Tetrahedron Letters 53 (2012) 5293–5296

Contents lists available at SciVerse ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

The use of the 1,2,4-triazine method of pyridine ligand synthesis for the preparation of a luminescent Pt(II) labeling agent

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ARTICLE INFO

Article history:
Received 5 June 2012
Revised 28 June 2012
Accepted 19 July 2012
Available online 27 July 2012

Keywords:
1,2,4-Triazine
Pyridine
Ligand
Platinum(II)
Luminescence

ABSTRACT

The 'triazine' methodology for the synthesis of functionalized pyridine ligands proved to be a convenient method for the preparation of a luminescent Pt(II) complex. The key ligand can be assembled easily starting from readily accessible reagents. Further cycloplatination and post-functionalization led to the ready-to-go luminescent 'tag' **2** for peptide labeling.

Luminescent complexes are of significant interest because of their applications as molecular probes in biomedical,[1] sensing,[2] or imaging areas,[3] and as light-emitting devices.[4] Traditionally, lanthanide chelates, and in particular, Eu(III) complexes with long-life intense luminescence in aqueous solutions, have been widely used as luminescent labels for time-resolved immunoassays.[5] But recently, due to their greater stability and more intense emissions, the attention of researchers has turned to cyclometallated platinum(II) and iridium(III) complexes.[6–8] These materials demonstrate high quantum yields, large Stokes shifts, and relatively long lifetimes for easy detection in naturally fluorescent biological media. In addition, the design of lanthanide chelates is a real challenge. A ligand should protect the lanthanide center from quenching by water molecules, provide strong metal binding to prevent release of the lanthanide ion, and act as a sensitizer to enable energy transfer to the metal center.[9] To fulfill these requirements the ligand should be nine-coordinating with strong chelating groups (e.g., carboxylic), and also provide participation in the coordination by the aromatic part, for example, a pyridine or polypyridine.[10] The fine balance between the chromophore and the coordinating sphere leads to complex ligands such as pyridine derivatives functionalized with polyaminoacetate arms and also bearing a spacer with a group for bioconjugation (e.g., complex **1**). From this point of view, ligands for cyclometallated complexes of platinum metals seem to be more accessible, as the aromatic system is simple and is the only metal binding unit. Typical cyclometallated phenylpyridine (ppy) or related complexes of Pt(II) or Ir(III) with functionalized auxiliary ligands are available in a few synthetic steps and are easily isolated and purified.[11,12] Thus, a ppy complex of Ir(III) with a bipyridine auxiliary ligand bearing an amino group was successfully used for the preparation of labeling reagents for biological substrates.[13]

We envisaged that the introduction of a group suitable for peptide labeling directly into the cyclometallating ligand (e.g., 2-thienylpyridine) would make the design of the complex easier. In this case, a very accessible reagent such as acetylacetone can be used as an auxiliary ligand in the cyclometallated complex. Only the main cyclometallating ligand with a 2-arylpyridine fragment bearing a carboxylic or amino group connected through a spacer is required. The rational design of such a ligand led us to choose 2-(2-thienyl)pyridine (thpy) as a cyclometallating system. It is reactive toward cyclometallation and the cyclometallated thpy complexes of Pt(II) exhibit increased lifetimes.[14] The same effect can be achieved by the expansion of the aromatic system by introduction of an aryl substituent at position 5 of pyridine.[15] In addition, it can act as a spacer-bearing functionality to expedite binding to biological targets. For example, the 4-methoxyphenyl substituent can be easily modified by consecutive demethylation and alkylation.[16] This allows introduction of an acetate moiety, which can be further activated for peptide binding by typical procedures, For example, formation of an *N*-succinimide ester.[17] In this way, the design led us to complex **2**, and the key feature of this work was the synthesis of the cyclometallating ligand.

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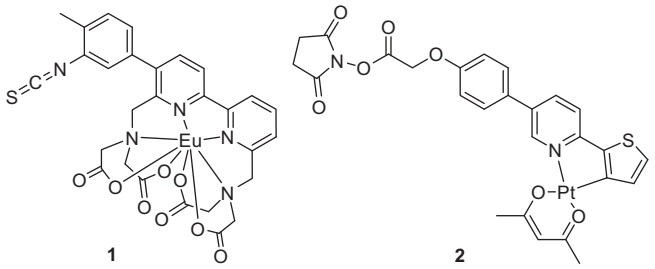
0040-4039/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tetlet.2012.07.090

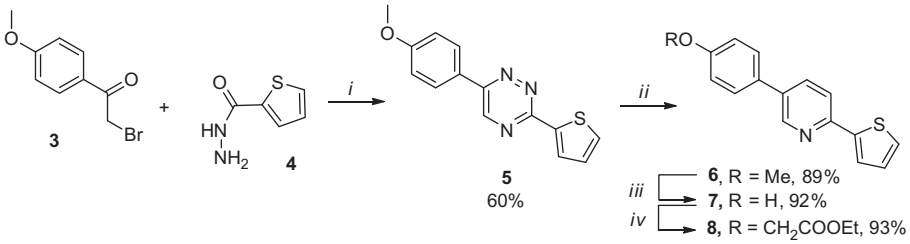


A convenient method for the preparation of substituted pyridines involves transformation of 1,2,4-triazines in cycloaddition reactions with various nucleophilic dienophiles.[18–20] We previously reported a 1,2,4-triazine method of bipyridine ligand synthesis for the preparation of new luminescent Eu(III) labeling reagents, for example, **1**.[21] The methodology included an easy synthesis of substituted 1,2,4-triazine followed by transformation into the corresponding pyridine via an inverse electron demand Diels-Alder reaction, and a further relatively long sequence of modifications. We now propose an extension of the methodology for a simple preparation of a pyridine ligand for luminescent Pt(II) labeling agent **2**, starting from very accessible reagents in a few steps. The usual approach for the synthesis of cyclometallating pyridine ligands includes consecutive introduction of aromatic substituents into the pyridine core by cross-coupling reactions with the stepwise formation of an aromatic system,[22] in contrast, the '1,2,4-triazine' method allows rapid formation of the entire aromatic system with the required substituents.[23–25]

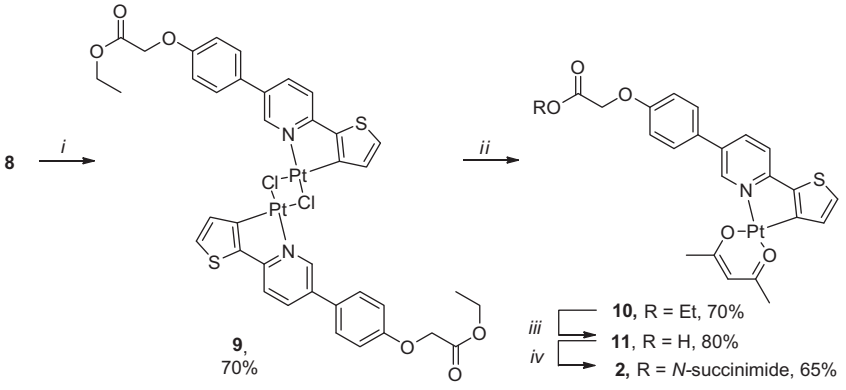
The aromatic part of the ligand was assembled according to the '1,2,4-triazine' strategy starting from 2-bromo-4′-methoxyacetophenone (**3**), thiophene-2-carbohydrazide (**4**), and 2,5-norbornadiene as the main building blocks. Cyclization of the bromoacetophenone with 2 equiv of the hydrazide[26] resulted in the formation of 1,2,4-triazine **5**. Next, cycloaddition of 2,5-norbornadiene to the triazine led to the substituted pyridine **6** via a cascade involving a Diels-Alder reaction with inverse electron demand, a retro Diels-Alder reaction with elimination of a nitrogen molecule, and a retro Diels-Alder reaction with elimination of cyclopentadiene. Phenol **7** was obtained by demethylation of the methyl ether **6** either with boron tribromide or pyridine hydrochloride. In the first case, the conditions were milder and the yield was higher. The final ligand **8** was prepared by alkylation of phenol **7** with ethyl bromoacetate in acetonitrile (Scheme 1).[27]

The ligand was initially cyclometallated according to the typical procedure in refluxing acetic acid containing potassium tetrachloroplatinate. The reaction was complicated by significant reduction of platinum with the formation of platinum black, and therefore proceeded in low yield to give dimeric complex **9**. Next, we changed the solvent to acetonitrile. The reaction was clean and proceeded without any trace of platinum black in spite of the longer reaction time, yielding 70% of complex **9** (Scheme 2). The dimeric complex was cleaved with dimethylsulfoxide to form an intermediate monomeric DMSO complex, PtL(DMSO)Cl. Neither the dimeric dichloro complex nor the monomeric DMSO complex was emissive, but the DMSO ligand was very labile and could be easily substituted by another auxiliary ligand. Hence the DMSO complex was not purified and was directly converted into complex **10** by treatment with an excess of sodium acetylacetonate in acetone.[28] Hydrolysis of the ethyl ester in 1 N LiOH solution gave the corresponding acid **11**.[29] Finally, this was activated with *N*-hydroxysuccinimide in the presence of dicyclohexylcarbodiimide according to the standard protocol to give the target platinum label **2**.[30]

The absorption and luminescence spectra of **2** in aerated CH_2Cl_2 at room temperature are presented in Figure 1. The photophysical



Scheme 1. Ligand synthesis. Reagents and conditions: (i) NaOAc, AcOH/EtOH, reflux, 10 h; (ii) 2,5-norbornadiene, *o*-xylene, reflux, 48 h; (iii) BBr_3, CH_2Cl_2, 0 °C, 4 h or Py·HCl, 150 °C, 10 h; (iv) $BrCH_2COOEt$, K_2CO_3, MeCN, reflux, 10 h.



Scheme 2. Complex preparation. Reagents and conditions: (i) K_2PtCl_4, AcOH, reflux, 24 h or K_2PtCl_4, MeCN, reflux, 48 h; (ii) (1) DMSO, reflux, 5 min; (2) Na-acac, acetone, reflux, 5 h; (iii) 1 N LiOH, H_2O/THF, reflux, 10 h; (iv) *N*-hydroxysuccinimide (NHS), DCC, Et_3N, DMF, 60 °C, 24 h.

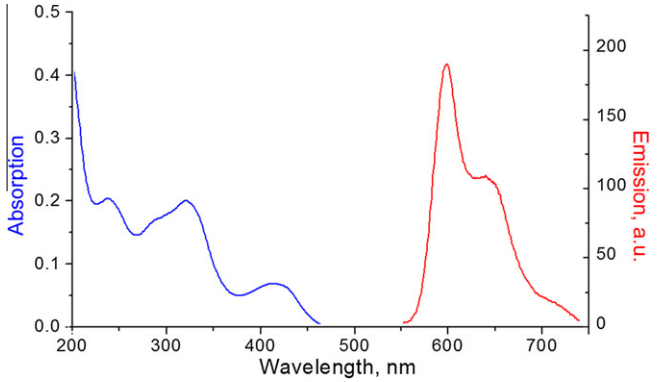


Figure 1. Absorption and emission spectra of **2** in aerated dichloromethane solution.

Table 1
Photophysical parameters of **2**

λ_{abs} [a] (nm)	ε [b] ($dm^3\ mol^{-1}\ cm^{-1}$)	λ_{em} [c] (nm)	Φ_{em} [d]
237	8793	597	0.01
320	8594		
415	3500		

[a] λ_{abs} — absorption band maximum, in aerated CH_2Cl_2 solution at 298 K.
[b] ε — molar absorptivity (±5%).
[c] λ_{em} — emission maximum, in aerated CH_2Cl_2 solution at 298 K.
[d] Φ_{em} — photoluminescence quantum yield relative to $Ru(bipy)_3Cl_2$ in aerated water solution (±20%).[31]

data are collected in Table 1. The absorption spectrum is typical for cyclometallated arylpyridine complexes of platinum(II).[14] The moderate absorption band at 415 nm was assigned to a metal-to-ligand charge transfer (MLCT) transition. More intense absorption bands at 280–320 nm corresponded to ligand-centered (LC) π,π^*-transitions. Upon photoexcitation at the lowest energy band, the complex was moderately emissive in aerated CH_2Cl_2 solution at room temperature and exhibited orange luminescence with a Stokes shift of about 180 nm. The emission band at 597 nm showed some resolved vibrational modes with 1200 cm^{-1} progression. Based on previous studies of complexes of this type, the emission origin was assumed to be a result of ligand-centered $^3\pi,\pi^*$-transition from a triplet state.[15] The emission lifetime in ambient conditions corresponds to tens of microseconds. The quantum yield of the phosphorescence was about 0.01 at room temperature in aerated CH_2Cl_2 solution.

In conclusion, an efficient and straightforward '1,2,4-triazine' methodology for the synthesis of a pyridine ligand has been successfully applied for the preparation of a luminescent cyclometallated platinum(II) labeling agent. The methodology allows easy construction of the platinum(II) luminophore bearing an NHS-activated carboxylic group for peptide binding, starting from readily available reagents in a few steps. The key step is the preparation of an appropriately substituted cyclometallating pyridine ligand via the corresponding 1,2,4-triazine. The preliminary results revealed that the luminophore **2** prepared in this way was a 'ready-to-go' luminescent label, which can be excited with cheap lasers and possesses sufficiently intense long-lived luminescence with a strong Stokes shift for time-resolved detection. Further studies of bioconjugation and luminescent visualization are in progress.

Acknowledgements

The authors thank Ural Federal University and the President of Russian Federation (grant МК-1103.2012.3) for financial support.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2012.07.090.

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27. *Preparation of ligand **8***: The starting phenol **7** (600 mg, 2.37 mmol) and K_2CO_3 (3.2 g, 23.68 mmol) were suspended in MeCN (35 mL) and ethyl bromoacetate (435 mg, 2.6 mmol) was added. The mixture was stirred for 10 h at reflux. The solvent was removed, H_2O (50 mL) was added to the residue, and the white solid product **8** was filtered and recrystallized from EtOH. Yield 750 mg, 2.2 mmol, 93%. ^1H NMR (400 MHz, $CDCl_3$, ppm): 1.30 (t, J = 7.1 Hz, 3H, Et), 4.27 (q, J = 7.1 Hz, 2H, Et), 4.65 (s, 2H, CH_2), 6.99 (m, 2H, Ar), 7.11 (dd, J_1 = 4.8 Hz, J_2 = 3.7 Hz, 1H, thienyl), 7.38 (dd, J_1 = 4.8 Hz, J_2 = 1.1 Hz, 1H, thienyl), 7.51 (m, 2H, Ar), 7.58 (dd, J_1 = 3.7 Hz, J_2 = 1.1 Hz, 1H, thienyl), 7.67 (d, J = 8.2 Hz, 1H, Py), 7.81 (d, J_1 = 8.2 Hz, J_2 = 2.2 Hz, 1H, Py), 8.74 (d, J = 2.2 Hz, 1H, Py). Found: C, 67.15; H, 5.01; N, 3.95. Calcd for $C_{19}H_{17}NO_3S$: C, 67.24; H, 5.05; N, 4.13.
28. *Preparation of complex **10***: The starting ligand **8** (750 mg, 2.2 mmol) was suspended in MeCN and pulverized potassium tetrachloroplatinate powder (917 mg, 2.2 mmol) was added with vigorous stirring. The mixture was stirred and refluxed for 2 d. The solvent was removed and the resulting complex **9** was treated with 3 ml of DMSO. The mixture was heated for 5 min at reflux, and then the DMSO was removed under vacuum. The residue was dissolved in acetone (50 mL) and sodium acetylacetonate (3.1 g, 22.10 mmol) was added, and the suspension stirred under reflux for 5 h. The solvent was removed, and the product **10** was isolated by column chromatography on silica with CH_2Cl_2 as the eluent. The crude product was recrystallized from acetone to give orange crystals of **10**. Yield 680 mg, 1.07 mmol, 49%. ^1H NMR (400 MHz, $CDCl_3$, ppm): 1.31 (t, J = 7.1 Hz, 3H, Et), 1.97 (s, 3H, acac), 1.99 (s, 3H, acac), 4.28 (q, J = 7.1 Hz, 2H, Et), 4.67 (s, 2H, CH_2), 5.48 (s, 1H, acac), 7.00 (m, 2H, Ar), 7.19 (d, J = 4.5 Hz, 1H, thienyl), 7.31 (d, J = 8.2 Hz, 1H, Py), 7.49 (m, 3H, Ar + thienyl), 7.82 (d, J_1 = 8.2 Hz, J_2 = 2.2 Hz, 1H, Py), 9.00 (dd, J = 2.2 Hz, J_{HPt} = 43 Hz, 1H, Py). HRMS (ESI): $C_{24}H_{23}NO_5PtS$ requires M+H, 633.1023, found 633.1082.
29. *Preparation of complex **11***: Complex **10** (198 mg, 0.312 mmol) was suspended in THF (2.5 mL) and 1 N aq LiOH solution was added. The mixture was refluxed for 10 h. The solvents were condensed in vacuo, and the solids filtered. The remaining solution was acidified with 1 N HCl to pH 1–2. A yellow precipitate of **11** was formed and removed by filtration. This was dried and used for next

step without further purification. Yield 56 mg, 0.250 mmol, 80%. ^{1}H NMR (400 MHz, DMSO-d_6, ppm): 1.95 (s, 3H), 2.01 (s, 3H), 4.63 (s, 2H), 5.54 (s, 1H), 7.01 (d, ^3J = 4.8 Hz, 1H), 7.02 (m, 2H), 7.44 (d, ^3J = 8.4 Hz, 1H), 7.52 (d, ^3J = 4.8 Hz, 1H), 7.57 (m, 2H), 8.09 (dd, ^3J = 8.4 Hz, ^4J = 2 Hz, 1H), 8.93 (d, ^4J = 2 Hz, 1H). HRMS (ESI): $C_{22}H_{19}NO_5PtS$ requires M+H, 605.0710, found 605.0716.

30. *Preparation of the labeling agent* **2**: The complex **11** (180 mg, 0.30 mmol) was dissolved in dry DMF 30 mL and then dicyclohexylcarbodiimide (38 mg, 0.33 mmol) and *N*-hydroxysuccinimide (68 mg, 0.33 mmol) were added. The mixture was stirred at 60 °C for 24 h under a nitrogen atmosphere. The reaction progress was monitored by TLC. The solvent was removed under reduced pressure and the final product **2** was isolated by column chromatography (silica, CH_2Cl_2, R_f = 0.5) as orange crystals. Yield 135 mg, 0.19 mmol, 65%. The product was unstable toward water and should be kept cold under an inert atmosphere. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): 1.97 (s, 3H, Acac), 2.00 (s, 3H, Acac), 2.86 (s, 4H, NHS), 5.03 (s, 2H, CH_2), 5.48 (s, 1H, Acac), 7.06 (m, 2H, Ar), 7.20 (d, J = 4.5 Hz, 1H, thienyl), 7.32 (d, J = 8.2 Hz, 1H, Py), 7.49 (d, J = 4.5 Hz, 1H, thienyl), 7.55 (m, 2H, Ar), 7.83 (d, J_1 = 8.2 Hz, J_2 = 2.2 Hz, 1H, Py), 9.03 (dd, J = 2.2 Hz, J_{HPt} = 43 Hz, 1H, Py). HRMS (ESI): $C_{26}H_{22}N_2O_7PtS$ requires M+H, 702.0874, found 702.0802.

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