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Synthesis and spectroscopic characterization of enantiopure protected *trans*-4-amino-1-oxyl-2,2,6,6-tetramethyl piperidine-3-carboxylic acid (*trans* β-TOAC)

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Abstract—Enantiomerically pure (3*R*,4*S*) and (3*S*,4*R*) protected 4-amino-1-oxyl-2,2,6,6-tetramethylpiperidine-3-carboxylic acids were synthesized by reduction of the enamines resulting from the condensation of 3-carboxymethyl-1-oxyl-2,2,6,6-tetramethyl-4-piperidone with (*R*) or (*S*)-α-methylbenzylamine. While $NaBH_3CN/CH_3COOH$ reduction gave predominantly a mixture of the two possible *cis*-diastereomers, the use of $NaBH_4/(CH_3)_2CHCOOH$ resulted in a mixture of *only* one *trans*- and one *cis*-diastereomer. Removal of the chiral auxiliary from the separated diastereoisomers by hydrogenolysis and regeneration of the nitroxide radical gave the desired β-amino esters. The ESR spectrum of the (3*R*,4*S*)-enantiomer is also reported.

Nitroxide free radicals have found applications in a variety of fields because of their significant stability, for example, (i) as spin labels in the study of conformation and structural mobility of biological systems,[1] including previous work from 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)[2] (Fig. 1), (ii) as spin traps of other radical species,[3] and (iii) as oxidizing agents.[4] Optically active nitroxides have been employed as enantioselective oxidizing agents and for stereoselective coupling with prochiral radicals.[5] We recently reported the synthesis of both enantiomers of *cis*-β-TOAC (*cis*-4-amino-1-oxyl-2,2,6,6-tetramethylpiperidine-3-carboxylic acid), a cyclic β-amino acid bearing a nitroxide group.[6] However, we were drawn towards the synthesis of the *trans*-enantiomers of these compounds, given their similarity to the β-amino acids *trans*-ACHC (2-aminocyclohexane-1-carboxylic acid),[7] and *trans*-APiC (4-aminopiperidine-3-carboxylic acid).[8] Gellman and co-workers had demonstrated that oligomers of *trans*-ACHC fold to preferentially form a helical secondary structure, the 3_{14}-helix,[7]

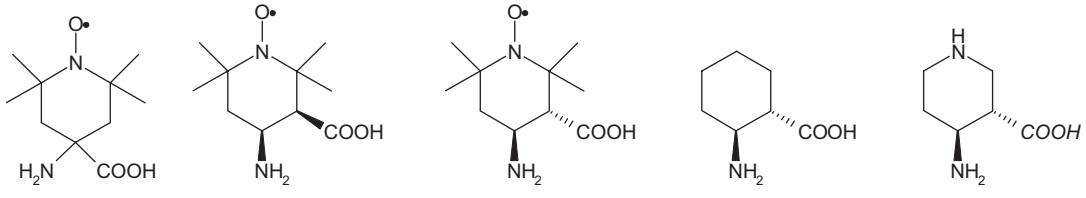

Figure 1. Chemical structures of the amino acids TOAC, β-TOAC, ACHC and APiC.

Keywords: Chiral nitroxides; Modified β-amino acids; Spin-labelled amino acids; *trans*-β-TOAC.
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in which *trans*-APiC residues can be incorporated.[8] Similar β-peptide helical structures were later shown to be stable in both organic and aqueous solvents, and resistant to enzymic hydrolysis and microbial digestion.[9]

We were interested in the incorporation of the *trans*-β-TOAC residue into a β-peptide, and the examination of the resulting secondary structure using ESR spectroscopy in the same way that we had used TOAC to probe 3_{10}-helical forms in α-peptides.[2] With this aim in mind, we needed a synthetic route to *trans*-β-TOAC that would furnish us with a sufficient quantity of nitroxide β-amino acid to allow subsequent peptide synthesis.

Our initial intention was to use the synthetic pathway that we had already developed to obtain the *cis*-β-TOAC diastereomers **3** (Fig. 2),[6a] and then to epimerize these compounds under basic conditions to obtain the corresponding *trans*-diastereomers, in the same manner as the route reported by Gellman and co-workers for the synthesis of *trans*-ACHC and *trans*-APiC.[8] Amination of 3-carboxymethyl-1-oxyl-2,2,6,6-tetramethyl-4-piperidone **1** with either (*R*)- or (*S*)-α-methyl-benzylamine gave the corresponding enamine (*R*)-**2** or (*S*)-**2** in 87% and 82% yield, respectively. The enamine (*R*)-**2** or (*S*)-**2** was reduced in the presence of $NaBH_3CN$ and acetic acid to give a 1:1 mixture of the *cis*-diastereomers **3**. Under these conditions, only traces of each of the two *trans*-diastereomers could be detected by ^1H NMR in the crude reduction product.[10,11] The *cis*-diastereomers **3** were separated, as previously described, by formation of their HCl salts and selective crystallization from MeCN.[6a] Selective epimerization at carbon-3 of these products in the presence of NaOMe gave 1:1 cis/trans mixtures as determined by ^1H NMR analysis of the crude products.[10,11] However, while *trans* (1′*S*,3*R*,4*S*)-**3** could be isolated in 43% yield from the epimerization of *cis* (1′*S*,3*S*,4*S*)-**3**, the diastereomer *trans* (1′*S*,3*S*,4*R*)-**3** could not be separated by column chromatography from *cis* (1′*S*,3*R*,4*R*)-**3**.

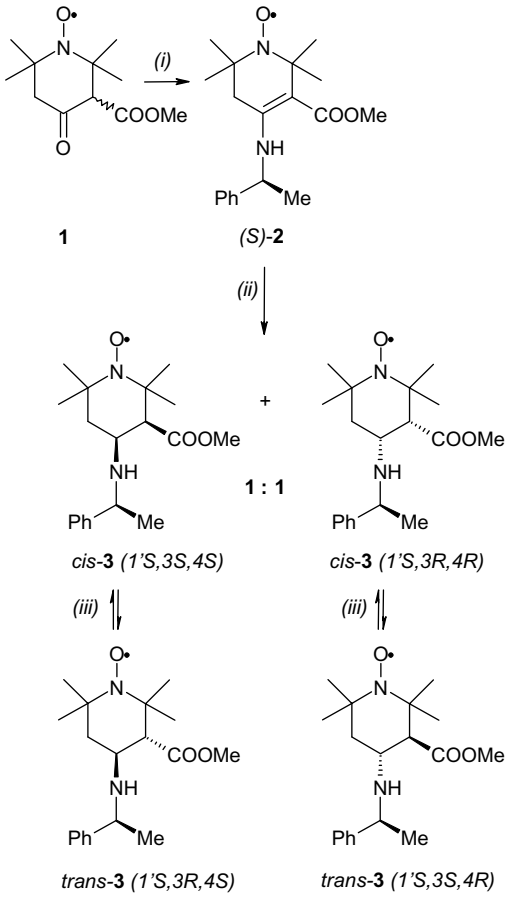


Figure 2. (i) (*R*)-α-Methyl-benzylamine; AcOH; EtOH; 4 Å-MS; rt; 48 h, 87%; (ii) $NaBH_3CN$; AcOH; EtOH; 75 °C; 2 h; then HCl/EtOAc; 0 °C; filtration and recrystallization from MeCN, (1′*S*,3*S*,4*S*)-**3** 31%, (1′*S*,3*R*,4*R*)-**3** 26%; (iii) NaOMe; MeOH; 70 °C; 18 h, *trans* (1′*S*,3*R*,4*S*)-**3** 43%, *cis* (1′*S*,3*S*,4*S*)-**3** 47%.

To obtain greater quantities of a single diastereomer of *trans*-**3** we reinvestigated the reduction of enamine (*S*)-**2**, applying the optimized reaction conditions ($NaBH_4$ in the presence of a bulky organic acid) previously developed by Xu and et al.[12] for the synthesis of ethyl 2-amino-1-cyclohexanecarboxylates by asymmetric reduction of the corresponding α-methylbenzylamine derived enamine. However, when using $NaBH_4$ in the presence of excess isobutyric acid and toluene as co-solvent, we were surprised by the results obtained: ^1H NMR analysis of the crude reaction product revealed the presence of only one *cis* (1′*S*,3*S*,4*S*)-**3** and one *trans* (1′*S*,3*R*,4*S*)-**3** diastereomer in a ratio of ca. 1:1 (Fig. 3), with no trace of the other two diastereomers.[10,11] Chromatographic separation afforded *cis* (1′*S*,3*S*,4*S*)-**3** [6a] in 29% yield and *trans* (1′*S*,3*R*,4*S*)-**3**[13,14] in 30% yield.

At this stage we are unable to explain the selectivity of the reduction of enamine (*S*)-**2** in the presence of isobutyric acid. In any case, we were able to take advantage of this finding to shorten the synthetic path leading to *trans*-β-TOAC. Hydrogenation of the hydrochloride salt of *trans* (1′*S*,3*R*,4*S*)-**3** over Pd/C for 30 min, followed by regeneration of the nitroxide radical from the resulting hydroxylamine intermediate with a catalytic amount of $Cu(OAc)_2$ in the air for 7 days,[15,16] gave the desired *trans* H-(3*R*,4*S*)-β-TOAC-OMe **4** diastereomer[13] in 41% yield (Fig. 4). This compound is indeed ESR-active and its spectrum in MeOH solution shows a three line pattern typical of mono-nitroxide radicals (Fig. 5). Sim-

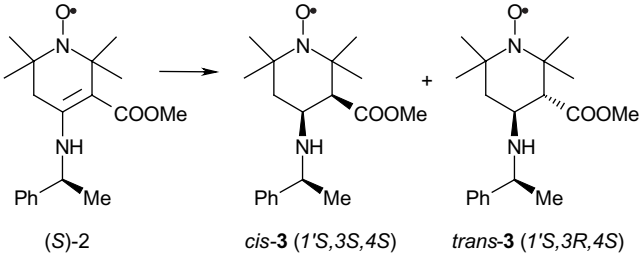
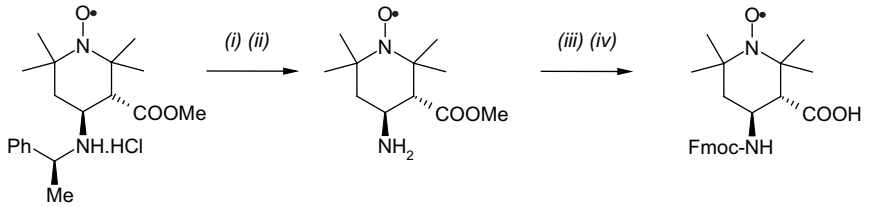


Figure 3. Reduction of the enamine (*S*)-**2** with $NaBH_4$, $(CH_3)_2$-CHCOOH, toluene, 0 °C to rt, 24 h; *cis* (1′*S*,3*S*,4*S*)-**3** 29%, *trans* (1′*S*,3*R*,4*S*)-**3** 30%.



trans-**3** (*1'S,3R,4S*) *trans* H-(*3R,4S*)-β-TOAC-OMe **4** *trans* Fmoc-(*3R,4S*)-β-TOAC-OH **5**

Figure 4. Removal of the chiral auxiliary from **3**, followed by C-deprotection/N-protection of the resulting β-amino ester enantiomer **4**. (i) Pd/C 10%; 95% EtOH; rt; 30 min. (ii) $Cu(OAc)_2$; MeOH; air; rt; 7 days, 41%; (iii) NaOH (aq); MeOH; reflux; 5 h. (iv) Fmoc-succinimidyl carbonate; $NaHCO_3$; acetone/water 2:1; rt; 18 h, 40%.

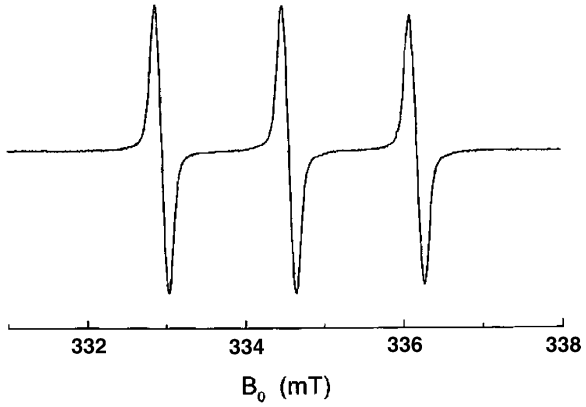


Figure 5. ESR spectrum of *trans* H-(3*R*,4*S*)–TOAC-OMe **4** in a 0.5 mM MeOH solution at room temperature.

ilar treatment of *trans* (1′*R*,3*S*,4*R*)-**3**[13] obtained by a similar $NaBH_4$ reduction of enamine (*R*)-**2** in an isobutyric acid/toluene solution, gave the *trans* H-(3*S*,4*R*)-β-TOAC-OMe **4**[13] enantiomer (not shown). Saponification of the methyl ester function of *trans* H-(3*R*,4*S*)-β-TOAC-OMe **4** and *trans* H-(3*S*,4*R*)-β-TOAC-OMe **4** followed by protection of the amino function with the Fmoc group gave the β-amino acid derivatives *trans* Fmoc-(3*R*,4*S*)-β-TOAC-OH **5** and its (3*S*,4*R*) **5** enantiomer (Fig. 4), suitable for use in peptide synthesis.

In conclusion, the two enantiomers of the *trans*-β-TOAC residue, bearing a nitroxide function, have been synthesized in enantiopure form and in a reasonable yield. Their synthesis was facilitated by an unexpected selectivity in the reduction of an enamine intermediate. Derivatives **4** and **5** of *trans* (3*R*,4*S*)-β-TOAC are currently being used, in combination with (1*S*,2*S*)-ACHC, for the synthesis and 3D-structural analysis of a designed β-hexapeptide.

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10. Well resolved ^{1}H and ^{13}C NMR spectra could only be obtained by preliminary reduction of the nitroxide function with sodium dithionite.[11]
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13. All new compounds gave satisfactory analytical data ($^{1}H/^{13}C$ NMR, C,H,N analysis and/or ESI/MS). Further experimental details of syntheses will be given in a full account of this study. Optical rotations $[\alpha]_{546}^{25}$ are as follows: (1′*S*,3*R*,4*S*)-**3**: −52 (*c* 0.27; MeOH). (1′*R*,3*S*,4*R*)-**3**: +48 (*c* 0.28; MeOH). (3*R*,4*S*)-**4**: −24 (*c* 0.1; MeOH). (3*S*,4*R*)-**4**: +24 (*c* 0.1; MeOH).
14. Synthesis of (1′*S*,3*R*,4*S*)-**3**: Isobutyric acid (3.5 mL, 30 mmol) was cooled to 0 °C and $NaBH_4$ (288 mg, 7.6 mmol) was added slowly in portions over 40 min. The mixture was then stirred at rt for 30 min. The enamine (*S*)-**2** (497 mg, 1.5 mmol) was dissolved in toluene (4 mL) and added dropwise to the mixture. Further portions of $NaBH_4$ (57 mg, 1.5 mmol) were added to the mixture after 2, 4 and 12 h. After 20 h, the mixture was diluted with CH_2Cl_2 (40 mL) and washed with saturated $NaHCO_3$ solution (2 × 30 mL) and with brine (30 mL). The organic phase was dried over $MgSO_4$, filtered and concentrated. Column chromatography using cHex/EtOAc 2:1 as eluent gave successively the enamine (*S*)-**2** (67 mg, 13%), (1′*S*,3*R*,4*S*)-**3** (151 mg, 30%) and (1′*S*,3*S*,4*S*)-**3** (149 mg, 29%). The *trans*-diastereomer (1′*S*,3*R*,4*S*)-**3** was converted to its HCl salt by addition of a solution of HCl in EtOAc for characterization. ^{1}H NMR (D_2O) 7.42 (m, 5H, ArH), 4.52 (q, 1H, PhC*H*CH_3), 3.78 (m, 1H, H-4), 3.72 (s, 3H, OCH_3), 2.97 (d, 1H, H-3, $J_{3,4}$ = 11.8 Hz), 1.70, 1.40 (2m, 2H, H-5), 1.58 (d, 3H, PhCHC*H*$_3$), 1.46, 1.25, 1.16, 1.04 (4s, 12H, 4CH_3); ^{13}C NMR 173.1 (C=O), 151.9, 137.9, 133.1, 132.5, 130.9 (ArC), 62.7, 61.2 (C_2, C_6), 58.8 (*C*HPh), 56.4 (OCH_3), 55.1 (C_4), 53.3 (C_3), 39.8 (C5), 31.5, 31.3, 25.6, 24.5, (CH_3), 21.0 (Ph*C*H$_3$); ES-MS *m*/*z* (%) 335.2 (100) $[M+2H]^+$, 334.2 (61) $[M+H]^+$.
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16. The extent of oxidation of the intermediate hydroxylamine to the nitroxide was monitored by thin layer chromatography.