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First access to the spin-labelled β-amino acid POAC in an enantiopure state by resolution through its binaphthyl esters

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Abstract—Resolution of *trans* 3-(9-fluorenylmethyloxycarbonylamino)-1-oxyl-2,2,5,5-tetramethylpyrrolidine-4-carboxylic acid (Fmoc-POAC-OH) was quickly achieved upon esterification with (*aR*)-1,1′-binaphthyl-2,2′-diol, chromatographic separation of the obtained diastereomers, and facile saponification of the aryl ester function with removal of the chiral auxiliary.

Stable nitroxide free radicals are of continuing interest for use as spin labels in the study of conformation and structural mobility of biological systems,[1a–d] as spin traps of other radical species[1e–h] and as oxidizing agents.[1i–k] Optically active nitroxides have also been developed as enantioselective oxidizing agents, and for stereoselective coupling with prochiral radicals.[2] As far as amino acids are concerned, the nitroxide bearing, achiral $C^{\alpha,\alpha}$-disubstituted glycine 4-amino-1-oxyl-2,2,6,6-tetramethylpiperidine-4-carboxylic acid residue (TOAC) (Fig. 1),[3] has been widely used to label peptides at N-terminal and internal positions for biological studies and conformational analysis by ESR methods.[4] The TOAC residue has also been previously employed in our groups as a probe for structural investigations involving intramolecular energy transfer (fluorescence quenching) and intramolecular spin polarization (CIDEP) effects in designed, rigid, peptide-based systems.[5] However, the TOAC structure presents a few disadvantages: its achiral character may hinder access to stereochemical information, and its tetrasubstituted α-carbon induces a reduced reactivity of the amino function which may be problematic if the residue is to be placed at an internal position of a peptide. Accordingly, we have been interested by the prospect of creating *spin-labelled cyclic β-amino acids that could be obtained in enantiopure form*. The cyclic structure was designed to provide molecular rigidity, allowing a topologically well defined placement of the nitroxide function, while the choice of a β-amino acid structure was expected to allow easier peptide couplings than the TOAC structure. With this aim, we have recently reported the synthesis of enantiopure 4-amino-1-oxyl-2,2,6,6-tetramethylpiperidine-3-carboxylic acid (*cis*-β-

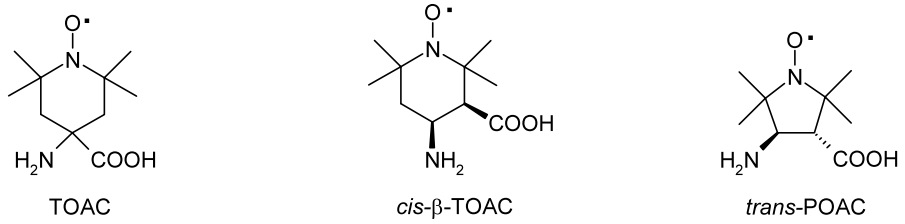

Figure 1. Chemical structures of the spin-labelled amino acids TOAC, β-TOAC and POAC.

Keywords: binaphthol esters; chiral nitroxides; modified β-amino acids; spin-labelled amino acids; POAC; resolution.
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TOAC),[6] and we now wish to present our preliminary results relative to the practical resolution of 3-amino-1-oxyl-2,2,5,5-tetramethylpyrrolidine-4-carboxylic acid (*trans*-POAC) (Fig. 1).

The interest in the *trans* POAC residue, first described by Rassat and Rey,[3a] was recently highlighted by Nakaie and co-workers,[7] who repeated Rassat's synthesis in order to obtain *trans*-**5** (Fig. 2), and then prepared its N^{α}-Fmoc (9-fluorenylmethyloxycarbonyl) protected derivative *trans*-**6** for use in solid-phase peptide synthesis in view of its incorporation into an angiotensin II analogue. In that study it was noted that coupling of the next amino acid after the POAC residue proceeded smoothly, whereas the equivalent coupling after the TOAC residue required a large excess of reagent and repeated coupling steps.[7,8] Another potential utility of POAC concerns the field of β-peptides, which has been the subject of increasing interest in recent years, after it was demonstrated that oligomers of β-amino acids may fold into new stable helical conformations.[9] Therefore, as it represents a valuable additional spin-labelled β-amino acid, POAC is likely to be used as a tool for conformational studies of β-peptides by ESR methods, in the same way that TOAC has been used in the α-amino acid series.

To our knowledge, POAC was synthetically obtained only as a mixture of the two *trans* enantiomers and used as such.[3a,7] The access to both its enantiomers in enantiopure form appeared to us to be highly desirable: (*i*) in order to avoid separation problems related to the formation of diastereomers in the couplings with chiral α- or β-amino acids, and (*ii*) in view of investigations into β-peptide helix conformations, as it has been shown that absolute configuration at C^{α} and C^{β} in β-amino acids influences helical screw sense.[9,10]

We began by exploring alternative pathways for the asymmetric synthesis of *trans*-POAC by Michael addition of (*R*)-(+)-α-methylbenzylamine to 3-carbomethoxy-2,2,5,5-tetramethylpyrroline **3a**[11] (Fig. 3), its *N*-Boc protected derivative **3b**,[12] 3-cyano-1-oxyl-2,2,5,5-tetramethylpyrroline **3c**,[13] a synthetic intermediate in the preparation of *trans*-**5** (Fig. 2), and its 3-carbomethoxy-1-oxyl 2,2,5,5-tetramethylpyrroline analogue **3d**,[13] both readily obtained from the carboxamide **1**.[13] We had confidence in this strategy, as not only was it the key step in the synthesis of racemic *trans*-POAC **5** via Michael addition of ammonia to the α,β-unsaturated nitrile **3c**,[3a,7] but also because Gellman and co-workers have recently achieved an asymmetric synthesis of *trans*-3-aminopyrrolidine-4-carboxylic acid by Michael addition of (*R*)-α-methylbenzylamine to the corresponding α,β-unsaturated ester.[14]

However, in our hands, none of the substrates **3a**–**d** provided the corresponding Michael adduct, whatever the applied activation modes (Fig. 3):[15] use of water,[14] or ytterbium triflate catalysis,[16] or combined use of pressure and microwave activation.[17,18] Even Michael addition of aqueous ammonia on the methyl ester **3d**, which was attempted in anticipation of a considered asymmetric addition on chiral α,β-ethylenic esters, failed when performed under the same experimental conditions as those employed in the reaction of **3c**.

Having met with failure in our attempts at direct asymmetric synthesis, we switched to the search for a practical resolution procedure of racemic POAC, that we

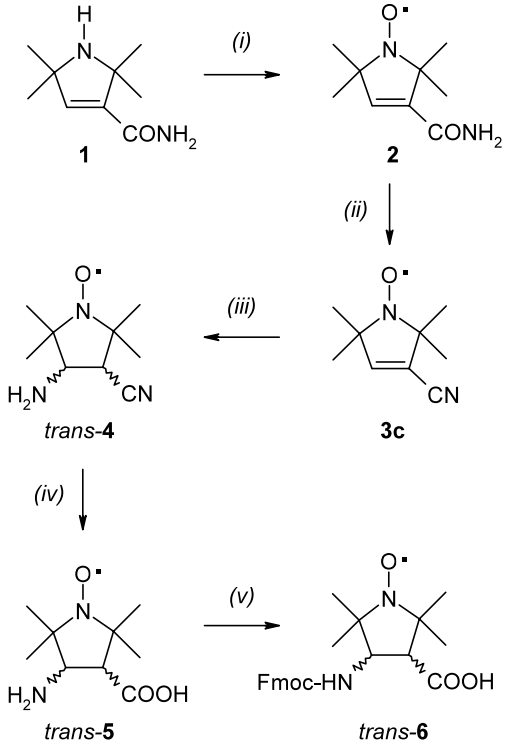

Figure 2. Synthetic path for the preparation of racemic *trans*-POAC **5**[3a,7] and *trans*-Fmoc-POAC-OH **6**[7] (the *trans* spatial relationship between the C^4–N bond and the C^3–CN or C^3–COOH bond of compounds **4**, **5** and **6** is not represented to avoid confusion with enantiomerically pure compounds). (*i*) $NaWO_4{\cdot}2H_2O$; H_2O_2 35%; $NaHCO_3$; MeOH/MeCN; 0°C to rt; 24 h. (*ii*) TsCl; pyridine; rt; 36 h. (*iii*) NH_3 (aq); rt; 5 days. (*iv*) $Ba(OH)_2$; H_2O; reflux; 48 h. (*v*) Fmoc-OSu (Fmoc-succinimidyl carbonate); $NaHCO_3$; acetone:H_2O 2:1; rt; 18 h.

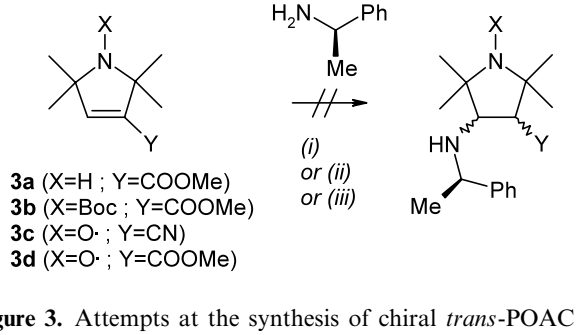

Figure 3. Attempts at the synthesis of chiral *trans*-POAC by asymmetric Michael addition to the α,β-unsaturated esters or nitrile **3a**–**d**. (*i*) H_2O; 55°C; 8 days. (*ii*) $Yb(OTf)_3$; toluene; 50°C; 5 days. (*iii*) Microwave activation, (a) no solvent; 150°C; 10 min (1.6 bar), or (b) KF/alumina; no solvent; 150°C; 10 min, or (c) H_2O; 150°C; 10 min (6 bar), or (d) H_2O:THF 2:1; 100°C; 10 min (3 bar), or (e) xylene; 150°C; 10 min (1.6 bar).

synthesized by Rassat's method. The nitrile **3c** was reacted with aqueous ammonia at room temperature and atmospheric pressure, to provide the *trans*-β-amino nitrile **4**[3a] in 40% yield, accompanied by 32% of the amide **2** which could be recycled in later runs (Fig. 2). The ^{1}H NMR spectrum of **4**, performed after sodium dithionite reduction of the nitroxide group,[19] confirmed that only the *trans* enantiomers were formed under these conditions, as previously demonstrated by X-ray diffraction analysis.[7] Basic hydrolysis of *trans*-**4** provided the free amino acid *trans*-POAC **5**,[3a] which was immediately N^{α}-protected upon treatment with Fmoc-OSu, to afford racemic *trans*-Fmoc-POAC-OH **6**[7] in 58% overall yield.

For resolution of *trans*-**6** we discarded the methods involving amide bond formation, with either amines or amino acid derivatives as chiral auxiliaries, because of the harsh acidic conditions required for removal of the chiral auxiliary from the separated diastereomers, incompatible with the presence of the nitroxide group of POAC.[4a] Rather, we considered resolution through formation of diastereomeric pairs of amino esters by reaction with chiral alcohols, only a few examples of which, notably using menthol as auxiliary, are known.[20] For this purpose, we selected 2,2′-dihydroxy-1,1′-binaphthyl (binaphthol) as a new and potentially efficient chiral auxiliary, taking into account the known resolution of related dihydroxy-1,1′-binaphthyl compounds by means of their esterification with chiral amino acids (the reverse as in the present case).[21,22] Indeed, while parallel attempts of esterification with (−)-menthol, (−)-10-dicyclohexylsulfamoyl-(D)-isoborneol and 1,2:3,4-di-*O*-isopropylidene galactose either did not work or did not provide separable diastereomeric esters, the use of (*aR*)-binaphthol to form the mono-esters (Fig. 4) allowed the easy separation of the two obtained diastereomers **7a**[12] and **7b**,[12] isolated in 40 and 41% yield (80 and 82% of theoretical yield), respectively, by standard column chromatography on silica gel. Advantageously, for resolution up to a gram-scale, it was also possible, prior to chromatography, to partially purify the mixture by crystallization, as the diastereomer with a lower R_f (**7b**) had an appreciably poorer solubility in non-polar solvents than that with a higher R_f (**7a**). Unfortunately, the obtained crystals were not suitable for X-ray diffraction analysis, which prevented the determination of the absolute configuration of **7a** and **7b**, either (*aR*,3*R*,4*R*) or (*aR*,3*S*,4*S*) (Fig. 4).

Alkaline hydrolysis of the esters **7a** and **7b** resulted in partial cleavage of the Fmoc protecting group, as expected.[23] Subsequent re-protection of the amine function gave the desired Fmoc-amino acids (+)-**6**[12] and (−)-**6**,[12] in 57 and 43% yield, respectively. The two enantiomers were examined by chiral HPLC using a Chiralcel OD-RH column,[24] which demonstrated the *ee* of each to be >99.5%. We are currently pursuing the search for a crystalline derivative of (+)-**6** and (−)-**6** to allow the assignment of their absolute configuration by X-ray diffraction analysis.

Altogether, the present study provides two conclusive results: (*i*) the access to both enantiomers of POAC in an enantiomerically pure state is now open for the first time, and (*ii*) the straightforward resolution through separation of the diastereomeric binaphthyl monoesters of Fmoc-POAC-OH, represents a new procedure which in our view deserves to be further investigated as a general method for the separation of β- or α-amino acid enantiomers.

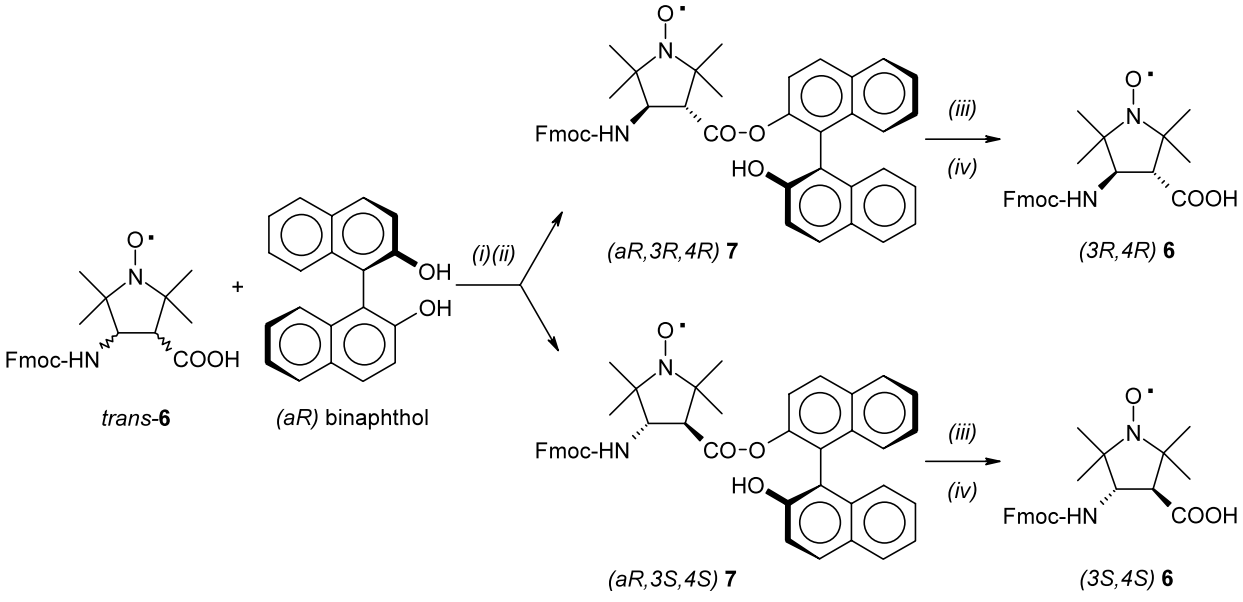

Figure 4. Resolution of *trans* Fmoc-POAC-OH through its (*aR*) binaphthyl mono-esters. (*i*) EDC [1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride]; DMAP (4-dimethylaminopyridine); CH_2Cl_2:MeCN 1:1; 0°C; 2 h. (*ii*) Crystallization CH_2Cl_2:Et_2O 1:1 then chromatography *c*Hex:EtOAc 7:3. (*iii*) NaOH; H_2O; MeOH; 40°C; 6 h. (*iv*) Fmoc-OSu; $NaHCO_3$; acetone:H_2O 2:1; rt; 18 h.

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