Frey, H.; Weener, J. W.; Meijer, E. W.; Paulus, W.; Duncan, R. *J. Controlled Release* **2000**, *65*, 133−148.

(7) Kitchens, K. M.; El-Sayed, M. E. H.; Ghandehari, H. *Adv. Drug Delivery Rev.* **2005**, *57*, 2163−2176.

(8) (a) Padilla De Jesus, O. L.; Ihre, H. R.; Gagne, L.; Fréchet, J. M. J.; Szoka, F. C. *Bioconjugate Chem.* **2002**, *13*, 453−461. (b) Feliu, N.; Walter, M. V.; Montanez, M. I.; Kunzmann, A.; Hult, A.; Nystrom, A.; Malkoch, M.; Fadeel, B. *Biomaterials* **2012**, *33*, 1970−1981.

(9) (a) Lee, C. C.; MacKay, J. A.; Fréchet, J. M. J.; Szoka, F. C. *Nat. Biotechnol.* **2005**, *23*, 1517−1526. (b) Gillies, E. R.; Dy, E.; Fréchet, J. M. J.; Szoka, F. C. *Mol. Pharmaceutics* **2005**, *2*, 129−138. (c) Almutairi, A.; Akers, W. J.; Berezin, M. Y.; Achilefu, S.; Fréchet, J. M. J. *Mol. Pharmaceutics* **2008**, *5*, 1103−1110. (d) Guillaudeu, S. J.; Fox, M. E.; Haidar, Y. M.; Dy, E. E.; Szoka, F. C.; Fréchet, J. M. J. *Bioconjugate Chem.* **2008**, *19*, 461−469. (e) Ye, M. Z.; Qian, Y.; Shen, Y. Q.; Hu, H. J.; Sui, M. H.; Tang, J. B. *J. Mater. Chem.* **2012**, *22*, 14369−14377.

(10) Ihre, H.; Hult, A.; Soderlind, E. *J. Am. Chem. Soc.* **1996**, *118*, 6388−6395.

(11) (a) Ihre, H.; Hult, A.; Fréchet, J. M. J.; Gitsov, I. *Macromolecules* **1998**, *31*, 4061−4068. (b) Ropponen, J.; Tuuttila, T.; Lahtinen, M.; Nummelin, S.; Rissanen, K. *J. Polym. Sci., Polym. Chem.* **2004**, *42*, 5574−5586. (c) Malkoch, M.; Malmstroem, E.; Hult, A. *Macromolecules* **2002**, *35*, 8307−8314. (d) Fréchet, J. M. J.; Ihre, H.; De Jesus, O. L. P. *J. Am. Chem. Soc.* **2001**, *123*, 5908−5917. (e) Parrott, M. C.; Marchington, E. B.; Valliant, J. F.; Adronov, A. *J. Am. Chem. Soc.* **2005**, *127*, 12081−12089.

(12) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004−2021.

(13) (a) Walter, M. V.; Malkoch, M. *Chem. Soc. Rev.* **2012**, *41*, 4593−4609. (b) Iha, R. K.; Wooley, K. L.; Nystrom, A. M.; Burke, D. J.; Kade, M. J.; Hawker, C. J. *Chem. Rev.* **2009**, *109*, 5620−5686. (c) Wu, P.; Feldman, A. K.; Nugent, A. K.; Hawker, C. J.; Scheel, A.; Voit, B.; Pyun, J.; Fréchet, J. M. J.; Sharpless, K. B.; Fokin, V. V. *Angew. Chem., Int. Ed.* **2004**, *43*, 3928−3932. (d) Franc, G.; Kakkar, A. K. *Chem. Soc. Rev.* **2010**, *39*, 1536−1544. (e) Ledin, P. A.; Friscourt, F.; Guo, J.; Boons, G. J. *Chem.—Eur. J.* **2011**, *17*, 839−846. (f) Chen, Z.; Jackson, A. C.; Tang, G. Q.; Shen, Y.; Wang, X. *Sci. Sin. Chim.* **2011**, 281−303.

(14) Antoni, P.; Nystroem, D.; Hawker, C. J.; Hult, A.; Malkoch, M. *Chem. Commun.* **2007**, *22*, 2249−2251.

(15) (a) Hoyle, C. E.; Bowman, C. N. *Angew. Chem., Int. Ed.* **2010**, *49*, 1540−1573. (b) Killops, K. L.; Campos, L. M.; Hawker, C. J. *J. Am. Chem. Soc.* **2008**, *130*, 5062−5064. (c) Lowe, A. B.; Hoyle, C. E.; Bowman, C. N. *J. Mater. Chem.* **2010**, *20*, 4745−4750.

(16) (a) Chen, G.; Kumar, J.; Gregory, A.; Stenzel, M. H. *Chem. Commun.* **2009**, *41*, 6291−6293. (b) Montanez, M. I.; Campos, L. M.; Antoni, P.; Hed, Y.; Walter, M. V.; Krull, B. T.; Khan, A.; Hult, A.; Hawker, C. J.; Malkoch, M. *Macromolecules* **2010**, *43*, 6004−6013.

(17) Antoni, P.; Robb, M. J.; Campos, L.; Montanez, M.; Hult, A.; Malmstrom, E.; Malkoch, M.; Hawker, C. J. *Macromolecules* **2010**, *43*, 6625−6631.

(18) Ropponen, J.; Nummelin, S.; Rissanen, K. *Org. Lett.* **2004**, *6*, 2495−2497.

(19) (a) Jin, R.; Dijkstra, P. J.; Feijen, J. *J. Controlled Release* **2010**, *148*, e41−e43. (b) Dondoni, A.; Marra, A. *Chem. Soc. Rev.* **2012**, *41*, 573−586. (c) Wang, N.; Dong, A.; Tang, H.; Van Kirk, E. A.; Johnson, P. A.; Murdoch, W. J.; Radosz, M.; Shen, Y. *Macromol. Biosci.* **2007**, *7*, 1187−1198.

(20) (a) Lowe, A. B.; Hoyle, C. E.; Bowman, C. N. *J. Mater. Chem.* **2010**, *20*, 4745−4750. (b) Syrett, J. A.; Jones, M. W.; Haddleton, D. M. *Chem. Commun.* **2010**, *46*, 7181−7183.

(21) Ma, X.; Tang, J.; Shen, Y.; Fan, M.; Tang, H.; Radosz, M. *J. Am. Chem. Soc.* **2009**, *131*, 14795−14803.

(22) (a) Ono, Y.; Shikata, T. *J. Am. Chem. Soc.* **2006**, *128*, 10030−10031. (b) Shen, Y.; Ma, X.; Zhang, B.; Zhou, Z.; Sun, Q.; Jin, E.; Sui, M.; Tang, J.; Wang, J.; Fan, M. *Chem.—Eur. J.* **2011**, *17*, 5319−5326.

(23) Haba, Y.; Harada, A.; Takagishi, T.; Kono, K. *J. Am. Chem. Soc.* **2004**, *126*, 12760−12761.

(24) Parrott, M. C.; Valliant, J. F.; Adronov, A. *Langmuir* **2006**, *22*, 5251−5255.

(25) (a) Danhier, F.; Lecouturier, N.; Vroman, B.; Jerome, C.; Marchand-Brynaert, J.; Feron, O.; Preat, V. *J. Controlled Release* **2009**, *133*, 11−17. (b) Tong, R.; Cheng, J. *Polym. Rev.* **2007**, *47*, 345−381.