Syntheses of bifunctional molecules containing [12]aneN₃ and carbazol moieties as effective DNA condensation agents

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Bifunctional molecules containing macrocyclic polyamine [12]aneN₃ and carbazol units, 1–4, have been efficiently synthesized and fully characterized. Through gel electrophoresis, atomic force microscopy, and dynamic light scattering experiments, compounds 3 and 4b bearing both [12]aneN₃ and carbazol moieties showed effective DNA condensation ability at the concentration of 80 μM. Investigations from EB displacement fluorescence spectra, viscosity titration, and ionic strength effects revealed that the effective DNA condensation comes from the appropriate combination of carbazol and [12]aneN₃ units in the bifunctional molecules, and the DNA condensation process is reversible. The incorporation of triazole units in the molecules clearly reduced the cytotoxicity.

DNA condensation, macrocyclic polyamines, carbazoles, triazole, gel electrophoresis, AFM

1 Introduction

The condensation of DNA, a process in which the extended DNA chains are collapsed into tightly packed state, has received considerable attention due to its biological importance in DNA transcription, replication, and gene therapy [1–4]. Various compounds including cationic lipids, polymers, dendrimers, peptides, chitosan, and metal complexes have been synthesized as DNA condensing agents and used as non-viral gene vectors in in vitro experiments and clinic trials for the last two decades [5–12]. Compared to viral gene vectors, these synthetic agents have shown the advantages including biocompatibility, non-immunogenicity, convenience for modification, and potential for large-scale production. Tremendous progress has been achieved in the design and synthesis of non-viral vectors, some common design principles and structure-property trends have emerged. For example, almost all synthetic gene vectors contain amine moieties which can be protonated to afford electrostatic binding with DNA. The structure features and charge density of the condensers have remarkable effects on the kinetic process of DNA condensation and the size of the nanoparticles formed [5, 13–17]. However, there are still several barriers to be overcome before the synthetic non-viral vectors can be put into practical application. These include the lack of reproducible and scalable formulation, low stability in biological fluids, proneness to aggregation, long term storage, DNA size-dependent delivery, and properly reproducible and efficient transfection. Thus it is desirable to explore new and novel DNA condensing agents as potential non-viral gene vectors.

The interactions between DNA and small molecules are categorized into three main modes: electrostatic binding, groove-binding, and intercalative-binding. For most organic condensing agents, the presence of positively charged moieties such as amine or guanidine groups is prerequisite. Recently, it was found that the intercalative binding could be the major contribution in the condensation process for the metal complexes condensing agents [18, 19], which was
also true for the early discovered dye-type condensers [20, 21]. Based on the above consideration, we are currently working on the design and synthesis of bifunctional molecules as effective DNA condensing agents [22, 23]. Herein, we report the synthesis of a series of bifunctional molecules bearing macrocyclic polyamine \([12\text{aneN}_3]\) and carbazole moieties (Figure 1) and their application in the condensation of DNA. Macrocyclic polyamines have been widely applied in the design and synthesis of artificial nucleases and non-viral gene vectors [12, 24–26], serving as electrostatic binding unit. Carbazole derivatives are often used as the intercalating molecules with DNA and for photo-physical studies [27, 28], with a large planar aromatic ring promoting the condensation process.

2 Experimental

2.1 Materials and methods

All solvents and reagents were of analytical grade and were used as received. Ultrapure milli-Q water (18.25 MΩ) was used in all DNA condensation assays. DNA was purchased from Solarbio company. The concentrations of CT-DNA used in all DNA condensation assays. DNA was purchased from Solarbio company. The concentrations of CT-DNA were determined by UV spectroscopy at 260 nm, taking 6600 M\(^{-1}\) cm\(^{-1}\) as the molar absorption coefficient. \(^1\)H and \(^13\)C NMR spectra were obtained on an Advance Bruker 400 MHz spectrometer at 25 °C. Chemical shifts (in ppm) were reported relative to internal tetramethylsilane (TMS) or residual solvent peaks. IR spectra were recorded on a Nicolet 380 spectrometer in the range of 4000–400 cm\(^{-1}\). The suspensions were stirred at 90 °C for 24 h, the reaction was cooled to room temperature, then compound 7 (0.80 g, 2.0 mmol), CuSO\(_4\) (64 mg, 0.40 mmol) and sodium ascorbate (160 mg, 0.80 mmol) were added. The resulting mixture was stirred at room temperature under argon for 12 h. Then the mixture was poured into water and filtered. The solid was washed with water and dried in vacuo, after which the solid was added into HCl solution (Prepared by slowly adding 0.5 mL of acetyl chloride into 8 mL of methanol and then stirring for 5 min at 0 °C) and stirred for 1 h. Then the solvents were removed in vacuo and the resulting solid was dried to give 1 as its hydrochloric salt (0.54 g, 70%). \(^1\)H NMR (400 MHz, D\(_2\)O) \(\delta\) 8.05 (s, 1H), 4.34 (q, \(J = 7.4\) Hz, 2H), 4.23 (s, 2H), 3.22 (dd, \(J = 12.8, 6.3\) Hz, 8H), 3.13 (s, 4H), 2.19–2.11 (m, 2H), 2.06 (s, 4H), 1.37 (t, \(J = 7.4\) Hz, 3H); \(^13\)C NMR (101 MHz, D\(_2\)O) \(\delta\) 137.92, 126.22, 48.41, 47.93, 46.01, 42.86, 41.65, 19.91, 18.68, 14.48; IR (KBr): \(\nu\) 751 (w), 855 (w), 906 (w), 1002 (w), 1055 (m), 1152 (w), 1218 (w), 1364 (w), 1458 (m), 1587 (m), 2757 (s), 2954 (s), 1259 (w), 1197 (m), 1079 (w), 906 (w), 751 (w) cm\(^{-1}\); HRMS (ES+) calcd. for C\(_{14}\)H\(_{29}\)N\(_6\) (M+H\(^+\))\(^{+}\): 281.2452, found 281.2452.

\(N\)-(2-azidoethyl)carbazol (6a)

To a solution of 5a (0.262 g, 0.956 mmol) in DMF (8 mL) was added sodium azide (0.620 g, 9.56 mmol). The suspension was stirred at 80 °C for 8 h. After being cooled down to room temperature, the mixture was poured into water (100 mL), stirred for 5 min and then filtered. The solid was dried in vacuo (0.210 g, 93%) and used without any further purification. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.10 (d, \(J = 7.8\) Hz, 2H), 7.47 (m, 4H), 7.26 (t, \(J = 6.4\) Hz, 2H), 4.48 (t, \(J = 6.2\) Hz, 3H), 3.73 (t, \(J = 6.2\) Hz, 2H); \(^13\)C NMR (101 MHz, CDCl\(_3\)) \(\delta\) 140.16, 125.93, 123.16, 120.54, 119.47, 108.39, 49.88, 42.36; MS (ES+): \(m/z\) = 259.5 [M+Na\(^+\)]

\(N\)-(3-azidopropyl)carbazol (6b)

To a solution of 5b (0.288 g, 1 mmol) in DMF (8 mL) was added sodium azide (0.650 g, 10 mmol). The suspension was stirred at 80 °C for 6 h. After being cooled to room temperature, a saturated aqueous NaCl (30 mL) was added and the solution was extracted with ethyl acetate (3 × 20 mL). The combined organic extracts were washed with

![Figure 1](image_url)