NMR Studies of metal complexes and DNA binding of the quinone-containing antibiotic streptonigrin

Xiangdong Wei and Li-June Ming *

Department of Chemistry and Institute for Biomolecular Science, University of South Florida, Tampa, Florida 33620-5250, USA

Optical and $^1$H NMR techniques have been applied to the study of a few metal complexes (Co$^{2+}$, Fe$^{2+}$, and Yb$^{3+}$) of the antitumor antibiotic streptonigrin (SN) produced by Streptomyces flocculus to elucidate the structure of the complexes. The hyperfine-shifted $^1$H NMR signals of these paramagnetic complexes were fully assigned by means of relaxation and two-dimensional NMR techniques. These studies revealed that SN binds transition metal and lanthanide ions and forms stable metal–drug complexes, with the metal located at the quinolinequinone–picolinate site. This configuration requires a $\pm 180^\circ$ twist of the C2–C2' bond in the crystal structure of the drug. The hyperfine-shifted $^1$H NMR signals of the Co$^{2+}$–SN complex are significantly changed upon addition of calf thymus DNA or poly[dA–dT], indicating direct binding of Co$^{2+}$–SN complex with DNA.

Streptonigrin (SN, also known as rufomycin and bruneomycin) is a metal-dependent quinone-containing antibiotic produced by Streptomyces flocculus (Fig. 1). This antibiotic has been shown to exhibit active inhibition toward several tumors and cancers (e.g. lymphoma, melanoma, and breast and cervix cancers) as well as viruses in some early in vitro and clinical observations. However, the high toxicity and serious side effects of this drug reduce its clinical value, and its use is even limited to in vivo studies. Streptonigrin is a quinone moiety, it may share some common mechanistic characteristics with other quinone-containing antibiotics such as the anthracyclines in inhibition of cancer growth. Two mechanisms for this action have been proposed: (1) by way of interference with cell respiration and (2) through disrupting cell replication and transcription. A key step in this action is reflected by the induction of severe irreversible damage to DNA and RNA in vitro and in vivo in the presence of reducing agents.

Streptonigrin is able to bind several different metal ions, and requires metal binding for full antibiotic and antitumor activity. The transition metal ions Cu$^{2+}$ and Fe$^{2+}$ have been known to accelerate SN-mediated DNA scission in the presence of NADH (reduced nicotinamide adenine dinucleotide), thus enhancing the antitumor activity of this antibiotic. This antibiotic also exhibits a strong EPR signal upon reduction in the presence of a bound metal ion, indicating the formation of a metal–semiquinone form of this drug. These results indicate that metal ions are directly involved in the action of SN. Metal–SN complexes can be reduced to their semiquinone forms by NADH to induce cleavage of DNA. This reduction process is accelerated by oxidizing agents.

Other optical techniques have been applied to the study of a few metal complexes (Co$^{2+}$, Fe$^{2+}$, and Yb$^{3+}$) of the antitumor antibiotic streptonigrin (SN) produced by Streptomyces flocculus to elucidate the structure of the complexes. The hyperfine-shifted $^1$H NMR signals of these paramagnetic complexes were fully assigned by means of relaxation and two-dimensional NMR techniques. These studies revealed that SN binds transition metal and lanthanide ions and forms stable metal–drug complexes, with the metal located at the quinolinequinone–picolinate site. This configuration requires a $\pm 180^\circ$ twist of the C2–C2' bond in the crystal structure of the drug. The hyperfine-shifted $^1$H NMR signals of the Co$^{2+}$–SN complex are significantly changed upon addition of calf thymus DNA or poly[dA–dT], indicating direct binding of Co$^{2+}$–SN complex with DNA.

Fig. 1 (A) The molecular structure of streptonigrin based on the crystallographic study. (B) The molecular structure of a metal complex of streptonigrin based on the NMR studies discussed in this report. The metal is put in the quinolinequinone–picolinate site according to the results from the NMR studies. This structure requires a $\pm 180^\circ$ rotation of the bipyridine C2–C2' bond in the crystal structure of the drug (A). The numbering of SN follows the nomenclature: 3-amino-2-(7-amino-6-methoxy-5-hydroxy-3,4-dimethoxyphenyl)-5-methylpyridine bound through the quinolinequinone–amine functionalities based on the crystal structure; and via the quinolinequinone–picolinate functionalities which requires a significant twist of the crystal structure.

We report here a study of the binding of SN with paramagnetic metal ions, including the transition metal ions Co$^{2+}$ and Fe$^{2+}$, and the lanthanide Yb$^{3+}$. Since the chemical shift and the relaxation times of paramagnetic molecules are very sensitive to structural changes, they can be utilized as very sensitive ‘probes’ for the studies of molecular structures and interactions. The paramagnetically shifted $^1$H NMR signals of the metal–SN complexes have been fully assigned and their relaxation times measured, which afford an accurate determination of their structures in solution. The interaction of the
shifted the signals due to the drug are paramagnetically shifted to the complex in methanol is shown in Fig. 3 (spectrum B), in which the absorption at 420 nm with an affinity constant of 5.43 × 10^6 (CH3CN), respectively.

Co^2+–SN complex with DNA has also been monitored by the use of optical and NMR spectroscopies. A direct interaction was observed, where a significant change of the hyperfine-shifted 1H NMR signals of the complex was detected in the presence of DNA. These paramagnetic metal–SN complexes can serve as prototypical model systems for future investigation of other paramagnetic metal–drug complexes and their binding with DNA.

Results and Discussion

Titration of streptonigrin with metal ions

A freshly prepared methanol solution of SN gives a deep brown solution with λmax = 392 nm. An optical titration shows Co^2+ ion can bind SN tightly in methanol to form a very stable 1:1 Co^2+–SN complex (λmax = 404 nm, Fig. 2). A fitting of the change of the absorption at 404 nm of SN with respect to the amount of Co^2+ gives an affinity constant of 3.30 × 10^6 M⁻¹ for the simple equilibrium Co^2+ + SN ⇌ Co^2+–SN (inset, Fig. 2). Similarly, the addition of Fe^2+ to SN in methanol under argon shifts the electronic absorption of the drug to 400 nm upon the formation of a 1:1 Fe^2+–SN complex with an affinity constant of 5.43 × 10^6 M⁻¹. Upon the addition of Yb^3+ to SN in CH3CN the λmax shifts to 410 nm (greenish yellow) with an affinity constant 1.58 × 10^6 M⁻¹ for the formation of a 1:1 Yb^3+–SN complex. The change of the electronic transition in SN upon the binding of these three metal ions (cf. Fig. 2) is similar to that observed previously for Cu^+ and Zn^2+ binding to the drug.⁶

¹H NMR of ytterbium(III)–streptonigrin complex

The ¹H NMR spectrum of a freshly prepared 1:1 Yb^3+–SN complex in methanol is shown in Fig. 3 (spectrum B), in which the signals due to the drug are paramagnetically shifted to the region of 6 5 to -10. Since the Yb^3+-bound SN is undergoing chemical exchange with the free drug, signal assignment of the Yb^3+–SN complex can be achieved by the use of saturation transfer two-dimensional EXchange SpectroscopY (EXSY) on a sample with both the free drug and the complex present (Fig. 4).¹⁴ The paramagnetically shifted signals in an EXSY spectrum can thus show cross-peaks with their diamagnetic counterparts of the free drug, which can easily be assigned on the basis of chemical shift and COSY (Fig. 3A). For example, the signal at δ = 8.9 (which integrates to 3 protons with T1 = 114.5 ms) is assigned to 5-CH3 on the picolinate ring (Fig. 1), and the signals at δ = -5.5 (73.7) and -1.8 (167.9 ms) are assigned to quinolonequinone 3'-H and 4'-H protons, respectively (Fig. 4).

Since the relaxation time T1 of a proton in paramagnetic molecules is proportional to the sixth power of the proton–metal distance (i.e. T1 ∝ r^6), it is therefore extremely sensitive to structural changes. Thus, it can be taken as a ‘ruler’ for the measurement of the proton–metal distances in paramagnetic molecules. The three most upfield shifted signals in the spectrum of the complex with the shortest relaxation times are attributable to the protons closest to the paramagnetic Yb^3+ center. The large paramagnetic shift and short T1 value of the 5-CH3 protons suggest that they are close to the bound Yb^3+. This T1 value is shorter than that of the 6'-H protons (Table 1), indicating that the 5-CH3 protons are closer to the metal. This is
consistent with binding of the Yb\textsuperscript{3+} at the quinolinequinone–picolinate site as shown in Fig. 1B. The \( T_1 \) values of other signals are also consistent with this binding mode for this complex (Table 1).

Since Yb\textsuperscript{3+}, like alkaline earth metal ions,\textsuperscript{9} prefers an oxygen-rich ligand binding environment with little covalency, the ethylenediamine diacetate-like binding mode shown in Fig. 1B is presumably the preferred binding mode for the biologically relevant Ca\textsuperscript{2+} and Mg\textsuperscript{2+} ions as well. As transition metal ions have been proposed to be involved in the binding of SN to DNA and cleavage of DNA by the drug, the study of metal–SN complexes is important to provide further mechanistic information about SN action.\textsuperscript{6,8} However, because the ligand binding preferences between transition metal ions and the lanthanides (and the alkaline earth metals) are very different, whether or not this Yb\textsuperscript{3+} binding mode is applicable to transition metal–SN complexes cannot be answered at this stage.

\textbf{\( ^1\)H NMR of iron(II)–streptonigrin complex}

The redox-active Fe\textsuperscript{2+} ion has been shown to enhance the activity of SN.\textsuperscript{5} Hence, it is important to reveal the exact binding mode of Fe\textsuperscript{2+} with this antibiotic and solve the structure of the Fe\textsuperscript{2+}–SN complex in order to gain further insight into the mechanism of SN action and the role of metal ion in the action. Because Fe\textsuperscript{2+} can afford relatively sharp hyperfine-shifted \( ^1\)H NMR signals,\textsuperscript{11} the Fe\textsuperscript{2+} complex of SN can be thoroughly analysed by means of NMR techniques. The \( ^1\)H NMR spectrum of a 1:1 Fe\textsuperscript{2+}–SN complex shows several well defined hyperfine-shifted signals (Fig. 3C). The ‘clean’ spectrum indicates that there is only one Fe\textsuperscript{2+}–SN complex formed under the experimental conditions. The binding mode of Fe\textsuperscript{2+} can be determined when signal assignment is achieved, as discussed below.

All the \( ^1\)H NMR signals of the Fe\textsuperscript{2+}–SN complex can be assigned by means of two-dimensional NMR techniques (COSY and EXSY, Fig. 5) and \( T_1 \) measurement (Table 1). The most downfield-shifted signal at \( \delta \) 65.6 (7.5 ms) can be assigned to the 3'-H proton, which is four bonds away from the bound Fe\textsuperscript{2+} and is the closest to the metal. Therefore, it should gain the largest through-bond contact shift and shortest relaxation time compared to all other protons. The rest of the hyperfine-shifted signals can be assigned based on their correlations with their diamagnetic counterparts of the free drug in the EXSY spectrum. The only COSY cross-peaks of the complex (inset, Fig. 5) are associated with the phenyl ring protons 5'- and 6'-H at \( \delta \) 5.78 and 5.22, respectively. A complete signal assignment is shown in Table 1. The 15.7 ms \( T_1 \) value of the 5-CH\textsubscript{3} signal at \( \delta \) 1.44 is shorter than that of the 6'-H signal at \( \delta \) 5.22 (35.6 ms). This indicates that Fe\textsuperscript{2+} is bound to SN at the quinolinequinone–picolinate site (Fig. 1B, Model I in Table 1), similar to that in Yb\textsuperscript{3+}–SN. This binding mode requires a \( \pm 180^\circ \) rotation of the bipyridine C2–C2' bond in the crystal structure (Fig. 1A).

Another configuration with the metal bound through the 3-NH\textsubscript{2} nitrogen of SN has been proposed in previous studies\textsuperscript{40} based on the crystal structure of the free drug\textsuperscript{2} (Model II, Table 1). This alternative would afford a Fe–H (5-CH\textsubscript{3}) distance much longer than the Fe–H\textsubscript{6} distance, thus a longer \( T_1 \) value for the 5-CH\textsubscript{3} protons than the 6'-H proton. This configuration can be discarded based solely on the \( T_1 \) values reported in our study (Table 1). The Fe\textsuperscript{2+}–SN complex is presumably the iron-bound form of the drug under the reduction conditions in the cells. The unambiguous assignment of the \( ^1\)H NMR signals and the determination of the structure of this complex described here provide an important step for further study of the interaction of this complex with biomolecules and cell components.

The NMR results also indicate that the formation of a Fe\textsuperscript{2+}–SN (semiquinone) complex \textit{via} electron transfer from Fe\textsuperscript{2+} to SN is not likely to occur. This is because: (1) there is no indication of a high unpaired electron density on the quinone ring (as a result of the free radical on a semiquinone moiety),
which would afford a large contact shift and an even shorter relaxation time on the 6'-OCH$_3$ protons; and (2) there is no sign of a larger magnetic moment and longer electronic relaxation time due to the $S = \frac{3}{2}$ Fe$^{3+}$ (relative to the $S = 2$ Fe$^{2+}$) in a Fe$^{2+}$–SN (semiquinone) complex, which would afford faster relaxing and broader hyperfine-shifted $^1$H NMR signals.

$^1$H NMR of cobalt(II)–streptogin complex

A 1:1 Co$^{2+}$–SN complex is formed upon the addition of 1 equivalent Co$^{2+}$ to SN in methanol as shown by its electronic and $^1$H NMR spectra (Figs. 2 and 3E). The hyperfine-shifted $^1$H NMR signal at $\delta$ 87 ($T_1 = 13.8$ ms) can be assigned to the 3'-H proton which is four bonds away from the metal and is the proton closest to the metal. The signal at $\delta 29.6$ (40.8 ms) can be assigned to 4'-H five bonds away from the metal that gains significant contact shift via the aromatic pyridine ring. The assignment of most hyperfine-shifted signals can be achieved by the use of the EXSY technique to reveal saturation transfer between the complex and free drug (Fig. 6, Table 1). For example, the 5-CH$_3$, 5'-H, and 6'-H are found at $\delta$ 16.2 (38.8), 10.24 (291.4), and 13.41 (87.0 ms), respectively. The shortest $T_1$ value of the 5-CH$_3$ protons among all protons reflects that these protons have the shortest distance to the Fe$^{2+}$. The signal assignment and the $T_1$ values of the hyperfine-shifted signals of Co$^{2+}$–SN (Table 1) are consistent with the structure shown in Fig. 1B, with the Co$^{2+}$ bound to the quinolinequinone-picolinate function groups (Model I, Table 1) rather than to the quinolinequinone and the 3-NH$_2$ groups (Model II, Table 1). This binding mode is similar to that found in the Fe$^{2+}$–SN complex. Again, this indicates that the bipyridine C2–C2' bond of the free drug in the crystal structure has to rotate by $\pm 180^\circ$ upon metal binding.

The electronic ($\lambda_{\text{max}} = 370$ nm) and NMR (Fig. 3D) spectra of the Co$^{2+}$–SN complex observed in borate buffer solution at pH 8 are similar to those acquired in methanol solution. The acquisition of the NMR spectrum of a metal–SN complex in aqueous solution is important for further study of its interaction with DNA (see below). This complex shows broader isotropically shifted $^1$H NMR signals in water than in methanol, possibly attributable to a coagulation of this hydrophobic drug in aqueous solution. The broadness of $^1$H NMR signals in aqueous solution has also been observed for metal–anthracene complexes which also contain an extended hydrophobic ring system. The virtually identical spectral features of the Co$^{2+}$–SN complex in water and methanol, however, indicate the formation of the same complex in these two solutions. This suggests that the structural information acquired in methanol can assist the assignment of the structure and better understanding of the action of metal–SN complexes in aqueous solutions under physiological conditions.

Interaction of Co$^{2+}$–SN complex with poly[dA-dT]

The air-sensitive Fe$^{3+}$–SN complex is difficult to handle when sample transfer is necessary during experiments. The binding mode of Co$^{2+}$ with this drug is similar to that of Fe$^{2+}$, suggesting that the more air-resistant Co$^{2+}$–SN complex can serve as a substitute for Fe$^{2+}$–SN, and as a good model system to provide molecular information and DNA-binding property of metal–SN complexes. Moreover, the sensitivity of hyperfine-shifted signals toward subtle structural changes also suggests that the paramagnetic Co$^{2+}$–SN complex can serve as a good probe for monitoring the binding of metal–SN complexes with DNA.

Previous studies showed that SN exhibited a preferred cleavage site at cytosine bases adjacent to purine bases in DNA. Moreover, addition of poly[dA-dT] to the complex Cu$^{2+}$–SN was previously observed to cause small perturbation of the drug signals (0.22 to 0.31 ppm), which was suggested to be due to the binding of this complex to poly[dA-dT]. Upon addition of 10 units of poly[dA-dT] to Co$^{2+}$–SN in borate buffer $D_2$O solution at pH 8.0 three new $^1$H NMR signals appear, one sharp peak at $\delta$ 16.8 and two broad peaks at $\delta = 15$ (overlapped) and 127 (Fig. 7D), with concomitant disappearance of the downfield hyperfine-shifted signals of the Co$^{2+}$–SN complex (Fig. 7A). This significant change of the paramagnetically shifted signals suggests that Co$^{2+}$–SN complex is bound to poly[dA-dT], forming a ternary Co$^{2+}$–SN–poly[dA-dT] complex.
binding of metal ion to DNA). This observation clearly indicates that Co$^{2+}$ remains bound to SN upon binding of the Co$^{2+}$–SN complex to large pieces of native calf thymus DNA. Since irradiation of these signals does not reveal any noticeable exchange with the two hyperfine-shifted 3'- and 4'-H signals of the Co$^{2+}$–SN complex under the experimental conditions. This indicates that this complex binds calf thymus DNA to form a kinetically inert Co$^{2+}$–SN–DNA ternary complex. The results presented here clearly reveal the binding of the complex Co$^{2+}$–SN with poly[dA-dT]$_2$ and native calf thymus DNA.

Conclusion

Optical titration and one- and two-dimensional NMR techniques have been applied to the study of the few metal complexes (Co$^{2+}$, Fe$^{2+}$, and Yb$^{3+}$) of the antitumor antibiotic streptonigrin. These studies reveal that SN binds transition metal and lanthanide ions with the metal located in the quinolinequinone–picolineate site, which affords a configuration that requires a ≈180° rotation of the bipyridine C2–C2' bond in the crystal structure. The Co$^{2+}$–SN complex shows different isotropically shifted $^1$H NMR signals upon addition of calf thymus DNA and poly[dA-dT], indicating direct binding of the complex with DNA. These studies provide the foundation for future investigation of the interactions between metal–SN complexes and different oligonucleotide sequences to reveal detailed information about the mechanism of SN action and the structures of metal–SN–DNA ternary complexes. This report also demonstrates that NMR can be a versatile tool for the study of paramagnetic metal–DNA complexes.

Experimental

Chemicals and sample preparations

Streptonigrin was purchased from Sigma Co., and was also supplied as a gift by Rhône-Poulenc Rorer, Recherche- Développement Laboratories (Pairs) and by the National Cancer Institute (Drug Synthesis & Chemistry Branch, Division of Cancer Treatment). The drug is soluble in some organic solvents such as 1,4-dioxane, pyridine, dimethylformamide, dimethyl sulfoxide, and slightly soluble in alcohol and CHCl$_3$. It is barely soluble in aqueous solution at pH < 7, but is slightly soluble at higher pHs to low mM levels. However, it is unstable and photosensitive at pH > 8.5. This low solubility in water causes difficulty for NMR studies. To overcome this problem, SN was first dissolved in aqueous solution at high pH and then adjusted to the desired pH value. The drug solution was prepared just before the experiments, and the concentration of the drug was determined by using $\varepsilon_{260}$ = 14 200 M$^{-1}$ cm$^{-1}$ at pH 7.2. The metal complexes of SN were prepared by direct addition of stoichiometric amount of metal ions to the SN solutions.

All metal salts were obtained as the highest grade. Metal ion concentrations were determined by edta titration with xylene orange as indicator. All the organic solvents used in the experiments were HPLC grade. The DNA solutions were prepared by dissolving, respectively, 1 mg soluble calf thymus DNA (Sigma Chemical Co.) and 10 units polydeoxyadenylic acid–thymidylic acid (poly[dA-dT], Sigma) in borate buffer D$_2$O solution at pH 8.0 and stored at 4 °C. One unit of poly[dA-dT] yields $A_{260}$ = 1.0 in 1.0 mL water (at 1 cm path length). The Co$^{2+}$ and Fe$^{2+}$ samples were prepared under anaerobic conditions, and transferred to an optical cell or an NMR tube under argon using a gas-tight syringe.

The electronic spectra were acquired on a Hewlett Packard 8452A diode array spectrophotometer using a quartz cell of 1 cm path length. Metal titrations were performed by continuous addition of metal ions to SN solutions (e.g. 0.033 mM in the case of Co$^{2+}$ titration shown in Fig. 2). The spectra were recorded and calibrated against dilution factors. The affinity constant can be obtained by fitting the change in the absorptions (i.e. $\Delta A = A_{M\cdot SN} - A_{SN}$) with respect to the metal concentration according to the equilibrium $M + SN \rightleftharpoons M\cdot SN$.

Nuclear magnetic resonance experiments

The metal complex concentrations in organic solvents for NMR studies were about 4 mM, whereas those in aqueous solutions were about 2 mM. All $^1$H NMR spectra were acquired on a Bruker AMX360 spectrometer at 360.13 MHz. The $^1$H chemical shift was referenced to external tetramethylsilane to avoid the effect on the chemical shift of an internal reference by the paramagnetism of the metal complexes. A 90° pulse with pre-saturation for solvent suppression was used for the acquisition of one-dimensional $^1$H NMR spectra (8K data points). A line-broadening factor of 10–30 Hz was introduced to the spectra via exponential multiplication prior to Fourier transformation to enhance the signal-to-noise ratio.

In the presence of chemical exchange (such as an equilibrium $M + L \rightleftharpoons M\cdot L$), saturation transfer can occur between counterparts, such as between the paramagnetically shifted signals in M–L and their diamagnetic counterparts in L in NMR experiments. This can be conveniently studied by the saturation transfer techniques used for detection of the nuclear Overhauser effect (NOE), such as one-dimensional difference spectroscopy with the decoupler set on and off the signal of interest and the two-dimensional EXSY pulse sequence ($D_{90°} - T_{1}$–90°–T$_{90°}$–free induction decay). Owing to the fast nuclear relaxation rates and the fast molecular rotational correlation time, NOE cannot be detected in small paramagnetic complexes. The cross-peaks observed in the EXSY spectra of the M–SN complexes are thus due to chemical exchange of the drug between its free and complexed forms. The EXSY spectra were acquired with presaturation for solvent suppression and 1024 × 512 data points. A 45–60° shifted sine-squared–bell window function was applied in both dimensions prior to Fourier transformation in phase sensitive EXSY spectra. Magnitude-COSY spectra of the complexes were acquired for the elucidation of through-bond proton couplings as shown in Fig. 5. The spectra were acquired with 1024 × 256 data points, and then a 0°-shifted sine-squared–bell window function was applied to both dimensions and processed in magnitude mode.

Proton spin–lattice relaxation times ($T_1$) for all the metal complexes were determined by the use of the inversion-recovery method ($D_{180°}$–$T_{90°}$–free induction decay) with 16 different $\tau$ values and a recycle time of ≈5$T_1$. The peak intensities were fitted against the $\tau$ values by a three-parameter fitting program on the spectrometer to give the $T_1$ values. Since nuclear relaxation in paramagnetic molecules is dependent upon the metal–nucleus distance, relative distances can be obtained with respect to a reference nucleus [i.e. $T_{M\cdot H} = (T_{MM}L_{\text{metal}})^{1/6} \cdot T_{L\text{metal}}$]. The proton $4\cdot H$ ($T_{M\cdot H} = 5.97 \pm 0.7$) was chosen as the reference proton. Since dipolar relaxation in paramagnetic metal–pyridine complexes has been demonstrated to be the predominant contribution to nuclear relaxation, the contact contribution to the nuclear relaxation was not taken into consideration in this study. In most cases, paramagnetic relaxation is the predominant contribution to nuclear relaxation. To demonstrate this, a 0.5 ppm diatmic contribution was considered in the calculation of the distance $r_{M\cdot H}$. There is no significant difference in the calculated $r_{M\cdot H}$ with or without considering the diamagnetic contribution.

Acknowledgements

This work has been partially supported by a University of South Florida (USF) Research and Creative Scholarship Award, and by the Florida Division American Cancer Society.
Edward L. Cole Research Grant (F94USF-3) on antitumor antibiotics X. W. acknowledges a summer research fellowship (1995) awarded by the Institute for Biomolecular Science at USF. The gift of streptonigrin by Rhône Poulenc Laboratories and by the National Cancer Institute (Drug Synthesis and Chemistry Branch, Developmental Therapeutics Program, Division of Cancer Treatment) is gratefully acknowledged.

References


Received 5th March 1998; Paper 8/01841C