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Synthesis of substituted terpyridine ligands for use in protein purification

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ABSTRACT

A number of 4,4″-disubstituted terpyridines bearing a 4′-thioethanamine linker, and regioisomers thereof, have been synthesised from 4-substituted picolinates. The substituted terpyridines were immobilised onto epoxy-activated Sepharose™ FF gel, creating functionalised solid supports for the fractionation of proteins—including antibodies—by mixed mode affinity chromatography. The metal chelating properties of the immobilised ligand render the stationary phase also amenable for use in immobilised metal-ion affinity chromatography (IMAC), and have been demonstrated with the purification of suitably tagged green fluorescent protein (GFP).

1. Introduction

Affinity chromatography in its various forms is an important tool for the analytical and preparative purification of proteins and other biomolecules.[1–6] In recent years there has been a considerable interest in the development of low molecular weight affinity ligands for the functionalisation of support materials for the chromatographic purification of proteins.[7–10] Such efforts typically seek to address limitations in protein purification such as specificity, capacity, ligand toxicity and leaching.[11,12] The development of synthetic ligands for use in affinity chromatography has utilised traditional ligand discovery approaches ranging from ligand-based design, structure based design and ligand screening.[13]

The use of low molecular weight pyridine-based ligands in protein purification is well documented.[7–10] Such ligands present opportunities for protein binding through a number of interactions such as hydrogen bonding, hydrophobic interactions, ionic interactions, thiophilic interactions, etc., and are therefore amenable to various forms of chromatography. A recent study has reported the synthesis of an array of pyridine based ligands for use in mixed mode affinity chromatography.[10] The pyridine nucleus in these ligands contained a thioethanamine unit to create both a thiophilic centre[10] and an easy modality for immobilisation onto suitably activated support materials and solid phase surfaces. Both the nature and arrangement of the substituents on the pyridine nucleus of these ligands were demonstrated to affect their binding capacities and specificities for cell culture derived monoclonal antibodies (mAbs).

As part of an ongoing research program into the synthesis of hetero-aromatic ligands for use in protein separation,[10,14,15] a range of substituted terpyridines bearing a thioethanamine functionality have now been prepared (Scheme 1). These ligands incorporate the terpyridine skeleton as a structural motif with substituents on the peripheral terpyridine rings to influence the steric and electronic properties of the ligand. Starting from the appropriately 4-substituted alkyl picolinates **1**, the 4,4″-disubstituted terpyridines of interest **2** were prepared via a number of alternative synthetic strategies building upon modifications to procedure of Constable et al..[16] The advantages of these synthetic approaches will be discussed, and applications documented of the target compounds as ligands used to prepare novel chromatographic supports for the separation of antibodies and suitably tagged proteins.

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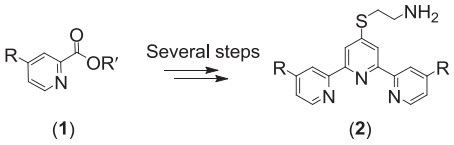
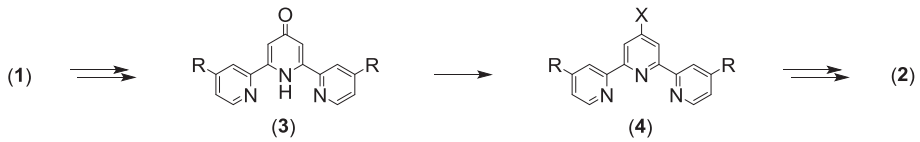


Scheme 1. Terpyridine targets of interest, where R is a range of substituents having varying steric and electronic properties, i.e., H, Me, OMe, Cl, Br, NO_2.

2. Results and discussion

The target compounds **2** were prepared via the C4′-halogenated terpyridine **4** as the key intermediate (Scheme 2). This intermediate was accessed from the picolinates (**1**) via the terpyridone **3**.



Scheme 2. Key intermediates in the synthesis of target compounds **2**.

2.1. Terpyridone formation

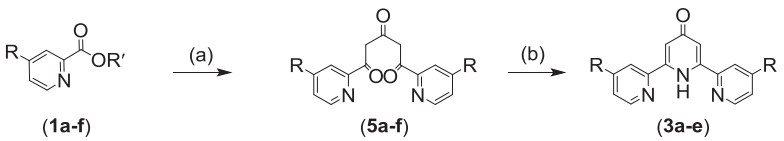
Based on a two-step procedure established by Constable and Ward for the preparation of terpyridin-4′-ones,[16] the 4-substituted picolinates **1a**–**f** were reacted with acetone in a Claisen-like condensation to give the triketones **5a**–**e**, followed by ring closure with ammonium acetate to furnish the symmetrical 4,4″-disubstituted terpyridones **3a**–**e**, (Scheme 3). Constable and Ward performed the first step of this procedure using 3 or more equivalents of the picolinate **1a** and 5 equiv of sodium hydride to 1 equiv of acetone.[16] These investigators reported an initial exothermic reaction occurred, which, once settled, was refluxed for 6 h. In earlier studies, we have found that the number of equivalents of the picolinate can be reduced to 2.25 equiv.[17] Furthermore, we have found that good yields of the triketone **5a** can be obtained by performing the reaction for as little as 2 h at room temperature, as similarly reported by Wieprecht et al. for the chloro analogue **5d**.[18]

Table 1
Terpyridone formation via Claisen-like condensation of 4-substituted picolinates with acetone

Entry	Picolinate			Conditions for step a Scheme 3 [a,b]	Terpyridone		
		R	R′			R	Yield[c]
1	**1a**	H	Et	2 h at 30 °C.	**3a**	H	53%
2	**1b**	Me	Me	18 h at 30 °C.	**3b**	Me	43%
3	**1c**	OMe	Me	18 h at 30 °C.	**3c**	OMe	58%
4	**1d**	Cl	Me	3 h at 30 °C.	**3d**	Cl	58%
5	**1e**	Br	Et	2 h at 30 °C.	**3e**	Br	48%

[a] 2.5 equiv of picolinate used in all instances.
[b] Step b) 18 h, reflux.
[c] Represents the overall yield from the corresponding picolinate.

The unsubstituted terpyridone **3a** was, however, consistently isolated as a stable 1:1 adduct of the terpyridone **3a** with acetic acid (Fig. 1), as evidenced by NMR spectroscopy and a crystal structure.[17]



Scheme 3. Preparation of symmetrical 4,4″-disubstituted terpyridones from 4-substituted picolinates. (a) NaH, DME, acetone; 2–18 h, 30 °C. (b) NH_4OAc, EtOH; 18 h, reflux.

With the exception of the 4-nitropicolinate **1f**, the 4-substituted picolinates **1a**–**1e** reacted readily in the Claisen condensation reaction. The 4-nitropicolinate **1f** did not react and was recovered as starting material. Extended reaction times (18 h at 30 °C) or heating under reflux for 2 h did not change this outcome with **1f**. The 4-chloropicolinate **1d** was prone to substitution at C-4 by methoxide liberated from the ester group of the picolinate **1d** in the course of the reaction, and therefore it was necessary to shorten the reaction time considerably. Reaction at 30 °C for 18 h or refluxing for 6 h lead to the complete displacement of the chloro substituent at C-4 of the picolinate **1d**. However, performing the reaction for 3 h at 30 °C yielded the 4,4″-dichloro-triketone **5d** successfully. The 4-bromopicolinate **1e** did not undergo displacement of the halogen by the ethoxide leaving group, although the Claisen condensation reaction did require gentle heating to allow the reaction to proceed as mentioned above. The triketones **5a**–**e** were isolated as solids that were used without purification. This was not only desirable from a Green Chemistry perspective, but the triketones were brightly coloured and very pervasive and therefore handling was kept to a minimum to avoid contamination of glassware.

Treatment of the triketones **5a**–**e** with ammonium acetate (Scheme 3) lead to ring closure in a condensation reaction that gave the terpyridones **3a**–**e** as crystalline solids in moderate to good overall yields (Table 1). The terpyridone **3d** was obtained in 58% yield containing a trace (ca. 5%) of the 4,4″-dimethoxyterpyridone **3c** (by ^1H NMR spectroscopy) resulting from some dimethoxy triketone **5c** formed during the initial Claisen reaction. This impurity could be readily removed by recrystallisation but for convenience could be carried through to the next step for subsequent removal.

This acetic acid adduct was employed in subsequent reactions without causing any side reactions or decrease in product yield. The other 4,4″-disubstituted terpyridones **3b**–**e** were isolated as their free bases, and not as acetic acid adducts.

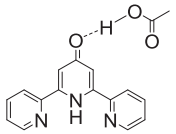


Fig. 1. Acetic acid adduct of (**3a**).

2.2. Halogenation at C-4′

2.2.1. Iodination via an O-triflate. A number of methods were investigated for the C-4′ halogenation of the terpyridones **3a**–**e**. Whilst Constable and Ward[16] have reported the use of $POCl_3/PCl_5$ as