

Synthesis and resolution of new paramagnetic α -amino acids

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Dedicated to Professor Csaba Szántay on the occasion of his 80th birthday

Abstract

New, paramagnetic unnatural α -amino acids were synthesized by the O'Donnell method. In the new amino acids nitroxide is condensed with thiophene, benzene, and tetrahydroisoquinoline ring, or linked through a methylene, benzyl or propargyl spacer. Some of the racemic paramagnetic α -amino acid esters described earlier or in this work were resolved by fractional crystallization of diastereomeric salts. Another approach for optically active paramagnetic amino acids is the modification of *S*-tyrosine derivatives with Pd-catalyzed cross-coupling reactions with paramagnetic acetylene and with a paramagnetic boronic acid.

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Keywords: Amino acid; Nitroxides; O'Donnell reaction; Pd-catalyzed cross-coupling; Resolution

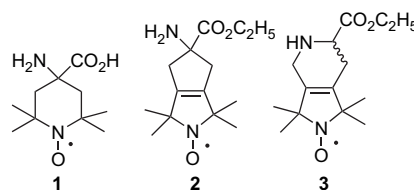
1. Introduction

Proteins, consisting of 20 natural amino acids are responsible for the majority of functional attributes of living systems from viruses to mammals. These biopolymers are formed by natural biosynthetic pathways at the ribosome. The physical and chemical properties of proteins are a reflection of the side chains of each of the constituent amino acids. However, for studying function and structure of proteins by various spectroscopic methods it is often necessary to have special groups on the amino acids with various selected physical and chemical properties, such as fluorescent-, NMR-, EPR-probe group and crosslinker.¹

One of the most convenient and economical methods for studying a protein is the chemical or biochemical modification of it, mainly at the amino acid side chain,² however, this method requires high function- and regioselectivity. The other approach is the incorporation of amino acids into the protein by classical Merrifield amino acid synthesis or by solid-phase peptide synthesis.³ Recent development in incorporating

unnatural amino acids into proteins in vivo opens up entirely new pathways for determining protein function and structure. The molecular engineering of the translational machinery that consists of transfer RNA and aminoacyl tRNA synthetase to recognize unnatural amino acids makes possible the incorporation of an unnatural amino acid into a specific protein site during translation by applying amber codon as the genetic codon for unnatural amino acids.⁴ This new method induced a renaissance in synthesizing various unnatural amino acids.

A helicogenic paramagnetic amino acid, TOAC (**1**)⁵ (Scheme 1) has been widely used and incorporated into many peptides such as alamethicin,⁶ phospholamban,⁷ and melanocortin peptides⁸ just to mention a few recent examples.



Scheme 1. Structures of TOAC (**1**) and PTOAC (**2**) and paramagnetic homo-proline (*rac*)-(**3**).

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From our laboratory a new pyrroline nitroxide based paramagnetic achiral amino acid ester, so called PTOAC (**2**) with a rigid side chain has been reported.⁹ However, faced with difficulties of a many-step synthesis^{9,10} and low yield because of the concurrent formation of compound (*rac*)-**3**, we returned to the synthesis of α -amino acids with flexible chains by the O'Donnell synthesis,^{11,12} alkylating *N*-dibenzylidene glycine ester with various paramagnetic allylic bromides yielding paramagnetic amino acids with different size, polarity, and side-chain length.¹³

Our intention on to continue this research was inspired by the fact that cysteine and tyrosine modified with a spin label were incorporated into *Xenopus oocytes* using the nonsense suppression technique¹⁴ and compound (*rac*)-**25** was incorporated into Ras-binding domain of c-Raf 1 protein by using an expressed protein ligation.¹⁵ The drawback of the O'Donnell synthesis is that racemic amino acids are formed, so we thought that their resolution would be desirable as well as the synthesis of new paramagnetic amino acids, which mimic more precisely the natural amino acids. Several methods have been used for the resolution of amino acids.¹⁶ In the present study we used the chiral recognition between *N*-acetyl amino acids and amino acid esters.¹⁷ We proposed that amino acid esters, similarly to phenylalanine, could be resolved by enantiomers of *N*-acetylphenylglycine.¹⁸

In this paper we report the synthesis of paramagnetic amino acids and the resolution of new and known compounds as well as modification of natural, amino acids with nitroxides by Pd-catalyzed C–C bond forming reactions. These new amino acids with a flexible side chain can find different applications

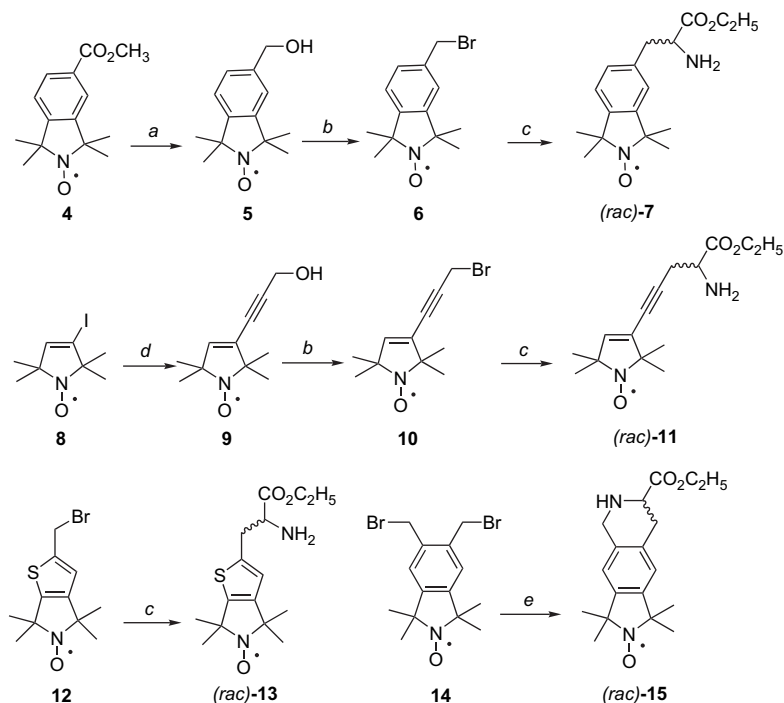
than TOAC, because as non-helicogenic amino acids can be incorporated into β -strands. TOAC is capable of studying the backbone dynamics, while newly synthesized amino acids with paramagnetic side chains designed to mimic salient features of the native side chains they replace, moreover a pyrroline nitroxide is more stable toward chemical manipulations than a piperidine nitroxide.

2. Results and discussion

2.1. Synthesis of new paramagnetic amino acids

For the synthesis of a paramagnetic amino acid containing an isoindoline side chain, ester (**4**)¹⁰ was reduced to alcohol (**5**) by refluxing with a suspension of NaBH₄ in *tert*-BuOH, while MeOH was added dropwise¹⁹ (Scheme 2). Treatment of alcohol (**5**) with methanesulfonyl chloride in the presence of Et₃N gave the mesylate, which was converted to bromo compound (**6**) with LiBr in acetone.²⁰ Alkylation of *N*-diphenylmethylene glycine ethyl ester under phase transfer conditions followed by hydrolysis of the Schiff base resulted in the formation of racemic amino acid ethyl ester (*rac*)-(**7**).¹²

To get an amino acid with a rigid spacer between the nitroxide ring and α -carbon atom we introduced a propargylic spacer. Sonogashira reaction²¹ seemed the most reasonable solution for this problem, therefore treatment of vinylic iodide **8**²² with a *tert*-butyldimethyl-(2-propynyloxy)silane in the presence of CuI and PdCl₂(Ph₃P)₂ catalysts in Et₃N as a solvent under N₂, yielded the protected propargyl alcohol derivative, which was deprotected with tetrabutylammonium



Scheme 2. (a) NaBH₄ (7.0 equiv), MeOH (excess), *tert*-BuOH, 3 h, reflux, 61%; (b) MsCl (1.1 equiv), Et₃N (1.1 equiv) CH₂Cl₂, 0 °C, 1 h, then LiBr (2.0 equiv), acetone, reflux, 30 min (45–69%); (c) Ph₂C=NCH₂CO₂Et (1.0 equiv), 10% aq NaOH, CH₂Cl₂, Bu₄NHSO₄ (0.5 equiv), rt, 2 h, then 5% aq H₂SO₄, EtOH, 30 min, rt, then solid K₂CO₃ to pH=8, 15–52%; (d) HC≡CHCH₂OTBDMS (2.0 equiv), CuI (0.05 equiv), PdCl₂(PPh₃)₂ (0.1 equiv), Et₃N, under N₂, rt, 5 h, then Bu₄NF (1 equiv), THF, rt, 30 min, 58%.

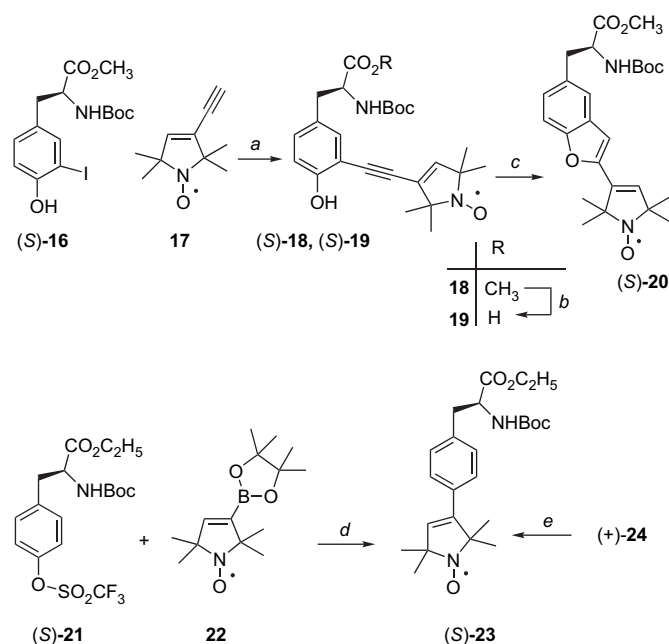
fluoride to yield compound **9**. This compound was then converted into propargylic bromide (**10**) derivative via the mesylate. By utilizing the O'Donnell procedure as above, compound (*rac*)-**11** was achieved after hydrolysis of the imine. The same O'Donnell method was applied for synthesis of amino acid ester (*rac*)-(**13**) with a thieno[2,3-*c*]pyrrole moiety containing side chain starting from bromo compound²³ (**12**) as well as for the synthesis of tetrahydroisoquinoline amino acid (*rac*)-**15** starting from dibromo compound (**14**).²⁴ Under these conditions the dialkylation of α -carbon atom does not occur, while monoalkylation of α -carbon atom and nitrogen alkylation under work-up conditions has been observed instead.¹⁰ 1,2,3,4-Tetrahydroisoquinoline-3-carboxylic acid is a widely used unnatural amino acid building block occurring in ACE inhibitor Quinapril²⁵ and in opioid peptide antagonists,²⁶ therefore its paramagnetic analogue may find important applications.

2.2. Synthesis of new paramagnetic amino acids from *S*-tyrosine derivatives

A convenient approach for the synthesis of chiral paramagnetic amino acid is the modification (alkylation) of the side chain of a natural amino acid.¹³ The disadvantage of this approach is that modification of the heteroatom may lead to the loss of bioactive properties. It was obvious that the carbon–carbon bond is required, however, under mild conditions, which are compatible with ester and protected nitrogen. After considering the possibilities of amino acid modifications^{27,28} we decided a utilization of the Pd-catalyzed cross-coupling reactions.²⁹ The Sonogashira reaction of *N*-protected *S*-3-iodo-tyrosine ester³⁰ (*S*)-**16** with **17** paramagnetic acetylene¹⁰ under conditions mentioned above yielded (*S*)-**18** paramagnetic amino acid ester, which could be hydrolyzed to (*S*)-**19** carboxylic acid with NaOH in aq methanol (Scheme 3). It was obvious that compound (*S*)-**18** as a 2-alkynylphenol offers the construction of a benzo[*b*]furan side chain. Of the possible cyclization techniques³¹ we used the treatment with tetrabutylammonium fluoride in THF³² to get the benzofuran side chain containing amino acid (*S*)-**20**. The Suzuki reaction was also a useful tool in the synthesis of paramagnetic phenylalanine (*S*)-**23** of which racemic form was reported earlier from our laboratory.¹³ Treatment of *N*-protected *S*-tyrosine ethyl ester *O*-triflate²⁴ (*S*)-**21** with paramagnetic boronic acid ester **22**³³ in dioxane/aq Na₂CO₃ solution in the presence of Pd(PPh₃)₄ catalyst afforded paramagnetic phenylalanine (*S*)-**23**, with a pyrroline nitroxide ring in the 4-position of the aromatic ring. The specific rotation of (*S*)-**23** was +3.3 with 0.2 less than the resolved authentic sample (with ee 99%) indicating that minimal racemization had occurred during the Suzuki reaction conditions.

2.3. Resolution of some racemic, paramagnetic amino acids

Four amino acid esters ((*rac*)-**7**, (*rac*)-**13**, (*rac*)-**24**, (*rac*)-**25**) were resolved with (*R*)-*N*-acetylphenylglycine. In all



Scheme 3. (a) **17** (1.5 equiv), CuI (0.05 equiv), PdCl₂(PPh₃)₂ (0.1 equiv), Et₃N, under N₂, rt, 5 h, 39%; (b) 1.1 equiv NaOH, MeOH/water, rt, 2 h, then H⁺, 78%; (c) Bu₄NF, THF, reflux, 1 h, 39%; (d) **21** (1.0 equiv), dioxane, Pd(PPh₃)₄, 10% aq Na₂CO₃, reflux, 2 h, 31%; (e) Boc₂O (1.25 equiv), Et₃N (1.25 equiv), CH₂Cl₂, rt, 2 h, 82%.

four cases amino acid esters were treated with half an equivalent resolving agent in ethyl acetate. The precipitated diastereomer salts were resuspended in ethyl acetate and heated to reflux. The diastereomeric salts of (*rac*)-**7**, (*rac*)-**13**, and (*rac*)-**24** were recrystallized in the presence of triethylamine equivalent with the less stable diastereomer, while (*rac*)-**25** was filtered after cooling and 30 min stirring. This procedure resulted in high enantiomer excess (ee > 96%) for amino acids **24** and **25**, in the case of compound **7** limited enantiomer excess (ee 48%) and low enantiomer excess (16%) for compound **13** were achieved. The absolute configuration in case of (+)-**24** was found to be (*S*), in other cases only the direction of rotation is indicated (Scheme 3).

3. Conclusion

In conclusion, we have reported the synthesis of a new series of paramagnetic amino acids. In the case of the O'Donnell synthesis racemic amino acids were formed. Some of them could be resolved by fractional crystallization of diastereomeric salts from excellent to moderate enantiomeric excess. The other method for achieving paramagnetic chiral amino acids is further derivatization of natural amino acid derivatives with Pd-catalyzed cross-coupling reactions, although this method was limited to amino acids with an aromatic side chain. The improvement of resolution techniques for paramagnetic amino acids containing annellated pyrroline ring as well as incorporation of optically active amino acids into proteins is in progress.

4. Experimental

4.1. General

Melting points were determined with a Boetius micro melting point apparatus. Elemental analyses (C, H, N, S) were performed on Carlo Erba EA 1110 CHNS elemental analyzer. Mass spectra were recorded on an Automass Multi instrument in the EI mode (70 eV, direct inlet). ESI-TOF MS measurements were performed with a BioTOF II instrument (Bruker Daltonics, Billerica, MA). IR spectra were taken on Specord M85 instrument. ESR spectra were obtained from 10^{-5} M solutions (CHCl_3), using a Magnettech MS200 spectrometer. Preparative flash column chromatography was performed on Merck Kieselgel 60 (0.040–0.063 mm). Optical purity was determined by HPLC (Jasco UV-1575 detector, Jasco PU-1580 pump) containing Chiralpack AD column at $T=20^\circ\text{C}$ with 0.8 mL/min flow rate. The mobile phase consisted of 75% hexane and 25% EtOH and UV detection was performed at 256 nm. Optical rotations were recorded at 20°C , with a Perkin–Elmer 343 polarimeter. Qualitative TLC was carried out on commercially prepared plates ($20\times 20\times 0.02$ cm) coated with Merck Kieselgel GF₂₅₄. Dibenzylidene glycine ethyl ester¹¹ and compounds **1**,⁵ **2**,⁹ **3**,⁹ **4**,¹⁰ **8**,²² **12**,²³ **14**,²⁴ **16**,³⁰ (the same as the reported *R* isomer), **17**,¹⁰ **21**,²⁷ **22**,³³ **23**,¹³ and **24**¹² were prepared as described earlier. *tert*-Butyldimethyl(2-propynyloxy)silane and all other reagents and compounds were purchased from Aldrich or Fluka. Triethylamine (Et_3N) was distilled from CaH_2 prior to use.

4.2. 5-Hydroxymethyl-1,1,3,3-tetramethyl-1,3-dihydro-2H-isoindol-2-yloxy radical (**5**)

To a stirred refluxing suspension of **4** ester (2.48 g, 10.0 mmol) and NaBH_4 (2.64 g, 70.0 mmol) in anhyd *tert*-BuOH (20 mL) a mixture of anhyd *tert*-BuOH/MeOH (12:8 mL) was added dropwise over 3 h under N_2 . After cooling, the mixture was concentrated in vacuo, quenched with water (30 mL), extracted with CHCl_3 (2×20 mL), dried (MgSO_4), filtered, and evaporated. The crude alcohol was purified by flash chromatography (hexane/EtOAc) to yield compound **5** (1.34 g, 61%) as a yellow solid. Mp $108\text{--}110^\circ\text{C}$, R_f 0.20 (hexane/EtOAc 2:1). EA calcd for $\text{C}_{13}\text{H}_{18}\text{NO}_2$: C, 70.88; H, 8.24; N, 6.36. Found: C, 70.73; H, 8.15; N, 6.26. IR Nujol (ν): 3420 (OH), 1600, 1550 ($\text{C}=\text{C}$). MS (EI) (m/z): 220 (M^+ , 61), 205 (81), 190 (72), 105 (100).

4.3. General procedure for synthesis of bromo compounds (**6**,**10**)

To a solution of alcohol **5** or **9** (10.0 mmol) and Et_3N (1.11 g, 11.0 mmol) in CH_2Cl_2 (20 mL) methanesulfonyl chloride (1.25 g, 11.0 mmol) was added dropwise at 0°C and the mixture was stirred at ambient temperature for 60 min. Then the mixture was washed with water (15 mL), the organic phase

was separated, dried (MgSO_4), filtered, and evaporated. The residue was dissolved in anhyd acetone (20 mL), LiBr (1.74 g, 20.0 mmol) was added and the mixture stirred and refluxed for 30 min. After cooling, the acetone was evaporated off and the residue was partitioned between water (10 mL) and EtOAc (30 mL). The organic phase was separated, dried (MgSO_4), filtered, and evaporated. The residue was purified by flash column chromatography (hexane/ Et_2O) to give compounds **6** and **10**.

4.3.1. 5-Bromomethyl-1,1,3,3-tetramethyl-1,3-dihydro-2H-isoindol-2-yloxy radical (**6**)

Yield 1.95 g (69%). Mp $108\text{--}110^\circ\text{C}$, R_f 0.30 (hexane/ Et_2O 2:1). IR Nujol (ν): 1690, 1550 ($\text{C}=\text{C}$). EA calcd for $\text{C}_{13}\text{H}_{17}\text{BrNO}$: C, 55.14; H, 6.05; N, 4.95. Found: C, 55.20; H, 6.00; N, 5.86. MS (EI) (m/z): 284/282 (M^+ , 91/91), 269/267 (38) 254/252 (17/17), 188 (100).

4.3.2. 3-(3-Bromoprop-1-ynyl)-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-1-yloxy radical (**10**)

Yield 1.15 g (45%). Mp $82\text{--}83^\circ\text{C}$, R_f 0.39 (hexane/ Et_2O 2:1). EA calcd for $\text{C}_{11}\text{H}_{15}\text{BrNO}$: C, 51.38; H, 5.88; N, 5.45. Found: C, 51.23; H, 5.72; N, 5.43. IR Nujol (ν): 1600, 1555 ($\text{C}=\text{C}$). MS (EI) (m/z): 258/256 (M^+ , 20/20), 243/241 (26/26), 228/226 (50), 162 (65), 41 (100).

4.4. 3-(3-Hydroxyprop-1-ynyl)-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-1-yloxy radical (**9**)

The mixture of **8** iodo compound (1.33 g, 5.0 mmol), $\text{PdCl}_2(\text{PPh}_3)_2$ (350 mg, 0.5 mmol), and CuI (48 mg, 0.25 mmol) in anhyd Et_3N (15 mL) was stirred and deoxygenated with N_2 for 15 min. Then *tert*-butyldimethyl(2-propynyloxy)silane (1.73 g, 10.0 mmol) dissolved in anhyd Et_3N (5 mL) was added dropwise causing an immediate brown precipitation from the yellow solution. Within 10 min the mixture became a thick slurry and was vigorously stirred for 5 h at ambient temperature. The mixture was diluted with EtOAc (20 mL), filtered through a Celite pad, washed with EtOAc (5 mL) then the solvents were evaporated off. The residue was partitioned between Et_2O (30 mL) and water (10 mL), the organic phase was separated, dried (MgSO_4), filtered, and evaporated. The crude product was dissolved in anhyd THF (20 mL) and tetrabutylammonium fluoride (5 mL, 5.0 mmol) in THF (1.0 M stock solution) was added and the mixture was stirred at ambient temperature for 30 min. Then THF was evaporated off, the crude product was dissolved in CHCl_3 (20 mL), washed with water (10 mL), the organic phase was separated, dried (MgSO_4), filtered, and evaporated. The residue was purified by flash column chromatography (hexane/EtOAc) to yield **9** alcohol as a yellow solid (970 mg, 58%). Mp $82\text{--}84^\circ\text{C}$, R_f 0.26 (hexane/EtOAc 2:1). EA calcd for $\text{C}_{11}\text{H}_{16}\text{NO}_2$: C, 68.01; H, 8.30; N, 7.21. Found: C, 68.12; H, 8.28; N, 7.15. IR Nujol (ν): 3320 (OH), 1600, 1540 ($\text{C}=\text{C}$). MS (EI) (m/z): 194 (M^+ , 32), 179 (71), 164 (100).

4.5. General procedure for synthesis of amino acid esters (*rac*)-**7**, -**11**, -**13**, -**15**

To stirred solution of *N*-diphenylmethylene glycine ethyl ester (801 mg, 3.0 mmol) and compound **6** or **10** or **12** or **14** (3.0 mmol) in CH₂Cl₂ (20 mL) and aq 10% NaOH (3 mL or 6 mL in case of **14**) was added followed by addition of Bu₄NHSO₄ (508 mg, 1.5 mmol) and the mixture was stirred at rt for 2 h. The organic phase was separated, dried (MgSO₄), filtered, and evaporated to give the crude imine, which was immediately subjected to acidic hydrolysis. The residue was dissolved in EtOH (20 mL) and 5% aq H₂SO₄ (5 mL) was added and the mixture was allowed to stand at ambient temperature and the mixture was monitored by TLC. After consumption of Schiff base (ca. 30 min) water (10 mL) was added and the pH=8 was adjusted by adding solid K₂CO₃. The mixture was extracted with CHCl₃ (2×20 mL), then organic phase was separated, dried (MgSO₄), filtered, evaporated, and the residue was purified by flash column chromatography (CHCl₃/MeOH) to give racemates of **7**, **11**, **13**, **15** amino acid esters (15–52%) as yellow oils.

4.5.1. (*rac*)-2-Amino-3-(2-oxyl-1,1,3,3-tetramethyl-1,3-dihydro-2H-isoindol-5-yl)propionic acid ethyl ester radical (**7**)

Yield 476 mg (52%), yellow oil, *R*_f 0.08 (CHCl₃/Et₂O 2:1). EA calcd for C₁₇H₂₅N₂O₃: C, 66.86; H, 8.25; N, 9.17. Found: C, 66.80; H, 8.14; N 9.01. IR neat (*ν*): 3380, 3310 (NH₂), 1730 (C=O), 1610 (C=C). MS (EI) (*m/z*): 305 (M⁺, 33), 290 (4), 232 (37), 189 (100).

4.5.2. (*rac*)-2-Amino-5-(1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-3-yl)-pent-4-ynoic acid ethyl ester radical (**11**)

Yield 335 mg (40%), yellow oil, *R*_f 0.10 (CHCl₃/Et₂O 2:1). EA calcd for C₁₅H₂₃N₂O₃: C, 64.49; H, 8.30; N, 10.03. Found: C, 64.44; H, 8.32; N, 10.00. IR neat (*ν*): 3380, 3300 (NH₂), 2210 (C≡C), 1730 (C=O), 1610 (C=C). MS (EI) (*m/z*): 279 (M⁺, 28), 264 (3), 249 (1), 234 (7), 102 (100).

4.5.3. (*rac*)-2-Amino-3-(5-oxyl-4,4,6,6-tetramethyl-4,6-dihydro-5H-thienof[2,3-*c*]pyrrol-2-yl)propionic acid ethyl ester radical (**13**)

Yield 354 mg (38%), yellow oil, *R*_f 0.11 (CHCl₃/Et₂O 2:1). EA calcd for C₁₅H₂₃N₂O₃S: C, 57.85; H, 7.44; N, 9.00; S, 10.30. Found: C, 57.70; H, 7.28; N, 8.98; S, 10.21. IR neat (*ν*): 3390, 3310 (NH₂), 1730 (C=O), 1600 (C=C). MS (EI) (*m/z*): 311 (M⁺, 2), 281 (63), 238 (6), 179 (100).

4.5.4. (*rac*)-2-Oxyl-1,1,3,3-tetramethyl-1,3,5,6,7,8-hexahydro-2H-pyrrolo[3,4-*g*]isoquinoline-7-carboxylic acid ethyl ester radical (**15**)

Yield 142 mg (15%), yellow oil, *R*_f 0.38 (CHCl₃/Et₂O 2:1). EA calcd for C₁₈H₂₅N₂O₃: C, 68.11; H, 7.94; N, 8.83. Found: C, 68.01; H, 7.92; N, 8.85. IR neat (*ν*): 3410 (NH), 1740 (C=O), 1620, 1555 (C=C). MS (EI) (*m/z*): 317 (M⁺, 30), 287 (6), 244 (74), 229 (100).

4.6. (*S*)-2-*tert*-Butoxycarbonylamino-3-[4-hydroxy-3-(1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-3-ylethynyl)phenyl]propionic acid methyl ester radical (**18**)

A solution of (*S*)-*N*-*tert*-butoxycarbonyl-3-iodo-tyrosine methyl ester (842 mg, 2.0 mmol), PdCl₂(PPh₃)₂ (140 mg, 0.2 mmol), and CuI (19 mg, 0.1 mmol) in anhyd Et₃N (10 mL) was stirred and deoxygenated with N₂ for 15 min. Then compound **17** (492 mg, 3.0 mmol) dissolved in anhyd Et₃N (5 mL) was added dropwise causing an immediate brown precipitation from the yellow solution. Within 10 min the mixture became a thick slurry and was vigorously stirred for 5 h at ambient temperature. The mixture was diluted with EtOAc (20 mL), filtered through a Celite pad, washed with EtOAc (5 mL) then the solvents were evaporated off. The residue was partitioned between Et₂O (30 mL) and water (10 mL), the organic phase was separated, dried (MgSO₄), filtered, and evaporated. The residue was purified by flash column chromatography (hexane/EtOAc) to give the title compound **18** (356 mg, 39%) as a beige solid. Mp 126–128 °C, *R*_f 0.18 (hexane/EtOAc 2:1), [α]_D +5 (*c* 0.21, MeOH). EA calcd for C₂₅H₃₃N₂O₆: C, 65.63; H, 7.27; N, 6.12. Found: C, 65.59; H, 7.22; N, 6.05. IR Nujol (*ν*): 3300, 1730, 1680 (C=O), 1580, 1540 (C=C). MS (EI) (*m/z*): 457 (M⁺, 3), 427 (3), 371 (4), 57 (100).

4.7. (*S*)-2-*tert*-Butoxycarbonylamino-3-[4-hydroxy-3-(1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-3-ylethynyl)phenyl]propionic acid radical (**19**)

To a solution of **18** ester (228 mg, 0.5 mmol) in MeOH (5 mL) and NaOH (22 mg, 0.55 mmol) dissolved in water (5 mL) was added in one portion, the mixture was allowed to stand at rt and monitored by TLC. After consumption of the ester (~2 h), water (10 mL) was added, the MeOH was evaporated off. The aqueous phase was cautiously acidified to pH=5 with 5% aq H₂SO₄. The turbid solution was extracted with CHCl₃ (2×10 mL), the organic phase was separated, dried (MgSO₄), filtered, and evaporated. The residue was purified further with flash chromatography to give **19** acid as a pale yellow solid (172 mg, 78%). Mp 178–180 °C, *R*_f 0.68 (CHCl₃/MeOH, 4:1), [α]_D +21 (*c* 0.21, MeOH). EA calcd for C₂₄H₃₁N₂O₆: C, 64.99; H, 7.05; N, 6.32. Found: C, 65.02; H, 7.01; N, 6.25. IR neat (*ν*): 3300 (OH), 1670 (C=O), 1570, 1550 (C=C). MS (ESI) (*m/z*): 442 (M–H)[–].

4.8. (*S*)-2-*tert*-Butoxycarbonylamino-3-[2-(1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-3-yl)-benzofuran-5-yl]propionic acid methyl ester radical (**20**)

A solution of **18** (457 mg, 1.0 mmol) and tetrabutylammonium fluoride (1.0 M stock solution, 2 mL, 2.0 mmol) in THF (10 mL) was refluxed for 1 h. The solvent was evaporated off, the residue was dissolved in EtOAc (10 mL), washed with water (10 mL), the organic phase was dried over MgSO₄, filtered, evaporated, and the residue was purified by flash column chromatography to give the title compound (178 mg, 39%) as

a pale brown solid. Mp 44–47 °C, R_f 0.37 (hexane/EtOAc, 2:1), $[\alpha]_D^{20} +2$ (c 0.20, MeOH). EA calcd for $C_{25}H_{33}N_2O_6$: C, 65.63; H, 7.27; N, 6.12. Found: C, 65.59; H, 7.22; N, 6.05. IR neat (ν): 3320 (NH), 1740, 1780 (C=O), 1610 (C=C). MS (EI) (m/z): 457 (M^+ , 2), 427 (3), 371 (15), 304 (85), 57 (100).

4.9. Synthesis of (*S*)-2-*tert*-butoxycarbonylamino-3-[4-(1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-3-yl)phenyl]propionic acid ethyl ester (**23**)

4.9.1. Method A

To a stirred solution of (*S*)-2-*tert*-butoxycarbonylamino-4-[(trifluoromethyl)sulfonyl]oxy]benzenepropionic acid ethyl ester (882 mg, 2.0 mmol) in dioxane (10 mL) and after deoxygenation with N_2 for 10 min. $Pd(PPh_3)_4$ (100 mg, 0.086 mmol) was added, followed by addition of compound **22** (532 mg, 2.0 mmol), aq 10% Na_2CO_3 (5 mL) and the mixture was stirred and refluxed for 2 h. After cooling, the solvents were evaporated off, the residue was partitioned between EtOAc (20 mL) and water (10 mL). The organic phase was washed with saturated aq sodium bicarbonate solution (10 mL), aq 10% citric acid (10 mL), and saturated sodium chloride solution (10 mL). The organic phase was separated, dried ($MgSO_4$), filtered, and evaporated. The residue was purified by flash column chromatography (hexane/EtOAc) to give (267 mg, 31%) yellow semisolid. R_f 0.41 (hexane/EtOAc, 2:1), $[\alpha]_D^{20} +3.3$ (c 0.5, methanol). EA calcd for $C_{24}H_{35}N_2O_5$: C, 66.80; H, 8.17; N, 6.49. Found: C, 66.75; H, 8.19; N, 6.42. IR neat (ν): 3320 (NH), 1730, 1680 (C=O), 1610 (C=C). MS (EI) (m/z): 431 (M^+ , 28), 401 (3), 360 (22) 199 (40), 57 (100).

4.9.2. Method B

To a stirred solution of (+)-**24** amino acid ester (66 mg, 0.2 mmol) and Et_3N (25 μ L, 0.25 mmol) in CH_2Cl_2 (5 mL) di-*tert*-butoxycarbonate (53 mg, 0.25 mmol) was added in one portion and the mixture was stirred for 2 h at rt. Then the solvent was washed with saturated aq $NaHCO_3$ (5 mL), water (5 mL), dried ($MgSO_4$), filtered, and evaporated. The residue was purified by flash column chromatography (hexane/EtOAc) to give (71 mg, 82%) yellow semisolid. R_f 0.41 (hexane/EtOAc, 2:1), $[\alpha]_D^{20} +3.5$ (c 0.51, methanol). EA calcd for $C_{24}H_{35}N_2O_5$: C, 66.80; H, 8.17; N, 6.49. Found: C, 66.74; H, 8.10; N, 6.39. All the spectroscopic data were identical with the sample obtained by method A.

4.10. (+)-2-Amino-3-[4-(1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-3-yl)phenyl]propionic acid ethyl ester radical (**24**)

(*rac*)-2-Amino-3-[4-(1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-3-yl)phenyl]propionic acid ethyl ester radical (**24**) (0.815 g, 2.46 mmol) and (*R*)-*N*-acetylphenylglycine (0.240 g, 1.23 mmol) were dissolved in hot EtOAc (5.6 mL). After cooling and seeding it was left at rt overnight. It was then filtered and suspended and stirred twice with hot EtOAc

(5 mL and 5 mL+30 μ L Et_3N , respectively) to give yellowish powder (0.289 g, 0.54 mmol, 44.1%), $[\alpha]_D^{20} -59.4$ (c 0.5, MeOH). To this salt CH_2Cl_2 (5 mL) and saturated aq sodium bicarbonate and sodium chloride solution (1 mL) were added and it was stirred for 5 min. After separation of the aq phase, it was further extracted by CH_2Cl_2 (2 $\times$ 5 mL). The organic phase was dried over Na_2SO_4 and solvent was evaporated to give (+)-2-amino-3-[4-(1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-3-yl)phenyl]propionic acid ethyl ester radical (0.179 g 43.9%), $[\alpha]_D^{20} +12.7$ (c 0.5, methanol), ee 99%. Retention time for (+)-**24**: 11.33 min and (–)-**24**: 12.57 min.

4.11. (+)-2-Amino-3-(1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-3-yl)propionic acid ethyl ester radical (**25**)

(*rac*)-2-Amino-3-(1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-3-yl)propionic acid ethyl ester radical (**25**) (1.600 g, 6.27 mmol) and (*R*)-*N*-acetylphenylglycine (0.605 g, 3.13 mmol) were dissolved in hot EtOAc (11 mL). After cooling and seeding it was left at rt overnight. It was then filtered and suspended and stirred twice with hot EtOAc (10 and 9 mL, respectively) to give yellowish powder (0.500 g, 1.1 mmol, 35.0%), $[\alpha]_D^{20} -69.3$ (c 0.5, MeOH). To this salt CH_2Cl_2 (5 mL) and saturated aq sodium bicarbonate and sodium chloride solution (1 mL) were added and it was stirred for 5 min. After separation of the aq phase, it was further extracted by CH_2Cl_2 (2 $\times$ 5 mL). The organic phase was dried over Na_2SO_4 and solvent was evaporated to give (+)-2-amino-3-(1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-3-yl)propionic acid ethyl ester radical (0.273 g, 34.1%), $[\alpha]_D^{20} +11.5$ (c 0.5, methanol), ee 96%. Retention time for (+)-**25**: 6.11 min and (–)-**25**: 7.03 min.

4.12. (+)-2-Amino-3-(2-oxyl-1,1,3,3-tetramethyl-1,3-dihydro-2*H*-isoindol-5-yl)propionic acid ethyl ester radical (**7**)

(*rac*)-2-Amino-3-(2-oxyl-1,1,3,3-tetramethyl-1,3-dihydro-2*H*-isoindol-5-yl)propionic acid ethyl ester radical (**7**) (0.747 g, 2.45 mmol) and (*R*)-*N*-acetylphenylglycine (0.236 g, 1.22 mmol) were dissolved in hot EtOAc (5 mL). After cooling and seeding it was left at rt overnight. To this suspension giving diethyl ether (5 mL) and it was then filtered. Then it was suspended and stirred twice with hot EtOAc (5 mL+50 μ L Et_3N and 5 mL+10 μ L Et_3N , respectively) to give yellowish powder (0.353 g, 0.71 mmol, 57.7%), $[\alpha]_D^{20} -66.4$ (c 0.5, MeOH). To this salt CH_2Cl_2 (5 mL) and saturated aq sodium bicarbonate and sodium chloride solution (1 mL) were added and it was stirred for 5 min. After separation of the aq phase, it was further extracted by CH_2Cl_2 (2 $\times$ 5 mL). The organic phase was dried over Na_2SO_4 and solvent was evaporated to give (+)-2-amino-3-(2-oxyl-1,1,3,3-tetramethyl-1,3-dihydro-2*H*-isoindol-5-yl)propionic acid ethyl ester radical (0.216 g, 57.7%), $[\alpha]_D^{20} +7.7$ (c 0.5, methanol). Retention time for (+)-**7**: 5.91 min and (–)-**7**: 6.45 min.

4.13. (–)-2-Amino-3-(5-oxyl-4,4,6,6-tetramethyl-4,6-dihydro-5H-thieno[2,3-c]pyrrol-2-yl)propionic acid ethyl ester radical (**13**)

(rac)-2-Amino-3-(5-oxyl-4,4,6,6-tetramethyl-4,6-dihydro-5H-thieno[2,3-c]pyrrol-2-yl)propionic acid ethyl ester radical **13** (103 mg, 0.33 mmol) and (R)-N-acetylphenylglycine (31.9 mg, 0.16 mmol) were dissolved in hot EtOAc (1.3 mL). After cooling and seeding it was left at rt overnight. To this suspension giving diethyl ether (1 mL) and it was then filtered. Then it was suspended and stirred twice with hot EtOAc (1.3 mL+4 μ L Et₃N and 1.3 mL+4 μ L Et₃N, respectively) to give yellowish powder (28.4 mg, 0.06 mmol, 33.6%), [α]_D²⁰ –72.3 (c 0.5, MeOH). To this salt CH₂Cl₂ (5 mL) and saturated aq sodium bicarbonate and sodium chloride solution (1 mL) were added and it was stirred for 5 min. After separation of the aq phase, it was further extracted by CH₂Cl₂ (2 $\times$ 5 mL). The organic phase was dried over Na₂SO₄ and solvent was evaporated to give (–)-2-amino-3-(5-oxyl-4,4,6,6-tetramethyl-4,6-dihydro-5H-thieno[2,3-c]pyrrol-2-yl)propionic acid ethyl ester radical (15.6 mg, 30.3%), [α]_D²⁰ –0.7 (c 0.5, methanol), ee 16%. Retention time for (+)-**13**: 8.28 min and (–)-**13**: 14.64 min.

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