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Novel design of a pentacyclic scaffold as structural mimic of saframycin A

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Abstract—The design, synthesis and evaluation of a pentacyclic scaffold, **CWO-324** to mimic saframycin A is described. **CWO-324** is readily synthesized in five steps from 1,4-diacetyl-piperazine-2,5-dione and 2,5-dimethoxybenzaldehyde. **CWO-324** was found to scission DNA, binds to bases 69–83(5′-GCAGTCAGG CACCGT-3′) of Hind III/Rsa I from plasmid pBR322 DNA in a foot-printing study and possesses anti-tumor activity.
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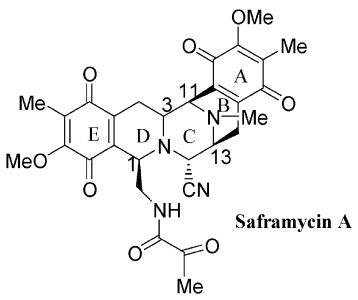
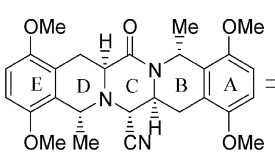
1. Introduction

Saframycins,[1] natural antiproliferative agents with a novel molecular architecture containing two units of 7-methoxy-6-methyl-1,2,3,4-isoquinoline-5,8-dione connected through the fifth ring to give the central piperazine ring, and bearing a methylene group substituted with a pyruvamide group has been an attractive target for total synthesis[2a–g] due to both its structural complexity and excellent biological activity. The promising clinical efficacy of members of this series for the treatment of solid tumors has recently led to the preparation of a large number of diverse analogs with amendable structural modifications devoted to exploring variation in the nature of the side chains.[3] The chemistry and biology for this class of tetrahydroisoquinoline antitumor antibiotics have been reviewed.[4]

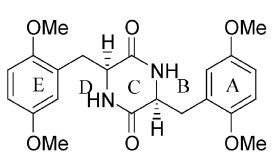
We have approached the design of saframycin from a different angle, and our strategy is to simplify the structural complexity of the natural product basic skeleton but to retain the biological activity. We identified one particular bond in saframycin A for disconnection, i.e. the C-3 and C-11 bond, since this bond connects the two isoquinoine-5,8-dione units to form the bridging ring B that gives saframycin its complex skeletal framework. The heart of our design is to reassemble the two isoquinoline units of saframycin through the fifth ring by forming the novel 5a,12a-diazapentacene ring system, **CWO-324**, a rather novel simplified scaffold. The designed molecule, **CWO-324**, is similar to saframycin A in that it also has a pentacyclic structure with a central ring containing an amino-nitrile functional group. It is important to note that the designed molecule **CWO-324** has a much greater degree of symmetry as compared to the latent symmetry present in saframycins. Thus **CWO-324** now renders itself to an aesthetically pleasing disconnection whereby compound **1** becomes a precursor. Our design is perhaps attractive in that it requires only a simple synthesis.



Saframycin A



CWO-324



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Keywords: 5a,12a-diazapentacene; saframycin model; DNA binding and scissioning.

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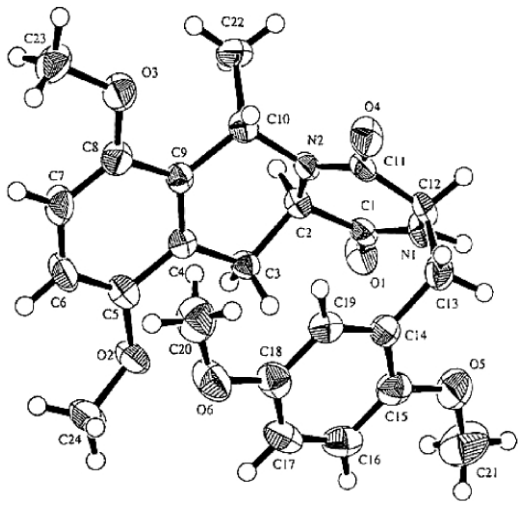


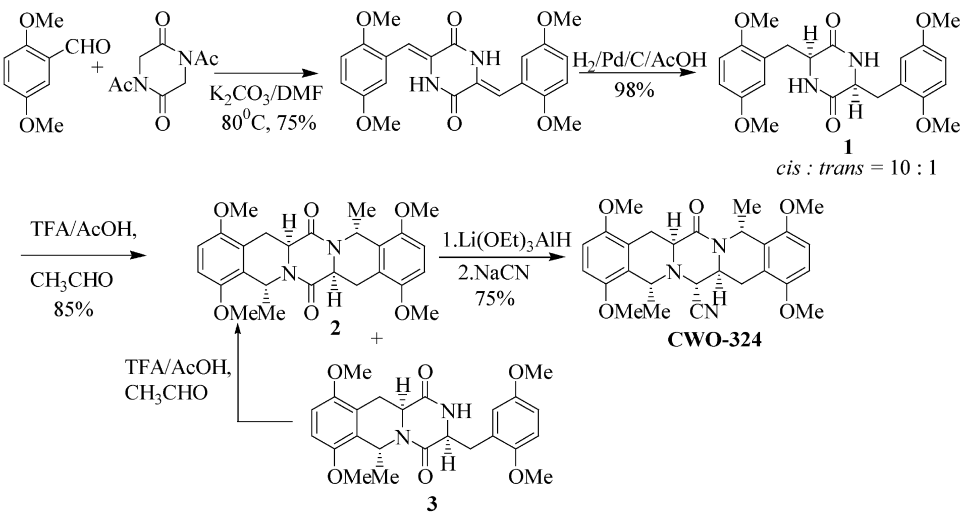
Figure 1. X-ray structure showing the relative stereochemistry of **3**.

2. Results and discussions

Here, we report the synthesis, DNA binding and scission properties, and cytotoxic activity of **CWO-324**. The 3,6-bis(2,5-dimethoxyphenyl)methylpiperazine-2-5-dione **1** was prepared as a 10:1 mixture of *cis* and *trans* isomers from the condensation of 1,4-diacetyl-piperazine-2,5-dione with 2,5-dimethoxybenzaldehyde (K_2CO_3, DMF, 75% yield), and subsequent catalytic hydrogenation (Pd/C, H_2, 98% yield). Catalytic reduction of bis-arylidenepiperazine-diones has been reported to give bis(aryl)methyl compound with the predominant product as the *cis* isomer.[5] The *cis* isomer of **1** can be obtained by rigorous washing with cyclohexane as reported by Lieberskind.[5] Myers[3b] has recently evaluated the versatility of structural modification at C-1 by variation of a wide range of aliphatic and aromatic aldehydes in the Pictet–Springler cyclization reaction. We have previously described the Pictet–Springler cyclization of mono-arylidenepiperazinediones with aldehyde that yields the reverse stereochemistry to saframycin. In this study, acetaldehyde was chosen to reduce any unfavorable impact on the binding to DNA due to the incorrect stereochemistry of the methyl group. The reaction of **1** with a large excess of acetaldehyde in the presence of trifluoroacetic acid in acetic acid provided the desired pentacyclic product **2** in 85% yield, together with some mono-cyclized product **3**. We were able to obtain a crystal of **3** suitable for an X-ray crystallographic analysis and this proved unequivocally its stereochemistry (Fig. 1). The reaction of **3** with acetaldehyde in the presence of trifluoroacetic acid in acetic acid also gave **2**. The course of the reaction has been reported to proceed through an *E*-iminium intermediate that cyclizes stereoselectively from the less hindered α-face.[2b,6] Selective mono-reduction of **2** with lithium triethoxyaluminum hydride in THF to the corresponding cyclic aminal followed by addition of sodium cyanide gave **CWO-324** in 75% yield after flash chromatography on silica gel (Scheme 1).[2]

It is particularly important that the analog **CWO-324** should retain a certain degree of similar characteristics as saframycin A for host molecular recognition in the non-covalent binding to the minor groove of DNA. The DNA binding site of **CWO-324** was determined by foot-printing experiments using a minor-groove cleaving agent ruthenium (III)–picen complexes, performed on a 135-bp ^{32}P-end-labeled DNA obtained from a restriction fragment of Hind III/Rsa I from plasmid pBR322 DNA.[7] This restriction fragment has been used to foot-print the binding site of the antibiotic saframycin.[8] The foot-printing of compound **CWO-324** does not show many binding sites except preferential binding sequence at bases 69–83(5′-GCAG**TCAGG CACCGT**-3′) (Fig. 2). This is in good agreement with the binding sequence of saframycin A reported by Lown et al.[8] at bases 73–85 (**TCAGGCACCGT**GT) with Hind III/Rsa I from plasmid pBR322 DNA. This result strongly suggests that the average orientation/location of the simplified scaffold of **CWO-324** within the base pair pocket of DNA has not been altered. Clearly, the good mimic of our simplified pentacyclic **CWO-324** to the parent saframycin scaffold is noteworthy for the future design of saframycin analogs.

Computer modeling studies with Cerius II program for the most stable conformation of the design **CWO-324** (total energy, 138.355 J) and saframycin A (total energy



Scheme 1. Synthesis of **CWO-324**.

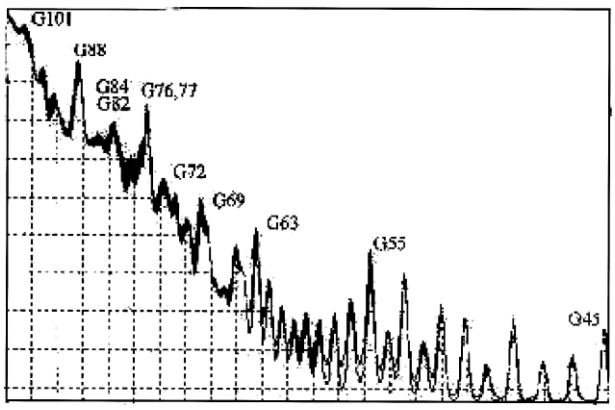


Figure 2. Densitometry of autoradiograph of a 10% denaturing polyacrylamide gel showing foot-printing of **CWO-324** in a 135-bp restriction fragment (45–101) induced by ruthenium-(III)–picen complexes and H_2O_2. The upper and lower region represents different cleavage intensity in the absence (upper) and presence (lower) of **CWO-324**.

118.106 J) showed reasonable compatibility in shape (Fig. 3). The two quinone rings of saframycin has been reported to be at 75° to each other,[9] a close resemblance to that obtained by our simulation. Overlay of **CWO-324** and saframycin A showed an optimal overlap for ring D and E, but ring A, B and C are in a slightly different orientation. The presence of a shallow-cleft within **CWO-324** suggests that it is indeed mimicking the shape of saframycin A and this further support the similarity in their binding sites.

The saframycin analog **CWO-324** was tested in the NCI human cancer cell panel,[10] and the in vitro inhibitory activities, LC_{50} (μM), are represented in Table 1. Of the sixty cell lines tested, **CWO-324** showed selective biological activities against non-small cell lung, colon, CNS, melanoma, ovarian, renal and prostrate cell lines. Unfortunately, it displayed a much lower activity relative to saframycin A (Table 1). However, the absence of the quinonoid moiety in **CWO-324** provides evidence that it is not a prerequisite for cytotoxic activity. Ecteinascidins,[11] a non-quinonoid natural product has recently been found to be more cytotoxic than saframycin A.

It has been proposed that the cytotoxicity of saframycin A is due to DNA alkylation, and it has been demonstrated, for instance, that saframycin causes PM2-CCC DNA cleavage in the presence of reducing agents.[12] Our investigation on the simplified model **CWO-324** revealed a similar ability to the scission of ϕ-174 DNA, although at a much higher concentration (Fig. 4—concentrations from 50 μM to 1 mM were found to cause increasing single strand cleavage of ϕ-174 DNA into the open-circular Form II). The mechanism of DNA cleavage by **CWO-324** may be similar to that reported for quinocarcin[13] that mediates DNA cleavage, and addition of dithiothreitol (DDT) enhanced DNA cleavage. Since the structure of quinocarcin differs from saframycins in that it does not possess a quinoid moiety, the mechanism by which it mediates DNA cleavage has not been apparent.

3. Conclusion

In summary, a strategy of saframycin A mimicry based on the 5a,12a-diazapentacene scaffold has been successfully developed. Considering the exceptional similarity in binding sites to DNA of **CWO-324**, this new scaffold can be

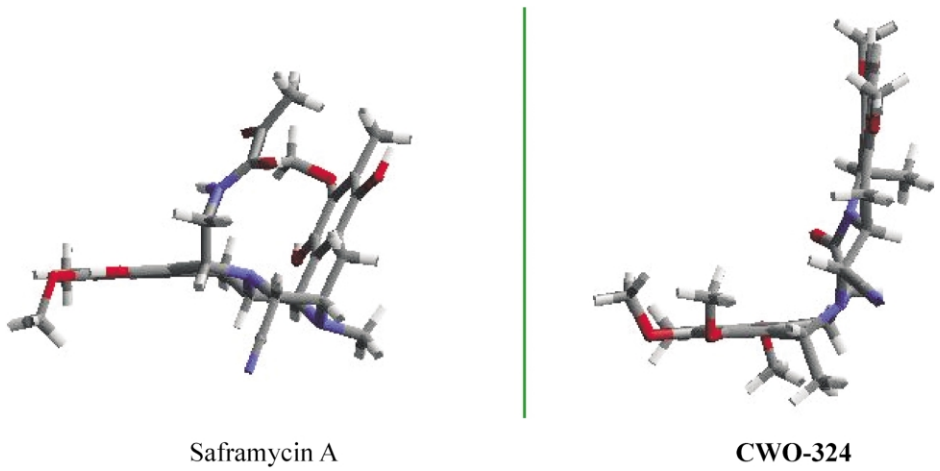


Figure 3. Computer simulation of the most stable conformation of saframycin A and **CWO-324** whereby ring A is placed perpendicular to the plane of the paper.

Table 1. Inhibition of in vitro human tumor cell growth by **CWO-324** (μM)

	NCI-H522	COLO-205	U251	SK-MEL-2	OVCAR-8	UO-31	PC-3	BT549	SR
GI_{50}	1.74	1.85	1.72	1.73	1.71	1.90	2.91	2.40	1.60
LC_{50}	5.75	6.46	5.89	5.89	7.76	6.76	29	6.31	>50
TGI	3.09	3.47	3.24	3.24	5.44	3.46	8.71	3.09	5.89

GI_{50}, LC_{50}, TGI values are from the National Cancer Institute of USA. Cell type: NCI-H522, non-small cell lung cancer line; COLO 205, colon cancer cell line; U251, CNS cancer cell line; SK-MEL-2, melanoma cell line; OVCAR-8, ovarian cancer cell line; UO-31, renal cancer cell line; PC-3, prostate cancer; BT594, breast cancer cell line; SR, leukemia cell line.

Figure 4. Agarose gel electrophoresis of ϕ174 (RF1) DNA(100 μM per base pair) treated with **CWO-324** and incubated in 50 mM Tris HCl buffer, pH 8.5 and 1 mM DTT at 37°C for 14 h. Lane 1. DNA plasmid as received. Lanes 2–7. 10, 50, 100, 250, 500 and 1000 μM of **CWO-324**, respectively.

considered a new prototype for the development of novel potential anti-tumor agents. The ease in the synthesis of this new scaffold provides a practical and scalable synthesis for related analogs in the future. Further works are in progress to improve the cytotoxicity and the preparation of enantiomerically pure compound.

4. Experimental

4.1. General procedure

4.1.1. 3,6-Bis[(2,5-dimethoxyphenyl)methylene]-2,5-piperazinedione. A mixture of 1,4-diacetylpiperazinedione (5.7 g, 50 mmol), 2,5-dimethoxybenzaldehyde (20.00 g, 0.12 mol) and potassium carbonate (14.65 g, 0.12 mol) in dry dimethylformamide (150 mL) was stirred under a nitrogen atmosphere in an oil bath at 80°C overnight. The reaction mixture was cooled and most of the solvent evaporated in vacuo. Water was added to the oily residue to give a yellow solid. The crude product was further washed with ether to give the title compound (15.36 g, 75%) as yellow solid; mp 285°C (decomposed); ν_{max} ($CHCl_3$) 1680, 1623 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 9.29 (2H, br, N*H*), 6.94–7.06 (6H, m, Ar*H*), 6.77 2 H, s, C=C*H*×2), 6.28, 3.80 (6H, s, OCH_3×2), 3.74 (6H, s, CH_3×2); *m/z* (EI, 70 eV) 410 (55, M^+), 379 (70%), 348 (65%); HRMS (ESI, MeOH): MH^+ found 411.1554. $C_{22}H_{23}N_2O_6$ requires 411.1551.

4.1.2. 3,6-Bis[(2,5-dimethoxyphenyl)methyl]-2,5-piperizenedione (1). This was prepared according to the method of Liebeskind.[5] A mixture of 3,6-bis[(2,5-dimethoxyphenyl)methylene]-2,5-piperazinedione (9.5 g, 23 mmol) and 10% palladium on charcoal (1 g) in acetic acid (150 mL) was shake under a hydrogen atmosphere (Parr hydrogenator) at 80°C for 6 h. The clear and colorless reaction mixture was filtered through Celite and concentrated. Water was added and the white solid was collected by filtration and dried in vacuo (9.2 g, 98%). The white solid obtained was triturated with hot cyclohexane several times to give **1** (8.4 g, 89%) as a white solid; mp 179–181°C; ν_{max} ($CHCl_3$) 1670 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 7.90 (2H, br, N*H*), 6.70–6.85 (6H, m, Ar*H*), 4.23 (2H, br dd, *J*=3.9, 9.0 Hz), 3.80 (6H, s, OCH_3), 3.75 (6H, s, OCH_3), 3.85 (2H, dd, *J*=3.9, 13.5 Hz, CH_2), 2.51 (2H, dd, *J*=9.0, 13.5 Hz, CH_2); *m/z* (EI, 70 eV) 414 (65, M^+), 383 (75%); HRMS (EI): M^+, found 414.1793. $C_{22}H_{26}N_2O_6$ requires 414.1791.

4.1.3. 1,4,8,11-Tetramethoxy-5,12-dimethyl-5,6a,7,12,13a,14-octadydro-6,13-dioxo-5a,12a-diazapentacene (2) and 3-(3,5-dimethoxybenzyl)-7,10-dimethoxy-6-methyl-2,3,11,11a-tetrahydro-5*H*-pyrazino(1,2-*b*)-isoquinoline-1,4-diones (3). To a solution of compound **1** (6.02 g, 14.50 mmol) in acetic acid/trifluoroacetic acid mixture (50 mL in a 1:4 mixture) was added acetaldehyde (8.41 mL, 145.00 mmol) and the mixture stirred at room temperature for 1 h followed by heating to reflux for 5 h. The reaction was cooled, diluted with cold water and extracted with chloroform. The organic layer was washed with sodium bicarbonate and brine, dried ($MgSO_4$) and the solvent evaporated in vacuo. Purification of the crude product by column chromatography on silica gel (ethyl acetate/hexane 1:1) gave **2** (5.82 g, 85%) as a white solid; mp 231–232°C. [Found: C, 66.86; H, 6.52; N, 6.03. $C_{26}H_{30}N_2O_6$ requires C, 66.94; H, 6.48; N, 6.00%]; ν_{max} ($CHCl_3$) 1650 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 6.68 (4H, d, *J*=5.8 Hz, Ar*H*), 6.00 (2H, q, *J*=6.6 Hz, C*H*×2), 4.38 (2H, dd, *J*=4.5, 12.6 Hz, C*H*), 3.78–3.81 (12H, close s, OCH_3×4), 3.11 (2H, dd, *J*=4.5, 12.6 Hz, CH_2), 2.65 (2H, dd, *J*=12.6, 17.4 Hz, CH_2), 1.44 (6H, d, *J*=6.6 Hz, CH_3×2); δ_C (75 MHz, $CDCl_3$) 163.7, 150.8 149.7 126.5, 121.9, 108.0, 107.9 55.6, 55.5, 50.2, 44.6, 29.6, 18.7; *m/z* (EI, 70 eV) 466 (100, M^+), 451 (90), 423 (70%). **3a** (minor, 320 mg, 5%); mp 201–202°C. [Found: C, 65.18; H, 6.42; N, 6.32. $C_{24}H_{28}N_2O_6$ requires C, 65.44, H, 6.41; N, 6.36%]; ν_{max} ($CHCl_3$) 1678, 1652 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 6.66–6.80 (4H, m, Ar*H*), 6.62 (1H, d, *J*=1.2 Hz, Ar*H*), 6.10 (1H, br s, N*H*), 5.94 (1H, q, *J*=6.6 Hz, C*H*), 4.38 (1H, dd, *J*=4.8, 5.6 Hz, CH), 4.23 (1H, dd, *J*=4.8, 12.4 Hz, C*H*), 3.82 (3H, s, OCH_3), 380 (3H, s, OCH_3), 3.74 (3H, s, OCH_3), 3.37 (1H, dd, *J*=4.8, 13.8 Hz, C*H*), 3.28 (3H, s, OCH_3), 3.09 (1H, dd, *J*=4.8, 17.6 Hz, C*H*), 2.99 (1H, dd, *J*=5.6, 13.8 Hz, C*H*), 1.48 (1H, dd, *J*=12.4, 17.6 Hz, C*H*), 1.41 (3H, d, *J*=6.6 Hz, CH_3); *m/z* (EI, 70 eV) 440 (100, M^+), 425 (60), 397 (20%). *Crystallographic data*. M_r: 439.49 g/mol; crystal system: monoclinic; space group: $P2_1/n$; *a*: 12.576(2) Å; *b*: 10.899(2) Å; *c*: 15.596(2) Å; α=90.00°, β=90.66(1)°, γ=90.00°, *V*: 2137.6(5) $Å^3$; *Z*: 4; *D*: 1.278 g/cm^3; crystal size: 0.40×0.40×0.60 mm^3; *R*=0.046, R_w=0.039, GOF=8.80 for 3003 reflections with $I>3.00\sigma(1)$; radiation type: Cu K_α wavelength: 1.54178 Å; diffractometer: Rigaku AFC6S; CCDC-number: 213200.

4.1.4. 1,4,8,11-Tetramethoxy-5,12-dimethyl-5,6a,7,12,13a,14-octadydro-13-dioxo-5a,12a-diaza-pentac ene-6-carbonitrile (CWO-324). Compound **2** (1 mmol)

in THF was added dropwise to a solution of lithium triethoxyaluminum hydride (0.6 mmol) in THF and the mixture was allowed to react for 3 h at −10°C under nitrogen. To this reaction mixture was then added a concentrated solution of sodium cyanide (1.5 mmol) and the mixture was stirred for 5 h at room temperature. Purification of the crude product by flash column chromatography on silica gel (ether/hexane as eluent, 3:1) gave the title compound **CWO-324** in 75% yield as a light yellowish brown solid; mp 164–165°C. [Found: C, 68.09; H, 6.30; N, 8.93. $C_{27}H_{31}N_3O_5$ requires C, 67.91; H, 6.54, N 8.80%]; ν_{max} ($CHCl_3$) 2198, 1624 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 6.72 (2H, *J*=6.6 Hz, Ar*H*), 6.62 (2H, d, *J*=6.6 Hz, Ar*H*), 5.90 (1H, q, *J*=5.8 Hz, C*H*), 5.50 (1H, d, *J*=3.6 Hz, CH), 4.35 (1H, q, *J*=5.2 Hz, C*H*), 4.23–4.16 (1H, m, C*H*), 3.80 (6H, s, OCH_3), 3.71 (6H, s, OCH_3), 3.70 (1H, dd, *J*=5.8, 15 Hz, C*H*), 3.43–3.36 (2H, m, CH_2), 2.82 (2H, m, CH_2), 1.42 (3H, d, *J*=5.8 Hz, CH_3), 1.38 (3H, d, *J*=5.2 Hz, CH_3); δ_C (75 MHz, $CDCl_3$) 18.3, 20.0, 21.6, 22.9, 44.1, 51.4, 53.7, 55.4, 55.5, 55.6, 55.8, 107.0, 107.5, 108.1, 108.9, 110.8, 116.3, 122.8, 123.6, 128.3, 128.9, 149.2, 149.5 (close doublet), 151.2, 164.5; *m/z* (EI, 70 eV) 450 (100, M^+-CN), 435 (80).

4.2. Scissioning and footprinting experiments

λ-Phage ϕX-174 supercoiled plasmid DNA was purchased from Life Technologies (Gibco BRL). No purification was needed prior to use for scisioning assay.

A 135-bp DNA was isolated from the plasmid pBR 322 using restrictive endonuclease *Hind III*/*Rsa I* (New England Biolabs) and purified for footprinting experiment by a sequential procedure described.[7]

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