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Simple and Dendritic Cyclam Derivatives. Photophysical Properties, Effect of Protonation and Zn^{2+} Coordination, Preliminary Screening as Inhibitors of Tumour Cell Growth

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Dedicated to Professor Dr Michael Veith on the occasion of his 60th birthday.

We have synthesized two novel dendrimers (BG1 and BG2) consisting of a 1,4,8,11-tetraazacyclotetradecane (cyclam, 1) core with appended four dimethoxybenzene and eight benzyl units (BG1) and twelve dimethoxybenzene and sixteen benzyl units (BG2). The absorption and luminescence spectra of these compounds and the changes taking place upon protonation and Zn^{2+} coordination of their cyclam core have been investigated in acetonitrile–dichloromethane 1:1 v/v solution. For comparison purposes, the absorption and luminescence spectra of 1,4,8,11-tetrabenzyl-cyclam (2), and dendrons BD1 and BD2, model compounds of the branches of BG1 and BG2 respectively, have also been studied. BD1, BD2, BG1, and BG2 exhibit the absorption and emission spectra of their 1,3-dimethoxybenzene unit, but in the two dendrimers the emission intensity is quenched by the cyclam amine groups and increases upon protonation and metal coordination. In order to test if these cyclam derivatives have an antitumour effect, we have studied their action on proliferation in the human neuroblastoma TS12 cell line. Screening experiments have shown that cell proliferation was (i) strongly reduced by the tetrabenzyl substituted cyclam 2, and (ii) unaffected by cyclam and the benzo dendrimers BG1 and BG2. Antitumour screening experiments have also been performed on the tetranaphthyl substituted cyclam 3 and the naphtho-dendrimer NG2, whose photophysical properties have been previously studied. Cell proliferation came out to be moderately reduced by 3, whereas dendrimer NG2 had no effect, similar to dendrimers BG1 and BG2.

Keywords: Cyclam; Dendrimers; Zn(II) coordination; Photophysics; Antitumour activity

INTRODUCTION

1,4,8,11-Tetraazacyclotetradecane (cyclam, **1**) is one of the most extensively investigated ligands in coordination chemistry [1,2]. Both the cyclam and its 1,4,8,11-tetramethyl derivative in aqueous solution can be protonated and can coordinate metal ions such as Co(II), Ni(II), Cu(II), Zn(II), Cd(II), and Hg(II) with very large stability constants [3–6]. Cyclam-based ligands are of interest in fields as diverse as catalysis [7–10], selective metal uptake and transport [11–13], and luminescent sensors [14–17]. Protonated cyclam has recently been used to assemble two different metal complexes for energy transfer purposes [18,19]. Cyclam and its derivatives have also important applications in medicine as agents for tumour cell growth inhibition [20], as inhibitors of the Human Immunodeficiency Virus (HIV) [21–23] and imaging applications [24,25]. A compound consisting of two cyclam units connected by

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a *p*-phenylenebis(methylene) linker is a one of the most potent anti-HIV agents known [26,27] and its Zn^{2+} complex has recently been found to be ten times more active than the free ligand [28]. In some cases, the cyclam derivatives contain pendant functionalities to increase complex stabilities or to allow attachment of other chemical species to the macrocyclic structure.

Cyclam [29,30] can be used as a core to construct dendrimers, tree-like macromolecules that exhibit a well defined chemical composition and can contain selected chemical functions at predetermined sites of their structure [31,32]. Dendrimer chemistry is a rapidly expanding field for both basic and applicative reasons [33–39], including medical applications [40–43].

Continuing our studies on dendrimers, we have recently investigated the photophysical properties of some cyclam-based dendrimers and the effects of protonation [29] and metal coordination on these systems [44–46]. In view of the above mentioned biological activity of cyclam derivatives, we thought that it was worthwhile to perform screening experiments on the antitumour properties of some cyclam dendrimers and related compounds.

This paper reports the synthesis, photophysical characterization, protonation and Zn^{2+} coordination of two novel cyclam dendrimers, **BG1** and **BG2**, consisting of a cyclam core appended with four dimethoxybenzene and eight benzyl units, **BG1**, and twelve dimethoxybenzene and sixteen benzyl units, **BG2** (Scheme 1). For comparison purposes, the properties of 1,4,8,11-tetrabenzyl-cyclam **2**, and dendrons **BD1** and **BD2** (Scheme 1), model compounds of the branches of **BG1** and **BG2** respectively, have also been studied. We have also performed experiments on the effect of the simple and dendritic cyclam derivatives on the inhibition of cell growth in the human neuroblastoma TS12 line.

RESULTS AND DISCUSSION

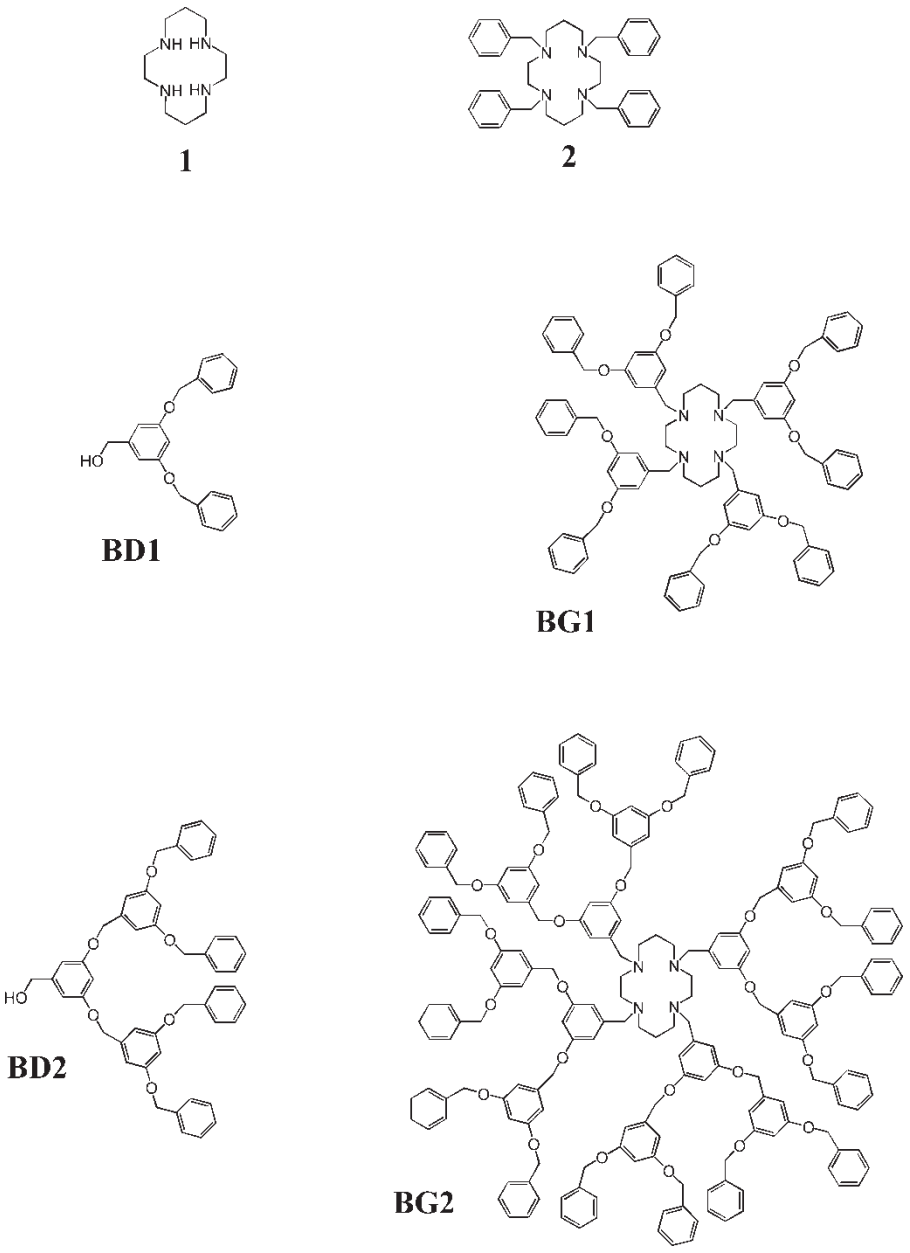
Photophysical Properties

All the compounds are soluble in acetonitrile–dichloromethane 1:1 v/v, which has therefore been chosen as the solvent for our experiments. A summary of the results obtained is given in Table I.

Cyclam (**1**) shows only a weak absorption tail in the 240–270 nm region. The absorption spectrum of **2** is quite similar to that of cyclam, except for the presence of a superimposed weak structure, typical of benzene units [47]. The absorption and emission spectra of **BD1**, **BD2**, **BG1** and **BG2** are displayed in Fig. 1. The absorption spectra are dominated by the bands of their 1,3-dimethoxybenzene chromophoric groups. The molar absorption coefficients of these bands increase linearly with increasing number of 1,3-dimethoxybenzene units contained in the three compounds (Table I). In compound **2**, the expected benzene-like emission [47] is almost completely quenched, presumably by an electron-transfer process involving the amine units of the cyclam ring. The emission band exhibited by **BD1**, **BD2**, **BG1**, and **BG2** can be safely assigned to 1,3-dimethoxybenzene, which shows an emission band in the same spectral region, but with a higher quantum yield (for example, five times higher than **BD1**). The lower emission intensity observed in the case of **BD1** and **BD2** compared to 1,3-dimethoxybenzene can be attributed to intrachromophoric interactions in the dendrons, in particular to the presence of excimers (between two dimethoxybenzene units) or exciplexes (between a dimethoxybenzene and a benzene unit), that occur to a different extent in the two dendrons. The presence of two emitting species in the dendrons, namely the 1,3-dimethoxybenzene chromophore and excimer or exciplex, is supported by the presence of two lifetimes (<0.5 and 3.5 ns) and by a red tail in the emission band of both **BD1** and **BD2** compared to pristine 1,3-dimethoxybenzene. This intrachromophoric interaction was not observed in the case of dimethoxybenzene dendrons terminated by methyl instead of benzyl units [48]: in particular, no difference in the emission quantum yield of increasing generation dendrons was shown. Therefore, in our case the different emission intensities of **BD1** and **BD2** is likely due to exciplex interactions occurring to a larger extent in **BD2** compared to **BD1**. The emission of the peripheral benzyl units cannot be seen, as expected because of the presence of the lower lying dimethoxybenzene excited state. The emission intensity of the dendrimers **BG1** and **BG2** (solid lines in Fig. 1) is much lower than that of **BD1** and **BD2**, respectively. This result can be attributed to a quenching of the fluorescent excited state of dimethoxybenzene units by an electron transfer process in the two cyclam dendrimers. One can observe (Fig. 1) that the extent of quenching of dimethoxybenzene fluorescence is less pronounced in **BG2** with respect to **BG1**: this can probably be ascribed to the larger distance of some of the dimethoxybenzene units from the cyclam core in **BG2** compared to **BG1**.

Protonation Behaviour

The addition of trifluoroacetic acid up to 5 equivalents to a solution of **BD1** or **BD2** does not cause any appreciable change in the absorption and emission spectra, as expected because of the absence of basic sites. Addition of trifluoroacetic acid, however, causes noticeable changes in the absorption and emission spectra of **2**, **BG1** and **BG2**, which contain the cyclam



SCHEME 1

TABLE I Absorption and luminescence properties in acetonitrile/dichloromethane 1:1 v/v solution at 298 K

	Absorption		Emission	
	λ_{max} (nm)	ε ($M^{-1}\,cm^{-1}$)	λ_{max} (nm)	I_{rel}
2	258	1670	–	–
2·$2H^+$	258	1200	286	55
2·Zn^{2+}	258	900	286	45
BD1	282	2300	312	200
BD2	282	7100	312	100
BG1	282	9800	312	15
BG1·$2H^+$	283	11700	314	75
BG1·Zn^{2+}	283	11800	315	145
BG2	282	31900	312	50
BG2·$2H^+$	283	34400	314	75
BG2·Zn^{2+}	282	34700	315	100

unit. In the case of **2**, a substantial decrease in the intensity of the absorption tail in the 230–280 nm region is accompanied by the appearance of the typical benzene emission at 286 nm. In the case of **BG1** and **BG2** (Fig. 1), acid titration caused an increase in intensity and a red shift of the absorption band at 280 nm, with isosbestic points at 238 and 274 nm, and a substantial increase in the emission intensities (excitation at 274 nm) with a moderate red-shift of the emission maximum (Table I). The changes of absorption and emission intensities for **BG1** and **BG2** upon acid addition are linear and reach a plateau at 2 equivalents, suggesting that, under the experimental conditions used, two protons are sufficient to engage the four nitrogen lone pairs of the cyclam unit,

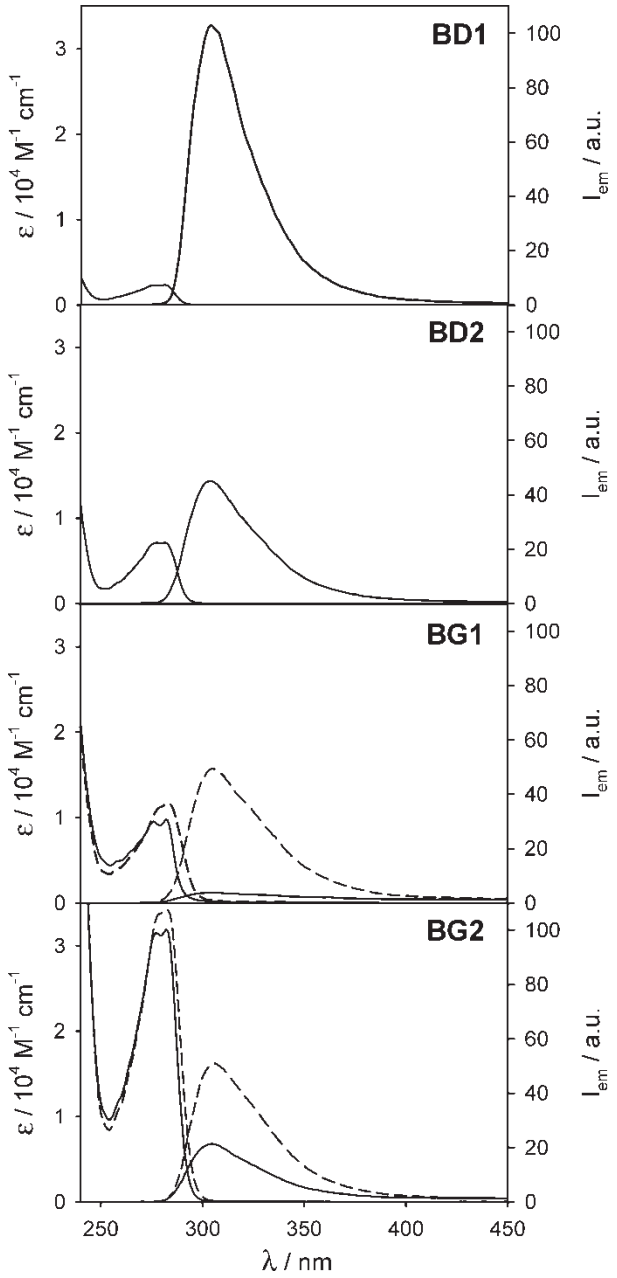
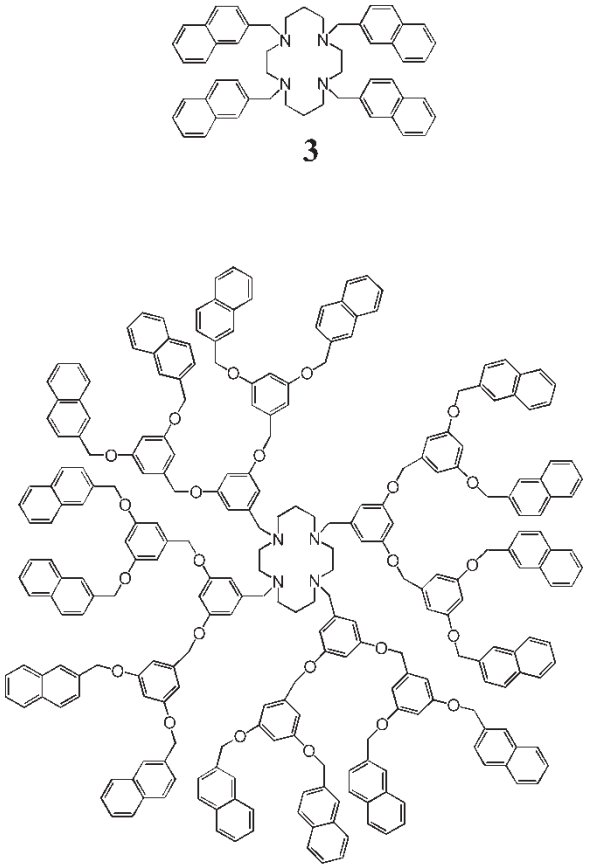


FIGURE 1 Absorption and emission spectra (solid lines) in acetonitrile–dichloromethane 1:1 v/v solution at 298 K and effect caused by addition of 3 equivalents of CF_3COOH (dashed lines) to **BG1** and **BG2**.

thereby preventing electron transfer quenching of the dimethoxybenzene excited state. At the end of the acid titration, however, the relative emission quantum yields of the **BG1** and **BG2** dendrimers are lower than that of the **BD1** and **BD2** dendrons, respectively (Table I). This result can be accounted for by either (i) the presence of a residual electron transfer quenching of the excited dimethoxybenzene units by the diprotonated cyclam core, or (ii) an electrostatic effect of the protonated core on the radiative and/or radiationless deactivation of the dimethoxybenzene excited state. The first hypothesis seems unlikely on the basis of the results previously obtained for



SCHEME 2

the naphtho-dendrimer **NG2** (Scheme 2) [29]: the diprotonated species of **NG2** has a quantum yield identical to that of the corresponding dendron. The second hypothesis is consistent with the slight red-shift of both the absorption and emission spectra of the protonated species compared with the non protonated ones, which indicates that the fluorescent excited state is indeed perturbed by the presence of the positive charges in the core.

Complexation of Zn^{2+}

Cyclam is a well known ligand for complexation of Zn^{2+} ions [2,3]. Titration of compounds **2** (Fig. 2), **BG1** and **BG2** (Fig. 3) with $Zn(NO_3)_2{\cdot}6H_2O$ caused strong spectral changes. The changes observed in the absorption spectra are quite similar to those caused by protonation. For compound **2**, coordination of the metal ion to the cyclam nitrogens caused a substantial decrease of the absorption tail in the 230–280 nm region (Fig. 2). Therefore, the structured absorption bands of the benzene becomes more evident and the typical benzene-like emission band ($\lambda_{max} = 286$ nm) appears. Titration plots (Fig. 2, inset) show that a 1:1 complex is formed with formation constant $\log K = 6.0 \pm 0.3$. In the case of **BG1** and

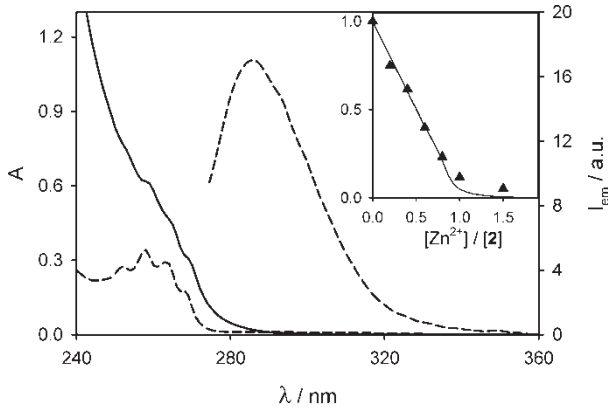


FIGURE 2 Absorption and emission spectra of **2** in acetonitrile–dichloromethane 1:1 v/v solution at 298 K upon addition of 0 (solid line) and 3 equivalents (dashed line) of $Zn(NO_3)_2{\cdot}6H_2O$. The inset shows the normalized titration plot obtained monitoring changes in absorption at 258 nm upon addition of $Zn(NO_3)_2{\cdot}6H_2O$.

BG2, titration with $Zn(NO_3)_2{\cdot}6H_2O$ caused a strong increase in intensity of the dimethoxybenzene emission band (Fig. 3), since the lone pairs of the amine units of the cyclam core are engaged in metal coordination. The emission intensity at the end of the titration is higher than that obtained upon protonation, demonstrating that structural rearrangements caused by metal coordination play a role in determining the emission intensity. The titration plots (Fig. 3, inset) showed that **BG1** and **BG2** form complexes with 1:1 metal/ligand stoichiometry. The corresponding formation constants are high: $\log K = 7.1 \pm 0.7$ and 6.7 ± 0.3 for **BG1** and **BG2**, respectively.

Biological Effects

In order to see whether the compounds discussed above exhibit antitumour activity, their effects on proliferation were evaluated on neuroblastoma TS12 cell line with respect to untreated exponentially growing controls.

Figure 4(a) shows that cyclam (**1**) itself has no significant effect on cell proliferation with respect to controls after six days of incubation. Figure 4(b) shows that tetrabenzyl-cyclam **2**, exhibits drastically different biological properties. Such a compound reduces proliferation in a dose-dependent manner, reaching an inhibition of 90% at the 15 μM dose after six days of incubation. The positive antitumour activity can probably be ascribed to an increased lipophilicity of **2** with respect to **1**. As recently reported [20], lipophilicity should indeed facilitate the transport of cyclam derivatives across the membrane, into the cell. Perhaps surprisingly, dendrimers **BG1** and **BG2**,

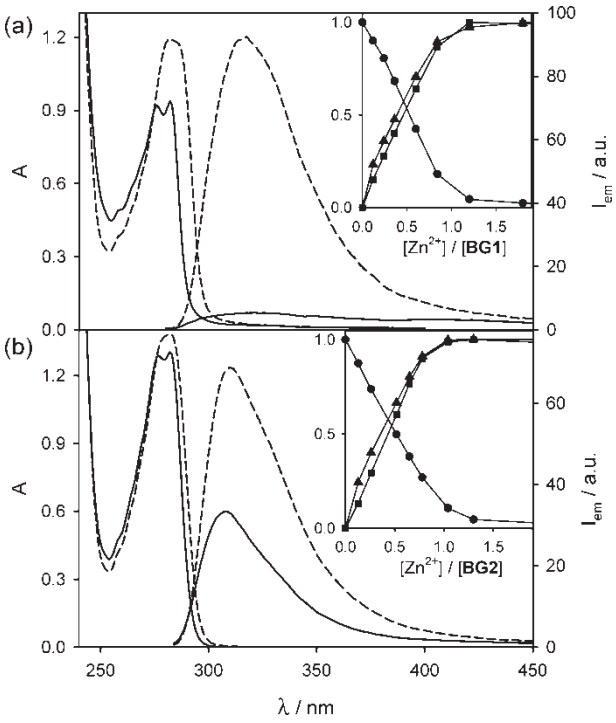


FIGURE 3 Absorption and emission spectra (solid lines) of (a) **BG1** and (b) **BG2** in acetonitrile–dichloromethane 1:1 v/v solution at 298 K and effect caused by addition of 3 equivalents of $Zn(NO_3)_2{\cdot}6H_2O$ (dashed lines). The insets show the normalized titration plots obtained monitoring changes in absorption at 254 (●), 282 nm (■) and emission at 308 nm (▲) upon addition of $Zn(NO_3)_2{\cdot}6H_2O$.

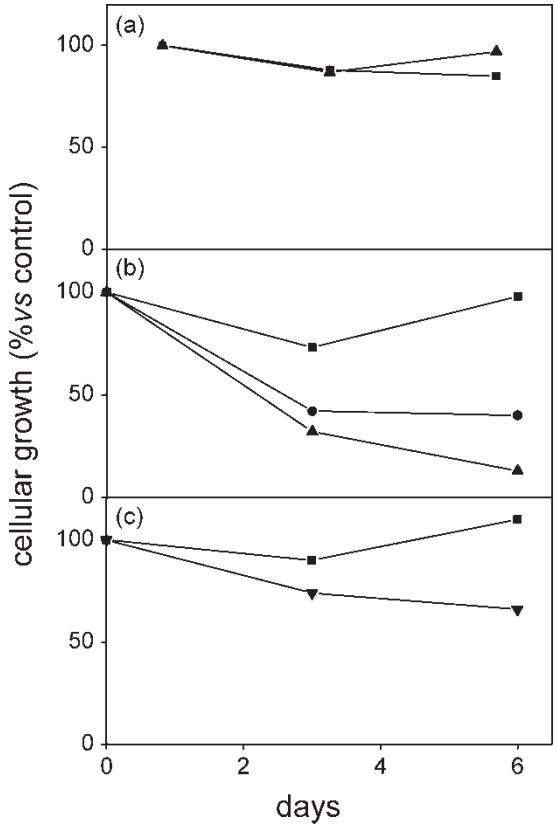


FIGURE 4 Effect on human neuroblastoma TS12 cell growth of compounds **1** (a), **2** (b) and **3** (c), at different concentrations: 1 (■), 5 (●), 15 (▲), 20 μM (▼).

which contain a larger number of benzyl units at the periphery, do not exhibit any antiproliferative capacity (data not shown).

Effects on proliferation on neuroblastoma TS12 cell line were also evaluated for the tetranaphthyl substituted cyclam **3** and the naphtho-dendrimer **NG2** (Scheme 2), whose photophysical properties have been previously reported [29,44–46]. Compound **3**, in which four naphthyl units are appended to the cyclam core, reduces cell growth, but to a lesser degree with respect to compound **2** (Fig. 4(c)). Compound **NG2**, which carries four dimethoxybenzene dendrons terminated by naphthyl groups, does not exhibit any cell growth inhibition (data not shown).

Conclusions

We have described a new family of cyclam cored dendrimers and their reaction with H^+ and Zn^{2+}. In both cases there is a strong increase in the fluorescent emission due to the engagement of the nitrogen lone pairs of the cyclam amines.

Since cyclam is used as an antitumour agent, we have studied the inhibition effect of the investigated cyclam derivatives on human neuroblastoma TS12 cell proliferation. We have found that the inhibition effect is: (i) practically absent in the case of cyclam and dendrimers **BG1**, **BG2**, **NG1** and **NG2**, (ii) moderate for tetranaphthyl substituted cyclam **3**, (iii) strong for tetrabenzyl substituted cyclam **2**. The antiproliferative activity is, of course, the result of several factors, including the membrane crossing ability, which can be low for long branched [40] or highly rigid [49] macromolecules, and specific effects on signal transduction mechanisms, connected with the coordination ability of cyclam. The difficulty to rationalize the results obtained on the antiproliferative activity is therefore not surprising. A small hint on the complexity of the system is given by the experiments on Zn^{2+} coordination [44–46], which show that different appended branches can affect not only the complexation constant, but even the stoichiometry of the complex formed.

EXPERIMENTAL SECTION

General Remarks

3,5-Dihydroxybenzyl alcohol, benzyl bromide, $Zn(NO_3)_2{\cdot}6H_2O$, CF_3COOH and 1,4,8,11-tetraazacyclotetradecane were purchased from Aldrich. 3,5-Bis(benzyloxy)benzyl bromide and 3,5-bis[3′,5′-bis(benzyloxy)benzyloxy]benzyl bromide were synthesized according to literature procedures [50]. NMR spectroscopic data were obtained at 400 MHz with a Bruker AM 400 spectrometer (for ^1H and ^{13}C NMR spectra, the $CDCl_3$ signals were used as an internal reference; shifts are quoted with respect to tetramethylsilane, TMS). MALDI-TOF mass spectra were obtained with a TofSpec E&SE instrument from Micromass, Manchester; FAB mass spectra were obtained with Concept 1H of Kratos Analytical Ltd., Manchester, with *m*NBA as Matrix. EI–MS were obtained with MS-50 from A.E.I., Manchester.

Synthesis

All synthetic experiments were routinely carried out under dry argon. To the mixture of 1 equivalent of the starting 1,4,8,11-tetraazacyclotetradecane (cyclam) and a 40–50-fold excess of potassium carbonate in dry chloroform (100 ml), a solution of 4.4–5 equivalents of the corresponding benzyl bromide in 30 ml dry chloroform was added dropwise. The mixture was stirred for 2–3 days under reflux and after filtering off the undissolved K_2CO_3, the solvent was removed *in vacuo*. After collecting the residue in dichloromethane and washing with water, aq. Na_2CO_3 and again with water, the organic phase was dried with Na_2SO_4. Further purification was achieved by column chromatography yielding a bright yellow solid.

1,4,8,11-Tetrabenzyl-1,4,8,11-tetraazacyclotetradecane (2)

Reaction procedure as described above. Amounts: 1,4,8,11-tetraazacyclotetradecane (0.50 g, 2.49 mmol), benzyl bromide (1.79 g, 10.45 mmol) and potassium carbonate (13.82 g, 0.1 mol). The compound was recrystallized from hot tetrahydrofuran (THF) yielding 0.44 g (31%) of white solid; mp 151–153°C; TLC (SiO_2): $R_f = 0.43$ (dichloromethane/methanol 10:1 v/v); ^1H NMR: (400 MHz, $CDCl_3$, 25°C), δ [ppm] = 1.75 (t, 4 H, CH_2), 2.42 (t, 8 H, CH_2N), 2.53 (s, 8 H, CH_2N), 3.47 (s, 8 H, Ar–CH_2), 7.08–7.23 (m, 20 H, CH_{ar}); ^{13}C-NMR: (100.6 MHz, $CDCl_3$, 25°C), δ [ppm] = 23.9 (CH_2), 50.5 (CH_2N), 51.5 (CH_2N), 59.5 (Ar–CH_2), 126.6, 128.0, 128.9, 140.1; EI–MS: m/z (%): 560.4 (25, $M + H^+$), 469.5 (45, $M - C_7H_7^+$); $C_{38}H_{50}N_4$: (562.8).

1,4,8,11-Tetrakis[3,5-bis(benzyloxy)benzyl]-1,4,8,11-tetraazacyclotetradecane (BG1)

Reaction procedure as described above. Amounts: 1,4,8,11-tetraazacyclotetradecane (0.1 g, 0.49 mmol), 3,5-bis(benzyloxy)benzyl bromide (0.82 g, 2.15 mmol) and potassium carbonate (2.63 g, 19.06 mmol). Column chromatography (SiO_2, 40–63 μm, 1: dichloromethane/methanol 30:1; 2: dichloromethane/methanol 20:1 v/v) yielding 0.34 g (49%) of a bright yellow solid; mp 45–46°C; TLC (SiO_2): $R_f = 0.37$ (dichloromethane/methanol 20:1 v/v); ^1H NMR: (400 MHz, $CDCl_3$, 25°C), δ [ppm] = 1.75

(t, 4 H, CH_2), 2.43 (t, 8 H, CH_2N), 2.53 (s, 8 H, CH_2N), 3.29 (s, 8 H, Ar–CH_2), 4.75 (s, 16 H, Ph–CH_2O–Ar), 6.32 (d, $^4J_{HH} = 2.2$ Hz, 4 H, CH_{ar}), 6.61 (d, $^4J_{HH} = 2.2$ Hz, 8 H, CH_{ar}), 7.21–7.35 (m, 40 H, CH_{Ph}): ^{13}C NMR: (100.6 MHz, $CDCl_3$, 25°C), δ [ppm] = 24.7 (CH_2), 50.2 (CH_2N), 51.6 (CH_2N), 59.1 (Ar–CH_2), 69.8 (Ph–CH_2O–Ar), 100.7 (CH_{ar}), 107.6 (CH_{ar}), 127.6 (CH_{Ph}), 127.9 (CH_{Ph}), 128.5 (CH_{Ph}), 137.0 (C_{Ph}), 143.2 (C_{ar}), 159.8 (C_{ar}); FAB − MS: m/z (%): 1410.7 (70, $M + H^{\oplus}$), 1320.6 (15, $M - C_7H_7^{\oplus}$); $C_{94}H_{96}N_4O_8$: (1409.8).

1,4,8,11-Tetrakis[3,5-bis[3′,5′-bis(benzyloxy)benzyloxy]benzyl]-1,4,8,11 Tetraazacyclotetradecane (BG2)

Reaction procedure as described above. Amounts: 1,4,8,11-tetraazacyclotetradecane (0.09 g, 0.45 mmol), 3,5-bis[3′,5′-bis(benzyloxy)benzyloxy]benzyl bromide (1.81 g, 2.24 mmol) and potassium carbonate (3.28 g, 23.73 mmol). Column chromatography (SiO_2, 40–63 μm, dichloromethane/methanol 40:1 v/v) yielding 0.73 g (52%) of bright yellow solid; mp 49°C; TLC (SiO_2): $R_f = 0.32$ (dichloromethane/methanol 40:1 v/v); ^1H NMR: (400 MHz, $CDCl_3$, 25°C), δ [ppm] = 1.75 (br, t, 4 H, CH_2), 2.43 (br, t, 8 H, CH_2N), 2.51 (br, s, 8 H, CH_2N), 3.29 (br, s, 8 H, Ar–CH_2), 4.63 (br, s, 16 H, 2 Ar–CH_2O–Ar), 4.85 (br, s, 32 H, 4 Ph–CH_2O–Ar), 6.25 (br, t, 4 H, CH_{ar}), 6.41 (br, t, 8 H, CH_{ar}), 6.51 (br, d, 16 H, CH_{ar}), 6.58 (br, d, 8 H, CH_{ar}), 7.13–7.28 (br, m, 80 H, CH_{Ph}); ^{13}C NMR: (100.6 MHz, $CDCl_3$, 25°C), δ [ppm] = 50.4 (CH_2N), 51.6 (CH_2N), 59.4 (Ar–CH_2), 69.9 (Ar–CH_2O–Ar), 70.0 (Ph–CH_2O–Ar), 101.2, 101.5, 106.4, 108.2 (CH_{ar}), 127.6, 127.9, 128.5 (CH_{Ph}), 136.8, 139.5, 139.7, 159.8, 160.1 (C_{ar}); MALDI–TOF–MS: m/z (%): 3107.8 (60, $M^{\oplus}$); $C_{206}H_{192}N_4O_{24}$: (3107.7).

Photophysical Experiments

The luminescence spectra were carried out in air-equilibrated acetonitrile/dichloromethane 1:1 v/v solution at 298 K with a Perkin Elmer LS50 spectrofluorimeter. Stability constants of the Zn^{2+} complexes were obtained by implementing the spectra into the SPECFIT software [51,52]. Fluorescence lifetimes were measured by time-correlated single-photon counting (0.5 ns time resolution) with an Edinburgh Instruments FLS920 equipment (D_2 lamp, $\lambda_{ex} = 275$ nm). The estimated experimental errors are: ±2 nm on the band maximum, ±5% on the molar absorption coefficient, ±5% on the fluorescence lifetime and ±5% on the log K values.

Biological Experiments

Cell Line

The TS12 cell line was derived from a human neuroblastoma biopsy obtained from a patient of the Paediatric Oncology Department at the Paediatric Clinic of the University of Bologna, Italy. The cells were maintained in RPMI 1640 culture medium (GIBCO, Grand Island, NY, USA), supplemented with 10% fetal bovine serum (GIBCO), at 37°C in a 5% CO_2 humidified atmosphere, as previously described [53].

Cell Treatments

A 2 mM solution of each of the compounds in dimethyl sulfoxide (DMSO) containing 8 mM HCl were added to the culture medium. Control experiments showed that, in the absence of the examined compounds, the DMSO–HCl mixture did not affect cell proliferation under the experimental condition used. The final concentrations of the examined compounds were in the range 1–50 μM.

Growth Inhibition

The effect of the compounds on cell proliferation was evaluated by the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) Assay [54], based on the reduction of tetrazolium salt MTT, taken as an index of the number of the metabolically active cells and the results were expressed as a percentage of the controls. Results from 6 replicate cultures from 3 independent experiments were averaged with a corresponding experimental error lower than ± 5% in all cases.

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References

[1] Lukeš, I.; Kotek, J.; Vojtíšek, P.; Hermann, P. *Coord. Chem. Rev.* **2001**, *216–217*, 287.
[2] Hay, B. P.; Hancock, R. D. *Coord. Chem. Rev.* **2001**, *212*, 61.
[3] Bianchi, A.; Micheloni, M.; Paoletti, P. *Coord. Chem. Rev.* **1991**, *110*, 17.
[4] Kimura, E. *Prog. Inorg. Chem.* **1994**, *41*, 443.
[5] Meyer, M.; Dahaoui-Gindrey, V.; Lecomte, C.; Guilard, R. *Coord. Chem. Rev.* **1998**, *178*, 1313.
[6] Fabbrizzi, L.; Licchelli, M.; Pallavicini, P.; Sacchi, D. *Supramol. Chem.* **2001**, *13*, 569.
[7] Iranzo, O.; Kovalevsky, A. Y.; Morrow, J. R.; Richard, J. P. *J. Am. Chem. Soc.* **2003**, *125*, 1988.
[8] Lee, E. Y.; Hong, D.; Park, H. W.; Suh, M. P.; Eur *J. Inorg. Chem.* **2003**, *17*, 3242.
[9] Bonfa, L.; Gatos, M.; Mancin, F.; Tecilla, P.; Tonellato, U. *Inorg. Chem.* **2003**, *42*, 3943.

[10] Semones, M. A.; Peters, D. G. *J. Electrochem. Soc.* **2000**, *147*, 260.
[11] Alcock, N. W.; Clarke, A. J.; Errington, W.; Josceanu, A. M.; Moore, P.; Rawle, S. C.; Sheldon, P.; Smith, S. M.; Turonek, M. L. *Supramol. Chem.* **1996**, *6*, 281.
[12] Kimura, E. *Tetrahedron* **1992**, *48*, 6175.
[13] Kimura, E.; Kurogi, Y.; Takahashi, T. *Inorg. Chem.* **1991**, *30*, 4117.
[14] Sancenón, F.; Benito, A.; Hernández, F. J.; Lloris, J. M.; Martínez-Máñez, R.; Pardo, T.; Soto, J. *Eur. J. Inorg. Chem.* **2002**, *4*, 866.
[15] Padilla-Tosta, M. E.; Lloris, J. M.; Martínez-Máñez, R.; Pardo, T.; Sancenón, F.; Soto, J.; Marcos, M. D. *Eur. J. Inorg. Chem.* **2001**, *5*, 1221.
[16] Al Shihadeh, Y.; Benito, A.; Lloris, J. M.; Martínez-Máñez, R.; Pardo, T.; Soto, J.; Marcos, M. D. *Dalton Trans.* **2000**, 1199.
[17] Fabbrizzi, L.; Licchelli, M.; Pallavicini, P.; Perotti, A.; Taglietti, A.; Sacchi, D. *Chem. Eur. J.* **1996**, *2*, 75.
[18] Loiseau, F.; Marzanni, G.; Quici, S.; Indelli, M. T.; Campagna, S. *Chem. Commun.* **2003**, 286.
[19] Simpson, N. R. M.; Ward, M. D.; Farran Morales, A.; Ventura, B.; Barigelletti, F. *Dalton Trans.* **2002**, 2455.
[20] Sibert, J. W.; Cory, A. H.; Cory, J. C. *Chem. Commun.* **2002**, 154.
[21] Kimura, E.; Koike, T.; Inouye, Y. In *Perspective on Bioinorganic Chemistry*; Hay, R. W., Dilworth, J. R., Nolan, K. B., Eds.; JAI Press Inc. Stamford, CT, 1999; Vol. 14, p 145.
[22] Liang, X.; Weishäupl, M.; Parkinson, J. A.; Parsons, S.; McGregor, P. A.; Sadler, P. J. *Chem. Eur. J.* **2003**, *9*, 4709.
[23] Paisey, S. J.; Sadler, P. J. *Chem. Commun.* **2004**, 306.
[24] Brucher, E.; Sherry, A. D. In Chapter 6, *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*; Merbach, A. E., Toth, E., Eds.; John Wiley: New York, 2001.
[25] Caravan, P.; Ellison, J. J.; McMurry, T. J.; Lauffer, W. H. *Chem. Rev.* **1999**, *99*, 2293.
[26] Liang, X.; Parkinson, J.; Weishäupl, M.; Gould, R. O.; Paisey, S. J.; Park, H. S.; Hunter, T. M.; Blindauer, C. A.; Parson, S. A.; Sadler, P. J. *J. Am. Chem. Soc.* **2002**, *124*, 9105.
[27] De Clerck, E.; Yamamoto, N.; Pauwels, R.; Baba, M.; Schols, D.; Nakashima, H.; Balzarini, J.; Debyser, Z.; Murrer, B. A.; Schwartz, D.; Thornton, D.; Bridger, G.; Fricker, S.; Henson, G.; Abrams, M.; Picker, D. *Proc. Natl Acad. Sci.* **1992**, *89*, 5286.
[28] Esté, J. A.; Cabrera, C.; De Clerck, E.; Struyf, S.; Van Damme, J.; Bridger, G.; Skerlj, R. T.; Abrams, M. J.; Henson, G.; Gutierrez, A.; Clotet, B.; Schols, D. *Mol. Pharmacol.* **1999**, *55*, 67.
[29] Saudan, C.; Balzani, V.; Ceroni, P.; Gorka, M.; Maestri, M.; Vicinelli, V.; Vögtle, F. *Tetrahedron* **2003**, *59*, 3845.
[30] Enoki, O.; Imaoka, T.; Yamamoto, K. *Org. Lett.* **2003**, *5*, 2547.
[31] Newkome, G. R.; Moorefield, C.; Vögtle, F. *Dendrimers and Dendrons: Concepts, Syntheses, Perspectives*; VCH: Weinheim, 2001.
[32] *Dendrons and Other Dendritic Polymers*; Fréchet, J. M. J., Tomalia, D. A., Eds.; Wiley: New York, 2001.
[33] Caminade, A.-M.; Majoral, J.-P. *Acc. Chem. Res.* **2004**, *37*, 341.
[34] Special Issue on Dendrimers and Nanoscience; Astruc, D., Ed.; *Comp. Rend. Chimie* **2003**; *6* (8–10).
[35] Balzani, V.; Ceroni, P.; Maestri, M.; Saudan, C.; Vicinelli, V. *Top. Curr. Chem.* **2003**, *228*, 159.
[36] Oosterom, G. E.; Reek, J. N. H.; Kamer, P. C. J.; van Leeuwen, P. W. N. M. *Angew. Chem. Int. Ed.* **2001**, *40*, 1828.
[37] Tully, D. C.; Fréchet, J. M. J. *Chem. Commun.* **2001**, 1229.
[38] Hecht, S.; Fréchet, J. M. J. *Angew. Chem. Int. Ed.* **2001**, *40*, 74.
[39] Schlüter, A. D.; Rabe, J. P. *Angew. Chem. Int. Ed.* **2000**, *39*, 864.
[40] Boas, U.; Heegaard, P. M. H. *Chem. Soc. Rev.* **2004**, *33*, 43.
[41] Fuchs, S.; Kapp, T.; Otto, H.; Schöneberg, T.; Franke, P.; Gust, R.; Schlüter, A. D. *Chem. Eur. J.* **2004**, *10*, 1167.
[42] Stiriba, S.-E.; Frey, H.; Haag, R. *Angew. Chem. Int. Ed.* **2002**, *41*, 1329.
[43] Patri, A. K.; Majoros, I. J.; Baker, Jr., J. R. *Curr. Opinion Chem. Biol.* **2002**, *6*, 466.
[44] Saudan, C.; Balzani, V.; Gorka, M.; Lee, S.-K.; Maestri, M.; Vicinelli, V.; Vögtle, F. *J. Am. Chem. Soc.* **2003**, *125*, 4424.
[45] Saudan, C.; Balzani, V.; Gorka, M.; Lee, S.-K.; van Heyst, J.; Maestri, M.; Ceroni, P.; Vicinelli, V.; Vögtle, F. *Chem. Eur. J.* **2004**, *10*, 899.
[46] Saudan, C.; Ceroni, P.; Vicinelli, V.; Maestri, M.; Balzani, V.; Gorka, M.; Lee, S.-K.; van Heyst, J.; Vögtle, F. *Dalton Trans.* **2004**, 1597.
[47] Berlman, I. B. *Handbook of Fluorescence Spectra of Aromatic Molecules*; Academic Press: London, 1965.
[48] Kimata, S.-I.; Jiang, D.-L.; Aida, T. *J. Polym. Sci., Part A: Polym. Chem.* **2003**, *41*, 3524.
[49] Dennig, J. *Top. Curr. Chem.* **2003**, *228*, 227.
[50] Hawker, G. J.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **1990**, *112*, 7638.
[51] Binstead, R. A. *SPECFIT*; Spectrum Software Associates: Chapell Hill, NC, 1996.
[52] Gampp, H.; Maeder, M.; Meyer, C. J.; Zuberbulher, A. *Talanta* **1985**, *32*, 257.
[53] Bartolini, G.; Orlandi, M.; Ammar, K.; Magrini, E.; Ferreri, A. M.; Rocchi, P. *Anticancer Res.* **2003**, *23*, 1495.
[54] Denizot, F.; Lang, R. *J. Immunol. Methods* **1986**, *89*, 271.