Journal of Labelled Compounds and Radiopharmaceuticals
J Label Compd Radiopharm 2007; **50**: 370–374.
Published online in Wiley InterScience
(www.interscience.wiley.com). DOI: 10.1002/jlcr.1268

JLCR

Short Research Article

Towards stereoselective radiosynthesis of α-[^{11}C]methyl-substituted aromatic α-amino acids – a challenge of creation of quaternary asymmetric centre in a very short time†

ALEXANDER POPKOV[1,5,*], MILAN NÁDVORNÍK[2], PAVLA KRUŽBERSKÁ[3], ANTONÍN LYČKA[4], SZABOLCS LEHEL[5] and NICHOLAS GILLINGS[5]

[1]Faculty of Health and Social Studies, University of South Bohemia, Branišovská 31, České Budějovice 370 05, Czech Republic
[2]Department of General and Inorganic Chemistry, Faculty of Chemical Technology, Pardubice University, Pardubice 532 10, Czech Republic
[3]Department of Analytical Biochemistry, Institute of Entomology, Branišovská 31, České Budějovice 370 05, Czech Republic
[4]Research Institute for Organic Syntheses, Rybitví 296, Pardubice 20 532 18, Czech Republic
[5]PET & Cyclotron Unit, Copenhagen University Hospital, Blegdamsvej 9, Copenhagen 2100, Denmark

Received 29 August 2006; Revised 6 January 2007; Accepted 20 January 2007

Abstract: In positron emission tomography (PET) α-methyl amino acids have two potential applications: As analogues of neutransmitter precursors for the study of neurodegenerative diseases, and as non-metabolised analogues of proteinogenic amino acids for the study of amino acid uptake into normal and cancer cells.

Clinical applications of such amino acids are strongly limited due to their poor availability. We carried out [^{11}C]methylation of metalocomplex synthons derived from protected DOPA or tyrosine. For [^{11}C]methylation, sodium hydroxide (5 mg of fine dry powder) was sealed in a vial, which was flushed with dry nitrogen before addition of a solution of the complex (10 mg) and $^{11}CH_3I$ in 1,3-dimethylimidazolidin-2-one (300 μl). After 10 min at 25°C, a 9% radiochemical yield (decay-corrected) of a mixture of the diastereomeric α-[^{11}C]methylDOPA complexes or a 7% radiochemical yield of a mixture of the diastereomeric α-[^{11}C]methyltyrosine complexes was achieved. Individual diastereomers were successfully separated by preparative HPLC, diluted with excess of water and extracted on C18 cartridges. Optimisation of the procedure including hydrolysis of the complexes (hydrolytic deprotection of enantiomerically pure amino acids) and subsequent purification of the enantiomers of α-[^{11}C]methylDOPA and α-[^{11}C]methyltyrosine is underway. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: asymmetric synthesis; α-methyl amino acids; carbon-11; [^{11}C]methylation; DOPA; tyrosine; nickel; complexes

Introduction

α-Amino acids bearing an α-methyl group are widely used for replacement of proteinogenic amino acids with their α-methylated analogues in peptides. Such modification of peptides introduces restriction to conformational freedom and increases stability of the peptides towards various enzymes. In positron emission tomography (PET) α-[^{11}C]methyl amino acids could play a dual role:

1. precursors of neurotransmitters analogues for the study of neurodegenerative diseases;
2. non-metabolised analogues of proteinogenic amino acids for the study of amino acids uptake into normal and cancer cells.[1]

Evaluation of the clinical usefulness of such amino acids is limited by the lack of reliable preparative approaches to these compounds. An industrial procedure was adopted for the synthesis of the only enantiomerically pure ^{11}C-labelled α-methyl amino acid, α-[^{11}C]methyltryptophan.[2] All attempts to prepare enantiomerically pure α-[^{11}C]methylated tyrosine failed.[3] The only published synthesis of ^{11}C-methyl-

*Correspondence to: Alexander Popkov, Faculty of Health and Social Studies, University of South Bohemia, Branišovská 31, České Budějovice 370 05, Czech Republic. E-mail: sasha@jcu.cz
Contract/grant sponsor: the Ministry of Education, Youth and Sports of the Czech Republic; contract/grant number: MSM 6007665808
†Proceedings of the Ninth International Symposium on the Synthesis and Applications of Isotopically Labelled Compounds, Edinburgh, 16–20 July 2006.

labelled α-metyltyrosine utilised a very original combined chemical and enzymatic approach.[3a] The amino acid core was built by malonic ester chemistry; dimethyl 2-(4-methoxybenzyl)malonate was methylated with [^{11}C]methyliodide. Hydrolysis of the prochiral diester using pig liver esterase (EC 3.1.1.1) led to the enantiomerically enriched monoester. After transformation of the free carboxylic group into an amino group via isocyanate and deprotection, the labelled α-metyltyrosine was obtained in 62% e.e. The decay-corrected radiochemical yield was 12–20% in a synthesis time of 45–50 min. However, low enantiomeric excess and long synthesis do not allow the use of this approach for routine clinical production of the amino acid. Except for ^{11}C-labelled α-methyltryptophan and several ^{14}C-labelled α-methyl amino acids (α-methyltyrosine[4] and α-methylDOPA), no other enantiomerically pure radiolabelled α-methyl amino acid have been used for *in vivo* investigations in humans (α-[^{11}C]methyltryptophan) or laboratory animals.

Our efforts concentrated on adaptation of known metallocomplex amino acids synthons widely used for preparation of non-labelled α-methyl amino acids.[5] Ni(II) complexes of Schiff bases of (*S*)-*N*-benzylproline (2-benzoylphenyl)amide (BPB) and α-amino acids were developed as artificial analogues of pyridoxal 5′-phosphate (PLP)-dependent enzymes. BPB was designed as a re-usable enzyme-like chiral auxiliary. Their preparative applications for stoichiometric asymmetric synthesis of α-amino acids are being perfected by a number of groups worldwide.[6]

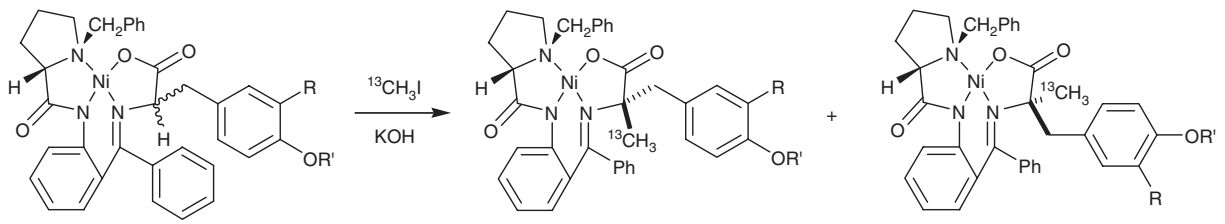
Results and discussion

(^{13}C)Methylation of sterically hindered complexes derived from protected tyrosine and protected DOPA ((*S*,*S*)-TyrK, (*S*,*S*)-TyrK(OBu-*t*) and (*S*,*S*)-DOPAK, Scheme 1) by fivefold excess of $^{13}CH_3I$ was chosen as a model reaction. Alkylation in the aprotic solvent DMI run as expected for alkylation of sterically hindered tertiary carbon. Yields varied from experiment to experiment depending mostly on particle size and dryness of the KOH used.

For [^{11}C]methylation of (*S*,*S*)-DOPAK and (*S*,*S*)-TyrK(OBu-*t*), dry fine powdered KOH was sealed in a vial, the vial was flushed with dry argon followed by addition of a solution of the complex and $^{11}CH^3I$ in DMI (300 μl). After 10 min at 25°C, the 4% radiochemical yield of (*S*,*S*)-α-[^{11}C]methylDOPAK and 5% radiochemical yield of (*S*,*R*)-α-[^{11}C]methylDOPAK was achieved. Yield of diastereomers of α-[^{11}C]MeTyrK(OBu-*t*) was 7%. Low radiochemical yield was observed due to two reasons:

1. (*S*,*S*)-α-[^{11}C]MeDOPAK is a minor diastereomer. Sterically preferred *si*-attack leads to unwanted (*S*,*R*)-α-[^{11}C]MeDOPAK. This disadvantage might be overcome by application of the starting complex with opposite configuration of the chiral centres. Usage of the complexes derived from a new generation chiral auxiliaries[7] instead of BPB should further increase the yield of the desired diastereomer;
2. slow alkylation of the sterically hindered α-carbon allows [^{11}C]methyl iodide to be mostly hydrolysed by KOH.

Chromatographic properties of (*S*,*S*)-α-MeTyrK and (*S*,*R*)-α-MeTyrK are very similar. Their separation on a 4 × 150 mm C18 column takes 50 min, too long a time for preparation of ^{11}C-labelled compounds. While useless for radiochemical syntheses, the complex was a convenient model for assignment of stereochemistry of the products of (^{13}C)methylation. Diastereomers of MeTyrK(OBu-*t*) or diastereomers of α-MeDOPAK are easily separable (Figure 1). The retention times of (*S*,*S*)-α-MeDOPAK and starting (*S*,*S*)-DOPAK are so close that the mixture of these compounds appears as a single peak on a chromatogram. This was elucidated by application of the reconstructed ion current technique



(*S*,*S*)-TyrK R=H R'=Me
(*S*,*S*)-TyrK(OBu-t) R=H R'=Bu-*t*
(*S*,*S*)-DOPAK R=OMe R'=Me

(*S*,*S*)-MeTyrK R=H R'=Me
(*S*,*S*)-MeTyrK(OBu-t) R=H R'=Bu-t
(*S*,*S*)-MeDOPAK R=OMe R'=Me
minor isomer

(*S*,*R*)-MeTyrK R=H R'=Me
(*S*,*R*)-MeTyrK(OBu-t) R=H R'=Bu-*t*
(*S*,*R*)-MeDOPAK R=OMe R'=Me
minor isomer

Scheme 1

DOI: 10.1002.jlcr

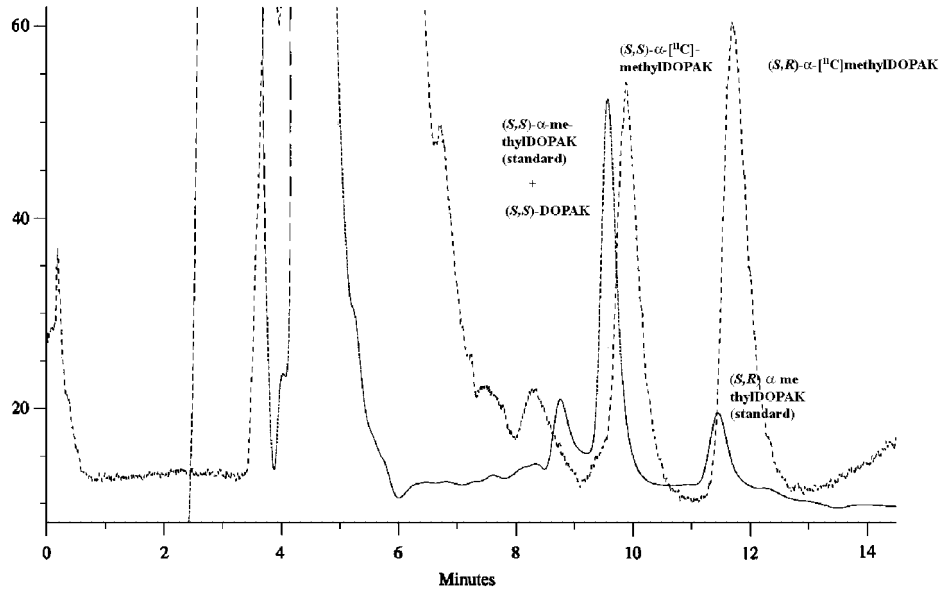


Figure 1 HPLC separation of diastereomers of α-[^{11}C]MeDOPAK (dotted line, detection by γ-detector); added standards – α-methylDOPAK diastereomers and (*S*,*S*)-DOPAK were detected by UV-detector (solid line).

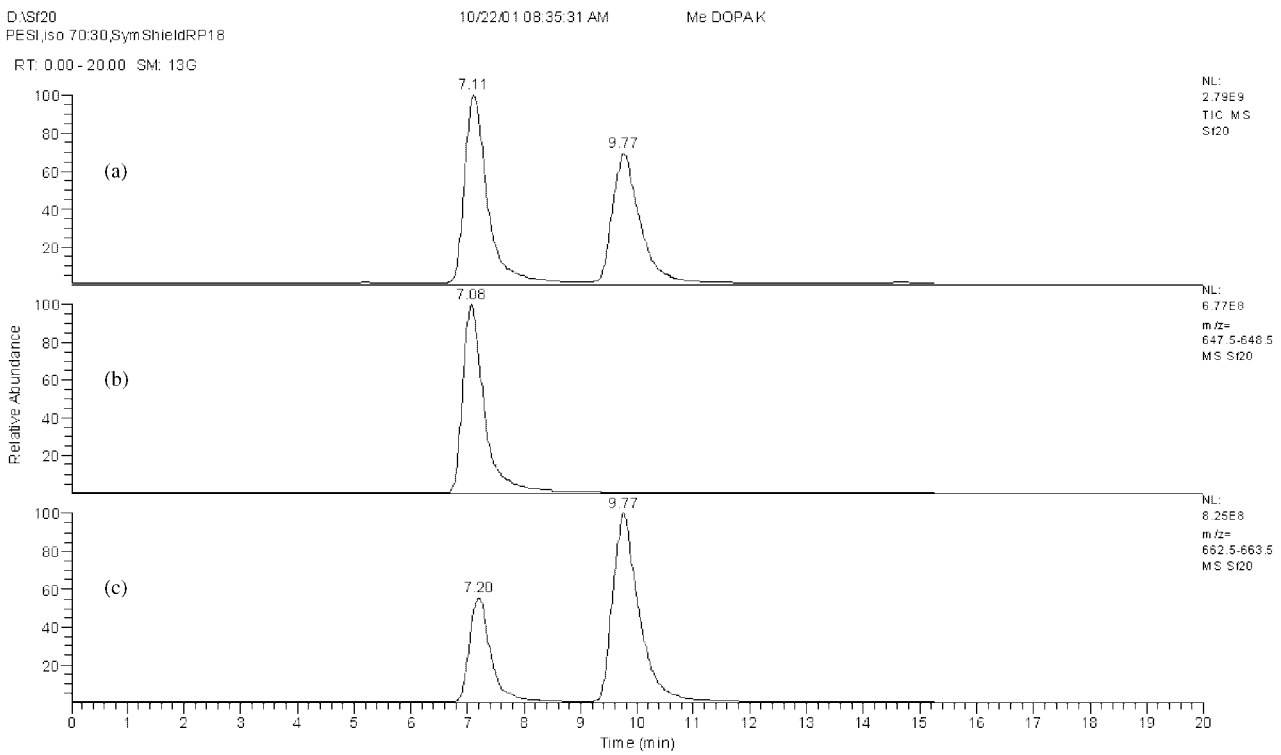


Figure 2 Reconstructed ion current (RIC) chromatogram during HPLC–ESI-MS separation of (*S*,*S*)-DOPAK ([M]$^+$=647) and both diastereomers of α-(^{13}C)MeDOPAK ([M]$^+$=663): (a) total ion current chromatogram (similar to HPLC analysis with UV–VIS detection); (b) RIC chromatogram (*M*=647.5–648.5). Retention time 7.08 min corresponds to (*S*,*S*)-DOPAK; (c) RIC chromatogram (*M*=662.5–663.5). Retention time 7.20 min corresponds to (*S*,*S*)-α-(^{13}C)MeDOPAK; retention time 9.77 min corresponds to (*S*,*R*)-α-(^{13}C)MeDOPAK.

during HPLC–ESI-MS separation of a mixture of starting (*S*,*S*)-DOPAK and both (*S*,*R*)-α-(^{13}C)MeDOPAK and (*S*,*S*)-α-(^{13}C)MeDOPAK (Figure 2).

Stereochemistry of the diastereomers of α-(^{13}C)Me-TyrK was assigned by combined application of ^{13}C NMR and circular dichroism (CD) spectroscopy:

DOI: 10.1002.jlcr

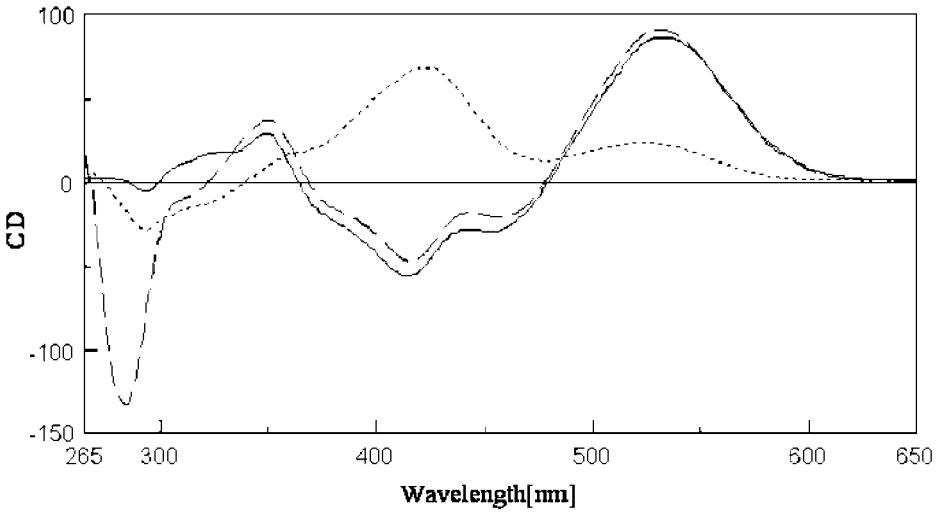


Figure 3 CD spectra of (*S,S*)-TyrK (———), the first fraction, (*S,S*)-α-(^{13}C)MeTyrK (— — —) and the second fraction (*S,R*)-α-(^{13}C)MeTyrK (· · · · · · ·).

1. the reaction mixture after alkylation of (*S,S*)-TyrK with $^{13}CH_3I$ gave two predominant peaks in the ^{13}C NMR spectrum: minor at 29.3 ppm and major at 28.5 ppm. The diastereomeric excess was 7%;
2. preparative TLC separation of the reaction mixture gave two fractions. In the ^{13}C NMR spectrum of the first fraction a single predominant peak at 29.3 ppm was recorded. The first fraction was associated with minor diastereomer. Similarly, in the ^{13}C NMR spectrum of the second fraction a single predominant peak at 28.5 ppm was recorded. The second fraction was associated with the major diastereomer;
3. circular dichroism spectra of starting (*S,S*)-TyrK and both fractions (diastereomers) of α-(^{13}C)MeTyrK were recorded. Cotton effects in the spectra of both (*S,S*)-TyrK and the first fraction (minor diastereomer) were similar in both areas (650–480 and 480–360 nm). Cotton effect in the spectrum of the second fraction (major diastereomer) in the range 480–360 nm had an opposite sign (Figure 3). Based on these CD data, the *SS* configuration was assigned to the first fraction (minor diastereomer) and the *SR* configuration was assigned to the second fraction (major diastereomer). This assignment is consistent with the proposed predominance of *si*-alkylation leading to (*S,R*)-α-(^{13}C)MeTyrK as the major product (Scheme 1).

The predominant signals in the ^{13}C NMR spectra of diastereomers of α-MeTyrK(OBu-*t*) and α-(^{13}C)MeDOPAK were assigned by analogy. In the case of α-MeDOPAK the major peak at 28.9 ppm was assigned to (*S,R*)-α-(^{13}C)MeDOPAK, the minor peak at 27.7 ppm was assigned to (*S,S*)-α-(^{13}C)MeDOPAK. The diastereomeric excess of the methylation reaction was 12%. Higher diastereomeric excess is probably due to additional steric hindrance introduced by the second methoxy group in the amino acid part of the complex.

Individual diastereomers of α-[^{11}C]MeTyrK(OBu-*t*) and α-[^{11}C]MeDOPAK were successfully separated by preparative HPLC, diluted with excess of water and extracted on C18 cartridges. Optimisation of the procedure including hydrolysis of the complexes (hydrolytic deprotection of enantiomerically pure amino acids) and subsequent purification of the enantiomers of α-[^{11}C]methylDOPA and α-[^{11}C]methyltyrosine is underway.

Conclusion

A synthetic procedure suitable for the routine preparation of (*S*)-α-[^{11}C]methylDOPA and (*S*)-α-[^{11}C]methyltyrosine was developed, final radiochemical synthetic steps are now being optimised.

Acknowledgements

Access to Jasco J-715 is acknowledged (grant MSM 6007665808).

REFERENCES

1. Brogden RN, Heel RC, Speight TM, Avery GS. *Drugs* 1981; **21**: 81.
2. (a) Crich D, Davies JW. *J Chem Soc, Chem Commun* 1989; 1418–1419; (b) Bourne GT, Crich D, Davies JW, Horwell DC. *J Chem Soc, Perkin Trans* 1 1991; 1693–1699; (c) Plenevaux A, Lemaire C, Delfiore G, Comar D. *Appl Radiat Isot* 1994; **45**: 651.

3. (a) Gee AD, Långström B. *J Chem Soc, Perkin Trans* 1 1991; 215–218; (b) Rajagopal S, Venkatachalam TK, Conway T, Diksic M. *Appl Radiat Isot* 1992; **43**: 979.
4. Cumming P, Venkatachalam TK, Rajagopal S, Diksic M, Gjedde A. *Synapse* 1994; **17**: 125.
5. Belokon YN, Bakchmutov VI, Chernoglazova NI, Kochetkov KA, Vitt SV, Garbalinskaya NS, Belikov VM. *J Chem Soc, Perkin Trans* 1 1988; 305–312.
6. (a) Belokon YN, Bulychev AG, Vitt SV, Struchkov YT, Batsanov AS, Timofeeva TV, Tsyryapkin VA, Ryzhov MG, Lysova LA, Bakhmutov VI, Belikov VI. *J Am Chem Soc* 1985; **107**: 4252; (b) Belokon YN. *Pure Appl Chem* 1992; **64**: 1917; (c) Collet S, Bauchat P, Danion-Bougot R, Danion D. *Tetrahedron: Asymmetry* 1998; **9**: 2121; (d) Belokon YN, Tararov VI, Maleev VI, Saveleva TF, Ryzhov MG. *Tetrahedron: Asymmetry* 1998; **9**: 4249; (e) Qiu W, Soloshonok VA, Cai C, Tang X, Hruby VJ. *Tetrahedron* 2000; **56**: 2577; (f) Tang X, Soloshonok VA, Hruby VJ. *Tetrahedron: Asymmetry* 2000; **11**: 2917; (g) Cai C, Soloshonok VA, Hruby VJ. *J Org Chem* 2001; **66**: 1339; (h) Belokon YN, Kochetkov KA, Ikonnikov NS, Strelkova TV, Harutyunyan SR, Saghiyan AS. *Tetrahedron: Asymmetry* 2001; **12**: 481; (i) Debache A, Collet S, Bauchat P, Danion D, Euzenat L, Hercouet A, Carboni B. *Tetrahedron: Asymmetry* 2001; **12**: 761; (j) Popkov A, Císařová I, Sopková J, Jirman J, Lyčka A, Kochetkov KA. *Collect Czech Chem Commun* 2005; **70**: 1397.
7. (a) Popkov A, Gee A, Nádvorník M, Lyčka A. *Trans Metal Chem* 2002; **27**: 884; (b) Saghiyan AS, Hambardzumyan HH, Manasyan LL, Petrosyan AA, Maleev VI, Peregudov AS. *Synth Commun* 2005; **35**: 449.

DOI: 10.1002.jlcr