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4-Amino-1-oxyl-2,2,6,6-tetramethylpiperidine-3-carboxylic acid (β-TOAC), the first spin-labelled, cyclic, chiral β-amino acid resolved in an enantiomerically pure state

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Abstract—Amination of 3-carboxymethyl-1-oxyl-2,2,6,6-tetramethyl-4-piperidone with (*R*)-α-methylbenzylamine, $NaBH_3CN$ reduction of the resulting enamine and removal of the chiral auxiliary from the separated diastereoisomers, led to enantiomerically pure (3*S*,4*S*) and (3*R*,4*R*) methyl 4-amino-1-oxyl-2,2,6,6-tetramethylpiperidine-3-carboxylates. © 2003 Elsevier Science Ltd. All rights reserved.

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] Moreover, optically active nitroxides were developed as enantioselective oxidizing agents and for stereoselective coupling with prochiral radicals.[2] Following on from previous work in our groups with the nitroxide-based, achiral, C^{α}-tetrasubstituted α-amino acid TOAC (4-amino-1-oxyl-2,2,6,6-tetramethylpiperidine-4-carboxylic acid),[3] we were interested by the prospect of creating a new chiral, spin-labelled amino acid.

The TOAC residue[4] (Fig. 1) was used to label peptides at *N*-terminal and internal positions for conformational and biological studies.[5] The tetrasubstituted α-carbon of TOAC is responsible for its ability to induce β-turn or 3_{10}/α-helical structures in peptides, but also for the reduced reactivity of its amino group, which may be problematic if the residue is to be placed at an internal position of a peptide.[6] The β-amino acid POAC (3-amino-1-oxyl-2,2,5,5-tetramethylpyrrolidine-4-carboxylic acid), first described by Rassat and Rey,[4a] was recently incorporated by solid phase synthesis into an angiotensin-II analogue.[7] 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] However, to our knowledge, the POAC residue was synthesized as a mixture of the two *trans* enantiomers, and used as such.[9] It appeared

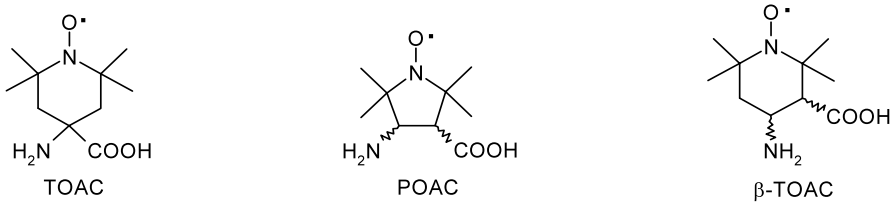

Figure 1. Chemical structures of the spin-labelled amino acids TOAC, POAC and β-TOAC.

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to us to be desirable to design a cyclic derivative that could be obtained in enantiopure form, while keeping the β-amino acid structure that allows easy peptide coupling. The compound which we named β-TOAC (Fig. 1) was the chosen target.

The β-amino acids have been the subject of much synthetic effort in recent years, particularly after it was demonstrated that their oligomers may fold into stable helical conformations.[10] Gellman and co-workers successfully developed asymmetric routes to *trans*-3-aminopyrrolidine-4-carboxylic acid and *trans*-2-aminocyclopentanecarboxylic acid.[11] The key steps in their syntheses were the reductive amination of a β-ketoester with either (*R*)- or (*S*)-α-methylbenzylamine in the presence of $NaBH_3CN$, and subsequent selective crystallisation of the HCl salts of the obtained β-amino esters, to provide either *trans* enantiomer. A similar method for the preparation of *cis*-2-aminocyclohexanecarboxylic acid had previously been described by Xu et al.[12] who performed the reduction step using $NaBH_4$ and an organic acid, and isolated the major diastereomer formed as its HBr salt. This pathway was used recently by Gellman and co-workers[13] who eventually obtained *trans*-2-aminocyclohexanecarboxylic acid by epimerization of the *cis* isomer. We decided to attempt such a procedure applied to 3-carboxymethyl-1-oxyl-2,2,6,6-tetramethyl-4-piperidone **2** (Fig. 2) as a route to enantiopure β-TOAC.

Commercially available 2,2,6,6-tetramethyl-4-piperidone was oxidized by a described procedure to its 1-oxyl derivative **1** (Fig. 2).[14] The efficiency of carboxylation of this compound with carbon dioxide in the presence of potassium phenoxide following the published method[15] proved to be variable in our hands; the yield of esterified product **2** obtained after reaction with diazomethane (lit. 35%) varied between 15 and 50% over different runs performed under apparently the same conditions. Amination of **2** with (*R*)-α-methylbenzylamine in the presence of acetic acid proceeded smoothly to provide the desired products (*R*)-**3**[16] in 61% yield. The reaction was also performed with (*S*)-α-methylbenzylamine, to give (*S*)-**3**[16] in 66% yield (not shown). The enamine structure of **3** was determined from its ^1H NMR spectrum.[17]

The isolated enamine (*R*)-**3** was reduced in the presence of $NaBH_3CN$ and acetic acid. These conditions are known not to affect the nitroxide group.[19] The mixture of reduced products **4** was purified by column chromatography. The desired HCl salts were prepared by adding a solution of HCl in EtOAc to a cold solution of **4** in EtOAc. The precipitate formed was collected and recrystallised from MeCN to give (1′*R*,3*S*,4*S*)-**4**[16] as a sole diastereomer as judged from its ^1H NMR spectrum. The other diastereomer (1′*R*,3*R*,4*R*)-**4**[16] was obtained from the EtOAc mother liquor. The same reaction sequence was followed starting from the enamine (*S*)-**3**, to give (1′*S*,3*R*,4*R*)-**4**[16] as crystals and (1′*S*,3*S*,4*S*)-**4**[16] from the EtOAc mother liquor (not shown).

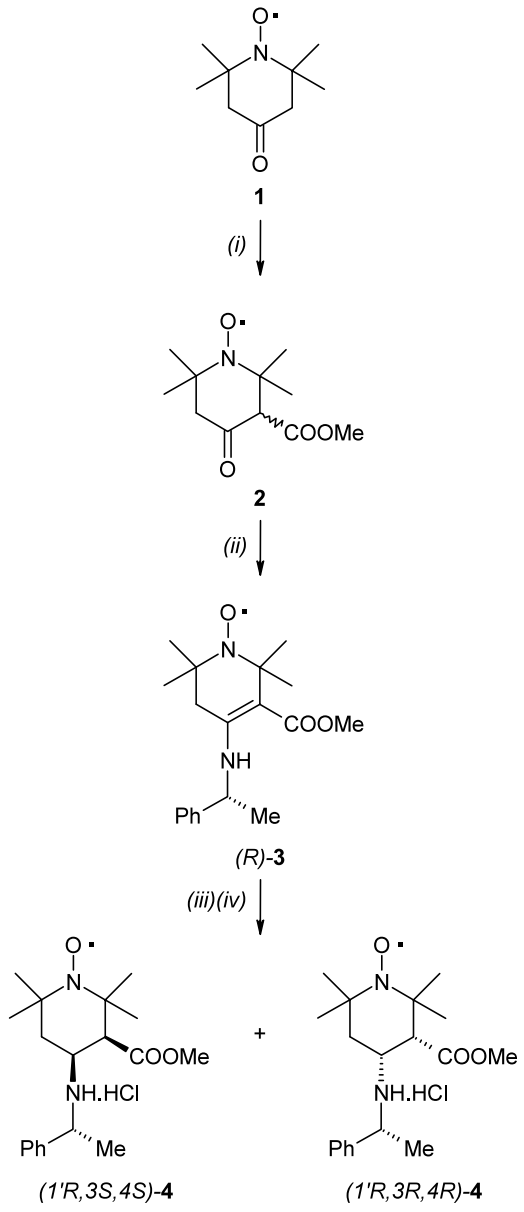

Figure 2. Synthetic path for amination of 3-carboxymethyl-2,2,6,6-tetramethyl-4-piperidone **2** by (*R*)-α-methylbenzylamine, followed by reduction of the resulting enamine (*R*)-**3**. (i) KOPh; CO_2; DMF; rt; 2 h, then CH_2N_2; Et_2O. (ii) (*R*)-α-methylbenzylamine; AcOH; EtOH; 4Å MS; rt; 48 h. (iii) $NaBH_3CN$; AcOH; EtOH; 75°C; 2 h. (iv) HCl/EtOAc; 0°C; filtration and recrystallisation from MeCN.

The derivative (1′*S*,3*R*,4*R*)-**4** was obtained after recrystallisation from MeCN as large orange crystals suitable for X-ray diffraction analysis (Fig. 3),[20] allowing the assignment of the absolute configuration at C_3 and C_4.

The crystal structure confirmed the *cis* configuration of the β-amino ester, which had been suggested by the NMR spectra. This result is in agreement with the findings of Cimarelli and Palmieri,[21] who obtained *cis* products from $NaHB(OAc)_3$ reduction of cyclic β-enamino esters. It is also consistent with the already mentioned results of Xu et al.[12] and Gellman and co-workers[13] in the case of six-membered rings. How-

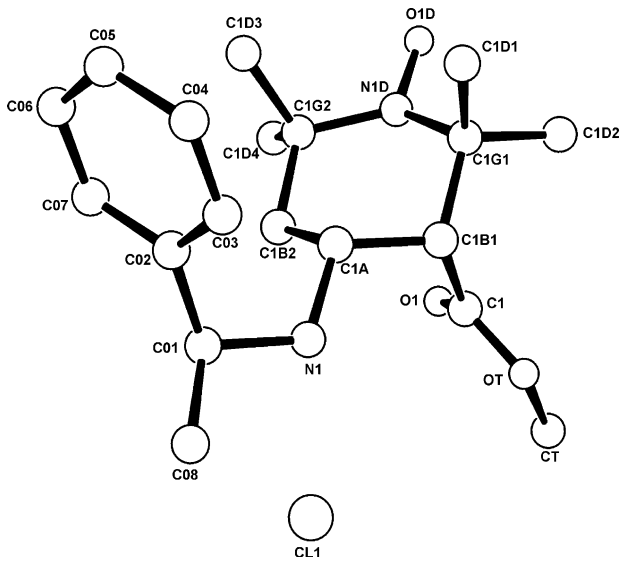

Figure 3. X-Ray diffraction structure of (1′*S*,3*R*,4*R*)-**4**·HCl.[20]

ever, it is at variance with that of Gellman and coworkers,[11] who had obtained the *trans* reduction products from a pyrrolidine substrate when $NaBH_3CN$ was used as the reducing agent.

Removal of the chiral auxiliary from **4** by hydrogenation over Pd/C proved to be a little delicate. While it is known that the nitroxide group may be completely reduced under these conditions to the secondary amine,[22] it is also possible to obtain an intermediate *N*-hydroxy compound, from which the nitroxide function can be regenerated.[23,24] Our initial hydrogenation attempts carried out at a pressure of 50 psi for several hours resulted in complex mixtures of products. We found that hydrogenation at atmospheric pressure and for only a short period (<30 min) was enough to allow efficient cleavage of the α-methylbenzyl group, while avoiding over-reduction of the nitroxide group to the amine and subsequent decomposition. The nitroxide function was regenerated by stirring the crude hydrogenated product open to the air in the presence of $Cu(OAc)_2$ for 7 days. In this way compounds (3*S*,4*S*)-**5**[16] and (3*R*,4*R*)-**5**[16] were obtained in 54 and 62% yield, respectively (Fig. 4). A derivative suitable for peptide synthesis was prepared from (3*S*,4*S*)-**5** by saponification of the methyl ester and Fmoc (9-fluorenylmethyloxycarbonyl) protection of the amino group, to provide the building block (3*S*,4*S*)-**6**[16] in 45% yield. The other enantiomer (3*R*,4*R*)-**6**[16] was prepared in the same way from (3*R*,4*R*)-**5** in 83% yield.

HPLC analysis of (3*S*,4*S*)-**6** and (3*R*,4*R*)-**6** on a Chiralcel OD-RH column[25] showed the enantiomeric excess of each to be >99.5%.

In conclusion, the two enantiomers of the *cis* β-TOAC residue, bearing a nitroxide function, have been synthesized in enantiopure form and in a reasonable yield. We are currently investigating epimerization of the major *cis* amination products **4** to their *trans* isomers, as a

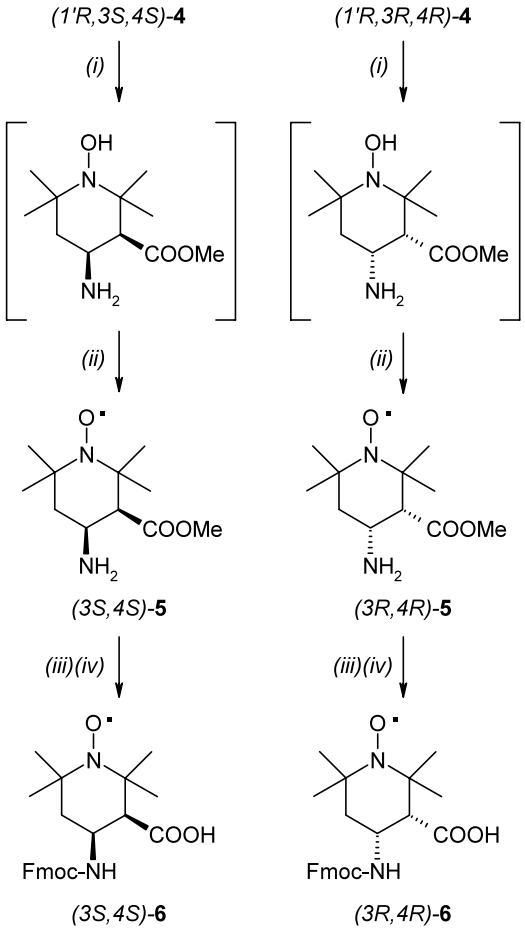

Figure 4. Removal of the chiral auxiliary from diastereoisomers **4**, followed by *C*-deprotection/*N*-protection of the resulting β-amino ester enantiomers **5**. (i) Pd/C 10%; 95% EtOH; rt; 30 min. (ii) $Cu(OAc)_2$; MeOH; air; rt; 7 days. (iii) NaOH (aq); MeOH; reflux; 5 h. (iv) Fmoc-succinimidyl carbonate; $NaHCO_3$; acetone/water 2:1; rt; 18 h.

route to the *trans* β-TOAC enantiomers. We believe that these novel spin-labelled, rigid and chiral β-amino acids will find applications in peptidomimetic conformational and biological studies.

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