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Synthesis and antitumour activity of medium molecular weight phthalimide polymers of camptothecin

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Abstract: Attachment of anticancer agents to polymers has been demonstrated to improve their therapeutic profiles. A new monomer containing camptothecin, 5-norbonene-*endo*-2,3-dicarboxylimidoundecanoyl-camptothecin (NDUCPT) and its homopolymer and copolymer with acrylic acid (AA) were synthesized and spectroscopically characterized. The NDUCPT content in poly(NDUCPT-*co*-AA) obtained by elemental analysis was 51%. The average molecular weights of the polymers determined by gel permeation chromatography were as follows: $M_n = 12\,100$, $M_w = 23\,400\ g\,mol^{-1}$, $M_w/M_n = 1.93$ for poly(NDUCPT), $M_n = 15\,400$, $M_w = 28\,300\ g\,mol^{-1}$, $M_w/M_n = 1.83$ for poly(NDUCPT-*co*-AA). The IC_{50} value of NDUCPT and its polymers against U937 cancer cells was larger than that of CPT. The *in vivo* antitumour activity of all polymers in Balb/C mice bearing the sarcoma 180 tumour cell line was greater than that of CPT at a dose of $100\ mg\,kg^{-1}$.

Keywords: 5-norbonene-*endo*-2,3-dicarboxylimidoundecanoyl camptothecin (NDUCPT); poly(NDUCPT-*co*-AA); photopolymerization; *in vitro* cytotoxicity; *in vivo* antitumour activity

INTRODUCTION

One of the major challenges of chemotherapy is to suppress the nonspecific toxicity of the existing drugs against normal cells without diminishing their therapeutic activity.[1–3] The issue could be addressed by conjugating the drugs to polymeric matrices, thereby increasing both the site specificity of the drug and its concentration at tumour sites. In comparison to low molecular weight anticancer drugs, polymer-based therapeutics have been found to accumulate more in tumour tissues due to an enhanced permeability and retention effect at these sites.[4,5] Moreover, they can prolong the antitumour activity of the parent drug by releasing it at a controlled rate at the targeted site.[6]

Over the last 20 years, this type of research has led to the development of several polymeric drugs with improved therapeutic profile, including polymers of doxorubicin,[7,8] taxol,[9,10] neocarzinostatin,[11] podophyllotoxin[12] and camptothecin (CPT).[13–16] Among these compounds the camptothecin conjugates are particularly interesting, because the parent drug represents an ideal candidate for conjugation to polymers: CPT is a rather easily available natural product with a well understood mechanism of action *in vitro*. More specifically, experiments *in vivo* have also shown that CPT displays a significant antitumour activity in nude mice bearing human lung, ovarian, breast, pancreas and stomach cancers.[17] It has also been demonstrated that CPT is an antimitotic drug acting at the S-phase (DNA synthesis phase) of the cell cycle and as such it has low toxicity against normal resting cells.[18] The mechanism of action of this drug involves stabilization of the topoisomerase-I-induced DNA strand breaks, thereby preventing subsequent strand religation and leading ultimately to apoptotic cell death.[19] Consequently, prolonged inhibition of topoisomerase I was postulated to be an important factor for the therapeutic activity of CPT. However, in biological media, CPT was found to undergo an unfavorable chemical modification, during which the active δ-lactone form present at pH 4–5 is converted to an inactive open ring structure. This low stability together with the poor aqueous solubility of CPT have

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led to unpredictable toxic effects and have slowed the clinical development of this drug.

The limitations of CPT can be addressed by attaching it to a polymeric support that could act as a transport form for this drug and enhance its biodistribution while keeping intact its therapeutic profile. Both water soluble and insoluble CPT polymers have been reported and have interesting pharmacological properties. While the water soluble conjugates promise to solve the poor solubility of the natural product,[14] the water insoluble polymers exhibit a superior antitumor activity against *in vitro* human cancers and *in vivo* animal xenografts.[20]

Inspired by the clinical potential and current limitations of the camptothecin-based therapeutics, we sought to construct and study phthalimide-based polymers of CPT. Such polymers have been found in our laboratories to be efficient carriers of 5-fluorouracil (5-FU), retaining the antitumour activity while decreasing the toxicity of the parent molecule. Moreover, tetrahydrophthalic-acid-based polymers showed a strong antitumour activity against cancer cell lines,[21–24] while they displayed very low toxicity against normal cells.[25]

We report herein the synthesis and biological evaluation of new antitumour active polymers of CPT based on the 2,5-methano-1,2,5,6-tetrahydrophthalimide template. The monomeric unit was built by conjugating CPT to tetrahydrophthalic anhydride using 11-aminoundecanoic acid. Its homopolymer and copolymer with acrylic acid (AA) were prepared by photopolymerization. The obtained monomeric unit and its polymers were identified by ^{1}H NMR and ^{13}C NMR spectroscopy, and elemental analysis. The average molecular weights of the polymers were measured by gel permeation chromatography (GPC). The *in vitro* cytotoxicities were evaluated with mouse mammary carcinoma (FM3A) and human histiocytic lymphoma (U937) as cancer cell lines, and mouse liver cells (AC2F) as a normal cell line. The *in vivo* antitumour activities of the synthesized polymers against mice bearing the sarcoma 180 tumour cell line were evaluated. Our data indicate that the synthesized polymers exhibit higher antitumour activities than monomer (NDUCPT) and CPT. In addition, they display a low cytotoxicity against normal cell lines, suggesting that they can be used for further pharmacological and clinical studies.

EXPERIMENTAL

Materials

CPT (Aldrich, Wisconsin, USA), *cis*-5-norbonene-*endo*-dicarboxylic anhydride (NDA; Aldrich, Wisconsin, USA), 11-aminoundecanoic acid (Aldrich, Wisconsin, USA), 2,2-dimethoxy-2-phenylacetophenone (DMP; Aldrich, Wisconsin, USA), dimethylaminopyridine (DMAP; Aldrich, Wisconsin, USA), 1-[3-(dimethylaminopropyl)]-3-ethylcarbodiimide hydrochloride (EDC; Aldrich, Wisconsin, USA), and triethylamine (TEA; Aldrich, Wisconsin, USA) were used without further purification. Acrylic acid (AA; Junsei, Tokyo, Japan) was dried with NaCl and distilled under vacuum (7 mmHg, 45 °C).

For the *in vitro* tests, FM3A, P388 and U937 were used as cancer cell lines and AC2F was used as a normal cell line. For the *in vivo* test, Balb/C mice and sarcoma 180 cell line were purchased from the Center of Genetic Engineering (Korea Institute of Science and Technology) at 6–8 weeks of age. Mice were maintained under specific pathogen-free conditions at 22 ± 1 °C and 55 ± 5% humidity. They were fed a Purina chow diet and water *ad libitum* during the experiment. Animal experiments were approved by the Ethical Committee for Animal Experimentation of Kosin University and performed according to the NIH Guide for the Care and Use of Laboratory animals.

Instruments

IR spectra were recorded on a Perkin-Elmer 397 spectrometer (Norwalk, USA) using KBr pellets. ^{1}H and ^{13}C NMR spectra were measured on a FT-400 MHz Varian Gemini 2000 spectrophotometer (Palo Alto, USA). DMSO-d_6 was used as the solvent. Elemental analyses were carried out with an elemental analyser (Carlo Erba, model EA 180, Peapack, USA). Number- and weight-average molecular weights (M_n and M_w) and polydispersity (M_w/M_n) were estimated by gel permeation chromatography (GPC; Waters 410 differential refractometer, Massachusetts, USA).

Synthesis of monomer

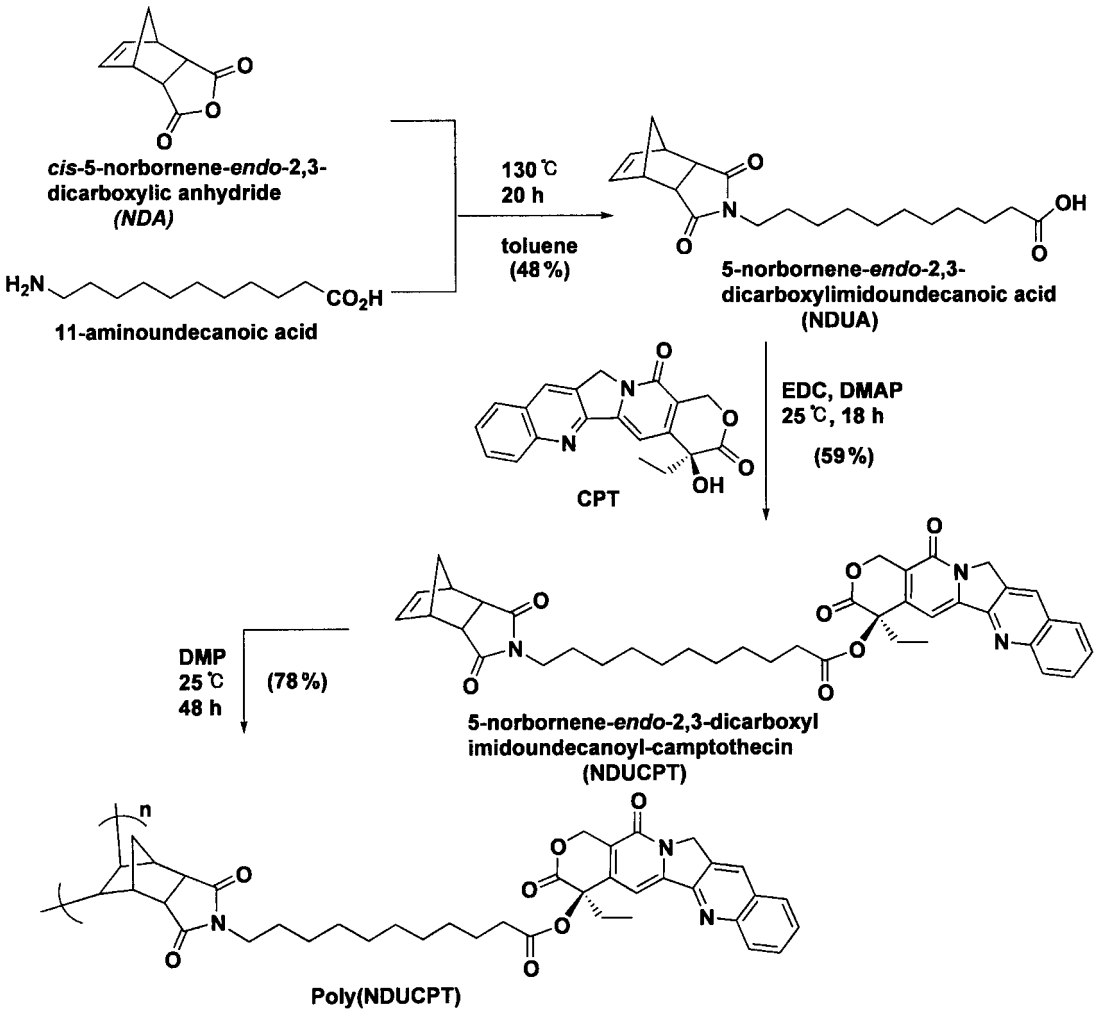
The NDUCPT monomer was synthesized according to Scheme 1.

5-Nornonene-*endo*-2,3-dicarboxylimidoundecanoic acid (NDUA)

11-Aminoundecanoic acid (6.1 g, 30.4×10^{-3} mol), MPA (4.9 g, 30×10^{-3} mol) and TEA (1 ml) were refluxed in toluene (100 ml) for 22 h with azeotropic removal of water via a Dean–Stark apparatus. After evaporation of the solvent, the residue was triturated with 0.1 N HCl (25 ml), and dissolved in saturated aqueous $NaHCO_3$ (20 ml). The aqueous solution was washed with ethyl acetate (60 ml), acidified to pH 2 by adding conc HCl and extracted twice with chloroform (2×30 ml). The chloroform layers were dried over anhydrous $MgSO_4$ and evaporated to obtain pure ETPUA in 48% yield. ^{1}H NMR (DMSO-d_6): δ(ppm) = 11.89 (s, 1H, COO*H*), 6.00 (s, 2H, HC=CH of MPA), 3.27 (s, 2H, CONCH_2CH$_2$—), 3.20–3.13 (m, 4H, COC*H*C*H*CH$_2$C*H*C*H*CO), 2.15 (t, 2H, CH$_2$CH_2COOH), 1.44–1.53 (m, 4H, CH_2CHCHCO of MPA and CH_2CH$_2$COOH), 1.12–1.29 (m, 14H).

5-Nornonene-*endo*-2,3-dicarboxylimidoundecanoyl-camptothecin (NDUCPT)

A mixture of ETPUA (416 mg, 1.2×10^{-3} mol), CPT (348 mg, 1×10^{-3} mol), DMAP (144 mg, 1.2×10^{-3} mol), and EDC (229 mg, 1.2 mol) in anhydrous dichloromethane (5 ml) was stirred at room



Scheme 1.

temperature. After 18 h stirring, the reaction mixture was diluted with dichloromethane (150 ml) and washed with 0.1 N HCl (30 ml), saturated aqueous $NaHCO_3$ (30 ml), and water (30 ml). The organic layer was dried over anhydrous $MgSO_4$ and evaporated under reduced pressure to give a pale yellow solid. The product was purified by flash silica gel column chromatography using CH_2Cl_2–MeOH (97:3, v/v) as eluent to yield a yellow solid (480 mg, 59%). ^{1}H NMR (DMSO-d_6): δ(ppm) = 8.65 (s, 1H, H-7), 8.12 (d, 1H, H-12), 8.08 (d, 1H, H-9), 7.83 (t, 1H, H-10), 7.70 (t, 1H, H-11), 7.02 (s, 1H, H-14), 5.99 (s, 2H, *H*C=C*H* of MPA), 5.48 (d, 2H, H-17), 5.24 (d, 2H, H-5), 3.28 (t, 2H, CONCH_2CH_2), 3.20 (s, 2H, CHC*H*CON of MPA), 3.11 (t, 2H, C*H*CHCON of MPA), 2.15 (t, 2H, CH_2CH_2COO), 1.50–1.54 (m, 4H, CH_2CHCHCON of MPA and H-19), 0.97–1.22 (m, 14H, NCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH$_2$ CH$_2$COO), 0.92 (t, 3H, H-18); ^{13}C NMR (DMSO-d_6): δ(ppm) = 177.4, 172.1, 167.2, 156.6, 152.2, 147.9, 146.0, 145.4, 134.2, 131.6, 130.4, 130.0, 129.8, 128.9, 128.5, 128.0, 127.7, 119.0, 94.7, 75.6, 66.3, 51.8, 50.2, 45.1, 44.2, 37.4, 33.1, 30.3, 28.76, 28.73, 28.68, 28.4, 28.3, 27.1, 26.1, 24.4, 7.5. HRMS: calculated, $[M + H]^+$ = 678.3174; found, 678.3165.

Synthesis of Poly(NDUCPT)

A solution of NDUCPT (0.88 g, 1.3×10^{-3} mol) and DMP (0.02 g, 0.065×10^{-3} mol) as an initiator in dry acetone (10 ml) was introduced into a dry Pyrex polymerization tube. The tube was flushed twice with N_2 gas, sealed and placed in a photochemical chamber where it was irradiated at 313 nm (115 V, 60 Hz power supply) at 25 °C for 48 h. After polymerization, the tube was opened and the viscous liquid obtained was slowly precipitated into a large excess of n-hexane (300 ml). The precipitated polymer was collected by filtration and washed several times with acetone. The obtained homopolymer was dried under reduced pressure to a constant weight. The conversion was 78%.

Syntheses of Poly(NDUCPT-*co*-AA)

A solution of NDUCPT (0.88 g, 1.3×10^{-3} mol) and AA (0.1 ml, 1.3×10^{-3} mol) with DMP (0.04 g, 0.13×10^{-3} mol) as an initiator in dry acetone (10 ml) was introduced into a dry Pyrex polymerization tube. The tube was sealed after flushing twice with bubbling purified N_2 gas. The preparation procedure for poly(NDUCPT-*co*-AA) was the same as that described for the homopolymerization of NDUCPT except for the monomer pairs. The copolymerization conversion of NDUCPT with AA was 75%.

Measurements of average molecular weight and compositions

To compare the average molecular weights of the synthesized polymers, we determined the apparent molecular weights by GPC using a microstyragel column and low polydispersity polystyrenes as a standards at 40 °C. Dimethylformamide was used as an eluent. The contents of NDUCPT moiety in the copolymers were calculated from C, H and N data obtained by elemental analysis.

Biological Activity Tests

In vitro cytotoxicities of NDUCPT and its polymers

The cytotoxicities of NDUCPT and its polymers against three cancer cell lines *in vitro* were determined by the MTT colorimetric method.[26] The MTT assay is based on the reduction of the soluble 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) into a blue–purple formazan product, mainly by mitochondrial reductase activity inside living cells. The cells used in cytotoxicity assay were cultured in RPMI 1640 medium supplemented with 10% foetal calf serum. Cells suspended in the medium were plated in 96-well culture plates and incubated at 37 °C in a 5% CO_2 incubator for 24 h. The test samples were dissolved in the minimum quantity of dimethylsulfoxide (DMSO) and diluted with phosphate-buffered saline just before use. The solution was added to the cells (2×10^4) in 96-well plates and cultured at 37 °C for 3 days. The cultured cells were mixed with 20 µl of MTT solution and incubated for 4 h at 37 °C. The supernatant was carefully removed from each well and 100 µl of DMSO was added to each well to dissolve the formazan crystals which were formed by the cellular reduction of MTT. After mixing with a mechanical plate mixer, the absorbance was measured at 570 nm using a microplate reader. All experiments were carried out in triplicate. Cytotoxic activities were expressed as IC_{50} values, the drug concentrations required for 50% inhibition of cell growth. There was a good reproducibility between replicate wells with standard errors below ±10%.

In vivo antitumour activity test

In vivo evaluation of the antitumour activity of NDUCPT and its polymers was performed on sarcoma 180 cells which were kept as an ascitic tumour in Balb/C mice with weekly transplants. The cells (1×10^6) were injected intraperitoneally (i p) into Balb/C mice (6 weeks old, 25 g). The test samples in physiological saline were given i p to mice once a day for four consecutive days starting from 24 h after the cell injection. The different doses tested were 10 and 100 mg kg^{-1}. Groups of ten animals were used. For comparison, the antitumour activity of CPT was also tested by the same method. The control group was divided into two groups: one subgroup was treated with sarcoma 180 cells together with neat saline by replacing the sample solution; the other subgroup was treated with sarcoma 180 cells alone. Antitumour activity was evaluated by the median survival time of the experimental group and was expressed as the treated/control (T/C) value.

RESULTS AND DISCUSSION

Identification of monomer and polymers

The structures of the synthesized monomer and polymers were confirmed by IR, ^{1}H NMR and ^{13}C NMR spectroscopies. The FTIR characteristic absorption peaks for NDUCPT appeared at 1752 and 1693 cm^{-1} (C=O stretching), and 1670 cm^{-1} (C=C stretching). The ^{1}H NMR spectrum of NDUCPT is shown in Fig 1. The peaks of vinyl protons in NDUCPT appeared at 6.0 ppm and ethyl proton of CPT moiety in NDUCPT indicated at 1.5 ppm and at 0.9 ppm, respectively.

Poly(NDUCPT) was synthesized via radical polymerization through the carbon–carbon double bond on the norbornene moiety. The FTIR spectrum of poly(NDUCPT) showed the characteristic absorption peaks at 1752 and 1693 cm^{-1} with disappearance of vinyl absorptions at 1670 cm^{-1} which appeared in NDUCPT monomer. The characteristic peaks for the methine protons of the polymer backbone of poly(NDUCPT) were observed at 0.7 ppm in the ^{1}H NMR spectrum. The peak for the vinyl protons of monomeric NDUCPT at 6.0 ppm was not observed. The FTIR spectrum of poly(NDUCPT-*co*-AA) indicated absorption at 3400–2800 cm^{-1} (COOH stretching of AA moiety) and 1750–1700 cm^{-1} (C=O stretching). The absorption peaks caused by protons of NDUCPT moiety in poly(NDUCPT-*co*-AA) were assigned to the same as those of poly(NDUCPT). In the ^{1}H NMR spectrum, the proton peaks for the carboxylic acid, methine and methylene protons of the AA moiety in poly(NDUCPT-*co*-AA) were observed at 12.0, 1.0 and 1.2 ppm, respectively. The characteristic peaks for the methine proton on the NDUCPT moiety were observed at 0.9 ppm. The peak assigned to the olefinic proton of NDUCPT and AA moiety at 6.0 and 7.1 ppm disappeared, respectively.

Solubility of NDUCPT and its polymers

NDUCPT and its polymers were soluble in acetone, 2-butanone, DMF, DMSO, EtOH, MeOH and THF, but they were poorly soluble or insoluble in water, acetic acid, n-hexane and diethyl ether.

Average molecular weights and compositions of polymers

The average molecular weights and polydispersity indices of the polymers are listed in Table 1. The number average molecular weights (M_n) of the polymers we synthesized using a previously reported method[21–25] ranged from 12 100 to 18 300 g mol^{-1} and molecular weight distributions (M_w/M_n) were 1.83–1.93. The polymers met the minimum chain length requirement to exhibit antitumour activity

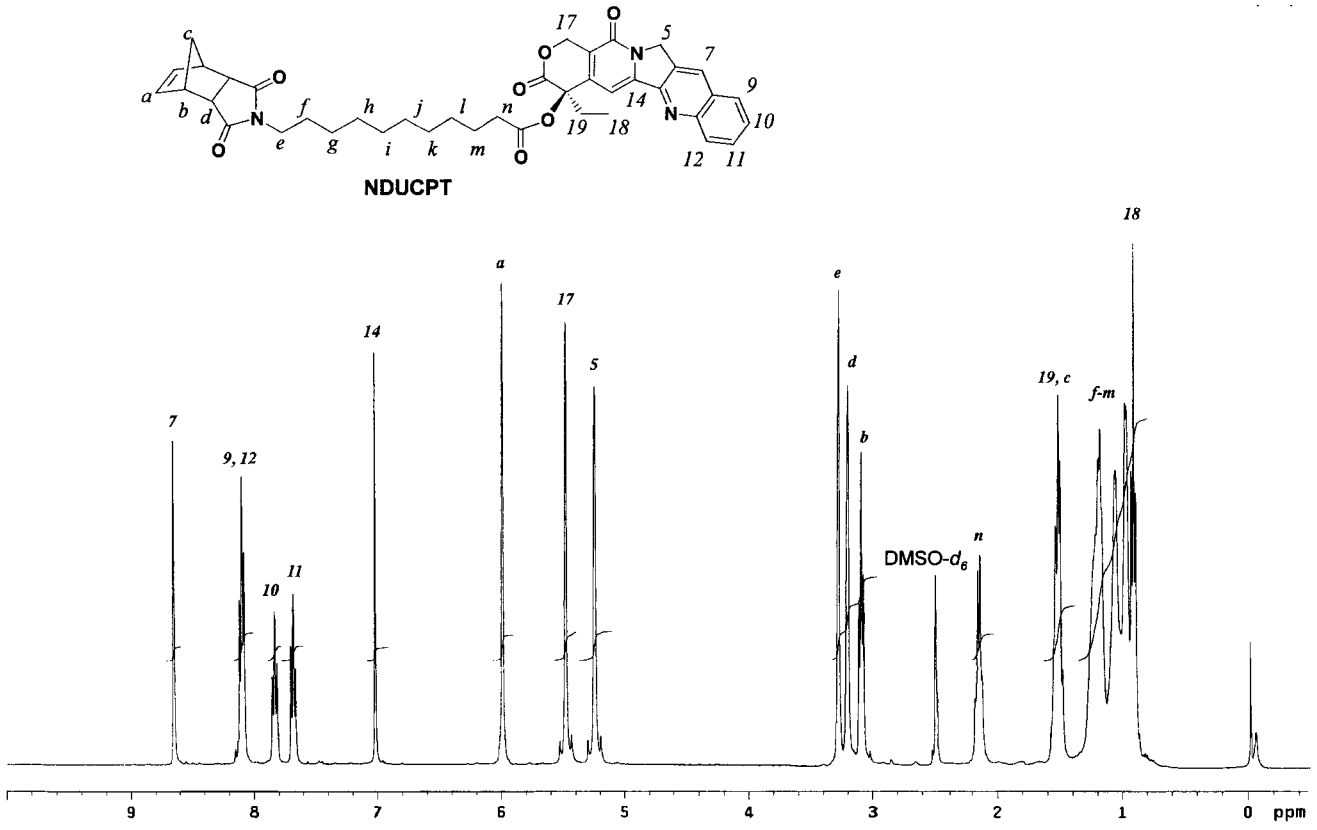


Figure 1. ^{1}H NMR spectrum of NDUCPT.

based on Ottenbrite's findings.[27] The elemental analysis of poly(NDUCPT-*co*-AA) is as follows: C, 46.34; H, 8.17; N, 2.93. The NDUCPT composition in poly(NDUCPT-*co*-AA) was determined by the elemental analysis and was 51%

Table 1. Average molecular weights[a] and polydispersity of the polymers

Polymer	M_n (g mol^{-1})	M_w (g mol^{-1})	M_w/M_n
Poly(NDUCPT)	12 100	23 400	1.9
Poly(NDUCPT-*co*-AA)	15 400	28 300	1.8

[a] Molecular weights were determined by GPC in DMF.

In vitro cytotoxicity of NDUCPT and its polymers

The *in vitro* cytotoxicity of NDUCPT and its polymers was evaluated against two cancer cell lines and one normal cell line. As shown in Table 2, the values of 50% cytotoxicity (IC_{50}) for NDUCPT and its polymers were in the range 12–66 ng ml^{-1} against cancer cell lines. The cytotoxicity of monomer and its polymers were lower than that of free CPT, because the drugs attached on monomer and its polymers are not active and they take a certain period to release the active parent drug. The cytotoxicity of NDUCPT against U937 cell was higher than those of poly(NDUCPT) and poly(NDUCPT-*co*-AA), but its cytotoxicity against FM3A cells was lower than those of poly(NDUCPT) and poly(NDUCPT-*co*-AA). The cytotoxicity of

Table 2. *In vitro* cytotoxicity of NDUCPT and its polymers against cell lines

	IC_{50} (ng ml^{-1}) for cell lines[a]			
	Cancer cells		Normal cells	
Sample	FM3A[b]	P388[c]	U937[d]	AC2F[e]
CPT	0.05 ± 0.004	0.22 ± 0.01	0.21 ± 0.02	0.04 ± 0.002
NDUCPT	28 ± 1.7	34 ± 0.1	13 ± 0.3	72 ± 5.0
Poly(NDUCPT)	12 ± 0.6	31 ± 0.5	17 ± 0.1	10 ± 0.4
Poly(NDUCPT-*co*-AA)	13 ± 1.1	66 ± 1.9	37 ± 1.5	20 ± 1.3

[a] 50% growth inhibition.
[b] Mouse mammary carcinoma cell.
[c] Mouse leukaemia cell.
[d] Human histiocytic lymphoma cell.
[e] Mouse liver cell.

NDUCPT and its polymers against P388 cells increased in the following order: NDUCPT = NDUCPT > poly(NDUCPT-*co*-AA). In a normal cell line (AC2F), the cytotoxicity of NDUCPT and its polymers were much lower than that of free CPT.

In vivo antitumour activity of NDUCPT and its polymers

The antitumour effects of NDUCPT and its polymers against sarcoma 180 injected i p in mice are shown in Table 3 together with that of CPT for comparison. Mortality was recorded and mean survival time was calculated for each compound. The activity of polymers was expressed as a survival effect (*T*/*C*), where *T* is the mean survival time of mice treated with sample and *C* is the survival time of mice in a control group.

As shown in Table 3, entries 4, low dosage of CPT (10 mg kg^{-1}) led to good antitumour activity (625% increase compared to the control group). However, increase of drug dosage to 100 mg kg^{-1} led to a sharp decrease of the mean survival time (33% as compared to the control group). This may be attributed to the inherent toxicity of CPT.

The anticancer activity of the monomeric unit (NDUCPT) at different dosages is shown in Table 3, in entries 5 and 6. At low and high drug dosages (10 and 100 mg kg^{-1}) we observed a significant increase (above 472%) of the mean survival time *versus* that of the control group. This suggests that at a high concentration the monomer displays low toxicity and at a low dosage it led to a statistically significant prolongation of the life of mice beyond the experimental period (95 days).

Evaluation of poly(NDUCPT) at two different dosages is presented in Table 3, entries 7 and 8. High drug dosage (100 mg kg^{-1}) led to an increase of the mean survival time by about 600% *versus* that of the control group. At this dosage, nine out of the ten mice survived the experimental period of 95 days, indicating that the polymer has a low toxicity level. However, at low drug dosage (10 mg kg^{-1}) the mean survival time was only slightly increased (166%) and no mice survived the experimental time of 95 days. This may be due to the low concentration of CPT in the polymer.

As indicated in Table 3, entries 9 and 10, treatment of mice with poly(NDUCPT-*co*-AA) resulted in a substantial increase of the mean survival time both at high and low drug dosage. At high dosage (100 mg kg^{-1}) we observed an increase in mean survival time by 625% leading to a 100% survival of treated mice beyond the experimental period of 95 days. At low drug dosage the mean survival time was 344% and only four mice survived beyond the 95 days. These results demonstrate that this polymer has a very low toxicity even at high dosages and can thus overcome the inherent toxicity associated with high doses of CPT.

From the data of Table 3 it is interesting to note that the monomer conjugate NDUCPT displays a similar antitumour activity with poly(NDUCPT-*co*-AA) and slightly better than poly(NDUCPT). Nonetheless, if we consider that the content of NDUCPT in the poly(NDUCPT-*co*-AA) is 51 mol% we can conclude that this copolymer doubles the overall efficiency of the monomeric unit. Thus, given the high cost of CPT, its polymer or copolymer administrations may be useful in decreasing the overall cost while maintaining the efficiency of the parent drug.

CONCLUSIONS

A new monomer, 5-norbonene-*endo*-2,3-dicarboxylimidoundecanoyl-camptothecin (NDUCPT), was synthesized from camptothecin (CPT) and 5-nornonene-*endo*-2,3-dicarboxylimidoundecanoic acid (NDUA). Its homopolymer and copolymer with acrylic acid (AA) were prepared by photopolymerization and were identified by ^{1}H NMR and ^{13}C NMR spectroscopy. The polydispersity indices of all synthesized polymers ranged from 1.8 to 1.9. The content of NDUCPT in poly(NDUCPT-*co*-AA) was found to be 51 mol%.

Table 3. *In vivo* Antitumour activity of NDUCPT and its polymers

Entry	Sample	Dose (mg kg^{-1})	Survival time (days)[a]	*T*/*C* (%)[b]	*S*/*E*[c]
1	Control	—	14.7 ± 2.3	100	0/10
2		Saline	15.7 ± 0.5	100	0/10
3	CPT	100	5.00 ± 0.00	33	0/10
4		10	95.0 ± 0.0	625	10/10
5	NDUCPT	100	95.0 ± 0.0	625	10/10
6		10	71.7 ± 13.6	472	6/10
7	Poly(NDUCPT)	100	90.3 ± 1.55	594	9/10
8		10	25.3 ± 5.86	166	0/10
9	Poly(NDUCPT-*co*-AA)[d]	100	95.0 ± 0.0	625	10/10
10		10	52.3 ± 10.4	344	4/10

[a] Mean survival time of animals dying within the experimental period of 95 days.
[b] *T/C (%) represents the ratio of the survival time of the mice treated with a sample (T) to the control (C) mice* × 100.
[c] *S/E denotes the ratio of the number of survival mice (S) to that of experimental mice (E) after the experimental period of 95 days.*
[d] *For poly(NDUCPT-co-AA), the NDUCPT composition is 51% which means that the drug composition is 26%.*

IC_{50} values obtained from the *in vitro* test for NDUCPT, poly(NDUCPT) and poly(NDUCPT-*co*-AA) were 12–66 ng ml^{-1} against cancer cell lines, which were much higher than those of CPT. In a normal cell line, the cytotoxicities of NDUCPT and its polymers were much lower than that of CPT.

On the basis of mean survival times in mice bearing the sarcoma 180 tumour cell line, the synthesized monomer and its polymers exhibited excellent antitumour activity (about 600% *T*/*C*) and reduced toxicity as compared with CPT administration at high drug dosage (100 mg kg^{-1}). However, at low drug concentration (10 mg kg^{-1}) we observed no significant increase in the antitumour activities of NDUCPT and its homopolymer and copolymers as compared to treatment with monomeric CPT. Treatment of mice with CPT led to a 100% survival beyond the experimental period. From these data, we may suggest that administration of CPT in a form of polymer or conjugate is more efficient when high drug dosages are needed.

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