



Sodium and potassium benzeneazophosphonate complexes with crown ethers: Solid-state microwave synthesis, characterization and biological activity

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ABSTRACT

The eco-friendly synthesis, spectroscopic (IR, MS, ¹H and ¹³C NMR) study and biological (cytostatic, antiviral) activity of sodium and potassium benzeneazophosphonate complexes, obtained by reaction in the solid state under microwave irradiation of the alkali salts of ethyl [α -(4-benzeneazoanilino)-*N*-benzyl]phosphonic acid and [α -(4-benzeneazoanilino)-*N*-4-methoxybenzyl]phosphonic acid with crown ethers containing 18-membered (dibenzo-18-crown-6 and bis(4'-di-*tert*-butylbenzo)-18-crown-6), 24-membered (dibenzo-24-crown-8) and 30-membered (dibenzo-30-crown-10) macrocyclic rings, have been described. The simple work-up solvent free reaction is an efficient green procedure for the formation of mononuclear crown ether complexes in which the sodium/potassium ion is bound to oxygen atoms of the macrocycle and the phosphonic acid oxygen. The free crown ethers, alkali benzeneazophosphonate salts and their complexes were evaluated for their cytostatic activity *in vitro* against murine leukemia L1210, murine mammary carcinoma FM3A and human T-lymphocyte CEM and MT-4 cell lines, as well as for their antiviral activity against a wide variety of DNA and RNA viruses. The investigated compounds showed no specific antiviral activity, whereas all the free crown ethers and their complexes demonstrated cytostatic activity, which was especially pronounced in the case of bis(4'-di-*tert*-butylbenzo)-18-crown-6 and its complexes.

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1. Introduction

There is currently great interest in alkali metal chemistry, particularly of sodium and potassium ions because of their importance in natural systems. The study of the complexation of these biologically important cations was advanced enormously by the discovery of crown ethers, the first neutral synthetic heterocyclic compounds with powerful non-covalent cation binding properties [1,2]. Crown ethers, macrocyclic polyethers with a hydrophobic ethylenic ring surrounding a hydrophilic cavity of ether oxygen atoms, are able to bind selectively a range of inorganic and organic ions and neutral species [3–5], but their greatest affinity is for the alkali and alkaline earth cations [6]. The fact that the small and hydrated metal ions become large and lipophilic as crown ether complexes provides increased metal salt solubility and increased anion reactivity in aprotic organic solvents, which is widely used in studies of mediated ion transport, solute separations and anion-activated catalysis [7,8]. Transport of cations across a phase boundary is one of the most studied applications in the crown ether area. One approach to elucidate the highly selective complexation and transport of metal ions by biomolecules is through the study of

metal–crown complexes. Crown ethers, due to their ionophoric properties, resemble biologically important ionophores such as the macrocyclic antibiotics gramicidin, valinomycin and nonactin, which are known to make cell membranes selectively permeable to alkali cations [9,10]. The mechanism by which crown ethers facilitate ion transport through cell membranes is very complicated. Generally, their effectiveness for this purpose is determined by the degree of lipophilicity they possess and by the stability of the complexes they form. The rate of cation transport is greatly influenced also by the solvent media and the nature of the anion which accompanies the cation–crown ether complex.

Following our study on complexes of macrocyclic compounds with alkali salts of biologically interesting phosphonic acid derivatives, we recently reported the synthesis of a sodium ethyl 4-benzeneazophosphonate complex obtained by the reaction with a 15-membered mixed dioxo–diaz heteromacrocyclic compound under microwave conditions, as the first example of microwave-promoted synthesis of an alkali metal complex of a macrocyclic compound as well as of alkali metal macrocyclic complexation in the solid-state [11]. In the literature there are known coordination compounds, mostly of transition metal ions, which have been synthesized by microwave-assisted reactions in solution [12–14], but among them there are only a few examples of those derived from macrocyclic ligands [15,16]. Continuing our research in the field of microwave

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coordination chemistry, in the present report we describe the formation of sodium and potassium complexes by the solid-state microwave reaction of alkali salts of ethyl [α -(4-benzeneazoanilino)-*N*-benzyl]phosphonic acid (Na/KA₁) and ethyl [α -(4-benzeneazoanilino)-*N*-4-methoxybenzyl]phosphonic acid (Na/KA₂) with crown ethers containing 18-, 24- and 30-membered macrocyclic rings. As a preliminary screening for their biological activity, the complexes were evaluated for their cytostatic activity *in vitro* against murine leukemia L1210, murine mammary carcinoma FM3A and human T-lymphocyte CEM and MT-4 cell lines, and the results thus obtained were compared with those of the free crown ethers and discussed in terms of their binding behaviour and structure–activity relationships. The antiviral activity of the compounds was evaluated against a number of DNA and RNA viruses.

Similarly to natural cationic ionophores, crown ether compounds were also found to be toxic in prokaryotic and eukaryotic cellular systems. Toxicological research was done in various microorganisms and higher organisms such as mice, rats and dogs, and the results obtained in this field, along with biomedical potentials of crown ethers, have been summarized in our recently published review [17]. In most cases the proposed mechanism for the toxicity of crown ethers is connected with their ability to transport ions, most probably by inserting and integrating into membrane bilayers and conducting cations, as expected for carriers [18,19]. In addition, we reported the antiproliferative activity of several crown ethers and their derivatives *in vitro* against several tumor cell lines and compared their activity to that of valinomycin, an antibiotic known as a selective potassium ionophore [20]. This was the first systematic study of the cytostatic activity of non-functionalized crown compounds. Some crown ethers showed marked tumor cell growth inhibitory activity, presumably as the result of their activity as membrane-active potassium ion transporters, which may promote K⁺ efflux. No interaction with double-stranded DNA was detected. There are substantial differences between normal and tumor cells with respect to the membrane potentials of K⁺ transport regulation [21,22]. It is worth noting that some functionalized crown ethers, such as acridine, anthraquinone, bis(propargylic)sulfone and cisplatin derivatives, were designated to act either as DNA intercalators or DNA metal chelators [17,23–26].

2. Experimental

2.1. Materials and methods

Crown ethers dibenzo-18-crown-6 (DB18C6), bis(4'-*tert*-butylbenzo)-18-crown-6 (mtbDB18C6), dibenzo-24-crown-8 (DB24C8) and dibenzo-30-crown-10 (DB30C10), obtained commercially or prepared according to published methods [2], were purified by repeated recrystallizations from the appropriate solvents: acetone (DB18C6), methanol (mtbDB18C6, DB24C8), and *n*-hexane (DB30C10), respectively. Sodium and potassium salts of monoethyl [α -(4-benzeneazoanilino)-*N*-benzyl]phosphonic acid (NaA₁, KA₁) and [α -(4-benzeneazoanilino)-*N*-4-methoxybenzyl]phosphonic acid (NaA₂, KA₂) were prepared and purified following previously described procedures [27,28]. All compounds were dried on P₂O₅ in a vacuum at room temperature and stored in a dry-box before use. The solvents used (AR grade) were purified and dried by standard procedures. Microwave experiments were performed in a microwave oven in an open porcelain vessel at 700 W. Melting points were determined on a hot stage microscope and are uncorrected. FTIR spectra were recorded on an ABB Bomen MB102 spectrometer using KBr pellets. LDI mass spectra were performed on a Finnigan FT/MS 2001 DD instrument (Finnigan, Mad-

ison, WI) operating in the linear positive ion mode. The one- and two-dimensional ¹H and ¹³C NMR spectra (in CDCl₃) including ¹H, ¹³C, APT, ¹H–¹H COSY, ¹H–¹³C COSY, HMQC and HMBC experiments, were recorded with a Bruker XWIN-600 Fourier-transform spectrometer at ambient temperature. The ¹H and ¹³C chemical shifts (δ) were referred to SiMe₄. All two-dimensional experiments were performed by standard pulse sequences, using Bruker XWIN-NMR software Version 3.5. Elemental analyses (CHN) were performed on a Perkin–Elmer Analyser PE 2400 Series 2. The phosphorus content was determined by the vanadomolybdatophosphoric acid spectrophotometric method, and water by thermogravimetric analysis carried out on a Cahn RG electromicrobalance in an air atmosphere applying a heating rate of 4 °C min⁻¹.

2.2. Preparations of the complexes

The crown ether complexes were prepared by a conventional thermal method in solution and by microwave-promoted solid-state synthesis. Preparation of complexes of DB18C6 (**1–4**) and DB24C8 (**7–10**) by heating in solution was described earlier [29–31].

2.2.1. Conventional thermal method in solution

The reactions were in general carried out by dissolving an equimolar amount of the crown ether and sodium (NaA₁, NaA₂) and potassium (KA₁, KA₂) salt, respectively, of the corresponding monoethyl ester of benzeneazophosphonic acid in acetonitrile, then refluxing for 2–6 h with vigorous stirring. Only for complex **7** was dry ethanol used as the solvent. The clear hot solution was filtered and allowed to stand at room temperature. Most of the complexes were gradually formed by removal of solvent. The precipitate was filtered off, washed with a small amount of cold reaction solvent, and dried. Complexes of mtbDB18C6 (**5** and **6**) and DB30C10 (**11**) were obtained as a syrup after all the solvent was removed, which solidified slowly on standing, and were washed with cold CH₂Cl₂. All the complexes obtained were hygroscopic compounds, as were the free sodium and potassium phosphonate salts, and were dried under high vacuum at ambient temperature without decomposition, giving stable anhydrous complexes. Only complex **1** was obtained as a monohydrated complex, which was confirmed by thermogravimetric analysis. The purity of the complexes was checked by elemental, thermogravimetric and spectral analyses. Only elemental data for the newly synthesized complexes of mtbDB18C6 and DB30C10 are given.

Na(mtbDB18C6)A₁ (**5**): M.p. 104–106 °C. *Anal.* Calc. for C₄₉H₆₁N₃O₉PNa: C, 66.13; H, 6.91; N, 4.72; P, 3.49. Found: C, 66.313; H, 6.78; N, 4.51; P, 3.27%.

K(mtbDB18C6)A₁ (**6**): M.p. 96–98 °C. *Anal.* Calc. for C₄₉H₆₁N₃O₉PK: C, 64.95; H, 6.79; N, 4.64; P, 3.42. Found: C, 64.78; H, 6.99; N, 4.54; P, 3.21%.

K(DB30C10)A₁ (**11**): M.p. 93–95 °C. *Anal.* Calc. for C₄₉H₆₁N₃O₁₃PK: C, 60.67; H, 6.34; N, 4.33; P, 3.19. Found: C, 60.47; H, 6.59; N, 4.21; P, 3.09%.

2.2.2. Focused solid-state microwave irradiation

Equimolar amounts of the crown ether and the appropriate alkali salt (ca. 0.5 mmol) in a porcelain mortar were ground together until a fine homogenous powder was obtained. This mixture was then irradiated either for 6 min (3 × 2) in the case of complexes **1–6** or 4 min (2 × 2) for complexes **7–11**, using a 700 W microwave. After the reaction was completed, the liquefied product was allowed to cool down, washed with small amount of cold CH₂Cl₂ and dried *in vacuum* for 24 h giving an orange solid (Yield 92–95%).

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