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Original article

Synthesis, anti-*Trypanosoma cruzi* activity and micelle interaction studies of bisguanylhydrazones analogous to pentamidine

Márcia Narcizo Borges [a,b], Jorge Cardoso Messeder [a], José Daniel Figueroa-Villar [a,*]

[a] *Departamento de Química, Instituto Militar de Engenharia, Praça General Tibúrcio 80, 22290-270 Rio de Janeiro, Brazil*
[b] *Departamento de Química Orgânica, Instituto de Química, Campus do Valonguinho, Universidade Federal Fluminense, Niterói, Rio de Janeiro, Brazil*

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Abstract

Three new bisguanylhydrazones analogous to pentamidine were synthesized, fully characterized and tested as anti-*Trypanosoma cruzi* candidates. Contrary to literature reports, that bicationic compounds are more active than monocationic compounds against *Trypanosoma brucei*, it was found that these bisguanylhydrazones are much less effective against *T. cruzi* than simple aromatic monoguanylhydrazones, thus suggesting different mechanism of action for both parasites. Spin-spin nuclear relaxation studies of the interaction of these compounds with SDS and CTAB micelles showed that only the most trypanocidal compound displays significant discrimination between anionic and cationic micelles.

Keywords: Bisguanylhydrazones; Micelle; Chagas disease; NMR relaxation

1. Introduction

Bicationic antibiotics, especially bisamidines [1,2] and bisguanylhydrazones [3] have been shown to be active against African trypanosomiasis. Among the bicationic antibiotics, until 2002 only the bisamidines have been tested against *Trypanosoma cruzi*, the causative agent of American trypanosomiasis [4,5]. It was only recently that biguanidines and "reversed" diamidino compounds were also tested against *T. cruzi* and *L. donovani* [5]. We have previously shown that aromatic guanyl hydrazones exhibit in vitro anti-*T. cruzi* activity [6]. The mechanism of action of these compounds is not clear yet, but it has been suggested that it may involve their interaction with the parasite DNA and/or its cellular membrane [7,8]. A molecular modeling study using docking methodologies showed that monoguanylhydrazones have great potential to interact with DNA, and that they possibly do that at the DNA minor groove, with some specificity for AT rich regions [7]. These molecular modeling results agree with experimental DNA binding studies carried out with African trypanocidal bicationic compounds berenil and pentamidine, which have been shown to be minor groove binders [9–12]. On the other hand, using NMR, it has been shown that the in vitro anti-*T. cruzi* activity of the aromatic guanyl hydrazones is related to their selectivity to bind to anionic SDS micelles in relation to cationic CTAB micelles, which were used as a membrane models [8]. That work showed that the differences in spin–spin relaxation times between the conditions with the presence of SDS or CTAB micelles are directly related to the IC_{50} values of the guanylhydrazones [8], thus strongly suggesting that the mechanism of anti-*T. cruzi* activity of these compounds involves interaction with the parasite membrane. Also, guanylhydrazones, being more active than gentian violet against the blood forms of *T. cruzi* in contaminated blood, and not having deleterious effects on the blood cells at the used concentrations [6], have also been shown to interact weakly with plasmatic proteins [12,13], therefore, making them promising agents for the chemical prophylaxis of blood in blood banks. In this work, we have prepared and tested against *T. cruzi* three novel bisguanylhydrazones analogous to pentamidine and studied their interaction with SDS and CTAB micelles.

2. Chemistry

The compounds were prepared by alkylation of 4-hydroxybenzaldehyde with the appropriate dibromoalkane

* Corresponding author.
E-mail address: figueroa@ime.eb.br (J.D. Figueroa-Villar).

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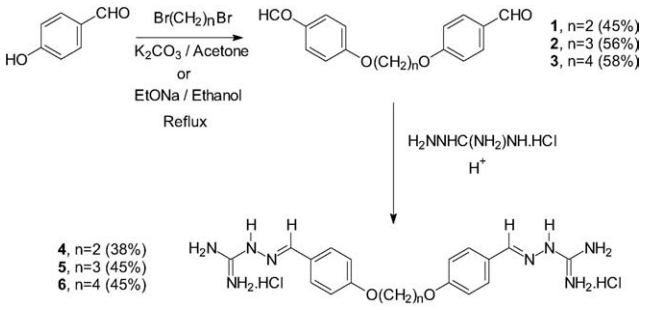

Fig. 1. Synthesis of the bisguanilhydrazones.

followed by conversion of the resulting dialdehyde to the respective bisguanylhydrazone by treatment with aminoguanidine hydrochloride, as shown in Fig. 1.

The alkylation of 4-hydroxybenzaldehyde with the dibromoalkanes, using K_2CO_3 in methanol, was appropriate for the preparation of compounds **2** and **3** [14]. However, in order to obtain **1** it was necessary to use sodium ethoxide in ethanol as base. Dialdehydes **1** and **2** were easily converted to the respective bisguanylhydrazones by treatment with aminoguanidine hydrochloride in 95% ethanol containing a few drops of fuming hydrochloric acid [3,6]. On the other hand, the conversion of dialdehyde **3** to the respective bisguanylhydrazone required the use of a Dean–Stark apparatus using toluene as solvent and a catalytic amount of *p*-toluenesulfonic acid [6]. The bisguanylhydrazones are insoluble in all common organic solvents and sparingly soluble in water. Their purification was better accomplished by repeatedly washing with an appropriate solvent. The purity of the samples obtained in this way was checked out by ^1H and ^{13}C NMR using concentrated samples (0.1 M) in DMSO-d_6 and elemental analysis.

3. Pharmacology and discussion

Once the final products were purified and fully characterized, we carried out in vitro bioassays using 7 days trypomastigote forms of Y-strain *T. cruzi* [15]. These tests were conducted using the procedure developed by Brener [16] and afforded the inhibitory concentration as summarized in Table 1.

The data in Table 1 show that the compound with three CH_2 at the middle chain, compound **5**, is the most active. This result with *T. cruzi* follows the same trend as those found in the results obtained for pentamidine and analogues against African trypanosomiasis, where the compounds with an odd number of CH_2 groups in the middle chain ($n = 3$ and $n = 5$) are more active than the compounds with an even number of CH_2 groups [17]. Interestingly, the bisguanylhydrazones tested here are less active against *T. cruzi* than most monoguanylhydrazones tested in our previous work [6], where some compounds displayed IC_{50} values as low as 17 μM. This is in opposition to literature reports on the behavior of cationic antibiotics when tested against *Trypanosoma brucei*, where an increase in the number of positively charged groups leads to increased anti-parasite activity [3]. It has been shown that bisamidines active against *T. brucei* exert their action by interaction with the parasite DNA [18]. This difference in behavior regarding the biological activity of bicationic compounds, when comparing the literature results obtained with African trypanosomiasis with our results obtained for American trypanosomiasis, suggests that their mechanism of action against *T. cruzi* is different, and possibly related to the interaction of the compounds with the parasite membrane, instead of interaction with the DNA [8].

In order to model the interaction of the cationic compounds with membranes we carried out NMR longitudinal relaxation studies [8] in the presence of cationic (CTAB) and anionic (SDS) micelles as membrane models. The relaxation studies were done using 2.5 mM samples of the products, to make sure that the measurements were carried out with stable solutions of all the compounds. Also, this concentration is close to the determined IC_{50} values. For these studies, the chosen tensoactive agent concentration was 20 mM, to ensure that we were working well above the critical micellar concentration (CMC) of SDS and CTAB [19]. The longitudinal relaxation (T_1) values for all observed hydrogens of the bisguanylhydrazones are summarized in Table 2 and Fig. 2.

It must be noticed that we were not able to measure the relaxation time of the hydrogens of the junction alkyl chain of the bicationic compounds because their signals are obscured or superimposed with either the water signal or the tensoactive agent signals. The data from Table 2 show that the bisguanylhydrazones interact more strongly with the SDS micelles than with the CTAB micelles. This is somehow expected, as the interaction should be much favored by the electrostatic attraction between the cationic compounds and the anionic micelles. However, in our previous work we showed that some monoguanylhydrazones, despite their positive charge, do not interact preferentially with anionic SDS micelles as compared to cationic CTAB micelles [8]. A better way to compare the relaxation behavior of the different compounds is using Fig. 2, which shows the behavior of all the observed hydrogens for the three bisguanylhydrazones under all the studied conditions.

Table 1
Trypanocidal activity for the bisguanylhydrazones against the trypomastigote forms of Y-strain *T. cruzi*

Compound	Concentration (mg/ml)				IC_{50} (mM)
	5.0	2.5	0.50	0.25	
4	3.5	0	0	0	>100
5	100	93	46	3.6	1.2 ± 0.1
6	95	56	5.5	0	5.6 ± 0.4

Table 2
^{1}H T_1 values (in seconds) for all the bisguanylhydrazones (2.5 mM) in D_2O and in D_2O containing SDS or CTAB (20 mM)

Compound	Solutions	T_1 (s)				
		H-2	H-3	H-5	H-6	H-7
4	D_2O	1.895	1.311	1.311	1.895	2.486
	SDS + D_2O	0.882	0.632	0.632	0.882	0.972
	δT1 (SDS)	1.013	0.679	0.679	1.013	1.514
	CTAB + D_2O	1.442	1.343	1.343	1.442	1.966
	δT1 (CTAB)	0.453	–0.032	–0.032	0.453	0.520
5	D_2O	1.295	1.222	1.222	1.295	1.654
	SDS + D_2O	0.722	0.570	0.570	0.722	0.908
	δT1 (SDS)	0.573	0.652	0.652	0.573	0.746
	CTAB + D_2O	1.327	1.053	1.053	1.327	1.576
	δT1 (CTAB)	–0.032	0.169	0.169	–0.032	0.078
6	D_2O	1.313	0.791	0.791	1.313	1.627
	SDS + D_2O	0.599	0.467	0.467	0.599	0.781
	δT1 (SDS)	0.714	0.324	0.324	0.714	0.846
	CTAB + D_2O	1.096	1.104	1.104	1.096	1.237
	δT1 (CTAB)	0.217	–0.313	–0.313	0.217	0.390

Found average error = 0.03%.

Considering the data from Table 2 and Fig. 2, it can be observed that all the bisguanylhydrazones interact with the SDS micelles. In fact, the relaxation time changes in SDS are greater for **4**, which is the less active compound. Using the relaxation measurements for H-7, which is the non-exchangeable hydrogen closest to the cationic terminal, the relaxation changes induced by the SDS micelles follow the order **4** > **6** > **5** and do not show any correlation to the IC_{50} values. However, it can be observed that compound **5** does not interact significantly with the cationic micelles, as its values for T_1 are not affected by the presence of CTAB above the CMC. Interestingly, this is not the case for compounds **4** and **6**, which also show a reasonable degree of interaction with the cationic micelles. Despite the reduced number of examples used in this work, the interaction results suggest that compounds that interact with the anionic micelles but do not so with the cationic micelles, as it is the case of **5**, are more active that the compounds that, even interacting more strongly with SDS micelles, do interact with both types of micelles, as it is the case for compounds **4** and **6**. This observation seems to be in agreement with our previous demonstration that simple guanylhydrazones are more active when they are able to discriminate better between both types of micelles [8].

The normal variation of T_1 when there is an increase in the degree of intermolecular interaction is to decrease, as the interaction leads to more efficient relaxation pathways. However, for compound **6**, it is observed that T_1 actually increases for hydrogens H-3 and H-5, suggesting a decrease on the correlation time of those hydrogens.

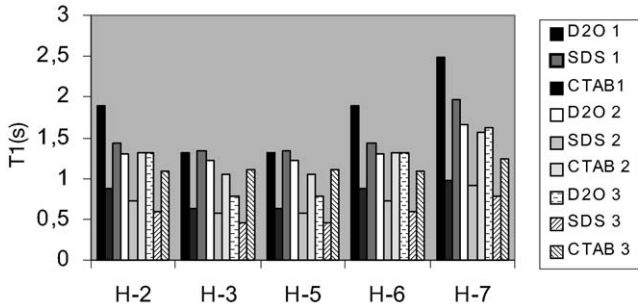

Fig. 2. Differences between the relaxation times (T_1) of the bisguanylhydrazones hydrogens in D_2O and D_2O + CTAB solutions.

4. Experimental protocols

4.1. Chemistry

All the organic starting materials used in this work were Aldrich, and were used as purchased, without previous purification. The solvents were distilled and dried previous to their use. Melting points were determined on a Fisher–Johns apparatus and are uncorrected. Infrared spectra were obtained on Perkin-Elmer, Models 1710 and 1420, spectrophotometers. The bisguanylhydrazones were prepared by reaction of the respective aldehyde with aminoguanidine hydrochloride in the presence of an acid catalyst [3,6].

4.1.1. 1,2-Bis(4-carboxyaldehydephenoxy)ethane *1*

Dialdehyde **1** was prepared by reaction of 4-hydroxybenzaldehyde (5.0 g, 40 mmol) with 1,2-dibromoethane (2.0 ml, 18 mmol) and sodium ethoxide (0.9 g, 40 matg of sodium) in refluxing ethanol (25 ml) for 3 h. The crude product obtained after cooling of the reaction mixture was filtered by suction and washed with water to eliminate the formed sodium bromide and the remaining solid was recrystallized from 95% ethanol to afford pure **1** in 45% yield: m.p. 110–112 °C; IR (KBr) ν_{max} 2950, 1760, 1670, 1300, 1240 and 1150 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.80 (s, 2H), 7.91 (d, 9.7 Hz, 4H), 7.20 (d, 9.7 Hz, 4H) and 4.51 (s, 4H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 191.3, 163.1, 131.8, 129.8, 115.0 and 66.6.

4.1.2. 1,3-Bis(4-carboxyaldehydephenoxy)propane *2*

Dialdehyde **2** was prepared by refluxing a mixture of 4-hydroxybenzaldehyde (4.0 g, 30 mmol), 1,3-dibromo-

propane (1.5 ml, 15 mmol) and K_2CO_3 (4.1 g, 30 mmol) in dry acetone (30 ml). After the completion of the reaction (18 h), the acetone was evaporated under vacuum and the resulting solid washed with water to extract the formed KBr. The crude solid product was then recrystallized from 95% ethanol to afford pure **2** in 56% yield: m.p. 125–129 °C; IR (KBr) ν_{max} 2950, 1700, 1600, 1460, 1240 e 1155 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 9.88 (s, 2H), 7.95 (d, 8.9 Hz, 4H), 7.25 (d, 8.9 Hz, 4H), 4.44 (t, 6.5 Hz, 4H) and 2.3 (quintet, 6.4 Hz, 2H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 190.8, 162.9, 131.3, 129.2, 114.5, 64.3 and 27.8.

*4.1.3. 1,4-Bis(4-carboxyaldehydephenoxy)butane **3***

Dialdehyde **3** was prepared in the same manner as **2**, but using 4-hydroxybenzaldehyde (4.0 g, 30 mmol), 1,4-dibromobutane (2.4 ml, 17 mmol), K_2CO_3 (4.1 g, 30 mmol) and a reaction time of 24 h. The crude product was purified by recrystallization from carbon tetrachloride to afford pure **3** in 58% yield: m.p. 95–99 °C; IR (KBr) ν_{max} 2950, 1700, 1675, 1600, 1440, 1240 and 1150 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 9.92 (s, 2H), 7.88 (d, 9.0 Hz, 4H), 7.15 (d, 9.0 Hz, 4H), 4.18 (bs, 4H) and 1.88 (bs, 4H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 190.8, 163.0, 131.3, 129.5, 114.5, 67.2 and 24.7.

*4.1.4. 1,2-Bis(4-guanylhydrazonephenoxy)ethane hydrochloride **4***

Compound **4** was prepared by refluxing a mixture of aldehyde **1** (0.38 g, 2.0 mmol) and aminoguanidine hydrochloride (0.46 g, 4.1 mmol) in 95% ethanol (20 ml) containing a few drops of fuming HCl during 4 h. The solid obtained after eliminating the solvent under vacuum was washed with boiling chloroform and filtered to afford pure **4** in 38% yield: m.p. 300–305 °C (dec.); IR (KBr) ν_{max} 3360, 1670, 1620, 1254, 1172 and 1047 cm^{-1}, ^1H NMR (DMSO-d_6, 300 MHz) δ 11.82 (bs, 2H), 8.18 (s, 2H), 7.85 (d, 8.2 Hz, 4H), 7.72 (bs, 8H), 7.08 (d, 8.2 Hz, 4H) and 4.42 (s, 4H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 159.7, 154.7, 146.3, 128.9, 125.8, 114.3 and 66.1; EILRMS *z/m* (int. %) 381 (M^+, 17) and 324 (100). Anal. Calc. for $C_{18}H_{24}N_8O_2Cl_2$: C, 47.56; H, 5.33; N 24.67%. Found: C, 47.38; H, 5.24; N 24.46%.

*4.1.5. 1,3-Bis(4-guanylhydrazonephenoxy)propane hydrochloride **5***

Compound **5** was prepared in the same manner as **4** but using a reaction time of 3 h and the following amounts of reagents: aldehyde **2** (0.8 g, 2.95 mmol), aminoguanidine hydrochloride (0.7 g, 6.4 mmol) and 95% ethanol (30 ml) containing a few drops of fuming HCl. The crude product was washed with boiling 95% ethanol and filtered to afford pure **5** in 45% yield: m.p. 300–310 °C (dec.); IR (KBr) ν_{max} 3410, 1680, 1630, 1514, 1250 and 1170 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 11.96 (bs, 2H), 8.08 (s, 2H), 7.82 (d, 9.0 Hz, 4H), 7.55 (bs, 8H), 7.15 (d, 9.0 Hz, 4H), 4.22 (t, 6.5 Hz, 4H) and 2.20 (quintet, 6.5 Hz, 2H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 160.4, 155.3, 146.6, 129.3, 126.1, 114.7, 64.5 and 28.5; EILRMS *z/m* (int. %) 396 (M^+, 15) and 338 (100). Anal. Calc. for $C_{19}H_{26}N_8O_2Cl_2$: C, 48.70; H, 5.60; N 23.93 %. Found: C, 48.54; H, 5.53; N 23.78%.

*4.1.5. 1,4-Bis(4-guanylhydrazonephenoxy) hydrochloride **6***

Compound **6** was prepared by reaction of dialdehyde **3** with aminoguanidine hydrochloride in refluxing toluene with *p*-toluenesulfonic acid in a Dean–Stark apparatus during 1 h. The crude product was washed with boiling 95% ethanol and filtered to afford pure **6** in 45% yield: m.p. 226–234 °C (dec.); IR (KBr) ν_{max} 3300, 1680, 1610, 1515, 1255 and 1170 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 11.92 (bs, 2H), 8.08 (s, 2H), 7.85 (d, 7.6 Hz, 4H), 7.8 (bs, 8H), 7.05 (d, 7.5 Hz, 4H), 4.15 (bs, 4H) and 1.95 (bs, 4H); EILRMS *z/m* (int. %) 410 (M^+, 8) and 352 (100). Anal. Calc. for $C_{20}H_{28}N_8O_2Cl_2$: C, 49.77; H, 5.85; N 23.23 %. Found: C, 49.70; H, 5.79; N 23.15 %.

4.2. NMR drug-micelle interaction

4.2.1. Solutions preparations

The relaxation measurements for the pure bisguanylhydrazones were carried using 2.5 mM solutions of the drugs in D_2O. The measurements with the micelles were carried out using solutions with a drug concentration of 2.5 mM and a tensoactive agent concentration of 20 mM, which is above the CMC for either SDS (8.0×10^{-3} M at 25 °C) or CTAB (9.2×10^{-4} M at 25 °C) [11].

4.2.2. NMR measurements

All the NMR measurements were carried out in a Varian UNITY-300 NMR spectrometer (300 MHz) using 5 mm sample tubes. The chemical shifts were determined relative to the HOD signal (δ 4.80 ppm) [20] of the solvent (D_2O 98% D, Aldrich). The T_1 relaxation experiments were carried out using the inversion recovery pulse sequence with presaturation of the water signal with a decoupling power of 0.75 W. The width of the 90° pulse for hydrogen was calibrated before every set of measurements and was always close to 15 μs. The other pertinent acquisition parameters were: acquisition time 3.774 s, spectral width 4000 Hz and 29952 data points for every transient. All the experiments were conducted in triplicate at 36.0 ± 0.1 °C using a relaxation delay of 12 s and collecting 68 transients for each FID.

4.3. Pharmacology

4.3.1. Bioactivity tests

Bloodstream trypomastigotes forms of Y-strain *T. cruzi* [12] were obtained from Swiss albino female mice, weighing 18–20 g, at the peak of parasite concentration, 7 days after intra-peritoneal inoculation. To determine the in vitro activity, the desired amount of each compound was dissolved in 1000 μl of DMSO, and 10 μl of this solution was mixed with 390 μl of blood from the acutely infected mice. The maximum amount of DMSO used (2.5% in volume) did not have

any significant effect on trypomastigote growth. One hundred and fifty microliter of the suspension was distributed into a sterile multi-well plate, which was incubated for 24 h at 4 °C. After the incubation, 5 μl of the suspension were examined with a microscope for the presence of motile organisms: 50 microscopic fields were examined at 400 magnification according to the method of Brener [16]. The number of parasites still present and their morphology were noted and scored against a negative control (parasite suspension and parasite suspension plus 2.5% DMSO solution), and a positive control (parasite suspension and 18 μM gentian violet). The values of ID_{50}, the drug concentration (μM) necessary to kill 50% of the parasites, were obtained by linear and polynomial regression analysis [21].

5. Conclusions

Bisguanylhydrazones are much less active anti-*T. cruzi* compounds than simple guanylhydrazones. This result being is in opposition to what have been reported in the literature for cationic antibiotics used against *T. brucei*, where an increase in the number of positively charged groups increases the activity [1]. This result strongly suggests that the mechanism of action of this type of compound is different for both parasites. We believe that in the case of American trypanosomiasis the most likely mechanism is the interaction of the cationic compounds with the parasite membrane, disrupting it or changing its permeation properties, rather than interaction with the parasite DNA [8], as its have been shown to be the case for bicationic compounds in African trypanosomiasis. Longitudinal relaxation studies indicate that bisguanylhydrazones do interact with the SDS micelles used as models for the parasite membrane, with the most active compound being the one whose relaxation properties are less affected by the presence of cationic CTAB micelles.

One important conclusion of this paper is that the work on the development of bicationic compounds active against *T. cruzi* seems to be less likely to produce useful results for the chemotherapy of Chagas disease than the development of new monocationic antibiotics.

References

[1] P. Ulrich, A. Cerami, J. Med. Chem. 27 (1984) 35–40.
[2] M. Cory, R.R. Tidwell, T.A. Fairley, J. Med. Chem. 35 (1992) 431–438.
[3] P. Ulrich, R.W. Grady, A. Cerami, Drug Dev. Res. 2 (1982) 219–228.
[4] J.R. Baker, L.F. Selden, Trans. Roy. Soc. Trop. Med. Hyg. 75 (1981) 80–85
W.E. Gutteridge, Trop. Disease Bull. 73 (1976) 699.
[5] C.E. Stephens, R. Brun, M.M. Salem, K.A. Werbovetz, F. Tanious, W.D. Wilson, D.W. Boykin, Bioorg. Med. Chem. Lett. 13 (2003) 2065–2069.
[6] J.C. Messeder, L.W. Tinoco, J.D. Figueroa-Villar, E.M. Souza, R. Santa-Rita, S.L. de Castro, Bioorg. Med. Chem. Lett. 5 (1995) 3079–3084.
[7] O. Santos-Filho, M.T. Araujo, J.D. Figueroa-Villar, Bioorg. Med. Chem. Lett. 7 (1997) 1797–1802.
[8] M.N. Borges, J.D. Figueroa-Villar, Biopolymers (Biospectroscopy) 62 (2001) 9–14.
[9] T.C. Jenkins, A.N. Lane, S. Neidle, D.G. Brown, Eur. J. Biochem. 213 (1993) 1175–1184.
[10] K.J. Edwards, T.C. Jenkins, S. Neidle, Biochem. 31 (1992) 7104–7109.
[11] M. Durand, E. Seche, J.C. Maurizot, Eur. Bioph. J. Bioph. Lett. 30 (2002) 625–630.
[12] L.W. Tinoco, J.D. Figueroa-Villar, J. Braz. Chem. Soc. 10 (1999) 281–286.
[13] L.W. Tinoco, J.D. Figueroa-Villar, Spectrosc. Lett. 32 (1999) 941–951.
[14] C.F.H. Allen, J.W. Gates Jr., Org. Synt. Coll. 3 (1966) 140–143.
[15] L.H.P. Silva, V. Nussenzweig, Folia Clin. Biol. 20 (1953) 191–208.
[16] Z. Brener, Rev. Inst. Med. Trop. 4 (1962) 389–396.
[17] I.O. Donkor, S.K. Jones, R.R. Tidwell, Bioorg. Med. Chem. Lett. 3 (1993) 1137–1140.
[18] A. Kumar, D.W. Boykin, W.D. Wilson, S.K. Jones, B.C. Bender, C.C. Dykstra, J.E. Hall, R.R. Tidwell, Eur. J. Med. Chem. 31 (1996) 767–773.
[19] J.H. Fendler, Membrane Mimetic Chemistry, John Wiley and Sons, New York, 1982.
[20] B. Almanac (Ed.), Bruker Analytical Instruments 1993, Germany.
[21] S. Chaterjee, B. Price, Regression Analysis by Example, John Wiley & Sons, New York, pp. 270–274.