Novel Approaches to Polynuclear Platinum Pro-Drugs. Selective Release of Cytotoxic Platinum−Spermidine Species through Hydrolytic Cleavage of Carbamates

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BBR3464 is a novel trinuclear platinum drug currently in Phase II clinical trials. Polyamine-bridged dinuclear platinum compounds as represented by [{*trans*-Pt(NH$_3$)$_2$Cl}$_2$-μ-spermidine-N^1,N^8]Cl$_3$ (**1**) are highly interesting second-generation analogues of BBR3464 because the hydrogen-bonding and electrostatic contributions of the central platinum−amine group in BBR3464 are replicated by the free, noncoordinated "central" quaternary nitrogens of the linear polyamine linker while the presence of two separate Pt−Cl bonds maintains the bifunctional binding mode on the DNA adducts. Preclinical investigations confirm the potency of these species with cytotoxicity in the nanomolar range. This remarkable potency results in a relatively narrow therapeutic index. To enhance the therapeutic index of these drugs, we investigated the potential for "pro-drug" delivery of less toxic and better tolerated derivatives such as the compounds [{*trans*-Pt(NH$_3$)$_2$Cl}$_2$-μ-N^4-R-spermidine-N^1,N^8]Cl$_2$ where N^4-R represents BOC (*tert*-butyl), CBz (benzyl), and Fmoc (fluorenylmethyl) carbamate blocking groups, **2**−**4**, respectively. The bulky Fmoc derivative showed evidence for conformational isomers by ^{1}H NMR spectroscopy due to the inequivalence of the two *n*-propyl and *n*-butyl side chains of the spermidine moiety. The rate constants for hydrolysis and release of **1** were calculated. Release of cytotoxic **1** at physiologically relevant pH followed the order **4** > **2** > **3**. The calculated values for 4 (pH 5, 6.0(±3.9) × 10^{-10} s^{-1}; pH 6, 6.5(±0.2) × 10^{-9} s^{-1}; pH 7, 6.0(±0.2) × 10^{-8} s^{-1}; pH 8, 1.6(±0.1) × 10^{-7} s^{-1}) show a more pronounced pH dependence compared to **2** (pH 5, 4.6(±0.1) × 10^{-8} s^{-1}; pH 6, 4.2(±0.1) × 10^{-8} s^{-1}; pH 7, 3.2(±0.1) × 10^{-8} s^{-1}). Preliminary biological assays of cellular uptake and cytotoxicity confirm the utility of the pro-drug concept. While blocked-polyamine compounds such as **2**−**4** are, in general, 2−3 orders of magnitude less cytotoxic than **1**, there is significant cell type variability. Specifically, the Fmoc derivative **4** showed significantly enhanced cytotoxicity warranting further study of the pro-drug concept for greater selectivity and/or oral delivery.

Introduction

Polynuclear platinum complexes represent a discrete class of anticancer agents, distinct in biological activity from the mononuclear *cis*-DDP and its congeners.[1] Within this class of compounds, a variety of structural types differing in geometry and coordination type is possible.[2] The first compound to enter clinical trials from this new structural class is the trinuclear compound designated BBR3464.[3] The general formula for this structural class is shown in Figure 1 and may comprise either dinuclear or trinuclear compounds as indicated.

Polyamine-bridged dinuclear platinum compounds are highly interesting second-generation analogues of BBR3464 because the hydrogen-bonding and electrostatic contributions of the central platinum-amine group in BBR3464 is replicated by the free, noncoordinated "central" quaternary nitrogens of the linear polyamine linker while the presence of two separate Pt−Cl bonds maintains the bifunctional binding mode on the DNA adducts.[4] Preclinical investigations confirm the potency of these species with cytotoxicity in the nanomolar range.[5] The cyto-

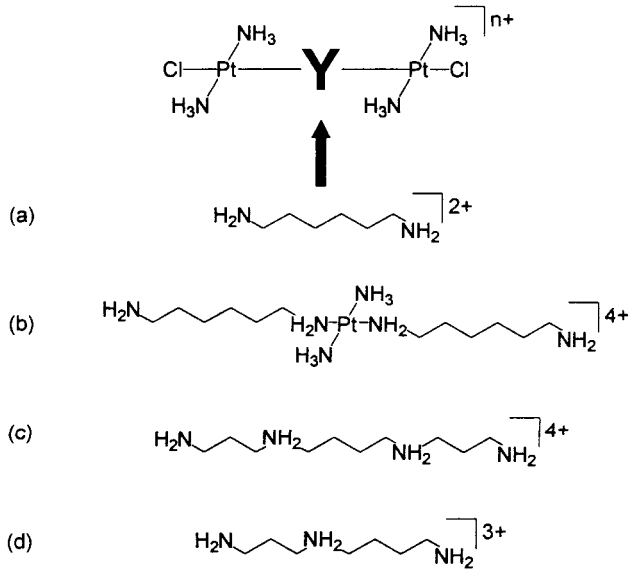

Figure 1. General structure of di- and trinuclear linear Pt polyamine compounds: (a) 1,1/*tt*; (b) 1,0,1/*ttt* (BBR3464); (c) 1,1/*tt*-spermine; (d) 1,1/*tt*-spermidine. The abbreviations refer to the number of leaving groups and the configuration of each Pt center, e.g., one leaving group trans to the linker chain.

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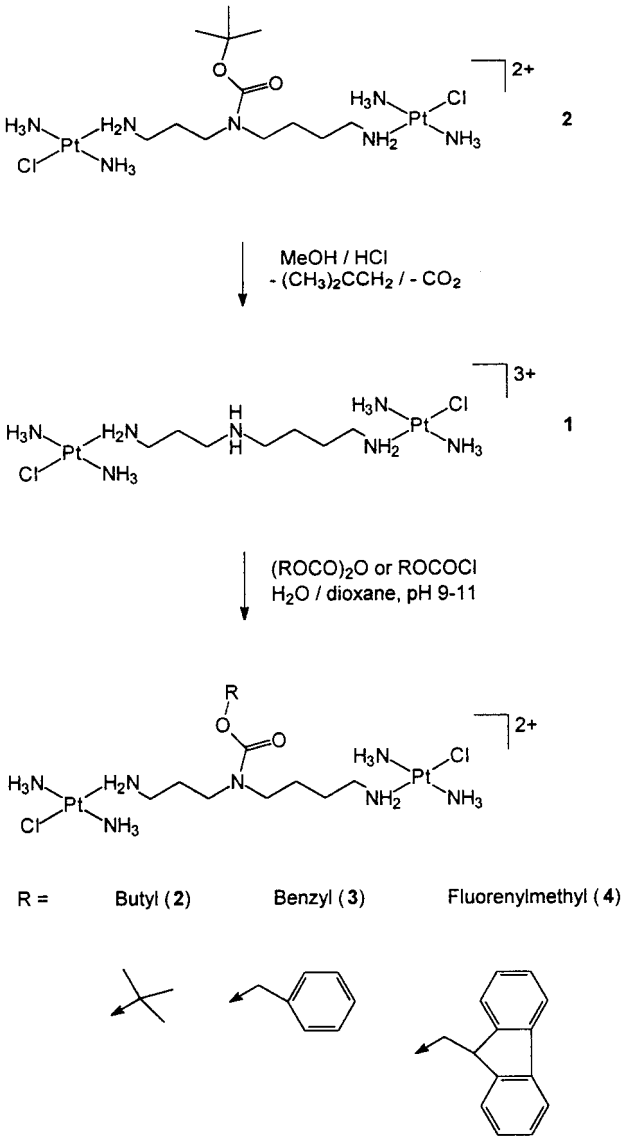

Figure 2. Synthesis scheme for compounds **1**−**4**. Complex **1** is prepared from **2** by acidic hydrolysis of the BOC protection group. Reintroduction of a blocking group leads to the formation of the various protected amines **2**−**4**.

toxicity and antitumor activity is a function of the specific linking polyamine.

The remarkable potency of polyamine-bridged dinuclear platinum complexes results in a relatively narrow therapeutic index. To enhance the therapeutic index of these drugs, we have begun to investigate the potential for "pro-drug" delivery of less toxic and better tolerated derivatives. Linear polyamine-bridged dinuclear platinum complexes are prepared by the scheme exemplified for spermidine of Figure 2. Because there are a number of potential binding sites, first a selectively blocked polyamine with the central nitrogens containing the *N*-BOC group is prepared. Then upon platination and production of the linear blocked polyamine-bridged platinum compound, the BOC group is removed by mild acid giving the polyamine-bridged compound with the protonated "central" amines.[6]

An interesting feature of the structure−activity relationships within the general structure represented in Figure 1 is that the possibility of hydrogen-bonding and electrostatic interactions in the linker greatly enhances the cellular uptake, cytotoxicity and antitumor activity in comparison to a simple diamine linker such as $H_2N(CH_2)_nNH_2$. In agreement with this observation, all blocked polyamine-bridged compounds are 2−3 orders of magnitude less cytotoxic.[6,7] Since the only difference is the charge on the compound and the presence of the "central" protonated but nonplatinated amine, it is reasonable to assume that these features account for the potent cytotoxicity.

The use of blocking carbamates for designed and controlled synthesis is not restricted to the BOC group. A range of carbamates differing in acid susceptibility is available. It was therefore of interest to explore how changes in the structure and acid susceptibility of the blocking group could influence the biological activity of the polyamine-bridged compounds. This paper presents our chemical and initial biological studies on the use of designed blocked polyamines capable of selective release of highly cytotoxic species.

Experimental Section

Starting Materials. [{*trans*-$Pt(NH_3)_2Cl\}_2$-μ-spermidine-N^1,N^8]Cl_3 (**1**) was prepared according to the published method.[6] Di-*tert*-butyl dicarbonate, benzylchloroformate, and fluorenylmethylchloroformate were purchased from Aldrich and used without further purification.

Instrumentation. ^{1}H NMR spectra were measured in D_2O solution on a Varian Mercury 300 MHz spectrometer using sodium (trimethylsilyl)propionsulfonate (TSP, δ = 0.00 ppm relative to TMS) as internal reference. 2D DQF-COSY, ROESY, and TOCSY NMR spectra were recorded in D_2O on a Varian Unity 500 MHz spectrometer at 22 °C. The data were processed with Felix 97 on an Indigo2 SGI workstation. The ROESY spectrum was collected with a mixing time of 400 ms and a total recycle delay of 1.5 s, 32 transients, 512 time increments with 2048 complex points.

pH measurements were taken on a Corning 340 pH meter with combined glass electrode. pD values in deuterated solutions were obtained by addition of 0.4 units to the meter reading. Extinction coefficients were determined with a JASCO V-550 UV/VIS spectrophotometer using 1 cm cuvettes.

Analyses of the products and hydrolysis studies were carried out on an analytical Beckman System Gold Nouveau HPLC instrument with UV detection at 215 nm. A Lichrosphere RP-8 column (5 mm particle size, dimensions 250 mm × 4 mm) was used with a solvent gradient from water/methanol 97:3 (0.05 M $NaClO_4$, 1% NaCl) to water/methanol 70:30 (0.05 M $NaClO_4$, 2% NaCl).

Hydrolysis Study. For the hydrolysis experiments, 10^{-3} mmol of complex was dissolved in 1 mL of Nanopure water. The pH of the solutions was adjusted by addition of HNO_3 (0.1 M, 0.01 M) and NaOH (0.1 M, 0.01 M), respectively. The samples were incubated in a water bath at 37 °C, and aliquots of 20 μL were taken from the bulk solution for HPLC analysis. The pH values of the samples were controlled in regular intervals and readjusted if necessary.

Preparations. [{*trans*-$Pt(NH_3)_2Cl\}_2$-μ-N^4-BOC-spermidine-N^1,N^8]-Cl_2 (2). To a solution of 0.1 mmol of **1** in 7 mL of H_2O was added 0.25 mmol of di-*tert*-butyl dicarbonate in 3 mL of dioxane. NaOH (1 M) was added dropwise to reach pH 10−11. The solution was stirred for 24 h at ambient temperature; the pH was readjusted to 10 approximately 3 h after the start of the reaction. The clear solution was then evaporated to dryness, and the remaining colorless residue was redissolved in 40 mL of methanol. The insoluble starting compound was removed by filtration, and the filtrate was concentrated by rotary evaporation until a precipitate formed. LiCl (5 mmol) dissolved in methanol was added, and the mixture was cooled to 4 °C overnight.

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The colorless product was collected by filtration in 62% yield. Anal. Calcd for $C_{12}H_{39}N_7O_2Cl_4Pt_2$: C, 17.05; H, 4.65; N, 11.60; Cl, 16.77. Found: C, 16.79; H, 4.63; N, 11.24; Cl, 16.70.

[{*trans*-Pt(NH$_3$)$_2$Cl}$_2$-μ-*N*^{4}-CBz-spermidine-*N*^{1},*N*^{8}]Cl$_2$ (3). Compound **1** (0.25 mmol) was dissolved in 20 mL of H_2O and was combined with 0.75 mmol of benzylchloroformate in 10 mL dioxane at 0 °C. The mixture was allowed to come to room temperature and subsequently brought to pH 10 by means of 1 M NaOH. The clear solution was stirred for 24 h, and the pH was periodically controlled and if necessary readjusted to 10−11. A small amount of a black precipitate was filtered off, and the filtrate was concentrated to dryness. The solid was dissolved in 60 mL of boiling methanol, and the solution was filtered to remove the insoluble starting compound. The methanolic solution was concentrated to ca. 20 mL, and a 3−5-fold excess of LiCl in methanol was added. The mixture was allowed to crystallize at 4 °C overnight yielding 81% colorless crude product, which was recrystallized from water or methanol. The final yield was 70−75%. Anal. Calcd for $C_{15}H_{37}N_7O_2Cl_4Pt_2$: C, 20.49; H, 4.24; N, 11.15; Cl, 16.12. Found: C, 20.54; H, 4.28; N, 10.92; Cl, 16.15.

[{*trans*-Pt(NH$_3$)$_2$Cl}$_2$-μ-*N*^{4}-Fmoc-spermidine-*N*^{1},*N*^{8}]Cl$_2$ (4). A solution of 0.4 mmol of **1** in 25 mL of H_2O was adjusted to pH 9−10 with 2 M NaOH. Fluorenylmethylchloroformate (0.5 mmol)in 15 mL dioxane was added with stirring at 0 °C, then the solution was allowed to come to room temperature. The pH was readjusted to 9−10, and the mixture was stirred at ambient temperature for 4 h. The solution was concentrated in a vacuum to approximately 5 mL volume and then cooled to 4 °C. A colorless precipitate was collected by filtration and washed with dioxane and diethyl ether. The crude product was recrystallized from water and subsequently from methanol giving **4** in 58% yield. Anal. Calcd for $C_{22}H_{41}N_7O_2Cl_4Pt_2$: C, 27.31; H, 4.27; N, 10.13; Cl, 14.66. Found: C, 27.49; H, 4.36; N, 10.61; Cl, 14.95.

Biological Assays. Cell Culture. A2780, A2780/CDDP, CH1, and CH1/CDDP cell lines were used in this study and maintained according to published procedures.[8,9]

Growth Inhibition Assay. The Sulforhodamine B (SRB) assay was used to determine growth inhibition potency of platinum drugs.[10] The cells were seeded in 96-well microtiter plates at (3−8) × 10^3 cells/well in 160 μL growth medium and allowed to attach overnight. Platinum agents were then added after serial dilution in quadruplicate wells and exposed to cells for 2 or 96 h. After the 2 h drug incubations were complete, plates were washed free of drug with phosphate-buffered saline (PBS) and then refed with normal growth medium for a further 94 h. Quantitation of cell growth in treated and control wells was then assessed using 0.4% SRB dissolved in 1% acetic acid. IC_{50} values were determined graphically.

Cellular Accumulation. The cellular accumulation assays followed published procedures.[7] Briefly, cells were resuspended at 10^7/mL in media supplemented with 25 μM HEPES. Platinum complexes were added, and samples were incubated at 37 °C in 5% CO_2. At 0 and 2 h, aliquots were removed for determination of cell concentrations and for measurement of platinum content. For the latter, aliquots were washed three times in cold phosphate buffered saline, resuspended in 1% Triton-X in water, and sonicated. Platinum content was measured by flameless atomic absorption spectroscopy.

Results

Acidity of the N4 Position of Platinum-Bound Spermidine. Three blocking groups differing in acid stability were examined: BOC, CBz, and Fmoc. To find the optimal conditions for the synthetic procedure, the pK_a of the N4 site of the unprotected Pt spermidine complex **1** was determined prior to the protection reactions. A pH titration was carried out and monitored by ^{1}H NMR spectroscopy. Only one of the four signals of the spermidine linker chain could be followed over the entire pH range without significant overlap with other signals (see signal c in Chart 1 and Table 1). The pD dependence of this resonance is shown in Figure 3. For the present situation with only a single deprotonation site present the relationship between the observed chemical shifts δ and the pD value is given by the following equation:[11]

$$\delta = \frac{\delta_B + \delta_{BH}\, 10^{pK_a - pD}}{1 + 10^{pK_a - pD}}$$

In this equation, δ_B and δ_{BH} represent the chemical shifts of the deprotonated and the protonated species, respectively. The

Chart 1

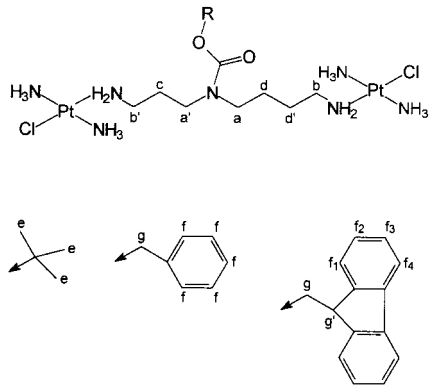

Table 1. ^{1}H NMR Chemical Shifts (ppm) of Compounds **1**−**4** (All Signals Are Multiplets except Where Indicated)

	δ/ppm				
	1[a]	**2**	**3**	**4a**	**4b**
a/a′	3.12	3.30	3.42	2.59/3.16	3.08/2.67
b/b′	2.77	2.71	2.67	2.37/2.46	2.60/2.04
c	2.11	1.91	1.89	1.66	1.42
d/d′	1.77	1.67	1.66	1.00/1.22	1.42/1.49
e[b]		1.50			
f			7.50		
f_1/f_2 [c]				7.75/7.51	7.75/7.51
f_3/f_4 [d]				7.56/8.00	7.56/8.00
g/g′			5.19[b]	4.88[e]/ 4.39[f]	4.88[e]/ 4.39[f]

[a] Reference 6. [b] Singlet. [c] Doublet each, ^3J = 6.6 Hz. [d] Doublet each, ^3J = 7.2 Hz. [e] Doublet, ^3J = 19.5 Hz. [f] Triplet, ^3J = 19.5 Hz.

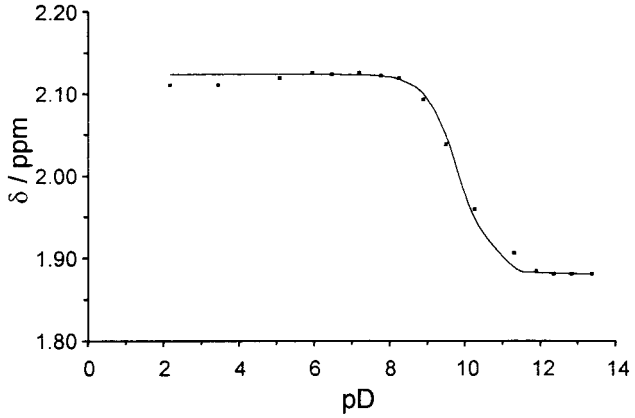

Figure 3. pD dependence of the chemical shift of one methylene resonance (signal c in Chart 1) of compound **1**. The curve shows the computer best fit (see text and ref 11 for details).

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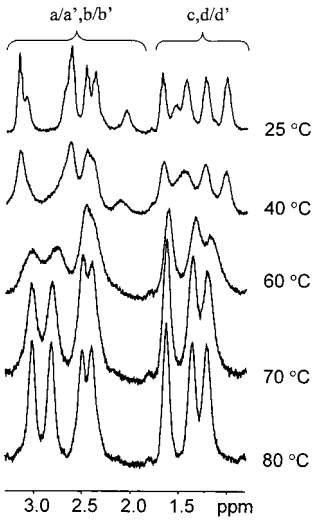

Figure 4. Temperature dependence of the aliphatic section of the ^{1}H NMR spectrum of **4**.

conversion from the deuterated to normal aqueous solution follows the formula $pK_{D_2O} = (1.015pK_{H_2O}) + 0.45$.[12] The obtained pK_a value of 9.24 ± 0.05 is significantly lower compared to values of various secondary aliphatic amines which range from 10.5 to 11.0.[13] The increased acidity is most likely a consequence of the electron withdrawing effect of the coordinated platinum centers.

Synthesis and Characterization of Compounds 2−4. The synthesis of the blocked spermidine compounds was achieved in a water/dioxane mixture at pH values between 9 and 11. These conditions provided a sufficient percentage of the deprotonated amine which reacted as a nucleophile during the protection step. The synthesis is a modification of a reaction pathway applied for the protection of the amino function of amino acids.[14] The compounds were isolated as chloride salts and were characterized by HPLC and ^{1}H NMR spectroscopy. The BOC-protected Pt spermidine complex was synthesized earlier as an intermediate during the preparation of **1**, and characterized as a mixed chloride/nitrate salt.[6] Compound **2** shows an identical HPLC profile and ^{1}H NMR spectrum as the precursor compound of **1**, confirming that the BOC protection group could be reintroduced to the N4 position via the described pathway (Figure 2). The synthetic procedure was then used to obtain a series of carbamates which varied only in the aliphatic or aromatic residue on the protection group.

The ^{1}H NMR spectra of compounds **2** and **3** are, except for the signals of the protection groups, nearly superposable. The alkyl chain gives rise to four major sets of multiplets for the methylene protons, a pattern also found in the spectrum of **1**. The chemical shifts are summarized in Table 1. The assignment of the signals is in agreement with a COSY spectrum taken of **3** as well as the previously published spectrum of **2**.[6] The Fmoc protected complex **4** displays a markedly different ^{1}H NMR spectrum with at least 11 different resonances being resolved in the aliphatic range of the spectrum between 1.00 and 3.16 ppm at room temperature. The temperature dependence of the ^{1}H NMR spectrum (Figure 4) shows coalescence at elevated temperature, resulting in seven signals of equal intensity at 70 °C. As a consequence of the bulky residue of the Fmoc protecting group the proton signals of each of the seven methylene groups are chemically inequivalent in compound **4**. The room temperature spectrum is further complicated by the fact that the multiple proton signals do not integrate in a strictly 1:1 intensity ratio, as would be expected for protons of the same methylene group in different chemical environments. The spectrum, rather, indicates the presence of two conformational isomers which interconvert slowly on the NMR time scale. These conformational changes do not seem to affect the protecting group since only one set of resonances is detected for the flourenylmethyl residue.

To examine this point further a series of 2D NMR spectra were recorded for **4**. In the COSY spectrum at least 13 different resonances are resolved in the aliphatic range (Figurer 5A). For a complete assignment of all resonances 2D ROESY and TOCSY spectra were recorded at room temperature. The TOCSY spectrum, Figure 5B, allows assignment of every resonance to one of the two aliphatic (*n*-butyl or *n*-propyl) chains of the spermidine linker, because only signals within the same chain are connected by cross-peaks. However, the signal at 1.42 ppm shows cross-peaks between resonances in both the *n*-butyl and *n*-propyl chains. This leads to the conclusion that two signals, one from the *n*-propyl and one from the *n*-butyl chain, are coincidentally superimposed at this frequency. Correlation of the TOCSY spectra with COSY and ROESY spectra then allowed full assignment of the signals, as summarized in Table 1. The presence of strong exchange cross-peaks (EXSY peaks) in the ROESY spectrum confirms the presence of two conformational isomers (Figure 5C).[15] The assignment of the aromatic protons of the Fmoc group, Figure 5D, is in agreement with the ROESY spectrum, since strong NOEs are detected between the resonance of f_1 and both g and g′ signals, but none of the latter shows NOE cross-peaks with the f_4 resonance. The assignment is illustrated in Chart 1.

According to the integration of the ^{1}H NMR spectrum, the major conformer **4a** contributes to ca. 65% of the overall intensity, while the minor conformer **4b** accounts for the remaining 35%. The origin of the conformational isomerism is most likely to arise from the relative positions of the bulky Fmoc group and the inequivalent alkane chains of the linker. Compared to the BOC and CBz analogues the chemical shifts of the methylene protons of the linker chain in both Fmoc conformers appear at higher field. The upfield shift is more pronounced for the *n*-butyl chain in **4a** and the *n*-propyl chain in **4b**, respectively. The increased shielding of these protons results most likely from an interaction with the two aromatic rings of the fluorenyl moiety. The rotation of this group around the nitrogen−carbonyl bond is expected to be slow on the NMR time scale due to the high double bond character of this bond.

Hydrolysis Studies. The hydrolysis of the protective groups of compounds **2**−**4** was monitored at 37 °C at various pH values. By the start of the reaction the HPLC chromatograms of all samples displayed only signals of the protected species. In addition, the chromatogram of **2** contained a peak of a minor impurity (ca 0.4%) which remained unchanged during the time course of the reaction. Deprotection of the secondary amino function leads to the appearance of a signal for the unprotected spermidine complex **1** that, as a consequence of the increased cationic charge, is well separated from the signal of the N4 blocked precursor. The area percentage of the integrated signal of the unprotected species is taken as an estimate for the amount of hydrolysis at a given timepoint. To allow for a more accurate

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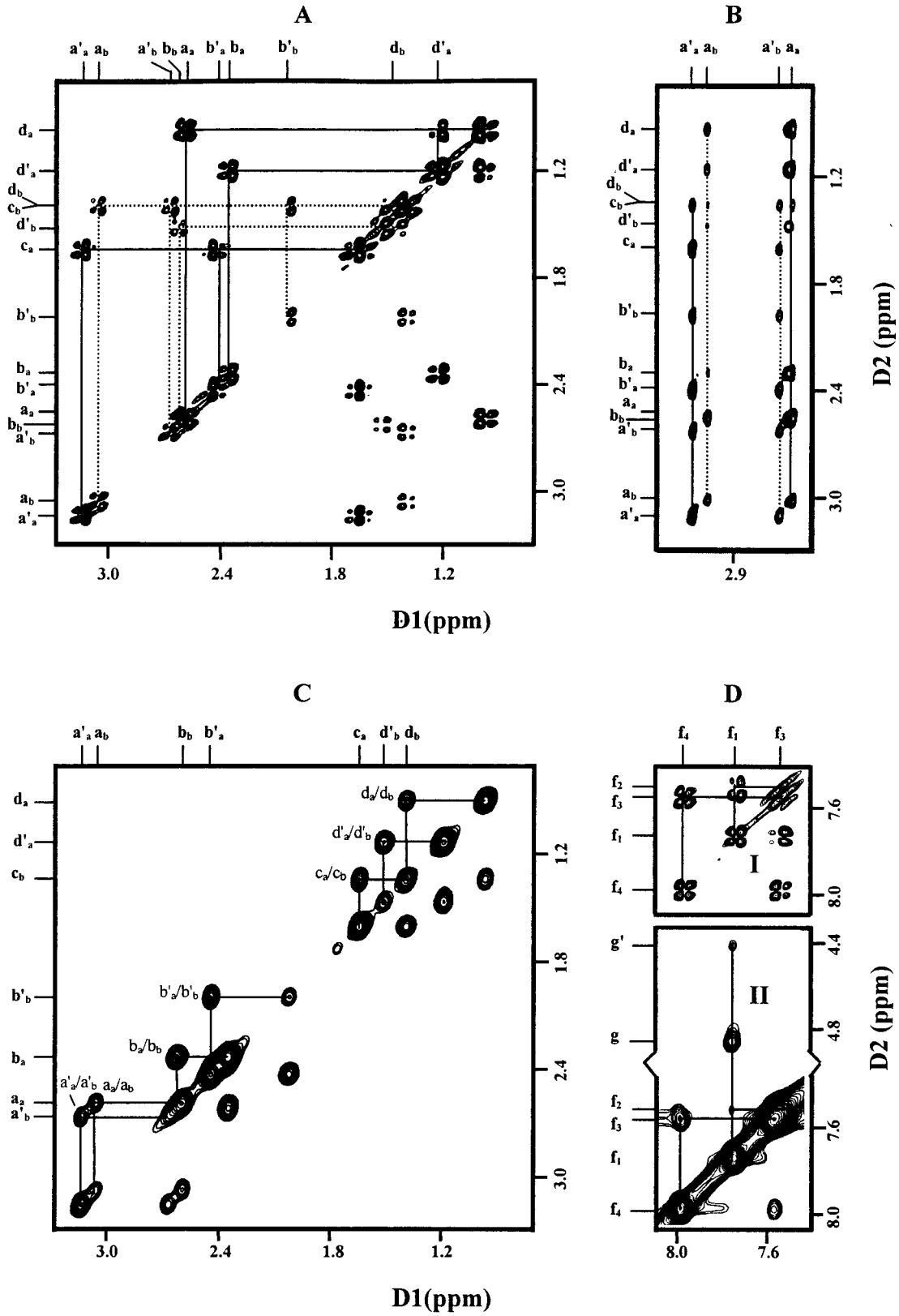

Figure 5. Aliphatic section of 2D DQF-COSY (A) and TOCSY (B) spectra of **4**, showing the two conformational isomers: a, —; b, ···. Exchange cross-peaks between the two isomers are shown in the ROESY spectrum (C); the weaker NOEs are omitted for clarity. D: Aromatic section of the DQF-COSY (I) and ROESY (II) spectra of **4**.

quantitative comparison of the results, the extinction coefficients for compounds **1**−**4** have been determined at 215 nm (Table 2). The integral values of the blocked compounds have been corrected for the higher absorbance of these species at the observed wavelength compared to the unprotected species **1**. Table 3 provides a summary of the amount of hydrolysis for all samples before and after the correction. Rate constants (see below) were calculated based on the corrected integral values.

The hydrolysis profile of compound **2** in the pH range between 5 and 7 is depicted in Figure 6A. Although the BOC

Table 2. Extinction Coefficients of Compounds **1**−**4** at 215 nm and Correction Factors To Account for Different Absorption of the Compounds at This Wavelength

	$\epsilon_{215}/10^3$ ($L \cdot mol^{-1} \cdot cm^{-1}$)	correction factor $\epsilon_{215}/\epsilon_{215}$ (**1**)
1	3.64	1
2	3.90	1.07
3	8.69	2.39
4	25.0	6.87

Table 3. Percentage of Deprotected Species **1** Found in the Samples of Compounds **2**−**4** at Various pH Values

	2		**3**		**4**	
pH	% **1**[a,c]	time (d)[b]	% **1**	time (d)	% **1**	time (d)
5	14.3 [15.1]	42	0.05 [0.13]	30	0.14 [1.0]	41
6	13.0 [13.8]	42	0.05 [0.12]	42	0.45 [3.0]	41
7	6.2 [6.6]	25			3.4 [19.6]	41
8					7.3 [35.2]	30

[a] Percentage of **1** of the overall integration in the chromatogram after incubation. [b] Time of incubation at 37 °C. [c] Values in square brackets are corrected for the different extinction coefficients at 215 nm.

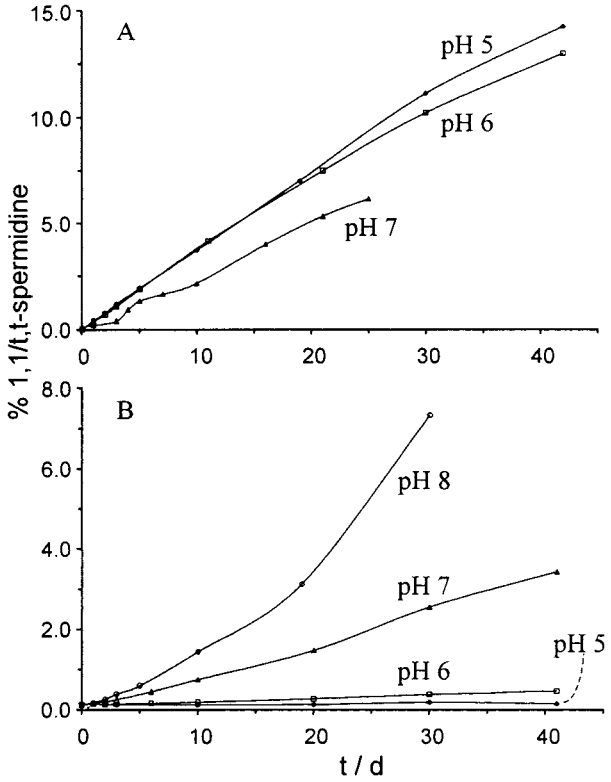

Figure 6. Percentage of unprotected 1,1/*tt*-spermidine (**1**) found in the HPLC chromatograms of compounds **2** (A) and **4** (B), respectively, over time at different pH values. The results are not corrected for the different extinction coefficients of compounds **1**−**4**.

protecting group is regarded as stable in neutral and moderately acidic aqueous solutions,[16] a slow release over a time period of several days is evident from the data. After 25 days at neutral pH the signal of the unprotected Pt spermidine complex contributes approximately 6% to the overall integration of the chromatogram. At lower pH values more hydrolysis product is detected, but the rate of cleavage is still low throughout the examined pH range. Approximately 13% of the free spermidine compound, **1**, is released within 42 day at pH 6 compared to little more than 14% at pH 5. Slightly higher values for the amount of **1** result if the corrected integral values are used (see paragraph above and Table 3). Rapid and complete acidic hydrolysis of the BOC group is known to take place at pH < 2 and is commonly used for the deprotection of the amino function in *tert*-butylcarbamates.[16]

Benzylcarbamates show in general higher stability toward acidic hydrolysis and are usually removed by catalytic hydrogenolysis rather than by acid or base-catalyzed cleavage.[16] Therefore, it is not surprising that no significant amounts of spontaneous deprotection is evident from the HPLC profile of 3 at pH 5 and 6. Over the complete timecourse, the amount of **1** detected in the samples is well below 0.1%.

The HPLC profile of compound 4 at pH 5−8 is displayed in Figure 6B. Similar to **3**, only minor amounts of hydrolyzed species are observed at pH 5 and 6, proving the excellent acid stability of the Fmoc protection group. However, a steady increase in concentration of the unprotected complex is detected, meaning that slow decomposition of the carbamate is taking place under these conditions. Higher pH values strongly favor the deprotection of the complex and considerable amounts of free spermidine complex are released at pH 7 and 8 (Table 3). The reason for the reversed pH dependence of the hydrolysis reaction compared to complex **2** lies in the structure of the flourenylmethyl residue. The aromatic rings stabilize a dibenzocyclodienylanion, and therefore the reaction is believed to commence via a β-elimination process,[17] allowing cleavage of fluorenylmethylcarbamates under mild basic conditions.

In the range between pH 5 and 6 compound **2** clearly shows the highest hydrolysis rate in this series. However, at physiological pH the Fmoc complex **4** obviously undergoes faster deprotection than **2**. Although no data were obtained for **3** at this pH, it is a reasonable assumption that the benzylcarbamate will become more stable with increasing pH.[16] First-order rate constants were calculated for the deprotection of compounds **4** and **2**. The rate constants obtained for **4** (pH 5, $6.0(\pm 3.9) \times 10^{-10}$ s^{-1}; pH 6, $6.5(\pm 0.2) \times 10^{-9}$ s^{-1}; pH 7, $6.0(\pm 0.2) \times 10^{-8}$ s^{-1}; pH 8, $1.6(\pm 0.1) \times 10^{-7}$ s^{-1}) show a more pronounced pH dependence compared to **2** (pH 5, $4.6(\pm 0.1) \times 10^{-8}$ s^{-1}; pH 6, $4.2(\pm 0.1) \times 10^{-8}$ s^{-1}; pH 7, $3.2(\pm 0.1) \times 10^{-8}$ s^{-1}), where all values are found to be in the same order of magnitude. However, the very slow nature of the hydrolysis process and the inherent inconsistencies in calculating absolute concentrations while continually calibrating HPLC conditions result in large standard deviations for some of the values, especially in the pH range where the compounds exhibit great stability. Therefore, no attempts were made to calculate rate constants for complex **3** and we interpret the calculated rate constants with caution.

Biological Activity. A preliminary comparison of the pharmacological properties of the blocked polyamine compounds was undertaken to examine the pro-drug potential in a biological setting. In L1210 murine leukemia the BOC-spermidine compound showed intermediate potency between that of a standard 2+ compound, 1,1/*t*,*t* (n = 6), and the "parent" 1,1/*t*,*t*-spermidine carrying a 3+ charge, as previously reported,[7] Table 4. Incorporation of charge and hydrogen-bonding capability into the linking diamine or polyamine has been shown to dramatically enhance cellular accumulation in polynuclear platinum complexes.[7,18] The cellular uptake of the 1,1/*t*,*t*-spermidine compound (overall charge is 3+) is known to be high and

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Table 4. Growth Inhibition and Accumulation of Pt Complexes in L1210 Cell Lines after 2 h of Exposure

	growth inhibition[a]			accumulation[b]	
	L1210/0	L1210/DDP	RF[c]	L1210/0	L1210/DDP
cisplatin	1.3 (0.37)	59 (6.0)	44	5.0 (0.89)	1.5 (0.76)
1	0.75 (0.29)	0.26 (0.23)	0.35	17 (3.4)	20 (2.1)
2	1.25 (0.13)	4.6 (1.0)	3.7	5.0 (0.63)	4.8 (0.75)
1,1/*tt*	3.7 (0.37)	16 (2.8)	4.3	3.8 (0.70)	0.67 (0.21)

[a] IC_{50} (mM) mean (±SE) for three experiments of two determinations each. [b] Attamol Pt complex/cell (±SE) for three experiments of two determinations each. [c] Resistance factor [(IC_{50} L1210/platinum complex)/(IC_{50} L1210/0)]. See ref 7 for details.

significantly enhanced over "simple" dinuclear compounds such as [*trans*-{$PtCl(NH_3)_2$}$_2H_2N(CH_2)_6NH_2$]$^{2+}$ (overall charge is 2+).[18] Interestingly, cellular accumulation of the BOC-spermidine compound in the L1210/DDP (the sub-line resistant to cisplatin) was intermediate between that observed for the 2+ and 3+ compounds, Table 4. Based solely on charge considerations the BOC-spermidine with a charge of 2+ should have cellular uptake similar to 1,1/*t*,*t* ($n = 6$). The enhanced uptake could be explained by some hydrolysis in media or plasma producing small amounts of the protonated 1,1/*t*,*t*-spermidine. Considering the potency of polynuclear-polyamine compounds, a small percentage of hydrolysis could have a significant impact on in vivo activity. In a panel of human ovarian cancer cell lines, the three blocked polyamine compounds showed different patterns of cytotoxicity amongst themselves and also in comparison to the "free" polyamine compound, Table 5. Of especial interest is the remarkably low value for **4** (Fmoc) in A2780 cells. Overall, the results suggest that the activity of the blocked polyamine-platinum compounds may be cell line specific, and thus the possibility for drug development in selected tumors or drug delivery is seen. For example, the stability of the Fmoc complex at pH 5 and 6 could allow for oral delivery, stabilizing the compound to the acid of the stomach but releasing the active species at pH 7−8.

Table 5. IC_{50} (mM) Values in Human Ovarian Carcinoma Cell Lines Sensitive and Resistant to Cisplatin[a]

	1	**2**	**3**	**4**	cisplatin
A2780	<0.25	2.1	24.0	0.84	1.6
A2780CisR	<0.25	19.0	100.0	65.0	12.0
CH1	0.43	8.0	25.0	46.0	0.34
CH1CisR	0.35	11.0	43.0	62.0	1.1

[a] See refs 2 and 8−10 for details.

In summary, we report a method for selectively activating blocked-polyamines to produce the highly potent "free" polyamine-bridged platinum compounds. This method has utility for enhancing the Therapeutic Index and oral bioavailability of drugs, and may also have utility for tumor-specific development of the drugs based on enzymatic activation. The method is applicable to a wide range of spermidine and spermine derivatives.[5]

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