

Solid-Phase Synthesis of Tris-heteroleptic Ruthenium(II) Complexes and Application to Acetylcholinesterase Inhibition

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A synthetic route with two consecutive coordination chemistry steps on a solid support affords tris-heteroleptic ruthenium(II) polypyridyl complexes with high purity and in good yields. As an application we report the identification of a nanomolar acetylcholinesterase inhibitor from a small ruthenium complex library synthesized on Lanterns.

Solid-phase synthesis has the attractive feature that it allows one to perform multiple consecutive reaction steps without any intermediate purification and reactions can be driven to completion by using excess of reagents. Furthermore, solid-phase synthesis is a core technology for combinatorial chemistry in which, for example, large libraries are accessible in an economical fashion by split-pool synthesis. Whereas solid-phase organic chemistry is a mature field, solid-phase synthesis of metal complexes is much less developed.^{1–3}

Here we disclose a reaction sequence that enables the synthesis of tris-heteroleptic ruthenium(II) polypyridyl (pp) complexes⁴ on resins, and we apply it to the combinatorial synthesis of acetylcholinesterase (AChE) inhibitors.

Our solid-phase synthetic strategy draws from a solution synthesis method developed by Mann and Freedman,⁵ in which a mixture of $[\text{Ru}(\text{pp})(\text{CH}_3\text{CN})_2\text{Cl}_2]$ and $[\text{Ru}(\text{pp})(\text{CH}_3\text{CN})_3\text{Cl}]\text{Cl}$, prepared by the photolysis of $[(\eta^6\text{-C}_6\text{H}_6)\text{Ru}(\text{pp})\text{Cl}]\text{Cl}$, is reacted consecutively with two pp ligands to form a tris-heteroleptic ruthenium complex. A representative example of our reaction sequence is shown in Scheme 1. First, 1,10-phenanthroline-5-carboxylic acid (**1**)⁶ was immobilized on a solid support through a Rink amide linker. For this, 4-[(2,4-dimethoxyphenyl)(Fmoc-amino)methyl]phenoxyacetyl-modified TentaGel [poly(ethylene glycol) grafted on low cross-linked polystyrene] macrobeads (**2**) were Fmoc-deprotected with 20% piperidine in dimethylformamide (DMF), and the resulting free amine (**3**) reacted with carboxylic acid **1** at 70 °C in the presence of the coupling reagent PyBOP to yield the immobilized phenanthroline-5-carboxamide **4**.⁷ Thereafter, as a precaution, small amounts of potentially unreacted amino groups were capped with Ac_2O and Hünig's base. In the following key step, the 1,10-phenanthroline-derivatized beads **4** were treated with 1.5 equiv of $[\text{Ru}(\text{bpy})(\text{CH}_3\text{CN})_2\text{Cl}_2]/[\text{Ru}(\text{bpy})(\text{CH}_3\text{CN})_3\text{Cl}]\text{Cl}$ (**5**; bpy = 2,2'-bipyridine) at a concentration of 20 mM in DMF for 3 h at 80 °C. This route is based on the assumption that, because of the spacial separation of the

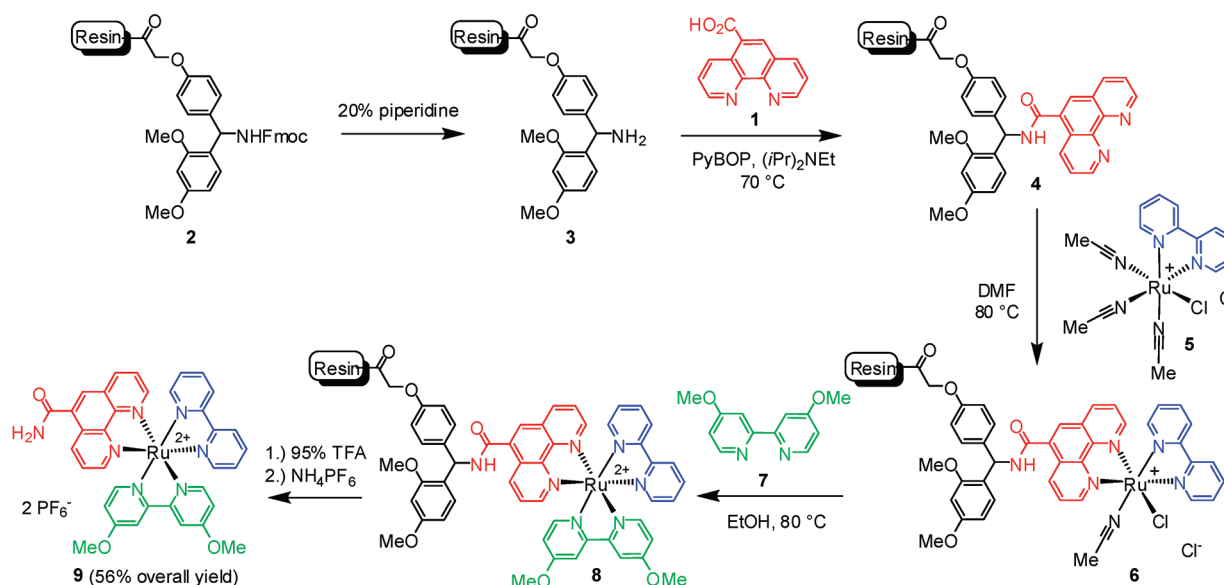
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Scheme 1. Example of the Solid-Phase Synthesis of a Tris-heteroleptic Ruthenium(II) Complex^a

^a PyBOP = (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate, Ac₂O = acetic anhydride, and TFA = trifluoroacetic acid. Complexes **5**, **6**, **8**, and **9** are mixtures of isomers, but only one structure is shown.

ligand **4** on the resin, the ruthenium precursor **5** will mainly react with only 1 equiv of **4** to form **6**, without any risk of further reaction with a second immobilized phenanthroline. We observed that the extent of this side reaction depends on the solvent, with DMF giving the most satisfactory results with little or no cross reaction.⁸

Next, after washing the resin, 4,4'-dimethoxy-2,2'-bipyridine (**7**) was added as an ethanol solution, and the resulting solution was heated to 80 °C. The formation of the tris-heteroleptic ruthenium complex **8** in this step was apparent from luminescence of the beads upon exposure to UV light. The reaction was driven to completion by an extended reaction time (16 h) and an excess of ligand (10 equiv). Finally, the complex was released from the resin by treatment with 95% aqueous trifluoroacetic acid (TFA). The ¹H NMR spectrum of the crude material after evaporation of the TFA solution is shown in Figure 1 and demonstrates the high purity of the crude material without having any significant amounts of side products. Silica gel purification of this material followed by precipitation with NH₄PF₆ yielded complex **9** as a mixture of diastereomers in an overall 56% yield over five reaction steps.

In order to test the generality of this reaction sequence, we synthesized a set of six additional tris-heteroleptic ruthenium(II) polypyridyl complexes on TentaGel macrobeads as shown in Table 1. We used 1,10-phenanthrolines with carboxylic acids at either the 4 or 5 position,^{6,9} two different ruthenium precursor complexes [Ru(pp)(CH₃CN)₂Cl₂]/[Ru(pp)(CH₃CN)₃Cl]Cl (pp = bpy and phen), and three different ligands for the 3 position. The overall yields of the purified products ranged from 34 to 56% (Table 1), and in all instances, the desired tris-heteroleptic ruthenium com-

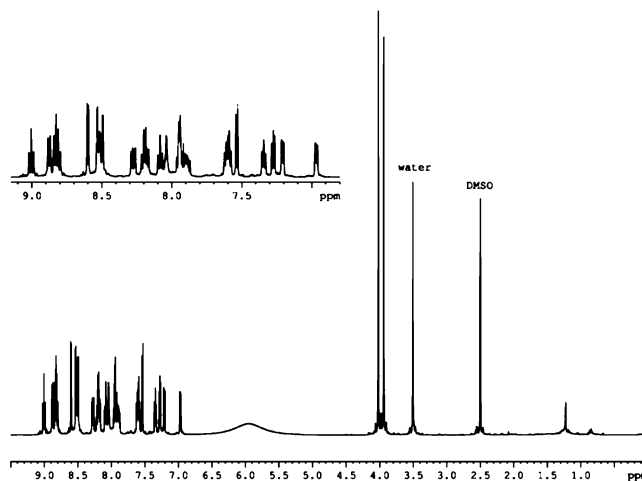


Figure 1. 500 MHz ¹H NMR spectrum (DMSO-*d*₆) of crude [Ru(bpy)-(phen-5-CONH₂)(4,4'-MeO₂bpy)](CF₃CO₂)₂ after cleavage from a solid support with 95% aqueous TFA and evaporation.

Table 1. Solid-Phase Synthesis of Different Tris-heteroleptic Ruthenium(II) Polypyridyl Complexes^a

entry	first ligand	precursor 5	third ligand	yield (%)
1	phen-5-CONH ₂	bpy	(MeO) ₂ bpy	56
2	phen-5-CONH ₂	bpy	Me ₂ bpy	41
3	phen-5-CONH ₂	bpy	Me ₄ phen	36
4	phen-5-CONH ₂	bpy	Me ₂ phen	39
5	phen-4-CONH ₂	phen	(MeO) ₂ bpy	48
6	phen-4-CONH ₂	phen	Me ₄ phen	45
7	phen-4-CONH ₂	phen	Me ₂ phen	34

^a See the Supporting Information for a detailed procedure (method A). Isolated as PF₆ salts. phen-5-CONH₂ = 1,10-phenanthroline-5-carboxamide, phen-4-CONH₂ = 1,10-phenanthroline-4-carboxamide, bpy = 2,2'-bipyridine, phen = 1,10-phenanthroline, Me₂bpy = 4,4'-dimethyl-2,2'-bipyridine, Me₄phen = 3,4,7,8-tetramethyl-1,10-phenanthroline, Me₂phen = 5,6-dimethyl-1,10-phenanthroline, and (MeO)₂bpy = 4,4'-dimethoxy-2,2'-bipyridine.

plexes were the main products and formed as mixtures of two diastereomers.¹⁰

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Table 2. Different Solid Supports for the Synthesis of Complex **9**

entry	solid supports	loading (mmol/g)	methods ^a	yields (%)
1	TentaGel macrobeads ^b	0.18	A	56
2	TentaGel regular resin ^b	0.26	A	42
3	polystyrene Lantern ^c	35 ^e	B (A, C)	41 (22, 66)
4	polystyrene macrobeads ^b	0.65	D	25
5	polystyrene regular resin ^d	0.20	D	29

^a The methods differ only in the number of equivalents and concentrations of **5**: (A) 1.5 equiv, 20 mM; (B) 2.0 equiv, 71 mM; (C) 1.5 equiv, 195 mM; (D) 5 equiv, 130 mM. ^b Rapp Polymere GmbH. ^c Mimotopes Pty Ltd. ^d Advanced ChemTech. ^e Loading in $\mu\text{mol/Lantern}$

We next investigated the influence of the resin itself on the overall yield of the ruthenium complex syntheses. Table 2 lists a selection of different solid supports used for the synthesis of complex **9**, which consisted of polystyrene regular resin, macrobeads, and SynPhase Lanterns,¹¹ in addition to TentaGel macrobeads and regular TentaGel resin. With our standard conditions (Table 2, method A), using only a slight excess of 1.5 equiv of the precursor complex **5** at 20 mM in the first coordination chemistry step **4** + **5** → **6** (Scheme 1), TentaGel resins and especially the macrobeads gave the best results (Table 2, entries 1 and 2). However, the yields on the polystyrene resins, in particular the polystyrene Lanterns (Table 2, entry 3), could be improved significantly by increasing the concentration and number of equivalents of **5** in the first coordination step **4** + **5** → **6**. For example, increasing the number of equivalents of **5** from 1.5 to 2 and 5, and the concentration from 20 to 71 and 195 mM, improved the overall yields of complex **9** from 22 to 41 and 66%, respectively. Apparently, this coordination chemistry step proceeds more sluggishly on polystyrene and must be pushed by increasing the concentration of **5**. In contrast, raising the temperature from 80 to 90 °C in this reaction step resulted in highly diminished yields, most likely due to the decomposition of the precursor complex **5**, which has a limited stability and therefore requires a reasonably quick reaction kinetic even at 80 °C.¹² Overall, it can be concluded that the polar TentaGel resin, especially as macrobeads, is superior in atom economy to hydrophobic polystyrene supports for this reaction sequence, and the coordination chemistry step **4** + **5** → **6** in particular. This is maybe not surprising because the coordination chemistry steps involve charged species that should be more compatible with polar resins.¹³

Finally, we were seeking to apply this new methodology to the development of a ruthenium enzyme inhibitor, and we chose AChE as a proof-of-principle target because it has already been demonstrated that the $[\text{Ru}(\text{phen})_3]^{2+}$ dication binds with micromolar affinity to AChE.¹⁴ Accordingly, a

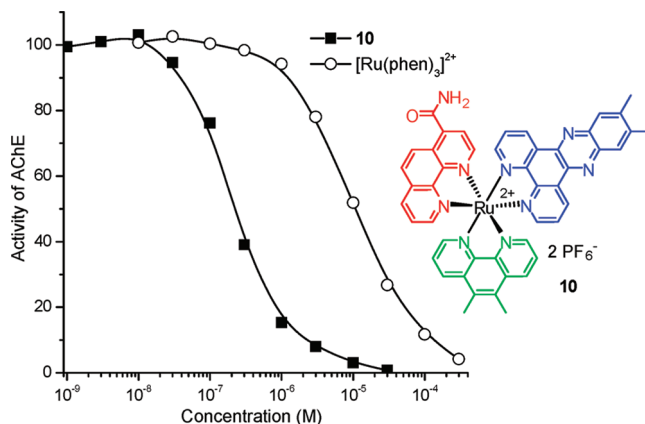


Figure 2. IC₅₀ curves against AChE from *Electrophorus electricus* of the diastereomerically pure complex **10** and a comparison with $[\text{Ru}(\text{phen})_3]\text{Cl}_2$ (both complexes racemic). The activity of AChE as a function of the concentration of ruthenium inhibitors was measured by a standard colorimetric method using acetylthiocholine as a substrate. See the Supporting Information for more details.

small 28-membered library of ruthenium complexes synthesized on Lanterns, followed by a brief structure–activity relationship, led us to the identification of one complex, bearing as ligands 1,10-phenanthroline-4-carboxamide, 5,6-dimethyl-1,10-phenanthroline, and 7,8-dimethyldipyrido[3,2-*a*:2',3'-*c*]phenazine, as a nanomolar inhibitor for AChE (see the Supporting Information for more details). The two diastereomers of this compound were subsequently resolved by reversed-phase high-performance liquid chromatography and the relative stereochemistry assigned by NMR (see the Supporting Information). The two complexes differed in binding affinity to AChE by a factor of 2, with the more potent diastereomer **10** displaying an IC₅₀ of 200 nM (Figure 2). With this, **10** is around 500-fold more potent than the parent complex $[\text{Ru}(\text{phen})_3]\text{Cl}_2$ (IC₅₀ = 10 μM), demonstrating the power of this solid-phase combinatorial approach to access modified tris-heteroleptic complexes in a rapid fashion.

In conclusion, we have developed a simple and convenient solid-phase synthetic route to tris-heteroleptic ruthenium(II) polypyridyl complexes. The strength of this methodology lies in the rapid access to new complexes without the need for multiple purification steps and the ability to employ standard combinatorial chemistry protocols, as has been demonstrated for the development of a nanomolar AChE inhibitor.

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Supporting Information Available: Experimental details and spectroscopic data. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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