Pergamon

Tetrahedron: *Asymmetry* 11 (2000) 3535–3541

TETRAHEDRON: *ASYMMETRY*

Efficient enantiorecognition of ruthenium(II) complexes by silica-bound teicoplanin

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Received 28 June 2000; accepted 25 July 2000

Abstract

A series of chiral tris-diimine ruthenium(II) complexes have been resolved by HPLC on a chiral stationary phase. The stationary phase (**CSP1**) was prepared by covalent attachment of the glycopeptide antibiotic teicoplanin to isocyanate activated silica gel. **CSP1** selectively retains the enantiomers of $[Ru(L)_3]^{2+}$ (L = 2,2′-bypyridine (bpy), 1,10-phenanthroline and 4,7-diphenyl-1,10-phenanthroline), with a preference for the Δ isomer. For the mixed-ligand complexes $[Ru(bpy)_2pztr]^+$ and $[Ru(bpy)_2pytr]^+$ (Hpztr = 3-(pyrazin-2-yl)-1,2,4-triazole, Hpytr = 3-(pyridin-2-yl)-1,2,4-triazole), where the triazole unit is bound to the metal centre either through the N^2 or the N^4 nitrogen of the ring, **CSP1** discriminates both the enantiomers and the regioisomers. Diastereo- and enantioselective association was also observed between **CSP1** and the stereoisomers of the dinuclear complex $((Ru(bpy)_2)_2bpt]^{3+}$ (Hbpt = 3,5-bis(pyridin-2-yl)-1,2,4-triazole), with differences in binding affinities of 1.4 kJ/mol between the homochiral enantiomers.

1. Introduction

Transition metal complexes with polycyclic heteroaromatics have been extensively studied for their unique photochemical, photophysical and molecular recognition properties.[1–4]

Enantioselective interactions are often observed between these chiral transition metal complexes and organized biological media, such as nucleic acids or oligonucleotides. The well defined, stereostable three-dimensional structure and the emissive properties of chiral tris-chelated metal complexes make them powerful tools in the elucidation of structural requirements, energetics and dynamics of DNA recognition.

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0957-4166/00/$ - see front matter
PII: S0957-4166(00)00292-5

It has been reported that the Δ and Λ enantiomers of $[Ru(phen)_3]^{2+}$ (phen = 1,10-phenanthroline) and of related mixed ligand ruthenium complexes bind with different affinities and/or geometries to DNA.[5–15] Despite the extensive application of chiral Ru(II) complexes as enantioselective probes to elucidate noncovalent interactions of small bioactive molecules with DNA, no highly efficient, general method is so far available for the direct separation and quantitation of the enantiomers of these compounds. Low efficiency chromatographic resolutions, based on chiral mobile phase additives,[16,17] on preformed diastereomeric species,[18] or using DNA-hydroxyapatite columns[19] have been described. Recently, some examples of direct resolutions using a polysaccharide HPLC chiral phase have been described.[20] Here we report the highly efficient chromatographic resolution of a set of chiral mono- and dinuclear Ru(II) complexes, including the prototypical ones containing phen and bpy (bpy = 2,2′-bypyridine) as ligands, on a silica-bound teicoplanin HPLC stationary phase.

2. Results and discussion

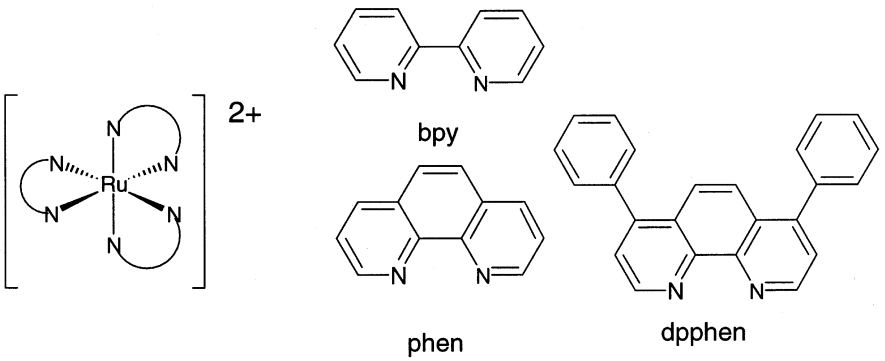
Teicoplanin is a member of the vancomycin glycopeptide family of antibiotics. Its aglycon skeleton consists of four interlocked cyclic peptides forming a cup-shaped structure that incorporates conformationally fixed biaryl and biaryl ether units.[21]

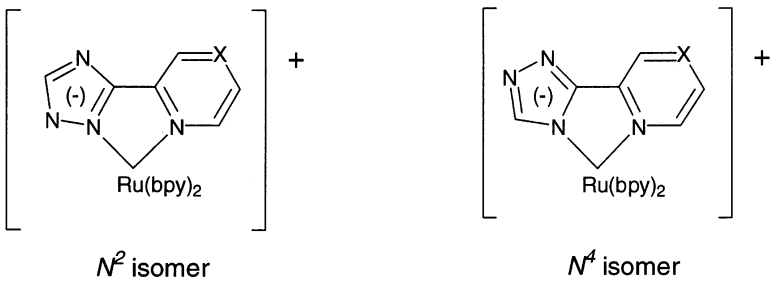
Teicoplanin has been used, after immobilization onto silica gel microparticles, as a HPLC chiral selector with excellent recognition abilities and a wide application range.[22,23]

We have recently prepared a new glycopeptide-bound stationary phase (**CSP1**) by treating isocyanate activated aminopropyl silica gel with a pyridine solution of teicoplanin for 12 h at 70°C. In the resulting chromatographic material the teicoplanin is tethered to the silica surface through a six carbon aliphatic spacer.[24]

After packing the modified silica gel (**CSP1**) into a 250×4 mm stainless steel column, we used the mono- and dinuclear Ru(II) complexes $[Ru(bpy)_3]^{2+}$, **1**, $[Ru(phen)_3]^{2+}$, **2**, $[Ru(dpphen)_3]^{2+}$ (dpphen = 4,7-diphenyl-1,10-phenanthroline), **3**, $[Ru(bpy)_2pztr]^{+}$ (Hpztr = 3-(pyrazin-2-yl)-1,2,4-triazole), **4**,[25,26] $[Ru(bpy)_2pytr]^{+}$ (Hpytr = 3-(pyridin-2-yl)-1,2,4-triazole), **5**[27,28] and $[(Ru(bpy)_2)_2bpt]^{3+}$ (Hbpt = 3,5-bis(pyridin-2-yl)-1,2,4-triazole), **6**[29] (see Scheme 1) to investigate the enantioselective binding properties of **CSP1**. HPLC runs were carried out with MeOH/MeCN eluents to which 0.1 or 0.5 M $AcONH_4$ (pH 7.0) was added using UV or simultaneous UV and CD detections to monitor separations. As evident from the results collected in Table 1, the silica-bound teicoplanin does bind Ru(II) complexes with a marked dependence upon their stereochemistry, regiochemistry and ligand structure. Enantioselectivity values (α in Table 1) in the range 1.03–1.74, combined with good chromatographic efficiency resulted in complete baseline resolutions in all cases with the exception of **3** and the heterochiral isomer of complex **6** (see below). Overall retention of the enantiomers of **1**–**6** is controlled by electrostatic forces: single charged mixed ligand complexes **4** and **5**, where the triazole units are likely to be deprotonated under our experimental conditions,[30] are less retained than double charged ones **1**–**3**, and the triple charged dinuclear complexes **6** required a more concentrated buffer for column elution. Indeed, using eluent A (Table 1) **4** and **5** are not retained on **CSP1**, while **6** is not eluted. Size and shape of the ligands controls the affinity of complexes with the same net charge for the immobilized teicoplanin: flat and large aromatic ligands favor binding, with $[Ru(phen)_3]^{2+}$ showing a twofold increase in retention compared with $[Ru(bpy)_3]^{2+}$. However, $[Ru(dpphen)_3]^{2+}$, **3** is less retained than either **1** or **2**, indicating that the steric bulk of the phenyl

rings at the distal sites on **3** reduces the interaction with the immobilized teicoplanin. Enantioselectivity follows a similar trend, with $[Ru(phen)_3]^{2+}$ and $[Ru(dpphen)_3]^{2+}$ showing the highest and lowest values, respectively. Substitution of one bpy unit in **1** for pztr or pytr also increases enantioselectivity. As reported before,[25–28] different regioisomers are expected for compounds **4** and **5** where the triazole ring is bound to the metal centre either through the N^2 or the N^4





N^2 isomer

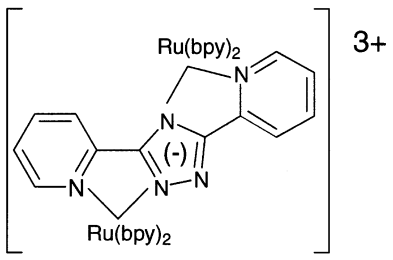
X = N
(**4**) $[Ru(bpy)_2pztr]^+$

X = C
(**5**) $[Ru(bpy)_2pytr]^+$

N^4 isomer

X = N
(**4**) $[Ru(bpy)_2pztr]^+$

X = C
(**5**) $[Ru(bpy)_2pytr]^+$



(**6**) $[(Ru(bpy)_2)_2bpt]^{3+}$

Scheme 1. Top: structure of homoleptic polypyridyl complexes. N–N equals bpy, phen or dpphen (complexes **1**, **2** and **3**, respectively). Central: structure of coordination isomers of pyrazine triazole (X = N, **4**) and pyridine triazole (X = C, **5**) containing $Ru(bpy)_2$ complexes. Bottom: structure of dinuclear complex **6**

Table 1
Chromatographic data for complexes **1**–**6** on **CSP1**

Compd		K'_1[a]	α[b]	Config.[c]	Eluent
1	$[Ru(bpy)_3]Cl_2$	12.56	1.10	Δ	A
2	$[Ru(phen)_3]Cl_2$	18.58	1.28	Δ	A
3	$[Ru(dpphen)_3]Cl_2$	7.84	1.03	Δ	A
4	$[Ru(bpy)_2(pztr)]PF_6$ N^2-bound	3.33	1.20		B
4	$[Ru(bpy)_2(pztr)]PF_6$ N^4-bound	3.67	1.18		B
5	$[Ru(bpy)_2(pytr)]PF_6$ N^2-bound	3.22	1.17		C
5	$[Ru(bpy)_2(pytr)]PF_6$ N^4-bound	2.94	1.25		C
6	$[(Ru(bpy)_2)_2(bpt)](PF_6)_3$ *homo*	6.28[d]	1.74[d]	Δ,Δ	D
6	$[(Ru(bpy)_2)_2(bpt)](PF_6)_3$ *hetero*	7.88[d]	1.07[d]		D

[a] Retention factor at 25°C of the first eluted enantiomer, defined as $(t_1-t_0)/t_0$, where t_1 is the elution time of the first enantiomer and t_0 is the elution time of a non-retained sample.
[b] Enantioselectivity factor defined as k'_2/k'_1.
[c] Configuration of the more strongly retained enantiomer.
[d] Temperature: 35°C. Eluents: (A) $CH_3CN/CH_3OH/AcONH_4$ 0.1 M 60/20/20; (B) $CH_3CN/CH_3OH/AcONH_4$ 0.1 M 30/50/20; (C) $CH_3CN/CH_3OH/AcONH_4$ 0.1 M 40/40/20; (D) $CH_3CN/CH_3OH/AcONH_4$ 0.5 M 60/20/20.

nitrogen atom of the ring. Interestingly, for both **4** and **5**, **CSP1** selectively retains each enantiomer of the N^2 and N^4 bound regioisomers allowing a complete resolution of the four isomers of each complex in a single run. Such a finding implies a remarkable regio- and enantioselectivity considering the small structural differences, confined to a single ligand, between the regioisomeric complexes. Elution order for the enantiomers of **1**–**3** was established by on-line CD detection at the ligand-centered transitions wavelength:[31] in all cases **CSP1** preferentially retains the Δ enantiomers (see Fig. 1).

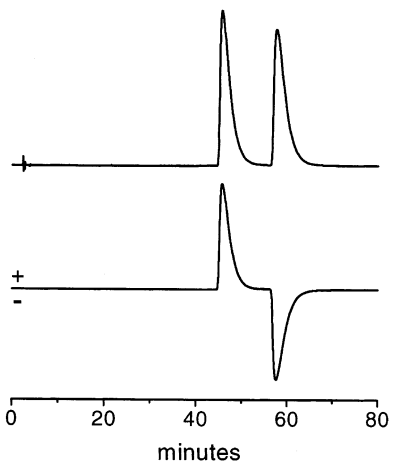


Figure 1. Enantioselective chromatography of $[Ru(Phen)_3]^{2+}$ **2** on **CSP1**, 250×4 mm ID column, eluent flow rate = 1.3 mL/min. UV (top) and CD (bottom) detections at 267 nm. CD trace is vertically expanded by a factor of 4

Marked selectivity was also observed toward the stereoisomers of the dinuclear complex **6**. For this species one of the two metals is N^2 and the other is N^4 bound to the central triazole unit. Consequently **6** exists as a couple of diastereomers, each of which entails a pair of

enantiomers: in one diastereomer the two metals have the same configuration (ΔΔ or ΛΛ, homochiral), while in the other they have the opposite configuration (ΔΛ or ΛΔ, heterochiral). All the expected stereoisomers of **6** are selectively retained by the immobilized teicoplanin: the four peaks observed in the HPLC trace (see Fig. 2) were assigned to the homo- (first and fourth eluted) and heterochiral (second and third) species on the basis of relative peak area, CD detection and comparison of the chromatographic behaviour with that of the parent mononuclear species **1**. The homochiral isomer of **6** is in fact expected to behave like a bis-analyte, one enantiomer of which has both metals with a chirality matching that of the stationary phase (more retained, configuration tentatively assigned as ΔΔ) and the other enantiomer has a mismatched chirality (less retained). Conversely, the heterochiral complexes are expected to show intermediate affinity for the stationary phase and lower enantioselectivity compared with the homochiral ones. For the heterochiral species CD signals are oppositely signed but very weak compared with those of the homochiral complex.

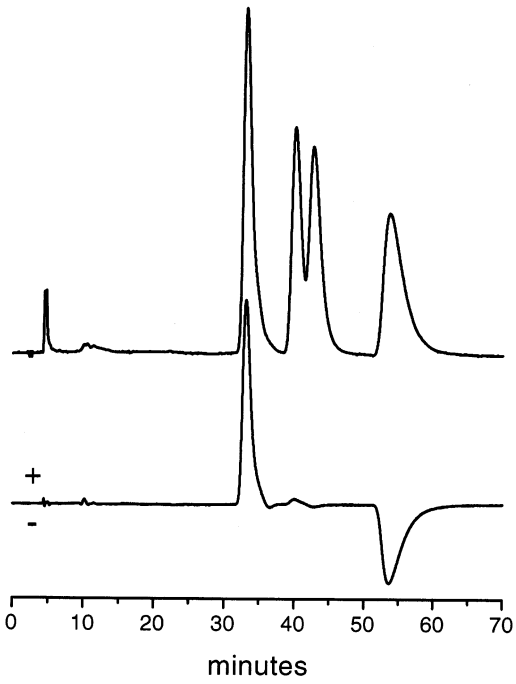


Figure 2. Enantio- and diastereoselective chromatography of $[(Ru(bpy)_2)_2(bpt)]^{3+}$ **6** on **CSP1**, 250×4 mm ID column, eluent flow rate = 1.3 mL/min. UV (top) and CD (bottom) detections at 289 nm. CD trace is vertically expanded by a factor of 10

We also examined a related stationary phase having the surface-linked teicoplanin aglycon[32] as a chiral unit (**CSP2**). We found that under similar experimental conditions, **CSP2** shows the same level and sense of enantioselectivity, but greater retention for complexes **1**–**6** compared with **CSP1**. For example the enantiomers of $[Ru(phen)_3]^{2+}$ are selected by **CSP2** with $\alpha = 1.27$ using eluent D of Table 1. Thus the recognition site seems to be located within the cyclopeptide backbone of teicoplanin, with the sugar residues playing a minor role in reducing the number of potential sites for nonselective interaction.

Interestingly, differences in binding affinities between enantiomeric Ru(II) complexes and silica-bound teicoplanin are of the same order of magnitude as those reported for larger biomolecules. Thus, in hydroorganic eluents containing ammonium acetate in the 20–100 mM range, teicoplanin binds the enantiomers of **2** and those of homochiral-**6** with $\Delta\Delta G_{25°C}$ of 0.6 and 1.4 kJ/mol, respectively,[†] while calf tymus DNA in 50 mM NaCl, 5 mM Tris buffer, pH 7.1 binds the enantiomers of **2** with $\Delta\Delta G_{20°C} = 0.3$ kJ/mol and the enantiomers of $[Ru(phen)_2dppz]^{2+}$ (dppz = dipyrido[3,2-*a*:2′3′-*c*]phenazine) with $\Delta\Delta G_{20°C} = 1.6$ kJ/mol.[5] DNA and teicoplanin also show similar structural and stereochemical requirements for binding, in that both select the Δ enantiomers of **1** and **2**, while they have no preference for the enantiomers of **3**.

The above results show that **CSP1** is capable of binding the stereoisomers of a variety of Ru(II) complexes with high regio-, diastereo- and enantioselectivity. While the structural details of teicoplanin binding selectivity are not yet experimentally defined, we believe that some characteristics of **CSP1** (conformational homogeneity and the presence of a cleft-like cavity with aromatic portion available for π-stacking in the peptide backbone, inherent high chromatographic efficiency deriving from the grafting mode[24]) make it a highly selective system for the discrimination of Ru(II) complexes.

Given the broad range of selectivities shown by **CSP1**, we anticipate it will find additional analytical and preparative applications in the field of chiral transition metal complexes.

3. Experimental

Synthesis of the chiral stationary phases[24,32] and of the Ru(II) complexes have been described elsewhere.[25–30] Liquid chromatography was performed on a Waters chromatograph equipped with a Rheodyne Model 7725i 20 μL injector and two Model M510 solvent-delivery systems. CD and UV detections were obtained using a Model CD-995 detector (Jasco Europe, Italy). Chromatographic data were collected and processed using the Millenium 2010 Chromatography Manager software (Waters Chromatography).

Acknowledgements

The authors wish to acknowledge financial support from Italian MURST and CNR and from the EU TMR Programme Grant Nr CT96-0076.

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[†] Calculated from $\Delta\Delta G = -RT \ln \alpha$, where R is the gas constant and T is the column temperature.

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